

## Supplemental Material

### Genetic Reduction of VEGFR2 Rescues Aberrant Angiogenesis Caused by Epsin Deficiency

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Running Title: Reducing VEGFR2 Corrects Abnormal Angiogenesis

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## Supplemental Figure Legend

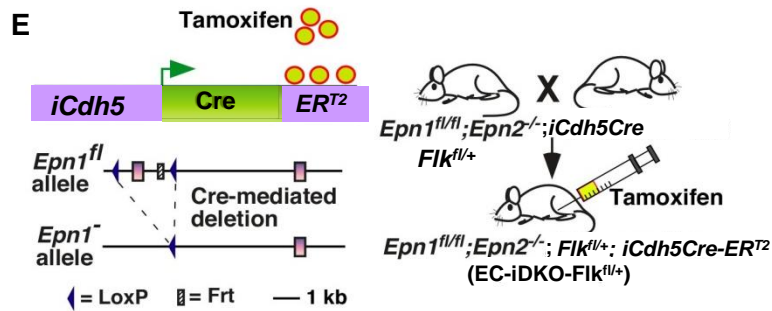
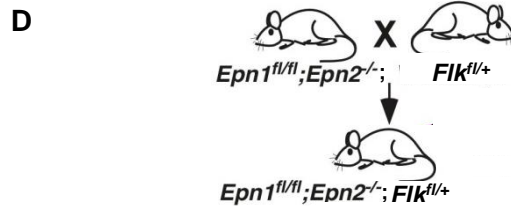
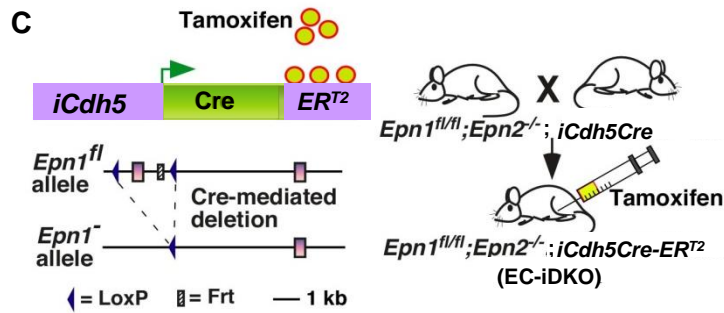
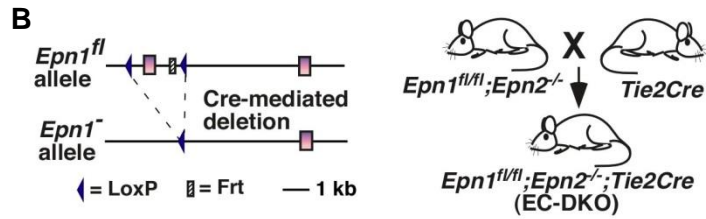
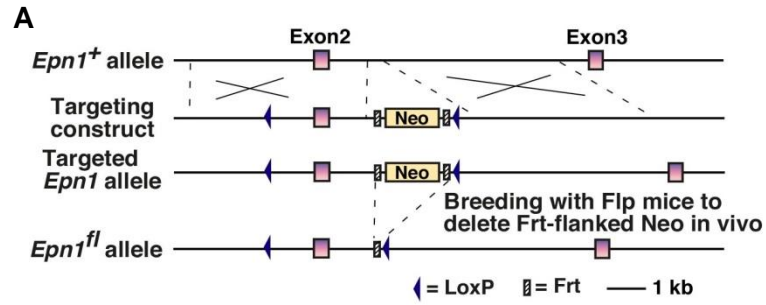
**Supplemental Figure I. Generation of EC-DKO, EC-iDKO and EC-iDKO-Flk<sup>fl/+</sup> mice.** **A**, Diagram shows homologous recombination of the floxed gene-targeting vector at the *Epn1* locus. **B**, Strategy to generate constitutive endothelial cell-specific epsin DKO (EC-DKO) mice by crossing *Epn1<sup>fl/fl</sup>; Epn2<sup>-/-</sup>* mice with *Tie2 Cre deleter* mice. **C**, Strategy to generate tamoxifen inducible endothelial cell-specific epsin DKO (EC-iDKO) mice by crossing *Epn1<sup>fl/fl</sup>; Epn2<sup>-/-</sup>* mice with *iCDH5 Cre deleter* mice. **D**, Strategy to generate *Epn1<sup>fl/fl</sup>; Epn2<sup>-/-</sup>; Flk<sup>fl/+</sup>* mice by crossing *Epn1<sup>fl/fl</sup>; Epn2<sup>-/-</sup>* mice with *Flk<sup>fl/+</sup>* mice. **E**, Strategy to generate inducible endothelial cell-specific epsin DKO, VEGFR2 heterozygous (EC-DKO-Flk<sup>fl/+</sup>) mice by crossing the *Epn1<sup>fl/fl</sup>; Epn2<sup>-/-</sup>; Flk<sup>fl/+</sup>* mice with *iCDH5 Cre deleter* mice.

