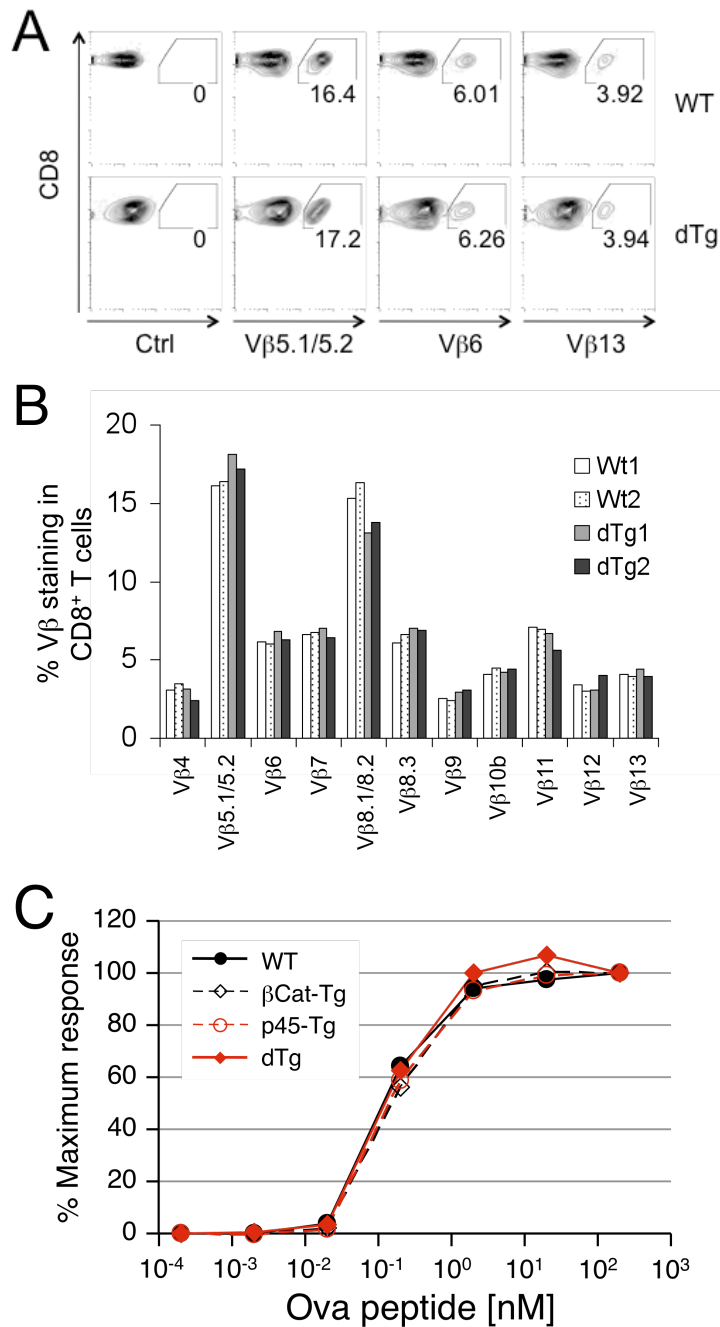


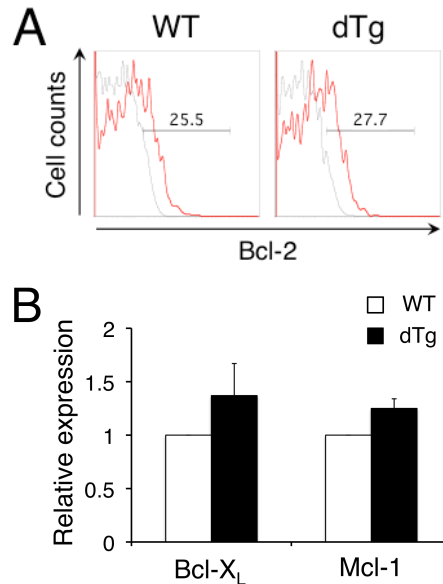
Zhao et al., Supplemental Figure 1



**Supplemental Figure 1. βCat and p45 transgenes did not cause apparent changes in TCR repertoire.** (A) and (B) Detection of TCR Vβ subtypes on splenic CD8 T cells. Splenocytes from dTg and WT littermate controls were stained for CD4 and CD8 along with TCR Vβ screening panel antibodies (BD Biosciences). Representative flow cytometric data for selected Vβ subtypes are shown in (A). Note that the complete Vβ subtype antibodies are only available in FITC-conjugated format, and that the low

