

Supplementary information for:

TGF- β regulates phosphorylation and stabilization of Sox9 protein in chondrocytes through p38 and Smad dependent mechanisms.

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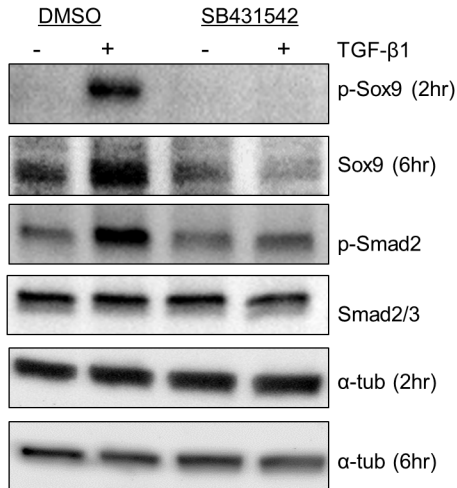
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Supplementary Table S1- Conserved Sox9 Serine 211 p38 phosphorylation motif

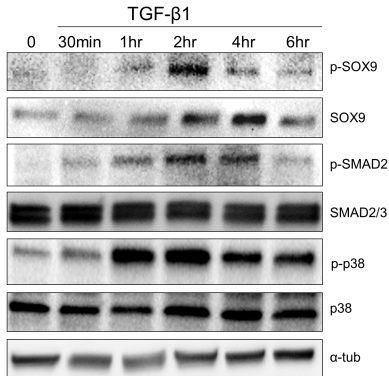
Species	Sequence
	211
Human	FKALQAD <u>S</u> PHSSSGM
Chimpanzee	FKALQAD <u>S</u> PHSSSGM
Mouse	FKALQAD <u>S</u> PHSSSGM
Bovine	FKALQAD <u>S</u> PHSSSGM
Platypus	FKALQAD <u>S</u> PHSSSGM
Chicken	FKALQAD <u>S</u> PHSSSGM
Frog	FKALQAD <u>S</u> PHSSSGI

Supplementary Table S2. Primer Sequences for qPCR

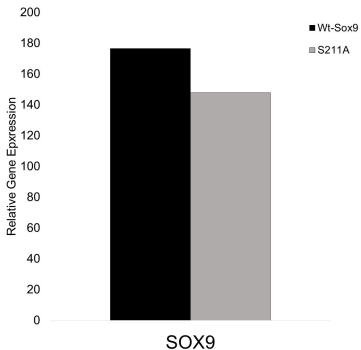
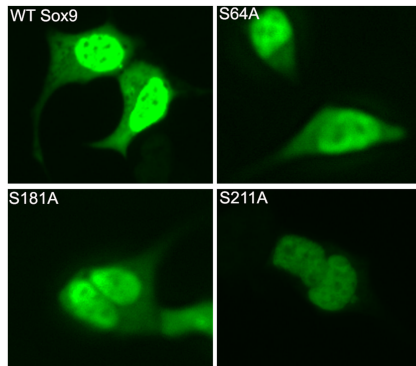
Gene	Forward Primer	Reverse Primer
<i>Mouse Sox9</i> CCA GA	AGG AAG TCG GTG AAG AAC GG	GGA CCC TGA GAT TGC
<i>Mouse Papss2</i> TCC	CCC GTG ATG GAG TCA ACA TGA G	GTG CTT TGC AGT GGG TGT
<i>Mouse PPIA</i> ⁵⁶ GCA CAT	CGC GTC TCC TTC GAG CTG TTT G	TGT AAA GTC ACC CTG



Supplementary Figure S1: SB431542 blocks TGF- β 1 mediated upregulation of p-Sox9 and Sox9 proteins. ATDC5 cells were pre-treated with SB431542 or DMSO control for 2 hours, then treated with TGF- β 1 or vehicle control for 2 or 6 hours. Cell lysates were collected and used for immunoblotting for p-Sox9, Sox9, p-Smad2, and Smad2/3. To ensure equal loading, α -tubulin was used as loading control.

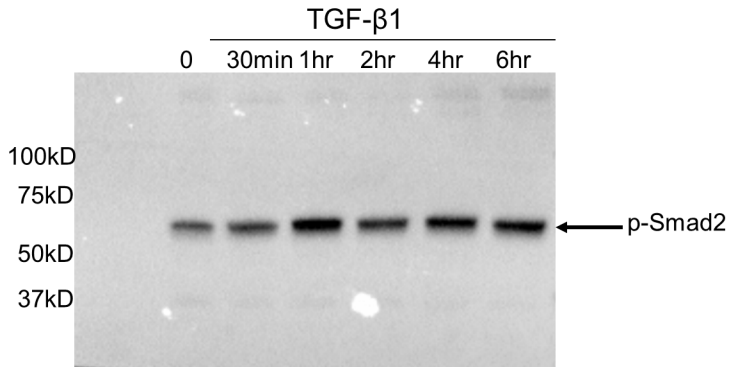


Supplementary Figure S2: SOX9 phosphorylation and protein levels are up-regulated by TGF- β 1 in bovine articular chondrocytes. A) Bovine articular chondrocytes were treated with TGF- β 1 for varying times as indicated at which point protein lysates were collected. SOX9, p-SOX9, SMAD2/3, p-SMAD2, p38, and p-p38 protein levels were determined by immunoblot. α -tubulin was used as a loading control. N=3.

