Supplementary Figure legends

Figure S1. **The overall survival rates of melanoma patients with different expression levels of genes listed in a Venn diagram in Figure 1D**. **(A)** The overall survival rates of melanoma patients with different *BIRC5* expression were not significantly different (*p*-value = 0.08). **(B)** The overall survival rates of melanoma patients with different *BUB1* expression were not significantly different (*p*-value = 0.91). **(C)** The overall survival rates of melanoma patients with different *CDCA5* expression were not significantly different (*p*-value = 0.24). **(D)** The overall survival rates of melanoma patients with different *CENPN* expression were not significantly different (*p*-value = 0.64). **(E)** The overall survival rates of melanoma patients with different *NDC80* expression were not significantly different (*p*-value = 0.62). **(F)** The overall survival rates of melanoma patients with different *NUF2* expression were not significantly different (*p*-value = 0.54).

Figure S2. **Knocking down expression of *AURKB* mediates melanoma cell cycle.** Knocking down expression of *AURKB* of **(A)** A375 and **(B)** A375R was established. The cells (2.5×10^5/well) were seeded into 60-mm dishes. Samples were analyzed by using a FACSCalibur flow cytometer.

Figure S3. Knocking down of *AURKB* mediates expression of c-caspase 3 and c-PARP in xenograft melanoma specimens. Melanoma specimens from **(A)** A375, **(B)** A375R, **(C)** M249 and **(D)** M249R xenograft models were prepared for c-caspase

3 and c-PARP analysis by IHC staining; scale bars = 100 μm. Statistical significance was determined by one-way ANOVA and the asterisks indicate a significant decrease compared with vehicle control mice (***, $p < 0.001$).

Figure S4. Knocking down expression of *AURKB* mediates EGF associated BRAF/MEK/ERKs and PI3-K/AKT pathways in melanoma. (A) The GSEA enrichment plot of the ERBB signaling pathway is associated with expression of *AURKB*; the expression data was from GSE: 4587. **(B)** Normalized expression profile with different *AURKB* expressing samples. Heat map was generated by GSEA. **(C, D)** Knocking down expression of *AURKB* decreased EGF level by ELISA kit in M249 and M249R. **(E)** Knocking down expression of *AURKB* increases in levels of cleaved PARP, cleaved caspase 3, Bax and decreases in Bcl-2 by Western blot. **(F, G)** Knocking down expression of *AURKB* mediates activation of histone H3, BRAF/MEK/ERKs and PI3-K/AKT in M249 and M249R cell lines. Statistical significance was determined by one-way ANOVA and the asterisks indicate a significant change compared with the control group (*, $p < 0.05$; **, $p < 0.01$).

Figure S5. **HI-511 synthesis.** The synthesis of HI-511 and NMR spectrum of the products.

Figure S6. HI-511 inhibits cell growth and mediates cell cycle in both A375 and A375R cells. (A) A375 **(B)** A375R (8×10^3/well) cells were seeded into 96-well

