Supplementary Data

Supplementary Methods:

Cell viability assay. Cells were seeded overnight at a density of 2,000 cells per well in 96-well plates in RPMI with 10% FBS and then treated with the relevant reagents for 3 days. For MP08 and MP41 cells, the primary cells were first established in conditioned media (The American Journal of Pathology, 180, 2, 599, 2012) and then transferred to 10% FBS RPMI for viability studies. Viable cell numbers were determined using MTS assay kit according to the manufacturer's protocols (Promega). Each assay consisted of three replicates and was repeated at least twice. Data were expressed as percentages of control cells, calculated from the absorbance corrected for background.

Caspase 3/7 assay. Caspase activity was detected by using Caspase-Glo® 3/7 assay kit (Promega). Briefly, cells ($2X10^4$ cells/well) were seeded in a luminometer white 96 plate (Thermo scientific) and incubated for 24 hr at 37°C. Cells were treated with vehicle or with Erlotinib for 5 hrs. One hundred microlitre of caspase 3/7 reagents were added to each well and incubated for 1 hr on rotary shaker at room temperature. Luminescence for each well was recorded using GLOMAX+ instrument (Promega).

Cell cycle analysis by Flow cytometry. Cells were exposed to Erlotinib for 24 hr. Approximately 1.5 X 10^5 cells were collected and centrifuged at 1,000 rpm for 5 min. DNA staining was performed by adding 500 μl propidium iodide (200 μg/mL in Triton X-100, 0.2% v/v saline, Final concentration: 100 μg/mL) and 50 μL RNAase (final concentration: 40 μg/mL) to 1.5 X 10^5 cells. Cellular DNA content was measured by Becton Dickinson FACStar Plus dual laser system and a FACSort system. Approximately 10,000 cells were analyzed for each DNA content histogram. The samples were excited at 380–410 nm and the resulting fluorescence measured at wavelengths >550 nm. Analysis of the percentage of

cells in G1, S and G2 /M phases of the cell cycle was made by the ModFit LT program (Verify Software Home).

Western blot analysis. Cell lysates were prepared using a RIPA buffer (Sigma) according to the manufacturer's instruction. Protein samples were applied to the wells of NuPAGE 4-20% Tris-Gly gel, electrophoresed in SDS running buffer (Invitrogen), and transferred to Nitrocellurose membranes using the iBlot transfer apparatus (Invitrogen). Membranes were blocked in Tris-buffered saline containing 0.5% Tween 20 (TBS-T) and 5% BSA for 1 hr at room temperature followed by incubation with primary antibody overnight at 4°C. After membranes were washed three times for 10 min each in TBS-T, HRP-conjugated secondary antibody (Bio-Rad) in TBS-T containing 2% BSA was applied for 1 hour at room temperature. Proteins were visualized using G-box Chemi Systems (SynGene).

Quantitative RT-PCR. Quantitative RT-PCR was performed on RNA isolated from NSCLC cells and patient samples. Total RNA was extracted using RNeasy mini kit (Qiagen), RNeasy FFPE kit (Qiagen) and RNAlater RNA stabilization reagent (Qiagen), according to the manufacturer's instructions. cDNA was synthesized with High Capacity cDNA Reverse transcription Kit (Applied Biosystems) and Taqman microRNA Reverse Transcription Kit (Applied Biosystems). RT-PCR was performed on a LightCycler with SYBR Green system (Roche) using 7900HT Fast Real-Time PCR system (Applied Biosystems). GAPDH expression was used as an internal reference to normalize input cDNA. Primer sequences used for real-time RT-PCR and quantitative RT-PCR of Cripto-1, Cripto-3 and GAPDH are: (1) Cripto-1: Forward 5'-TCCAGTTTCCCCTGGAC G-3' and Reverse 5'-TTTAAGAAGAAAATGAGAAATTCGTT-3'; (2) Cripto-3: Forward 5'-TGTAAAACGACGGCCAGTTCTACCCGGCTGTGATGGCC-3' and Reverse 5'-CAGGAAACAGCTATGACCGAGGTAAGAAGTAGTTAGTTCTGT-3'; (3) GAPDH: Forward 5'-TGATGACATCAAGAAGGTGG-3' and Reverse 5'-TTTC TTACTCCTTGGAGGCC-3'. The specificities of Cripto-1 and -3 specific primers were validated in Cripto-1-positive/Cripto-3-negative (H727) and Cripto-3-

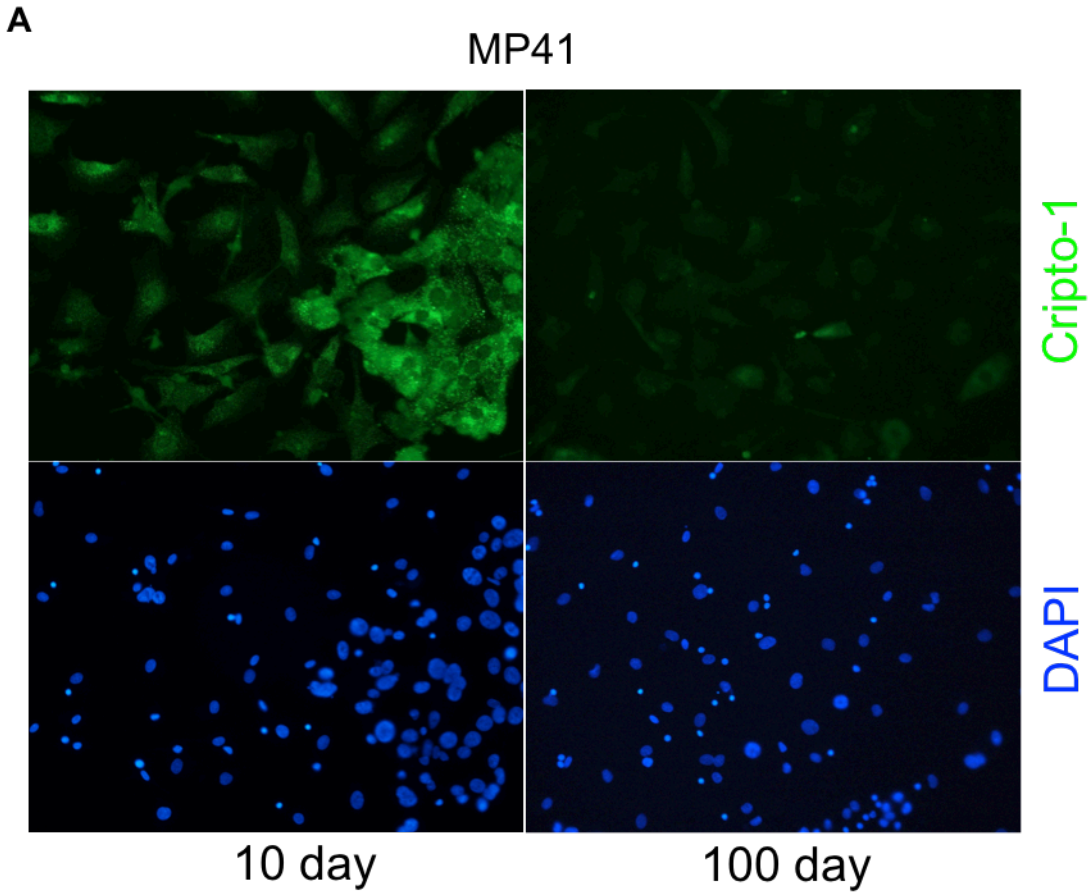
positive/Cripto-1-negative (H69) cells (Biochem Biophys Res Commun. 377(1):215-20, 2008) (See Figure 1C). Taqman gene expression assays (Src: Hs01082246_m1; GAPDH: Hs02758991_g1; miR-205: has-miR-205-5p) were purchased from Invitrogen.

