SUPPLEMENTAL MATERIAL

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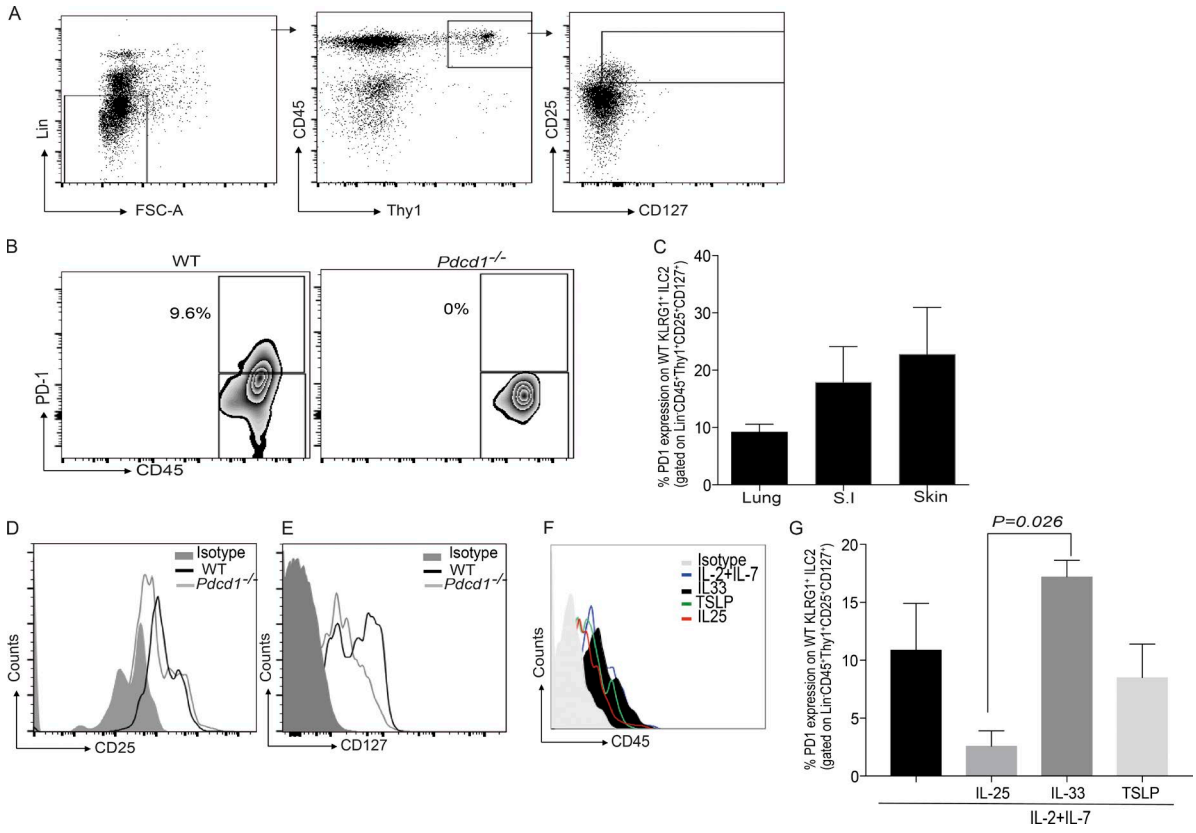

Figure S1. **Characterization of ILC-2 subsets in WT and *Pdcd1*$^{-/-}$ mice.** ILC-2 subsets were characterized by flow cytometry. Lin$^-$ was defined as CD3$^-$, CD4$^-$, CD8$^-$, NK1.1$^-$, GR1$^-$, Ter119$^-$, CD5$^-$, CD11b$^-$, CD11c$^-$, F4/80$^-$, CD45R/B220$^-$, and CD19$^-$. ILC-2s were defined as Lin$^-$CD45$^+$ Thy1$^+$CD127$^+$ CD25$^+$ST2$^+$KLRG1$^+$. Representative flow plots of gating strategy in the lungs (A) and flow plots for PD-1 expression (B). Summary of PD-1 expression in KLRG1$^+$ILC-2 subsets in the various organs in WT mice (C). Cytokine receptor expression, i.e., CD25 and CD127, in KLRG1$^+$ILC-2 subsets in WT and *Pdcd1*$^{-/-}$ lungs were analyzed using flow cytometry (D and E). Lung KLRG1$^+$ILC-2 from WT mice were stimulated with various cytokines, i.e., IL-2, IL-7 alone, or IL-7 in combination with IL-33 or IL-25 or TSLP for 48 h, and then PD-1 expression was measured by flow cytometry (F and G). Experiments were repeated at least three times, and each experiment had $n = 5$ mice. Data are shown as mean ± SEM. A one-way ANOVA analysis followed by a multiple comparison test (Tukey) was performed to determine statistical significance between the various cohorts in C and G. $P \leq 0.05$ was considered significant. Significant p-values are denoted in the figures.

