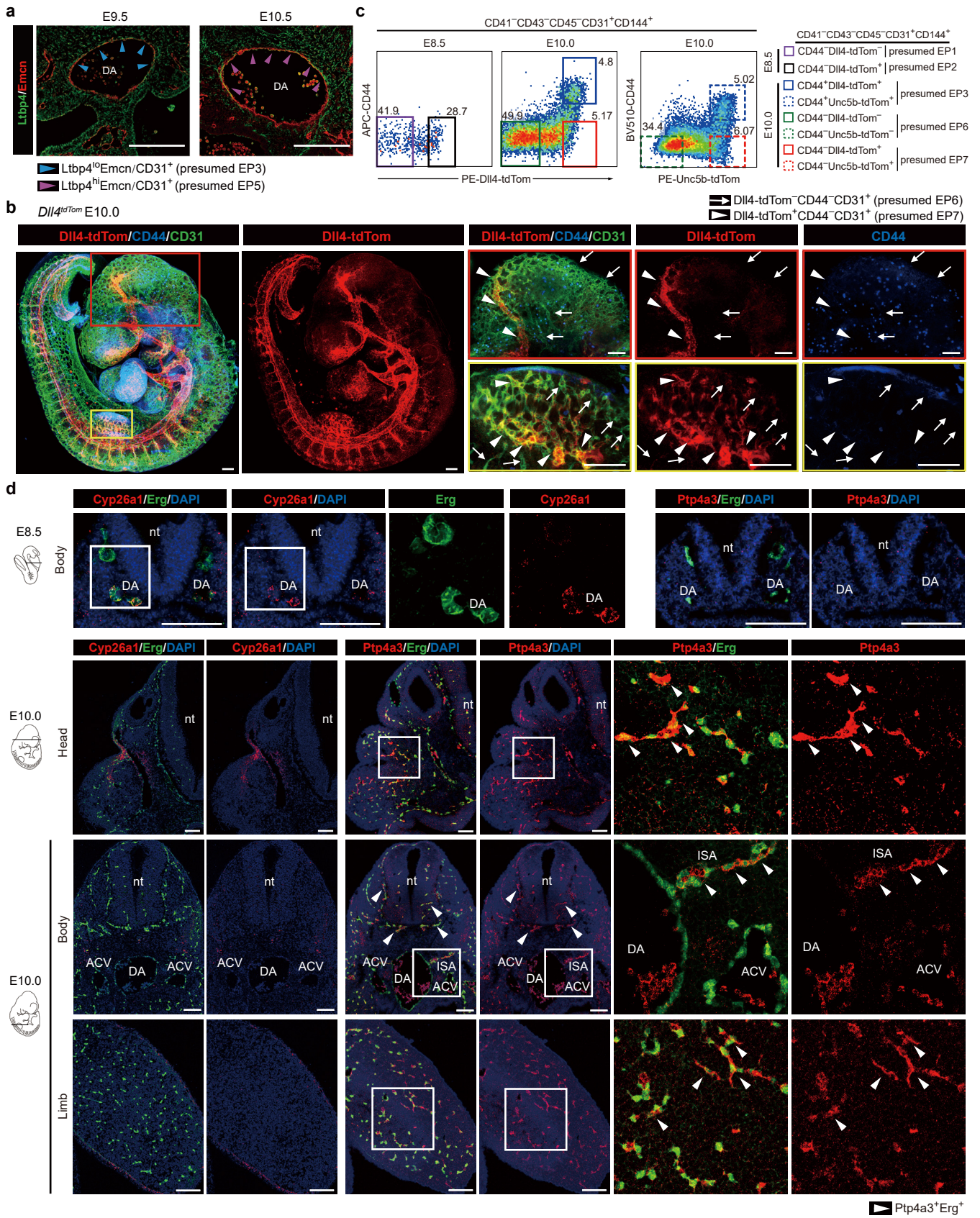


**Fig. S5**



**Fig. S5. Identification of distinct VEC populations by whole-mount immunostaining and RNAscope.**

**a**, Representative immunostaining on cross sections of aortic region of mouse embryos at different developmental stages as indicated. Note the inner layer of E10.5 and E11.0 aortas (purple arrowheads) show higher  $Ltbp4^+$  fluorescent signals than that of E9.5 (blue arrowheads), indicative of its mature arterial VEC characteristics. DA, dorsal aorta; Scale bars, 100  $\mu\text{m}$ .

**b**, Whole-mount immunostaining of E10.0 *Dll4-tdTomato* embryos with the antibodies to RFP (red), major artery endothelial marker CD44 (blue) and pan-endothelial marker CD31 (green). Arrowheads indicate the presumed EP7 located at the branches of internal carotid arteries and in the limb bud capillaries. Arrows indicate the presumed EP6 without *Dll4-tdTom* expression. Red boxes, reconstructions with a total of 40  $\mu\text{m}$  thickness; yellow boxes, reconstructions with a total of 30  $\mu\text{m}$  thickness. Scale bars, 100  $\mu\text{m}$ .

**c**, Representative FACS plots for cell sorting. Single cell suspensions were prepared from *Dll4-tdTomato* and *Unc5b-tdTomato* embryos respectively at different developmental stages as indicated. Cell populations representing different presumed VEC clusters are denoted as colored boxes and were isolated for scRNA-seq.

**d**, RNAscope assay of the indicated mRNAs (red) coupled with immunostaining of *Erg* (green) on cross sections of E8.5 (upper) and E10.0 (lower) mouse embryos. Note the expression of *Cyp26a1* specifically in the aortic VECs at E8.5 but not in the VECs at E10.0. Arrowheads denote  $Ptp4a3^+$  VECs, which location is restricted to some small vessels, including intersegmental branches from the dorsal aorta, in accordance with that of EP7, but not expressed at major vessels including DAs and ACVs. Images in white boxes show inserts at high magnification. The diagrams to the left indicate the positions of sections. nt, neural tube; DA, dorsal aorta; ACV, anterior cardinal vein. ISA, intersegmental artery. Scale bars, 100  $\mu\text{m}$ .