Figure S1

(A) Increased migration of MCF7 and MTflEcad cells upon loss of E-cadherin (shEcad and MTdeltaEcad, respectively). *p<0.0001 and **p= 0.0236, unpaired t-test. Data are shown as mean±SD.
(B) shRNA-mediated depletion of E-cadherin (shEcad) induces invasion of MCF7 cells. ***p=0.0186, two sided t-test. Data are shown as mean±SD.
(C) Epithelial-mesenchymal transition reduces the proliferation rates of NMuMG, MCF7 and MTflEcad NMuMG cells. Cell numbers were measured using an MTT assay.

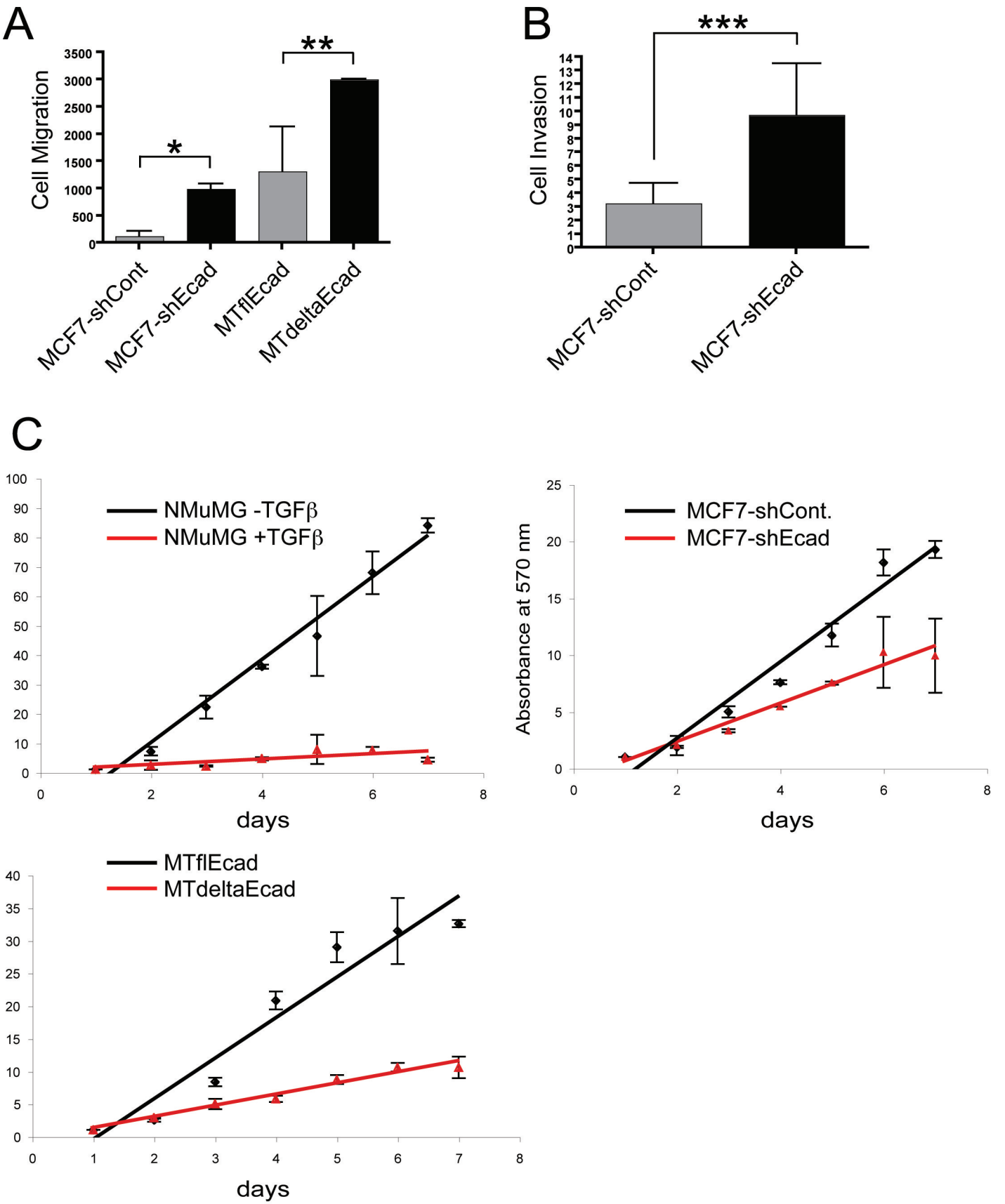


Figure S2

NCAM promoter activity is increased upon loss of E-cadherin.

