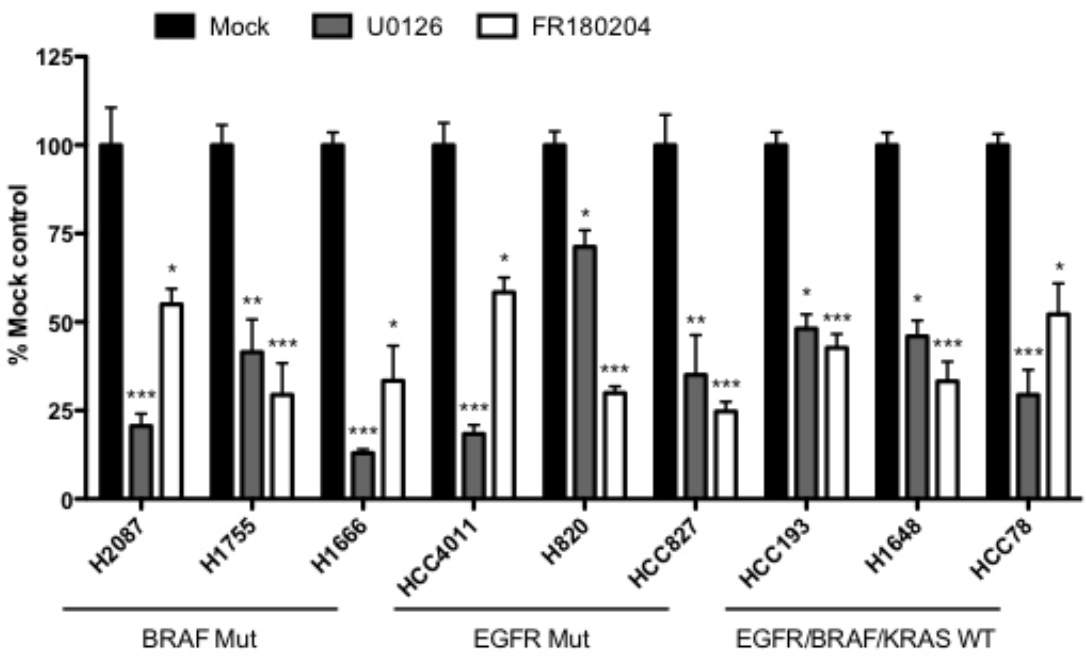
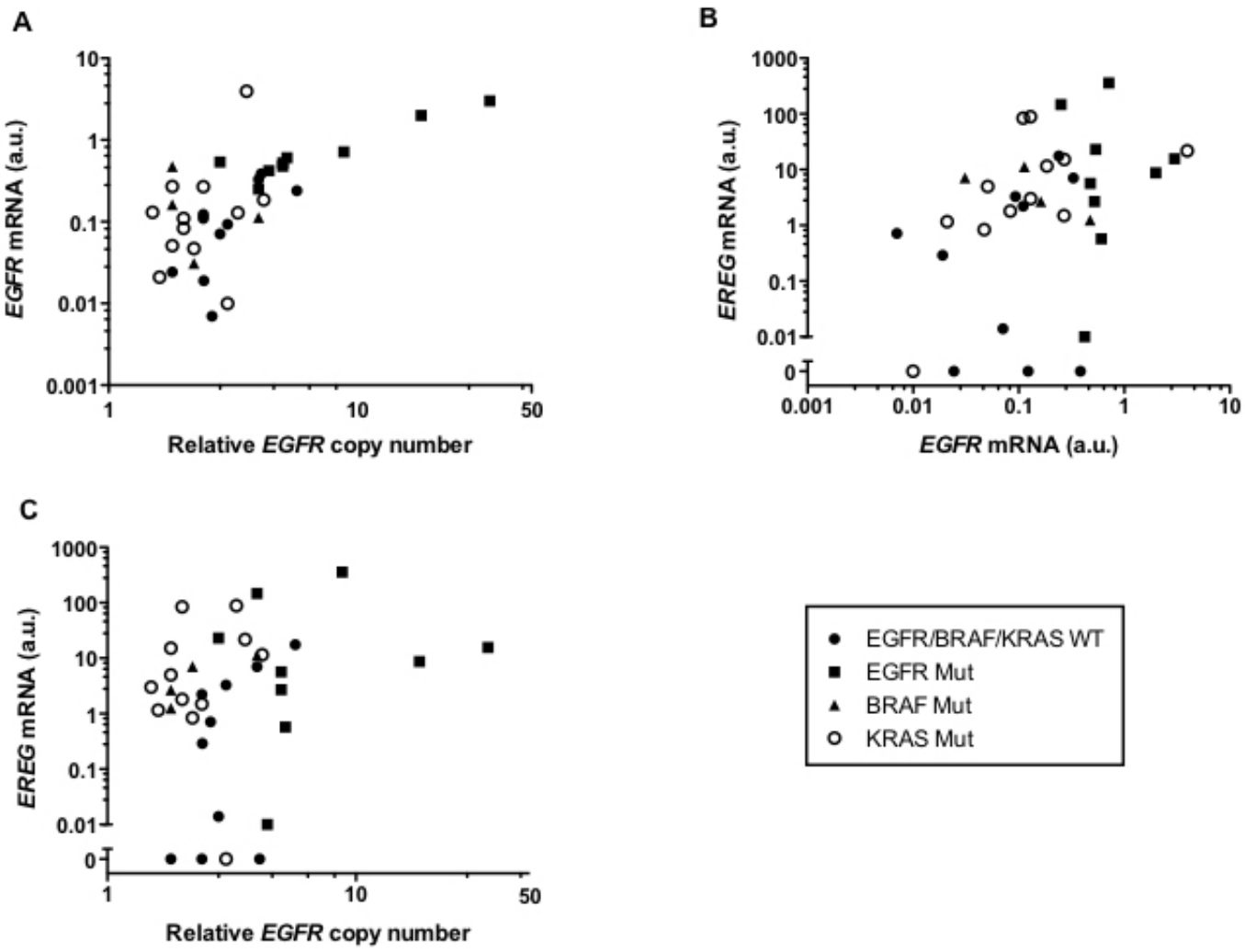


