

Supplemental Figures.

Kohlhapp et al. 11-01580-FLR Revision.

CD8+ T cells Sabotage their own Memory Potential through IFN- γ -dependent modification of the IL-12/IL-15R α axis on Dendritic Cells

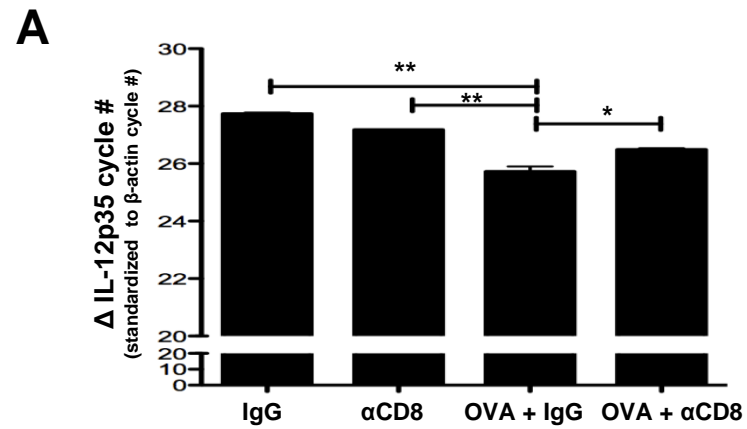
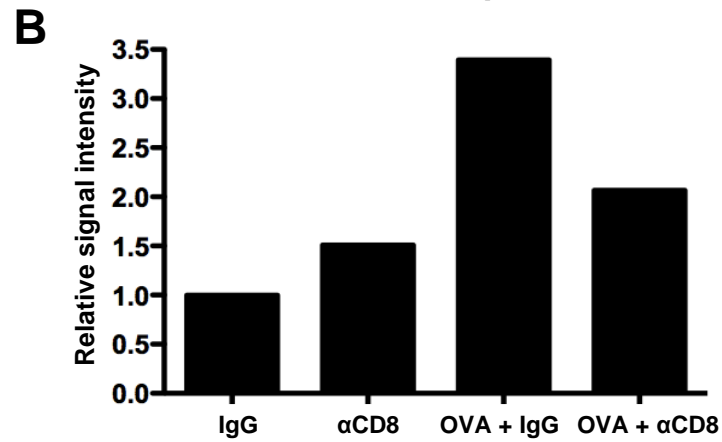
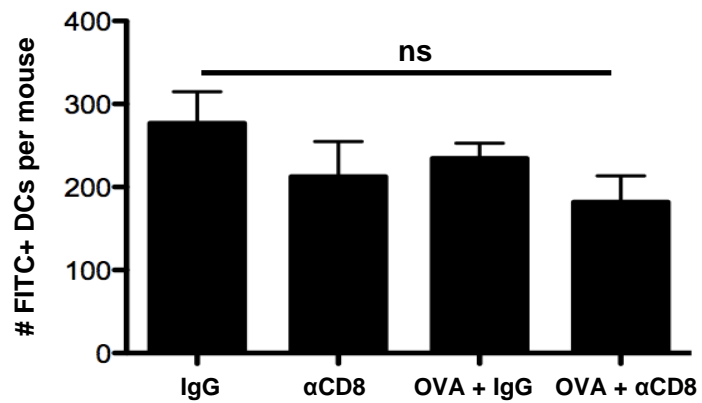
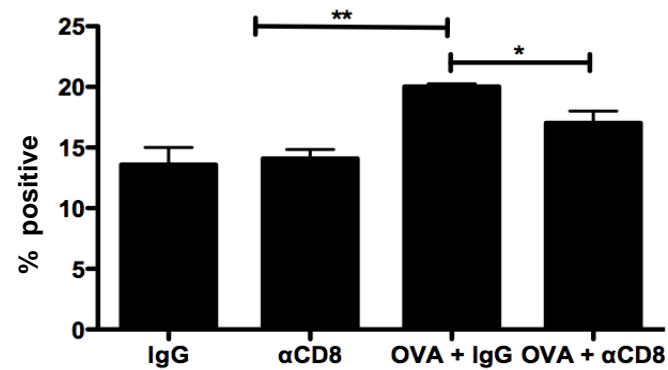
Figure S1**IL-12p35****IL-12p35****C** **Dendritic Cells****D** **IL-12p40**