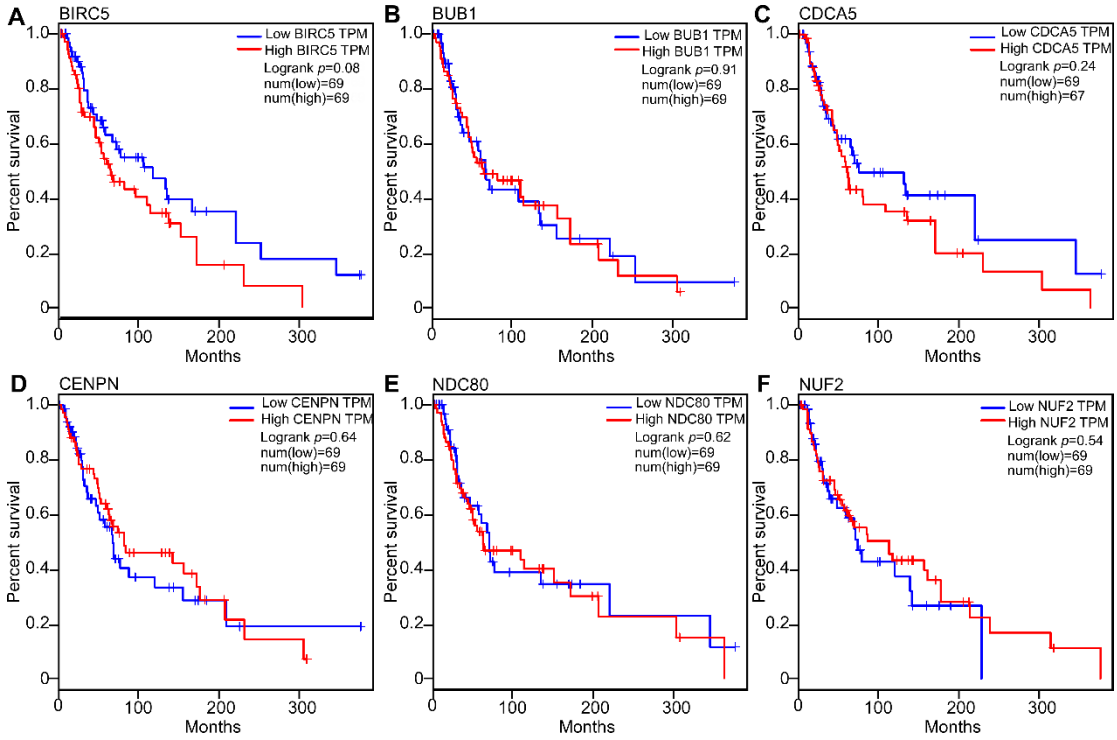
plates. After incubation overnight, cells were treated with the indicated concentrations of HI-511 and incubated for 48 h. Viability was estimated using the MTS assay as described in Materials and Methods. **(C)** A375 and **(D)** A375R cells were seeded (2.5 × 10^5/well) into 60-mm dishes. Treatment with DMSO or 5 μM HI-511 for 48 h. Samples were analyzed by using a FACSCalibur flow cytometer. Statistical significance was determined by one-way ANOVA and the asterisks indicate a significant change compared with the control group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure S7. **HI-511, a dual-target inhibitor against both AURKB and BRAF V600E, affects growth, apoptosis, and protein expression levels in M249 and M249R melanoma cells. (A, B)** HI-511 inhibits anchorage-independent cell growth. Cells (8 × 10^3/well) were seeded into 6-well plates with 0.3% Basal Medium Eagle agar containing 10% FBS and different concentrations of HI-511 and then cultured for 2 weeks. Colonies were scored under a microscope by using the Image-Pro PLUS (v6.) software program. Effect of HI-511 and the activation of histone H3, MEK, ERKs, and AKT was detected by Western blot in **(C)** M249 and **(D)** M249R cells. **(E, F)** M249 and M249R cells (2.5 × 10^5/well) were incubated with HI-511 (1.25, 2.5, or 5 μM) or vehicle control for 48 h. Cells were collected and apoptosis was detected by flow cytometry with Annexin V and propidium iodide staining. **(G, H)** The cells were incubated with HI-511 or vehicle control for 48 h, then the effect of HI-511 on apoptosis-associated protein expression was determined by Western blot. Statistical

significance was determined by one-way ANOVA. The asterisks indicate a significant change compared with untreated control cells (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Vemu: vemurafenib.

Figure S8. HI-511 binds to and suppresses the activation of wild type BRAF. (A) Computer docking model of HI-511 binding with wild type BRAF. **(B)** HI-511 inhibits wild type BRAF *in vitro*. Active kinase wild type BRAF and HI-511 (0, 1, 5, 10 μM) or vemurafenib (5 μM) were mixed with the substrate phosphatidylinositol. **(C)** Knocking down of *AURKB* inhibits BRAF wild type melanoma cell (SK-MEL-31) growth. **(D)** HI-511 suppresses BRAF wild type melanoma cell (SK-MEL-31) growth. Statistical significance was determined by one-way ANOVA. The asterisks indicate a significant change compared with untreated control cells (**, $p < 0.01$; ***, $p < 0.001$).

