Supplemental figure legends

Figure S1. Generation of EAT-2A-/- mice

A. The organization of EAT-2A [Sh2d1b1] genomic locus is shown (top). Exons are filled boxes. In the targeting vector (middle), *LoxP* flanking *Neo* replaced exon 1. The thymidine kinase gene (TK) lies external to the 3' genomic DNA fragment. The structure of the targeted allele is shown (bottom). *B*. DNA from EAT-2A+/+, EAT-2A-/+ and EAT-2A-/- mice was subjected to southern blot with external probe, which is green box. *C*. The deletion of *Neo* in pups from EAT-2A-/+ x Cre deleter transgenic mice was confirmed by PCR with two sets of primer pairs (P1F - P1R and P2F-P2R).

Figure S2. Generation of EAT-2B-/- mice

A. The organization of EAT-2B genomic locus is shown (top). The targeting vector was generated using the pKO-Scrambler *LoxP* vector and replacing a 1-kb region encompassing exon 1 of EAT-2B with the neomycin resistance gene (*Neo*). The recombination vector contained 3 kb and 7.6 kb of homology upstream and downstream, respectively, of *LoxP-Neo*. *B*. Southern blot analysis of EAT-2B+/+, EAT-2B-/+ and EAT-2B-/- mice after DNA digestion with SacI. Map location of external probe is shown in green box (See S2A). *C*. As shown in S1, the deletion of *Neo* in pups from EAT-2B-/+ x Cre deleter transgenic mice was confirmed by PCR with two sets of primer pairs (P1F-P1R and P2F-P2R).

Figure S3. Generation of mice lacking both EAT-2A and EAT-2B.

A. Schematic outline of the EAT-2A and EAT-2B targeting.

To generate EAT-2A and EAT-2B double mutant ES cell clones, the first exon of EAT-2A was replaced by the *Neo* flanked by two *LoxP* sites in the first targeting vector. The second targeting vector with *LoxP-Hygromycin* cassette was used to delete the first exon of EAT-2B in one of EAT-2A-targeted ES clones. ES cell clones that carried both targeted mutants on the same chromosome were identified by Cre-mediated recombination in which the genomic DNA fragment flanked by two Lox P sites was excised. The arrows indicate the location of two sets of primer pairs (P1F-P1R and P2F-P2R) for PCR detection of deletion. 600 bp or 650 bp PCR

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amplicons were amplified from two hygromycin susceptible ES cell clones (1D5H and 2E2A), but not from hygromycin resistant clones (1C3B and 2G1F).

B. Expression analysis.

RNA was isolated from *B6*, EAT-2A−/−, EAT-2B−/− and EAT-2A/B−/− NK cells. The expression of EAT-2A, EAT-2B or both transcripts was determined by RT-PCR.

Figure S4. EAT-2A−/− NK cells, but not EAT-2B−/− NK cells, are unable to eliminate RMA-S/CD48+ tumor cells.

$3x10^6$ CFSE-labeled RMAS/CD48+ or RMAS/CD48− tumor cells were injected into the peritoneum of *B6*, EAT-2A−/− or EAT-2B−/− mice. After 18 hours, the cells were recovered from peritoneum and the number of tumor cells was determined based on the percentage of CFSE+ cells by flow cytometry.

Figure S5. Impaired clearance of RMAS/CD48+ tumor cells in NK cell-depleted EAT-2A/B-/- and *B6* mice.

NK cell-depletion of EAT-2A/B-/- and *wt B6* mice was accomplished by injecting 100 µg anti-NK1.1 mAb *i.p.* on days –2 and day 0. CFSE-labeled RMAS/CD48+ cells were injected i.p. in EAT-2A/B-/- or *wt* mice. As described in Fig. S4, the total number of tumor cells was calculated based on the percentage of CFSE+ cells.

Figure S6. Defective killing of CD48+ P815 cells by EAT-2A-/- but not by EAT-2B-/- NK cells.

NK cells were isolated from *B6*, EAT-2A-/- or EAT-2B-/- mice and cultured, as described in *Materials and Methods*. Cytolytic activity was determined in a 4 h [^{51}Cr] -release assay.

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Figure S7. Cytotoxicity directed against YB2/0, Yac1 and CHO targets is not impaired in EAT-2A-/-, EAT-2B-/- or EAT-2A/B-/- mice.

EAT-2A-/-, EAT-2B-/-, EAT-2A/B-/- or *B6* NK cells were cultured in IL-2 containing medium for 7 days before use in a 4 h killing assay against :

A YB2/0 cells ($[^{51}]$Cr-release assay)

B YAC-1 cells ($[^{51}]$Cr-release assay)

C CHO cells (LDH release assay)

Figure S8. Impaired cytotoxic acitivity mediated by αCD244 antibody, but not by αLy49D and αNKG2D, in EAT-2A-/-.and EAT-2B-/- mice.

