

### **Supplementary Figure legend.**

#### **Figure S1 Blocking IL-10 signalling at the time of immunization increases both antigen specific IFN $\gamma$ secreting CD8 $^+$ T and CD4 $^+$ T cells responses**

Group of 3 C57BL/6 mice were primed with 50  $\mu$ g of OVA, 15  $\mu$ g of LPS at 14 days apart with or without 500  $\mu$ g of anti IL10R Ab or normal rat serum (NRS). Six days after final examination, spleen cells from immunized mice were collected. ELISA, ELISPOT assay and Intracellular Staining for IFN $\gamma$  was performed as described in materials and methods. A: Left: ELISPOT assay for CD8 $^+$  T cell responses to a MHC I restricted peptide SIINFKEL. Middle: Percentage of CD8 $^+$  IFN $\gamma$  $^+$  T cells by flow cytometry analysis. Splenic cells pulsed with SIINFKEL overnight, and stained with CD4, CD3 and IFN $\gamma$ . CD3 $^+$ , CD4 $^-$ . CD3 $^+$  cells were gated and CD3 $^+$ CD4 $^-$ IFN $\gamma$  $^+$  cells are shown. Right: Splenic cells pulsed with SIINFKEL overnight, supernatants were measured for the presence of IFN $\gamma$  by ELISA. Results throughout are the mean $\pm$ 1SEM of three individual mice and represent one of two independent experiments. B: Splenic cells pulsed with OVA overnight, and stained with CD4, CD3 and IFN $\gamma$ . CD3 $^+$  cells were gated and CD3 $^+$ CD4 $^+$ IFN $\gamma$  $^+$  cells are shown. Left: FACS profile; Right: Percentage of CD4 $^+$ IFN $\gamma$  $^+$  T cells.

#### **Figure S2. Blocking IL-10 signalling at the time of OVA/LPS immunization increases the numbers of IL-10 producing cells.**

Group of 3 C57BL/6 mice were immunized with 50  $\mu$ g of OVA and 15  $\mu$ g of LPS with or without 500  $\mu$ g of anti IL10R Ab twice at 14 days apart. Six days after final

immunization, spleen cells from immunized mice were collected for surface staining and intracellular staining for IFN $\gamma$  and IL10. A: Splenic cells were collected and pulsed with 1 $\mu$ g/ml of OVA overnight, supernatants were measured for IL-10 by ELISA (Left). Or CD3<sup>+</sup> lymphocytes were gated. CD4 and IL10 were stained as described in materials and methods. B: CD4, GITR and IL10 were stained. Left: FACS profile of CD4<sup>+</sup>GITR<sup>+</sup>IL-10<sup>+</sup> cells, Right: summarised data of CD4<sup>+</sup>GITR<sup>+</sup>IL10<sup>+</sup> cells. C: splenic cells were cultured overnight in the presence of 1 $\mu$ g/ml of OVA; cells were stained for CD4, IL10 and IFN $\gamma$ . Left: FACS profile. Right: CD4<sup>+</sup>INF $\gamma$ <sup>+</sup>IL10<sup>+</sup> cells. Results throughout are the mean and 1 SEM of three individual mice and represent one of two independent experiments.

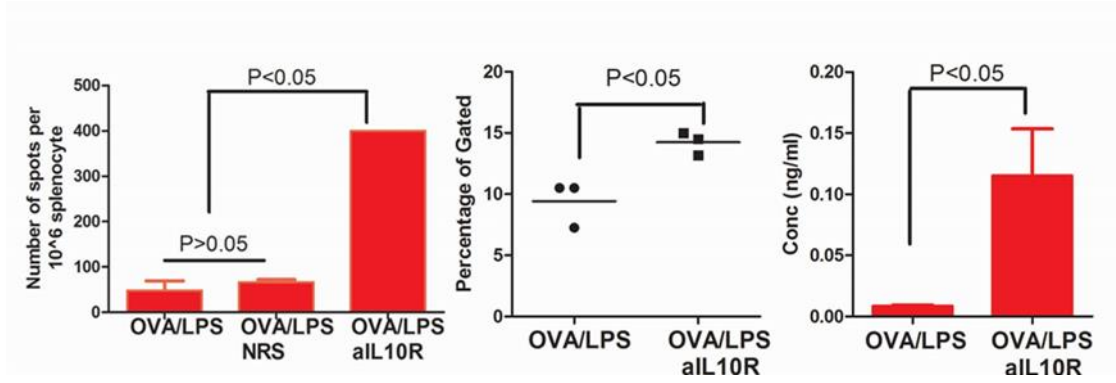
**Figure S3 Blocking IL-10 signalling at the time of OVA/LPS immunization in mice does not**

