

## Supplemental figure legends

### Figure S1. Generation of EAT-2A<sup>-/-</sup> mice

A. The organization of EAT-2A 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<sup>+/+</sup>, EAT-2A<sup>-/+</sup> and EAT-2A<sup>-/-</sup> mice was subjected to southern blot with external probe, which is green box. C. The deletion of *Neo* in pups from EAT-2A<sup>-/+</sup> 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<sup>-/-</sup> 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<sup>+/+</sup>, EAT-2B<sup>-/+</sup> and EAT-2B<sup>-/-</sup> 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<sup>-/+</sup> 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 *LoxP* 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

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<sup>-/-</sup>, EAT-2B<sup>-/-</sup> and EAT-2A/B<sup>-/-</sup> NK cells. The expression of EAT-2A, EAT-2B or both transcripts was determined by RT-PCR.

### **Figure S4. EAT-2A<sup>-/-</sup> NK cells, but not EAT-2B<sup>-/-</sup> NK cells, are unable to eliminate RMA-S/CD48<sup>+</sup> tumor cells.**

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

### **Figure S5. Impaired clearance of RMA-S/CD48<sup>+</sup> tumor cells in NK cell-depleted EAT-2A/B<sup>-/-</sup> and *B6* mice.**

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

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

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

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

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

- A YB2/0 cells (<sup>51</sup>Cr-release assay)
- B YAC-1 cells (<sup>51</sup>Cr-release assay)
- C CHO cells (LDH release assay)

**Figure S8. Impaired cytotoxic activity mediated by  $\alpha$ CD244 antibody, but not by  $\alpha$ Ly49D and  $\alpha$ NKG2D, in EAT-2A<sup>-/-</sup> and EAT-2B<sup>-/-</sup> mice.**

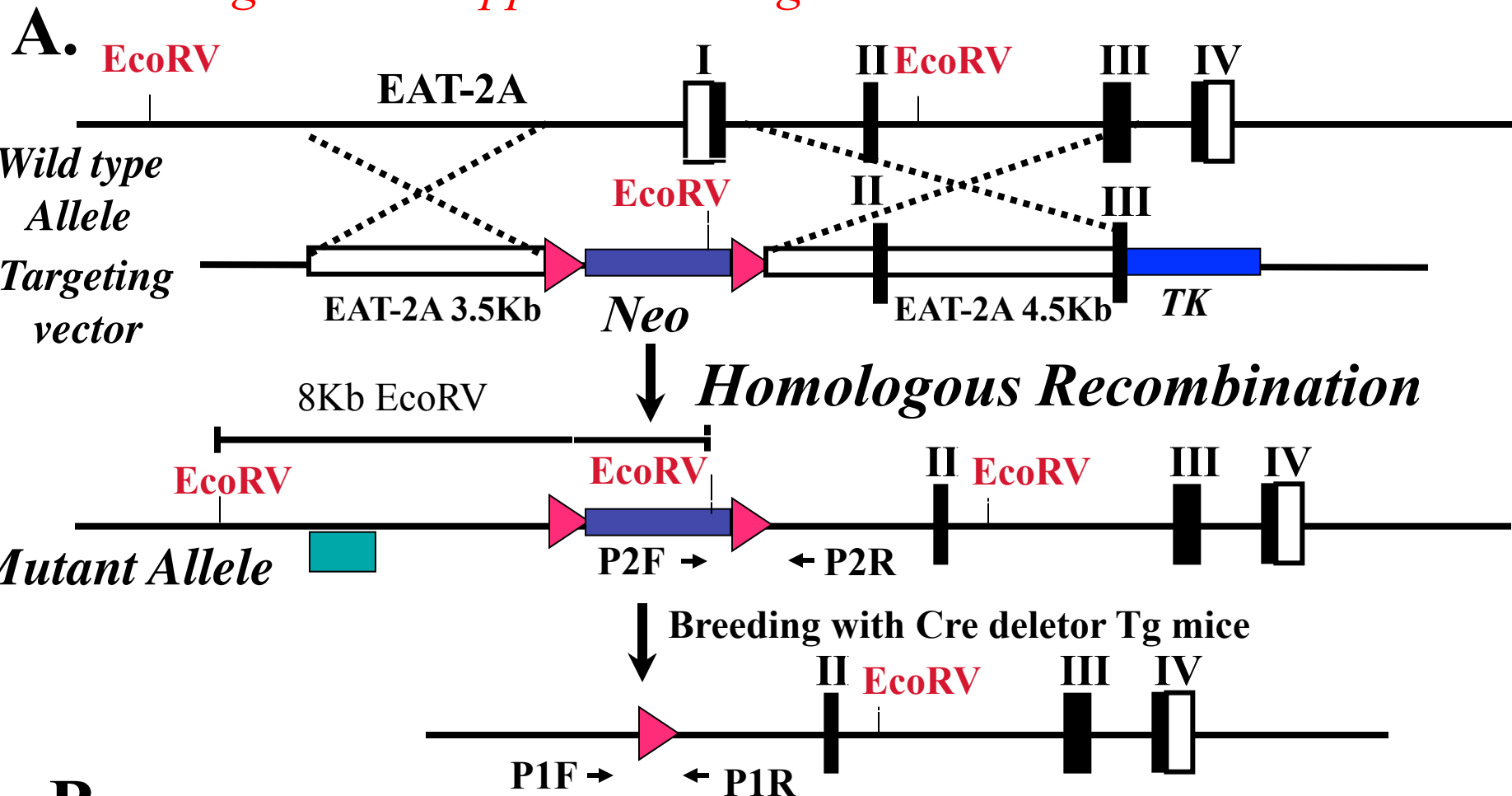
IL-2-activated NK cells from EAT-2A<sup>-/-</sup>, EAT-2B<sup>-/-</sup> or *B6* mice were tested for antibody redirected cytotoxic activity against P815 tumor cells in a 4h [<sup>51</sup>]Cr-release assay.

