

Supplementary Tables

Supplemental Table S1: Primer sequences for RT-qPCR

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Product length(bp)
<i>IL-6</i>	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG	131
<i>TNF-α</i>	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG	89
<i>Cxcl1</i>	CTGGGATTACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC	117
<i>Abca1</i>	AGTGATAATCAAAGTCAAAGGCACAC	AGCAACTTGGCACTAGTAACTCTG	136
<i>β-Actin</i>	TTCCAGCCTTCCTTCTTG	GGAGCCAGAGCA GTAATC	182
<i>Axl</i>	CCAGGACACCCCAAGGTGCTAAT	TGGTGGACTGGCTGTGCTTGC	189
<i>Cd36</i>	GATGACGTGGCAAAGAACAG	TCCTCGGGTCTCTGAGTTAT	107
<i>Ctsh</i>	TACAACAAGGGCATCATGGA	TTCTTGACGAATGCAACAGC	98
<i>Ctsk</i>	CAGCAGAGGTGTGTACTATG	GCGTTGTTCTTATTCCGAGC	174
<i>Ctsl</i>	TCTCACGCTCAAGGCAATCA	AAGCAAAATCCATCAGGCCTC	52
<i>Ctss</i>	ATAAGATGGCTGTTTTGGATG	TTCTTTTCCAGATGAGACGC	164
<i>ItgaV</i>	CTCATCGTTTCTATCCCACC	TTGAGTCCAGCCTTCATCG	150
<i>Lxrα</i>	CCTGATGTTTCTCTGACTC	TGACTCCAACCCTATCCTTA	147
<i>Mertk</i>	GTGGCAGTGAAGACCATGAAGTTG	GAAGTCCGGGATAGGGAGTCAT	568
<i>Mfge8</i>	ATCTACTGCCTCTGCCCTGA	CCAGACATTTGGCATCATTG	103
<i>Msr1</i>	TGGAGGAGAGAATCGAAAGCA	CTGGACTGACGAAATCAAGGAA	140
<i>Sectm1a</i>	CAGTGATGACCTGTAACATCTC	CAAGTATATCCCTGTGTGGTCG	197
<i>Sectm1b</i>	GAGAAGCAGGTAAGAAGCTGGAG	CAGTTCACACCGAAGAACCC	95
<i>Sectm1a</i> (Genotyping)	CATTCTCTCCATACAGGCTGG	CTTGAAGTGGAGCTCCCAC	KO: 127; WT: 211

Supplemental Table S2: Antibodies and Reagents used for flow cytometry analysis

Reagent name	Manufacturer	Clone	Catalog#	Dilution
CD11b (BV510)	Biolegend	M1/70	101245	1:50
CD11b (BV650)	Biolegend	M1/70	101259	1: 50
CD11b (Alexa Fluor-594)	Biolegend	M1/70	101254	1: 50
CD11b-PE	Biolegend	M1/70	101208	1:50
CD16/32	eBioscience	93	101302	1: 100
CD45 (Alexa Fluor-488)	Biolegend	S18009D	160306	1: 50
CD45.2 (Alexa Fluor-488)	Biolegend	104	109816	1: 50
F4/80 (APC)	Biolegend	BM8	123116	1: 50
F4/80 (BV421)	Biolegend	BM8	123132	1: 50
F4/80 (PE)	Biolegend	BM8	123110	1: 50
Ly6C (PE/Dazzle 594)	Biolegend	HK1.4	128044	1: 50
Ly6G (BV421)	Biolegend	1A8	127628	1: 50
Ly6G (APC)	Biolegend	1A8	127614	1: 50
MerTK (PE/Cyanine7)	Biolegend	2B10C42	151522	1: 50
Fixation Buffer	Biolegend	/	420801	/
Live/Dead Fixable Blue Dead Cell Stain Kit	Thermo Fisher	/	L34962	1: 100

Supplemental Table S3: Echocardiographic measurements of cardiac function in WT and Sectm1a-KO mice at 1-month after cardiac I/R

Parameter	Units	WT-Sham (n = 4)	KO-Sham (n = 5)	WT-I/R (n = 6)	KO-I/R (n = 6)
Heart Rate (HR)	bpm	567 ± 28	572 ± 33	558 ± 34	549 ± 37
Diameter;s	mm	2.41 ± 0.22	2.57 ± 0.17	2.86 ± 0.17*	3.83 ± 0.52 #
Diameter;d	mm	3.75 ± 0.23	3.92 ± 0.14	4.03 ± 0.29	4.65 ± 0.33 #
Volume;s	μL	21.04 ± 4.31	26.51 ± 3.54	31.41 ± 4.60*	54.84 ± 4.73 #
Volume;d	μL	60.88 ± 8.65	66.10 ± 9.33	75.41 ± 3.58*	93.68 ± 10.86 #
Ejection Fraction (EF)	%	66.16 ± 3.19	62.78 ± 2.26	58.36 ± 3.33*	38.49 ± 10.21 #
Fractional Shortening (FS)	%	36.10 ± 2.39	34.64 ± 2.43	28.82 ± 2.18 *	17.62 ± 5.20 #

*, $p < 0.05$, when comparing WT-I/R to WT-Sham; #, $p < 0.05$, when comparing KO-I/R to WT-I/R. Data are presented as Mean ± SEM, 2-way ANOVA.

