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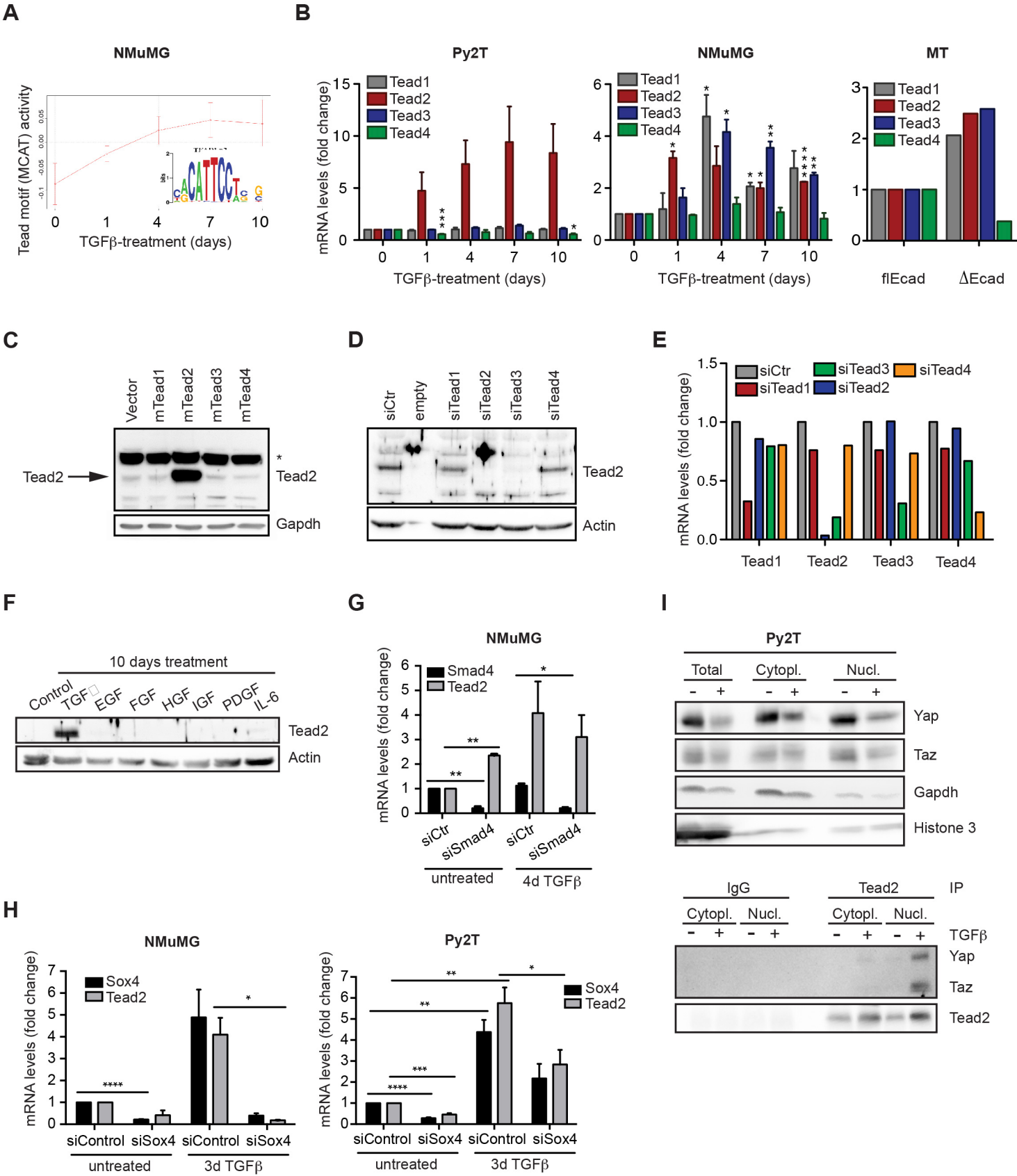
Supplementary material Fig. S1



