Supplemental Data

Bernier-Latmani et al., "DLL4 promotes continuous lacteal regeneration in the adult small intestine".

6 Supplemental Figures

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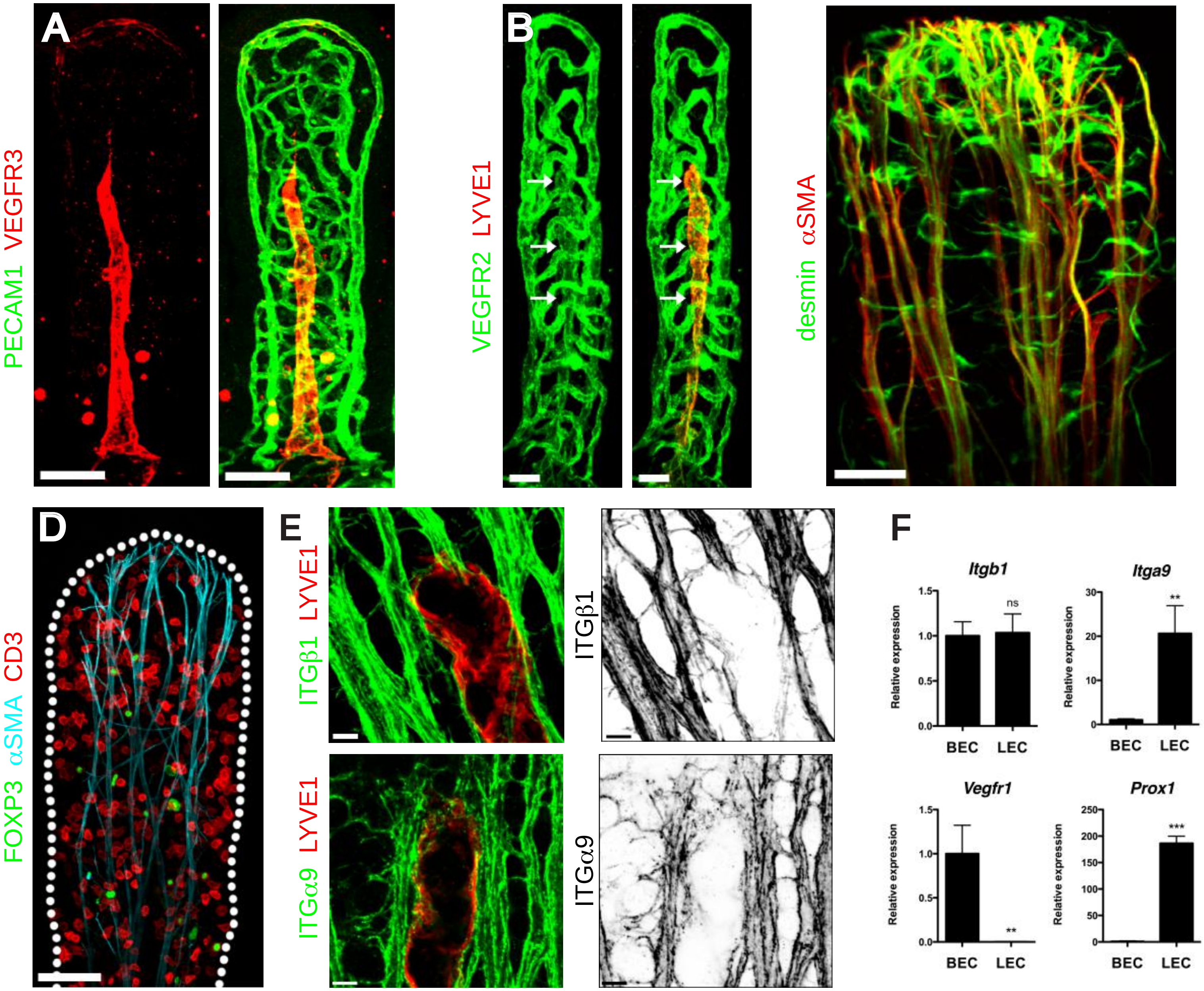
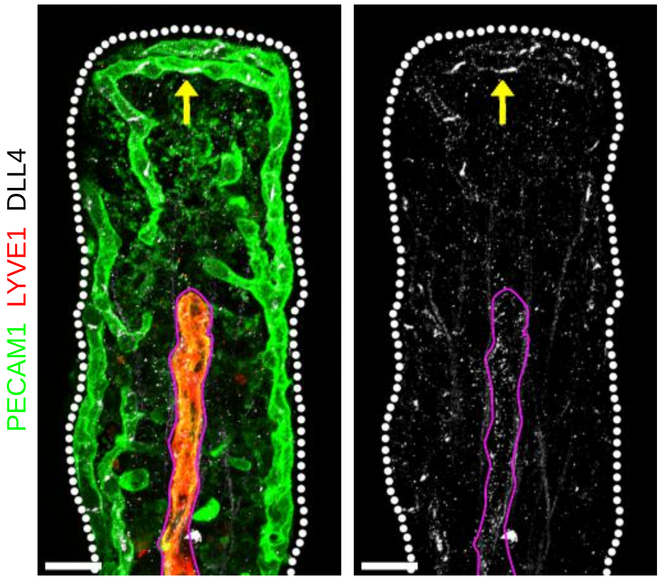
Supplemental Figure 1