Supplemental Table S4: Echocardiographic measurements of cardiac function in rSectm1a- and control IgG2a-treated mice at 1-month after cardiac I/R

Parameter	Units	IgG2a-I/R (n = 6)	rSectm1a-I/R (n = 6)
Heart Rate (HR)	bpm	550 ± 31	561 ± 23
Diameter; s	mm	2.55 ± 0.19	2.36 ± 0.12
Diameter; d	mm	3.54 ± 0.21	3.72 ± 0.13
Volume; s	μL	25.07 ± 4.68	20.16 ± 2.52
Volume; d	μL	54.58 ± 7.71	59.96 ± 4.91
Ejection Fraction (EF)	%	56.04 ± 2.36	65.98 ± 2.01*
Fractional Shortening (FS)	%	27.97 ± 1.42	36.56 ± 1.75*

*, $p < 0.05$, when comparing rSectm1a-I/R to IgG2a-I/R. Data are presented as Mean ± SEM, Student's *t* test.

Figure S1A-C

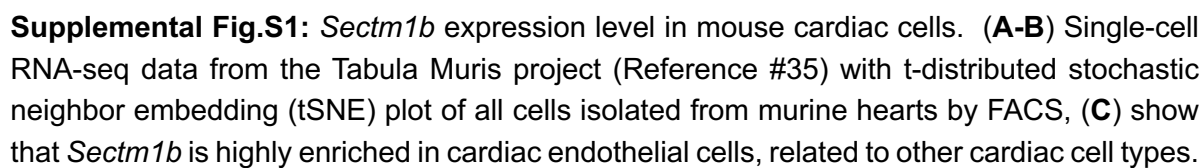
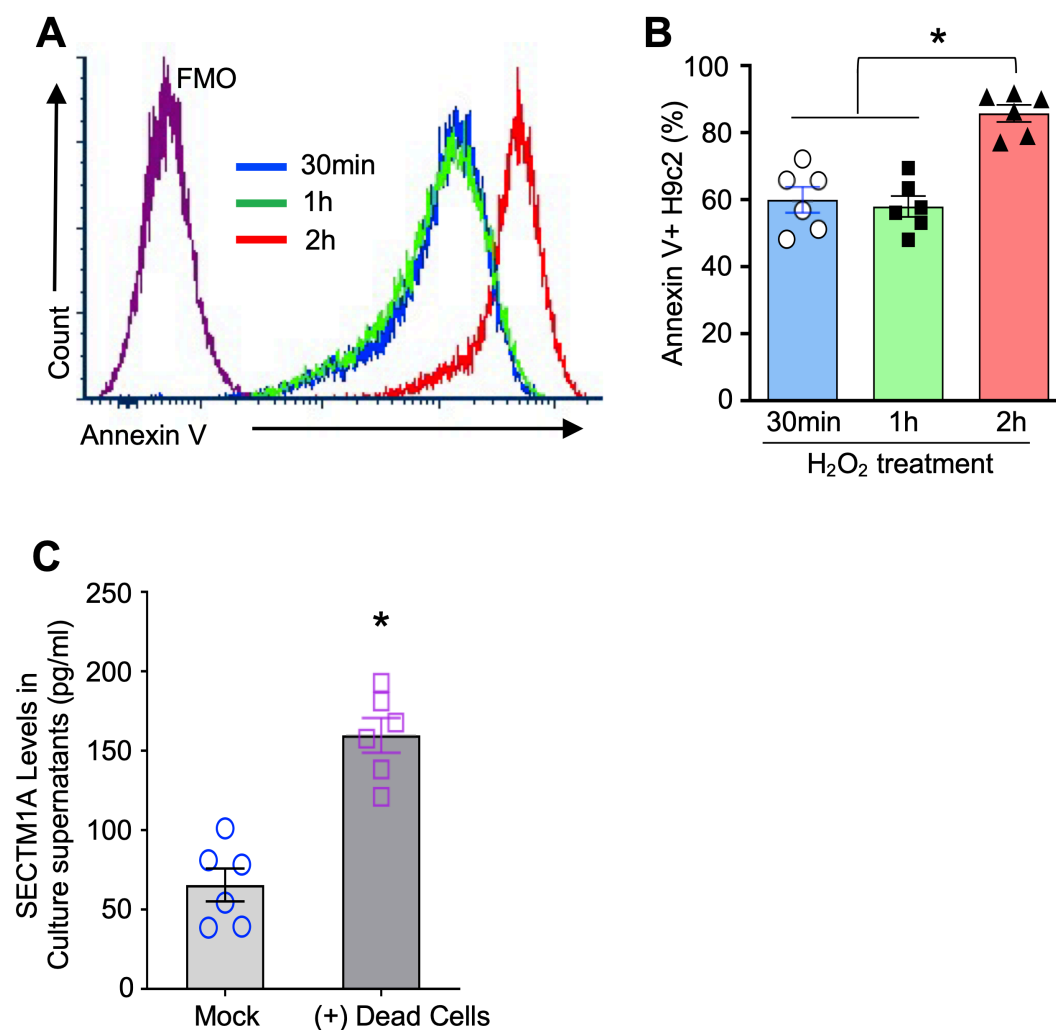
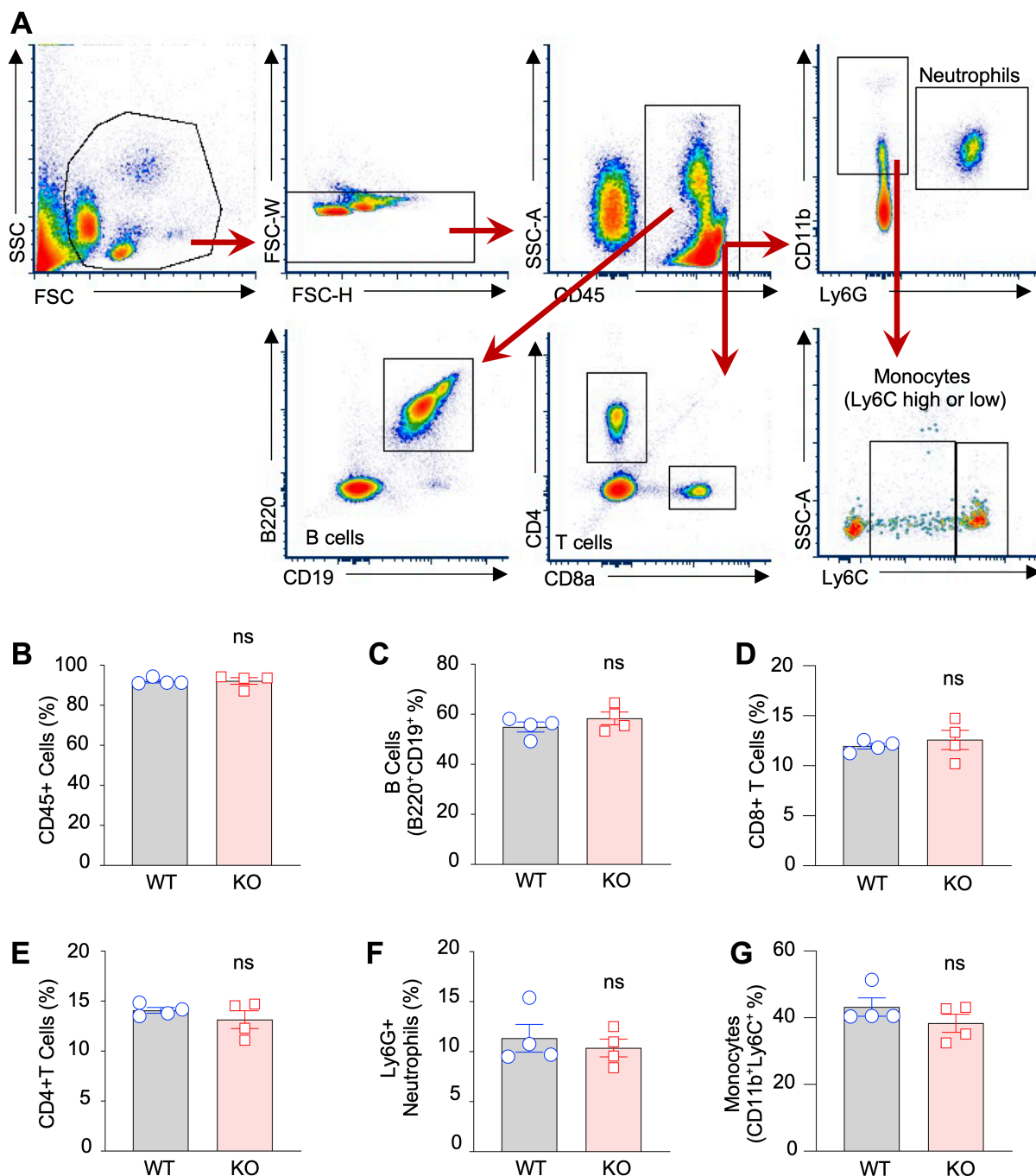


