Antagonistic regulation of EMT by TIF1γ and Smad4 in mammary epithelial cells

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SUPPLEMENTARY INFORMATION

Methods

***Microarray Analysis*.** Transcriptomic microarray analysis was performed on HMEC-TR cells silenced for TIF1γ or Smad4 (si-control, si1-TIF1γ or si1-Smad4). The regulation of EMT by TGFβ1 involves several cellular responses; we consequently examined changes in gene expression at 24 hours after TGFβ1 treatment. Efficient TIF1γ and Smad4 silencing was checked by quantitative RT-PCR and Western Blotting (Fig S1). RNA quality was assessed using an Agilent Bioanalyzer (model 2100; Agilent Technologies). Before hybridization, RNA was amplified to obtain cRNA and was labeled using One-Cycle cDNA Synthesis Kit (Affymetrix) according to the manufacturer's instructions. The stained arrays were scanned on an Affymetrix GeneChip Scanner 3000-7G. The raw signal was extracted and normalized using GeneChip Operating Software version 1.4 (GCOS1.4). All quality controls were in the expected range indicated by Affymetrix. A given mRNA transcript was considered differentially expressed in a comparison of any two samples if the difference between expression levels had a p value of ≤ 0.05 in the Welch Anova parametric test using a multiple test correction (Benjamini and Hochberg false discovery rate). TIF1γ and Smad4 target genes were classified in three subgroups by GeneSpring filtering using a combination of three cut-offs as shown in Table S1. Genes whose expression was modified by TIF1γ or Smad4 knockdown after TGFβ1 treatment were identified from the list of TGFβ1-dependent genes based on the average ratios of si-TIF1γ+/ctrl+ (>1.5) and si-Smad4+/ctrl+ (>1.5) respectively (Table S1). Both siTIF1γ+/ctrl- and si-Smad4+/ctrl- ratios were calculated and are presented in comparison with the ctrl+/ctrl- ratio. We identified 1047 genes whose expression was exclusively modified by Smad4 inhibition (Smad4-dependent and TIF1γ-independent genes, SDTI), 457 genes whose expression was modified by the down-regulation of both proteins (Smad4-dependent and TIF1γ-dependent genes, SDTD) and 250 genes exclusively regulated by TIF1γ knockdown (Smad4-independent and TIF1γ-dependent genes, SITD). The three complete lists are shown in Supplementary data (Table S2).

***Immunoprecipitation and western blot analysis*.** TIF1γ and/or flag-Smad3 expression vectors were transfected, either alone or in combination, into HMEC-TR cells using FuGENE HD (Roche). The cells were then incubated for 48h and treated or not with TGFβ1 for 2h, and total cell lysates were collected. The proteins were immunoprecipitated with anti-Flag (Euromedex) or anti-TIF1γ (Bethyl) antibodies and Protein A/G–agarose (Fast Flow, Millipore), separated by SDS/PAGE (10%), electroblotted onto PVDF transfer membrane, stained with the specific primary antibodies anti-flag EL1B11 (Euromedex), anti-Smad4 (SantaCruz biotechnology), anti-TIF1γ (Euromedex) and horseradish-peroxidase-linked secondary antibodies, and then detected with ECL plus reagent (Roche).

***Luciferase assays*.** HMEC-TR cells were transfected or not with siRNAs in 24-well-plates, cultured in complete medium for 24h and then co-transfected with 150 ng of $(CAGA)_9$-MLP-Luc (Dennler *et al.*, 1998) or PAI-1 promoter (p800-Luc) luciferase constructs and 50 ng of the indicated construct (pSG5-TIF1γ or Smad4) expressing vectors. The pRL-TK vector (25 ng; Promega) was used as internal control for transfection efficiency. After transfection, the cells were cultured for an additional 24h in complete medium with or without TGFβ1. Transfected cells were then washed and collected. Luciferase activity was measured in equivalent amounts of each lysate using the dual luciferase kit (Promega). Luciferase firefly activity was normalized to the *Renilla* luciferase activity of the pRL-TK vector. Experiments were performed in triplicate and each set was repeated at least three times.

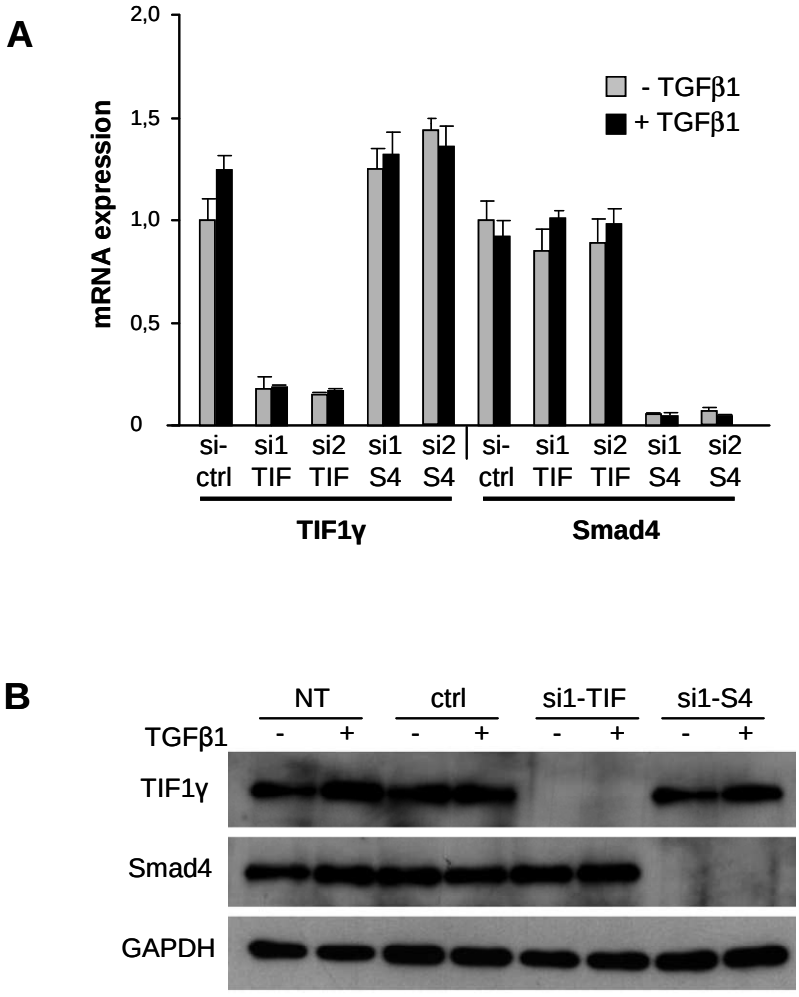

Fig S1: Validation of TIF1γ and Smad4 transient down regulation. HMEC-TR cells transfected with control siRNA (ctrl) or two siRNA sequences targeting human TIF1γ or Smad4 mRNA were treated with TGFβ for 24h. (**A**) Expression of TIF1γ and Smad4 was determined by quantitative PCR. Values were normalized to the amount of HPRT mRNA and expressed relative to the value obtained in control TGFβ1-untreated cells. The experiment shown is representative of five separate experiments performed in triplicate. Error bars represent s.d. (**B**) The levels of endogenous TIF1γ, Smad4 and GAPDH were determined by immunoblotting. ctrl, control; TIF, TIF1γ; S4, Smad4.

