Supplemental Material

To accompany manuscript:

Preservation of Post-Infarction Cardiac Structure and Function via Long-Term Oral Formyl Peptide Receptor Agonist Treatment

(Short title: FPR Agonist & Cardiac Changes Post-MI)

DETAILED METHODS

FPR1 AND FPR2 KNOCKOUT. Formyl peptide receptor (FPR) 1 gene knockout (KO) mice were generated by homologous recombination targeting of 129/SvEvTac embryonic stem (ES) cells through replacement of the Fpr1 coding sequence TACATCGTTCTGGATGTCTTCTCATATTTGATCTTTGCCGTCACATTTGTCCTTGGGGTTCTG GGCAACGGGCTCGTGATCTGGGTGGCTGGTTTCCGCATGAAACACACTGTCACCACCATC TCTTACTTGAACTT with a LacZ-Neo cassette according to methods previously described (1). Correctly targeted ES cells were selected by G418 and confirmed by polymerase chain reaction (PCR) as well as Southern blot analysis. FPR1 gene KO mice could be genotyped by PCR analysis using primers for the Neo cassette GGGTGGGATTAGATAAATGCCTGCTCT and target allele ACCAAATGGCCAATGTCCTCCCATG, whereas the wild-type (WT) mouse could be genotyped with primers for the Neo cassette and the endogenous allele GATGGACACCAACATGTCTCTCCTC. FPR1 gene KO mice were backcrossed to over 99.5% C57BL6/J background using the Speed Congenics service from the Jackson Laboratory.

The FPR2 KO was created to match the published FPR2 KO mouse as described (2), which was deficient in FPR2 and FPR3. Deletion of Fpr2/Fpr3 genes was done by homologous recombination targeting of Taconic Artemis C57BL/6N Tac ES cells. First, the targeting vector was constructed with the Fpr3 gene fragment of the bacterial artificial chromosome (BAC) clones from the C57BL/6J RPCIB-731 BAC library. The Fpr3 gene exon (including 5' untranslated region [UTR] and 3'UTR) was replaced by a LoxP-recombination-site-flanked mouse Fpr3 exon plus Fpr3-recombination-site-flanked positive selection cassette (puromycin resistance). The puromycin-resistance gene expression is under the control of a eukaryotic promoter and contains a polyadenylation signal. Correctly targeted ES cell clones were selected by puromycin resistance and screened by Southern blot analysis. Secondly, the Fpr3 floxed ES cells were targeted again for Fpr2 gene KO. Fpr2 targeting vector was constructed with Fpr2 gene

fragment of BAC clones from the C57BL/6J RPCIB-731 BAC library. The Fpr2 gene exon 2 (5'UTR and coding sequence from ATG to STOP codons) was replaced by an Flp-recombination-site-flanked positive selection cassette (Neomycin resistance) which is expressed under the control of a eukaryotic promoter and contains a polyadenylation signal. The Fpr2 3'UTR containing the polyadenylation site was preserved to prevent any risk of transcriptional read through and putative splicing to downstream elements. Correctly targeted ES cell clones were selected by G418 resistance and screened by Southern blot analysis using probes from PCR primer pairs: (i) sense TCTGGGAATCTTAGGAAACCAAGAG/antisense GAAGTGTCCATCACATATAACAGC; (ii) sense GAAAATCAGGACAGTCTTAGGGTGC/antisense GTTCTAACTGAGAGCCTGCATAGG. After blastocyst injection and chimera breeding, founder mice were identified by PCR analysis using the following genotyping primers: genotyping primers sense GAAACCACACTTATACATTAACAAGG/antisense GAAGTGGAATGAAAACCATCC would detect a 391bp PCR product of the KO allele. Primers sense GTTTCCCTTTCAGCTTGTGG/antisense GAAGTGGAATGAAAACCATCC would detect a 329bp PCR product of the WT allele. The Fpr2/Fpr3 double-targeted mice were first bred with FlpE transgene mice to achieve Fpr2 gene KO, and subsequently bred with Cre transgene mice to achieve Fpr3 gene KO.

COMPOUND SYNTHESIS AND FORMULATION. Compound 43 (Cmpd43, 1-(4-chlorophenyl)-3-(5-isopropyl-1-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea), was synthesized as described (3). Cmpd43 was formulated in suspension vehicle composed of 0.5% carboxymethylcellulose in de-ionized water. Stock suspensions were made by vortexing the compound vigorously with glass beads and sonicating for 20 min. Aliquots for daily use were made and stored at −20°C. Thawed suspensions were vortexed vigorously before administration. Dosing suspension concentration was calculated assuming 100% active ingredient.

