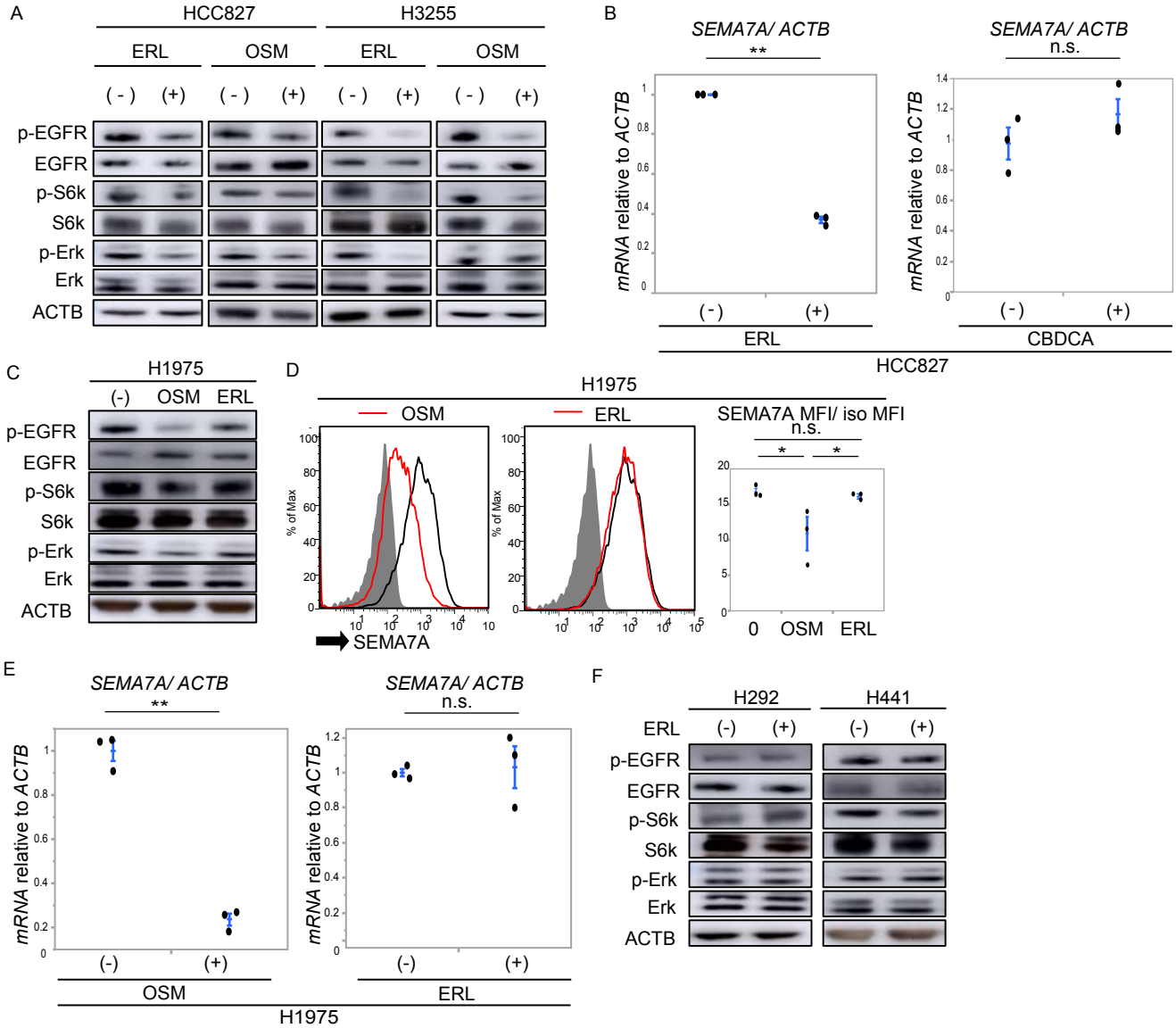


Supplemental Figure 1. EGFR signals regulate SEMA7A expression in lung adenocarcinoma cells.

(A) Representative immunoblots showing downregulation of p-EGFR, p-S6k, and p-Erk in HCC827 and H3255 24 hours after treatment with erlotinib (ERL; 10 nM).

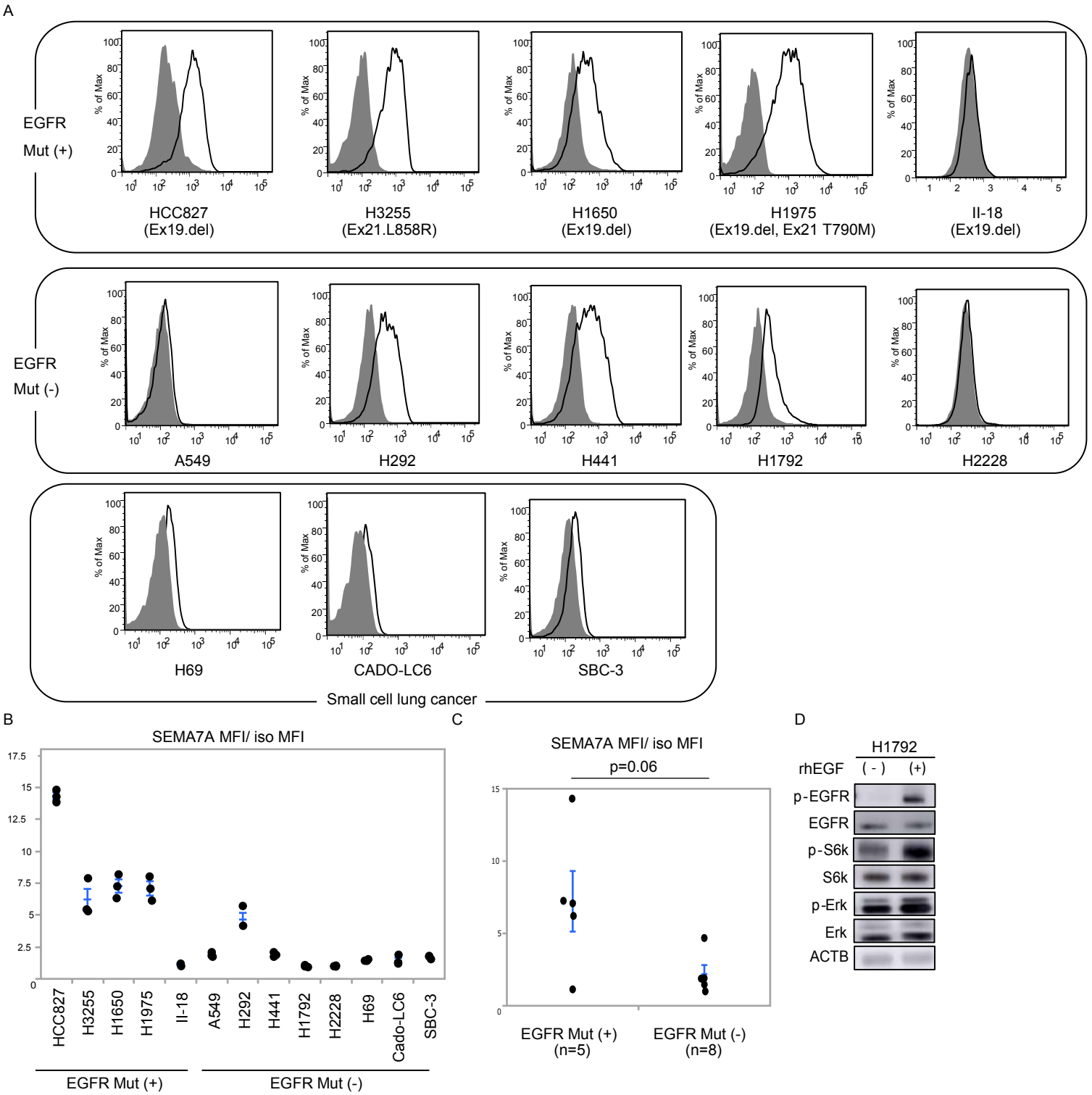
(B) qRT-PCR analyses showing expression of SEMA7A 4 hours after treatment with erlotinib (ERL; 10 nM) or carboplatin (CBDCA; 500 μM). Data (means ± SE) are representative of three independent experiments. The two-sample t test was used to evaluate significance. **p<0.01; n.s.: not significant.