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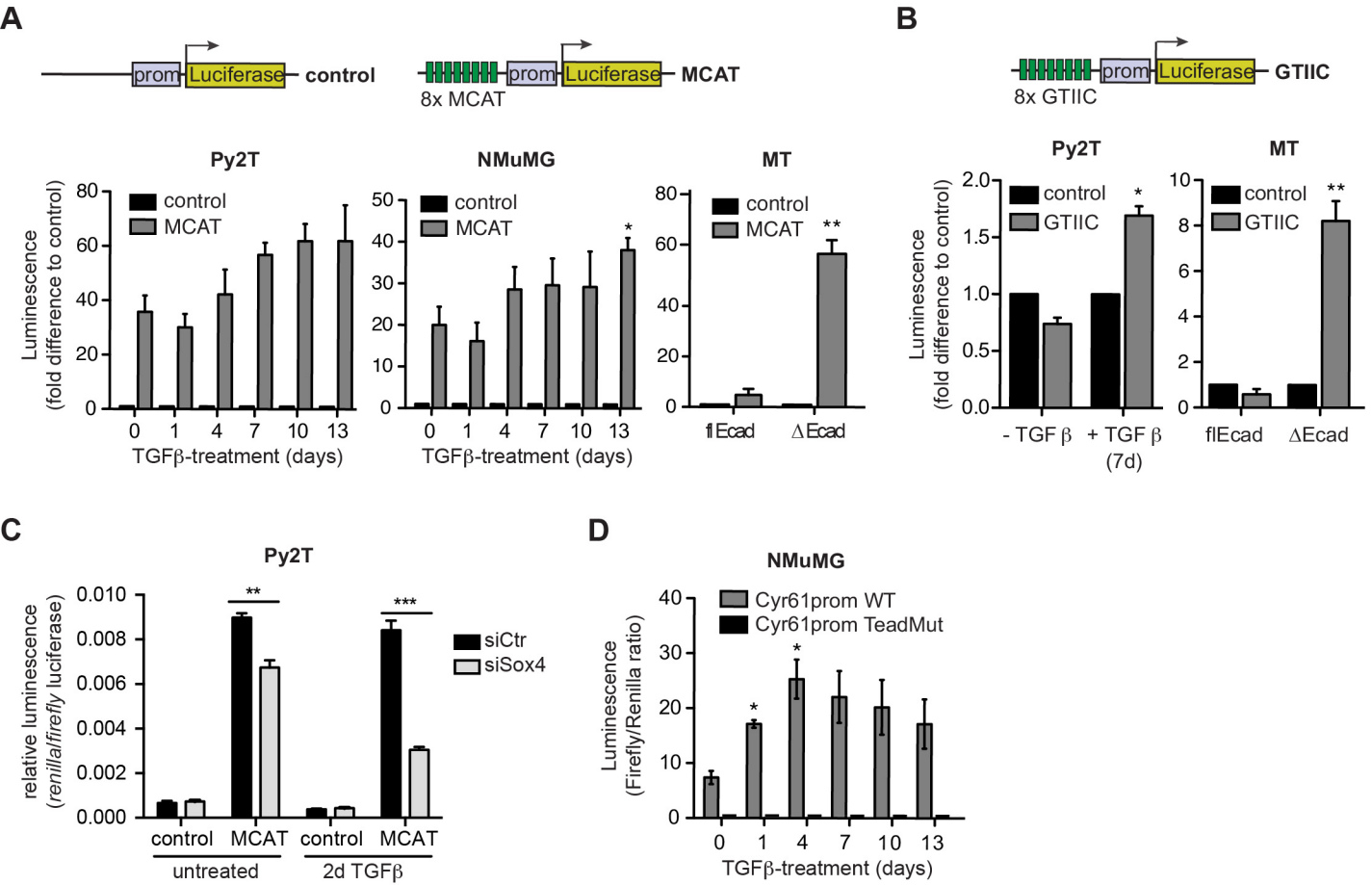
Supplementary material Fig. S2



