Supplemental data

Temporal and Spatial cooperation of Snail1 and Twist1 during Epithelial-Mesenchymal Transition predicts for human breast cancer recurrence

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Supplemental Figure Legends

Figure S1, related to Figure 1: MCF10A cells undergo EMT in response to both continuous and transient TGFβ1 treatment, and Twist1 is predominantly nuclear in resting MCF10A cells.

(**A**) Western blotting for indicated proteins of WCLs from MCF10A cells treated with 2 ng/ml TGFβ1 continuously and cells collected at indicated times. (**B**) Western blotting for indicated proteins of WCLs from MCF10A cells treated with 2 ng/ml TGFβ1 transiently for indicated durations and cells collected at indicated times. (**C**) Scratch wound migration assay of MCF10A either untreated or treated with transient TGFβ1 for 4h. Phase contrast photographs of the assay at indicated times are shown. Representatives of 2 independent experiments are shown. (**D** and **E**) Graphs of densitometric intensity of RT-PCRs of indicated mRNAs from MCF10A cells

treated with 2 ng/ml TGFβ1 either continuously (**D**) or transiently for 4h (**E).** (**F**) Western blotting for indicated proteins of whole cell lysates from MCF10A cells treated transiently with 2 ng/ml TGFβ1 for 4h and cells collected at indicated times.

Figure S2, related to Figure 1: **Similar transient TGFβ1-induced EMT is also observed in HMLER and A549 cells.**

(**A**-**C**) Western blotting for indicated proteins of WCLs (**A**), RT-PCR for indicated mRNA of total RNA (**B**) and phase contrast photographs (**C**) of HMLER cells treated with 2 ng/ml TGFβ1 either continuously or transiently for 4h, and cells collected at indicated times. Representatives of 2 independent experiments are shown. (**D** and **E**) Western blotting for indicated proteins of WCLs (**D**) and photographs of phase contrasts and immunofluorescent staining for indicated proteins (**E**) of A549 cells treated with 2 ng/ml TGFβ1 either continuously or transiently for 4h, and cells collected or imaged at indicated times. DAPI (blue) was used to detect nuclei in all panels. Representatives of 3 independent experiments are shown. (**F**) Scratch wound migration assay of A549 cells either untreated or treated with transient TGFβ1 for 4h. Phase contrast photographs of the assay at indicated times are shown. Representatives of 2 independent experiments are shown.

Figure S3, related to Figure 2: EMT initiation is not dependent on Snail2, Twist2, and Zeb1.

(**A** to **C**) Upper panels: Western blotting for indicated proteins of whole cell lysates from MCF10A cells transduced with a lentivirus expressing the control luciferase shRNA or Snail2

(**A**), Twist2 (**B**), or Zeb1 (**C**) shRNA, then treated with 2 ng/ml TGFβ1 continuously, and cells collected at indicated times. Lower panels: RT-PCR for indicated mRNA of total RNA from the same untreated cells as in the upper panels, showing at least >85% siRNA-mediated depletion of targeted mRNA. Gapdh was used to verify equal total RNA inputs.

Figure S4, related to Figure 2: Snail1 depletion results in decreased cell numbers in culture without significantly affecting the expression patterns of other EMT factors.

(**A**) Cell counts in triplicates in a 24-well plate at indicated times from seeding of MCF10A cells transduced with a lentivirus expressing either the control luciferase or Snail1 shRNA, then treated with 2 ng/ml TGFβ1 continuously, and numbers of live cells determined by Trypan blue dye exclusion at indicated times. Error bars represent mean +/- s.e.m.. Representatives of 3 independent experiments are shown. (**B**) RT-PCRs for indicated mRNAs of total RNA from the same MCF10A cells as in (**A**).

Figure S5, related to Figure 3: Snail2 is not required to maintain E-cadherin downregulation in transient TGFβ1-induced EMT.

Western blotting for E-cadherin and β-Actin of whole cell lysates from MCF10A cells transduced with a lentivirus expressing either the control luciferase or Snail2 shRNA, then treated with 2 ng/ml TGFβ1 either continuously or transiently for 4h, and cells collected at indicated times. Representatives of 3 independent experiments are shown.

Figure S6, related to Figure 3: Snail1 and Twist1 are required for EMT induction and maintenance, respectively, in HMLER and A549 cells.

(**A**) Western blotting for indicated proteins of WCL from HMLER cells transduced with a lentivirus expressing either the control luciferase shRNA, Snail1 shRNA or Twist1 shRNA, and treated with 2 ng/ml TGFβ1 either continuously or transiently for 4h, and cells collected at indicated times. Representatives of 2 independent experiments are shown. (**B**) Western blotting for indicated proteins of WCL from A549 cells transduced with a lentivirus expressing either the control luciferase shRNA, Snail1 shRNA, or Twist1 shRNA, and treated with 2 ng/ml TGFβ1 either continuously (left panel) or transiently for 4h (right panel), and cells collected at indicated times. Representatives of 2 independent experiments are shown. See also related Figure S7.

Figure S7, related to Figure 3: Snail1 and Twist1 are required for initiation and maintenance, respectively, of EMT-induced morphological changes in HMLER cells.

(**A** to **F**) Phase contrast photographs of HMLER cells transduced with indicated shRNA and then treated with 2 ng/ml TGFβ1 either continuously or transiently as indicated, and cells imaged at day 12.

Figure S8, related to Figure 4: TGFβ1-dependent Snail1 binding to human E-cadherin promoter used as a positive control.

Chromatin immunoprecipitation using a Snail1 monoclonal antibody in nuclear extracts from MCF10A cells either untreated or treated with 2 ng/ml TGFβ1 for 2 days. Immunoprecipitated chromatins were subjected with PCR for 30 cycles using a primer set specific for a known Snail1-binding site on the human E-cadherin promoter[1].

Figure S9, related to Figure 5: Snail1, Snail2, and Twist1 mRNA profiles in human primary breast cancer and BM DTCs

Total RNAs were isolated from 92 primary breast tumours (56 non-recurrent and 36 recurrent patients) and 20 paired primary breast tumours and BM DTCs and subjected to microarray analyses, which were normalized against Gapdh mRNA.

(A) Snail1 microarray signals in primary tumours correlated with distant recurrences (p=0.0015).

(B) Twist1 microarray signals in primary tumours did not correlate with distant recurrences.

(C) Twist1:Snail1 ratio in primary tumours did not correlate with distant recurrences.

(D) Snail2:Snail1 ratio in primary tumours did not correlate with distant recurrences.

(**E** and **F**) Snail2:Snail1 ratio in primary tumours (**E**) and BM DTCs (**F**) from paired samples did not correlate with distant recurrences.

Figure S10, related to Figure 6: Twist1 is required to maintain low ERK:p38 signalling ratio in response to transient, but not continuous, TGFβ1 treatment.

Western blotting for indicated proteins of WCLs from MCF10A cells transduced with a lentivirus expressing Twist1 shRNA, then treated with 2 ng/ml TGFβ1 either continuously or transiently for 4h, and cells collected at indicated times.

Figure S11, related to Figure 6: Twist1-induced growth arrest is dependent on a low ERK:p38 activity ratio.

(**A** and **B**) Western blotting for indicated proteins of WCL (**A**) from MCF10A cells stably expressing either the empty vector pCI-Neo or Flag-tagged Twist1 (2 independent stable pools A and B were obtained). The ratio of p-ERK1/2 normalized to total ERK1/2 over p-p38 normalized

to total p38 was determined by densitometry and shown as percent of the ratio in the empty vector control cells (**B**). The error bars represent mean +/- s.e.m. from 3 independent experiments. Student's T-test, * p=0.02; ** p<0.01. (**C**) Cell counts in triplicate in a 24-well plate at indicated times from seeding of the same cell pools as in **A**. Error bars represent mean +/- s.e.m. Representatives of 3 independent experiments are shown. * p<0.001; ** p<0.0001. (**D**) The same cell lines as in **A** were treated 1 day after seeding with the solvent DMSO or the specific p38 inhibitor SB203580 at indicated concentrations and cell counts determined on day 4 from seeding. Drugs were replenished daily. Fold changes in cell counts in SB203580 over DMSO are shown with the counts in DMSO set at 1. Error bars represent mean +/- s.e.m. Representatives of 3 independent experiments are shown. *, ** p<0.05.

Figure S12, related to Figure 6: Acute depletion of Twist1 in Twist1-overexpressing MCF10A cells results in high ERK:p38 signalling ratio and reverts growth arrest.

(**A**) Upper panel: Western blotting for indicated proteins of WCLs from 2 independent pools of Twist1-overexpressing MCF10A cells transduced with a lentivirus expressing either the control luciferase or Twist1 shRNA. Greater than 90% depletion of both endogenous and exogenous Twist1 was achieved. Lower panel: The ratio of p-ERK1/2 normalized to total ERK1/2 over p-p38 normalized to total p38 was determined by densitometry of the respective panels in the upper panel and expressed as fold change over the ratio in luciferase shRNA control cells, which is set at 1. Representative results of 2 independent experiments are shown.

(**B**) Cell counts in duplicate in a 24-well plate at indicated times from seeding of the same cell lines as in (**A**). Error bars represent mean +/- s.e.m. Representatives of 2 experiments are shown. * p=0.02; **p<0.01.

Figure S13: Twist1 is nuclear in untreated MCF10A cells.

