SUPPLEMENTAL DATA

Gene expression analysis

The expression of target genes was assessed with reverse transcription and quantitative PCR (RT-qPCR). Total RNA was extracted with the PerfectPure RNA Cultured Cell Kit (5 Prime, Hamburg, Germany), reverse transcribed into cDNA with random hexamer primers, and amplified with real-time qPCR on the Applied Biosystems 7500 Fast Real-Time PCR system (Applied Biosystems, Darmstadt, Germany). The reaction was carried out in 14 µl reaction volumes containing 2 µl of cDNA (20 ng), specific sense and anti-sense primers (250 nM each), and 7 µl Power SYBR Green PCR Master Mix (Applied Biosystems). PCR primers were synthesized by TIB Molbiol (Berlin, Germany) and their sequences were as follows:

- *VEGF* (GenBank NM_001171623.1):

  forward (5'-AAGGAGGAGGGCAGAATCAT-3'),

  reverse (5'-ATCTGCATGGTGATGTTGGA-3');
- *STAT3* (GenBank NM_139276.2):

  forward 5'- GGCCATCTTGAGCACTAAGC-3'),

  reverse (5'-CGGACTGGATCTGGGTCTTA -3');
- *SP4* (GenBank NM_003112.3):

  forward 5'-TCAGCAGCAAGGACAAGATG -3'),

  reverse (5'- AAGCCTCTTGCCAGGTTGTA -3');
- *β2M* (GenBank NM_004048.2):

  forward (5'- GTGCTCGCGCTACTCTCTCT-3'),

  reverse (5'- CGGCAGGCATACTCATCTTT -3').

Primers for murine targets were as follows:

- *Sp4* (GenBank NM_009239.4):

  forward (5'- GCCTGCTCCTGTCCTAACTG -3'),

  reverse (5'- CTCGGAGGTGAGAGGTCTTG -3');

- *Vegf* (GenBank NM_001025250.3):

  forward (5'- CAGGCTGCTGTAACGATGAA -3'),

  reverse (5'- GCATTCACATCTGCTGTGCT -3').

- *β2m* (GenBank NM_009735.3):

  forward (5'- GAAATCCAAATGCTGAAGAACG-3'),

  reverse (5'- CAAATGAATGTTCAGAGCATCATG -3')

- *Pecam1* (GenBank NM_008816.2):

  forward (5'- AGAGACGGTCTTGTCGCAGT -3'),

  reverse (5'- TACTGGGCTTCGAGAGCATT -3');

- *VE-Cad* (GenBank NM_009868.4):

  forward (5'- ACCGAGAGAAACAGGCTGAA -3'),

  reverse (5'- AGACGGGGAAGTTGTCATTG -3');

- *Vegfr2* (GenBank NM_010612.2):

  forward (5'- GGCGGTGGTGACAGTATCTT -3'),

  reverse (5'- GTCACTGACAGAGGCGATGA -3');

- *Vegfr3* (GenBank NM_008029.3):

  forward (5'- GCTGTTGGTTGGAGAGAAGC -3'),

  reverse (5'- GAGCCACTCGACACTGATGA -3');

- *Efnb2* (GenBank NM_010111.5):

  forward (5'- CTCAACTGTGCCAGACCAGA -3'),

  reverse (5'- CTTGTTGGACCGTGATTCCT -3');

- *Nrp1* (GenBank NM_008737.2):

  forward (5'- GGAGCTACTGGGCTGTGAAG -3'),

  reverse (5'- ACCGTATGTCGGGAACTCTG -3');

- *Pdpn* (GenBank NM_010329.3):

  forward (5'- GCCAGTGTTGTTCTGGGTTT -3'),

  reverse (5'- AGAGGTGCCTTGCCAGTAGA -3');

After an initial activation step for 2 min at 50°C and denaturation at 95°C for 10 min, 40 cycles of 15 sec at 95°C, and 1 min at 60°C were performed. Specificity of the reaction was verified by melting curve analysis at the end of each series of assays. The relative amount of gene transcript was calculated by the cycle threshold method using the Applied Biosystems 7500 System v.1.2.3 software and normalized for the endogenous reference (β2-microglobulin).