Figure S9. **HI-511 mediates the expression of apoptosis markers c-caspase 3 and c-PARP.** Melanoma specimens from **(A)** A375, **(B)** A375R, **(C)** M249 and **(D)** M249R xenograft models were prepared for IHC staining for c-caspase 3 and c-PARP; scale bars = 100 μm. Statistical significance was determined by one-way ANOVA and the asterisks indicate a significant decrease compared with vehicle control mice (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Vemu: vemurafenib.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Low BIRC5 TPM
High BIRC5 TPM
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num(high)=69
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BUB1
Low BUB1 TPM
High BUB1 TPM
Logrank p=0.91
num(low)=69
num(high)=69
C
CDCA5
Low CDCA5 TPM
High CDCA5 TPM
Logrank p=0.24
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D
CENPN
Low CENPN TPM
High CENPN TPM
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E
NDC80
Low NDC80 TPM
High NDC80 TPM
Logrank p=0.62
num(low)=69
num(high)=69
F
NUF2
Low NUF2 TPM
High NUF2 TPM
Logrank p=0.54
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Percent survival
Months
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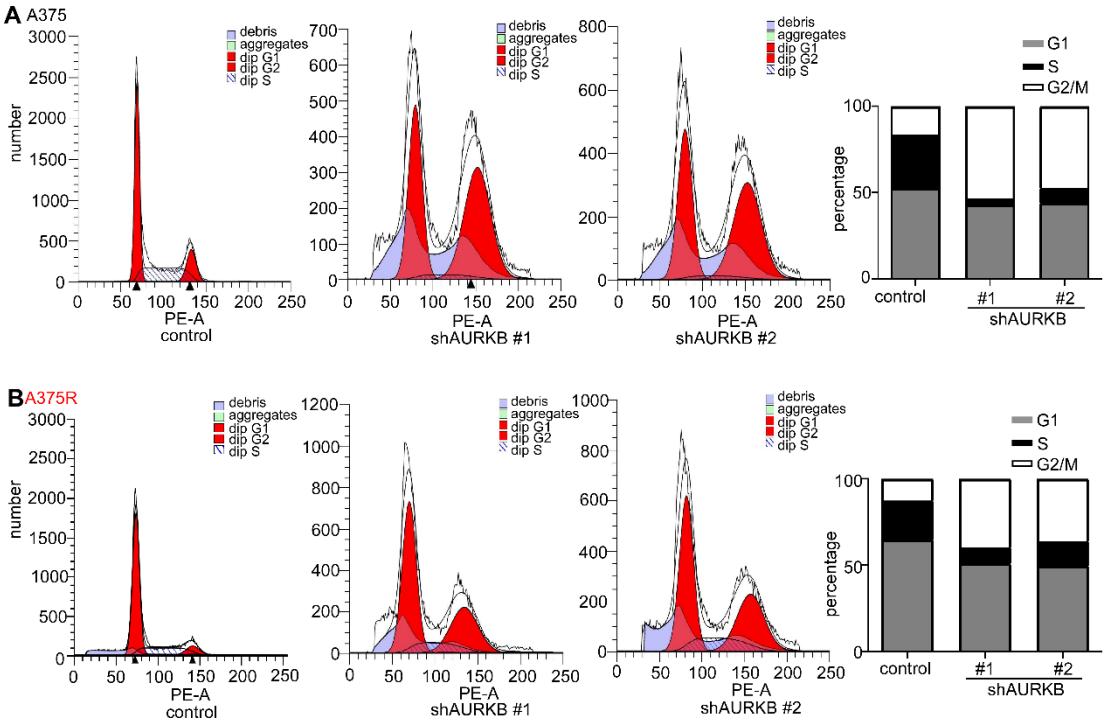
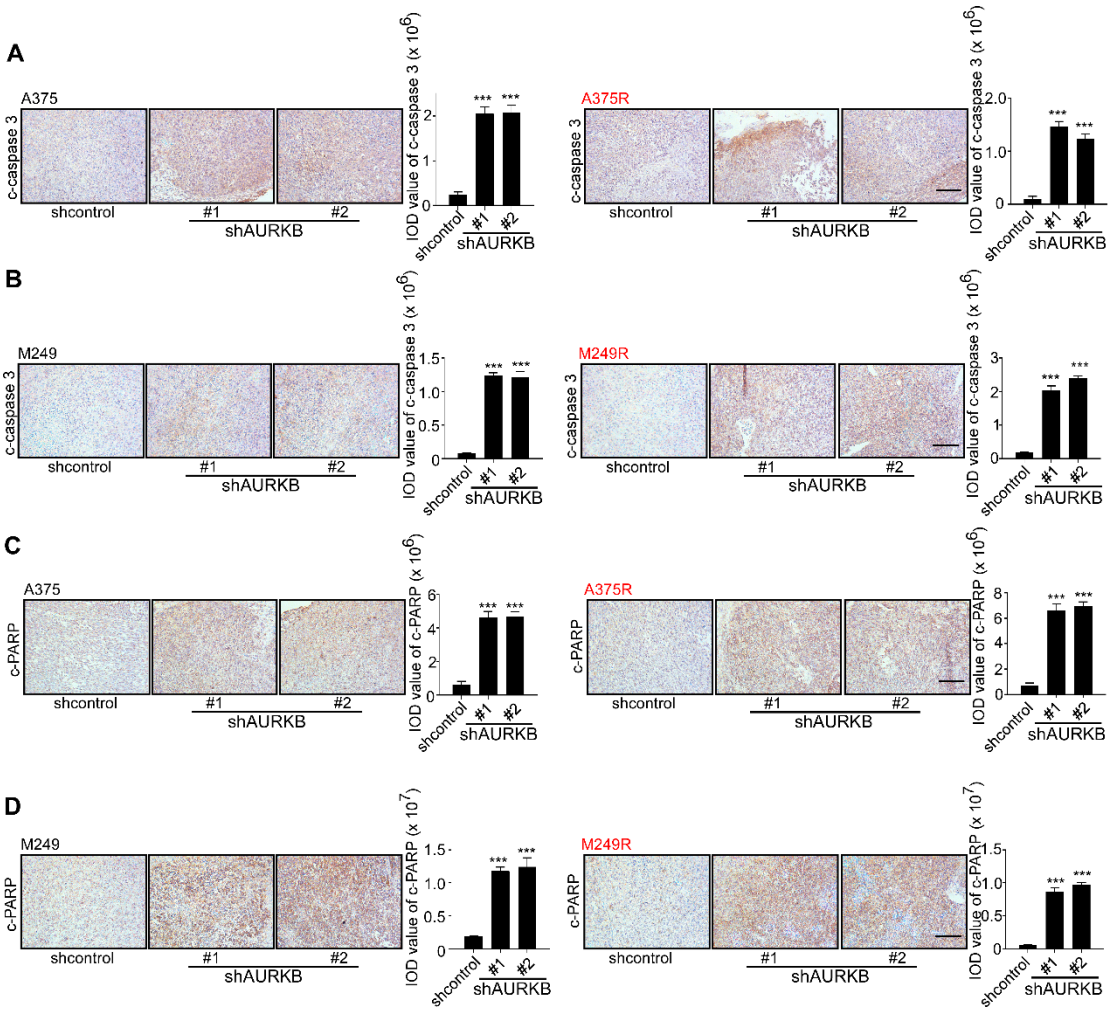
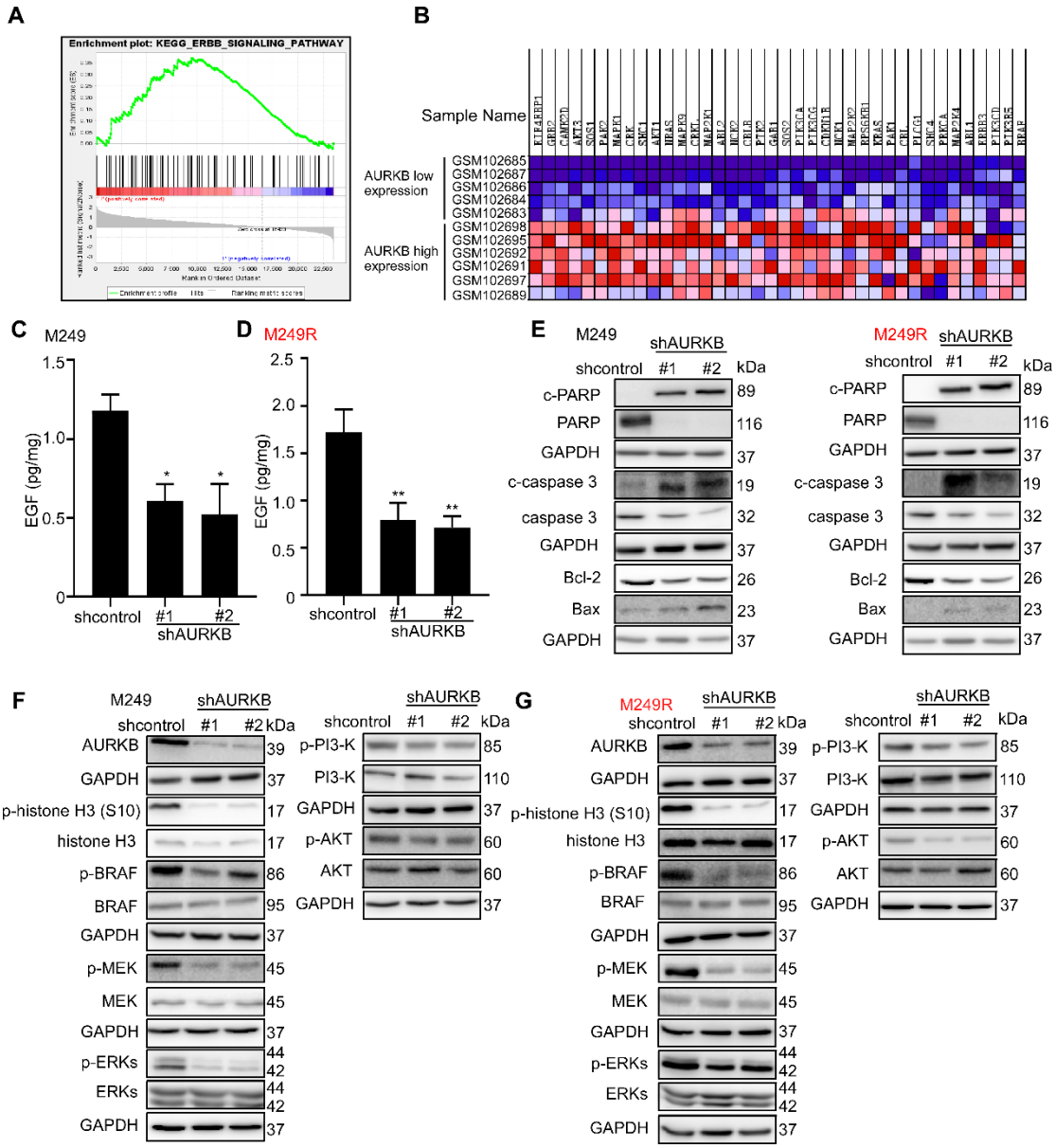
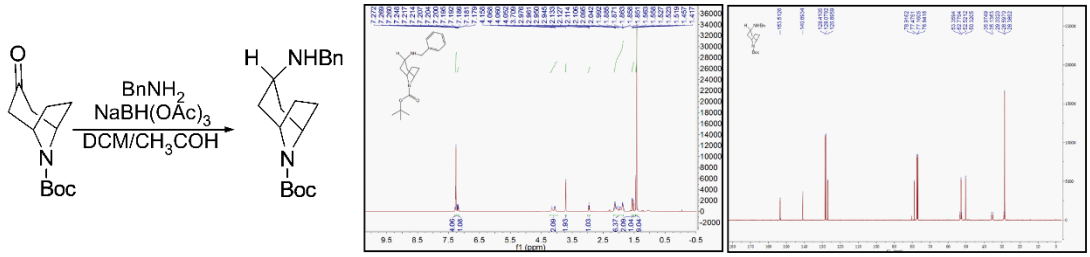
0.2
0.4
0.6
0.8
1.0
100
200
300


