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Supplemental Information

GM-CSF and IL-4 Fusion Cytokine Induces B Cell-Dependent Hematopoietic Regeneration

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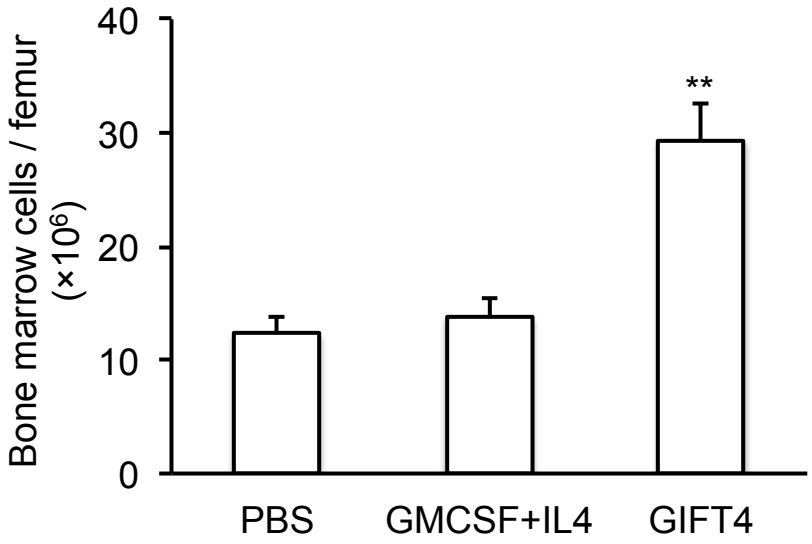
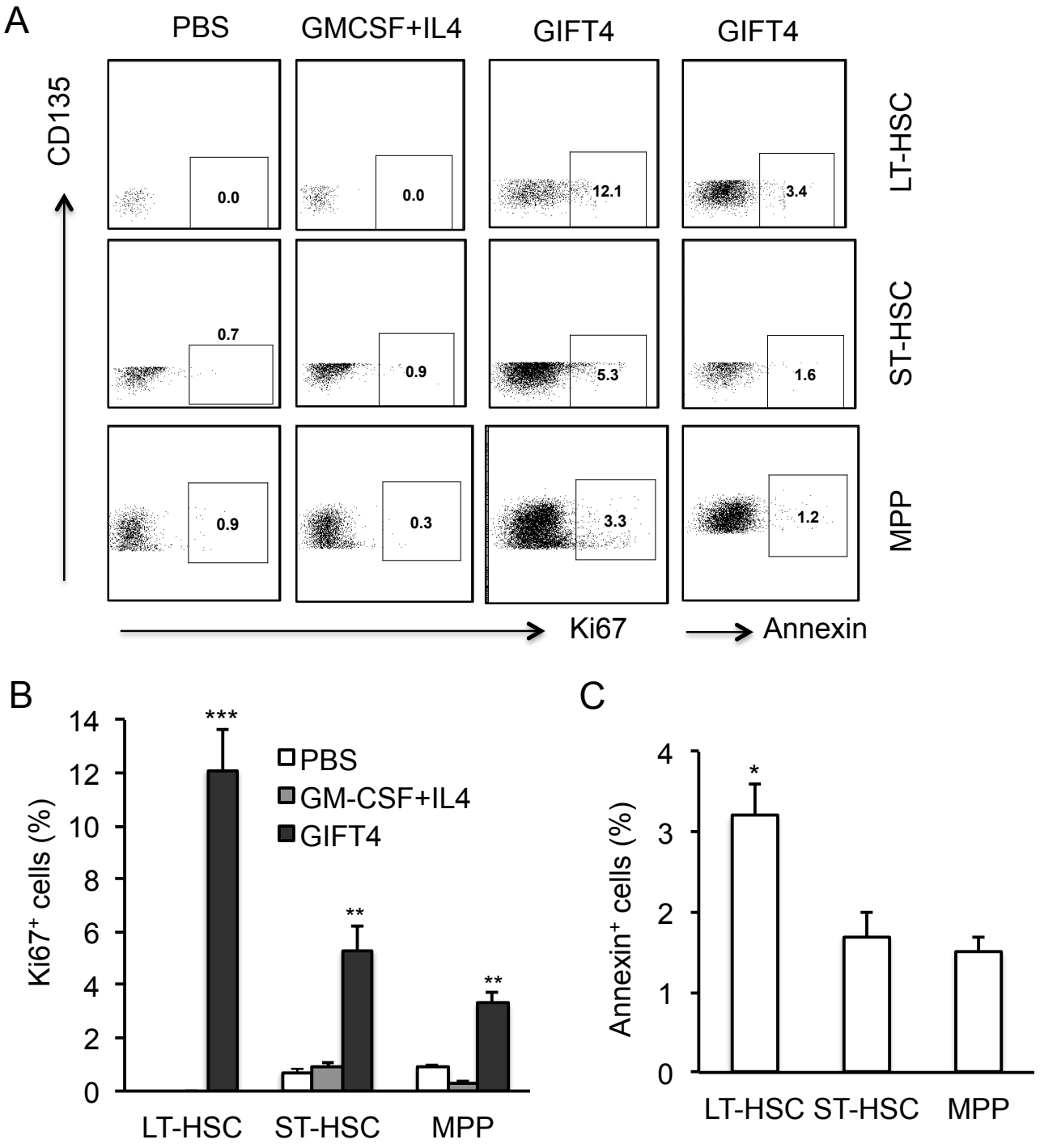
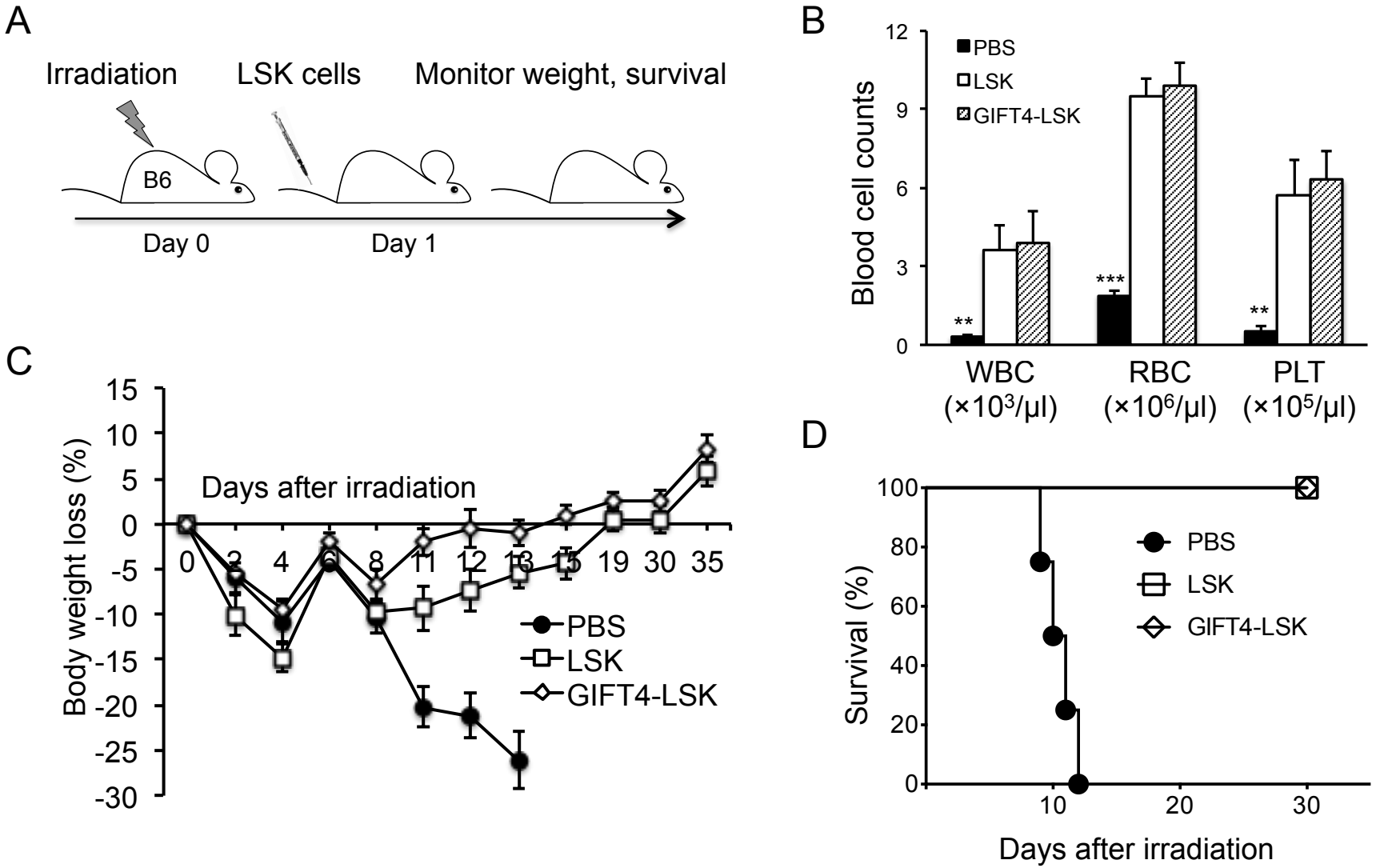


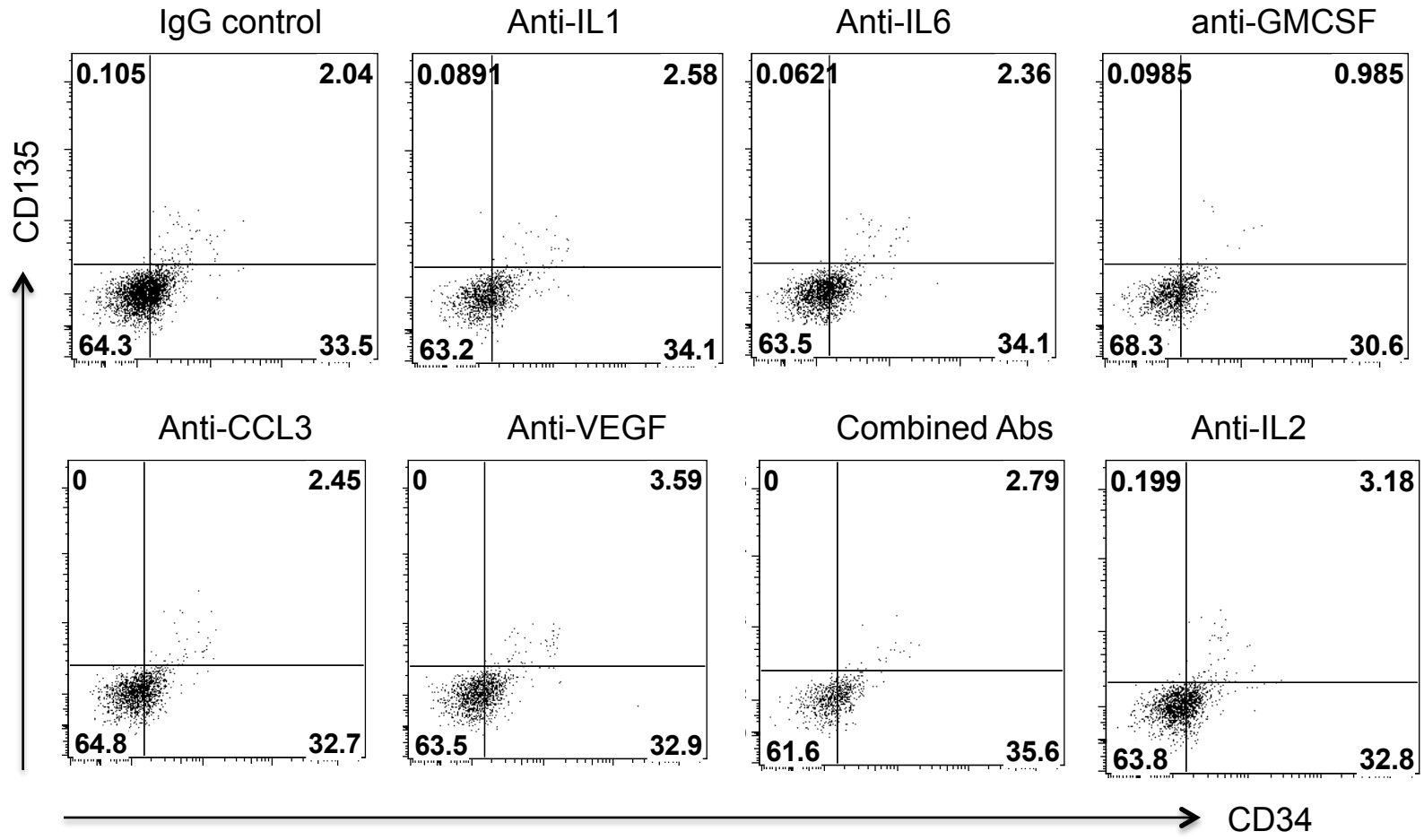
