**Materials and Methods:**

Statement of scientific and experimental rigor: Mice were randomized into treatment groups and measurements were performed in a blinded fashion. Investigators and Mouse Metabolic Phenotyping Core technicians were blinded to treatment conditions and researchers were not made aware of the treatment allocations until all data have been collected and analyzed. Echo data analyses and statistical analysis were independently performed by two 'blinded researchers' using one-way ANOVA with repeated measures. Surgeries were performed by a highly experienced surgeon who has done over 2,000 of these surgeries.

**Reporter assay**

Cardiac fibroblasts were plated in six well plates and transfected with a GAL4 responsive luciferase reporter (pG5-luc) and expression vectors for a GAL4 DNA binding domain (GAL4-DBD) full length SRC-1, -2 or -3 fusion construct (pBIND-SRC-1, pBIND-SRC-2 or pBIND-SRC-3) or pBIND control using Lipofectamine 3000 as per the manufacturer's protocol (Invitrogen). 24 hours post transfection, cells were treated with 6 μM MCB-613 or DMSO and incubated overnight. Post treatment cells were lysed and total protein was isolated using the Promega luciferase assay system according to the manufacturer's protocol. Protein concentration was measured using a Bradford assay (Biorad). Relative light units were measured and normalized to total protein concentration.

**Model of heart failure in adult mice**

Adult (8-10 week old) ICR (CD1) mice were used for all studies. To induce MI in eight-to ten-week-old mice, the left anterior descending (LAD) artery was permanently ligated

by an experienced mouse cardiac surgeon (over 2,500 ligations) as previously described (22). Briefly, mice were anesthetized with 2% isoflurane and then intubated. The heart was exposed by performing a thoracotomy through the fourth or fifth intercostal space and an 8-0 nylon suture was tied around the LAD. The initial dose of MCB-613 or vehicle control was administered intraperitoneally at 20 mg/kg, two hours after surgery in a blinded fashion. 20 mg/kg MCB-613 was previously shown to prevent breast cancer tumor growth *in vivo* (1). Subsequent injections at the same time of the day and same dose were given for six additional days then repeated doses were given for three days at week eight. Echos were measured in blinded fashion at baseline, 24 hours, 14 days, 56 days, 70 days, and 80 days post-MI. Echo values at day 70 were elevated 2 standard deviation away from mean value from 24 hour, 14, 56, and 80 days for both the control and drug-treated groups suggesting a systemic error and data points were not included in our analysis (2). In addition, no user or data collection errors could be identified. Mice were harvested at the indicated time points for analyses. For all experiments, investigators and Mouse Metabolic Phenotyping Core technicians were blinded to treatment conditions and researchers were not made aware of the treatment allocations until all data had been collected and analyzed. All mice in this study survived starting at the time of the drug or control injections. All mice had an immediate drop in ejection fraction post-MI and visual evidence of an intact ligation thread and local cardiac tissue blanching at the time of harvest indicating successful coronary artery ligation in all.

**Echocardiography**

Cardiac function was determined by echocardiography (VisualSonics, Vevo 2100, 40Mhz-550S probe). After alignment in the transverse B-mode with the papillary muscles,

cardiac function was measured on M-mode images.  Echo measurements and ejection fraction measurements were performed by the Mouse Phenotyping Core and obtained in a blinded fashion.

**Histological analysis**

Whole hearts were fixed with 10% formalin, embedded in paraffin, and sectioned at 7μm intervals. Each slide had three to 10 sections, which started at the apex and ended at the suture ligation site (approximately 30-50 slides). Sections 1, 5, 10, 15 and 30 were stained with Picrosirius red to calculate average percent fibrosis area of the LV myocardium (3).

**Electron Microscopy**

Mice were sacrificed and hearts were quickly removed and placed directly into cold primary fix (2% paraformaldehyde + 2.5% glutaraldehyde + 2mM $CaCl2$ in 0.1M cacodylate buffer, pH 7.4) where they were sliced in cross-section then held in cold primary fix for 4 days. After fixation, tissues were stained with 0.1% tannic acid in 0.1M cacodylate buffer, rinsed and osmicated for one hour, after which the tissue was rinsed in $dH2O$ and counter-stained in aqueous uranyl acetate. Once again the tissues were rinsed in $dH2O$ and then dehydrated in a gradient series of ethanol (50, 70, 80, 90, 95 and 100%). Tissues were slowly infiltrated over a period of four days with increasing dilutions of plastic resins to ethanol, respectively, until 100% plastic was reached. After a full day of infiltration in three changes of 100% plastic, the tissues were embedded in freshly made Spurr's Low Viscosity resin (45) and polymerized at 60oC overnight.  Ultra-thin sections of 55-65 nm were cut with a Diatome Ultra45 diamond knife, using a Leica UC7 ultramicrotome. Sections were collected on 150 hex-mesh copper grids and viewed

on a Hitachi H7500 transmission electron microscope. Images were captured using an AMT XR-16 digital camera and AMT Image Capture, v602.600.51 software.

**Isolation of cardiac cells**

Mice were placed under the surgical plane of anesthesia before cervical dislocation. The hearts were removed and cells were isolated by Langendorff retrograde perfusion of calcium-free pH 7.4 Tyrodes solution (130 mM NaCl, 74.55 mM KCl, 0.5 mM MgCl, 0.33 mM NaH2PO4, 0.25 mm HEPES, 22 mM glucose) with collagenase, 1 mg/mL for 15 minutes. The hearts were then removed from the apparatus, and finely minced in the same Tyrodes buffer with 15mg/mL BSA before trituration with a glass pipette. Cardiomyocytes were then pelleted by differential centrifugation, 300 RPM for 3 minutes. Following the protocol from Skelly et al., (29) the supernatant containing the noncardiomyocyte population of cells was then filtered through a 70 micron filter and pelleted at 750g, and resuspended in 1.1mL 2% FBS in PBS. 0.1mL was removed for the "no stain control" for FACS. The other 1 mL was incubated with 4 $\mu$g/mL Calcein Blue and 10 $\mu$M DyeCycle Ruby and incubated at 37o C for 10 minutes. The cells were then spun down at 600G, and resuspended in 0.5mL 2% FBS/PBS containing Sytox Green (30 nM). Cells were then sorted for: Sytox Green-, Calcein+, DyeCycle Ruby+ into 0.4% FBS in PBS using a FACS Aria ii cell sorter. They were then pelleted and re-suspended in 100 $\mu$L 0.4% FBS in PBS, counted, and passed through the 10x Genomics Chromium system.

