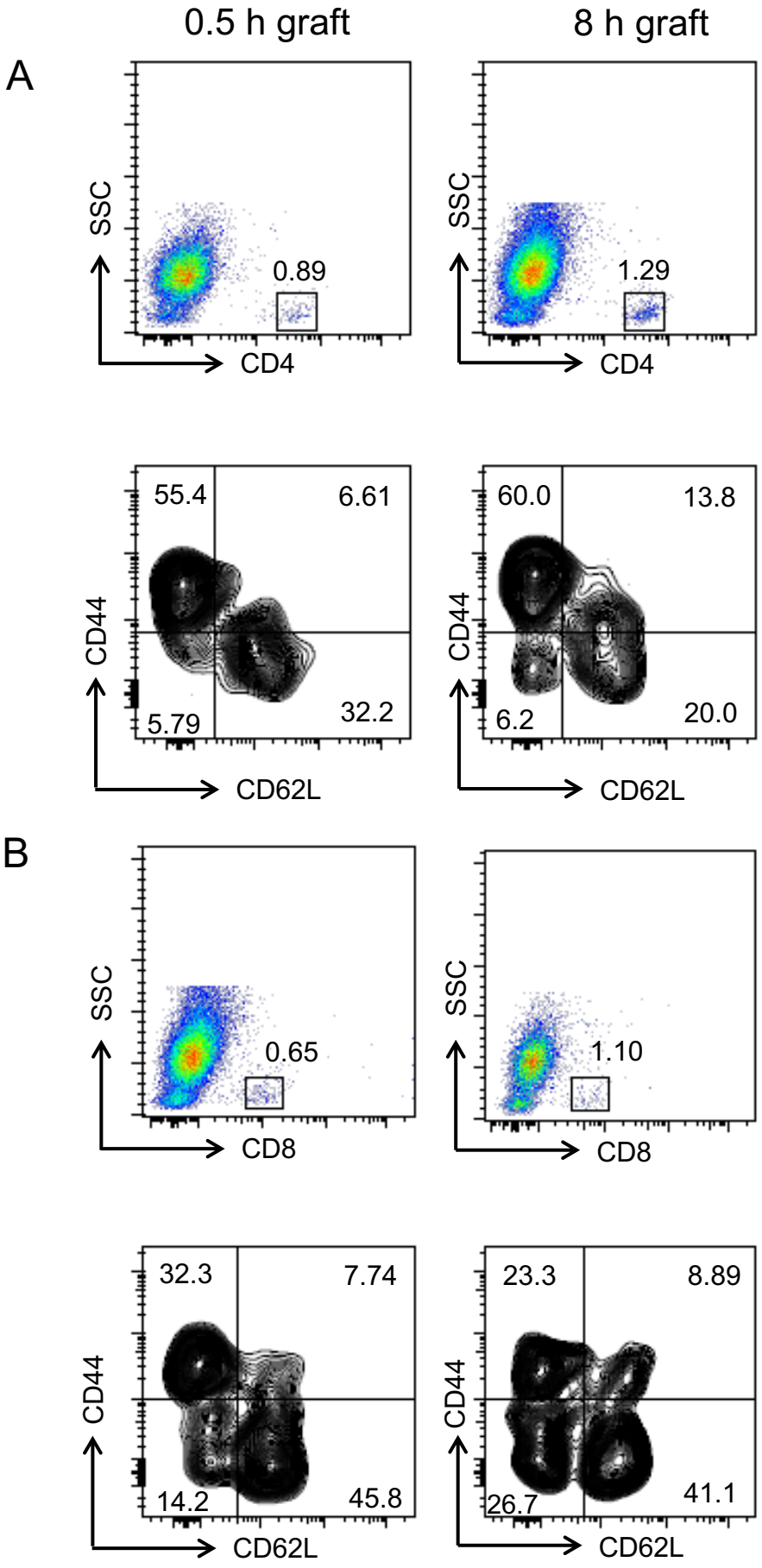
