Leukocyte-Expressed β_2-Adrenergic Receptors Are Essential for Survival After Acute Myocardial Injury

BACKGROUND: Immune cell–mediated inflammation is an essential process for mounting a repair response after myocardial infarction (MI). The sympathetic nervous system is known to regulate immune system function through β-adrenergic receptors (βARs); however, their role in regulating immune cell responses to acute cardiac injury is unknown.

METHODS: Wild-type (WT) mice were irradiated followed by isoform-specific βAR knockout (βARKO) or WT bone-marrow transplantation (BMT) and after full reconstitution underwent MI surgery. Survival was monitored over time, and alterations in immune cell infiltration after MI were examined through immunohistochemistry. Alterations in splenic function were identified through the investigation of altered adhesion receptor expression.

RESULTS: β_2ARKO BMT mice displayed 100% mortality resulting from cardiac rupture within 12 days after MI compared with ≈20% mortality in WT BMT mice. β_2ARKO BMT mice displayed severely reduced post-MI cardiac infiltration of leukocytes with reciprocally enhanced splenic retention of the same immune cell populations. Splenic retention of the leukocytes was associated with an increase in vascular cell adhesion molecule-1 expression, which itself was regulated via β-arrestin–dependent β_2AR signaling. Furthermore, vascular cell adhesion molecule-1 expression in both mouse and human macrophages was sensitive to β_2AR activity, and spleens from human tissue donors treated with β-blocker showed enhanced vascular cell adhesion molecule-1 expression. The impairments in splenic retention and cardiac infiltration of leukocytes after MI were restored to WT levels via lentiviral-mediated re-expression of β_2AR in β_2ARKO bone marrow before transplantation, which also resulted in post-MI survival rates comparable to those in WT BMT mice.

CONCLUSIONS: Immune cell–expressed β_2AR plays an essential role in regulating the early inflammatory repair response to acute myocardial injury by facilitating cardiac leukocyte infiltration.

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Clinical Perspective

What Is New?

- Using chimeric mice, we demonstrate that immune cell–specific β_2-adrenergic receptor (β_2AR) expression is essential to the repair process after myocardial infarction. In the absence of β_2AR, vascular cell adhesion molecule-1 expression is increased in leukocytes, inducing their splenic retention after injury and leading to impaired scar formation, followed by rupture and death.
- Vascular cell adhesion molecule-1 expression is regulated dynamically by βAR ligands, including β-blockers, in both mouse and human tissues. Splenectomy partially restores β_2AR-deficient leukocyte infiltration into the heart after injury, and gene therapy to rescue leukocyte β_2AR expression completely restored all injury responses to that observed in normal mice.

What Are the Clinical Implications?

- βARs regulate cardiac function and remodeling after injury, classically through their effects in cardiomyocytes, and are targeted by β-blockers to help prevent detrimental myocardial remodeling. However, our findings indicate that inhibition/deletion of immune cell–expressed β_2AR causes leukocyte dysfunction and altered immunomodulatory responses to acute injury.
- These results have important clinical implications because β-blockers are used frequently in patients around the time of myocardial infarction and perioperatively for noncardiac surgeries with uncertain mortality risk.
- Thus, understanding the essential role for β_2AR in mediating immune cell responses will inform strategies for β-blocker, or βAR agonist, administration after acute injury.

Inflammation is critical for initiating reparative processes after ischemic injury.[1] After myocardial infarction (MI), an intense inflammatory response is initiated, leading to recruitment of proinflammatory leukocytes, including monocytes, neutrophils, and mast cells.[1–6] Secreted factors from these proinflammatory cell populations recruit and activate reparative cell populations to promote extracellular matrix deposition and vascularization.[7,8] This rapid inflammatory response is necessary for healing and preserving the structure of the left ventricle (LV) after MI because dysregulation of this process results in increased cardiomyocyte death and degradation of the extracellular matrix.[9]

Sympathetic nervous system regulation of immune responses is well established,[10] and β-adrenergic receptor (βAR) expression has been reported on virtually all immune cell types. All 3 βAR subtypes are expressed on various hematopoietic cell-derived immune cell populations, with βAR subtype expression varying widely between populations and immune cell activation states.[11] Although the role of β_1AR in the immune system is not well established, β_1AR expression has been shown to be limited primarily to cells of the innate immune system, where it regulates inflammatory mediator production.[12,13] β_2AR is the most highly and widely expressed βAR isoform,[10,14] with similar levels of immune cell expression in rodents and humans,[10,14] and is known to regulate a number of functions, including hematopoiesis, lymphocyte homing, and immune cell maturation.[10] However, the focus of many of these studies involved the effect of β_2AR on adaptive immune responses, whereas its involvement in mediating early, innate immune responses and initiation of inflammation remains unclear.[10,15,16] β_3AR has been shown to be important in early stages of hematopoiesis for mediating immune cell mobilization and egress from the bone marrow (BM).[17–19] Although βAR subtype expression and function vary in the immune system, the role of immune cell–expressed βAR in the acute inflammatory response after MI has yet to be elucidated.

In the present study, the impact of immune cell–specific βAR expression on cardiac inflammation and remodeling after MI was investigated through the use of chimeric mice that lack specific βAR isoforms on cells of hematopoietic origin. We demonstrate that β_2AR is essential in initiating early immune responses after acute cardiac injury and that targeting immune cell–expressed β_2AR may provide a novel therapeutic strategy for preventing adverse effects after MI.

METHODS

Rationale and Study Design

The purpose of this study was to investigate the impact of βAR in regulating immune responses after MI. To differentiate the effects of immune cell–expressed βAR from cardiac-expressed βAR, we generated chimeric mice using a BM transplantation (BMT) approach in which wild-type (WT) recipient mice received WT control or βAR subtype–specific knockout (KO) BM to produce immune cell– and βAR isoform–specific KO mice. These mice were subjected to sham or MI surgery, and survival outcome and immune responses were examined, along with the mechanisms of observed changes.

BM Transplantation

WT C57BL/6 recipient mice (male; age, 8 weeks) were lethally irradiated with 950 rads using x-ray irradiation to remove endogenous BM cells. Donor BM isolated from the femurs of β_1ARKO, β_2ARKO, β_3ARKO, or WT C57BL/6 mice was introduced by retro-orbital injection (1×10^7 cells) within 24 hours of irradiation. BM was allowed to reconstitute for 1 month before MI surgery. Reconstitution was confirmed at the conclusion of the study for each mouse with reverse transcription–quantitative polymerase chain reaction (RT-qPCR) analysis for β_1AAR, β_2AR, and β_3AR expression on recipient BM. All animal procedures were performed in accordance with the Institutional Animal Care and Use

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Committee at Temple University and the National Institutes of Health's *Guidelines on the Use of Laboratory Animals*.

Coronary Artery Occlusion Surgery

Myocardial infarction was induced as previously described.[20] Mice were anesthetized with 2% isoflurane inhalation. A small skin incision was made, and the pectoral muscles were retracted to expose the fourth intercostal space. A small hole was made, and the heart was popped out. The left coronary artery was sutured ≈3 mm from its origin, and the heart was placed back into the intrathoracic space, followed by closure of muscle and skin. Animals received a single dose (0.3 mg/kg) of buprenorphine immediately after surgery.

Splenectomy Surgery

Mice were anesthetized as above, and a small incision was made in the left subcostal abdominal wall. Sutures were placed around the splenic vasculature, and the spleen was removed. The incision was closed in 2 layers, peritoneum and skin, with suture. Animals received a single dose (0.3 mg/kg) of buprenorphine immediately after surgery.

Echocardiography

Cardiac function was assessed via transthoracic 2-dimensional echocardiography performed at baseline and at weekly intervals after MI with a 12-mHz probe on mice anesthetized with isoflurane (1.5%). M-mode echocardiography was performed in the parasternal short-axis view to assess several cardiac parameters, including LV end-diastolic dimension, wall thickness, LV fractional shortening, and LV ejection fraction. Percent fractional shortening was calculated from the following equation: [(LVID;d−LVID;s)/LVID;s]×100%, where LVID;d is LV end-diastolic dimension and LVID;s is LV end-systolic dimension. Percent ejection fraction was calculated from the following equation: [(LV vol;d−LV vol;s)/LV vol;d]×100%, where LV vol;d is LV end-diastolic volume and LV vol;s is LV end-systolic volume.

Lentivirus Infection of BM

BM isolated from the femurs of mice was transduced with lentiviral vectors for 3XFlag–β_2AR–red fluorescent protein or green fluorescent protein (GFP) using a multiplicity of infection of 100. Transductions were performed in modified Eagle medium plus 10% fetal bovine serum (FBS) in the presence of 5 μg/mL Polybrene (Sigma-Aldrich). For in vitro experiments, media was changed 24 hours after infection to complete media (modified Eagle medium+10% FBS) and incubated an additional 24 hours before experiments. For generation of BM-derived macrophages, isolated BM was cultured in 10% L929-conditioned modified Eagle medium plus 10% FBS for 1 week before lentiviral infection with GFP control, WT β_2AR, β_2AR^{TYY}, or β_2AR^{GRK-} constructs.[21,22] For in vivo experiments, BM was rinsed 1 hour after infection and transplanted into irradiated mice via retro-orbital injection. BM was allowed to reconstitute for 1 month.

Human Macrophage Cell Culture

THP-1 cells (American Type Culture Collection), a human monocytic cell line, were cultured in modified RPMI-1640 media containing 2 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mmol/L HEPES, and 1 mmol/L sodium pyruvate supplemented with 10% FBS under standard cell culture growth conditions (37°C/5% CO_2/95% humidified air). THP-1 cells were differentiated into macrophages with the use of 200 nmol/L phorbol-12-myristate-13-acetate 48 hours before to all experiments. Cells were washed with complete media and treated for 24 hours with vehicle (PBS), 0.1 μmol/L salbutamol, or 0.1 μmol/L ICI-118,551.

Human Spleen Samples

Spleen samples from deceased human tissue donors who had been administered metoprolol long term or age- and sex-matched subjects not treated with metoprolol were procured by the National Disease Research Interchange with support from National Institutes of Health grant 2 U42 OD011158 (control subjects: n=5; age, 74.6±15.5 years [mean±SD]; 1 male and 4 female subjects; metoprolol subjects: n=6; age, 77.5±8.4 years; 1 male and 5 female subjects).

RT-qPCR Analysis

cDNA was synthesized from the total RNA of BM and spleen with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). RT-qPCR was performed with the SYBR Select Master Mix (Applied Biosystems) in triplicate for each sample using the primers listed in Table I in the online-only Data Supplement at an annealing temperature of 60.1°C. RT-qPCR data were analyzed with the Applied Biosystems Comparative CT Method (ΔΔCT), with GAPDH, TPT-1, and 18s rRNA used to normalize the expression of genes of interest and to calculate relative quantitation (RQ) and $RQ_{min/max}$ values for each.

Immunoblot

BM and spleen samples were homogenized in radioimmunoprecipitation assay buffer containing 1× HALT protease inhibitor cocktail (78437; Thermo Scientific) and phosphatase inhibitor cocktail set IV (524628; Calbiochem, USA). Equal amounts of lysates were resolved by SDS-PAGE (10% gels) and transferred to Immobilon-PSQ polyvinylidene fluoride 0.2-μm pore size membranes (Millipore). Odyssey Blocking Buffer (LI-COR Biosciences) was used to prevent nonspecific binding. Immunoblotting was performed overnight at 4°C with diluted antibodies against Flag M2 (1:10,000; Sigma-Aldrich), GFP (1:1000; Cell Signaling), vascular cell adhesion molecule-1 (VCAM-1; 1:1000; Santa Cruz Biotechnologies), β-tubulin (1:1000; Cell Signaling), β-actin (1:1000; Santa Cruz), or GAPDH (1:1000; Cell Signaling). After being washed with TBS-T, membranes were incubated at room temperature for 60 minutes with the appropriate diluted secondary antibody (IRDye680 donkey anti-rabbit IgG [H+L] at 1:20 000; IRDye800CW goat anti-mouse IgG [H+L] at 1:15 000; LI-COR Biosciences; IRDye680 donkey anti-goat IgG [H+L] at 1:20 000). Bound antibody was detected with the LI-COR Biosciences Odyssey System (LI-COR Biosciences). Intensities were normalized to corresponding GAPDH, β-tubulin, and β-actin intensities.

Histological Analysis

Excised hearts were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5-μm thickness. Deparaffinized sections were stained for hematoxylin-eosin (Sigma-Aldrich).

NIS Elements software was used to measure infarct size and to visualize cell infiltration and morphology.

Immunohistochemistry was performed on deparaffinized sections to examine the infiltration of various immune cell types. Antigens were retrieved with a citrate-based antigen-unmasking solution (Vector Laboratories). Hearts were blocked (10% FBS/PBS), and a 0.3% H_2O_2 solution was used to block endogenous peroxide activity in sections used for immunohistochemical staining. Hearts were incubated with antibodies against CD3 (1:100; Abcam), CD68 (1:100; Abcam), major basic protein (obtained from Nancy and Jamie Lee Laboratories; Mayo Clinic), mast cell tryptase (1:100; Abcam), myeloperoxidase (1:100; Santa Cruz), or VCAM-1 (1:100; Santa Cruz). Washed slides were incubated with the appropriate secondary antibodies, anti-mouse horseradish peroxidase (1:1000; GE Healthcare), anti-goat horseradish peroxidase (1:1000; Santa Cruz), anti-goat Alexa Fluor 647 (1:1000; Invitrogen), and anti-rabbit Alexa Fluor 647 (1:1000; Invitrogen), followed by staining with DAPI for immunofluorescence or hematoxylin for immunohistochemical staining. Immunofluorescent stained hearts were mounted with the Prolong Gold Antifade Reagent (Invitrogen). Immunohistochemically stained hearts were developed with a DAB Substrate Kit (Vector Laboratories) and mounted with Permount Mounting Media (Thermo Scientific). Staining was visualized on a Nikon Eclipse microscope at ×20 magnification, and NIS Elements software was used for recording images and image analysis. Images were quantified as the number of positive cells per area.

