Supplemental Data

Regulation of Activated CD4$^+$ T Cells by NK Cells via the Qa-1–NKG2A Inhibitory Pathway

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Supplemental Reference

Gorman, J.R., van der Stoep, N., Monroe, R., Cogne, M., Davidson, L., and Alt, F.W. (1996). The Ig(kappa) enhancer influences the ratio of Ig(kappa) versus Ig(lambda) B lymphocytes. Immunity. *5*, 241-252.

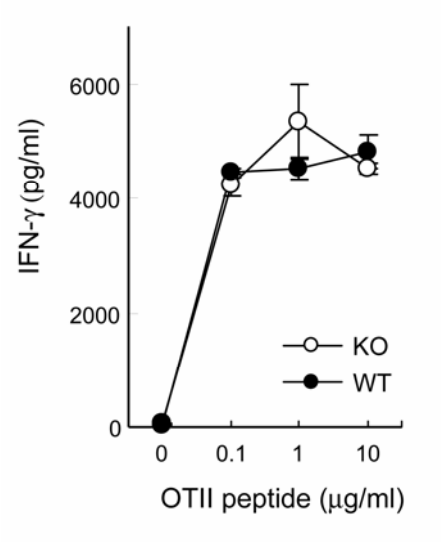


Figure S1. Activation of Qa-1-Deficient OTII CD4 Cells in *Rag2$^{-/-}$Prf1$^{-/-}$* Hosts
OTII CD4$^+$ T cells (1×10^6) purified from both Qa-1 wild type (WT) and Qa-1-deficient (KO) mice were transferred i.v. into *Rag2$^{-/-}$Prf1$^{-/-}$* hosts followed by immunization with 50 μg OVA peptide emulsified in CFA. Spleen and draining LN were collected 14 days after transfer and 1×10^5 pooled lymph node and spleen cells were restimulated with OVA peptide. IFN-gamma secretion was measured 72 hours after stimulation. Data shown represents mean $\pm$ s.d. (n=3).

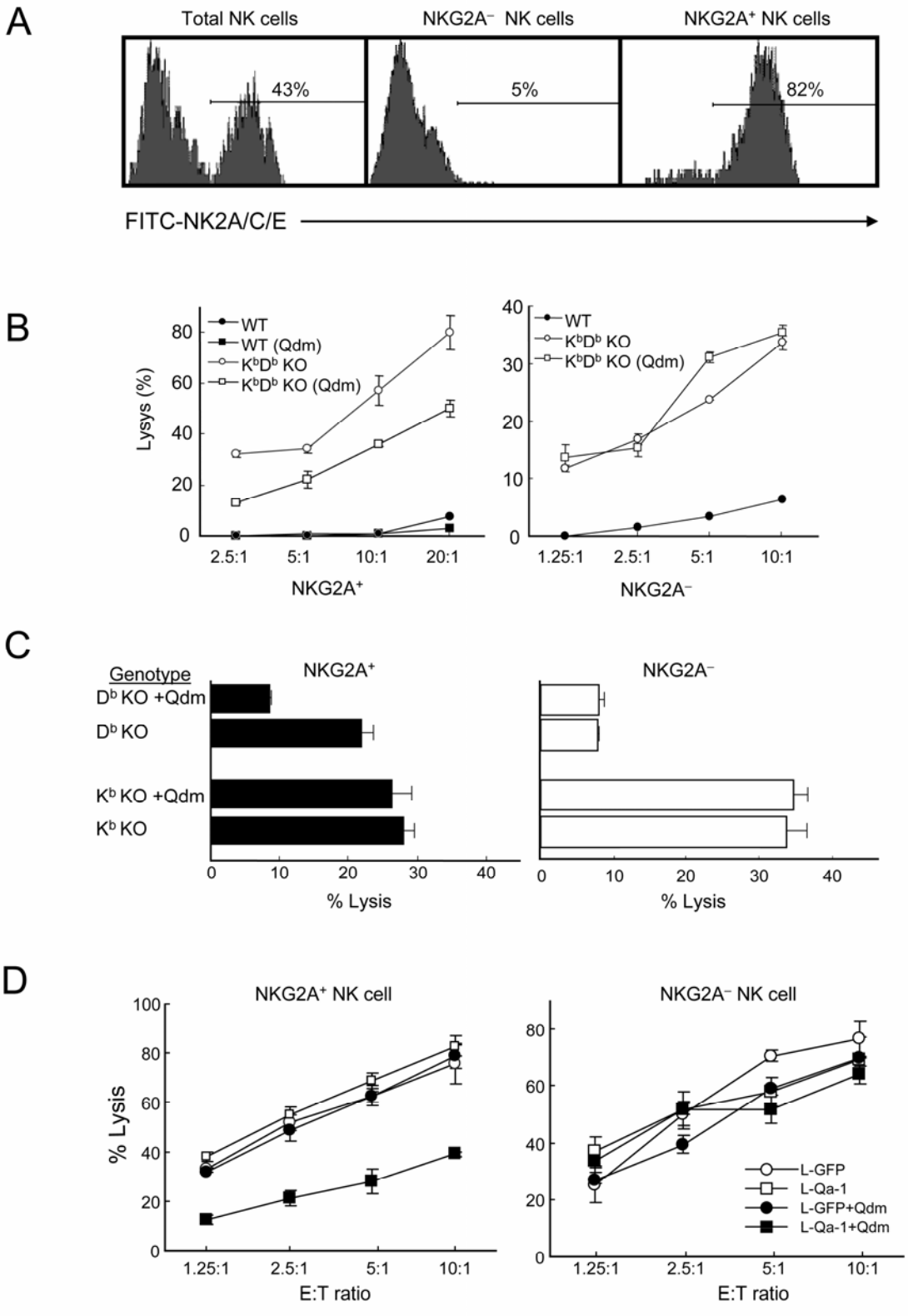
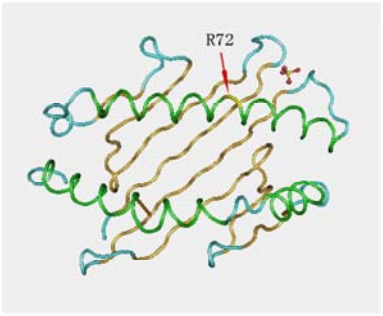