(A) MCF7 cells expressing E-cadherin or depleted for E-cadherin expression were transfected with a CAT reporter plasmid containing 1000 bp (ΔSsp) or 647 bp (ΔNde) of the NCAM promoter sequence, and CAT activity was determined 48 hours after transfection (*p = 0.0114 and **p = 0.0374). Data are shown as mean±SD.

(B) MTflEcad cells carrying floxed alleles of the E-cadherin gene were treated with purified recombinant Cre-recombinase fused to a HIV-Tat entry peptide to ablate the E-cadherin alleles by recombination/excision. RNA was extracted at the time points of treatment as indicated, and the relative expression levels of NCAM and E-cadherin were determined by quantitative RT-PCR.

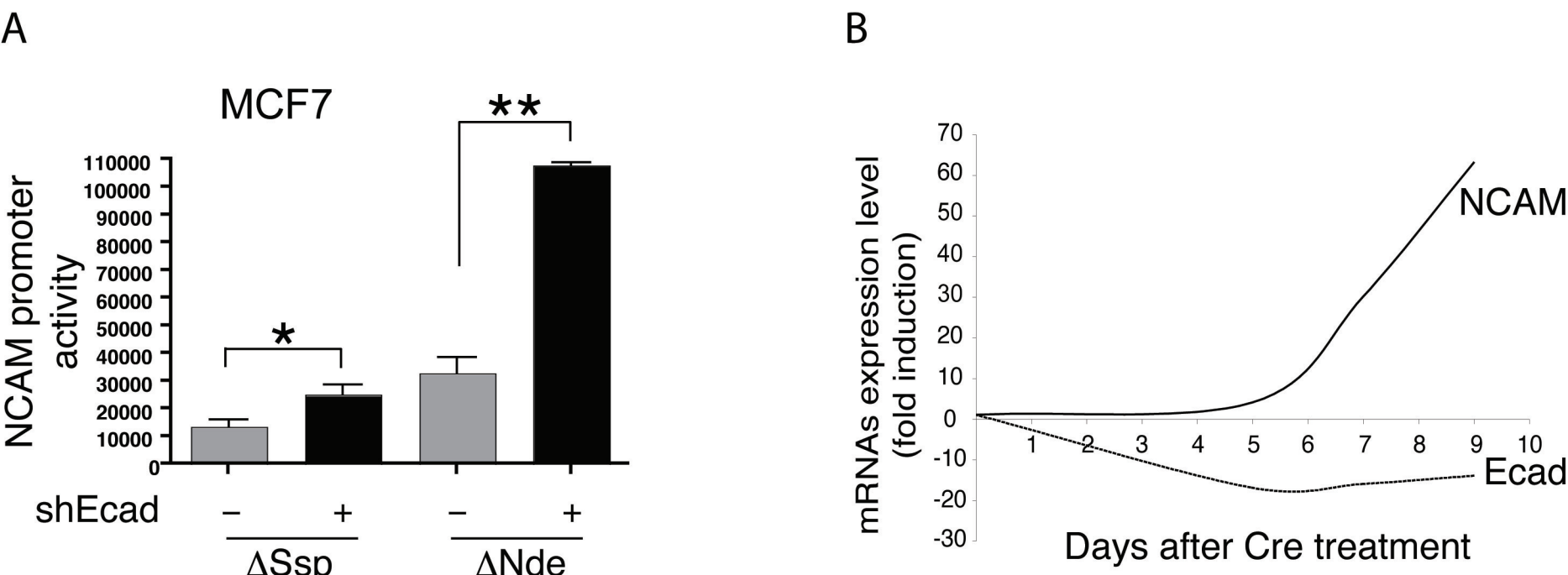


Figure S3

Loss of E-cadherin expression in HEK293-shEcad cells.

