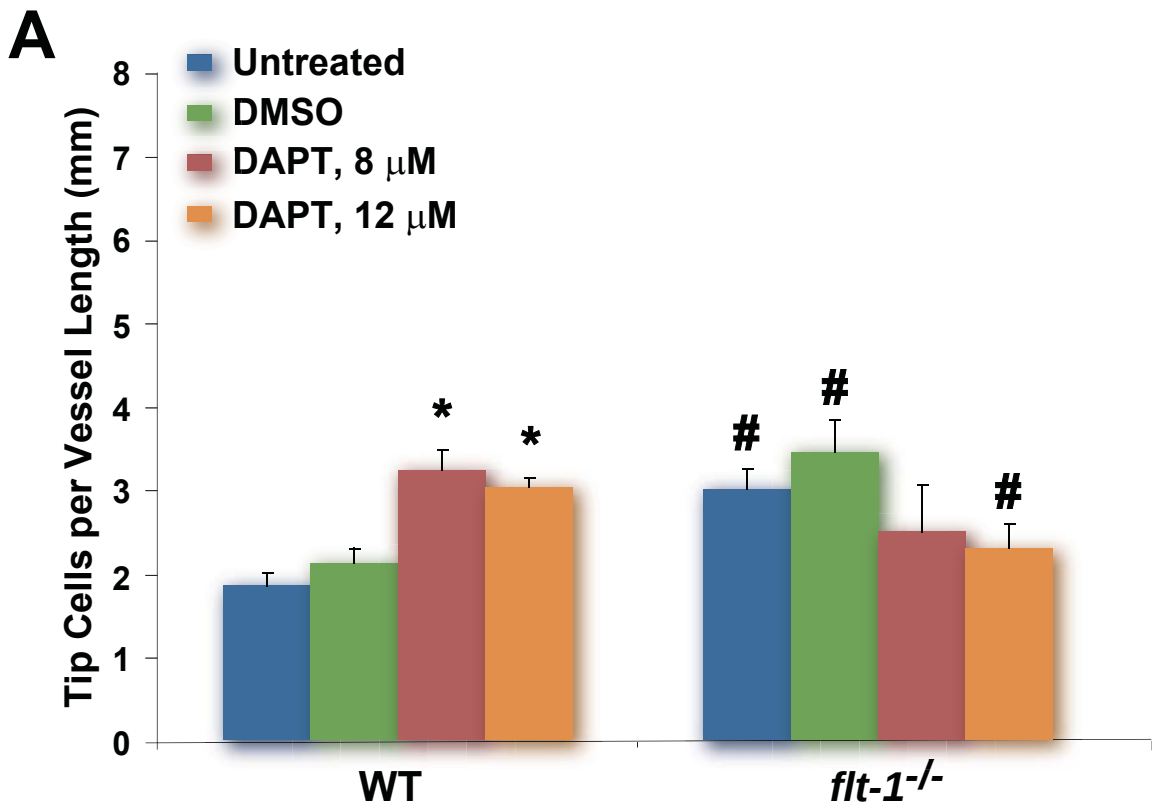
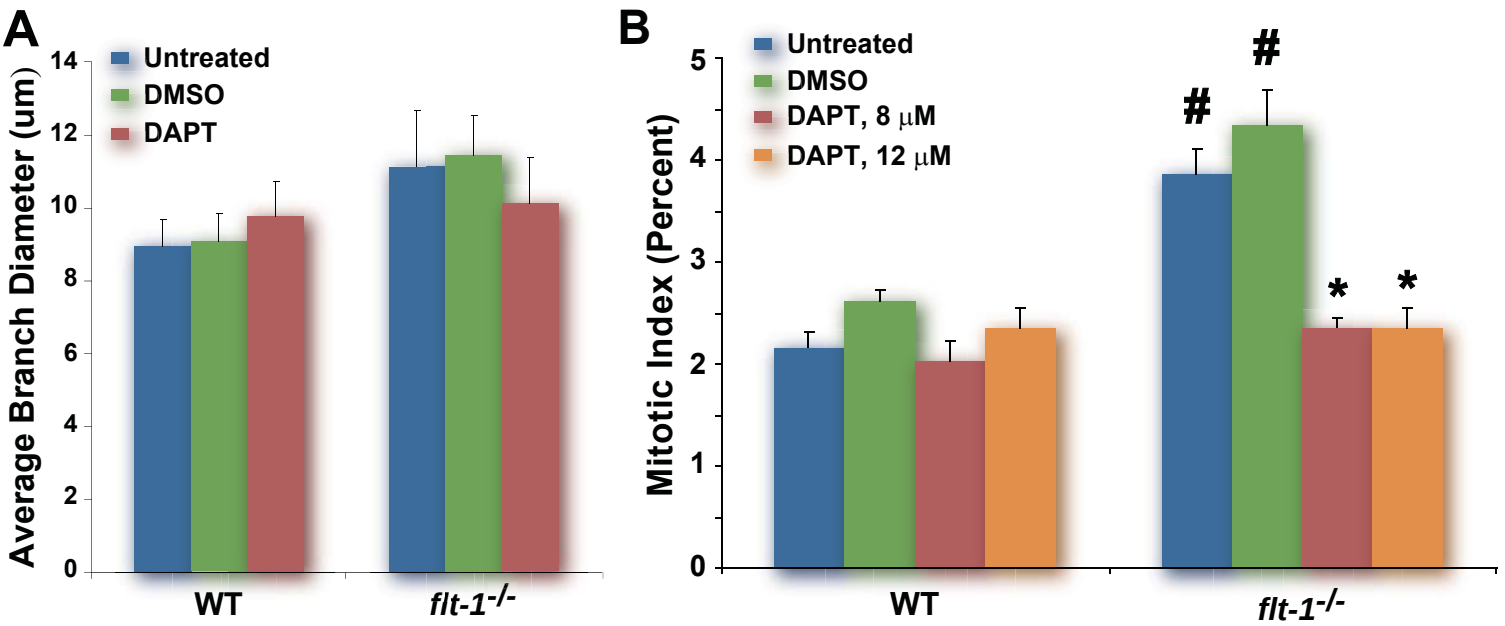


