Supplemental Table 1. Flow cytometry antibodies and related reagents used in this study.

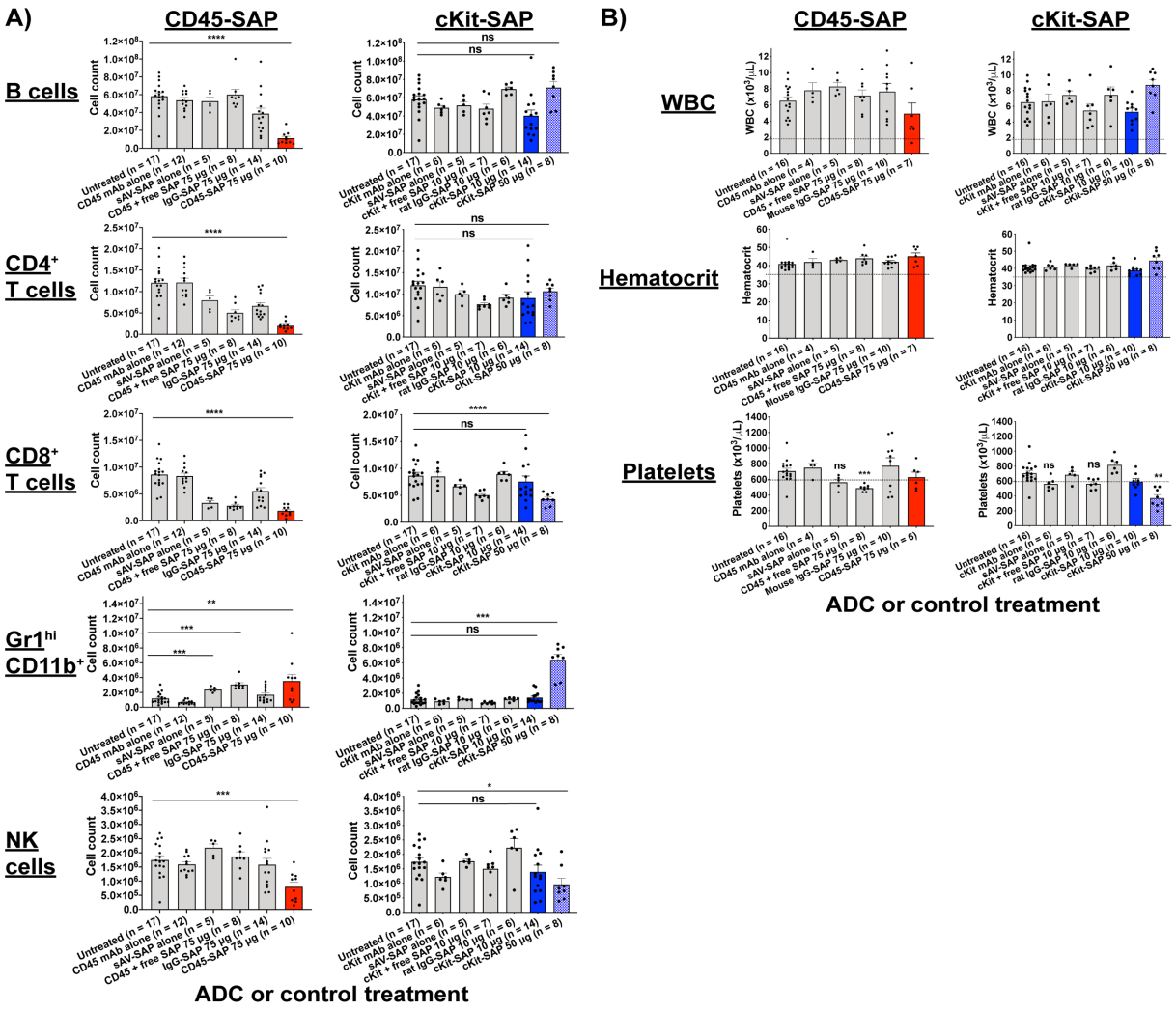
Reagent name or target	Species reactivity	Clone	Fluorophore	Vendor(s)
Anti IgG F(ab')2	Rabbit	Polyclonal	Alexa Fluor 647	Cell Signaling
CD3	Mouse	145-2C11	FITC	BioLegend
			APC	BioLegend
			PerCP-Cyanine5.5	BioLegend
			Purified – LEAF	BioLegend
CD4	Mouse	GK1.5	FITC	BioLegend
			PE-Cyanine7	BioLegend
			APC	BioLegend
			APC-eFluor 780	eBioscience
			eFluor 450	eBioscience
			Biotin	BioLegend
			Purified – InVivoPlus	BioXcell
CD4	Mouse	RM4-4	FITC	BioLegend
CD4	Mouse	RM4-5	PerCP-Cyanine5.5	BioLegend
CD4	Human	RPA-T4	FITC	BioLegend
CD5	Mouse	53-7.3	PE	BioLegend
CD8	Mouse	53-6.7	FITC	BioLegend
			APC	BioLegend
			APC-eFluor 780	eBioscience
			eFluor 450	eBioscience
			Brilliant Violet 510	BioLegend
			BioLegend	BioLegend
			Purified – InVivoPlus	BioXcell
CD11b	Mouse	M1/70	FITC	BioLegend
			PerCP-Cyanine5.5	BD
			APC	BioLegend
CD11c	Mouse	N418	PE	BD
			PE-Cyanine7	BD

CD16/32	**Mouse**	**93**	**Purified – Fc Block** **PE-Cyanine7**	**BioLegend** **BioLegend**
CD19	**Mouse**	**1D3**	**PerCP-Cyanine5.5**	**BioLegend**
CD25	**Mouse**	**3C7**	**PE**	**BD**
CD28	**Mouse**	**37.51**	**Purified - LEAF**	**BioLegend**
CD34	**Mouse**	**HM34**	**FITC**	**BioLegend**
CD44	**Mouse/human**	**IM7**	**Brilliant Violet 421**	**BioLegend**
CD45	**Mouse**	**30-F11**	**PE**	**BD**
CD45	**Human**	**HI30**	**Purified** **Biotin**	**BioLegend** **BioLegend**
CD45	**Human**	**BC8**	**Purified** **Biotin**	**Leinco** **Leinco**
CD45.1	**Mouse**	**A20**	**PE** **PE-Cyanine7** **eFluor 450**	**BioLegend** **BioLegend** **eBioscience**
CD45.2	**Mouse**	**104**	**Biotin** **Brilliant Violet 421** **Brilliant Violet 605** **Purified – InVivoPlus**	**BioLegend** **BioLegend** **BioLegend** **BioXcell**
CD45R (B220)	**Mouse/human**	**RA3-6B2**	**FITC** **PerCP-Cyanine5.5** **APC** **APC-eFluor 780** **eFluor 450** **Brilliant Violet 605**	**BioLegend** **BD** **BioLegend** **eBioscience** **eBioscience** **BioLegend**
CD48	**Mouse**	**HM48-1**	**APC**	**BioLegend**
CD49b	**Mouse**	**DX5**	**FITC**	**BD**
CD62L	**Mouse**	**MEL-14**	**APC** **Brilliant Violet 650**	**BioLegend** **BioLegend**
CD80	**Mouse**	**16-10A1**	**BV421**	**BioLegend**

