Figure S1. Organization of adult DRG peripheral axons, Related to Figure 1
(A) Lateral view of the adult trunk. Note the absence of the somatosensory axon reporter [*Tg(p2rx3a>mCherry)*] along the posterior lateral line nerve (pLLn), a sensory nerve that innervates neuromasts along the trunk. Arrows, superficial axon bundles. Dashed line, scale margin. (B-C) Maximum intensity projections through the basal epidermis of scales isolated from indicated transgenic lines that mark sensory neurons. Arrows, superficial axon bundles. (D) Maximum intensity projections through the basal epidermis of isolated scales immunostained as indicated. (E) Transverse vibratome section through the adult mid-trunk. Arrows, DRG cell bodies. Yellow arrowheads, DRG peripheral axons between myosepta. Dashed box, area of magnification. Double-headed arrows, DRG central axons within the dorsal spinal cord (sc). Dashed lines, spinal cord boundary. Transgenes: (A) somatosensory axons [*Tg(p2rx3a>mCherry)*] and myelinating Schwann cells [*Tg(cldnk:GAL4);Tg(14xUAS:MA-GFP)*] (Münzel et al., 2012); (B) *Tg(Tru.Gap43:EGFP)* (Udvadia, 2008); (C) *TgBAC(trpa1b:EGFP)* (Pan et al., 2012); (E) *Tg(p2rx3a>mCherry)*. Staining: (D) axons (zn-12) (Metcalfe et al., 1990), axons (acTubulin), and nuclei (DAPI); (E) nuclei (DAPI). Scale bars, 250 μm (A,E), 25 μm (B-D) and 100 μm (E'').

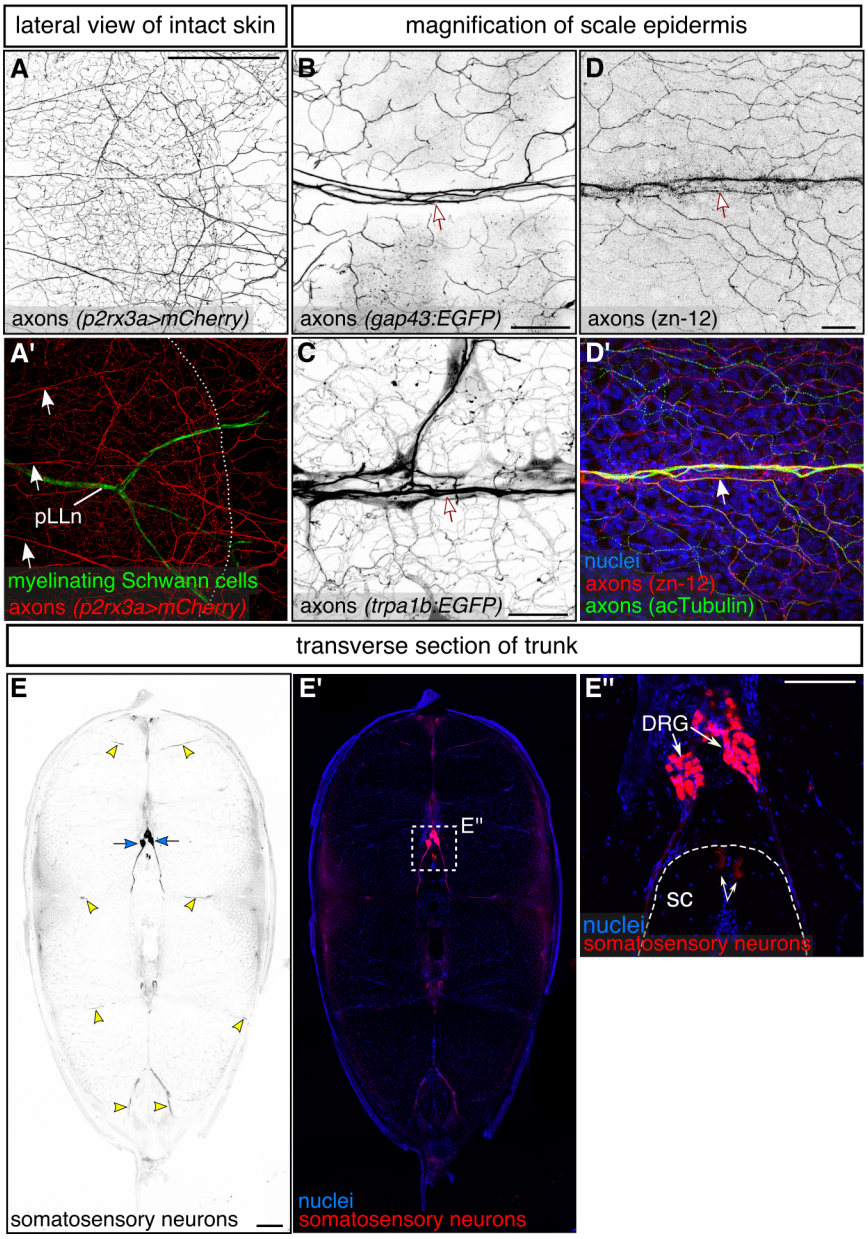

lateral view of intact skin
magnification of scale epidermis
A
axons (p2rx3a>mCherry)
B
axons (gap43:EGFP)
D
axons (zn-12)
A'
pLLn
myelinating Schwann cells
axons (p2rx3a>mCherry)
C
axons (trpa1b:EGFP)
D'
nuclei
axons (zn-12)
axons (acTubulin)
transverse section of trunk
E
somatosensory neurons
E'
E''
nuclei
somatosensory neurons
E''
DRG
SC
nuclei
somatosensory neurons