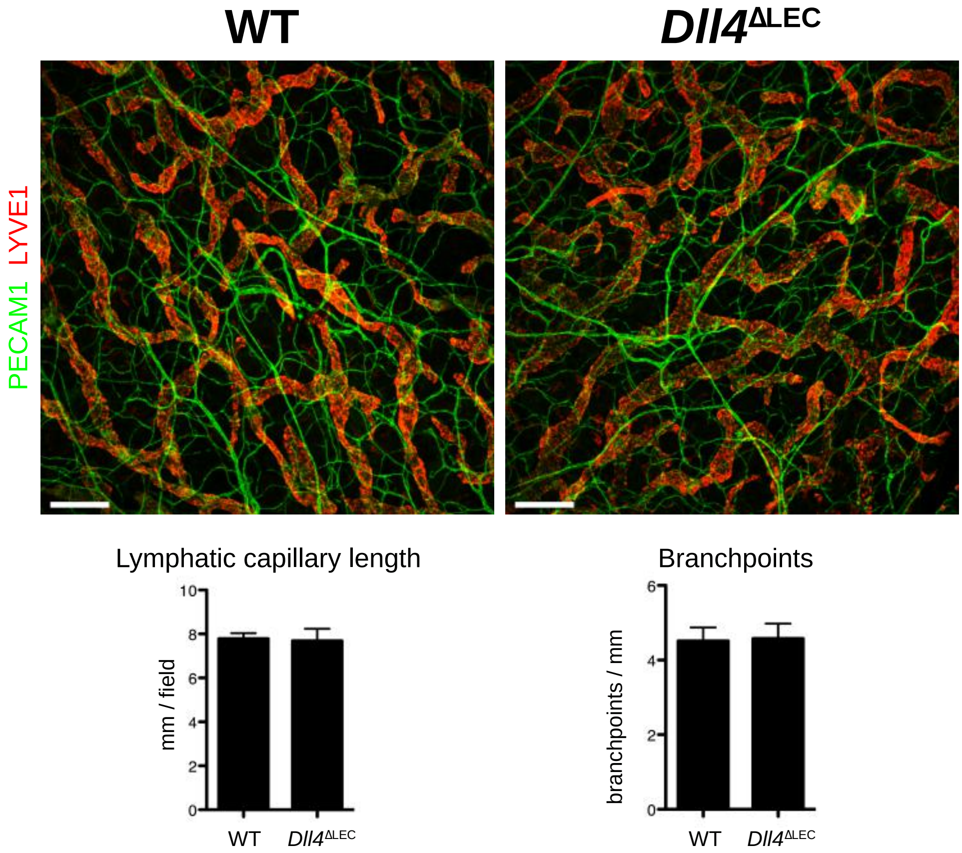
Supplemental Figure 1: Characterization of small intestinal stroma. (A) VEGFR3 (red) is expressed at high levels in adult lacteals and on blood capillaries (PECAM1, green) at the villus tip (yellow). (**B**) VEGFR2 (green) is expressed at high levels on villus blood capillaries and at lower, but detectable, levels on lacteals (arrows). **(C)** Desmin (green) is co-expressed with αSMA (red) on villus SMCs and expressed by a population of αSMA^{neg} pericyte-like cells. (**D**) FOXP3^{+} regulatory T cells (FOXP3, green nuclei; CD3, red membrane) are closely associated with villus SMCs (αSMA, cyan). (**E**) Whole-mount immunostaining for ITGβ1 and ITGα9 (green, black) and lacteals (LYVE1, red) in adult intestinal villi. (**F**) RT-qPCR analysis of *Itgb1* and *Itga9* expression in sorted intestinal LECs and BECs. Mean ± s.d. of relative expression, *n*=3. Isolation purity was confirmed by analyzing expression of BEC marker *Vegfr1* and LEC marker *Prox1*. Scale bars: 50 μm A, D; 25 μm B; 20 μm C; 5 μm F. ** $P <$ 0.01, ***$P < 0.001$, two-tailed unpaired Student's *t*-test.

