Supplementary Information

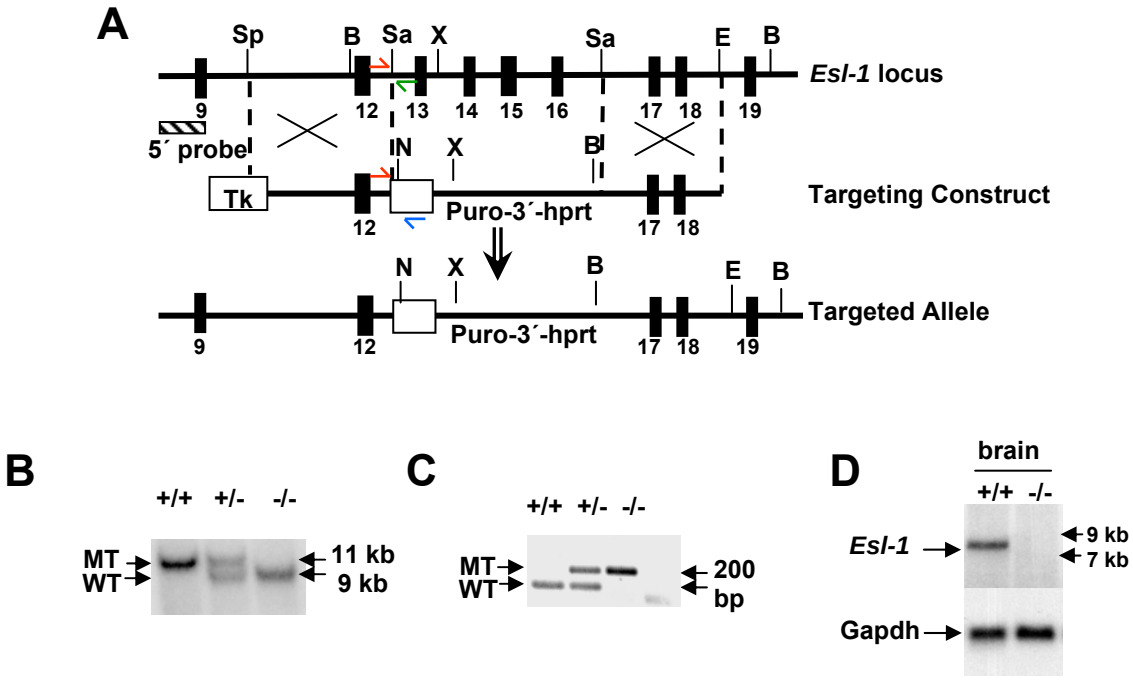

Figure S1. Generation of *Esl-1* knock-out mice. A. The schematic drawing of the genomic locus of *Esl-1* gene, the targeting construct, and the targeted allele. The position of 600bp 5' probe for Southern analysis is shown. The positions of PCR primers are designated as with red, green and blue arrows respectively. Abbreviations: B, BamHI; E, EcoRI; N, NotI; Sa, SacI; Sp, SpeI; Puro, puromycin; TK, herpes simplex viral thymidine kinase. **B.** Southern Blot analysis of genomic DNA from the knock-out mice using a 5' probe. Genomic DNA was digested with Not1 and XbaI. The 11-kb fragment was detected in the wild type *Esl-1* allele and a 9-kb in the disrupted allele. ***C.*** genotyping strategy with the three primers denoted in (**A**). **C.** PCR screening strategy for wildtype and knockout allele in genomic DNA. **D.** The *Esl-1* gene in the wt brain yields a ~7.5kb transcript in the brain, but the knock out sample yield no stable transcript.