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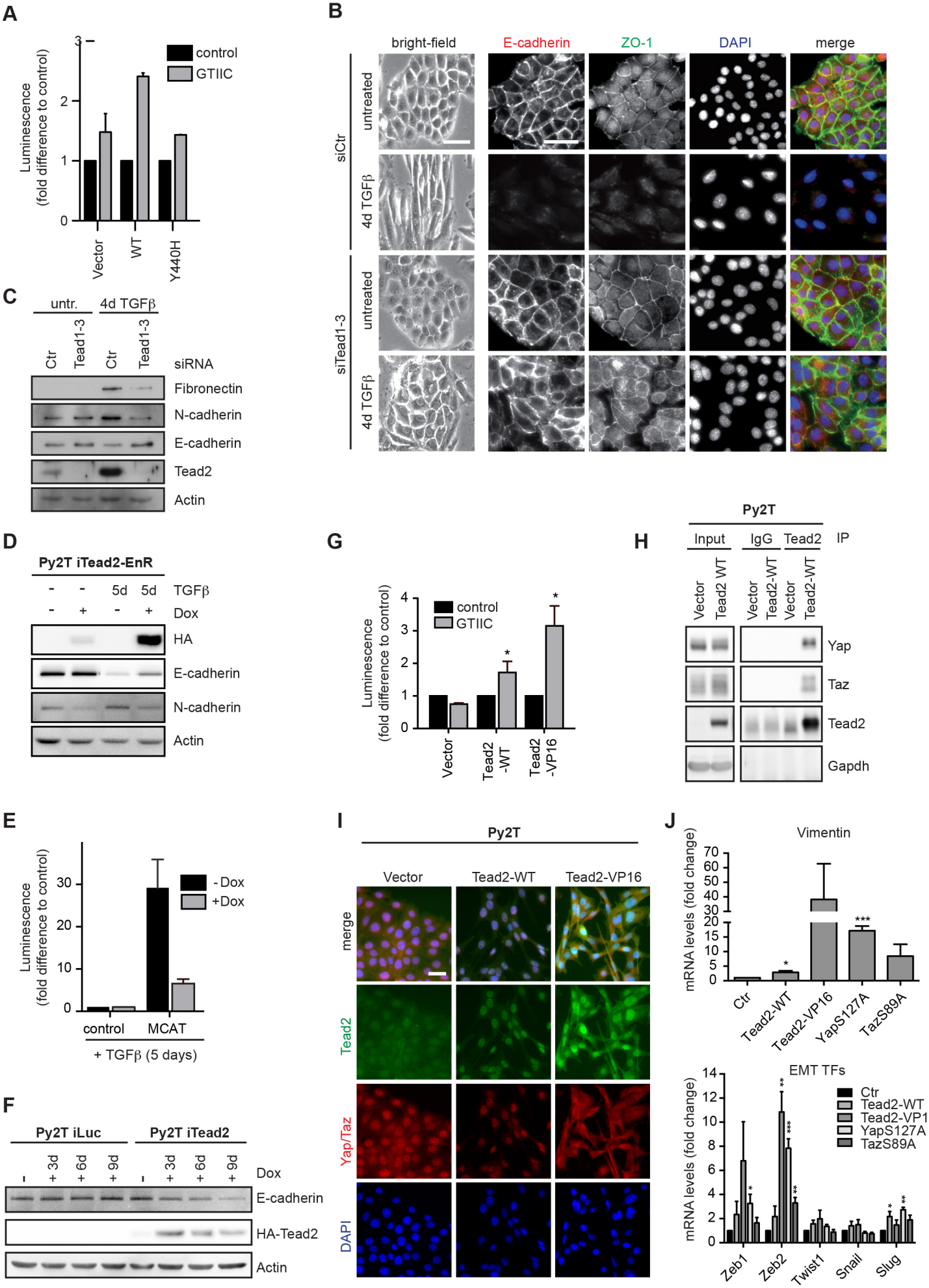
Supplementary material Fig. S3