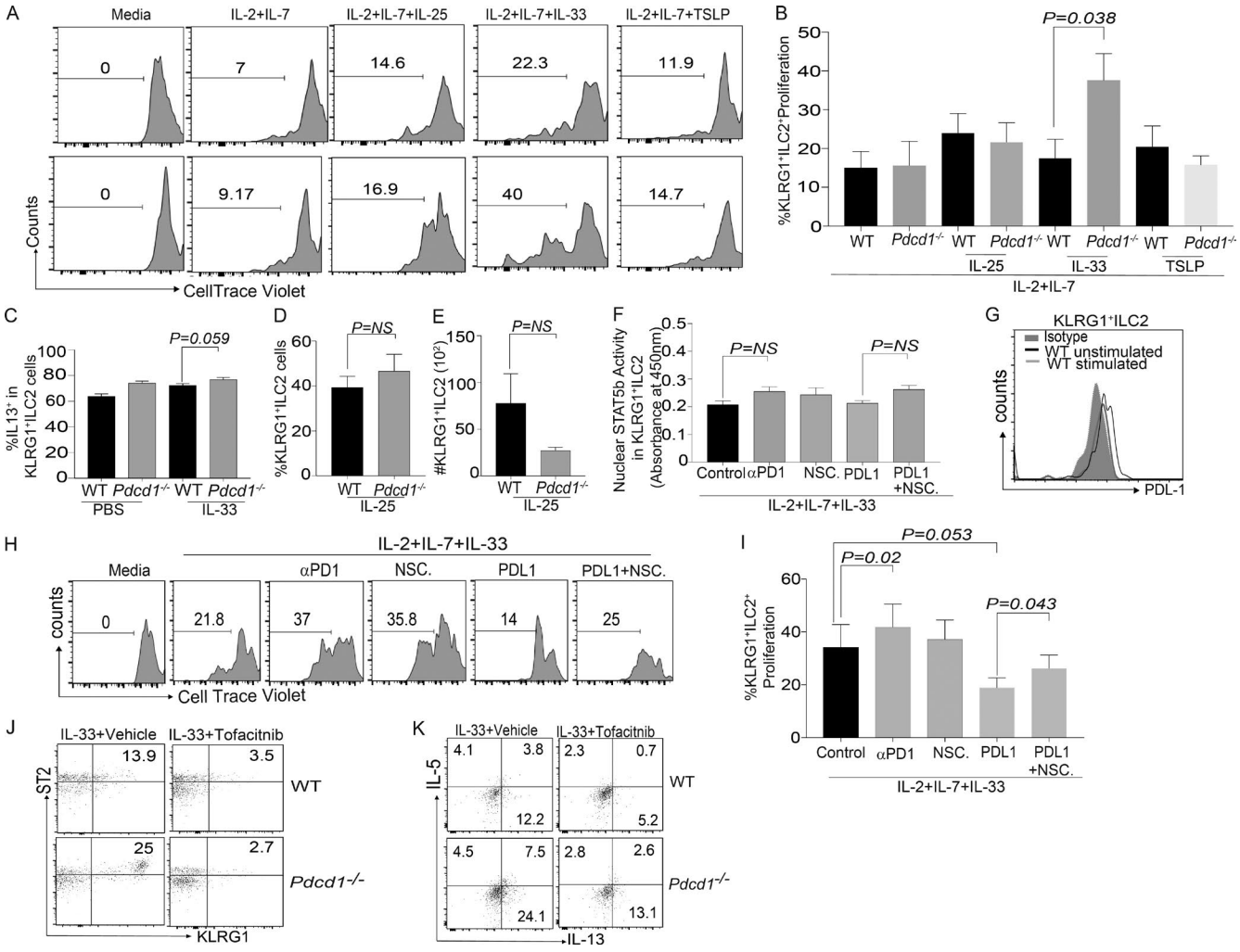

Figure S2. **PD-1 inhibits proliferation of KLRG1⁺ILC-2s.** ILC-2s from WT and *Pdcd1*$^{-/-}$ mice were stimulated with IL-2, IL-7, and IL-33, or IL-25 or TSLP, for 3 d. Proliferation of WT and *Pdcd1*$^{-/-}$ ILC-2 was monitored by flow cytometry by gating on Lin^{-}CD45^{+} Thy1^{+}CD127^{+}CD25^{+}ST2^{+}KLRG1^{+} (A and B). WT and *Pdcd1*$^{-/-}$ mice were either treated with PBS or IL-33 (200 ng/mice) for 3 d. Cytokine expression of ILC-2 subsets were measured by stimulating the cells with cell stimulation cocktail for 4 h, and cytokine expression was measured by flow cytometry (C). WT and *Pdcd1*$^{-/-}$ mice were treated with IL-25 (200 ng/mice) for 3 d. ILC-2 frequency and absolute numbers in the lungs were evaluated by flow cytometry at day 3 (D and E). Experiments were repeated where WT mice were treated with IL-33 (200 ng/mice) for 3 d and lung ILC-2s were stimulated for 72 h with IL-2, IL-7, and IL-33. In addition, cohorts were treated with Isotype control antibody, αPD-1 antibody, NSC87877, PDL-1 fc, or PDL-1 fc plus NSC8787. After stimulation, cells were restimulated with IL-2 (100 ng/ml), and then nuclear lysates were tested for STAT5b (F). WT and *Pdcd1*$^{-/-}$ mice were treated IL-33 (200 ng/mice) for 3 d, and then lung KLRG1^{+} ILC-2s were stimulated with IL-2, IL-7, and IL-33. After 72 h of culture, PDL-1 expression was evaluated in Lin^{-} CD45^{+} Thy1^{+}CD127^{+}CD25^{+}ST2^{+}KLRG1^{+} cells (G). Representative flow plots showing the proliferation of CD45^{+} Thy1^{+}CD127^{+}CD25^{+}ST2^{+}KLRG1^{+} (H) and a summary of proliferation in n = 5 mice (I). Mechanistic experiments were set up with WT and *Pdcd1*$^{-/-}$ mice where cohorts were treated with rmIL-33 + Vehicle (DMSO) or with rmIL-33 + Tofacitinib (15 mg/kg/d/mouse). Representative flow plot of KLRG1^{+}ILC-2s in the different cohorts were measured by flow cytometry (J), and functional cytokine expression was monitored in the various cohorts (K). All experiments were repeated three times, and animals per cohort was n = 5. Data shown are mean ± SEM. A One-way ANOVA analysis followed by a multiple comparison test (Tukey) was performed to determine statistical significance between the various cohorts in B, C, F, and I. A Student's t test was performed for determining statistical significance in D and E. $P \leq 0.05$ was considered significant. Significant p-values are denoted in the figures.