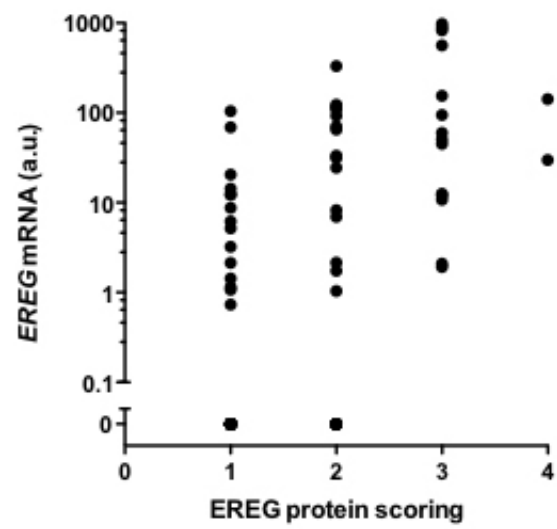
Supplementary Fig. 1



Supplementary Fig. 2

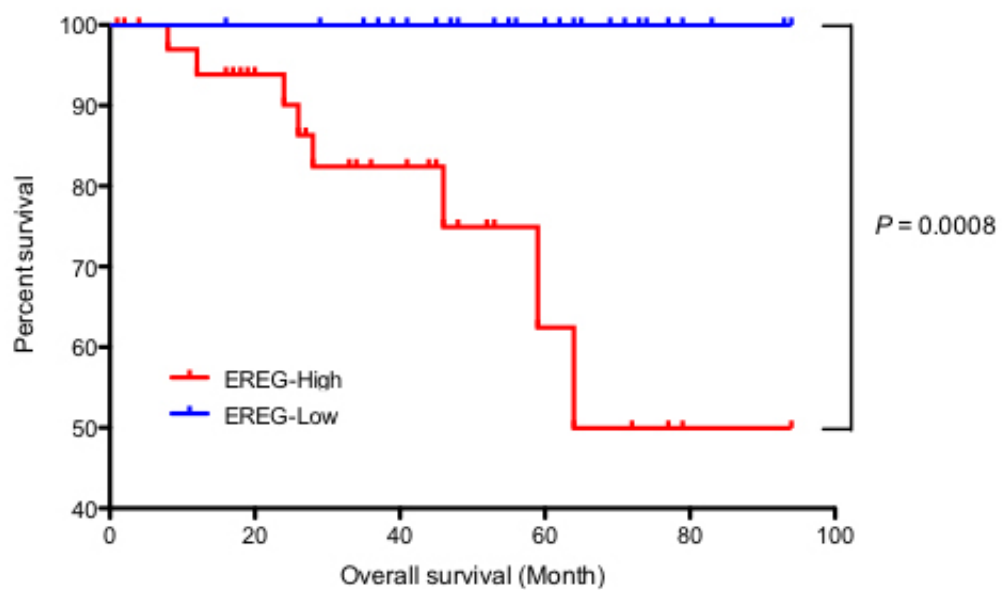


Supplementary Fig 3



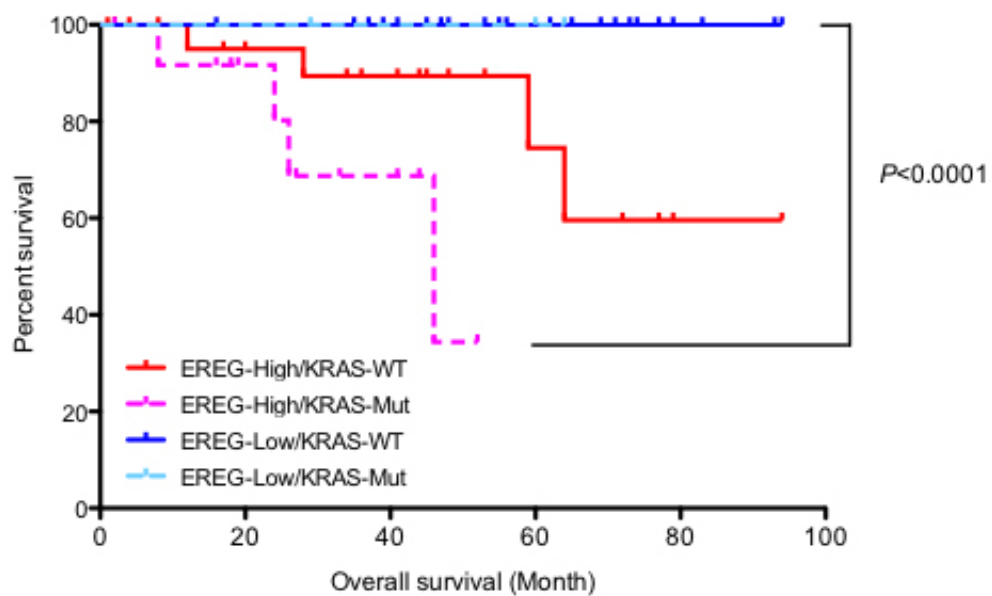
Supplementary Fig 4

A

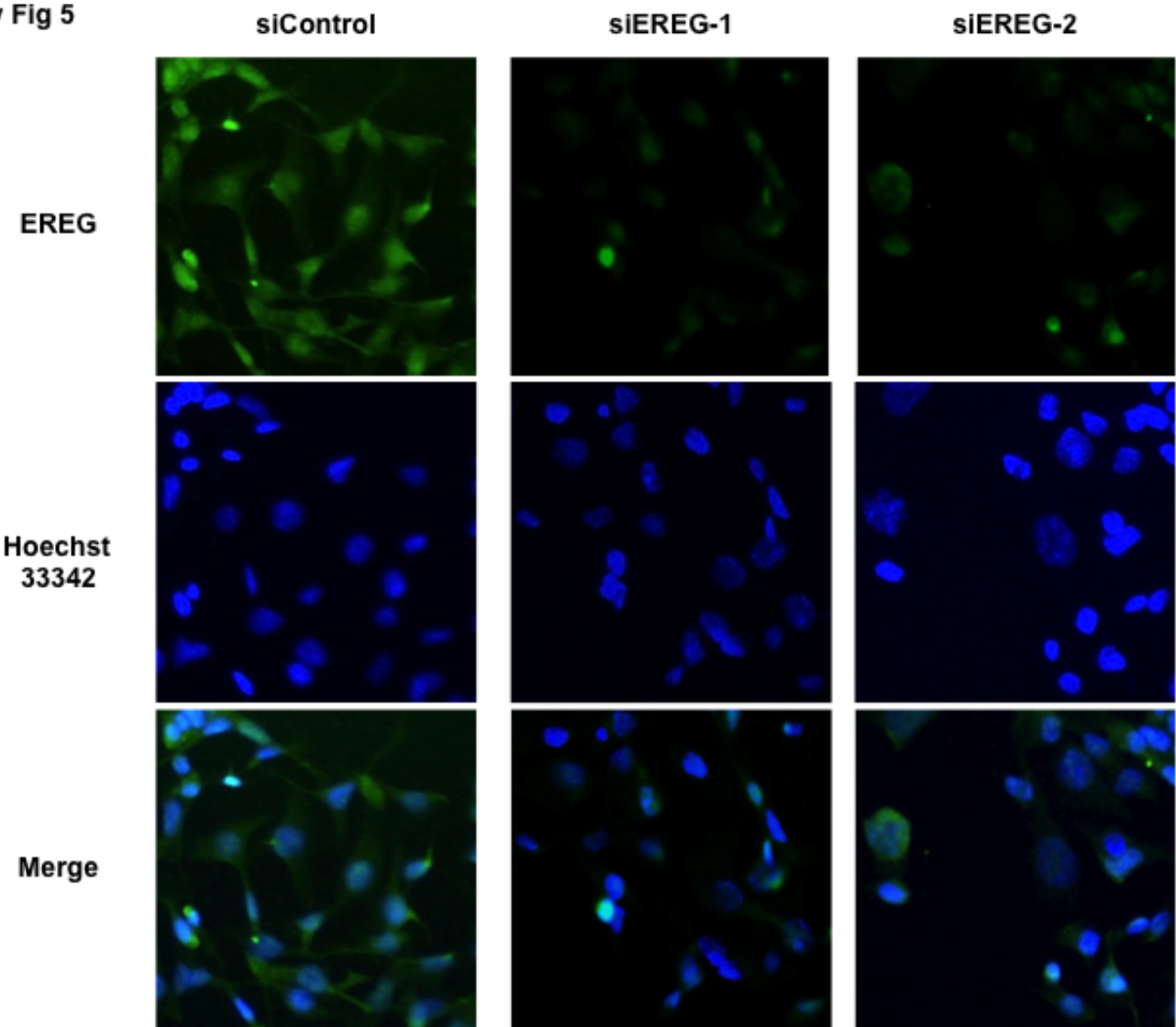


Supplementary Fig 4

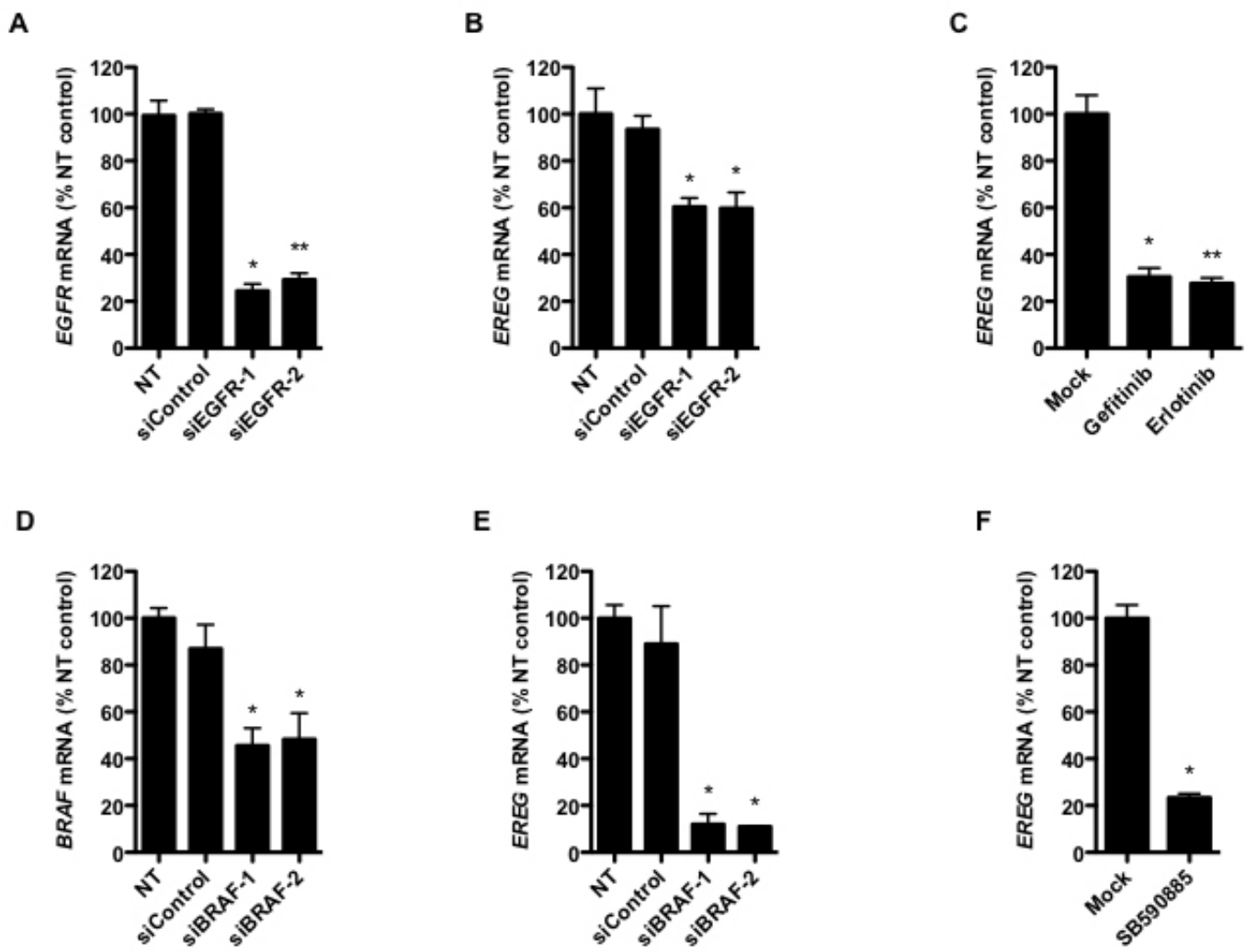
B



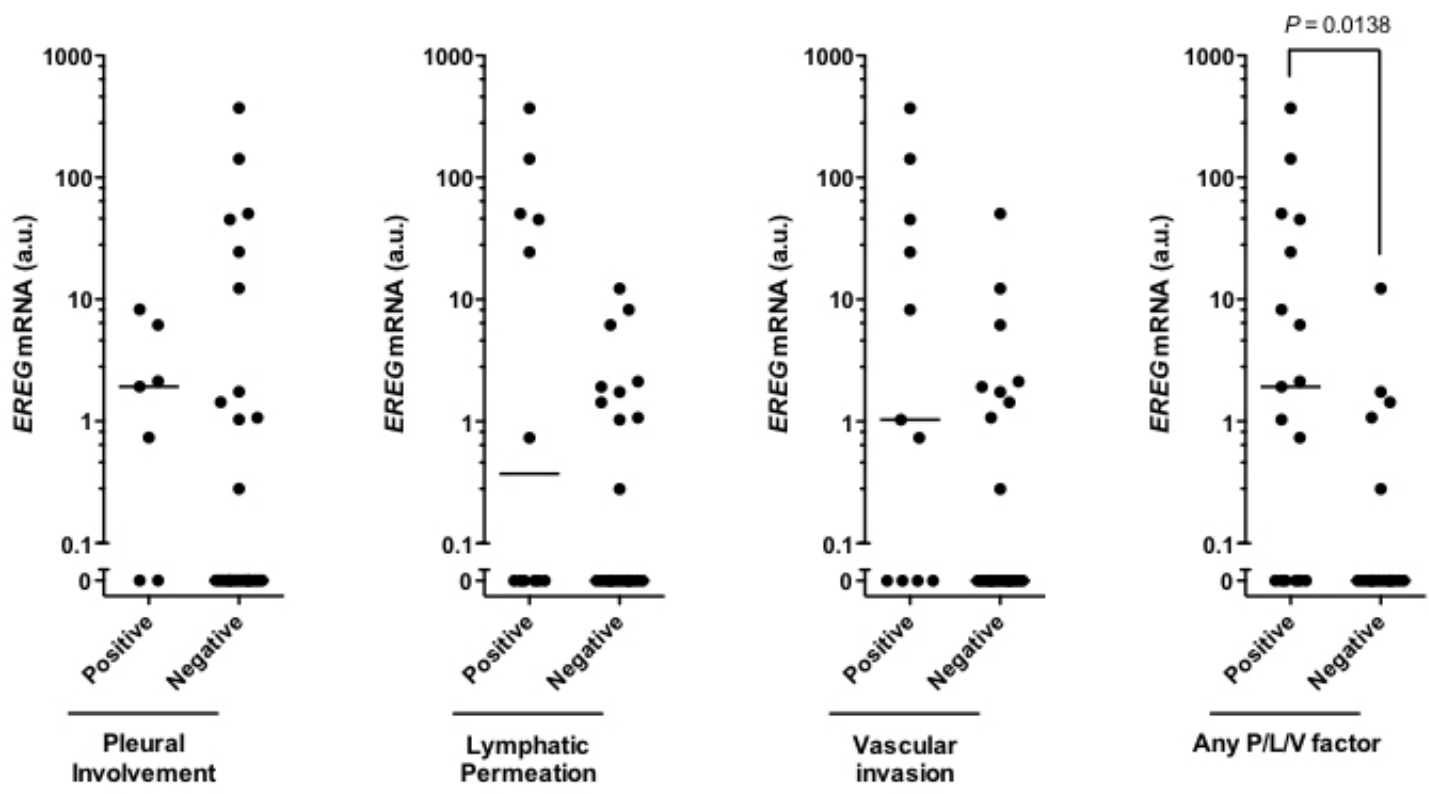
Supplementary Fig 5



Supplementary Fig 6



Supplementary Fig 7



