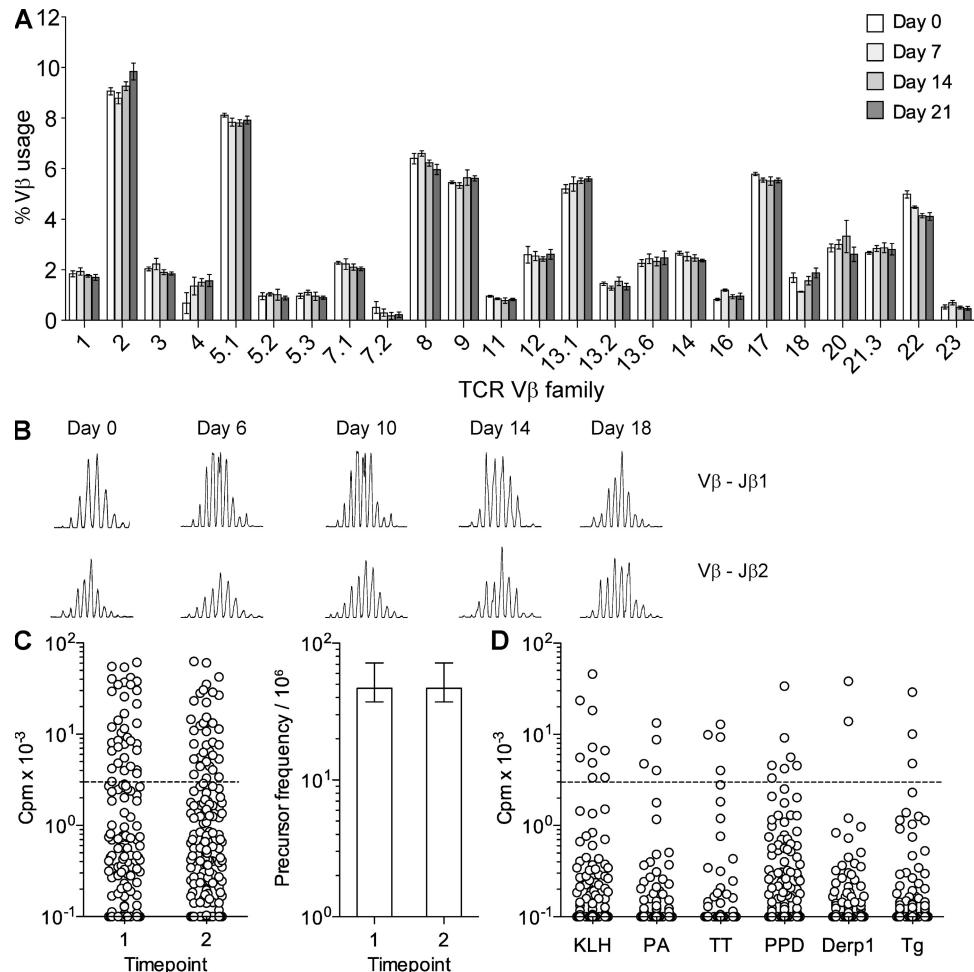
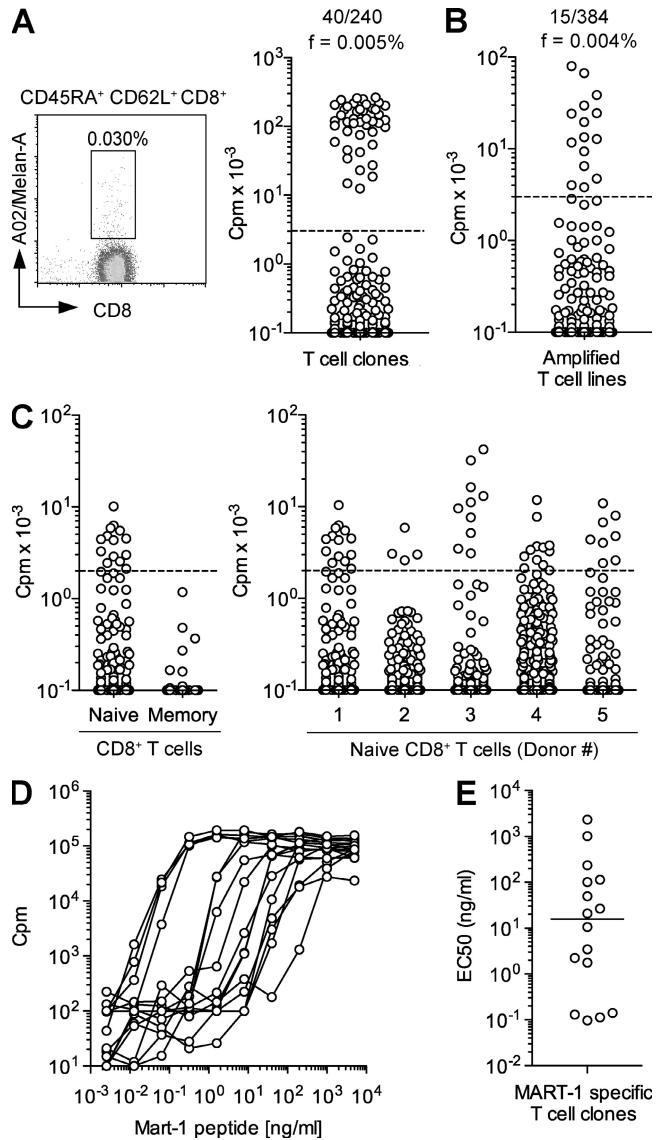


