Supplemental material

Title

Enhanced ISGylation via USP18 Isopeptidase Inactivation Fails to Mitigate the Inflammatory or Functional Course of Coxsackievirus B3-Induced Myocarditis.

Running title:
USP18 Inactivation Does Not Alter Inflammation or Function in CVB3 Myocarditis

Authors

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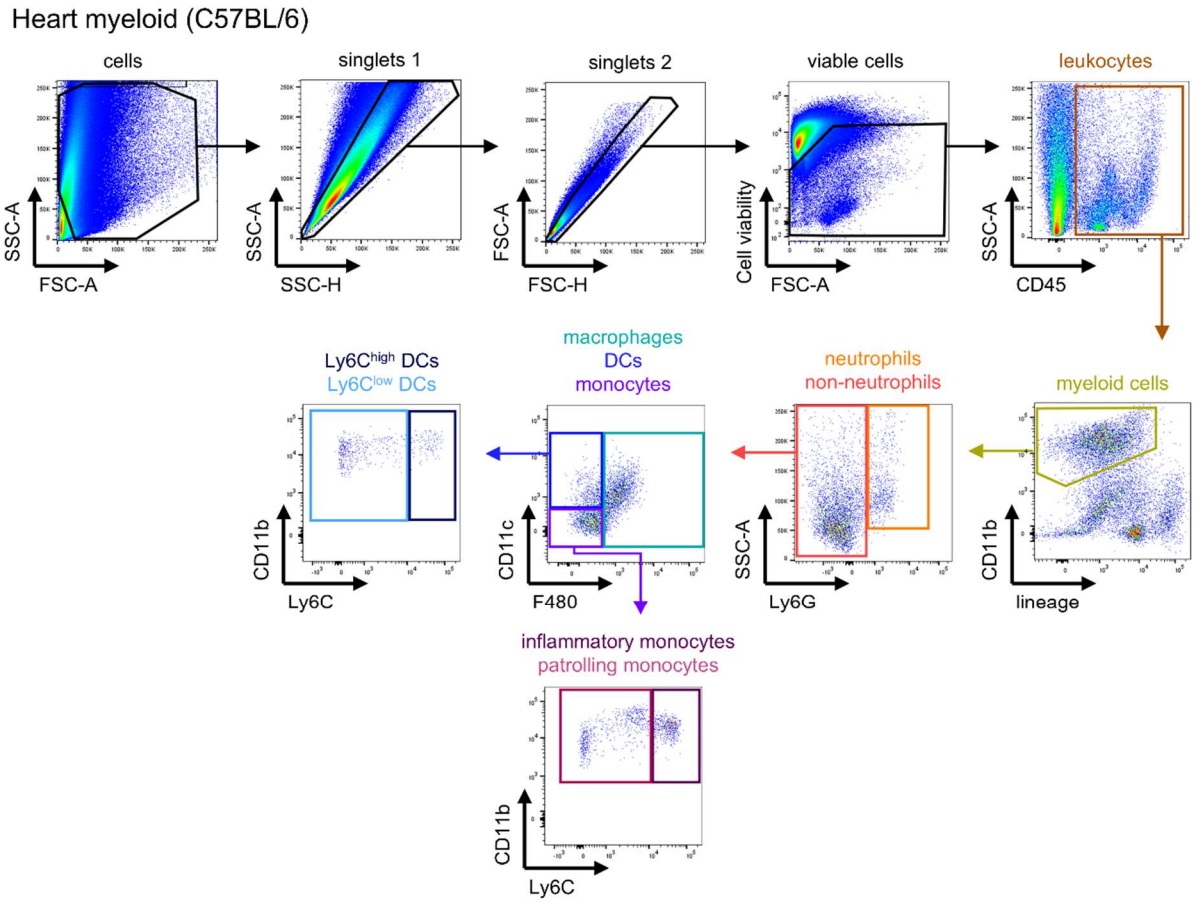
Supplemental figures



