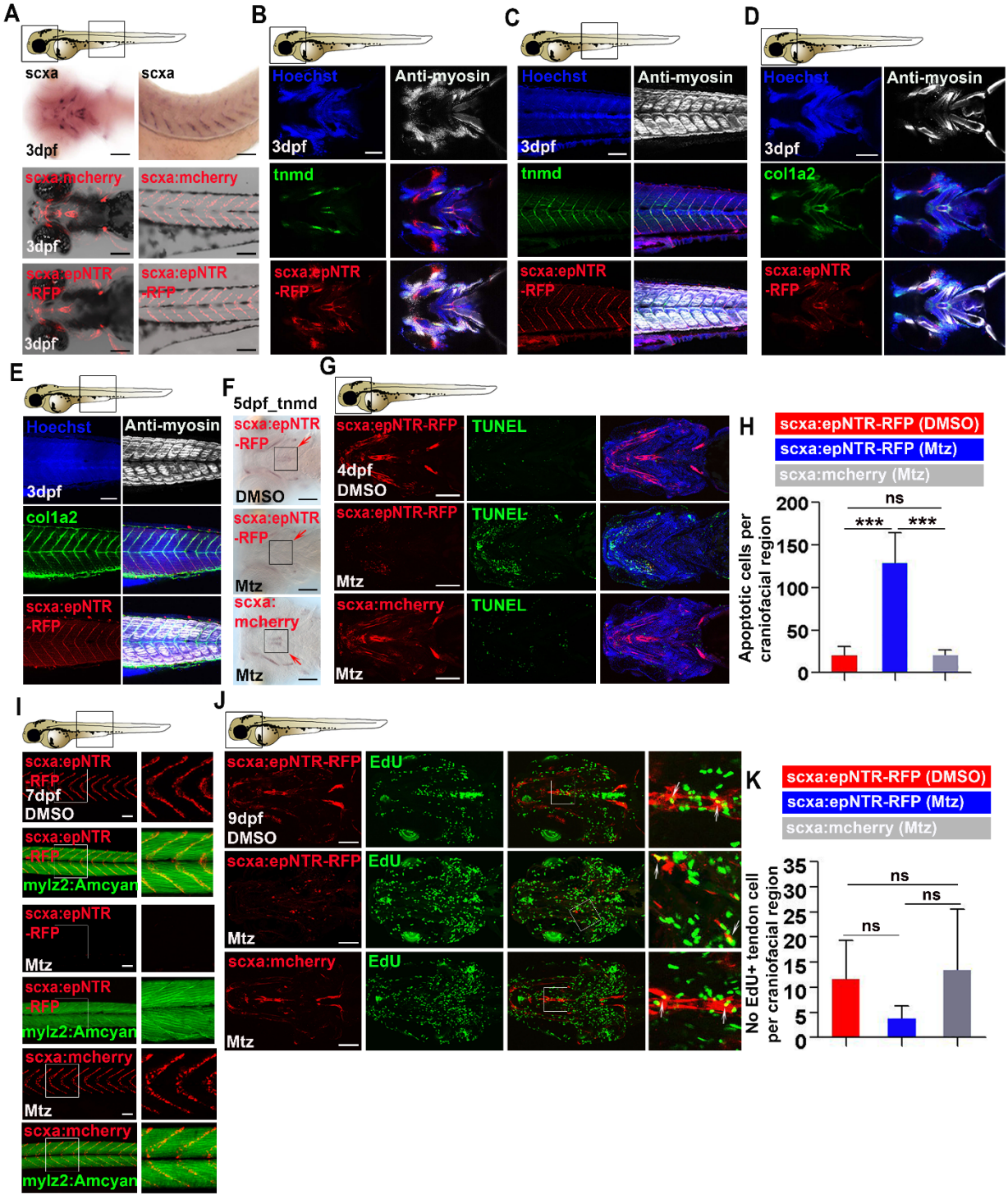

A
scxa
scxa
3dpf
scxa:mcherry
scxa:mcherry
3dpf
scxa:epNTR
-RFP
scxa:epNTR
-RFP
3dpf
B
Hoechst
Anti-myosin
3dpf
tnmd
scxa:epNTR
-RFP
C
Hoechst
Anti-myosin
3dpf
tnmd
scxa:epNTR
-RFP
D
Hoechst
Anti-myosin
3dpf
col1a2
scxa:epNTR
-RFP
E
Hoechst
Anti-myosin
3dpf
col1a2
scxa:epNTR
-RFP
F
5dpf_tnmd
scxa:epNTR
-RFP
DMSO
scxa:epNTR
-RFP
Mtz
scxa:
mcherry
Mtz
G
scxa:epNTR-RFP
TUNEL
4dpf
DMSO
scxa:epNTR-RFP
TUNEL
Mtz
scxa:mcherry
TUNEL
Mtz
H
scxa:epNTR-RFP (DMSO)
scxa:epNTR-RFP (Mtz)
scxa:mcherry (Mtz)
Apoptotic cells per craniofacial region
200
150
100
50
0
ns
***
***
I
scxa:epNTR
-RFP
7dpf
DMSO
scxa:epNTR
-RFP
mylz2:Amcyan
scxa:epNTR
-RFP
Mtz
scxa:epNTR
-RFP
mylz2:Amcyan
scxa:mcherry
Mtz
scxa:mcherry
mylz2:Amcyan
J
scxa:epNTR-RFP
EdU
9dpf
DMSO
scxa:epNTR-RFP
EdU
Mtz
scxa:mcherry
EdU
Mtz
K
scxa:epNTR-RFP (DMSO)
scxa:epNTR-RFP (Mtz)
scxa:mcherry (Mtz)
No EdU+ tendon cell per craniofacial region
35
30
25
20
15
10
5
0
ns
ns
ns

Figure S1. Characterization of a tendon cell ablation line and tendon cell proliferation, Related to Figure 1.

(A) Expression of endogenous *scxa* transcript, *Tg(scxa:mcherry)*, and *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* transgene in the craniofacial (left, ventral views) and trunk region (right, lateral views). Scale bar, 100 μm.

(B) Optical section of *tnmd* transcript (green) and tendon (red), and muscle (white) in craniofacial region in *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish. Scale bar, 100 μm.

(C) Confocal images of tendon cells (red), *tnmd* transcript (green) and muscle (white) in the trunk region in *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish, lateral view. Scale bar, 100 μm.

(D) Optical sections showing tendon cells (red), *col1a2* transcript (green) and muscle (white) in the craniofacial region in *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish, ventral views. Scale bar, 100 μm.

(E) Confocal images of tendon cells (red), *col1a2* transcript (green) and muscle (white) in the trunk region in *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish, lateral views. Scale bar, 100 μm.

(F) Craniofacial expression of *tnmd* transcripts in unablated (top and bottom panels) and ablated (middle panel) embryos at 5dpf. Tendon cells were ablated at 3-5dpf. Red arrow, SH tendon region. Scale bar, 100 μm.

(G) Confocal images of apoptotic cells in the craniofacial region in unablated (top and bottom panels) and ablated (middle panels) embryos. Tendon cells were ablated at 3-4dpf. Scale bar, 100μm.

(H) Quantification of apoptotic cell number in unablated (red, n=6 and grey, n=5) and ablated (blue, n=6) embryos. One-way ANOVA with tukey's post-hoc analysis and data were mean $\pm$ s.d. ***, $p<0.001$. ns, no significance.

