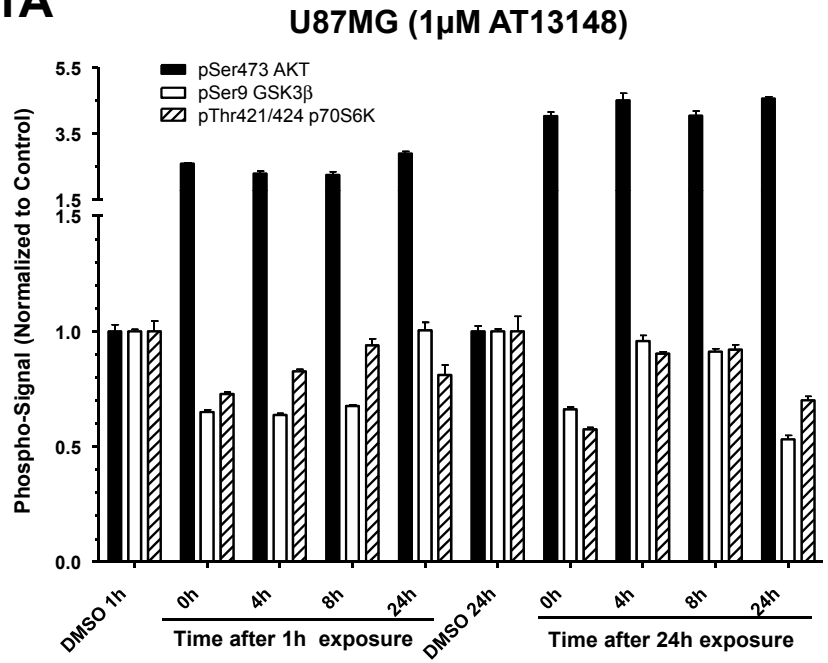
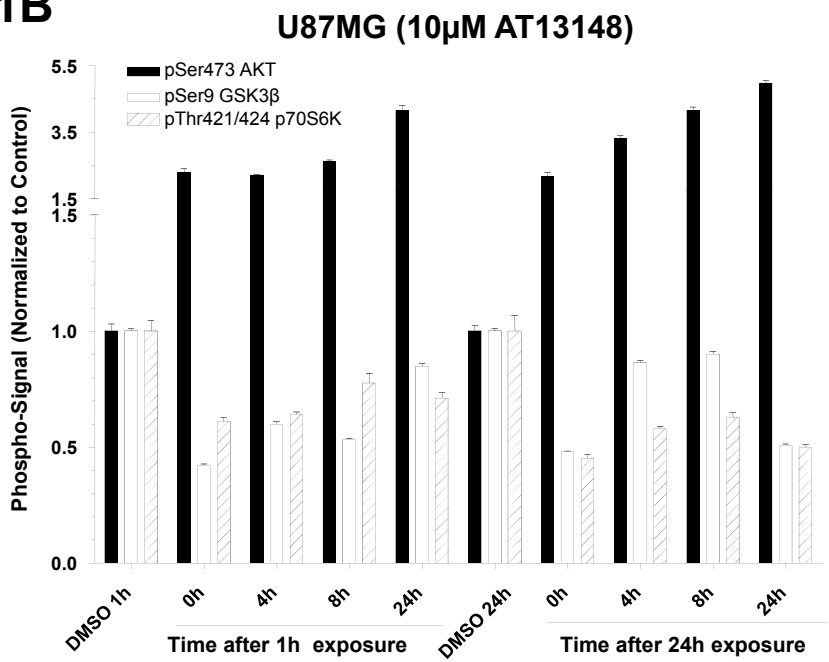
Supplementary Figures and Tables

