

LEGENDS FOR SUPPLEMENTARY FIGURES AND TABLES

Supplementary figure S1. TGF- β -induced EMT involves stepwise transcriptome reprogramming. **(A)** Immunofluorescence microscopy for changes in the localization and expression levels of marker proteins during different time points (untreated (d0), day1 (d1), day 4 (d4) and day 7 (d7)) of TGF- β induced EMT). Staining was performed with antibodies against the epithelial markers E-cadherin and ZO-1, the mesenchymal markers N-cadherin and Fibronectin 1, with Phalloidin to visualize the actin cytoskeleton and against Paxillin to detect focal adhesion plaques. Scale bar, 100 μ m, 63X magnification. Treatment scheme is depicted above. **(B)** Phase contrast images of NMuMG cells pretreated with DMSO, Actinomycin D or Cycloheximide 30 min before TGF- β stimulation for 24 hours. Scale bar, 100 μ m, 20X magnification. **(C)** GO enrichment analysis was performed for genes upregulated during EMT shown in Fig. 1B with DAVID functional classification tool; the plot shows representative categories of the top 5 clusters. **(D)** Same as C for Fig. 1C downregulated genes. **(E-F)** RNA represented as normalized tag counts for hallmark epithelial (E) and mesenchymal (F) markers. **(G)** Similar analysis as shown in (Figure 1A), but limited to lincRNA differentially expressed compared to day 0. **(H-I)** Same analysis as shown in Figure 1B and C, but only for lincRNAs. **(J)** Normalized tag counts for a known EMT driving lincRNA (Malat1) during EMT time course. **(K)** mRNA levels for several genes identified as deregulated in the RNA-Seq analysis of TGF β -induced EMT in NMuMG cells and performed RT-qPCRs in NMuMG cultured under similar conditions but without exposure to TGF β were measured by qRT-PCRs relative to Ctcf and plotted on the y-axis. Mean and SEM is plotted from three independent biological replicates. * $p < 0.05$ Student's *t*-test.

Supplementary figure S2. Smad signaling but not JNK pathway is required for the onset of EMT. **(A)** Immunoblots for total JNK and Smad as well as their phosphorylated forms in human epithelial (MCF7) and mesenchymal (BT549) breast cancer cells. Lamin B serves as loading control. **(B)** Similar analysis as in Fig 1G was performed in non-invasive tumor

samples (n=14). **(C)** Bar plot showing the ratio between p-Smad2 and p-JNK in non-invasive (n=15) and invasive (n=34) tumor samples that was derived using a relative score assigned to p-JNK and p-Smad2 in each clinical sample based on the IHC staining intensity (score ranges from 0 to 3 where 0 is lowest and 3 is highest; however, to facilitate calculating the ratio, instead of 0 we have assigned a very small value (0.001)). y axis represents relative staining intensity. $p=0.06$ (Student's *t*-test). **(D)** Similar analysis as in (C) but plotted values are scores for p-Smad2 and p-JNK in non-invasive and invasive tumor samples. **(E)** Similar analysis as in (C) but plotted values are scores for p-JNK in low and high grade non-invasive and invasive tumor samples **(F)** Representative immunoblots (n=2) showing JNK and phosphorylation state of JNK in NMuMG cells depleted with Smad4 for four days and induced with TGF- β for 24 hours. Actin serves as loading control. The right panel depicts the quantification of immunoblots showing a decrease in JNK signaling upon Smad4 knockdown during TGF- β -induced EMT in NMuMG cells. **(G)** Similar analysis as in (F), but instead of Smad4 depletion, cells were pretreated with PI3-Kinase inhibitor and Akt inhibitor. These results show increased JNK activity upon Akt/PI3 Kinase inhibition. **(H)** Representative immunoblots (n=2) showing JNK and phosphorylation state of JNK in NMuMG cells treated with ERK inhibitor, PI3-Kinase inhibitor and Akt inhibitor for 24 hours in the absence of TGF- β . Actin serves as loading control. This analysis shows that Akt inhibition results in the induction of JNK signaling in epithelial cells. **(I)** Immunofluorescence microscopy in Smad4 depleted cells for changes in the localization and expression levels of depicted marker proteins after 1 day of TGF- β -induced EMT. Scale bar, 100 μ m, 40X magnification. **(J)** NMuMG cells pretreated for 30 minutes with DMSO or JNK inhibitor were stimulated with TGF- β for 1 day and immunofluorescence assay was performed for marker proteins. Scale bar, 100 μ m, 40X magnification. * $p<0.05$ Student's *t*-test.

Supplementary figure S3. Alternate ways of interfering with JNK signaling validate the significance of JNK pathway for mesenchymal fate. **(A)** NMuMG cells treated for four or seven days with TGF- β and DMSO or JNKi were stained for the phosphorylated form of the

JNK target c-Jun (p c-Jun). Scale bar 100µm, 63X magnification. **(B)** HMEC cells treated for four days with TGF-β in the presence of DMSO or JNKi were stained for the phosphorylated form of the JNK target ATF4 (pATF4). Scale bar 100µm, 63X magnification. **(C)** mRNA levels of JNK1 and JNK2 were measured by qRT-PCR relative to Ctcf after seven days of JNK1 and JNK2 double knock down in NMuMG cells and plotted on the y-axis. Mean and SEM is plotted from three independent biological replicates. **(D)** Immunofluorescence analysis of cells treated in (C) for EMT marker proteins. Scale bar 100µm, 63X magnification. **(E)** Immunofluorescence analysis for EMT marker proteins of NMuMG cells treated with JNK-IN-8 or DMSO for seven days during TGF-β induced EMT. Scale bar 100µm, 63X magnification. **(F)** Immunofluorescence analysis in cells treated as in (E) for phospho c-Jun (p c-Jun). Scale bar 100µm, 63X magnification. **(G)** Representative immunoblots (n=2) showing phosphorylation state of JNK substrate c-JUN during TGF-β-induced EMT upon chemical inhibition of JNK activity in NMuMG cells. B- Actin serves as loading control. **(H)** Quantification of immunoblots in (G) and its graphical representation. Y axis represents intensity normalized to c-Jun level. Mean and SEM is plotted from two independent biological replicates. ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test.

Supplementary figure S4. Inhibition of JNK signaling significantly reverts gene expression changes acquired during TGF-β-induced EMT. **(A)** RNA levels represented by normalized tag counts derived from RNA-seq data for key epithelial genes that are upregulated upon JNKi at day 7 (d7). **(B)** GO enrichment analysis using DAVID functional classification tool for genes upregulated following JNK inhibition at day 7. Top enriched categories are represented with corresponding P-values. The numbers of genes that are downregulated during EMT and are reversed by JNK inhibitor are depicted in the second last and last columns respectively. **(C)** Same as in (A) for key mesenchymal genes that are upregulated during EMT and downregulated upon JNKi at day 7 (d7). **(D)** Same as in (B) for genes that are upregulated during EMT and downregulated upon JNKi at day 7 (d7). **(E-F)** Venn diagrams same as Figure 2B and C, but for lincRNAs. **(G)** RNA levels (normalized tag

counts) for genes depicted in Fig. 2E and F. **(H)** mRNA levels for genes depicted in Fig. 2E and F were measured by qRT-PCR relative to Ctcf after seven days of TGF- β induced EMT with JNKi or DMSO treatment starting at later time points, i.e. 1 day or 3 days after initial TGF- β induction. Mean and SEM is plotted from three independent biological replicates. **(I)** Immunofluorescence for EMT marker proteins of cells treated as in (H). Scale bar 100 μ m, 63X magnifications. **(J)** Same as in Fig. 2E and F for a control locus (Gata4 promoter) that is not changing in RNA levels during EMT.

Supplementary figure S5. Regulatory factors employed by JNK signaling regulate mesenchymal fate. **(A-B)** GO term analysis of genes upregulated upon JNKi and downregulated during normal EMT (A) as well as genes downregulated upon JNKi and upregulated during normal EMT (B). GO-term enrichment analysis was performed as in Supplementary Figure 4B. **(C)** mRNA levels for factors depicted in Fig. 3A were measured by qRT-PCR relative to Ctcf in NMuMG cells treated for seven days with TGF- β and DMSO or JNKi. Mean and SEM is plotted from two independent biological replicates. **(D)** Same as in (C) for factors in Figure 3B. Mean and SEM is plotted from two independent biological replicates. * $p < 0.05$, Student's *t*-test.

Supplementary figure S6. JNK-induced factors partly implicated in EMT also critically regulate EMT. **(A)** mRNA levels for transcription factors were measured by qRT-PCR relative to Ctcf to access knock down efficiency in NMuMG cells used for experiments depicted in Fig. 3C. **(B)** mRNA levels for classical EMT markers in cells treated as in Fig 3C were measured by qRT-PCR relative to Ctcf and SEM is plotted from 3 independent biological replicates. **(C)** Representative bright field images and immunofluorescence microscopy analysis of changes in the localization and expression levels of marker proteins after four days (d4) of siRNA mediated depletion of three transcription factors (Etv6, Tcf12 and Hoxb9) using single siRNAs during TGF- β -induced EMT. Scale bar, 100 μ m, 40X magnification. **(D)** RT-qPCR for knock down efficiency of analyzed transcription factors in same cells as in (C).

(E) mRNA levels for classical EMT markers in cells treated as in (C) were measured by RT-qPCR relative to Ctcf and plotted on the y-axis. Mean and SEM is plotted from two independent biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test.

Supplementary figure S7. Additional validation of JNK induced factors by alternate siRNA pool. **(A)** Immunofluorescence analysis as shown in Fig. 3C but knock down of candidate factors was performed using SMART pool siRNAs instead of single siRNAs. **(B)** mRNA levels of EMT markers were measured by qRT-PCR relative to Ctcf for cells treated as in Fig S7A. Mean and SEM is plotted from two independent biological replicates. **(C-D)** Similar validation as in A and B was performed for JNK-induced factors partly implicated in EMT. * $p < 0.05$, Student's *t*-test.

