SUPPLEMENTARY METHODS

Mice

C57BL/6 (B6) mice (all H-2^b and Thy1.1; B6.CD45.1, B6.CD45.2, luciferase expressing transgenic B6.luc$^+$, and B6.GFP) and AKR/b mice (H-2^b) were used as HSC donors for B6 (B6.CD45.2 or B6 Thy1.2 CD45.1; albino B6 [Thy1.2; CD45.2]; B6 Jα18$^{-/-}$ [Thy1.2; CD45.1]; B6.Rag2γc$^{-/-}$ BALB.B (H-2^b, Thy1.2, CD45.2, CD229.1$^+$), and BALB/c (H-2^d, Thy1.2, CD45.2, CD229.1$^+$) recipient mice. B10.D2 (H-2^d, Thy1.1, CD45.2) were used as donors for BALB/c mice. HSC donors were 6-10 weeks, and recipients $\geq$8 weeks old. Mice were bred and maintained at the Stanford University Research Animal Facility. Albino B6 mice were purchased from the Jackson Laboratories. All studies were approved by the Stanford University Administrative Panel on Laboratory Animal Care (APLAC).

Total lymphoid irradiation and total body irradiation

Irradiation was performed with a Philips x-ray unit (200 kV, 10 mA; Philips Electronic Instruments, Rahway, NJ) at a rate of 84 cGy/minute with a 0.5-mm Cu filter.

Staining for flow cytometry

For FACS-identification of HSC antibodies against c-Kit (2B8), Thy1.1 (OX-7), Sca-1 (D7), and the lineage markers CD3ε (145-2C11 or 17A2), CD4 (GK1.5), CD5 (53-7.3), CD8α (53-6.7), B220 (RA3-6B2), Gr1 (RB6-8C5), Mac1 (M1/70) and TER-119 (TER-119) were used. To distinguish long-term and short-term HSC antibodies against CD34 (RAM34), Flt3 (CD135: A2F10), and SLAM (CD150: mShad150) were added. To label Tregs CD25 (PC61.5) and FoxP3 (FJK-16s) antibodies were used in addition to CD4. Phenotype analysis was performed using B220 (RA3-6B2), Thy1.1 (OX-7), Thy1.2 (53-2.1), CD3ε or TCRβ (H57-597), CD4, CD8 (5H10), proliferative activity using Ki-67 (SolA15). Donor versus host cells were identified by Thy1.1, Thy1.2, CD45.1 (A20) and CD45.2 (104), or GFP. Antibodies were from eBioscience, Biolegend, Invitrogen, or BD Biosciences Pharmingen.

After surface-staining specimens were fixed and permeabilized using the Foxp3 Staining Buffer Set (eBioscience) and antibody-stained for intracellular markers (FoxP3, Ki-67). For cell cycle studies the Vybrant DyeCycle stain (Invitrogen) was used according to the manufacturer's instructions, respectively. Propidium iodide staining, ethidium monoazide, or a viability kit (LIVE⁄DEAD® Fixable Aqua Dead Cell Stain Kit, Invitrogen) were used to exclude dead cells.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Basic transplant schema of TLI/ATG.

(A) Anesthetized mice are placed into customized lead jigs that expose cervical and mesenteric lymph nodes (LN), thymus, and spleen. (B) In humans irradiation is given to a mantle field and an inverted Y, exposing cervical, supra-clavicular, mediastinal, axillary, periaortic, iliac, inguinal and femoral LN, spleen, (and the thymus). (C) TLI begins on d-23 prior to transplantation. 17 doses of 240 cGy are given with 2 days of break after each block of 5 daily doses. Intraperitoneal ATG injections are given on d-12, -10, -8. HCT of 5,000-10,000 donor KTLS-HSC is performed on d0.

Supplemental Figure 2. Lymphoablation after TBI vs TLI/ATG.

Lymphoid organs of wild-type controls, mice at 24 hrs post lethal TBI vs the last TLI dose were compared by absolute cell counts and FACS analysis. (A) Absolute spleen cell numbers revealed no significant difference between both groups, but counts were ~15x lower than in wild-type controls. (B) Absolute cell numbers per thymus were lower in TLI- vs TBI-treated animals, and ~80x lower compared to wild-type controls. (C) Absolute cell numbers of cervical (cer), mesenteric (mes), and axillary (ax) LN. Cell counts of cerLN were ~23x lower after TLI/ATG than after TBI, and ~100x reduced compared with those of wild-type mice. Cell counts of mesLN were ~15x lower after TLI/ATG than after TBI, and ~50x reduced compared with those of wild-type mice. Cell counts of axLN were ~5x lower after TLI/ATG than after TBI, and ~88x reduced compared with those of wild-type mice.

