SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1

Presence of NK and T cells and expression of cytotoxic genes in livers of infants with biliary atresia. Panel **(A)** depicts representative sections of livers from an unaffected control and from an infant at the time of diagnosis of biliary atresia immunostained with anti-CD56 antibody (to detect NK cells: red color, arrows) and anti-CD3 antibody (to detect T cells: green color, arrowheads) after nuclear counterstaining with DAPI (blue). Photograph represents an overlay of images captured with individual filters; white bar = 50 μm. Panel **(B)** shows the hepatic mRNA expression for NK cell-enriched cytotoxic genes in livers of infants with biliary atresia. mRNA was quantified by real-time PCR and expressed as a ratio to human *HPRT* (mean ± S.D.). N=9 for biliary atresia and N=7 for controls; *P<0.05.

Supplementary Figure 2

Lysis of epithelial cells by RRV-primed NK cells. Mean ± S.D. of percent of ^{51}Cr release by the cholangiocyte line mCL, hepatocyte line H2.35, lung epithelial line MLE-12, and breast carcinoma lines 4T1-MZ and 67-NR after 5 hours of co-culture with hepatic NK cells purified 7 days after injection of RRV into 1 day old mice. Ratios represent target (mCL, H2.35, MLE-12, 4T1-MZ, 67-NR) to effector (NK) cells. N=3 wells per group; results are representative of 2 independent experiments, with hepatic NK cells obtained from pools of 15-20 livers (*P<0.01 when cytolytic activities are compared among the different ratios; **P<0.01 when comparisons are from mCL or H2.35 to other cells).

Supplementary Figure 3

Expression of Nkg2d ligands. Flow cytometry histogram of mCL cells labeled with anti-panRae1 antibodies or isotype control (**A**; number represents median fluorescence intensity of panRae1; representative figure of 3 different experiments). In panel **B**, the hepatic mRNA expression of the *Rae1* family of genes, *Mult1*, and *H60* after 7 days following saline or RRV injection is depicted as a ratio to *Gapdh*. *P<0.02 for RRV versus saline groups; N=3-4 per group per time point.

Supplementary Figure 4

Number of hepatic NK cells and the expression of CD69 after RRV inoculation. (**A**) Total number of hepatic CD49b+ cells based on flow cytometry 3-14 days after RRV injection in the first day of life with or without daily administration of blocking anti-Nkg2d antibodies. (**B**) Percentage of CD49b+ liver cells also expressing CD69. N=3-4 per group and per time point; *P<0.05.

Supplementary Figure 5

Expression of cytokines and chemokines after RRV inoculation and anti-Nkg2d antibodies. Hepatic mRNA expression for *Ifnγ, Cxcl9, Cxcl10, perforin and granzymes A and B* at 3-14 days after RRV injection to Balb/c mice in the first day of life and in a group of mice that also received daily administration of blocking anti-Nkg2d antibodies. The baseline level of mRNA expression is shown in saline-injected controls as interrupted black lines. N=3-4 per group and per time point; *P<0.05.

Supplementary Table 1

Average number of mononuclear cells per unit of gall bladder/extrahepatic bile duct harvested en bloc from neonatal mice at 7 and 14 days after saline or RRV inoculation in the first day of life. Cells were identified and quantified by flow cytometric analysis. N=15-20 for each group and time point.

	Day 7: saline	Day 7: RRV	Day 14: saline	Day 14: RRV
CD3/CD4	1	24	1	7
CD3/CD8	0	12	0	0
CD19	7	0	0	0
CD11b/Gr1	0	22	0	5
CD11b/F4-80	2	104	1	5
CD3/CD49b	1	20	0	1
CD49b	71	1456	62	1019

CD19=B cells; CD11b/Gr1=neutrophils; CD11b/F4-80=macrophages; CD3/CD49b=NKT cells; CD49b=NK cells

Supplementary Table 2

Oligonucleotide primer sequences and PCR product sizes used in real-time PCRs to quantify the expression of cytokines, chemokines, cytotoxic mediators, and NK cell-enriched genes.