(**A**) Immunostaining of untreated MCF10A cells using anti-Twist1 monoclonal antibody. Nuclear counterstaining was done with DAPI. (**B**) Untreated MCF10A cells were fractionated into nuclear and cytoplasmic fractions as confirmed by Lamin A/C and β-Tubulin antibodies, respectively. The same fractions were then subjected to Western blotting for Twist1.

Table S1: Sequences of all primers and shRNAs used

Name of primers	Sequences
Ecad-promoter-Fw	aatcagaaccgtgcaggtcc
Ecad-promoter-Rv	acaggtgctttgcagttccg
ChIP Twist1-Fw1	gtagaatcagttgagtttatg
ChIP Twist1-Rv1	cataaatagtgcacaggagc
ChIP Twist1-Fw2	ggagtgttcaaagcaagatg
ChIP Twist1-Rv2	cacactatagaatggttagcc
ChIP Twist1-Fw3	cctgacctgtcaacaagt
ChIP Twist1-Rv3	ggctcttgaactgttccgg
ChIP Twist1-Fw4	ccggaacagttcaagagcc
ChIP Twist1-Rv4	caggggtcattagccagt
ChIP Twist1-Fw5	gagatttctgcagccacgttc
ChIP Twist1-Rv5	gcagagttgatcccttacc
ChIP Twist1-Fw6	cagacaattttacagtaaggcagc
ChIP Twist1-Rv6	cttctcagtacggactcg
ChIP Twist1-Fw7	ctgcttccagtttgccag
ChIP Twist1-Rv7	gttcactcttcggtggaa
TWIST1-P1-Fw	atgagacaggactgcagggctagcgtagaatcagttgagtttatg
TWIST1-P2-Fw	atgagacaggactgcagggctagccctgacctgtcaacaagt
TWIST1-P3-Fw	atgagacaggactgcagggctagcgagatttctgcagccacgttc
TWIST1-P4-Fw	atgagacaggactgcagggctagcggtaagggatcaactctgc
TWIST1-P1-4-Rv	gtaagcaaaggccactggagatctctctcgagcggcgacgcgtgg
Snail1-RTPCR-Fw	accttccagcagccctacgacc
Snail1-RTPCR-Rv	gtgtggcttcggatgtgcatc
Snail2-RTPCR-Fw	gcagtaatacaatgcccctcc
Snail2-RTPCR-Rv	ggcgtggctattaaccgtacc
Twist1-RTPCR-Fw	ggcacccagtcgctgaacg
Twist1-RTPCR-Rv	gacgcggacatggaccagg
Twist2-RTPCR-Fw	gccgccaggtacatagactt
Twist2-RTPCR-Rv	ccccaaacataagacccaga
Zeb1-RTPCR-Fw	cgagtcagatgcagaaaatgagcaaaac
Zeb1-RTPCR-Rv	acccagactgcgtcacatgtctt
Foxc2-RTPCR-Fw	gcctaaggacctggtgaagc
Foxc2-RTPCR-Rv	ttgacgaagcactcgttgag
Gapdh-RTPCR-Fw	gcgtcttcaccaccatgg
Gapdh-RTPCR-Rv	tgacacgttggcagtggg
Snail1 shRNA	gcaggactctaatccagagtt
Snail2 shRNA	cagaaggtcatcatctgccat
Twist1 shRNA	gctggactccaagatggcaag
Twist2 shRNA	agcaagaagtcgagcgaagat
Zeb1 shRNA	ccagatgttgtggtagctcaa
Luc shRNA	ccggttgccaagaggttccat

Table S2: Normalized Quantitative RT-PCR Data for BM DTCs

Study No.	Grade	ER	PR	Her-2	Recurr-ence	Twist1	Snail1	Snail2	Twist1/Snail1 Ratio	Snail2/Snail1 Ratio
1	II	neg	neg	pos	Yes	**7.01**	**0.26**	16.56	**27.10**	64.00
2	II	pos	pos	neg	Yes	**5.31**	**0.28**	13.55	**18.77**	47.84
3	III	neg	neg	pos	Yes	**0.32**	**0.19**	1.87	1.68	9.92
4	III	neg	neg	neg	Yes	**9.00**	**1.60**	142.02	**28.44**	88.65
5	III	neg	pos	pos	Yes	**1.29**	**2.06**	N/A	0.63	N/A
6	III	neg	neg	neg	Yes	**0.41**	**0.18**	6.36	2.27	35.26
7	III	pos	neg	neg	Yes	**5.21**	**0.27**	60.55	**19.29**	224.41
8	III	neg	neg	pos	Yes	**3.89**	**0.32**	28.84	**12.04**	89.26
9	III	neg	neg	neg	Yes	**0.41**	**1.00**	2.91	0.41	2.91
10	II	pos	neg	neg	Yes	**6.45**	**0.51**	1.52	**12.55**	2.95
11	II	Pos	Neg	Neg	Yes	**2.50**	**0.3**	3.01	8.28s	9.99
12	II	neg	neg	neg	Yes	**1.20**	**0.31**	0.37	2.51	1.20
13	III	neg	neg	neg	Yes	**0.95**	**0.33**	0.55	2.89	1.68
14	III	neg	pos	neg	Yes	***3.07***	0.87	3.20	8.34	3.68
15	II	pos	pos	neg	Yes	**0.69**	**0.46**	N/A	**14.22**	N/A
16	III	neg	neg	pos	No	**0.85**	**0.42**	2.25	2.03	5.36
17	III	pos	neg	pos	No	**0.52**	**0.95**	2.35	0.55	2.48
18	III	neg	neg	neg	No	**0.47**	**0.26**	N/A	1.83	N/A
19	III	neg	neg	neg	No	**0.91**	**0.37**	10.93	2.43	29.24
20	III	neg	neg	neg	No	**0.34**	**1.10**	2.68	0.31	2.43
21	III	neg	neg	pos	No	**0.47**	**1.16**	2.01	0.40	1.73
22	III	pos	pos	neg	No	**0.84**	**0.61**	11.39	1.39	18.77
23	III	neg	neg	neg	No	**0.56**	**0.61**	62.68	1.27	103.25
24	II	pos	pos	neg	No	**0.95**	**0.45**	9.51	2.10	20.97
25	III	neg	neg	neg	No	**0.31**	**0.28**	2.53	1.14	9.19
26	III	neg	neg	neg	No	**0.62**	**0.71**	23.43	0.88	33.13
27	II	pos	neg	neg	No	**1.09**	**0.47**	1.57	2.35	3.36
28	II	pos	pos	neg	No	**0.21**	**3.01**	N/A	0.07	N/A
29	II	pos	pos	neg	No	**0.19**	**0.14**	N/A	1.39	N/A
30	II	neg	neg	neg	No	**0.33**	**0.38**	0.48	0.88	1.27

Supplemental experimental procedures

Antibodies and miscellaneous reagents.

Antibodies to E-cadherin, Vimentin, N-cadherin, Snail1, phospho-p38 (T180/Y182), pan-p38, phospho-ERK1/2, pan-ERK1/2, p21 (Cell Signaling), Twist1 (Abcam), p53 (Santa Cruz), Lamin A/C (Santa Cruz), Tubulin and Actin (Sigma) were used accordingly to the manufacturers' recommendations. G418 (Invitrogen) was used at 500 mg/ml (active fraction) for selection of MCF10A cells stably expressing the pCI-Flag-Neo plasmid.

Western blotting

Cells were lysed directly on their culturing plates with RIPA buffer (150 mM NaCl, 50 mM TrisCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitors (1 mM PMSF, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM sodium pervanadate, 1mM sodium fluoride) for 10 min on ice. Cells were scraped off plates and spun at 14000 rpm for 10 min to pellet insoluble materials. Clear whole cell lysates were subjected to concentration determination using Bradford reagents and boiled for 10 min with 1x Lammeli buffer and 5% 2-mercaptoethanol (Sigma). Equal protein amounts were resolved on 10-12% polyacrylamide gels and transferred onto PDVF membranes (Millipore). Membranes were blocked with 5% dry milk in 1xTBS with 0.1% Tween (1xTBST), incubated with desired primary antibodies at $4^{o}C$ overnight with gentle shaking, washed 3 times with 1xTBST, incubated with appropriate HRP-conjugated secondary antibodies for 2 hrs at room temperature, washed 3 times with 1xTBST, and subjected to light development with ECL reagents (Pierce).

Cell viability/proliferation assay

Cells were plated onto 24-well plates in triplicates. Each time when the media was changed, supernatant was collected and live floating cells (if any) were determined using Trypan blue dye and a hemocytometer, and the count was added to the final count. At indicated times, cells were trypsinized and live cells counted as above.

Chromatin precipitation

ChIP assay was performed according to Upstate Biotechnology's ChiP kit. For human Twist1 promoter primer sets, we performed 32 cycles. For the human E-cadherin promoter primer set, we performed 30 cycles. Both PCR sets were done at the annealing temperature of 50^{o}C.