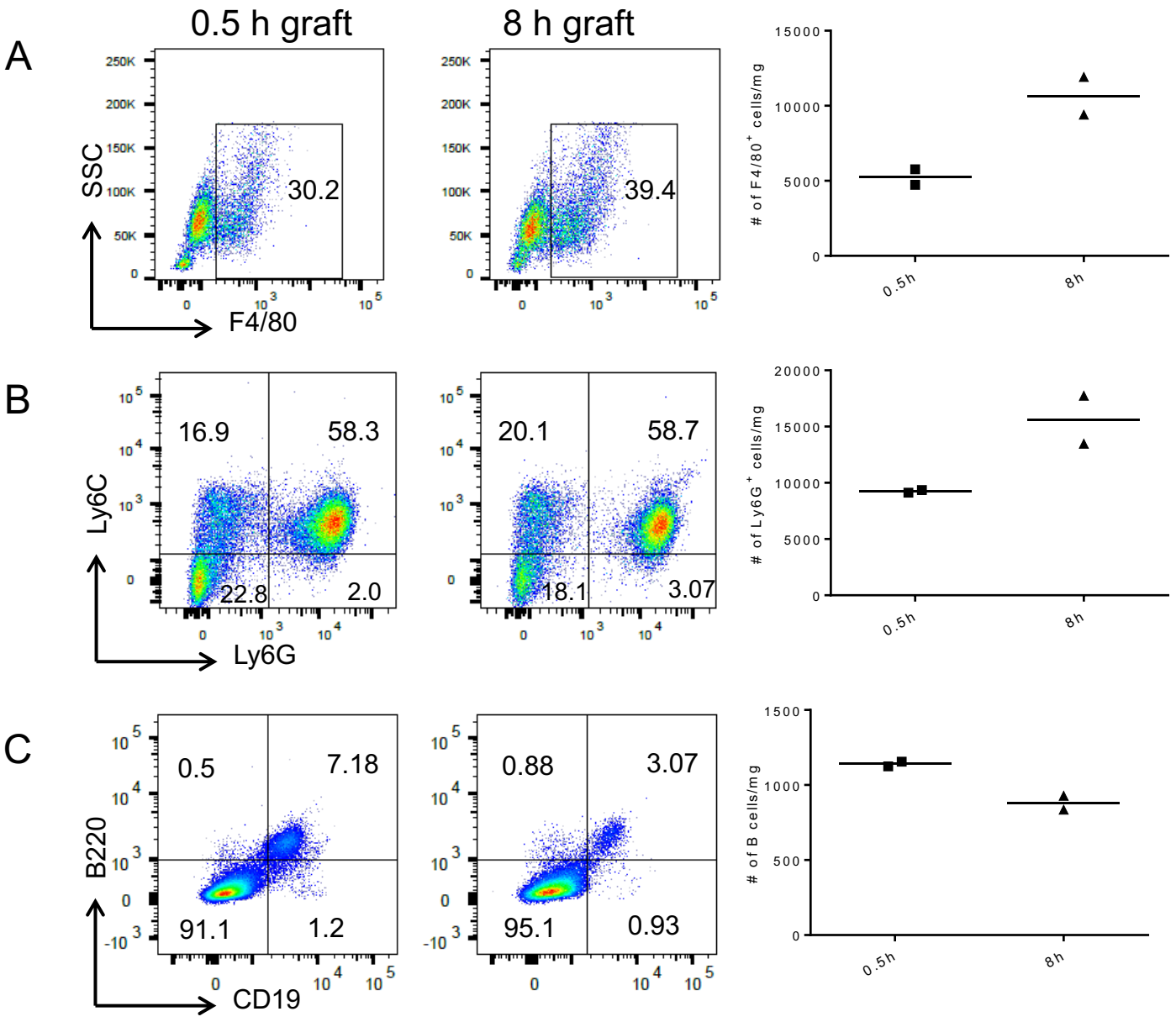
Supplemental Figure 1



