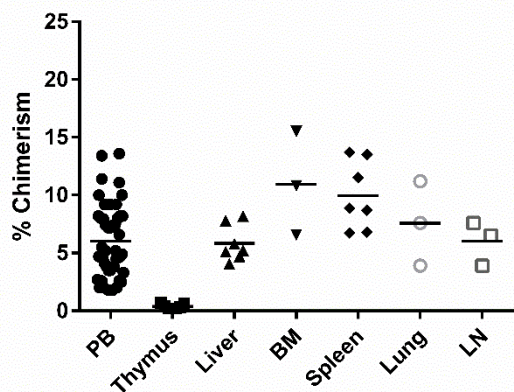


**Extrinsic allospecific signals of hematopoietic origin  
dictate iNKT cell lineage-fate decisions during  
development.**

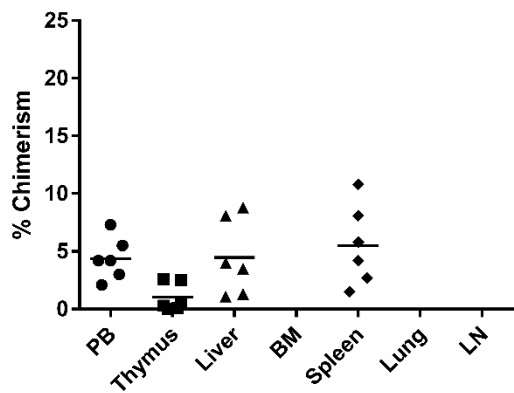
Beverly SI Strong, Tess J Newkold, Amanda E Lee, Lucas  
E Turner, Amir M Alhajjat, Jonathan W Heusel, Aimen F  
Shaaban

Supplemental Figure 1

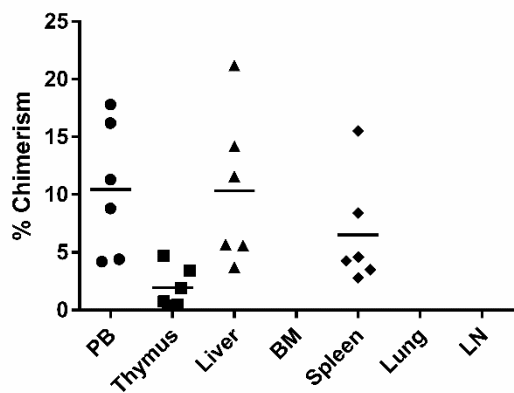
**Balb/c → B6 Chimera**



**B10.D2 → B6 Chimera**

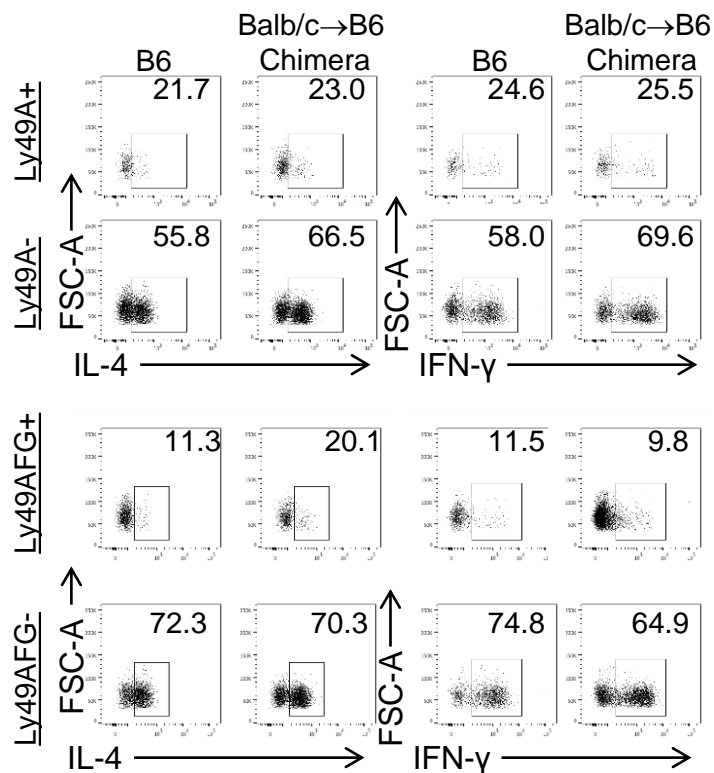
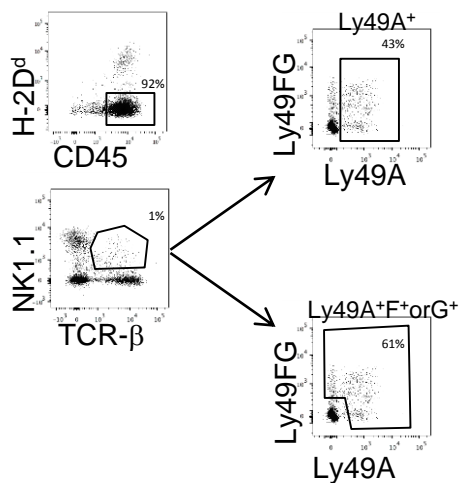