**Single Cell RNA-seq Analysis**

We captured 4,382 cells from two saline-treated mice, 8,204 cells from two MCB-613 treated mice 24 hours post-MI, 24,154 cells from two saline-treated mice and 25,859 cells from two MCB-613 treated mice 12 weeks post-MI for analysis. Illumina's Raw Fastq files were processed using 10x Genomics CellRanger (version 3.1.0) with default settings. Aligned Data was analyzed using Seurat v 3.1 integrated vignette (4, 5) for all samples and time points. Each data set was subject to SCTransform vignette before starting integrated vignette. For FindVariableFeatures, command was set to find 15000 nfeatures for each sample. We used both JackStraw (6) and a heuristic approach to determine optimal number of Components for downstream analysis, and UMAP graph-based visualization. To avoid over clustering, resolution during FindClusters was initially set to 0.1 to broadly identify major cell populations and separated for further analysis. Cells markers from previous single cell analysis of non-cardiomyocytes in mouse hearts were used to label major cell types (7, 8), Chi-square statistical analysis for cluster composition and visualization was done as described before (9). Differentially expressed genes were determined for each cell type between Drug treated and control using Seurat, FindMarkers. Genes with log fold change greater that 0.75 or less than -0.75 was used to order cells for Pseudotime analysis using Monocle2 (10-12). For the ligand receptor analysis we used significant (<0.05, qvalue) differentially expressed ligands that changed in response to Drug treatment, and the median expression of receptors of each cell cluster. We used R package iTALK to analyze and visualize potential changes of cell to cell communication in response to drug treatment (13).

**Granulocyte isolation**

Bone marrow cells were isolated from the rear legs of mice treated with control or MCB-613 for 24 hours. Rear legs were removed and placed in ice-cold Hanks balanced salt solution (HBSS) (without Ca/Mg) plus 2% FBS. Both ends of the bone were cut and the bone marrow was flushed with ice cold HBSS with 2% FBS using a 26G needle. Clumps were broken up with an 18G needle and filtered through a 70 μm filter and centrifuged at 400g for 10 min at 4°C. The pellet was suspended in RBC lysis buffer (BD Pharmigen) and incubated at room temperature for two minutes. 8 ml of HBSS buffer was added and spun at 400g for 10 mins at 4°C. Viable cells were counted by trypan blue exclusion and bone marrow granulocytes were isolated using a mouse Neutrophil Isolation Kit from Miltenyi Biotec according to manufacturer's instructions. Total RNA was isolated from granulocytes using the Qiagen RNA isolation kit according to the manufacturer's protocol. cDNA was prepared with the VILO master mix reagent.

**Western blots**

For cell lysates, NETN buffer with 10% glycerol was used to lyse cells and isolate total protein. All lysis buffers were supplemented with protease and phosphatase inhibitors. 50-70 μg of cell lysate protein was loaded onto a 4-15% gradient gel (Biorad) and transferred onto a PVDF membrane. Immunoblotting was carried out using antibodies for SRC-1, SRC-2 (Abcam), SRC-3 (Cell Signaling), and Hsp90 (Cell Signaling). HRP conjugated anti-rabbit and anti-mouse secondary antibodies were used at dilutions of 1:2,500. Pierce ECL was used for chemiluminescent detection.

**Immunostaining**

Hearts were perfused with cardioplegic 20 mM KCL-PBS and then with 10% neutral buffered formalin before drop fixing and then processing into paraffin wax. 5 micron sections were then cut and placed onto slides. Lyz1 immunofluorescence was performed by first removing the paraffin and then rehydrating the sections. After that, we performed antigen retrieval (Antigen unmasking solution, Tris-based, Vector Labs cat#H-3301). Sections were permeablized with 0.1% tween20-PBS, blocked with 10% goat serum in 1% tween20-PBS, and then incubated with primary antibody in blocking solution (1:200 Rabbit anti-Lysozyme, Abcam cat#AB108508, alpha-SMA Abcam cat# ab5694), followed by secondary (1:200 Goat anti-Rabbit, Alexa 488), and then Rhodamine-conjugated WGA (1:250 Vector Labs Cat#RL-1022) and DAPI (1:500 Thermo Fisher Scientific Cat#62248). Images were taken on a Zeiss LSM780 confocal microscope. LYZ+ cells were counted manually from random images spanning the entire myocardium of the left ventricle below the left anterior descending coronary artery occlusion surgery. n = 3 hearts/group, > 10mm2 imaged/heart, 24 hours post-MI surgery. Cleaved Caspase-3 was detected with Cleaved Caspase-3 (Asp175) Antibody (Alexa Fluor® 488 Conjugate, Cell Signaling cat #9669). Cleaved caspase-3 + cells were counted in 6 images per heart at 60x from 3 control and 3 MCB-613 hearts 24 hours and 72 hours post-MI and presented as percent of cells per image.

**RAW264.7 treatment with MCB613**

RAW264.7 cells were cultured in complete DMEM (4.5 g/L glucose supplemented with 10% FBS, 1 mM Na Pyruvate and 1% penicillin/streptomycin). Cells were split using Corning$^{TM}$ Cell stripper solution and plated on 100 mm standard tissue culture dishes at 2 x $10^6$ cells per plate. Next day plates were either left untreated or stimulated with LPS

(100 ng/mL) and IFN-y (100 ng/mL) to induce an M1 pro-inflammatory phenotype. Cells were then incubated for 8-hrs to allow for activation. Immediately after polarization, cells were either left untreated or administered 5 $\mu$M or 10 $\mu$M MCB-613 and allowed to incubate overnight. The following day cells were collected for RNA isolation with the RNeasy Mini Kit (Qiagen).

