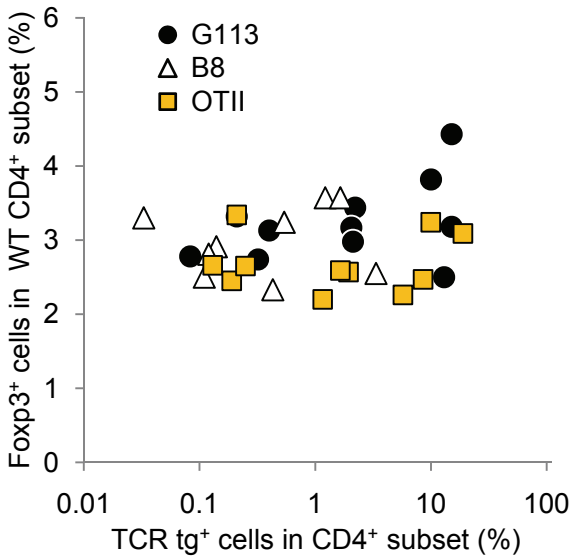
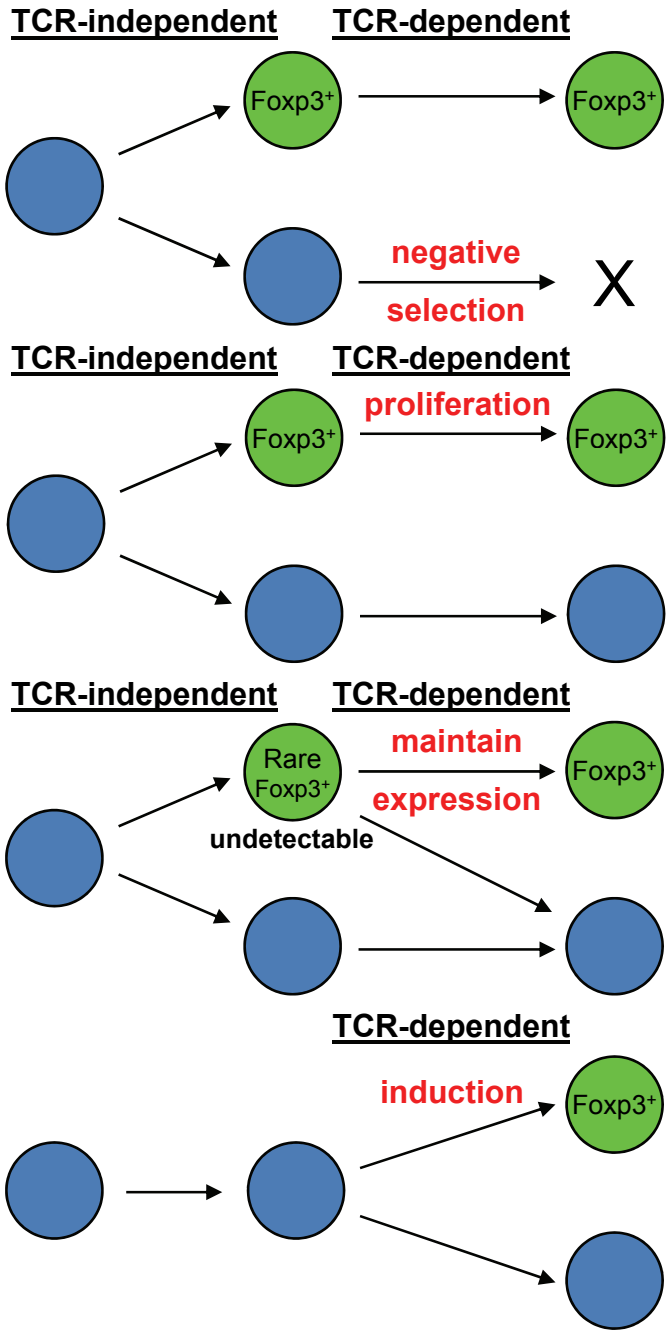
Intraclonal competition limits regulatory T cell fate determination in the thymus

Jhoanne L. Bautista, Chan-Wang J. Lio, Stephanie K. Lathrop, Katherine Forbush, Yuqiong Liang, Jingqin Luo, Alexander Y. Rudensky, and Chyi-Song Hsieh

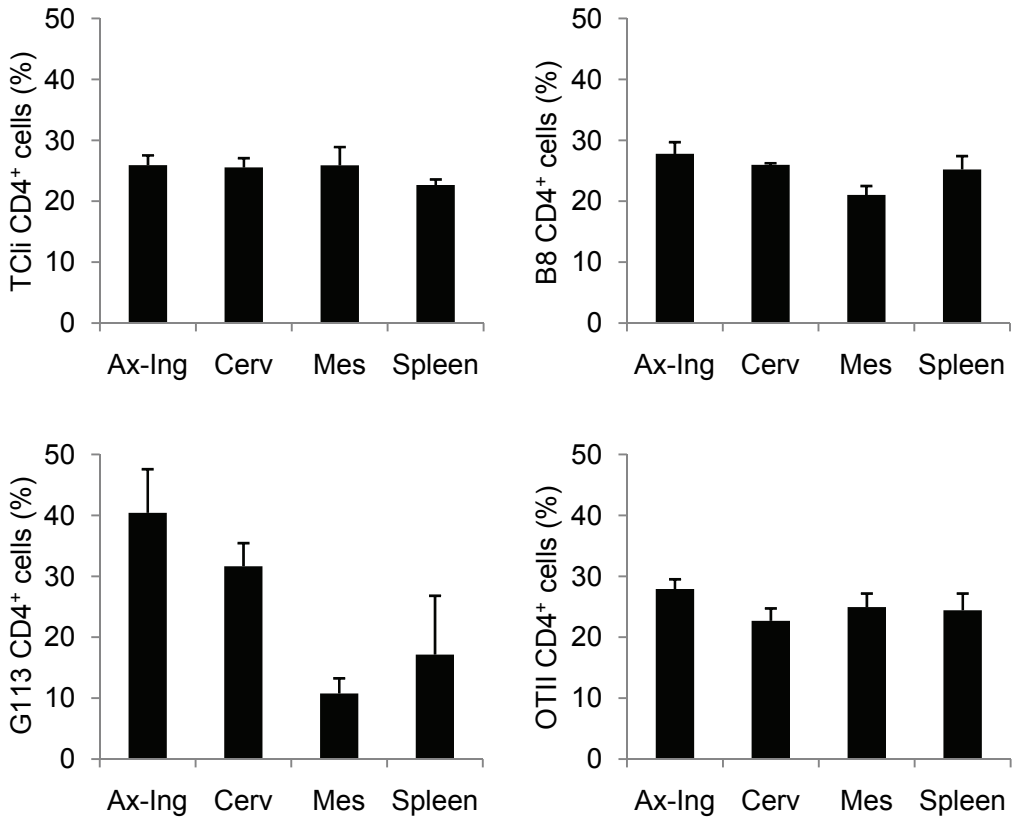
Supplementary Figures 1-6



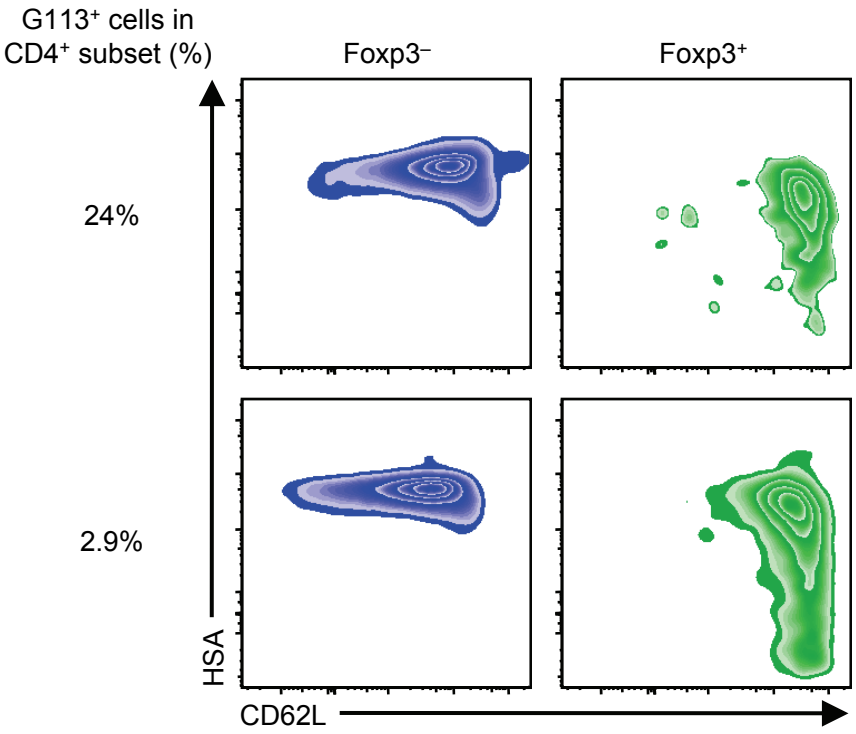
Supplementary Fig. 1. T_{reg} cell development of wild-type congenic thymocytes is not markedly affected by the presence of TCR transgenic T cells. Radiation BM chimeras were generated as per **Fig. 2b-c**. The frequency of Foxp3⁺ cells in the wild-type CD45.1⁺ CD4⁺ subset are shown indexed to the frequency of TCR transgenic cells within the entire CD4⁺ thymocyte subset.



