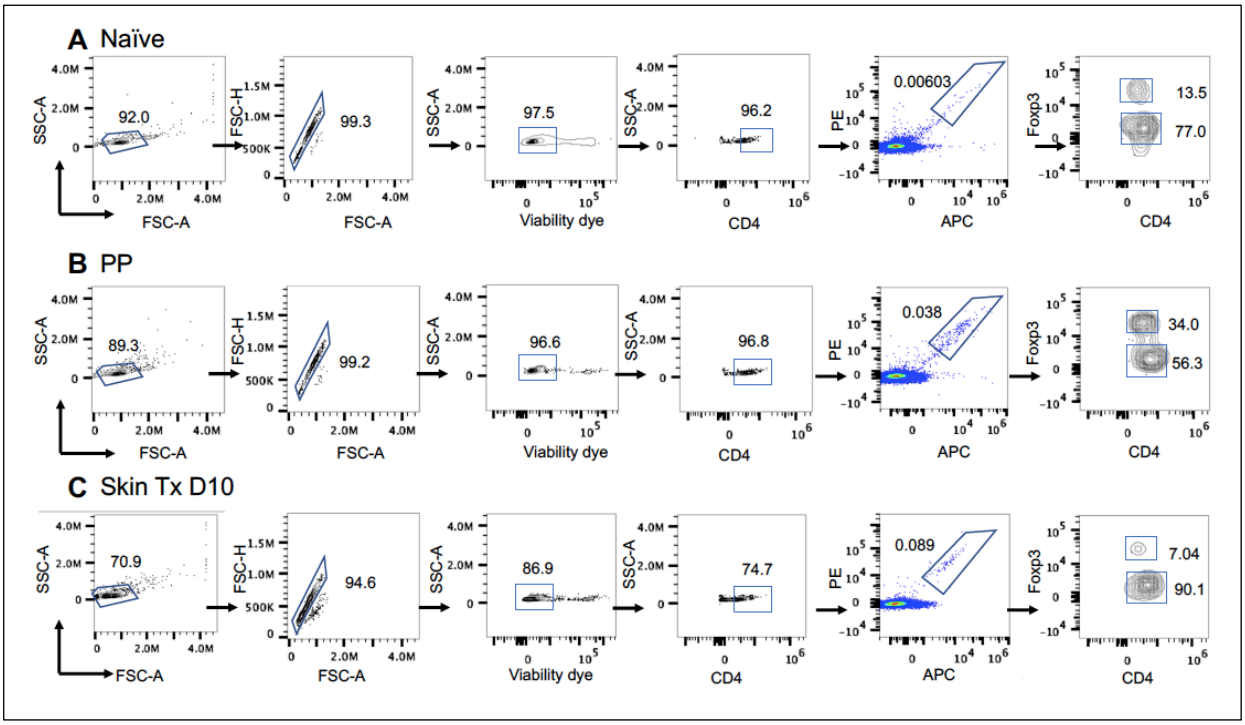
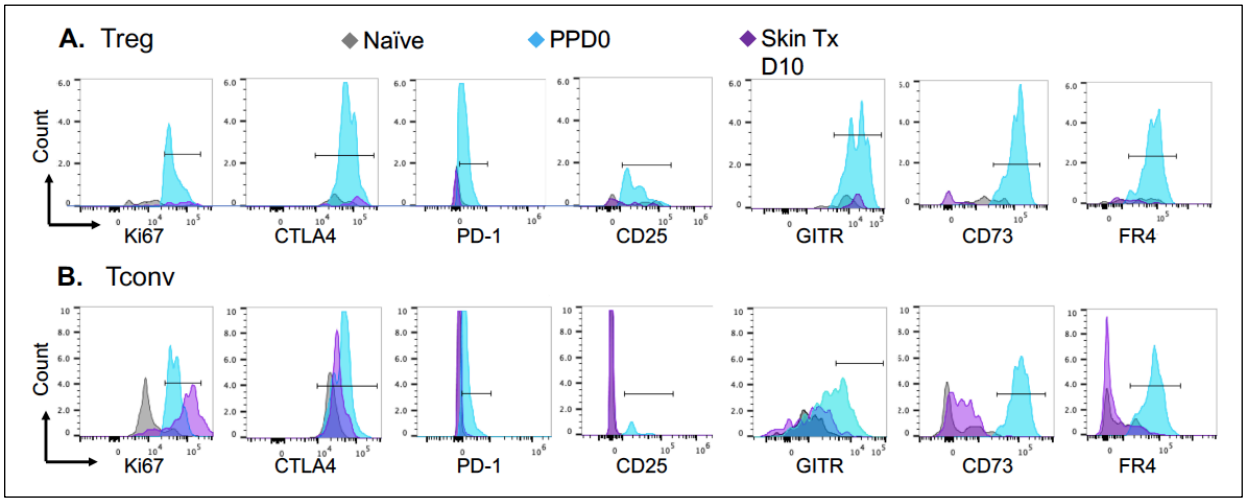
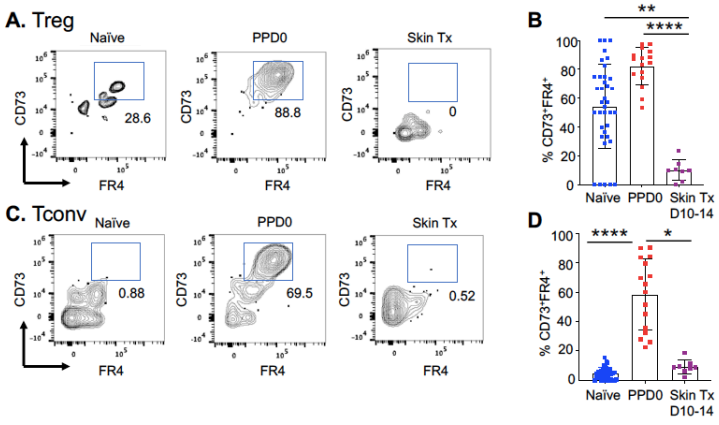
Supplemental Figures