**Quantitative PCR (qPCR)**

For granulocytes, qPCR analysis was carried out using the Taqman kit with primers for Tlr7, Lcn2 and 18s.  For cardiac fibroblasts and macrophages, first-strand synthesis was performed using the SuperScript VILO MasterMix cDNA synthesis kit (ThermoFisher) per the manufacturer's instructions. Quantitative gene expression was performed using SYBR Green or Taqman reagents (Applied Biosystems) using gene-specific primers. Melt curves were performed for each primer sets that were designed to be gene specific. Data were graphed relative to an internal standard (Tbp or 18s) using the $\Delta\Delta$Ct method.

**VO2 and VCO2 measurements**

VO2 and VCO2 were measured by indirect calorimetry using a progressive maximal exercise test until mice reached exhaustion.  Measurements were obtained by the Mouse Phenotyping Core facility in a blinded fashion.

**Statistics**

Results are reported as the mean ± SEM. The statistical significance of the difference between means was assessed using the unpaired 2-tailed Student's *t* test (for the comparison of 2 groups).   *P* less than 0.05 was considered significant. We expect based on our previous study measuring MCB-613 effects on ejection fraction, N=30 mice will be

required to evaluate significance to detect a difference between means for ejection fraction measurements. All Echo data analyses and statistical analysis were independently performed by two 'blinded researchers' using one-way ANOVA with repeated measures.

## Study Approval

All animal studies and protocols were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine and conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

## Data Availability

Data are available in the Gene Expression Omnibus (GEO) under accession number GSE157542.

