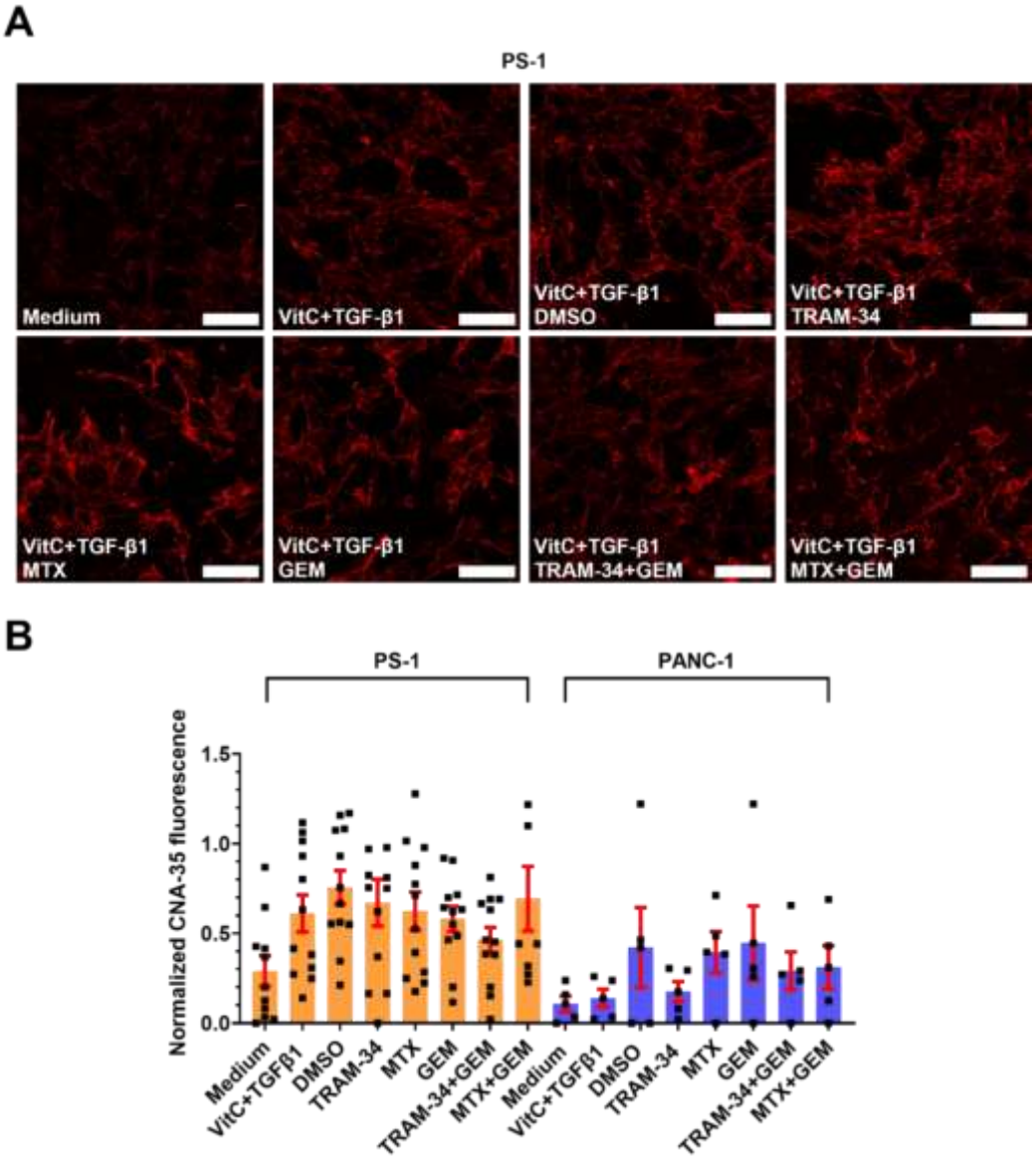
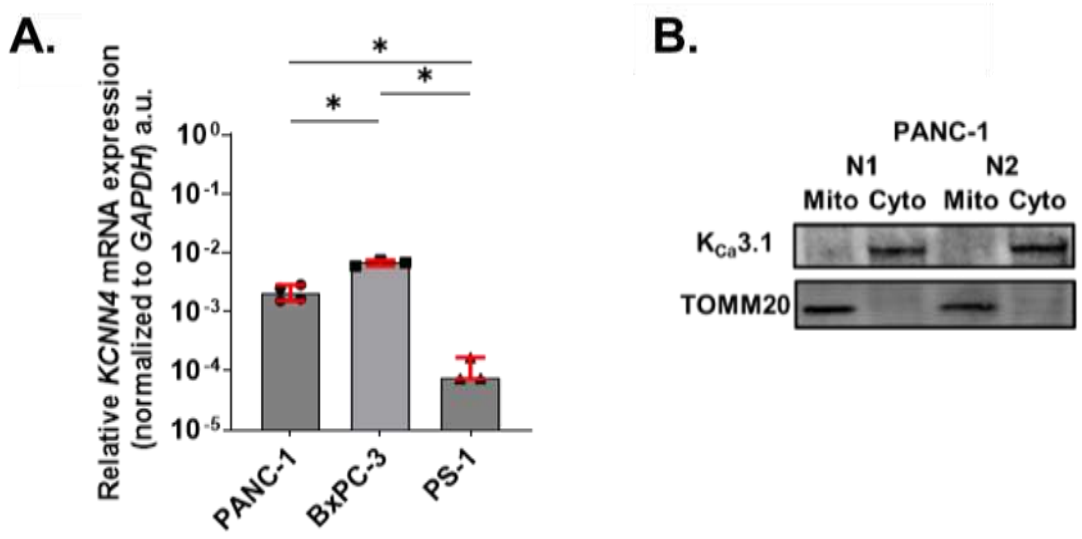
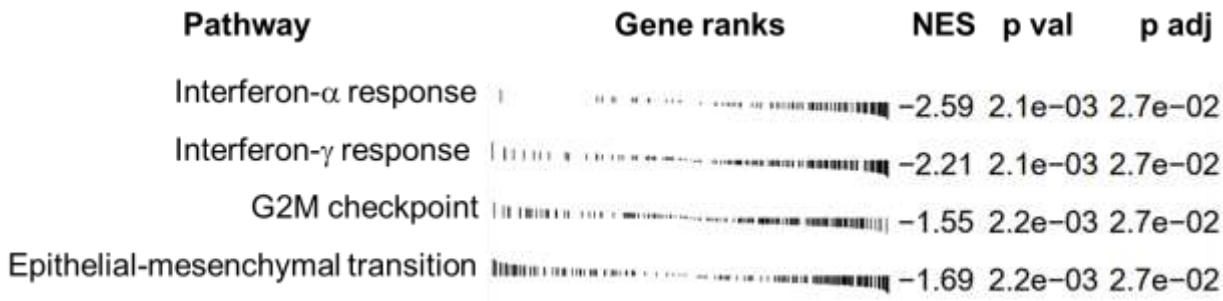
## Supplemental material