(I) Live images of tendon cells (red) and trunk muscle (green) in unablated (top and bottom panels) and ablated (middle panels) embryos. Insets show magnified view at the right. Scale bar, 100μm.

(J) Confocal images of tendon cells (red) and EdU^+ cells (green) in control (top and bottom panels) and regenerated (middle panels) fish. Insets show magnified view shown at the right. White arrow, EdU^+ tendon cells. Scale bar, 200 μm.

(K) Quantification of EdU^+ tendon cell in control (red, n=3 and grey, n=5) and regenerated (blue, n=6) fish. One-way ANOVA with tukey's post-hoc analysis and data were as mean $\pm$ s.d. ns, no significance. Blue color in (B-E, G) was Hoechst staining. All images, anterior to the left.

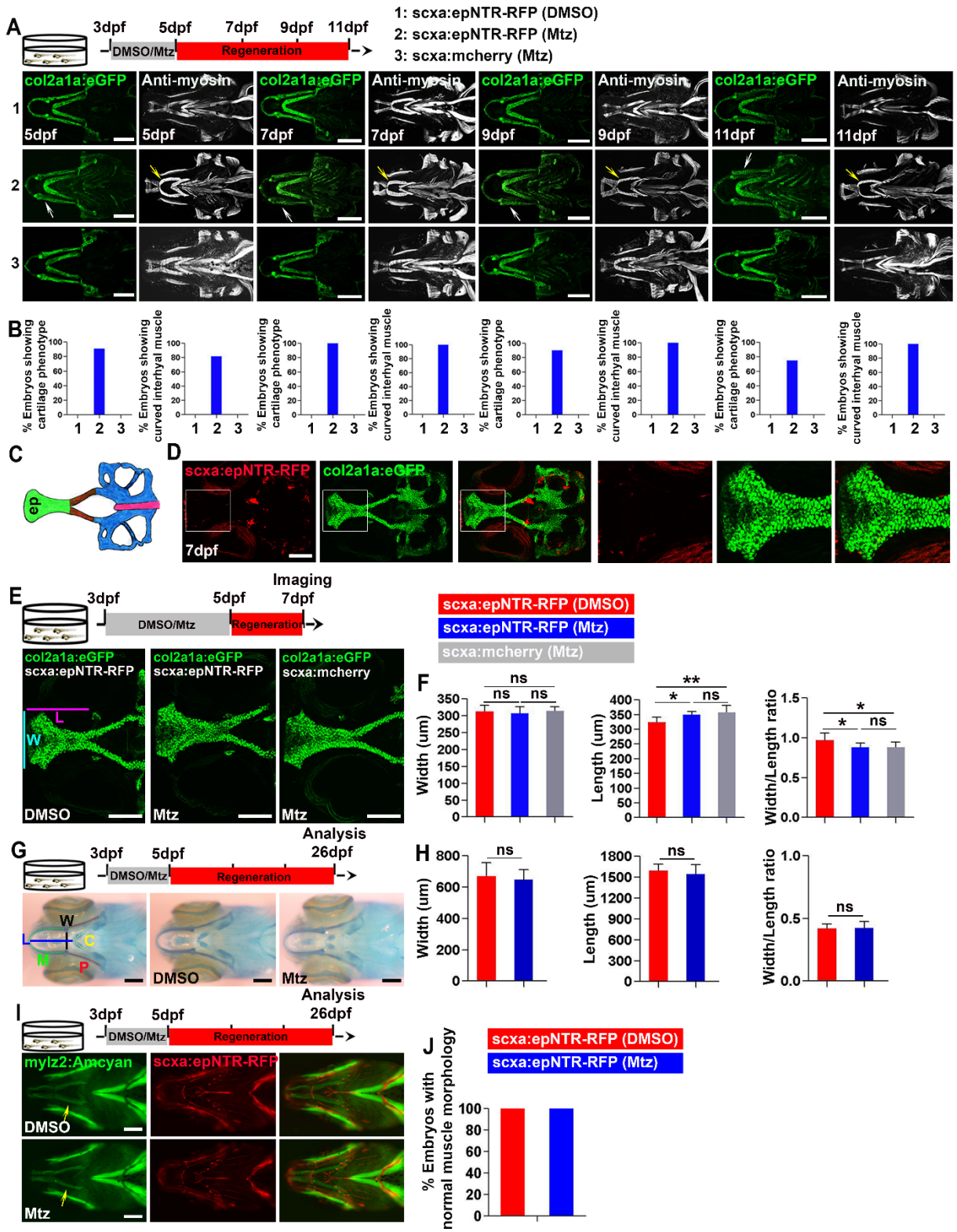

A
3dpf
5dpf
7dpf
9dpf
11dpf
DMSO/Mtz
Regeneration
1: scxa:epNTR-RFP (DMSO)
2: scxa:epNTR-RFP (Mtz)
3: scxa:mcherry (Mtz)
col2a1a:eGFP
Anti-myosin
B
% Embryos showing cartilage phenotype
% Embryos showing curved interhyal muscle
C
ep
D
scxa:epNTR-RFP
col2a1a:eGFP
7dpf
E
Imaging
scxa:epNTR-RFP (DMSO)
scxa:epNTR-RFP (Mtz)
scxa:mcherry (Mtz)
scxa:epNTR-RFP
scxa:mcherry
DMSO
Mtz
F
Width (um)
Length (um)
Width/Length ratio
ns
G
Analysis
26dpf
H
I
mylz2:Amcyan
J
% Embryos with normal muscle morphology

Figure S2. Tendon cell ablation disrupts the morphology of pharyngeal cartilage and muscle, but not the ethmoid plate, Related to Figure 1.

(A) Confocal images of cartilage (green) and muscle (white) in unablated (top and bottom panels) and ablated (middle panels) embryos at indicated time points. White arrow, dysmorphic palatoquadrate cartilage. Yellow arrow, curved interhyal muscle. Scale bar, 200 μm.

(B) Quantification of cartilage and muscle phenotypes in unablated (Row 1, n=6 for 5 dpf, n=5 for 7 dpf, n=5 for 9 dpf, n=4 for 11 dpf and Row 3, n=4 for 5 dpf, n=4 for 7 dpf, n=5 for 9 dpf, n=3 for 11 dpf) and ablated (Row 2, n=11 for 5 dpf, n=4 for 7 dpf, n=10 for 9 dpf, n=4 for 11 dpf) embryos at indicated time points.

(C) A cartoon of zebrafish neurocranial cartilage. ep, ethmoid plate.

(D) Confocal images of tendon cells (red) and neurocranial cartilage (green) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; col2a1a:eGFP)* fish. White box showed magnified view of ethmoid plate shown to the right. Scale bar, 200 μm.

(E) Confocal images of ethmoid plates in unablated (left and right panels) and ablated (middle panel) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; col2a1a:eGFP)* fish. Scale bar, 200 μm.

(F) Quantification of width (left), length (middle), and width/length ratio (right) in unablated (red, n=9 and grey, n=9) and ablated (blue, n=10) fish. One-way ANOVA with tukey's post-hoc analysis and data were mean $\pm$ s.d. *, $p<0.05$. **, $p<0.01$. ns, no significance.

