**Supplementary Information**

**Supplementary Figures 1-7 and Legends**

# IL-33 induces thymic involution-associated naive T cell aging and impairs host control of severe infection

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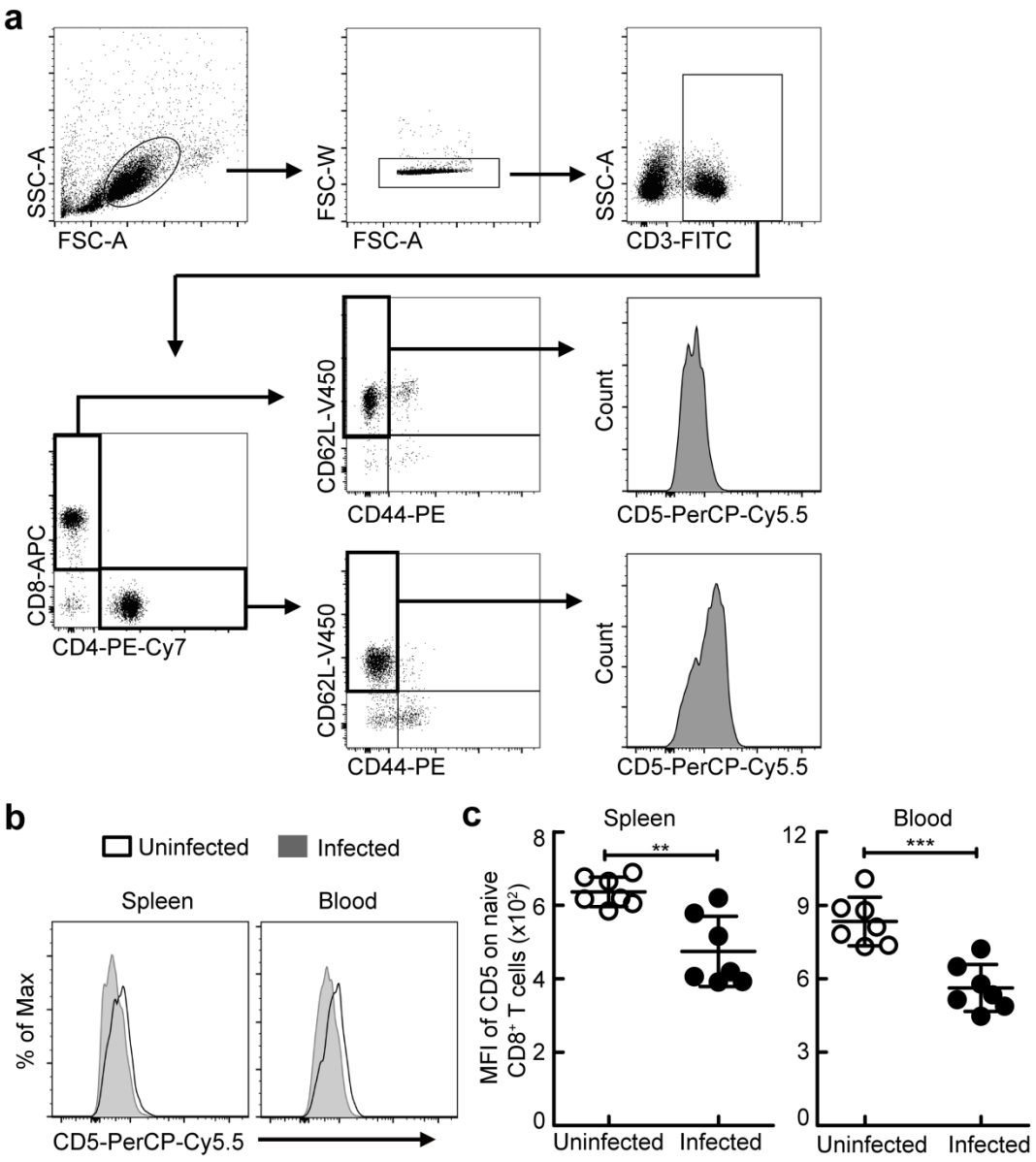


**Fig. S1. Schistosome infection decreases the expression of CD5 in naïve CD8$^+$ T cells. (a)** Gating strategy of CD5 expression on naïve CD4$^+$ T cells or naïve CD8$^+$ T cells; (**b, c**) Samples were obtained from uninfected mice or mice 8 weeks after schistosome infection. Representative and quantified flow cytometry of CD5 MFI on naive CD8$^+$ T cells from the spleen or peripheral blood (n = 7 mice, pool of two independent experiments). P = 0.0014 (Spleen), P = 0.0002 (Blood), Unpaired two-tailed Student's t-test. All data are shown as the mean ± s.d. **P < 0.01, ***P < 0.001. Source data are provided as a Source Data file.

