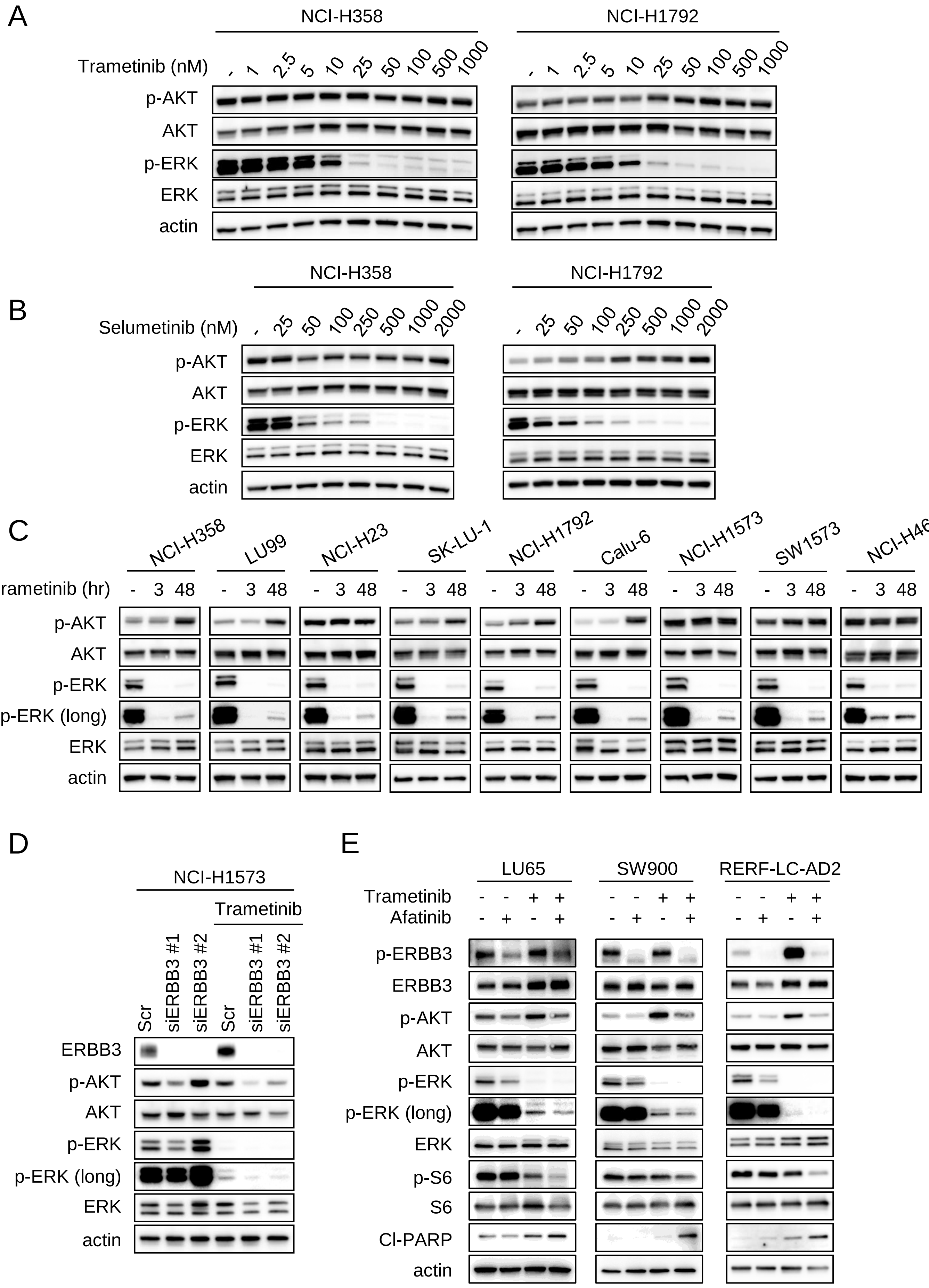
Supplementary Data

Epithelial-to-mesenchymal transition defines feedback activation of receptor tyrosine kinase signaling induced by MEK inhibition in *KRAS* mutant lung cancer

Hidenori Kitai, Hiromichi Ebi, Shuta Tomida, Konstantinos V Floros, Hiroshi Kotani,