(C) Representative immunoblots showing expression of p-EGFR, p-S6k, and p-Erk in H1975 24 hours after treatment with erlotinib (ERL; 500 nM) or osimertinib (OSM; 15 nM).

(D) Change of SEMA7A expression 24 hours after treatment with osimertinib (15 nM) or erlotinib (500 nM). H1975 harbors both activating and T790M mutations, and osimertinib is effective against the T790M mutation. Gray areas indicate isotype control, and black lines indicate the non-treatment cells. Left, representative of three independent experiments. Right, data (means ± SE) are representative of three independent experiments. MFI: median fluorescence intensity; iso: isotype control. To evaluate significance, ANOVA followed by Dunnett' s Multiple Comparison test was used. *p<0.05; n.s.: not significant.

(E) qRT-PCR analyses showing expression of SEMA7A 4 hours after treatment with osimertinib (OSM; 15 nM) or erlotinib (ERL; 500 nM). Data (means ± SE) are representative of three independent experiments. The two-sample t test was used to evaluate significance. **p<0.01; n.s.: not significant.

(F) Representative immunoblots showing no downregulation of p-EGFR, p-S6k, or p-Erk in H292 and H441 cells 24 hours after treatment with erlotinib (ERL; 10 nM).

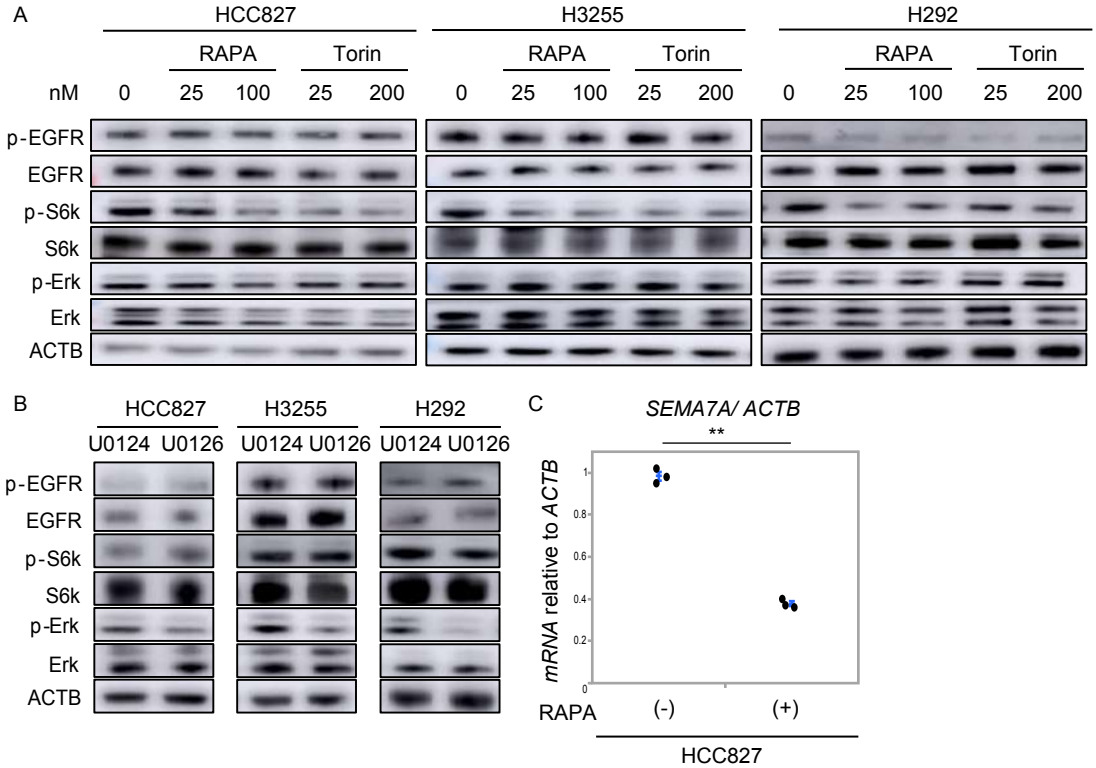


Supplemental Figure 2. SEMA7A expression in various human lung cancer cell lines.

(A, B) SEMA7A expression in human lung cancer cell lines, tested by FCM. Gray areas indicate isotype control, and black lines indicate the parental cells. FCM data are representative of three independent experiments. Data in dot plots (means ± SE) are representative of three independent experiments. MFI: median fluorescence intensity; iso: isotype control.

(C) Correlation between SEMA7A expression and EGFR mutation status in human lung cancer cell lines. The two-sample t test was used to evaluate significance.

(D) Representative immunoblots showing upregulation of p-EGFR, p-S6k, and p-Erk in H1792 cells 1 hour after treatment with recombinant human EGF (rhEGF; 10 nM).



Supplemental Figure 3. mTOR inhibitors decrease p-S6k expression, and MEK inhibitors decrease p-Erk expression, in lung adenocarcinoma cells.

(A) Representative immunoblots showing downregulation of p-S6k in lung adenocarcinoma cells 1 hour after treatment with rapamycin (RAPA) or Torin at the indicated concentrations.