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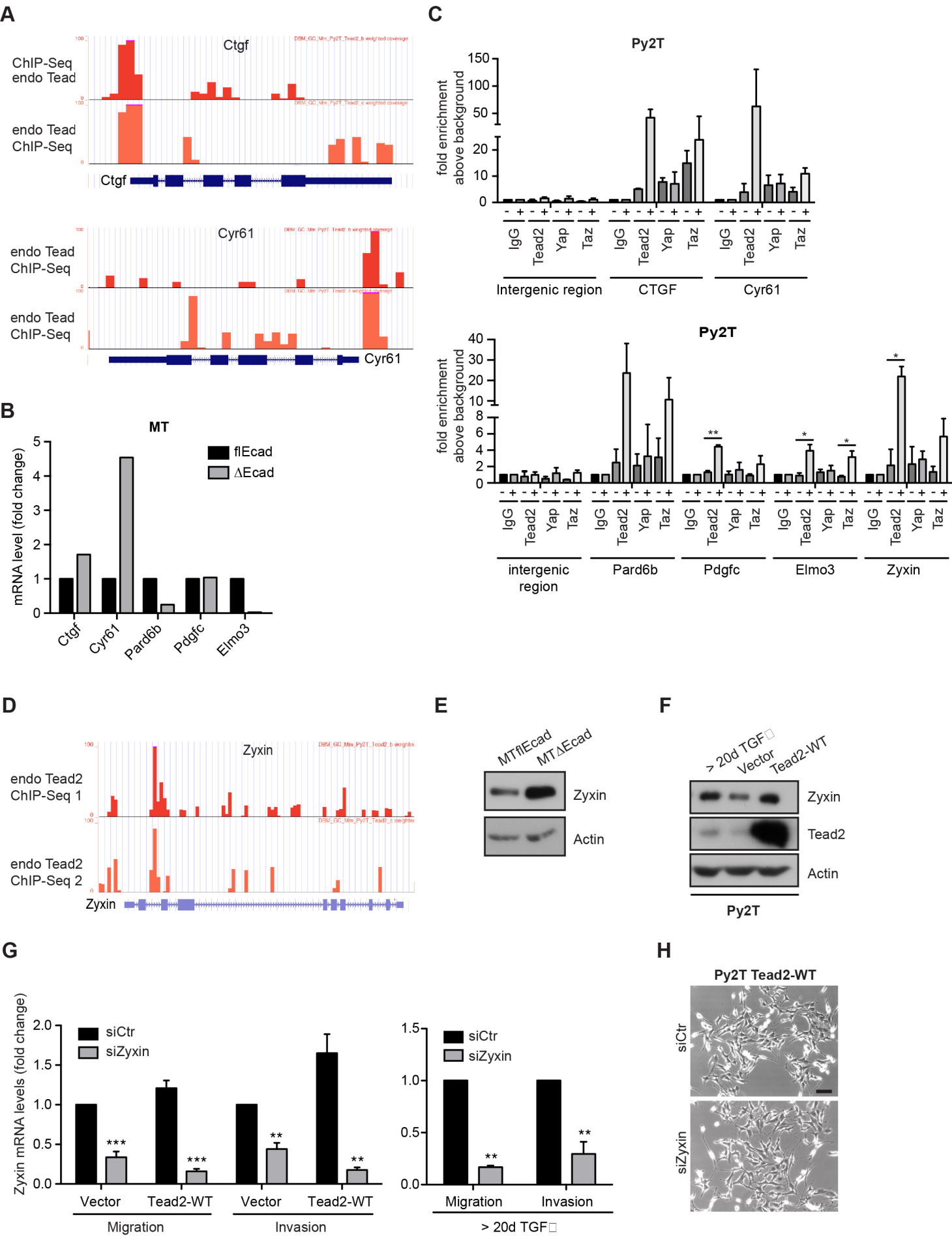
Supplementary material Fig. S4



Tead2 expression levels control Yap/Taz subcellular distribution, zyxin expression, and epithelial-mesenchymal transition

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Supplemental material Figure Legends

Fig. S1. Tead2 levels are upregulated in three EMT models.

(A) Motif Activity Response Analysis (MARA) predicts Tead activity during EMT in NMuMG cells. Shown is the Tead consensus binding motif (MCAT) used for the identification of Tead-regulated genes and the increase in the expression of Tead-regulated genes (activity) during TGFβ-induced EMT in NMuMG cells.

(B) Changes in mRNA expression of Tead1-4 during EMT. Time course experiments of TGFβ-induced EMT were performed as described in Fig. 1B and mRNA levels were determined by quantitative RT-PCR. Results are presented as mean fold difference ± s.e.m. (n = 3; *p < 0.05, **p < 0.01, ***p < 0.001; Py2T and NMuMG). Expression changes in MT cells were determined once in triplicates.

(C, D) Anti-Tead2 antibodies do not detect family members other than Tead2. Murine Tead 1-4 were transiently overexpressed in HEK293 cells (C) and siRNA pools against the different family members were transfected in NMuMG cells (D). Cell lysates were probed with α-Tead2 by immunoblotting. The asterisk marks a band that is not specific for murine Tead2.

(E) Quantification of siRNA efficiency in the experiment described in (D) by quantitative RT-PCR. The downregulation of Teads mRNA levels was specific for the targeted family members, with the exception of siTead3, which depleted the expression of Tead2 and Tead3.

(**F**) TGFβ-signaling induces the expression of Tead2. Py2T cells were treated or not with different growth factors for ten days followed by immunoblotting analysis for Tead2. Actin was used as a loading control. (EGF: epidermal growth factor; FGF: fibroblast growth factor, HGF: hepatocyte growth factor; IGF: insulin-like growth factor; PDGF: platelet derived growth factor; IL-6: interleukin 6).

(**G**) Tead2 upregulation via non-canonical TGFβ-signaling. NMuMG cells were transfected with siRNA pools against Smad4 or control siRNA and treated with TGFβ as indicated. mRNA levels of Smad4 and Tead2 were determined by RT-qPCR. Results are presented as mean ± s.e.m. (n = 2; *p < 0.05, **p < 0.01).

