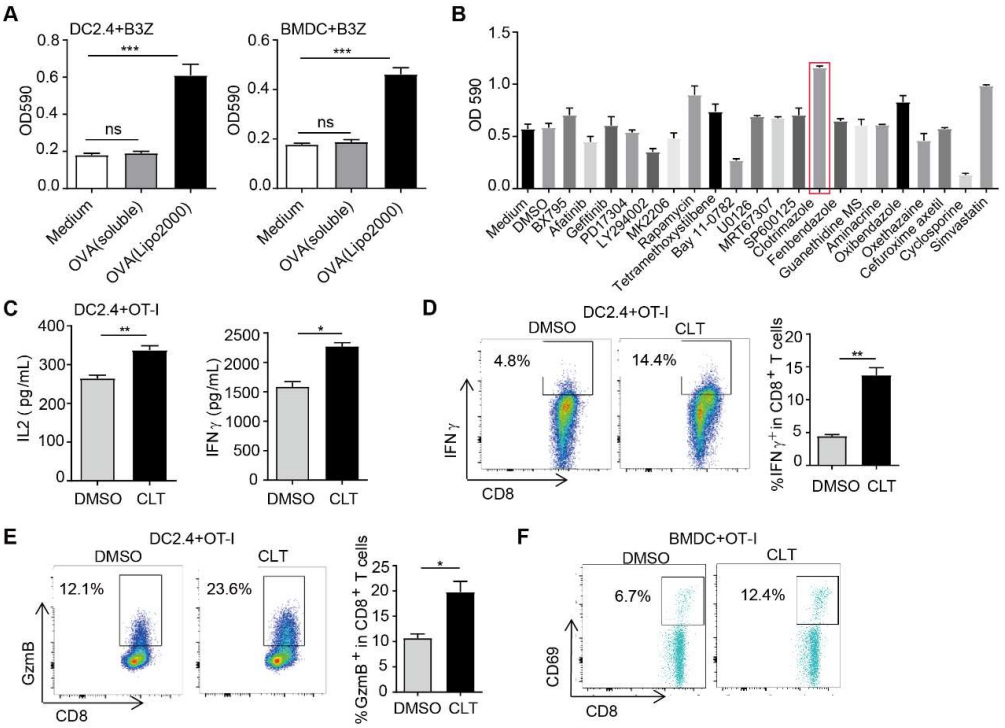
Supplementary Figures



Supplementary Figure 1. Clotrimazole enhanced DC-induced T cell activation.