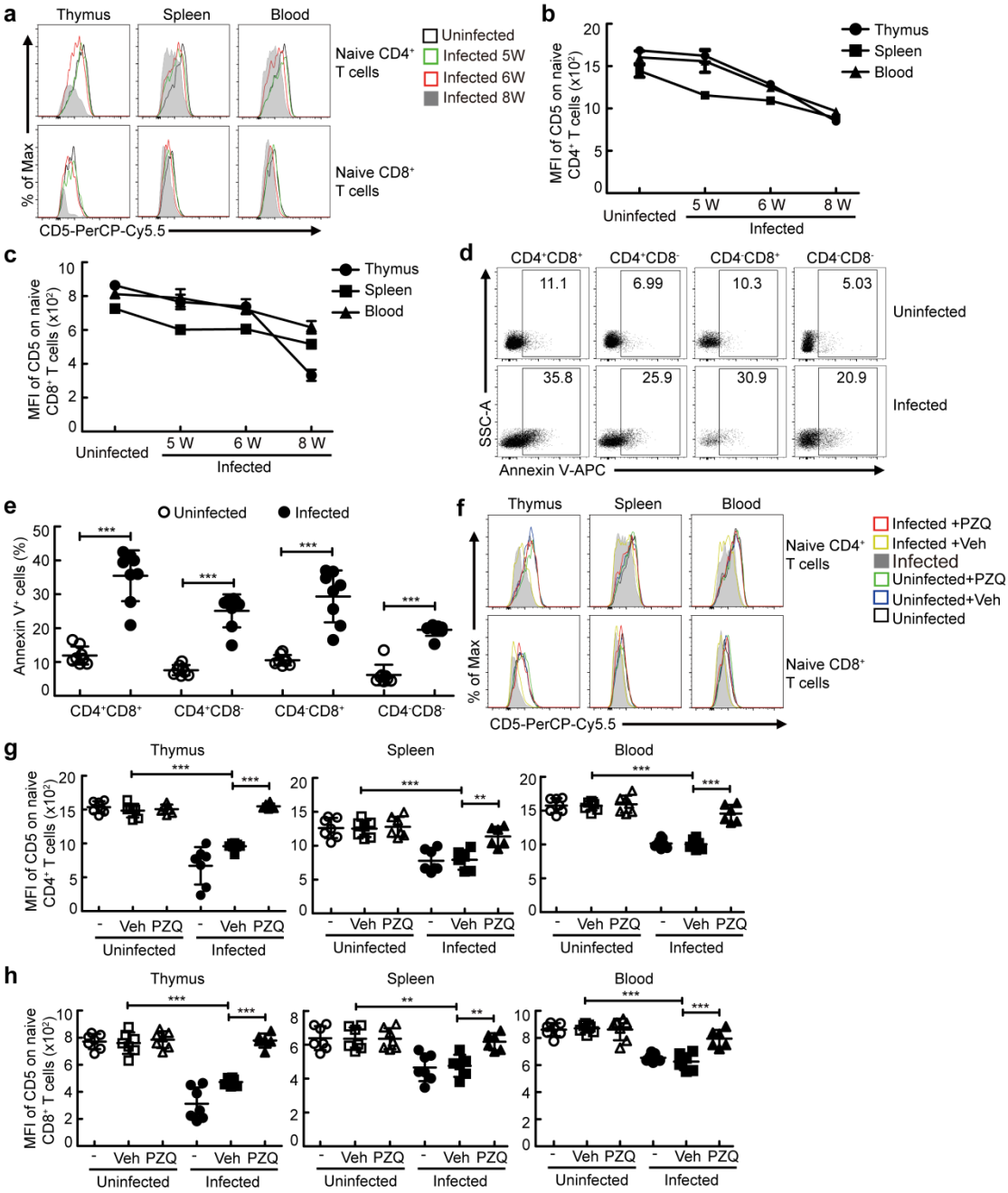


**Fig. S2. T cell aging is linked to thymic involution during schistosome infection.** (**a-c**) Cells were from uninfected mice or mice 5, 6, or 8 weeks after schistosome infection. Representative and quantified flow cytometry of CD5 MFI on naïve $CD4^+$ T cells or naïve $CD8^+$ T cells from the thymus, spleen, or peripheral blood (Infected 8 W, n = 3 mice; other groups, n = 4 mice). (**d, e**) Samples were obtained from uninfected mice or mice 8 weeks after schistosome infection. Representative and quantified flow cytometry of annexin $V^+$ cells gated on $CD4^+CD8^+$DP, CD4SP, CD8SP, or $CD4^-CD8^-$ DN thymocytes in the thymus (n = 8 mice, pool of two independent experiments). $P < 0.0001$ for all thymocyte subsets, One-way

ANOVA with Tukey's multiple comparisons. (**f-h**) Mice were treated with PZQ at week 8 after infection and sacrificed 7 weeks after PZQ treatment. Representative and quantified flow cytometry of CD5 MFI on naive $CD4^+$ T cells or naïve $CD8^+$ T cells from the thymus, spleen, or peripheral blood (Infected+Veh or Infected+PZQ, n = 6 mice; other groups, n = 7 mice; pool of two independent experiments). $CD4^+$ T cells: Infected+Veh versus Uninfected+Veh, $P < 0.0001$ (Thymus), $P < 0.0001$ (Spleen), $P < 0.0001$ (Blood); Infected+PZQ versus Infected+Veh, $P < 0.0001$ (Thymus), $P = 0.0035$ (Spleen), $P < 0.0001$ (Blood). $CD8^+$ T cells: Infected+Veh versus Uninfected+Veh, $P < 0.0001$ (Thymus), $P = 0.0017$ (Spleen), $P < 0.0001$ (Blood); Infected+PZQ versus Infected+Veh, $P < 0.0001$ (Thymus), $P = 0.0094$ (Spleen), $P < 0.0001$ (Blood). One-way ANOVA with Tukey's multiple comparisons. All data are shown as the mean ± s.d; *P < 0.05, **P < 0.01, ***P < 0.001. Source data are provided as a Source Data file.

