In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target

Robert T. Manguso1,2,3, Hans W. Pope1,3, Margaret D. Zimmer1,3, Flavian D. Brown1,2, Kathleen B. Yates1,3, Brian C. Miller1,3,4, Natalie B. Collins1,3, Kevin B1,3, Martin W. LaFleur1,2, Vikram R. Juneja6, Sarah A. Weiss1, Jennifer Lo7, David E. Fisher7, Diana Miao2,3, Eliezer Van Allen2,3, David E. Root3, Arlene H. Sharpe5,8, John G. Doench3 & W. Nicholas Haining1,3,5

Immunotherapy with PD-1 checkpoint blockade is effective in only a minority of patients with cancer, suggesting that additional treatment strategies are needed. Here we use a pooled in vivo genetic screening approach using CRISPR–Cas9 genome editing in transplantable tumours in mice with immunotherapy to discover previously undescribed immunotherapy targets. We tested 2,368 genes expressed by melanoma cells to identify those that synergize with or cause resistance to checkpoint blockade. We recovered the known immune evasion molecules PD-L1 and CD47, and confirmed that defects in interferon-γ signalling caused resistance to immunotherapy. Tumours were sensitized to immunotherapy by deletion of genes involved in several diverse pathways, including NF-κB signalling, antigen presentation and the unfolded protein response. In addition, deletion of the protein tyrosine phosphatase PTPN2 in tumour cells increased the efficacy of immunotherapy by enhancing interferon-γ-mediated effects on antigen presentation and growth suppression. In vivo genetic screens in tumour models can identify new immunotherapy targets in unanticipated pathways.
Defects in the IFN-γ pathway induces resistance
We next analysed genes that, when deleted, become significantly enriched in immunotherapy-treated tumours, as these might represent resistance mechanisms. We observed that sgRNAs targeting five genes required for sensing and signalling through the IFN-γ pathway (Stat1, Jak1, Ifngr2, Ifngr1 and Jak2) were significantly enriched in immunotherapy-treated mice (Fig. 1e; FDR < 0.001). We confirmed this finding in an in vivo competitive assay that compared the relative growth of mixtures of isogenic Stat1-null or control B16 cells in animals treated with immunotherapy (Fig. 1f). In wild-type mice treated with GVAX and anti-PD-1 immunotherapy, the relative proportion of Stat1-null cells increased significantly (Fig. 1g, h; P < 0.01, Student’s t-test), suggesting that Stat1-null cells have a marked growth advantage over wild-type tumour cells when under immune attack. Similar results were obtained for Jak1-null and Ifngr1-null cells (Fig. 1h). Consistent with this finding, tumours deficient in Stat1 or Ifngr1 (Extended Data Fig. 4a, c) grew significantly faster than wild-type tumours when treated with immunotherapy (Extended Data Fig. 2c; P < 0.05, Student’s t-test). Similar trends were observed for Jak1-null tumours (Extended Data Fig. 2c), and in the BrafV600E/Pten−/− (Braf/Pten) mouse melanoma line (J.L. et al., submitted). Mice injected with Braf/Pten melanoma cells deficient in Stat1, Jak1 or Ifngr1 (Extended Data Fig. 4b, d) had significantly larger tumours and shorter survival than mice with wild-type tumours when treated with PD-1 blockade (Extended Data Fig. 2d; P < 0.05, Student’s t-test; P < 0.0001, log-rank test). Therefore, the efficacy of immunotherapy in both melanoma models depends in part on the sensing of IFN-γ by tumour cells.

We next sought to determine why IFN-γ-pathway mutant tumours were resistant to immunotherapy. IFN-γ-pathway-deficient B16 tumour cells had a significant growth advantage over wild-type tumour cells when exposed to IFN-γ or IFNβ (Extended Data Fig. 2e, P < 0.001, Student’s t-test). In addition, tumour cells deficient in Stat1, Jak1 or Ifngr1 failed to upregulate MHC-I presentation molecules after stimulation with IFN-γ (Extended Data Fig. 2f). Indeed, co-culture of wild-type and Stat1-null tumour cells that had been engineered to express the model antigen ovalbumin (OVA) with antigen-specific OT-I CD8+ T cells resulted in a significantly larger fraction of Stat1-null B16 cells surviving relative to wild-type B16 cells (Extended Data Fig. 2g; P < 0.001, Student’s t-test), suggesting decreased sensitivity of Stat1-null tumour cells to the effects of cytotoxic T cells. Thus the screening platform recovered both known immune evasion molecules and mechanisms of resistance to immunotherapy.