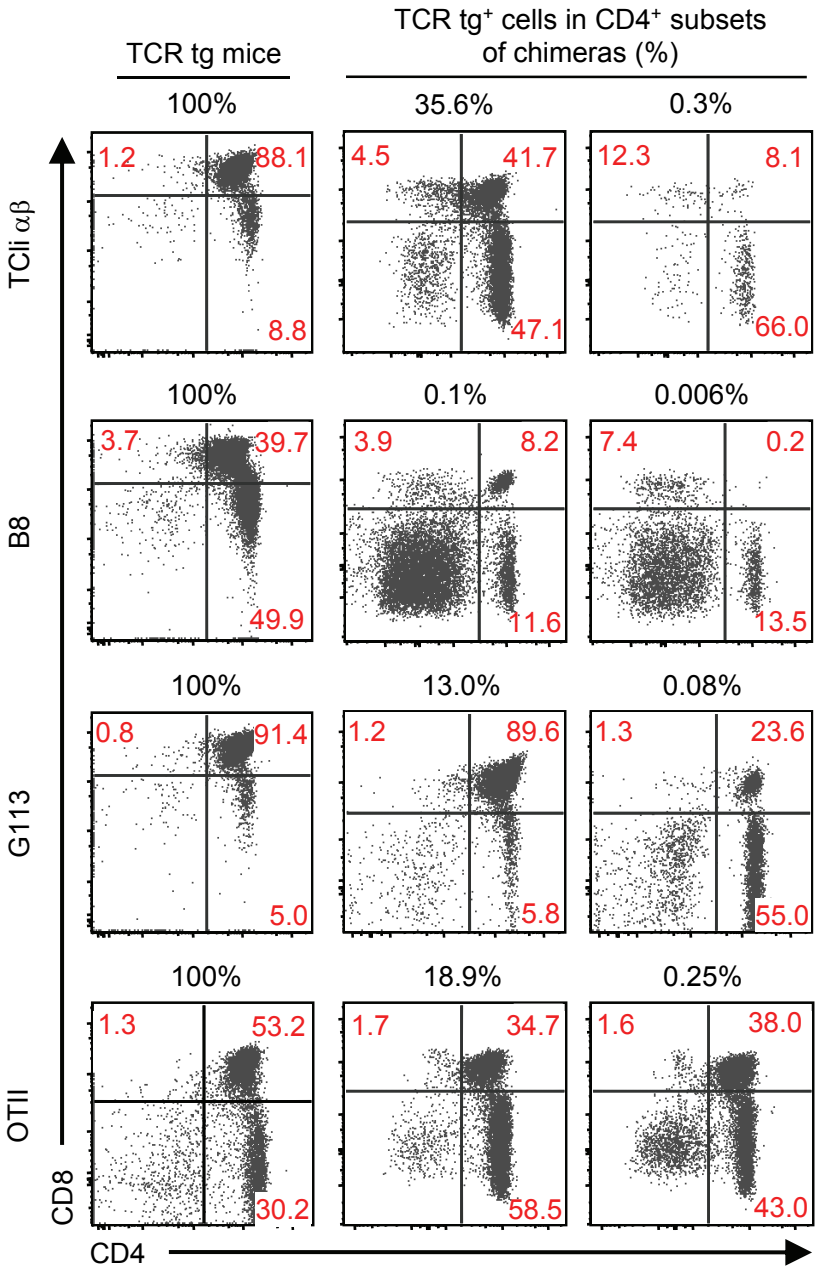
Supplementary Fig. 2. Models of thymic T_{reg} cell development. A stochastic-selective model (top) has been suggested[16] in which TCR-independent Foxp3 expression (green cells) is followed by TCR-driven negative selection of Foxp3$^-$ cells (blue). However, no Foxp3$^+$ cells were observed with OTII or B8 TCR transgenic cells at any clonal frequency (**Fig. 2c**), arguing against this possibility. Another potential model would involve the TCR-dependent proliferation of cells which had upregulated Foxp3 in a TCR-independent manner. The lack of cell division during Foxp3 induction (**Fig. 6b**) suggests that this is unlikely. The inability to observe Foxp3$^+$ cells with certain TCRs could be explained by a third model in which TCR