

SUPPLEMENTAL MATERIAL

Schwartz et al., <https://doi.org/10.1084/jem.20170051>

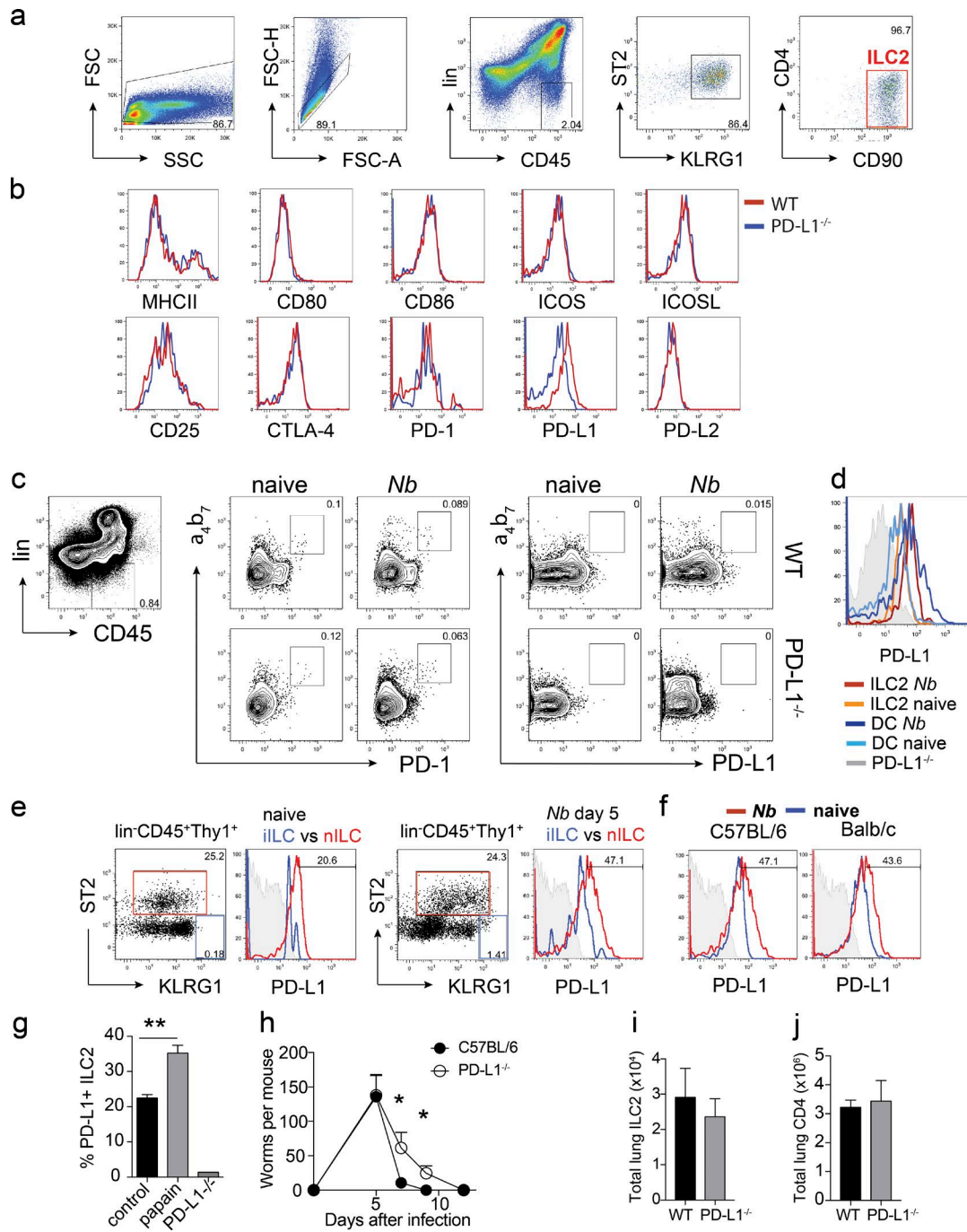


Figure S1. Gating strategy and expression of PD-L1 on ILC2s. (a) Gating strategy to identify ILC2s from mouse lungs. (b) Histograms show expression of indicated markers on lung ILC2s from *N. brasiliensis*-infected WT (red) or PD-L1^{-/-} mice. (c) PD-1 and PD-L1 expression on lin⁺CD45⁺α₄β₇⁺ ILC progenitor population in the bone marrow of WT and PD-L1^{-/-} mice before and 5 d after *N. brasiliensis* infection. Dot plots are representative of three individual mice. (d) Representative histogram overlay shows the expression of PD-L1 on ILC2s (orange and red) and DCs (light and dark blue) from naive and day 5 *N. brasiliensis*-infected mice. (e) Expression of PD-L1 on nILCs (red line; lin⁺CD45⁺KLRG1^{int}ST2⁺) and iILCs (blue line; lin⁺CD45⁺KLRG1^{int}ST2⁺) in naive (left) and infected (right) C57BL/6 mice. (f) Surface staining of PD-L1 on ILC2s from lungs of naive (blue line) and infected (red line) C57BL/6 (left) or BALB/c (right) mice. (g) WT and PD-L1^{-/-} mice were challenged on three consecutive days with papain or PBS intranasally, and ILC2s were analyzed for PD-L1 expression on day 4. Bar graphs show the mean + SEM of six mice per group from two pooled experiments. (h) Kinetics of worm burden on indicated days after infection with *N. brasiliensis*. Mean + SEM from five to nine mice per time point from three experiments. (i and j) Total numbers of ILC2s (i) and CD4⁺ T cells (j) in the lungs of *N. brasiliensis*-infected WT and PD-L1^{-/-} mice. Bar graphs show the mean + SEM of five to eight mice from three experiments. Student's *t* test was used to analyze data, and *p*-values <0.05 were considered statistically significant. *, *P* < 0.05; **, *P* < 0.01.