Supplemental Figure 3. Splenic NK and NKT cells after TLI/ATG versus TBI.

(A) NK cells in the spleens were determined by their co-expression of the surface markers Dx5 and CD122. Co-staining of CD3 identifies NKT cells. (B) The proportion of NK cells was increased in the spleen after TBI, but not after TLI. In LN proportions of NK cells were increased both after TBI and TLI/ATG compared with wild-type mice.

Supplemental Figure 4. Engraftment across genetic barriers after TLI/ATG vs TBI.

10,000 KTLS-HSC from B6.luc^{+} mice (Thy1.1, CD45.1; H2^{b}) were infused into albino B6 (Thy1.2, CD45.2; H2^{b}), BALB.B (Thy1.2, CD45.2; H2^{b}), and BALB/c (Thy1.2, CD45.2; H2^{d}) mice. Recipients were prepared either with lethal TBI (950 cGy for B6, 800 cGy for BALB) or TLI/ATG 17x240 cGy. Percent donor chimerism of blood B cells (left), T cells (middle), and granulocytes (right) after TBI (symbols in black) vs TLI/ATG (blue [congenic], red [miAg-mismatched, green [MHC-mismatched]) at 1 month (solid symbol) and 3 months (framed symbol) post-HCT. TBI conditioned recipients were mostly donor- type in the B cell and granulocyte lineage, but mixed chimeric for T cells. TLI/ATG- conditioned congenic and miAg-mismatched recipients were mixed chimeras in all lineages. The level of donor chimerism did not increase over time. MHC-mismatched BALB/c recipients had no evidence of donor cells in the blood.

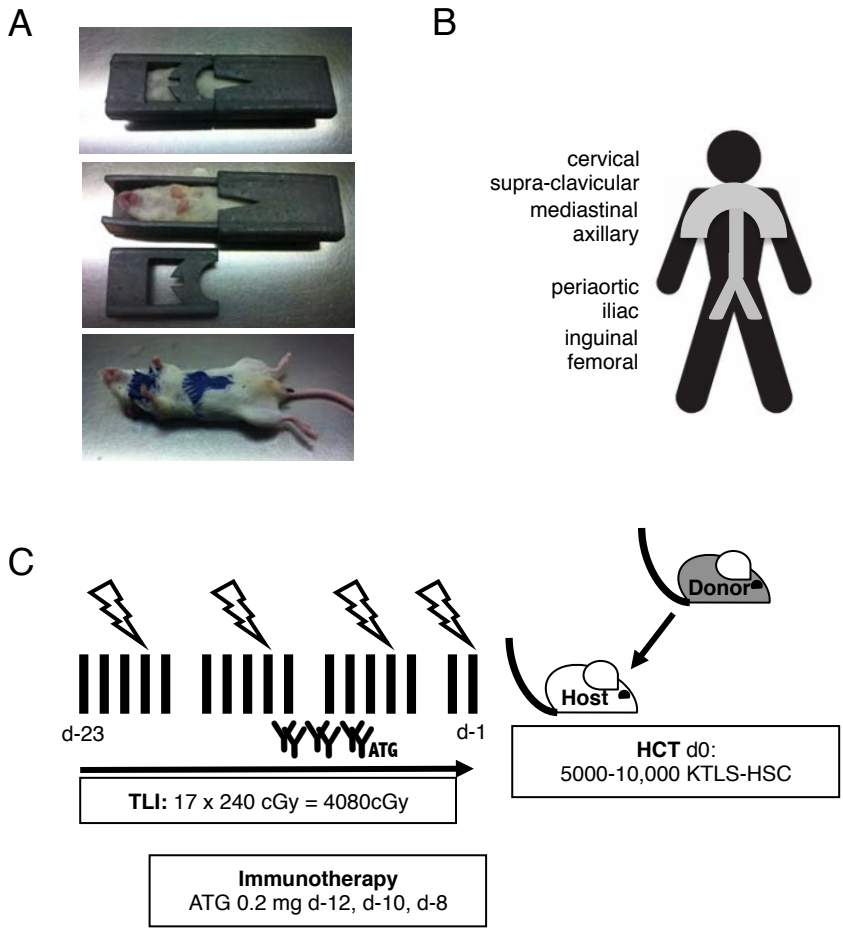
Supplemental Figure 5. Donor chimerism post TLI +/- ATG vs sublethal TBI using HSC vs whole BM grafts.