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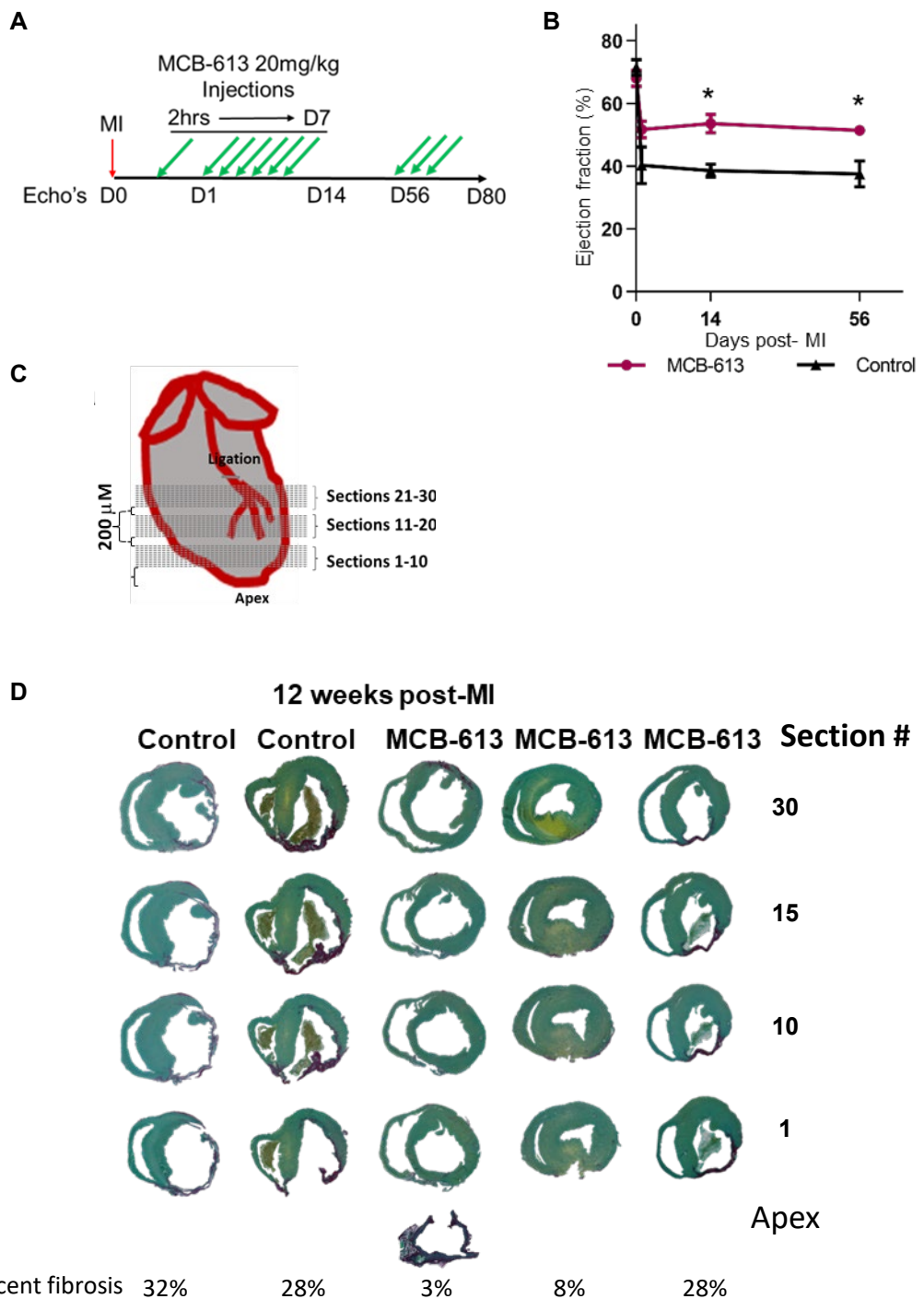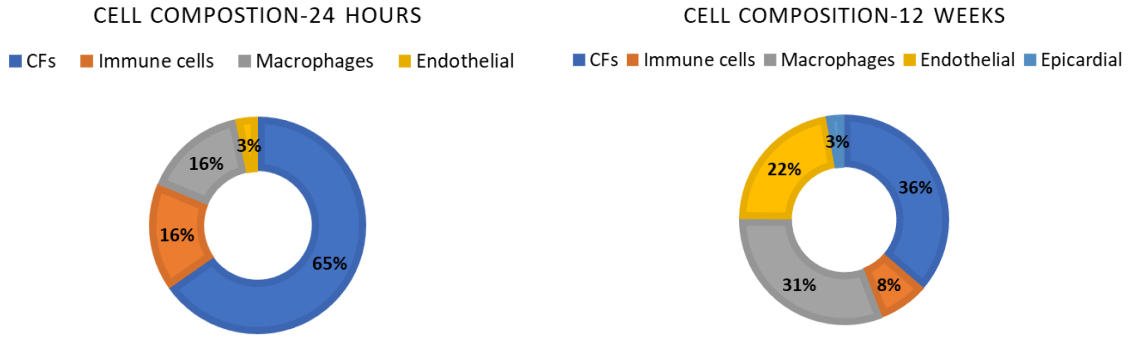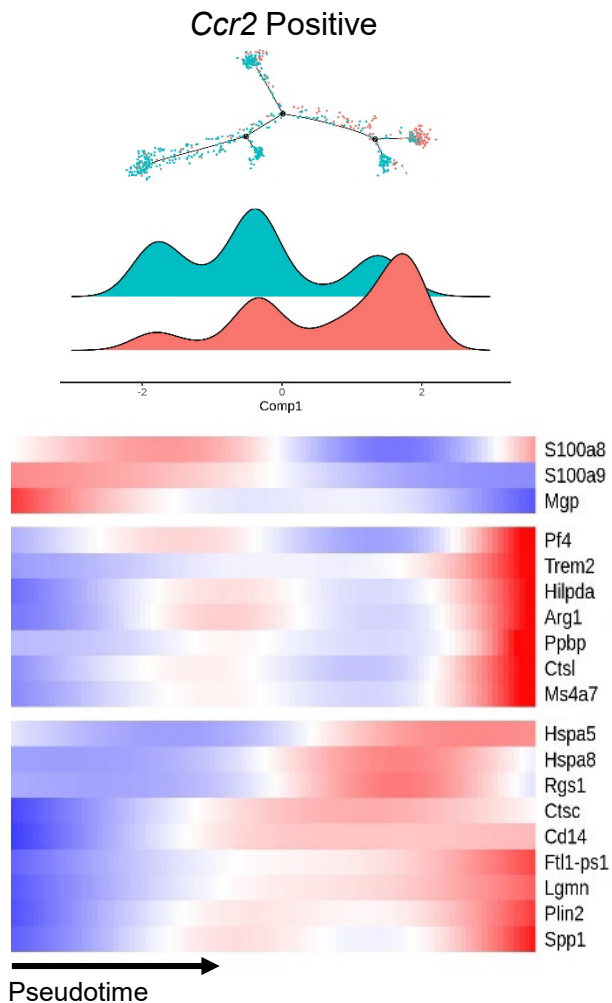
**Supplemental Figure 1.** (**A**) Schematic representation of experimental procedures. Mice were treated with MCB-613 or control two hours after permanent ligation of the left anterior descending coronary artery and for three additional days and at week eight. (**B**) 20 mg/kg MCB-613 or vehicle control was injected IP two hours after surgery and at days two and four. Ejection fraction was measured by echocardiography at the indicated times. Values shown are mean ± SEM; Student's t-test = *P n=6 14 days *P< 0.006; 6 weeks *P< 0.006. (**C**) Diagram of heart tissue sectioning. The apex was removed and discarded, except for one heart as shown, due to the expected presence of severe fibrosis at the apex following MI in all heart samples. Following removal of the apex, three groups of ten 5 μM cross sections toward the ligation, including the papillary muscle region, were placed onto slides for histologic analysis. (**D**) Picrosirius red stain of sections 1, 10, 15 and 30 from hearts of control and MCB-613- treated mice 12 weeks post-MI.
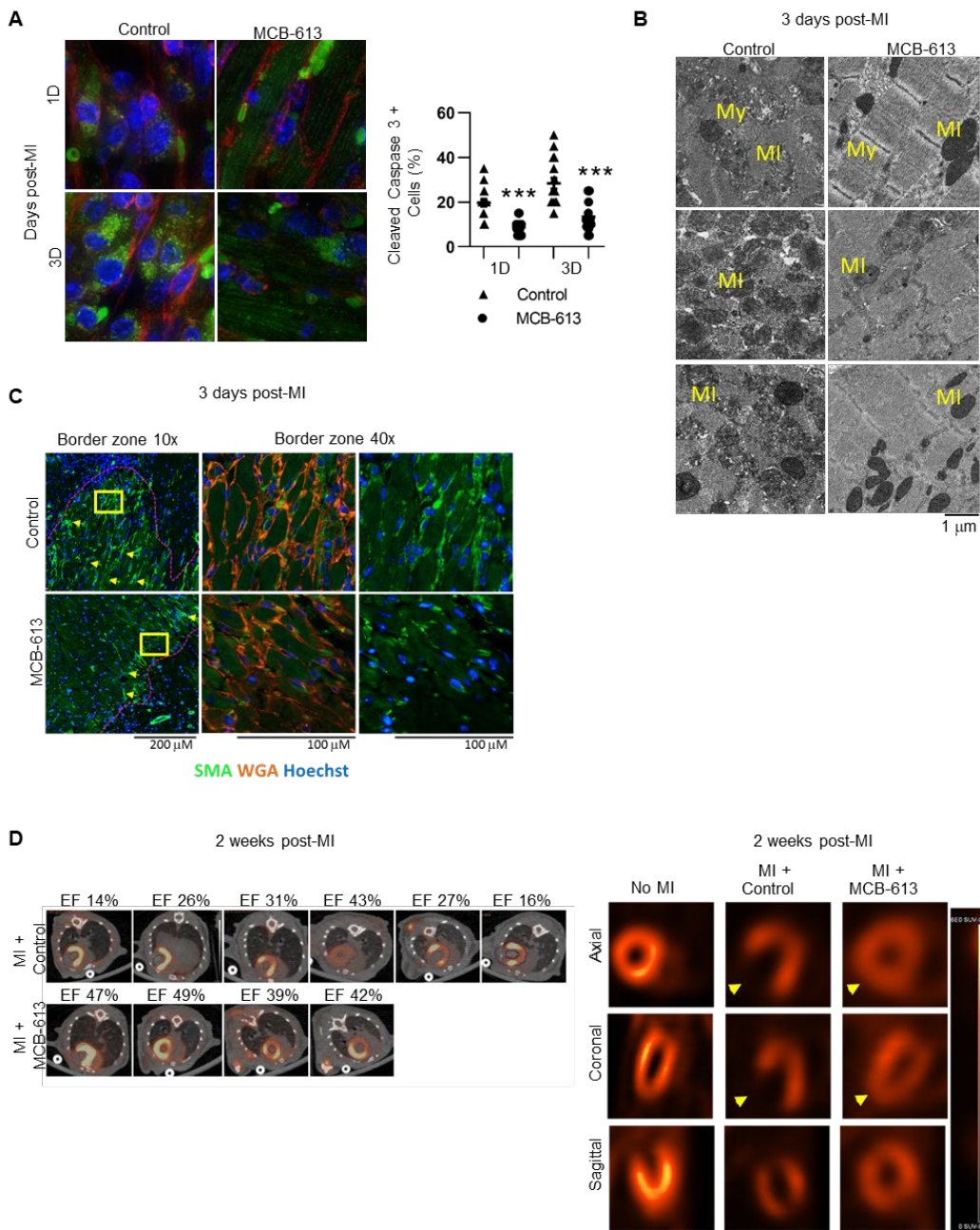