**Supplementary Fig. 1: PSC-derived matrix quantity remains unchanged upon $K_{Ca}$3.1 inhibition.** (**A**) Representative fluorescence images of CNA-35-tdTomato-labeled extracellular collagen (red) in PS-1 cells. The treatment conditions are indicated: control (Medium), VitC+TGF-β1. Moreover, PS1 cells stimulated with VitC+TGF-β1 were treated in addition with DMSO, TRAM-34, maurotoxin (MTX), gemcitabine (GEM), or combinations of either inhibitor with gemcitabine (TRAM-34+GEM; MTX+GEM). Scale bar: 200 µm. (**B**) Quantification of CNA-35-tdTomato fluorescence intensity in PS-1 and PANC-1 cells following stimulation: medium (PS-1: n/N=11/4; PANC-1: n/N=5/4); VitC+TGF-β (PS-1: n/N=12/4; PANC-1: n/N=5/4), VitC+TGF-β+DMSO (PS-1: n/N=12/4; PANC-1: n/N=5/4), VitC+TGF-β+TRAM-34 (PS-1: n/N=12/4; PANC-1: n/N=5/4), VitC+TGF-β+maurotoxin (MTX: PS-1: n/N=12/4; PANC-1: n/N=5/4), VitC+TGF-β+gemcitabine (GEM: PS-1: n/N=12/4; PANC-1: n/N=5/4); VitC+TGF-β+TRAM-34+GEM (PS-1: n/N=12/4; PANC-1: n/N=5/4); VitC+TGF-β+MTX+GEM (PS-1: n/N=12/4; PANC-1: n/N=5/4). N: number of experiments. Data points correspond to individual replicates (n), while bar charts represent the mean ± SEM.