Figure S2. Erbb3b and Adgra2 are required for the development of most skin-innervating DRG neurons, Related to Figure 1

(A) Lateral views of the larval spinal cord. Arrowheads, DRG cell bodies. Brackets, RB cell bodies. (B,C) Quantification of the total number of *Tg(-17.6isl2b:GFP)*-expressing DRG neurons (B) and dorsal root ganglia (C) per one side of the fish. *n*=12-15 fish per genotype. Black bars, mean ± SEM. *, p<0.01, Wilcoxon rank sum test. (D,E) Dorsolateral brightfield and fluorescent images of juvenile fish. Dashed boxes indicate regions of magnification. *mitfa*$^{w2/w2}$ animals lack melanophores (Lister et al., 1999) and were used to aid visualization of the spinal cord. (F,G) Fluorescent images along the rostral spinal cord. Dashed boxes indicate regions of magnification. Arrowheads indicate the bilateral ganglia in F''' and unilateral ganglion in G'''. (H) Images of multiple regions of lateral trunk skin from individual *erbb3b*$^{-/-}$ and sibling adults. Insets, regions imaged. Note the non-uniform skin innervation in *erbb3b*$^{-/-}$ animals. (I,J) Quantification of *Tg(p2rx3a>mCherry)* axon density in lateral trunk skin. Imaged regions indicated in panel H. *n*=5 fish per genotype, twelve separate 0.25 mm^2 regions were quantified per fish. Black bars, mean ± SEM. *, p<0.01, Wilcoxon rank sum test. (K-N) Projections through the epidermis of scales isolated from *adgra2*$^{-/-}$ and sibling adults. Note the sparse innervation by NC-derived axons in the *adgra2*$^{-/-}$ epidermis. These remaining axons are likely not nociceptive, because they do not express the nociceptive marker *Tg(p2rx3a>mCherry)* (see Figure 1J). Based on the distribution and morphology of these remaining endings, they do not penetrate to superficial strata nor do they appear to innervate Merkel cells. Arrows in panels L and N indicate Schwann cell bodies. (O) Quantification of epidermal axon length based on tracings of the neural crest lineage reporter in the indicated genotypes. *n*=13-15 scales isolated from *n*=3 fish per genotype. Axon length is expressed as mm/0.1 mm^2. Black bars, mean ± SEM. *, p<0.01, Wilcoxon rank sum test. Transgenes: (A) *Tg(-17.6isl2b:GFP*) (Pittman et al., 2008); (D,E) *Tg(p2rx3a>mCherry)*; (F,G) *Tg(-17.6isl2b:GFP)* and *Tg(p2rx3a>mCherry)*; (H) *Tg(p2rx3a>mCherry)*; (L,N) *Tg(-28.5Sox10:Cre);Tg(ubb:GswitchR)* (red channel only). Staining: (K,M) axons (acTubulin) and nuclei (DAPI). Scale bars, 100 μm (A, F''', G'''), 2 mm (D,E), 250 μm (F,G,H) and 25 μm (K-N).

