Supplementary Materials for

Soluble Vascular Endothelial Growth Factor Receptor-3 (sVEGFR-3) is Essential for Corneal Alymphaticity

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Figs. S1 to S4

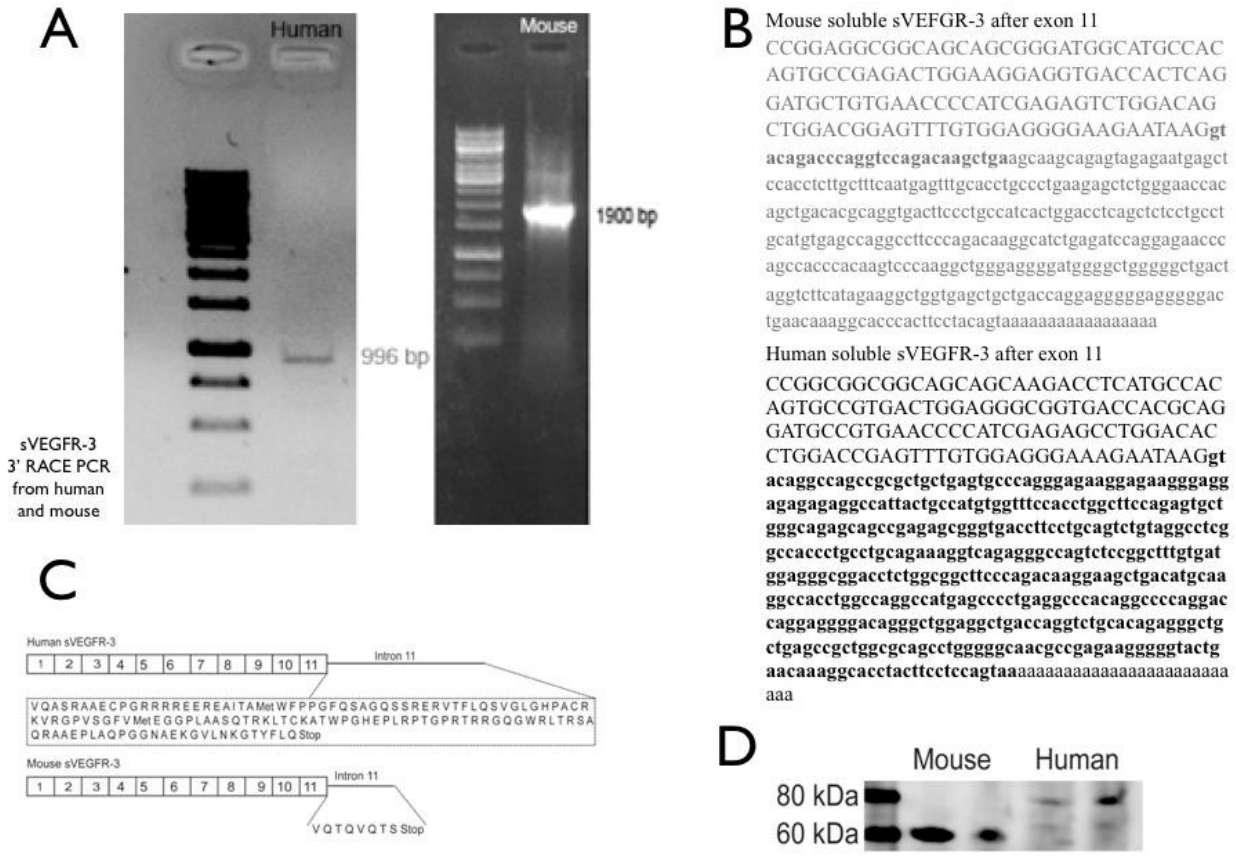

Fig. S1. Identification of human and mouse sVEGFR-3 using 3' RACE. (**A**) 3'RACE PCR for human sVEGFR-3, human; using forward primer spanning exon 7-8 boundary (Hu Exon 7-8 Primer: GCACCGAGGTCATTGTGCATGAAA) and Reverse primer (ACTGGAGGAAGTAGGTGCCTTTGT) and mouse exon 1-2 boundary (GACTCCTCCAAGGCCTGGCAAAT) and Q1 primer (GAGGACTCGAGCTCAAGC). Once 3' end sequence was known, the full-length sVEGFR-3 of Human and mouse was cloned using exon 1 and 3' terminal region primers. (**B**) Mouse and human sVEGFR-3 onward exon 11, exon (capital letters) and intron region (small letters), bold letters of intron showing the region that is translated. Mouse sVEGFR-3 mRNA is 1992bp long. Mouse mRNA has 338 bp

unique intron derived tail from intron 11, out of which first 27bp are translated into protein. Mouse sVEGFR-3 has a coding region of 1575 bp encoding 59.5 kDa protein. Human sVEGFR-3 mRNA consists of 2067 bp and 414 bp unique intron derived tail from intron 11 coding for 137 unique amino acids. Human sVEGFR-3 has a coding region of 1962 bp encoding 73.19 kDa protein. (**C**) Representative diagram showing the unique amino acids derived from intron 11 of human and mouse sVEGFR-3. (**D**) Representative western blot of mouse and human cornea with antibody specific to anti N-terminal end of VEGFR-3 demonstrates sVEGFR-3 at 60 and 73 kDa, respectively (n=5).

