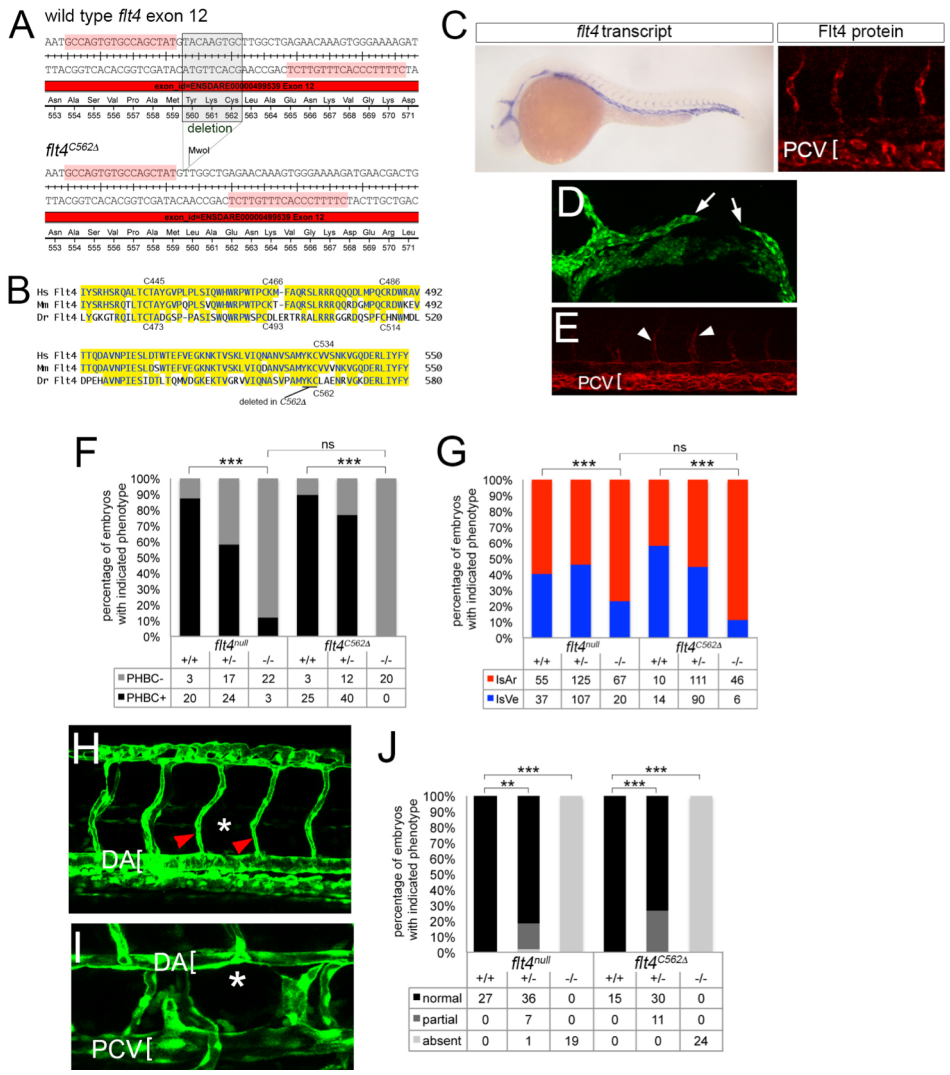

Figure S1. Generation and characterization of *flt4*$^{C562\Delta}$. (A) TALEN target sequences (highlighted in pink) in *flt4* exon 12 flanking Cysteine 562. Boxed sequence indicates amino acids deleted in *flt4*$^{C562\Delta}$ mutants. The nine nucleotide deletion creates an MwoI site used for genotyping. (B) Alignment of region of Flt4 extracellular domains from human (Hs), mouse (Mm), and zebrafish (Dr) containing cysteines implicated in disulfide linkage between cleaved extracellular domain and remaining Flt4 protein. Position of amino acids deleted in *flt4*$^{C562\Delta}$ is indicated. (C) *Left*, whole mount *in situ* hybridization using a digoxigenin labeled antisense *flt4* riboprobe on *flt4*$^{C562\Delta}$ mutant embryos at 25 hpf. *Right*, whole mount immunostaining using a polyclonal antibody against zebrafish Flt4 at 30 hpf; *flt4* transcript and Flt4 protein are detectable in *flt4*$^{C562\Delta}$ mutant embryos. (D) Confocal image of *flt4*$^{C562\Delta}$ mutant *Tg(fli1a:egfp)*^{y1} embryo at 26 hpf; incompletely formed primordial hindbrain channel is indicated by white arrows. (E) Tie2 immunostained *flt4*$^{C562\Delta}$ mutant embryo showing ISVe loss. White arrowheads denote weak staining in ISAs (F) Percentage of embryos with delayed PHBC formation of indicated genotype. (G) Percentage of ISA and ISVe connections in embryos of indicated genotype at 72 hpf. (F, G) Data from *flt4*^{null} are same as those shown in main figures. (H) *Tg(fli1a:egfp)*^{y1} embryo mutant for *flt4*$^{C562\Delta}$ at 48 hpf showing lack of parachordal cells (PACs; normal position indicated by asterisk); ISAs indicated by red arrowheads, dorsal aorta denoted by bracket. (I) Loss of thoracic duct in *Tg(fli1a:egfp)*^{y1} embryo mutant for *flt4*$^{C562\Delta}$ at 5 dpf. Normal position of thoracic duct denoted by asterisk; dorsal aorta (DA) and posterior cardinal vein (PCV) are indicated by brackets. (J) Percentage of embryos of indicated genotype with normal, partial, or absent thoracic duct at 5 dpf. Data from *flt4*^{null} are the same as those shown in Figure 2.

