Supplementary Material

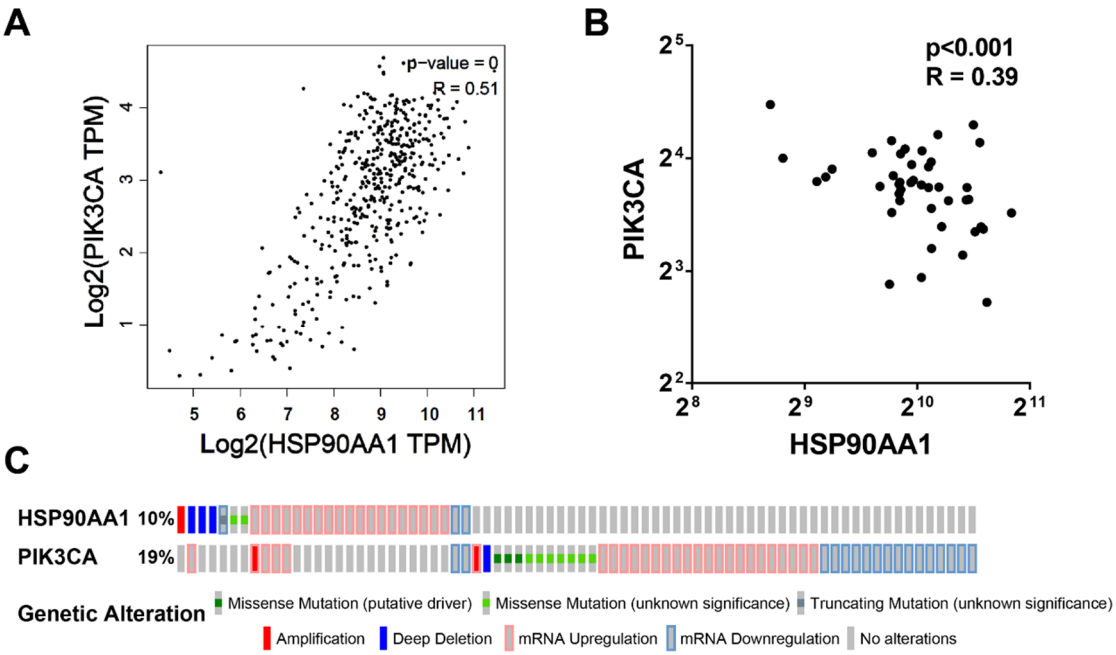


Figure S1. (A) The correlation of expression profiles on PIK3CA and HSP90AA1 in melanoma cohort of TCGA database. (B) The correlation of expression profiles on PIK3CA and HSP90AA1 in normal human skin tissues in TCGA and GTEx databse. (C) The mutations of HSP90AA1 and PIK3CA on melanoma patients in TCGA databse.

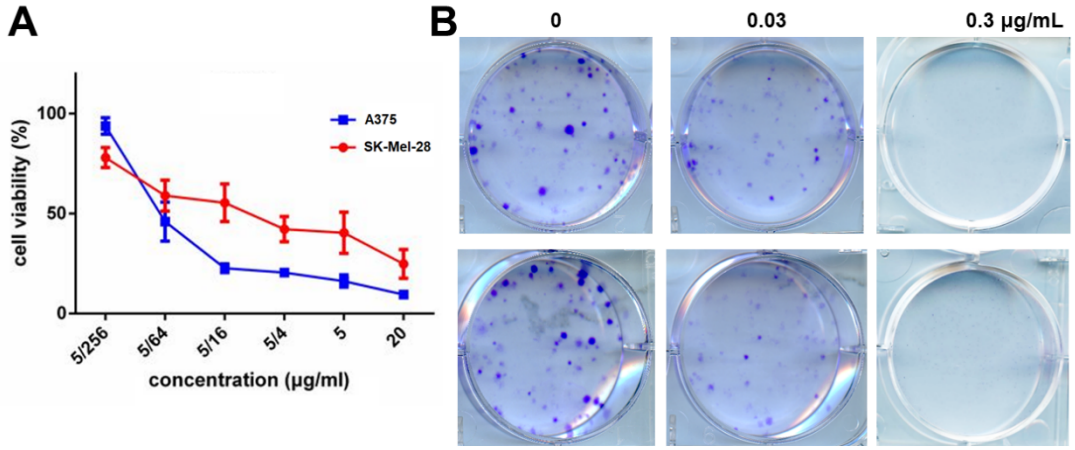


Figure S2. (A) The inhibition curves of cell viabilities with DHP1808 incubation, (B) The replicated results of colony formation assays, A375 cells were incubated with various concentrations (0.03 or 0.3μg/ml) of DHP1808 for 5 days.