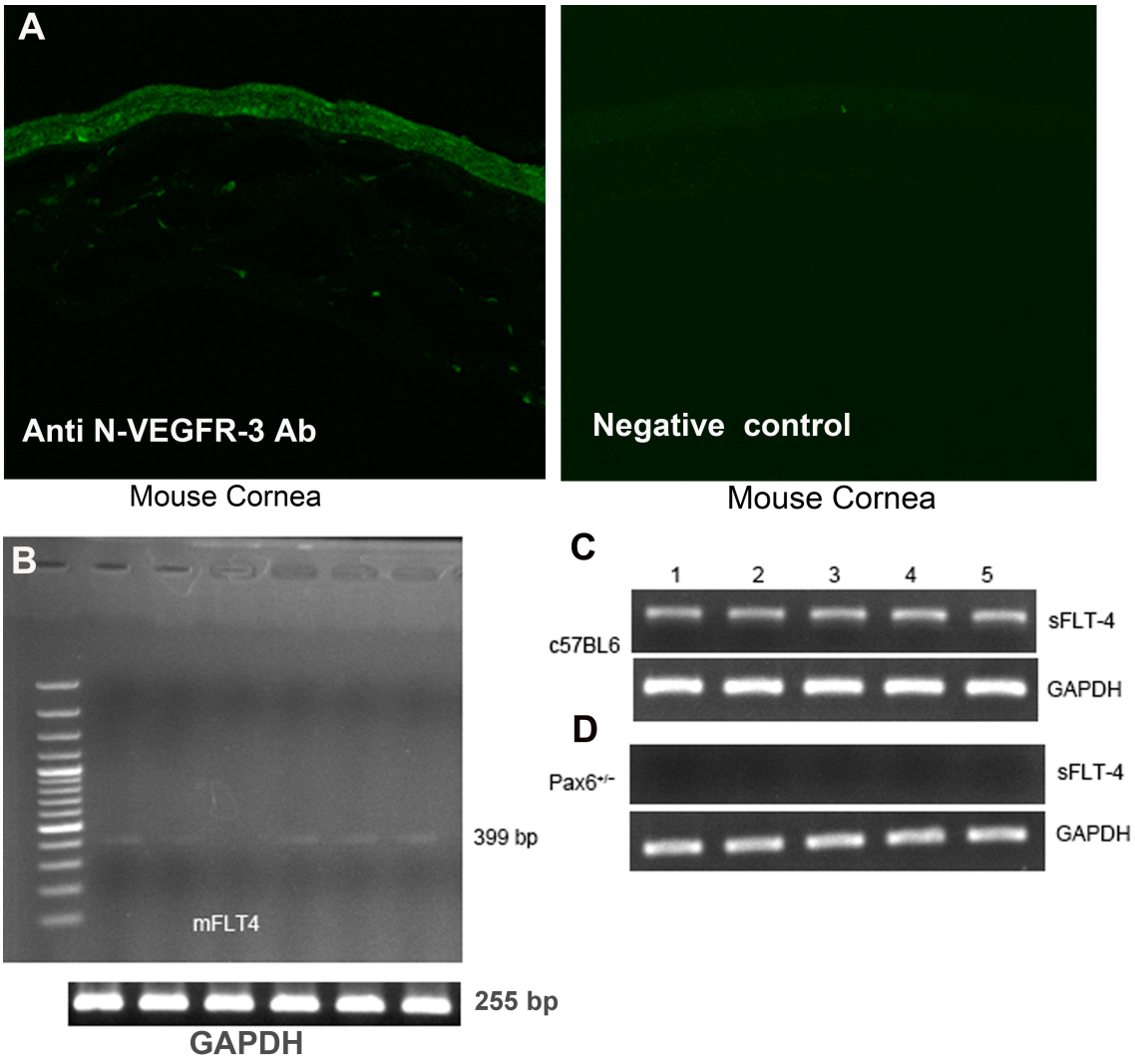

Fig. S2. Cornea expresses soluble VEGFR-3 and no or minimal levels of membrane VEGFR-3, while *Pax6*$^{+/-}$ mice, the strain spontaneously develop blood and lymphatic vessels in cornea, do not express detectable levels of sVEGFR-3. (**A**) Immunofluorescent detection of sVEGFR-3 using anti N-terminal end antibody specific to VEGFR-3 reveals sVEGFR-3 localization in C57BL/6 mouse corneal epithelium. Isotype-negative control antibody was used to control for nonspecific staining (**B**) RT PCRs from cornea total RNA using primers specific to exon 14 onward (present only in membrane VEGFR-3 only) show minimal to no expression of mVEGFR-3 in cornea (n=6). (**C**) RT PCR'S, with reverse primer in unique intron tail and

forward primers at exon-exon juntions, show sVEGFR-3 in Normal C57BL/6J mice cornea. (**D**) RT PCR for sVEGFR-3 show absence of sVEGFR-3 in *Pax6*$^{+/-}$ mice cornea (*n*=5).