Supplementary figure legends

Supplementary Fig. 1. The effects of U0126 and FR180204 on *EREG* expression in *EREG*-overexpressing NSCLC cells with wild-type *KRAS* (*BRAF* mutants, *EGFR* mutants, and NSCLCs with wild-type *EGFR/BRAF/KRAS*) as evaluated by the same methods in Fig. 2. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for comparison with mock treatment (DMSO alone) by the Kruskal-Wallis test with Dunn's Multiple Comparison.

Supplementary Fig. 2. (A) Correlations between *EGFR* expression and *EGFR* copy number (Pearson $r = 0.6450$, $P < 0.0001$ in all NSCLCs; Pearson $r = 0.9841$, $P < 0.0001$ in *EGFR* mutants; Pearson $r = 0.7448$, $P = 0.0135$ in NSCLCs with wild-type *EGFR/BRAF/KRAS*). (B) Correlations between *EREG* expression and *EGFR* expression. (C) Correlations between *EREG* expression and *EGFR* copy number (Pearson $r = 0.8455$, $P = 0.0021$ in NSCLCs with wild-type *EGFR/BRAF/KRAS*).

Supplementary Fig. 3. A significant correlation was observed between *EREG* mRNA expression and *EREG* protein expression scoring in lung adenocarcinomas (Spearman $r = 0.5265$, $P < 0.0001$).

Supplementary Fig. 4. Kaplan-Meier analysis of overall survival (month) in lung adenocarcinoma patients who had not received EGFR-TKI therapy. The patients were classified (A) according to the *EREG* expression levels or (B) according to *EREG* expression levels and *KRAS* mutational status.

Supplementary Fig. 5. siRNA-mediated *EREG* silencing in H358 cells as evaluated by Immunofluorescent staining. After 72 h of siRNA transfection, the cells were stained with the anti-*EREG* antibody (green) and Hoechst 33342 (blue) and analyzed by fluorescent microscopy. siControl: treatment with *Tax* siRNA. si*EREG*-1 and si*EREG*-2: treatment with *EREG* siRNAs.

