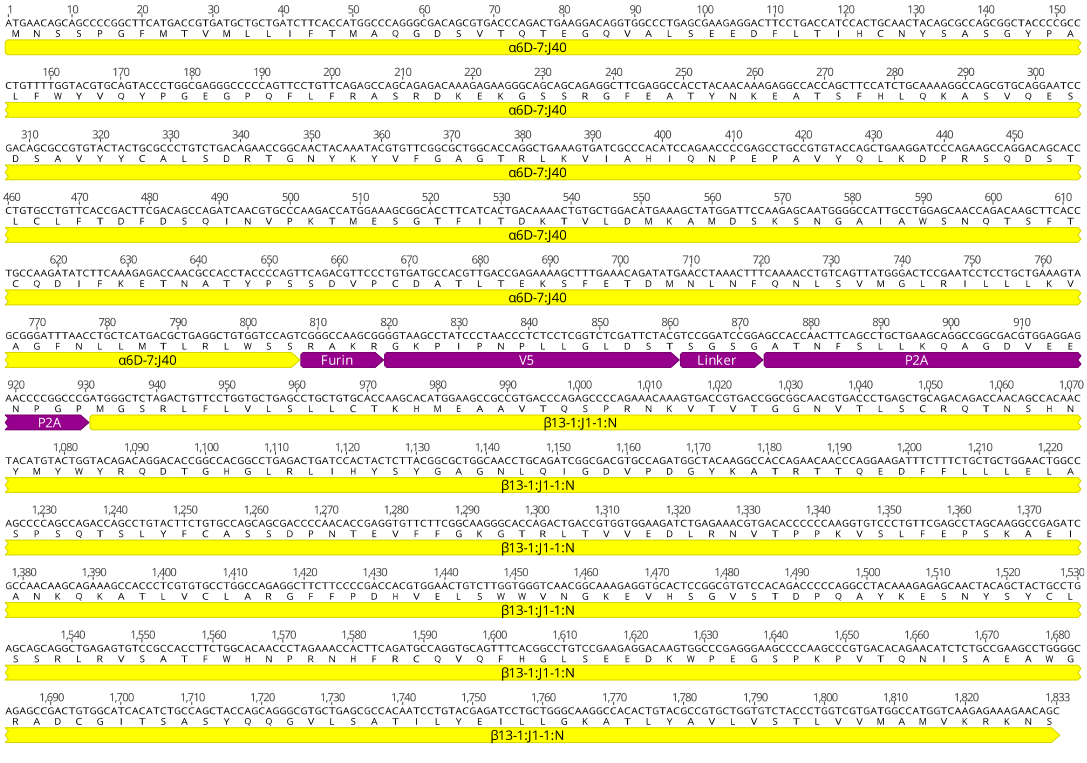
Supplemental Table 1. GUCY2C-Specific VDJ Usage in *Gucy2c*$^{-/-}$ and Retrogenic Mice.