Figure S2A-C



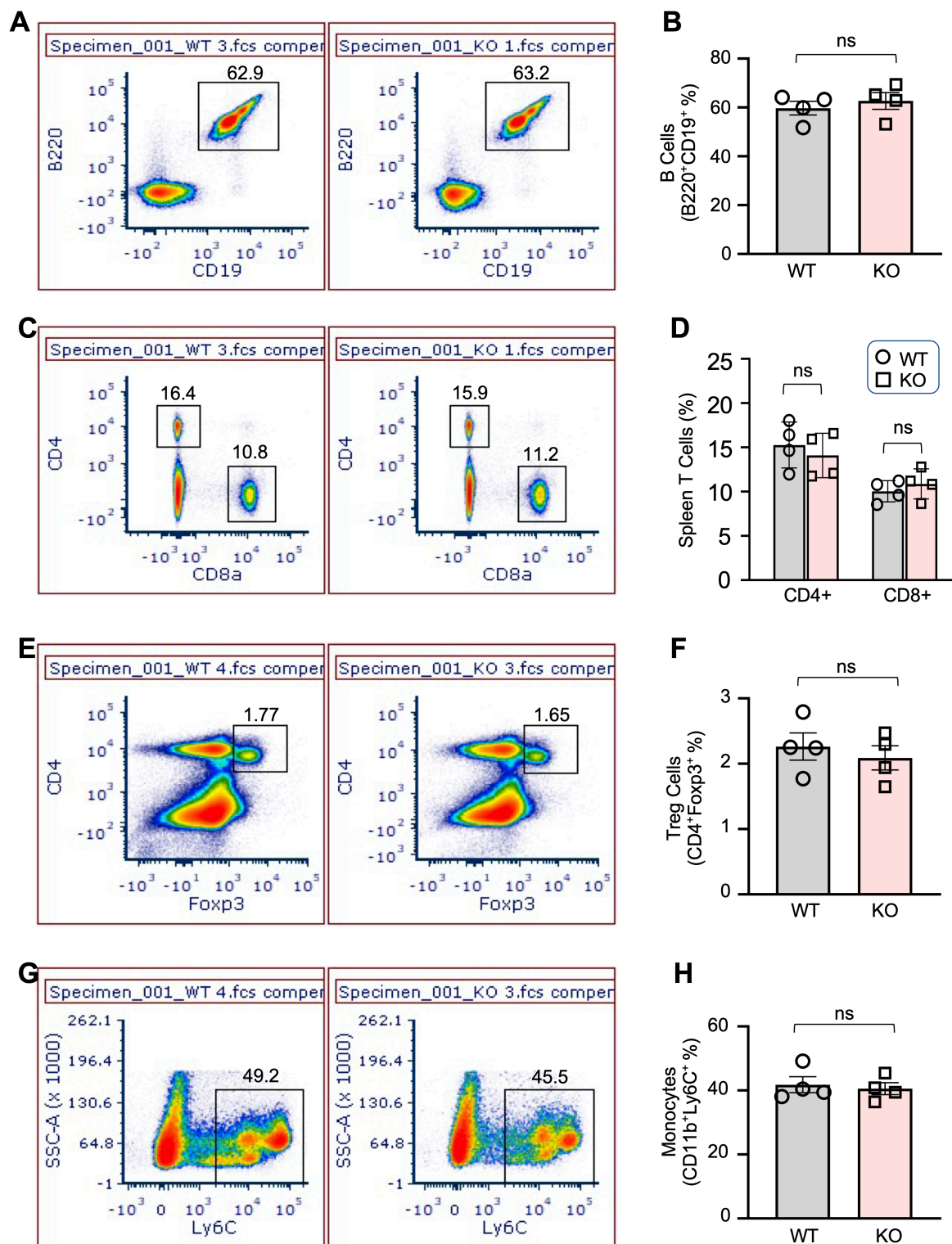
Supplemental Fig.S2: (A/B) Flow cytometry analysis with Annexin V staining. Representative flow cytometry histograms (A) and their quantification results (B) showing the apoptosis of H9c2 cells that is induced by H₂O₂ treatment for the indicated time points (n=6, **p* < 0.05). **(C)** The concentration of SECTM1A was measured in culture supernatants collected from cultured BMDMs in the absence (mock control) and the presence of dead cells for 6h (n=6, **p* < 0.05). All results are presented as mean ± SEM and analyzed by one-way ANOVA (A/B) or Student's *t* test (C).