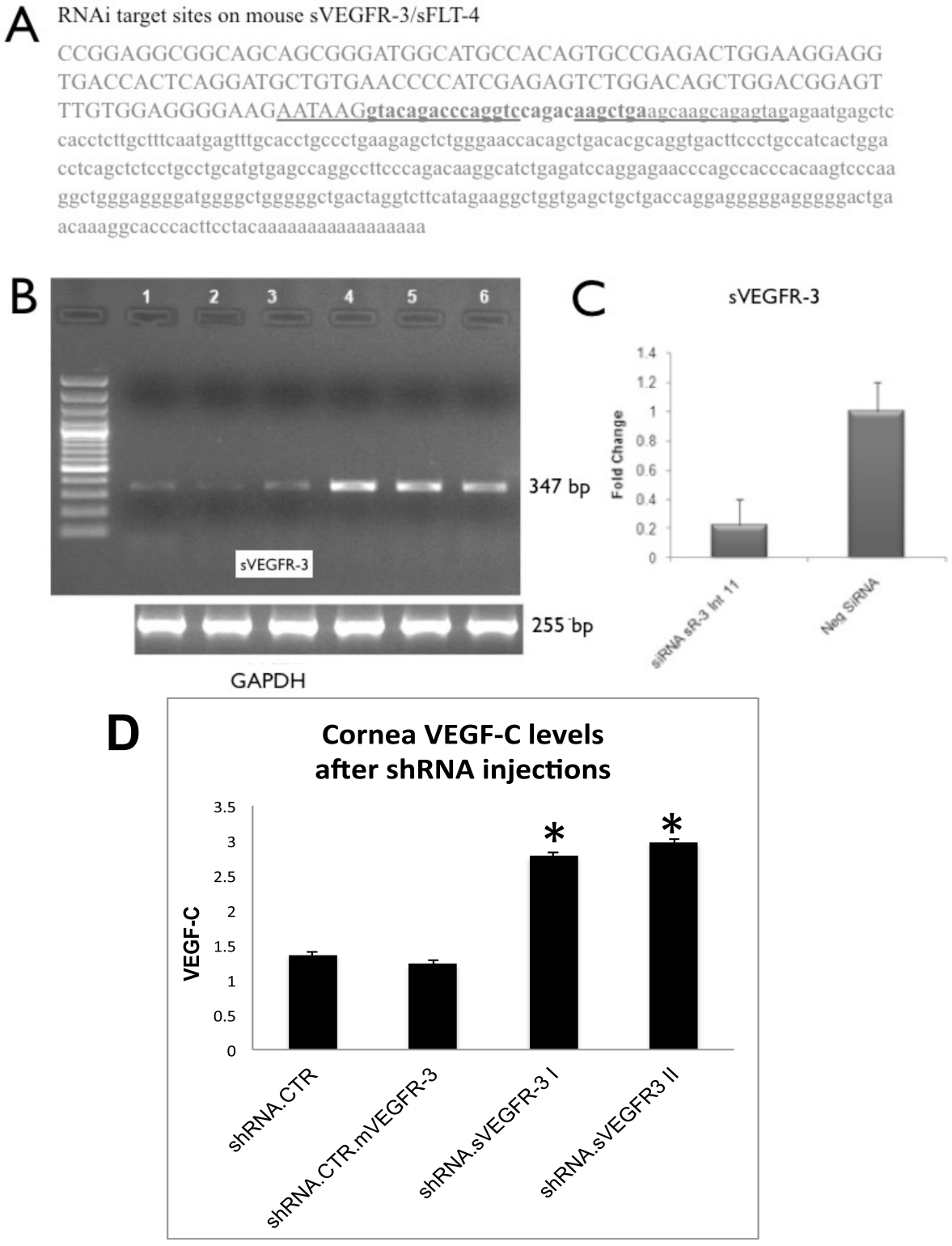

Fig. S3. Target sites on sVEGFR-3 used to design siRNA and shRNA. (**A**) Mouse exon 11 (capital letters) and intron 11 (lower case) of sVEGFR-3 showing the target sites (underlined)

for RNAi. (**B**) RT PCR for sVEGFR-3 on day 3 of mouse cornea injected with pshRNA-sVEGFR-3 (Lane 1-3) and pshRNA-CTR (Lane 4-6) showing knockdown of sVEGFR-3 in mouse cornea. (**C**) SVEC4-10 cell line transected with siRNA-sVEGFR-3 and siRNA-CTR show knockdown of sVEGFR-3 up to 80% after 48 hrs of transfection. (**D**) ELISA for VEGF-C demonstrates that mouse corneas injected with shRNA against sVEGFR-3 express higher free VEGF-C levels after 48 hrs of injection.

