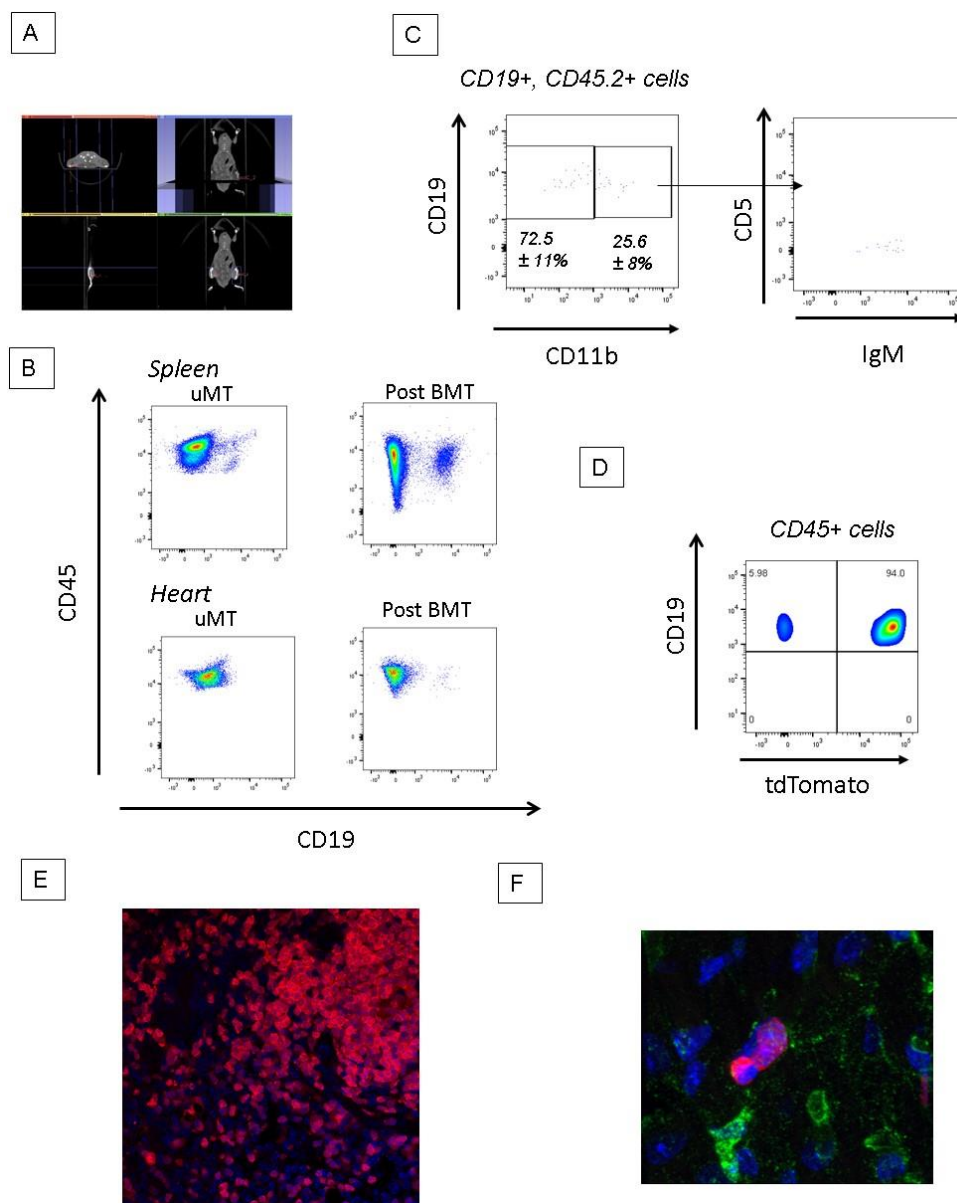
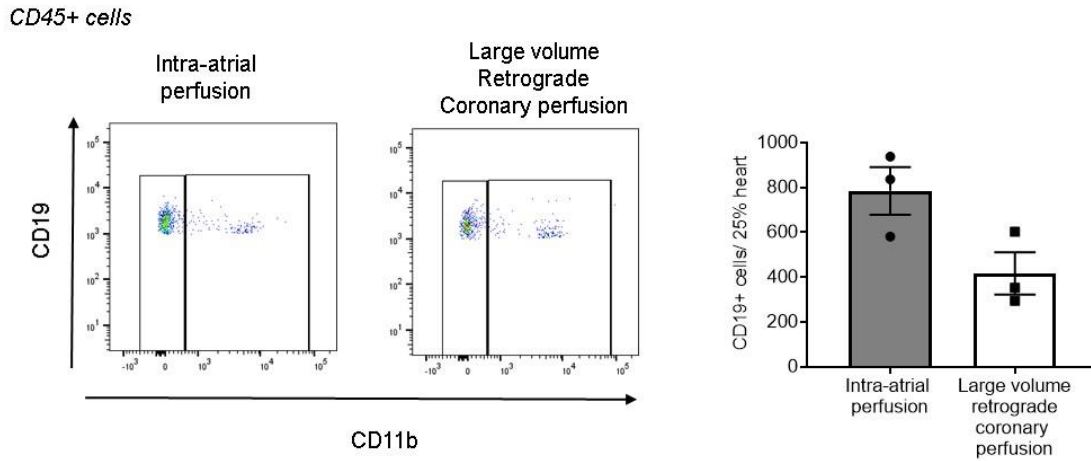