**Supplemental Figure 2. MCB-613 protects myocardium post-MI.** (**A**) Representative images of cleaved caspase -3 immunostaining one and three days post-MI. Values shown are mean ± SEM (n = 3); Student's t-test (*P = 0.02, * P = .005). (**B**) Representative electron micrographs of cardiac tissue from the left ventricle infarct zone 72 hours post –MI. n=3 hearts/group. Arrows indicate Z lines, mitochondria (Mi); myofibrils (My). Hearts from saline-treated mice contain randomly distributed mitochondria and disorganized myofibrils. Hearts from MCB-613-treated mice contain regular and continuous sarcomeres with intervening mitochondria with normal cristal architecture. Scale bars = 1000nm. (**C**) Representative SMA staining from control and MCB-613 treated hearts three days post-MI. Left panels are low magnification with the infarct border zone demarcated with a dashed line. Right panels are high magnification. n=3 hearts/group. (**D**) Composite PET images normalized for signal and infarct region from 6 control and 4 drug-treated animals (left panel). Representative image of center slice of mouse heart in axial (short axis), coronal (long axis), and sagittal views showing differences in morphology and $^{18}$F-FDG uptake between control (no MI), MI, and MI plus MCB-613 at two weeks post-MI (right panel). Arrow indicates infarct zone. *n* = 6 control no MI.

