SUPPLEMENTAL FIGURES

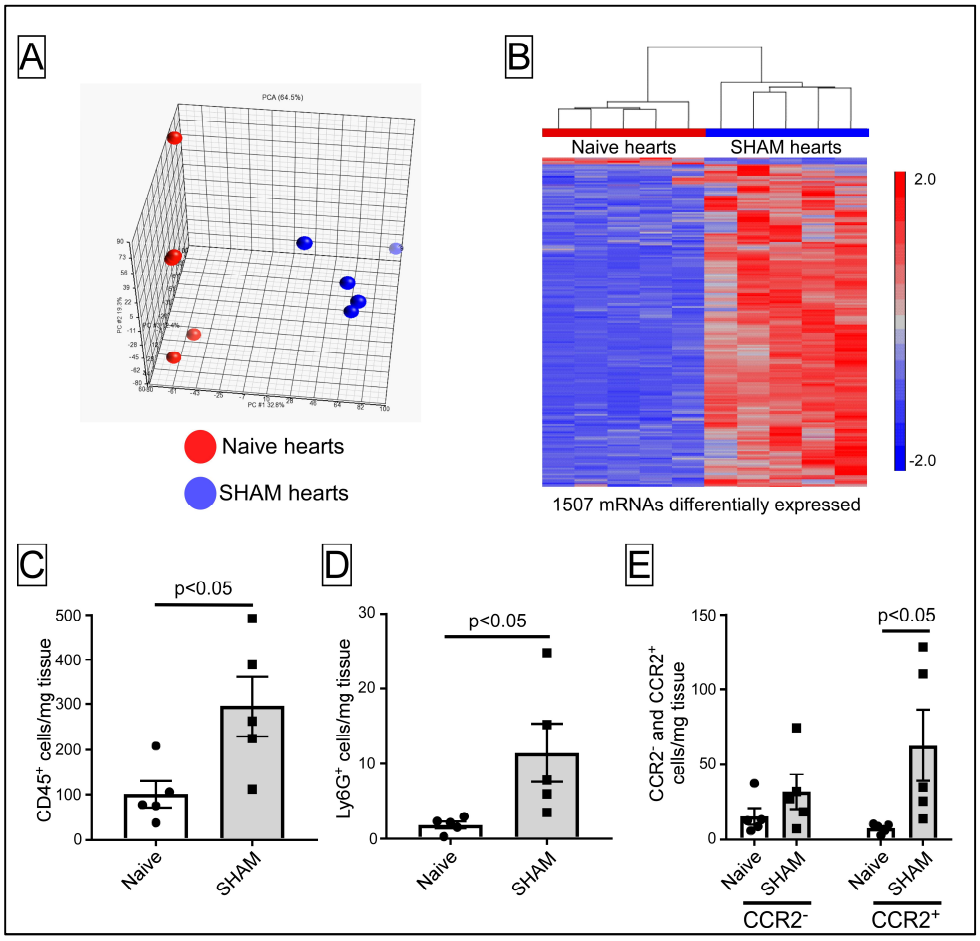

Figure S-1: Activation of the cardiac inflammatory response in the hearts of SHAM mice. Male C57BL/6J (10 weeks) were subjected to open-chest I/R (ischemia/reperfusion) SHAM procedure in order to examine the effects of this surgery in cardiac inflammation. The procedure consisted of thoracotomy and a suture passed around LAD (Left Anterior Descending coronary). The mouse chest was kept open for approximately 45 min. We chose to study an open-chest I/R SHAM because this is the most common surgical procedure used to study cardiac inflammation. Mice were sacrificed 5 days after the procedure and the hearts were removed and used in RNAseq and flow cytometry experiments. The naïve group consists of male littermate controls (C57BL/6J) not subjected to surgery. **A)** Principal component analysis of a total of 15,835 mRNAs from Naïve (n=5) and SHAM hearts (n=5) showed two distinct populations. **B)** Hierarchical clustering based on significantly expressed genes in the SHAM *vs* Naïve hearts (|fold-change|≥2, FDR<0.01; 1,507 mRNAs). KEGG pathway analyses of the differentially expressed genes marked dysregulation of several pathways related to inflammation (TNF signaling pathway, Chemokine signaling pathway, Phagosome, Hematopoietic cell lineage, Cytokine-Cytokine receptor interaction, Fc gamma R-mediated phagocytosis, Toll-like receptor signaling pathway, Natural Killer cell mediated cytotoxicity, B cell receptor signaling pathway, NOD-like receptor signaling pathway, Leukocyte transendothelial migration, NF-kappa B signaling pathway). The data is available on the NCBI GEO repository, accession number GSE120867. **C-E)** Flow cytometry analyses showed that the number of $CD45^+$ cells (p=0.03, leukocytes), $CD45^+Ly6G^+$ cells (p=0.04, neutrophils) and $CD45^+Ly6G^-CD64^+CCR2^+$ cells (p=0.04, $CCR2^+$ macrophages) was significantly increased in the hearts of SHAM mice as compared to naïve hearts n=5 hearts/condition. *P* values were calculated with Student's *t*-test.