Yuta Adachi, Satoshi Oizumi, Masaharu Nishimura, Anthony C Faber, Seiji Yano

- Supplementary Figures S1-S14
- Supplementary Tables S1-S5


A
NCI-H358
NCI-H1792
Trametinib (nM)
-
1
2.5
5
10
25
50
100
500
1000
p-AKT
AKT
p-ERK
ERK
actin
B
NCI-H358
NCI-H1792
Selumetinib (nM)
-
25
50
100
250
500
1000
2000
p-AKT
AKT
p-ERK
ERK
actin
C
NCI-H358
LU99
NCI-H23
SK-LU-1
NCI-H1792
Calu-6
NCI-H1573
SW1573
NCI-H460
Trametinib (hr)
-
3
48
p-AKT
AKT
p-ERK
p-ERK (long)
ERK
actin
D
NCI-H1573
Trametinib
Scr
siERBB3 #1
siERBB3 #2
Scr
siERBB3 #1
siERBB3 #2
ERBB3
p-AKT
AKT
p-ERK
p-ERK (long)
ERK
actin
E
LU65
SW900
RERF-LC-AD2
Trametinib
Afatinib
- - + +
- + - +
p-ERBB3
ERBB3
p-AKT
AKT
p-ERK
p-ERK (long)
ERK
p-S6
S6
Cl-PARP
actin

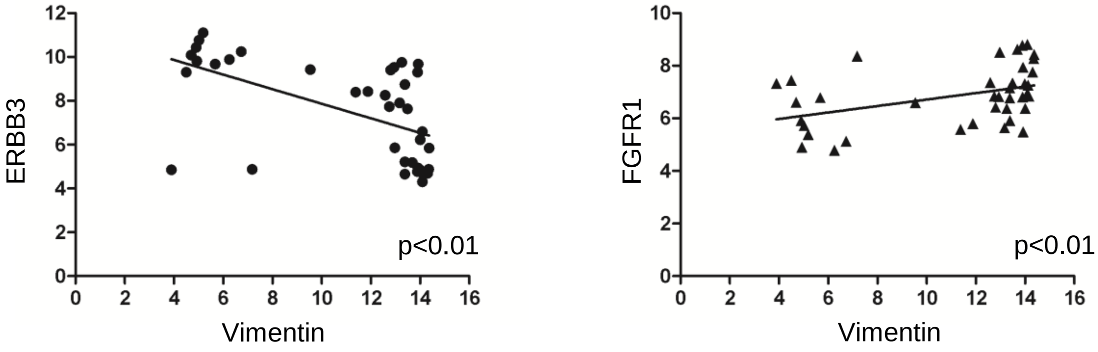
Supplementary Figure S1. Feedback activation of ERK signaling and upregulation of AKT signaling following MEK inhibitor treatment in *KRAS* mutant lung cancers.

(A, B) Trametinib (A) and Selumetinib (B) inhibit ERK phosphorylation in a dose-dependent manner. NCI-H358 and NCI-H1792 *KRAS* mutant cells were treated with the indicated drugs for 3 hours and probed with the indicated antibodies.

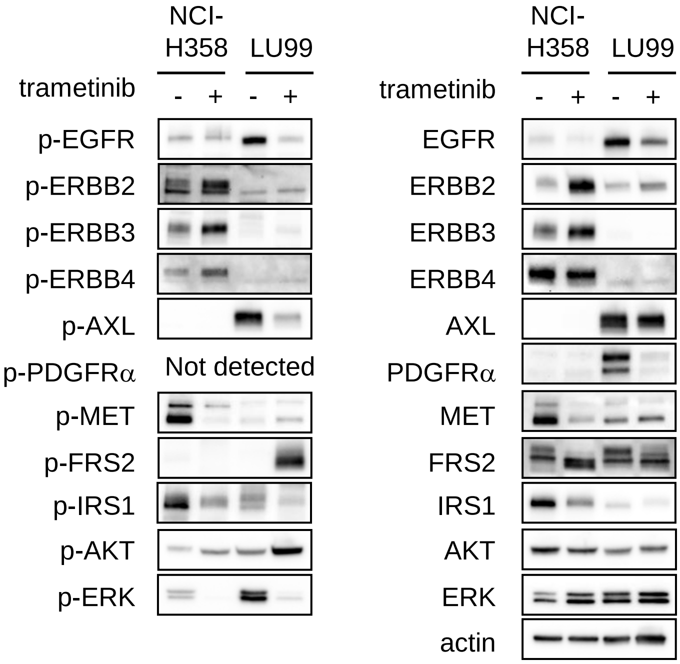
(C) *KRAS* mutant lung cancer cell lines were treated with 50 nM trametinib for 3 or 48 hours, and lysates were probed with the indicated antibodies.

(D) NCI-H1573 cells were transfected with two different siRNAs designed against ERBB3 or scramble siRNA and cultured for 48 hours. Afterwards, the media was replaced with or without 50 nM trametinib and cells were treated for an additional 72 hours. Lysates were probed with the indicated antibodies. All immunoblots are representative of three independent experiments.