(G) Alcian blue staining of lower jaw cartilage in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish at 26 dpf. Scale bar, 1mm.

(H) Quantification of width (left), length (middle), and width/length ratio (right) in unablated (red, n=5) and ablated (blue, n=4) fish at 26 dpf. Two-tailed, unpaired Student's t-test and data were mean $\pm$ s.d. ns, no significance.

(I) Live images of craniofacial muscle (green) and tendon (red) in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; mylz2:Amcyan)* fish. Yellow arrow, interhyal muscle. Scale bar, 1 mm.

(J) Quantification of fish with normal muscle morphology in unablated (red, n=15) and ablated (blue, n=10) condition. L, length; W, width; M, meckel's cartilage. P, palatoquadrate cartilage. C, ceratohyal cartilage. dpf, days post fertilization. All images, ventral view, anterior to the left.

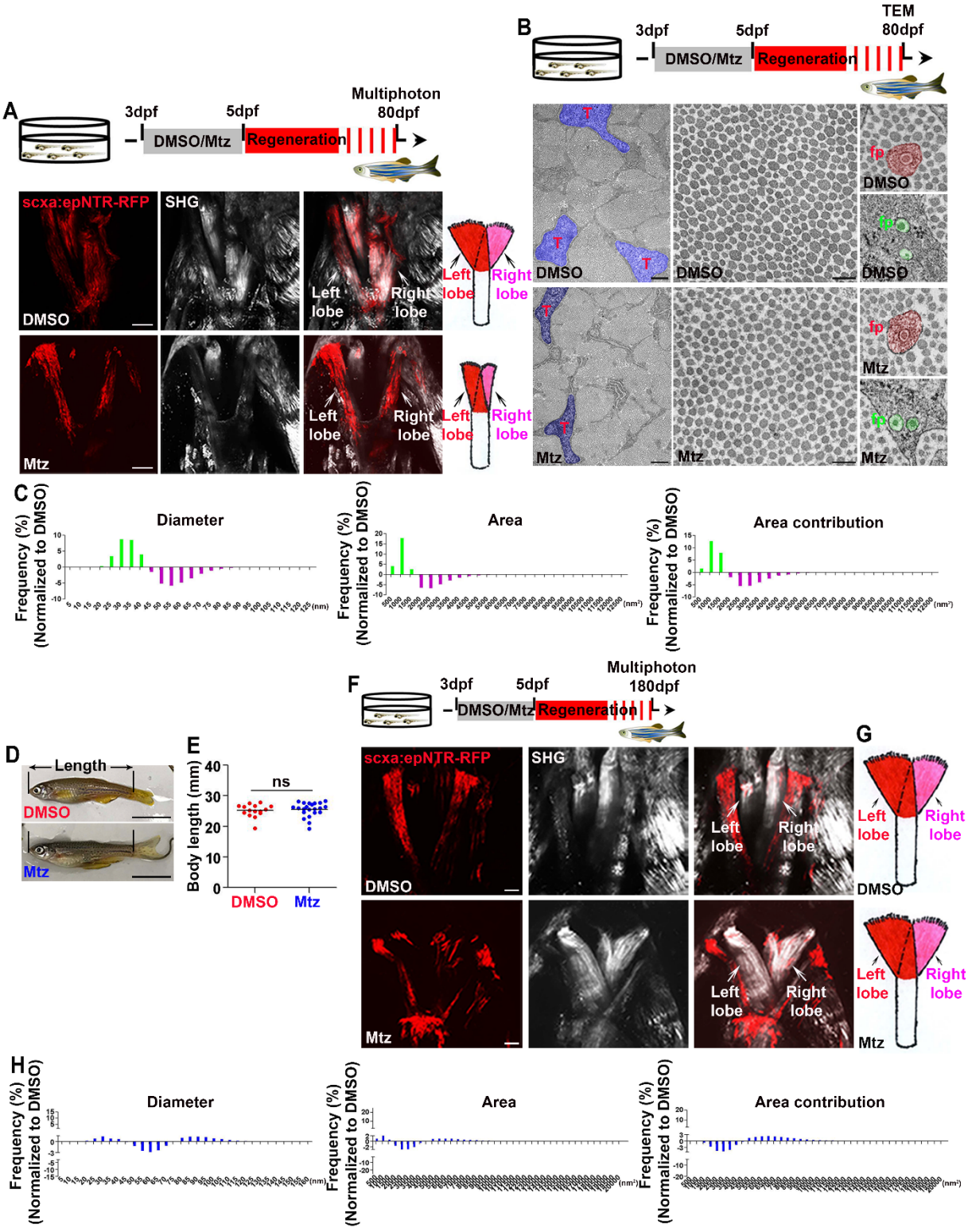

A
3dpf
5dpf
DMSO/Mtz
Regeneration
Multiphoton
80dpf
scxa:epNTR-RFP
SHG
DMSO
Mtz
Left lobe
Right lobe
B
3dpf
5dpf
DMSO/Mtz
Regeneration
TEM
80dpf
T
fp
DMSO
Mtz
C
Diameter
Area
Area contribution
Frequency (%) (Normalized to DMSO)
(nm)
(nm²)
D
Length
DMSO
Mtz
E
Body length (mm)
ns
DMSO
Mtz
F
3dpf
5dpf
DMSO/Mtz
Regeneration
Multiphoton
180dpf
scxa:epNTR-RFP
SHG
Left lobe
Right lobe
DMSO
Mtz
G
Left lobe
Right lobe
DMSO
Mtz
H
Diameter
Area
Area contribution
Frequency (%) (Normalized to DMSO)
(nm)
(nm²)

Figure S3. SH tendon structure in regenerating juveniles and adults, Related to Figure 2.
(A) Multiphoton images of SH tendon in control (DMSO) and regenerated (Mtz) fish at 80 dpf. Ventral views, anterior to the top. SHG, second harmonic generation. Cartoons in the right show SH tendon lobes (black arrow). Scale bar, 100 μm.
(B) Transmission electron microscopy analysis of SH tendon collagen fibril in control (DMSO) and regenerated (Mtz) at 80 dpf. Low (left panels) and high (middle panels) magnification views of transverse-sectioned SH tendon. Right panels (red fp, fibripositor, extension outside the tendon cell body. green fp, fibripositor with collagen fibril inside the tendon cell body). T, tendon cell. Scale bar, 1 μm (left panels) and 0.2μm (middle panels).
(C) Distribution of collagen fibril diameter (left), area (middle) and area contribution (right) in regenerated SH tendon at 80 dpf after normalizing to control SH tendon. Green bars showed the increase in small diameter, area, and area contribution. Purple bars showed the decrease in large diameter, area and area contribution.
(D) Representative images for control (DMSO) and regenerated (Mtz) fish at 180 dpf. Scale bar, 1cm.
(E) Quantification of body length at 180 dpf for control (DMSO, n=15) and regenerated (Mtz, n=22) fish. Each dot represents the body length of individual fish. Two-tailed, unpaired Student's t-test and data were mean. ns, no significance.
(F) Multiphoton images of SH tendon in control (DMSO) and regenerated (Mtz) fish at 180 dpf. Ventral views, anterior to the top. SHG, second harmonic generation. White arrows, SH tendon lobes. Scale bar, 100 μm.
(G) Cartoons for SH tendon lobes in control (DMSO) and regenerated (Mtz) SH tendons at 180 dpf.
(H) Distribution of collagen fibril diameter, area, and area contribution in regenerated SH tendon at 180 dpf after normalizing to control SH tendon. dpf, days post fertilization. nm, nanometer.

