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Supplemental information

Macrophage IL-1β promotes arteriogenesis by autocrine STAT3- and NF-κB-mediated transcription of pro-angiogenic VEGF-A

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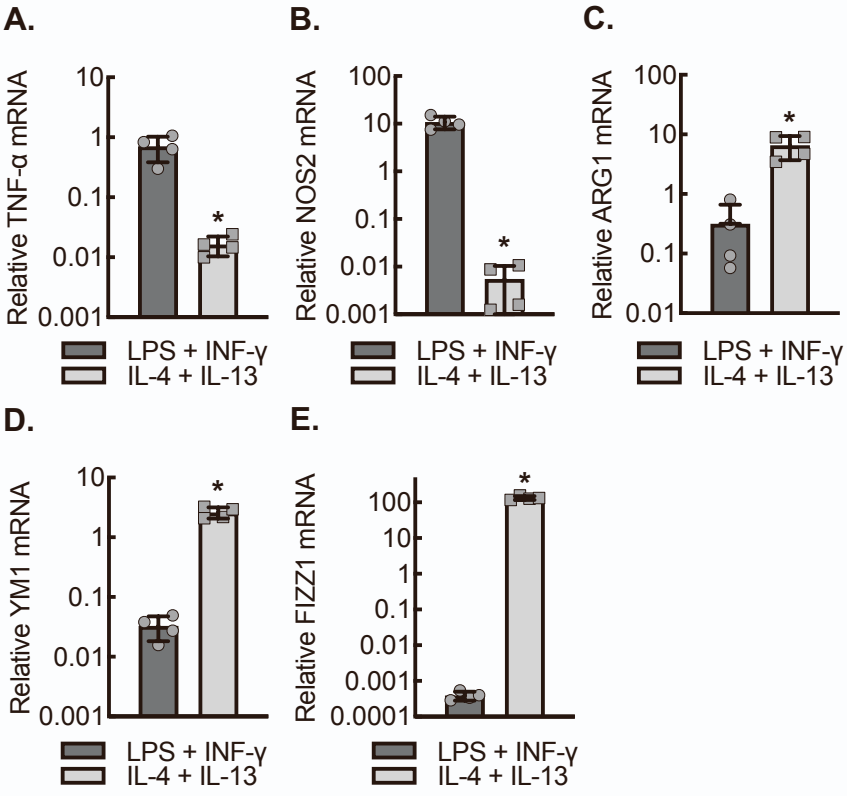
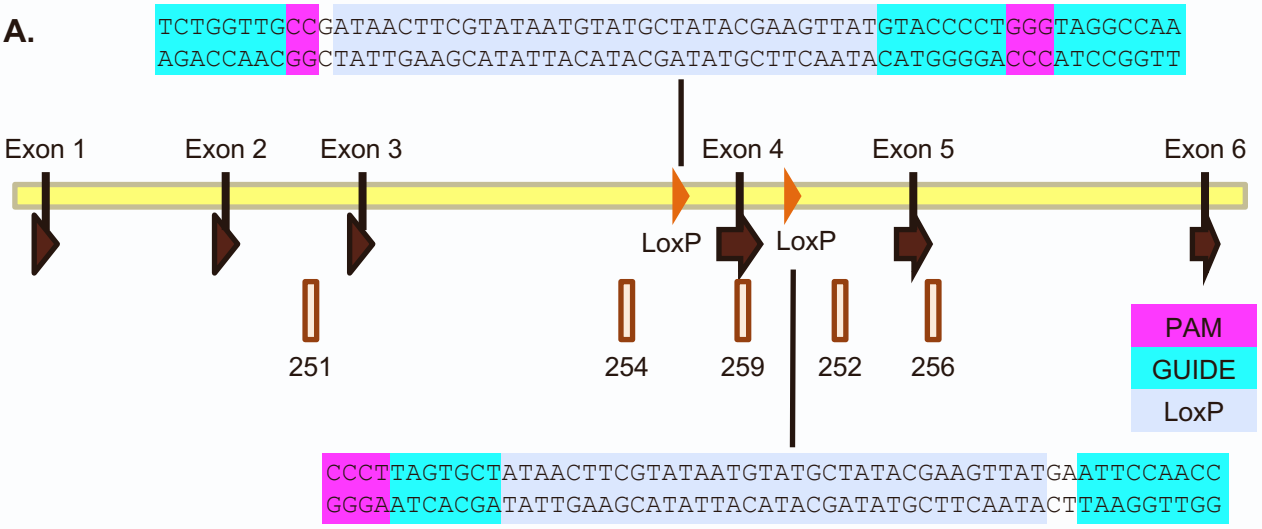
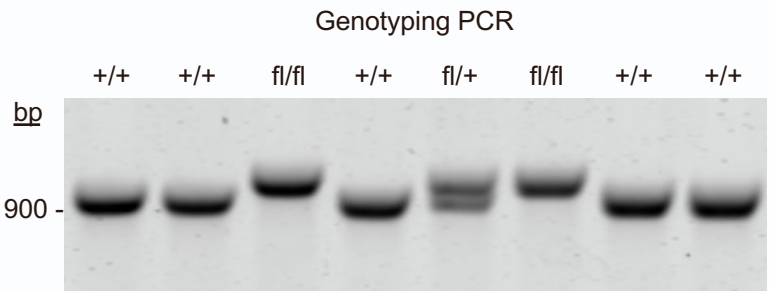