- A  $\alpha$ CD244.
- B  $\alpha$ Ly49D.
- C  $\alpha$ NKG2D.

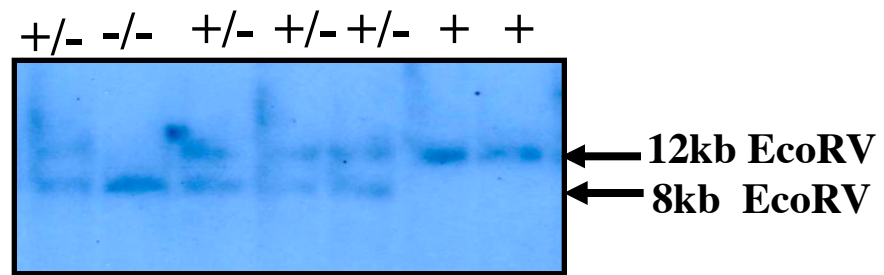
**Figure S9. Defective CD244-mediated IFN- $\gamma$  production in EAT-2A<sup>-/-</sup> NK cells.**

NK cells isolated from EAT-2A<sup>-/-</sup>, EAT-2B<sup>-/-</sup> or *B6* mice were cultured, as described in Materials and Methods. The NK cells were stimulated with  $\alpha$ NKG2D or  $\alpha$ Ly49D mAb for 24 hours. The supernatant were measured using ELISA.