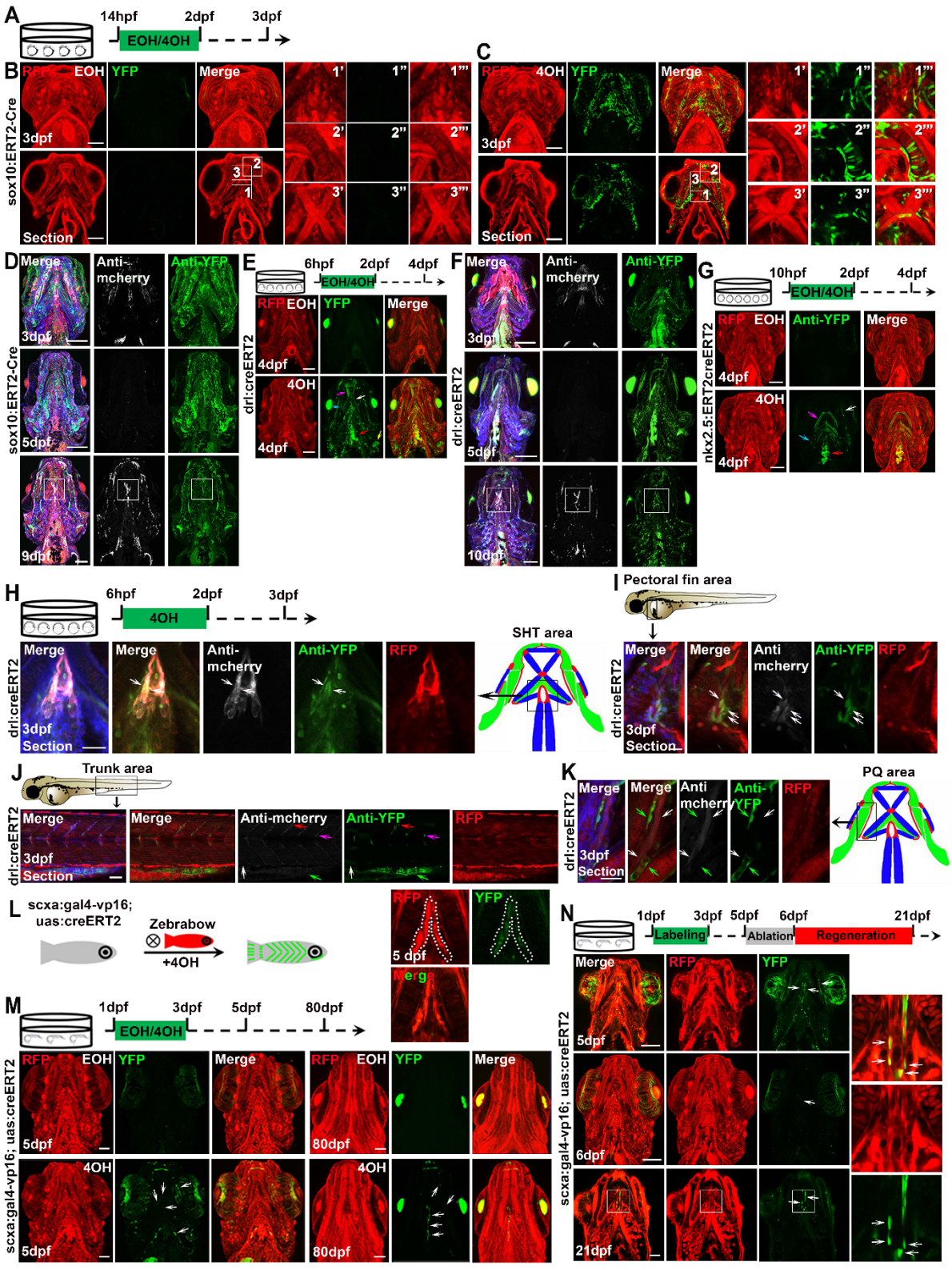

A
14hpf
2dpf
3dpf
EOH/4OH
B
sox10:ERT2-Cre
RFP EOH YFP Merge
3dpf
Section
1'
1"
1"'
2'
2"
2"'
3'
3"
3"'
C
RFP 4OH YFP Merge
D
Merge
Anti-mcherry
Anti-YFP
3dpf
5dpf
9dpf
E
6hpf
2dpf
4dpf
EOH/4OH
drl:creERT2
RFP EOH YFP Merge
4dpf
4OH
F
10dpf
G
10hpf
nkx2.5:ERT2creERT2
H
6hpf
2dpf
3dpf
4OH
RFP
Section
SHT area
I
Pectoral fin area
J
Trunk area
K
PQ area
L
scxa:gal4-vp16;
uas:creERT2
Zebrabow
+4OH
5 dpf
M
1dpf
3dpf
5dpf
80dpf
N
Labeling
Ablation
Regeneration
21dpf

Figure S4. CreER-loxP mediated strategy for SH tendon cell lineage tracing, Related to Figure 3 and Figure 4.

(A-C) Recombination test of *Tg(sox10:ERT2-Cre; ubi:zebrabow)* line with Ethanol (EOH, B) and 4-hydroxytamoxifen (4OH, C). Insets show higher magnification view shown to the right. White box 1-1’’’, SH tendon region. 2-2’’’, Meckel's cartilage region. 3-3’’’, mandibulo-hyoid junction region. Scale bar, 100 μm.

(D) *sox10:ERT2-Cre* based cell lineage tracing of regenerated SH tendon cells (white box in bottom panels) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; uas-E1b:NfsB-mCherry; sox10:ERT2-Cre; ubi:zebrabow)* fish. 4OH treatment was performed at 14hpf-2dpf and tendon cells were ablated at 3-5dpf. Scale bar, 100 μm.

(E) Recombination test of *Tg(drl:creERT2; ubi:zebrabow)* line with Ethanol (EOH) and 4-hydroxytamoxifen (4OH). Recombination could be observed in interhyal muscle (white arrow), intermandibularis posterior muscle (purple arrow), heart (red arrow), thymus (yellow arrow), and vasculature (cyan arrow). Scale bar, 100 μm.

(F) *drl:creERT2* based cell lineage tracing for regenerated SH tendon cells (white box in bottom panels) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; uas-E1b:NfsB-mCherry; drl:creERT2; ubi:zebrabow)* fish. 4OH treatment was performed at 6hpf- 2dpf and tendon cells were ablated at 3-5dpf. Scale bar, 100 μm.

(G) Recombination test of *Tg(nkx2.5:ERT2creERT2; ubi:zebrabow)* line with Ethanol (EOH) and 4-hydroxytamoxifen (4OH). Recombination could be observed in interhyal muscle (purple arrow), hypobranchial artery (white arrow), heart (red arrow), and vasculature (cyan arrow). Scale bar, 100 μm.