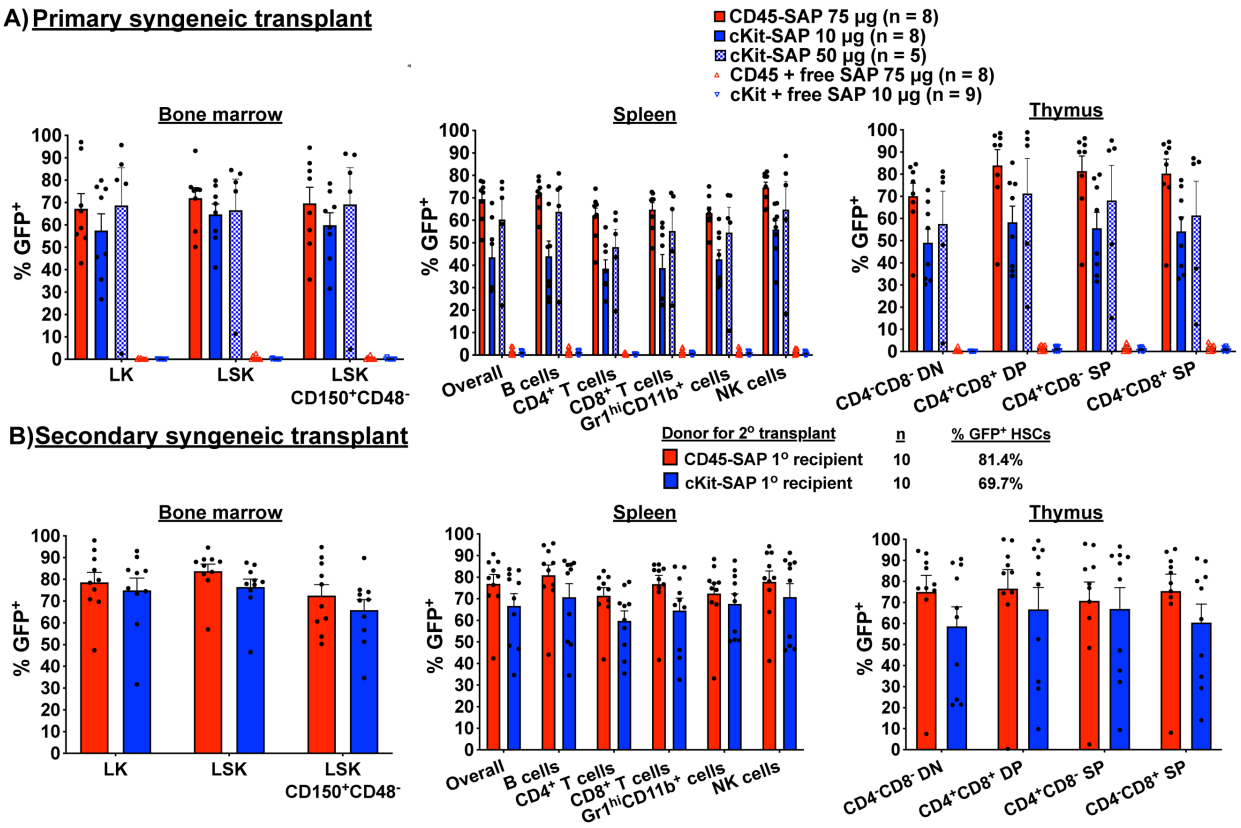
CD86	**Mouse**	**GL-1**	**APC-eFluor 780**	**eBioscience**
CD117 (c-Kit)	**Mouse**	**2B8**	**Biotin**	**BioLegend**
CD117 (c-Kit)	**Mouse**	**ACK2**	**PE** **APC-eFluor 780**	**BioLegend** **eBioscience**
CD135 (Flt3)	**Mouse**	**A2F10**	**PE**	**BioLegend**
CD150	**Mouse**	**TC15-12F12.2**	**PE-Cyanine7**	**BioLegend**
CD161 (NK1.1)	**Mouse**	**PK136**	**PE** **APC** **PE-Cyanine7** **Purified – InVivoPlus**	**BioLegend** **BioLegend** **BioLegend** **BioXcell**
CD172 (SIRPα)	**Mouse**	**P84**	**APC Fire 750**	**BioLegend**
CD184 (CXCR4)	**Mouse**	**L276F12**	**Alexa Fluor 647**	**BioLegend**
CD274 (PD-L1)	**Mouse**	**MIH1**	**APC-eFluor 780**	**eBioscience**
CD336 (NKp46)	**Mouse**	**29A1.4**	**PE** **PerCP-Cyanine5.5**	**BioLegend** **BioLegend**
CFSE	**N/A**	**N/A**	**CFSE**	**BioLegend**
F4/80	**Mouse**	**BM8**	**Brilliant Violet 421**	**BioLegend**
FcεRIα	**Mouse**	**1-Mar**	**PE-Cyanine7**	**BioLegend**
FoxP3	**Mouse**	**FJK-16**	**APC**	**eBioscience**
Granzyme A	**Mouse**	**3G8.5**	**PE**	**BioLegend**
Granzyme B	**Mouse/human**	**GB11**	**Pacific Blue**	**BioLegend**
IgG2a,κ Isotype, rat	**N/A**	**2A3**	**Purified – InVivoPlus**	**BioXcell**
IgG2a,κ Isotype, mouse	**N/A**	**C1.18.4**	**Purified – InVivoPlus**	**BioXcell**
IgG2b,κ Isotype, rat	**N/A**	**LTF-2**	**Purified – InVivoPlus**	**BioXcell**
H-2D^{d}	**Mouse**	**34-2-12**	**Biotin** **Brilliant Violet 421**	**BioLegend** **BD**
H-2K^{b}/D^{b}	**Mouse**	**28-8-6**	**PE-Cyanine7**	**BioLegend**
H-2K^{b}	**Mouse**	**AF6-88.5**	**FITC** **PE** **PE-Cyanine7**	**BD** **BioLegend** **BioLegend**

H-2K^{d}	Mouse	SF1-1.1	FITC APC Brilliant Violet 421	BioLegend BioLegend BioLegend
I-A^{b}	Mouse	AF6-120.1	PE	BioLegend
IFNγ	Mouse	XMG1.2	APC	BioLegend
Lineage cocktail (CD3, Gr1, B220, TER-119, CD11b)	Mouse	17A2, RB6-8C6, RA3-6B2, TER-119, M1/70	Pacific Blue	BioLegend
Ly-6A/E (Sca1)	Mouse	D7	FITC PE Brilliant Violet 605	BioLegend BD BioLegend
Ly-6G/Ly-6C (Gr1)	Mouse	RB6-8C5	FITC APC APC-eFluor 780	BD BioLegend eBioScience
			PerCP-Cyanine5.5	BioLegend
Ly-6C	Mouse	HK1.4	Brilliant Violet 421	BioLegend
Ly-6G	Mouse	1A8	PerCP-Cyanine5.5	BioLegend
			APC-Cy7	BioLegend
Perforin	Mouse	S16009A	APC	BioLegend
Phospho-Stat1 (Y701)	Mouse	58D6	Purified	Cell Signaling
Phospho-Stat3 (Y705)	Mouse	13A3-1	Brillliant Violet 421	BioLegend
Phospho-Stat5 (Y694)	Mouse	47	PE	BD
Siglec F	Mouse	S17007L	PE	BioLegend
Streptavidin	N/A	N/A	Alexa Fluor 488 Brilliant Violet BV421	Invitrogen BD
TCRβ	Mouse	H57-597	PE-Cyanine7	BioLegend
TCRγδ	Mouse	GL3	Brilliant Violet 421	BioLegend
TER-119	Mouse	TER-119	FITC	BioLegend