VEGF promoter, EMSA oligonucleotide sequences and site-directed mutagenesis primer

-267
GGGCGCGTGTCTCTGGACAGAGTTTCCGGGGGCGGATGGGTAATTTTCAGGCTGTGAACCTTGGTG
GGGGTCGAGCTTCCCCTTCATTGCGGCGGGCTGCGGGCCAGGCTTCACTGAGCGTCCGCAGAGCCC
GGGCCCGAGCCGCGTGTGGAAGGGCTGAGGCTCGCCTGTCCCCGCCCCCCGGGGCGGGCCGGGGGG
CGGGGTCCCGGCGGGGCGGAGCCATGCGCCCCCCCCTTTTTTTTTTAAAAGTCGGCTGGTAGCGGGG
AGGatcgcggaggcttggggcagccgggtagctcggaggtcgtggcgctgggg+50

EMSA oligo sequences for SP1 and SP4
GCGGGCCGGGGGCGGGGTCCCGGC SP4 -59 to -82
TCGCCTGTCCCCGCCCCCCGGGGC SP1 -80 to -103

Site directed mutagenesis of SP4 sequences

GGGGCGGGCCaaaGGCGGGGTCCC site directed mutagenesis forward Primer

Nucleotides written in CAPITAL LETTERS correspond to VEGF promoter sequence -267 to 0. Nucleotides written in small letters correspond to VEGF gene sequence +1 to+50.

A sequence in dark green was used as a synthethic oligo for SP1 EMSA experiments

A sequence in light green was used as a synthethic oligo for SP4 EMSA experiments. Mutated SP4 sequence is written in small blue letters.

Fig. S1 Effect of SP4 on VEGF-mediated tube formation by human dermal microvascular endothelial cells (HDMEC). Cells were transiently transfected with either *SP4* siRNA or scrambled (Scramb) siRNA, and stimulated for 16 hrs with conditioned medium (10% v/v) from HPMC treated without or with IL-6+sIL-6R (both at 100 ng/ml) for 24 hours. *P<0.05 vs. cells stimulated with IL-6+sIL-6R in the absence of siRNA, n=4. Representative phase contrast images (magnification 100 x)

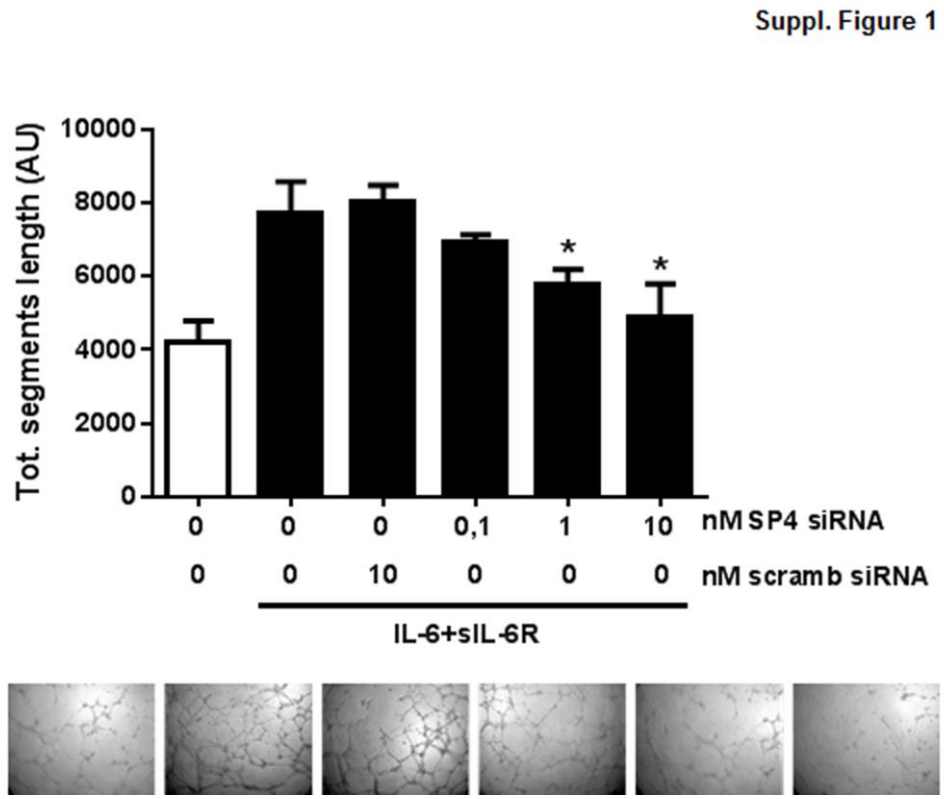
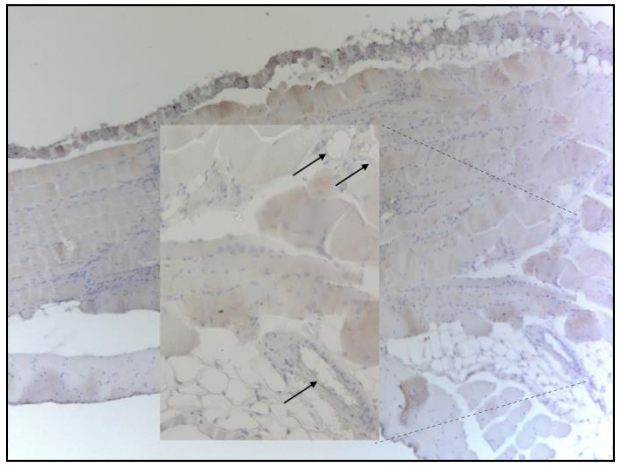


