CD8+ T cells that respond to chronic viral infections or cancer are characterized by the expression of inhibitory receptors such as programmed cell death protein 1 (PD-1) and by the impaired production of cytokines. This state of restrained functionality—which is referred to as T cell exhaustion—is maintained by precursors of exhausted T (TPEX) cells that express the transcription factor T cell factor 1 (TCF1), self-renew and give rise to TCF1- exhausted effector T cells. Here we show that the long-term proliferative potential, multipotency and repopulation capacity of exhausted T cells during chronic infection are selectively preserved in a small population of transcriptionally distinct CD62L+ TPEX cells. The transcription factor MYB is not only essential for the development of CD62L+ TPEX cells and maintenance of the antiviral CD8+ T cell response, but also induces functional exhaustion and thereby prevents lethal immunopathology. Furthermore, the proliferative burst in response to PD-1 checkpoint inhibition originates exclusively from CD62L+ TPEX cells and depends on MYB. Our findings identify CD62L+ TPEX cells as a stem-like population that is central to the maintenance of long-term antiviral immunity and responsiveness to immunotherapy. Moreover, they show that MYB is a transcriptional orchestrator of two fundamental aspects of exhausted T cell responses: the downregulation of effector function and the long-term preservation of self-renewal capacity.
Fig. 1 | CD62L marks transcriptionally distinct and functionally superior TPEX cells during chronic infection. a–c, Naive wild-type mice were infected with LCMV-Cl13 and TPEX-cell-enriched (PD-1+ Tim-3+) CD8+ T cells were sorted and subjected to scRNA-seq at 30 dpi. The resulting data were combined with publicly available scRNA-seq datasets from mouse exhausted CD8+ T cells11,19 and analysed. a, Uniform manifold approximation and projection (UMAP) plot of 15,743 single exhausted T cells coloured according to cluster classification. b, Normalized gene expression of Tcf7, Sell, Gzmb and Cx3cr1 projected onto the UMAP. c, Heat map showing the expression of all identified cluster signature transcripts. d, Congenically marked naive P14 cells were transferred into recipient mice, which were subsequently infected with LCMV-Docile and analysed at 21 dpi. Flow cytometry plots show the expression of PD-1, Ly108 and CD62L in splenic P14 T cells. e, UMAP plot showing two predicted developmental trajectories generated using Slingshot analysis. Cells are colour-coded on the basis of pseudotime prediction. f–k, Congenically marked naive P14 T cells were transferred into primary recipient (R1) mice, which were then infected with LCMV-Cl13. The indicated subsets of P14 T cells were sorted at 28 dpi and $3 \times 10^4$ cells were re-transferred to infection-matched secondary recipient (R2) mice. Splenic P14 T cells of R2 mice were analysed at day 21 after re-transfer. f, Schematic of the experimental set-up. g, Flow cytometry plots (g) and cell numbers (h) of recovered progenies at day 21 after re-transfer (gated on CD4+ CD19+ cells). i–k, Flow cytometry plots (i), numbers (j) and average percentages (k) of recovered CD62L+ TPEX, CD62L+ TPEX and TEX cells per spleen in R2 mice. Cells were gated on P14 cells (day 21 after re-transfer). Dots in graphs represent individual mice (i,h,j); horizontal lines and error bars of bar graphs indicate mean and s.e.m., respectively. Data are representative of at least two independent experiments. P values are from Mann–Whitney tests (h,j); $P > 0.05$, not significant (NS).
TCR signals\textsuperscript{1,14}, both CD62L\textsuperscript{+} and CD62L\textsuperscript{−} TPEX cells expressed higher levels of the TCR-induced transcriptional regulator NUR77 than TPEX cells (Extended Data Fig. 1k–p). There were no major differences in cytokine production between the two TPEX subsets, but IFNγ cells were enriched among CD62L\textsuperscript{+} TPEX cells (Extended Data Fig. 2a,b). CD62L\textsuperscript{−} TPEX cells were also found among endogenous gp33-specific and among polyclonal antigen-responsive PD-1\textsuperscript{+} CD8\textsuperscript{T} T cells in LCMV-Docile-infected mice (Extended Data Fig. 2c–e). Notably, CD62L\textsuperscript{−} TPEX cells were transcriptionally distinct from both naive and memory T cells derived from acute LCMV infection (Extended Data Fig. 2f,g).

Sloping analysis of our scRNA-seq data revealed a developmental trajectory that began with CD62L\textsuperscript{+} TPEX cells and progressed into CD62L\textsuperscript{−} TPEX cells, from which it bifurcated into either CX3CR1\textsuperscript{+} or CX3CR1\textsuperscript{−} TPEX cells (Fig. 1e). Similar results were obtained when we sorted P14 TPEX cells based on a Tcf7\textsuperscript{GFP} reporter from LCMV-Cl13-infected mice and performed scRNA-seq followed by RNA velocity analysis (Extended Data Fig. 2h–j). Overall, these data suggest a one-way developmental trajectory that originates from CD62L\textsuperscript{+} TPEX cells. To test this model experimentally, we sorted CD62L\textsuperscript{+}, CD62L\textsuperscript{−}, and P14 cells on day 28 after infection with LCMV-Cli3, separately re-transferred them into congenically marked infection-matched hosts and analysed this three weeks later (Fig. 1f–k). Compared with CD62L\textsuperscript{+} TPEX and TPEX cells, CD62L\textsuperscript{−} TPEX cells showed a superior repopulation capacity (Fig. 1g,h) and were able to efficiently self-renew and give rise to both CD62L\textsuperscript{+} TPEX and TPEX cells (Fig. 1i–k). These characteristics were maintained even after repetitive adoptive transfers (Extended Data Fig. 3a–f). By contrast, the few CD62L\textsuperscript{−} TPEX cells that were detected in the P14 compartment (around 1–2%) did not expand or generate progeny efficiently (Extended Data Fig. 3g–i).

We confirmed the superior developmental properties of CD62L\textsuperscript{+} TPEX cells using single T cell transfer and fate-mapping via retrogenic colour barcoding\textsuperscript{22–24} (Extended Data Fig. 4). Notably, single CD62L\textsuperscript{+} TPEX cells exhibited self-renewal and multipotent repopulation capacity, akin to single naive T cells (Extended Data Fig. 4a–h). In line with the epigenetic imprint of exhaustion\textsuperscript{20–29}, progeny derived from single CD62L\textsuperscript{+} TPEX cells maintained high levels of PD-1 expression compared to their naive-derived counterparts (Extended Data Fig. 4d,g,i). The CD62L\textsuperscript{−}TPEX linked developmental hierarchy uncovered here is unrelated to previously proposed TPEX cell subsets based on differential CD69 expression\textsuperscript{20} (Extended Data Fig. 5a–j). Together, these results show that CD62L\textsuperscript{+} TPEX cells represent a transcriptionally distinct population with stem-like developmental capacity that maintains the responses of exhausted CD8\textsuperscript{T} T cells during chronic infection.

**MYB governs exhausted T cell function and longevity**

Functional annotation of our scRNA-seq data identified Myb, encoding the transcription factor MYB (also called c-Myc), as specifically enriched among CD62L\textsuperscript{+} TPEX cells (Fig. 2a,b and Supplementary Table 1). MYB has important roles in the self-renewal of haematopoietic stem cells and cancer cells\textsuperscript{30}, T cell leukaemia\textsuperscript{31} and CD8\textsuperscript{T} memory T cells\textsuperscript{32,33}. To characterize the dynamics of Myb expression in chronic infection, we infected Myb\textsuperscript{fl/fl} reporter mice\textsuperscript{14} with LCMV-Docile (Fig. 2c and Extended Data Fig. 5k), and found that the expression of Myb was highest in CD62L\textsuperscript{+} TPEX cells (Fig. 2c and Extended Data Fig. 5k). Myb expression in CD8\textsuperscript{T} T cells responding to LCMV-Docile infection was significantly higher than in those responding to LCMV-Armstrong infection (Extended Data Fig. 5i), and was further enhanced by the inhibition of PD-1 signalling in vivo (Extended Data Fig. 5m–o). Moreover, in vitro TCR stimulation induced the expression of Myb in a dose-dependent manner (Extended Data Fig. 5p). Finally, the proportions of CD62L\textsuperscript{+} antigen-specific CD8\textsuperscript{T} T cells were 10-fold higher in LCMV-Docile versus LCMV-Armstrong infection (Extended Data Fig. 5q,r). Together, these data indicate that strong and persistent TCR stimulation favours the sustained expression of MYB and retention of CD62L\textsuperscript{+} TPEX cells during chronic infection.

To study the role of MYB in CD8\textsuperscript{T} T cells during viral infection, we infected Myb\textsuperscript{fl/flCd4cre} mice\textsuperscript{14} (which lack MYB specifically in T cells) and Myb\textsuperscript{fl/fl}control littersmates with LCMV-Docile or LCMV-Armstrong (Fig. 2d,f). Before infection, Myb\textsuperscript{fl/flCd4cre} mice showed no major abnormalities in the thymic and mature CD8\textsuperscript{T} cell compartments (Extended Data Fig. 6). LCMV-Armstrong-infected Myb\textsuperscript{fl/flCd4cre} mice mounted CD8\textsuperscript{T} cell responses that were similar to those of controls, and showed no overt signs of disease (Fig. 2e,f and Extended Data Fig. 7a–d). By contrast, LCMV-Docile-infected Myb\textsuperscript{fl/flCd4cre} but not control mice exhibited signs of severe immunopathology and most became moribund within 10 dpi (Fig. 2g and Extended Data Fig. 7e–i). Depletion of CD8\textsuperscript{T} T cells averted these symptoms and protected LCMV-Docile-infected Myb\textsuperscript{fl/flCd4cre} mice (Extended Data Fig. 7j,k), indicating that MYB-deficient CD8\textsuperscript{T} T cells mediated the fatal immunopathology in chronic LCMV infection. In line with these findings, splenic gp33\textsuperscript{+} CD8\textsuperscript{T} T cells accumulated at increased frequencies in Myb\textsuperscript{fl/flCd4cre} mice at 8 dpi (Fig. 2h).