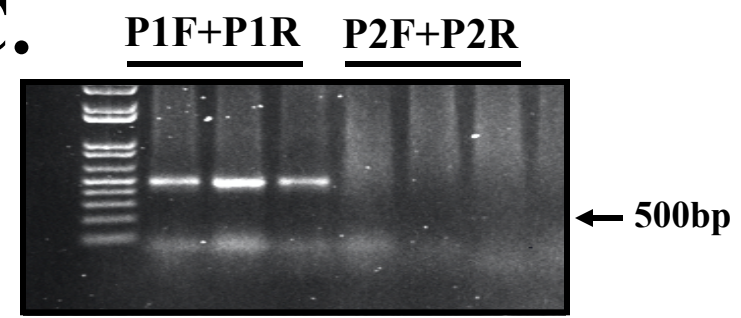
# **Supplementary Figures**



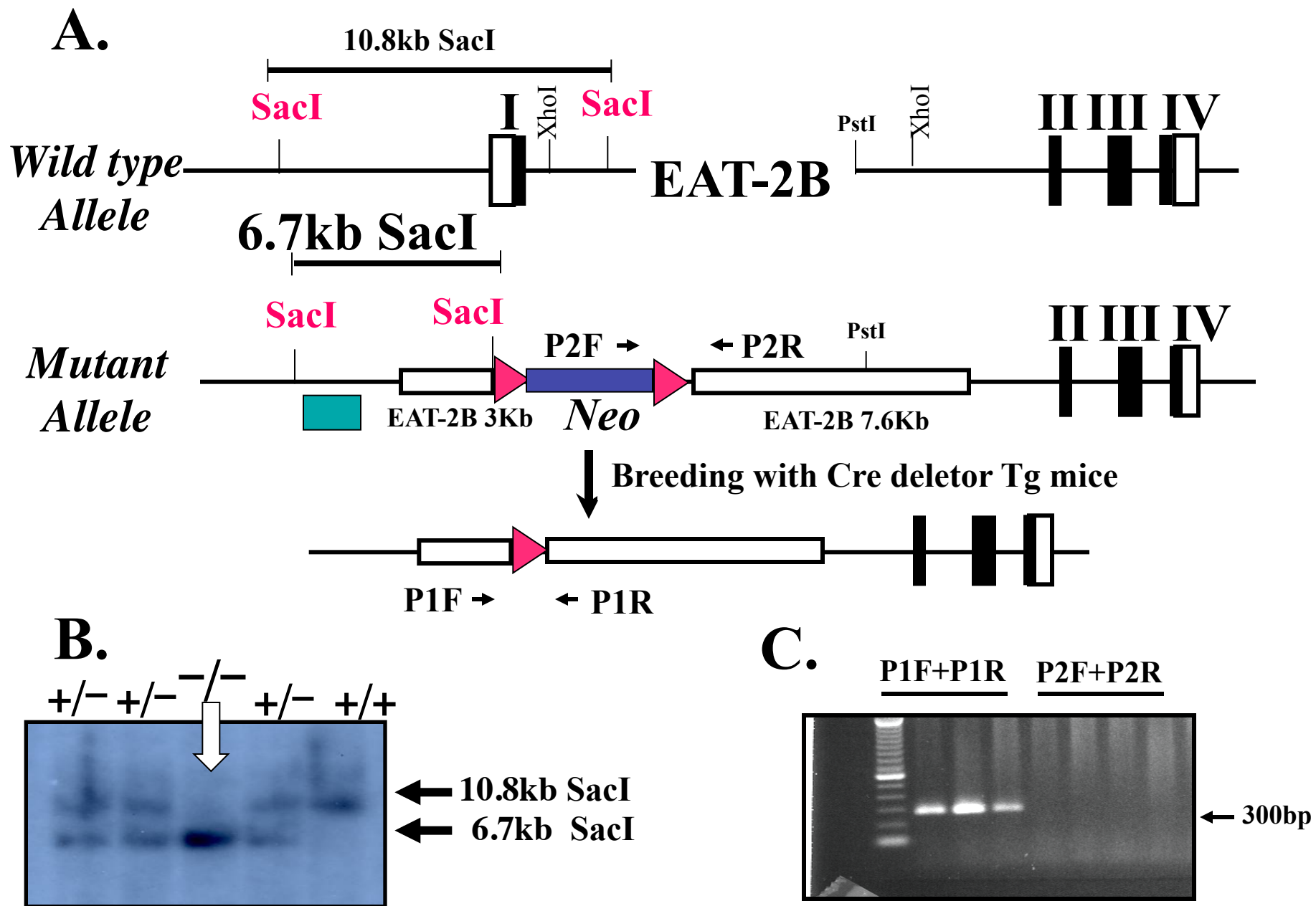
