

Online Figure I: PlexinA receptor expression in mouse mesenteries.

In situ hybridization with antisense riboprobes against the indicated PlexinA-type receptors. Only *Plexna1* hybridizes with mesenteric tissue, and its expression is restricted to valves (arrows, bottom row). Scale bars: 100um (A-D), 20um (E-H).

Online Figure II: Normal lymphatic valve development in *Nrp2*^{-/-} mice.

Staining of wild type (A) and *Nrp2*^{-/-} (B) mesenteries with the indicated markers. Anti-Foxc2 (A, B, green) and anti-Itga9 (A', B', red) staining shows normal valves (arrows) in mesentery lymphatics of wild type and *Nrp2*^{-/-} littermates. (C) No difference was observed in Itga9-positive valve area between wild type and *Nrp2*^{-/-} mice. Quantification was performed using ImageJ (Mann-Whitney U-test $p=0.85$). Scale bars: 90 μ m.

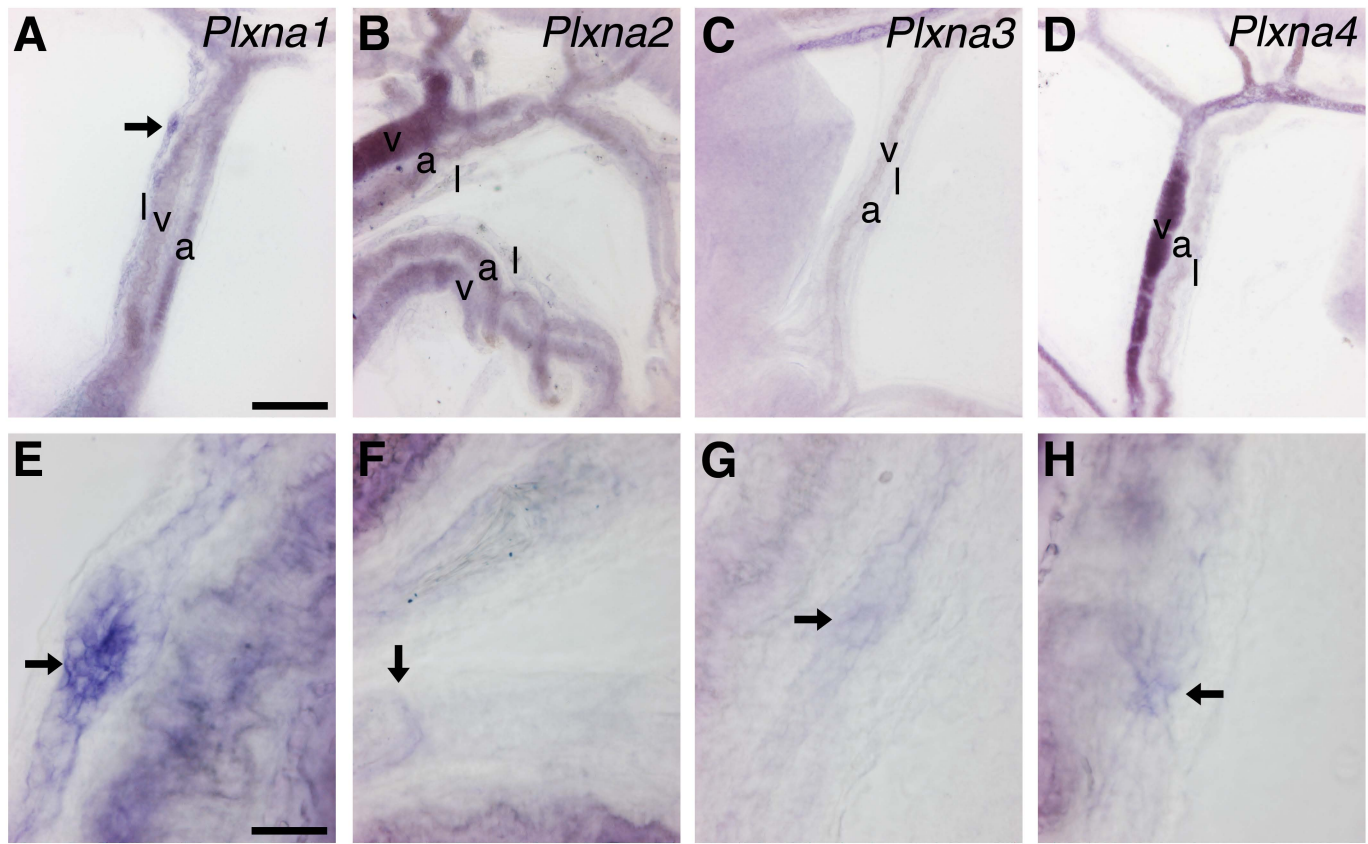
Online Figure III: Normal vascular development in *Sema3a*^{-/-} mice.

(A, B) Confocal images of whole-mount skin from neonatal wild type (A) or *Sema3a*^{-/-} (B) mice stained with anti-CD31. Note the normal development of the vascular plexus in the *Sema3a*^{-/-} mice. Scale bars: 112um.