Development • Supplementary information

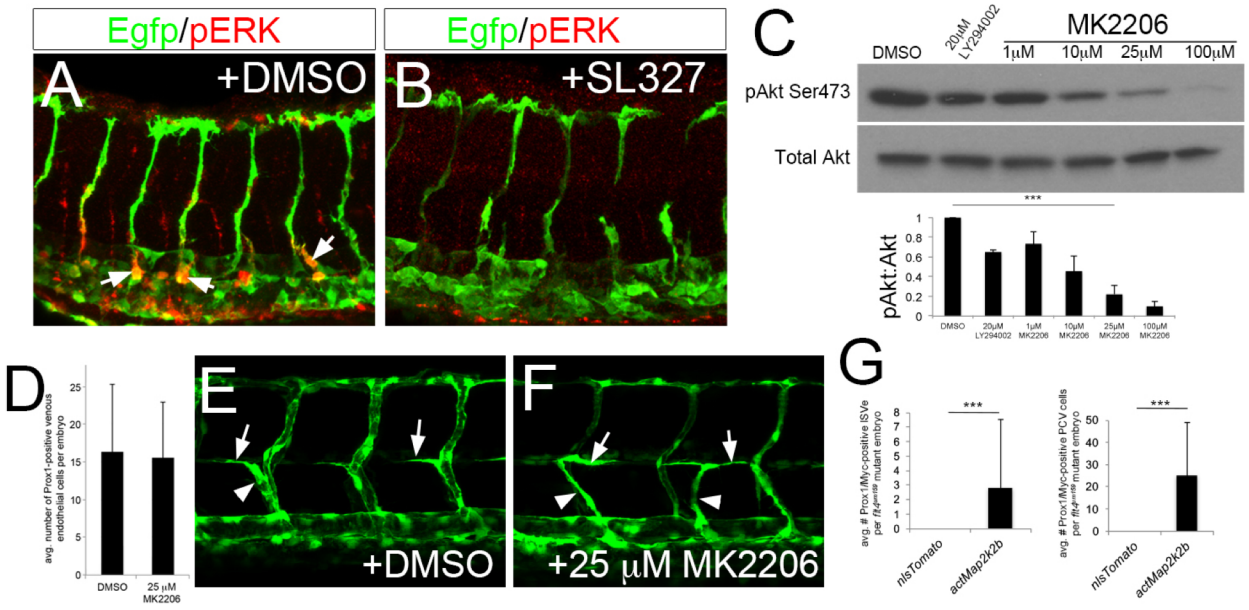

Figure S2. **Akt is dispensable for early lymphatic development.** (A, B) Wild type *Tg(fli1a:egfp)*^{y1} at 36 hpf immuonstained for pERK (red) and EGFP (Green). Embryos were treated with (A) DMSO or (B) 15 μM SL327 starting at 28 hpf. (C) pAkt and total Akt levels in whole embryo lysates treated with indicate compound at indicated concentration. Graph shows quantification of Akt phosphorylation intensity from Western normalized to total Akt levels. ***p<0.001, error bars±S. D. 25 μM MK2206 significantly reduce pAkt levels by 80% and was used for subsequent studies. (D) Quantification of Prox1-positive endothelial cells in embryos treated with DMSO or 25 μM MK2206 between 28 and 38hpf. (E, F) Confocal microscopy of live *Tg(fli1a:egfp)*^{y1} at 49 hpf treated with (E) DMSO or (F) 25 μM MK2206 from 20 hpf. Parachordal cells (arrows) and lymphatic sprouts (arrowheads) are apparent in both DMSO and MK2206-treated embryos. (G) Quantification of lymphatic sprouts and Prox1/Myc-positive posterior cardinal vein (PCV) endothelial cells in *flt4*$^{C562\Delta}$ mutant embryos injected with indicated transgene.

Development • Supplementary information

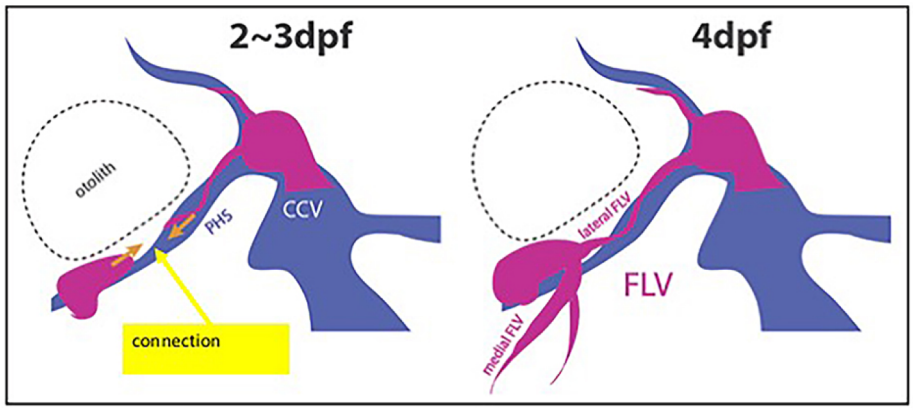

Figure S3. Schematic of facial lymphatic development at 2 and 4 dpf.