Gene	Primer Sequences	Temp. (˚C)	Size (bp)
Ifnγ *	For: 5'-GGCTGTCCCTGAAAGAAAGC-3' Rev: 5'-GAGCGAGTTATTTGTCATTCGG-3'	52	100
Il12p40	For: 5'-AAAGGCTGGGTATCGGTGG-3' Rev: 5'-ACTGGCTGTGCTGGAACTCC-3'	52	118
Cxcl9	For: 5'-GAGCTAGATAGACCTCACCAAG-3' Rev: 5'-CCATTAGCACCATCTCTGA-3'	52	101
Cxcl10	For: 5'-TCGCTCAAGTGGCTGGGATG-3' Rev: 5'-TAGGGAGGACAAGGAGGGTGTG-3'	57	117
Perforin	For: 5'-GGGTTTATCAGTTGTGCCGTC-3' Rev: 5'-TAGCAGATGGACAGGGGTGTAG-3'	53	128
Granzyme-A	For: 5'-CTCACTCAATCAATAAGGAGCCAG-3' Rev: 5'-CTGTTGCTTTTTTCTTTAGCCG-3'	51	129
Granzyme-B	For: 5'-GGAACACCTCTTCTGCCACC-3' Rev: 5'-AGCATTAGATAACATTCTCGGGG-3'	54	148
Rae1α	For: 5'-GCGAAGTGCTTAGTGGATGAAATAC-3' Rev: 5'-GGTTGTGTCAAACATTCCCCC-3'	53	122
Rae1β	For: 5'-GAAGAAATGTTTGACACAACCTCTG-3' Rev: 5'-CCTGGCTTTGCGGATAAATC-3'	52	137
Rae1γ	For: 5'-CGATGATGGGGACCTTGTGC-3' Rev: 5'-GGTGATACTGGGGGACCTTGAG-3'	52	124
Rae1δ	For: 5'-ACCATCAAGGCTCCTACCCC-3'	53	130

	Rev: 5'-CTTCAGTGGCATTTGCTGTCTC-3’		
Rae1ε	For: 5'-GAAAGATGATGGGGACCTTGTG -3’ Rev: 5'-TGATACTGGGGGACCTTGAGG -3’	52	125
H60	For: 5'-GTCACTGCCTCAACAAATCGTC-3’ Rev: 5'-GTCGGAAGTTATTCACTGTAGGATG-3’	52	123
Mult1	For: 5'-TCCTCTTGCTCTGTTCCTTTCC-3’ Rev: 5'-TGCTGCTTGTAAAATGGAGTCAC-3’	52	136
Gapdh	For: 5'-TGGTTTGACAATGAATACGGCTAC-3’ Rev: 5'-GGTGGGTGGTCCAAGGTTTC-3’	52	92
*CD69***	For: 5'-GACTTCAGCCCAAAATGCTTG-3’ Rev: 5'-TCCAACCCAGTGTTCCTCTCTAC-3’	51.6	115
CRTAM	For: 5'-GCAACTCCTTTCAAGCCAATCC-3’ Rev: 5'-CAAGTAGCCAGGTTATCTGCGG-3’	53.5	124
SLAM	For: 5'-TATGCCCCCATTCTGGAGAG-3’ Rev: 5'-CACAGTGGAGTAAACCGTATTTGC-3’	51.3	101
GRANZYME-A	For: 5'-ATGGTTTGTGCTGGAAGCCTCC-3’ Rev: 5'-GGGTCTCCGCATTTATTTTCAAGG-3’	56.0	128
GRANZYME-B	For: 5'-ACCATTGAGTTGTGCGTGGG-3’ Rev: 5'-GCCATTGTTTCGTCCATAGGAG-3’	54.7	123
NKG7	For: 5'-ATTTCTGGTTTGAGGCTGTGG-3’ Rev: 5'-CATAATGCTGAAGGTCTGCGTC-3’	55.0	116
FCGR3B	For: 5'-CTGACTTCCACATTCCAAAAGC-3’ Rev: 5'-TGCCAAACCTTGAGTGATGG-3’	52.7	122
KLRC2	For: 5'-ACTGCCACCTCCAGAGAAGC-3’ Rev: 5'-TGCTCCAGGAAAGGAATAAGAAC-3’	51.9	105
KLRC4	For: 5'-CAAAGAGGCAGCAAAGGAAAC-3’ Rev: 5'-CATTCCCTTGATGATCCGAAG-3’	49.8	117
ULBP1	For: 5'-ATGTACTGGGAACAAATGCT-3’	53.0	127