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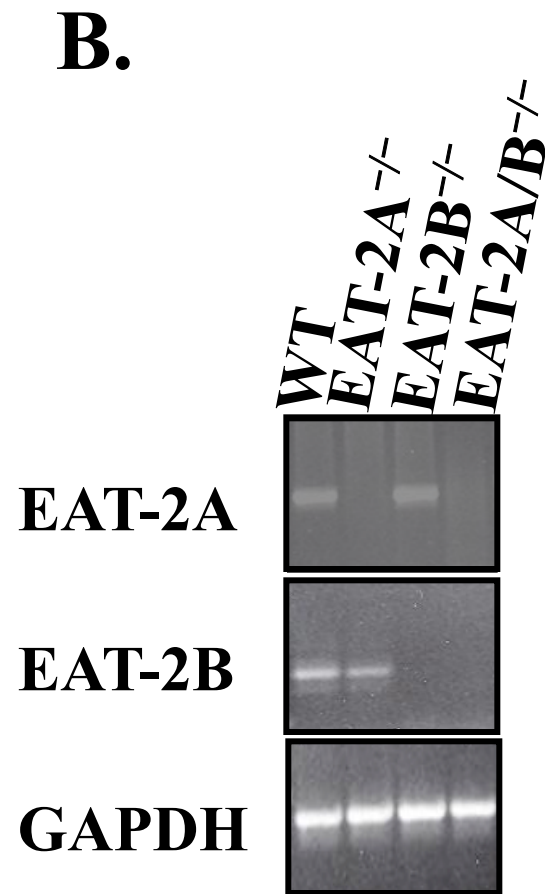
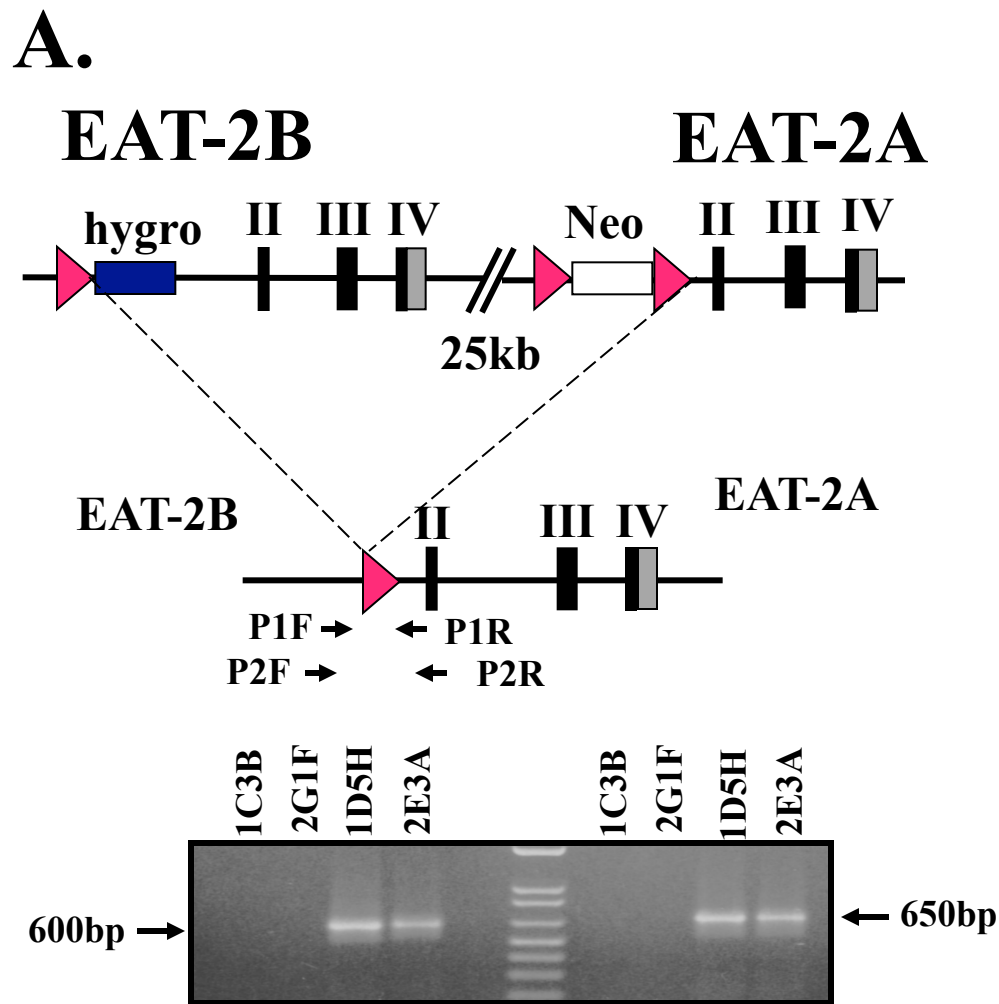