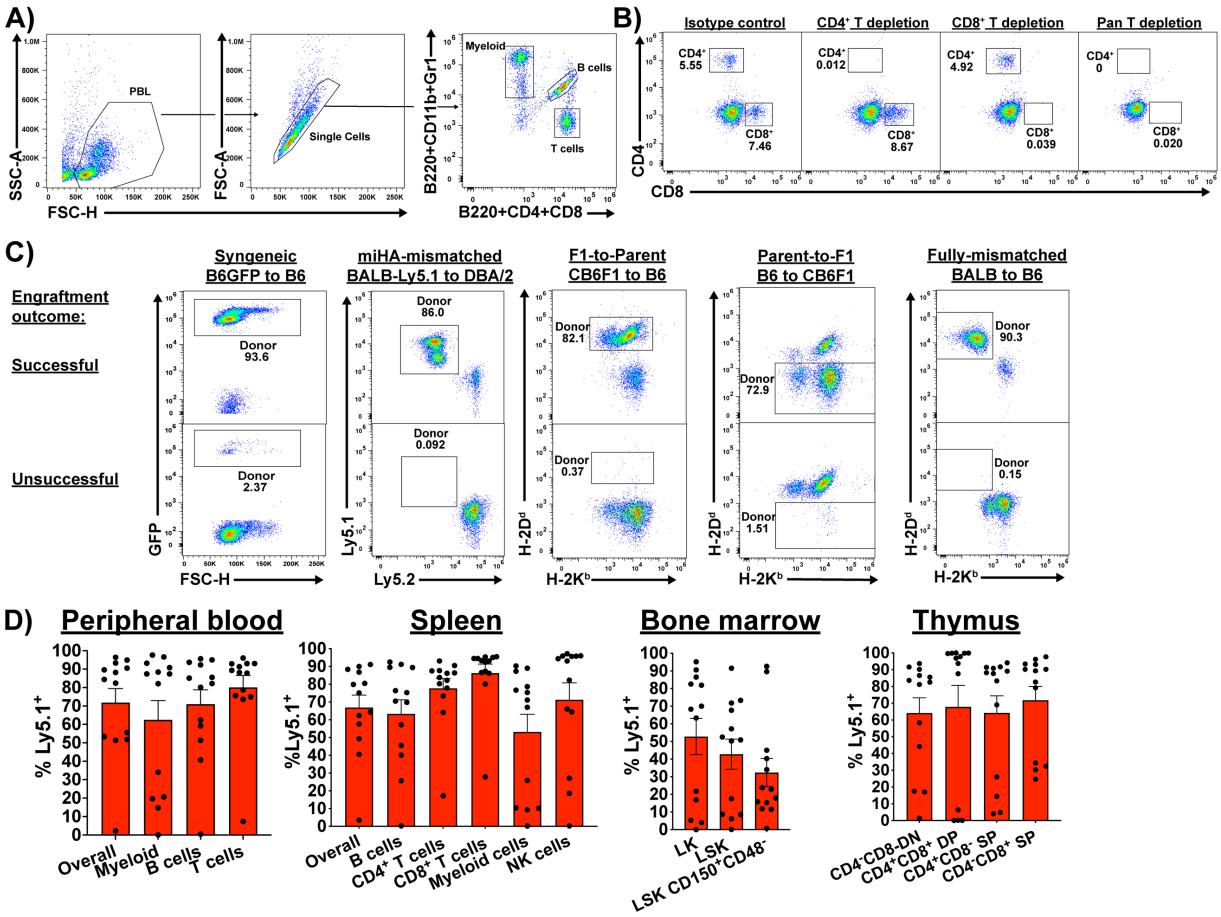
Viability stains	**N/A**	**N/A**	**7-AAD** **Zombie Aqua** **Zombie Violet**	**BioLegend** **BioLegend** **BioLegend**



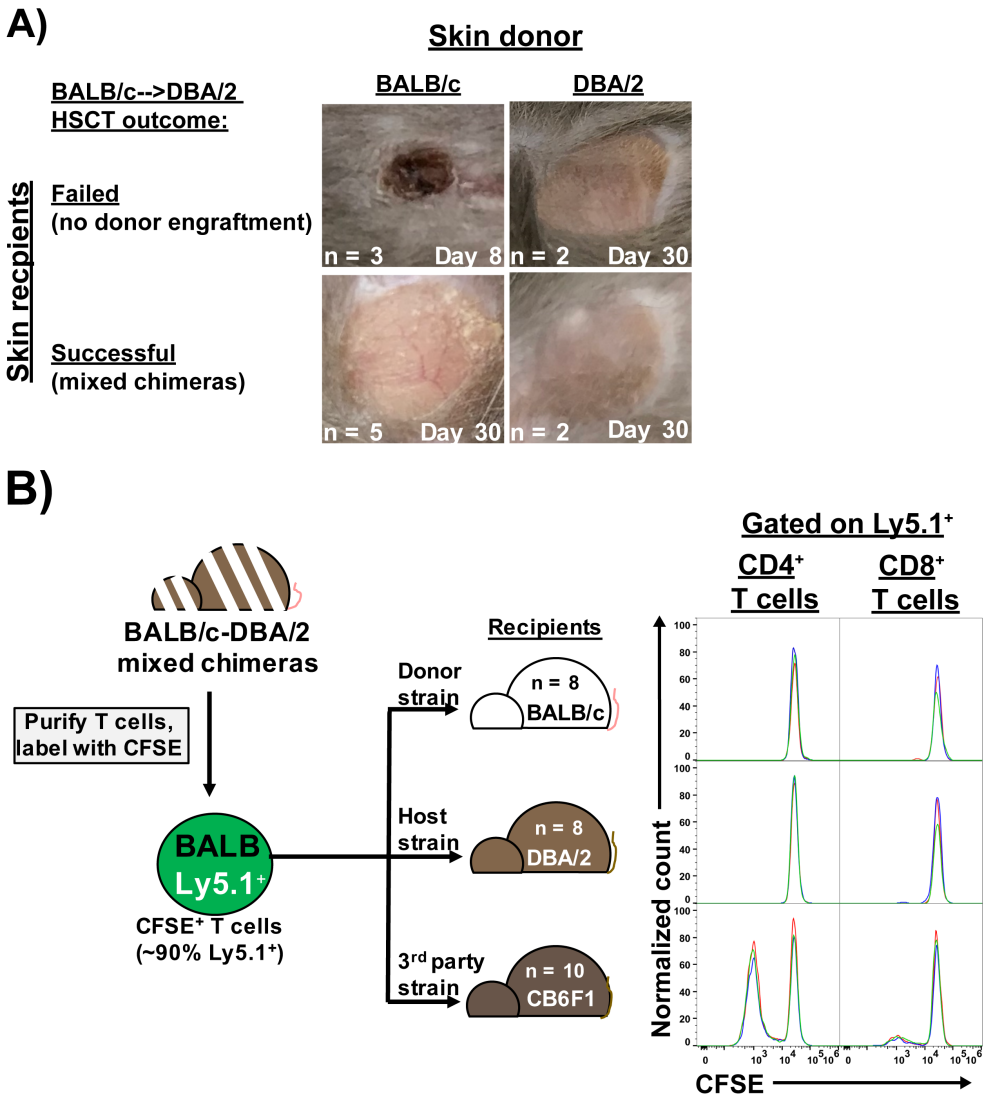
Supplemental Figure 1. Acute hematologic effects of cKit-SAP and CD45-SAP conditioning. (A and B) Absolute leukocyte counts by subset in mouse spleen (A) and CBCs (B) 7 days after administration of CD45-SAP, cKit-SAP, or control conjugates and treatments. Dotted lines in Panel B are the lower reference limits for the for the CBC assays; groups whose means were statistically below the lower reference limit are indicated. Please note that the same cohort of untreated mice was used to compare with mice in the CD45-SAP and cKit-SAP groups. Data are presented as mean ± SEM of the indicated numbers of mice pooled from 2-4 experiments. One-way ANOVA (Panel A – all cKit groups and CD45-SAP - Myeloid group) and Student's *t* test (all other groups) were used for statistical comparisons; ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, and **** = $p<0.0001$.



