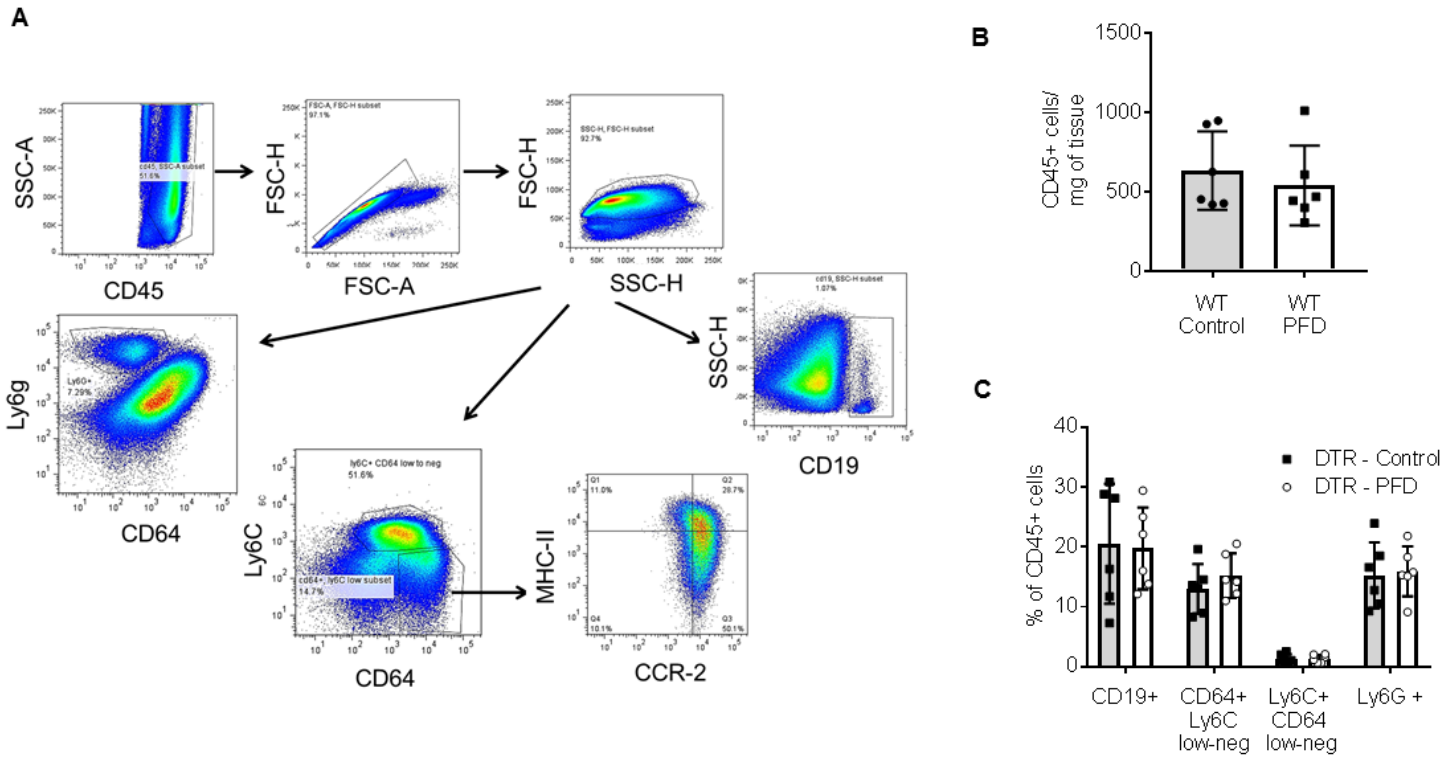


Figure S-1. A) Gating strategy for the flow cytometric analysis used to identify the different subpopulations of myocardial CD45+ cells. B-C) Wild type mice were fed either chow enriched with Pirfenidone (WT PFD) or regular chow (WT control). After 7 days, hearts were collected and analyzed via flow cytometry. n=6/group..

