Harnessing Endogenous Stem/Progenitor Cells for Tendon Regeneration: Lee et al., 2015

Supplementary Figures

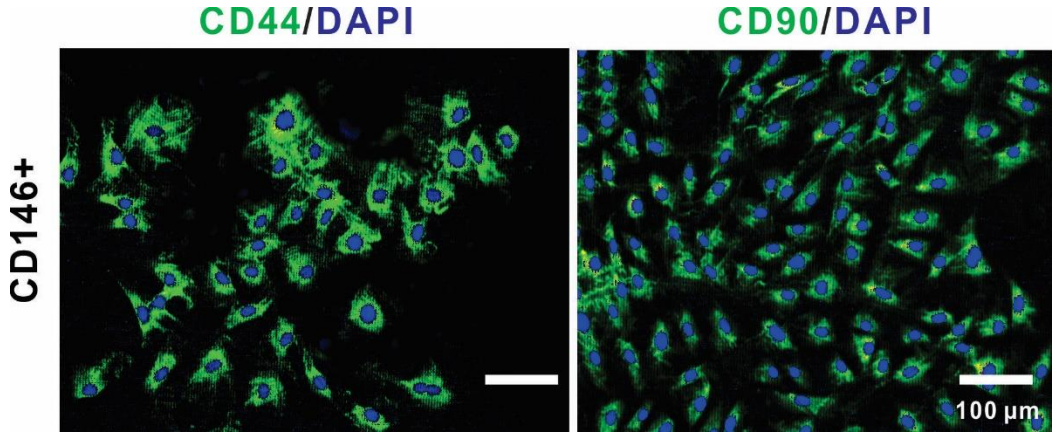

Figure S1. Expression of CD44 and CD90 in sorted $CD146^+$ tendon cells. $CD146^+$ cells isolated from rat PT were sorted by FACS. After 4 – 6 hours culture, the cells were fixed in formalin, and immunofluorescence was performed for CD44 and CD90. Images are selected as the representatives of 6 replicates total.

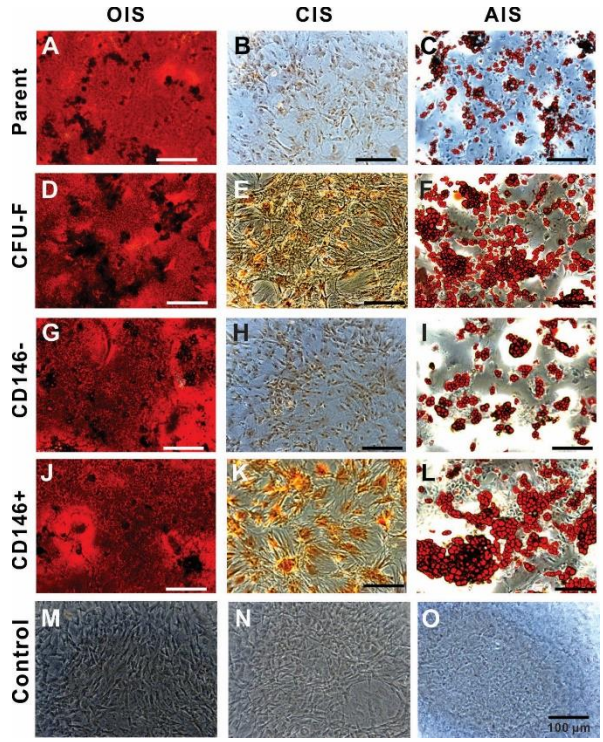

Figure S2. Multilineage differentiation of rat PT cells: Alizarin Red (AR) (A, D, G, J, M), Safranin O (Saf-O) (B, E, H, K, N), and Oil Red O (ORO) (C, F, I, L, O). Heterogeneous PT cells (A - C) and sorted $CD146^-$ cells (G - I) were able to differentiate into osteogenic (A, G) and adipogenic lineages (C, I) but failed to differentiate into chondrogenic lineage (B, H). However, single cell-derived clones (D - F) and sorted $CD146^+$ cells (J - L) were able to differentiate into all three lineages tested, with higher potential for adipogenic differentiation (F, L) as compared to heterogeneous and $CD146^-$ cells (C, I). Control groups without differentiation media show negative staining (M - O) (OIS: osteogenic induction supplements, CIS: chondrogenic induction supplements, AIS: adipogenic induction supplements). Scale: 100 µm.