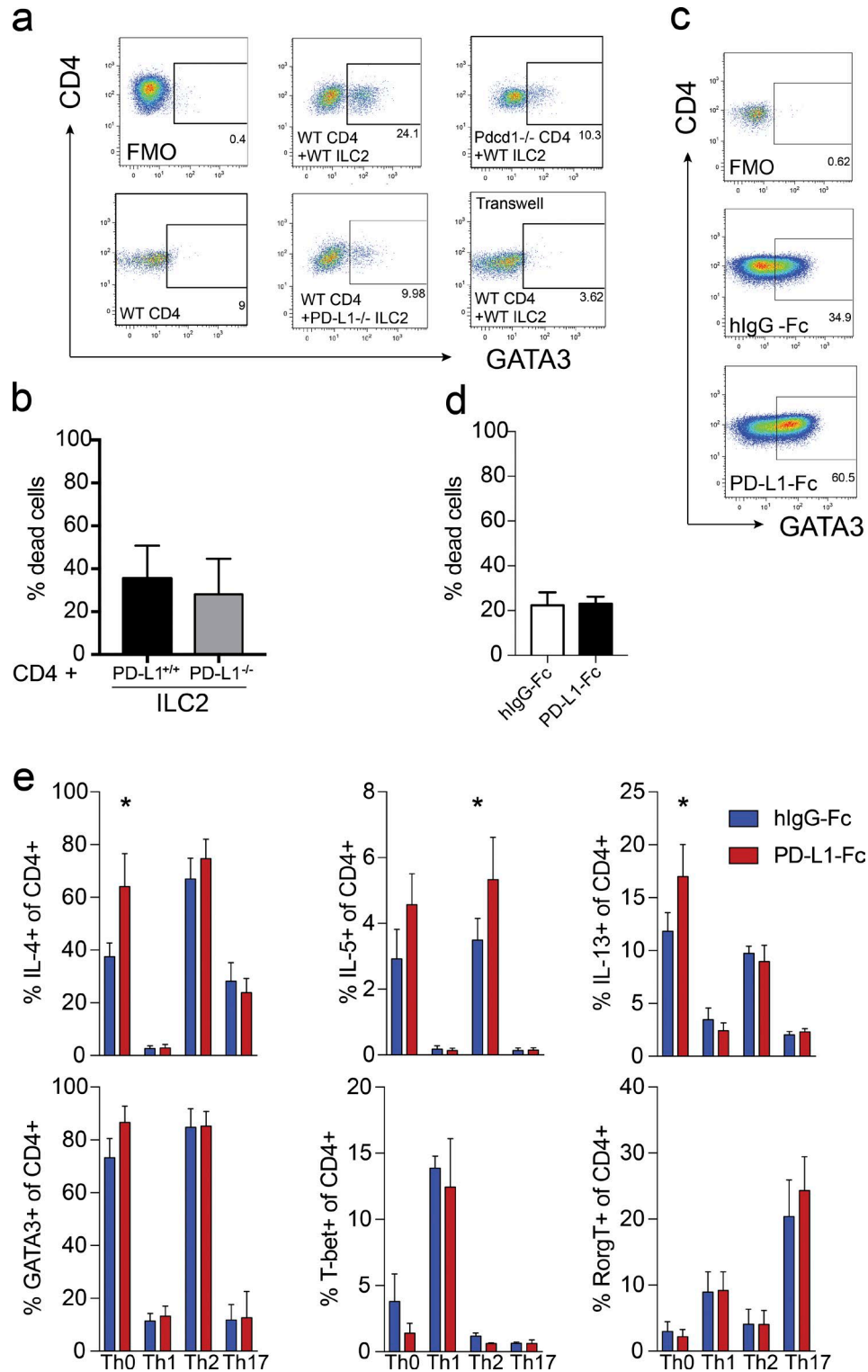


Figure S2. **Analysis of CD4⁺ T cells in vitro.** (a) Representative dot plots for the intranuclear GATA3 staining in hlgG-Fc- and PD-L1-Fc-stimulated CD4 T cells. Plots are gated on live, CD3⁺CD4⁺ lymphocytes. (b) Bar graphs show the frequency of Live/Dead aqua-positive cells 5 d after the start of co-culture. Mean + SEM from six replicates. (c) Representative dot plots for the intranuclear GATA3 staining in indicated co-cultured CD4 T cells. Plots are gated on live, CD3⁺CD4⁺ lymphocytes. (d) Bar graphs show the frequency of Live/Dead aqua-positive cells 24 h after culture setup. Mean + SEM from six replicates. (e) Bar graphs show the mean + SEM of six replicates per group from two experiments of Fig. 3 h. Student's *t* test was used to analyze data, and *p*-values <0.05 were considered statistically significant. *, *P* < 0.05.