**A**

CELL COMPOSTION-24 HOURS

■ CFs ■ Immune cells ■ Macrophages ■ Endothelial

CELL COMPOSITION-12 WEEKS

■ CFs ■ Immune cells ■ Macrophages ■ Endothelial ■ Epicardial



**B**

*Ccr2* Positive



Pseudotime

**Supplemental Figure 3. (A)** Cell count composition by major cell type at 24 hours and 12 weeks. **(B)** Pseudo-time trajectory of Ccr2 positive macrophages (top). Density plot of Ccr2p macrophages across the pseudotime trajectory (bottom). Heatmap showing dynamics of gene expression for Ccr2p macrophages across the pseudotime trajectory.
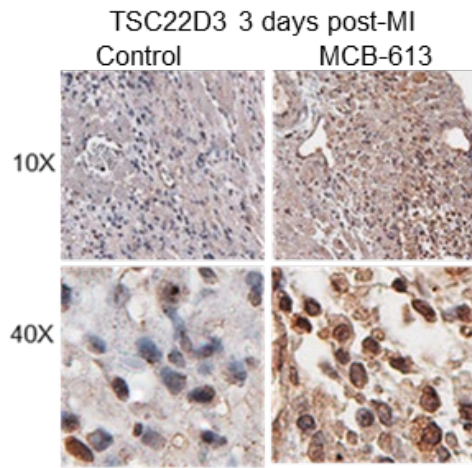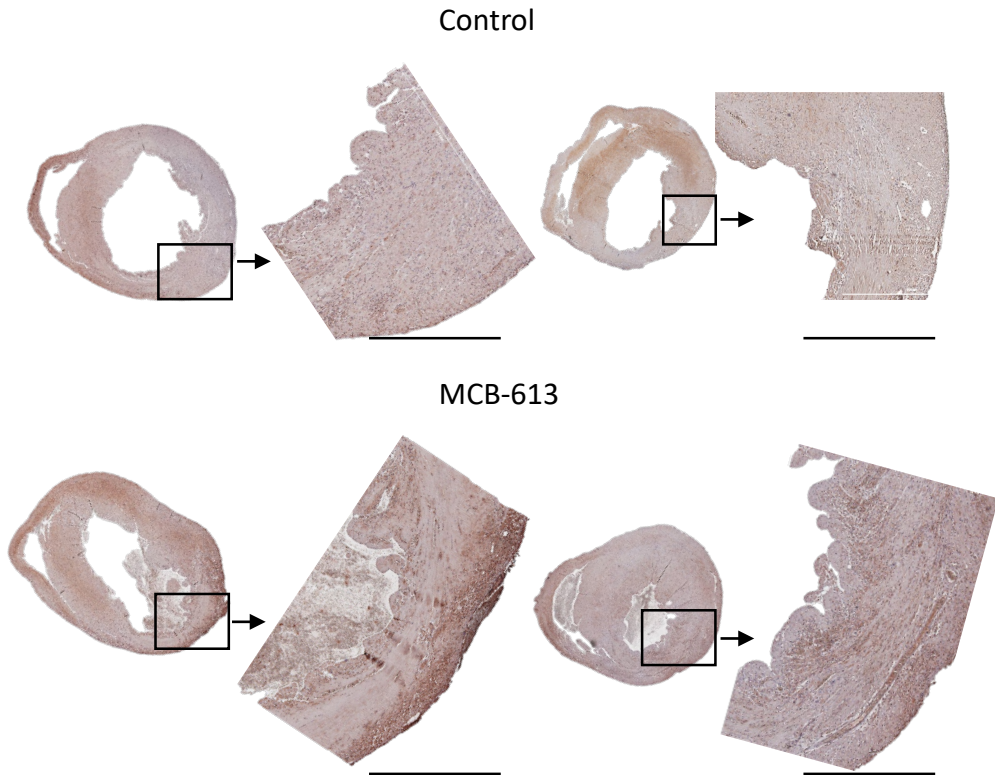
**Supplemental Figure 4. (A)** Representative TSC22D3 immunostaining of 3 heart tissue sections from control and MCB-613-treated mice 3 days post-MI. **(B)** Replicate TSC22D3 staining from control and MCB-613 treated hearts 3 days, post-MI. Low magnification from endocardium to epicardium at border zone area and 10X magnification of boxed in area. Scale bar 1000 μm.
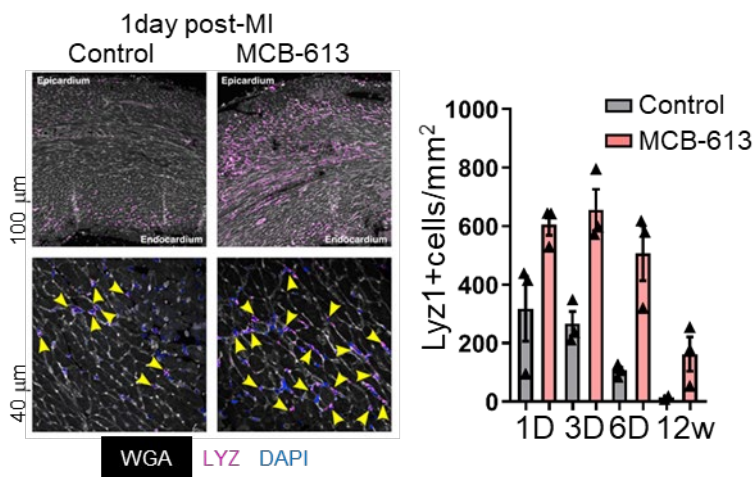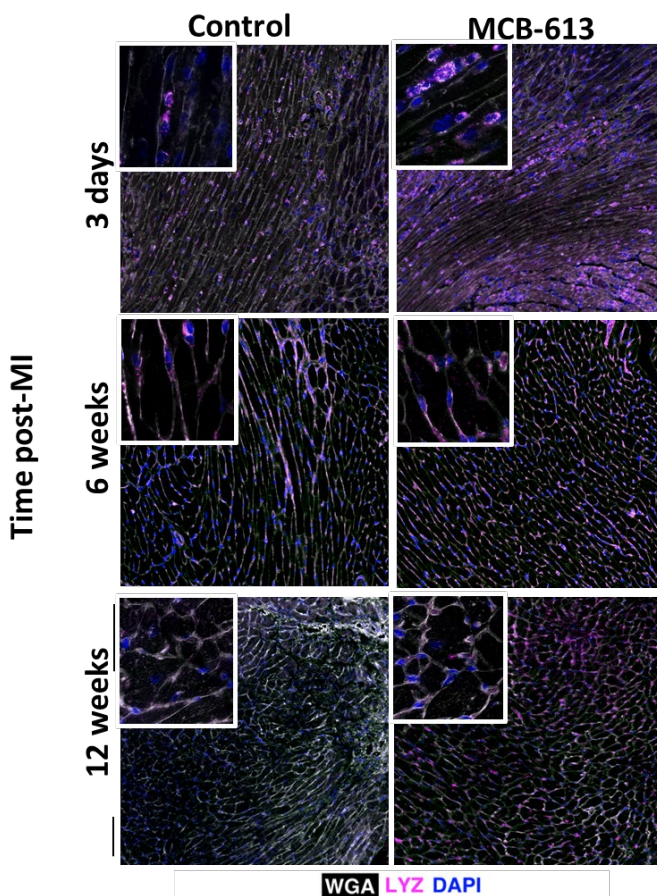