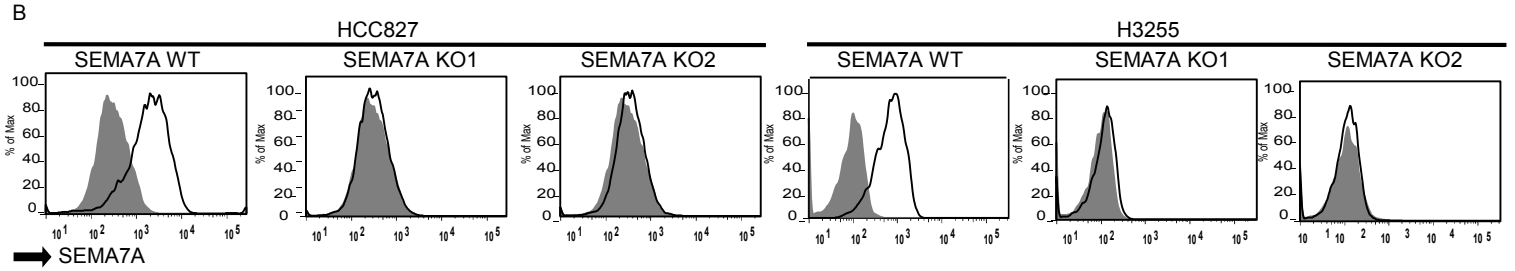
(B) Representative immunoblots showing downregulation of p-Erk in lung adenocarcinoma cells 24 hours after treatment with U0126 (2 μM).

(C) qRT-PCR analyses showing expression of SEMA7A 4 hours after treatment with rapamycin (RAPA; 100 nM). Data (means ± SE) are representative of three independent experiments. The two-sample t test was used to evaluate significance. **p<0.01.

A

Target primer in exon 1	Sequence
Forward	5’ - CACCGGCAGCTCCTCTGTGTGGGT-3’
Reverse	5’ - AAACACCCACACAGAGGAGCTGCC-3’
Target primer in exon 4	Sequence
Forward	5’ -CACCGTCCCTCGGTTCCGCCGCATC-3’
Reverse	5’ - AAACGATGCGGCGGAACCGAGGGAC-3’

B



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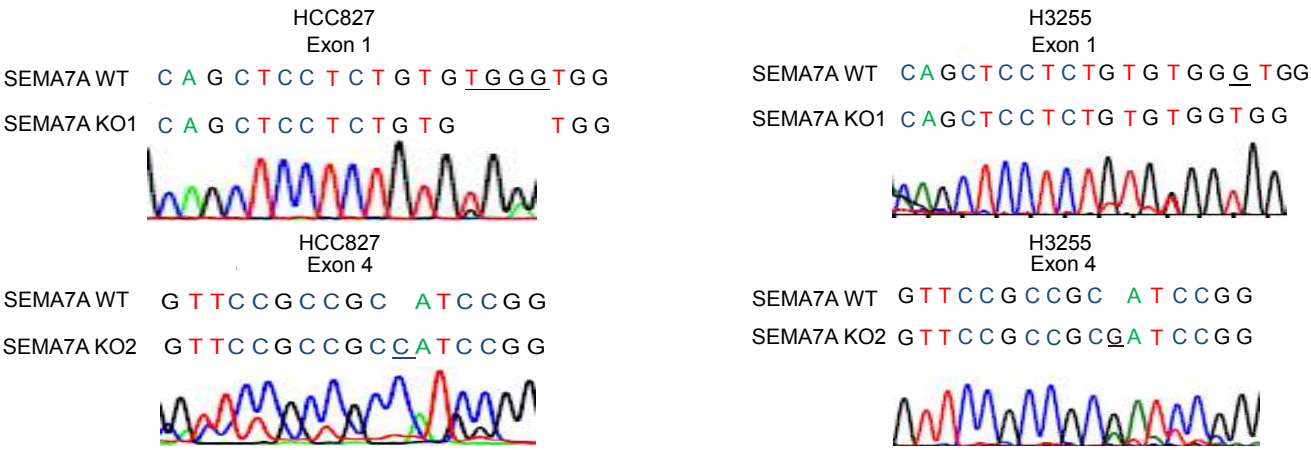


D

Primers spanning exon 1	Sequence
Forward	5’ - GTTCCAGTGTGCCATGTGGAG - 3’
Reverse	5’ - GTCTCATATCCAGAGCTGAGGCC- 3’
Primers spanning exon 4	Sequence
Forward	5’ -ACAGGTGAATGGCACTGTGGTG-3’
Reverse	5’ - CTGCTATACCTTGTCTGTTCCTCTG-3’

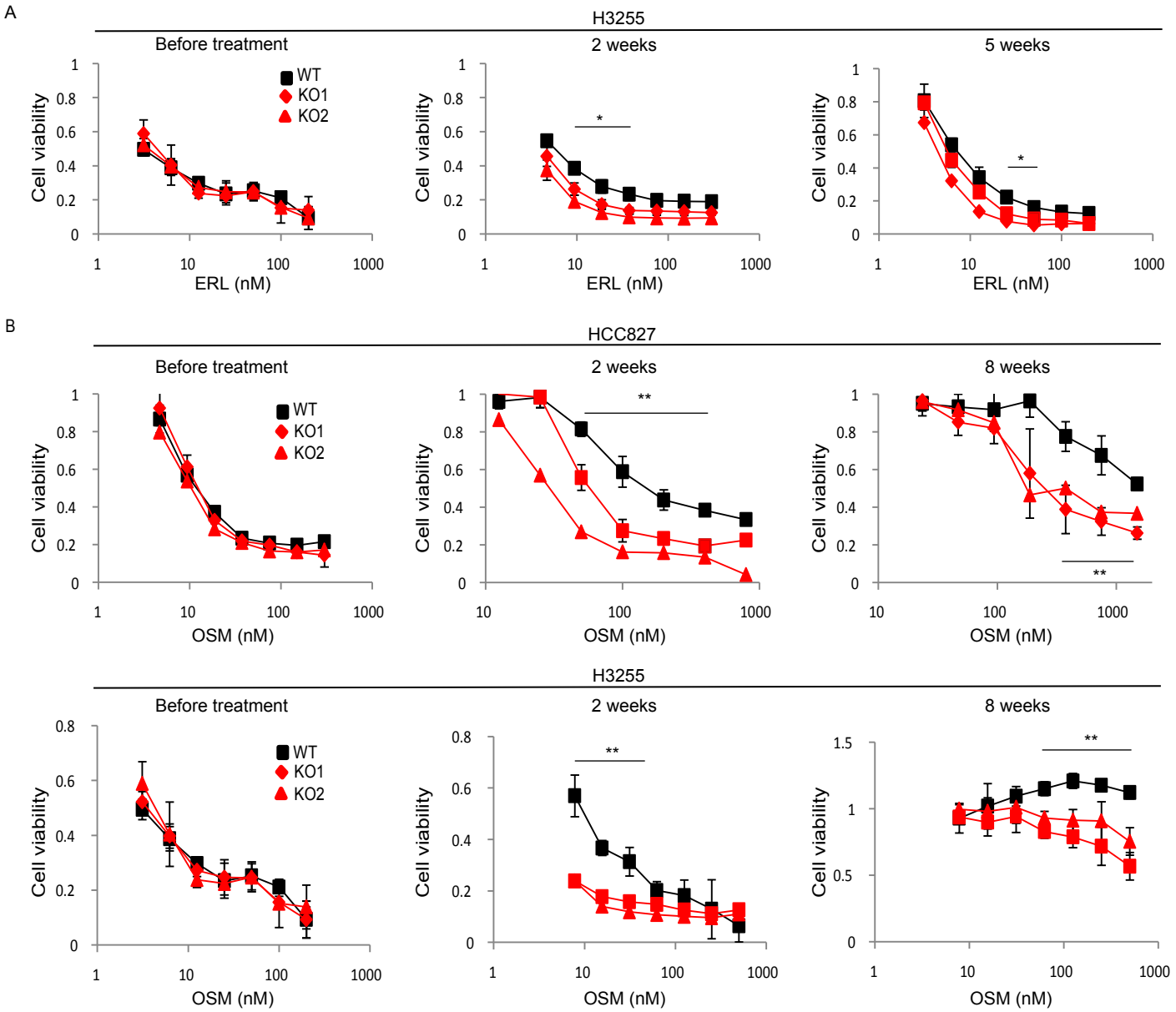
Supplemental Figure 4. SEMA7A knockout cells generated by CRISPR/Cas9.