Supplemental Figure 1. GIFT4-enhanced Bone Marrow Cellularity. Naïve B6 mice (n=10 per group) were intravenously injected with GIFT4, GM-CSF and IL-4 (20ng/mouse/day) or PBS for 6 days. Bone marrow cells were isolated from the femur of treated mice on day 7, and counted under a microscopy. Data were from three independent experiments. ** $P<0.01$.


A
PBS
GMCSF+IL4
GIFT4
GIFT4
CD135
LT-HSC
ST-HSC
MPP
0.0
0.0
12.1
3.4
0.7
0.9
5.3
1.6
0.9
0.3
3.3
1.2
Ki67
Annexin
B
Ki67+ cells (%)
PBS
GM-CSF+IL4
GIFT4
***
**
**
LT-HSC
ST-HSC
MPP
C
Annexin+ cells (%)
*
LT-HSC
ST-HSC
MPP

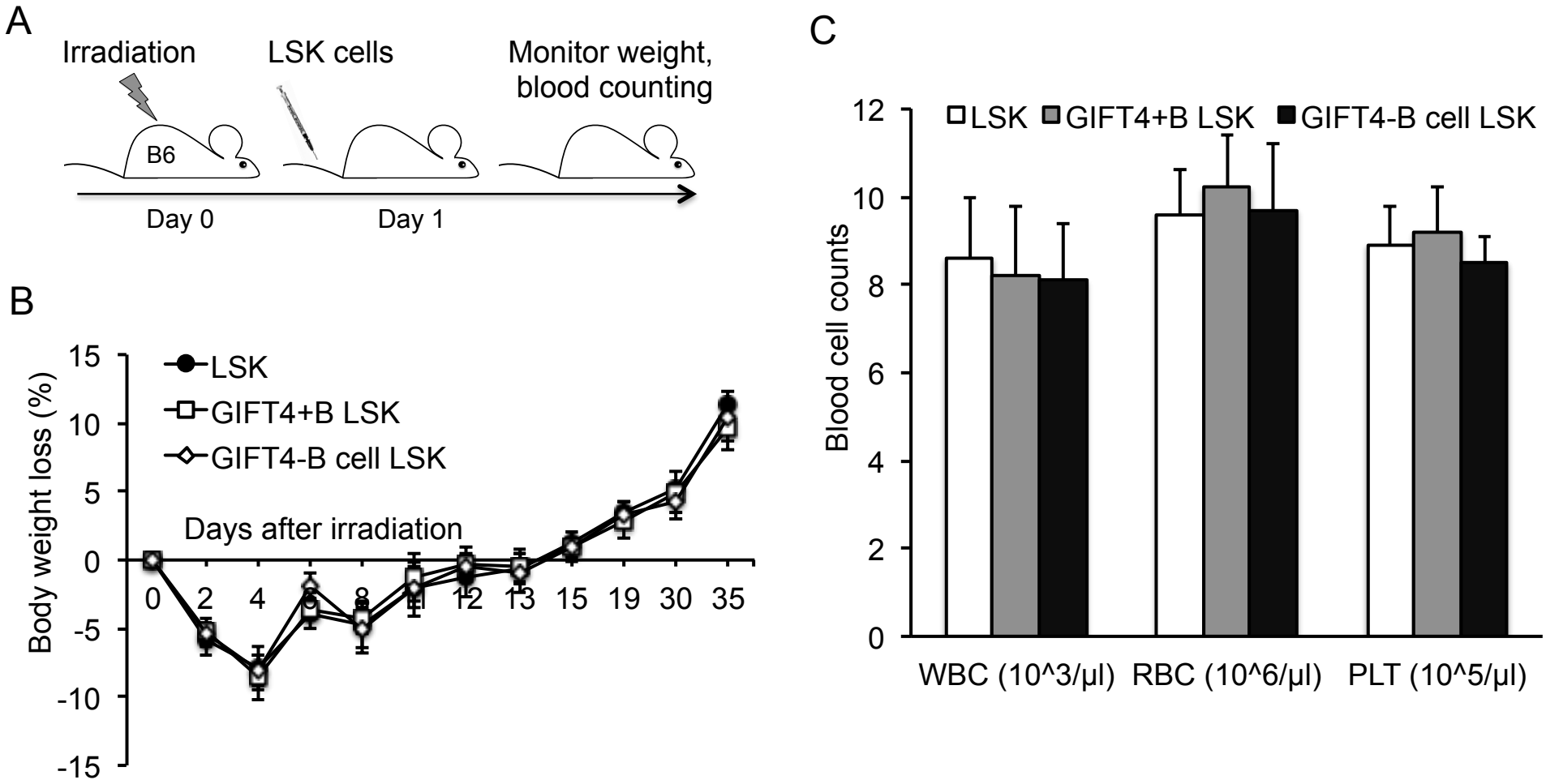
Supplemental Figure 2. GIFT4-triggered Proliferation of Bone Marrow HSC and MPP. Bone marrow cells isolated from GIFT4, GM-CSF and IL-4 or PBS treated B6 mice (n=10 per group) were subjected to surface staining with anti-mouse Sca-1, c-Kit, CD34 and CD135 antibodies, followed by Annexin-V staining or intracellular staining with anti-Ki67 antibodies. The cells were finally analyzed by FACS. (A) Data were representatives of LT-HSC, ST-HSC and MPP gated on $Lin^-Sca\text{-}1^+c\text{-}Kit^+CD34^-CD135^-$, $Lin^-Sca\text{-}1^+c\text{-}Kit^+CD34^+CD135^-$ and $Lin^-Sca\text{-}1^+c\text{-}Kit^+CD34^+CD135^+$ cells respectively. (B) Percentage of $Ki67^+$ LT-HSC, ST-HSC and MPP in bone marrow LSK compartment in the three groups of mice or (C) $Annexin^+$ LT-HSC, ST-HSC and MPP in bone marrow LSK population in GIFT4-treated mice were calculated and presented. Data were from three independent experiments. * $P<0.05$, ** $P<0.01$; *** $P<0.001$.


