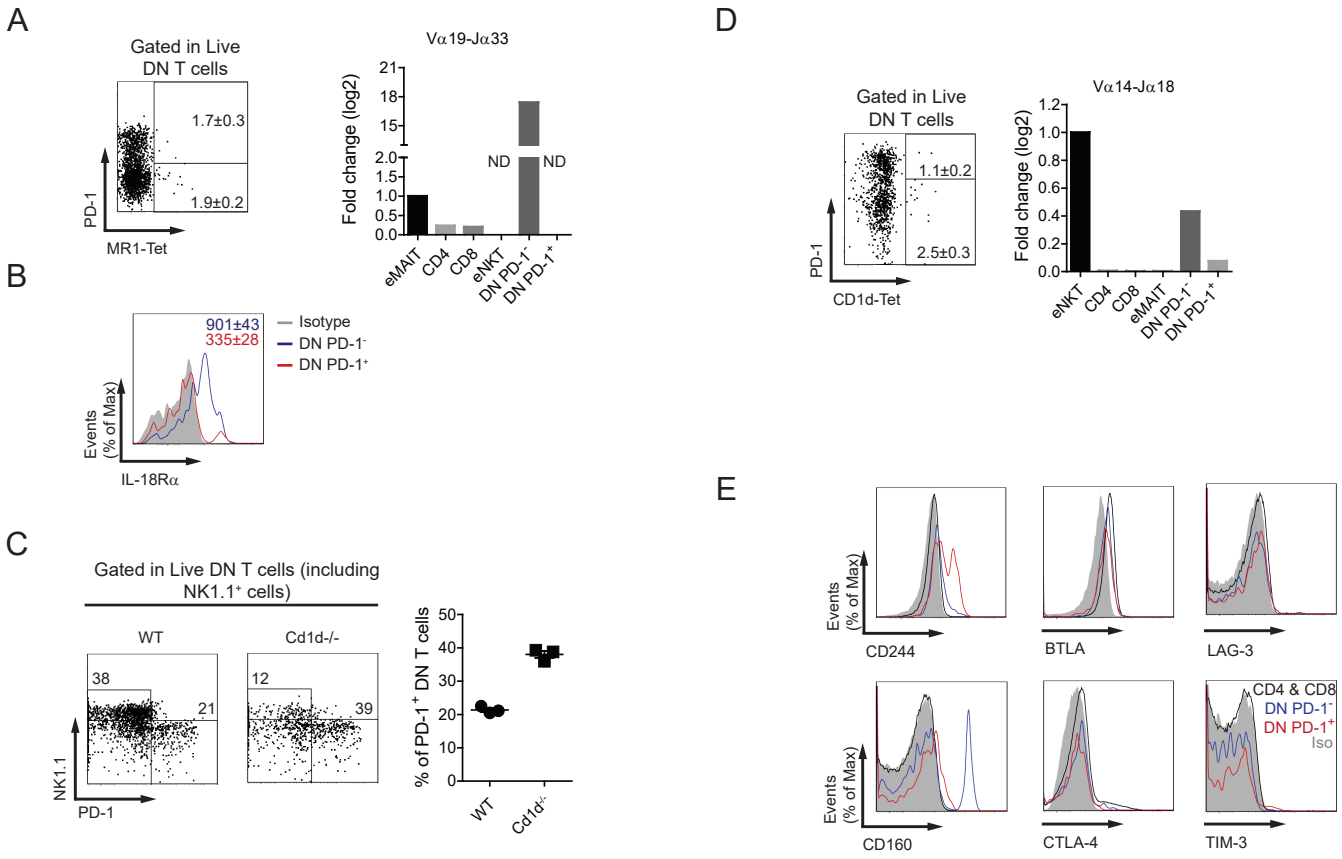
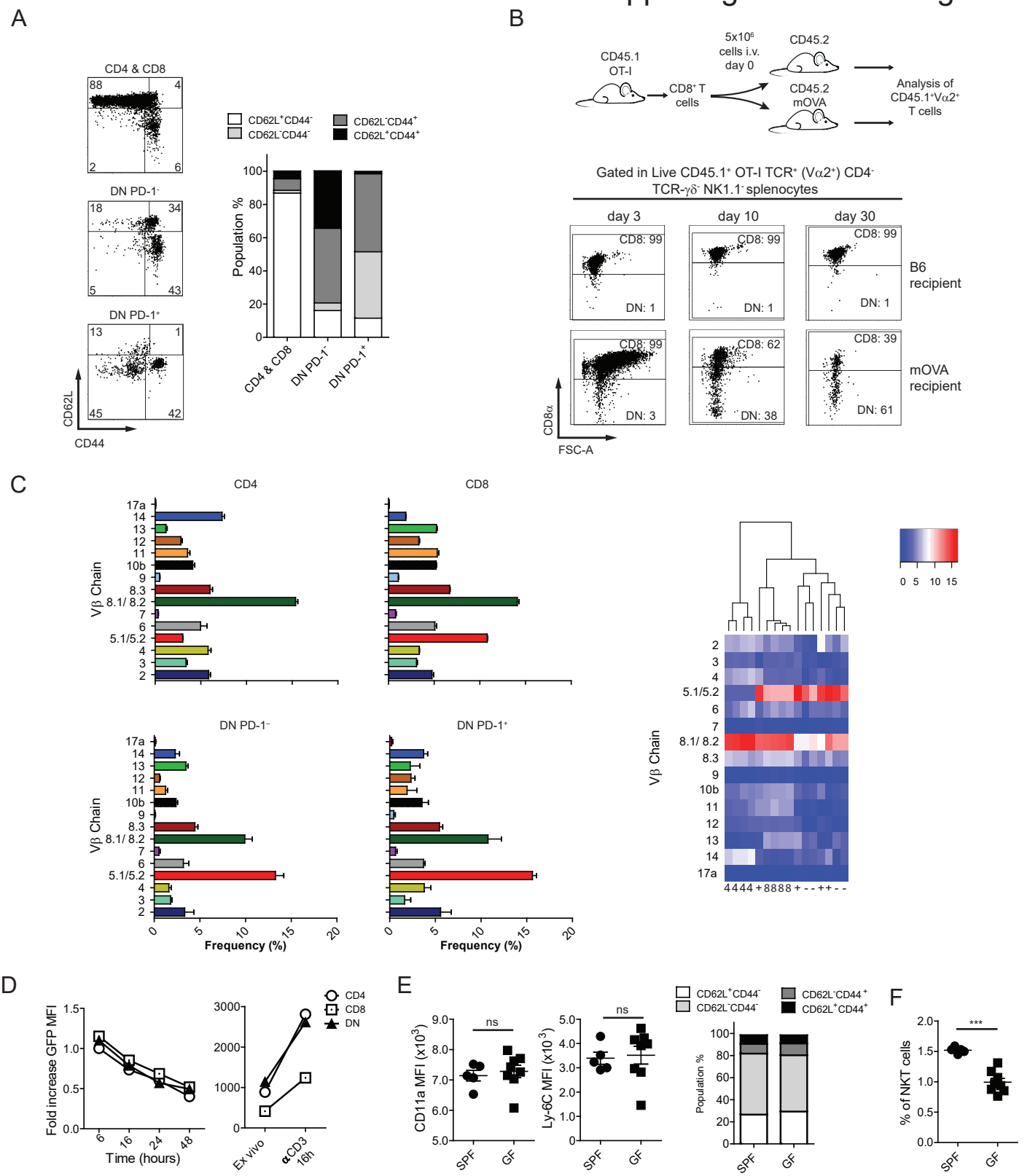
Supporting Information Figure 1



