Online Resources

A transmembrane c-terminal fragment of syndecan-1 is generated by the metalloproteinase ADAM17 and promotes lung epithelial tumor cell migration and lung metastasis formation

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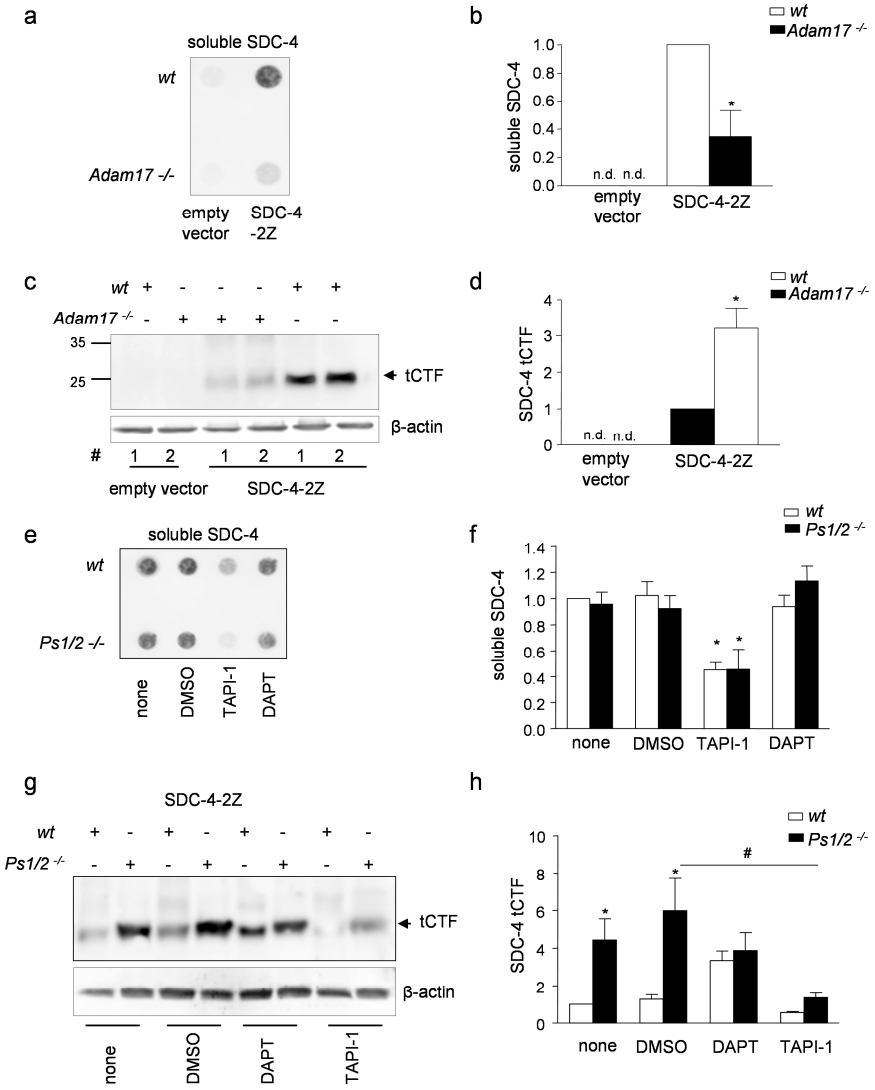
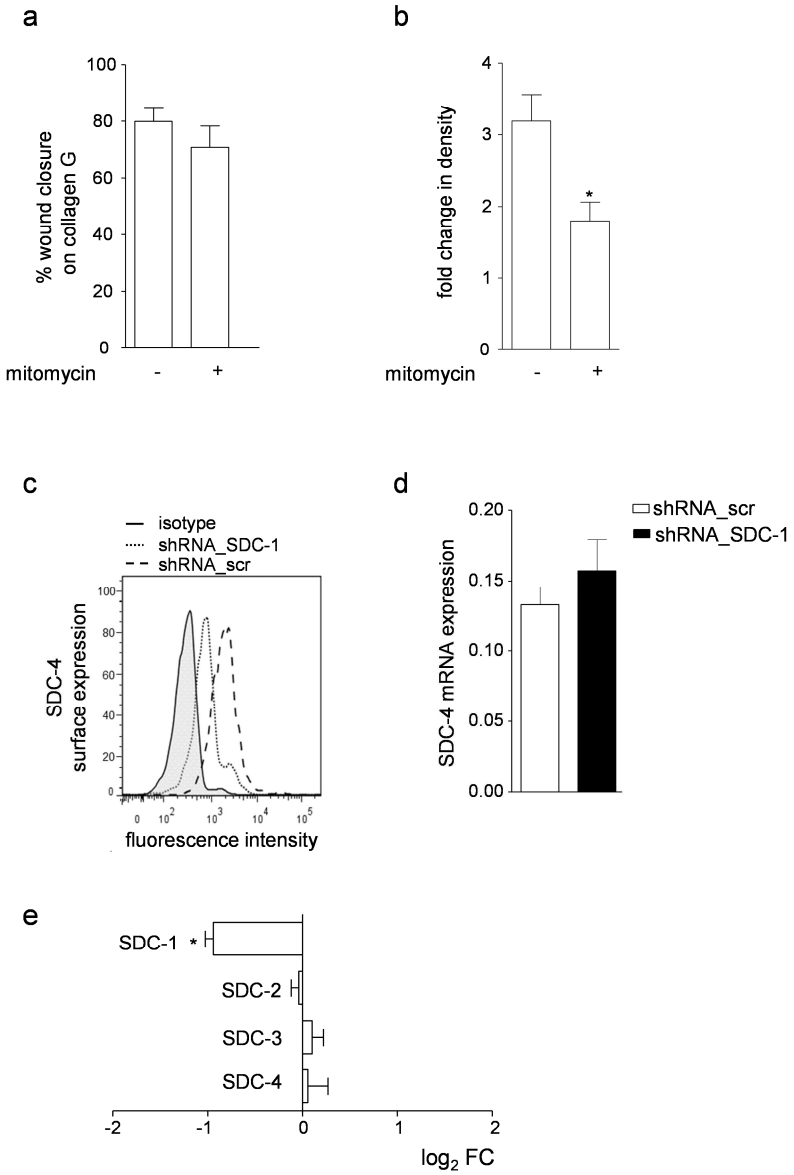