**increases the numbers of CD4<sup>+</sup>Foxp3<sup>+</sup> T cells.**

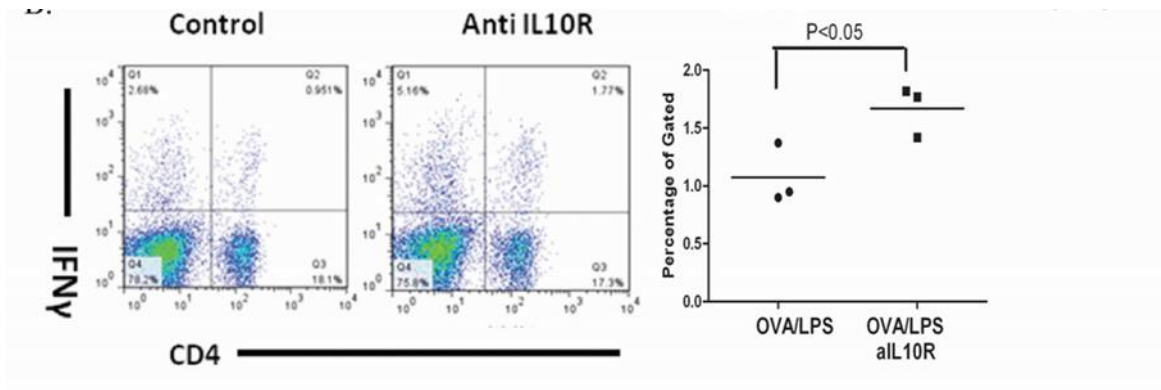
Group of 3 C57BL/6 mice were immunized with 50  $\mu$ g of OVA and 15  $\mu$ g of LPS with or without 500  $\mu$ g of anti IL10R Ab twice at 14 days apart. Six days after final immunization, spleen cells from immunized mice were collected for surface staining and intracellular staining of CD4 and Foxp3.

**Figure S1**

**A: CD8+ cells**

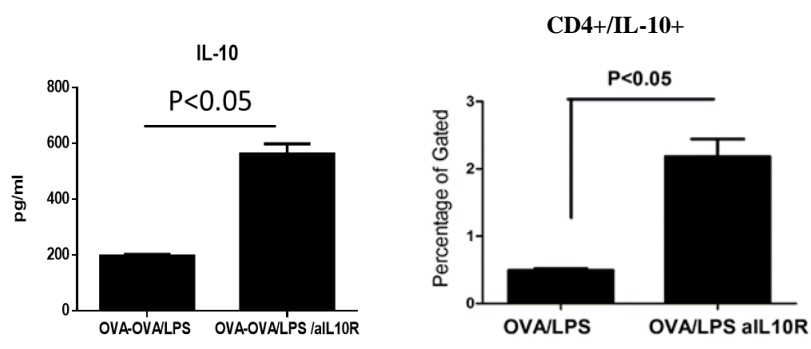


**B: CD4+ cells**

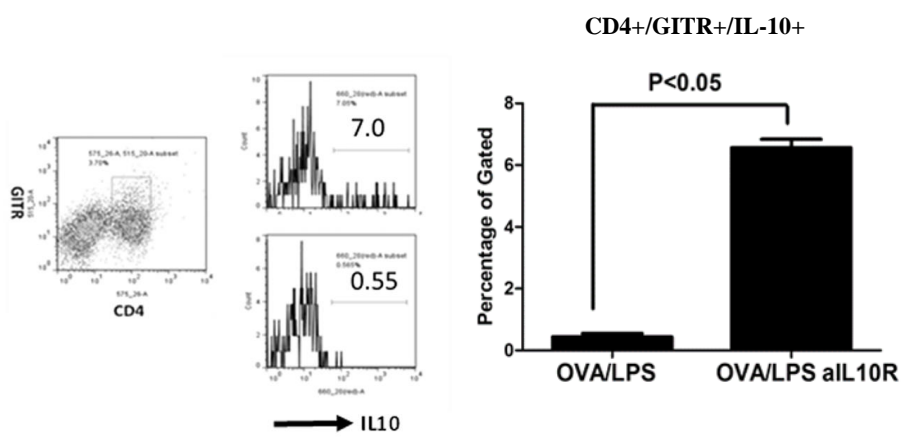


**Figure S2**

**A**



**B**



**C**

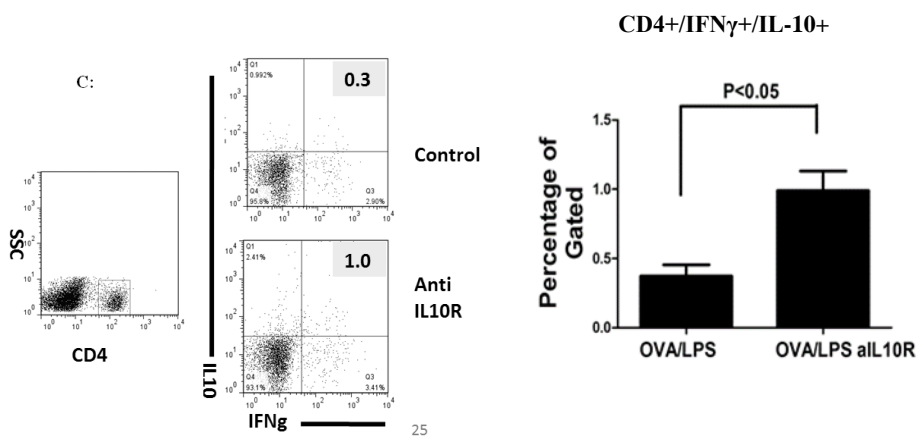


Figure S3

