

## **Supplementary Information**

**TGF- $\beta$  signalling and PEG10 are mutually exclusive and inhibitory in chondrosarcoma cells**

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## Supplementary Table S1

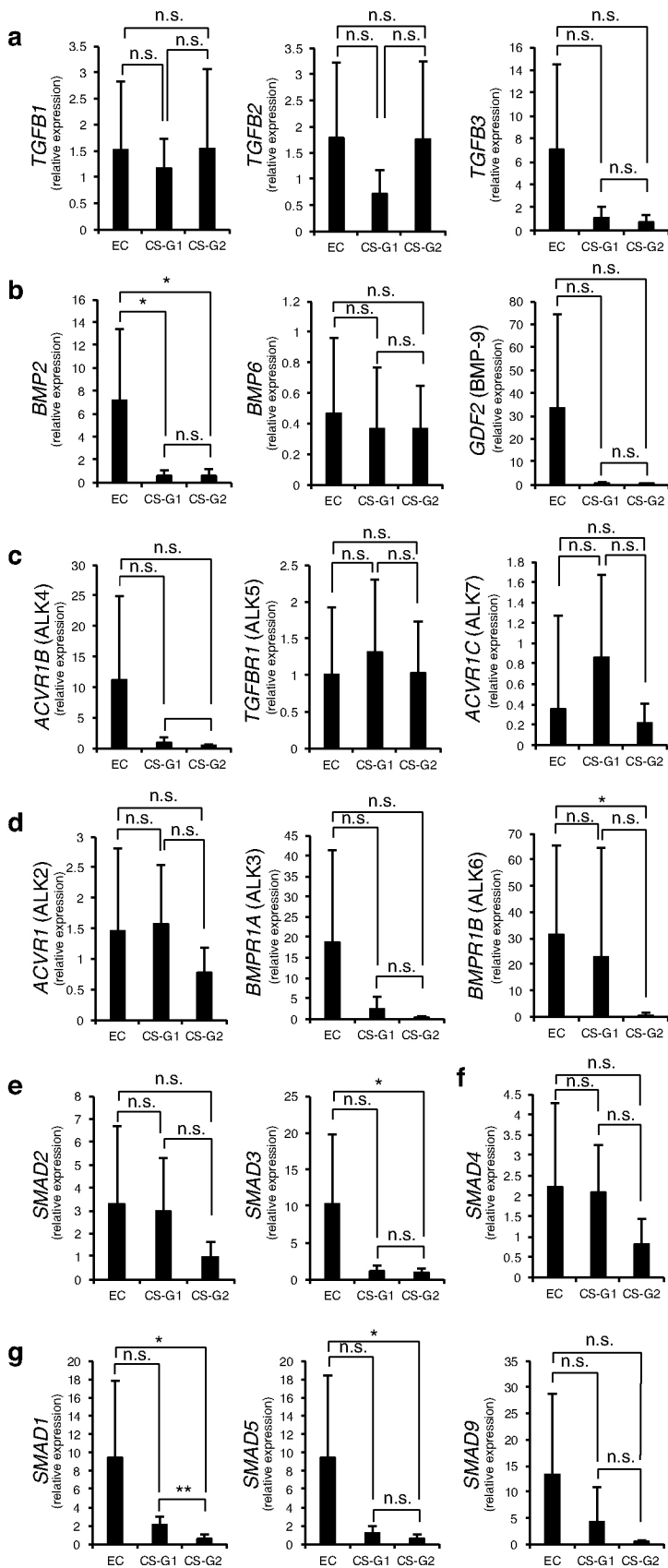
Primer sequences utilized in this study (5' to 3')