Percent donor chimerism of B cells (top), T cells (middle), and myeloid (Mac1^{+}) cells (bottom) at 1, 2, and 3 months post-HCT in recipients conditioned with TLI +/- ATG given pure HSC (left), or whole bone marrow (WBM; middle), and in recipients conditioned with 400 cGy (sublethal) total body irradiation (TBI) given pure HSC (right). TBI-conditioned recipients achieved the highest level of donor chimerism in all lineages. TLI-conditioned recipients of WBM achieved higher levels of donor chimerism as compared to HSC recipients. The level of donor chimerism in TLI- recipients did not increase significantly from 1 to 3 months post-HCT. The addition of ATG did not influence donor chimerism significantly in any treatment group. (B) Compiled chimerism levels displayed as the median percent donor cells per lineage at 1-3 months post-HCT.

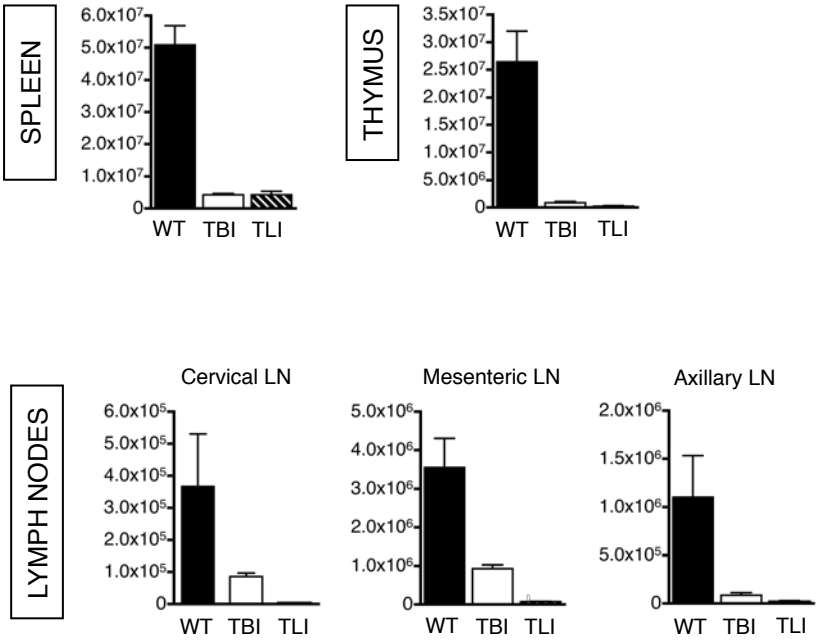
Supplemental Figure 6. T regulatory cells influence HSC activity.

BALB.K ($H2^k$) recipients underwent sublethal 400 cGy TBI and were given 3000 AKR/J ($H2^k$) KTLS-HSC, HSC+$3x10^5$ $CD4_{conv}$ or HSC+$2x10^5$ Treg. Left graph: compiled data on percent B cells of all blood cells at 4 weeks post-HCT revealing higher proportions in HSC+Treg as compared to HSC or HSC+$CD4_{conv}$ recipients. Right graph: Cumulative proportions of blood T cells at 6 weeks post-HCT with higher levels in HSC and HSC+Treg compared with HSC+$CD4_{conv}$ recipients.