Flow Cytometry

Flow cytometry analysis of immune cell populations was performed on cells isolated from blood and BM. Immune cells were separated with an antibody against CD45-FITC (BD Biosciences) and sorted on an LSRII flow cytometer for size and granularity by forward scatter and side scatter. Analysis was performed with Flowjo software.

Statistical Analysis

Data presented are expressed as mean±SD for continuous variables and as count and/or percentage for categorical variables. Comparisons of a continuous variable between different treatment groups were performed with the nonparametric Kruskal-Wallis test for ≥3 groups and the exact Wilcoxon rank-sum test for 2 groups because of the small group sizes to guard against possibly nonnormally distributed data. Comparisons of a survival end point between treatment groups were performed with the log-rank test. When data were collected over time on the same set of animals such as fractional shortening in Figure 1B, they were analyzed with a mixed-effects model to take into account the correlation among repeated measures and the potential nonconstant variability over time across different groups. Multiple pairwise comparison adjustments were made with Bonferroni or Dunnett correction as appropriate. Values of $P<0.05$ were considered statistically significant. P values and n (group size) values are reported in the figure legends. All statistical analyses were performed with SAS version 9.3 software (SAS Institute Inc).

RESULTS

Lack of Immune Cell–Expressed β_2AR Increases Mortality After Acute Myocardial Injury

To examine the contribution of immune cell–specific βAR subtype expression on survival and cardiac function after MI, chimeric animals were generated via WT, β_1ARKO, β_2ARKO, or β_3ARKO BMT into irradiated recipient WT mice. β_1AR, β_2AR, and β_3AR expression was examined by RT-qPCR on reconstituted BM from all animals (Figure 1A), confirming that β_1AR, β_2AR, and β_3AR were knocked out in their respective BM with no differences in the other 2 βAR subtypes. After reconstitution, the BMT mice underwent sham or MI surgery, and cardiac function was monitored via echocardiography (Figure IA and Table II in the online-only Data Supplement). LV contractility, wall thickness, and cardiac dimensions were significantly altered in each MI group, as shown by decreased percent fractional shortening (Figure 1B), increased LV hypertrophy, and increased LV dilation relative to sham animals, although no differences were evident between WT, β_1ARKO, β_2ARKO, and β_3ARKO BMT groups. However, mortality rates among the BMT mice differed significantly after MI. All sham BMT mice displayed 100% survival, and WT BMT mice exhibited ≈20% mortality by 2 weeks after MI (Figure 1C), consistent with prior studies in non-BMT mice.[20] β_1ARKO and β_3ARKO BMT animals had a small but nonsignificant increase in mortality after MI compared with WT BMT animals. Strikingly, β_2AR BMT mice displayed 100% mortality after MI as a result of cardiac rupture, with death observed 4 to 12 days after MI. Although infarct size 1 day after MI was not different between WT and β_2ARKO chimeric mice (Figure IB and IC in the online-only Data Supplement), hematoxylin-eosin staining revealed wall thinning and weakening in β_2ARKO BMT mice 4 days after MI compared with WT BMT hearts (Figure 1D), suggesting an impairment in early repair mechanisms.

Lack of Immune Cell β_2AR Expression Impairs Leukocyte Infiltration After Acute Myocardial Injury

A number of immune cell populations, including monocytes/macrophages, neutrophils, mast cells, and T cells, are known to be important for the initiation of wound healing and cardiac remodeling after MI. Because of the high mortality via cardiac rupture observed in β_2AR BMT mice after MI, which could reflect decreased immune cell–initiated repair, we assessed whether WT and β_2ARKO BMT mice display differences in MI-induced cardiac immune cell population infiltration. Immunostaining was performed to identify cells of monocyte/macrophage lineage (CD68), mast cells (tryptase), neutrophils (myeloperoxidase), eosinophils (major basic protein), and T cells (CD3) in sham BMT hearts and in the remote,

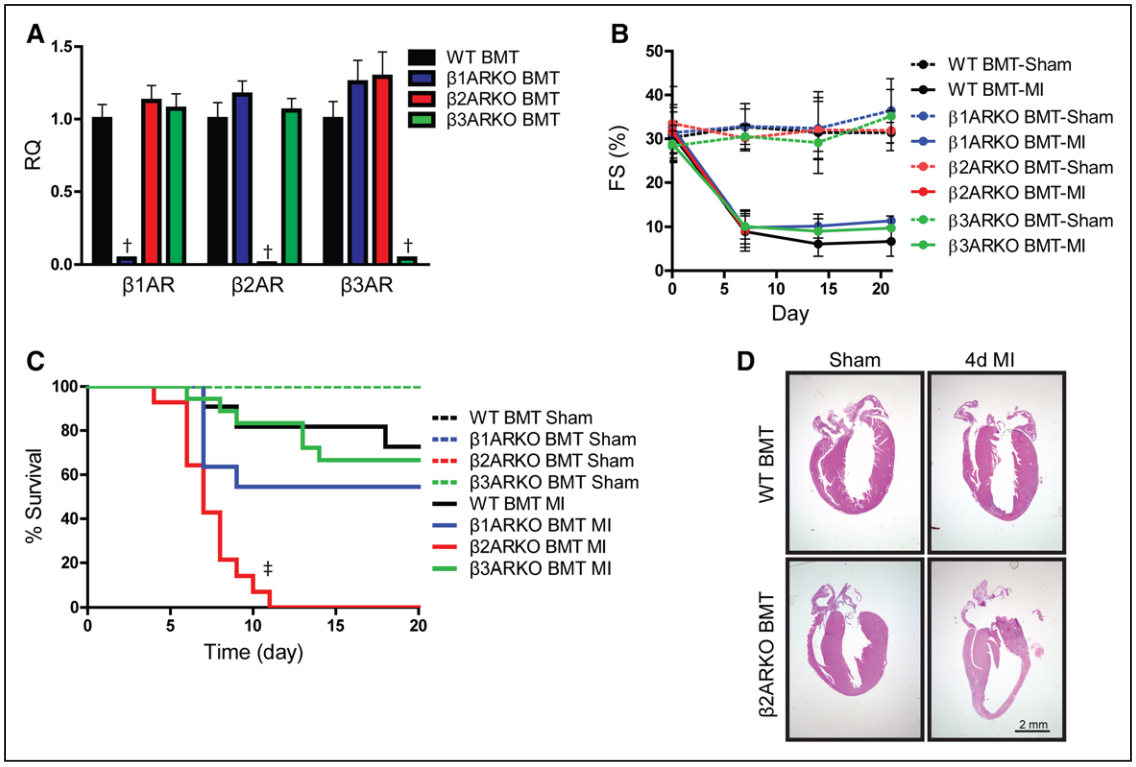

Figure 1. Effects of hematopoietically expressed β-adrenergic receptor (βAR) subtypes on cardiac survival and function after myocardial infarction (MI).

A, C57BL/6 mice receiving wild-type (WT), β_1AR knockout (KO), β_2ARKO, or β_3ARKO bone marrow transplantation (BMT) were subjected to sham or MI surgery. Expression of β_1AR, β_2AR, and β_3AR was assessed by reverse transcription–quantitative polymerase chain reaction on reconstituted WT, β_1ARKO, β_2ARKO, and β_3ARKO BM and presented as relative quantitation (RQ)+RQ_{max}. n=6 for all groups. Exact Wilcoxon rank-sum tests with multiple-comparison adjustment (3 comparisons). †P<0.01 versus WT. **B**, Left ventricular fractional shortening (FS) was measured at the short axis from the M mode with Visual Sonic Analysis software. Mixed-effects modeling for repeated-measures data with multiple-comparison adjustments was performed and indicated no significant differences compared with WT BMT. **C**, WT (n=9 for sham, n=11 for MI), β_1ARKO (n=7 for sham, n=11 for MI), β_2ARKO (n=10 for sham, n=14 for MI), or β_3ARKO (n=7 for sham, n=18 for MI) BMT mice were monitored daily for survival. Log-rank tests with multiple-comparison adjustment (3 comparisons). ‡P<0.001 versus WT BMT MI. All sham groups had 100% survival after surgery. **D**, Hematoxylin-eosin staining for sham and 4-day post-MI hearts from WT and β_2ARKO BMT mice.

border, and infarct zones of BMT hearts after MI (Figure 2 and Figures II and III in the online-only Data Supplement). Compared with WT BMT hearts, β_2ARKO BMT hearts had significantly less monocyte/macrophage, mast cell, and neutrophil infiltration into both the border and infarct zones (Figure 2A). Quantification of the staining demonstrated that decreased monocyte/macrophage (Figure 2B and 2C), mast cell (Figure 2D and 2E), and neutrophil (Figure 2F and 2G) recruitment to β_2ARKO BMT mouse hearts was maintained over time after MI compared with WT BMT mice. Not all immune cell populations were affected, however; eosinophil and T-cell infiltration into the hearts of both WT BMT and β_2ARKO BMT mouse hearts was not different (Figure III in the online-only Data Supplement). Flow cytometric comparative analysis of immune cell populations in the BM or blood of WT and β_2ARKO BMT animals showed no difference in granulocyte, monocyte, or lymphocyte populations (Figure IV in the online-only Data Supplement). Therefore, despite having similar levels of hematopoietic-derived cells compared with WT BMT mice, those with immune cell–specific deletion of β_2AR have impaired leukocyte recruitment to the heart after acute injury.

Mice Lacking Immune Cell–Expressed β_2AR Have Increased Splenic Retention of Leukocyte Populations

Because overall immune populations are similar between WT and β_2ARKO BMT mice but decreased leukocyte populations are observed in β_2ARKO BMT hearts after MI, we aimed to determine whether splenic retention of leukocytes could play a role in this phenotype. Sham β_2ARKO BMT mice had an increased spleen size compared with their WT BMT counterparts (Figure 3A), which was maintained after MI (Figure 3B). Leukocyte levels in spleen sections from WT or β_2ARKO BMT mice were examined to determine whether an increase in leukocytes within the

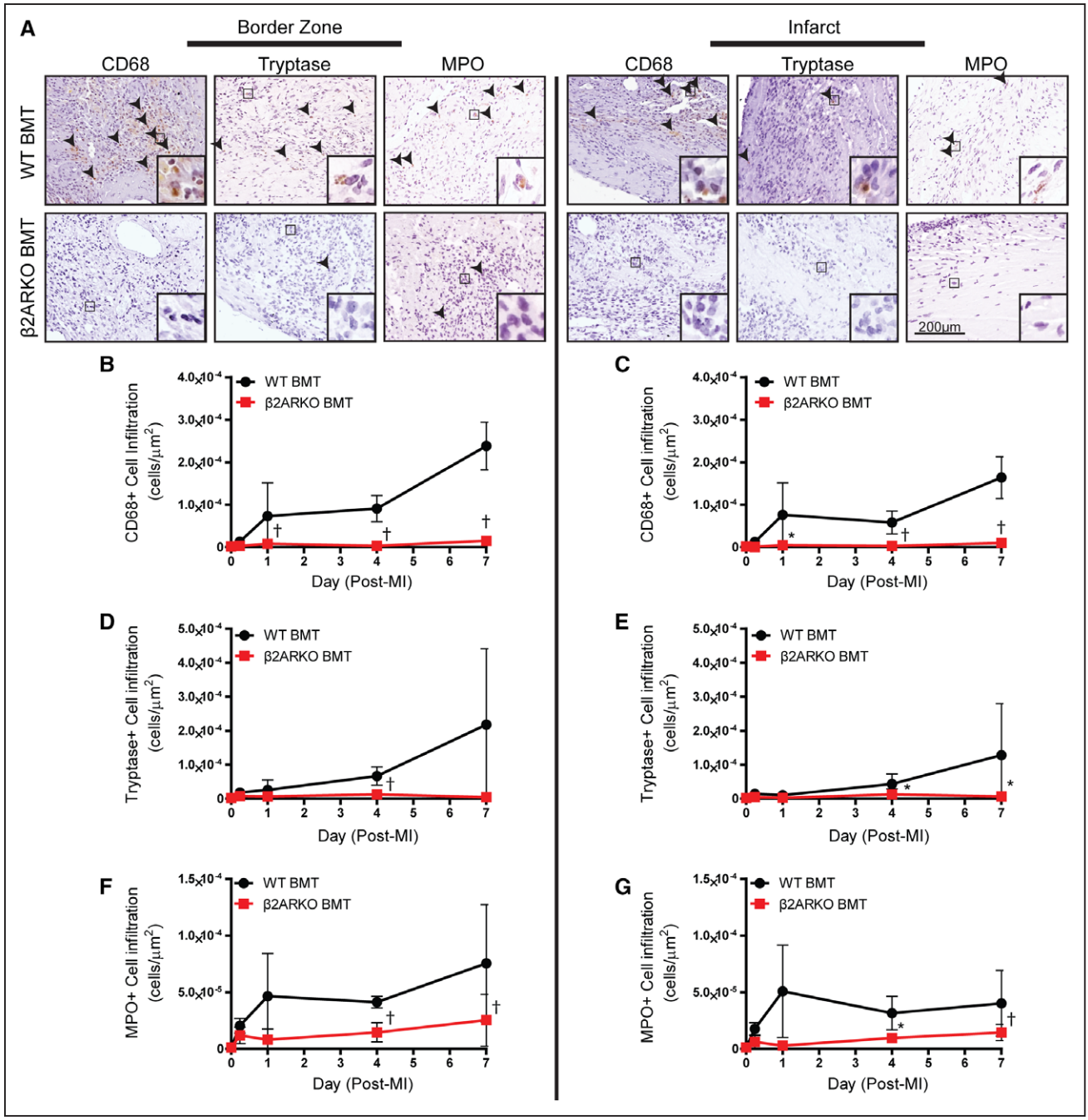

Figure 2. Effect of hematopoietic β-adrenergic receptor (βAR) expression on immune cell infiltration after myocardial infarction (MI).

A, Representative CD68, tryptase, and myeloperoxidase (MPO) staining of the border or infarct zones of hearts after MI surgery in wild-type (WT) bone marrow transplantation (BMT) or β_2AR knockout (KO) BMT mice. Quantification of CD68 (**B** and **C**), tryptase (**D** and **E**), and MPO (**F** and **G**) staining in the border and infarct zones of WT and β_2ARKO BMT mouse hearts. n=4 for WT BMT sham; n=5 for β_2ARKO BMT sham; n=3 for WT BMT 6 hours; n=3 for β_2ARKO BMT 6 hours; n=4 for WT BMT 1 day; n=6 for β_2ARKO BMT 1 day; n=5 for WT BMT 4 days; n=5 for β_2ARKO BMT 4 days; n=6 for WT BMT 7 days; n=6 for β_2ARKO BMT 7 days. Exact Wilcoxon rank-sum tests. **P*<0.05. †*P*<0.01 versus WT BMT.