activation provides an instructive signal to maintain Foxp3 expression induced in a TCR-independent manner on a rare subset of thymocytes at a frequency below the limit of detection. However, the simplest model is one in which TCR-dependent signals ultimately leads to the induction of Foxp3. We consider both TCR-dependent maintenance or induction of Foxp3 to be TCR-instructive models of thymic T_{reg} cell development.



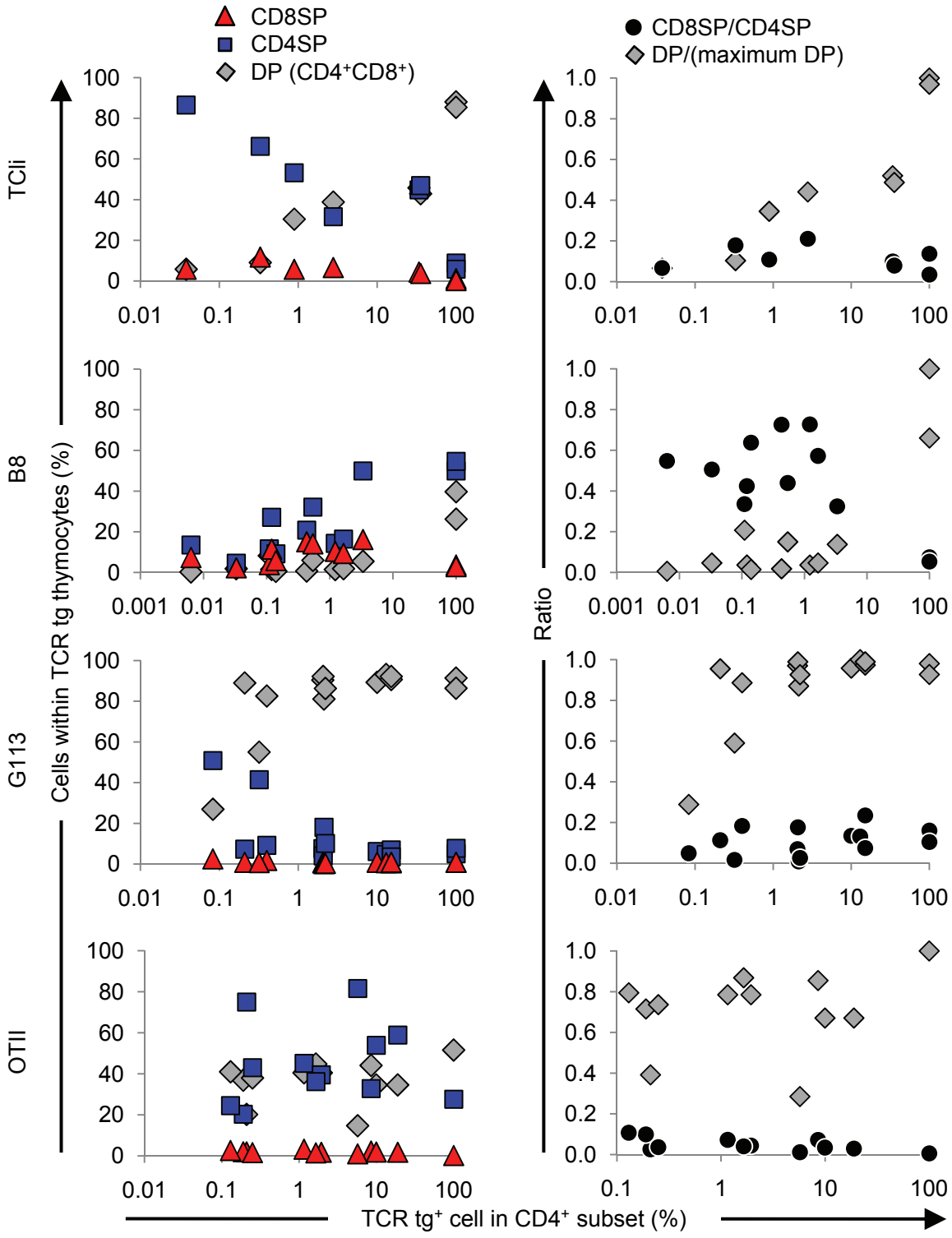
Supplementary Fig. 3. Distribution of TCR transgenic CD4$^+$ T cells in the secondary lymphoid tissues of mixed BM chimeras. The relative frequency was calculated from the percentages of TCR transgenic T cells in the CD4$^+$ subset for each of the indicated secondary lymphoid organs divided by the summed percentages from