IL-2-activated NK cells from EAT-2A-/-. EAT-2B-/- or *B6* mice were tested for antibody redirected cytotoxic activity against P815 tumor cells in a 4h $[^{51}]$Cr-release assay.

A αCD244.

B αLy49D.

C αNKG2D.

Figure S9. Defective CD244-mediated IFN-γ production in EAT-2A-/- NK cells.

NK cells isolated from EAT-2A-/-, EAT-2B-/- or *B6* mice were cultured, as described in Materials and Methods. The NK cells were stimulated with αNKG2D or αLy49D mAb for 24 hours. The supernatant were measured using ELISA.

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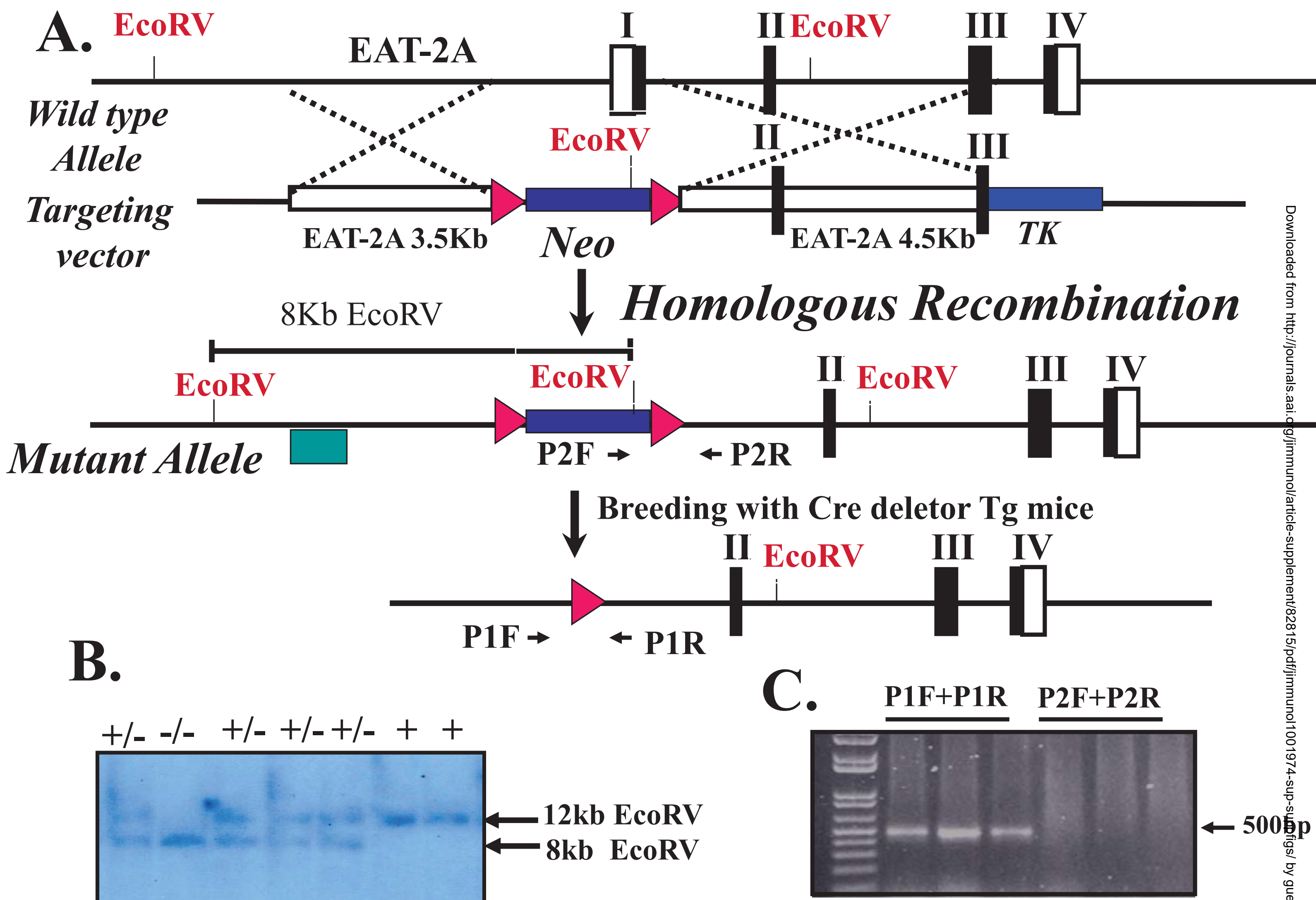

