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# Supplementary Materials for

## Targeting the NF-κB signaling pathway in chronic tendon disease

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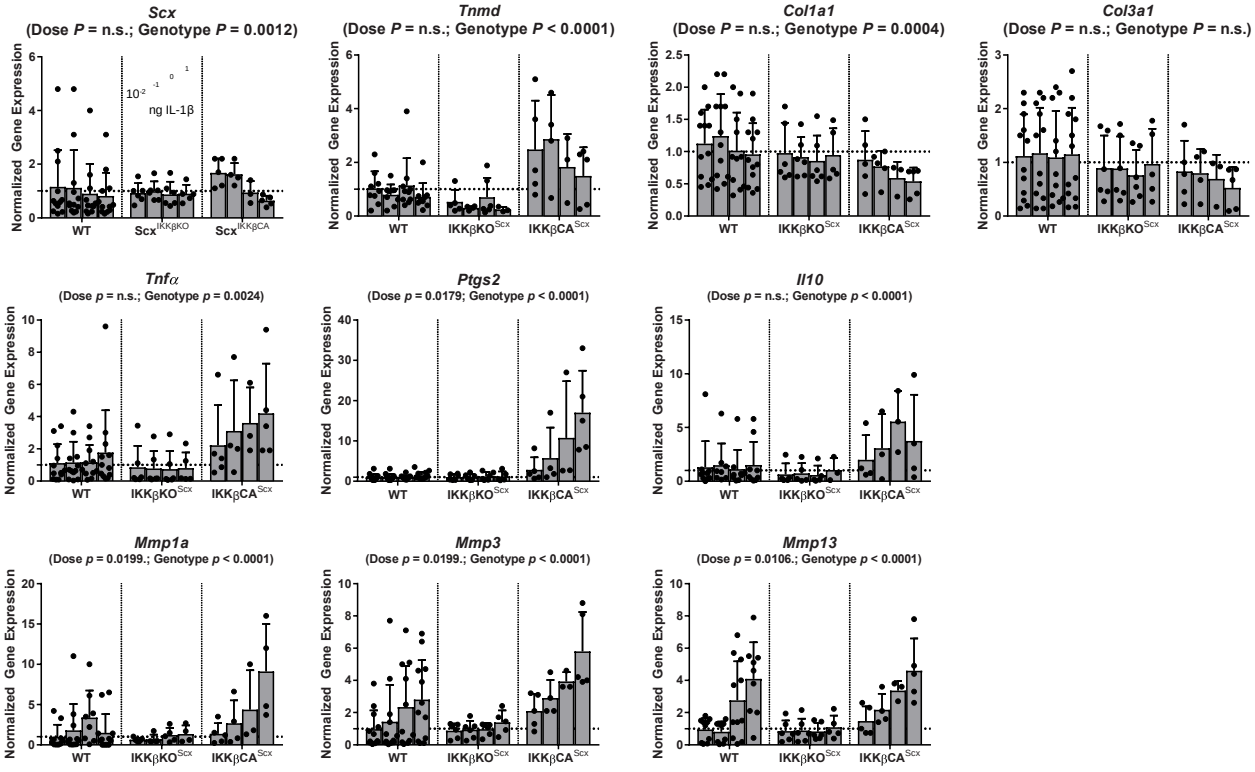


**Fig. S1. Gene expression of cultured tendon fibroblasts in response to varying doses of IL-1β.** Fibroblasts were isolated from tail tendons from wildtype (WT, $n = 10$), tendon-specific IKKβ knockout ($IKK\beta KO^{Scx}$, $n = 6$), and constitutively active IKKβ ($IKK\beta CA^{Scx}$, $n = 5$) mice. Gene expression is normalized to *GAPDH*. Data are shown as mean ± SD. Statistically significant differences were calculated using two-way ANOVA (genotype, dose) with Tukey's post-hoc test.

