Supplementary Material

**Supraphysiological levels of IL-2 in Jak3-deficient mice promote strong proliferative responses of adoptively transferred naive CD8+ T cells**

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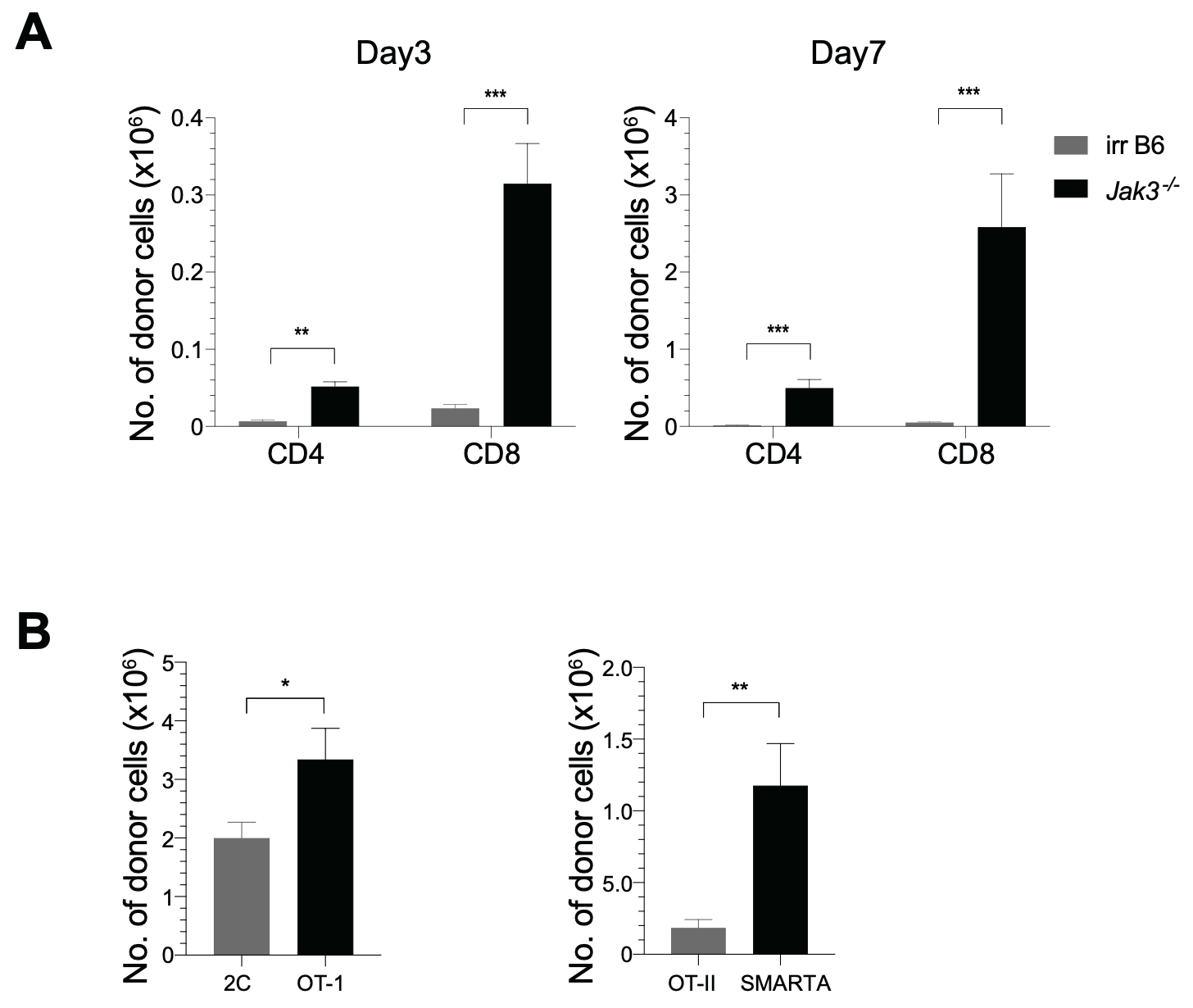
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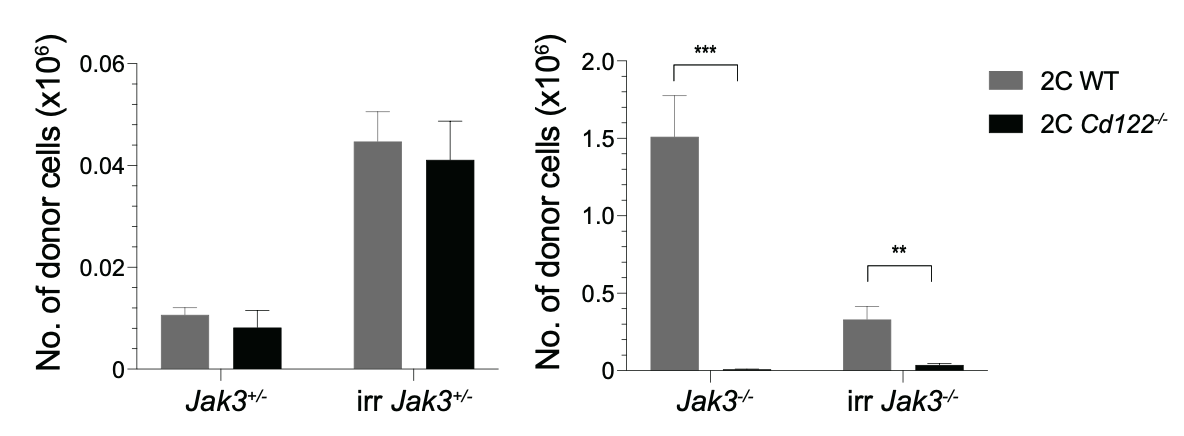
**Supplementary Figures**