(A) Target sequences for human SEMA7A in the CRISPR/Cas9 system.

(B) SEMA7A expression in SEMA7A WT or KO HCC827 or H3255 cells, tested by FCM. Data are representative of three independent experiments. Gray areas indicate isotype control.

(C) CRISPR/Cas9 target regions in exons 1 and 4 of SEMA7A. SEMA7A KO cells have a 1- or 4-nucleotide deletion or a 1-nucleotide insertion leading to a frameshift.

(D) Primers used for sequencing.

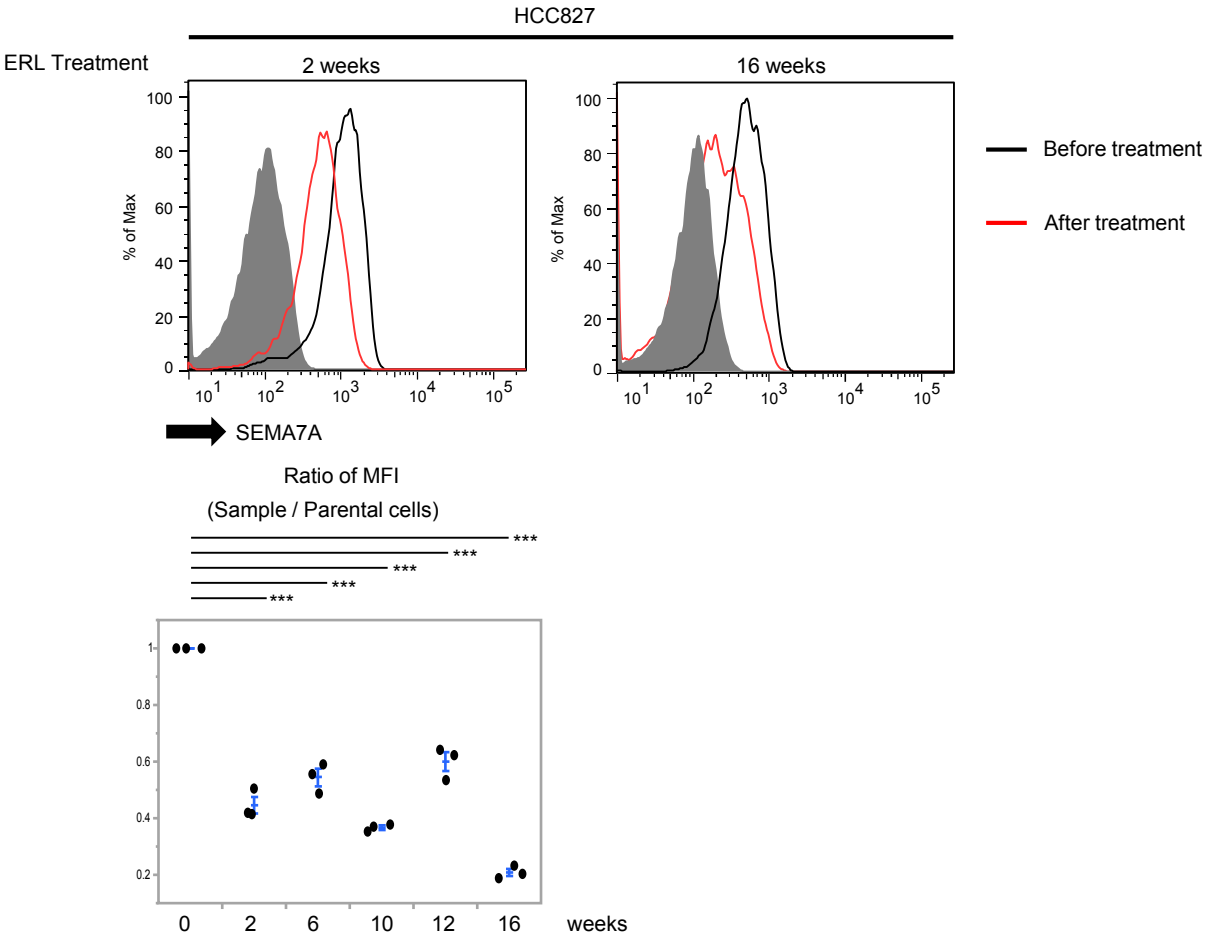


Supplemental Figure 5. SEMA7A expressing lung adenocarcinoma cells become resistant to EGFR-TKI.

(A) SEMA7A WT and KO H3255 cells were treated with low-dose erlotinib for 0, 2, or 5 weeks. Treated cells were passaged and cultured on 96-well plates for 24 hours, exposed to erlotinib for 72 hours at the indicated concentrations, and then subjected to the modified MTT assay.