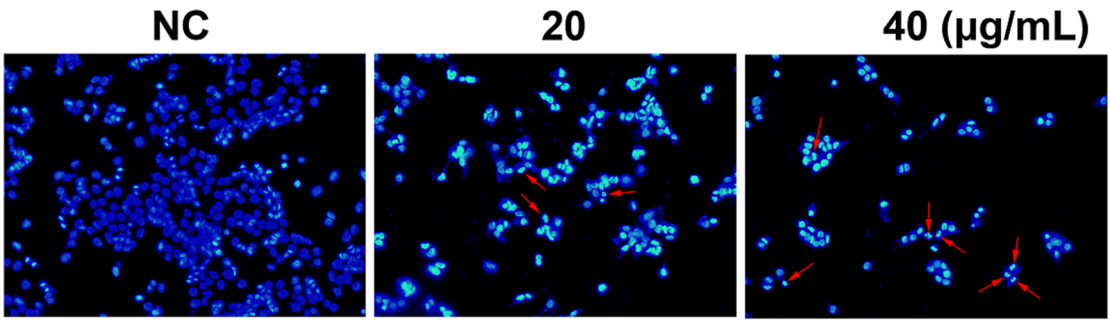


Figure S3. A375 cells were incubated with various concentrations (0, 20 or 40μg/ml) of DHP1808 for 24 h. The cellular morphology was observed under fluorescent microscopy after Hoechst 33258 staining.