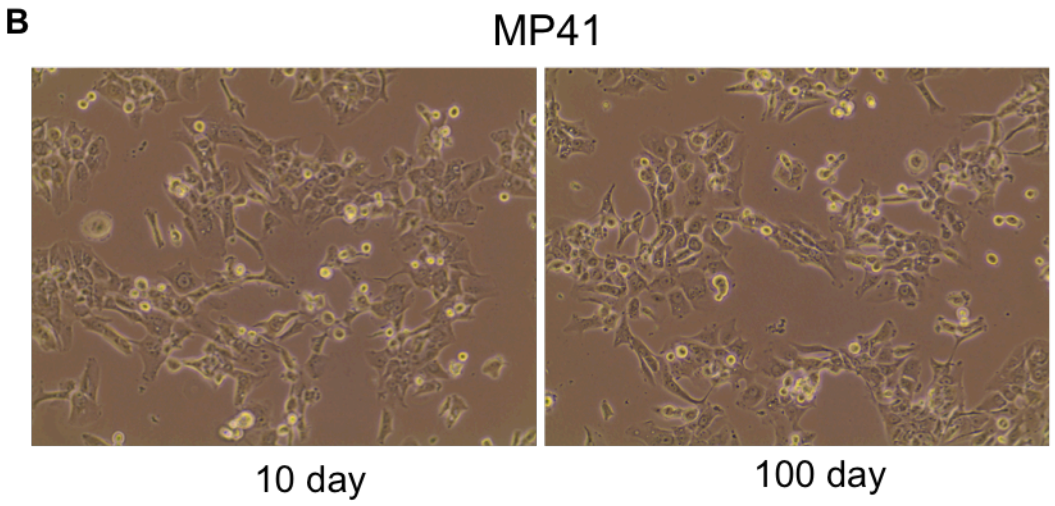
siRNA- and shRNA-mediated knockdown. Transfection of a small interfering RNA (siRNA) duplex targeting human Cripto-1 (Invitrogen, Stealth TDGF1-HSS144243), human SRC (Thermo, ON-TARGET Human SRC) and miR-205 inhibitor (Ambion, AM11015) were carried out using PepMutTM siRNA transfection reagent (SignaGen), according to the manufacturer's instructions. Briefly, cells were seeded at a density of $6x10^5$ cells/well in a 6-well tissue culture dish and incubated overnight. Cells were then transfected with the siRNA-PepMute mixture (200 pmol siRNA and 2.4 μl PepMute reagent) for 3 days. As a negative control, we used Stealth Negative control siRNA (Invitrogen). In the shRNA experiments, cells were infected with ZEB1 shRNA (Open Biosystems shRNA Library) retrovirus in the presence of 1 μg/ml polybrene, and were selected for 48 hrs with 1 μg/ml of puromycin 48 hrs after infection. Expression was measured by western blot 48–72 h later.

Cripto-1 and miR-205 overexpression studies. To establish stable cell lines, the pCI-neo-hCripto-1 (Normanno et al., 2004) or miR-205 (HmiR0026-MR04, GeneCopoeiaTM) expression vectors were transfected using lipofectaminTM 2000 plus (Invitrogen) following the manufacturer's instruction. All vectors were linearized by AhdI (NEB) enzyme to increase efficiency of plasmid integration. Cells (70-80% confluence) were washed with Opti-MEM (Invitrogen), and incubated with a DNA-lipofectamine mixture (2 μg DNA and 3 μl lipofectamine reagent). For stable transfection, the transfected cells were selected with 500 μg/ml of the antibiotic G418 (Gibco). The parental cells transfected with empty vectors were generated as controls. Cripto-1 over-expression was detected using an anti-human Cripto-1 rabbit monoclonal antibody (Epitomics) by western blot and miR-205 expression was measured by RT-PCR.

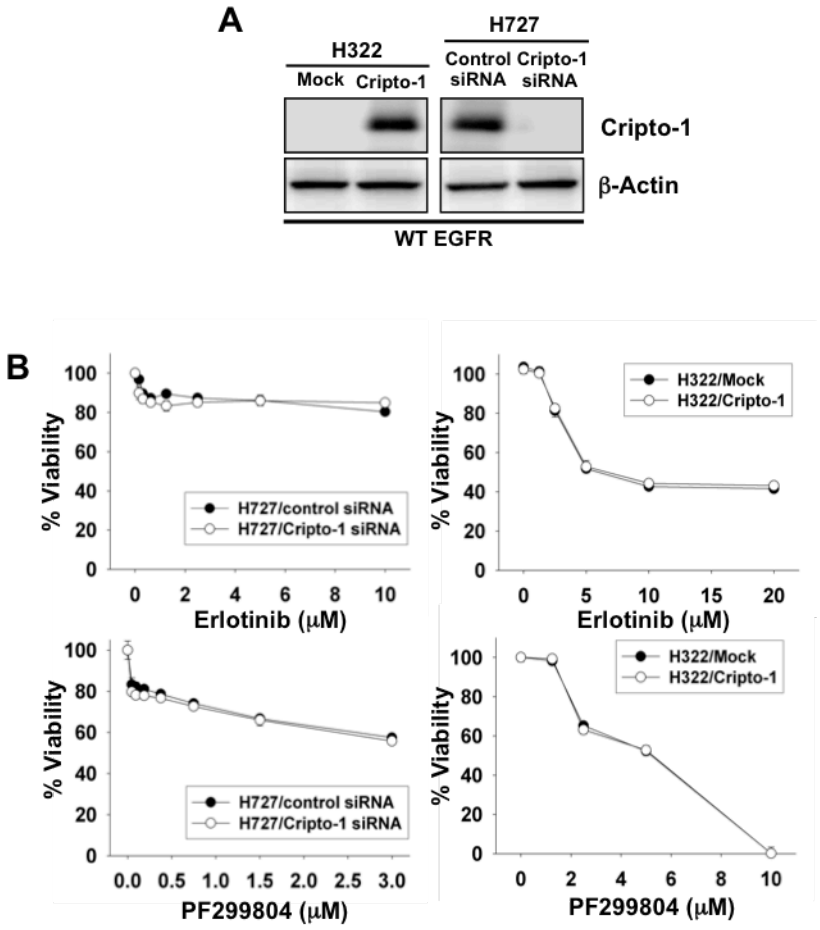
Migration and invasion assay. CytoSelect™ 24-Well Cell Invasion and migration Assay kits were purchased from Cell Biolabs. According to the manufacturer's instructions, cells were serum-starved overnight and $5x10^5$ cells in 300 μl of medium without FBS were placed in the upper chamber of transwell plates and the lower chamber was filled with 0.5 ml of medium supplemented with 10% FBS. To quantify the migrated and invaded cells, crystal violet-stained cells were extracted by extraction buffer followed by spectrophotometer measurement at 560 nm.

