PTPN2 regulates the generation of exhausted CD8+ T cell subpopulations and restrains tumor immunity

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CD8+ T cell exhaustion is a state of dysfunction acquired in chronic viral infection and cancer, characterized by the formation of Slamf6+ progenitor exhausted and Tim-3+ terminally exhausted subpopulations through unknown mechanisms. Here we establish the phosphatase PTPN2 as a new regulator of the differentiation of the terminally exhausted subpopulation that functions by attenuating type 1 interferon signaling. Deletion of Ptpn2 in CD8+ T cells increases the generation, proliferative capacity and cytokotoxicity of Tim-3+ cells without altering Slamf6+ numbers during lymphocytic choriomeningitis virus clone 13 infection. Likewise, Ptpn2 deletion in CD8+ T cells enhanced Tim-3+ anti-tumor responses and improved tumor control. Deletion of Ptpn2 throughout the immune system resulted in MC38 tumor clearance and improved programmed cell death-1 checkpoint blockade responses to B16 tumors. Our results indicate that increasing the number of cytotoxic Tim-3+ CD8+ T cells can promote effective anti-tumor immunity and implicate PTPN2 in immune cells as an attractive cancer immunotherapy target.

Results

Loss of Ptpn2 promotes the early proliferation of CD8+ T cells during LCMV clone 13 infection. We recently conducted a pooled in vivo loss-of-function screen and identified PTPN2 as a candidate regulator of CD8+ T cell responses16. To examine the role of PTPN2 in LCMV clone 13 infection, we created bone marrow chimera using CHIME (CHimeric IMMune Editing), a chimeric-based CRISPR-Cas9 delivery method (Fig. 1a)28 to delete Ptpn2 in hematopoietic cells from P14 TCR transgenic mice (specific for the LCMV CD8 epitope, GP33-41). We confirmed the efficient deletion (~80%) of Ptpn2 in naive P14 CD8+ T cells using the tracking of indels by decomposition (TIDE) assay29 (Fig. 1b). To evaluate cell-intrinsic functions of PTPN2 in CD8+ T cells, we cotransferred congenically marked naive P14 CD8+ T cells using the tracking of RNA-containing (Ptpn2-deleted) and control sgRNA-containing (control) CD8+ T cells to wild-type recipient mice and subsequently...
infected them with LCMV clone 13. Given that Ptpn2 deficiency leads to alterations in thymocyte maturation and aberrant T cell activation at homeostasis, we transferred only naive CD44−CD62L+ cells before LCMV infection (Supplementary Fig. 1a). These cells did not express effector-related molecules, such as granzyme B (Supplementary Fig. 1b) or markers of cell proliferation, such as Ki-67 or 5-bromo-2-deoxyuridine (BrdU) incorporation (Supplementary Fig. 1c,d). Following LCMV infection, there was a significant increase in the Ptpn2-deleted cells compared to control cells at 8d and 15d after infection, but not 30d after infection (Fig. 1c−e and Supplementary Fig. 1e). The increased cell numbers at these time points were due, in part, to increased proliferation of Ptpn2-deleted cells (Fig. 1f). Deletion of Ptpn2 also increased the percentage of granzyme B+ cells at early time points (Fig. 1g,h) but did not affect the percentage of IFN-γ+ TNF+ cells (Supplementary Fig. 1f). Thus, Ptpn2 deletion provides CD8+ T cells with a transient advantage early during LCMV clone 13 infection but does not prevent contraction at later time points.

Deletion of Ptpn2 enhances formation of the Tim-3+ terminally exhausted subpopulation during LCMV clone 13 infection. The changes in granzyme B expression prompted us to examine the impact of Ptpn2 deletion on the generation of the Slamf6+ and Tim-3+ subpopulations. Ptpn2 deletion increased the ratio of Tim-3+ to Slamf6+ cells at 8, 15 and 22d after infection (Fig. 2a,b), despite transferring only Slamf6+Tim-3− cells (Supplementary Fig. 2a).
**Fig. 2** | Deletion of Ptpn2 enhances formation of the Tim-3+ terminally exhausted subpopulation during LCMV Clone 13 infection. 

**a.** Representative flow cytometry plots of Tim-3 and Slamf6 expression on control or Ptpn2-deleted P14 T cells in the spleen 8 d after LCMV clone 13 infection. Data are representative of eight independent experiments, n ≥ 4 mice. 

**b.** Ratio of Tim-3+/Slamf6+ control or Ptpn2-deleted P14 T cells in the spleen 8, 15, 22 and 30 d after LCMV clone 13 infection. Data are representative of two independent experiments, n ≥ 4 mice. 

**c.** Number of Tim-3+ control or Ptpn2-deleted P14 T cells in the spleen 8, 15, 22 and 30 d after LCMV clone 13 infection. Data are representative of two independent experiments, n ≥ 4 mice. 

**d.** Number of Slamf6+ control or Ptpn2-deleted P14 T cells in the spleen 8, 15, 22 and 30 d after LCMV clone 13 infection. Data are representative of two independent experiments, n ≥ 4 mice. 

**e.** Quantification of granzyme B expression for Tim-3+ control or Ptpn2-deleted P14 T cells in the spleen 8, 15, 22 and 30 d after LCMV clone 13 infection. Data are representative of two independent experiments, n ≥ 4 mice. 

**f.** Number of transferred cells per spleen. 

**g.** Frequency of BrdU incorporation for Slamf6+ cells following Ptpn2 deletion (Fig. 2c), whereas there was no difference in the number of Slamf6+ cells (Fig. 2d), nor CXCR5+ cells (Supplementary Fig. 2e). Furthermore, in non-TCR transgenic chimeras, Ptpn2 deletion resulted in an increase in the percentage of Tim-3+ cells and a decrease in the percentage of Slamf6+ cells compared to control cells.

Analysis of the populations using Tim-3 and a distinct progenitor marker (CXCR5) (refs. 4,16,17) gave identical results (Supplementary Fig. 2b,c). Moreover, following Ptpn2 deletion, we observed a significant reduction in expression of two additional markers of progenitor exhausted cells, CD127 and TCF1 (Supplementary Fig. 2d). The increase in the Tim-3+ to Slamf6+ ratio was driven by a specific increase in the number of Tim-3+ cells following Ptpn2 deletion (Fig. 2c), whereas there was no difference in the number of Slamf6+ cells (Fig. 2d), nor CXCR5+ cells (Supplementary Fig. 2e). Furthermore, in non-TCR transgenic chimeras, Ptpn2 deletion resulted in an increase in the percentage of Tim-3+ cells and a decrease in the percentage of Slamf6+ cells compared to control cells.
Thus, deletion of *Ptpn2* enhances formation of the Tim-3⁺ subset during LCMV clone 13 infection.

To determine whether changes in granzyme B expression and BrdU incorporation (Fig. 1f–h) were intrinsically changed in the Tim-3⁺ subpopulations, we compared cotransferred control and *Ptpn2*-deleted cells and found only a minimal increase in granzyme B expression in the *Ptpn2*-deleted Tim-3⁺ cells (Fig. 2e). In addition, we found a nominal increase in BrdU incorporation between control and *Ptpn2*-deleted Tim-3⁺ cells (Fig. 2f), indicating a similar increase in actively proliferating cells. We found no differences in granzyme B expression nor BrdU incorporation in Slamf6⁺ control and *Ptpn2*-deleted cells (Fig. 2g,h). These findings demonstrate that *Ptpn2* deletion leads to a specific increase in the generation of the Tim-3⁺ subpopulation, while preserving the number of Slamf6⁺ cells, and that the altered ratio of Tim-3⁺ to Slamf6⁺ cells is primarily responsible for the observed increase in granzyme B expression and BrdU incorporation.