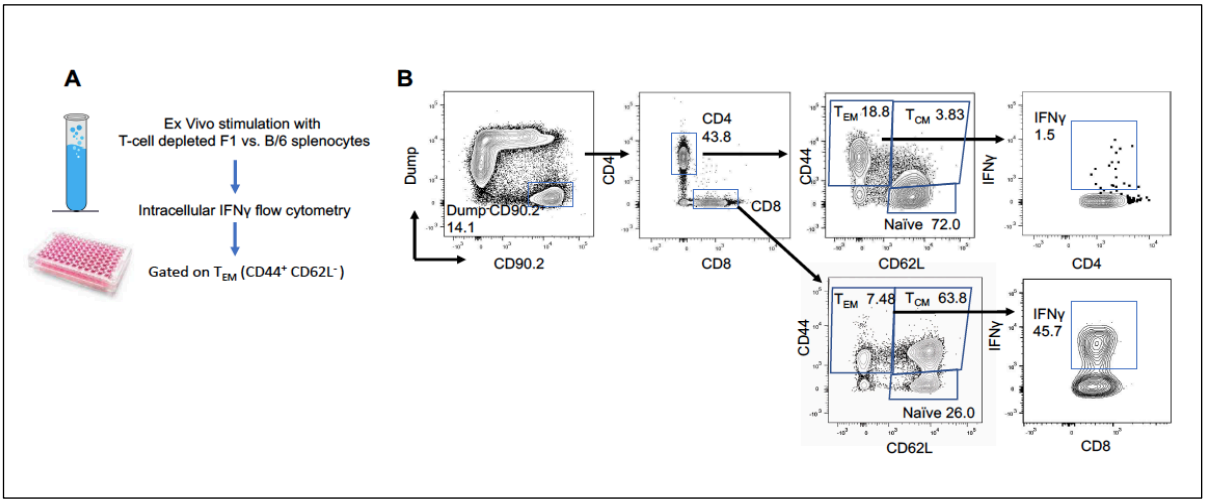
Supplemental Figure 1. Gating strategy for 2W:I-A^b CD4$^+$ T conventional (Tconv) and T regulatory (Tregs) cells. Single cell suspension from murine spleen and LNs of naïve, post-partum (PP) day 0, and skin transplanted mice (post-operative day (POD) 10) were magnetically enriched for CD4$^+$ lymphocytes and stained for 2W:I-A^b tetramer. T cells were identified as CD4$^+$ cells after gating on single-cell and live lymphocytes. Dead cells were excluded using zombie NIR Fixable viability kit. 2W**:**IA^b specific CD4$^+$ T cells were gated, then sub-gated into Tconv (CD4$^+$Foxp3$^-$) and Tregs (CD4$^+$Foxp3$^+$) from **(A)** naïve, **(B)** PP, and **(C)** Skin Tx D10 mice.