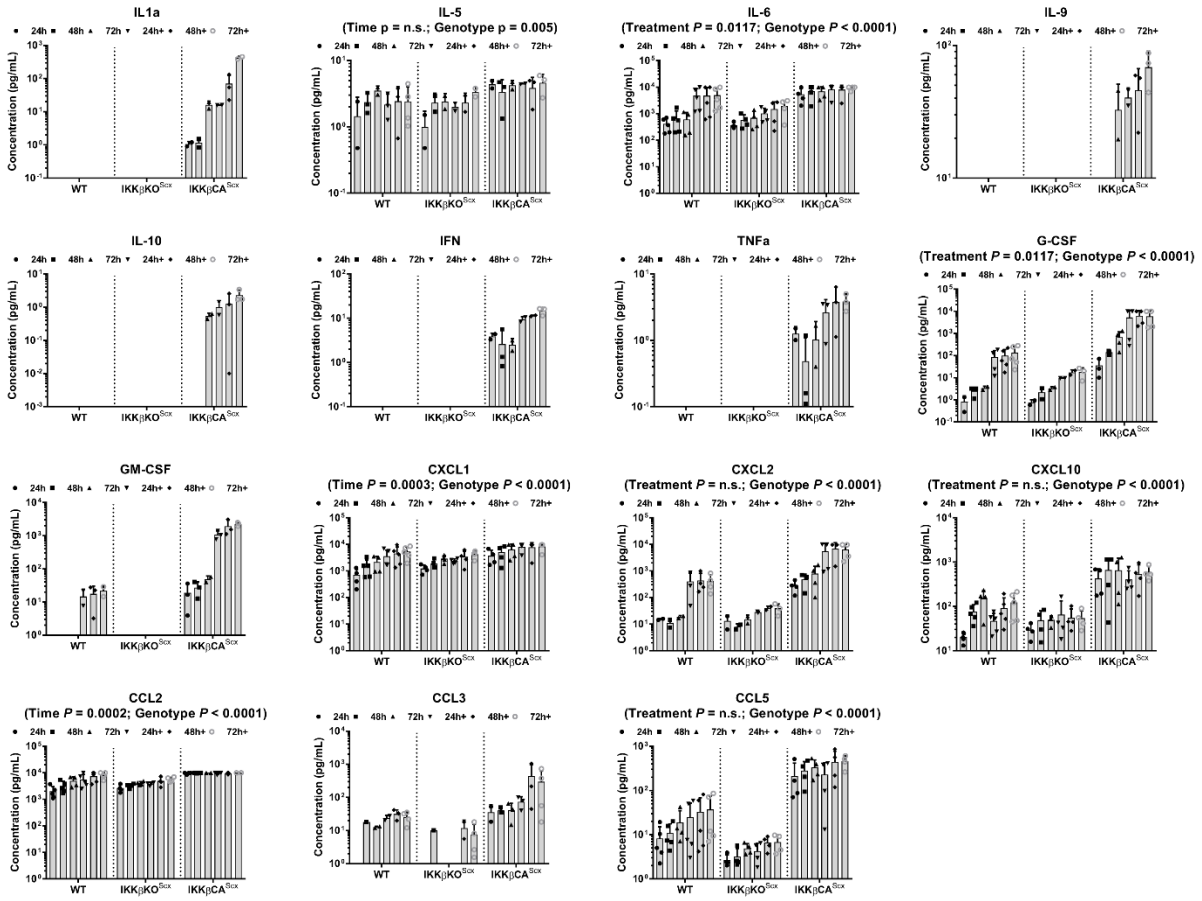


**Fig. S2. Multiplex ELISA of cultured tendon fibroblasts in response to 10 ng IL-1β over the 72-hour period.** Fibroblasts were isolated from tail tendons from wildtype (WT, $n = 5$), tendon-specific IKKβ knockout ($IKK\beta KO^{Scx}$, $n = 5$), and constitutively active IKKβ ($IKK\beta CA^{Scx}$, $n = 5$) mice. Bars represent mean + SD. Statistically significant differences were calculated using two-way ANOVA (genotype, treatment) with Tukey's post-hoc test. Datasets without reported *P*-values were not compared due to undetectable baseline cytokine concentrations in WT controls.