expression of GFP in mature CD8 T cells accompanying  $\beta$ -catenin transgene did not affect the detection of most of V $\beta$  subtypes that are expressed at high levels. The results from 2 independent experiments are collectively shown in (B). V $\beta$ 2, V $\beta$ 3, V $\beta$ 14, and V $\beta$ 17<sup>a</sup> subtypes are less abundant and expressed weaker, and no apparent differences have been noted. (C) Functional avidity of antigen-specific T cells in the transgenic strains. Splenocytes from *actA*LM-Ova-infected mice were isolated on day 7 post-infection and incubated with the Ova<sub>257-264</sub> peptide at indicated concentrations for 6 hours. Fractions of IFN- $\gamma$ -producing cells were determined by intracellular staining. The response to 200 nM Ova peptide in each mouse strain was arbitrarily set to 100%, and responses to other concentrations of Ova peptide in the same strain were normalized. Data are average of 2 independent measurements.

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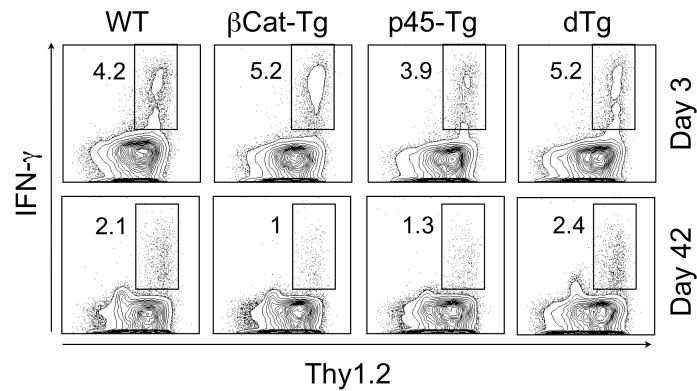


**Supplemental Figure 2. Similar expression levels of pro-survival Bcl-2 family members in antigen-specific effector CD8 T cells in WT and dTg mice.**

(A) Detection of Bcl-2 protein in Ova-specific effector CD8 T cells. WT and dTg mice were infected with *actA*LM-Ova, and on day 7 post-infection, expression of Bcl-2 in Ova-specific CD8 T cells were detected by simultaneous intracellular staining for Bcl-2 and IFN- $\gamma$ . Gray lines denote staining with isotype control for Bcl-2, and red lines denote Bcl-2 staining. The percentages of Bcl-2<sup>+</sup> cells are marked.

(B) Detection of Bcl-X<sub>L</sub> and Mcl-1 transcripts in Ova-specific effector CD8 T cells. Antigen-specific CD8 T cells were detected and labeled with MHC I/SIINFEKL tetramer, and the tetramer-positive cells were purified by FACS sorting on days 7 and 42, respectively. Total RNAs were prepared and relative expression of Bcl-X<sub>L</sub> and Mcl-1 was determined by quantitative reverse transcription PCR. Results are means  $\pm$  s.e.m. of duplicate measurement of 2 independent samples.

Zhao et al., Supplemental Figure 3



**Supplemental Figure 3. Secondary expansion of antigen-specific CD4 T cells in WT and transgenic strains.** Mice of indicated genotypes were first infected with *actA*<sup>-</sup>LM-Ova, and 45 days later infected with virulent LM-Ova. On days 3 and 42 after the second challenge, antigen-specific CD4 T cells in splenocytes were detected by ICS for IFN- $\gamma$  after 6-hour incubation of the splenocytes with LLO<sub>190-201</sub> peptides. Percentages of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> cells are shown. Data are representative of 2 independent experiments with at least 6 mice analyzed for each genotype.