Supplementary figures

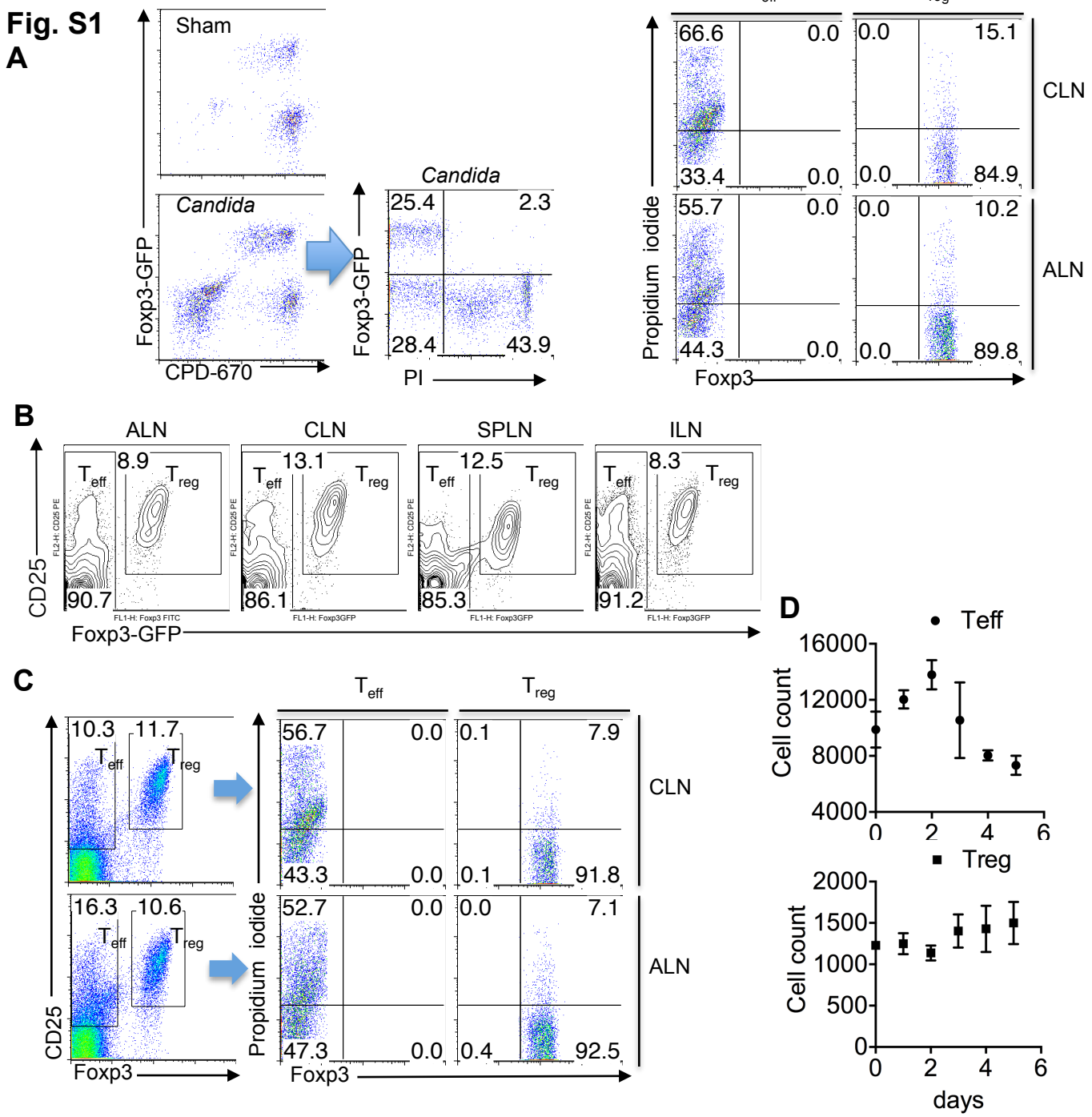

Fig. S1. A. Foxp3^{+} T$_{regs}$ do not show increased proliferation, but increased viability on d5 after OPC infection. We adoptively transferred cell proliferation dye-670 (CPD-670) labeled CD4^{+} cells isolated from CD45.2 Foxp3^{GFP} reporter mice, into CD45.1 *Rag1*$^{-/-}$ mice four days before infection. We infected the mice, and assessed the proliferation by CPD-670 dilution, and viability by PI staining (gated on CD45.2+ CD4+ cells) on d5 after OPC infection (**left panel**). Foxp3^{GFP} reporter mice were infected as in Fig 1A, and viability was measured using PI staining on day 5 after primary infection (**right panel**). **B,C. Gating strategy to assess viability of CD4 cells in *C.albicans* infected Foxp3^{GFP} reporter mice.** Flow cytometric contour plots of CD25 and Foxp3-GFP expression of the cells, isolated from the spleen (SPLN) or lymph nodes (LN) on day 1 after reinfection. Plots show CD4 gated cells, with further gating on Foxp3 - (T_{eff}) or CD25+ Foxp3+ (T_{reg}) cells in **B**, or CD25+Foxp3- (T_{eff}) or CD25+Foxp3+ (T_{reg}) cells. We refer to the non