

Figure S1. (a) Representative immunostain images of PCNA from samples collected at P4, P9, P33, and P60. (b) The quantitative data showed a progressive decrease in PCNA levels in fibrocartilage during humerus head development.



Figure S2. The SHG images provided a valuable tool to distinct unique distributions of type I collagen in tendon, fibrocartilage (FC), and bone. The combination of SHG and Scx^{Lin} tracing images further support the FC cell origin from tendon without further differentiation into bone cells.



Figure S3. DTA-ablation of Scx^{Lin} cells led to significant decrease in expression levels of Sox9 and Runx2 in fibrocartilage cells with tamoxifen administration from postnatal day(P) 3&P4 (once daily for 2 consecutive days) and harvest at P30. (**a**) The co-immunostain of Sox9 showed a sharp decrease of Sox9 expression in the Scx^{Lin} fibrocartilage cells in the DTA-treated group, which is significantly different from the control (n = 4; p < 0.001); and (**b**) The co-immunostain of Runx2 displayed a drastic reduction of Runx2 expression in the Scx^{Lin} fibrocartilage cells in the DTA-treated group, which is significantly different from the control (n = 4; p < 0.001); and (**b**) The co-immunostain of Runx2 displayed a drastic reduction of Runx2 expression in the Scx^{Lin} fibrocartilage cells in the DTA-treated group, which was significantly different from the control (n = 4; p < 0.01).



Figure S4. The SHG images in the background of the 3.2 Col 1^{Lin} displayed a close association of the 3.2 Col 1⁺-FC cell numbers and the expansion of type I collagen mass during postnatal development. (**a**) 3.2 Col 1^{Lin} mice were induced at P3 and harvested at P4, P9, P33 and P60, respectively; and (**b**) There was a continuous increase in the 3.2 Col 1⁺-FC cell numbers and the expansion of type I collagen mass during postnatal development at the stages of P33 and P60 with no 3.2 Col 1⁺ cells in the FC adjacent to tendon at P60, indicating a cell migration.



Figure S5. (a) Representative TUNEL assay data showed no TUNEL signals among Scx+ fibrocartilage cells during early developmental stages (P19 and P33) but a sharp increase in the TUNEL+/Scx+ fibrocartilage cells at the stage of P60; and (b) there was a significant difference of TUNEL levels between P33 and P60 (p < 0.001; n = 4).