MOUSE MYOCARDIAL INFARCTION STUDIES. Male C57BL/6 mice were purchased from the Jackson Laboratory and were 10 to 12 weeks of age at the time of surgery. Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending coronary artery using 2 procedures. For 28-day studies (UC San Diego, CA), mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (8 mg/kg) given via the intraperitoneal route followed by oral intubation with a modified endotracheal tube. Mice were placed in a right lateral position on a circulating-water heating pad for maintenance of normal body temperature and mechanically ventilated at a tidal volume of ~0.5 ml and ~105 respirations per min. The neck and chest areas were shaved and prepped with a 70% isopropyl alcohol followed by betadine solution. After draping for aseptic surgery, the heart was exposed via a left thoracotomy in the fourth intercostal space, and the pericardium incised. The descending left coronary artery was regionally located (*ramus interventricularis paraconalis*). Animals were randomized into treatment groups. The coronary artery was permanently ligated with a silk suture with a tapered curved 6-mm needle. Complete coronary occlusion was confirmed visually by noting the prompt and sustained pallor of the anterior wall distal to the ligation site. For animals designated as sham, the snare was placed but not tightened. The chest was closed in layers and the pneumothorax evacuated. For pain management, a local anesthetic (bupivacaine) was infiltrated subcutaneously along the edges of the incision sites, and buprenorphine (0.05 to 0.1 mg/kg) administered immediately postoperatively. Animals were returned to their cage, given water and standard rodent chow ad libitum, and monitored daily until the terminal procedure. For 3-day studies (Bristol-Myers Squibb), mice were infarcted using a rapid method without the need of artificial ventilation during the surgery. Mice were anesthetized with 2% to 2.5% isoflurane inhalation and the chest cavity was exposed at the 4th intercostal space; the heart was exposed and moved through the incision site by gently squeezing the chest cavity. The coronary artery was ligated using a 6-0 silk suture. Successful artery ligation was

verified by noting the prompt and sustained pallor of the anterior wall distal to the ligation site. The heart was immediately repositioned in the chest cavity, and the incision was closed. Animals were allowed to recover in a warm box and returned to their home cages. Pain management was as described for the 28-day procedure. Mice were dosed daily (QD) with Cmpd43 or with vehicle for 28 days. Dosing was initiated 24 h post-MI. Dosing route was oral via gavage at a dose volume of 5 ml/kg. All dosing was carried out in the morning between 8 and 10 AM.

RAT ISCHEMIA–REPERFUSION STUDY. Male Sprague–Dawley rats with body weight averaging ~225 g at the time of surgery were anesthetized with ketamine (50 to 100 mg/kg) and xylazine (5 to 10 mg/kg) cocktail via intraperitoneal injection. Rats were intubated via a 20-gauge catheter, placed on a mechanical ventilator and ventilated with room air. The fur on the left side of the chest was removed with a clipper and the skin was disinfected with a series of chlorhexidine, alcohol, and betadine scrubs according to standard aseptic procedure. The rat was placed in a supine position on a surgical tray and a 2-cm skin incision was made at the level of the 4th and 5th intercostal space. The pectoral muscles were separated on the left side of the animal. A loose purse string pocket surrounding the incision was formed by using a 3-0 Vicryl suture with a taper needle (Ethicon part number J311H) threaded through the skin and muscle layers. A hole was made in the intercostal space between the 4th and 5th ribs, and the heart was exposed via a small left thoracotomy. The region of the left anterior descending coronary artery was identified (*ramus interventricularis paraconalis*) and transiently ligated (45 min). After release of the ligature, the chest wall and incision were sutured closed; animals were weaned from the respirator and allowed to recover. Sham-operated animals underwent the same procedure without coronary artery ligation. Post-surgical analgesia was given during the recovery phase. Rats were dosed QD with Cmpd43 or with vehicle for 6 weeks after ischemia–reperfusion. Dosing route was oral via gavage at a dose volume of 2 ml/kg. All dosing was carried out in the morning between 8 and 10 AM.

EX VIVO PASSIVE MECHANICS. Following exposure of the heart via thoracotomy, the heart was arrested in diastole by rapid intracardiac infusion of ice-cold cardioplegic solution containing high potassium and 2,3 butanedione monoxime. The heart was excised, the aorta cannulated and the heart perfused with cardioplegia to flush out residual blood. The atrial appendages were trimmed, the hearts weighed, and a cardiac balloon inserted into the left ventricle across the mitral valve. The hearts were mounted onto a pressure–volume (PV) measurement stand and multiple epicardial surface markers placed onto the anterior wall. A digital camera was positioned to enable capture of video images of the anterior surface of the heart. PV curves were obtained by digitally acquiring left ventricular pressure and volume during balloon inflations. There were 2 to 3 conditioning runs followed by 2 to 3 data acquisition runs with maximal left ventricular pressure of ~50 mmHg. Synchronized video images were obtained during each data run. Following acquisition of the PV data, the hearts were perfusion-fixed for 5 to 10 min by infusion of 10% formalin into the aortic cannula while maintaining left ventricular balloon pressure at 10 to 15 mmHg. Hearts were stored in 10% formalin at room temperature for subsequent histologic processing.

