# 21st SIBBM Seminar

# Frontiers in Molecular Biology

## *Molecular Mechanisms of Cell Response to Biological Sensing*

# *Abstracts*

Siena, Italy • 16-18 June 2026

https://SIBBM2026.azuleon.org

## With the support of

CNR - IFC

EMBL

Francesco Amaldi's Family

NAnA every child a genius

Riccardo Cortese's Family

Università di Siena 1240

## Platinum

GSK

## Gold

TEMA RICERCA

Promega

## Silver

Agilent Trusted Answers

DASIT GROUP | CARLO ERBA REAGENTS

MCE MedChemExpress

MERCK

starlab

## Bronze

BIO CLASS

Sial

Vinci-Biochem

ZANICHELLI

# Speakers' Abstracts

in alphabetical order of presenting author
(presenting authors are shown underlined)

## SMAPs: a new weapon of the CTL killing arsenal that can be tailored for cancer cell targeting

Cosima T. Baldari
University of Siena, Italy

Cytotoxic T cells (CTLs) are the main cellular mediators of the adaptive immune defenses against intracellular pathogens and malignant cells. Upon recognition of specific antigen on their cellular target, CTLs assemble an immunological synapse where they mobilise their killing machinery that is released into the synaptic cleft to orchestrate the demise of their cell target. The arsenal of CTLs is stored in lysosome-like organelles that undergo exocytosis in response to signals triggered by the T cell antigen receptor following antigen recognition. These organelles include lytic granules carrying a cargo of cytotoxic proteins packed on a proteoglycan scaffold, multivesicular bodies carrying the death receptor ligand FasL, and the recently discovered supramolecular attack particles (SMAPs) that carry a core of cytotoxic proteins encased in a non-membranous glycoprotein shell. I will summarise our current understanding of the mechanisms that orchestrate SMAP biogenesis and our efforts to explore their translational potential for cancer cell targeting by grafting a cancer specificity determinant onto a protein component of their outer shell. Exploiting engineered SMAPs as tumour-specific cytotoxic agents introduces a fundamentally new immunotherapeutic paradigm where we decouple cytotoxic activity from living immune cells and open new therapeutic avenues beyond the constraints of current cell-based immunotherapies.

## The genetic and biochemical basis of human leading strand synthesis

Roberto Bellelli
Queen Mary University of London, United Kingdom

Genomic instability is a hallmark of cancer and understanding its nature has provided avenues for cancer therapy. The most prominent example has been the successful use of PARP inhibitors (PARPi) for the treatment of BRCA1/BRCA2-mutant cancers. We have previously shown that loss of the POLE3-POLE4 subunits of DNA Polymerase Epsilon (Pole) sensitizes cancer cells to PARPi by unleashing replicative gap accumulation (**Hill, Ozgencil et al., Cell Reports 2024**). More recently, we have discovered a synthetic lethality between loss of POLE3-POLE4, and the alternative clamp loader complex, CHTF18-DSCC1-CHTF8-RFC2/5 (hereafter CHTF18-RFC) that unveiled the mechanism of leading strand DNA synthesis. By studying this genetic interaction indeed, we discovered two parallel mechanisms that supports efficient leading strand synthesis by Pole, CHTF18-dependent loading of PCNA at leading strands and POLE3-POLE4-dependent binding of nascent dsDNA (**Agnarelli, Buckley-Benbow et al.**, **Nature Communications 2025**). Consistently with loss of Pole processivity being crucial for sensitization to PARPi, we then show that deletion of CHTF18 sensitizes cancer cells to PARPi, highlighting a novel strategy to sensitize cancer cells to these compounds (**Buckley-Benbow et al., under review**). Finally, by studying synthetic lethality in POLE3-POLE4 and CHTF18 KO cells we present unpublished evidence on the existence of additional layers of regulation of processive leading strand synthesis in human cells.

## Targeting RNA methylases in cancer therapy: a novel approach to revolutionize treatment

Sandra Blanco
CIC-IBMCC, University of Salamanca, Spain

RNA modifications, collectively known as the epitranscriptome, constitute an additional and dynamic layer of gene regulation with profound implications for cellular identity, tissue homeostasis, and disease. Although more than 170 RNA modifications have been described to date, their functional relevance in cancer biology is only beginning to be understood. Emerging evidence indicates that non-mutational events, particularly post-transcriptional RNA modifications, play central roles in tumor initiation, progression, metastasis, and therapy response.

Our research aims to decipher how epitranscriptomic marks, including m6A, m5C, m7G, and m1G modifications in tRNA and rRNA, reshape the translational landscape to promote cancer cell adaptation. Using transcriptome-wide sequencing, epitranscriptomic profiling, CRISPR/Cas9-based genetic screens, proteomics, 3D culture systems, mouse models, and patient samples, we investigate how RNA “writers,” “erasers,” and “readers” regulate proliferation, stress responses, metabolic adaptation, immune evasion, and cancer stem cell maintenance.

We identify tRNA and rRNA methylation as critical regulators of translational control and tumor progression. Overexpression of specific RNA methyltransferases correlates with poor prognosis. Loss of tRNA and rRNA methylation reprograms the translation of cell cycle, metabolic, invasion, and inflammatory genes, reshaping tumor fitness and the microenvironment while impairing tumor growth and metastasis in vivo.

Together, our findings show that RNA modifications fine-tune the translational machinery to enable tumor plasticity. Targeting RNA-modifying enzymes represents a promising therapeutic strategy, alone or combined with metabolic or immunomodulatory approaches, opening new avenues for epitranscriptome-based cancer therapies.

## METTL16-mediated inhibition of MXD4 promotes acute myeloid leukemia through activation of MYC-MAX axis

Guglielmo Bove[1], M. Babaei[1], A. Bueno-Costa[3], S. Amin[1], N. Simonelli[1], R. Benedetti[1,10], C. Dell'Aversana[1,9], M. Conte[1], L. Montella[12], V. Summa[13], M. Brindisi[13], M.R. Del Sorbo[1], G. Favale[1], N. Profitos-Peleja[5], V. Carafa[1,2], G. Roué[5], F. Ciardiello[1], H.G. Stunnenberg[4,11], W.L. Megchelenbrink[1,4], A. Nebbioso[1,10], M. Esteller[3,6,7,8], L. Altucci[1,2,9,10], N. Del Gaudio[1]

[1]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
[2]BIOGEM, Via Camporeale Area P.I.P., Ariano Irpino (AV), Italy
[3]Cancer Epigenetics Group, Josep Carreras Leukaemia Research Institute (IJC), IJC Building, Germans Trias I Pujol, Ctra de Can Ruti, Cami de Les Escoles S/N, Badalona, Barcelona, Catalonia, Spain
[4]Prinses Máxima Centrum, Utrecht, The Netherlands
[5]Lymphoma Translational Group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain
[6]Centro de Investigación Biomédica en Red Cancer (CIBERONC), Madrid, Spain
7Instituciò Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain
[13]Department of Pharmacy, School of Medicine and Surgery, University of Naples "Federico II", Naples, Italy

N6-methyladenosine ($m^6A$) is an RNA modification that governs multiple aspects of RNA metabolism, including splicing, translation, stability, decay, and the processing of marked transcripts. Although accumulating evidence suggests that the $m^6A$ writer METTL16 is involved in leukemia, the molecular pathway(s) by which it contributes to leukemogenesis remain unexplored. In this study, we shed light on a novel molecular mechanism whereby METTL16 plays a role in acute myeloid leukemia (AML) progression in an $m^6A$-dependent manner. Our investigations revealed that METTL16 is overexpressed in primary AMLs. Genetic depletion of METTL16 or its pharmacological inhibition strongly affected the proliferation of AML cells, eventually triggering apoptosis. Transcriptome-wide analysis identified mRNA of MAX Dimerization Protein 4 (MXD4), a MYC pathway regulator, as the downstream target of METTL16. Mechanistically, we showed that METTL16 controls the stability of MXD4 mRNA, resulting in a reduction in MXD4 protein levels that indirectly activates the MYC-MAX axis, essential for leukemogenesis. Strikingly, suppression of MXD4 rescued the expression levels of MYC target genes, restoring AML cell survival. Our findings unveil a novel METTL16-MXD4 oncogenic axis crucial for AML progression, suggesting the potential use of METTL16 as a novel therapeutic target in AML and providing a new strategy to target MYC activity in cancer.

## Rewiring tau splicing in FTDP-17 through targeted exon 10 Skipping

Giuseppina Covello[1,2], I. Brentari[1], E. Callegaro[1], L. Cantergiani[1],
D. Perrone[3], M.A. Denti[1]
[1]Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy
[2]Department of Biology, University of Padova, Padova, Italy
[3]Department of Biology and Evolution, University of Ferrara, Ferrara, Italy

The neuropathological aggregation of microtubule (MT)-associated tau protein as Neurofibrillary Tangles (NFTs) in the brain is a pathological hallmark of several neurodegenerative disorders, collectively known as “Tauopathies”, including which Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17), a rare autosomal dominant condition. Among the genes linked to FTDP-17, *MAPT* encodes the tau protein, and approximately half of its mutations affect the alternative splicing of exon 10 (E10). Aberrant inclusion of E10 leads to an imbalance in tau isoforms and promotes pathological aggregation of the tau protein as NFTs in neurons.
We explored the feasibility of an Antisense (as-) RNA-based gene therapy to correct tau splicing in FTDP-17.
Here, we investigated the feasibility of an antisense RNA-based therapeutic approach to correct tau mis-splicing in FTDP-17. Specifically, we assessed whether 2'-O-methyl phosphorothioate antisense oligonucleotides (ASOs) can modulate E10 alternative splicing. We show that ASO transfection modulates tau exon 10 splicing with variable efficiency depending on oligonucleotide concentration and target sequence, both in the endogenous transcript of rat pheochromocytoma (PC-12) cells and in human tau pre-mRNA expressed in HeLa and SK-N-BE cells. We identified two AONs capable of inducing exon 10 skipping. The results were confirmed using scrambled control oligonucleotide.
Building on these findings, we are currently performing proof-of-concept studies, *in vitro,* in orthogonal models of FTLD disease using human *hiPSCs*-FTDP-17-derived neurons.

**Keywords:** RNA Therapeutics, ASOs; *MAPT*; Exon 10 Skipping; Tauopathies; FTDP-17.

## CDK-driven phosphorylation of TRAIP ubiquitin ligase is essential for mitotic replisome disassembly and mitotic DNA synthesis (MiDAS)

Aga Gambus
University of Birmingham, United Kingdom

Disassembly of the replication machinery (replisome) from chromatin is an active process driven by two ubiquitin ligases $Cul2^{LRR1}$ and TRAIP, which both target Mcm7 subunit of the replicative helicase for ubiquitylation. Uncontrolled unloading of replisomes during S-phase would be disastrous for genome stability and cell viability. On the other hand, replisomes retained on under-replicated DNA in mitosis require removal to allow access and processing of the DNA before cell division. $Cul2^{LRR1}$ ubiquitylates helicases only in S-phase and its interaction is restricted to only terminated helicase. TRAIP ubiquitylates replisomes in mitosis but can also act in specific situations during S-phase. However, we do not know how TRAIP's activity is regulated to stop uncontrolled replisome unloading in S-phase but allow all replisome unloading in mitosis.
We have shown that TRAIP activity towards replisomes is not regulated at the level of interaction with the substrate: it interacts with terminated replisomes in S-phase without ubiquitylation. However, in mitosis, TRAIP is phosphorylated by cyclin dependent kinases (CDKs) and this phosphorylation is essential for mitotic replisome unloading. CDK phosphorylation of TRAIP stimulates its autoubiquitylation activity and ubiquitylation of replisomes isolated from mitotic chromatin. The phosphorylation of TRAIP is also important in human cells for TRAIP functions during MiDAS and to stimulate genomic stability. Although essential during mitosis, the CDK-driven phosphorylation of TRAIP is not sufficient to activate uncontrolled unloading of replisomes in S-phase. Altogether, TRAIP is emerging as an important factor of mitotic DNA repair, which is regulated analogously to other mitotic repair factors.

## Characterization of *NFYA* 3′UTR landscape reveals alternative polyadenylation as a targetable vulnerability in prostate cancer

G. Pagani[1], C. Casirati[1], S. Fornezza[1], C. Priami[2], G. Fois[1], S. Bisi[3], A. Mironov[4,5], R. Cacioppo[6], N. Manfrini[1,7], M. Zavolan[4,5], F. Nicassio[3], E. Migliaccio[2], Paolo Gandellini[1]
[1]University of Milan, Milan, Italy
[2]European Institute of Oncology, Milan, Italy
[3]Italian Institute of Technology, Milan, Italy
[4]Biozentrum University of Basel, Basel, Switzerland
[5]Swiss Institute of Bioinformatics, Basel, Switzerland
[6]MRC Laboratory of Molecular Biology, Cambridge, UK
[7]Istituto Nazionale Genetica Molecolare Romeo ed Enrica Invernizzi, Milan, Italy

Alternative polyadenylation (APA) is emerging as a key post-transcriptional mechanism reshaping oncogene regulation and promoting aggressive cancer phenotypes. Here, we investigated whether *NFYA*, encoding the regulatory subunit of the oncogenic transcription factor NF-Y, undergoes APA in prostate cancer. Re-annotation of the *NFYA* 3′UTR—through interrogation of bulk, single-cell, and 3′-end RNA-sequencing datasets, followed by 3′RACE validation—identified four functional isoforms, one of which represents the canonical form in both normal and tumor cells.
Integrated transcriptomic and proteomic analyses across prostate cancer cell lines and patient samples revealed pervasive *NFYA* 3′UTR shortening, which correlated with higher tumor grade, castration-resistant phenotype, increased NF-YA protein levels, and enhanced proliferation. Conversely, *NFYA* 3′UTR lengthening was associated with cellular quiescence. Mechanistically, reporter assays demonstrated that 3′UTR lengthening reduces NF-YA protein expression through decreased mRNA stability, impaired translation efficiency, and enhanced nuclear retention, rather than increased miRNA-mediated repression. Importantly, enforced reprogramming of *NFYA* APA—achieved via CRISPR/Cas9-mediated deletion or antisense oligonucleotide (ASO)-based masking of polyadenylation signals—induced 3′UTR lengthening, reduced NF-YA protein abundance, and attenuated aggressive traits of prostate cancer cells both in vitro and in vivo.
Collectively, these findings establish *NFYA* APA as a critical determinant of oncogenic NF-YA activity and a previously unrecognized driver of prostate cancer progression. Moreover, they provide proof-of-concept for gene-specific, APA-directed therapeutic strategies. ASO-mediated modulation of polyadenylation emerges as a clinically translatable approach to fine-tune oncogene expression, opening new avenues for RNA-based precision therapies in aggressive prostate cancer.

## Extended promoter architecture governs *VDAC3* expression: insights into mitochondrial gene regulation and oxidative stress sensing

Francesca Guarino, S. Reina, G. Nicotra, X. Pappalardo, V. De Pinto
Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

*VDAC3* (Voltage-Dependent Anion Channel 3) gene encodes a mitochondrial outer membrane protein involved in energy metabolism and oxidative stress regulation, yet its transcriptional control remains poorly understood. This study aimed to define the regulatory architecture of the *VDAC3* promoter within the context of cellular sensing and redox-responsive gene regulation. An integrative systems biology approach was applied, combining genomic, epigenomic, and computational analyses. Our analysis shows that the previously described a minimal core promoter, whereas transcription is governed by an extended “maxi promoter region” spanning both upstream and downstream of the transcription start site, consistent with GeneHancer annotations. This region displays hallmarks of long-range chromatin interactions such as strong promoter signals, CpG island, proximal and distal enhancers, active histone marks. Transcription factor binding site analysis revealed a distributed regulatory architecture, with key oxidative stress–responsive factors, including *NRF1*, *BACH1*, and *FOXA2*, predominantly located outside the core promoter, indicating regulatory layers not captured by minimal promoter models. Moreover, the upstream regulatory region displays a high density of evolutionary conserved TCT motifs commonly found in genes involving in mTOR pathway and adaptive responses to metabolic and oxidative stress. This feature suggests that *VDAC3* transcription may be finely tuned through alternative initiation mechanisms, enabling rapid and context-dependent gene expression in response to cellular cues. Overall, this multi-layered analysis identifies previously unrecognized regulatory modules and positions *VDAC3* as a dynamically regulated hub within cellular sensing networks. These findings provide a framework for understanding mitochondrial gene regulation in response to oxidative stress and support future functional validation.

## SINEUPs are a functional class of antisense long non-coding RNAs enhancing translation

Stefano Gustincich[1,2]
[1]Center for Human Technologies, Non-coding RNAs and RNA-based therapeutics, Istituto Italiano di Tecnologia (IIT), Genova, Italy
[2]Center for Clinical and Computational Genomics, Non-coding Genomics, IIT, Aosta, Italy

SINEUP RNAs are a class of natural and synthetic antisense non-coding RNAs that enhance protein expression of target mRNAs. Their activity relies on two functional domains: the Binding Domain, which confers target specificity, and the Effector Domain, which upregulates mRNA translation. This modular architecture enables the rational design of synthetic SINEUPs capable of selectively increasing the translation of virtually any gene of interest. Here, we present data on the biology of natural SINEUPs, their mechanism of action, the design principles of synthetic SINEUPs and representative examples of their therapeutic applications.

## Noncoding RNA roles in the coordination of DNA replication in human cells

Maite Huarte[1,2]
[1]Center for Applied Medical Research, University of Navarra, Pamplona, Spain
[2]Institute of Health Research of Navarra (IdiSNA), Pamplona, Spain

Nuclear RNAs, whether coding or noncoding, are integral components of the chromatin environment, where they intervene in diverse genome-associated processes. While their roles in transcriptional control are well established, the impact of these RNAs on the mechanics of DNA replication and the maintenance of genomic stability remains poorly understood. I will present our latest findings uncovering the multifaceted roles of RNAs in coordinating the duplication of the human genome. We have found that the presence of specific RNAs at transcription start sites is linked to the initiation of replication, where they influence the behavior of the Origin Recognition Complex (ORC). Moving beyond initiation, our research highlights how long noncoding RNAs (lncRNAs) function as essential mediators at the replication fork. We demonstrate that specific lncRNA structural domains are required to engage replication factors, such as the DDX11 helicase, to ensure the correct establishment of sister chromatid cohesion. Furthermore, we characterize a distinct class of repetitive lncRNAs that are enriched at active forks and modulate the efficiency of DNA synthesis while favoring proper chromatin packaging. Collectively, these studies reveal that nascent RNAs provide a critical regulatory layer that ensures the spatial and temporal coordination of transcription and replication in human cells.

## The dark side of Medulloblastoma

A. Belvedere[1], P. Tollis[2], F. A. Mangone[1], T. Santini[1], A. Setti[1], G. Esposito[1], M. Celardo[1], M Ballarino[1], A. Grandioso[1,3], Pietro Laneve[1,3]
[1]Department of Biology and Biotechnologies “C. Darwin”, Sapienza University, Rome, Italy
[2]Center for Life Nano-& Neuro-Science, Fondazione Italian Institute of Technology, Rome, Italy
[3]Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy

Medulloblastoma (MB) is the most common pediatric brain tumor. Despite improved survival, treatments still impose long-term sequelae, underscoring the need for molecularly informed therapies. Although integrated omics has defined four MB molecular subgroups with distinct signatures and drivers, the contribution of long non-coding RNAs (lncRNAs) to subgroup-specific programs remains insufficiently explored. We focused on Group 3 (G3) MB, the most aggressive subgroup, marked by high metastatic burden, poor outcome and frequent MYC amplification. Building on our previous identification of MYC-dependent lncRNAs in G3 MB (Rea et al., 2021), we prioritized lncMB1 and lncMB3 for functional investigation.
LncMB3 emerged as a pro-survival factor, as its depletion induces apoptosis. Mechanistically, lncMB3 modulates the TGF-β pathway through RNA-RNA interactions involving HMGN5, reshaping a photoreceptor-like transcriptional program linked to apoptotic control in G3 MB cells. This mode of action has recently been defined (Grandioso et al., 2025), together with translational efforts including combination of lncRNA targeting with chemotherapy and nanocage-based RNA drug delivery. In parallel, competitive ASOs are being developed to disrupt pathological complexes in G3 MB cells. LncMB1 is transcribed antisense to RHOT1, a key regulator of microtubule-dependent mitochondrial (MT) trafficking. Its depletion reduces RHOT1 mRNA and protein abundance, downregulates gene sets associated with MT metabolism, and causes fragmentation and redistribution of the MT network. Ongoing RNA pulldown RNA-seq, mass spectrometry, and untargeted metabolomics aim to define the basis through which lncMB1 preserves MT homeostasis.
Overall, our findings identify lncMB3 and lncMB1 as core MYC-dependent lncRNAs controlling complementary hallmarks of G3 MB aggressiveness, apoptotic signaling and MT dynamics, and support their candidacy as RNA-based therapeutic vulnerabilities in the deadliest MB subgroup.

## An RNA-extension activity of pol η contributes to the resolution of transcription–replication conflicts during HU-induced replication stress

L. Grasso[1,5], E. Galati[1], G.M. Nava[1], L. Atzeni[1,6], R. Quadri[1], A. Chakraborty[1], G.W. Brown[2,3] F. Zambelli[1,4], M. Muzi-Falconi[1], Federico Lazzaro[1]

Transcription-replication conflicts (TRCs) and R-loops are prominent sources of replication stress and genome instability. They are driven by transcription-replication interference and exacerbated by deoxyribonucleotide depletion, as upon Hydroxyurea (HU) treatment. In this study, we characterize a novel, translesion synthesis-independent role of DNA polymerase η (pol η) under HU-induced replication stress in *Saccharomyces cerevisiae*. We demonstrate that, during HU-challenged S phase, pol η is recruited to a subset of active replication origins in the proximity of highly transcribed genes, independently of the orientation of collisions, suggesting a general role in TRCs resolution. Our data support a model where pol η contributes to overcoming TRCs by elongating nascent RNA transcripts and incorporating RNA into DNA. The resulting RNA:DNA hybrid structures cannot be processed in the absence of RNase H activity, leading to checkpoint activation, persistent stress, and cell death. The toxic effects of pol η are prevented by overexpression of Sen1 or RNase H1 or by inhibition of transcriptional elongation. These findings imply that the RNA-extension capacity of pol η facilitates TRCs bypass by remodeling and/or stabilizing RNA:DNA hybrids at stalled forks. Our work provides insights into how cells cope with TRCs, even when canonical R-loop resolution pathways are compromised.

## Dysregulation of centrosomal protein STARD9 as a novel therapeutic vulnerability in ER+ breast cancer

Ylenia Martinini[1,*], A. Duatti[1,*], E. Pardella[1,*], A. Smiriglia[1], G. Comito[1], M. Mangoni[1,2], S. Bianchi[3], L. Visani[2], I. Meattini[1,2], G. Giurato[4], C. Ferravante[4], A. Salvati[4], G. Nassa[4], L. Livi[1,2], A. Morandi[1]
[1]Department of Experimental and Clinical Biomedical Sciences “Mario Serio”, University of Florence, Florence, Italy
[2]Radiation Oncology Unity, Department of Oncology, AOU Careggi, Florence, Italy
[3]Department of Health Science, Division of Pathological Anatomy, University of Florence, Florence, Italy
[4]Department of Medicine, Surgery and Dentistry “Scuola Medicina Salernitana”, University of Salerno, Baronissi, Italy
*These authors contributed equally

ER+/HER2- breast cancer (BC) is the most frequently diagnosed subtype and is often associated to favorable outcomes. However, late recurrence and metastatic progression remain critical challenges, with long-term survival in the metastatic setting still limited. Here, we performed genomic and transcriptomic profiling of ER+/HER2- metastatic BC patients who, despite an initially poor prognosis, exhibited an unexpectedly prolonged survival for >10 years after diagnosis, markedly exceeding typical clinical expectations. These patients received heterogeneous treatments, with CDK4/6 inhibitors commonly administered. Cross-analysis of paired primary and relapsed tissues identified *STARD9* as the only commonly gene mutated and upregulated in relapsed tumors. *STARD9 encodes a* poorly characterized centrosomal protein involved in spindle pole assembly and mitotic stability. To investigate its role, we overexpressed the kinesin motor domain of STARD9 in ER+/HER2- BC cell lines, observing reduced cell viability and increased sensitivity to CDK4/6 inhibitors. Mechanistically, confocal microscopy and comet assay revealed that *STARD9* overexpression increases DNA damage and micronuclei formation. We also observed disruption of mitotic checkpoints (TTK and AURKA) and key cell cycle regulators (Rb, CCND1 and CCNB1), suggesting a role for STARD9 in chromosomal instability and mitotic catastrophe, ultimately leading to apoptosis. Further, combining *STARD9* overexpression with docetaxel (microtubule-disrupting agent), alisertib (AURKA inhibitor), or luvixasertib (TTK inhibitor) markedly reduced cell viability. Finally, we supported our *in vitro* findings with immunohistochemistry and Kaplan-Meier analyses in ER+/HER2- BC patients, showing that higher *STARD9* expression correlates with improved survival. Overall, our findings identify *STARD9* as a novel prognostic and therapeutic biomarker in BC, with potential utility in stratifying patients who may especially benefit from targeted therapies.

## Targeting the m6A pathway in ALK-driven anaplastic large cell lymphoma

Sara Milioni[1], L. J. Mun[2], V. Miano[2], S. Maestri[3], M. Pelizzola[3], S. Turner[2], I. Barbieri[1,2]
[1]University of Turin, Department of molecular biotechnology and health sciences, Molecular Biotechnology Center, Italy
[2]Division of cellular and molecular pathology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom
[3]Center for Genomic Science of IIT@SEMM, Fondazione Istituto Italiano di Tecnologia (IIT), Milan, Italy

Methyltransferase 3 (METTL3)-mediated mRNA-m$^6$A methylation is emerging as a druggable target in haematologic malignancies, yet its regulation and therapeutic value in other haematological malignancies such as anaplastic large cell lymphoma (ALCL) remain undefined. Here, we show that METTL3 expression correlates with a clinically aggressive subset of the peripheral T cell lymphoma, anaplastic lymphoma kinase (ALK)-positive ALCL whereby METTL3 expression distinguishes a population of patients that relapse early and have a lower event free survival. To uncover the mechanism driving this phenotype, we show using a CUT&RUN assay that the ALCL-defining oncoprotein nucleophosmin1-anaplastic lymphoma kinase (NPM1-ALK) is present on chromatin in the region of transcriptional start sites (TSS), notably at the METTL3 promoter containing a conserved STAT motif. Disrupting this complex removes H3K4me3 and Pol II-Ser5P from the METTL3 locus, reducing METTL3 expression and destabilising METTL14/WTAP proteins leading to decreased global m$^6$A levels. Furthermore, MeRIP-seq after ALK-inhibition identified hypomethylated transcripts related to chromatin remodelling and kinase pathways. These data suggest that METTL3 drives an aggressive phenotype through enhanced transcriptional activity in the cells via these pathways.
It follows that METTL3 knockdown and/or functional inhibition with STM2457 reduced cell proliferation and enhanced cytotoxicity particularly when administered in combination with ALK inhibitors, which *in vivo*, lead to tumour regression.
Collectively, our data position catalytic NPM-ALK as a direct transcriptional activator of METTL3, establishing METTL3-dependent m$^6$A wiring as a downstream effector of aberrant ALK signalling providing a pre-clinical rationale for dual ALK/METTL3 targeting in relapsed and/or resistant ALK-positive ALCL.