**C.**

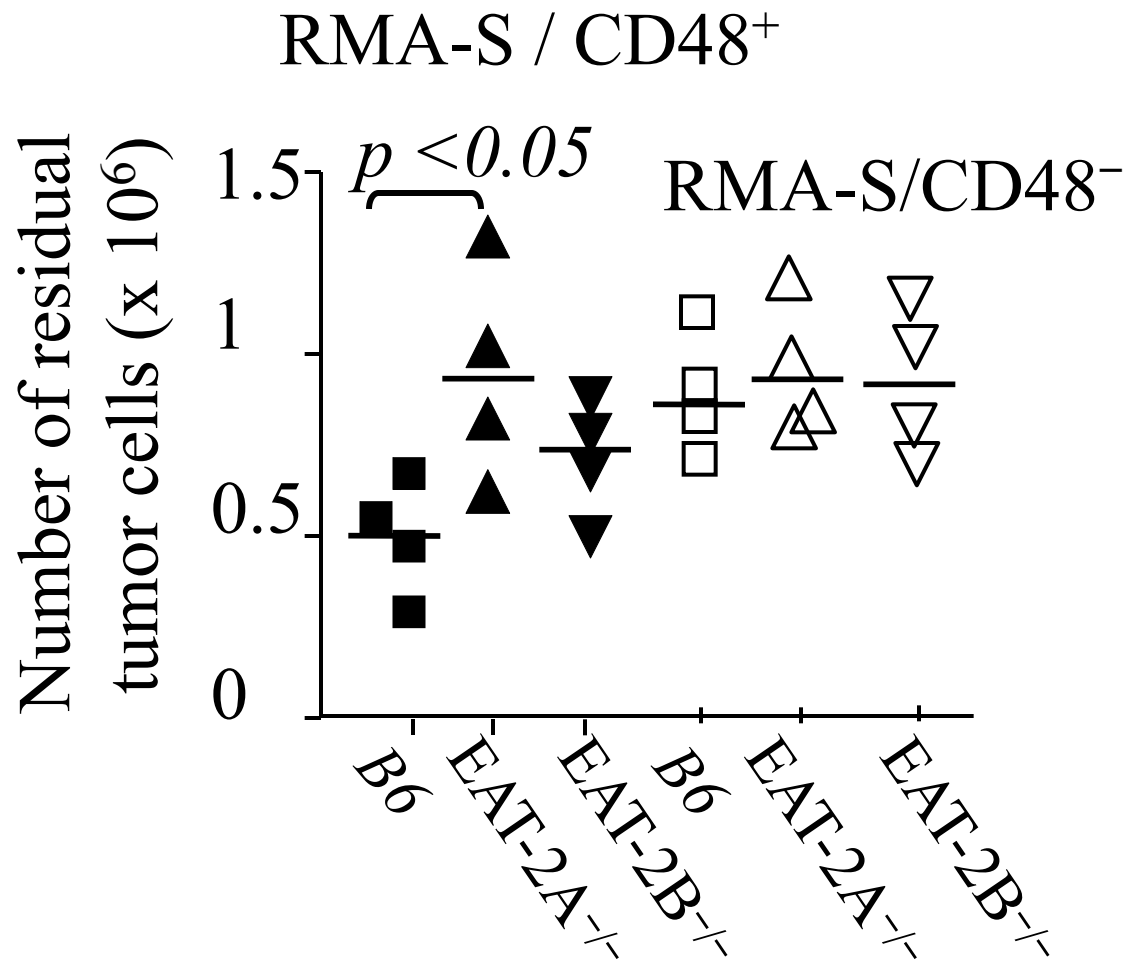


*N. Wang et al. Supplemental Figure S2*



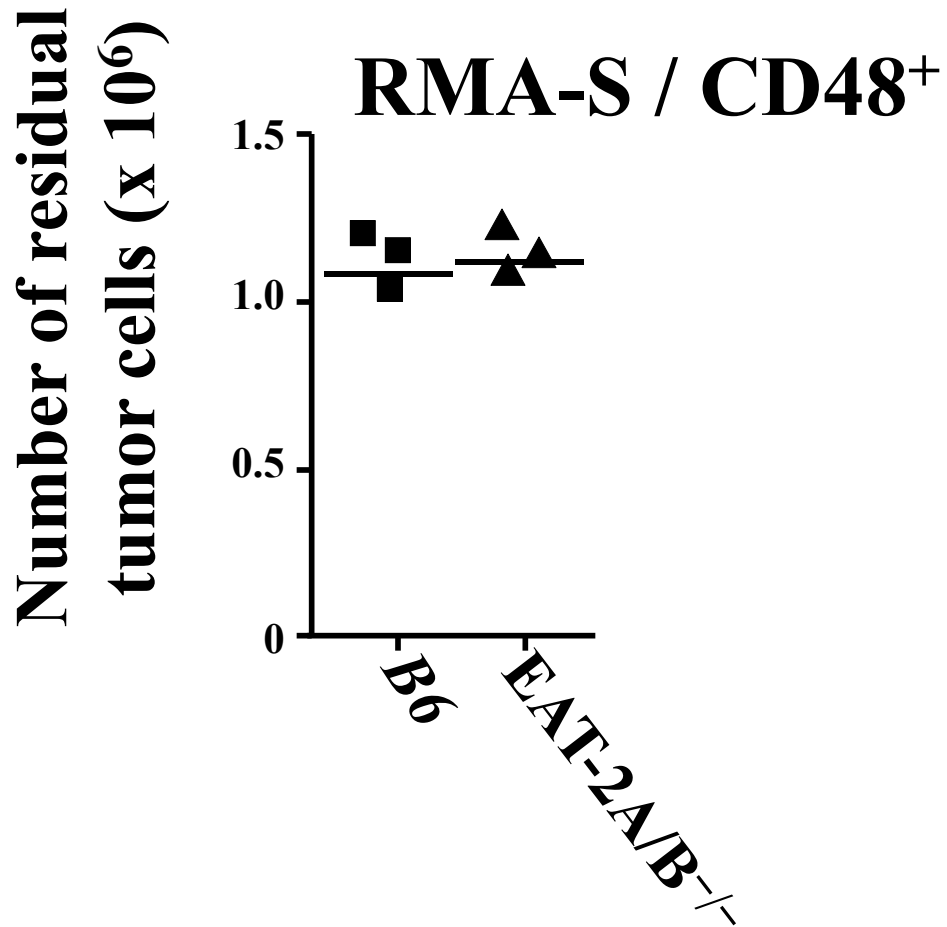


*N.Wang et al. Supplemental Figure S4*