Figure S2. Analysis of NKG2A$^+$ and NKG2A$^-$ NK Cells

(**A**) Separation of NKG2A$^+$ and NKG2A$^-$ NK cells. NK cells were enriched by negative selection before NKG2A$^+$ and NKG2A$^-$ NK cells were sequentially separated by positive selection using

NKG2AB6 and DX5 antibody, respectively, as described in Experimental Procedures. Purified cells were incubated with IL-2 for 5 days. FACS analysis of NKG2A expression is shown.
(**B**) Lysis of H-2K^{b}D^b-deficient CD4 target cells by NKG2A$^+$ and NKG2A$^-$ NK cells.
H-2K^{b}D^b-deficient CD4 cells were activated by Concanavalin A (ConA) for 48 hour and labeled with ^{51}Cr before use as targets of NKG2A$^+$ and NKG2A$^-$ NK cells. The percentage of killing at the indicated E:T ratios is shown.
(**C**) H-2K^b-deficient or H-2D^b-deficient CD4 T cells were used as targets for NK cells and the percentage of lysis at an E:T ratio of 10:1 is shown. Data shown represents mean $\pm$ s.d. (n=3).
(**D**) L cells infected with lentivirus expressing either GFP (control) or Qa-1 (as indicated) were subject to *in vitro* killing by IL-2-activated NKG2A$^+$ (left panel) or NKG2A$^-$ (right panel) NK cells with or without addition of Qdm peptide (30 μM). Lysis (%) is shown at the indicated E:T ratios. Data shown represents mean $\pm$ s.d. (n=3).