**Ptpn2** deletion promotes effector-skewed Slamf6⁺ and Tim-3⁺ subpopulations during LCMV infection. We next performed single-cell RNA-sequencing (RNA-seq) on control and *Ptpn2*-deleted cells 30 d after LCMV infection, because the canonical features of exhaustion are present at this time point⁵⁻⁹. Unsupervised clustering of the cells revealed six subpopulations, which we identified by marker gene expression and previously defined signature enrichment (Fig. 3a,b) and Supplementary Fig. 3a,b. We recapitulated the previously described terminally exhausted, progenitor exhausted, proliferating and effector-like populations⁴, and identified a distinct IFN-responsive cluster that contained both progenitor exhausted and terminally exhausted cells, suggesting that the cluster represented cells that were actively responding to IFN-α (Fig. 3b and Supplementary Fig. 3b). Further analysis of the distribution of the control or *Ptpn2*-deleted cells across the clusters revealed a significant skewing of the control cells into the progenitor exhausted cluster and the *Ptpn2*-deleted cells into the effector-like, proliferating and terminally exhausted clusters (Fig. 3c,d), consistent with our flow cytometry data (Supplementary Fig. 3c). We also noted that within a subpopulation, the *Ptpn2*-deleted cells or control CD8⁺ T cells tended to cluster together (Supplementary Fig. 3d). Differential expression analysis between *Ptpn2*-deleted and control cells within the progenitor and terminally exhausted clusters revealed that the *Ptpn2*-deleted cells had increased expression of genes associated with terminally exhausted or effector cells such as *Gzma*, *Cd160*, *Cd8* and *Gzci* (adjusted *P* values <0.005), consistent with an enrichment of effector-related gene signatures (Fig. 3e,f). Thus, at 30 d after LCMV infection, *Ptpn2*-deleted progenitor and terminally exhausted cells have increased transcription of effector-related genes.

We next asked whether *Ptpn2* deletion impacted the cell states of Slamf6⁺ and Tim-3⁺ subpopulations at an earlier time point after LCMV infection. We performed RNA-seq on cotransferred *Ptpn2*-deleted or control CD8⁺ T cells 8 d after LCMV clone 13 infection (Supplementary Table 1) and found that the Slamf6⁺ and Tim-3⁺ subpopulations clustered together regardless of *Ptpn2* deletion (Supplementary Fig. 3e). Gene set enrichment analysis (GSEA) revealed that both control and *Ptpn2*-deleted Slamf6⁺ cells were significantly enriched for the LCMV Slamf6 versus Tim-3 Up signature, whereas the control and *Ptpn2*-deleted Tim-3⁺ cells were significantly enriched for the LCMV Slamf6 versus Tim-3 Down signature (Supplementary Fig. 3f). Consistent with this, *Ptpn2* deletion had almost no effect on the epigenetic state of either the Slamf6⁺ or Tim-3⁺ populations ([<0.25% of open chromatin regions differentially expressed] 8 d after infection (Supplementary Fig. 3g and Supplementary Table 2). Both control and *Ptpn2*-deleted CD8⁺ T cells still showed characteristic differences between the Slamf6⁺ and Tim-3⁺ populations (Supplementary Fig. 3h). In addition, both control and *Ptpn2*-deleted cells showed chromatin-accessible regions at the *Tox* locus (Supplementary Fig. 3i), characteristic of T cell exhaustion⁴, indicating the cells were differentiating into the exhausted subpopulations.

Although the general states of the Slamf6⁺ and Tim-3⁺ cells were maintained at day 8, GSEA of the Slamf6⁺ and Tim-3⁺ *Ptpn2*-deleted cells compared to control cells revealed an enrichment for effector-related gene signatures (Fig. 3g,h). Within both the Slamf6⁺ and Tim-3⁺ subpopulations, *Ptpn2*-deleted cells had increased expression of effector-related genes pertaining to cytotoxicity (Gzmn, Lamp2, Serpina3g) and proliferation (Stmn1, Tkl1, Tpil1), compared to control cells (Fig. 3i,j). Thus, while *Ptpn2* deletion does not fundamentally change the Slamf6⁺ and Tim-3⁺ subpopulations, it does lead to increases in Tim-3⁺ cell numbers and robustly increases effector-related genes both early and late after LCMV infection.

Consistent with the increase in effector-like profiles in the *Ptpn2*-deleted cells, *Ptpn2*-deleted CD8⁺ T cells had increased cytotoxic potential (Fig. 3k). This increased killing potential was due to an intrinsic change within the Tim-3⁺ cells, as *Ptpn2*-deleted Tim-3⁺ cells showed increased killing of target cells when compared with control cells (Fig. 3l). Overall, these findings validate the observed enrichment in cytotoxic genes seen by RNA-seq and demonstrate that *Ptpn2* deletion improves the cytotoxic function of CD8⁺ T cells.

**Ptpn2** deletion increases Tim-3⁺ cell differentiation and proliferation. The increase in Tim-3⁺ cell numbers at 8 d after LCMV infection is prominen...
infection, coupled with the enhanced effector-related gene signatures, led us to ask whether PTPN2 was impacting the differentiation of these subpopulations. We found that Ptpn2-deleted CD8+ T cells had a marked competitive advantage over control cells 4 d after infection (Fig. 4a) and enhanced differentiation into Tim-3+ cells (Fig. 4b). Ptpn2 deletion resulted in a decreased percentage of Slamf6+Tim-3– cells and an increased percentage of Slamf6+Tim-3+ cells and Slamf6–Tim-3+ cells (Fig. 4c), as well as an increase in granzyme B expression (Fig. 4d). We then asked whether the increase in the number and percentage of Tim-3+ cells was owing to (1) increased conversion from Slamf6+ cells4,16,17; (2) increased proliferative capacity of either population; or (3) increased persistence (Fig. 4e). First, to test conversion, we isolated antigen-experienced control or Ptpn2-deleted Slamf6+ cells (Fig. 4e) and restimulated them in vitro with signals 1–3 (anti-CD3, anti-CD28 and IFN-α together)31, as well as IL-2, and found that Ptpn2-deleted Slamf6+ cells preferentially formed Slamf6+Tim-3+ and Slamf6–Tim-3+ subsets compared to control cells (Fig. 4f). Second, we tested...
Fig. 4 | Ptpn2 deletion increases Tim-3+ cell differentiation and proliferation. a, Quantification of frequencies of cotransferred control or Ptpn2-deleted CD8+ T cells 4 d after LCMV clone 13 infection. Frequencies at day 4 were normalized to input frequencies at day 0. Data are representative of two independent experiments, n = 5 mice. b, Representative flow cytometry plots of Slamf6 and Tim-3 expression on splenic CD8+ T cells 4 d after LCMV clone 13 infection for cotransferred control and Ptpn2-deleted cells. Data are representative of two independent experiments, n = 5 mice. c, Quantification of Slamf6+ Tim-3+ and Slamf6 Tim-3− and Slamf6 Tim-3− subsets in b. Data are representative of two independent experiments, n = 5 mice. D, Quantification of expression of granzyme B in day 8 Tim-3−, Slamf6−, Slamf6+ Tim-3−, and Slamf6+ Tim-3+ cells. Data are representative of two independent experiments, n = 5 mice. e, Schematic of in vitro conversion and CTV proliferation assays using cotransfected control or Ptpn2-deleted CD8+ T cells isolated at 8 d after LCMV clone 13 infection. f, Quantification of Slamf6+ Tim-3+, Slamf6+ Tim-3− and Slamf6+ Tim-3− subsets following in vitro stimulation (anti-CD3/CD28 with IL-2 and IFN-α) of control or Ptpn2-deleted CD8+ T cells isolated at 8 d after LCMV clone 13 infection. g, Quantification of frequency of divisions following in vitro stimulation (anti-CD3/CD28 with IL-2) of CTV-labeled Slamf6− (g) or CTV-labeled Tim-3− (h) control or Ptpn2-deleted CD8+ T cells isolated at 8 d after LCMV clone 13 infection. Data are representative of two independent experiments, n = 5 mice. i, Schematic of in vivo persistence assay of Tim-3+ cells. j, Quantification of frequency of divisions following in vitro stimulation (anti-CD3/CD28 with IL-2 and IFN-α) of control or Ptpn2-deleted CD8+ T cells isolated at 8 d after LCMV clone 13 infection. Data are representative of two pooled experiments, n = 8 mice. Bar graphs represent the mean and error bars represent the s.d. Statistical significance was assessed by two-sided Student’s paired t-test (a, c, d, f, h) or two-sided Student’s unpaired t-test (j) (NS, P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). See also Supplementary Fig. 4. Panels e,i adapted from ref. 28, Springer Nature Ltd.
proliferative capacity following restimulation and found that antigen-experienced Ptpn2-deleted Slamf6+ cells underwent more rounds of division than control Slamf6+ cells (Fig. 4g and Supplementary Fig. 4a,b). When BrdU incorporation was assessed previously, there was comparable incorporation between control and Ptpn2-deleted Slamf6+ cells, indicating similar active proliferation (Fig. 2h). Here, the increase in CellTrace Violet (CTV) dilution signifies enhanced proliferative capacity of the Ptpn2-deleted Slamf6+ cells compared to control cells. We also compared the proliferative capacity of antigen-experienced control and Ptpn2-deleted Tim-3+ cells following restimulation and found that Ptpn2-deleted Tim-3+ cells also underwent more rounds of division than control Tim-3+ cells (Fig. 4h and Supplementary Fig. 4c,d). Thus, while the Ptpn2-deleted Slamf6+ and Tim-3+ cells have a similar frequency of actively proliferating cells compared to control cells (Fig. 2f,h), they had the greatest difference in proliferative capacity (Fig. 4g,h).

