SUPPLEMENTARY INFORMATION

WHOLE-MOUNT ADULT EAR SKIN IMAGING REVEALS DEFECTIVE NEURO-VASCULAR BRANCHING MORPHOGENESIS IN OBESE AND TYPE 2 DIABETIC MOUSE MODELS

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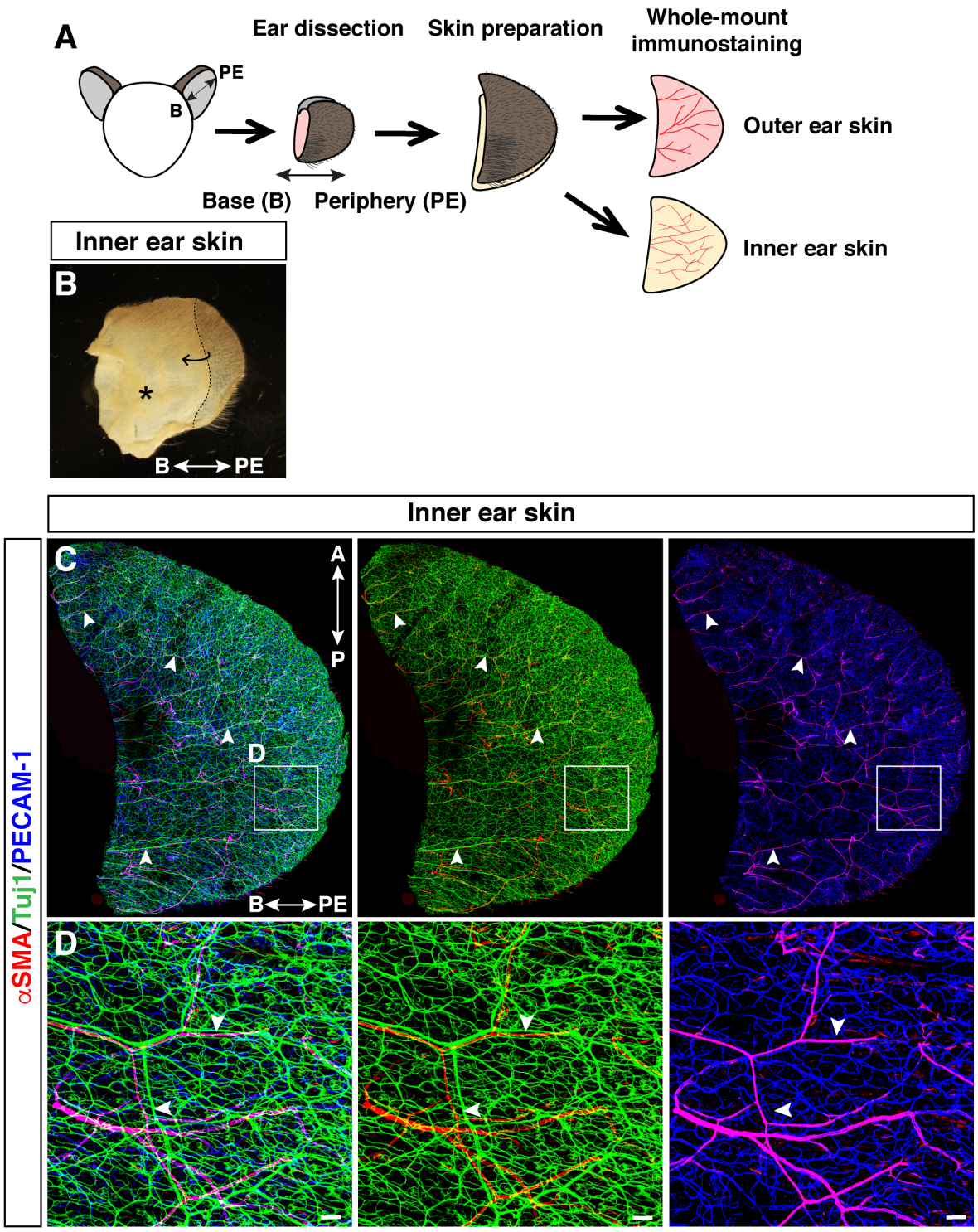
A
Ear dissection
Skin preparation
Whole-mount immunostaining
PE
B
Outer ear skin
Base (B)
Periphery (PE)
Inner ear skin
Inner ear skin
B
B ⟷ PE
Inner ear skin
αSMA/Tuj1/PECAM-1
C
A
P
D
B ⟷ PE
D

Supplementary Figure 1. Whole-mount immunofluorescence confocal microscopy of adult inner ear skin

(A) Diagram of dissection of outer and inner parts of ear skin. (B) First, outer part of the ear skin was peeled away from the cartilage (not shown). Next, the cartilage (asterisk) was peeled off from inner skin before staining. (C) Inner ear skin was stained with antibodies to a VSMC marker αSMA (red), a pan-neuronal marker Tuj1 (green), and a pan-endothelial marker PECAM-1 (blue). The boxed regions of (C), peripheral regions of the inner ear skin, are magnified in (D). Arrowheads indicate the alignment of small-diameter nerves and VSMC-covered remodeled blood vessels. The orientation of base (B)-periphery (PE) axis and anterior (A)-posterior (P) axis is shown. Scale bars, 100 μm.