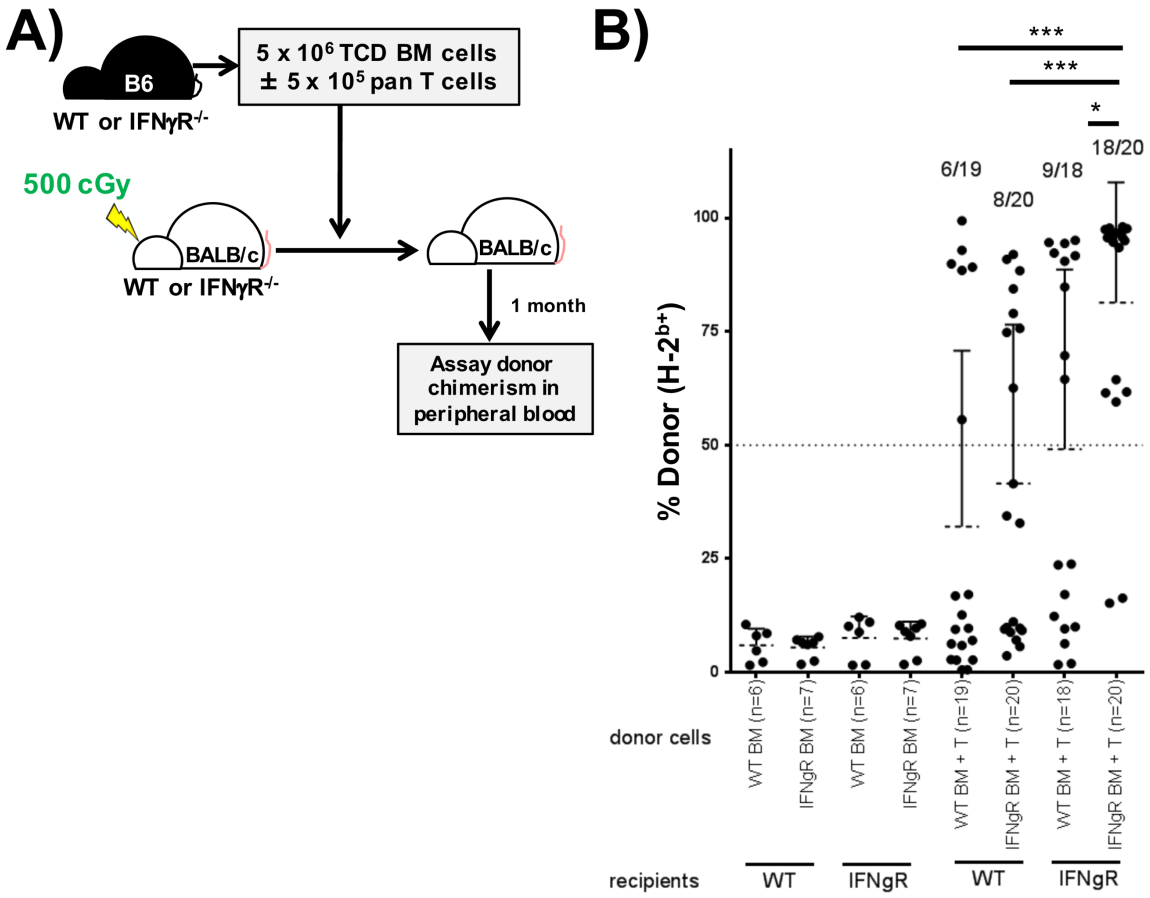
Supplemental Figure 2. Donor engraftment in lymphoid organs of primary and secondary syngeneic HSCT recipients. **(A)** Donor chimerism by cell subset in bone marrow, spleen and thymus 6 months after primary syngeneic (B6-GFP→B6) HSCT; these data correspond to the peripheral blood donor chimerism shown in Figure 1C, and include mice pooled from 2-3 experiments. For thymus, DN = double negative. DP = double positive, SP = single positive. **(B)** Donor chimerism in bone marrow, spleen, and thymus 4 months after secondary transplantation of bone marrow obtained from B6-GFP→B6 primary recipients to a new cohort of lethally-irradiated B6 mice. These data correspond to the peripheral blood donor chimerism shown in Figure 1E, and include mice pooled from 2 experiments. The percentages of GFP^+ HSCs (donor-derived HSCs from primary transplant recipients) that were infused into the secondary recipients is provided. Data points and error bars represent mean ± SEM.



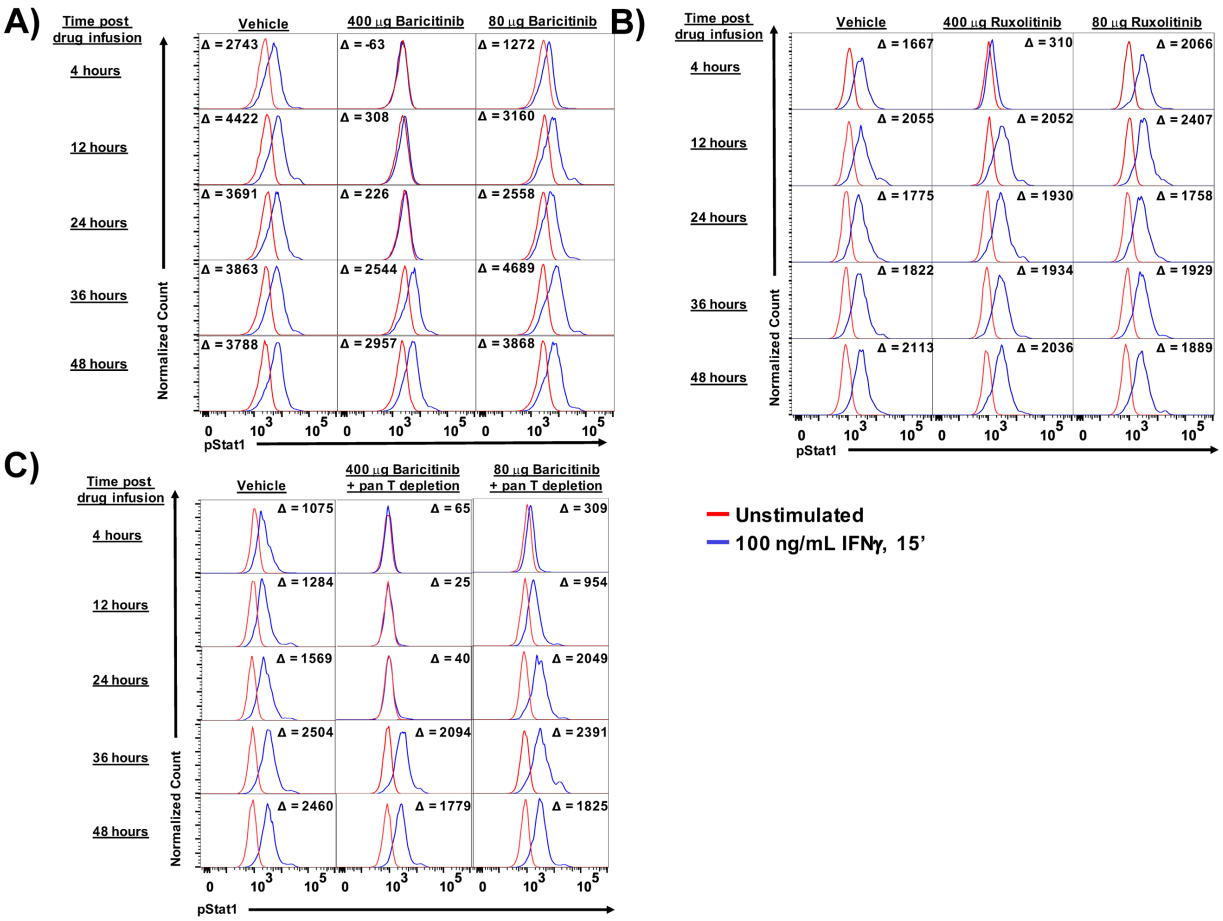
Supplemental Figure 3. Donor engraftment in lymphoid organs of CD45-SAP conditioned, miHA-mismatched transplant recipients. (A) Gating strategy for identifying B, T, and myeloid lineage cells in donor chimerism analysis. (**B**) Confirmation of pre-transplant T cell depletion in peripheral blood of mice receiving 250 μg αCD4, αCD8, or both depleting antibodies per day for three days. (**C**) Sample FACS plots illustrating identification of donor and recipient derived peripheral blood cells for all HSCT models used in this study. (**D**) 10^7 whole bone marrow cells isolated from BALB/c-Ly5.1→DBA/2 mixed chimeras were infused to a new cohort of lethally-irradiated DBA/2 mice. The percentage of $Ly5.1^+$ HSCs (donor-derived HSCs from primary recipients) that were infused into secondary recipients was ~70% (76% and 64.5% for two independent serial transfer experiments). Donor chimerism in peripheral blood, bone marrow, spleen, and thymus were assessed at 4 months post-transplant. Data in Panel D represent mean ± SEM of 13 recipient mice pooled from 2 experiments.