MYB-deficient TPEX and TPEX cells expressed significantly higher levels of IFNγ and TNF, whereas TPEX cells also expressed more granzyme B and underwent increased proliferation (measured by the expression of Ki67) compared to controls (Fig. 2i and Extended Data Fig. 7l–o), despite the viral titres being similar in both groups of mice (Extended Data Fig. 7p). MYB-deficient gp33\textsuperscript{+} CD8\textsuperscript{T} T cells showed increased expression of the inhibitory receptors PD-1 and TIM-3 compared to control cells (Extended Data Fig. 7q), which suggests that the increased function and proliferation of effector cells was not due to impaired expression of inhibitory receptors. Notably, CD62L\textsuperscript{−} TPEX cells were specifically lost in the absence of MYB (Fig. 2j) and Extended Data Fig. 7r,s).

To longitudinally examine the cell-intrinsic role of MYB in the absence of potentially confounding immune pathology, we generated mixed bone marrow chimeric mice that contained small numbers of Myb\textsuperscript{fl/flCd4cre} (10–20%) and congenically marked control CD8\textsuperscript{T} T cells and infected them with LCMV-Docile (Fig. 2k–n and Extended Data Fig. 8). Consistent with our observations in non-chimeric mice, MYB-deficient antigen-specific CD8\textsuperscript{T} T cells proliferated more and exhibited increased expression of effector molecules compared to controls (Extended Data Fig. 8a–e). Similarly, the MYB-deficient CD8\textsuperscript{T} T cell compartment was devoid of CD62L\textsuperscript{−} TPEX cells (Extended Data Fig. 8f,g). Although MYB-deficient TCF1\textsuperscript{+} TPEX cells initially developed, they were poorly maintained (Fig. 2l and Extended Data Fig. 8h–j). This observation concurred with a premature termination of cell-cycle activity in MYB-deficient TPEX and TPEX cells and a marked contraction of the entire antigen-specific compartment (Fig. 2m,n and Extended Data Fig. 8k–n).

Similar results were obtained from adoptively transferred MYB-deficient and control P14 T cells (Extended Data Fig. 9a–g). Thus, MYB mediates the development of CD62L\textsuperscript{+} TPEX cells and functional exhaustion of CD8\textsuperscript{T} T cells during the acute phase, sustains long-term proliferative capacity and prevents the attrition of antigen-specific T cells during the chronic phase of infection.

**MYB orchestrates exhausted T cell transcription**

We next sorted MYB-deficient and control P14 TPEX cells from LCMV-Docile-infected mice and performed transcriptional profiling by RNA-seq (Extended Data Fig. 10a). The analysis showed that there was a loss of the CD62L\textsuperscript{+} TPEX cell signature in MYB-deficient compared with control TPEX cells (Extended Data Fig. 10b), confirming that MYB deficiency resulted in the loss of CD62L\textsuperscript{−} TPEX cells and not merely CD62L\textsuperscript{+} TPEX expression. We next performed RNA-seq of control and MYB-deficient P14 TPEX cells and TPEX cells sorted for differential expression of CD62L\textsuperscript{+} (Fig. 3a). The analysis revealed transcriptional divergence between all subsets and identified S84 differentially expressed genes (P < 0.05) between control CD62L\textsuperscript{−} and CD62L\textsuperscript{+} TPEX cells (Fig. 3b, Extended Data Fig. 10c and Supplementary Table 2). CD62L\textsuperscript{−} TPEX cells expressed higher levels of transcripts that encode molecules related to lymph node homing (for example, Sell, Ccr7 and Slpr1), and higher levels of the cell-cycle...
Fig. 2 | The transcription factor MYB is required for the generation of CD62L+ TPEX cells and the functional exhaustion of T cells during chronic infection. a, Normalized gene expression of Myb projected onto the UMAP plot. b, Violin plots showing normalized expression of Sell and Myb. c, Myb reporter mice were infected with LCMV-Docile and splenic CD8+ T cells were analysed at the indicated time points after infection. Left, representative flow cytometry plots showing the expression of CD62L and Myb–GFP among naive (CD44hi) and gp33+ CD8+ T cells. Right, quantification showing the geometric mean fluorescence intensity (GMFI) of Myb–GFP among CD62L+ TPEX, CD62L− TPEX and TRES cells as fold change over naive CD8+ T cells. d–h, Mixed bone marrow chimeric mice containing Myb–cKO and Cd44–GFP control T cells were infected with either LCMV-Armstrong (LCMV-Arm) or LCMV-Docile (LCMV-Doc). d, Schematic of the experimental set-up. e–h, Flow cytometry plots and quantification showing the expression of IFNγ and TNF after gp33 peptide restimulation (i) and the frequencies of CD62L+ TRES cells (j) (Myb–cKO (n = 8); Ctrl (n = 8)). Cells were gated on gp33+ cells; 8 dpi. k, Mixed bone marrow chimeric mice containing Myb–cKO and Cd44–GFP control T cells were infected with LCMV-Docile and analysed at the indicated time points. k, Schematic of the experimental set-up. i–n, Quantifications show the frequencies of gp33+ TRES cells (l), Kif14+ cells (m) and gp33+ cells (n). Dots represent individual mice; symbols and error bars represent mean and s.e.m., respectively; box plots indicate minimum and maximum values (whiskers), interquartile range (box limits) and median (centre line). Data are representative of all analysed mice (e–g), two (c, f, j, l–n) or three independently performed experiments (h). P-values are from two-tailed unpaired t-tests (c, f, h–j) and Mann–Whitney tests (i–n).

Inhibitors Cdkn1b and Cdkn2d and the quiescence factors Kif2 and Kif3, compared with CD62L TPEX cells. Genes that were upregulated in CD62L TPEX cells included those that encode positive cell-cycle regulators (E2f1, Cdc6, Skp2, Cdc25a and Kif14), metabolic enzymes (P2rx7, Hk2, Pfkm, Pkm and Gpd2) and nutrient transporters (Slc7a5, Slc19a2 and Slc25a10) (Extended Data Fig. 10c and Supplementary Table 2). A comparison of MYB-deficient and control CD62L TPEX cells identified 580 differentially expressed genes (Supplementary Table 2), including genes that encode molecules related to T cell exhaustion and TPEX Cell identity (Lef1, Eomes, Cita2a, Ifr4, Klf2, Ntsf and Cd160), cell-cycle regulation and stem cell renewal (E2f1, Rbl2, Klf14, Cdc25b, Bmp7 and Wnt3) (Fig. 3c). Consistent with the impaired expression of transcripts related to cell migration and lymph node homing (Ccr7, Cxcr5, Slpr1, Itgb1 and Itgb3), MYB-deficient antigen-specific CD8+ T cells were largely excluded from the lymph nodes (Extended Data Fig. 10d,e). We also observed increased expression of Kit—encoding KIT, which is involved in haematopoiesis and T cell activation in MYB-deficient versus wild-type TPEX cells (Fig. 3c). Indeed, KIT was exclusively expressed in CD62L TPEX cells and was highly upregulated in MYB-deficient TPEX cells (Extended Data Fig. 10f,g). A comparison of MYB-deficient and control TPEX cells revealed further significant transcriptional changes (1,532 differentially expressed genes), including the upregulation of transcripts that encode cytotoxic molecules (Gzma, Gzmc and Gzme) or that are related to terminal exhaustion transcripts (C3, Cd244a, Cd160, Entpd1, Id2 and Cd101), and the downregulation of transcripts related to CX3CRI T RES cells, which have been shown to be more effective in controlling viral burden compared to their CX3CRI counterparts (Extended Data Fig. 10h–j and Supplementary Table 2). Flow cytometric analysis revealed a lack of CX3CRI+ cells and an increase in terminally exhausted CD101+ cells among MYB-deficient T RES cells compared to controls (Extended Data Fig. 10k–l). Consistent with accelerated differentiation into terminally differentiated cells, T RES–Cell-related transcripts, including Tcf7, Slamf6, Lef1 and Xcl1, were more strongly
downregulated in MYB-deficient than in control TEX cells (Extended Data Fig. 10h,j). Many of the genes that were dysregulated in the absence of MYB, including Tcf7, Kit, Slamf6, Lef1, Klf2, Slpr1, Icos, E2f1, Gzma, Gzmrc and Myb itself, contained MYB-binding regions in human T cells (Supplementary Table 3) and were conserved and aligned with open chromatin regions in mouse exhausted T cells (Fig. 3e,f, Extended Data Fig. 11a–d and Supplementary Table 3). Together, our results show that MYB is a central transcriptional orchestrator of T cell exhaustion that mediates the development of CD62L+ TPEX cells and restrains the terminal differentiation of exhausted T cells.