Supplementary figure S8. Alternate interference with JNK signaling validates the transcriptional dependence of transcription factors on JNK. **(A)** ChIP assay using anti-ATF2 antibody following JNK inhibition in NMuMG cells undergoing EMT for four days was performed. Quantitative PCRs were performed for promoters of JNK-dependent transcription factors and enrichments are plotted on the y-axis as ratio of precipitated DNA (bound) to total input DNA and then further divided by the same obtained in the empty vector transfected cells. Similar analysis for control gene promoters such as Hoxb8 and Hprt showed no detectable enrichment signal. SEM is derived from independent biological replicates. **(B)** NMuMG cells treated for four days with TGF- β in the presence of DMSO or JNKi as in (A) were stained for the ATF2 localization. Zoomed nuclei in the right part were shown for visualization purpose. Scale bar 100 μ m, 63X magnification. **(C)** NMuMG cells were treated with DMSO or JNK inhibitor JNK-IN8 for seven days during TGF- β induced EMT. mRNA levels for transcription factors were measured by RT-qPCR relative to Ctcf and plotted on the y-axis. Mean and SEM is plotted from two independent biological replicates. **(D)** NMuMG cells were transfected with siRNA SMART pools for JNK1 and JNK2 or NTC and treated with TGF- β for 7 days. mRNA levels for transcription factors were measured by RT-qPCR relative

to Ctcf and plotted on the y-axis. Mean and SEM is plotted from three independent biological replicates. **(E)** Bright field images of epithelial NMuMG cells (d0), cells that have undergone TGF- β -induced EMT for 8 days (d8) and cells that have reverted to an epithelial state from d8 mesenchymal state after TGF- β withdrawal for 22 days (d8-22) (MET). Scale bar, 100 μ m, 20X magnification. **(F)** Immunoblot for total JNK and phospho-JNK (pJNK) levels in cells shown in (E). Lamin B serves as loading control. **(G)** mRNA levels for transcription factors in cells treated as in (Figure 3B) were measured by qRT-PCRs relative to Ctcf and plotted on the y-axis. Mean and SEM is plotted from three independent biological replicates. * $p < 0.05$, ** $p < 0.01$, Student's *t*-test.

Supplementary figure S9. Newly identified transcription factors PRKRIR, PCBP4 and TSC22D1 rescue TGF- β induced cell cycle arrest. Lineage trees tracked by live cell imaging on NMuMG cells under control, Non treated control (NTC) and siRNA-mediated depletion of factors (X cell death, ? Cell could not be tracked till the end).

Supplementary figure S10. siRNA-mediated efficient knockdown of transcription factors results in transcriptional and phenotypic remodeling without affecting cell-cycle or cell viability. **(A)** Scratch assay in MDA-MB-231 cells treated for four days with DMSO or TGF- β receptor blocker. Scale bar, 200 μ m; 20 X magnification. Mean and SEM is plotted from three independent biological replicates. **(B)** Representative immunoblots (n=3) showing JNK and phosphorylation state of JNK in MDA MB 231 treated similarly as (A). β -actin serves as loading control. On right quantification of immunoblots and its graphical representation showing decreased JNK activation upon Smad4 knock down during TGF- β -induced EMT in NMuMG. **(C)** mRNA levels of TSC22D1 was measured by RT-qPCRs relative to CTCF and plotted on the y-axis from two epithelial cell lines MCF7 and MDA MB 361 and two mesenchymal cell lines MDA MB 231 and BT549. Mean and SEM is plotted from two independent biological replicates. **(D)** Following transfection with control (NTC) or siRNAs against the four candidates (PRKRIR, NFIL3, PCBP4 and TSC22D1) for four days in MDA-

MB-231 cells, mRNA levels of the target genes were measured by RT-qPCRs relative to Ctcf and plotted on the y-axis. Mean and SEM is plotted from two independent biological replicates. **(E)** Same analysis as in (D) for cells transfected with single siRNAs. Mean and SEM is plotted from two independent biological replicates. **(F)** Same analysis as in Fig. 5E for cells transfected with single siRNAs. Mean and SEM is plotted from two independent biological replicates. **(G)** Cell cycle was monitored in MDA-MB-231 cells that were non-treated or transfected with siRNAs as in (D) by measuring BrdU incorporation (x-axis) and total DNA content determined by 7-AAD (y-axis). Percentage of cells in G0/G1-phase, S-phase and G2-M-phase are presented in the plots. **(H)** Representative histograms displaying number of cells (y-axis) with respective DNA content determined by 7-AAD (x-axis) for cell cycle analysis described in (D). **(I)** Bar plots showing percentage for cell populations depicted in (G). Mean and SEM is plotted from three independent biological replicates. **(J)** Induction of apoptosis was determined in MDA-MB-231 cells treated like described in (G) by annexin V staining. Bar plots show percentage for cell populations positive for annexin V only (early apoptotic), for annexin V and PI DNA counterstain (late apoptotic), only for PI (necrotic) or negative for both stainings (non-apoptotic). Mean and SEM is plotted from three independent biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test.

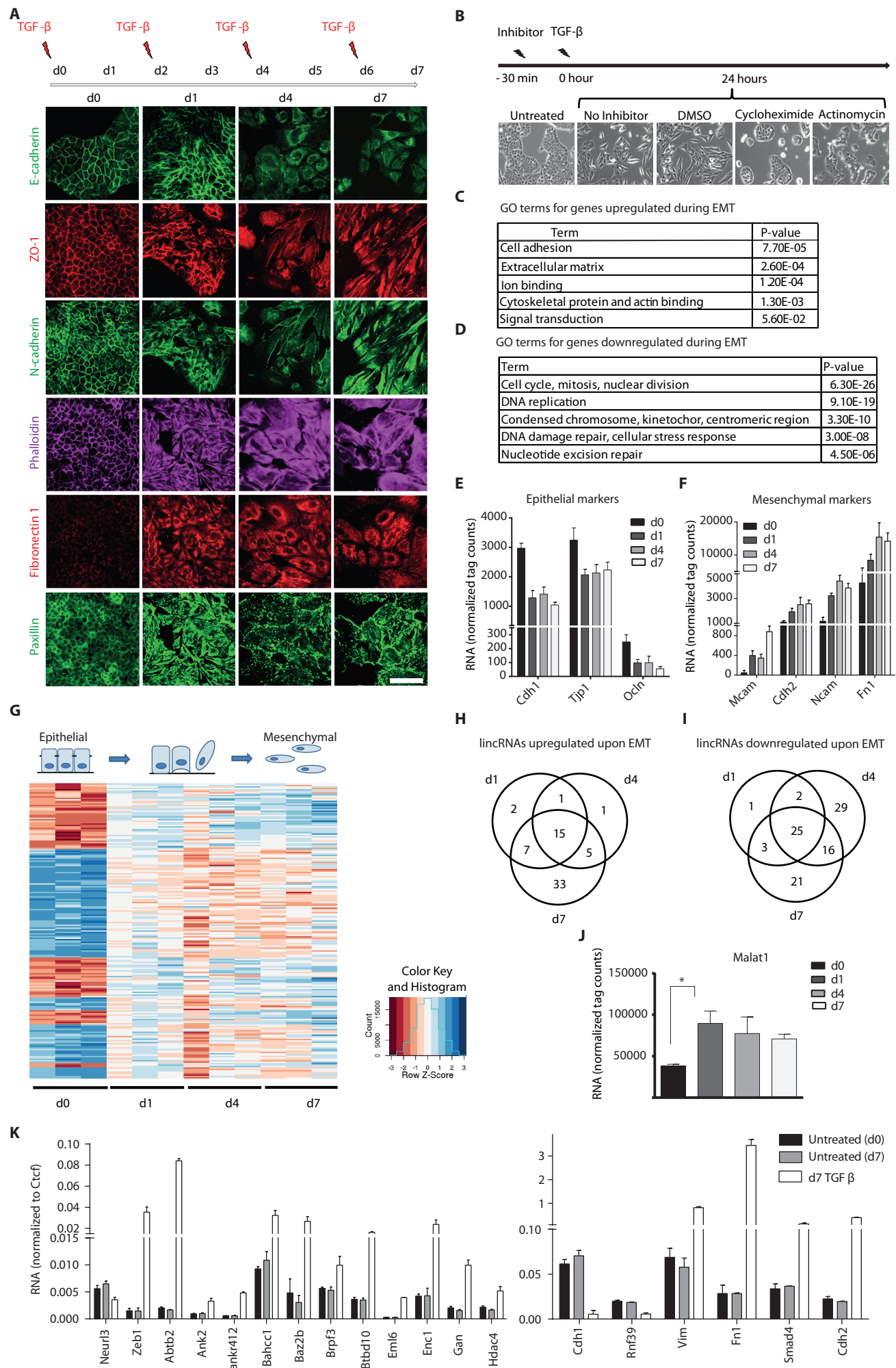
Supplementary figure S11. Expression of TSC22D1-target genes is downregulated upon TSC22D1 knockdown in MDA MB 231 cells. **(A)** RNA levels of TSC22D1 and its target genes is represented by normalized tag counts (y-axis) derived using RNA-seq data from TSC22D1 depleted MDA MB 231 cells. **(B)** Similar analysis as in (Figure 7I) but with respect to Satb2 staining. Error bars represent SEM from independent biological replicates. ** $p < 0.01$, Student's *t*-test.