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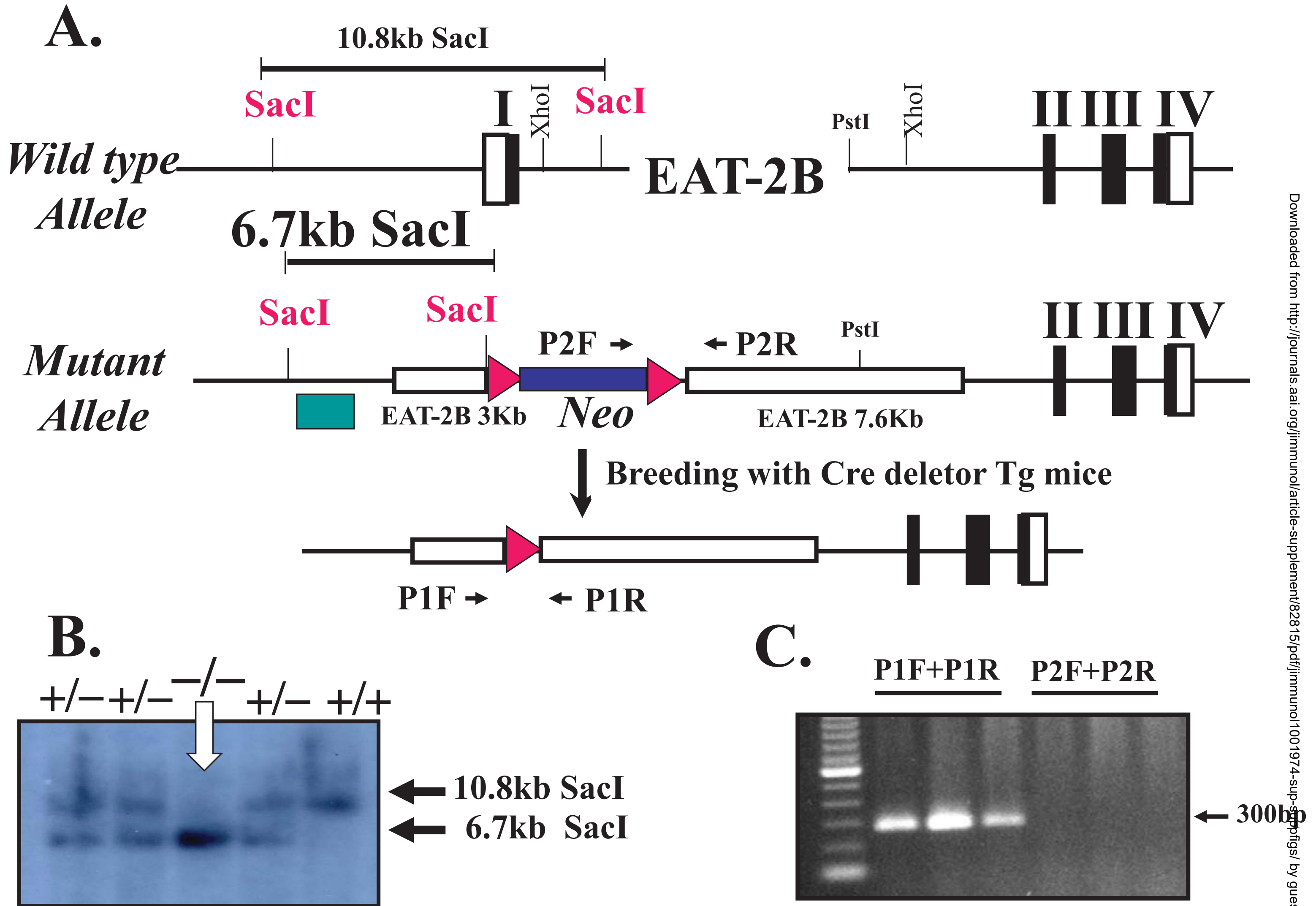