Gene targets that increase efficacy of immunotherapy
Among the 50 most-depleted genes in immunotherapy-treated mice (all with an FDR < 0.08), we identified four prominent biological pathways. sgRNAs targeting genes involved in: (i) TNF signalling/NFκB activation; (ii) antigen processing and presentation; (iii) inhibition of kinase signalling; or (iv) the ubiquitin–proteasome pathway were markedly depleted in mice treated with GVAX and PD-1 blockade (Fig. 2a) relative to growth in Tcra−/− mice. In many cases, multiple members of the same pathway (for example, Erap1, Tap2, Calr) or even the same multi-protein complex (for example, Rbck1, Rnf31, Bin2) were depleted under immune selective pressure, underscoring the importance of these diverse biological pathways.

We selected representative genes from each of these four groups to validate based on their highest cumulative score as ranked by the STARS algorithm (see Methods). These genes were Ptpn2, a phophatase involved in multiple signalling processes; H2-T23, a non-classical MHC-I gene; Ripk1, a kinase that regulates cell death and inflammation; and Stubby, an E3 ubiquitin ligase involved in the regulation of the unfolded protein response. In vivo competition assays showed that tumour cells deleted of each of the four genes were strongly selected against in wild-type animals treated with immunotherapy, but grew at equivalent rates to control tumour cells in vitro and in Tcra−/− mice (Fig. 2b, c; P < 0.01, Student’s t-test). This suggests
that loss of function of these genes renders tumour cells more sensitive to immunotherapy, but does not alter their cell growth or survival in the absence of T cells.

H2-T23 encodes Qa-1b (HLA-E in humans), a non-classical MHC molecule that binds the inhibitory receptor NKG2A on T cells and natural killer (NK) cells. We confirmed that loss of function of Qa-1b enhanced the efficacy of immunotherapy by comparing the growth of H2-T23-null B16 melanoma cells (Extended Data Fig. 4g) to control B16 tumours in mice treated with GVAX and PD-1 blockade. Immunotherapy in control tumours eradicated only 1 out of 10 tumours. By contrast, immunotherapy of H2-T23-null tumours was curative in all 10 animals (Fig. 2d, e; P < 0.05, Student’s t-test). These results indicate that Qa-1b functions an immune evasion molecule in tumours and that loss of function of H2-T23 improves tumour immunity.

Figure 2 | Loss-of-function screening identifies targets that increase the efficacy of immunotherapy. a, Frequency histograms of enrichment or depletion of sgRNAs targeting genes indicated. b, Competition assays of tumour cells lacking Ptpn2, H2-T23, Ripk1 or Stub1. c, Ratios of tumour cells lacking the indicated genes. n = 3–13 mice per group; representative of two independent experiments. d, e, Tumour volume (d) and survival analysis (e) of H2-T23-null (red) or control (grey) B16 tumours. Data are mean ± s.e.m.; n = 10 mice per group; representative of two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Figure 3 | Deletion of Ptpn2 sensitizes tumours to immunotherapy. a, b, Tumour volume for control tumours or those with gene deletions as indicated in B16 melanoma (a; n = 20 animals per group; representative of three independent experiments) and Braf/PTEN melanoma (b; n = 10 animals per group; representative of two independent experiments; data are mean ± s.e.m.). c, d, Flow plots (c) and change in ratios (d) of Ptpn2-null, Ptpn2 rescued or overexpressing B16 cells relative to control B16 cells. n = 5 animals per group. Data are mean ± s.d. *P < 0.05; **P < 0.01; ***P < 0.001.