Luciferase reporter assay

Twist1 promotor mutants were generated using long-template PCR system (Roche) in the presence of an enhancer (Invitrogen), inserted into the luciferase reporter plasmid pGL2 (Promega), and cotransfected in triplicate into HEK293 cells with the pCMV-Flag-Snail1 plasmid or the empty vector. Renilla luciferase plasmid was also co-transfected for transfection efficiency normalization. The empty pGL2 vector was used to determine background luciferase activity. The human E-cadherin promotor pGL2 plasmid was used as a positive control for a Snail1-repressed promotor. 10^5 HEK293 cells per well were plated onto a 96-well plate and allowed to grow for 24 hrs prior to transfection. We used the TransIT-LT1 transfection reagent (Mirus) accordingly to the manufacturer's recommendation. 48 hrs after transfections, cells were subjected to luciferase assay using the Dual-Glo luciferase assay system (Promega) according to the manufacturer's recommendation. Firefly luciferase activity was normalized to renilla luciferase activity and expressed as fold change over the background empty pGL2 vector.

Immunoflourescent staining

Cells were seeded onto sterile glass cover slips placed in culture plates 24 hrs prior to immunostaining. The entire procedure was performed at room temperature. Cells were washed twice with PBS, fixed for 10 min with 4% paraformaldehyde, permeabilized for 20 min with 0.5% Triton X-100 in PBS, and blocked with 3% BSA in PBS for 10 min. Permeabilized cells were then incubated with either anti-Twist1 monoclonal antibody (Abcam) at 1:50 dilution or mouse IgG in dilution buffer (DB: 1% BSA in PBS) for 1 hr, washed 3 times with washing buffer (WB: 0.1% Triton X-100 in PBS), incubated with Alexa Flour 488-conjugated rabbit anti-mouse polyclonal antibodies (Molecular Probes) at 1:500 dilution in DB for 1hrs, and washed 2 times with WB. For nuclear detection, cells were counterstained with DAPI (Sigma) in PBS for 15 min and washed 3 times with WB. Cover slips were mounted with an anti-fade mounting buffer (Vectorshield), and fluorescence was detected with a fluorescent microscope.

Scratch wound migration assay

On day 0, cells were seeded so that they will be >90 confluent on the day of imaging. On day 1, cells were treated transiently with 2ng/mol TGFβ1 for 4h. Scratch wounds were created using a p200 tip on day 5 (MCF10A cells) or day 3 (A549 cells). Phase contrast photographs were taken at the same location of a scratch wound at times 0h, 8h and 24h after the scratch wound was created.

Subcellular fractionation

Cells were grown on 10-cm plate overnight, then trypsinized and centrifuged at 800g. The cell pellet was washed once with ice-cold PBS and re-suspended in 300 μl ice-cold buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDA, 0.1 mM EGTA, 0.1% NP-40, 1mM DTT, 1mM PMSF and 1x protease inhibitor cocktail from Roche). Cell slurry was centrifuged at 10,000g for 10 min at 4^{o}C to pellet nuclei. Supernatant (cytoplasmic fraction) was collected and protein concentration measured. The pellet (nuclear fraction) was washed 3 times with 1ml of buffer A, re-suspended in 100 μl buffer B (20 mM HEPES, 0.4 mM NaCl, 1 mM EDTA, 1mM EGTA, 1 mM DTT, 1 mM PMSF and 1x protease inhibitor cocktail from Roche) and mixed vigorously at 4^{o}C for 20 min. Nuclear lysate was then sonicated at output 2.5 (Fisher Scientific Sonic Dismembrator 550) for 10 sec and centrifuged at 10,000g for 10 min at 4^{o}C. Supernatant was collected and protein concentration measured. Equal protein amounts of cytoplasmic and nuclear fractions were resolved on 12% polyacrylamide gel and subjected to Western blotting for Twist1, Lamin A/C (nuclear marker) and β-tubulin (cytoplasmic marker).

BM DTC Isolation

For detailed experimental procedures, see [2, 3]. Briefly, the human protocol was approved by the IRB of Washington University and all enrolled patients signed written informed consent. All samples were obtained prior to the initiation of chemotherapy. All patients were subsequently treated with the same regimen consisting of four cycles of epirubicin/Taxotere with or without zoledronic acid. All patients were treated at the Siteman Cancer Center at Washington University and follow-up data were obtained prospectively. The mean follow-up time was 5 years.

20ml of BM were collected from breast cancer patients and 5 healthy volunteers and subjected to

hypotonic RBC lysis. ~5 x 10^7 nucleated cells were incubated with EpCAM immunomagnetic beads (DAKO) for 1 h at 4°C and the entire procedure repeated 5 times. Beads were washed with cold PBS/1% FCS, and isolated using a magnetic particle concentrator (Dynal MPC). Captured cells were then snap-frozen for RNA isolation using the standard TRIzol reagent (Invitrogen). Total RNA was used for two-cycle biotinylated cRNA target synthesis (Affymetrix). Samples yielding >15 μg of cRNA were used for GeneChip Affymetrix microarray hybridization following standard protocols and array images were processed using the Affymetrix Microarray Analysis Suite (MAS5) statistical algorithm.

For quantitative RT-PCR, first strand cDNAs were synthesized from 2 μg total RNA using Omniscript reverse transcriptase (Qiagen) and random hexamers. Primer/probe sets for indicated transcripts were purchased from Applied Biosystems and reactions were carried out per manufacteurer's standard protocol using an ABI 7500 FAST Sequence Detection System.

Reference for supplemental data:

1. Hou, Z. *et al.* The LIM protein AJUBA recruits protein arginine methyltransferase 5 to mediate SNAIL-dependent transcriptional repression. *Mol Cell Biol* 28, 3198-3207 (2008).
2. Lin, Y. *et al.* A gene expression signature that predicts the therapeutic response of the basal-like breast cancer to neoadjuvant chemotherapy. *Breast Cancer Research and Treatment* (2009).

3. Watson, M.A. *et al.* Isolation and molecular profiling of bone marrow micrometastases identifies TWIST1 as a marker of early tumor relapse in breast cancer patients. *Clin Cancer Res* 13, 5001-5009 (2007).