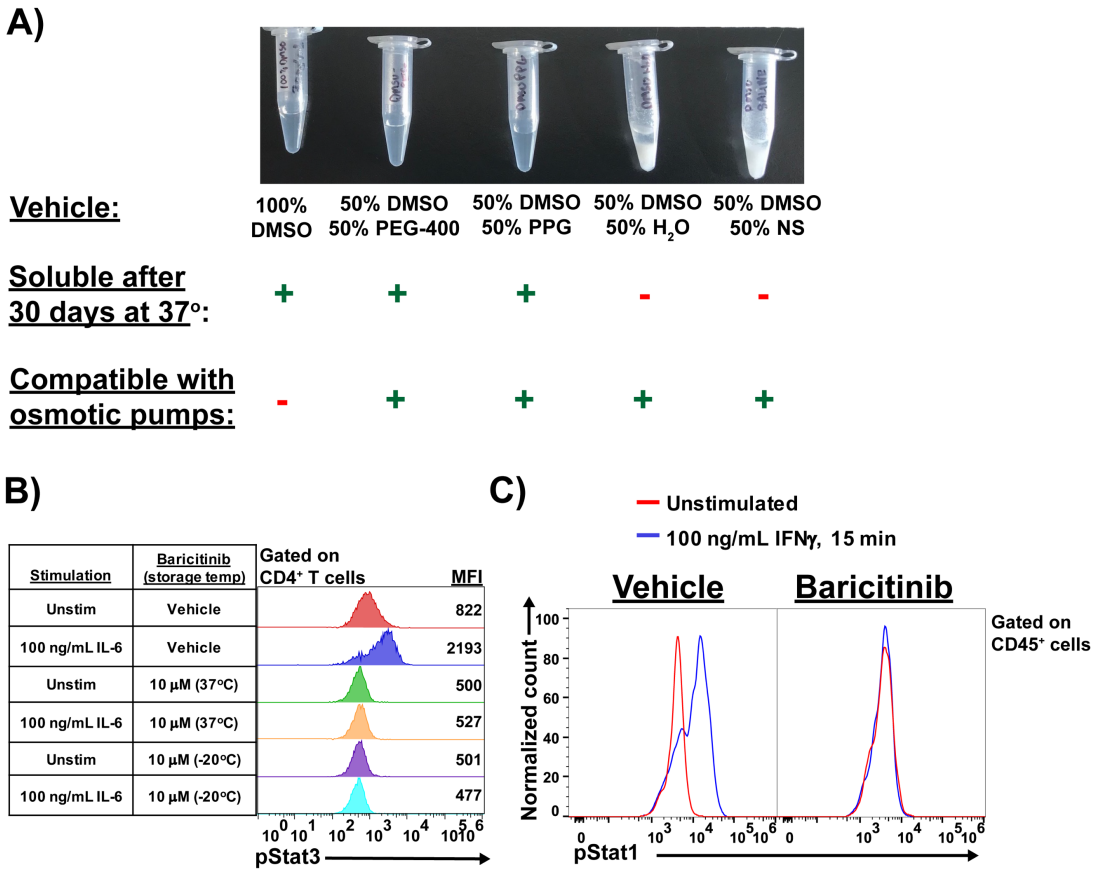
Supplemental Figure 4. BALB/c→DBA/2 mixed chimeras are cross-tolerant to donor and recipient-derived antigen. (A) BALB/c or DBA/2 ear skin was surgically engrafted at 6 months post-HSCT to CD45-SAP-conditioned, BALB/c→DBA/2 recipients that had either rejected or successfully engrafted donor HSCs. Skin grafts were monitored 30 days for signs of rejection; inset text indicates the number of skin graft recipients analyzed across two experiments (lower left) and the time post-skin graft when images were acquired (lower right). **(B)** In vivo MLRs in which CFSE labeled T cells from BALB/c-DBA/2 mixed chimeras were infused to BALB/c, DBA/2, or CB6F1 mice. The indicated numbers of recipient mice per group were analyzed across two experiments, with CFSE histograms from three representative mice per group presented.



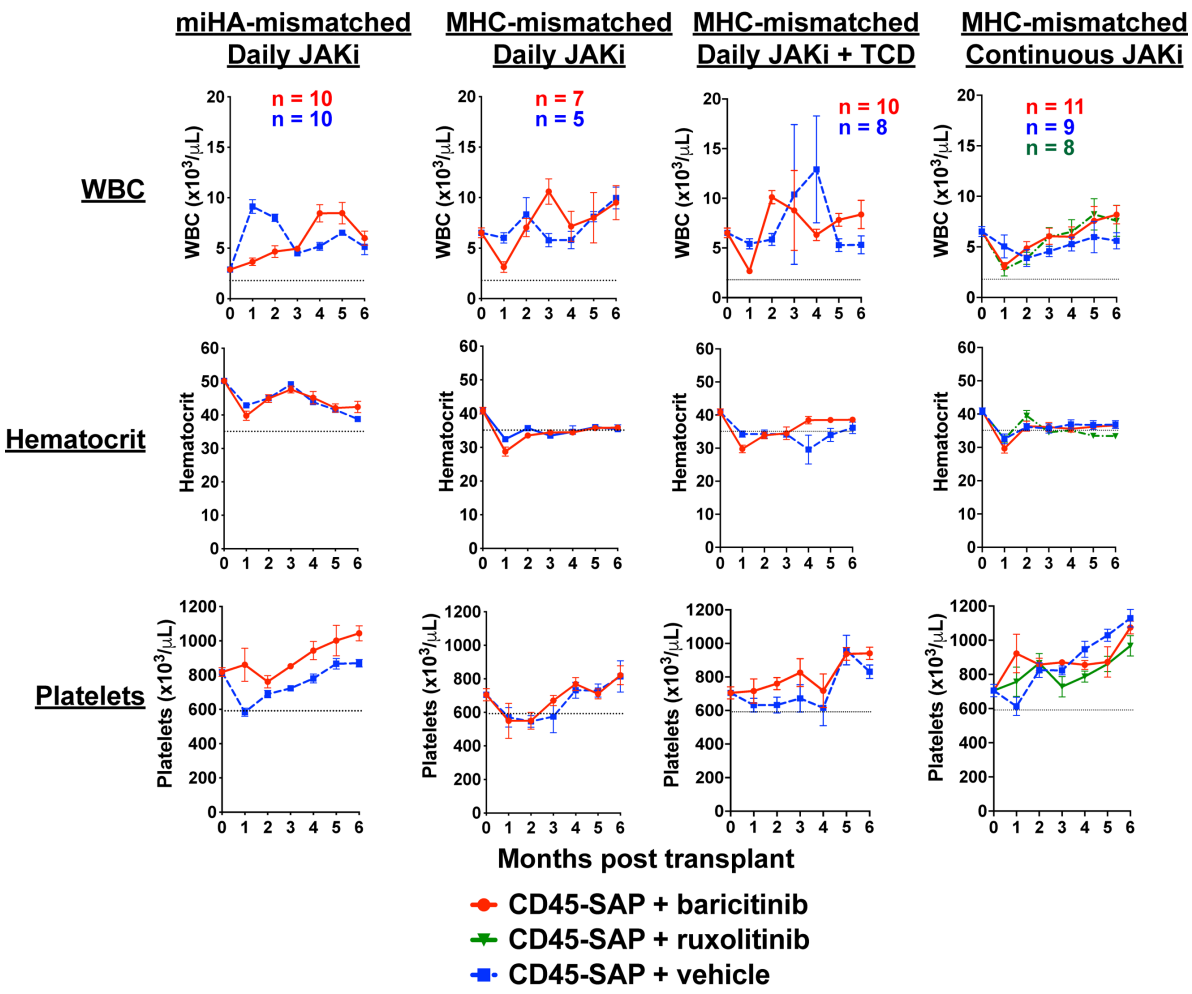
Supplemental Figure 5. Deficiency of IFNγ signaling permits engraftment of fully-mismatched HSCs in allo-HSCT with reduced intensity conditioning. (A) Schema for a RIC allo-HSCT model in which WT or IFNγR$^{-/-}$ BALB/c recipients were sublethally irradiated then transplanted with bone marrow with or without T cells from WT or IFNγR$^{-/-}$ B6 mice. **(B)** Peripheral blood chimerism 1 month post-HSCT. Results are pooled from three independent experiments; the frequency of mice in each group with greater than 50% donor chimerism is indicated above each dataset. One-way ANOVA was used for statistical comparisons, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, and **** = $p<0.0001$



