Supplementary Figure Legends

Supplementary Figure 1: Few CD11c^{Hi}B220^{+} conventional dendritic cells are present in mid-gestation fetuses. Gating strategy (**A**) and average percentage (**B**) of various leukocyte populations among E14.5 fetal liver mononuclear cells prior to transplantation. Two staining panels were used to phenotype the cells. Panel A: live/dead marker, CD45, Gr-1, CD3, B220, CD11, Class I, and Class II. Panel B: live/dead marker, CD45, Gr-1, F4/80, CD11, NK1.1, Class I, and Class II. Few dendritic cells were detected. n= 4 in two independent experiments.

Supplementary Figure 2: Analysis of dendritic cell maturation in non-chimeric animals. Relative mean fluorescence intensity (MFI) of Class II, B7-1, B7-2, and CD40 on host-derived APCs in spleens of chimeric and injected non-chimeric animals was calculated relative to a baseline population (CD11c^{-}B220^{-}) to account for experimental variability. No significant differences were observed in any comparison. Chimera n=7, injected non-chimera n=3.

Supplementary Figure 3: Lack of semi-direct antigen presentation early after in utero transplantation in chimeric animals. A. Representative flow cytometry plots of chimeric animals 2-3 weeks after allogeneic IUHCTx (B6 donor cells (H-2K^{b}, I-A^{b}) into BALB/c hosts (H-2K^{d}, I-A^{d}). After gating on live CD45+ leukocytes, the expression of donor and host-specific Class II antigens was analyzed on donor and host-derived antigen presenting cells in spleen, lymph nodes, and bone marrow (spleen shown). Conventional dendritic cells (CD11c^{Hi}B220^{Low}, cDC), plasmacytoid DC (CD11c^{dim}B220^{Hi}, pDC), and

B cells ($CD11c^{-}B220^{Hi}$, B cell) were analyzed separately for the Class II expression. There was no detectable expression of donor Class II on host APCs at this time point. Chimera n=4, naïve n=4 in two independent experiments.

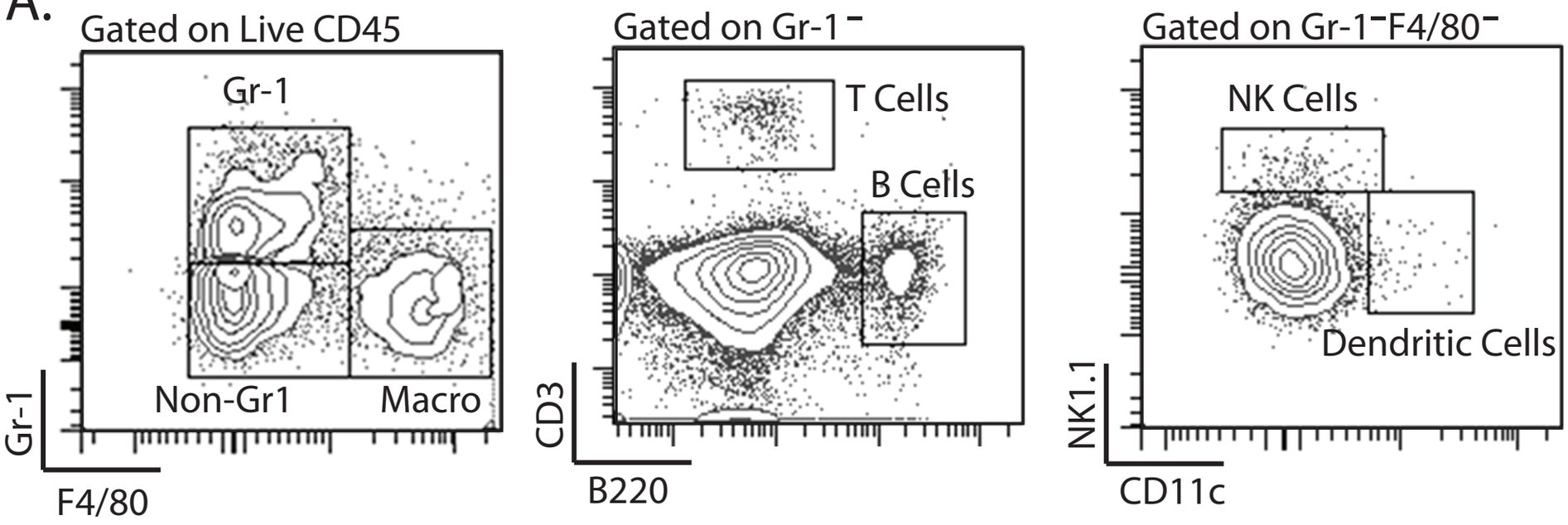
B. Representative flow cytometry plots demonstrating donor-derived APCs ($CD11c^{+}$, H-$2Kb^{+}$) in the thymus of chimeric animals.