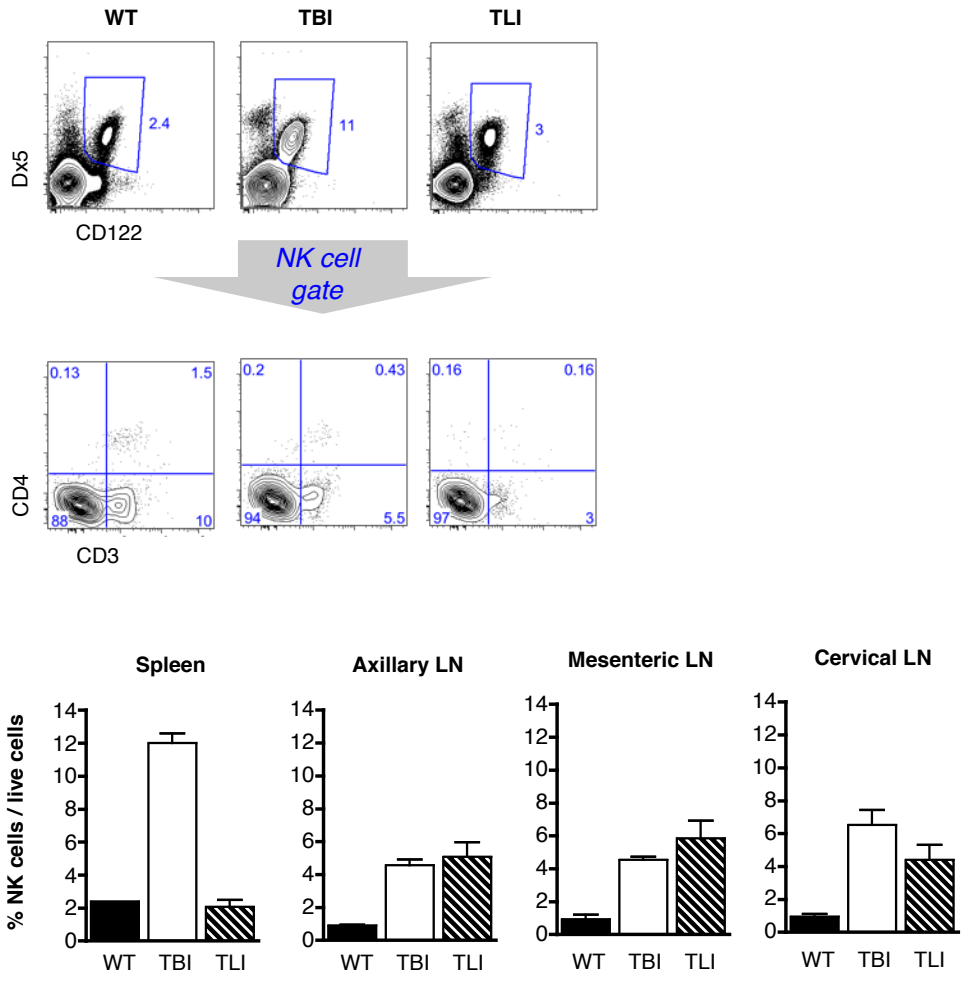
Supplemental Figure 1. Basic transplant schema of TLI/ATG.



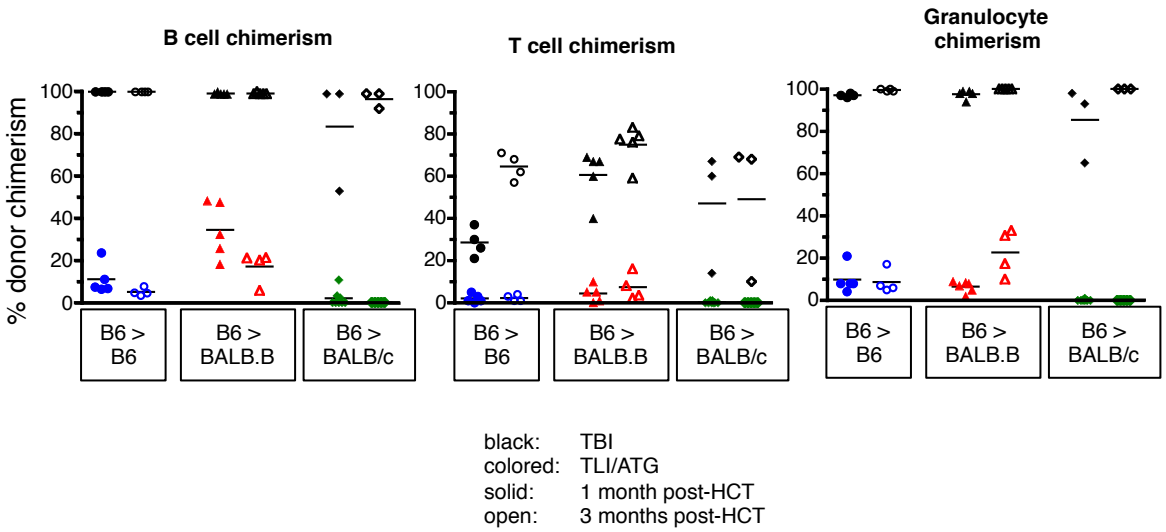
Supplemental Figure 2. Lymphoablation after TBI vs TLI/ATG.