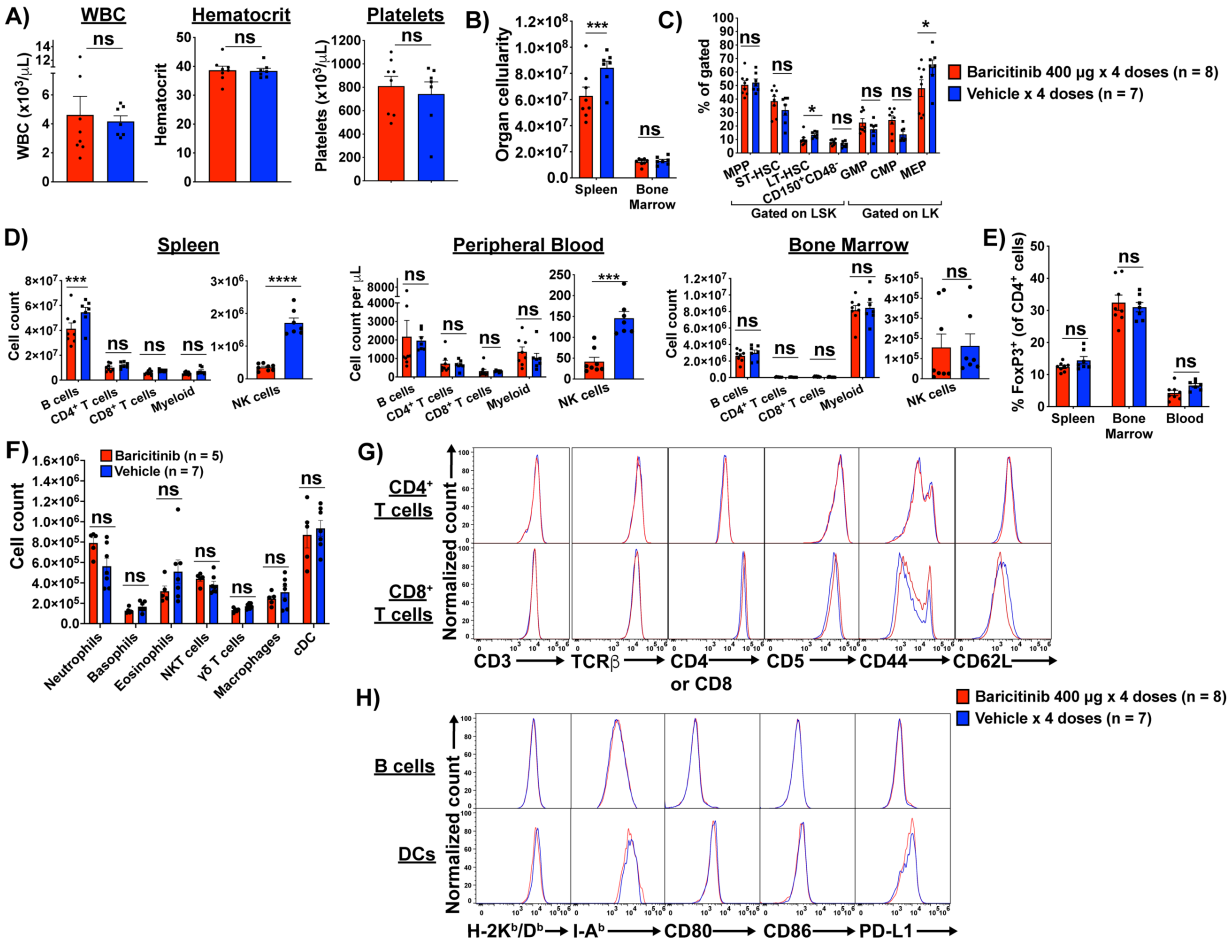
Supplemental Figure 6. Pharmacodynamics of subcutaneously-administered baricitinib and ruxolitinib. (A and B) B6 mice received a single subcutaneous injection of baricitinib (A) or ruxolitinib (B) at 400 µg or 80 µg per mouse, and Stat1 phosphorylation of whole blood leukocytes ($CD45^+$ gated) in response to IFNγ stimulation was assayed at the indicated times post-drug infusion. **(C)** Mice received 250 µg of both αCD4 and αCD8 depleting antibodies per day for three days prior to baricitinib injection, then analyzed as in Panel A. For all panels, data from a single mouse in each treatment group are displayed and are representative of 2 (vehicle groups) or 4 (baricitinib and ruxolitinib groups) mice analyzed over two experiments. Inset numbers are the MFI differences between IFNγ-stimulated and unstimulated samples.