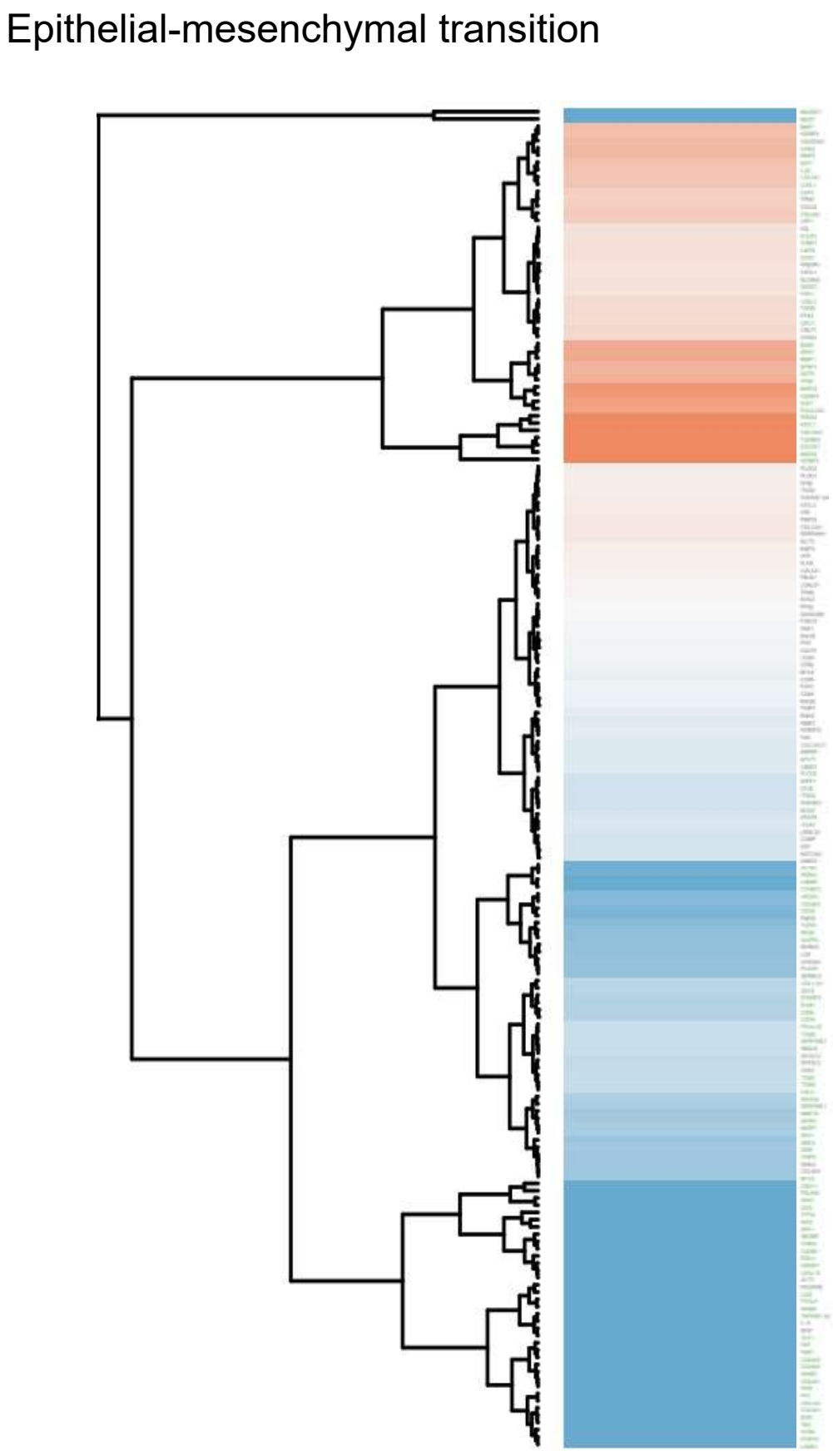

**Supplementary Fig. 2: $K_{Ca}$3.1 is expressed in the investigated cell lines.** (**B**) Quantification of $K_{Ca}$3.1 mRNA expression assessed by RT-qPCR. Data points reflect different passages, with each point being the average of n=3 technical replicates. Bar charts present the median ± 95% CI. *: $p < 0.05$, Kruskal-Wallis test with Dunn's post hoc test. (**B**) Western blot probing the presence of $K_{Ca}$3.1 channels in mitochondria (Mito) of PANC-1 cells. TOMM20 served as a marker protein of mitochondria. $K_{Ca}$3.1 channel proteins were hardly detected in mitochondrial lysates as opposed to cytosolic ones (Cyto).



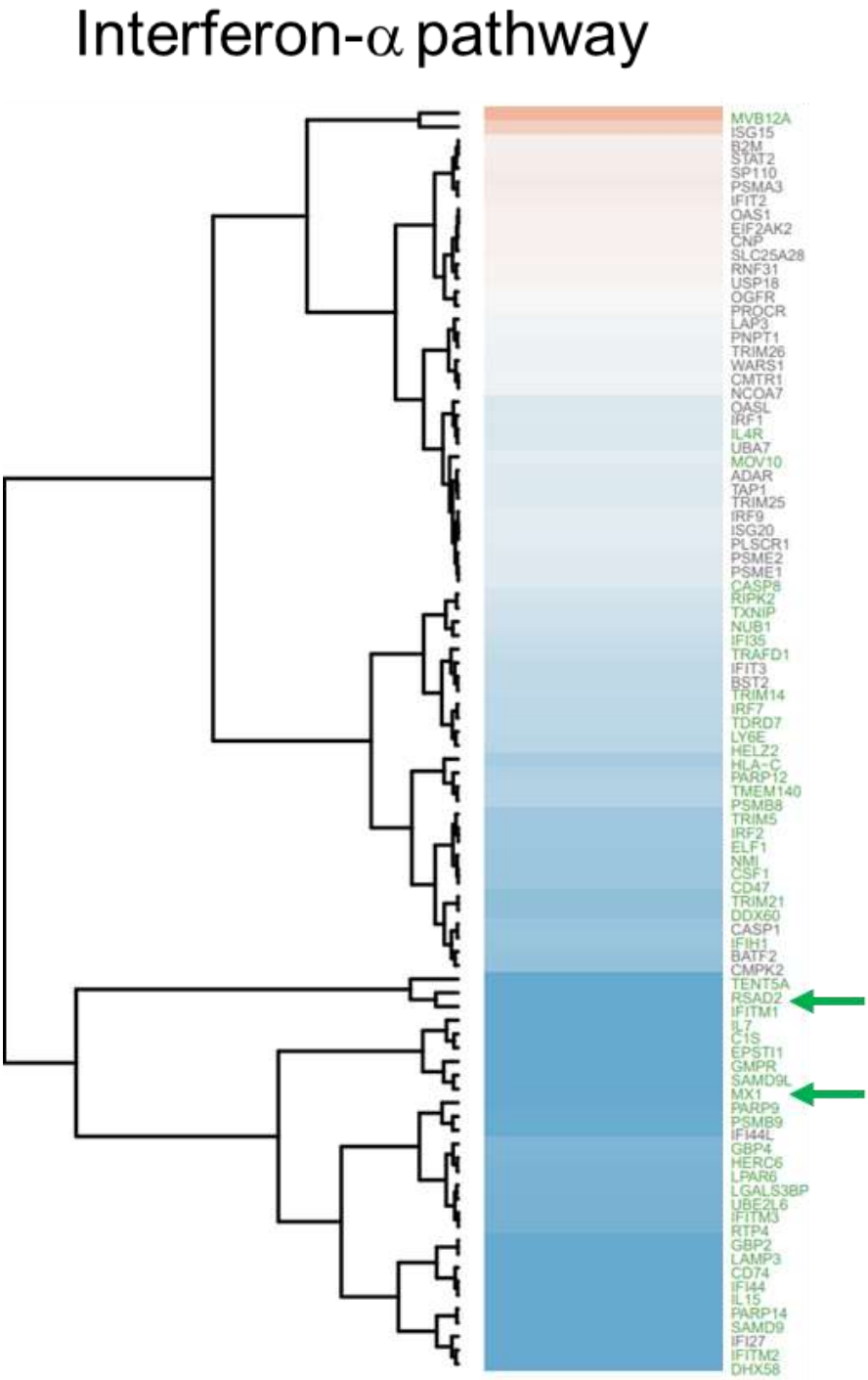

**Supplementary Figure 3:** Enrichment plots from fgsea output show the top differentially regulated hallmark pathways. The left half of the barcode pattern indicates enrichment of the set at the top of the ranked list of genes (upregulated) and most bars towards the right indicate enrichment at the bottom of the list (downregulated). The normalized enrichment score (NES) with P values reflects the degree to which the genes are enriched at the top or bottom of the list. A negative value means down-regulation of the respective gene set.

