The transcription factor PREP1 induces EMT and metastasis by controlling the TGF-beta-SMAD3 pathway in non-small cell lung adenocarcinoma.

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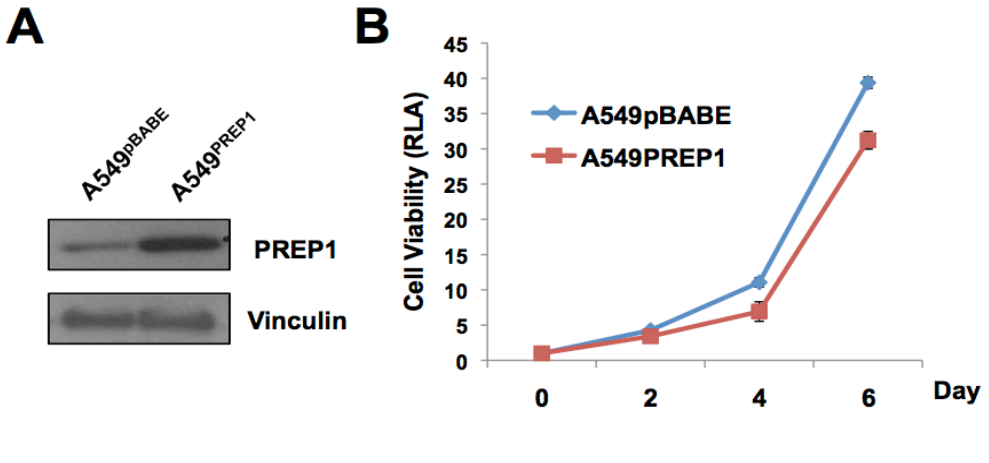
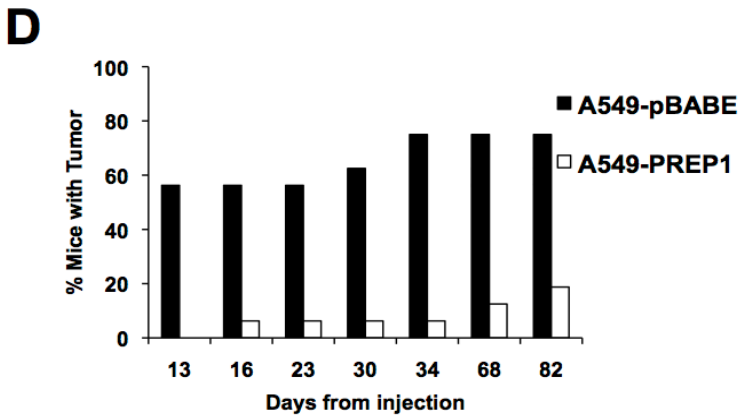
Inventory of Supplementary Information

Supplementary Figure S1. Effects of ectopic expression of PREP-1 on A549 cell proliferation, apoptosis and xenografts growth. (A) Western blot analysis of PREP1 in A549^{PREP1} vs A549^{pBABE} cells. Anti-vinculin was used to normalize for protein loading **(B)** Cell viability of A549^{PREP1} vs A549^{pBABE} cells was determined by luminometric detection of ATP content. RLA, Relative Luciferase Activity. **(C)** Apoptosis in A549^{PREP1} vs A549^{pBABE} cells was determined by luminometric assays of caspase 3/7 activity. RLA, relative luciferase activity. Error bars represent the mean ± standard deviation (SD) of three independent experiments. **(D)** Diagram showing the percent of mice with tumor at different times from the subcutaneous injection of A549^{PREP1} vs A549^{pBABE} cells. **(E)** Table: number of tumor masses/number of injected mice, average size of the masses and volume range. Picture: Representative tumor masses explanted at day 111 after injection.