S1A

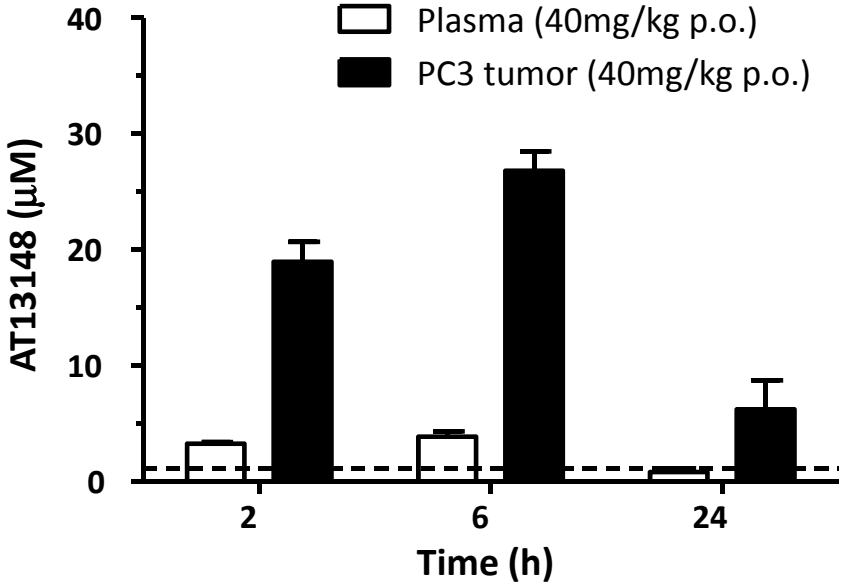


S1B



Supplementary Figure 1

S2A



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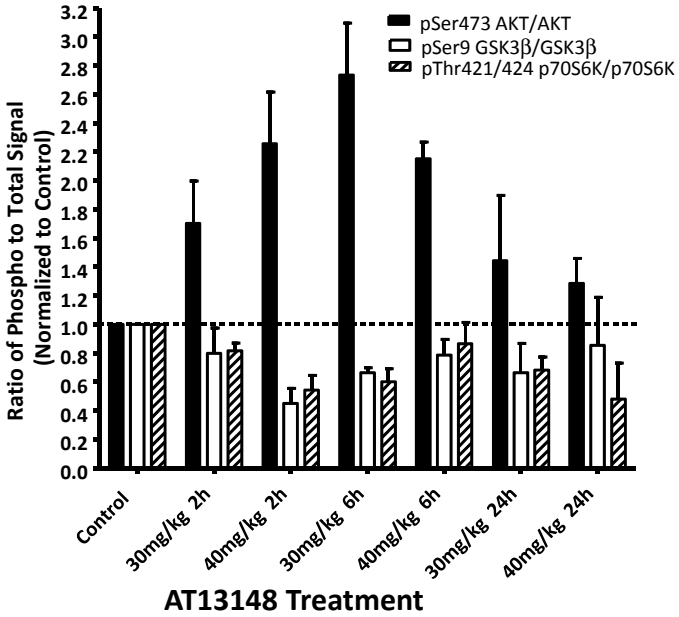

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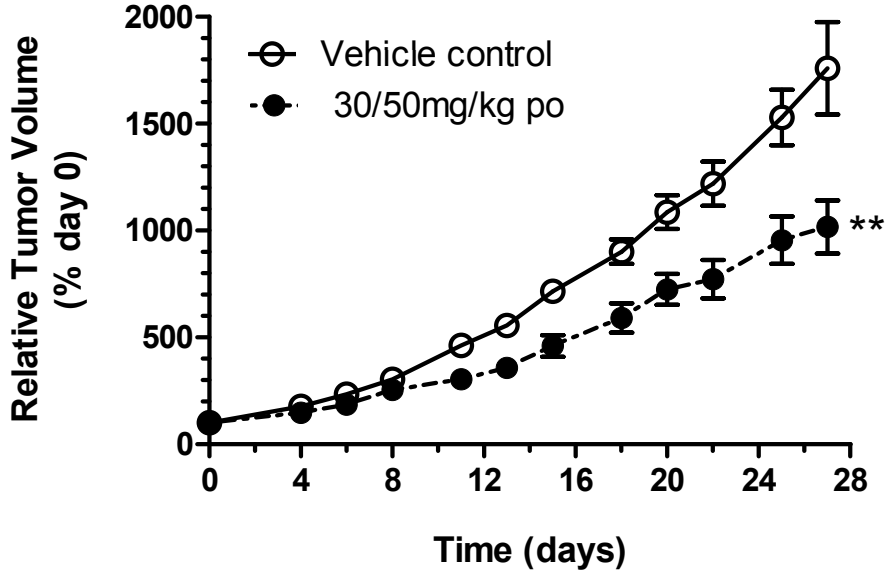