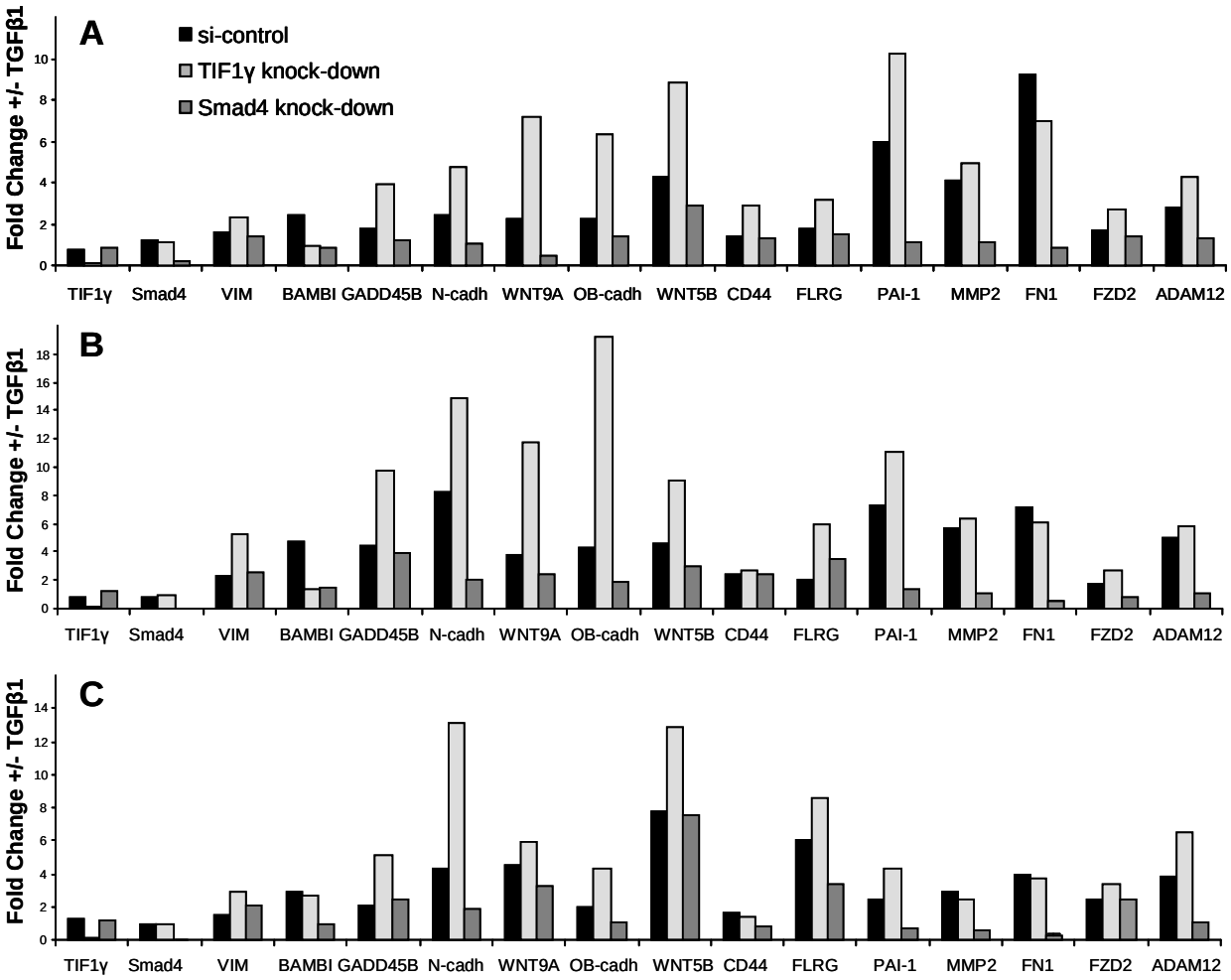

Fig S2: Validation of microarray results. (**A)** Results from microarray experiments. (**B)** The two independent RNA extractions performed for microarray experiments were used to quantify the expression of 16 selected genes by qRT-PCR. (**C)** Two additional and independent RNA extractions from HMEC-TR cells were used to quantify the expression of selected genes. All values were normalized to the amount of HPRT mRNA and expressed relative to the value obtained in control TGFβ1-untreated cells. For B and C, the data shown correspond to one of two independent experiments performed in triplicate, with comparable results. VIM, Vimentin; cadh, cadherin. ctrl, control; TIF, TIF1γ; S4, Smad4.