Development • Supplementary information

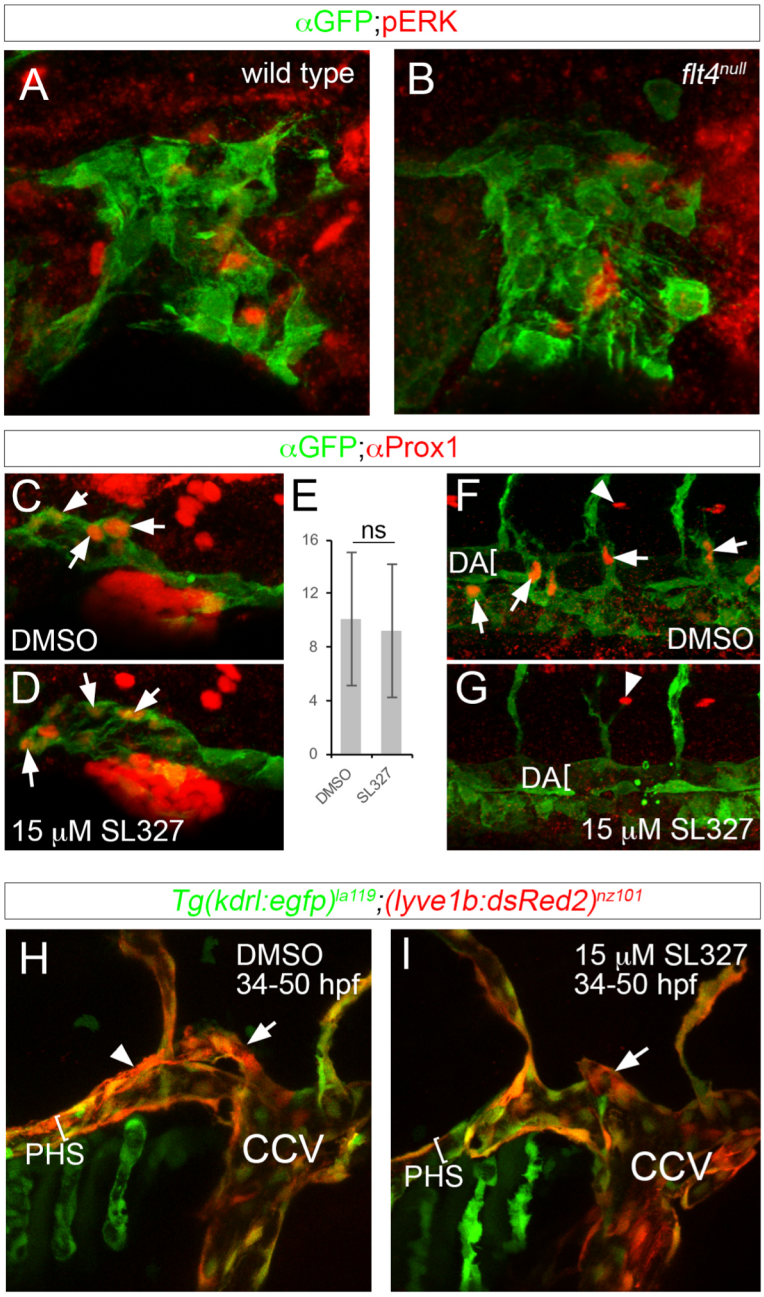

Figure S4. Flt4 and ERK are dispensable for initial facial lymphatic progenitor specification and sprouting. (A-D, F-I) Confocal images, lateral views, dorsal is up, anterior to the left. (A, B) *Tg(fli1a:egfp)*^{y1} of the indicated genotypes immunostained with anti-GFP and anti-pERK antibodies at 36hpf (C, D, F, G) *Tg(fli1a:egfp)*^{y1} immunostained with anti-GFP and anti-Prox1 antibodies at 36hpf. (C, F) Embryo treated with DMSO; images from same embryo. (F, G) Embryo treated with 15μM SL327starting at 28hpf; images from same embryo. (E) Average number of Prox1-positive CCV endothelial cells per embryo at 36hpf; ns – not statistically significant, error bars are ± S.D. (H, I) Live *Tg(kdr:egfp)*^{la119}*;(lyve1b:DsRed2)*^{nz101} at 50hpf treated with (H) DMSO or (I) 15μM SL327 starting at 28hpf. Lumen of primary head sinus denoted by a bracket. Position of facial lymphatic vessel derived from PHS is indicated by arrowhead; brach derived from common cardinal vein (CCV) is denoted by an arrow.

Development • Supplementary information