Human

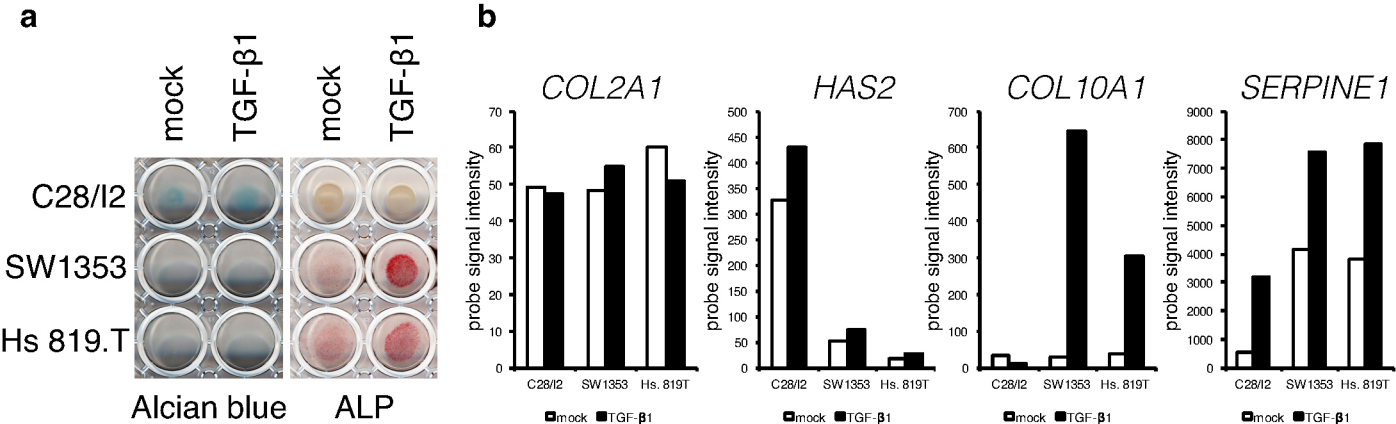
Gene	Forward	Reverse
<i>SOX9</i>	AGTACCCGCACTTGCACAAC	TTCAGAAGTCTCCAGAGCTTGC
<i>COL2A1</i>	TCTGGGTTCCAGGGACTTCCT	GAACACCCTGGTCACCTGGTTTT
<i>PEG10</i>	CGAAGAAGGGACGAGCTCTCTG	CCTCCGACTGCTTCATGACCTT
<i>TGFB1</i>	AATTGAGGGCTTTCGCCTTAGC	AACCCTGCGTTGATGTCCACTT
<i>TGFB2</i>	CAAAATAGACATGCCGCCCTTC	CCATTGCTGAGACGTCAAATCG
<i>TGFB3</i>	AGCACAACGAACTGGCTGTCTG	GGACACATTGAAGCGGAAAACC
<i>BMP2</i>	CATGATGGAAAAGGGCATCCTC	TTAAGGCGTTTCCGCTGTTTGT
<i>BMP6</i>	TGGAGAATGCTCCTTCCCCTC	CGGGGTTTCATAAGGTGAACCAA
<i>GDF2 (BMP-9)</i>	GGTCCAGAAACCTGCCCTTCT	ATGGCTGATCATCTCCCTCAGC
<i>TGFB1 (ALK5)</i>	TCCAAACCACAGAGTGGGAACA	TCGTCGAGCAATTTCCAGAAT
<i>ACVR1 (ALK2)</i>	GGGCCTTTGGACTTGTTTTGTG	CACATCGTAGAACGGTGGCTTG
<i>ACVR1B (ALK4)</i>	GAGGCATCTGCATGGGTCTTCT	CCCTCCACCCTGATCAGACAGT
<i>ACVR1C (ALK7)</i>	GGCGCTCTCAATTGCTAGTGGT	ACCTAAGTCCGCTATGGCACA
<i>BMPR1A (ALK3)</i>	ATGCGTGAGGTTGTGTGTGTC	GGGCCAGCATTCTGACATTAG
<i>BMPR1B (ALK6)</i>	GTGCCAGTGACCCCTCTTATG	GGGGCGTAACTTCTTGATGCAC
<i>SMAD1</i>	AGAGGAGATGTTTCAGGCGGTTG	ACTTGTGGAGGAGGCATGGAAC
<i>SMAD2</i>	TGAAAGGGTGGGAGCAGAATA	ATGCTTGAGCAACGCACTGAAG
<i>SMAD3</i>	CATGACTGTGGATGGCTTCACC	GCTGCATTCTGTTGACATTGG
<i>SMAD4</i>	TGGGTCAACTCTCCAATGTCCA	TCCAAC TGACACCTTTGCCTA
<i>SMAD5</i>	ACCTGAGCCACAATGAACCACA	GAAGGGGGATAAGGGCTGTTTG
<i>SMAD9</i>	GCAGAGCCGGAAGTCAACTAT	AGCTGAGCGAAGAGCTGGTTGT
<i>GAPDH</i>	CACCATCTTCCAGGAGCGAGAT	TGGACTCCACGACGTA CT CAGC