**Supplementary Figure 1**

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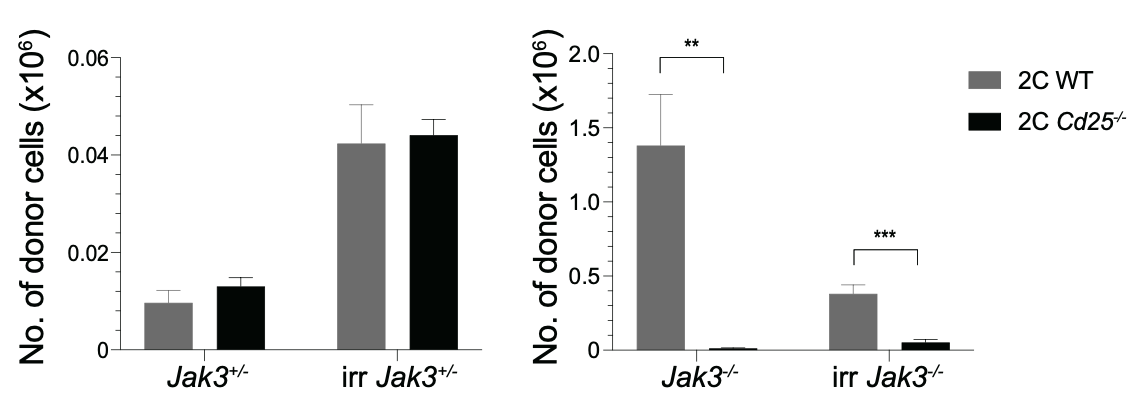
**Supplementary Figure 1**. **Robust proliferation of naive T cells adoptively transferred into *Jak3−/−* mice.** **(A)** A mixture of CFSE-labeled naive CD4+ and CD8+ T cells purified from B6 mice (Thy1.1) was co-injected i.v. into either irradiated (700 rad) B6 mice or unmanipulated *Jak3−/−* mice (1 × 106 cells for each donor per mouse; *n* = 3-5 mice). Spleen cells of the recipient mice were analyzed on days 3 (left) and 7 (right) by flow cytometry for total donor cell recovery. Data shown are the mean ± SD (*n* = 3-5 mice per group). **(B)** A mixture of FACS-purified CFSE-labeled either naive 2C (Ly5.1) and OT-I (Thy1.1) CD8+ or naive OT-II (Thy1.1) and SMARTA (Ly5.1) CD4+ T cells was co-injected i.v. into *Jak3−/−* mice (0.5-1 × 106 cells for each donor per mouse; *n* = 2-3 mice).Spleen cells of the recipient mice were analyzed on day 7 by flow cytometry for total donor cell recovery. Data shown are the mean ± SD (*n* = 2-3 mice per group). \* *P < 0.05*, \*\* *P < 0.01*, \*\*\* *P < 0.001.*

