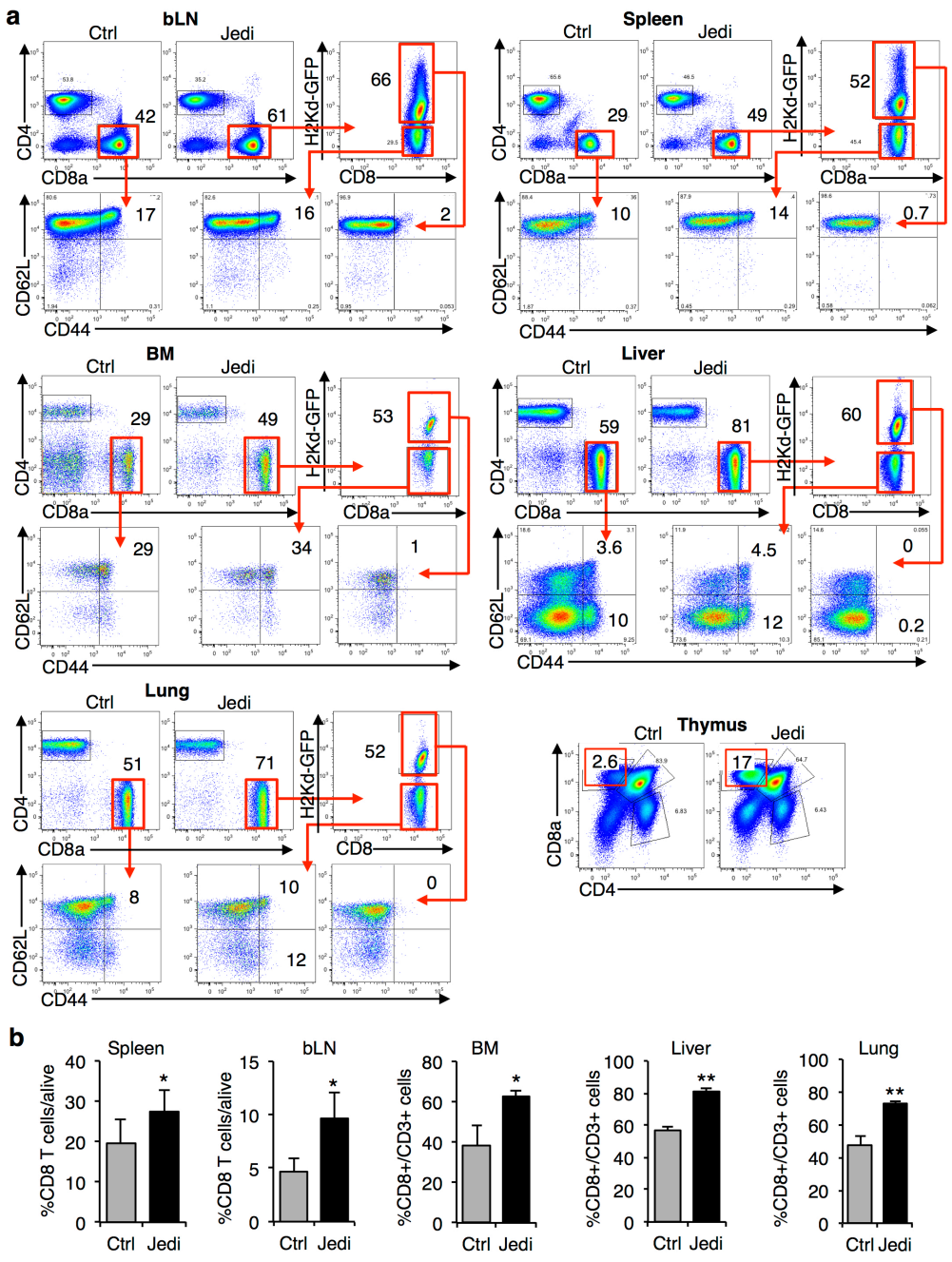

Supplementary Figure 1

Immune cell characterization of the Jedi mice and sequence of the Jedi T cell receptor.