## A novel class of proteinaceous particles as both therapeutic targets and delivery platforms for glioblastoma treatment

Stella Nappi[1], C. Staton[2,3], C. Cassioli[1], N. Capitani[1], A. Hoyle[2], I. Vendrell[4,5], R. Fischer[5], M.L. Dustin[2], C. Ulivieri[1]*, C.T. Baldari[1]*
[1]Department of Life Sciences, University of Siena, Italy
[2]Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK
[3]Institute for Developmental and Regenerative Medicine, Department of Paediatrics, University of Oxford, Oxford, UK
[4]CAMS Oxford Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[5]Target Discovery Institute, Centre for Medicines Discovery, University of Oxford, Oxford, UK
*Shared senior authorship

Glioblastoma (GBM) is the most aggressive and therapy-resistant brain tumor in adults. Despite aggressive standard treatment based on surgical resection followed by radiotherapy and chemotherapy, patient outcomes remain poor, and immunotherapy approaches, including immune checkpoint inhibitors and engineered T cells, have shown limited efficacy. This resistance is largely driven by the complex tumor microenvironment (TME) and a still incomplete understanding of the mechanisms underlying bidirectional communication between GBM cells and their surrounding immune and stromal components.
Intercellular communication within the TME is increasingly recognized to be mediated by extracellular particles. Here we identify a previously uncharacterized subpopulation of membrane-free extracellular particles (~100 nm) in the secretome of U373 glioblastoma cells, termed Thrombospondin-Encapsulated Dense Particles (ThrEDs). These entities are characterized by a thrombospondin glycoprotein shell and have a protein composition enriched in proteins associated with metabolic reprogramming and cellular motility.
Functional in vitro analysis revealed that tumor derived-ThrEDs, unlike canonical membrane-bound extracellular vesicles, significantly promote migration of low-grade glioma cell model and suppress T cell activation by altering cytoskeletal dynamics.
Notably, smaller non vesicular extracellular particles, such as exomeres and supermeres, have been recently shown to cross the blood brain barrier, highlighting their potential as vehicles for brain-targeted therapies.
Together, our findings uncover a novel mechanism of immune modulation in GBM and provide a foundation for exploring ThrEDs as both therapeutic targets and delivery platforms.

## From planarian neoblasts to mammalian stem cells: a cross-species screen for conserved and novel molecular regulators

L. Spagnoletti[1], A. Codino[1], A. Simi[1], A. Cuomo[2], G. Millán-Zambrano[3], I. Barbieri[4], F. Cremisi[5], S. Gustincich[1], Luca Pandolfini[1]
[1]Center for Human Technologies, Istituto Italiano di Tecnologia (IIT), Genoa, Italy
[2]Istituto Europeo di Oncologia (IEO), Milan, Italy
[3]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Seville, Spain
[4]University of Turin, Turin, Italy
[5]Scuola Normale Superiore di Pisa, Pisa, Italy

Planarians are freshwater flatworms renowned for their extraordinary regenerative capabilities, which are sustained by neoblasts, a population of adult pluripotent stem cells capable of generating every differentiated cell type. For decades, they have served as a powerful model for the molecular dissection of regeneration, tissue homeostasis, and stem cell biology.
Recently, my lab contributed to establishing high-quality, chromosome-level reference genomes and annotations for multiple planarian species[1]. Beyond cementing planarians as a premier model for regeneration, these resources position them as an evolutionary reservoir for probing protein modularity. A notable fraction of planarian genes encode orphan proteins, while others fuse conserved domains with sequences of unknown structure and function. This offers a unique window into how modular shuffling drives evolutionary innovation.
To explore these features in a more experimentally tractable setting, we strieved to integrate this model with our expertise in mouse developmental biology and *in vitro* neural stem cell differentiation[2]. We cloned an expression library from planarian neoblasts and used it to perform a cross-species functional screen, introducing planarian coding sequences into a mammalian neural stem cell system to identify inducers of neuralization. I will present the results of this screen, which uncover both deeply conserved regulators of stem cell behavior and planaria-specific protein innovations with no vetebrate counterpart.
Together, these findings establish cross-species functional genomics as a strategy to mine divergent genomes for novel biology, and they highlight planarians as a source of previously inaccessible protein functions relevant to stem cell regulation and development.

[1] Ivanković et al. *Nat Commun* **15**, 8215 (2024) doi:10.1038/s41467-024-52380-9
[2] Codino *et al. Nat Commun* **16**, 7051 (2025). 10.1038/s41467-025-62414-5

## Tunneling nanotubes provide a route for SARS-CoV-2 spreading

Anna Pepe, M. Zhang, D. Valente, C. Zurzolo
Institut Pasteur, Paris, France

Neurological manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection represent a major issue in long coronavirus disease. How SARS-CoV-2 gains access to the brain and how infection leads to neurological symptoms are not clear because the principal means of viral entry by endocytosis, the angiotensin-converting enzyme 2 receptor, are barely detectable in the brain. We report that human neuronal cells, nonpermissive to infection through the endocytic pathway, can be infected when cocultured with permissive infected epithelial cells. SARS-CoV-2 induces the formation of tunneling nanotubes (TNTs) and exploits this route to spread to uninfected cells. In cellulo correlative fluorescence and cryo–electron tomography reveal that SARS-CoV-2 is associated with TNTs between permissive cells. Furthermore, multiple vesicular structures such as double-membrane vesicles, sites of viral replication, are observed inside TNTs between permissive and nonpermissive cells. Our data highlight a previously unknown mechanism of SARS-CoV-2 spreading, likely used as a route to invade nonpermissive cells and potentiate infection in permissive cells.

## 3D Blueprints of malignancies

Stefano Piccolo
University of Padua, Padua, Italy

The tissue-level processes underpinning metastatic outgrowth remain unclear. We combined single-cell RNA sequencing, spatial transcriptomics, AI-supported 3D imaging and topology metrics in human breast cancer with functional investigations in mice to uncover a 3D morphogenetic process essential for macrometastatic expansion. I will present evidence that metastases redeploy developmental morphogenetic programs to build macrometastases as a 3D trabecular lattice of epithelial cords. I will discuss the role of metastatic cells within primary tumors destined to metastasize, and, more generally, the connection between tissue form and function in malignancies.

## Targeting METTL3 induces homologous recombination defects and sensitizes tumors to PARP inhibition across solid cancers

Fabio Piscopo[1], B. Cesaro[1] , V. La Monica[2,3], A. Ferri[2,3], G. Maroni[1], L. Del Giacco[4], D. Rotili[5], C. Sette[6], A. Marzio[2,3,7], A. Fatica[1]
[1]Department of Biology and Biotechnology “Charles Darwin”, Sapienza University of Rome, Rome, Italy
[2]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, Weill Cornell Medicine, New York, NY, USA
[3]Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA
[4]Dept. of BioSciences, University of Milan, Milan, Italy
[5]Department of Science, Roma Tre University, Rome, Italy
[6]GSTeP Organoids Research Core Facility, Fondazione Policlinico A. Gemelli IRCCS, Rome, Italy
[7]Parker Institute for Cancer Immunotherapy (PICI), Weill Cornell Medical College, Weill Cornell Medicine New York, New York, NY, USA

Aberrant m$^6$A RNA methylation, driven by METTL3, has been implicated in DNA damage responses and cancer cell adaptation. However, whether this pathway can be effectively exploited for therapeutic intervention remains unclear. Here, we show that pharmacological inhibition of METTL3 induces homologous recombination defects and creates a therapeutically actionable vulnerability across different solid tumors. In triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and ovarian cancer (OC), METTL3 inhibition reduces proliferation and enhances sensitivity to DNA-damaging agents. Notably, METTL3 inhibition synergizes with PARP1/2 inhibitors across models, including patient-derived organoids with homologous recombination-proficient (HRP) status, typically resistant to PARP inhibition. Mechanistically, METTL3 inhibition impairs homologous recombination, as shown by reduced chromatin-bound RAD51 following DNA damage, further enhanced by PARP trapping. *In vivo*, METTL3 targeting suppresses tumor growth and potentiates response to DNA damage-based therapies. Together, our findings suggest METTL3 as a therapeutic target in DNA repair and support its inhibition as a strategy to induce synthetic lethality and improve PARP inhibitor efficacy in cancer.

## Accelerating drug discovery of mitochondria diseases with brain organoid models

Alessandro Prigione
Heinrich Heine University, Düsseldorf, Germany

Mitochondrial diseases, caused by pathogenic variants in the nuclear or mitochondrial DNA, represent a major therapeutic challenge due to the paucity of effective model systems. One of the most severe forms of mitochondrial disease is Leigh syndrome (LS), a devastating neurological disease with no cure that leads to early death in children. New Approach Methodologies (NAMs), such as induced pluripotent stem cells (iPSCs) and derived organoids, offer unprecedented opportunities for building alternative human-relevant models. In this talk, I will present our ongoing efforts in advancing the understanding and therapies for LS using patient-derived iPSCs and brain organoids as NAMs. Examples of innovative interventions for LS are shown based on iPSC-driven deep learning or high-content based screening. We discovered Sildenafil as a new repurposable drug that has received the designation of Orphan Drug from the European Medicines Agency (EMA) for the treatment of LS. For this, a clinical trial is currently under development. Overall, we show how brain organoids can be harnessed as NAMs to accelarate the development of innovative therapies for incurable pediatric mitochondrial diseases with highly unmet medical needs.

## A Haspin-Fab1 axis modulates TORC1 localization and activity in budding yeast

Roberto Quadri, Z. Turan, E. Galati, T. Vaccari, M. Muzi-Falconi
Dept. of Biosciences, University of Milan, Milan, italy

The highly conserved eukaryotic kinase mTOR is a central player in the regulation of cell metabolism and proliferation. Its pivotal role is to integrate a plethora of stimuli ranging from nutrient availability to energy levels and stresses into cell growth control. Although intense research in the field has elucidated an intricate network of mTOR regulators, the picture is far from being complete and novel players are continuously emerging. Recently, a tight interplay between this protein and the lipid kinase Fab1 has been reported in budding yeast, showing that TORC1, one of the two budding yeast mTOR complexes, phosphorylates Fab1 to promote its own relocalization to specialized endosomes (SE) overall dampening TORC1 activity.
Here we show that yeast Haspin acts through a similar, Fab1-dependent mechanism to modulate TORC1 distribution and activity. *sc*Haspin paralogues physically interact with Fab1 and mediate TORC1 accumulation on SE, driving a reduction in its activity as well as overseeing vacuolar dynamics. Remarkably, Haspin and TORC1 are not part of the same signaling axis converging on Fab1 to modulate TORC1 activity, as they respond to distinct stimuli. The nature of Haspin-transduced signal presumably corresponds to a mitotic stimulus whose nature is currently under investigation. This pathway is likely conserved to human cells, as human Haspin and Fab1 physically interact, and Fab1 is phosphorylated by Haspin and might represent a valuable tool to modulate mTOR in the context of cancer therapy as well as ageing. Indeed, preliminary experiments show a reduced chronological lifespan of yeast Haspin mutants.

## Genome-scale cancer dependency maps reveal specific vulnerabilities in MYCL-amplified small cell lung cancer

Alessandro Restellini[1], I. Cassotti[1], T. Mora[1], G. Fois[1], D. Tolomeo[2], C.T. Storlazzi[2], P. Gandellini[1]
[1]Department of Biosciences, University of Milan, Milan, Italy
[2]Department of Biosciences, University of Bari Aldo Moro, Bari, Italy

Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine tumor (~15% of lung cancers) that lacks effective therapies. *MYC* paralogs (*MYC*, *MYCL*, *MYCN*) are frequently amplified in SCLC in a mutually exclusive manner, with *MYCL* amplification (MYCL+) being the most common. Although these paralogs have been considered functionally equivalent, most studies have focused on MYC.

Here, interrogation of genome-scale loss-of-function screens (DepMap) revealed that *MYC* and *MYCL* display distinct co-dependency profiles, with *MAX* emerging as a MYC-specific co-dependency. Consistently, MYCL+ SCLC cells express higher levels of a shorter *MYCL* isoform lacking the MAX dimerization domain, raising concerns about the efficacy of MYC–MAX targeted therapies in MYCL+ patients. Moreover, MYCL+ cells do not exhibit *MYC* oncogene addiction, unlike MYC-amplified (MYC+) cells, while being modestly vulnerable to *MYCL* inhibition.

Marked SCLC plasticity has so far limited the identification of effective targets derived from transcription-based molecular classification. Notably, we found that genome-scale RNAi dependency profiles group SCLC cells more coherently by paralog amplification status than by molecular subtype, suggesting that these genomic alterations define distinct and unrecognized essentialities.

We identified specific vulnerabilities of MYCL+ SCLC cells, which were enriched for protein–protein interactions and clustered in defined chromosomal regions. These included genes rarely mutated or deleted in MYCL+ cells, supporting their role as context-specific dependencies. Among these, *E2F1*, *TFDP1*, and *KDM1A* showed increased essentiality in MYCL+ models regardless of their molecular subtype, a finding validated *in vitro* by siRNA-mediated knockdown followed by growth assays.

Overall, this study identifies candidate precision therapy targets for MYCL+ SCLC and suggests that shifting from subtype-based to paralog-driven classification may uncover novel therapeutic opportunities.

## Top1cc-driven genome instability imprints distinct indel and structural variant signatures in colorectal cancer

Marco Russo[1,2], G. Grottini[1], S. Morelli[3], F. Martínez-Jiménez[4,5], J. Marinello[1,2], G. Capranico[1,2]
[1]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
[2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[3]School of Medicine, Vita-Salute San Raffaele University, Milan, Italy
[4]Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain
[5]Hartwig Medical Foundation, Amsterdam, Netherlands

Topoisomerase I (Top1) regulates DNA topology during transcription and replication by forming transient Top1–DNA cleavage complexes (Top1ccs), which are stabilized by camptothecin (CPT) analogs, increasing Top1cc levels, disrupting nucleic acid synthesis, and promoting Top1 degradation; we showed that these stabilized Top1ccs enhance transcription–replication conflicts, inducing DNA double-strand breaks at highly transcribed early-replicating genes and driving chromosomal instability (PMID: 38787953), suggesting that persistent Top1ccs may reshape the cancer genome through site-specific mutations and rearrangements.
Because the mutational footprint of Top1 poison therapy remains poorly defined, we performed an exploratory analysis of mutational signatures in metastatic colorectal cancer whole-genome sequencing data from the Hartwig Medical Foundation cohort. Among insertion/deletion signatures, only de novo ID6 (mainly characterized by 1 bp deletions at C/T nucleotides) showed specificity for Top1 poison treatment. In parallel, analysis of structural variants revealed an increased tumor mutational burden (TMB) of SVs in treated samples, with enrichment of a de novo SV6 signature characterized by tandem duplications.
To validate these observations, we generated WGS data from colorectal cancer cell lines (HCT116 and HT29) treated with CPT and compared them with untreated controls, observing an increased structural variant mutational burden alongside a slight increase in indel signatures, consistent with the cohort-derived results.
Overall, these results identify a Top1 poison–associated mutational phenotype in colorectal cancer, encompassing both indel and structural variant features, and suggest that Top1cc-driven genome stress promotes distinct and recurrent genomic alterations. Ongoing work will refine the definition of these signatures and elucidate the mechanisms linking Top1cc stabilization to localized indel formation and structural variation.

## Determinants and consequences of the pattern of DNA replication

Julian E. Sale
Division of Protein & Nucleic Acid Chemistry, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The spatial and temporal organisation of DNA replication is a defining feature of genome function yet the extent to which it is encoded by DNA sequence versus shaped by chromatin context remains incompletely resolved. I will discuss our work over the past four years integrating methods for quantitative mapping of replication origin, modelling and cross-species analyses to define the determinants and consequences of replication programme organisation in mammalian cells.
Our method for simultaneously mapping origin location and efficiency, Ini-seq 2, demonstrated that the most efficient sites of replication initiation are organised at the borders of broad initiation zones. These maps allowed us to show that replication initiation is an important determinant and modulator of mutational patterns both in the population and in cancer.
Recent work has shown that origin activity can be quantitatively predicted from distributed sequence features, supporting a model in which replication emerges from a probabilistic, sequence-encoded initiation landscape that is modulated, but not necessarily specified, by chromatin state. However, we have also found that the organisation of the replication programme breaks down when genomic segments are isolated in extrachromosomal circular DNA molecules, which are frequently found in cancer cells, suggesting that context does play a significant role in determining replication patterns.
I will discuss very recent work that directly explores this interplay between sequence and context by examining the replication programme of human chromosomes when transferred out of their native environment into mouse cells using methods developed as part of the Synthetic Human Genome project. Understanding the relationship between sequence and cellular / chromatin context in determining the pattern and timing of replication will be essential in the design and writing of artificial genomes that have predictable, and non-deleterious, replication dynamics.

## Promoting neural stem cell maturation via mechanical forces for next-generation neurodegenerative disease therapies

Laura Talarico[1], A. Zanelli[1], Y. Horiguchi[2], F. Coppedè[3], J. Takahashi[2], M. Onorati[1], F. Raudzus[2], V. Raffa[1]
[1]Department of Biology, University of Pisa, Italy
[2]Department of Clinical Application, Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan
[3]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy

Neurodegenerative and traumatic diseases, such as spinal cord injury (SCI) and Parkinson's disease (PD), share common mechanisms including neuronal degeneration and cell loss. Stem cell–based replacement therapies have therefore become a key strategy, as stem cells can generate new neurons capable of restoring damaged circuits. However, their therapeutic efficacy critically depends on achieving proper neuronal maturation and functional integration.
To address this challenge, our laboratory has developed nano-pulling, a technique to apply remote mechanical stimulation to neural cells. Mechanical cues are emerging as potent regulators of neuronal development, and nano-pulling has been shown to induce morphological and functional changes in neural precursors. Thus, nano-pulling may represent a promising tool to enhance neuronal maturation.
Here, we investigated the long-term effects of nano-pulling on human spinal cord neuroepithelial stem (SC-NES) cells transplanted into mouse spinal cord organotypic slices. Mechanically stimulated samples showed increased expression of pre-synaptic markers and enhanced neuronal network formation, indicating accelerated maturation. To explore underlying mechanisms, we analyzed the epigenetic state of genes involved in neuronal differentiation in *in vitro* SC-NES cultures. Preliminary data on SOX2, NEUROG2 and Nestin revealed methylation trends consistent with increased maturation via nano-pulling.
Eventually, we extended our analyses to human iPSC-derived 3D models by generating induced dopaminergic (iDA) progenitor neurospheres and transplanting them into mouse organotypic brain slices. The neurospheres successfully engrafted and extended neuronal processes into the surrounding tissue.
These findings support the potential of nano-pulling to improve the maturation and functional integration of stem-cell-derived neurons, advancing the development of effective cell transplantation therapies for neurodegenerative and traumatic diseases.

## Choosing to be different: mapping neural stem cell fate to organelle function in brain development

Elena Taverna
Human Technopole, Milan, Italy

Neurons in the developing human brain arise from two main classes of neural progenitors—apical and basal progenitors (APs and BPs)—which differ markedly in polarity, morphology, and proliferative behavior. Understanding how these distinct identities are established and maintained remains a central question in developmental neurobiology.
Our work addresses whether organelle function contributes to neural stem cell identity and fate decisions, with a particular focus on the Golgi apparatus. As the central hub of cellular glycosylation, the Golgi is critically linked to human neurodevelopment, and its dysfunction is associated with primary microcephaly, a disorder characterized by altered neural progenitor behavior and lineage progression.
We find that the Golgi apparatus undergoes a striking reorganization during the transition from APs to BPs, particularly in its spatial relationship with the centrosome, pointing to a remodeling of intracellular architecture along the centrosome–Golgi axis during fate specification. Strikingly, perturbing Golgi positioning and function is sufficient to alter AP-to-BP fate transitions, demonstrating that organelle organization is not merely a consequence of cell identity but can actively instruct it.
Together, our findings uncover a reciprocal interplay between genetic programs and intracellular organization in neural stem cells and support a model in which organelles act as regulators of cell fate decisions during brain development.

## Genotype-phenotype single-cell transcriptomics for massive parallel assessment of genetic variants

Lorenzo Vaccaro[1,2,$], R. De Santis[1,2,$], F. Panariello[1], A. Grimaldi[1], G.Gandolfo[1], M. Massa[1,2], T. Fusco[1,2], M. Salvi[1,2], M. Bamundo[3], G. Gambardella[4], R. Arnese[4], E. Agolini[5], C. Cesario[5], I.M. Abdelrazek[6], H. AbdelGhany[6], A. Novelli[5], M. Cesana[7], C. Ziegenhain[8], C. Missero[3,9], D. Cacchiarelli[1,2,10]
[1]Telethon Institute of Genetics and Medicine (TIGEM), Armenise/Harvard Laboratory of Integrative Genomics, Pozzuoli, Italy
[2]Department of Translational Medicine, University of Naples “Federico II”, Naples, Italy
[3]CEINGE Biotecnologie Avanzate Franco Salvatore, Naples, Italy
[4]Telethon Institute of Genetics and Medicine (TIGEM)
[5]Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[6]Department of Human Genetics, Medical Research Institute, Alexandria University, Alexandria, Egypt
[7]Department of Advanced Biomedical Sciences, University of Naples “Federico II”, Naples, Italy
[8]Division of Medical Systems Bioengineering, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
[9]Department of Biology, University of Naples Federico II, Naples, Italy
[10]Scuola Superiore Meridionale (SSM, School of Advaced Studies), Naples, Italy
[$]Co-first authors

Predicting the pathogenic effect of rare genetic variants remains a significant challenge, hindered by the limited availability of diagnosed patient cohorts and the difficulty in systematically evaluating the impact of both existing and novel variants across diverse functional contexts. To address this issue, we first leveraged a large-scale protein Multiplexed Assay of Variants Effect (MAVE) to functionally assess ~2,300 missense variants of the TP63 gene, which are strongly associated with autosomal dominant developmental disorders. Variant activity was precisely measured using an optimized fibroblast-to-keratinocyte conversion protocol, providing an in-vivo-like functional readout. This comprehensive MAVE effort yielded a clinically actionable dataset, reclassifying hundreds of common variants and definitively resolving a Variant of Unknown Significance as pathogenic in a patient with ectodermal dysplasia. However, classical MAVE methodologies are fundamentally constrained by the requirement for specific, predefined phenotypic assays. To overcome this bottleneck and enable systematic, future-proof variant screening, we developed SCRAMseq (Single Cell RNAseq Associated with MAVEs by sequencing). This generalizable platform leverages full-length scRNAseq for high-throughput, single-cell genotype-to-phenotype mapping, utilizing the entire transcriptome as the functional readout. Extensive validation across diverse biological contexts, including different genes and distinct disease-driving pathways, confirms SCRAMseq’s superior reproducibility and accuracy (also compared with bulk assay based on cell conversion). SCRAMseq is a robust and user-friendly workflow set to accelerate the discovery and dissection of gene variant effects on any potential disease-driving pathway, enabling the application of MAVEs to any possible disease driving gene.

# Poster Abstracts

(presenting authors are shown underlined)

P.1

## A novel molecular mechanism driven by Cystatin B during early stages of human neuronal development

Natalia Abate[1], M.V. Pravatà[2], N. Conte[1], E. Penna[1], F. Barracca[1], F. Ragona[3], L. Canafoglia[3], S. Cappello[2], M. Crispino[1], R. Di Giaimo[1]
[1]Department of Biology, University of Naples Federico II, Naples, Italy
[2]Biomedical Center, Faculty of Medicine, Ludwig-Maximilians-University, Munich, Germany
[3]Integrated Diagnostics for Epilepsy, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Loss-of-function mutations in Cystatin B (CSTB) cause EPM1, a progressive myoclonus epilepsy characterized by early-onset neurodegeneration. CSTB roles in neurodevelopment have been demonstrated using human cerebral organoids (COs). Single-cell RNA sequencing analysis of ventral EPM1-COs revealed an abnormal specification of progenitor fate, with a shift toward dorsal neuron identities. This misspecification resulted from impaired extracellular vesicle (EV) dynamics and altered protein cargo, establishing CSTB as a safeguard of ventral patterning. Here, we investigated the molecular mechanisms altered in pathology, by looking at CSTB interactors during human neurodevelopment. Using COs patterned toward ventral identity, we identified a strong interaction between CSTB and the TRiC/CCT chaperonin complex, a key regulator of many cellular processes. To determine the pathological relevance of this interaction, we examined whether its disruption contributes to EPM1 phenotypes. Remarkably, ventral EPM1 neurons display early and persistent impairment of protein synthesis, emerging at the onset of neuronal differentiation. This is accompanied by cytoskeletal disorganization and fragmentation, altered endosomal maturation and consequent defective EV biogenesis and secretion, indicating a broad impairment of cellular processes underlying neuronal development, many of which depend on CCT-complex. Intriguingly, restoring CSTB protein level in EPM1 cells rescues protein synthesis defects. However, simultaneous silencing of one of the CCT-complex components, via CRISPR-i, abolishes this rescue, demonstrating that CSTB functions critically depend on its interaction with the CCT complex.
Together, these findings identify CSTB-CCT cooperation as a key regulatory mechanism linking proteostasis, cytoskeletal dynamics and vesicle-mediated signaling, revealing a novel pathogenic pathway underlying EPM1.

## Turning TRPM8 and PSMA against prostate cancer by exploiting convergent lethality

Nicole Annesi, M. Cortese, F. Lorenzini, E. Marmocchi, M. Cantarelli, R. Begaj, M. C. Mione, A. Alaimo, A. Lunardi
Department of Cellular, Computational and Integrative Biology - CIBIO, University of Trento, Trento, Italy

Prostate cancer (PCa) is the most common non-cutaneous malignancy in men. Current treatments for metastatic PCa target the androgen pathway, however, almost invariably, tumor progresses to the lethal castration resistant (mCRPC) stage of the disease. Defining new therapeutic strategies for advanced PCa remains an unmet clinical need.
In this study, we investigated the over activation of two highly expressed markers in PCa: the Transient Receptor Potential Melastatin 8 (TRPM8) and the Prostate-Specific Membrane Antigen (PSMA). TRPM8 is an ion channel whose overexpression supports tumorigenesis through $Ca^{2+}$ signaling. However, it has been shown that administration of TRPM8 agonists strikes cancer cells in combination with sublethal dose of standard therapies. PSMA, instead, acts as a plasma membrane enzyme that releases glutamate from folates (Vitamin B9). This glutamate activates metabotropic receptors (mGluR1/5), triggering pro-tumoral PI3K and MAPK pathways and inducing $Ca^{2+}$ release from intracellular stores. Here we show that concomitant administration of the TRPM8 agonist D-3263 and Vitamin B9 in PCa cells co-expressing TRPM8 and PSMA results in a convergent lethal effect most likely due to $Ca^{2+}$ cytotoxicity. This combined treatment showed superior efficacy compared to monotherapies also *in vivo*, in zebrafish xenografts co-expressing TRPM8 and PSMA, resulting in a significant slowdown in tumor growth. Since both TRPM8 and PSMA are detectable in extracellular vesicles released by PCa cells, liquid biopsy represents a promising tool for stratifying mCRPC patients who may benefit most from this convergent lethality strategy. In conclusion, our data suggest that the TRPM8/PSMA axis represents a specific metabolic vulnerability that can be exploited against PCa, offering a novel and effective therapeutic opportunity for the treatment of advanced stages of the disease.

