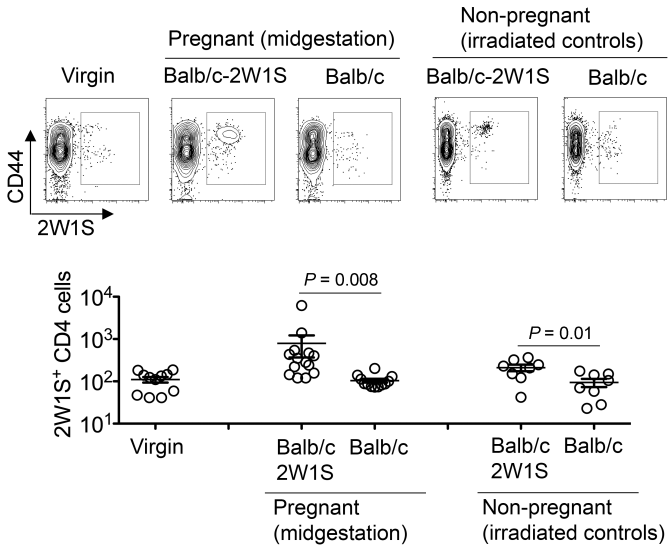
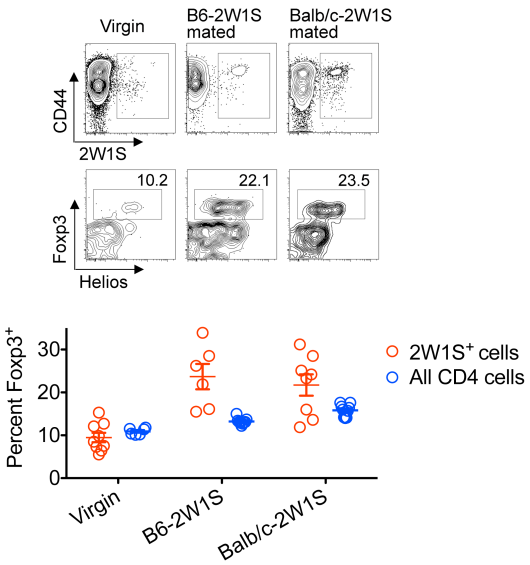
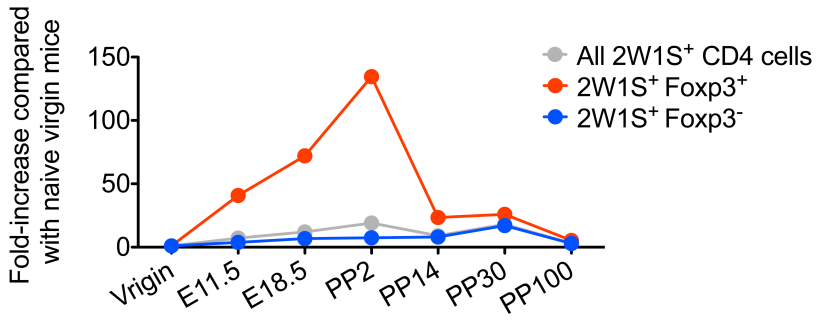


Supplementary Figure 1. Expansion of maternal CD4 cells with fetal-2W1S-specificity is specific to mating with 2W1S-expressing males. Representative FACS plots (top) and cumulative data illustrating the accumulation of $2W1S^+$ CD4 cells in virgin, non-irradiated B6 females midgestation (E11.5) after mating with either 2W1S-expressing or control Balb/c males, and irradiated (100 rads) non-pregnant B6 females after continuous mating with 2W1S-expressing or control Balb/c males. These data are representative of two independent experiments each with similar results. Bar, mean ± one standard error.

