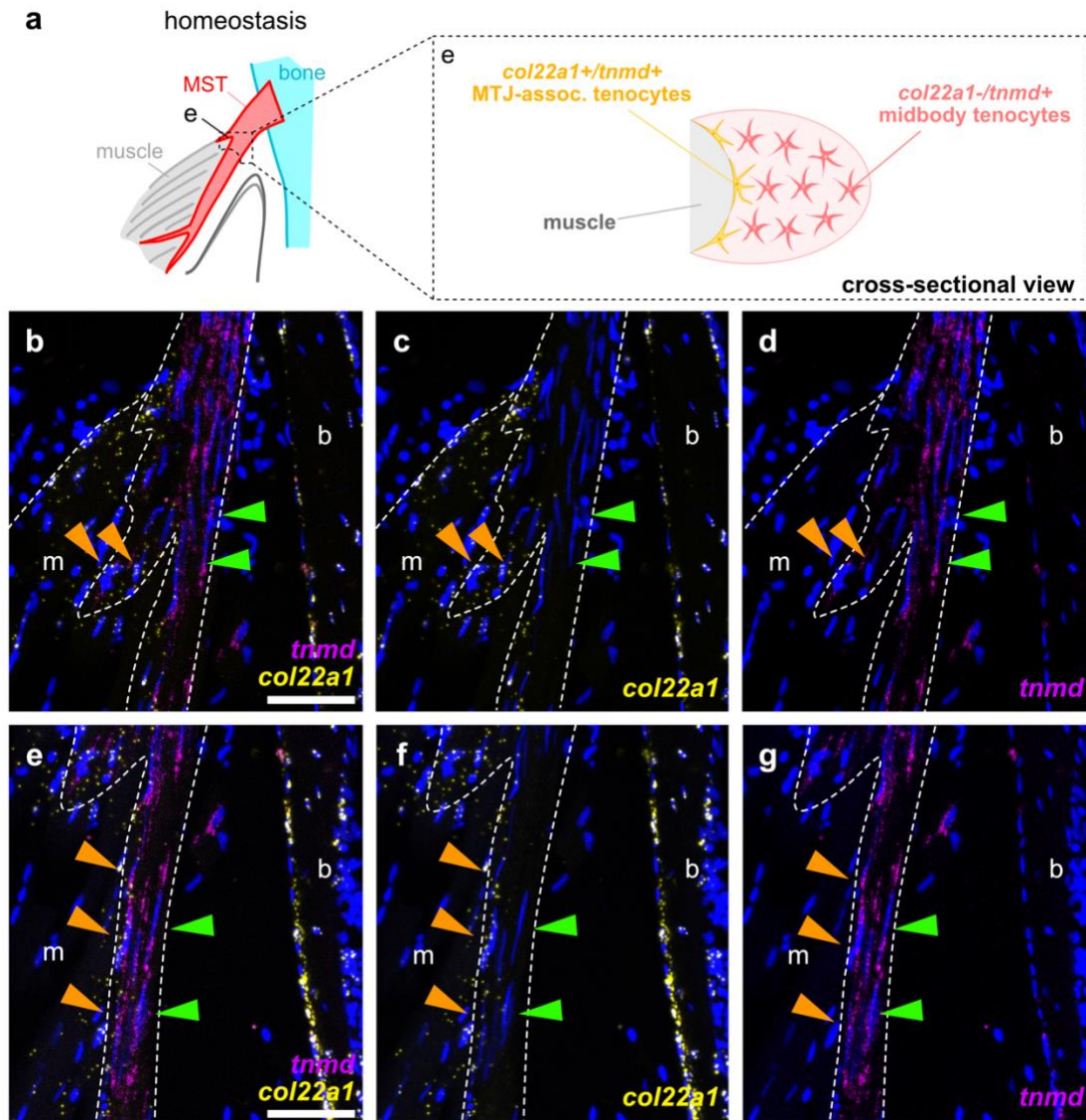
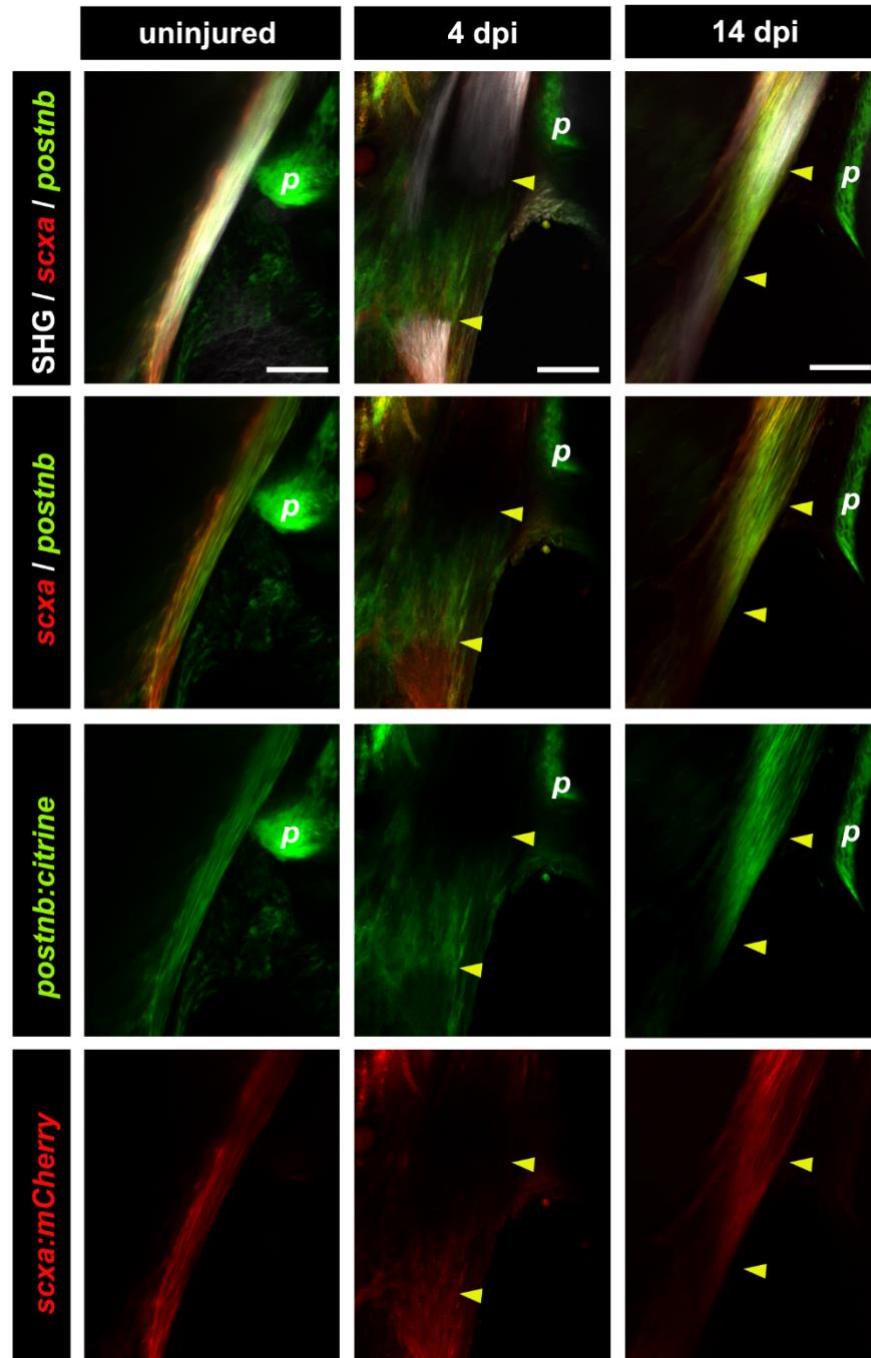


