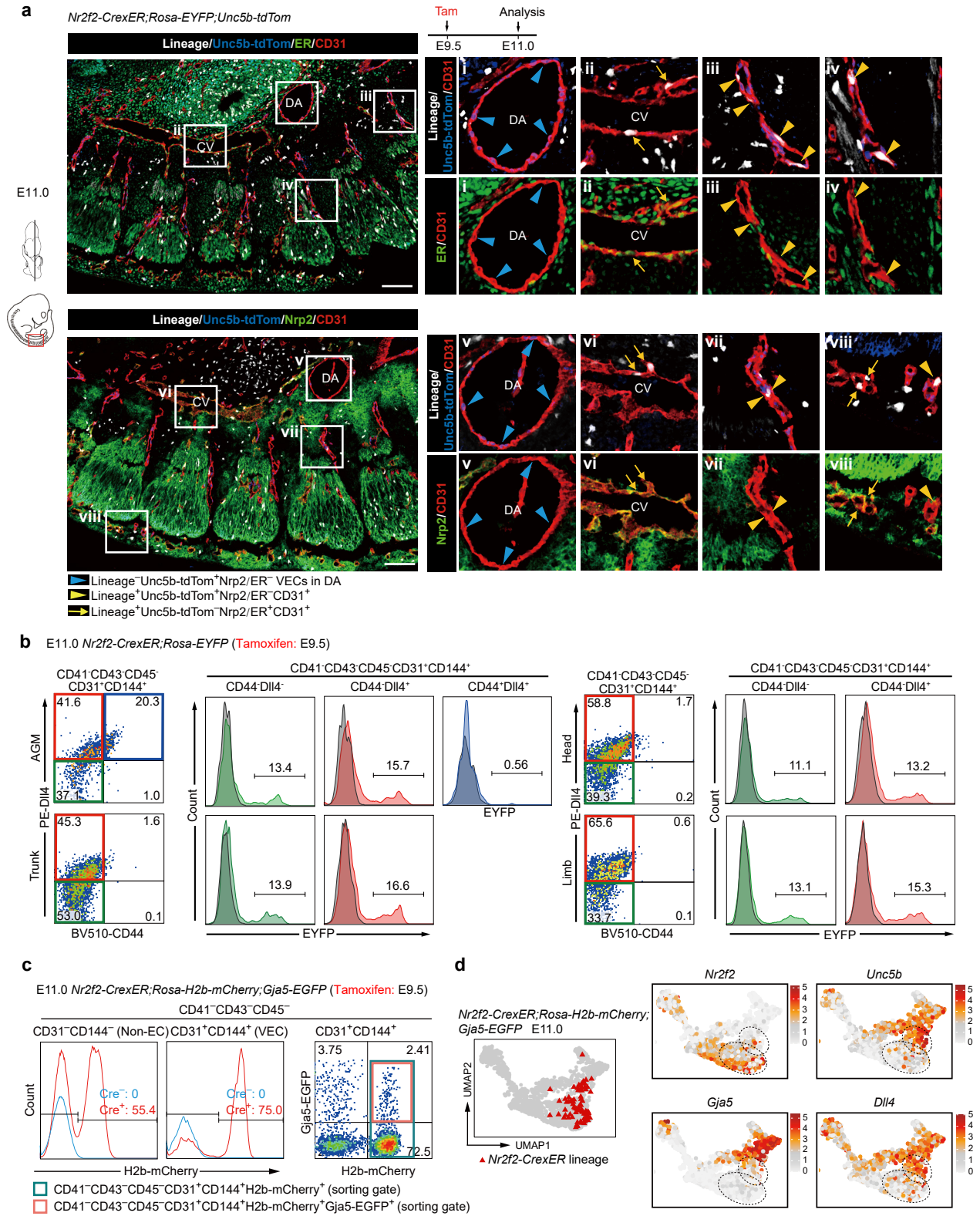


**Fig. S11**



**Fig. S11. Capillary arterialization from venous-featured plexus VECs in mid-gestational embryos revealed by genetic lineage tracing.**

**a,** Representative immunostaining on sagittal sections of E11.0 *Nr2f2-CrexER;Rosa-EYFP;Unc5b-tdTomato* embryos after single dose of tamoxifen induction at E9.5. The *Nr2f2-CrexER* lineage-labeled VECs are observed in the *Unc5b-tdTom<sup>+</sup>Nrp2/ER<sup>-</sup>* artery plexus VECs (yellow arrowheads) in addition to *Unc5b-tdTom<sup>-</sup>Nrp2/ER<sup>+</sup>* venous VECs (yellow arrows), but not in the DA (blue arrowheads). Images to the right show inserts at high magnification. The diagrams to the left indicate the positions of the sections and imaging. DA, dorsal aorta. CV, cardinal vein. Scale bars, 100  $\mu$ m.

**b,** Representative FACS analysis of different regions of E11.0 *Nr2f2-CrexER;Rosa-EYFP* embryos after single dose of tamoxifen induction at E9.5. Data are representative of 2 independent experiments. The presumed EP6 (vein & venous plexus VECs, green boxes), EP7 (arterial plexus VECs, red boxes), and major artery VECs (*CD44<sup>+</sup>Dll4<sup>+</sup>* in AGM region, blue box) are indicated. Histograms to the right indicate the EYFP labelling in different populations. Littermate *Rosa-EYFP* embryos are used as the negative controls. AGM, aorta-gonad-mesonephros.

**c,** Representative FACS plots for analysis and cell sorting. Single cell suspensions were prepared from E11.0 *Nr2f2-CrexER;Rosa-H2b-mCherry;Gja5-EGFP* embryos after single dose of tamoxifen induction at E9.5. Histograms show the lineage labeling in both non-ECs and VECs. Cell populations isolated for scRNA-seq are denoted as colored boxes (red for lineage<sup>+</sup>*Gja5-EGFP<sup>+</sup>* VECs and blue for lineage<sup>+</sup> VECs). Littermate *Rosa-H2b-mCherry* embryos are used as the negative control.

**d,** UMAP plots of the integration data of sequenced *Nr2f2-CrexER* lineage labeled cells and original VECs with the expression of indicated arteriovenous marker genes mapped on. Note that most lineage and *Gja5* double-positive VECs show clear arterial genes but a loss of *Nr2f2* expression. Dashed outlines of EP6 and EP7 are indicated.