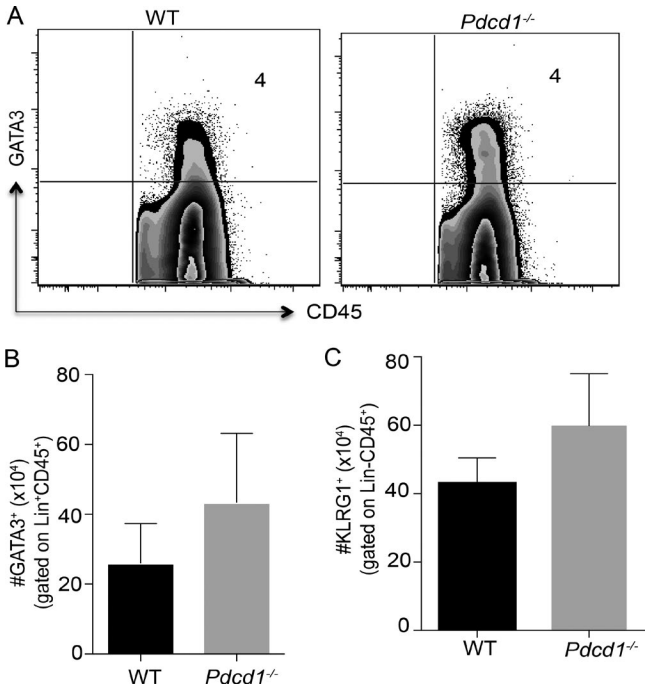

Figure S3. **KLRG1$^+$ ILC-2 cells from the lungs of WT and *Pdcd1*$^{-/-}$ mice after infection show no differences in frequency and number.** WT or *Pdcd1*$^{-/-}$ mice were infected with *N. brasiliensis*, and then KLRG1$^+$ ILC-2 numbers in the lungs were evaluated by flow cytometry. Representative flow plots showing GATA3$^+$ ILC-2 numbers in the WT and *Pdcd1*$^{-/-}$ (A). Summary of absolute numbers of GATA3$^+$ILC-2$^+$ cells (B). Summary of absolute numbers of KLRG1$^+$ILC-2$^+$ cells (C). Animals per cohort was $n = 5$. Data shown is cumulative of at least two repeats. A Student's *t* test analysis was performed to determine statistical significance between the various cohorts. $P \leq 0.05$ was considered significant. Significant p-values are denoted in the figures.

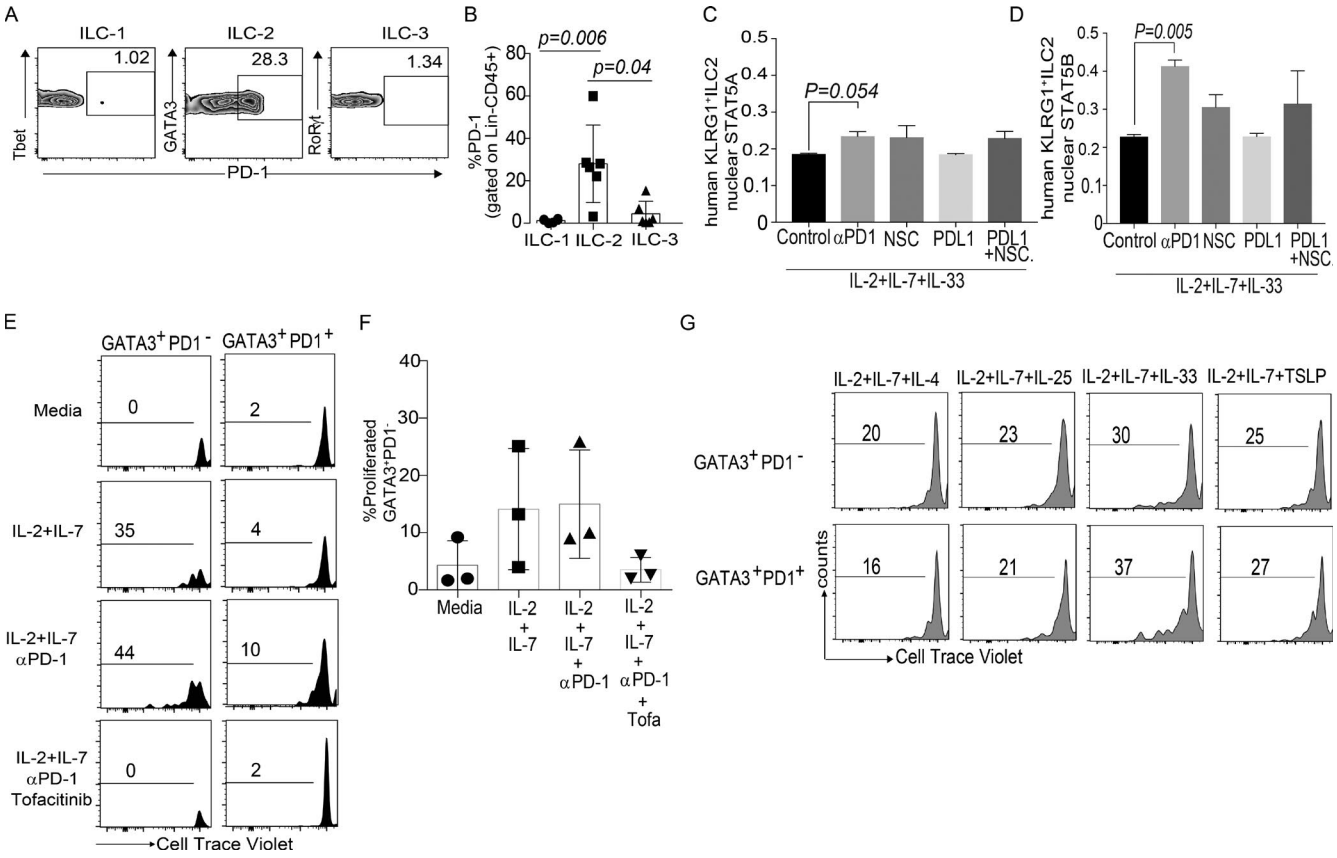

Figure S4. **PD-1 regulates human KLRG1$^+$ILC-2s.** PD-1 expression was analyzed in ILC subsets in PBMCs. Cells were gated on Lin$^-$CD45$^+$, and then PD-1 versus T-bet, GATA3, and RORγT was analyzed in $n = 5$ donors (A and B). Leukapheresis product was obtained from a normal donor, and KLRG1$^+$ ILC-2s were stimulated for 72 h with IL-2, IL-7, and IL-33 in triplicates. In addition, cohorts were treated with Isotype