Figure S1. DC expression of IL-12 and DC number. (A and B) B6 mice were OVA vaccinated and CD8 depleted (250 μ g at day 0 and 100 μ g at day 5). RT-PCR was performed on mRNA isolated from pooled draining inguinal lymph nodes (n=5) on day 6. Primers specific for IL-12p35 and β -actin were used. Cycle numbers for IL-12p35 standardized to the cycle numbers of β -actin are shown (A). Signal intensities relative to β -actin and then compared to input values for IgG-treated mice are shown (B). (C) . B6 mice were OVA vaccinated and CD8 depleted (250 μ g at day 0 and 100 μ g at day 5). Mice were painted with a FITC solution (2%) on day 5 (n= 3-5). Draining inguinal lymph nodes were harvested on day 7. (D) B6 mice (n= 3-5) were footpad vaccinated with CFA and OVA vaccination at day 0. IL-12 production was assessed 4 days after vaccination in the draining inguinal lymph nodes. All data are representative of results from 2-3 independent experiments. *p < 0.05, **p < 0.01.

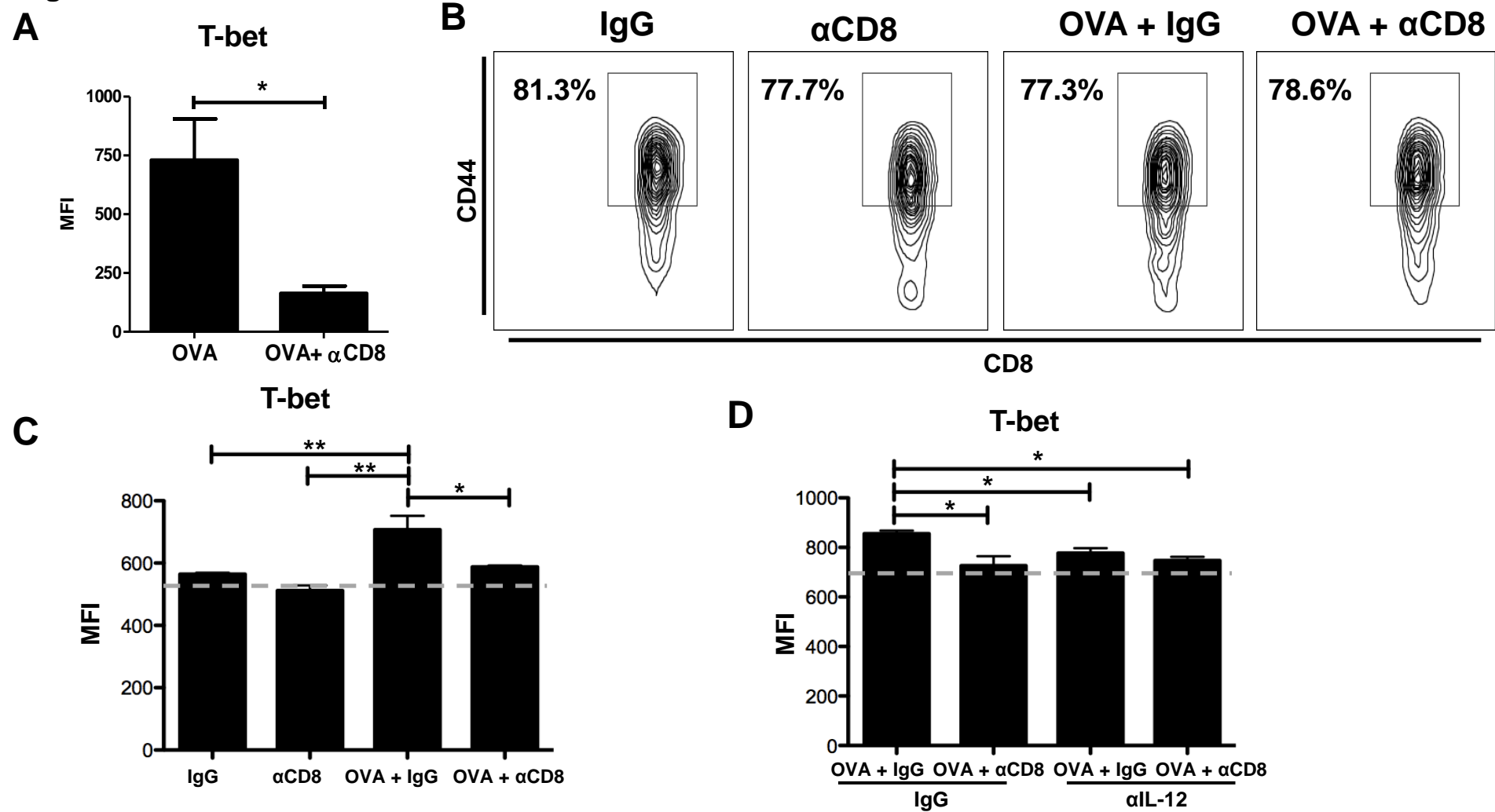
Figure S2

Figure S2. OT-I CD8⁺ T cell activation. (A) B6 mice were OVA vaccinated (days 0, 5, and 10) and treated with either IgG or anti-CD8 antibody (250 μ g at day 0; 100 μ g at day 5). OT-I CD8⁺ T cells were adoptively transferred at day 10. The draining inguinal lymph nodes were harvested 5 days after adoptive cell transfer. Cumulative bar graph showing intracellular T-bet