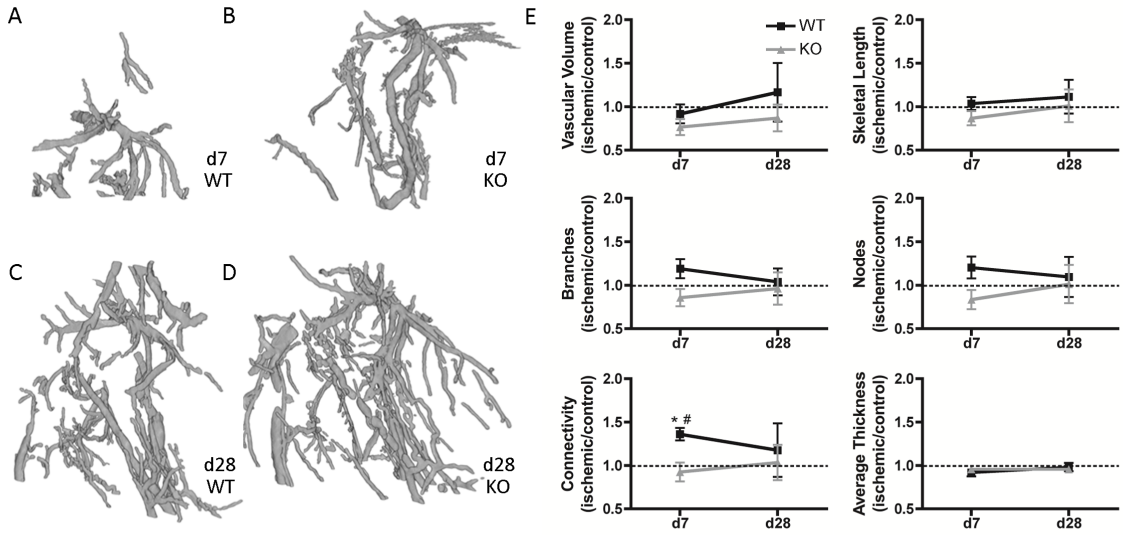
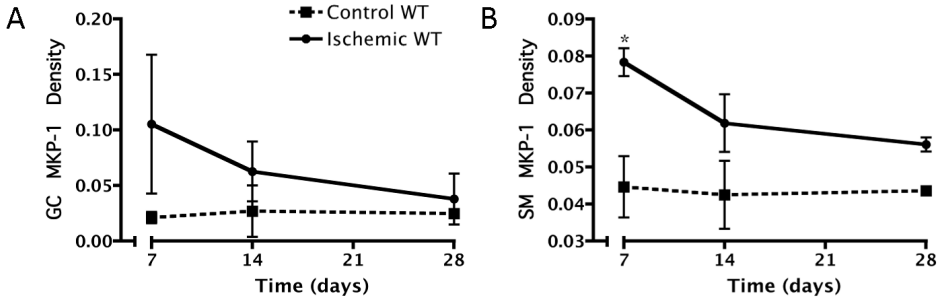
SUPPLEMENTAL DATA