Figure S1. Polarization of macrophages. Related to Figure 1. Quantitative RT-PCR for relative mRNA expression of TNF-α (**A**) NOS2 (**B**), ARG1 (**C**), FIZZ1 (**D**), YM1 (**E**), as representative inflammatory (IL-1β, TNF-α, NOS2) or alternatively activated (ARG1, FIZZ1, YM1) markers from lysates of BMDMs treated with either LPS+INF-γ or IL-4+IL-13 for 24 hours (*, P< 0.05 by t-test; *n*=4 mice total, 2 males and 2 females). Data, mean ± SD.



B.



C.

Short- and Long-Range PCR Primers

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251: CCAGAGCACCCACTTACCTG
252: GTGGTCCTTGCATGAAACG
254: GTATGGGAAGCCATTGAGAGC
256: TGCTCCTACTTACCTTGTTGGT
259: CACTCATTGTGGCTGTGGAG
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Figure S2. Diagram of IL-1β Gene Editing and Genotyping PCR identifying *IL-1β*$^{fl/fl}$ Mice. Related to Figure 2. (**A**) IL-1β Gene Editing Strategy. Two recognized guide sites in intron 3 and 4. Aqua shaded letters represent the guide sites (GUIDE). Magenta shaded letters represent the protospacer adjacent motif (PAM) of 5'-NGG-3'. After the CRISPR/Cas9 system introduced a double strand break in two targeted sites of the IL-1β allele, the homologous donor vector recombined, which resulted in the insertion of the two LoxP sequences, shaded with light blue. LoxP insertion sights illustrated by orange triangles. Primer sites (251, 254, 258, 252, and 256) for PCR genotyping of the floxed *IL-1β* allele are also illustrated. Cre-mediated excision leads to deletion of 67 amino acids and frame-shift/premature termination of translation in exon 5. (**B**) Genotyping PCR products, using primers 254 and 252 for forward and reverse primers respectively, were then resolved by electrophoresis on 1% agarose gel in order to identify alleles with LoxP sequences flanking exon 4. Wild-type (+) allele is 901 bp and LoxP flanked allele is 969 bp. (**C**) The sequences of the short- and long-range PCR primers.