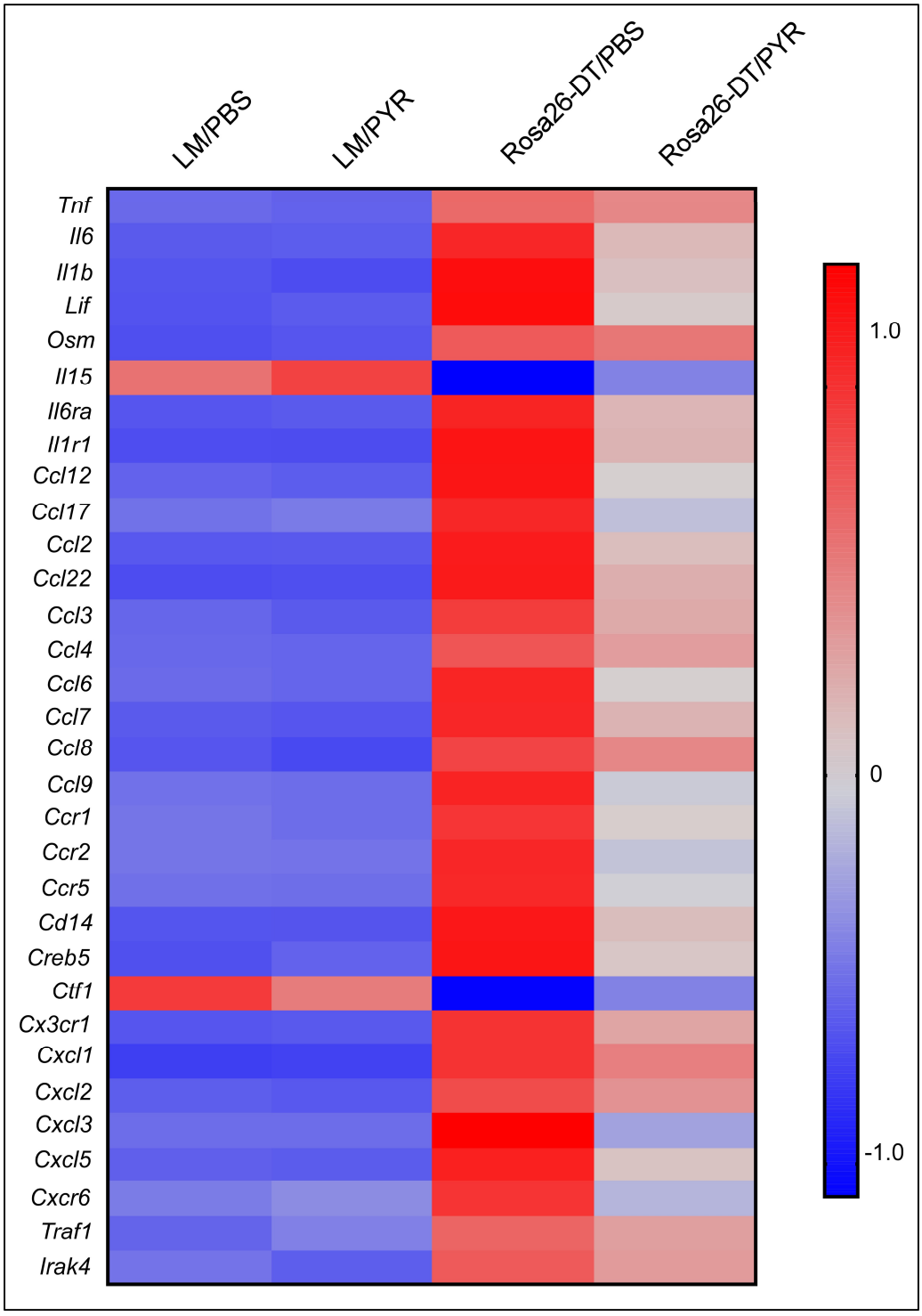

Figure S-2: Heat map of the PYR-induced reversed genes at Day 5. Cytokines, chemokines and receptors of cytokines and chemokines differentially expressed in Rosa26-DT$^{Mlv2c\text{-}Cre}$ (Rosa26-DT)/PYR hearts in comparison to Rosa26-DT$^{Mlv2c\text{-}Cre}$/PBS. Specific genes were identified in the top 4 high-scored inflammatory KEGG pathways (|fold-change|≥1.5 and FDR<0.05; n= 6 hearts/condition).