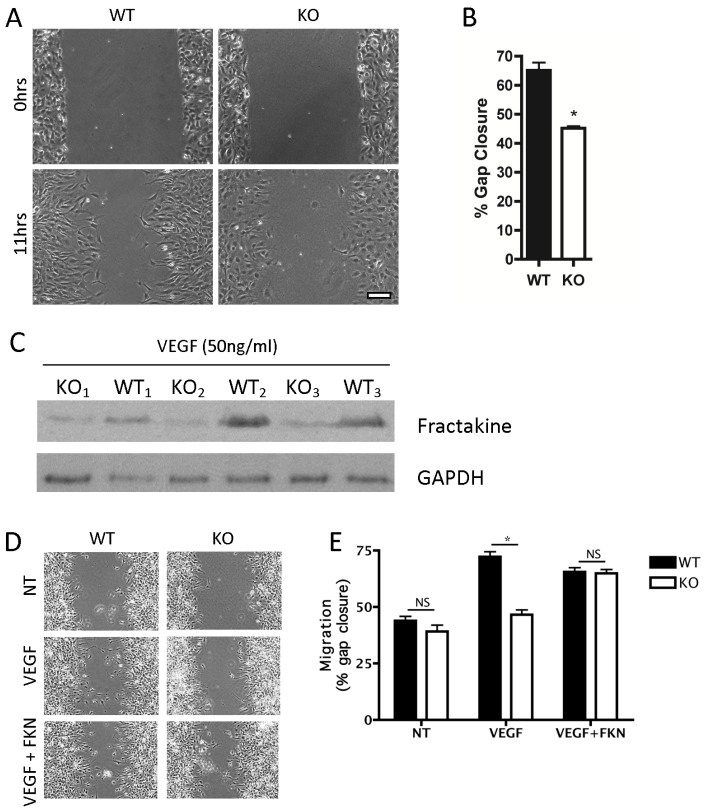
MKP-1 PROMOTES NEOVASCULARIZATION AND ANGIOGENIC GENE EXPRESSION
Boerckel et al. ATVB



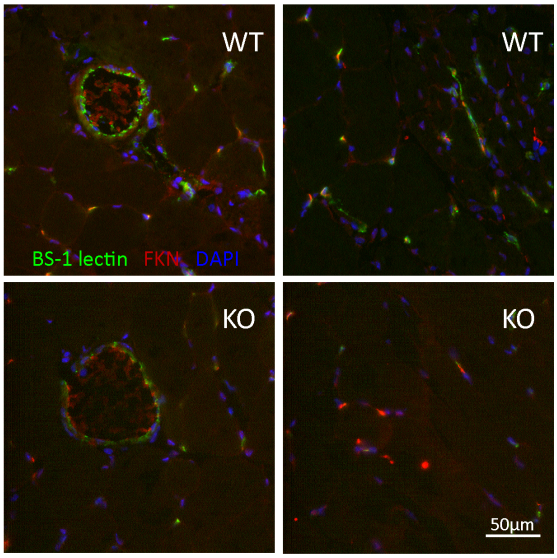
Supplemental Figure I. **MicroCT angiography of proximal hindlimb region of interest.** 3D reconstructions of WT and KO vascular structures in the thigh region at day 7 (A, C) and day 28 (B, D) post-surgery (N = 4-9 per time point per genotype). Quantification of vascular network parameters (E) revealed no significant differences in arteriogenic network formation between WT and KO mice, except for connectivity at day 7 (*$p \leq 0.05$ vs. KO at same time point, #$p \leq 0.05$ vs. control at same time point). At day 28, there were no significant differences between genotypes or compared with controls.