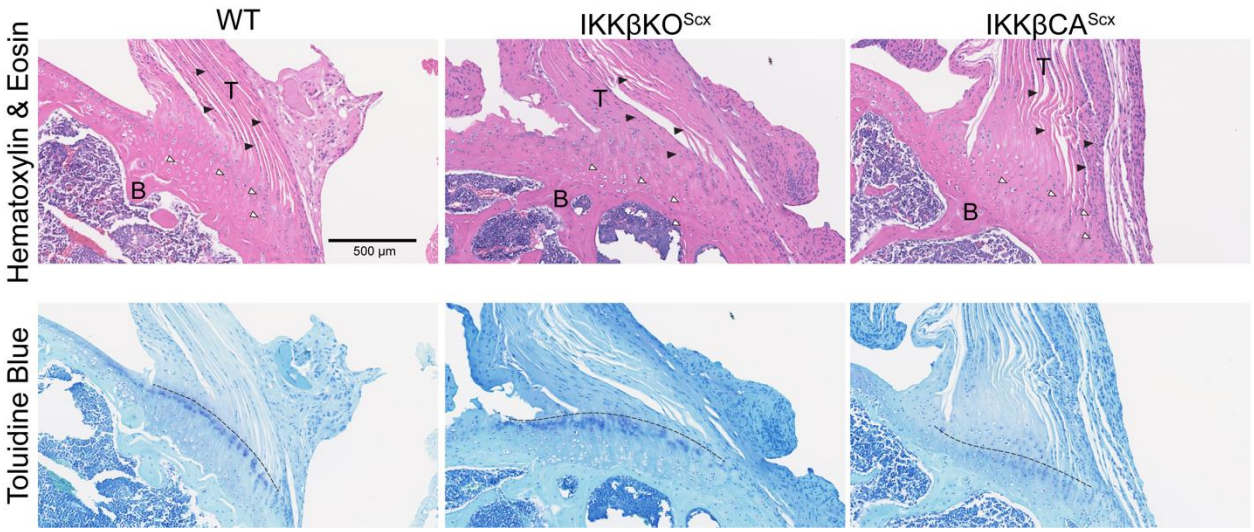


**Fig. S3. H&E- and Toluidine blue–stained section of tendons and tendon entheses of WT, IKKβKO$^{Scx}$, and IKKβCA$^{Scx}$ mice.** Black arrowheads: spindle shaped tendon fibroblasts indicated, white arrowheads: enthesis chondrocytes; metachromasia demonstrating fibrocartilage interface can be seen below the dashed line in Toluidine Blue-stained sections.