Figure S1, related to Figure 1

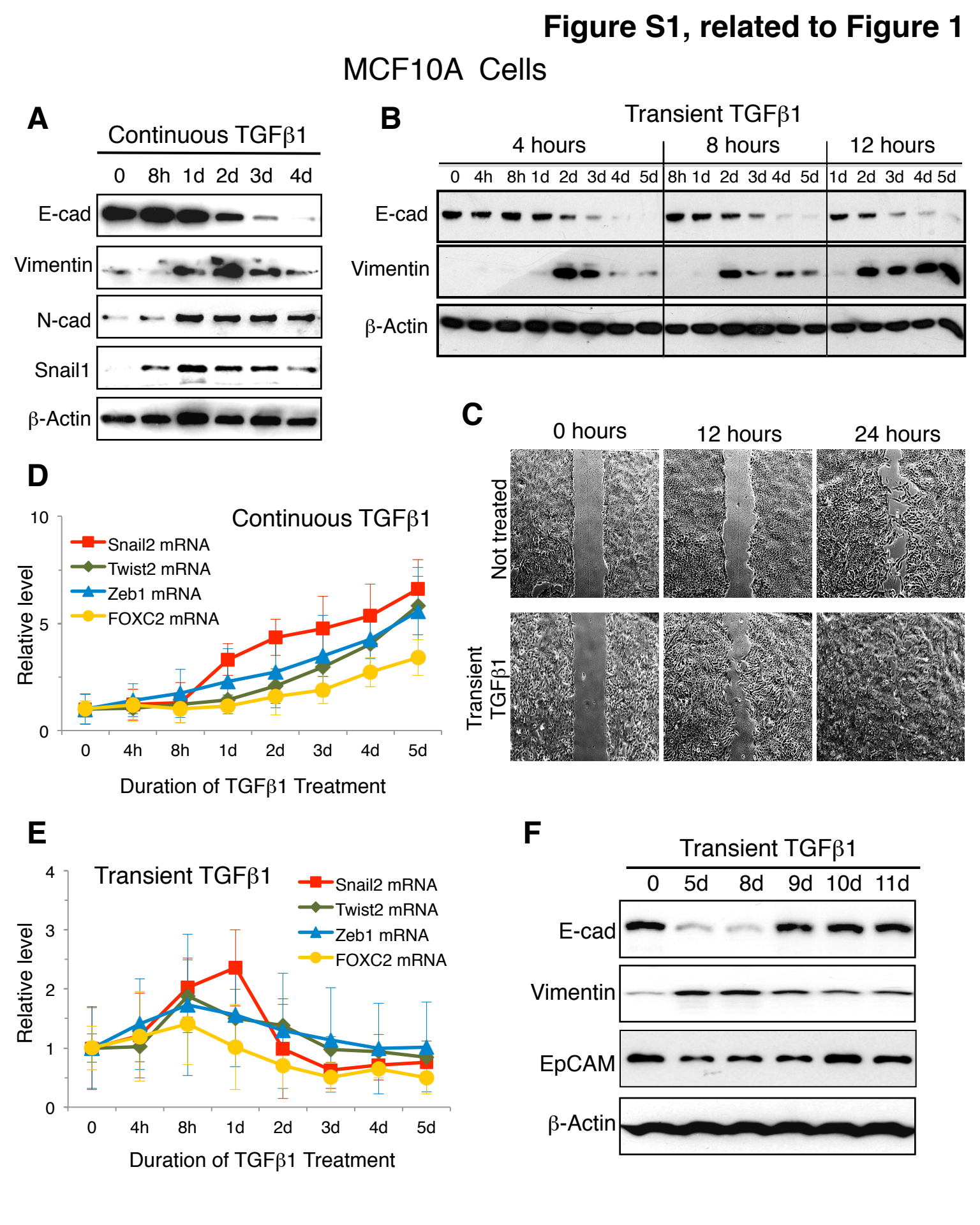


Figure S2, related to Figure 1

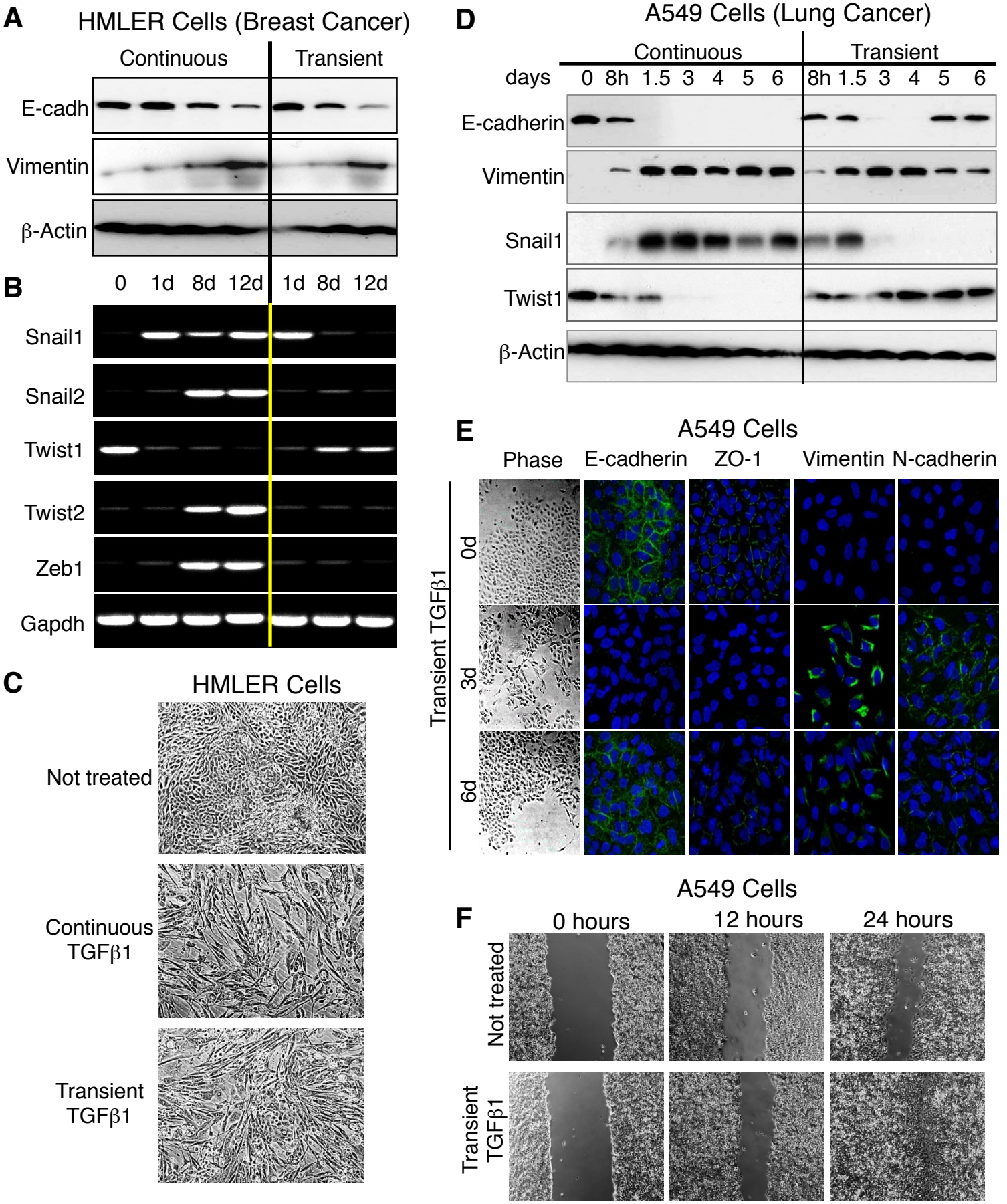
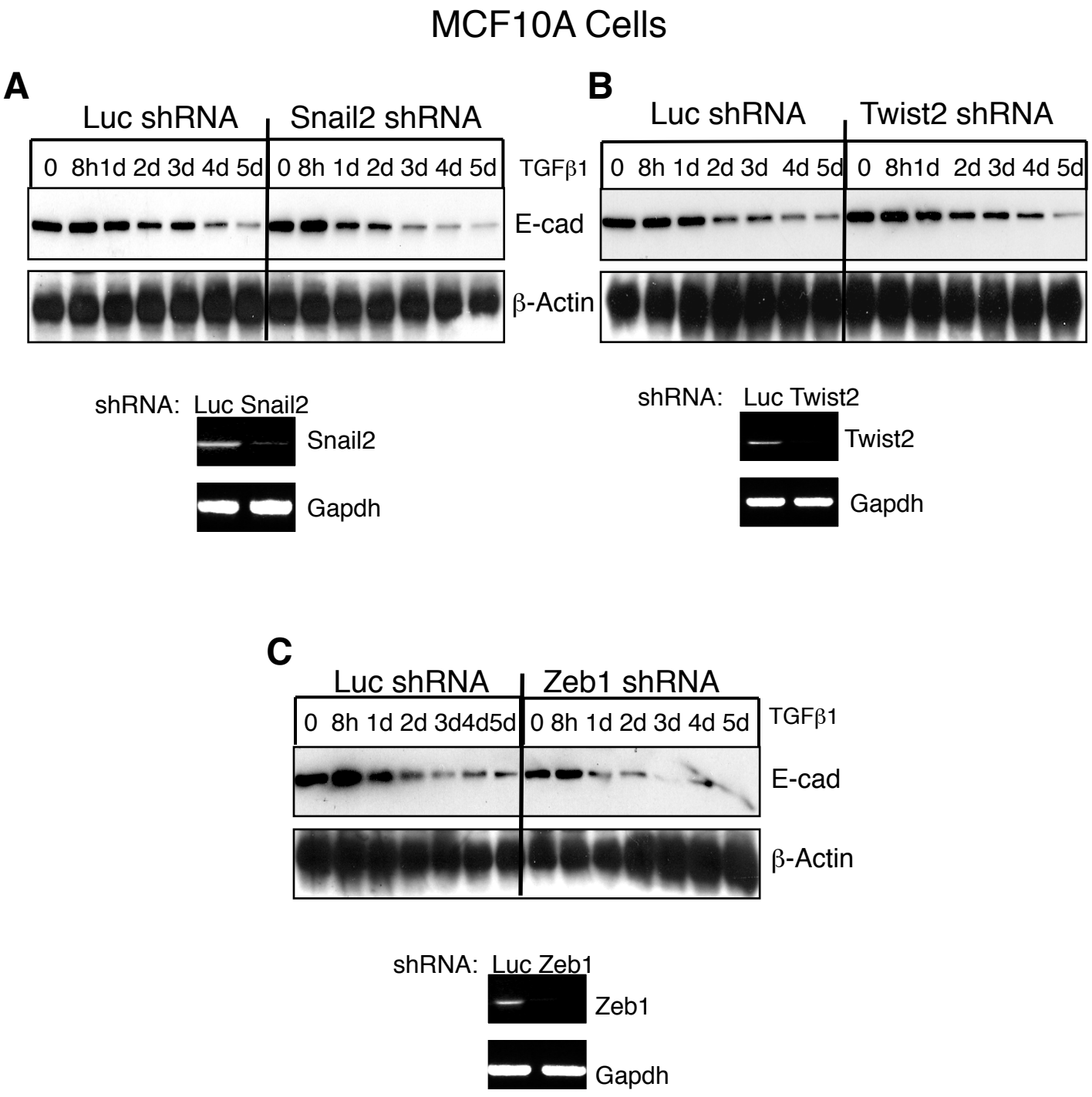