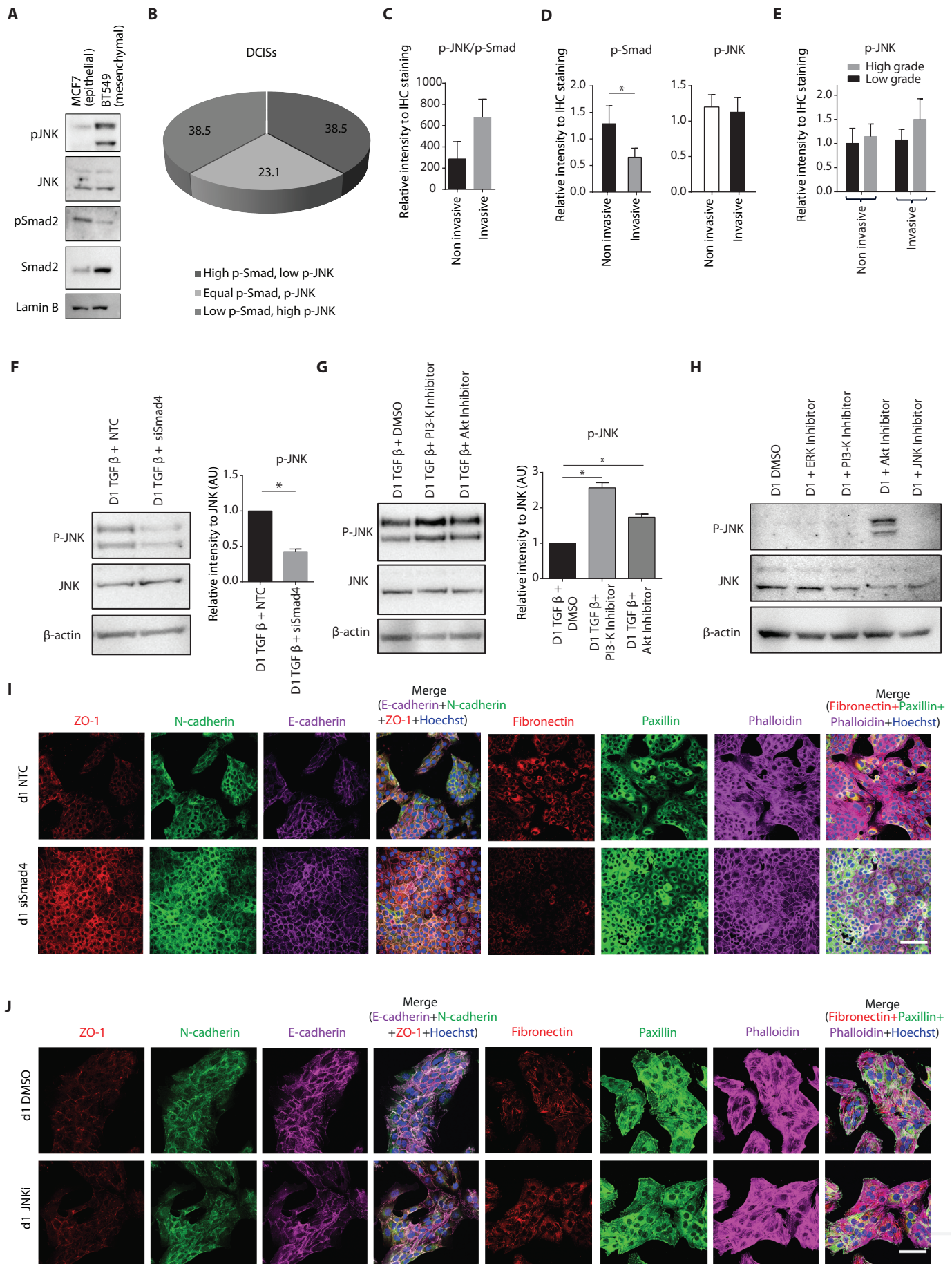
Supplementary Table S1. Detailed description of clinical samples (age, sex, tumor size, grades, lymph node migration status, ER PR HER status etc.) used in this study for immunohistochemistry.

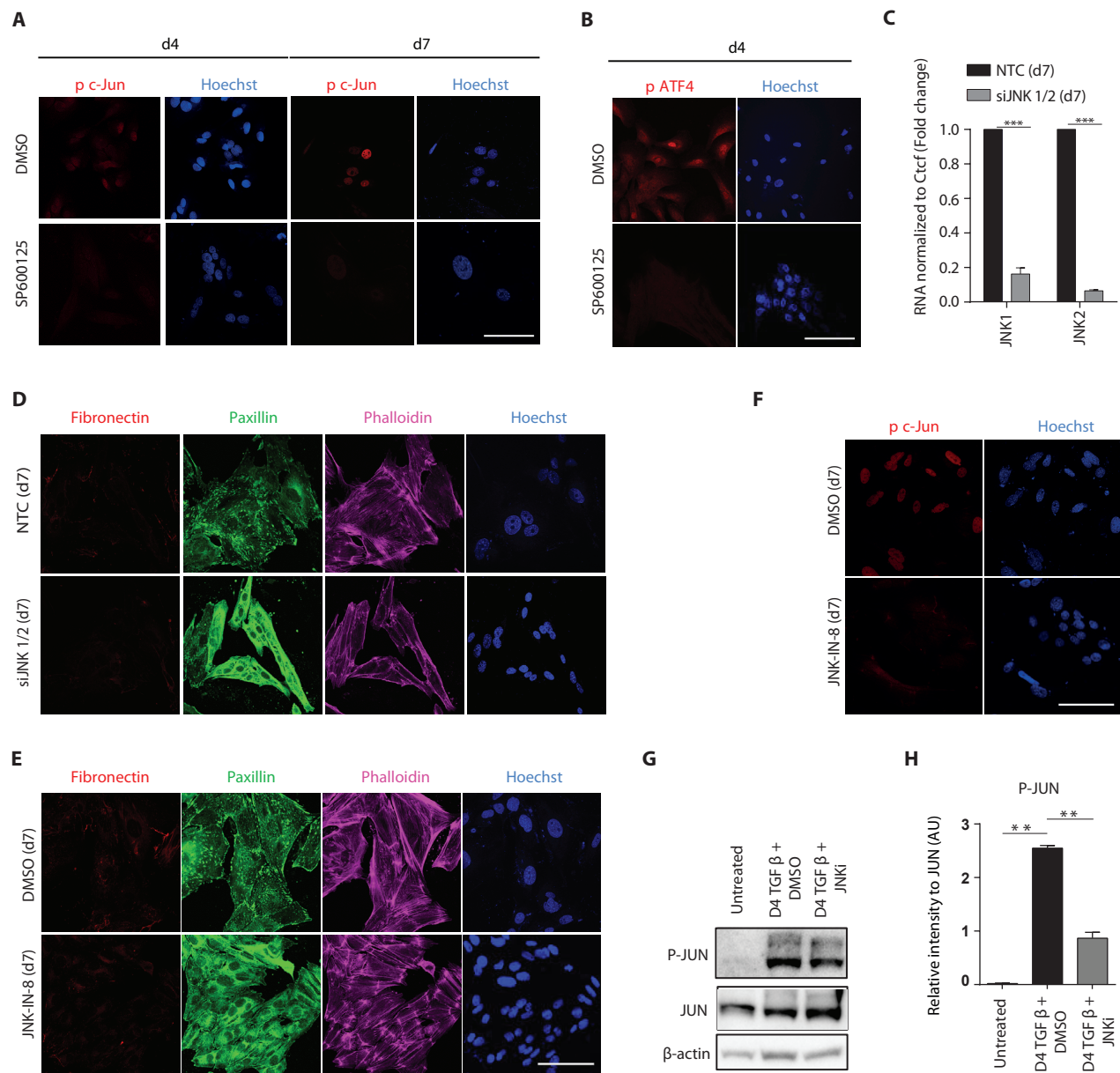
Supplementary Table S2. A number of genes differentially expressed upon depletion of novel transcription factors in mesenchymal breast cancer cells line (MDA-MB-231) are previously implicated in cancer biology in similar contexts.

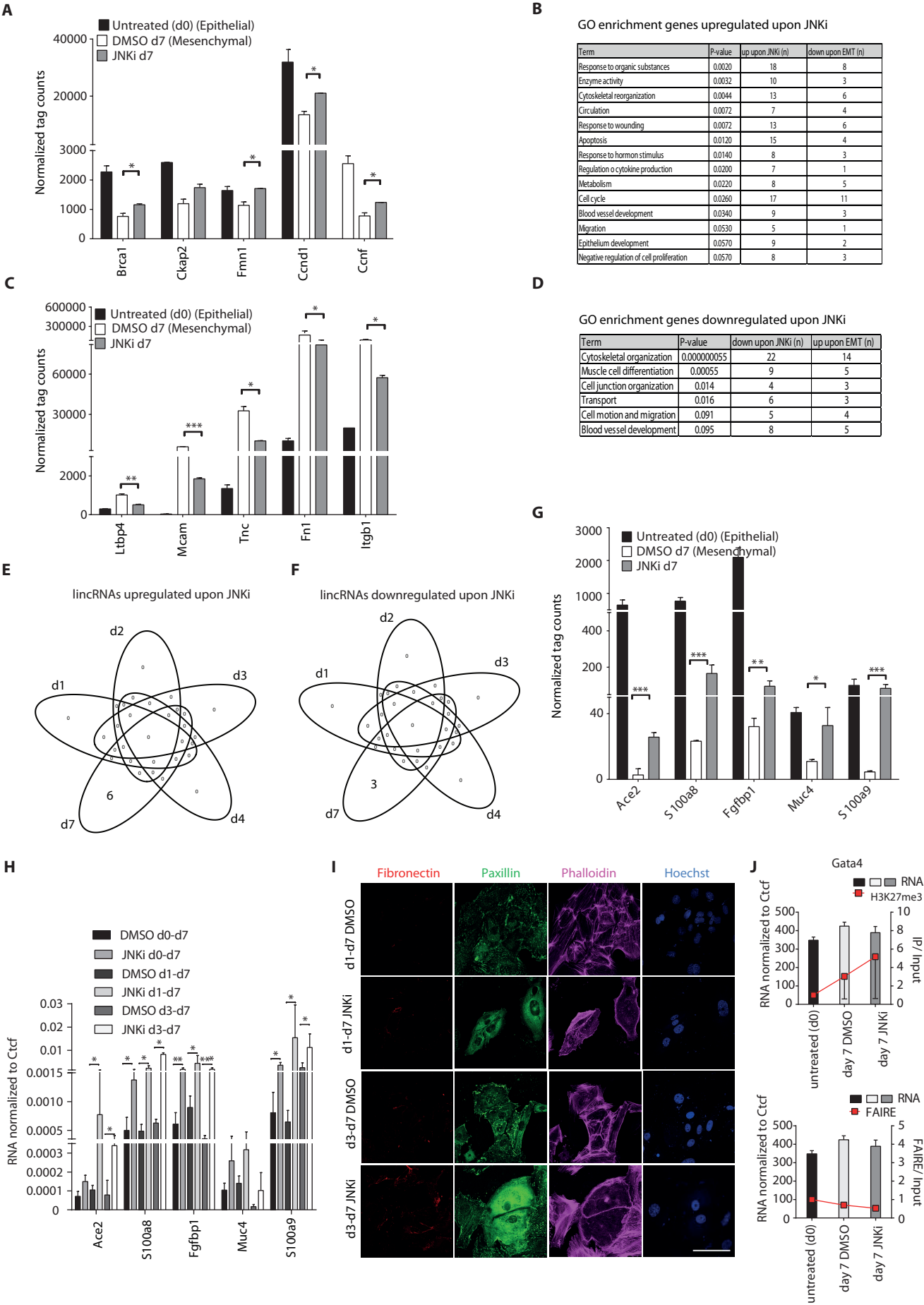
Supplementary Table S3. The table contains details of all siRNAs employed in this study.

