Fig. S1

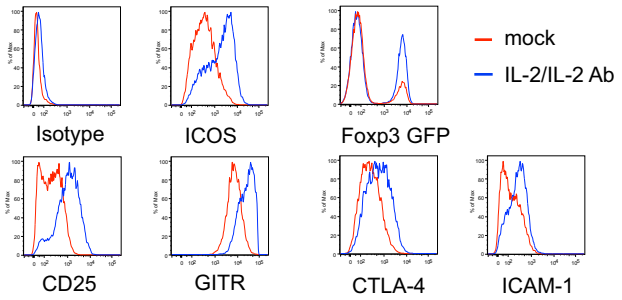
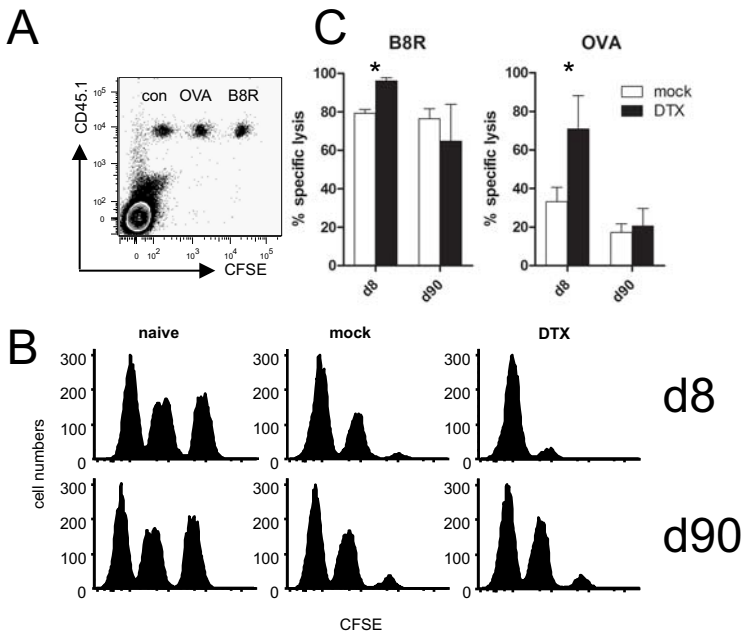