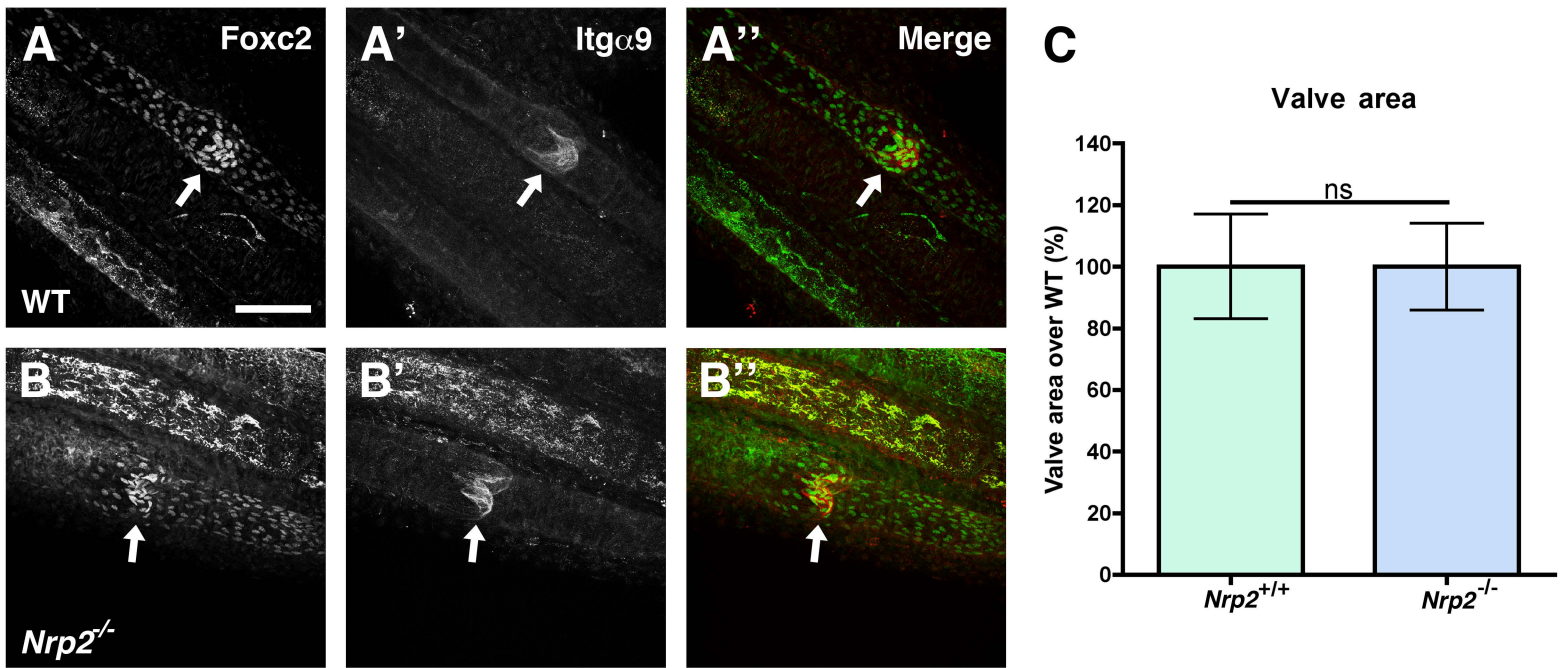
Online Figure IV: Abnormal collecting lymphatics in *Sema3a*^{-/-} mice.

(A-B) Collecting lymphatic vessels in wild type (A) and *Sema3a*^{-/-} (B) mice after FITC-dextran or rhodamine dextran perfusion in the anterior footpad at P6. (C) Quantification of the perfused length of lymphatics in wild type and *Sema3a*^{-/-} mice. $n=10$ mice/genotype, 1 image per footpad. Quantification was performed using ImageJ (Mann-Whitney U-test $p<0.0001$). (D,F) Rhodamine dextran or (E,G) YFP expression on *Vegfr3::YFP,WT* (D,E) or *Vegfr3::YFP,Sema3a*^{-/-} mice (F,G). Small lymphatic valves located at branchpoints in the mutant collecting lymphatics are insufficient to prevent backflow of the lymph into the side branch (F,G orange arrows) compared to non-perfused side branches in wild type (D,E, black arrows), suggesting that defective valve formation in *Sema3a* mutant mice may cause aberrant lymph drainage. Red arrows indicate direction of lymph flow. Boxed areas in A,B are shown at higher magnification in D-G. Scale bars: 715um (A,B), 375um (F-G).