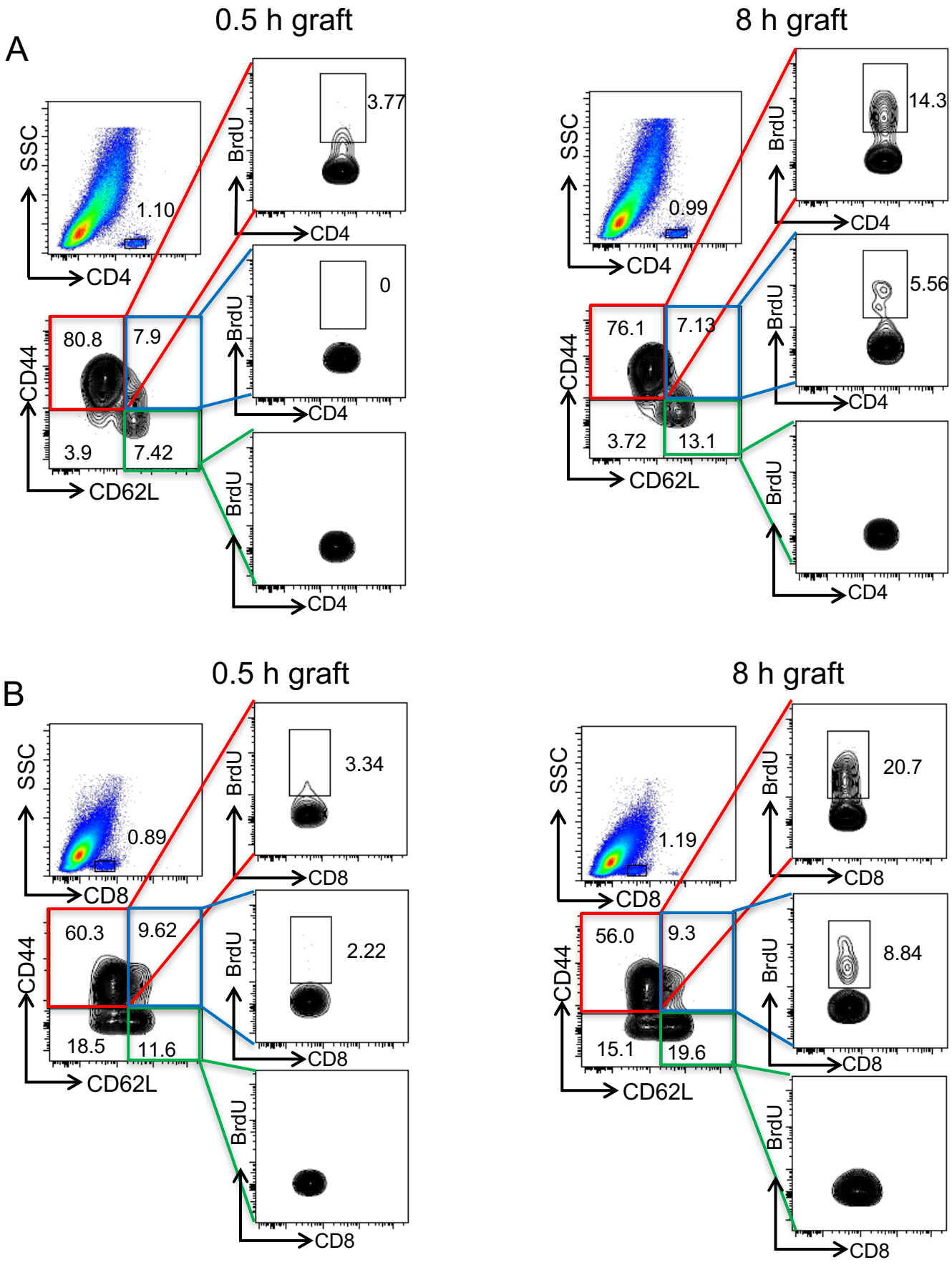
Supplemental Figure 1: Endogenous T cell populations infiltrating heart allografts subjected to minimal and prolonged cold ischemic storage at early times post-transplant. A/J hearts subjected to 0.5 or 8 hours CIS were transplanted to C57BL/6 mice and on day 2 post-transplant the allografts were harvested, digested to prepare single cell suspensions and aliquots were stained with fluorochrome labeled mAb and analyzed by flow cytometry with gating of **(A)** CD4 and **(B)** CD8 T cells to detect (naïve ($CD62L^{low}CD44^{low}$ and $CD62L^{high}CD44^{low}$), central memory ($CD62L^{high}CD44^{high}$) and effector memory ($CD62L^{low}CD44^{high}$) populations within the grafts. The numbers presented within each quadrant of the plots indicates the percentage of CD4 and CD8 T cells expressing CD62L and CD44. Results shown are representative of CD4 and CD8 T cells in two individual allografts.