Figure S3A-G



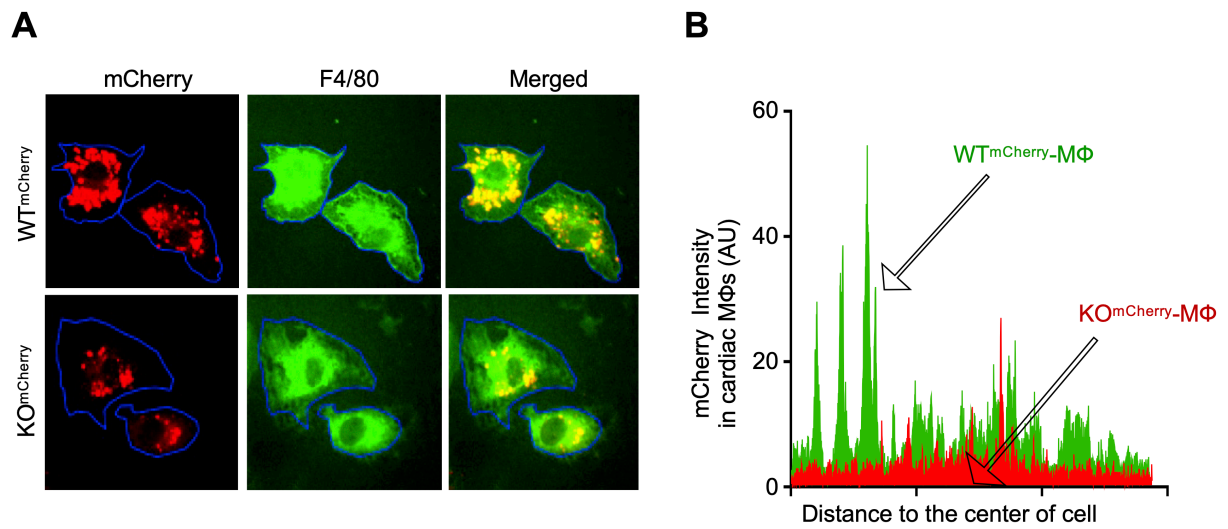
Supplemental Fig.S3: Analysis of immune cell populations in the blood of WT^{Cherry} and KO^{Cherry} mice. (A) Representative flow cytometry plots showing the gating strategy for different immune cell populations in murine peripheral blood. (B-G) Quantifications of different immune cell populations which show no significantly difference between two groups (n=4; ns, not significant vs. WT). Results are presented as mean $\pm$ SEM and analyzed by Student's *t* test (B-G).