Supplementary Figures and Legends

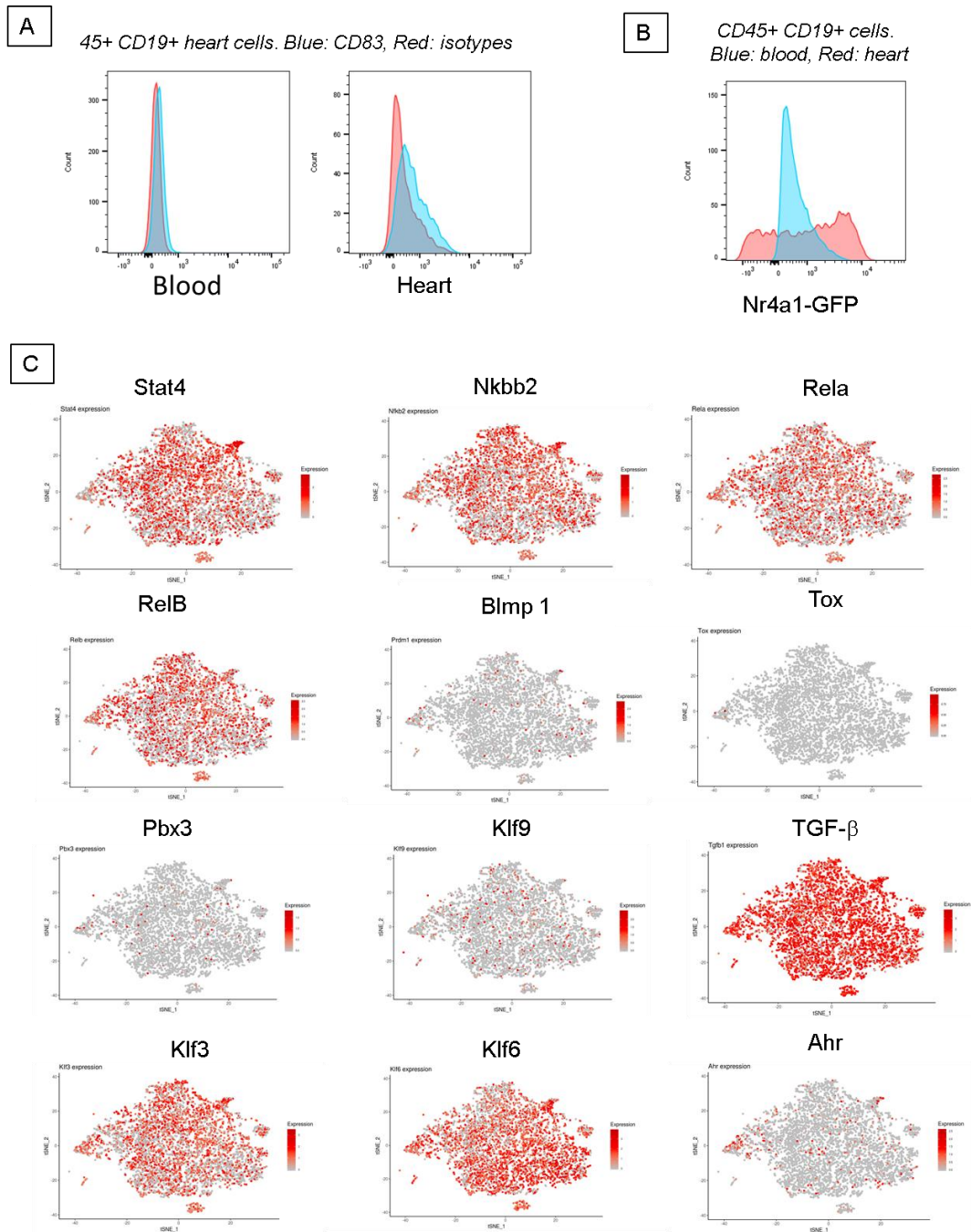


Supplementary Figure 1: A) Summary of irradiation plan for treated animals. 10 grays of radiation were delivered only to the bilateral femurs with no expected spillover irradiation to the mediastinum. B) Representative plots of splenic and myocardial CD45⁺ CD19⁺ cells in μ MT B cell deficient animals before and after bone marrow transplant with targeted radiation. Post bone marrow transplant (BMT) a clear population of B cells is visible in both the spleen and the heart. n=3. Each FACS plot reports average % of cells \pm standard deviation next to each gate. C) Flow cytometric analysis of donor derived CD45.2⁺ CD19⁺ B cells in the heart. Donor