Supporting information for

Inhibition of Histone Deacetylases Sensitizes EGFR-TKI-Resistant Non-Small Cell Lung Cancer Cells to Erlotinib *In Vitro* and *In Vivo*

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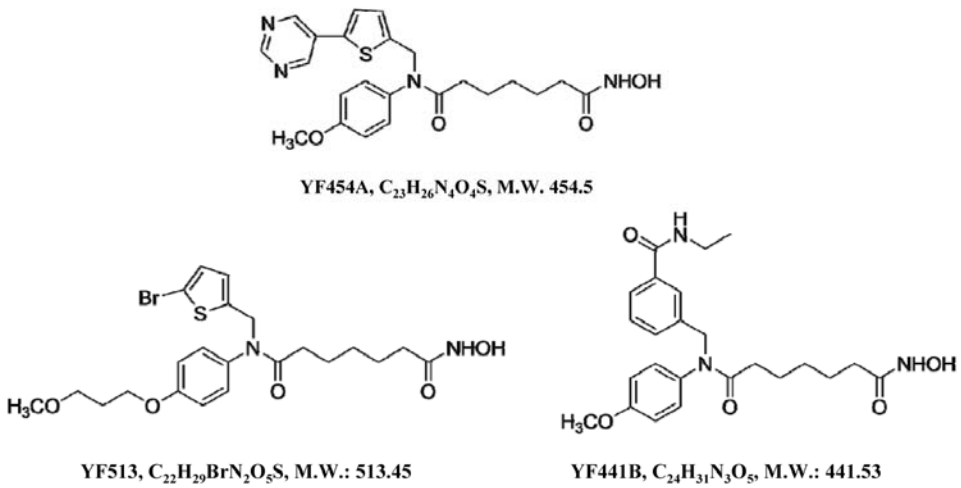
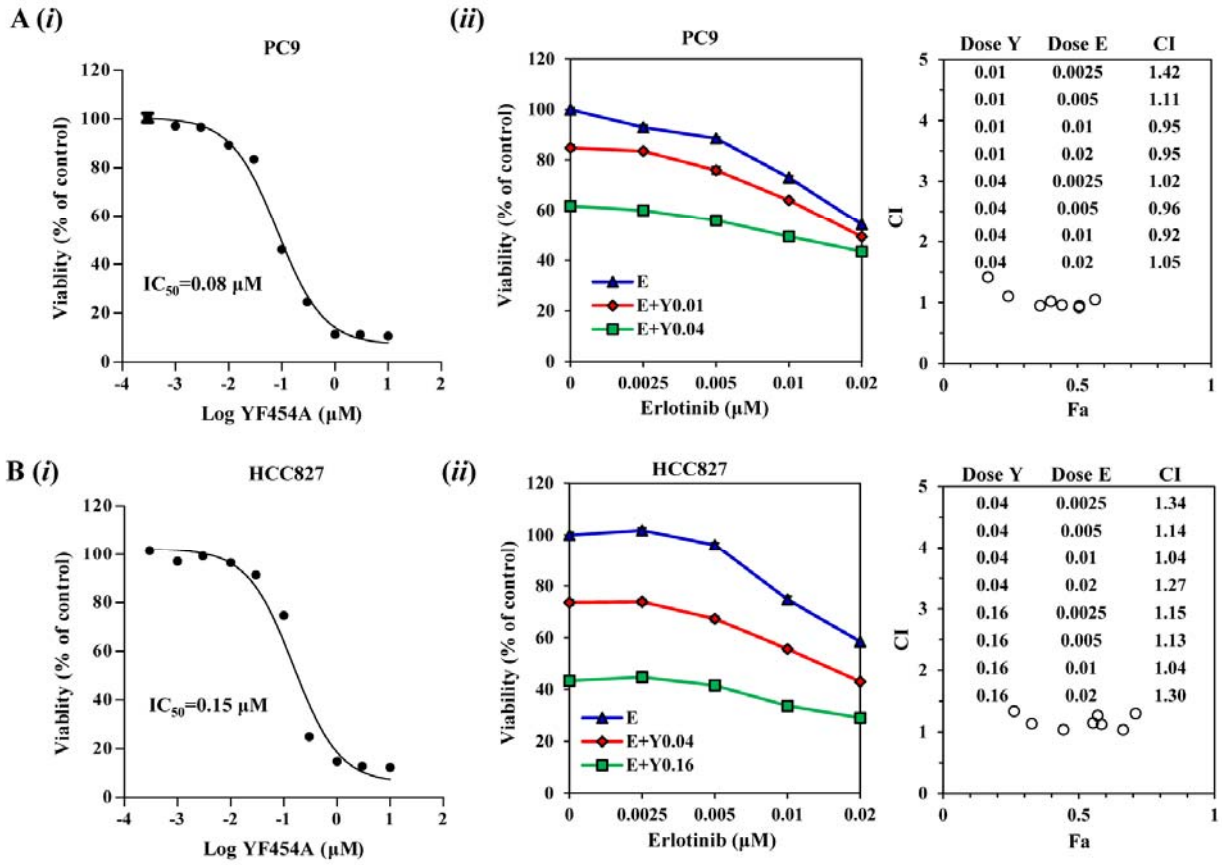
Supplementary Figure 1



Supplementary Figure 1. Chemical structures of YF-454A, YF513 and YF441B.