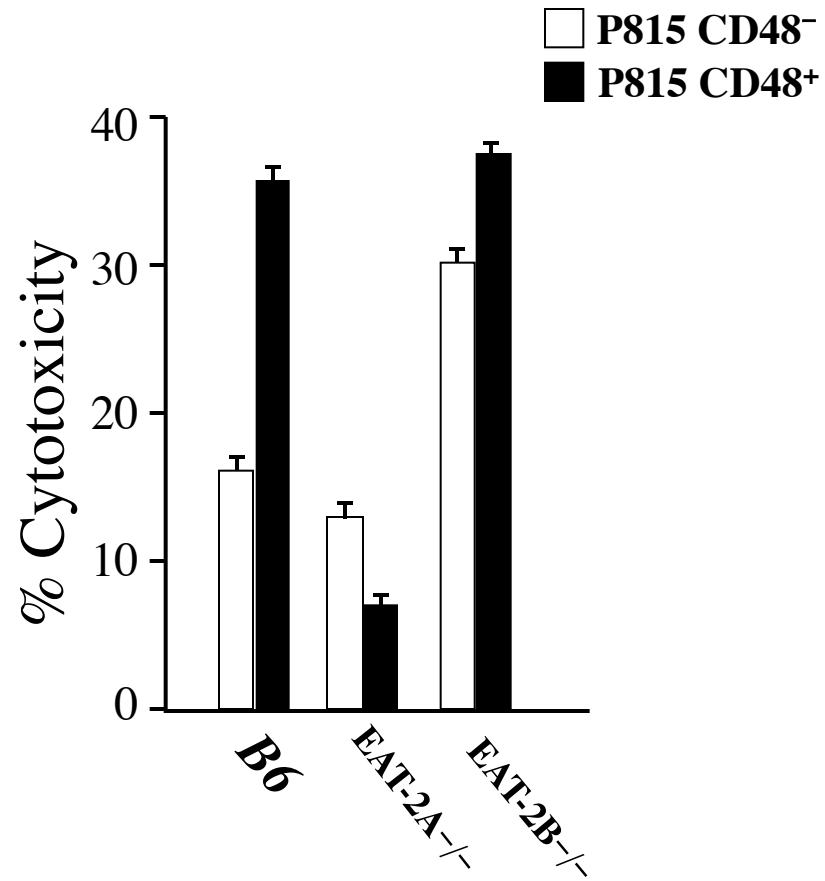




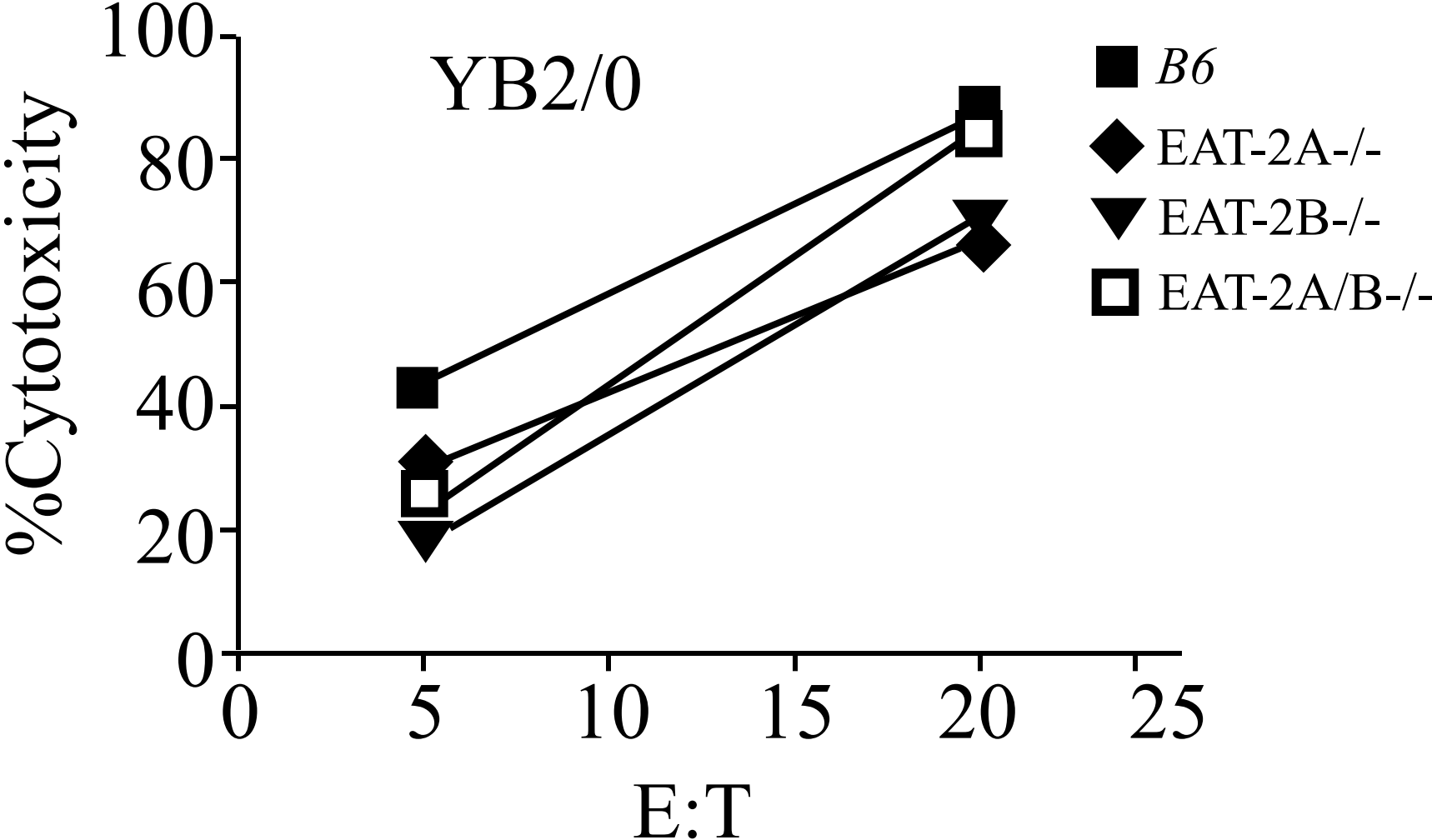
*N.Wang et al. Supplemental Figure S5*

