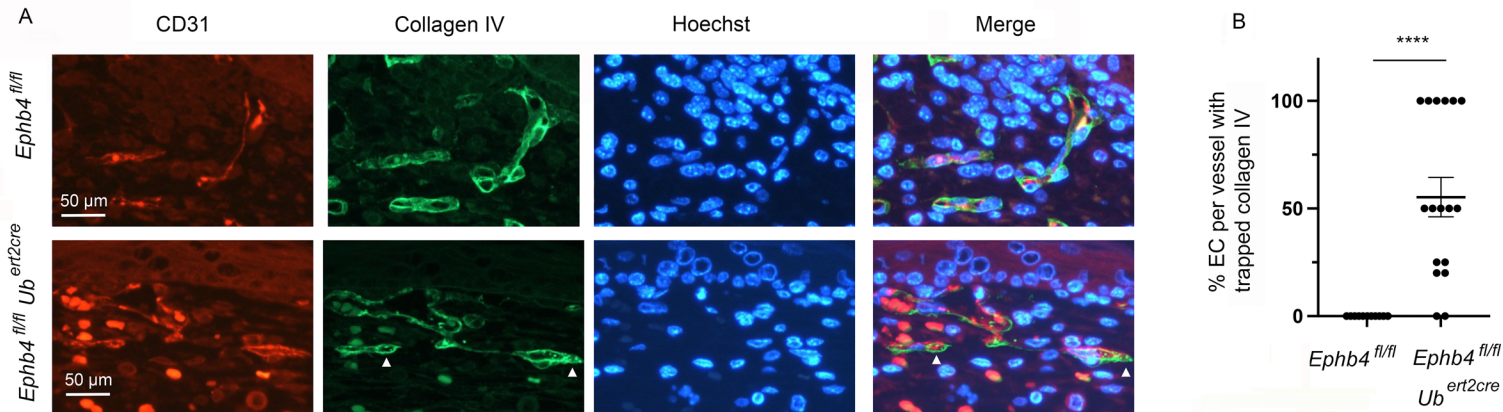


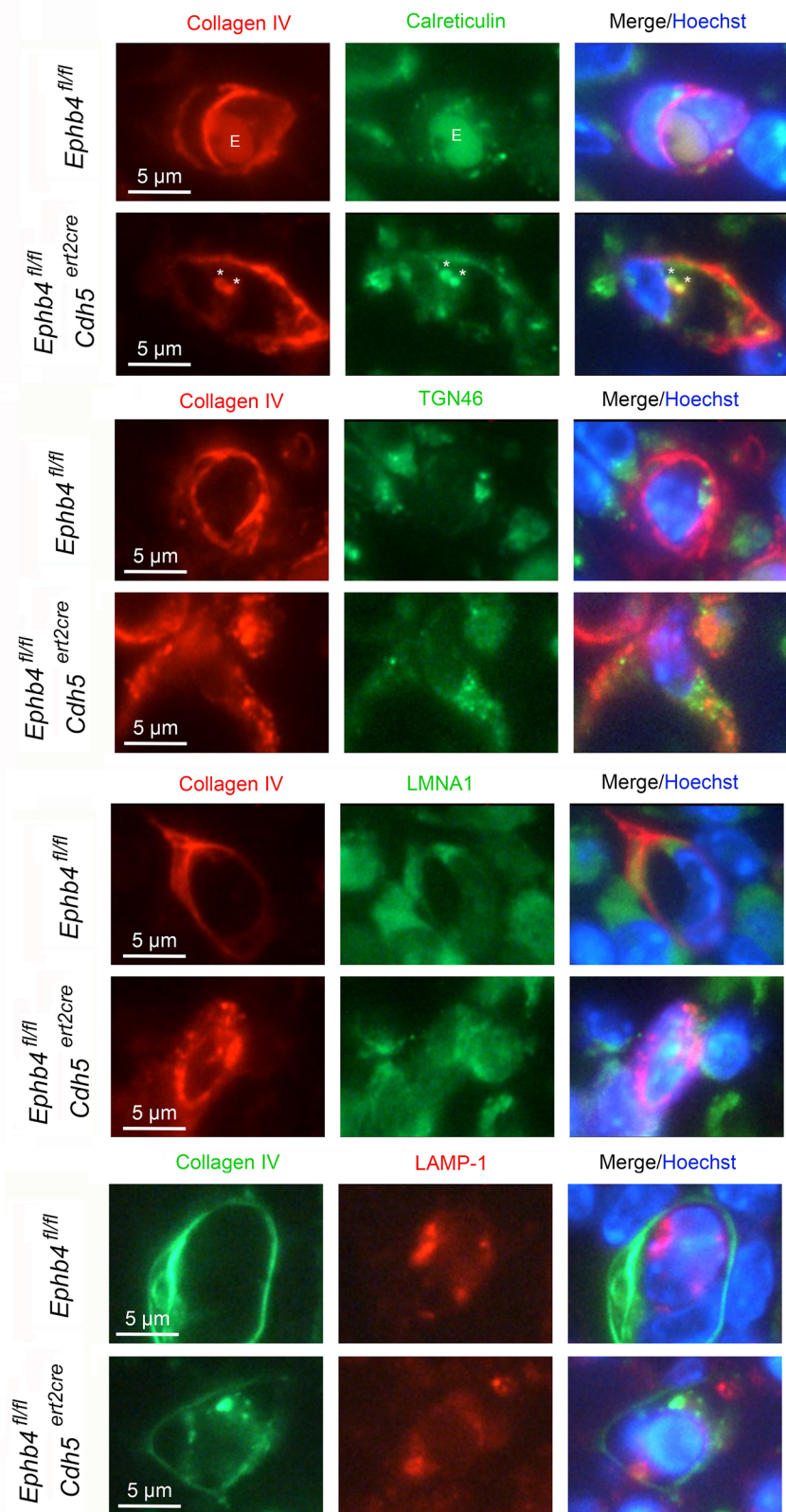
### Supplemental Figure 1. Hemorrhage, EC apoptosis and reduced lymphatic vessels

following induced global disruption of *Ephb4* during developmental angiogenesis. TM was administered to *Ephb4<sup>fl/fl</sup>* and *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos at E13.5 and embryos were harvested at E18.5. (A) Images at left show extensive cutaneous hemorrhage and an edematous appearance of *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos. H&E staining of skin sections confirmed vascular hemorrhage and combined CD31 and LYVE-1 staining of sections revealed a reduced number of intact CD31<sup>lo</sup>LYVE-1<sup>+</sup> initial LV in *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos (shown with arrowheads in images of *Ephb4<sup>fl/fl</sup>* sections). (B) Plot shows the number of identified CD31<sup>lo</sup>LYVE-1<sup>+</sup> LV in randomly selected 200 μm x 200 μm areas of skin of *Ephb4<sup>fl/fl</sup>* and *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos. Bars show the mean +/- 1 SEM of LV/field (*Ephb4<sup>fl/fl</sup>*, n=9; *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>*, n=16). (C) Skin sections were stained with anti-CD31 and anti-activated caspase 3 antibodies and Hoechst to identify apoptotic EC. Examples of activated caspase 3-positive EC with fragmented nuclei in images of *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* sections are indicated with arrowheads. (D) Plot shows the percentage of apoptotic cells in individual CD31<sup>+</sup> BV in skin of *Ephb4<sup>fl/fl</sup>* and *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos selected from multiple randomly chosen areas. Bars show the mean +/- 1 SEM of percentage apoptotic EC per vessel (*Ephb4<sup>fl/fl</sup>*, n=15; *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>*, n=10). \*\*\*\*,  $P < 0.0001$ ; Mann Whittney test.