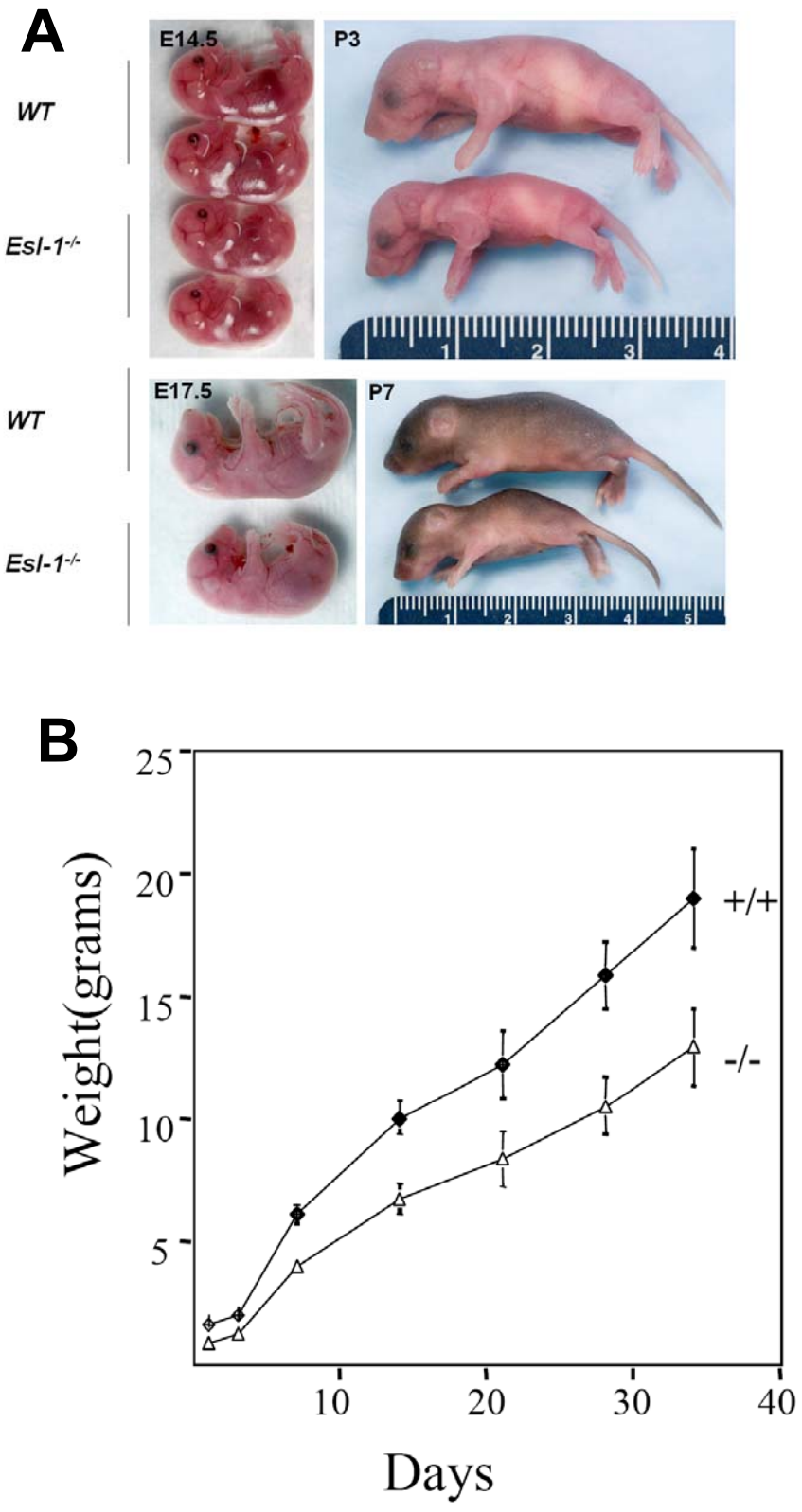

Figure S2. A. *Esl-1*$^{-/-}$ embryos and neonates (E14.5, E17.5, P3 and P7) are smaller than the WT littermates. B. The growth curve of *Esl-1*$^{-/-}$ mice from newborn to 5 wk.

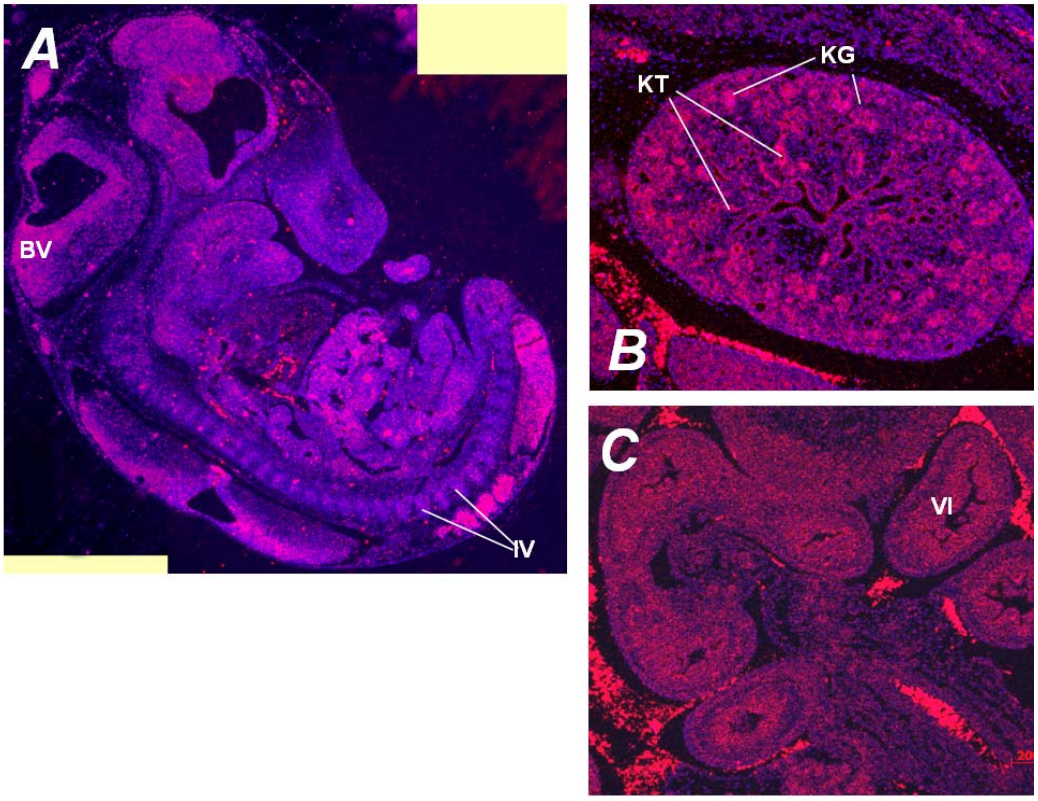

Figure S3. Expression pattern of *Esl-1* in the E12.5 WT mouse Embryos (A) and in E16.5 kidney (B) and intestine (C). *Esl-1* is specifically expressed in intervertebral tissue (IV), brain ventricle (BV), kidney tubules (KT), kidney glomeruli (KT) and intestinal villi (VI).