**B6 → Balb/c Chimera**



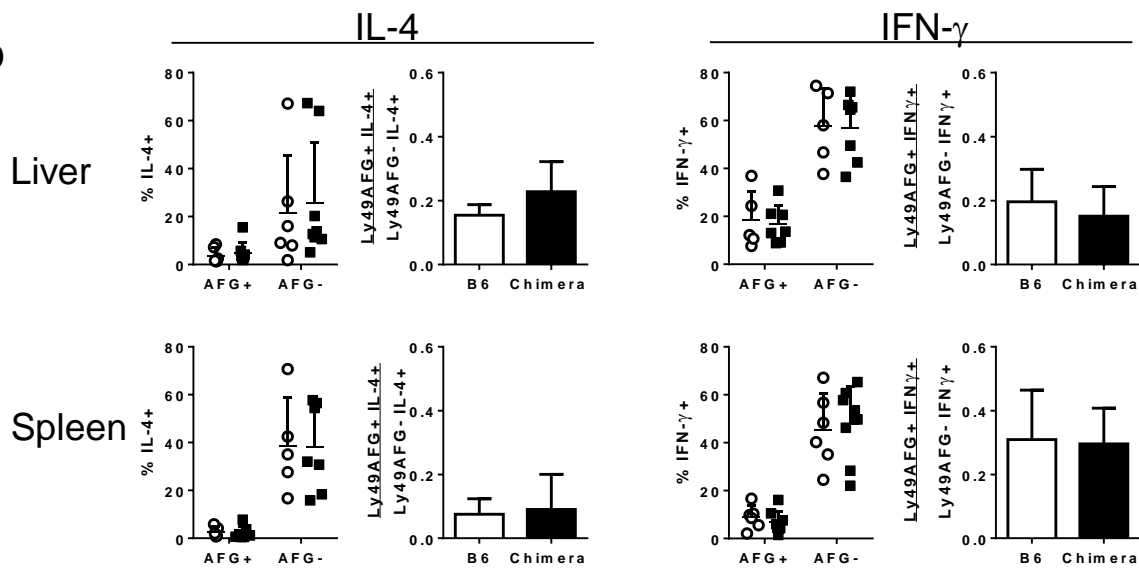
Supplemental Figure 2

**a**

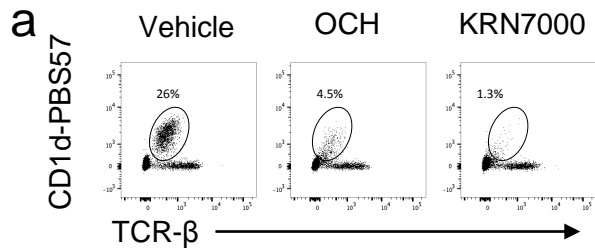


□ B6      ■ Balb/c → B6 Chimera

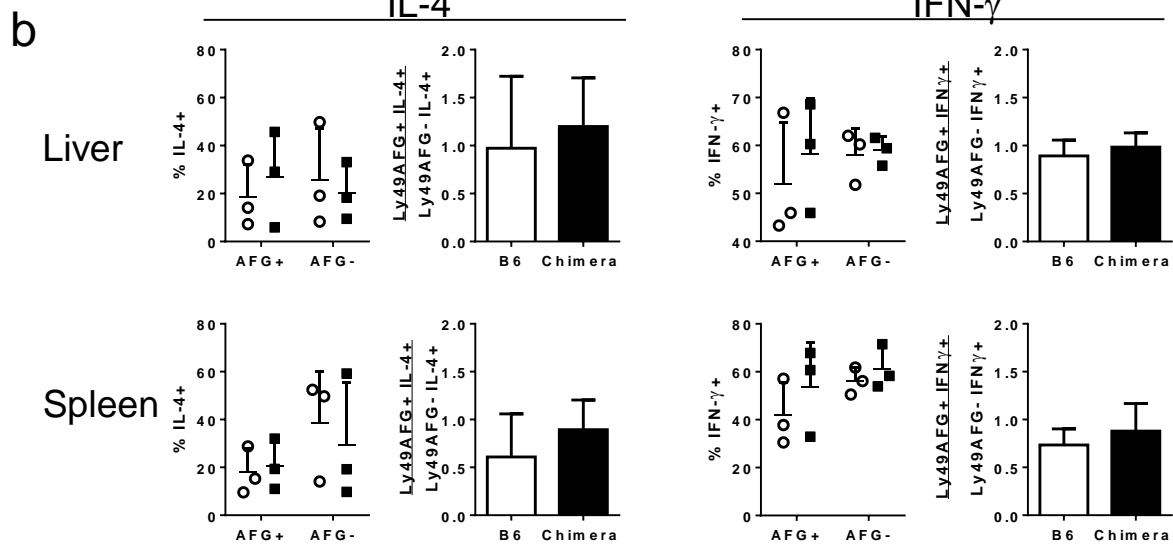
**b**



# Supplemental Figure 3



□ B6      ■ Balb/c  $\rightarrow$  B6 Chimera



**Supplemental Figure 1. Chimerism levels in all tissues tested.** Chimerism levels for each tissue tested. Chimerism calculated as percent of CD45+ events expressing donor MHC class I (H-2K<sup>d</sup> for Balb/c→B6, H-2K<sup>d</sup> for B10.D2 → B6, H-2K<sup>b</sup> for B6 → Balb/c). Each data point is representative of one animal.

**Supplemental Figure 2. Functional responsiveness of iNKT cells in prenatal chimeras.** B6 control and Balb/c→B6 chimeras were injected intravenously with KRN7000 and NKT cells were analyzed two hours later for intracellular cytokine production. (A) iNKT cells were identified by co-expression of TCR- $\beta$  and NK1.1 rather than CD1d-PBS57 tetramer due to down-regulation of TCR following stimulation. Representative dot plots showing IFN- $\gamma$  and IL-4 production by Ly49A<sup>+</sup> vs Ly49A<sup>-</sup> or Ly49AFG<sup>+</sup> vs