leukocytes:	CD45$^+$
myeloid cells:	CD45$^+$CD11b$^+$lineage$^-$
neutrophils:	CD45$^+$CD11b$^+$lineage$^-$Ly6G$^+$
macrophages:	CD45$^+$CD11b$^+$lineage$^-$ Ly6G$^-$CD11c$^{+/-}$F4/80$^+$
dendritic cells (DCs):	CD45$^+$CD11b$^+$lineage$^-$ Ly6G$^-$CD11c$^+$F4/80$^-$
Ly6C^{high} dendritic cells:	CD45$^+$CD11b$^+$lineage$^-$ Ly6G$^-$CD11c$^+$F4/80$^-$Ly6C^{high}
Ly6C^{low} dendritic cells:	CD45$^+$CD11b$^+$lineage$^-$ Ly6G$^-$CD11c$^+$F4/80$^-$Ly6C^{low}
monocytes:	CD45$^+$CD11b$^+$lineage$^-$Ly6G$^-$CD11c$^-$F4/80$^-$
inflammatory monocytes:	CD45$^+$CD11b$^+$lineage$^-$Ly6G$^-$CD11c$^-$F4/80$^-$Ly6C^{high}
patrolling monocytes:	CD45$^+$CD11b$^+$lineage$^-$Ly6G$^-$CD11c$^-$F4/80$^-$Ly6C^{low}
lineage$^-$:	Ter-119$^-$ B220$^-$ CD49b$^-$ NK1.1$^-$ CD90$^-$

Figure S1: Representative gating strategy used in flow cytometry analysis to quantify cardiac myeloid immune cell numbers in C57BL/6 mice. Immune cells were isolated from cardiac tissue, stained with an antibody-fluorochrome cocktail against myeloid surface markers and measured by flow cytometry. The respective gating strategy is shown. First, cell debris and cell doublets were removed using FSC-A/SSC-A, SSC-H/SSC-A and FSC-H/FSC-A gates. Next, dead cells were removed by gating with a cell viability dye. Finally, leukocytes were gated, and distinct myeloid subpopulations were identified using a combination of cell surface markers. The name of each myeloid subpopulation is written above the plot in the same color as its final gate. The complete gating criteria are indicated below the representative gating strategy.

