

Supplemental Figure S1

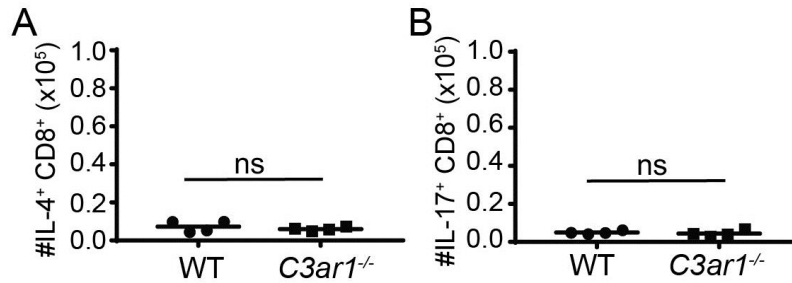


Figure S1. Absence of recipient C3aR1 does not result in IL-17 or IL-4 production by CD8⁺ T cells. Spleen cells were isolated from B6 WT and *C3ar1*^{-/-} recipients of BALB/c heart grafts isolated on day 6 post-transplant (as in Figure 1), stimulated with BALB/c (donor-origin) APCs, and stained for intracellular expression of IL-4 (A) and IL-17 (B) and analyzed by flow cytometry gated on recipient CD8⁺ T cells. Total numbers of cytokine producing cells per spleen are shown. n=4/group; ns= not significant.

Supplemental Figure S2

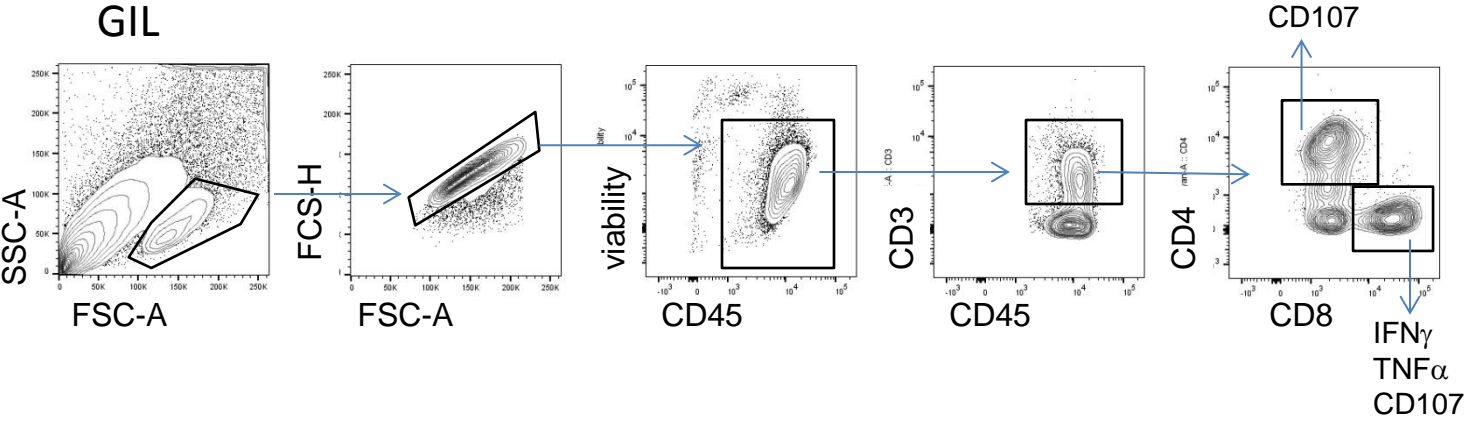
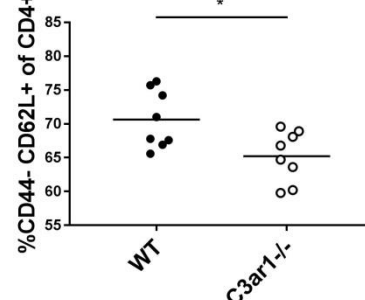
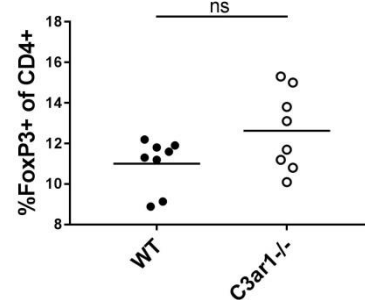
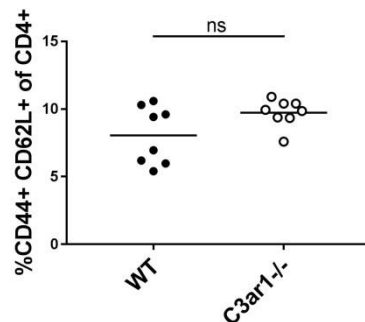
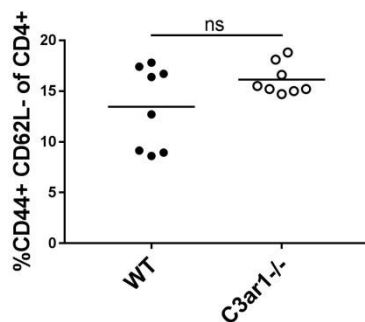
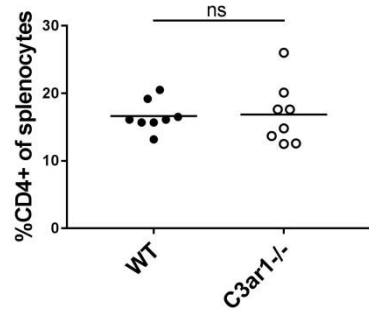