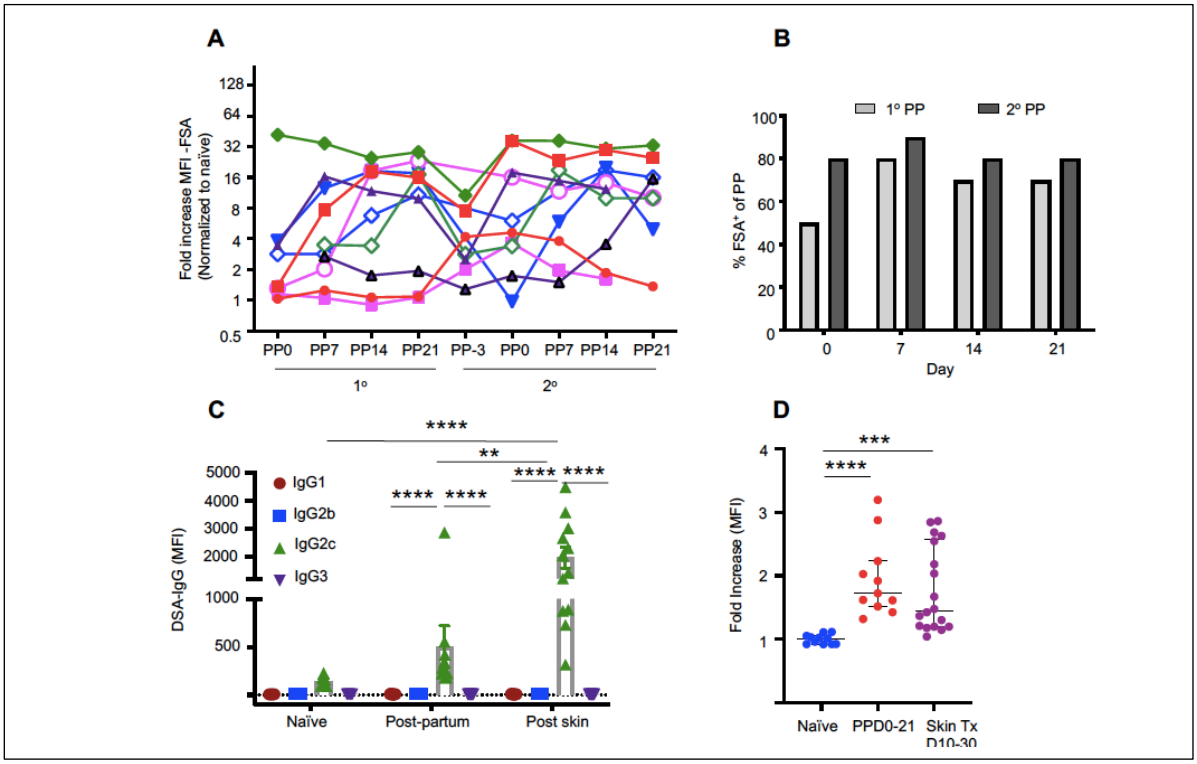
Supplemental Figure 2. Phenotype of Treg and Tconvs. Spleens, and inguinal, axillary and brachial LNs were harvested from naïve, PPD0 and POD10 skin transplanted (Tx) recipients, and analyzed for surface and intracellular markers expression. Representative histograms of markers for Ki67, CTLA4, PD-1, CD25, GITR expressed by 2W:IA^{b} -specific **(A)** Tregs, and **(B)** Tconv cells respectively.