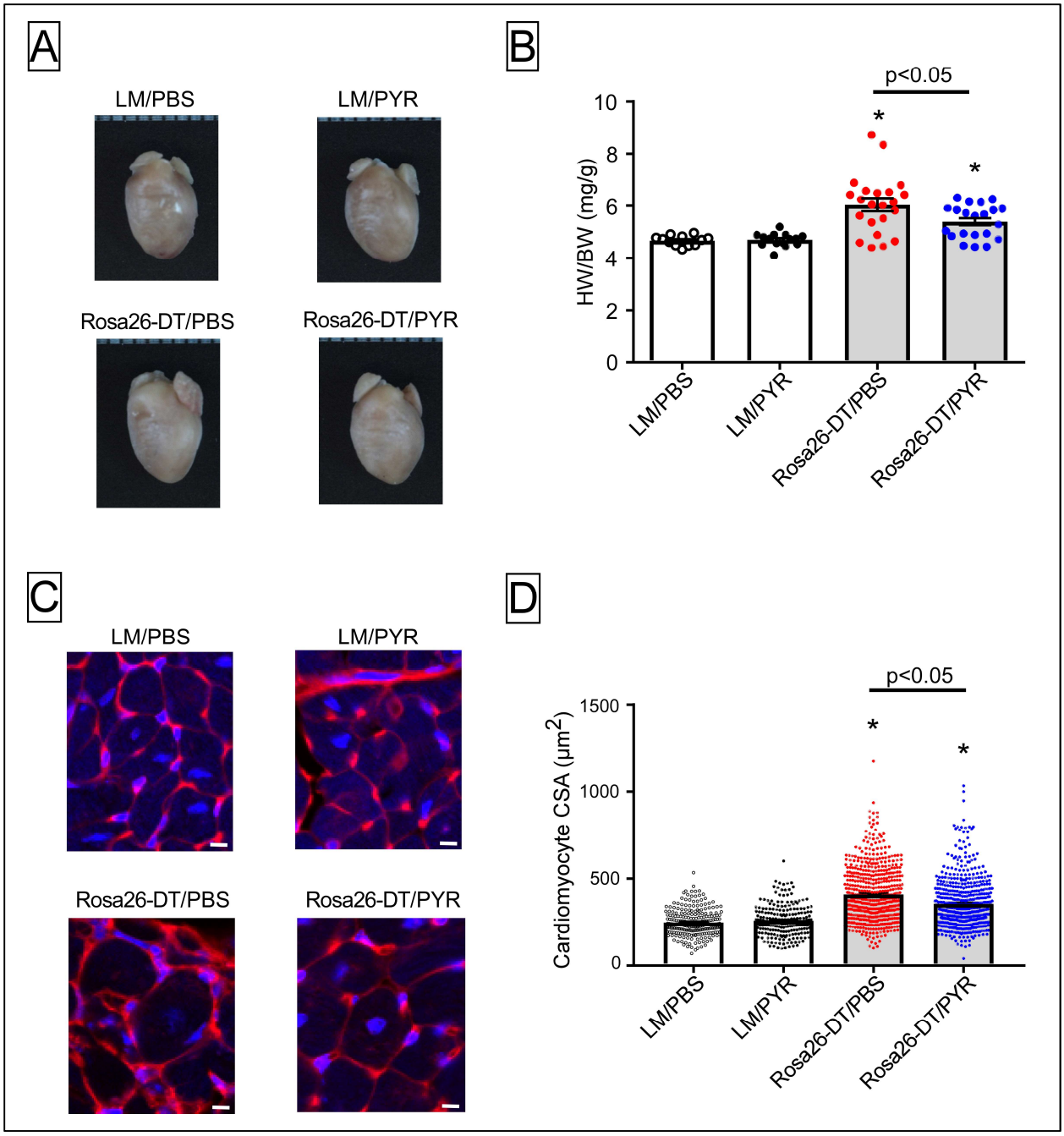

Figure S-3: Cardiac hypertrophy is significantly attenuated by PYR in injured hearts at Day 5 post DT administration. A) Heart representative pictures. **B)** Heart Weight/Body Weight ratio in LM and Rosa26-DT$^{Mlv2c\text{-}Cre}$ (Rosa26-DT) mice diluent- or PYR-treated. n=12-22 hearts/condition. * = p<0.05 in comparison to LM hearts. **C)** Representative WGA stained hearts. Scale bar: 10μm. **D)** Cardiomyocyte cross-sectional area (CSA) is increased following injury. PYR treatment significantly inhibited the increase in cardiomyocyte CSA induced by injury. Data obtained from 4 hearts. n= ~250-500 cells analyzed per condition. * = p<0.05 in comparison to LM hearts. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test.

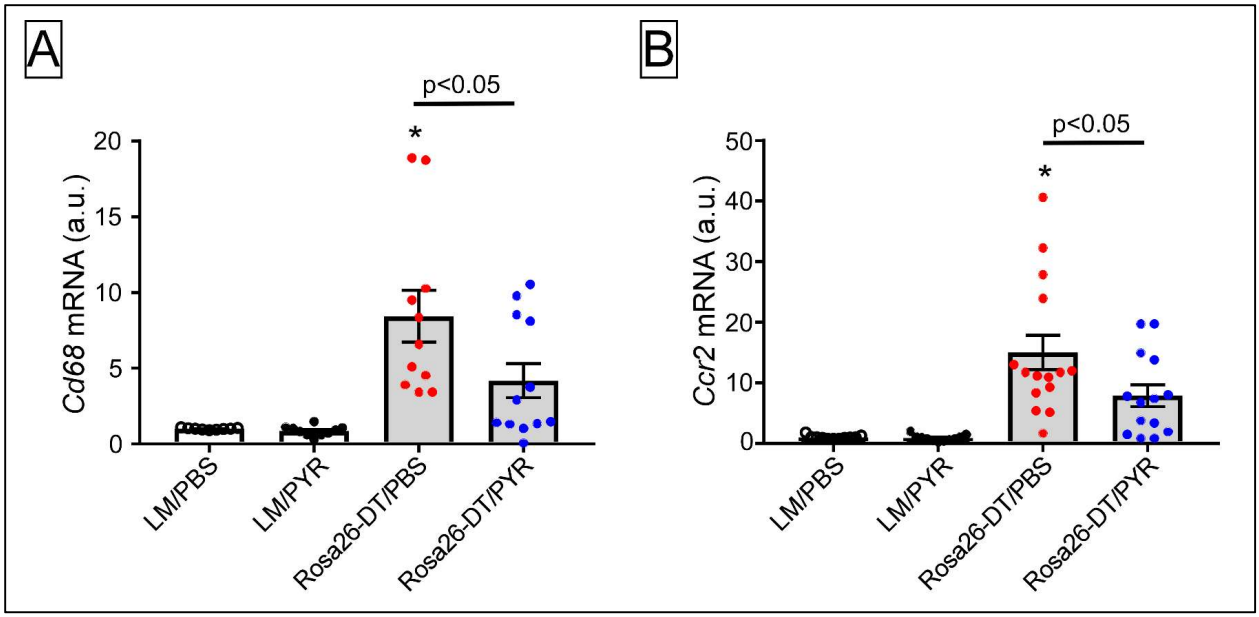

Figure S-4: RNA expression of macrophage markers in the heart at Day 5 post-injury. A-B) Bar-graph showing the expression of *Cd68* and *Ccr2* mRNAs in hearts from LM and Rosa26-DT$^{MIv2c\text{-}Cre}$ (Rosa26-DT) treated or not with PYR. Injury leads to an increase in the mRNA of these markers. PYR treatment significantly reduces the expression of *Cd68* and *Ccr2* in injured hearts (n=9-15 hearts per condition.). * = $p<0.05$ in comparison to LM hearts. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test.

