Supplemental Data

RB1CC1 suppresses type II collagen synthesis in chondrocytes, and causes dwarfism

Ichiro Nishimura^{1,2,*}, Tokuhiro Chano^{1,*}, Hiroko Kita¹, Yoshitaka Matsusue², and Hidetoshi Okabe¹

Departments of ¹Clinical Laboratory Medicine, and ²Orthopaedic Surgery, Shiga University of Medical Science, Tsukinowa-cho, Seta, Otsu, Shiga 520-2192, Japan.

Supplemental Figure 1. Col2-RB1CC1 causes dwarfism in mice.

A. Col2-RB1CC1 (*RB1CC1/Cre*) mice had lower body weights than the other types (*RB1CC1/wt*, *wt/Cre* and *wt/wt*) of mice. Horizontal numbers indicate mouse ages (weeks old: wo). *B & C. RB1CC1/Cre* mice (24 wo) had significantly shorter tibial (B) and cranio-sacral (C) lengths than the other types. The values indicate the means from both #44 and #84 of transgenic *CAG-floxed-Neo-RB1CC1* mouse lines. One-way factorial ANOVA and multiple comparison tests accompanied by Scheffe's significance were used to evaluate the relationships between genotypes and body sizes (*; p<0.05, statistically significant).

Supplemental Figure 2. RB1CC1 cannot inhibit synthesis of aggrecan, COMP and sGAG, but that of type II collagen.

A. ATDC5 cells were differentiated to chondrocytes for 0-96 hr after the transduction of *RB1CC1* cDNA or the empty vector *control* (1µg/well), and the lysates were analyzed by Western blots with the indicated antibodies. D & numbers indicate hours fed by the differentiation medium. Pre-Diff: pre-transduced ATDC5. D96 cont: non-transduced & 96 hr differentiated ATDC5. *B*. The sulfated glycosaminoglycan (sGAG) amount in culture medium of 96 hr differentiated ATDC5 chondrocytic cells was not significantly changed irrespective of *RB1CC1* cDNA transduction (0-2µg/well). The means \pm standard errors were from quadruplicate experiments, and were statistically analyzed by the Kruskal-Wallis test (p=0.401).

Supplemental Figure 3. Activated NFkB or accumulated p62/SQSTM1 cannot be detected in Col2-RB1CC1 mice.

Neither phosphorylated NFkB (536) nor p62/SQSTM1 was detected in the chondrocytes of d18.5 *RB1CC1/Cre* and *wt/wt* embryos. Scale bar: 100µm.