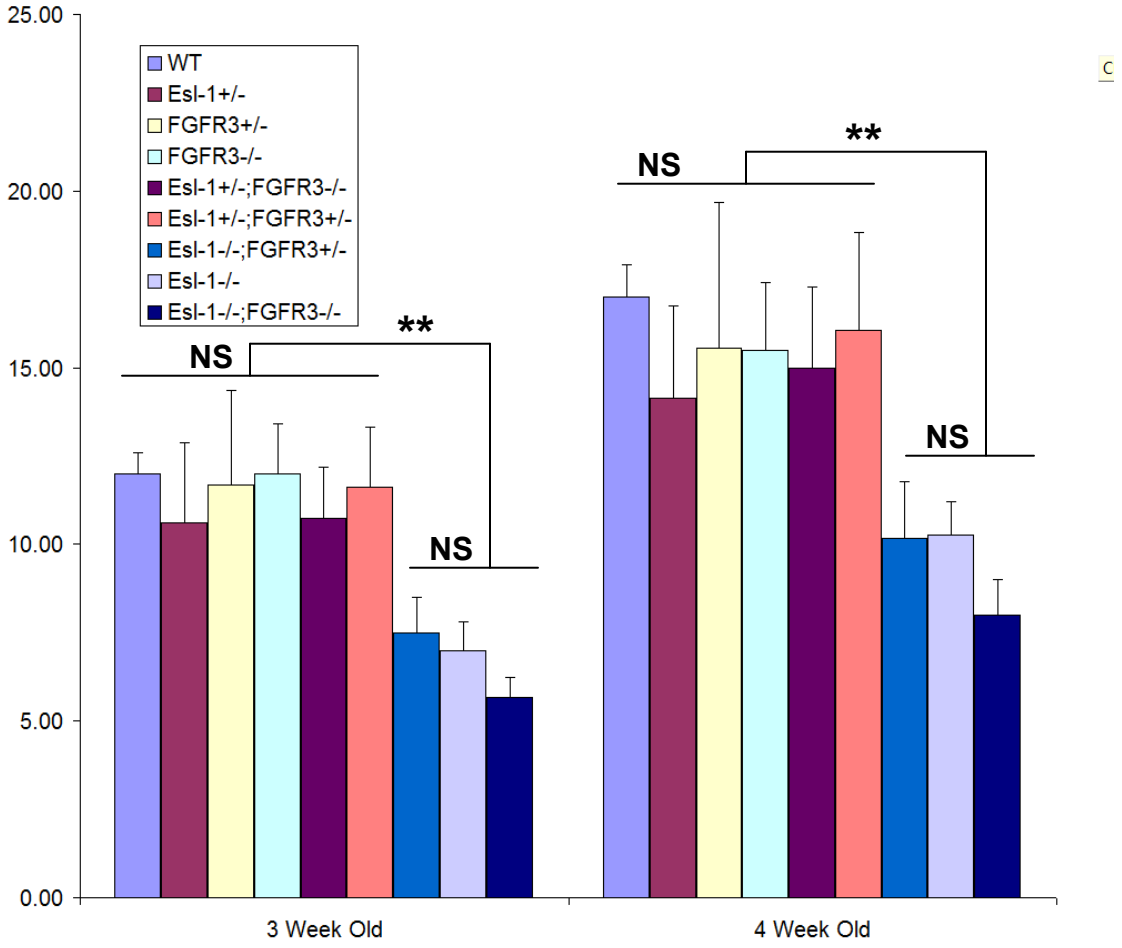

Figure S4. The comparison of the body weight of *Esl-1*$^{-/-}$, *FgfR3*$^{-/-}$ and double knockout mice at 3 wks and 4 wks old. NS: not significant; **: P<0.01

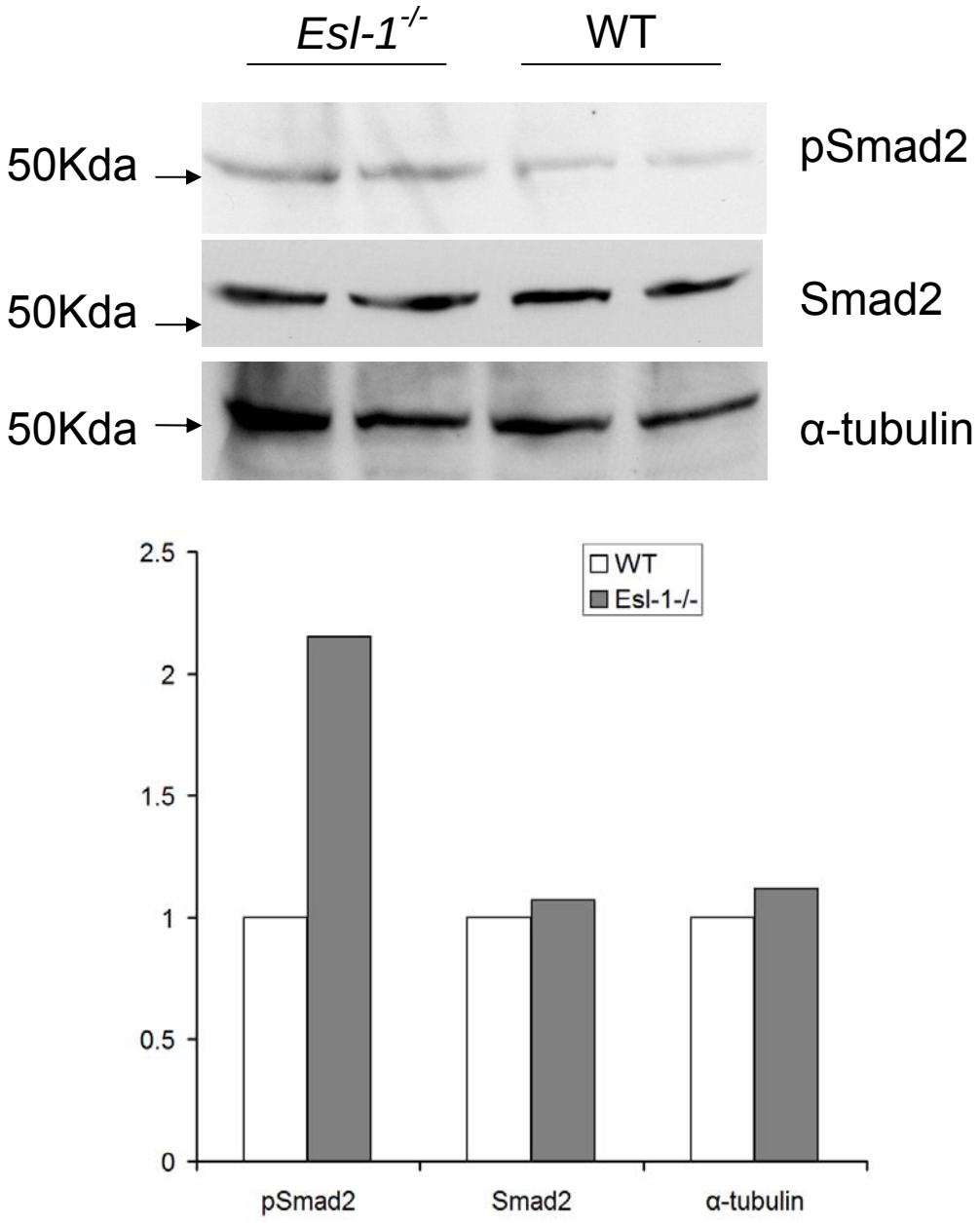

Figure S5. pSmad2 status in *Esl-1*$^{-/-}$ cartilage. The pSmad2 in the *Esl-1*$^{-/-}$ rib cartilage is increased, while total Smad2 is unchanged. α-Tubulin is used as control. Quantification is shown in the histogram.