	Rev: 5'-GAATGAAGCAGAGGAAGATG-3’		
ULBP2	For: 5'-ATCATGGACCCAATAGCTC-3’ Rev: 5'-ATCAGGTAGCACCAAGAGAA-3’	47.3	105
ULBP3	For: 5'-AGGTCAGGATGTCTTGTGAG-3’ Rev: 5'-TGTCCACTTTCTGTTGTTTG-3’	50.5	113
KLRF1	For: 5'-GGATTGGGCTTAACTTTACCTCC-3’ Rev: 5'-TGCTTTCCTTAATGGCAGCAC-3’	51.4	129
MIC-B	For: 5'-TCAAGGACCAGAAAGGAGGC-3’ Rev: 5'-AGGTTTTGGGAGAGGAAGAGC-3’	54.6	127
NCR1	For: 5'-CAGAAATGTATGACACACCCACC-3’ Rev: 5'-TTCCCTCCTTGAGCAGTAAGAAC-3’	54.1	126
NCR2	For: 5'-TGTGTTCTGTGGACTCCTCGTAG-3’ Rev: 5'-TGGCAGGTGGCTTTTTGG-3’	55.9	126
NCR3	For: 5'-TGGTGGTGGAGAAAGAACATCC-3’ Rev: 5'-CATTTGCCCTGGTAATAGACGG-3’	55.7	124
HPRT	For: 5'-CCAGTCAACAGGGGACATAAAAG-3’ Rev: 5'-GACCAAGGAAAGCAAAGTCTGC-3’	50.9	127

