Supplemental Material

Supplemental Materials and Methods

Mouse experiments. Tissues from all irradiated mice were histologically/pathologically evaluated (blinded) by a Board Certified Veterinary Pathologist (KLB); the tumor types that arose and the causes of illness were determined. For competitive bone marrow transplants, ~6 week-old female CD45.1 C57Bl/6 mice (NCI mouse repository, Frederick, MD) were lethally irradiated (9 Gy, ^{137}Cs) 24 hours before transplantation. Bone marrow from unirradiated CD45.2 donor *Smarcal1*$^{+/+}$, *Smarcal1*$^{+/\Delta}$ and *Smarcal1*$^{\Delta/\Delta}$ littermates was mixed 1:1 with bone marrow from unirradiated, wild-type CD45.1 mice, and injected ($2x10^6$ cells, intravenously) into lethally irradiated CD45.1 recipients. Peripheral blood leukocytes from recipient mice were analyzed every 2 weeks for CD45.1/CD45.2 expression using flow cytometry (see below).

Flow cytometry. Biotinylated antibodies were recognized by streptavidin conjugated to Pacific Blue (Invitrogen). Fluorescent antibodies against c-Kit (ACK2; eBioscience), Sca-1 (D7; BD Biosciences), CD48 (HM48-1; BD Biosciences) and CD150 (TC15-12F12.2; BioLegend) were used for HSPC identification. Thymic T cells were evaluated with fluorescently labeled antibodies against CD4 (GK1.5, BD Biosciences), CD8 (53-6.7, BD Biosciences), and Thy1.2 (53-2.1, BD Biosciences). Following competitive bone marrow transplantation, peripheral leukocyte CD45 expression was determined using fluorescently labeled antibodies against CD45.1 (A120, BD Biosciences) and CD45.2 (104, BD Biosciences).

Immunofluorescence. Cells were adhered to poly-L-lysine coated coverslips, fixed in ice-cold 2% paraformaldehyde/PBS (pH 7.4) for 10 minutes, permeabilized with 0.5% Triton X-100/PBS for 5 minutes, and blocked for 1-2 hours with 5% BSA/PBS (all steps at room temperature).

Anti-γH2AX (clone JBW-301 from Millipore or clone 20E3 from Cell Signaling) diluted 1:500 in 10% normal goat or donkey serum, respectively, in PBS was incubated overnight at 4°C. Goat anti-mouse or donkey anti-rabbit Alexa Fluor 488 (Invitrogen) diluted 1:500 in 5% normal goat or donkey serum, respectively, in PBS was incubated at room temperature for 1 hour. Coverslips were washed with PBS and then ToPro3 iodide or DAPI was added to stain nuclei. Coverslips were mounted with VectaShield to slides. Samples were blinded for analysis.