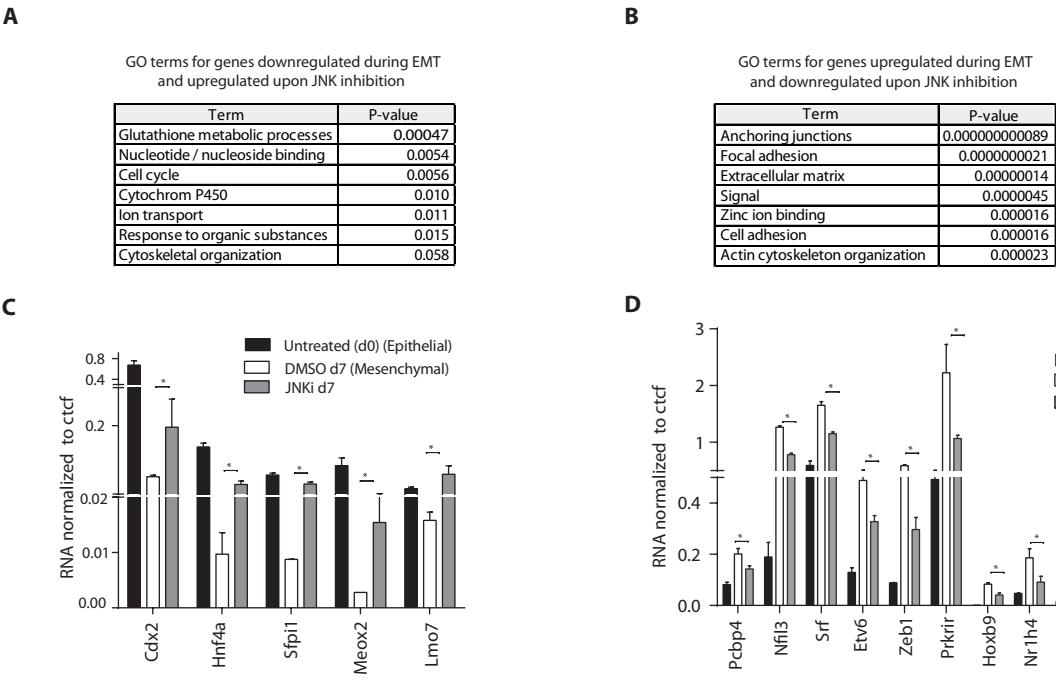
Supplementary Table S4. This table provides details of all primers used in this study.