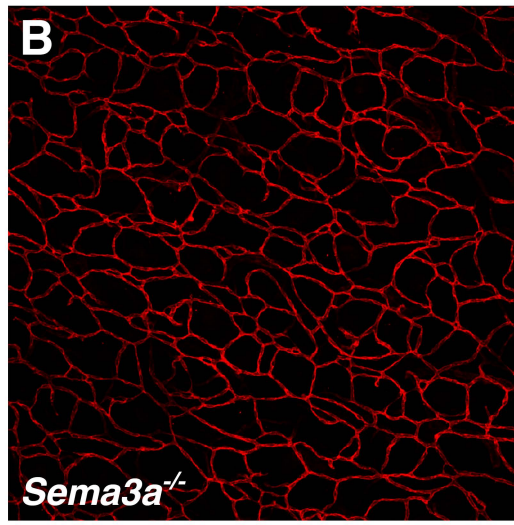
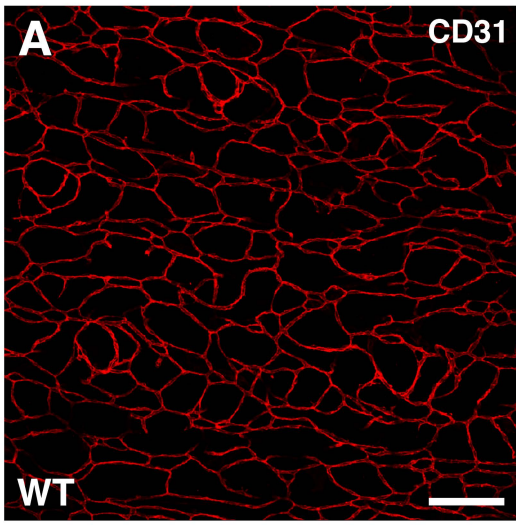
Supplementary Material



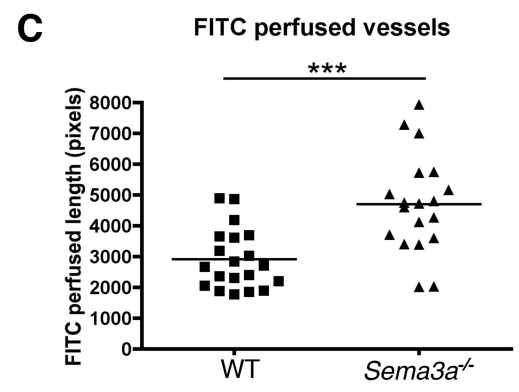
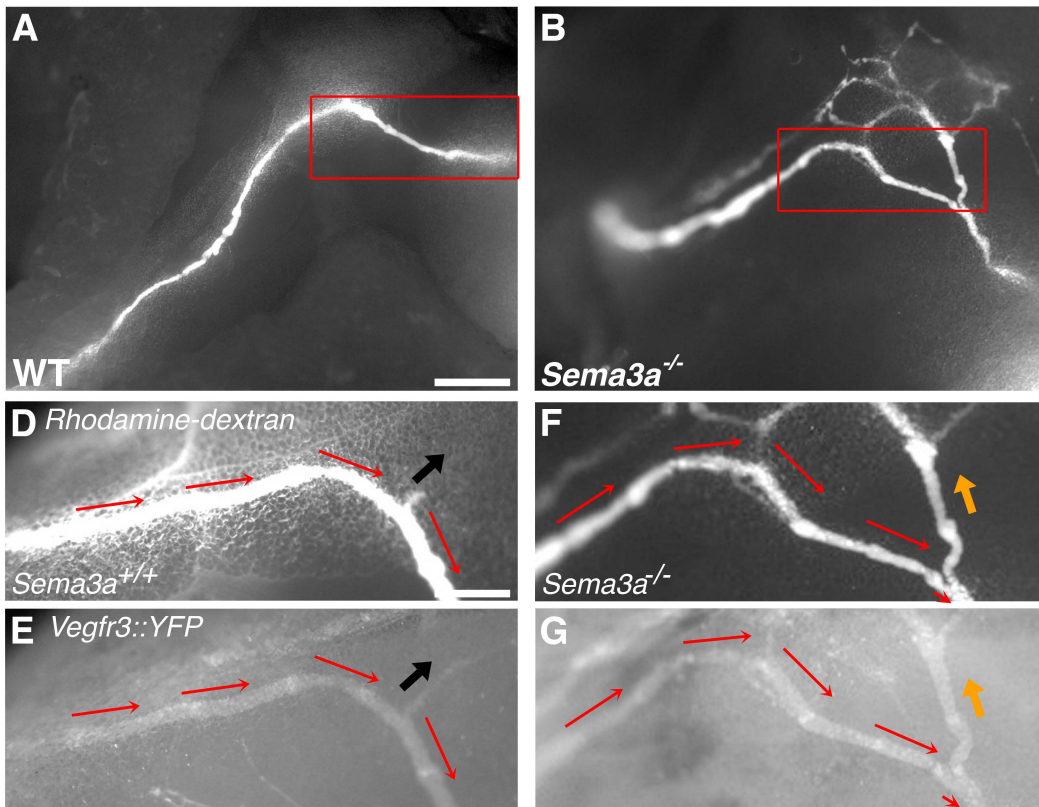
Online Figure I



Online Figure II



Online Figure III



Online Figure IV