Mouse

Gene	Forward	Reverse
<i>Peg10</i>	AGCACGCATGACCCTCACATTA	TACGGATGCAAATTCGGTTGTG
<i>Hprt1</i>	CGTTGGGCTTACCTCACTGCTT	CAAAAAGCGGTCTGAGGAGGAA

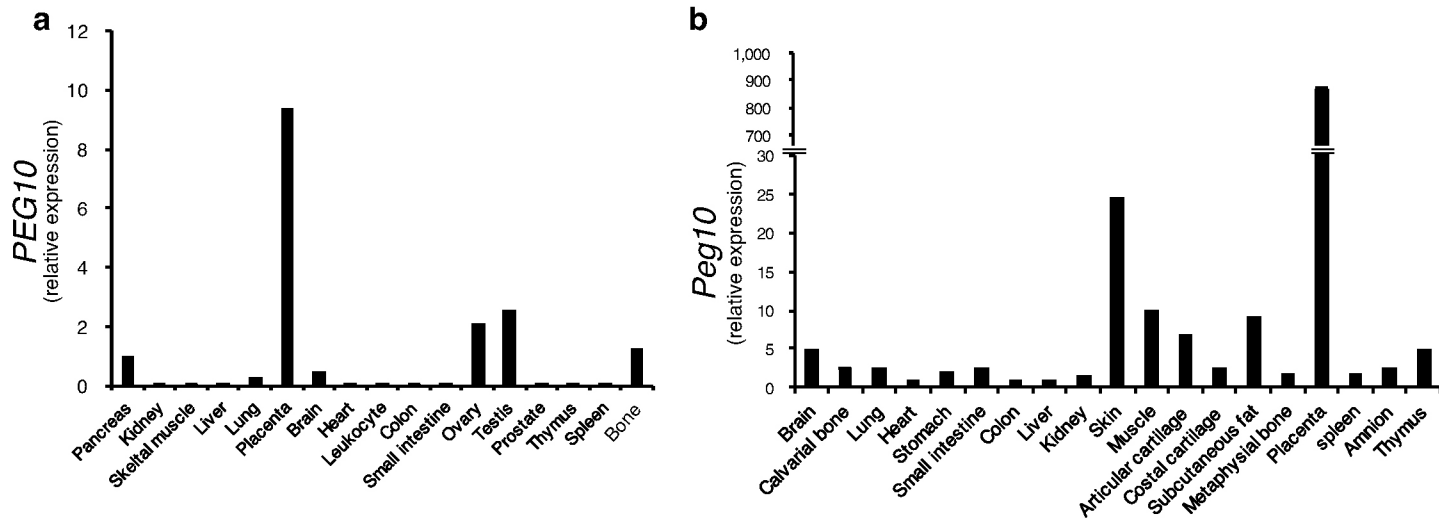


**Supplementary Figure S1. Expression of genes encoding signalling components of TGF- $\beta$  and BMP pathways.** mRNA samples from tumours were examined by RT-qPCR. EC, enchondromas (n = 6); CS-G1, grade 1 chondrosarcomas (n = 5); CS-G2, grade 2 chondrosarcomas (n = 6). \* $P < 0.05$ ; \*\* $P < 0.01$ ; n.s., not significant.