P.3

## Novel strategies to reactivate p53 and overcome drug resistance in tumors

Ylenia Antonacci[1,3], F. Marzano[1], D. I. Abbrescia[1], B. Balech[1], C. Pousis[1], C. Telegrafo[1], M. F. Caratozzolo[1], A. M. D'Erchia[3], P. Zanfardino[4], V. Petruzzella[4], L. Guerrini[5], E. Sbisà[2], G. Pesole[1,3], A. Tullo[1]
[1]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, Bari Italy
[2]Institute of Biomedical Technologies Consiglio Nazionale delle Ricerche Bari, Italy
[3]Department Biosciences, Biotechnologies and Environment, University of Bari A. Moro, Bari, Italy
[4]Department of Translational Biomedicine and Neuroscience-DiBraiN, University of Bari A. Moro, Bari, Italy
[5]Department of Biosciences, University of Milano, Italy

Chemoresistance is one of the major limitations affecting the effectiveness of cancer therapies. In tumor cells, mutations or alterations in the p53 tumor suppressor pathway can impair tumor cell elimination induced by chemotherapy. The *TP53* gene plays a key role in several biological processes, including cell cycle arrest, apoptosis, senescence, DNA repair, and autophagy.
We identified *TRIM8* as a direct target gene of p53. Under genotoxic stress conditions, p53 induces *TRIM8* expression, which in turn stabilizes p53 through a positive feedback loop, thereby promoting cell cycle arrest and DNA repair mechanisms.
Furthermore, *TRIM8* has been found to be downregulated in several cancers, including clear cell renal cell carcinoma (ccRCC) and colorectal carcinoma (CRC), leading to impaired activation of p53. This downregulation is caused by the upregulation of two microRNAs, miR-17-5p and miR-106b-5p, whose expression is controlled by the oncoprotein N-MYC. Restoration of TRIM8 expression decreases the levels of these microRNAs through p53-dependent overexpression of miR-34a, which suppresses the oncogenic activity of N-MYC. Consequently, stabilization of p53 restores the sensitivity of tumor cells to chemotherapeutic agents Among the mechanisms underlying chemoresistance are the altered expression of ABC (ATP-Binding Cassette) and SLC (Solute Carrier) transporters, which regulate the intracellular availability of chemotherapy drugs. We are currently investigating the transcriptomic profile of colorectal cancer cells with p53 dysfunction caused by TRIM8 deficiency, to determine whether TRIM8 restoration can activate p53-dependent pathways, such as modulation of ABC and SLC transporters, thereby increasing tumor cell responsiveness to chemotherapy and potentially reversing the malignant phenotype.

P.4

## REP-1 deficiency induces aberrant mitochondrial metabolic rewiring from glycolysis to lipid oxidation in CHM disease

Chiara Barone[1], I. Carratù[1], S. Buonocore[1], G. Trinchese[1], G.A. Vitale[1], G. Fasciolo[1], M. Ziaco[2], P. Venditti[1], A. Fontana[1,2], M.P. Mollica[1], D. Antonini[1] , G. Giamundo[1], I. Conte[1]
[1]Department of Biology, University of Naples Federico II, Naples, Italy
[2]Institute of Biomolecular Chemistry ICB, National Research Council CNR, Pozzuoli, Italy

Choroideremia (CHM) is an X-linked retinal degenerative disorder characterized by progressive dysfunction of the retinal pigment epithelium (RPE) and photoreceptors, for which no therapy currently exists. While the genetic basis of CHM is well established, the metabolic mechanisms driving disease progression remain incompletely understood. In this study, we investigated the impact of REP-1 deficiency on retinal metabolism using a combination of immunofluorescence, cell culture and pharmacological treatments, RNA sequencing with computational analysis, mitochondrial morphology assessment, Seahorse metabolic profiling, and in vivo medaka models. Our investigation uncovered a previously unrecognized role of REP-1 in regulating GLUT-1 and GLUT-4 membrane trafficking, thereby controlling glucose uptake and mitochondrial metabolic programming. We found that REP-1 deficiency induced a chronic metabolic shift toward lipid oxidation, resulting in reduced glycolytic flux, elevated oxidative stress, and impaired ATP production, alterations which ultimately culminated in progressive retinal dystrophy. Importantly, pharmacological restoration of GLUT trafficking via leptin administration re-established glucose uptake and mitochondrial function, rescuing cellular energetics both in vitro and in vivo. These findings identify REP-1 as a critical regulator of retinal metabolic homeostasis, suggesting that targeting glucose–lipid metabolic rewiring represents a promising therapeutic strategy for CHM and potentially other inherited retinal dystrophies.

P.5

## Identifying RNA Binding Protein regulators of TIGIT expression in Natural Killer cells

Marina Benitez Ramos[1], A. Bresciani[1], N. Perotti[1], B. Popović[2], E. Verhoeyen[3,4], M. Wolkers[2], A. Provenzani[1]
[1]Department of Cellular, Computational and Integrative Biology (CIBIO); University of Trento, Italy
[2]Department of Hematopoiesis, Sanquin Research, 1066 CX Amsterdam, the Netherlands
[3]Université Côte d'Azur, INSERM, C3M, Nice, France
[4]CIRI, Université de Lyon, INSERM U1111, ENS de Lyon, Université Lyon 1, CNRS UMR, Lyon, France

Natural Killer (NK) cells are emerging as promising therapeutic effectors due to their rapid cytotoxicity against cancer cells; however, their activity is often impaired within the tumor microenvironment (TME), leading to a dysfunctional state. A deeper understanding of the regulatory pathways that drive immune checkpoint expression and lead to NK cell dysfunction is still needed. RNA Binding Proteins (RBP) are important, dynamic regulators of mRNA fate, determining their stability, translation, transportation, and decay. Their rapid activation in immune regulation, i.e. by regulating cytokines' expression, is well documented, and their dysregulation has also been linked to the development of malignancies due to their important role in maintaining cell homeostasis. However, RBPs' role in regulating immune checkpoint expression remains largely unexplored. By using a pooled CRISPR library targeting 1,078 different RBPs, we have found that the expression of the immune checkpoint TIGIT is regulated by RBPs in NK-92 cells. Following transduction, we observed a shift from the characteristically homogeneous TIGIT expression seen in wild-type NK-92 cells to a distinct, heterogeneous expression profile, indicating RBP-mediated modulation. Our screen identified 22 potential RBP regulators of TIGIT expression: 9 negative regulators (whose knockouts increased TIGIT expression) and 13 positive regulators (whose knockouts decreased TIGIT expression). The relevance of these hits in NK-92 cancer cell cytotoxicity will be tested in an in vitro model of coculture with melanoma cells. Further, we established an exhaustion-like protocol by repeatedly exposing cancer cells to NK-92 and evaluated the resilience of NK cells to enter a dysfunctional state. These studies will help to uncover novel regulatory pathways of TIGIT regulation in NK cells. The identification of RBP-driven mechanisms can support the discovery of novel gene regulatory pathways regulating NK cell dysfunction.

# TGS1-mediated 5′ cap trimethylation regulates translation of target mRNAs in AML

Maxim Bouvet[3], V. Miano[1], G. Carrà[2], J. Lj Miljkovic[4], V. Paupe[4], C.S. Yu[4], D. Ignoti[3], S. Milioni[3], D. Viavattene[3], A.M. Benedetti[1], S.-L. Luan[1], D. Acquarone[3], V. Poli[3], P.E. Porporato[3], S. Gustincich[5], S.D. Turner[1,6], M.P. Martelli[7], A. Menga[8], L. Brunetti[7,9], M.P. Murphy[4,10], L. Pandolfini[5], I. Barbieri[1,3]
[1]Division of Cellular and Molecular Pathology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom
[2]San Luigi Gonzaga Hospital, Orbassano, Italy; Department of Clinical and Biological Sciences, University of Turin, Italy
[3]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy
[4]MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom
[5]Central RNA Lab, Istituto Italiano di Tecnologia, Genoa, Italy
[6]Faculty of Medicine, Masaryk University, Brno, Czech Republic
[7]Department of Medicine and Surgery, Hematology, Center for Hemato-Oncological Research (CREO), University of Perugia, Perugia, Italy
[8]Department of Health Sciences, University of Eastern Piedmont, Novara, Italy
[9]Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy
[10]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

The vast majority of primary RNA transcripts undergo extensive post-transcriptional maturation, including 5′-capping, splicing, and chemical modifications that influence RNA fate and function. Among these, 5′-cap hypermethylation by Trimethylguanosine Synthase 1 (TGS1) produces 2,2,7trimethylguanosine ($m^{2,2,7}G$), a modification historically associated with snRNA metabolism. However, the role of TGS1 and $m^{2,2,7}G$ in mRNA regulation remains largely unexplored. Here, we identify a novel function of TGS1 in the translation of nuclear-encoded mitochondrial mRNAs. The short TGS1 isoform, generated via proteolysis, localizes to both the cytoplasm and nucleus, while the long isoform is cytoplasmic. Fractionation and immunoblotting confirmed isoform distribution at the interface between ER and mitochondria.
Transcriptomic analyses revealed a significant enrichment of mitochondrial proteins among TGS1 targets, with one-third of them encoding OXPHOS components. These mRNAs showed reduced abundance in mitochondrial fractions of TGS1-depleted cells, without affecting mitochondrial mass. APEX2 assays excluded a role for TGS1 in mRNA localization to the outer mitochondrial membrane (OMM), pointing instead to translational regulation. Despite reduced cytoplasmic $m^{2,2,7}G$, EIF3b localization near the OMM remained unaltered, while CLIP-qPCR revealed increased EIF3b binding to TGS1-target mRNAs upon TGS1 knockdown, suggesting impaired release during elongation. Translation of mitochondrial proteins occurs at the OMM, where their co-translational import is tightly regulated. We show that although mRNA positioning is unaffected, TGS1 is essential for efficient translation dynamics at this site.
Mechanistically, TGS1 mediated 5′-cap hypermethylation promotes translation of TCA and OXPHOS genes, sustaining mitochondrial respiration and limiting ROS accumulation. This work uncovers a previously unrecognized role for TGS1 in mitochondrial metabolism and cancer.

P.7

## Unraveling the role of PTBP1 in regulating alternative splicing of *MAPT* in a patient-derived midbrain organoid model of Parkinson's disease

Maria Giusy Bruno[1], N. Urrestizala-Arenaza[2,3,4], P. Ramos-Gonzalez[2,3], F. Belpinati[1], G. Menichetti[1], A. Valentino[1], E. Trabetti[1], C. Bombieri[1], F. Cavaliere[2,3], M. G. Romanelli[1]
[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2]Achucarro Basque Center for Neuroscience, Leioa, Spain
[3]Basque Biomodel Platform for Human Research (BBioH), Leioa, Spain
[4]University of the Basque Country UPV/EHU, Leioa, Spain

The polypyrimidine-tract-binding protein 1 (PTBP1) is a well-known ribonucleoprotein that plays a key role in regulating alternative splicing (AS) during brain development. Dysregulation of splicing has been increasingly implicated in neurodegenerative diseases, including Parkinson's disease (PD), although the specific contribution of PTBP1 remains largely unexplored. PTBP1 has been shown to regulate the AS of the MAPT gene, generating distinct isoforms of the microtubule-associated protein Tau. Imbalance of Tau isoforms—particularly the 3R/4R ratio—is a hallmark of tauopathies. Moreover, PD forms carrying the LRRK2 G2019S mutation show a strong link with Tau pathology. This study aims to characterize PTBP1 expression in a PD organoid model and its association with Tau isoforms dysregulation, to clarify the contribution of this RNA-binding protein to disease pathogenesis.
PD human midbrain organoids (hmO) were generated from induced pluripotent stem cells (iPSCs) carrying the LRRK2 G2019S mutation. Organoids were collected at 20 and 40 days of maturation to analyze gene expression of PTBP1, total Tau, Tau exon 10 and 6 by qPCR. Immunofluorescence staining of organoids was performed to evaluate neuronal differentiation and Western blot analysis validated qPCR results.
PTBP1 was found to be overexpressed in PD hmO at 40 days compared to controls. Tau isoform expression was shifted toward increased inclusion of exon 10 and exon 6 in PD hmO, consistent with a pathological enrichment of 4R-Tau. Immunofluorescence analysis confirmed the presence of neuronal and midbrain markers, including MAP2, FOXA2, and TH.
These preliminary findings indicate an upregulation of PTBP1 in PD hmO, suggesting it may contribute to disease mechanisms. Further investigation of the interplay between PTBP1 and Tau isoforms expression in LRRK2 G2019S-associated PD could clarify the role of AS in neurodegeneration.

## A CD44 aptamer-guided therapeutic strategy to block crosstalk between tumor cells and tumor-associated macrophages in chemoresistant triple-negative breast cancer

Alessandra Caliendo[1,2], A. Caianiello[1], S. Camorani[1], A. Corteggio[3], D. Melillo[3], A. Zannetti[4], M. Fedele[1], P. Italiani[3], L. Cerchia[1]

[1]Institute of Endotypes in Oncology, Metabolism and Immunology "Gaetano Salvatore", National Research Council, Naples, Italy

[2]Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy

[3]Institute of Biochemistry and Cell Biology, National Research Council, Naples, Italy

[4]Institute of Biostructures and Bioimaging, National Research Council, Naples, Italy

**Introduction**: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype characterized by high heterogeneity and a strongly immunosuppressive tumor microenvironment (TME), which contribute to therapy resistance. Within the TME, tumor-associated macrophages (TAMs) play a pivotal role in TNBC progression. Although TAM-targeting strategies are emerging, limited specificity often causes off-target effects and toxicity. Nuclease-resistant RNA aptamers offer a promising strategy for selective targeting of tumor-TME interactions.

**Materials and methods**: TAMs were generated by exposing PMA-primed human THP-1 cells to conditioned media (CM) from mesenchymal-like TNBC cell lines (MDA-MB-231, BT-549) and cisplatin-resistant MDA-MB-231 cells (Cis-Pt-R), or by Transwell coculture. TAMs phenotype was assessed by immunoblotting, confocal microscopy, and ELISA/ELLA assays. TNBC-induced TAMs modulation was evaluated through migration/invasion and spheroid formation assays. Aptamer binding to TAMs was analyzed by flow cytometry using a fluorescent anti-CD44 sTN58 2′F-Py RNA aptamer.

**Results and Discussion**: TAMs generated by TNBC-CM or Transwell coculture significantly enhanced TNBC migration and invasion and displayed a hybrid TAM-like phenotype with both pro-inflammatory and immunoregulatory markers. Notably, TAMs educated by Cis-Pt-R cells, but not parental cells, showed increased CD44 expression and were efficiently bound by the sTN58 aptamer, which we previously validated as a targeting reagent for chemoresistant TNBC overexpressing CD44. Importantly, sTN58 binding to both tumor cells and TAMs significantly reduced TNBC spheroid growth and impaired tumor-macrophage interactions.

**Conclusion**: Here, we show that chemoresistant TNBC cells actively reshape the TME by promoting macrophage differentiation toward a pro-tumoral phenotype and provide preliminary evidence of aptamer-based therapeutic approaches targeting the crosstalk between tumor cells and TAMs.

P.9

## N-Glycosylation modulates receptor binding and neutralization sensitivity of Influenza A H5N1 hemagglutinin

Alessia Campese[1,2], K. Da Costa[2], J. Farias[1,3], M. Castelli[4], S. Grappi[5], A. Manenti[5], E. Montomoli[5,6], N. J. Temperton[2], A. Ruggiero[1]
[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2]Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent and Greenwich Chatham Maritime, Kent, UK
[3]Department of Microbiology, Vaccine Development Laboratory, Biomedical Sciences Institute, University of São Paulo, Sao Paulo, Brazil
[4]Università Vita Salute San Raffaele, Milano, Italy
[5]VisMederi srl, Siena, Italy
[6]Department of Molecular and Developmental Medicine, University of Siena, 53100 Siena, Italy

**Background:** Highly pathogenic avian influenza A viruses (H5N1) pose a persistent global threat due to their rapid evolution, expanding host range, and zoonotic potential. The hemagglutinin (HA) glycoprotein is a major determinant of viral entry, host adaptation, and immune recognition. N-glycosylation of HA can modulate receptor binding and shield antigenic epitopes, yet the functional role of specific glycosylation sites in emerging H5N1 strains remains incompletely defined. This study examines the impact of altered HA N-glycosylation on viral infectivity and neutralisation in the H5N1 strain A/dairy cow/Texas/24-008749-002.
**Methods:** Eight HA expression plasmids carrying targeted mutations at conserved N-glycosylation sites were generated. Lentiviral pseudotypes were produced to assess viral entry. Titration assays were performed on cells lines that display different patterns of sialic acids. Neutralisation assays were conducted using reference animal antisera and convalescent human sera from individuals previously infected with H5N1 A/Vietnam/1194/2004 strain.
**Results:** Altered HA N-glycosylation significantly affected viral infectivity. Removal of glycosylation at residues 26–27 and the combined mutation at residues 499 and 558 markedly impaired viral entry across multiple cell lines. Neutralisation assays revealed differential sensitivity among mutants despite high sequence conservation. The N181Q mutant consistently showed increased susceptibility to both animal antisera and human sera, indicating reduced antigenic shielding.
**Conclusions:** Hemagglutinin N-glycosylation critically influences viral entry and antibody recognition. Specific glycosylation sites contribute to infectivity and immune evasion, underscoring their relevance to host adaptation and antigenic evolution. Importantly, human sera retained neutralising activity against newer variants, suggesting potential cross-protective immunity.

P.10

## Development of flexible heterotypic 3D models for live imaging study of anti-tumor immune strategies in prostate cancer

Marta Cantarelli[1], R. Begaj[1], E. Marmocchi[1], N. Annesi[1], D. Bressan[1], V. Poli[2], F. Chiacchiera[1], A. Lunardi[1]
[1]Department of Cellular, Computational and Integrative Biology-CIBIO, University of Trento, Trento, Italy
[2]Department of Molecular Biotechnology and Health Science, University of Torino, Torino, Italy

Prostate cancer (PCa) remains largely unresponsive to immunotherapy due to its “cold” tumor microenvironment, characterized by low immune infiltration and immunosuppressive signaling. Understanding the molecular mechanisms underlying tumor–stroma–immune interactions is essential to improve therapeutic strategies.
Here, we developed and characterized three-dimensional (3D) homotypic and heterotypic spheroid models to investigate prostate tumor biology in a physiologically relevant context. Prostate cell lines displayed distinct aggregation behaviors: DU145 cells formed compact spheroids, RWPE1 showed delayed compaction, whereas PC3 failed to generate stable structures, reflecting intrinsic biological differences. To model PCa-relevant molecular alterations, we exploited inducible expression of ERG, a key oncogenic transcription factor frequently overexpressed in PCa. ERG induction impaired spheroid compaction in epithelial cells, consistent with its role in promoting epithelial-to-mesenchymal transition (EMT) and altering cell adhesion programs. Transcriptomic profiling further revealed ERG-dependent transcriptional reprogramming associated with EMT-related pathways.
Co-culture with prostate normal fibroblasts (NF) or cancer-associated fibroblasts (CAFs) enhanced spheroid compaction and modulated epithelial–stromal organization, highlighting the contribution of stromal components to tumor architecture and signaling. What's more, the integration of Natural Killer (NK) cells enabled the study of immune cell interactions and cytotoxic responses in a 3D tumor context.
Overall, this heterotypic 3D spheroid system provides a versatile platform to dissect molecular mechanisms of tumor–microenvironment crosstalk and to evaluate novel immunotherapeutic strategies.

P.11

## Adipose mesenchymal stem cell-derived extracellular vesicles as potential therapeutic biologic agents for damaged motoneurons in Amyotrophic Lateral Sclerosis

Mauro Caprioli, D. Sganzerla, I. Scambi, A. D'Alberto, S. De Marchi, E. Pelullo, F. Virla, E. Turano, R. Mariotti
Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Italy

Extracellular vesicles derived from adipose mesenchymal stem cells (ASC-EVs) are emerging as promising biologics due to their neuroprotective and immunomodulatory properties, supporting their potential application for the treatment of neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS). Intranasal delivery is a non-invasive route to directly target the central nervous system (CNS), enabling nanosized therapeutic agents to cross the blood–brain barrier. The study aimed to evaluate the neuroprotective potential of ASC-EVs on injured motor neuron-like NSC-34 cells and to establish an in vitro epithelial barrier model to explore the molecular mechanisms underlying their migration across it. Molecular characterization of ASC-EVs typical protein markers was performed by Western Blot analysis. An *in vitro* epithelial barrier model was developed using nasal epithelium carcinoma RPMI 2650 cells cultured on transwell system, while NSC-34 cells grew on the basolateral compartment of the chamber. Motor neuron cells were exposed to hydrogen peroxide to induce oxidative stress and subsequently treated with ASC-EVs.  EVs migration mechanisms across the epithelial barrier was evaluated using endocytosis inhibitors, and the vesicles protective potential was assessed through immunofluorescence analysis. Our findings demonstrate that ASC-EVs express canonical protein markers and are able to cross the epithelial barrier to reach injured motor neurons, where they are internalized and exert a significant neuroprotective effect, preserving mitochondrial membrane potential. Moreover, ASC-EVs migration involves both transcellular and paracellular routes, with a predominant contribution from the transcellular pathway mediated by clathrin-dependent endocytosis.
These findings demonstrate that ASC-EVs exert a robust neuroprotective effect on damaged motoneurons, supporting their potential as non-invasive therapeutic biologics for ALS.

P.12

## Identification of a novel functional axis SLC-VDAC for Glioblastoma multiforme potential treatment

Roberta Caschetto[1], C. Caruso[1], P. Caruso[1], R. Fallica[1], M.Scalise[2], F. Marzano[3], X.G. Pappalardo[1], A. Magrì[4]
[1]Dept. of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[2]Dept. DiBEST (Biologia, Ecologia, Scienze della Terra), Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Arcavacata di Rende, Italy
[3]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, Bari, Italy
[4]Dept. of Biological, Geological and Environmental Sciences, University of Catania, Catania, Italy

Glioblastoma multiforme (GBM) remains one of the most aggressive and therapeutically refractory brain tumors, largely due to its marked molecular heterogeneity, metabolic plasticity, and the absence of a unified classification capturing dynamic transitions between proneural and mesenchymal states. Emerging evidence identifies metabolic reprogramming as a central determinant of GBM progression, highlighting the need to elucidate how tumor cells sense and adapt to metabolic cues. In this context, we investigated the role of plasma membrane solute carrier (SLC) transporters and voltage-dependent anion channel (VDAC) proteins located in the outer mitochondrial membrane as key mediators of cellular metabolic regulation. These protein families are strategically positioned to coordinate extracellular nutrient uptake with mitochondrial function, thereby shaping cellular responses to metabolic stress. To dissect their functional interplay, we applied an integrative multi-omics computational framework across two complementary levels: (i) protein–protein interaction network analysis to evaluate whether transcriptional associations are reflected in functional protein-level connectivity, and (ii) in silico analysis of publicly available RNA-seq datasets. Our findings converge on a coordinated regulatory axis involving SLC7A11 and VDAC2, suggesting a functional coupling between cystine-glutamate transport, redox homeostasis, and mitochondrial activity. Overall, our results provide a multi-layered perspective on how membrane transport systems integrate metabolic signals in GBM and highlight potential targets for metabolically informed therapeutic strategies.  Additional experimental validation will be essential to elucidate the underlying signaling mechanisms and to assess their potential as targets for precision therapies.

The authors acknowledge PRIN 2022 (P2022RLLBB), funded by the European Union NextGeneration EU.

P.13

## SupraMolecular Attack Particles (SMAPs): a new weapon against virus-infected cells

Maria Carmela Cavallo[1], C. Cassioli[1], A. Miccoli[1], M.L. Dustin[2], N. Capitani[1], C.T. Baldari[1]
[1]Dept of Life Sciences, University of Siena, Siena, Italy
[2]Dept of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

Upon antigen recognition on target cells, cytotoxic T lymphocytes (CTLs) form a highly organized structure known as immunological synapse (IS), which mediates target cell killing through rapid exocytosis of lytic granules (LGs) and engagement of the Fas/FasL signaling pathway. Recently, a third cytotoxic mechanism has been identified involving the release at the IS of particles known as SupraMolecular Attack Particles (SMAPs). SMAPs are characterized by a central *core* region containing cytotoxic components such as granzymes (Gzms) and perforin (Prf), and an outer glycoprotein shell, with thrombospondin-1 (TSP-1) identified as the most representative component. We characterized first the expression levels of TSP-1 in CD8+ T lymphocytes during their *in vitro* differentiation to CTLs and we identified, alongside the canonical full-length TSP-1 protein (FL), a protein form of about 60kDa, that is the only expressed in mature CTLs. This 60 kDa form represents the C-terminal portion of the protein, likely resulting from the cleavage of the FL protein. We moreover demonstrate by immunofluorescence analysis that 60 kDa TSP-1 contains all molecular determinants for proper localization to LGs. Furthermore, we decided to engineer TSP-1 with the aim to generate artificial SMAPs specifically targeting virally infected cells. The engineering process involved the 60 kDa C-terminal portion of TSP-1 by removing two adhesive sites of the protein: the CD47 binding domain and the integrin binding site (RGD motif). We first demonstrated that adhesion mutants correctly localize to LGs, with no differences compared to the *wild-type* protein. In addition, using TIRF confocal microscopy, we assesed that CTLs expressing TSP-1 60kDa mutants were able to release SMAP-like particles as well as the *wild-type* 60kDa TSP-1 form. The next step of the project will be to evaluate the cytotoxic activity of engineered SMAPs and, as a final goal, to introduce specificity against virus-infected cells.

P.14

## Exploring the transcriptome of Pediatric Myelodysplastic Syndrome (PMDS)

Marianna Ciuffreda[1], A. Mattè[1], V. De Santis[1], C. Valentini[1], R. Bertorelli[1], T. Lammens[2], P. Macchi[1], T. Tebaldi[1]
[1]Department of Cellular, computational and integrative biology - CIBIO
[2]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium

Pediatric myelodysplastic syndrome (PMDS) is a rare preleukemic condition characterized by genetic aberrations in Hematopoietic Stem and Progenitor Cells (HSPCs), resulting in ineffective hematopoiesis and accumulation of immature blasts (Patel 2021). While hematopoietic cell transplantation (HCT) remains the only curative option for PMDS, it is not effective in all cases (Smith et al. 2013). The main limitation is that the current literature has extensively mapped the genetic and transcriptomic landscape of PMDS using whole bone marrow (WBM) analysis (Pastor et al. 2017), which often masks subtle molecular changes within the HSPC compartment, underscoring the need for cell-specific data to identify novel therapeutic targets. This study aims to define the malignant landscape of sorted CD34+ HSPCs to discover therapeutically exploitable vulnerabilities, integrating PMDS profiles with public healthy adult dataset (GSE118944). Preliminary results reveal a "metabolically primed but replicationally arrested" phenotype: PMDS progenitors show an upregulation of cytoplasmic translation machinery (*EIF4A1, RPL22, RACK1*) alongside markers of mitotic failure (*GINS1, MCM4, TK1*). Furthermore, we observed significant dysregulation of the m6A epitranscriptomic pathway, specifically, downregulation of the writer *METTL3* and upregulation of readers (*YTHDF2*) and erasers (*FTO, ALKBH5*). This suggests *METTL3* acts as a "gatekeeper" in maintaining the immature state. To strengthen these findings, we are currently expanding the cohort by 11 samples. A key future perspective of this work is the integration of Whole Exome Sequencing (WES) to correlate somatic mutations with these observed transcriptomic and epitranscriptomic signatures. By pinpointing the dysregulation of translation and m6A pathways, this study provides high-resolution insights into the drivers of pediatric pre-leukemia and identifies specific, targetable vulnerabilities for future therapeutic intervention.