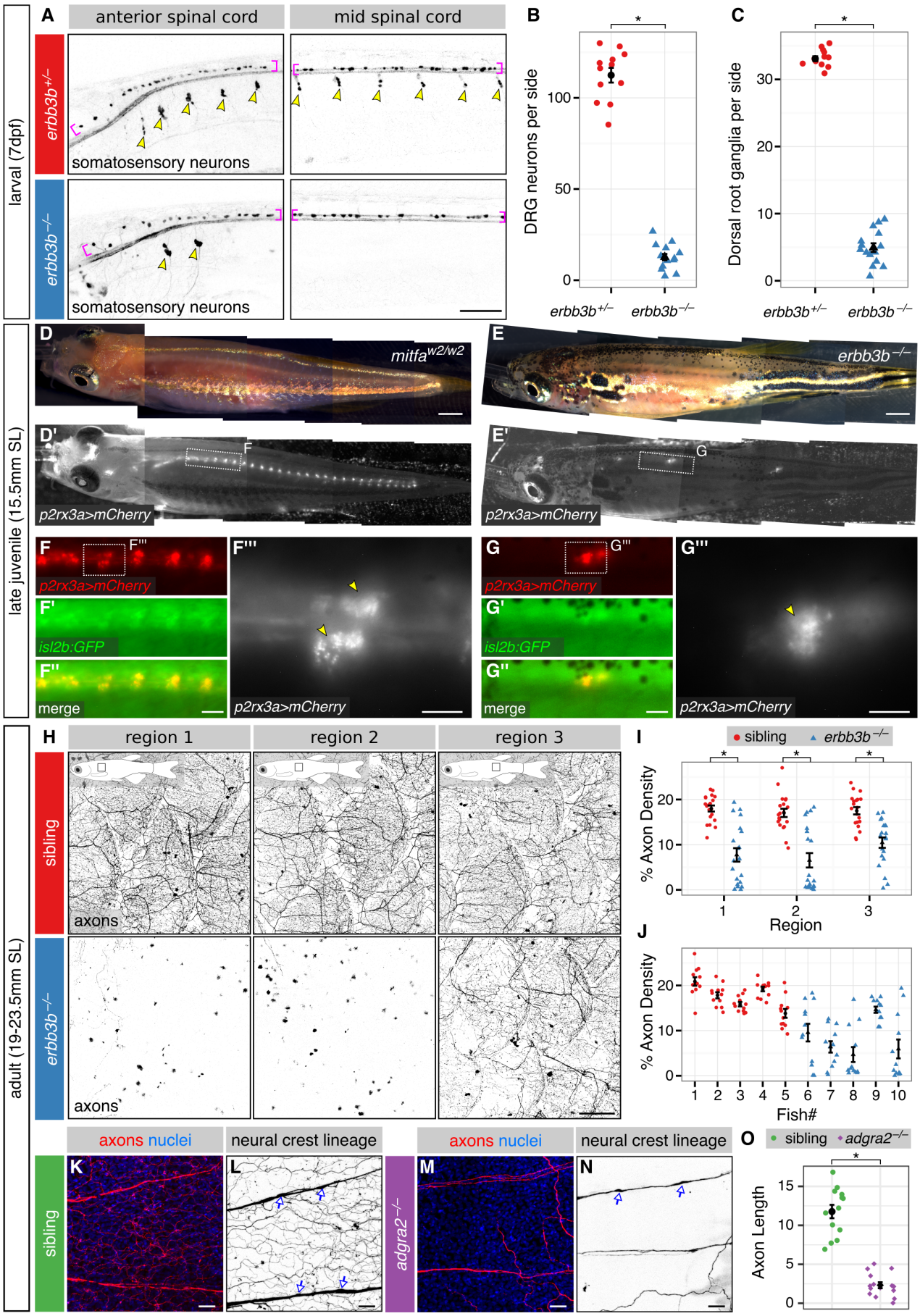

larval (7dpf)
A
anterior spinal cord
mid spinal cord
erbb3b+/–
somatosensory neurons
erbb3b–/–
somatosensory neurons
B
DRG neurons per side
100
50
0
erbb3b+/–
erbb3b–/–
C
Dorsal root ganglia per side
30
20
10
0
erbb3b+/–
erbb3b–/–
late juvenile (15.5mm SL)
D
mitfaw2/w2
D'
F
p2rx3a>mCherry
E
erbb3b–/–
E'
G
p2rx3a>mCherry
F
F'''
p2rx3a>mCherry
F'
isl2b:GFP
F''
merge
F'''
p2rx3a>mCherry
G
G'''
p2rx3a>mCherry
G'
isl2b:GFP
G''
merge
G'''
p2rx3a>mCherry
adult (19-23.5mm SL)
H
region 1
region 2
region 3
sibling
axons
erbb3b–/–
axons
I
sibling
erbb3b–/–
% Axon Density
20
10
0
1
2
3
Region
J
% Axon Density
20
10
0
1 2 3 4 5 6 7 8 9 10
Fish#
axons nuclei
neural crest lineage
axons nuclei
neural crest lineage
sibling
K
L
adgra2–/–
M
N
O
sibling
adgra2–/–
Axon Length
15
10
5
0

Figure S3. DRG axons innervate the epidermis during late juvenile stages, Related to Figure 2
(A) Repeated imaging of a single lateral skin region during scale formation. Arrowheads, individual axons extending along the scale surface. Dashed lines, scale margins. cc, club cells. (B) Lateral view of three dorsal root ganglia from the same fish as shown in panel A. (C) Isolated scales imaged at the indicated stages. Arrows, presumptive Schwann cells. Transgenes: (A) *Tg(-28.5Sox10:Cre); Tg(ubb:GswitchR)*; (B) *Tg(-28.5Sox10:Cre);Tg(ubb:GswitchR)* (red channel only); (C) axons [*Tg(p2rx3b:EGFP)*], and keratinocytes and Schwann cells [*Tg(-28.5Sox10:Cre);Tg(actb2:BswitchR)*]. Scale bars, 50 μm.

