PTENα functions as an immune suppressor and promotes immune resistance in $PTEN$-mutant cancer

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$PTEN$ is frequently mutated in human cancers and $PTEN$ mutants promote tumor progression and metastasis. $PTEN$ mutations have been implicated in immune regulation, however, the underlying mechanism is largely unknown. Here, we report that PTENα, the isoform of PTEN, remains active in cancer bearing stop-gained $PTEN$ mutations. Through counteraction of CD8$^+$ T cell-mediated cytotoxicity, PTENα leads to T cell dysfunction and accelerates immune-resistant cancer progression. Clinical analysis further uncovers that PTENα-active mutations suppress host immune responses and result in poor prognosis in cancer as relative to PTENα-inactive mutations. Furthermore, germline deletion of $Pten$ in mice increases cell susceptibility to immune attack through augmenting stress granule formation and limiting synthesis of peroxidases, leading to massive oxidative cell death and severe inflammatory damage. We propose that PTENα protects tumor from T cell killing and thus PTENα is a potential target in antitumor immunotherapy.
The importance of phosphatase and tensin homolog deleted on chromosome ten (PTEN) in tumorigenesis is underscored by its frequent mutations, including missense, nonsense, frameshift, and large deletions. The tumor-suppressive activity of PTEN largely depends on its lipid phosphatase activity, which antagonizes PI3K/AKT activation. Accordingly, a significant fraction of PTEN missense mutations is identified in human cancers, which selectively abolish its phosphatase activity. Additionally, more than half of PTEN mutations result in protein truncation and instability, which are functionally comparable to PTEN loss. In spite of the dysregulation of PTEN-AKT signaling in cancers with PTEN truncated or missense mutations, the outcomes of tumors with different types of PTEN mutations are distinct. Therefore, an in-depth understanding of the biological functions of various PTEN mutations in tumorigenesis is still in need, and crucial for cancer treatment.

Through restoring or enhancing the effector function of CD8+ tumor-infiltrating T cells, immunotherapy has revolutionized the treatment of patients with advanced-stage cancers. However, this therapy is limited to a minority of patients with cancer, and the clinical efficacy is often compromised along with tumor development. Previous studies have revealed that tumor cells can hijack strategies developed to limit immune responses, thereby affecting priming, activation, and recruitment of T cells, causing tumor immune escape. Apart from these processes of T cells, effective anti-tumor immunity also requires cytotoxic T lymphocytes (CTLs)-mediated cell killing. Researches have shown that the function of CTLs depends on the ROS production in the target cells, and recent studies further uncover that CTLs cause different types of cell death depending on the context of the target cells. These phenomena lead to the hypothesis that, instead of affecting T cell function, tumor-intrinsic factors may directly affect the susceptibility of tumor cells to CTLs. To date, researches on tumor immune escape still mainly focus on the effects of tumor cells on T cells, while little is known about whether tumor cells can escape from CTLs-mediated killing through modulating their own metabolic state.

PTENα (also known as PTEN-L) is the first identified isoform of PTEN, which contains an additional disordered 173-amino acid N terminus. This region confers functions of PTENα that are distinct from those of PTEN. The role of PTENα in tumors is complicated, which limits tumor growth in glioblastoma cells, while promotes carcinogenesis in liver cancer. Up to now, the effects of PTENα in anti-tumor immunity remain elusive. In this study, we find that PTENα acts as an immune suppressor that restricts CD8+ T cell response against PTEN mutant cancers, consequently resulting in poor outcome of the disease. Rather than directly affecting T cell priming or differentiation, PTENα promotes the cell resistance to T cell-mediated cytotoxicity and results in tumor immune escape. Reciprocally, loss of PTENα down-regulates translation of proteins associated with the oxidation-reduction process, and potentiates ferroptosis upon incubation with cytotoxic T cells, exacerbating inflammatory responses and promoting immune clearance. Our data thus demonstrate that PTENα acts as a suppressor of T cell cytotoxicity-induced oxidative cell death, and highlight the importance of targeting PTENα in antitumor immune therapy.

Results
PTENα remains active in PTEN-mutant cancer. Loss-of-function mutations in the PTEN gene are frequent in human cancer. In addition to phosphatase-inactive mutations, abundant stop-gained mutations were detected in exons encoding the C-terminal domain of PTEN. Through induction of PTEN instability, stop-gained mutations restrict the function of phosphatase PTEN, whose effects are largely identical to those of phosphatase-inactive mutations. However, through analyzing the survival of patients with tumor-bearing PTEN mutations, we found that the outcome of patients with stop-gained mutations was poorer than those with phosphatase-inactive mutations (Fig. 1b and Supplementary Fig. 1a). We next analyzed differentially expressed genes in cancers with these two-type mutations based on the matched diagnosis age (40–60 years), aneuploidy score (<3), and neoplasm histologic grade (G3). Utilizing gene set enrichment analysis (GSEA), we found that genes related to adaptive immune response were selectively enriched in cancers with phosphatase-inactive mutations as compared with those with stop-gained mutations (Fig. 1c). Similar results were also detected between the two-type mutations in a broader context (Fig. 1d). These results lead to the hypothesis that, aside from tumor-promoting effects, stop-gained mutations of PTEN restrict host immune response against cancer.

As an isoform of PTEN, PTENα is initially translated from a CUG codon upstream of and in-frame with the coding region of canonical PTEN. As shown in Supplementary Fig. 1b, phosphatase-inactive mutations impaired the phosphatase activity of PTEN and its isoform PTENα. To assess the effects of stop-gained mutations on PTEN isoforms, we analyzed the protein stability of the PTEN family with or without stop-gained mutations. As shown in Fig. 1e, f and Supplementary Fig. 1c, in contrast to the identical half-life between wild-type PTEN and PTENα, stop-gained mutations accelerated the degradation of PTEN rather than PTENα. To further confirm this result, we utilized the Jurkat and MOLT4 cells, which have been reported to carry stop-gained PTEN mutations. In accordance with the bioinformatics data, we found that MOLT4 cells contain PTENC267Rf*9 mutation and Jurkat cells contain PTENP234Afs*1 and PTENP247* mutations by DNA-sequencing analysis (Supplementary Fig. 1d). We then employed an anti-PTEN antibody to assess the status of endogenous PTEN and PTENα in Jurkat and MOLT4 cells. As shown in Supplementary Fig. 1d, compared with wild-type PTEN and PTENα in HEK-293T, truncated PTENα rather than PTEN can be detected in both Jurkat and MOLT4 cells. Our data thus indicate that PTENα remains active in cancer with stop-gained PTEN mutations (Supplementary Fig. 1b).