(**H**) Tead2 expression during EMT is dependent on Sox4. NMuMG (*left*) and Py2T (*right*) cells were transfected with siRNA pools against Sox4 or control siRNA. mRNA levels for Sox4 and Tead2 were determined by RT-qPCR. Results are presented as mean ± s.e.m. (n = 2 (NMuMG), n = 3 (Py2T); *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

(**I**) Subcelluar distribution of Yap and Taz and their interaction with Tead2 during EMT. Py2T cells were cultured in the presence (5 days) and absence of TGFβ. Nuclear/cytoplasmic fractions were prepared (*top*; 15% SDS-PAGE) followed by co-immunoprecipitation experiments (*bottom*; 10% SDS-PAGE) with an antibody against Tead2 or IgG (negative control). Levels of Yap, Taz, Tead2, Gapdh (cytoplasmic marker) and histone 3 (nuclear marker) were determined by immunoblotting analysis.

Fig. S2. Tead2 transcriptional activity increase during EMT.

(A,B) Measurement of Tead activity in the three cellular EMT systems by luciferase reporter assays. *Top panels:* Scheme of the luciferase reporter constructs. Eight repeats of the core MCAT (A) or GTIIC (B) Tead DNA binding motifs were cloned in front of a basal promoter followed by the *Firefly* luciferase gene. A construct lacking a Tead-binding motif served as control. *Bottom panels:* NMuMG and Py2T cells were treated with TGFβ for the indicated durations prior to luminescence analysis. Reporter constructs were transfected along with a *Renilla* luciferase construct for normalization. Results are shown as mean ± s.e.m. (NMuMG: n = 3; Py2T: n = 5; MT: n = 2; *p < 0.05; **p < 0.01).

(**C**) Analysis of Tead transcriptional activity after Sox4 depletion. Py2T cells were transfected with siRNA pools against Sox4 or a control and treated with TGFβ as indicated. Relative Tead activity was determined by using the Tead-responsive MCAT luciferase reporter construct as described in supplementary material Fig. S2A. Results are shown as mean ± s.e.m. (n = 3; **p < 0.01, ***p < 0.001).

(D) Tead activity was determined using a luciferase reporter construct harboring the promoter of the Cyr61 gene (Cyr61prom WT). The same construct with a mutated Tead binding site (Cyr61prom TeadMut) was used as control. Results are shown as mean ± s.e.m. (n = 3; *p < 0.05).

Fig. S3. Tead2 upregulation is required for EMT.

(A) Effect of Tead2 WT or Tead2 Y440H overexpression on Tead activity. Measurement of Tead activity in NMuMG cells stably expressing wildtype Tead2 (WT) or a point mutant unable to interact with Yap/Taz (Y440H) using the Tead-responsive GTIIC luciferase reporter construct as described in supplementary material Fig. S2B. Data represent mean ± s.e.m. (n =2).

(B) Effect of Tead knockdown on cell morphology and the disassembly of adherens and tight junctions. NMuMG cells were transfected with siRNA pools targeting each Tead1, 2, and 3. One day after transfection, cells were treated or not with TGFβ as indicated. Overall cell morphology (phase contrast) and immunofluorescence staining of adherens junctions (E-cadherin) and tight-junctions (ZO-1) are shown. DAPI staining visualizes cell nuclei. Scale bars, 50 μm.

(C) Immunoblotting analysis for the expression of the mesenchymal markers Fibronectin and N-cadherin and the epithelial marker E-cadherin in the experiment described in (B). Immunoblotting for Tead2 was used to assess knockdown efficiency, and immunoblotting for actin was used as a loading control.

(D) Interference with Tead transcriptional activity in Py2T cells undergoing EMT. A HA-tagged, dominant-negative version of Tead2 (Tead2-EnR) was expressed in Py2T cells in a doxycycline-inducible fashion (Py2T-iTead2-EnR). The Tead2-EnR construct encompasses the Tead2 DNA-binding domain fused to the transcriptional repression domain of *Drosophila* Engrailed. Py2T-iTead2-EnR cells were induced to undergo EMT by TGFβ treatment for five days in the presence or absence of doxycycline (Dox), and the expression of Tead2-EnR (HA) and of the EMT markers E-cadherin and N-cadherin were analyzed by immunoblotting. Actin was used as loading control.

(E) In the experiment described in (D), Tead transcriptional activity was determined by using the MCAT *firefly* luciferase reporter as described in supplemental material Fig. S1F. Results are presented as mean ± s.e.m. (n = 2).

