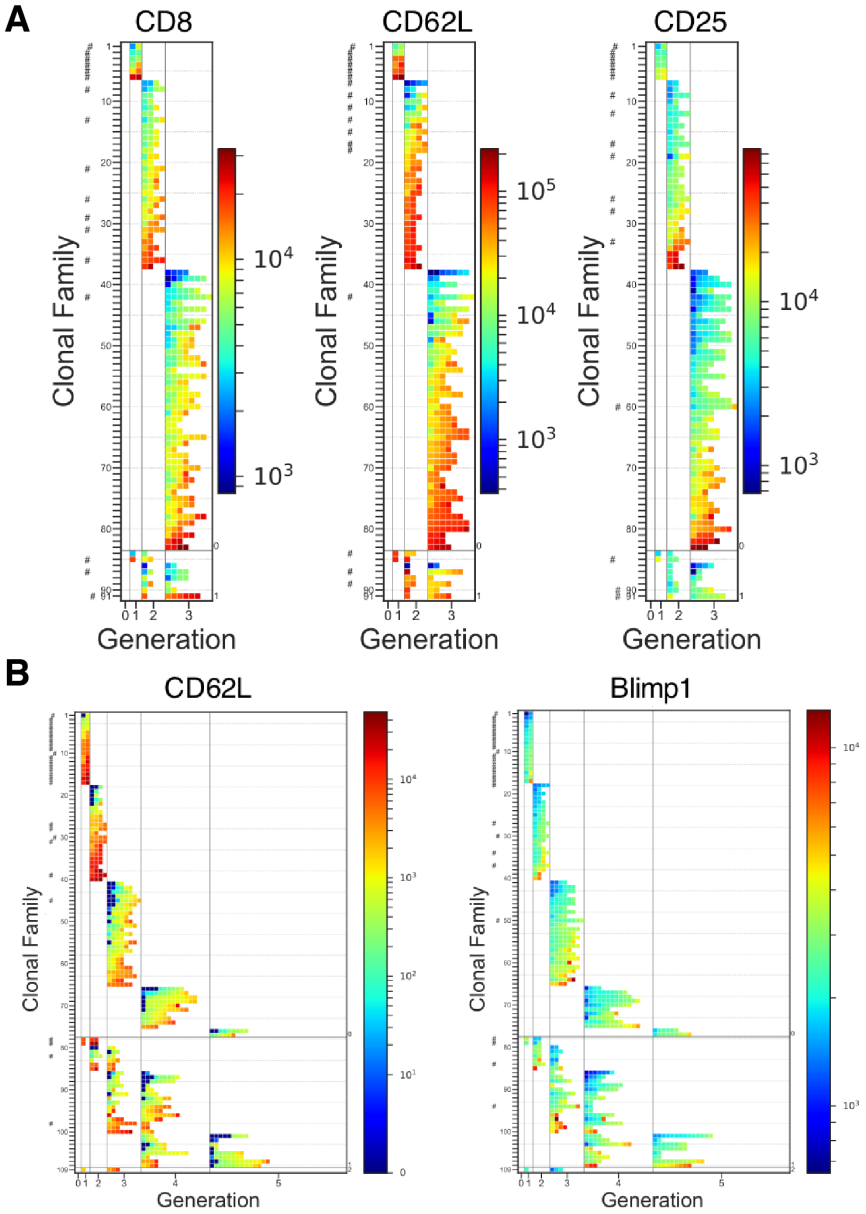
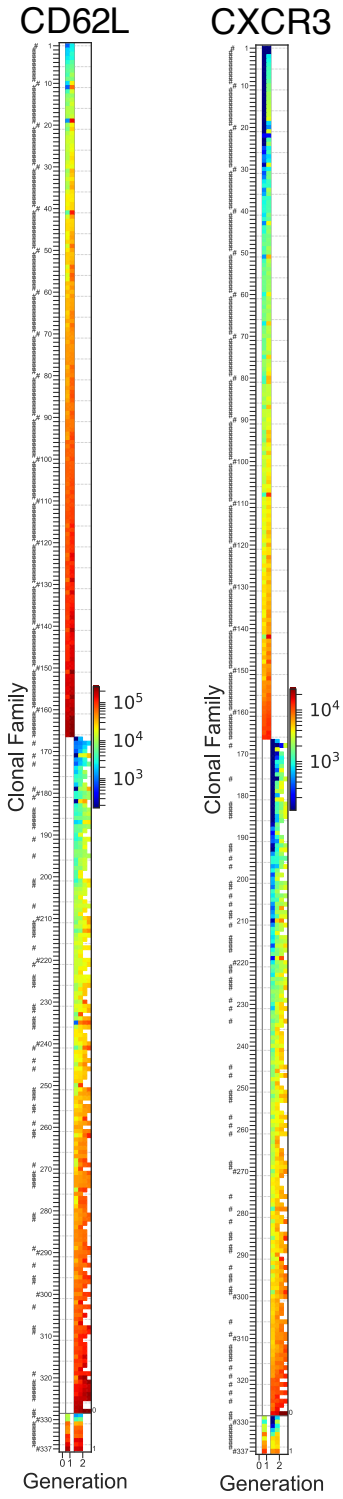
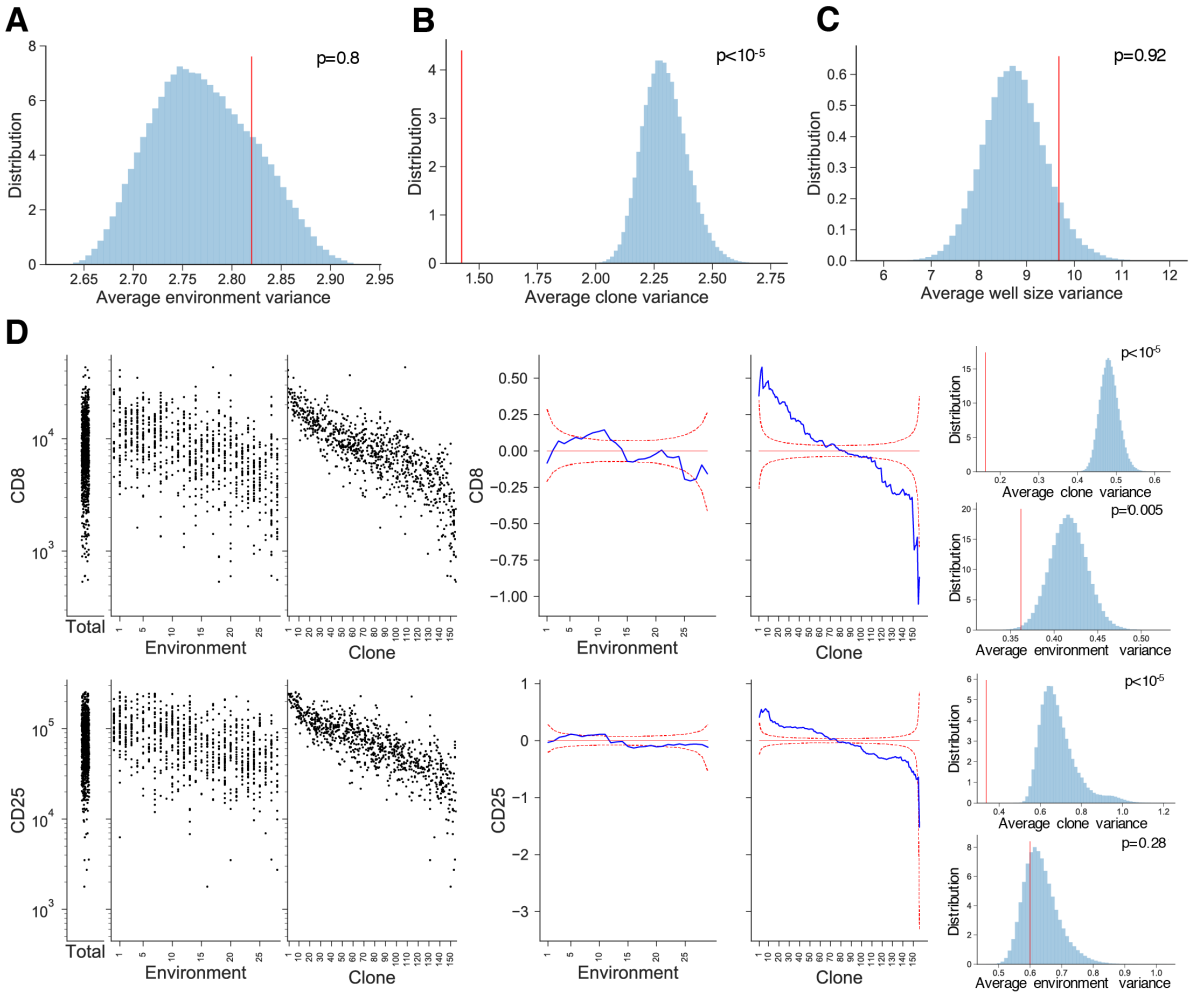
**Supplemental information:**



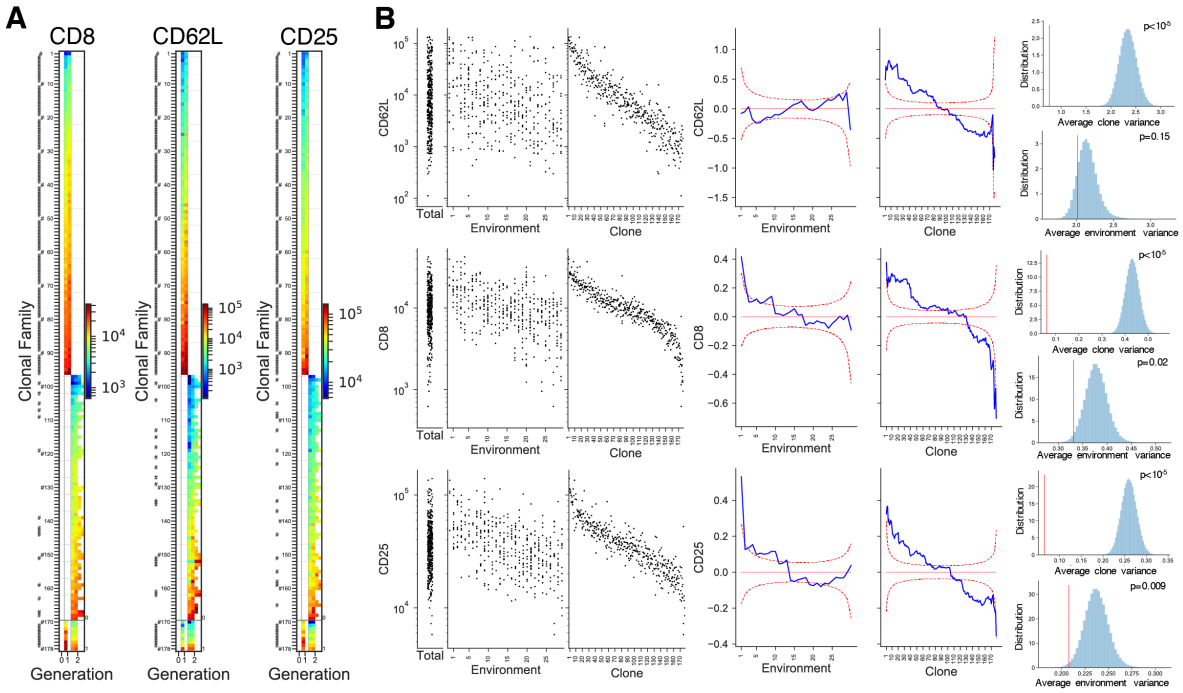

**SUPPLEMENTAL FIGURE 1.** (**A**) Multiplexing tracking dyes to determine clonal membership, generation number and cell phenotype is reproducible across time points**.** Purified murine CD8+ T cells were multiplex dye labelled as in Fig 1. These cells were stimulated with anti-CD3 (10 µg $ml^{-1}$), anti-CD28 (2 µg $ml^{-1}$) and rhIL-2 (31.6 U $ml^{-1}$) for 24h in the presence of the anti-mouse IL-2 blocking antibody clone S4B6 (25 µg $ml^{-1}$). Single cells