**Supplementary Figure 2**



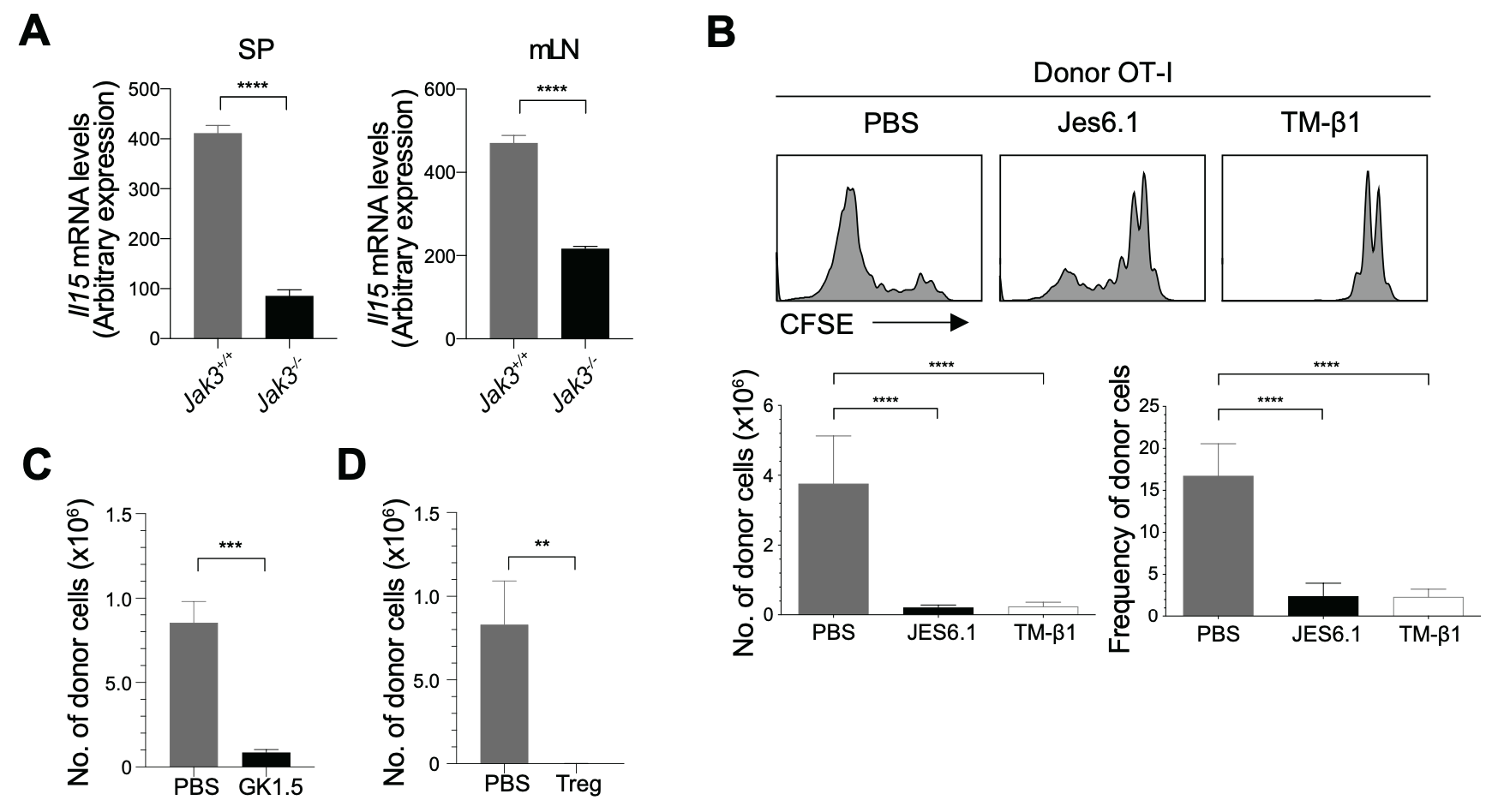
**Supplementary Figure 2**. **Role of IL-2/IL-15Rβ expression for inducing donor T cell expansion in *Jak3−/−* hosts.** A mixture of FACS-purified CFSE-labeled naive WT (Ly5.1) and CD122(IL-2/IL-15Rβ)-deficient 2C CD8+ T cells was co-injected i.v. into either irradiated (700 rad) or unmanipulated *Jak3−/−* and as a control *Jak3+/−* mice (0.5 × 106 cells for each donor per mouse; *n* = 2-3 mice). Spleen cells of the recipient mice were analyzed on day 7 by flow cytometry for total donor cell recovery. Data shown are the mean ± SD (*n* = 2-3 mice per group). \*\* *P < 0.01*, \*\*\* *P < 0.001*.

