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Supplementary Materials for

Primary cilia as the nexus of biophysical and hedgehog signaling at the tendon enthesis

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Supplementary Materials

Table S1. Primers for Real-time RT-PCR assay.

Gene	Primer sequence
GAPDH	Sense: 5'-TGTGTCCGTCGTGGATCTGA-3'; Antisense: 5'-TTGCTGTTGAAGTCGCAGGAG-3';
IFT88	Sense: 5'-TGGCCAACGACCTGGAGATTAACA-3'; Antisense:5'-ATAGCTGCTGGCTTGGGCAAATTC-3';
IFT80	Sense: 5'-AAGGAACCAAAGCATCAAGAATTAG-3'; Antisense: 5'-AGATGTCATCAGGCAGCTTGAC-3';
Dync2li1	Sense: 5'-ACCTTAGCGTTGGAGTACAC-3'; Antisense: 5'-TAAGTCCAGCAAGGAGGTTC-3';
Kif3a	Sense: 5'-GCTATAGACAGGCCGTCAGC-3'; Antisense: 5'-GTCTTTGGAGGTTCGTTGGA-3';
Ptch1	Sense: 5'-TGCTGTGCCTGTGGTCATCCTGATT-3'; Antisense: 5'-CAGAGCGAGCATAGCCCTGTGGTTC-3';
Gli1	Sense: 5'-GGTCTCGGGGTCTCAAACTG-3'; Antisense: 5'-CCATTCTCTGGTGGGGTTCC-3';
Gli2	Sense: 5'- AACTTTTGTCTCCTCGGGTCC-3'; Antisense: 5'-CTGCTGTCCTCCAAGAGACC-3';
Gli3	Sense: 5'-AAGCCCATGACATCTCAGCC-3'; Antisense: 5'-CTCGAGCCCACTGTTGGAAT-3';

Table S2. Key reagents and their sources.

Reagent	**Source**	**Catalog No.**	**Usage**
Monoclonal acetylated tubulin	Sigma	T7451	1:300
Arl13b	Proteintech	17711-1-AP	1:50
IFT88		13967-1-AP	1:100
Percentrin	Abcam	ab4448	1:200
Smoothened	LSBio	LS-A2666	1:100
Gli1	Abcam	ab49314	1:500
Goat anti-mouse IgG, Alexor Fluor 488	ThermoFisher	A-11001	1:1000
Goat anti-mouse IgG, Alexor Fluor 568		A-11031	
Goat anti-mouse IgG, Alexor Fluor 647		A-32733	
Goat anti-rabbit IgG, Alexor fluor 488		A-11008	
Goat anti-rabbit IgG, Alexor Fluor 568		A-11011	
Goat anti-rabbit IgG, Alexor Fluor 647		A-21244	
DAPI		D1306	

Table S3. Sample sizes for assays.

		Sample size				
		Histology and IHC	RT-PCR	Gait analysis	μCT	Biomechanics
C57BL/6J mice	P1-W13	5				
	Control	4	4			
	Overloaded	5	4			
	Unloaded	5				
$Gli1CreER^{T2}$;$Rosa26^{mT/mG}$		4-5	6-7			
ScxCre;$IFT88^{fl/fl}$ or WT		4-5		5-9	5-11	5-12
ScxCre; $Smo^{fl/fl}$ or WT	Control	5-7		7-8	7-9	7-9
	Overloaded	5		8	8-9	7-8
	Unloaded	4-5		8	8	8

Note: Sample sizes were balanced between male and female mice; μCT, microcomputed tomography.