(A) Phase contrast micrographs of HEK293 cells stably expressing a control shRNA (shCont, left panel) or an E-cadherin-specific shRNA (right panel).

(B) Immunoblotting analysis of E-cadherin (Ecad), NCAM, β-catenin, and vimentin expression in HEK293-shCont and HEK293-shEcad cells. Immunoblotting for vinculin was used as loading control.

(C) Scratch wounding assay on confluent layers of HEK293-shCont and HEK293-shEcad cells. 42 hours after wounding, phase contrast microphotographs were taken. Note that within this period, HEK293-shEcad cells had almost closed the gap, while HEK293-shCont cells had migrated less.

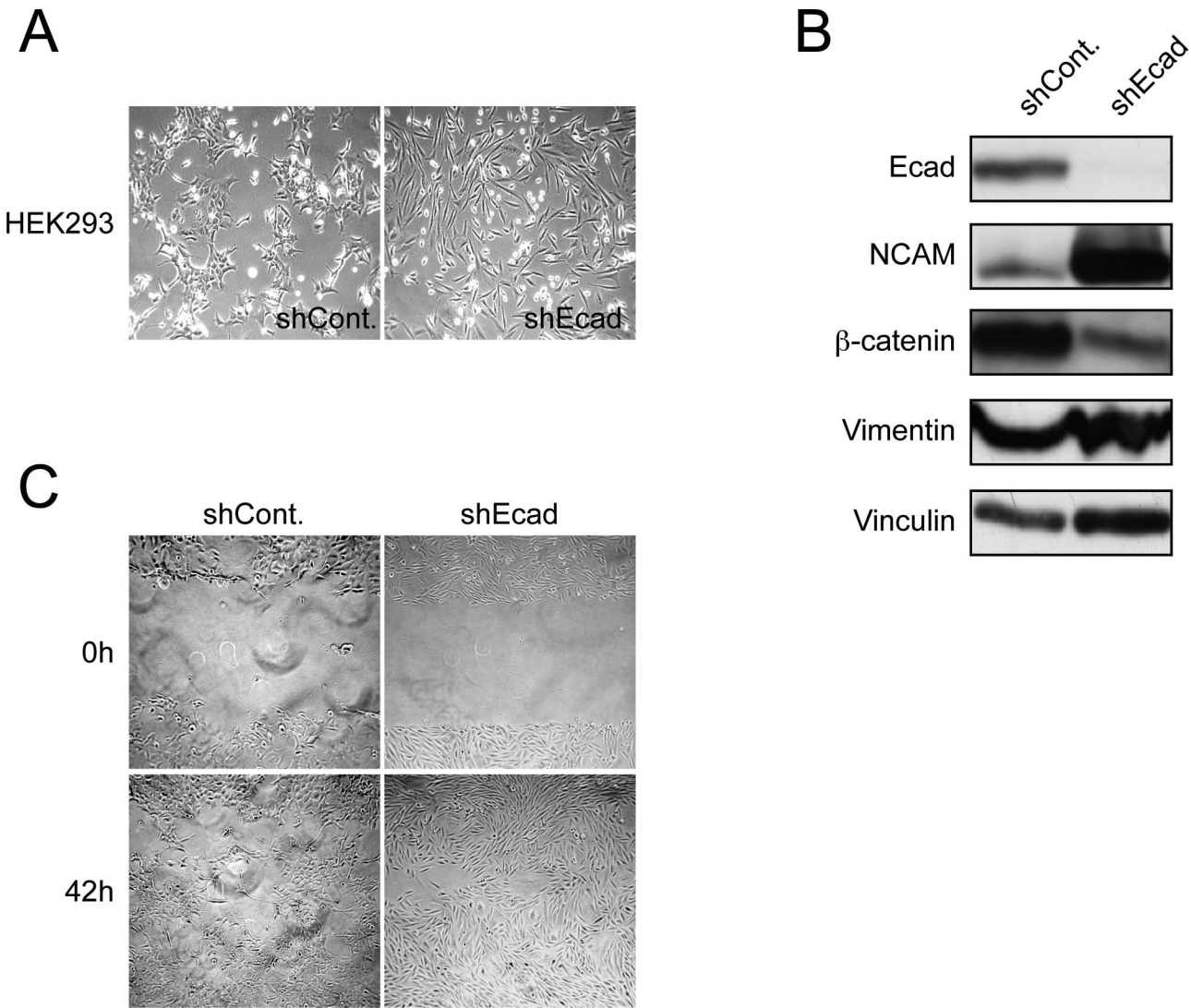


Figure S4

E-cadherin deletion in β cells of the islets of Langerhans leads to increased NCAMexpression in normal tissues. Immunofluorescence staining for E-cadherin **(green; A, D, E)** and for NCAM **(red; C, D, F)** in a pancreatic section from a E-cad F/F;RipCre mouse as indicated. E and F represent higher magnifications of the boxes in panels A and C, respectively. Single β cells that have lost E-cadherin expression and gained high levels of NCAM expression are indicated by arrows. Nuclei are stained with DAPI (blue).