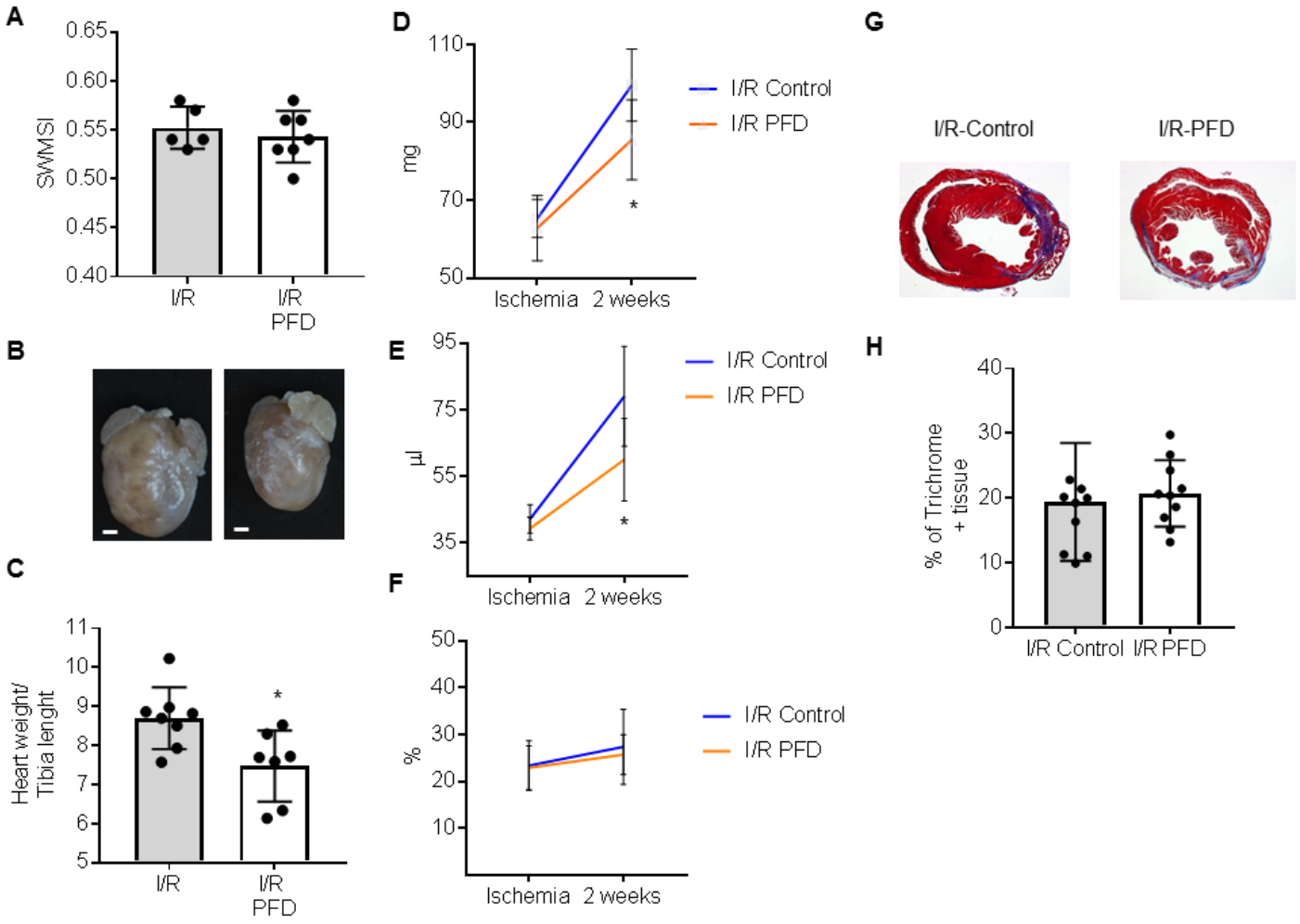


Figure S-2. Effects of Pirfenidone administered immediately after I/R injury.