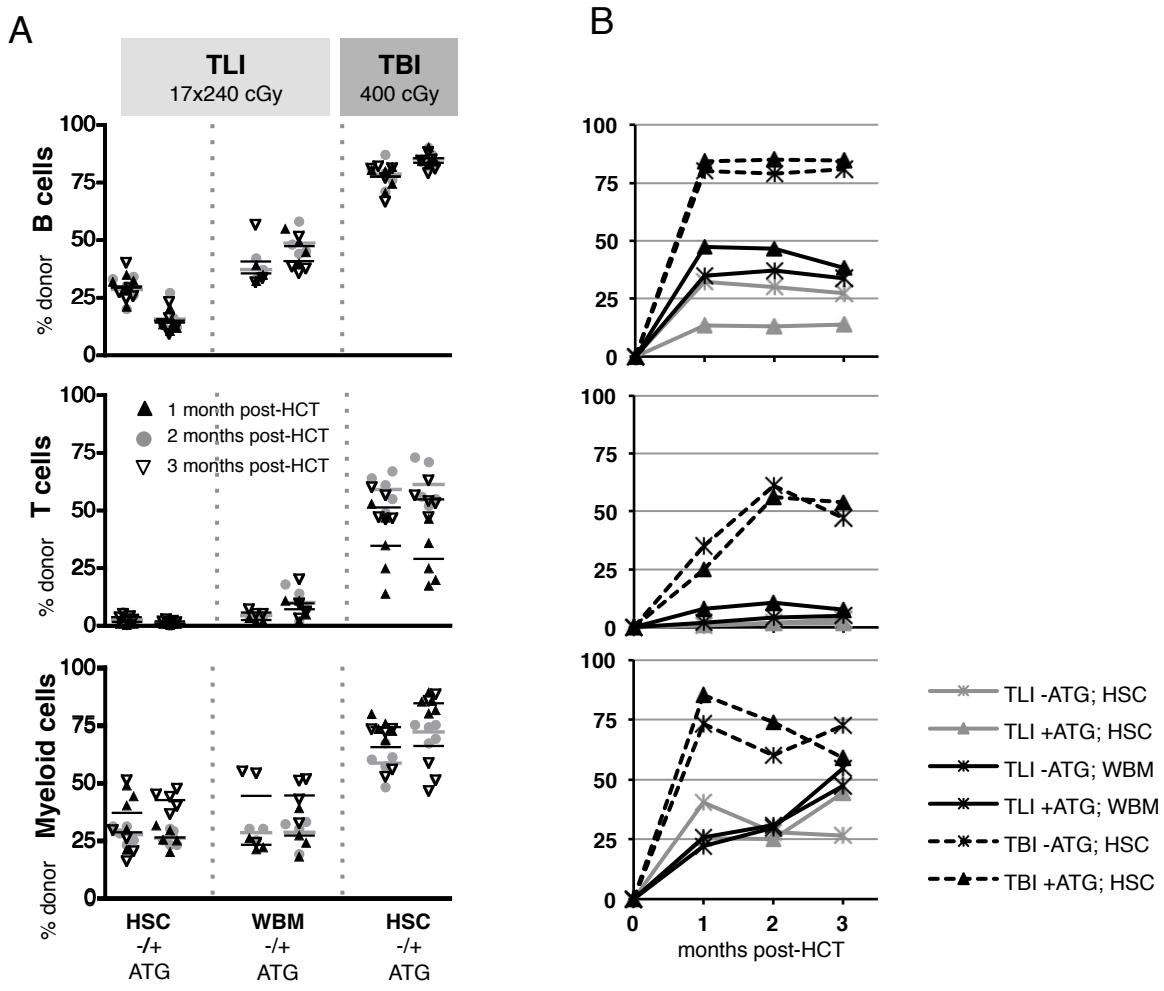
Supplemental Figure 3. Splenic NK cells at d0 post-TLI/ATG vs TBI.



Supplemental Figure 4. Engraftment across genetic barriers after TLI/ATG vs TBI.



Supplemental Figure 5.
Donor chimerism post TLI +/- ATG vs sublethal TBI using HSC vs whole BM grafts.



Supplemental Figure 6. Lymphocyte reconstitution after non-myeloablative TBI