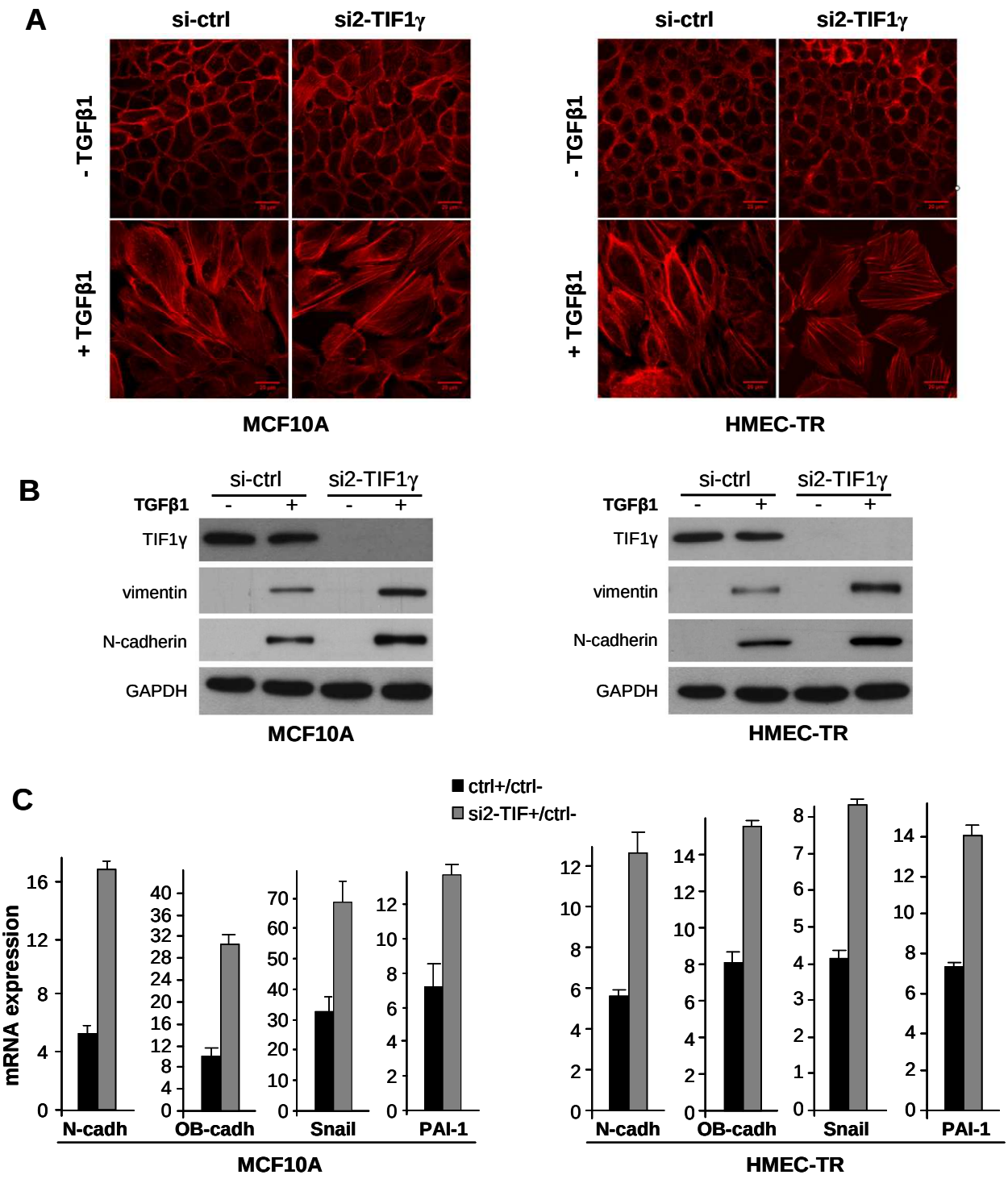

Fig S3: Inactivation of TIF1γ expression enhances TGFβ1-induced EMT. MCF10A and HMEC-TR cells transfected with control siRNA (ctrl) or siRNA#2 targeting human TIF1γ were treated with TGFβ for 96h. (**A**) Immunofluorescence confocal acquisitions showing actin subcellular localization detected by phalloidin. Bars = 20 µm. (**C**) Endogenous TIF1γ, Vimentin and N-cadherin levels were determined by immunoblotting. GAPDH served as a loading control. (**D**) MCF10A and HMEC-TR cells transfected with control siRNA (ctrl) or siRNA#2 targeting human TIF1γ were treated with TGFβ for 9h. Expression of N-Cadherin (N-cadh), OB-Cadherin (OB-cadh), Snail and PAI-1 was determined by quantitative RT-PCR. Values were normalized to the amount of HPRT mRNA and expressed relative to the value obtained in TGFβ1-untreated controls. Error bars represent s.d. The experiment shown is representative of at least two separate experiments performed in triplicate.

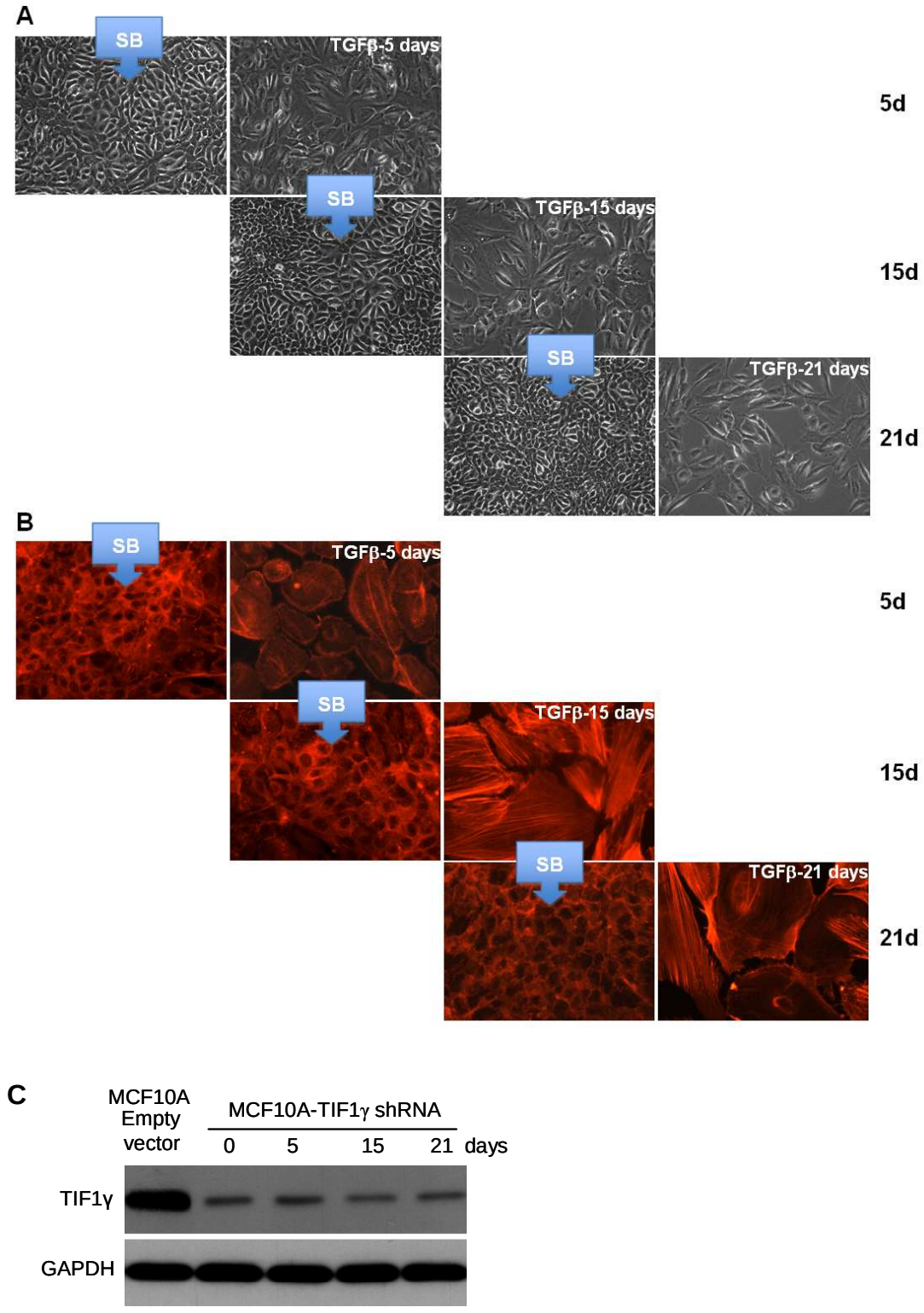

Fig. S4: MCF10A cells stably infected with sh(TIF1γ) retrovirus (Open Biosystems) were exposed to TGFβ1 during 21 days. Cells gradually reverted back to epithelial cells resembling parental MCF10A cells after TGFβ1 removal and adjunction of TβRI kinase inhibitor (SB-431542). (**A)** Phase contrast images (x10) and (**B**) immunofluorescence acquisitions showing actin subcellular localization detected by phalloidin (x40). SB, SB-431542; d, days. (**C**) TIF1γ levels were determined by immunoblotting in parental MCF10A cells and in MCF10A cells stably infected with sh(TIF1γ) retrovirus. GAPDH served as a loading control.