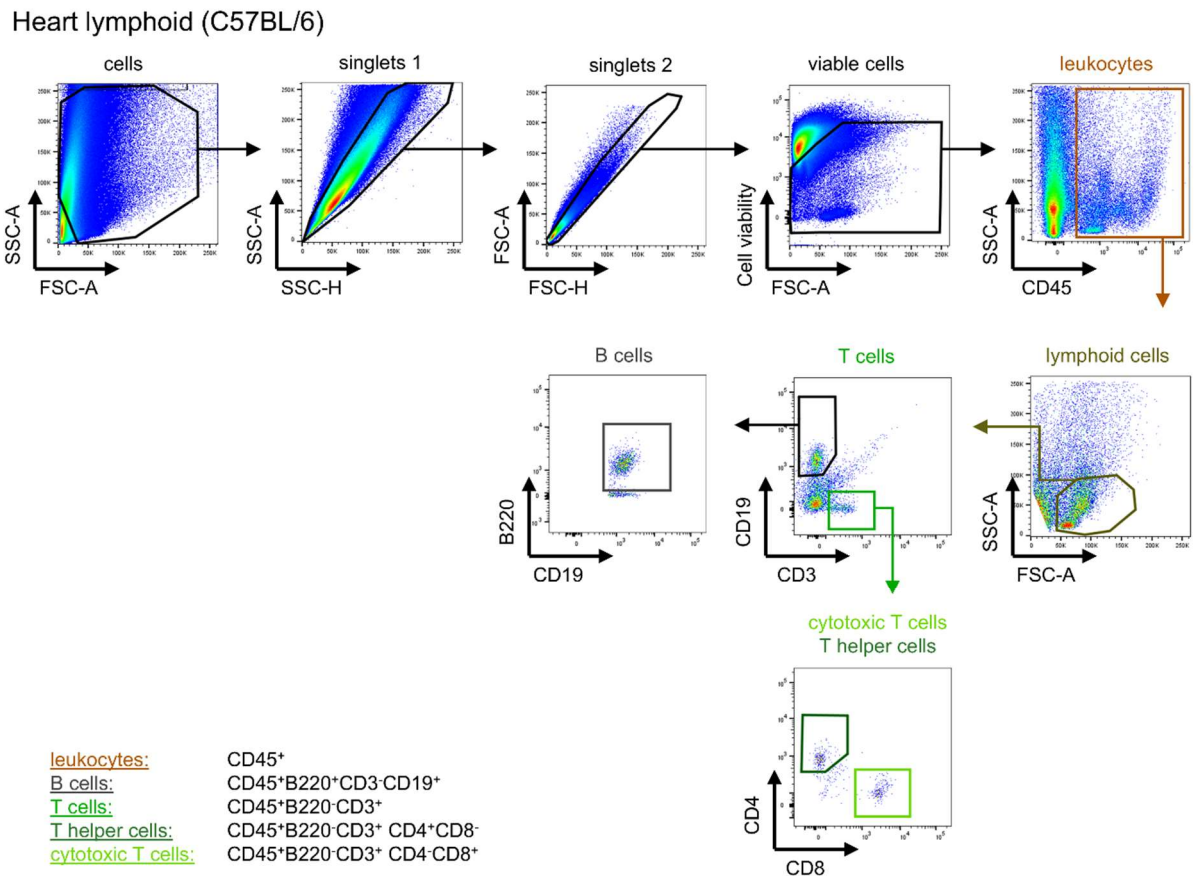
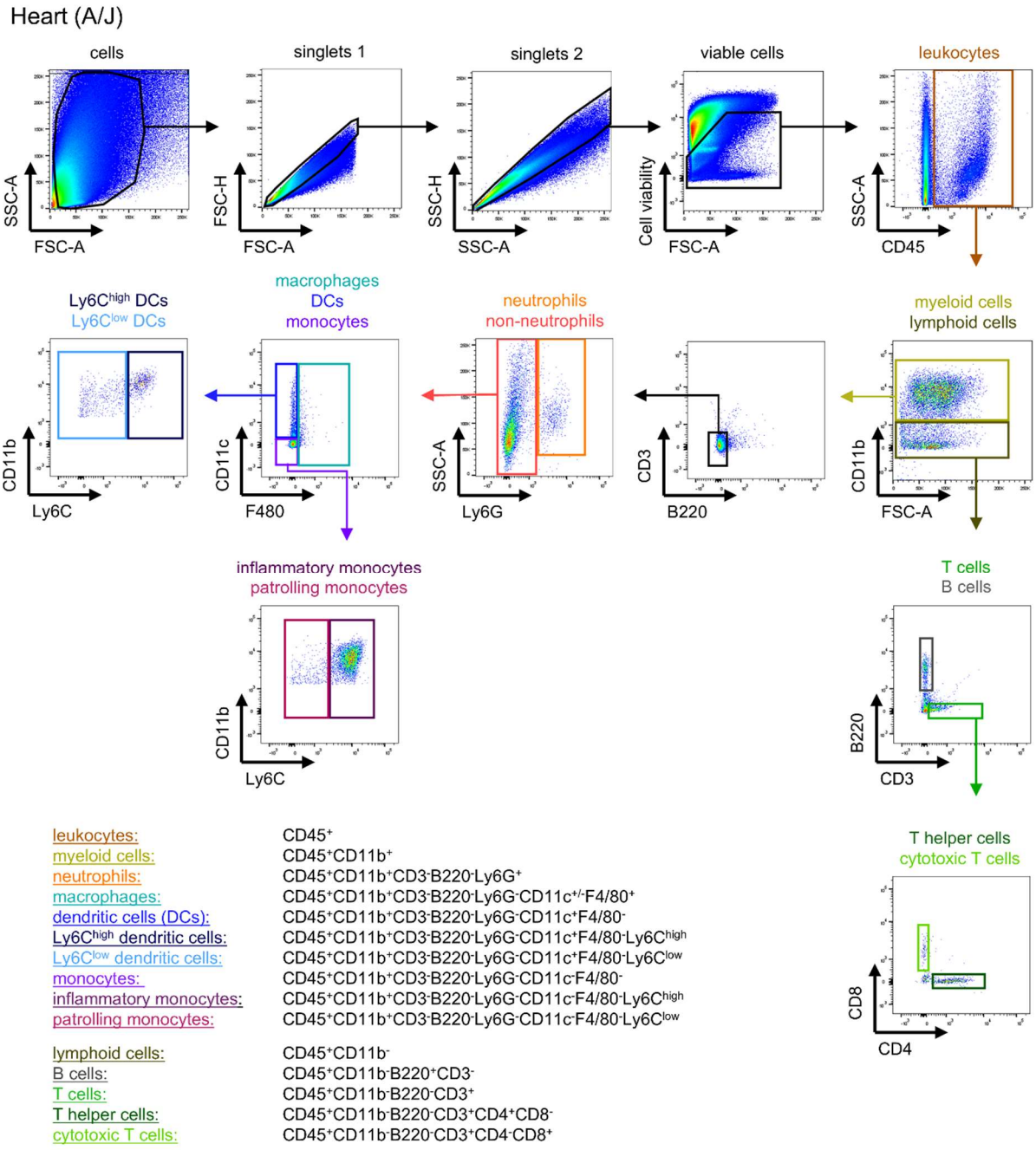


