Supporting Information

Development and validation of novel Z-360-based macromolecules for the active targeting of CCK2-R

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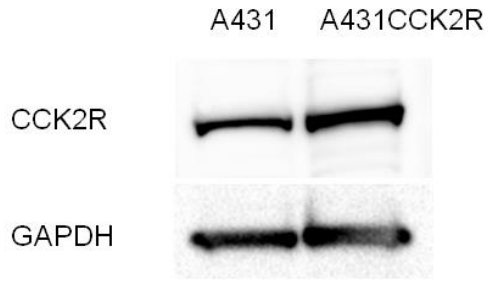

Figure S1. Western blot analysis for CCK2-R in A431 WT and A431-CCK2-R^{+} cell lines. A total of 40 µg protein of total cell lysate from each cell line was subjected to analysis.

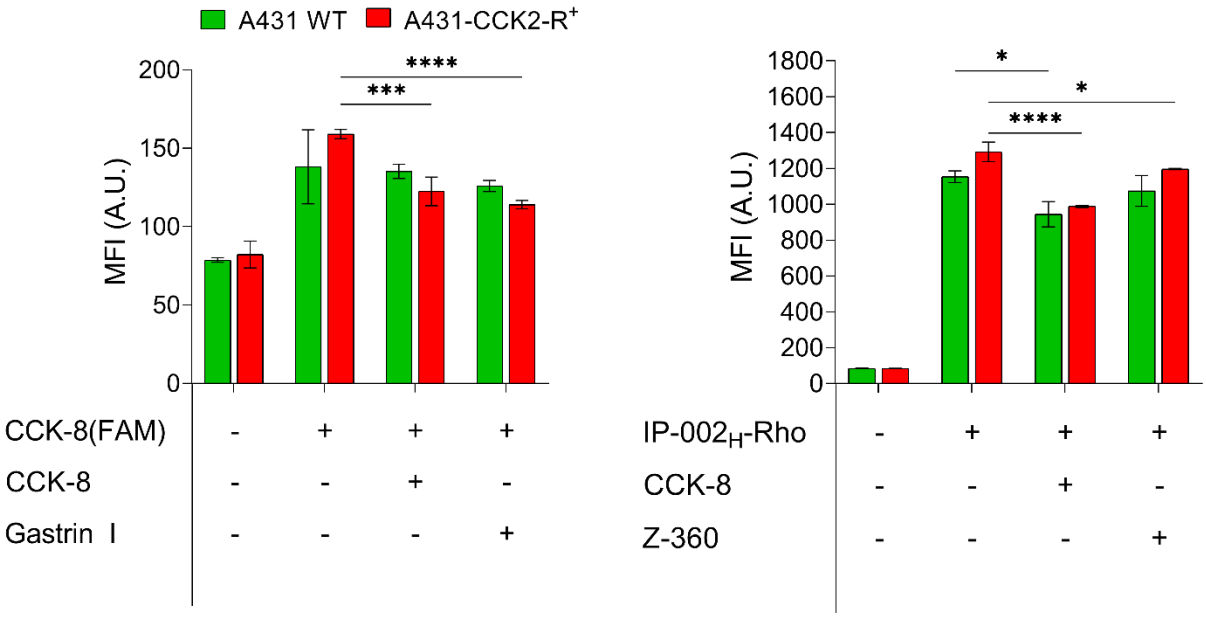

Figure S2. Mean fluorescence intensity (MFI) from FC analysis of A431 WT (green bars) and A431-CCK2-R^{+} (red bars) cell lines after 30 min incubation with (A) 5 µg/mL CCK-8(FAM) or (B) 0.5 µg/mL IP-002$_{H}$-Rho, with or without 30 min pre-incubation with (A) 7.7 µg/mL CCK-8, 30 µg/mL gastrin I, or (B) 5 µg/mL CCK-8 and Z-360. Values are expressed as mean ± SD of 2 independent experiments performed in duplicate.