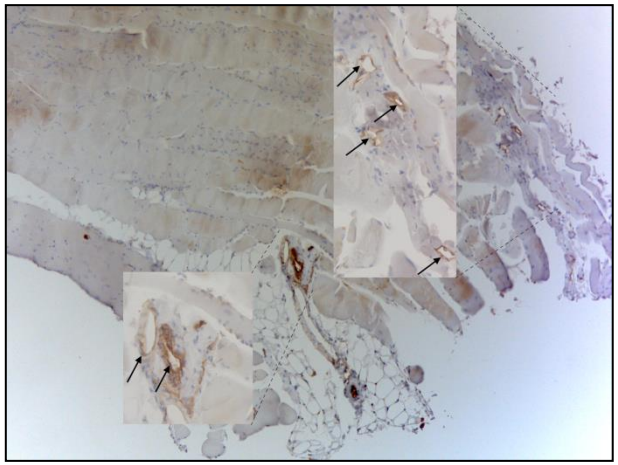
Fig. S2 Effect of IL-6 signaling in mice on peritoneal vasculature following repeated peritonitis. WT and IL-6-/- mice received 4 consecutive doses of SES (i.p.) administered at 7-day intervals. Histological evaluation was recorded on day-49 post first SES challenge. Sections were stained for either PECAM-1 (CD31) (A, B) or podoplanin (C, D). Representative 40x magnification (insert panels: 400x magnification) fields are shown. Scores displayed in Panels B and D reflect digital assessments of immunohistochemistry staining as described in Materials and Methods.