Supplementary Figure 4: Deletion and selective Treg survival depend on continued chimerism. Despite successful engraftment in 2-3 week-old 4C mice, all recipients analyzed at 5 weeks after IUHCTx had low (<1%) levels of engraftment. **(A)** In non-chimeric animals, there was no deletion in the thymus or the spleen. **(B)** There was no increase in the percentage of Tregs in thymus or spleen in injected non-chimeric animals but a decrease in %Tregs in spleen. Uninjected n=5, non-chimera n=5. * $p<0.05$ by Student's t-test

Supplementary Figure 5: Increase in percent but not number of splenic Tregs in wild-type chimeras. Percentage **(A)** and absolute number **(B)** of $CD4^{+}Foxp^{+}CD25^{+}$ Tregs among CD4 cells in thymi and spleens of wild-type chimeric and uninjected mice at 2 weeks after in utero transplantation. Uninjected n=6, chimera n=5. *$p<0.005$ by Student's t-test.

Supplementary Figure 1

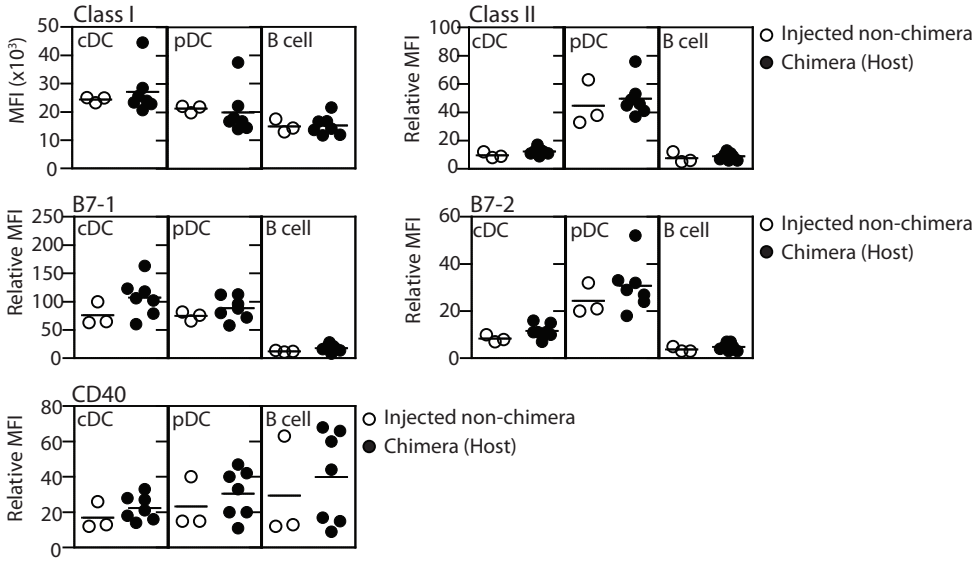
A.