Supplementary Fig. 6. (A) siRNA-mediated *EGFR* knockdown in HCC827 NSCLC cells harboring *EGFR* mutations (E746-A750 deletion). NT: treatment with medium alone; siControl: treatment with *Tax* siRNA; si*EGFR*-1 and si*EGFR*-2: two siRNAs targeting different sites of *EGFR* mRNA. * $P < 0.001$; ** $P < 0.05$ for comparison with NT. **(B)** siRNA-mediated *EGFR* knockdown reduces *EREG* mRNA expression in HCC827 cells. * $P < 0.001$ for comparison of NT. **(C)** Treatment with gefitinib or erlotinib down-regulates *EREG* expression in HCC827 cells. After treatment with gefitinib (1 μM) or erlotinib (1 μM) for 24 h, the cells were harvested for subsequent quantitative RT-PCR analysis. * $P < 0.01$; ** $P < 0.001$ for comparison with mock treatment (DMSO alone). **(D)** siRNA-mediated *BRAF* knockdown in H1666 NSCLC cells harboring *BRAF* mutations (G466V point mutation). NT: treatment with medium alone; siControl: treatment with *Tax* siRNA; si*BRAF*-1 and si*BRAF*-2: two siRNAs targeting different sites of the *BRAF* mRNA. * $P < 0.001$ for comparison with NT. **(E)** siRNA-mediated *BRAF* knockdown reduces *EREG* mRNA expression in H1666 cells. * $P < 0.001$ for comparison with NT. **(F)** The *BRAF* inhibitor SB590885

down-regulates *EREG* expression in H1666 cells. After treatment with SB590885 (10 μ M) for 24 h, the cells were harvested for subsequent quantitative RT-PCR analysis. * P <0.001 for comparison with mock treatment (DMSO alone). In Fig. 2A-G, the columns represent the mean \pm SD (*bars*) in eight determinations from two independent experiments, and NT was set at 100%. All experiments were performed by quantitative RT-PCR analysis. The differences between two groups were analyzed by the Mann-Whitney test, and the differences between more than two groups were analyzed by the Kruskal-Wallis test with Dunn's multiple comparison.

Supplementary Fig. 7. Comparisons of *EREG* mRNA expression levels between tumors with or without pleural involvement (P), between tumors with or without lymphatic permeation (L), between tumors with or without vascular invasion (V), and between tumors with any P/L/V factor-positive or without such characteristics. The differences between groups were analyzed by the Mann-Whitney test.

Supplementary Table 1. Characteristics of tumor specimens of non-small cell lung cancer.

Parameter		No.	(%)
Gender	Male	45	(51)
	Female	44	(49)
Age	≤70	44	(49)
	>70	45	(51)
Smoking history	Smoker	48	(54)
	Non-smoker	41	(46)
Stage	I	57	(64)
	II	11	(12)
	III	20	(23)
	IV	1	(1)
Pathology	Adenocarcinoma	77	(87)
	Squamous cell carcinoma	12	(13)
Pleural involvement	+	33	(37)
	-	56	(63)
Lymphatic permeation	+	40	(45)
	-	49	(55)
Vascular invasion	+	37	(42)
	-	52	(58)
<i>KRAS</i> gene	Wild-type	72	(81)
	Mutation	17	(19)
<i>EGFR</i> gene	Wild-type	55	(62)
	Mutation	34	(38)

Supplementary Table 2. Cell lines used in the present study.