Wild type mice were subjected to 90 minutes closed-chest ischemia reperfusion (I/R) injury. Mice were fed either chow enriched with Pirfenidone or regular chow. While in the experiment reported in Figure 3 the animals were randomized to Pirfenidone enriched diet or control diet 3 days prior to I/R injury, in this experiment mice were given Pirfenidone or vehicle intraperitoneally after I/R injury and were randomized to Pirfenidone enriched or control diet only after I/R injury. A) Area at risk during closed chest-ischemia as determined by the simplified segmental wall motion score index (SWMSI) at time of ischemia. B) Representative pictures of hearts harvested from control mice (left) and Pirfenidone treated animals (right) 2 weeks post I/R injury. Scale bar = 1mm. C) Gravimetric analysis of hearts harvested from control mice (I/R control) and Pirfenidone treated animals (I/R-PFD). n = 8 control, n=7 Pirfenidone. .D-F) echocardiographic assessment of myocardial function at the time of ischemia and 2 weeks after I/R injury, n=5 I/R control, n=7 I/R-PFD. D) LV mass (LVM) by 2-D echocardiography, E) LV end-diastolic volume (LVEDV). F) LV ejection fraction (LVEF). *=$p<0.05$. Bars represent average. Error bars represent standard deviation. G) Representative trichrome staining of histological sections of hearts from control animals (left panel) and Pirfenidone treated animals (right panel). 1.25X magnification H) Quantitative assessment of the % of trichrome positive staining, 2 sections analyzed per each heart, n=10 sections/group. * = $p< 0.05$. Bars represent mean, error bars represent standard deviation. P values were calculated with Student's T test.