A**B**

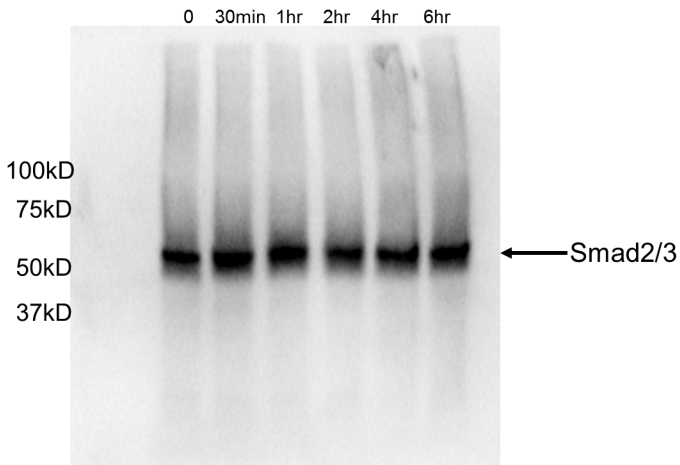
Supplementary Figure S3: Sox9 S211A mutant mRNA is transcribed and protein can localize to the nucleus. A) QPCR was used to show that the Sox9 S211A mutant was transcribed at similar levels to the WT-Sox9. B) HEK 293 cells were transfected with HA-tagged WT Sox9 or HA-tagged mutants (S64A, S181A, and S211A). Immunofluorescence staining was performed using anti-HA antibody and Alexa-Fluor 488 conjugated secondary antibody (green). Representative fluorescence images show Wt-Sox9 and Sox9 mutants are able to translocate to the nucleus. No signal was observed in samples incubated with secondary antibody alone (not shown).

Supplementary Figure 4-1 to 10: Examples of uncropped western blots.

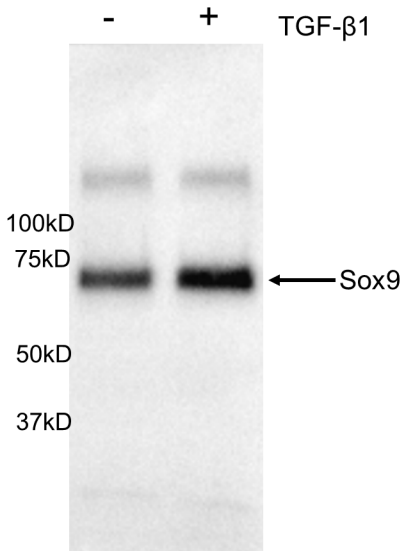


Example of p-Smad2 uncropped western blot, from Figure 1D

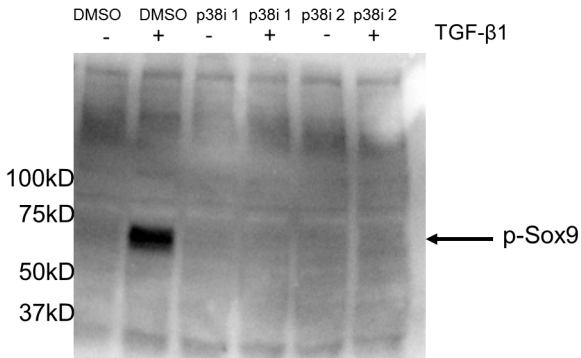
TGF- β 1



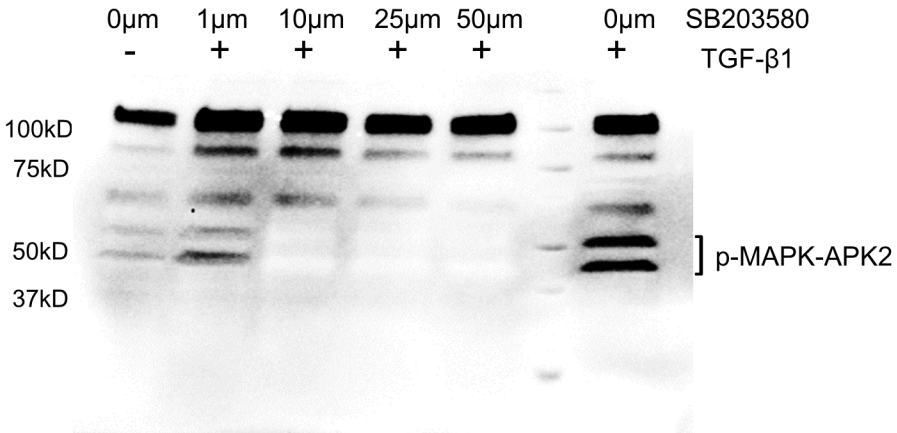
Example of Smad2/3 uncropped western blot, from Figure 1D