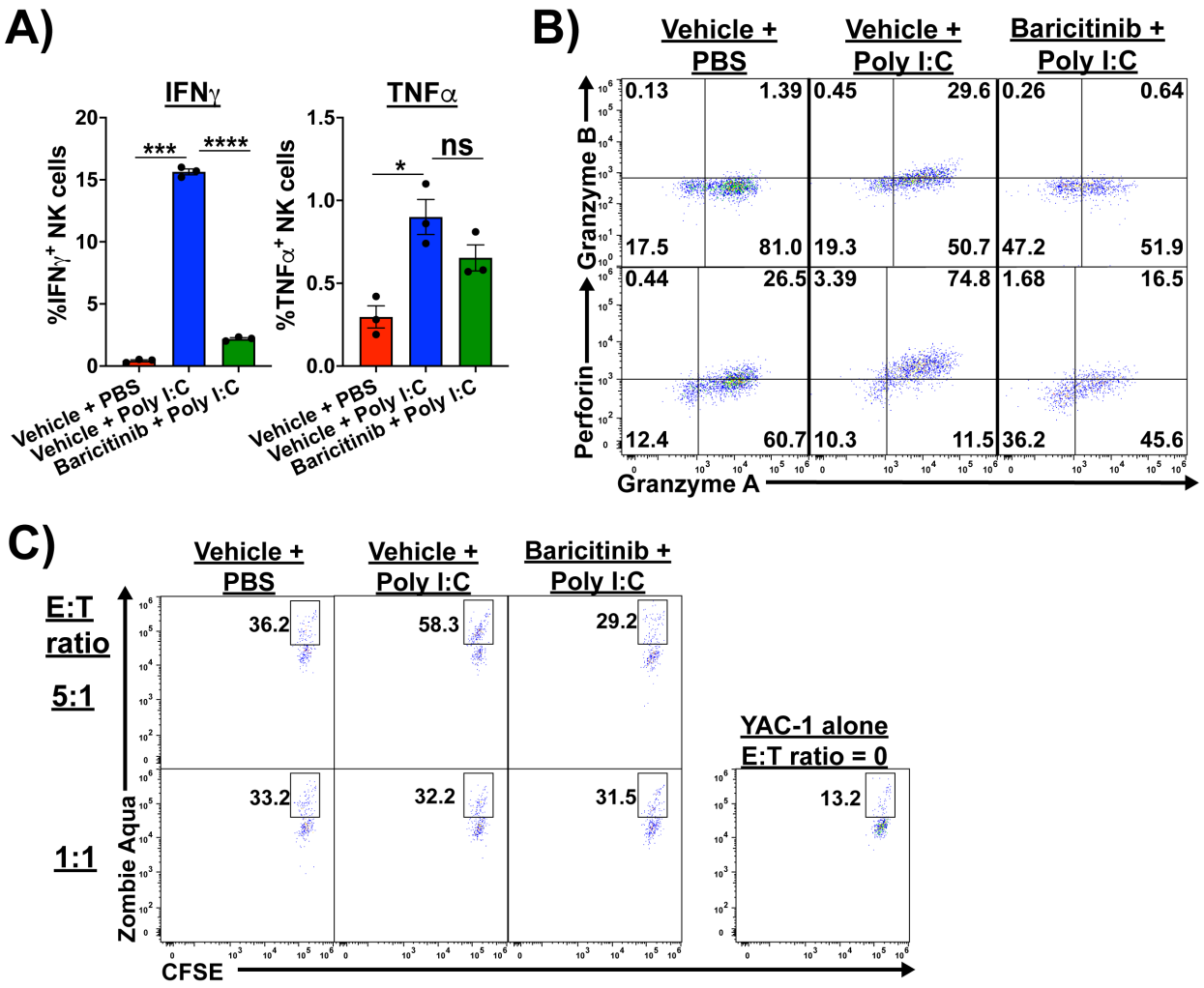
Supplemental Figure 7. Baricitinib is compatible with in vivo drug delivery via osmotic pump. (A) Solubility of baricitinib (70 mg/mL) in various solvents after 30 days incubation at 37°C. Vehicle compatibility with osmotic pumps is as per the manufacturer. PEG = polyethylene glycol; PPG = polypropylene glycol; NS = normal saline (0.9% NaCl). **(B)** Baricitinib in 50% DMSO/50% PEG-400 that had been incubated at 37°C for 30 days was then tested for inhibitory activity against IL-6-induced Stat3 phosphorylation in human peripheral blood $CD4^+$ T cells. **(C)** B6 mice implanted with baricitinib- or vehicle-loaded osmotic pumps were assayed immediately prior to HSCT (four days post-pump implantation) for IFNγ-induced Stat1 phosphorylation using a whole blood assay.



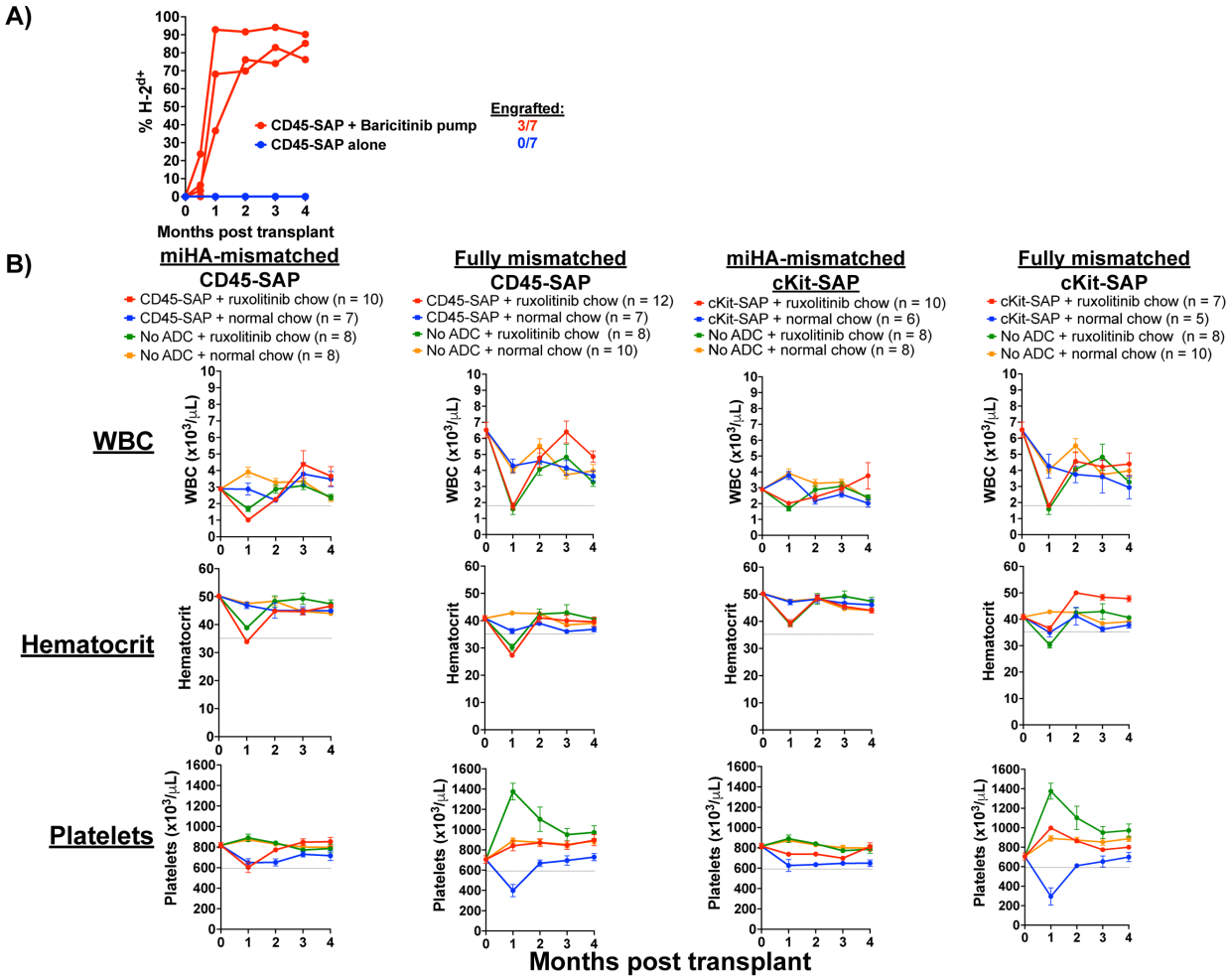
Supplemental Figure 8. Complete blood counts in miHA-mismatched and MHC-mismatched (F1-to-parent) HSCT models conditioned with CD45-SAP and JAK1/2 inhibitors. WBC, hematocrit and platelet counts for miHA- and MHC- mismatched allo-HSCT models dosed daily with baricitinib (left two columns), MHC-mismatched allo-HSCT receiving pre-transplant $CD4^+$ and $CD8^+$ T cell depletion (TCD) plus daily baricitinib (third column), and MHC-mismatched allo-HSCT receiving continuously infused baricitinib or ruxolitinib via osmotic pump (right column). Dotted lines indicate the lower reference limits for the CBC assays. Insets indicate the numbers of mice analyzed per group, which were pooled from 2-3 experiments. Data points and error bars represent mean ± SEM.