PTENα promotes tumor immune escape. To investigate the role of PTENα in tumorigenesis, we used CRISPR/Cas9 technology to knock out the endogenous PTEN in the B16-F10 cell line, which also abolished the expression of PTENα, and subsequently re-enforced the expression of PTENα in Pten−/− cell line. As shown in Supplementary Fig. 2a–c, ectopic expression of PTENα elicited little effects on cell proliferation in vitro. In light of the impaired immune response in cancer with stop-gained mutations, we thus employed irradiated B16-F10 cell line as tumor vaccine to assess the role of PTENα in modulation of host immune response against cancer (Fig. 2a). As shown in Fig. 2b, in contrast to the identical volume of tumor with or without PTENα in unimmunized C57BL/6 mice, overexpression of PTENα weakened the effectiveness of cancer vaccination. Through analysis of tumor-infiltrating lymphocytes (TIL) by flow cytometry, less amounts of CD45+ immune cells were detected in PTENα-expressing tumors as relative to control tumors (Fig. 2c).

To study the status of immune cells in PTENα-expressing tumors, we applied scRNA-seq methods to investigate CD45+ immune cells isolated from PTENα-expressing or control tumors with two replicates. To remove batch-effect, data integration and batch-effect correction were applied to the data. Utilizing graph-based clustering to analyze the tumor-infiltrating immune cells,
we identified 12 clusters of immune cells for 10× data. We then defined the clusters based on the exclusive expression of canonical marker genes. As shown in Supplementary Fig. 2d, e, and Supplementary Table 1, major immune cell types in cancers including CD4\(^+\) T, CD8\(^+\) T, Treg, NK, monocyte, macrophages, neutrophil, B, plasma B, plasmacytoid dendritic cells (pDCs), and conventional DCs (cDCs). Compared with more than 80% of lymphoid cells in the control tumor, the percentage of T
PTEN promotes T cell exhaustion in a T cell-extrinsic manner. Since the presence of PTENα in cancers drives CD8+ T cell dysfunction, we employed Pten−/− mice to further study the role of PTENα in the modulation of T cell fate. Although PTENα was expressed in both lymphoid and non-lymphoid organs (Supplementary Fig. 4a), Pten−/− mice developed normally, and no spontaneous tumor was detected within 2-year old. In order to determine whether loss of PTENα affects the proliferation, differentiation, and survival of T cells, we firstly employed a CFSE-staining assay to measure the T cell expansion during activation. As shown in Supplementary Fig. 4b, c, deletion of PTENα elicited little effects on T cell expansion during T cell priming. Besides, identical ratio of iTreg cell (CD4+CD25+Foxp3+), Th1 (CD4+IFNγ+), Th17 (CD4+IL-17A+) and Tr1 (CD4+IL-10+) cells were induced under various T-cell polarizing conditions between wild-type and Pten−/− mice (Supplementary Fig. 4d). Finally, we used Annexin V/7-AAD staining to assess cell death during T cell activation. As shown in Supplementary Fig. 4e, no significant difference was detected between wild-type and Pten−/− T cells.

We next utilized lymphocytic choriomeningitis virus clone 13 (LCMV-C13) to induce T cell exhaustion. As shown in Fig. 3a, b, high viral loads were found in the lungs in wild-type mice, whereas Pten−/− mice showed a much-reduced viral burden on day 30 post infection. Compared with wild-type mice, the frequency of virus-specific CD8+ T cells (GP33-41+) was increased in the lungs from Pten−/− mice (Fig. 3c and Supplementary Fig. 5a). Consistent with published studies18, we observed that high levels of inhibitory receptors including PD-1, TIM-3, and LAG-3 were expressed on the surface of virus-specific wild-type CD8+ T cells rather than Pten−/− CD8+ T cells during chronic infection (Fig. 3d). Moreover, we stimulated CD8+ GP33-41+ T cells with virus-specific peptides to induce the production of effector cytokines in wild-type and Pten−/− T cells. As shown in
Supplementary Fig. 5b, Ptenα−/− T cells produced greater amounts of effector cytokines than wild-type T cells upon exposure to viral peptide. Similar results were also observed in virus-specific T cells from spleens (Supplementary Fig. 5c–e). These data thus indicate that deletion of PTENα blocks T cell dysfunction.

We next sought to determine whether T cell polarization is dependent on T cell-intrinsic PTENα. To this end, we used a bone marrow (BM) transplantation model in which Ptenα−/− or wild-type mice were lethally irradiated and transplanted with wild-type or Ptenα−/− BM and subsequently infected with LCMV-C13. As shown in Fig. 3e, on day 30 post-infection, the numbers of virus-
specific T cells were identical between Pten"−/− recipients of wild-type BM and those of Pten"−/− BM. Of note, compared with wild-type donor mice, greater amounts of virus-specific T cells were detected in the two types of Pten"−/− recipient mice (Fig. 3e). Conversely, LCMV-Cl13 infection resulted in lower amounts of virus-specific T cells in wild-type recipients, no matter which kind of BM is adoptive transferred (Fig. 3f). Consistent results were also detected by assessment of inhibitory receptor expression and effector cytokine production (Supplementary Fig. 6a–d). Combined with the data that PTENα is also expressed in non-lymphoid organs (Supplementary Fig. 4a), our data thus demonstrate that T cell-extrinsic PTENα drives T cell exhaustion.

PTENα promotes cell resistance to T cell cytotoxicity. To investigate the mechanism by which PTENα promotes T cell exhaustion, we analyze the epithelial-immune cell interaction. As shown in Fig. 4a, loss of PTENα triggered stronger inflammatory response in the early stage of higher titers of LCMV-Cl13 infection, causing massive congestion and edema in the lungs of Pten"−/− mice upon viral infection. Moreover, greater amounts of death cells were detected in Pten"−/− lungs assessed by TUNEL staining (Fig. 4b). Besides, we performed mass spectrometry analysis of tissue interstitial fluid (TIF) in the lungs during inflammation, and found that a series of nuclear proteins were released in extracellular matrix of lungs from Pten"−/− mice at the early stage of viral infection, which acts as danger-associated molecular patterns (DAMPs) and activates immune response (Fig. 4c). Consistently, loss of PTENα increased the amounts of HSP70, HSP90, HMGB1, and eATP (extracellular ATP) in TIF in the lung during viral infection (Figs. 4d, e). These results thus indicate that deletion of PTENα promotes immunogenic cell death upon LCMV infection.

Since LCMV-Cl13 is a non-cytolytic virus that hardly induces cell death in vitro, we hypothesized that T cell-mediated cytotoxicity mainly contributed to the severe cell death. To this end, we performed in vitro killing assay to investigate whether PTENα affects target cell responses to CTLs. We pulsed Mock or PTENα expressing B16-Pten"−/− cells with LCMV-GP33-41 peptide and incubated with CD8+ T cells isolated from spleens of C57BL/6 mice untreated or infected with LCMV-Cl13. Notably, incubation of CD8+ T cells from the infected mice induced significantly lower cell lysis rates in PTENα-expressing B16 cells relative to those control cells (Fig. 4f). Moreover, compared with PTENα-expressing cells, the T cell-mediated cytotoxicity of Mock B16-Pten"−/− cells induced high level of ATP release and intracellular ROS accumulation, which indicated that loss of PTENα resulted in non-apoptotic cell death under immune attack (Fig. 4g, h). Similar results were also detected in Pten"+/+ and Pten"−/− MEFs (Supplementary Fig. 7a–c). Our data thus demonstrate that CTLs triggers immunogenic death of Pten"−/− cells that exacerbates inflammatory damage upon exposure to pathogens. The existence of PTENα correspondingly limits antigen clearance, resulting in T cell exhaustion.