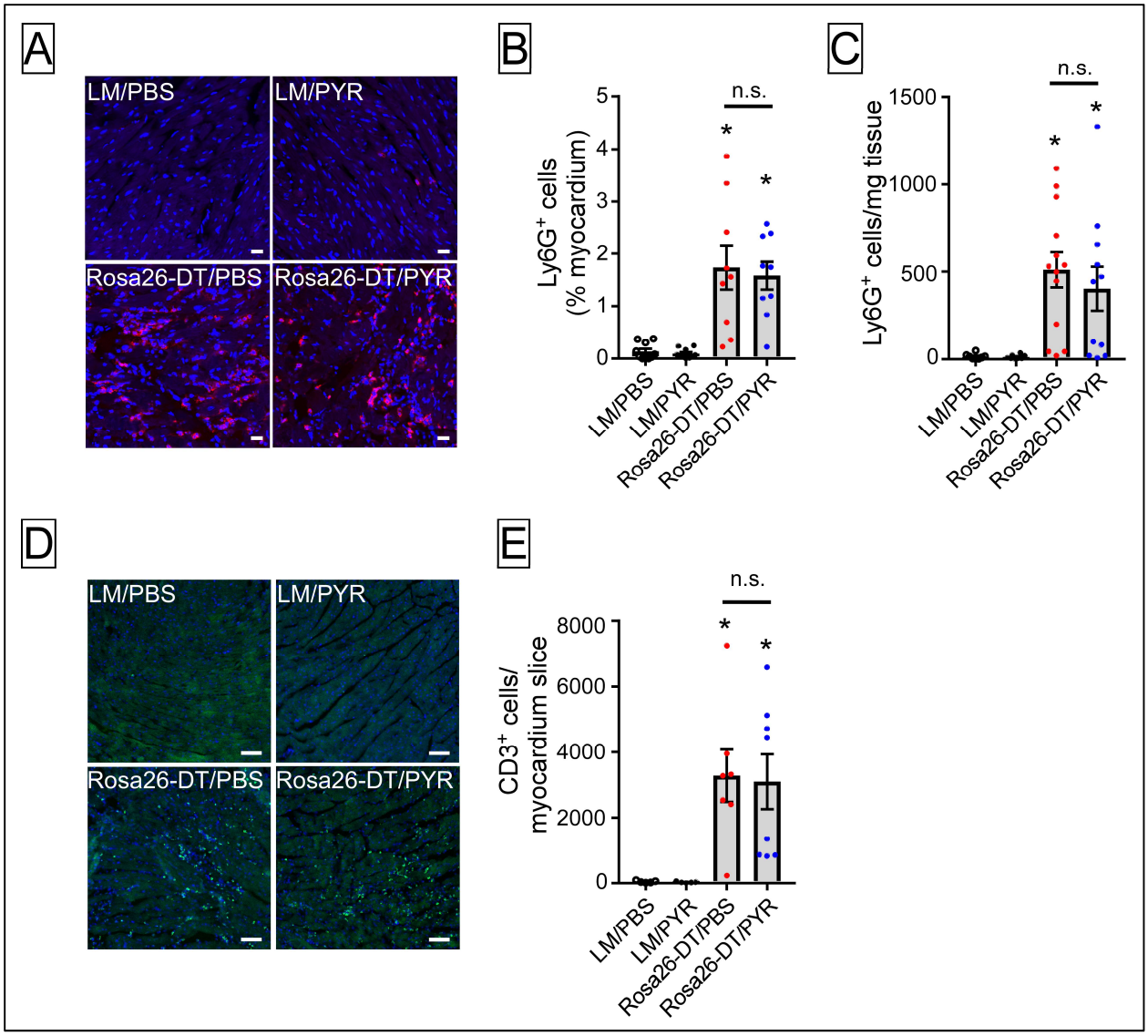

Figure S-5: PYR does not change the number of Ly6G$^+$ cells and CD3$^+$ cells in injured hearts (Day 5). A) Representative pictures of Ly6G$^+$ cells in the heart. Scale bar=20µm. **B)** Bar-graph showing the Ly6G stained area. n=9-10 myocardium sections obtained from 5 hearts per condition. **C)** Flow cytometry analyses showing the number of CD45$^+$Ly6G$^+$ cells/mg of cardiac tissue (n=8-13 hearts per condition). **D)** Representative pictures of CD3$^+$ cells in the heart (Scale bar= 50µm). **E)** Quantification of CD3$^+$ cells per myocardium slice. n=6-8 hearts per condition. * = $p<0.05$ in comparison to LM hearts; n.s.: not significant. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test.

