

Supplemental Figure 1. In vivo MLR T-cell subsets, sequestration and cytokines. (A) Frequency of non-chimeric alloreactive lymphocytes by T-cell subset and tissue compartment. Number of CFSE labeled lymphocytes in peripheral blood (PB), bone marrow (BM) and thymus were negligible. (B) Serum cytokine levels for naïve mice, E14 injected dams, and non-chimeric pups. Levels of IL-4 were undetectable. Each data point represents at least 3 independent experiments. Error bars represent ± SEM.



Supplemental Figure 2. Assessment of chimeric and fostered cells for anergy and/ or suppression. CD4⁺, CFSE labeled BALB/c lymphocytes co-cultured with (**A**) syngeneic BALB/c splenocytes, (**B**) third party Swiss Webster splenocytes, and (**C**) B6 splenocytes before and (**D**) after addition of IL-2. Each tracing is representative of at least 3 independent experiments.



Supplemental Figure 3. Relationship between magnitude of maternal humoral response and loss of chimerism. Fold increase in maternal humoral response was 138.7 ± 15.4 (n=23) for non-chimeric pups as compared to 32.7 ± 13.8 (n=10) for chimeric pups (p=0.0002). Error bars represent \pm SEM.



Supplemental Figure 4. Impact of fetal IUHCT and fetal loss on maternal humoral response. Magnitude of maternal humoral response in each injected dam versus (**A**) the total number of fetuses injected per litter (n=23, r=0.546, p=0.0070) and (**B**) the number of aborted fetuses per litter (n=24, r=0.547, p=0.0057).



Supplemental Figure 5. Long term chimerism levels of fostered and F1 mice.

Fostered mice are BALB/c mice injected on E14 with 20 million GFP+ B6 BM cells and nursed by non-injected BALB/c surrogate mothers. Frequency of chimerism was 100%, N=22 at 1 month and N=13 at 2, 4 and 6 months). F1 mice are F1 progeny of B6 dams and BALB/c fathers, injected on E14 with 20 million GFP+ B6 BM cells. Frequency of chimerism was 100%, N=20 at 1 month and N=15 at 2, 4 and 6 months. Error bars represent ± SEM.



Supplemental Figure 6. Tissue compartment analysis of allo-reactive maternal lymphocytes in non-chimeric F1 pups. (A) CD4⁺, H2Kb⁺ lymphocytes from F1 fetal recipients (B) CD4⁺, H2Kb⁻ lymphocytes from E14 injected BALB/c dams. H2Kb⁻ cells derived from F1 recipients in the (C) peripheral blood, (D) bone marrow, (E) lymph nodes, and (F) thymus. Each tracing represents at least 5 independent experiments.