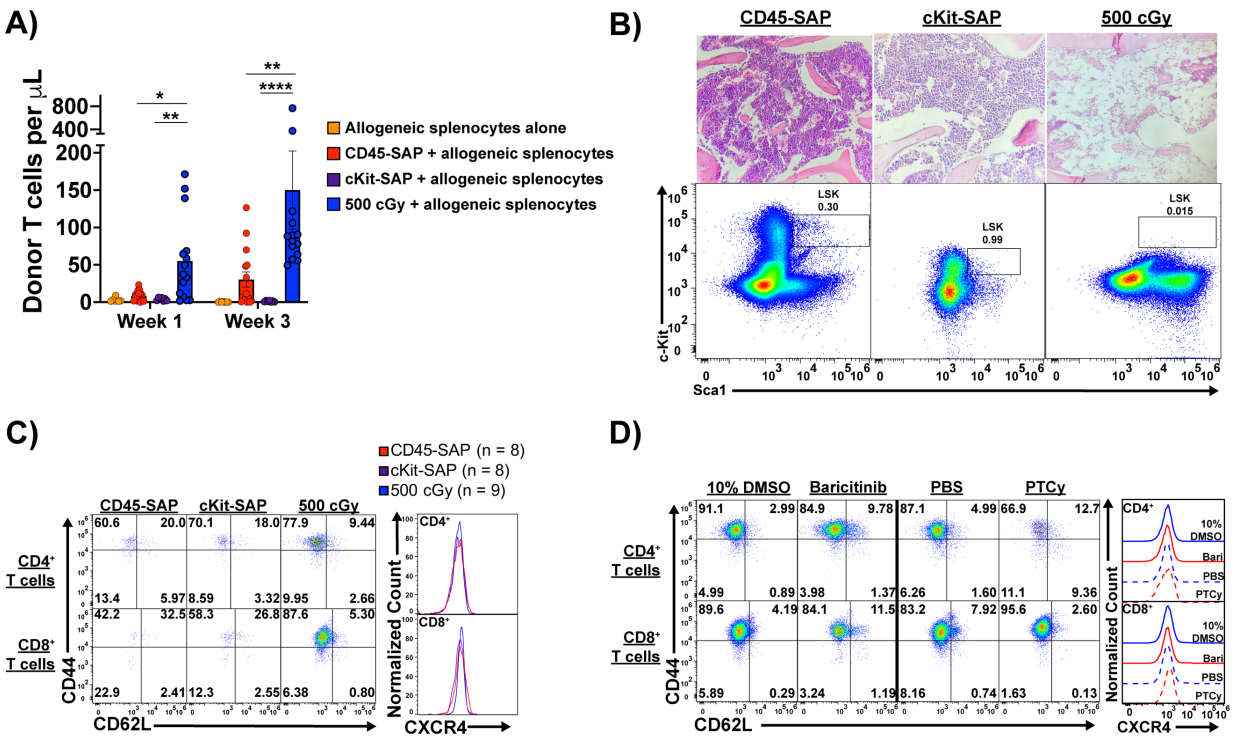
Supplemental Figure 9. Acute effects of baricitinib on the pre-HSCT recipient. B6 mice were treated daily with baricitinib or vehicle for 4 days prior to analysis of blood and lymphoid organs. The color scheme in the legend (upper-right) is used throughout the figure. **(A and B)** CBCs (A) and organ cellularity (B). **(C)** Proportions of HSPC subsets in bone marrow. **(D)** Absolute B, T, myeloid ($Gr1^+$ and/or $CD11b^+$), and NK cell counts in spleen, blood, and bone marrow. **(E)** Frequencies of $FoxP3^+$ Tregs (of total $CD4^+$ T cells) in spleen, blood and bone marrow. **(F)** Absolute counts of splenic neutrophils ($Ly6G^{hi}Siglec\ F^-$), eosinophils ($Ly6G^{lo}Siglec\ F^+$), basophils ($DX5^+Fc\varepsilon RI\alpha^+$), NKT cells ($CD3^+NK1.1^+$), γδ T cells ($CD3^+TCR\gamma\delta^+$), macrophages ($CD11b^+F4/80^+$), and conventional dendritic cells (cDC; $B220^-CD11c^+MHCII^{hi}$) **(G and H)** Splenic T cell (G) and APC (H) phenotyping. Data points and error bars represent mean ± SEM, with the indicated numbers of mice pooled across 3 experiments. The Mann-Whitney *U* test (Panel A – WBC and Platelets, Panel C – LT-HSC group, Panel D – Peripheral Blood group) or Student's *t* test (all other groups) were used for statistical comparisons: ns = not significant, * is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$, and **** is $p < 0.0001$.



Supplemental Figure 10. In vivo baricitinib treatment inhibits NK cell cytokine production and cytotoxicity. (**A-C**) Functional assays of NK cells stimulated in vivo with Poly I:C (or receiving PBS as a control) after four doses of baricitinib or vehicle: intracellular cytokine staining after a 4-hour stimulation (A; three technical replicates per group) and cytolytic enzyme expression plus cytotoxicity assay after a 24-hour stimulation (B and C). For Panel A, data from one representative mouse of 2 (PBS group) or 5 (Poly I:C groups) mice from three experiments are presented. For Panels B and C, data from one representative mouse of 3 (PBS group) or 6 (Poly I:C groups) mice from three experiments are presented. Data points and error bars represent mean ± SEM. One-way ANOVA was used for statistical comparisons; ns = not significant, * is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$, and **** is $p < 0.0001$.



Supplemental Figure 11. Donor chimerism in fully MHC-mismatched HSCT with baricitinib pumps and CBCs after conditioning with ADCs plus ruxolitinib chow. (A) B6 recipient mice were conditioned with CD45-SAP plus baricitinib infused continuously via osmotic pumps (drug dosing as per Figure 3E), then transplanted with $20x10^6$ BALB/c bone marrow cells. Each line represents one transplant recipient. **(B)** Serial CBCs for allo-HSCT experiments described in Figure 6. Dotted lines indicate the lower reference limits for the CBC assays, and data points and error bars represent mean ± SEM. For both panels, legends indicate the numbers of mice analyzed per group, which were pooled from 2-3 experiments.



Supplemental Figure 12. Circulating T cell counts, bone marrow analysis, and splenic donor T cell phenotyping in graft-versus-host alloreactivity model. (**A**) Circulating donor T cells at Weeks 1 and 3 post-splenocyte infusion in CB6F1 recipients receiving TBI- versus ADC-based conditioning. **(B)** Histology (upper panels; 10X objective) and flow cytometry (lower panels; gated on viable Lin$^-$ cells) of bone marrow from CD45-SAP or cKit-SAP conditioned mice 8 weeks after allogeneic splenocyte infusion, compared with that of an irradiated mouse that succumbed at 3 weeks post-splenocyte infusion. **(C and D)** CD44, CD62L, and CXCR4 expression on donor T cells 1 week post-splenocyte infusion in recipients conditioned with TBI or ADCs as per Figure 7 (C), or in sublethally irradiated recipients treated with the indicated drugs as per Figure 8 (D). The Kruskal-Wallis test was used for statistical comparisons; ns = not significant, * is $p<0.05$, ** is $p<0.01$, *** is $p<0.001$, and **** is $p<0.0001$.