Loss of Ptpn2 sensitizes tumours to immunotherapy

We next focused on Ptpn2, and confirmed that loss of Ptpn2 sensitized tumours to immunotherapy in vivo competition assays with two additional sgRNAs (Extended Data Fig. 3a–c; P < 0.01, Student’s t-test; all sgRNAs shown). Ptpn2-null B16 tumours were significantly more sensitive to immunotherapy (Fig. 3a; P < 0.01, Student’s t-test; Extended Data Fig. 3d; P < 0.001, log-rank test), but did not show any growth disadvantage in the absence of T-cell-mediated immunity or immunotherapy (Extended Data Fig. 5a–d). Loss of Ptpn2 also increased sensitivity to T-cell immunity in the Braf/PTEN melanoma model (Fig. 3b and Extended Data Fig. 4f), and the MC38 colon carcinoma model (Extended Data Fig. 5). Thus, loss of Ptpn2 sensitizes tumour cells to the effect of immunotherapy.

Enforced expression of PTPN2 in Ptpn2-null tumour cells (that is, Ptpn2 rescue) abrogated the sensitivity to immunotherapy in vivo (Fig. 3c, d; P < 0.05, Student’s t-test), suggesting that off-target effects of gene editing were not likely to be the cause of the increased sensitivity. Moreover, overexpression of Ptpn2 in control B16 tumour cells (that is, Ptpn2 overexpression) led to an outgrowth of tumour cells in immunotherapy-treated mice (Fig. 3c, d; P < 0.001, Student’s t-test), indicating that increased Ptpn2 expression renders tumour cells resistant to the effect of immunotherapy.

To determine whether PTPN2 amplification was associated with immunotherapy resistance in human cancer, we examined exome-sequencing data from patients treated with blockade of PD-1, PD-L1 or CTLA-4. PTPN2 amplification occurred in 20 patients, but the majority of these events were chromosomal or arm-level amplifications. Focal amplification occurred in only two patients, a number too small to conclusively determine whether PTPN2 amplification results in a mechanism of resistance (Extended Data Fig. 6).

Ptpn2 loss increases antigen presentation by tumours

To identify the mechanisms by which loss of Ptpn2 enhanced the efficacy of immunotherapy, we compared the composition of immune cell subsets in the tumour microenvironment of control and Ptpn2-null
tumours using flow cytometry. We observed no difference in the total number of CD45+ cells, NK cells, CD4+ T cells, FoxP3+ regulatory T cells, or cells in the myeloid compartment in Ptpn2-null tumours relative to wild-type tumours (Fig. 4a and Extended Data Figs 7a–d, 8a, b). However, Ptpn2-null tumours contained a significantly greater number of CD8+ T cells and γδ+ T cells (Fig. 4a; P < 0.05, Student’s t-test). We confirmed this observation with immunohistochemistry staining and found a threefold increase in the number of CD8+ cells, which diffusely infiltrated Ptpn2-deficient tumours (Fig. 4b; P < 0.01, Student’s t-test) treated with immunotherapy. Further flow cytometry analysis revealed that Ptpn2-null tumours contained an increased fraction of CD8+ T cells expressing granzyme B (Fig. 4c; P < 0.01, Student’s t-test). Thus, Ptpn2 loss increased the number of activated, cytotoxic CD8+ T cells in tumours.

We reasoned that the increase in number of cytotoxic CD8+ T cells in Ptpn2-null tumours could be due to improved recognition of tumour cells through increased antigen presentation. To test this, we expressed full-length OVA in Ptpn2-null or control B16 cells. Staining with a monoclonal antibody specific to the SIINFEKL epitope from OVA in the context of H2K(b) was significantly higher in Ptpn2-null cells, suggesting that loss of Ptpn2 increased the levels of antigen-loaded MHC-I on the surface of tumour cells (Fig. 4d; P < 0.001, Student’s t-test). Additionally, Ptpn2-deficient cells had increased total MHC-I