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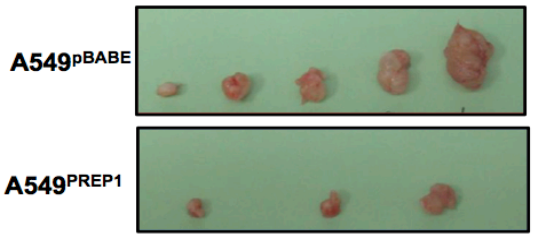
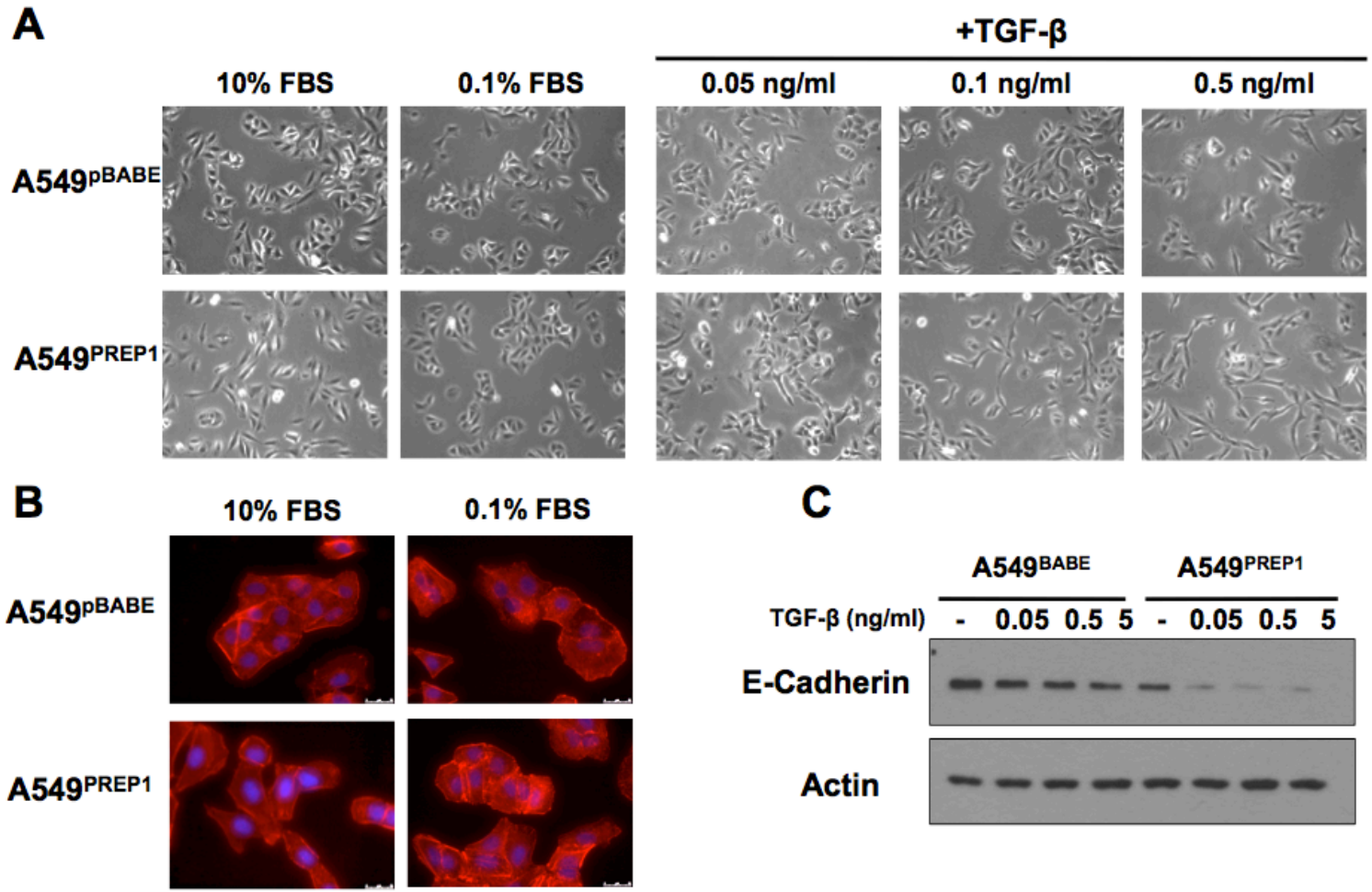
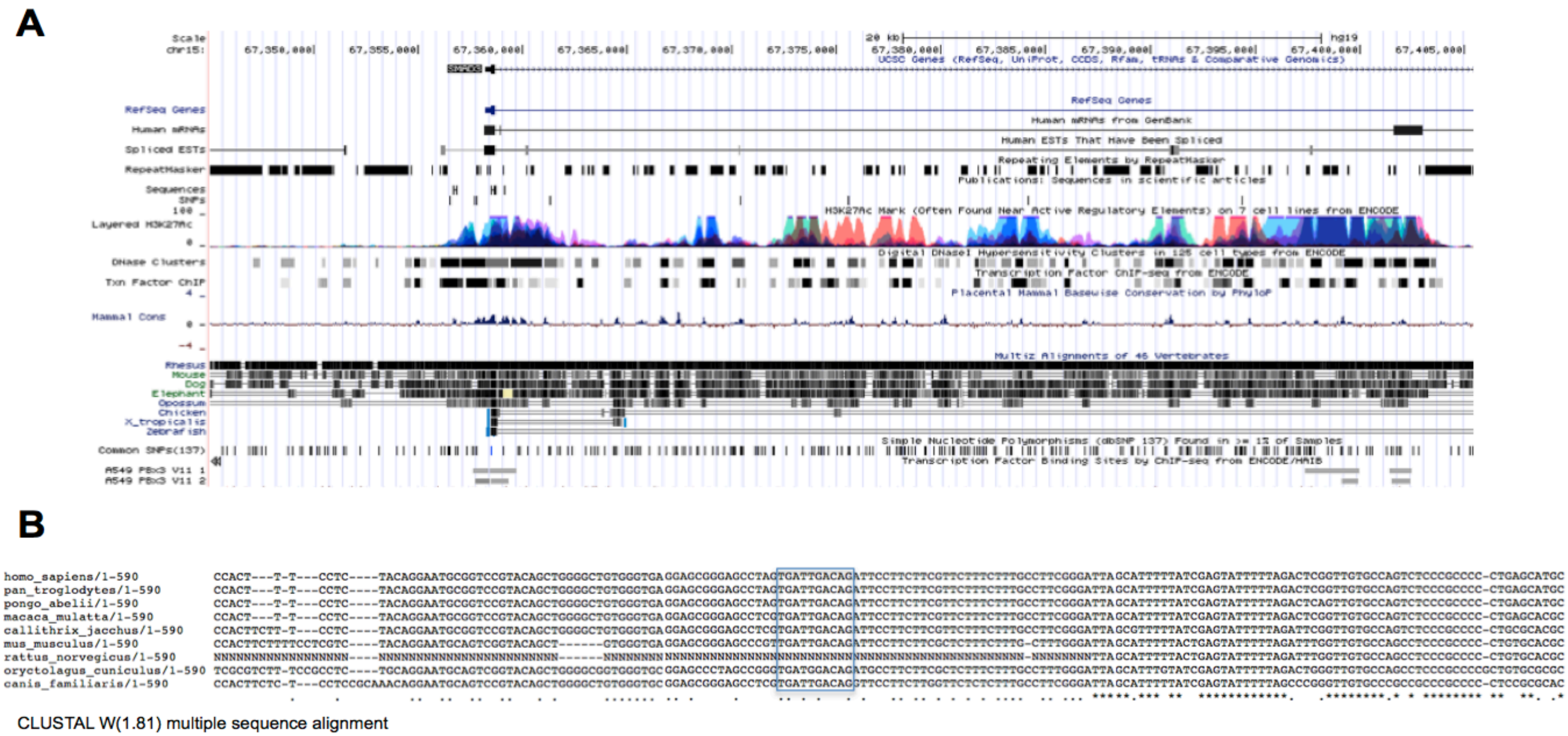
	N° Tumor Mass	Average Tumor Mass	Volume Range
A549^{pBabe}	12/16	198,31 mm^3	20-568 mm^3
A549^{Prep1}	3/16	53,9 mm^3	20-78 mm^3



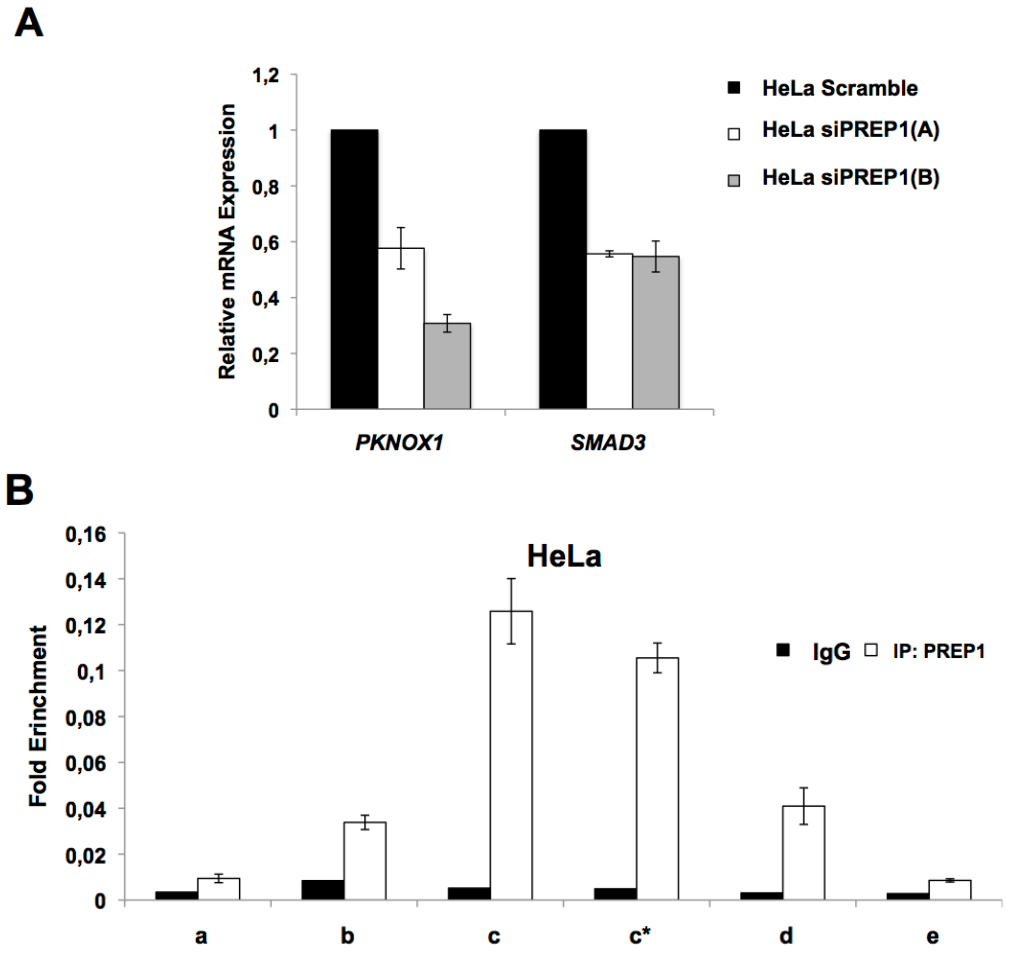
Supplementary Figure S2. PREP1 increases the A549 cells responsiveness to TGF-beta. (**A**) Bright field and immunofluorescence analysis of A549^{pBABE} and A549^{PREP1} cells, grown in presence of two different fetal bovine serum concentrations (0.1% and 10%) or increasing concentrations of TGF-beta in 0.1% FBS. (**B**) Immunofluorescence analysis (phalloidin and DAPI staining) of A549^{pBABE} and A549^{PREP1} cells grown in presence of the indicated TGF-beta concentrations in serum-deprived medium (0.1% FBS). (**C**) Immunoblotting analysis of E-cadherin expression in A549^{pBABE} and A549^{PREP1} cells, grown in presence of the indicated concentrations of TGF-beta. Beta actin was used to normalize for equal loading.