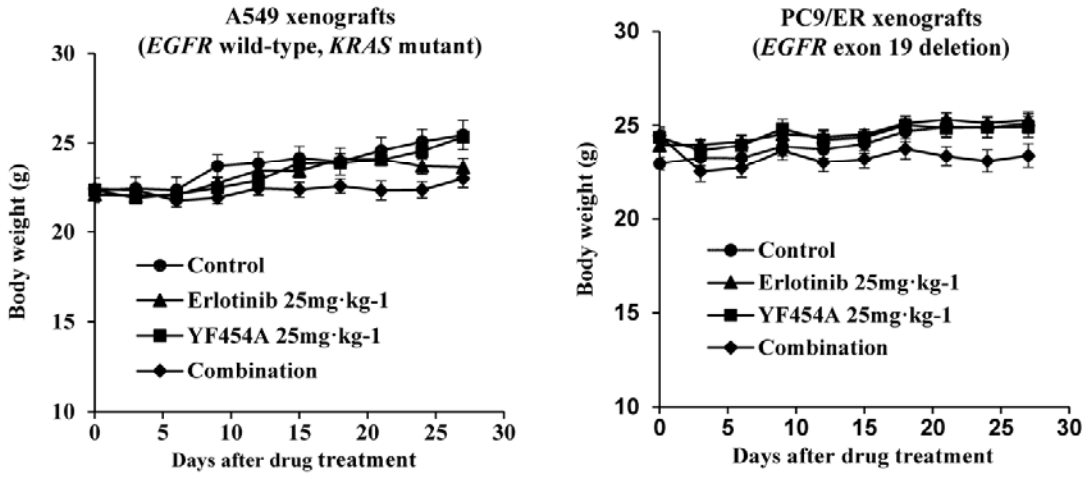
Supplementary Figure 2



Supplementary Figure 2. YF454A and erlotinib are not synergistic in non-EGFR-TKI-resistant NSCLC cells. Non-EGFR-TKI-resistant NSCLC PC9 (**A**) and HCC827 (**B**) cells

were respectively treated with YF454A (Y) alone or in combination with erlotinib (E) for 72h. Cell viability was measured and IC_{50}s of YF454A in PC9 [**A(*i*)**] and HCC827 [**B(*i*)**] cells were calculated. The interaction of the drug pair was analyzed using the CI equation and presented with Fa (fraction affected by the dose) in the Fa-CI plots [**A(*ii*)** and **B(*ii*)**]. CI values were calculated using the Calcusyn software package (Biosoft, Cambridge, UK). All results were represented as the mean ± SEM (n = 6 per group).

Supplementary Figure 3



Supplementary Figure 3. The combined treatment of YF454A and erlotinib has little effect on body weight in mice. The mouse body weight was recorded every three days during treatments. *Dots*, mean; *bars*, SEM. In A549 xenograft mouse model, there were 8 mice in each treatment group. In PC9/ER xenograft model, there were 11 mice in the control group, Erlotinib group and YF454A group, and there were 10 mice in the combination group).

Supplementary Table 1. Sequence of primers for PCR amplification.