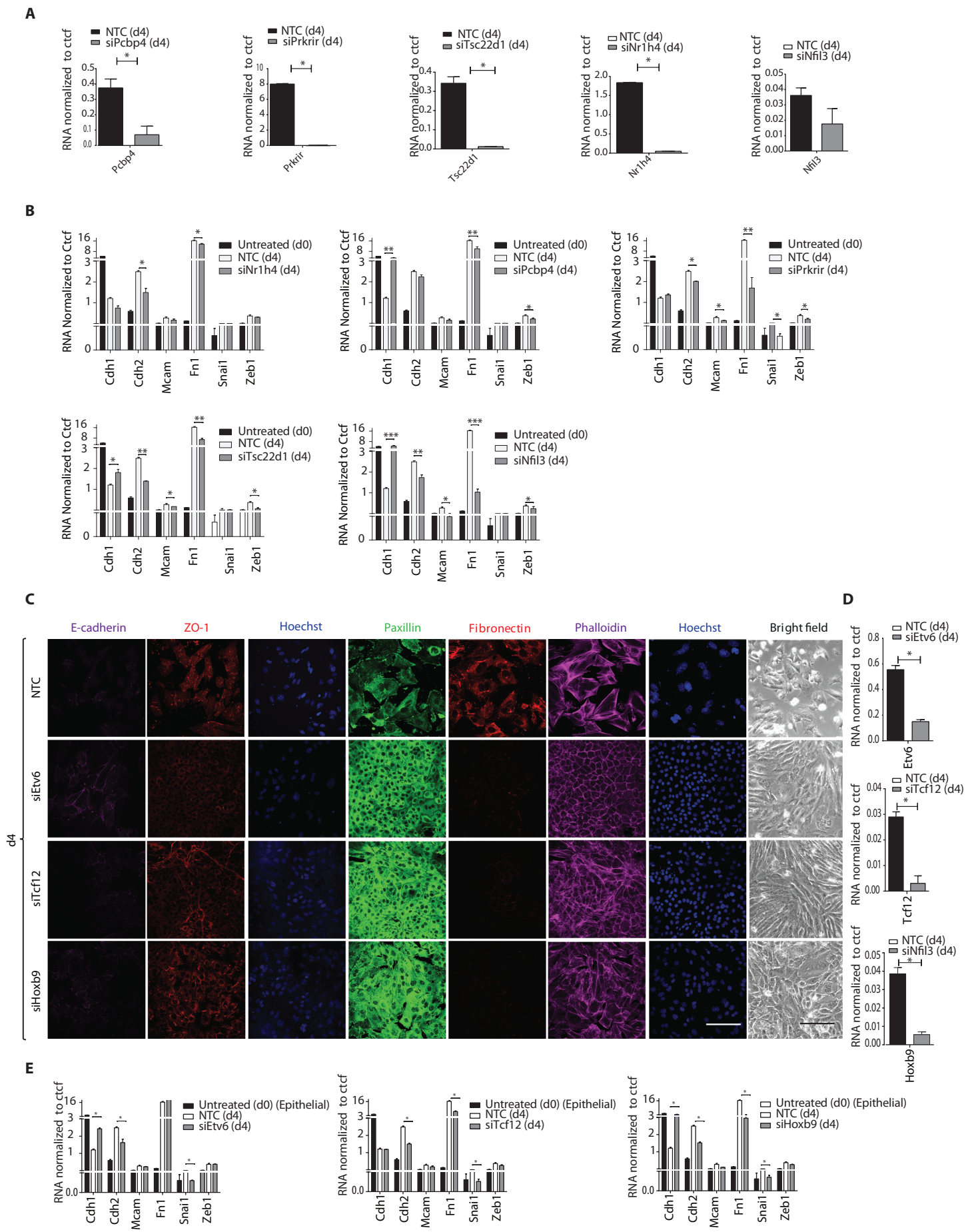


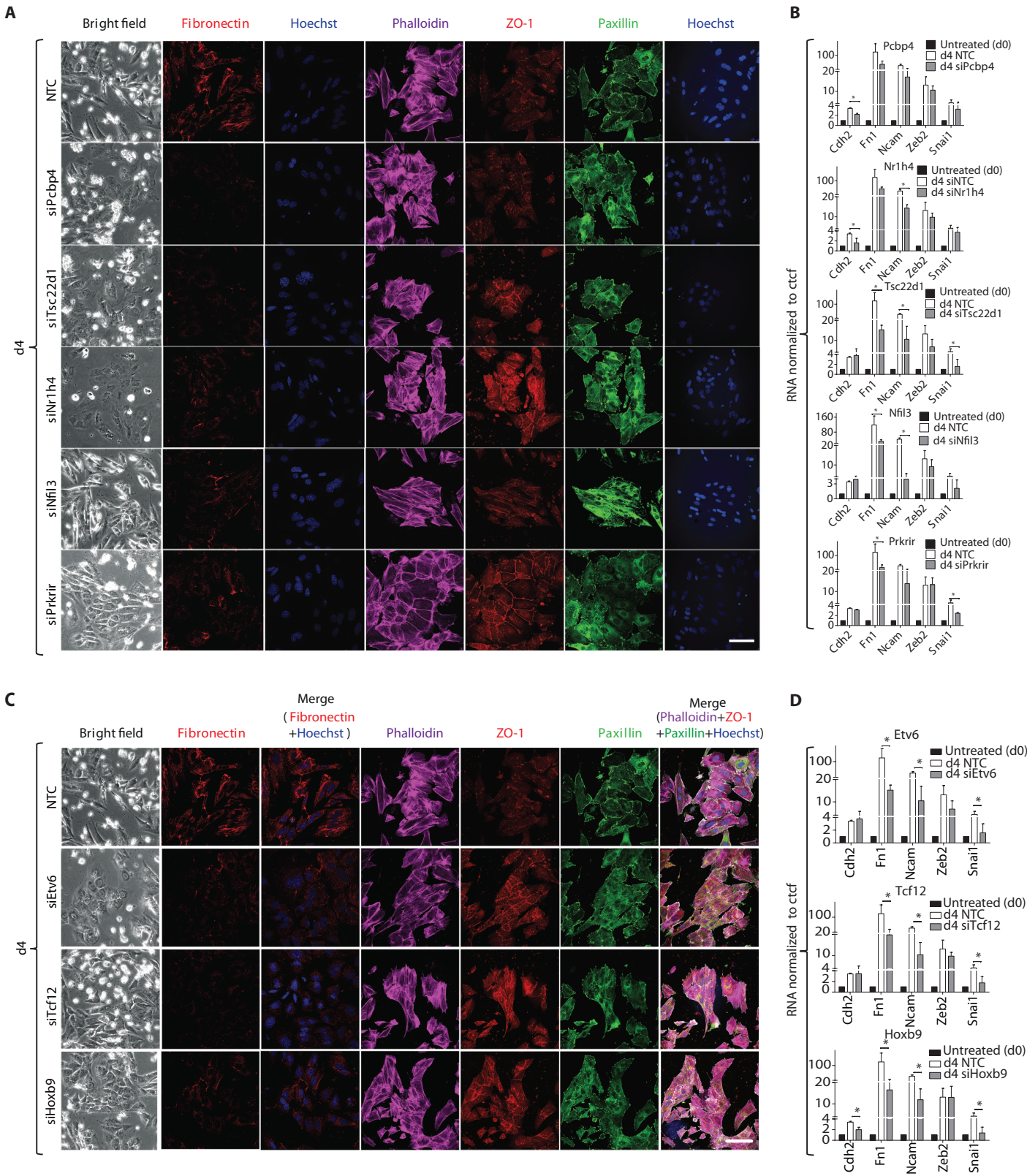


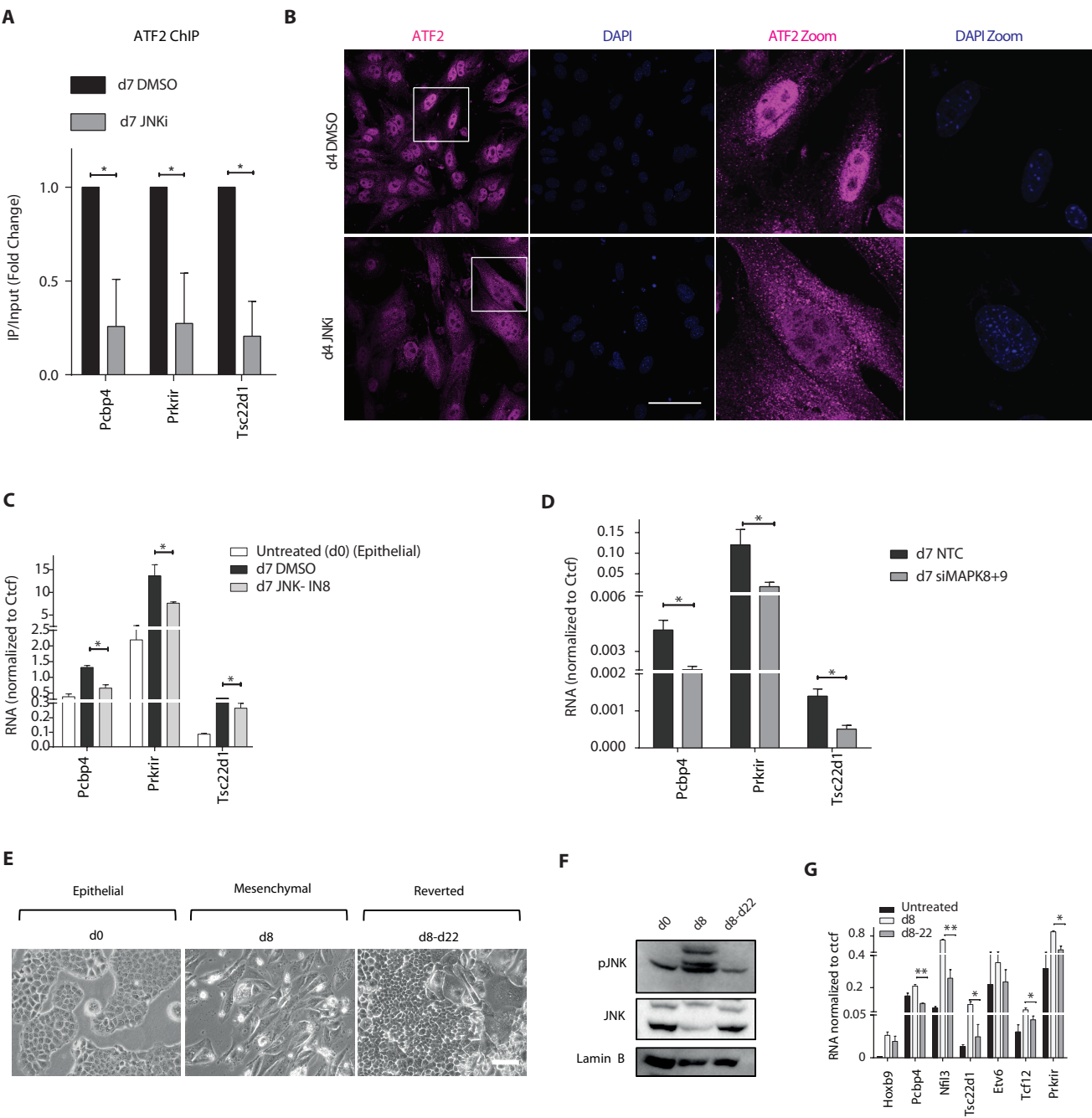




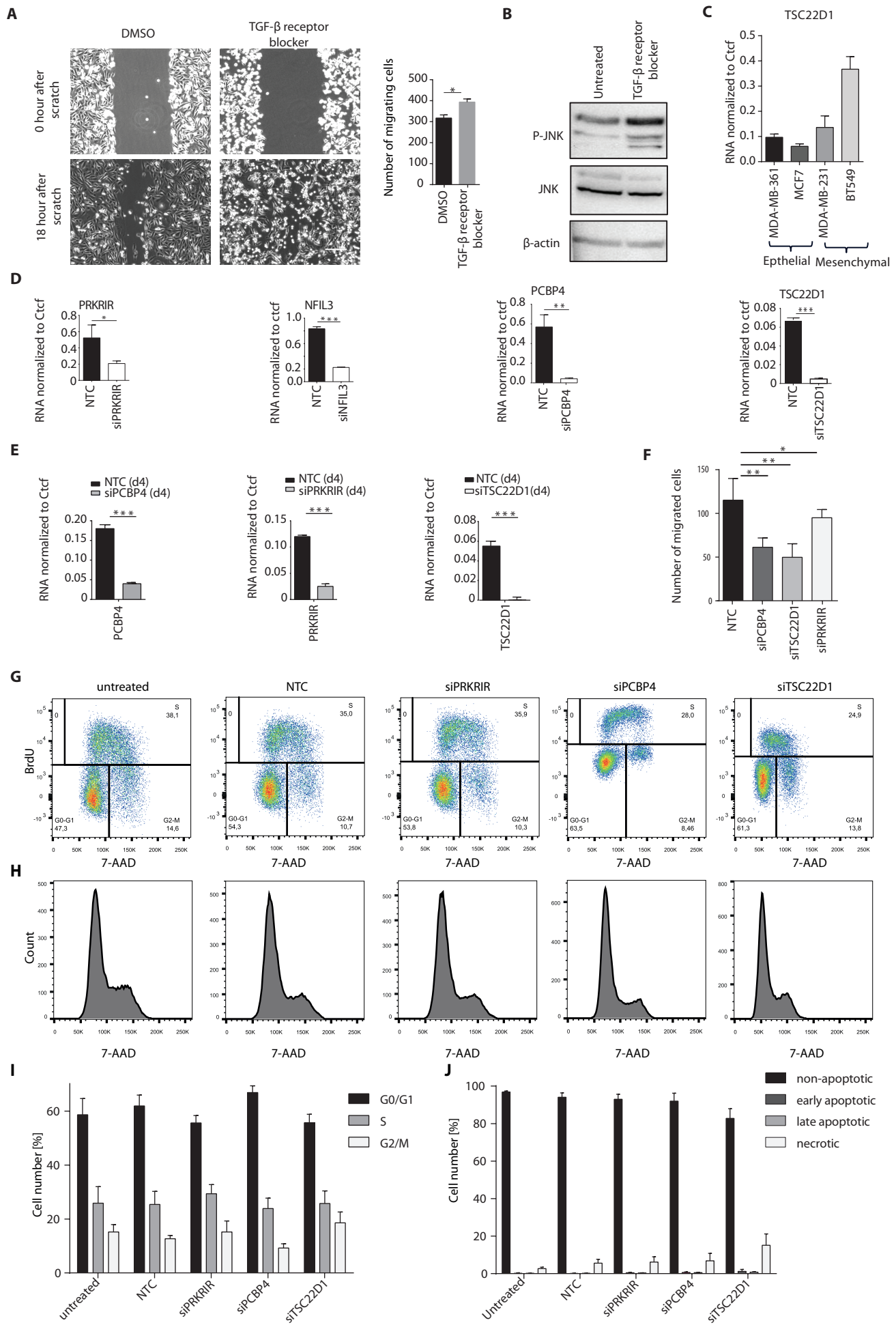


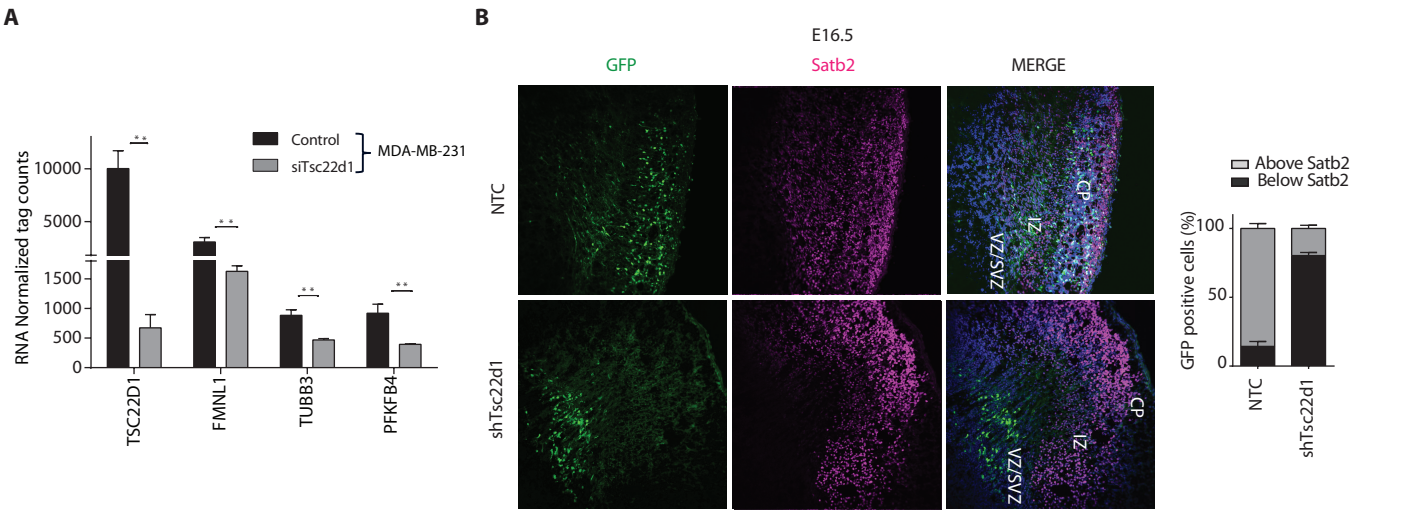












Supplementary Table S1. Detailed description of clinical samples (age, sex, tumor size, grades, lymph node migration status, ER PR HER status etc.) used in this study for immunohistochemistry.

Histo-Nr.	p-JNK	p-SMAD2	age	histology	Grade (G)	size (mm)	T	N	ER	PR	HER2
980155	0	0	60	ductulo-lobular	2	16	1c	0	+	+	+
980805	3	0	48	ductal	2	16	1c	0	+	+	+
971606	1	0	64	ductal	2		2	0	+	+	+
980809	2	0	50	ductulo-lobular	2	15	2	0	+	+	-
990994	2	1	73	ductal	2	16	2	0	+	+	-
980316	1	0	50	ductal	3	35	4	0	-	-	-
970901	3	0	63	ductal	2	25	2	0	+	+	-
970068	3	1	68	tubular	1	7	1b	0	+	+	-
970068	2	1	68	tubular	1	7	1b	0	+	+	-
980873	3	0	72	tubular	1	15	1c	0	+	+	-
960847	1	0	80	tubular	1	20	1c	0	+	+	-
960847	1	0	80	tubular	1	20	1c	0	+	+	-
20522	3	1		ductal	2	35	2	0	-	+	-
851004	0	1	43	ductal	2	10	1b	0	+	+	-
851940	1	0	46	ductal	2	10	1b	0			
860369	0	0	39	ductal	2	15	1c	0	-	+	-
861947	0	2	66	ductal	2	19	1c	0	+	-	-
871140	2	0	39	ductal	2	25	2	0	+	+	-
871240	0	3	61	ductal	2	26	2	0	-		
871840	0	0	39	ductal	2	25	2	0	+	+	-
881424	0	3	83	tubulär	1	17	1c	0	+	+	
901882	0	2	47	ductal	2	15	1c	0	-	-	+
910969	0	1	70	ductal	1	10	1b	0	+	+	-
930593	3	3	69	ductal	3	15	1c	0	-	-	-
931486	1	1	61	ductal	3	18	1c	0	+	+	-
940414	1	0	70	ductal	2	12	1c	0	+	+	-
940453	0	0	79	ductal	1	24	2	0	+	+	-
940880	2	1	56	ductal	3	28	2	0	-	-	-
941306	0	0	47	lobular	2	23	2	0	+	+	-
950460	0	0	78	ductal	2	40	2	0	+	+	-
970813	0	0	67	ductal	2	10	1b	0	+	+	-
971606	2	0	64	ductal	2	20	1c	0	+	+	-
980316	2	0	49	ductal	3	35	2	0	+	+	-
981842	0	1	56	ductal	3						
20639	0	0		DCIS			is				
20772	2	3		DCIS	low-grade	22	is		+	+	+
20785	2	1		DCIS	high-grade	15	is		+	+	-
20945	0	0		DCIS	low-grade	11	is				
21074	2	1		DCIS	high-grade	44	is		+	+	-
21305	2	0		DCIS							
11241	1	3	59	DCIS	high-grade	25	is		-	-	+
10948	1	0		DCIS	high-grade	23	is				
11241	1	2		DCIS	high-grade	25	is		-	-	+
20421	1	3		DCIS	low-grade	12	is		+	-	-
10254	1	3	71	DCIS	high-grade	16	is		+	+	-
11234	1	1	45	DCIS	Low-grade	40	is		+	+	-
31388	1	0	46	DCIS	low-grade	5	is		+	+	-
30826	0	0	67	DCIS	high-grade	9	is		-	-	-

Supplementary Table S2. Genes differentially regulated upon PRKRIR knockdown in MDA-MB-231 cell line,

id	log2FoldChange	Reference
IL8	-1.951702107	1: Fernando RI, Castillo MD, Litzinger M, Hamilton DH, Palena C. IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells. Cancer Res. 2011 Aug 1;71(15):5296-306. doi: 10.1158/0008-5472.CAN-11-0156. Epub 2011 Jun 8. PubMed PMID: 21653678; PubMed Central PMCID: PMC3148346.
CSF3	-1.908163695	Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, Yu L, Ross J, Korsisaari N, Cao T, Bou-Reslan H, Kallop D, Weimer R, Ludlam MJ, Kaminker JS, Modrusan Z, van Bruggen N, Peale FV, Carano R, Meng YG, Ferrara N. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. Proc Natl Acad Sci U S A. 2010 Dec 14;107(50):21248-55. doi: 10.1073/pnas.1015855107. Epub 2010 Nov 16. PubMed PMID: 21081700; PubMed Central PMCID: PMC3003076.
FRS3	-1.784673809	Valencia T, Joseph A, Kachroo N, Darby S, Meakin S, Gnanapragasam VJ. Role and expression of FRS2 and FRS3 in prostate cancer. BMC Cancer. 2011 Nov 11;11:484. doi: 10.1186/1471-2407-11-484. PubMed PMID: 22078327; PubMed Central PMCID: PMC3231952.