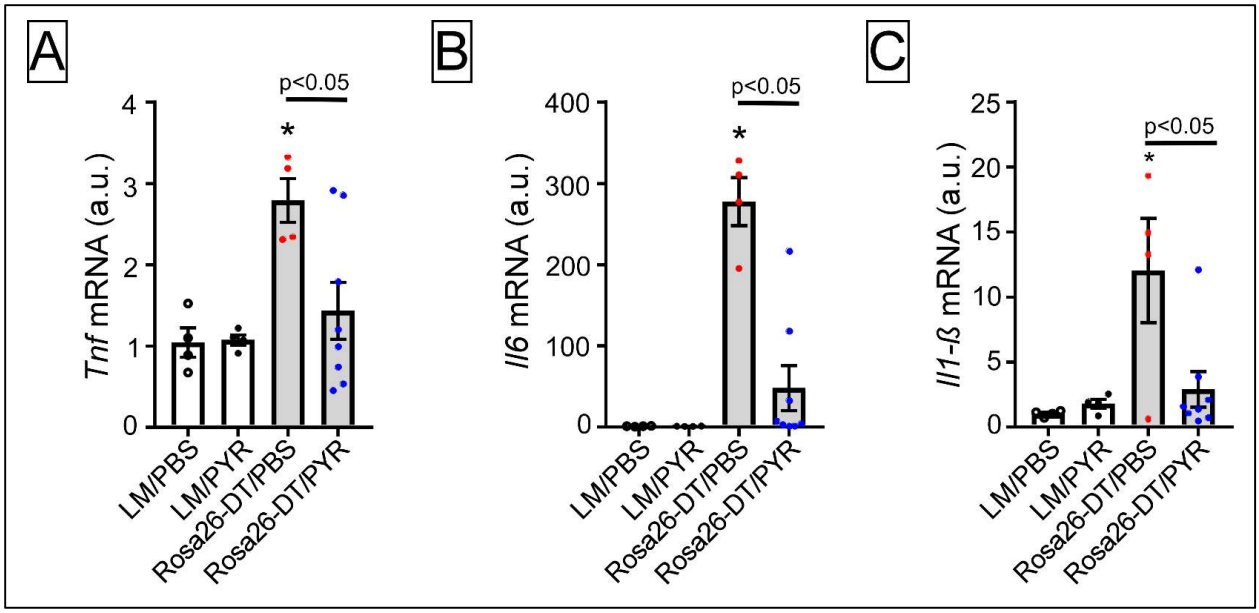

Figure S-6: PYR treatment inhibits the expression of pro-inflammatory cytokines in the heart at Day 3 post DT injection. A-C) Pro-inflammatory cytokines mRNA expression in the heart at Day 3. *Tnf*, *Il6* and *Il-1b* mRNAs are increased in the hearts of Rosa26-DT$^{Mlv2c\text{-}Cre}$ (Rosa26-DT)/PBS mice. In PYR-treated mice, the expression of these cytokines is significantly reduced. * = p<0.05 in comparison to LM hearts. n=4-8 hearts/condition. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test.

