Supplemental Figures

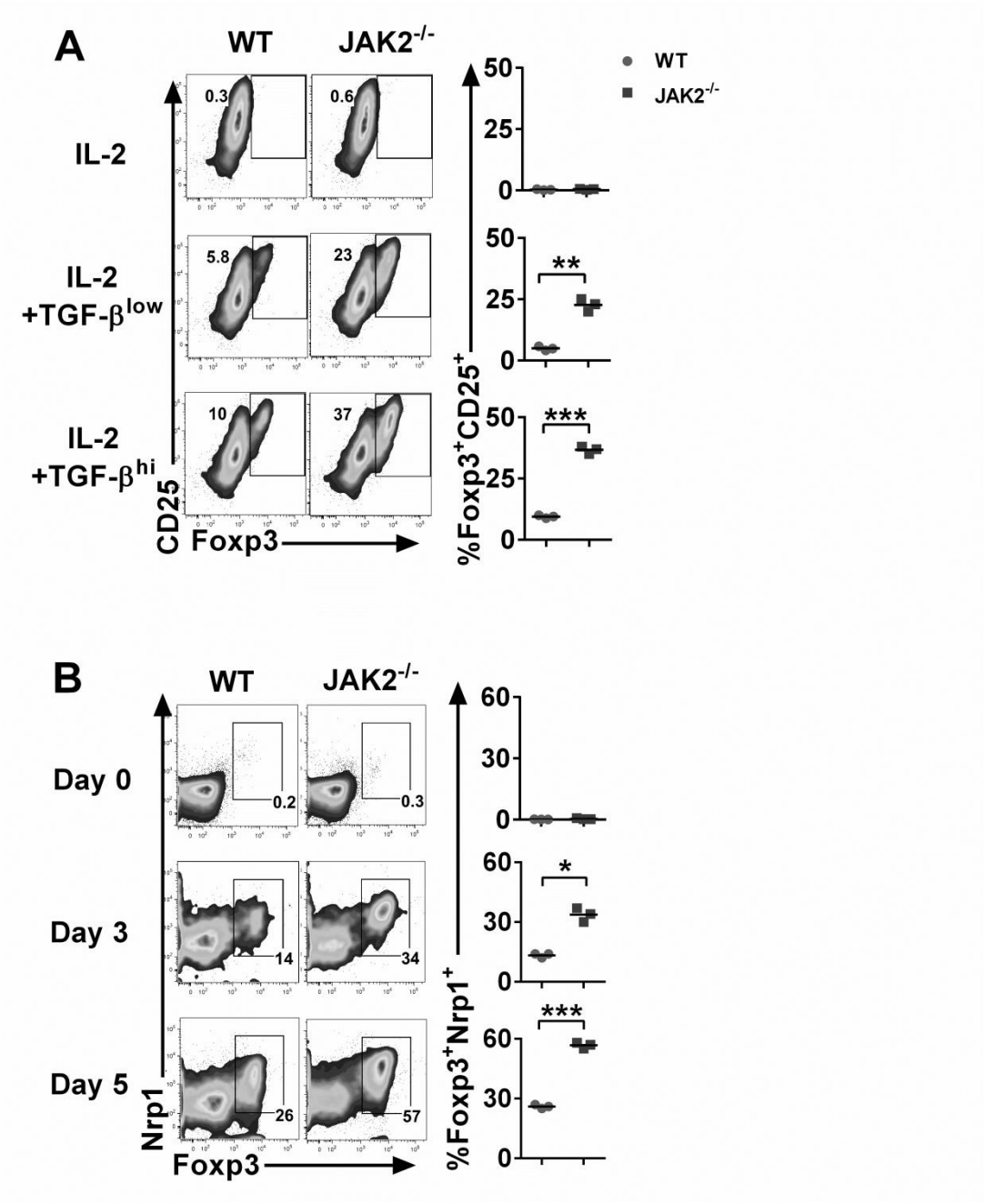

Figure S1. Generation and phenotype of CD8$^+$ iTregs.
Allogeneic CD8$^+$ iTregs were generated *in vitro* as described in figure 1. At day 5, expression of Foxp3$^+$ was analyzed by flow cytometry. **(A)** FACS plots and graphs show %Foxp3$^+$ expression among WT and JAK2$^{-/-}$ CD8$^+$ cells (n=3/group). **(B)** %Foxp3$^+$ Nrp1$^+$ on day 0, 3 and 5 of iTreg generation from WT and JAK2$^{-/-}$ CD8$^+$ cells (n=3/group). Student's *t*-test was used to compare the data. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001. Data represent the mean ± SEM.