(a) Brachial lymph nodes (bLN), spleen, bone marrow (BM), liver, lung and thymus from Jedi mice and control littermates were stained with CD3e, CD4, CD8a antibodies, and an H-2K^{d}-GFP$_{200\text{-}208}$ pentamer to measure the frequency of EGFP-specific T cells. bLN, spleen, BM, liver and lung were also stained with CD62L and CD44 antibodies to determine the percentage of naïve and memory CD8 T cells. Graphs present the mean±s.d. of the frequency of CD8+ T cells relative to the total live cells in bLN and spleen from the Jedi mice. Data are representative of 2 and 3 experiments (n=4-8 mice/group). **(b)** Graphs show the percentage of CD8+ T cells compared to total liver or total CD3e+ cells in the bLN, spleen, BM, liver and lung (n=4-8 mice/group). *P<0.05 vs WT, **P<0.01 vs WT.

a

trav7-4, traj30, trac

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trbv2, trbj1.6, trbc1

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b

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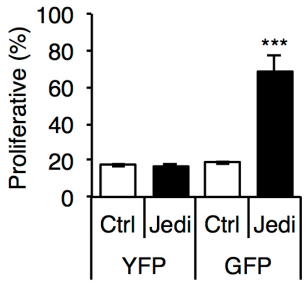
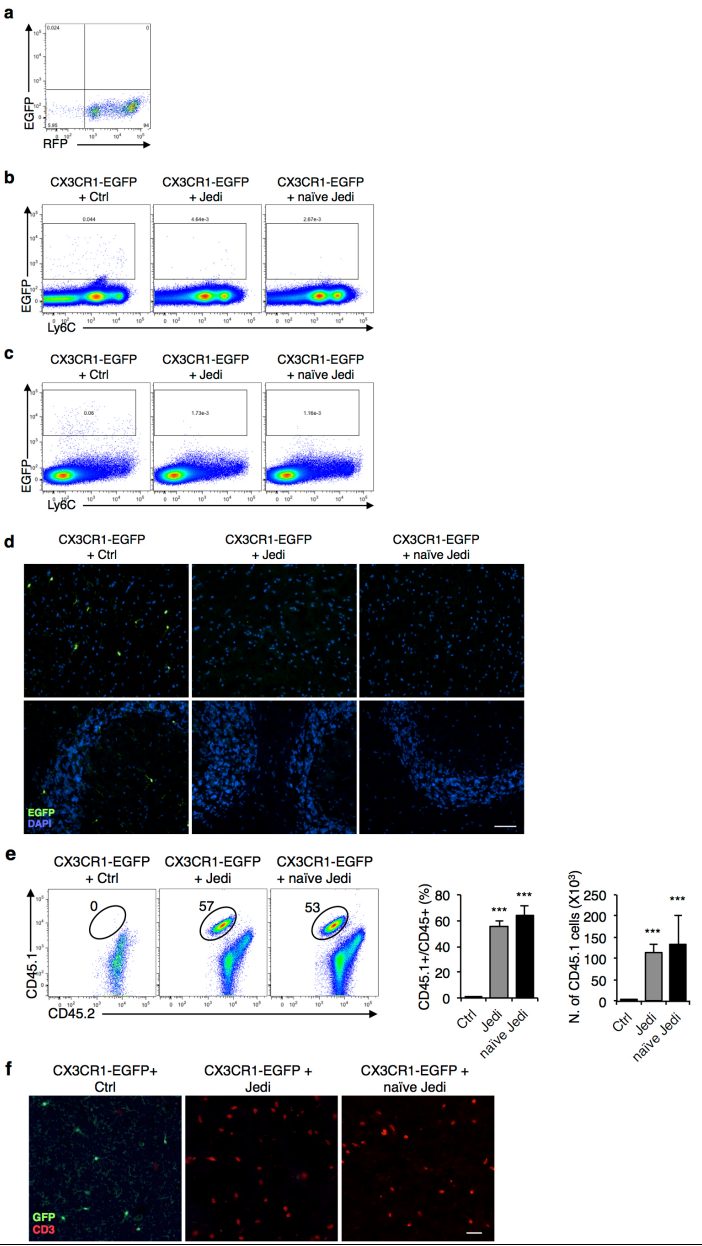
c