RANBP3	-1.028845602	Dai F, Shen T, Li Z, Lin X, Feng XH. PPM1A dephosphorylates RanBP3 to enable efficient nuclear export of Smad2 and Smad3. EMBO Rep. 2011 Oct 28;12(11):1175-81. doi: 10.1038/embor.2011.174. PubMed PMID: 21960005; PubMed Central PMCID: PMC3207100.
FLOT2	-1.022561755	Berger T, Ueda T, Arpaia E, Chio II, Shirdel EA, Jurisica I, Hamada K, You-Ten A, Haight J, Wakeham A, Cheung CC, Mak TW. Flotillin-2 deficiency leads to reduced lung metastases in a mouse breast cancer model. Oncogene. 2013 Oct 10;32(41):4989-94. doi: 10.1038/onc.2012.499. Epub 2012 Nov 12. PubMed PMID: 23146906.
TWF1	-1.012274777	Bockhorn J, Dalton R, Nwachukwu C, Huang S, Prat A, Yee K, Chang YF, Huo D, Wen Y, Swanson KE, Qiu T, Lu J, Park SY, Dolan ME, Perou CM, Olopade OI, Clarke MF, Greene GL, Liu H. MicroRNA-30c inhibits human breast tumour chemotherapy resistance by regulating TWF1 and IL-11. Nat Commun. 2013;4:1393. doi: 10.1038/ncomms2393. PubMed PMID: 23340433; PubMed Central PMCID: PMC3723106.

ARNTL2	-0.990786259	Mazzocchi G, Pazienza V, Panza A, Valvano MR, Benegiamo G, Vinciguerra M, Andriulli A, Piepoli A. ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer. J Cancer Res Clin Oncol. 2012 Mar;138(3):501-11. doi: 10.1007/s00432-011-1126-6. Epub 2011 Dec 24. PubMed PMID: 22198637.
CXCL2	-0.963136522	Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, Norton L, Brogi E, Massagué J. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2012 Jul 6;150(1):165-78. doi: 10.1016/j.cell.2012.04.042. PubMed PMID: 22770218; PubMed Central PMCID: PMC3528019.
HTRA1	-0.935380065	Esposito V, Campioni M, De Luca A, Spugnini EP, Baldi F, Cassandro R, Mancini A, Vincenzi B, Groeger A, Caputi M, Baldi A. Analysis of HtrA1 serine protease expression in human lung cancer. Anticancer Res. 2006 Sep-Oct;26(5A):3455-9. PubMed PMID: 17094466. ; Mullany SA, Moslemi-Kebria M, Rattan R, Khurana A, Clayton A, Ota T, Mariani A, Podratz KC, Chien J, Shridhar V. Expression and functional significance of HtrA1 loss in endometrial cancer. Clin Cancer Res. 2011 Feb 1;17(3):427-36. doi: 10.1158/1078-0432.CCR-09-3069. Epub 2010 Nov 23. PubMed PMID: 21098697; PubMed Central PMCID: PMC3057564.
GPR116	-0.922929776	Tang X, Jin R, Qu G, Wang X, Li Z, Yuan Z, Zhao C, Siwko S, Shi T, Wang P, Xiao J, Liu M, Luo J. GPR116, an adhesion G-protein-coupled receptor, promotes breast cancer metastasis via the Gαq-p63RhoGEF-Rho GTPase pathway. Cancer Res. 2013 Oct 15;73(20):6206-18. doi: 10.1158/0008-5472.CAN-13-1049. Epub 2013 Sep 5. PubMed PMID: 24008316.
FASN	-0.920360159	Agostini M, Almeida LY, Bastos DC, Ortega RM, Moreira FS, Seguin F, Zecchin KG, Raposo HF, Oliveira HC, Amoêdo ND, Salo T, Coletta RD, Graner E. The Fatty Acid synthase inhibitor orlistat reduces the growth and metastasis of orthotopic tongue oral squamous cell carcinomas. Mol Cancer Ther. 2014 Mar;13(3):585-95. doi: 10.1158/1535-7163.MCT-12-1136. Epub 2013 Dec 20. PubMed PMID: 24362464.
SRGN	-0.918073523	Li XJ, Ong CK, Cao Y, Xiang YQ, Shao JY, Ooi A, Peng LX, Lu WH, Zhang Z, Petillo D, Qin L, Bao YN, Zheng FJ, Chia CS, Iyer NG, Kang TB, Zeng YX, Soo KC, Trent JM, Teh BT, Qian CN. Serglycin is a theranostic target in nasopharyngeal carcinoma that promotes metastasis. Cancer Res. 2011 Apr