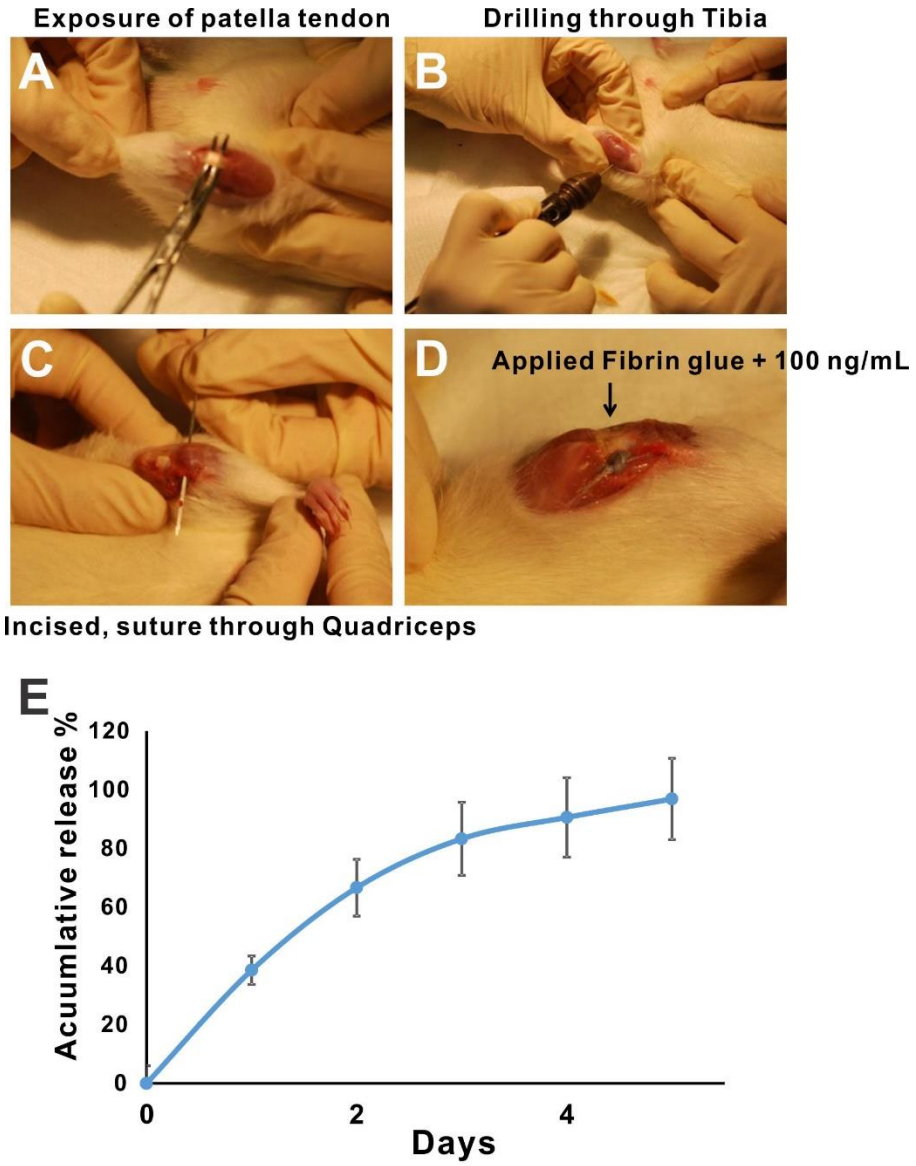

Figure S3. Surgical procedure for the rat PT repair. Upon exposure of the rat PT (A), a full-transection was made using a surgical blade. After creating a hole in the tibia (B), a cerclage suture was made through the tibia and quadriceps (C, D). Then we delivered 100 ng/mL CTGF in a 200 μL fibrin gel (50 mg/mL fibrinogen + 50 U/mL thrombin). *In vitro* release test showed that CTGF loaded in fibrin gel was 100% released in 5 days (E).