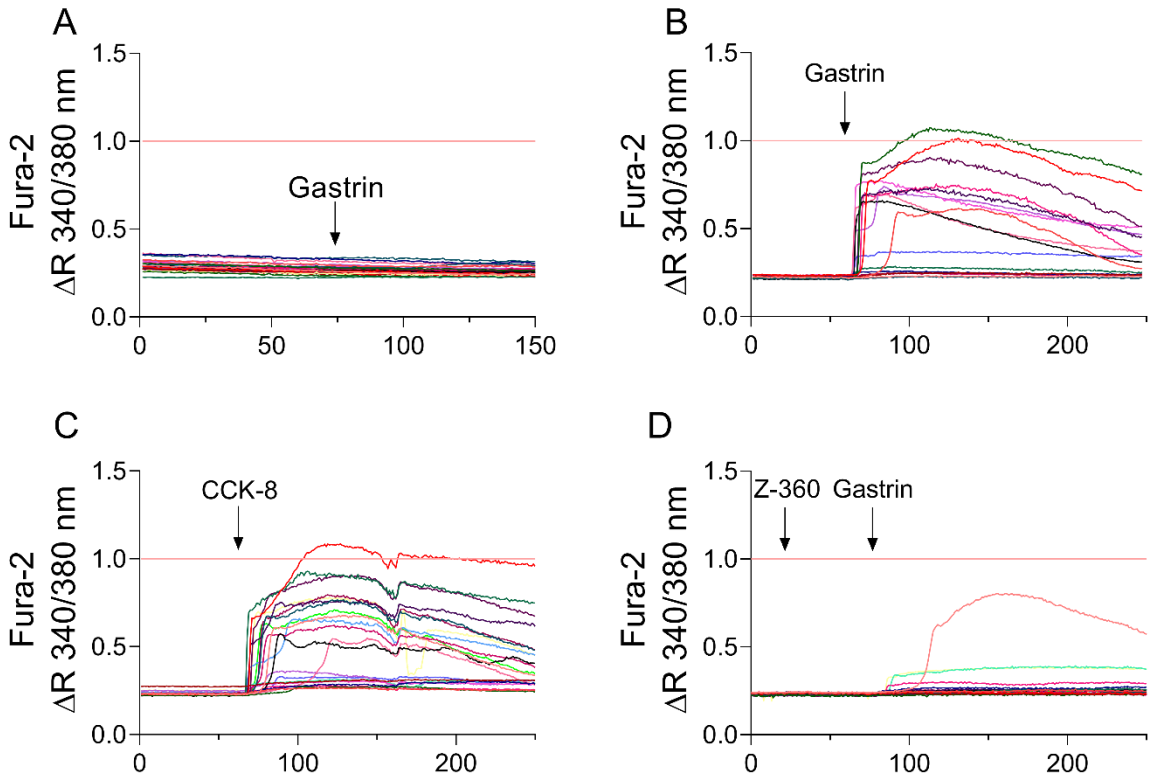

Figure S3. Representative traces of cytosolic Ca^{2+} measurements (expressed as Fura-2 340/380 nm ratio) in A431 WT (A), A431-CCK2-R^+ (B-D) cells, upon stimulation with 10 µM Gastrin I (A-B, D) or 10 µM CCK-8 (C). Each trace represents a cell. The absence of Ca^{2+} peaks indicate the lack of G-protein coupled CCK2 receptors in A431 WT cell lines.