		15;71(8):3162-72. doi: 10.1158/0008-5472.CAN-10-3557. Epub 2011 Feb 2. PubMed PMID: 21289131.
NET1	-0.915848912	Gilcrease MZ, Kilpatrick SK, Woodward WA, Zhou X, Nicolas MM, Corley LJ, Fuller GN, Tucker SL, Diaz LK, Buchholz TA, Frost JA. Coexpression of alpha6beta4 integrin and guanine nucleotide exchange factor Net1 identifies node-positive breast cancer patients at high risk for distant metastasis. Cancer Epidemiol Biomarkers Prev. 2009 Jan;18(1):80-6. doi: 10.1158/1055-9965.EPI-08-0842. PubMed PMID: 19124484; PubMed Central PMCID: PMC2669542.
SDC1	-0.896291757	Péterfia B, Füle T, Baghy K, Szabadkai K, Fullár A, Dobos K, Zong F, Dobra K, Hollósi P, Jeney A, Paku S, Kovalszky I. Syndecan-1 enhances proliferation, migration and metastasis of HT-1080 cells in cooperation with syndecan-2. PLoS One. 2012;7(6):e39474. doi: 10.1371/journal.pone.0039474. Epub 2012 Jun 26. PubMed PMID: 22745764; PubMed Central PMCID: PMC3383727.
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Genes differentially regulated upon PCBP4 knockdown in MDA-MB-231 cell line

id	log2FoldChange	Reference
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EBPL	0.824555421	

CMTM4	0.853446723	Wang Y, Li J, Cui Y, Li T, Ng KM, Geng H, Li H, Shu XS, Li H, Liu W, Luo B, Zhang Q, Mok TS, Zheng W, Qiu X, Srivastava G, Yu J, Sung JJ, Chan AT, Ma D, Tao Q, Han W. CMTM3, located at the critical tumor suppressor locus 16q22.1, is silenced by CpG methylation in carcinomas and inhibits tumor cell growth through inducing apoptosis. <i>Cancer Res.</i> 2009 Jun 15;69(12):5194-201. doi: 10.1158/0008-5472.CAN-08-3694. Epub 2009 Jun 9. PubMed PMID: 19509237.
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Genes differentially regulated upon TSC22D1 knockdown in MDA-MB-231 cell line

id	log2FoldChange	Reference
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DBF4	-1.299525234	Nambiar, S., Mirmohammadsadegh, A., Hassan, M., Mota, R., Marini, A., Alaoui, A., ... & Hengge, U. R. (2007). Identification and functional characterization of ASK/Dbf4, a novel cell survival gene in cutaneous melanoma with prognostic relevance. <i>Carcinogenesis</i> , 28(12), 2501-2510.

ADAM10	-1.309098448	Gavert, N., Sheffer, M., Raveh, S., Spaderna, S., Shtutman, M., Brabletz, T., ... & Ben-Ze'ev, A. (2007). Expression of L1-CAM and ADAM10 in human colon cancer cells induces metastasis. <i>Cancer research</i> , 67(16), 7703-7712.
WNT9A	-1.309618635	Person, A. D., Garriock, R. J., Krieg, P. A., Runyan, R. B., & Klewer, S. E. (2005). Frzb modulates Wnt-9a-mediated β -catenin signaling during avian atrioventricular cardiac cushion development. <i>Developmental biology</i> , 278(1), 35-48.
TNFRSF11A	-1.322120035	Palafox, M., Ferrer, I., Pellegrini, P., Vila, S., Hernandez-Ortega, S., Urruticoechea, A., ... & González-Suárez, E. (2012). RANK induces epithelial–mesenchymal transition and stemness in human mammary epithelial cells and promotes tumorigenesis and metastasis. <i>Cancer research</i> , 72(11), 2879-2888.
PKN3	-1.350450711	Unsal-Kacmaz, K., Ragunathan, S., Rosfjord, E., Dann, S., Upeslakis, E., Grillo, M., ... & Klippel, A. (2012). The interaction of PKN3 with RhoC promotes malignant growth. <i>Molecular oncology</i> , 6(3), 284-298.
PBK	-1.536166529	Ayllon, V., & O'connor, R. (2007). PBK/TOPK promotes tumour cell proliferation through p38 MAPK activity and regulation of the DNA damage response. <i>Oncogene</i> , 26(24), 3451-3461.
CMKLR1	-1.575483083	Yoshimura, T., & Oppenheim, J. J. (2011). Chemokine-like receptor 1 (CMKLR1) and chemokine (C–C motif) receptor-like 2 (CCRL2); Two multifunctional receptors with unusual properties. <i>Experimental cell research</i> , 317(5), 674-684.
NGFR	-1.685707784	Isohata, N., Aoyagi, K., Mabuchi, T., Daiko, H., Fukaya, M., Ohta, H., ... & Sasaki, H. (2009). Hedgehog and epithelial-mesenchymal transition signaling in normal and malignant epithelial cells of the esophagus. <i>International Journal of Cancer</i> , 125(5), 1212-1221.
EGR1	-1.929342421	Tarcic, G., Avraham, R., Pines, G., Amit, I., Shay, T., Lu, Y., ... & Yarden, Y. (2012). EGR1 and the ERK-ERF axis drive mammary cell migration in response to EGF. <i>The FASEB Journal</i> , 26(4), 1582-1592.
FOXM1	-2.002905673	Bao, B., Wang, Z., Ali, S., Kong, D., Banerjee, S., Ahmad, A., ... & Sarkar, F. H. (2011). Over-expression of FoxM1 leads to epithelial–mesenchymal transition and cancer stem cell phenotype in pancreatic cancer cells. <i>Journal of cellular biochemistry</i> , 112(9), 2296-2306.
S100A2	-2.214097398	Naz, S., Bashir, M., Ranganathan, P., Bodapati, P., Santosh, V., & Kondaiah, P. (2014). Protumorigenic actions of S100A2 involve regulation of PI3/Akt signaling and functional interaction with Smad3. <i>Carcinogenesis</i> , 35(1), 14-23.

CTBP1	-0.816191774	Zhang, X. L., Huang, C. X., Zhang, J., Inoue, A., Zeng, S. E., & Xiao, S. J. (2013). CtBP1 is involved in epithelial-mesenchymal transition and is a potential therapeutic target for hepatocellular carcinoma. <i>Oncology reports</i> , 30(2), 809-814.
CTNNB1	-0.80314676	Stewart, C. A., Wang, Y., Bonilla-Claudio, M., Martin, J. F., Gonzalez, G., Taketo, M. M., & Behringer, R. R. (2013). CTNNB1 in Mesenchyme Regulates Epithelial Cell Differentiation during Müllerian Duct and Postnatal Uterine Development. <i>Molecular Endocrinology</i> , 27(9), 1442-1454.

Supplementary Table S3. Sequences of siRNAs used in this study

Gene names	<i>SMART pools</i> siRNA sequences
Nr1h4	UGCCAGGAGUGCCGGCUAA GAUUUGUGCCGGACGGGAU GUGUAAAUCUAAACGGCUA GAAACUUCCUGCCGGACAU GCACAUUUUAAGAUCGGGA
Pcbp4	GGGAGAGACUGUAAAGCGA GGAUGCUGAUGCACGGGAA AAAUCAAGGAGAUCCGAGA AAGUUGAGAAUGAGCGCUA
Prkrir	ACAAAUGUUAGGUGGAUA GGGAUUGAAUAUGGAGUAU AUUUAAUGGUGGACACUA GCAUUAAGCAGGAACGAU
Etv6	UGAUGAGAGUCACGUGCUA GAUCGACAUUAAAGCGACA CCAAAGAGGAUUUCCGCUA CCGCACAAGCUUCGGAUUA
Nfil3	GGAAGUUGCAUCUCAGUCA CAAAGACGGUGGCUAUAAG AGAAAGACGCCAUGUAUUG GCUGAAGUCUUGCGAAGUA
Tsc22d1	GUAUAGUCGUUCUGCGCAU GCUCAGGAUCAACCGCAUA GUGUGGUAGCUAUCGACAA UCAGGUGGCUGGUCGGAAA
Smad4	GAGUGCAGUUGGAAUGUAA GCAAUUGAGAGUUUGGUAA UCAGUAUGCGUUUGACUUA CUAUCCAUGUGCUACGAAA
Tcf12	CUUGAAUGACAGUCGAUUA GCAGCAACUUCACGAGCAU ACACAAGUCUUCGCGCAAU CCAAGUUUCCUUCGGGCCA
Hoxb9	UCAUAAGUCACGAGAGCGA GUUAGAAGCUAGCUAUACU GGACAAUAAAAUUUGCGAA