β_2ARKO BMT spleens could account for the splenomegaly observed between the 2 groups in sham animals (Figure V in the online-only Data Supplement) and 4 days after MI (Figure 3C). Increased levels of monocyte/macrophages, mast cells, and neutrophils (Figure 3D) were observed in β_2ARKO BMT spleens compared with WT BMT mice, suggesting that β_2ARKO leukocytes have an impaired ability to mobilize from the spleen to the heart after injury.

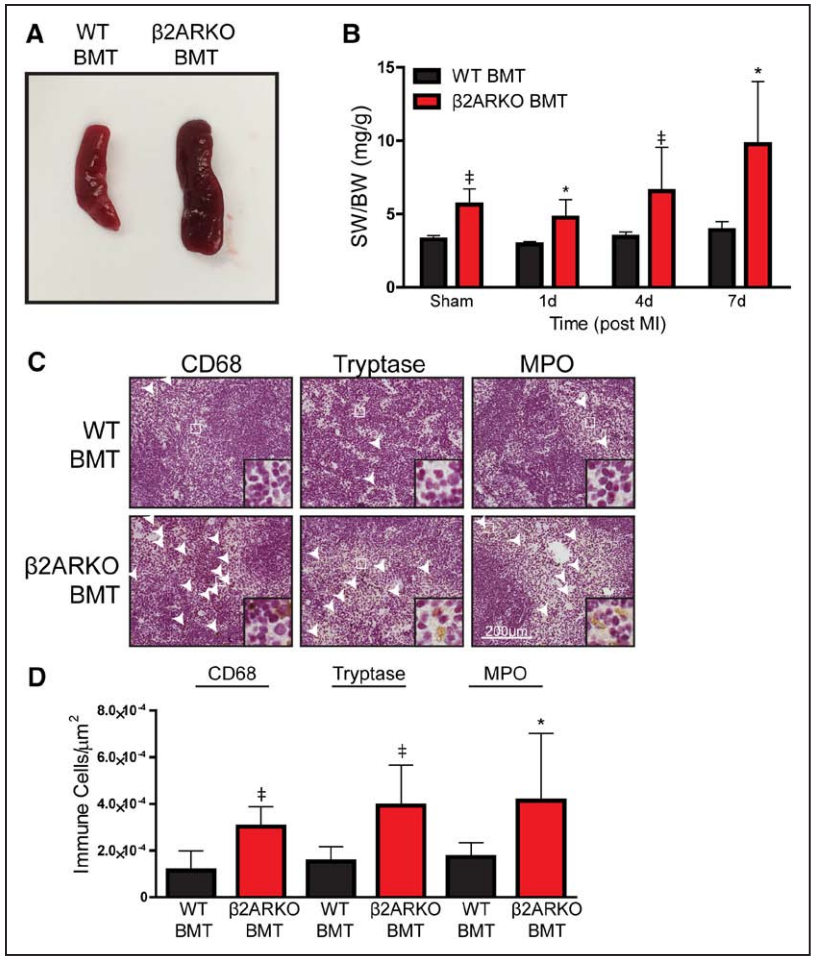

Figure 3. β-Adrenergic receptor (β_2AR) knockout (KO) mice have splenomegaly and retention of leukocyte populations. **A**, Representative images of spleens from wild-type (WT) and β_2ARKO bone marrow transplantation (BMT) animals. **B**, Gravimetric analysis of spleen weight to body weight (SW/BW) of spleens from WT and β_2ARKO BMT sham and myocardial infarction (MI) animals. n=6 for WT BMT sham; n=8 for β_2ARKO BMT sham; n=4 for WT BMT 1 day; n=4 for β_2ARKO BMT 1 day; n=10 for WT BMT 4 days; n=7 for β_2ARKO BMT 4 days; n=7 for WT BMT 7 days; n=5 for β_2ARKO BMT 7 days. Exact Wilcoxon rank-sum tests. *P<0.05. ‡P<0.001 versus WT BMT. **C**, Representative CD68, tryptase, and myeloperoxidase (MPO) staining from 4-day post-MI spleens of WT or β_2ARKO BMT mice. **D**, Quantification of CD68, tryptase, and MPO staining from WT and β_2ARKO BMT spleens 4 days after MI surgery. n=10 for WT BMT; n=8 for β_2ARKO BMT. Exact Wilcoxon rank-sum tests. *P<0.05. ‡P<0.001 versus WT BMT.

Splenic and Macrophage VCAM1 Expression Is Sensitive to β_2AR Activity and Expression in Mice and Humans

VCAM-1 expression on splenic macrophages has recently been identified as a hematopoietic stem cell retention factor important for splenic myelopoiesis.[23] To determine whether VCAM-1 levels were increased in the spleens of β_2ARKO BMT mice, leading to the retention of myeloid populations, its expression was assessed in the spleens of WT or β_2ARKO BMT mice. Immunostaining indicated increased splenic VCAM-1 expression with localization in the red pulp, where macrophages reside (Figure 4A and 4B). Protein levels of VCAM-1 were confirmed to be elevated in β_2ARKO chimeric mouse spleens compared with WT BMT mice via immunoblotting analysis (Figure 4C and 4D). Furthermore, transcript expression of VCAM-1 was increased in β_2ARKO BMT spleens both basally and 4 days after MI (Figure 4E).

To determine whether β_2AR stimulation alters VCAM-1 expression at a cellular level, WT BM-derived macrophages were treated with the β_2AR-selective agonist salbutamol, which decreased VCAM-1 expression (Figure 4F). Strikingly, salbutamol also decreased VCAM-1 expression in a human macrophage cell line (Figure 4F), confirming that β_2AR-mediated alterations in VCAM-1 are translatable between species. Because VCAM-1 was decreased by β_2AR stimulation, we next tested whether pharmacological inhibition of β_2AR could reciprocally increase VCAM-1 expression. Indeed, treatment of human macrophages with the β_2AR-selective antagonist ICI-118,551 (Figure 4G) increased VCAM-1 expression. Furthermore, VCAM-1 expression was significantly increased in the spleens of human subjects treated with the β-blocker metoprolol compared with age- and sex-matched subjects who had not taken a β-blocker (Figure 4H), demonstrating the clinical relevance of our findings.

Proximal β_2AR signaling through either G protein– or β-arrestin (βARR)–dependent pathways has been shown to exert distinct cellular effects.[21,22] Thus, to determine the proximal mechanism through which β_2AR controls VCAM-1 expression, lentiviral constructs were generated containing either β_2AR^{TYY}, which is unable to couple to $G\alpha_s$,[22] or β_2AR^{GRK-}, which cannot be phosphorylated by GRK,[21] thereby preventing the recruitment of βARRs. Using BM-derived macrophages from WT or β_2ARKO mice, we showed VCAM-1 transcript expression to be increased in β_2ARKO macrophages (Figure 5A). Lentivirus-mediated

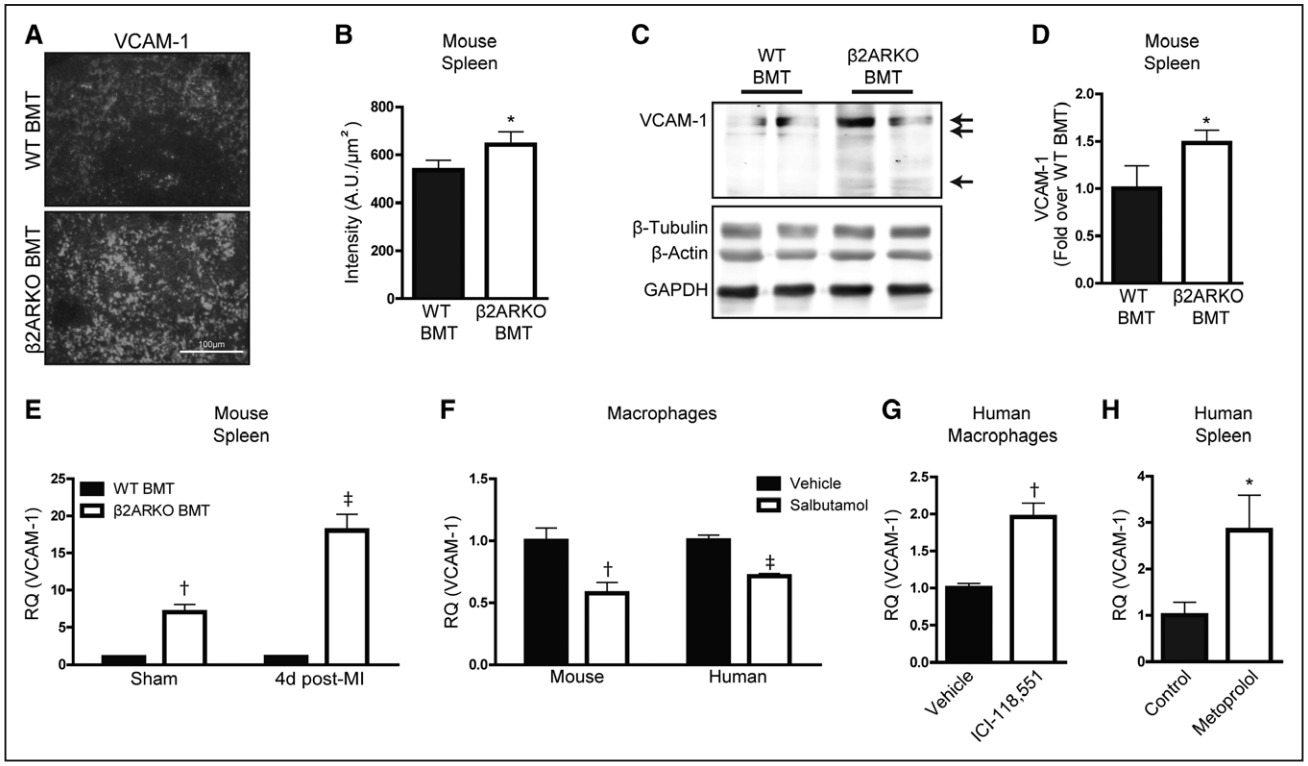

Figure 4. Vascular cell adhesion molecule-1 (VCAM-1) is increased in β-adrenergic receptor (β_2AR) knockout (KO) bone marrow transplantation (BMT) spleens.

A, Immunohistochemistry for VCAM-1 (white) showing levels and localization of VCAM-1 expression in wild-type (WT) and β_2ARKO BMT spleens. **B**, Quantification of the intensity of VCAM-1 staining. n=5 for WT BMT; n=5 for β_2ARKO BMT. Exact Wilcoxon rank-sum test. **P*<0.05 versus WT BMT. **C**, Representative immunoblot showing VCAM-1 expression in WT and β_2ARKO BMT spleens. **Arrows** indicate the 3 isoforms of VCAM-1. B-tubulin, β-actin, and GAPDH are shown as loading controls. **D**, Quantification of VCAM-1 immunoblot expression from the mice: n=12 for WT BMT, n=12 for β_2ARKO BMT. Exact Wilcoxon rank-sum test. ‡*P*<0.001. **E**, Reverse transcription–quantitative polymerase chain reaction (RT-qPCR) was used to measure VCAM-1 expression in WT or β_2ARKO BMT spleens and presented as relative quantitation (RQ)+RQ_{max}. n=8 for WT BMT sham; n=6 for WT BMT myocardial infarction (MI); n=6 for β_2ARKO BMT sham; n=8 for β_2ARKO BMT MI. Exact Wilcoxon rank-sum tests. †*P*<0.01. ‡*P*<0.001 versus WT BMT. **F**, RT-qPCR was used to measure VCAM-1 expression in mouse (BM-derived macrophages [BMDMs]) or human (THP-1 derived) macrophages and presented as RQ+RQ_{max}. n=7 for mouse vehicle; n=10 for mouse salbutamol; n=9 for human vehicle; n=10 for human salbutamol. Exact Wilcoxon rank-sum tests. †*P*<0.01. ‡*P*<0.001 versus vehicle. **G**, VCAM-1 expression in human macrophages treated with vehicle or ICI-118,551 was quantified by RT-qPCR and presented as RQ+RQ_{max}. Exact Wilcoxon rank-sum test. †*P*<0.01 versus vehicle. **H**, RT-qPCR was used to measure VCAM-1 expression in human spleens from control or metoprolol-treated patients and presented as RQ+RQ_{max}. n=5 for control; n=6 for metoprolol. Exact Wilcoxon rank-sum test. **P*<0.05 versus control. A.U. indicates arbitrary units.

restoration of β_2AR expression in β_2ARKO macrophages (Figure VE in the online-only Data Supplement) decreased VCAM-1 expression to that in WT macrophages (Figure 5A), whereas a GFP control lentivirus had no effect on VCAM-1 expression. Mechanistically, β_2ARKO BM-derived macrophages transduced with β_2AR^{GRK-} had elevated expression of VCAM-1, whereas β_2AR^{TYY} had decreased VCAM-1 similar to WT levels (Figure 5A), indicating that GRK-dependent β_2AR signaling is required for regulation of VCAM-1 expression in macrophages. In support of this observation, βARR2KO mice had splenomegaly similar to β_2ARKO mice (Figure 5B) with retention of monocytes/macrophages, mast cells, and neutrophils (Figure 5C and 5D). Interestingly, βARR1KO mice had normal splenic size and leukocyte levels, indicating that β_2AR regulates VCAM-1 expression selectively via βARR2 signaling.

Splenectomized WT and β_2ARKO BMT Mice Have Similar Levels of Leukocyte Infiltration After Acute Myocardial Injury

To confirm whether splenic retention of β_2ARKO leukocytes is primarily responsible for their decreased infiltration into the heart after MI, we examined leukocyte recruitment to the heart in splenectomized WT and β_2ARKO BMT animals receiving sham or MI surgery (Figure 6A and Figure VI in the online-only Data Supplement).