**Figure 4 | Deletion of Ptpn2 improves antigen presentation and T cell stimulation.** a. Quantification of immune cells in Ptpn2-null (red) or control (grey) B16 tumours, n = 8–10 animals per group; data from two experiments. b. Immunohistochemistry for CD8α in control or Ptpn2-null tumours. n = 4–5 animals per group. c. Granzyme B (Gzmb) expression by CD8+ T cells in Ptpn2-null or control B16 tumours. n = 8–10 animals per group; data from two experiments. d. SIINFEKL-H2K(b) presentation by Ptpn2-null or control B16 cells. MFI, mean fluorescence intensity. e. Intracellular IFNγ and TNF staining in CD8+ T cells after restimulation with Ptpn2-null or control B16 cells. f. Ratios of B16 cells lacking the indicated genes after co-culture with antigen-specific T cells. n = 3; representative of two independent experiments. Data are mean ± s.d. *P < 0.05; **P < 0.01; ***P < 0.001.
OT-I CD8+ T cells that recognize the SIINFEKL epitope. T cells cultured with Ptpn2-null OVA-B16 cells were significantly more activated as measured by intracellular IFNγ- and TNF staining (Fig. 4e; P < 0.001, Student's t-test). To test whether Ptpn2-null tumour cells were more sensitive to the presence of T cells, we co-cultured mixed populations of OVA-expressing Ptpn2-null or wild-type B16 cells with antigen-specific CD8+ T cells for six days. Ptpn2-deficient tumour cells were preferentially depleted from the co-culture (Fig. 4f; P < 0.05, Student's t-test). Thus, loss of Ptpn2 in tumour cells increased antigen presentation and sensitivity to cytotoxic CD8+ T cells.

**Loss of Ptpn2 increases IFNγ-sensing by tumour cells**

Ptpn2 negatively regulates IFNγ-sensing by dephosphorylating Jak1 and STAT1 (refs 17, 18). We tested whether Ptpn2 loss sensitized tumours to immunotherapy by increasing IFNγ-sensing. We first assayed STAT1 activity in Ptpn2-null cells in response to IFNγ stimulation. Ptpn2-null B16 cells showed increased phosphorylated (p-)STAT1 after treatment with IFNγ while overexpression of Ptpn2 repressed this phosphorylation (Fig. 5a).

Transcriptional analysis of the Ptpn2-null and control B16 tumour cells exposed to IFNγ, TNF or both indicated that loss of Ptpn2 caused a marked change in the expression profile of IFNγ response genes following IFNγ exposure, but did not change gene expression in unstimulated conditions or after TNF stimulation alone (Fig. 5b; Supplementary Information 2). Co-stimulation of IFNγ and TNF had a synergistic effect on Ptpn2-null cells, because IFNγ stimulation appeared to sensitize B16 cells to TNF (Extended Data Fig. 9b). Loss of function of PTPN2 in four human tumour cell lines also increased expression of IFNγ response genes following stimulation with IFNγ (Fig. 5c; FDR < 0.001). Several T cell chemokines were upregulated in Ptpn2-null cells (Cxc9, Cxc10, Cxc11 and Ccl5; Fig. 5b), which might increase infiltration of T cells into tumours. Members of the antigen processing and presentation pathway, such as Tap1, Tapbp, and B2m were also upregulated in Ptpn2-null cells (Fig. 5b), consistent with our previous observation of enhanced antigen presentation in Ptpn2-deficient cells.

Cell-cycle regulators such as Cdkn1a and several genes involved in apoptosis, such as Casp4, Casp8, Iftl2, Ripk1 and Bak1, were significantly upregulated in Ptpn2-null tumour cells treated with cytokines relative to wild-type tumour cells. Consistent with this finding, exposure to IFNγ alone or in combination with IFNγ or TNF significantly reduced the growth of Ptpn2-null mouse (Fig. 5d; P < 0.001, Student's t-test) and human (Fig. 5e; P < 0.01, Student's t-test) tumour cell lines in vitro relative to wild-type tumour cells. These results demonstrate that IFNγ alone is sufficient to cause a growth disadvantage in both mouse and human tumour cells that lack Ptpn2/PTPN2.