T_{reg} cells activated by the infection *in vivo* as effector cells (T_{eff}). (**C**). **D)** Mice were infected as in Fig 1A. At indicated time-points after primary infection, cells were harvested and stained for flow cytometry. To determine their absolute numbers, CD4+T cells were counted by adding 10000 fluoresbrite beads in 500ul of single-cell suspensions, and counting 5000 fluoresbrite beads using flow cytometry.

Supplementary figures

Fig. S2

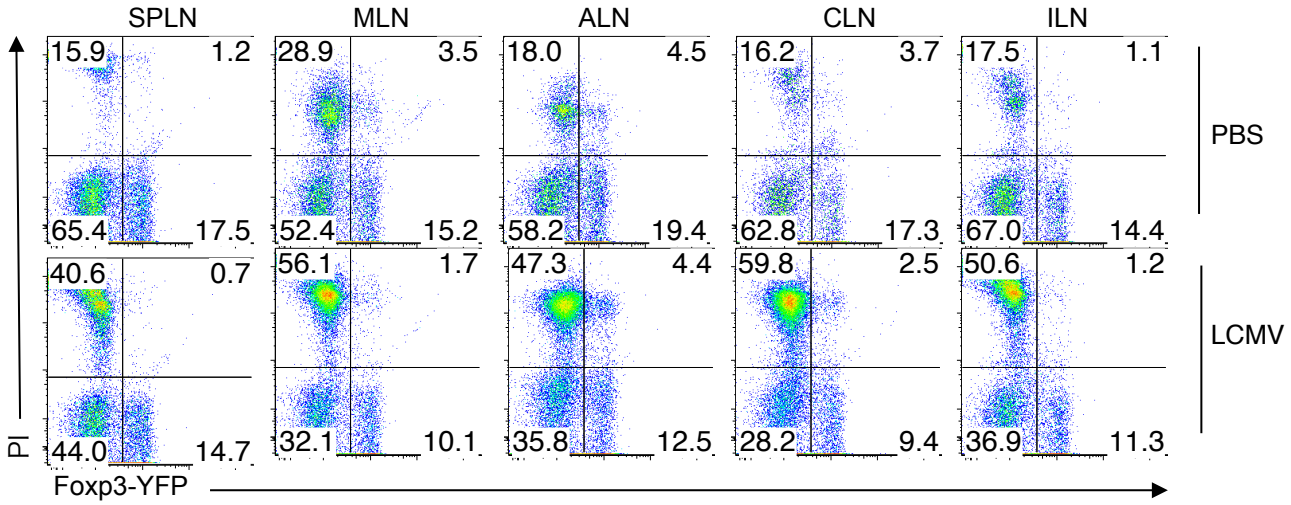

Fig. S2. T_{regs} show increased viability during chronic LCMV (clone 13) infection in mice. $Foxp3^{YFP}$ mice (n = 3/group) were infected with LCMV as described in methods. Cells from indicated lymphoid organs were isolated on day 22 after infection and apoptosis of YFP^+ T_{regs} and YFP^- effector cells was assessed using PI staining. Flow cytometric contour plots of PI staining (y-axis) and Foxp3-YFP (x-axis) expression of the cells isolated from the spleen (SPLN), mesenteric lymph nodes (MLN), ALN, CLN and ILN (all gated on $CD4^+$ cells). Data are representative of the results from two experiments each with viability measured at 3 different time-points.