**CD62L+ TPEX cells fuel therapeutic reinigoration**

To test the functional potential of MYB-dependent CD62L+ TPEX cells, we separately transferred CD62L+ and CD62L− TEX cells as well as TEX cells into either wild-type (Extended Data Fig. 11e–g) or T-cell-deficient Tcra−/− mice (Fig. 4a,b), which were then infected with LCMV-Armstrong. Although all subsets maintained high expression of PD-1 (Extended Data Fig. 11f,g), progeny that were derived from CD62L+ TPEX cells expanded more efficiently (Fig. 4b and Extended Data Fig. 11f,g), contained more KLRG1+ effector cells and provided significantly enhanced viral control compared to the other exhausted T cell subsets (Fig. 4b). CD62L+ TPEX cells also gave rise to more CX3CR1+ TEX cells (Extended Data Fig. 11h,j), altogether indicating that they have a superior potential to generate functional effector cells as compared to their CD62L− counterparts. We next tested the role of PD-1 and therapeutic PD-1 checkpoint blockade in the generation and function of CD62L+ TPEX cells. To this end, we generated P14 T cells that lack functional Pdcd1 (encoding PD-1) using CriespR–Cas9 (Extended Data Fig. 12a–f). Similar to previous studies (39–41), PD-1-deficient P14 T cells exhibited increased clonal expansion in response to LCMV-Docile, as compared with control cells (Extended Data Fig. 12b). Although the frequencies of TPEX and TEX cells were unaffected by the loss of PD-1 (Extended Data Fig. 12c), the frequencies—but not the numbers—of CD62L+ TPEX cells were markedly decreased compared to control P14 T cells (Extended Data Fig. 12d). This was due to a concurrent increase in the proportions and the absolute numbers of Kit+ TPEX cells and TEX cells (Extended Data Fig. 12e,f). These results indicate that PD-1 signalling does not affect the development or maintenance of CD62L+ TPEX cells but limits their differentiation into CD62L− TEX cells. In line with this conclusion, the numbers of CD62L+ TEX cells remained stable during PD-1 checkpoint inhibition, whereas the overall population of antigen-responsive PD-1− CD8+ T cells expanded robustly (Extended Data Fig. 12g–p). To directly test the role of CD62L+ TPEX cells in checkpoint blockade, we performed adoptive transfer
Exhausted T cell subsets were sorted at 28 dpi and 1.0 × 10^4–2.5 × 10^4 cells or no therapy. Together, our results reveal that MYB-dependent CD62L+ TPEX cells, did not expand in response to PD-1 checkpoint inhibition in response to checkpoint blockade. Consistent with this conclusion, no apparent proliferative response (Fig. 4d,e), which indicates that experiments in the context of therapeutic PD-1 inhibition (Fig. 4c–e). Re-transferred CD62L+ TPEX cells proliferated strongly in response to PD-1 checkpoint inhibition and generated larger progenies compared with untreated controls, while undergoing concurrent self-renewal (Fig. 4d,e). In stark contrast, both CD62L- TPEX and TEx cells showed no apparent proliferative response (Fig. 4d,e), which indicates that CD62L+ but not CD62L- TPEX cells fuel the generation of effector cells in response to checkpoint blockade. Consistent with this conclusion, MYB-deficient antigen-responsive PD-1+ CD8+ T cells, which lack CD62L+ TPEX cells, did not expand in response to PD-1 checkpoint inhibition (Fig. 4f–h). Together, our results reveal that MYB-dependent CD62L+ TPEX cells exclusively fuel the proliferative burst in response to PD-1 checkpoint inhibition and therefore dictate the success of therapeutic checkpoint blockade.

Overall, our data show that the CD8+ T cell response in chronic infection is maintained by a small population of distinct TPEX cells that co-express TCF1, CD62L and the transcription factor MYB. These cells, which we term stem-like exhausted T (T_{SLEX}) cells here, possess superior self-renewal, multipotency and long-term proliferative capacity compared to their TCF1+ but CD62L- descendants. Loss of MYB abrogated the differentiation of T_{SLEX} cells and severely impaired the persistence of the entire TCF1+ TPEX cell compartment, ultimately resulting in the collapse of the complete CD8+ T cell response. MYB also mediates functional exhaustion during chronic infection by restricting the initial expansion and effector function of antigen-responsive CD8+ effector T cells. As a result, mice that lacked MYB in their T cells succumbed to chronic but not acute viral infection, highlighting that T cell exhaustion is an essential adaptation to chronic infection. Thus, MYB represents a transcriptional checkpoint that instructs the differentiation and function of CD8+ T cells in response to severe or chronic infection. Our data also show that T_{SLEX} cells are exclusively required to mediate the response to PD-1 checkpoint inhibition. These findings not only advance our understanding of the mechanisms of T cell re-invigoration in the context of checkpoint inhibition, but also emphasize the need for new therapeutic strategies that target T_{SLEX} cells to harness the full potential of T cell-mediated immunotherapy. Furthermore, the superior proliferative and developmental potential of T_{SLEX} cells makes them prime targets of adoptive T cell transfer and chimeric antigen receptor (CAR) T cell therapies. Finally, our results show that two central but seemingly unrelated properties of exhausted T cells—limited function and longevity—are intimately linked by a single transcription factor MYB; this is a notable example of evolutionary parsimony, which ensures ongoing T cell immunity during chronic infection while preventing collateral damage to the host.
1. Hashimoto, M. et al. CD8 T cell exhaustion in chronic infection and cancer: opportunities for interventions. Annu. Rev. Med. 69, 301–318 (2018).
2. Mclane, L. M., Abdel-Hakeem, M. S. & Wherry, E. J. CD8 T cell exhaustion during chronic viral infection and cancer. Annu. Rev. Immunol. 37, 457–495 (2019).
3. Im, S. J. et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature 537, 417–423 (2016).
4. Kallies, A., Zehn, D. & Utschneider, D. T. Precursor exhausted T cells: key to successful immunotherapy? Nat. Rev. Immunol. 20, 128–136 (2020).
5. Utschneider, D. T. et al. T Cell factor 1-expressing memory-like CD8+ T cells sustain the immune response to chronic viral infections. Immunity 45, 415–427 (2016).
6. Wu, T. et al. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and prevent T cell death. Cell Rep. 18, 109–120 (2017).
7. Siddiqui, J. et al. Intratumoral Tcf1+PD-1+CD8+ T cells with stem-like properties promote tumor cell growth and induce programmed cell death. Cancer Immunol. Res. 7, 506–516 (2019).
8. Gabriel, S. S. et al. Transforming growth factor-β-regulated mTOR activity preserves cellular metabolism to maintain long-term T cell responses in chronic infection. Immunity 60, 212–227 (2022).
9. Miller, B. C. et al. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. Nat. Immunol. 20, 326–336 (2019).
10. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat. Rev. Immunol. 20, 651–668 (2020).
11. Utschneider, D. T. et al. Early precursor T cells establish and propagate T cell exhaustion in chronic infection. Nat. Immunol. 21, 1256–1266 (2020).
12. Alfei, F. et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. Nature 571, 265 (2019).
13. Chen, Z. et al. Transforming growth factor β-regulated mTOR activity preserves cellular metabolism to maintain long-term T cell responses in chronic infection. Immunity 54, 1698–1714 (2021).
14. Graef, F. et al. Serial transfer of single-cell-derived immune competence reveals stemness of CD8+ central memory T cells. Immunity 41, 116–126 (2014).
15. Grassmann, S. et al. Distinct surface expression of activating receptor Ly49H drives differential expansion of NK cell clones upon murine cytomegalovirus infection. Immunity 50, 1389–1400 (2019).
16. Grassmann, S. et al. Early emergence of T central memory precursors programs clonal dominance during chronic viral infection. Nat. Immunol. 21, 1563–1573 (2020).
17. Abdel-Hakeem, M. S. et al. Epigenetic marking of exhausted T cells hinders memory differentiation upon eliminating chronic antigenic stimulation. Nat. Immunol. 22, 1008–1019 (2021).
18. Yates, K. B. et al. Epigenetic scars of CD8+ T cell exhaustion persist after cure of chronic infection in humans. Nat. Immunol. 22, 1020–1029 (2021).
19. Beltra, J.-C. et al. Developmental relationships of exhausted CD8+ T cell subsets reveal underlying transcriptional and epigenetic landscape control mechanisms. Immunity 52, 825–841.e8 (2020).
20. Ramsay, R. G. & Gonda, T. J. Myb function in normal and cancer cells. Nat. Rev. Cancer 8, 523–534 (2008).
21. Lahortiga, I. et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat. Genet. 39, 593–595 (2007).
22. Chen, Z. et al. miR-150 regulates memory CD8 T cell differentiation via c-Myb. Cell Rep. 20, 2584–2597 (2017).
23. Gautham, S. et al. The transcription factor c-Myb regulates CD8+ T cell stemness and antitumor immunity. Nat. Immunol. 20, 337–349 (2019).
24. Dias, S. et al. Effecter regulatory T cell differentiation and immune homeostasis depend on the transcription factor Myb. Immunity 46, 78–91 (2017).
25. Emambokus, N. et al. Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. EMBO J. 22, 4478–4488 (2003).
26. Edling, C. E. & Hallberg, B. c-Kit—a hematopoietic cell essential receptor tyrosine kinase. Int. J. Biochem. Cell Biol. 39, 1995–1998 (2007).
27. Frumento, G. et al. CD117 (c-Kit) is expressed during CD8+ T cell priming and stratifies sensitivity to apoptosis according to strength of TCR engagement. Front. Immunol. 10, 468 (2019).
28. Mansour, M. R. et al. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science 346, 1373–1377 (2014).
29. Frebel, H. et al. Programmed death 1 protects from fatal circulatory failure during systemic virus infection of mice. J. Exp. Med. 209, 2485–2499 (2012).
30. Odonzor, P. M., Pauken, K. E., Paley, M. A., Sharpe, A. & Wherry, E. J. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8 T cells. J. Exp. Med. 212, 1125–1137 (2015).
31. Nüssing, S. et al. Efficient CRISPR/Cas9 gene editing in uncultured naive mouse T Cells for in vivo studies. J. Immunol. 204, 2308–2315 (2020).

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Methods

Mice and generation of mixed bone marrow chimeric mice
All mice used in this study were on a C57BL/6J background. Age- and sex-matched mice were used for experiments and allocated to experimental groups without further randomization or blinding. CD45.1 or CD45.2 mice were obtained from the Australian Resources Centre or were purchased from Envigo at 6–8 weeks of age. Id3<sup>−/−</sup> mice expressing the P14 TCR transgene (JAX: Tg(TcrLCMV)327Sdz) were used in some experiments as described. <sup>11</sup> Myb<sup>−/−</sup> mice and Myb<sup>−/−</sup>CD<sup>4<sup>+</sup></sup> mice were sorted previously. <sup>13</sup> Myb<sup>−/−</sup>CD<sup>4<sup>+</sup></sup>CD<sup>44<sup>−</sup></sup> mice were crossed to include the P14 TCR transgene for some experiments. Littermate Myb<sup>−/−</sup> mice were used as controls. Mixed bone marrow chimeric mice were generated by irradiating CD45.1 host mice (2 × 5.5 Gy), before reconstitution with a mix of CD45.1/CD45.2 CD<sup>4<sup>+</sup></sup> bone marrow and CD45.2 CD<sup>4<sup>−</sup></sup>CD<sup>44<sup>−</sup></sup> bone marrow. Mice were left to recover for 6 to 8 weeks before further experiments. P14 transgenic Tcf7<sup>gfp</sup> mice on a CD45.1 background were generated in the laboratory of D.Z. by inserting a GFP expression construct into the Tcf7 gene locus and will be described in detail elsewhere. P14 mice expressing diverse combinations of the congenic markers CD45.1/2 and Thy1.1/2, as well as Tcra<sup>−/−</sup> mice were bred under specific-pathogen-free conditions at the mouse facility of the Institute for Medical Microbiology, Immunology and Hygiene at the Technical University of Munich. All mice were maintained and used in accordance with the guidelines of the University of Melbourne Animal Ethics Committee or the district government of upper Bavaria (Department 5—Environment, Health and Consumer Protection).