all four organs. The percentages of CD4$^+$ transgenic cells were determined by flow cytometry from the spleen and following lymph nodes: axillary and inguinal (Ax-Ing), cervical (Cerv), and mesenteric (Mes). Data shown are the mean ± s.d. of 8 mice from 2 experiments per TCR transgenic line.



Supplementary Fig. 4. Foxp3^{-} CD4SP G113 thymocytes are phenotypically at an earlier stage of development. Representative FACS plot of Foxp3^{-} and Foxp3^{+} TCRβ^{hi}CD4SP G113 *Rag1*$^{-/-}$ thymocytes from mixed BM chimeras (**Fig. 2c**) are shown.



Supplementary Figure 5. The effect of clone size on the CD4 by CD8 profile. Representative CD4 versus CD8 FACS plots of transgenic CD45.2^{+}CD45.1^{-} thymocytes from TCR transgenic *Foxp3*^{gfp} *Rag1*$^{-/-}$ mice (left, TCR tg mice) or irradiation mixed BM chimeras (**Fig. 2c**) are shown. The frequencies of the TCR transgenic cells in the CD4SP thymocyte subset are also shown (top of each plot) as an indication of the percentage of chimerism. Data are summarized in **Supplementary Fig. 6** online.



Supplementary Fig. 6. Relative increase in CD8SP frequency for B8 TCR transgenic cells. Data shown on the left are the frequency of the various thymic subsets (CD4SP, CD8SP, and DP) of the TCR transgenic cells in radiation mixed BM chimeras (**Fig. 2b-c**, **Supplementary Fig. 5** online). On the right, the ratio of DP cells to the maximum value for each TCR transgenic line and the CD8SP to CD4SP ratio are shown. For consistency, the data are indexed to the % of the TCR transgenic cells in the CD4SP thymocyte subset.