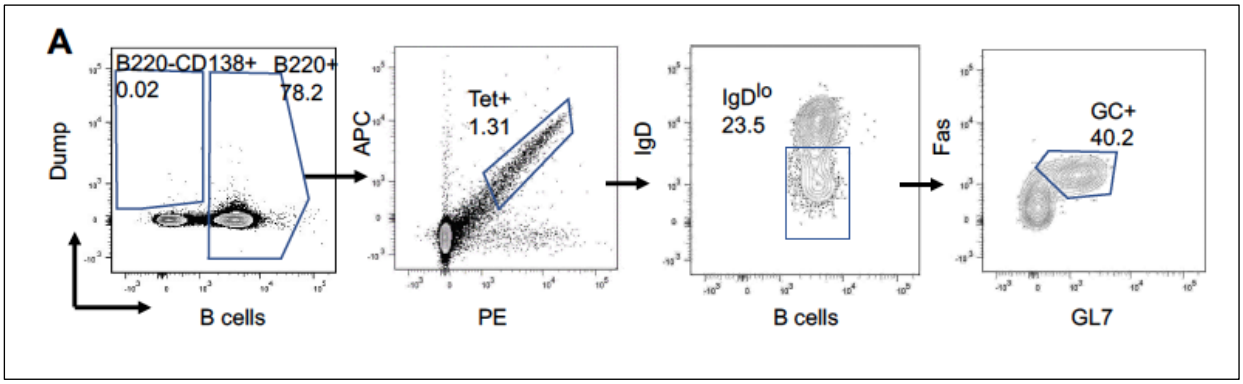
Supplemental Figure 3. Gating strategy for Treg and Tconv cells co-expressing CD73 and FR4. **(A)** Representative flow cytometry plots of CD73 and FR4 expressed by 2W:IA^{b} Tregs **(B)** % CD73^{hi}FR4^{hi} of 2W:IA^{b} Treg in naïve, PP0 and skin transplanted POD10-14 recipients, *n=8-37/group.* **(C)** Representative flow cytometry plots of CD73 and FR4 expressed by 2W:IA^{b} Tconv cells, and **(D)** % CD73^{+}FR4^{+} of 2W:IA^{b} Tconv in naïve, PPD0 and skin transplanted POD10-14 recipients, *n=8-37/group*. Data are pooled from ≥ 2 independent experiments. Data represent mean ± SEM. **p<0.05, **p<0.01, ****p<0.0001* by Kruskal-Wallis test with Dunn's post hoc test.



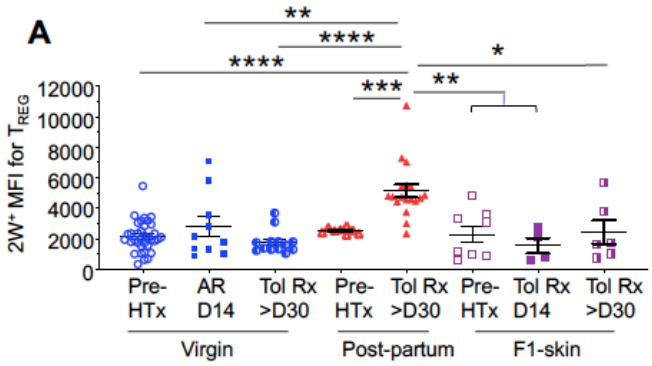
Supplemental Figure 4. Gating strategy for donor-specific $CD4^+$ and $CD8^+$ T cells producing IFNγ. (A) $1x10^6$ responder T cells (C57BL/6) incubated with $5x10^6$ T cell-depleted splenocyte stimulators (C57BL/6xBALB/c) in triplicate in 96-well plates, followed by intracellular IFNγ staining. **(B)** Representative flow cytometry plot showing $Dump^-CD90^+$ cells that were then separated into $CD4^+$ and $CD8^+$ gates. $CD4^+$ and $CD8^+$ T cells were examined for CD44 and CD62L expression to define the T_{EM} ($CD44^+CD62L^-$), T_{CM} ($CD44^+CD62L^+$), naïve ($CD44^-CD62L^+$) subsets.