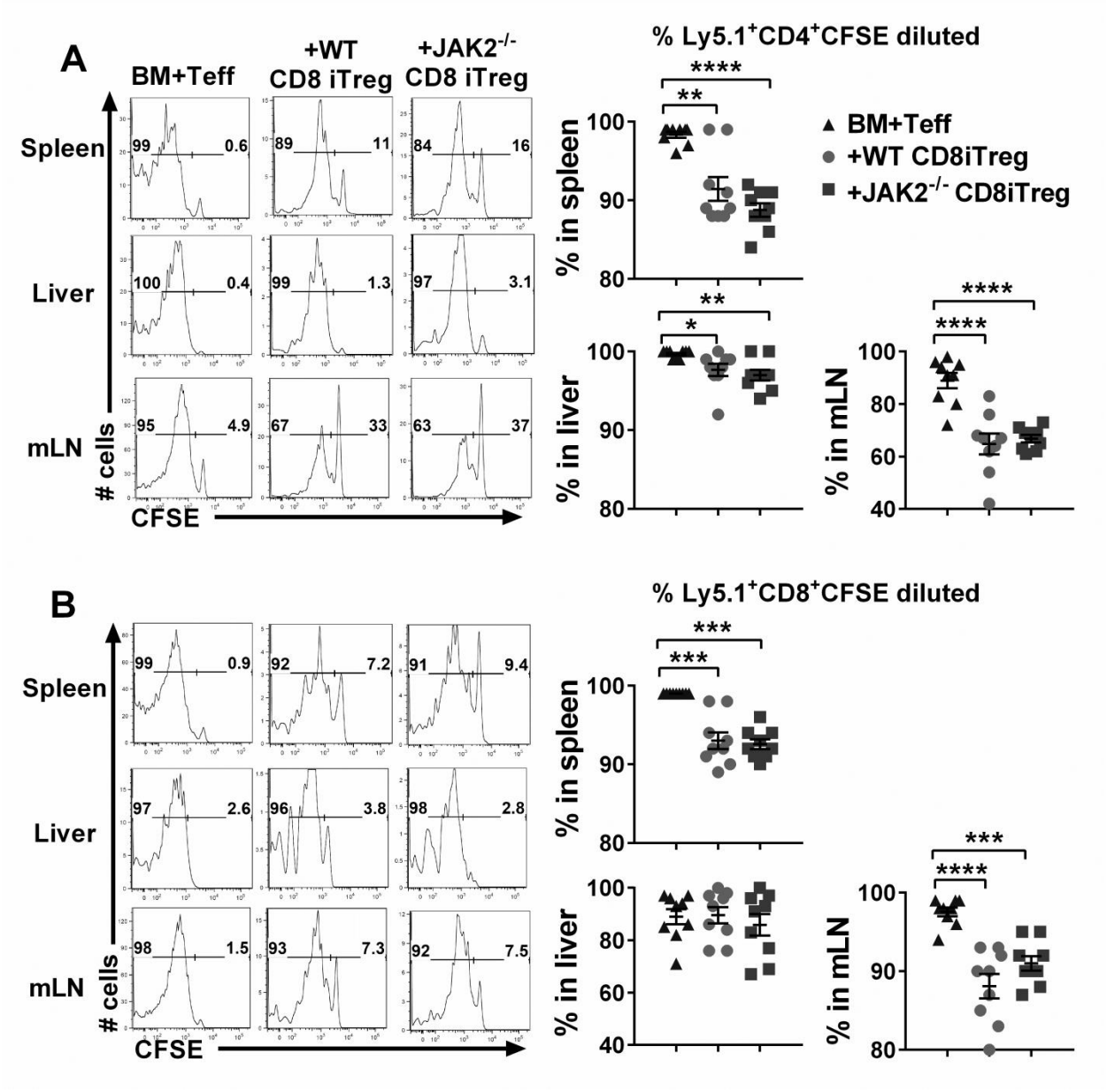

Figure S2. Suppressive activity of CD8 iTregs *in vivo*.
Lethally irradiated BALB/c mice were adoptively transferred as shown in figure 4A. Seven days after BMT, CSFE profile was shown among $H2^{b+}Ly5.1^{+}$Teff cells. Representative %CFSE-diluted cells on gated **(A)** $CD4^{+}$ and **(B)** $CD8^{+}$ Teffs are shown. Data are replicate of 2 independent experiments (n=9/group). A One- way ANOVA was used to compare the data. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ and, ****$p \leq 0.0001$. Data represent the mean ± SEM.

