Id3 and Id2 act as a dual safety mechanism in regulating the development and population size of innate-like γδ T cells

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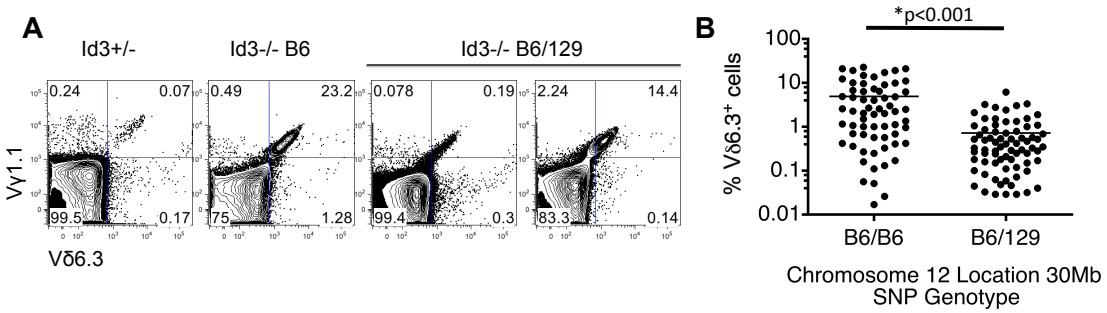

Figure S1. Phenotype of Vγ1.1Vδ6.3 T cells. **(A)** Representative FACS analysis of thymocytes with Vγ1.1 and Vδ6.3 markers. Two B6/129 F2 backcrossed mice along with controls were shown. **(B)** B6 homozygosity of one SNP marker (rs29198984) on chromosome 12 was associated with the expansion of Vδ6.3$^+$ γδ T cells in Id3$^{-/-}$ B6/129 F2 mice. The SNP marker overlaps with the restriction enzyme site for ApaLI, which is used to separate the 129 and B6 alleles after ApaLI digestion of the PCR products. 138 Id3$^{-/-}$ B6/129 F2 mice were analyzed for their genotype of the SNP marker and their percentage of Vδ6.3$^+$ γδ T cells in the thymus. Those with B6/B6 genotype had significantly higher percentage of Vδ6.3$^+$ γδ T cells compared to those with B6/129 genotype. Each dot represent one mouse.

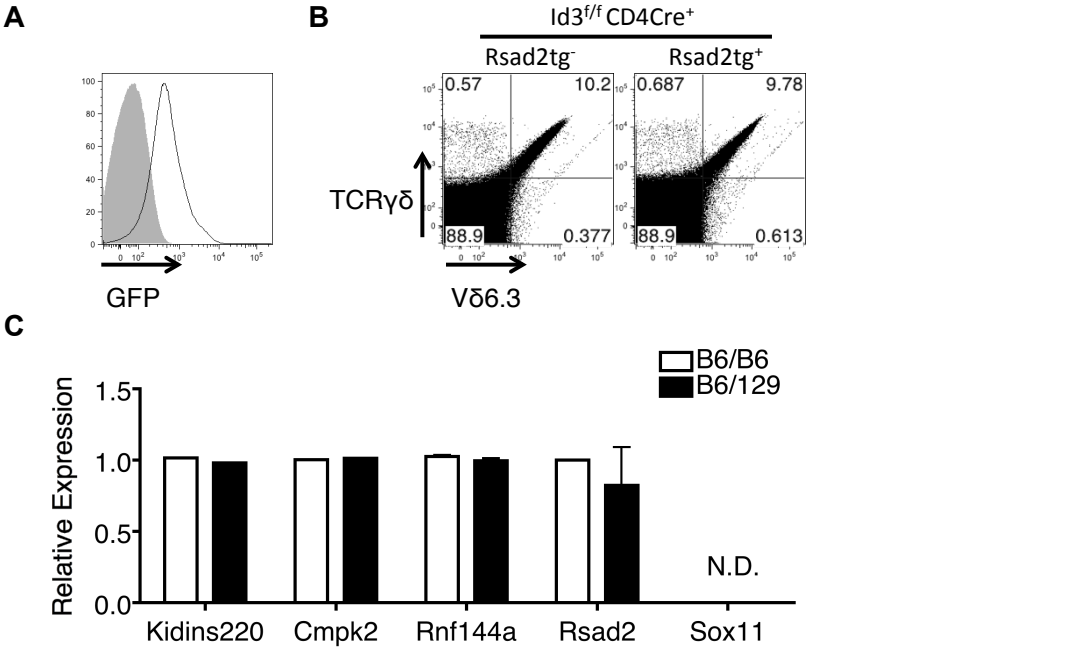

Figure S2. Exploring the role of the candidate genes on mouse chromosome 12 in the expansion of Vδ6.3$^+$ γδ T cells. **(A)** A transgenic mouse carrying a cassette consisting of CAG promoter, GFP, loxP-STOP-loxp and *Rsad2* cDNA of the 129 genetic background was created on the B6 background. The expression of GFP from the transgene cassette was demonstrated in this representative plot of total thymocytes. **(B)** Expression of *Rsad2* from the 129 genetic background failed to suppress the accumulation of Vδ6.3$^+$ γδ T cells in mice with Id3 deficiency. Data representative of multiple experiments combining the *Rsad2* transgene with Id3 floxed alleles and CD4Cre, LckCre or CMVCre. **(C)** Screening QPCR analysis of Vδ6.3$^+$ γδ T cells with either B6/B6 homozygosity or B6/129 heterzygosity on chromosome 12 location 25.16Mb-28.02Mb showed that the five known protein-coding candidate genes within or near this genomic interval are either expressed to a similar extent in both groups or not expressed in these cells. n=1 to 3 in each group. Error bars indicate SD.