PTENα restricts oxidative cell death. Previous studies have revealed that ROS is essential for CTLs-mediated cytotoxicity. In consideration of the higher ROS level in PTENα-deletion cells induced by CTLs, we treated Pten"−/− and Pten"+/− MEFs with hydrogen peroxide (H2O2) to mimic CTLs stimulation and investigated the nature of cell death caused by PTENα deficiency. As shown in Supplementary Fig. 8a, Pten"−/− deficiency markedly increased cell susceptibility to ROS-mediated cell death. Moreover, using a series of cell death inhibitors, we found that the ferroptosis inhibitor ferrostatin-1 (Fer-1) was the only one completely abolishing the cell death induced by H2O2 (Supplementary Fig. 8c). Consistent results were also observed in Mock or PTENα-expressing Pten"−/− B16 cells (Fig. 5a–c). Besides, treatment of iron chelator deferamamine (DFO) and α-TOC (a most abundant form of vitamin E) significantly rescued Pten"−/− MEFs from H2O2-induced cell death (Supplementary Fig. 8d, e). Reciprocally, supplementation of the ferroptosis activator erastin and RSL3 significantly promoted cell death compared with other cell death agonists did (Supplementary Fig. 8f, g). To further confirm that loss of PTENα selectively induces ferroptosis, we measured the status of GPX4, which is a key regulator of ferroptosis. As shown in Supplementary Fig. 8h, the protein levels of GPX4 were reduced in lungs from Pten"−/− mice compared with wild-type mice upon exposure to the virus.

In order to ascertain that suppression of ferroptosis mainly contributes to the oncogenic function of PTENα, we used α-TOC which is a radical scavenger and lipid peroxidation inhibitor, to suppress ferroptosis commitment. Considering only a portion of α-TOC dosage can reach the tumor by oral administration and its water-insoluble characteristic, we exploited a molecular-matched strategy to prepare α-TOC-loading TPGS nanoparticles (NP-VE) with extremely high drug loading levels (up to 10 mg/ml), which can effectively limit ROS production (Supplementary Fig. 8i, j). In vitro assays showed that α-TOC treatment weakened the difference of cell death between PTENα-expressing cells and controls cells exposure to cytotoxic T cells (Fig. 5d). Besides, we also used BODIPY-C11 dye to analyze the level of lipid ROS. Analog to the intracellular ROS level, the presence of PTENα limited the level of lipid ROS in B16 cells (Pten"−/−) under T cell attack (Fig. 5e). Moreover, treatment of NP-VE in the cancer vaccine model promoted the progression of Mock-expressing B16-Pten"−/− cells to a similar level of PTENα expressing B16-Pten"−/− cells (Fig. 5f). Flow cytometry analysis of the infiltrating
immune cells revealed that injection of NP-VE restricted the amount of CD45^+ immune cells infiltrating in tumors as compared with untreated Mock tumors (Supplementary Fig. 9a). Besides, the presence of PTENα exhibited little effects on the recruitment of immune cells in tumors treated with NP-VE (Supplementary Fig. 9a), suggesting that blockade of oxidative cell death is essential for the immunosuppressive effects of PTENα. Further flow cytometry analysis demonstrated that NP-VE treatment or PTENα presence restricted both T and B cell recruitment in tumors (Supplementary Fig. 9b, c). In accordance with the suppressive effects of NP-VE treatment on T cell recruitment, increased expression of PD1 and reduced production
of IFNγ were also detected in CD8+ T cells isolated from mock tumors treated with NP-VE (Supplementary Fig. 9d, e). Taken together, our data demonstrate that PTENα increases cell resistance to CTLs-induced immunogenic cell death and consequently leads to cancer immune escape.

PTENα blocks stress granules formation during oxidative stress. To investigate the molecular mechanism by which PTENα modulates cell response to immune attack, we performed pull-down assays with FLAG-tagged proteins coupled with mass spectrometry under the condition of H2O2 treatment. Interestingly, we noticed that majority of PTENα-associated proteins were involved in stress granules assembly (G3BP1, G3BP2, etc.) and formation (eIF2α, PCBP1, PCBP2, ZC3HAV1, etc.) (Fig. 6a).

Subsequent co-immunoprecipitation and immunofluorescence assays further confirmed the association of PTENα with stress granules components (Fig. 6b, c). To determine whether PTENα also participates in this process, we co-transfected PTEN or PTENα with eIF2α in the presence or absence of H2O2 treatment. Compared with the stimulatory effect of H2O2 treatment on the association of PTENα with eIF2α (Fig. 6d, lane 3–4 versus lane 1–2), PTEN did not interact with stress granules-associated proteins (Fig. 6d, lane 7–8 versus lane 5–6 and Fig. 6e, lane 3 versus lane 1 and 2).

To determine whether PTENα influences stress granules formation, we transfected mock or PTENα into Pten−/− B16 cells followed by stimulating with H2O2, which is also a known inducer of stress granules. In spite of extensive co-localization of PTENα with intrinsic G3BP1 detected by immunofluorescence assay, overexpression of PTENα significantly inhibited H2O2-induced cytoplasmic foci formation compared with cells transfected with empty vectors (Fig. 6f, g). Together, these results indicate that PTENα selectively interacts with stress granules-associated proteins and blocks stress granules formation under stress conditions.

PTENα maintains protein synthesis of peroxidases. The phosphorylation of eIF2α is crucial for stress granules formation24. Given that PTENα possesses the canonical phosphatase activity and interacts with eIF2α, we hypothesized that PTENα modulates eIF2α phosphorylation under multiple kinds of stress conditions. As anticipated, stimulated by H2O2, overexpression of PTENα in Pten−/− B16 cells inhibited eIF2α phosphorylation (Fig. 7a, lanes 4 and 6 versus lanes 3 and 5). Similar results were also observed in MEFs (Supplementary Fig. 10a, lanes 4 and 6 versus lanes 3 and 5).

Additionally, unlike PTENα, enforced expression of PTEN or the phosphatase-dead PTENα-C297S mutant did not significantly influence the eIF2α phosphorylation status in Pten−/− MEFs upon H2O2 exposure (Supplementary Fig. 10a, lanes 4 and 6 versus lanes 3 and 5). Besides, we also performed in vitro phosphatase assay and found that PTENα directly dephosphorylates eIF2α in vitro (Fig. 7b, lane 3 versus lane 2). Our data thus confirm that PTENα acts as a phosphatase on eIF2α.