Fig. S3

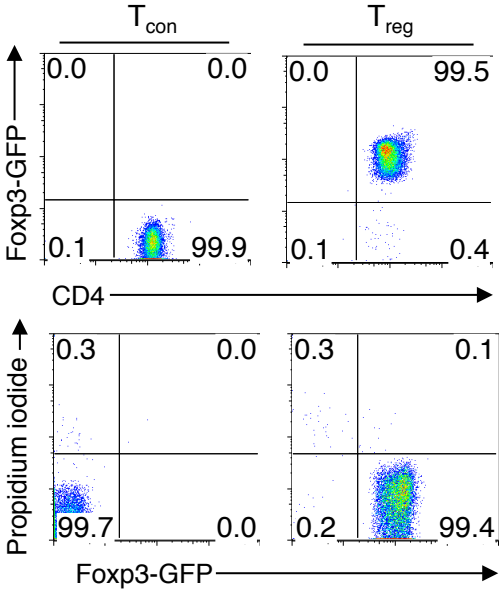

Fig.S3. *Ex vivo* sorted T_{cons} and $T_{regs.}$ Flow cytometric dot plots of CD4 and Foxp3GFP expression (upper panel), and Foxp3GFP expression and propidium iodide (PI) staining (lower panel) of mouse T_{cons} (left) and mouse T_{regs} (right), enriched using magnetic isolation and further purified by FACS.

Supplementary figures

Fig. S4

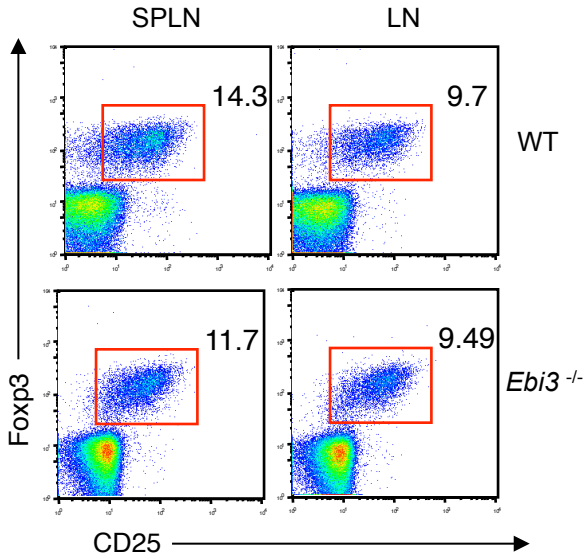

Fig.S4. T_{reg} frequency in WT and *Ebi3*$^{-/-}$ mice *ex vivo*. Flow cytometric dot plots of CD25 and Foxp3 expression of WT mice (upper panel), and *Ebi3* $^{-/-}$ mice (lower panel). Plots show CD4 gated cells.

Fig. S5

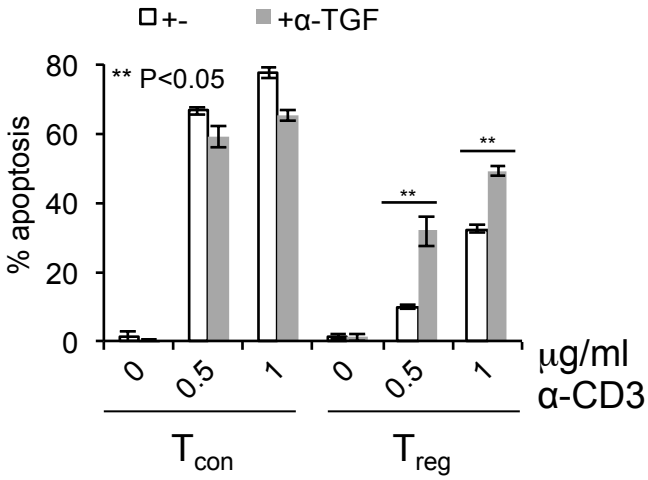

Fig.S5. Pre-blocking TGF-β partially abrogates RICD resistance in $T_{regs.}$ Percentage apoptosis of mouse T_{cons} and T_{regs} (right), which were restimulated under RICD conditions for 18 hours in the presence or absence of TGF-β blocking antibody (clone 1d11). We pre-blocked TGF-β by adding 50 µg/ml of anti-TGF-β antibody both during primary stimulation and RICD induction.