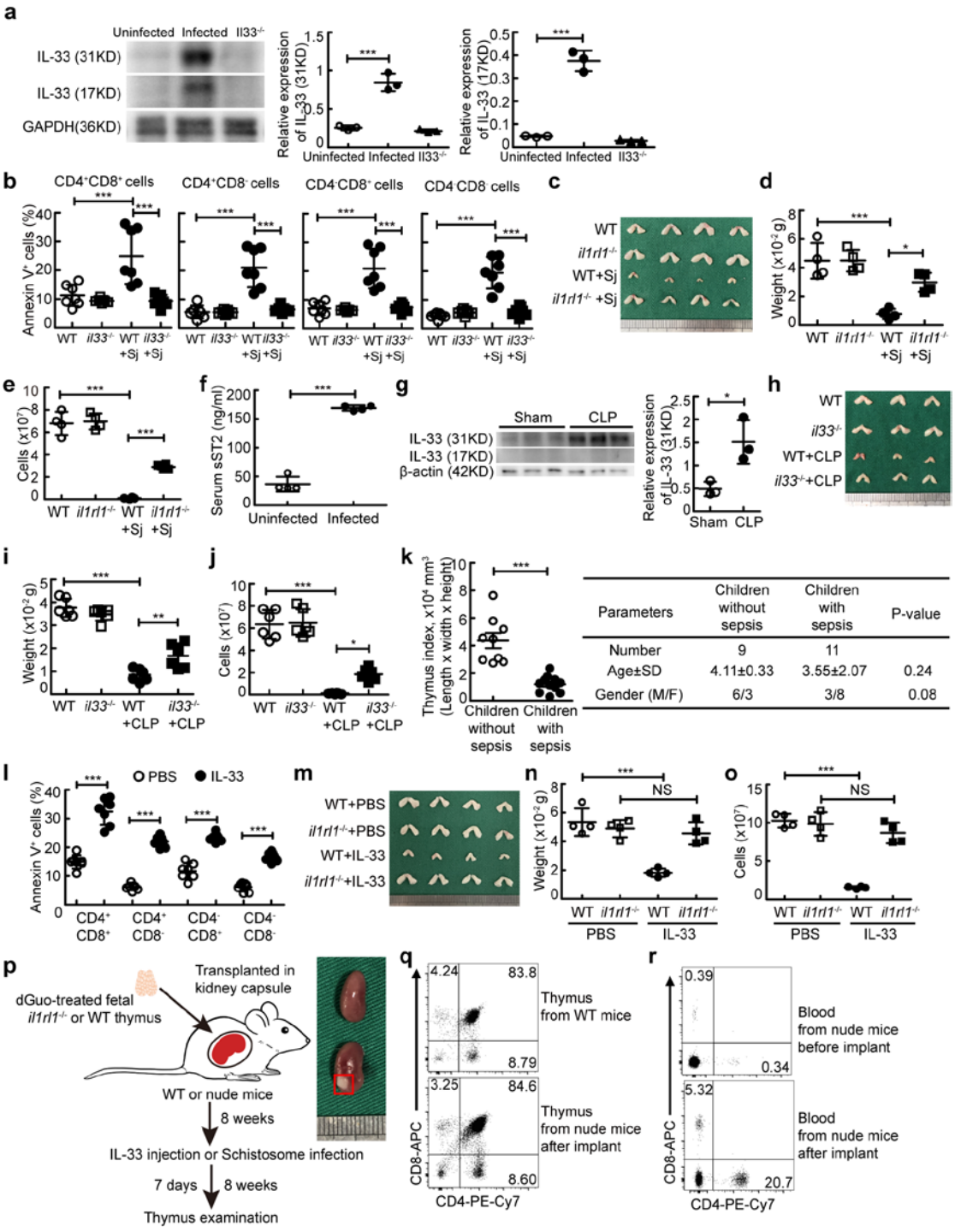


**Fig. S3. IL-33 leads to thymic involution during severe infection.** (**a**) Western blots of IL-33 in the liver after schistosome infection (n = 3 mice). P = 0.0001 (31KD), P < 0.0001 (17KD). (**b**) Annexin $V^+$ cells in thymocytes after schistosome infection (n = 7 mice, pool of two independent experiments). WT+Sj versus WT, P < 0.0001 for all subsets; *il33*$^{-/-}$+Sj versus WT+Sj, P < 0.0001 for all subsets. (**c**-**e**) Morphology, weight and cellularity of thymus from schistosome-infected mice (n = 4 mice). WT+Sj versus WT, P = 0.0002 (weight), P < 0.0001

