

## Supplemental Material

# B cells require type 1 interferon to produce alloantibodies to transfused KEL-expressing RBCs in mice

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## Supplemental Data

### Figure Legends

#### **Supplemental Figure 1: IFNAR expression is required for alloimmunization to KEL RBCs.**

Data show experimental replications of Figure 1. Peripheral blood of KEL-expressing transgenic mice was transfused into recipients. Serum anti-KEL IgG was measured by flow cytometric crossmatch. (A) Anti-KEL IgG in serum of WT or IFNAR1<sup>-/-</sup> mice 28 days after transfusion. (B) Serum anti-KEL IgG of transfused WT mice injected i.p. with anti-IFNAR1 blocking antibody (MAR1-5A3), an isotype control IgG1 antibody (MOPC-21), or PBS on Day -1, +2, and +7, relative to transfusion on Day 0. (C) Anti-KEL IgG of transfused WT, IFNAR1<sup>-/-</sup>, and bone marrow chimeric mice. Recipients were irradiated and reconstituted with donor bone marrow cells, 8 weeks prior to transfusion. “n/a”; non applicable. \*p<0.05 by (A, C) Mann Whitney U test and (B) Kruskal-Wallis test with a Dunn’s post-test.

#### **Supplemental Figure 2: IFNAR expression by B cells is required for KEL RBC alloimmunization.**

Data show experimental replications of Figure 2D (A), 3D (B), and 4D (C). (A) Mixed chimeras were generated by reconstituting irradiated CD45.2<sup>+</sup> WT recipients with a mixture of Zbtb46-DTR (CD45.1<sup>+</sup>, CD45.2<sup>+</sup>) and either IFNAR1<sup>-/-</sup> (CD45.2<sup>+</sup>) or WT (CD45.1<sup>+</sup>) bone marrow. Serum anti-KEL IgG produced by indicated chimeras treated with PBS or DT prior to transfusion with KEL RBCs. Z46-DTR = Zbtb46-DTR. n.s., not significant by Kruskal-Wallis test with a Dunn’s post-test. (B, C) Anti-KEL IgG in serum of indicated chimeras following transfusion with KEL RBCs. \*p<0.05, \*\*p<0.01 and n.s., not significant, by Mann Whitney U test. (B) Mixed chimeras were generated by reconstituting irradiated IFNAR1<sup>-/-</sup> (CD45.2<sup>+</sup>) recipients with a mixture of TCR $\alpha$ <sup>-/-</sup> (CD45.2<sup>+</sup>) and either IFNAR1<sup>-/-</sup> (CD45.2<sup>+</sup>) or WT (CD45.1<sup>+</sup>) bone marrow. (C) Mixed chimeras were generated by reconstituting irradiated WT (CD45.2<sup>+</sup>) recipients with a mixture of muMT<sup>-</sup> (CD45.2<sup>+</sup>) and either IFNAR1<sup>-/-</sup> (CD45.2<sup>+</sup>) or WT (CD45.1<sup>+</sup>) bone marrow. Chimeras reconstituted with only IFNAR1<sup>-/-</sup> bone marrow (left) served as negative controls for alloimmunization.