control antibody, α–PD-1 antibody, NSC87877, PDL-1 fc, or PDL-1 fc plus NSC87877, and then nuclear lysates were tested for STAT5a and STAT5b (C and D). PBMCs were labeled with Cell Trace Violet, and then ILC-2s were stimulated with rhIL-2 + rhIL-7 (40 ng/ml) + Vehicle (DMSO) + Isotype control or with rhIL-2 + rhIL-7 + Vehicle + αPD-1 or with rhIL-2 + rhIL-7 + Tofacitinib + αPD-1. Dilution of Cell Trace Violet as a measure of proliferation was performed at day 5 after stimulation (E and F). Experiments were repeated with $n = 3$ donors. Data are represented as mean ± SEM. PBMCs were labeled with Cell Trace Violet, and then ILC-2s were stimulated with rhIL-2 + rhIL-7 (40 ng/ml) + rhIL-4 (40 ng/ml) or with rhIL-2 + rhIL-7 + IL-25 (40 ng/ml) or with rhIL-2 + rhIL-7 + IL-33 (40 ng/ml) or rhIL-2 + rhIL-7 (40 ng/ml) + rhTSLP (40 ng/ml). Dilution of Cell Trace Violet as a measure of proliferation was performed at day 5 after stimulation (G). A one-way ANOVA analysis followed by a multiple comparison test (Tukey) was performed to determine statistical significance between the various cohorts in B and F. A Student's *t* test was used to measure differences between isotype and αPD-1 antibody in C and D. $P \leq 0.05$ was considered significant. Significant p-values are denoted in the figures.