(E) Cells were treated with 1uM of the pan-EGFR inhibitor afatinib, 50 nM trametinib, or the combination of these two drugs for 48 hours, and lysates were probed with the indicated antibodies.

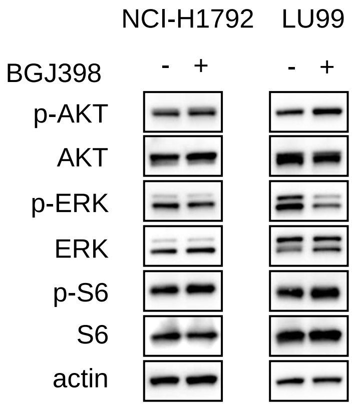


Supplementary Figure S2. Scattered plot analysis showing inverse relationship between ERBB3 and Vimentin and positive correlation between FGFR1 and Vimentin. $p < 0.01$, both by linear regression analysis. Axes are in log2.



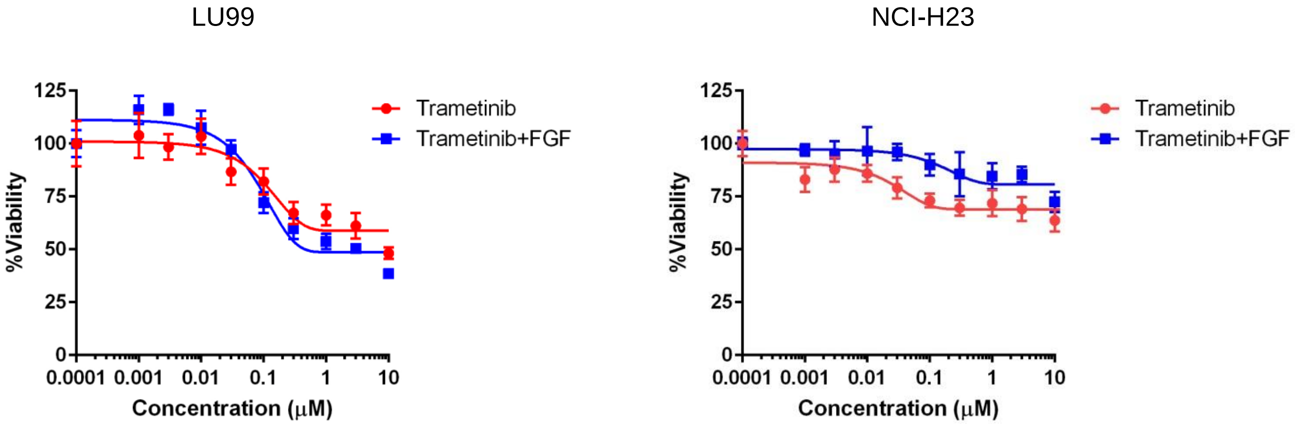
Supplementary Figure S3. MEK inhibition promotes activation of FRS2 in mesenchymal-like *KRAS* mutant lung cancer cells.

NCI-H358 epithelial-like cells and LU99 mesenchymal-like cells were treated with 50 nM trametinib for 72 hours. Cell lysates were immunoblotted with indicated antibodies.



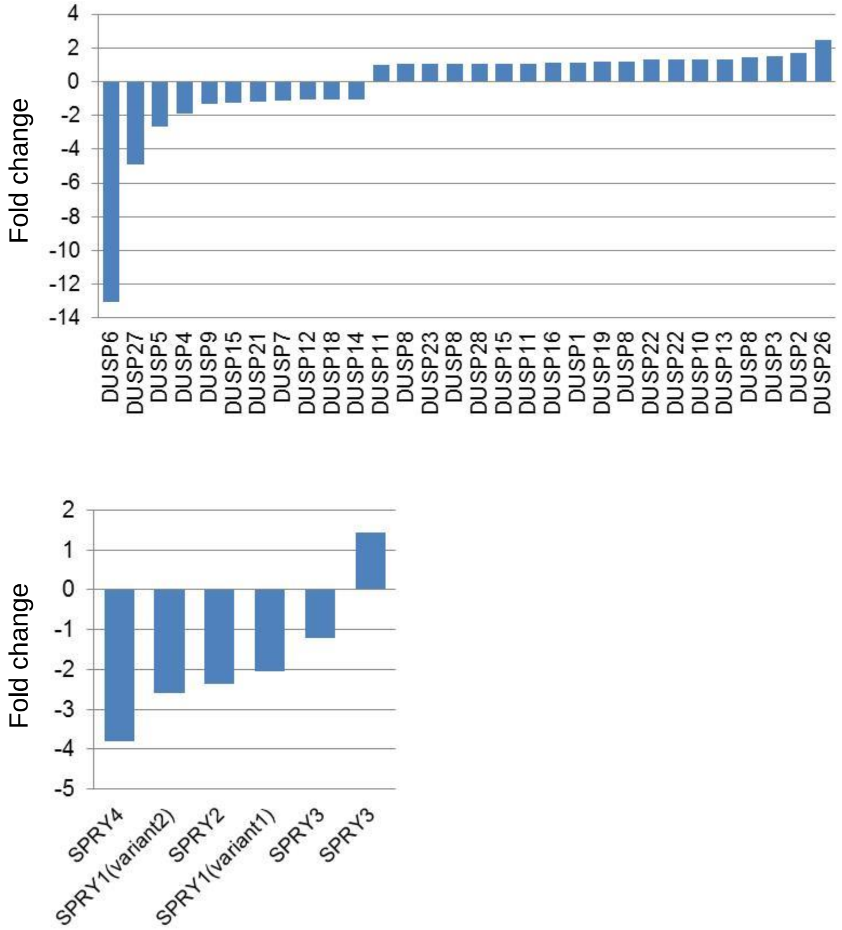
Supplementary Figure S4. FGFR inhibition has minimal effect on downstream signaling in mesenchymal-like *KRAS* mutant cancer cell lines.