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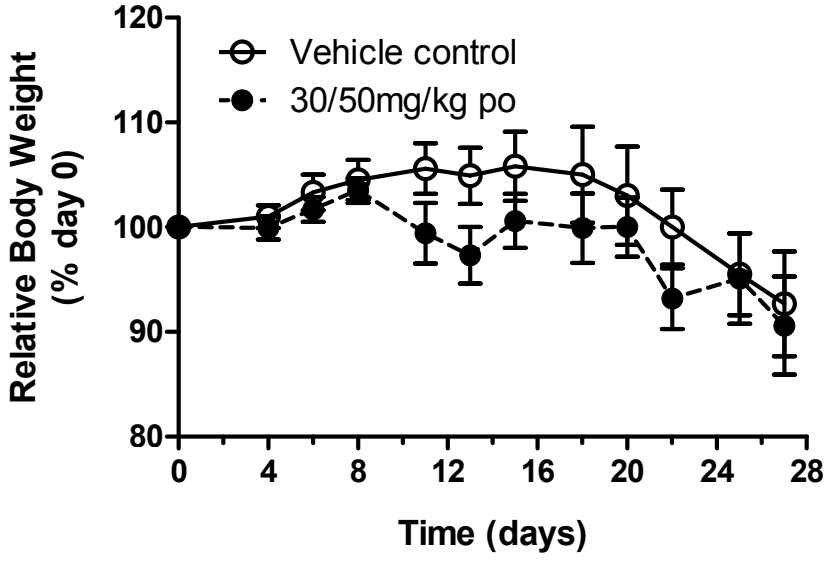