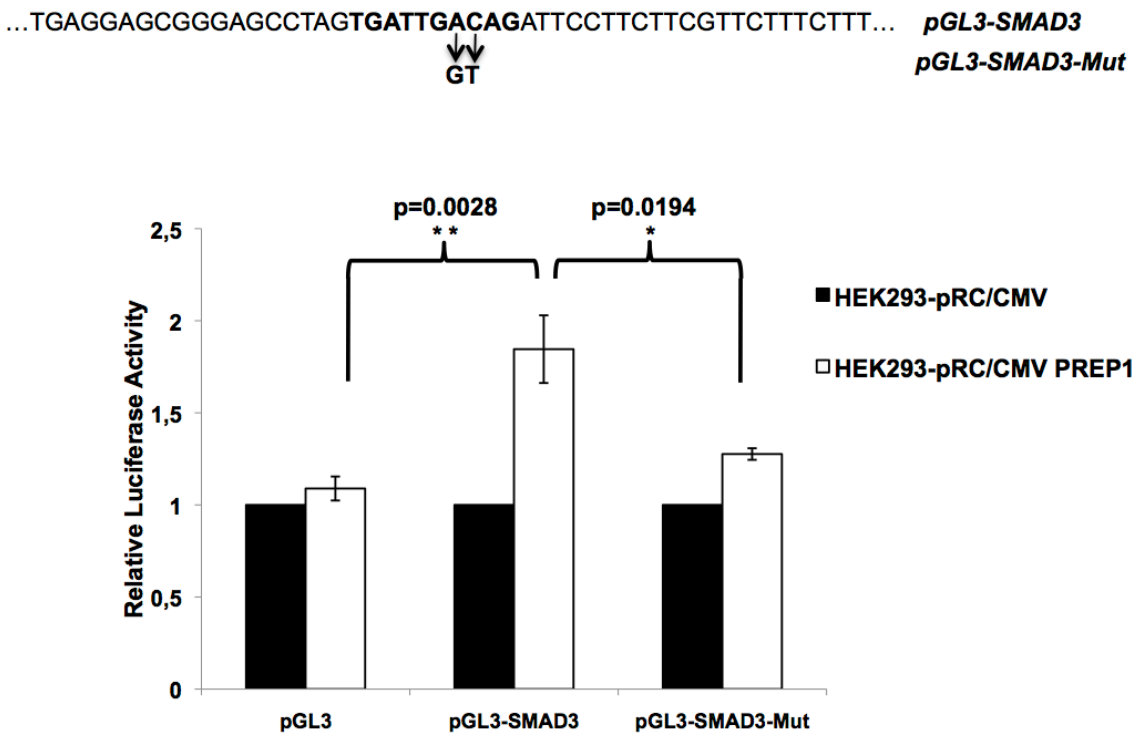
Supplementary Figure S3. Identification of a PREP1 binding site in the human *SMAD3* first intron. (A) Scheme (UCSC Genome Browser hg19) of the human genomic region encompassing the promoter, first exon and first intron of *SMAD3*. The peaks represent the regions of histone H3-K27 acetylation**,** according to ENCODE. **(B)** Inter-species alignement of the region containing the decameric PREP1 binding site (position +1002 from TSS) identified by analyzing the *SMAD3* genomic sequence (131433 bp, along with 20Kb of the 5' flanking region), for the presence of sequences matching the PREP1 consensus binding site established from genome-wide ChIPseq analysis (Penkov et al., 2013). The PREP1 site is localized within a highly conserved 600bp region, embedded in a larger region (from about 2Kb upstream to the TSS to approximately 3Kb within the first intron), characterized by high density of histone H3-K27 acetylation and TF binding sites.



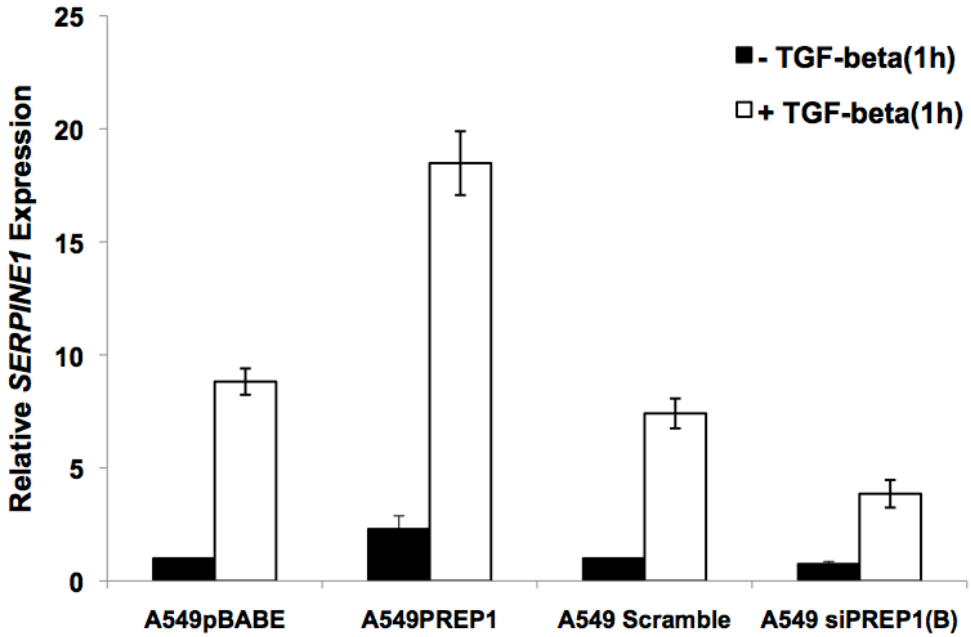
Supplementary Figure S4. PREP1-dependent regulation of *SMAD3* in HeLa cells. (A) Q-RT-PCR analysis of the PREP1 and SMAD3 transcripts in HeLa cells transfected with PREP1-specific siRNAs. *GAPDH* was used as internal control. Bars represent the mean ±SD of three independent experiments (**B**) Real-time PCR analysis of PREP1 binding to the *SMAD3* first intron in HeLa cells. Two overlapping amplicons (c and c*), containing the putative PREP1 site, were analyzed. Fold enrichment represents the percent of the signal detected vs the input chromatin. Bars represent the mean ±SD of three independent experiments.



