

European Journal of Immunology

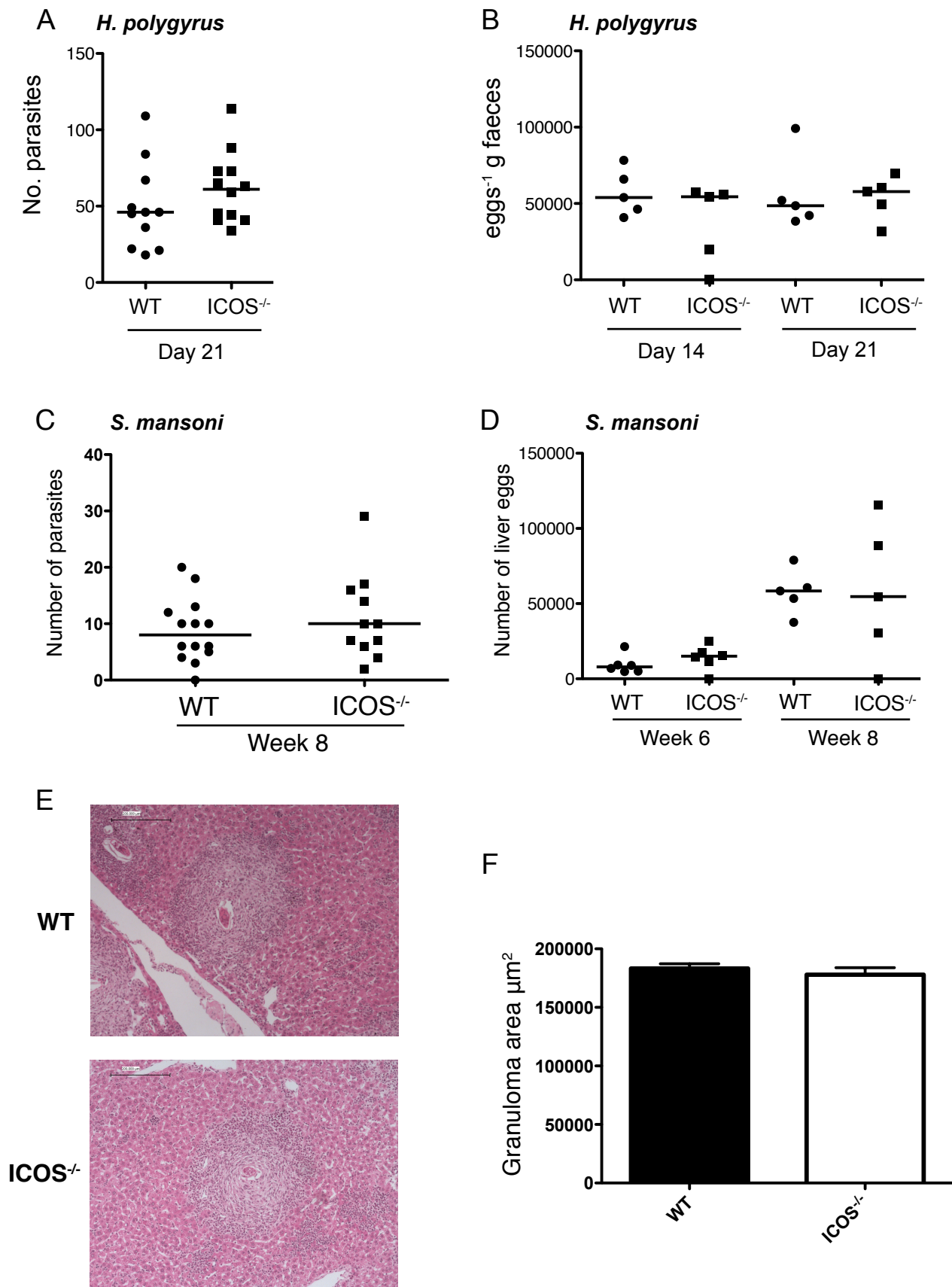
Supporting Information

for

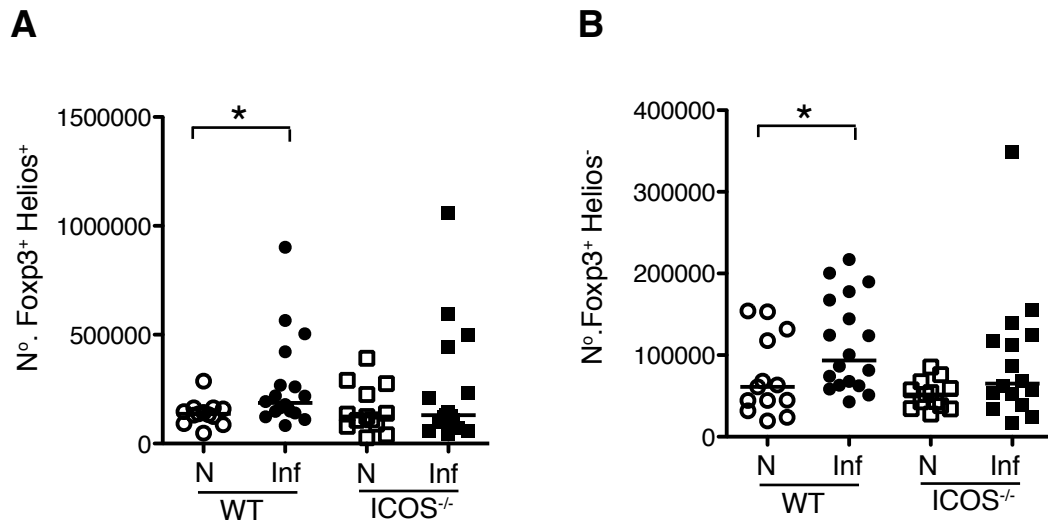
DOI 10.1002/eji.201242794

Stephen A. Redpath, Nienke van der Werf, Ana M. Cervera, Andrew S. MacDonald,
David Gray, Rick M. Maizels
and Matthew D. Taylor