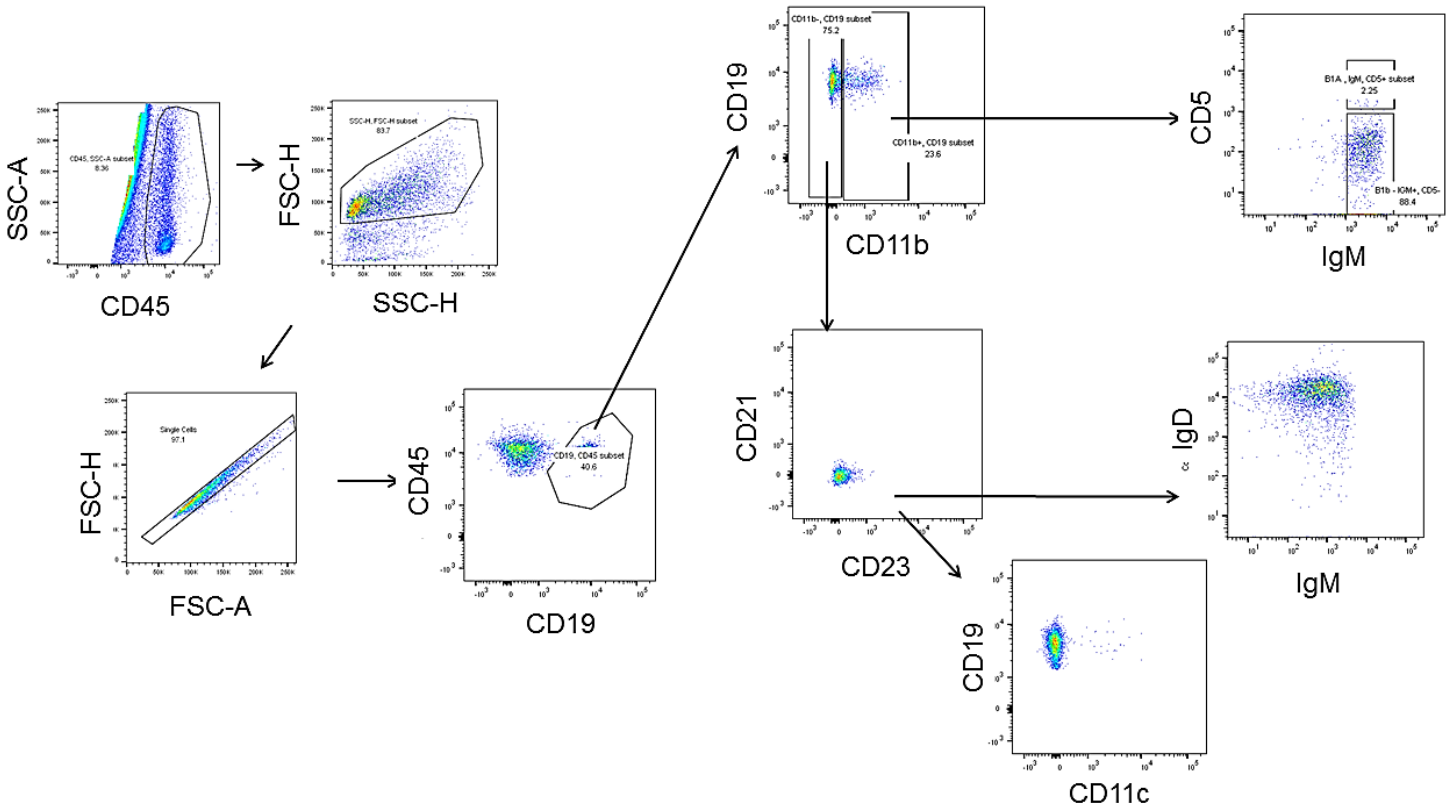


Figure S-3. Gating strategy used to study the different subpopulations of myocardial CD19+ cells. The scale of IgM staining is consistent across different graphs