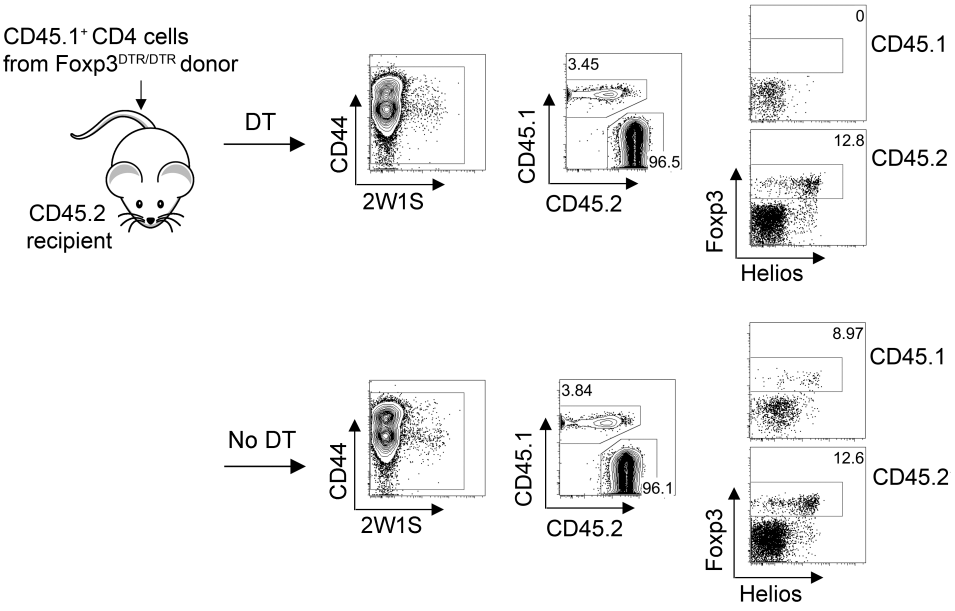


Supplementary Figure 2. Expansion of maternal CD4 and Foxp3$^+$ regulatory cells with fetal-2W1S-specificity in allogeneic compared with syngeneic pregnancy. Representative FACS plots (top) and cumulative data illustrating the accumulation of 2W1S$^+$Foxp3$^+$ (red) or total Foxp3$^+$ CD4 cells (blue) in virgin or pregnant midgestation (E11.5) B6 mice impregnated by 2W1S-expressing B6 or 2W1S-expressing Balb/c males. These data are representative of two independent experiments each with similar results. Bar, mean ± one standard error.

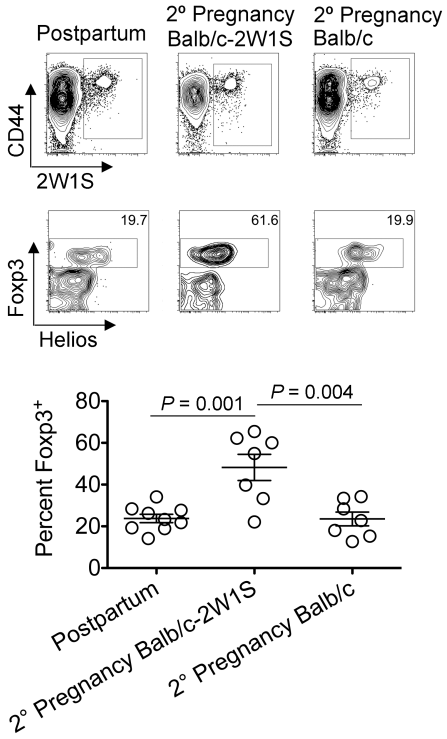
	All 2W1S$^+$	Fold-increase	P valve	2W1S$^+$ Foxp3$^+$	Fold-increase	P valve	2W1S$^+$ Foxp3$^-$	Fold-increase	P valve
Virgin	110 ± 16			10 ± 2			100 ± 17		
E11.5	791 ± 424	7.2	0.0011	408 ± 356	40.8	0.0001	383 ± 103	3.8	0.0024
E18.5	1334 ± 380	12.1	0.0058	720 ± 236	72.0	0.0001	680 ± 162	6.8	0.0027
PP2	2096 ± 677	19.0	0.0011	1346 ± 499	134.6	0.0006	741 ± 247	7.4	0.0111
PP14	995 ± 212	9.0	0.0011	234 ± 59	23.4	0.0002	801 ± 171	8.0	0.0026
PP30	1975 ± 1085	18.0	0.0003	259 ± 97	25.9	0.0003	1716 ± 1052	17.2	0.0003
PP100	338 ± 76	3.1	0.0042	53 ± 10	5.3	0.0011	286 ± 70	2.9	0.0077