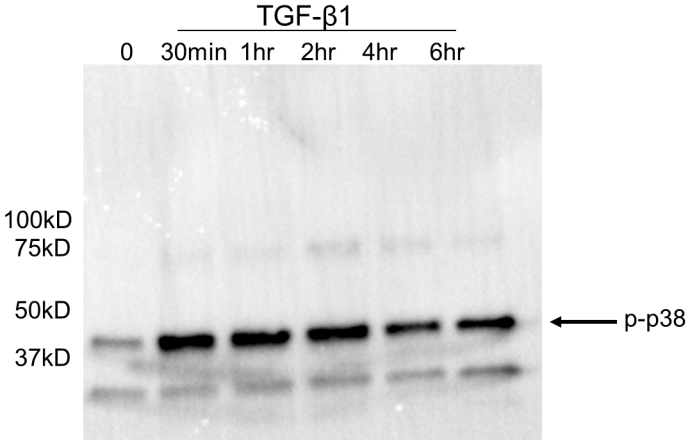
Example of Sox9 uncropped western blot, from Figure 1B



Example of p-Sox9 uncropped western blot, from Figure 3C



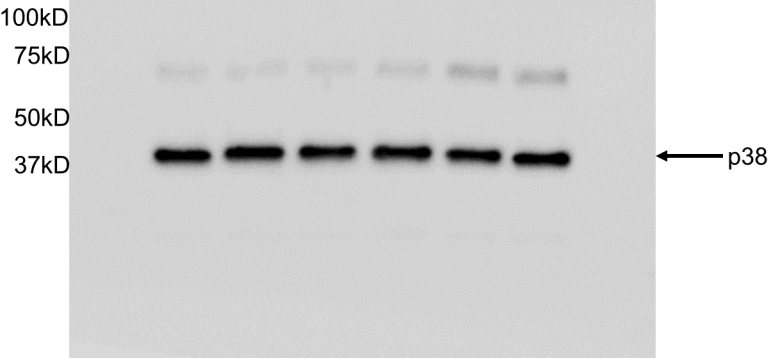
Example of p-MAPK-APK2 uncropped western blot, from Figure 3A



Example of p-p38 uncropped western blot, from Figure 1D

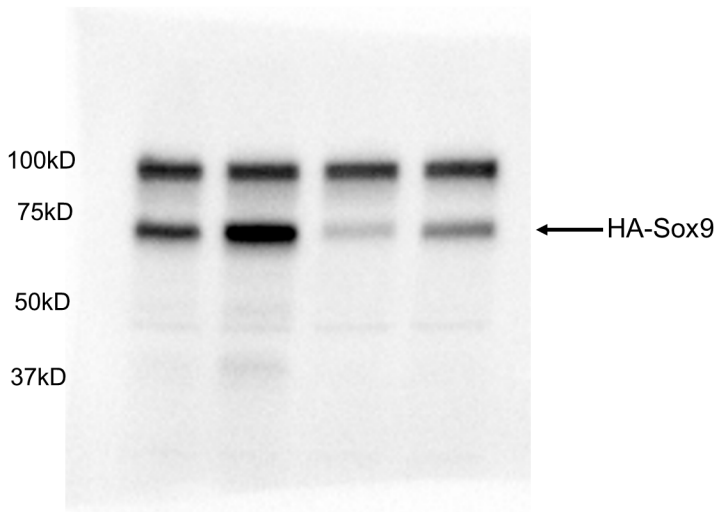
TGF- β 1

0 30min 1hr 2hr 4hr 6hr

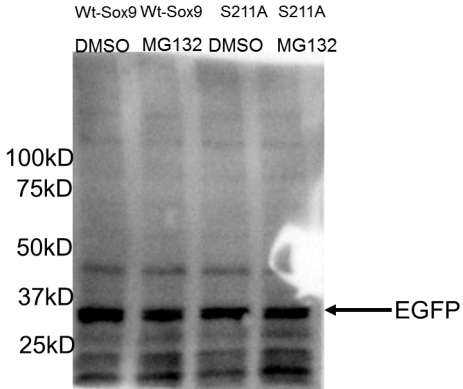


Example of p38 uncropped western blot, from Figure 1D

Wt-Sox9 Wt-Sox9 S211A S211A
DMSO MG132 DMSO MG132



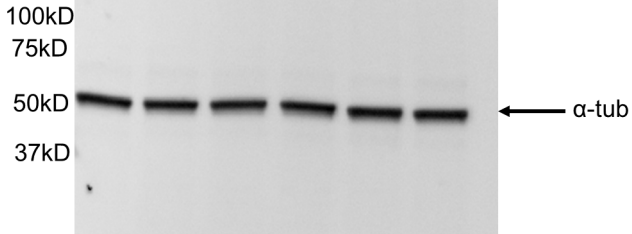
Example of HA-Sox9 uncropped western blot, from Figure 2C



Example of EGFP uncropped western blot, from Figure 2C

TGF- β 1

0 30min 1hr 2hr 4hr 6hr



Example of α -tub uncropped western blot, from Figure 1D