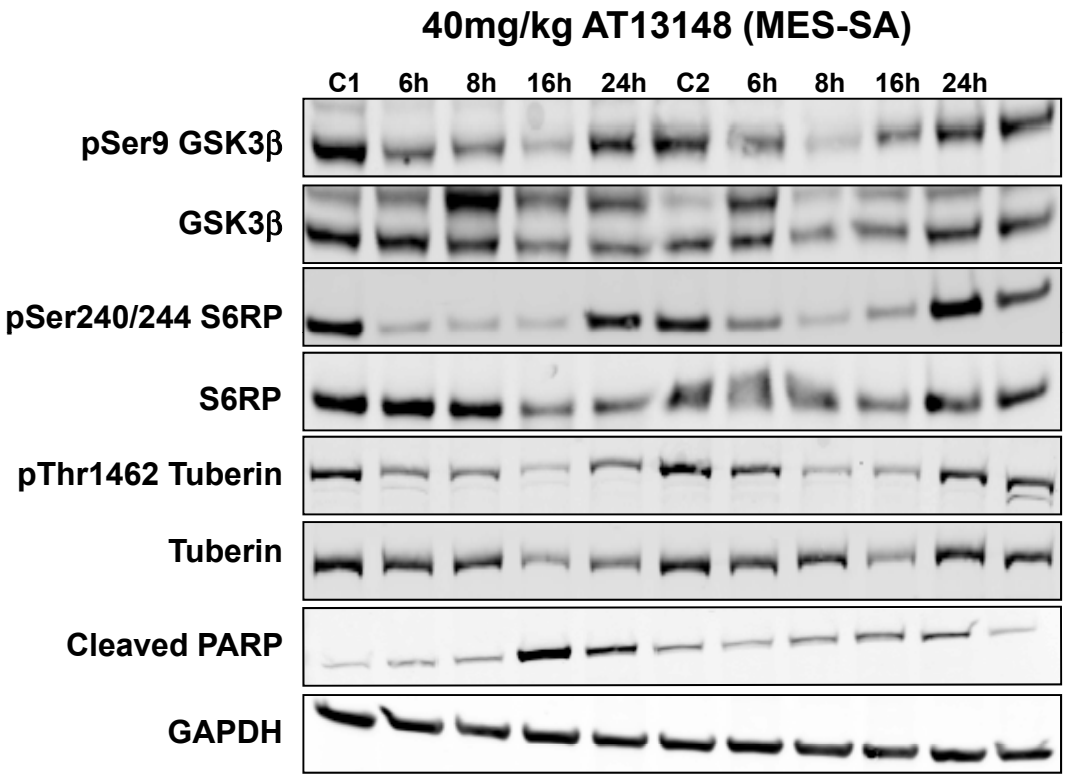
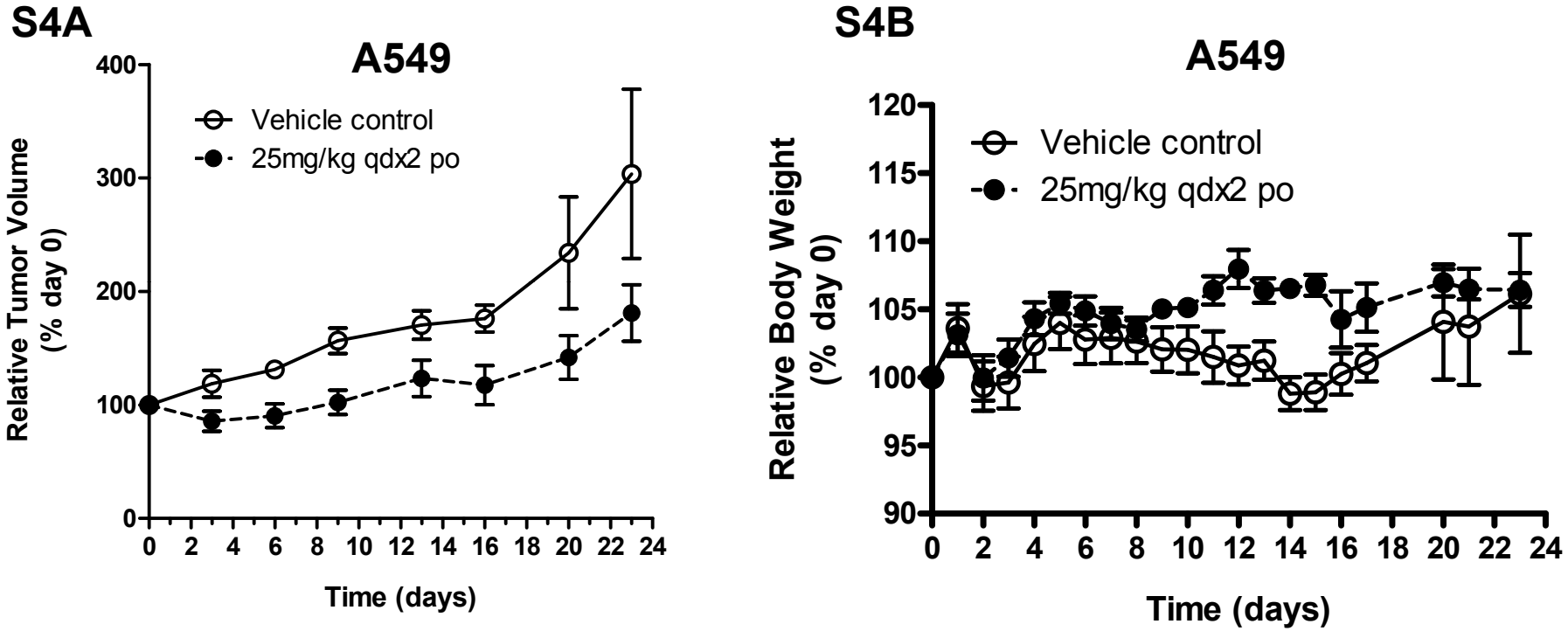