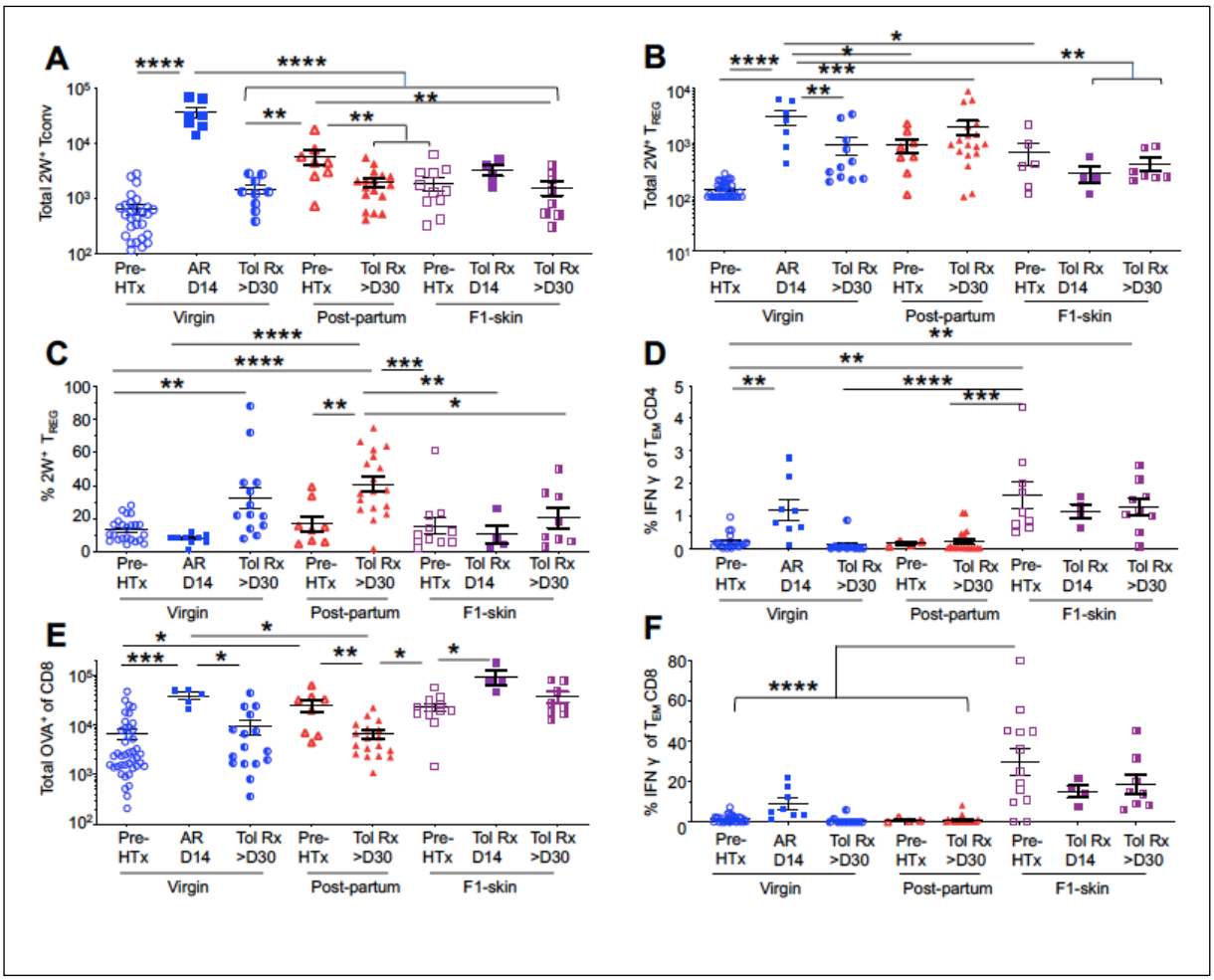
Supplemental Figure 5. Fetus-specific antibodies (FSA) in post-partum mice after primary and secondary pregnancy. (A) Fold increase MFI of FSA from individual mice after primary (1°) and secondary (2°) pregnancies. Each dot indicates individual mice. **(B)** % of FSA^{+} (>2-fold increase over virgin levels) of 1° and 2° post-partum (PP) days 0, 7, 14 and 21. **(C)** Isotypes (IgG1, IgG2b, IgG2c, IgG3) of FSA collected on PP45-60, DSA from recipients of BALB/c skin POD 14) or serum from naïve mice, *n=6-12/group*. **(D)** 2W-OVA-specific IgG (Fold increase in MFI) of naïve, PP0-21(1:5 dilution) and skin transplanted recipients on POD10-30 (1:10 dilution), *n=11-18/group.* Data are pooled from ≥ 2 independent experiments. Data represent mean ± SEM. ***p<0.01*, ****p<0.001, ****p<0.0001* by Kruskal-Wallis test with Dunn's post hoc test.