Figure S2. Gating strategy for detection of cytokine producing and CD107a/b⁺ CD8⁺ T cells from within graft infiltrating lymphocytes (GIL).

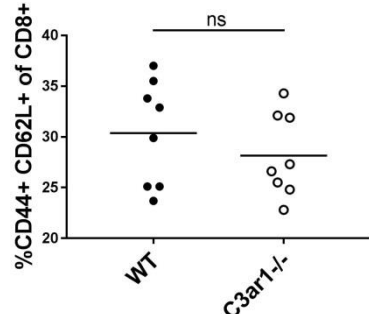
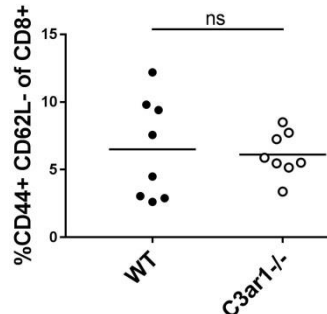
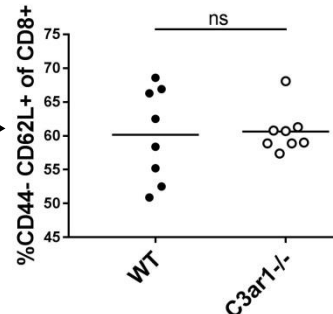
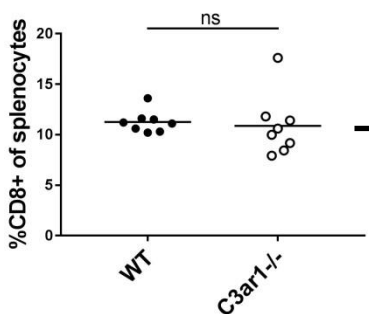
Supplemental Figure S3

(legend on next page)

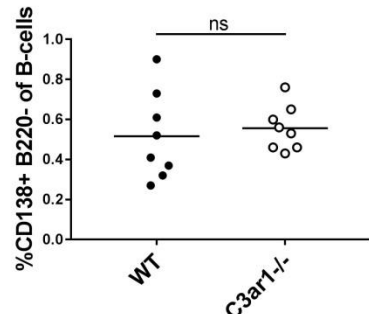
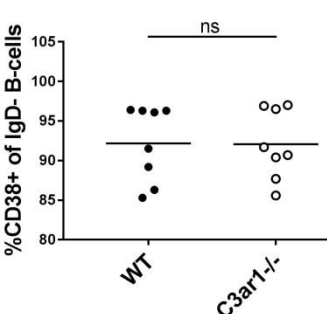
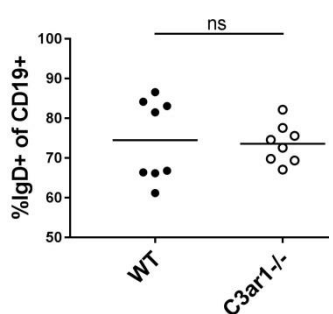
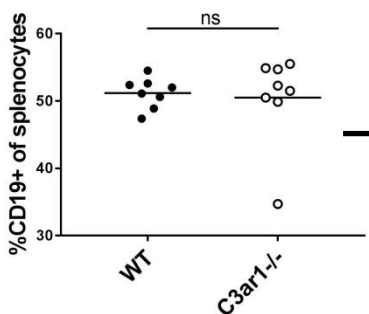
A