Supplementary Figures:

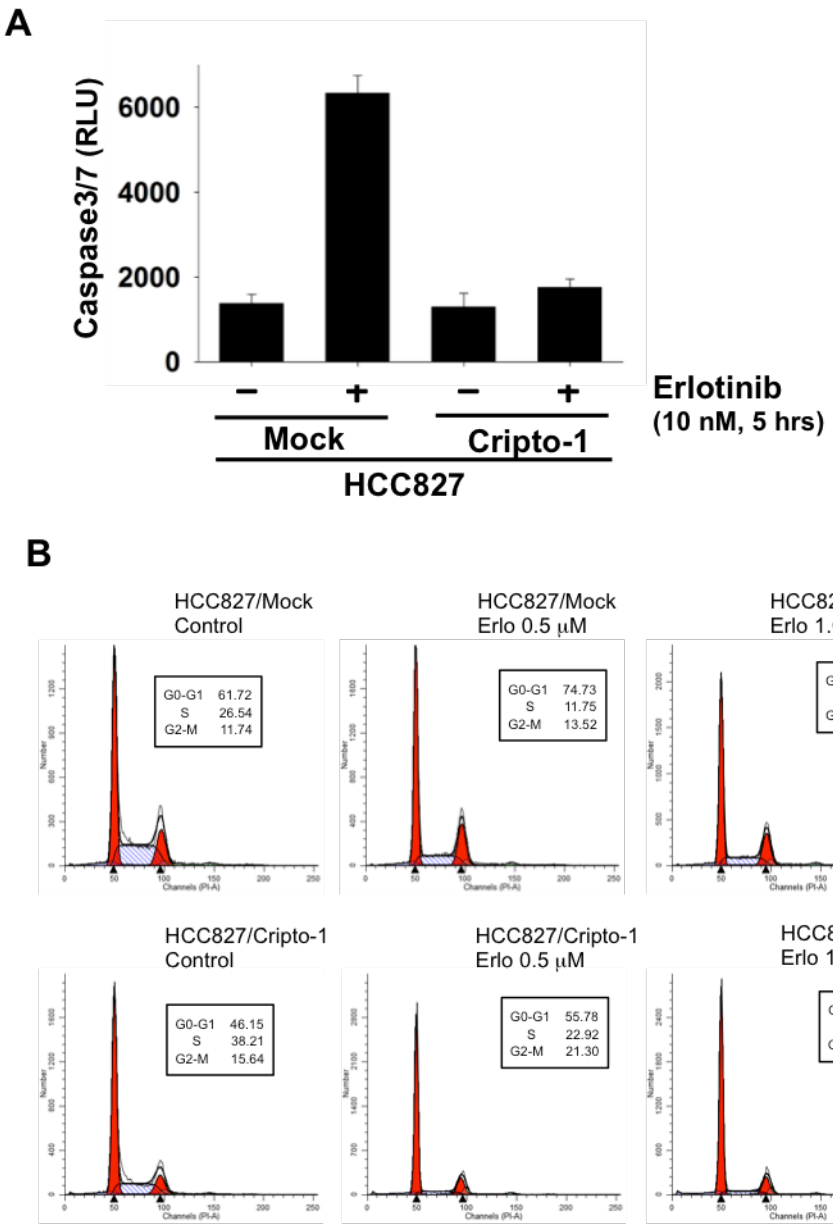




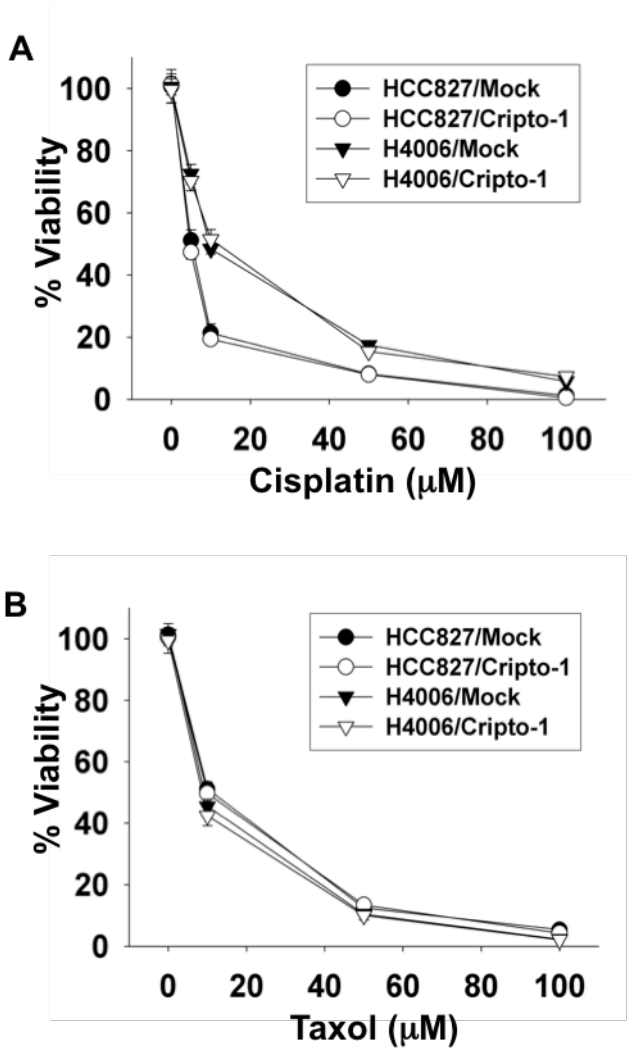
Supplementary Figure 1 (A) Cripto-1 immunochemistry of MP41 cells. Cripto-1 is expressed in the early passaged cells (day 10) and loss of Cripto-1 expression occurs in all cells, not just a subpopulation of the late passaged (day 100) MP41 cells. Cripto-1 was stained with Cripto-1 antibody (Rockland) and DNA by DAPI. Magnification: x200 (B) Cell morphology (phase contrast images) of early (10 day) and late (100 day) passaged MP41 cells. Magnification: x200



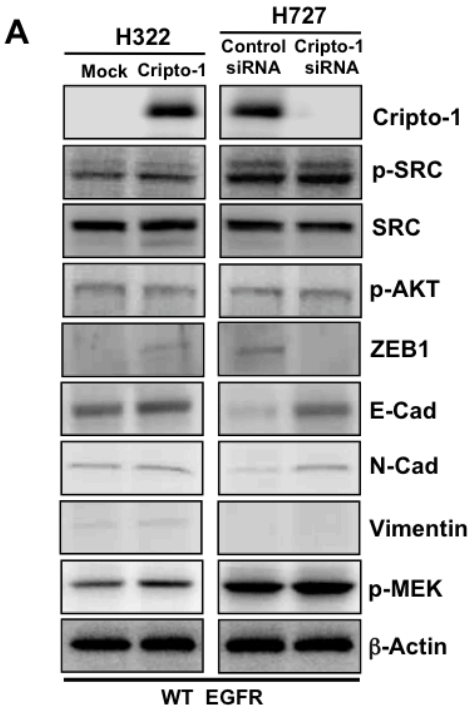
Supplementary Figure 2. Cripto-1 does not affect sensitivity of EGFR WT NSCLC cells to EGFR-TKIs. (A) Western blot analysis of Cripto-1 expression in Cripto-1 and Cripto-1 siRNA transfected NSCLC cells. WT, wild-type. (B) Cripto-1 does not affect erlotinib and dacomitinib sensitivity of NSCLC cells harboring wild-type EGFR.

