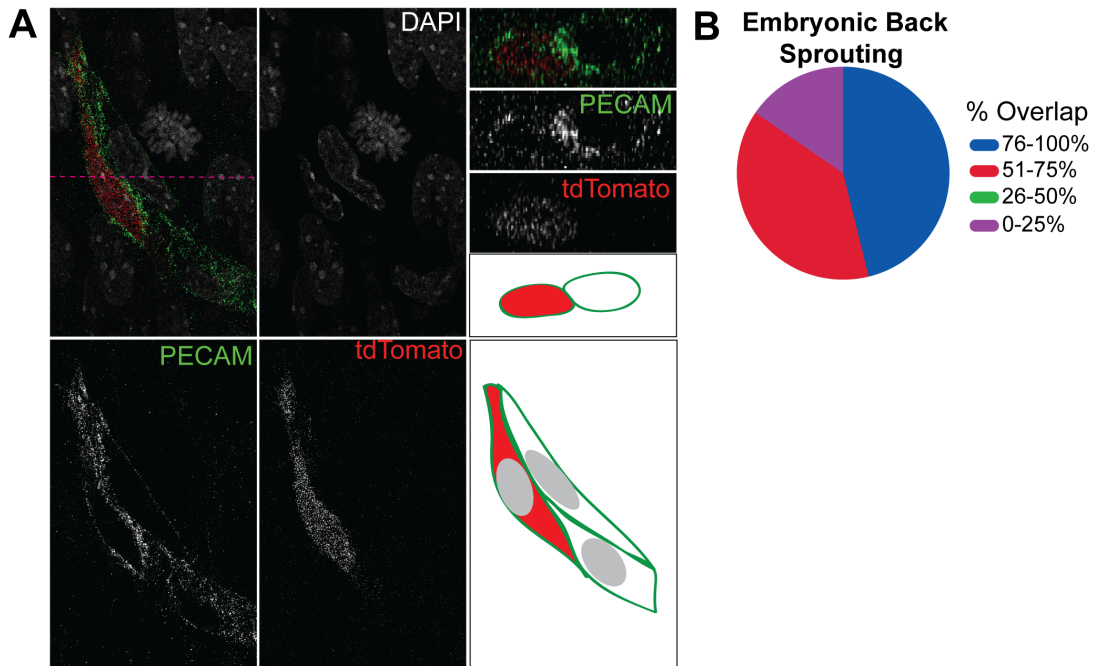


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## SUPPLEMENTAL MATERIAL

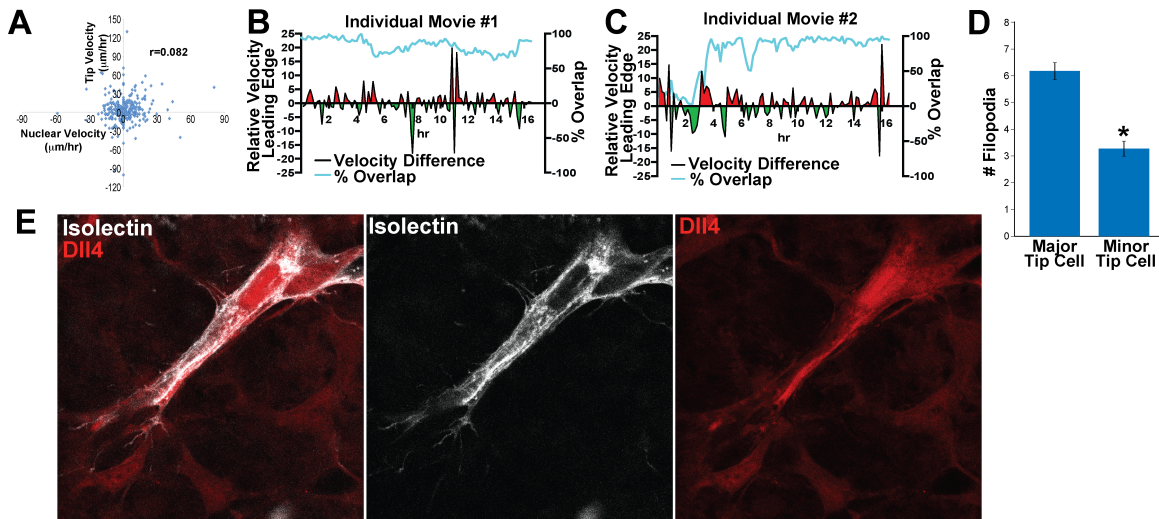
## SUPPLEMENTAL FIGURES

## SuppFig 1

**Supplemental Figure 1. Multiple Endothelial Cells Occupy the Tip of Embryonic Back Vessel**

**Sprouts.** **A)** Left, Mosaic vessel sprout from E12.5 back of *VEcad-Cre* x *tdTomato<sup>flox/+</sup>* embryo expressing vascular *tdTomato* (red), and stained with PECAM (green, endothelial marker) and DAPI (white, nuclear marker). Right, orthogonal views and diagrams show overlap of endothelial cells near the tip. **B)** Frequency of topologies with indicated percent tip cell overlap in mouse embryonic back.