(H-K) Developmentally *draculin*$^{+}$ lineage derived-cells contributed to SH tendon (H), pectoral fin tendon (I), and myosepta tendon (J), but not to other craniofacial tendons/ligaments (K). SHT area (white arrows, *draculin*$^{+}$ lineage-derived SH tendon cells. Scale bar, 25μm). Pectoral fin area (white arrows, *draculin*$^{+}$ lineage-derived tendon cells, Scale bar, 25μm). Trunk area (white arrow, superficial trunk muscle. green arrow, caudal hematopoietic tissue. purple arrow, endothelial cell, red arrow, *draculin*$^{+}$ lineage-derived tendon cell. scale bar, 50μm). PQ area (white arrows, PQ ligament. green arrows, *draculin*$^{+}$ lineage-derived endothelial cells. Scale bar, 25μm).

(L) *Tg(uas:creERT2)* line generated for *scxa*$^{+}$ cell lineage tracing and percentage of YFP-labeled SH tendon cell after recombination.

(M) Recombination test of *Tg(scxa:gal4-vp16; uas:creERT2; ubi:zebrabow)* line with EOH and 4OH. White arrow, labeled craniofacial tendon cells. Scale bar, 100μm for 5 dpf and 500μm for 80 dpf.

(N) *uas:creERT2* based cell lineage tracing for regenerated SH tendon cells (white box in bottom panels and white arrow in magnified images shown to the right) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; uas:creERT2; ubi:zebrabow)* fish. Scale bar, 100 μm. In all Cre-mediated recombination tests, CFP and YFP expression were detected with an anti-GFP antibody. Blue color in (D, F, H-K) was Hoechst staining. hpf, hour post fertilization. dpf, day post fertilization. SHT, sternohyoideus tendon. PQ, palatoquadrate.

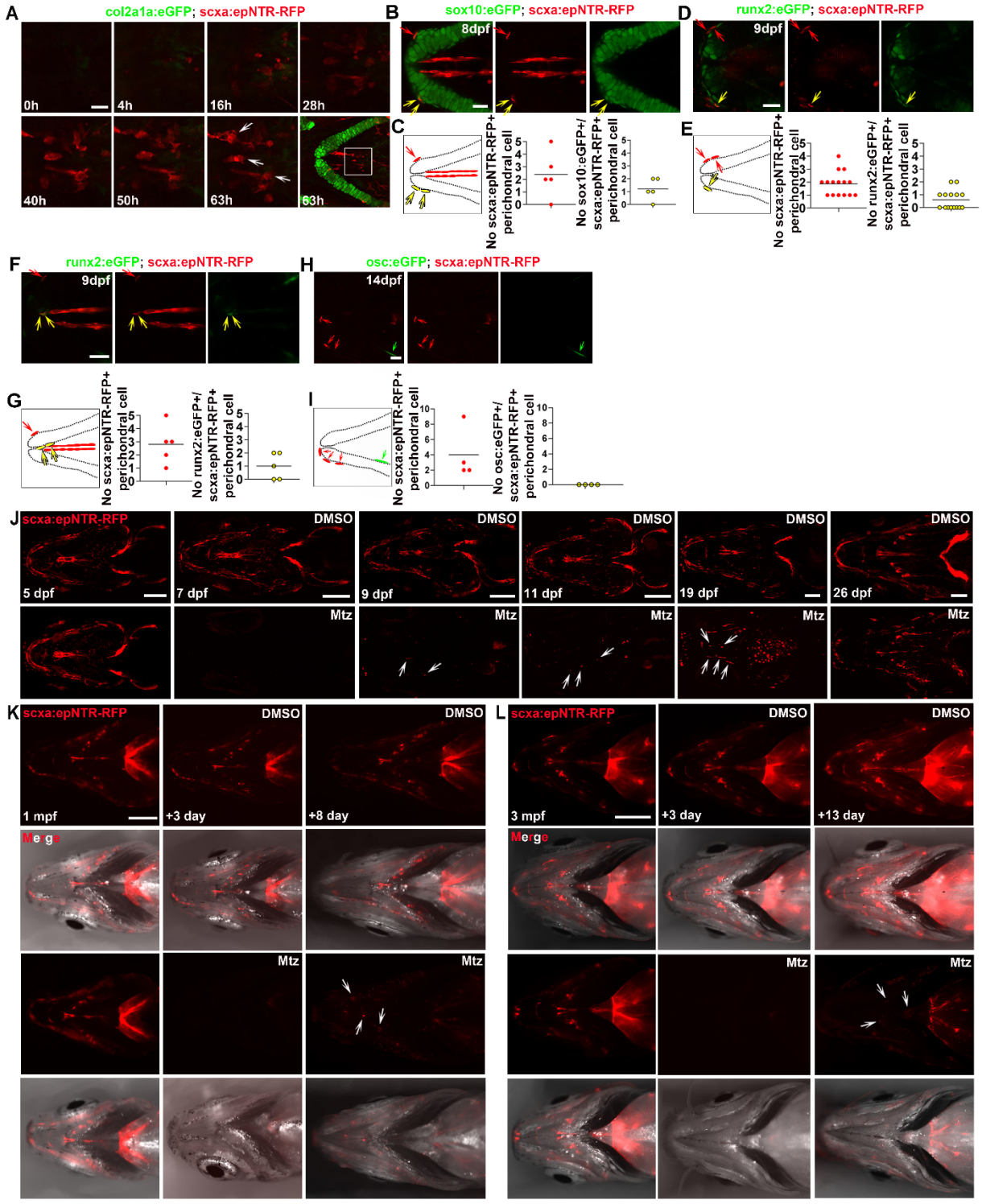

A
col2a1a:eGFP; scxa:epNTR-RFP
0h
4h
16h
28h
40h
50h
63h
63h
B
sox10:eGFP; scxa:epNTR-RFP
8dpf
C
No scxa:epNTR-RFP+ perichondral cell
No sox10:eGFP+/ scxa:epNTR-RFP+ perichondral cell
D
runx2:eGFP; scxa:epNTR-RFP
9dpf
E
No scxa:epNTR-RFP+ perichondral cell
No runx2:eGFP+/ scxa:epNTR-RFP+ perichondral cell
F
runx2:eGFP; scxa:epNTR-RFP
9dpf
H
osc:eGFP; scxa:epNTR-RFP
14dpf
G
No scxa:epNTR-RFP+ perichondral cell
No runx2:eGFP+/ scxa:epNTR-RFP+ perichondral cell
I
No scxa:epNTR-RFP+ perichondral cell
No osc:eGFP+/ scxa:epNTR-RFP+ perichondral cell
J
scxa:epNTR-RFP
5 dpf
DMSO
7 dpf
DMSO
9 dpf
DMSO
11 dpf
DMSO
19 dpf
DMSO
26 dpf
Mtz
Mtz
Mtz
Mtz
Mtz
K
scxa:epNTR-RFP
1 mpf
DMSO
+3 day
DMSO
+8 day
Merge
Mtz
Mtz
L
scxa:epNTR-RFP
3 mpf
DMSO
+3 day
DMSO
+13 day
Merge
Mtz
Mtz

Figure S5. Newly-induced tendon cells arise from musculoskeletal attachment sites and tendon cell ablation and regeneration at later stages. Related to Figure 3 and Figure 4.

(A) Live imaging of new cell induction in SH muscle attachment site during regeneration in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; col2a1a:eGFP)* fish. White arrow, newly induced tendon cell. White box in right merged image shows SH muscle attachment site analyzed. Scale bar, 25 μm.