Figure S4A-H



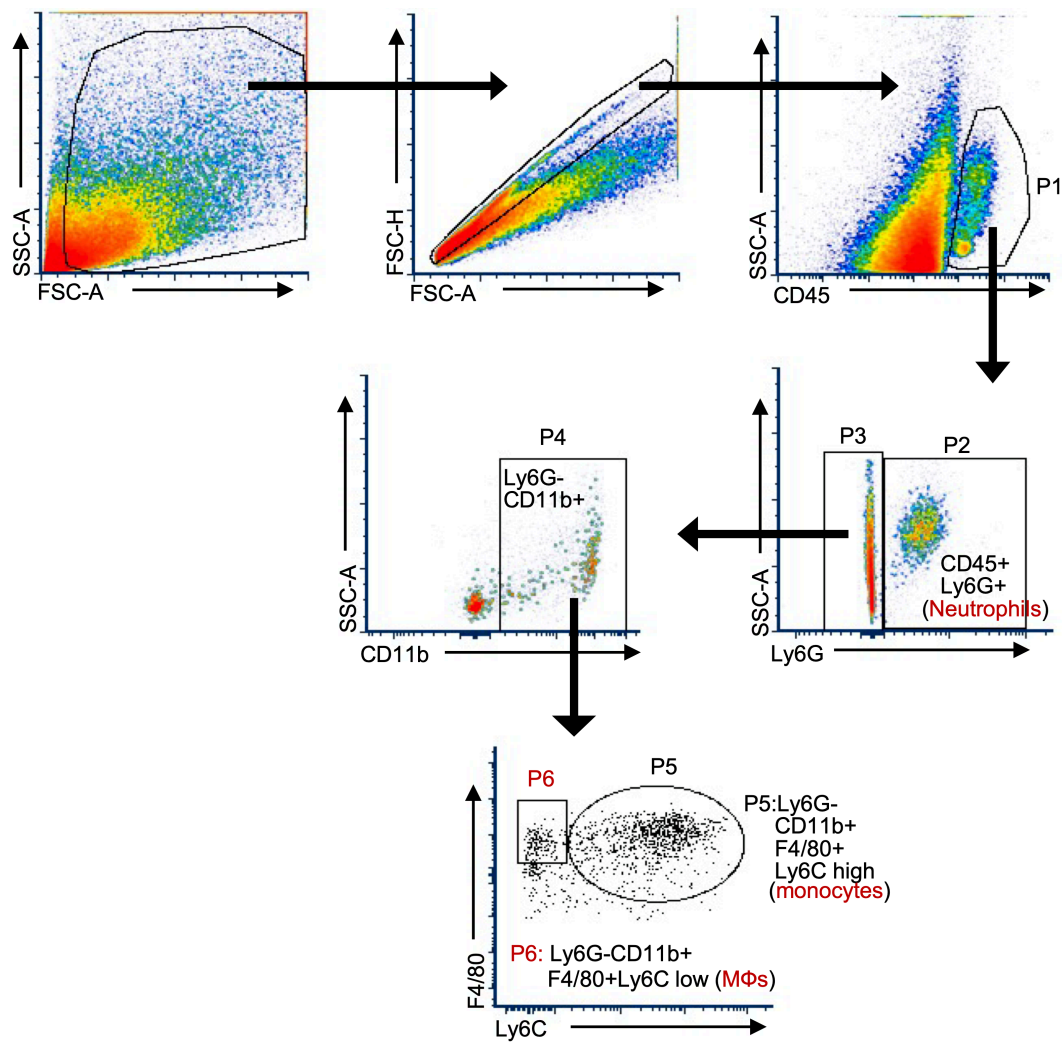
Supplemental Fig.S4: Analysis of immune cell populations in the spleen of WT^{Cherry} and KO^{Cherry} mice. (A, C, E, G) Representative flow cytometry plots and (B, D, F, G) their quantification results which show no significantly difference between two groups (n=4; ns, not significant.). Results are presented as mean ± SEM and analyzed by Student's *t* test (B, D, F, and H).

Figure S5A/B



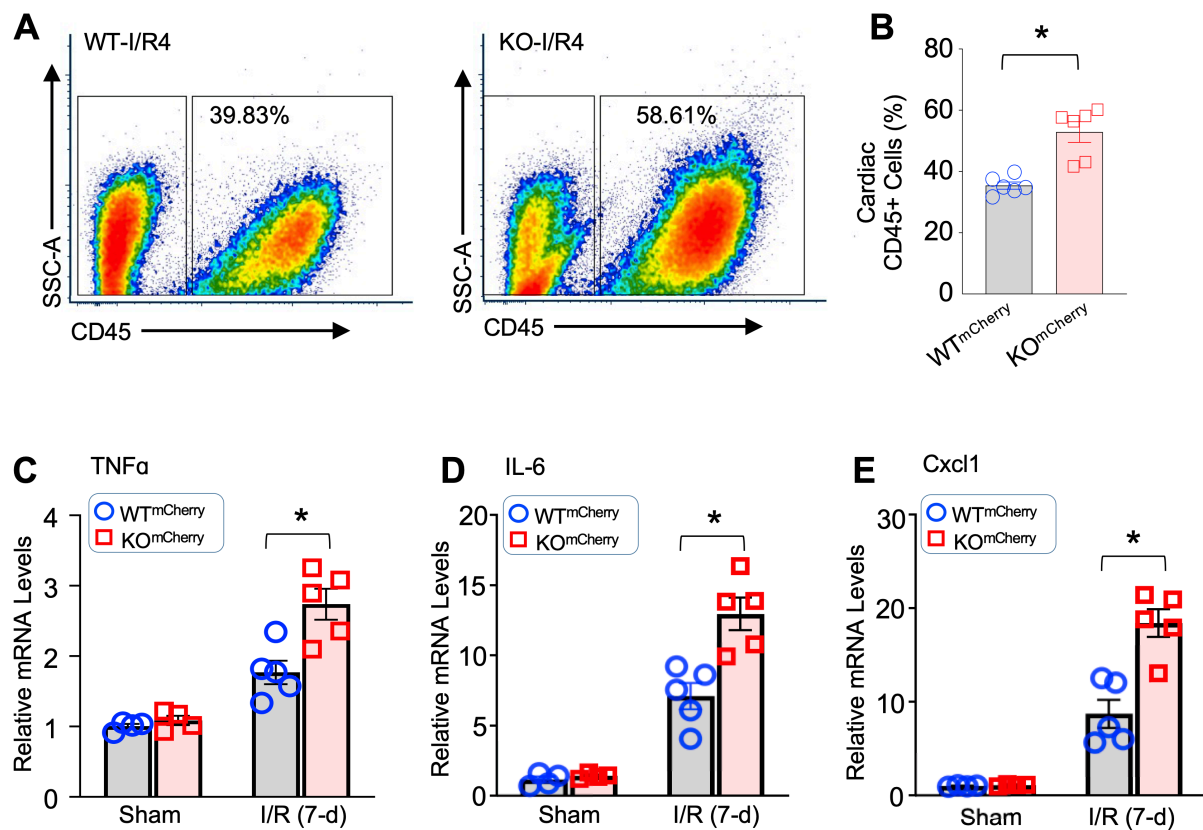
Supplemental Fig.S5: Cardiac MΦs isolated from WT^{mCherry} and KO^{mCherry} mice at Day 4 post-I/R and measured red mCherry intensity under confocal microscopy. (A) Representative images of mCherry-engulfing MΦs and (B) their quantification results.