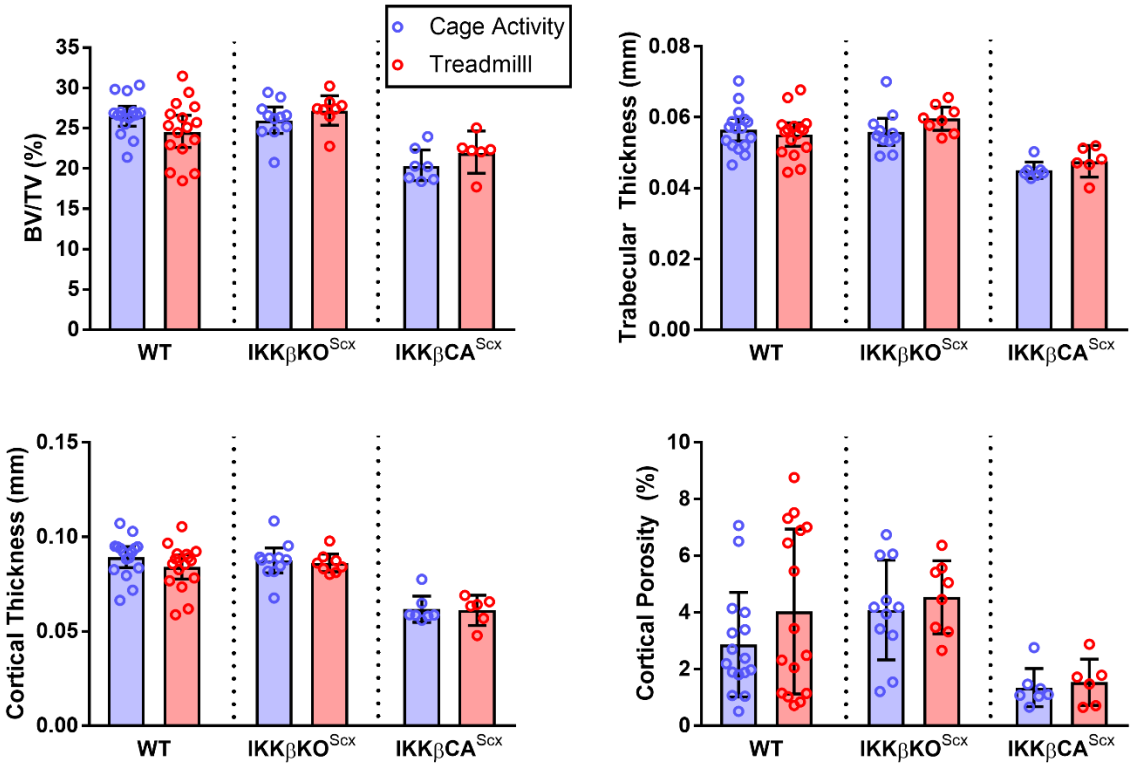


**Fig. S4. µCT results for treadmill overuse model.** 10-week-old mice were subjected to a chronic overuse protocol with 1 week of progressive training followed by 4 weeks of downhill running. Control mice were permitted normal cage activity. The humeral head was scanned at an energy of 55 kVP, intensity of 145 µA, and a resolution of 12.3 µm. Data are shown as mean ± SD with individual points representing biologically independent samples.