FLOW CYTOMETRY. Hearts for flow cytometry were processed for cell isolations. Mice were injected intraperitoneally with 100 units of heparin and then anesthetized with 5% isoflurane. Hearts were perfused with 10 ml cold 1×PBS via the apex of the left ventricle and removed from the chest wall. Connective tissue was removed and hearts were sliced in half along the long axis and rinsed in cold 1×PBS. Hearts were minced into small pieces (2 mm × 2 mm) and placed into a Miltenyi C Tube with 5 ml of digestion solution (472 units/ml collagenase, Type 4:1088 units/ml DNase 1 in 1×HBSS+/+, 2% FBS, 0.2% BSA, 10 mM HEPES). Digestion into a single cell suspension was accomplished using the Miltenyi GentleMACS™ Octo 8 tissue dissociator. The digestion took 35 min to complete. The cell

suspension was passed through a 40-µm cell strainer to remove tissue debris. Residual red blood cells were lysed with ACK Lysis Solution (Gibco Cat# A10492-01). Cell counts and viability were determined by acridine orange/propidium iodide staining on the Nexcelom Auto2000 cell counter. Cell preparations were analyzed for $CD206^{+}$ macrophage populations. For each sample, 2×10^{6} cells were blocked for 15 min in 60 µl of blocking buffer (1×PBS−/− containing 3% fetal bovine serum, 5% mouse serum, 5% rat serum, 1% Fc block, 10 mM EDTA, 10 mM HEPES, and 0.1% sodium azide). Surface markers were then stained by addition of 60 µl of antibody cocktail containing 0.25 µg CD45[PerCP], Clone 30-F11, 0.25 µg Ly-6G[APC-Cy7] Clone 1A8, 0.75 µg CD64[APC] Clone X54-5/7.1, and 0.15 µg CD206[PE-Cy7] Clone C068C2 in blocking buffer. After staining for 40 min on ice, cells were washed and suspended in 1×PBS containing 3% fetal bovine serum, 10 mM EDTA, 10 mM HEPES, 0.1% sodium azide, and 300 nM DAPI. Data were acquired on a Cytek DxP10 FACS Calibur. Compensation was performed using UltraComp antibody capture beads (eBioscience Cat#01-2222). To set gates, Fluorescence Minus One controls were performed for each antibody. Data were analyzed using FlowJo (Version 10). All antibodies were from BioLegend Inc.

PHAGOCYTOSIS. Mice were injected intraperitoneally with 1 ml of 2% BioGel P100 solution (BioRad, Inc.). After 4 days, the peritoneum was lavaged with PBS/2 mM EDTA. Residual BioGel particles were removed by passing the exudate through a 40-µm strainer. Cells were washed with PBS and then seeded into 96-well plates (Costar 3904) at a density of 1.2×10^{5} cells/well. After 90 min, non-adherent cells were removed by washing with PBS and the macrophages were incubated overnight in Macrophage-SFM media (ThermoFisher, Inc.). Macrophages were treated with test compound for 15 min and then fed opsonized FITC-labeled zymosan (1:8 ratio of cells to zymosan) for 45 min at 37°C. Cells were washed and extracellular fluorescence was quenched with 0.025% Trypan Blue. Phagocytosis was measured using a SpectraMAX Gemini EM plate reader (Molecular Devices, Inc.).

HL-60 KO LINES. FPR1- and FPR2-deficient HL-60 lines were created using CRISPR technology. PCR amplicons were designed to amplify and sequence the CRISPR targeted regions out of genomic DNA isolated from HL-60 cells. The following PCR primers were used: FPR1 forward primer: 5'-gctgttgtgggagctctagg; FPR1 reverse primer: 5'-tgtggatcttggtggcaata; FPR2 forward primer: 5'-tgggtctcctggtaggagtg; FPR2 reverse primer: 5'-catgccctttttgtggatct. Sanger sequencing of the PCR amplicons revealed 1 polymorphism in FPR1 compared with human genome build GRCh37, corresponding to SNP rs2070745, for which HL-60 cells were heterozygous G/C. No polymorphisms were found in the FPR2 amplicon. CRISPR guides were designed to not overlap the SNP found for FPR1. CRISPR gRNAs were designed and oligos synthesized, annealed, and cloned into the all-in-one Cas9-2A-GFP/sgRNA vector pXPR004 (BROAD Institute). FPR1 sgRNA sequence: 5'-GATGCAGGACGCAAACACAG; FPR2 sgRNA sequence: 5'-GATGCAGGACACAAATGCAG. NEON electroporation (THERMO) was used to deliver 1µg of the Cas9/sgRNA constructs into 2×10^5 HL-60 cells with the following settings: 1 pulse, 1350 V, and 35 msec. Three days post-transfection, GFP-positive cells were single-cell sorted into 96-well plates using a FACS Aria (B-D). Clones were expanded, then a portion removed and genomic DNA isolated. PCR was performed using the same primers as described above. The amplicons were sequenced by NGS using a MiSeq (Illumina). Clones were identified to be null on the basis of insertions and deletions that disrupted the reading frame for both alleles. The following null clones were selected: FPR1 clone #5: 100% NGS reads show +1bp insertion; FPR2 clone #4: 100% NGS reads show −13 bp deletion. Because only 1 allele was presumably identified by NGS, the PCR amplicons were also TOPO cloned and Sanger sequenced. FPR1 clone #5 sequence data showed the presence of both rs2070745 alleles, indicating that the +1 bp insertion was biallelic. TOPO clones of the FPR2 clone #4 amplicon showed only the presence of a −13 bp deletion, indicating that it too could be a biallelic, or potentially contains an allele with a much