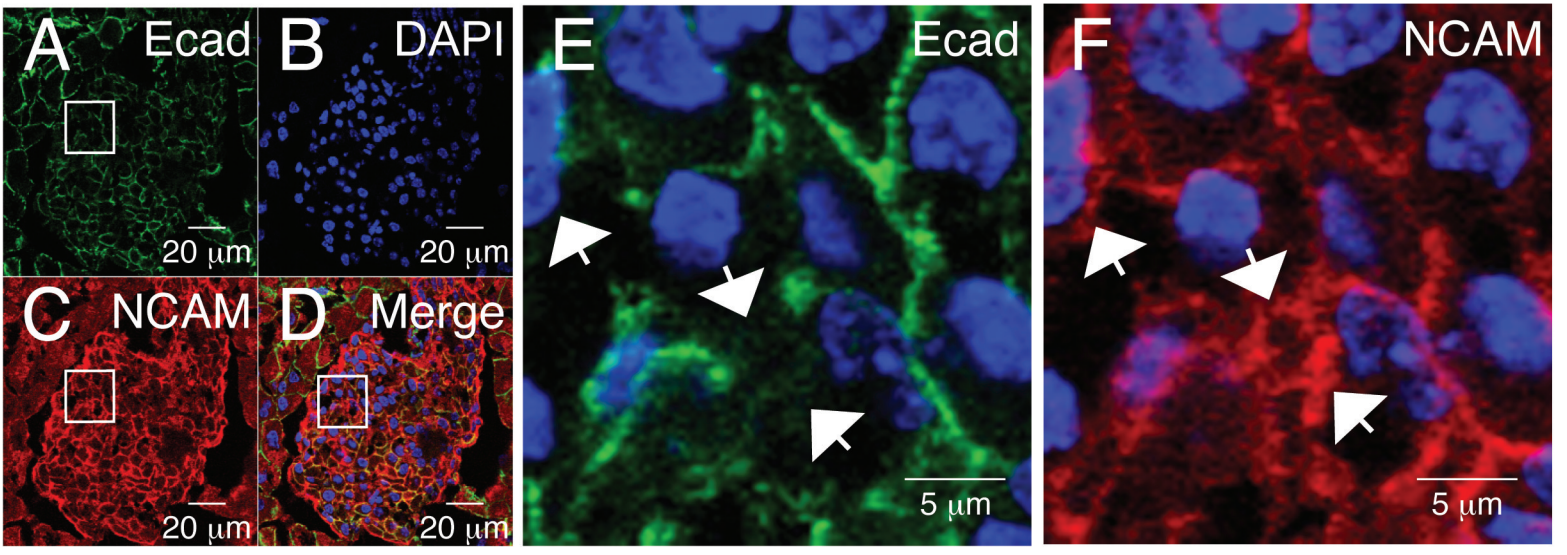


Figure S5

NCAM depletion increases cell migration

Scratch wounding assay on confluent layers of NMuMG cells transfected with siControl or siNCAM siRNA and treated with TGFβ. 24 hours after wounding, phase contrast microphotographs were taken. Note that within this time period, siNCAM-transfected cells had almost closed the gap, while the siControl cells had migrated less.

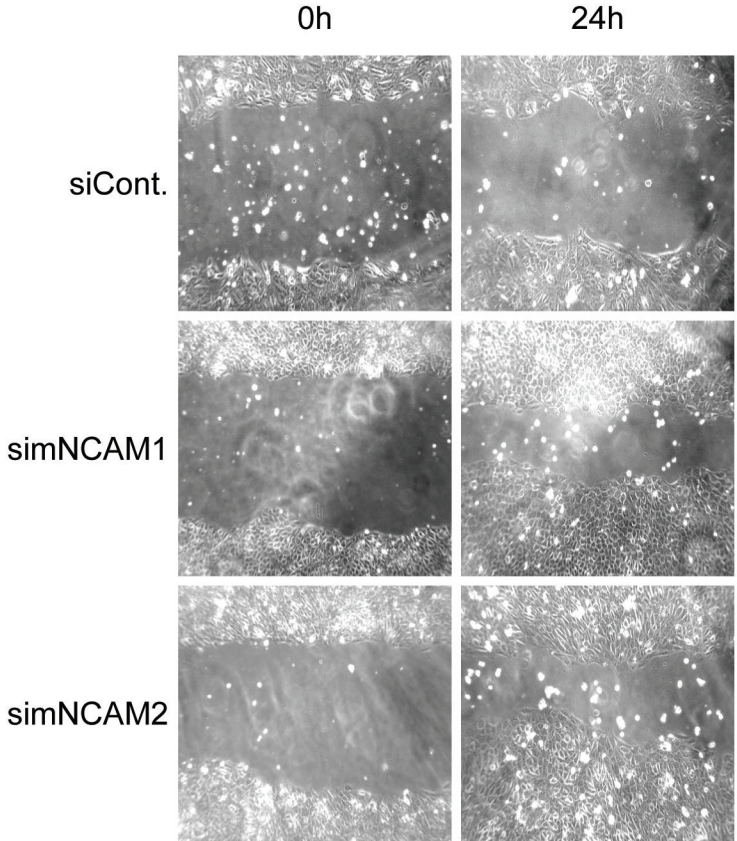


Figure S6

Characterization of siRNA targeting mouse and human NCAM.

(A) Immunoblotting analysis of MTdeltaEcad cells transfected for 48 hours with mouse NCAM-specific siRNA targeting NCAM or with mismatch control siRNA. Immunoblotting for vinculin was used as loading control.

(B and **C)** HEK293 and HEK293-shEcad and MCF7-shEcad cells **(D)** were transfected for the indicated times with 3 independant siRNA targeting human NCAM or with mismatch control siRNA (siCont) and analyzed by immunoblotting (upper panels) or quantitative RT-PCR (lower panels) for NCAM expression. Mouse riboprotein L19 primers were used for normalization, and fold induction was calculated against siControl-treated cells using the comparative Ct method (ΔΔCt). *p<0.01, unpaired t-test. Data are shown as mean±SD.