Genes	Forward (5'-3')	Reverse (5'-3')
EGFR	ACGAGTAACAAGCTCACGCA	CAGCTCCTTCAGTCCGGTTT
HER2	GGTGGTCTTTGGGATCCTCA	ACCTTCACCTTCCTCAGCTC
AXL	GGTGGCTGTGAAGACGATGA	CTCAGATACTCCATGCCA
MET	CCACCCTTTGTTCAGTGTGG	AGTCAAGGTGCAGCTCTCAT
IGF1R	GACAACCAGAACTTGCAGCA	CCCTTTAGTCCCCGTCACTT
KAT2B	GTCACCTGCCAGCAAAAGAA	CGCAGTCTTCGTTGAGATGG
CCND1	GCATGTTCGTGGCCTCTAAG	CGTGTTTGCGGATGATCTGT
FOXA1	AAGACTCCAGCCTCCTCAAC	CGTATGCCTTGAAGTCCAGC
MCM7	CATGCCTCTGATCATGTGCC	AGCACCGTGATACTACGAGG
TIPIN	GGAGAATGGCGTGATTGACC	TCAGGCTCAGTTCCTTCACC
MSH6	AATGCTGAAGAACGGAGGGA	TTCGTAATGCAAGGATGGCG
SUV39H2	GGCCCACCTTCAGACTTCTA	CCACGTCCATTGCTAGTTCG
E2F3	ATCCCTAAACCCGCTTCCAA	AGGGGAGGCAGTAAGTTCAC
CHK1	TCATGGCAGGGGTGGTTTAT	GTTGCCAAGCCAAAGTCTGA
CDC25A	CTACTGATGGCAAGCGTGTC	TACCCAGGCGATCTCTCTCT
E2F1	CTTCGTAGCATTGCAGACCC	GGAGATGATGGTGGTGGTGA
RB	TCACATTCCTCGAAGCCCTT	ACGGTCGCTGTTACATACCA
P107	GAGGTGGTGATCCGCTCAGA	TAGAGGAGACATTGGCATCAG
DP1	AACTCGGCTCAGGAATGTCA	CGATGACCGTCTTCTTGCTG
β-Actin	GTACGCCAACACAGTGCTG	CGTCATACTCCTGCTTGCTG

Supplementary Table 2. Cytotoxicity of novel HDAC inhibitors in EGFR-TKI-resistant NSCLC cells.

Cell line	Characteristics	IC_{50} (μM)				
		YF454A	YF513	YF441B	SAHA	Erlotinib
A549	*EGFR* wild-type, *KRAS* mutant	0.87±0.11	4.46±0.39	5.36±0.19	5.34±0.23	>10
H1299	*EGFR* wild-type, *NRAS* (Q16K)	3.50±0.45	6.03±0.84	10.06±0.60	11.73±0.86	>10
H1975	*EGFR* (L858R, T790M)	0.15±0.02	0.73±0.01	1.80±0.14	1.59±0.12	>10
PC9/ER	*EGFR* exon 19 deletion	0.20±0.02	1.78±0.13	2.91±0.05	4.25±0.11	5.05±0.48
HCC827/ER	*EGFR* exon 19 deletion	0.21±0.02	1.18±0.09	1.80±0.14	2.11±0.25	>10

Cytotoxicity of YF454A, YF513, YF441B, SAHA and erlotinib in EGFR-TKI resistant NSCLC cell lines was shown. The cytotoxicity of compounds was determined by the cell viability assays, and the IC_{50} values were expressed as mean±SEM. The mean of three independent experiments were shown (n = 3 per

treatment group).

Supplementary Table 3. Genes that were significantly affected by the combined treatment of YF454A and erlotinib.

GO.ID	Term	*P*-value
GO:0006260	DNA replication	4.95736E-07
GO:0090304	nucleic acid metabolic process	5.11466E-07
GO:0007049	cell cycle	1.54993E-06
GO:0022402	cell cycle process	2.17026E-06
GO:0006974	cellular response to DNA damage stimulus	3.92178E-06
GO:0000278	mitotic cell cycle	3.99921E-06
GO:0044770	cell cycle phase transition	4.29028E-06
GO:0031570	DNA integrity checkpoint	5.32336E-06
GO:1901576	organic substance biosynthetic process	1.01810E-05
GO:0044772	mitotic cell cycle phase transition	1.06287E-05
GO:0000077	DNA damage checkpoint	1.71877E-05
GO:0006139	nucleobase-containing compound metabolic process	2.09767E-05
GO:0009058	biosynthetic process	2.29972E-05
GO:0009059	macromolecule biosynthetic process	2.85468E-05
GO:0000082	G1/S transition of mitotic cell cycle	2.94935E-05

Supplementary Table 4. Differential expression analysis for five selected cell cycle-related genes.

Gene name	Differential expression		Survival analysis	
	Log2 (fold change)	Adjusted *P*-value (q)	HR	*P*-value
MSH6	0.833	7.9×10^{-19}	2.4 (1.86-3.1)	4.5×10^{-12}
MCM7	1.37	1.95×10^{-24}	2.05 (1.6-2.62)	4.8×10^{-9}
E2F3	1.45	2.82×10^{-44}	0.85 (0.67-1.07)	0.17
TIPIN	0.742	2.10×10^{-12}	0.83 (0.65-1.04)	0.11
SUV39H2	1.42	2.85×10^{-33}	0.99 (0.79-1.25)	0.949

We performed differential expression analysis by using patient RNA-seq data from the Cancer Genome Atlas (http://cancergenome.nih.gov) and gene microarray expression data from GEO (http://www.ncbi.nlm.nih.gov/geo/).