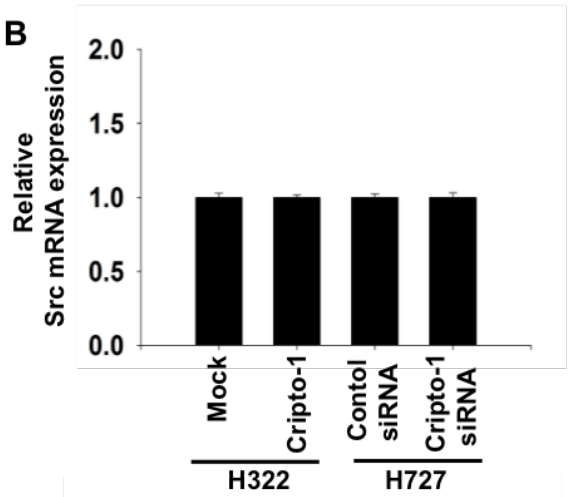


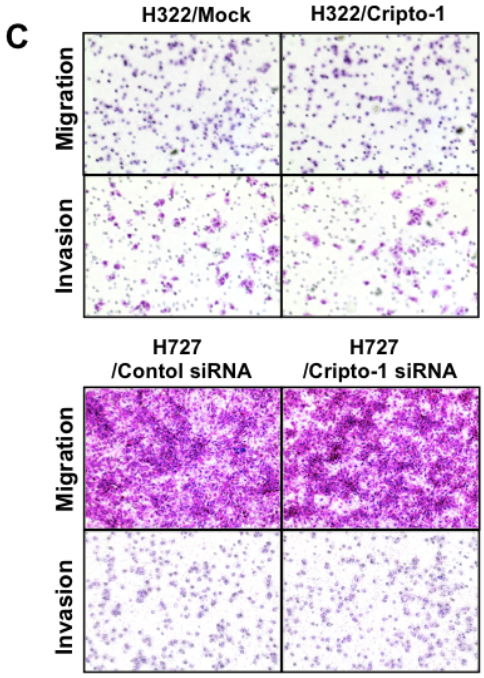
Supplementary Figure 3. Cripto-1 attenuates EGFR inhibitor-induced apoptosis. (A) Caspase 3/7 activity was measured in HCC827/Mock and HCC827/Cripto-1 cells in the presence or absence of erlotinib as indicated. RLU, Relative Luciferas e Unit. (B) Cell cycle analysis in erlotinib-treated HCC827/Mock and HCC827/ Cripto-1 cells by FACS.

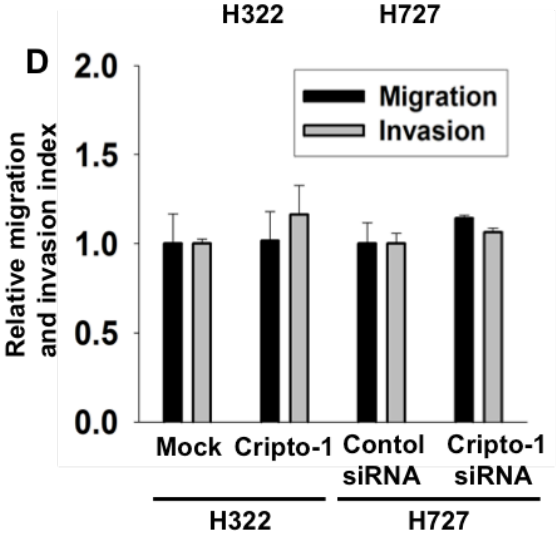


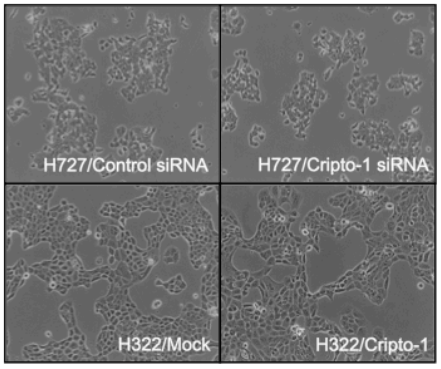
Supplementary Figure 4. MTS assay on the effect of Cisplatin (A) and Taxol (B) in HCC827/Cripto-1 and H4006/Cripto-1 cells.


A
H322
H727
Mock
Cripto-1
Control siRNA
Cripto-1 siRNA
Cripto-1
p-SRC
SRC
p-AKT
ZEB1
E-Cad
N-Cad
Vimentin
p-MEK
β-Actin
WT EGFR

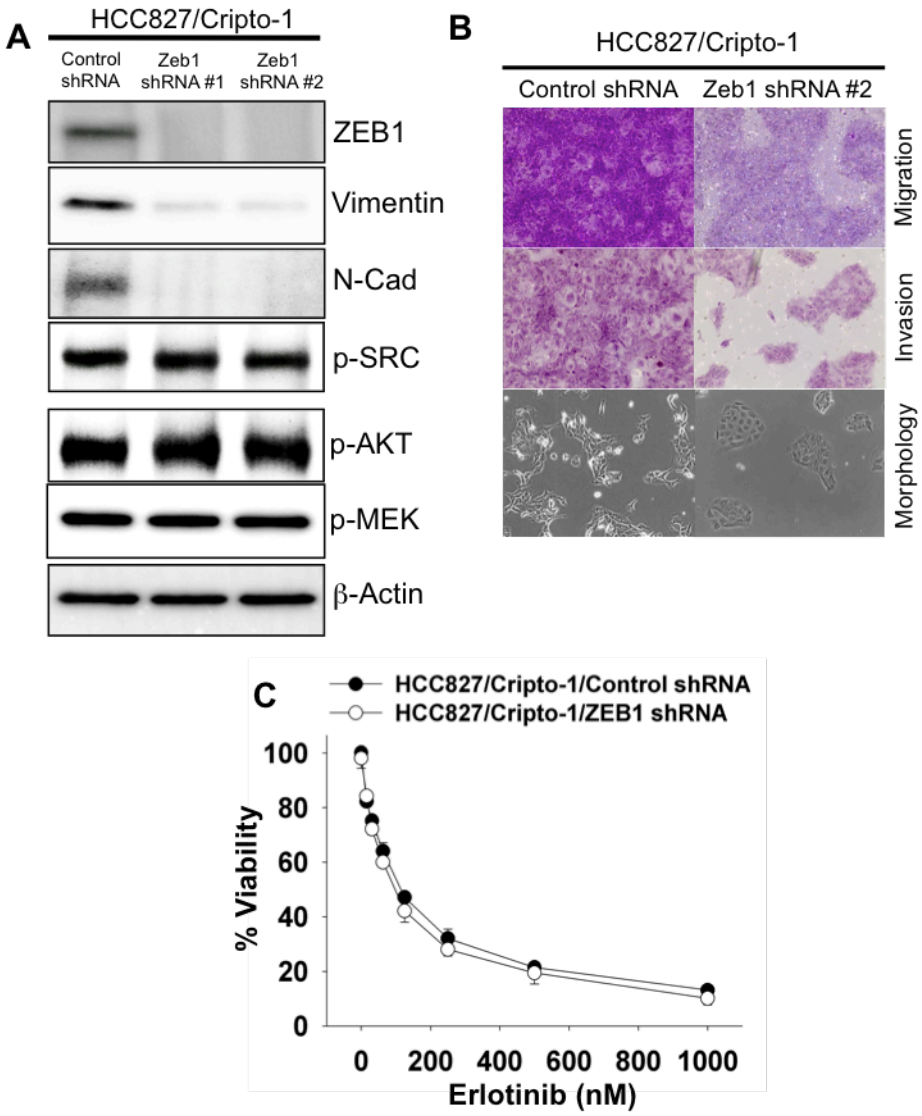

B
Relative Src mRNA expression
2.0
1.5
1.0
0.5
0.0
Mock
Cripto-1
Contol siRNA
Cripto-1 siRNA
H322
H727