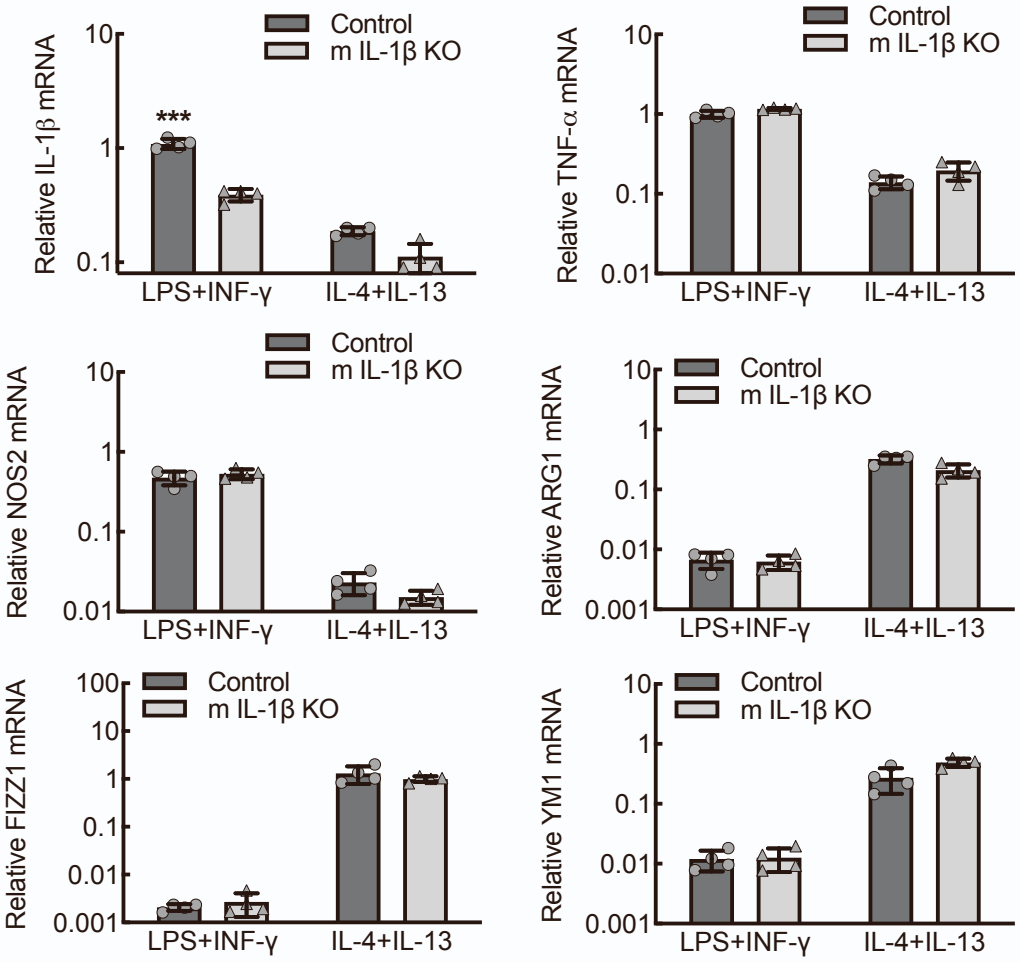

Figure S3. Beyond IL-1β expression, BMDMs from myeloid *IL-1β*-deleted mice do not reveal significant differences in polarization markers after stimulation with either LPS+INF-γ or IL-4+IL-13. Related to Figure 2. Quantitative RT-PCR for relative expression of indicated inflammatory (IL-1β, TNF-α, NOS2) or alternatively activated markers (ARG1, FIZZ1, YM1) from lysates of primary mouse BMDMs treated with either LPS+INF-γ or IL-4+IL-13 for 24 hours (***, P<0.0001 compared to all others by ANOVA; *n*=4 mice total, 2 males and 2 females). Data, mean ± SD.