(**F**) Impact of prolonged Tead2 expression on E-cadherin protein levels. Py2T cells were induced to express HA-tagged Tead2-WT (iTead2) or *firefly* luciferase (iLuc) as a control upon doxycycline treatment for indicated periods. Tead2 (HA) and E-cadherin expression are shown by immunoblotting analysis. Actin served as loading control.

(G) Analysis of Tead transcriptional activity in Py2T cells expressing wildtype Tead2 (Tead2-WT) or a constitutively active version of Tead2 (Tead2-VP16) using the GTIIC Tead-responsive *Firefly* luciferase reporter construct (supplemental material Fig. S1G). The results shown represent mean ± s.e.m. ($n = 2$; $*p < 0.05$).

(H) Determination of the amount of Yap/Taz/Tead2 complexes in control (vector) and Tead2-expressing Py2T cells (Tead2-WT). Lysates were subjected to immunoprecipitation with a Tead2 antibody or with irrelevant IgG as negative control (IP). The presence of Tead2 and of co-immunoprecipitated Yap and Taz was analyzed by immunoblotting. The levels of these proteins in total cell lysates were also assessed (Input). Gapdh was used as loading control.

(I) Immunofluorescence staining of Yap/Taz and Tead2 in Py2T cells expressing wildtype Tead2 (Tead2-WT), constitutively active Tead2 (Tead2-VP16) or transduced with an empty vector control in the absence of TGFβ. Scale bar, 25 μm.

(J) Analysis of mRNA expression levels of vimentin and EMT transcription factors in Py2T cells stably expressing wildtype Tead2 (Tead2-WT), constitutively active Tead2 (Tead2-VP16) or the Hippo-insensitive mutants of Yap (Yap S127A) and Taz (Taz S89A). The results shown represent mean ± s.e.m. ($n = 3$; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Fig. S4. Identification and validation of direct Tead2 target genes.

(A) Genome browser view of Tead2 ChIP-Seq data showing the binding of Tead2 at promoter regions of the known Tead target genes *Ctgf* and *Cyr61*. Py2T cells were treated with TGFβ for five days, chromatin was prepared and subjected to chromatin immunoprecipitation (ChIP) using a Tead2 antibody. Precipitated chromatin was then subjected to next generation sequencing. Data of two independent biological replicates are shown on separate wiggle-tracks.

(B) Expression of Tead2 target genes in the MTflEcad/MTΔEcad model of EMT as determined by quantitative RT-PCR.

(**C**) Binding of Yap and/or Taz to direct Tead2 target genes. Py2T cells were treated with TGFβ for five days, chromatin was isolated and subjected to ChIP using a Tead2, Yap, Taz or IgG antibody as negative control. ChIP-qPCR analysis was performed as shown in Fig. 4E. Data are presented as mean ± s.e.m. (n = 2, *p < 0.05; **p < 0.01).

(D) Genome browser view of the Tead2 ChIP-Seq data showing the binding of Tead2 at an intronic region in the zyxin gene (*Zyx*).

(E) Immunoblotting analysis of zyxin expression levels in the MTflEcad/MTΔEcad model. Actin served as a loading control.

(F) Immunoblotting analysis of zyxin and Tead2 expression levels in Py2T cells transduced with an empty vector (Vector) or overexpressing wildtype Tead2 (Tead2-WT), and Py2T cells treated with TGFβ for at least 20 days (> 20d TGFβ).

(G) Efficiency of siRNA-mediated ablation of zyxin expression. Quantification of zyxin mRNA levels by quantitative RT-PCR in control Py2T cells (Vector) or cells expressing wildtype Tead2 (Tead2-WT; *left*) and in Py2T cells treated with TGFβ for at least 20 days (> 20d TGFβ; *right*) and treated with siRNA against zyxin (siZyxin) or control siRNA (siCtr). Measurements were performed in parallel to the transwell migration/invasion assays shown in Fig. 5K. (n = 3 mean ± s.e.m.; **p < 0.01, ***p < 0.001)

(**H**) Cell morphology of Py2T cells stably overexpressing Tead2-WT after siRNA-mediated depletion of zyxin. Phase-contrast images of Py2T cells overexpressing Tead2-WT two days after transfection of siRNA pools against zyxin or a control siRNA. Scale bar, 100 μm.

Table S1. Tead2 ChIP Sequencing Analysis

Py2T cells were treated with TGFβ for five days and subjected to chromatin immunoprecipitation using the Tead2 antibody followed by next generation sequencing (ChIP-Seq; n = 2). The Excel file shows a comprehensive list of Tead2-bound genes and their expression [log2 fold change (FC)] during TGFβ-induced EMT.