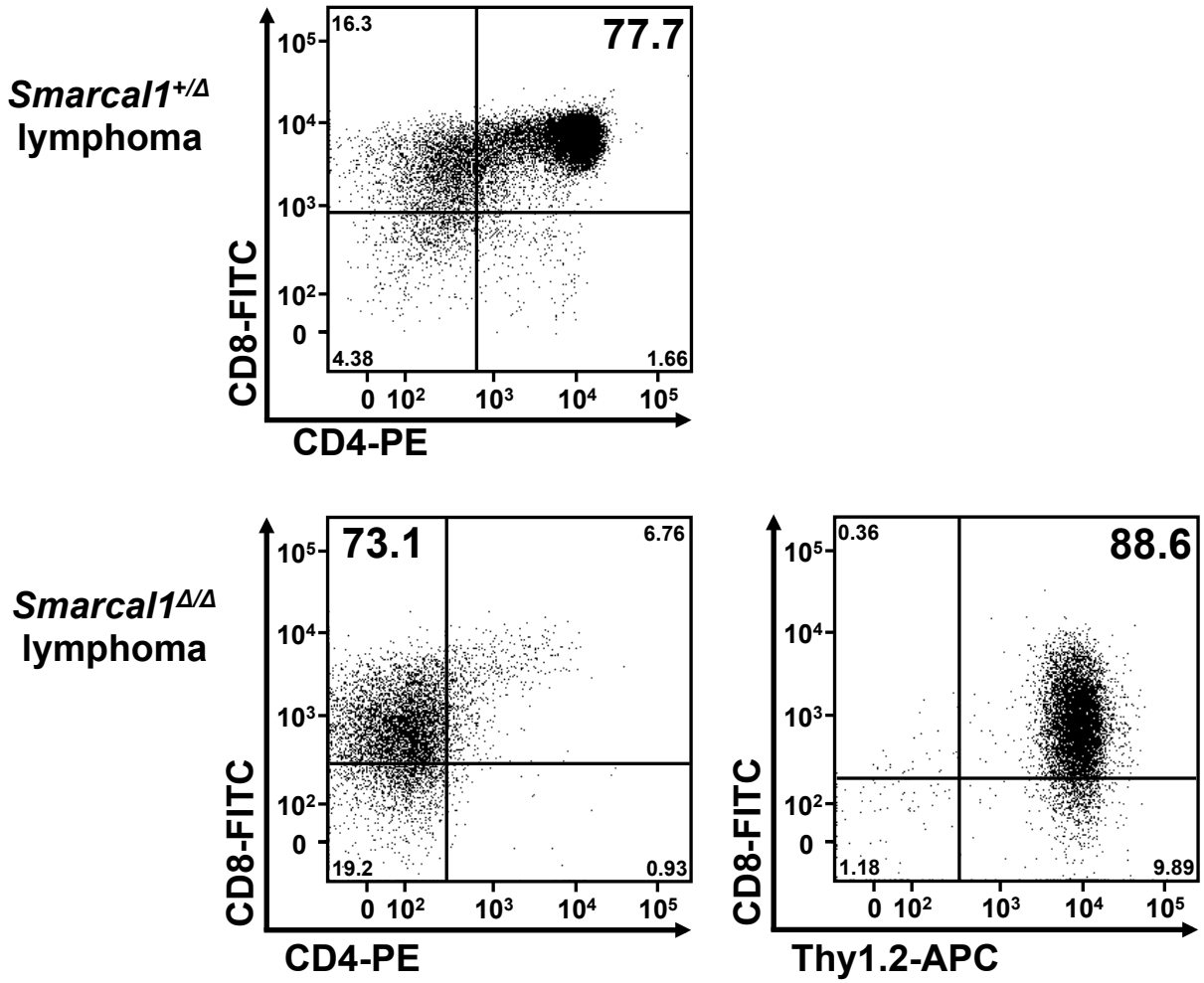
Supplemental Figure Legends

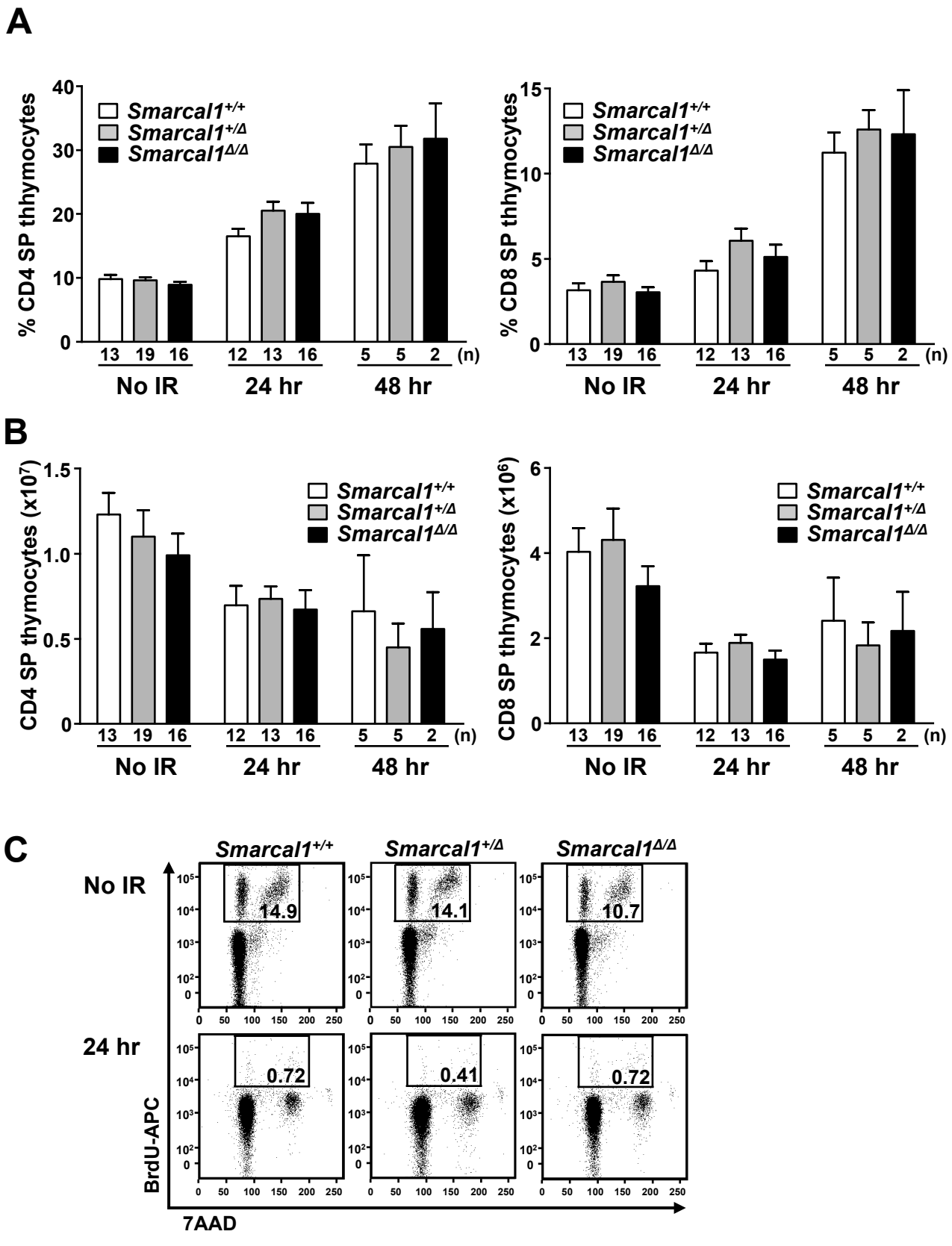
Figure 1. T cell lymphoma development in Smarcal1-deficient mice. Representative dot plots of CD4+/CD8+ or CD8+/Thy1.2+ lymphomas that developed in the irradiated cohort of mice; genotype indicated.

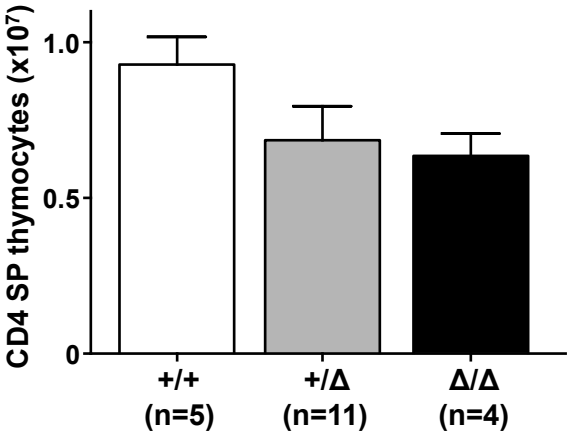
Figure 2. Loss of Smarcal1 does not alter sensitivity to ionizing radiation. A, B) Percentages (A) or total numbers (B) of CD4 SP (left) or CD8 SP (right) thymocytes without irradiation or at the indicated intervals following IR. Data are the mean of seven independent experiments with littermates; error bars are SEM. The number of mice denoted by n. C) Representative dot plots of BrdU incorporation in thymocytes from littermates without IR and 24 hours after IR.