A

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HLA-E 61 DRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQW
Qa-1  61 ERETWKARDMGRNFRVNLRTLLGYYNQSNDESHTLQW
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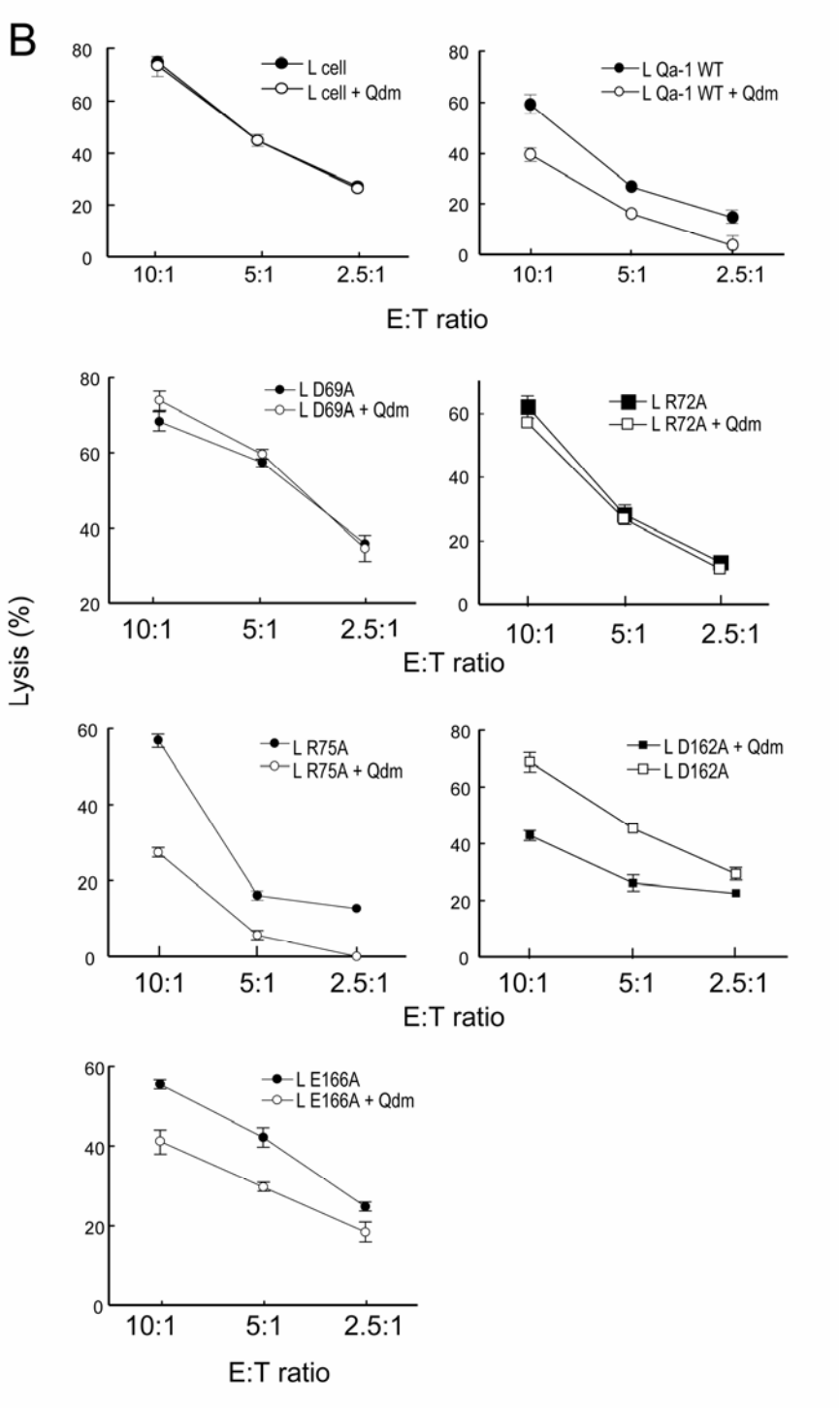
Mutation	WT	D69A	R72A	R75A	D162A	E166A
Expression on L cells	+	-	+	+	+	+
Protect from NKG2A^{+} NK cell killing	+	-	-	+	+	+


B
L cell
L cell + Qdm
L Qa-1 WT
L Qa-1 WT + Qdm
L D69A
L D69A + Qdm
L R72A
L R72A + Qdm
L R75A
L R75A + Qdm
L D162A + Qdm
L D162A
L E166A
L E166A + Qdm
Lysis (%)
E:T ratio
10:1
5:1
2.5:1