**Supplementary Figure 4:** Heatmap pages of EGSEA output show the fold changes of gene expression for the respective hallmark gene sets. Red indicates up-regulation, while blue indicates down-regulation. Gene names are colored based on their statistical significance in the limma differential expression analysis. Genes whose upregulation was verified with qPCR are marked with green arrows.

**4A.**



**4B.**


Interferon-α pathway
MVB12A
ISG15
B2M
STAT2
SP110
PSMA3
IFIT2
OAS1
EIF2AK2
CNP
SLC25A28
RNF31
USP18
OGFR
PROCR
LAP3
PNPT1
TRIM26
WARS1
CMTR1
NCOA7
OASL
IRF1
IL4R
UBA7
MOV10
ADAR
TAP1
TRIM25
IRF9
ISG20
PLSCR1
PSME2
PSME1
CASP8
RIPK2
TXNIP
NUB1
IFI35
TRAFD1
IFIT3
BST2
TRIM14
IRF7
TDRD7
LY6E
HELZ2
HLA-C
PARP12
TMEM140
PSMB8
TRIM5
IRF2
ELF1
NMI
CSF1
CD47
TRIM21
DDX60
CASP1
IFIH1
BATF2
CMPK2
TENT5A
RSAD2
IFITM1
IL7
C1S
EPSTI1
GMPR
SAMD9L
MX1
PARP9
PSMB9
IFI44L
GBP4
HERC6
LPAR6
LGALS3BP
UBE2L6
IFITM3
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SAMD9
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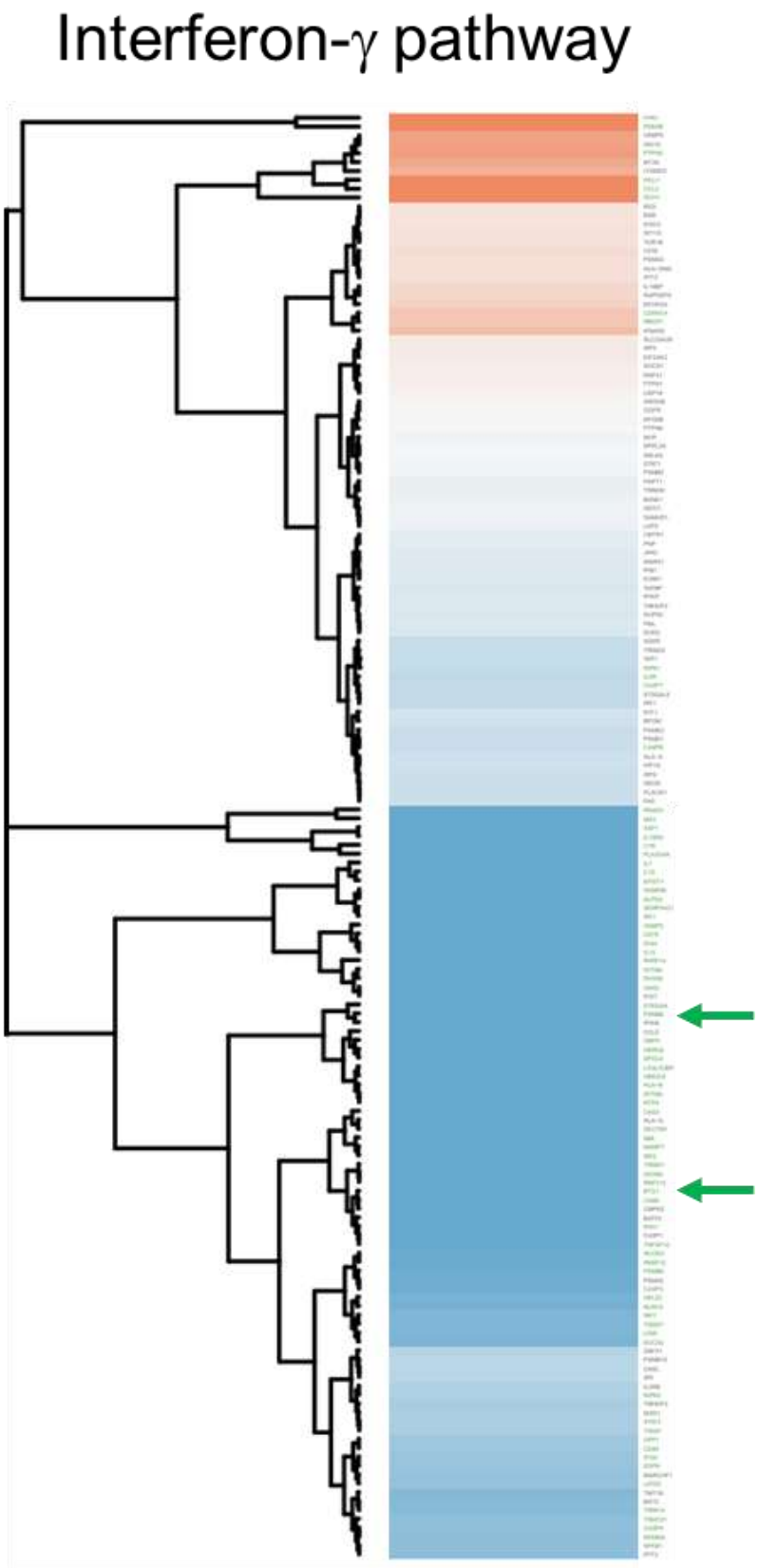
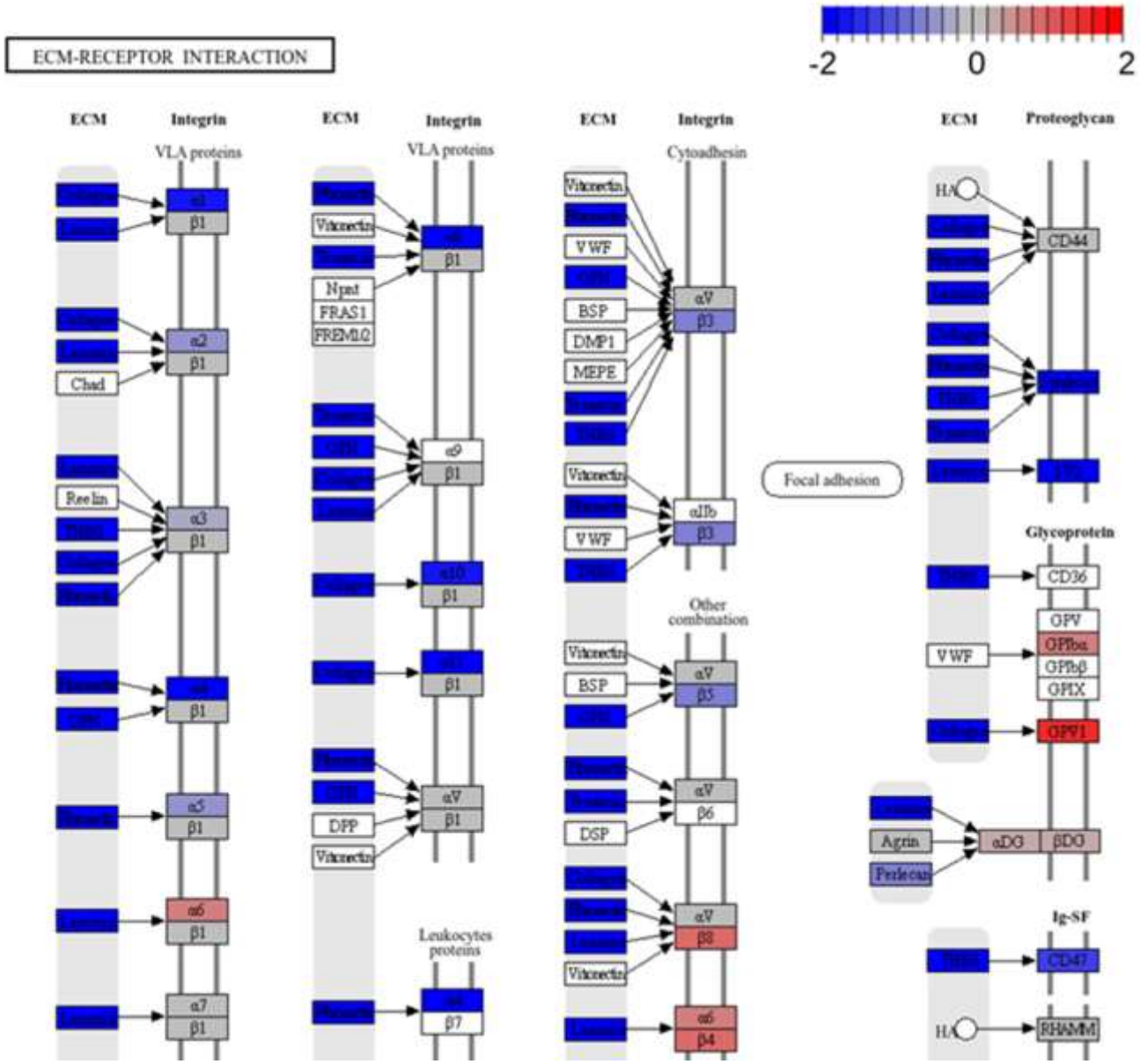
IFITM2
DHX58