**Supplemental Figure II. Epsin deficiency promotes VEGF-dependent *in vivo* angiogenesis.** **A**, CD31 immunostaining of intestine of E18 WT or EC-iDKO embryos. Scale bars: 100  $\mu$ m. **B**, CD31-positive surface area in **A** was quantified by SlideBook software. Error bars indicate the mean  $\pm$  s.e.m.  $n > 5$ , \* $p < 0.05$ .

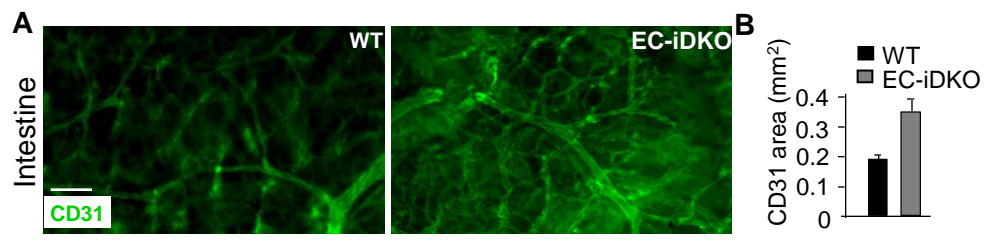
**Supplemental Figure III. Epsin deficiency impairs internalization and colocalization of phosphorylated VEGFR2 to EEA1 endosomes.** WT or DKO MECs stimulated with 50 ng/mL VEGF-A for 0, 1 and 10 min were fixed and stained using the specified antibodies. Scale bar: 10  $\mu$ m.

**Supplemental Figure IV. Genetic reduction of VEGFR2 expression rescues proliferation caused by epsin deletion.** Quantification of *in vivo* BrdU incorporation in intestines isolated from BrdU injected WT, EC-iDKO, or EC-iDKO-Flk<sup>fl/+</sup> mice shown in Figure 3E. Error bars indicate the mean  $\pm$  s.e.m.  $n > 5$ , \* $p < 0.05$ .