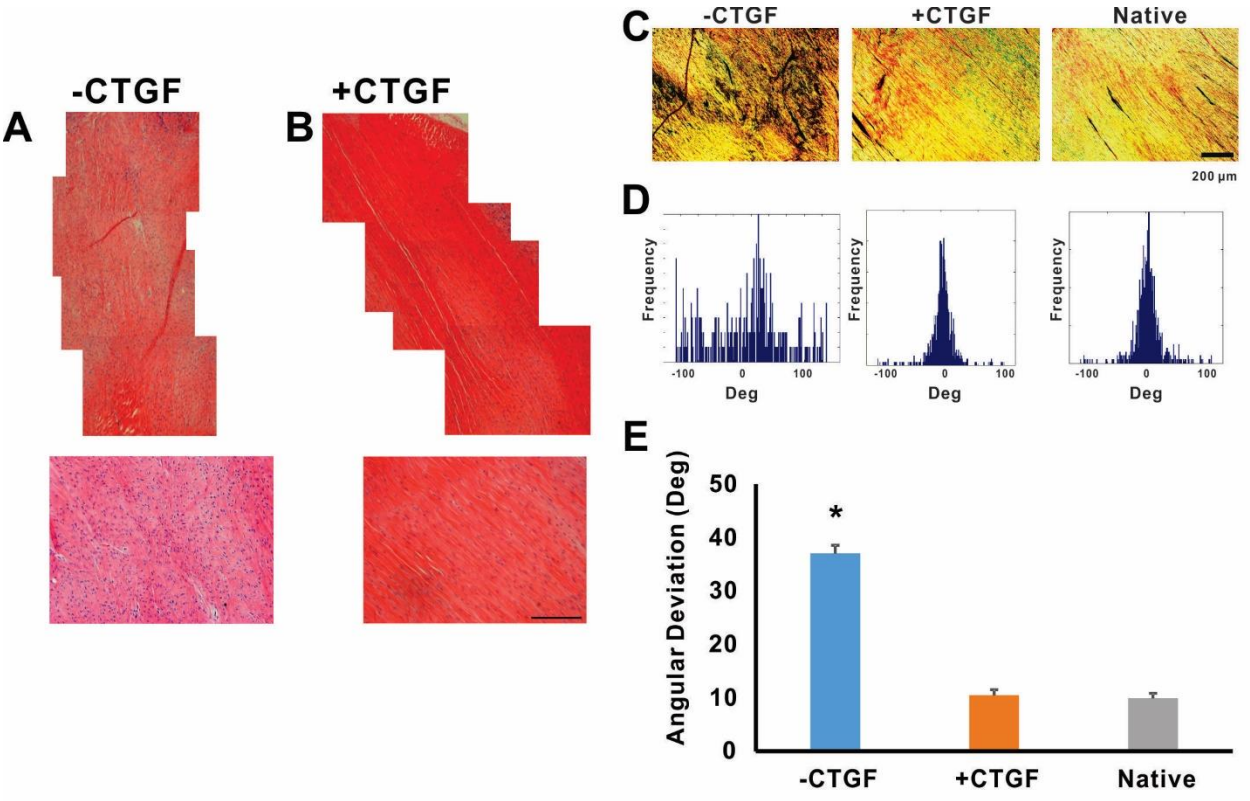

Figure S4. Histology of the regenerated PT at 4 wks. Tendon injury resulted in scar-like disorganized matrix with hypercellularity without CTGF for 4 wks (A), whereas CTGF delivery yielded native-like dense and aligned fibrous structure (B), consistent with data by 2 wks (**Fig. 3**). Collagen fiber orientation was assessed by Picrosirious Red staining (C), followed by an automated digital image processing for local directionality and angular deviation (AD) (D - E). Alignment of collagen fibers in the CTGF-regenerated tendon was similar to that of the native tendon, in contrast to disoriented fibers in scar-like tendon without CTGF (D). Quantitatively, the AD of fibers in samples without CTGF was significantly larger than the AD of CTGF-regenerated and native tendons (E). n = 6 per samples, *:$p<0.001$ compared to +CTGF and native, One way ANOVA with post-hoc Tukey HSD.