Supplementary Figure 1. The MST contains cells expressing midbody tenocyte and myotendinous junction markers.

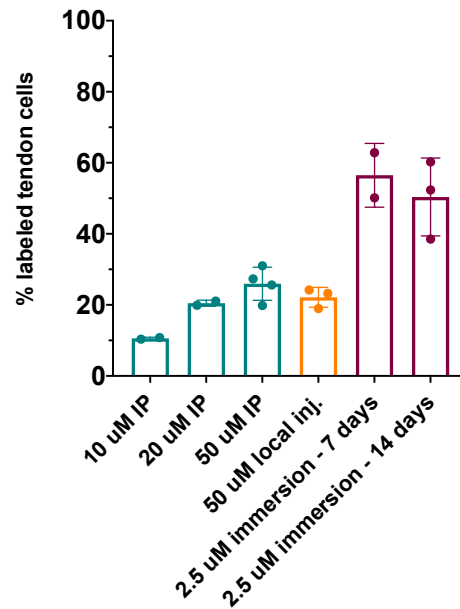
(b-g) Section RNAscope *in situ* hybridization of *tnmd* and *col22a1* on an uninjured adult control MST showing expression along two different regions of the tendon. Panels b-d show a region at the beginning of the MTJ and the midbody/MTJ section. Panels e-g show the section of the



midbody that contains MTJ cells along the length of the tendon. *Col22a1* expression is specifically localized to the tendon cells that are in direct contact (*tnmd+/col22a1+*) with the muscle (m) (orange arrowheads), while *tnmd+/col22a1-* cells are present in tenocytes not directly in contact with the muscle along the length of the tendon (green arrowheads). *Col22a1+* cells can also be seen in periosteal cells lining the maxillary bone (b).



Supplementary Figure 2. *Postnb:citrine* expression during tendon regeneration. Z-projections of sub-slices from 2-photon stacks of uninjured and regenerating (4 and 14 days post-injury (dpi)) MSTs in *postnb:citrine;scxa:mCherry* zebrafish overlaid with second harmonic generation (SHG) signal. Yellow arrowheads denote the severed tendon ends and the boundaries of the defect. p, periosteum. Scale bar, 100 μ m.