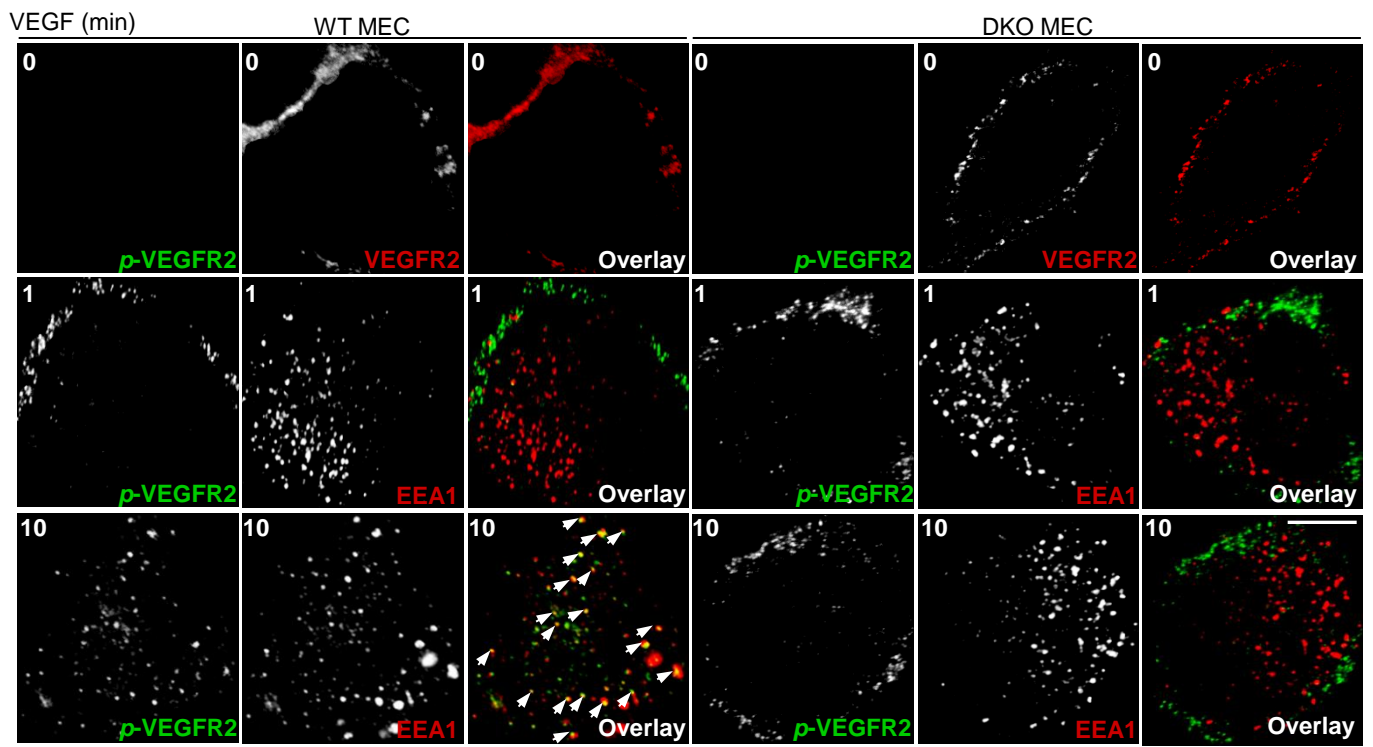
**Supplemental Figure V. Genetic reduction of VEGFR2 expression rescues *in vitro* angiogenesis caused by epsin deletion.** Quantification of specified immunoblots of whole cell lysates from WT, DKO or DKO-Flk<sup>fl/+</sup> MECs stimulated with 50 ng/mL VEGF-A shown in Figure 4A. Error bars indicate the mean  $\pm$  s.e.m.  $n > 5$ , \* $p < 0.05$ .



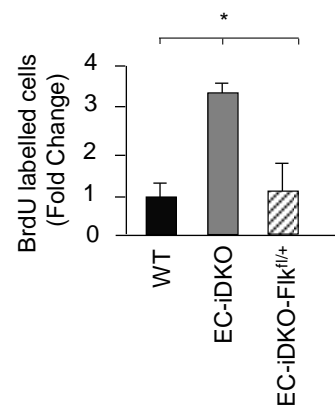
Supplemental Figure I



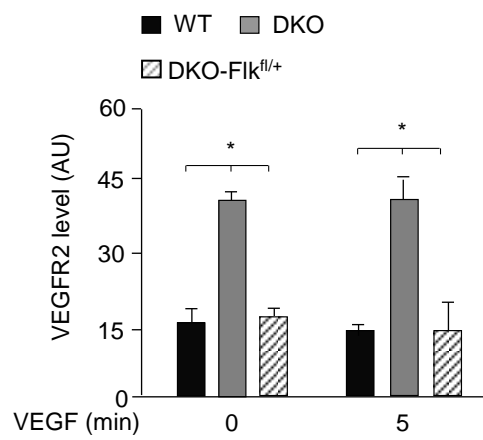
Supplemental Figure II



Supplemental Figure III



Supplemental Figure IV



Supplemental Figure V