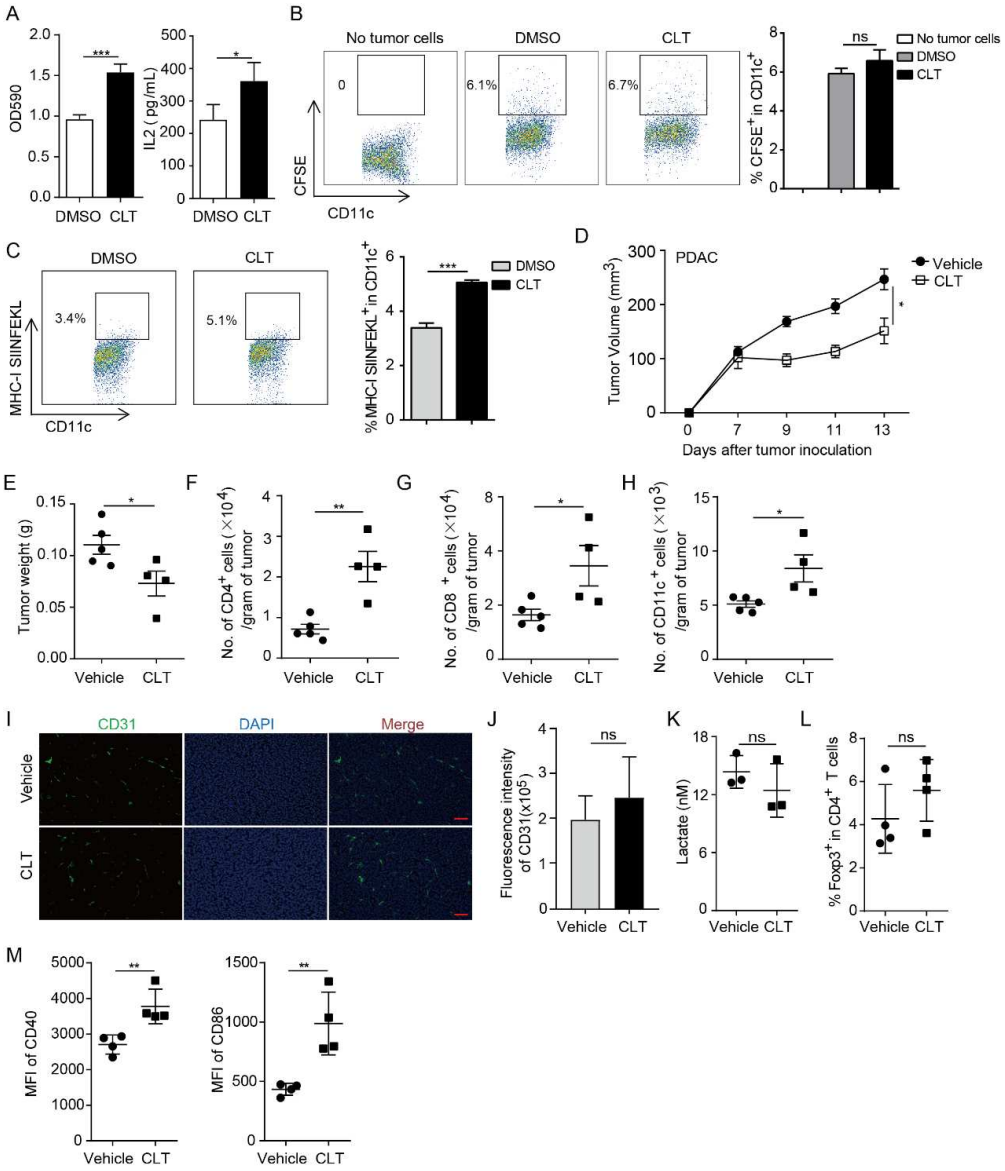
A. OVA (100 μg/mL) was added or transfected by Lipo2000 into DC2.4 or BMDCs for about 20 hrs, followed by co-cultured with B3Z for additional 24 hrs, after which B3Z activation was measured by LacZ activity.

B. DC2.4 cells were pretreated with different drugs or inhibitors for 2 hrs, and transfected with OVA (100 μg/mL) for 20 hrs, followed by co-culture with B3Z cells for an additional 24 hrs, after which LacZ activity in B3Z cells were measured. Clotrimazole pretreated DCs induced the highest LacZ activity in B3Z cells.

C-E. DC2.4 cells were treated with clotrimazole or DMSO, then transfected with OVA (100 μg/mL) for 20hrs, and co-cultured with OT-I cells for additional 24 hrs, after which OT-I cells activation were measured by IL-2 production, IFNγ production(**C**), and intracellular expression of IFNγ(**D**) and GZMB(**E**).

F. BMDCs were treated with clotrimazole or DMSO, then transfected with OVA (100 μg/mL) for 20 hrs, and co-culture with OT-I cells for additional 24 hrs, after which OT-I cells activation were measured by CD69 expression.

Data in **A-F** are the representative results of 3 repeated experiments. **P*<*0.05,* ***P* < 0.01, ****P* < 0.001 and ns=not significant, by one-way ANOVA with Bonferroni's post-test.



Supplementary Figure 2. Clotrimazole inhibited tumor growth and induced immune cell infiltration into tumor microenvironment.

A. The mixture of BMDCs and live B16-OVA cells were treated with clotrimazole (10 μM) or DMSO for 20 hrs, then co-cultured with B3Z cells for additional 24 hrs. The LacZ activity and IL-2 production were measured.

B. BMDCs were pretreated with Clotrimazole or DMSO, then co-cultured with CSFE-labeled B16-OVA cells for 2hrs; after extensive wash, the CFSE signal in $CD11c^+$ cells were detected by flow cytometry.

C. BMDCs were pretreated with Clotrimazole or DMSO, then co-cultured with B16-OVA cells for 24 hrs, after which the MHC-I-SIINFEKL expression on DCs were detected by flow cytometry.

