

Runx2 mediated Induction of Novel Targets ST2 and Runx3 Leads to Cooperative Regulation of Hypertrophic Differentiation in ATDC5 Chondrocytes

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Supplementary Figures

Fig. 1

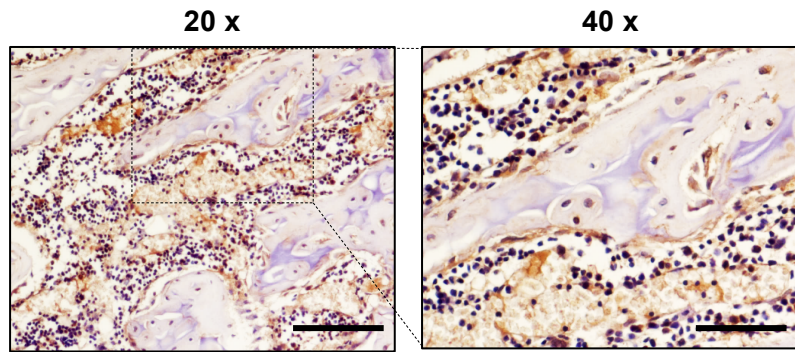


Fig. 2

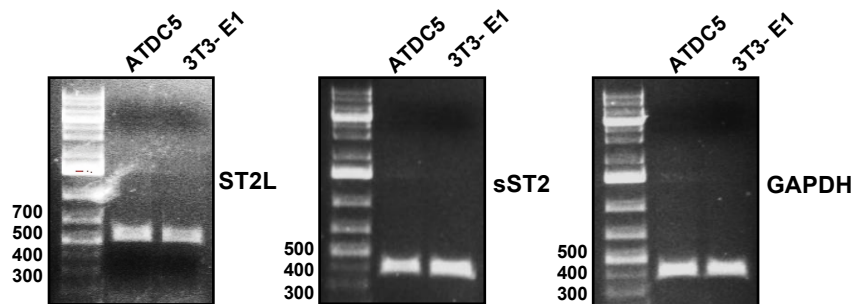


Fig. 3 A

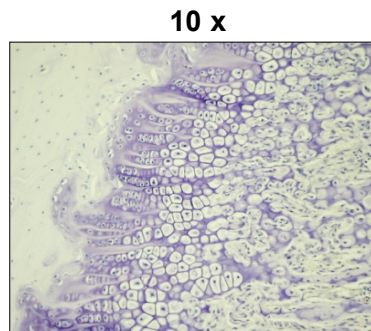


Fig. 3 B

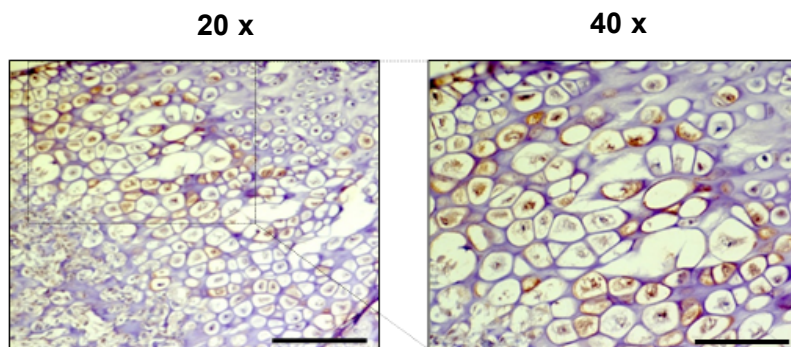


Fig. 4 A

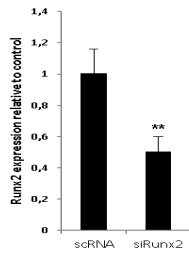


Fig. 4 B

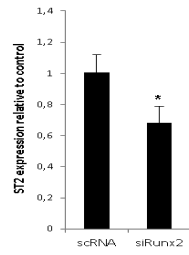


Fig. 5

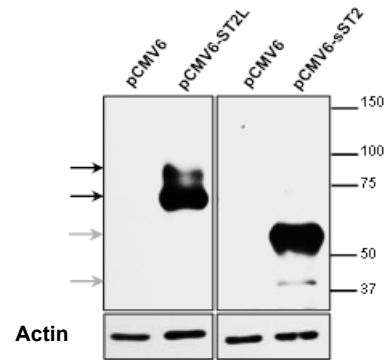


Fig. 6

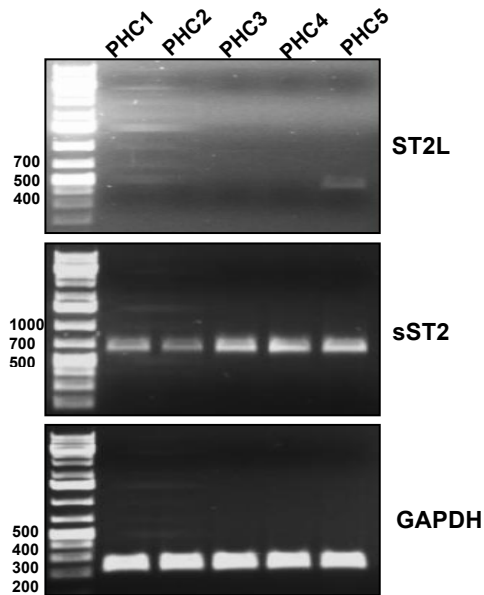


Fig. 7

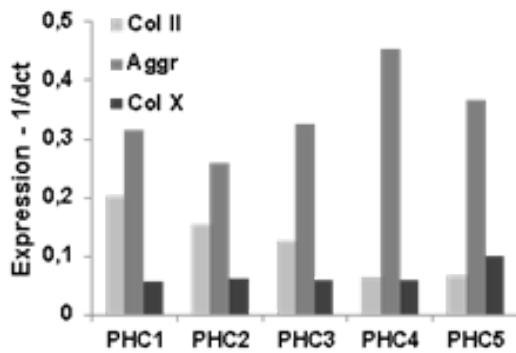
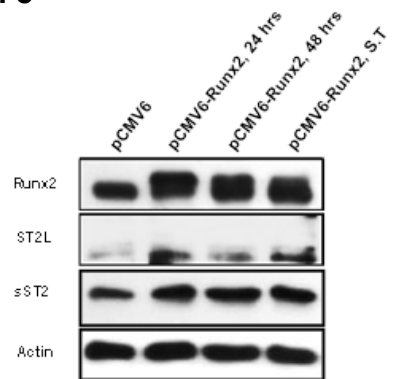


Fig. 8



Supplemental Figure Legends

Supplemental figure 1. Immunohistochemical staining of ST2 in the trabecular bone of tibia of three week old mice. Magnification of 20x and 40x are shown. Scale bar is 100 μ m for 20x and 50 μ m for 40x pictures.

Supplemental figure 2. Full gels of the endogenous expression of ST2L, sST2 and GAPDH in ATDC5 and MC3T3- E1 cells.

Supplemental figure 3. (A) Negative control staining, in tibial growth plate. (B) Immunohistochemical staining of Runx2 in the tibial growth plate at the age of three weeks. Magnification of 20x and 40x are shown. Scale bar is 100 μ m for 20x and 50 μ m for 40x pictures.