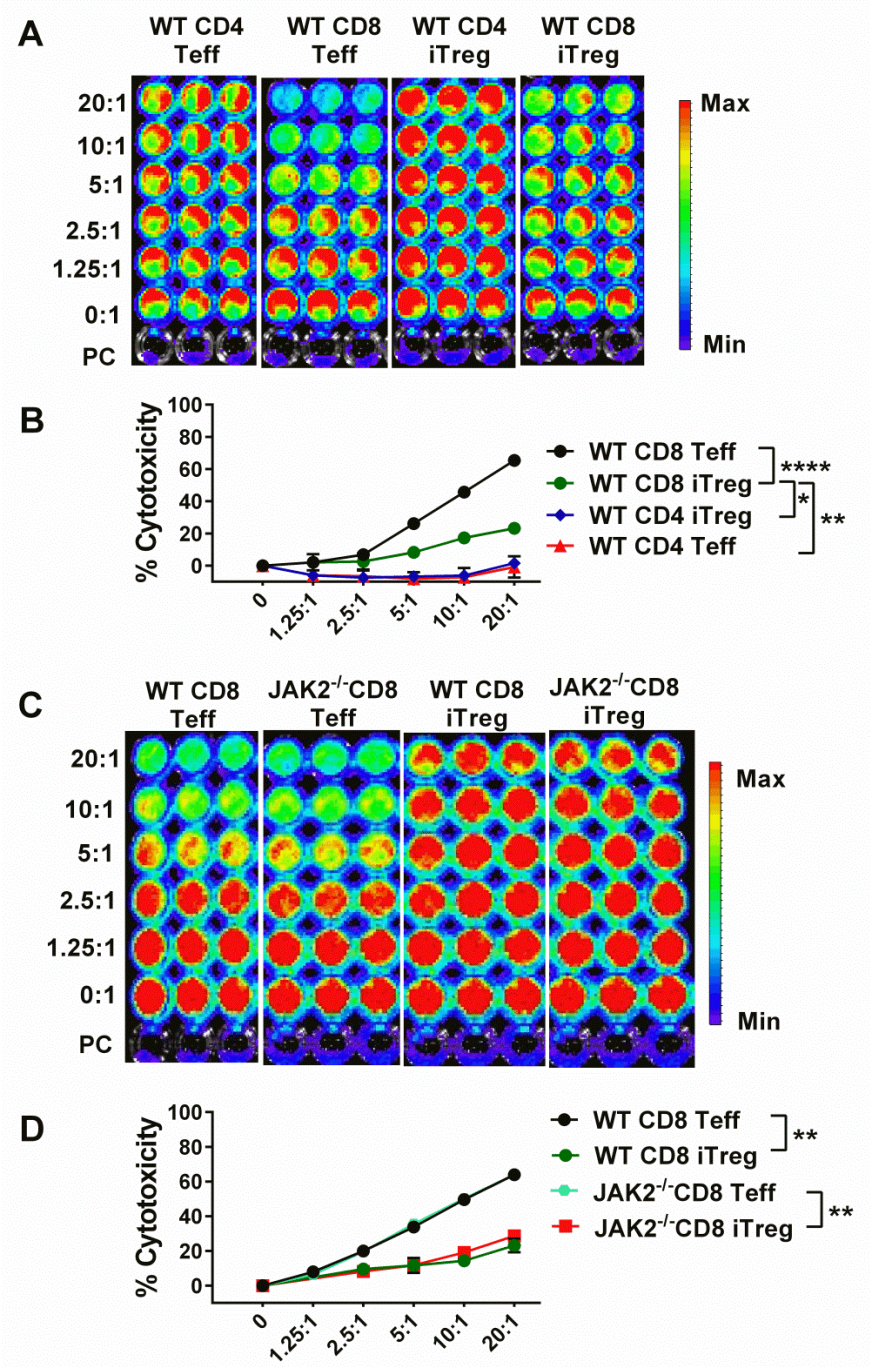

Figure S3. Cytotoxic activity of CD8$^+$ iTregs *in vitro*.
Various subsets of T cells as effectors (E) were co-cultured with luciferase-expressing allogeneic P815 mastocytoma targets (T) at 37°C for 4 hrs. After incubation, luciferin was added to the culture. Bioluminescent imaging data were analyzed and quantified using Living Imager Software. **(A and C)** Luminescence images of 96-well plates showing viable tumor cell in Red. **(B and D)** Graph depicting %cytotoxic activity in different E: T ratio is shown. Student's *t*-test was used to compare % cytotoxicity. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001. Data represent the mean ± SEM.