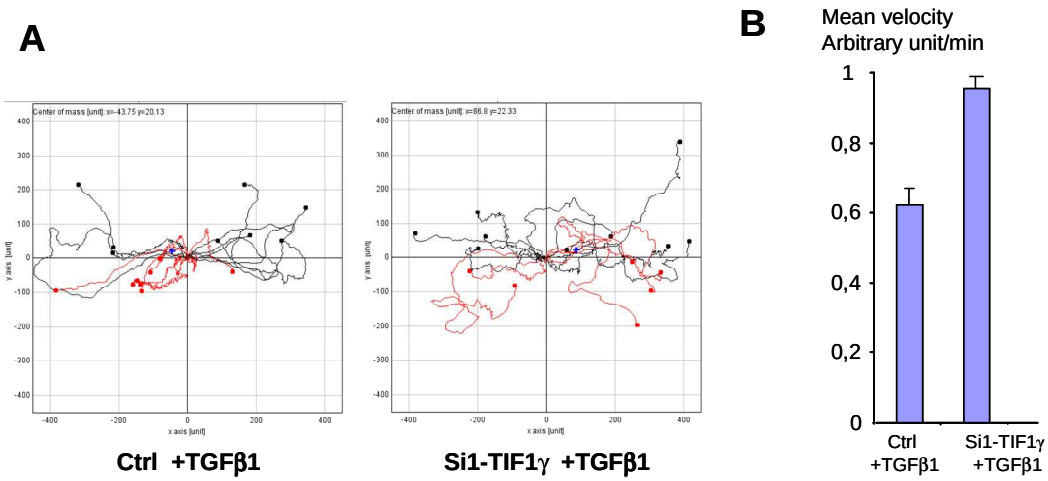

Fig S5: Inactivation of TIF1γ increases TGFβ1-induced cell motility. MCF10A cells were grown to confluence with or without addition of TGFβ1. Medium was aspirated and a gel filter tip was drawn carefully through the monolayer to create a wound between cells. Cells were then washed with PBS and fresh medium was added, with or without TGFβ1. The healing process was observed by timelapse video microscopy. The migration patterns of individual cells were determined by timelapse on duplicates. In cells inactivated or not for TIF1γ and treated with TGFβ, the migration path of 8 cells from the wound edge was tracked with the Manual Tracking plug-in developed by F. Cordelières (Orsay, France). The traces were reported on a diagram (**A**). (**B**) The mean velocity was calculated on the 16 tracks with the Chemotaxis Tool from Ibidi (G. Trapp). Error bars represent s.d.

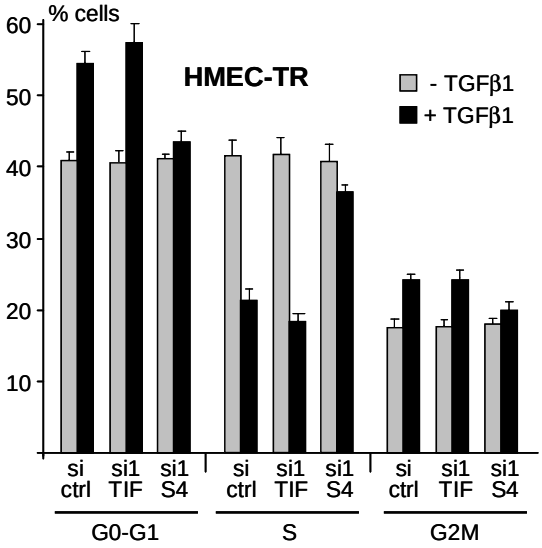

Fig S6: Inactivation of TIF1γ expression had no effect on cell cycle distribution. Cell cycle distribution of TIF1γ or Smad4-inactivated cells cultured or not with TGFβ1 for 96h. Harvested cells were fixed in 75% ethanol at 4°C, then treated with RNase III and stained with propidium iodide. Cell cycle distribution was analyzed using a FACS Canto II (Becton Dickinson).