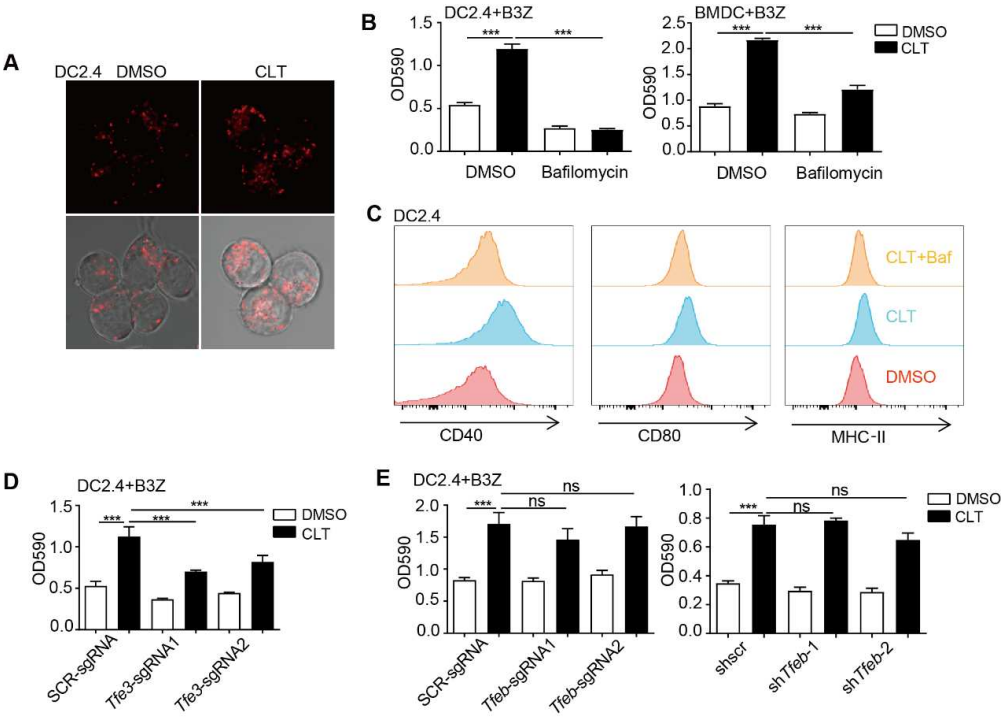
D. B6 mice with established PDAC tumors were treated with clotrimazole or vehicle

on day 5-9 (40 mg/kg, i.p.), and tumor growth was monitored. Tumor volume is shown as mean ± SD. n = 5 for vehicle group and n=4 for clotrimazole group.

E-H. B6 mice with established PDAC tumors were with clotrimazole or vehicle on day 5-9 (40 mg/kg, i.p.). Tumors were isolated on day 13, and tumor-infiltrating immune cells were analyzed by flow cytometry. Shown are tumor weight (**E**), numbers of tumor-infiltrating $CD4^+$ T cells (**F**), $CD8^+$ T cells (**G**), and $CD11c^+$ dendritic cells (**H**), n=5 for vehicle and n=4 for clotrimazole group.

I-M. C57/B6 mice with established MC38 tumors were treated with vehicle or clotrimazole (40mg/kg, day 3-7, i.p.). Tumors were harvested at day 11. The expression pattern of CD31 in tumors was detected by immunofluorescence (**I, J**), lactate concentration was measured (shown as per gram tumor) (**K**), the infiltrated FoxP3+ cells and co-stimulatory molecule expression on $CD11c^+$ cells were detected by flow cytometry (**L-M**). n=3 for (**I, J**), n=4 for (**L, M**). Scale bar=50 μm in **I**.

Tumor volume is presented as mean ±SD, $*P < 0.05$, $**P < 0.01$, by unpaired Student's *t* test (**A-H, J-M**) or two-way ANOVA with Bonferroni's post-test (**D**).



Supplementary Figure 3. Clotrimazole induced DC activation by lysosome-associated pathway.

A. DC2.4 cells were treated with clotrimazole or DMSO for 24 hrs, the PE-labeled lysosome tracker was added into the medium for another 30 mins. The fluorescence signal was detected by fluorescent microscopy.