Since eIF2α phosphorylation restricts host translational machinery21, we incubated Pten+/+ and Pten−/− MEFs in medium containing 35S isotope-labeled methionine (35S-Met) and then measured 35S-labeled protein synthesis. Relative to Pten+/+ MEFs, a substantial decrease in protein synthesis was observed in Pten−/− MEFs after exposure to H2O2 or TG (Fig. 7c). Consistent with these findings, puromycin incorporation (as a measure of protein synthesis) into nascent cells was also significantly decreased under stress conditions in Pten−/− MEFs compared with Pten+/+ MEFs, as revealed by immunofluorescence and immunoblotting analysis (Supplementary Fig. 10b, lanes 4–6 versus lanes 1–3, and Supplementary Fig. 10c). Similar results were also detected in Mock or PTENα-expressing Pten−/− B16 cells (Fig. 7d, e, lanes 4–6 versus lanes 1–3). Together, our data thus indicate that deletion of PTENα promotes eIF2α-mediated translation inhibition.

Stress granules are known to be related with selective translation22–24. To determine which proteins are regulated by eIF2α phosphorylation and consequent stress granules assembly in Pten−/− MEFs, we performed a mass spectrometry analysis of Pten−/− and Pten−/− MEFs under oxidative stress. As shown in Fig. 7f and Supplementary Fig. 10d, loss of PTENα selectively affected the synthesis of proteins associated with translation and oxidation–reduction processes. Ensued immunoblotting analysis confirmed that protein level of GPX4 rather than its mRNA level was significantly decreased in Ptenα-null cells under oxidative stress condition (Fig. 7g, lane 4 and lane 6 versus lane 3 and lane 5, Supplementary Fig. 10e, lane 4 and lane 6 versus lane 3 and lane 5, and Supplementary Fig. 10f). Accordingly, intracellular ROS levels in H2O2-treated PTENα-expressing B16 cells were diminished relative to control cells (Fig. 7h).
the effectiveness of tumor vaccination (Fig. 7i). Our data thus demonstrate that Gpx4 largely contributes to the stimulatory role of PTENα in cancer immune escape.

Collectively, we identify PTENα acts as phosphatase of eIF2α, which in turn promotes synthesis of peroxidases and blocks oxidative cell death, eventually ameliorating cancer immune evasion.
Fig. 4 PTENα promotes cell resistance to T cell cytotoxicity. a-e Wild-type and Pten−/− mice were i.v. infected with 1 × 10^6 PFU LCMV-Cip3. On day 7 post-infection, lungs were harvested from the mice. a The lungs were subjected to hematoxylin and eosin (H&E) staining. The image is representative of four mice with similar results. Scale bar: 200 μm. b TUNEL staining of lungs from wild-type and Pten−/− mice on day 7 post-infection. The image is representative of four mice with similar results. The red arrow indicates TUNEL positive cells. c The tissue interstitial fluids were isolated from the lungs and subjected to MS analysis. The differential proteins were analyzed using GSEA with GO gene sets. ES is an abbreviation of enrichment score, and NES represents normalized enrichment score. KO refers to Pten−/−. Each sample is a mixture of fluids from three mice. d and e The interstitial fluids were isolated from the lungs, and used for immunoblot analysis with indicated antibodies (d), and ATP content assay (e) (n = 4 mixed samples from three mice, mean ± SD, ***P = 0.0002). For immunoblot analysis, equal amounts of loading proteins were showed by the Ponceau S staining. The relative ratios of Hsp70, Hsp90, and HMGB1 to Poncea S-stained total proteins were indicated. UT, untreated. f-h In vitro cytotoxicity assay of B16 cells. Mock or Ptenα expressing Pten−/− B16 cells pulsed with Gp33–41 peptide were used as target cells. Effector cells and target cells were incubated at indicated ratios. Effector-uninfection refers to T cells from uninfected mice and acts as a negative control. f 20 h post-incubation, B16 cells were washed to remove lymphocytes and counted using CCK-8 (n = 3 cell cultures, mean ± SD, ***P (2) = 0.0006, ***P (25) = 0.0003, ***P < 0.0001). g Culture medium was collected 20 h post-incubation and centrifuged to remove cells and debris. The supernatant was subjected to ATP content assay (n = 3 cell cultures, mean ± SD, ns, not significant, **P = 0.0078, ****P < 0.0001). h Untreatment control refers to pulsed B16 cells without effector incubation. h 12 h post-incubation, B16 cells were harvested and stained with DCFDA, using for flow cytometry analysis. Cells with higher intensity of FSC and SSC were gated and considered as B16 cells. Mean fluorescence intensity (MFI) of DCFDA was used for statistical analysis (n = 3 cell cultures, mean ± SD, **P = 0.0255). Untreatment control refers to pulsed B16 cells without effector incubation. Statistical significance was assessed by two-tailed unpaired Student’s t test (h) or one (e) or two (f and g). *way ANOVA followed by Tukey’s multiple comparisons test. Data are representative of two (a, d–h) independent experiments. Source data are provided as a Source Data file. See also Supplementary Fig. 7.

Discussion

Despite the wide application of immunotherapy in the treatment of cancers, only a subset of patients responds to the treatment, and the clinical efficacy is often compromised along with tumor development10. Notably, a recent study uncovers that PTEN mutations are frequently observed in immune-resistant cancers25. Although the combination of immunotherapies and PI3K-AKT pathway inhibitors elicits partially effective to reinvigorate tumor-infiltrating T cells26, the immunosuppressive tumor environment can hardly be transformed, which suggests that other signaling pathways are also triggered in addition to PTEN dysfunction. Here we identify that presence of PTENα in PTEN-mutant cancers counteracts immune attack and promotes tumor immune escape (Supplementary Fig. 10 g). Rather than affecting T cell priming, tumor-intrinsic PTENα restricts T cell-mediated cytotoxicity through limiting ferroptosis, which attenuates immunogenic cell death and maintains immunosuppressive niche. Our data thus indicate that PTENα can be a potential target of immune therapy to regulate the susceptibility of tumor cells to immune responses.

As an N-terminally extended isoform of PTEN, PTENα translates through a CUG codon upstream of and in-frame with the coding region of canonical PTEN15. Accompanied with PTEN, the phosphatase-inactive mutations also impair the phosphatase activity of PTENα. However, we find that the stop-gained mutations, which result in PTEN instability and degradation, hardly affect PTENα stabilization. Notably, the presence of stop-gained mutations of PTENα is correlated with less T-cell infiltration and worse patient outcome, suggesting the oncogenic role of PTENα in tumor development. Interestingly, the role of PTENα in tumors seems complicated due to the opposite effects of PTENα on tumor growth in glioblastoma and liver cancer14,16. Although clinical analysis reveals that sustained expression of PTENα in liver cancer tissue is correlated with accelerated tumor progression, both types of research use the model of nude mice bearing tumor xenograft and fail to investigate the role of PTENα in anti-tumor immunity14,16. Herein, we identify the immunosuppressive function of PTENα, which attenuates cancer cell susceptibility to T cell-mediated cytotoxicity through limiting oxidative cell death, thereby promoting cancer escape from immune eradication.