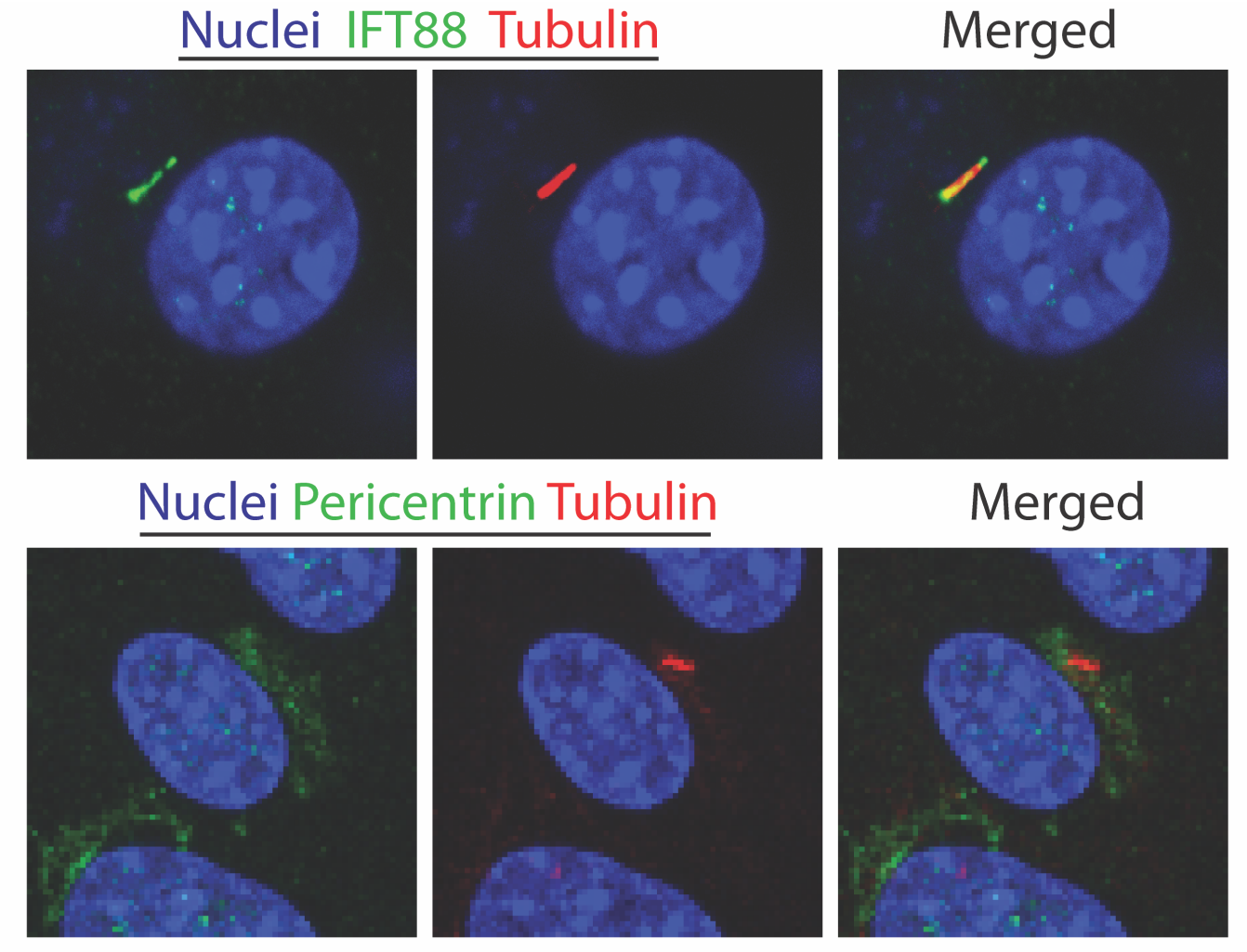

Fig. S1. Representative images showing the presence of primary cilia at the tendon enthesis. Cilia are visualized by ciliary markers IFT88 (green, top) and acetylated tubulin (red) and the centrosome marker pericentrin (green, bottom).

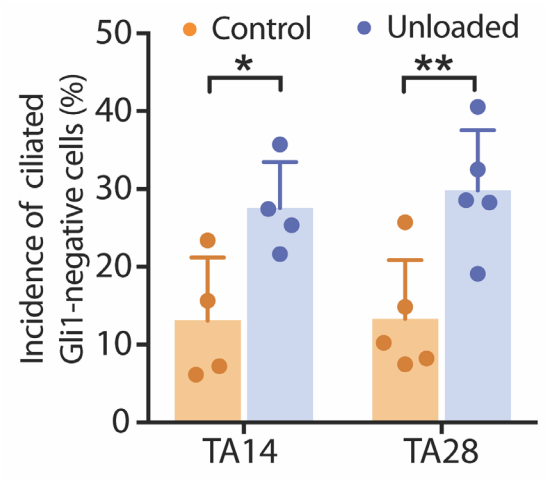

Fig. S2. Quantification of incidence of ciliated Gli1-negative cells, normalized by the number of Gli-negative cells. Incidence was determined from immunofluorescent images of tendon entheses of GliCreER^{T2};Rosa26$^{mT/mG}$ mice with tamoxifen injection at P14 or P28 (labelled TA14 and TA28, respectively) and by neonatal BtxA-induced paralysis (unloaded) or saline injection (control) at the time points indicated. TA, tamoxifen injection. *p<0.05, **p<0.01.