larger deletion that extends beyond 1 of the primer binding sites.

OXIDATIVE BURST ASSAY. HL-60 parental cells or FPR1/2 KO lines were differentiated in 1.3% DMSO for 5 days and used for oxidative burst experiments. A volume of 100 µl of HBSS buffer (Invitrogen, 14025), containing 400 µM of luminol (Sigma Cat# A4685), was used to seed ~100,000 cells/well using 96-well Opti-Plates (Perkin Elmer Cat#6005290). Approximately 2 µl of 50×-concentrated test compound was prepared in assay buffer (1×HBSS/20 mM HEPES/0.1% fatty acid free BSA/DMSO 0.2%) and gently mixed. After a 15-min incubation at room temperature, 3 µl of 1 mg/ml zymosan (opsonized in charcoal-stripped human AB serum) was added to a final concentration of 30 µg/ml. Luminol bioluminescence was recorded every 2.5 min for 60 min at 37°C using LMaxII 384 plate reader; data were analyzed using data at the 30-min time point, which is in the dynamic range of the assay.

CHEMOTAXIS ASSAY. HL-60 cells were differentiated for 5 days in 1.2% DMSO. The assay medium was phenol red-free RPMI with 0.2% fatty acid free BSA. Approximately 1×10^6 cells were added to the upper chamber of a Transwell plate (Corning #3387). Migration was induced by placing chemoattractant in the bottom chamber and the differentiated HL-60 cells in the top chamber. Following migration, differentiated HL-60 cells in the lower chamber (migrated fraction) were quantitated using a luminescence cell-viability assay (Promega, G7571).

BRET-BASED SIGNALING. Several of the plasmid constructs used in the bioluminescence resonance energy transfer (BRET) experiments have been previously described. Briefly, the coding sequence of β-arrestin1 and β-arrestin2 were fused to *Renilla* luciferase II to generate β-arrestin1-Rluc and β-arrestin2-Rluc (4). The membrane targeting motif from K-Ras, CAAX motif (GKKKKKKSKTKCVIM), was

fused to *Renilla* GFP to create rGFP-CAAX plasmid (5). The *Renilla* luciferase II was inserted at specific positions in the Gα proteins to generate Gα-Rluc constructs: Gαi1-Rluc (position 91), Gαi2-Rluc (position 100), Gαi3-Rluc (position 100), GαoA-Rluc (position 99), GαoB-Rluc (position 99), Gα12-Rluc (position 84), and Gα13-Rluc (position 1; N-terminal fusion) (4,6). The Gγ1 protein was tagged with GFP10 to obtain GFP-Gγ1, using the same strategy described previously for GFP-Gγ2 (7). Concerning P115 and PDZ-RhoGEF, the Gα12/Gα13-binding domain of P115 (amino acids 1 to 244) was fused to GFP10 to create P115-GFP, while rGFP was added in the N-terminus of the Gα12/Gα13 binding domain of PDZ-RhoGEF (amino acids 281 to 483) to generate rGFP-PDZ-RhoGEF.

Plasmid constructs encoding FPR1 or FPR2 were generated by optimizing the coding sequence of human FPR1, human FPR2, rat FPR2, and mouse FPR2 (GeneOptimizer from ThermoFisher). An artificial cleavable signal peptide from bovine prolactin (MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS) was added to the N-terminus of the receptors to increase cell surface expression.