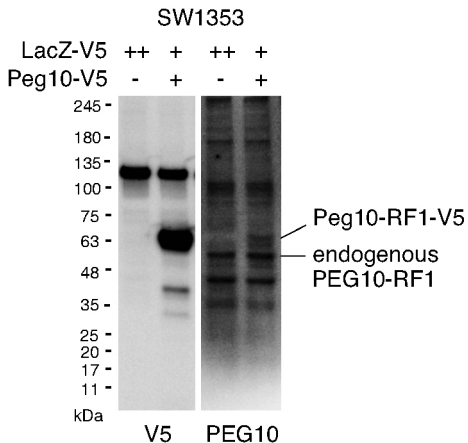
**Supplementary Figure S2. Abnormal differentiation of chondrosarcoma cells upon chondrogenic stimulation by TGF- $\beta$ 1.** (a) Micromasses of C28/I2, SW1353, and Hs 819.T cells were formed and then cultured further with or without TGF- $\beta$ 1 (1 ng/ml) for 7 days, followed by alcian blue and ALP staining. (b) Microarray analysis of *COL2A1*, *HAS2*, *COL10A1*, and *SERPINE1* expression in the experiment shown in Table 1.

## Supplementary Figure S3



**Supplementary Figure S3. Tissue distribution of PEG10 gene expression in humans and mice.** (a, b) A multiple tissue cDNA panel of humans (a) and mice (b) was subjected to RT-qPCR analyses of *PEG10* (human) and *Peg10* (mouse).

# Supplementary Figure S4

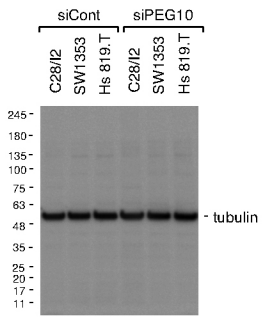


## Supplementary Figure S4. Induction of exogenous PEG10 in SW1353 cells.

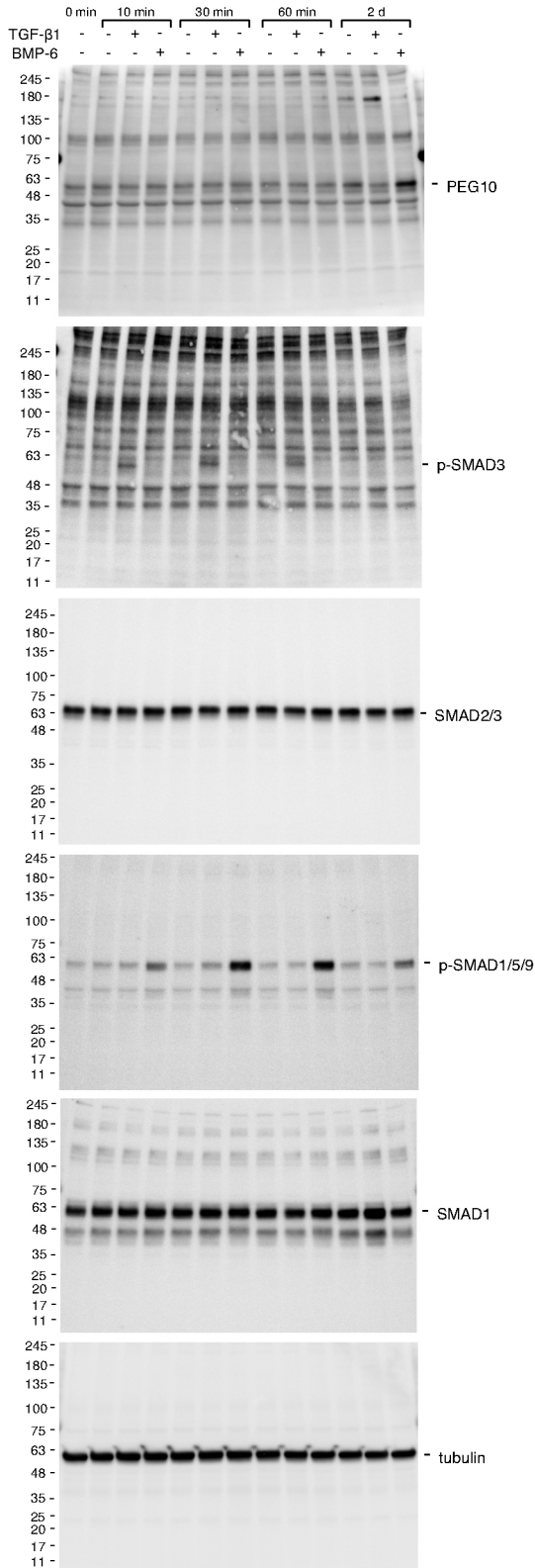
SW1353 cells were transfected with control LacZ or Peg10-V5 expression plasmids overnight, followed by immunoblotting with anti-V5 or anti-PEG10 antibodies.