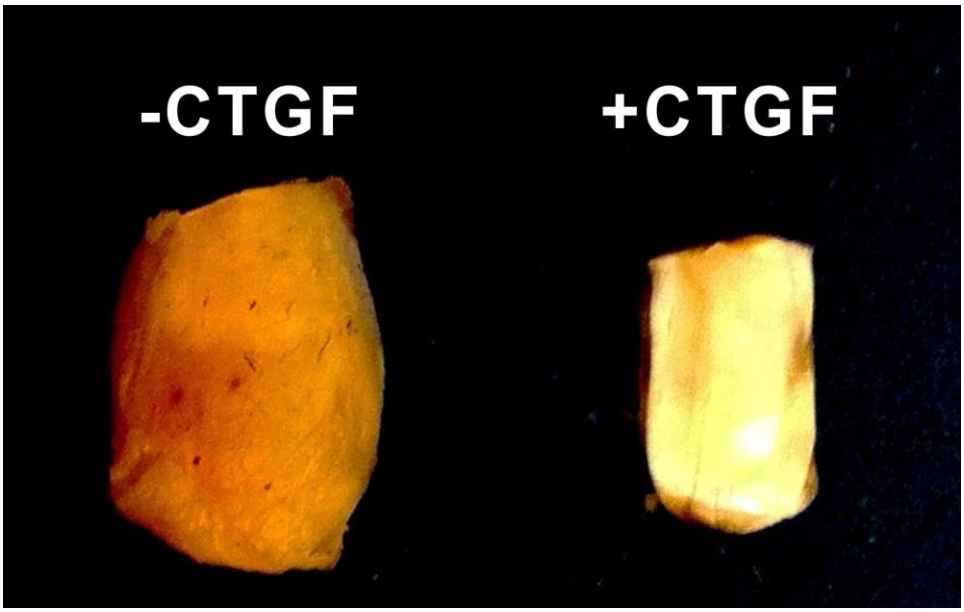

Figure S5. Rat PT tissues harvested at 4 wks post-op with or without CTGF delivery. Macroscopic images taken using a digital camera demonstrated an obvious volumetric increase in the healed PT without CTGF as compared to the CTGF-regenerated PT.

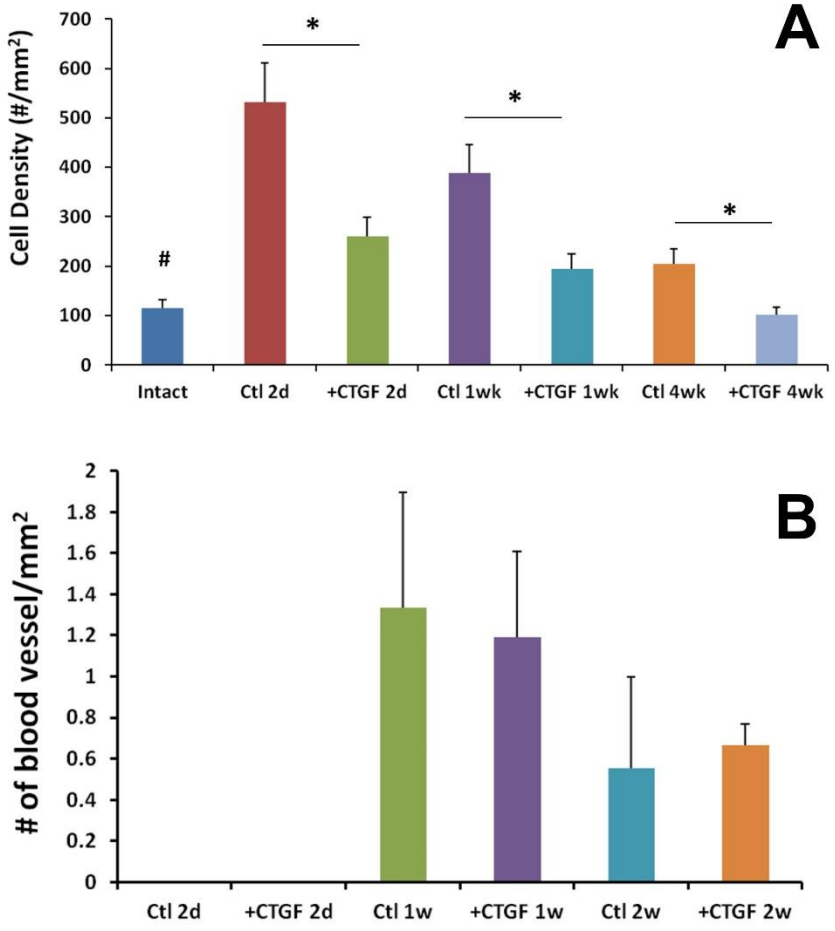

Figure S6. Quantitative analysis of the number of cells and blood vessels in the healing tendon. Total cell densities in the fibrin alone group were significantly higher than in the CTGF-delivered group by 4 wks (A). There was no significant difference in blood vessel number between the fibrin alone (control) and CTGF-delivered group (B). n = 10 randomly selected slides per group, *: $p<0.001$, One way ANOVA with post-hoc Tukey HSD.