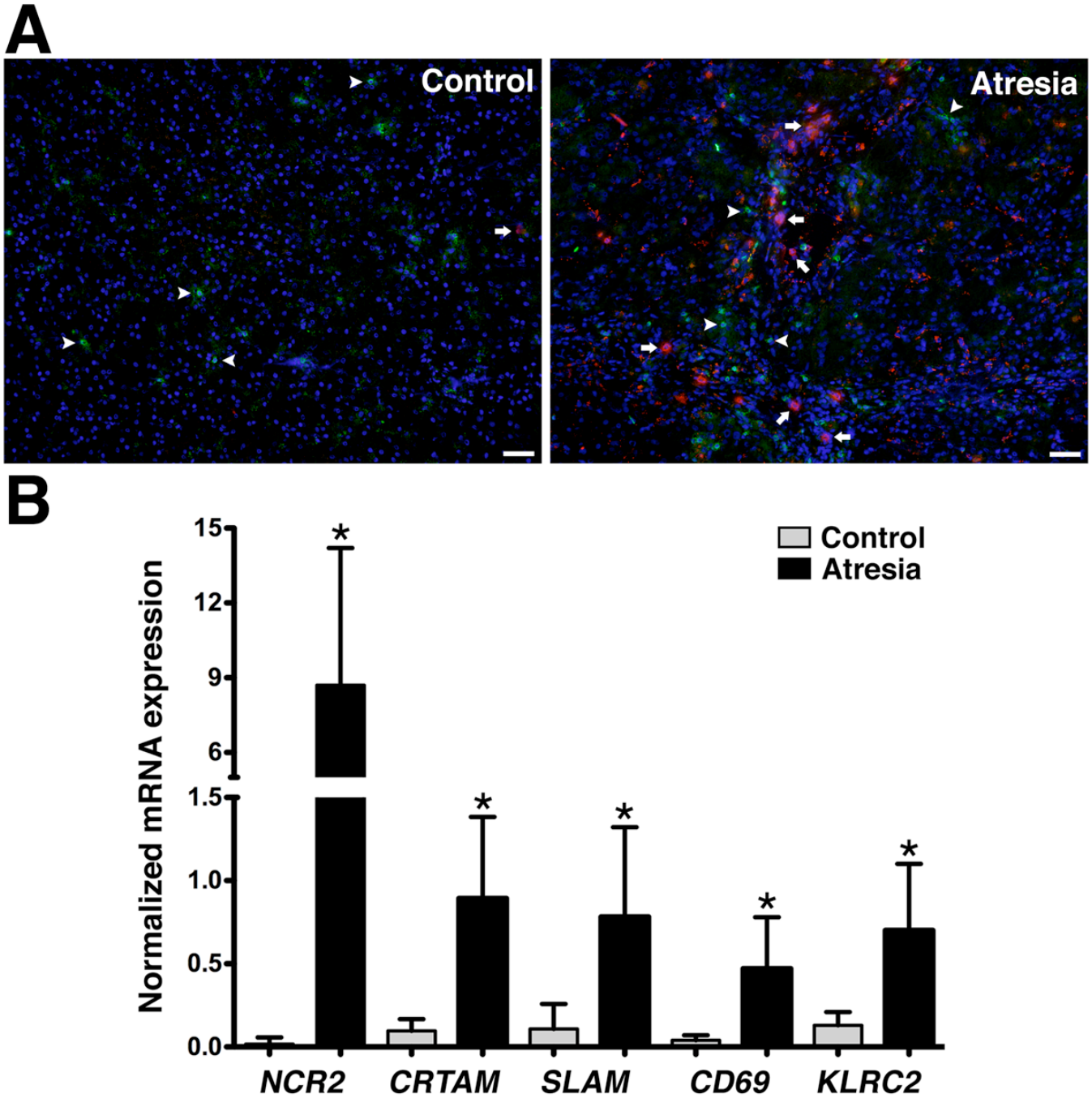
Temp: annealing temperature

Size (bp): product size in base pairs

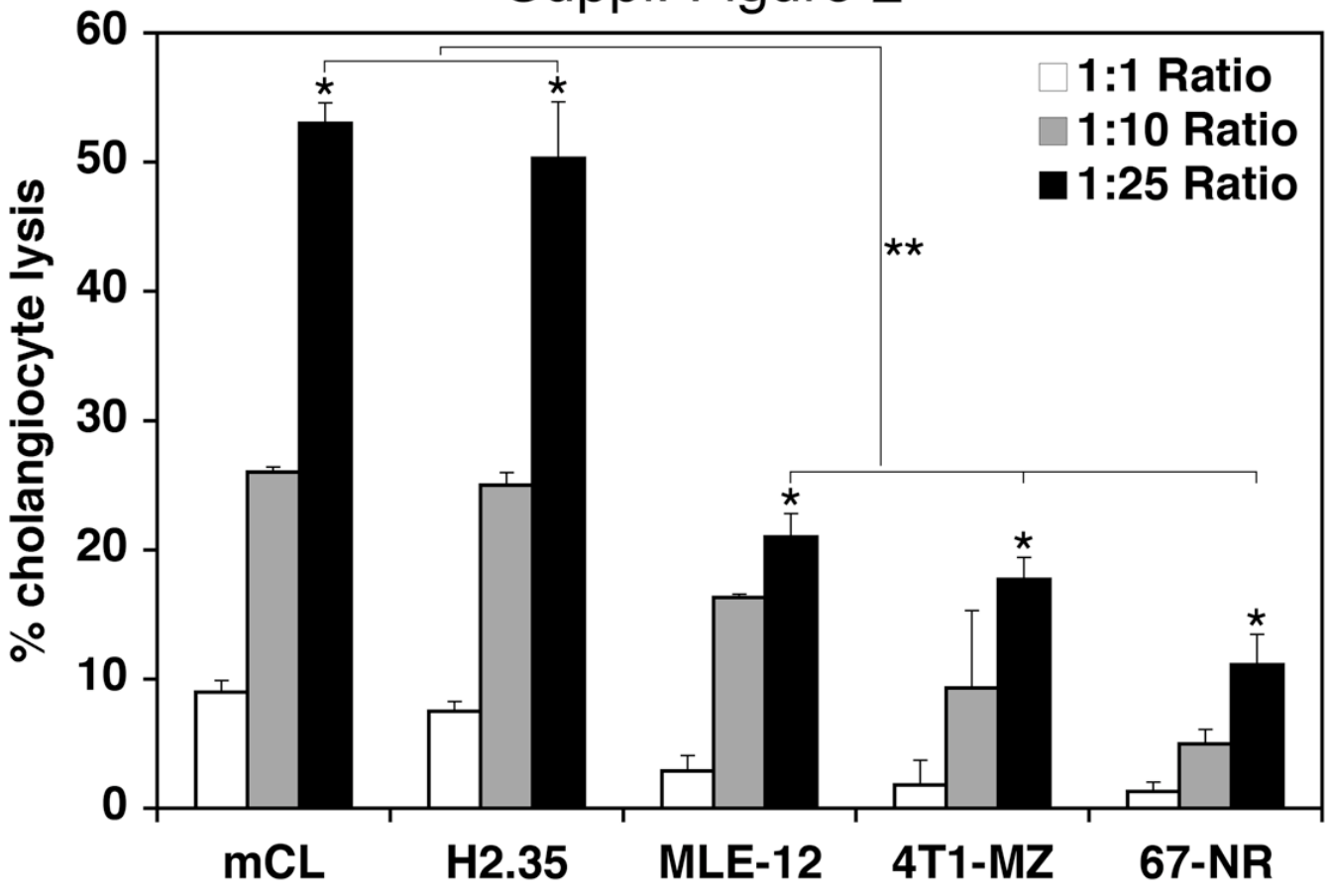
*Non-capital letters denote murine genes