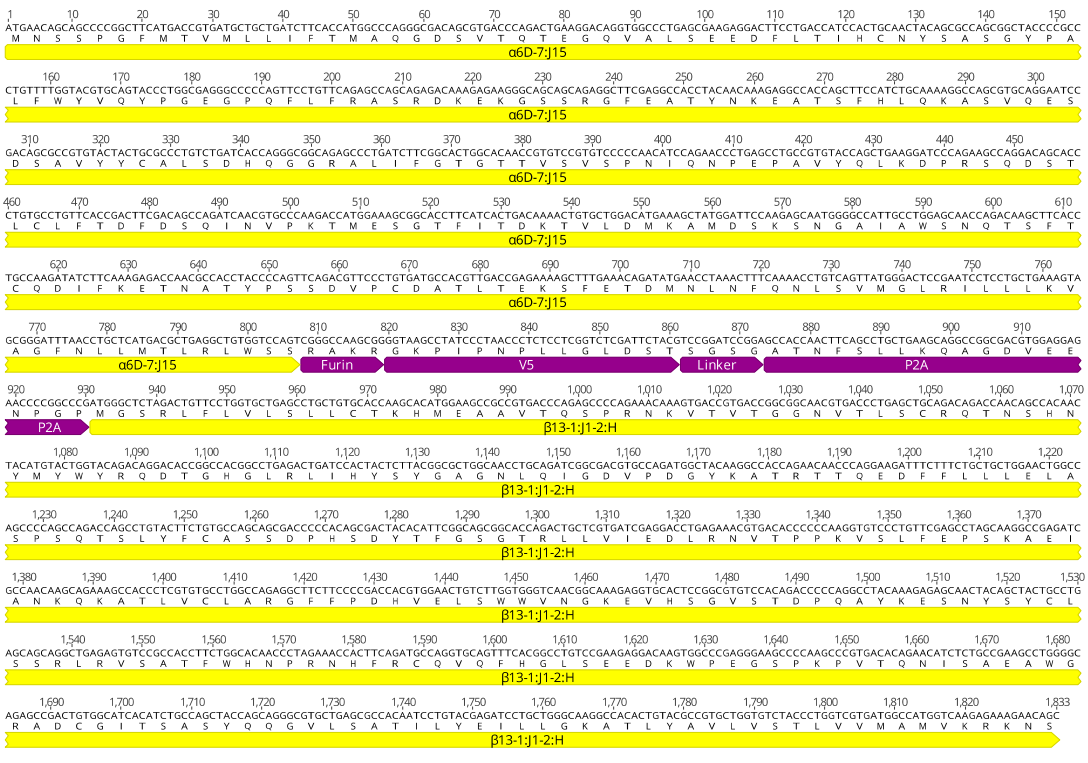
	***Gucy2c*$^{-/-}$ TCRα Usage**		***Gucy2c*$^{-/-}$ TCRβ Usage**	
Frequency Rank[a]	**CDR3**	**V-J Usage**	**CDR3**	**V-D-J Usage**
1	AVNMGYKLT	TRAV6-2 TRAJ9	ASSDPNSDYT	TRBV13-1 TRBJ1-2
2	ALSDRTGNYKYV	TRAV6D-7 TRAJ40	ASSDPNTEVF	TRBV13-1 TRBJ1-1
3	AAVTNAYKVI	TRAV4D-3 TRAJ30	ASSDPHSDYT	TRBV13-1 TRBJ1-2
4	ALSDHQGGRALI	TRAV6D-7 TRAJ15	ASGRGDTQY	TRBV29 TRBD1 TRBJ2-5
5	ALAPPSSAGNKLT	TRAV6D-5 TRAJ17	AWSRGTGGARGQDTQY	TRBV31 TRBD2 TRBJ2-5
TCR 4A and TCR 5B VDJ usage are indicated by color				

[a]The top 5 CDR3s ranked in Fig. 1 are shown with their V-J or V-D-J usage.