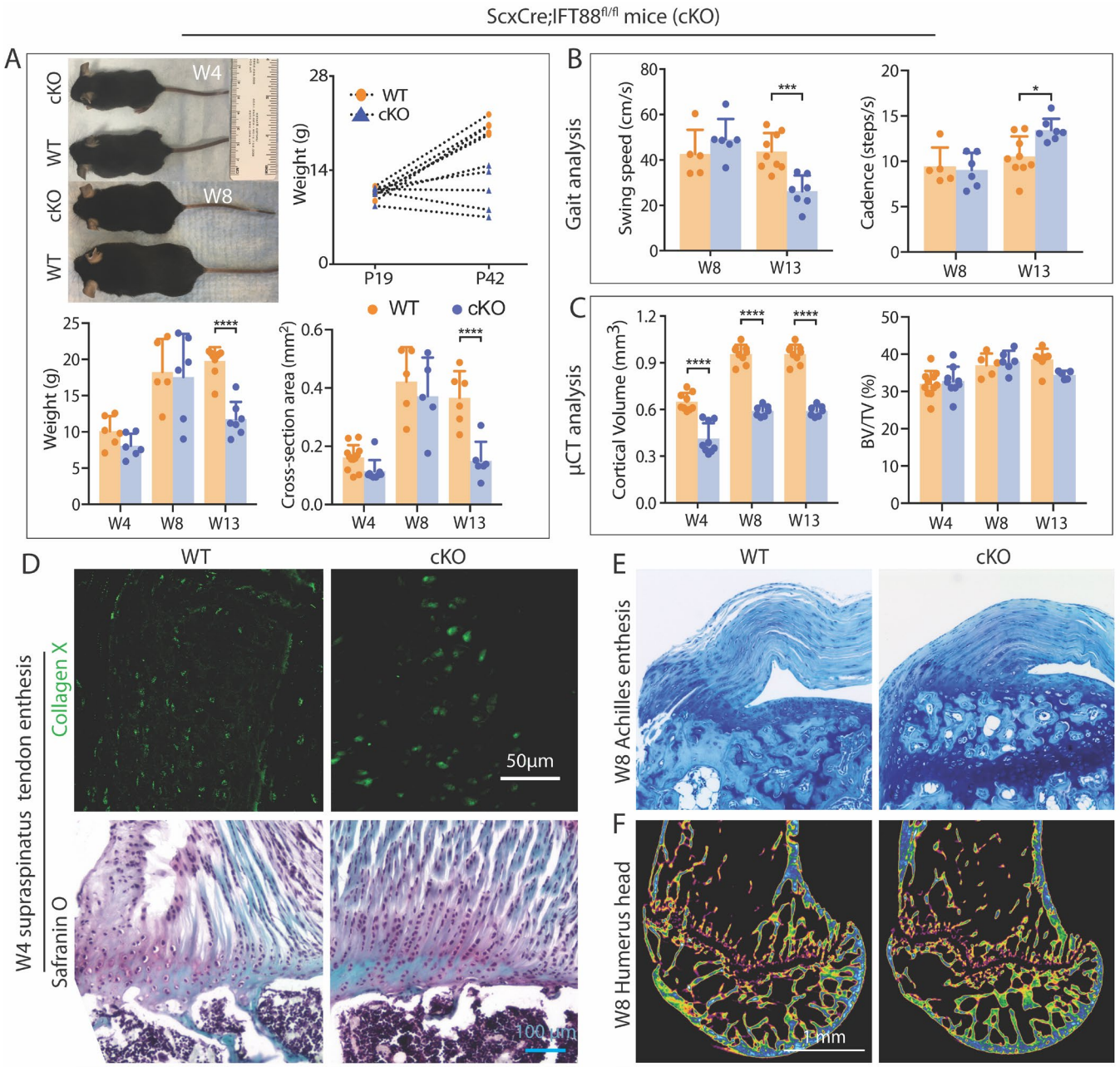

Fig. S3. Phenotype of cilia knockout ScxCre;IFT88$^{fl/fl}$ (cKO) mice.

(A) Mice with cilia deletion appeared normal at W4 but were smaller than WT controls by week 13. ScxCre;IFT88$^{fl/fl}$ mice had retarded growth rates after P19. Photo Credit: Fei Fang, Columbia University.

(B) Gait patterns, including swing speed and cadence, were changed in 13-week-old ScxCre;IFT88$^{fl/fl}$ mice compared to WT controls.

(C) Trabecular volume normalized to total volume (BV/TV) and cortical bone volume were decreased in ScxCre;IFT88$^{fl/fl}$ mice compared to WT controls.