To examine the persistence of Tim-3+ cells in vivo, we transferred antigen-experienced control or Ptpn2-deleted Tim-3+ cells separately to new recipients infected with LCMV clone 13 (Fig. 4i). We recovered an increased number of Ptpn2-deleted Tim-3+ cells compared to control cells in the spleen and liver (Fig. 4j and Supplementary Fig. 4e). We also found a decrease in survival of the recovered Ptpn2-deleted Tim-3+ cells (Supplementary Fig. 4f), suggesting that their increased cell number was likely owing to a proliferative advantage (Fig. 4h), consistent with the known role of PTPN2 in regulating the proliferative capacity of naive CD8+ T cells by dephosphorylation of kinases downstream of the TCR. Thus, these findings demonstrate that Ptpn2 deletion affects the generation of Tim-3+ cells in multiple ways: by enhancing conversion of Slamf6+ cells into Tim-3+ cells and increasing the proliferative capacities of both Slamf6+ and Tim-3+ cells.

**Ptpn2 deletion increases Tim-3+ cell differentiation through enhanced IFN-α signaling.** We next determined the factors driving the increased differentiation of Slamf6+ cells into Tim-3+ cells following Ptpn2 deletion. We evaluated the necessity of IL-2 and IFN-α, as these cytokines have important roles in the differentiation of the Tim-3+ subpopulation during LCMV clone 13 infection and lead to phosphorylation of STAT5 and STAT1, respectively, both known targets of PTPN2 (refs. 5,8). We stimulated control or Ptpn2-deleted naive CD8+ T cells with anti-CD3/CD28 plus IL-2, IFN-α, both IL-2 and IFN-α, or blocking antibodies to abolish IL-2 and IFN-α signaling. Stimulation with IL-2, IFN-α and IL-2 plus IFN-α led to increased CD25 expression on Ptpn2-deleted cells compared to control cells (Supplementary Fig. 5a), indicating increased activation. Consistent with antigen-experienced Slamf6+ cells, stimulation of naive Slamf6+ cells with IL-2 plus IFN-α resulted in a decreased percentage of Slamf6+Tim-3+ cells and an increased percentage of Slamf6+Tim-3+ and Slamf6+ Tim-3+ cells in the Ptpn2-deleted cells, compared to control cells (Figs. 5a-c and Supplementary Fig. 5b). However, the addition of IL-2 or IFN-α separately was unable to recapitulate the marked increase of Slamf6+Tim-3+ cells observed following treatment with the combination (Fig. 5c). Moreover, CD28 costimulation was also required for the differentiation of Slamf6+Tim-3+ cells (Supplementary Fig. 5c). We also ruled out the contribution of soluble factors, as conditioned supernatant from Ptpn2-deleted cells did not increase the percentage of Slamf6+Tim-3+ cells compared to control cells (Supplementary Fig. 5d). Thus, IL-2, IFN-α and CD28 were all required for the enhanced generation of Tim-3+ cells in Ptpn2-deleted CD8+ T cells in our in vitro stimulation assay.

Given the requirement for IFN-α in vitro and the known role for IFN-I signaling in the regulation of Tim-3+ and Slamf6+ subpopulations, we next investigated the impact of Ptpn2 deletion on STAT1 phosphorylation. Ptpn2-deleted cells had an increased percentage and duration of pSTAT1 expression (Fig. 5d), despite comparable levels of the type 1 interferon receptor (IFNAR1) (Supplementary Fig. 5e). This increase in pSTAT1 was observed in both Slamf6+ and Tim-3+ subsets (Fig. 5e,f). Consistent with this, IFN-I signaling was required for the early expansion of Ptpn2-deficient cells (Fig. 5g) and the increased differentiation of Ptpn2-deleted cells into Tim-3+ cells (Fig. 5h). Given PTPN2 regulates common signaling molecules, such as STAT1 and JAK1, within the type 1 and 2 IFN pathways, we examined whether IFN-γ was required for the increased Tim-3+ subset differentiation of Ptpn2-deleted CD8+ T cells during LCMV clone 13 infection. Following IFN-γ blockade, Ptpn2-deleted CD8+ T cells still had significantly increased expansion and elevated Slamf6+ to Tim-3+ subset differentiation compared to control cells (Supplementary Fig. 5f and Fig. 5i), but were decreased in percentage compared to isotype-treated Ptpn2-deleted cells, indicating a nonessential role for IFN-γ in the generation of Tim-3+ cells (Supplementary Fig. 5g). Overall, these findings indicate that IFN-I signaling plays a crucial role in the differentiation of Ptpn2-deleted cells into Tim-3+ cells.

**Loss of Ptpn2 enhances Tim-3+CD8+ T cell differentiation in tumors.** Given the importance of dysfunctional T cell subpopulations in tumors, we next asked whether Ptpn2 also regulates the balance and functions of CD8+ T subpopulations in responses to tumors. We cotransferred congenically marked naive OT-1 TCR transgenic (specific for the ovalbumin (OVA) CD8 epitope OVA257–264) Ptpn2-deleted and control CD8+ T cells to wild-type recipients and subsequently challenged these mice with MC38-OVA tumors. Consistent with chronic LCMV infection, Ptpn2-deleted OT-1 CD8+ T cells significantly outcompeted control CD8+ T cells at day 7 in the tumor (Fig. 6a,b) and Supplementary Fig. 6a). Ptpn2 deletion also led to an increase in CD25 and a decrease in CD127 expression in transferred CD8+ T cells in the tumor-draining lymph node (Supplementary Fig. 6b,c), indicating increased activation of these cells. In addition, Ptpn2-deleted cells had increased generation of IFN-γ+TNF+ cells following peptide restimulation in vitro (Supplementary Fig. 6d). Transcriptional profiling of control or Ptpn2-deleted CD8+ T cells revealed that Ptpn2-deleted CD8+ T cells were significantly enriched for the tumor infiltrating lymphocytes (TIL) Tim-3+ signature, whereas control cells were enriched for the TIL Slamf6+ signature (Fig. 6e and Supplementary Table 3) (ref. 9). In addition, Ptpn2-deleted cells were significantly enriched for nTORC1 signaling and several other effector-related signatures that were also enriched in the Ptpn2-deleted cells in the LCMV model (Fig. 6d). Consistent with this, Ptpn2 deletion increased the percentages of granzyme B–expressing OT-1 CD8+ T cells in the tumor, draining lymph node and spleen, compared to control OT-1 CD8+ T cells (Fig. 6e). To determine the functional effect of these cells, we transferred control or Ptpn2-deleted OT-1 CD8+ T cells to recipient mice implanted with B16-OVA tumors (Fig. 6f). Ptpn2-deleted CD8+ T cells had a significant effect on B16-OVA tumor growth and led to clearance of 25% of the tumors (Fig. 6g,h). Moreover, when control and Ptpn2-deleted cells were cotransferred in the B16-OVA model, Ptpn2-deleted OT-1 T cells had an increased percentage of the Slamf6+Tim-3+ subset compared to control cells (Fig. 6i). Thus, concordant with the LCMV clone 13 model, Ptpn2-deleted CD8+ T cells outcompete control cells in the tumor, have elevated Tim-3 and granzyme B expression and possess increased effector function.