(cells); *il33*$^{-/-}$+Sj versus WT+Sj, P = 0.0115 (weight), P = 0.0002 (cells). (**f**) The level of sST2 was determined by ELISA (n = 4 mice), P < 0.0001. (**g**) Western blots of IL-33 in the thymus after CLP induction (n = 3 mice), P = 0.0204. (**h**-**j**) Morphology, weight, and cellularity of thymus after CLP induction (n = 6 mice, pool of two independent experiments). WT+CLP versus WT, P < 0.0001 (weight or cells); *il33*$^{-/-}$+CLP versus WT+CLP, P = 0.0069 (weight), P = 0.0213 (cells). (**k**) Relative thymus size (n = 9 patients for children without sepsis, n = 11 patients for children with sepsis), P < 0.0001. (**l**) Annexin V$^{+}$ cells in thymocytes after IL-33 treatment (n = 7 mice, pool of two independent experiments). P < 0.0001 for all thymocyte subsets. (**m**-**o**) Morphology, weight, and cellularity of thymus after IL-33 treatment (n = 4 mice). WT+IL-33 versus WT+PBS, P < 0.0001 (weight or cells); *il33*$^{-/-}$+IL-33 versus *il33*$^{-/-}$+PBS, P = 0.9063 (weight), P = 0.4730 (cells). (**p**) Morphology of the thymus in the kidney capsule after thymus transplantation. (**q**) Representative flow cytometry of thymocytes after thymus transplantation. (**r**) Representative flow cytometry of T cells in peripheral blood after thymus transplantation. (**a**, **b**, **d**, **e**, **i**, **j**, **l**, **n**, **o**) One-way ANOVA with Tukey's multiple comparisons. (**f**, **g**, **k**) Unpaired two-tailed Student’s t-test. All data are shown as the mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant. Source data are provided as a Source Data file.

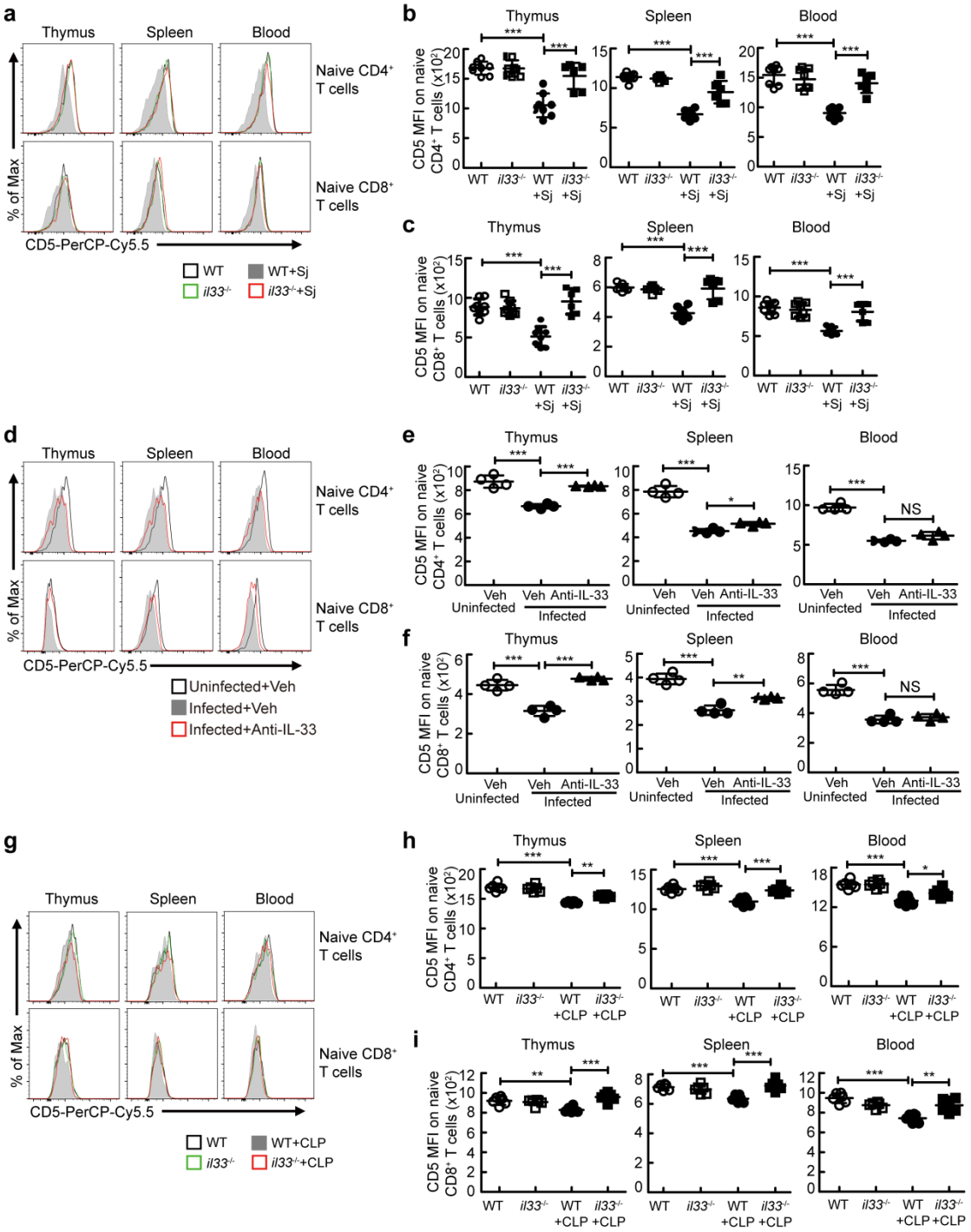


**Fig. S4. Loss of IL-33-mediated thymic involution reverses T-cell immunity during severe infection.** (**a-c**) Samples were from uninfected or schistosome-infected WT or *il33*$^{-/-}$ mice. CD5 MFI on naive CD4$^{+}$ T cells or naïve CD8$^{+}$ T cells from the thymus, spleen, or peripheral blood (*il33*$^{-/-}$+Sj, n = 6 mice, other groups, n = 8 mice, pool of two independent experiments). CD4$^{+}$ T cells: WT+Sj versus WT, $P < 0.0001$ (Thymus, Spleen, or Blood); *il33*$^{-/-}$+Sj versus WT+Sj, $P < 0.0001$ (Thymus, Spleen, or Blood). CD8$^{+}$ T cells: WT+Sj versus