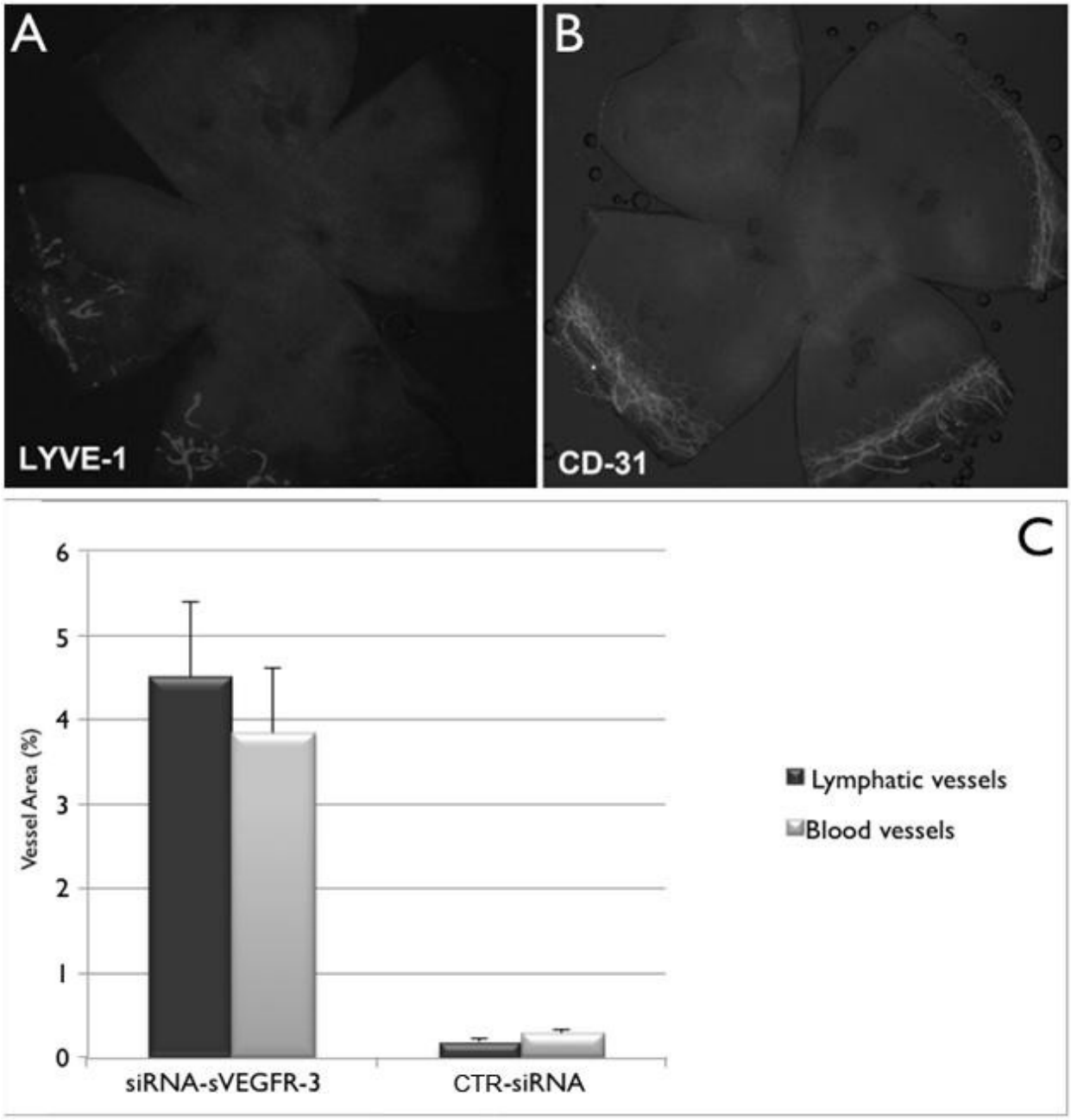

Fig. S4. Injection of cornea with siRNA sVEGFR-3 mouse cornea flatmounts stained with LYVE-1 (**A**) and CD-31 (**B**) staining, injected with two injections of siRNA-sVEGFR-3 and control siRNA after every 3 days lead to increase of (**C**) 4.5% lymphatic and 3.8% blood vessel area. Corneas harvested after 7 days of injections (*n*=10).