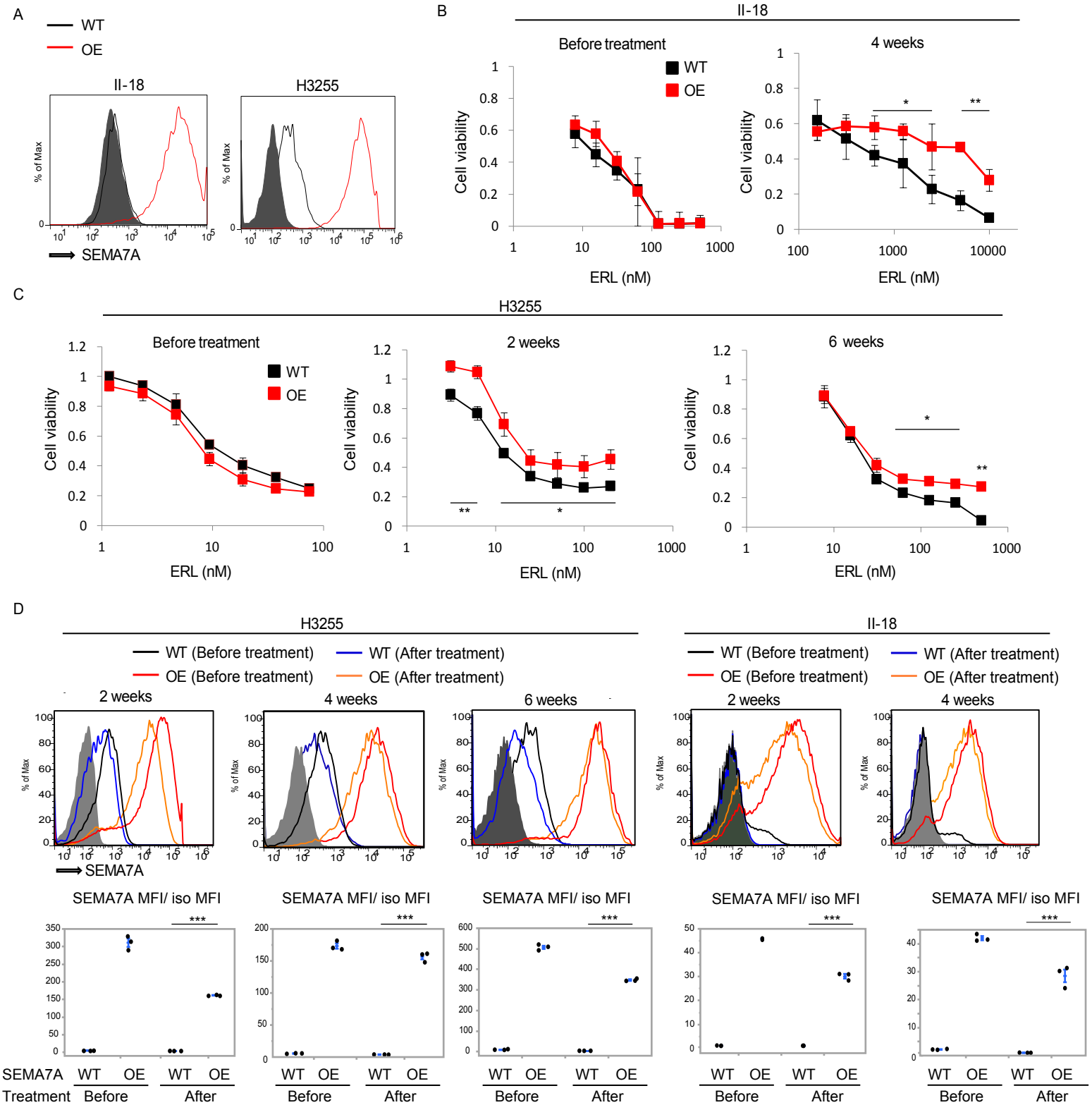
(B) SEMA7A WT and KO HCC827 or H3255 cells were treated with low-dose osimertinib for 0, 2, or 8 weeks. Treated cells were passaged and cultured on 96-well plates for 24 hours, exposed to osimertinib for 72 hours at the indicated concentrations, and then subjected to the modified MTT assay.

Data (means ± SE) are representative of three independent experiments. To evaluate significance, ANOVA followed by Dunnett' s Multiple Comparison test was used. *p<0.05, **p<0.01; n.s.: not significant.



Supplemental Figure 6. SEMA7A expression is partially downregulated by long-term exposure to EGFR-TKI.

Reduced expression of SEMA7A in Mut-EGFR lung cancer cell lines after long-term exposure to EGFR-TKI. Gray areas indicate isotype control. FCM data are representative of three independent experiments. Data in dot plots (means ± SE) are representative of three independent experiments. MFI: median fluorescence intensity. To evaluate significance, ANOVA followed by Dunnett' s Multiple Comparison test was used. ***p<0.001.



Supplemental Figure 7. SEMA7A-overexpressing lung adenocarcinoma cell lines become resistant to EGFR-TKI.

(A) SEMA7A expression in SEMA7A WT (empty vector) or OE II-18 and H3255, as determined by FCM. Data are representative of three independent experiments. Gray areas indicate isotype control.

(B) II-18 WT or OE cells after treatment with low-dose erlotinib for 0 or 4 weeks were passaged and cultured on 96-well plates for 24 hours, exposed to erlotinib for 72 hours, and then subjected to the modified MTT assay.

(C) H3255 WT or OE cells after treatment with low-dose erlotinib for 0, 2, or 6 weeks were passaged and cultured on 96-well plates for 24 hours, exposed to erlotinib for 72 hours, and then subjected to the modified MTT assay.

Data in dot plots (means ± SE) are representative of three independent experiments. The two-sample t test was used to evaluate significance. *p<0.05, **p<0.01, ***p<0.001.

(D) SEMA7A expression in SEMA7A WT (empty vector) or OE II-18 and H3255 after long-term exposure to erlotinib, as determined by FCM. Data are representative of three independent experiments. Gray areas indicate isotype control. Data in dot plots (means ± SE) are representative of three independent experiments. The two-sample t test was used to evaluate significance. ***p<0.001.

HCC827 ; Figure 4A Erlotinib (nM)

	0 week	5 weeks	10 weeks	15 weeks
WT	2.26	53.6	84.6	>4000
KO1	2.21	21.3	27.2	500
KO2	4.03	27.6	17.1	296

HCC827 ; Figure 6D Erlotinib (nM)

	6 weeks
WT+U0124	56.3
WT+U0126	14.2
KO2+U0124	28.3
KO2+U0126	14.1

H3255 ; Figure S5A Erlotinib (nM)

	0 week	2 weeks	5 weeks
WT	3.12	6.66	7.16
KO1	3.53	3.43	5.59
KO2	4.36	2.17	4.39

HCC827 ; Figure S5B Osimertinib (nM)

	0 week	2 weeks	8 weeks
WT	14.8	150	>1500
KO1	12.3	57.5	250
KO2	10.4	29.3	375

H3255 ; Figure S5B Osimertinib (nM)

	0 week	8 weeks
WT	5.1	9.2
KO1	3.2	3.4
KO2	4.0	4.2

Il-18 ; Figure S7B Erlotinib (nM)

	0 week	4 weeks
WT	11.7	347
OE	21.3	1930

H3255 ; Figure S7C Erlotinib (nM)