Supplementary Figure 2

Jedi T cells specifically recognize EGFP

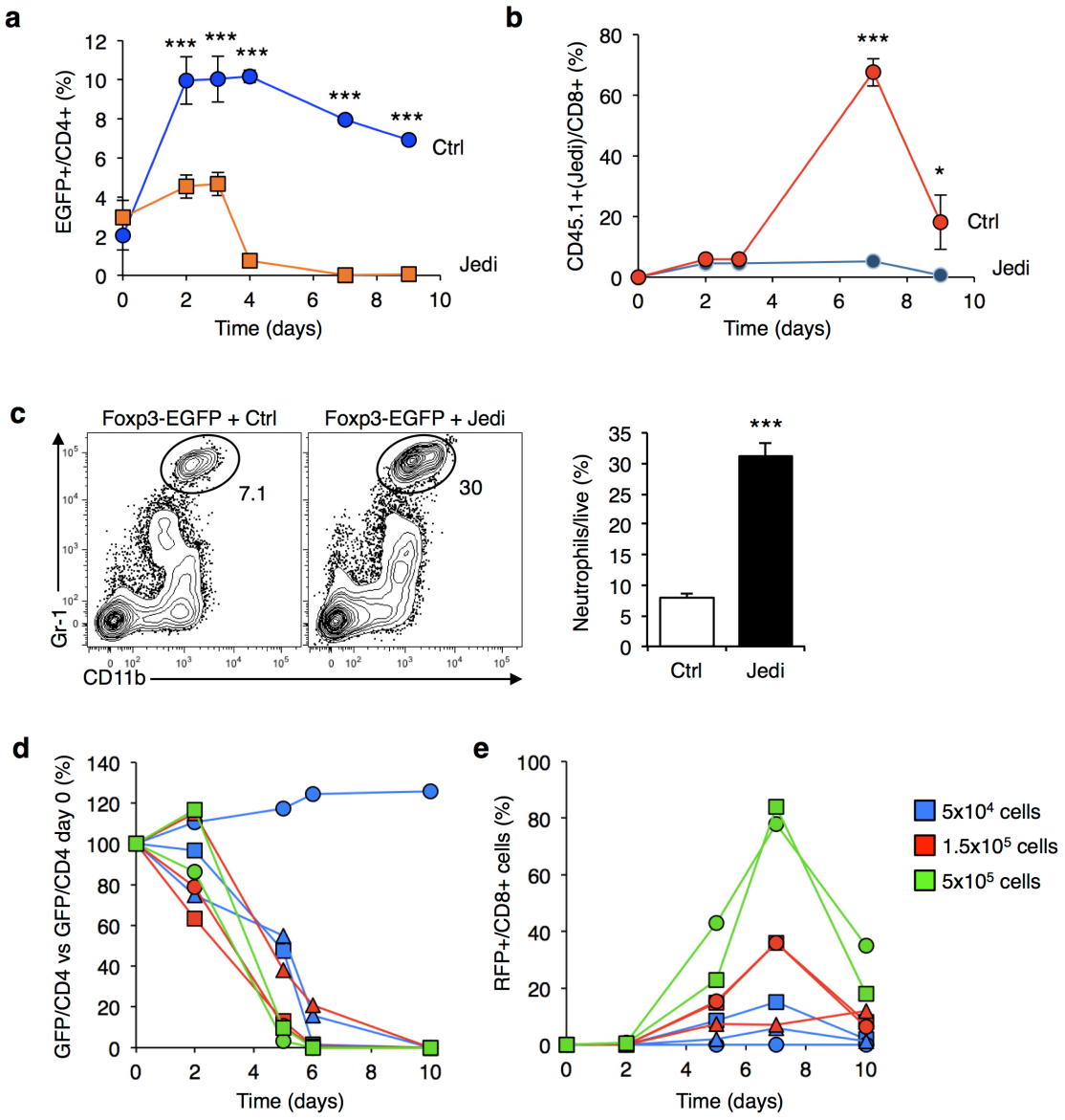
(a) Sequence of the rearranged alpha and beta chains in the Jedi mice. **(b)** Comparison of the amino acid sequence between similar fluorescent proteins, namely Yellow Fluorescent Protein (eYFP), mCitrine, Green Fluorescent Protein (eGFP) and mCerulean. Highlighted in bold is the immune epitope of eGFP. **(c)** Bone marrow cells were transduced with a lentivirus expressing YFP or EGFP and cultured in the presence GM-CSF to generate dendritic cells (BMDCs). Isolated Jedi or B10D2 CD8+ T cells were stained with Violet Cell Proliferation dye and added to BMDCs. EGFP-specific cells were identified by H-2K^{d}-GFP$_{200\text{-}208}$ pentamer staining. Graph present the mean±s.d. of the frequency of cells that have proliferated (n=3 wells/group). ***P<0.001 vs each group.