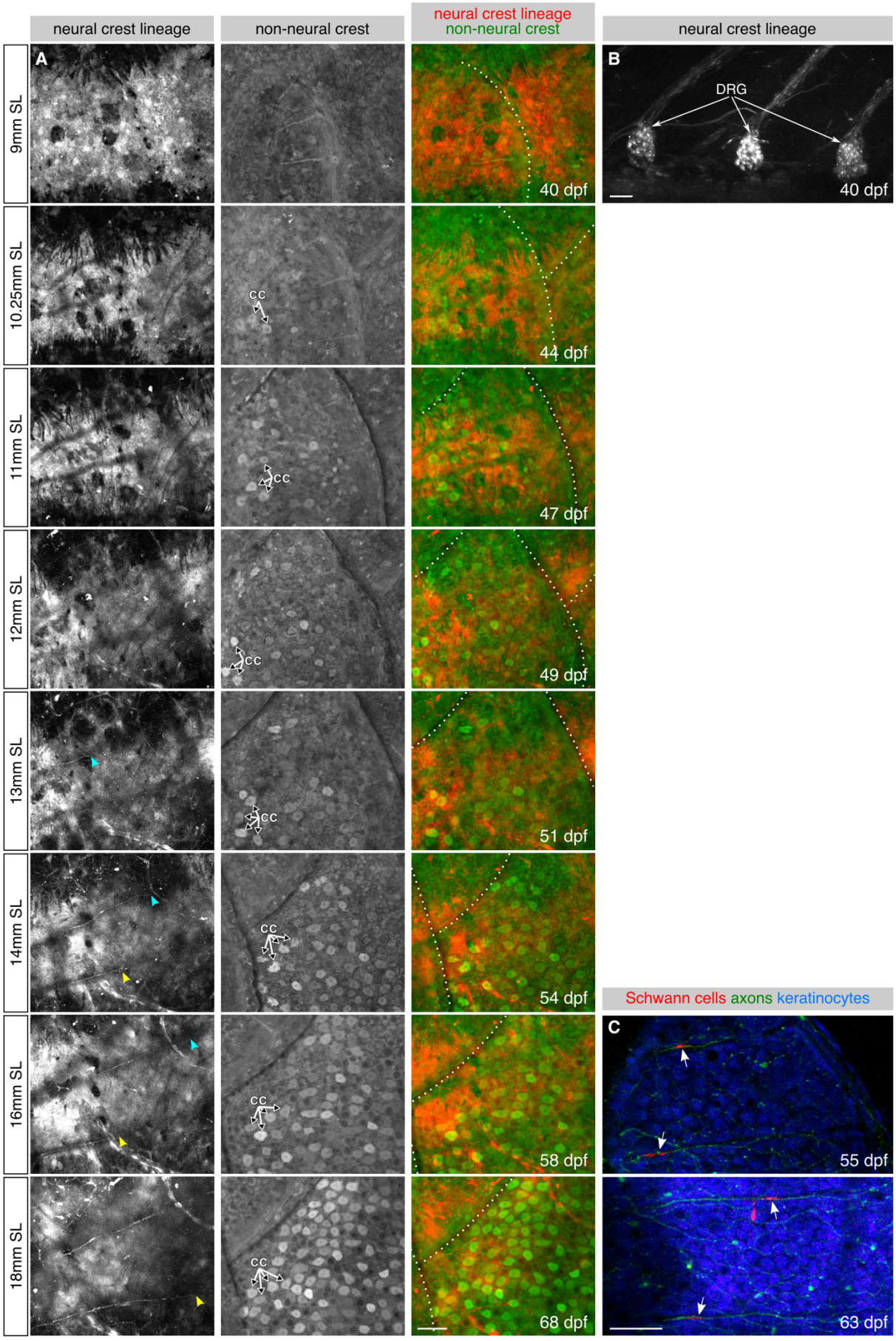

neural crest lineage
non-neural crest
neural crest lineage
non-neural crest
neural crest lineage
A
B
9mm SL
10.25mm SL
11mm SL
12mm SL
13mm SL
14mm SL
16mm SL
18mm SL
DRG
CC
40 dpf
44 dpf
47 dpf
49 dpf
51 dpf
54 dpf
58 dpf
68 dpf
40 dpf
Schwann cells axons keratinocytes
C
55 dpf
63 dpf

Figure S4. Development of skin vascularization, Related to Figure 4
(A-D) Lateral images of skin vasculature in fish expressing the pan-endothelial marker *Tg(fli1a:EGFP)* and stained with alizarin red S to label mineralized scales during late juvenile stages. Different individuals were imaged for each panel of A-C, whereas the same individual was repeatedly imaged in D. In panel A, note the large caliber vessels beneath each scale that connect to vessels beneath adjacent dorsal and ventral scales. In panels B and C, note the elaboration of the dermal network. In panel D, note that capillaries first extend along the radii above scales at ~18 mm SL. Arrows, vessel extending along scale surface. Yellow lines, planes of orthogonal sections. (E) Representative lateral view of the juvenile trunk in an animal expressing *Tg(fli1a:EGFP)* to label endothelium and *Tg(p2rx3a>mCherry)* to label axons. Arrow, tip of vessel growing along scale surface. Arrowheads, superficial scale nerves. Dashed lines, scale margins. Scale bars, 250 μm.