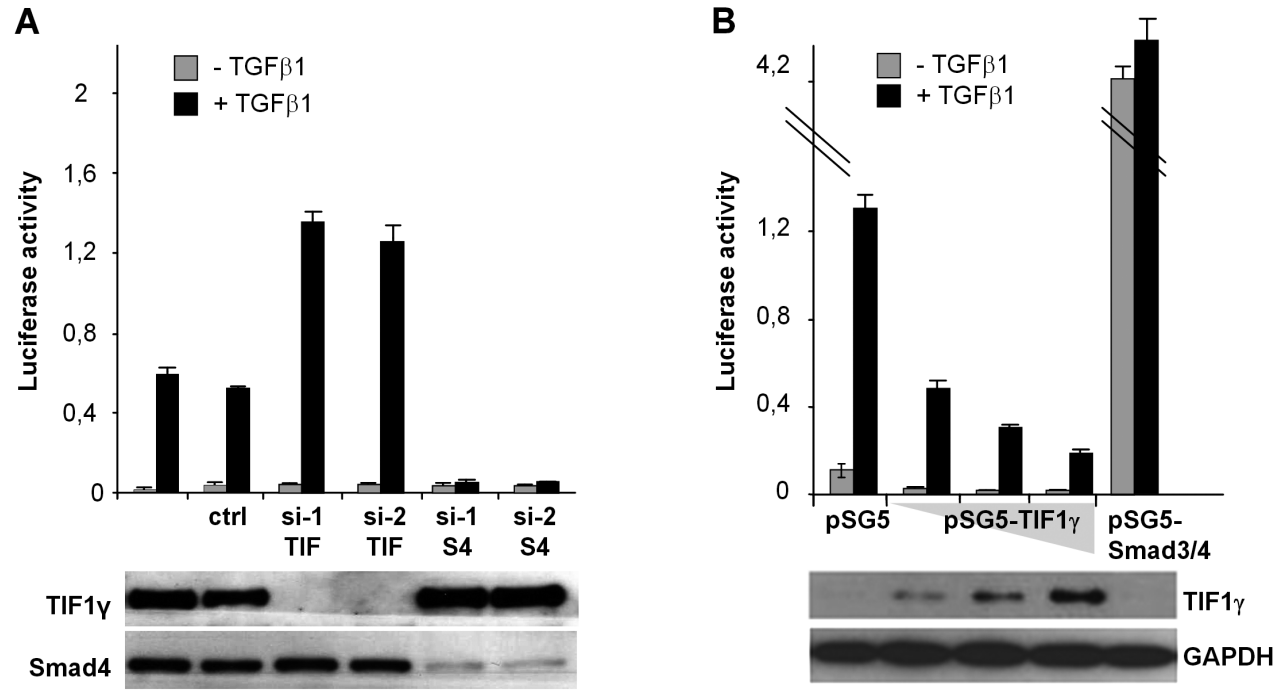

Fig S7: TIF1γ inhibits TGFβ1 signaling. HMEC-TR were cotransfected with the pGL3(CAGA)-Luc reporter vector (known to be driven by the activated Smad complex) together with the pRL-CMV internal control vector and si-RNA (**A**) or the expression vectors indicated (**B,C**). Twenty-four hours before harvesting, transfected cells were either stimulated by TGFβ1 or left unstimulated, then luciferase activity was determined. The relative luciferase activity is given as the mean +/- s.d. of one experiment performed in triplicate, representative of three experiments. Expression levels of TIF1γ, Smad4 and GAPDH proteins are shown. ctrl, control; TIF, TIF1γ; S4, Smad4. Isolated TIF1γ domains (TRIM, nt 1-1368; Middle, nt 1369-2664; PHD/Br, nt 2665-3390) were cloned in pSG5-flag expression vector. As previously shown (Dupont *et al*., 2005; 2009), inactivation of TIF1γ enhanced the TGFβ1-transcriptional response of the artificial (CAGA)9-MLP-Luc reporter (A). In addition, TIF1γ was found to inhibit the transcriptional activity of the reporter construct in a dose-dependent manner (B). Isolated domains of TIF1γ (TRIM, middle or PHD/Br) are void of biological effect on luciferase reporter construct (C).

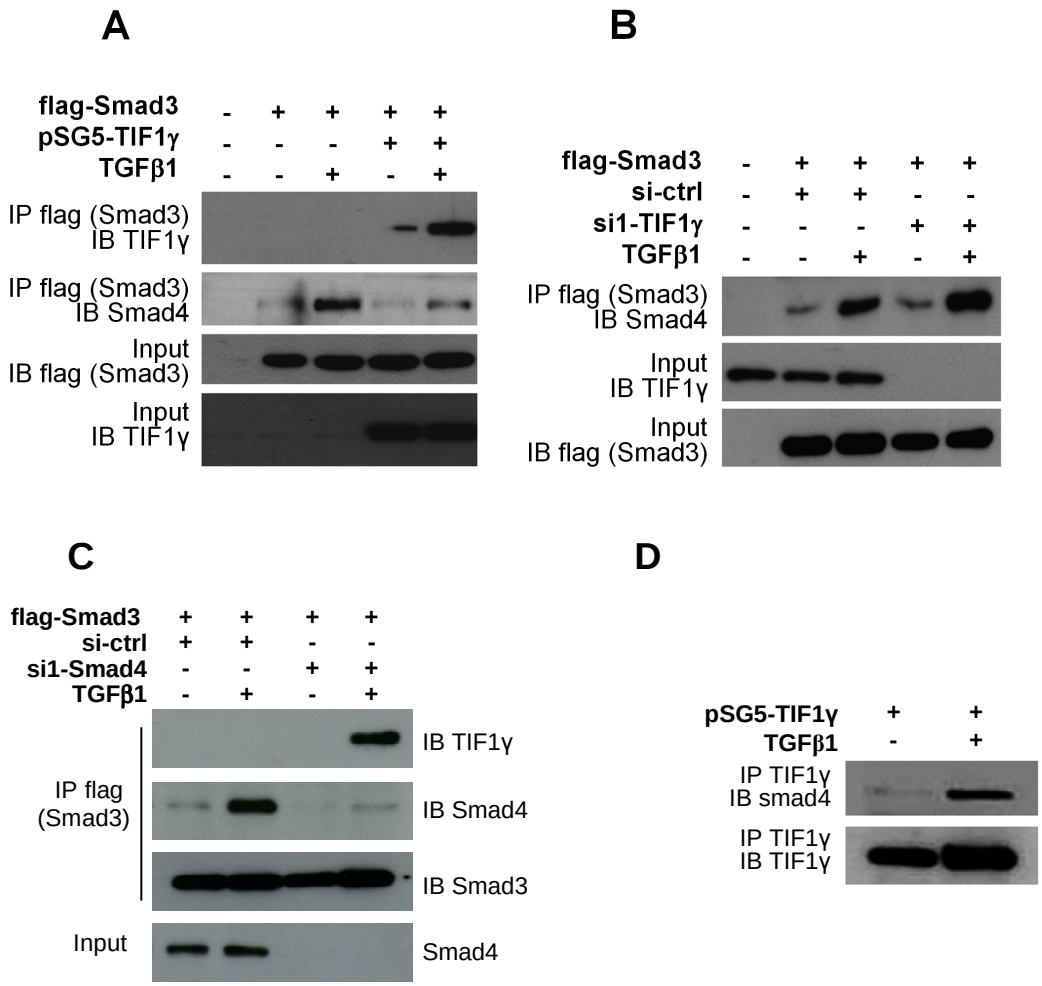

Fig S8: TIF1γ inhibits Smad3/4 complexes. HMEC-TR cells were transfected with the indicated vectors, control siRNA (ctrl) or siRNA targeting human TIF1γ mRNA. 48h post-transfection, the cells were treated or not with TGFβ1 for 2h. Lysates were immunoprecipitated (IP) and immunoblotted (IB) as indicated. Protein expression was monitored by immunoblot analysis of total cell extracts (Input). As previously shown (He *et al.*, 2006), over-expression of TIF1γ in HMEC-TR cells decreased Smad3/Smad4 interaction (A) whereas TIF1γ down-regulation favored the formation of Smad3/Smad4 complex (B). In cells overexpressing TIF1γ, TIF1γ could be coimmunoprecipitated with Smad3, this interaction being enhanced by TGFβ1 treatment (A). In cells inactivated for Smad4, the Smad3/TIF1γ complex was more abundant (C). TIF1γ has been shown to interact with Smad4 and to act as a RING-finger ubiquitin ligase for Smad4, regulating Smad4 localization (Dupont *et al.*, 2005; 2009). We found that Smad4 could be coimmunoprecipitated with TIF1γ in HMEC-TR cells, this interaction being enhanced by TGFβ1 treatment (D).