Figure S3, related to Figure 2



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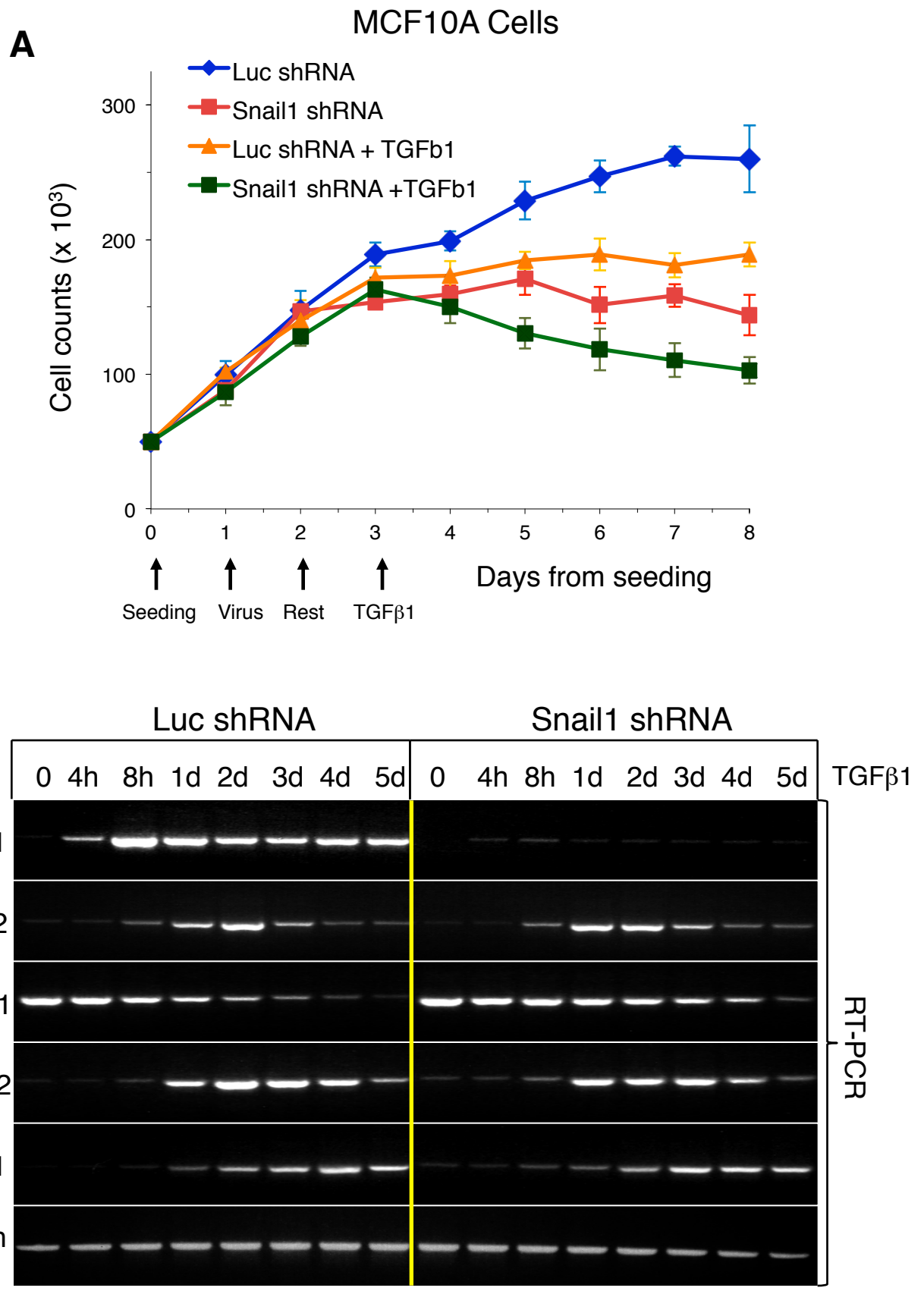

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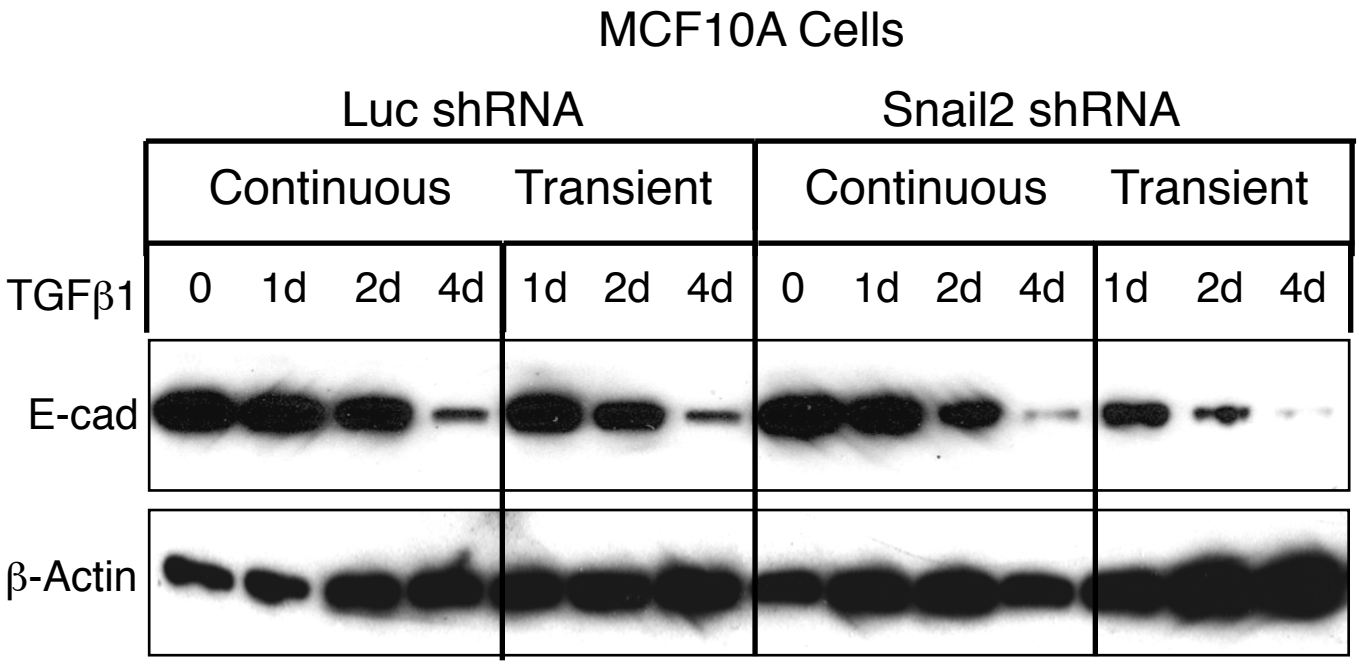

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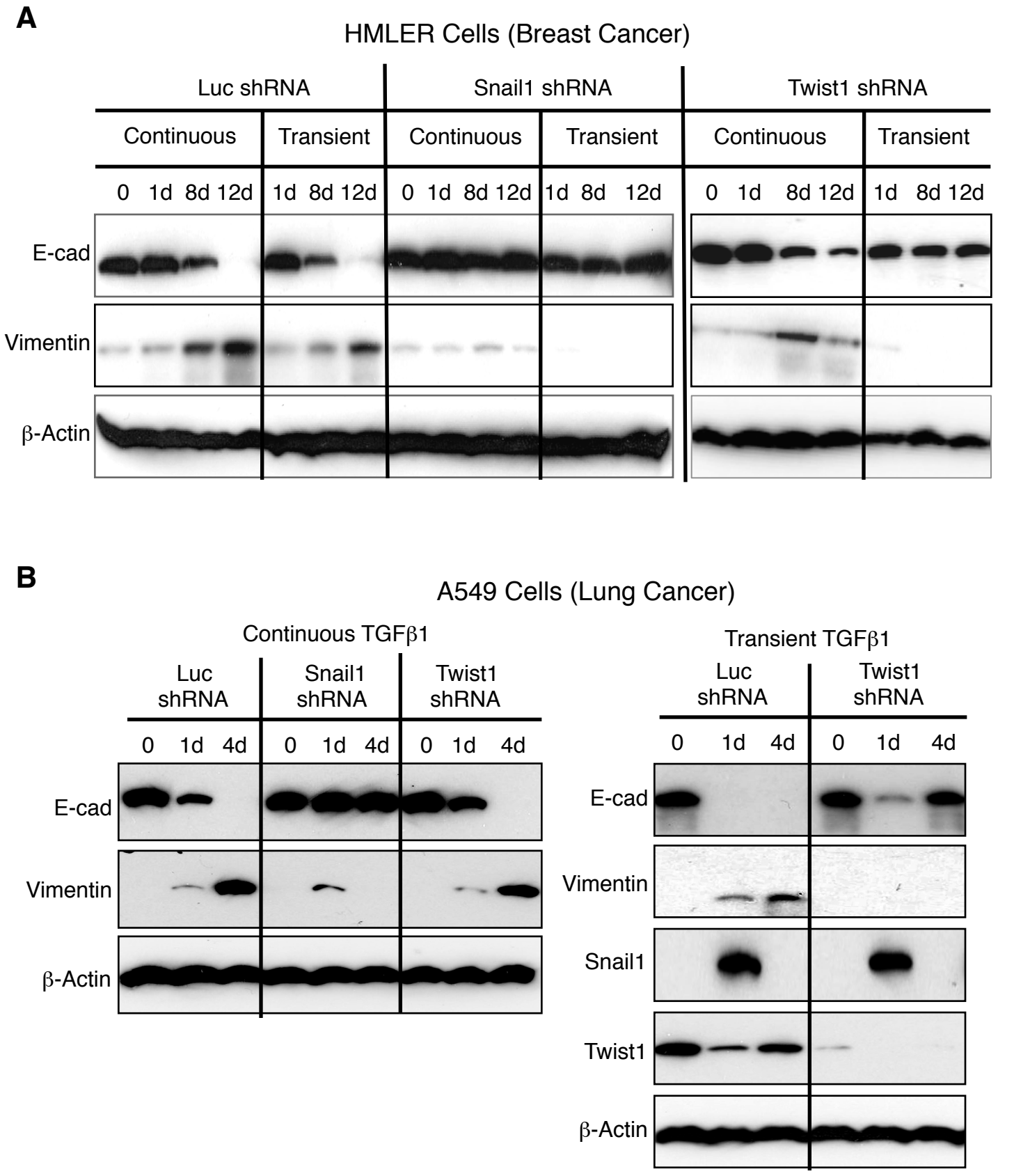
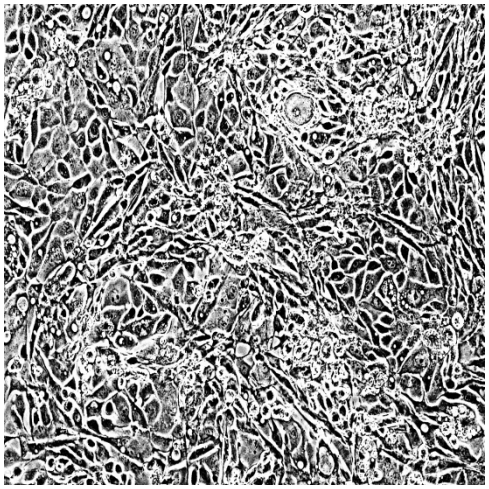


