

Supplementary Information for

Myeloid ALX/FPR2 regulates vascularization following tissue injury

Brian E. Sansbury^a, Xiaofeng Li^a, Blenda Wong^a, Andreas Patsalos^b, Nikolas Giannakis^c, Michael J. Zhang^d, Laszlo Nagy^{b,c} and Matthew Spite^a

^aCenter for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

^bDepartments of Medicine and Biological Chemistry, Johns Hopkins University of School of Medicine and Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA.

^cDepartment of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen H-4032, Hungary.

^dDepartment of Medicine, University of Minnesota Medical School, Minneapolis, MN, USA.

Address correspondence to: Matthew Spite, Ph.D. Hale Building for Transformative Medicine, Room 3016K, Boston, MA 02115; Ph: 617-525-5133; E-mail: <u>mspite@bwh.harvard.edu</u>

This PDF file includes:

Figures S1 to S4



Figure S1. Validation of *Alx/Fpr2* gene deletion and its effect on perfusion recovery in female mice and RvD1 biosynthesis in ischemia. Expression of *Alx/Fpr2* and *Hprt* in BMDM from wild type (WT) and *Alx/Fpr2^{-/-}* mice was measured by qRT-PCR (*A*) shown in relative fluorescence units (RFU) and number of cycles required for signal amplification (Mean Cq) and (*B*) by gel electrophoresis. n=4 per genotype. (*C*) Quantification of perfusion recovery measured by laser speckle contrast perfusion imaging in WT and *Alx/Fpr2^{-/-}* female mice following HLI. n=5-7 per genotype. (*D*) Quantification of RvD1 measured by LC-MS/MS in ischemic limb skeletal muscle of male *Alx/Fpr2^{-/-}* undergoing HLI. n=3 per group at each time point. Graphs represent mean ± SEM; (*D*) unpaired Student's *t*-test vs. Sham, **p*<0.05.



Figure S2. Systemic administration of RvD1 improves perfusion recovery while local delivery does not affect macrophage recruitment. Quantification of perfusion recovery measured by laser speckle contrast perfusion imaging in WT mice following HLI with daily intraperitoneal administration of RvD1 (4µg/kg; i.p.). n=4 per group. (*B*) Levels of CD45⁺F4/80⁺ leukocytes in ischemic skeletal muscle of WT mice on day 3 of HLI after daily subcutaneous administration of RvD1 (4µg/kg; s.c.). n=4 per group. (*A*) two-way ANOVA, **p*<0.05.



Α

Figure S3. Validation of gene expression changes by qRT-PCR and RvD1-induced expression changes in immunity/host defense-related genes. BMDM were stimulated with RvD1 (10nM; 6 hours) then subjected to qRT-PCR or RNA-Sequencing. (*A*) Correlation plot showing fold change (FC) of gene expression in RvD1-stimulated BMDM as measured by qRT-PCR vs. RNA-Seq. The component genes that make up each of the immunity/host defenserelated GO pathways that were upregulated (*B*) or downregulated (*C*) by RvD1 as determined by RNA-Seq analysis are shown in heatmaps listed by decreasing normalized expression. n=6 for qRT-PCR; n=3 for RNA-Seq.





Day Post-HLI

Figure S4. Validation of myeloid-specific *hALX/FPR2* gene deletion and its effect on perfusion recovery in female mice. (*A*) Representative PCR of DNA from tails of *LysM-cre;FPR2*^{fl/fl} and h*FPR2*^{fl/fl} mice. (*B*) Flow cytometric analysis of GFP expression in peripheral blood leukocytes from *LysM-cre;hFPR2*^{fl/fl} and control (*hFPR2*^{fl/fl}) mice. (*C*) Quantification of perfusion recovery measured by laser speckle contrast perfusion imaging in *hALX/FPR2*^{fl/fl} and *hALX/FPR2*^{fl/fl} and mice following HLI. n=5-7 per genotype.