Supplementary figures

Fig. S6

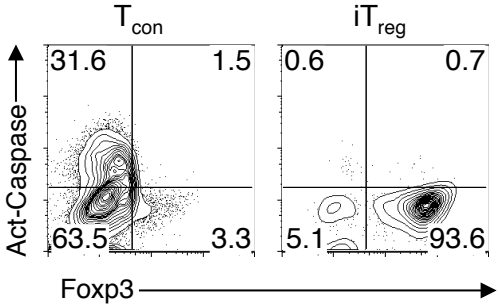

Fig.S6. Activated Caspase-9 staining in T_{cons} and iT_{regs}. Flow cytometric contour plots showing Foxp3 and active caspase-9 expression of mouse T_{cons} (left) and mouse iT_{regs} (right), which were restimulated under RICD conditions for 8 hours.

Fig. S7

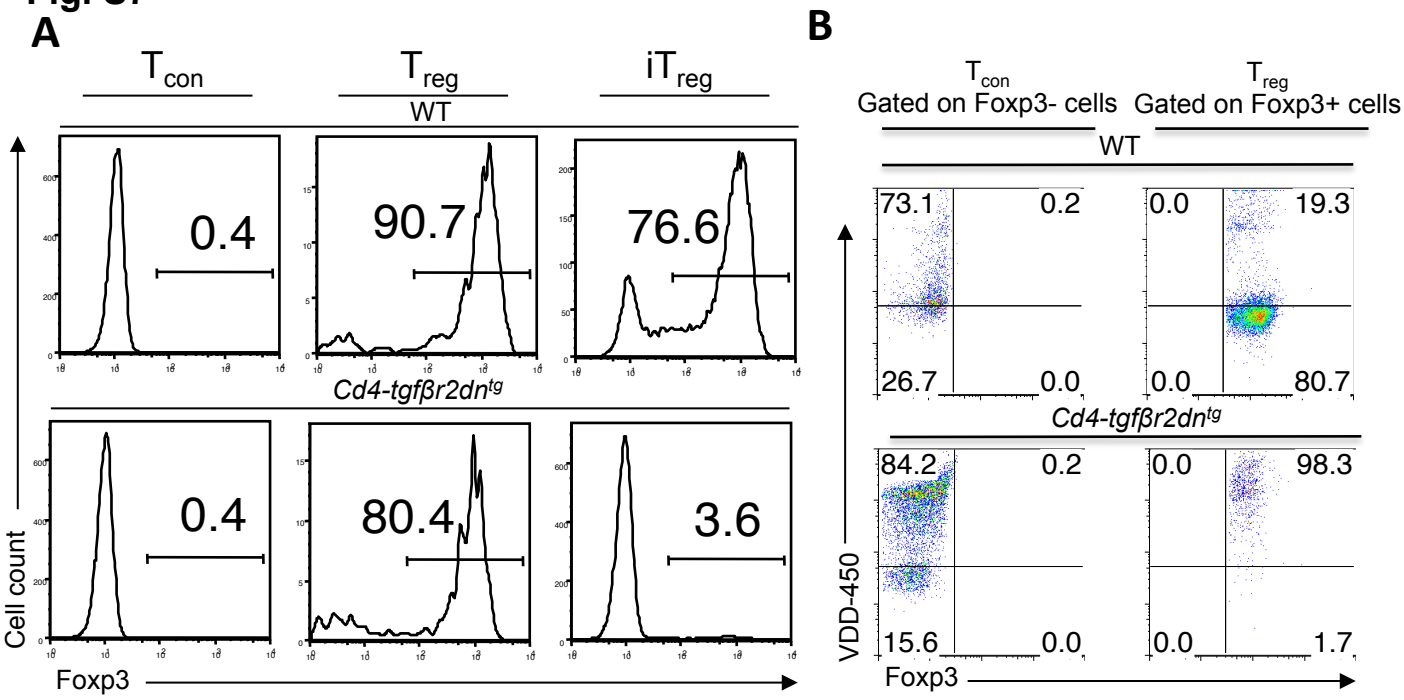

Fig.S7. Foxp3 expression in T_{cons}, T_{regs} and iT_{regs}. **A)** Flow cytometric histogram plots showing Foxp3 expression of T_{cons} (left), T_{regs} (middle) and iT_{regs} (right) from WT (above) or *Cd4-tgfβr2dn*^{tg} (below) mice, which were stimulated for 4 days, and before restimulation under RICD conditions. **B)** Cells were stimulated and restimulated as in A. Viability of the cells from WT (above) or *Cd4-tgfβr2dn*^{tg} (below) mice, as measured by viability dye 450 (VDD 450) gating on Foxp3+ (intracellularly stained) cells.