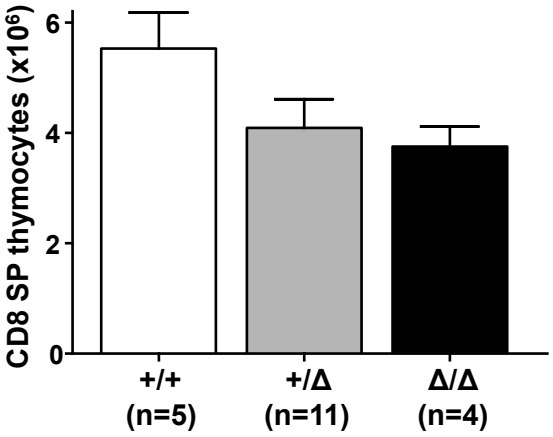
Figure 3. Smarcal1-deficient mice have reduced numbers of SP thymocytes 72 hours after IR. Total numbers of CD4 SP (left) or CD8 SP (right) thymocytes from littermates 72 hours following IR. Mean values graphed are from 3 independent experiments; error bars are SEM.

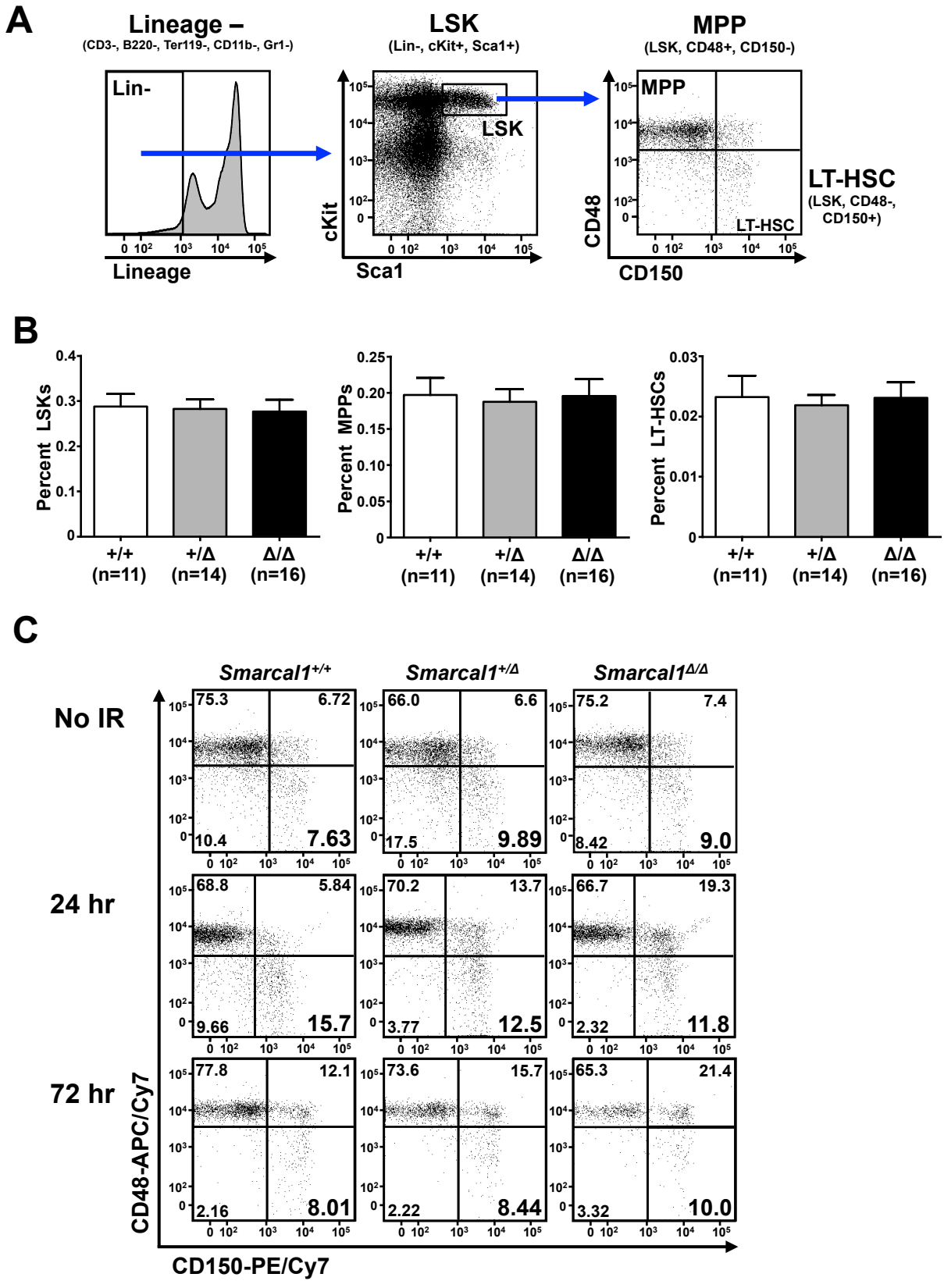
Figure 4. HSPC phenotyping. A) Schematic demonstrating the gating used to identify specific HSPC populations. B) Percentage of LSKs, MPPs, and LT-HSCs in the bone marrow of unirradiated littermates. Error bars are SEM; data from 5 independent experiments. C) Representative dot plots of MPPs and LT-HSCs from littermates not subjected to IR or analyzed at the indicated time after IR.