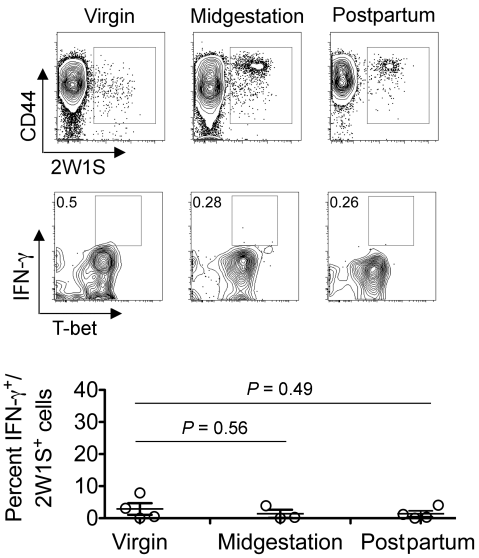
Supplementary Figure 3. Accumulation of maternal Foxp3$^+$ regulatory and Foxp3$^-$ CD4 cells with fetal-2W1S-specificity throughout gestation and following parturition. Table (top) illustrating number and fold-expansion of CD4 cells with fetal-2W1S-specificity at each pregnancy (E) or postpartum (PP) time point compared with virgin controls, and composite graph (bottom) illustrating the accumulation of all fetal-2W1S-specific CD4 cells (gray), 2W1S-specific Foxp3$^+$ (red), and 2W1S-specific Foxp3$^-$ (blue) CD4 cell subsets.



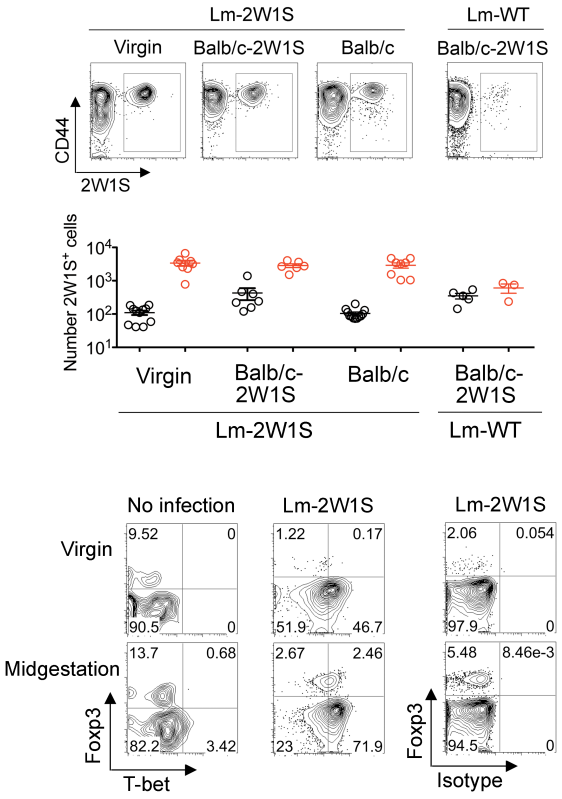
Supplementary Figure 4. Foxp3^{+} Tregs are efficiently eliminated among Foxp3$^{DTR/DTR}$ donor cells in Foxp3$^{WT/WT}$ recipient mice. Representative FACS plots illustrating the percent Foxp3^{+} among each subset of CD4 cells twelve days after adoptive transfer. Purified CD4 cells from CD45.1^{+} Foxp3$^{DTR/DTR}$ mice were adoptively transferred into naïve CD45.2^{+} recipients that were administered diphtheria toxin (two 0.5 μg doses 8 hours apart) (top) or no DT controls (bottom).



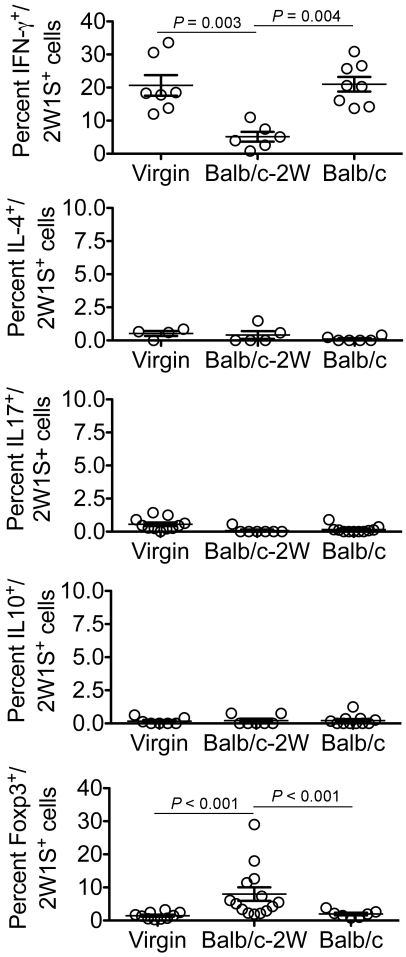
Supplementary Figure 5. Accelerated expansion of maternal Foxp3$^+$ CD4 cells with fetal-2W1S-specificity occurs in an antigen-specific fashion during secondary pregnancy. Representative FACS plots (top) and composite data (bottom) illustrating percent Foxp3$^+$ among 2W1S$^+$ CD4 cells after primary pregnancy by Balb/c-2W1S males (postpartum), or midgestation (E11.5) during secondary pregnancy by Balb/c-2W1S or Balb/c control males. These data are representative of two independent experiments each with similar results. Bar, mean ± one standard error.