Cell line	Histological type	Mutation
NHBE	NHBEC	<i>KRAS/BRAF/EGFR</i> Wild-type
SAEC	NHBEC	<i>KRAS/BRAF/EGFR</i> Wild-type
BEAS-2B	NHBEC	<i>KRAS/BRAF/EGFR</i> Wild-type
HBEC3	NHBEC	<i>KRAS/BRAF/EGFR</i> Wild-type
HBEC4	NHBEC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H157	NSCLC	<i>KRAS</i> Mutation
NCI-H358	NSCLC	<i>KRAS</i> Mutation
NCI-H441	NSCLC	<i>KRAS</i> Mutation
NCI-H460	NSCLC	<i>KRAS</i> Mutation
NCI-H1264	NSCLC	<i>KRAS</i> Mutation
NCI-H1792	NSCLC	<i>KRAS</i> Mutation
NCI-H2009	NSCLC	<i>KRAS</i> Mutation
NCI-H2122	NSCLC	<i>KRAS</i> Mutation
NCI-H2126	NSCLC	<i>KRAS</i> Mutation
HCC44	NSCLC	<i>KRAS</i> Mutation
HCC515	NSCLC	<i>KRAS</i> Mutation
HCC4017	NSCLC	<i>KRAS</i> Mutation
NCI-H1395	NSCLC	<i>BRAF</i> Mutation
NCI-H1666	NSCLC	<i>BRAF</i> Mutation
NCI-H1755	NSCLC	<i>BRAF</i> Mutation
NCI-H2087	NSCLC	<i>BRAF</i> Mutation
NCI-H820	NSCLC	<i>EGFR</i> Mutation
NCI-H3255	NSCLC	<i>EGFR</i> Mutation
NCI-H1975	NSCLC	<i>EGFR</i> Mutation
HCC827	NSCLC	<i>EGFR</i> Mutation
HCC2279	NSCLC	<i>EGFR</i> Mutation
HCC2935	NSCLC	<i>EGFR</i> Mutation
HCC4006	NSCLC	<i>EGFR</i> Mutation
HCC4011	NSCLC	<i>EGFR</i> Mutation
PC9	NSCLC	<i>EGFR</i> Mutation
NCI-H661	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H838	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H1299	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H1437	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H1648	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
NCI-H1819	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
HCC15	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
HCC78	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
HCC95	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type
HCC193	NSCLC	<i>KRAS/BRAF/EGFR</i> Wild-type

NHBEC: Noncancerous human bronchial epithelial cell line

NSCLC: Non-small cell lung cancer

Supplementary Table 3. Univariate and multivariate analysis in patients with non-small cell lung cancer.

Prognostic marker	Hazard ratio	95% CI	P value
Univariate analysis			
Age (>70 vs ≤70)	1.392	0.504 – 3.846	0.5241
Gender (male vs female)	1.490	0.539 – 4.123	0.4419
Smoking history (smoker vs non-smoker)	1.852	0.657 – 5.221	0.2439
Pathology (adeno vs squamous)	0.678	0.190 – 2.422	0.5499
Stage (I-II vs III-IV)	1.219	0.343 – 4.329	0.7596
<i>KRAS</i> gene (mutation vs wild-type)	2.244	0.709 – 7.108	0.1693
<i>EGFR</i> gene (mutation vs wild-type)	0.616	0.210 – 1.803	0.3762
<i>EREG</i> expression (as a continuous variable)	1.002	1.000 – 1.004	0.0239
Multivariate analysis			
Pathology (adeno vs squamous)	0.629	0.165 – 2.399	0.4970
Stage (I-II vs III-IV)	1.578	0.497 – 5.012	0.4388
<i>KRAS</i> gene (mutation vs wild-type)	1.584	0.470 – 5.345	0.4583
<i>EREG</i> expression (as a continuous variable)	1.002	1.000 – 1.004	0.0346

Supplementary Table 4. Univariate and multivariate analysis in adenocarcinoma patients.

Prognostic marker	Hazard ratio	95% CI	P value
Univariate analysis			
Age (>70 vs ≤70)	1.245	0.394 – 3.938	0.7088
Gender (male vs female)	1.493	0.467 – 4.777	0.4995
Smoking history (smoker vs non-smoker)	1.948	0.617 – 6.151	0.2556
Stage (I-II vs III-IV)	1.176	0.316 – 4.372	0.8086
<i>KRAS</i> gene (mutation vs wild-type)	3.329	0.982 – 11.292	0.0536
<i>EGFR</i> gene (mutation vs wild-type)	0.632	0.200 – 1.997	0.4338
<i>EREG</i> expression (as a continuous variable)	1.002	1.000 – 1.004	0.0133
Multivariate analysis			
Stage (I-II vs III-IV)	1.912	0.439 – 8.329	0.3878
<i>KRAS</i> gene (mutation vs wild-type)	2.852	0.768 – 10.595	0.1175
<i>EREG</i> expression (as a continuous variable)	1.002	1.000 – 1.004	0.0366