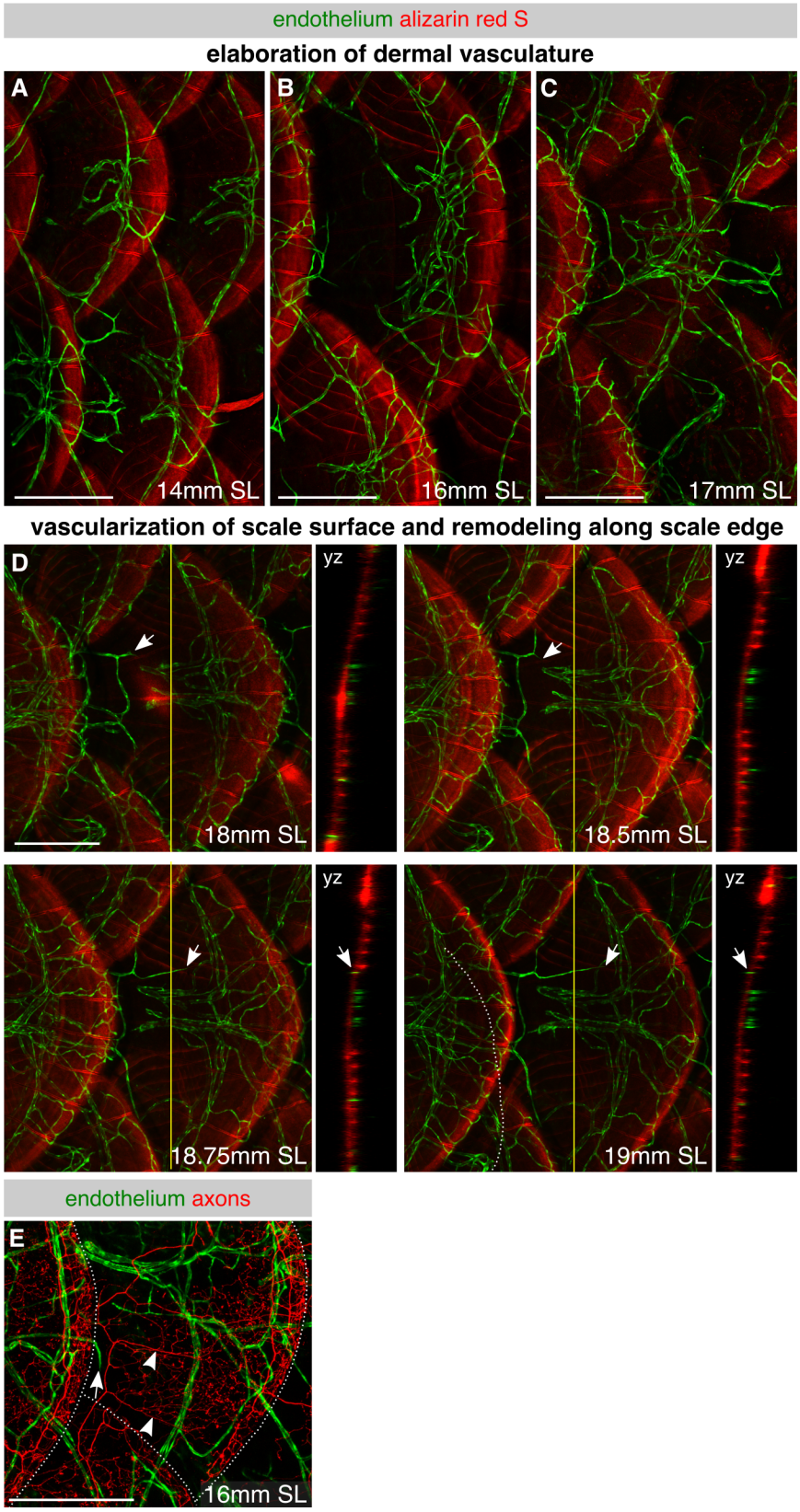

endothelium alizarin red S
elaboration of dermal vasculature
A
14mm SL
B
16mm SL
C
17mm SL
vascularization of scale surface and remodeling along scale edge
D
yz
18mm SL
yz
18.5mm SL
yz
18.75mm SL
yz
19mm SL
endothelium axons
E
16mm SL

Figure S5. The role of osteoblasts and osteoclasts in axon patterning, ECM formation and scale regeneration, related to Figures 5 and 6

(A) Transverse TEM of scale radius. Note that the position of the nerve is variable with respect to other radial cell types (compare to Figure 3E). en, endothelium; ob, osteoblast; bc, basal cell; mc, mucus secreting cell; n, nerve; r, radius; ms, mineralized scale. (B) Lateral DIC and fluorescent images of an isolated scale. f, focus. r, radius. Dashed boxes indicate regions of magnification. Inset, a single radius. (C) Scale nerve and radial patterns in *csf1ra* mutants. (D) Lateral view of intact skin. (E) Posterior edge of an isolated scale immunostained as indicated. (F) Single z-plane near the posterior edge of an isolated scale immunostained as indicated. Due to the curvature of the scale, the z-plane is an oblique section through the epidermis. In panel F'' the laminin channel has been over-exposed (o.e.) to highlight the intracellular puncta within basal cells. Basal cells are distinguishable by their characteristic nuclear size and cell borders. ep, episquamal osteoblasts; hyp, hyposquamal osteoblasts; bc, basal cell. Dashed boxes indicate region of magnification. Yellow line, plane of orthogonal section. (G) Brightfield and fluorescent images of control and osteoblast-ablated adult animals 7 days after removal of two dorsolateral rows of anterior scales (brackets). Note the lack of scale regeneration in osteoblast-ablated animals compared with robust scale regeneration in control animals (asterisks). Transgenes: (B) endothelium [*Tg(fli1a:EGFP)*] and axons [*Tg(p2rx3a>mCherry)*]; (D) *Tg(sp7:mCherry-nfsB)*; (G) all cells [*Tg(actb2:BswitchR)*] and osteoblasts [*Tg(sp7:mCherry-nfsB)*]. Staining: (C) axons (acTubulin) and nuclei (DAPI); (E) laminin (anti-laminin), F-actin (phalloidin) and nuclei (DAPI); (F) laminin (anti-laminin), osteoblasts (zns-5), F-actin (phalloidin) and nuclei (DAPI). Scale bars, 1 μm (A), 250 μm (B), 50 μm (C-F), 10 μm (F') and 500 μm (G).

