Figure S1. Models predicting effector and memory T cell lineage relationship. Schematic representation of four of the proposed models describing the lineage relationship of effector (E) and memory (M) T cells. Dendritic cells with different stimulating capacities are depicted in light and dark blue. Naive T cells are shown in pink. Note that other (variations on these) models have been proposed, but the fundamental distinguishing feature of the different models for the current study is whether they predict that single naive T cells produce single or multiple subsets.

Figure S2. Phenotypic characterization of barcode-labeled and unlabeled OT-I T cells after LM-OVA infection. CD45.2+ B6 2° recipient mice were injected with naive, barcode-labeled (bc) CD8+CD45.1+ OT-I cells or unmanipulated (−) CD8+CD45.1+ OT-I cells (n = 7 and 2, respectively). Mice were subsequently infected i.v. with LM-OVA. At day 7 after infection, blood cells were stained for the indicated surface markers and analyzed by flow cytometry. Circles represent individual mice; bars indicate group averages. Data are representative of two independent experiments.
Figure S3. CD127loKLRG-1hi and CD127hiKLRG-1lo cells are related. CD45.2+ B6 2° recipient mice (n = 4) were injected with naive, barcode-labeled CD8+CD45.1+ OT-I cells and subsequently infected i.v. with LM-OVA. 10 d after infection, spleen and lymph node cells were isolated, enriched for CD8+ cells, and sorted into CD8+, CD45.1+, CD127loKLRG-1hi and CD8+, CD45.1+, CD127hiKLRG-1lo cells. Data are derived from four mice analyzed within one experiment. (A) Flow cytometric sorting plot gated on CD8+CD45.1+GFP+ cells; sorting gates are indicated. (B and C) Both sorted populations, abbreviated as CD127lo and CD127hi, were divided into two halves (samples A and B) that were independently analyzed for barcode content. CD127hi half-samples were separately expanded for 3 d in vitro in the presence of OVA257-264–loaded splenic dendritic cells at a T cell/dendritic cell ratio of 10:1 before barcode analysis. Barcodes with a p-value <0.0001 were considered to be present above background. On average, 430 different barcodes were detected per mouse. (B) Representative 2-D plots of barcode comparisons. Numbers indicate the correlation between signals from samples A and B. (C) Correlation analysis of barcodes present in samples A and B. Data from four mice analyzed within one experiment are depicted.
Figure S4. Barcode data analysis in a case of suboptimal sampling. CD45.2+ B6 2° recipient mice were injected with naive, barcode-labeled CD8+CD45.1+ OT-I cells and subsequently received an i.v. infection with LM-OVA at days 0 and 30. 5 d later, genomic DNA was isolated from the spleen of two 2° recipient mice. (A and B) To determine how suboptimal sampling would affect correlation analyses on cell populations of which one is sampled less efficiently than the other, 1.5% of the spleen 1 DNA was diluted in four steps of twofold dilutions (dilutions I–V, with I being the highest and V being the lowest concentration). For each dilution, two samples were independently analyzed for barcode content. Barcodes with a p-value of <0.0001 were considered to be present above background. (A) The correlation of intradilution (red squares) and interdilution comparisons is depicted in a heat map. (B) The correlation of different interdilution comparisons (orange horizontal bars) is depicted in relation to the correlation of the two corresponding interdilution comparisons and their mean correlation (highest, lowest, and middle black horizontal bars, respectively). Note that when correlation analyses are performed for two samples that have substantially different intrasample correlation values (e.g., I vs. IV, I vs. V, and II vs. V), this value approximates that of the poorest intrasample comparison. (C and D) To test whether the exclusion of barcodes detected with low confidence would facilitate the detection of barcodes that are differentially present, part of the spleen 1 DNA was divided in two samples (S1 1 and S1 2) and part of the spleen 1 DNA was mixed with part of the spleen 2 DNA (S1+2). All three samples were diluted and split in two halves (samples A and B) for independent barcode analysis. Standard barcode analysis is shown in the first row. To statistically determine which barcodes are likely to be outliers (i.e., detected with low confidence), a linear model was fitted through the barcode signals from the intrasample comparisons and the studentized residuals were calculated per barcode. Barcodes with residuals ≥3 (97.5% chance of being an outlier) in the intrasample comparisons were either colored (second row) or excluded (third row). Barcodes with a p-value of <0.0005 were considered to be present above background. (C) Barcode comparison between two related samples (S1 1 vs. S1 2) in relation to the corresponding intrasample comparisons. (D) Barcode comparison between two partially unrelated samples (S1 1 vs. S1+2) in relation to the corresponding intrasample comparisons. (E) Barcode comparison between CD127loKLRG-1hi (CD127lo) and CD127hiKLRG-1lo (CD127hi) cells from the experiment shown in Fig. S3. Standard barcode analysis is shown in the first row. Barcodes with residuals ≥3 in the intrasample comparisons were either colored (second row) or excluded (third row), as described for C and D. MPEC, memory precursor effector cell (CD127hiKLRG-1lo); SLEC, short-lived effector cell (CD127loKLRG-1hi).
Figure S5. Memory CD8+ T cells present in different organs harbor the same set of barcodes. CD45.2+ B6 2° recipient mice (n = 4) were injected with naive, barcode-labeled CD8+CD45.1+ OT-I cells and subsequently received an i.v. infection with LM-OVA. Barcode analysis was performed on blood, spleen, bone marrow, and lymph node cells isolated at day 28 after infection. All samples were divided into two halves (samples A and B) that were independently analyzed for barcode content. Barcode comparisons are made between samples A and B taken from the same mouse (within one mouse) or from two different mice (between two mice). Barcodes with a p-value <0.005 were considered to be present above background. (A) Representative 2-D plots of barcode comparisons. Numbers indicate the correlation between signals from samples A and B. (B) Correlation analysis of barcodes present in samples A and B isolated from blood, spleen, bone marrow, and lymph nodes. Data from four mice analyzed within one experiment are depicted.
Figure S6. Relatedness of effector and memory T cells in an oligoclonal antigen-specific T cell response. B6 2° recipient mice were injected with barcode-labeled Ltd cells and subsequently infected i.v. with LM-OVA. 27 d later, mice were rechallenged with LM-OVA. Barcode analysis was performed on a 250–300-μl blood sample drawn at day 8 after infection (effector phase), and on a spleen sample isolated at day 5 after rechallenge (2° expansion) from the same mice. Blood and spleen samples were divided into two halves (samples A and B) that were independently analyzed for barcode content. Barcodes with a p-value < 10^{-8} were considered to be present above background. On average, 25 barcodes were detected per mouse. 2-D plots of barcode comparisons are shown for two mice within one experiment. All barcodes present in more than one mouse were excluded. To limit analysis to barcodes detected with high reliability, barcodes with residuals ≥3 (97.5% chance of being an outlier) in the intrasample comparisons (depicted in purple in the intrasample comparisons) were excluded in the intersample comparisons, as described for Fig. S3 (C and D). Note that this filtering procedure precludes the calculation of correlation values. Data from one experiment are depicted.