Supplementary Information

Supplementary Methods

Assessment of clinical characteristics of the single-agent anti-PD-1 cohort

Clinical data were retrospectively collected from patient record, including age, gender, stage of disease and serum lactate dehydrogenase (LDH) prior treatment, and target tumor sizes before and after treatment. Summary statistics (e.g., mean, SEM, number in the group) are reported in **Supplementary Table S1**. Wilcoxon rank-sum test or Chi-squared test were used to compare listed measurements between PTEN-present and PTEN-absent patients.

Gene expression analysis

RNA was isolated from confirmed FFPE metastatic melanoma samples using an RNeasy FFPE kit (QIAGEN, Venlo, Netherlands) according to the manufacturer's protocol. The expression profile of 594 genes included in GX Human Immunology V2 kit (Nanostring Technologies) and 30 candidate genes potentially associated with immune resistance described in our previous study, was determined for each sample using the Nanosting nCounter analysis system. Normalization of the raw Nanostring data was conducted using the expression of 15 reference genes by nSolver Analysis Software v1.1 (Nanostring Technologies). For the preclinical study, fresh tumor tissues were collected from 14-day tumor-bearing mice, disrupted using a rotor-stator homogenizer, followed by RNA isolation using RNeasy mini kit (Qiagen). cDNA synthesis was then carried out using a cloned AMV first-strand cDNA synthesis kit (Invitrogen). Quantitative real-time PCR was carried out using a C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA) employing SYBR Green technology with a total volume of 15 μl. The primers for genes of interest were listed in **Supplementary Table S2**. The mRNA expression levels of genes of interest were normalized relative to GAPDH transcript expression levels.

Establishment of Lentiviral vectors for gene overexpression or silencing

GIPZ viral particles containing shRNAs targeting human PTEN (Target sequences: 5'-GGCGCTATGTGTATTATTA-3' for 17 and 5'-CGGGAAGACAAGTTCATGT-3' for 60) and non-silencing shRNA were purchased from Open Biosystems (Huntsville, AL). PTEN-silenced and corresponding control cell lines were generated according to the manufacturer's protocol. Full-length, sequence-verified cDNAs encoding autophagy-related genes were obtained from the human Ultimate open reading frame (ORF) collection (Life Technologies) and transferred via Gateway recombination cloning into a modified pHAGE-EF1a-IRES-EGFP lentiviral vector (provided by Dr. Darrell Kotton, Boston University School of Medicine). The Clone IDs of autophagy-related genes in the ORF collection were listed in **Supplementary Table S3**. To overexpress *MAP1LC3B* in PTEN-silenced tumor cells, *MAP1LC3B* (clone#IOH61641) cDNA was cloned into a modified pCDH-CMV-IRES-RFP (System Biosciences). To knockdown the expression of autophagy related genes in melanoma cells, lentiviral shRNA constructs were obtained from the RNAi Consortium shRNA library (Sigma-Aldrich).

Generation of ORF-expressing and shRNA-expressing tumor cells for *in vitro* T cell-killing assay

Viral particles containing either ORF or shRNAs of gene-of-interest were used to infect patient-derived melanoma cells. To test the effect of overexpression of autophagy related genes, freshly transduced cells were co-cultured autologous tumor-reactive T cells. ORF-positive cells were gated based on the expression of tagged GFP using flow cytometry analysis. Stable cell lines expressing shRNAs targeting autophagy-related genes were generated by 2-week puromycin treatment after viral transduction. These established stable cell lines were used for *in vitro* T cell assay as described above.

Multiplex protein analysis.

14-day established tumors in B6 nude mice were resected, homogenized, and sonicated in a lysis buffer containing a protease inhibitor cocktail (Roche, Basel, Switzerland). 25µl of a 4 mg/ml tumor lysate for each sample was assayed for MILLIPLEX human cytokine/chemokine panels I (premixed 41-plex) and II (premixed 23-plex) according to the manufacturer's protocol (EMD Millipore).

Animal experiments for supplementary figures

Tyr:CreER; $PTEN^{lox/lox}$; $BRAF^{V600E/+}$ mice on a C57BL/6 background (6-8 weeks of age) were treated with 4-hydroxytamoxifen for tumor induction. Similar treatment schedule was applied for the combination therapy using GSK2636771 and anti-CTLA-4(9H10, BioXcell) as previously described. The dose of anti-CTLA-4 antibody is 200 µg/per mouse. For the transplanted tumor model, $1x10^6$ BP cells were implanted subcutaneously into C57BL/6 mice. 7 days after tumor challenge, tumor-bearing mice were treated with GSK2636771 and/or anti-PD-1 Ab as previously described. The relevant solvent and control rat IgG antibody (Sigma) were administered to control animals.