Among the inhibitory effects of PTENα on host antitumor immune response, we regard its negative role in oxidative cell death as the core mechanism in vivo. First, the tumor vaccination model reveals that tumor-intrinsic rather than T cell-intrinsic PTENα mainly contributes to cancer immune evasion. Second, both in vitro killing assay and T cell adoptive transfer model demonstrate that reduction of immunogenic cell death and ensued DAMPs release is the cause of limited recruitment of immune cell. Third, chronic viral infection and BMT models demonstrate that reduced cell death and consequently persistent antigenic stimulation induced by PTENα drive exhausted T cell formation. Above all, our results demonstrated that, through protecting tumor cells from T cell killing, PTENα limits the infiltration of leukocytes in tumors and impairs T cell effector function, eventually leading to tumor immune escape.

The physiological function of PTENα remains largely unknown. Here we demonstrate that, as a phosphatase of eIF2α, PTENα maintains host protein synthesis, and limits stress granule formation. Stress granules have a great impact on cells’ adaption to environmental changes through selective modulation of host protein synthesis under various stress conditions23,24. We found that PTENα restricts stress granule formation and maintains translation of proteins associated with oxidation–reduction processes, which in turn limits oxidative cell death and ameliorates the inflammatory response by blocking DAMPs release. In accordance with viral infection, it has been reported that Pseudomonas aeruginosa infection triggers severe immunopathology in mice without PTEN-L (PTENα)27. Our work thus supports the notion that the presence of PTENα is critical for host protection from pathogen-induced tissue damage. In PTENα mutant cancer, this process is hijacked, thereby promoting the adaption of tumor cells to T cell cytotoxicity, resulting in tumor immune escape.

Ferroptosis has been implicated in a variety of pathological disorders, such as neurodegenerative events, ischemia/reperfusion injury, and bacterial infection28. Besides, a previous study has revealed that CTLs can kill cancer cells by inducing ferroptotic cell death12. A recent study also uncovers that the sensitivity of tumor cells to oxidative stress and ferroptosis limits their metastasis through blood29. In this study, our results identify the inhibitory role of PTENα in the modulation of ferroptosis. Through neutralizing intracellular ROS, PTENα enhanced cancer cell resistance against T cell killing. Moreover, the presence of PTENα promoted cancer metastasis through blood. Therefore, these results further confirm the importance of PTENα in cancer immune escape and highlight the potential of targeting PTENα to be a promising way in cancer treatment.
Methods

Mice. *Pten*−/− mice (C57BL/6J background) were generated and reported in our previous study30. Balb/c and Balb/c nude mice were purchased from Charles River Laboratories. 6–8 weeks old male mice were used for the study. All animals were maintained under a specific pathogen-free condition at the Department of Laboratory Animal Science of Peking University Health Science Center and reared in standard conditions with controlled temperature (20–26 °C), humidity (40–70%), and 12/12-hour dark/light cycle. The animal experimental protocols were approved by the Ethics Committee of Peking University Health Science Center, and the animal testing and research comply with all relevant ethical regulations.

Cells. B16-F10, HEK-293T, Molt4, Jurkat, CT26, and HeLa cells were from American Type Culture Collection (ATCC). MEFs were obtained through mincing...
Fig. 5 Pten restrains oxidative cell death. a Microscopy analysis of H_2O_2 (500 μM) treated Mock or Pten-positive expressing B16-Pten^−/− cells. UT untreated. Scale bar: 100 μm. b Mock or Pten-negative expressing B16-Pten^−/− cells were treated with 500 μM H_2O_2 and the percent cytotoxicity was assessed using LDH release assay (n = 4 cell cultures, mean ± SD, ***P < 0.0001). UT untreated. c Mock or Pten-positive expressing B16-Pten^−/− cells were treated with 500 μM H_2O_2 in the absence or presence of 1 μM Fer1. Cells were stained with propidium iodide (PI), followed by flow cytometric analysis. All cells were gated. The death cell rates were used for statistical analysis (n = 3 cell cultures, mean ± SD, ***P < 0.0001). UT untreated. d and e in vitro cytotoxicity assay for B16 cells. Mock or Pten-positive expressing B16-Pten^−/− cells pulsed with GP33–41 peptide were used as target cells. Effector cells and target cells were incubated at indicated ratios. Effector-uninfection refers to non-antigen-specific T cells and acts as a negative control. Before incubation, CD8^+ T cells were stained with CFSE, then the effector cells and target cells were incubated in the presence or absence of 20 mM NP-VE. 20 h post-incubation, the cells were harvested and subjected to PI staining. Death cells in the CFSE− cells were assessed by flow cytometric analysis (n = 3 cell cultures, mean ± SD, ns not significant, *P = 0.0188, ****P < 0.0001). Gating strategies were shown. e 12 h post-incubation, cells were harvested and stained with BODIPY-C11, followed by flow cytometry analysis. The gating strategy was identical to that in Fig. 4h. MFI of the target cells was used for statistical analysis (n = 3 cell cultures, mean ± SD, *P = 0.0207). f Mock or Pten-negative transfected Pten^−/− B16 cells were subjected to the cancer vaccination model and subcutaneously injected in the presence or absence of 50 μL of 5 mM NP-VE. Tumor volumes were monitored over time, and the statistical significance between the Mock groups were indicated (Pten^+ vs NP-VE, n = 7 mice; other groups, n = 6 mice, mean ± s.e.m., *P = 0.0465, **P (d20) = 0.0015, **P (d20) = 0.0019). Statistical significance was assessed by two-tailed unpaired Student’s t test (b, e, f) or one (d) or two (c, way) ANOVA followed by Tukey’s multiple comparisons test. Data are representative of two (a–f) independent experiments. Source data are provided as a Source Data file. See also Supplementary Figs. 8 and 9.

13.5-day embryos, digesting with trypsin, and filtering through a 70 μm strainer. For the construction of B16-Pten^−/− cell lines, the CRISPR-Cas9 system was used. In brief, the guide sequence “TGGCATATTATAGCATG” targeting Pten was cloning into the PX330 plasmid. The PX330 plasmid was co-expressed with Cas9 in the B16 cells and selected with G418. The cells were seeded in a 96-well plate at the density of 20 cells per well. After 2–3 weeks of expansion, the clones were harvested and examined with sequencing and western blot.