Supplementary Figure 3

Jedi T cells cross the blood brain barrier and kill EGFP-expressing microglia.

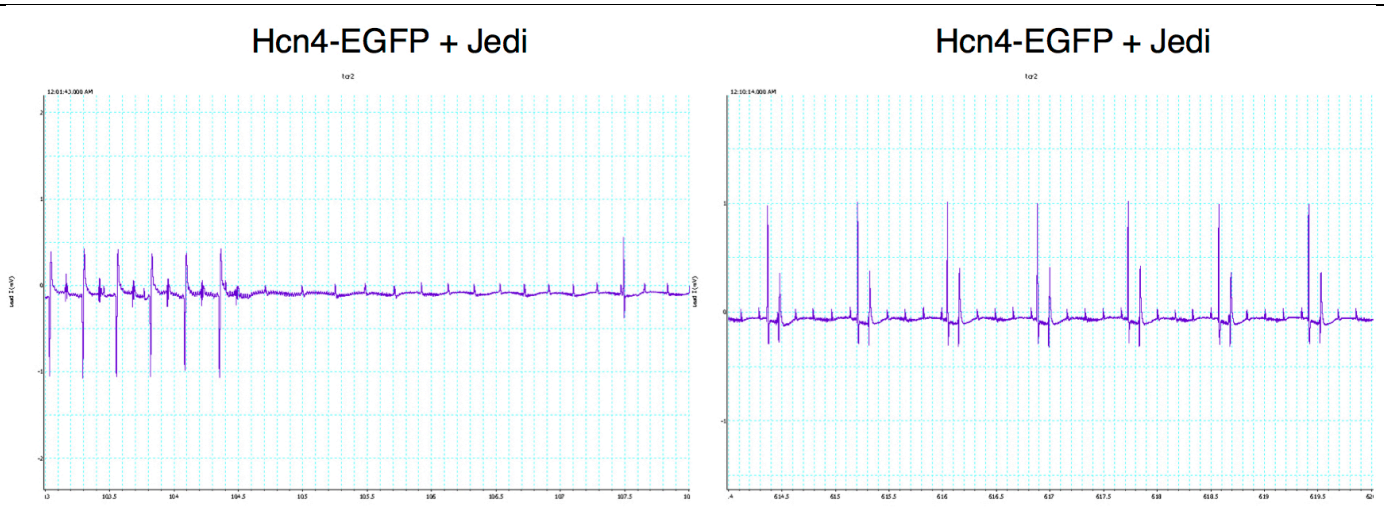
(a) Blood from bone marrow transplanted CX3CR1-EGFP mice was obtained 10 weeks after the transplant to confirm bone marrow engraftment prior to the transfer of the Jedi T cells. **(b)** Representative flow cytometry plots showing EGFP expression in the bone marrow (BM) of BM transplanted CX3CR1-EGFP mice 1 week after transferring Jedi T cells. **(c)** Representative flow cytometry plots showing EGFP expression in the lymph nodes (LN) of BM transplanted CX3CR1-EGFP mice 1 week after transferring Jedi T cells. **(d)** Representative images of the brain showing EGFP expression (signifying microglia) on tissue sections in different regions of the brain (20x magnification, bar represents 50 μm). **(e)** Representative flow cytometry plots showing CD45.1 Ctrl or Jedi T cells in the brain of BM transplanted CX3CR1-EGFP mice 1 week after transferring the indicated type of T cells. Graphs show the percentage of CD45.1+ cells compared to total CD45+ cells and the total number of CD45.1+ cells in the brain (n=3 mice/group). ***P<0.001 vs Control-treated. **(f)** Representative images of the brain showing EGFP expression (signifying microglia) and T cells (by CD3 staining) on tissue sections (20x magnification, bar represents 50 μm).