Download Table S1

Table S2. Genes that are directly regulated by Tead2 during EMT

Gene	log2 FC	pValue
Il11	5.56	9.21E-04
Serpine1	3.35	1.51E-03
Olfm2	3.04	5.49E-03
Pdgfc	2.98	1.43E-03
Pgf	2.84	1.58E-03
Itga5	2.80	1.06E-03
Marcksl1	2.54	5.21E-03
Clu	2.53	2.12E-03
Adamts4	2.07	1.47E-03
9930111J21Rik2	2.01	6.11E-03
Hbegf	1.89	2.94E-03
Kctd11	1.87	1.13E-02
Cyr61	1.82	1.71E-03
Rhob	1.76	1.34E-02
Sfn	1.73	3.53E-03
Sfn	1.72	6.36E-03
Ccrn4l	1.61	2.52E-03
Mir181b-2	1.50	2.99E-02
Nppb	1.47	1.90E-02
Ctgf	1.36	2.49E-03
Itpripl2	1.33	2.52E-03
Tnfaip6	1.33	3.53E-03
Rnf122	1.33	3.09E-02
Cd97	1.32	1.21E-02
Ctla2b	1.31	1.89E-02
Tmprss11f	1.30	1.07E-02
Myo7a	1.22	8.21E-03
Smad7	1.21	4.44E-03
Limk1	1.21	7.45E-03
Itpripl2	1.20	6.44E-03
Atg4c	1.19	7.18E-03
Creb3l2	1.12	2.01E-02
Cttnbp2nl	1.08	4.88E-03
Tmcc3	1.08	3.54E-02
Zyx	1.04	2.84E-02
Plaur	1.04	6.24E-03
Ier3	1.03	6.21E-03
Mfhas1	1.03	4.02E-03
Cnn3	1.01	6.17E-03
Podnl1	1.01	9.48E-03
Klk9	0.99	2.75E-02
Atp11a	0.99	5.39E-03
Amotl2	0.96	1.21E-02
Schip1	0.94	6.33E-03

Fbln2	0.94	9.52E-03
Sdcbp2	0.92	1.94E-02
Klf6	0.91	8.44E-03
Zfp451	0.90	2.90E-02
Soga1	0.89	1.35E-02
Egr3	0.89	1.41E-02
Gne	0.87	3.17E-02
Acvr1	0.86	8.82E-03
Adcy7	0.86	2.19E-02
Cnn3	0.83	5.74E-03
Lrrc8c	0.82	1.19E-02
Trim13	0.81	2.25E-02
Mir22hg	0.80	3.80E-02
B230120H23Rik	0.80	1.73E-02
Zfp658	0.79	9.73E-03
Ugdh	0.78	1.43E-02
Loxl3	0.76	1.36E-02
E030011O05Rik	0.75	1.69E-02
Synpo	0.75	1.89E-02
Ercc1	0.75	6.86E-03
Ncor2	0.74	4.56E-02
Csrp1	0.73	2.74E-02
Atp6v1g1	0.72	4.26E-02
H2-Ab1	0.72	1.30E-02
Tmem150a	0.71	3.27E-02
Ankrd1	0.71	9.96E-03
Sept9	0.69	1.50E-02
Zfp568	0.68	4.68E-02
Mapk6	0.66	1.37E-02
Atp6v1g1	0.66	3.74E-02
Fxr2	0.66	1.48E-02
Lamb1	0.63	2.60E-02
Mat2a	0.62	3.97E-02
Cdc42ep1	0.61	2.42E-02
Fpgt	0.60	1.45E-02
Zkscan5	0.59	3.11E-02
Dok1	0.58	2.64E-02
Pcif1	-0.59	1.81E-02
Ect2	-0.62	1.69E-02
Tnfrsf1b	-0.63	1.19E-02
Zfp672	-0.67	2.64E-02
Tnni2	-0.68	3.15E-02
Ift80	-0.68	1.07E-02
Cep68	-0.69	1.05E-02
Tmem106b	-0.70	1.90E-02
Zcwpw1	-0.70	1.77E-02
Ahnak	-0.70	9.37E-03