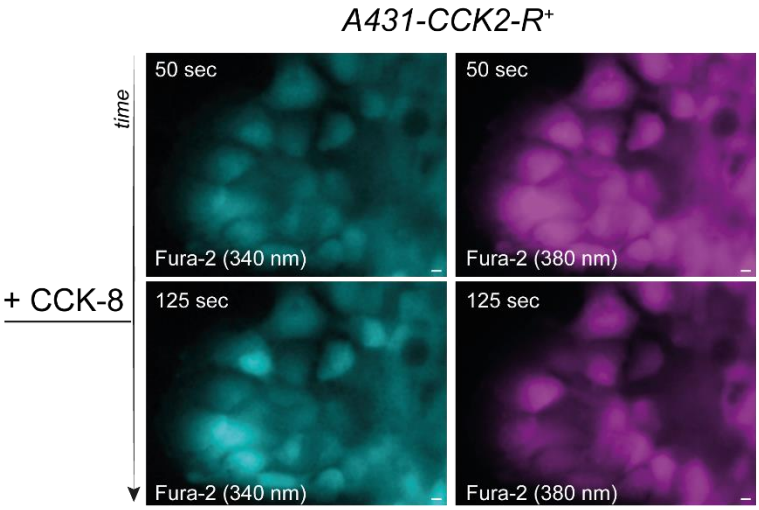

Figure S4. Representative images of Fura-2 AM calcium imaging (performed as in Figure 4) on CCK-8-stimulated A431-CCK2-R^+ cells. Scale bar: 10 µm.

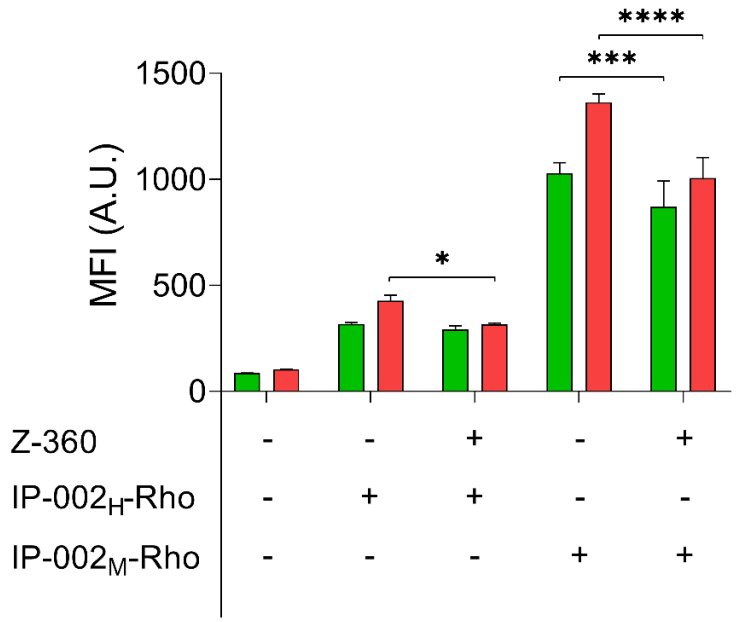

Figure S5. Association of IP-002$_H$-Rho and IP-002$_M$-Rho with A431 WT (green bars) and A431-CCK2-R$^+$ (red bars) cell lines in presence or absence of the natural ligand Z-360 (0.136 µg/mL, 262 nM) as assessed by Flow Cytometry (FC) analysis. Cells were pre-incubated with Z-360 or medium alone for 30 min and then exposed to IP-002$_H$-Rho (0.36 µg/mL, 262 nM) or IP-002$_M$-Rho (0.5 µg/mL, 262 nM). The association was assessed after further 30 minutes. MFI: Mean fluorescence intensity. Values are expressed as mean of 3 independent experiments performed in duplicate ± SD (*= $p < 0.05$; ***= $p< 0.001$ **** = $p < 0.0001$).