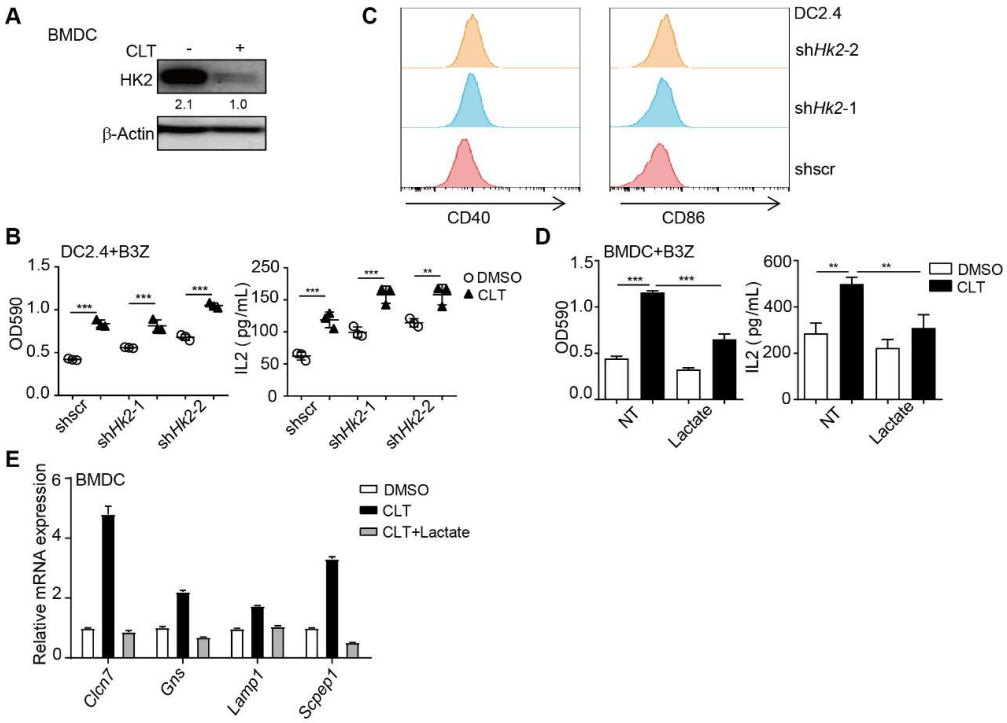
B. BMDC or DC2.4 cells were treated with DMSO, clotrimazole, bafilomycin or clotrimazole+bafilomycin, then transfected with OVA (100 μg/mL) for 20 hrs, and co-culture with B3Z for additional 24 hrs, after which B3Z cells activation were measured by LacZ activity.

C. DC2.4 cells were treated with DMSO, clotrimazole, clotrimazole+bafilomycin for 24 hrs, the expression of CD40, CD80 and MHC-II were measured by flow cytometry.

D. DC2.4 cells were treated clotrimazole or DMSO, then transfected with OVA (100 μg/mL) for 20 hrs, and co-cultured with B3Z for additional 24 hrs, after which B3Z cells activation were measured by LacZ activity.

E. DC2.4 cells were treated with clotrimazole or DMSO for 20 hrs, then transfected with OVA (100 μg/mL) for 20 hrs, and co-cultured with B3Z for additional 24 hrs, after which B3Z cells activation were measured by LacZ activity.

Data in **A-E** are the representative result of 3 repeated experiments. **P*<*0.05,* ***P* < 0.01, ****P* < 0.001 and ns=not significant, by one-way ANOVA with Bonferroni's post-test.



Supplementary Figure 4. Clotrimazole inhibited lactate production by targeting HK2 in DCs.