expression on OT-I CD8⁺ T cells. (B) CD11c⁺ DCs were magnetically purified from inguinal DLNs, pooled (n=10), and analyzed in triplicate. Purified CD11c⁺ DCs (10⁴) were loaded with OVA₂₅₇₋₂₆₄ and co-cultured for 6 hours with magnetically-purified OT-I CD8⁺ T cells (10⁵). (B) CD11c⁺ DCs were magnetically purified from inguinal DLNs of mice treated with either IgG or anti-CD8 antibody (250 μ g at day 0; 100 μ g at day 5), pooled (n=10), and run in triplicate, with some mice receiving OVA vaccination. Purified CD11c⁺ DCs (10⁴) were loaded with OVA₂₅₇₋₂₆₄ peptide and co-cultured for 6 hours with magnetically-purified naïve OT-I CD8⁺ T cells (10⁵). Bar graph showing the difference in intracellular T-bet expression in OT-I Thy1.1⁺ CD8⁺ T cells compared to T-bet expression of unstimulated OT-I CD8⁺ T cells (MFI = 550) shown with gray dotted line cultured in the absence of DCs. (C) Co-culture was performed as in (B) with either control IgG (1 μ g/mL) or IL-12 blocking antibody (1 μ g/mL) at the beginning of the co-culture. T-bet expression was determined as in (B). Bar graph showing the difference in intracellular T-bet MFI in OT-I Thy1.1⁺ CD8⁺ T cells compared to T-bet expression of unstimulated OT-I CD8⁺ T cells (MFI = 720) shown with gray dotted line cultured in the absence of DCs. All data are representative of results from 2-3 independent experiments. *p < 0.05, **p < 0.01.