Unc13d	-0.77	3.39E-02
A430105I19Rik	-0.78	7.18E-03
Tspan4	-0.78	9.58E-03
Ly6f	-0.80	1.91E-02
Rere	-0.83	9.26E-03
Arrdc1	-0.83	9.26E-03
Sh3rf2	-0.88	1.91E-02
Myo6	-0.91	1.83E-02
Nbeal2	-0.93	4.17E-03
Hist2h3c2	-0.99	4.65E-02
Pkhd1l1	-1.00	1.56E-02
Tnfrsf26	-1.02	1.82E-02
Rnf13	-1.04	1.33E-02
Malat1	-1.05	2.49E-02
Yipf2	-1.11	4.02E-02
March9	-1.14	4.55E-02
Tmtc2	-1.15	4.09E-03
Entpd3	-1.16	4.66E-02
Jag2	-1.16	4.07E-03
Tinagl1	-1.18	4.29E-03
Airn	-1.20	6.26E-03
Prss22	-1.20	7.39E-03
Pard6b	-1.21	1.45E-02
Syt8	-1.27	4.40E-03
Capn5	-1.27	1.59E-02
H1f0	-1.31	4.77E-03
Emp2	-1.35	4.61E-02
Clip4	-1.36	4.28E-03
Tlr5	-1.40	3.10E-03
Dcaf4	-1.45	5.04E-03
Atp6v0a1	-1.45	1.33E-02
Esrp2	-1.54	6.11E-03
Ckb	-1.68	5.39E-03
Uap1l1	-1.73	3.75E-02
Atp1b1	-1.82	1.43E-03
Blnk	-1.84	2.04E-03
Rbm47	-2.00	2.87E-02
Mal	-2.07	3.83E-03
Gpr97	-2.08	1.34E-02
Elmo3	-2.12	4.99E-03
Tenc1	-2.18	1.43E-03
Afap1l2	-2.39	2.30E-03
Klk10	-3.24	3.83E-03
Cldn9	-3.89	9.11E-04
Cp	-4.01	3.45E-02

Listed are genes, which are directly bound by Tead2 and change their expression after five days of TGF -induced EMT in Py2T cells. Genes are represented by their gene names, their log2 fold-changes (log2FC) and by statistical significance (p-value).

Table S3. Primer sequences used for RT-pPCR

Name	**Sequence (5'- 3')**
Tead1	ccaggatcctcacaagacg
	gaatgggggctgtgactg
Tead2	ctgaggacagggaagacgag
	cttcgagccaaaacctgaat
Tead3	gagctgattgcccgctac
	tgtatgtggctggacacctg
Tead4	tcaaaacacctaccctgtcca
	gccctgcaggagactcaa
Zyxin	aagagaagcagcacccaca
	ctctacctccttcagggtcaag
E-cadherin	cgaccctgcctctgaatcc
	tacacgctgggaaacatgagc
Cyr61	ctggcatctccacacgagttac
	tgccctttttaggctgctg
Rpl19	ctcgttgccggaaaaaca
	tcatccaggtcaccttctca
Vimentin	ccaacctttttcttccctgaa
	ttgagtgggtgtcaaccaga
Zeb1	gccagcagtcatgatgaaaa
	tatcacaatacgggcaggtg
Zeb2	ggaggaaaaacgtggtgaactat
	gcaatgtgaagcttgtcctctt
Twist	gccggagacctagatgtcattg
	cacgccctgattcttgtgaa
Snail	ctctgaagatgcacatccgaa
	ggcttctcaccagtgtgggt
Slug	tgtgtctgcaagatctgtggc
	tccccagtgtgagttctaatgtg

Ctgf	ctctcgtcgcctctgcac
	cagtcctggcccatagca
Pard6b	tgggctacgcagatatcca
	ggcactgtagtcagcttcttcc
Elmo3	ctcagcactgccccagat
	gcacccttcacgacttgtatt
Pdgfc	tgtgtcccacgtaaagttacaaa
	tcagtgagtgacttatgcaatcc

Table S4. Primer sequences used for ChIP-qPCR

Name	**Sequence (5'- 3')**
Intergenic region	gctccgggtcctattcttgt
	tcttggtttccaggagatgc
Ctgf	caatccggtgtgagttgatg
	ggcgctggcttttatacg
Cyr61	ctctgatggatctgagaagagg
	gccctttataatgcctgccta
Pard6b	ccacctactttccagcagaga
	gctattttgaggcattactcagc
Elmo3	tgcctctgagaatagctgga
	ggagatccagaactgaaagagaa
Pdgfc	tgctctgtggattgcttcac
	ctccaagaagaagcctgtcc
Zyxin	ccctgtcctgagcagatgtt
	agaacgagccaggttgaaga