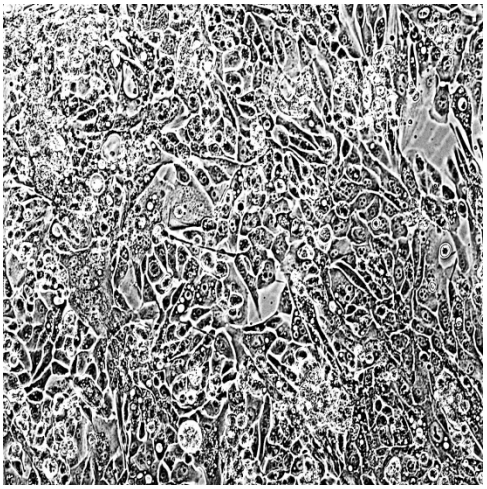
Figure S7, related to Figure 3

HLMER Cells

A Luc shRNA
Not treated



C Snail1 shRNA
Continuous TGFβ1



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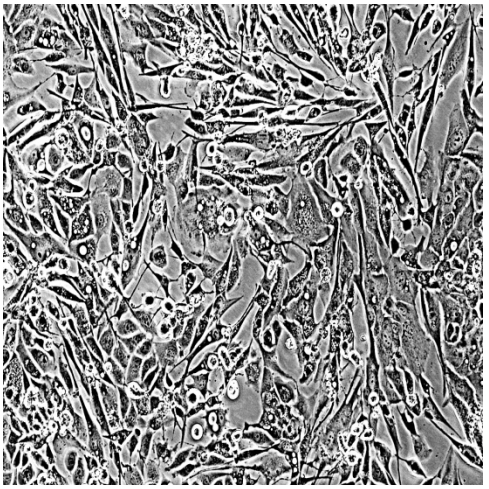
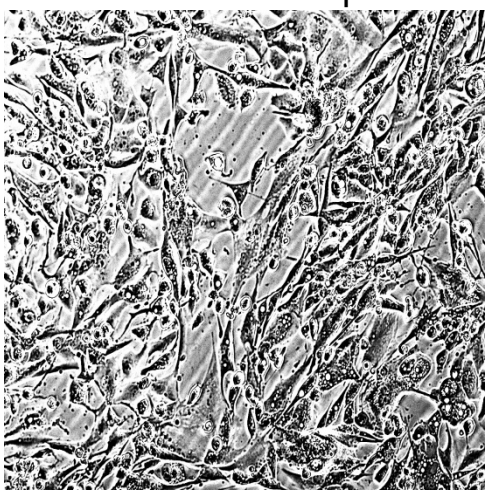
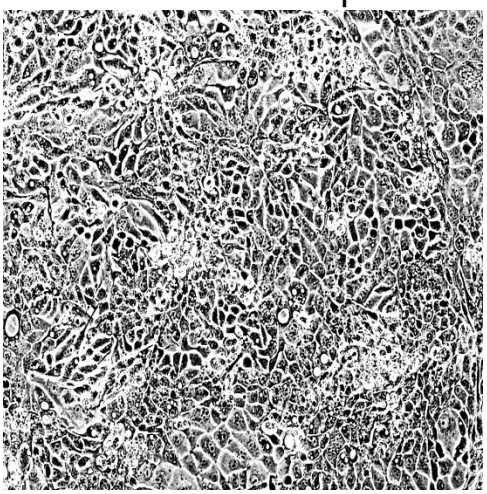
Continuous TGFβ1