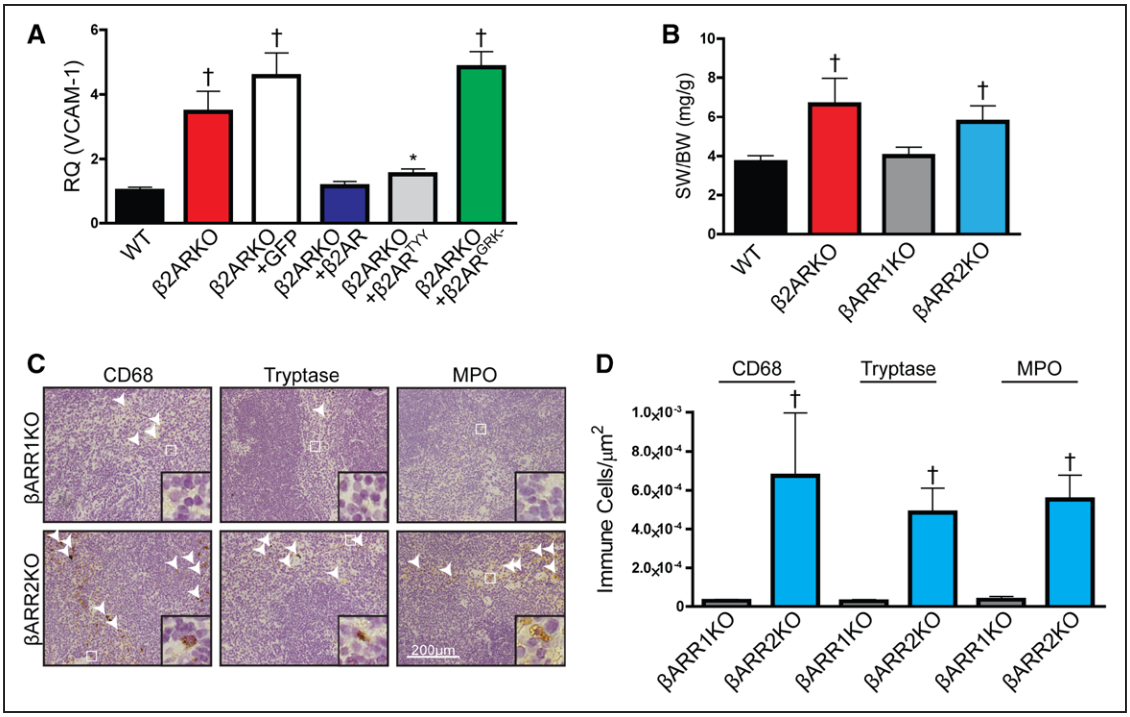

Figure 5. β-Adrenergic receptor (βAR) regulates vascular cell adhesion molecule-1 (VCAM-1) through β-arrestin (βARR)–dependent mechanisms.

A, Reverse transcription–quantitative polymerase chain reaction was used to measure VCAM-1 expression in bone marrow–derived macrophages (BMDMs) from wild-type (WT) or β_2AR knockout (KO) mice and β_2ARKO BMDMs transduced with green fluorescent protein (GFP), β_2AR, β_2AR^{TYY}, or β_2AR^{GRK-} lentivirus and presented as relative quantitation (RQ)+RQ$_{max}$. n=9 for WT; n=6 for β_2ARKO; n=6 for β_2ARKO+β_2AR; n=6 for β_2ARKO+GFP; n=6 for β_2ARKO+β_2AR^{TYY}; n=6 for β_2ARKO+β_2AR^{GRK-}. Exact Wilcoxon rank-sum tests with multiple-comparison adjustment (5 comparisons). *P<0.05. †P<0.01 versus WT. **B**, Gravimetric analysis of spleen weight to body weight (SW/BW) of spleens from WT, β_2ARKO, βARR1KO, and βARR2KO animals. n=7 for WT; n=6 for β_2ARKO; n=6 for βARR1KO; n=6 for βARR2KO. Exact Wilcoxon rank-sum tests with multiple comparison adjustment (3 comparisons). †P<0.01 versus WT. **C**, Representative CD68, tryptase, and myeloperoxidase (MPO) staining of spleens from βARR1KO and βARR2KO mice. **D**, Quantification of CD68, tryptase, and MPO staining from βARR1KO and βARR2KO spleens. n=6 for βARR1KO; n=4 for βARR2KO. Exact Wilcoxon rank-sum tests. †P<0.01 versus βARR1KO.

Splenectomy in WT BMT animals decreased MI-induced infiltration of monocytes/macrophages (Figure 6B) and neutrophils (Figure 6D) into the border zone by ≈50%, with less impact on mast cells (Figure 6C). Conversely, splenectomy of β_2ARKO BMT animals increased cardiac infiltration of monocytes/macrophages, neutrophils, and mast cells to levels not different from those observed in splenectomized WT BMT mice. Together, these results confirm that β_2AR-deficient leukocytes accumulate in the spleen, where they remain after MI, but have the capacity to infiltrate the heart after injury in the absence of the spleen, similar to spleen-independent leukocyte infiltration levels attained in WT BMT mice.

Restoration of β_2AR Expression Reverses Leukocyte Dysfunction and Restores Survival Rates After MI

To determine whether restoration of β_2AR expression in β_2ARKO BM could revert the β_2AR BMT phenotype to the WT BMT phenotype after MI, β_2ARKO BM was transduced with the WT β_2AR lentivirus construct or GFP control lentivirus before transplantation. Immunoblotting was used to confirm that the protein expression of GFP in control and Flag-tagged β_2AR expression for lentivirus-transduced reconstituted BM (Figure 7A) and β_2AR expression in β_2ARKO BM after reconstitution were ≈95% of endogenous levels (Figure 7B). Similar to β_2ARKO BMT mice, mice receiving β_2ARKO BM transduced with GFP control lentivirus displayed 100% mortality after MI, with all mice dying between days 4 and 14 of cardiac rupture (Figure 7C); however, restoration of β_2AR expression in β_2ARKO BM increased survival after MI to close to WT BMT levels (Figure 7D). Reconstitution with β_2AR-infected β_2ARKO BM also reduced both spleen size (Figure 7E) and VCAM-1 expression (Figure 7F and 7G) compared with GFP-transduced β_2ARKO BMT mice.

Accordingly, restoration of β_2AR in β_2ARKO BM also reduced levels of the leukocyte populations in the spleen (Figure 8A and 8B) to those not different from WT BMT mice (Table III in the online-only Data Supplement). Con-

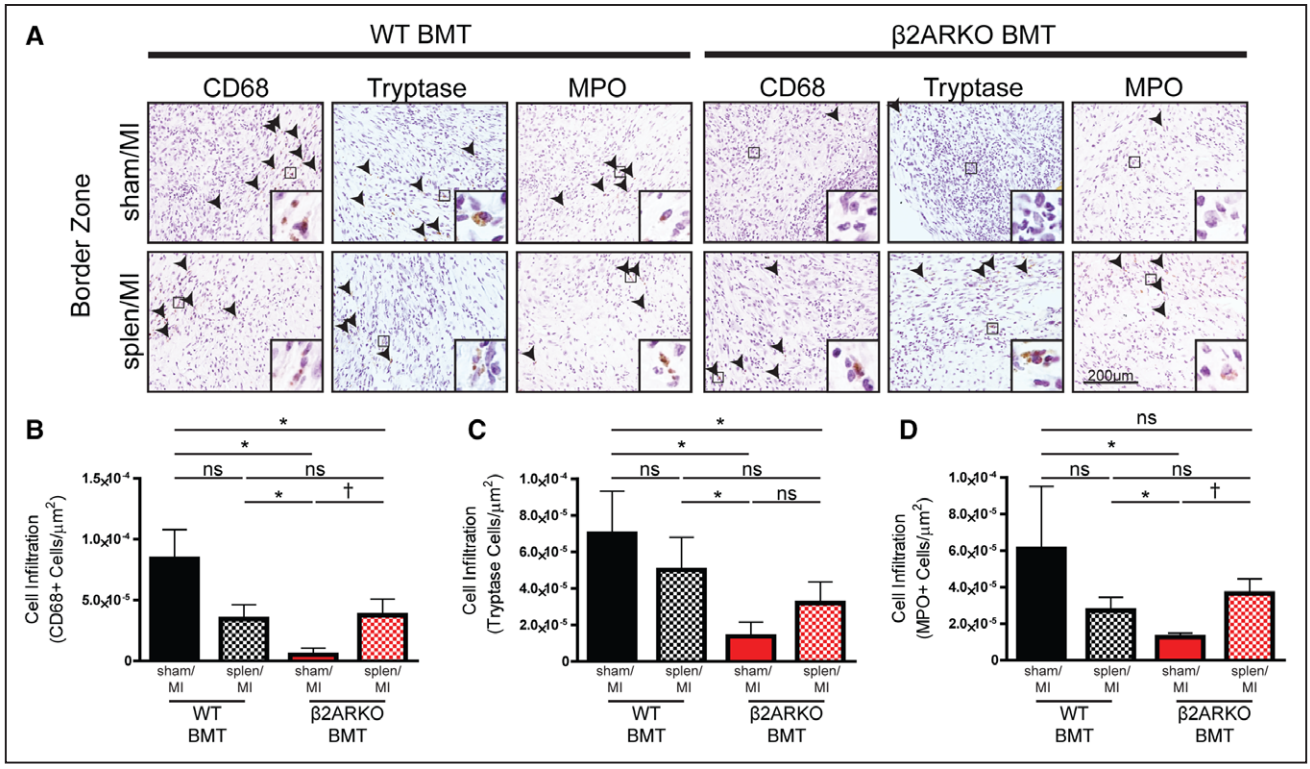

Figure 6. Splenectomy restores β-adrenergic receptor (β_2AR) knockout (KO) leukocyte infiltration into the heart after myocardial infarction (MI).

A, Representative CD68, tryptase, and myeloperoxidase (MPO) staining of the border or infarct zones of hearts 4 days after MI surgery in wild-type (WT) or β_2ARKO bone marrow transplantation (BMT) that received sham and splenectomy surgery. Quantification of CD68 (**B**), tryptase (**C**), and MPO (**D**) staining in the border and infarct zones of 4-day post-MI hearts from sham and splenectomy WT and β_2ARKO BMT mice. n=6 for WT BMT sham/MI; n=5 for WT BMT splenectomy/MI; n=6 for β_2ARKO BMT sham/MI; n=7 for β_2ARKO BMT splenectomy/MI. Exact Wilcoxon rank-sum tests with multiple-comparison adjustment (6 comparisons). *P<0.05. †P<0.01.

versely, immunohistochemistry for leukocyte infiltration in sham (Figure VII in the online-only Data Supplement) and injured myocardium of β_2AR-rescued β_2ARKO BMT mice revealed the reciprocal results. Thus, leukocyte infiltration was increased in the border (Figure 8C) and infarct (Figure VIII in the online-only Data Supplement) zones of the heart in β_2ARKO BMT mice transduced with β_2AR versus GFP lentivirus, including monocytes/macrophages, mast cells, and neutrophils (Figure 8D), which were restored to WT BMT levels (Table III in the online-only Data Supplement).

DISCUSSION

Inflammatory responses are critical for wound healing after MI.[1] All 3 βAR isoforms have been shown to mediate a number of effects in the immune system, including hematopoiesis, lymphocyte homing, and cytokine/chemokine production. However, little is known about how they regulate immune cell responses after acute cardiac injury.[10,14] To investigate the immune cell–specific impact of βARs on cardiac survival and remodeling after MI, we generated chimeric mice lacking β_1AR, β_2AR, or β_3AR expression on cells of hematopoietic origin. The most striking outcome was observed in β_2ARKO BMT animals, which displayed 100% mortality resulting from cardiac rupture, in contrast to their WT counterparts, which had a ≈20% mortality rate. β_2AR chimeric mice had decreased infiltration of leukocyte populations compared with their WT counterparts, demonstrating impaired innate immune responses. Recent findings have shown the importance of proinflammatory monocytes in initiating early immune cell–dependent reparative responses after MI.[2] Thus, it is likely that the inability of leukocyte populations to traffic to the heart acutely after MI in β_2ARKO chimeric mice impairs early repair processes, contributing to scar instability, cardiac rupture, and death.