Supplemental Figure 2



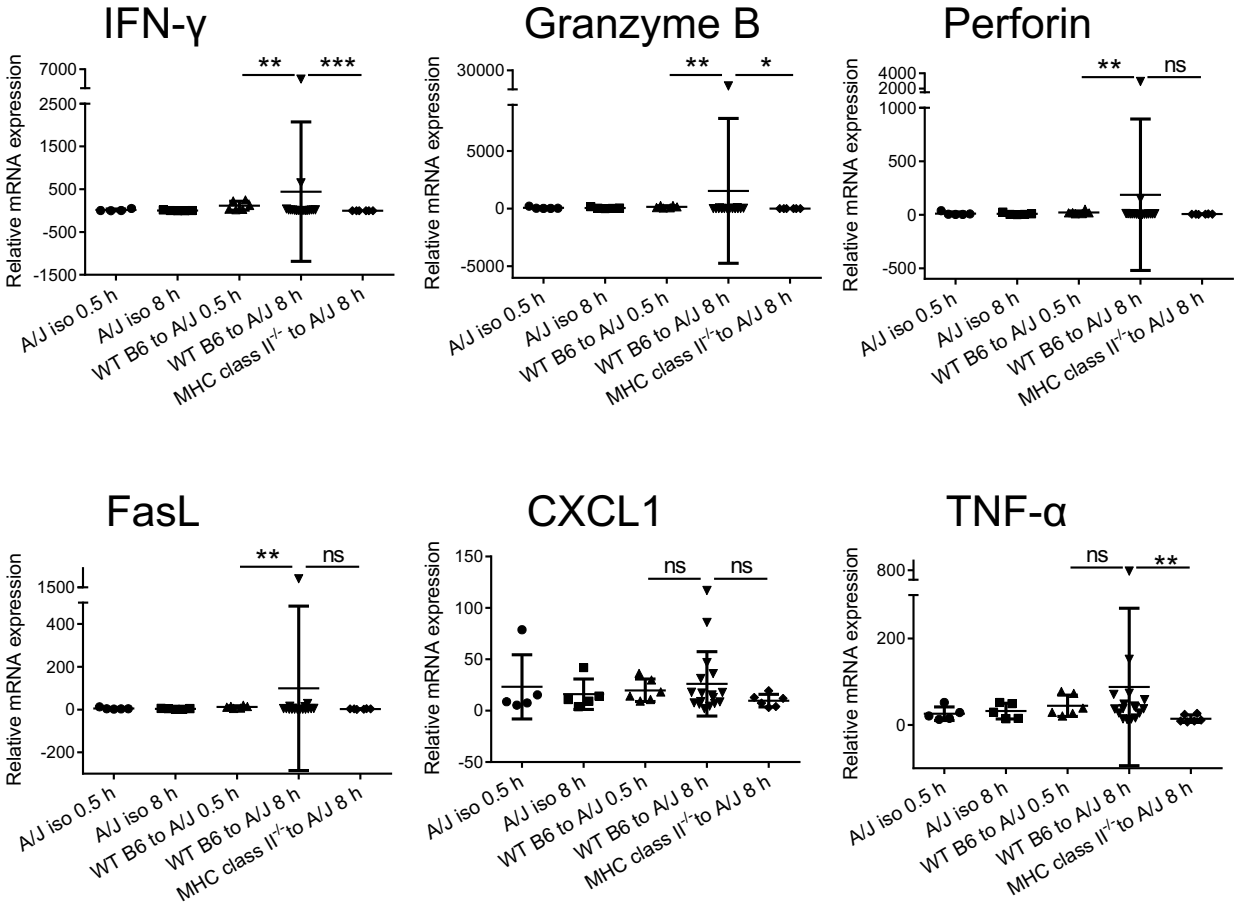
Supplemental Figure 2: Endogenous macrophage, neutrophil and B cell populations infiltrating heart allografts subjected to minimal and prolonged cold ischemic storage at early times post-transplant. A/J hearts subjected to 0.5 or 8 hours CIS were transplanted to C57BL/6 mice and on day 2 post-transplant the allografts were harvested, digested to prepare single cell suspensions and aliquots were stained with fluorochrome labeled mAb and analyzed by flow cytometry to detect and quantitate (A) macrophages, (B) neutrophils, and (C) B lymphocytes in the grafts.