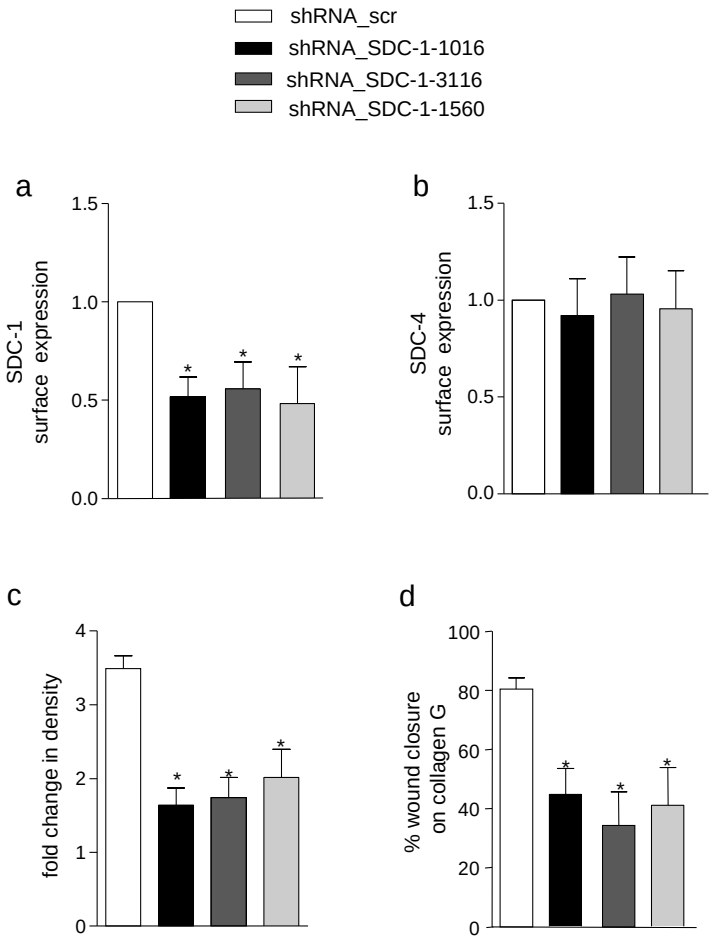
Supplementary Fig.1 Sequential processing of syndecan-4 by ADAM17 and γ-secretase.

a-d) Wild type (wt) and *Adam17*$^{-/-}$ MEF cells were transfected with syndecan-4-2Z (SDC-4-2Z) or empty vector as control. After 48 h, conditioned media were harvested and subsequently assayed for the release of syndecan-4 (SDC-4) by dot blotting using an antibody against the ectodomain of SDC-4 (a). The signal for released soluble SDC-4 was quantified by densitometry and expressed in relation to the signal of the wild type control (b, n.d.: not detectable). Cell lysates were subjected (two independent experiments are marked with lane 1 or 2) to Western blotting using rabbit IgG, which allows direct detection of the 2Z-tag (c) and quantified by densitometry (d). The arrow indicates the signal for the transmembrane C-terminal fragment (tCTF), which was quantified by densitometry (d, n.d.: not detectable). e-h) Presenilin1/2$^{-/-}$ (*Ps1/2* $^{-/-}$) and wild type MEF cells, transfected with SDC-4-2Z expression vector, were treated for 16 h with metalloproteinase inhibitor TAPI-1 (10 µM), γ-secretase inhibitor DAPT (5 µM) or DMSO (vehicle, 0.1%). The release of soluble SDC-4 was examined by dot blotting (e) and quantified by densitometry expressed in relation to the untreated control (f). Cell lysates were subjected to Western blotting (g) and the tCTF was quantified by densitometry expressed in relation to the untreated control (h). Data are shown as representative experiments and means + SD calculated from three independent experiments. Statistically significant differences compared to the respective wild type control are indicated by asterisks, and statistical differences compared to the DMSO control are indicated by hashes ($p < 0.05$).