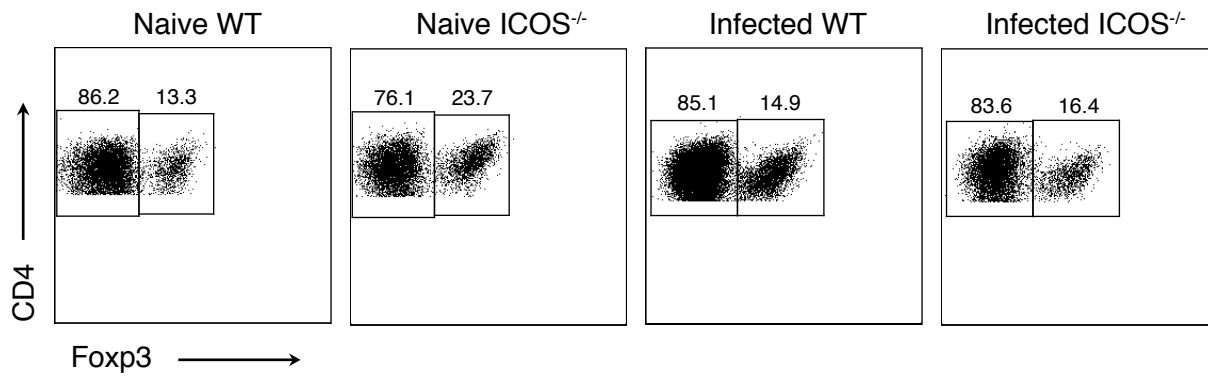
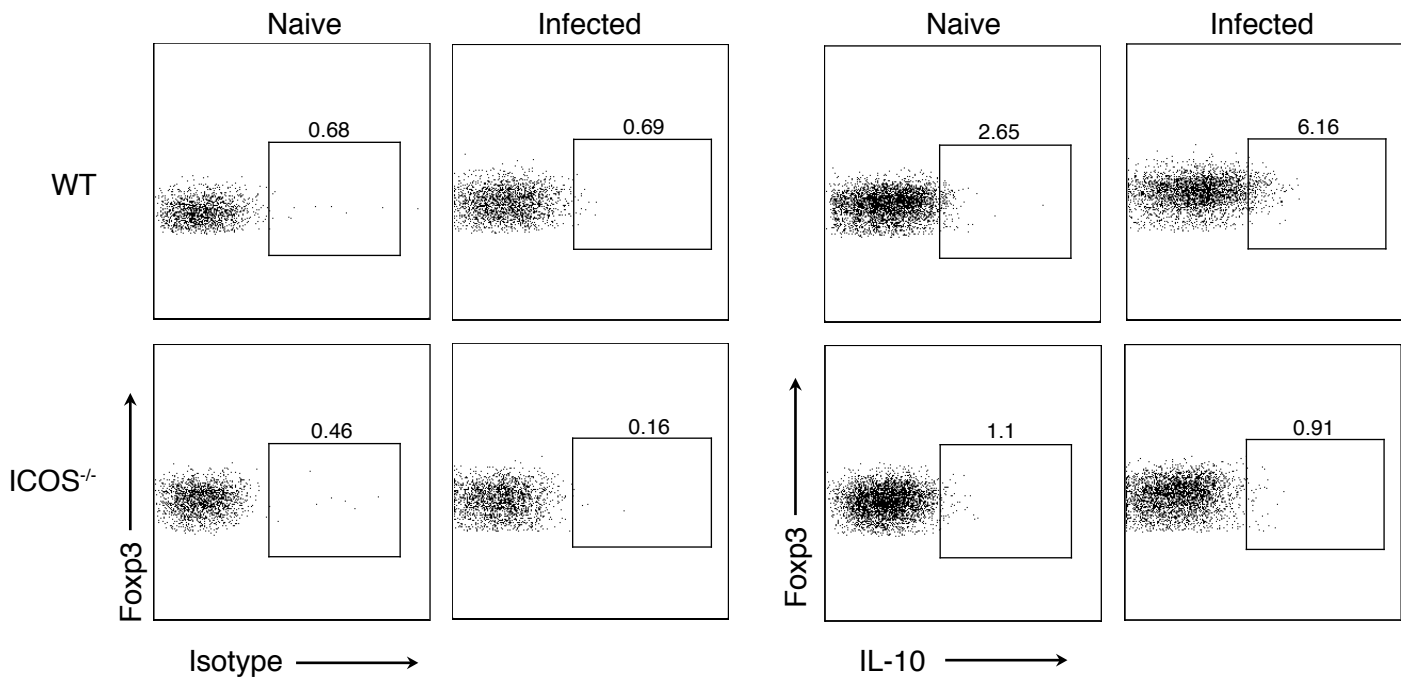
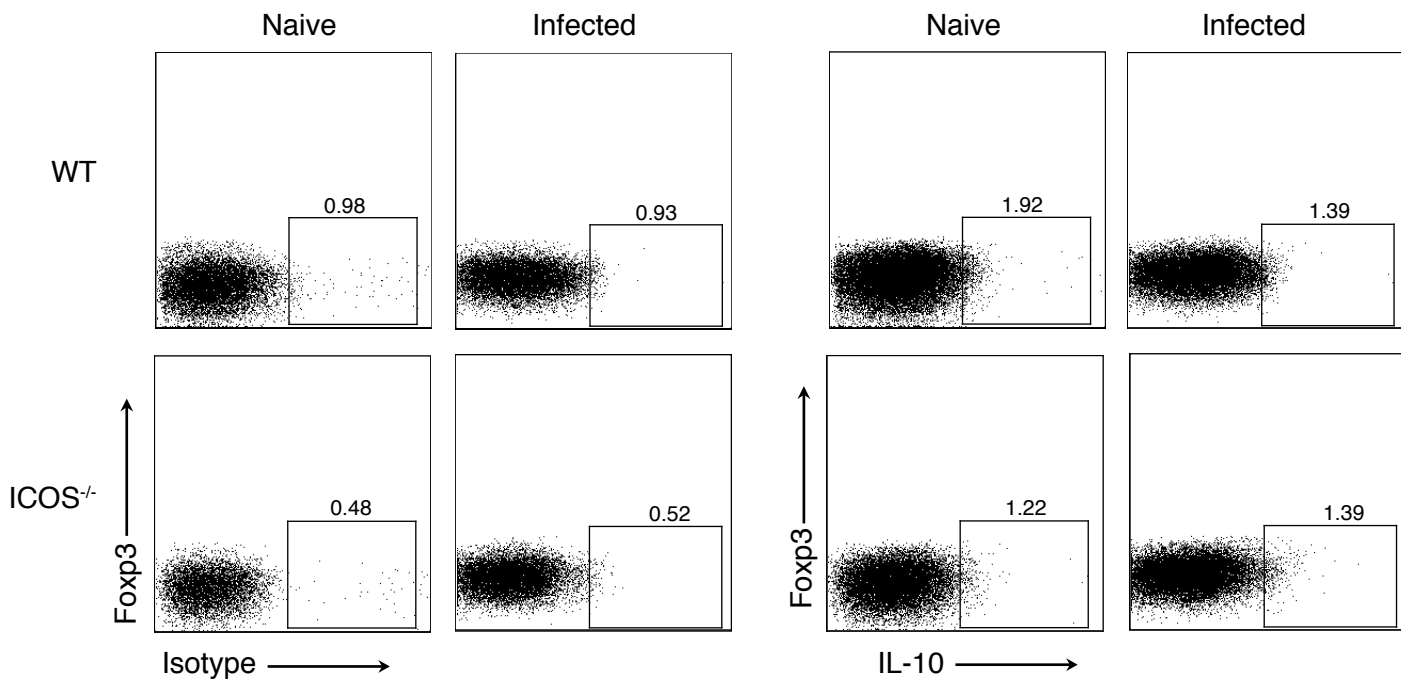
**ICOS controls Foxp3⁺ regulatory T-cell expansion, maintenance and IL-10
production during helminth infection**