Supplemental Figure 3



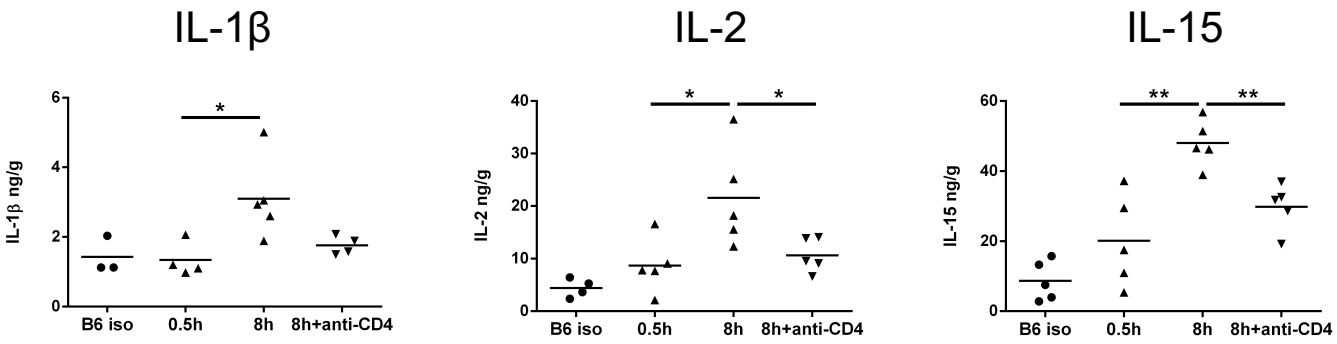
Supplemental Figure 3: Endogenous CD4 and CD8 T cell proliferation in allografts subjected to minimal vs. prolonged CIS. A/J hearts subjected to 0.5 or 8 hours CIS were transplanted to C57BL/6 mice. On days 0 and 1 post-transplant, all mice were injected with 100 µg BrdU i.p. and the next day allografts were harvested, digested, and aliquots of single cell suspensions were stained with antibody and gated to detect **(A)** CD4 and **(B)** CD8 T cells expressing CD44 and CD62L indicating naïve (green), central memory (blue) and effector (red) memory phenotypes. The stained cells were analyzed by flow cytometry with examples of gating as shown for each allograft sample to assess the BrdU incorporation of each of the T cell populations within the grafts. The numbers presented within each quadrant of the CD62L x CD44 plots indicates the percentage of graft infiltrating CD4 and CD8 T cells expressing naïve and central and memory T cell phenotypes. Numbers in the BrdU incorporation plots indicates the percentages of $BrdU^+$ CD4 and CD8 T cells for each of the gated naïve (green) and central (blue) and (red) effector memory T cell phenotypes. Results shown are representative of CD4 and CD8 T cells in two individual allografts.