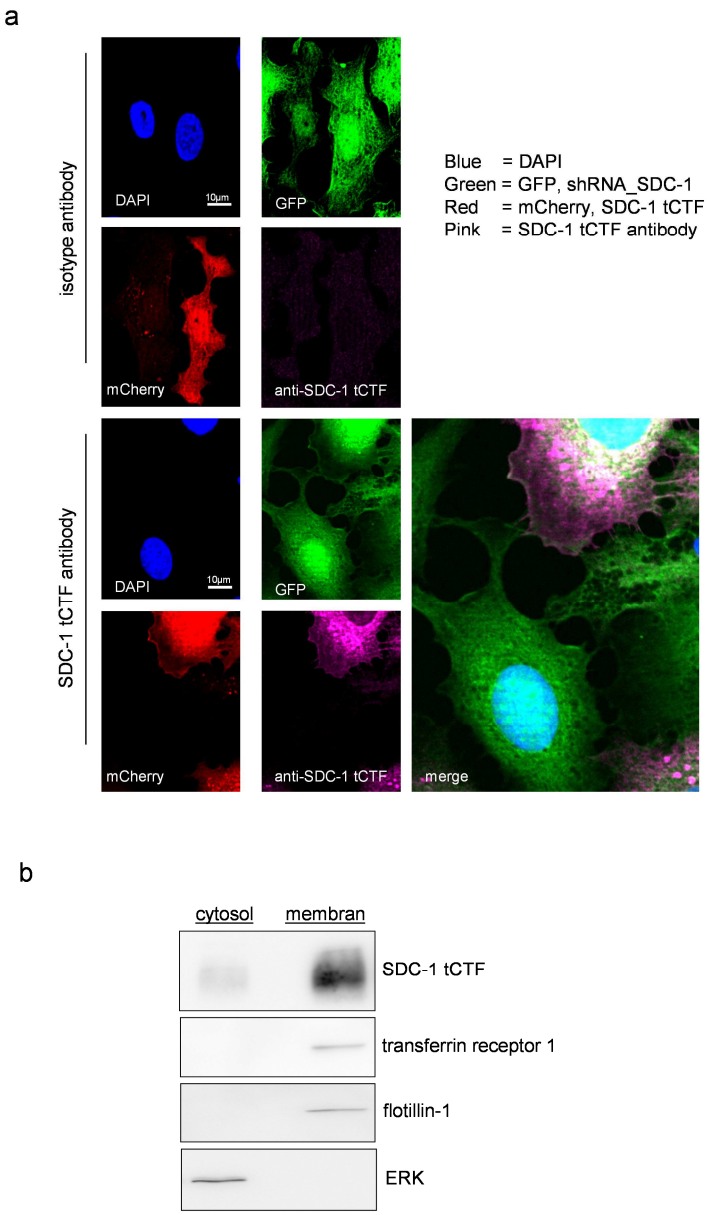
Supplementary Fig.2 Effect of mitomycin on proliferation and wound closure and influence of shRNA mediated syndecan-1 knockdown on other syndecans.

a) Wild type A549 cells were grown to confluency, pretreated with 10 µg/ml mitomycin for 2 h, washed twice and wounded by a defined scratch. Wound closure was monitored for 24 h and quantified using the IncuCyte ZOOM system. (b) Wild type A549 cells were pretreated with or without 10 µg/ml mitomycin for 2 h, washed twice and assayed for proliferation over 48 h by measuring changes in confluence using the IncuCyte ZOOM software. c-d) A549 cells were transduced with lentivirus encoding scr or SDC-1 shRNA (SDC-1). The influence of SDC-1 knockdown on SDC-4 expression was controlled by flow cytometry (c) and qPCR (d). Data represent means + SD of three independent experiments and statistically significant differences were indicated by asterisks ($p<0.05$). Changes in expression on RNA level for SDC-1, -2, -3 and -4 are illustrated for shRNA_SDC-1 cells in comparison to shRNA_scr cells. Shown are the $\log_2$ fold change (FC) values with the mean + SD of 3 independent array experiments. Bars to the left of the middle line indicate that a given gene is downregulated, while bars to the right depict upregulation of a gene. SDC: syndecan; (*): significantly changed with an ANOVA raw p-value of $5x10^{-5}$(e).