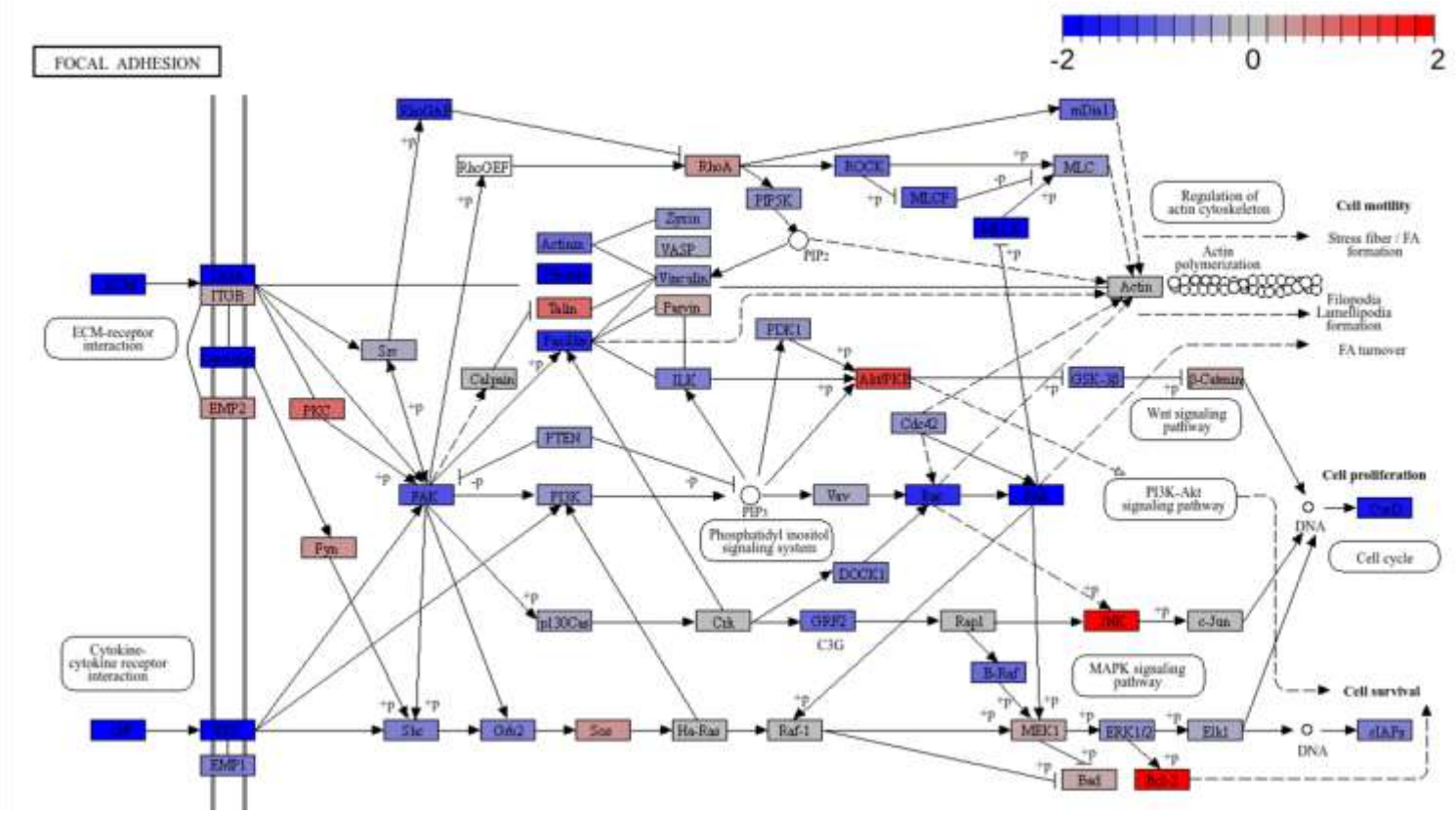
**4C.**

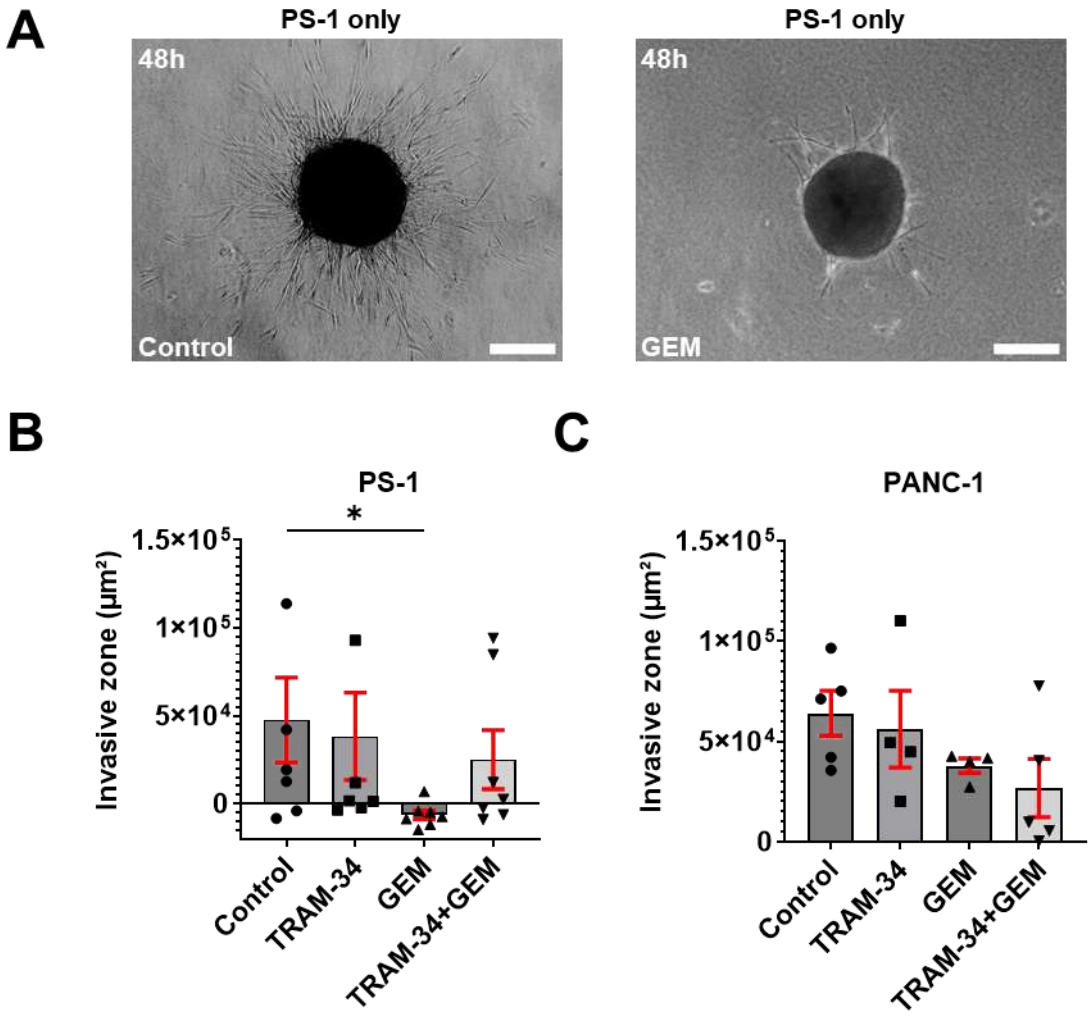


**Supplementary Figure 5A:** KEGG pathway analysis of the gene set for ECM-receptor interactions. The fold changes of gene expression are color coded: blue for down-regulation and red for up-regulation.