Supplemental Figure 4



Supplemental Figure 4: Endogenous memory CD8 T cell activation in allografts subjected to prolonged CIS induces effector T cell gene expression. At 48 hours post-transplant, total RNA was isolated from iso- and allo-grafts in Figure 4A and the expression of mRNA encoding the indicated genes was tested by qPCR. Results for individual graft sample RNA are expressed as relative mRNA expression vs. mRNA expression in the heart of a naive C57BL/6 mouse. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significantly different.

Supplemental Figure 5



Supplemental Figure 5: Elevated production of proinflammatory cytokines in allografts subjected to 8 hours CIS. Groups of C57BL/6 (n = 5/group) mice received C57BL/6 isografts subjected to 8 hours CIS or A/J cardiac allografts subjected to either 0.5 or 8 hours CIS. The indicated recipients of 8 hour CIS allografts were treated with 200 μg anti-CD4 mAb on days -3, -2 and -1 prior to transplant and then received A/J cardiac allografts subjected to 8 hours CIS. On day 2 post-transplant, grafts were harvested and lysates prepared and quantities of IL-1b IL-2 and IL-15 were tested by ELISA. *$p < 0.05$.

Supplementary Table1: Antibodies used in this study

Antibody	Host	Clone	Catalog number	Source
anti-CD4	rat	YTS191	BE0119	Bio X Cell
anti-CD4	rat	GK1.5	BE0003-1	Bio X Cell
anti-CD154	rat	MR1	BE0017-1	Bio X Cell
CTLA-4 Ig	rat	CTLA-4	BE0099	Bio X Cell
anti-mouse CD40	rat	FGK4.5	BE0016-2	Bio X Cell
anti-mouse IL-12/IL-23p40	rat	C17.8	505305	BioLegend
Control IgG	rat	-	I4131	Sigma-Aldrich
FITC anti-mouse CD45R/B220	rat	RA3-6B2	553088	BD Biosciences
FITC anti-mouse CD62L	rat	MEL-14	553150	BD Biosciences
PE anti-mouse CD4	mouse	RM4-5	553049	BD Biosciences
PE anti-mouse Ly6C	rat	1A8	551461	BD Biosciences
PE anti-mouse CD44	rat	IM7	553134	BD Biosciences
PE anti-mouse CD45.2	mouse	104	12-0454-83	eBioscience
PE anti-mouse IL-12Rβ1	mouse	114	551974	BD Biosciences
PE anti-mouse IL-12Rβ2	mouse	aa24-622	LS-C125812	LifeSpan BioSciences
APC anti-mouse CD4	rat	RM4-5	17-0042-82	eBioscience
APC anti-mouse CD8a	mouse	53-6.7	553035	BD Biosciences
APC anti-mouse CD62L	rat	MEL-14	553152	BD Biosciences
PE-Cyanine7 anti-mouse CD45	rat	30-F11	20-0451-81	eBioscience
APC-Cy7 anti-mouse CD4	rat	GK1.5	552051	BD Biosciences
APC-Cy7 anti-mouse Ly6C	rat	HK1.4	128025	BioLegend
APC/Fire 750 anti-mouse CD8a	mouse	53-6.7	100765	BioLegend
PerCP-Cy5.5 anti-mouse CD19	rat	1D3	551001	BD Biosciences
PE CF-594 anti-mouse CD44	rat	IM7	562464	BD Biosciences