Of great importance, the β_2ARKO BMT mice had decreased leukocyte infiltration into the heart after MI, with a reciprocal increase in spleen size and leukocyte retention, suggesting an impairment in immune cell egress from the spleen to the heart after acute cardiac injury. Thus, splenectomy of the β_2ARKO BMT mice restored cardiac leukocyte infiltration responses to those of splenectomized WT BMT mice. Recently, the spleen has been shown to be an important monocyte reservoir, holding active monocytes for release on inflammatory injury,[6,24] and has been demonstrated to be of particular importance after MI and during heart failure when there

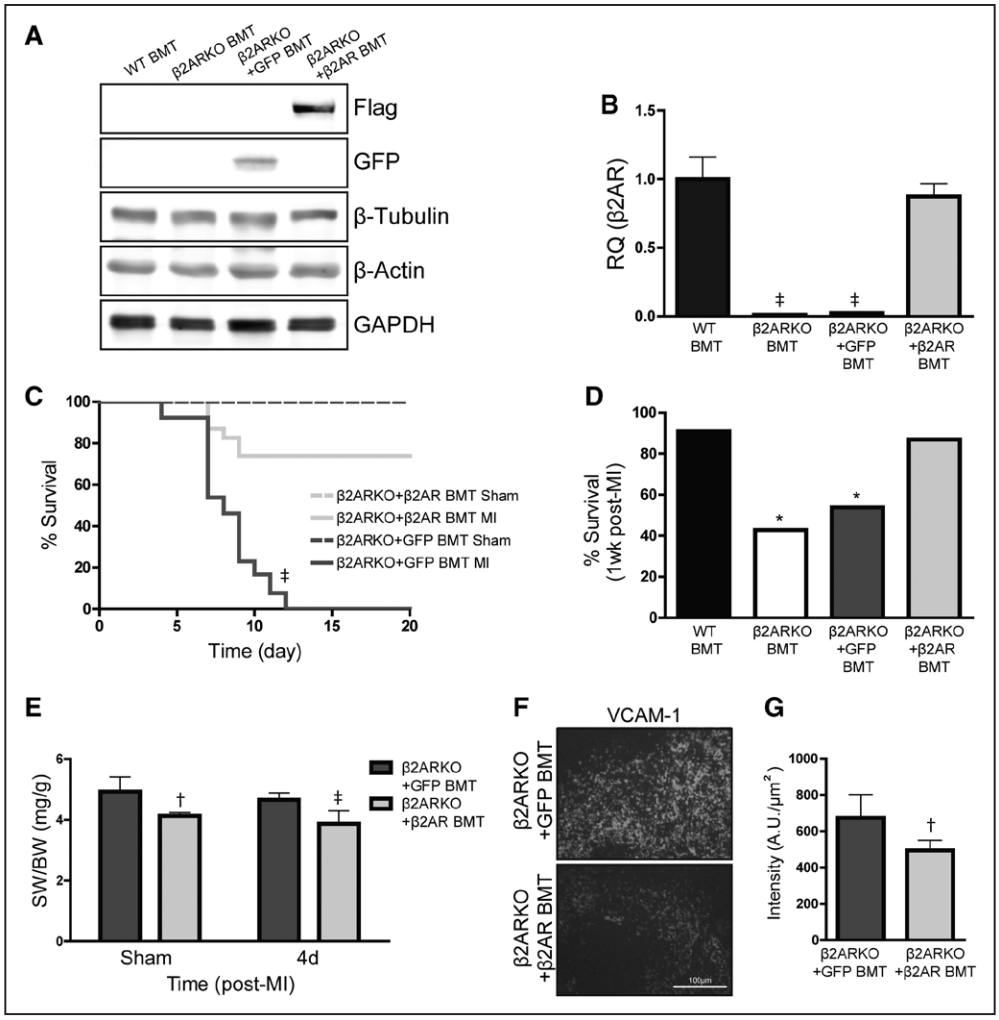

Figure 7. Restoration of β-adrenergic receptor (βAR) expression in β_2AR knockout (KO) bone marrow (BM) restores survival after myocardial infarction (MI).

A, Representative immunoblot showing protein expression of green fluorescent protein (GFP) and Flag in reconstituted BM from β_2ARKO, β_2ARKO+GFP, and β_2ARKO+β_2AR BM transplantation (BMT) mice. β-tubulin, β-actin, and GAPDH are shown as loading controls. **B**, β_2AR expression was measured by reverse transcription–quantitative polymerase chain reaction in reconstituted BM from wild-type (WT) and β_2ARKO mice and reconstituted BM from mice that had GFP or β_2AR transduced into β_2ARKO BM by lentivirus before transplantation. Values are presented as relative quantitation (RQ)+RQ_{max} expressed relative to WT BMT. n=10 for WT; n=10 for β_2ARKO BMT; n=9 for β_2ARKO+GFP BMT; n=11 for β_2ARKO+β_2AR BMT. Exact Wilcoxon rank-sum tests with multiple-comparison adjustment (3 comparisons). ‡P<0.001. **C**, β_2ARKO+GFP and β_2ARKO+β_2AR BMT mice were subjected to sham or MI surgery and monitored daily for survival. All sham groups had 100% survival after surgery. n=6 for β_2ARKO+GFP; n=7 for β_2ARKO+β_2AR BMT sham; n=27 for β_2ARKO+GFP MI; n=26 for β_2ARKO+β_2AR BMT MI. Log-rank test. ‡P<0.001 versus β_2ARKO+β_2AR BMT. **D**, Percent survival of β_2ARKO+GFP and β_2ARKO+β_2AR BMT mice 1 week after MI. Log-rank tests with multiple-comparison adjustment (3 comparisons). *P<0.05 versus WT BMT MI. **E**, Gravimetric analysis of spleen weight to body weight (SW/BW) of spleens from β_2ARKO+GFP and β_2ARKO+ β_2AR BMT mice. n=7 for β_2ARKO+GFP sham; n=6 for β_2ARKO+ β_2AR sham; n=8 for β_2ARKO+GFP MI; and n=10 for β_2ARKO+ β_2AR MI. Exact Wilcoxon rank-sum tests. †P<0.01. ‡P<0.001 versus β_2ARKO+GFP BMT. **F**, Representative vascular cell adhesion molecule-1 (VCAM-1) staining for β_2ARKO+GFP and β_2ARKO+β_2AR BMT spleens 4 days after MI. **G**, Quantification of VCAM-1 intensity from immunohistochemistry of β_2ARKO+GFP (n=6) and β_2ARKO+β_2AR BMT (n=7) spleens. Exact Wilcoxon rank-sum test. †P<0.01 versus β_2ARKO+GFP BMT.

is increased antigen processing and adaptive immune system activation.[25] These processes are regulated through a variety of signals.[6] One molecular mechanism of leukocyte egress from the spleen through macrophage expression of VCAM-1 has recently been identified.[23] Our results demonstrate an increase in VCAM-1 in the macrophage-containing red pulp region of spleens from β_2ARKO BMT animals, resulting in the retention of leukocyte populations in the spleens of these animals. VCAM-1 was also increased with a β_2AR antagonist in human macrophages, with a reciprocal decrease in expression after β_2AR stimulation. Interestingly, macrophage

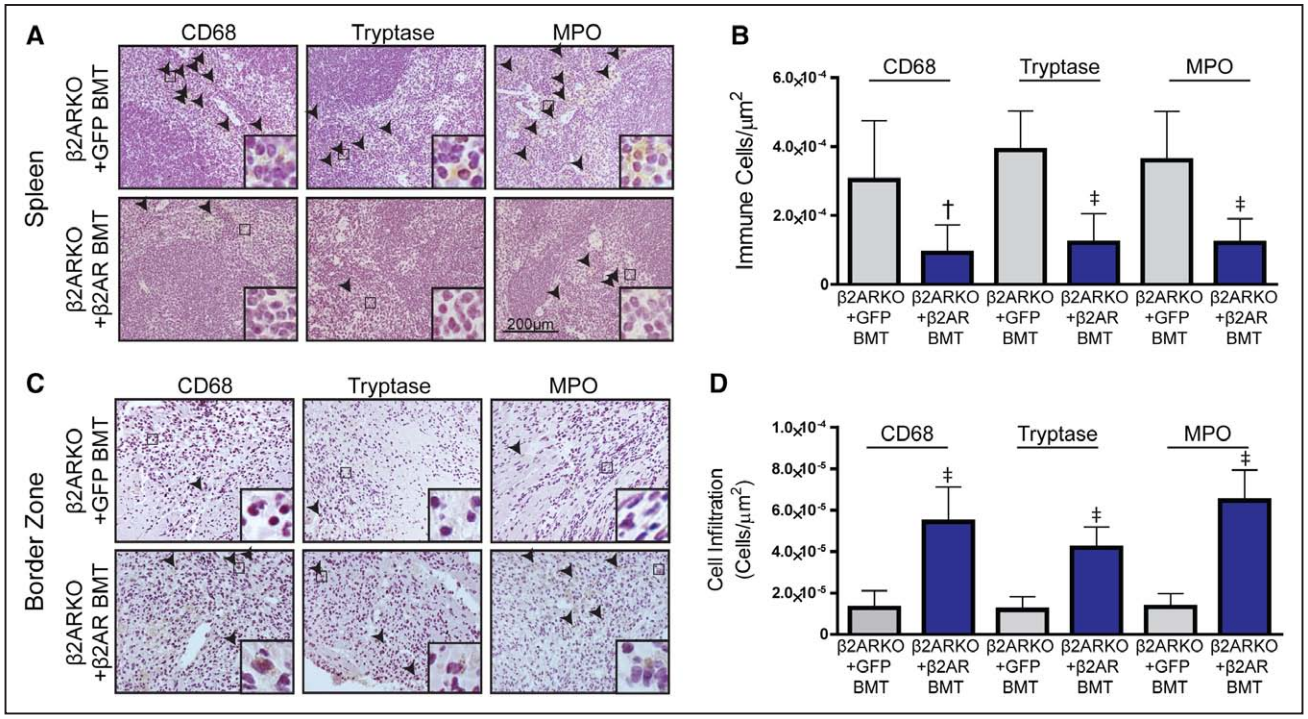

Figure 8. β-Adrenergic receptor (βAR) re-expression on reconstituted β_2AR knockout (KO) bone marrow (BM) reverses splenic retention of leukocytes.

A, Representative CD68, tryptase, and myeloperoxidase (MPO) staining of spleens from β_2ARKO+green fluorescent protein (GFP) and β_2ARKO+β_2AR BM transplantation (BMT) mice 4 days after myocardial infarction (MI). **B**, Quantification of CD68, tryptase, and MPO staining from the spleens from **A**. n=8 β_2ARKO+GFP BMT; n=8 β_2ARKO+β_2AR BMT. Exact Wilcoxon rank-sum tests. †P<0.01. ‡P<0.001 versus β_2ARKO+GFP BMT. **C**, Representative CD68, tryptase, and MPO staining of the border zone of hearts from β_2ARKO+GFP and β_2ARKO+β_2AR BMT animals 4 days after MI surgery. **D**, Quantification of CD68, tryptase, and MPO staining from the border zone of 4-day post-MI hearts from β_2ARKO+GFP (n=8) and β_2ARKO+β_2AR BMT (n=6) mice. Exact Wilcoxon rank-sum tests. ‡P<0.001 versus β_2ARKO+GFP BMT.

VCAM-1 expression appears to be regulated within this context in a β_2AR/βARR2–dependent manner.

A common limitation of studies in mice is that the findings do not always translate to human pathophysiology. However, βAR activation has been implicated in reducing spleen size and release of certain immune cell populations in a number of different species, including murine and swine models and humans.[26–30] These changes were independent of alterations in blood flow, although the mechanism was never identified. Furthermore, β-blocker administration was shown to prevent the splenic release of immune populations, similar to our present study using β_2ARKO chimeric animals, and this response was amplified when combined with an inflammatory stimulus.[31] Mechanistically, our study demonstrates increased levels of leukocyte populations in the spleens of β_2ARKO BMT mice both before and after MI, effects that are clearly independent of vascular or splenic β_2AR expression. Importantly, inhibition of leukocyte egress from the spleen and decreased infiltration of these populations into the heart can be reversed by restoring β_2AR expression in the BM before transplantation using a lentiviral construct, confirming the specificity of the response and demonstrating the ability to modulate hematopoietic cell receptor expression using gene therapy approaches. In addition, our data in human macrophages are consistent with those in mouse macrophages, and we also observed increased VCAM-1 expression in the spleens of human donors who had taken the β-blocker metoprolol. Although metoprolol has preference for β_1AR, it loses its selectivity at the higher doses often used clinically[32] that would also antagonize β_2AR. This could account for the retention of immune cell populations observed in other studies and confirmed in our chimeric mouse model, providing further clinical relevance. Interestingly, increases in circulating immune cells with epinephrine or isoproterenol are greatly diminished in splenectomized patients,[33,34] and multiple long-term studies examining the effects of splenectomy in humans have demonstrated an increased incidence in MI and heart failure with worsened prognosis after such events.[35,36]

Although many of the benefits of β-blockers are thought to be mediated through their actions on β_1AR in cardiomyocytes,[37] immune cell–expressed βAR and β-blocker therapy have been suggested to play roles in the regulation of immune responses during heart failure.[38–42] Our findings suggest that the administration of β-blockers with selectivity to β_2AR around the time

of MI could diminish leukocyte egress from the spleen and subsequent cardiac immune cell–dependent remodeling. The impact of such a process on overall cardiac remodeling would likely depend on the severity of β_2AR inhibition; a short-term decrease in activity may simply dampen the inflammatory response but not ultimately prevent it, whereas long-term inhibition of leukocyte-expressed β_2AR could negatively affect post-MI repair processes. Interestingly, it has recently been suggested that perioperative use of β-blockers may actually increase cardiovascular events, including MI,[43] and, according to the American Heart Association/American College of Cardiology, continues to have an uncertain mortality risk.[44] A link between β_2AR inhibition in leukocytes and these clinical observations has not been demonstrated but warrants further investigation.

Unlike inhibition, short-term β_2AR agonist administration during the inflammatory phase after MI in combination with long-term β_1AR antagonist administration may be an improved therapeutic strategy to prevent detrimental remodeling and to preserve cardiac function after cardiac injury. Several studies investigating the use of β_2AR agonists in the treatment of heart failure have found beneficial effects, which were attributed to the promotion of cardiomyocyte survival. However, the long-term benefits of βAR blockade in heart failure have contraindicated the use of β_2AR agonists.[45–50] In many of these studies, β_2AR agonist administration began at later time points, thus missing the acute inflammatory phase. Regardless, in animal models, β_2AR agonists have been shown to improve cardiac remodeling after MI or ischemia/reperfusion to a greater extent than that achieved by β_1AR antagonists,[46,49,50] whereas cardiac function and survival were further improved with a combined β_1AR blocker/β_2AR agonist strategy.[46–48] Remarkably, a single dose of the β_2AR agonist clenbuterol before ischemic insult was shown to decrease the resulting cardiac injury.[45] However, these studies did not assess the contribution of the early immune cell responses to their outcomes.

Conclusions

Using a chimeric mouse approach, we identified a critical role for hematopoietic cell–expressed β_2AR in the regulation of acute cardiac inflammation and remodeling after MI. β_2AR-deficient immune cells displayed impaired recruitment to the injured myocardium after MI, with reciprocal leukocyte retention within the spleen that was maintained after MI. Lentiviral-mediated re-expression of β_2AR in β_2ARKO BM before transplantation restored BM migration, splenic retention levels of leukocyte populations, and leukocyte infiltration into the heart after injury. Together, our results highlight an immunomodulatory role for β_2AR that could be targeted to promote early leukocyte-dependent reparative processes after MI, with negligible or even beneficial effects on cardiomyocytes, while avoiding issues inherent to the promotion of prolonged inflammatory events.

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DISCLOSURES

Drs Tilley and Koch have equity in Renovacor, Inc, which has neither funded this study nor has a relevant product related to this study. The other authors report no conflicts.

AFFILIATIONS

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FOOTNOTES

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Leukocyte-Expressed β_2-Adrenergic Receptors Are Essential for Survival After Acute Myocardial Injury

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Primer sequences used for RT-PCR.