A. BMDCs were treated with clotrimazole or DMSO for 24 hrs, then HK2 expression was detected by WB.

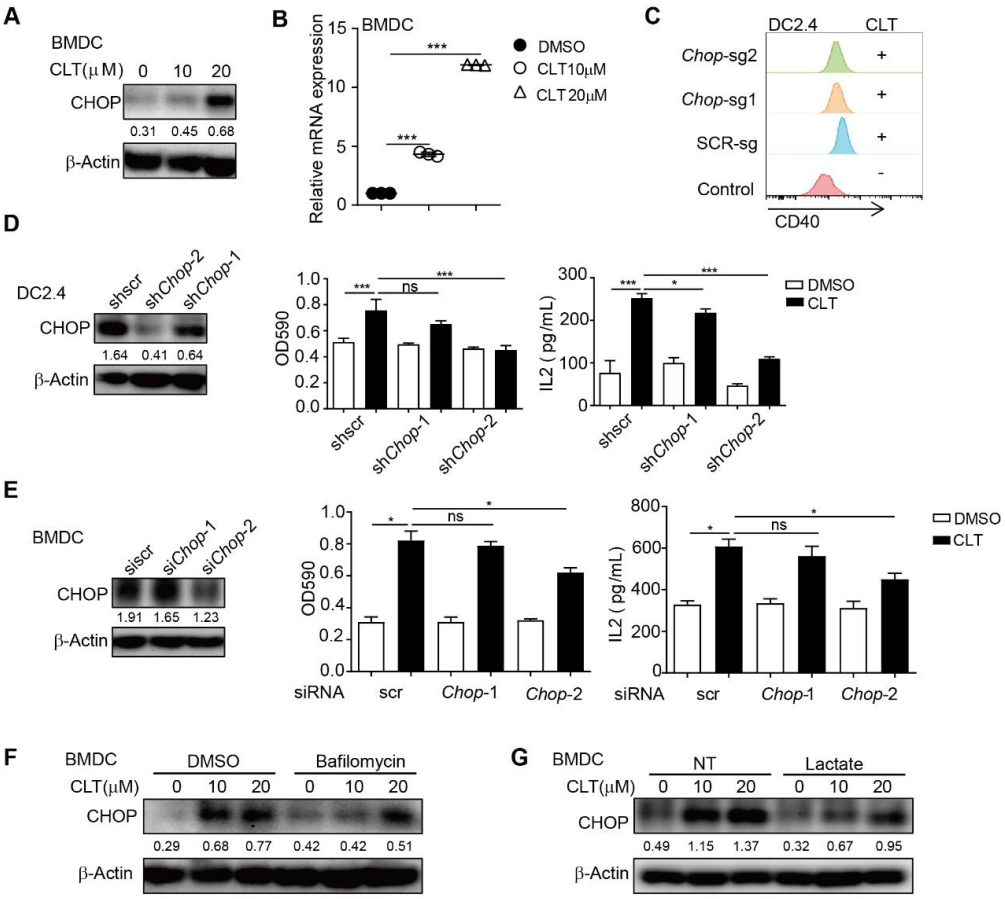
B. The expression of CD40 and CD86 in DC2.4 cells were detected by flow cytometry.

C. DC2.4 cells were treated with clotrimazole or DMSO, then transfected with OVA (100 μg/mL) for 20 hrs, then co-cultured with B3Z for additional 24hrs, after which B3Z cells activation was measured by LacZ activity and IL-2 production.

D. BMDCs were treated with DMSO, clotrimazole, lactate or clotrimazole+lactate, then transfected with OVA (100 μg/mL) for 20 hrs, then co-cultured with B3Z for additional 24 hrs, after which B3Z cells activation was measured by LacZ activity and IL-2 production.

E. BMDCs were treated with DMSO, clotrimazole, lactate or clotrimazole+lactate for 24hrs. The expression of lysosome-associated genes was measure by qPCR.

Data in **A-D** are the representative result of 3 repeated experiments. Data in **E** are shown as mean ± SD of 3 replicates from 1 representative experiment. $^{**}P < 0.01$, $^{***}P < 0.001$, by one-way ANOVA with Bonferroni's post-test.



Supplementary Figure 5. Clotrimazole induced DC activation depending on CHOP.

A-B. BMDCs were treated with clotrimazole or DMSO for 24 hrs, and the expression of CHOP was detected by WB (**A**) or qPCR (**B**).

C. DC2.4 cells were treated with clotrimazole or DMSO for 24hrs, after which the expression of CD40 were detected by flow cytometry.