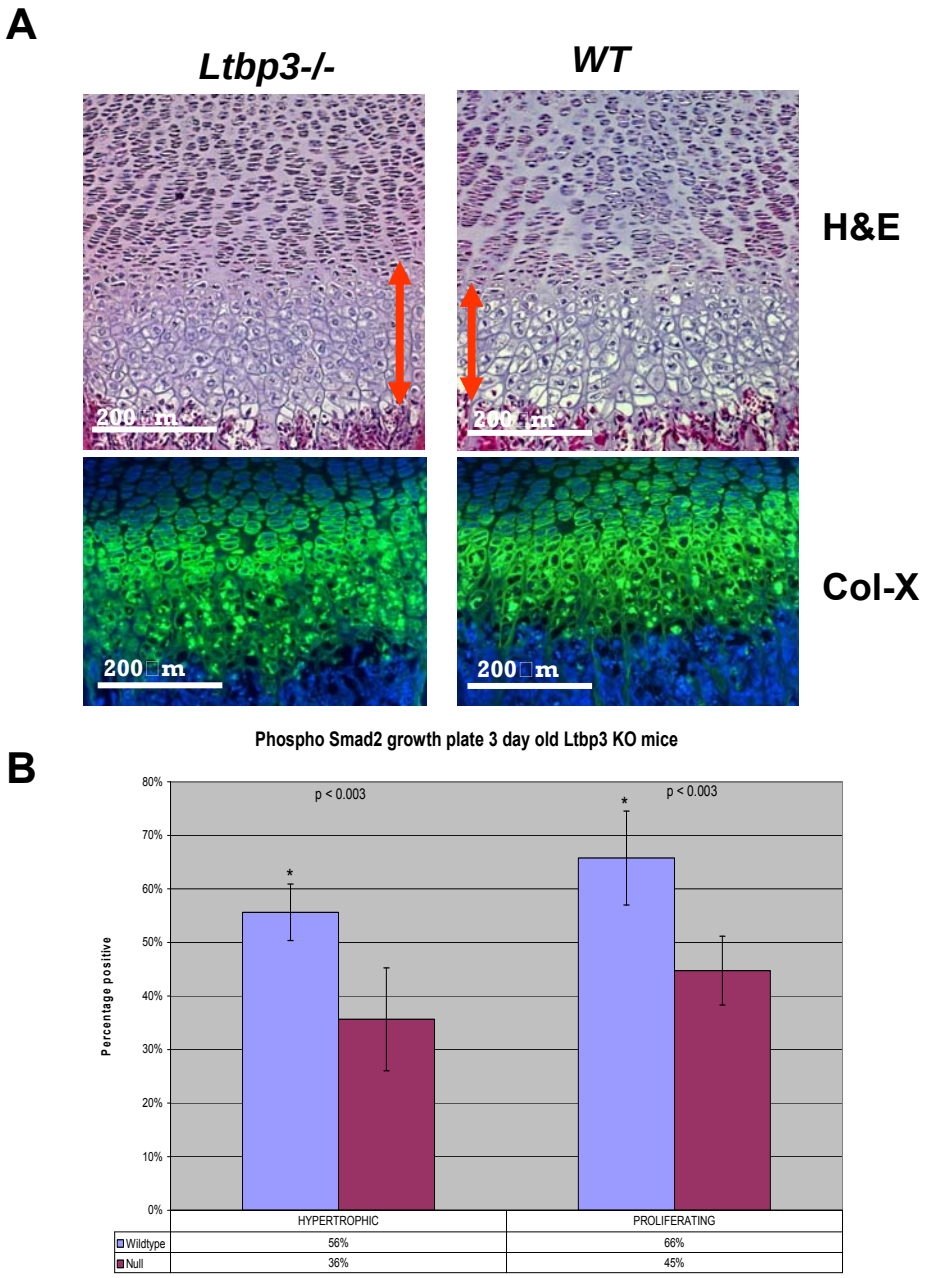

Figure S6. ***LTBP3*** $^{-/-}$ **mice growth plate phenotype.** **A.** H&E and Collagen X staining of P3 growth plates from WT and *Ltbp3* $^{-/-}$ mice (n=4) shows higher cell density in the PZ and a longer HZ (red arrows) in *Ltbp3* $^{-/-}$ mice as compared to WT littermates. **B.** P3 *Ltbp3* $^{-/-}$ growth plate contains less p-Smad2 positive cells in both PZ and HZ.