Gene Name	Reference Sequence	Sequence
Mouse ADRB1	NM_007419	Forward 5'-GCT GAT CTG GTC ATG GGA TT-3' Reverse 5'-CGT CAC ACA CAG CAC ATC TA-3'
Mouse ADRB2	NM_007420	Forward 5'-AAG AAT AA GCC CGA GTG GT-3' Reverse 5'-GTA GGC CTG GTT CGT GAA GA-3'
Mouse ADRB3	NM_013462	Forward 5'-TGT TCC TTT GCC TCC AAC A-3' Reverse 5'-CAA CGA ACA CTC GAG CAT AGA-3'
Mouse GAPDH	NM_001289726	Forward 5'-AAC AGC AAC TCC CAC TCT TC-3' Reverse 5'-CCT GTT GCT GTA GCC GTA TT-3'
Human GAPDH	NM_001256799	Forward 5'-GGT GTG AAC CAT GAG AAG TAT GA-3' Reverse 5'-GAG TCC TTC CAC GAT ACC AAA G-3'
Mouse TPT-1	NM_009429	Forward 5'-ATC ATC TAC CGG GAC CTC ATC-3' Reverse 5'-CCC TCT GTT CTA CTG ACC ATC T-3'
Human TPT-1	NM_001286272	Forward 5'-GGG CTG CAG AAC AAA TCA AG-3' Reverse 5'-CAT CCT CAC GGT AGT CCA ATA G-3'
Mouse VCAM-1	NM_011693	Forward 5'- GCA CTC TAC TGC GCA TCT T-3' Reverse 5'- CAC CAG ACT GTA CGA TCC TTT C-3'
Human VCAM-1	NM_001069	Forward 5'-GAT TGG TGA CTC CGT CTC ATT-3' Reverse 5'-CCT TCC CAT TCA GTG GAC TAT C-3'
Mouse 18s rRNA	NR_003278	Forward 5'-GCA ATT ATT CCC CAT GAA CG-3' Reverse 5'-GGC CTC ACT AAA CCA TCC AA-3'
Human 18s rRNA	NR_003286	Forward 5'-CTG AGA AAC GGC TAC CAC ATC-3' Reverse 5'- GCC TCG AAA GAG TCC TGT ATT G-3'

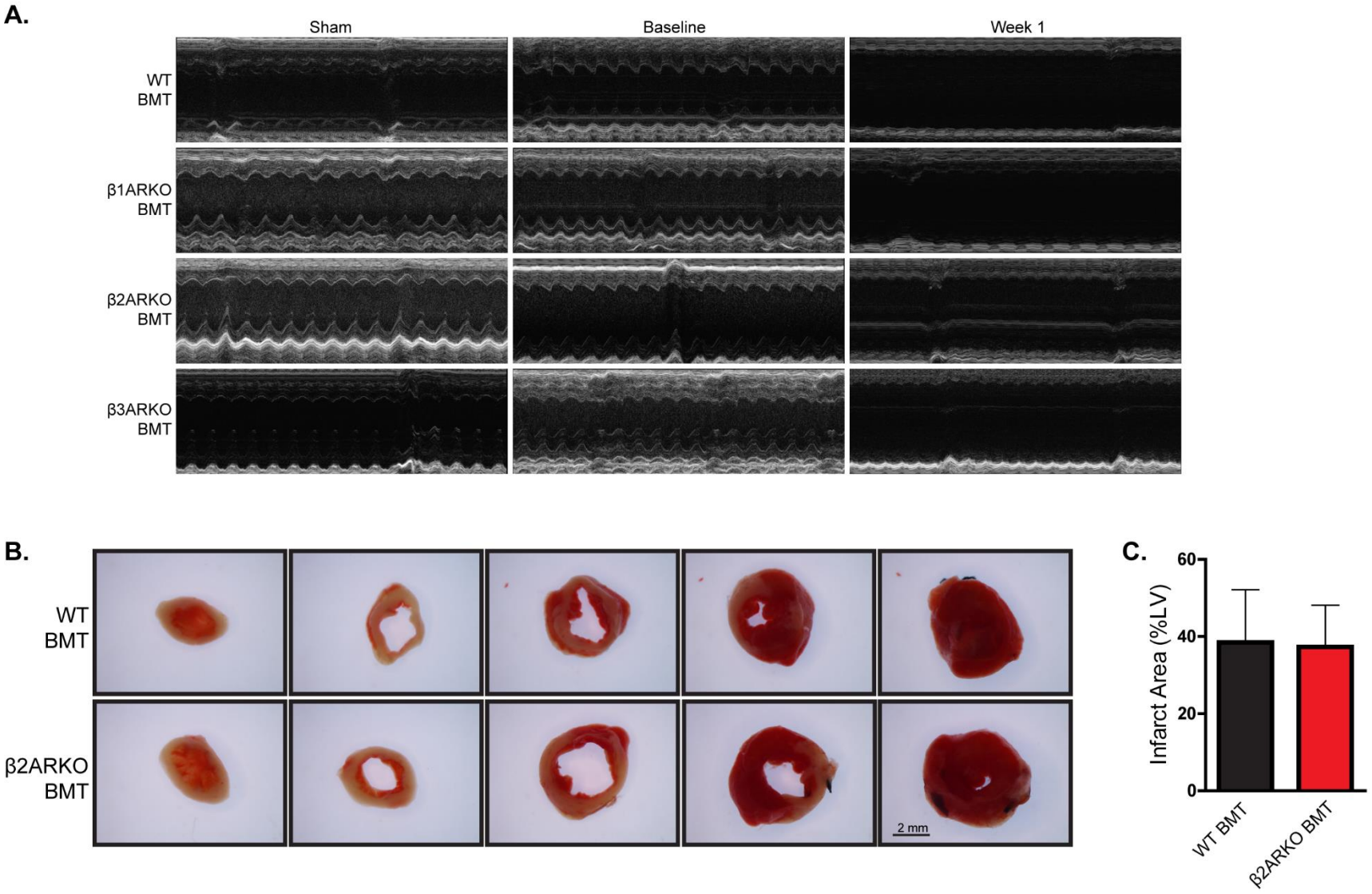
Supplemental Table 2: Echocardiography Measurements

	WT BMT Sham		WT BMT MI		β1ARKO BMT Sham		β1ARKO BMT MI		β2ARKO BMT Sham		β2ARKO BMT MI		β3ARKO BMT Sham		β3ARKO BMT MI	
	Baseline (n=14)	1 Week (n=14)	Baseline (n=14)	1 Week (n=14)	Baseline (n=4)	1 Week (n=4)	Baseline (n=14	1 Week (n=8)	Baseline (n=16)	1 Week (n=16)	Baseline (n=16)	1 Week (n=16)	Baseline (n=4)	1 Week (n=4)	Baseline (n=14	1 Week (n=8)
Fractional Shortening (%)	29.6±3.4	32.8±4.0	31.0±3.0	8.9±3.6	31.4±4.8	32.9±5.2	32.6±4.5	9.9±3.7	34.9±8.4	30.1±2.8	34.2±6.1	9.2±4.7	28.4±3.4	30.5±2.8	28.9±4.3	10.1±2.9
Ejection Fraction (%)	57.6±5.3	61.7±6.8	58.4±4.5	19.8±6.6	59.5±6.9	62.0±7.9	61.6±6.5	21.4±7.8	61.3±5.7	57.1±5.4	63.8±9.4	19.7±3.5	55.1±5.4	58.4±4.3	55.9±6.2	21.9±5.9
LV Volume-diastolic (µL)	61.2±10.3	47.2±11.4	66.8±7.3	144.2±38.3	56.0±6.4	56.7±14.2	57.2±8.7	120.4±35.9	51.0±8.3	60.8±10.1	54.0±16.8	155.0±53.3	67.9±9.6	70.5±7.1	63.0±13.3	129.9±33.4
LVAW-diastolic (mm)	0.60±0.07	0.67±0.16	0.63±0.11	0.43±0.20	0.71±0.03	0.90±0.13	0.78±0.11	0.48±0.11	0.70±0.12	0.68±0.13	0.74±0.23	0.41±0.15	0.70±0.07	0.75±0.08	0.72±0.07	0.42±0.08
LVPW-diastolic (mm)	0.63±0.06	0.80±0.02	0.60±0.08	0.27±0.08	0.80±0.09	0.77±0.10	0.75±0.12	0.54±0.21	0.69±0.09	0.59±0.10	0.70±0.14	0.34±0.14	0.68±0.05	0.72±0.06	0.70±0.05	0.42±0.12
LVID-diastolic (mm)	3.85±0.30	3.37±0.38	3.83±0.21	5.41±0.64	3.64±0.17	3.71±0.32	3.47±0.80	5.00±0.62	3.30±0.36	3.81±0.26	3.51±0.48	5.57±0.78	3.94±0.24	4.01±0.17	3.81±0.34	5.17±0.60
LV Volume-systolic (µL)	24.6±6.1	17.7±5.7	27.4±4.5	116.4±35.3	22.7±6.9	22.3±10.5	22.9±6.7	96.3±37.0	18.8±8.3	23.4±10.1	19.6±16.8	127.8±54.7	30.7±6.4	29.4±5.3	28.3±8.3	102.8±30.8
LVAW-systolic (mm)	1.03±0.22	1.03±0.32	1.00±0.12	0.57±0.28	0.95±0.08	1.29±0.17	1.09±0.11	0.54±0.14	1.07±0.22	1.10±0.20	1.13±0.22	0.67±0.27	1.07±0.09	1.10±0.11	1.10±0.07	0.61±0.11
LVPW-systolic (mm)	0.98±0.10	1.20±0.11	0.98±0.16	0.40±0.07	1.10±0.30	1.02±0.07	2.48±0.17	4.63±0.31	1.02±0.21	0.97±0.08	1.14±0.18	0.48±0.13	0.94±0.09	0.99±0.09	1.08±0.55	0.53±0.13
LVID-systolic (mm)	2.74±0.22	2.27±0.32	2.64±0.21	4.92±0.63	2.07±0.91	2.46±0.45	2.48±0.30	4.63±0.69	2.22±0.32	2.71±0.26	2.37±0.40	5.09±0.93	2.84±0.26	2.78±0.20	2.56±0.63	4.66±0.64
Heart Rate (beats/min)	434.1±34.2	537.6±15.0	455.1±16.5	511.4±26.1	464.6±19.4	477.6±45.2	466.3±15.6	475.5±23.2	465.4±6.3	507.4±17.1	469.2±17.2	502.0±14.5	515.8±19.4	522.5±15.5	486.4±15.3	535.2±10.6

Supplemental Table 3: Comparison of immune cell infiltration. Immune cell counts (4d post-MI) expressed as percent of WT.

	β2ARKO BMT-BZ	β2ARKO BMT-Infarct	β2ARKO BMT-Spleen	β2ARKO +GFP BMT-BZ	β2ARKO +GFP BMT-Infarct	β2ARKO +GFP BMT-Spleen	β2ARKO +β2AR BMT-BZ	β2ARKO +β2AR BMT-Infarct	β2ARKO +β2AR BMT-Spleen
CD68	3.5%	4.6%	513.9%	1.4%	7.4%	474.8%	105.2%	56.2%	143.6%
Tryptase	35.4%	30.6%	149.2%	47.4%	25.6%	139.0%	101.1%	95.4%	63.2%
MPO	15.9%	29.1%	203.9%	20.03%	20.2%	173.3%	95.4%	83.2%	86.2%

Supplemental Figure 1:



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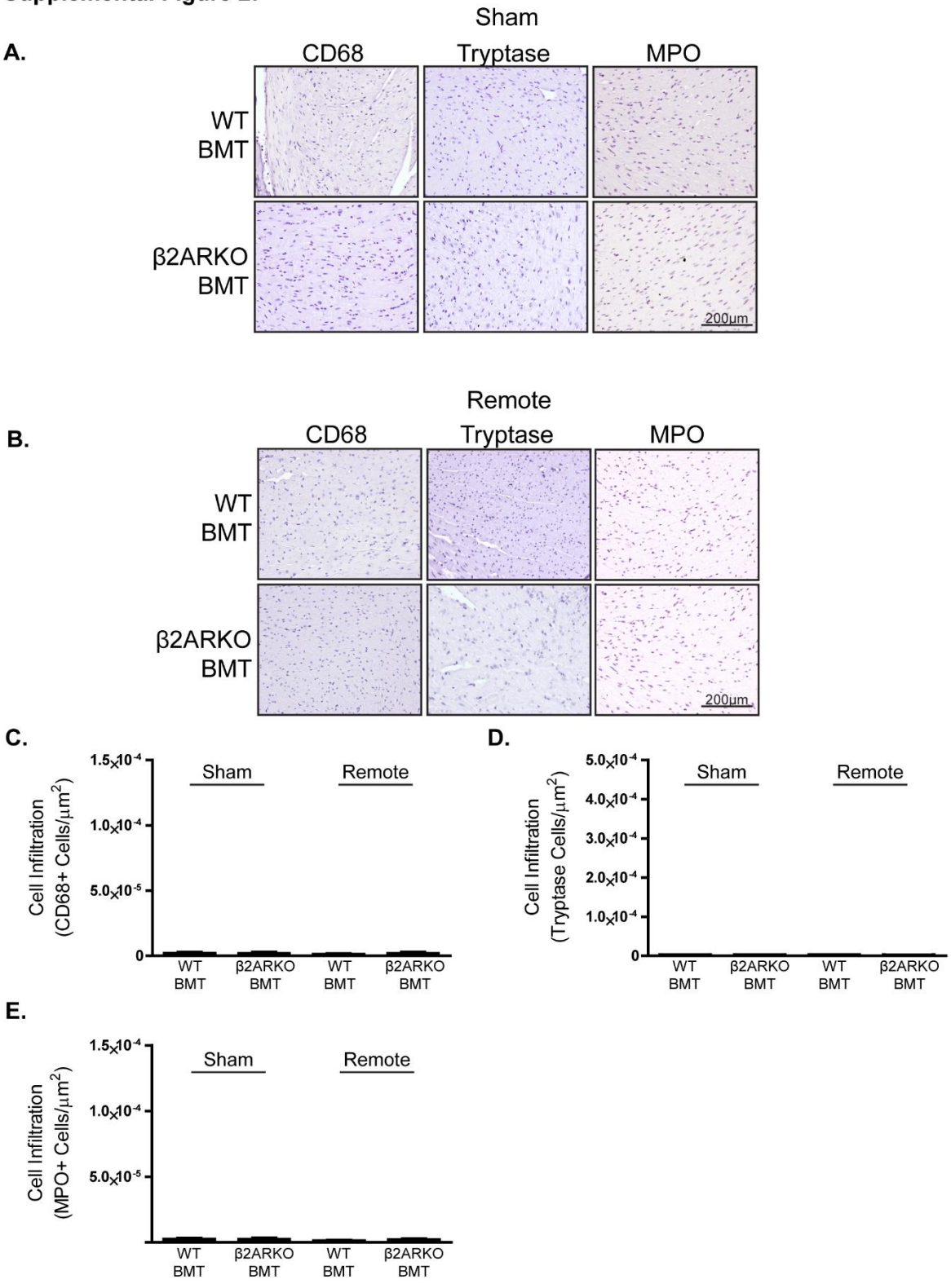