Generation of colour-barcoded P14 cells
Retrogenic colour-barcoding was used to heritably label individual P14 cells and their progeny for in vivo single-cell fate-mapping experiments, as previously described. <sup>24,25</sup> In brief, bone marrow was collected from congenically marked (CD45.1<sup>+</sup> or CD90.1<sup>+</sup>) P14 donor mice and stained with Ly6A/E (<i>Sca-1</i>), anti-mouse CD3 and CD19 antibodies, together with propidium iodide for live or dead discrimination. Haematopoietic stem cells (HSCs) were then sorted as live CD3<sup>−</sup>CD19<sup>−</sup>Sca-1<sup>+</sup> cells and cultured at 37 °C in cDMEM (DMEM [Life Technologies], supplemented with 10% FCS, 0.025% l-glutamine, 0.1% HEPES, 0.1% gentamicin and 1% penicillin/streptomycin), supplemented with 20 ng ml<sup>−1</sup> mouse IL-3, 50 ng ml<sup>−1</sup> mouse IL-6 and 50 ng ml<sup>−1</sup> mouse SCF, for three to four days in tissue-culture-treated 48-well-plates. Expanded stem cells were then retrovirally transduced with constructs encoding the fluorescent proteins GFP, YFP, BFP, CFP and T-Sapphire by spinoculation. After two days in culture, the transduced HSCs were then sorted according to their unique congenic or retrogenic colour barcode and their CD62L/Ly108 phenotype into a 96-well V-bottom plate containing a pellet of 4 × 10<sup>5</sup> C57BL/6 splenocytes. The unique congenic and retrogenic colour barcodes of sorted cells enabled the simultaneous transfers of multiple individual cells for fate-mapping. After sorting, the whole content of each well was injected into separate C57BL/6 recipients.

Gene deletion by CRISPR – Cas9–sgRNA complex electroporation
Pdcd1 gene deletion was conducted as reported previously. <sup>41</sup> In brief, P14 cells were purified using an EasySep mouse CD8<sup>+</sup> T cell isolation kit (STEMCELL Technologies) according to the manufacturer’s instructions, after which cells were electroporated (Pulse DN100) with a complex of Alt-R S.p. Cas9 Nucleic (Integrated DNA Technologies) and a previously described Pdcd1-targeting sgRNA (Synthego) <sup>40</sup> using the P3 primary cell 4D-Nucleofector X kit (Lonza). Mice were rested in fully supplemented RPMI medium (see above) at 37 °C for 10 min, after which P14 cells were counted, and 5,000 P14 cells were injected intravenously into recipient mice before infection with LCMV.

LCMV infections and checkpoint blockade
LCMV-Docile, LCMV-CI13 and LCMV-Armstrong were propagated and quantified as previously described. <sup>26</sup> For LCMV-Docile and LCMV-CI13 infection, frozen stocks were diluted in PBS and 2 × 10<sup>5</sup> PFU were injected intravenously. For LCMV-Armstrong infection, frozen stocks were diluted in PBS and 2 × 10<sup>6</sup> PFU were injected intraperitoneally. For infection of Tcra<sup>−/−</sup> mice, a dosage of 2 × 10<sup>5</sup> PFU was used. For CD4<sup>+</sup> T cell depletion, mice were injected twice intraperitoneally with 200 μg per mouse of anti-CD4 monoclonal antibody (GK1.5, BioXCell) on days 1, 3 and 5 of infection. For PD-1 blockade, monoclonal anti-PD-1 antibodies (B7-HL, BioXCell) were injected intraperitoneally at 200 μg per mouse at the specified days after infection.

In vitro culture of naive CD8<sup>+</sup> T cells
Cell-culture 48-well or 96-well plates were prepared by coating with anti-CD3 at various concentrations for at least 2 h at 4 °C. Control wells were coated with PBS for the same duration. The wells were washed twice using PBS. Enriched naive CD8<sup>+</sup> T cells were seeded in the wells and were cultured in RPMI medium supplemented with 10% FCS,
55 μM β-mercaptoethanol, 2 mM Glutamax, 25 mM HEPES buffer and 100 U ml⁻¹ penicillin and 10 μg ml⁻¹ streptomycin for three days in a humidified incubator at 37 °C with 5% CO₂.

**Surface and intracellular antibody staining of mouse cells**

Surface staining was performed for 30 min at 4°C in PBS supplemented with 2% FCS (FACS buffer) with the following antibodies: CD8α (53-6.7, BD), CD44 (IM7, BD), CD45.1 (A20, BD or Biologend), CD45.2 (104, BD), CD90.1 (HS52, Thermo Fisher Scientific) CX3CR1 (SA011F11, Biologend), PD-1 (RMP1-30 or 29F.1A2, Biologend), CD62L (MEL-14, Biologend), TIM-3 (RMT7-23, Biologend), CD103 (Moushil101, Thermo Fisher Scientific), Ly108 (eBio13G3-18D, BD), CD117 (KIT) (ACK2, Thermo Fisher Scientific), CD244 (2B4) (eBio244F4, Thermo Fisher Scientific), CD160 (eBioCXN46-3, Bioscience), TGF (GID7, Thermo Fisher Scientific) and KLRG1 (2F.1, Biologend). LCMV derived D³/gp33-41 tetramers were obtained from the NIH Tetramer Facility; tetramer staining was performed for 30–60 min at 4°C in FACS buffer. Each cell staining reaction was preceded by a 10-min incubation with purified anti-mouse CD16/32 Ab (FcγRII/III block; 2.4G2) and (fixable) viability dye (Thermo Fisher Scientific).

For intracellular cytokine staining, splenocytes were ex vivo restimulated with gp33-41 (gp33) peptide (5 μM) for 5 h in the presence of brefeldin A (Sigma) for the last 4.5 h, fixed and permeabilized using the Cytofix/Cytoperm (BD) or transcription factor staining kit (Bioscience) and stained with anti-IFNγ (XMG1.2, Thermo Fisher Scientific), Tnf (MP6-XT22, Thermo Fisher Scientific). Other intracellular staining was performed with the Foxp3 transcription factor staining kit (Bioscience) and the following antibodies: TCF1 (C63D9, Cell Signaling), GZMB (MHGB04, Thermo Fisher Scientific) and Ki67 (FM264G, BD).

**In vitro activation of T cells**

CD8⁺ T cells were isolated using the CD8⁺ T cell enrichment kit (Miltenyi Biotech) and, in some instances, CTV labelled. Wild-type cells were stimulated with plate-bound anti-CD3 at the indicated concentration and in fully supplemented tissue-culture medium (RPMI plus 10% FCS, 2 mM Glutamax, 1 mM pyruvate, 55 μM mercaptoethanol, 100 U ml⁻¹ penicillin, 10 μg ml⁻¹ streptomycin) and 100 U ml⁻¹ IL-2.

**Histology**

For immunofluorescence, spleens were embedded and frozen in OCT, sectioned at 15 μm and mounted on SuperFrostPlus Adhesion glass (Thermo Fisher Scientific). Sections were dehydrated using silica beads, fixed with 4% paraformaldehyde for 10 min and washed with PBS. Samples were blocked using 5% normal goat serum for 2 h before staining. Samples were incubated with antibodies against B220 (RA3-6B2, eBioscience), CD3 (17A2, eBioscience) and F4/80 (BM8, Biologend) diluted in 5% NGS for 2 h at room temperature in the dark. After staining, samples were washed with PBS at least three times. Samples were then mounted using ProLong Gold Antifade Mountant (Invitrogen) and imaged using an inverted LSM780 microscope (Carl Zeiss) and a plan apochromat 63× NA 1.40 oil-immersion objective (Carl Zeiss). For haematoxylin and eosin (H&E) staining, organs were collected and fixed in 10% formalin. Fixed samples were embedded in paraffin and sectioned at 10 μm, mounted on SuperFrostPlus Adhesion glass and stained using H&E. Mounted samples were imaged using a Nikon SMZ1270 Stereo Microscope. Imaging data were analysed using Fiji (ImageJ) software (NIH).

**scRNA-seq and analysis**

Relating to the dataset introduced in Fig. 1, TₚЄX cell-enriched CDS⁺ T cells were sorted as CD8⁺ PD⁻1 TIM⁻3⁻⁴⁺⁺ from the spleens of chronically infected mice (LCMV-CI3) using a FACSAria III (BD Biosciences). Afterwards, the single cells were encapsulated into droplets with the Chromium TM Controller (10X Genomics) and processed following the manufacturer’s specifications. Bead captured transcripts in all encapsulated cells were uniquely barcoded using a combination of a 16-bp 10X barcode and a 10-bp unique molecular identifier (UMI). The Chromium Single Cell 3’ Library & Gel Bead Kit v2 for the wild-type untreated sample or v3 for wild type treated with 200 μg of anti-PD-L1 antibody (10E9G2, BioXCell) for 24 h were used to generate cDNA libraries (10X Genomics) following the protocol provided by the manufacturer. Libraries were quantified by QubitTM 3.0 Fluometer (Thermo Fisher Scientific) and quality was checked using 2100 Bioanalyzer with High Sensitivity DNA kit (Agilent). For library sequencing the NovaSeq 6000 platform (51 Cartridge, Illumina) in 50-paired end mode was used. The sequencing data were demultiplexed using CellRanger software (v.2.0.2) and the reads were aligned to the mouse mm10 reference genome using STAR aligner. Aligned reads were used to quantify the expression level of mouse genes and generate the gene-barcode matrix. Subsequent data analysis was performed using Seurat R package (v.3.2)⁴³. The sequencing data are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE168282 (ref. ⁴³). For further analysis in this study, datasets GSM5133522 and GSM5133523 (ref. ¹⁹) were combined with another publicly available scRNA-seq dataset of mouse exhausted CD8⁺ T cells, accessed from GSM122712 (ref. ²⁹) and analysed using Seurat R package (v.3.2)⁴³. The 2,000 most variable genes were included for the anchoring process and used for downstream analysis to calculate principal components of log-normalized and scaled expression data. On the basis of the principal component analysis (PCA), a UMAP of the identified clusters was visualized. Cluster-specific genes were identified with the FindAllMarker function in Seurat with parameters min.pct = 0.25, logfc.threshold = 0.25. Trajectories were predicted using the Slingshot 1.4.0 package⁴⁴, using the function slingshot with default settings and starting with the CD62L⁺ T cells cell cluster. The functional annotation tool DAVID (LHRI) was used to interrogate gene sets to identify transcription factors of interest. Selected lists of genes were then further explored using enrichment analyses against existing RNA-seq-datasets³²,³⁰.