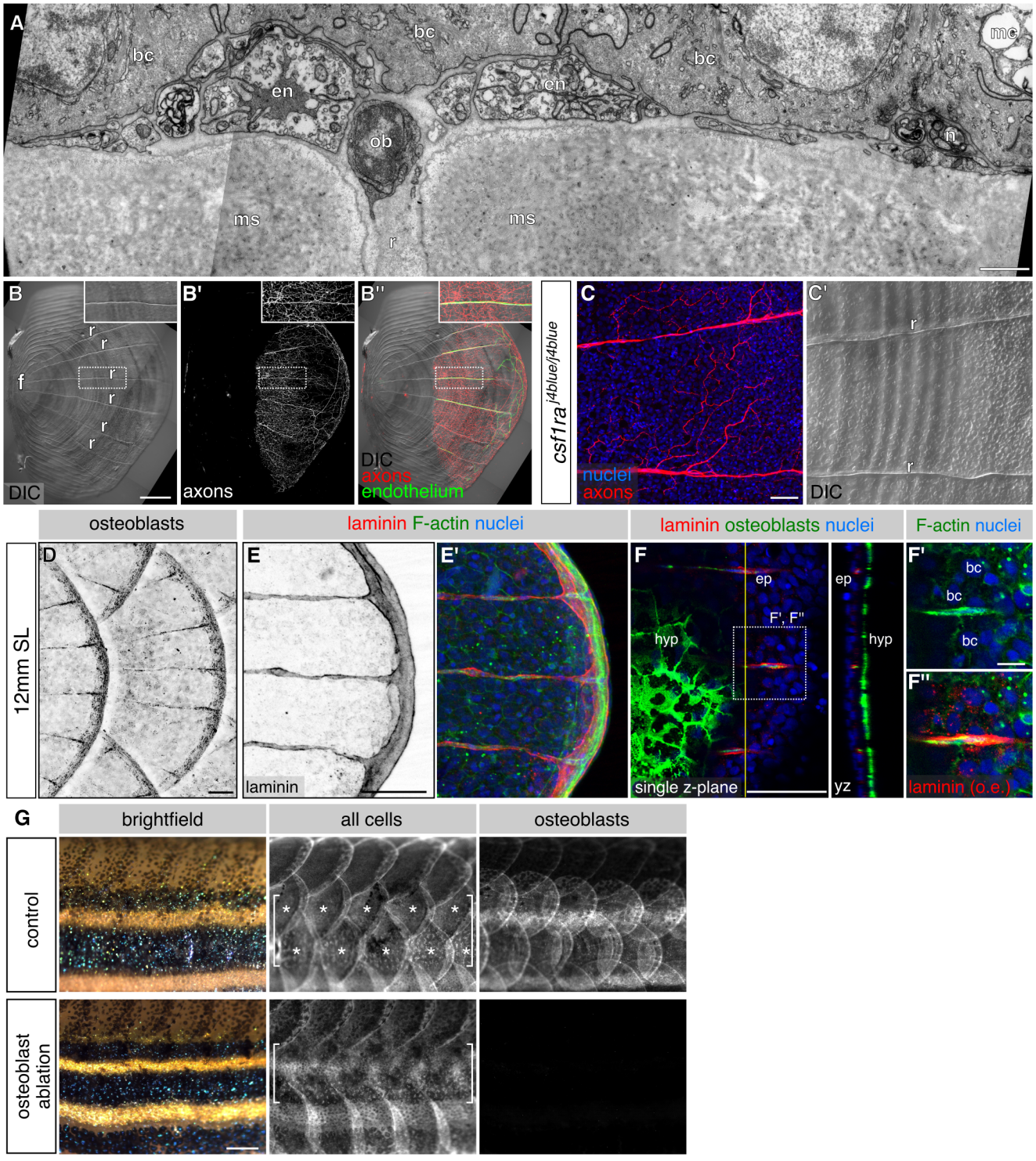

A
bc
bc
bc
mc
en
en
ob
n
ms
ms
r
B
B'
B''
r
f
DIC
axons
DIC
axons
endothelium
csf1ra j4blue/j4blue
C
C'
nuclei
axons
r
DIC
osteoblasts
laminin F-actin nuclei
laminin osteoblasts nuclei
F-actin nuclei
12mm SL
D
E
E'
F
F'
F''
laminin
ep
hyp
F', F''
single z-plane
yz
bc
laminin (o.e.)
G
brightfield
all cells
osteoblasts
control
osteoblast ablation