#### **Supplemental Figure 3. IFNAR expression promotes germinal center B cell development.**

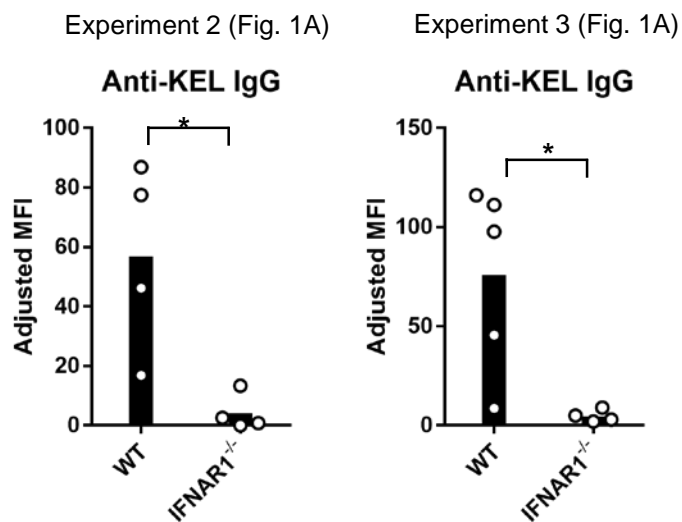
Data show experimental replications of Figure 5. IFNAR1<sup>-/-</sup> and WT mice were transfused with KEL RBCs. (A-C) Spleen GC B cells (CD19<sup>+</sup>IgD<sup>lo</sup>Fas<sup>+</sup>GL7<sup>hi</sup>) from (A) naïve or transfused mice (B) 8 or (C) 36 days after transfusion were quantified by flow cytometry as percent of CD19<sup>+</sup> B cells (left) and cell number (right). (D) Serum anti-KEL IgG measured by flow cytometric crossmatch 36 days after transfusion. (C, D) Mice received a second transfusion 28 days following the first transfusion. Data are from one of two independent experiments with 3-5 mice per group. \*p<0.05, n.s., not significant, by Mann Whitney U test. Experiments with 3 mice per group were not tested for statistical significance.

#### **Supplemental Figure 4. IFNAR expression promotes plasma cell differentiation.**

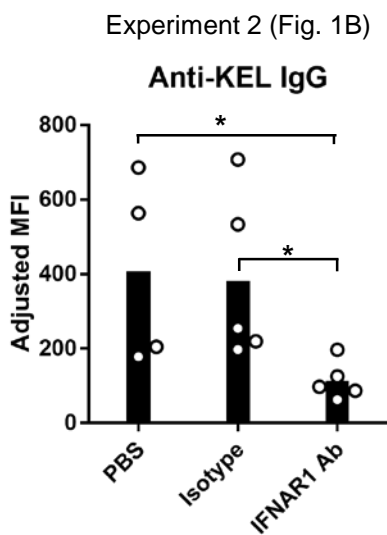
Data show experimental replications of Figure 6B-C. WT and IFNAR1<sup>-/-</sup> mice were transfused 28 days following an initial transfusion, and plasma cells were analyzed 14 days later. (A) Bone marrow plasma cells (CD19<sup>+</sup>IgD<sup>lo</sup>B220<sup>lo</sup>CD138<sup>+</sup>) were quantified by flow cytometry as (B) percent of CD19<sup>+</sup> B cells and (C) cell number. Data show one of two independent experiments with 4 mice per group. \*p<0.05, n.s. not significant, by Mann Whitney U test.

# Supplemental Figure 1

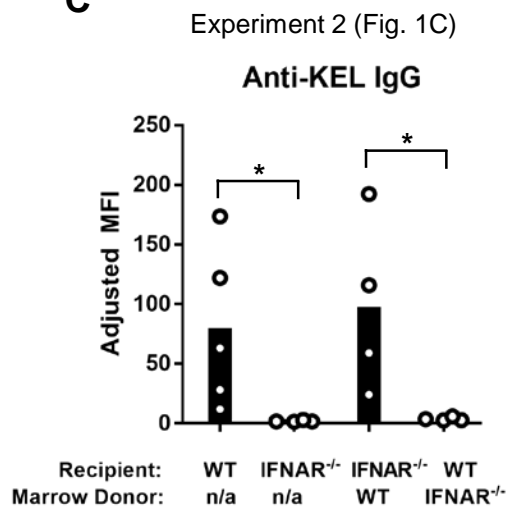
**A**



**B**



**C**

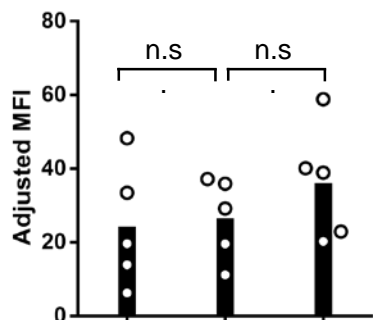


# Supplemental Figure 2

## A

Experiment 2 (Fig. 2D)