For the dataset used in Extended Data Fig. 2h–j, TₓЄX cells were sorted as live CD8⁺ PD⁻1 CD45.1⁺ TC⁻/F⁻ GFP⁻ cells from the spleens of chronically infected mice (LCMV-CI3, 28 dpi) using a MoFlo Astrios cell sorter (Beckman Coulter) and processed using the 10X Genomics technology, according to the manufacturer’s protocol (Chromium Single Cell 3 GEM v3 kit). Quality control was performed with a High Sensitivity DNA Kit (Agilent 5067-4626) on a Bioanalyzer 2100, as recommended in the protocol. Libraries were quantified with the Qubit dsDNA HS Assay Kit (Life Technologies Q32851). All steps were performed using RPT filter tips (Starlab) and LoBind tubes (Sigma). The library was sequenced with 20,000 reads per cell. Illumina paired-end sequencing was performed with 150 cycles on a Novaseq 6000. Annotation of the sequencing data was performed using CellRanger software (v.5.0.0) against the mouse reference genome GRCm38 (mm10-2020-A). All subsequent analysis was performed using SCAPNY (v.1.6)⁴⁶. After general pre-processing (less than 15% mitochondrial genes, regressing out cell cycle, filtering mitochondrial genes and total counts), the data were count-normalized per cell and logarithmized. RNA velocities were calculated using Velocyto⁴⁷ and analysed with scVelo⁴⁸. The sequencing data are available at the NCBI GEO (http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE205608.

**Bulk RNA extraction, sequencing and analysis**

Relating to the dataset in Extended Data Fig. 2f, g, the indicated subsets of exhausted P14 cells (CD62L⁻ TₓЄX, CD62L⁺ TₓЄX, TₓЄX, TₓЄX) were sorted from the spleen of chronically infected mice (LCMV-CI3) at 28 dpi. As a comparison, memory P14 cells were sorted from the spleen of LCMV-Armstrong-infected mice at 28 dpi according to the following phenotypes: CD62L⁺Ly108⁻ (CD62L⁺ ‘memory’), CD62L⁺Ly108⁻ (CD62L⁻ memory) and CD62L⁺Ly108⁻ (effector). In addition, naive P14 cells were
included for the analysis. RNA extraction from sorted P14 T cells was performed using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s instructions. Each sample group consisted of two to three biological replicates. Sequencing was performed on an Illumina Novaseq by Novogene, generating 150-bp paired-end reads. RNA-seq reads were aligned to the mouse reference genome GRCh38/mm10 using STAR (v.2.5.4). Read counts per gene locus were obtained with htseq-count (v.0.11.4). Statistical analysis was performed in R (v.3.6.3). Genes with total reads lower than 200 across all samples were excluded. Normalization and differential gene expression analysis was performed using DESeq2 (v.1.26.0). Batch effects were identified using sva (v.3.34.0) and subsequently modelled in the DESeq2 design formula. Genes were considered differentially expressed when they achieved an FDR of less than 0.05 and a log2-transformed fold change of greater than 1. The sequencing data are available at the NCBI GEO (http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE205608.

Relating to the data in Fig. 3 and Extended Data Fig. 10, RNA extraction from sorted P14 T cells was performed using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s instructions. Each sample consisted of two experimental replicates. All samples were sequenced on an Illumina NextSeq500 generating 80-bp paired-end reads. RNA-seq reads were aligned to the mouse reference genome GRCh38/mm10 using the Subread aligner (Rssubread v.2.2.6). Gene-level read counts were obtained by running featureCounts. A read count summarization program within the Rsubread package and the inbuilt Rssubread annotation that is a modified version of the NCBI RefSeq mouse (mm10) genome annotation build 38.1. Pseudogenes, or genes that did not meet a counts per million reads (CPM) cut-off of 0.5 in at least two libraries were excluded from further analysis. Read counts were converted to log2(CPM, quantile normalized and precision weighted with the voom function of the limma package after accounting for batch effects. A linear model was fitted to each gene, and the empirical Bayes moderated t-statistic was used to assess differences in expression. Raw P-values were adjusted to control the false discovery rate across all comparisons using the Benjamini–Hochberg procedure. Enrichment analysis of Gene Ontology (GO) terms on the differentially expressed genes was performed using the g:Profiler function within the limma package. Pathway enrichment against the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways on the differentially expressed genes was performed using the keggapi function also implemented in the limma package. Gene set enrichment analysis was performed using Gene Set Enrichment Analysis (GSEA) software (v.4.0.3).

**ChIP–seq analysis**

Previously published raw ChIP–seq data for the MYB transcription factor were downloaded from the NCBI GEO with accession number GSE59657. Reads were mapped to the human genome (GRCh38) using the align function in Rsubread (refs. 50–53). Peak calling was performed using Homer (v.4.11) with an FDR set to 1 x 10^-6. In brief, tags for the aligned libraries were first created using the makeTagsDirectory function within Homer then followed by peak calling using the ‘style’ factor parameter with called peaks annotated to the nearest genes. Overlap between differentially expressed genes from the RNA-seq data (mouse) and ChIP–seq data (human) was performed by first transforming the human genes associated with each annotated peak to their corresponding mouse homologues using information available in the Ensembl database through the bioMart database package (v.4.0.3).

**Analysis of evolutionary conservation**

Genomic conservation data for the human and mouse genomes were obtained from UCSC Genome Browser (https://genome.ucsc.edu). Annotated tracks of human ChIP data were manually aligned to annotated tracks of mouse ATAC data using conserved loci of 100 vertebrates against the human genome and conserved loci of 30 mammals against the mouse genome as reference points.

**Flow cytometry**

Flow cytometry was performed using a Fortessa or Cytoflex LX (Beckman Coulter) and sort purification was performed on a BD FACSAria Fusion or MoFlo Astrios (Beckman Coulter). All data were analysed using FlowJo 10 (Tree Star). Graphs and statistical analyses were done with Prism 7 (GraphPad Software).

**Statistics**

A paired or unpaired Student’s t-test (two-tailed), Welch’s t-test, Mann–Whitney U test or one-way ANOVA was used to assess significance. Statistical methods to predetermine sample size were not used.

**Data availability**

Most of the sequencing data generated for this study have been deposited in the NCBI GEO database with accession number GSE188526. The sequencing data shown in Extended Data Fig. 2f–j have been deposited with accession number GSE205608. Source data are provided with this paper.

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**Author contributions** C.T., L.K., V.R.B. and A.K. designed the study and wrote the manuscript. C.T., L.K. and S.R. performed core experimental work and data analysis. S.S.G. performed initial key experiments. D.C., K. Knöpper, Y.L., W.S., L.K. and S.J. performed computational analyses. K. Kanev, W.K., D.T.U., S.N., T.M., S.V.T., S.A.W., I.A.P., S.J. and J.L. performed supporting experimental work and data analysis. S.L.N., K. Kanev and D.Z. contributed new reagents or analytic tools.

**Competing interests** The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Isolation of polyclonal exhausted T cells for scRNA-seq and phenotypic characterization of CD62L− TPEX cells in chronic infection. (a, b) CD4+ T cell-depleted naive mice were infected with LCMV-CI13, treated with or without anti-PD-L1, and exhausted PD-1+TIM-3lo T cells were sorted at >day 30 post-infection as described19. (a) Schematic of the experimental set-up. (b) Flow cytometry plots showing the sorting strategy. (c–j) Naive congenically marked (CD45.1+) Id3-GFP P14 cells were transferred to naive recipients (Ly.5.2), which were then infected with LCMV-Docile. Splenic P14 T cells were analysed at the indicated time points after infection. (c) Schematic of the experimental set-up. (d) Flow cytometry plots showing the expression of Id3-GFP, TCF1 and CD62L among splenic P14 T cells at 7 and 21 dpi. (e) Quantification showing absolute numbers of splenic CD62L− TPEX, CD62L+ TPEX, and total P14 cells (left) and frequencies of CD62L− cells among TPEX cells (right) at the indicated time points after infection (f) Flow cytometry plots showing the expression of Ly108 and CD62L and quantification of CD62L− TPEX cells among P14 T cells in the spleen, lymph nodes, blood, bone marrow and liver at day 31 post LCMV-Docile infection. (g–j) Histograms (g, h) and quantification (i, j) of expression of molecules as indicated in P14 T cell subsets and naive CD8+ T cells. (k–p) Congenically marked naïve Nur77-GFP reporter P14 T cells were transferred into naive (k–m) or CD4+ T-cell-depleted (n–p) recipient mice, which were subsequently infected with LCMV-CI13. Nur77-GFP expression was analysed at indicated time points post-infection. (k, n) Schematics of the experimental set-up. Histograms (l, o) and quantifications (m, p) showing Nur77-GFP expression in the indicated P14 T cell subsets at 8 and 21 dpi. GMFI, geometric mean fluorescence intensity. Dots in graphs represent individual mice; box plots indicate range, interquartile and median; horizontal lines and error bars of bar graphs indicate mean and s.e.m. Data are representative of two independent experiments (e, f, i, j) and all analysed mice (m, p). P values are from two-tailed unpaired t-tests (e, i, j), two-way ANOVA (f), and one-way ANOVA (m, p); P > 0.05, not significant (n.s.).
Extended Data Fig. 2 | Functional and transcriptional profiling of exhausted T cell subsets and RNA velocity analysis showing that differentiation streams originate from CD62L+ TPEX cells. (a, b) Congenically marked naive P14 T cells were adoptively transferred into naive recipient mice, which were then infected with LCMV-CI13. Splenic P14 T cells from each group were sorted at day 28 post-infection and restimulated independently using gp33-pulsed splenocytes in vitro. (a) Schematic of the experimental set-up. (b) Quantifications showing cytokine production of each subset after restimulation. (c–e) Wild-type mice were infected with LCMV-Docile and splenic CD8+ T cells were analysed at the indicated time points after infection. (c) Schematic of the experimental set-up. (d) Flow cytometry plots showing the expression of CD62L and CD8+ T cells among endogenous gp33-specific CD8+ T cells. (e) Quantification showing the proportions of CD62L-expressing cells among gp33+ TPEX (left) and polyclonal PD-1+ TPEX cells (right) at the indicated time points after infection. (f, g) Congenically marked naive P14 T cells were adoptively transferred into naive recipient mice, which were then infected with LCMV-CI13 or LCMV-Armstrong. Splenic P14 compartments from each group were sorted at 28 dpi and processed for bulk RNA-seq. (f) Schematic of the experimental set-up. (g) Principal component plot showing the transcriptional landscapes of sorted populations as indicated. (h–j) Congenically marked naive Tcfl7-GFP P14 T cells were adoptively transferred into naive mice, which were then infected with LCMV-CI13. P14 TPEX cells were sorted at 28 post-infection based on the expression of Tcfl7-GFP. (h) Schematic of the experimental set-up. (i) Flow cytometry plots showing the sorting strategy and post-sort purity. (j) RNA velocity analysis showing developmental trajectories of TPEX cells, together with the expression of Tcfl7 (left) and Sell (right). Horizontal lines and error bars of bar graphs indicate mean and s.e.m., respectively. Data are representative of two independent experiments (b) and all analysed mice (e). P values are from Mann–Whitney tests (b) and two-tailed unpaired t-tests (e); P > 0.05, not significant (n.s.).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Capacity for self-renewal and multipotent differentiation is restricted to the CD62L+ TPEX cell compartment.