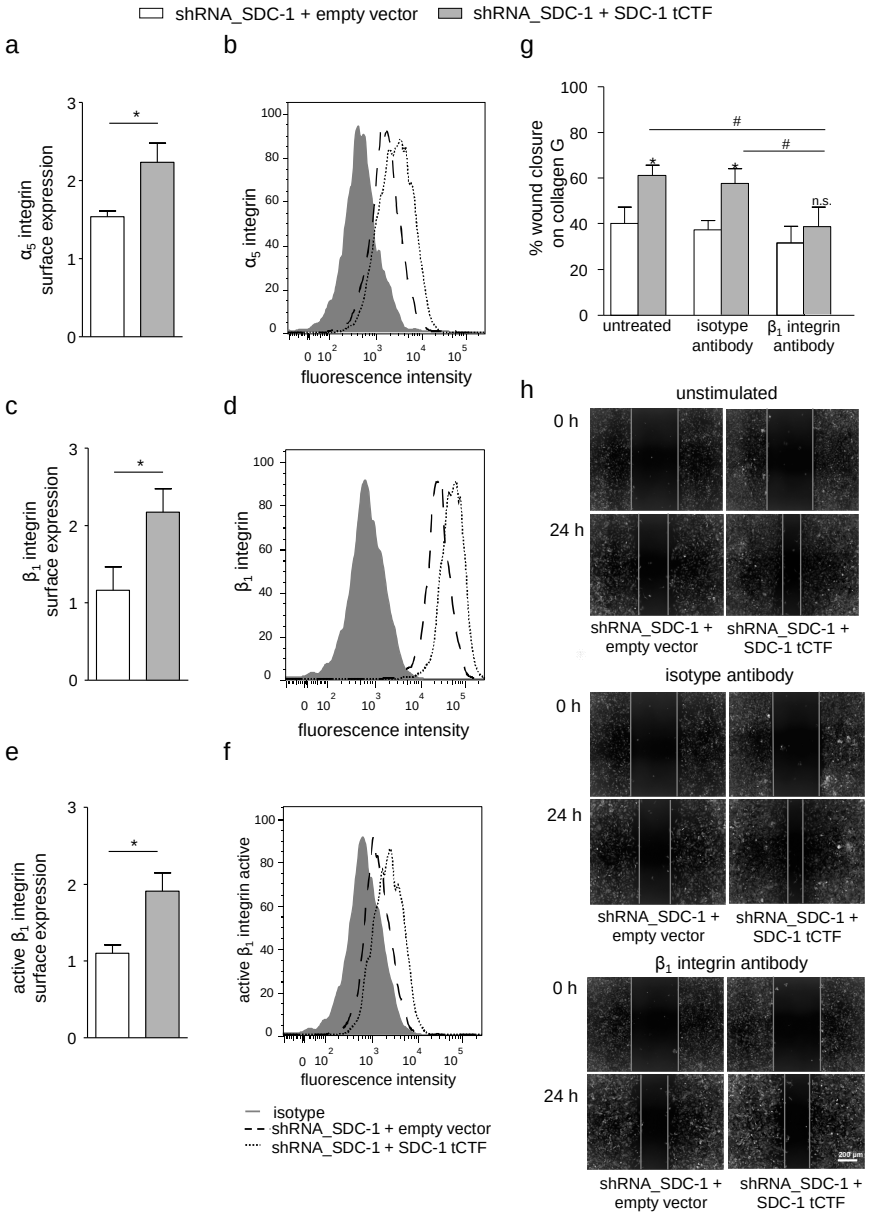
Supplementary Fig.3 Knockdown with different shRNA sequences confirms the role of syndecan-1 in cell proliferation and migration.

A549 cells were transduced with lentivirus encoding scr shRNA or different shRNA sequences for SDC-1. a-b) Efficiency of SDC-1 surface expressed downregulation (a) and the influence on SDC-4 expression were controlled by flow cytometry (b). c-d) Transduced cells were assayed for proliferation over 48 h by measuring changes in confluence using the IncuCyte ZOOM system (c). Transduced A549 cells were grown to full confluency on coated collagen G and wounded by a defined scratch. Wound closure was monitored from 0 h after scratch induction to 24 h and quantified using the IncuCyte ZOOM system (d). Data represent means + SD of three independent experiments. Statistically significant differences compared to the scr control are indicated by asterisks ($p < 0.05$).



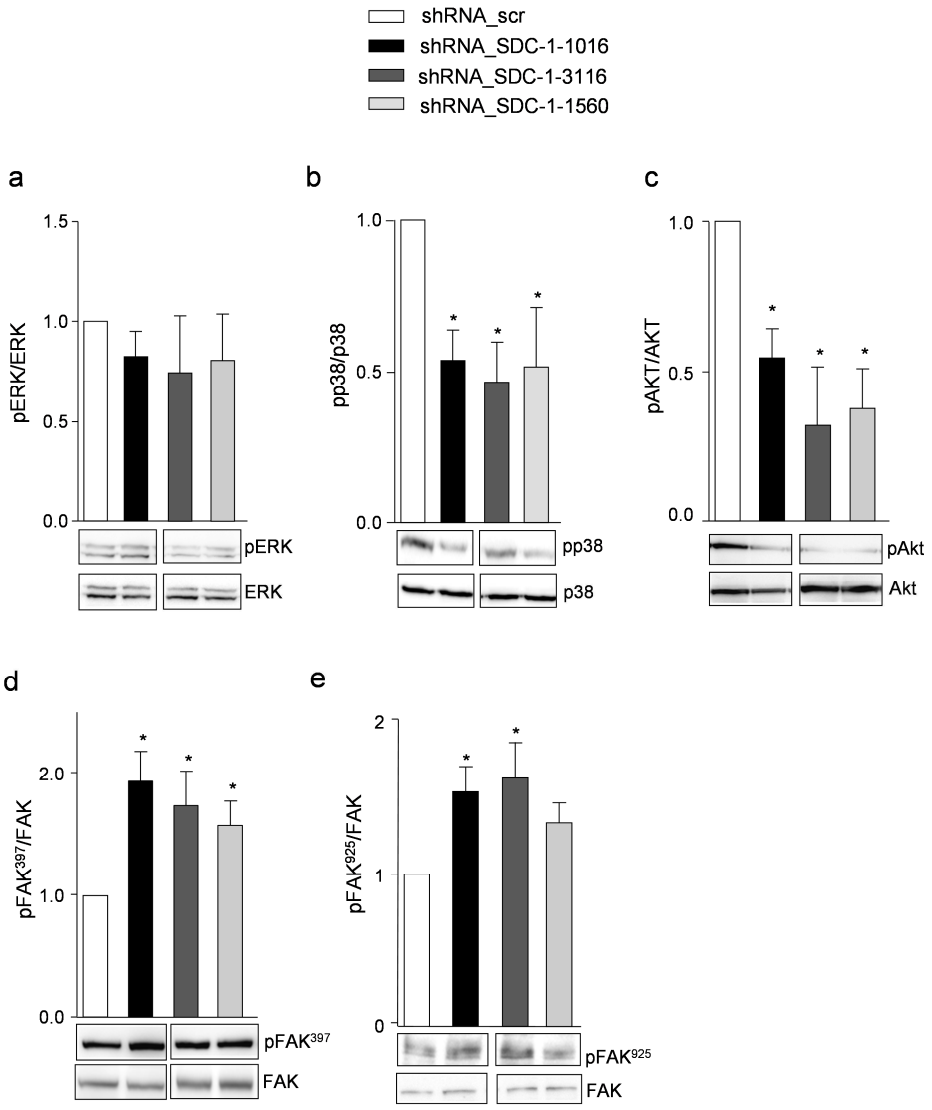
Supplementary Fig.4 Immunocytochemistry and cytosol-membrane fractions of syndecan-1-tCTF expressing A549 cells.