derived CD19⁺ cells are both CD11b⁺ and CD11b⁻. Within the CD11b⁺ compartment all cells are IgM⁺ CD5⁻. Plots are representative of the results from 3 independently irradiated/ transplanted animals. D) Flow cytometry of splenocytes from F1 reporter mouse generated

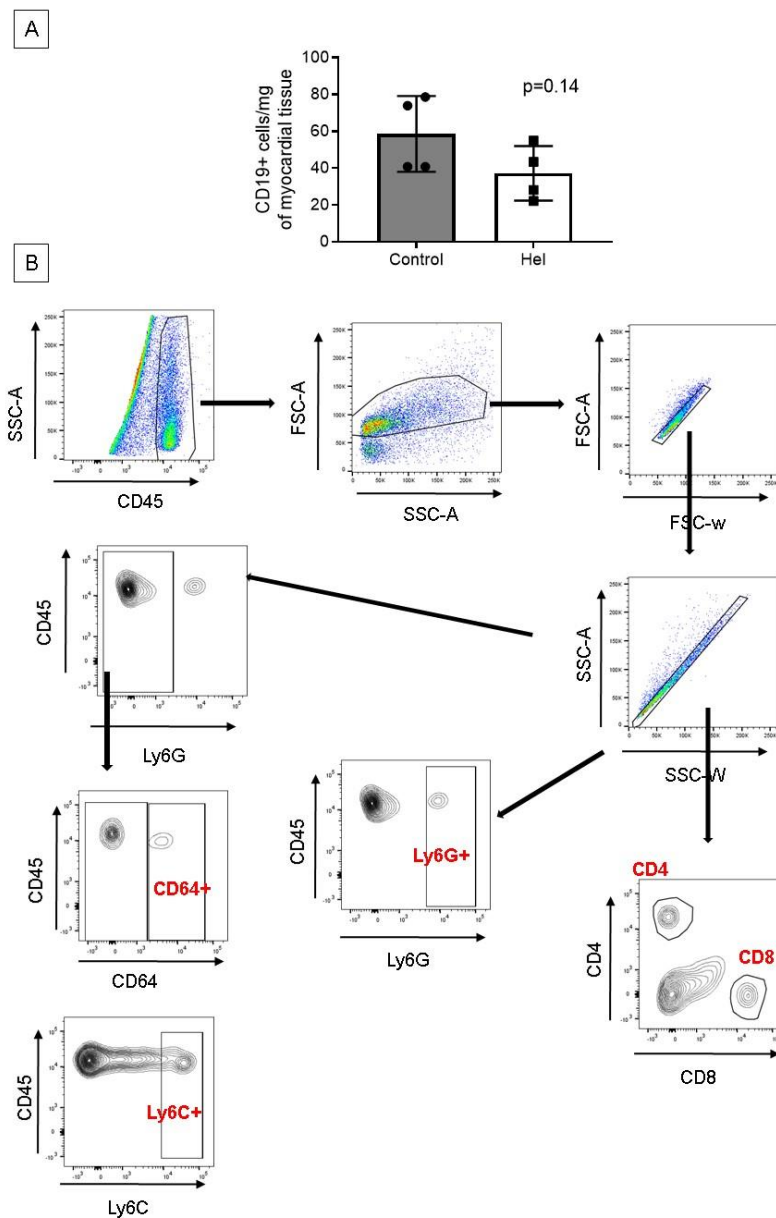
crossing a CD19-Cre animal with a ROSA-TdTomato Lox animal shows that B cells express TdTomato with high efficiency. E) Confocal image of spleen from CD19-tomato reporter animal, 40X. F) Confocal image of murine myocardium from CD19-tomato reporter animal. Occasional clusters of 3 B cells were found in the myocardial parenchyma. 120 X Z-stack projection, Olympus. Red = tomato+ B cell, green= CD31, Blue= DAPI.