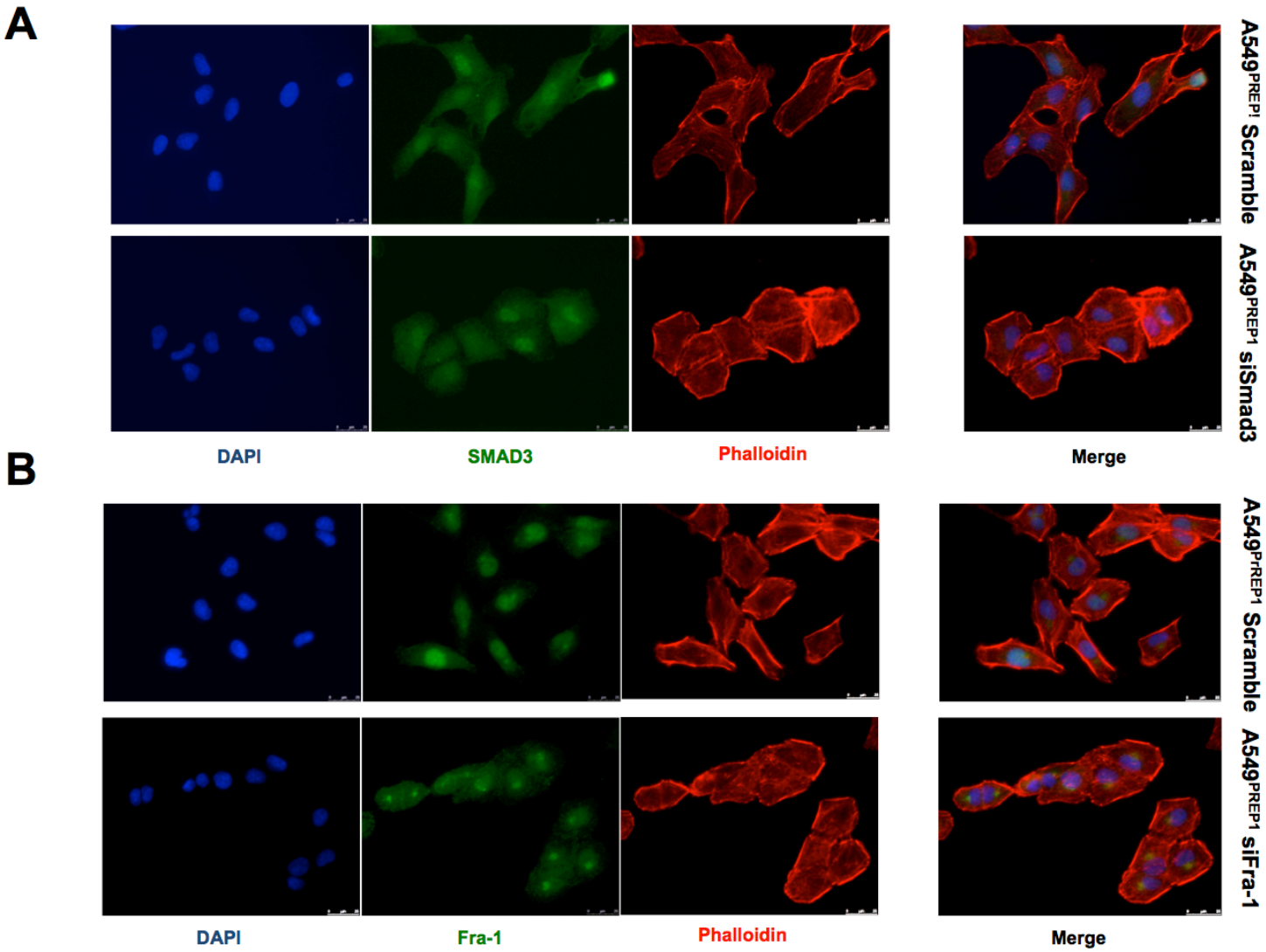
Supplementary Figure S5. PREP1-mediated transactivation of a *SMAD3* reporter construct. A human *SMAD3* 247bp fragment containing the wild-type or mutated PREP1 binding site was cloned in the pGL3-promoter luciferase vector. HEK293 cells were cotransfected with the pGL3-*SMAD3* reporter along with the PREP1 expression vector (pRC/CMV-PREP1) or empty control vector (pRC/CMV). Firefly luciferase activity was normalized by cotransfecting a Renilla luciferase reporter. Error bars represent the mean ±SD of three independent experiments. The asterisks show statistically significant differences: (*, p<0.05; **, p<0.01).



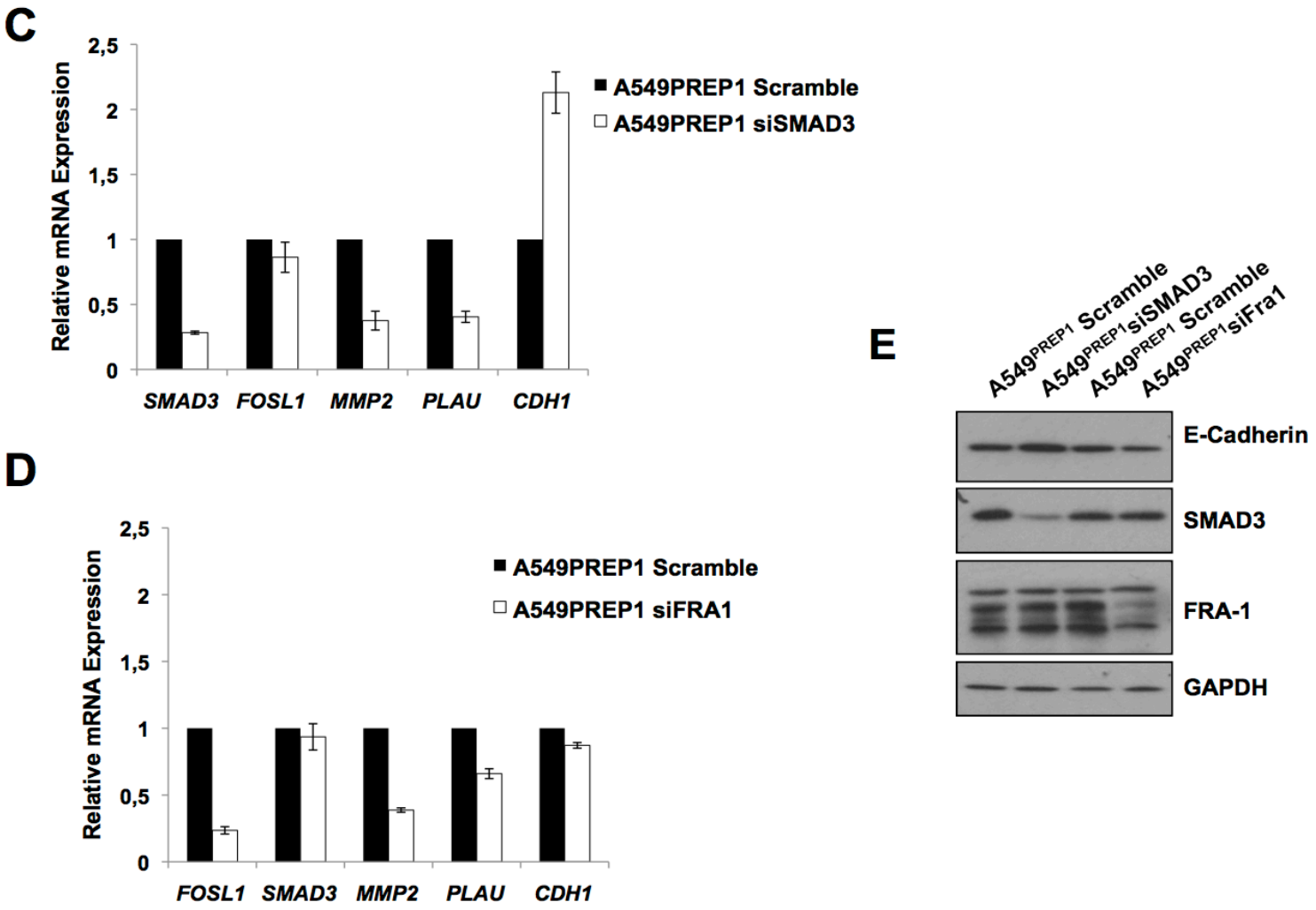
Supplementary Figure S6. PREP1 affects the TGF-beta-mediated induction of the PAI-1 transcript. Real-time RT-PCR analysis of PAI-1 (*SERPINE1*) mRNA in A549^{pBABE} vs A549^{PREP1} cells. *GAPDH* was used as internal control. Error bars represent the mean ±SD of three independent experiments.