Antibodies and reagents. Following commercial antibodies were used in this study. clone number, catalog number and dilutions were shown in turn: anti-Pten (139G6, 9539, 1:1000), anti-elF2α (D7D3, 5324, 1:1000) and antibody to phosphorylated elF2α (Ser51) (119A11, 3597, 1:1000) (all from CST); anti-GAPDH (1C4, KM9002T, 1:5000) and anti-GFP (9F6, KM8009, 1:5000) (both from Sun-bio). anti-FLAG (M2, F3165, 1:5000, Sigma-Aldrich); anti-a-tubulin (29P, P29, M175-3, 1:5000), H-2D^b LCMV gp33 Tetramer-KAVYNFATC (TB-5002-1, 1:250) (both from MBL); anti-puromycin (17H1, MABE341, 1:1000) (all from Merck-Millipore); anti-nicotinamide N-acetyltransferase (N-2000, 1:2500) and anti-IFN-α (RMT3-23, 25-5870-82, 1:250), anti-LAG3 (C9B7W, 17-2231-82, 1:250), anti-IL-10 (JES5-16E3, 12-7101-41, 1:250), anti–IL-17A (eBio17B7, 12-7177-82, 1:250), anti-IL-10 (JESS-1663, 12-7110-41, 1:250), anti-Foxp3 (FJK-16-16, 17-5773-82, 1:250), anti-PD-1 (1J43, 46-9985-82, 1:250), anti-TIM3 (RM3-23, 25-5870-82, 1:250), anti-LAG3 (C9B7W, 17-2231-82, 1:250), anti–CD45 (30-F11, 25-0451-81, 1:250), anti-CD25 (PD25S, 12-0521-83, 1:250), anti-body to IFN-γ (XM1.2, 17-7311-82, 1:250) and antibody to TNF-α (MPE6-X22, 25-7321-82, 1:250) (all from eBioscience); Anti-CD4 (GK1.5, 13-0041-U500, 1:250) and anti-CD8 (138G6, 9559, 1:1000), anti-eIF2α (D7D3, 5324, 1:1000) and antibody to phospho-eIF2α (D7D3, 5324, 1:1000) (all from CST); anti-GAPDH (1C4, KM9002T, 1:5000) and anti-GFP (9F6, KM8009, 1:5000) (both from Sun-bio).

12 weeks post-bone marrow transplantation, the mice were euthanized with carbocain. The spleens of the infected mice, and the CD8^+ T cells were isolated using Magni-萘x Mouse CD8 Positive Selection Kit (eBioscience). The CD8^+ T cells were transferred into the tumor-bearing nude mice on day 10, 14, and 18 post tumor inoculation. Tumor volumes were monitored overtime.

Viral infection. For LCMV chronic infection mice model, 6–8 week-old mice were infected with v.i. infection with indicated titers of LCMV-C13.

Quantitative real-time PCR (qRT-PCR). mRNA extraction (reintron) were used to isolate total RNA from cells or tissues, followed by reverse transcribing with GoScriptTM Reverse Transcription System (Promega). TransStart Top Green qPCR SuperMix (TransGen Biotech) was used for real-time PCR. qRT-PCR was performed using Applied Biosystems 7500 Fast & 7500 Real-Time PCR System and 7500 Software v2.3 (Applied Biosystems) (all primers are listed in Supplementary Table 2).

Bone marrow transplantation (BMT). To obtain bone marrow cells, donor mice were sacrificed, and the femurs and tibias were harvested. The bone marrow canals were washed out with RPMI1640 medium containing 1% (vol/vol) FBS, and the bone marrow cells were collected, followed by red blood cell (RBC) lysis. Recipient mice were lethally irradiated (960 cGy/mouse), and i.v. injected with 5 x 10^6 bone marrow cells. Experiments on transplanted mice were performed after a latency of 30 days to ensure bone marrow reconstitution.

In vitro cytotoxic assay. C57BL/6 mice were untreated or i.v. infected with 1 x 10^6 PFU LCMV-C13. On day 7 post infection, lymphocytes were isolated from spleens of the infected mice, and the CD8^+ T cells were isolated using MagniSort™ Mouse CD8 Positive Selection Kit (eBioscience). The CD8^+ T cells were incubated with target cells pulsed with 2 μg/ml GP33-41 peptide at indicated ratios. 8 h post incubation, target cells were collected and stained with DCFDA, using for flow cytometry analysis. For percent cell lysis rate, target cells were washed to remove lymphocytes, and live cells were measured using Cell counting Kit-8 (CCK-8, Dojindo) following the manufacturer’s instructions.

Detection of ROS production. ROS-sensitive fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) was used to detect generation of ROS in cultured cells. Briefly, cells were washed with PBS, followed by staining with 100 mM DCFDA for 30 min at 37°C. After washing with FACs (PBS + 1% (vol/vol) FBS), stained cells were subjected to flow cytometry analysis. For detection of lipid peroxidation, cells were collected, and stained with 2 μM BODIPY-C11. Lipid ROS level was determined by flow cytometry analysis with excitation at 488 nm.
**TUNEL staining.** For the detection of Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) positive cells, the in-situ cell death detection kit (Roche) was used according to the manufacturer’s instructions.

**Confocal microscopy.** Cells were seeded on cover glasses. 12 h later, cells were fixed with 100% acetone, permeabilized with 0.5% (vol/vol) Triton X-100 and then blocked using 1% (wt/vol) BSA dissolved in PBS. Primary antibody was applied for at least 1 h.
followed by incubation with fluorophore-conjugated secondary antibody for 1 h. DAPI (BioDee Biotechnology) was used to indicate nuclear, and cells were evaluated with fluorescence microscopy using NIS-Elements AR Analysis 4.20.00 64 bit software (Nikon). NIS Elements Viewer 4.20 was used to analyze the confocal microscopy data.

LDH release assay. The CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega) was used to detect lactate dehydrogenase (LDH) release and cell death rates. In brief, cells were seeded in 96-well plates and treated with indicated reagents. The experimental LDH release and maximum LDH release were determined according to the manufacturer’s instructions, and the percentage of cytotoxicity was calculated by dividing experimental LDH release by maximum LDH release.

Interstitial fluid isolation. Tissue was harvested from mice. Bulk tissue was placed on a 40 µm cellulose, followed by centrifuging at 45 × g for 5 min at 4 °C to remove surface fluid. Then the tissue was centrifuged at 500 × g for 10 min at 4 °C, and the interstitial fluid was collected. After spinning at 10,000 × g for 10 min at 4 °C to remove insoluble particles, the supernatant was subjected to immunoblot analysis and ATP content assay.

Ponceau S staining. Protein samples were subjected to immunoblot analysis. After the electrophoresis and transmembrane, polyvinylidene fluoride membrane was stained by Ponceau S staining solution (Beyotime) for 30 min. The membrane was washed and used for scanning.

ATP content assay. ATP contents in the interstitial fluids were detected using ATP assay kit (Nanjing Jiancheng) according to the manufacturer’s instructions.

Propidium iodide (PI) staining. Cultured cells were digested and fixed with 100% ethanol, followed by staining with 1.1 mg/ml PI containing 1% (vol/vol) Triton-X100. Stained cells were used for flow cytometry analysis. For cell cycle analysis, peaks of cells with diploid DNA content were analyzed. Determination of cell death was performed by analyzing the sub-G1 peak.