(a–f) Congenically marked naive P14 T cells were transferred into primary recipient mice (R1), which were then infected with LCMV-Cl13. The indicated subsets of P14 T cells were sorted at 21 dpi and $4 \times 10^4$ cells were re-transferred to infection-matched secondary recipient mice (R2). The indicated subsets of P14 T cells were sorted from R2 mice at 35 dpi and $1-3 \times 10^5$ cells were re-transferred to infection-matched tertiary recipient mice (R3). Splenic P14 T cells of R3 mice were analysed at day 14 post re-transfer. (a) Schematic of the experimental set-up. (b) Representative flow cytometry plots showing the sorting strategy and post-sort purities. Flow cytometry plots (c) and calculated fold expansion (d) of recovered P14 progenies at day 14 after secondary and tertiary re-transfers. Flow cytometry plots and quantifications showing expression of Ly108 and CD62L of splenic P14 cells in R2 and R3 mice (e) and average percentages of recovered CD62L+ TPEX, CD62L− TPEX, CD62L+ TEX and CD62L− TEX cells per spleen in R2 and R3 mice (f) at day 14 post re-transfer, respectively. (g–l) Congenically marked naive P14 T cells were transferred into primary recipient mice (R1), which were then infected with LCMV-Cl13. The indicated subsets of P14 T cells were sorted at 28 dpi and $3-30 \times 10^3$ cells were re-transferred to infection-matched secondary recipient mice (R2). Splenic P14 T cells of R2 mice were analysed at day 14 post re-transfer. Of note, maximum cell numbers attainable for each subset were transferred to allow for reliable evaluation of phenotypic diversification in expanded progenies. Fold expansion of recovered progenies was then normalized to distinct input numbers. (g) Schematic of the experimental set-up. (h) Representative flow cytometry plots showing the sorting strategy and post-sort purities. Flow cytometry plots (i) and fold expansion (j) of recovered progenies at day 14 post re-transfer. Flow cytometry plots and quantifications showing expression of Ly108 and CD62L of splenic P14 cells of R2 mice (k) and average percentages of recovered CD62L+ TPEX, CD62L− TPEX, CD62L+ TEX and CD62L− TEX cells per spleen (l) in R2 mice at day 14 post re-transfer. Dots in graphs represent individual mice; horizontal lines and error bars of bar graphs indicate mean and s.e.m., respectively. Data are representative of two independent experiments (b, c, e, h, i, k) and all analysed mice (d, f, j, l). P values are from Mann–Whitney tests (d, j); P > 0.05, not significant (n.s.).
Extended Data Fig. 4 See next page for caption.
Extended Data Fig. 4 | Single CD62L+ TPEX cells show a stem-like capacity for self-renewal and multipotent differentiation. (a–d) Single naive colour-barcoded P14 T cells were transferred to primary recipient mice, which were then infected with LCMV-Armstrong. Splenic P14 T cells were analysed at day 8 post LCMV-Armstrong infection. (a) Schematic of the experimental set-up for the naive P14 single-cell transfer. (b) Flow cytometry plots showing expression of GFP and YFP (left) or BFP/CFP and CFP/T-Sap (right) in peripheral blood of retrogenic P14 donor mice (pre-gated on CD8+CD44loCD45.1+). (c) Tracking of colour-barcoded single-cell-derived progenies at 8 dpi in the spleens of three representative recipient mice. Recovered progenies were distinguished according to their combinatorial expression of GFP and YFP into populations I, II, III, IV and V, which were further subdivided by their expression of T-Sapphire, CFP, and BFP into progenies characterized by their unique combinatorial colour barcode. Note: in the display used, CFP emission appears on the diagonal between the BFP (x-axis) and T-Sapphire signal (y-axis) and is therefore indicated on both axes. (d) Flow cytometry plots depicting combined staining of CD45.1 and Thy1.1 with KLRG1 (upper row), or CD62L with PD-1 (lower row) for three progenies derived from adoptively transferred single naive P14 cells (grey: endogenous CD4−CD19− cells). (e–i) Colour-barcoded naive P14 T cells were transferred into primary recipient mice (R1), which were subsequently infected with LCMV-CI13. P14 T cells were sorted at 28 dpi and single CD62L+ or CD62L− TPEX cells were re-transferred into naive secondary recipient mice (R2), which were subsequently infected with LCMV-Armstrong. Splenic P14 T cells were analysed at day 8 post LCMV-Armstrong infection. (e) Schematic of the experimental set-up. (f) Percentages of transferred single cells of CD62L+ TPEX, CD62L− TPEX cell or naive phenotype from which progenies were recovered at 8 dpi. (g) As in (d), but for adoptively re-transferred single CD62L+ TPEX cells. (h) Size of single-T-cell-derived progenies and frequencies of CD62L+, KLRG1+ and (i) PD-1+ cells therein. Dots in graphs represent individual clones derived from a single transferred cell. Horizontal lines and error bars of bar graphs indicate mean and s.e.m., respectively. Data show all analysed mice (h, i). *P* values are from Mann–Whitney tests (h–i); *P* > 0.05, not significant (n.s.).
Extended Data Fig. 5 See next page for caption.
Extended Data Fig. 5 | CD69 expression in T$_{PEx}$ cells does not correlate with CD62L expression and does not predict developmental and repopulation potential; chronic LCMV infection and strong TCR stimuli favour MYB expression and the formation of stem-like CD62L$^+$ T$_{PEx}$ cells. (a) Normalized expression of Cd69 projected on the UMAP of scRNA-seq data as in Fig. 1. (b) Enrichment of Ly108$^+$CD69$^+$ ("T$_{PEx}$ prog1") and Ly108$^+$CD69$^-$ ("T$_{PEx}$ prog2") signatures at single-cell and cluster levels. (c) Flow cytometry plots and quantification showing CD69 expression in CD62L$^+$ and CD62L$^-$ P14 T$_{PEx}$ cells on day 28 post LCMV-CI13 infection. (d–j) Congenically marked naive P14 T cells were transferred into primary recipient mice (R1), which were then infected with LCMV-CI13. The indicated subsets of P14 T cells were sorted at 28 dpi and re-transferred to infection-matched secondary recipient mice (R2). Splenic P14 T cells of R2 mice were analysed at day 14 post re-transfer. (k–l) Naive MybGFP reporter mice were infected with either LCMV-Docile or LCMV-Armstrong and CD8$^+$ T cells were analysed at the indicated time points after infection. (k) Representative flow cytometry plots depict Ly108 and Myb-GFP expression among antigen-specific (gp33$^+$) CD8$^+$ T cells. (l) Histograms (filled) show Myb-GFP expression of gp33$^+$ CD8$^+$ T cells in mice infected with LCMV-Docile (top) and LCMV-Armstrong (bottom). Empty histograms depict Myb-GFP expression in naive CD8$^+$ T cells in the same samples. Corresponding quantification show the fold change of geometric mean fluorescence intensity (GMFI) of Myb-GFP in the indicated populations. (m–o) LCMV-Docile-infected Myb$^{GFP}$ reporter mice were treated with or without anti-PD-L1. Splenic CD8$^+$ T cells were analysed at 6 dpi. (m) Schematic of the experimental set-up. (n) Flow cytometry plots and quantification showing frequencies of splenic gp33$^+$ CD8$^+$ T cells in anti-PD-L1-treated and untreated control mice at 6 dpi. (o) Naive Myb$^{GFP}$ and wild-type (non-reporter, control) CD8$^+$ T cells were stimulated and cultured in vitro using plate-bound anti-CD3. Representative histogram and normalized quantification show GMFI of Myb-GFP expression in CD8$^+$ T cells stimulated with plate-bound anti-CD3 at the indicated concentrations. (p) Flow cytometry plots and quantification show the frequencies of Ly108$^+$ and CD62L$^+$ T cells among splenic antigen-specific (gp33$^+$) T cells in wild-type mice at day 8 post LCMV-Docile or LCMV-Armstrong infection. (q) Congenically marked P14 T cells were adoptively transferred into naive recipient mice, which were then infected with either LCMV-Docile or LCMV-Armstrong. Splenic P14 T cells were analysed at 8 dpi. Flow cytometry plots and quantification show the frequencies of Ly108$^+$ and CD62L$^+$ T cells among splenic P14 T cells. Dots in graphs represent individual mice (c, f, l, o, q, r) and individual wells (p); box plots indicate range, interquartile and median; horizontal lines and error bars in indicate mean and s.e.m., respectively. Data are representative of two independent experiments (c, l, o, p) and all analysed mice (f, q, r). P values are from two-tailed unpaired t-tests (c, l, o–r) and Mann-Whitney tests (f); P > 0.05, not significant (n.s.).
Extended Data Fig. 6 | Development of mature CD8+ T cells is largely normal in Myb<sup>fl/fl</sup>Cd4<sup>cre</sup> mice. Adult 8–12 weeks Myb<sup>fl/fl</sup>Cd4<sup>cre</sup> (Myb-cKO) and littermate Myb<sup>fl/fl</sup> control (Ctrl) mice were euthanized, and T cell populations were analysed in the thymus, spleen and lymph nodes. Flow cytometry and quantifications showing (a–c) frequencies of thymocyte subsets, (d–f) frequencies and abundance of mature splenic CD8<sup>+</sup> T cells, (g) surface expression of CD127 (IL-7R), CCR7 and CD25 (IL-2R) of splenic CD8<sup>+</sup> T cells and (h, i) frequencies of mature CD8<sup>+</sup> T cells residing in lymph nodes. (h). Dots in graphs represent individual mice; box plots indicate range, interquartile and median. All data are representative of two independent experiments. P values are from two-tailed unpaired t-tests (d-h) and Mann–Whitney tests (a, c, e, i); P > 0.05, not significant (n.s.).
Extended Data Fig. 7 | See next page for caption.
**Extended Data Fig. 7 | MYB is required to limit CD8+ T cell expansion and cytotoxicity in response to chronic infection.** (a–s) Mybfl/fl Cd4Cre+ (Myb-cKO) mice and littermate Mybfl/fl control mice (Ctrl) were infected with either LCMV-Armstrong (a–d) or LCMV-Docile (e–s). (a–b) Flow cytometry plots showing (a) splenic antigen-specific (gp33+) CD8+ cells and (b) expression of CD62L and KLRG1 among antigen-specific cells in Myb-cKO and control mice at indicated time points post LCMV-Armstrong infection. (c) Quantification of central memory (Tcm), effector memory (Tem), CX3CR1+ and KLRG1+ cells among gp33+ CD8+ cells in Myb-cKO and control mice at indicated time points post LCMV-Armstrong infection. (d) Numbers of splenic gp33+CD8+ T cells in Myb-cKO and control mice at indicated time points post LCMV-Armstrong infection. (e) Box plots showing the weights of spleens (left) and the total numbers of splenocytes (right) in Myb-cKO and control mice at day 8 post LCMV-Docile infection. (f) Images showing the infiltration of immune cells (arrows) in livers (middle) and lungs (right) in Myb-cKO and control mice at 8 dpi. (g) Confocal images of F4/80 and B220 expression in frozen spleen sections and (h) quantification showing the cellular organization and area of lymphoid regions in Myb-cKO and control mice at day 8 post LCMV-Docile infection. (i) Confocal images of CD3 and B220 expression in frozen spleen sections showing the distribution of B and T cells in the spleens of Myb-cKO and control mice at day 8 post LCMV-Docile infection. (j) Image and box plot showing the size and weights of spleens in untreated and CD8+ T-cell-depleted Myb-cKO mice at day 8 post LCMV-Docile infection. (k) Survival curve of CD8-depleted Myb-cKO mice post LCMV-Docile infection. (l) Proportion of cytokine-producing antigen-specific Tem and Tcm cell subsets after gp33 peptide restimulation of Myb-cKO and control mice at day 8 post LCMV-Docile infection. (m, n) Quantification of IFNγ expression (m), and granzyme B (GZMB) expression in Tem and Tcm cells (n) in Myb-cKO and control mice at day 8 post LCMV-Docile infection. (o) Flow cytometry plots and quantification showing the proportions of Ki67+ within the gp33+ compartment in Myb-cKO and control mice at day 8 post LCMV-Docile infection. (p) Box plots showing viral titres in the kidneys of Myb-cKO and control mice at day 8 post LCMV-Docile infection. (q) Box plots showing the expression of PD-1 (left) and TIM-3 (right) among gp33+ CD8+ T cells of control and Myb-cKO mice at day 8 post LCMV-Docile infection. (r) Flow cytometry plots and quantification show the frequencies of Tcf1+Tim3− (TCF1+TIM-3−) and Tcf1−Tim3+ (TCF1−TIM-3+) among splenic gp33+ CD8+ T cells of Myb-cKO and control mice. (s) Quantification showing the absolute numbers of splenic CD8+ gp33+ CD62L+ Tcf1+Tim3− (Tcf1+TIM-3−) and Tcf1−Tim3+ (TCF1−TIM-3+) cells in Myb-cKO and control mice at day 8 post LCMV-Docile infection. Data are representative of two independent experiments (c–e, k–r) or all mice (j, s) and images (h) analysed; P > 0.05, not significant (n.s.).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | MYB limits CD8+ T cell expansion and cytotoxicity during chronic infection in a cell-intrinsic manner. (a–n) Naive CD45.1 mice were lethally irradiated and reconstituted using a mixture of Mybfl/flCd4Cre−/− (Myb−cKO) and Cd4Cre+ or littermate Mybfl/fl control (Ctrl) bone marrow. Chimeric mice were subsequently infected with LCMV- Docile and analysed at the indicated time points after infection. Quantification showing the frequencies of (a) polyclonal antigen-specific gp33+ cells among Myb−cKO and control CD8+ T cells at 8 dpi. (b) Flow cytometry plots and quantification of IFNγ+ cells among Myb−cKO and control CD8+ T cells after peptide restimulation in vitro at 8 dpi. (c) Quantification of GZMB expression among gp33+ cells of the indicated genotypes. (d, e) Flow cytometry plots and quantification showing the frequencies of (d) Ki67+ cells and (e) annexin-V+ cells among Myb−cKO and control antigen-responsive CD8+ T cells. (f, g) Flow cytometry plots and quantification showing the frequencies of TCF1+ TPEX cells among antigen-specific T cells (f) and CD62L+ cells among TPEX cells (g) in Myb−cKO and control compartments at 8 dpi. (h) Flow cytometry plots showing the frequencies of TPEX cells among gp33+ cells at 49 dpi. (i–j) Flow cytometry plots (i) and quantification (j) showing kinetics of splenic polyclonal PD-1+ TPEX cells among Myb−cKO and control CD8+ T cells after infection. (k–l) Flow cytometry plots (k) and quantification (l) showing the frequencies of the entire antigen-responsive PD-1+ cell compartment among Myb−cKO and control CD8+ T cells at indicated time points after infection. (m, n) Flow cytometry plots and quantifications showing the frequencies of Ki67+ cells among Myb−cKO and control polyclonal TPEX (m) and TPEX (n) cells at indicated time points after infection. GMFI, geometric mean fluorescence intensity. Dots in graphs represent individual mice; box plots indicate range, interquartile and median. Symbols and error bars represent mean and s.e.m., respectively. All data are representative of two independent experiments. P values are from two-tailed unpaired t tests (a–g) and Mann–Whitney tests (j–n); P > 0.05, not significant (n.s.).
Extended Data Fig. 9 | MYB limits proliferation and cytotoxicity and sustains the long-term self-renewal of exhausted CD8+ T cells.