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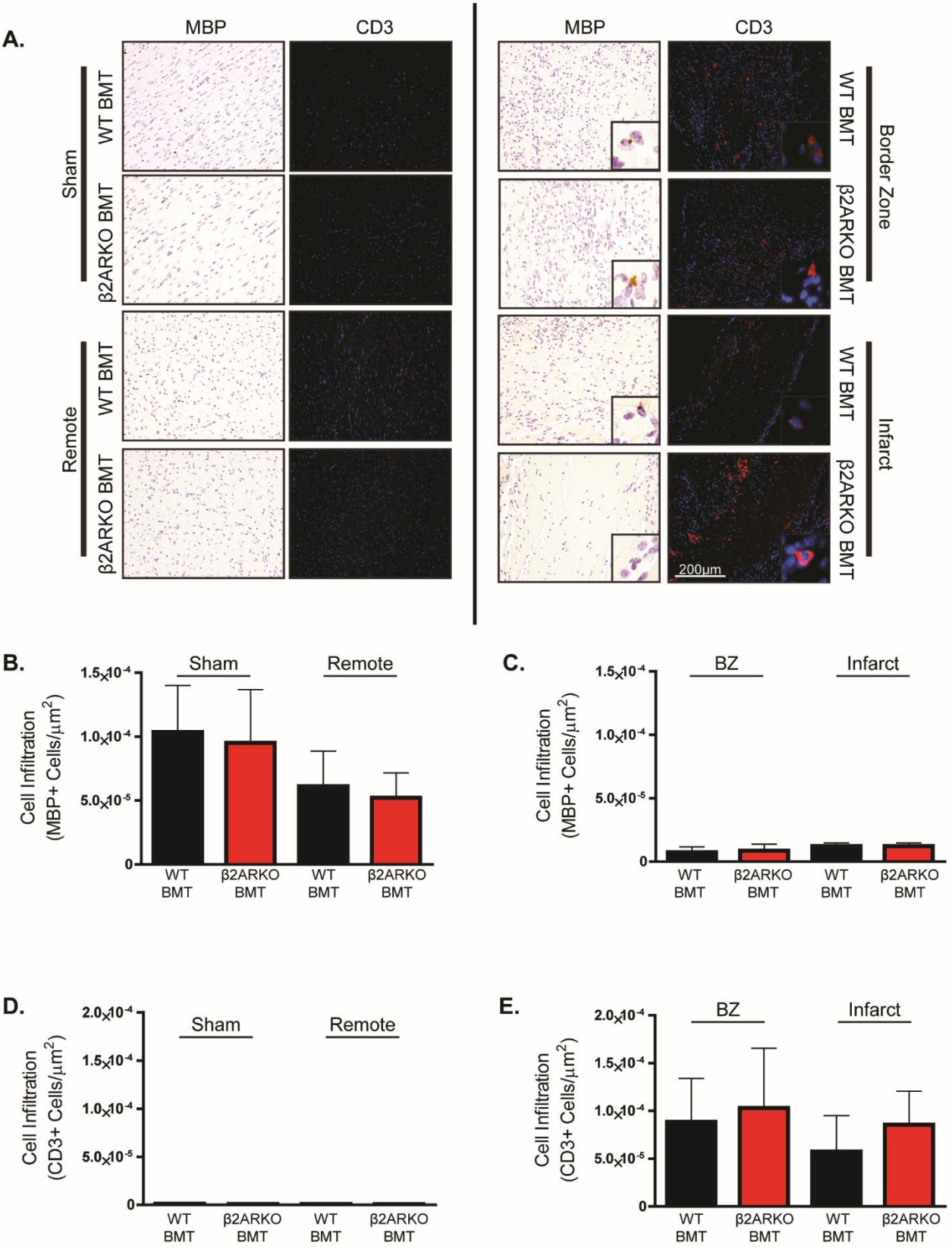

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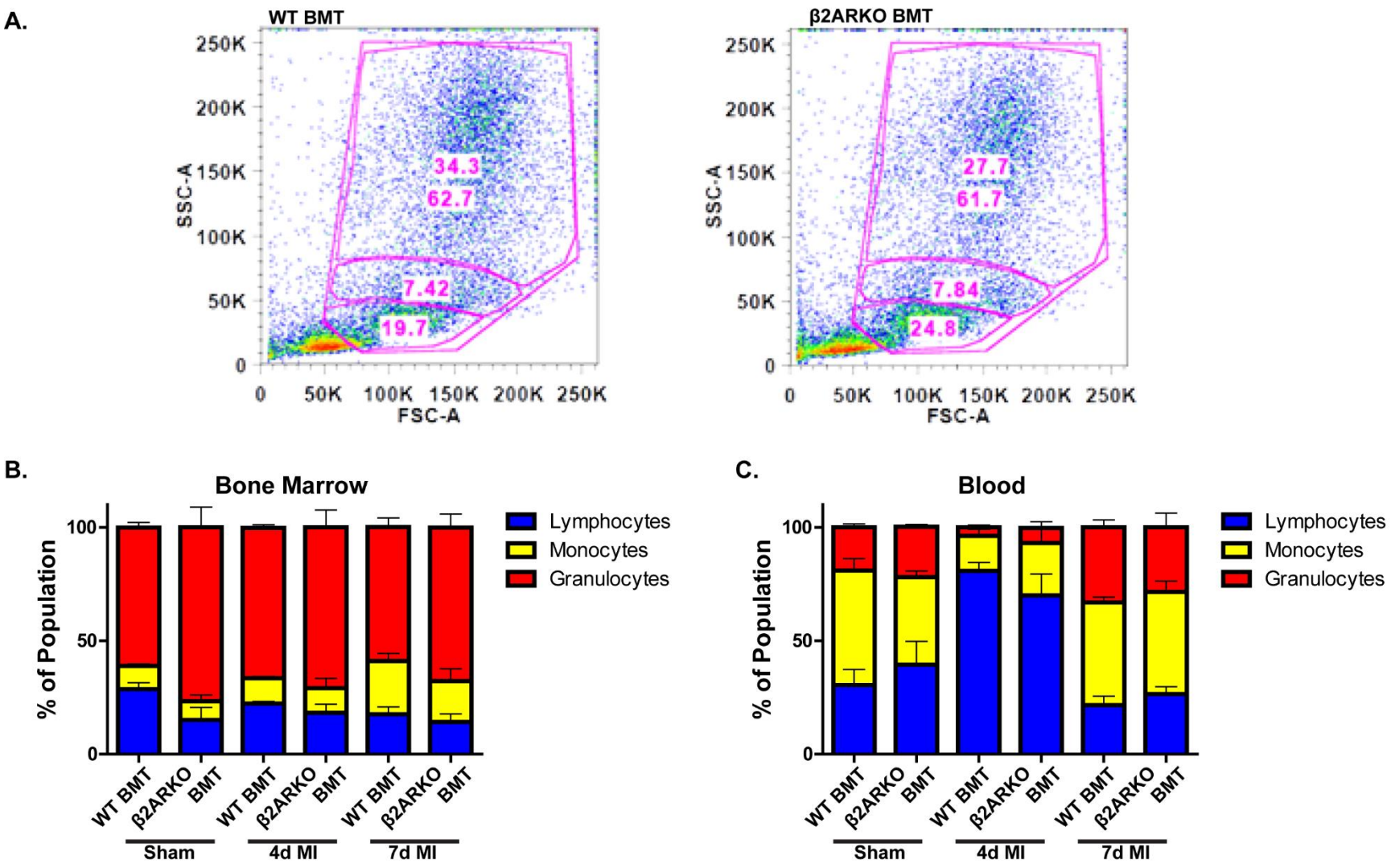

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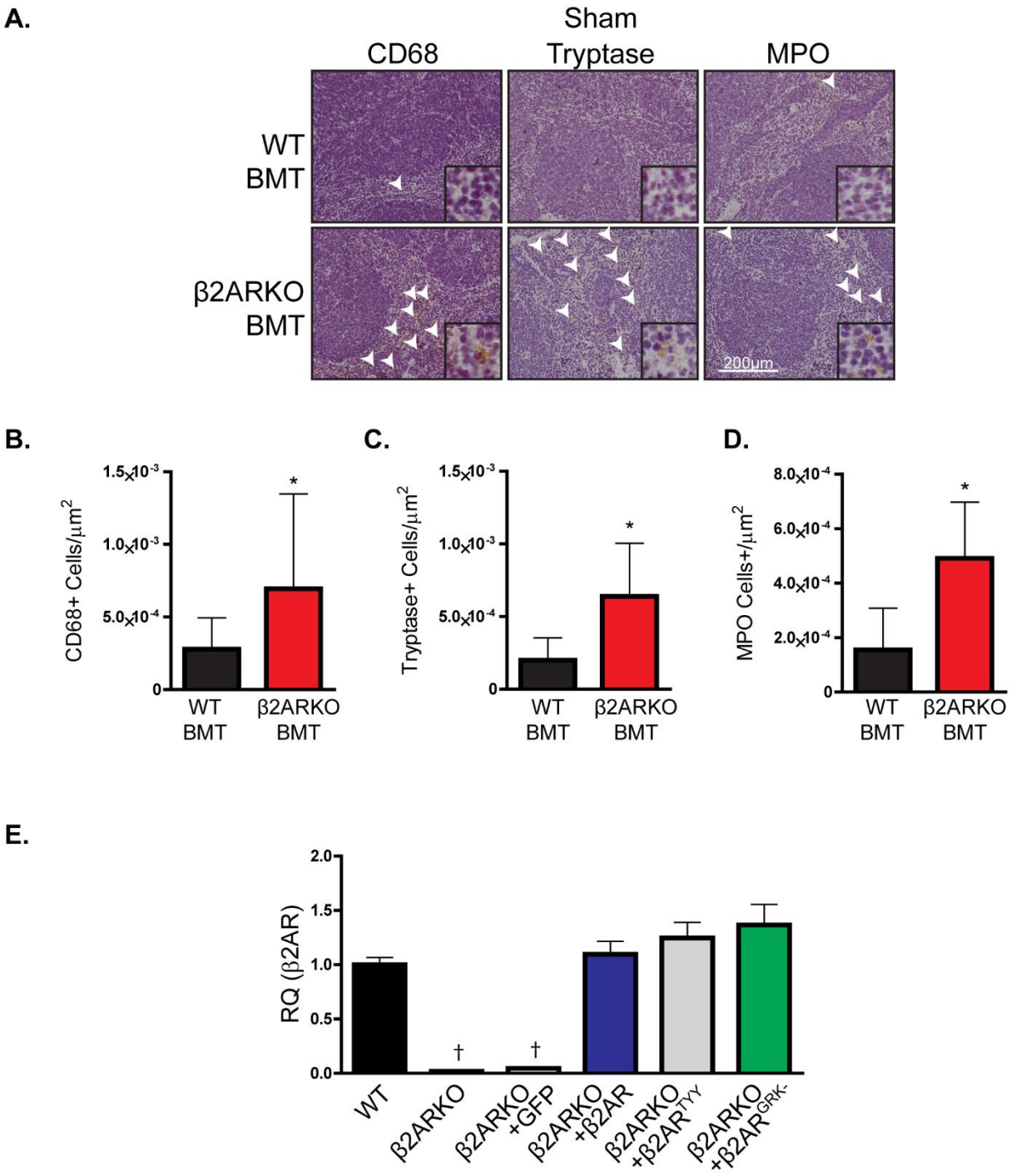