BRET-based biosensor assays were carried out using HEK293 cells transiently transfected with the plasmids encoding FPR1 or FPR2 and a given biosensor. HEK293 cells were cultured in DMEM supplemented with penicillin-streptomycin and 10% fetal bovine serum. Two days before the BRET experiment, HEK293 cells were transfected with either FPR1 or FPR2 and the different BRET biosensor components (see below*), using linear polyethylenimine 25 kDa (PEI) at a PEI to DNA ratio of 3:1. Transfected cells were directly seeded in 96-well plates pretreated with poly-D-lysine, at a density of 35,000 cells/well, and maintained in culture for the next 48 h. BRET experiments were carried out using the following procedures: cells were washed once with Tyrode's buffer (137 mM NaCl, 1 mM $CaCl_2$, 0.9 mM KCl, 1 mM $MgCl_2$, 3.6 mM NaH_2PO_4, 5.5 mM glucose, 12 mM $NaHCO_3$, and 25 mM HEPES, pH 7.4) and 90 µl of Tyrode's buffer was added to each well. Cells were equilibrated in their new buffer at room temperature for at least 30 min. After the equilibration period, 10 µl of 10× prolume purple

coelenterazine was added to each well (final concentration of 2 μM). Test compounds were subsequently added to each well using the HP D300 digital dispenser from Tecan (12 singlicate concentrations) and cells were incubated with the compounds for 15 min at room temperature. BRET readings were then collected using a Synergy Neo Multi-Mode reader from BioTek with BRET2 filters 410/515. The BRET signal was determined by calculating the ratio of the light emitted by GFP (515 nm) over the light emitted by the Rluc (410 nm). The ligand-induced change in BRET signal was determined by subtracting the signal obtained in absence of ligand from the values obtained after incubation with the test compounds. Sigmoidal concentration–response curves were generated with those values using a 4-parameter logistic equation (GraphPad Prism software), to determine EC_{50} values of the different compounds.

*Biosensor components co-transfected with FPR1 or FPR2:

- Gαi1 biosensor: Gαi1-Rluc, Gβ1 and GFP-Gγ1
- Gαi2 biosensor: Gαi2-Rluc, Gβ1 and GFP-Gγ1
- Gαi3 biosensor: Gαi3-Rluc, Gβ1 and GFP-Gγ1
- GαoA biosensor: GαoA-Rluc, Gβ1 and GFP-Gγ1
- GαoB biosensor: GαoB-Rluc, Gβ1 and GFP-Gγ1
- Gα12 biosensor: Gα12-Rluc, P115-GFP, Gβ1 and Gγ1
- Gα13 biosensor: Gα13-Rluc, rGFP-PDZ-RhoGEF, Gβ1 and Gγ1
- β-arrestin1 biosensor: β-arrestin1-Rluc and rGFP-CAAX
- β-arrestin2 biosensor: β-arrestin2-Rluc and rGFP-CAAX

RNA ISOLATION AND RT-PCR. Peritoneal macrophages were collected by lavage as described in the preceding section. Cells were lysed directly in Tri-Reagent and RNA was prepared using MagMax-96 (Ambion Cat#AM1839) and analyzed by SYBR-Green RT-qPCR as follows. Total RNA (250 to

500 ng) was reverse transcribed in a 20-µl reaction volume using Q-Script™ reverse transcriptase and a blend of oligo(dT) and random primers according to manufacturer's instructions (Quanta Biosciences, Inc. # 95047). Following cDNA synthesis, each reaction was diluted with 50 µl of nuclease-free water. For each sample, 4 µl was subjected to real-time qPCR in a 20-µl reaction volume using Power SYBR® Green PCR Master Mix according to the manufacturer's instructions (Applied Biosystems Inc.). Each sample was amplified in duplicate for each gene analyzed. Ribosomal protein L30 was used as an invariant control to normalize for input RNA. Gene-specific forward and reverse primer sets were used at final concentrations of 500 nM each and their specificity validated by dissociation analysis. Data were analyzed using the comparative Ct method (2ΔΔCt).

IN SITU HYBRIDIZATION. Affymetrix ViewRNA™ sequential branched-chain-DNA amplification method was used. Proprietary probe sets were designed for mouse arginase-1 (NM_007482, Cat# VB1-17389-01) by the manufacturer. The arginase-1 probe set consists of a series of 20 oligonucleotide pairs, which hybridize to the target RNA of interest. Signal amplification and visualization were achieved by sequential hybridization to a series of branched DNA structures concluding with hybridization to a Type-6 specific alkaline phosphatase probe and Fast Blue substrate for Ubiquitin C followed by hybridization to a Type-1 specific alkaline phosphatase probe and Fast Red substrate for arginase-1. The target mRNAs were visualized using a standard bright field microscope and scanned at 20× using eSlide Manager™ (Leica Biosystems Imaging, Inc., Vista, CA) followed by quantitation of target using the HALO™ Digital image analysis program (Indica Labs, Corrales, NM).