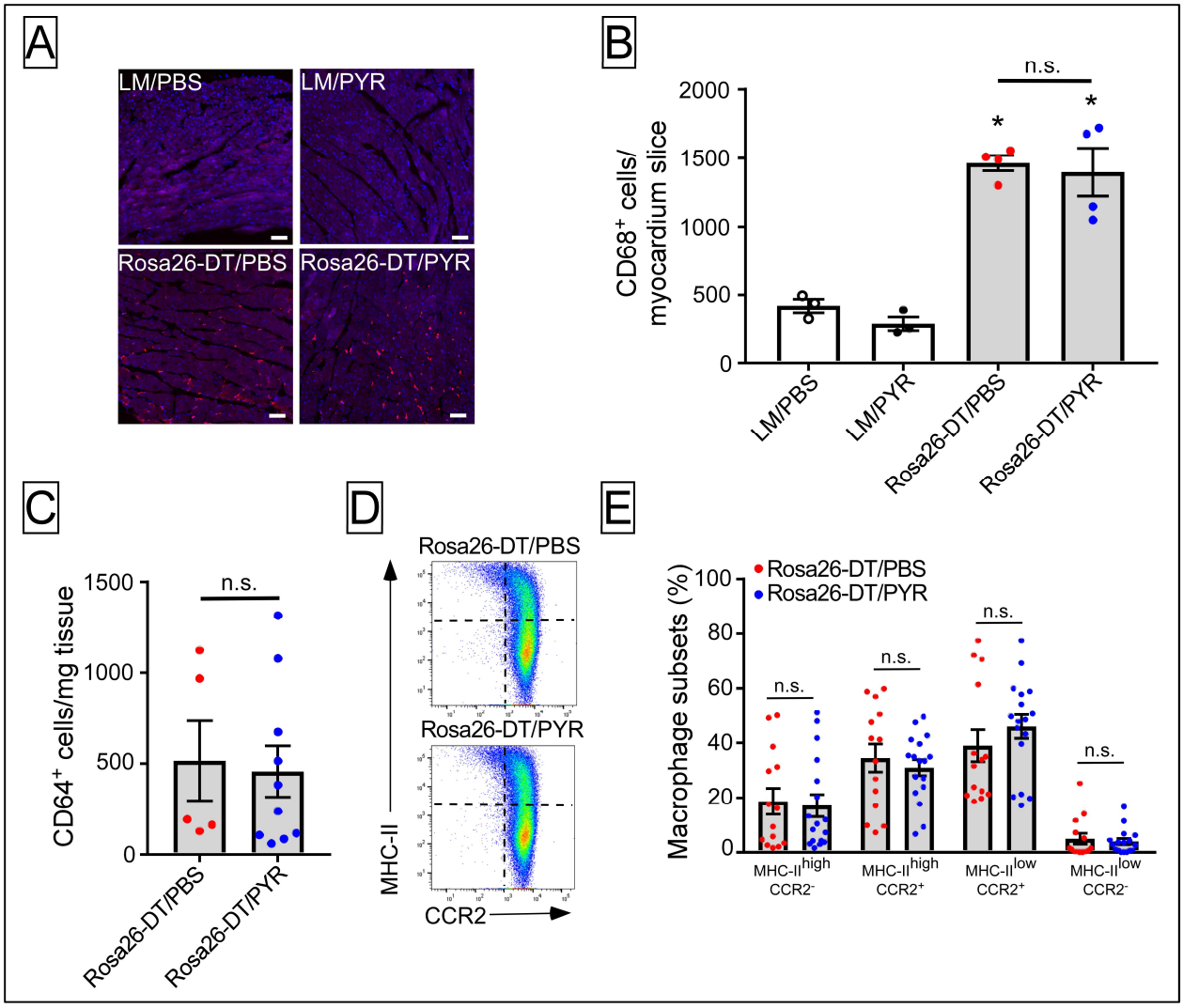

Figure S-7: PYR treatment does not change the number of CD68$^+$ cells in the heart at Day 3. A) Representative pictures of CD68$^+$ cells in the heart at Day 3 (Scale bar= 50µm). **B)** Quantification of the number of CD68$^+$ cells in the heart. n= 3-4 myocardium slices obtained from 3-4 hearts per condition. **C)** Quantification of the number of CD45$^+$Ly6G$^-$CD64$^+$ cells in the hearts of Rosa26-DT$^{Mlv2c\text{-}Cre}$ (Rosa26-DT)/PBS and Rosa26-DT$^{Mlv2c\text{-}Cre}$ /PYR mice by flow cytometry (n=5-10 hearts/condition). **D-E)** Macrophage subsets composition (CD45$^+$Ly6G$^-$CD64$^+$MHC-II$^{high/low}$CCR2$^{+/-}$) in the injured hearts treated or not with PYR at day 3 (n=14-17 hearts/condition). * = $p<0.05$ in comparison to LM hearts; n.s. = not significant. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test for panel (**B**) and Student's *t*-test for panels (**C**) and (**E**).

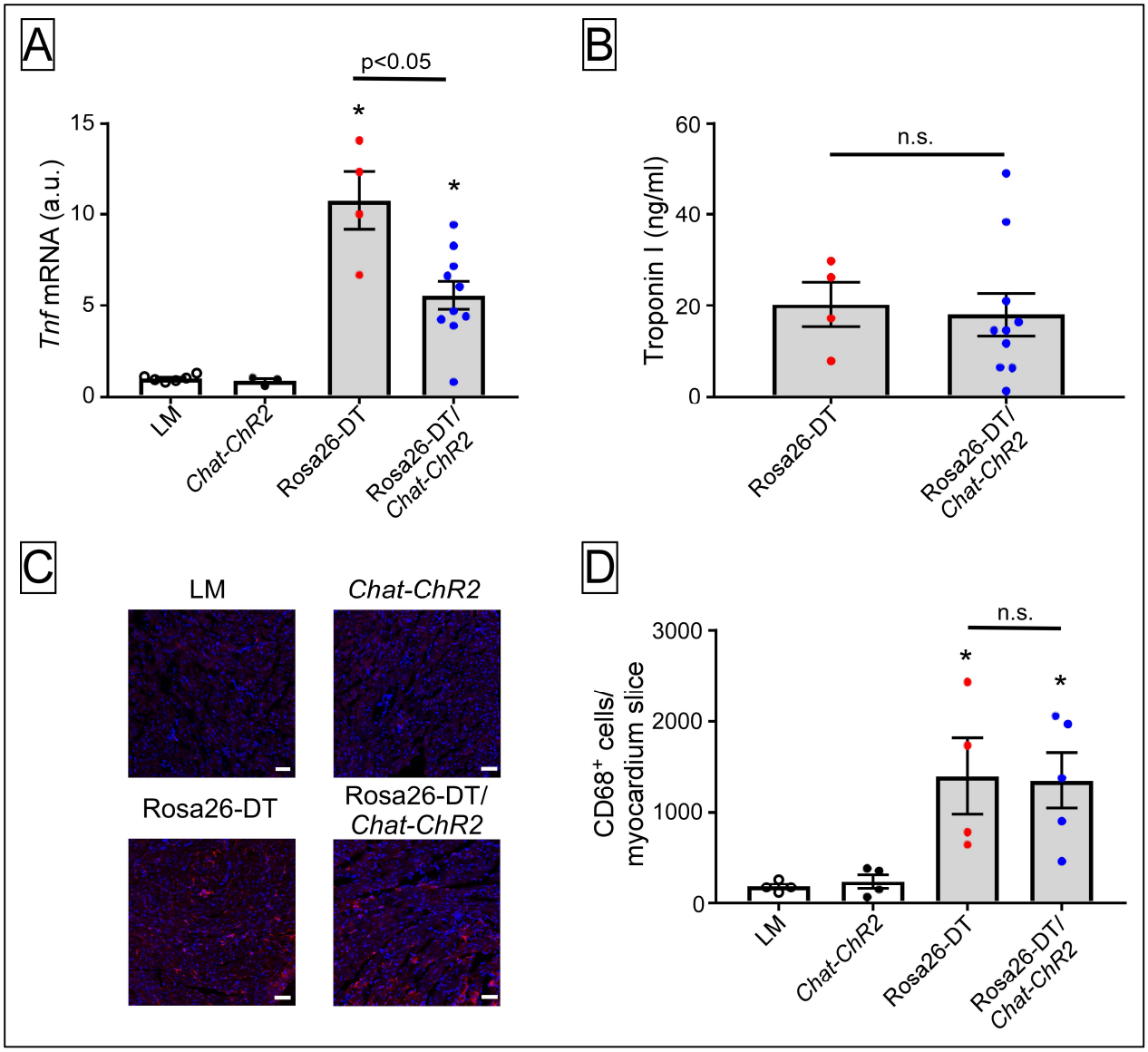

Figure S-8: Characterization of the Rosa26-DT$^{Mlv2c\text{-}Cre}$/*Chat-ChR2* model at Day 3 post-DT. A) *Tnf* mRNA expression in the hearts of LM, *Chat-ChR2,* Rosa26-DT$^{Mlv2c\text{-}Cre}$ and Rosa26-DT$^{Mlv2c\text{-}Cre}$/*Chat-ChR2* mice on day 3 (n=3-10 hearts per condition). **B)** Troponin I levels in the serum of Rosa26-DT$^{Mlv2c\text{-}Cre}$ (Rosa26-DT) and Rosa26-DT$^{Mlv2c\text{-}Cre}$/*Chat-ChR2* (n=4-10 samples) mice. **C)** Representative fluorescence images of CD68^{+} staining in the hearts of LM, *Chat-ChR2,* Rosa26-DT$^{Mlv2c\text{-}Cre}$ and Rosa26-DT$^{Mlv2c\text{-}Cre}$/*Chat-ChR2* mice on day 3 (Scale bar= 50µm). **D)** Quantification of CD68^{+} cells in the heart (n=4-5 myocardium sections obtained from 4-5 hearts/condition). * = p<0.05 in comparison to LM hearts; n.s = not significant. *P* values were calculated with one-way ANOVA followed by the Tukey post hoc test for panel (**B**) and (**D**) and Student's *t*-test for panels (**A**).