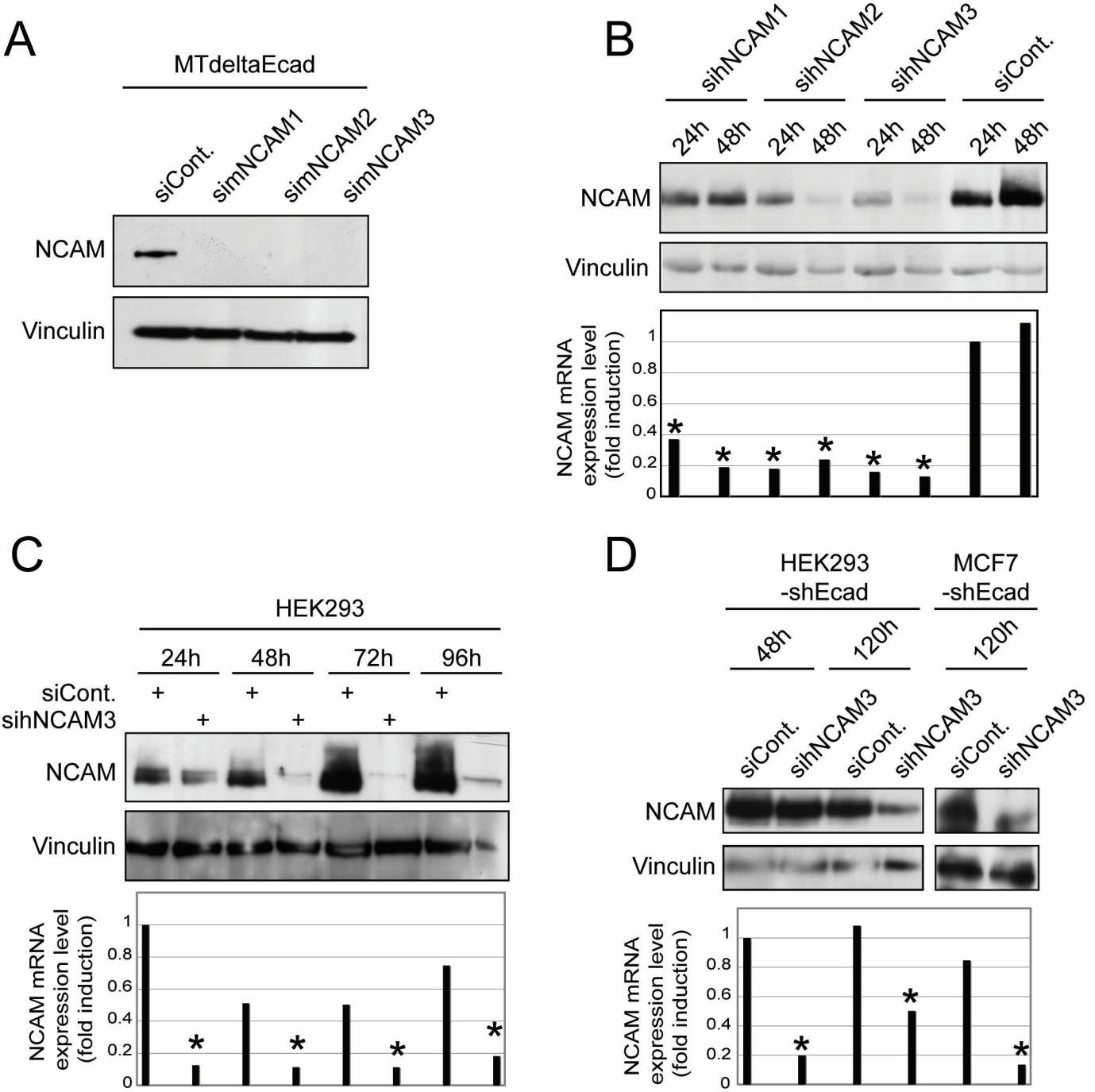