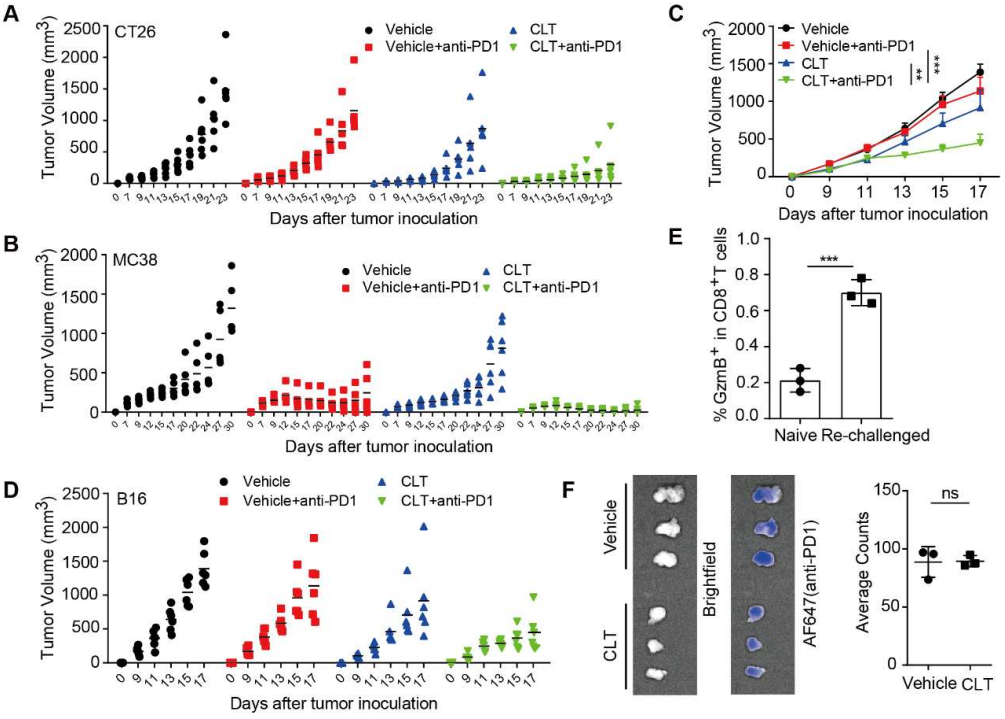
D. DC2.4 cells were treated with clotrimazole or DMSO, then transfected with OVA (100 μg/mL) for 20 hrs, followed by co-culture with B3Z for additional 24 hrs, after which B3Z cells activation was measured by LacZ activity and IL-2 production. WB detected the knockdown efficiency of CHOP by shRNA.

E. BMDCs were transfected with CHOP-siRNA or NC-siRNA, then treated with clotrimazole or DMSO, and transfected with OVA (100 μg/mL) for 20 hrs, followed by co-cultured with B3Z for additional 24 hrs, after which B3Z cell activation were measured by LacZ activity and IL-2 production. WB detected the knockdown efficiency of CHOP by siRNA.

F. BMDCs were treated with DMSO, clotrimazole, bafilomycin or clotrimazole+bafilomycin for 24 hrs, the expression CHOP was detected by WB.

G. BMDCs were treated with DMSO, clotrimazole, lactate or clotrimazole+lactate for 24 hrs, the expression CHOP was detected by WB.

Data in **A, C, D, E, F, G** are the representative result of 3 repeated experiments. Data in **B** are shown as mean ± SD of 3 replicates from 1 representative experiment. **P*<*0.05,* ****P* < 0.001 and ns=no significant, by one-way ANOVA with Bonferroni's post-test.



Supplementary Figure 6. Clotrimazole potentiated the efficacy of anti-PD1 therapy in multiple mouse tumor models.

A. Individual tumor growth curve of each mouse in CT26 tumor model which were treated with clotrimazole, anti-PD1, or clotrimazole in combination with anti-PD1.

B. Individual tumor growth curve of each mouse in MC38 tumor model which were treated with clotrimazole, anti-PD1, or clotrimazole in combination with anti-PD1.