**Sensitivity of Ptpn2–null tumours depends on IFNγ**

To determine whether the mechanism of increased immunotherapy sensitivity of Ptpn2-null tumour cells depends on IFNγ sensing and signalling, we generated B16 tumour cell lines in which Ptpn2 had been knocked out and Stat1, Jak1, Ifngr1 or Ifnar2 had also been knocked out (Fig. 5f). We then tested the growth of these double-null cells relative to tumour cells lacking only Stat1, Jak1, Ifngr1 or Ifnar2 in animals treated with GVAX and anti–PD-1 immunotherapy. Loss of Ptpn2 alone was associated with a significant growth disadvantage in the presence of immunotherapy as expected (Fig. 5g, h). However, simultaneous loss of function of any of the genes necessary for the sensing of IFNγ abolished the growth disadvantage of Ptpn2-null cells (Fig. 5g, h; P < 0.01, Student's t-test). Loss of Ifnar2, a gene that senses type I interferon, did not abrogate the sensitivity of Ptpn2-null tumours to GVAX and anti–PD-1 immunotherapy (Fig. 5h). This genetic epistasis experiment indicates that in tumour cells that are unable to respond to IFNγ signalling, Ptpn2 deficiency cannot sensitize tumour cells to immunotherapy. Thus, the mechanism by which Ptpn2 deficiency sensitizes tumour cells to immunotherapy is dependent on the sensing of IFNγ.

**Discussion**

We used pooled loss-of-function genetic screens in vivo to discover multiple genes in diverse biological processes that sensitize tumours to immunotherapy, providing a new approach for immuno-oncology target discovery. Deletion of Ptpn2 markedly increased the response of tumours to immunotherapy. Ptpn2 encodes a protein tyrosine phosphatase that regulates a range of intracellular processes, including IFNγ signalling, which it can inhibit by dephosphorylating STAT1 and JAK1 (refs 17–21). Loss of Ptpn2 in tumour cells increased IFNγ signalling and antigen presentation to T cells, and amplified growth arrest in response to cytokines, suggesting that its therapeutic inhibition may potentiate the effect of immunotherapies that invoke an IFNγ response.

Loss of function of Qa-1b (the non-classical MHC molecule encoded by H2-T23, or HLA-E in humans) increased sensitivity of tumour cells to immune attack. The function of Qa-1b-HLA-E in tumour immunity has been unclear, because it can bind to both activating and inhibitory receptors expressed by T cells and NK cells6,22, and because it has been variously reported to be associated with good or bad clinical outcomes23,24. Loss of function of Qa-1b may increase the immune response to tumours by de-repression of T- or NK-cell function25,26 or by limiting the stimulation of CD8+ T regulatory cells27, either of which would make blockade of HLA-E an attractive immunotherapeutic approach.

Tumours were also sensitized to immunotherapy by deletion of Ripk1, as well as by loss of function of members of the linear ubiquitin assembly complex (LUBAC), Bir2, Rbck1 or Rnf31, that responsible for ubiquitination and stabilization of the signalling complex that includes Ripk1. Ripk1 has a complex role in the response to inflammation, including activation of NF-κB-mediated survival pathways28. Strategies to alter the stability of Ripk1 in tumours may provide additional therapeutic avenues to enhance tumour immunity.

Functional genomic approaches using genome editing have to date focused on identifying genes required by tumour cells for the cancer hallmarks of growth, metastasis and drug resistance29.11,12,29. Our study extends this approach to interrogate the interaction of the tumour cell with the immune system. We anticipate that it can be broadly applied to systematically define genes that govern interactions between cancer cells and the immune system.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**METHODS**

*In vivo CRISPR screening in B16 tumour cells.* We created a Cas9-expressing version of the B16 melanoma cell line and confirmed that it could edit DNA efficiently with CRISPR using sgRNAs targeting the PD-1 gene. For screening the B16-Cas9 cell line, we created a library of 9,992 optimized sgRNAs targeting 2,398 genes, which were selected from the Gene Ontology (GO) term categories: kinase, phosphatase, cell surface, plasma membrane, antigen processing and presentation, immune system process, and chromatin remodelling. The transcript abundance of the genes in these categories were then filtered to include only those that were expressed >RPKM(log2) = 0.9. These genes were then ranked for expression in the B16 cell line using RNA-sequencing to select for the top 2,398 expressed genes. The library was divided into four sub-pools, each containing one sgRNA per gene and 100 non-targeting control sgRNAs. The four sub-pools were screened individually and sgRNAs were delivered to B16-Cas9 cells via lentiviral injection at an infection rate of 30%. Transduced B16 cells were purified using an hCD19 reporter by positive magnetic selection (Miltenyi) and then expanded in vitro before being implanted into animals. For each sub-pool, B16 cells were implanted into 10 Tcrα−/− mice, 10 wild-type mice treated with GVAX, and 10 wild-type mice treated with GVAX and PD-1 blockade (see below for treatment protocols). B16 cells transduced with libraries were also grown in vitro at approximately 2,000 × library coverage for the same time period as the animal experiment. Mice were euthanized 12–14 days after tumour implantation and tumour genomic DNA was prepared from whole tumour tissue using the Qiagen DNA Blood Midi kit. PCR was used to amplify the sgRNA region and sequencing to determine sgRNA abundance was performed on an Illumina HiSeq. Significantly enriched or depleted sgRNAs from any comparison of conditions were identified using the STARS algorithm.*