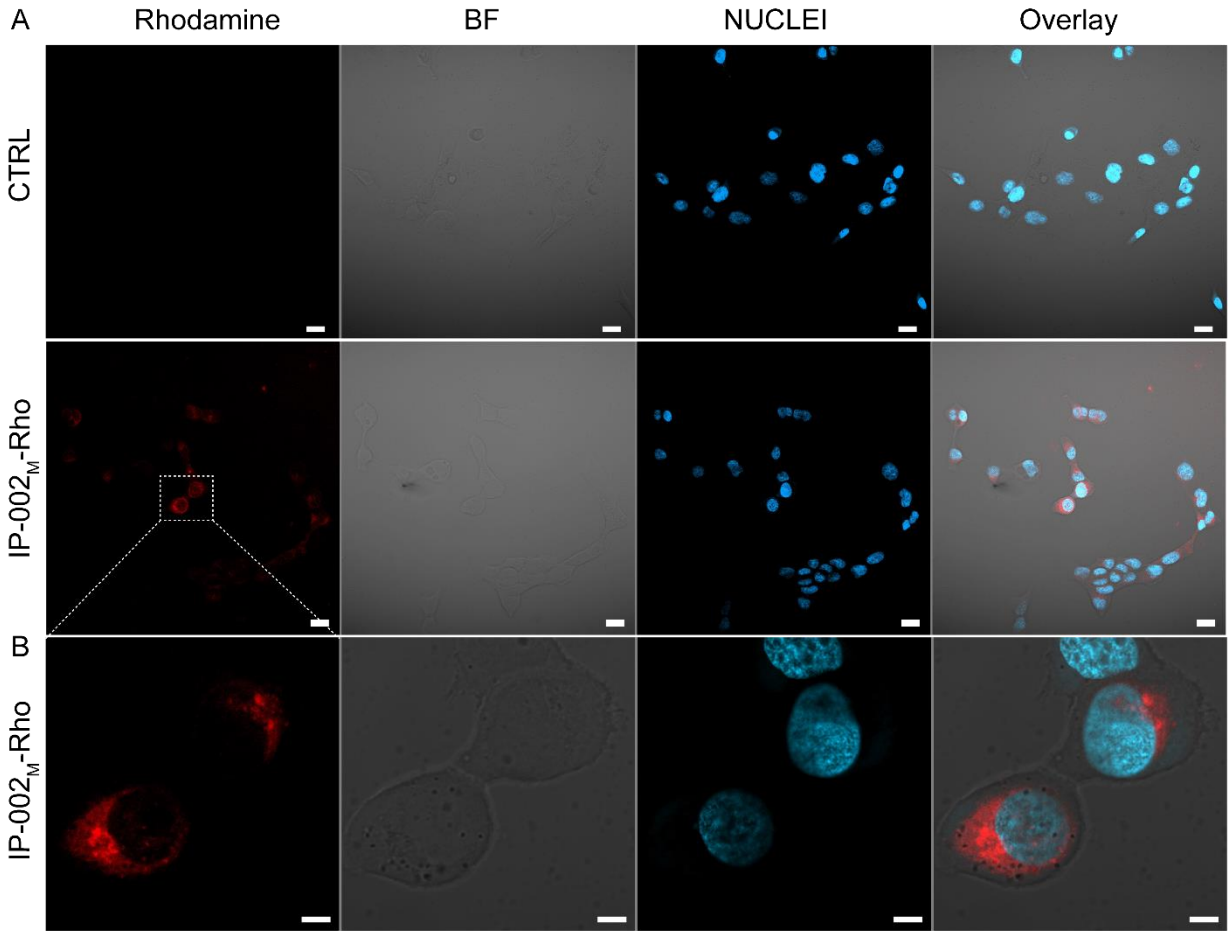

Figure S6. Confocal laser scanning microscope images of A431-CCK2-R$^+$ cells (A) incubated for 30 minutes with medium only (CTRL) or medium containing 2 µg/mL IP-002$_M$-Rho. Cells were visualized for IP-002$_M$-Rho (red), Hoechst 33258-stained nuclei (blue), and bright field (BF). The merged images are presented (overlay). Images were acquired with a 40x objective. Scale bar: 20 µm. (B) Magnified view of small ROI (63x objective). Scale bar: 5 µm.