Table S1: SITD, SDTD and SDTI groups of TGFβ1-dependent genes were identified based on the indicated combination of 3 cut off values shown in the table. FC, Fold Change.

	ctrl+/ctrl-	**si-Smad4+/ctrl+**	**si-TIF1γ+/ctrl+**
Smad4 Independent and TIF1γ Dependant (SITD)	FC>1.3 and FC<0.77	0.66<FC<1.5	FC>1.5 and FC<0.66
Smad4 Dependent and TIF1γ Dependent (SDTD)	FC>1.3 and FC<0.77	FC>1.5 and FC<0.66	FC>1.5 and FC<0.66
Smad4 Dependent and TIF1γ Independent (SDTI)	FC>1.3 and FC<0.77	FC>1.5 and FC<0.66	0.66<FC<1.5

Table S2: Three complete lists are shown in the excel file:
SDTI: Smad4-dependent and TIF1γ-independent probes
SDTD: Smad4-dependent and TIF1γ-dependent probes
SITD: Smad4-independent and TIF1γ-dependent probes

Table S3: TGFβ1 target genes regulated by TIF1γ and potentially involved in EMT process. Genes showing an opposite response to Smad4 or TIF1γ down-regulation are presented in bold. * Target genes for which quantitative RT-PCR was performed. S4, Smad4; TIF, TIF1γ

Gene symbol	Gene name	ctrl+/ctrl-	si-S4+/ctrl-	si-TIF+/ctrl-
Cell adhesion/ cell-surface proteins				
ADAM12 *	a disintegrin and metalloproteinase domain 12 (meltrin alpha)	2.83	1.31	**4.25**
CDH11 *	cadherin 11. type 2. OB-cadherin (osteoblast)	2.25	1.42	**6.29**
CDH2 *	cadherin 2. type 1. N-cadherin (neuronal)	2.38	1.05	**4.76**
CELSR2	cadherin. EGF LAG seven-pass G-type receptor 2	1.38	0.86	0.81
CLDN14	claudin 14	3.38	1.45	1.39
CLDN4	claudin 4	3.15	0.89	1.43
CLU	clusterin	2.03	1.56	1.24
CNTNAP3	cell recognition molecule CASPR3	1.58	1.44	0.90
DSG3	desmoglein 3 (pemphigus vulgaris antigen)	1.52	0.83	0.54
EPPK1	epiplakin 1	1.81	0.61	0.71
JPH1	junctophilin 1	0.41	0.76	0.27
MLLT4	mixed-lineage leukemia (trithorax homolog. Drosophila)	1.44	0.88	0.92
OCLN	occludin	1.60	0.95	0.80
PDGFC	platelet derived growth factor C	1.45	1.22	2.36
PKP1	plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)	2.13	1.16	**3.66**
PODXL	podocalyxin-like	2.73	0.43	1.65
PVRL4	poliovirus receptor-related 4	1.95	1.00	1.21
SELL	selectin L (lymphocyte adhesion molecule 1)	1.63	1.1	1.04
STYK1	protein kinase STYK1	1.47	1.1	0.60
HNT	neurotrimin	2.65	0.52	0.68
KAL1	Kallmann syndrome 1 sequence	2.13	0.91	1.20
CD58	CD58 antigen. (lymphocyte function-associated antigen 3)	3.92	1.90	1.77
TSPAN-2	tetraspan 2	3.64	0.85	2.33
Matrix extra cellular proteins				
COL1A1	collagen. type I. alpha 1	6.54	0.19	0.41
COL5A1	collagen. type V. alpha 1	5.59	1.60	**9.40**
COL5A2	collagen. type V. alpha 2	2.29	0.64	1.10
ECM1	extracellular matrix protein 1	1.80	0.36	0.60
MMP10	matrix metalloproteinase 10 (stromelysin 2)	4.44	0.15	0.48
MMP28	matrix metalloproteinase 28	1.49	0.48	0.60
SERPINB1	serine (or cysteine) proteinase inhibitor. clade B. member 1	1.59	0.39	0.43
SERPINB13	serine (or cysteine) proteinase inhibitor. clade B. member 13	1.63	0.38	0.21
SERPINB5	serine (or cysteine) proteinase inhibitor. clade B. member 5	1.72	0.84	1.04
SERPINE1 *	plasminogen activator inhibitor type 1 (PAI-1)	5.95	1.14	**10.26**
SPP1	secreted phosphoprotein 1 (osteopontin. bone sialoprotein I)	1.69	0.35	0.45
TIMP3	tissue inhibitor of metalloproteinase 3	1.58	0.62	0.52
TNC	tenascin C (hexabrachion)	1.79	0.51	0.56
Cytoskeleton				
FLNC	filamin C. gamma (actin binding protein 280)	1.80	1.48	1.17
KRT16	keratin 16	2.13	0.4	0.61
KRT9	keratin 9 (epidermolytic palmoplantar keratoderma)	1.43	0.78	0.94
KRTHA4	keratin. hair. acidic. 4 (intermediate filament)	5.78	2.28	**12.02**
MYLIP	myosin regulatory light chain interacting protein	0.67	0.64	0.42
MYLK	myosin. light polypeptide kinase	1.38	1.24	0.76
MYO10	myosin X	1.59	1.62	2.77
MYO5B	myosin VB	1.42	0.69	0.61
NEXN	nexilin (F actin binding protein)	0.73	0.6	0.34
NRAP	nebulin-related anchoring protein	1.75	1.43	1.10
TPM1	tropomyosin 1 (alpha)	4.55	1.18	2.0
Microtubule/Microtubule associated protein/Spindle				
BPAG1	bullous pemphigoid antigen 1. 230/240kDa	0.58	0.33	0.16
EML1	echinoderm microtubule associated protein like 1	1.83	0.80	**2.75**
KIF11	kinesin family member 11	0.56	0.55	0.33