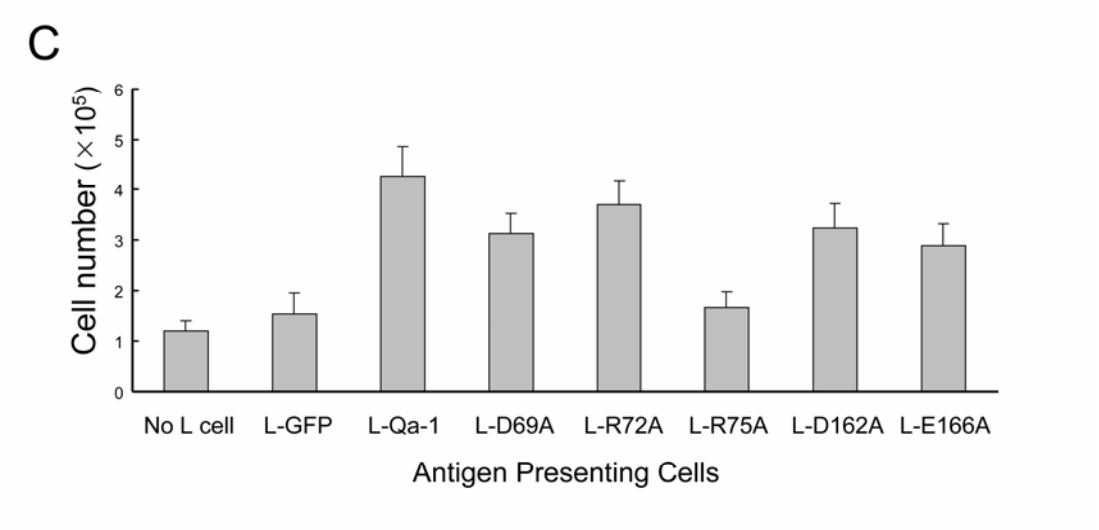


Figure S3. The R72A Mutation Disables the Qa-1 Molecule in Protecting L Cells from NK Cell Killing

(**A**) The top panel shows the position of R72 in the alpha 1 domain of the Qa-1 molecule. The sequence alignment (lower panel) between HLA-E and Qa-1 shows conservation around the alpha 1 region. The potential NKG2A-binding residues (arrows) are conserved between HLA-E and Qa-1. The selected mutations screened are listed in the lower table.

(**B**) L-cells were infected with lentivirus expressing either WT or Qa-1 mutants (as indicated) and used as target cells of IL-2-activated NKG2A$^+$ NK cells with or without addition of Qdm peptide (30 μM). Percentage of lysis is shown at the indicated E:T ratios.

(**C**) L-cells expressing different Qa-1 mutants were used as stimulators to CD8 cells from B6.Tla mice immunized with Qa-1 ConA blasts. Expansion of CD8 cells after 3 days is shown. Data shown represents mean $\pm$ s.d. (n=3).