Supplemental Figure I. DAPT treatment induces more tip cells from WT vessels but not *flt-1*$^{-/-}$ vessels, and an increased DAPT concentration does not alter these responses. Dy 7 tip cells per vessel length (A). *, p≤0.004 vs. WT/untreated or WT/DMSO. #, p≤0.03 vs. WT of same treatment group. Values are averages +/- standard error of the mean (SEM).



Supplemental Figure II. Vessel diameter is unaffected by Notch inhibition, and endothelial cell proliferation is unaffected by an increased dose of DAPT. Dy 8 vessel networks assessed for average vessel branch diameter (A). Dy 7 vessel mitotic indicies were quantified by counting PH3+/PECAM-1+ cells and normalizing to total PECAM-1+ cells (B). #, $p \leq 0.04$ vs. WT of same treatment group. *, $p \leq 0.05$ vs. *flt-1*$^{-/-}$/untreated or *flt-1*$^{-/-}$/DMSO. Values are averages +/- standard error of the mean (SEM).

Data Supplement

Methods

ES Cell Culture and In Vitro Differentiation
Wild-type (WT) and *flt-1*$^{-/-}$ (gift of G.H. Fong) mouse ES cell maintenance and differentiation was as described.[1] The γ-secretase inhibitor IX, DAPT (N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-S-phenylglycine t-Butyl Ester, Santa Cruz Biotechnology, Inc.), was resuspended in DMSO (Sigma), and added to ES cell cultures at 8 and 12 μM on dy 5 and 7.

ES Cell Antibody Staining and Quantitative Analysis
Fixation and staining of dy 7 and 8 ES cell cultures was as described.[2] Primary antibodies used were: rat anti-mouse PECAM-1 (BD Biosciences) at 1:1000, and rabbit polyclonal anti-phospho-Histone H3 (ser10, EMD Millipore) at 1:500. Secondary antibodies were donkey anti-rat AlexaFluor488 (IgG; H+L) at 1:1000 (Invitrogen), and donkey anti-rabbit AlexaFluor568 (IgG; H+L) at 1:500 (Invitrogen). Incubation with DAPI for 30 min followed all staining.

ES cell cultures were imaged with a confocal microscope (Leica TCS SP5) using a ×40 objective (HCX PL APO oil), and 6-10 z-axis confocal scans were acquired and flattened when necessary. Vessel images were traced and processed using ImageJ plugins (BoneJ/Skeletonise 3D, Skeleton/AnalyzeSkeleton (2D/3D)) to generate vessel length values. A tip cell was defined as a cell oriented away from a parent vessel, extending filopodia but not obviously engaged with a target cell, and emerging from a parent vessel with its nucleus clearly beyond the axis of the parent vessel. For dy 7 cultures from each treatment group/dose and cell type, tip cells were counted and normalized to vessel length. For these same dy 7 cultures, ImageJ was used to count phospho-Histone H3+ (PH3+) mitotic endothelial cells and total endothelial cells, to yield a mitotic index (PH3+ and PECAM+ cells per total PECAM+ cells) for each image. Vessel diameter for dy 8 cultures was also measured using ImageJ by determining the midpoint of a vessel between two branch points and measuring the vessel width perpendicular to the vessel axis.

Statistics
Statistical comparison of ES cell-derived vessel diameter and tip cell numbers was done using the Student's two-tailed t-test. Mitotic index values were compared using χ^2 analysis.

References

1. Kearney JB, Bautch VL. In vitro differentiation of mouse ES cells: hematopoietic and vascular development. *Methods Enzymol.* 2003;365:83-98.

2. Kappas NC, Zeng G, Chappell JC, Kearney JB, Hazarika S, Kallianos KG, Patterson C, Annex BH, Bautch VL. The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching. *J Cell Biol.* 2008;181:847-858.

Supplemental Figure Legends

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