C
H322/Mock
H322/Cripto-1
Migration
Invasion
H727 /Contol siRNA
H727 /Cripto-1 siRNA
Migration
Invasion

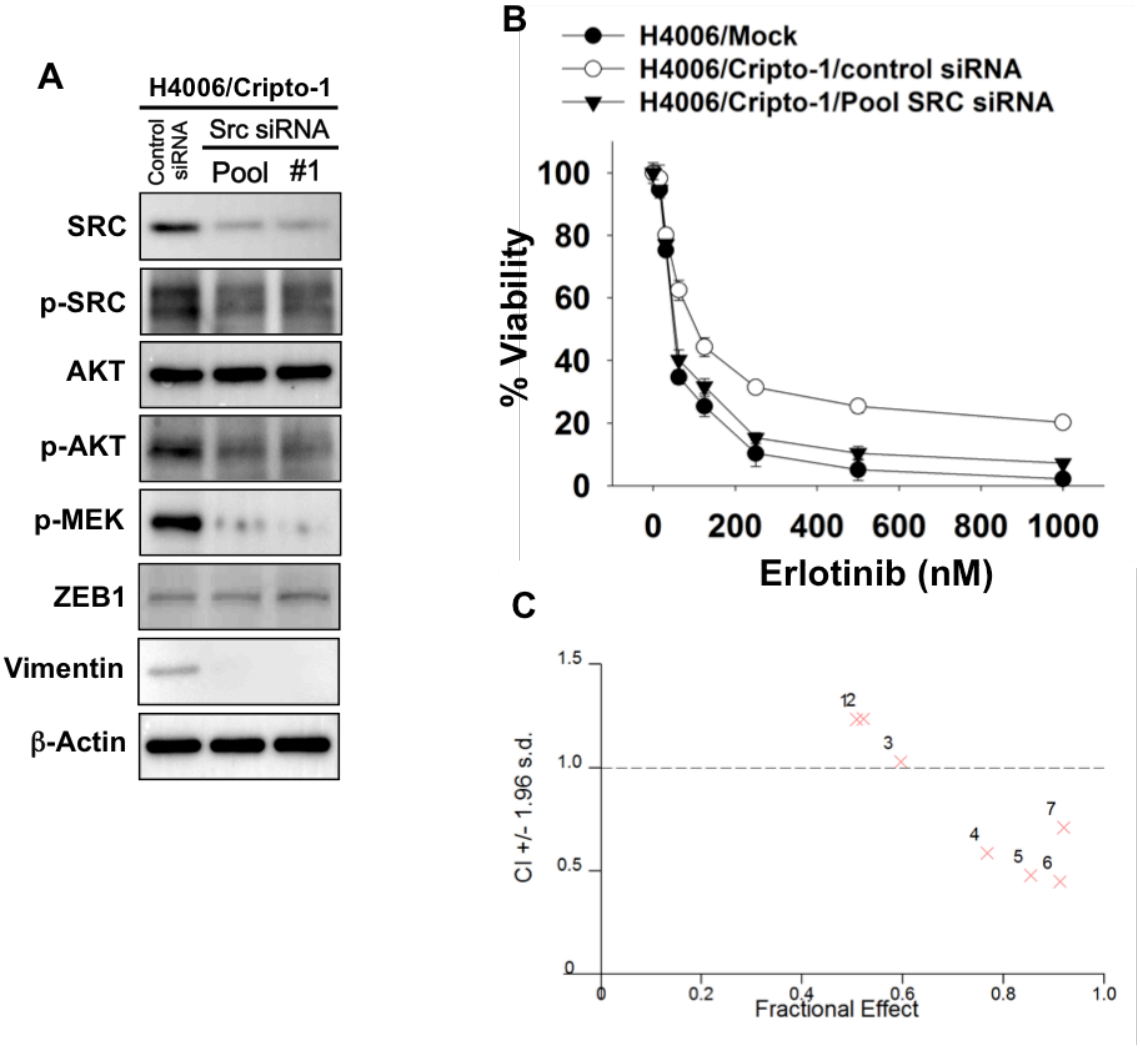

D
Relative migration and invasion index
2.0
1.5
1.0
0.5
0.0
Migration
Invasion
Mock
Cripto-1
Contol siRNA
Cripto-1 siRNA
H322
H727