**Deletion of Ptpn2 enhances CD8+ T cell responses to tumors and checkpoint blockade efficacy.** PTPN2 is ubiquitously expressed in the hematopoietic compartment and has roles in myeloid, T and B cell development and function. Thus, therapeutic targeting of PTPN2 could potentially affect multiple immune subtypes. To model this, we investigated whether Ptpn2 deletion in all hematopoietic cells would attenuate tumor growth by subcutaneously implanting MC38 tumors directly into the chimeras (Fig. 7a
Fig. 5 | *Ptpn2 deletion increases Tim-3+ cell differentiation through enhanced IFN-α signaling. a–c, Quantification of Slamf6+Tim-3+ (a), Slamf6+Tim-3+ (b) and Slamf6–Tim-3+ (c) subsets following in vitro stimulation (anti-CD3/CD28) of control or *Ptpn2-deleted naive CD8+ T cells in the presence of indicated cytokines or blocking antibodies. Data are representative of two pooled experiments, n ≥ 4 technical replicates. d, Quantification of pSTAT1 expression in Slamf6–Tim-3–, Slamf6+Tim-3+ and Slamf6–Tim-3+ cells following ex vivo stimulation with IFN-α. Data are representative of two independent experiments, n = 5 biological replicates. e, Quantification of pSTAT1 in Slamf6–Tim-3–, Slamf6+Tim-3+ and Slamf6–Tim-3+ cells following ex vivo stimulation with IFN-α. Data are representative of two independent experiments, n = 5 biological replicates. f, Quantification of frequencies of cotransferred control and *Ptpn2-deleted cells following ex vivo stimulation (anti-CD3/CD28) of control or *Ptpn2-deleted naive CD8+ T cells in the presence of indicated cytokines or blocking antibodies. Data are representative of two pooled experiments, n ≥ 4 technical replicates. g, Quantification of pSTAT1 in Slamf6–Tim-3–, Slamf6+Tim-3+ and Slamf6–Tim-3+ cells following ex vivo stimulation (anti-CD3/CD28) of control or *Ptpn2-deleted naive CD8+ T cells in the presence of indicated cytokines or blocking antibodies. Data are representative of two pooled experiments, n ≥ 4 technical replicates. h, Quantification of frequencies of cotransferred control and *Ptpn2-deleted cells following ex vivo stimulation (anti-CD3/CD28) of control or *Ptpn2-deleted naive CD8+ T cells in the presence of indicated cytokines or blocking antibodies. Data are representative of two pooled experiments, n ≥ 4 technical replicates. i, Quantification of pSTAT1 in Slamf6–Tim-3–, Slamf6+Tim-3+ and Slamf6–Tim-3+ cells following ex vivo stimulation (anti-CD3/CD28) of control or *Ptpn2-deleted naive CD8+ T cells in the presence of indicated cytokines or blocking antibodies. Data are representative of two pooled experiments, n ≥ 4 technical replicates. Bar graphs represent the mean and error bars represent the s.d. Statistical significance was assessed by two-way ANOVA (a–c) or two-sided Student’s paired t-test (d–i) (NS, P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). See also Supplementary Fig. 5.
Fig. 6 | Loss of Ptpn2 enhances Tim-3+ CD8+ T cell differentiation in tumors. a, Representative flow cytometry plot of control or Ptpn2-deleted OT-1 T cells in the tumor 7 d after MC38-OVA injection. Data are representative of two independent experiments, n = 4 biological replicates. b, Quantification of frequencies of cotransferred control or Ptpn2-deleted CD8+ T cells 7 d after MC38-OVA injection. Frequencies at day 7 were normalized to input frequencies at day 0. Data are representative of two independent experiments, n = 4 biological replicates. c, d, GSEA TIL Slamf6+ versus Tim-3+ Up top 50 and TIL Slamf6+ versus Tim-3+ Down top 50 signature enrichment (c) and GSEA effector signatures for cotransferred control or Ptpn2-deleted OT-1 T cells in MC38-OVA tumors (d) 7 d after injection. Data are representative of one experiment, n = 3 pooled mice and two technical replicates. e, Quantification of granzyme B expression in cotransferred OT-1 CD8+ T cells 7 d after MC38-OVA implantation in the tumor, draining lymph node and spleen for control and Ptpn2-deleted cotransferred mix as in a. Data are representative of two independent experiments, n ≥ 3 mice. f, Schematic of adoptive transfer of either control or Ptpn2-deleted naive OT-1 CD8+ T cells separately to mice challenged with B16-OVA 1 d after transfer of T cells. g, Tumor growth curves for B16-OVA tumors following transfer of naive OT-1 control or Ptpn2-deleted CD8+ T cells separately into wild-type recipients that were implanted with B16-OVA cells. Data are representative of two independent experiments, n = 8 mice. h, Survival curves of mice in g. i, Quantification of Slamf6+Tim-3+; Slamf6+Tim-3+ and Slamf6+Tim-3+ subsets 9 d after B16-OVA implantation in the tumor for cotransferred control and Ptpn2-deleted cells as in a. Data are representative of two independent experiments, n = 7 mice. Bar graphs represent the mean and error bars represent the s.d. (except for g, where error bars represent the s.e.m.). Statistical significance was assessed by a two-sided Student’s paired t-test (b,e,i), two-sided Kolmogorov-Smirnov test (c,d), two-way ANOVA (g) or two-sided log-rank Mantel-Cox test (h) (NS, P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). See also Supplementary Fig. 6. Panel f adapted from ref. 21, Springer Nature Ltd.

Supplementary Fig. 7a). Given PTPN2 has a role in myeloid and regulatory T cells5,34, we examined these cell types in the blood of the chimeras at steady state and found similar frequencies of myeloid and regulatory T cell subsets in the blood, but elevated major histocompatibility complex (MHC)-II+ monocytes in the Ptpn2-deleted chimeras, compared to controls (Supplementary Fig. 7b). In addition, the Ptpn2-deleted chimeras did not have elevated inflammatory cytokine levels compared to controls (Supplementary Fig. 7c).

We next evaluated tumor growth in these chimeras and found that deletion of Ptpn2 led to complete MC38 tumor clearance in all Ptpn2-deleted chimeras, whereas there was progressive tumor growth in control chimeras (Fig. 7b and Supplementary Fig. 7d). There were no differences in the absolute numbers of CD4+ T cells, CD8+ T cells and myeloid cells in the MC38 tumors before clearance (Supplementary Fig. 7e). However, the frequency and number of Slamf6+Tim-3+CD8+ T cells was increased in the tumors of Ptpn2-deficient chimeras compared to control chimeras (Fig. 7c,d).
Fig. 7 | Deletion of Ptpn2 enhances CD8⁺ T cell responses to tumors and checkpoint blockade efficacy. a. Schematic for MC38-WT tumor challenge in chimeric mice where approximately 50% of immune cells express a control sgRNA or a Ptpn2-targeting sgRNA. WT, wild type. b. Tumor growth curves for control or Ptpn2-deleted chimeric mice following 1 × 10⁶ cell MC38-WT challenge. Representative of two independent experiments, n ≥ 8 mice. c,d. Quantification of frequency of Tim-3⁺, Slamf6⁻ and Tim-3⁻, Slamf6⁺ subsets in CD8⁺ T cells infiltrating day 9 MC38 tumors in control or Ptpn2-deleted bone marrow chimeras. Data are representative of two independent experiments, n ≥ 9 mice. e,f. Quantification of frequency of granzyme B⁺ CD8⁺ T cells infiltrating day 9 MC38 tumors implanted in control or Ptpn2-deleted bone marrow chimeras. Data are representative of two independent experiments, n ≥ 9 mice. g,h. Quantification of frequency of granzyme B⁺ CD8⁺ T cells from control or Ptpn2-deleted bone marrow chimeras 14 d after MC38 tumor implantation, pre-gated on CD8⁺Vex⁺ cells. Data are representative of two independent experiments, n = 5 mice. h,i. Tumor growth curves for control or Ptpn2-deleted bone marrow chimeras challenged with 1 × 10⁶ B16 tumor cells treated with GVAX (green triangles) on days 1, 4 and anti-PD-1 (black triangles) on days 12, 14, 16, 18, 20, 22, 24 and 26. Data are representative of two independent experiments, n ≥ 9 mice. j. Quantification of frequency of granzyme B⁺ CD8⁺ T cells from the blood of chimeras in i, 14 d after B16 tumor implantation, pre-gated on CD8⁺Vex⁺ cells. Data are representative of two independent experiments, n = 5 mice. Bar graphs represent the mean and error bars represent the s.d. (except for h,i where error bars represent the s.e.m.). Statistical significance was assessed by two-way ANOVA (b,d,h,i) or a two-sided Student's unpaired t-test (e-g,j) (NS, P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). See also Supplementary Fig. 7. Panel a adapted from ref. 28, Springer Nature Ltd.
whereas the number of Slamf6+Tim-3 CD8+ T cells was unchanged. Furthermore, the frequency and number of granzyme B+ CD8+ T cells was also increased in tumors of Ptpn2-deficient chimeras compared to control chimeras (Fig. 7c,f). In addition, peripheral blood CD8+ T cells of Ptpn2-deleted chimeras had significantly more granzyme B+ cells, fewer CD127+ cells, more Slamf6+Tim-3+ cells and more CD44+CD62L− cells (Fig. 7g and Supplementary Fig. 7f–i). CD8+ T depletion studies revealed that CD8+ T cells are required for clearance of MC38 tumors in Ptpn2-deleted mice (Fig. 7h and Supplementary Fig. 7j). Furthermore, Ptpn2-deleted chimeras that completely eliminated primary tumors could clear a larger secondary challenge of MC38 tumor cells in a CD8+ T cell–dependent manner (Supplementary Fig. 7k,l).