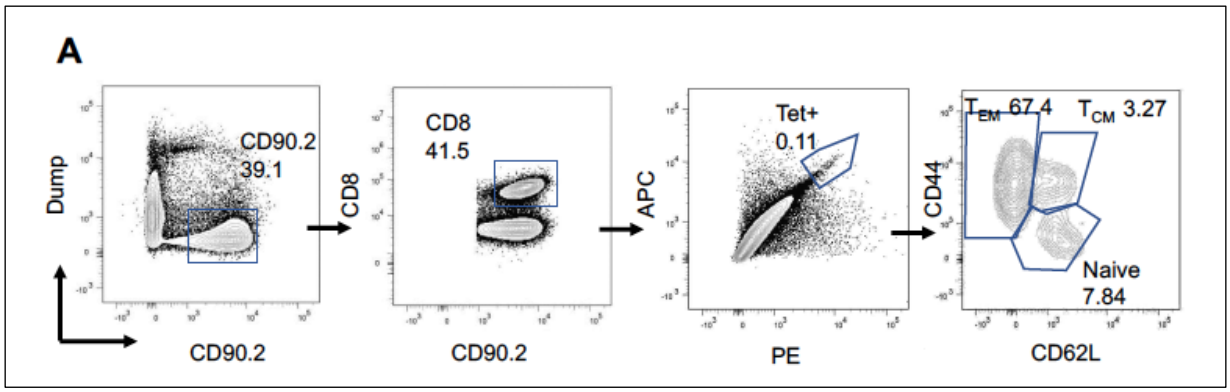
Supplemental Figure 6. Gating strategy to identify donor MHC-specific B cells with germinal center phenotype. (A) Representative flow plots showing gating on $B220^+$ B cells, followed by tetramer (αK^d, αL^d, $\alpha I\text{-}E^d$)-binding, IgD^{lo} B cells, and GC B cells were defined by Fas^+GL7^+ expression.



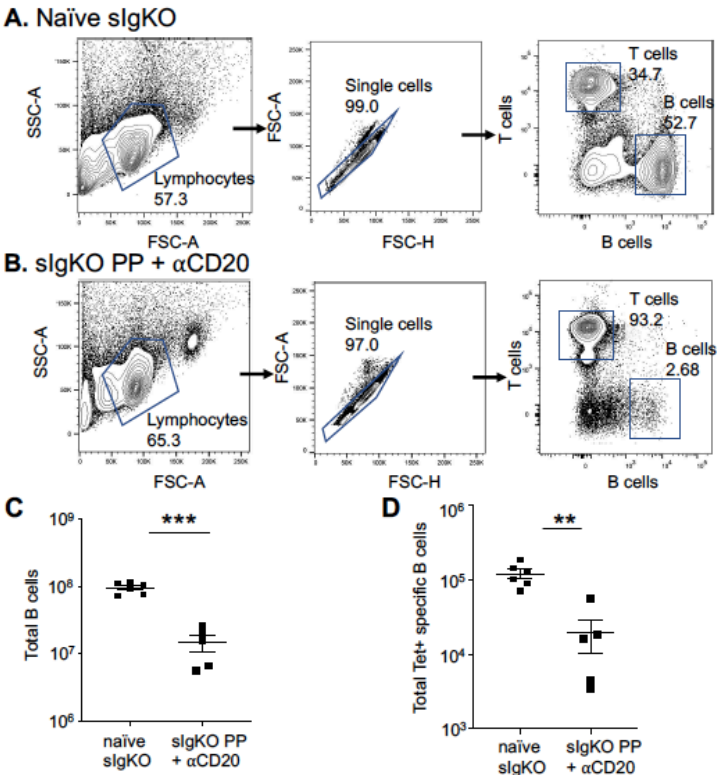
Supplemental Figure 7. MFI of 2W:I-A^b-binding on Tregs. Spleens, and inguinal, axillary and brachial LN were harvested from virgin, post-partum or F1-skin sensitized C57BL/6 mice that received fetus-matched heart allograft (HTx) and treated with CoB/DST (Tol Rx). HTx recipients were examined on days 14 or >30 post-HTx. Mean channel fluorescence (MFI) of 2W:IA^b tetramer binding to Tregs, *n= 4-35/group*. Data are pooled from ≥ 2 independent experiments. Data represent mean ± SEM. **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001* by Kruskal-Wallis test with Dunn's post hoc test.