Supplemental Figure 2



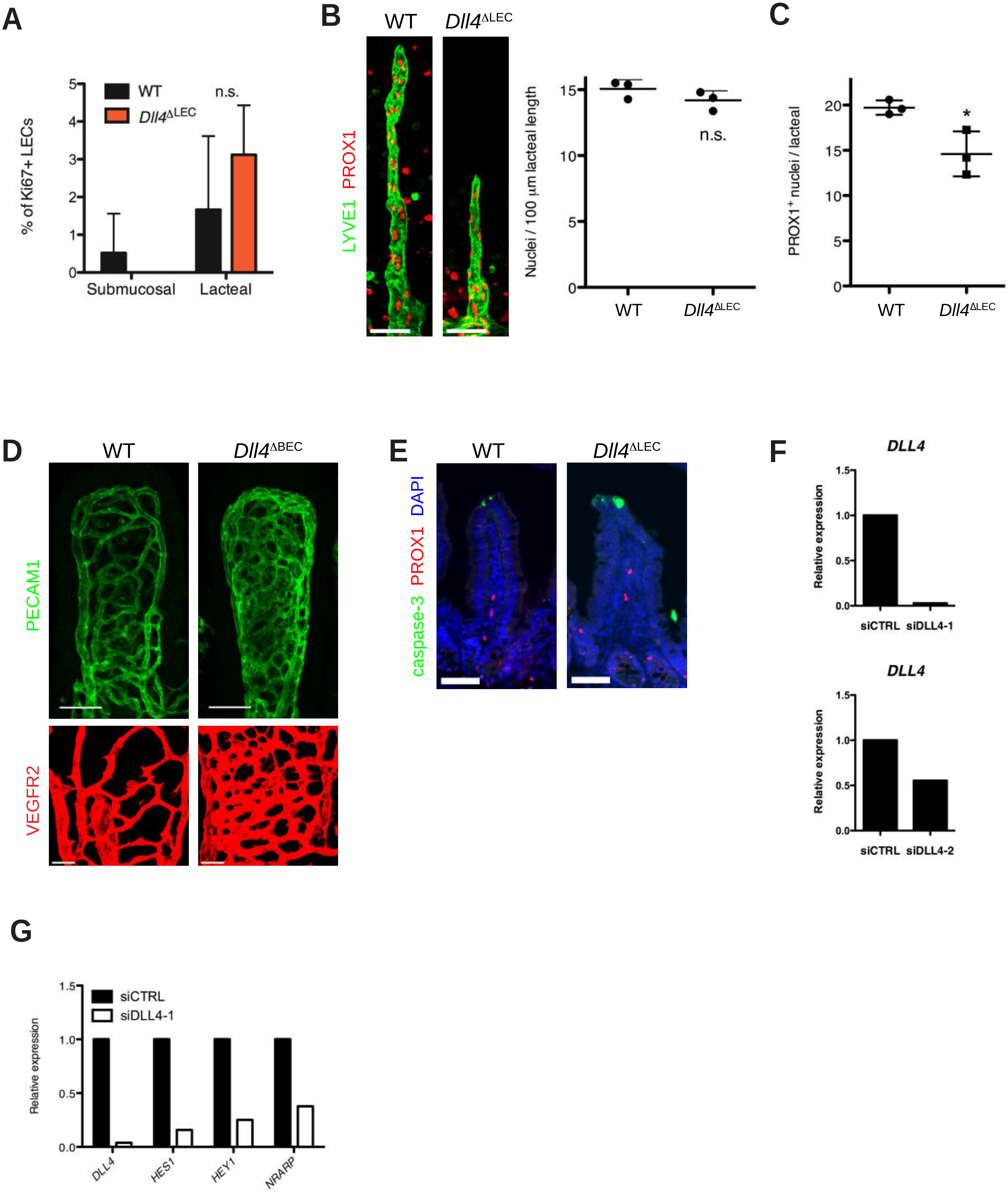
Supplemental Figure 2: DLL4 (white) displays perinuclear localization in both villus arterioles (PECAM1, green, arrow) and lacteals (pink outline). Scale bar: 20 μm.