Smarcal1+/Δ lymphoma
16.3
77.7
4.38
1.66
CD8-FITC
CD4-PE
Smarcal1Δ/Δ lymphoma
73.1
6.76
19.2
0.93
0.36
88.6
1.18
9.89
Thy1.2-APC


A
% CD4 SP thhymocytes
Smarcal1+/+
Smarcal1+/Δ
Smarcal1Δ/Δ
13 19 16
12 13 16
5 5 2
(n)
No IR
24 hr
48 hr
% CD8 SP thhymocytes
B
CD4 SP thymocytes (x10^7)
CD8 SP thhymocytes (x10^6)
C
No IR
24 hr
14.9
14.1
10.7
0.72
0.41
0.72
BrdU-APC
7AAD


CD4 SP thymocytes (x10^7)
1.0
0.5
0
+/+
(n=5)
+/Δ
(n=11)
Δ/Δ
(n=4)


CD8 SP thymocytes (x10^6)
6
4
2
0
+/+
(n=5)
+/Δ
(n=11)
Δ/Δ
(n=4)


A
Lineage –
(CD3-, B220-, Ter119-, CD11b-, Gr1-)
LSK
(Lin-, cKit+, Sca1+)
MPP
(LSK, CD48+, CD150-)
Lin-
Lineage
cKit
Sca1
LSK
MPP
LT-HSC
CD48
CD150
LT-HSC
(LSK, CD48-, CD150+)
B
Percent LSKs
Percent MPPs
Percent LT-HSCs
+/+ (n=11)
+/Δ (n=14)
Δ/Δ (n=16)
C
Smarcal1+/+
Smarcal1+/Δ
Smarcal1Δ/Δ
No IR
24 hr
72 hr
75.3
6.72
10.4
7.63
66.0
6.6
17.5
9.89
75.2
7.4
8.42
9.0
68.8
5.84
9.66
15.7
70.2
13.7
3.77
12.5
66.7
19.3
2.32
11.8
77.8
12.1
2.16
8.01
73.6
15.7
2.22
8.44
65.3
21.4
3.32
10.0
CD48-APC/Cy7
CD150-PE/Cy7