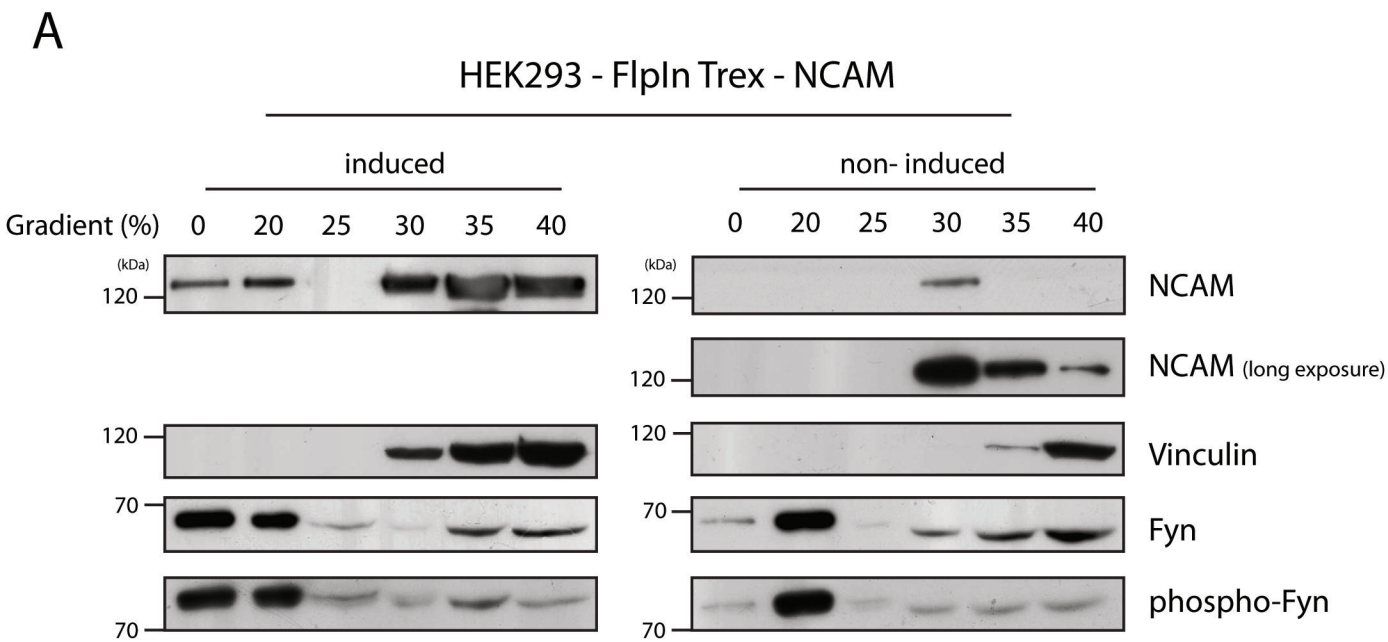