Supplementary Figure 2

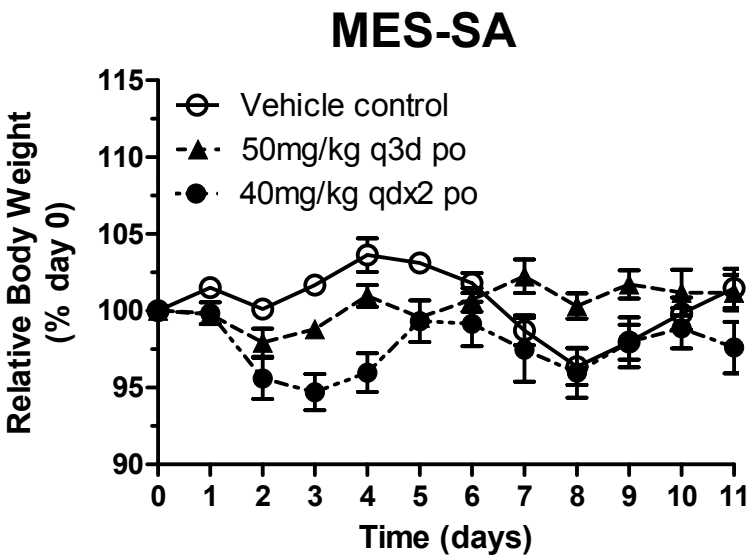


Supplementary Figure 3

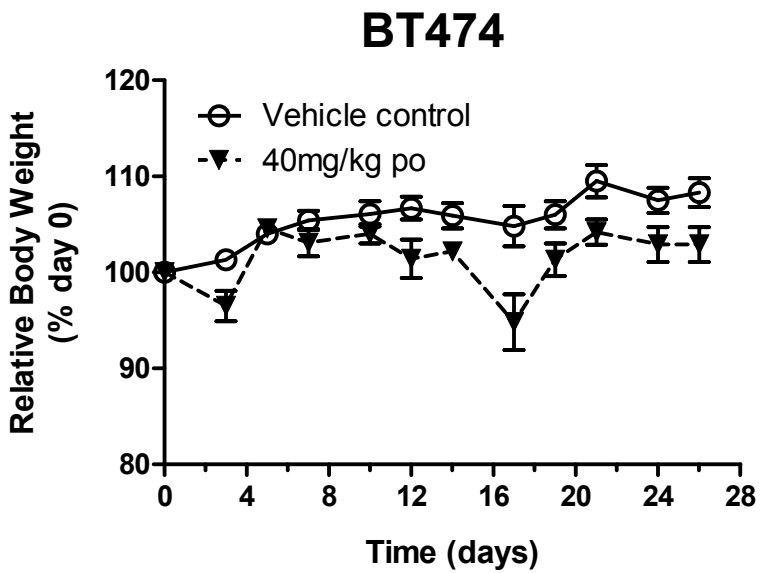


Supplementary Figure 4

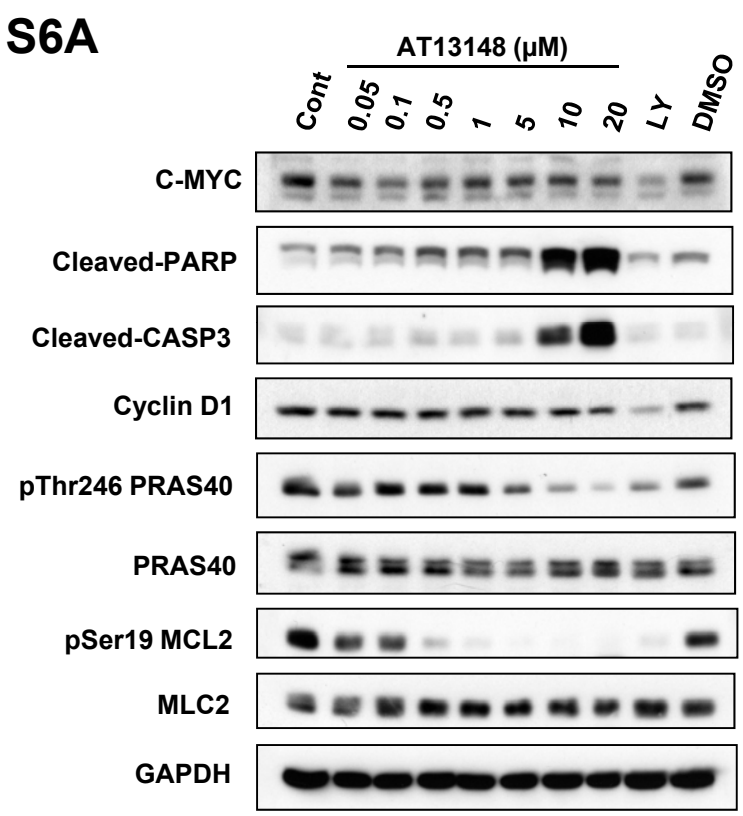
S5A