WT, P < 0.0001 (Thymus, Spleen, or Blood); *il33*$^{-/-}$+Sj versus WT+Sj, P < 0.0001 (Thymus, Spleen, or Blood). One-way ANOVA with Tukey's multiple comparisons. (**d**-**f**) Samples were from uninfected or schistosome-infected mice treated with anti-IL-33. CD5 MFI on naive CD4$^{+}$ T or naïve CD8$^{+}$ T cells from the thymus, spleen, or peripheral blood (n = 4 mice). CD4$^{+}$ T cells: Infected+Veh versus Uninfected+Veh, P < 0.0001 (Thymus, Spleen, or Blood); Infected+anti-IL-33 versus Infected+Veh, P < 0.0001 (Thymus), P = 0.0439 (Spleen), P = 0.0899 (Blood). CD8$^{+}$ T cells: Infected+Veh versus Uninfected+Veh, P < 0.0001 (Thymus, Spleen, or Blood); Infected+anti-IL-33 versus Infected+Veh, P < 0.0001 (Thymus), P = 0.00559 (Spleen), P = 0.7075 (Blood). One-way ANOVA with Tukey's multiple comparisons. (**g**-**i**) Samples were from WT or *il33*$^{-/-}$ mice and WT or *il33*$^{-/-}$ mice after CLP induction. CD5 MFI on naive CD4$^{+}$ T or naïve CD8$^{+}$ T cells from the thymus, spleen, or peripheral blood (n = 6 mice, pool of two independent experiments). CD4$^{+}$ T cells: WT+CLP versus WT, P < 0.0001 (Thymus, Spleen, or Blood); *il33*$^{-/-}$+CLP versus WT+CLP, P = 0.0034 (Thymus), P < 0.0001 (Spleen), P = 0.0175 (Blood). CD8$^{+}$ T cells: WT+CLP versus WT, P = 0.0018 (Thymus), P = 0.0007 (Spleen), P < 0.0001 (Blood); *il33*$^{-/-}$+CLP versus WT+CLP, P < 0.0001 (Thymus), P = 0.0002 (Spleen), P = 0.0012 (Blood). One-way ANOVA with Tukey's multiple comparisons. Data are shown as the mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant. Source data are provided as a Source Data file.