Supplementary Figure 1. ICOS deficiency does not affect susceptibility to *H. polygyrus* or *S. mansoni* infections, or *S. mansoni*-mediated pathology. (A-B) WT and ICOS^{-/-} mice were infected with 200 *H. polygyrus* L3 larvae and the parasite burdens were assessed at d 21 (A), and the number of *H. polygyrus* eggs in the faeces were quantified at d 14 and d 21 (B). (C-F) WT and ICOS^{-/-} mice were infected with 70 *S. mansoni* cercariae and the number of adult parasites quantified at wk 8 of infection (C), and the number of *S. mansoni* eggs in the liver were quantified at wk 6 and wk 8 pi (D). To measure granuloma size liver sections were stained with H & E and the area of immune infiltrate surrounding individual eggs was measured using a compound microscope and Leica analysis software. (E) Representative micrographs of *S. mansoni* egg induced granulomas. Scale bar = 200 μm (F) Area in μm^2 of hepatic granulomas. (A-D) Closed circles denote *H. polygyrus* or *S. mansoni* infected WT mice and closed squares denote *H. polygyrus* or *S. mansoni* infected ICOS^{-/-} mice. Data points represent individual mice and bars show median values. (F) Bars depict mean and standard deviation. A & C show pooled data from 3 independent experiments. B shows data from one experiment. D - F show representative data from three independent experiments, two at wk 6, and one at wk 8.



Supplementary Figure 2. *H. polygyrus* infection expands both Helios⁺ and Helios⁻ Foxp3⁺ Tregs in the MLN. WT (circles) and ICOS^{-/-} (squares) mice were infected with *H. polygyrus* and the number of Helios⁺Foxp3⁺ (A) and Helios⁻Foxp3⁺ Tregs (B) quantified in the MLN at d 7 pi. Open and closed symbols represent naive and infected mice respectively. Panels show combined data from three independent experiments. Lines denote means. * p < 0.05 (Tukey's HSD, ANOVA performed on combined data from three independent experiments).

A LP CD4⁺ T cells**B** LP CD4⁺Foxp3⁺ T cells**C** LP CD4⁺Foxp3⁻ T cells

Supplementary Figure 3. Representative FC staining for Foxp3 and IL-10 on LP CD4⁺ T cells. LP cells were isolated from naive and *H. polygyrus* infected mice 7 d pi, stimulated with PMA and ionomycin, and stained for CD4, Foxp3, and IL-10. (A) Gating strategy identifying CD4⁺Foxp3⁺ Tregs and CD4⁺Foxp3⁻ Teff cell populations. (B) Representative staining for Foxp3 versus IL-10 on CD4⁺Foxp3⁺ Tregs. (C) Representative staining Foxp3 versus IL-10 on CD4⁺Foxp3⁻ Teff cells.