SUPPLEMENTAL REFERENCES

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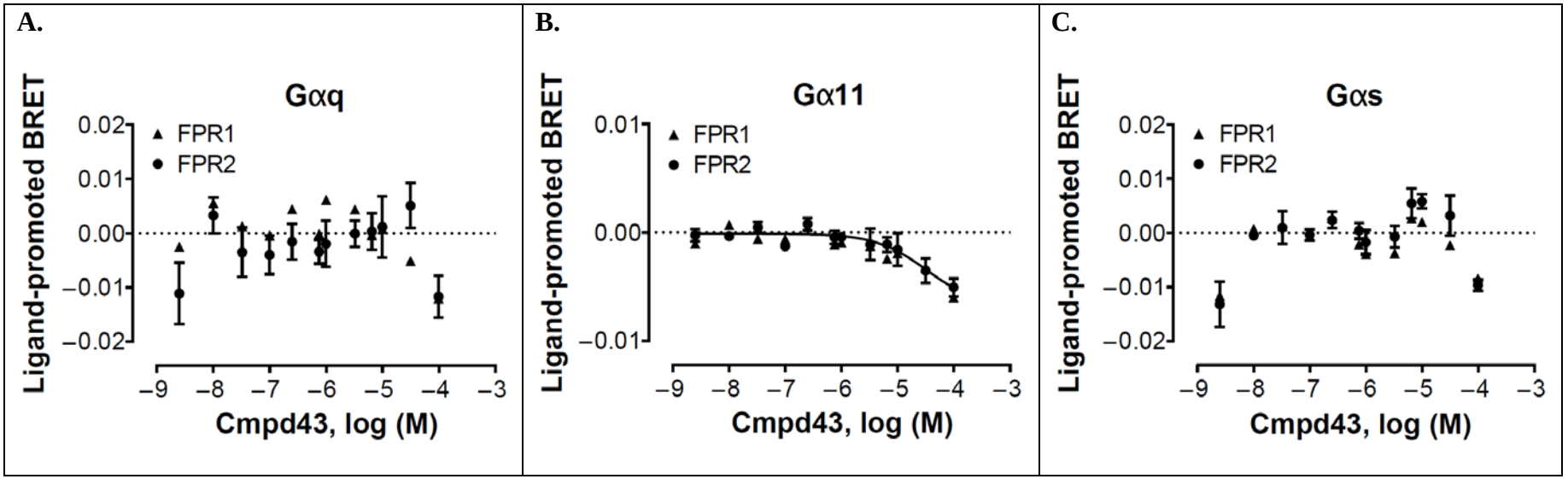
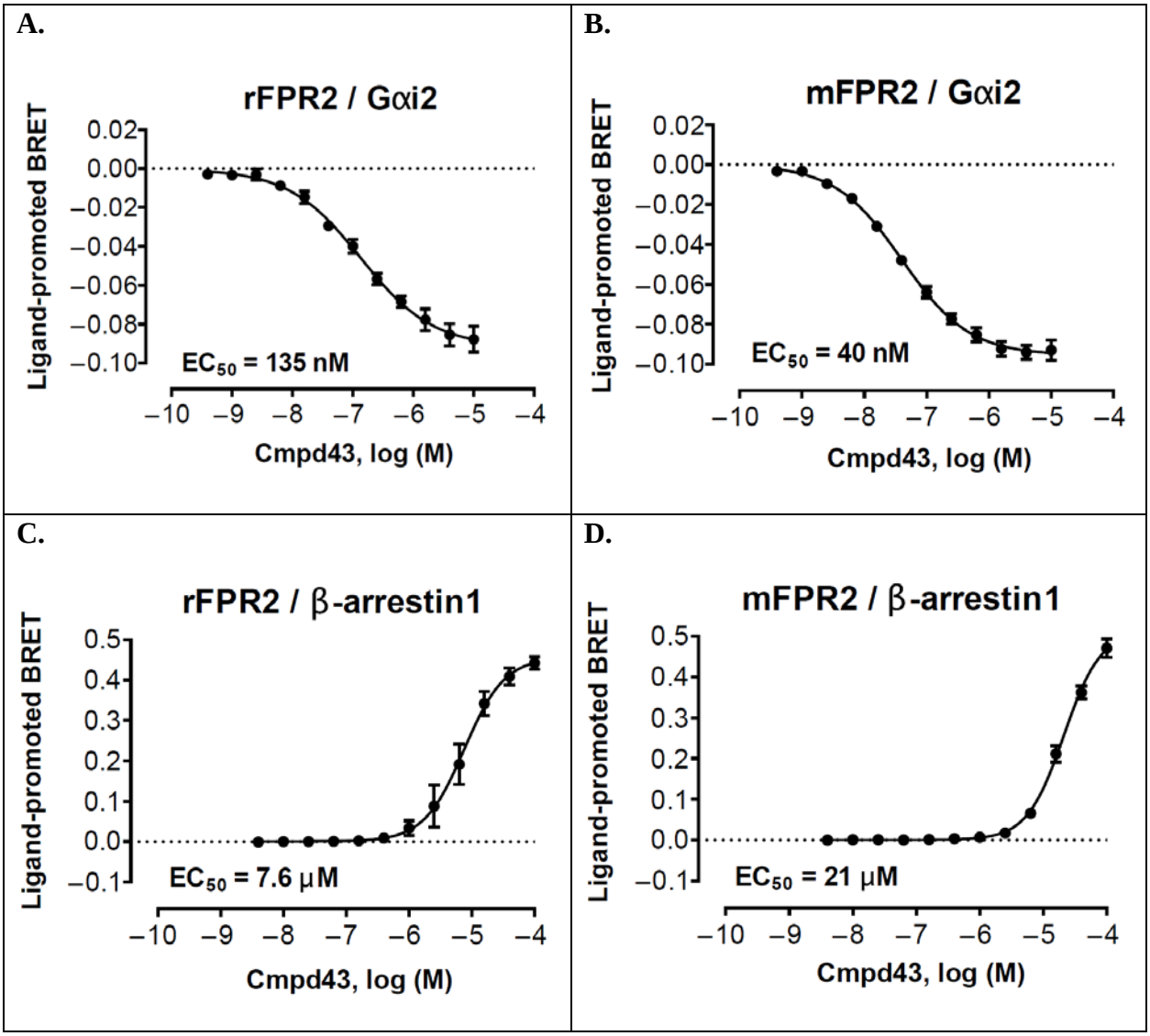
7. Gales C, Rebois RV, Hogue M et al. Real-time monitoring of receptor and G-protein interactions in living cells. Nat Methods 2005;2:177-84.