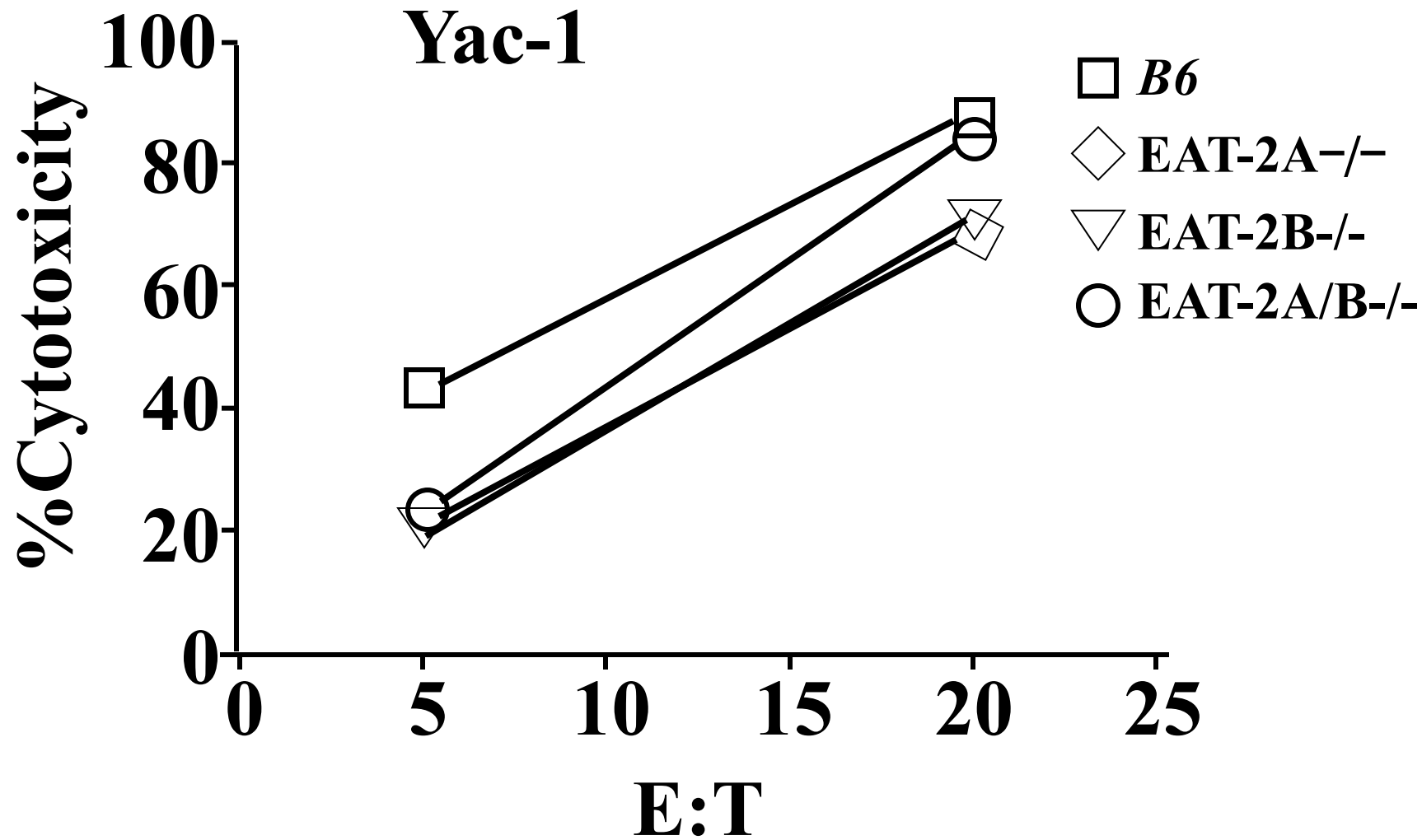


*N.Wang et al. Supplemental Figure S6*

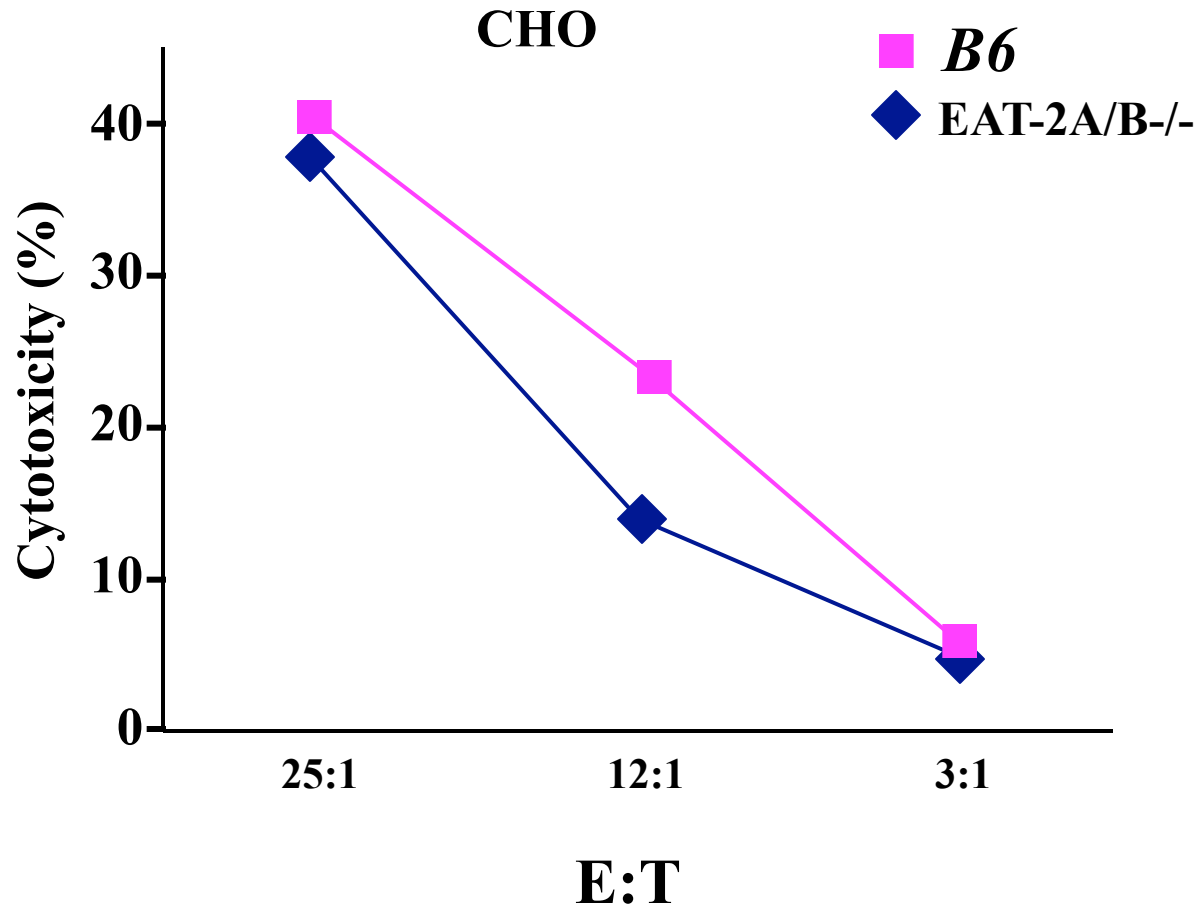


*N.Wang et al. Supplemental Figure S7A*