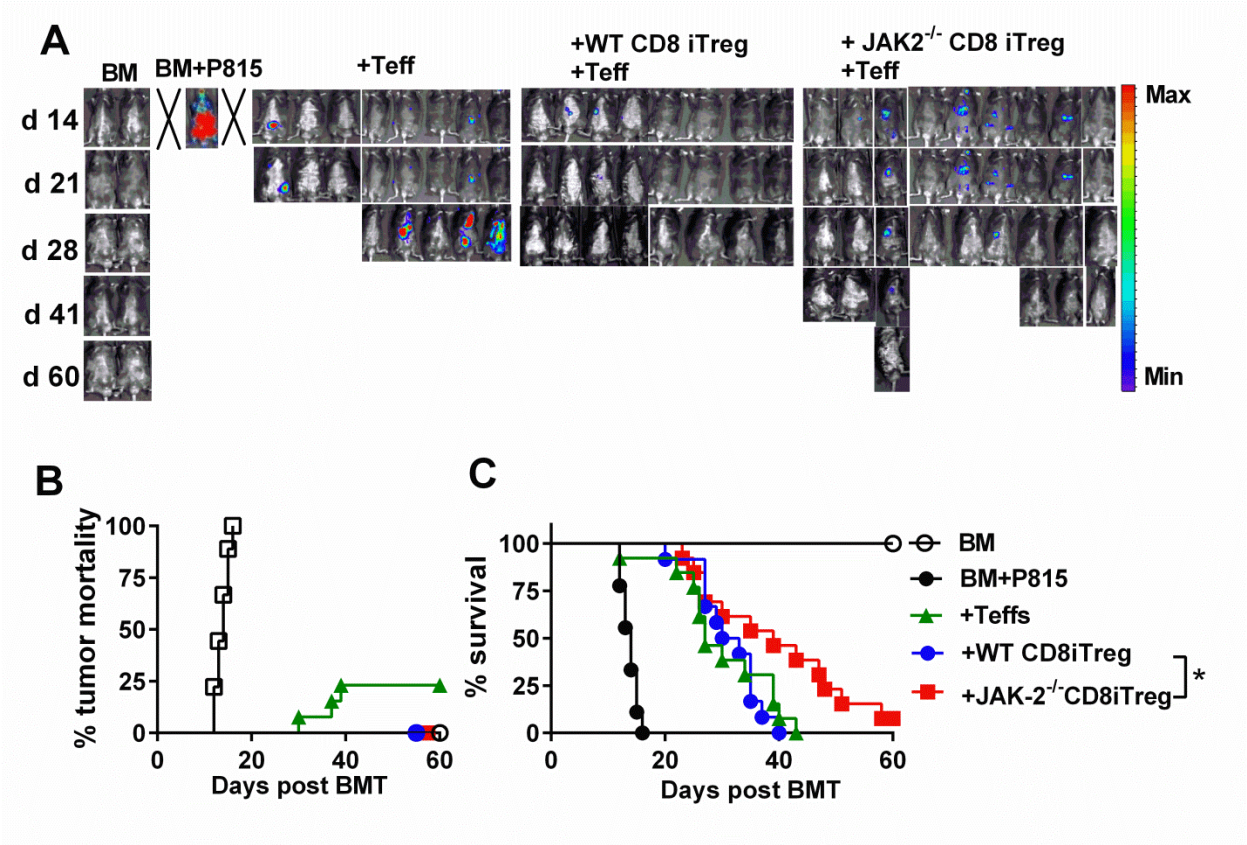

Figure S4. Effects of CD8 iTregs on GVHD and GVL responses after allo-BMT. Lethally irradiated BDF1 mice were adoptively transferred with $2x10^6$ $CD8^+$ iTregs, $5x10^6$ WT-TCD BM, and $5x10^3$ P815 mastocytoma. Three days later, $3x10^6$ CD25-depleted T-cells were i.v injected to induce GVHD. Recipients were monitored for **(A)** tumor burden, **(B)** tumor mortality and **(C)** survival until day 60. Data are combined from 2 independent experiments (n=8-9/group).*$p \leq 0.05$. Data represent the mean ± SEM. Log-rank (Mantel-Cox) test was used to compare the tumor mortality and survival.