A549 cells were transduced with lentivirus encoding GFP and SDC-1 shRNA. Overexpression of SDC-1 tCTF was carried out by second transduction of A549 cells with lentivirus encoding mCherry and SDC-1 tCTF. Subsequently cells were fixed, permeabilized and stained with isotype control (clone 20102) (upper panel) or SDC-1 tCTF antibody (clone 2E9) (lower panel). GFP (green) and mCherry (red) signal were used as transduction controls and double transduced cells expressing the SDC-1 tCTF showed a pink signal. Exemplary images of three independent experiments are shown (a). Cytosol-membrane fractions were subjected to Western blotting and assessed for SDC-1 tCTF, transferrin receptor 1, flotillin-1 and ERK expression (b).



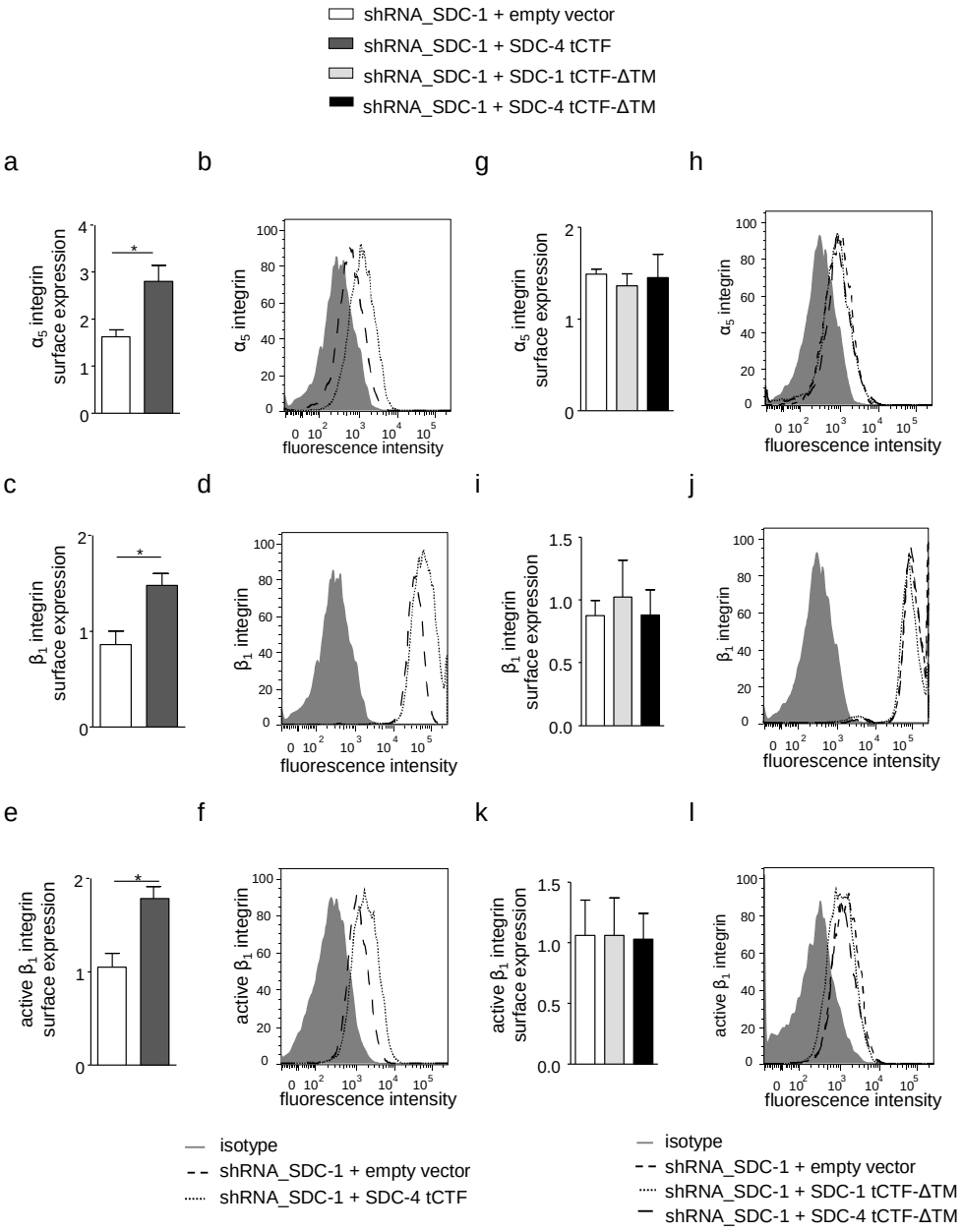
Supplementary Fig.5 Syndecan-1 tCTF modulates integrin surface expression and restoration of cell migration is blocked by β_1 integrin inhibition.