B



C



D

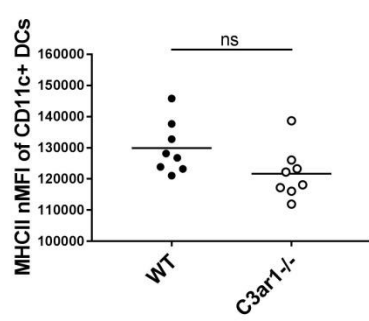
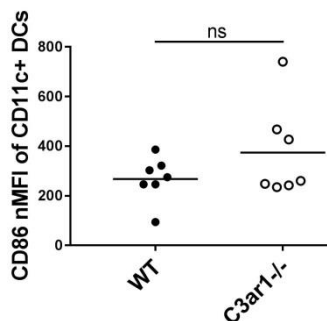
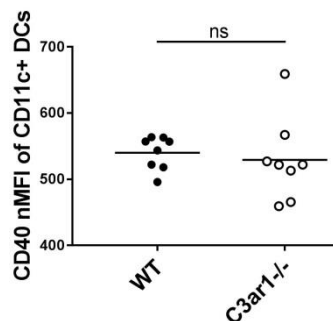
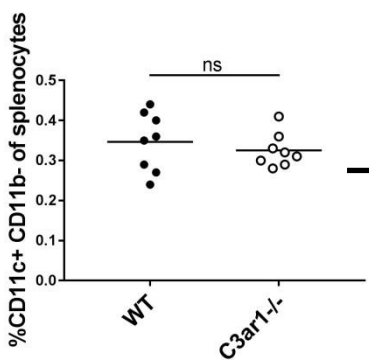


Figure S3. Immunophenotyping of naïve B6 WT and *C3ar1*^{-/-} mice. Animals are bred at Mount Sinai in the same room and cohoused. Spleen cells were obtained from male and female mice 6-12 weeks of age and analyzed by flow cytometry for CD4⁺ T cells and subsets (A), CD8⁺ T cells and subsets (B), B cell subsets (C) and costimulatory molecule expression on CD11c^{hi}CD11b⁻ DCs (D). n=eight per group, ns=not significant, *p<0.05

Supplemental Figure S4

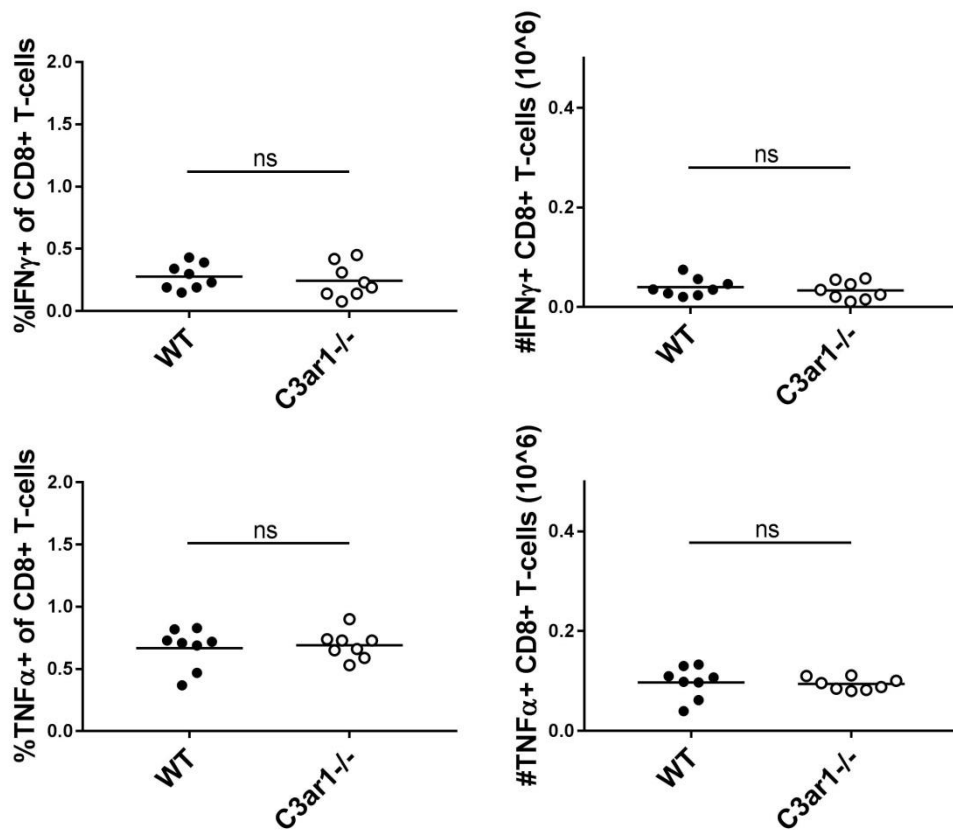


Figure S4. Baseline frequencies of cytokine producing alloreactive CD8⁺ T cells are similar in B6 WT and C3ar1^{-/-} mice. Splens cells from naïve animals analyzed in Supplemental Figure S3 were stimulated in vitro with allogeneic BALB/c APCs, stained for intracellular IFN γ or TNF α and analyzed by flow cytometry. Graphs depict percentages (left panels) and total numbers of donor-reactive IFN γ - (top) and TNF α - (bottom) producing CD8⁺ T cells per spleen. n=8/group, ns=not significant.