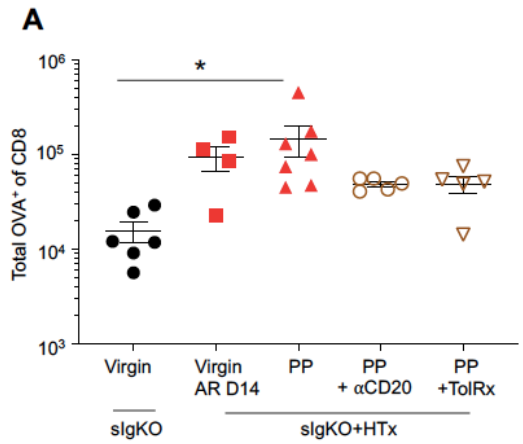
Supplemental Figure 8. Quantification of 2W:I-A^b and OVA:K^b and donor-specific IFNγ$^+$ CD4$^+$ T cells and CD8$^+$ T cells. Spleens, and inguinal, axillary and brachial LN were harvested from virgin, post-partum mice or F1-skin sensitized C57BL/6 mice that received fetus-matched heart allograft (HTx) and treated with CoB/DST (Tol Rx), and analyzed on the indicated days post-HTx. **(A)** Total number of 2W:IA^b specific Tconv CD4$^+$ T cells, *n=4-30/group*. **(B)** Total number of 2W:IA^b specific Treg cells, *n=4-38/group*. **(C)** % of 2W:IA^b specific Treg of CD4$^+$ cells, *n=4-23/group*. **(D)** % IFNγ$^+$ of T_{EM} CD4$^+$ cells, *n=4-20/group*. **(E)** Total number of OVA$^+$ of CD8$^+$ cells, *n=4-42/group*. **(F)** % IFNγ$^+$ of T_{EM} CD8$^+$ cells, *n=4-29/group*. Data are pooled from ≥ 2 independent experiments. Data represent mean ± SEM. **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001* by Kruskal-Wallis test with Dunn's post hoc test. Data for the Virgin and post-partum groups are from Figure 2.