**Capital letters denote human genes

Suppl. Figure 1



Suppl. Figure 2



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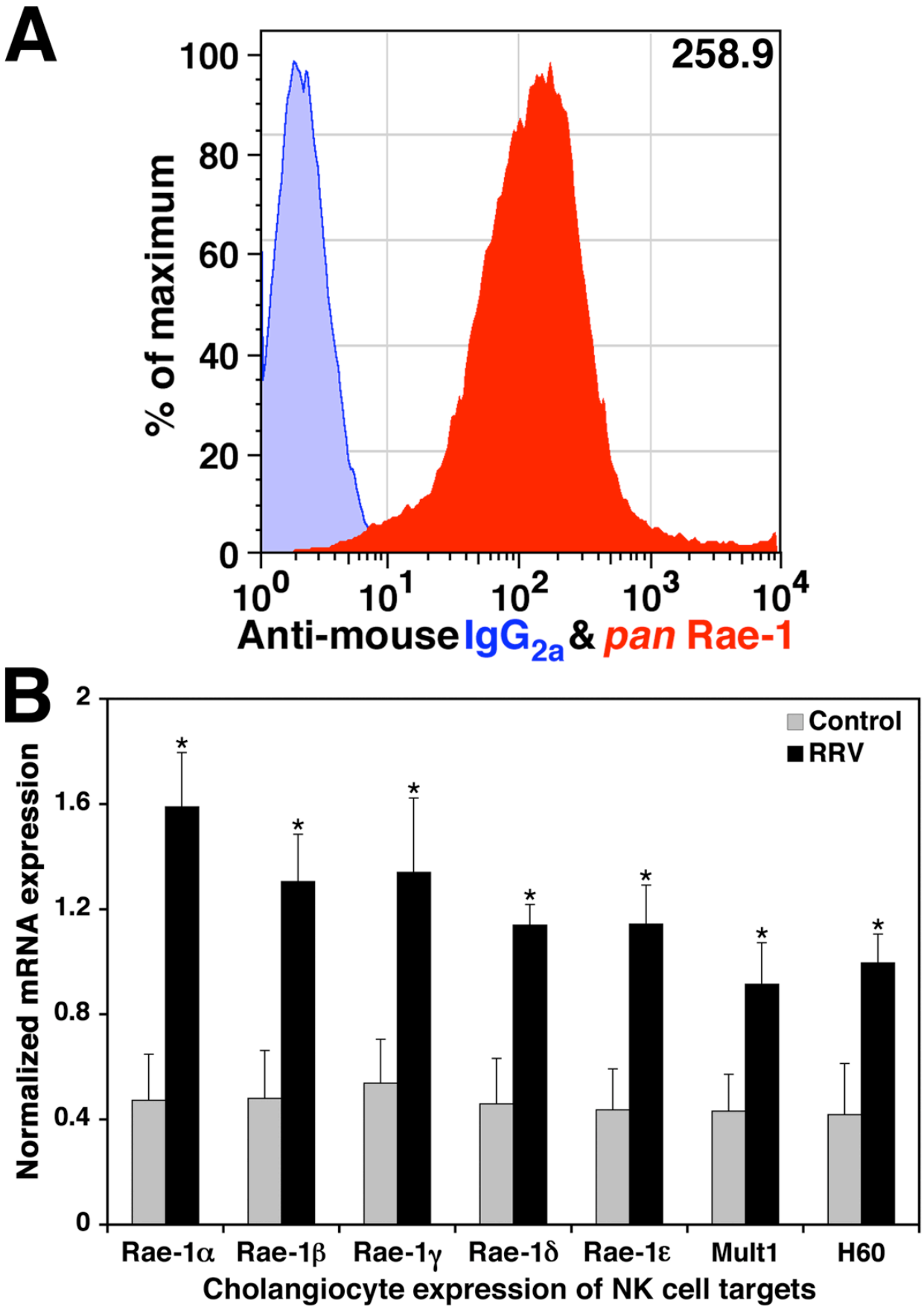