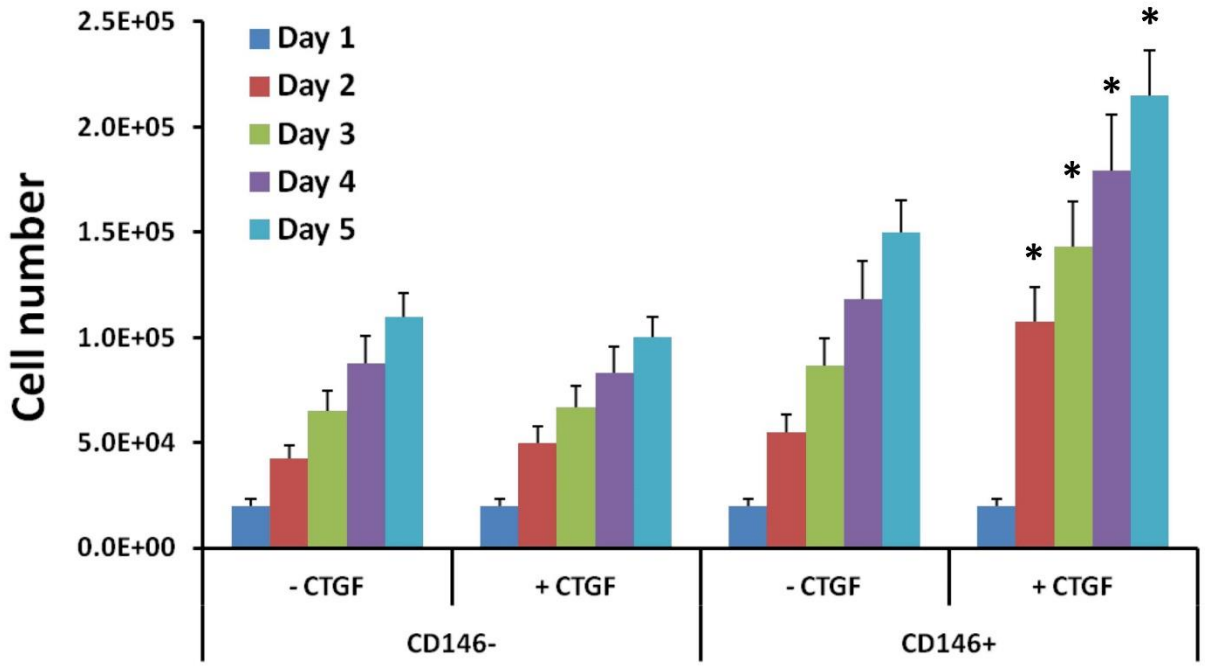

Figure S7. Cell proliferation promoted by CTGF. Isolated and sorted CD146$^{+/-}$ tendon cells were plated on 6 wells (20,000 cells/well) and cultured up to 5 days with or without 100 ng/mL CTGF. By 5 days, CTGF treatment significantly increased the total number of CD146^{+} cells as compared to without CTGF. However, CTGF failed to affect the proliferation of CD146^{-} cells. n = 6 biological replicates per group. *: $p<0.001$ compared to untreated group, One way ANOVA with post-hoc Tukey HSD.