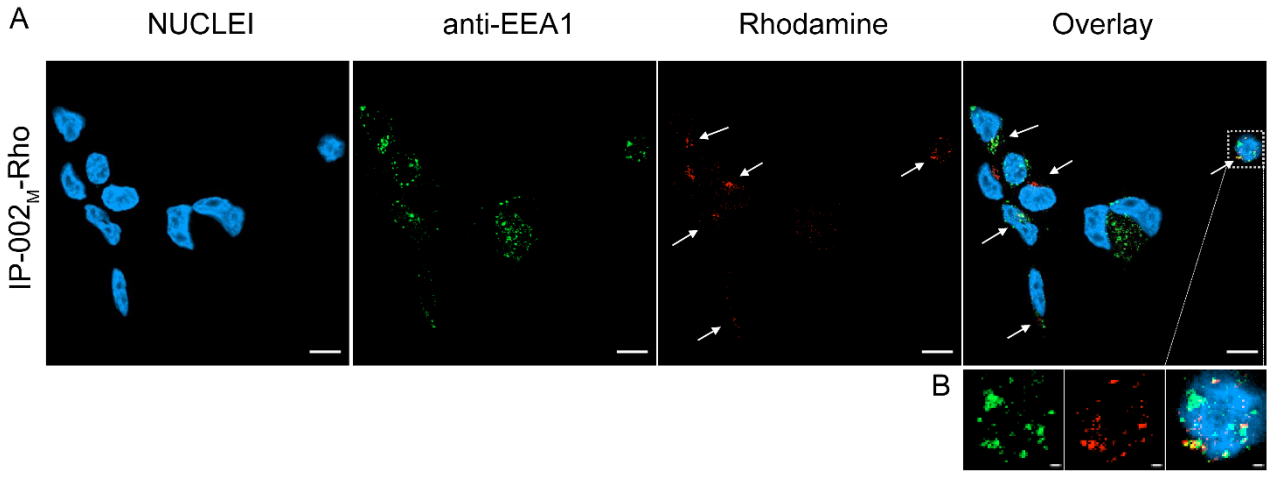

Figure S7. (A) Confocal microscopy images of A431-CCK2-R$^+$ cells after 1 h of incubation with IP-002$_M$-Rho (red, indicated by white arrows) and anti-EEA-1 antibody (green). Nuclei were visualized using Hoechst 33258. Scale bar: 10 µm. (B) Magnification of a single cell, indicated by a

white square in panel E. Colocalization of IP-002$_M$-Rho with the endosomal marker EEA-1 is displayed in yellow. Scale bar: 1 µm.