Statistical analysis of the Nanostring data

In the univariate analysis, the Wilcoxon rank-sum test was conducted for each of the 609 genes in the Nanostring data to compare between patients with and without PTEN expression. For multiple testing controls, genes with a false discovery rate no larger than 0.05 were considered statistically significant. In the multivariate analysis, a leave-one-out cross-validation analysis was conducted for the Nanostring data as follows: At each time, data excluding a single patient is used as the training set to build a predictive model for patient PTEN status, for which the Sure

Independence Screening method combined with SCAD-penalized multivariate logistic regression model are used for variable selection from the 609 genes. The selected model can be used to predict the PTEN status for the left-out patient. The procedure was repeated for all the patients and yielded a predictive model each time, which can obtain the prediction error rate based on the status prediction of excluded patients. Importantly, this enables assessment of the uncertainty of the prediction importance of a gene in terms of the frequency of a gene being selected among all the predictive models obtained with a patient excluded; the results of 5 genes with the highest selection frequency are reported.

Supplementary Figure Legends:

Supplementary Figure S1. PTEN loss in melanoma promotes resistance to T cell-mediated anti-tumor activity in the context of concurrent a selective inhibitor for mutant *BRAF*. (A) Illustration of the principle of the modified xenograft model. (B) PTEN-silenced or PTEN-WT A375/GH cells were treated with PLX4720, a selective BRAF inhibitor (BRAFi), at indicated concentrations for 48 hours. The number of live tumor cells was determined using CellTiter-Blue Cell Viability assay. (C) PTEN-silenced or PTEN-WT A375/GH cells were treated with 1nM PLX4720 for 10 hours, followed by co-culture with pmel-1 T cells for additional 3 hours. The percentage of cleaved caspase-3^+ tumor cells was determined by flow cytometry. (D) B6 nude mice were challenged with PTEN-silenced or PTEN-WT A375/GH cells. 7 days after tumor challenge, luciferase-expressing pmel-1 T cells were transferred into tumor-bearing mice. After T cell transfer, experimental mice received PLX4720 for 3 days. The luciferase intensity at the tumor site was determined by Bioluminescence imaging 6 days after T cell transfer. (E) Tumor growth curve and (F) Kaplan-Meier survival curves of tumor-bearing B6 nude mice treated with BRAFi in combination with T cell transfer. Similar results were obtained in repeated experiments. In C-F, N=3-5 per group. * $P<0.05$.

Supplementary Figure S2. Lymphocyte infiltration status and the expression level of MHC class I in tumors from melanoma patients

(A) Representative CD8 staining of tumors from PTEN absent and PTEN present patients with stage IIIB/C melanoma. (B) Correlation of $CD8^+$ T cell-infiltration with PTEN expression status and *BRAF*/*NRAS* mutation status of stage IIIB/C melanoma patient tumors. (C) The percentage of $CD8^+$ T cell infiltrated areas in tumor samples obtained from stage IIIB/C melanoma patients with different *BRAF*/*NRAS* mutation status. (D) Comparison of immune cytolytic activity at tumor sites between groups of patients with different *PTEN* CNs. Cytolytic activity was defined by the log-average of *GZMA* and *PRF1* mRNA expression. (E) Association of AKT activation with reduced Lscore. Patients were stratified based on the level of $p\text{-}AKT^{S472}$ as determined by RPPA in TCGA of SKCM (cut off, $\leq$ median). (F) The expression of MHC class I in tumor tissues obtained from stage IIIB/C melanoma patients with different PTEN expression status.

Supplementary Figure S3. Examples of increased VEGF expression in regions with loss of PTEN in melanoma patients with heterogeneous PTEN expression.

Supplementary Figure S4. List of genes which are differentially expressed in melanomas with PTEN loss. (A) Hierarchical clustering of genes differentially expressed in advanced melanoma patients with and without PTEN expression (unadjusted $P < 0.05$). Total RNAs were isolated from FFPE tumor tissues of advanced melanoma patients. The gene expression profiles were determined by NanoString Technologies. (B) Genes most frequently selected by predictive models identified in leave-one-out cross-validation analysis demonstrate supportive evidence of association with PTEN expression status. The selected frequency of distinct models of each gene

was listed in the column of number. (C) The receiver operating characteristic curve using the classifier constructed with these genes was plotted. The area under the curve was displayed as the number in the plot.