Figure S6. Nerves and capillaries extend along radii of scales isolated from diverse body locations, Related to Figure 5

(A) Schematic indicating the different regions of the trunk from which scales were isolated in panel B. (B) DIC and fluorescent images of scales isolated from various regions of the trunk of adult female and male animals (25-27 mm SL). White lines and dashed boxes in top panels indicate scale margins and areas of magnification for lower panels, respectively. Arrows, superficial nerves associated with vasculature. Note several examples of radii that are occupied by nerves but not vasculature (arrowheads). Transgenes: (B) endothelium [*Tg(fli1a:EGFP)*] and axons [*Tg(p2rx3a>mCherry)*]. Scale bars, 500 μm (top panels) and 100 μm (lower panels).

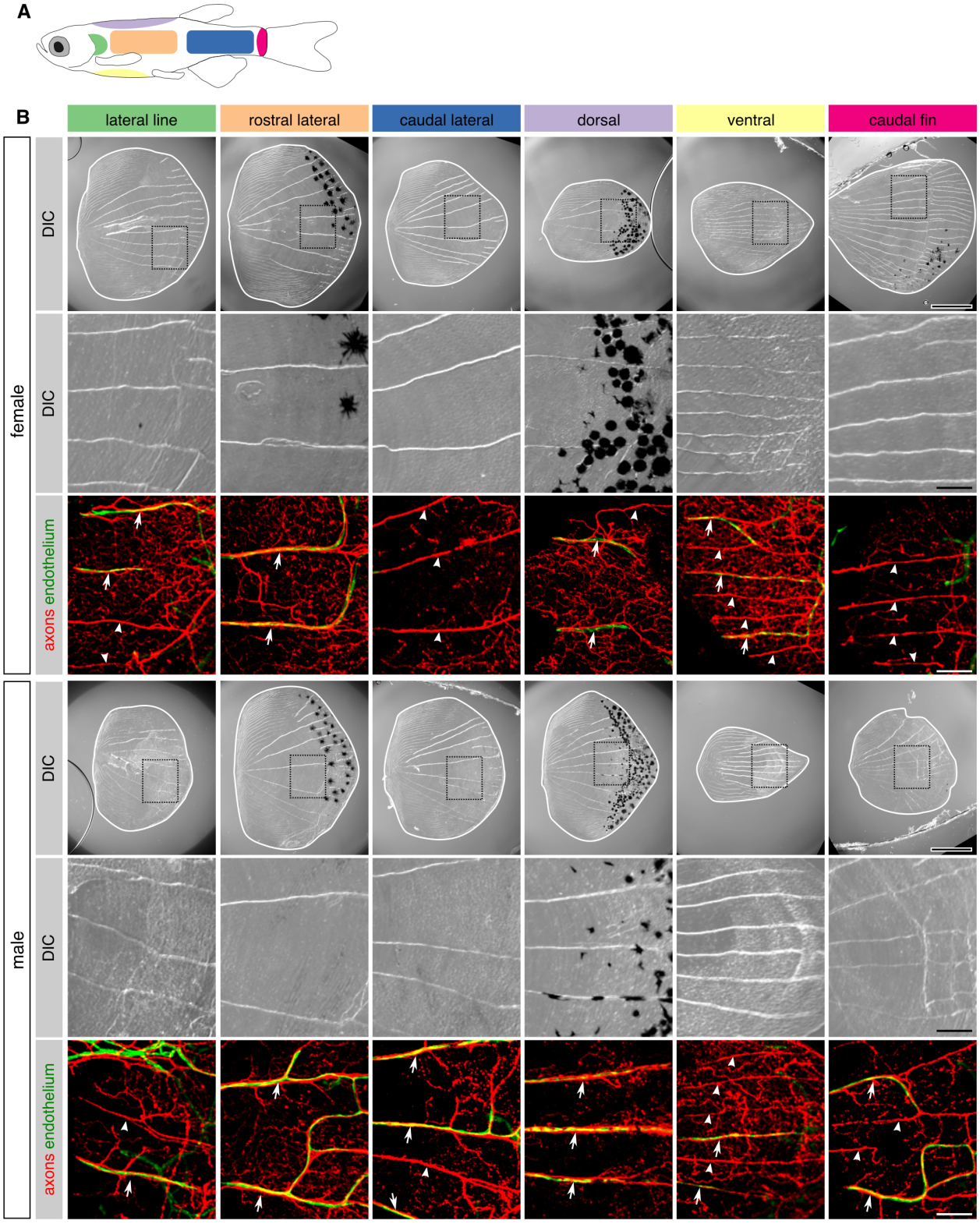
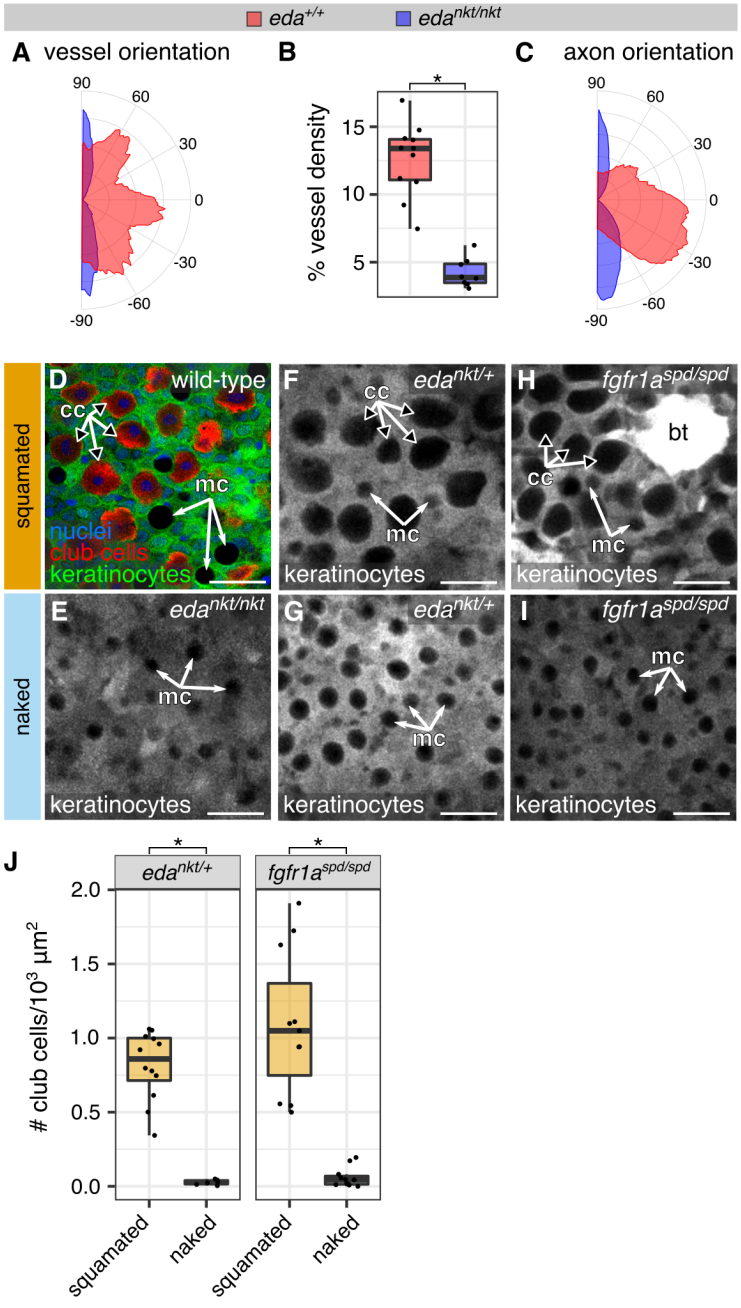