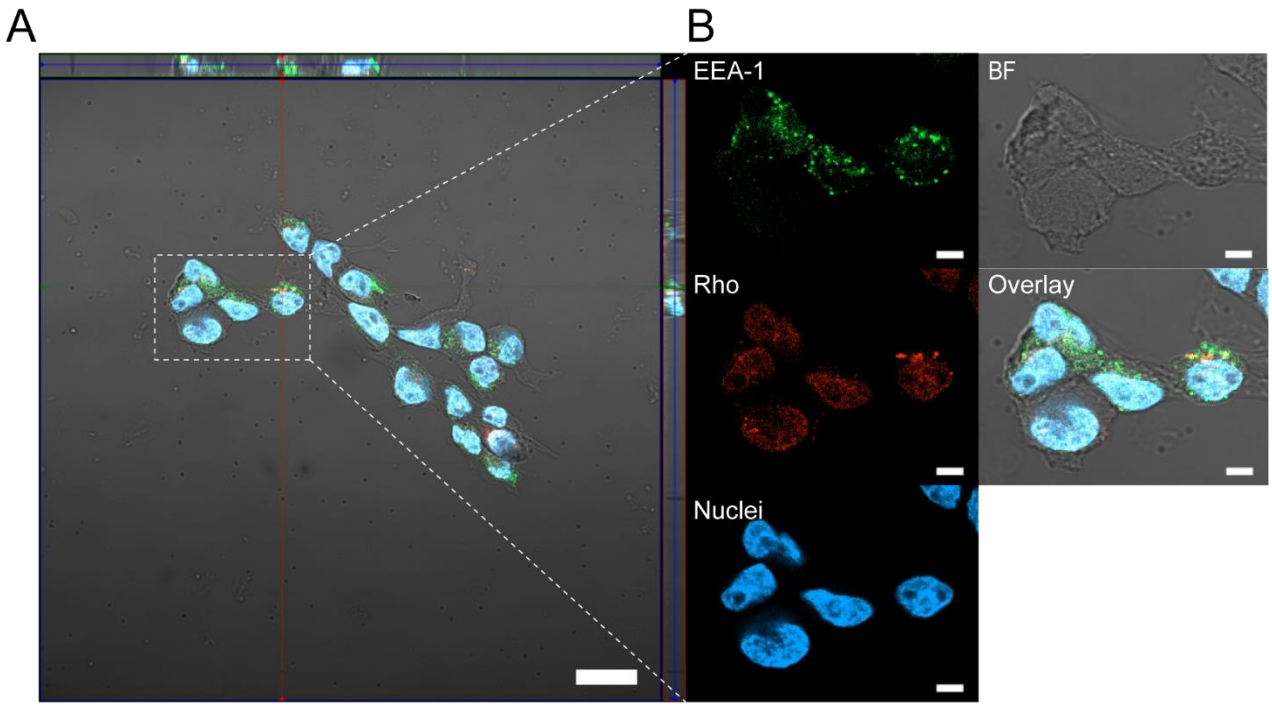

Figure S8. Confocal laser scanning microscope images of A431-CCK2-R$^+$ cells after 1 h incubation with IP-002$_M$-Rho (red) and stained with anti-EEA-1 antibody (green). Cells were visualized with Hoechst 33258-stained nuclei (blue) and bright field (BF). (A) Orthogonal projection on confocal section. Scale bar: 20 µm. (B) Magnified view of small ROI showing separate channels and overlay. Scale bar: 5 µm.

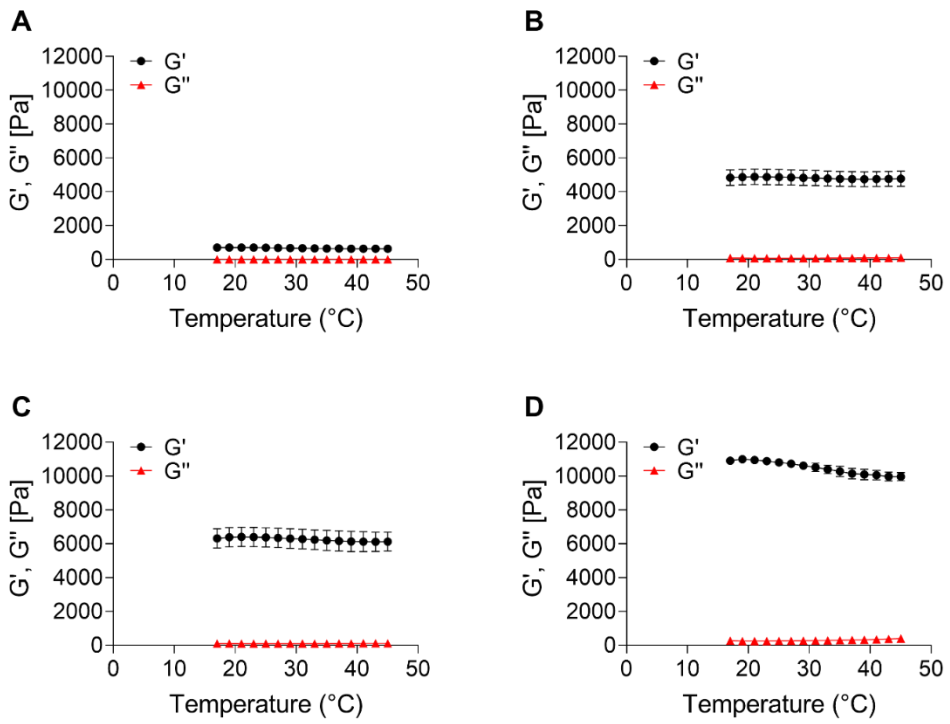

Figure S9. Temperature dependence of the storage modulus (G', black circles) and loss modulus (G'', red triangles) of **(A)** 10%, **(B)** 16%, **(C)** 20% and **(D)** 25% w/V GelMA scaffold.