(B) Confocal images of ceratohyal cartilage attachment site in unablated *Tg(scxa:gal4-vp16; uas:epNTR-RFP; sox10:eGFP)* fish. Yellow arrow, *sox10:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells. Red arrow, *scxa:epNTR-RFP*$^{+}$ cells. Scale bar, 25 μm.

(C) Cartoon and quantification of *scxa:epNTR-RFP*$^{+}$ and *sox10:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells in ceratohyal cartilage attachment site at 8dpf. In cartoon, yellow arrow shows *sox10:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells, red arrow shows *scxa:epNTR-RFP*$^{+}$ cells. Black dashed line in cartoon demarcated ceratohyal cartilage.

(D) Confocal images of ceratohyal cartilage attachment site in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; runx2:eGFP)* fish. Yellow arrow, *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$cells. Red arrow, *scxa:epNTR-RFP*$^{+}$ cells. Scale bar, 25 μm.

(E) Cartoon and quantification of *scxa:epNTR-RFP*$^{+}$ and *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells in ceratohyal cartilage attachment site at 9dpf. In cartoon, yellow arrow shows *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$cells, red arrow shows *scxa:epNTR-RFP*$^{+}$ cells. Black dashed line in cartoon demarcated ceratohyal cartilage.

(F) Confocal images of ceratohyal cartilage attachment site in unablated *Tg(scxa:gal4-vp16; uas:epNTR-RFP; runx2:eGFP)* fish. Yellow arrow, *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$cells. Red arrow, *scxa:epNTR-RFP*$^{+}$ cells. Scale bar, 25 μm.

(G) Cartoon and quantification of *scxa:epNTR-RFP*$^{+}$ and *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells in ceratohyal cartilage attachment site at 9dpf. In cartoon, yellow arrow shows *runx2:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells, red arrow shows *scxa:epNTR-RFP*$^{+}$ cells. Black dashed line in cartoon demarcated ceratohyal cartilage.

(H) Confocal images of ceratohyal cartilage attachment site in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; osc:eGFP)* fish. Red arrow, *scxa:epNTR-RFP*$^{+}$ cells. Green arrow, *osc:eGFP*$^{+}$ osteoblast. Scale bar, 25 μm.

(I) Cartoon and quantification of *scxa:epNTR-RFP*$^{+}$ and *osc:eGFP*$^{+}$*/scxa:epNTR-RFP*$^{+}$ cells in ceratohyal cartilage attachment site at 14dpf. In cartoon, red arrow shows *scxa:epNTR-RFP*$^{+}$ cells, green arrow shows *osc:eGFP*$^{+}$ osteoblast. Black dashed line in cartoon demarcated ceratohyal cartilage.

(J) Tendon cell regeneration upon ablation at 5-7 dpf. White arrow indicates new cell. Scale bar, 200 μm.

(K) Tendon cell regeneration upon ablation at 30-33 dpf. White arrow indicates new cell. Scale bar, 0.5 mm.

(L) Tendon cell regeneration upon ablation at 90-93 dpf. White arrow indicates new cell. Scale bar, 0.2 cm.

dpf, days post fertilization. mpf, month post fertilization. For (A, D, E, H, I), tendon cell was ablated at 3-5dpf. All image, ventral view, anterior to the left.