We next asked whether Ptpn2 deficiency in the immune system could improve PD-1 ICB responses to a more immunorefractory model, B16 melanoma. Treatment of B16-challenged Ptpn2-deficient chimeras with PD-1 ICB and therapeutic vaccination (GVAX) resulted in attenuated tumor growth and increased survival compared to control chimeras (Fig. 7i and Supplementary Fig. 7m). In addition, 26% of the anti-PD-1-treated Ptpn2-deleted chimeras completely cleared their tumors, in contrast to progressive tumor growth in all control chimeras. This enhanced response to B16 melanoma was accompanied by an increase in granzyme B+ CD8+ T cells in the peripheral blood (Fig. 7j). These findings demonstrate that Ptpn2 deficiency in the immune system increases the cytotoxic CD8+ T cell response in the tumor and ultimately leads to a CD8+ T cell–dependent clearance of MC38 tumors and improved PD-1 ICB responses to B16 tumors.

**Discussion**

The mechanisms that govern the generation and balance of the terminally exhausted and progenitor exhausted subpopulations in chronic infection and cancer remain unknown. Here we demonstrate that deletion of Ptpn2 in CD8+ T cells enhances anti-tumor immunity by increasing the formation of the Tim-3+ subset. We find that early and late time points that Ptpn2 deletion promotes effector-related gene expression in both the progenitor (Slamf6+) and terminally exhausted (Tim-3+) subsets, indicating Ptpn2 deletion alters both the balance and functionality of the exhausted subpopulations. Deletion of Ptpn2 also promotes IFN-α signaling, which accelerates Tim-3+ cell differentiation at an early time point. Furthermore, deletion of Ptpn2 in the immune system leads to complete clearance of MC38 tumors and improves PD-1 ICB responses to B16 tumors. Overall, these findings improve our understanding of the differentiation and role of the Tim-3+ subpopulation during anti-tumor immune responses.

Our work implicates PTPN2 as a new regulator of the balance between the Tim-3+ and Slamf6+ populations. PTPN2 has a crucial effect on this balance primarily at early time points during LCMV clone 13 infection, where the Tim-3+ and Slamf6+ subpopulations differentiate from early effector cells into exhausted cells. PTPN2 has a multitude of targets to dephosphorylate within the TCR, IL-2, IL-7 and IFN signaling cascades. Here we show that Ptpn2 deletion leads to enhanced IFN-I signaling, which is required for the early competitive advantage seen in Ptpn2-deleted CD8+ T cells, as well as the enhanced early differentiation of Slamf6+Tim-3+ cells into Slamf6+Tim-3+ and Slamf6+Tim-3+ cells. These findings are consistent with IFN-I signaling attenuating the TCF1–Bcl6 axis during LCMV infection, resulting in an increase in the percentage of Tim-3+ cells, and highlight a crucial role for IFN-I signaling early in the differentiation of terminally exhausted cells. We also find a nonessential role for IFN-γ signaling in promoting the differentiation of Tim-3+ cells in Ptpn2-deleted CD8+ T cells, as would be expected given its shared signaling pathway members with IFN-I. Additional studies will be needed to determine whether IFN-γ has a direct role on CD8+ T cells or an indirect role on MHC-I expression in the host. Moreover, we find an essential role for IL-2 in vitro for enhancing Tim-3+ subset formation in Ptpn2-deleted cells, where it likely works in conjunction with IFN-α to support proliferation. This is consistent with a requirement for proliferation of CXCR5+ progenitor cells to differentiate into Tim-3+ terminally exhausted cells during LCMV clone 13 infection. Overall, these findings help to elucidate the molecular mechanisms controlling CD8+ T cell fate decisions into progenitor or terminally exhausted subpopulations in response to LCMV infection.

Currently, it is believed that an increase in the progenitor exhausted subpopulation promotes the efficacy of PD-1 ICB in chronic infection and cancer. However, the Tim-3+ subpopulation is the primary cytotoxic population, and thus also plays an important role in immune responses. It is likely that both progenitor exhausted and terminally exhausted cells are required for an effective immune response that balances cytotoxic potential and longevity. Our work represents a scenario where Ptpn2 deletion causes an early increase in the cytotoxic Tim-3+ subpopulation without altering the number of progenitor Slamf6+ CD8+ T cells. This occurs because: (1) Ptpn2-deleted Slamf6+ cells have increased conversion into Tim-3+ cells; (2) Ptpn2-deleted Slamf6+ cells have increased proliferative capacity and thus can replenish the Slamf6+ cell pool; and (3) Ptpn2-deleted Tim-3+ cells have increased proliferative capacity, which further expands their numbers. This early increase in the number of cytotoxic cells and their inherent cytotoxic potential results in clearance of MC38 tumors and improved responses of B16 tumors to PD-1 blockade. These findings can be reconciled with data showing that an increase in the progenitor subpopulation improves checkpoint blockade responses by considering that following PD-1 blockade, the progenitor exhausted subpopulation expands and converts into terminally exhausted cytotoxic Tim-3+ cells. Our findings suggest that both the progenitor and terminally exhausted subpopulations can promote anti-tumor immunity and that Ptpn2 deletion leads to improved tumor immunity through an increase in Tim-3+ cytotoxic cells, without depleting the progenitor subpopulation.

Finally, this work supports the development of PTPN2 inhibitors for cancer immunotherapy and the deletion of PTPN2 in chimeric antigen receptor T cell–based therapies. PTPN2 has a cell-intrinsic role in CD8+ T cells in tumors, limiting their accumulation and expression of granzyme B, consistent with Ptpn2-deficient CD8+ T cell responses in the LCMV model and the RIP-mOVA model of diabetes. Moreover, adoptive transfer of Ptpn2-deleted CD8+ T cells potently attenuates the growth of B16-OVA tumors, demonstrating that the increase in Tim-3+ cells following Ptpn2 deletion has a therapeutic benefit on tumor immunity. Furthermore, deletion of Ptpn2 in the whole hematopoietic compartment leads to a CD8+ T cell–dependent complete clearance of MC38 tumors, accompanied by a significantly elevated systemic cytotoxic CD8+ T cell response, which could be beneficial for enhancing immunity to disseminated metastatic disease. In the immune system also improves PD-1 ICB responses to B16 tumors, indicating its potential use as a combination therapy with PD-1 blockade.