**Supplemental Figure 3. Disruption of an exon 1 floxed *Ephb4* allele during developmental angiogenesis results in accumulation of collagen IV in EC.** *Ephb4<sup>fl/fl</sup>* and *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos in which exon 1 of *Ephb4* was floxed were administered TM at E13.5 and harvested at E18.5. (A) Skin sections were stained with anti-CD31 and anti-collagen IV antibodies and Hoechst. Note intracellular accumulation of collagen IV in EC of *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>* embryos (examples highlighted with arrowheads). (B) Plot shows the percentage of EC with intracellular collagen IV punctae in individual CD31+ BV in skin of embryos selected from multiple randomly chosen areas. Bars show the mean  $\pm$  1 SEM of percentage EC with collagen IV accumulation (*Ephb4<sup>fl/fl</sup>*, n=11; *Ephb4<sup>fl/fl</sup> Ub<sup>ert2cre</sup>*, n=17). \*\*\*\*,  $P < 0.0001$ ; Mann Whitney test.



**Supplemental Figure 4. Collagen IV is retained within the ER of induced EPHB4-deficient EC during developmental angiogenesis.** TM was administered to *Ephb4*<sup>fl/fl</sup> and *Ephb4*<sup>fl/fl</sup> *Cdh5*<sup>ert2cre</sup> embryos at E13.5 and embryos were harvested at E18.5. Skin sections were stained with anti-collagen IV antibodies and antibodies against calreticulin (ER), LMNA1 (ERGIC), TGN46 (Golgi) or LAMP-1 (lysosome). Representative images of individual BV are shown. Note colocalization of collagen IV punctae with calreticulin in *Ephb4*<sup>fl/fl</sup> *Cdh5*<sup>ert2cre</sup> embryos (asterisks). Note lack of colocalization of collagen IV punctae with other organelle markers in *Ephb4*<sup>fl/fl</sup> *Cdh5*<sup>ert2cre</sup> embryos. E, erythrocyte.



Figure 6C

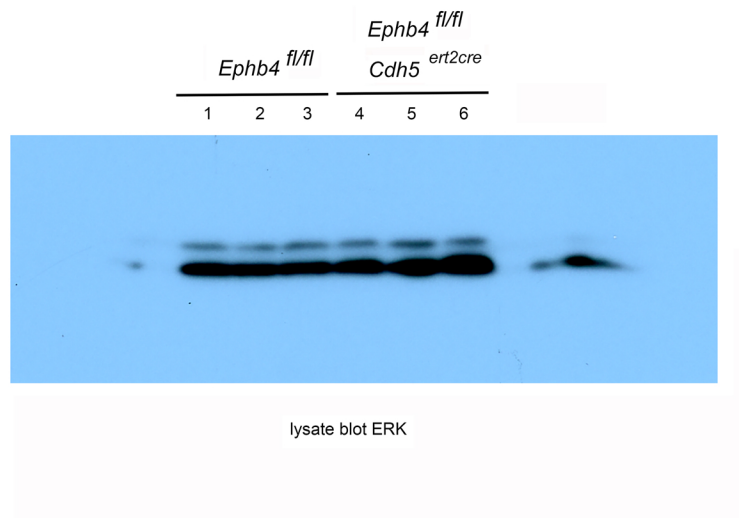
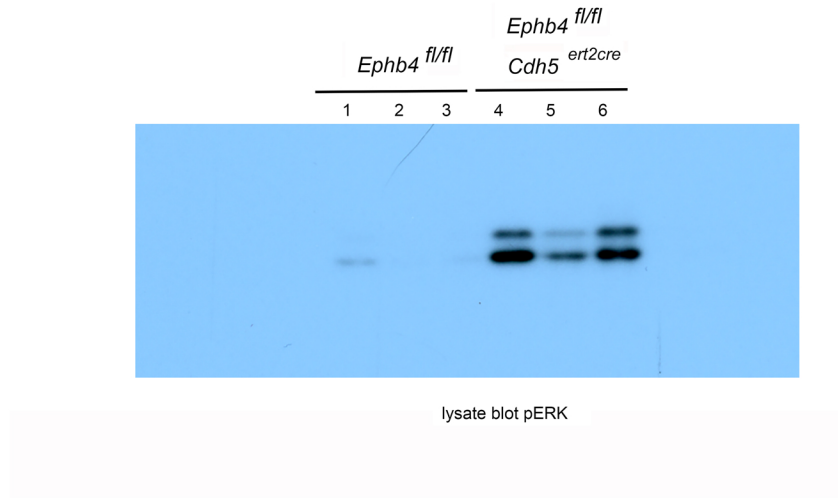