Supporting information Figure 1. (A) Frequency of MR1-Tet$^+$ cells among PD-1$^-$ o PD-1$^+$ DN T cells (left) and relative expression (over eMAIT) of the TCR rearrangement Vα19-Jα33 RNA in the indicated populations (right). (B) Levels (MFI) of IL-18Rα in PD-1$^-$ and PD-1$^+$ DN T cells. (C) Analysis of PD-1 and NK1.1 expression within T cells from WT and *Cd1d*$^{-/-}$ mice. DN T cells were defined as in Fig. 1A but including NK1.1$^+$ cells. (D) Frequency of CD1d-Tet$^+$ cells among PD-1$^-$ o PD-1$^+$ DN T cells (left) and relative expression (over eNKT) of Vα14-Jα18 rearrangement RNA in indicated populations (right). (E) Expression of inhibitory coreceptors in PD-1$^-$ and PD-1$^+$ DN T cells compared to CD4 and CD8 counterparts. ND: non detectable. Data is expressed as mean ± SEM and representative of 1-3 experiments (n=3-5). Cumulative data come from pooling results from several experiments.

Supporting Information Figure 2



Supporting information Figure 2. Phenotype of DN T cells. (A) Distribution of CD4$^+$ together with CD8$^+$ (CD4 & CD8), PD-1$^-$ DN and PD-1$^+$ DN T cells in the spleen according to CD44 and CD62L expression. Representative plots (left panels) and cumulative data are shown (right). (B) Outline showing the experimental design (top) and the evolution in time of the adoptively transferred cells (bottom) in the spleen of recipient mice. Five million CD8$^+$ T cells (purity > 95-97 %) from CD45.1 OT-I mice were injected i.v. into congenic CD45.2 mOVA mice. Donor cells were analyzed at days 3, 10 and 30 after transfer. (C) TCR Vβ repertoire (left panels) and cluster analysis (right panel) of CD4$^+$ (4), CD8$^+$ (8), PD-1$^-$ (-) and PD-1$^+$ (+) DN T cells. (D) Change of GFP levels over time in CD4$^+$, CD8$^+$ or DN T cells from Nur77-GFP mice seeded in plates and left unstimulated (left) or stimulated with αCD3 for 16 hours (right). (E) Activation phenotype of PD-1$^+$ DN T cells in SPF and GF mice. (F) Percentage of NKT cells among splenocytes in SPF and GF mice. Data are expressed as mean ± SEM and representative of one experiment (n=3-8).

Supporting information. Antibodies used for flow cytometry: anti-CD3ε (145-2C11 and 17A2), anti-TCR-β (H57-597), anti-TCR-γδ (GL3), anti-CD8α (53-6.7), anti-CD4 (GK1.5), anti-NK1.1 (PK136), anti-CD49b (DX5), anti-B220 (RA3-6B2), anti-PD-1 (29F.1A12), anti-Helios (22F6), anti-Ly-6C (HK1.4), anti-CD62L (MEL-14), anti-CD44 (IM7), anti-CD69 (H1.2F3), anti-CD11a (2D7), anti-CD43 (1B11), anti-CD25 (PC61 and 3C7), anti-CD122 (TM-β1), anti-CD127 (A7R34), anti-CD27 (LG.3A10), anti-CTLA4 (UC10-4B9), anti-CD160 (eBioCNX46-3 and 7H1), anti-BTLA (8F4), anti-2B4 (m2B4 (B6)458.1), anti-LAG-3 (C9B7W), anti-TIM-3 (B8.2C12), anti-Vα2 (B20.1), anti-CD45.1 (A20), anti-IL18Rα (FAB1216F), anti-IL-2 (JES6-5H4), anti-TNF-α (MP6-XT22), anti-IL-17A (TC11-18H10.1), anti-IFN-γ (XMG1.2), anti-ROR-γt (AFKJS-9) and anti-T-bet (4B10)