	0 week	2 weeks	6 weeks
WT	11.6	12.3	215
OE	8.24	21.3	238

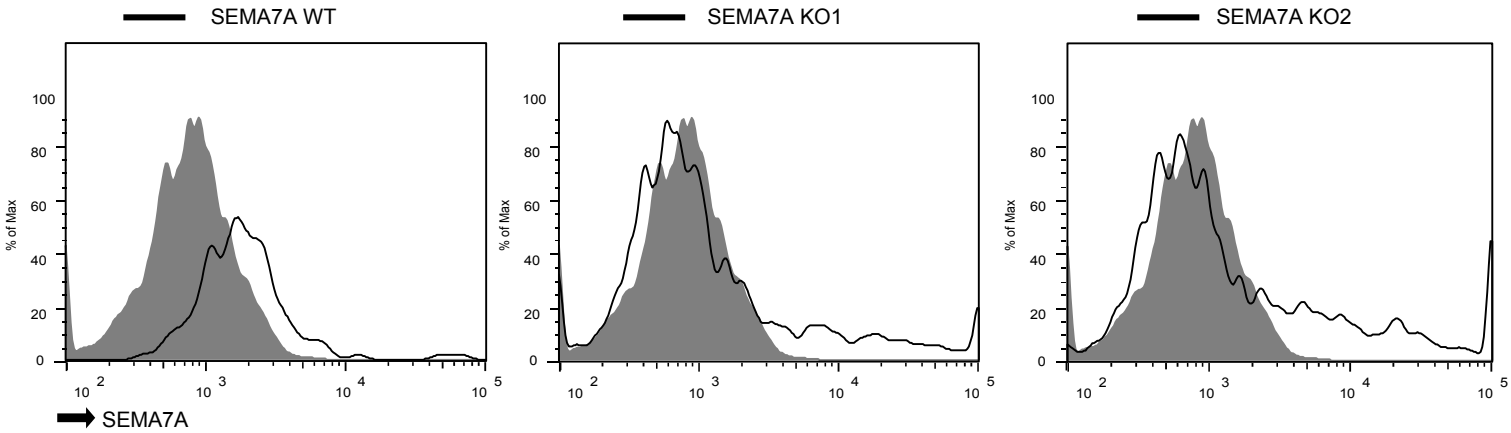
Supplemental Figure 8. IC50 of each EGFR-mutant cell line after treatment with EGFR-TKI.

‘WT’ indicates cell lines with empty vector.

‘KO’ indicates SEMA7A knockout cell lines.

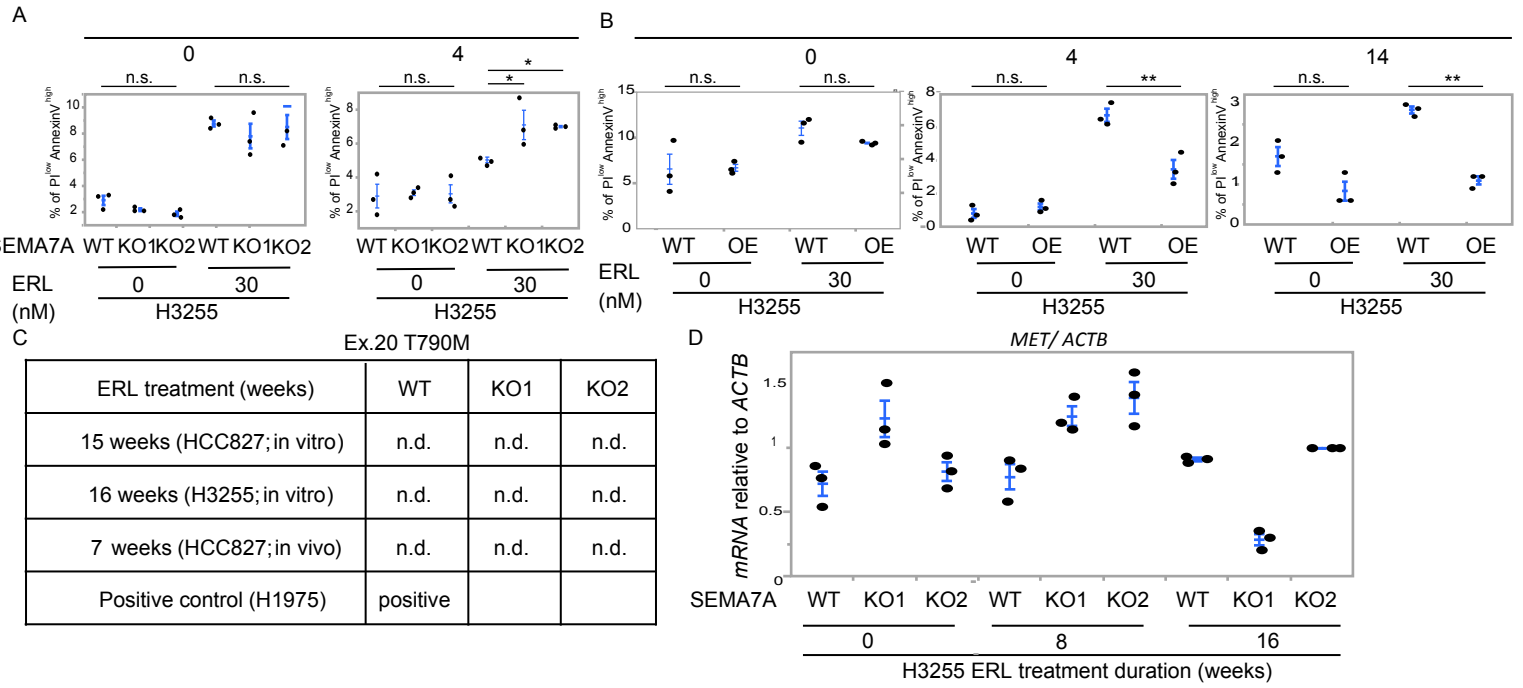
‘OE’ indicates SEMA7A-overexpressing cell lines.

‘Week’ indicates exposure time to EGFR-TKI.



Supplemental Figure 9. Expression of SEMA7A in xenografts.