Full-length blots of immunoblots illustrated in Figures 3 and 4.

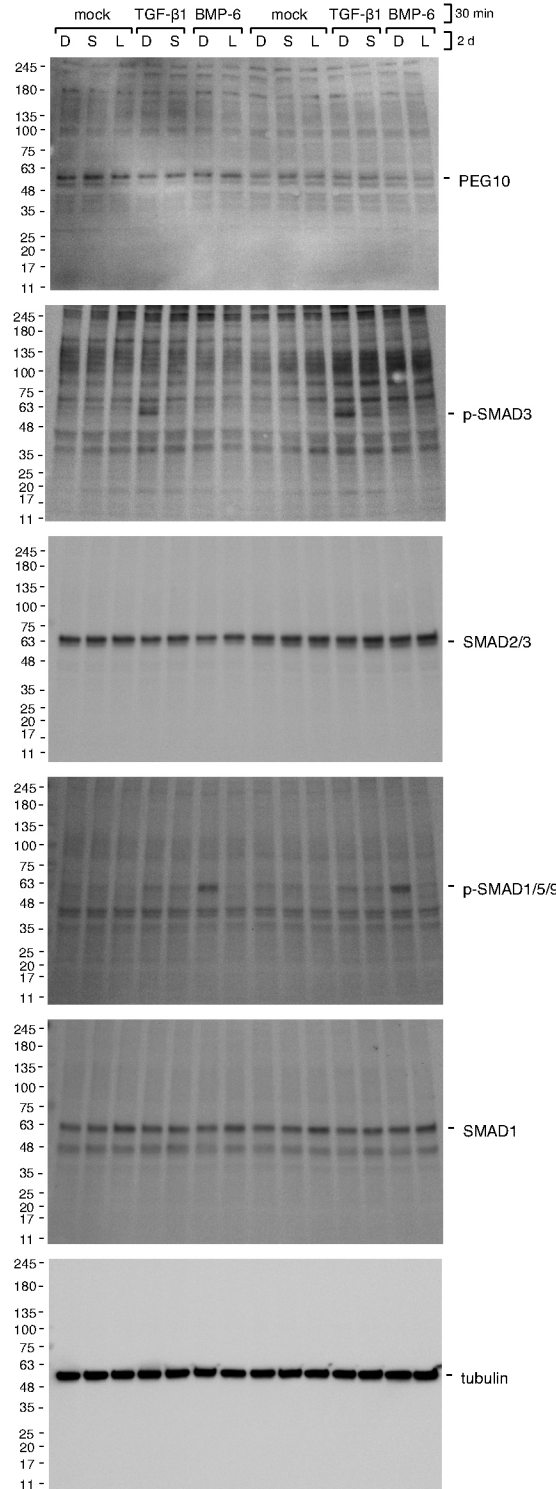
**Fig.3b**



**Fig.4d**



**Fig.4f**



# Supplementary Figure S6

## Full-length blots of immunoblots illustrated in Figure 5c