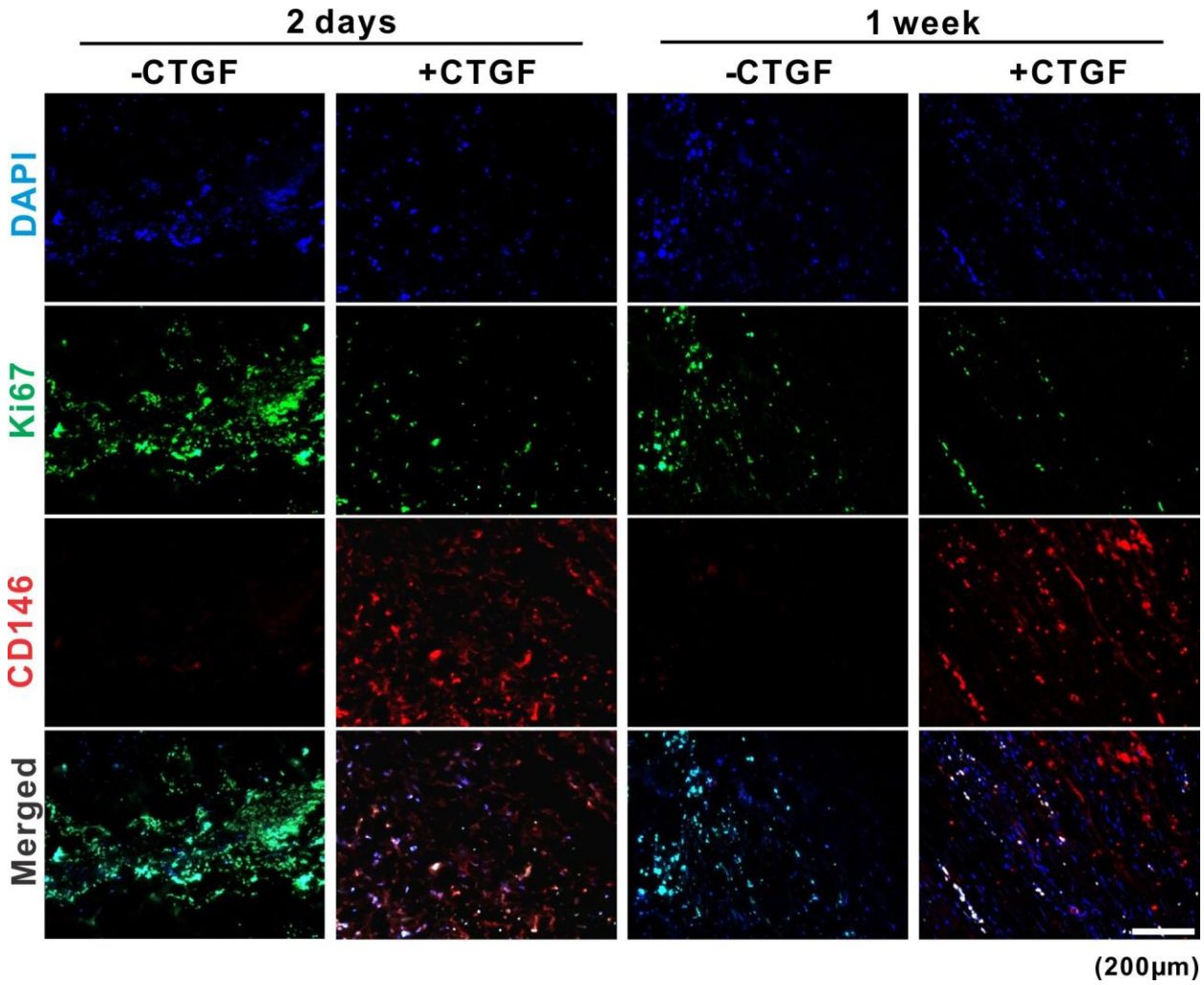

Figure S8. Ki67^{+} proliferative cells in the healing tendon with or without CTGF delivery. Abundant Ki67^{+} cells were found in the tendon healing region without CTGF by 2 days that diminished by 1 wk. The majority of CD146^{-} cells were Ki67^{+} 2 days post-op, whereas over 65% (**Fig. 6A**) of CD146^{+} cells were Ki67^{+}. Images are selected as the representatives of 6 replicates total.