A
Irradiation
LSK cells
Monitor weight, survival
B6
Day 0
Day 1
B
Blood cell counts
PBS
LSK
GIFT4-LSK
**
***
**
WBC (×10³/μl)
RBC (×10⁶/μl)
PLT (×10⁵/μl)
C
Body weight loss (%)
Days after irradiation
PBS
LSK
GIFT4-LSK
D
Survival (%)
PBS
LSK
GIFT4-LSK
Days after irradiation

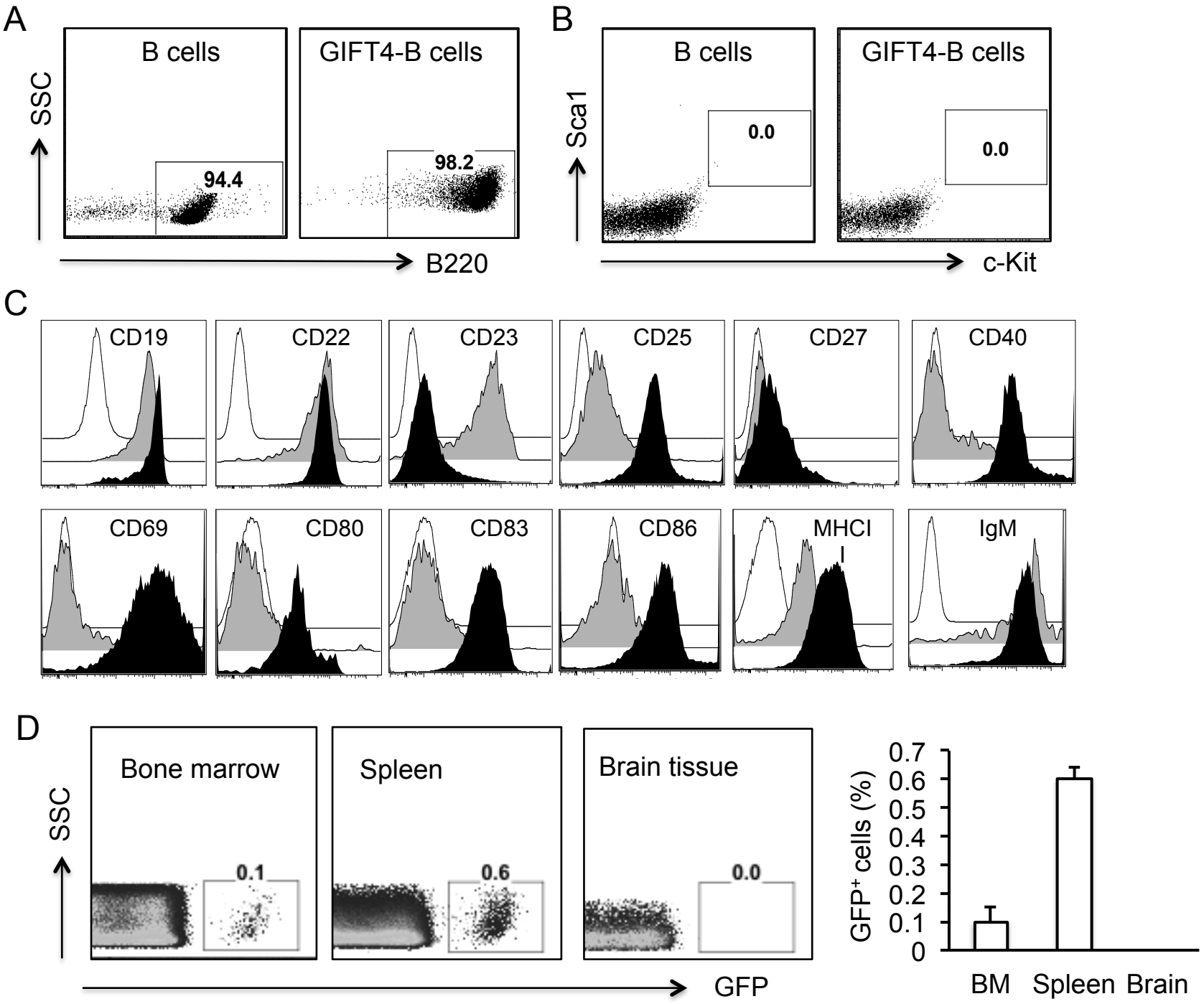
Supplemental Figure 3. Hematopoiesis of GIFT4-augmented LSK Cells in a Murine Model of Irradiation-induced Bone Marrow Failure. (A) Naive B6 mice (n=10 per group) received a dose of 11Gy total body irradiation. On the next day, the irradiated mice were intravenously transplanted with sorted bone marrow LSK cells (10^4/mouse) from naïve (LSK) or GIFT4-treated B6 mice (GIFT4-LSK). Mice treated with PBS served as control. (B) Peripheral blood was harvested from the three groups of mice on day 14 after irradiation, and the numbers of white blood cells (WBC), red blood cells (RBC) and platelets (PLT) per microliter peripheral blood were measured on a blood counter. (C) Mouse body weight was measured with a digital scale, and body weight loses after irradiation was calculated basing on the initial weight before irradiation. (D) Mouse death after irradiation was monitored and percentage of survival was calculated. Data were from two independent experiments.