A
A375
debris
aggregates
dip G1
dip G2
dip S
number
PE-A
control
shAURKB #1
shAURKB #2
G1
S
G2/M
percentage
control
#1
#2
shAURKB
B
A375R


A
A375
c-caspase 3
shcontrol
#1
#2
shAURKB
IOD value of c-caspase 3 (x 10^6)
A375R
B
M249
M249R
C
c-PARP
IOD value of c-PARP (x 10^6)
D
IOD value of c-PARP (x 10^7)
***


A
Enrichment plot: KEGG_ERBB_SIGNALING_PATHWAY
B
Sample Name
AURKB low expression
AURKB high expression
GSM102685
GSM102687
GSM102686
GSM102684
GSM102683
GSM102698
GSM102695
GSM102692
GSM102691
GSM102697
GSM102689
C
M249
EGF (pg/mg)
shcontrol
#1
#2
shAURKB
D
M249R
E
M249
M249R
shAURKB
shcontrol
kDa
c-PARP
PARP
GAPDH
c-caspase 3
caspase 3
Bcl-2
Bax
F
M249
AURKB
p-histone H3 (S10)
histone H3
p-BRAF
BRAF
p-MEK
MEK
p-ERKs
ERKs
p-PI3-K
PI3-K
p-AKT
AKT
G
M249R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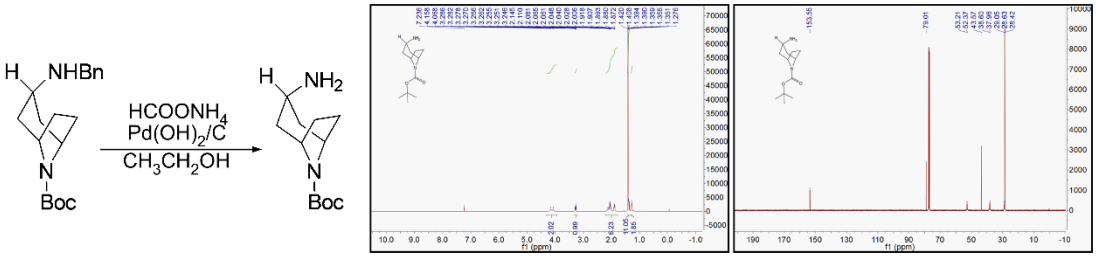
NaBH(OAc)3
DCM/CH3COH
H
NHBn
N
Boc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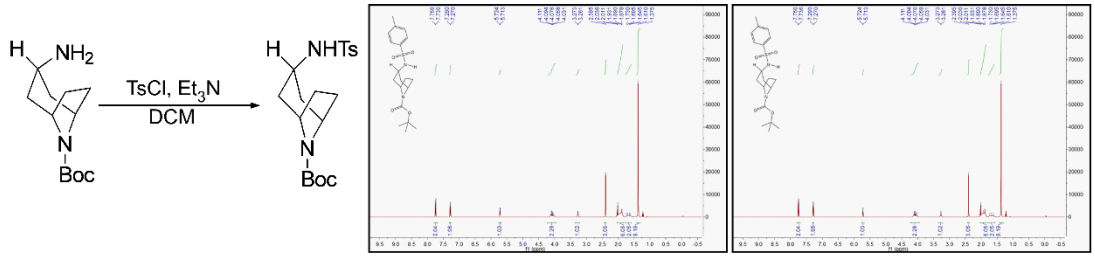
HCOONH4
Pd(OH)2/C
CH3CH2OH
NH2
N
Boc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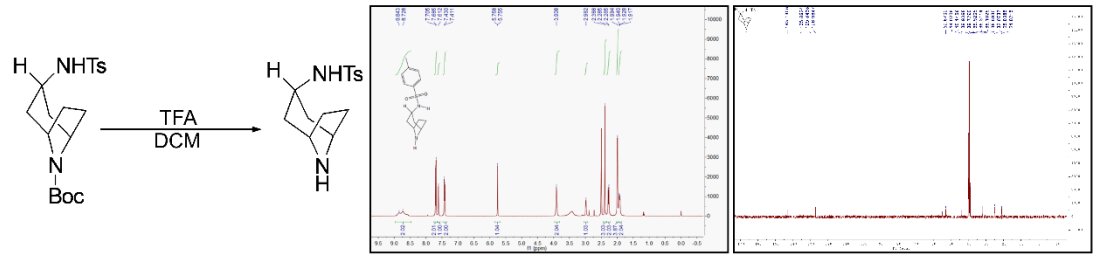
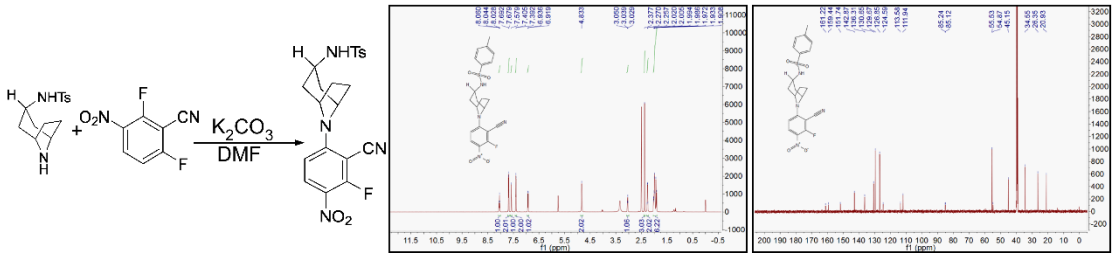
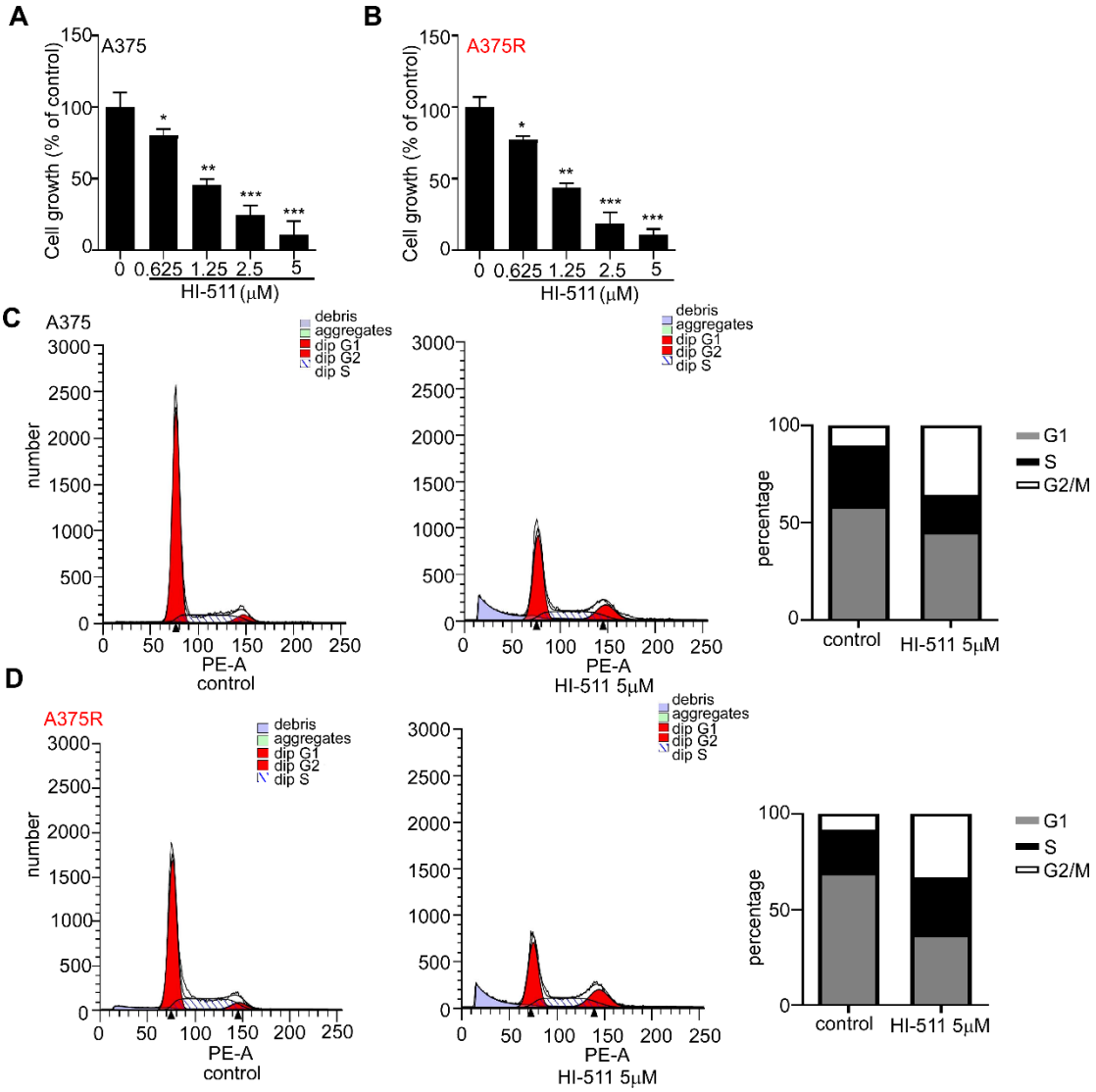
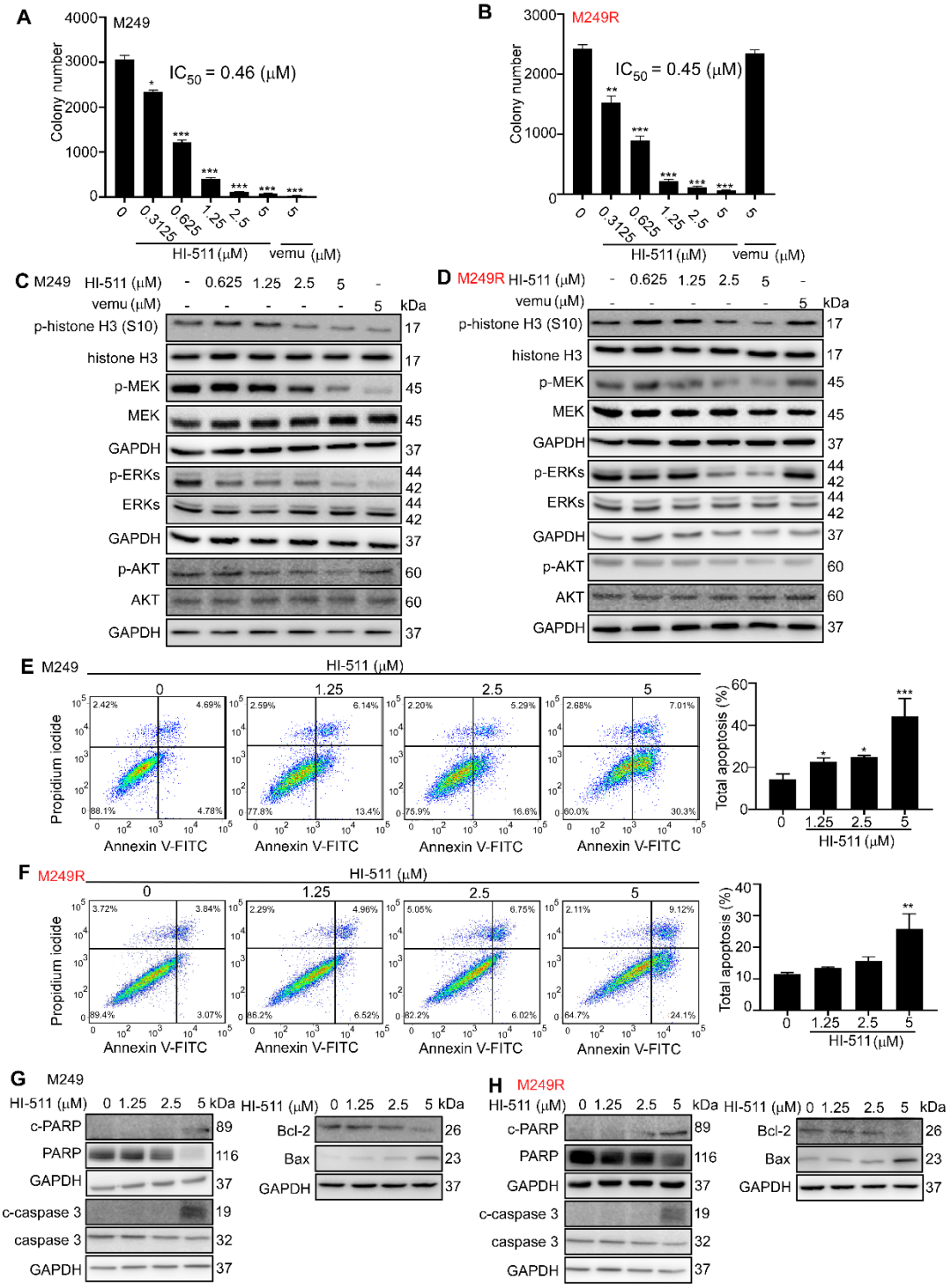
NH2
TsCl, Et3N
DCM
NHTs
N
Boc