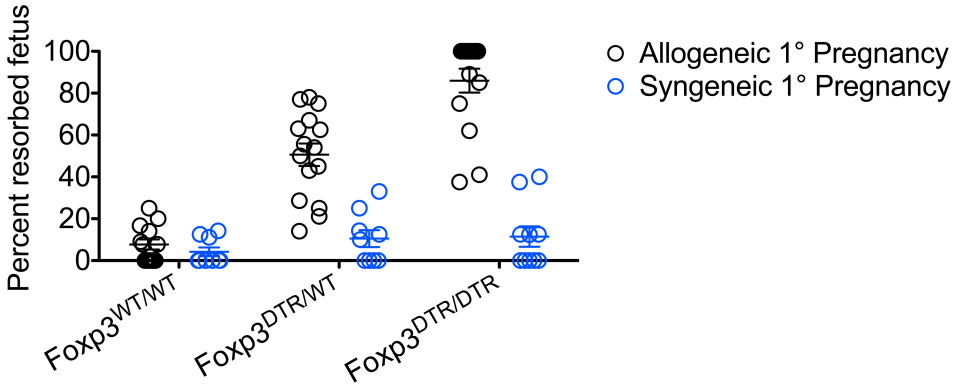
Supplementary Figure 6. Maternal CD4 cells with 2W1S-specificity in virgin, pregnant, and postpartum mice do not produce IFN-γ after *ex vivo* stimulation. Representative FACS plots (top) and cumulative data illustrating percent IFN-γ producing $2W1S^{+}$ CD4 cells after stimulation with PMA/Ionomycin for five hours in cultures supplemented with brefeldin A. These data are representative of two independent experiments each with similar results. Bar, mean ± one standard error.