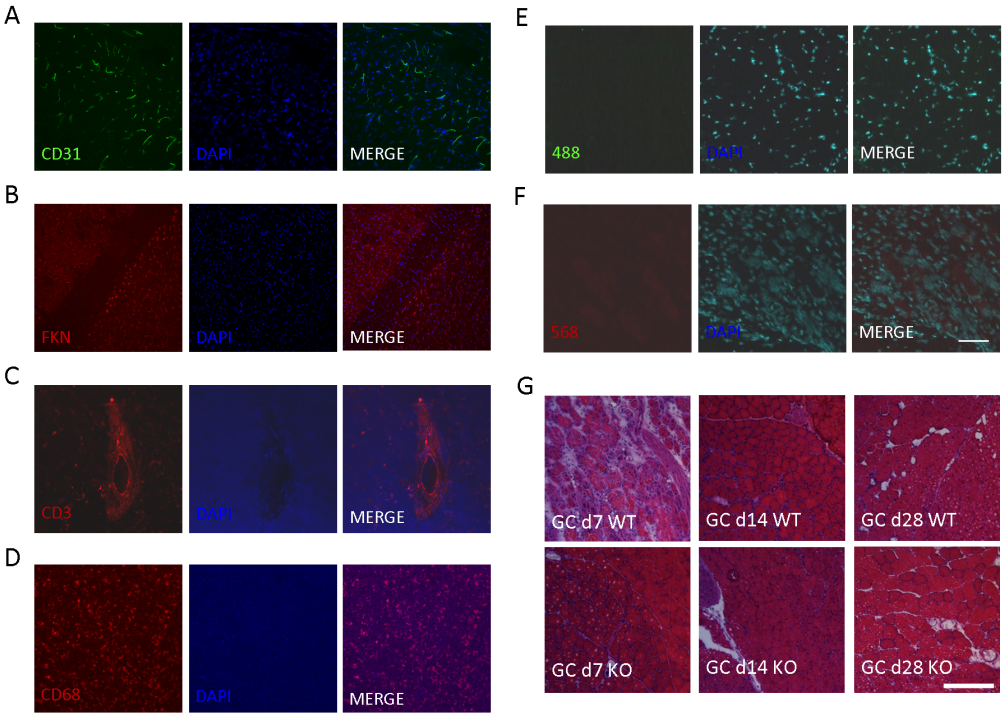
Supplemental Figure II. **Quantification of MKP-1 immunostaining in WT hindlimbs.** Gastrocnemius (GC; A) and soleus (SM; B) muscle sections from WT mice were stained for MKP-1 at 7, 14 and 28 days. MKP-1 was elevated at day 7 post surgery, and expression decreased over time. *$p \leq 0.05$ vs control limb at same time point.



Supplemental Figure III. **Mouse aortic endothelial cell (MAEC) migration and fractakline gene expression.** Serum starved MAEC were scratched, treated with 50 ng/ml VEGF, and cells were allowed to migrate for 11 hrs (A). MKP-1 KO cells featured significantly reduced migration into the gap compared with cells from WT mice (B). Scale bar: 50 μm. Serum-starved MAEC, treated for 6 hrs with 50ng/ml, were then immunoblotted for fractalkine and GAPDH expression (C). MAEC were taken from 6 independent mice (3 WT, 3 KO). Fractalkine expression was elevated in WT mouse cells compared to KO. MAEC were then treated with vehicle, VEGF (50ng/ml), or VEGF (50ng/ml) + recombinant mouse fractalkine (100ng/ml), and migration was evaluated over 11 hrs (D, E). Fractalkine treatment rescued the effect of MKP-1 deletion. *$p \leq 0.05$, NS = not significant.



***Supplemental Figure IV*. Fractalkine & endothelial cell co-localization.** To demonstrate that ischemic hindlimb endothelial cells express fractalkine, sections were co-stained with BS-1 lectin (green) and fractalkine (red). Nuclei were co-stained with DAPI (blue). Red and green signal co-localized in both patent vessels (left column) and microvessels (right column), though fractalkine staining was also evident in hematocytes, consistent with the known function of fractalkine as a cleaved signaling molecule that interacts with CX3CR1-expressing cells. Despite positive staining in ECs, fractalkine expression was also markedly lower in KO mice compared to WT littermates, indicative of MKP-1 regulation of fractalkine gene expression. Scale bar: 50 µm.



Supplemental Figure V. **Immunofluorescent tissue staining: positive and negative controls.** Verification of antibody staining was evaluated by positive control tissue staining for CD31 and fractalkine in WT mouse brain tissue (A, B) and CD3 and CD68 in WT mouse spleen tissue (C, D). Lack of non-specific antibody-tissue binding was verified using negative controls of adjacent sections of WT gastrocnemius muscle lacking primary antibody but incubated with secondary Alexa Fluor 488 and 568 (E, F). H&E staining of GC sections illustrated the extent of inflammatory cell infiltration in WT tissue, but not KO (G). Scale bar: 50 µm.