Supplementary Figure S5. Autophagy activity regulated by the PI3K pathway plays a critical role in T cell-induced tumor apoptosis. (A) Expression of LC3 I and LC3 II in fresh tumor lysates from melanoma patients with *BRAF* mutation. (B) Patient-derived melanoma cell lines Mel2338 and Mel2549 were transduced with lentiviral vectors encoding the ORFs of several autophagy-related genes. The percentage of killed (cleaved casp-3^{+}) cells among ORF^{+} cells before and after co-culture with paired autologous T cells was determined using flow cytometry. A comboscore was calculated as described in the methods section and used to evaluate the effect of overexpression of ORFs in tumor cells on sensitivity to T cell-mediated killing. (C) The Mel 2400 cell line overexpressing *MAP1LC3B* was established by cell sorting using flow cytometry. The percentage of killed *MAP1LC3B*-overexpressing tumor cells was determined as previously described. GFP-expressing tumor cells were served as control. (D) Expression of LC3II and p62 in *MAP1LC3B*-overexpressing and *MAP1LC3B*-silenced tumor cells with or without the bafilomycin A1 (Baf1) treatment. *MAP1LC3B*-overexpressing (O/E) and *MAP1LC3B*-silenced (shLC3B) Mel 2400 cell lines were generated after viral transduction. Mel 2400 lines expressing either GFP or shRNA targeting luciferase were served as control cell lines for overexpressing or silencing experiments, respectively. 100nM of Baf1 treatment for 4 hours was used to determine the autophagic flux. The intensity of western blot band was measured and used for plotting. Both the western blot image and the intensity of each band were shown. Numbers in parentheses in the density figure indicate the lane positions in the western

blot imagine. The change of autophagic flux (ΔAF) of p62 was determine by the difference of cells with or without Baf1 treatment. The changes of LC3II, p62 and ΔAF of p62 were summarized in the listed table. (E) The patient-derived melanoma cell line Mel2400 was treated with 1 μm HCQ for 24h. Pretreated tumor cells were co-cultured with autologous TILs for 3 hours. Representative contour plots of the expression of cleaved caspase-3 in tumor cells under different treatment conditions were shown. (F) The patient-derived melanoma cell line Mel2400 was treated with 1 μm or 10 μm HCQ for 10 hours, and followed by co-culturing with autologous TILs for 3 hours. The T cell-mediated tumor killing was determined as previously described. Similar results were obtained in repeated experiments.

Supplementary Figure S6. The effect of PI3Kβ inhibitor on melanoma with PTEN loss. Human PTEN-loss melanoma cell lines, A2058, WM1799, WM2644 and UCSD354L cells, were used. (A) Decreased activation of Akt in PTEN-loss melanoma cells treated with a PI3Kβ inhibitor for 3 hours. (B) The inhibition of growth of PTEN loss melanoma cells treated with a PI3Kβ inhibitor. Melanoma cell lines were treated with a PI3Kβ inhibitor at indicated concentrations for 72 hours. The number of live tumor cells was determined using CellTiter-Blue Cell Viability assay. (C) Increased T cell-induced tumor apoptosis by a PI3Kβ inhibitor. PTEN loss melanoma cell lines expressing gp100 and murine MHC class I were generated and treated with a PI3Kβ inhibitor for overnight. Pretreated tumor cells were co-cultured with pmel-1 T cells for 3 hours (E:T=10:1). The percentage of cleaved caspase-3^+ in tumor cells was determined and used to calculate the Comboscore as previously described. Any treatment that enhances the sensitivity of a tumor to T cell-mediated killing generates a comboscore greater than 1. (D) The numbers of white blood cells (WBCs) in the blood from mice treated the PI3K inhibitors.

C57BL/6 mice were injected with the splenocytes from Pmel-Thy1.1 mice, followed by vaccination of gp100 peptide. Vaccinated mice received either vehicle, GSK2636771 (30mg/kg/d) or BKM120 (60mg/kg/d) for 5 days. 5 days after vaccination, the blood samples were harvested to measure WBC counts. (E) The status of Akt activation in tumor tissues from mice bearing PTEN loss tumor after PI3K inhibitor treatment. Melanoma was initiated in a group of Tyr:CreER; $PTEN^{lox/lox}$; $BRAF^{V600E/+}$ mice. Mice with measureable tumors were randomized and treated with either vehicle, GSK2636771 (30mg/kg/d) or BKM120 (60mg/kg/d) for 5 days. Protein lysates from treated tumors were harvested and used to determine the levels of p-Akt and total Akt.