PTPN2 is a particularly attractive cancer immunotherapy target given its established tumor-intrinsic role in restraining anti-tumor immunity. Inhibition of PTPN2 in a tumor-bearing host would enhance IFN-γ signaling within tumor cells, thereby increasing MHC-I expression and sensitivity to IFN-γ-mediated apoptosis. Increased MHC-I expression would also promote TCR-driven differentiation of exhausted T cells into the Tim-3+ population. In addition, PTPN2 inhibition in CD8+ T cells would increase IFN-I signaling and further enhance the formation and effector function of the cytotoxic Tim-3+ population. Overall, these findings provide the rationale for combining PTPN2 inhibition and PD-1 ICB for cancer immunotherapy and for deletion of PTPN2 in chimeric antigen receptor T cell therapies.
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Competing interests
A.H.S. has patents on the PD-1 pathway licensed by Roche/Genentech and Novartis, consults for Novartis, is on the scientific advisory boards for Surface Oncology, Sqz Biotech, Ellstar Therapeutics, Elpiscience, Selecta and Monopteros and has research funding from Merck, Novartis, Roche, Ipsen, UCB and Quark Ventures. W.N.H. has a patent application on T cell exhaustion-specific enhancers held by the Dana-Farber Cancer Institute and now is employed by Merck. W.N.H. is also a founder of Arsenal Biosciences. A.H.S. and W.N.H. have a patent application on PTPN2 as a therapeutic target held/submitted by the Dana-Farber Cancer Institute. G.J.F. has a consulting or advisory role for Novartis, Lilly, Roche/Genentech, Bristol-Myers Squibb, Bethyl Laboratories, Xiao Therapeutics, Quiet Therapeutics and Seattle Genetics; patents, royalties or other intellectual property from Novartis, Roche/Genentech, Bristol-Myers Squibb/Medarex, Amplimmune/Astrazeneca, Merck, EMD Serono and Boehringer Ingelheim and research funding from Bristol-Myers Squibb. The remaining authors declare no competing interests.

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Methods

Mouse breeding and production. Seven- to ten-week-old female or male mice were used for all experiments and 7- to 14-week-old female or male mice were used as donors for bone marrow chimera experiments. Wild-type C57BL/6J mice were purchased from the Jackson Laboratory. LoxP-STOP-loxP donor and LSK recipient mice (B6.129(6N)-Gt(ROSA) 26Sortm1(CAG-cas9*,-EGFP)Fehz/J) were a generous gift from F. Zhang, Massachusetts Institute of Technology. These mice were bred to Zp3-Cre mice (C57BL/6-Tg(Zp3-Cre)Gwh/J) to induce the loxP-STOP-loxP in the female germline. The resulting Cas9-expressing strain was then bred to OT1 (C57BL/6-Tg(Tcrz/rb1)100Mbj/J) or P14 (Taconic B6-Cg-Tcrz/rb1Mom Tg(Tcrz/LCMV)327Sdz backcrossed ten generations to Jackson C57BL/6J) TCR transgenic mice on the CD4.1 (B6.SJL-Plpaca Pepck/Bly) congenic background. All strains were backcrossed at least ten generations to Jackson C57BL/6J. The sample size was chosen to ensure the possibility of statistical analysis and minimize the use of animals. Data exclusion was not used.

E.J. Wherry, University of Pennsylvania) were cultured in EMEM supplemented with 10% FBS and 1% penicillin/streptomycin. All cell lines were confirmed as wild-type recipients.

Seven- to ten-week-old female or male mice (B6J.129(B6N)-Gt(ROSA) 26Sortm1(CAG-cas9*,-EGFP)Fezh/J) were a generous gift from F. Zhang, Massachusetts Institute of Technology. These mice were bred to Zp3-Cre mice (C57BL/6-Tg(Zp3-Cre)Gwh/J) to induce the loxP-STOP-loxP in the female germline. The resulting Cas9-expressing strain was then bred to OT1 (C57BL/6-Tg(Tcrz/rb1)100Mbj/J) or P14 (Taconic B6-Cg-Tcrz/rb1Mom Tg(Tcrz/LCMV)327Sdz backcrossed ten generations to Jackson C57BL/6J) TCR transgenic mice on the CD4.1 (B6.SJL-Plpaca Pepck/Bly) congenic background. All strains were backcrossed at least ten generations to Jackson C57BL/6J. The sample size was chosen to ensure the possibility of statistical analysis and minimize the use of animals. Data exclusion was not used.

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OVA experiments mice were challenged with 2.5×10^5 B16-OVA tumor cells subcutaneously. Tumors were measured every 2–3 days once palpable by a caliper. Tumor volume was determined by the formula for an ellipsoid: 1/2 × D1 × D2 where D is the longer diameter and d is the shorter diameter. Mice were euthanized when tumors ulcerated, reached 2 cm^3 or a body condition score >2 was observed.

To deplete CD8^+ T cells, mice were injected intraperitoneally with 100μg of anti-CD8\(\text{mAb}\) (53-5.8 BioXCell catalog no. BE0223) or isotype (HRPBN BioXCell catalog no. BE0088) on days –3, 0, 3, 6 and 9 and 200μg of anti-CD6 (2.43 BioXCell catalog no. BE0061) or isotype (LT-2 BioXCell catalog no. BE0090) on days 12, 15, 18, 21 and 24 (relative to MC38 tumor injection on day 0). For depletion of CD8^+ T cells in secondary rechallenge experiments, mice were injected intraperitoneally with 100μg of anti-CD8\(\text{mAb}\) (53-6.7 BioXCell catalog no. BE0004-1) or isotype (2A3 BioXcell catalog no. BE0089) on days –3, 0, 3 and 6 (relative to MC38 rechallenge on day 0).

Tumor infiltrating lymphocyte isolation. Tumors were excised and minced mechanically when tumors ulcerated, reached 2 cm^3 or a body condition score >2. Tumors were then digested in collagenase for 10 min at 37°C. Lymphocytes were enriched using an Optiprep gradient (Sigma-Aldrich catalog no. D1556).

Monitoring of T cell responses in the blood. To monitor the stability of Vex transduction, mice were bled via the tail vein. Blood was then lysed twice using ACK lysis buffer, stained and analyzed by flow cytometry. For monitoring of T cell responses to LCMV infection or tumor, mice were anesthetized with isoflurane (Henyx, 4% Isoflurane in oxygen, 0.3L/min, respiratory rate 150 bpm). After sacrifice, tumors and spleens were isolated by centrifugation at 400g on a histopaque-1083 gradient (Sigma-Aldrich catalog no. 10831-6X100ML) and stained for flow cytometry.

In vitro T cell differentiation assay using naive CD8^+ T cells. Naive CD8^+ T cells were obtained from spleens of control and Ptpn2-deleted chimeric mice as described above. CD4^− T cells were then activated on plate-bound anti-CD3 (OKT-3, BioXCell catalog no. BE0001) (5 μg/ml) with or without anti-CD28 (37.51, BioXCell catalog no. BE00015) (5 μg/ml) and supplemented with 200 μU/ml IL-2 (R&D Systems catalog no. 202-IL), 1000 μU/ml IFN-α (PBL assay Science catalog no. 12105-1), 50μg/ml anti-IL-2 (S486-1, JS661A2, BioXCell catalog no. BE0043-1, BE0043) or 50 μg/ml anti-IFNAR blocking antibodies for 72h. For supernatant transfer experiments, supernatant was isolated from wells of control or Ptpn2-deleted stimulated T cells (72h poststimulation as above). The supernatant was added to wild-type naive CD8^+ T cells activated on plate-bound anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml) and supplemented with 200 μU/ml IL-2, 1000 μU/ml IFN-α, 50 μg/ml anti-IL-2 or 50 μg/ml anti-IFNAR blocking antibodies for 72h.

In vitro cytotoxicity assay using antigen-experienced Tim-3^− CD8^+ T cells. Naive P14 control and Ptpn2-deleted CD8^+ T cells were cotransferred to wild-type recipient mice (500:500 mix) on day –1, and the mice were infected with LCMV clone 13 on day 0 (as above). CD4^− T cells were depleted on days –1 and 1 (as above). Splenocytes were isolated on day 6 after infection and enriched for CD8^+ T cells using CD8\(\text{mAb}\) + MACS kit. CD8^+ T cells were stimulated on plate-bound anti-CD3 (10μg/ml) and anti-CD28 (10μg/ml) with 1000 μU/ml IFN-γ for 0, 2, 5, 10, 15 and 30 min at 37°C. After stimulation, cells were pelleted at 800g, fixed in 2% methanol-formaldehyde (Cell Signaling Technology catalog no. 12606) and permeabilized with ice-cold 90% methanol for 20 min on ice. Cells were then washed with MACS buffer and stained with pSTAT1 antibody.