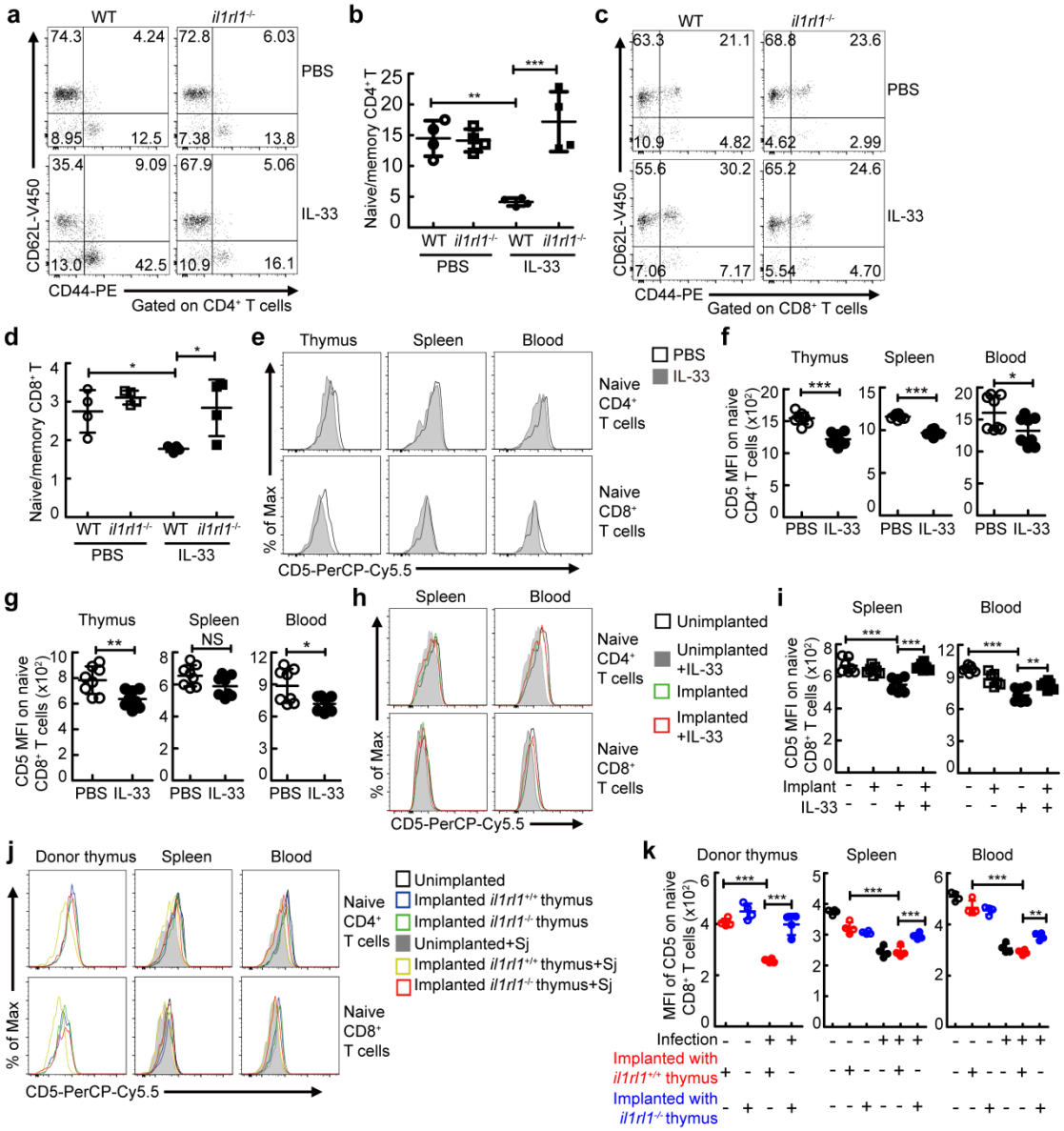


**Fig. S5. IL-33 results in thymic involution-mediated T cell aging.** (**a-d**) Naïve/memory naïve $CD4^+$ T cells or $CD8^+$ T cells in the spleen from WT or *il1rl1*$^{-/-}$ mice after IL-33 treatment (n = 4 mice). WT+IL-33 versus WT+PBS, P = 0.0018 ($CD4^+$ T cells), P = 0.0271 ($CD8^+$ T cells); *il1rl1*$^{-/-}$+IL-33 versus WT+IL-33, P = 0.0002 ($CD4^+$ T cells), P = 0.0237 ($CD8^+$ T cells). (**e-g**) Samples were from mice after IL-33 treatment. CD5 MFI on naive $CD4^+$ T cells or $CD8^+$ T cells from the thymus, spleen, or peripheral blood (n = 8 mice, pool of two independent experiments). $CD4^+$ T cells, P < 0.0001 (Thymus or Spleen), P = 0.0392 (Blood); $CD8^+$ T cells, P = 0.0057 (Thymus), P = 0.0676 (Spleen), P = 0.0162 (Blood); Unpaired two-tailed Student's t-test. (**h**, **i**) Mice were injected with IL-33 at week 8 after transplantation with *il1rl1*$^{-/-}$ thymus. (**h**) Representative flow cytometry of CD5 MFI on naive $CD4^+$ T cells

or $CD8^+$ T cells in the spleen or peripheral blood. (**i**) CD5 MFI on naive $CD8^+$ T cells in the spleen or peripheral blood (PBS, n = 7 mice; IL-33, n = 8 mice; pool of two independent experiments). WT+IL-33 versus WT+PBS, P < 0.0001 (Spleen), P < 0.0001 (Blood); *il1rl1*$^{-/-}$+IL-33 versus WT+IL-33, P < 0.0001 (Spleen), P = 0.0028 (Blood). (**j, k**) Mice were infected with schistosome at week 8 after transplantation with *il1rl1*$^{+/+}$ or *il1rl1*$^{-/-}$ thymus. (**j**) Representative flow cytometry of CD5 MFI on naive $CD4^+$ T cells or $CD8^+$ T cells in donor thymus, spleen, or peripheral blood. (**k**) CD5 MFI on naive $CD8^+$ T in donor thymus, spleen, or peripheral blood (n = 4 mice). Infected+*il1rl1*$^{+/+}$ thymus versus Uninfected+*il1rl1*$^{+/+}$ thymus, P < 0.0001 (Donor thymus, Spleen, or Blood); Infected+*il1rl1*$^{+/+}$ thymus versus Infected+*il1rl1*$^{-/-}$ thymus, P < 0.0001 (Donor thymus), P = 0.0008 (Spleen), P = 0.0017 (Blood). (**b**, **d**, **i**, **k**) One-way ANOVA with Tukey's multiple comparisons. Data are shown as the mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001. Source data are provided as a Source Data file.