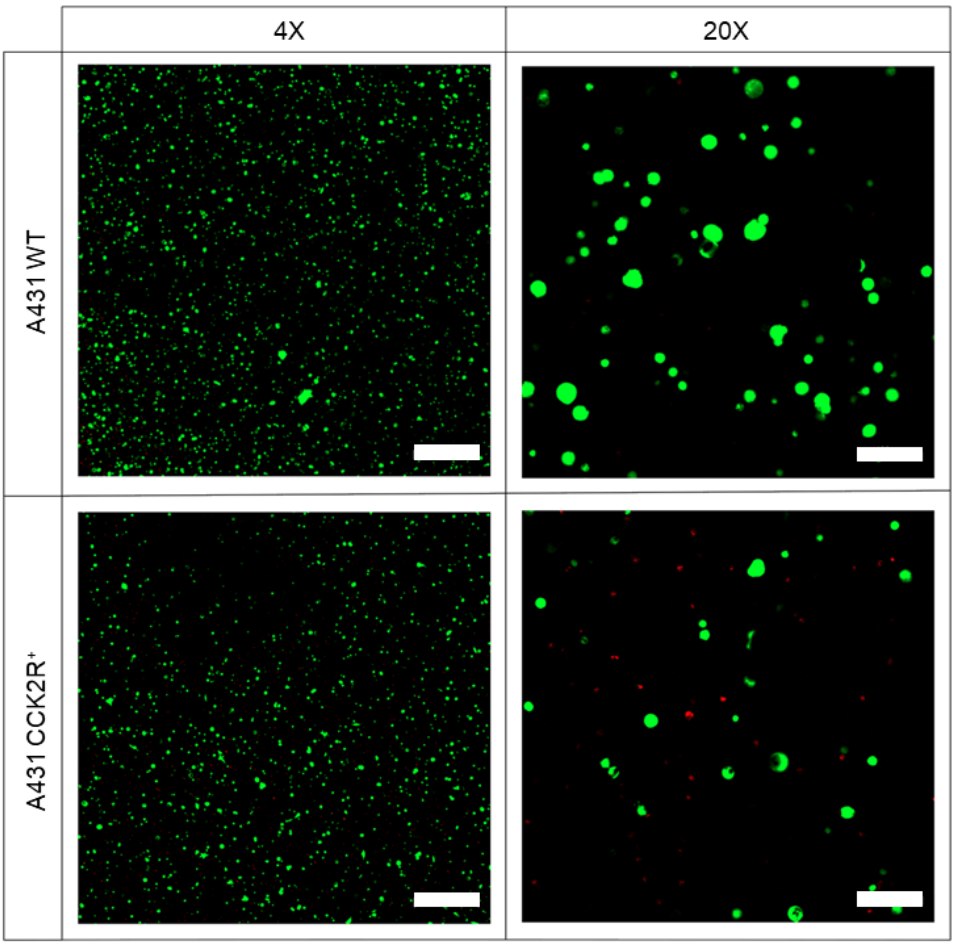

Figure S10. Confocal microscopy images of A431-WT (top) and A431-CCK2-R^+ (bottom) cells live/dead assay within the 3D scaffolds. Green indicates live cells and red indicates dead cells. Scale bars: 500 µm (left) or 100 µm (right).