## miR-214-driven-MFN2-downregulation promotes mitochondria dysfunction and it affects melanoma progression

Sara Cozzubbo[1,2], S. Cucinellli[1,2], A. Larcher[1,2], M. Coco[1,2], I. C. Salaroglio[3], J. Kopecka[3], G. Morciano[4,5], K. Hodge[6], P. Pinton[4], S. Zanivan[6], C. De Pittà[7] , C. Riganti[3], F. Orso[8], D. Taverna[1,2]
[1]Molecular Biotechnology Center “Guido Tarone”, University of Torino, Torino, Italy
[2]Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy
[3]Department of Oncology, University of Torino, Torino, Italy
[4]Laboratory for Technologies of Advanced Therapies (LTTA), Section of Experimental Medicine, Departement of Medical Science, University of Ferrara, Ferrara, Italy
[5]Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", Italy
[6]Cancer Research UK Beatson Institute, Glasgow, UK
[7]Department. of Biology, University of Padova, Padova, Italy
[8]Department. of Translational Medicine (DIMET), Università del Piemonte Orientale, Novara, Italy

Metabolic rewiring is a cancer hallmark that can be tightly regulated by microRNAs (miRs), orchestrators of tumor progression. miR-214 is a small RNA often overexpressed in malignant melanomas and breast cancers able to promote dissemination and metastasis formation, however not impacting on cell proliferation. Specifically, miR-214 silences Transcription Factors, such as TFAP2C, suppresses miR-148b and upregulates adhesion molecules (ALCAM, ITGA5). Here, based on *in vitro* and *in vivo* investigations, we previously showed the capability of miR-214 to interfere with metabolic features by inducing a Warburg effect, coinciding with increased glycolysis and reduced mitochondrial activity. Consistently, defective TCA, ETC enzyme activities and ATP production were observed. Focusing on mitochondria, altered mitochondria morphology and ultrastructure, in mitochondria-ER contact sites, were observed as well as abnormal Ca2+ flux, membrane polarization and ROS production. Hence, we hypothesized that miR-214 could affect metabolism mainly by inducing mitochondria dysfunction. We proved that MFN2, which controls mitochondria fusion, cell metabolism and ER-mitochondria tethering, is a direct miR-214 target in our melanoma models. Notably, MFN2 overexpression in miR-214-rich cells restored several mitochondrial functions *in vitro* and *in vivo*. Furthermore, MFN2’s levels restoration reduced cell migration, transendothelial migration *in vitro*, and extravasation capacity *in vivo*, suggesting a critical role in regulating miR-214-driven metastatic traits. Collectively, these data provide a rationale for considering MFN2 as a key mediator of miR-214-induced mitochondrial dysfunction and metabolic rewiring. However, further studies are required to fully elucidate the underlying molecular mechanisms and to exploit therapeutically this novel tumor metabolic liability.

P.16

## Establishing METTL16 as a key regulator of GBM carcinogenesis

MarcoCrepaldi[1], G. Bove[1], M. Babaei[1], S. Amin[2], G. Favale[1], V. Carafa[1,2], A. Nebbioso[1,3], P. Peruzzi[4,5], N. Del Gaudio[1,6], L. Altucci[1,2,3]
[1]Department of Precision Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy
[2]BIOGEM, Via Camporeale Area P.I.P., Ariano Irpino (AV), Italy
[3]Program of Medical Epigenetics, Vanvitelli Hospital, Naples, Italy
[4]Harvey Cushing Neuro-Oncology Laboratories, Department of Neurosurgery, Mass General Brigham, Boston, MA, USA
[5]Harvard Medical School, Boston, MA, USA
[6]Department of Life Sciences, Health and Health Professions, Link Campus University, Rome, Italy

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults, characterized by rapid proliferation, high heterogeneity, and poor prognosis, with a median survival of ~15 months despite standard therapy. Increasing evidence highlights the role of N6-methyladenosine (m6A) RNA modification in cancer biology. While METTL3 has been extensively studied, the contribution of the m6A writer METTL16 to GBM remains largely unexplored.
This study aims to define the role of METTL16 in GBM progression, evaluate its impact on tumor cell survival and proliferation, and investigate the underlying molecular mechanisms, assessing its potential as a therapeutic target. METTL16 expression was analyzed using TCGA, GTEx, and CPTAC datasets and validated in GBM cell lines (U87, T98G) and patient-derived glioma stem-like cells. Loss-of-function studies were performed via shRNA-mediated knockdown, and pharmacological inhibition targeted METTL16 enzymatic activity. Functional assays included proliferation, clonogenicity, apoptosis (PI staining, PARP cleavage), and cytotoxicity.
Transcriptomic changes were assessed by RNA-seq and Gene Set Enrichment Analysis, followed by RT-qPCR and immunoblot validation. In addition, m6A RNA immunoprecipitation sequencing (MeRIP-seq) was used to identify methylated transcripts and distinguish direct METTL16-dependent regulation from indirect transcriptional effects.
Results: METTL16 is significantly overexpressed in GBM tissues and cell models; its depletion reduces proliferation, clonogenicity, and metabolic activity while inducing apoptosis, effects recapitulated by pharmacological inhibition. Transcriptomic analysis revealed downregulation of mTOR, MYC, and E2F signaling pathways. Mechanistically, METTL16 targeting disrupts the mTOR/S6K axis, and mTOR expression positively correlates with METTL16, suggesting a functional link.
Overall, METTL16 emerges as a key regulator of GBM cell survival and proliferation, and integration of RNA-seq with MeRIP-seq enables identification of direct epitranscriptomic targets, supporting METTL16 as a promising therapeutic strategy warranting further validation.

P.17

## Neuronal late endosomes serve as selective RNA hubs disrupted by ALS-linked FUS mutation

A. Setti[1], L. Stufera Mecarelli[2], T. Santini[1], Angelo D'Angelo[1], V. Padovano[1], E. Vitiello[3], F. Castagnetti[4], J. Martone[5], D. Mariani[1,2,6], I. Bozzoni[1,2,6]
[1]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Italy
[2]Center for Human Technologies@Istituto Italiano di Tecnologia (IIT), Genoa, Italy
[3]Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Heidelberg 69102, Germany
[4]Karlsruher Institute für Technologie (KIT), Karlsruhe, Germany
[5]Institute of Molecular Biology and Pathology, National Research Council of Italy, Rome, Italy
[6]Center for Life Nano- & Neuro-Science@Sapienza, Fondazione Istituto Italiano di Tecnologia (IIT), Rome, Italy

Neurons depend on tightly regulated positioning of cellular components to maintain long-distance signaling, but the mechanisms guiding specific mRNAs to distant regions remain unclear. Here we show that late endosomes function as selective RNA carriers in human motor neurons and uncover the molecular logic guiding their loading. Using APEX2-mediated proximity labeling, we identify the external transcriptome of RAB7A-positive endosomes and find a specific population of mRNAs enriched for endosomal, axonal and synaptic functions. We find that mRNAs enriched in RAB7A endosomes contain evolutionarily conserved 5′UTRs which act as localization signals, and that the RNA-binding protein GEMIN5 interacts with these regions to promote endosomal RNA recruitment. Finally, we show that in ALS-associated conditions there is a conspicuous loss of endosome-associated transcripts and the mislocalization of GEMIN5 from endosomes. These findings uncover fundamental principles of RNA compartmentalization and highlight endosomal mRNA loading as a vulnerable axis in neuronal homeostasis.

P.18

## Pirin as a novel therapeutic target in hepatocellular carcinoma through modulation of the BCL3–tmTNF-α juxtacrine signaling axis

B.D.C. Stanca[1], L. Giannotti[2*], P. Alifano[2], S. Resta[2], M. Calcagnile[3], M. Massaro[4], S. Quarta[4], C. Demitri[2], E. Stanca[2], L. Siculella [2], Fabrizio Damiano[2]
[1]Institute of Polymers, Composites and Biomaterials - CNR, Naples, Italy.
[2]Dept Experimental Medicine, Univ. of Salento, Lecce, Italy
[3]Dept Biological and Environmental Sciences and Technologies, Univ. of Salento, Lecce, Italy
[4]Institute of Clinical Physiology (IFC) -CNR, Lecce, Italy

Human pirin (PIR) is an oncogene implicated in a wide range of malignancies, including melanoma, hepatocellular, colorectal, and lung cancers, where it regulates critical cellular processes such as cell cycle progression, senescence, ferroptosis, epithelial–mesenchymal transition (EMT), and chemoresistance. Although PIR has been associated with NF-κB–mediated inflammatory signalling and tumour progression, the precise molecular mechanisms underlying these interactions remain poorly understood. In this study, we examined the impact of PIR knockdown on cell viability, oxidative homeostasis, and NF-κB signalling in the HepG2 hepatocarcinoma cell line. Silencing of PIR markedly reduced cell proliferation and survival, disrupted cellular redox homeostasis, induced mitochondrial dysfunction, and caused genomic DNA damage, ultimately leading to cell death. Mechanistically, downregulation of PIR affected the nucleocytoplasmic localisation of BCL3, a regulator of the NF-κB transcription factor RelA. This alteration resulted in increased expression of TNF-α, particularly its transmembrane form (tmTNF-α), which is known to promote deleterious juxtacrine signalling. Notably, attempts to inhibit tmTNF-α-mediated signalling revealed that treatment with soluble TNF-α partially mitigated the inflammatory dysregulation observed in PIR-silenced cells, whereas administration of an anti-TNF-α antibody proved more effective. These interventions also contributed to restoring cellular redox equilibrium, preserving mitochondrial integrity, and improving cell viability. Overall, our findings support a model in which PIR knockdown induces cancer cell death through a juxtacrine signalling mechanism driven by BCL3 dysregulation and increased pro-inflammatory tmTNF-α expression, highlighting PIR as a potential therapeutic vulnerability in cancer.

P.19

## The role of ERG transcription factor in the modulation of immuno-regulatory protein CD47

Pierpaolo De Iaco[1,2], D. Rosini[1], I. Cosi[1]. C. Nannelli[1], R. Notaro[1], M. De Angioletti[1,3]
[1]Laboratory of Cancer Genetics, Core Research Laboratory, Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO), Florence
[2]Doctorate School GENETICS, ONCOLOGY and CLINICAL MEDICINE (GenOMeC), University of Siena, Siena, Italy
[3]Istituto di Chimica dei Composti Organometallici (ICCOM), National Council of Research, Sesto Fiorentino, Florence, Italy

Immune checkpoints are key regulatory molecules that maintain the balance between immune activation and inhibition, preventing autoimmunity while ensuring effective responses against infections and tumor cells. The CD47–SIRPα axis is a critical innate immune checkpoint: CD47, a transmembrane protein broadly expressed on healthy cells, interacts with SIRPα, an inhibitory receptor on myeloid cells, such as macrophages and dendritic cells. This interaction generates a "don't eat me" signal that inhibits phagocytosis. Therefore, CD47 is a potential target for immunotherapy, also in “cold” tumors, which are poorly infiltrated by immune cells and resistant to conventional immunotherapies. Prostate cancer (PCa) is a cold tumor in which the role of therapy with immune checkpoint inhibitors is controversial. Half of PCa patients overexpress the transcription factor ERG, which contributes to tumor onset. We are investigating whether ERG regulates CD47 expression by using two cell lines with high ERG expression levels: the prostatic VCaP and ovarian HeLa cells. Specifically, we are assessing the levels of CD47 mRNA (quantitative real-time PCR), proteins (western blotting), and CD47 expression on cell membrane (flow cytometry) in these two cancer cell lines before and after ERG silencing. Preliminary results have shown that ERG upregulates CD47 expression at both mRNA and protein levels, resulting in the increase of CD47 molecules on cell membranes. Next, we will investigate whether this ERG-mediated increase of CD47 expression is able to affect the ability of macrophages to control cancer cells. The definition of role of ERG in regulating CD47 expression and, eventually, in modulating tumor immune evasion, may contribute in designing novel therapeutic approaches to make effective immune therapy also in "cold tumor" such as prostate cancer.

## HMGB1 regulates nuclear properties and cell migration through Lamin A/C transcriptional modulation

Alessio De Magis[1,2], G. Gallo[1,2], L. Sassi[1], M. Chighizola[3,4], H. Holuigue[3,5], L. Dal Fabbro[3], A. Podestà[3], M.E. Bianchi[1,2]
[1]Chromatin Dynamics Unit, San Raffaele Hospital, Milan, Italy
[2]School of Medicine, Vita-Salute San Raffaele University, Milan, Italy
[3]Physics Dept., University of Milan, Milan, Italy
[4]Present affiliation: Dynamo Group, INSERM, Marseille, France
[5]Present affiliation: Mechanobiology Institute (MBI), National University, Singapore

The spacial organization and mechanical properties of the nucleus are critical determinants of cell function, yet the contribution of chromatin architectural proteins to nuclear mechanics remains largely unexplored. Here, we investigate the role of HMGB1, a chromatin-associated protein, in regulating nuclear morphology, stiffness, and cell migration. Using HMGB1 knockout mouse embryonic fibroblasts (MEFs), we show that loss of HMGB1 leads to a mild increase in nuclear size and a marked reduction in nuclear stiffness, as measured by atomic force microscopy. A chimeric *Hmgb1* KO/wt embryo model demonstrated that HMGB1-deficient cells display enlarged nuclei across multiple tissues.
Functionally, HMGB1 loss enhances cell migration, as shown by Boyden chamber and wound-healing assays, suggesting that nuclear softening facilitates movement through confined environments. Mechanistically, these effects are independent of senescence, CTCF levels, HP1 expression, and global histone depletion. Instead, transcriptomic and biochemical analyses identify c-JUN and Lamin A/C as key downstream effectors. HMGB1 deficiency reduces LMNA mRNA through decreased c-JUN levels and binding to the *Lmna* promoter, leading to lower Lamin A/C abundance and altered nuclear lamina organization. Importantly, partial Lamin A/C depletion mimics HMGB1 loss, recapitulating nuclear enlargement, reduced stiffness, and increased migration.
Together, our findings uncover a previously unrecognized role for HMGB1 in maintaining nuclear integrity and mechanics via Lamin A/C regulation and establish a mechanistic link between chromatin architecture and nuclear lamina architecture and function, with implications for development, aging, and migration-dependent processes.

P.21

## Functional interplay between ACOT8 and Nef enhances HIV-1 infectivity and remodels the viral envelope

Carola De Martinis[1], M. Bissoli[1], M. Voi[1], E. Orlandi[1], M. Belay[2], A. Ghignone[2], E. Marengo[2], A. Ruggiero[1], E. Robotti [2], D. Zipeto[1]
[1]Department Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2]Department of Sciences and Technological Innovation, University of Piemonte Orientale, Alessandria, Italy

**Background:** ACOT8 is an acyl-CoA thioesterase involved in lipid metabolism, and it has been implicated in the HIV-1 replication cycle through interactions with the viral protein Nef. Here, we aimed to study the nature of this interaction by investigating the lipid composition of the virus envelope in the ACOT8-deficient condition and how this affects infectivity.
**Materials and methods**: To assess the role of ACOT8 in HIV-1 infectivity, we generated ACOT8 knockout (KO) HEK293T cells using CRISPR/Cas9. Editing was confirmed by Sanger sequencing and Western blot. HIV-1 pseudotyped viral particles (PVPs) were produced in wild-type (WT) and ACOT8 KO cells, as well as in the presence of Nef variants that either have the capacity or do not interact with ACOT8. Infectivity was evaluated in TZM-bl cells using equivalent p24 input. HIV-1 envelope lipids were extracted with methanol/MTBE and analyzed by uHPLC/MS.
**Results:** PVPs generated in ACOT8 KO cells showed a significant reduction in infectivity compared to WT-derived particles. This effect was envelop-specific, as VSV-G PVPs were unaffected by the absence of ACOT8. Re-expression of ACOT8 fully restores HIV-1 PVPs infectivity. Notably, enhanced infectivity was observed in PVPs produced in the presence of a Nef variant that interacts with ACOT8. In addition, Nef–ACOT8 interaction significantly altered HIV-1 lipid composition, increasing phospholipids (PC, PE) and glycosphingolipids (HexCer), consistent with substantial lipid remodeling.
**Conclusions:** These findings identify ACOT8 as a host factor required for optimal HIV-1 infectivity and further indicate that the Nef-ACOT8 interaction enhances the production of highly infectious viral particles. Lipidomic analysis suggests that the Nef–ACOT8 interaction reshapes the HIV-1 envelope by enriching lipid molecules associated with membrane fluidity and lipid raft organization, potentially promoting a more fusion-competent membrane and thereby increasing viral infectivity.

## Identification of bioactive compounds activating Mtf1

Gaia De Rigo[1], S. Ciaco[2], A.M. Gambetta[3], G. Martello[1]
[1]Department of Biology, University of Padua, Padova, Italy
[2]Department of Biotechnologies, Chemistry and Pharmaceutics, University of Siena, Siena, Italy
[3]Department of Clinical and Experimental Medicine, University Magna Graecia, Catanzaro, Italy

Huntington's Disease is an incurable autosomal dominant neurodegenerative disease caused by trinucleotide repeat expansion in the huntingtin gene (HTT), which results in the production of a mutant form of HTT protein. Mutant HTT (mHTT) forms aggregates and the main hallmark of HD is the degeneration of medium spiny neurons in the striatum, leading to neuronal degeneration.
Metal regulatory transcription factor 1 (MTF1) is a transcription factor with a key role in regulating metal homeostasis and in detoxification systems, and it has been identified as a potential suppressor of mHTT toxicity.
Activating endogenous MTF1 represents a promising therapeutic strategy in neurodegenerative diseases.
In this study, mouse embryonic stem cells were leveraged as a biological model to perform a screening of possible bioactive compounds predicted to activate Mtf1, and Metallothioneins (Mts) were used as readouts to get insights on the induction of Mtf1. Furthermore, neural precursor cells were employed to validate the compounds that elicited the most robust activation.
From the results, various compounds emerged for their ability to activate Mtf1 and may help reduce the toxic effects of mHTT.
These findings contribute to the development of novel therapeutic strategies targeting MTF1, with broader implications for the treatment of HD and different neurodegenerative disorders.

## Reprogramming-to-organoids: how to cost-efficiently leverage hIPSCs to model human diseases

De Santis Rosa[3,4,*],O. Gagliano[1,2,*], S. Angiolillo[1,2,*], A. Gesualdo[1,2], R. Frison[1,2], W. Qin[1,2,5], A. Maset[1,2], B. Auletta[6,7,8], A. Grimaldi[3,4], F. Panariello[3,4], L. Santarelli[3,9], P. Grumati[3,9], A. Urciuolo[6,7,8], D. Cacchiarelli[3,4], N. Elvassore[1,2]
[1]Venetian Institute of Molecular Medicine (VIMM), Padova, Italy
[2]Department of Industrial Engineering (DII), University of Padova, Padova, Italy;
[3]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli
[4]Department of Translational Medicine, University of Naples "Federico II", Naples, Italy
[5]Shanghai Institute for Advanced Immunochemical Studies (SIAIS), ShanghaiTech University, Shanghai, China
[6]Department of Molecular Medicine, University of Padova, Padova, Italy
[7]Neuromuscular Engineering lab, Istituto di Ricerca Pediatrica, Città della Speranza, Padova, Italy
[8]University College London Great Ormond Street Institute of Child Health, London, United Kingdom
[9]Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy
*Contributed equally

Human-induced pluripotent stem cells (hiPSCs) are essential for personalized and regenerative medicine. However, their application in patient-derived organoid (PDO) modeling is limited by high costs, labor-intensive procedures, and the risk of cytogenetic instability during extended culture. Recently, we developed a direct reprogramming-to-organoid method that uses "nascent" hiPSCs (freshly reprogrammed cells, not expanded) to produce 3D epiblast-like cysts within 14 days, avoiding traditional expansion steps.
To characterize the molecular basis of this accelerated reprogramming process, we performed single-cell multi-omic analysis (scRNA-seq and scATAC-seq) on pooled hiPSC lines at specific reprogramming stages. Integrating transcriptomic and chromatin accessibility data, we reconstructed the pluripotency gene regulatory network (GRN) and identified key transcription factors, including *OCT4*, *SOX2*, and *NANOG*, whose coordinated activation marks the early attainment of full pluripotency, supporting the direct differentiation of nascent hiPSCs into organoids without traditional expansion.
To evaluate reproducibility and disease relevance, we analyzed brain organoids from six different lines, including three healthy controls and three Fragile X syndrome (FXS) carriers. Single-cell data validated consistent cortical specification and maturation among the lines and identified transcriptional changes specific to FXS. Additionally, we used the same protocol on heart, muscle, and liver organoids, confirming proper germ-layer differentiation in all cases.
This study demonstrates that single-cell multi-omics can effectively support and confirm a rapid, scalable process for creating patient-specific organoids, while maintaining genomic integrity and allowing disease modeling.

P.24

## EGFR ligand Angiogenin predicts response to TGFβ Inhibition in Pancreatic Cancer via a TNF-α Paracrine Axis in Tumor-Associated Macrophages

Veronica De Vita[1], A. Bonato[1], A. Aparo[2], D. Mangiameli[1], S. Casalino[3], A. Quinzii[3], S. Pietrobono[1], D. Melisi[1,3]
[1]Department of Medicine, Digestive Molecular Clinical Oncology Research Unit, University of Verona, Verona, Italy
[2]Research Center LURM (Interdepartmental Laboratory of Medical Research), University of Verona, Verona, Italy
[3]Investigational Cancer Therapeutics Clinical Unit, Azienda Ospedaliera Universitaria Integrata, Verona, Italy

Transforming growth factor-β receptor (TGFβR) inhibition has shown promise in pancreatic ductal adenocarcinoma (PDAC), but predictive biomarkers remain undefined. We identify angiogenin (ANG) as a negative prognostic yet positive predictive biomarker for TGFβRI inhibition using galunisertib in combination with gemcitabine-based chemotherapy. In the randomized phase II H9H-MC-JBAJ trial, high baseline ANG predicted poor survival with gemcitabine alone but significant benefit from galunisertib addition. Mechanistic studies revealed that tumor-derived ANG binds epidermal growth factor receptor (EGFR) on tumor-associated macrophages (TAMs), activating RhoA-dependent cytoskeletal remodeling and autocrine TGFβ signaling. This drives M2-like polarization and Smad2-mediated transcription of tumor necrosis factor-α (Tnf-α), which activates Nf-κB in neighboring tumor cells, conferring chemoresistance. TGFβRI inhibition suppressed TAM-derived Tnf-α, reduced M2 polarization, prevented Nf-κB activation, and restored chemosensitivity in ANG-high models. Clinically, elevated ANG correlated with systemic TNF-α, and galunisertib reduced TNF-α exclusively in ANG-high patients, with reductions associated with markedly improved survival. These findings define an ANG–EGFR–TGFβ–TNF-α axis in TAMs as a stromal driver of PDAC chemoresistance, and provide a mechanistic rationale for the development of combination strategies targeting TGFβ signaling in ANG-high PDAC patients.

P.25

## Prenatal exposure to THC and CBD alters endocannabinoid and dopaminergic gene expression in the rat prefrontal cortex: epigenetic insights across preadolescence and adolescence

Martina Di Bartolomeo[1], S. Di Martino[2], F. Gilardini[1,3], A.G. Ventura[2], M. Kuchar[4], F. Drago[2], V. Micale[2,5], C. D'Addario[1,6]
[1]Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
[2]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[3]School of Advanced Studies, Center for Neuroscience, University of Camerino, Camerino, Italy
[4]Forensic Laboratory of Biologically Active Substances, Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic
[5]Oasi Research Institute - IRCCS, Troina, Italy
[6]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Prenatal cannabis exposure is an increasing public health concern, yet the long-term consequences of individual cannabinoids — Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) — on neurodevelopment remain poorly understood. This study investigated the effects of prenatal THC and CBD exposure on gene expression and DNA methylation of endocannabinoid and dopaminergic system genes in the rat prefrontal cortex (PFC) at preadolescent and adolescent time points.
Pregnant Sprague-Dawley rats received daily subcutaneous injections of THC (2 mg/kg) or CBD (10 mg/kg) from gestational day 5 to 20. Offspring PFC tissue was collected at preadolescence and adolescence. Gene expression was assessed by qRT-PCR while DNA methylation was analyzed by Pyrosequencing at selected CpG sites within the respective gene promoters.
In preadolescents, THC increased *Cnr1*, *Dagl-α* expression and reduced *Faah* expression, with a near-significant trend toward Drd2 upregulation; no methylation changes were observed. CBD increased *Cnr1* and *Dagl-α* expression without methylation alterations. In adolescents, THC upregulated *Cnr1* alongside reduced methylation at CpG site 1 and across the average of four analyzed sites, and also increased *Cnr2*, *Dagl-α*, and *Drd2* expression without further methylation changes. CBD selectively increased *Dagl-α* expression with no methylation modifications.
Prenatal cannabinoid exposure produces age-dependent and compound-specific transcriptional changes in the rat PFC. The epigenetic regulation of *Cnr1* observed exclusively in adolescent THC-exposed animals suggests a delayed, methylation-mediated mechanism that may contribute to neurodevelopmental vulnerability during critical maturational windows.
Acknowledgments: This work was supported by the Italian Minister of the University and Research, PRIN 2022 Project code: 2022K7YKTY, CUP: E53D23011400006 to S.D.M., V.M., and C.D.D.

# ASO-mediated p63 isoform switching as therapeutic strategy for AEC syndrome

Daniela Di Girolamo[1,2], E. Zagaria[2], E.C. Liao[3], M-G. Alameh[3], C. Missero[1,2]
[1]CEINGE Biotecnologie Avanzate Franco Salvatore, Napoli, Italy
[2]Biology Department, University of Naples Federico II, Napoli, Italy
[3]Center for Craniofacial Innovation, Children's Hospital of Philadelphia Research Institute, Philadelphia, USA

The *TP63* gene, a key member of the p53 family, is a master regulator of epidermal commitment and homeostasis. The ΔNp63α isoform, which is predominant in the epidermis, contains a C-terminal region harboring the Sterile Alpha Motif (SAM) and Transactivation Inhibitory Domain (TID). In Ankyloblepharon-Ectodermal defects-Cleft lip/palate (AEC) syndrome, heterozygous missense mutations within these domains, encoded by exon 13 and 14, trigger protein misfolding and aggregation. These pathological aggregates exert a toxic gain-of-function, sequestering p63 and disrupting the core transcriptional program required for skin integrity.
Since the p63β isoform naturally lacks exon 13 and is not subject to aggregation, we explored an isoform-switching strategy to bypass the molecular defect. Using a conditional mouse model, we demonstrated that p63β is functionally capable of sustaining epidermal stratification and adult skin homeostasis. Consequently, we developed a splice-switching strategy using antisense oligonucleotides (ASOs) designed to induce exon 13 skipping, thereby converting the pathogenic p63α into the functional p63β isoform.
We generated and screened multiple ASOs in immortalized human keratinocytes (NHEK N/TERT-2G) to evaluate their exon 13 skipping efficiency, isoform conversion rates, and cytotoxicity profiles. The lead ASO candidates demonstrated high efficiency in shifting the p63α-to-β ratio without compromising cell viability. Furthermore, in primary keratinocytes derived from AEC patients (carrying the I537T mutation), ASO treatment significantly reduced the p63 pathogenic aggregation and restored the expression of key p63-dependent transcriptional targets.
In conclusion, our findings establish ASO-mediated p63 isoform switching as a potent therapeutic approach for AEC syndrome, offering a rational strategy to restore epidermal function by avoiding toxic protein aggregates.