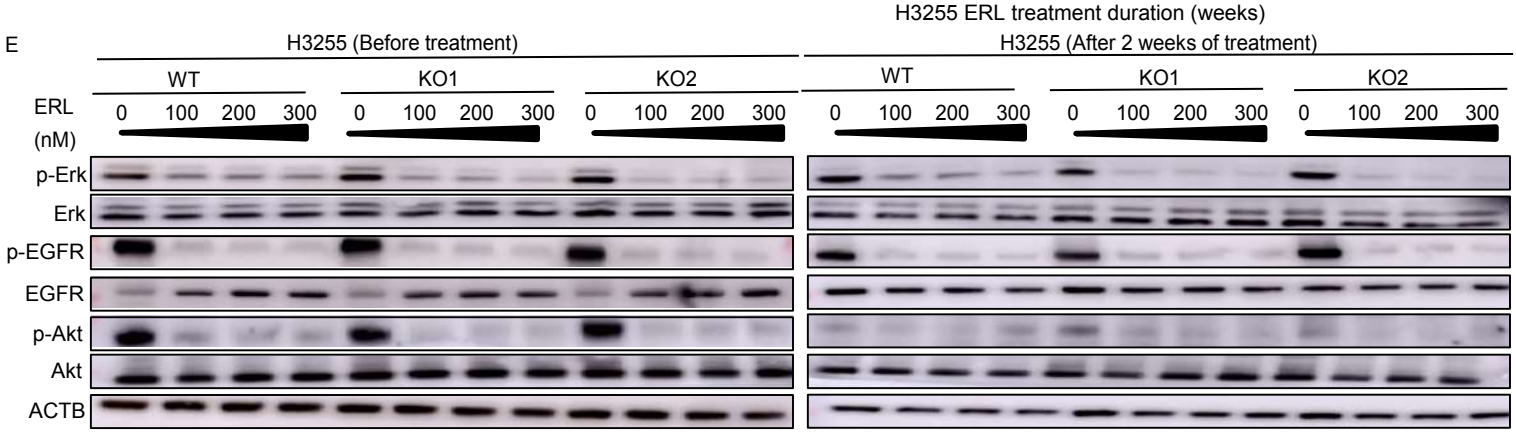
FCM evaluating SEMA7A expression of a SEMA7A WT or KO HCC827 xenograft after 3 weeks of treatment with erlotinib. Gray areas indicate isotype control.



C

Ex.20 T790M

ERL treatment (weeks)	WT	KO1	KO2
15 weeks (HCC827; in vitro)	n.d.	n.d.	n.d.
16 weeks (H3255; in vitro)	n.d.	n.d.	n.d.
7 weeks (HCC827; in vivo)	n.d.	n.d.	n.d.
Positive control (H1975)	positive		



Supplemental Figure 10. SEMA7A inhibits apoptosis induced by erlotinib and maintains Erk phosphorylation in SEMA7A WT cells.

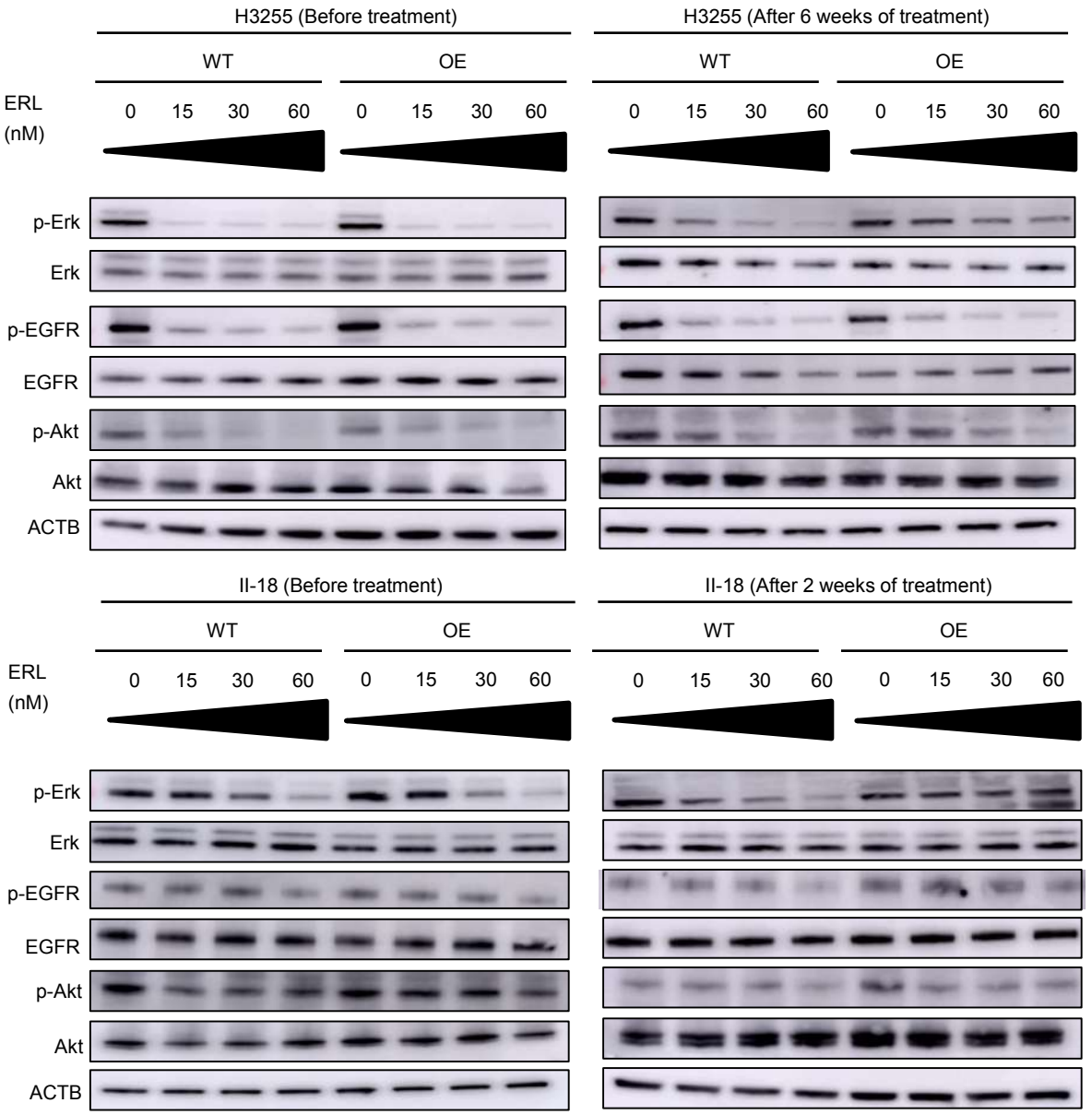
(A) SEMA7A WT and KO H3255 cells were exposed to erlotinib at the indicated concentrations for 48 hours, before and after treatment with low-dose erlotinib for 4 weeks. Graphs indicate the proportion of PI^{low} and Annexin V^{high} cell populations, expressed as percentages. Data (means ± SE) are representative of three independent experiments. To evaluate significance, ANOVA followed by Dunnett' s Multiple Comparison test was used. *p<0.05. n.s.: not significant.

(B) SEMA7A WT and OE H3255 cells were exposed to erlotinib at the indicated concentrations for 48 hours, before and after treatment with low-dose erlotinib for 4 and 14 weeks. Graphs indicate the proportion of PI^{low} and Annexin V^{high} cell populations, expressed as percentages. Data (means ± SE) are representative of three independent experiments. The two-sample t test was used to detect significance. **p<0.01. n.s.: not significant.