Figure S3

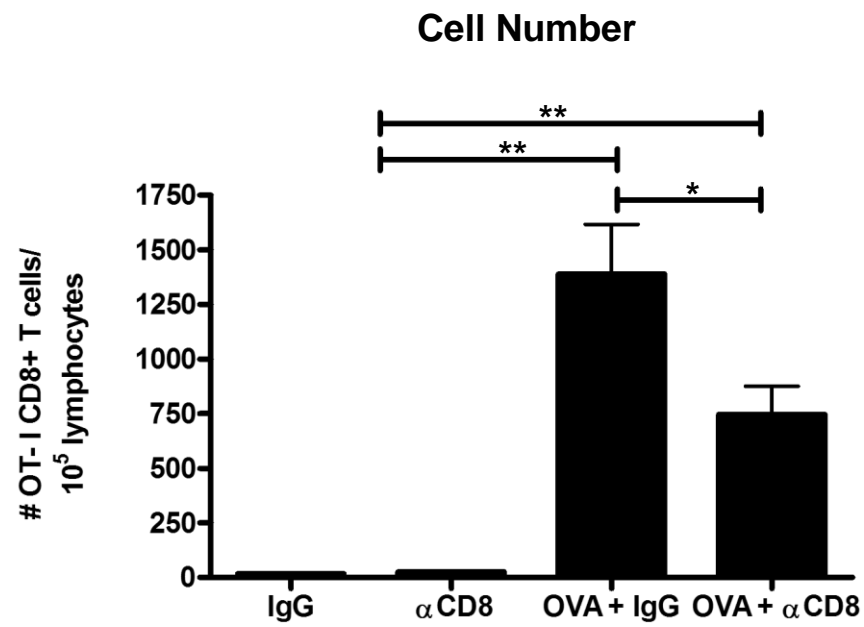


Figure S3. Number of OT-I CD8+ T cells at the effector phase. B6 mice were OVA vaccinated and CD8 depleted or IgG treated (250 μ g at day 0 and 100 μ g at day 5) and received adoptively-transferred OT-I Thy1.1+ CD8+ T cells (10⁵) at day 10. Draining inguinal lymph nodes were harvested 5 days later and OT-I CD8+ T cells were enumerated. All data shown are representative of results from 2-3 independent experiments with n=3-5 per group. *p < 0.05, **p < 0.01

Figure S4

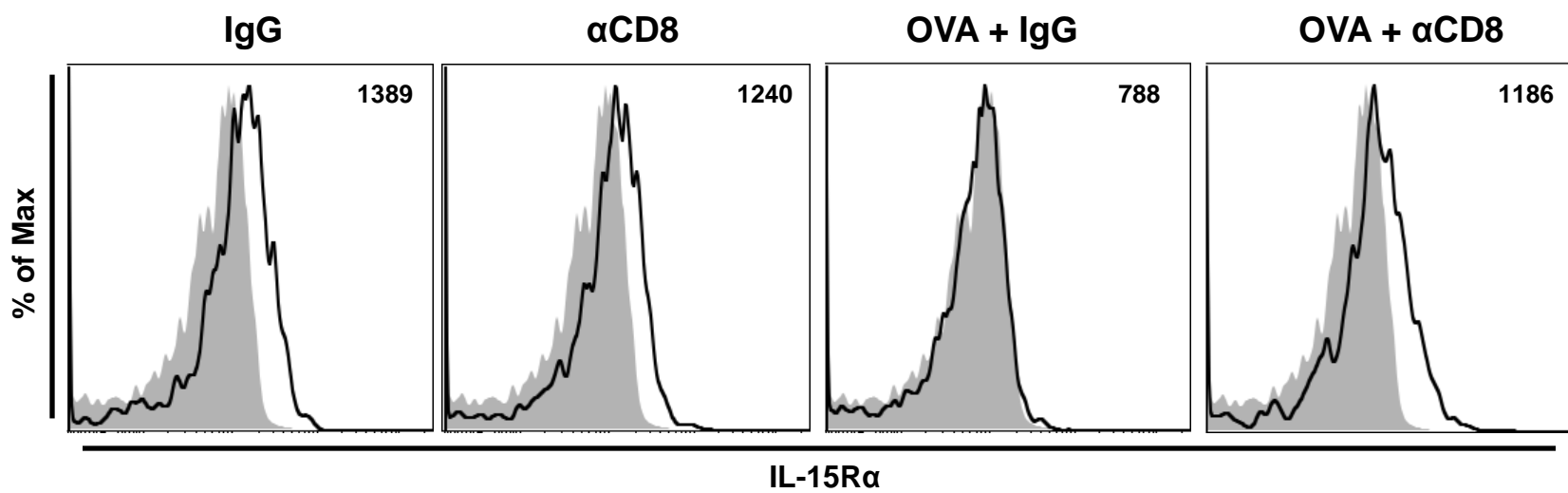


Figure S4. IL-15R α expression on DCs at day 14. B6 mice were OVA vaccinated and CD8 depleted or IgG treated (250 μ g at day 0 and 100 μ g at day 5) and adoptively transferred OT-I Thy1.1+ CD8+ T cells (10^5) at day 10. Draining inguinal lymph nodes were harvested 14 days later and IL-15R α expression on DCs was analyzed. Numbers listed indicate MFI. All data shown are representative of results from 2-3 independent experiments with n=3-5 per group.