E
H727/Control siRNA
H727/Cripto-1 siRNA
H322/Mock
H322/Cripto-1

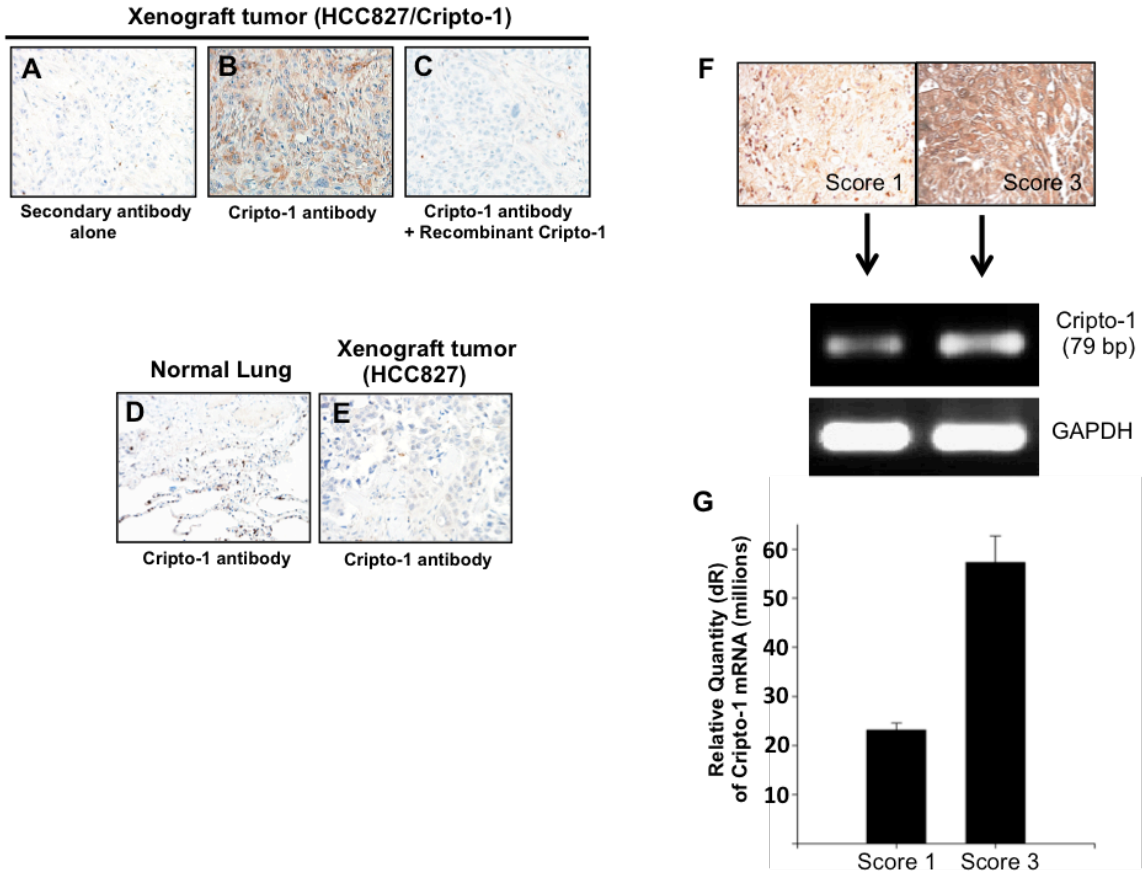
Supplementary Figure 5. Effect of Cripto-1 on SRC signaling and EMT. (A) Western blot analysis of both SRC and EMT signaling protein expression in EGFR WT NSCLC cell lines either stably-transfected with Cripto-1 or knocked-down by Cripto-1siRNA. Cripto-1 blot is the same with supplementary figure 2A. (B) The level of SRC mRNA in Cripto-1 stably-transfected and siRNA knockdown NSCLC cell lines by RT-PCR. (C and D) Cell migration and invasion were measured as described in Materials and Methods. Data are representative of three independent experiments. (E) Phase-contrast images of Cripto1-transfected and -knockdown NSCLC cell lines. Note no morphology changes in H727/Cripto-1 siRNA and H322/Cripto-1 cells; Magnification, 200X.



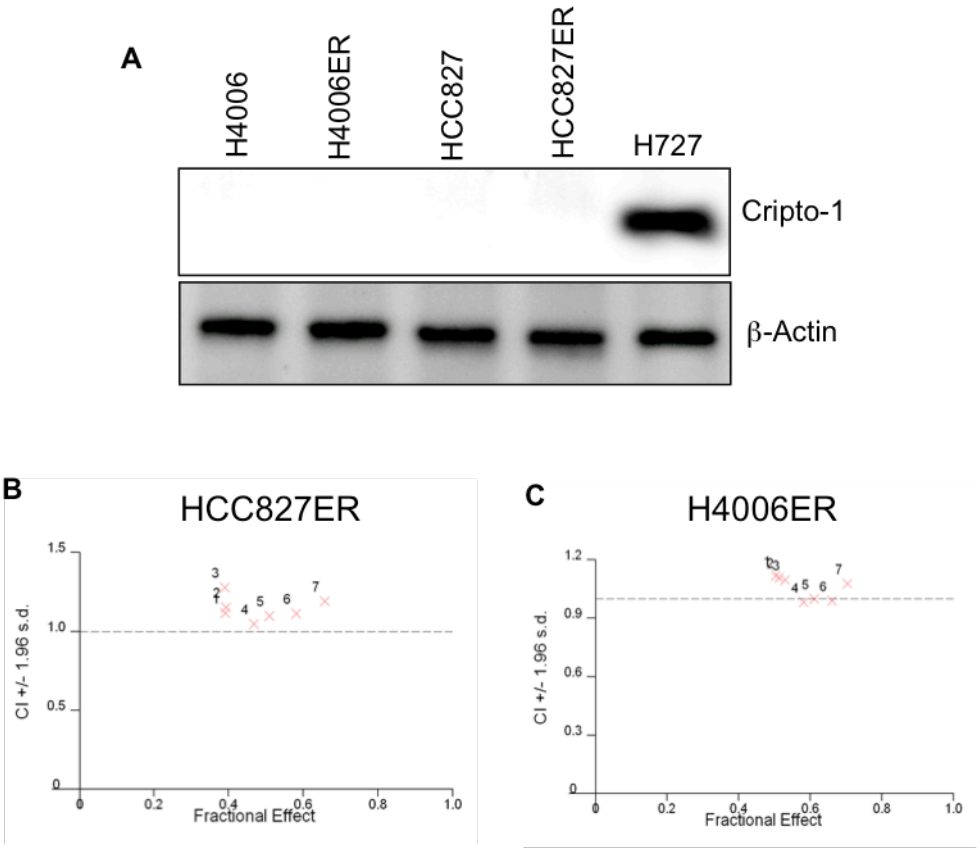
Supplementary Figure 6. Cripto-1-induced EGFR-TKI resistance is not ZEB1-dependent. (A) Western blot analyses of ZEB1, Vimentin, N-Cadherin and pSRC in HCC827/Cripto-1/ZEB1 shRNA cells. (B) ZEB1 knockdown reversed the EMT phenotype but (C) failed to restore erlotinib sensitivity of HCC827/Cripto-1 cells. Magnification, 200X.



Supplementary Figure 7. Cripto-1-induced EGFR-TKI resistance is SRC dependent. (A) Western blot analysis of HCC4006/Cripto-1 cells 72 hrs after SRC siRNA transfection. β-actin was used as loading control. (B) SRC siRNA reinstated erlotinib sensitivity of HCC827/Cripto-1 cells. Two days after transfection, the indicated cell lines were treated with erlortinib for 3 days, followed by MTS assay. Data represent triplicate experiments. (C) Synergistic effect of AZD0530 and erlotinib combination. MTS assays were performed in HCC827/Cripto-1 cells treated with 150 nM of erlotinib plus increasing concentrations of AZD0530 (15.625, 31.25, 62.5, 125, 250, 500 and 1000 nM) for 3 days. Fa, fraction affected; CI, combination index.



Supplementary Figure 8. Specificity of Cripto-1 antibody. Immunohistochemical staining of HCC827/Cripto-1 xenograft tumors with (A) secondary antibody alone, (B) Cripto-1 antibody (Rockland) and (C) Cripto-1 antibody blocked with recombinant human Cripto-1 protein (R&D systems). Cripto-1 immuno histochemical staining of (D) human normal lung tissue and (E) HCC827 xenograft tumors. (F) RT-PCR verification of Cripto-1 expression (bottom) on human NSCLC samples that express Cripto-1 protein at different levels (See Figure 6A and B) quantified by immunohistochemistry using Cripto-1 specific antibody (top). (G) Similar experiments to Figure S8F were performed by real-time PCR using different Cripto-1 specific primers. Note that the RNA was extracted from tumor cells (marked by a pathologist) scraped out of H&E-stained FFPE slides. Magnification, 400X



Supplementary Figure 9. AZD0530 and erlotinib combination elicits no synergy in acquired erlotinib-resistant NSCLC cells. (A) Western blot analysis. Cripto-1 expression is not elevated in acquired erlotinib-resistant (ER) NSCLC cell lines. (B and C) MTS assays were performed in acquired erlotinib-resistant cells treated with erlotinib at IC50 concentration (40 μM for HCC827ER and 20 μM for H4006ER) plus increasing concentrations of AZD0530 (0.15625, 0.3125, 0.625, 1.25, 2.5, 5 and 10 μM) for 3 days. CI, combination index.