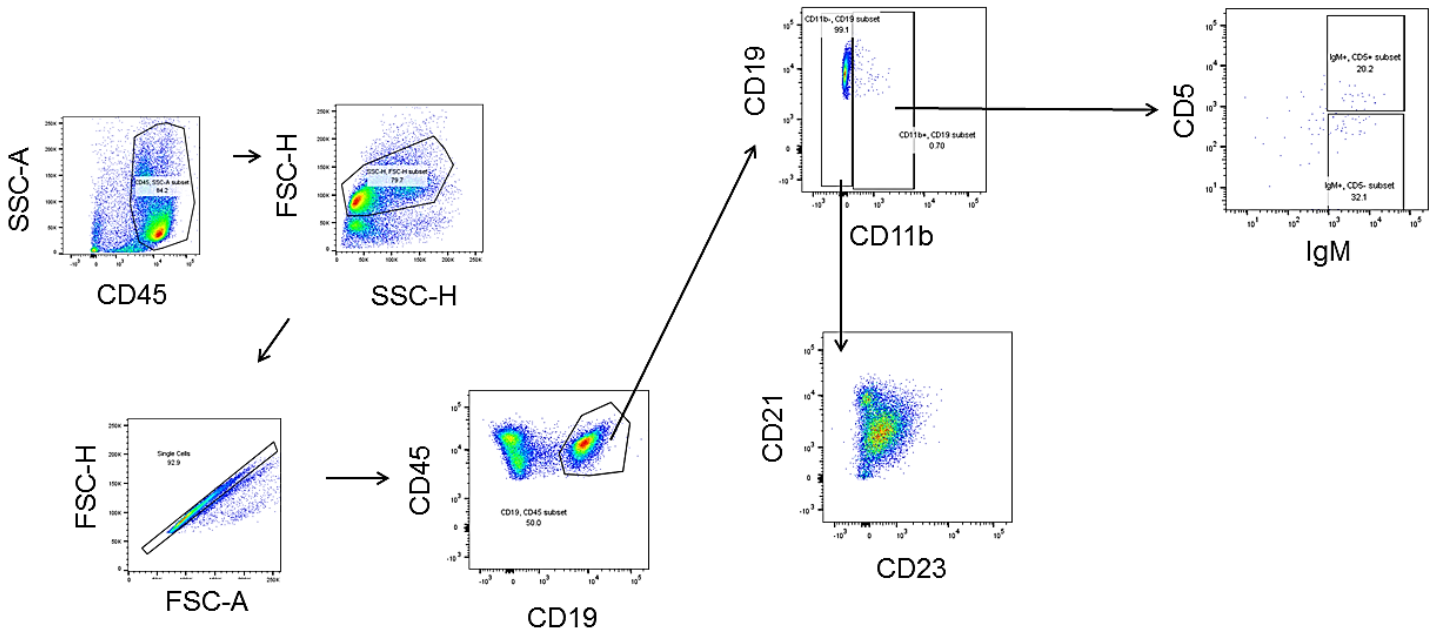


Figure S-4. Staining of splenic CD19+ cells (provided for reference)

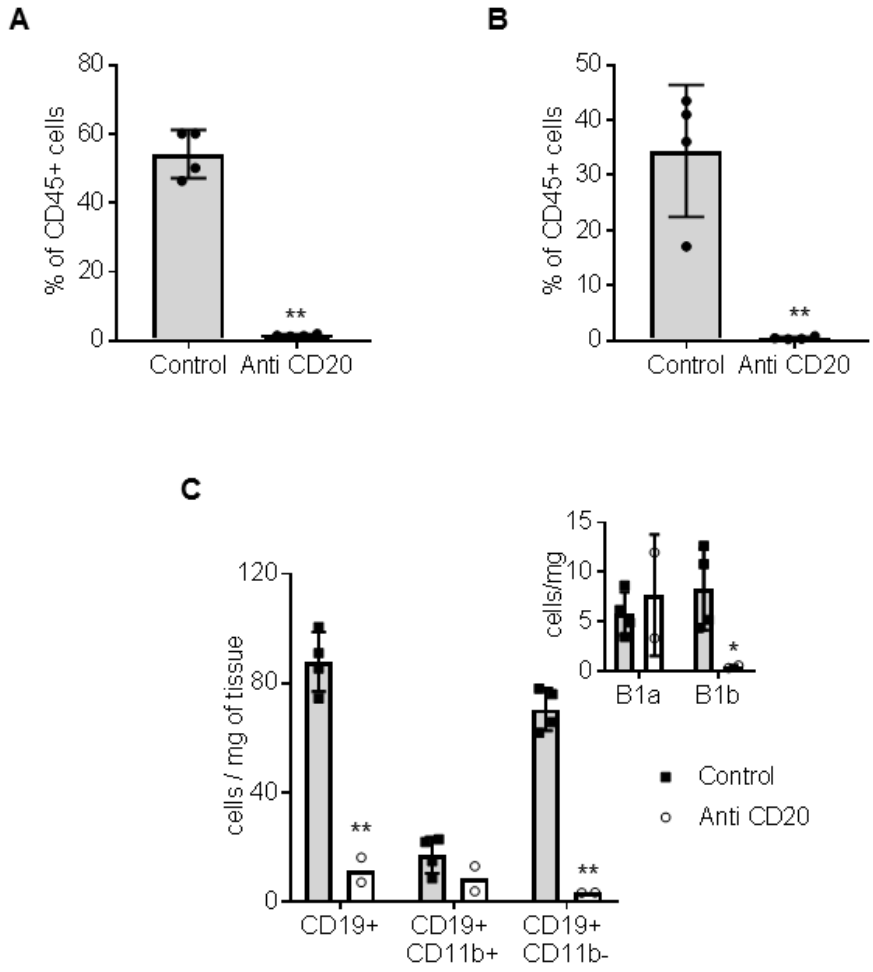
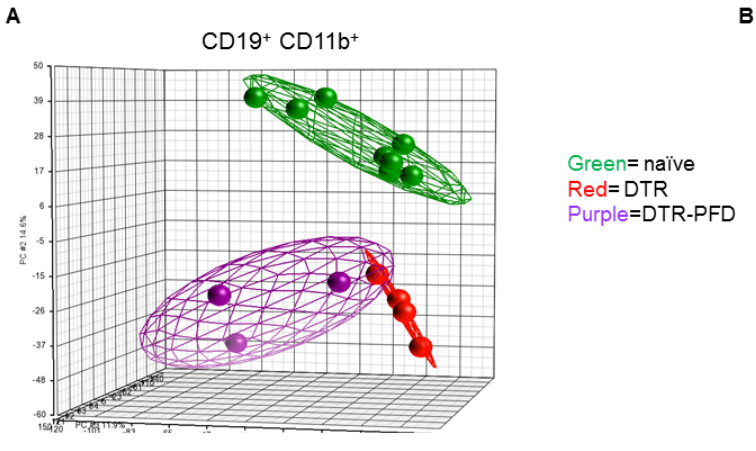