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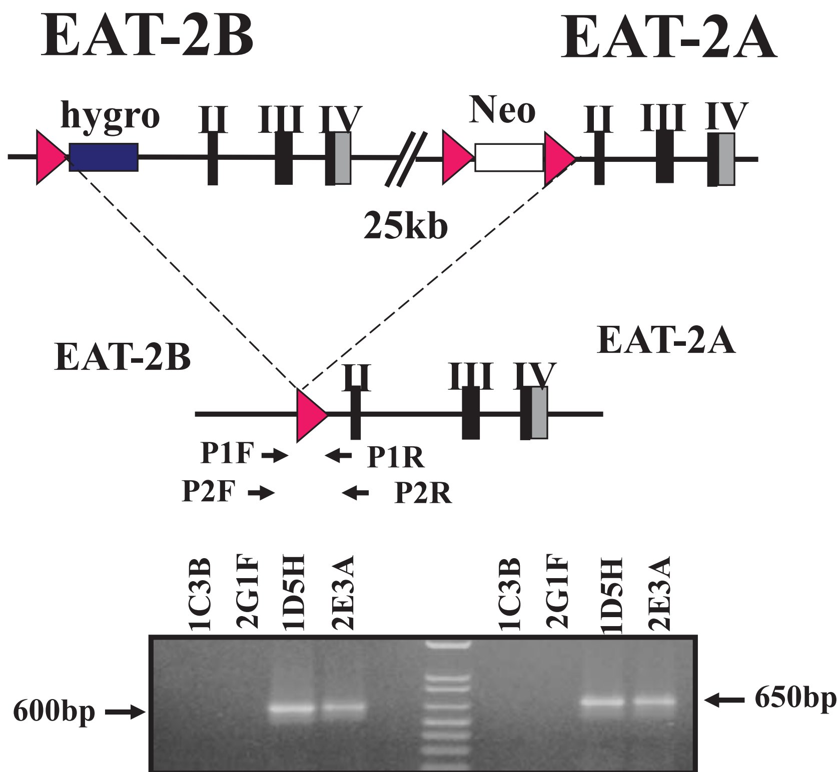
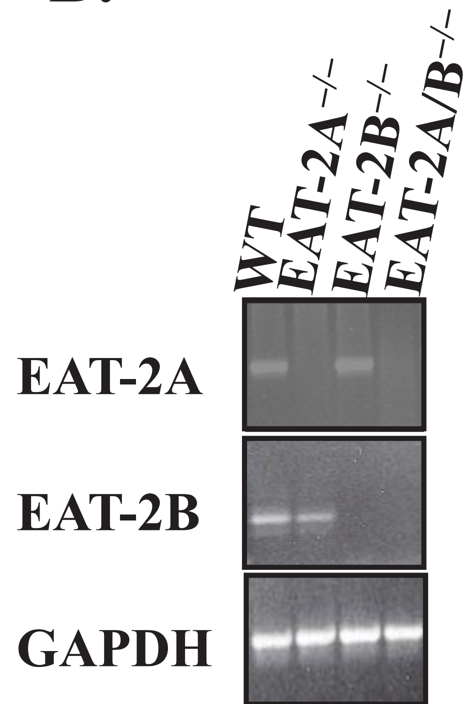
Supplemental Figure S3





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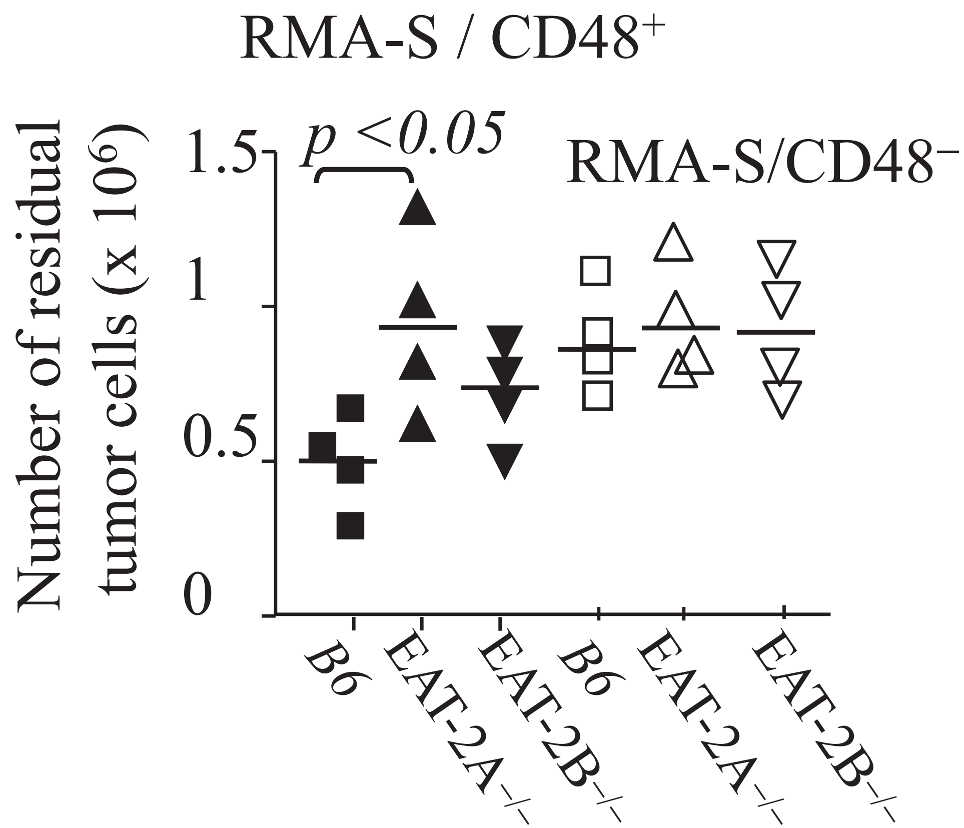