Supplemental Figure 4. HSC and MPP Profile in GIFT4-treated Bone Marrow Cells. Bone marrow cells isolated from naïve B6 mice were stimulated with GIFT4 protein in the presence of anti-mouse IL-1, IL-2, IL-6, GM-CSF, CCL3 or VEGF specific neutralizing antibodies or the combination of those antibodies, anti-mouse IL-2 neutralizing antibody, or control IgG (5μg/ml) for 4 days. The cells were then subjected to FACS analyses with anti-mouse Lin, Sca-1, c-Kit, CD34 and CD135 antibodies. Data were a representative of three independent experiments.


A
Irradiation
LSK cells
Monitor weight, blood counting
B6
Day 0
Day 1
B
Body weight loss (%)
LSK
GIFT4+B LSK
GIFT4-B cell LSK
Days after irradiation
0 2 4 6 8 11 12 13 15 19 30 35
15 10 5 0 -5 -10 -15
C
Blood cell counts
LSK
GIFT4+B LSK
GIFT4-B cell LSK
12 10 8 6 4 2 0
WBC (10^3/μl)
RBC (10^6/μl)
PLT (10^5/μl)

Supplemental Figure 5. Hematopoiesis of LSK cells from Mice Treated with GIFT4 and B Cells or GIFT4-B cells in vivo. (A) Naive B6 mice (n=10 per group) were irradiated with a dose of 11Gy. On the next day, the irradiated mice were intravenously transplanted with sorted bone marrow LSK cells (10^4/mouse) from naïve mice (LSK) or mice treated with GIFT4 and B cells (GIFT4/B-LSK) or GIFT4-B cells (GIFT4B-LSK). (B) Mouse body weight was measured with a digital scale, and body weight loses after irradiation was calculated basing on the initial weight before irradiation. (C) Peripheral blood was harvested from the three groups of mice on day 35 after irradiation, and the numbers of white blood cells (WBC), red blood cells (RBC) and platelets (PLT) per microliter peripheral blood were measured on a blood counter. Data were from two independent experiments.


A
B cells
GIFT4-B cells
SSC
94.4
98.2
B220
B
B cells
GIFT4-B cells
Sca1
0.0
0.0
c-Kit
C
CD19
CD22
CD23
CD25
CD27
CD40
CD69
CD80
CD83
CD86
MHCI
IgM
D
Bone marrow
Spleen
Brain tissue
SSC
0.1
0.6
0.0
GFP
GFP+ cells (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BM
Spleen
Brain

Supplemental Figure 6. Bio-distribution of GIFT4-B Cells in vivo. (A) Naïve B cells were purified from splenocytes of GFP^+ transgenic mice with B cell enrichment kit by negative selection. Purified B cells were stimulated with GIFT4 protein for 5 days. The purity of naïve B cells or GIFT4-B cells was analyzed by FACS with anti-mouse B220 antibody. (B) The presence of LSK cells in purified naïve B cells or GIFT4-B cells was examined by FACS with anti-mouse Sca-1 and c-Kit antibodies. (C) The phenotype of GIFT4-B cells (Black) or purified B cells (Gray) was profiled with a panel of B cell markers. IgG isotype served as antibody control (White). (D) GIFT4-B cells were intravenously injected into naïve B6 mice. On day 2 after injection, splenocytes, bone marrow cells and brain mononuclear cells were isolated from treated mice with lymphocyte isolation medium. GIFT4-B cells were gated on GFP^+ cells. Percentage of GIFT4-B cells in spleen, bone marrow or brain was calculated and presented. Data were from three independent experiments.