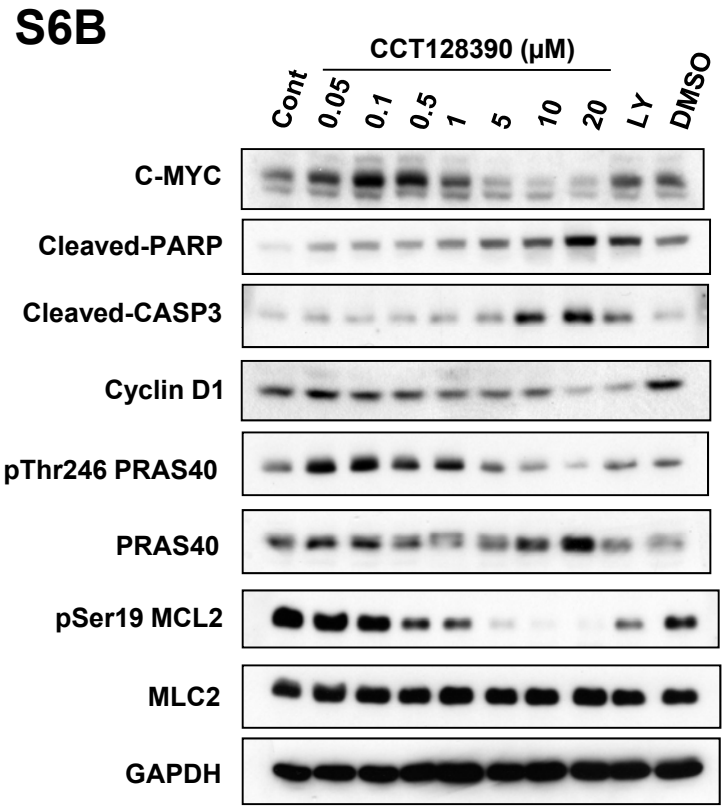


S5B



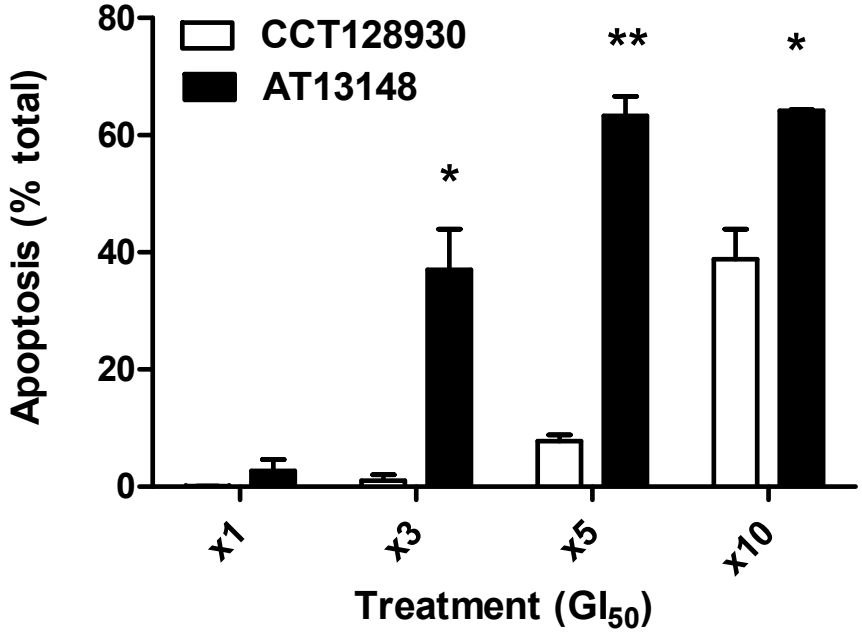
Supplementary Figure 5





Supplementary Figure 6

S6C



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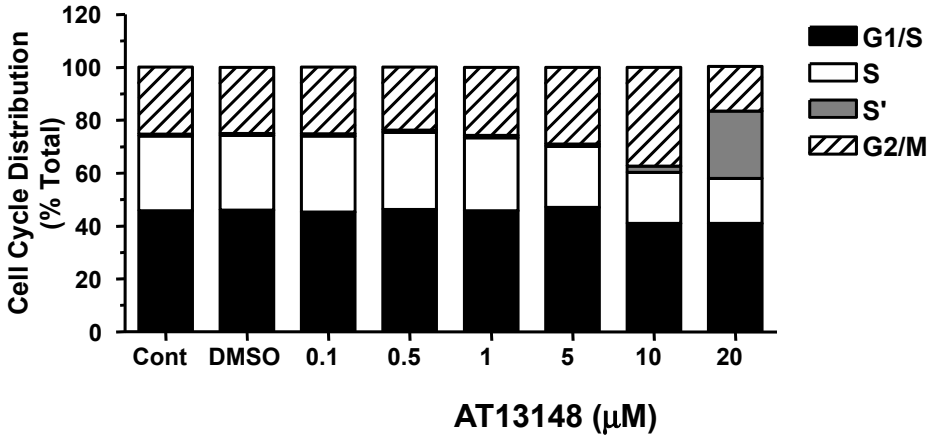