Supplemental Figure 3



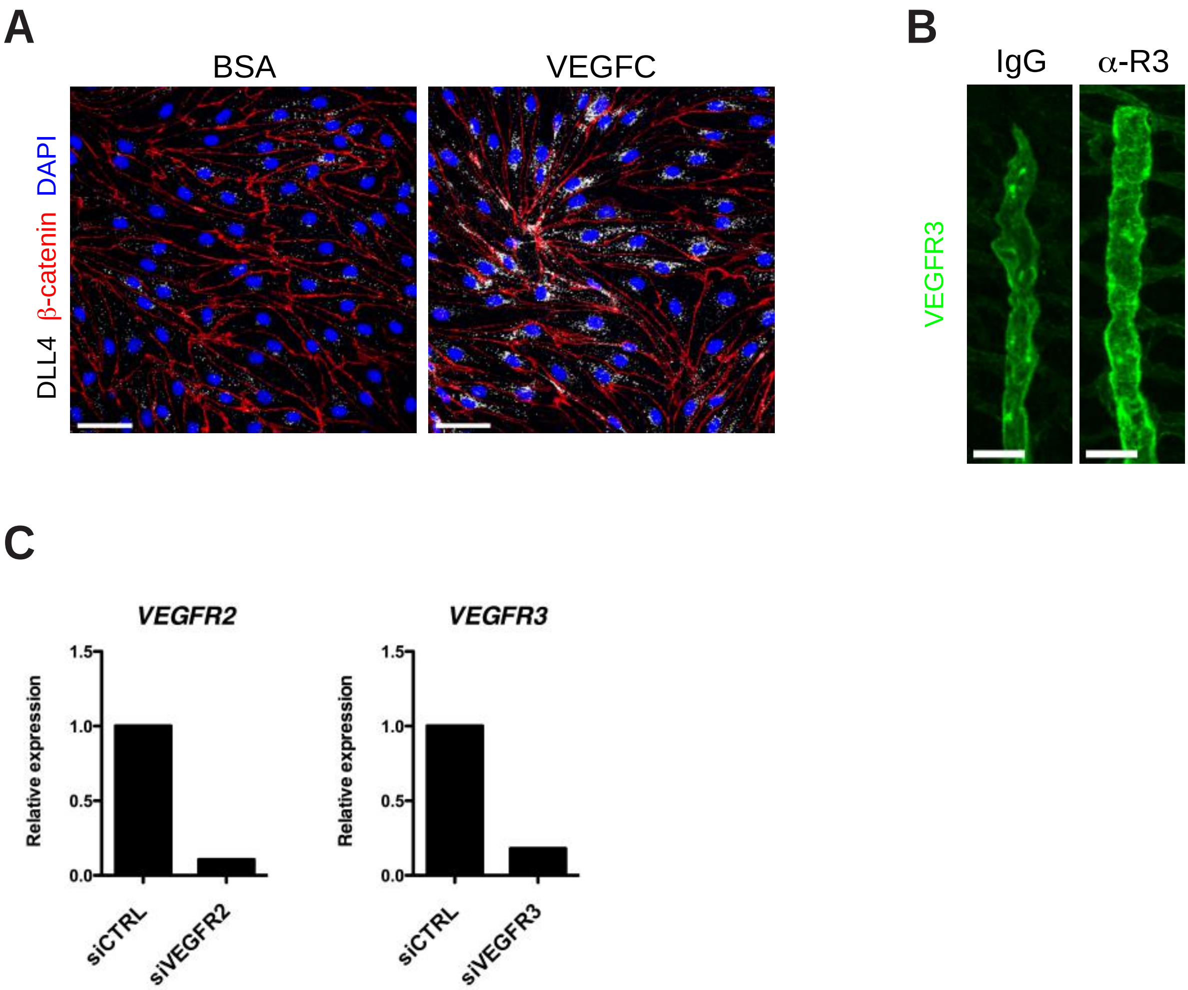
Supplemental Figure 3: Dermal lymphatic capillary (LYVE1, red) length and branching are unchanged in *Dll4*$^{\Delta LEC}$ mice (PECAM1, green) 5 weeks after tamoxifen injection. Mean ± s.d. of lymphatic capillary length and number of branchpoints in control and *Dll4*$^{\Delta LEC}$ mice, n=5. Scale bar: 200 μm.