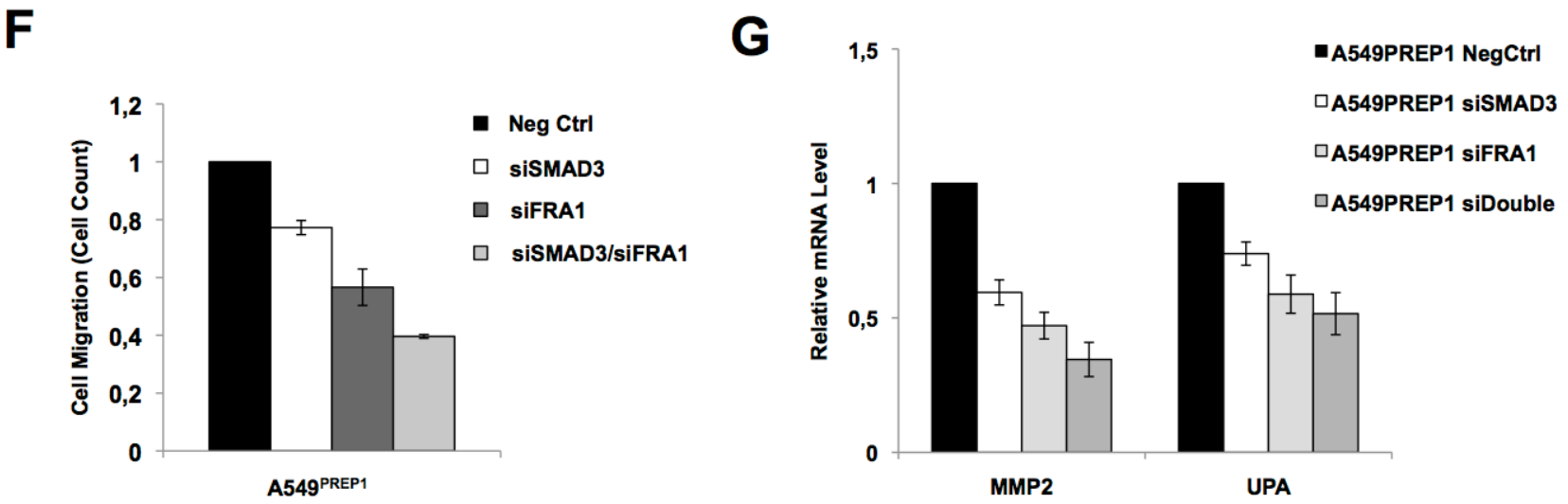
Supplementary Figure S7. SMAD3 and FRA-1 collaborate in response to PREP1 for the induction of EMT, cell motility and ECM-degrading proteases. A549^{PREP1} cells were transfected with the SMAD3- or FRA-1- specific vs control (scramble) siRNAs. (**A**) and (**B**) Immunofluorescence analysis. Actin filaments were stained by rodhamine-conjugated phalloidin. SMAD3 and FRA-1 were visualized by FITC immunostaining. Cell nuclei were visualized by DAPI staining.



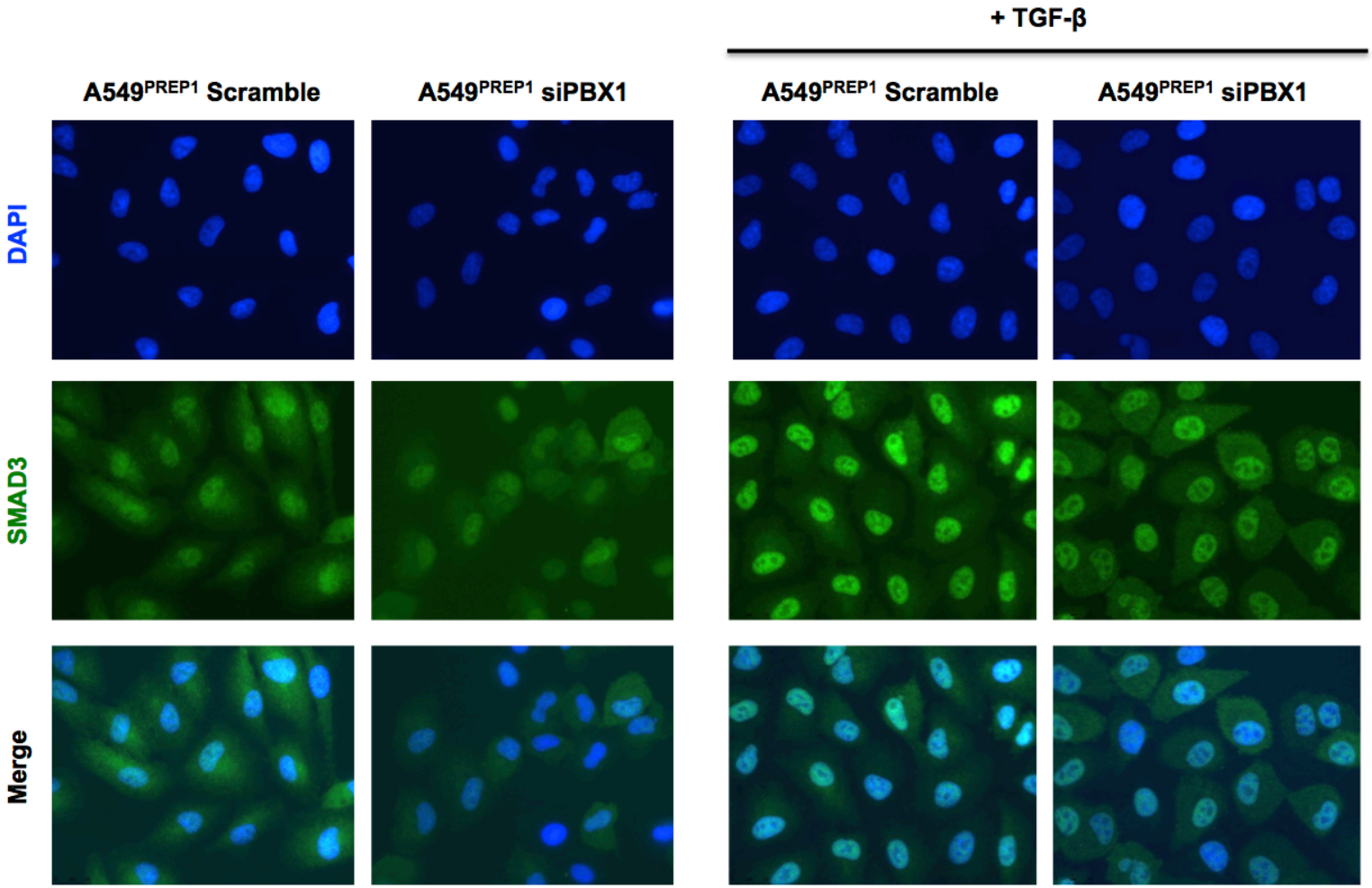
Supplementary Figure S7. SMAD3 and FRA-1 collaborate in response to PREP1 for the induction of EMT, cell motility and ECM-degrading proteases. (**C**) and **(D)** Real-time RT-PCR analysis of *SMAD3*, *FOSL1* (FRA-1), *MMP2, PLAU* (uPA) and *CDH1* (E-cadherin). *GAPDH* was used as internal control. Error bars represent the mean ±SD of three independent experiments. (**E**) Western blotting analysis. The extent of siRNA-mediated knockdown was verified by analyzing both FRA-1 and SMAD3 expression. GAPDH was used to normalize for equal loading.