Hematoxylin and eosin (H&E) staining. Tissue specimens were fixed in 10% (vol/vol) neutral buffered formalin followed by paraffin-sectioning and H&E staining.

Preparation of lymphocytes. Lymphocytes in spleen were isolated by grinding the tissues in PBS containing 1% (vol/vol) FBS, followed by filtering through a 75 µm strainer. To isolate lymphocytes infiltrating in the lung, minced tissues were digested with 0.5 mg/ml Collagenase D (Roche) and 25 µg/ml DNase I (Sigma) at 37 °C for 30 min. Then the tissues were grind and filtered through a 75 µm strainer. A 40% (10 ml)/70% (5 ml) Percoll gradient (GE Healthcare) was used to isolate the tissues by centrifuging 800 × g for 20 min. Cells at the inter-layer were collected and counted for further operation. For isolation of TIL, tumors were minced, grind and filtered just like the lungs mentioned above. Then the cells were resuspended in 5 ml FACS buffer, adding to a 40% (5 ml)/80% (5 ml) Percoll gradient. The gradient was centrifuged at 400 × g for 45 min, and the cells at the inter-layer of 40%/80% Percoll gradient was collected for further operation.

In vitro T cell differentiation assay. Naïve CD4+ T (CD4+CD44low) cells from spleens of C57BL/6 mice were sorted, and activated with plate-bound 2 µg/ml anti-CD3 (145-2C11; BioLegend) and 1 µg/ml anti-CD28 (37-31; BioLegend) antibodies. The cells were treated with various cytokines or antibodies for polarization. For Th1 cell differentiation, 10 µg/ml anti-IL-4 (11B11; BioLegend) antibody and 10 ng/ml IL-12 (R&D) were used. For Th17 polarization, 20 ng/ml IL-6 (R&D), 5 ng/ml TGF-β (R&D), 10 µg/ml anti-IFN-γ (XMG1.2; BioLegend) and 10 µg/ml anti-IL-4 antibodies were used. For Tr1 cell differentiation, cells were treated with 50 ng/ml IL-27 (eBioscience), 10 µg/ml anti-IFN-γ and 10 µg/ml anti-IL-4 antibodies. For iTreg differentiation, 1 ng/ml TGF-β, 4 ng/ml IL-2 (R&D), 10 µg/ml anti-IFN-γ and 10 µg/ml anti-IL-4 antibodies were used. The cells were cultured at 37 °C for 48 or 72 h, followed by flow cytometry analysis.

For detection of activation induced cell death, Naïve CD4+ T cells were activated with plate-bound 2 µg/ml anti-CD3 and 1 µg/ml anti-CD28 antibodies. 72 h later, cells were collected, and subjected to Annexin V/7-AAD staining.

CFSE staining. For CFSE staining, splenocytes were harvested, and washed twice with PBS, followed by staining with CFSE at dark for 10 min. Then the cells were washed twice with PBS containing 5% (w/v) FBS, and subjected to flow cytometry analysis.

Annexin V/7-AAD staining. Annexin V/7-AAD staining was performed using Annexin V Apoptosis Detection Kit (eBioscience), following the manufacturer’s instructions.

Flow cytometry. For surface staining, cells were incubated with specific antibodies for 30 min at room temperature. The flow cytometry analyzer (BD Biosciences), FACSuite Software Bundle v1.0 (BD Biosciences), and FACSDiva Software v6.1 (BD Biosciences) were used for acquiring and analyzing the cells. The FACS data were analyzed with FlowJo v7.6.1 software. To perform intracellular cytokine staining, cells were stimulated with 100 ng/ml PMA and 500 ng/ml ionomycin, or 2 µg/ml GP3.41 peptide together with GolgiPlug and GolgiStop (BD) for 5 h. Subsequently, cells were fixed and permeabilized, followed by staining with specific anti-cytokine antibodies.

Pulldown assay and mass spectrometry. H1299 cells were seeded and transfected with FLAG-tagged-PTEEnx. The whole-cell extracts were incubated with FLAG-beads (Sigma-Aldrich), followed by washing four times using PBS-N (PBS with 0.1% (vol/vol) NP40). Immunoprecipitated proteins were eluted using the 3x FLAG-peptide and subjected to SDS-PAGE. After staining with Coomassie Brilliant blue, the gel was excised and subjected to in-gel trypsin digestion and dried. 10 µl 0.1% formic acid was used to dissolve the peptides, and the peptides were sampled onto a 100 µm × 10 cm fused silica emitter packed with reversed-phase Reprosil-Pur C18-AQ resin (3 µm and 120 Å; Ammerbuch). Linear gradients of 5–32% acetonitrile in 0.1% formic acid were used to elute the sample at a flow rate of 300 nl/min for 50 min. An LTQ Orbitrap Elite mass spectrometer (Thermo-Fisher) equipped with a nanoelectrospray ion source (ProxeonBiosystems) was used to acquire the mass spectra data. Collision-induced dissociation (normalized collision energy, 35%; activation Q, 0.25; activation time, 10 ms) was used to perform fragmentation in the LTQ with a target value of 3000 ions. To search the raw files, the SEQUEST engine against a database from the Uniprot protein sequence database was used. Parameters were set as follows: protein modifications (carbamidomethylation (C) (fixed), oxidation (M)), variable), and phosphorylation (S, T, Y) (variable); the enzyme specificity was set to trypsin; a maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 0.5 Da.
Millipore) for at least 4 h. The precipitants were washed with PBS-N (PBS with 0.1% (vol/vol) NP40) four times, using for immune blotting analysis.

**In vitro phosphatase assay.** HEK293T cells were transfected to express FLAG-tagged eIF2α and treated with 100 μM H2O2 to induce phosphorylation of eIF2α. Then the phosphorylated eIF2α was precipitated with FLAG beads, and incubated with precipitated FLAG-tagged PTENα in phosphatase buffer (20 mM Hepes pH = 7.2, 1 mM DTT, 1 mM MgCl2, and 1 mM EDTA) for one hour. The proteins were eluted with FLAG peptide and subjected to immunoblot analysis.

**Puromycin incorporation assay.** Indicated cells were treated with H2O2 (100 μM) for indicated hours, followed by pulsing with puromycin (50 μg/mL) for 30 min. Puromycin-labeled proteins were identified with immunoblot analysis or confocal microscopy.

**35S-metabolic labeling.** Ptenα+/+ and Ptenα−/− MEFs (5 × 105) were treated with TG (500 nM) or H2O2 (100 μM) for 2 h. After starvation with methionine-free DMEM medium containing 2% (vol/vol) dialyzed fetal calf serum for 30 min, 20 μCi [35S]-labeled methionine were added. 1 h later, cells were lysed, and labeled proteins were subjected to SDS–PAGE analysis. 35S-labeled proteins were detected with a PhosphorImager.