Supplemental Figure 4



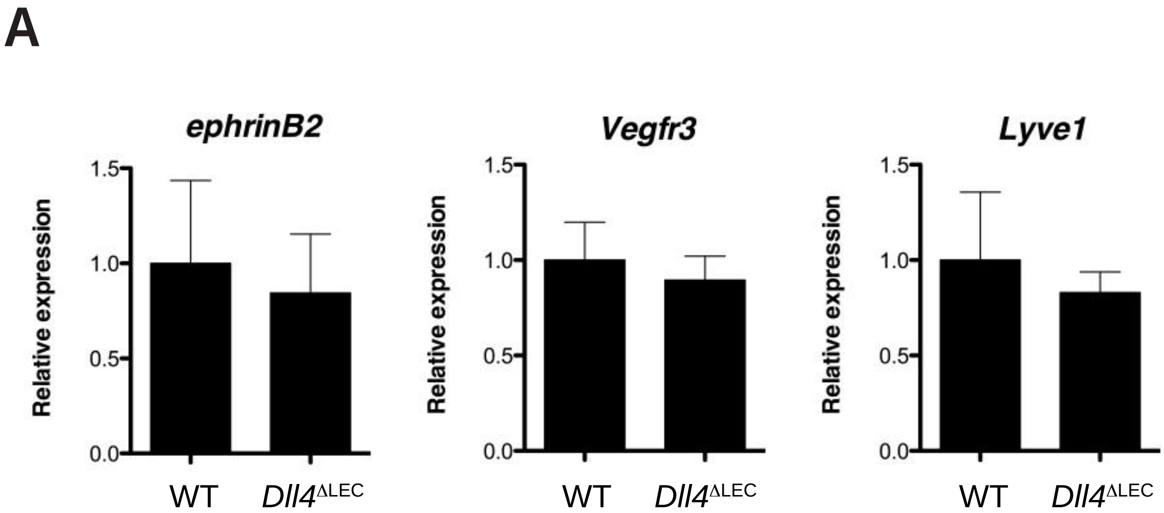
Supplemental Figure 4: Further characterization of DLL4 signaling in lymphatic and blood vessels. **(A)** Proliferation rate of intestinal LECs is comparable between *Dll4*$^{\Delta LEC}$ and control mice. Mean ± s.d. % Ki67^{+} LECs in control and *Dll4*$^{\Delta LEC}$ submucosal and lacteal lymphatic vessels, *n*=4-6. **(B)** The number of LECs (PROX1, red) per lacteal length (LYVE1, green) is similar between *Dll4*$^{\Delta LEC}$ and control mice. Mean ± s.d. of PROX1^{+} nuclei/ 100 μm lacteal length, *n*=3. **(C)** Quantification of total LEC number in *Dll4*$^{\Delta LEC}$ and control lacteals. Mean ± s.d. of PROX1^{+} nuclei/ lacteal, *n*=3. (**D**) Intestinal blood vessel *Dll4* deletion increases the number of filopodia and vessel branches. Representative whole-mount images of villus blood capillaries (top, PECAM1, green; bottom, VEGFR2, red) in control and *Dll4*$^{\Delta BEC}$ mice. **(E)** Immunostaining for PROX1 (red) and caspase-3 (green) in intestinal villi from control and *Dll4*$^{\Delta LEC}$ mice. Virtually no PROX1^{+}/ caspase-3^{+} cells were detected in mice of either genotype. (**F**) siRNA-mediated knockdown of *DLL4* in LECs 24 hours after transfection, analyzed by RT-qPCR. Mean of relative expression. Data shown are representative of two independent experiments. (**G**) LEC expression of Notch target genes *HES1*, *HEY1* and *NRARP* following transfection with either control or *DLL4*-specific siRNA, analyzed by RT-qPCR. Data shown are representative of two independent experiments. Scale bars: 50 μm B, D (top), E; 20 μm D (bottom). *$P < 0.05$, two-tailed unpaired Student’s *t*-test.