Fig. 10 A

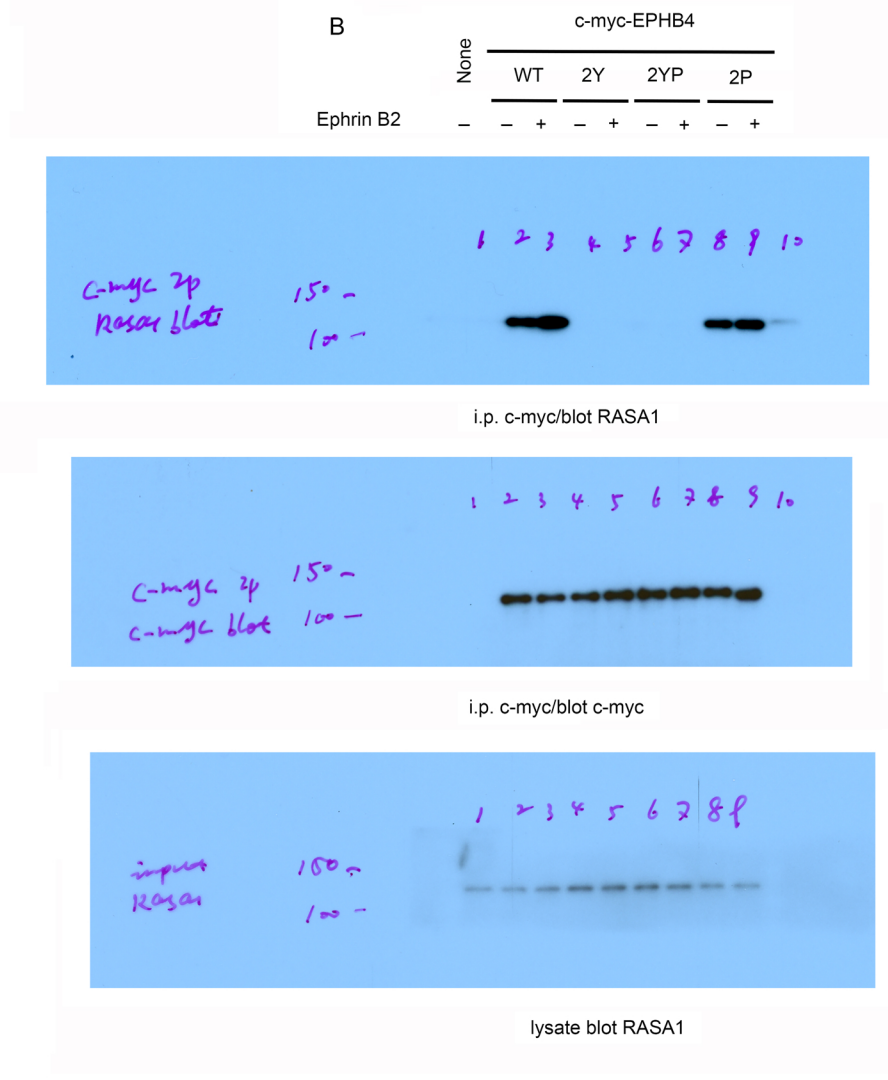


Fig. 10 B