A pan-FGFR inhibitor, NVP-BGJ398 (1uM), was used to treat NCI-H1792 and LU99 cells for 48 hours. Lysates were probed with the indicated antibodies. Independent experiments were performed three times, and a representative result is shown.



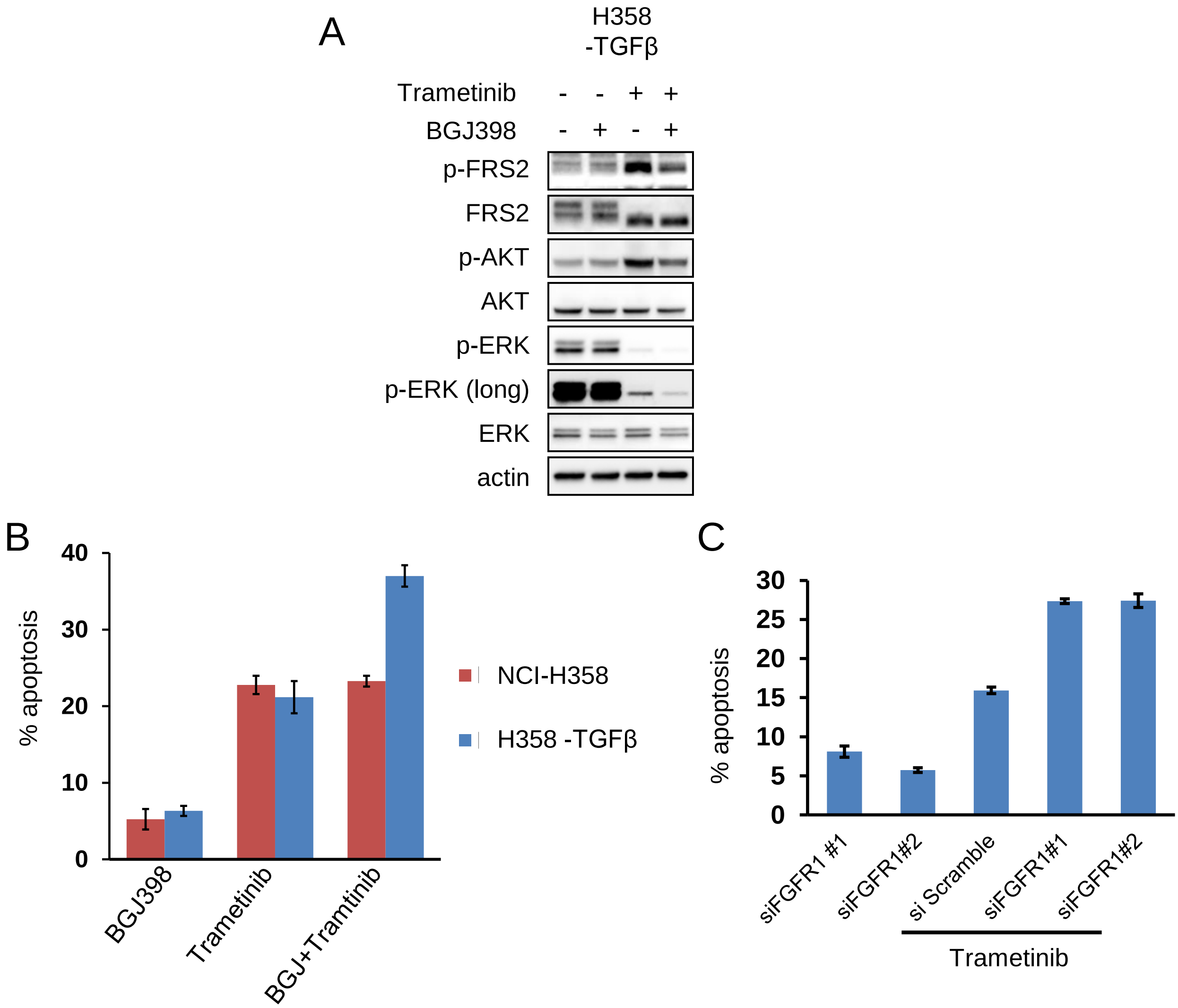
Supplementary Figure S5. FGF modestly induces trametinib resistance in mesenchymal-like *KRAS* mutant lung cancer cell lines.