(a–g) Congenically marked naive control (Cd4cre+) and Mybfl/fl Cd4cre (Myb-cKO) P14 T cells were adoptively transferred into naive recipient mice, which were subsequently infected with LCMV-Docile. Splenic P14 T cells were analysed at indicated time points post-infection (p.i.). (a) Schematic of the experimental set-up. (b) Box plot showing PD-1 expression of transferred P14 cells at 8 dpi. (c) Flow cytometry plots and quantification showing frequencies of CD62L+ cells among Myb-cKO and control P14 TPEX cells. (d) Flow cytometry plots and quantification showing the expression of granzyme B (GZMB) in Myb-cKO and control TPEX cells at 8 dpi. (e) Flow cytometry plots and quantifications showing the production of cytokines as indicated from Myb-cKO and control P14 T cells after gp33 peptide restimulation at 8 dpi. (f) Flow cytometry plots and quantification showing the frequencies of Treg cells among Myb-cKO and control P14 T cells at the indicated time points after infection. (g) Flow cytometry plots and quantification showing the frequencies of Ki67+ cells among Myb-cKO and control P14 T cells at indicated time points after infection. GMFI, geometric mean fluorescence intensity. Dots in graphs represent individual mice; box plots indicate range, interquartile and median; Data are representative of two independent experiments (b–g). P-values are from two-tailed unpaired t-tests; P > 0.05, not significant (n.s.).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | MYB regulates the expression of multiple genes that are critical to exhausted T cell function and maintenance. (a, b) Congenically marked MybrflflCd4Cre (Myb-cKO, CD45.2+) and Cd4Cre (Ctrl, CD45.2+ or CD45.2+CD45.1+) P14 T cells were adoptively transferred into separate naive recipient (CD45.1) mice, which were then infected with LCMV-Docile. Splenic P14 T\textsubscript{ex} cells were sorted at day 7 post-infection and processed for bulk RNA-seq. (a) Schematic of the experimental set-up. (b) Gene set enrichment analysis showing loss of CD62L\textsuperscript{+} T\textsubscript{ex} transcriptional signature in Myb-cKO T\textsubscript{ex} cells compared to control T\textsubscript{ex} cells. (c) Volcano plots highlighting genes differentially expressed (FDR < 0.15) between control CD62L\textsuperscript{+} T\textsubscript{pe} and CD62L\textsuperscript{−} T\textsubscript{pe} cells. (d–e) Mixed bone marrow chimeric mice containing congenically marked Myb-cKO and control CD8\textsuperscript{+} T cells were infected with LCMV-Docile. Flow cytometry plots (d) and quantification (e) showing the frequencies of the entire antigen-responsive PD-1\textsuperscript{+} cell compartment among Myb-cKO and control CD8\textsuperscript{+} T cells in the spleen and lymph nodes at day 70 post-infection. (f, g) Congenically marked Myb-cKO and CtrlP14 T cells were adoptively transferred into separate naive recipient mice, which were then infected with LCMV-Docile. Splenic P14 T\textsubscript{ex} cells were analysed at day 8 post-infection. (f) Schematic of the experimental set-up. (g) Quantification showing the abundances of the indicated P14 subsets per spleen. (h) Heat map depicting genes differentially expressed (FDR < 0.15, FC > 1) between control CD62L\textsuperscript{+} T\textsubscript{ex} and CD62L\textsuperscript{−} T\textsubscript{ex} cell or Myb-cKO and control T\textsubscript{ex} cells, with genes of interest annotated. (i) Gene set enrichment analysis showing loss of CX3CR1\textsuperscript{+} T\textsubscript{ex} transcriptional signature in P14 Myb-cKO T\textsubscript{ex} cells compared to control T\textsubscript{ex} cells. (j) Volcano plot highlighting genes differentially expressed (FDR < 0.15) between control and Myb-cKO T\textsubscript{ex} cells with genes of interest annotated. (k) Flow cytometry plots and quantification show the frequencies of CX3CR1\textsuperscript{+} cells among control and Myb-cKO T\textsubscript{ex} P14 T cells at day 8 post LCMV-Docile infection. (l) Flow cytometry plots and quantifications showing CX3CR1 and CD101 expression in Myb-cKO and control T\textsubscript{ex} cells at the indicated time points after infection. Dots in graph represent individual mice; box plots indicate range, interquartile and median. Symbols and error bars in (l) represent mean and s.e.m., respectively. Data are representative of two independent experiments (e, g, k, l). P values are from two-tailed unpaired t-tests; P > 0.05, not significant (n.s.).
Extended Data Fig. 11 | See next page for caption.
Extended Data Fig. 11 | MYB directly regulates target gene expression, and CD62L+ TPEX cells have a superior potential to give rise to CX3CR1+ TEX cells. (a–d) Representative tracks showing MYB ChIP–seq peaks in the LEF1 (a), E2F1 (b), GZMA (c), and MYB (d) gene loci of human Jurkat T cells and ATAC-seq peaks of TPEX and TEX cells in the corresponding mouse gene loci aligned according to the sequence conservation. (e–g) Congenically marked naive P14 cells were transferred to primary recipient mice (R1), which were subsequently infected with LCMV-CI13. The indicated subsets of P14 cells were sorted at 28 dpi and re-transferred to naive secondary recipient mice (R2), which were then infected with LCMV-Armstrong. Splenic P14 T cells in R2 mice were analysed at 8 dpi. (e) Schematic of the experimental set-up. (f) Flow cytometry plots of progenies recovered at 8 dpi. (g) Cell numbers (left) and quantification of PD-1 expression (right) in P14 T cell populations derived from the indicated transferred subsets at 8 dpi. (h, i) Congenically marked naive P14 T cells were transferred into primary recipient mice (R1), which were then infected with LCMV-Docile. The indicated subsets of P14 T cells were sorted at 7 dpi and 7.5×10⁴ cells were re-transferred to infection-matched (LCMV-Docile) secondary recipient mice (R2). Splenic P14 T cells in R2 mice were analysed at day 28 post re-transfer. (h) Schematic of the experimental set-up. (i) Flow cytometry plots and box plots showing the frequencies of CX3CR1+ and CD101+ cells among recovered TEX cells derived from the indicated re-transferred TEX subsets at day 28 post re-transfer. Data are representative of two independent experiments. Pvalues are from Mann–Whitney tests (g) and two-tailed unpaired t-test (i); P> 0.05, not significant (n.s.).
Extended Data Fig. 12 | See next page for caption.
Extended Data Fig. 12 | Effect of PD-1 signalling on CD62L+ T\textsubscript{res} cells.

