## **Supporting Information**

## Cozzo Picca et al. 10.1073/pnas.1103810108



**Fig. S1.** 6.5<sup>hi</sup>CD4<sup>+</sup>CD25<sup>+</sup> T cells exhibit Treg cell function. Representative dot plots (n = 3) showing IFN- $\gamma$  production by CFSE-labeled CD4<sup>+</sup> LN cells from TS2 mice following in vitro culture with (A) S2 peptide only or (B) with S2 peptide, 6.5<sup>hi</sup>CD4<sup>+</sup>CD25<sup>+</sup> LN cells from TS1 × HA28 mice, and indicated amounts of S1 peptide. Percentages of IFN- $\gamma^+$  TS2 cells are indicated. Data are representative of three independent experiments.



**Fig. 52.**  $V\alpha 8.3^*V\beta 10^*CD4^*CD25^*$  T cells exhibit Treg cell function. Representative dot plots (n = 3) showing IFN- $\gamma$  production by CFSE-labeled CD4<sup>+</sup> LN cells from TS2 mice following in vitro culture with (A) S2 peptide only or (B) with S2 peptide,  $V\alpha 8.3^*V\beta 10^*CD4^*CD25^*$  LN cells from TS1(SW) × Pev(SW)HA mice, and indicated amounts of S1(SW) peptide. Percentages of IFN- $\gamma^*$  TS2 cells are indicated. Data are representative of three independent experiments.



**Fig. S3.** Enhanced thymocyte deletion does not promote V $\alpha$ 8.3<sup>+</sup> Treg cell formation in TS1(SW) × HACII mice. Representative dot plots show CD4 versus CD8 expression (*Left*), CD25 versus Foxp3 expression on CD4<sup>+</sup>CD8<sup>-</sup> events (*Center*), and V $\alpha$ 8.3 versus V $\beta$ 10 expression on CD4<sup>+</sup>CD8<sup>-</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> events (*Right*). Thymocytes (*Upper*) and splenocytes (*Lower*) from TS1(SW), TS1(SW) × Pev(SW)HA, and TS1(SW) × HACII mice. Mean percentages ± SEM in indicated gates are shown. *n* = 4–6 for each condition.

DNAS Nd

S A



## Thymocytes

**Fig. S4.** Agonist-induced IL-2 production does not promote Treg cell formation in TS1 × HA28 and TS1 × PevHA mice. Representative dot plots (n = 3) show Foxp3 versus 6.5 staining of CD4SP thymocytes (*Upper*) and of CD4<sup>+</sup> splenocytes (*Lower*) obtained from TS1, TS1 × HA28, TS1 × PevHA, and TS1 × Pev(SW)HA mice either without IL-2 treatment ("No IL2") or 5 d after injection of IL-2:anti-IL2 complexes ("+IL-2"). The percentages and numbers of cells ± SEM in the indicated quadrants are shown.

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