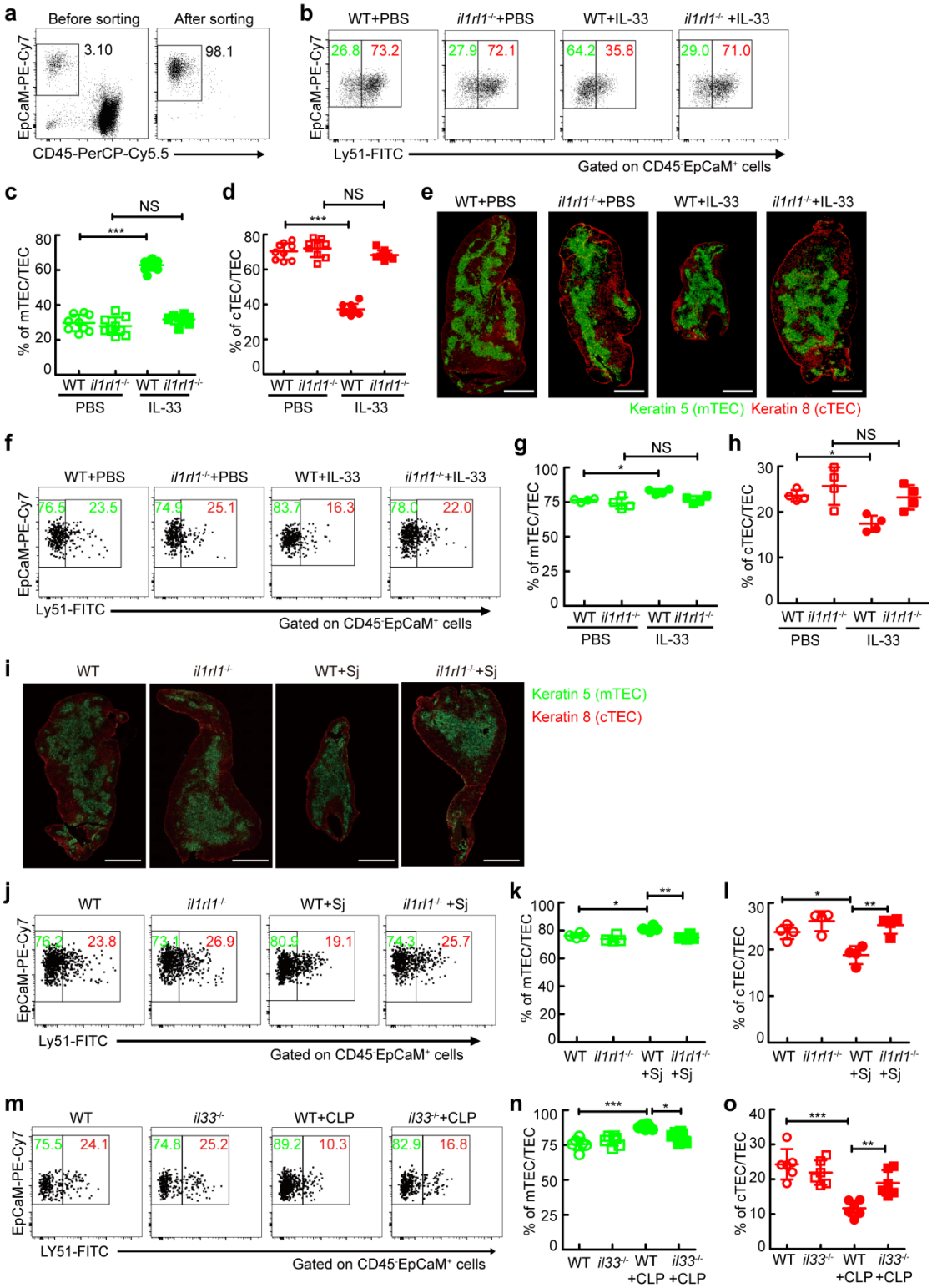


**Fig. S6. IL-33 perturbs the compartment of TECs.** (**a**) Representative flow cytometry of TECs before or after being sorted by MACS from the thymus cultured in FTOCs. (**b-d**) Representative and quantified flow cytometry of mTECs and cTECs in FTOC treated with

IL-33 (n = 9 biologically independent samples of fetal thymic lobes, pool of three independent experiments). WT+IL-33 versus WT+PBS, P < 0.0001 (mTEC or cTEC); *il1rl1*$^{-/-}$+IL-33 versus *il1rl1*$^{-/-}$+PBS, P = 0.2001 (mTEC), P = 0.2018 (cTEC); One-way ANOVA with Tukey's multiple comparisons. (**e**) Histology of the thymus from mice after IL-33 treatment; Green, Keratin 5; red, Keratin 8; Scale bar, 1000 μm. (**f-h**) Representative and quantified flow cytometry of cTECs or mTECs in WT or *il1rl1*$^{-/-}$ mice after IL-33 treatment (n = 4 mice). WT+IL-33 versus WT+PBS, P = 0.0314 (mTEC or cTEC); *il1rl1*$^{-/-}$+IL-33 versus *il1rl1*$^{-/-}$+PBS, P = 0.5786 (mTEC or cTEC); One-way ANOVA with Tukey's multiple comparisons. (**i**) Histology of the thymus from WT or *il1rl1*$^{-/-}$ mice after schistosome infection; Green, Keratin 5; red, Keratin 8; Scale bar, 1000 μm. (**j-l**) Representative and quantified flow cytometry of cTECs or mTECs in WT or *il1rl1*$^{-/-}$ mice after schistosome infection (n = 4 mice). WT+Infected versus WT+Uninfected, P = 0.0115 (mTEC), P = 0.0113 (cTEC); *il1rl1*$^{-/-}$+Infected versus WT+Infected, P = 0.0023 (mTEC), P = 0.0020 (cTEC); One-way ANOVA with Tukey's multiple comparisons. (**m-o**) Representative flow cytometry and quantification of flow cytometry of cTECs or mTECs in WT or *il33*$^{-/-}$ mice after CLP induction (n = 6 mice, pool of two independent experiments). WT+CLP versus WT, P < 0.0001 (mTEC or cTEC); *il1rl1*$^{-/-}$+CLP versus WT+CLP, P = 0.0111 (mTEC), P = 0.0094 (cTEC); One-way ANOVA with Tukey's multiple comparisons. Data are shown as the mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant. Source data are provided as a Source Data file.