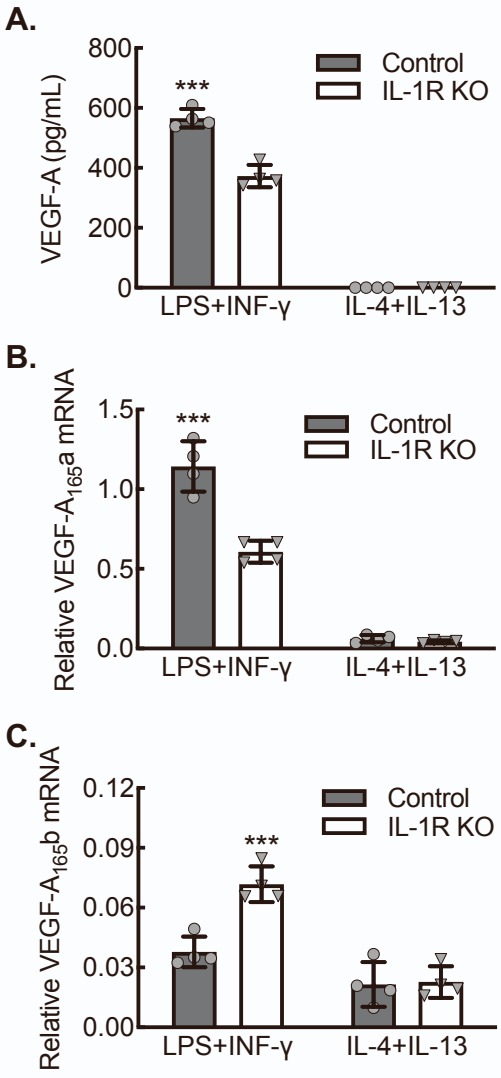

Figure S4. Macrophage VEGF-A expression is critically dependent on autologous IL-1R expression in the context of inflammatory stimuli. Related to Figure 2. (**A**) Primary mouse BMDMs from control or *IL-1R*-deleted mice (IL-1R KO) were treated with either LPS+INF-γ or IL-4+IL-13 for 24 hours, followed by ELISA on culture supernatants for secreted VEGF-A protein (***, P<0.0001 compared to all others by ANOVA; n=4 mice total, 2 males and 2 females). (**B**) BMDMs treated as in (**A**) followed by quantitative RT-PCR for relative VEGF-A_{165}a mRNA expression (***, P<0.0001 compared to all others by ANOVA; n=4 mice total, 2 males and 2 females) (**C**) BMDMs treated as in (**A**) followed by quantitative RT-PCR for relative VEGF-A_{165}b mRNA expression (***, P<0.0001 compared to all others by ANOVA; n=4 mice total, 2 males and 2 females). Data, mean ± SD.