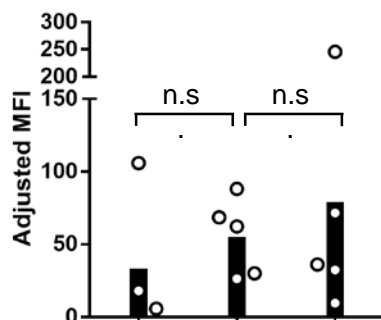
Anti-KEL IgG



BM donor 1: Z46-DTR Z46-DTR Z46-DTR  
 BM donor 2: IFNAR1<sup>-/-</sup> IFNAR1<sup>-/-</sup> WT  
 Treatment: PBS DT DT

Experiment 3 (Fig. 2D)

Anti-KEL IgG

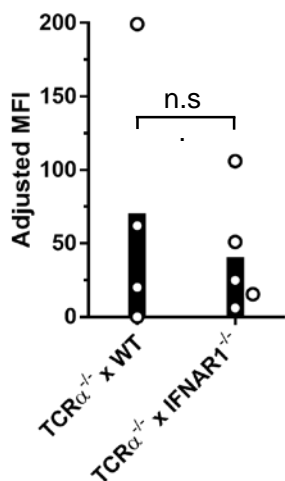


BM donor 1: Z46-DTR Z46-DTR Z46-DTR  
 BM donor 2: IFNAR1<sup>-/-</sup> IFNAR1<sup>-/-</sup> WT  
 Treatment: PBS DT DT

## B

Experiment 2 (Fig. 3D)

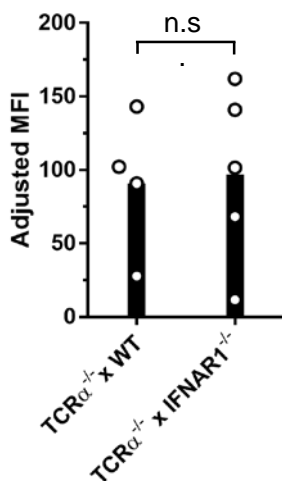
Anti-KEL IgG



TCR $\alpha^+$  x WT  
 TCR $\alpha^+$  x IFNAR1<sup>-/-</sup>

Experiment 3 (Fig. 3D)

Anti-KEL IgG

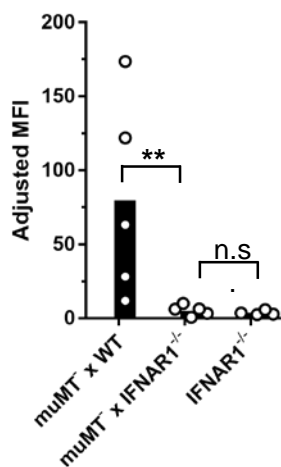


TCR $\alpha^+$  x WT  
 TCR $\alpha^+$  x IFNAR1<sup>-/-</sup>

## C

Experiment 2 (Fig. 4D)

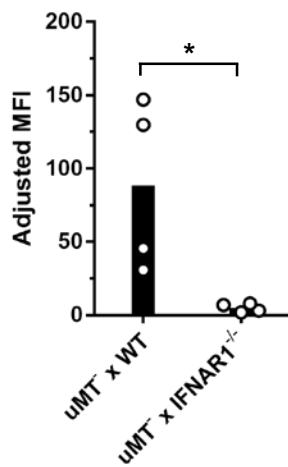
Anti-KEL IgG



muUMT x WT  
 muUMT x IFNAR1<sup>-/-</sup>  
 IFNAR1<sup>-/-</sup>

Experiment 3 (Fig. 4D)