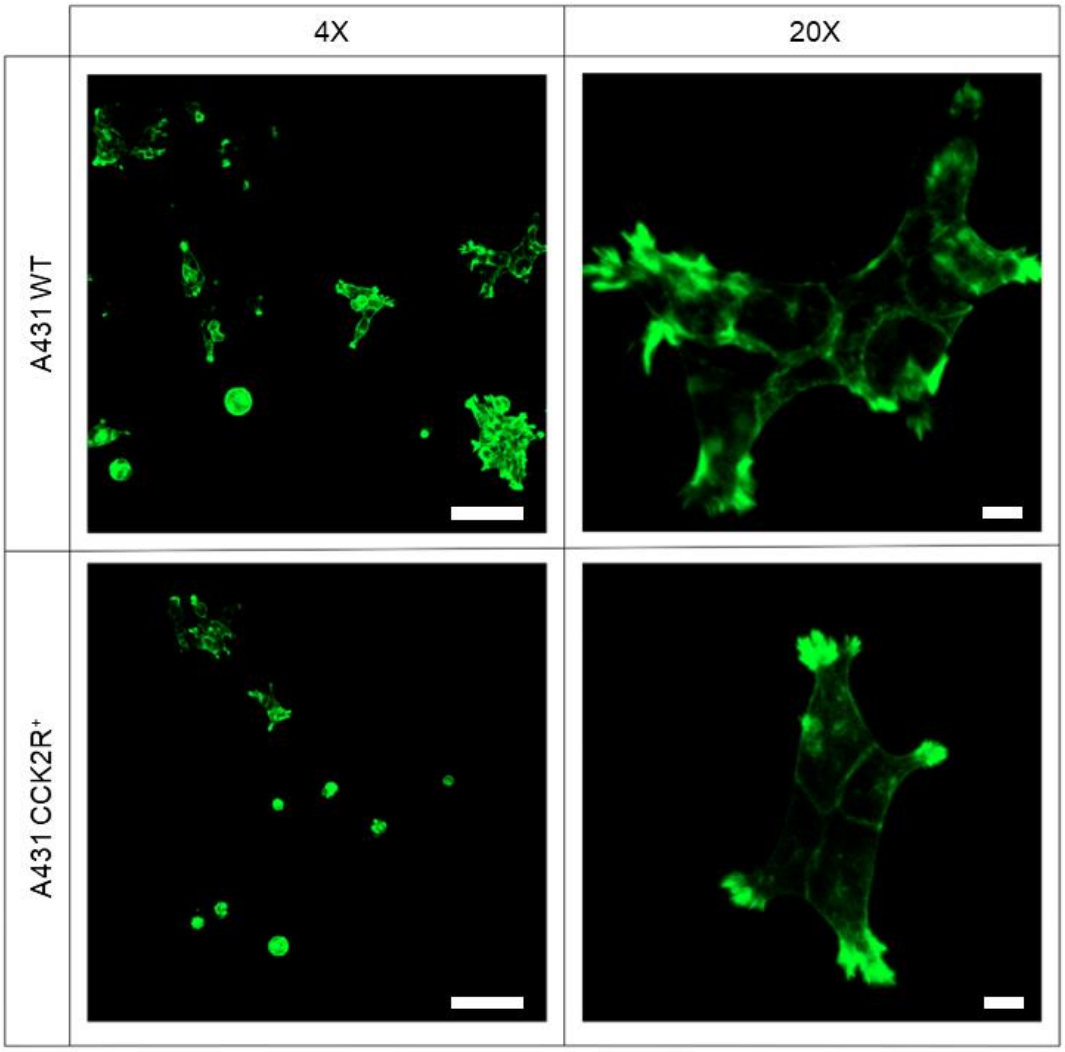

Figure S11. Confocal microscopy analysis of A431-WT (top) and A431-CCK2-R^+ (bottom) cells morphologies after Oregon Green 488 phalloidin staining within the 3D scaffolds. Scale bars: 100 µm (left) or 10 µm (right).