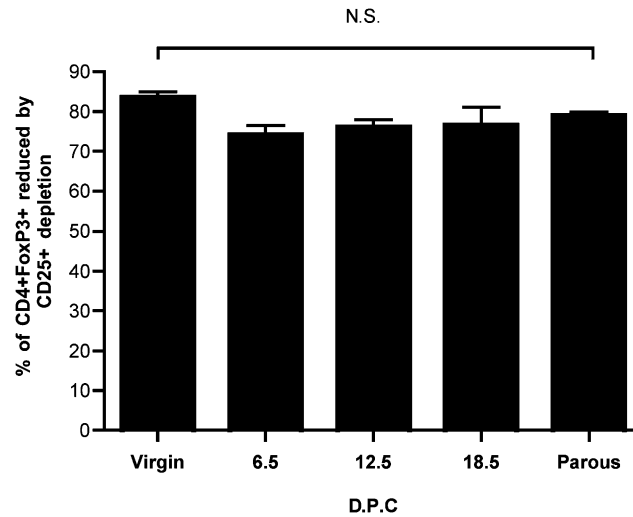
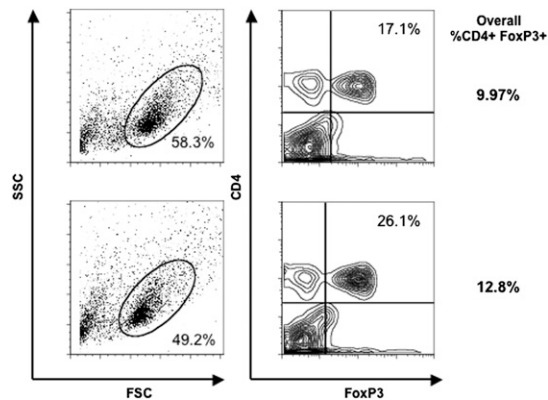


# Supporting Information

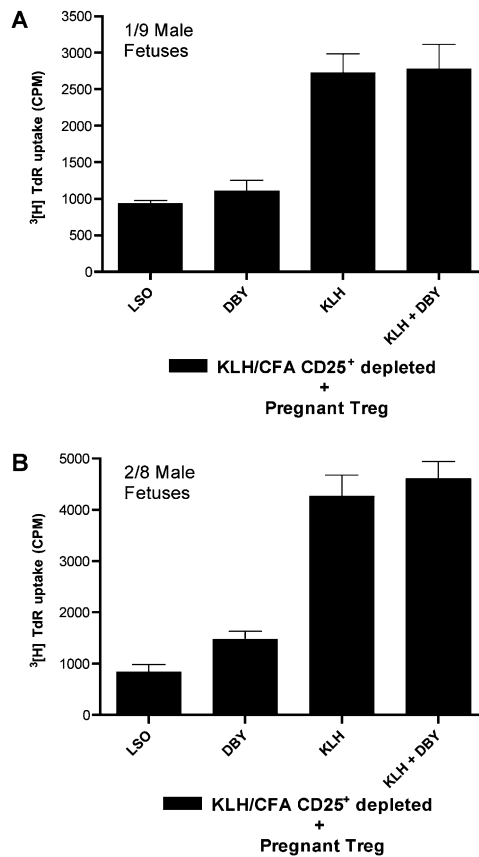
Kahn and Baltimore 10.1073/pnas.1003909107



**Fig. S1.** Effect of magnetic bead separation of CD25<sup>+</sup> cells on reduction on Tregs in whole splenocytes in vivo. Splenocytes were from virgin, timed first pregnancy of C57BL/6 × C57BL/6 (8 weeks old, *n* = 4 each time point) dpc 6.5, 12.5, or 18.5, or 14-week-old retired breeder (parous). Depletion of CD25<sup>+</sup> cells (Treg) by magnetic bead. Whole splenocytes and CD25<sup>+</sup>-reduced splenocytes evaluated for percentage reduction in CD4<sup>+</sup>FoxP3<sup>+</sup> population. Statistical difference determined by one-way ANOVA with Bonferroni–Dunn posttest (N.S., nonsignificant).



**Fig. S2.** Quantification of viable Tregs added to mixed suppression assays. Splenocytes harvested from a timed first pregnancy of 6-week-old C57BL/6 × C57BL/6 male at dpc 15.5 (three male fetuses) as well as virgin 6-week-old C57BL/6 female immunized with KLH/CFA 7 days prior. FACS performed on CD25<sup>+</sup> splenocytes isolated by magnetic bead separation to determine the percentage of cells that represent viable Tregs (CD4<sup>+</sup>FoxP3<sup>+</sup>) added to the cocultures in Fig. 4D.



**Fig. S3.** Pregnancy-induced, antigen-specific T regulatory cells fail to develop with limited numbers of male fetuses. Splenocytes were harvested from a timed first pregnancy of 6-week-old C57BL/6  $\times$  C57BL/6 male at dpc 12.5 (one male fetuses) as well as virgin 6-week-old C57BL/6 female immunized with KLH/CFA 7 days prior. Cellular proliferation assay was performed using  $1 \times 10^6$  cells per mL with stimuli indicated as the CD4<sup>+</sup> T cell peptide epitope of the H-Y antigen presented by I-A<sup>b</sup> (10  $\mu$ M *Dby*), the I-A<sup>b</sup> presented lysteriolysin peptide epitope (10  $\mu$ M *Lso*), or KLH (1  $\mu$ g/mL). CD25<sup>+</sup>-reduced splenocytes from KLH/CFA-immunized mouse cocultured with the CD25<sup>+</sup> (Treg-enriched population) isolated by magnetic bead positive selection from splenocytes harvested from a timed first pregnancy of 6-week-old C57BL/6  $\times$  C57BL/6 male at dpc 12.5 (one male fetus) pregnant mouse at a ratio of 4:1. Cultures were pulsed with 1  $\mu$ Ci per well  $^3\text{H}$ TdR for the final 18 h of a 72-h culture. (A) 1/9 male fetuses. (B) 2/8 male fetuses.