A549 cells were transduced with lentivirus encoding scr or SDC-1 shRNA. Subsequently, a second transduction was carried out with virus for overexpression of SDC-1 tCTF, or with empty vector control virus. a-f) Double transduced A549 cells expressing SDC-1 tCTF or empty vector were investigated for α_5 integrin (a, quantification; b, representative histogram), β_1 integrin (c and d) or active β_1 integrin (e and f) surface expression. g-h) Double transduced A549 cells were grown to confluency on collagen G coated wells, wounded by a defined scratch and treated with antibodies against β_1 integrin (clone 4B7R) (20 µg/ml), isotype (clone 11711) (20 µg/ml) as control or left untreated. Wound closure was monitored for 24 h and quantified using the IncuCyte ZOOM software (g). Exemplary images of three independent blocking experiments are shown (h). Data represent means + SD of three independent experiments and representative histograms and pictures are shown. Statistically significant differences are indicated by asterisks ($p<0.05$).



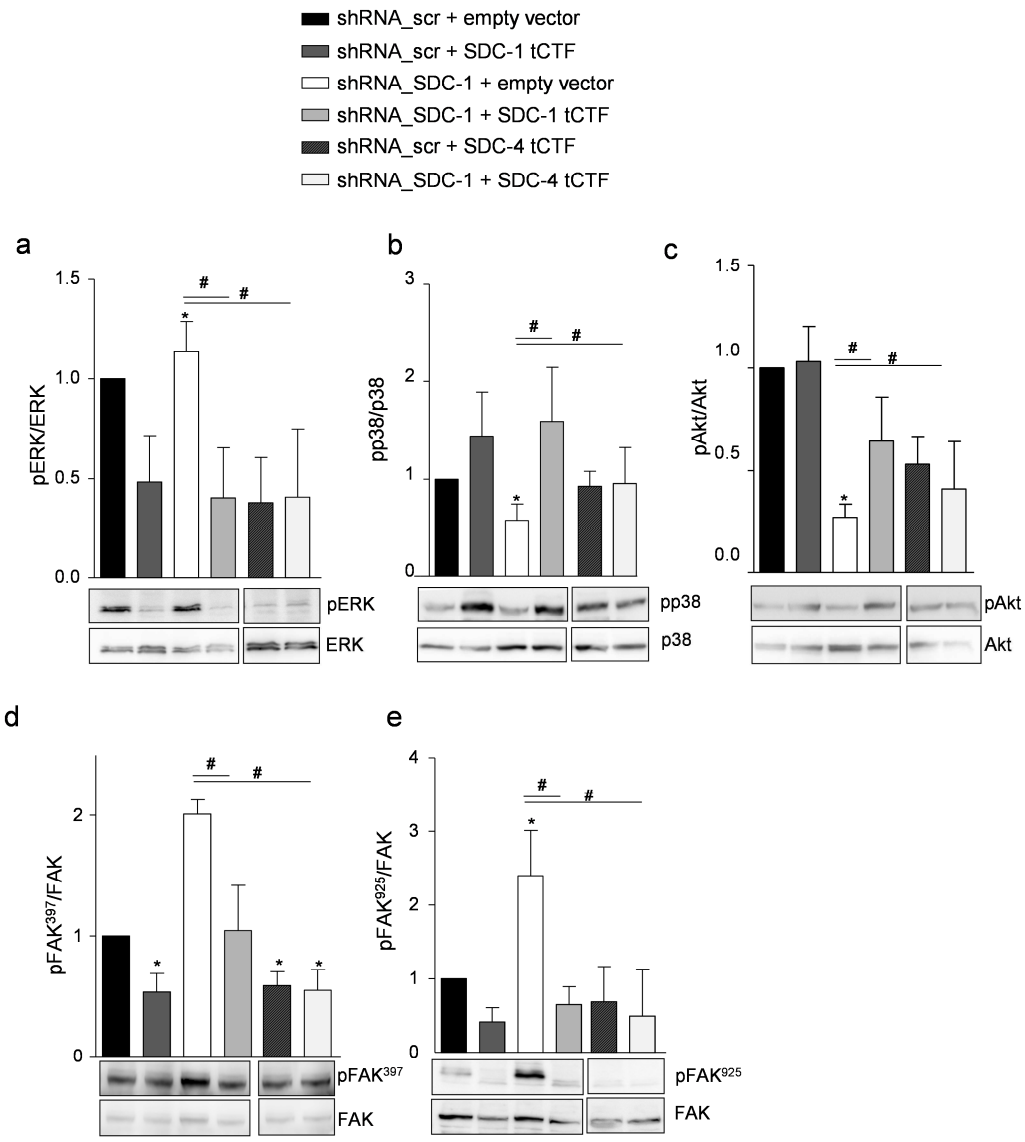
Supplementary Fig.6 Syndecan-1 knockdown with different shRNA sequences confirms the modulation of intracellular signaling pathways.