Figure S-5. B cell depletion with anti-CD20 antibody. A-B) Mice were injected with Anti-CD20 antibody (Anti CD20) or isotype control (Control). 7 days after injection the spleen (panel A, n=3/group) and the heart (panel B, n=3 group) were collected and analyzed via flow cytometry. C) Mice were injected with Anti-CD20 antibody or isotype control (Control). On day 7 post injection, mice were subjected to closed chest ischemia-reperfusion injury. On day 4 post ischemia-reperfuion injury the animals were sacrificed and the myocardium was analyzed via flow cytometry. n=2 Anti CD20, n=3 Control. Bars represent average, error bars represent standard deviation.



C

CD19+ CD11b+

Pathway	Differentially regulated: DTR vs naive	Differentially regulated: DTR-PFD vs naive
Hematopoietic cell lineage	✓	
Cytokine-cytokine receptor interaction	✓	
B cell receptor signaling pathway	✓	
Cell adhesion molecules (CAMs)	✓	
Antigen processing and presentation	✓	
MAPK signaling pathway	✓	

D

CD19+ CD11b-

Pathway	Differentially regulated: DTR vs naive	Differentially regulated: DTR-PFD vs naive
Cytokine-cytokine receptor interaction	✓	
Hematopoietic cell lineage	✓	✓
Toll-like receptor signaling pathway	✓	
Chemokine signaling pathway	✓	
TNF signaling pathway	✓	

Figure S-6. Transcriptional profiling of CD19$^+$CD11b$^+$ and CD19$^+$CD11b$^-$ cells. Mice expressing the diphtheria toxin receptor in the myocardium were either left untreated (naive) or exposed to diphtheria toxin and fed chow enriched with Pirfenidone (DTR-PFD) or regular chow (DTR)**.** Mice were sacrificed at day 4 post diphtheria toxin (DT) injection and the heart was collected for analysis via flow cytometry. CD19$^+$CD11b$^+$ and CD19$^+$CD11b$^-$ cells were FACS sorted from naïve hearts, and hearts from DT-treated mice fed regular diet (DTR) Pirfenidone enriched diet (PFD) and transcriptional profiling was performed using RNAseq. A) Principal component analysis of myocardial CD19$^+$CD11b$^+$ lymphocytes isolated from naive mice (green), DTR treated mice (red) and DTR treated mice exposed to Pirfenidone (purple). B) Principal component analysis of myocardial CD19$^+$CD11b$^-$ lymphocytes isolated from naive mice (green), DTR treated mice (red) and DTR treated mice exposed to Pirfenidone (purple). C-D) KEGG pathway analysis of genes with differential expression between DTR injured and naïve mice (left column) and DTR injured+Pirfenidone and naïve mice (right column) in CD19$^+$CD11b$^+$ lymphocytes (C) and CD19$^+$ CD11b$^-$ lymphocytes (D). Further details provided in the text

Supplementary Tables

Table S-1 – KEGG analysis of genes with differential expression ≥ 2 folds or ≤ 2 folds in $CD19^+$ $Cd11b^+$ cells sorted from DTR injured hearts vs naïve hearts. List of genes highlighted within KEGG pathways indicated in figure S-6 C (Pathway followed by differentially expressed genes within that pathway)