C. B6 mice were subcutaneously injected with B16 tumors cells and divided into 4 groups stochastically, then treated with clotrimazole (40 mg/kg, day3-7), anti-PD1 (7.5mg/kg, day 9/12), or clotrimazole in combination with anti-PD1 at indicated time points. Tumor volume was shown as mean ± SD. n=6 for each group.

D. Individual tumor growth curve of each mouse in B16 tumor model which were treated as described in **C**.

E. $CD8^+$ T cells from the mice lymph node which previously rejected MC38 tumors upon combination therapy co-culture with MC38 tumor cells for 24 hrs, the intracellular GZMB production were detected by flow cytometry.

F. Left panel: B6 mice were subcutaneously injected with MC38 tumor cells and then treated with clotrimazole (40 mg/kg) or vehicle on day 3-7; AF647 labeled anti-PD1 (5 mg/kg) were injected on day 9. Tumors were harvested 24 hrs later, and the tumor distribution of anti-PD1 antibody was measured by IVIS Lumina III imaging system (Perkin-Elmer). Right panel: the average fluorescence of each tumor was quantitated. n=3 for each group.

Tumor volume is presented as mean ±SD, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns=not significant, by two-way ANOVA with Bonferroni's post-test (**C**) or unpaired Student's *t* test (**E-F**).

Supplementary Table 1.

The sequences of primers used for qPCR

Actin-FP	5'-AGAGGGAAATCGTGCGTGAC-3'
Actin-RP	5'-CAATAGTGATGACCTGGCCGT-3'
Clcn7-FP	5'-CGCCAGTCTCATTCTGCACT-3'
Clcn7-RP	5'-GCTTCTCGTTGTGTGGAATCT-3'
Gns-FP	5'-CGGTGTGCGGCTATCAGAC-3'
Gns-RP	5'-CAGGGCATACCAGTAACTCCA-3'
Lamp1-FP	5'-CAGCACTCTTTGAGGTGAAAAAC-3'
Lamp1-RP	5'-ACGATCTGAGAACCATTCGCA-3'
Naglu-FP	5'-ACCGCTATTACCAGAATGTGTG-3'
Naglu-RP	5'-GTGTGCAAGTTACCCATGCG-3'
Scpep1-FP	5'-CTGCTGCTCCTATCGTTCTTAC-3'
Scpep1-RP	5'-CCTTTCGGACAGTCACATAATCC-3'
Tap1-FP	5'-GGACTTGCCTTGTTCCGAGAG-3'
Tap1-RP	5'-GCTGCCACATAACTGATAGCGA-3'
Tap2-FP	5'-CTGGCGGACATGGCTTTACTT-3'
Tap2-RP	5'-CTCCCACTTTTAGCAGTCCCC-3'
Earp1-FP	5'-TAATGGAGACTCATTCCCTTGGA-3'
Earp1-RP	5'-AAAGTCAGAGTGCTGAGGTTTG-3'
B2m-FP	5'-TTCTGGTGCTTGTCTCACTGA-3'
B2m-RP	5'-CAGTATGTTCGGCTTCCCATTC-3'
Chop-FP	5'-GTCCCTAGCTTGGCTGACAGA-3'
Chop-RP	5'-TGGAGAGCGAGGGCTTTG-3'
CD86-FP	5'-ACGTATTGGAAGGAGATTACAGCT-3'
CD86-RP	5'-TCTGTCAGCGTTACTATCCCGC-3'

Supplementary Table 2.

The list of drugs/inhibitors used for screening

Drugs/inhibitors	Target/Bioactivity	Drugs/inhibitors	Target/Bioactivity
BX795	PDK1	Tetramethoxystilbene	antineoplastic
Afatinib	EGFR/ErbB	Clotrimazole	antifungal
Gefitinib	EGFR	Fenbendazole	anthelmintic
PD17304	FGFR1	Guanethidine monosulfate	antihypertensive
LY294002	PI3K	Aminacrine	local antiseptic
MK2206	AKT	Oxibendazole	anthelmintic
Rapamycin	mTOR	Oxethazaine	anesthetic
SP600125	JNK	Cefuroxime axetil	antibacterial
Bay 11-0782	IKKβ	Cyclosporine	immunosuppressant
U0126	MEK	Simvastatin	Cholesterol-lowering
MRT67307	IKKε/TBK1		