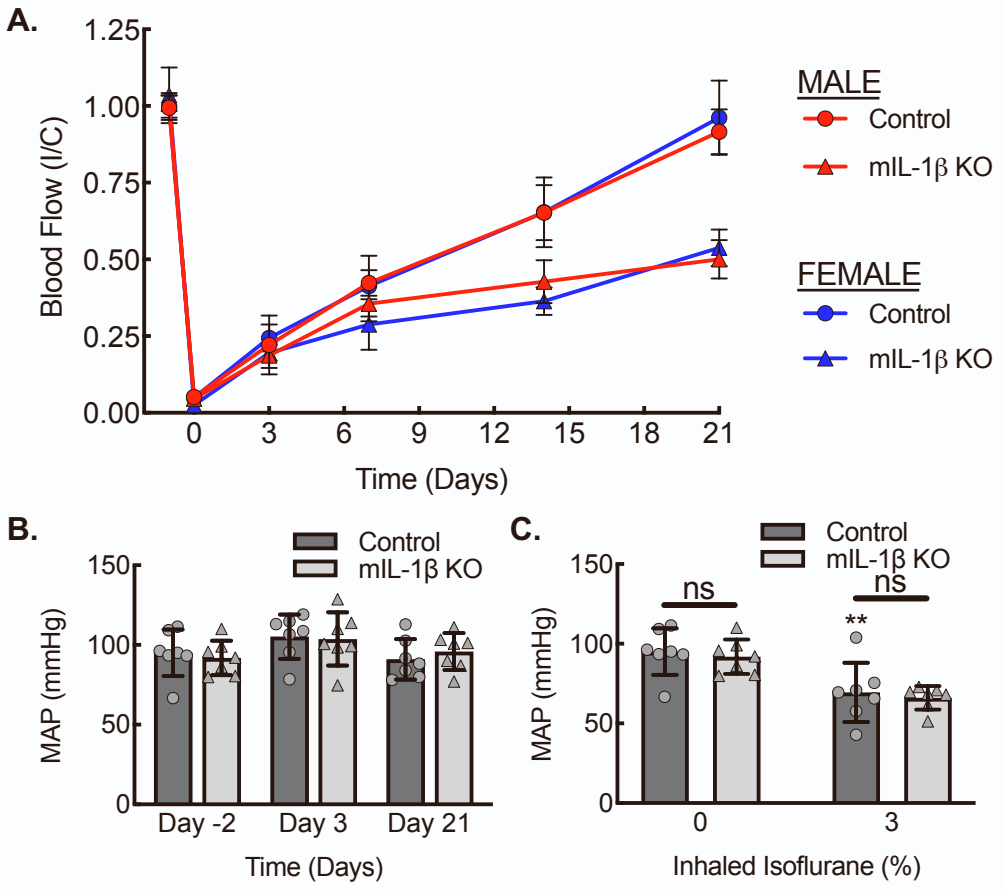

Figure S5. Control and myeloid *IL-1β*-deleted mice do not demonstrate significant sex-specific differences in recovery of blood flow nor significant differences in mean arterial pressures during hind limb ischemia. Related to Figure 5. (**A**) Quantitative analysis from laser Doppler blood flow imaging of both control or myeloid *IL-1β*-deleted (mIL-1β KO) mice at indicated time points before and after femoral artery ligation (comparison by ANOVA; *n*=6 males or 6 females in each group). (**B**) Mean arterial blood pressures (MAPs) by tail-cuff for alert/awake control or mIL-1β KO mice before (Day -2) and after (Days 3 and 21) femoral artery ligation (*n*=6 mice total, 3 males and 3 females). (**C**) MAPs using tail-cuff for control or mIL-1β KO mice that were alert/awake (0% isoflurane) or anesthetized (3% isoflurane) (**, P=0.0082 compared to alert/awake control; *n*=6 mice total, 3 males and 3 females). Data, mean ± SD.