## Inside and beyond muscle: lncRNA-mediated regulation of intrinsic myogenic programs and paracrine crosstalk

Daniele Durante, M. Simula, S. Capurso, G. Buonaiuto, M. Ballarino
Department of Biology and Biotechnologies “Charles Darwin” Sapienza University of Rome, Rome, Italy

LncRNAs have long been appreciated for their cell-type-specific expression, which enables them to act as pivotal regulators of cellular development and physiology. Along these lines, our laboratory has identified several examples of lncRNAs with active roles in the precise fine-tuning of complex biological processes, such as myogenesis, thereby orchestrating muscle stemness [1] or differentiation [2], [3], [4]. Among them, the best-characterized example is *Charme*, a lncRNA which displays muscle-specific expression and that in humans has been implicated in cardiomyogenesis [5]. Interestingly, expanding upon its cell-intrinsic role, we have recently shown that its function extends beyond cardiomyocytes as its absence profoundly impairs the maturation and paracrine activity of resident cardiac fibroblasts, thereby disrupting extracellular matrix homeostasis and intercellular crosstalk [6]. Given that the lncRNA is also expressed in human adult skeletal muscle, we hypothesized that it may fulfill a comparable role both within the fibers and across the extracellular space. Indeed, our results demonstrate that the lncRNA is not only essential for the differentiation of human primary myoblasts, but also influences the surrounding extracellular environment. Considering the importance of muscle crosstalk in neuromuscular diseases, we are currently investigating the role of this lncRNA in muscle-nerve communication, with the aim of identifying new avenues for targeted therapeutic strategies.

[1] Cipriano A, et al. Elife. 2021;10:e54782. doi:10.7554/eLife.54782.
[2] Ballarino M, et al. EMBO J. 2018;37(18). doi:10.15252/embj.201899697.
[3] Desideri F, et al. Cell Rep. 2020;33(12). doi:10.1016/j.celrep.2020.108548.
[4] Taliani V, et al. Elife. 2023;12:e81360. doi:10.7554/eLife.81360.
[5] Buonaiuto G, et al. Nat Commun. 2025;16(1). doi:10.1038/s41467-025-62754-2.
[6] Floris E, et al. Cell Death Dis. 2026; In press.

## Cannabidiol effect on preventing Parkinson’s Disease pathogenesis in iPSC-derived dopaminergic neurons from sporadic patients

Claudia Esposito[1], M. Talarico[1], An. Quattrone[2,3], G.L. Benedetto[1], Raffaele Covello[1], A.l. Quattrone[3], E.I. Parrotta[2], G. Cuda[1]
[1]Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro
[2]Department of Medical and Surgical Sciences, University "Magna Graecia" of Catanzaro
[3]Neuroscience Research Centre, University "Magna Graecia" of Catanzaro

Cannabidiol (CBD) is a natural cannabinoid found in the Cannabis sativa plant. Unlike tetrahydrocannabinol (THC), CBD does not cause psychoactive effects. Several studies demonstrated the therapeutic benefits of CBD as antioxidant, anti-inflammatory, anticonvulsant, antiemetic, and sleep-inducing. Moreover, pre-clinical, *in vivo* and *in vitro* studies on Parkinson’s disease (PD) demonstrated the neuroprotective effect of CBD through different mechanisms of action and molecular targets. The aim of this study was to evaluate the CBD effects in iPSC-derived mesencephalic dopaminergic neurons (mDA) from sporadic PD patients. We established an *in vitro* model by the reprogramming of iPSCs from lymphocytes of three patients, clinically diagnosed as sporadic PD. Then, we induced mesencephalic dopaminergic differentiation applying a well-established protocol (Fedele et al.). The three individual cell lines were pooled at DIV20 to generate pooled-PD mDA, to mitigate biological variability, minimize line-specific biases, enhance experimental strength, and provide a more physiologically relevant context for drug screening. On DIV30, two groups were randomly established: i) untreated PD, as control group; ii) CBD treated PD. On DIV60, all experimental conditions were harvested to perform downstream analysis. The MTT assay revealed an increase in cell viability in the treated group compared to the control group. Immunofluorescence analysis confirmed an increase in MAP2- and TH-positive neurons, and a statistically significant overall improvement in neurite branching. The pathological α-synuclein protein, evaluated through Jess assay (Simple Western), decreased in the treated group. Overall, these results demonstrate an ameliorative effect of CBD in an *in vitro* model of sporadic PD, paving the way for further studies on the neuroprotective role of CBD in this established model.

P.29

## OncomiR-driven metastatic phenotype associates with chromatin changes in drug-resistant melanoma

A. Ortolano[1], R. Frigerio[2], D. Liguoro[3], F. Busu[4], F. Di Modugno[5], S. Roca[6], F. Fierro[3], M. Fanciulli[3], G. Ciliberto[7], R. Mancini[4,8], Luigi Fattore[3,9]
[1]Department of Anatomy, Histology, Forensic-Medicine and Orthopedics, Sapienza University of Rome, Rome, Italy
[2]Department of Experimental and Clinical Medicine, “Magna Graecia” University of Catanzaro, Catanzaro, Italy
[3]Gene Expression and Cancer Models Unit, IRCCS-Regina Elena National Cancer Institute, Rome, Italy
[4]Faculty of Medicine and Psychology, Department Clinical and Molecular Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy
[5]Tumor Immunology and Immunotherapy Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[6]Experimental Pharmacology Unit, Istituto Nazionale Tumori - IRCCS - Fondazione G. Pascale, Naples, Italy
[7]Takis s.r.l., Rome, Italy
[8]Scuola Superiore Di Studi Avanzati Sapienza (SSAS), Sapienza University of Rome, Rome, Italy.
[9]Department of Life Health Sciences and Health Professions, Link Campus University, Rome, Italy

Metastatic melanoma is a highly aggressive disease with rising global incidence. Although BRAF/MEK inhibitor therapy improves survival in BRAF-mutant patients, acquired resistance remains a major clinical challenge. Increasing evidence indicates that miRNAs contribute to this process. We identified two oncomiRs, miR-4443 and miR-4488, markedly upregulated in drug-resistant melanoma cells, where they drive a migratory and invasive phenotype associated with an elongated morphology. Mechanistically, both miRNAs directly target the intermediate filament nestin. LNA-modified antimiRs, highly stable in vitro and in vivo, were used to inhibit miR-4443/miR-4488. Their inhibition reduced migration and invasion across multiple drug resistant melanoma cell lines and promoted reversion of the elongated phenotype. Western blot and confocal analyses showed restoration of nestin expression, while flow cytometry confirmed efficient uptake of fluorescent LNAs. Importantly, in vivo administration of these LNAs reduced the metastatic capacity of resistant melanoma cells, supporting the therapeutic potential of this strategy. Given the morphological differences between sensitive and resistant cells and the link between cytoskeletal organization and chromatin structure, we investigated whether these phenotypes were also associated with epigenetic changes. ATAC-seq revealed a global reduction in chromatin accessibility in resistant versus sensitive cells. Notably, nestin was among the genes epigenetically downregulated in resistant cells, suggesting dual control: direct translational repression by miR-4443/miR-4488 and indirect modulation through reduced promoter accessibility. Ongoing experiments aim to further dissect this regulation. Overall, miR-4443 and miR-4488 shape cytoskeletal and chromatin features in resistant melanoma cells, and their inhibition with LNA-antimiRs represents a promising approach to limit metastatic traits and restore features compatible with drug sensitivity.

## Integrative epigenetic analysis of BDNF: PBMC DNA methylation and salivary miRNA expression in bipolar disorder

Beatrice Feliciani[1], F. Martella[1], C. Cavallotto[2], C. Marrangone[2], R. Allegretti[2], N. Girone[3], S. Saluzzi[4], M. Pucci[1], A. Sabatucci[1], M. Pettorruso[2,5], A. Di Giorgio[4], G. Martinotti[2,5,6], B. Dell'Osso[3,7,8], C. D'Addario[1,9]
[1]Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
[2]Department of Neuroscience, Imaging, and Clinical Science, University "G. d'Annunzio" of Chieti Pescara, Chieti, Italy
[3]University of Milan, Department of Mental Health, Department of Biomedical and Clinical Sciences Luigi Sacco, Milan, Italy
[4]Department of Mental Health and Addictions, ASST Papa Giovanni XXIII, Bergamo, Italy
[5]Department of Mental Health, ASL 2 Abruzzo Lanciano-Vasto-Chieti, Chieti, Italy
[6]Psychopharmacology, Drug Misuse and Novel Psychoactive Substances Research Unit, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom
[7]"Aldo Ravelli" Center for Neurotechnology and Brain Therapeutic, University of Milan, Milan, Italy
[8]Department of Psychiatry and Behavioral Sciences, Bipolar Disorders Clinic, Stanford University, CA, USA
[9]Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

Bipolar disorder is a complex psychiatric condition characterized by dysregulation of molecular pathways involved in neuroplasticity, stress response, and neuroimmune signaling. In this study, we investigated the epigenetic regulation of BDNF through an integrative approach combining DNA methylation analysis and microRNA (miRNA) expression profiling across distinct peripheral biological matrices. DNA was extracted from PBMCs to assess methylation levels at CpG sites within key BDNF promoter regions, while salivary samples were used to evaluate the expression of selected BDNF-targeting miRNAs.
Our results confirmed a significant reduction in BDNF gene expression in PBMCs from bipolar patients compared to healthy controls. We focused on CpG sites within promoter regions I and IV. While promoter IV did not appear to play a major role in transcriptional regulation, promoter I exhibited increased methylation in patients, with a sex-specific effect emerging after stratification, particularly in males.
To explore post-transcriptional regulation, four salivary exosomal miRNAs targeting BDNF were selected based on literature evidence. Among them, miR-16-5p was significantly upregulated in bipolar patients.
Overall, our findings support a multilevel epigenetic modulation of BDNF involving both DNA methylation and miRNA-mediated mechanisms across peripheral compartments. These results suggest that peripheral cells may act as dynamic sensors of biological signals, providing insights into the molecular mechanisms underlying bipolar disorder.

P.31

## ISG20 as a key exoribonuclease driving inflammation-induced 3′end miRNA trimming in pancreatic β-cells

Daniela Fignani[1,2], E. Aiello[1,2], G.E. Grieco[1,2], E. Pedace[1,2], S. Farooq[3,6], K. Preethi[3], G. Claudiane[4], C. Evans-Molina[3], R. Regazzi[4], F. Dotta[7], G. Sebastiani[1,2,5]
[1]Diabetes Unit-Dept. of Medicine, Surgery and Neuroscience, University of Siena, Italy
[2]Umberto Di Mario Foundation ONLUS, Toscana Life Science, Siena, Italy
[3]Departments of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA
[4]Department of Fundamental Neurosciences, University of Lausanne, Switzerland
[5]Regional Centre for Precision Medicine (CReMeP), Siena, Italy
[6]Department of Diabetes-Immunology, Beckman Research Institute, Duarte, CA, USA
[7]Department of Biomedical Sciences, National Research Council, Rome

IsomiRs are miRNA sequence variants arising from alternative processing, with 3′end trimming representing a dynamic isoform class. Inflammation-mediated enzymatic isomiR remodelling remain poorly understood. Here we show that inflammatory stress in autoimmune type 1 diabetes activates a pancreatic β cell-specific 3′-end trimming program which shapes their miRnome.
Small-RNA sequencing datasets from human pancreatic islets and human β-cell lines exposed to proinflammatory cytokines were analysed. IsomiRs and canonical miRNAs were quantified to assess changes in proportions across different isomiR classes in response to inflammation. RNA sequencing of cytokines-treated islets was performed on human β-cell lines. To identify sequence motifs determinants of 3′end trimming, K-mer enrichment analysis was performed on inflammation-trimmed miRNAs. IsomiR composition was also profiled in a psoriasis cohort of biopsied skin (n=49 samples) to explore whether inflammation-driven isomiR remodelling is conserved across autoimmune diseases.
In human islets, 31 and 61 miRNAs at 24h and 48h showed increased 3′Trim proportions under inflammation. In β-cells, 45 miRNAs showed increased 3′Trim at 48h. The exoribonucleases ISG20 was the most significantly upregulated after cytokine treatment and showed the strongest correlation with 3′Trim proportions. K-mer analysis identified the trinucleotide UGC as significantly enriched in all shared trimming miRNAs (FDR=0.003). In psoriatic skin, differential proportion analysis revealed an opposite significant reduction in 3′Trim and an increase in 3′Extension and canonical miRNA proportions respect to normal skin.
In conclusion, in pancreatic β-cells, miRNAs undergo a specific 3′end trimming during inflammation indicating a novel miRNAs remodeling mechanism in T1D. ISG20 emerges as a candidate enzyme linking inflammation to isomiR remodelling, and the trinucleotide UGC represents a putative cis-regulatory motif for the generation of trimmed miRNAs.

P.32

## Human neural stem cell fate is controlled by the alternative splicing regulator PTBP1

Ermes Filomena[1], C. Lasconi[1], D.L. Capobianco[1], F. Di Palma[1], C. Pousis[2], P. Spadavecchia[1], L. Simeone[1], F. Proto[1], D.C. Profico[3], M. Gelati[4], E. Picardi[1], G. Pesole[1], A.L. Vescovi[5,6], M. Svelto[1], L. Simone[7], F. Pisani[1], A.M. D'Erchia[1]
[1]Dipartimento di Bioscienze Biotecnologie e Ambiente, Università degli Studi di Bari Aldo Moro, Bari
[2]Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, CNR, Bari
[3]ProPharma LLC, Leiden, The Netherlands
[4]Fondazione Istituto Neurologico "C.Besta" Milano
[5]Università degli Studi Link Campus University, Roma
[6]Abu Dhabi Stem Cell Centre, Abu Dhabi, United Arab Emirates
[7]Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia

During fetal brain development, temporally defined alternative splicing (AS) programs control human neural stem cell (hNSC) self-renewal and differentiation, thereby regulating neurogenesis and gliogenesis. Polypyrimidine tract-binding protein 1 (PTBP1) is a well-characterized AS-regulator that has been largely investigated for its role in controlling the fate of NSCs and embryonic stem cells in mice through multiple NSC-related pathways (PMID: 38701759, 36626906, 27926877, 22655688, 17606642, 28990924, 25800779). Interestingly, PTBP1 can also shuttle between the nucleus and cytoplasm (PMID: 11739782), where it regulates mRNA stability and translation (PMID: 31729427, 35643957). However, its functional role in cell fate decisions of primary, non-immortalized hNSCs from the subventricular zone remains unknown.
We found that PTBP1 regulates hNSC fate at multiple levels, from cell proliferation to cellular metabolism, by controlling mitochondria and lipid droplets, to gene expression. RNA sequencing analysis revealed that PTBP1 depletion affects the expression profile of hundreds of coding and non-coding genes and produces a wide range of splicing changes, the majority of which are represented by skipped exons. RNA-seq data were also analyzed to characterize the expression profile of PTBP1 in hNSCs, identifying three PTBP1 transcripts expressed at higher levels. Consistent with the RNA-seq results, hNSCs exhibited two main translated proteins corresponding to the *isoforms a* and *c* and we found that the overexpression of *isoform a* alters the endogenous PTBP1 isoform pattern and changes hNSC fate, disrupts the endogenous isoform balance, decreases proliferation and stemness markers, and induces metabolic changes consistent with early differentiation.
Taken together, our results demonstrate that PTBP1 preserves the stemness state of hNSCs by controlling the expression profile of genes involved in orchestrating a neuronal differentiation program.

P.33

## Assessing gut microbiome health using integrative omics data analysis and machine learning

B. Kumar[1,2,3], E. Lorusso[3], Bruno Fosso[3], G. Pesole[3,4]
[1]European School of Molecular Medicine (SEMM), Milan, Italy
[2]Dipartimento di Oncologia ed Emato-Oncologia, Università degli Studi di Milano, Milan, Italy
[3]Department of Biosciences, Biotechnology and Environment (DBBA), University of Bari A. Moro, Bari, Italy
[4]National Research Council, Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM), Bari, Italy

The human gut microbiome plays a key role in maintaining host health. Imbalances in its structure and function, known as dysbiosis, have been linked to many diseases. Microbiome features can serve as indicators of gut health, and machine learning (ML) enables large-scale modelling of such data . However, robust classifiers capable of reliably distinguishing healthy from diseased individuals remain limited. Here, we developed and validated a binary ML model to predict gut health status, defined as healthy (no clinically diagnosed disease) or non-healthy (with diagnosed disease), using species-level gut microbiome profiles. We retrieved 7,452 stool shotgun metagenomes data from 32 studies across 12 disease conditions from public repositories. Samples were grouped as non-healthy (n = 3,987) or healthy (n = 3,465) according to the original study annotations.  A dedicated computational pipeline processed over 55 TB of sequencing data and generated species-level profiles. The workflow included batch correction for inter-study technical variation and log-ratio transformation. The dataset was split into training and test sets. Sixteen supervised models, combining four algorithms with three feature selection methods, were evaluated using F1 score and ROC–AUC. All models performed well on the test set, with F1 scores ranging from 78% to 86%, and ROC–AUC from 89% to 95%. The SVM-RBF model combined with permutation-based feature selection achieved the best performances (F1 = 86.6%). External validation on 642 samples from six independent studies confirmed model generalizability (overall F1 = 70.6%).  The model also performed well on previously unseen disease cohorts, including Clostridioides difficile infection  and type 2 diabetes.  This approach provides a scalable, non-invasive framework for microbiome-based health status prediction and highlights the potential of ML-driven gut microbiome diagnostics.

P.34

## MAPK15 regulates iron homeostasis through modulation of IRP2 stability

Lorenzo Franci[1,2], G. Vallini[3], D. Bonente[4], S. Gargiulo[1], L. Gherardini[1], G. Inzalaco[5], M. Taranta[1], E. Bertelli[4], V. Barone[4], M. Chiariello[1,6]
[1]Istituto di Fisiologia Clinica (IFC), Consiglio Nazionale delle Ricerche (CNR), Siena, Italy
[2]Dept. of Industrial Chemistry "Toso Montanari", University of Bologna, Bologna, Italy
[3]Section of Dermatology, Dept. of Medical, Surgical and Neurological Science, University of Siena, Italy
[4]Dept. of Molecular and Developmental Medicine, University of Siena, Siena, Italy
[5]Istituto degli Endotipi in Oncologia, Metabolismo ed Immunologia (IEOMI), Consiglio Nazionale delle Ricerche (CNR), Napoli, Italy
[6]Istituto per lo Studio, la Prevenzione e la Rete Oncologica, Siena, Italy

MAPK15 is an atypical MAP kinase involved in the regulation of cellular responses and metabolic adaptation to stress, although its role in iron metabolism remains unclear. Here, we investigated whether MAPK15 modulates iron homeostasis through reactive oxygen species (ROS). Given the central role of the liver in systemic iron metabolism and the emerging function of MAPK15 as a regulator of hepatic functions, we employed Huh-7 cells as a relevant in vitro model to investigate the ability of this MAP kinase to control hepatocellular iron metabolism, particularly in cancer. We found that MAPK15 depletion induces ROS accumulation and increases intracellular free iron, which are associated with reduced IRP2 levels and altered iron homeostasis. These changes were accompanied by dysregulation of pathways controlling iron uptake, storage, and export. In vivo analyses in MAPK15 knockout mice confirmed increased iron accumulation and reduced IRP2 levels, while IRP1 remained unaffected. Consistently, decreased expression of iron metabolism regulators, including FTH1, TFR1, and SLC40A1, was observed, supporting a systemic alteration of iron handling. Notably, these findings suggest that oxidative stress may contribute to the disruption of canonical IRP2-dependent regulation of iron metabolism. Overall, our findings identify MAPK15 as a key regulator of iron homeostasis, possibly through a ROS-dependent pathway controlling IRP2 stability, ultimately highlight a functional link between redox balance and iron metabolism.

P.35

## Regulatory landscape of *VDAC1*: integrating metabolic and transcriptional signals in cancer

Gaia Fusto[1], S. D'Amato[1], X. G. Pappalardo[1], A. Messina[2], V. De Pinto[1], F. Guarino[1]
[1]Dept of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania, Catania, Italy
[2]Dept of Geological and Biological Sciences (DSBGA), University of Catania, Catania, Italy

VDAC1 encodes the predominant isoform of the voltage-dependent anion channel family, serving as a crucial regulator of mitochondrial permeability, metabolism, and cell survival. While previous studies have characterized the basal trascriptional activity of VDAC genes, the broader regulatory architecture governing VDAC1 expression remains largely unexplored. This study aims to extend the analysis of epigenetic features by investigating chromatin accessibility, histone modifications, DNA methylation patterns, and regulatory landscapes across the VDAC1 locus, including promoter, intronic, and intergenic regions, in two cancer-derived cell lines. Particular attention will be given to map transcription factor binding sites within the gene body and to relate their activity with key cellular metabolic signaling pathways. Preliminary epigenomic analyses reveal a complex regulatory landscape at the VDAC1 locus, including association with several transcription factors involved in proliferation, viability and cell metabolism. The locus features CpG islands, active chromatin marks, multiple enhancer clusters including super-enhancers, and RNA elements like lncRNAs, piRNA, pseudogenes, all potentially involved in cancer progression. To link these regulatory elements to functional outcomes, the GeneXplain software suite will be employed to integrate multiple databases, enabling the reconstruction of transcriptional and signaling networks and the identification of potential upstream regulators. Overall, this work is expected to demonstrate that VDAC1 is regulated by a complex, multilayered regulatory architecture involving promoter, intronic, and enhancer elements, as well as non-coding RNAs. This approach is anticipated to provide new insights into its role in cancer-associated transcriptional programs and its involvement in integrating proliferative signals with metabolic pathways.

P.36

## Indirect mechanisms of T cell and CAR T cell suppression by SARS-CoV-2 in patients with classical Hodgkin lymphoma

Gatta Matteo[1], L. Lopresti[1], V. Tatangelo[1], A. Onnis[1], E. Andreano[2], R. Rappuoli[2], L. Patrussi[1], C.T. Baldari[1]
[1]Department of Life Sciences, University of Siena, Siena, Italy
[2]Fondazione Biotecnopolo di Siena, Siena, Italy

Classical Hodgkin lymphoma (cHL) is characterized by a profoundly immunosuppressive tumor microenvironment (TME) that impairs T cell function and promotes immune escape. Receptor/ligand interactions, including immune checkpoints, are well-established mediators of T cell inhibition in cHL. In other hematological malignancies soluble mediators play a central role in shaping T cell exhaustion and dysfunction, suggesting that similar mechanisms may operate in cHL. Here we investigated whether and how tumor-derived soluble factors contribute to T cell dysfunction cHL. To this aim, cHL cell-derived culture media were used to condition $CD4^+$ and $CD8^+$ T cells and CAR-T cells from healthy donors. Our results demonstrated a marked impairment of T cell activation and cytotoxic capacity, accompanied by altered differentiation profiles. Mechanistically, exposure to tumor-derived soluble factors resulted in defective immunological synapse formation and impaired lytic granule polarization, as assessed by confocal microscopy. Notably, cHL supernatants induced upregulation of exhaustion markers, including PD-1 and LAG-3, and increased expression of their transcription factor TOX. These changes were associated with a distinct cytokine profile identified by ELISA and qPCR. Ongoing studies are aimed at dissecting the molecular pathways underlying these effects, with a focus on signaling networks governing T cell exhaustion and immune suppression. By integrating functional assays with molecular profiling of the cHL secretome, we seek to identify key soluble mediators and signaling axes responsible for T cell dysfunction.
This work provides a mechanistic framework to understand how the cHL microenvironment enforces T-cell exhaustion and suppresses anti-tumor immunity, with potential implications for improving the efficacy of cell-based therapies in patients with hematological malignancies.

P.37

## Title Integrated multi-omics reveals tissue-specific host–microbiome interactions and cross-site microbial signatures in pediatric IBD and FGID

Antonio Grimaldi[1,2], R. Capuana[2], T. Fusco[3], R.De Santis[3], M. Salvi[3], L. Vaccaro[3], G. Gandolfo[2], M. Massa[3], D. Sgambato[4], V. Rainone[4], M. Simonetti[3], M. Creoli[3], C. Chiantese[3], M. Martinelli[3], E.Miele[3], A. Staiano[3], D. Cacchiarelli[1,2,3]

[1]Genomics and Experimental Medicine Program, Scuola Superiore Meridionale (SSM School of Advanced Studies), Naples, Italy

[2]Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

[3]Department of Translational Medicine, University of Naples "Federico II", Naples, Italy

[4]Department of Chemical, Materials and Industrial Productions Engineering

Pediatric Inflammatory Bowel Diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), and Functional Gastrointestinal Disorders (FGIDs) are chronic conditions with rising incidence and significant clinical impact, yet their molecular determinants remain incompletely understood. We performed an integrative multiomic analysis in a pediatric cohort comprising IBD (CD and UC), FGID patients, age-matched healthy controls, and family members. Over 250 biospecimens were collected from multiple intestinal regions (ileum, ascending, transverse, descending colon, and rectum), as well as stool and oral swabs. Host mucosal transcriptomes were profiled using 3' digital RNA sequencing, while microbial composition and functional potential were assessed through shotgun metagenomics of stool and oral samples, complemented by shallow host genotyping. Transcriptomic analyses identified robust, tissue-specific differential gene expression signatures distinguishing IBD from controls, and molecular profiles capable of discriminating CD and UC across intestinal segments. Microbiome profiling revealed marked alterations in community structure and metabolic pathways in IBD, with UC showing pronounced dysbiosis and reduced diversity, while FGID displayed subtler shifts. Integration of host and microbial datasets uncovered significant correlations between differentially expressed genes and specific bacterial taxa, highlighting candidate host–microbiome interaction axes underlying disease phenotypes. Cross-site integration further demonstrated that gut microbial dysbiosis is partially mirrored in the oral microbiome, indicating systemic microbial alterations beyond the intestinal niche. Altogether, this study provides a high-resolution, multi-layered view of host–microbiome dynamics across the pediatric gastrointestinal tract, identifying disease-associated pathways and candidate biomarkers with potential for patient stratification and non-invasive monitoring.