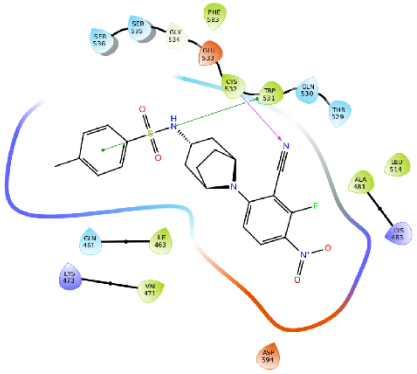

H
NHTs
TFA
DCM
N
Boc
N
H


H
NHTs
N
H
O2N
F
CN
K2CO3
DMF
NO2


A
A375
Cell growth (% of control)
150
100
50
0
0 0.625 1.25 2.5 5
HI-511 (μM)
B
A375R
C
A375
debris
aggregates
dip G1
dip G2
dip S
number
PE-A
control
HI-511 5μM
percentage
G1
S
G2/M
D
A375R


A
M249
Colony number
IC50 = 0.46 (μM)
HI-511 (μM)
vemu (μM)
B
M249R
IC50 = 0.45 (μM)
C M249
D M249R
p-histone H3 (S10)
histone H3
p-MEK
MEK
GAPDH
p-ERKs
ERKs
p-AKT
AKT
kDa
E M249
F M249R
Propidium iodide
Annexin V-FITC
Total apoptosis (%)
G M249
H M249R
c-PARP
PARP
c-caspase 3
caspase 3
Bcl-2
Bax