LU99 and NCI-H23 cells were treated with indicated dose of trametinib in the absence or presence of 20 ng/mL of FGF2 for 72 hours. Error bars are ± SD, n = 6. Independent experiments were performed twice and a representative result is shown. Note that FGF induced modest resistance under low dose trametinib treatment was consistently observed in LU99 cells. For the viability assays sparsely seeded cells were plated in a 96-well plate and the next day trametinib and FGF2 were added. 72 hours later, Cell Counting kit (Dojindo) was added per well and luminescence was recorded by iMark Microplate Reader (Bio-Rad).



Supplementary Figure S6. Results of microarray analysis showing strong suppression of DUSP6 and SPRY4 mRNA levels following trametinib treatment.

NCI-H1792 cells were treated with trametinib for 48 hours. mRNA was extracted and analyzed as described in materials and methods section. Data were derived from average of two independents.

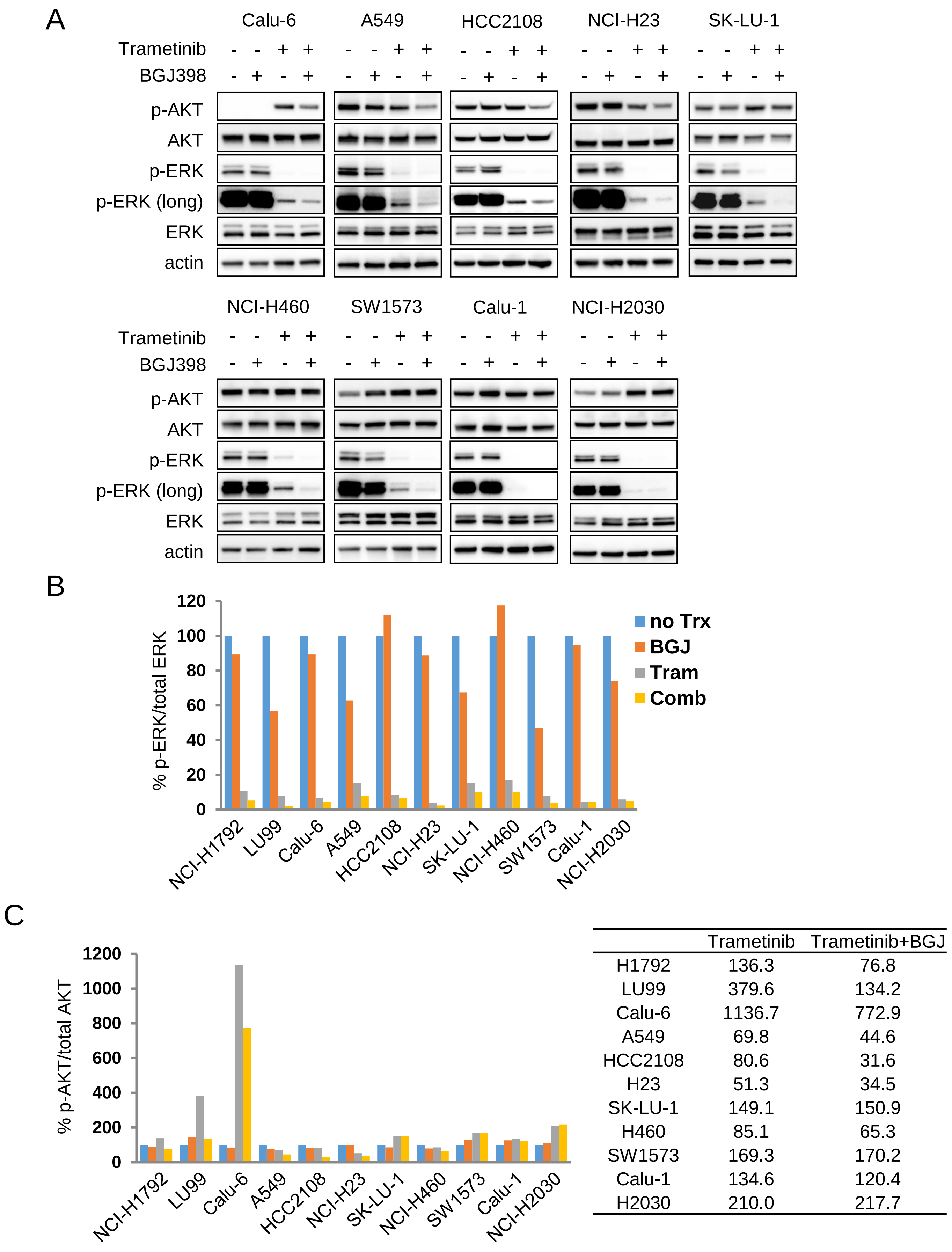


Supplementary Figure S7. FGFR1-FRS2 pathway is involved in feedback activation of MAPK signaling in an EMT induced NCI-H358 epithelial-like *KRAS* mutant cancer cells.

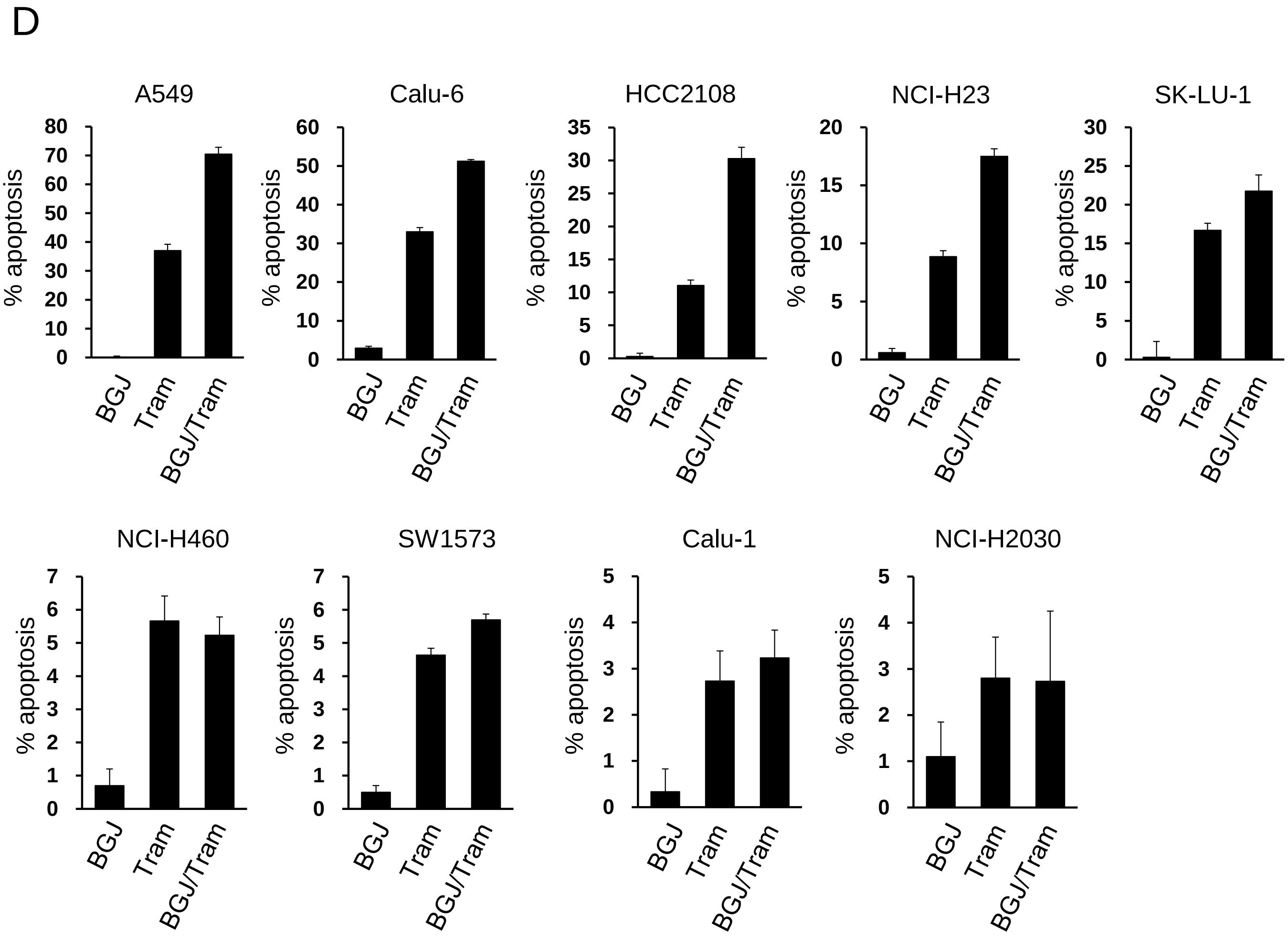
(A) NCI-H358 cells were treated with TGFβ1 (4 ng/mL) for 14 days in order to induce EMT. Then EMT-induced cells (H358-TGFβ) were treated with 1uM pan-FGFR inhibitor NVP-BGJ398, 50 nM trametinib, or the combination of these two drugs for 48 hours, and lysates were probed with the indicated antibodies. Independent experiments were performed three times, and a representative result is shown.