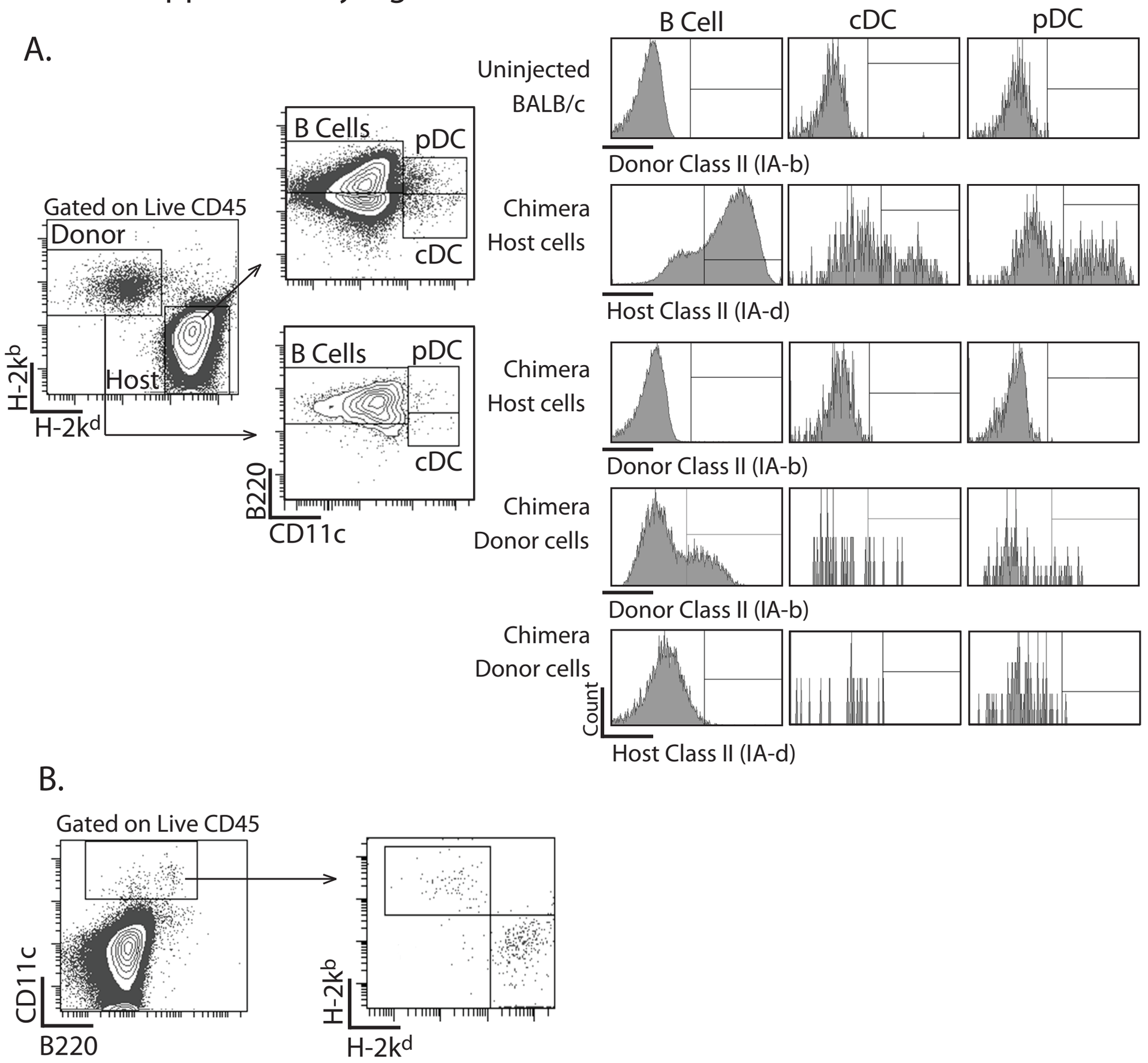
B.

Cell Type	% CD45 (SEM)
Granulocytes	23.9 (2.2)
Macrophages	15.0 (1.4)
NK Cells	10.0 (3.2)
B Cells	3.3 (0.5)
T Cells	2.9 (0.6)
Dendritic Cells	0.3 (0.1)

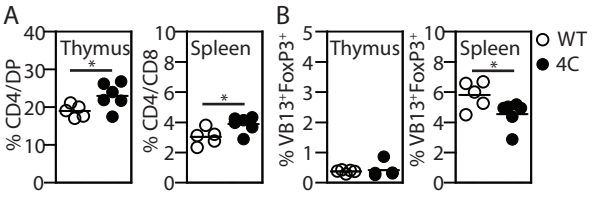
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5