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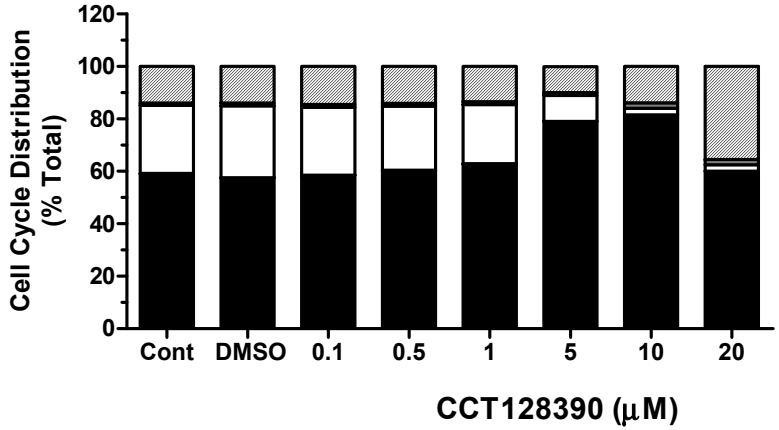
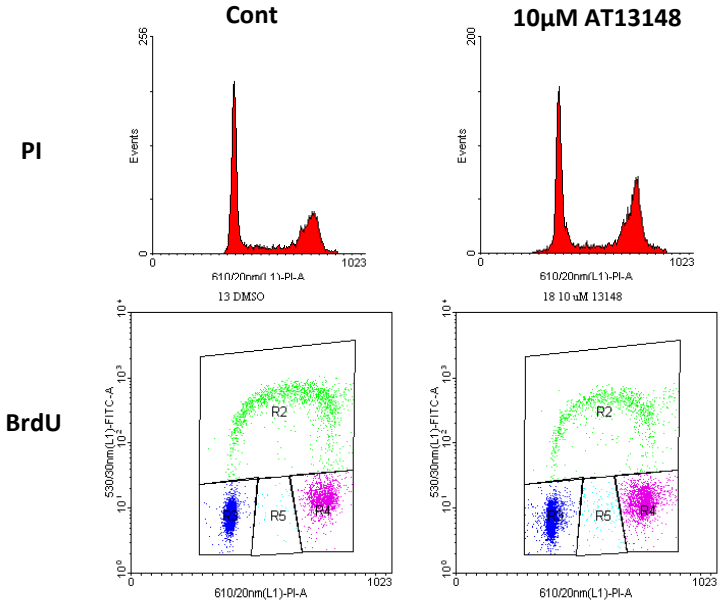
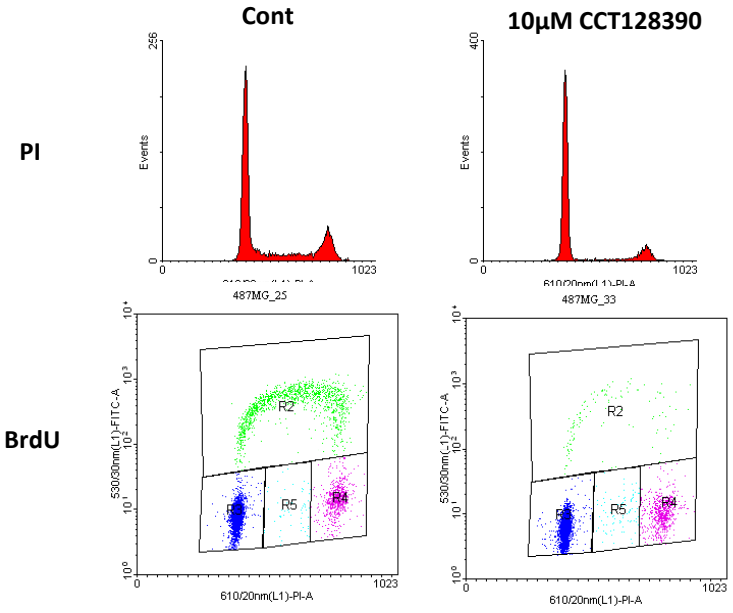