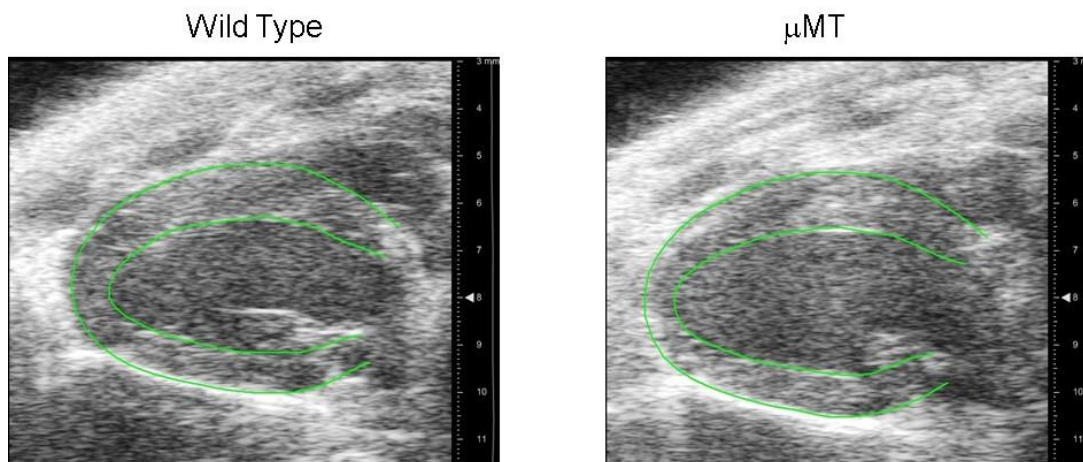
Supplementary Figure 2: A) Flow cytometric analysis of myocardial B cells of hearts cleaned via intra-atrial injection of 3 mls of high pressure PBS without calcium or magnesium and myocardial B cells from hearts cleaned through aortic cannulation and large volume (2mls) retrograde coronary perfusion with 2 mls of Krebs-Henseleit buffer. Representative plots from 3 independent animals (left) and quantitative data in histogram format (right) representing mean \pm standard deviation.



Supplementary Figure 3: A) Flow cytometric analysis of total CD83 in circulating (blood) CD19⁺ cells (left panel) and myocardial CD19⁺ cells (right panel). Isotype control staining is reported in red and CD83 staining is reported in light blue. B) Flow cytometric analysis of Nr4a1-GFP expression in CD19⁺ B cells from the circulating blood (blue) and from the heart (red). C) TSNE plot of gene expression of specific genes in myocardial B cells. Each dot represents a cell. Cells expressing the gene indicated above each graph are marked in red. Higher levels of gene expression are represented with a darker shade of red. Grey color indicates no detectable expression of the indicated gene.