B Luc shRNA
Transient TGFβ1



D Snail1 shRNA
Transient TGFβ1



F Twist1 shRNA
Transient TGFβ1

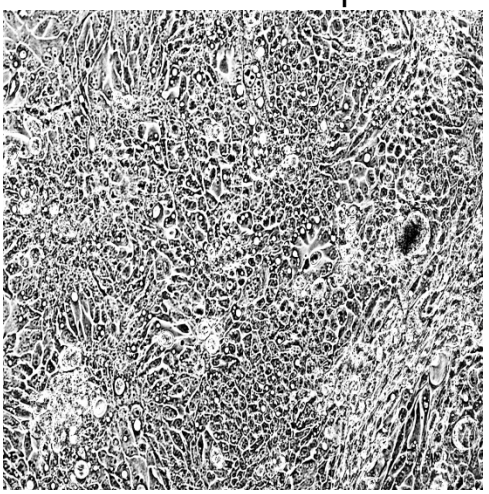


Figure S8, related to Figure 4

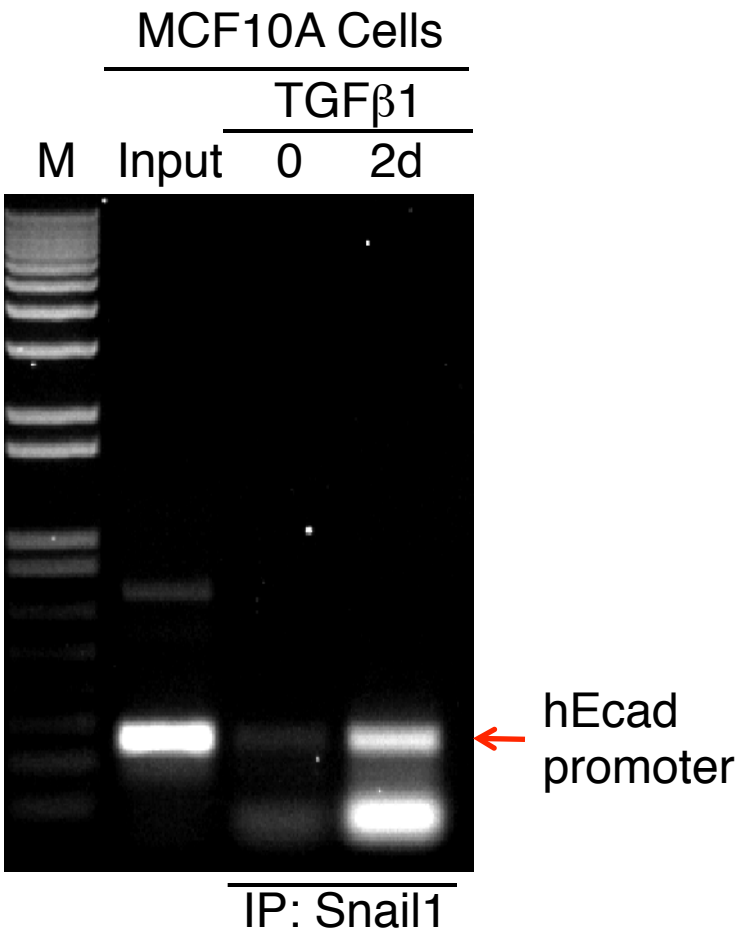


Figure S9, related to Figure 5

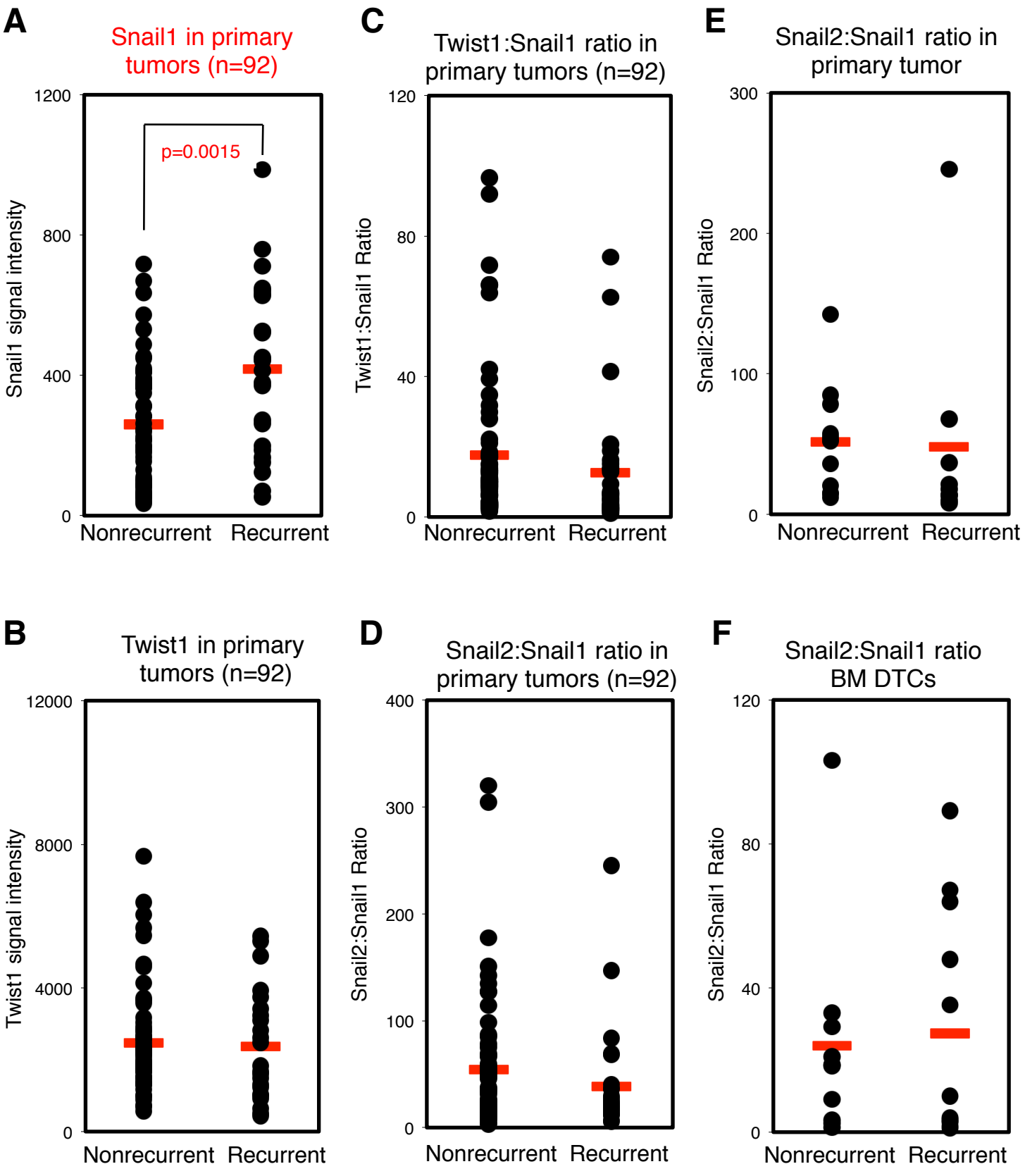


Figure S10, related to Figure 6

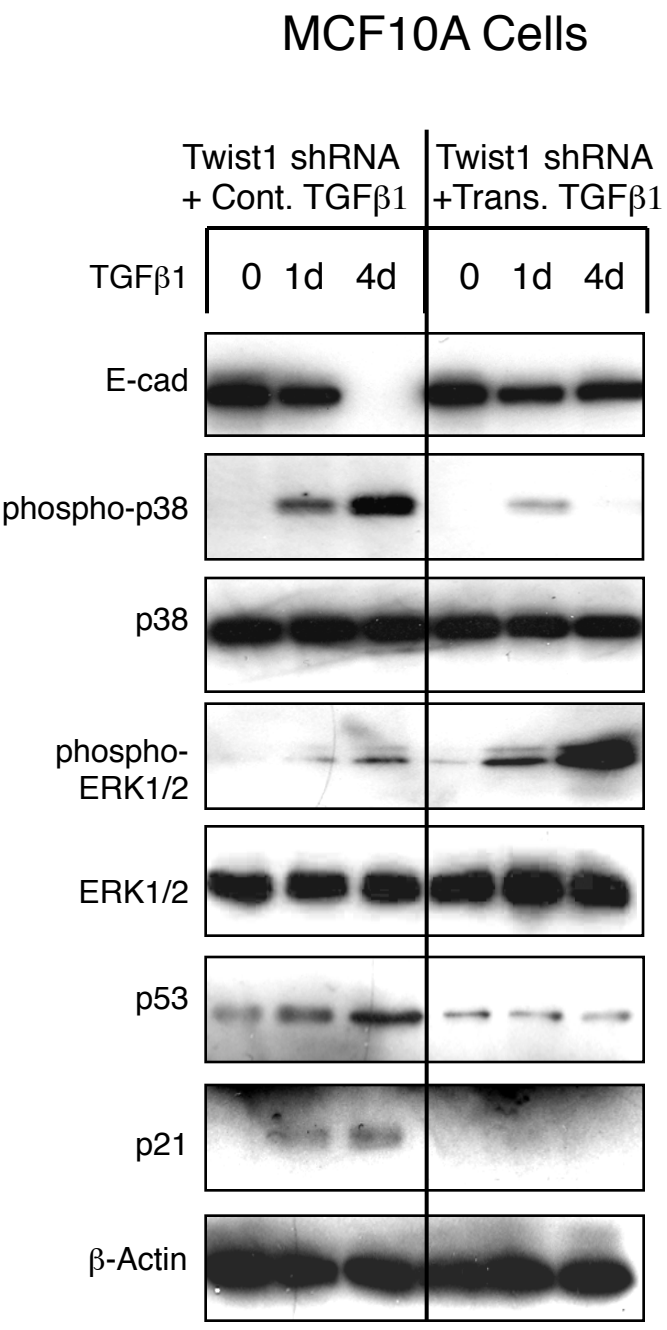
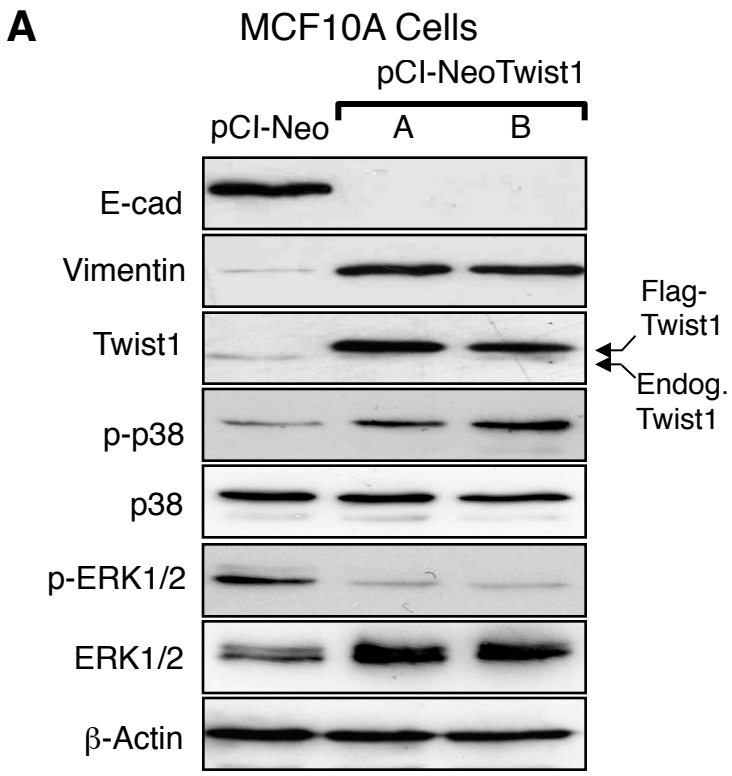
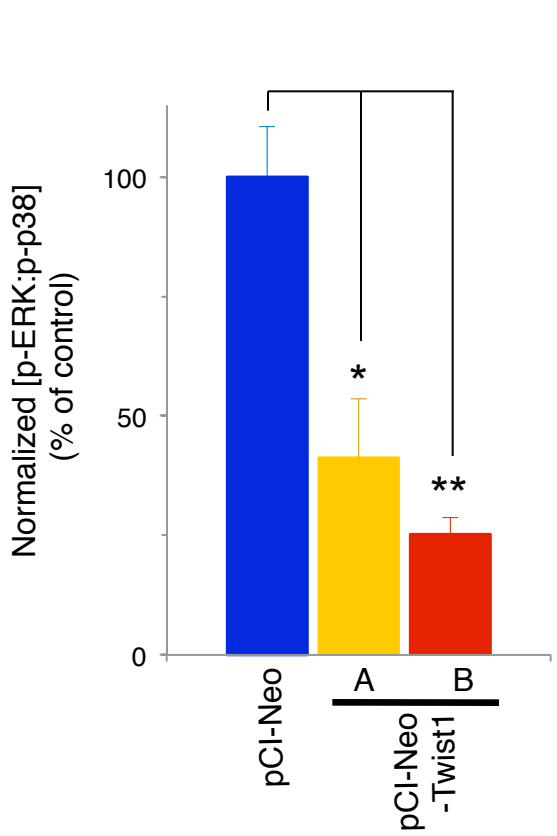
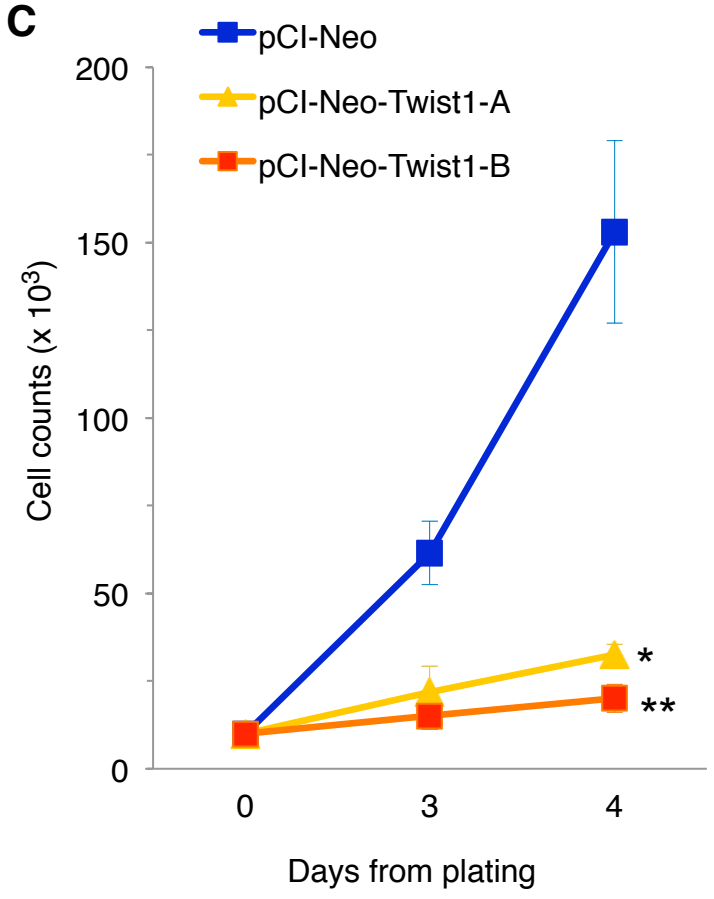