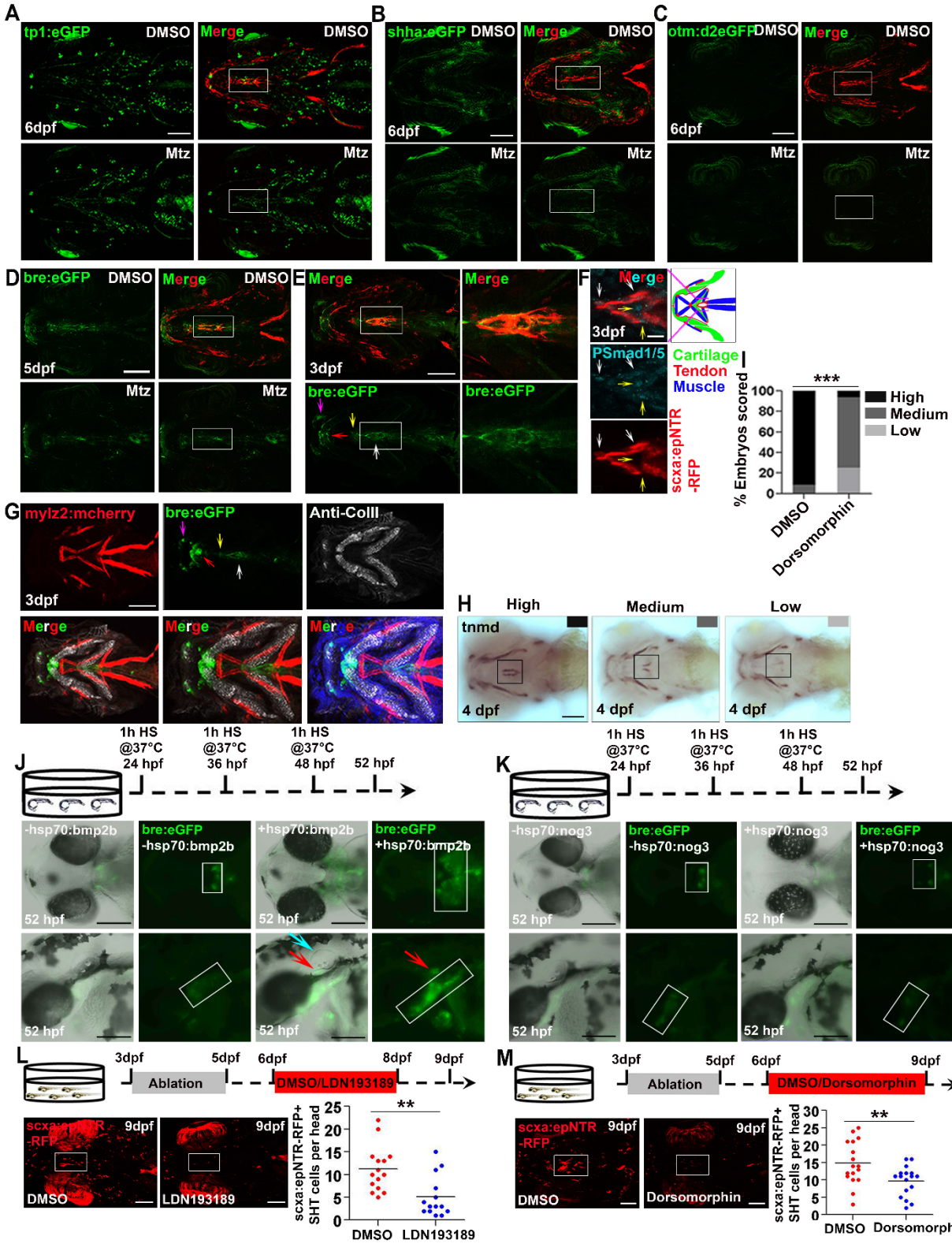

A
tp1:eGFP DMSO
Merge DMSO
6dpf
Mtz
B
shha:eGFP DMSO
Merge DMSO
6dpf
Mtz
C
otm:d2eGFP DMSO
Merge DMSO
6dpf
Mtz
D
bre:eGFP DMSO
Merge DMSO
5dpf
Mtz
E
Merge
3dpf
bre:eGFP
F
Merge
3dpf
PSmad1/5
Cartilage
Tendon
Muscle
scxa:epNTR
-RFP
% Embryos scored
***
High
Medium
Low
DMSO
Dorsomorphin
G
mylz2:mcherry
bre:eGFP
Anti-ColII
3dpf
Merge
H
High
Medium
Low
tnmd
4 dpf
1h HS @37°C 24 hpf
1h HS @37°C 36 hpf
1h HS @37°C 48 hpf
52 hpf
J
-hsp70:bmp2b
bre:eGFP -hsp70:bmp2b
+hsp70:bmp2b
bre:eGFP +hsp70:bmp2b
52 hpf
K
-hsp70:nog3
bre:eGFP -hsp70:nog3
+hsp70:nog3
bre:eGFP +hsp70:nog3
52 hpf
L
3dpf
5dpf
6dpf
8dpf
9dpf
Ablation
DMSO/LDN193189
scxa:epNTR -RFP
9dpf
DMSO
LDN193189
scxa:epNTR-RFP+ SHT cells per head
**
DMSO
LDN193189
M
3dpf
5dpf
6dpf
9dpf
Ablation
DMSO/Dorsomorphin
scxa:epNTR -RFP
9dpf
DMSO
Dorsomorphin
scxa:epNTR-RFP+ SHT cells per head
**
DMSO
Dorsomorphin

Figure S6. Identification of the BMP pathway as being involved in tendon development and regeneration, Related to Figure 5.

(A) Live images of tendon cells (red) and cells responding to Notch signaling (green) in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; tp1:eGFP)* fish. White box, SH tendon region. Scale bar, 200 μm.

(B) Live images of tendon cells (red) and cells responding to Sonic hedgehog signaling (green) in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; shha:eGFP)* fish. White box, SH tendon region. Scale bar, 200 μm.

(C) Live images of tendon cells (red) and cells responding to Wnt signaling (green) in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; otm:d2eGFP)* fish. White box, SH tendon region. Scale bar, 200 μm.

(D) Live images of tendon cells (red) and cells responding to BMP signaling (green) in unablated (DMSO) and ablated (Mtz) *Tg(scxa:gal4-vp16; uas:epNTR-RFP; bre:eGFP)* fish. White box, SH tendon region. Scale bar, 200μm.

(E) Confocal images of tendon (red) and BMP signaling (green) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP; bre:eGFP)* fish. White box showed magnified SH tendon region in the right panels. White arrow, *bre:eGFP* expression in SH tendon. Purple arrow, *bre:eGFP* expression surrounding the mouth. Red arrow, *bre:eGFP* expression surrounding Meckel's cartilage. Yellow arrow, *bre:eGFP* expression in the mandibulo-hyoid junction. White box, SH tendon region. Scale bar, 100 μm.

(F) Confocal images of SH tendon (red) and Phospho-Smad1/5 (cyan) in *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish. Images were from purple boxed area shown to the right cartoon. Active P-Smad1/5 signals are seen in tendon cells (white arrows) and non-tendon cells (yellow arrows). Scale bar, 25 μm.

(G) Confocal images of muscle (red), *bre:eGFP* expression (green), and cartilage (white) in *Tg(mylz2:mcherry; bre:eGFP)* fish. Inset shows magnified views shown at the bottom right. White arrow, *bre:eGFP* expression in SH tendon. Purple arrow, *bre:eGFP* expression surrounding the developing mouth. Red arrow, *bre:eGFP* expression surrounding Meckel's cartilage. Yellow arrow, *bre:eGFP* expression in the mandibulo-hyoid junction. Blue color was Hoechst staining. Scale bar, 100um.

(H) Craniofacial *tnmd* expression upon DMSO and Dorsomorphin treatment. Treatment was performed at 1-3dpf. Black box, SH tendon region. Scale bar, 100 μm.

(I) Quantification of embryos with high, medium and low expression of *tnmd* in SH tendon region upon DMSO and Dorsomorphin treatment. n=12 for DMSO, n=16 for Dorsomorphin. Chi test. ***, $p<0.001$.

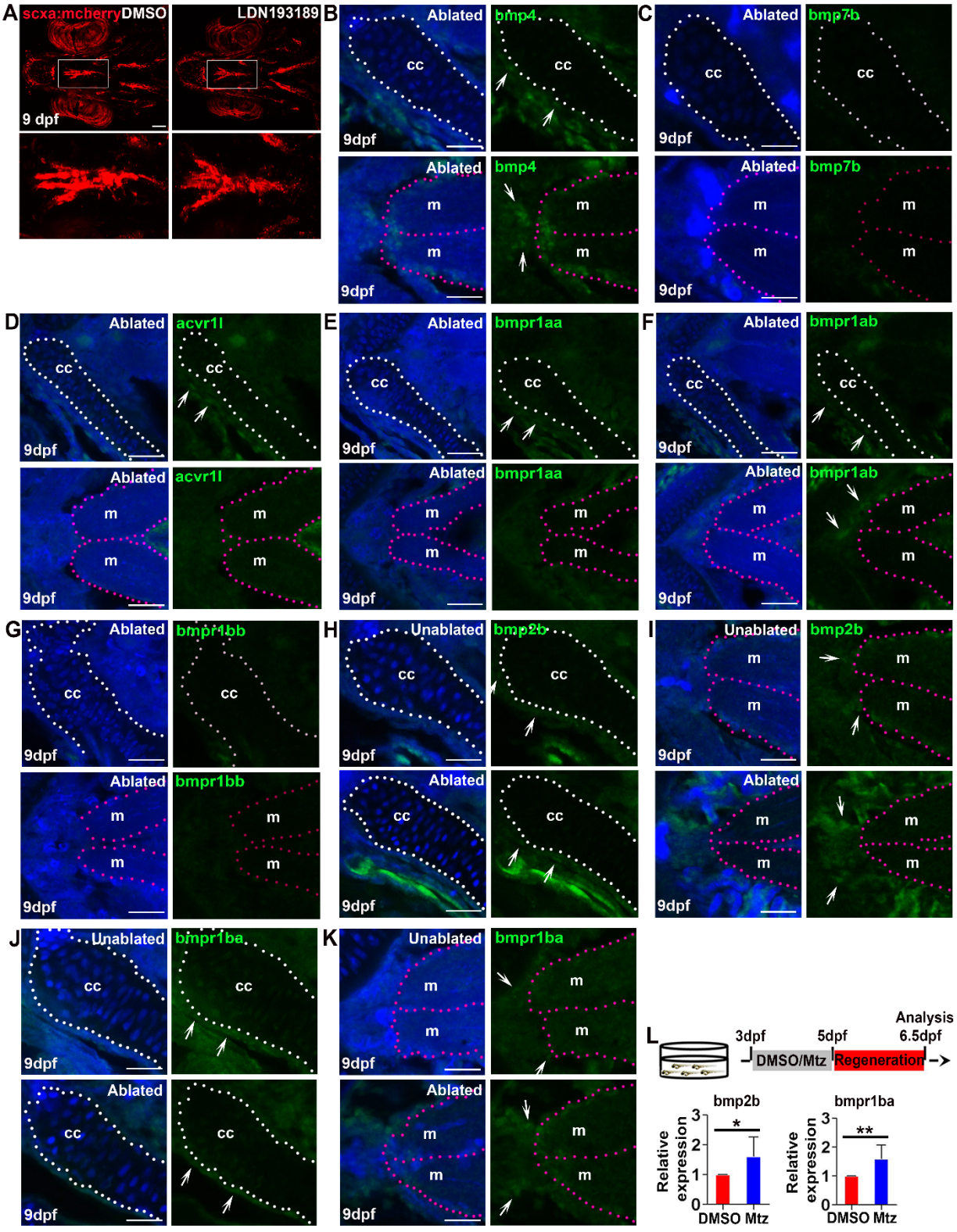
(J) Live images of *Tg(bre:eGFP)* fish at 52 hpf with (+*hsp70:bmp2b*) and without (-*hsp70:bmp2b*) *bmp2b* over expression. Enhanced signal could be observed in the lower jaw region (white box), and region (red arrow) closely adjacent to utricle of inner ear (cyan arrow). Scale bar, 100 μm. Top panels show ventral view, bottom panels show lateral view.

