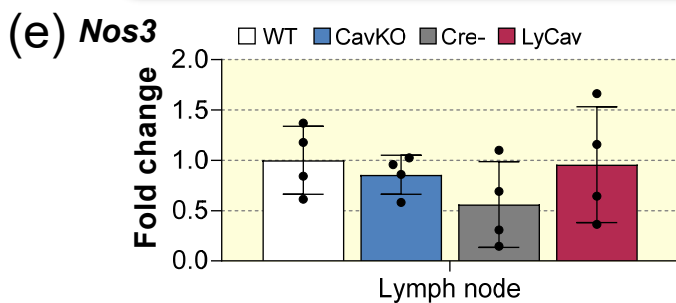
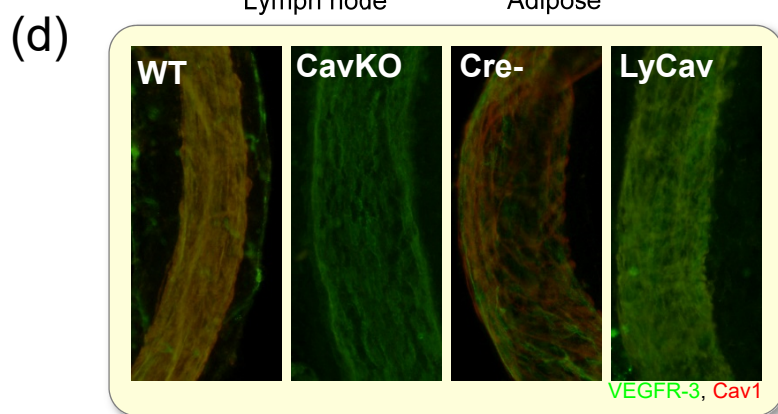
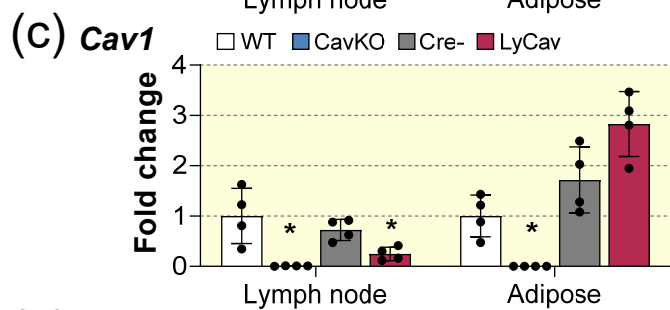
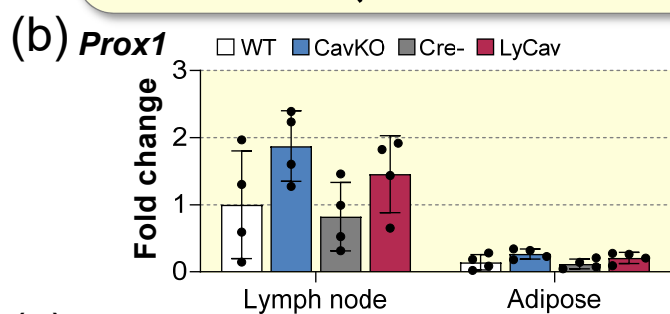
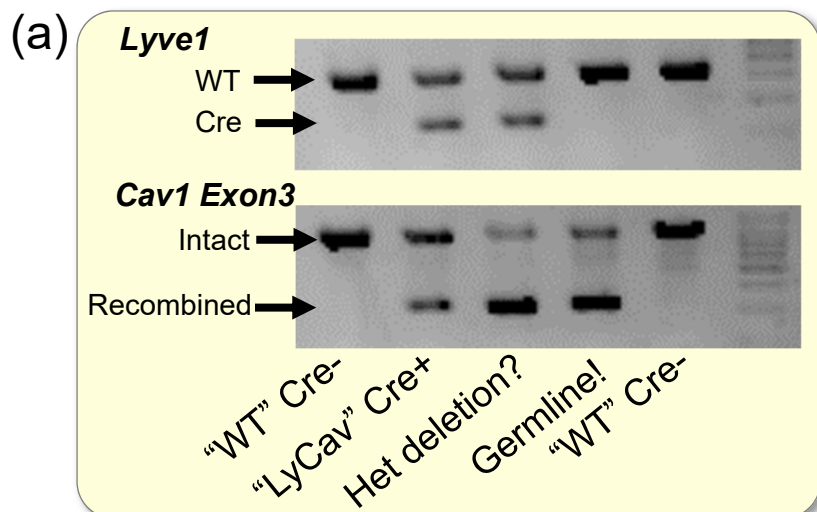