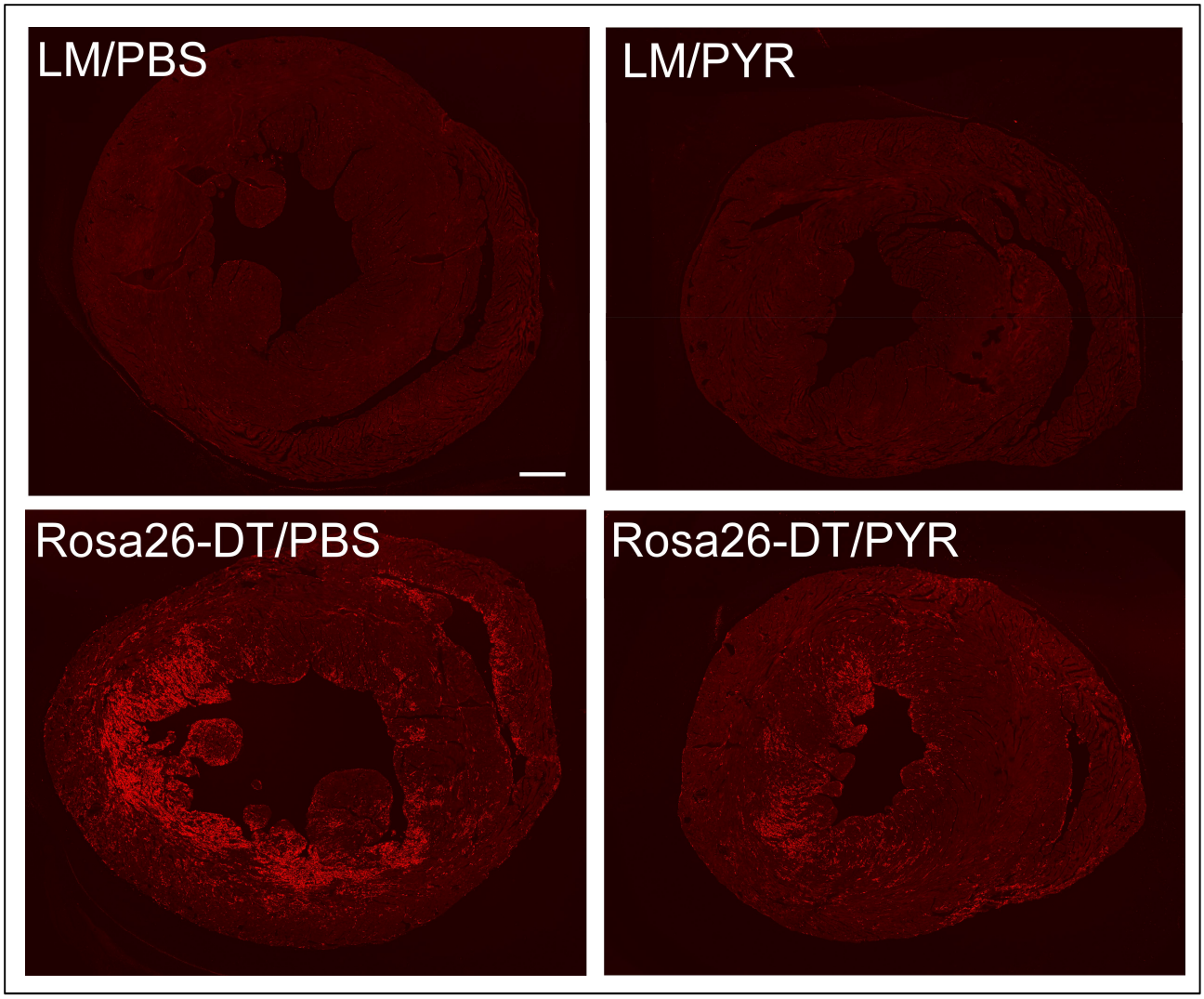

Figure S-9: Representative fluorescent microscopic images of myocardial sections (~70-130 per section) from 10X tile scanning of the entire heart at the mid-papillary level using a confocal microscopy (see methods for details). Scale bar = 500μm.