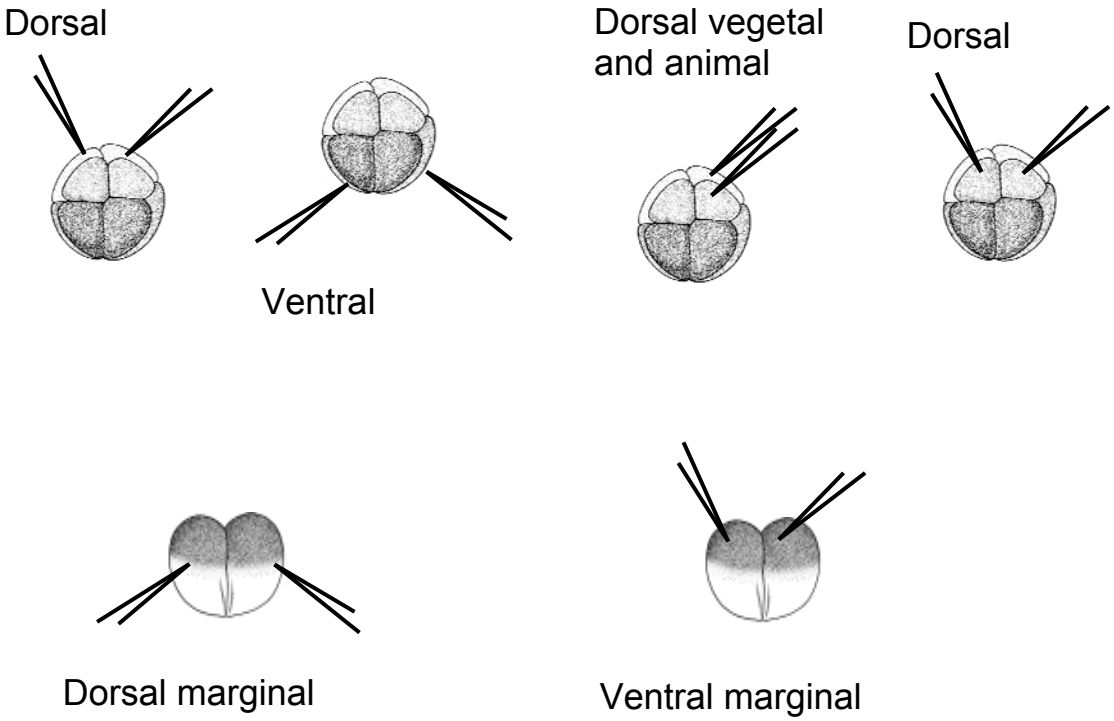

Figure S7. Schematic representation of different injection sites and different stages of embryos used in these studies.

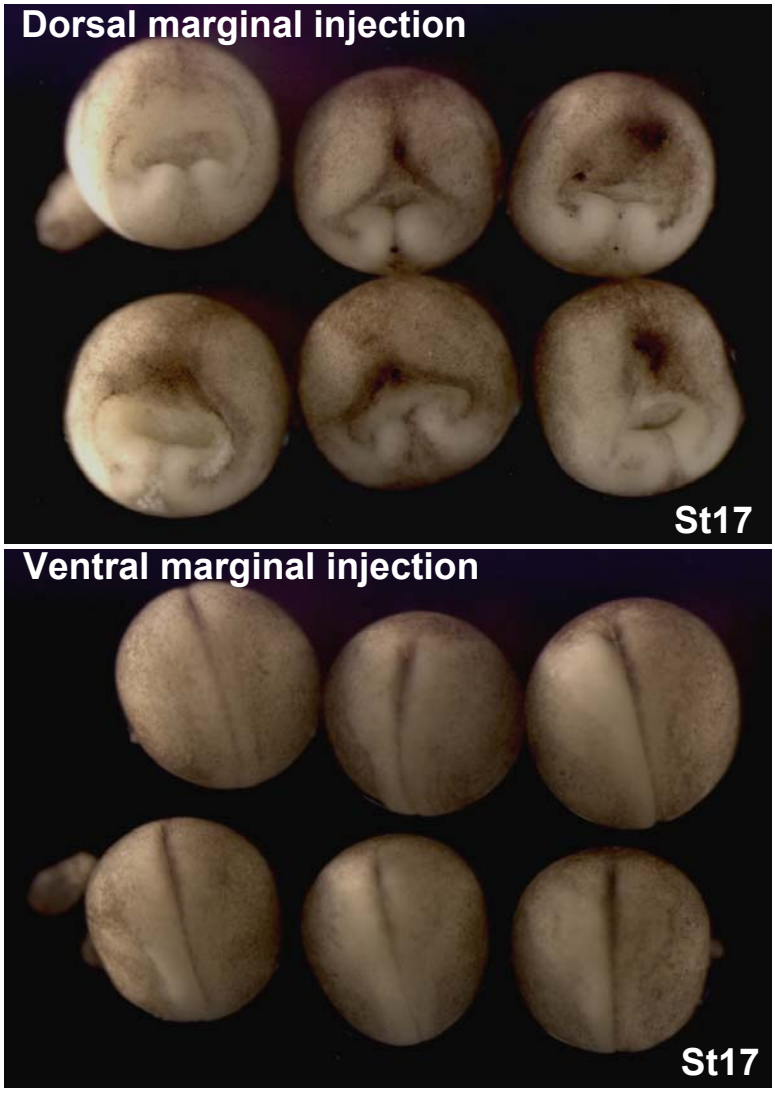

Figure S8. ESL-1 modulates body axis formation, during early embryogenesis.

Dorsal view at stage 17. Two cell-stage embryos were injected with mRNAs encoding with full-length *xEsl-1* (460 pg). Embryos (upper panel) that were injected in the dorsal marginal zone developed shortened body axes and open neural folds while control embryos (lower panel) that were injected in the ventral marginal zone developed normally.

Supplemental Table 1. Effect of xEsl-1 RNA injection and sites of injection

xEsl-1 RNA	Stage of injection	Site of injection	No. of embryos	Normal (%)	Abnormality (%)
460 pg	2 cell	Dorsal marginal zone*	28	6 (22%)	22 (78%)
460 pg	2 cell	Ventral marginal zone*	30	30 (100%)	0 (0%)
230 pg	8 cell	2 Dorsal vegetal*	20	12 (60%)	8 (40%)
230 pg	8 cell	2 Ventral vegetal*	20	20 (100%)	0 (0%)
230 pg	8 cell	1 Dorsal vegetal and 1 Dorsal animal*	14	4 (29%)	10 (71%)
230 pg	8 cell	2 Dorsal animal*	21	4 (19%)	17 (81%)
115 pg	8 cell	2 Dorsal animal*	23	19 (83%)	4 (17%)
230 pg	8 cell	2 Dorsal vegetal*	33	12 (60%)	18 (54%)
230 pg	8 cell	2 Ventral vegetal*	13	13 (100%)	0 (0%)