**Supplemental Figure 5. (A)** Representative LYZ staining from control and MCB-613 treated hearts 24 hours post-MI. Top is low magnification from endocardium to epicardium. Bottom is high magnification of sub-endocardial regions. Yellow arrows indicate LYZ+ cells. Quantification of LV density of LYZ+ cells. n=3 hearts/group, > 10mm$^2$ imaged/heart, 24 hours post-MI surgery. P < *0.039, *0.009, *0.01. **(B)** Replicate LYZ staining from control and MCB-613 treated hearts 3 days, 6 weeks and 12 weeks post-MI. Low magnification from endocardium to epicardium. Image inset is high magnification. Scale bar
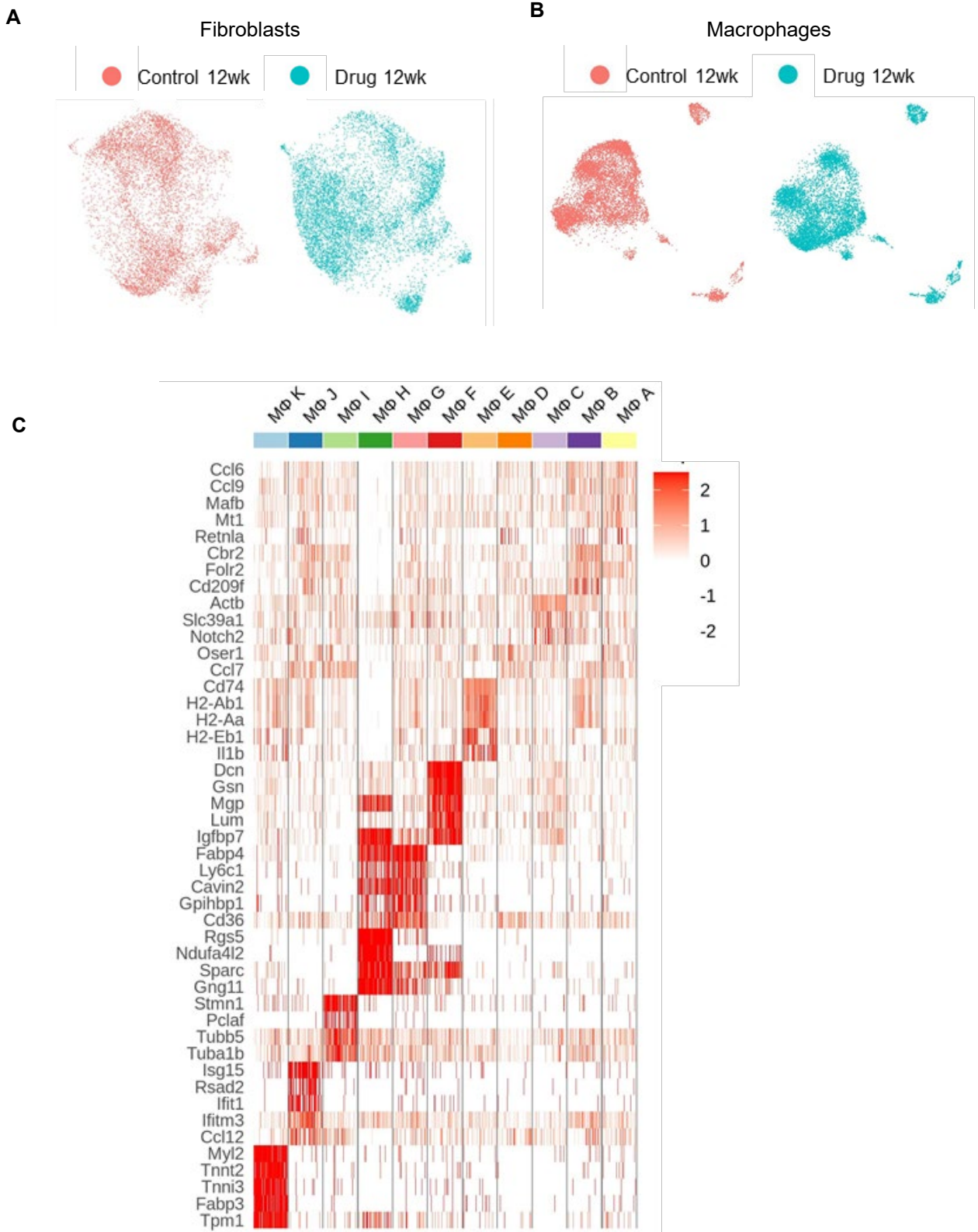
**Supplemental Figure 6. (A)** UMAP plot of fibroblasts from control (salmon) and MCB-613 (aqua) 12 weeks post-MI. **(B)** UMAP plot of macrophages from control (salmon) and MCB-613 (aqua) 12 weeks post-MI. **(C)** Heat map showing top 5 differentially expressed genes in macrophages 12 weeks post-MI.
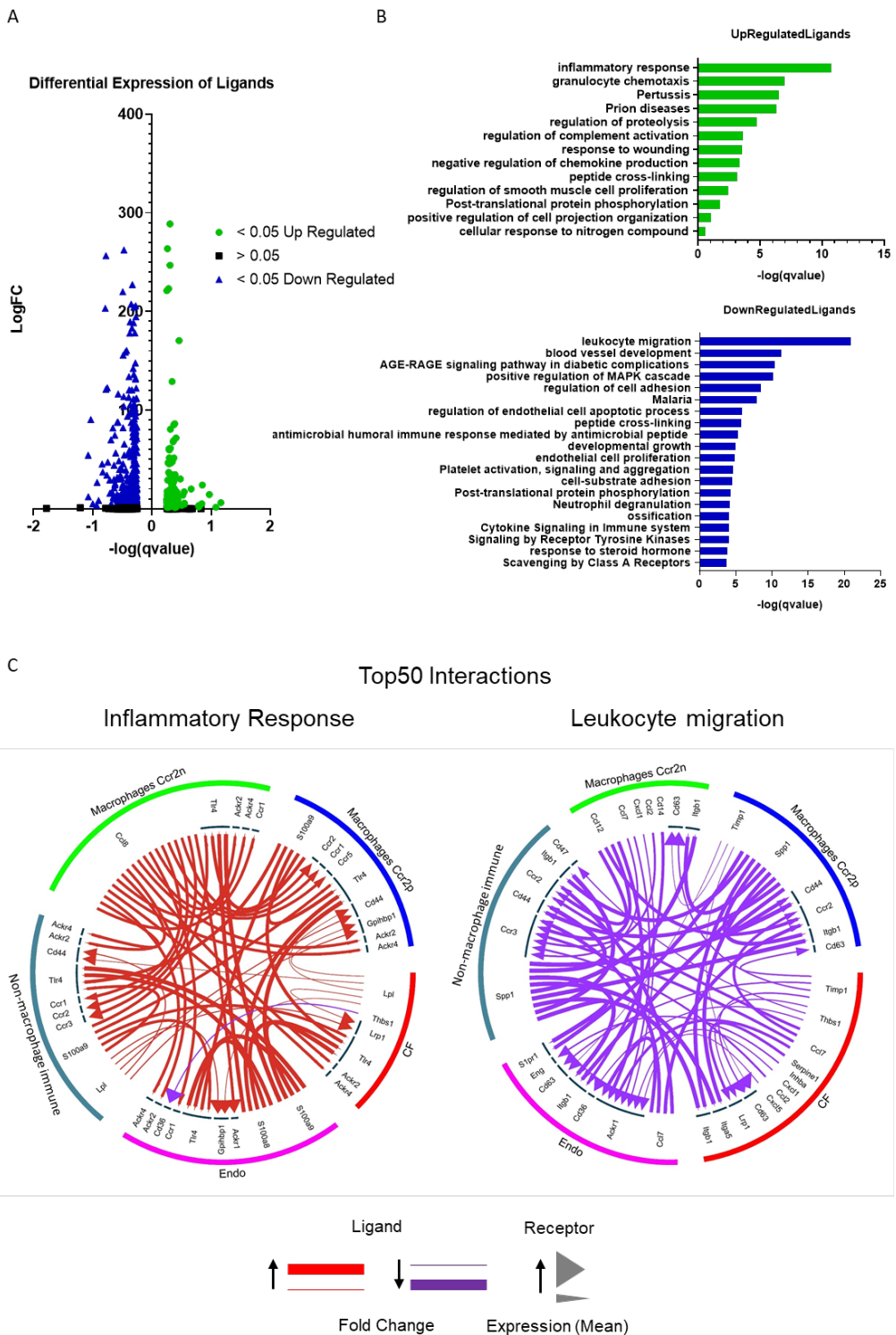