Supplementary Figure 4: A) Hearts from HEL mice and syngeneic aged/sex matched wild type controls were dissociated in single cells and analyzed via flow cytometry to quantify the number of CD19⁺ cells/mg of myocardial tissue. The HEL mice had a number of myocardial B cells comparable to that of wild type controls. Bars represent mean \pm standard deviation B) Gating strategy used to identify specific subpopulation of myocardial leukocytes.



Supplementary Figure 5: Representative echocardiographic images from wild type mice (left) and B cell deficient μ MT mice (right).

Supplementary Video 1: Z-stack of myocardial section from CD19-tomato reporter mouse stained with CD31 (green) and DAPI (blue). A cluster of 3 extravascular CD19⁺ cells is visible. 120X, Olympus.

Supplementary Video 2: Animation of 3 dimensional rendering of confocal imaging from CD19-tomato reporter mouse stained with CD31 (green) and DAPI (blue.) A B cell in transit through the endothelium of a blood vessel is clearly visible. The blood vessel is likely a post capillary venule.

Supplementary Video 3: Modified Langendorf perfusion system used to perfuse μ MT B cell deficient hearts with wild type blood.

Supplementary Video 4: Intravital microscopy of B cells in a transplanted heart. The heart was removed from a wild type animal and immediately transplanted in the neck of a syngeneic CD19-tomato reporter animal with cold ischemic time <30 minutes. The transplanted heart was imaged 24 hours post-transplant. Blood vessels were marked with intravenous injection of high molecular weight fluorescent dextran. B cells are visible in green, while blood vessels are visible in red. Some B cell pass rapidly across the visual field. However, several B cells are seen stopped on the endothelium. Some other B cells are seen as they arrive and pause on the microvascular endothelium. In the center of the visual field some B cells are seen as they move across the endothelium with a movement pattern that is reminiscent of patrolling monocytes.

Supplementary tables

GENE	AVG_LOGFC	% HEART	% BLOOD
NR4A1	3.28	0.96	0.01
VPS37B	2.18	0.90	0.18
PIM1	1.24	0.84	0.31
CD83	1.09	0.85	0.33
ATF4	0.92	0.82	0.31

Supplementary table 1: List of top 5 differentially expressed genes between myocardial and circulating B cells that were detected in more than 80% of myocardial B cells and in less than 35% of circulating (blood) B cells. AVG_LOGFC = Average Log fold change in the comparison heart vs blood.

	HR	EDV	ESV	EF	SV	dV/dt-s / EDV	dV/dt-d / ESV	LVM
WT	626.4	28.5	7.4	73.7	21.1	0.8	27.5	73.4
μ MT	627.0	28.7	5.8	79.9	22.9	0.8	28.4	68.1
ttest	0.93	0.88	0.06	<0.01	0.10	0.45	0.48	<0.01

	LVPWd	IVSd	LVIDd	LVPWs	IVSs	LVIDs	E	A	E/A	E'	E/E'
WT	0.9	1.0	3.0	1.4	1.4	1.5	766.9	738.8	1.1	30.9	25.2
μ MT	0.8	0.9	3.0	1.3	1.4	1.4	814.6	729.3	1.1	23.5	36.0
ttest	0.11	0.05	0.96	0.55	0.29	0.22	0.30	0.59	0.48	0.01	0.01

Supplementary table 2- Echocardiographic measurements in wild type and μ MT animals. BW= body weight, HR= heart rate, EDV= end diastolic volume, ESV = end systolic volume, EF= ejection fraction, SV= stroke volume, dV/dt-s = rate of change of LV volume during systole, dV/dt-s/ EDV = rate of change of LV volume during systole indexed to end diastolic volume, dV/dt-d= rate of change of LV volume during diastole, dV/dt-d/ ESV = rate of change of LV volume during diastole indexed to end systolic volume, LVM= left ventricular mass, LVPWd= left ventricular posterior wall in diastole, IVSd= interventricular septum in diastole. LVIDd= left ventricular internal diameter in diastole, LVPWs= left ventricular posterior wall in systole, IVSs= interventricular septum in systole. LVIDs= left ventricular internal diameter in systole, E= peak velocity of early left ventricular filling, A= peak velocity of ventricular filling secondary to atrial contraction, E'= peak mitral annular velocity in early diastole. T-test = unpaired two way t test for difference between WT and μ MT. N=7/group.