SUPPLEMENTAL FIGURE S1 Lack of Cmpd43 Activity on Gαq, Gα11, and Gαs Signaling Pathways, in HEK293 Cells Expressing Human FPR1 and FPR2 Receptors



The ability of Compound 43 (Cmpd43) to engage different signaling pathways was assessed using bioluminescence resonance energy transfer (BRET) biosensors detecting the activation of Gαq **(A)**, Gα11 **(B)**, and Gαs **(C)**. HEK293 cells expressing human FPR1 or FPR2 were stimulated with Cmpd43, and modulation of the BRET signals from the different biosensors was recorded. Data represent the average ± SEM of 3 independent experiments. FPR = formyl peptide receptor; SEM = standard error of the mean.

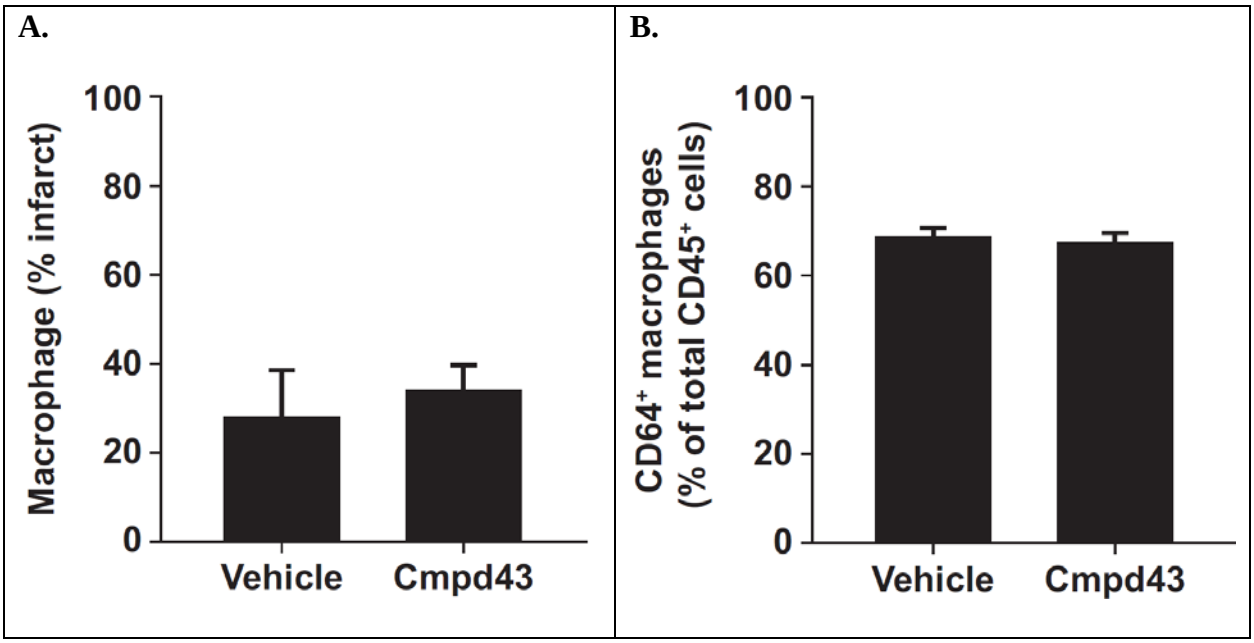
SUPPLEMENTAL FIGURE S2 Activation of Gi Protein and β-Arrestin Recruitment After Stimulation of Rat and Mouse FPR2 with Cmpd43