Fig. S2



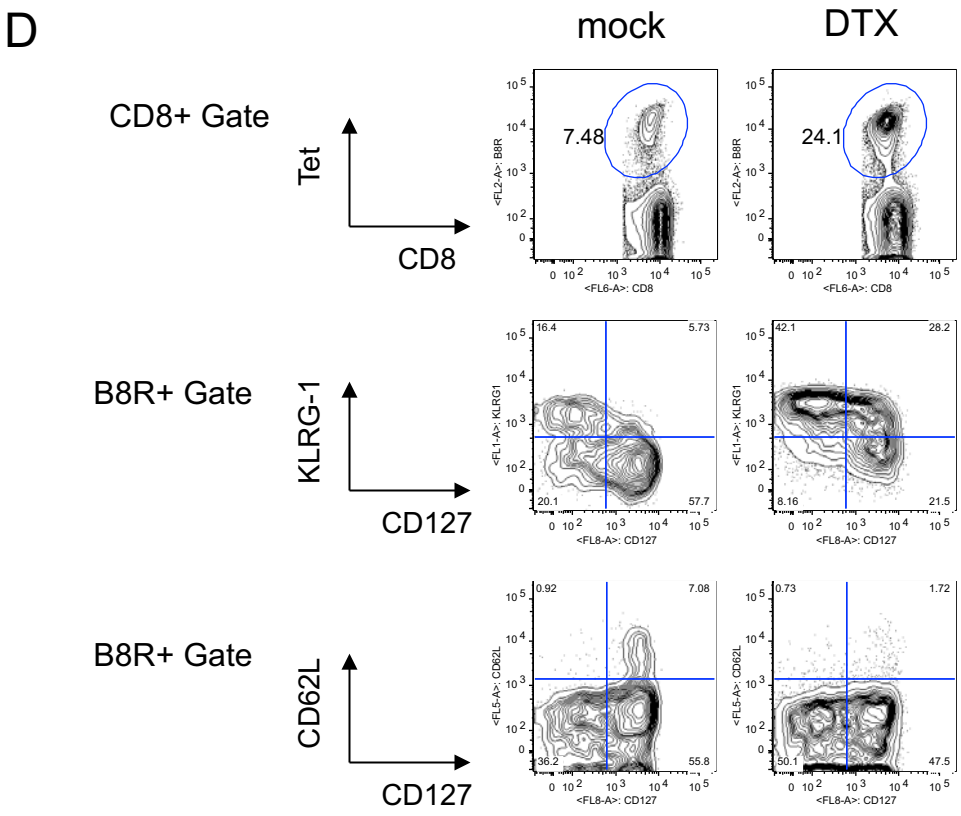
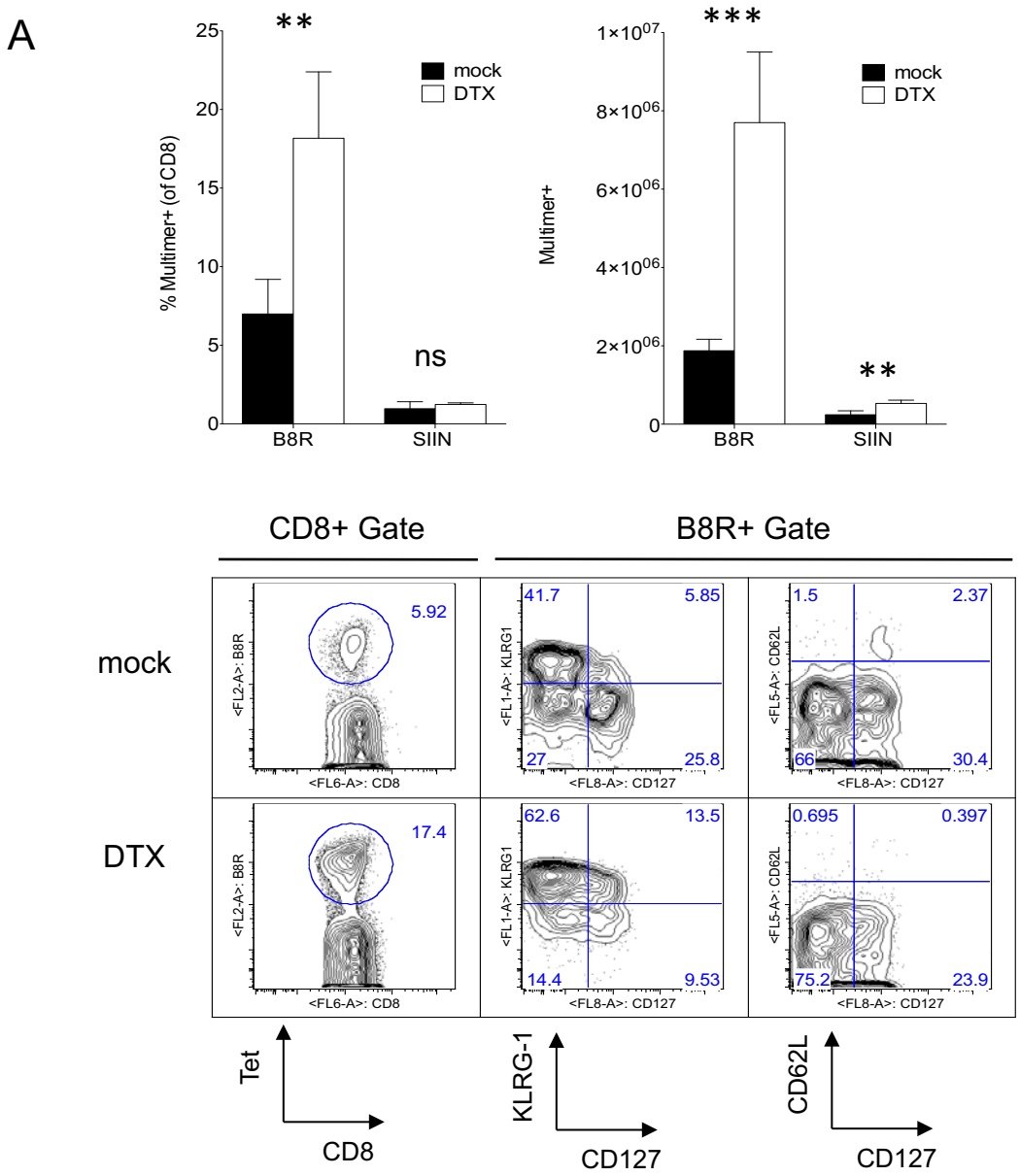