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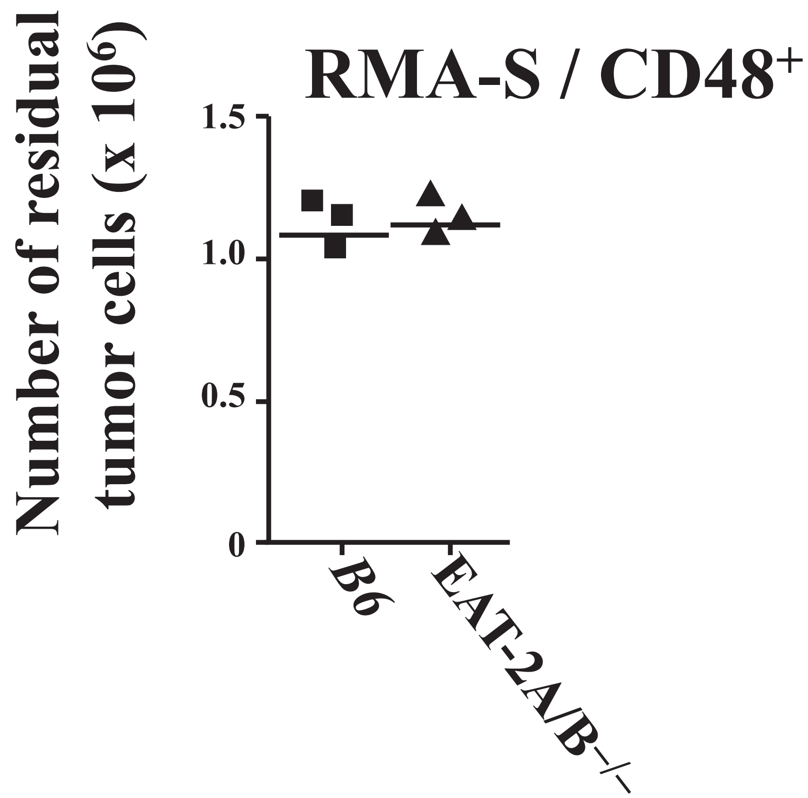

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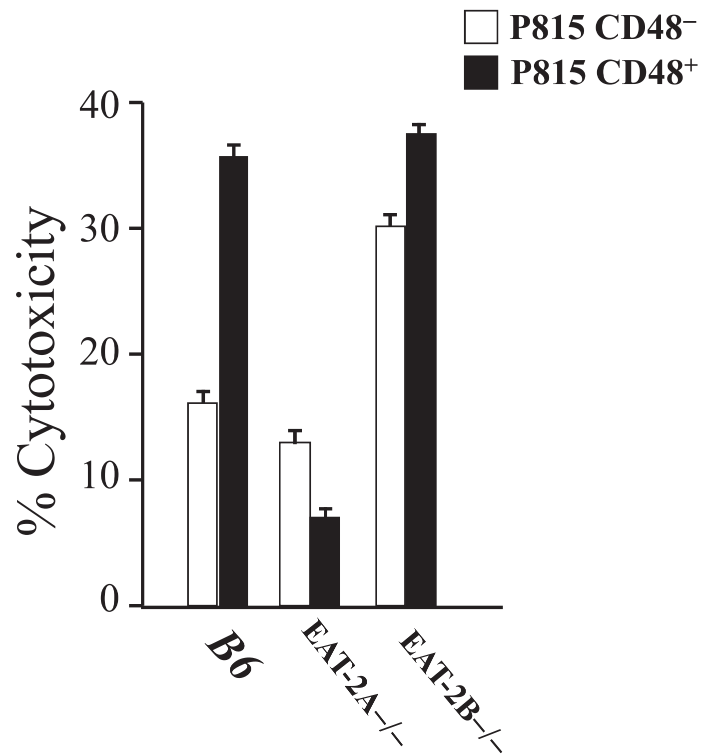