Figure S6



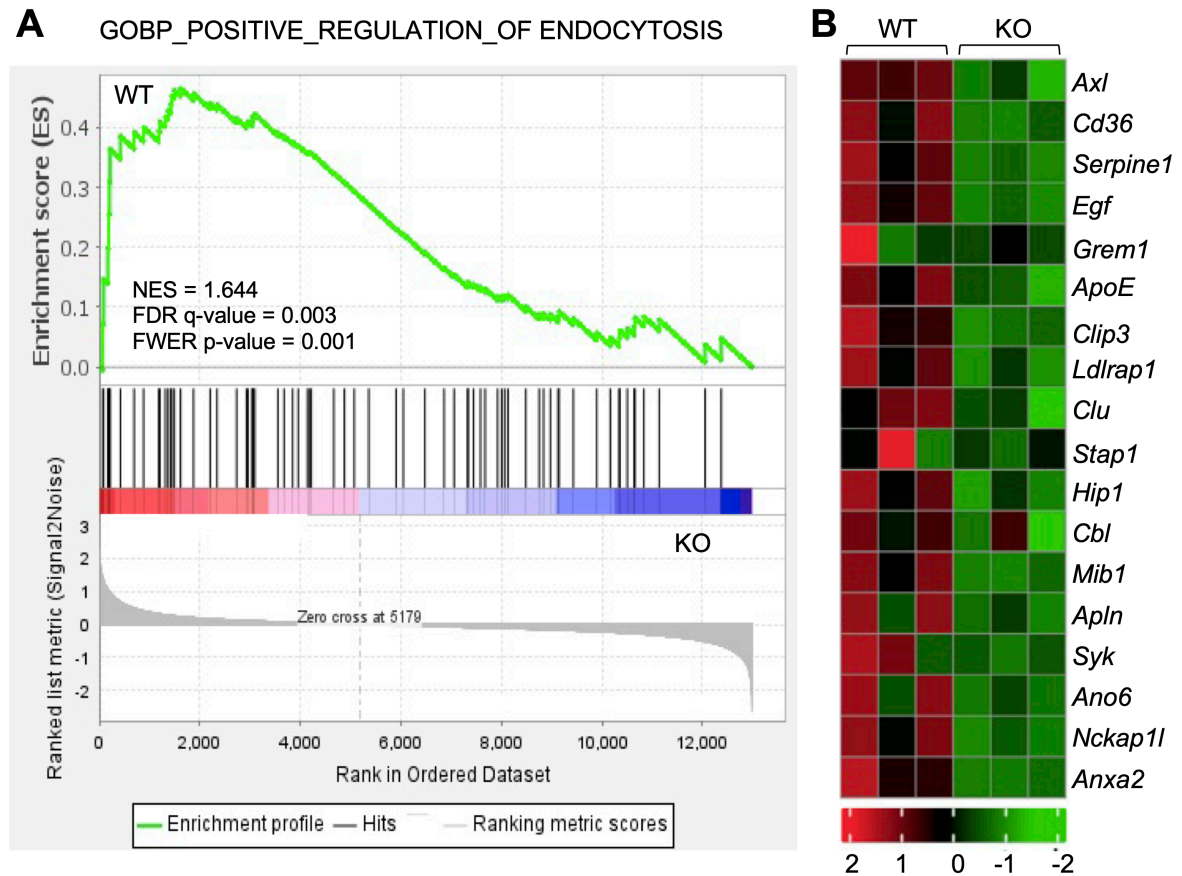
Supplemental Fig.S6: Representative flow cytometry plots showing the gating strategy for different types of immune cells isolated from murine hearts.