from each of the 10 combinations were sorted and pooled into 20 individual wells followed by culture for a further 27h. Generation number and fluorescence intensity of CD8 (APC-Cy7), CD62L (PE) and CD25 (PE-Cy7) expression were determined by flow cytometry 51h post-stimulation. Image displays data pooled from all wells. Of 160 clones initially seeded, 91 families were detected with at least 2 members, yielding a recovery of 56.9%. (**B**) Distinct tracking dyes can be multiplexed to allow for compatibility with fluorescent reporters. Purified $Blimp^{gfp/+}$ CD8+ T cells were multiplex dye labelled with CellTrace Yellow (CTY), CTV and CPD, resulting in 6 profiles. These cells were stimulated with anti-CD3 (10 µg $ml^{-1}$), rhIL-2 (31.6 U $ml^{-1}$) and mIL-12 (10 ng $ml^{-1}$) for 24h in the presence of the anti-mouse IL-2 blocking antibody clone S4B6 (25 µg $ml^{-1}$). Single cells from each of the 6 combinations were sorted and pooled into 49 individual wells followed by culture for a further 42h. Generation number and fluorescence intensity of CD62L (APC-Cy7) and Blimp1 (GFP-reporter) expression were determined by flow cytometry 66h post-stimulation. Image displays data pooled from all wells. Of 360 clones initially seeded, 109 families were detected with at least 2 members, yielding a recovery of 30.3%.