Fig.S3



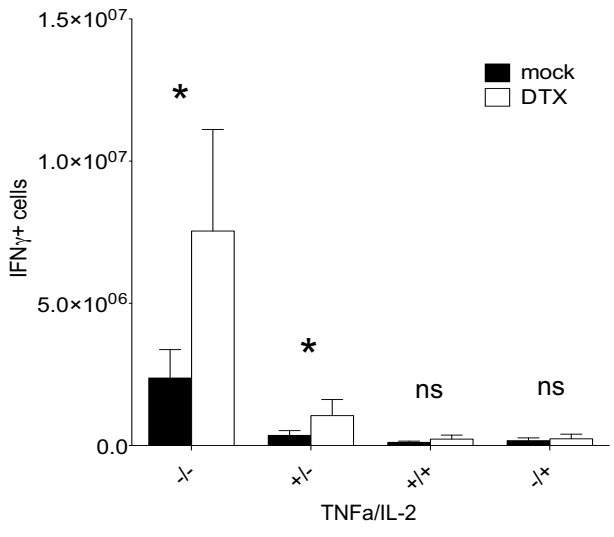
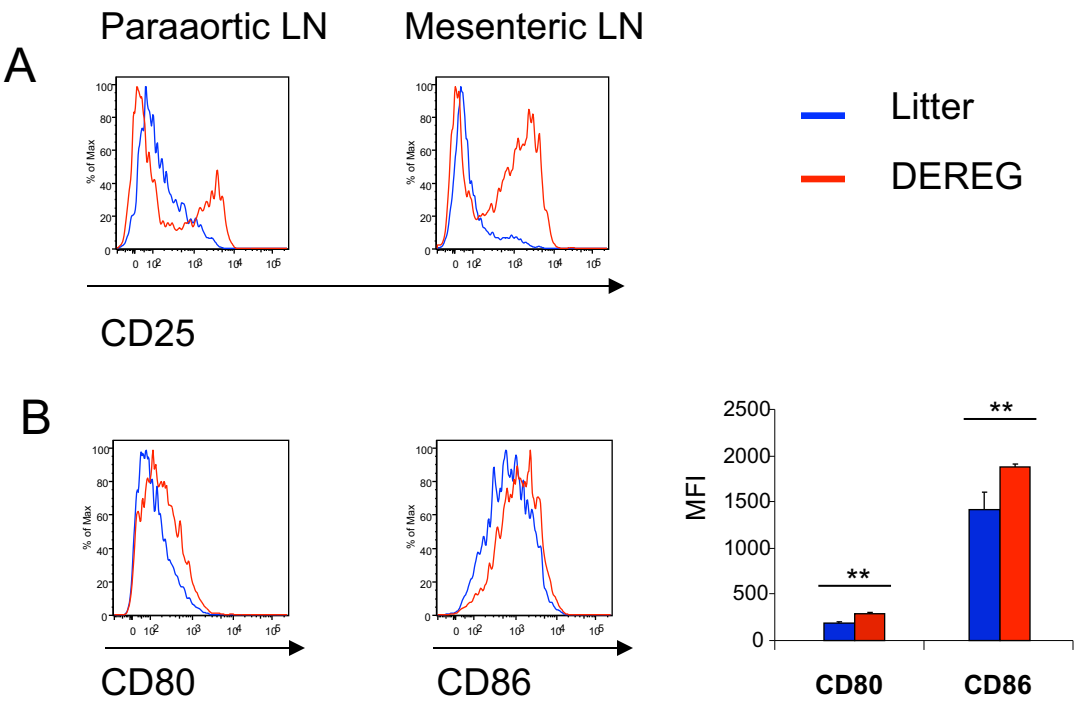


Fig. S4



Supplemental Material:

Figure S1

IL-2/IL-2 antibody complexes induce proliferation and activation of Treg *in vivo*. Foxp3-reporter mice (n=2) were treated with IL-2/IL-2 antibody complexes or mock for three consecutive days. One day later splenocytes were isolated and Treg were analyzed using several surface markers.

Figure S2

Enhanced *in vivo* killing after priming in the absence of Treg is observed in the acute but not in the memory phase.

Groups of DEREG mice (n=3) were immunized with MVA OVA i.p. and treated with DTX or mock on d1- d3. Congenic splenocytes were stained with different concentrations of CFSE and pulsed with B8R (bright), OVA_{257} (intermediate) or control peptide (dim) before transfer (A). As control and for assessment of relative killing rates, stained and pulsed splenocytes were transferred into naïve and immunized previously depleted of Treg or mock-treated mice (B). On day 8 or d60 post prime, mice were analyzed for their capacity to lyse peptide pulsed splenocytes *in vivo (C).* Data are representative of three independent experiments. Bars show mean values, error bars show SEM, $* p< 0.05$.

(D) Treg control the size of the monofunctional, short-lived effector T cell pool: Groups of DEREG mice (n=4) were immunized with MVA OVA i.p., treated with DTX or mock-treated and analyzed on d8. Representative plots show relative distribution of KLRG-1, CD62L, and/or CD127 expression of T cells binding to B8R loaded multimers.

Figure S3

Treg control the size of the monofunctional, short-lived effector T cell pool during infection with replicating Vaccinia Virus

Groups of DEREG mice (n=4) were immunized with 2x10e6 pfu VV OVA i.p., treated with DTX or mock-treated on d1- d3 and analyzed on d8. A shows representative plots and graphs with relative and absolute numbers of KLRG-1, CD62L and/or CD127 expression of multimer binding CD8 T cells. B shows absolute numbers of IL-2 and/or TNFα producing, IFNγ+ cells after stimulation with B8R peptide. Data are presented as mean +SD and are representative of 3 independent experiments. ns= not significant, *=$p<0.05$, **= $p<0.01$, ***=$p<0.001$

Figure S4

Treg control CD25 expression on T cells and CD80/CD86 expression on DC and CD86 KO mice recapitulate Treg mediated suppression.

Groups of DEREG mice or littermates were immunized with MVA OVA i.p. treated with DTX on d1- d3 and analyzed on d3.5. OT-1 T cells were transferred on d-1 (4x 10e5) and analyzed on d3.5 in para-aortic or mesenteric LN. (A) shows representative histograms of CD25 expression on OT-1 T cells. B shows representative histograms and bar graphs of CD80/CD86 expression on DC harvested from mesenteric LN on d3.5.