A549 cells transduced to express scr shRNA or different SDC-1 shRNA sequences were analyzed for phosphorylation status of ERK1/2 (a), p38 (b), Akt (c), and FAK at sites Tyr397 (d) and Tyr925 (e) by Western blotting. Signals were quantified by densitometry as phosphorylated or activated versus total forms and calculated in relation to the controls expressing scramble shRNA. All samples were run on the same gel but for display the order was changed. Data represent means + SD of three independent experiments and representative Western blots are shown. Statistically significant differences are indicated by asterisks ($p<0.05$).



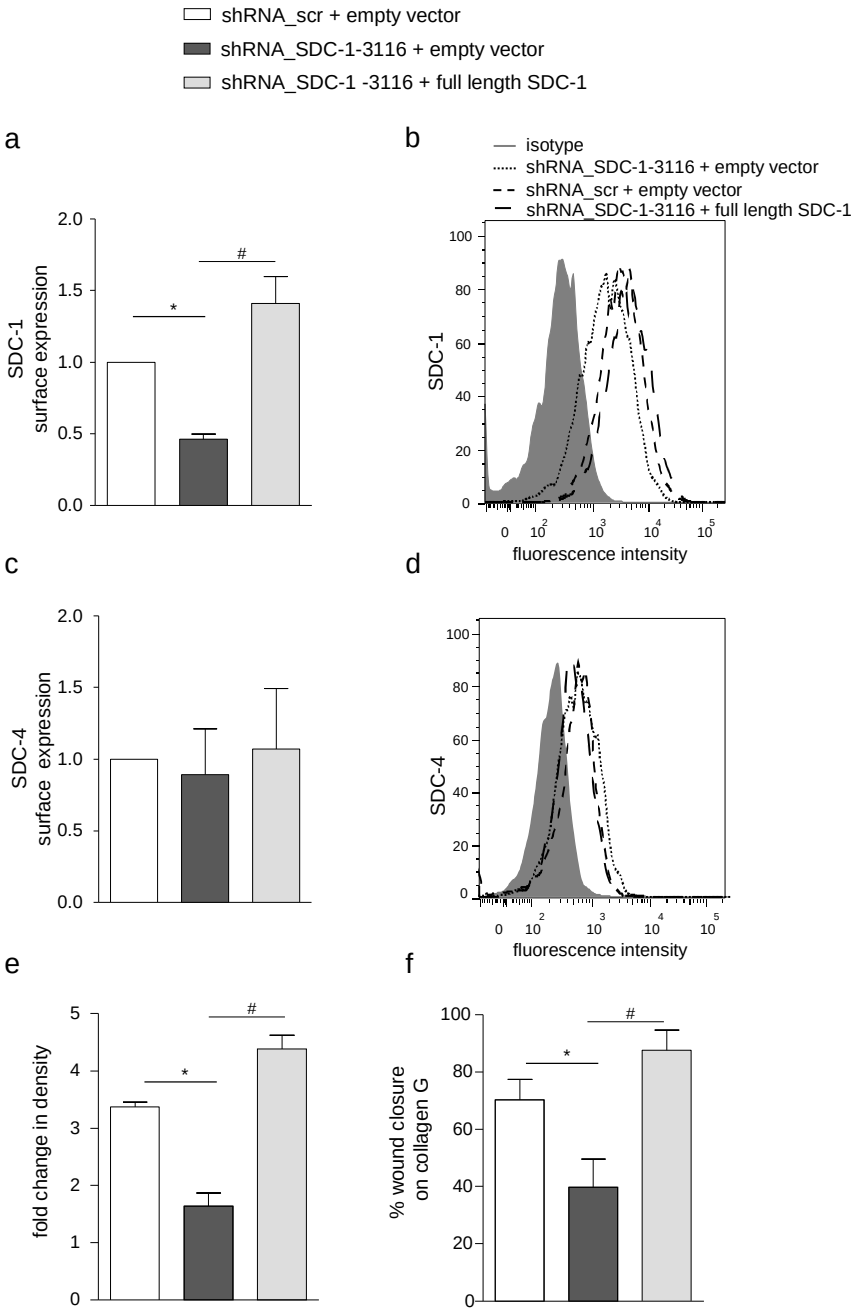
Supplementary Fig.7 Syndecan-4 tCTF modulates integrin surface expression and is depending on the transmembrane domain of Syndecan-1 or Syndecan-4.

A549 cells were transduced with lentivirus encoding scr or SDC-1 shRNA. Subsequently, a second transduction was carried out with virus for overexpression of SDC-4 tCTF, SDC-1 tCTF-ΔTMD, SDC-4 tCTF-ΔTMD or with empty vector control virus. a-l) Double transduced A549 cells were investigated for α_5-integrin (a, quantification; b, representative histogram and g, quantification; h, representative histogram), β_1 integrin (c-d and i-j) or active β_1 integrin (e-f and k-l) surface expression. All samples were run on the same gel but for display the order was changed. Data represent means + SD of three independent experiments and representative Western blots are shown. Statistically significant differences are indicated by asterisks ($p<0.05$).