Supplementary Figure 4

Jedi T cell can stably deplete a renewing cell population and can be used to phenocopy Foxp3-DTR mice.

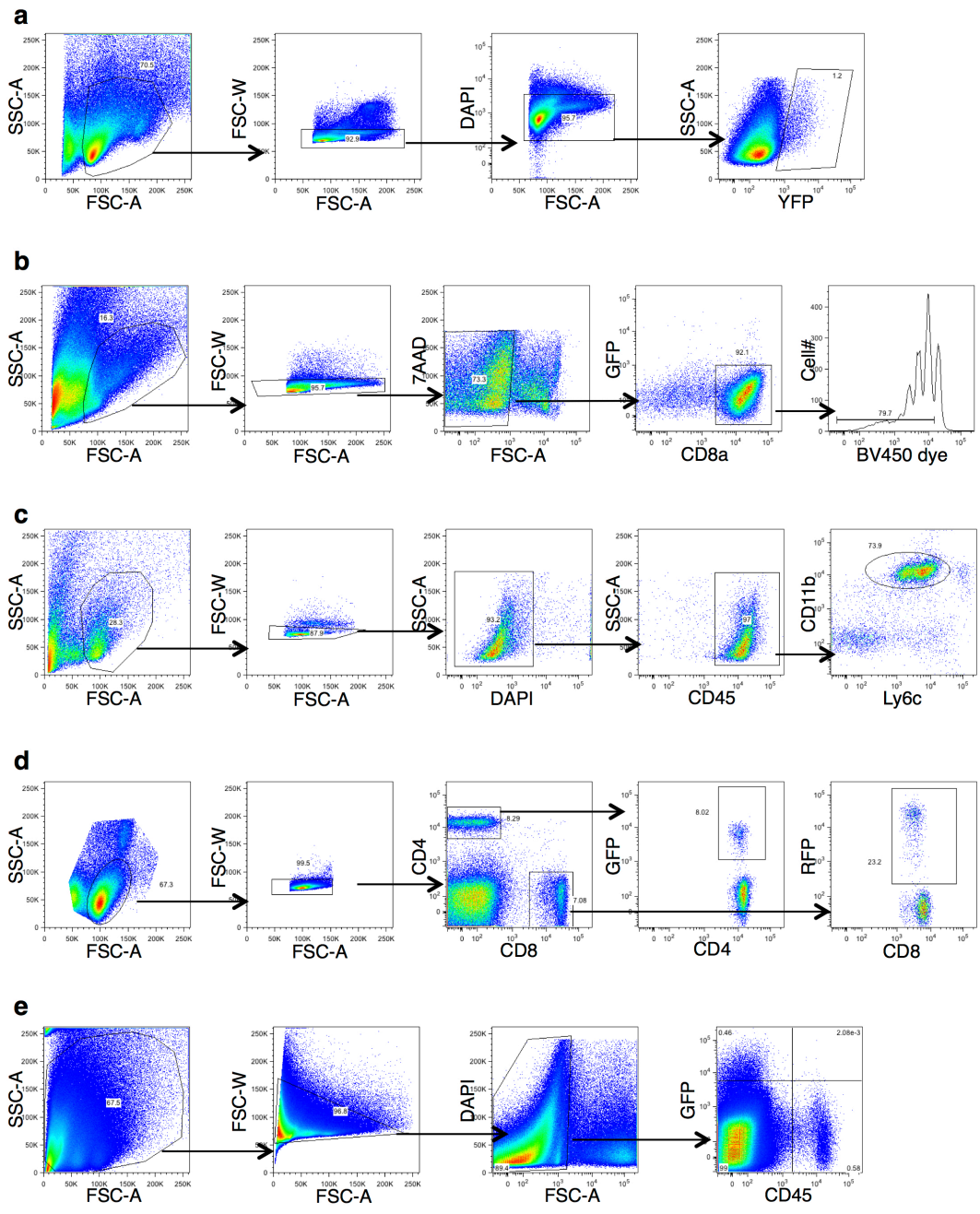
(a) Foxp3-EGFP male mice were injected with either control (Ctrl) or Jedi T cells. Percentage of EGFP+ cells among CD4 T cells was determined in the blood at the indicated time points (n=3-4 mice/group). **P<0.01 vs Control-treated, ***P<0.01 vs Control-treated. **(b)** Percentage of Jedi T cells among CD8+ T cells was determined in the blood at the indicated time points from the mice described in (a) (n=3-4 mice/group). The Jedi mice and control littermates were CD45.1 while the recipient Foxp3-EGFP mice were CD45.2, which permitted the cells to be detected by flow cytometry analysis. **(c)** Representative flow cytometry plots showing neutrophils in the blood of Ctrl or Jedi-treated male Foxp3-EGFP mice 9 days after the treatment. Graphs show the percentage of neutrophils (CD11b+ $Gr1^{hi}$ $SSC\text{-}A^{hi}$) compared to total live cells in the blood (n=3-4 mice/group). ***P<0.001 vs Control-treated. **(d)** Male Foxp3-EGFP mice were injected with either $5x10^4$, (blue), $1.5x10^5$ (red), or $5x10^5$ (green) RFP+ CD8+ Jedi T cells and vaccinated against EGFP. The percentage of EGFP+ cells among CD4 T cells was determined in the blood at the indicated time points. The graph represents the percentage of GFP+/CD4+ cells compared to the initial time point prior to the injection of the T cells at day 0. Each line represents one