The ability of Compound 43 (Cmpd43) to stimulate rat FPR2 (rFPR2) **(A, C)** and mouse FPR2 (mFPR2) **(B, D)** was assessed using bioluminescence resonance energy transfer (BRET) biosensors detecting the activation of Gαi2 **(A, B)** and the recruitment of β-arrestin1 **(C, D)**. HEK293 cells expressing either rFPR2 or mFPR2 were stimulated with Cmpd43, and modulation of the BRET signals from the different biosensors were recorded. Data represent the mean ± SEM of 3 to 4 independent experiments. FPR = formyl peptide receptor; SEM = standard

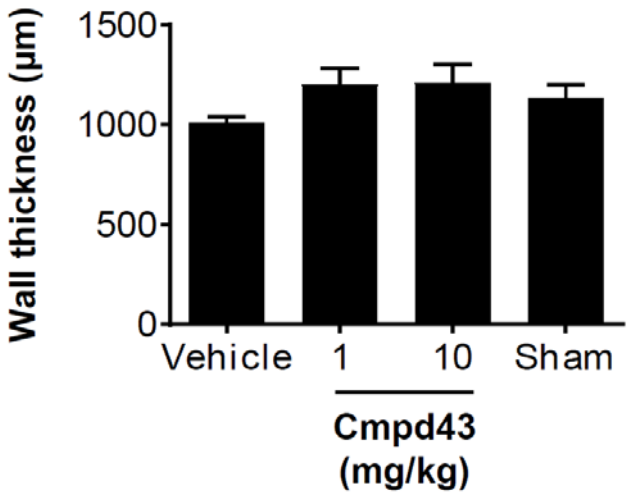
error of the mean.

SUPPLEMENTAL FIGURE S3 Macrophage Levels in the Mouse Heart ~3 Days Post Myocardial Infarction



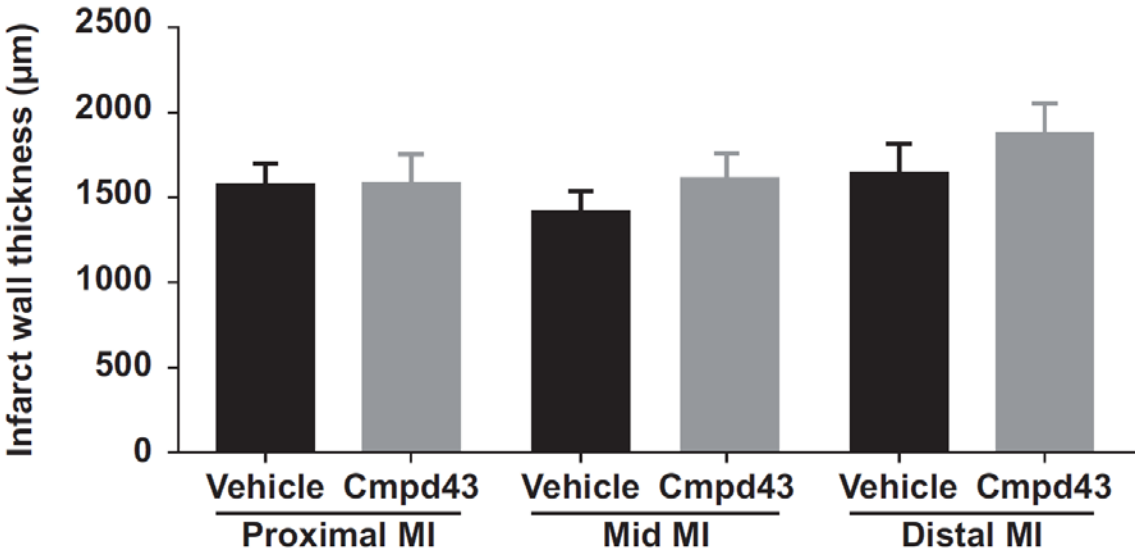
Inflammatory cell content was measured histologically in the infarct border zone of mice ~3 days post myocardial infarction. (**A**) Paraffin-embedded specimens of the left ventricle (n = 3/group) were processed with hemotoxylin and eosin, and the peri-infarct zone was analyzed for inflammatory cell content. Macrophages were identified by cellular morphology and quantified. (**B**) Flow cytometry of $CD64^+$ macrophages as a percentage of total immune cells ($CD45^+$) is shown. Cells were gated on $CD45^+$ cells > viable cells > single cells. No statistically significant differences with Cmpd43 treatment relative to vehicle were detected via histology or flow cytometry. Cmpd43 = Compound 43.

SUPPLEMENTAL FIGURE S4 Contralateral Wall Thickness



Transmural wall thickness was measured in the contralateral septal wall across from the mid-infarct zone. No statistically significant differences from vehicle were detected. Cmpd43 = Compound 43.

SUPPLEMENTAL FIGURE S5 Transmural Wall Thickness



Transmural wall thickness was measured at various regions across the infarct zone. No statistically significant differences in wall thickness between vehicle- and Cmpd43-treated rats were detected in any region. Cmpd43 = Compound 43; MI = myocardial infarction.