Supplemental figure 4. Runx2 regulates ST2 expression in differentiating ATDC5. (A) Runx2 knockdown in differentiating ATDC5. (B) Expression of total ST2 in Runx2 silenced ATDC5 cells in a differentiation condition. Gene silencing analysis was performed 48 hrs post transfection.

Supplemental figure 5. Post transcriptional modification of ST2L and sST2 isoforms. Immunoblotting of ATDC5 protein lysates transiently transfected with pCMV6, murine ST2L (PCMV6-ST2L) or murine sST2 (PCMV6-sST2) cDNA vectors. Transfer to polyvinylidene difluoride membrane was performed and ST2L (black arrows) and sST2 (gray arrows) bands were detected by anti-DKK antibodies.

Supplemental figure 6. Full gels of the endogenous expression of ST2L, sST2 and GAPDH in 5 PHCs.

Supplemental figure 7. Chondrogenic phenotype of PHCs was assessed by the qPCR evaluation of Col II, Aggrecan and Col X expression.

Supplemental figure 8. Runx2 regulates ST2L and sST2 expression in ATDC5 chondrocytes. A full blot of the effect of forced Runx2 expression on ST2L and sST2 splice variants expression. ATDC5 cells were transiently (24 hrs, lane 2 and 48 hrs, lane 3) or stably (S.T, lane 4) transfected with pCMV6 or pCMV6-Runx2 vectors. After transfer to polyvinylidene difluoride membrane, expression level of Runx2, ST2L, sST2 and β -actin were assessed by respective antibodies.

References of RT-PCR and qPCR primers used in this study.

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