P.38

## Deciphering the crosstalk between miR-200C and NF-YA splicing in cancer cells

S. Nikolli[1], C. Mottini[1], I. Manni[1], G. Piaggio[1], Aymone Gurtner[1,2]
[1]Gene Expression and Cancer Models Unit, IRCCS-Regina Elena National Cancer Institute, Rome, Italy
[2]Institute of Translational Pharmacology (IFT), National Research Council (CNR), Rome, Italy

miRNAs act as post-transcriptional regulators via mRNA degradation and/or translational repression, and emerging evidence indicates additional nuclear functions. The miR-200 family plays a central role in epithelial-to-mesenchymal transition (EMT), a key process in tumor metastasis. An unbiased screening of the EMT splicing program identified NF-YA, a master regulator of cell proliferation, as a major splicing event: the short isoform (exon 3 skipping) is associated with an epithelial phenotype, whereas the long isoform correlates with a mesenchymal phenotype. Accordingly, in a panel of colon cancer cell lines we observed that the short isoform is enriched in epithelial cells, while the long isoform predominates in mesenchymal ones. The ability of miR-200c to induce E-cadherin is impaired by dnNF-YA, suggesting that its role in EMT partly depends on NF-YA.
We hypothesize that, beyond known mechanisms maintaining the epithelial phenotype, miR-200 family members directly modulate splicing. miR-200c overexpression selectively increases endogenous short NF-YA mRNA and protein in both epithelial-like and mesenchymal-like colon cancer cells. It does not influence exogenous NF-YA isoforms, indicating a requirement for intronic regions. This effect is also observed in non-colon cancer cells.
Computational analyses identify multiple predicted miR-200c binding sites across NF-YA genomic regions, including exon 3. miR-200 family members localize in the nucleoplasm, where they associate with AGO1/2. Notably, both miR-200c and AGO1/2 bind NF-YA pre-mRNA within regions encompassing intron 2, intron 3, and exon 3.
Finally, NF-YA regulates miR-200 transcription by binding miRNA promoters, and its reduction decreases both primary and mature miR-200 levels, supporting a positive feedback loop that may contribute to maintenance of the epithelial phenotype. Overall, these findings reveal a previously unrecognized interplay between miR-200c and NF-YA isoforms relevant to EMT.

P.39

## The TDP43-ASRGL1 functional interaction governs the progression of neurodegeneration in *Drosophila*

Ciro Iaccarino[1], M. Fais[1,2], E.R. Simula[1], M. Galioto[1], C. Crosio[1], L.A. Sechi[1,2]
[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[2]Azienda Ospedaliera Universitaria di Sassari, Microbiology and Virology Unit 07100, Sassari, Italy

Amyotrophic lateral sclerosis (ALS) is characterized by the pathological accumulation of TDP43 showing different post-translational modifications including isoaspartate formation. In humans, the Asparaginase-like 1 protein (ASRGL1) is the sole protein possessing beta-aspartyl peptidase activity. Critically, a reduction in ASRGL1 function seems to increase isoaspartate formation in TDP43, which triggers its aggregation, possibly revealing a key mechanism in neurodegeneration. In fact, utilizing patient tissues and in vitro cell lines, it has been recently demonstrated that ASRGL1 silencing or overexpression triggers neuronal death or neuroprotection, respectively, in TDP43 proteinopathy models. However, the functional role of ASRGL1 in TDP43 associated proteinopathy and toxicity, remains poorly understood. We generated a *Drosophila melanogaster* model, expressing an RNAi for CG8860 the sole ortholog of human ASRGL1, and hTDP43 in neurons using the Elav-GAL4 driver. Survival analysis revealed that the combined genotype exhibited a marked reduction in lifespan compared with UAS-hTDP43 control flies This severe phenotype was accompanied by an increase in TDP43 proteinopathy regarding protein accumulation and aggregates formation. Interestingly, the double transgenic flies are also characterized by the enhanced expression of mdg4, an endogenous retrovirus–related gene. This study establishes a robust Drosophila model linking reduced ASRGL1 activity to TDP43 proteinopathy and neurodegeneration largely recapitulating findings from mammalian cell models.

P.40

# Metabolic CRISPR screen identifies novel synthetic lethal partner of *TGS1* in acute myeloid leukemia

Domenico Ignoti[1], D. Dellantonio[1], V. Miano[2], M. Bouvet[1], G. Carrà[3], L. Lopez[4], I. Barbieri[1,2]
[1]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy
[2]Division of Cellular and Molecular Pathology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom
[3]San Luigi Gonzaga Hospital, Regione Gonzole, Orbassano, Italy; Department of Clinical and Biological Sciences, University of Turin, Italy
[4]Andalusia Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain

Acute myeloid leukaemia (AML) is an aggressive hematological malignancy with a 5-year survival rate of approximately 20%. Despite initial responses to chemotherapy, most patients relapse, highlighting the urgent need for new therapeutic strategies.
AML cells display a strong dependence on oxidative phosphorylation (OXPHOS) to sustain proliferation and maintain an undifferentiated state. However, clinical application of OXPHOS inhibitors has been limited by toxicity, suggesting that combination approaches are required to exploit this vulnerability.
We recently characterized RNA 5′ cap trimethylguanosine synthase (TGS1) as a key regulator of AML metabolism and survival. TGS1 enhances the translation of mRNAs encoding mitochondrial proteins involved in OXPHOS and the tricarboxylic acid cycle. Accordingly, TGS1 depletion impairs mitochondrial respiration and increases oxidative stress, creating exploitable metabolic liabilities.
To identify such vulnerabilities, we performed CRISPR-Cas9 dropout screens in wild-type and TGS1-deficient AML cells using a library targeting ~3,000 metabolic genes. This analysis revealed multiple synthetic lethal interactions specific to TGS1 loss. Among these, KCNQ5, a subunit of the Kv7 potassium channel, emerged as a top dependency. KCNQ5 is exclusively expressed in AML and is not detected in other cancer types or normal hematopoietic cells, highlighting its potential as a highly selective therapeutic target.
We propose that KCNQ5 supports adaptation to metabolic stress induced by TGS1 depletion. Given the druggability of ion channels, KCNQ5 inhibition (e.g., XE991) may represent a novel therapeutic strategy.

P.41

## Capturing extracellular vesicles (EVs) in human ventral midbrain organoids

Giuliana La Rosa[1,2], C. Gabriele[3], E. Pascale[1], A. Barone[3], M.R. Iazzetta[4], L. Prestagiacomo[3], D. Paolino[3], M. Gaspari[3], E.I. Parrotta[2], A. Fiorenzano[1,5]
[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II," Naples, Italy
[2]Department of Medical and Surgical Sciences, Stem cell laboratory, University “Magna Graecia,” Catanzaro, Italy
[3]Department of Experimental and Clinical Medicine, University “Magna Graecia,” Catanzaro, Italy
[4]Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy
[5]Department of Experimental Medical Science, Developmental and Regenerative Neurobiology, Wallenberg Neuroscience Center, Lund Stem Cell Center, Lund University, Lund, Sweden

Human brain organoids represent a powerful model to recapitulate key features of neural development, where intrinsic programs and non-cell-autonomous signals orchestrate brain organization. Extracellular vesicles (EVs) have emerged as critical mediators of intercellular communication due to their bioactive cargo. We describe an innovative approach for EVs isolation from human ventral midbrain organoids (vMOs) using a heat shock protein-binding peptide that enables efficient EVs capture from complex 3D organoid systems, overcoming the limitations of conventional isolation methods. EVs isolated from day 25 vMOs were characterized confirming size distribution and marker expression. EVs cargo was profiled by mass spectrometry (nLC-MS/MS). In line with the developmental stage of vMOs, functional enrichment analysis of the EVs cargo revealed pathways associated with neurodevelopmental processes, including axon guidance, synaptic organisation and neuronal differentiation, as well as glycolysis, lipid metabolism and the oxidative stress response, alongside extracellular matrix organization and vesicle-mediated transport. These signatures reflect the high metabolic demands, tissue remodelling and active intercellular communication that characterise early-stage vMOs. Pathways related to protein folding and quality control also emerged, highlighting proteostasis and adaptive stress response mechanisms associated with rapid growth and differentiation. Network analysis further identified highly interconnected protein clusters involved in metabolic regulation and cellular stress, supporting the idea that the EVs cargo reflects coordinated biological programmes within the organoids. These findings establish a robust platform for the isolation of EVs from vMOs and provide insights into the molecular landscape of vesicle-mediated communication in human neural systems.

## The RNA modification m$^6$A facilitates poly-adenylation in the malaria parasite *Plasmodium falciparum*

Joshua Levendis[1], A. Distiller[1], S. Mussgnug[2], L. Fernando[1], S. Lancashire[1], N. Young[3], E. McHugh[1], S. Baumgarten[2], S. Ralph[1]
[1]Department of Biochemistry and Pharmacology, University of Melbourne, Victoria, Australia
[2]Institut Pasteur, Université Paris Cité, INSERM U1347, G5 Parasite RNA Biology Group, Paris, France
[3]Faculty of Veterinary Science, The University of Melbourne, Victoria, Australia

Advances in RNA modification detection have shown they can regulate gene expression by changing translation, stability, splicing and cellular localisation of mRNA. Knowledge about the nexus between the RNA modification pseudouridine and translational control has been used to make mRNA vaccines and therapeutics more effective, yet there are over 170 naturally occurring RNA modifications which are not intentionally being used in gene design. N$^6$-methyladenosine (m$^6$A) is the most abundant modification in eukaryotic mRNA and can change gene expression, but the molecular mechanisms of how it does this at an individual gene level are poorly understood. Our lab studies *Plasmodium falciparum* biology, a unicellular parasite which causes the deadliest form of malaria. *P. falciparum* has an abnormally high genomic AT content (80%) and employs transcriptional and post-transcriptional control over gene expression to differentiate into sexual and asexual stages in response to changing environments.
Here, we show using Nanopore direct RNA sequencing (SQK-RNA004) that the parasite contains complex 3'UTR m$^6$A patterns which are associated with alternative poly-adenylation. We were able to categorise individually sequenced RNA transcripts by methylation status and identify m$^6$A as a predictor of proximal poly-adenylation. While most m$^6$A are found in 3'UTRs, a handful of genes have intragenic methylation with alternative isoforms, including genes which have asexual or sexual stage specific expression. Finally, we used an inducible protein mislocalisation system to disrupt the methyltransferase which writes m$^6$A to mRNA and observed transcriptional readthrough and chimeric transcripts upon m$^6$A depletion. This work represents an effort to characterise what m$^6$A does to *P. falciparum* mRNA while working to understand if we can use m$^6$A to modulate timing or abundance of gene expression.

P.43

## GP130 signalling modulates the differentiation potential of naive human pluripotent stem cells

Nicolò Magri, G. Rossignoli, G. Panebianco, G. Zuffellato, G. Martello
Department of Biology, University of Padua, Padua, Italy

Naive human pluripotent stem cells (hPSCs), the *in vitro* counterpart of the pre-implantation epiblast, exhibit broad differentiation potential toward both embryonic and extraembryonic lineages, including the trophectoderm, which gives rise to the placenta. Given the established role of the GP130/JAK/STAT3 signalling pathway in maintaining pluripotency in mouse PSCs, we hypothesized that this pathway might contribute to the regulation of human pluripotency. Therefore, naive hPSCs were cultured and differentiated under different conditions that modulate the GP130/JAK/STAT3 pathway using activating and inhibitory approaches, both genetic (CRISPRi) and pharmacological. Short and long-term treatments were applied to evaluate pathway activity, as well as effects on proliferation, morphology, and expression profiles.
In maintenance and absence of pathway activation, a tendency toward differentiation is observed. In agreement, the over-activation of the GP130 signalling in naive hPSCs results in decreased efficiency toward the generation of the trophectoderm extraembryonic lineage. Overall, this indicates that GP130 signalling is involved in the remarkable ability to make extraembryonic tissues by playing an inhibitory role in the process. Further experiments will monitor the efficiency in the generation of embryonic lineages to evaluate fate-specific contribution. Furthermore, the dissection of GP130 downstream signalling will establish if JAK/STAT3 signalling and/or other pathways regulate naive hPSCs fate.

## EGLN1 stabilizes NRF2 to coordinate metabolism and oxidative stress

Federico Marchini[1], A. Iacopini[1], M. Chiariello[2], F. Galvagni[1]
[1]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy
[2]Istituto di Fisiologia Clinica (IFC), Consiglio Nazionale delle Ricerche (CNR) and Core Research Laboratory, Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO), Siena, Italy

Nuclear factor erythroid 2-related factor 2 (NRF2) is a key transcriptional regulator that controls the cellular response to oxidative stress. Under basal conditions, NRF2 is targeted for degradation by its negative regulator KEAP1 via the ubiquitin–proteasome pathway. In contrast, exposure to electrophilic or oxidative stress promotes NRF2 stabilization and nuclear translocation. Although NRF2 is widely recognized as a tumor suppressor under physiological conditions, its overexpression in cancers can support malignant cell survival by enhancing resistance to oxidative stress.
The aim of this study was to investigate a novel mechanism regulating NRF2 stability. We analyzed NRF2 protein expression in triple-negative breast cancer (TNBC) cells following proteasome inhibition with epoxomicin and found that NRF2 levels are modulated by cysteine independently of the KEAP1–proteasome axis. Exogenous NRF2 expression and RT-qPCR analyses confirmed that this regulation does not occur at the transcriptional level, suggesting the involvement of translational or post-translational mechanisms distinct from the canonical KEAP1-dependent pathway.
As a potential mediator of this regulation, we focused on EGLN1, a cellular sensor of oxygen and cysteine availability. EGLN1 is known to regulate hypoxia-inducible factor (HIF) by prolyl hydroxylation, promoting its proteasomal degradation under normoxic conditions. To test our hypothesis, EGLN1 activity was inhibited in TNBC cells using both RNA silencing and roxadustat, a selective EGLN1 inhibitor. Both approaches resulted in reduced NRF2 protein levels. Consistently, overexpression of wild-type EGLN1 increased NRF2 levels, whereas expression of a catalytically inactive EGLN1 mutant (D254H) led to decreased NRF2 expression. These findings reveal a functional link between NRF2 stability and EGLN1 prolyl hydroxylase activity, highlighting a previously unrecognized convergence between oxidative metabolism and the antioxidant response.

P.45

## Overcoming prostate cancer "coldness": a combinatorial approach using standard treatments and OMV-based vaccines

Elisa Marmocchi[1], R. Begaj[1], M. Cantarelli[1], D. Bressan[1], N. Annesi[1], M. Barbareschi[2], G. Bertalot[2], F. G. Carbone[2], A. Alaimo[1], M. Kruitof-de Julio[3], N. Ternette[4], U. Ala[5], F. Chiacchiera[1], G. Grandi[1], A. Lunardi[1]
[1]CIBIO Department, University of Trento, Trento, Italy
[2]Santa Chiara Hospital-APSS, Trento, Italy
[3]University Hospital of Bern, Bern, Switzerland
[4]University of Dundee, Dundee, Scotland
[5]University of Turin, Turin, Italy

Immunotherapies are ineffective against prostate cancer (PCa) due to its immunologically "cold" microenvironment: low mutational burden and scarce immune infiltration are the causes. To overcome this, we propose a combinatorial approach using repurposed Bacillus Calmette-Guerin (BCG) to recruit immune cells into the prostate, and the outer membrane vesicle (OMV) platform, engineered with newly discovered PCa epitopes, as a therapeutic vaccine. We established *in vitro* PCa cell models engineering four cell lines to express the transcription factor ERG (its overexpression characterizes half of PCa) upon doxycycline induction. The four cell lines represent different stages of aggressiveness of PCa, allowing us to selectively isolate ERG-dependent events when searching new epitopes. To identify them, deep RNA-sequencing and HLA-I immunoprecipitation coupled with mass spectrometry (MS) were performed. For *in vivo* preliminary testing, a castration-resistant PCa (CRPC) model was developed via subcutaneous injection of TRAMP-C2 cells in C57BL/6N mice. Efficacy tests vaccinating mice using empty OMVs was performed, followed by immunohistochemistry (IHC) on tumor sections. In silico RNA-Seq analysis uncovered the presence of some genes and relative isoforms specifically associated with ERG induction. These transcriptional events will be cross-referenced with the "unmatched epitopes" from MS. Shared candidates will be tested for their immunogenicity in the sera of PCa patients. IHCs of OMVs-treated tumors showed increased $CD8^+$ lymphocyte infiltration and reduced T-regulatory cells compared to PBS controls. OMVs treatment also induced neutrophil abscesses that surround necrotic areas. Furthermore, tumor collagen deposition recapitulates human CRPCs: its impact on immune infiltration is under investigation. We expect that the combination of immune cell recruitment and novel epitopes paves the way for tailored and affordable immunotherapies against PCa.

## Interaction between *Tbx1* and *Vegfr3* in cardiac morphogenesis

I. Aurigemma[1], M. Bilio[2], S. Cioffi[2], E. Illingworth[1], A. Baldini[2], Stefania Martucciello[1]
[1]Dept. of Chemistry and Biology, University of Salerno, Fisciano, Italy
[2]Institute of Genetics and Biophysics "ABT", Naples, Italy

Background: The transcription factor *TBX1*, a key gene implicated in the 22q11.2 deletion syndrome, plays a critical role in cardio-pharyngeal mesoderm (CPM) a population of progenitors that differentiate into different cell types, including endothelial cells, that contribute to the formation of the cardiac outflow tract (OFT). *VEGFR3* is a major lymphangiogenesis gene; *TBX1* activates *Vegfr3* in murine lymphatic endothelial cells by binding a putative enhancer in the endogenous *Vegfr3* gene and the two molecules interact strongly during cardiac lymphangiogenesis and in brain vascularization.
Objectives: We investigated whether *Tbx1* has a role in endothelial specification and whether *Tbx1-Vegfr3* interaction is required in cardiac progenitors in the CPM, or in their descendents, for development of the OFT.
Methods: We used a serum-free differentiation protocol in mouse ES cells (mESC) *WT* and *Tbx1* knockout and recently performed scRNA sequencing to characterize transient cellular states or differentiation dynamics. Furthermore we investigated *Tbx1-Vegfr3* interaction using mouse genetics approaches.
Results: We devised an EC differentiation model from cardiogenic mesoderm that resulted in approximately 95% VE-Cadherin-positive cells within 8 days. Furthermore, *Tbx1* was expressed in differentiating endothelial cells (from day4 to day8).
*In vivo*, combined *Tbx1-Vegfr3* mutants exhibited cardiac defects**,** including morphogenesis defects of the OFT.
Conclusions: Our findings reveal a previously underappreciated role of *TBX1* in endothelial differentiation and a *Tbx1-Vegfr3* interaction playing a critical role in cardiac morphogenesis.

P.47

## RBM20 is a novel regulator of alternative splicing in a neuronal model

Giacomo Menichetti, A. Valentino, A. Javaid, M. G. Bruno, F. Belpinati, E. Trabetti, M. G. Romanelli, C. Bombieri
Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona, Italy

**Background:** Protein Tau, encoded by the Microtubule-Associated Protein Tau (*MAPT*) gene, is essential for microtubule stabilization and cytoskeleton organization. The alternative splicing (AS) of *MAPT* gene generates multiple Tau isoforms with distinct structures and functions whose imbalance is associated with tauopathies, including Alzheimer's disease, Parkinson's disease, and frontotemporal dementia. Understanding the molecular mechanisms regulating MAPT AS is essential to elucidate neurodegenerative disease onset. Here, we investigate the role of the RNA-binding proteins Polypyrimidine-tract binding protein 1, PTBP1, and RNA-binding protein 20, RBM20, in the regulation of MAPT AS using a cellular model. Preliminary data indicated that PTBP1 and RBM20 interact with MAPT transcripts. PTBP1 knockdown was performed in SH-SY5Y cells to assess its role in MAPT splicing regulation.
**Materials and methods:** SH-SY5Y cells were transfected with siRNA targeting PTBP1. RNA and protein expression levels of PTBP1, MAPT splice variants, and RBM20 were analysed by RT-qPCR and western blot at different time points.
**Results and Conclusions:** PTBP1 silencing in SH-SY5Y cells resulted in a significant modulation of MAPT splicing, promoting the expression of Tau isoforms including exon 6 and exon 10, supporting a role for PTBP1 as a regulator of MAPT AS. Notably, PTBP1 knockdown was associated with increased RBM20 mRNA expression, suggesting a possible interplay in the fine-tuning of Tau isoform expression. Overall, these findings identify PTBP1 as a key regulator of MAPT exon splicing and provide preliminary evidence for RBM20 role in the regulation of neural splicing events.

## Exploring the regulative role of the long non-coding RNA AC064875.1 in Glioblastoma stem cells and its therapeutic potential

Gianluca Mordini[1], M. Russo[2], R. Appierdo[1], M. Helmer-Citterich[1], S.A. Ciaffrè[2], S. Galardi[1]
[1]Dept Biology, Tor Vergata Univ., Rome, Italy
[2]Dept Biomedicine and Prevention, Tor Vergata Univ., Rome, Italy

Long years of research about the origin of glioblastoma multiforme (GBM) have led to the general agreement that it likely arises from glioma stem-like cells (GSCs), which are also responsible for the high chemo- and radio-resistance of this tumour and of its recurrence (Lathia et al., 2015; Prager et al., 2020). Unravelling the non-coding transcriptome and molecular peculiarities of these cells should provide an inside view in GBM origin and new tools to defeat it.
Our research stems from an RNA-sequencing characterization that we have performed in a cohort of patient-derived GSC lines. We identified the extreme and specific overexpression of a novel lncRNA (AC064875.1) in GSCs versus healthy brain tissue, cultured healthy astrocytes and also versus human glioblastoma non-stem cell lines. AC064875.1 cytoplasmatic expression was found to be negatively correlated with patient survival and strongly reduced when GSCs are induced to differentiate. As a readout of the stemness ability of GSCs, we measured their capability to form new neurospheres and demonstrated that this function was inhibited after the stable knock-down of AC064875.1, suggesting a key role of AC064875.1 in maintaining stem cell-like characteristics, such as self-renewal ability and differentiation potential. Furthermore, transient silencing of this lncRNA leads to a reduction in the expression levels of some markers involved in maintaining the stemness of these cells. RNA pull-down experiments will identify the AC064875.1 molecular interactors, opening up the possibility of using this lncRNA as a target of differentiation therapy. Depleting the pool of tumour stem cells using this strategy will enable a drug repurposing approach to be employed, allowing these cells to be targeted in a more specific and effective way.

P.49

## Comprehensive isoform landscape of the LINP1 locus in LigI-defective fibroblasts under chronic DNA damage

Maria Chiara Mottola, P. Asti, S. Bione, G. Biamonti, A. Montecucco
Institute of Molecular Genetics “Luigi Luca Cavalli Sforza” (IGM) CNR, Pavia, Italy

Autosomal recessive partial DNA ligase I (LigI) deficiency has been linked to immunological disorders and increased susceptibility to cancer. 46BR.1G1 fibroblasts are derived from a patient with a biallelic hypomorphic mutation (R771W) in the *LIG1* gene. This defect impairs maturation of Okazaki fragments, with accumulation of single- and double-stranded breaks. Despite persistent damage, these cells retain proliferative capacity, a feature typical of preneoplastic lesions. We have previously studied protein coding gene expression in 46BR.1G1 and found alterations induced by the LigI defect that could explain the morphological features of these cells. Here we have exploited 46BR.1G1 to identify lncRNAs which could assist the proliferation of cells with chronic DNA damage.
RNA-seq was performed comparing 46BR.1G1 cells with LigI-rescued (7A3) and LigI wild-type cells (GM847). This analysis revealed numerous differentially expressed lncRNAs deregulated in tumours. Among these, transcripts of the LINP1 (LncRNA In Non-homologous end joining Pathway 1) genomic locus were selected for further analyses. Since numerous transcripts have been recently annotated on the LINP1 locus we performed a long-read RNA-seq to characterize the deregulated transcripts. The analysis identified 3 main transcripts overexpressed in 46BR.1G1 cells: LINP1-214, LINP1-202 (LINC00707), and a novel splicing variant of LINP1-202 with the retention of intron 4. These account for the 82% of the 41 transcripts expressed from LINP1 locus. LINP1-214 and LINP1-202 seem to be independently regulated since their selective downregulation does not impair the expression of the other one. Although mainly localized to the cytoplasm, LINP1-214 downregulation increases the markers of DNA damage and arrests proliferation of damaged but not of control cells, suggesting a major role of this transcript in the adaptation of 46BR.1G1 cells to persistent DNA damage.

P.50

## Vid22 counteracts G-quadruplex-induced genome instability

E. Galati[1], G.M. Bernini[1] , F.M. Scotti, D. Novarina[1], M. Chiara[1,2], A. Chakraborty[1,] S. Ricagno[1], L. Broggini[1], A. Chaves[1], C. Camilloni[1], S. Sertic[1], D.S. Horner[1,2], G. Pesole[2,3], G.W. Brown[4], F. Lazzaro[1], Marco Muzi-Falconi[1]
[1]Department of Biosciences, Università degli Studi di Milano, Milan, Italy,
[2]Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, Consiglio Nazionale delle Ricerche, Bari, Italy,
[3]Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università di Bari 'A. Moro', Bari, Italy
[4]Department of Biochemistry and Donnelly Centre, University of Toronto, Toronto, Canada

Genome instability is a pathological condition characterized by the accumulation of genetic alterations. It can cause cell death and, in higher eukaryotes, represents a hallmark of cancer cells. Using Synthetic Genetic Array (SGA) technology, we performed a yeast genetic screening to identify genes and pathways protecting from chronic DNA damage. Among the candidates, we focused on VID22, which encodes a putative transcription factor with an unclear role in the DSB repair pathway.
We found that vid22Δ cells show spontaneous genome instability and increased sensitivity to agents inducing replicative stress. Whole-genome sequencing of different vid22Δ mutants revealed an accumulation of gross chromosomal rearrangements, instability of repetitive sequences, and alterations at the rDNA locus. The affected regions are GC-rich and prone to form G-quadruplex (G4) structures. We demonstrated that Vid22 binds directly to G4 regions, protecting them from instability. Moreover, loss of Vid22 results in telomere length defects, characterized by enrichment of G4 motifs.
To dissect its function, we generated point mutants disrupting Vid22 homodimerization, DNA binding, or protein interactions. The characterization of these mutants will also be presented. Our data suggest that Vid22 is a novel regulator of G4 metabolism and genome integrity.

P.51

## Phytochemical-induced epigenetic reprogramming and cellular sensing: multi-omics dissection of *Broccolo nero* leaf extracts across developmental stages

Gaia Nicotra[1], S. Boninelli[1], D. Arena[2], G. Privitera[1], G. Fusto[1], M. Spatafora[2], S. Conti Nibali[1], XG. Pappalardo[1], S. Reina[1], F. Branca[2], V. De Pinto[1]
[1]Dept. of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[2]Dept. of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy

Broccoli novel foods (sprouts, microgreens, and baby leaves) represent a rich source of bioactive phytochemicals capable of modulating cellular responses to environmental and nutritional cues. While their anti-inflammatory properties are increasingly recognized, the molecular mechanisms underlying their impact on cellular sensing and gene regulation remain poorly defined. Supported by ELIXIRxNextGenIt-1st NOA Call, we employed a multi-omics strategy to dissect the epigenetic and transcriptional responses induced by *Broccolo nero* (BN) leaf extracts in tumorigenic (SKBR-3) and non-tumorigenic (NIH/3T3) cell models. By integrating transcriptomic profiling, DNA methylation analysis, and quantification of omega-3 fatty acids (linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid), we aimed to characterize how phytochemical exposure influences key regulatory networks involved in inflammation, oxidative stress, and cellular survival. Our results indicate that BN novel foods trigger distinct, developmental stage-dependent molecular signatures, reflecting differential activation of transcription factors, redox-sensitive pathways, and inflammatory mediators. This work contributes to a deeper understanding of the interplay between phytochemical signals of BN leaves and long-term gene expression reprogramming. Further investigation is needed to elucidate the precise signaling cascades, transcriptional regulators, and post-transcriptional mechanisms involved, as well as to explore bioavailability and synergistic interactions. Such insights may advance our understanding of cellular sensing processes and inform the development of targeted BN novel foods nutritional or therapeutic strategies.