Figure S2: Representative gating strategy used in flow cytometry analysis to quantify cardiac lymphoid immune cell numbers in C57BL/6 mice. Immune cells were isolated from cardiac tissue, stained with an antibody-fluorochrome cocktail against lymphoid surface markers and measured by flow cytometry. The respective gating strategy is shown. First, cell debris and cell doublets were removed using FSC-A/SSC-A, SSC-H/SSC-A and FSC-H/FSC-A gates. Next, dead cells were removed by gating with a cell viability dye. Finally, leukocytes were gated, and distinct lymphoid subpopulations were identified using a combination of cell surface markers. The name of each lymphoid subpopulation is written above the plot in the same color as its final gate. The complete gating criteria are indicated below the representative gating strategy.



leukocytes:	$CD45^+$
myeloid cells:	$CD45^+CD11b^+$
neutrophils:	$CD45^+CD11b^+CD3^-B220^-Ly6G^+$
macrophages:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^{+/-}F4/80^+$
dendritic cells (DCs):	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^+F4/80^-$
$Ly6C^{high}$ dendritic cells:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^+F4/80^-Ly6C^{high}$
$Ly6C^{low}$ dendritic cells:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^+F4/80^-Ly6C^{low}$
monocytes:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^-F4/80^-$
inflammatory monocytes:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^-F4/80^-Ly6C^{high}$
patrolling monocytes:	$CD45^+CD11b^+CD3^-B220^-Ly6G^-CD11c^-F4/80^-Ly6C^{low}$
lymphoid cells:	$CD45^+CD11b^-$
B cells:	$CD45^+CD11b^-B220^+CD3^-$
T cells:	$CD45^+CD11b^-B220^-CD3^+$
T helper cells:	$CD45^+CD11b^-B220^-CD3^+CD4^+CD8^-$
cytotoxic T cells:	$CD45^+CD11b^-B220^-CD3^+CD4^-CD8^+$

Figure S3: Representative gating strategy used in flow cytometry analysis to quantify cardiac immune cell numbers in A/J mice. Immune cells were isolated from cardiac tissue and stained with an antibody-fluorochrome cocktail containing both myeloid and lymphoid surface markers. Subsequently, immune cell counts and frequencies were measured by flow cytometry. The respective gating strategy is shown. First, cell debris and cell doublets were removed using FSC-A/SSC-A, SSC-H/SSC-A and FSC-H/FSC-A gates. Next, dead cells were removed by gating with a cell viability dye. Finally, leukocytes were gated, and the distinct myeloid and lymphoid subpopulations were identified using a combination of cell surface markers. For each immune cell subpopulation, the corresponding gating criteria is noted below the image of the representative gating strategy.