Supplementary Figure 6 (Continued)

S7A



S7B



Supplementary Figure 7

Supplementary Tables

Supplementary Table 1. Summary of the inhibitory activity of 10μM AT13148 against a panel of kinases *in vitro*.

	Kinase
>80% inhibition at 10 μM	AKT, PKA, ROCKII, p70S6K, MSK, RSK1/2, CHK2, SGK, PRK2, Aurora B
60-80% inhibition at 10 μM	AMPK, PKCα, MKK1, MARK3, CAMK1, IKKβ, DYRK1α, MNK1/2, MAPK/ERK, PKD1, ERK8
<60% inhibition at 10 μM	CHK1, SRC, PDK1, GSK3β, PLK1, NEK7, LCK, PBK, JNK, smMLCK, SRPK1, CK1/2, p38, MST2, CDK2, MAPKAP-K2, MAPKAP-K3, PRAK

Supplementary Table 2. Summary of the inhibitory activity of AT13148 against a panel of protein kinases

Kinase	Kinase inhibition IC_{50} (nM)
AKT1	38
AKT2	402
AKT3	50
p70S6K	8
PKA	3
ROCKI	6
ROCKII	4
RSK1	85
SGK3	63
CHK2	860
Aurora B	1840

Supplementary Table 3. Summary of the *in vitro* cytotoxicity of AT13148 against a panel of human tumor cell lines harboring different defects in the PI3K-AKT signaling pathway.

Cell line	Tumor	Genetic mutation	GI_{50} (μM)		
			Mean	SD	N
MES-SA	Uterine sarcoma	PTEN	1.54	0.78	8
MES-SA/Dx5	Uterine sarcoma (Multidrug-resistant)	PTEN	1.54	1.09	3
BT474*	Breast	PIK3CA, HER2 amplification	1.59	0.76	3
HCT-116	Colon	PIK3CA, KRAS	1.82	0.65	5
PC3*	Prostate	PTEN	1.87	0.76	3
A549	Lung	KRAS	2.65	0.04	4
SK-BR-3	Breast	HER2 amplification	2.77	0.66	3
MCF7	Breast	PIK3CA	2.10	0.80	3
U87MG*	Glioma	PTEN	3.27	0.90	3
MDA-MB-468	Breast	PTEN	2.90	2.02	3
DU-145	Prostate	PTEN	3.75	0.31	4
SK-OV-3	Ovarian	PIK3CA	3.77	0.40	3

Cytotoxicity was determined using a 72h Alamar Blue assay or a 96h SRB assay (*). Standard deviation (SD), number of assays (N).

Supplementary Table 4. Summary of the pharmacokinetics of AT13148 in mouse plasma and tissues following iv or oral administration.

Dose (mg/kg)	**Route**	**Tmax** (h)	**Cmax** (μM)	**T1/2** (h)	**AUCo-∞** (μMh)	**Cl** (L/h/kg)	**Vss** (L/kg)
5	i.v	0.083	1.55	2.83	6.75	1.68	9.05
5	p.o	0.250	0.962	10.3	9.37	1.20	
30	p.o	0.5	1.98	17.1	48.0	1.44	
40	p.o	4.0	2.48	33.2	108.0	0.84	
50	p.o	6.0	2.19	38.3	127.0	0.92	