Supplementary Figure 3. Optimizing 4OH-T delivery to obtain maximal tendon labeling

during homeostasis. Quantification of the percentage of CFP+ and/or YFP+ labeling in

scxa:creERT2;ubi:zebrabow zebrafish after various methods of 4OH-T delivery. For the IP

injections, ~10 μ L of 4OH-T at the various concentrations detailed was injected daily for 3

consecutive days. For the local injection, ~10 μ L 50 μ M of 4OH-T was injected locally into the

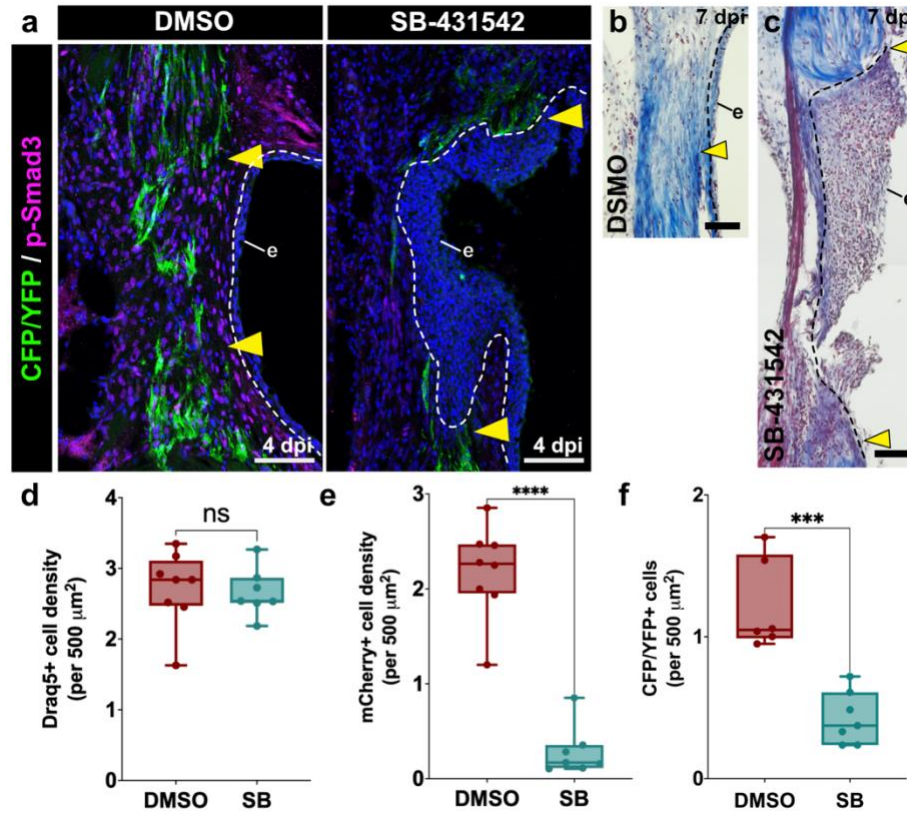
cavity between the skin and the jaw adductor muscle attached to the MST. For immersion,

zebrafish were incubated for 3 nights in 2.5 μ M of 4OH-T. For all conditions, the labeling was

analyzed 7 days after the last treatment, except for immersion which was assessed at both 7

and 14 days post-induction. IP, intraperitoneal; dps, days post-induction. Each point represents

a biological replicate from a different fish and the error bars depict mean $\pm$ standard deviation.



Supplementary Figure 4. Validation of TGF- β inhibition via SB-431542 treatment.

(a) Double immunostaining of CFP+ and/or YFP+ *scxa*-lineage cells (in green) with p-Smad3 (in magenta) at 4 days post-injury (dpi). Yellow arrowheads denote the boundary of the severed tendon ends. Scale bar, 100 μm .

(b-c) Masson's trichrome staining of DMSO- or SB-431542-treated tendons at 7 dpi when treated from 1-7 dpi. Yellow arrowheads denote the boundary of the severed tendon ends (Note: top stub was not captured in section shown in b). Black dotted lines denote the boundary of the epithelium. e, epithelial cells. Scale bar, 20 μm .

(d-f) Quantification of general cell density (d), *scxa:mCherry*+ cell density (e), and *scxa*-lineage cell density (f) in the injury site of regenerating tendons at 7 dpi in DMSO- and SB-431542 (SB) treated zebrafish (excluding skin) following a 1-7 dpi treatment. Unpaired two-tailed t-tests were performed between DMSO and SB-431542 conditions for statistical analysis (d-e: DMSO, N=8,

SB, N=7 in *scxa:mCherry* line; f: DMSO, N=6, SB, N=7 in *scxa:creERT2;ubi:zebrabow* line).

****p< 0.0001, ***p<0.001, ns, not significant.