P.52

## Dysregulation of microRNA biogenesis in cancer: the impact of mutant p53/Dicer complex

Sabela Nikolli[1,2], I. Manni[1], M.S. Roca[3], R. Lombardi[4], B. Pucci[3], L. Ronchetti[1], G. Grazi[5], G. Piaggio[1], A. Gurtner[1,6]
[1]UOSD SAFU, IRCCS Regina Elena National Cancer Institute, Rome Italy
[2]D'Annunzio University of Chieti, Pescara, Italy
[3]Experimental Pharmacology Unit, Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Naples, Italy
[4]Animal Facility-Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Naples, Italy
[5]Department of Clinical Experimental Oncology, IRCCS Regina Elena National Cancer Institute, Rome Italy
[6]Institute of Translational Pharmacology (IFT), National Research Council (CNR), Rome, Italy

The global miRNA deregulation observed in human cancers is often the result of defects in the miRNA biogenesis pathway. Still, the mechanisms through which miRNAs are regulated in cancer and the connection between oncogenes and miRNA biogenesis remain poorly understood. Only a handful of miRNAs have been identified as targets of mutant p53 (mutp53) at transcriptional level, and very few data about the role of mutp53 on deregulation of miRNA biogenesis are available yet.
In the process of studying the mutp53 involvement on the deregulation of miRNA biogenesis, we have obtained results that strongly suggest a new transcriptionally independent function of mutp53 in miRNA maturation, through a mechanism by which this oncogene is able to interfere with Dicer activity. The relevance of the proposed mechanism reside on the oncosuppressor functions of miRNAs down-regulated by mutp53, such as induced cell death in colon cancer cells and tumor growth inhibition in vivo.The KEGG pathway and GO analyses of putative target genes of the mutp53-dependent miRNAs identifies several pathways and genes related to cancer among which EGF, FGF signaling, cell cycle, mitosis, cell death and stress response. Interestingly, many of the genes belonging to these categories have oncogenic potential and are known as key regulators of altered molecular pathways in tumors. Our data support the idea that mutp53, inhibiting the expression of specific miRNAs, upregulates a subset of genes involved in tumor progression, including genes already targeted by molecular drugs. Finally, we performed immunoprecipitation of Dicer before (scr) and after (sh) p53 deletion, followed by mass spectrometry analysis. We identified 999 and 858 proteins in the cytoplasm of scr and shp53 cells, respectively, that bound significantly to Dicer (with a Mascot score >39). Bioinformatics analysis
indicate several regulatory networks in which the identified proteins are involved, such as cancer, cell cycle and cell death.

P.53

## WNT signaling activation promotes dopaminergic differentiation toward A10 fate

Erika Pascale[1], A. Bruzelious[2], G. La Rosa[1,3], E. Sozzi[4], R. Iazzetta[5], M. Parmar[2], A. Fiorenzano[1,2]
[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II," Naples, Italy
[2]Department of Experimental Medical Science, Developmental and Regenerative Neurobiology, Wallenberg Neuroscience Center, Lund Stem Cell Center, Lund University, Lund, Sweden
[3]Stem cell laboratory, Department of Medical and Surgical Sciences, University "Magna Graecia," Catanzaro, Italy
[4]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland
[5]Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy

Dopaminergic (DA) neurons comprise distinct subtypes, including A9 neurons involved in motor function and primarily affected in Parkinson’s disease, and A10 neurons, which regulate cognition and behavior and are implicated in multiple neuropsychiatric disorders. Despite sharing a common origin in the floor plate of the ventral midbrain and the same neurotransmitter identity, A9 and A10 dopaminergic neurons differ in morphology, projection patterns and selective vulnerability to oxidative stress, mitochondrial dysfunction and α-synuclein pathology. A key signaling pathway in midbrain DA (mDA) neuron development is WNT/β-catenin, which regulates anterior-posterior patterning, progenitor specification and neurogenesis. However, its contribution in specification of distinct DA neuronal subtypes remains incompletely unexplored. Single-cell RNA sequencing of an intranigral xenograft model revealed a selective enrichment of WNT signaling in A10 dopaminergic neurons compared to A9, suggesting a previously underappreciated role in subtype determination (Fiorenzano et al., PNAS 2024). To investigate this, we temporally modulated WNT signaling during dopaminergic differentiation. Strikingly, pathway activation within a defined developmental window (days 18–24) biased cell fate toward an A10 identity, whereas later stages activation had minimal impact. WNT activation further induced pronounced spatial reorganization, upregulation of A10-enriched molecular markers and increased axonal density and complexity. These effects were consistently observed across both 2D and 3D culture systems, underscoring a robust role for WNT signaling in dopaminergic patterning and maturation. Collectively, these findings establish WNT signaling as a key regulator of A10 dopaminergic neuron specification and identity, providing a framework for refining differentiation strategies to selectively generate A10 neurons and improve in vitro models of neuropsychiatric disorders involving A10 circuits.

P.54

## Machine learning-based assessment of the healthy human gut mycobiota landscape using ITS1 DNA metabarcoding data

G. Defazio[1], E. Lorusso[2], M. De Robertis[1,2], T. Mello[3], A. Galli[3], Graziano Pesole[1,2], B. Fosso[1]
[1]Department of Bioscience, Biotechnology and Environment, Università degli Studi di Bari Aldo Moro, Bari, Italy
[2]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, Bari, Italy
[3]Experimental and Clinical Biomedical Sciences “Mario Serio”, Università degli Studi di Firenze

Microbiome investigation[1] is a compelling research domain that magnifies composition and function of microorganisms populating our bodies as complex communities. However, defining the role of the human microbiome and its contribution in promoting host health and homeostasis is still challenging. In the human gut microbiome context, investigating links between microbiome compositional and functional profiles and host conditions may involve several covariates such as age, sex, BMI, and health status. Although statistically complex this challenge can be addressed by combining machine learning models with XAI (Explainable Artificial Intelligence). Currently, most studies available in the literature employ these tools by focusing on prokaryotes, which represent the most abundant component of the human gut microbiome. By contrast, very few studies have focused on fungi. To bridge the gap regarding the composition of the human mycobiome in health and disease, we present the methods and results described in our latest work[2]. In particular, we trained supervised learning models (e.g., Random Forest) achieving a predictive accuracy of 87% in health/disease classification based on compositional profiles from 1,500 freely available datasets. These are ITS1 DNA metabarcoding sequencing data related to 10 comparative health/disease study sesigns. In addition, we employed Shapley values to interpret the models and link several fungal genera to health/disease status. Furthermore, we also present unpublished results based on the inference of functional profiling of the investigated community using PICRUST2[3] combining compositional and functional profiles to train the models.

[1] PMID: 36270681.
[2] PMID: 41928315.
[3] https://doi.org/10.1038/s41587-020-0548-6

P.55

## Unraveling the role of the lncRNA MALAT1 in keratinocyte physiopathology

Fabio Petti[1], C. Pisani[2], A. Onori[2], L. Amicone[1], A. Marchetti[1], R. Maione[1], M. Di Rienzo[3], N. Corbi[2], C. Cicchini[1]
[1]Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
[2]CNR-Institute of Molecular Biology and Pathology, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
[3]Department of Epidemiology, Preclinical Research and Advanced Diagnostics, National Institute for Infectious Diseases "Lazzaro Spallanzani"-IRCCS, Rome, Italy

Keratinocytes serve as the primary cellular component of the epidermis, acting as the first line of defense against environmental insults. Dysregulation of the processes maintaining skin homeostasis is linked to various pathologies, including cutaneous squamous cell carcinoma (cSSC) and psoriasis, marked by aberrant keratinocyte proliferation and differentiation. Our work focuses on the role of the long non-coding RNA MALAT1 as a key player in keratinocyte biology under both physiological and pathological conditions. Literature evidence emphasizes its function as a molecular scaffold that promotes protein proximity to coordinate biological functions, furthermore it exerts multiple functions in controlling epithelial cell transdifferentiation and tumor progression. Based on bioinformatic predictions, we identified MALAT1 as a regulator of a novel interaction between the pro-autophagic protein AMBRA1 and the RNA-binding protein HAX1. We validated the MALAT1/HAX1 interaction via RNA Immunoprecipitation (RIP) assays in A431 cSSC cells. Notably, MALAT1 knockdown significantly altered the A431 phenotype, impairing both migratory capacity and the expression of differentiation markers. In Ker-CT cells, we further characterized the kinetics of keratinocyte differentiation following MALAT1 silencing via Antisense Oligonucleotides (ASO). Our results indicate that MALAT1 depletion impacts the differentiation program, a process linked to AMBRA1 downregulation and major cytoskeletal reorganization. Our objective is to expand the knowledge in this field, thereby uncovering novel molecular therapeutic targets for skin diseases.

# Abrogation of specific glycosylation sites on SARS-CoV2 Spike enhance virus infectivity and modulate antibody neutralizing activity

A. Campese[1], M. Bissoli[1], E. Orlandi[1], C. Bombieri[1], D. Zipeto[1], Piubelli C.[2], N. J. Temperton[3], M. Castelli[4], Alessandra Ruggiero[1]
[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2]Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy
[3]Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent and Greenwich Chatham Maritime, Kent, UK
[4]Università Vita Salute San Raffaele, Milano, Italy

**Background:** Post-translational modifications (PTMs) are involved in virus–host interactions, but little is known about SARS-CoV2. We studied how PTMs, particularly N-glycosylation, can modulate the structure and function of viral envelope proteins, influencing receptor binding, immune evasion, and infectivity.
**Methods:** By bioinformatics we identify the genomic sites associated with glycosylation and by site-directed mutagenesis, we generated 28 SARS-CoV-2 Spike (S) mutants in key domains (NTD, RBD, SD1–SD2, S2, and stalk) on a D614G virus backbone. Lentiviral pseudotypes were produced in HEK293T cells. Viral pseudotypes' infectivity was tested on CHO-ACE2-TMPRSS2 cells and normalized for infection assays in Caco-2 and Calu-3 cell lines. Neutralizing activity was assessed in sera from vaccinated individuals, stratified by prior infection status.
**Results:** Most mutants showed infectivity levels comparable to or lower than WT (measured as Relative Luminescence Unit, RLU), with two exceptions: N1074Q, targeting a glycosylation site in the S2 region and N331Q, located in the RBD, which had higher infectivity. Neutralization assays using IC50- derived serum dilutions revealed that several mutants were more efficiently neutralized than WT, particularly those affecting glycosylation sites in the NTD, S2, and stalk regions. Furthermore, sera from previously infected (PI) showed stronger neutralization capacity compared to naïve individuals across multiple mutants, including those in the NTD, RBD, S2, and stalk. In contrast, N1074Q was more efficiently neutralized by naïve sera ($p = 0.01$).
**Conclusion:** Collectively, these findings indicate that specific glycosylation patterns in the Spike protein play a key role in modulating both viral infectivity and antibody-mediated neutralization, highlighting their contribution to virus–host interactions and their potential relevance for the design of future vaccine and therapeutic strategies.

P.57

## Elucidating the role of GAT3 in Alzheimer’s disease

Alessandra Saitta[1,2], R. Basilotta[1], M. Lanza[1], G. Casili[1], S. Oddo[1], A. Caccamo[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[2]Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy

Alzheimer's disease (AD) is the most common neurodegenerative disorder and is characterized by progressive memory loss and cognitive decline. Alterations in multiple neurotransmitter systems contribute to AD-related deficits. Recently, increasing attention has been given to GABAergic system. During inhibitory neurotransmission, GABA released into the synaptic cleft is primarily cleared by the transporters GAT1 and GAT3. Reduced GAT3 activity, reported in postmortem AD brains, is thought to elevate extracellular GABA levels, thereby increasing tonic inhibition and impairing cognitive function. However, the relationship between GAT3 downregulation and AD-related dysfunction remains unclear. To address this, we selectively reduced GAT3 expression using CRISPR/Cas9 in APP/PS1 mice, a widely mouse model of AD. Three adeno-associated viral vectors (AAV5-GFAP-Cas9 carrying distinct gRNAs targeting GAT3) were injected into the cerebral ventricles of 6-month-old mice. Cognitive performance was assessed six months later using the novel object recognition test. Synaptic markers were evaluated by western blot analysis of PSD95 and synaptophysin, and GAT3 knockdown was confirmed by GFAP/GAT3 co-immunofluorescence. Immunofluorescence analysis confirmed a robust decrease in GAT3 expression in GFAP-positive astrocytes, indicating effective targeting. However, no significant differences were observed between wild-type and APP/PS1 mice in behavioral performance or synaptic protein levels following GAT3 reduction. These findings suggest that GAT3 loss alone may not drive AD-related dysfunction, or that compensatory mechanisms mitigate its effects.

## Methylomic profiling to uncover the pathological mechanisms of Marinesco-Sjögren syndrome

F. Bellia[1,2], L. Federici[1,2], Michele Sallese[1,2]
[1]Department of Innovative Technologies in Medicine and Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy
[2]Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

Marinesco-Sjögren syndrome (MSS) is a rare, early-onset neurodegenerative disease mainly affecting the cerebellum and skeletal muscles, with patients exhibiting cerebellar ataxia, muscle weakness, and congenital or early-onset cataracts. The syndrome is triggered by loss-of-function mutations in the *SIL1* gene in approximately 50-60% of the cases. In the woozy mouse, a model of MSS, Purkinje cells (PCs) localized in cerebellar lobules I–VIII are primarily affected, whereas those in lobules IX–X are spared. Similarly, oxidative muscles are the most affected, while glycolytic muscles are less involved.
To investigate the mechanisms underlying the selective degeneration of PCs, we performed DNA methylome profiling of cerebellar lobules I–VIII in wild-type (WT) and woozy (Wz) mice at 6 weeks of age, prior to overt PC neurodegeneration, enabling the identification of early epigenetic changes that may drive cell loss. High-coverage mapped reads were primarily located in key genomic features, including CGIs, CpG shores, promoters, and UTRs. Differentially methylated region (DMR) analysis revealed specific methylation differences between WT and Wz mice. In total, more than 700 DMRs were identified across CGIs, CpG shores, promoters, 5′ UTRs, exons and introns. Pathway enrichment analysis of DMR-associated genes showed significant enrichment in KEGG pathways related to the glutamatergic synapse, focal adhesion, long-term potentiation, and calcium, MAPK, Hippo, and Wnt signalling pathways.
In summary, Sil1 loss induces specific DNA methylation changes in gene regulatory regions associated with synaptic function and signaling pathways. These findings advance our understanding of MSS pathogenesis and may aid in identifying epigenetic biomarkers or therapeutic targets.
*We acknowledge the NextGenerationEU (PNRRMR1-2022-12375730) for funding, and the National Facility for Data Handling and Analysis at Human Technopole (Project ID 2154347) for their support.*

P.59

## A multi-omic atlas of transcription factor regulatory grammar in human fibroblasts

Marcello Salvi
Dept of Traslational Medicine, Università degli studi di Napoli Federico II

The rational design of transcription factor (TF) cocktails for cell fate conversion requires a mechanistic understanding of how individual TFs navigate and remodel the epigenome — knowledge that remains largely absent at proteome scale. Here we present **Project 300F**, a systematic perturbation atlas in which ~300 TFs were individually overexpressed in primary human fibroblasts and their regulatory impact resolved across five molecular layers: chromatin accessibility (ATAC-seq), DNA methylation at single-CpG resolution (WGBS and methylation arrays), histone modification landscapes (ChIP-seq), three-dimensional genome architecture (Hi-C compartments and TADs), and transcriptional output (RNA-seq).
As a reference coordinate system, we built a fibroblast T0 chromatin state atlas by decoding ChromHMM emission probabilities across 15 states into five functionally objective macrocategories — active promoters, active enhancers, poised/weak elements, Polycomb-repressed regions, and constitutive heterochromatin — each anchored to Hi-C PC1 compartment scores, TAD boundaries, and base-resolution CpG methylation. This multi-layer epigenome map defines the precise barriers each TF must overcome, quantifying remodeling events in fully methylated closed chromatin (>80% mCpG) versus pre-accessible loci.
Critically, all five data layers are fused into a unified regulatory graph in which nodes represent genomic loci and edges encode weighted relationships between ATAC-seq delta signals, RNA-seq log2 fold-changes, FIMO PWM binding scores, methylation deltas, ChromHMM state transitions, and TAD-constrained enhancer–promoter loops. Traversal of this graph enables mechanistic classification of each TF, pioneer factor, epigenetic rider, or indirect regulator and, crucially, the identification of non redudant TF combination. This atlas is designed as a predictive resource for the rational optimitation of reprogramming and trasdifferentation

P.60

## miRNA-mediated targeting of LSD1 and PRMT6 as a novel strategy to modulate androgen receptor signaling

Alfredo Sepe[1,2], A. Falconieri[1,2], F. Rosada[3], R. Borella[3], M. Basso[3], M. Pennuto[1,2]
[1]Department of Biomedical Sciences, University of Padova, Padova, Italy
[2]Veneto Institute of Molecular Medicine (VIMM), Padova, Italy
[3]Department of Cellular, Computational, and Integrative Biology (CIBIO), University of Trento, Trento, Italy

Clear cell renal cell carcinoma (ccRCC) is characterized by widespread epigenetic alterations and dysregulated transcriptional programs that contribute to tumor development and progression. Among the factors involved, lysine-specific demethylase 1 (LSD1) and protein arginine methyltransferase 6 (PRMT6) act as transcriptional co-regulators that can cooperatively enhance androgen receptor (AR) activity, with their effects further amplified under conditions that increase receptor activity and toxicity.
Previous studies on Spinal and Bulbar Muscular Atrophy (SBMA) models have shown that targeting these co-regulators can effectively modulate AR signaling. In particular, both pharmacological inhibition and genetic silencing of LSD1 and PRMT6 have been demonstrated to reduce AR transactivation and mitigate associated cellular toxicity. Furthermore, we observed that the inhibition of these targets can effectively reduce proliferation in PCa in vitro model. Notably, microRNA (miRNA)-mediated silencing of these factors has emerged as a promising strategy to regulate their expression and improve disease-related phenotypes in preclinical settings.
Building on these observations, we propose to extend this approach to ccRCC. We hypothesize that modulating overexpressed co-regulators such as LSD1 and PRMT6 through miRNA-based strategies may help control aberrant AR-driven transcriptional programs in this tumor type. Such an approach could attenuate excessive AR activity while preserving its physiological functions, potentially offering a more balanced therapeutic strategy.
In conclusion, applying miRNA-mediated targeting of epigenetic co-regulators represents a promising avenue in ccRCC and warrants further investigation to evaluate its potential for translational applications.

## Modeling early neuromuscular co-development: a 3D Neuromuscular organoids platform to unveil lncRNA dynamics in lineage bifurcation

Marco Simula[1], P. Tollis[2], D. Durante[1], S. Capurso[1], A. Setti[1], W. Visciglio[1], F. Mirabella[4], P. Laneve[3], M. Ballarino[1]
[1]Department of Biology and Biotechnologies “Charles Darwin” Sapienza University of Rome, Rome, Italy
[2]Center for Life Nano- & Neuro-Science of Istituto Italiano di Tecnologia (IIT), Rome, Italy
[3]Institute of Molecular Biology and Pathology CNR-IBPM, Rome, Italy
[4]Light imaging facility, Human Technopole, Milan, Italy

Many neuromuscular disorders originate from defects during early embryogenesis, highlighting the critical need to understand the mechanisms governing neuro-muscular co-development. The proper bifurcation of progenitor cells into interacting neuroectodermal and mesodermal lineages is a highly coordinated morphogenetic event, essential for the subsequent assembly of the neuromuscular system.Because traditional 2D cultures fail to capture the spatial and cellular complexity of this cross-talk, advanced 3D human Neuro-Muscular Organoids (hNMOs) have emerged as an invaluable platform for modeling physiological co-development [1]. By combining this model system with single-cell RNA sequencing (scRNA-seq), we demonstrated that these organoids successfully give rise to all key lineage-specific subtypes - including muscle, neurons, glia, sclerotome, and epithelium - which collectively contribute to the proper development and formation of the muscle-nerve interaction. Within this framework, we identified highly promising, long noncoding RNA (lncRNA) candidates whose expression is tightly restricted to either the neural or mesodermal developmental trajectories. To dissect their functional relevance, we are generating specific lncRNA-knockout hiPSC lines, which will be used to establish KO-NMO models. Using these targeted organoid models, we will investigate how ablating cell-type specific lncRNAs alters early lineage commitment. Ultimately, we plan to elucidate the noncoding regulatory landscape underlying neuromuscular tissue assembly, with a particular focus on lncRNAs of interest in the lab, either muscle-specific [2], [3] or neural-enriched [4].

[1] Martins F, et al. Cell Stem Cell. 2020 doi:10.1016/j.stem.2019.12.007
[2] Ballarino M, et al. EMBO J. 2018 doi:10.15252/embj.201899697.
[3] Buonaiuto G, et al. Nat Commun. 2025 doi:10.1038/s41467-025-62754-2.
[4] Tollis P, et al. Cell Death Dis 2023 doi:10.1038/s41419-023-06196-y.

P.62

## Blocking the TGF-β pathway attenuates neurodegeneration and tau pathology in a model of progressive supranuclear palsy

Mariagrazia Talarico[1], C. Esposito[1], An. Quattrone[2,3], G. L. Benedetto[1], R. Covello[1], M. Martini[4], V. Zuccalà[4], Al. Quattrone[3], E. I. Parrotta[2], G. Cuda[1]
[1]Department of Experimental and Clinical Medicine, University "Magna Graecia", Catanzaro, Italy
[2]Department of Medical and Surgical Sciences, University "Magna Graecia", Catanzaro, Italy
[3]Neuroscience Research Centre, University "Magna Graecia", Catanzaro, Italy
[4]Department of Human Pathology of Adult and Developmental Age "Gaetano Barresi", Section of Pathology, University of Messina, Italy

Progressive supranuclear palsy (PSP) is a devastating 4R-tauopathy with no approved disease-modifying treatment. Building on recent observation of markedly increased Transforming Growth Factor-beta (TGF-β) levels in PSP patient-derived ventral midbrain organoids (vMOs) and in serum neuronally derived exosomes in patients, this study aimed to investigate whether TGF-β inhibitors could mitigate PSP pathology in vMOs. Eighty-eight vMOs were generated from induced pluripotent stem cells of two PSP-Richardson's syndrome patients and two healthy controls (HC), respectively. PSP organoids were cultured under various conditions: (a) treated from day60 to day90 with TGF-β receptor-I inhibitor (SB431542), (b) exposed to recombinant TGF-β1, β2 and β3, and (c) untreated. At day90, tau phosphorylation and 4R-tau levels were assessed by immunoblotting. Neuronal (Tyrosine hydroxylase, TH, neurofilament light chain, NfL) and astrocytic (Glial Fibrillary Acidic Protein, GFAP) markers were analysed by immunofluorescence, and histopathological hallmarks by immunohistochemistry. At day90, untreated PSP-derived vMOs exhibited higher levels of phosphorylated tau (Ser396, Thr181, Ser202/Ser205 [AT8]), 4R-tau, and NfL than HC-derived vMOs, accompanied by astrocytic reactivity (increased GFAP), dopaminergic neuron loss (reduced TH), and PSP characteristic lesions with tau deposition in neurons and astrocytes. These alterations were significantly reduced in PSP-derived vMOs treated with TGF-β receptor-I inhibitor. Conversely, exposure to TGF-β exacerbated tau hyperphosphorylation, neurodegeneration and astrocytic reactivity. TGF-β inhibition prevented tau accumulation, dopaminergic loss and reactive astrogliosis in patient-derived PSP organoids. Monoclonal antibodies targeting the TGF-β pathway demonstrated acceptable safety profiles in humans in clinical trials, and our pre-clinical findings support a trial in early-stage PSP patients.

P.63

## CYP1A1 regulates estrogen receptor activity and response to CDK4/6 inhibitors in estrogen receptor-positive breast cancer cells

Gabriele Tani[1], A. Smiriglia[1], M. Bacci[1], N. Lorito[1], F. Bonechi[1], A. Santi[1], M. Gonzàlez-Fernàndez[2,3], S. Rottenberg[2,3], P. Francica[2,3], A. Morandi[1]
[1]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
[2]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[3]Cancer Therapy Resistance Cluster, Department for BioMedical Research, University of Bern, Bern, Switzerland

Estrogen receptor alpha (ER)-positive tumors are the most commonly diagnosed breast cancer. The standard therapeutic approach combines endocrine therapy with CDK4/6 inhibitors (CDK4/6i). Despite its efficacy, resistance frequently occurs, and we have previously shown that metabolic reprogramming plays a key role, prompting further metabolic investigation. To explore this, we performed an shRNA metabolic loss-of-function screen in an endocrine therapy-resistant ER+ breast cancer model (MCF7-LTED), identifying cytochrome P450-1A1 (CYP1A1) knockdown as synthetically lethal in combination with CDK4/6i. This combination induces ROS-dependent cell death, which is rescued by N-acetylcysteine (NAC). Notably, CYP1A1 silencing reduced ER transcriptional activity and activating phosphorylation, suggesting a role in sustaining ER signaling. Indeed, ER activation rescued the synthetic lethal effect.
Mechanistically, CYP1A1 may produce cholesterol-derived oxysterols that support ER activity. Indeed, two potential CYP1A1-derived oxysterols, e.g., 5,6-Cholesterol Epoxides (EC) and 6-oxo-cholestane-3β,5α-diol (OCDO), rescued both synthetic lethality and ER activation in CYP1A1-silenced cells. Moreover, ER degradation abrogated OCDO-induced signaling, confirming ER dependency. We further identified cholesteryl ester hydrolysis within lipid droplets (LD) as a potential cholesterol source for CYP1A1, supported by increased cholesteryl ester accumulation upon CYP1A1 silencing. Consistently, inhibition of carboxylesterase 1, a key enzyme in cholesterol mobilization, induced synthetic lethality with CDK4/6i, which is rescued by exogenous cholesterol.
Overall, our findings uncover a metabolically driven mechanism underlying CDK4/6i response and identify CYP1A1 as a potential predictive biomarker and therapeutic target in ER+ breast cancer.