**Supplementary Figure 3**



**Supplementary Figure 3**. **Role of IL-2Rα expression for inducing donor T cell expansion in *Jak3−/−* hosts.** Schematic diagram for adoptive transfer experiments. A mixture of FACS-purified CFSE-labeled naive WT (Ly5.1) and CD25(IL-2Rα)-deficient 2C CD8+ T cells was co-injected i.v. into either irradiated (700 rad) or unmanipulated *Jak3−/−* and as a control *Jak3+/−* mice (0.5 × 106 cells for each donor per mouse; *n* = 2-3 mice). Spleen cells of the recipient mice were analyzed on day 7 by flow cytometry for total donor cell recovery. Data shown are the mean ± SD (*n* = 2-3 mice per group). \*\* *P < 0.01*, \*\*\* *P < 0.001*.

**Supplementary Figure 4**

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**Supplementary Figure 4**. **Effect of CD4+ T cells on high levels of *in vivo* IL-2 in *Jak3−/−* mice. (A)** Spleen and mesenteric lymph node (mLN) cells from *Jak3−/−* and *Jak3+/+* mice were analyzed for *Il15* mRNA by quantitative RT-PCR (the mean ± SD; *n* = 3-5 mice per group). **(B)** *Jak3−/−* mice were injected i.p. with either anti-IL-2 mAb (JES6.1) or anti-IL-2Rβ mAb (TM-β1) (on days -3 and 0; 100 μg per mouse; *n* = 2-3 mice). The mice were then injected i.v. with FACS-purified CFSE-labeled naive OT-I CD8+ T cells (Thy1.1; 1 × 106 cells per mouse; *n* = 2-3 mice). At day 7 after adoptive transfer, spleen cells of the recipient mice were analyzed by flow cytometry for CFSE dilution and donor cell recovery (the mean ± SD; *n* = 2-3 mice per group). **(C)** *Jak3−/−* mice were injected i.p. with either anti-CD4 mAb (GK1.5) or as a control isotype IgG (total 4 injections every 2 days; 100 μg per mouse; *n* = 2-4 mice). The mice were injected i.v. with FACS-purified CFSE-labeled naive OT-I CD8+ T cells (Thy1.1; 1 × 106 cells per mouse; *n* = 2-4 mice). At day 5 after adoptive transfer, spleen cells of the recipient mice were analyzed by flow cytometry for donor cell recovery (the mean ± SD; *n* = 2-4 mice per group). **(D)** FACS-purified CFSE-labeled naive OT-I CD8+ T cells (Thy1.1; 1 × 106 cells per mouse) were injected i.v. with or without CD4+ Tregs (~0.2 × 106 cells per mouse) purified from Foxp3-GFP mice into *Jak3−/−* mice (*n* = 2-4 mice). Spleen cells of the recipient mice were analyzed on day 5 by flow cytometry for donor cell recovery (the mean ± SD; *n* = 2-4 mice per group). \*\* *P<0.01*, \*\*\* *P < 0.001*,\*\*\*\* *P<0.0001.*