MICAL2	NIH_MGC_69 Homo sapiens cDNA clone IMAGE:3886131	1.58	1.78	2.78
MID1	midline 1 (Opitz/BBB syndrome)	1.45	1.13	0.64
SSX2IP	synovial sarcoma. X breakpoint 2 interacting protein	0.69	0.58	0.32
TPX2	TPX2. microtubule-associated protein homolog (Xenopus)	0.70	0.83	0.43
TTL	tubulin tyrosine ligase	0.71	2.0	1.34
GTPase signaling/Ras signaling				
ARF1	ADP-ribosylation factor 1	1.36	1.72	0.89
ARHGAP19	Rho GTPase activating protein 19	0.69	0.8	0.34
ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	1.90	1.37	0.87
ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	0.63	0.46	1.45
CD14	CD14 antigen	0.62	0.54	0.31
MLPH	melanophilin	2.07	2.85	9.46
MTSS1	metastasis suppressor 1	1.58	1.37	2.95
PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	1.57	0.52	0.31
PIK3CD	phosphoinositide-3-kinase. catalytic. delta polypeptide	2.48	1.35	**4.01**
PSCD2	pleckstrin homology. Sec7 and coiled-coil domains 2	1.43	1.39	2.47
RAB40C	RAB40C. member RAS oncogene family	1.93	1.37	2.25
RAB7	RAB7. member RAS oncogene family	1.84	1.75	2.12
RASA1	RAS p21 protein activator (GTPase activating protein) 1	1.53	0.81	0.99
RASA4	DNA directed RNA polymerase II polypeptide J-related gene	1.78	0.83	0.77
RHPN2	rhophilin. Rho GTPase binding protein 2	1.48	0.96	0.64
RICS	Rho GTPase-activating protein	1.36	0.72	0.61
SEC14L1	SEC14-like 1 (S. cerevisiae)	1.62	2.26	3.74
WASF3	WAS protein family. member 3	0.50	2.01	1.54
Hyaluronan/CD44 signaling				
ASB6	ankyrin repeat and SOCS box-containing 6	0.74	1.40	1.25
CALML3	calmodulin-like 3	1.79	1.43	0.85
CD44 *	CD44 antigen	1.44	1.34	2.89
FANK1	fibronectin type 3 and ankyrin repeat domains 1	2.27	0.66	0.82
HAS3	hyaluronan synthase 3	0.56	0.52	0.12
HMMR	hyaluronan-mediated motility receptor (RHAMM)	0.53	1.09	0.79
Integrin signaling				
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	2.32	1.25	1.27
ILK	integrin-linked kinase	1.39	1.39	2.26
ITGA4	integrin. alpha 4	0.55	1.18	0.35
ITGA5	integrin. alpha 5 (fibronectin receptor. alpha polypeptide)	1.93	1.37	3.71
ITGB3	integrin. beta 3 (platelet glycoprotein IIIa. antigen CD61)	8.26	1.26	3.89
ITGB8	integrin. beta 8	1.46	1.06	0.72
WNT Beta catenin signaling				
FZD2 *	frizzled homolog 2 (Drosophila)	1.63	1.35	2.66
INVS	inversin	0.61	0.74	0.40
SIAH1	seven in absentia homolog 1 (Drosophila)	1.59	1.45	0.70
SIP	Siah-interacting protein	0.63	1.45	1.14
TCF7L1	transcription factor 7-like 1 (T-cell specific. HMG-box)	1.56	0.79	0.51
WNT5A	wingless-type MMTV integration site family. member 5A	2.44	1.44	**3.90**
WNT5B *	wingless-type MMTV integration site family. member 5B	4.29	2.87	**8.87**
WNT9A *	wingless-type MMTV integration site family. member 9A	2.23	0.44	**7.17**
Cell Differentiation				
EDG4	endothelial differentiation. 4	0.57	1.24	2.05
SOCS2	suppressor of cytokine signaling 2	1.39	0.82	1.53
SPRR1B	small proline-rich protein 1B (cornifin)	1.50	0.40	0.24

Table S4: Functional classification of TGFβ dependent genes whose expression was modified by TIF1γ knock-down

Functional annotation	Number of genes	percent
EMT	96	13.56
Regulation of transcription	63	8.9
Cell proliferation	61	8.61
Metabolism	61	8.61
Apoptosis	24	3.39
Other functions	118	16.66
Unknown functions	285	40.25

Table S5: Genes exhibiting antagonistic responses to Smad4 and TIF1γ down-regulation.
* Target genes for which quantitative RT-PCR was performed.

Gene symbol	Description	ctrl+/ctrl-	Si-Smad4+/ctrl-	Si-TIF1γ+/ctrl-
Cytoskeleton/Cell Junction/Cell-Matrix Proteins				
ADAM12 *	a disintegrin and metalloproteinase domain 12 (meltrin alpha)	2.83	1.31	4.25
CDH11 *	cadherin 11. type 2. OB-cadherin (osteoblast)	2.25	1.42	6.29
CDH2 *	cadherin 2. type 1. N-cadherin (neuronal)	2.38	1.05	4.76
COL5A1	collagen, type V, alpha 1	5.59	1.60	9.40
EML1	echinoderm microtubule associated protein like 1	1.83	0.80	2.75
JPH1	junctophilin 1	0.41	0.76	0.27
KRTHA4	keratin. hair. acidic. 4	5.78	2.28	12.02
PIK3CD	phosphoinositide-3-kinase. catalytic. delta polypeptide	2.48	1.35	4.01
PKP1	plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)	2.13	1.16	3.66
SERPINE1*	plasminogen activator inhibitor type 1 (PAI-1)	5.95	1.14	10.26
WNT9A*	wingless-type MMTV integration site family. member 9A	2.23	0.44	7.17
WNT5A	wingless-type MMTV integration site family. member 5A	2.44	1.44	3.90
Metabolism				
GLDC	glycine dehydrogenase (decarboxylating; glycine decarboxylase)	0.52	0.85	0.26
GLUL	Homo sapiens glutamine synthetase pseudogene. complete sequence.	3.70	1.54	5.63
LOX	lysyl oxidase	1.86	1.03	3.55
NOX5	NADPH oxidase. EF hand calcium-binding domain 5	2.75	1.54	7.31
P4HA3	prolyl 4-hydroxylase alpha III	8.70	2.01	15.59
SLC27A2	solute carrier family 27 (fatty acid transporter). member 2	0.52	0.92	0.25
TGM 2	transglutaminase 2	3.18	1.15	5.55
Cell Proliferation				
GADD45B *	growth arrest and DNA-damage-inducible. beta	2.70	1.49	4.51
Unknown Function				
BC041029	MRNA; cDNA DKFZp686O13152	0.55	1.20	0.30
AW300131	Transcribed sequences (AW300131)	1.73	1.01	2.97
AW139053	Homo sapiens cDNA clone IMAGE:2718337 3'	0.61	0.35	1.31
AW338214	Clone IMAGE:5275753. mRNA	0.50	0.81	0.32
ANKRD22	Hypothetical protein MGC22805	0.54	0.85	0.19
BJ-TSA-9	Hypothetical protein MGC14128	1.65	0.89	2.65
C5orf13	Chromosome 5 open reading frame 13	1.80	1.07	3.39
FLJ23657	Hypothetical protein FLJ23657	10.82	2.25	18.51
FLJ36031	Hypothetical protein FLJ36031	1.47	0.83	2.52
FLJ90166	Hypothetical protein FLJ90166	1.68	0.77	7.80
KCTD11	Homo sapiens cDNA clone IMAGE:2426829 3'	1.83	0.92	2.95
LOC93109	Hypothetical protein BC007772	1.69	1.09	2.73
MGC22679	Homo sapiens cDNA clone IMAGE:3854163 5'	0.40	0.88	0.24
SDPR	serum deprivation response (phosphatidylserine binding protein)	0.59	1.10	0.37
TGM2	AL031651	4.33	1.15	9.05
Other functions				
PROC	protein C (inactivator of coagulation factors Va and VIIIa)	2.87	1.19	5.93
OPN3	opsin 3 (encephalopsin. panopsin)	2.24	1.03	3.38

Table S6: Genes involved in cell cycle and found in SDTI group. * Target genes for which quantitative RT-PCR was performed.