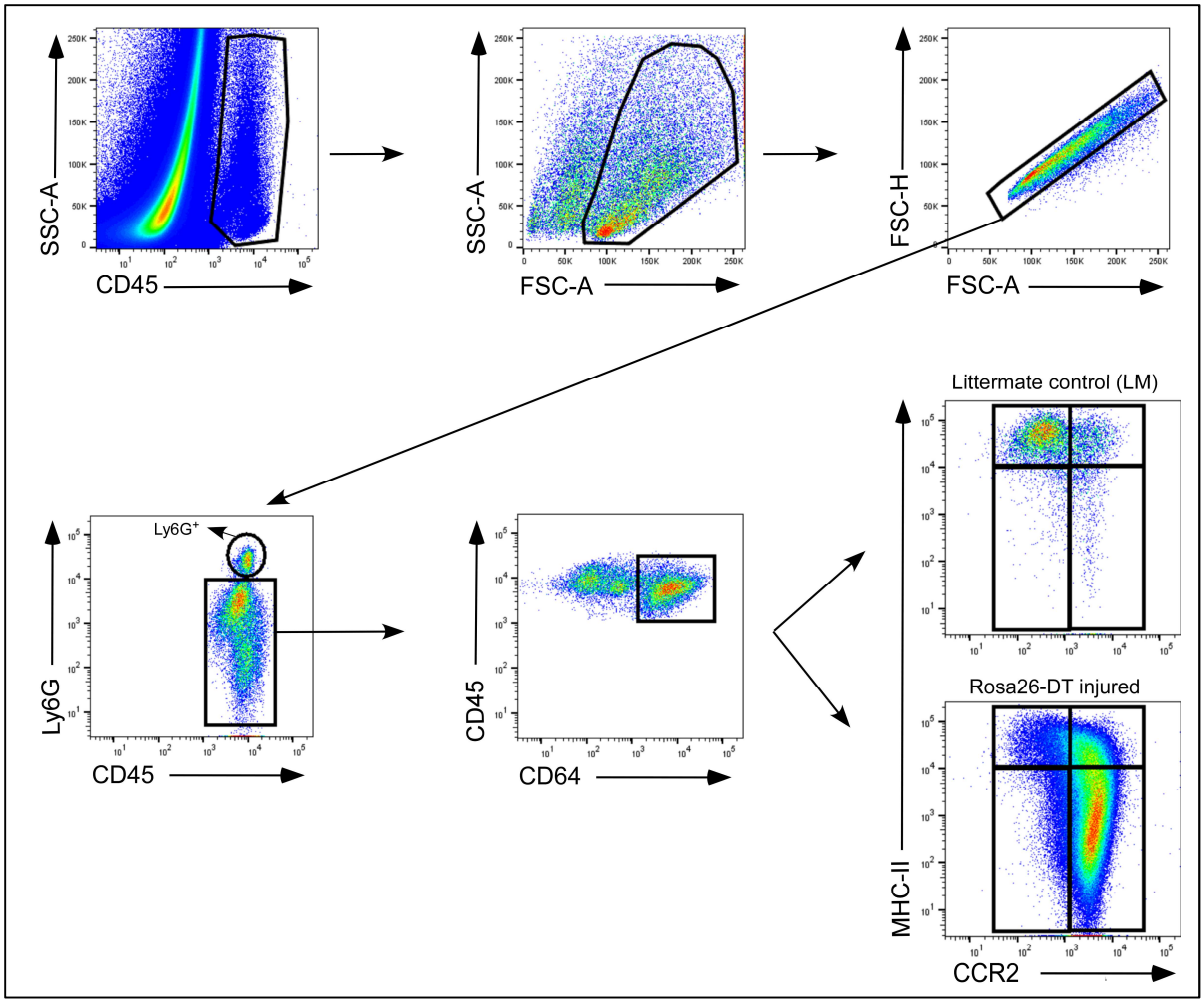

Figure S-10: Gating strategy for the FACS analysis used to identify the macrophage/monocyte subsets in the heart. Neutrophils were gated as $CD45^{+}Ly6G^{+}$ cells. Macrophages were gated as $CD45^{+}Ly6G^{-}CD64^{+}$ cells. $CD45^{+}Ly6G^{-}CD64^{+}$ macrophage/monocytes subsets were MHC-$II^{high}CCR2^{-}$, MHC-$II^{high}CCR2^{+}$, MHC-$II^{low}CCR2^{+}$ and MHC-$II^{low}CCR2^{-}$ cells.

SUPPLEMENTAL TABLES

Table S-1: List of primers used in the RT-PCR experiments.

Gene	**Brand**	**Assay identification**
Cd68	Integrated DNA Technologies	Mm.PT.58.32698807
Ccr2	Integrated DNA Technologies	Mm.PT.58.14116710
Tnf	Integrated DNA Technologies	Mm.PT.58.12575861
Il6	Integrated DNA Technologies	Mm.PT.58.10005566
Il1b	Integrated DNA Technologies	Mm.PT.58.41616450
Ccl2	Integrated DNA Technologies	Mm.PT.58.42151692
Ccl7	Integrated DNA Technologies	Mm.PT.58.17719534
Rplp0	Integrated DNA Technologies	Mm.PT.58.43894205
Slc18a3 (Vacht)	Applied Biosystems	Mm00491465_s1
Ccl2	Applied Biosystems	Mm00441242_m1
Ccl7	Applied Biosystems	Mm00443113_m1
Gapdh	Applied Biosystems	Mm99999915_g1
Rplp0	Applied Biosystems	Mm00725448_s1

Table S-2: List of antibodies used in the immunofluorescence experiments.

Antibody	**Brand**	**Proportion of Antibody**
Rat anti-mouse CD68 (MAC1957)	BioRad	1:400
Rat anti-mouse Ly-6G (1A8)	BD Biosciences	1:200
Anti-mouse CD3e FITC (145-2C11)	eBioscience	1:50
Rabbit anti-VAChT	Synaptic Systems	1:100
Rabbit anti-M3	Abcam	1:100
Rabbit anti-M1	Sigma-Aldrich	1:100
Rhodamine WGA	Vector Laboratories	1:200

Table S-3: List of antibodies used in the flow cytometry experiments.

Antibody	**Brand**	**Clone**	**Fluorophore**
CD45	BioLegend	30-F11	PerCP/Cy5.5
Ly-6G	BioLegend	1A8	FITC
CD64	BioLegend	X54-5/7.1	PE
F4/80	BioLegend	BM8	PE
I-A/I-E	BioLegend	M5/114.15.2	PE/Cy7
CD192 (CCR2)	BioLegend	SA203G11	Brilliant Violet 421
Ly-6C	BioLegend	HK1.4	APC/Cy7
CD3	BioLegend	17A2	APC
CD19	BioLegend	6D5	PE/Cy7