(a–f) Congenically marked PD-1-deficient (\textit{Pdcd1}\textsuperscript{−/−}) and control P14 T cells were transferred to naive mice, which were subsequently infected with LCMV-Docile. Splenic P14 T cells were analysed at 7 dpi. (a) Schematic of the experimental set-up. (b) P14 T cell frequencies and numbers of indicated genotypes. (c) Flow cytometry plots and frequencies of T\textsubscript{res} (Ly108\textsuperscript{hi}TIM-3\textsuperscript{lo}) and T\textsubscript{ex} (Ly108\textsuperscript{lo}TIM-3\textsuperscript{hi}) cells. (d) Box plots show frequencies and numbers of CD62L+ T\textsubscript{res} cells among control and PD-1-deficient P14 cells. Flow cytometry plots and box plots show (e) frequencies of KIT+ T\textsubscript{res} cells and (f) numbers of KIT+ T\textsubscript{res} and T\textsubscript{ex} cells per spleen. (g–k) Wild-type mice were infected with LCMV-Docile and treated with anti-PD-L1 at 200 μg/mouse at 1, 3 and 5 dpi. Splenic CD8+ T cells were analysed at 6 dpi. (g) Schematic of the experimental set-up. (h) Flow cytometry plots and quantification showing the frequencies of PD-1+ cells among splenic CD8+ T cells. (i–j) Flow cytometry plots (i) and quantification (j) showing expression of CD62L among polyclonal T\textsubscript{res} cells (Ly108\textsuperscript{hi}TIM-3\textsuperscript{lo}). (k) Quantification showing the population sizes of CD62L+ T\textsubscript{res}, CD62L− T\textsubscript{res} and T\textsubscript{ex} cells among total CD8+ T cells in untreated and anti-PD-L1-treated mice. (l–p) Wild-type mice were infected with LCMV-Docile and treated with anti-PD-L1 at 200 μg/mouse at 21, 23, 25, 27 and 29 dpi. Splenic CD8+ T cells were analysed at 31 dpi. (l) Schematic of the experimental set-up. (m–n) Flow cytometry plots (m) and quantification (n) showing the frequencies of the PD-1+ cells among splenic CD8+ T cells. (o) Flow cytometry plots and quantification showing the expression of CD62L among polyclonal T\textsubscript{res} cells (Ly108\textsuperscript{hi}TIM-3\textsuperscript{lo}). (p) Quantification showing the population sizes of CD62L+ T\textsubscript{res}, CD62L− T\textsubscript{res} and T\textsubscript{ex} cells among total CD8+ T cells in untreated and anti-PD-L1-treated mice. Dots in graphs represent individual mice; box plots indicate range, interquartile and median. Data are representative of at least two independent experiments. \textit{P} values are from two-tailed unpaired \textit{t}-tests; \textit{P} > 0.05, not significant (n.s.).
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- Flow Cytometry:
  - CytExpert (Beckman Coulter) v2.3.1.22

Data analysis

- Flow Cytometry:
  - FlowJo v10.6.1
- Data analysis and visualization:
  - Prism v7
- Visualization:
  - Adobe Illustrator v24.1.2
- Annotation of scRNA-seq data:
  - CellRanger v5.0.0
- scRNA-seq analysis:
  - SCANPY v1.6
  - Velocyto 0.17.17
  - scVelo v0.2.1

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample-size calculation was performed, the data presented in this study was collected in repeated independent experiments with at least 2-3 mice per group, as indicated in the Figure Legends. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analysis. |
| Replication | The presented data was successfully replicated, in some cases experiments were replicated by different authors. |
| Randomization | Age- and sex-matched mice were allocated to groups based on the experimental treatment (no randomization). |
| Blinding | Blinding was not performed, as data analysis was strictly quantitative and not subjective. Computational analysis was not blinded. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☐ ☒ Antibodies | ☒ ChiP-seq |
| ☒ Eukaryotic cell lines | ☒ Flow cytometry |
| ☒ Palaeontology and archaeology | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms | |
| ☒ Human research participants | |
| ☒ Clinical data | |
| ☒ Dual use research of concern | |

Antibodies

Antibodies used

Name, Clone, Supplier:
- CD45.1, A20, Bioreagent or BD Bioscience
- CD45.2, 104, BD Bioscience
- CD90.1, HISS1, Thermo Fisher Scientific
- CD4, RM4-5, Bioreagent
- CD4, GK1.5, Bioreagent
- CD44, IM7, BD Bioscience
- CD19, 605, Bioreagent
- CD117, ACK2, Thermo Fisher Scientific
- CD101, Moushi101, Thermo Fisher Scientific
- CD160, eBioCNX46-3, eBioscience
- CD244, eBio244F4, Thermo Fisher Scientific
Validation

All antibodies were obtained commercially and validation was based on the descriptions provided on the manufacturer’s homepage.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

- 6-8 weeks C57BL/6 mice were obtained from the Australian Resources Centre or Envigo
- P14 TCR transgenic mice expressing diverse combinations of the congenic markers CD45.1/2 and CD90.1/2, as well as TCRα knockout mice were bred at the mouse facility of Technische Universität München, München, Germany.
- Age- and sex-matched mice (all on C57BL/6 background) were used in this study and entered experiments at 6–8 weeks of age.
- MybGfp mice and Mybfl/flCd4Cre mice were bred and housed at the mouse facility of Peter Doherty Institute of Infection and Immunity

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected in the field.

Ethics oversight

Experimental protocols were approved by the committee for experimentation with laboratory animals of the district government of Upper Bavaria (Germany) or University of Melbourne Animal Ethics Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Single cell suspensions from different organs were obtained as described in the methods section.

Instrument

Cell sorting was performed on a MoFlo Astrios (Beckman Coulter). For data collection, a Cytoflex Lx cytometer (Beckman Coulter) was used.

Software

CytExpert (Beckman Coulter) v2.3.1.22 software was used for data collection. The acquired samples were analyzed using FlowJo software v10.6.1.

Cell population abundance

Sort purities were routinely confirmed, as assessed by post-sort measurements of the respective target cell populations.

Gating strategy

1. FSC/SSC gates were used to select lymphocyte populations.
2. FSC/FSC width gates were used to identify singlets.
3. Live/dead exclusion was performed using propidium iodide (PI) or eBioscience Fixable Viability Dye eF780.
4. Further gating of target-cell populations relied on the respective marker combinations, for cell surface or intracellular markers, used in the specific experiments.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.