## Gene therapy in Huntington’s disease: functional validation through early phenotype rescue and multi-omics profiling

Angela Toffano[1], R. Ghirardo[1], S. Amato[2], E. Motanova[3], A.M. Gambetta[4], G. Martello[1]
[1]Department of Biology, University of Padova, Padova, Italy
[2]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg
[3]Department of Biomedical Sciences, University of Padova, Padova, Italy
[4]Department of Clinical and Experimental Medicine, University Magna Graecia, Catanzaro, Italy

Huntington’s disease (HD) is an inherited and incurable neurodegenerative disorder caused by CAG repeat expansions in the huntingtin (HTT) gene. The resulting mutant HTT protein alters cellular physiology at the multiple levels, inducing toxicity and cell death. The mechanisms directly causing toxicity are partially understood, thus precluding the development of successful therapeutic strategies.
In order to implement an effective gene therapy, an unbiased genome-wide screening was performed to detect genes functionally involved in cytotoxicity induced by mutant HTT. This study enabled the identification of potential suppressors of mutant huntingtin, such as Mtf1, which were subsequently tested in vitro, in mouse ES cells, and in vivo, in zebrafish and mouse.
Currently, we are developing human in vitro models, including 2D striatal neurons and 3D organoids, to investigate HD pathology. In addition to these adult-stage models, recent studies have highlighted abnormalities arising during early development, such as defects in cell polarity, differentiation, and ciliogenesis. To address these early phenotypes, we employed neural progenitor cells derived from iPSCs carrying either 21 or 109 CAG repeats. The potential rescue effect of our candidate gene was then evaluated by measuring ROS levels and examining key molecular pathways through integrated transcriptomic and proteomic analyses.

## Compartmentalized control of Cdk1 activity drives mitosis exit

A.F. Serpico[1,2], Asia Trano[1], D. Grieco[1,2]
[1]DMMBM, University of Naples “Federico II”, Naples, Italy
[2]CEINGE Biotecnologie Avanzate “Franco Salvatore”, Naples, Italy

In mitosis, full activity of cyclin B-dependent kinase 1 (Cdk1), the major mitotic-driving kinase, is granted by positive feedback loops. Cdk1 antagonizes Wee1/Myt1, its inhibitory kinases, and stimulates Cdc25, its activating phosphatase. In addition, Cdk1 sustains the Spindle Assembly Checkpoint (SAC), the safeguard mechanism that prevents cyclin B degradation and chromosome segregation until spindle assembly [1,2]. Nevertheless, we have recently shown that spindle assembly relies on a spindle-localized fraction of Cdk1 that escapes the positive feedback loops, is inhibited by Wee1, and promotes spindle microtubule (MT) growth [3]. Here, through genetic and chemical means, we show evidence that inhibitory control of Cdk1 activity is also required to stabilize bipolar MT-chromosome attachments and to silence the SAC, promoting mitosis exit. Our findings have implication for chromosome stability and tumorigenesis.

[1] Hutter LH, et al. (2017). Interlinked bistable mechanisms generate robust mitotic transitions. Cell Cycle. 16(20):1885–1892.
[2] Serpico AF & Grieco D (2020). Recent advances in understanding the role of Cdk1 in the spindle assembly checkpoint. F1000Res. 9:F1000 Faculty Rev-57.
[3] Serpico AF, et al. (2022). Compartmentalized control of Cdk1 drives mitotic spindle assembly. Cell Rep. 38(4):110305.

P.66

## Integrated epigenetic and transcriptional profiling reveals microenvironment-dependent plasticity in pancreatic cancer models

S.N. Cox[1], D.I. Abbrescia[2], F. Fracasso[1], Y. Antonacci[1,2], C. Pousis[2], C. Lasconi[1], P. Spadavecchia[1], F. Marzano[2], A.M. D'Erchia[1], G. Pesole[1], S. J. Reshkin[1], R. Cardone[1], Apollonia Tullo[2]
[1]Department of Biosciences, Biotechnology and Environment, University of Bari, Italy
[2]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies – IBIOM - CNR – Bari, Italy

Pancreatic ductal adenocarcinoma (PDAC) progression is shaped by cross-talk among tumor cell types, including Cancer Stem Cells (CSCs), and their acidic tumor microenvironment, but the underlying transcriptomic and epigenetic mechanisms remain poorly understood. Here, we combined bulk RNA-seq and EPIC v2.0 DNA methylation profiling to characterize normal pancreatic epithelial (HPDE) cells, tumor parenchymal cells, and CSCs grown under different extracellular matrix (ECM) and extracellular pH (pHe) conditions. Transcriptomic analysis showed that ECM composition and extracellular acidosis strongly modulated gene expression programs, with the Collagen I-rich acidic condition (90%C, pH=6.7) producing the largest divergence from normal pancreatic epithelial cells. Furthermore, the CSCs displayed marked transcriptional rewiring, including repression of epithelial programs and induction of neural-associated and plasticity-related signatures. In parallel, methylation analysis revealed a clear separation of HPDE, the tumor parenchymal, and CSCs samples and showed a global reduction in mean beta values both in tumor-derived cells and CSCs compared with HPDE, consistent with widespread cancer-associated hypomethylation. Gene-focused analysis further supported a functional link between methylation and expression changes: hypomethylation of upstream CpG island probes was associated with increased FABP7 expression, whereas hypermethylation at the TGFBI-associated CpG island correlated with transcriptional repression in the CSCs. Overall, these results indicate that PDAC cellular plasticity is driven by the interplay between microenvironment-dependent transcriptional reprogramming and locus-specific epigenetic remodeling. This integrated framework highlights candidate biomarkers and regulatory mechanisms linked to stem-like features and tumor progression in pancreatic cancer.

P.67

## MiR-15b-5p: a newly identified tumor suppressor in colorectal adenocarcinoma

Danila Valletta, N. Mosca, M. Pezzullo, D. Diodati, A. Russo, N. Potenza
Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania “Luigi Vanvitelli”, Caserta, Italy

Colorectal cancer (CRC) is a leading cause of death for malignant tumors worldwide, with most patients developing CRC as a consequence of tumor progression from adenoma into colorectal adenocarcinoma (COAD). Much effort has been dedicated to uncovering genetic and epigenetic alterations driving the onset and progression of CRC; recently, microRNAs added new layers of complexity in the multistep process of carcinogenesis.
This study explores the role and molecular mechanisms of miR-15b-5p in COAD.
MiR-15b-5p was found to be strongly and significantly down-regulated in tumour samples in comparison to normal tissues (fold regulation: -6.5; p-value: $0.008 \times 10^{-17}$). The impact of miR-15b-5p on cancer hallmarks was then evaluated by mimic transfection and an array of biological assays performed on COAD cell lines models. Experimental results demonstrated that miR-15b-5p was able to inhibit cell proliferation, migration and invasion. Looking for molecular mechanism mediating the oncosuppressive effect of the miRNA, targetome and pathways enrichment analyses were performed, revealing that Wnt/beta-catenin pathway was one of the most significantly represented. Then, the functional relationship of miR-15b-5p with Wnt/beta-catenin pathway was investigated by TOP/FOP assay, demonstrating its ability in inhibiting the pathway. Thus, potential miRNA targets related to the Wnt/β-catenin pathway were extracted from the targetome by filtering for converse expression patterns in COAD biopsies and optimal miRNA–target pairing parameters. Their experimental validation as miRNA targets and functional assays are in progress.
Overall, the study could unveil novel regulatory circuits relying on miR-15b-5p contributing to COAD progression and may reserve new perspectives in diagnostics and therapeutics.

P.68

## Modelling early pre-cancerous stages *in vitro* using human gastric organoids and mucosoids

Antonia Voli[1], C. Centrella[1,2], D. Eletto[1], A. Porta[1], A. Tosco[1]
[1]Department of Pharmacy, University of Salerno, Fisciano (SA), Italy
[2]Ph.D. program in Drug Discovery and Development, University of Salerno, Fisciano (SA), Italy

Recent findings have refined the classical Correa model, repositioning gastric intestinal metaplasia (GIM) as a plastic and potentially reversible transitional state rather than an irreversible step in gastric carcinogenesis. This evolving paradigm suggests that distinct GIM subtypes, shaped by specific transcriptional programs, retain the capacity to revert under permissive conditions. The transition toward an intestinal phenotype is well established to be driven by chronic inflammation, most notably driven by *Helicobacter pylori* infection, together with sustained epithelial injury and genotoxic stress. Nevertheless, the molecular mechanisms governing epithelial reprogramming and the potential restoration of gastric identity remain to be fully elucidated.
In this study, we exploit the biomimetic properties of human gastric organoids and organoid-derived mucosoid cultures as advanced *in vitro* systems to model early pre-cancerous alterations. To recapitulate the pathological microenvironment driving GIM, these systems were challenged with inflammatory stimuli derived from *H. pylori*-conditioned peripheral blood mononuclear cells (PBMCs) to mimic a chronic inflammatory state, in combination with selected bile acids, which have been implicated in gastric epithelial injury and metaplastic transformation. Preliminary results indicate that while bile acids promote the expression of intestinal lineage markers, high-intensity inflammatory stimuli trigger the downregulation of gastric markers.
Overall, our findings highlight a complex crosstalk between inflammatory and metabolic associated pathways that shape gastric epithelial cell fate. This work contributes to the development of a physiologically relevant platform to dissect early events in gastric carcinogenesis and to explore the conditions under which metaplastic changes may be stabilized or potentially reversed.

## Impact of SARS-CoV-2 infection on neurodegeneration

Minxing Zhang[1], D. Valente[2], A. Fiorenzano[3], A. Pepe[1], C. Zurzolo[1]
[1]Unit of Membrane Traffic and Pathogenesis, Department of Cell Biology and Infection, Institut Pasteur, Paris, France
[2]Department of Experimental and Clinical Medicine, University “Magna Graecia” of Catanzaro, Catanzaro, Italy
[3]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy

Since the emergence of COVID-19 in 2019, many patients have reported long-term neurological symptoms, known as Long-COVID syndrome. Evidence suggests that SARS-CoV-2 can infect the human brain, potentially contributing to these symptoms. In addition, COVID-19 patients have been linked to a threefold increased risk of Alzheimer’s disease (AD) and a twofold increased risk of Parkinson’s disease (PD). However, the mechanisms by which the virus reaches the brain and its role in neurodegenerative diseases remain a critical area of ongoing investigation.
Previous research has shown that dopaminergic neurons are susceptible to SARS-CoV-2 infection in vitro, which might have detrimental consequences even years after the initial infection. Moreover, SARS-CoV-2 viral proteins can promote α-synuclein aggregation and amyloid fibril formation. Using 2D and 3D human cell cultures (organoids) and cryo-electron microscopy (cryo-EM) strategies, we will study SARS-CoV-2 neuroinvasion and its role in neurodegenerative diseases.
Our preliminary data showed that SARS-CoV-2 transfer from primary human nasal cells to human neuronal progenitor cells through Tunneling Nanotubes (TNTs), actin-membrane-based structures that connect distant cells. TNT-like structures positive for the SARS-CoV-2 N antigen were also observed, supporting the possibility of a cell-to-cell transmission route to neural cells. Furthermore, SARS-CoV-2-infected dopaminergic neuronal progenitor cells express higher levels of α-synuclein than uninfected progenitor cells. This study provides critical insights into the neuropathogenesis of SARS-CoV-2 and its potential contribution to long-term neurological symptoms.

# List of Participants

Natalia Abate
University of Naples Federico II
Naples, Italy
natalia.abate@unina.it

Vincenzo Aiello
Università Telematica San Raffaele
Rome, Italy
vincenzo.aiello@uniroma5.it

Nicole Annesi
University of Trento
Trento, Italy
nicole.annesi@unitn.it

Ylenia Antonacci
University of Bari
Bari, Italy
ylenia.antonacci@cnr.it

Cosima Baldari
University of Siena
Siena, Italy
baldari@unisi.it

Monica Ballarino
Sapienza University of Rome
Rome, Italy
monica.ballarino@uniroma1.it

Isaia Barbieri
University of Turin
Turin, Italy
isaia.barbieri@unito.it

Chiara Barone
University of Naples Federico II
Naples, Italy
chiarabarone36@gmail.com

Rubens Begaj
University of Trento
Povo, TN, Italy
rubens.begaj@unitn.it

Roberto Bellelli
Queen Mary University of London
London, United Kingdom
r.bellelli@qmul.ac.uk

Marina Benítez Ramos
University of Trento
Trento, Italy
marina.benitezramos@unitn.it

Sandra Blanco
CIC-IBMCC, University of Salamanca
Salamanca, Spain
sandra.blanco@usal.es

Alice Bonato
University of Verona
Verona, Italy
alice.bonato@univr.it

Laura Bottoni
Istituto Europeo di Oncologia
Milan, Italy
laura.bottoni@student.unisi.it

Maxim Bouvet
University of Turin
Turin, Italy
maxim.bouvet@unito.it

Guglielmo Bove
University of Campania Luigi Vanvitelli
Naples, Italy
guglielmo.bove@unicampania.it

Irene Bozzoni
Sapienza University of Rome
Rome, Italy
irene.bozzoni@uniroma1.it

Maria Giusy Bruno
University of Verona
Verona, Italy
mariagiusy.bruno@univr.it

Davide Cacchiarelli
TIGEM
Pozzuoli, NA, Italy
davide.kun@gmail.com

Livia Caizzi
University of Turin
Turin, Italy
livia.caizzi@unito.it

Alessandra Caliendo
National Research Council
Naples, Italy
alessandracaliendo@cnr.it

Alessia Campese
University of Verona
Verona, Italy
alessia.campese@univr.it

Marta Cantarelli
University of Trento
Povo, TN, Italy
martacantarelli2@gmail.com

Nagaja Capitani
Università degli Studi di Siena
Siena, Italy
ncapitani81@gmail.com

Giovanni Capranico
University of Bologna
Bologna, Italy
giovanni.capranico@unibo.it

Mauro Caprioli
University of Verona
Verona, Italy
mauro.caprioli@univr.it

Roberta Caschetto
University of Catania
Catania, Italy
roberta.caschetto@studium.unict.it

Maria Carmela Cavallo
University of Siena
Siena, Italy
mariacarmela1308@gmail.com

Laura Cerchia
CNR
Naples, Italy
laura.cerchia@cnr.it

Marcella Cesana
University of Naples Federico II
Naples, Italy
marcella.cesana@gmail.com

Ugo Chianese
University of Campania Luigi Vanvitelli
Naples, Italy
ugo.chianese@unicampania.it

Mario Chiariello
CNR
Siena, Italy
mario.chiariello@cnr.it

Marianna Ciuffreda
University of Trento
Trento, Italy
marianna.ciuffreda@unitn.it

Vittorio Colantuoni
University of Sannio
Benevento, Italy
vittorio.colantuoni@gmail.com

Ivan Conte
University of Naples Federico II
Naples, Italy
ivan.conte@unina.it

Giuseppina Covello
University of Padua
Padua, Italy
giuseppina.covello@unipd.it

Sara Cozzubbo
University of Turin
Turin, Italy
Sara.cozzubbo@unito.it

Marco Crepaldi
University of Campania Luigi Vanvitelli
Naples, Italy
Marco.crepaldi@unicampania.it

Angelo D'Angelo
Sapienza University of Rome
Rome, Italy
angelo.dangelo@uniroma1.it

Anna Maria D'Erchia
University of Bari
Bari, Italy
annamaria.derchia@uniba.it

Fabrizio Damiano
University of Salento
Lecce, Italy
fabrizio.damiano@unisalento.it

Pierpaolo De Iaco
University of Siena and ISPRO
Florence, Italy
p.deiaco@student.unisi.it

Alessio De Magis
Universitá Vita-Salute San Raffaele
Milan, Italy
demagis.alessio@hsr.it

Carola De Martinis
University of Verona
Verona, Italy
carola.demartinis@univr.it

Gaia De Rigo
University of Padua
Padua, Italy
gaia.derigo@unipd.it

Rosa De Santis
Telethon institute of genetics and medicine
Naples, Italy
r.desantis@tigem.it

Veronica De Vita
University of Verona
Verona, Italy
veronica.devita@univr.it

Nunzio Del Gaudio
Università degli studi LINK
Rome, Italy
n.delgaudio@unilink.it

Isabel Delany
GSK
Siena, Italy
Isabel.x.delany@gsk.com

Michela Denti
University of Trento
Trento, Italy
michela.denti@unitn.it

Simone Detassis
University of Trento
Trento, Italy
simone.detassis@unitn.it

Martina Di Bartolomeo
University of Teramo
Teramo, Italy
martina86@tin.it

Rossella Di Giaimo
University of Naples Federico II
Naples, Italy
digiaimo@unina.it

Daniela Di Girolamo
University of Naples Federico II
Naples, Italy
daniela.digirolamo@unina.it

Roberto Dinami
IRCCS – Regina Elena National Cancer Institute
Rome, Italy
roberto.dinami@ifo.it

Daniela Donna
University of Turin
Turin, Italy
daniela.donna@unito.it

Daniele Durante
Sapienza University of Rome
Rome, Italy
daniele.durante2@uniroma1.it

Claudia Esposito
University of Catanzaro
Catanzaro, Italy
claudia.esposito@studenti.unicz.it

Luigi Fattore
Link Campus University
Rome, Italy
l.fattore@unilink.it

Beatrice Feliciani
University of Teramo
Teramo, Italy
bfeliciani@unite.it

Daniela Fignani
University of Siena
Siena, Italy
daniela.fignani2@unisi.it

Ermes Filomena
University of Bari
Bari, Italy
ermes.filomena@uniba.it

Alessandro Fiorenzano
University of Naples Federico II
Naples, Italy
alessandro.fiorenzano@unina.it

Bruno Fosso
University of Bari
Bari, Italy
bruno.fosso@uniba.it

Lorenzo Franci
CNR Siena and University of Bologna
Siena, Italy
lorenzofranci4@gmail.com

Flavio Frezza
Università Telematica San Raffaele
Rome, Italy
flavio.frezza@uniroma5.it

Gaia Fusto
University of Catania
Catania, Italy
gaia.fusto@phd.unict.it

Silvia Galardi
University of Rome Tor Vergata
Rome, Italy
silvia.galardi@uniroma2.it

Federico Galvagni
University of Siena
Siena, Italy
federico.galvagni@unisi.it

Aga Gambus
University of Birmingham
Birmingham, United Kingdom
a.gambus@bham.ac.uk

Paolo Gandellini
University of Milan
Milan, Italy
paolo.gandellini@unimi.it

Luca Garelli
Università Telematica San Raffaele
Rome, Italy
luca.garelli@uniroma5.it

Raffaele Garraffo
Sapienza University of Rome
Rome, Italy
raffaele.garraffo@uniroma1.it

Matteo Gatta
University of Siena
Siena, Italy
mattgatt01@gmail.com

Marco Giovine
University of Genoa
Genoa, Italy
mgiovine@unige.it

Domenico Grieco
CEINGE and University of Naples Federico II
Naples, Italy
domenico.grieco@unina.it

Antonio Grimaldi
TIGEM
Pozzuoli, NA, Italy
a.grimaldi@tigem.it

Francesca Guarino
University of Catania
Catania, Italy
francesca.guarino@unict.it

Aymone Gurtner
CNR
Rome, Italy
Aymone.gurtner@ifo.it

Stefano Gustincich
Istituto Italiano di Tecnologia
Genoa, Italy
stefano.gustincich@iit.it

Maite Huarte
CIMA - Universidad de Navarra
Navarra, Spain
maitehuarte@unav.es

Ciro Iaccarino
University of Sassari
Sassari, Italy
ciaccarino@uniss.it

Aurora Iacopini
University of Siena
Siena, Italy
a.iacopini@student.unisi.it

Domenico Ignoti
University of Turin
Turin, Italy
domenico.ignoti@unito.it

Giovanni Inzalaco
CNR
Naples, Italy
giovanniinzalaco@cnr.it

Aqsa Javaid
University of Verona
Verona, Italy
aqsa.javaid@univr.it

Giuliana La Rosa
University of Naples Federico II
Naples, Italy
giuli.la.ro@gmail.com

Pietro Laneve
National Research Council of Italy
Rome, Italy
pietro.laneve@cnr.it

Federico Lazzaro
University of Milan
Milan, Italy
federico.lazzaro@unimi.it

Joshua Levendis
University of Melbourne
Parkville, Australia
jlevendis@student.unimelb.edu.au

Andrea Lunardi
University of Trento
Trento, Italy
andrea.lunardi@unitn.it

Nicolò Magri
University of Padua
Padua, Italy
nicolo.magri@studenti.unipd.it

Federico Marchini
University of Siena
Siena, Italy
federico.marchini@student.unisi.it

Elisa Marmocchi
University of Trento
Povo, TN, Italy
elisa.marmocchi@unitn.it

Graziano Martello
University of Padua
Padua, Italy
graziano.martello@unipd.it

Ylenia Martinini
University of Florence
Florence, Italy
ylenia.martinini@unifi.it

Stefania Martucciello
University of Salerno
Fisciano, SA, Italy
smartucciello@unisa.it

Giacomo Menichetti
University of Verona
Verona, Italy
giacomo.menichetti@univr.it

Annachiara Miccoli
University of Siena
Siena, Italy
annachiara.miccoli@student.unisi.it

Sara Milioni
University of Turin
Turin, Italy
sara.milioni@unito.it

Caterina Missero
CEINGE and University of Naples Federico II
Naples, Italy
missero@ceinge.unina.it

Andrea Morandi
University of Florence
Florence, Italy
andrea.morandi@unifi.it

Gianluca Mordini
University of Rome Tor Vergata
Rome, Italy
gianmordini@gmail.com

Maria Chiara Mottola
CNR
Pavia, Italy
mariachiaramottola@cnr.it

Marco Muzi Falconi
University of Milan
Milan, Italy
marco.muzifalconi@unimi.it

Luigi Naldini
San Raffaele Telethon Institute for Gene Therapy
Milan, Italy
naldini.luigi@hsr.it

Stella Nappi
University of Siena
Siena, Italy
stellanappi99@gmail.com

Francesco Nicassio
Istituto Italiano di Tecnologia
Milan, Italy
francesco.nicassio@iit.it

Gaia Nicotra
University of Catania
Catania, Italy
nctgai00r53c351i@studium.unict.it

Sabela Nikolli
IRCCS Regina Elena National cancer Institute
Rome, Italy
sabynikolli94@gmail.com

Salvatore Oliviero
University of Turin
Turin, Italy
salvatore.oliviero@unito.it

Francesca Orso
University of Piemonte Orientale
Novara, Italy
francesca.orso@uniupo.it

Simona Paladino
University of Naples Federico II
Naples, Italy
spaladin@unina.it

Luca Pandolfini
Istituto Italiano di Tecnologia
Genoa, Italy
luca.pandolfini@iit.it

Elvira Parrotta
University of Catanzaro
Catanzaro, Italy
parrotta@unicz.it

Erika Pascale
University of Naples Federico II
Naples, Italy
erika.pascale@unina.it

Laura Patrussi
University of Siena
Siena, Italy
patrussi2@unisi.it

Mattia Pelizzola
University Milano-Bicocca
Milan, Italy
mattia.pelizzola@unimib.it

Anna Pepe
Institut Pasteur
Paris, France
anna.pepe@pasteur.fr

Graziano Pesole
University of Bari and CNR-IBIOM
Bari, Italy
graziano.pesole@uniba.it

Fabio Petti
Sapienza University of Rome
Rome, Italy
f.petti@uniroma1.it

Stefano Piccolo
University of Padua
Padua, Italy
stefano.piccolo@unipd.it

Silvia Pietrobono
University of Verona
Verona, Italy
silvia.pietrobono@univr.it

Francesca Pisani
CNR
Naples, Italy
francesca.pisani@ibbc.cnr.it

Fabio Piscopo
Sapienza University of Rome
Rome, Italy
fabio.piscopo@uniroma1.it

Valeria Poli
University of Turin
Turin, Italy
valeria.poli@unito.it

Amalia Porta
University of Salerno
Fisciano, SA, Italy
aporta@unisa.it

Nicoletta Potenza
University of Campania Luigi Vanvitelli
Caserta, Italy
nicoletta.potenza@unicampania.it

Alessandro Prigione
Heinrich Heine University
Düsseldorf, Germany
alessandro.prigione@hhu.de

Alessandro Provenzani
University of Trento
Trento, Italy
alessandro.provenzani@unitn.it

Roberto Quadri
University of Milan
Milan, Italy
roberto.quadri@hotmail.it

Vittoria Raffa
University of Pisa
Pisa, Italy
Vittoria.raffa@unipi.it

Alessandro Restellini
University of Milan
Milan, Italy
alessandro.restellini@unimi.it

Alessandra Ruggiero
University of Verona
Verona, Italy
alessandra.ruggiero@univr.it

Marco Russo
University of Bologna
Bologna, Italy
marco.russo9@unibo.it

Lina Sabatino
University of Sannio
Benevento, Italy
sabatino@unisannio.it

Alessandra Saitta
University of Messina
Messina, Italy
alessandra.saitta@studenti.unime.it

Julian Sale
MRC Laboratory of Molecular Biology
Cambridge, United Kingdom
jes@mrc-lmb.cam.ac.uk

Michele Sallese
University of Chieti-Pescara
Chieti, Italy
michele.sallese@unich.it

Marcello Salvi
TIGEM
Pozzuoli, NA, Italy
m.salvi@tigem.it

Nicolo' Salvi
Sapienza University of Rome
Rome, Italy
n.salvi@uniroma1.it

Stefan Schoeftner
University of Trieste
Trieste, Italy
sschoeftner@units.it

Alfredo Sepe
University of Padua
Padua, Italy
alfredo.sepe@unipd.it

Angela Serpico
CEINGE
Naples, Italy
serpicoa@ceinge.unina.it

Adriano Setti
Sapienza University of Rome
Rome, Italy
adriano.setti@uniroma1.it

Marco Simula
Sapienza University of Rome
Rome, Italy
marco.simula@uniroma1.it

Paola Spadavecchia
University of Bari
Bari, Italy
paola.spadavecchia@uniba.it

Gaia Stortini
Italian Institute of Technology
Rome, Italy
gaia.stortini@iit.it

Laura Talarico
University of Pisa
Pisa, Italy
laura.talarico@phd.unipi.it

Mariagrazia Talarico
University of Catanzaro
Catanzaro, Italy
m.talarico@unicz.it

Gabriele Tani
University of Florence
Florence, Italy
gabriele.tani@unifi.it

Elena Taverna
Human Technopole
Milan, Italy
elena.taverna@fht.org

Toma Tebaldi
University of Trento
Trento, Italy
toma.tebaldi@unitn.it

Angela Toffano
University of Padua
Padua, Italy
angela.toffano@unipd.it

Alessandra Tosco
University of Salerno
Fisciano, SA, Italy
tosco@unisa.it

Asia Trano
University of Naples Federico II
Naples, Italy
asia.trano@unina.it

Apollonia Tullo
CNR
Bari, Italy
apollonia.tullo@cnr.it

Cristina Ulivieri
University of Siena
Siena, Italy
cristina.ulivieri@unisi.it

Lorenzo Vaccaro
TIGEM and University of Naples Federico II
Pozzuoli, NA, Italy
l.vaccaro1992@gmail.com

Danila Valletta
University of Campania Luigi Vanvitelli
Caserta, Italy
danila.valletta@unicampania.it

Giulia Vallini
University of Siena
Siena, Italy
giulia.vallini@student.unisi.it

Antonia Voli
University of Salerno
Fisciano, SA, Italy
avoli@unisa.it

Antonella Zannetti
CNR
Naples, Italy
antonella.zannetti@cnr.it

Minxing Zhang
Institut Pasteur
Paris, France
minxing.zhang@pasteur.fr