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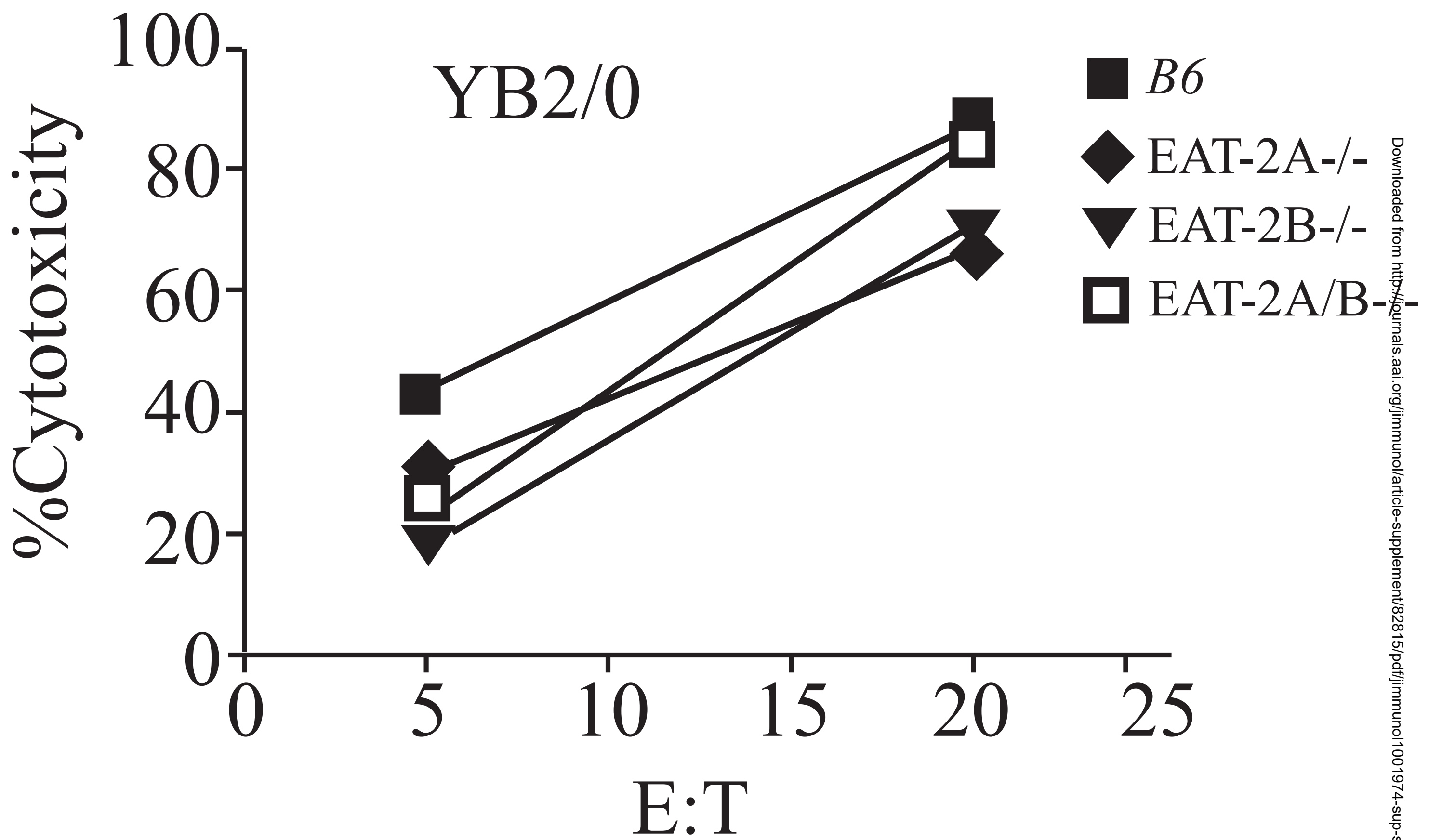
Supplemental Figure S7A



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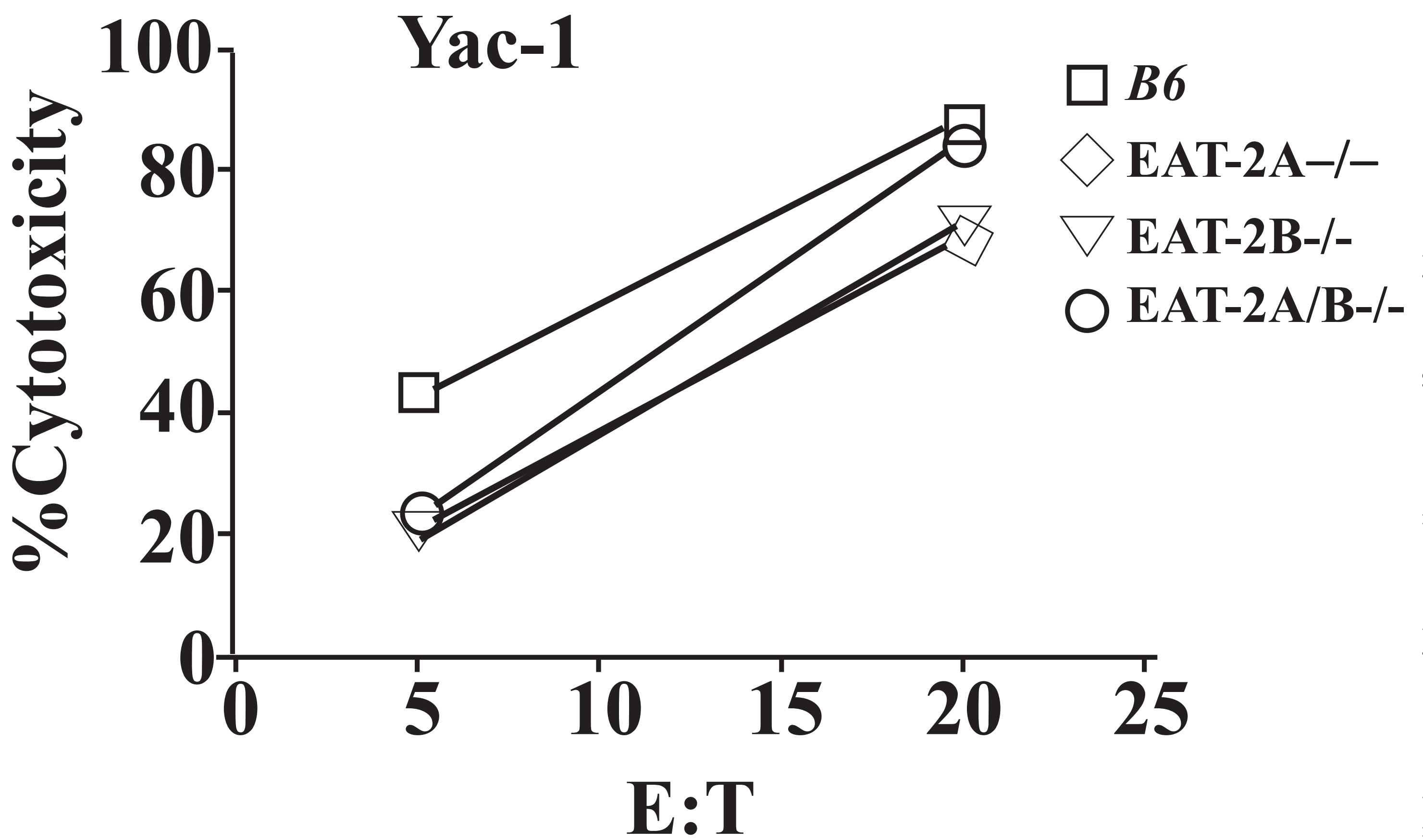
Supplemental Figure S7B