Supplementary Figure 1



Supplementary Figure Legend

Supplementary Figure 1. Validation of the mouse lines used in this study. **(a)** Genotyping results for Lyve1-Cre and Cav1^{flox/flox} genetics. For Lyve1, the lower band indicates the presence of the Cre insertion. All mice are used as Cav1^{flox/flox}, but the integrity of the “floxed” locus in exon 3 is verified. The upper band is the intact locus. The lower band indicates recombination as the “floxed” region is deleted, reducing the product size. WT mice are used as Cre⁻. A small amount of recombination is noted in LyCav mice that are Cre⁺ owing to the lymphatics in the skin. Some mice exhibit a potential heterozygous deletion of Cav1 systemically with strong recombination noted and the presence of an intact band in “het deletion” mice; these mice are not used. Germline deletion is seen in some mice lacking Cre; these mice are not used. **(b)** Relative *Prox1* RNA expression in isolated lymph nodes and adipose tissue of the mouse lines used. **(c)** Relative *Cav1* RNA expression in the isolated lymph nodes and adipose tissue of the mouse lines used. **(d)** VEGFR-3 (green) and Cav1 (red) immunofluorescence of on isolated lymphatic collecting vessels from the mouse lines used. **(e)** Relative endothelial nitric oxide synthase (*Nos3*) RNA expression in isolated lymph nodes of the mouse lines used. * $p < 0.05$ compared to respective control strain.

Supplementary Video Legends

Supp Video 1: Isolated, cannulated flank lymphatic vessel from a WT mouse. Video was taken for 5 minutes in drug-free buffer when 2 cmH₂O intraluminal pressure was applied.

Supp Video 2: Isolated, cannulated flank lymphatic vessel from a CavKO mouse. Video was taken for 5 minutes in drug-free buffer when 2 cmH₂O intraluminal pressure was applied.

Supp Video 3: Isolated, cannulated flank lymphatic vessel of a Cre⁺ mouse from the LyCav lineage. Video was taken for 5 minutes in drug-free buffer when 4 cmH₂O intraluminal pressure was applied.

Supp Video 4: Isolated, cannulated flank lymphatic vessel of a Cre⁺ mouse from the LyCav lineage. Video was taken for 5 minutes in buffer containing 100 μ M L-NAME when 4 cmH₂O intraluminal pressure was applied.

Supplementary Methods

Tissue RNA and qPCR

The inguinal lymph nodes and surround subcutaneous adipose tissue were isolated and separated. RNA was extracted with the Zymo Direct-zol RNA Miniprep Plus kit (Zymo Research, Irvine, CA). Reverse transcription of 1 µg RNA was performed with the iScript cDNA Synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA). qPCR was performed on an Applied Biosystems 7900T with *Ubc* used as the house keeping gene. Results are expressed as the $2^{\Delta\Delta CT}$ compared with wildtype mice. Primers used include *Ubc* (5'-GCCCAGTGTTACCACCAAGAAG-3' and 5'-GCTCTTTTGTAGATACTGTGGTGAGGAA-3'), *Prox1* (5'-AGAAGGGTTGACATTGGAGTGA-3' and 5'-TGCGTGTTGCACCACAGAATA-3'), *Cav1* (5'-CCAAGCATCTCAACGACGAC-3' and 5'-CTTCCAGATGCCGTCGAAAC-3'), and eNOS (*Nos3*) (5'-TTCCTGGACATCACTTCCCC -3' and 5'-CTTCCATTCTTCGTAGCGCC-3').