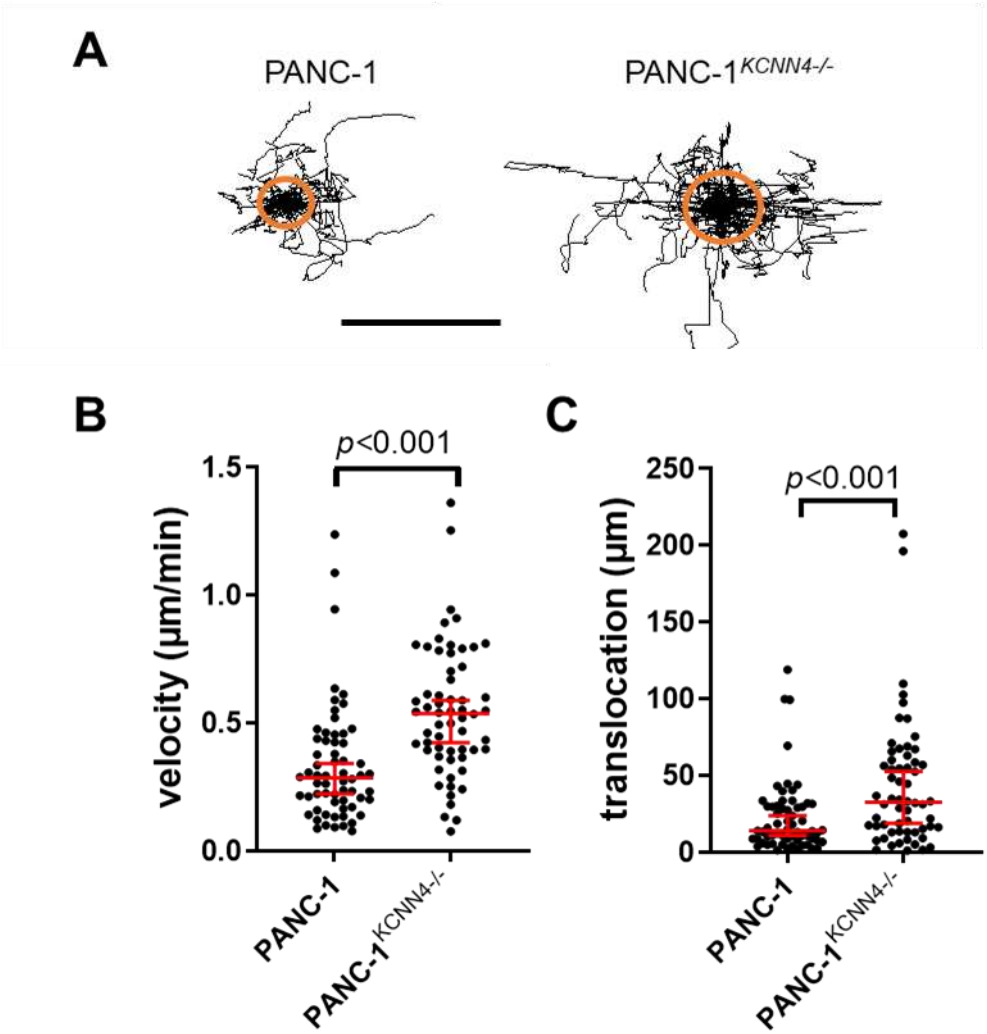


**Supplementary Figure 5B.** KEGG pathway analysis of the gene set for Focal Adhesion signaling. The fold changes of gene expression are color coded: blue for down-regulation and red for up-regulation.






**Supplementary Fig. 6: PS-1 cell spheroids are highly sensitive to gemcitabine.** (**A**) Representative images illustrating the migration pattern of PS-1 cell spheroids treated with either DMSO (Control, N=7) or gemcitabine (GEM, N=7). Scale bar: 200 µm. (**B**) Quantification of the invasive zones at t=48 h in PS-1 cell spheroids treated with DMSO (Control, N=7), TRAM-34 (N=7), gemcitabine (GEM, N=7), or a combination of TRAM-34 and gemcitabine (TRAM-34+GEM, N=7). Data points reflect individual spheroid invasive zones, while the bar charts present the mean ± SEM. *: $p < 0.05$, Kruskal-Wallis test with Dunn's post hoc test. (**C**) Quantification of the invasive zones at t=48 h in PANC-1 cell spheroids treated with DMSO (Control, N=5), TRAM-34 (N=4), gemcitabine (GEM, N=4), or a combination of TRAM-34 and gemcitabine (TRAM-34+GEM, N=5). Data points denote individual spheroid invasive zones, and the bar charts represent the mean.




**Supplementary Fig. 7: PANC-1$^{KCNN4-/-}$ cells migrated faster than wild-type cells.** (**A**) Trajectories of individual cells spontaneously migrating on a two-dimensional substrate. Trajectories are normalized to common starting points. The radii of the orange circles show the median translocation in both cases. (**B, C**) Quantification of cell velocity (B) and translocation (C) of individual cells. They were calculated from the trajectories for the 6h time span; N / n = 3 / ≥60; Kruskal-Wallis test with Dunn's post hoc test.