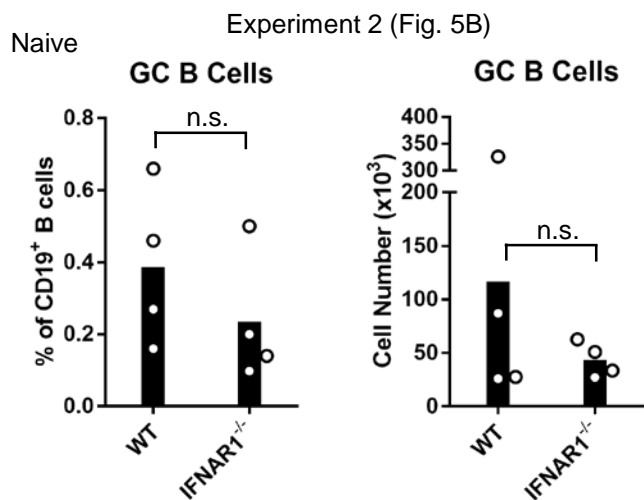
Anti-KEL IgG



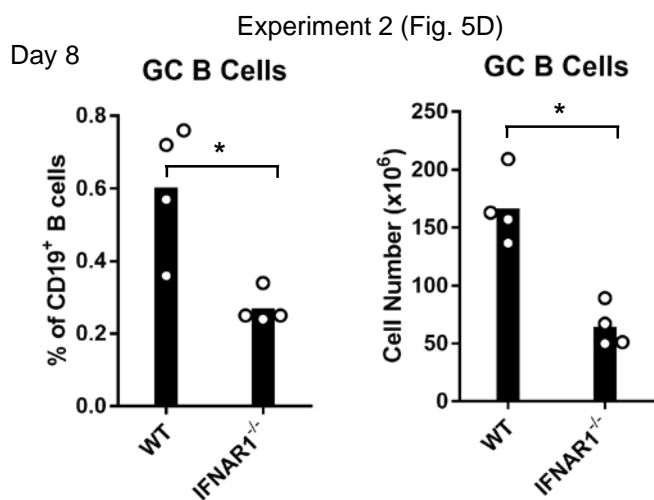
UMT x WT  
 UMT x IFNAR1<sup>-/-</sup>

# Supplemental Figure 3

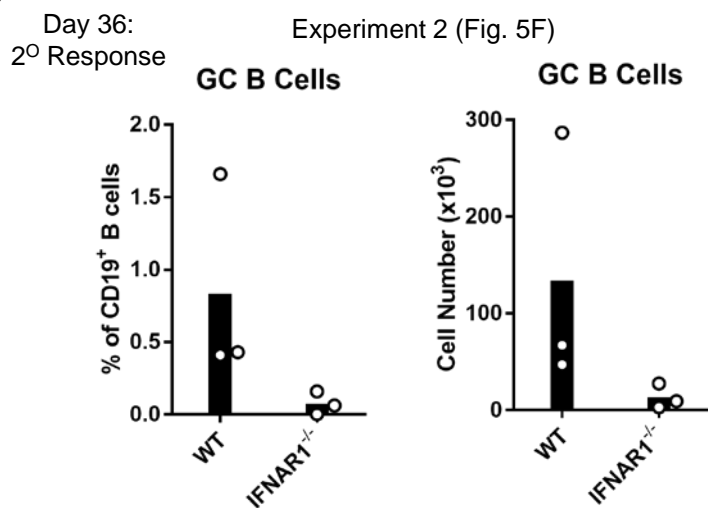
**A**



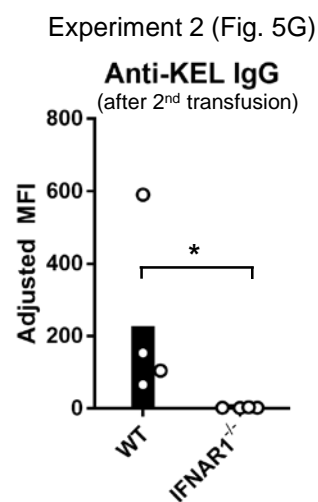
**B**



**C**



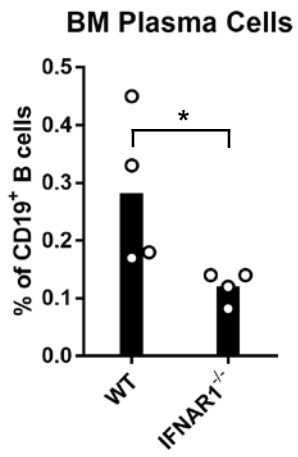
**D**



# Supplemental Figure 4

## A

Experiment 2 (Fig. 6B)



Experiment 2 (Fig. 6C)