* Embryos were injected in dorsal or ventral blastomeric embryos at the different stages and allowed to develop to stage 37. Embryos were scored for abnormality with dorsally curved trunk and shortened axis

Supplemental Table 2. Xnr3 rescues xEsl-1 phenotypes

No. of Experiments	xEsl-1 RNA	Xnr3 RNA	No. of embryos	Normal (%)	Abnormality (%)	Percentage of rescue
I	300 pg		25	15 (62%)	10 (40%)	
	300 pg	13 pg	25	21 (90%)	4 (16%)	60%
		13 pg	27	23 (91%)	4 (15%)	
II	300 pg		24	15 (62%)	9 (38%)	
	300 pg	13 pg	20	18 (90%)	2 (10%)	47%
		13 pg	23	21 (91%)	2 (9%)	
III	300 pg		23	4 (8%)	19 (92%)	
	300 pg	13 pg	22	11 (50%)	11 (50%)	45%
		13 pg	22	7 (32%)	15 (68%)	
IV	300 pg		16	4 (8%)	12 (75%)	
	300 pg	10 pg	17	9 (53%)	8 (47%)	37%
		10 pg	17	13 (76%)	4 (24%)	

•Embryos were injected in dorsal marginal zone embryos at the two-cell stage and allowed to develop to stage 18. Embryos were scored for abnormality with dorsally curved trunk and shortened axis. The percentage of rescue indicates change of abnormalities from the injection of xEsl1-1RNA to co-injection of xEsl-1 and Xnr3 RNA.

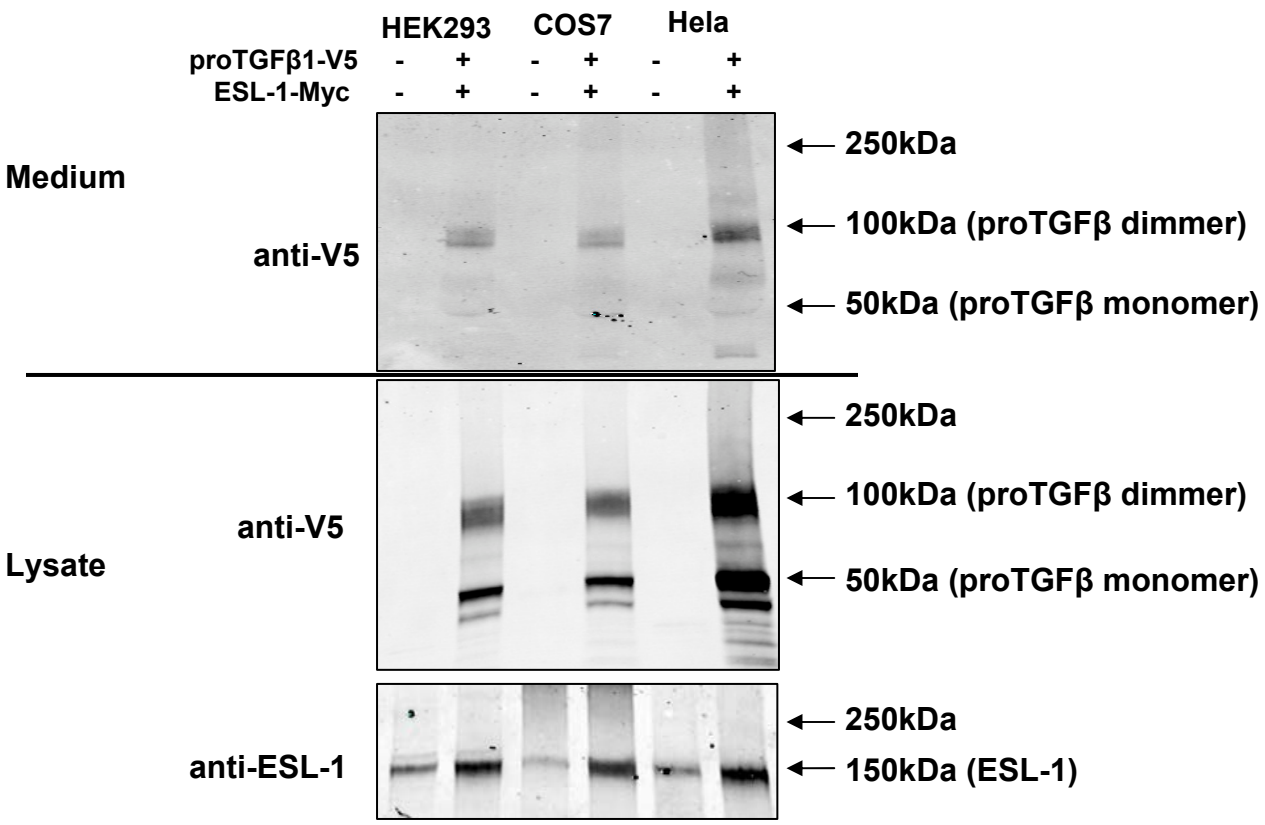

Figure S9. ESL-1 is neither secreted nor covalently bound to proTGFβ1 in HEK293, COS7, and Hela cells. Cells were transfected with V5-tagged proTGF β1 plasmid and Myc-tagged ESL-1 plasmid as shown on the top; antibodies for Western blot are shown on the left; molecular weights and description of the bands are shown on the right. Cells were lysed and the protein lysate electrophoresed in a non-reducing condition.