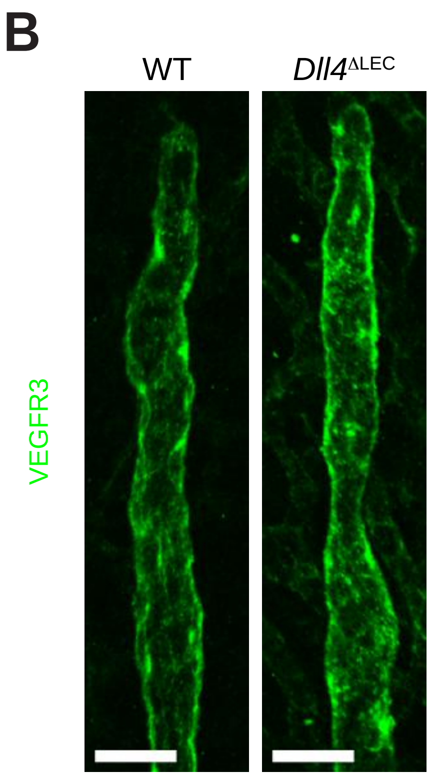
Supplemental Figure 5



Supplemental Figure 5: Regulation of DLL4 by VEGFR2 and VEGFR3 signaling. (A) VEGFC induces DLL4 in cultured LECs. Representative images of DLL4 (white) expression in cultured LECs (β -catenin, red; DAPI, blue) after 48 hours of BSA or VEGFC treatment. Data shown are representative of two independent experiments. (**B**) Increased membrane VEGFR3 (green) localization in lacteals after 7 days treatment with αVEGFR3. (**C**) Efficient siRNA-mediated knockdown of *VEGFR2* and *VEGFR3* in LECs 24 hours after transfection, analyzed by RT-qPCR. Mean of relative expression. Data shown are representative of two independent experiments. Scale bars: 50 µm A; 20 µm, B**.**

Supplemental Figure 6





Supplemental Figure 6: (A) Expression of intestinal LEC *ephrinB2*, *Vegfr-3* and *Lyve-1* are unchanged in *Dll4*$^{\Delta LEC}$ mice. Mean ± s.d. of relative expression as analyzed by qPCR. **(B)** Lacteal VEGFR3 is not decreased in *Dll4*$^{\Delta LEC}$ mice. Representative images of VEGFR3 (green) on lacteals from control and *Dll4*$^{\Delta LEC}$ mice. Scale bar: 20 μm.

Supplemental Movies 1-4: Individual LEC migration was tracked for 20 hours after a wound was made in a LEC monolayer transfected with either control or DLL4-specific siRNA and treated with BSA or VEGFC. Images were recorded every 15 min. Cell migration tracks are overlaid on movies for path visualization. Track colors are arbitrary.