Supplementary Figure S7. Improved antitumor activity can be achieved by combining the PI3Kβ inhibitor and immune checkpoint blockers. (A-C) Melanoma was initiated in a group of Tyr:CreER; $PTEN^{lox/lox}$; $BRAF^{V600E/+}$ mice. Mice with measureable tumors were randomized and treated with either vehicle plus isotype-matched control antibody, GSK2636771 (30mg/kg), anti-CTLA-4(200μg), or the combination of both GSK2636771 and anti-CTLA-4. (A) Schematic representation of anti-CTLA4 and the PI3Kβ inhibitor treatment protocol. (B) Tumor size in each of the treatment groups. Tumor growth was monitored every 3 days and plotted as means + SEM (N=4-8). (C) Kaplan-Meier survival curves of mice treated with GSK2636771 and/or anti-CTLA-4. Log-rank test demonstrates statistical significance ($P<0.05$): GSK2636771+ anti-CTLA-4 vs control, GSK2636771 or anti-CTLA-4. (D-E) The antitumor activity of the combination of GSK2636771 and anti-PD-1 in a transplanted tumor model. $1X10^6$ BP cells, murine melanoma cells bearing *BRAF* mutation and PTEN loss, were implanted subcutaneously to C57BL/6 mice. 7 days after tumor challenge, tumor-bearing mice were treated with GSK2636771 (30mg/kg) and/or anti-PD-1 Ab (100μg), as previously described. (D)

Schematic representation of anti-PD-1 and the PI3Kβ inhibitor treatment protocol. (E) Tumor size in each of the treatment groups (N=4-8).

Supplementary Table S1. Patient characteristics of the single-agent anti-PD-1 cohort.

		PTEN present (N=29)	PTEN absent (N=10)	P value
LDH(IU/L)		553.5 ± 57.06	1008 ± 327.5	0.13[a]
Baseline target tumor size		90.52 ± 13.43	71.93 ± 15.61	0.55[a]
Age		65.86 ± 2.537	61.30 ± 4.787	0.37[a]
Stage				0.43[b]
	III C	2	0	
	IVM1a	4	1	
	IVM1b	4	0	
	IVM1c	19	9	
Gender				0.79[b]
	Female	7	2	
	Male	22	8	

[a]Wilcoxon rank-sum test was used.
[b]Chi-squared test was used.

Supplementary Table S2. List of primers used for gene expression analysis by real-time PCR