Supplementary Fig.8 Restoration of intracellular signaling by syndecan-1 or -4 tCTF overexpression in syndecan-1 deficient A549 cells.

A549 cells were transduced with scr or SDC-1 shRNA vector. Subsequently, a second transduction was carried out with empty vector control virus or with virus for overexpression of SDC-1 tCTF, SDC-4 tCTF. Cell lysates of double transduced A549 cells were analyzed for phosphorylation or activation status of ERK1/2 (a), p38 (b), AKT (c), FAK at sites Tyr397 (d) and Tyr925 (e) by Western blotting. Signals were quantified by densitometry as phosphorylated or activated versus total forms and calculated in relation to the appropriate control cells. Data represent means + SD of three independent experiments. Statistically significant differences compared to shRNA_scr + empty vector or shRNA_SDC-1 + empty vector are indicated by asterisks or hashes, respectively ($p<0.05$).



Supplementary Fig.9 Syndecan-1 full length expression restores cell migration and proliferation

A549 cells that had been transduced with shRNA directed against the SDC-1 untranslated region (UTR) to downregulate endogenous SDC-1 expression were again transduced with lentivirus encoding full length SDC-1 or with empty vector. a-d) Double transduced A549 cells were investigated for surface expression of SDC-1 (a, quantification; b, representative histogram) or SDC-4 (c, quantification; d, representative histogram). e-f) Transduced cells were assayed for proliferation over 48 h by measuring fold change in density using the IncuCyte system (e). Transduced A549 cells were grown to confluency on collagen G and wounded by a defined scratch. Wound closure was monitored from 0 h after scratch induction to 24 h and quantified using the IncuCyte ZOOM system (f). Data represent means + SD of three independent experiments and representative histograms are shown. Statistically significant differences are indicated by asterisks and hashes ($p<0.05$).

Supplementary materials and methods:

GeneChip® hybridization

Gene expression in cells expressing shRNA against SDC-1 or shRNA scramble as control were analyzed using the GeneChip® Human Transcriptome Arrays 2.0 (Affymetrix, Santa Clara, CA, USA). Total RNA was isolated using RNeasy Kit (Qiagen) and quantified (Nanodrop 8000 (Thermo Scientific, Waltham, MA, USA)). Subsequently, RNA quality was assessed using RNA 6000 Nano Assay with the 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) to ensure that the samples had a RNA integrity number (RIN) of at least 9. Samples were prepared and hybridized to the GeneChip® Human Transcriptome Arrays 2.0 according to the GeneChip® WT Plus Reagent Kit user manual (P/N 703174 Rev. 2; Affymetrix). Briefly, for each sample, 250 ng of total RNA was reverse-transcribed into cDNA using a random hexamer oligonucleotide tagged with a T7 promoter sequence and stored at -20°C until further use. After second strand synthesis, double-stranded cDNA was used as a template for amplification with T7 RNA polymerase to obtain antisense cRNA. Random hexamers and dNTPs spiked with dUTPs were then used to reverse-transcribe the cRNA into single-stranded sense strand cDNA. The cDNA was then fragmented by uracil DNA glycosylase and apurinic/apyrimidic endonuclease 1. Fragment size was checked by using the 2100 Bioanalyzer (fragment size between 50 and 200 bp). Fragmented sense cDNA was biotin-endlabelled with Terminal Deoxynucleotidyl Transferase (TdT) and only stored overnight prior to array hybridization. Samples were hybridized to GeneChip® Human Transcriptome Arrays 2.0 at 45 °C for 16 h with 60 rpms. Hybridized arrays were then washed and stained on a Fluidics Station 450 (program: FS450 0001) and scanned on a GeneChip® Scanner 3000 7G (both Affymetrix). Raw image data were analyzed with Affymetrix® Expression Console™ Software, and gene expression intensities were normalized and summarized with robust multiarray average (RMA) algorithm (Irizarry et al., 2003). Statistical tests for differential expression and to identify differentially expressed genes was performed using Affymetrix® Transcriptome Analysis Console (TAC) Software, which computes and summarizes a traditional unpaired One-Way (single factor) Analysis of Variance (ANOVA) for each pair of condition groups. Transcripts that were expressed differently more than 1.5 fold with a raw ANOVA p-value lower than 0.05 between the analyzed sample groups were addressed as regulated.

Immunocytochemistry

For SDC-1 tCTF immunofluorescence staining, double transduced A549 cells were grown onto glass coverslips and fixed by incubating them in 4% paraformaldehyde for 20 min at room temperature. The cells were permeabilized with 0.1% Triton X-100 in PBS, incubated with the mAb SDC-1 tCTF antibody (2E9, 50 µg/ml) for 1 h at RT, followed by Alexa 657–conjugated goat anti-mouse for 1 h at RT. Slides were analyzed by fluorescence microscopy using an ApoTome.2 (Zeiss, Goettingen, Germany) with a Plan-Apochromat 63x/1.4 Oil objective (Zeiss) using AxioVision LE software (Zeiss).

Supplementary Reference

Ir zarry, RA; Hobbs, B; Collin, F; Beazer-Barclay, YD; Antonellis, KJ; Scherf, U; Speed, TP (2003). "Exploration, normalization, and summaries of high density oligonucleotide array probe level data" Biostatistics **4** (2): 249–64. doi:10.1093/biostatistics/4.2.249. PMID 12925520