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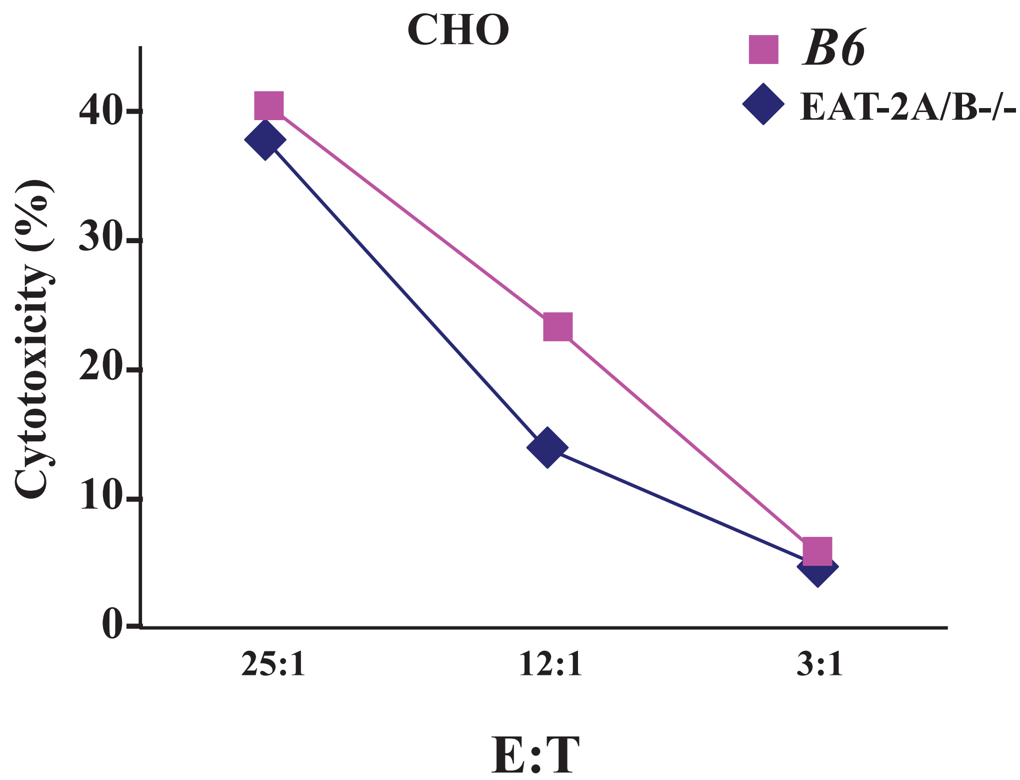
Supplemental Figure S7C