A

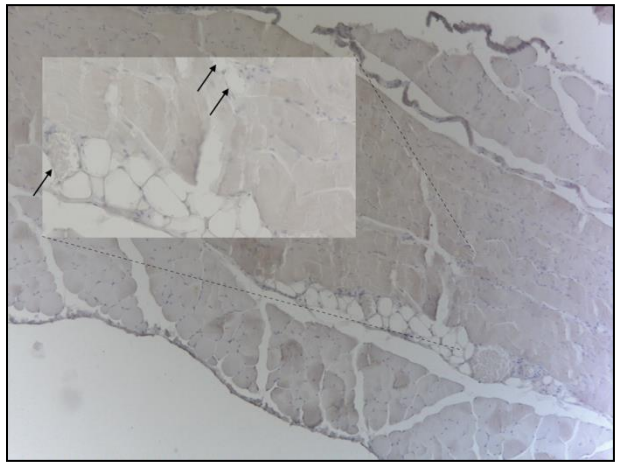
WT 4xSES d49 Isotype Control



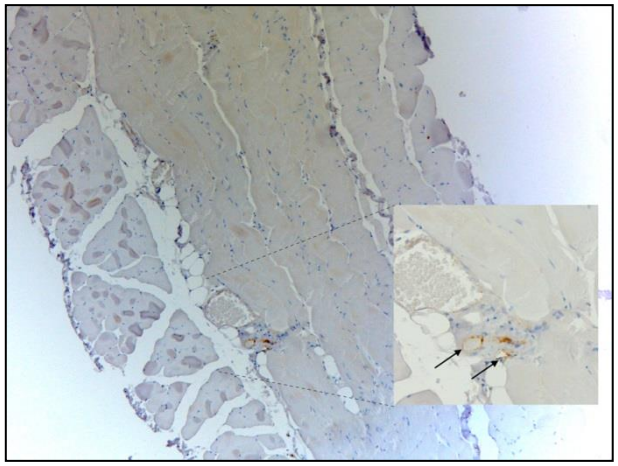
WT 4xSES d49 CD31



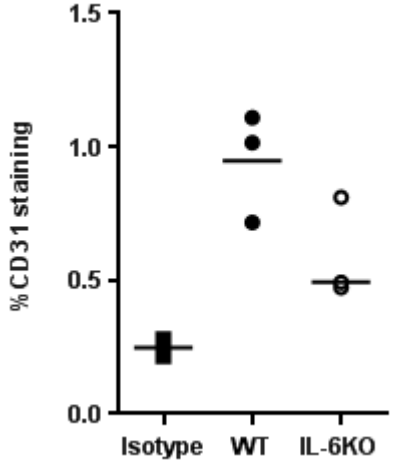
Il6$^{-/-}$ 4xSES d49 Isotype Control



Il6$^{-/-}$ 4xSES d49 CD31



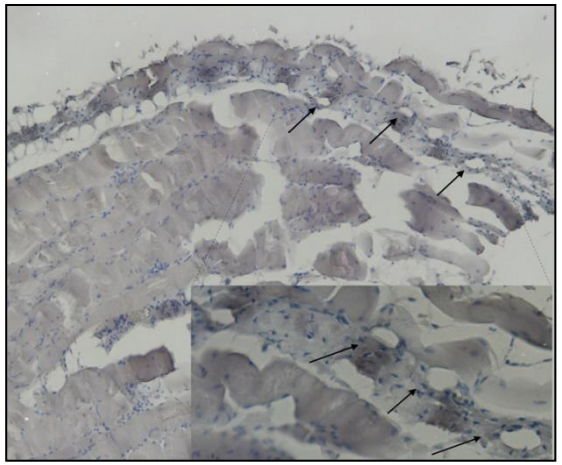
B



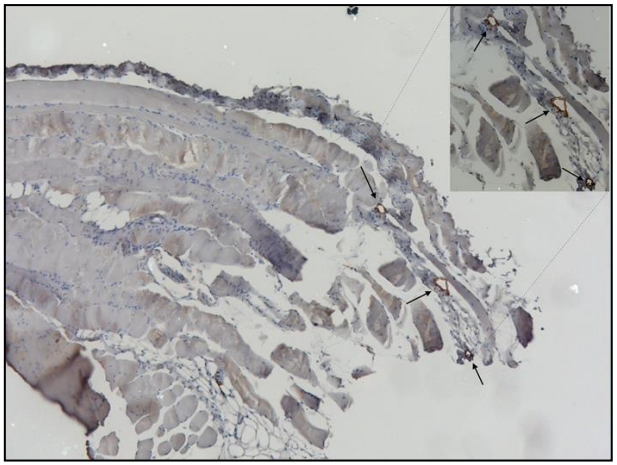
Suppl Fig. 2

C

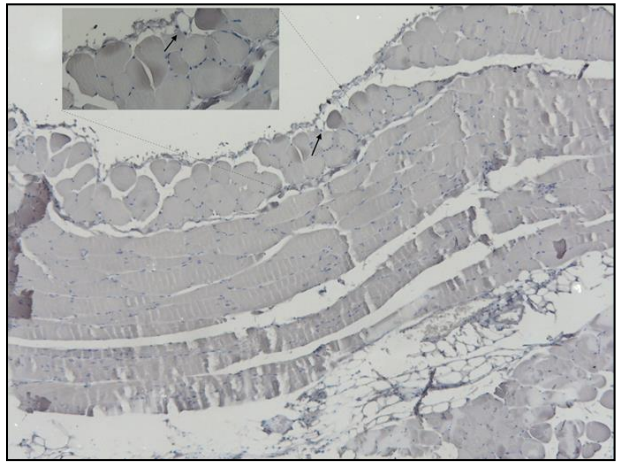
WT 4xSES d49 Isotype Control



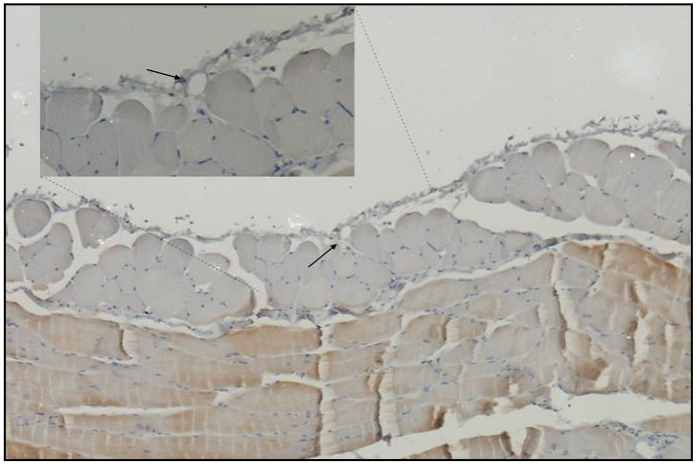
WT 4xSES d49 CD31



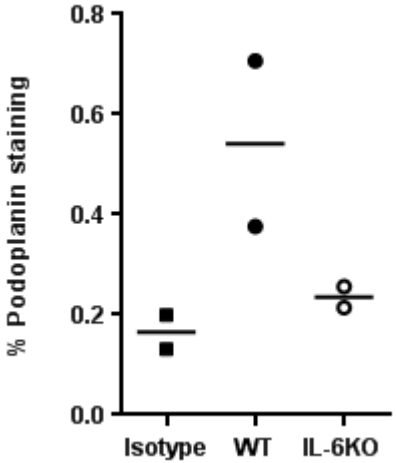
Il6$^{-/-}$ 4xSES d49 Isotype control



Il6$^{-/-}$ 4xSES d49 Pdpn



D



Suppl Fig. 2 cnt'd