Figure S7

High levels of NCAM140 associate with lipid rafts.

(A) HEK293 cells expressing NCAM under the control of the Tetracycline-inducible system (HEK293-FlpInTrex-NCAM) were either grown in the absence of Doxycycline (non-induced) or in the presence of Doxycycline (induced) to induce expression of NCAM. Lysates of the cells were fractionated by sucrose gradient centrifugation and analyzed by immunoblotting for NCAM, phosphorylated-p59Fyn, p59Fyn and vinculin, as indicated. In cells expressing high levels of NCAM (induced), NCAM, p59Fyn and FAK are present in lipid rafts (0 and 20% sucrose) as well as in the Triton X-100 soluble high-density fractions (30, 35, and 40% sucrose). In cells expressing low levels of NCAM, they are only detected in the high-density fractions (30, 35, and 40% sucrose).

(B) Expression levels of E-cadherin remain unchanged upon induced expression of NCAM. HEK293-FlpInTrex-NCAM and HEK293-FlpInTrex-GFP cells were induced with doxycycline to express NCAM and GFP, respectively, and NCAM and E-cadherin levels were determined by immunoblotting at the time points indicated. Tubulin served as loading control.



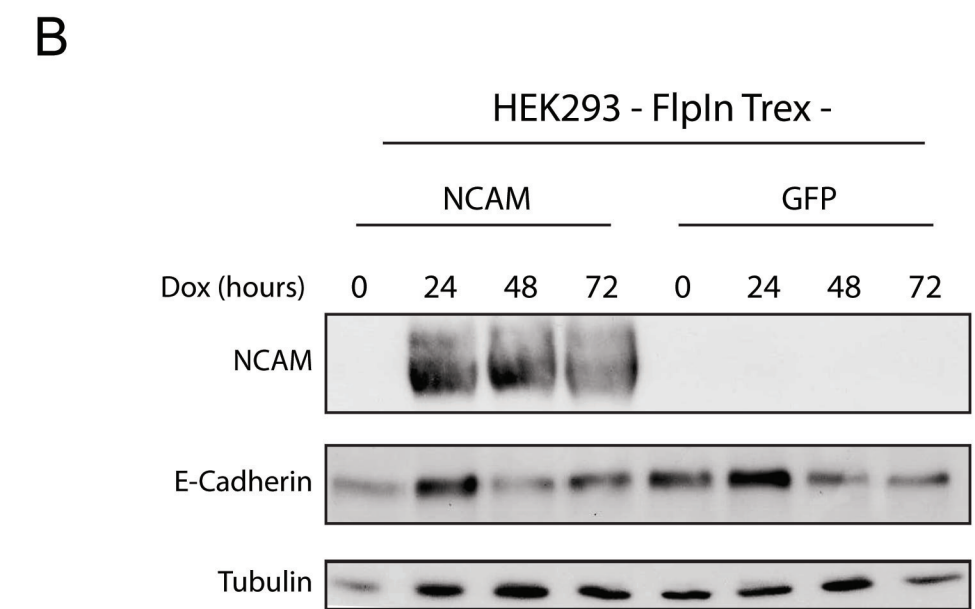


Table S1

Oligonucleotides used for quantitative SYBR green RT-PCR, siRNA and shRNA experiments.

qPCR primers	**Forward**	**Reverse**
human NCAM	ACCCCATTCCCTCCATCAC	GTGCCCATCCAGAGTCTCTTG
mouse NCAM	CAGGAGTCCTTGGAATTCATCC	TGGAGAAGACGGTGTGTCTGC
human RPL19	GATGCCGGAAAAACACCTTG	TGGCTGTACCCTTCCGCTT
siRNAs	**Sense**	**Anti-sense**
mNCAM1	GAACUGCAGUUUCCCUGCAGGUAGA	UCUACCUGCAGGGAAACUGCAGUUC
mNCAM2	CCGAAUACGUCUGCAUCGCAGAGAA	UUCUCUGCGAUGCAGACGUCUUCGG
mNCAM3	CCAUCAGACACUAUCUGGUCAAGUA	UACUUGACCAGAUAGUGUCUGAUGG
hNCAM1	UUCACUGCAGUACAGUUGUAGUUCC	GGAACUACAACUGUACUGCAGUGAA
hNCAM2	UACCUGACCAGAUAGUGUCUGAUGG	CCAUCAGACACUAUCUGGUCAGGUA
hNCAM3	UUGACCGCAAUGCACAUGAACAGGC	GCCUGUUCAUGUGCAUUGCGGUCAA
shRNA	**Sense**	**Anti-sense**
shEcad	GATCCCCATCTGAAAGCGGCTGATACTTCAAGAGAGTATCAGCCGCTTTCAGATTTTTTGGAAA	AGCTTTTCCAAAAAATCTGAAAGCGGCTGATATCTCTTGAAGTATCAGCCGCTTTCAGATGGG
shmNCAM control	GATCCCCGTACAAGGCTGAGTGGAAGTTCAAGAGACTTCCACTCAGCCTTGTACTTTTTGGAAA	AGCTTTTCCAAAAAGTACAAGGCTGAGTGGAAGTCTCTTGAACTTCCACTCAGCCTTGTACGGG