(K) Live images of *Tg(bre:eGFP)* fish at 52 hpf with (+*hsp70:nog3*) and without (-*hsp70:nog3*) *nog3* over expression. Reduced signal could be observed in lower jaw region (white box). Top panels show ventral view, bottom panels show lateral view. Scale bar, 100 μm.

(L) Live images (ventral view) of SHT cell regeneration in DMSO or LDN193189-treated *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish. White box showed SHT region analyzed. Quantification (right) of SHT cell number in DMSO (n=15, average =11 cells) or LDN193189 (n=14, average=5 cells) treatment. Each dot represents the cell number in individual fish. Two-tailed, unpaired Student's t-test and data were mean. **, $p<0.01$. Scale bar, 100 μm.

(M) Live images of SH tendon cell regeneration in DMSO or Dorsomorphin-treated *Tg(scxa:gal4-vp16; uas:epNTR-RFP)* fish. White box showed SH tendon region analyzed. Quantification (right) of SH tendon cells in DMSO (n=17, average = 14 cells) or Dorsomorphin (n=17, average = 9 cells) treatment. Each dot represents the cell number in individual fish. Two-tailed, unpaired Student's t-test and data were mean. **, $p<0.01$. Scale bar, 100 μm.

dpf, days post fertilization. hpf, hours post fertilization. SHT, sternohyoideus tendon. For (A-D, L, M), tendon cell was ablated at 3-5dpf. All images, ventral view, anterior to the left.


A
scxa:mcherry
DMSO
LDN193189
9 dpf
B
Ablated
bmp4
cc
m
9dpf
C
Ablated
bmp7b
cc
m
9dpf
D
Ablated
acvr1l
cc
m
9dpf
E
Ablated
bmpr1aa
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9dpf
K
Unablated
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bmpr1ba
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9dpf
L
3dpf
5dpf
Analysis
6.5dpf
DMSO/Mtz
Regeneration
bmp2b
bmpr1ba
Relative expression
*
**
DMSO
Mtz

Figure S7. LDN-193189 treatment in unablated fish and BMP ligand and receptor expression in ceratohyal cartilage and SH muscle attachment sites, Related to Figure 7.
(A) Live images of craniofacial tendon cell development in DMSO or LDN193189-treated *Tg(scxa:mcherry)* fish. White box indicated SH tendon region showed below. Treatment was performed at 6-8dpf.
(B) *bmp4* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(C) *bmp7b* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(D) *acvr1l* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(E) *bmpr1aa* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(F) *bmpr1ab* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(G) *bmpr1bb* expression (white arrow) in ceratohyal cartilage and SH muscle attachment site in ablated embryos.
(H) *bmp2b* expression (white arrow) in ceratohyal cartilage attachment site in unablated and ablated embryos.
(I) *bmp2b* expression (white arrow) in SH muscle attachment site in unablated and ablated embryos.
(J) *bmpr1ba* expression (white arrow) in ceratohyal cartilage attachment site in unablated and ablated embryos.
(K) *bmpr1ba* expression (white arrow) in SH muscle attachment site in unablated and ablated embryos.
(L) qPCR analysis of *bmp2b* and *bmpr1ba* expression in unablated (DMSO) and ablated (Mtz) fish at 6.5dpf. n=7 independent replicates. Two-tailed, unpaired Student's t-test and data were mean ± s.d. *, $p<0.05$. **, $p<0.01$.
Tendon cells were ablated at 3-5dpf. Blue color in (B-K) was Hoechst staining. cc, ceratohyal cartilage. m, SH muscle. White dashed line demarcated ceratohyal cartilage. Red dashed line demarcated SH muscle. Scale bar, 100 μm for (A) and 20 μm for (B-K). All images, ventral view, anterior to the left.

Fish line	Direction of primer	Primer sequence (5’-3’)
hsp70:bmp2b	forward	TCCCCGACGAGGTGTTTATTC
	Reverse	CGTGATGAAAACTTCGTATCGTGTTTG
hsp70:nog3	Forward	CGCAGGAAAGAACATGTGAGC
	reverse	CGGGTTGGACTCAAGACGATAG
uas:nfsb-mcherry	forward	TGAGCAAGGGCGAGGAGGATAACA
	reverse	CATGCCGCCGGTGGAGTGG

Table S1. Primers used for genotyping. Related to Figure 5

Gene	Direction of primer	Primer sequence (5’-3’)
bmp2b	forward	TCGGCGATGAGCGGATGACC
	reverse	CACGCCCACACCACCCACAGA
bmp4	forward	CGAGCCAACACCGTGAGAGGATT
	reverse	AGGCTTTGGGGATATTGGTGTTCA
bmp7b	forward	CCAGGAGCGCCGGGAGATGC
	reverse	CTTGGCGGTTTTGGAGCGGTTGG
bmpr1aa	forward	CACTGGCTGGCCTTTCTCATCTC
	reverse	CAGCAGGTGCCGTTTTTCTTG
bmpr1ab	forward	CTAGGCCGCTGGAGAGGAGAAA
	reverse	GAGGCGTGAGGCTGGATTGTG
bmpr1ba	forward	GATCGGGAAGGGTCGCTATGGAG
	reverse	TTTAACGCGGAGGGCTGTGAGAC
bmpr1bb	forward	TGCTGGGCGTCTTGTGGATTTATT
	reverse	CTGCCGGAGCTCTGCGACTG
acvr1l	forward	TGGCACGGAGGGCAAACC
	reverse	GCGCAGGGCAGTGAGACGAG

Table S2. Primers used for making BMP probes. Related to Figure S7 and STAR Methods

Gene	Direction of primer	Primer sequence (5’-3’)
bmp2b	forward	TCAGCCGCCTATACACAGAGATG
	reverse	ACCGGCCGTTCGTCAAGA
bmpr1ba	forward	CCTCAGCGCTTCCTTTGGTGTTAC
	reverse	GTGTTGGGTGCAGGTCTCGGTTAC
beta actin	forward	GATGCCCCTCGTGCTGTTTTC
	reverse	TCTCTGTTGGCTTTGGGATTCA

Table S3. Primers used for qPCR. Related to Figure S7 and STAR Methods