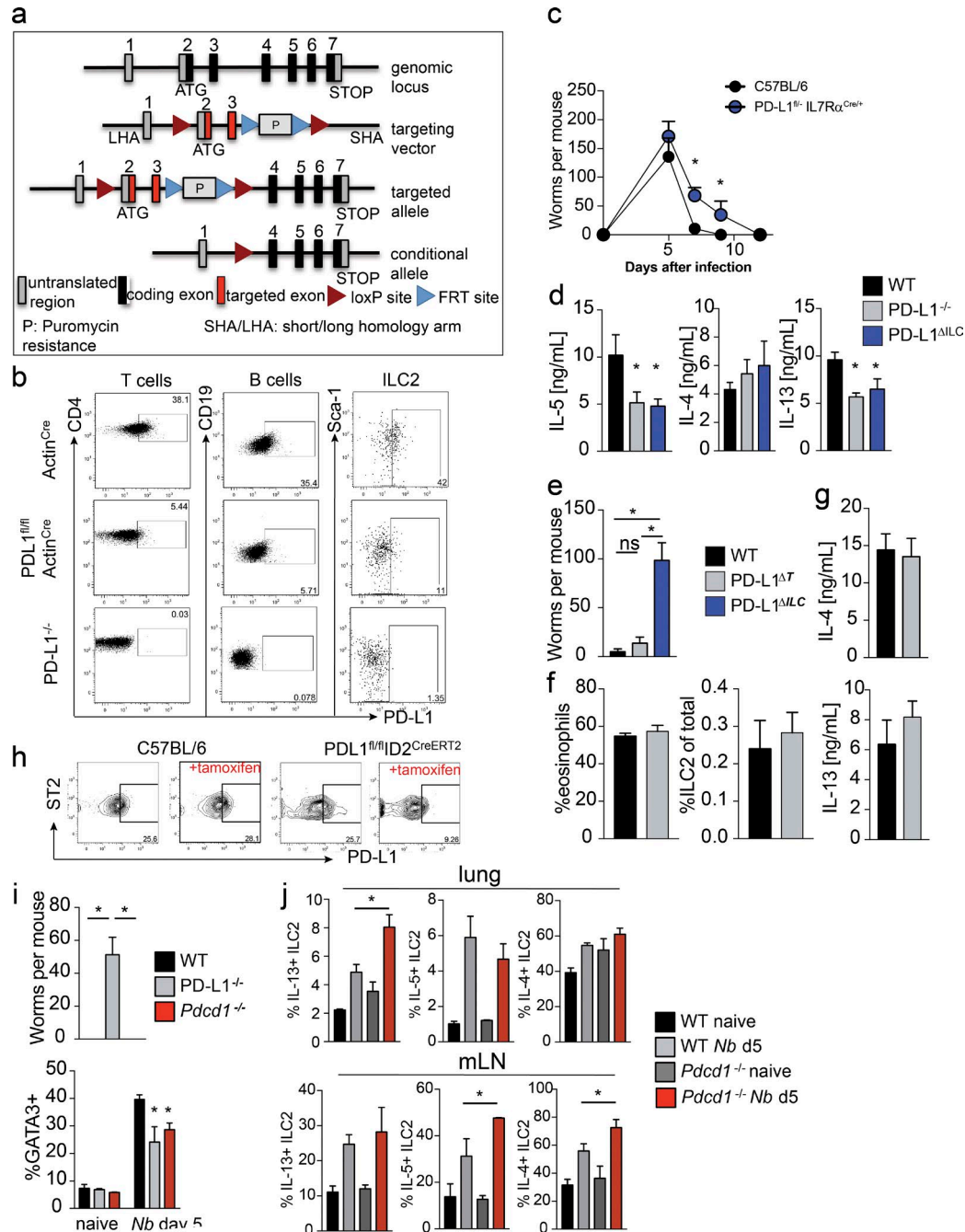


Figure S3. Generation of conditional PD-L1-knockout mice and analysis of *Pcd1*^{-/-} mice. (a) Targeting strategy to generate loxP-flanked PD-L1 mice. Mice were generated on a B6 background. (b) Efficiency of Cre-mediated PD-L1 deletion in ActinCre mice. Lymph node T cells, B cells, and lung ILC2s from control (top), PD-L1^{fl/fl}ActinCre⁺ (middle), and PD-L1^{-/-} (bottom) mice were analyzed for PD-L1 expression. (c) Kinetics of worm burden on indicated days after *N. brasiliensis* infection in WT and PD-L1^{fl/fl}IL7Rα^{Cre/+} mice. Mean + SEM from at least six mice per group per time point. (d) IL-5, IL-4, and IL-13 production from anti-CD3/anti-CD28-stimulated mLN cells from WT, PD-L1^{-/-}, and PD-L1^{fl/fl}IL7Rα^{Cre/+} mice measured by cytokine ELISA. Mean + SEM of six mice per group. (e) PD-L1^{fl/fl}CD4^{Cre/+}, PD-L1^{fl/fl}IL7Rα^{Cre/+}, and WT mice were infected with *N. brasiliensis* and analyzed for worm burden (e), lung eosinophilia (f), and IL-4 and IL-13 production from anti-CD3/anti-CD28-stimulated mLN cells (g) 7 d later. Mean + SEM from eight mice per group from two experiments. (h) Representative plot of PD-L1 expression on ILC2s from indicated mice. (i) WT, PD-L1^{-/-}, and *Pcd1*^{-/-} mice were infected with *N. brasiliensis*, and intestinal worm burden and T cell GATA3 expression were analyzed 7 and 5 d later, respectively. Mean + SEM from six mice per group from two experiments. (j) Expression of IL-4, IL-5, and IL-13 in lung and mLN ILC2s from naive or day 5 *N. brasiliensis*-infected WT or *Pcd1*^{-/-} mice measured by intracellular cytokine staining. Mean + SEM of six mice per group from two experiments. Student's *t* test was used to analyze data, and *p*-values < 0.05 were considered statistically significant. *, *P* < 0.05; ns, not significant.