(B) NCI-H358 or H358-TGFβ were treated with 1uM pan-FGFR inhibitor NVP-BGJ398, 50 nM trametinib, or the combination of these two drugs for 72 hours and analyzed by FACS to quantify annexin V positive cells. The average ± SD of 3 independent experiments is shown.

(C) H358-TGFβ cells were transfected with two different siRNAs designed against FGFR1 or scramble siRNA and cultured for 48 hours. Afterwards, media was replaced with or without 50 nM trametinib and cells were treated for additional 48 hours. The percentage of cells undergoing apoptosis, as measured by annexin V positivity by FACS analysis, is shown relative to scramble siRNA transfected cells. The average ± SD of 3 independent experiments is shown.



	Trametinib	Trametinib+BGJ
H1792	136.3	76.8
LU99	379.6	134.2
Calu-6	1136.7	772.9
A549	69.8	44.6
HCC2108	80.6	31.6
H23	51.3	34.5
SK-LU-1	149.1	150.9
H460	85.1	65.3
SW1573	169.3	170.2
Calu-1	134.6	120.4
H2030	210.0	217.7

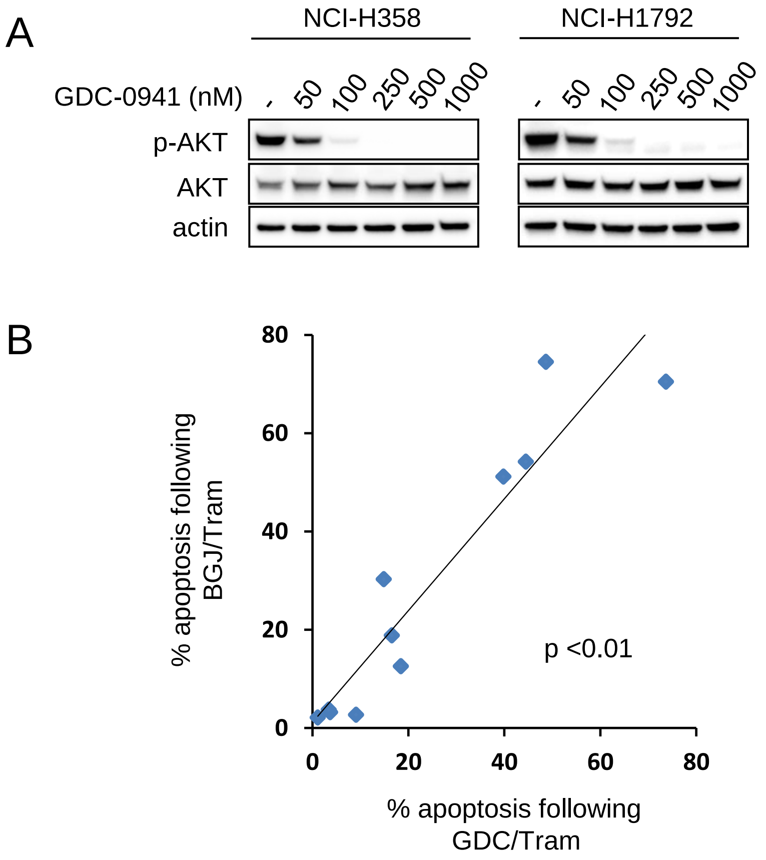


Supplementary Figure S8. Combination of MEK inhibition with FGFR inhibition leads to further ERK suppression and apoptosis in mesenchymal-like *KRAS* mutant lung cancer cell lines.

(A) Cells were treated with 1uM pan-FGFR inhibitor NVP-BGJ398, 50 nM trametinib, or the combination of these two drugs for 48 hours (SK-LU-1, NCI-H460, HCC2108, SW1573, and NCI-H2030) or 72 hours (A549, NCI-H23, Calu-6, and Calu-1), and lysates were probed with the indicated antibodies. Independent experiments were performed three times, and a representative result is shown.