(D) Immunostaining for collagen X and Safranin O staining of tendon entheses from 4-week-old mice showed minor changes in fibrocartilage formation in cKO mice compared to WT controls.

(E) Collagen organization of the Achilles tendon of 8-week old ScxCre;IFT88$^{fl/fl}$ mice was similar to WT controls.

(F) Bone morphology of humerus head from 8-week old cKO mice was similar to WT controls.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

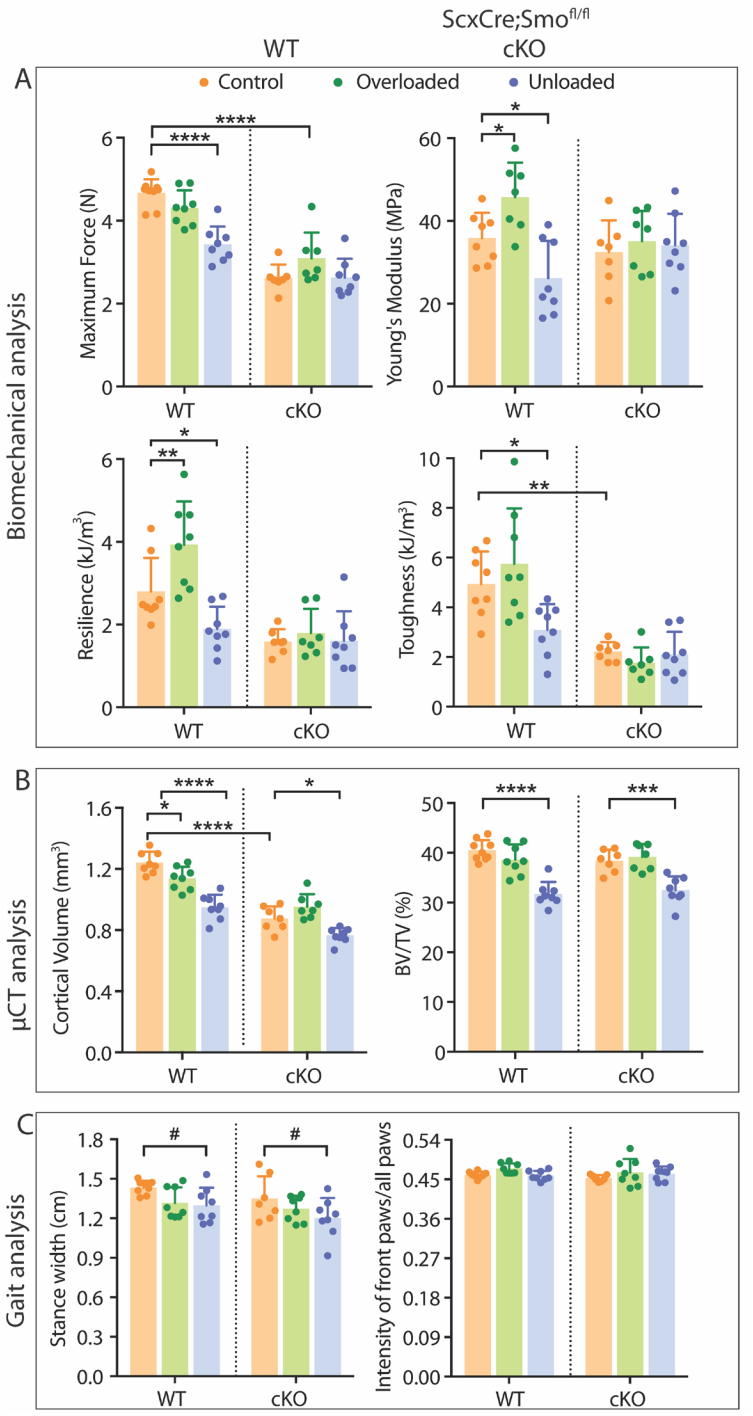

Fig. S4. Functional evaluation of Hh disrupted mice subjected to overloading and unloading treatments.

(A) Biomechanical analysis showed that WT mouse tendon entheses were responsive to changes in loading, whereas ScxCre;Smo$^{fl/fl}$ (cKO) tendon entheses were unresponsive to changes in loading.

(B) µCT analysis showed that humeral head cortical volume and BV/TV were similarly affected by changes to loading in WT and cKO mice.

(C) Gait analysis showed that walking posture and stance width were similarly affected to changes to loading in WT and cKO mice. Stance width: distance between the front paws; Intensity of front paws/all paws: average of the 15 most intense pixels from complete front paws divided by that from all four paws. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.