**Cell lines.** B16F10 melanoma and B16-GM-CSF were a gift from G. Dranoff. Braf/Pten melanoma were from D.E. MC38 colon carcinoma cells was a gift from A. Sharpe. A375 melanoma, A549 lung carcinoma and HT-29 colon carcinoma cells were purchased from ATCC. MelJuSo melanoma cells was a gift from the Cancer Cell Line Encyclopedia (Broad Institute). B16, MC38, A549 and HT-29 cells were all grown in DMEM (Gibco) with 10% fetal bovine serum (Gemini biosciences) and antibiotics. A375 and MelJuSo cells were grown in RPMI 1640 (Gibco) with 10% fetal bovine serum and antibiotics. All cell lines were subject to periodic testing for mycoplasma using the LookOut Mycoplasma PCR detection kit (Sigma).

**Animal treatment and tumour challenges.** The designs of these animal studies and procedures were approved by the Dana-Farber Cancer Institute IACUC committee. Dana-Farber Cancer Institute’s specific-pathogen free facility was used for the housing and care of all mice. Seven-week old wild-type female C57BL/6 mice were obtained from Jackson laboratories. A colony of B6.129S2-Tcratm1Mom/J Tcra−/− − mice, 10 wild-type mice treated with GVAX, and 10 wild-type mice treated with GVAX and PD-1, as above. Tumours were collected on day 12–13, weighed, mechanically diced, incubated with collagenase P (2 mg ml−1, Sigma–Aldrich) and DNase I (50 μg ml−1, Sigma–Aldrich) for 10 min, and pipetted into a single-cell suspension. After filtering through a 70-μm filter, cells were blocked with anti-mouse CD16/32 antibody (BioLegend) and stained with indicated antibodies for 30 min on ice. Dead cells were excluded using Aqua Live/Dead (1:1,000, ThermoFisher Scientific) added concurrently with surface antibodies. After washing, cells were fixed with Fixp3/Transcription Factor Staining Buffer Set (eBiosciences) as per manufacturer’s instructions, blocked with mouse and rat serum, then stained with intracellular antibodies. Spherotech AccuCount fluorescent particles were added for cell quantification before analysis on an LSR Fortessa using single-colour compensation controls and fluorescence-minus-one thresholds to set gate margins. Comparisons between groups were performed using Student’s t-tests.

**Flow cytometry analysis of B16 tumour cells.** B16 cells were trypsinized and washed in PBS + 2% FBS, stained with antibodies against cell surface proteins as per the manufacturer’s instructions and then analysed on an Accuri C6 flow cytometry system.

**Restimulation of T cells on B16 tumour cells.** OT-I T cells were isolated from the spleens of OT-I T cell receptor transgenic mice using the CD8α+ T cell isolation mouse kit (Miltenyi), as per manufacturer’s instructions. Purified OT-I T cells were then stimulated in 24-well plates with plate-bound anti-mouse CD3+ (1 μg ml−1), soluble anti-mouse CD28 (2 μg ml−1), Cell Signaling) and recombinant human IL-2 (100 U ml−1, DFCl supply centre). After 48h, activated OT-I T cells were transferred into fresh media containing recombinant human IL-2 and allowed to expand for 5–7 days. For restimulation, 1 × 106 OVA-expressing Ptpm2-null or control sgRNA-transfected B16 tumour cells were first plated in 24-well plates and stimulated with recombinant mouse IFNγ overnight to induce ovalbumin surface expression. 1 × 105 pre-activated OT-I T cells were then added to the wells on the next day and co-cultured with the B16 tumour cells for 2–3h. Subsequently, 1 × 106 brefeldin (eBiosciences) was added to the cultures to inhibit intracellular protein transport. Afterward, OT-I T cells were collected from each well, stained for surface markers and then fixed with Fixp3/Transcription Factor Staining Buffer Set (eBiosciences) as per the manufacturer’s instructions. Intracellular cytokine staining was then performed before analysis on an LSR Fortessa.