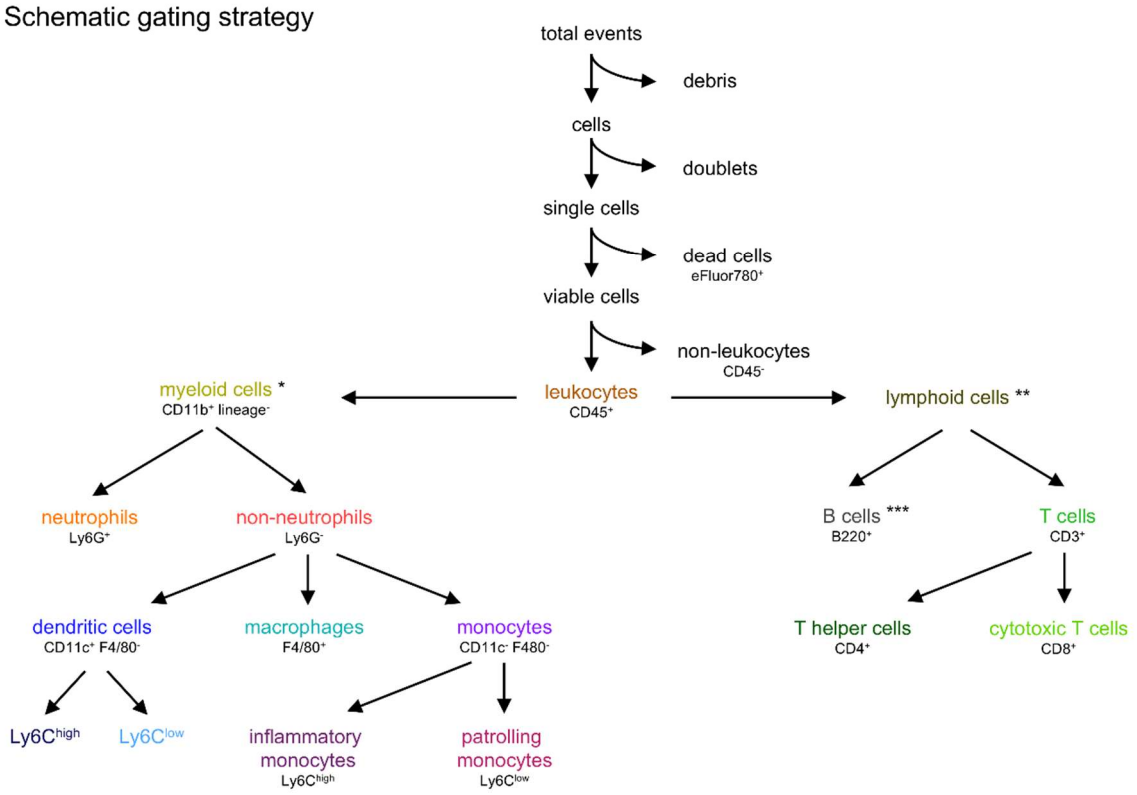


Figure S4: Schematic illustration of the flow cytometric gating strategy. This visualization illustrates the gating strategy applied to both the C57BL/6 and A/J mouse experiments and summarizes the representative gating plots presented in Figures S1–S3. Differences in the gating of immune cell populations between C57BL/6 and A/J mouse experiments are indicated with asterisks. * In C57BL/6 experiments lineage- includes the following markers: Ter-119- B220- CD49b- NK1.1- CD90-. In A/J experiments lineage- includes the following markers: CD3- B220-. ** In C57BL/6 experiments lymphoid cells are identified as SSC low. In C57BL/6 experiments lymphoid cells are identified as $CD11b^-$. *** In C57BL/6 experiments, B cells are additionally defined as $CD19^+$.

Supplemental table

Table S1: Details on the antibody fluorochrome conjugates used in flow cytometry experiments					
Antibodies used for flow cytometry of cardiac tissue isolated from C57BL/6 mice					
lineage	antibody - fluorochrome	dilution	company	clone	catalogue number
myeloid	B220 - PE	1:500	BD Bioscience	RA3-6B2	553090
	CD90 - PE	1:500		53-2.1	553005
	NK1.1 - PE	1:200		U5A2-13	550082
	Ter-119 - PE	1:400		TER-119	553673
	CD11b - PE CF594	1:300		M1/70	562317
	Ly6C - Pacific Blue	1:200	BioLegend	HK1.4	128013
	CD11c - BV510	1:150		N418	117353
	Ly6G - PerCp-Cy5.5	1:400		1A8	127615
	CD45.2 - BV711	1:200		104	109847
	F4/80 - APC	1:100		BM8	123115
	CD49b - PE	1:300	eBioscience (Life Technologies)	DX5	12-5971-82
lymphoid	CD8 - Pacific Blue	1:100	BD Bioscience	53-6.7	558106
	CD4 - V500	1:100		RM4-5	560783
	B220 - FITC	1:200	BioLegend	RA3-6B2	103205
	CD69 - PE	1:300		H1.2F3	104507
	CD3 - PerCp-Cy5.5	1:300		145-2C11	100327
	CD45.2 - BV711	1:200		104	109847
	CD19 - APC	1:500		6D5	115511
Antibodies used for flow cytometry of cardiac tissue isolated from A/J mice					
lineage	antibody - fluorochrome	dilution	company	clone	catalogue number
myeloid + lymphoid	B220 - BUV395	1:200	BD Bioscience	RA3-6B2	563793
	CD8 - PB	1:100		53-6.7	558106
	CD3 - BUV737	1:200		145-2C11	612771
	CD11b - BV510	1:300	BioLegend	M1/70	101245
	CD11c - PE dazzle	1:200		N418	117348
	Ly6G - BV605	1:400		1A8	127639
	F4/80 - APC	1:100		BM8	123116
	CD4 - PerCPCy5.5	1:300		RM4-5	100539
	CD45.2 - BV711	1:200		104	109847
	Ly6C - PeCy7	1:400		HK1.4	128018

Table S1: Details on the antibody fluorochrome conjugates used for flow cytometry. Dilution, company, clone and catalogue numbers are listed for all antibody - fluorochrome conjugates used to measure cardiac immune cell infiltration by multicolor flow cytometry.