Figure S11, related to Figure 6







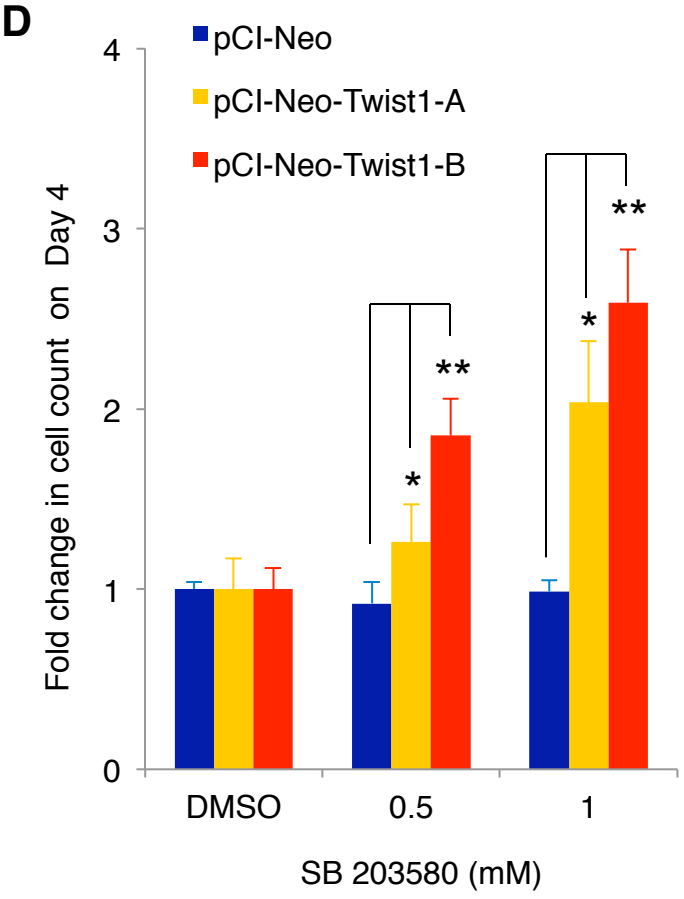


Figure S12, related to Figure 6

MCF10A cells

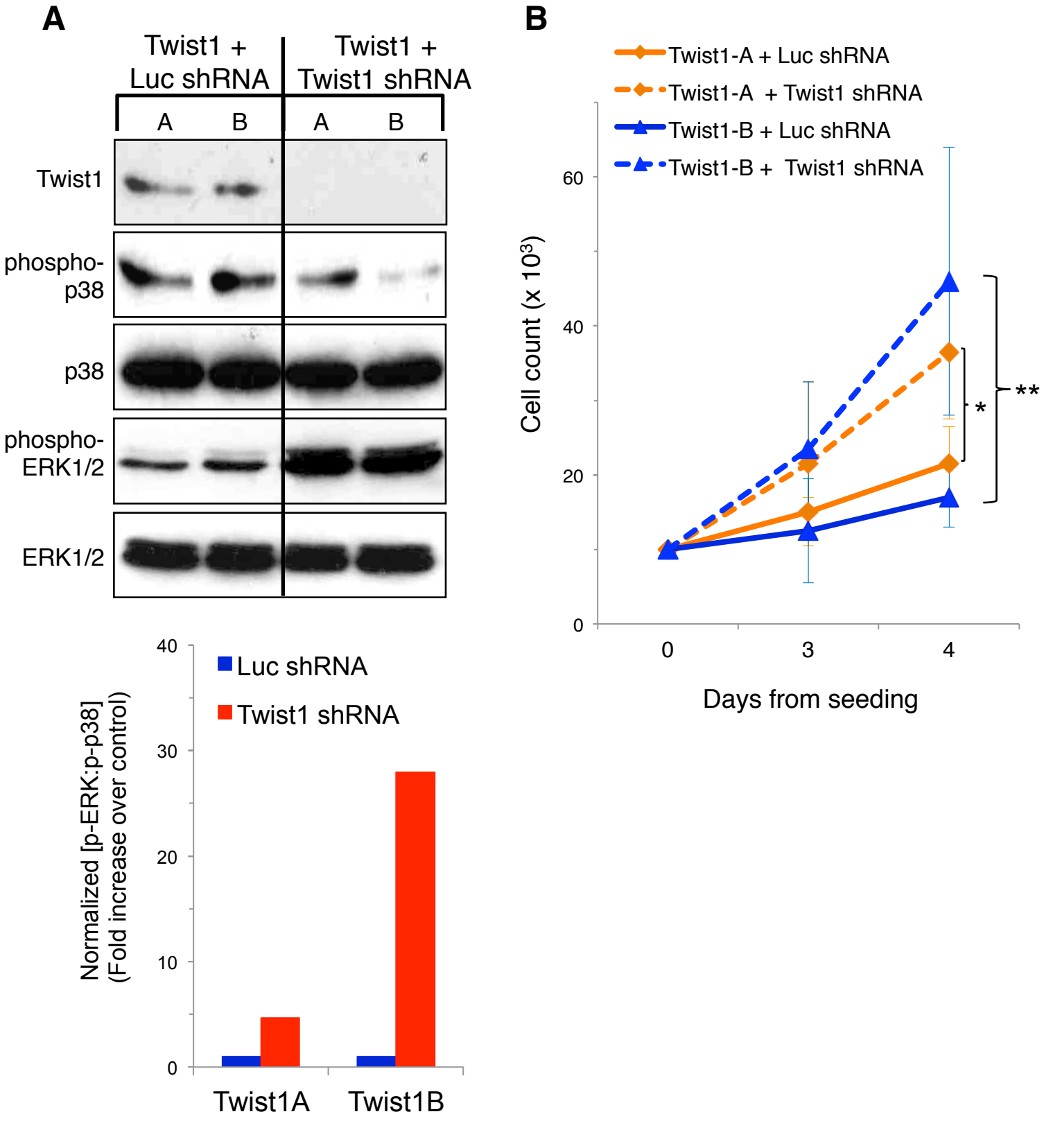
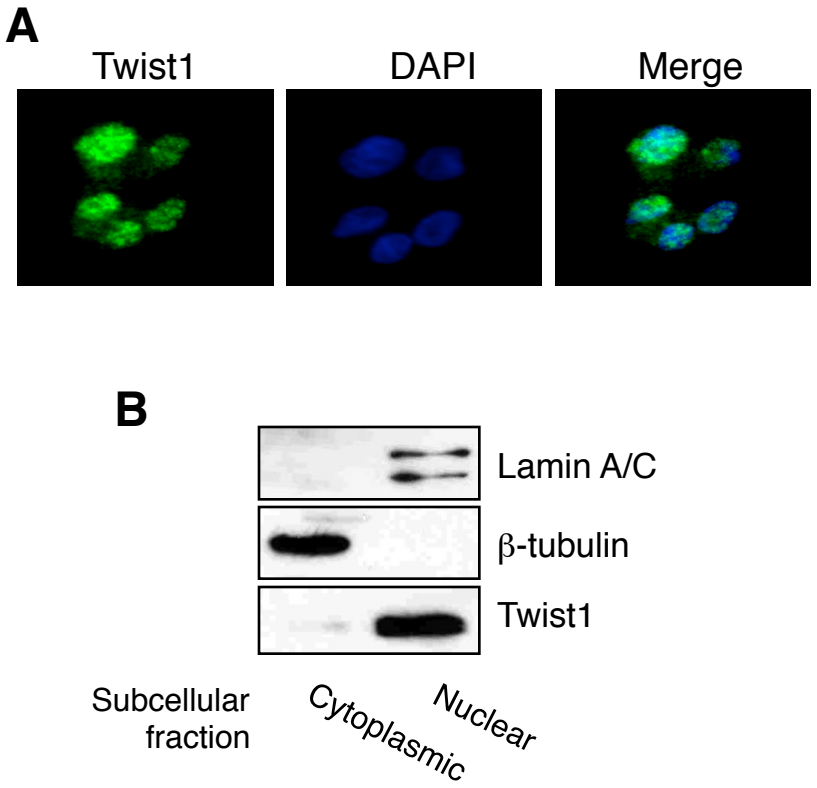


Figure S13


A
Twist1
DAPI
Merge
B
Lamin A/C
β-tubulin
Twist1
Subcellular fraction
Cytoplasmic
Nuclear