**RNA-seq sequencing analysis of tumour cells.** Ptpm2-null or control sgRNA-transfected B16 cells were stimulated with IFNγ (100 ng ml−1, Cell Signaling Technology), TNFα (10 ng ml−1, Peprotech) or both for 48h. RNA was extracted from cell pellets using the Qiagen RNeasy Mini kit according to the manufacturer’s instructions. First-strand Illumina-barcoded libraries were generated using the NEB 2×1015 Directional Kit according to the manufacturer’s instructions, including a 12-cycle PCR enrichment. Libraries were sequenced on an Illumina NextSeq 500 instrument using paired-end 37 bp reads. Data were trimmed for quality using the Trimmomatic pipeline with the following parameters: LEADING:15 SLIDINGWINDOW:4:15 MINLEN:16. Data were aligned to mouse reference genome mm10 using Bowtie2. HTSeq was used to map aligned reads to the target genes.
to genes and to generate a gene count matrix. Normalized counts and differential expression analysis was performed using the DESeq2 R package. We performed gene set enrichment analysis, as described previously32,33.

**Western blotting.** Whole cell lysates were prepared in lysis buffer (60 mM Tris HCl, 2% SDS, 10% glycerol, complete EDTA-free protease inhibitor (Roche)) and 500 U ml⁻¹ benzonase nuclease (Novagen). Samples were boiled at 100°C and clarified by centrifugation. Protein concentration was measured with a BCA protein assay kit (Pierce). Subsequently, 50–150 μg of protein was loaded on 4–12% Bolt Bis-Tris Plus gels (Life Technologies) in MES buffer (Life Technologies). Protein was transferred to 0.45-μm nitrocellulose membranes (Bio-Rad). Membranes were blocked in Tris-buffered saline plus 0.1% Tween 20 (TBS-T) containing 5% non-fat dry milk for 1 h at room temperature followed by overnight incubation with primary antibody at 4°C. Membranes were washed with TBS-T and incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. HRP was activated with Supersignal West Dura Extended Duration Substrate (Pierce) and visualized with a chemiluminescent detection system using Fuji ImageQuant LAS4000 (GE Healthcare Life Sciences). Blots were then analysed using ImageJ and Adobe Photoshop software.

**Immunohistochemistry.** Whole B16 tumours were fixed for 24 h in 10% neutral-buffered formalin and then permeabilized in 70% ethanol overnight. Fixed tissue was subsequently embedded in paraffin, sectioned and then mounted onto slides for staining against mouse CD8α (eBioscience, 14-0808-82). Slides were imaged on a Leica Scanscope XT and analysed using Aperio software.

**Exome-sequencing analysis.** All human studies were conducted in accordance with the Declaration of Helsinki and approved by the Dana-Farber Cancer Institute Institutional Review Board (Protocols 11-104, 02-180, 09-472, 02-021, 15-330). Informed consent was obtained from all subjects. Electronic medical charts were reviewed to assess best response by RECIST (version 1.1), duration of progression-free survival, duration of overall survival, patient demographic characteristics and other relevant clinical details (for example, smoking history). Patients from the Dana-Farber Cancer Institute with metastatic bladder cancer, head and neck squamous cell carcinoma, lung cancer and melanoma treated with anti-PD-1, anti-CD137, anti-CTLA-4, or a combination of these therapies were identified, and other relevant clinical details (for example, smoking history). Patients from these groups were then stratified by clinical benefit using the metric published previously35.