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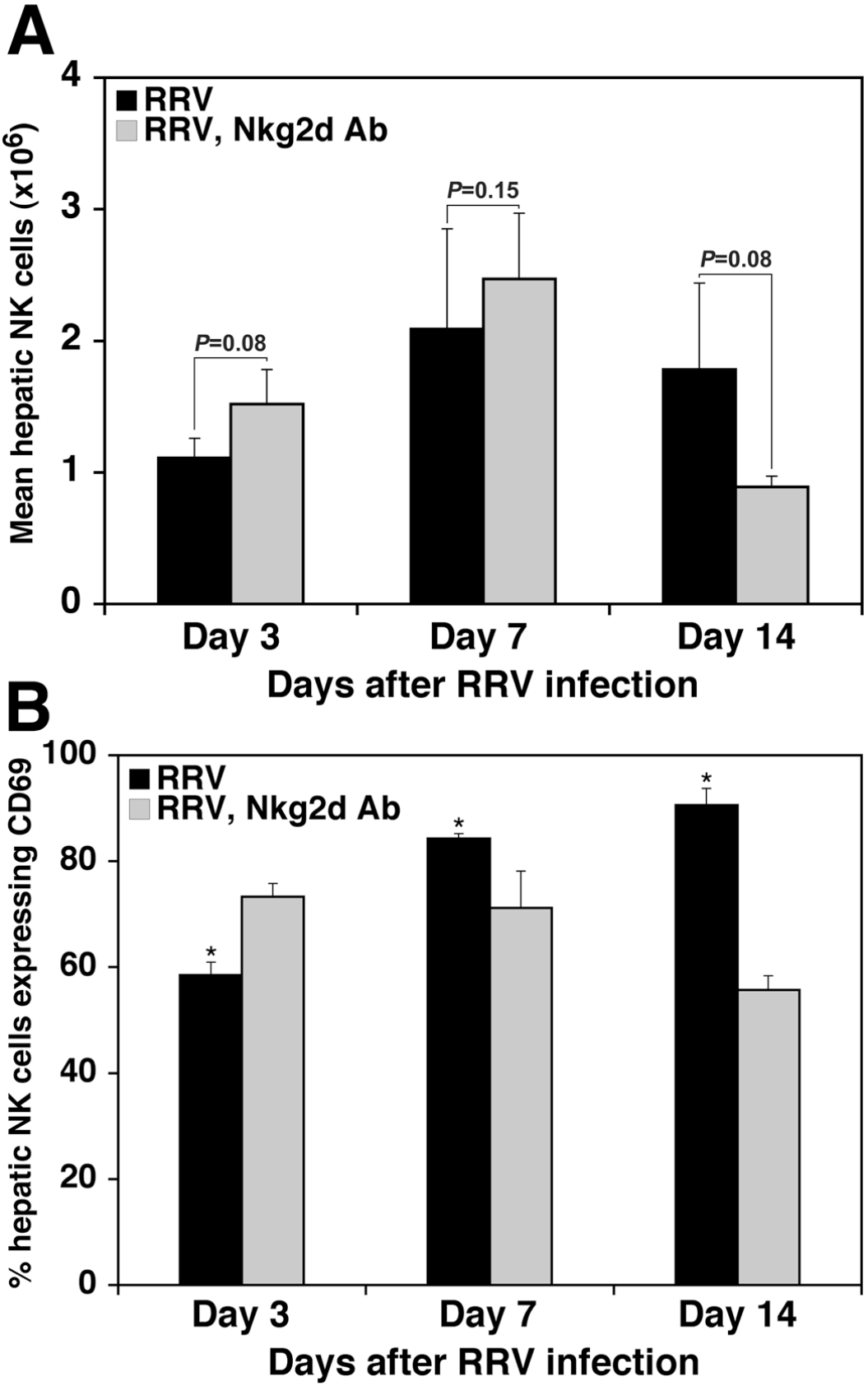

Suppl. Figure 5