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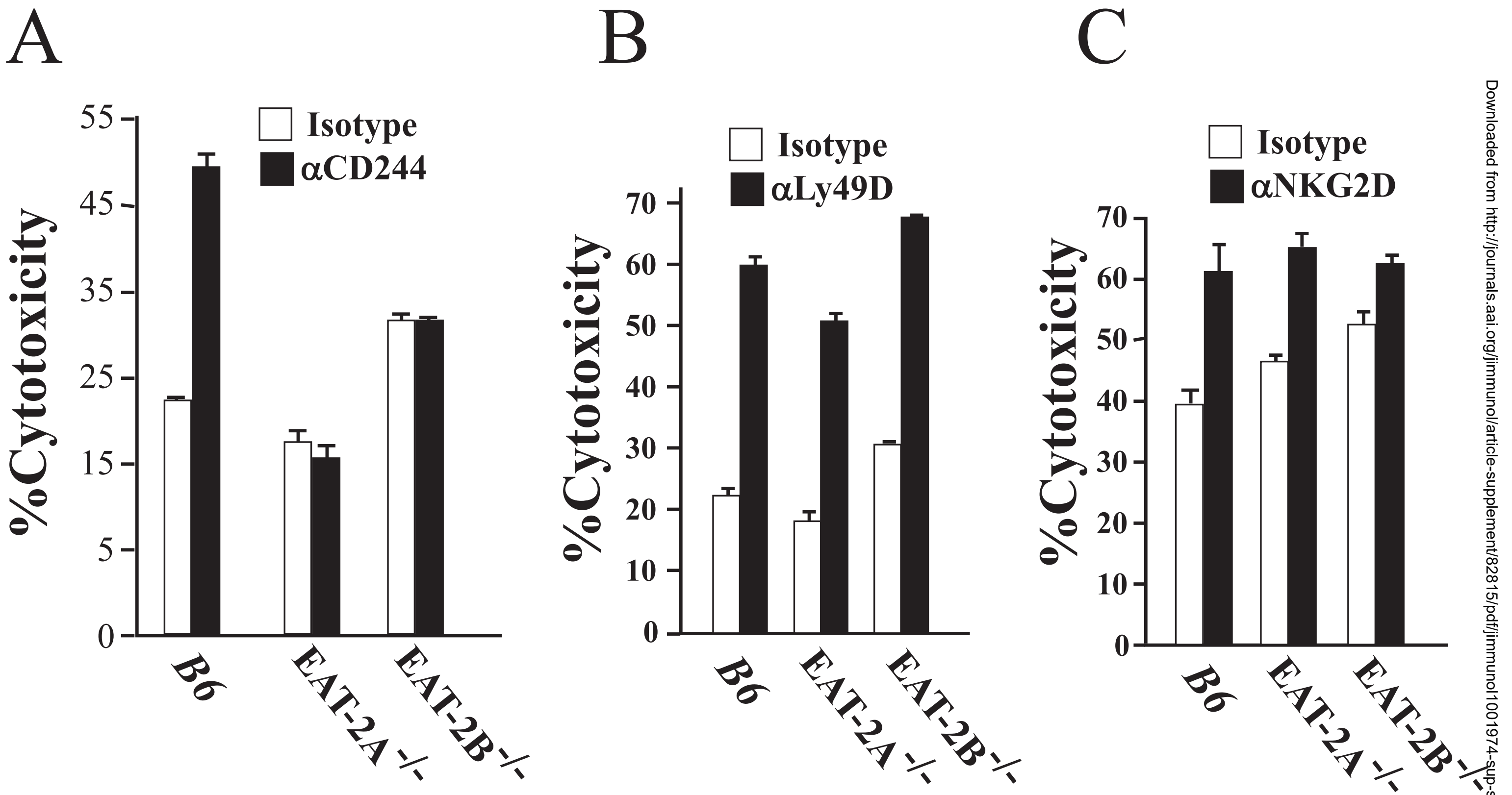
Supplemental Figure S8



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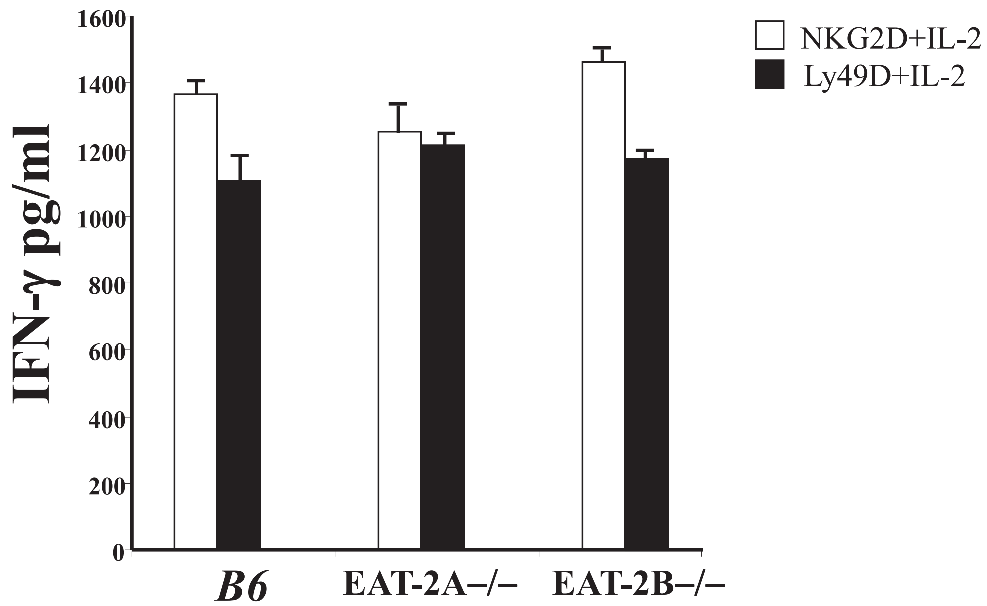
Supplemental Figure S9



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