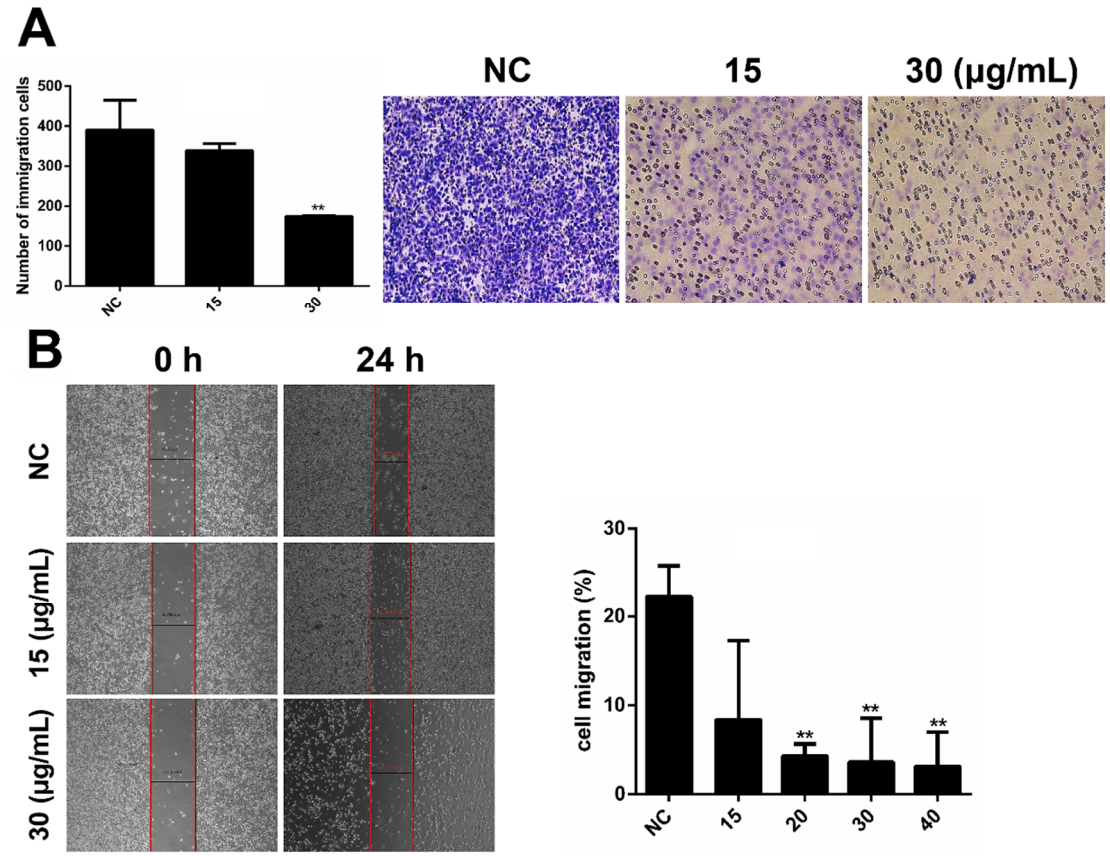


Figure S4. A. A375 cells were incubated with various concentrations (0, 15 or 30μg/ml) of DHP1808 for 24 h. The changes of cells invasion by transwell assay; B. A375 cells were incubated with various concentrations (0, 15 or 30μg/ml) of DHP1808 for 24 h. The changes of cells migration by wound healing assay;

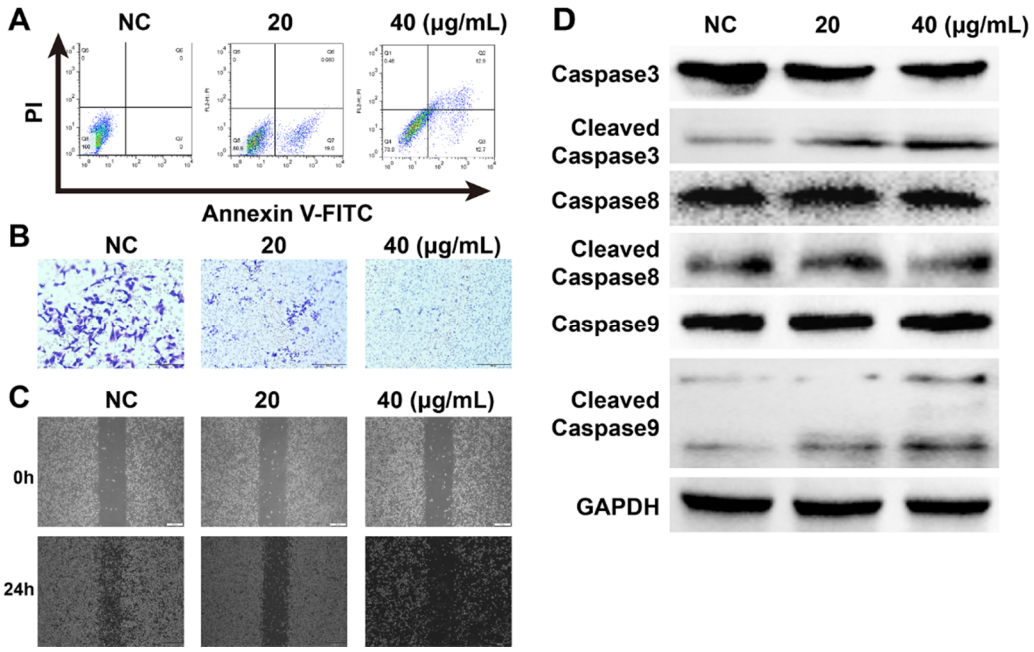


Figure S5. The results of Annexin V/PI staining (A), cell migration and invasion assays (B and C), caspase-3, caspase-8, caspase-9 immunoblot (D) with various concentrations (20 or 40μg/ml) of DHP1808 in SK-Mel-28 cells.

Table S1. Kinase Selectivity of DHP1808 against a panel of 97 kinases [a]

Compound Name	DiscoveRx Gene Symbol	Percent Control (%)	Compound Concentration (nM)
DHP1808	Abl	86	1000
DHP1808	Abl(T315I)	95	1000
DHP1808	ALK	83	1000
DHP1808	ARK5	120	1000
DHP1808	Aurora-A	97	1000
DHP1808	Axl	121	1000
DHP1808	Blk	113	1000
DHP1808	Bmx	118	1000
DHP1808	BRK	93	1000
DHP1808	B-Raf	94	1000
DHP1808	B-Raf(V599E)	103	1000
DHP1808	CDK1/cyclinB	108	1000
DHP1808	CDK2/cyclinA	112	1000
DHP1808	CDK5/p35	115	1000
DHP1808	CHK1	109	1000
DHP1808	CHK2	88	1000
DHP1808	CK1γ1	103	1000
DHP1808	CK1γ2	114	1000
DHP1808	CK1γ3	88	1000
DHP1808	cKit	89	1000