Supplemental Movie 1: siCTRL+BSA

Supplemental Movie 2: siDLL4+BSA

Supplemental Movie 3: siCTRL+VEGFC

Supplemental Movie 4: siDLL4+VEGFC

SUPPLEMENTAL TABLES

Supplemental Table 1: Antibodies used in the study

	Antibody	Supplier	Catalog number/clone
Wholemount	α-SMA-Cy3 (mouse)	Sigma	C6198
	β-catenin (rabbit)	Millipore	06-734
	CD3 (hamster)	Biolegend	100301
	CD11c (hamster)	BD Pharmingen	117313
	Desmin (rabbit)	Millipore	04-585
	Dll4 (goat)	R&D	AF1389
	E-cadherin (rabbit)	Cell Signaling	3195S
	F4/80 (rat)	Invitrogen	MF48000
	Fibronectin (rabbit)	Millipore	AB2033
	Foxp3 (rat)	eBioscience	14-5773
	GFP (rat)	Biolegend	338002
	Lyve-1 (rabbit)	AngioBio	11-034
	Lyve-1 (rat)	R&D	MAB2125
	NG2 (rabbit)	Millipore	AB5320
	Pecam-1 (rat)	BD Pharmingen	557355
	Periostin (goat)	R&D	AF2955
	Prox1 (goat)	R&D	AF2727
	Tenascin C (rat)	R&D	MAB 2138
	VE-cadherin (goat)	R&D	AF1002
	Vegfr-2 (goat)	R&D	AF644
	Vegfr-3 (goat)	R&D	MAB3491
	Vegfr-3 (rat)	Eli Lilly	clone mF4-31C1
Paraffin	Ki67 (mouse)	BD Pharmingen	556003
	Prox1 (goat)	R&D	AF2727
Cell sorting	CD31-PE	eBioscience	12-0311-81
	CD45-Apc-Cy7	eBioscience	25-0451-81
	EpCAM-eFluor 450	Biolegend	48-5791-80
	gp38-AlexaFluor 647	hybridoma	clone 8.1.1
Secondary Abs	Alexa Fluor 488	Invitrogen	
	Alexa Fluor 555	Invitrogen	
	Alexa Fluor 647	Invitrogen	

Supplemental Table 2. Primers used for RT-qPCR analysis

Gene	Species	Forward primer	Reverse primer
18S	Human/ mouse	5'-AGGAATTCCCAGTAAGTGCG	5'-GCCTCACTAAACCATCCAA
Dll4	mouse	5'-GGAACCTTCTCACTCAACATCC	5'-CTCGTCTGTTCGCCAAATCT
ephrinB2	mouse	5'-ATTATTTGCCCCAAAGTGGACTC	5'-GCAGCGGGGTATTCTCCTTC
Itgα9	mouse	5’-TGCTTTCCAGTGTTGACGAGA	5’-TTAAAGGACACGTTGGCATCATA
Itgβ1	mouse	5’-AATGCCAAATCTTGCGGAGAA	5’-TCTAAATCATCACATCGTGCAGAAGTA
Lyve-1	mouse	5'-TGGTGTTACTCCTCGCCTCT	5'-TTCTGCGCTGACTCTACCTG
Prox1	mouse	5'-AAGATATGTCCGACATCTCACCTTATTCAG	5'-CACGTCCGAGAAGTAGGTCTTCAG
Vegfr-1	mouse	5'-TGGCTCTACGACCTTAGACTG	5'-CAGGTTTGACTTGTCTGAGGTT
Vegfr-3	mouse	5'-CTGGCCAGAGGCACTAAGAC	5'-CAGGGTGTCCTCTGGGAATA
DLL4	human	5’-TGGGTCAGAACTGGTTATTGGA	5’-GTCATTGCGCTTCTTGCACAG
HES1	human	5’-AAGAAAGATAGCTCGCGGCAT	5’-CCAGCACACTTGGGTCTGT
HEY1	human	5’-AAGAAAGATAGCTCGCGGCAT	5’-CGTCAAAGTAACCTTTCCCTCCT
NRARP	human	5’-TCAACGTGAACTCGTTCGGG	5’-ACTTCGCCTTGGTGATGAGAT
VEGFR-2	human	5’-GGAACCTCACTATCCGCAGAGT	5’-CCAAGTTCGTCTTTTCCTGGGC
VEGFR-3	human	5’-GCACTGCCACAAGAAGTACCT	5’-GCTGCACAGATAGCGTCCC

Supplemental Table 3. siRNA used for in vitro mRNA silencing

Target Gene	Supplier	Reference number
siAllstar (siControl)	Qiagen	1027281
siDLL4	Life Technologies	132858
siDLL4	Life Technologies	132859
siDLL4 (pool)	Dharmacon	L-010490-00-0005
siVEGFR-2	Thermo Scientific	L-003148-00
siVEGFR-3	Thermo Scientific	L-003138-00