Figure S7A-E



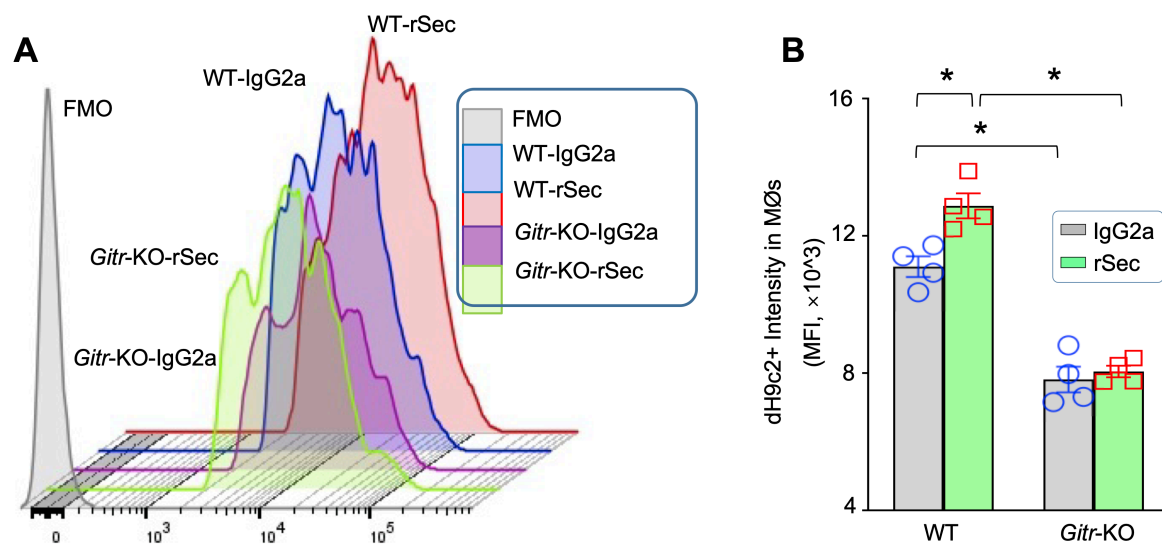
Supplemental Fig.S7: (A) Representative flow cytometry plots and (B) their quantification results showing higher number of CD45⁺ cells in KO^{Cherry}-hearts than WT^{Cherry}-hearts at Day 4 post-I/R (n=6; *, $p < 0.05$ vs. WT^{mCherry}). (C-E) The expression levels of pro-inflammatory cytokines (C) TNF α and (D) IL-6 as well as (E) chemokine Cxcl1 were measured in murine hearts after sham-operated and 7-day myocardial I/R (n=4 for sham group, n=5 for I/R group; *, $p < 0.05$ vs. I/R-WT^{mCherry}). Results are presented as mean $\pm$ SEM and analyzed by Student's *t* test (B-E).

Figure S8A/B



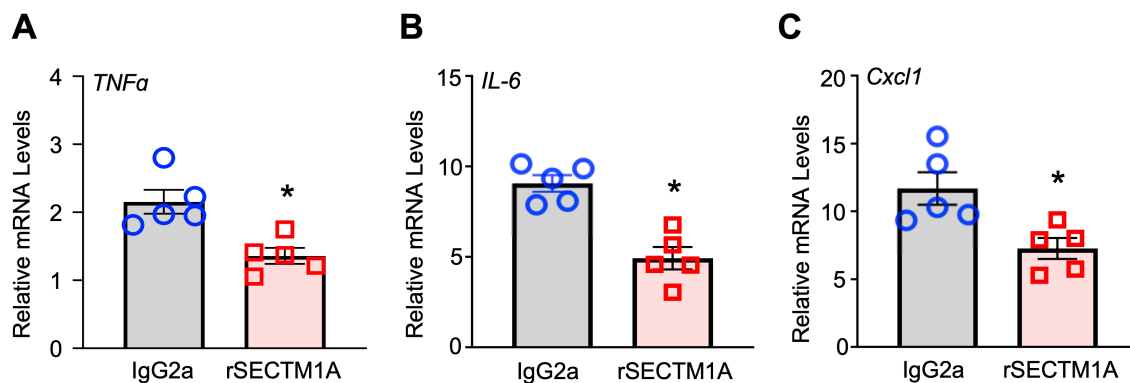
Supplemental Fig.S8: RNA-sequencing data assay. (A) Gene set enrichment analysis (GSEA) plot and (B) heatmap of positively regulated endocytosis genes in wild type (WT) MΦs compared to *Sectm1a*-knockout (KO) MΦs. Each square of the heatmap represents one biological replicate. Green and red color indicate down- vs. up-regulated genes, respectively.