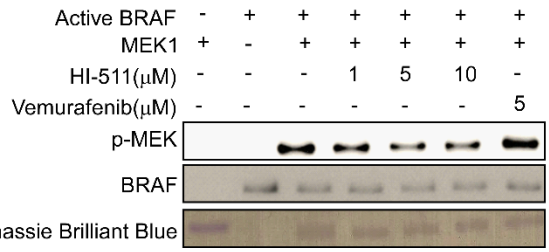
A



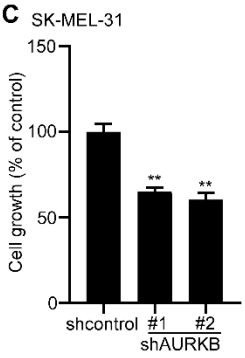
B

HI-511 binding with BRAF

BRAF binding residue	interaction	HI-511 binding area
Cys 532	H-bond	nitrile group
Trp 531	π-π stacking	phenyl ring



C



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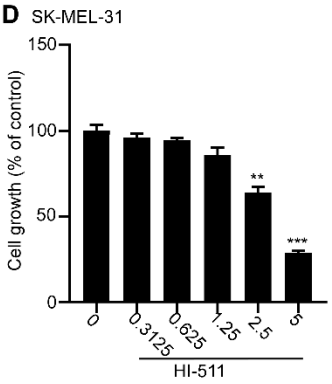

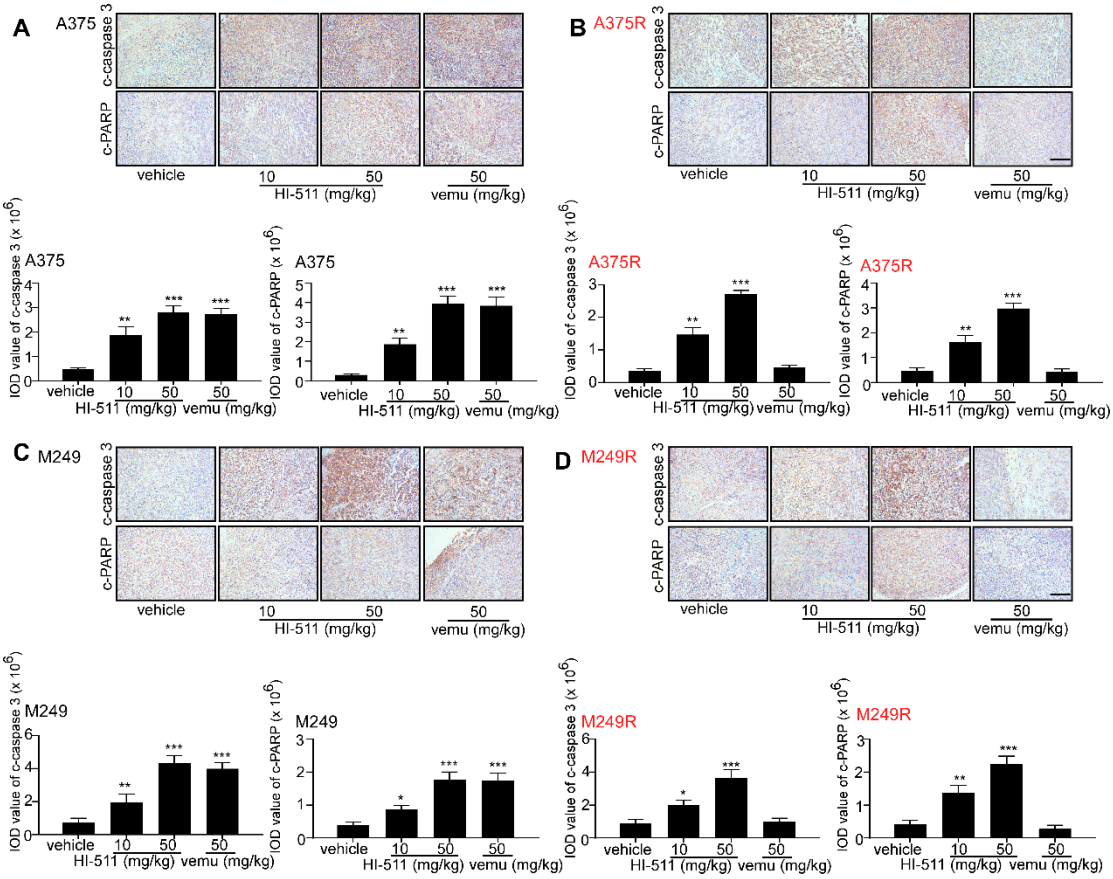
A
A375
c-caspase 3
c-PARP
vehicle
10
50
50
HI-511 (mg/kg)
vemu (mg/kg)
IOD value of c-caspase 3 (x 10^6)
IOD value of c-PARP (x 10^6)
B
A375R
C
M249
D
M249R
**
***
*