Gene symbol	Description	ctrl+/ctrl-	si-Smad4+/ctrl-	si-TIF1γ+/ctrl-
ASK	activator of S phase kinase	0.59	0.98	0.6
AURKB	aurora kinase B	0.57	0.93	0.82
BRRN1	barren homolog (Drosophila)	0.57	0.96	0.59
CCND2	cyclin D2	0.73	1.2	0.49
CCNF	cyclin F	0.56	0.98	0.79
CCNG2	cyclin G2	1.74	0.66	1.42
CDC25A	cell division cycle 25A	0.58	0.92	0.58
CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)	0.7	1.21	0.69
CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)	0.63	0.95	0.53
CDK4	cyclin-dependent kinase 4	0.66	1.16	0.92
CDKN1A *	cyclin-dependent kinase inhibitor 1A (p21. Cip1)	1.7	0.67	1.87
CDKN2B	cyclin-dependent kinase inhibitor 2B (p15. inhibits CDK4)	4.27	1.07	3.22
CHC1	chromosome condensation 1	0.61	1.28	0.77
DRIM	down-regulated in metastasis	0.51	1.12	0.67
DUSP22	dual specificity phosphatase 22	1.64	0.98	1.93
E2F5	E2F transcription factor 5. p130-binding	0.38	0.8	0.49
FANCD2	Fanconi anemia. complementation group D2	0.68	1.03	0.62
FGF2	fibroblast growth factor 2 (basic)	0.67	1.38	0.79
FGF5	fibroblast growth factor 5	2.03	0.96	2.48
GADD45A	growth arrest and DNA-damage-inducible. alpha	2.04	1.21	1.65
IFRD2	interferon-related developmental regulator 2	0.59	1.05	0.77
IGFBP7	insulin-like growth factor binding protein 7	1.89	0.89	2.15
IL15	interleukin 15	2	1	1.87
INHBA	inhibin. beta A (activin A. activin AB alpha polypeptide)	2.17	1.3	1.86
INSIG1	insulin induced gene 1	2.23	0.86	2.15
ISG20	interferon stimulated gene 20kDa	1.59	0.79	1.1
KRAS2	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	2.3	1.25	2.47
LRP1	low density lipoprotein-related protein 1	2.14	1.16	2.56
LRP5	low density lipoprotein receptor-related protein 5	0.61	1.04	0.84
MN1	meningioma (disrupted in balanced translocation) 1	1.98	0.66	2.71
MXD4	MAX dimerization protein 4	2.15	1	1.68
MYC *	v-myc myelocytomatosis viral oncogene homolog (avian)	0.6	0.95	0.55
NAP1L1	nucleosome assembly protein 1-like 1	0.65	1.02	0.68
NME1	non-metastatic cells 1	0.72	1.24	0.97
NOLC1	nucleolar and coiled-body phosphoprotein 1	0.57	1.07	0.52
PA2G4	proliferation-associated 2G4. 38kDa	0.6	0.92	0.8
PDGFA	platelet-derived growth factor alpha polypeptide	2.36	1.14	1.82
PLK1	polo-like kinase 1 (Drosophila)	0.5	1.05	0.61
POLD4	polymerase (DNA-directed). delta 4	2.26	0.72	2.58
PPP6C	protein phosphatase 6. catalytic subunit	0.6	1.44	0.78
PRIM1	nascent-polypeptide-associated complex alpha polypeptide	0.55	1.08	0.56
SASH1	SAM and SH3 domain containing 1	0.71	1.07	0.72
SKP2	S-phase kinase-associated protein 2 (p45)	0.42	0.67	0.48
SMAD3	MAD. mothers against decapentaplegic homolog 3	0.55	0.83	0.73
SPOCK	sparc/osteonectin (testican)	2.35	0.8	2.5
TCF8	transcription factor 8 (represses interleukin 2 expression)	2.62	0.93	2.24
TIEG	TGFB inducible early growth response	1.61	0.83	1.31
TNFSF13	tumor necrosis factor (ligand) superfamily. member 13	2.05	0.98	1.88
TNFSF9	tumor necrosis factor (ligand) superfamily. member 9	1.72	0.93	1.6

Table S7: si-RNAs used in the study.

Name	Sequence
si-TIF1γ#1	UUU CGC AGC ACA CAA GAG AUC UCC UCC GGA GGA GAU CUC UUG UGC UGC GAA A
si-TIF1γ#2	UUU CAC GGU GGA UAA AUC CAU UGG U ACCAAUGGA UUU AUC CAC CGU GAA A
si-Smad4#1	GCA AUU GAA AGU UUG GUA A U UAC CAA ACU UUC AAU UGC
si-Smad4#2	CCC ACA ACC UUU AGA CUG A U CAG UCU AAA GGU UGU GGG
Control	1027280 (Qiagen)

siRNA used for TIF1γ are stealth siRNA from Life technologies-Invitrogen

Table S8: Quantitative RT-PCR primers used in the study

Gene	Sequence
TIF1γ	PPH16274A (SA Biosciences)
Smad4	5'GTG GAA TAG CTC CAG CTA TC3' 5'CGG CAT GGT ATG AAG TAC TCC3'
Vimentin	5'CCA AAC TTT TCC TCC CTG AAC C3' 5'GTG ATG CTG AGA AGT TTC GTT GA3'
BAMBI	PPH01947E (SA Biosciences)
GADD45B	5'GTG GAT TAT AAT TGC AAC ATG ACG3' 5'TTG GCC GAC TCG TCA CCC3'
CDH2 (N-Cadherin)	5'GTG CAT GAA GGA CAG CCT CT3' 5'ATG CCA TCT TCA TCC ACC TT3'
WNT9A	5'CGC CTA CTT CGG GCT GAC3' 5'CTG CTT CCG CTC CAG CTT3'
CDH11 (OB-Cadherin)	5'CCC TGA AAT CAT TCA CAA TCC3' 5'AGT CCT GCT TCT GCC GAC T3'
WNT5B	5'GGA CAA CGC ATC TGT CTT TGG3' 5'GCT GAT GGC GTT GAC CAC3'
CD44	5'CTG GGG ACT CTG CCT CGT3' 5'GAG AGA TGC TGT AGC GAC CA3'
FLRG	5'CAC TCG TCG CGG TAG GTG3' 5'TTC CCT GCA AAG ATT CGT G3'
PAI-I	PPH00215E (SA Biosciences)
MMP2	PPH00151B (SA Biosciences)
FN1	PPH00143B (SA Biosciences)
FZD2	PPH02470A (SA Biosciences)
ADAM12	PPH07260E (SA Biosciences)
CDKN1A	5'GAC ACC ACT GGA GGG TGA CT3' 5'CCA CAT GGT CTT CCT CTG CT3'
c MYC	5'CTG CTG GGA GGA GAC ATG GT3' 5'TCC TGG ATG ATG ATG TTT TTG ATG3'
SNAIL	5'GTC GCA GGA CTC TAA TCC AGA3' ATC TCC GGA GGT GGG ATG
ZEB1	5'ATC CTG GGG CCT GAA GCT CAG G3' 5'TGG TGT GCC CTG CCT CTG GT3'
HMGA2	5'CCC AGG GGA AGA CCC AAA GGC3' 5'GTT GGC GCC CCC TAG TCC TCT3'
HPRT	5'TGA CCT TGA TTT ATT TTG CAT ACC3' 5'CGA GCA AGA CGT TCA GTC CT3'