A
B
lateral line
rostral lateral
caudal lateral
dorsal
ventral
caudal fin
female
male
DIC
DIC
axons endothelium
DIC
DIC
axons endothelium

Figure S7. Eda and Fgfr1a are required for cutaneous neurovascular polarization and maturation, related to Figure 7

(A-C) Orientation and density analysis of skin vasculature and innervation from projections through intact skin of animals expressing endothelial [*Tg(fli1a:EGFP)*] (A,B) and axonal [*Tg(p2rx3a>mCherry)*] markers (C). *, p<0.01, Wilcoxon rank sum test. *n*=8-11 skin regions of size 0.77-1.5 mm^2 from *n*=3-5 fish analyzed per genotype. (D) Single z-plane through the epidermis of an isolated scale immunostained as indicated. Note that club cells (cc) and mucus secreting cells (mc) do not express the keratinocyte marker *Tg(krt4:EGFP)* and can be distinguished by their characteristic shapes and sizes. (E-I) Single z-planes through the epidermis of intact fish of the indicated genotypes. Note the lack of club cells in the "naked" (non-squamated) skin regions. *Tg(krt4:EGFP)* is highly expressed in breeding tubercles (bt), male-specific keratin-rich structures visible in panel H. cc, club cells; mc, mucus secreting cells. (J) Quantification of club cell density based on the pattern of *Tg(krt4:EGFP)* expression in naked and squamated skin of fish of the indicated genotypes. *, p<0.01, Wilcoxon rank sum test. *n*=6-12 skin regions of size 0.1-1.2 mm^2 from *n*=4-6 fish analyzed per condition. Transgenes: (D-I) keratinocytes [*Tg(krt4:EGFP)*]. Staining: (D) club cells (CS-56) and nuclei (DAPI). Scale bars, 25 μm.


eda+/+
edankt/nkt
A vessel orientation
B
C axon orientation
% vessel density
squamated
naked
D wild-type
cc
mc
nuclei
club cells
keratinocytes
F edankt/+
H fgfr1aspd/spd
bt
E edankt/nkt
G edankt/+
I fgfr1aspd/spd
J
club cells/10^3 µm^2
squamated
naked