**Single-cell RNA sequencing sample preparation and data analysis.** For sample preparation, Mock or PTENα transfected Pten−/− B16 cells were subjected to the...
Fig. 7 PTE Nα maintains protein synthesis of peroxidases. a Mock or PTE Nα expressing Pten −/− B16 cells were treated with 100 μM H2O2 for indicated hours, followed by immunoblot analysis with anti-eIF2α and anti-phospho-eIF2α antibodies. Gray values of total eIF2α and phosphorylated eIF2α were determined, and the relative ratio of phosphorylated eIF2α to total eIF2α was indicated. b In vitro phosphatase assay of FLAG-tagged PTE Nα on phosphorylated eIF2α. Phosphorylation level of eIF2α was assessed by immunoblot analysis with anti-phospho-eIF2α antibodies. c δS-metabolic labeling analysis of Pten+/+ and Pten−/− MEFs treated with 100 μM H2O2 or 500 nM TG for 3 h. Gray values of autoradiographs were determined and used for statistical analysis (pooled samples, n = 3 independent experiments, mean ± SD, ***P = 0.0020, **P = 0.0007). d and e Puromycin incorporation assay of Mock or PTE Nα expressing Pten−/− B16 cells treated with 100 μM H2O2 for indicated hours. Puromycin-labeled proteins were identified with confocal fluorescence microscopy (d) and immunoblot analysis (e). Scale bars, 100 μm. f UT untreated, f Pten−/− and Pten+/− MEFs were treated with 100 μM H2O2, followed by mass spectrometry analysis. Proteins that were significantly decreased in Pten−/− MEFs were analyzed using DAVID with Gene Ontology (GO) terms. g Mock or PTE Nα expressing Pten−/− B16 cells were treated with 100 μM H2O2 for indicated hours, and expression level of GPX4 was assessed by immunoblot analysis with anti-GPX4 antibody. Gray values of GPX4 and α-tubulin were determined, and the relative ratio of GPX4 to α-tubulin was indicated. h Flow cytometry analysis of Mock or PTE Nα expressing Pten−/− B16 cells treated with H2O2 (100 μM) for indicated hours and stained with DCFDA. Mean fluorescence intensities of DCFDA were used for statistical analysis (n = 3 cell cultures, mean ± SD, ***P < 0.0001). All live cells were gated. UT, untreated. i Endogenous Gpx4 expression in B16-Pten−/− cells was knocked down using shRNA targeting Gpx4 or scramble shRNA. Then the cells were transfected to express Mock or PTE Nα and subjected to cancer vaccine model. The tumor volumes were monitored over time (Scramble, n = 5 mice; shRNA, n = 6 mice, mean ± s.e.m., *P = 0.0238, **P = 0.0158). Statistical significances between scramble groups were shown. Statistical significance was assessed by a two-tailed unpaired Student’s t test (c, h, i). Data are representative of two (a, b, d, e, g) independent experiments or pooled from three (c, i) independent experiments. Source data are provided as a Source Data file. See also Supplementary Fig. 10.

Cancer vaccination model. The tumors were collected when the tumor volumes of Mock groups reached 200 mm3. The TILs were isolated, and CD4+ 7AAD− cells were sorted, using for scRNA-seq with two replicates. Each sample is a mix of cells from two mice. Library construction and 10X scRNA-seq were performed by Berry Genomics. Sequencing was performed in different sequencing lanes in Illumina NovaSeq 6000. For raw data processing and quality control, Cell Ranger (version 6.0.1) was used to produce a raw unique molecular identifier (UMI) count matrix through processes including cellular barcode demultiplexing, mapping reads to the transcriptome and generating quantitative matrix. The matrix was converted into a Seurat object by the R package Cellranger. The functions and regulation of the PTEN suppressor. Loss of phosphatase activity mutations were from Uniprot database (https://www.uniprot.org/uniprot/P06484). Clinical data of the tumors patients carrying Pten mutations from the TCGA (UCGC, PanCancer Atlas) and Metastatic Colorectal Cancer databases (MSSCC, Cancer Cell 2018). The data were also available from the Allen Brain Atlas. The mass spectrometry proteome data generated in this study have been deposited in the GEO database with the accession code GSE17816.

Data availability
For raw data processing and quality control, Cell Ranger (version 6.0.1) was used to produce a raw unique molecular identifier (UMI) count matrix through processes including cellular barcode demultiplexing, mapping reads to the transcriptome and generating quantitative matrix. The matrix was converted into a Seurat object by the R package Cellranger. The functions and regulation of the PTEN suppressor. Loss of phosphatase activity mutations were from Uniprot database (https://www.uniprot.org/uniprot/P06484). Clinical data of the tumors patients carrying Pten mutations from the TCGA (UCGC, PanCancer Atlas) and Metastatic Colorectal Cancer databases (MSSCC, Cancer Cell 2018). The data were also available from the Allen Brain Atlas. The mass spectrometry proteome data generated in this study have been deposited in the GEO database with the accession code GSE178258. Sequencing data of PTEN and 4-kurtak cells have been deposited in the NCBI Genbank nucleotide database under accession code MZ615337, MZ615338 and MZ615339. Phosphatase-inactive PTE Nα mutations are from Uniprot database (https://www.uniprot.org/uniprot/P06484). Clinical data of the tumors patients carrying Pten mutations from the TCGA (UCGC, PanCancer Atlas) and Metastatic Colorectal Cancer databases (MSSCC, Cancer Cell 2018). The data were also available from the Allen Brain Atlas. The mass spectrometry proteome data generated in this study have been deposited in the GEO database with the accession code GSE178258. Sequencing data of PTEN and 4-kurtak cells have been deposited in the NCBI Genbank nucleotide database under accession code MZ615337, MZ615338 and MZ615339. Phosphatase-inactive PTE Nα mutations are from Uniprot database (https://www.uniprot.org/uniprot/P06484).

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Acknowledgements
This work was supported by grants including the National Key Research and Development Program of China (Grant 2016YFA05000302 to Y.Y.), the National Natural Science Foundation of China (Key grants 82030081 and 81874235 to Y.Y., and grant 82022032 and 81991505 to D.L.), the Lam Chung Nin Foundation for Systems Biomedicine, Clinical Medicine Plus X-Young Scholars Project, Peking University, the fundamental research funds for the Central Universities (No. PKU2021LCXQ026 to D.L.), and the Fundamental Research Funds for the Central Universities (No. BMU2018YJ003 to D.L.).

Author contributions
Y.S., D.L. and Yuxin Y. conceived the study and designed experiments. Y.S. and D.L. performed the most of experiments and analyzed the data. J.S., W.H., Yue Y. and F.Q. assisted in some experiments. Y.J. provided technical assistance. Xuyang Z. performed mass spectrometry analysis. Xin Z. and G.Z. analyzed the sc-RNAseq data. L.L., Z.L., Y.J., Y.L. and H.L. provided reagents. D.L. and Yuxin Y. supervised the research. Y.S., D.L. and Yuxin Y. wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-25417-6.

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Peer review information Nature Communications thanks Weiyi Peng and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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