Supplementary Table 1. IC50 of Cripto-1-overexpressed and Cripto-1 siRNA-transfected NSCLC cell lines harboring mutant (A, related to Figure 2B) and wild (B, related to Supplementary Figure 2B) EGFR. MTS assays were performed 72hrs after erlotinib and dacomitinib (PF299804) treatment. Data represent means ± SD of triplicate measurements relative to untreated cells.

A

Cell name	Erlotinib (IC50)	PF299804 (IC50)	EGFR
HCC827/Mock	10 nM	2 nM	Exon 19 del
HCC827/Cripto-1	75 nM	20 nM	
H4006/Mock	50 nM	10 nM	Exon 19 del
H4006/Cripto-1	100 nM	26.3 nM	
H3255/Mock	50 nM	1.5 nM	L858R

B

Cell name	Erlotinib (IC50)	PF299804 (IC50)	EGFR
H727/Control siRNA	> 10uM	3.01 uM	WT
H727/Cripto-1 siRNA	> 10 uM	2.98 uM	
H322/Mock	5.15 uM	5.51 uM	WT
H322/Cripto-1	5.03 uM	5.62 uM	

Supplementary Table 2. Combination treatment index related to Figure 5D (A) and Supplementary Figure 7C (B).

A

No.	Erlotinib (nM)	AZD0530 (nM)	Fa	CI
1	100	10	0.2723	0.549
2	100	50	0.3504	0.375
3	100	100	0.3506	0.601
4	100	500	0.5520	0.835
5	100	1000	0.6795	0.849

B

No.	Erlotinib (nM)	AZD0530 (nM)	Fa	CI
1	150	15.625	0.5087	1.233
2	150	31.25	0.52165	1.237
3	150	62.5	0.59585	1.031
4	150	125	0.7684	0.589
5	150	250	0.8551	0.478
6	150	500	0.9129	0.45
7	150	1000	0.9202	0.712

Supplementary Table 3. Cripto-1 immunohistochemistry scores and patient characteristics.

No.	Status	OS (months)	Stage	Sex	Smoking history	ECOG Performance status at initiation of EGFR-TKI	Histology	Previous chemotherapies	EGFR-TKI	Response to EGFR-TKI	PFS (months)	Mutation	IHC Score of Cripto-1
1	Dead	10.3	IV	M	Never	1	Ad	1	Gefitinib	PD	1.9	Exon 19 del	3
2	Dead	10.8	IIIb	M	Never	1	Ad	2	Erlotinib	PD	0.9	Exon 19 del	2
3	Dead	2.3	IV	M	Current	2	Ad	3	Erlotinib	PD	1.1	Exon 19 del	3
4	Dead	11.9	IV	M	Former	0	Ad	1	Gefitinib	SD	6.9	L858R	2
5	Dead	12.2	Recurrence	M	Current	1	Ad	2	Erlotinib	SD	4	Exon 19 del	1
6	Dead	16	IV	F	Never	0	Ad	1	Gefitinib	SD	4	L858R	1
7	Dead	13	IV	M	Never	1	Ad	1	Gefitinib	PD	1	L858R	1
8	Alive	11.3+	Recurrence	M	Former	1	Ad	3	Erlotinib	SD	10.5	L858R	0
9	Alive	13.1+	IV	M	Never	1	Ad	0	Erlotinib	SD	7.4	L858R	1
10	Dead	64.4	IIIb	F	Never	1	Ad	1	Gefitinib	PR	16.1	Exon 19 del	1
11	Dead	23.2	IV	F	Never	1	Ad	1	Gefitinib	PR	10.9	L858R	1
12	Dead	17.7	IV	F	Never	0	Ad	1	Gefitinib	PR	16.1	L858R	1
13	Alive	20.5+	IV	M	Former	1	Ad	4	Erlotinib	PR	14	Exon 19 del	1
14	Dead	12.6	IV	M	Current	1	Ad	3	Gefitinib	PR	7.6	Exon 19 del	2
15	Dead	11.1	IV	F	Never	1	Sq	0	Gefitinib	PR	10.9	L858R	1
16	Dead	9	IV	F	Never	1	Ad	1	Gefitinib	PR	5.8	Exon 19 del	1
17	Alive	45.6+	Recurrence	F	Never	0	Ad	0	Gefitinib	PR	24.6	Exon 19 del	0
18	Dead	20	IV	F	Never	1	Ad	0	Gefitinib	PR	10.3	Exon 19 del	0
19	Alive	51.3+	IV	F	Never	1	Ad	0	Gefitinib	PR	51.3+	Exon 19 del	2
20	Alive	19.2+	IV	F	Never	1	Ad	1	Gefitinib	PR	7.2	Exon 19 del	1
21	Dead	25.6	IV	F	Never	2	Ad	0	Gefitinib	PR	20.4	L858R	0
22	Alive	36.9+	Recurrence	F	Never	1	Ad	0	Gefitinib	PR	25.8	Exon 19 del	0
23	Dead	27.8	IV	F	Never	2	Ad	3	Erlotinib	PR	11.1	L858R /T790M	0
24	Alive	18.0+	IV	F	Never	0	Ad	1	Erlotinib	PR	10.1	Exon 19 del /T790M	0
25	Alive	9.8+	IV	F	Former	0	Ad	1	Erlotinib	PR	9.8	Exon 19 del	0
26	Alive	11.3+	IV	F	Never	1	Ad	1	Erlotinib	PR	10.2	L858R	1
27	Alive	72.6+	IV	F	Former	0	Ad	2	Erlotinib	PR	50.9	L858R	0
28	Alive	29.5+	IV	F	Former	1	Ad	2	Erlotinib	PR	13.2	Exon 19 del /T790M	2
29	Alive	16.1+	Recurrence	F	Never	1	Ad	1	Erlotinib	PR	16.1	Exon 19 del	0
30	Alive	30.5+	IV	M	Former	0	Ad	0	Erlotinib	PR	30.5+	Exon 19 del	0
31	Alive	23+	IV	M	Former	1	Ad	1	Erlotinib	PR	23+	Exon 19 del /T790M	0
32	Dead	2	IV	M	Never	1	BAC	0	Erlotinib	PD	1	L858R	3
33	Alive	17+	IV	M	Never	2	Ad	0	Erlotinib	PR	9	Exon 19 del	0
34	Dead	9	IV	M	Never	1	other	0	Erlotinib	PD	1	Exon 19 del	3
35	Alive	13+	IV	F	Never	1	Ad	0	Erlotinib	CR	13+	Exon 19 del	0
36	Alive	17+	IV	F	Never	0	BAC	1	Erlotinib	SD	17+	Exon 19 del	2
37	Alive	11+	IV	F	Never	2	Ad	1	Erlotinib	PR	11+	Exon 19 del	0
38	Alive	8+	IV	F	Never	1	Ad	0	Erlotinib	CR	8+	Exon 19 del	0
39	Alive	15+	IV	F	Never	0	Ad	0	Erlotinib	PR	15+	L858R /T790M	0
40	Alive	12+	IV	F	Never	1	Ad	1	Erlotinib	PR	12+	Exon 19 del /T790M	0

(Table 2 Cont.)