(C) Detection of the secondary mutation T790M by the PNA-LNA PCR-clamp method. The mutation was not detected either in vitro or in vivo after long-term erlotinib treatment. H1975 was used as a positive control. n.d.: not detected.

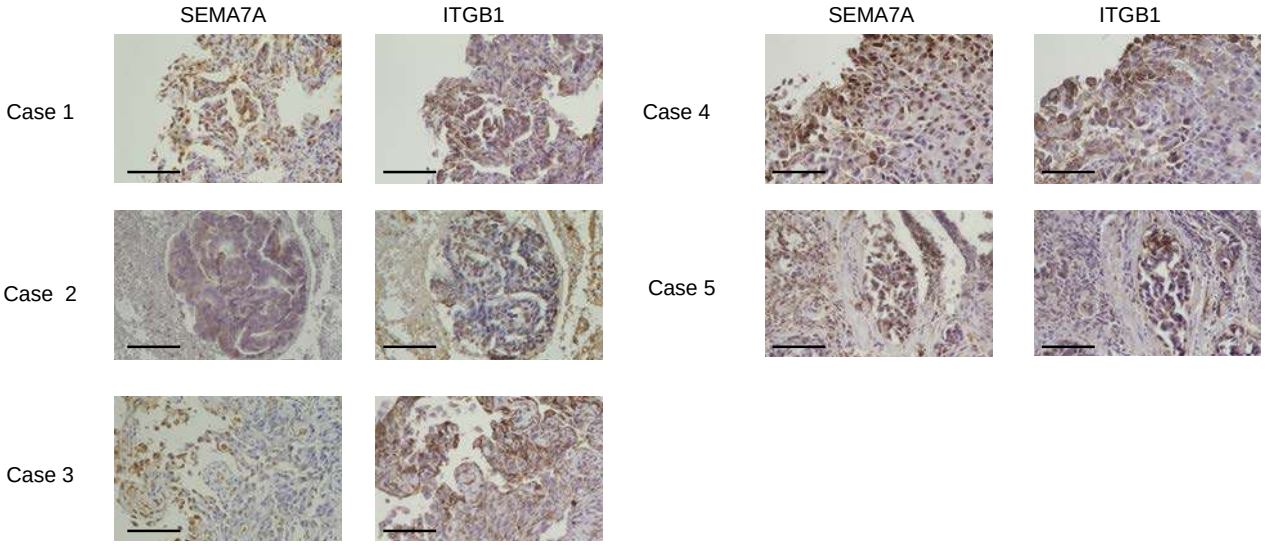
(D) Expression of *MET* in SEMA7A WT and KO H3255 cells was measured by qRT-PCR after exposure to low-dose erlotinib. Data (means ± SE) are representative of three independent experiments.

(E) Representative immunoblots showing p-EGFR, p-Akt, and p-Erk in H3255 cells 24 hours after treatment with erlotinib. After long-term treatment, p-Erk level was maintained in SEMA7A WT cells despite a reduction in the levels of p-EGFR and p-Akt by erlotinib.

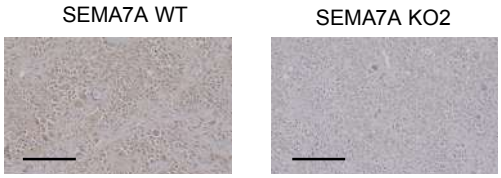


Supplemental Figure 11. SEMA7A promotes persistent ERK activation in response to long-term EGFR-TKI treatment.

Representative immunoblots showing p-EGFR, p-Akt, and p-Erk in H3255 and Il-18 cells 24 hours after treatment with erlotinib. After long-term treatment, p-Erk level was maintained in SEMA7A OE cells, despite a reduction in the levels of p-EGFR and p-Akt.



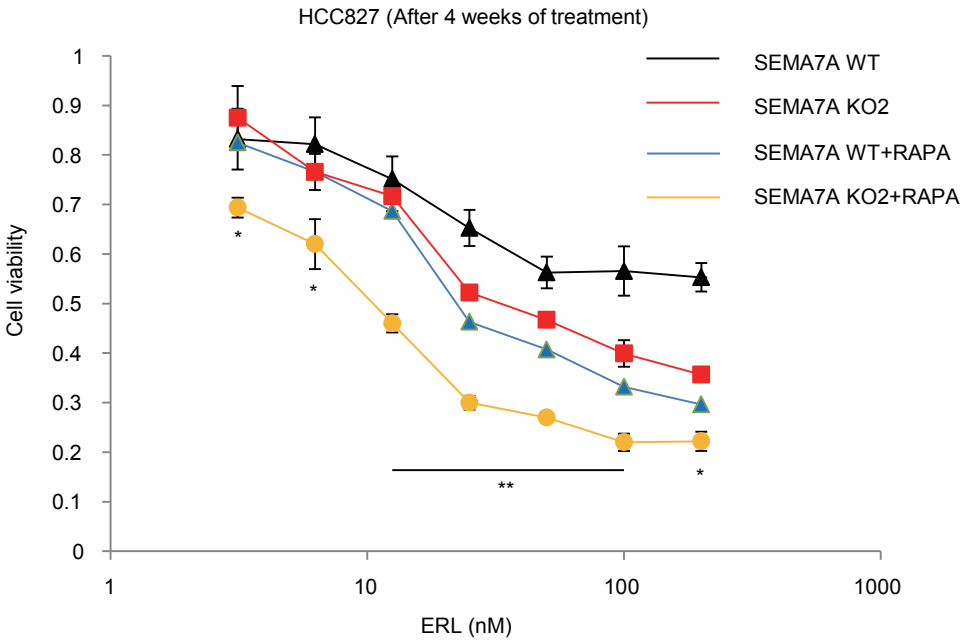
Supplemental Figure 12. Expression of SEMA7A and ITGB1 in clinical samples of EGFR mutant lung adenocarcinoma.
Representative SEMA7A and ITGB1 IHC of human lung adenocarcinoma samples. Scale bar, 50 μm.



Supplemental Figure 13. Expression of SEMA7A in xenografts.

Representative SEMA7A IHC of WT or KO HCC827 cell xenografts. Scale bar, 50 μm.

All data are representative of three independent biological experiments.



Supplemental Figure 14. Combination therapy with EGFR-TKI and an mTOR inhibitor resulted in higher sensitivity to EGFR-TKI in SEMA7A WT or KO cells than therapy with EGFR-TKI alone.

SEMA7A WT and KO HCC827 cells were treated with low-dose erlotinib for 4 weeks. Treated cells were passaged and cultured on 96-well plates for 24 hours, and exposed to erlotinib with or without rapamycin (RAPA) for 72 hours at the indicated concentrations, and then subjected to the modified MTT assay. Data (means ± SE) are representative of three independent experiments. To evaluate significance, ANOVA followed by Tukey−Kramer Multiple Comparison test was used. *p<0.05, **p<0.01.