Quantification of serum cytokines. Serum was collected from control or Ptpn2-deleted chimeric mice 1 week after reconstitution. Inflammatory cytokines (IL-6, IL-10, MCP-1, IFN-γ, TNF and IL-12p70) were measured using a BD Cytometric Bead Assay Mouse Inflammation Kit (BD Biosciences catalog no. 552364).

ATAC-seq library preparation and analysis. 50,000 cotransfected control or Ptpn2-deleted Slamf6^+ Tim-3^− P14 T cells per replicate were sorted from spleens of day 8 LCMV clone 13-infected mice into PBS with 10% FBS. Pelleted cells were incubated in 50μl of reaction mix (containing 2X Tagmentation DNA buffer, Tr5 enzyme and 2% digitonin in nuclease-free water) as previously described. The Tagmentation reaction was performed at 30°C for 10 min followed by 300 r.p.m. DNA was then purified using a MinElute Reaction Cleanup kit (Qiagen catalog no. 28206). A post-PCR cleanup was performed using Agencourt AMPure XP beads (Beckman Coulter catalog no. A63880) and library quality was verified using Tapestation analysis. Samples were sequenced on an Illumina NextSeq500 sequencing using 37 base-pair paired-end reads.

Quality trimming and primer removal within raw fastq files were done with Trimmomatic 0.33 using the following parameters: LEADING: 15, TRAILING: 15, SLIDINGWINDOW: 4:15, MINLEN: 36. Trimmed reads were aligned to mm9 with Bowtie2 v.2.2.4 using a maximum insert size of 1,000. Aligned bams were sorted, duplicates marked, and reads mapping to the blacklist region were removed. Peak-calling using MACS 2.1.1 was performed on merged bam files (Samtools v.1.3) from biological replicates using a q-value threshold of 0.001. Consensus peaks from all biological conditions were merged to create a single peak universe. Cut sites were extracted from each biological replicate and the number of cuts within each peak region were quantified to generate a raw count matrix. DESeq2 was used to normalize the counts matrix and perform differential accessibility analysis between all relevant comparisons. Tracks were visualized using Integrative Genomics Viewer v.2.3.77 (Broad Institute).

Bulk RNA-seq analysis of T cells. At day 7 or 8 after tumor or virus injection, T cells were isolated from spleens of control and Ptpn2-deleted CD8^+ T cells recruited from LCMV (as above) and replicates of 500 cells were sorted into 25 μl of buffer RT (QiAgen catalog no. 79216) + 1% beta-mercaptoethanol v/v. After flash-freezing on dry ice and storage at –80°C, lysates were converted to cDNA following capture with Agencourt RNAclean beads (Beckman Coulter catalog no. A63987) using the SmartSeq2 protocol as previously described. The cDNA was amplified using 16 PCR enrichment cycles before quantification and dual-index indexing with the Illumina Nexseq XT kit. The libraries were enriched with 12 cycles of PCR, then combined in equal volumes before final bead cleanup and sequencing on a Nexseq500 by 37 base-pair paired-end reads. After demultiplexing, low-quality base reads were trimmed with Trimmomatic using the following parameters: LEADING: 10, TRAILING: 10, SLIDINGWINDOW: 4:15, MINLEN: 16. Trimmed reads were then aligned to the mm10 mouse genome using Bowtie2. HTSeq was used to map aligned reads to genes and to generate a gene count matrix. Normalized counts and differential expression analysis were performed using the DESeq2 R package. We performed gene set enrichment analysis as previously described using signatures from the Hallmark database, the MsigDB collection and from our prior analysis of exhausted CD8^+ T cells from the spleens of LCMV clone 13 infected mice or from B16-OVA tumors.

Single-cell RNA-seq library preparation and analysis. Cotransfected control or Ptpn2-deleted P14 CD8^+ T cells were sorted from spleens of day 30 LCMV clone 13 infected, CD4-depleted mice on the basis of the markers CD8^+ CD4^− CD3^− CD45.2, Vex and fixable live/dead. Cells were counted and loaded onto the Chromium Controller (10X Genomics) for a target recovery of 5,000 single cells. Samples were processed per the manufacturer’s protocol and sequenced on an Illumina NextSeq500 sequencer using a 75-base-pair kit with paired-end reads. The Cell Ranger analysis pipeline v.1.2 was used for sample demultiplexing.
barcode processing, alignment, filtering, unique molecular identifier counting and aggregation of sequencing runs. The R Seurat package\(^5\) was used for downstream analyses. 

For each cell, two quality control metrics were calculated: (1) the total number of genes detected; and (2) the proportion of unique molecular identifiers contributed by mitochondrially encoded transcripts. Cells were excluded from downstream analysis if fewer than 200 or greater than 2,500 genes were detected and if mitochondrially encoded transcripts constituted greater than 5% of the total library, yielding an expression matrix of 7,027 cells by 13,133 genes. Each gene expression measurement was normalized by total expression within the corresponding cell and multiplied by a scaling factor of 10,000.

Mean and dispersion values were calculated for each gene across all cells; 1,829 genes were classified as highly variable. Highly variable genes were used for principal components analysis. Principal components were determined to be significant (\(P < 0.001\)) using the jackstraw method and ISNE was performed on these key principal components (1–17) using default parameters for 1,000 iterations for visualization in two dimensions. Unsupervised clustering was performed using a shared nearest neighbor modularity optimization-based algorithm\(^7\). Single-cell signature scoring using FastProject\(^7\) was performed with the Hallmark database from MSigDB and using signatures of the subpopulations derived from our prior analysis of exhausted CD8\(^+\) splenocytes from LCMV clone 13 infected mice\(^4\). Differential gene expression and signature enrichment analysis was performed using a two-sided Wilcoxon rank sum test. To determine the relative proportion of Ptpn2-deleted cells within each cluster, a two-sided binomial test was performed against the proportion of Ptpn2-deleted cells within the total dataset.

**Statistical analysis.** Statistical analyses were performed using GraphPad Prism 7 software or R 3.3.1. Data were considered statistically significant with \(P\) values <0.05 by a two-sided paired Student\'s \(t\)-test for comparing two groups in cotransfer experiments, two-sided unpaired Student\'s \(t\)-test for comparing two groups, one-way analysis of variance (ANOVA) for single comparisons with groups greater than two, two-way ANOVA for repeated measures comparisons or for multiple comparisons within groups, and a two-sided log-rank Mantel–Cox test for survival analysis. For GSEA of RNA-seq data a two-sided Kolmogorov–Smirnov test was used. For analysis of single-cell RNA-seq data, a two-sided Wilcoxon rank sum test was used for signature enrichments and a two-sided binomial test was used to determine proportional differences of control or Ptpn2-deleted cells in the clusters. For ATAC-seq analysis a hypergeometric test was used. Please see the Reporting Summary for additional details.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request. All sequencing data from this study has been deposited in the National Center for Biotechnology Information Gene Expression Omnibus and are accessible through the Gene Expression Omnibus Series accession code GSE134413.

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Reporting Summary

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Statistics

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
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- 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 wherever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- 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 analysis was performed using FACSDIVA version 8.0.1 (BD Pharmingen). Flow sorting was performed using FACSDIVA versions 6.1.3 or 8.0.2 (BD Pharmingen). Sequencing data was collected on a NextSeq500 (Illumina) or ABI3730xl DNA analyzer (Thermo Fisher Scientific).

Data analysis
All flow data was analyzed using FlowJo version 10.4.2. All statistical tests were run using R version 3.3.1. Sanger sequencing for the TIDE assay was analyzed on the online TIDE webtool. ATAC-seq data was processed/analyzed using Trimmomatic 0.33, Bowtie 2.2.4, MACS 2.1.1, Samtools 1.3, DESeq2, and Genomics Viewer 2.3.77. Bulk RNA-seq data was processed/analyzed using Trimmmomatic 0.36, Bowtie 2, HTSeq, and DESeq2. Single-cell RNA-seq was processed/analyzed using the Cell Ranger analysis pipeline 1.2, the R Seurat package 2.4, and FastProject 1.1.4.