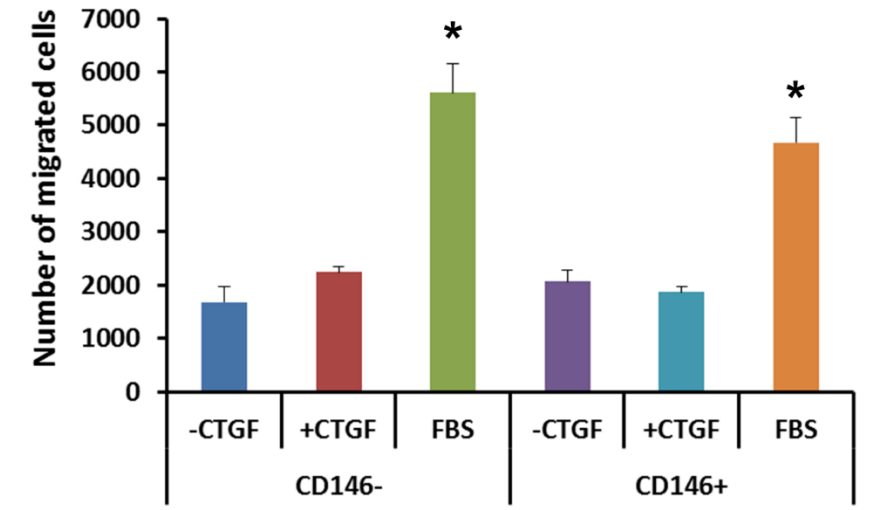

Figure S9. *In vitro* migration of CD146$^{+/-}$ tendon cells with CTGF. A boyden chamber-based migration assay (Cultrex® Cell Migration Assay, R&D Systems) was performed with and without 100 ng/mL CTGF with 10% FBS as a positive control. After 24 hours, the total number of migrated cells was not affected by the addition of CTGF both in CD146^{-} and CD146^{+} tendon cells. n = 6 per group, *:$p<0.0001$ compared to other groups, One way ANOVA with post-hoc Tukey HSD.