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SuppFig2

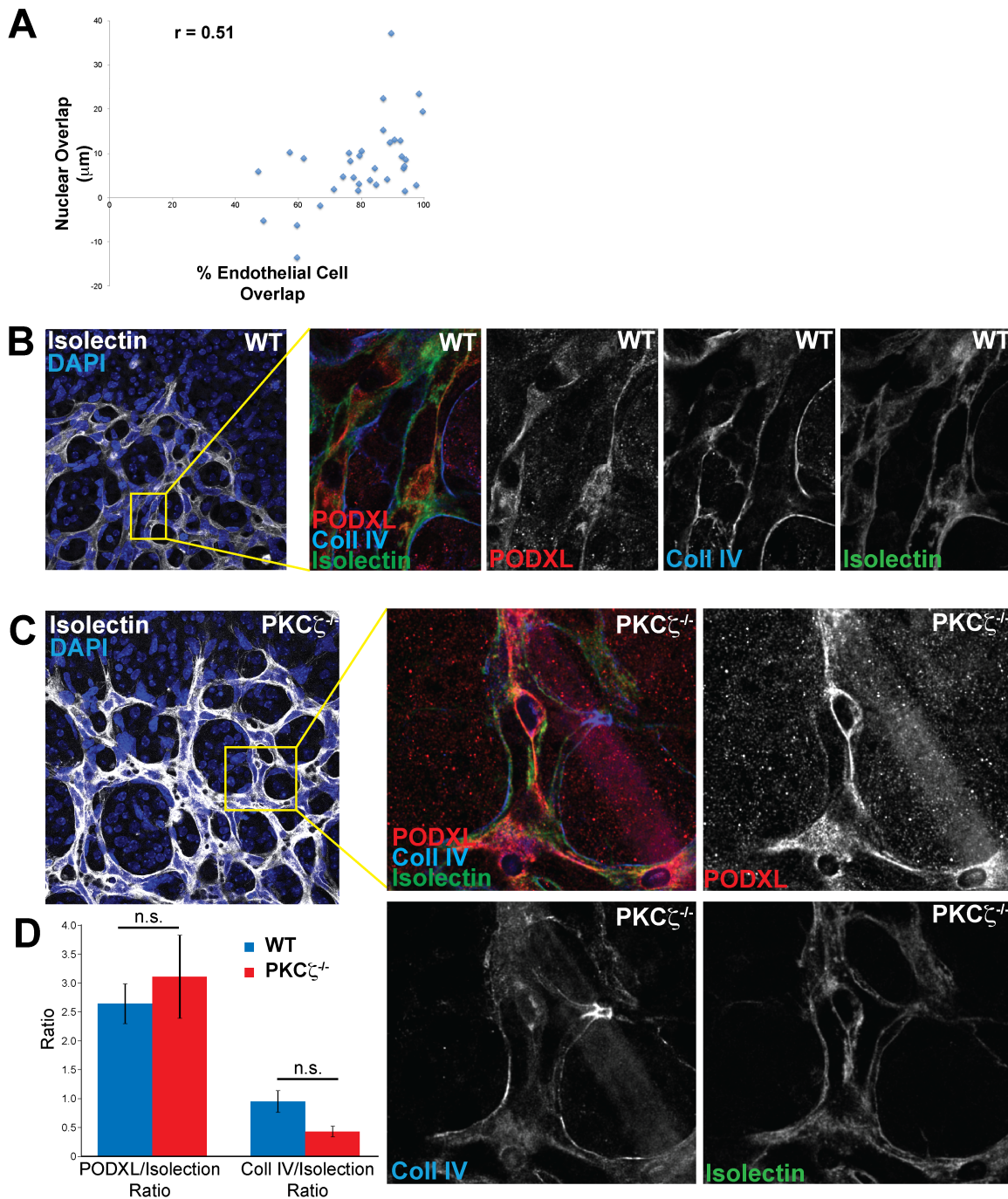


**Supplemental Figure 2. Endothelial Cell Dynamics and Notch Status at Sprout Tips. A)**

Relationship of velocities of tips of distal endothelial cells to nuclear velocities in sprouts ( $P$ = not significant). **B-C)** Graphs of two representative live-imaged sprouts, with relative velocities between two distal cells on the left and percent distal cell overlap on the right. **D)** Number of filopodia from major vs. minor tip cell in LifeAct mosaic HUVEC sprouts ( $n=117$  filopodia major;  $n=62$  filopodia minor). Statistics, Student's T-test, \*,  $p \leq 0.001$ . **E)** P6 Retinal sprout tip stained with Dll4 (red) and isolectin (white, endothelial cell marker). Right, individual channels as labeled.

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## Supp Fig 3

**Supplemental Figure 3. Overlap Correlation and Polarity of PKC $\zeta^{-/-}$  Retinal Vessels.**

A) WT P6 mouse retinas were mosaically labeled by tdTomato expression, stained with isolectin and DAPI, then processed and measured for both tip cell overlap (as shown in Fig. 1C) and nuclear overlap. The correlation suggests that, unlike nuclear switching, nuclear overlap is a surrogate for cellular overlap. ( $P \leq 0.002$ ). **B**) WT littermate control P6 retina stained for isolectin