# Supplemental methods

### 4.7. CNA-35-tdTomato production, purification and staining

We followed the protocol described previously (Schleinhege, 2025; Matrix Biol). CNA35-tdTomato was produced using competent *E. coli* BL21(DE3) bacteria (Novogen) transformed with the pET28a-tdTomato-CNA35 plasmid (Addgene plasmid #61606, RRID: Addgene_61606). *E. coli* were grown in LB medium (BD Bioscience) supplemented with 10 µg/mL kanamycin until reaching an optical density of 0.6 at 600 nm. Then protein expression was induced with 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG, AppliChem PanReac) at 37°C for 20 h with continuous shaking (220 rpm). Bacteria were then centrifuged (4,000 g at 4°C for 20 minutes), and the pellet was washed twice in a buffer containing 50 mM sodium phosphate and 300 mM NaCl, pH 7.0 followed by centrifugation (4,000 g at 4°C for 20 minutes). Bacteria were resuspended in lysis a buffer containing lysozyme (10 mg/mL) and protease inhibitor cocktail III (Calbiochem) and incubated on ice for 30 min. After adding Triton X-100 (0.1%; Sigma Aldrich), DNase I (1 mg/mL; Invitrogen), and RNase A (1 mg/mL; AppliChem PanReac) in the presence of 1 M $MgCl_2$, the pellet was sonicated on ice (three cycles of 60 s each). After sonication, mechanical lysis was performed using a French press. The lysate was clarified by centrifugation at 12,500 g at 4 °C for 45 min. The supernatant containing the target protein was purified using nickel-nitrilotriacetic acid (Ni-NTA) agarose beads according to the manufacturer's instructions (Qiagen). The bead-protein complexes were eluted from filter columns (Cytiva) with 250 mM imidazole. The eluate was concentrated using Amicon Ultra 0.5 ml 10k diafiltration filters, followed by an additional washing step. The filters were then inverted and centrifuged at 1,000 g for 2 min at 4°C to recover the concentrated CNA35-tdTomato protein. To ensure proper maturation of the fluorescent protein, the collected protein solution was incubated at 37°C overnight. The concentration of the purified CNA35-tdTomato (1.037 mg/mL) was assessed using the BCA Protein Assay Kit (Pierce). The protein solution was aliquoted and stored at -70 °C.

For evaluating the fibrosis of tumor nodes, tissue sections were stained with CNA-35-tdTomato {Schleinhege, 2025 #33}. Tissue sections were incubated with CNA-35-tdTomato (1:200) in PBS at 4°C overnight. Afterwards, samples were washed three times with PBS. Images were acquired with fluorescence confocal microscopy (Nikon ECLIPSE Ti2). We analyzed the wall thickness of tumor nodes and the intranodal CNA-35-tdTomato fluorescence intensity as a read-out of tumor fibrosis.

**Matrix production assay**

We followed the protocol described previously (Schleinhege, 2025; Matrix Biol). PS-1 or PANC-1 cells were seeded into a black-walled, clear-bottom 96-well plate (Thermo Fisher) at 80% confluence. Cells were cultured in DMEM/F12 (Sigma-Aldrich) supplemented with 0.5% FCS superior, vitamin C (284 µM, Sigma-Aldrich), and TGF-β1 (10 ng/mL, PeproTech) to stimulate matrix production, and treated with the $K_{Ca}$3.1 inhibitors and chemotherapeutic drug as indicated. After 24 h, the cells were washed twice with PBS. Newly produced collagen I was labeled with CNA35-tdTomato (1:200, diluted in PBS; {Schleinhege, 2025 #33}) at room temperature in the dark for 3 h. Fluorescence intensities were measured using a fluorescence plate reader (Fluoroskan II) and representative images were acquired by confocal microscopy (Nikon ECLIPSE Ti2 microscope). Fluorescence intensity values were blank corrected.

**Mitochondrial Western blot**

Mitochondrial proteins were isolated from PANC-1 cells using the QProteome Mitochondria Isolation Kit (Qiagen) according to the manufacturer's instructions. Protein fractions were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were probed with antibodies against the mitochondrial marker TOMM20 (F-10, sc-17764, Santa Cruz Biotechnology, 1:500) to confirm the enrichment of the mitochondrial fraction, and against $K_{Ca}$3.1 (AB_1852147, Sigma-Aldrich, 1:500) to assess mitochondrial $K_{Ca}$3.1 expression. Primary antibodies were incubated at 4°C overnight.

**Single cell migration experiments**

Migration of PANC-1 cells was monitored using time-lapse video microscopy as described previously (Dieterich et al., 2008). PANC-1 cells were seeded in pre-coated 12.5 $cm^2$ cell culture flasks. The coating matrix mimics the desmoplastic stroma of PDAC and is similar to the matrix used during spheroid migration experiments, containing the following: 40 µg/ml laminin (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), 40 µg/ml fibronectin (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), 800 µg/ml collagen I (Corning, New York, NY, USA), 12 µg/ml collagen III (Corning, New York, NY, USA), and 5.4 µg/ml collagen IV (BD Biosciences, Heidelberg, Germany). The next day cells were placed into temperature-controlled chambers (37°C) on the stage of microscopes.

Cell migration was recorded with CMOS cameras for 6 h at 5 min intervals using the MicroCamLab 3.1 software (Bresser, Rhede, Germany). Quantitative analysis of single cell migration was based on the segmentation of PANC-1 cell contours and centroids using the NIS software suite (Nikon, Inc., Tokio, Japan). The segmented cell contours allowed to

calculate cell velocity (µm/min) and translocation as described before (Storck et al., 2017). Migration velocity was defined as the displacement of the cell centroid as a function of time by applying a three-point difference quotient. Translocation represents the net distance covered during the entire experiment.

## References

Dieterich, P., Klages, R., Preuss, R., and Schwab, A. (2008). Anomalous dynamics of cell migration. *Proc. Natl. Acad. Sci. U.S.A.* 105, 459–463 doi: 10.1073/pnas.0707603105