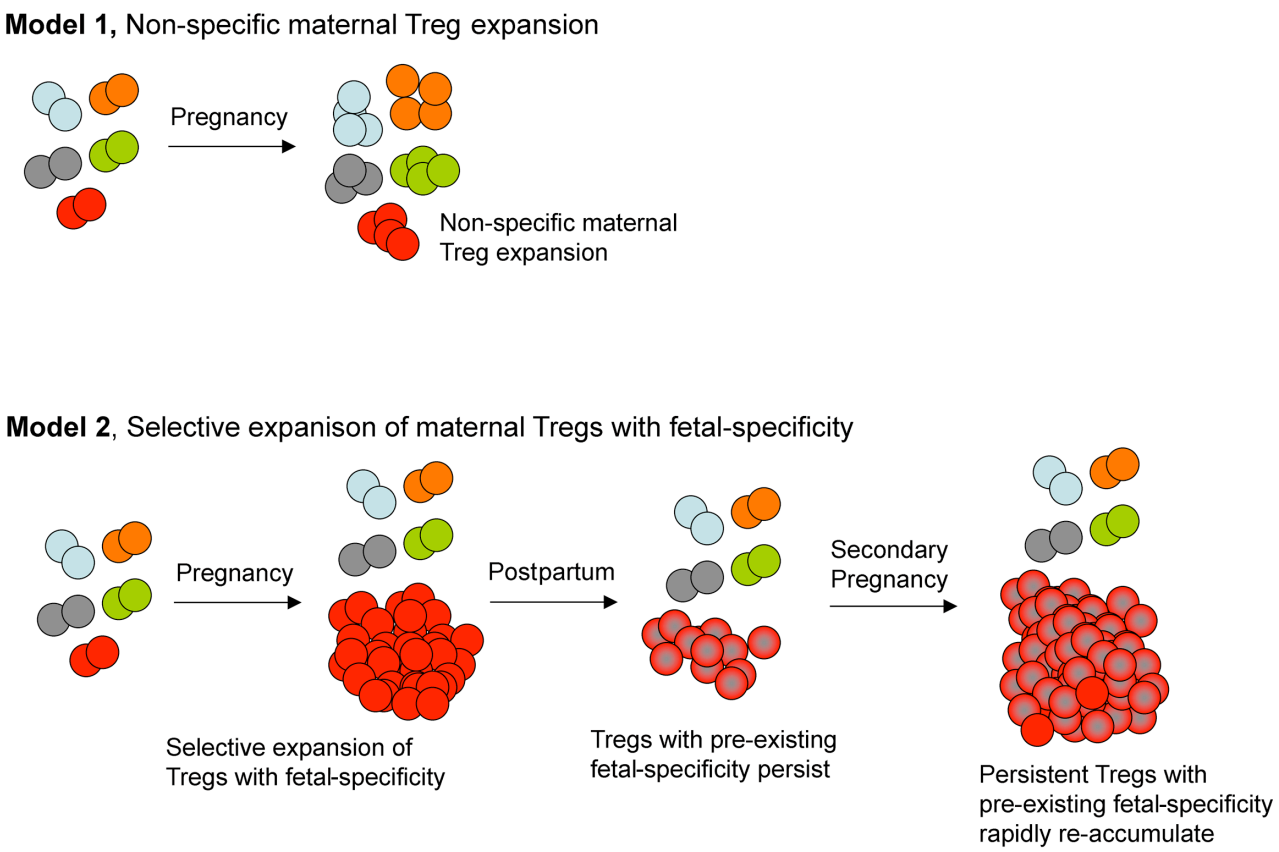
Supplementary Figure 7. Lm-2W1S stimulates expansion and T-bet expression among CD4 cells with 2W1S-specificity in virgin and pregnant mice. Representative FACS plots (top) and cumulative data illustrating number of $2W1S^+$ CD4 cells before (black) or five days after Lm-2W1S or non-recombinant Lm inoculation (red) for naïve virgin or pregnant B6 mice midgestation after mating with 2W1S-expressing or control Balb/c males (top). T-bet expression, compared with isotype staining controls, among $Foxp3^+$ $2W1S^+$ and $Foxp3^-$ $2W1S^+$ CD4 cells before or day 5 after Lm-2W1S inoculation in virgin or pregnant midgestation B6 mice impregnated by Balb/c-2W1S males (bottom). Bar, mean ± one standard error.



Supplementary Figure 8. Cytokine production and Foxp3 expression among CD4 cells with fetal-2W1S-specificity after Lm-2W1S inoculation in virgin and pregnant mice. Percent IFN-γ, IL-4, IL-17A, IL-10 and Foxp3 positive among $2W1S^+$ CD4 cells in virgin or pregnant midgestation B6 females impregnated by Balb/c-2W1S or Balb/c males 5 days after Lm-2W1S inoculation. These data are representative of three independent experiments each with similar results. Bar, mean ± one standard error.



Supplementary Figure 9. Fetal resorption triggered by maternal Treg ablation is sharply reduced in syngeneic compared with allogeneic pregnancy. Percent fetal resorption in allogeneic (black circles, impregnated by Balb/c males) or syngeneic (blue circles, impregnated by B6 males) for $Foxp3^{WT/WT}$, $Foxp3^{DTR/WT}$, or $Foxp3^{DTR/DTR}$ females each on the B6 background five days after the initiation of DT beginning midgestation. These data are representative of two independent experiments each with similar results. Bar, mean ± one standard error.



Supplementary Figure 10. Models comparing maternal regulatory CD4 cell accumulation. In model 1 (top), pregnancy stimulates non-specific expansion of maternal Foxp3^{+} regulatory T cells before investigation using antigen-specific tools. In model 2 (bottom), maternal regulatory T cells with fetal-antigen specificity selectively expand and accumulate during pregnancy. This model is supported by data presented in this paper where maternal regulatory CD4 cells with specificity for a single peptide antigen expressed by the developing fetus are found to expand greater than 100-fold, while Foxp3-expression among bulk maternal CD4 cells accumulate less than 2-fold. Furthermore, we show pregnancy-induced maternal regulatory T cells with fetal specificity are pheno-typically distinct, persist after delivery, and rapidly re-expand and provide protection from fetal resorption during secondary pregnancy.