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(white, endothelial), and DAPI (blue, nuclear). Yellow box shows area magnified on right panels, where channels for PODXL (red, apical), Collagen IV (blue, basal), and isolectin (green, endothelial) are shown. **C**) PKC $\zeta$ <sup>-/-</sup> P6 retina stained for isolectin (white, endothelial), and DAPI (blue, nuclear). Yellow box shows area magnified on right panels, where channels for PODXL (red, apical), Collagen IV (blue, basal), and isolectin (green, endothelial) are shown. **D**) Average ratio of apical to basal pixel intensity for PODXL (n=14), collagen IV (n=12) normalized to isolectin. Statistical comparisons vs. Isolectin by Student's T-test +/- s.e.m. n.s, not significant.

Supplementary Table 1. Antibodies Used in Study

<b>Antibody</b>	<b>Host</b>	<b>Species</b>	<b>Source</b>	<b>Catalog Number</b>	<b>Dilution</b>
PECAM-FITC	mouse	human	Invitrogen	MHCD3101	1:100
PODXL	goat	human	R & D Systems	AF1658	1:100
Collagen IV	rabbit	human	AbD Serotec	2150-0140	1:100
Dll4	rabbit	mouse	Abcam	#7280	1:50
$\beta$ 1 integrin	mouse	human	Abcam	ab27386	1:100
PODXL	goat	mouse	R & D Systems	AF1556	1:50
Collagen IV	rabbit	mouse	AbD Serotec	2150-1470	1:200
$\beta$ 1 integrin	rat	mouse	BD Pharmingen	#550531	1:50
546 Alexa Fluor	donkey	goat	Invitrogen	A-11056	1:200
647 Alexa Fluor	donkey	rabbit	Invitrogen	A-31573	1:200
594 Alexa Fluor	donkey	goat	Invitrogen	A-11058	1:200
546 Alexa Fluor	donkey	mouse	Invitrogen	A-10036	1:200
594 Alexa Fluor	donkey	rabbit	Invitrogen	A-21207	1:200
488 Alexa Fluor	donkey	rat	Invitrogen	A-21208	1:200