Hematopoietic cell lineage
• CD14 antigen
• CD19 antigen
• CD2 antigen
• CD22 antigen; hypothetical protein LOC100047973
• CD34 antigen
• CD36 antigen
• CD37 antigen
• CD55 antigen
• CD9 antigen
• Fc receptor, IgE, low affinity II, alpha polypeptide
• complement receptor 2
• interleukin 1 beta
• interleukin 1 receptor, type II
• interleukin 5 receptor, alpha
• membrane-spanning 4-domains, subfamily A, member 1
• tumor necrosis factor
Cytokine-cytokine receptor interaction
• CD27 antigen
• CD40 antigen
• Fas (TNF receptor superfamily member 6)
• chemochine (C-X-C motif) receptor 5
• chemokine (C-C motif) ligand 4
• chemokine (C-C motif) ligand 6
• chemokine (C-C motif) receptor 1
• chemokine (C-C motif) receptor 5
• chemokine (C-C motif) receptor 6
• chemokine (C-C motif) receptor 7
• chemokine (C-X-C motif) ligand 2
• colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
• interleukin 1 beta
• interleukin 1 receptor, type II
• interleukin 21 receptor
• interleukin 5 receptor, alpha
• interleukin 6 signal transducer
• predicted gene 614; interleukin 2 receptor, gamma chain
• tumor necrosis factor (ligand) superfamily, member 9
• tumor necrosis factor receptor superfamily, member 10b
• tumor necrosis factor receptor superfamily, member 12a
• tumor necrosis factor receptor superfamily, member 13c

• tumor necrosis factor
B cell receptor signaling pathway
• CD19 antigen
• CD22 antigen; hypothetical protein LOC100047973
• CD72 antigen
• CD79A antigen (immunoglobulin-associated alpha)
• CD79B antigen
• RAS, guanyl releasing protein 3
• caspase recruitment domain family, member 11
• complement receptor 2
• protein kinase C, beta
• protein tyrosine phosphatase, non-receptor type 6
• vav 2 oncogene
Cell adhesion molecules (CAMs)
• CD2 antigen
• CD22 antigen; hypothetical protein LOC100047973
• CD34 antigen
• CD40 antigen
• histocompatibility 2, O region alpha locus
• histocompatibility 2, O region beta locus
• histocompatibility 2, Q region locus 1
• histocompatibility 2, class II, locus Mb2
• integrin alpha L
• integrin beta 7
• intercellular adhesion molecule 1
• protein tyrosine phosphatase, receptor type, C
• selectin, lymphocyte
• similar to histocompatibility 2, T region locus 24
Antigen processing and presentation
• cathepsin B
• cathepsin L
• heat shock protein 1B; heat shock protein 1A; heat shock protein 1-like
• heat shock protein 90 alpha (cytosolic), class B member 1
• histocompatibility 2, O region alpha locus
• histocompatibility 2, O region beta locus
• histocompatibility 2, Q region locus 1
• histocompatibility 2, class II, locus Mb2
• legumain
• similar to histocompatibility 2, T region locus 24; histocompatibility 2, T region locus 24
MAPK signaling pathway
• CD14 antigen
• Fas (TNF receptor superfamily member 6)
• RAS guanyl releasing protein 1
• RAS, guanyl releasing protein 2
• RAS, guanyl releasing protein 3
• arrestin, beta 2
• calcium channel, voltage-dependent, R type, alpha 1E subunit
• calcium channel, voltage-dependent, alpha 2/delta subunit 4
• dual specificity phosphatase 6
• dual specificity phosphatase 7; similar to dual specificity phosphatase 7
• growth arrest and DNA-damage-inducible 45 gamma

• heat shock protein 1B; heat shock protein 1A; heat shock protein 1-like
• interleukin 1 beta
• interleukin 1 receptor, type II
• mitogen-activated protein kinase kinase 3
• mitogen-activated protein kinase kinase kinase kinase 2
• protein kinase C, beta
• ribosomal protein S6 kinase polypeptide 3
• tumor necrosis factor