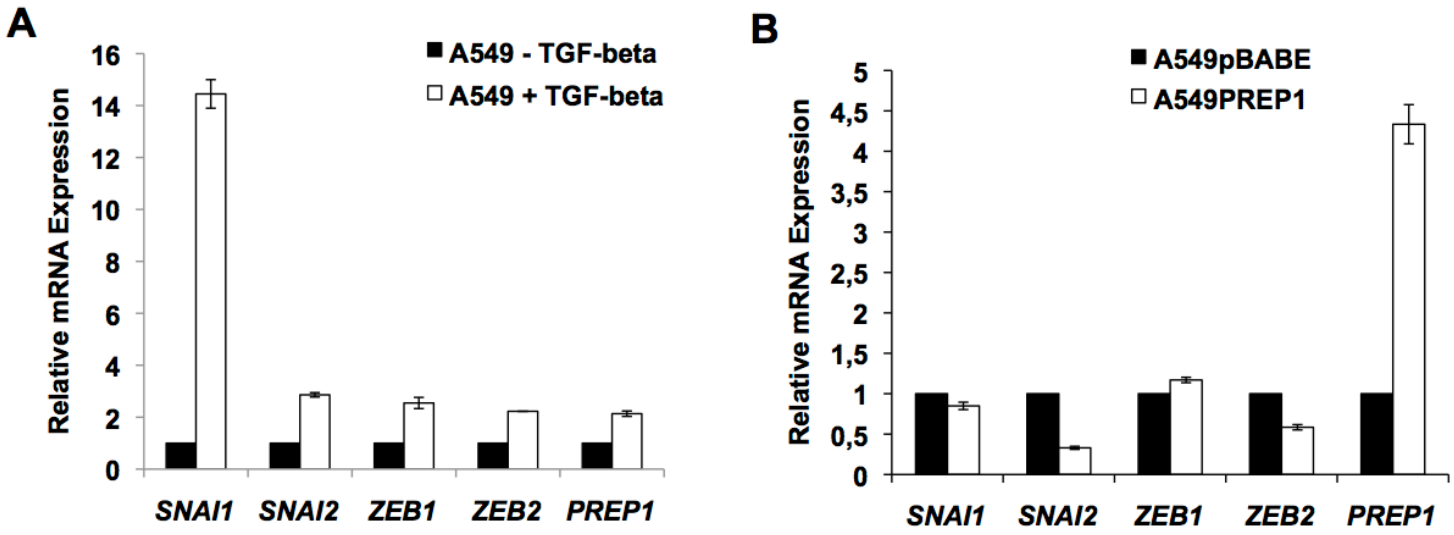
Supplementary Figure S7. SMAD3 and FRA-1 collaborate in response to PREP1 for the induction of EMT, cell motility and ECM-degrading proteases. (**F**) Cell migration. Transwell assays were performed as in Figure 3. The diagrams shows the cell count in five different fields. Error bars represent the mean ±SD of three independent experiments. (**G**) Q-RT-PCR of the MMP-2 (*MMP2*) and uPA (*PLAU)* transcripts 48h following transfection of SMAD3- or FRA-1- specific siRNAs in A549^{PREP1} cells. *Gapdh* was used as internal control. Bars represent the mean ±SD of three independent experiments.



Supplementary Figure S8. PBX1 knockdown inhibits both basal and TGF-beta-induced nuclear accumulation of SMAD3. Immunofluorescence analysis of SMAD3 in A549^{PREP1} vs A549^{pBABE} transfected with PBX1-specific vs control-siRNA, -/+ TGF-beta (5ng/ml) induction for 1 hour. SMAD3 was stained with FITC-conjugated antibodies. Cell nuclei were visualized by DAPI staining.



Supplementary Figure S9. PREP1 overexpression does not affect the transcripts encoding for EMT-inducing transcription factors. Q-RT-PCR analysis of the *SNAI1*, *SNAI2*, *ZEB1* and *ZEB2* transcripts: **(A)** in response to TGF-beta treatment (24h) of A549 cells; **(B)** in $A549^{PREP1}$ vs $A549^{pBABE}$ cells. *GAPDH* was used as internal control. Bars represent the mean ±SD of three independent experiments.



Supplementary Methods

Cell Culture procedures and transfection

A549, HEK293 and HeLa cells were obtained from ATCC and cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Invitrogen, Carlsbad, CA) and penicillin (100 U/mL)/streptomycin (100 U/mL) (Lonza, Basel, Switzerland) at 37°C, under 5% CO_2. Tgf-beta1 from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO).

The $A549^{pBABE}$ and $A549^{PREP1}$ stable transfectants were obtained by transfecting the pBABE-puro or pBABE-puro-PREP1 vector (Micali et al., 2009). Plasmid transfections were performed by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), accordingly to the manufacturer's instructions. Stable transfectants were isolated by puromycin (3μg/ml) (Sigma-Aldrich, St. Louis, MO) selection. The following siRNAs: PREP1(A) SASI_Hs01_00213040; PREP1(B) SASI_Hs02_00337905; SMAD3 and PBX1 (esiRNAs) and scrambled negative controls were from Sigma-Aldrich (MISSION, Sigma-Aldrich, St. Louis, MO). The FRA-1-specific siRNA (antisense sequence CUGACUGCCACUCAUGGUGdTdT) was from MWG-Biotech,Ebersberg, Germany. siRNAs were transfected by using INTERFERin (Polyplus-tranfection, Illkirch, France), according to the manufacturer's instructions.

Protein extraction and western blot analysis

Total extracts were prepared in Triton X-100 buffer (TrisHCl 10mM pH 7.5, NaCl 150mM, Triton X-100 1%, EDTA 1mM, glycerol 10%) containing either protease or phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and clarified by centrifugation. Nuclear and cytoplasmic extracts were fractionated according to (Longobardi and Blasi, 2003) Protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Protein extracts were resolved by 10% SDS-PAGE and blotted to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Membranes were first incubated with the primary antibody overnight and then with a peroxidase-conjugated secondary antibody. Peroxidase activity was measured by using an enhanced

chemiluminescence (ECL) kit (Pierce/Thermo Fisher Scientific, Rockford, IL), following the manufacturer's instructions. The antibodies used in this study were: Pbx1, Vimentin, JunB, Smad3, Smad2, phospho-Smad3 (Ser423/425), phospho-Smad2 (Ser465/467), phospho-ERK-1/2 (p42/44 MAPK) (Cell Signaling Technology, Danvers, MA); PREP1, GAPDH, , c-Jun, Fra-1, Pbx2, $p160^{MBP}$ (Santa Cruz Biotechnology, Santa Cruz, CA); E-cadherin (BD Biosciences, San Jose, CA); Actin and Vinculin (Sigma-Aldrich, St. Louis, MO).