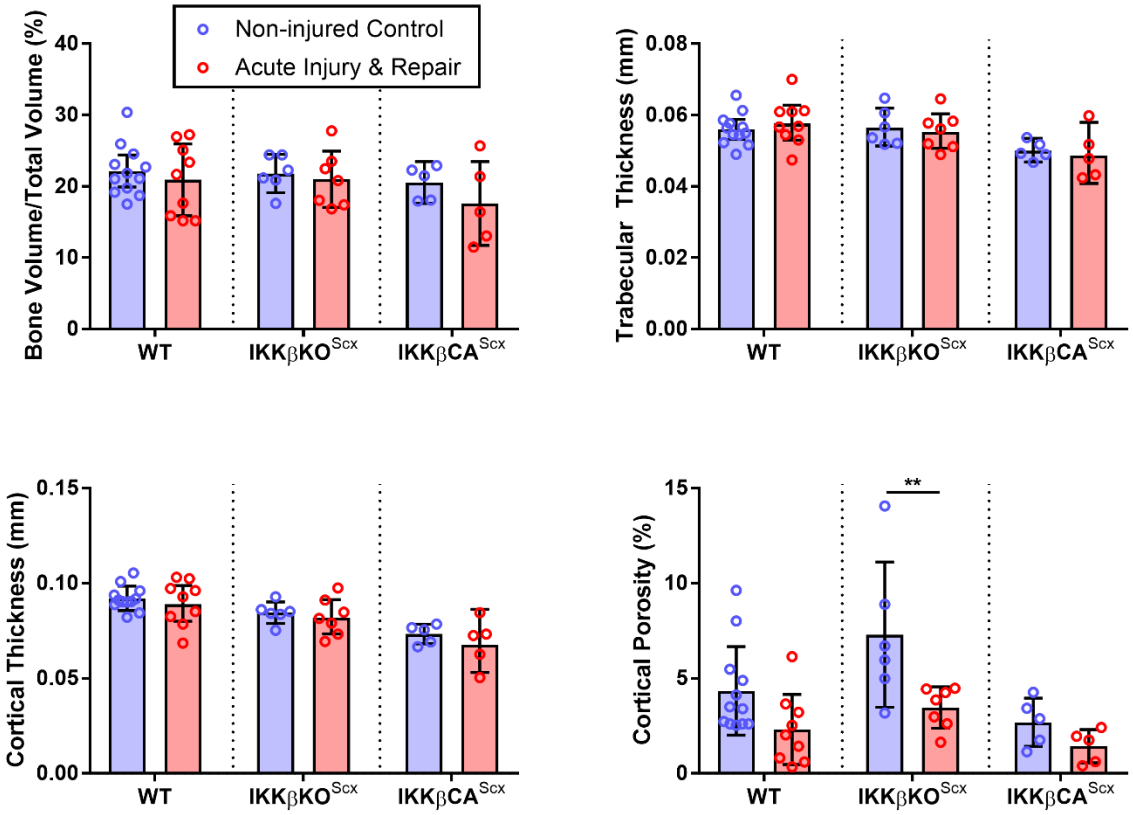


**Fig. S5. µCT results for acute injury and repair model.** 10-week-old mice were subjected to a unilateral acute injury of the supraspinatus tendon and immediate repair followed by 2 weeks of recovery. Sham operations were performed on contralateral limbs. The humeral head was scanned at an energy of 55 kVP, intensity of 145 µA, and a resolution of 12.3 µm. Data are shown as mean ± SD with individual points representing biologically independent samples. Statistically significant differences were calculated using One-way ANOVA (genotype, treatment) with Fisher's LSD post-hoc test ** - $P < 0.01$.

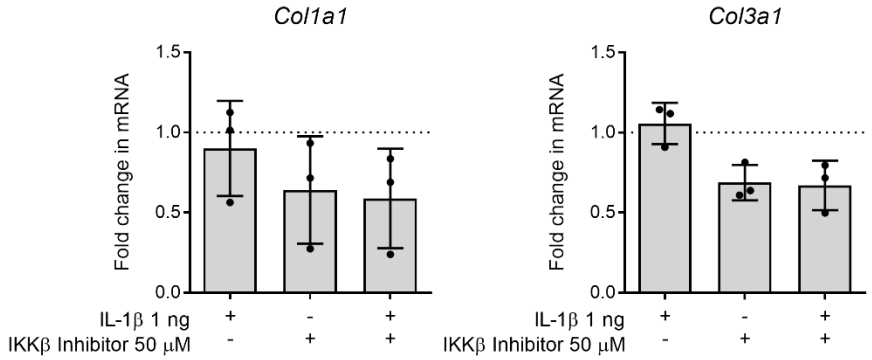


**Fig. S6. Gene expression of cultured human tendon fibroblasts in response to IL-1β and IKKβ inhibitor.** Data are shown as mean ± SD with individual points representing biologically independent samples. Statistically significant differences were calculated using One-way ANOVA (treatment) with Fisher's LSD post-hoc test.

