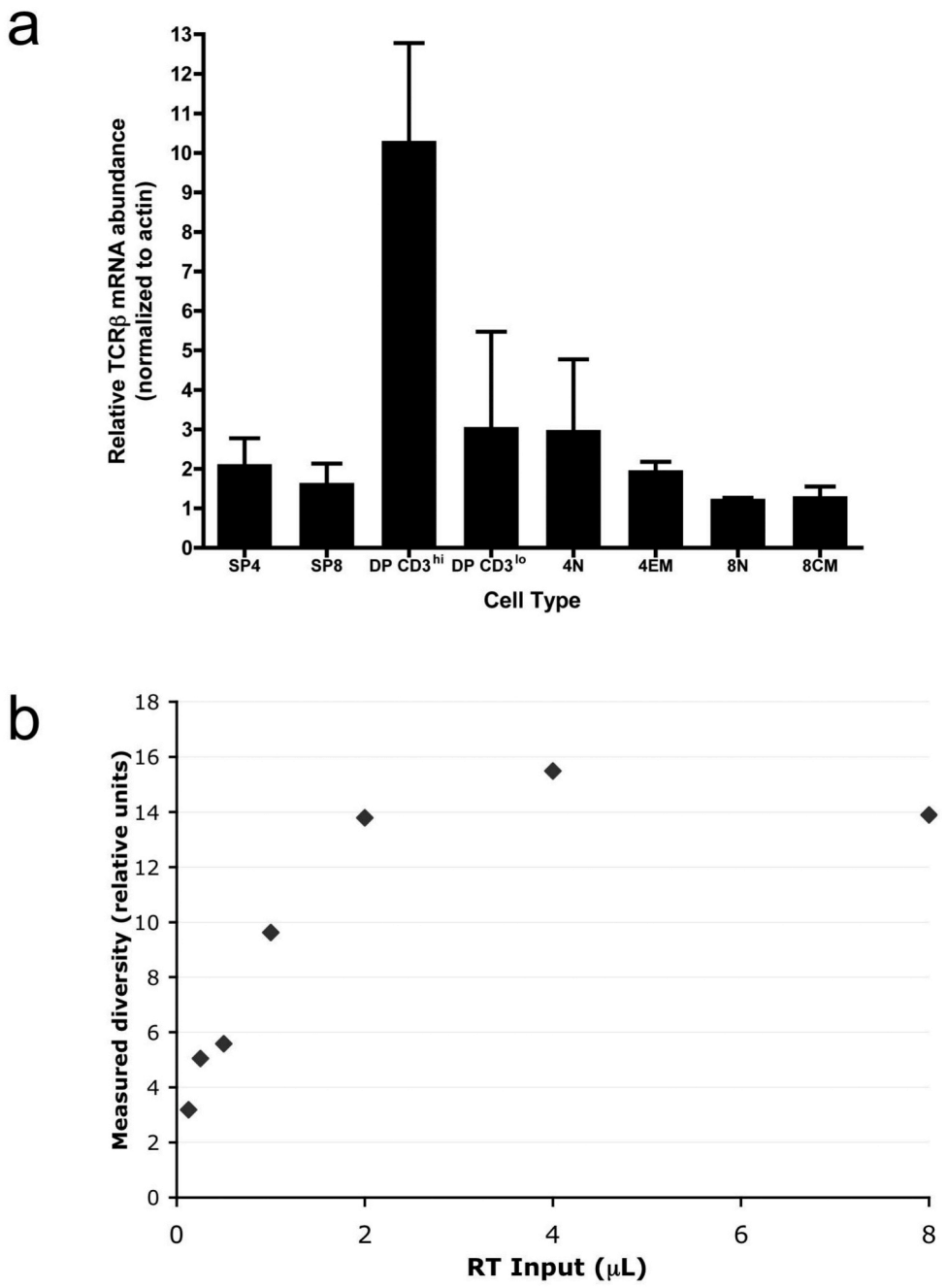

Supplementary Figure 1. T cell receptor mRNA expression.
(**a**) Generally similar (but not identical) TCR mRNA expression in different murine thymocyte and T cell subpopulations. From left to right are single-positive CD4+ thymocytes (SP4), single-positive CD8+ thymocytes (SP8), $CD3^{hi}$ double positive (DP) thymocytes, $CD3^{lo}$ double positive thymocytes, $CD4+CD44^{lo}CD62L^{hi}$ naïve (4N), $CD4+CD44^{hi}CD62^{lo}$ effector memory (4EM), $CD8+CD44^{lo}CD62L^{hi}$ naïve (8N), and $CD8+CD44^{hi}CD62L^{hi}$ central memory (8CM) splenic T cells. Bars represent the mean relative TCRβ mRNA expression levels, normalized to a beta actin standard, from 10^5 sort-purified cells of each phenotype from three mice; error bars denote standard

deviations. These data are consistent with a previous finding that expanded and non-expanded clones of circulating T cells express similar levels of TCR mRNA per cell[1]. Our data is also consistent with a previous report that CD4+ cells have more copies of TCR mRNA per cell than CD8+ cells do[2].

(**b**) Effect of input dilution on measured diversity.

Total RNA was isolated from $2x10^4$ T splenocytes (a small number of cells was used to minimize the chance that multiple cells with the same receptor could compensate for diversity loss). Half of the RNA was reverse transcribed in a 20 μL reaction. Varying amounts of this reaction were amplified by PCR and the relative sequence diversity of the PCR products was measured with AmpliCot. Diversity loss was observed if the sample represented less than $1/20^{th}$ of the starting RNA.

Using the estimates that T cells have roughly 400 copies of the TCRβ mRNA per cell[3] and that RT-PCR reactions may have less than 10% efficiency when a large amount of template is used[4] as well as the calculation from Poisson statistics that the experiment must sample an average of 3 mRNA copies per cell in order for 95% of the cells to have an mRNA included in the final sample, yields a prediction that diversity will be lost if less than $1/15^{th}$ of a cell's mRNA is sampled; this agrees well with our observations.

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