# Supporting Information

## Jang et al. 10.1073/pnas.0905039107

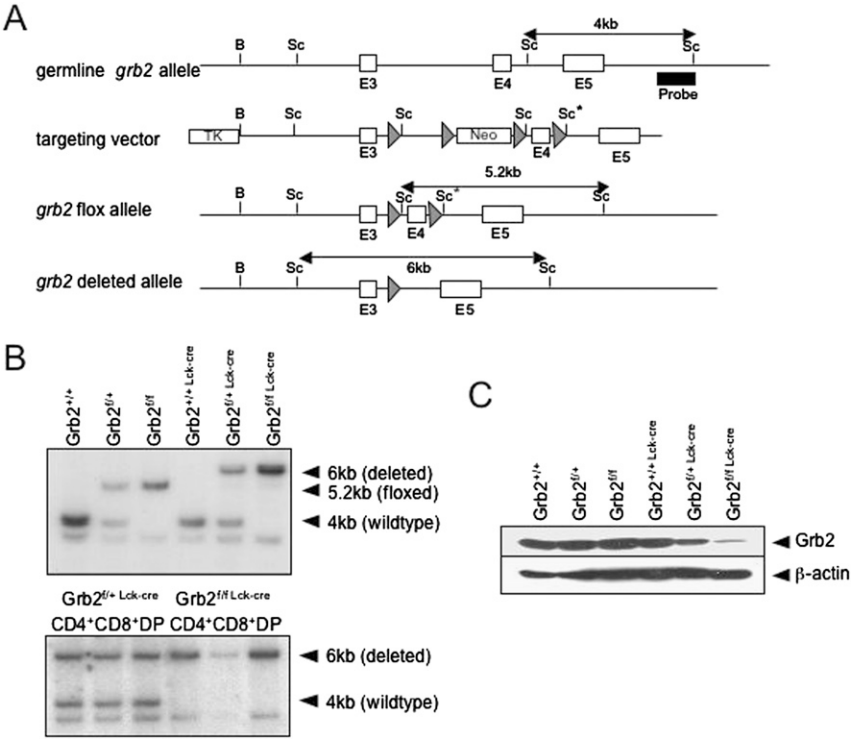


**Fig. S1.** Generation of Grb2$^{-/-}$(T) mice. (*A*) WT and mutant *grb2* gene loci. Shown from the top to bottom are germline, targeted, floxed, and deleted *grb2* alleles, respectively. Solid bar represents a probe for Southern blot analysis; triangles indicate loxP sites; open boxes indicate *grb2* exons. B, BamH1; Sc, SacI. (*B*) Southern blot analysis of the Grb2$^{-/-}$(T) mutation. Genomic DNA samples from total thymocytes (*Upper*) or purified thymocyte subsets (*Lower*) were digested with SacI and hybridized with the probe indicated in *A*. Genotypes of the samples are indicated at the top of the blot. The WT *grb2* allele (4 kb), *grb2* deleted allele (6 kb), and *grb2* floxed allele (5.2 kb) are indicated at the right side of the blot. (C) Western blot analysis of Grb2 expression in Grb2$^{-/-}$(T) thymocytes. Shown are levels of Grb2 protein in total thymocytes from mice of indicated genotypes.

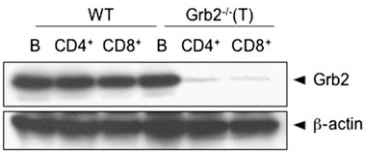


**Fig. S2.** Expression of Grb2 in peripheral lymphocytes. Western blot showing analysis of Grb2 expression in CD4 T, CD8 T, and B cells of WT and Grb2$^{-/-}$(T) mice. β-Actin was used as a loading control.

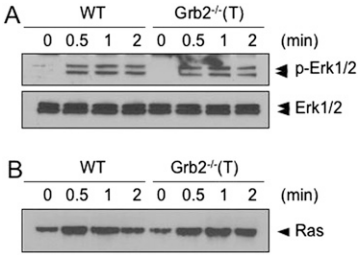


**Fig. S3.** Ras-Erk activation in Grb2$^{-/-}$(T) thymocytes after short periods of TCR stimulation. Total thymocytes were stimulated with anti-CD3ε and anti-CD4 for 0.5, 1, or 2 min, respectively. (*A*) Erk activation in thymocytes. Total cell lysates were resolved on a PAGE gel. Active form of Erk in the lysates was detected using an anti-active form Erk. (*B*) Ras pull-down assay. Cell lysates were directly used for Ras pull-down assay using a recombinant RBD of Raf as bait. Ras protein precipitated by the RBD of Raf was quantified by Western blot analysis.

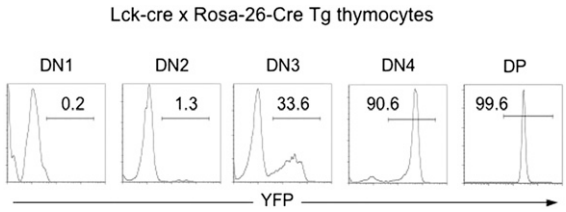


**Fig. S4.** Efficiency of loxP and Lck-Cre Tg–mediated recombination in thymocytes. Shown are histograms of FACS analysis of YFP$^+$ thymocytes in Lck-Cre × Rosa-26-YFP double transgenic mice. YFP-positive cells represent cells harboring a Rosa-26-YFP allele with Cre-loxP–mediated deletion.

**Table S1. Absolute numbers of thymocytes and peripheral T and B cells in WT and Grb2$^{-/-}$(T) mice**

| | Total cells ($\times 10^6$)* | |
|---|---|---|
| | WT | Grb2$^{-/-}$(T) |
| Thymus | | |
| Total | 192.2 ± 38.4 | 126.8 ± 34.5$^\dagger$ |
| DN | 5.1 ± 1.4 | 8.6 ± 3.4 |
| DP | 154.7 ± 28.2 | 102.2 ± 39.4$^\dagger$ |
| CD4$^+$ | 17.9 ± 4.7 | 4.0 ± 1.2$^\dagger$ |
| CD8$^+$ | 4.8 ± 1.0 | 2.8 ± 1.4$^\dagger$ |
| Spleen | | |
| Total | 106.2 ± 22.3 | 100.5 ± 21.5 |
| CD4$^+$ | 22.8 ± 6.0 | 6.5 ± 1.8$^\dagger$ |
| CD8$^+$ | 9.0 ± 2.8 | 4.8 ± 2.5$^\dagger$ |
| B220$^+$ | 60.7 ± 13.9 | 71.2 ± 17.6 |
| Lymph nodes | | |
| Total | 28.2 ± 4.1 | 15.8 ± 5.5$^\dagger$ |
| CD4$^+$ | 13.2 ± 2.5 | 1.9 ± 0.8$^\dagger$ |
| CD8$^+$ | 5.2 ± 1.6 | 2.3 ± 0.9$^\dagger$ |
| B220$^+$ | 7.6 ± 1.7 | 8.7 ± 3.5 |

*Values are mean ± SD of age-matched experiments. Results represent more than seven Grb2$^{-/-}$(T) and WT mice.

$^\dagger$Significant difference in comparison of WT and Grb2$^{-/-}$(T) mice (*t* test , $P < 0.01$).