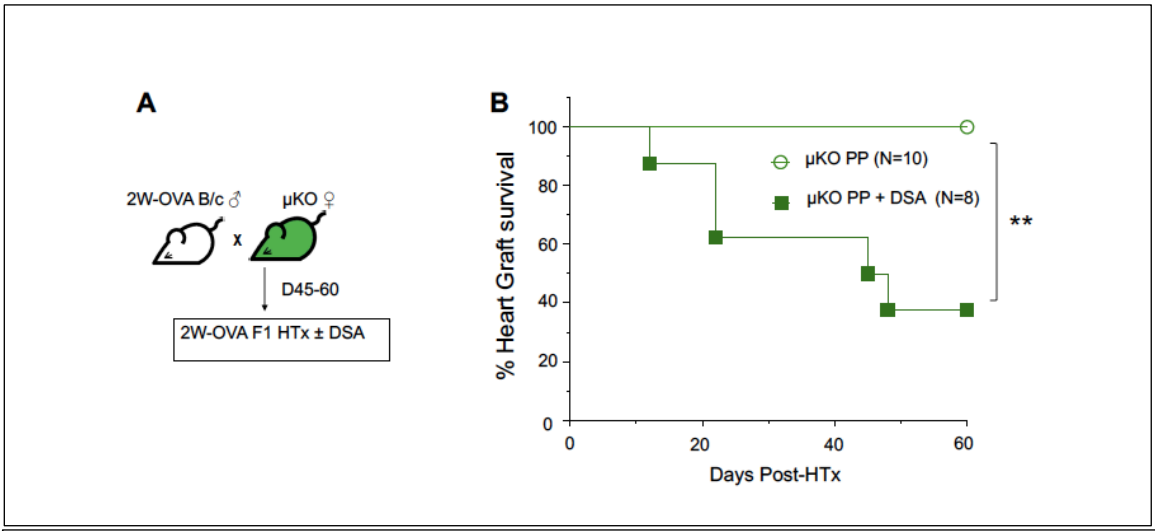
Supplemental Figure 9. Gating strategy for OVA:K^b $CD8^+$ cells. (A) Representative flow plots showing gating on $CD90.2^+$, K^b:OVA $CD8^+$ cells and for T_{EM} ($CD44^+CD62L^-$), T_{CM} ($CD44^+CD62L^+$), naïve ($CD44^-CD62L^+$) subsets.



Supplemental Figure 10. Treatment with αCD20 significantly reduced sIgKO B cells. For B cell depletion, sIgKO PP mice were injected with 250µg αCD20 (i.v.) on days -1 and 7 post 2W-OVA.F1 heart transplantation (HTx), and then one week later. Representative flow cytometry plots of B cells, by gating on total lymphocyte, single cell populations and $B220^+$ B cells from **(A)** naïve sIgKO, and **(B)** sIgKO PP + αCD20 treated mice were analyzed on day 30 post-HTx. **(C)** Total number of all B cells, *n=5-6/group*, and **(D)** total number of Tet^+ B cells, *n=5-6/group*, from pooled spleens and LNs. Data are pooled from 2 independent experiments. Data represent mean ± SEM. ***p<0.01, ***p<0.001* by Mann-Whitney *t* test.



Supplemental Figure 11. Depletion of B cells did not significantly reduce total number of OVA:K^b CD8$^+$ T cells. Spleens and inguinal, axillary, brachial LNs were harvested from sIgKO virgin, or allogeneic sIgKO PP mice received 2W:OVA.F1 HTx and treated with CoB/DST, and treated with αCD20 day -1 and 7 post-HTx. Mice were analyzed on day 30 post-HTx, and the total number of K^b:OVA specific CD8$^+$ cells quantified, *n=4-7/group*. Data are pooled from 2 independent experiments. Data represent mean ± SEM. **p<0.05* by Kruskal-Wallis test with Dunn's post hoc test.



Supplemental Figure 12. Immune serum at the time of offspring-matched heart transplant (HTx) overrides pregnancy-induced T cell tolerance. (A) Experimental design. Male 2W-OVA.BALB/c (♂) were mated with μKO.C57BL/6 (♀). PP μKO females were rested 45-60 days, then received 2W-OVA.F1 HTx and a single dose 200μL (i.v) of immune serum (DSA) on POD 0. Immune serum was pooled from mice that rejected 2W-OVA.F1 skin allografts (on POD 14-21), *n=8-10/group*. **(B)** % heart graft survival, *n=8-10/group*. Data are pooled from 2 independent experiments. ***p<0.01* by log rank test.

Supplemental Table

Recipient	Skin Tx	Skin Graft Survival
μKO	2W-OVA.B6	13 (x4)
μKO PP	2W-OVA.B6	13, 13, 15, 17
μKO	2W-OVA.F1	13 (x4)
μKO PP	2W-OVA.F1	13 (x4)

Supplemental Table 1. Rejection of 2W-OVA.B6 or 2W-OVA.F1 skin grafts by μKO or μKO post-partum (PP) recipients *(n=4 for each of 4 groups).*