No.	Status	OS (months)	Stage	Sex	Smoking history	ECOG Performance status at initiation of EGFR-TKI	Histology	Previous chemotherapies	EGFR-TKI	Response to EGFR-TKI	PFS (months)	Mutation	IHC Score of Cripto-1
41	Alive	13+	IV	M	Former	1	BAC	0	Erlotinib	PR	13+	Exon 19	0
42	Alive	7+	IV	M	Current	2	Ad	0	Erlotinib	PR	7+	L858R /T790M	0
43	Alive	10+	IV	F	Never	1	Ad	0	Erlotinib	PR	10+	Exon 19	0
44	Dead	9	IV	F	Former	0	Ad	1	Erlotinib	PR	9	Exon 19 del /T790M	0
45	Alive	4+	IV	M	Never	1	Ad	1	Erlotinib	PR	4+	L858R /T790M	0
46	Alive	8+	IV	F	Never	0	Ad	0	Erlotinib	PR	8+	Exon 19 del	0
47	Dead	5	IV	F	Never	2	Ad	0	Erlotinib	PR	5	Exon 19 del	0
48	Alive	7+	IV	F	Never	1	Ad	0	Erlotinib	PR	7+	L858R	0
49	Alive	7+	IV	F	Current	1	Ad	0	Erlotinib	PR	7+	Exon 19 del	0
50	Alive	11+	IV	M	Never	2	Ad	1	Erlotinib	PR	8	L858R	0
51	Alive	47+	IV	M	Current	1	Ad	1	Gefitinib	PD	1.1	Exon 19 del	2
52	Dead	44.3	IV	F	Never	1	Ad	1	Gefitinib	SD	9.6	L858R	1
53	Dead	19.2	IV	M	Never	1	Ad	1	Gefitinib	SD	3.5	L858R	2
54	Alive	34.3+	IV	F	Never	1	Ad	1	Gefitinib	SD	27.7	L858R	2
55	Alive	7+	IV	F	Never	1	Ad	1	Gefitinib +Erlotinib	PD	3.8	L858R	3
56	Alive	43+	IV	M	Never	1	Ad	1	Gefitinib	PR	18.4	G719C	0
57	Dead	30	IV	M	Former	1	Ad	0	Gefitinib	CR	11.8	Exon 19 del	0
58	Dead	16.5	IV	M	Never	1	Ad	0	Gefitinib	PR	3.9	Exon 19 del	0
59	Dead	21.1	IV	M	Current	1	Ad	0	Gefitinib	PR	1.6	G719	0
60	Dead	6	IV	F	Never	1	other	0	Gefitinib	PR	2	Exon 19 del	2
61	Dead	40.3	IV	F	Current	1	Ad	0	Gefitinib	PR	38.2	L858R	0
62	Alive	7.8+	IV	M	Current	1	Ad	0	Gefitinib	SD	2.5	Exon 19 del	1
63	Alive	4+	IV	M	Current	1	Ad	1	Gefitinib	SD	4.2	Exon 19 del	1
64	Alive	13+	IV	F	Current	1	Ad	1	Gefitinib	PR	11.1	Exon 19 del	0
65	Alive	124+	IV	F	Never	0	Ad	0	Gefitinib	SD	64.6	Exon 19 del	2
66	Dead	89.2	IIIA	M	Smoker	1	Ad	0	Gefitinib	SD	14.9	Exon 19 del	3
67	Dead	71.5	IIIA	M	Smoker	0	Ad	0	Gefitinib	SD	14.8	Exon 19 del	2
68	Dead	65.9	IIIA	M	Never	3	Ad	2	Gefitinib	PD	2.7	L858R	2
69	Dead	62	IV	F	Never	1	Ad	1	Gefitinib	PR	32.8	Exon 19 del	0
70	Dead	44.8	IIA	F	Never	1	Ad	2	Gefitinib	PR	5	Exon 19 del	0
71	Dead	82.8	IA	F	Never	1	Ad	0	Gefitinib	PR	18.8	Exon 19 del	0
72	Dead	80.2	IB	F	Smoker	1	Ad	2	Gefitinib	PR	4.8	Exon 19 del	0
73	Dead	84.4	IIIA	F	Never	1	Ad	0	Gefitinib	PR	8.8	Exon 19 del	0
74	Dead	38	IIIA	F	Never	0	Ad	0	Gefitinib	CR	9.9	L858R	0
75	Dead	69.2	IIB	F	Never	0	Ad	0	Gefitinib	PR	5.9	L858R	0
76	Dead	18.8	IIIB	M	Smoker	2	AdSq	1	Gefitinib	PR	8.6	Exon 19 del	0
77	Dead	57.7	IIB	M	Never	0	Ad	0	Gefitinib	PR	25.5	G719C	0
78	Dead	53.7	IIIA	M	Smoker	1	Ad	0	Gefitinib	CR	12.1	Exon 19 del	0
79	Dead	64.3	IIIA	M	Never	0	Ad	0	Gefitinib	PR	3.8	Exon 19 del	0
80	Dead	20.8	IIIB	M	Smoker	0	Ad	0	Gefitinib	PR	1.6	G719	0
81	Dead	6.2	IIIB	F	Never	2	Ad	0	Gefitinib	PR	1.9	Exon 19 del	0
82	Dead	14.3	IIIA	F	Smoker	1	Ad	2	Gefitinib	PR	10.9	Exon 19 del	0
83	Alive	42+	IV	M	Former	1	Ad	1	Erlotinib	PR	18	Exon 19 del	0
84	Dead	9	IV	F	Former	2	Ad	0	Erlotinib	PD	6	G719S	3
85	Alive	4+	IIIb	F	Never	0	Ad	0	Gefitinib	PD	2	Exon 19 del	2

OS = Overall survival
Stage: Recurrence = recurrence after surgical resection with or without adjuvant chemotherapy
Sex: M = male; F = female
Histology: Ad = adenocarcinoma; Sq = Squamous carcinoma; BAC = bronchoalveolar carcinoma
Response: PD = progressive disease; SD = stable disease; PR = partial response; CR = complete response
PFS = Progression-free survival

Supplementary Table 4. Combination treatment index related to HCC827ER (A) H4006ER (B) cells in Supplementary Figure 9B and 9C. Fa, fraction affected; CI, combination index.

A

No.	Erlotinib (uM)	AZD0530 (uM)	Fa	CI
1	40	0.15625	0.391	1.116
2	40	0.3125	0.39405	1.152
3	40	0.625	0.3895	1.278
4	40	1.25	0.46825	1.047
5	40	2.5	0.51005	1.1
6	40	5	0.58205	1.113
7	40	10	0.6587	1.191

B

No.	Erlotinib (uM)	AZD0530 (uM)	Fa	CI
1	20	0.15625	0.50335	1.12
2	20	0.3125	0.5133	1.11
3	20	0.625	0.5293	1.099
4	20	1.25	0.58107	0.982
5	20	2.5	0.6108	0.999
6	20	5	0.66085	0.989
7	20	10	0.7034	1.079