**Supplemental Figure 7. MCB-613 suppresses inflammatory response and leukocyte migration communication 24 hours post-MI. (A)** Analysis of up and down-regulated cytokines and receptor pairings of intercellular communication between cardiac cell types excluding cardiomyocytes. **(B)** GO-terms analysis of up and down-regulated ligands. **(C)** Ligand-receptor pairings between up and down-regulated ligands for identified inflammatory response and leukocyte migration categories. The line color indicates communication between the two cell types (up, red and down, purple). Directionality of the ligand-receptor pairing begins at the ligand and ends at the cognate receptor (arrowhead). The thickness of the line reflects the number of ligand-receptor pairings.
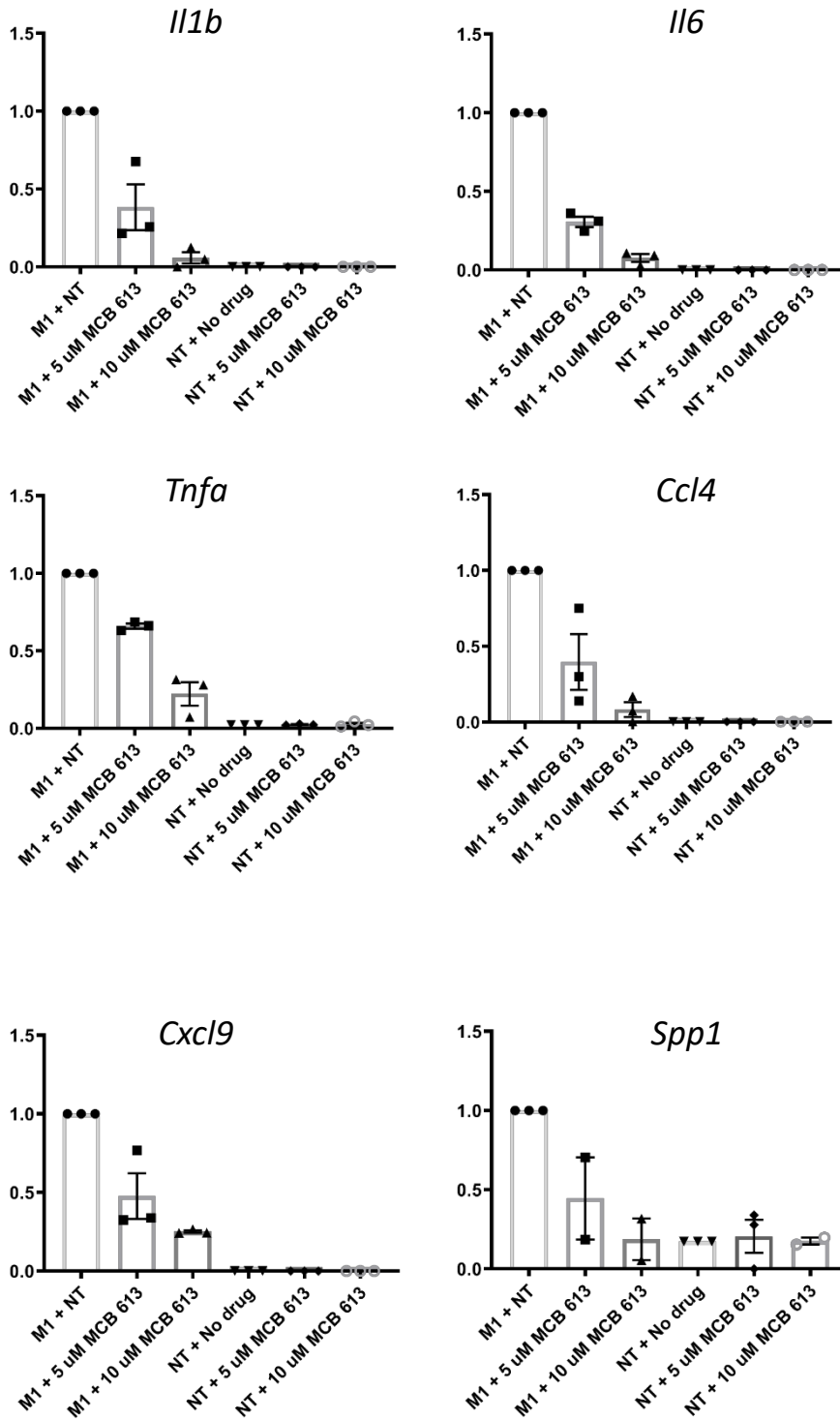
**Supplemental Figure 8. MCB-613 reduces pro-inflammatory signaling in macrophages had no effect on inflammatory gene expression in resting macrophages.** mRNA expression in RAW 264.7 macrophages stimulated with M1 reprogramming factors or no treatment (NT) for 8 hours followed by vehicle control or MCB-613 for an additional 24 hours.