Ly49AFG<sup>-</sup> iNKT cells. (B) Absolute and relative frequency of IFN- $\gamma$ <sup>+</sup> and IL-4<sup>+</sup> iNKT cells are shown in graphs. Relative frequency of cytokine expression was calculated for donor reactive versus donor irrelevant phenotypes (Ly49AFG<sup>+</sup> % Cytokine<sup>+</sup>/Ly49AFG<sup>-</sup> % Cytokine<sup>+</sup>). Each data point represents one animal.

**Supplemental Figure 3. Functional responsiveness of iNKT cells in response to OCH glycolipid in prenatal chimeras.** B6 control and Balb/c→B6 chimeras were injected intravenously with OCH and iNKT cells were analyzed two hours later for intracellular cytokine production. (A) iNKT cells were identified by co-expression of TCR- $\beta$  and CD1d-PBS57 tetramer. Representative dot plots the ability to gate on tetramer positive cells following OCH but not KRN7000 stimulation. (B) Absolute and relative frequency of IFN- $\gamma$ + and IL-4+ iNKT cells are shown in graphs. Relative frequency of cytokine expression was calculated for donor reactive versus donor irrelevant phenotypes (Ly49AFG+ % Cytokine+/Ly49AFG- % Cytokine+). Each data point represents one animal.