**SUPPLEMENTAL FIGURE 2.** Multiplexed tracking dyes to determine clonal membership and generation number can be adjusted to assess distinct components of cell phenotype. Purified OT-I/Bcl2l11$^{-/-}$ CD8+ T cells were multiplex dye labelled with CFSE, CTV and CPD, resulting in 10 profiles. These cells were stimulated with N4 peptide (0.01 µg ml$^{-1}$) and IL-4 (1,000 U ml$^{-1}$) for 24h in the presence of the anti-mouse IL-2 blocking antibody clone S4B6 (25 µg ml$^{-1}$). Single cells from each of the 10 combinations were sorted and pooled into 59 individual wells followed by culture for a further 22h. Generation number and fluorescence intensity of CXCR3 (PE-Cy7) and CD62L (PE) expression were determined by flow cytometry 46h post-stimulation. Image displays data pooled from all wells. Of 600 clones initially seeded, 337 families were detected with at least 2 members, yielding a recovery of 56.2%.




**SUPPLEMENTAL FIGURE 3. (A-C)** Testing null hypotheses of independence. Analysis of the CD62L data presented in Fig. 2. (**A**) To test the null hypothesis that each cell's fluorescence is independent of its membership of an environment, but potentially dependent on its generation, while also being cognizant that the sampling of clones in the same environment may lead to a coupling in their recovery, data permutation is restricted to clones that have the same generational structure and for whom all cells in each clone are measured (**Methods**). (**B**) To test the null hypothesis that each cell's fluorescence is independent of its clonal membership, but potentially dependent upon its generation, data permutation is restricted to cells across clones within the same generation (**Methods**). (**C**) To test for the null hypothesis that clonal expansion and recovery are independent of environmental membership, clones are permuted across environments (**Methods**). The vertical red line indicates the location of the data statistic of the originally ordered data. The histogram shows the density of the same statistic determined for 250,000 uniformly-at-random permutations of the data. The lower one-sided p-value is depicted in legend, resulting in rejection of the hypothesis in **B** and non-rejection of the hypotheses in **A** and **C** with a significance level of 0.05. (**D**) Visualization and testing for environment and clone independence. Analysis of the CD8 (top row) and CD25 (bottom row) data in Fig. 2. (Left column) The data are pooled, and fractionated by environment (i.e. well) and clone, and rank ordered from highest-to-lowest geometric mean. (Central column) The blue line is the difference between the rank ordered true data and the mean label-permuted data. Dashed red lines indicate 95% permuted data confidence intervals under the null hypothesis that expression is independent of membership label. (Right column) The vertical red line indicates the location of the data statistic and, with a null hypothesis as in Fig. 3C (top panel) or Fig. 3D (bottom panel), the histogram shows the density of the same statistic determined for 250,000 uniformly-at-random permuted assignments of cell-to-clone (top panel) or clone-to-environment (bottom panel). The lower one-sided p-value is depicted in legend.




**SUPPLEMENTAL FIGURE 4.** (**A**) Multiplexed tracking dyes to determine clonal membership, generation number and cell phenotype can identify clones as early as the first division. Purified murine CD8+ T cells were multiplex dye labelled with CFSE, CTV and CPD, resulting in 10 profiles. These cells were stimulated with anti-CD3 (10 μg $ml^{-1}$), anti-CD28 (2 μg $ml^{-1}$) and rhIL-2 (31.6 U $ml^{-1}$) for 24h in the presence of the anti-mouse IL-2 blocking antibody clone S4B6 (25 μg $ml^{-1}$). Single cells from each of the 10 combinations were sorted and pooled into 30 individual wells followed by culture for a further 18h. Generation number and fluorescence intensity of CD8 (APC-Cy7), CD62L (PE) and CD25 (PE-Cy7) expression were determined by flow cytometry 42h post-stimulation. Image displays data pooled from all wells. Of 300 clones initially seeded, 178 families were detected with at least 2 members, yielding a recovery of 59.3%. (**B**) Visualization and testing of environment and clonal independence. Analysis of CD62L (top row), CD8 (central row) and CD25 (bottom row) for the data presented in **A**. (Left column) The data are pooled, and fractionated by environment (i.e. well) and clone, and rank ordered from highest-to-lowest geometric mean. (Central column) The blue line is the difference between the rank ordered true data and the mean label-permuted data. Dashed red lines indicate 95% permuted data confidence intervals under the null hypothesis that expression is independent of membership label. (Right column) The vertical red line indicates the location of the data statistic and, with a null hypothesis as in Fig. 3C (top panel) or Fig. 3D (bottom panel), the histogram shows the density of the same statistic determined for 250,000 uniformly-at-random permuted assignments of clones to environment. The lower one-sided p-value is depicted in legend.