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request. All sequencing data from this study has been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through the GEO Series accession code GSE134413.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
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Life sciences study design

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

Sample size

Sample size was chosen to ensure the possibility of statistical analysis and to also minimize the use of animals in accordance with animal care guidelines from the Harvard Medical School Standing Committee on Animals and the National Institutes of Health. The results from previous results were also used to determine the sample size.

Data exclusions

For single-cell RNA-seq, two quality control metrics were calculated for each cell: (1) the total number of genes detected and (2) the proportion of UMIs contributed by mitochondrially encoded transcripts. Cells were excluded from downstream analysis if fewer than 200 genes or greater than 2500 genes were detected and if mitochondrially encoded transcripts constituted greater than 5% of the total library. These were pre-determined exclusion criteria based on standard Seurat parameters to exclude cells that had poor capture of transcripts (low recovery of genes), doublets (high recovery of genes), or were dying (high percentage of mitochondrially encoded transcripts). Data was not excluded for any other experiment.

Replication

Replicates were used in all experiments as noted in the text and figure legends. All experiments presented for which replication was attempted were successfully replicated.

Randomization

Age and sex-matched animals were used for each experiment. Animals were also co-housed when possible.

Blinding

LCMV Clone 13 infection and tumor experiments were blinded during data collection where possible (Figures 4j, 5g-5i, 6g-6h, 7b-7j and Supplementary Figures 2f, 4e-4f, 5f-5g, 7d-7m). All co-transfer experiments are inherently 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

- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Methods

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology         |
| ☒   | Animals and other organisms |
| ☒   | Human research participants |
| ☒   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging |

Antibodies

Antibodies and dyes were purchased from BD Biosciences (7-AAD Cat# 559925 (1:100 dilution), Slamf6 Clone 13G3 Cat# 561540 (1:100 dilution), BrDU Clone 3D4 Cat# 552598 (1:50 dilution), Ki67-PerCP-Cy5.5 Clone B56 Cat# 561284 (1:100 dilution)); Biolegend (B220 Clone RA3-6B2 Cat# 103208, 103226 (1:100 dilution), CD11b Clone M1/70 Cat# 101208, 101216 (1:100 dilution), CD127 Clone A7R34 Cat# 135014, 135014 (1:100 dilution), CD25 Clone PC61 Cat# 101904 (1:100 dilution), CD3ε Clone 17A2 Cat# 100220, 100328 (1:100 dilution), CD4 Clone RM4-5 Cat# 100516, 100531, 100543 (1:100 dilution), CD44 Clone IM7 Cat# 103008, 103028, 103030 (1:100 dilution), CD45.1 Clone A20 Cat# 110708, 110716, 110741 (1:100 dilution), CD45.2 Clone 104 Cat# 109824, 109832, 109830 (1:100 dilution), CD45 Clone 53-7.3 Cat# 100608 (1:100 dilution), CD62L Clone ME1-14 Cat# 104417 (1:100 dilution), CD80 Clone 53-6.7 Cat# 100737 (1:100 dilution), CD80 Clone YT556.7.7 Cat# 126600, 126608, 126610, 126620, 126664 (1:100 dilution), c-Kit Clone ACK2 Cat# 135108 (1:100 dilution), CXCR5 Clone L13D7 Cat# 145509 (1:50 dilution), Gr-1 Clone RB6-8C5 Cat# 108408 (1:100 dilution), Granzyme B Clone GB11 Cat# 515403, 515406 (1:100 dilution), I-A/I-E Clone MS/N1A 15.2 Cat# 107614 (1:100 dilution), IFN-y Clone XM51.2 Cat# 505810 (1:100 dilution), IFNAR1 Clone MAR1-5A3 Cat# 127314 (1:100 dilution), Ly-6c Clone HK1.4 Cat# 128007 (1:100 dilution), PD-1 Clone 29F.1A12 Cat# 135206, 135209 (1:100 dilution), Sca-1 Clone D7 Cat# 108128, 108128 (1:100 dilution), TCR Vα Clone MR9-4 Cat# 139506 (1:100 dilution), TCR Vβ8 Clone K16-133.18 Cat# 118406 (1:100 dilution), TCR-119 Clone TER-119 Cat# 136208 (1:100 dilution), TCR F1 Clone 7F11A10 Cat# 655208 (1:100 dilution), Tim-3 Clone RMT3-23 Cat# 119703, 119723 (1:100 dilution), TNF Clone MP6-AT22 Cat# 506322 (1:100 dilution), TruStain fcX Clone 93 Cat# 101320 (1:50 dilution), Rat IgG2a x Isotype Clone RK2758 Cat# 400508 (1:100 dilution), Rat IgG2b x Isotype Clone RTX4530 Cat#
**Eukaryotic cell lines**

**Policy information about cell lines**

**Cell line source(s)**

MC38-OVA, B16-OVA, and MC38-GP33-41 cells were created in the Sharpe and Haining labs. BHK and Vero cells were a gift from E. John Wherry. 293x cells were a gift from C. Kadoch. B16.F10 and B16/GMCSF cells were gifts from G. Dranoff.

**Authentication**

MC38 and B16 parental cells were validated through whole exome sequencing. MC38-OVA and B16-OVA cells were validated based on selection in puromycin. MC38 GP33-41 cells were validated by flow cytometry based on expression of the selectable marker GFP. B16/GMCSF cells were validated by ELISA.

**Mycoplasma contamination**

All cell lines were confirmed mycoplasma negative.

**Commonly misidentified lines**

293x (clone of HEK). These cells were used in the production of lentivirus which was validated by titering.

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**Animals and other organisms**

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

**Laboratory animals**

Female/male C57BL/6J mice were purchased from The Jackson Laboratory and used at age 7-10 weeks. C57BL/6-Tg(Zp3-cre)1Gwh/J, C57BL/6-Tg(TcraTcrb)1100Mjb/J, and B6.5IL-Ptpcrca Pecpb/BoyI mice were purchased from The Jackson Laboratory and bred to B6.129(B6N)-Gt(ROSA)26Sortm1(CAG-cas9*,-EGFP)Fz/hJ mice (a gift from F. Zhang). B6.Cg-Tcratm1Mom Tg(TcrLCMV)327Sdz mice were purchased from Taconic, backcrossed >10 generations to C57BL/6J from The Jackson Laboratory, and then bred to B6.129(B6N)-Gt(ROSA)26Sortm1(CAG-cas9*,-EGFP)Fz/hJ mice. These strains (both female and male) were used at 8-16 weeks of age for donor bone marrow to create bone marrow chimeras. Resulting bone marrow chimeras were used at 14-20 weeks of age. Within a given experiment all stages of the experiment were sex-matched (bone marrow donor, bone marrow recipient, and transferred cell recipient).

**Wild animals**

The study did not involve wild animals.

**Field-collected samples**

The study did not involve field-collected samples.

**Ethics oversight**

HMA Standing Committee on Animals

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**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**

Bones were excised, mechanically crushed, filtered to single-cell suspension, ACK lysed, c-Kit MACS purified, and sorted. Spleen and lymph nodes were dissected from mice, mechanically minced, and filtered to single-cell suspension. RBC lysis was performed on spleen samples that were not MACS purified. Blood samples were isolated by retro-orbital bleed and enriched for lymphocytes on a Histopaque-1083 gradient. Blood samples isolated via the tail vein were ACK lysed. Livers were excised, mechanically minced, filtered to single-cell suspension, and enriched for lymphocytes on a Percoll gradient. Tumors were dissected from the surrounding fascia, mechanically minced, treated with collagenase for 10 minutes at 37°C, and filtered to single-cell suspension. Tumor-infiltrating leukocytes were enriched using an Optiprep gradient. When necessary, cells were sorted on a FACS Aria II (BD Biosciences) to obtain greater than 95% purity.

**Instrument**

BD LSR II or BD Symphony A5 were used to collect data for analysis. BD FACS Aria II was used for cell sorting.
| Software                      | All flow data was collected using FACSDIVA versions 6.1.3, 8.0.1, or 8.0.2 (BD Pharmingen) and analyzed using FlowJo version 10.4.2. |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Cell population abundance    | All sorts had a purity > 95%, checked by post-sort re-sampling.                                                                       |
| Gating strategy              | Gating strategy summarized in Figure 2 and Supplementary Figures 1 and 6, with gates drawn based on single-stain and full-minus-one (FMO) controls. |

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