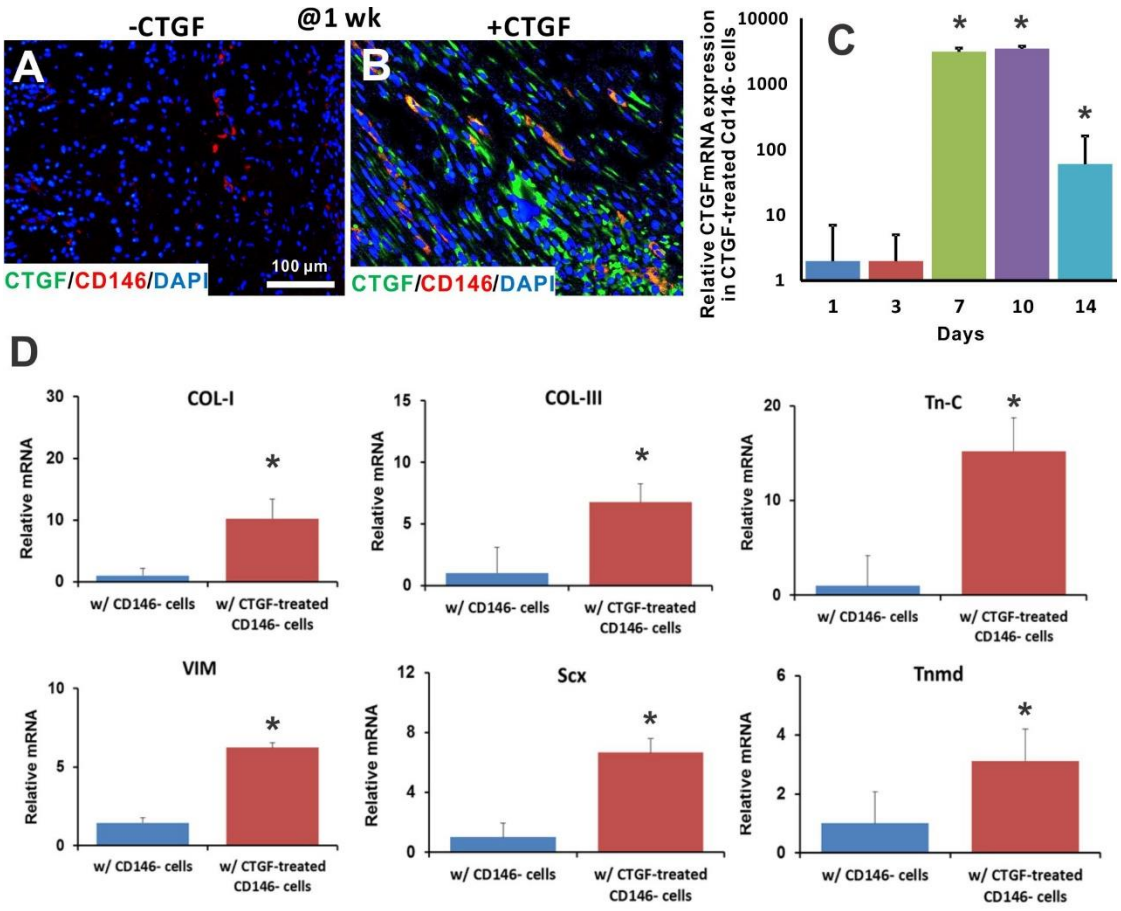

Figure S10. CTGF expression in CTGF-treated CD146^{-} cells both *in vivo* and *in vitro*. At 1-wk post-op, abundantly more CD146^{-} cells in the healing tendon with CTGF delivery expressed CTGF as compared to the healing tendon without CTGF delivery (A, B). *In vitro*, when CD146^{-} rat PT cells were treated by 100 ng/mL CTGF at day 1, CTGF mRNA expression showed an over 3000 fold increase from day 7, followed by a gradual decrease (C). n = 6 biological replicates per group. *: $p < 0.00001$ compared to the base line. When CTGF-once treated (1 day) CD146^{-} cells were co-cultured with CD146^{+} TSCs in 6 well Transwell® (Corning) system for 2 wks, tendon related gene expressions including COL-I and III, Tn-C, VIM, SCX, and Tnmd were significantly increased as compared to co-culturing with untreated CD146^{-} cells (D). n = 6 biological replicates per group. *: $p< 0.001$ compared to the control, One way ANOVA with post-hoc Tukey HSD.

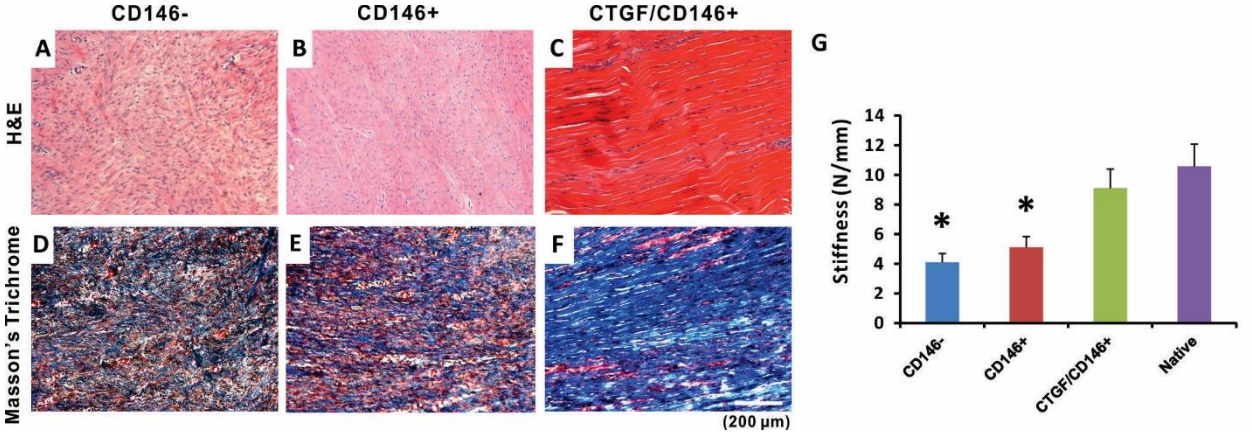

Figure S11. Tendon healing with cell transplantation. After 4 wks of cell transplantation (total 500,000 cells per sample), PT receiving $CD146^-$ cells (A, D) or untreated $CD146^+$ cells (B, E) showed unorganized scar-like tissue formation. However, transplantation of $CD146^+$ cells pretreated with CTGF for 1 wk resulted in reorganized collagen structure reminiscent of native tissue (C, F). Images are selected as the representatives of 6 replicates total. Tensile stiffness of healed tendon with CTGF-treated CD146+ cells was on the level of that of the native tendon, in contrast to tendons receiving $CD146^-$ cells or untreated $CD146^+$ cells (G) (n = 6 per group, *:$p<0.001$ compared to native; One way ANOVA with post-hoc Tukey HSD).