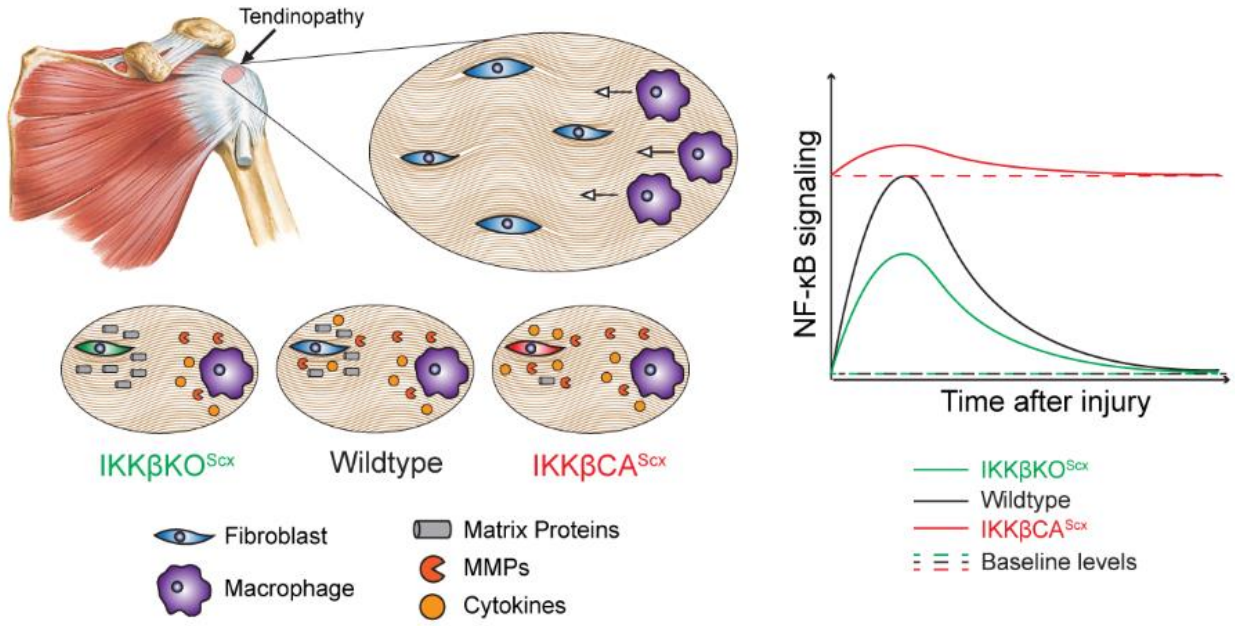


**Fig. S7. Schematic of how IKKβ/NF-κB drives chronic tendinopathy.** Schematic illustrating how injury-induced pro-inflammatory cytokines cause tendon fibroblasts to suppress tissue anabolism and increase matrix catabolism and cytokine production. NF-κB signaling within the tendon stromal and immune compartment increases during initial phases of healing. Constitutive activation of IKKβ (IKKβCA$^{Scx}$) chronically degrades the rotator cuff by synthesizing degenerative enzymes and pro-inflammatory cytokines. Fibroblasts without IKKβ (IKKβKO$^{Scx}$) remain agnostic to proinflammatory cytokines, maintain matrix production, and keep total NF-κB signaling lower. Dotted lines represent basal expression.

**Table S1. Semi-quantitative histological assessment of supraspinatus tendons in WT, IKKβKO$^{Scx}$, IKKβCA$^{Scx}$ mice.** Mast Cells, PMN Acute Inflammatory Cells, Monocytes: # of samples where present.

| | **WT (*n* = 8)** | **IKKβKO$^{Scx}$ (*n* = 4)** | **IKKβCA$^{Scx}$ (*n* = 4)** |
|---|---|---|---|
| **Mast Cells** | 1 of 8 | 3 of 4 | 1 of 4 |
| **PMN** | 0 of 8 | 0 of 4 | 0 of 4 |
| **Monocytes** | 8 of 8 | 4 of 4 | 4 of 4 |
| **Total Inflammatory Cells** | 1.00 (1,1) | 1.00 (1,1) | 2.00 (2,2) |
| **Total Cellularity** | 1.13 (1,2) | 1.75 (1,3) | 3.00 (3,3) |