Supplemental Figure S5

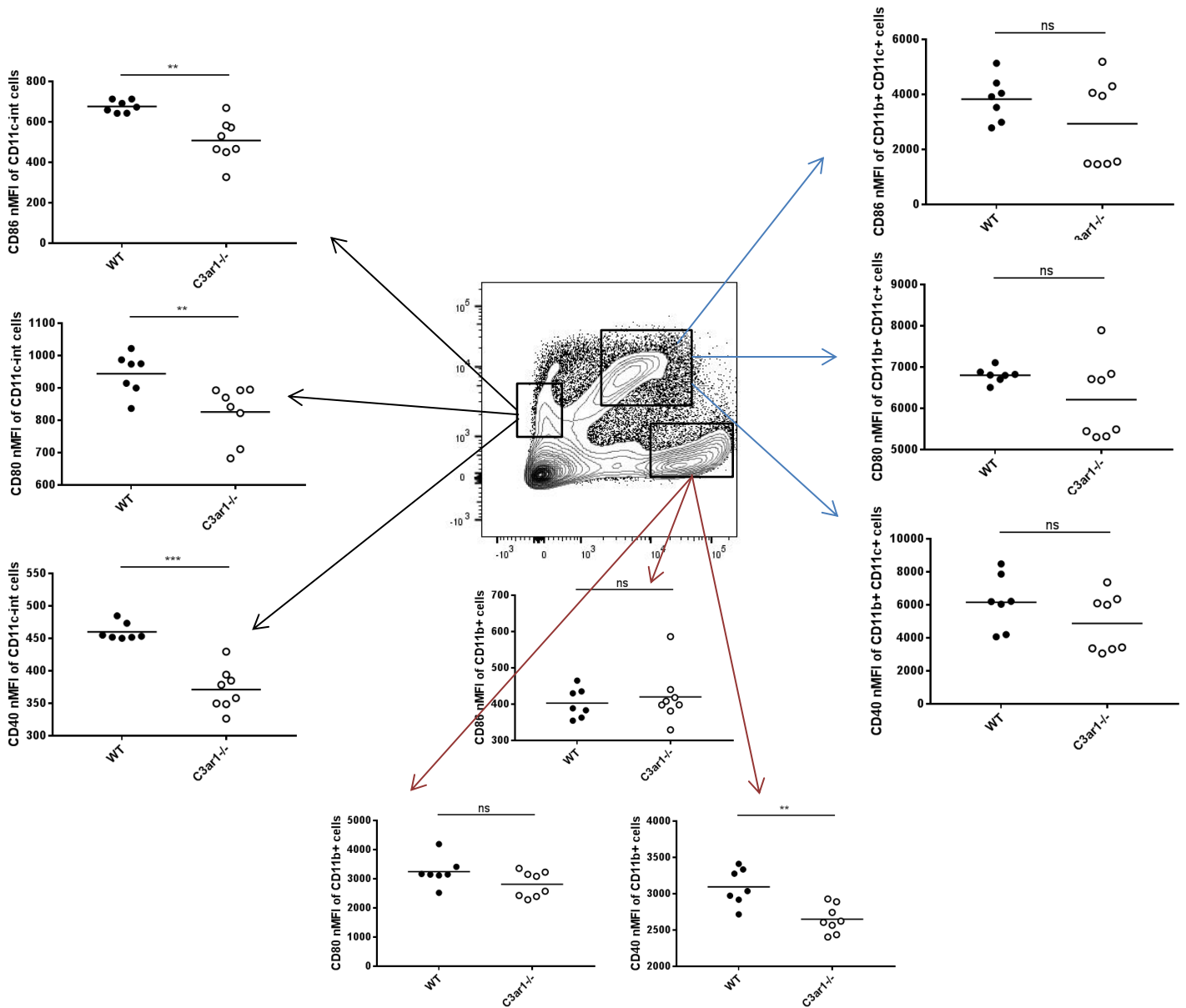


Figure S5. Costimulatory molecule expression on DC and monocyte subsets in transplanted WT and *C3ar1*^{-/-} mice. Quantification of MFI (bottom) showing expression of costimulatory molecules CD40 CD80, and CD86, gated on the splenic dendritic cell subsets in WT or *C3ar1*^{-/-} B6 recipients of BALB/c heart grafts 1 day post-transplantation from experiments depicted in Figure 6. n = 8/condition, ns=not significant, **p<0.01, ***p<0.001.