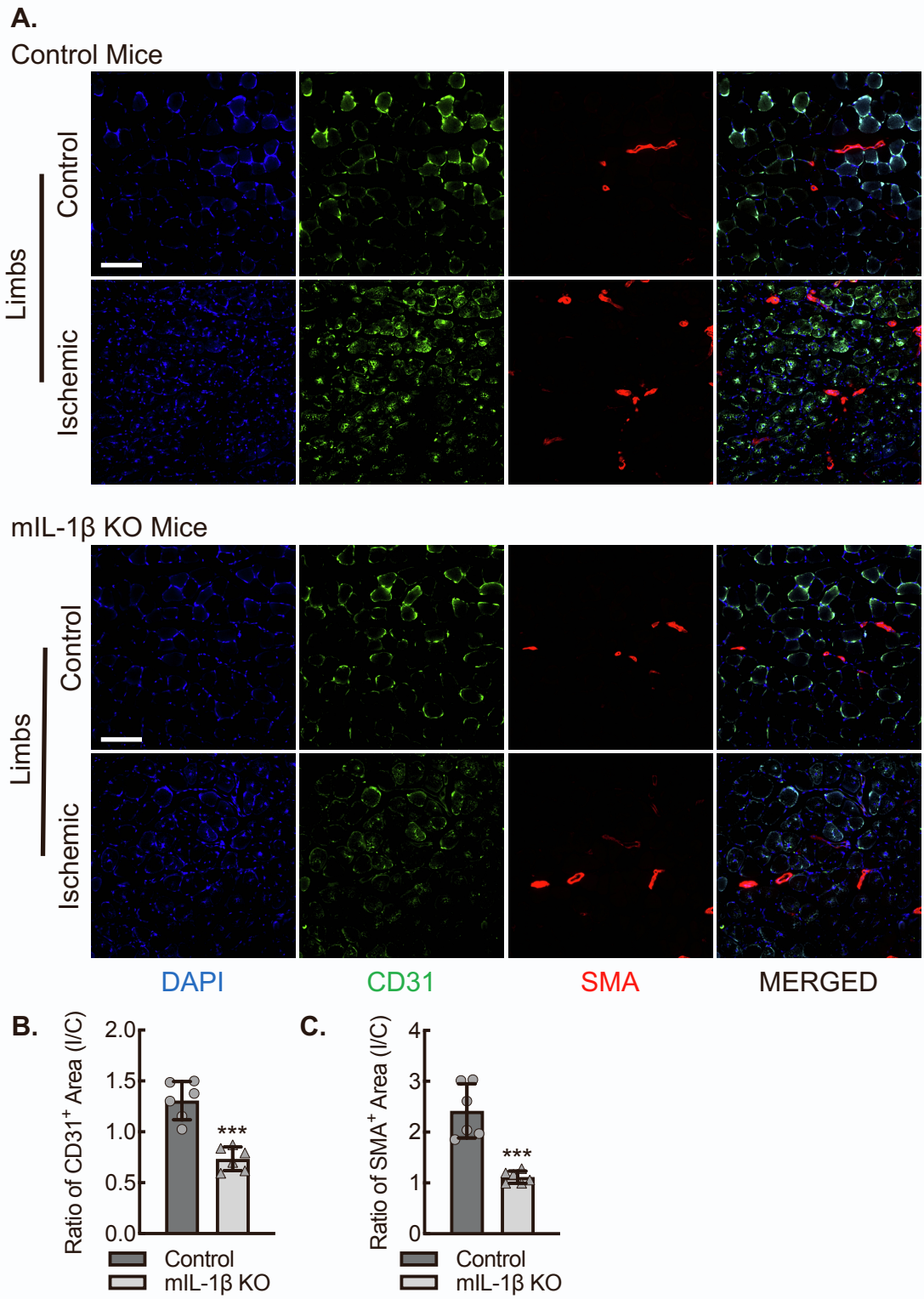

Figure S6. Angiogenesis and arteriogenesis are dependent on myeloid IL-1β expression. Related to Figures 5 and 6. (**A**) Immunofluorescence micrographs of ischemic and contralateral control muscle tissue at Day 21 post femoral artery ligation from either control or mIL-1β KO mice along with quantitation of the ratio of $CD31^+$ area (**B**) or SMA^+ area (**C**) between ischemic and contralateral control limbs (***, P≤0.0002 by t-test; *n*=6 mice total, 3 males and 3 females). Bar, 100 microns. Data, mean ± SD.