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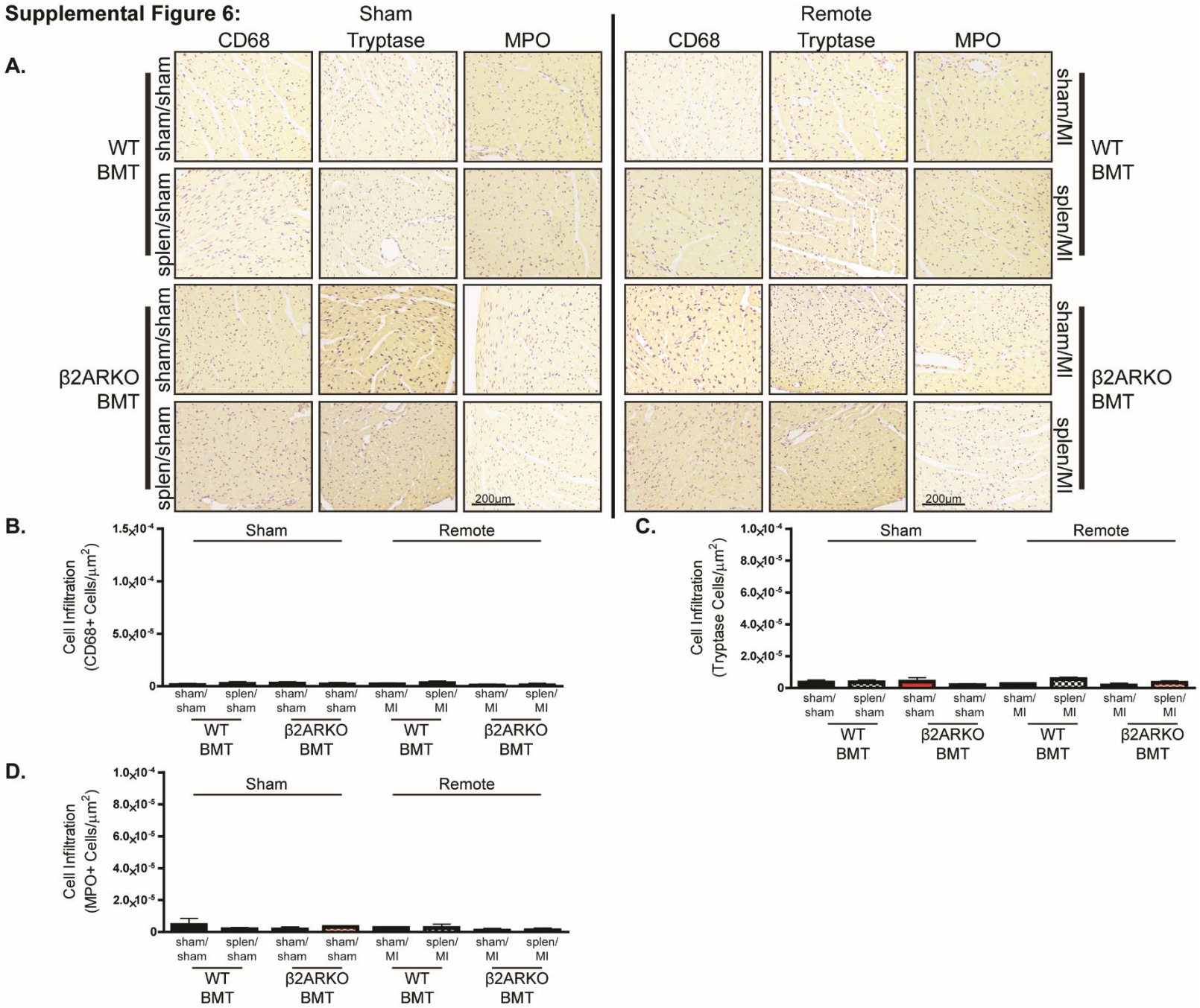

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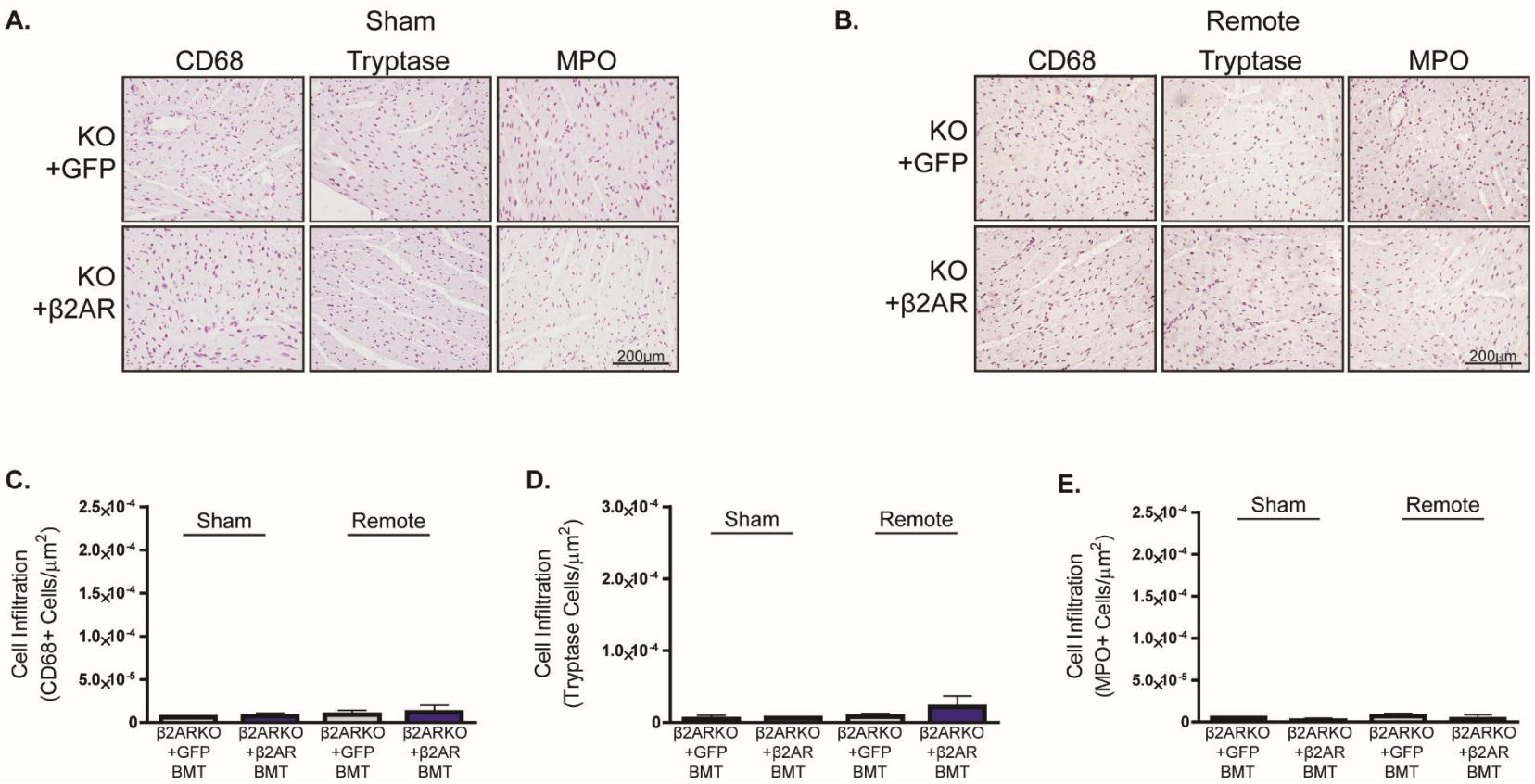

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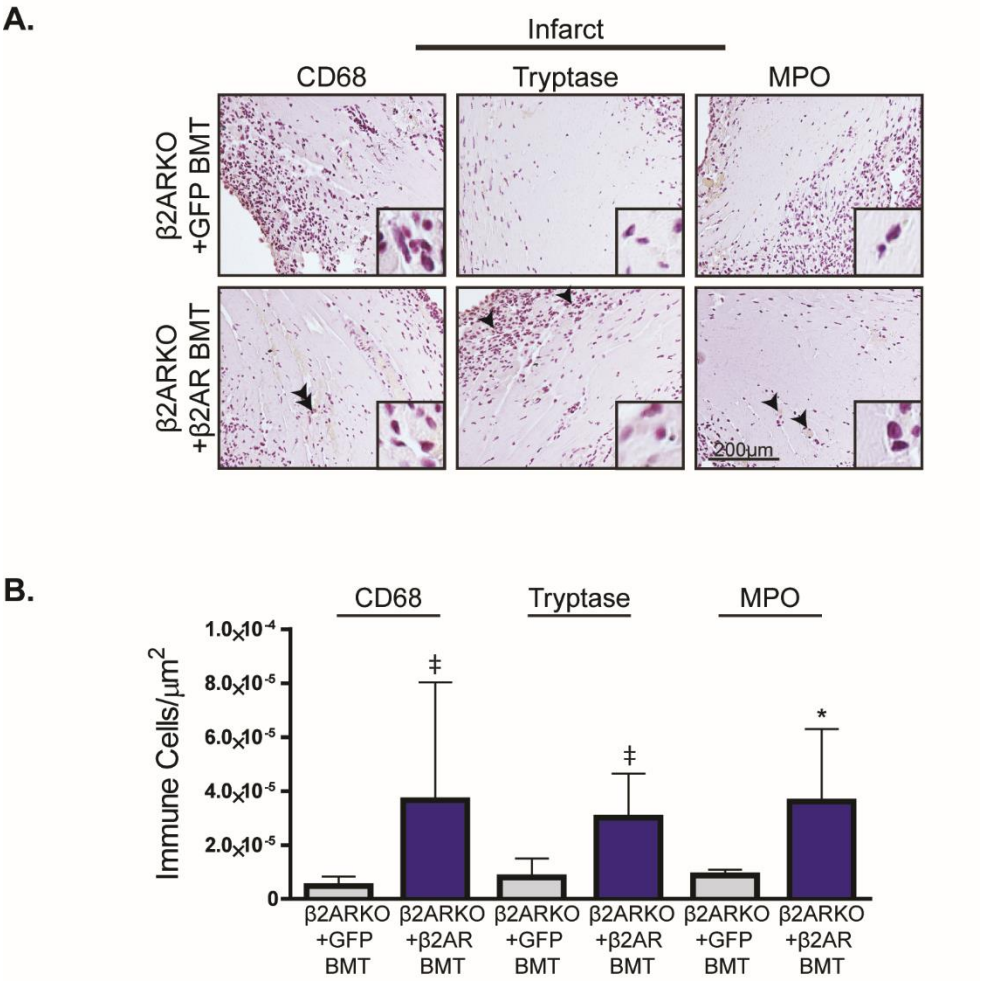

Supplemental Figure Legends:

Supplemental Figure 1. (A) Representative M-mode echocardiography from WT and β2ARKO BMT mice baseline and 1 week following sham or MI surgery. **(B)** Representative triphenyl tetrazolium chloride (TTC) staining for WT and β2ARKO BMT mouse hearts 24 h following MI surgery. Red indicates viable tissue while white shows necrotic tissues. **(C)** Quantification of infarct area from TTC staining was determined by calculating the volume of necrotic tissue compared to the total heart volume. n=4 for WT BMT and n=4 for β2ARKO BMT.

Supplemental Figure 2. Representative CD68, tryptase and MPO staining of sham **(A)** and the remote region **(B)** of hearts from WT and β2ARKO BMT animals 4d following surgery. Quantification of CD68 **(C)**, tryptase **(D)** and MPO **(E)** staining, n=4 for WT BMT sham, n=5 for β2ARKO BMT sham, n=5 for WT BMT 4d and n=5 for β2ARKO BMT 4d.

Supplemental Figure 3. (A) MBP and CD3 staining in sham hearts and the remote, border and infarct regions of WT and β2ARKO BMT mouse hearts following MI surgery. Quantification of MBP in sham hearts and remote region **(B)** and border and infarct regions **(C)**, and CD3 staining in sham hearts and remote region **(D)** and border and infarct regions **(E)** staining shows no significant differences. n=4 for WT BMT sham, n=4 for β2ARKO BMT sham, n=4 for WT BMT 4d and n=7 for β2ARKO BMT 4d.

Supplemental Figure 4. (A) Representative scatter plots for WT and β2ARKO BM separated by Forward Scatter (FSC) and Side Scatter (SSC). Flow cytometry of BM **(B)** and blood **(C)** was analyzed to determine immune cell subpopulations. Cells were sorted by size and granularity to identify temporal changes in the lymphocyte, monocyte and granulocyte populations following MI surgery in WT or β2ARKO BMT mice. **(B)** n=4 for WT BMT sham, n=4 for β2ARKO BMT sham, n=4 for WT BMT 4d, n=3 for β2ARKO BMT 4d, n=8 for WT BMT 7d

and n=4 for β2ARKO BMT 7d. **(C)** n=3 for WT BMT sham, n=3 for β2ARKO BMT sham, n=4 for WT BMT 4d, n=4 for β2ARKO BMT 4d, n=8 for WT BMT 7d and n=4 for β2ARKO BMT 7d.

Supplemental Figure 5. (A) Representative CD68, tryptase and MPO staining of spleens from WT and β2ARKO BMT sham animals. Quantification of CD68 **(B)**, tryptase **(C)** and MPO **(D)** staining from sham spleens. n=6 for WT BMT, n=8 for β2ARKO BMT, Exact Wilcoxon rank-sum test, * $P < 0.05$, † $p < 0.01$ vs WT. **(E)** β2AR expression was measured by RT-qPCR in BMDM from WT or β2ARKO mice or β2ARKO BMDM transduced with lentiviral constructs for GFP, β2AR, $\beta2AR^{TYY}$ or $\beta2AR^{GRK-}$. Values are presented as $RQ+RQ_{max}$ expressed relative to WT BMDM. n=9 for WT, n=6 for β2ARKO, n=6 for β2ARKO+β2AR, n=6 for β2ARKO+GFP, n= 6 for β2ARKO+$\beta2AR^{TYY}$, n=6 β2ARKO+$\beta2AR^{GRK-}$, Exact Wilcoxon rank-sum test with multiple comparison adjustment (5 comparisons), † $p < 0.01$ vs WT.

Supplemental Figure 6. Representative CD68, tryptase and MPO staining in sham-MI hearts and the remote region **(A)** of MI hearts from sham-splenectomy and splenectomy mice 4d following MI surgery. Quantification of CD68 **(B)**, tryptase **(C)** and MPO **(D)** staining, n=4 for WT BMT splen/sham, n=4 for β2ARKO BMT sham/sham, n=4 for sham/MI, n=5 for WT BMT splen/sham, n=5 for sham/MI, n=5 for β2ARKO BMT splen/sham, n=6 for WT BMT splen/MI, n=7 for β2ARKO BMT splen/MI.

Supplemental Figure 7. Representative CD68, tryptase and MPO staining of sham **(A)** and the remote region **(B)** of hearts from β2ARKO+GFP and β2ARKO+β2AR BMT mice 4d following surgery. Quantification of CD68 **(C)**, tryptase **(D)** and MPO **(E)** staining, n=5 for β2ARKO+GFP and β2ARKO+β2AR BMT sham, n=8 for β2ARKO+GFP BMT MI, n=6 for β2ARKO+β2AR BMT MI.

Supplemental Figure 8. Representative CD68, tryptase and MPO staining of the infarct zone **(A)** of hearts from β2ARKO+GFP and β2ARKO+β2AR BMT mice 4d following surgery. **(B)**

Quantification of CD68, tryptase and MPO staining. n=8 β2ARKO+GFP BMT and n=6 β2ARKO+β2AR BMT, Exact Wilcoxon rank-sum test, * $p < 0.05$, ‡ $p < 0.001$ vs β2ARKO+GFP BMT.