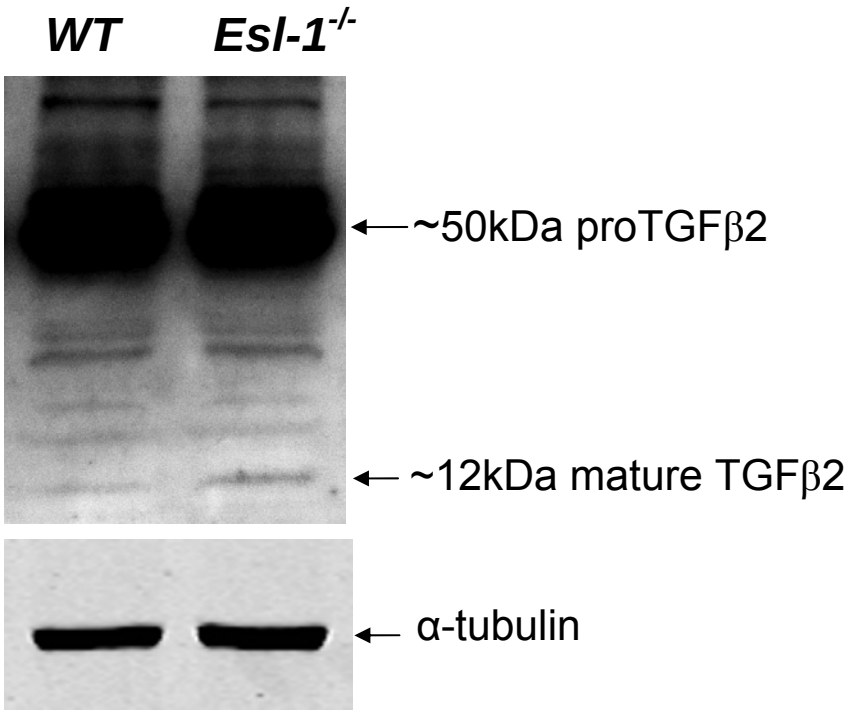

Figure S10. Increased amount of mature TGFβ2 is present in the *Esl-1*$^{-/-}$ primary chondrocyte lysate, compared to the WT one. α-Tubulin was used for loading control.

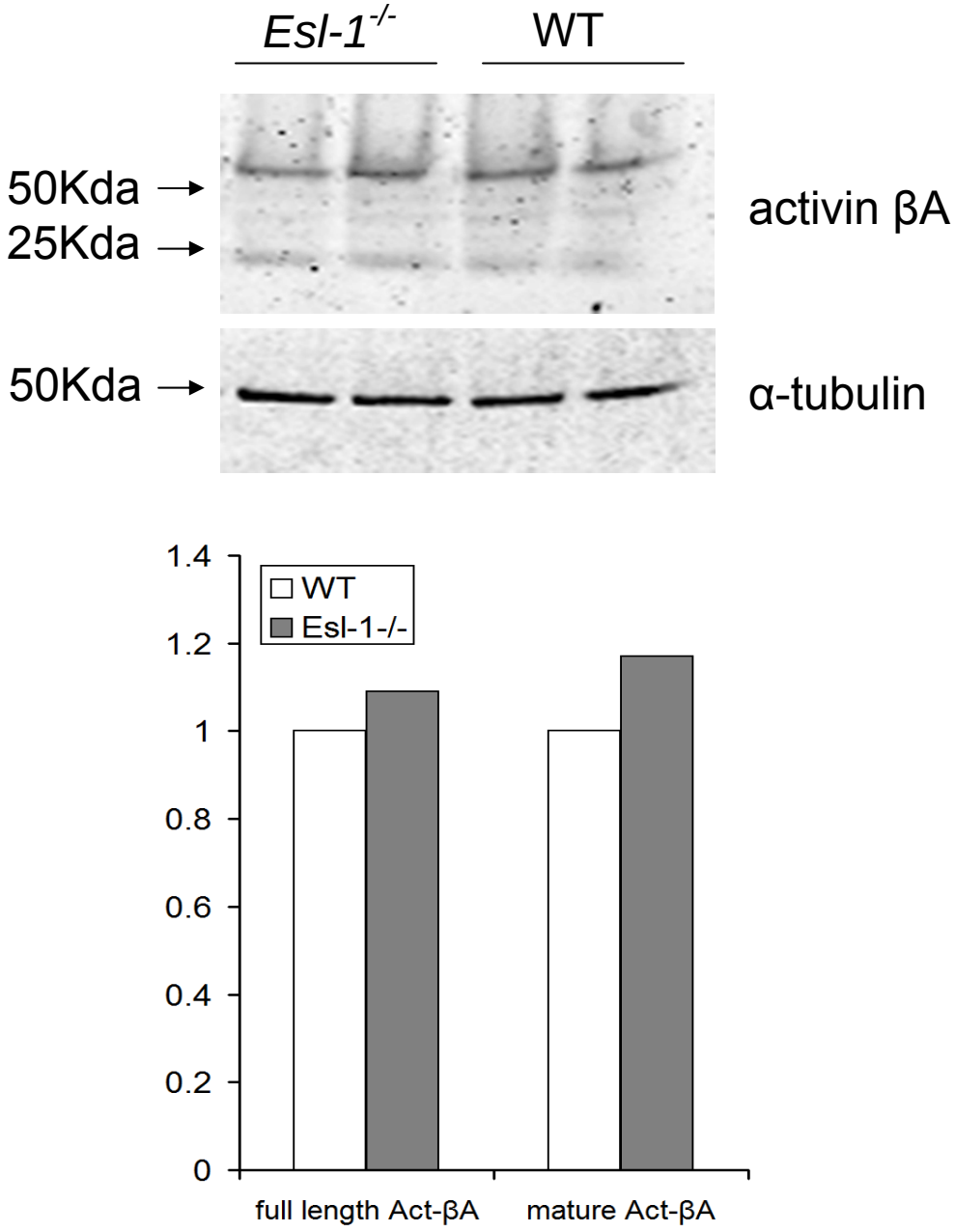

Fig. S11. Activin βA expression and maturation was not significantly changed in *Esl-1*$^{-/-}$ P3 rib cartilage protein comparing with WT sample.