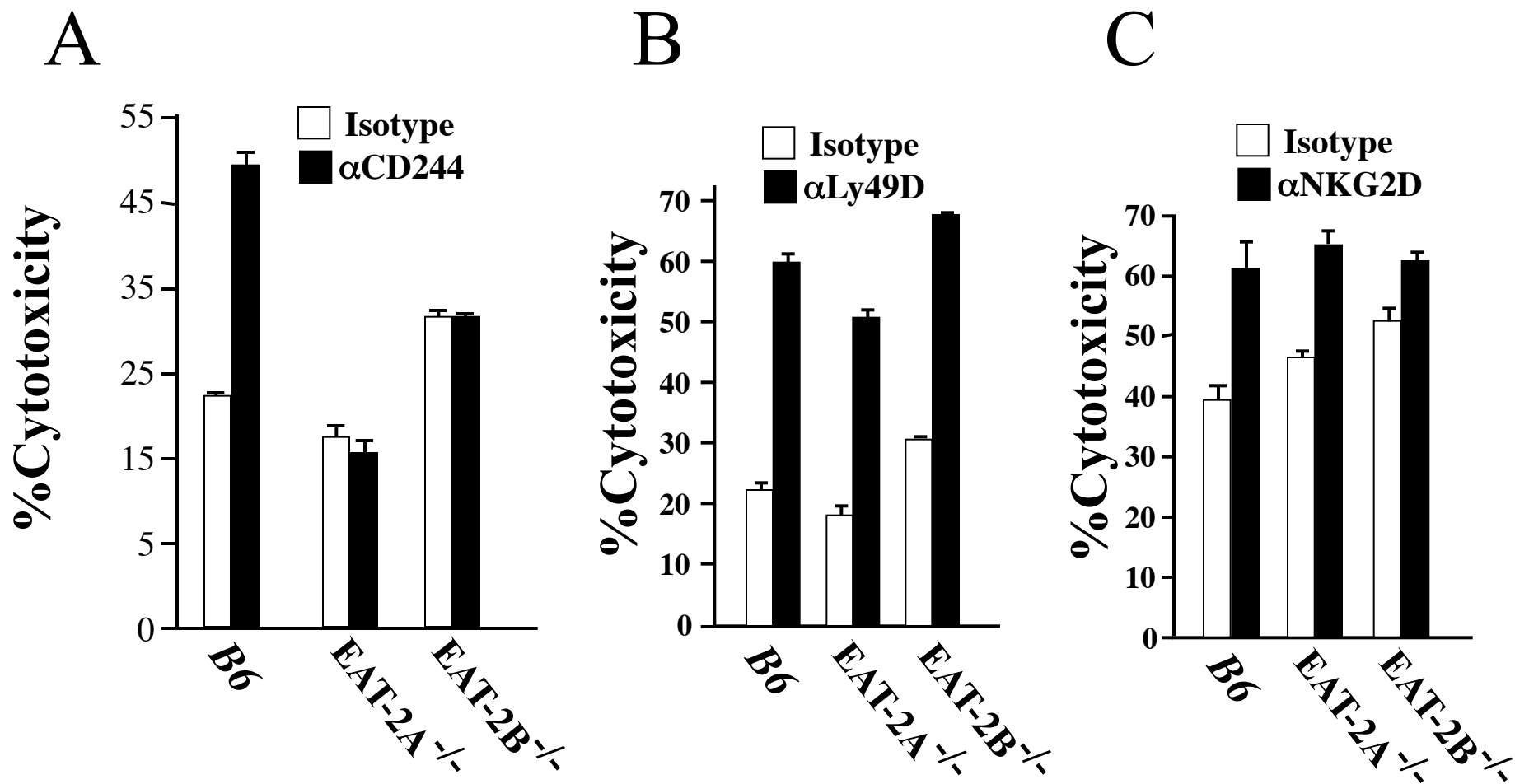




*N.Wang et al. Supplemental Figure S7C*



*N.Wang et al. Supplemental Figure S8*



*N.Wang et al. Supplemental Figure S9*