Real-time RT-PCR analysis

Total cellular RNA was isolated from A549 and HeLa cells using TRIzol reagent (Invitrogen, Carlsbad, CA), following the manufacturer's instructions. 1μg of RNA was reverse-transcribed using the QuantiTect Reverse Transcription Kit (Qiagen Sciences, Germantown, MD). Real time PCRs were carried out according to the CFX96TM Real Time PCR Detection Systems (Bio-Rad Laboratories, Hercules, CA) and analyzed by using IQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA).

Reactions were performed in triplicate and GAPDH was used as internal standard control. The relative level of expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The following primers were utilized:

	Forward	**Reverse**
PREP1(PKNOX1)	5′-GGCTCTGAAGGCACAACTTC-3′	5′-CAAGAAGATGAATGCGCAAA-3′
SMAD3	5′-CCCCAGAGCAATATTCCAGA-3′	5′-GGCTCGCAGTAGGTAACTGG-3′
PBX1	5′-CAGATGCAGCTCAAGCAGAG-3′	5′-CTCTTTGGCTTCCTCACTGG-3′
CDH1	5′-TGCCCAGAAAATGAAAAAGG-3′	5′-GTGTATGTGGCAATGCGTTC-3′
VIMENTIN	5′-CCCTCACCTGTGAAGTGGAT-3′	5′-CTCAATGTCAAGGGCCATCT-3′
PLAU	5′-ACAGCATTTTGGTGGTGACT-3′	5′-GCCATCCCGGACTATACAGA-3′
MMP2	5′-TTGACGGTAAGGACGGACTC-3′	5′-ACTTGCAGTACTCCCCATCG-3′
FOSL1	5′-ATCTGCAAAATCCCGGAAGG-3′	5′-AGTGCCTCAGGTTCAAGCACAG-3′
SERPINE1	5′-GGCTGACTTCACGAGTCTTTCA-3′	5′-ATGCGGGCTGAGACTATGACA-3′
SMAD2	5′-GTTCCTGCCTTTGCTGAGAC-3′	5′-TCTCTTTGCCAGGAATGCTT-3′
SMAD4	5′-TGTGCCTGGTTTGATGGTAA-3′	5′-GCCATTTTCCCAATCTGCTA-3′
SMAD1	5′-CTACCCTCACTCTCCCACCA-3′	5′-GCACCAGTGTTTTGGTTCCT-3′
SMAD5	5′-AACCTGAGCCACAATGAACC-3′	5′-GTGGCATATAGGCAGGAGGA-3′
SMAD7	5′-TACCGTGCAGATCAGCTTTG-3′	5′-AGTTTGAAGTGTGGCCTGCT-3′
SNAI1	5′-GGTTCTTCTGCGCTACTGCT-3′	5′-TAGGGCTGCTGGAAGGTAAA-3′
SNAI2	5′-TGGTTGCTTCAAGGACACAT-3′	5′-GTTGCAGTGAGGGCAAGAA-3′
ZEB1	5′-GTGCAGGAGGGACCTCTTTA-3′	5′-TCAAAAGGAAGTCAATGGACAA-3′

ZEB2	5′-CAAGAGGCGCAAACAAGC-3′	5′-GGTTGGCAATACCGTCATCC-3′
GAPDH	5′-GAAGGTGAAGGTCGGAGTC-3′	5′-GAAGATGGTGATGGGATTTC-3′

Cloning and site-specific mutagenesis

A 247bp fragmrnt encompassing the PREP1 binding site in the first intron of *SMAD3* was amplified from human genomic DNA by using the following primers:

FW 5'-*GGGGTACCCC*AACTCTCCGAAAACTCCTGTC-3';

RV 5'-*GGGGTACCCC*AAGTCAGAGCATTGCACTGCA-3'

KpnI restriction sites (underlined) were used for cloning into the pGL3-promoter (TK) vector (Promega, Madison, WI). Site specific mutations were introduced by PCR, by using the following primers:

FW 5'-CCTA**GT**GATTGGTAGATTCCTT-3'

RV 5'-AAGGAATCTACCAATC**AC**TAGG-3'

Luciferase assay

The AP-1 luciferase reporter (7x-TRE-Luc) was from Stratagene (Stratagene, La Jolla, CA) The pGL3 reporter and the internal control TK-promoter-Renilla (pRL-TK) used as internal control were from Promega (Promega, Madison WI). The pRC-CMV-PREP1 expression vector has been previously described (Berthelsen et al., 1998). 48hrs after transfections (in triplicate), cells were washed twice with phosphate-buffered saline (PBS) and lysed in Passive Lysis Buffer (Promega, Madison, WI). Lysates were analyzed by using the Dual-Luciferase Reporter Assay System in a GloMax 96 Microplate luminometer (Promega, Madison, WI).

Immunofluorescence microscopy

A549 cells were grown on sterile glass coverslips, washed twice with ice-cold PBS, fixed in parafolmaldehyde (4%) for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 10 minutes and blocked with PBS/BSA 5% for 30 minutes. Incubation with primary antibody was for 1.5 hours at room temperature, followed by 45 minutes incubation with secondary green-fluorescence dye-conjugated antibody (Alexafluor 488, Invitrogen, Carlsbad, CA) and/or 30 minutes with a Rhodamine Phalloidin conjugate (Molecular Probes, Invitrogen, Carlsbad, CA). VECTASHIELD mounting medium with DAPI (Vector Laboratories, Burlingame, CA) was used to stain the nuclei. Images were visualized using a Leica inverted fully automated microscope (DMI6000B) with digital camera DFC 420 RGB (Leica Microsystems, Wetzlar, Germany).

Migration and invasion assays

Migration assays. $4x10^4$ cells were resuspended in 100μl of complete medium and seeded into the upper chamber of a polycarbonate transwell insert (8.0 μm pore membrane size) (Corning inc., Corning, NY). Complete medium was placed in the lower well, and cells were incubated at 37°C in a 5% CO_2-supplied incubator for 5 hours. Cells migrated to the lower surface of the membrane were fixed in ice-cold methanol for 10 minutes and then stained with 1% crystal violet dye and counted under bright-field microscopy. In each experiment, the numbers of stained cells, in five independent image fields of the same size, were averaged and indicated as relative migration.

Invasion assay. The above-described procedure was used, except for the use of transwell inserts previously coated with a basement membrane matrix of Matrigel (BD Biosciences, San Jose, CA).

Wound healing assays. A549 cells were plated overnight to achieve a subconfluent cell layer in 6-well plates. A scratch was made on the cell layer with a micropipette tip and cultures were washed twice with PBS to remove floating cells. After the scratch, pictures of the cells were taken under bright field microscopy at different time points (0, 16 and 24 hours). Distance covered by the cells was determined by measuring the wound width at the different time point and subtracting it from the wound width at time 0. Three independent experiments were done in triplicates.