Single siRNAs

PRKRIR	GGACAACUCAAAUUCAAUA
TSC22D1	GUGCAAGUGUGGUAGCUAU
PCBP4	CCGAGCCUCUCCCUAGGUA
Prkrir	AAGUUGAGAAUGAGCGCUA
Tsc22d1	GCUGAAGUCUUGCGAAGUA
Pcbp4	GGGAGAGACUGUAAAGCGA
Nr1h4	GAUUUGUGCCGGACGGGAU
Nfil3	GGAAGUUGCAUCUCAGUCA
ON-TARGETplus Non-targeting Control	UGGUUUACAUGUCGACUAA

Supplementary Table S4. Primers used in this study

Gene name	Species	Forward (F) / Reverse (R)	Primer Sequence
Mouse RT Primers			
Ctcf	Mouse	F	GCGCTATCATGATCCCAACTTTGT
		R	GCATGTCTTGCCATTGTGTTCC
Fn1	Mouse	F	AGCAGAGGCTTATATGGGACA
		R	TGACGTCACCCGGACTCT
Cdh2	Mouse	F	GGTGGAGGAGAAGAAGACCAGGA
		R	TGGCATCAGGCTCCACAGTATCT
Ncam	Mouse	F	AAGCACACAGAGCCCAACGAG
		R	AAGGCAGCATGTCCTCGACAGT
Zeb2	Mouse	F	CCAGAGGAAACAAGGATTTCAAG
		R	CGGAGTCTGTCATGTCATCTAGGC
Snai1	Mouse	F	TCTCTAGGCCCTGGCTGCTTC
		R	CAGCAAAAGCACGGTTGCAGT
Nr1h4	Mouse	F	AGATGGGGATGTTGGCTGAATGTA
		R	CGTGCTGCTTCACATTTTTCCTTA
Pcbp4	Mouse	F	GGGGACAATGTGGGACTGAGC
		R	ACTGTGGCTCAGCTAGCTCTCG
Nfil3	Mouse	F	ACCAGGGAGCAGAACCACGAT
		R	TTGTCCGGCACAGGGTAAATCT
Srf	Mouse	F	TGACAGCAGTGGGGAAACCAA
		R	GTGCTGGGTGCTGTCTGGATT
Etv6	Mouse	F	TGATCCCTTTCGCTGTGAGACAT
		R	GCGTGTATGAAATTCGTTCTGCT
Tcf12	Mouse	F	CATTTGCAAGATGCAATGTCCTTC
		R	GTCTAAGCGGTCTTCCATTCGAGA
Prkrir	Mouse	F	GACCTGGCCTTCTTCAGGTTCC
		R	TGTTTGGCACAAAGTCGATAATGTT
Tsc22d1	Mouse	F	GGGAGTAGCAGCAGCCTAGCAG
		R	GCTACCACACTTGCACCAGAGC
Zeb1	Mouse	F	GTGATCCAGCCAAACGGAAACC
		R	TCTTTTTGGGTGGCGTGGAGT
Cdh1	Mouse	F	CCCGGGACAATGTGTATTACTATGA
		R	GCAGCTGGCTCAAATCAAAGTCC
Ocln	Mouse	F	CATGTCCGTGAGGCCTTTTGA
		R	TGGTGCATAATGATTGGGTTTGA
Smad4	Mouse	F	GAGAACATTGGATGGACGACT
		R	CACAGACGGGCATAGATCAC
S100a9	Mouse	F	GACACCCTGACACCCTGAG
		R	TGAGGGCTTCATTTCTCTTCTC
Muc4	Mouse	F	GAGAGTTCCTGGCTGTGTC
		R	ATTTGGGATGTTCTGGTGCT

Fgfbp1	Mouse	F	CTGCACACTCACAGAAAGGTG
		R	CTGAGAACGCCTGAGTAGCC
Ace2	Mouse	F	TGAAAAAGTGGTGGGAGATGA
		R	AACAGAGATGCAGGGTCACA
S100a8	Mouse	F	TCCTTGCGATGGTGATAAAA
		R	GGCCAGAAGCTCTGCTACTC
MAPK8	Mouse	F	AACTGTTCCCCGATGTGCT
		R	ACAAATCTCTTGCCTGACTGG
MAPK9	Mouse	F	TGATGACTCCCTATGTGGTAACTC
		R	ACCCACTGACCAGATATCAACA

Mouse ChIP Primers

Muc4	Mouse	R	CACAGACGGGCATAGATCAC
		F	CCTCACTCCCCATGCTCATTATAG
		R	GGGAAGAGCAGCACAGAGATAAAGA
Fgfbp1	Mouse	F	TGGGGCTCTGATACCACATGTCT
		R	ACAGGCGACATCATGCTAATGAA
S100a8	Mouse	F	ACAGGTGTGGCTTCCTCAAA
		R	CAGGCAGAGACCTACTGGTGAT
S100a9	Mouse	F	CGCCTGCCACCCTCTGTATATTA
		R	CTCACTTCCCTTCCATCCAACCT
Ace2	Mouse	F	GCCACAGGTATATTGTGATGCTT
		R	CGCTTTATTCTAAACCTGGGCAGA

Human RT Primers

CDH1	Human	F	TCTTGGAGGAATTCTTGCTTTGC
		R	ACCGCTCTCCTCCGAAGAAACA
CLDN3	Human	F	AACCTGCATGGACTGTGAAACCT
		R	GGTGGTCAAGTATTGGCGGTCAC
CDH2	Human	F	GGCCGTCATCACAGTGACAGAT
		R	CCCTGTTCTCAGGAACCTCACCA
ZEB1	Human	F	CCAACAGACCAGACAGTGTTACCAG
		R	TCTTGCCCTTCCTTTCTGTGT
MCAM	Human	F	CCCCATTCTCAAGTCATCTGGTA
		R	GTCTGGGACGACTGAATGTGGAC
HOHB9	Human	F	GACAAAGAGAGGCCGGATCAAAC
		R	TCTGGTATTTGGTGTAGGGACAGC
PCBP4	Human	F	CCAAGATCAAGGAGATCCGAGAGA
		R	TGTGGAGTTGGGGAGCAGGTC
NFIL3	Human	F	ATGCAGCTGAGAAAAATGCAGACC
		R	CCACATTGCTACTGGCATCAAGAG

SRF	Human	F	CTGCCTACCAGCTTCACCCTCAT
		R	GTGCACTTGAATGGCCTGCAC
ETV6	Human	F	CCCTGCGCCACTACTACAACTAAA
		R	TCATGATTTTCATCTGGGGTTTTCA
TCF12	Human	F	GAACTTTAATCGGGGTGGTTGGAT
		R	GTTGTTGCTGGGGATTTCATCTTC
PRKRIR	Human	F	GAAACAAATGACAAAGTTTGAATGGAA
		R	CACAAAGGAATGTAAACAGCAACGA
TSC22D1	Human	F	CTGACGACACCCCTGGTGGAT
		R	CGATTTTGTGTCAATAGCTACCACAC
CTCF	Human	F	GGGCTTGAGAGCTGGGTTCTATT
		R	CTTCGACTGCATCACCTTCCATT
DUSP2	Human	F	ACTCCAGGGCTCCTGTCTACGA
		R	CCTGCAGGTCTGACGAGTGACTION
CSF3	Human	F	CCAGGAGAAGCTGGTGAGTGAGT
		R	AGGGGATGCCCAGAGAGTGTC
FRS3	Human	F	AATAGCCACCCCGCTGAGCTT
		R	GCTGGAGACAGTGTAGCCTAGAGCA
IL6	Human	F	CCAGCTATGAACTCCTTCTCCACAA
		R	GAATCTTCTCCTGGGGGTACTGG
ADAMTS15	Human	F	GAAATACCGATCCTGCAATCTGGA
		R	ACTGCTCCTCCCGGAAGCTCT
IFIT2	Human	F	GTGGCAGAAGAGGAAGATTTCTGA
		R	TTTTAGTTGCCGTAGGCTGCTCTC
ELF3	Human	F	CCAGATGTCATTGGAGGGTACAGA
		R	ATCCAGTCCAGAACCTGCGTCTT
EGR1	Human	F	CTACGAGCACCTGACCGCAGA
		R	AGTGGTTTGGCTGGGGTAACTG
MAP3K3	Human	F	TGTTGTCCCAGGACAGAAACCATA
		R	ACCTGTCTGGACACCCCAGAGT
BTN3A2	Human	F	CCAAGATGGTGACTTCTATGAAAAAGC
		R	CATCCTCATAACCCTTCACTTCGAC
DPP4	Human	F	CTTAGTGGCACGGCAACACATTG
		R	CCATCAAGGGTAAAATGAGGTTCTG
RECK	Human	F	TCTGTCAAGTGTCTTCGCTCTTG
		R	GCACATAATGGGCAACAAGCAC
FOXM1	Human	F	TTTAGTGGCGATCTGCGAGATTTT
		R	CCCCTCCTCAGCTAGCAGCACT
NGFR	Human	F	CCTCATCCCTGTCTATTGCTCCA
		R	GTTCTGCTTGCAGCTGTTCCAC
FAM123B	Human	F	CAGGAATAGGATCCTTACCACCTGA
		R	CTGCTTCTGCAGCCACATATCCT
LHX6	Human	F	CAAGGAGATCTTCTGCAAGATGGAC
		R	CAGTCGCTGGCGTAGATCTGTC
LAMA1	Human	F	GGATGACCTCCATTCTGACTTACACA

CMKLR1	Human	R	AACCTTCCTTACATGGGCACTGAC
		F	ACTTCTTGATGGGAGGCGTGAC
CXCL3	Human	R	GTTGTCTGCAGCTCTCCAATGTGA
		F	CCATGGTTCAGAAAATCATCGAAA
FRS3	Human	R	TCTGGTAAGGGCAGGGACCAC
		F	AATAGCCACCCCGCTGAGCTT
ELF3	Human	R	GCTGGAGACAGTGTAGCCTAGAGCA
		F	CCAGATGTCATTGGAGGGTACAGA
		R	ATCCAGTCCAGAACCTGCGTCTT