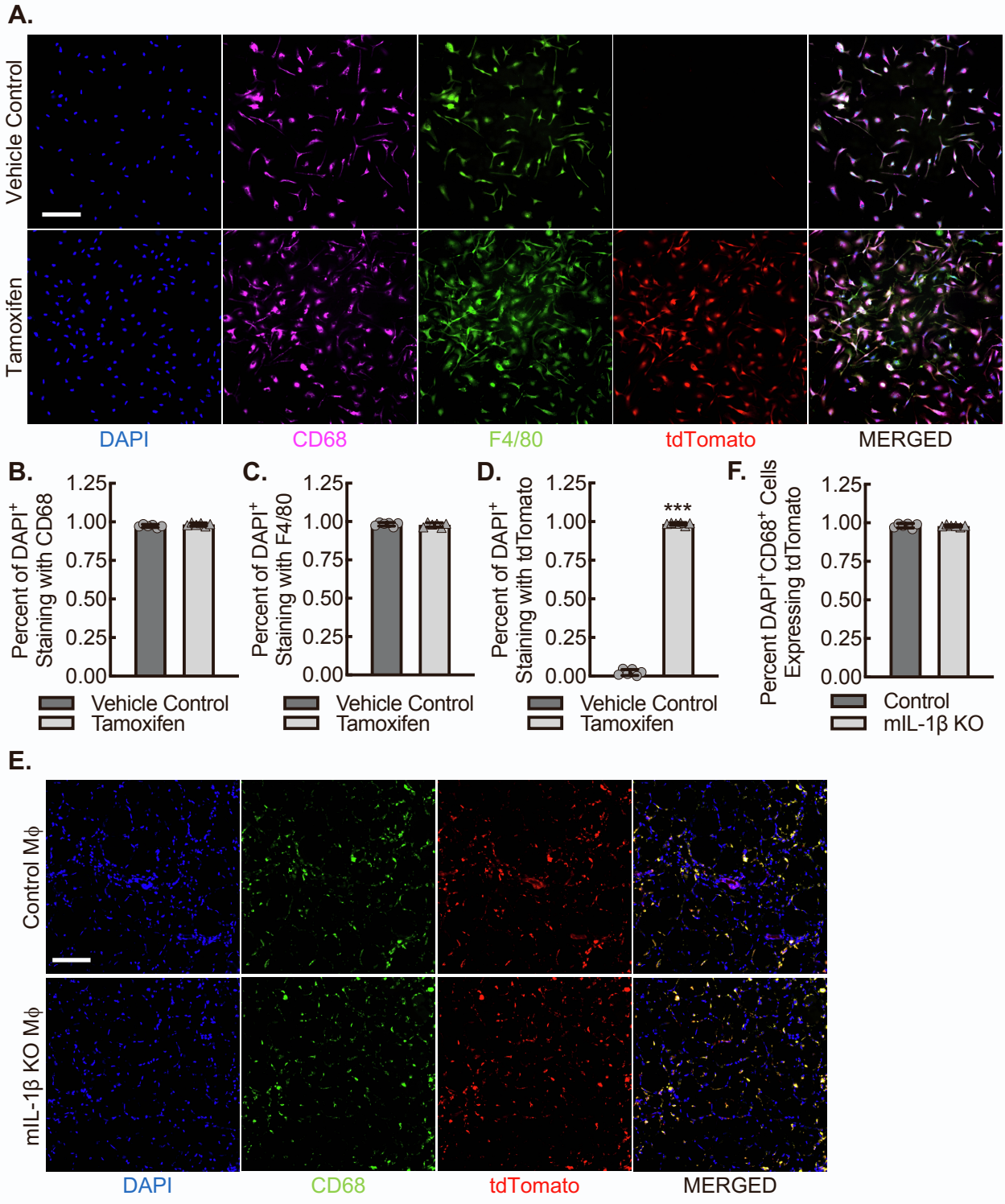

Figure S7. Validation of Ai9 mice and validation of CD68 as a marker for macrophages in the ischemic limb. Related to Figure 7. (**A**) Immunofluorescence micrographs of BMDMs from Ai9 mice treated with tamoxifen to induce tdTomato expression or vehicle control along with quantitation of DAPI$^+$CD68$^+$ cells (**B**), DAPI$^+$F4/80$^+$ cells (**C**), and DAPI$^+$ tdTomato$^+$ cells (**D**) (***, P<0.0001 by t-test; *n*=6 mice total, 3 males and 3 females). Bar, 100 microns. (**E**) Immunofluorescence micrographs of ischemic muscle tissue at Day 3 post femoral artery ligation from mIL-1β KO mice that underwent clodronate liposome macrophage depletion followed by transplant of tdTomato expressing BMDMs from either control or mIL-1β KO, Ai9 mice. (**F**) Quantitation of DAPI$^+$CD68$^+$ cells co-staining for tdTomato (*n*=6 mice total, 3 males and 3 females). Bar, 100 microns. Data, mean ± SD.