Table S-2 KEGG analysis of genes with differential expression ≥ 2 folds or ≤ 2 folds in $CD19^+$ $Cd11b^-$ cells sorted from DTR injured hearts vs naïve hearts. List of genes highlighted within KEGG pathways indicated in figure S-6 D (Pathway followed by differentially expressed genes within that pathway)

Cytokine-cytokine receptor interaction
• CD40 antigen(Cd40)
• chemokine (C-C motif) ligand 4(Ccl4)
• chemokine (C-C motif) receptor 1(Ccr1)
• chemokine (C-C motif) receptor 5(Ccr5)
• chemokine (C-X-C motif) receptor 2(Cxcr2)
• colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)(Csf2rb)
• colony stimulating factor 3 receptor (granulocyte)(Csf3r)
• interleukin 1 beta(Il1b)
• interleukin 1 receptor, type II(Il1r2)
• leukemia inhibitory factor receptor(Lifr)
• tumor necrosis factor receptor superfamily, member 13b(Tnfrsf13b)
Hematopoietic cell lineage
• CD14 antigen(Cd14)
• CD2 antigen(Cd2)
• CD55 molecule, decay accelerating factor for complement(Cd55)
• Fc receptor, IgE, low affinity II, alpha polypeptide(Fcer2a)
• colony stimulating factor 3 receptor (granulocyte)(Csf3r)
• complement receptor 2(Cr2)
• interleukin 1 beta(Il1b)
• interleukin 1 receptor, type II(Il1r2)
• tumor necrosis factor(Tnf)
Toll-like receptor signaling pathway
• CD14 antigen(Cd14)
• CD40 antigen(Cd40)
• chemokine (C-C motif) ligand 4(Ccl4)
• interleukin 1 beta(Il1b)
• mitogen-activated protein kinase kinase 1(Map2k1)
• secreted phosphoprotein 1(Spp1)
• signal transducer and activator of transcription 1(Stat1)
• tumor necrosis factor(Tnf)
Chemokine signaling pathway
• chemokine (C-C motif) ligand 4(Ccl4)
• chemokine (C-C motif) ligand 6(Ccl6)
• chemokine (C-C motif) receptor 1(Ccr1)

• chemokine (C-C motif) receptor 5(Ccr5)
• chemokine (C-X-C motif) ligand 2(Cxcl2)
• chemokine (C-X-C motif) receptor 2(Cxcr2)
• mitogen-activated protein kinase kinase 1(Map2k1)
• phospholipase C, beta 2(Plcb2)
• signal transducer and activator of transcription 1(Stat1)
• son of sevenless homolog 2 (Drosophila)(Sos2)
TNF signaling pathway
• B cell leukemia/lymphoma 3(Bcl3)
• CCAAT/enhancer binding protein (C/EBP), beta(Cebpb)
• activating transcription factor 6 beta(Atf6b)
• chemokine (C-X-C motif) ligand 2(Cxcl2)
• interferon gamma inducible protein 47(Ifi47)
• interleukin 1 beta(Il1b)
• mitogen-activated protein kinase kinase 1(Map2k1)
• mitogen-activated protein kinase kinase kinase 5(Map3k5)
• tumor necrosis factor(Tnf)

Specificity	Brand	Clone	Fluorophore
CD45	Biolegend	30-F11	PerCp/Cy5.5
IgM	Biolegend	RMM-1	Alexa 488
CD86	Biolegend	GL-1	Qdot 605, PE
CD21/CD35	Biolegend	7E9	BV421
CD11b	BD Horizon	M1/70	V500, FITC
CD5	Biolegend	53-7.3	PE/Cy7
IgD	BD Pharmigen	11-16c.2a	PE
CD23	Biolegend	B3B4	APC/Cy7
CD19	Biolegend	6D5	APC
CD64	Biolegend	X54-5/7.1	PE
Ly6G	Biolegend	1A8	FITC
MHC-II	Biolegend	M5/114.15	PE-Cy7
Ly6C	Biolegend	HK1.4	APC-/Cy7
CCR2	Biolegend	SA203G11	BV421

Supplementary Table 3 – list of antibodies used