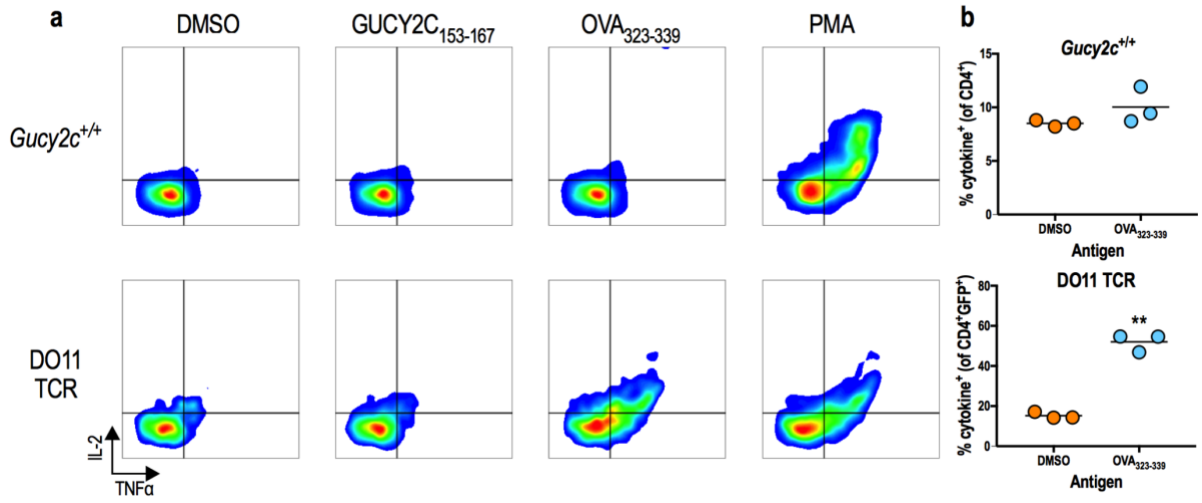
TCR 4A



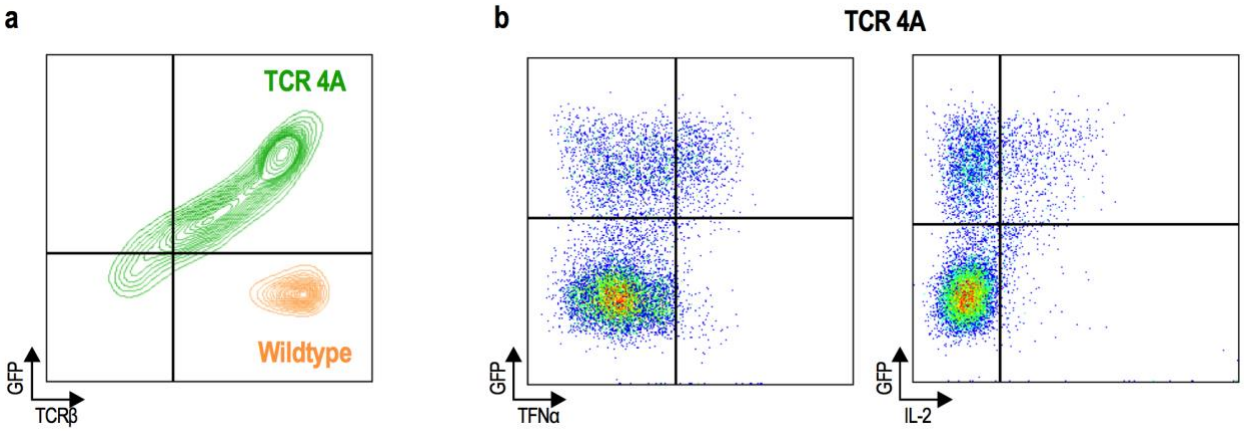
TCR 5B



Supplemental Figure 1. TCR 4A and 5B Sequences.



Supplemental Figure 2. DO11 Retrogenic Immune Responses. (a-b) Retrogenic mice produced with DO11 TCR and conventional *Gucy2c*$^{+/+}$ mice were immunized with Ad5-OVA. Splenocytes were collected 14 days later and analyzed by IL-2/TNFα ICS after stimulation with DMSO (vehicle), GUCY2C$_{153-167}$, OVA$_{323-339}$ or PMA/IONO (positive control). Data indicate responses by individual mice and are representative of two experiments with 3 mice/group. ** p <0.01, T-test.



Supplemental Figure 3. Relationship Between TCR Expression Levels and Cytokine Responses. (a) $CD4^+$ T cells from *Gucy2c*$^{-/-}$ retrogenic mice produced with TCR 4A and conventional *Gucy2c*$^{+/+}$ mice were stained with anti-GFP and anti-TCRβ antibodies, demonstrating that TCR 4A T cells possess a continuum of TCR levels, with the highest expressers being similar to conventional $CD4^+$ T cells. (b) $CD4^+$ T cells from Ad5-GUCY2C-immunized *Gucy2c*$^{-/-}$ TCR 4A retrogenic mice were stimulated with $GUCY2C_{153-167}$ peptide and stained with anti-GFP, anti-TNFα, and anti-IL-2 antibodies (data from **Fig. 5** gated on $CD4^+GFP^+$ cells). Here, data are gated on total $CD4^+$ T cells, demonstrating that cytokines are produced only by GFP^{hi} cells. Data indicate responses by individual mice and are representative of two experiments with 3-5 mice/group.