Gene	Forward	Reverse
CXCL1 (IL8RA)	CTGCGAGTGGCACTGCTG	CCTCCTCCCTTCTGGTCAGG
CXCL2 (IL8RB)	TCACCTCAAGAACATCCAAAGTGTG	CTTCAGGAACAGCCACCAATAAGC
CXCL3	ATCCAAAGTGTGAATGTAAGGTC	GCAGGAAGTGTCAATGATACG
CXCL5 (ENA-78/LIX),	CCGCTGCTGTGTTGAGAG	TCTGCTGAAGACTGGGAAAC
CXCL6 (GCP2)	GCTGAGAGTAAACCCCAAAAC	AACTGCTCCGCTGAAGAC
CXCL8	TCTGCAGCTCTGTGTGAAGG	ACTTCTCCACAACCCTCTGC
CXCL8	GTGGCTCTCTTGGCAGCCTTCCTGAT	TCTCCACAACCCTCTGCACCCAGTTT
CXCL9	CATGCTGGTGAGCCAAGCAGTTTGAA	CACTTCTGTGGGGTGTTGGGGACAAG
CXCL10	TGCAAGCCAATTTTGTCCACGTGTTG	GCAGCTGATTTGGTGACCATCATTGG
CXCL11 (I-TAC/IP-9)	ATGAGTGTGAAGGGCATGGC	TCACTGCTTTTACCCCAGGG
CXCL12 (SDF1)	CCCTCTGTGAGATCCGTCTTTGGCCT	TCTGATTGGAACCTGAACCCCTGCTG
CXCL13	TGATCGAATTCAAATCTTGCCCCGTG	AAGCTTGAGTTTGCCCCATCAGCTCC
CXCL16	ACTACACGAGGTTCCAGCTCC	CTTTGTCCGAGGACAGTGATC
CCL1 (I309),	AAGGCCCAAGCCAGACCAGAAGACAT	ATGTGGTTTCCAGAGCCCACAATGGA
CCL2 (MCP-1),	CCGAGAGGCTGAGACTAAC	CTTGCTGCTGGTGATTCTTC
CCL3 (MIP-1A),	CTCTGCATCACTTGCTGCTGACAC	CACTCAGCTCCAGGTCGCTGAC
CCL4 (MIP-1B),	ACCCTCCC CCGCCTGCTGC	CAGGTCATACACGTACTCC
CCL5 (RANTES),	CCTCGCTGTCATCCTCATTG	GGGTTGGCACACACTTGG
CCL7 (MCP-3),	AGAAGGACCACCAGTAGCC	AGAACCACTCTGAGAAAGGAC
CCL8 (MCP-2)	CGAGGAGCAGAGAGGTTGAG	GATGTTGGTGATTCTTGTGTAGC
CCL11 (Eotaxin),	ACCACCTCTCACGCCAAAGCTCACAC	CGGCACAGATATCCTTGGCCAGTTTG
CCL13 (MCP-4),	CAAACTGGGCAAGGAGATCTG	GGCCCAGGTGTTTCATATAATTCT
CCL14,	ATCTCCGTGGCTGCCATTCCCTT	CCACTTGTCACTGGGGTTGGTACA
CCL15 (MIP-1D),	TGGATCCCAGGCCCAGTTCATA	CCCCTTCTTGGTGAGGAATATG
CCL17 (TARC),	ACTGCTCCAGGGATGCCATCGTTTTT	ACAAGGGGATGGGATCTCCCTCACTG
CCL19,	CCAGCCTCACATCACTCACACCTTTGC	TGTGGTGAACACTACAGCAGGCACCC
CCL20 (MIP-3A),	TTTGCTCCTGGCTGCTTTGATGT	GTTTTGGATTTGCGCACACAGAC
CCL21 (MIP-2)	AACCAAGCTTAGGCTGCTCCATCCCA	TATGGCCCTTTAGGGGTCTGTGACCG
CCL22 (MDC),	AGGACAGAGCATGGCTCGCCTACAGA	TAATGGCAGGGAGGTAGGGCTCCTGA
CCL25 (TECK),	CCAAGGTGTCTTTGAGGACTGCTGCC	GGGAGACATTCCTCTTGCTGCTGCTG
CCL27,	CTACAGCAGCATTCCTACTGC	ATGGAGCTTTCTCTCTTGGTG
CCL28,	AGAAGCCATACTTCCCATTGC	AGCTTGCACTTTCATCCACTG
XCL1	ACACCATCACGGAAGGCTCCTTGAGA	AGTGAAATGAGCTGGCTGGCTGGAGA
XCL2	AGGAATTCATGAGTGAAGTCTCACATAG	TAACTCGAGCTAGCCAGTCAGGGTCACAG
CX3CL1	GCCATGTTCACCTACCAGAGCCTCCA	TGGAAGGTGGAGAATGGTCAAGGCTG
VEGFA	CGAAACCATGAACTTTCTGC	CCTCAGTGGGCACACACTCC
PD-L1	CCACCACCACCAATTCCAAGAG	GGACCCATCCAACTTGGCTG
GAPDH	CATCATCTCTGCCCCCTCT	GGTGCTAAGCAGTTGGTGGT

Supplementary Table S3. List of autophagy-related genes for overexpressing experiment

Gene Symbol	Entrez Gene ID	Clone ID
AMBRA1	55626	IOH27255
ATG10	83734	IOH22840
ATG12	9140	IOH10538
ATG12	9140	IOH13782
ATG12	9140	IOH40867
ATG13	9776	IOH6276
ATG13	9776	IOH3624
ATG3	64422	IOH13623
ATG4A	115201	IOH42076
ATG4B	23192	IOH4606
ATG4C	84938	IOH22446
ATG4D	84971	IOH6968
ATG5	9474	IOH5286
ATG7	10533	IOH52654
ATG9A	79065	IOH39945
BECN1	8678	IOH11006
MAP1LC3A	84557	IOH10043
MAP1LC3A	84557	IOH42908
MAP1LC3B	81631	IOH13267
MAP1LC3B	81631	IOH61641
MAP1LC3C	440738	IOH59918
ULK3	25989	IOH45122