## SUPPLEMENTAL MATERIAL

Geiger et al., <http://www.jem.org/cgi/content/full/jem.20090504/DC1>

**Figure S1.** TCR V $\beta$  repertoire maintenance, reproducibility, and parallel multiple screenings of T cell libraries. (A) Comparison of the V $\beta$  usage of expanded T cell cultures derived from naive CD4 $^{+}$  T cells. Cell lines were analyzed by flow cytometry for V $\beta$  expression on day 0 and during expansion over 3 wk (day 7, 14, and 21) after polyclonal stimulation with PHA, using a combination of 24 different V $\beta$ -specific antibodies. Results presented are mean  $\pm$  SD of triplicate wells. (B) T cells were activated and expanded as in A. At different time points (day 0, 6, 10, 14, and 18), RNA was extracted and reverse transcribed. Fragments covering the V $\beta$ -J $\beta$ 1 and V $\beta$ -J $\beta$ 2 regions were amplified by PCR. Data shown in A and B are representative of two different experiments. (C) A library was prepared from naive CD4 $^{+}$  T cells and tested for antigen-driven proliferation with TT at 5  $\mu$ g/ml at two different time points (10 d apart) during expansion (left). Dotted line represents the cut-off value. Precursor frequencies were then calculated for both time points (right). One experiment from one donor representative of five independent experiments performed with five different donors. Comparable results were obtained by varying the two time points in which the analysis was performed. (D) A naive CD4 $^{+}$  T cell library was simultaneously tested with different antigens (KLH, PA, TT, PPD, *Dermatophagoides pteronyssinus* major allergen I, and thyroglobulin). Each symbol represents one cell culture out of the 192 analyzed. Dotted line represents the cut-off value. Shown is one representative experiment of at least five performed.



**Figure S2. Frequencies of antigen-specific CD8<sup>+</sup> naive T cells measured using libraries of amplified T cells or staining with MHC class I multimers.** (A) Naive CD8<sup>+</sup> T cells were sorted from a healthy donor according to the expression of CD45RA and CD62L. A fraction of the naive CD8<sup>+</sup> T cells were stained with MHC class I Melan-A/MART-1 pentamers (A, left). The percentage of pentamer<sup>+</sup> T cells is indicated. Pentamer<sup>+</sup> T cells were sorted and cloned by limiting dilution. The proliferative response to Melan-A/MART-1 of the growing clones (A, right), the number of positive clones, and the calculated frequency (f) are shown. (B) A fraction of the sorted CD45RA<sup>+</sup> CD62L<sup>+</sup> naive T cells isolated from the donor in A were used to generate amplified libraries that were screened for their capacity to proliferate in response to monocytes pulsed with 5 µg/ml Melan-A/MART-1 peptide. After 3 d, proliferation was measured after 16-h pulse with [<sup>3</sup>H]thymidine. Shown are delta cpm values. Each symbol illustrates one culture out of the 384 screened. Calculated frequency (f) is shown. (C) Amplified libraries were prepared from naive and memory CD8<sup>+</sup> T cells isolated from a donor (left) or from naive CD8<sup>+</sup> T cells isolated from five donors and screened for their capacity to proliferate in response to Melan-A/MART-1 peptide. (D) Dose-response curves and (E) EC50 values of Melan-A/MART-1-reactive T cell clones. Bar indicates median value. Results in A and B are representative of two independent experiments performed with two different donors. Dotted lines represent cut-off values.

**Table S1.** V $\beta$  expression of antigen-specific T cell clones isolated from the same T cell culture

Donor (no.)	Cell culture	Antigen	Ag-specific clones/total clones screened	V $\beta$ usage <sup>a</sup>
2	1	KLH	16/30	V $\beta$ 2 (16/16)
	2	KLH	24/30	V $\beta$ 14 (24/24)
	3	KLH	9/35	V $\beta$ 5.3 (9/9)
	4	PA	40/43	V $\beta$ 17 (40/40)
5	1	KLH	20/56	V $\beta$ 12 (20/20)
	2	KLH	28/45	V $\beta$ 1 (28/28)
	3	PA	7/11	V $\beta$ 3 (7/7)
	4	PA	18/40	V $\beta$ 2 (18/18)

<sup>a</sup>V $\beta$  usage was determined by flow cytometry.

**Table S2.** Screening of PA-specific T cell cultures using overlapping peptides covering the entire PA sequence

Cell line (no.)	Peptides <sup>a</sup> (aa no.)	Amino acid sequence <sup>b</sup>
1	713–727	KLPLYISNPNEYKVNV
2	428–439	NQLSQILAPNNY
3	289–302	PIVHVDMENIILSKN
4	609–623	KIKLNAKMNILIRDK
5	441–455	PSKNLAPIALNAQDD
6	253–263	EKWSTASDPYS

<sup>a</sup>Peptide library of 15-mers with 11-aa overlap.