**Exome-sequencing analysis.** Copy-number alterations were called using a circular binary segmentation method34. Called copy-number ratio was corrected for tumour purity and pre-treatment tumour tissue and matched germline blood were obtained for each tumour that was sequenced. The amplified region containing the implicated gene was manually examined to determine whether the amplification was true or false-positive. For flow cytometry, the following anti-mouse fluorochrome-conjugated antibodies were used: H2K(b)/H2D(b) (clone 28-8-6, BioLegend), CD47 (clone miap301, BioLegend), SINIFEKHL2 (clone 25-12-14, BioLegend), granzyme B (clone GB11, BioLegend), TNF (clone MP6-XT22, BioLegend), IFNγ (clone XMG1.2, BioLegend), CD8α (clone 53-6.7, BioLegend), CD4 (clone RM4-5 or 2G15, BioLegend), TCRβ (clone H57-597, BioLegend), PD-1 (clone RPMI-30, BioLegend), Tim-3 (clone TM3R-2,3, BioLegend), CD45 (clone 104 or 30-F11, BioLegend), Ly6C (clone HK-1,4, BioLegend), 1-AI-E (clone M5/15.15.2, BioLegend), F4/80 (clone BM8, BioLegend), CD11c (clone N418, BioLegend), CD24 (clone M1/69, BioLegend), CD11b (clone M1/70, BioLegend), CD103 (clone 2E7, BioLegend), CD3ε (clone 145-2C11, BioLegend), TCRβ (clone Gl3, BioLegend), NK1.1 (clone PK136, BioLegend), CD44 (clone IM7, BioLegend), Ki-67 (clone B56, BD Biosciences), CD274 (clone MIH5, BD Biosciences), IFNγ (clone 2E2, Life Technologies), IFNAR2 (R&D Systems; FAB1083A), FOXP3 (clone FJK-16-1, eBioscience).

**CRISPR sgRNA sequences.** Gene name, sgRNA number and sequence were as follows: Cd274 sgRNA1, GCCTGTGTCACTTGTCAGC; Cd274 sgRNA2, AATCAACAGAATTTCGG; Cd274 sgRNA3, GGTCCACTCCTGGTCA; Cd274 sgRNA4, GTAGGACGCACTTGACGA; Cd47 sgRNA1, ATXAGGCTGAAAACCCGCA; Cd47 sgRNA2, CCACATTCAGCCAGA; Cd47 sgRNA3, AGTTTGAACGAAAGAAGG; Cd47 sgRNA4, GCAAGTGTATTTCCTACCAC; Stat1 sgRNA1, GATTCATTACACTGTCGTA; Stat1 sgRNA2, GTACGATGACAGTTCCCATC; Stat1 sgRNA3, GATACCATTTTCCCCCA; Jak1 sgRNA1, CAGCGGAGATATACCGCC; Jak1 sgRNA2, GAAGCGATCCTGACTCGG; Ifnfr1 sgRNA1, CGACTTCAGGGTGAAATACG; Ifnfr1 sgRNA2, GGTATCCTCACATGACACA; Ifnfr1 sgRNA3, GGTATCCTCACATGACACA; Ifnfr1 sgRNA4, GGTATCCTCACATGACACA; Ptpn2 sgRNA1, TCATTCAAGAACAGATGTA; Ptpn2 sgRNA2, ACACAAGCTGAGGACCCGA; H2-T23 sgRNA1, TACGCGACATTACCGG; H2-T23 sgRNA2, AGAGCTCTCGACAATACCCGG; H2-T23 sgRNA3, GGAACATTCAAGAGAATCCGG; Stub1 sgRNA1, GCATTGCTAAGAAGAACCCG; B2k1 sgRNA1, CACCGTGACGTCCACACGATTCC; control sgRNA1, CACCGTGACGTCCACACGATTCC; control sgRNA2, CCAGGAGATCCTTGACGACGG; control sgRNA3, ATGTTGAGTTCCCTTGCG; control sgRNA4, AGGTGTAAGGCGACCCG; control sgRNA5, ATTGTTGAGTTCCCTTGCG; human PTPN2 sgRNA1, TACGCGACATTACCGG; human PTPN2 sgRNA2, TACGCGACATTACCGG; human PTPN2 sgRNA3, ACAGGACGCTGAGCTGG; human PTPN2 sgRNA4, CACGCGAGATCCTGACCG; human PTPN2 sgRNA5, AGAGCTCTCGACAATACCCGG; human PTPN2 sgRNA6, AGAGCTCTCGACAATACCCGG; human PTPN2 sgRNA7, AGAGCTCTCGACAATACCCGG; human PTPN2 sgRNA8, AGAGCTCTCGACAATACCCGG.

**Data availability.** All data presented in this manuscript are available from the corresponding author upon reasonable request.