Figure S9A/B



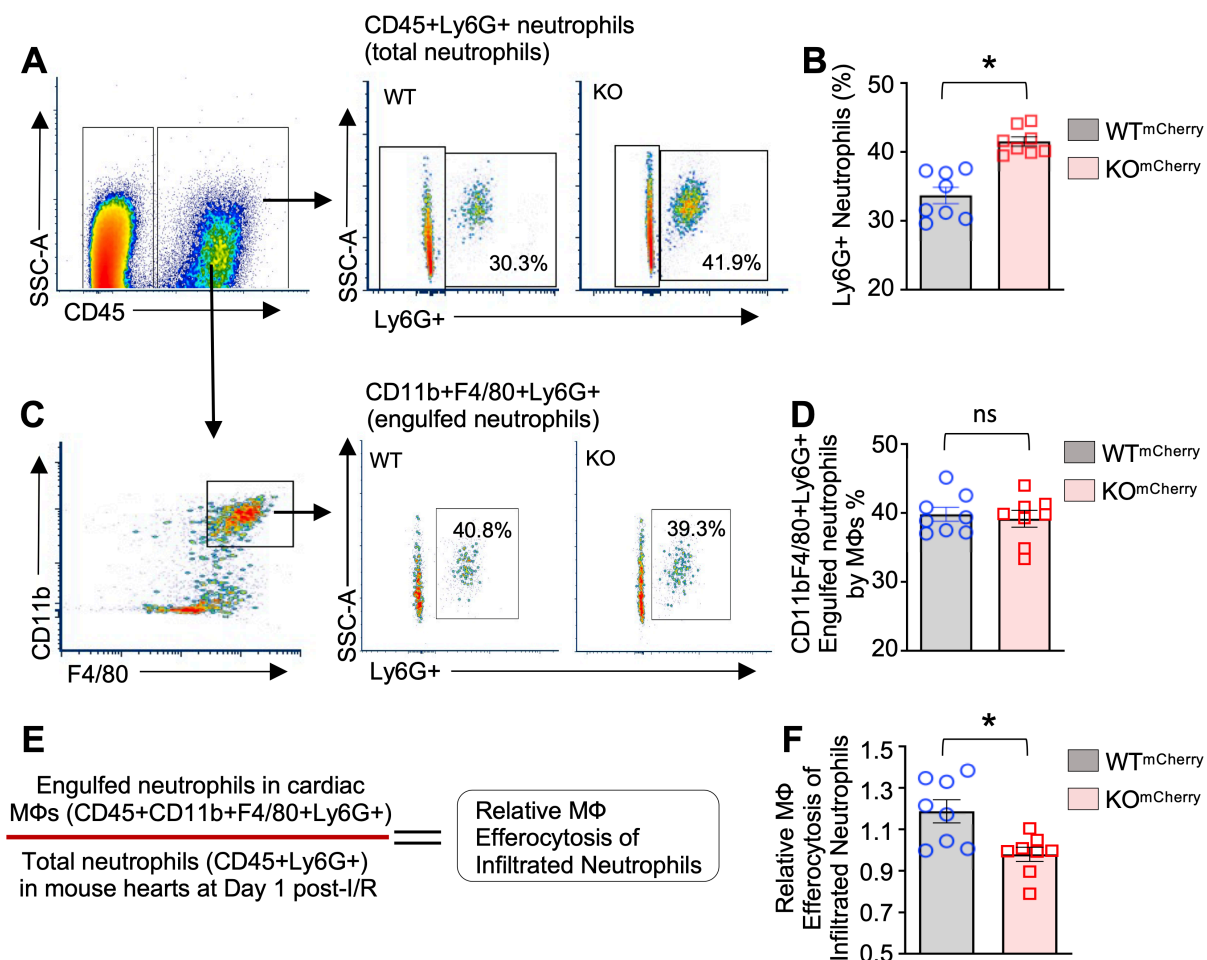
Supplemental Fig.S9: (A) Representative flow cytometry histograms and (B) their quantification of dead/dying H9c2-APC (deep red dye+) intensity (MFI) in MΦs ($n=4$; *, $p < 0.05$). Results are presented as mean $\pm$ SEM and analyzed by two-way ANOVA (B).

Figure S10A-C



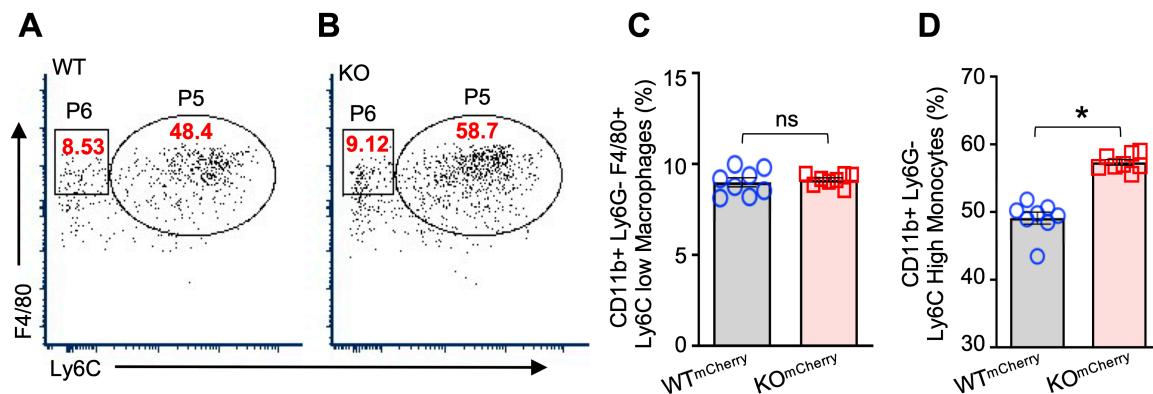
Supplemental Fig.S10: The expression levels of pro-inflammatory cytokines (A) $TNF\alpha$ and (B) $IL-6$ as well as (C) chemokine $Cxcl1$ were measured in IgG2a-treated and rSECTM1A protein-treated murine hearts after I/R (n=5; *, $p < 0.05$ vs. IgG2a-treated I/R hearts). Results are presented as mean ± SEM and analyzed by Student's t test (A-C).

Figure S11A-F



Supplemental Fig. S11: (A) Representative flow cytometry plots and (B) their quantification results showing higher number of Ly6G+neutrophils in KO^{Cherry}-hearts than WT^{Cherry}-hearts at Day 1 post-I/R. (C) Representative flow cytometry plots and (D) their quantification results showing the amount of cardiac MΦs in KO^{Cherry}-hearts than WT^{Cherry}-hearts at Day 1 post-I/R. (E/F) The ratio of engulfed to total neutrophils was calculated as relative MΦ efferocytosis of infiltrated neutrophils. (n=8; *, $p < 0.05$ vs. WT^{mCherry}). Results are presented as mean $\pm$ SEM and analyzed by Student's t test (B, D, and F). ns, not significant.

Figure S12A-D



Supplemental Fig. S12: (A/B) Representative flow cytometry plots that used gating strategy of Supplemental S6, and (C/D) their quantification results showing higher number of monocytes, whereas similar number of MΦs in KO^{Cherry}-hearts, compared to WT^{Cherry}-hearts at Day 1 post-I/R. (n=8; *, $p < 0.05$ vs. WT^{mCherry}). Results are presented as mean $\pm$ SEM and analyzed by Student's t test (C-D). ns, not significant.