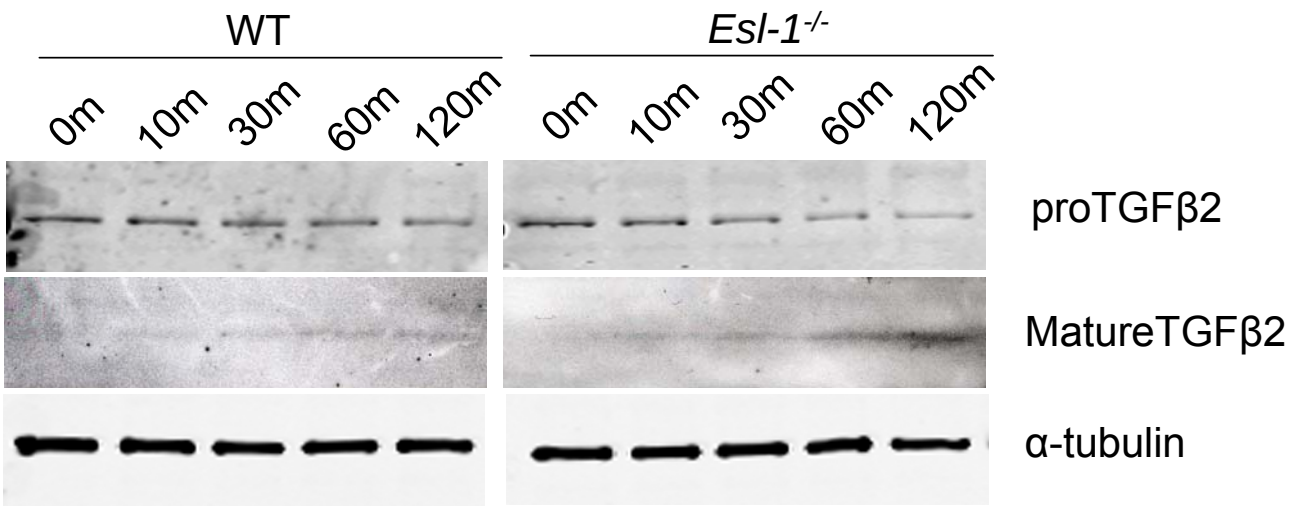

Figure S12. Loss of ESL-1 promotes maturation of proTGFβ2 in primary chondrocyte cultures by *in vitro* furin digestion. TGFβ2 antibody detection of the WT and Esl-1-/- primary chondryocte lysates digested by furin. Reaction time, genotypes, and identity of the bands are noted. These experiments were repeated three times and all three showed similar trends.

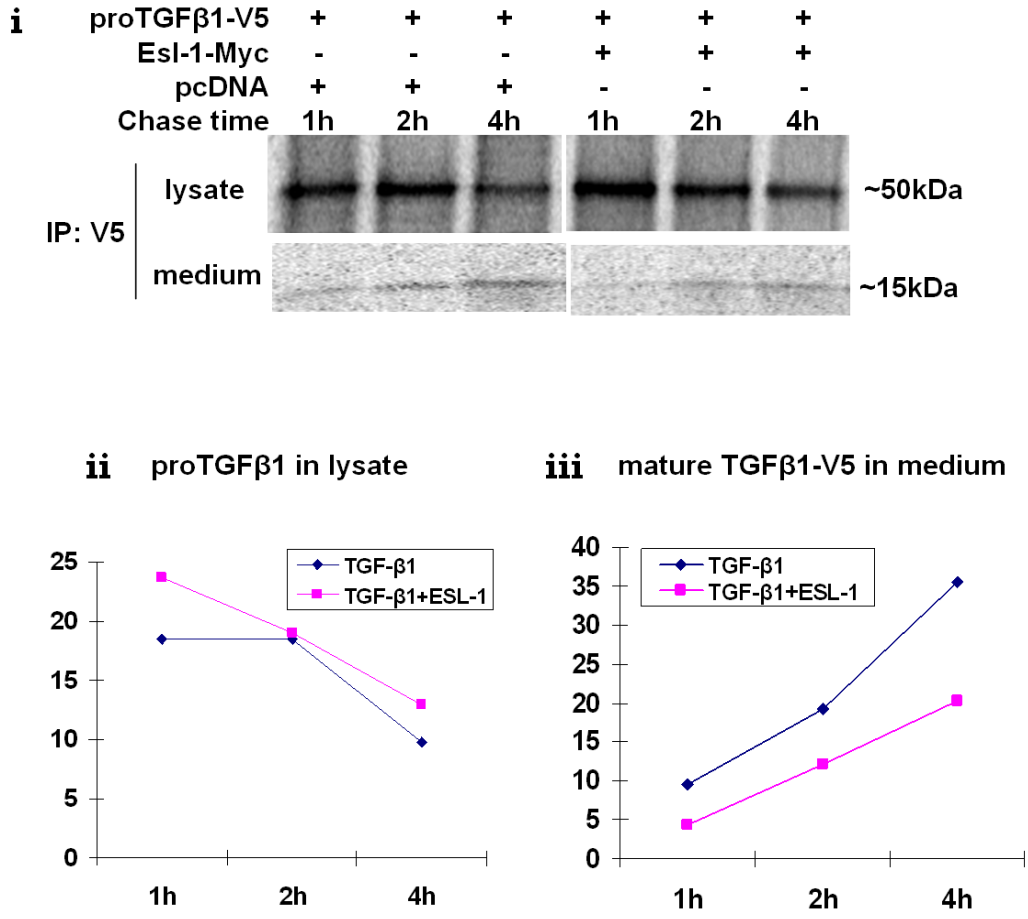

Figure S13. ESL-1 decreases the extracellular concentration of TGFβ1 by pulse chase studies. After 36hr transfection, the Hela cells were pulsed with S^{35}-Met/S^{35}-Cys for 2 hr, and then chased at different time points. Detailed scheme is shown on the top. Equal amounts of cell lysate and medium were immunoprecipitated with V5-antibody. **(i)** The autoradiography of IP products from pulse-chased cell lysate and medium. The quantified data from this panel were illustrated in panels **(ii)** and **(iii),** respectively, showing that in the presence of ESL-1, cells retained more TGFβ precursor and released less mature TGFβ ligand at all tested time points.