mouse. **(e)** Percentage of CD8+ Jedi T cells among total CD8+ T cells was determined in the blood at the indicated time points from the mice described in (d). The Jedi mice were RFP+, which permitted the cells to be detected by flow cytometry analysis. Each line represents one mouse.



Supplementary Figure 5

Jedi T cells can deplete rare cell populations to study their function.

Electrocardiograms (ECG) from additional mice treated as described in Figure 4. Images are representative of 2 experiments (n=4 mice/group).



Supplementary Figure 6

Gating strategies for flow cytometry analysis.

(a) Representative gating strategy of the spleen of B10D2 mice after injection of either LV.GFP or LV.YFP and adoptive transfer of either control or Jedi T cells. This gating strategy was used in Figure 1c and 1e. **(b)** Representative gating strategy of the *in vitro* cultures of BM-DCs and T cells to determine T cell proliferation. This gating strategy was followed in Supplementary Figure 2c. **(c)** Representative gating strategy of the brain from CX3CR1-GFP mice after adoptive transfer of either control or Jedi T cells. This gating strategy was used in Figure 2. **(d)** Representative gating strategy of the blood from FoxP3-GFP mice after adoptive transfer of either RFP+ control or RFP+ Jedi T cells. This gating strategy was used in Figure 3b and c. **(e)** Representative gating strategy of the heart from Hcn4-GFP mice after adoptive transfer of either control or Jedi T cells. This gating strategy was used in Figure 4d.