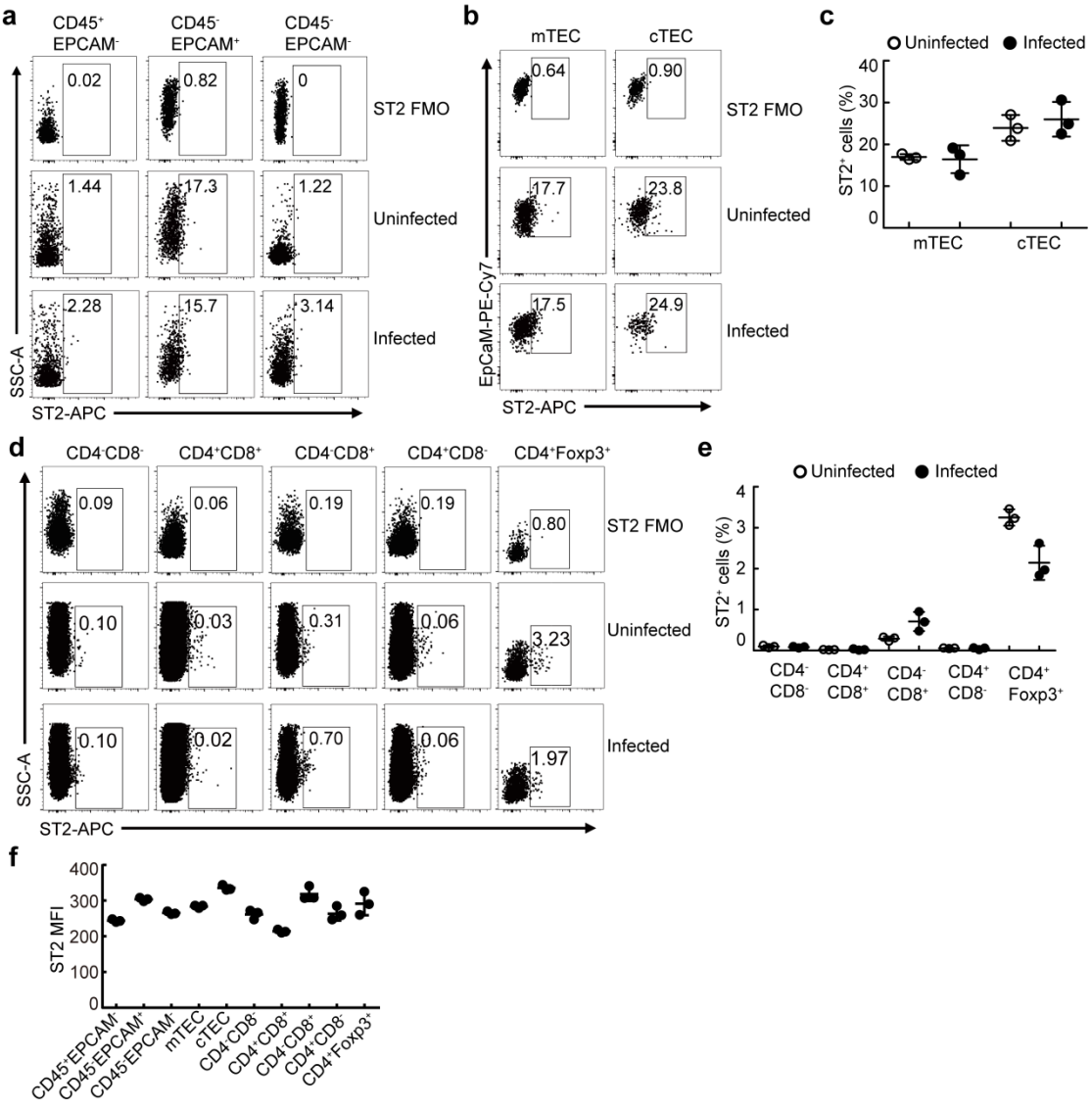


**Fig. S7. The expression of ST2 on cells in the thymus.** (**a-e**) Cells were from the thymus in uninfected or schistosome-infected mice. (**a**) Representative flow cytometry of $ST2^+$ cells gated on $CD45^+EpCaM^-$, $CD45^-EpCaM^+$, or $CD45^-EpCaM^-$ cells. (**b, c**) Representative and quantified flow cytometry of $ST2^+$ cells gated on mTECs or cTECs (n = 3 mice). (**d, e**) Representative and quantified flow cytometry of $ST2^+$ cells gated on $CD4^+CD8^+$DP, CD4SP, CD8SP, $CD4^-CD8^-$ DN, or Treg cells (n = 3 mice). (f) Quantified ST2 MFI of $ST2^+$ cells gated on $CD45^+EpCaM^-$, $CD45^-EpCaM^+$, $CD45^-EpCaM^-$, mTECs, cTECs, $CD4^+CD8^+$DP, CD4SP, CD8SP, $CD4^-CD8^-$ DN, or Treg cells in the thymus from schistosome-infected mice (n = 3 mice). Data are shown as the mean ± s.d. Source data are provided as a Source Data file.