DHP1808	cKit(D816H)	92	1000
DHP1808	cKit(V560G)	124	1000
DHP1808	CSK	91	1000
DHP1808	c-RAF	95	1000
DHP1808	cSRC	120	1000
DHP1808	DAPK1	121	1000
DHP1808	DCAMKL3	85	1000
DHP1808	DDR2	96	1000
DHP1808	DYRK2	106	1000
DHP1808	EGFR	105	1000
DHP1808	EGFR(L858R)	114	1000
DHP1808	EGFR(L861Q)	105	1000
DHP1808	EGFR(T790M)	115	1000
DHP1808	EGFR(T790M,L858R)	99	1000
DHP1808	EphA2	108	1000
DHP1808	EphA7	84	1000
DHP1808	EphB4	100	1000
DHP1808	ErbB2	91	1000
DHP1808	ErbB4	107	1000
DHP1808	FAK	115	1000
DHP1808	Fer	92	1000
DHP1808	Fes	120	1000
DHP1808	FGFR1	95	1000
DHP1808	FGFR2	113	1000
DHP1808	FGFR3	95	1000
DHP1808	FGFR4	106	1000
DHP1808	Flt1	109	1000
DHP1808	Flt3(D835Y)	95	1000
DHP1808	Flt3	105	1000
DHP1808	Flt4	114	1000
DHP1808	Fms	110	1000
DHP1808	Hck	95	1000
DHP1808	HIPK2	82	1000
DHP1808	HIPK3	96	1000
DHP1808	IGF-1R	104	1000
DHP1808	IKKα	121	1000
DHP1808	IKKε	93	1000
DHP1808	KDR	96	1000
DHP1808	LKB1	119	1000
DHP1808	MAPK2	122	1000
DHP1808	MEK1	100	1000
DHP1808	MELK	91	1000
DHP1808	Mer	90	1000
DHP1808	Met	88	1000

DHP1808	MST3	122	1000
DHP1808	NIM1	105	1000
DHP1808	p70S6K	91	1000
DHP1808	PAK1	118	1000
DHP1808	PAK4	118	1000
DHP1808	PDGFRα	101	1000
DHP1808	PDGFRα(D842V)	103	1000
DHP1808	PDK1	114	1000
DHP1808	Pim-1	93	1000
DHP1808	PKBα	116	1000
DHP1808	PKCα	90	1000
DHP1808	PKCε	120	1000
DHP1808	PKCη	102	1000
DHP1808	PKCι	117	1000
DHP1808	PKCμ	115	1000
DHP1808	PKCθ	103	1000
DHP1808	PKD2	100	1000
DHP1808	Ret	108	1000
DHP1808	ROCK-I	98	1000
DHP1808	Ron	85	1000
DHP1808	Ros	99	1000
DHP1808	Snk	84	1000
DHP1808	TAK1	110	1000
DHP1808	Tie2	121	1000
DHP1808	TrkA	122	1000
DHP1808	TrkC	106	1000
DHP1808	Wee1	115	1000
DHP1808	Yes	94	1000
DHP1808	ZAP-70	82	1000
DHP1808	ZIPK	104	1000
DHP1808	ATM	85	1000
DHP1808	ATR/ATRIP	87	1000
DHP1808	DNA-PK	86	1000

[a] Measurements were performed at a concentration of 1 μM of the inhibitor in duplicate. The % control means active kinase percentage in the well. The affinity was defined with respect to a DMSO control.

Experimental Section:

Colony formation assay

For the colony formation assay, a total of 300 cells were seeded in 6-well plates in triplicate and maintained in the complete medium for 7-12 days. The natural colonies were washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature. The colonies were then stained with Crystal Violet Staining Solution for 10 min, washed with water and air-dried. The total number of colonies with more than 50 cells was counted under fluorescence microscopy.

Transwell assay

For the invasion assay, we used Transwell chambers (8-μmpore size, Corning). Specifically, 1×10^5 of A375 cells were suspended in serum-free medium, and then loaded into the Matrigel precoated top-chambers (BD Bioscience, San Jose, CA, USA) and incubated with DMEM culture in the bottom chamber. Crystal violet staining was used to measure the invasiveness of A375 cells. Cell counts were performed in triplicate under a microscope.

Wound healing assay

Cell migration assay was performed using the agarose wound healing method. In brief, A375 cells were seeded in 6-well plate with DMEM for 1×10^5 cells per well and was further grown overnight. Three lines were cut in each well using a 200 ml pipette tip. Cells were then washed with PBS and serum-free DMEM including various concentrations of drugs or DMSO were added. Evaluation of the cell migration was determined under a light microscope between 0 h and 24 h of treatment.