Cell viability and caspase activity lumonimetric assay

Cell viability and Caspase 3/7 activities were assessed by using, respectively, the CellTiter-Glo luminescent cell viability assay kit (Promega, Madison, WI) and the Caspase-Glo assay kit (Promega, Madison, WI), according to manufacturer's instructions. Equal number of cells were seeded into 96-well plates. The CellTiter-Glo or the Caspase-Glo reagent were added to each well, and plates were incubated for 60 minutes at room temperature. Luminescence was quantitated by using a GloMax 96 Microplate luminometer (Promega, Madison WI). Experiments were performed in triplicate and repeated in three independent separately-initiated cultures.

Chromatin Immunoprecipitation

3 x 10^7 cells cells were fixed with formaldehyde 1% (v/v) for 10 minutes at room temperature. Glycine was then added to a final concentration of 0.125 M. Cells were washed twince with ice-cold PBS, harvested by scraping, pelleted at 1500rpm for 10 minutes, resuspend in SDS buffer (100 mM NaCl, 50 mM TrisHCl (pH 8), 5 mM EDTA (pH 8), 0,2% NaN_3, 0,5% SDS, protease inhibitors cocktail (Sigma-Aldrich, St. Louis, MO) and stored overnight at -80°C. Cell lysates were pelleted and resuspend in IP buffer (1 volume SDS buffer and 0,5 volume Triton Dilution buffer (100 mM TrisHCl (pH 8,6), 100 mM NaCl, 5 mM EDTA (pH 8), 0,2% NaN_3, 5% Triton X-100)). Samples were sonicated with a Sanyo MSE SoniPrep150 sonicator and immunoprecipitated overnight with 10 μg of PREP1 polyclonal antibody (PREP1 (N-15) sc-6245, Santa Cruz Biotechnology, Santa Cruz, CA) or unrelated IgG polyclonal antibody (Millipore, Billerica, MA) at 4°C. Immunoprecipitated complexes were collected by incubation with protein-A sepharose beads (GE Healthcare Life Sciences, Buckinghamshire, UK) rotating for 4 hours at 4°C and washed three times with Micelle Buffer (20 mM Tris HCl (pH 8), 150 mM NaCl, 5 mM EDTA, 5% w/v sucrose, 1% Triton X-100, 0,2% SDS), three times with Buffer 500 (500 mM NaCl, 1 mM EDTA, 50 mM HEPES (pH 7,5), 0,1% w/v deoxycholic acid, 1% Triton X-100) and twice with LiCl Buffer (250 mM LiCl, 10 mM TrisHCl (pH 8), 0,5% deoxycholic acid, 0,5% NP-40, 1 mM EDTA). The immunoprecipitated DNA was eluted with 100 μL of Elution Buffer (1% SDS, 100 mM $NaHCO_3$, 0,5 mg Protein-K), shaking overnight at 65°C, extracted with a PCR purification kit (Qiagen Sciences, Qiagen Sciences, Germantown, MD) and analyzed by real-time PCR. The enrichment of DNA was calculated in terms of %

input = $2^{-\Delta Ct}$, where ΔCt (threshold cycle) is determined by $Ct_{IP\ sample}$ - Ct_{Input} (the input was 1% of the chromatin amount immunoprecipitated). The following primers were utilized:

	Forward	Reverse
SMAD3a (-1074/-916)	5′-CCGATCCTTCTCTATCAGG-3′	5′-CTCTTGAGCCTTGTGAACCG-3′
SMAD3b (+663/+773)	5′-GTGCGTGTGTGTGAGAGTG-3′	5′-TGGAAGCCAGAGTGCCGCGT-3′
SMAD3c (+1002/+1150)	5′-GAATGCGGTCCGTACAGCTG-3′	5′-AGACTGGCACAACCGAGTCT-3′
SMAD3d (+1354/+1452)	5′-TTGAAACCGTGCAGAACGAG-3′	5′-ACCTAGCTAGCGCGCAACGG-3′
SMAD3e (+28456/+28565)	5′-GCATCAGCCGCTTCTCAAGT-3′	5′-ATCTCCCCACCATCACCTCC-3′
SMAD3c (+996/+1133)*	5′-TACAGGAATGCGGTCCGTAC-3′	5′-GACTGGCACAACCGAGTCTA-3′

Mice xenografts

Before implant $A549^{PREP1}$ and $A549^{pBABE}$ were evaluated for the vitality, counted and resuspended in PBS. 5 x 10^6 cells were suspended in 0.2 ml of PBS and inoculated subcutaneously in the right flank region. Mice were daily monitored for clinical signs and mortality. Body weight recording was carried out biweekly.

Tumor growth was monitored biweekly by caliper and recorded. At the end of the study mice were sacrificed by cervical dislocation. Tumor masses and lungs were collected in formalin 10% and then after 24 hours placed in EtOH 70%.

Immunohistochemistry

For immunohistochemistry, mouse tissues were formalin-fixed and paraffin embedded. Sections were stained with hematoxylin and eosin to assess the histological features. Immunohistochemical analyses of human (1:350, (Longobardi et al., 2010)) and human Nuclei (1:200, Millipore, Billerica, MA) was performed on paraffin-embedded sections. Paraffin was removed with xylene and the sections were rehydrated in graded alcohol. Antigen retrieval was carried out using preheated target retrieval solution (pH 6.0) for 45 minutes. Tissue sections were blocked with FBS in PBS for 90 min, incubated overnight with primary antibodies. HRP-Polymer Kit (Biocare Medical, Pike Lane Concord, CA) was used for the detection of anti human nuclei, and a biotinylated secondary antibody (1:200 Vector Lab, Burlingame, CA) was used for the detection of human PREP1, both followed by a diaminobenzidine chromogen reaction (Peroxidase substrate kit, DAB, SK-4100; Vector Lab, Burlingame, CA). All sections were counterstained with Mayer's hematoxylin and visualized under bright-field microscopy. Tissue microarrays were analyzed as described (Longobardi et al., 2010).

Supplementary References

Berthelsen, J., Zappavigna, V., Ferretti, E., Mavilio, F., and Blasi, F. (1998). The novel homeoprotein Prep1 modulates Pbx-Hox protein cooperativity. EMBO J *17*, 1434-1445.

Longobardi, E., and Blasi, F. (2003). Overexpression of PREP-1 in F9 teratocarcinoma cells leads to a functionally relevant increase of PBX-2 by preventing its degradation. J Biol Chem *278*, 39235-39241.

Longobardi, E., Iotti, G., Rosa, P. D., Mejetta, S., Bianchi, F., Fernandez-Diaz, L. C., Micali, N., Nuciforo, P., Lenti, E., Ponzoni, M.*, et al.* (2010). Prep1 (pKnox1)-deficiency leads to spontaneous tumor development in mice and accelerates EμMyc lymphomagenesis: A tumor suppressor role for Prep1. Molecular Oncology, 1-9.

Micali, N., Ferrai, C., Fernandez-Diaz, L. C., Blasi, F., and Crippa, M. P. (2009). Prep1 directly regulates the intrinsic apoptotic pathway by controlling Bcl-XL levels. Mol Cell Biol *29*, 1143-1151.

Penkov, D., Mateos San Martin, D., Fernandez-Diaz, L. C., Rossello, C. A., Torroja, C., Sanchez-Cabo, F., Warnatz, H. J., Sultan, M., Yaspo, M. L., Gabrieli, A.*, et al.* (2013). Analysis of the DNA-binding profile and function of TALE homeoproteins reveals their specialization and specific interactions with Hox genes/proteins. Cell Rep *3*, 1321-1333.