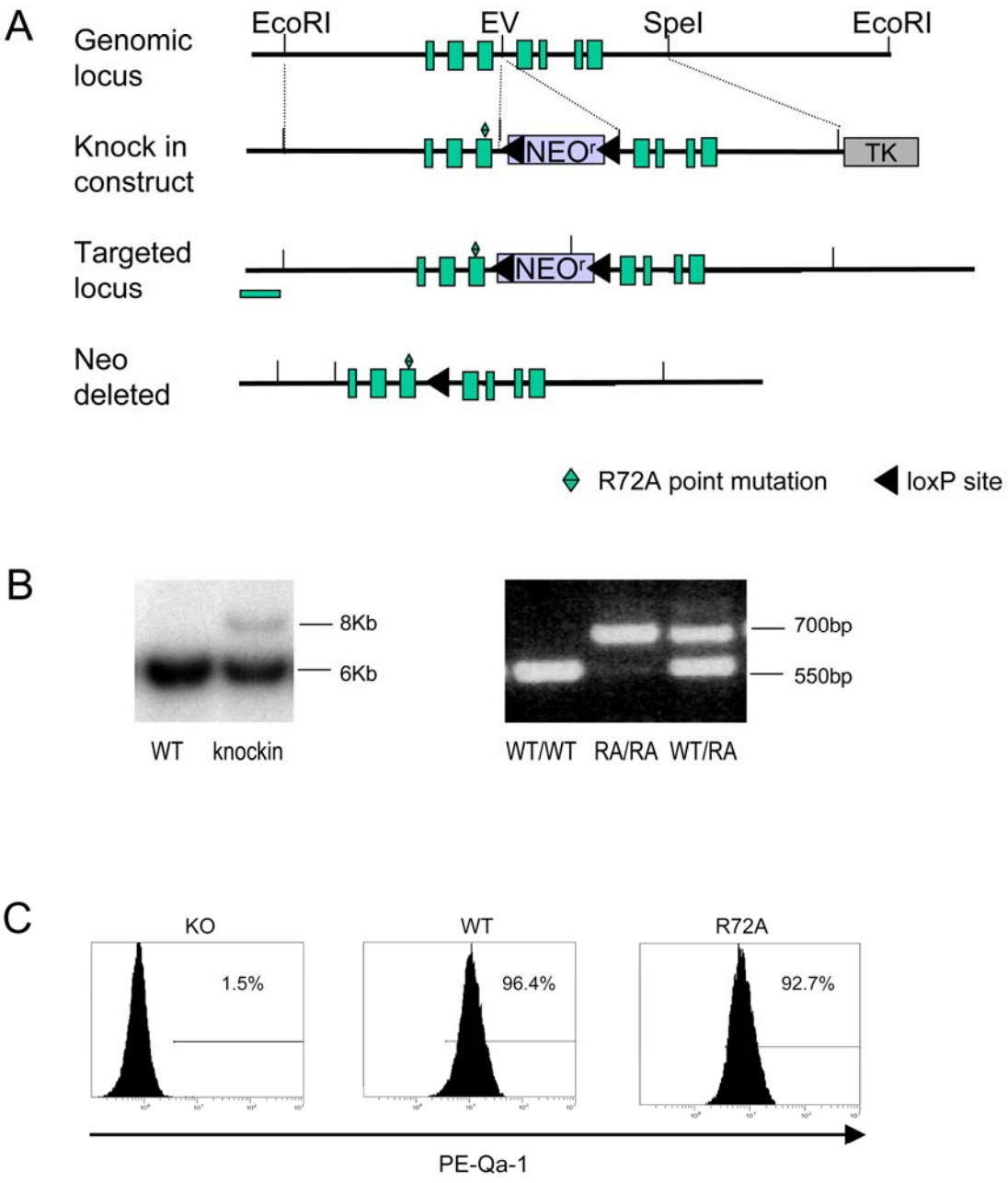


Figure S4. Generation of Qa-1 R72A Mutant Knockin Mice

A BAC clone containing an 11 kb DNA fragment including the Qa-1 gene (*H2-T23*) has been identified in the lab and fully mapped. LoxP flanked Neo^r was introduced into the EcoRV site in intron 3 of the Qa-1 gene without interrupting the splicing by avoiding the conserved splicing branch site. After cloning the 3.5 kb Qa-1-containing fragment (from EcoRI to EcoRV) into a cloning vector, the R72A mutation was introduced by site-directed mutagenesis and confirmed by sequencing. This fragment was cloned into the SalI site of pLNTK (Gorman et al., 1996) and the 2.7 kb fragment (EcoRV to SpeI) was then cloned into the XhoI site to complete the replacement vector. After TC1 embryonic stem cells were transfected with the targeting vector, positively-selected recombinants were identified by long-rang PCR screening and Southern blot. The Neo^r gene was deleted by crossing the germline transmitted litters to EIIα-CRE mice, which were then crossed to C57BL/6 mice for 5

generations. Homozygous R72A mutant mice were obtained by intercrossing the heterozygous littermates.
(A) Qa-1 Genomic Locus and R72A Targeting Strategy: DT, diphtheria toxin; *neo*^{r}, neomycin-resistance gene.
(**B**) Southern Blot of Embryonic Stem Cell Genomic DNA. The lower bands (6 kb) correspond to the wild type allele; the upper band (8 kb) represents the knockin allele. The right panel represents PCR genotyping of knockin mice. The WT (550bp) and knockin (700bp) products represent the addition of base pairs from the remaining loxP site and surrounding sequence (WT/WT, Qa-1 WT; RA/RA, homozygous mutant knockin mice; WT/RA, heterozygous).
(**C**) ConA-Induced Qa-1 Expression of Wild Type and R72A Mutant. Splenocytes from littermates (Qa-1-deficient [KO], Qa-1 wild-type [WT] and Qa-1 R72A knockin [R72A]) were individually stimulated with ConA for 40h and analyzed for surface Qa-1 expression by FACS analysis using Qa-1 antibody (BD Bioscience).