(B, C) The intensities of p-ERK (B) and p-AKT (C) following treatment with NVP-BGJ398, trametinib, or the combination of these drugs were quantified from each of the *KRAS* mutant cells (raw data in (A) and Figure 5A) and are presented as percentage relative to untreated cells.

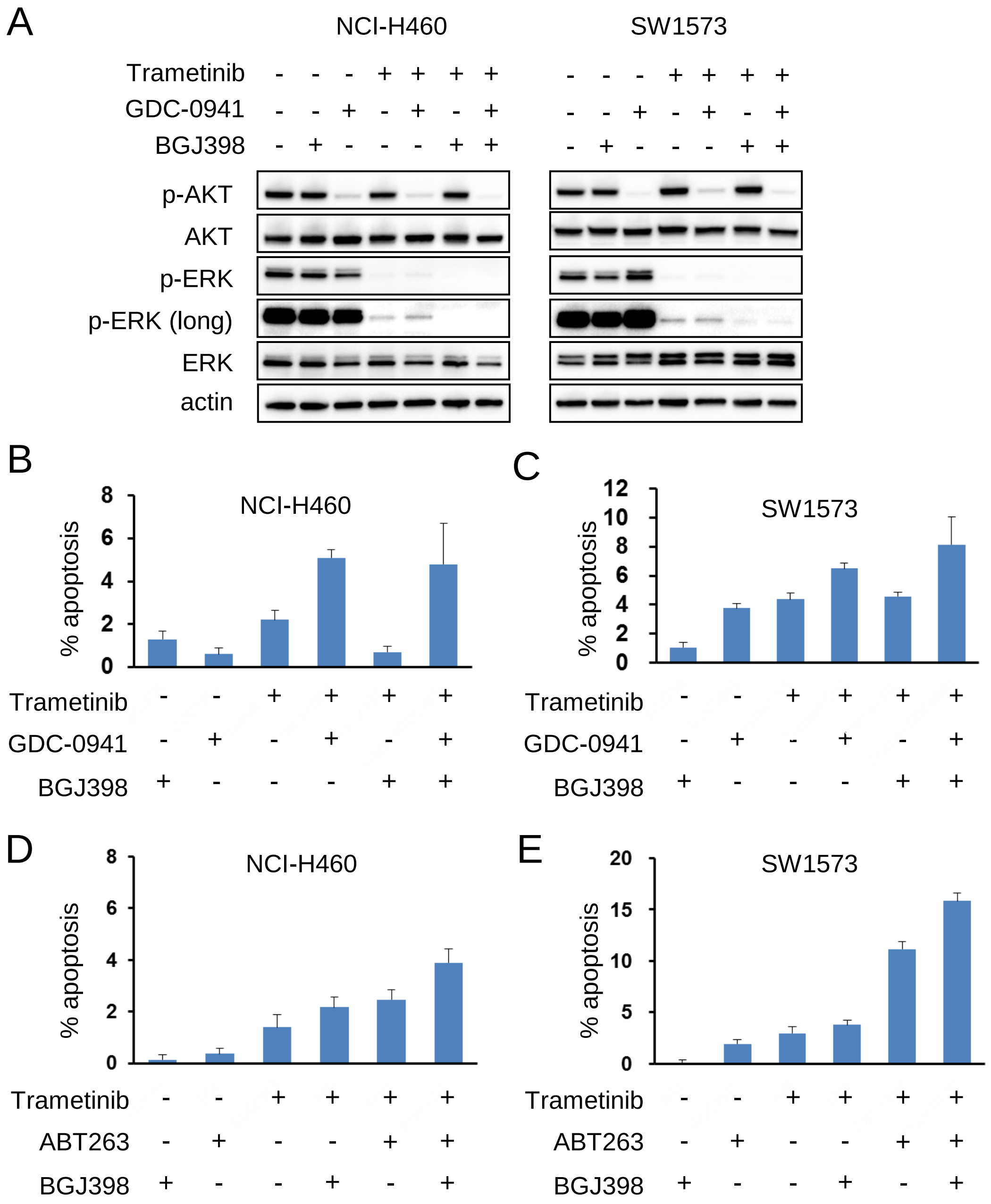
(D) Cells were treated with drug and drug combinations as in (A) for 72 hours and analyzed by FACS to quantify annexin V positive cells. The average ± SD of 3 independent experiments is shown.



Supplementary Figure S9. Magnitude of apoptosis induced by FGFR and MEK inhibition is related to that which is induced by PI3K and MEK inhibition in mesenchymal-like *KRAS* mutant lung cancer cell lines.

(A) A PI3K inhibitor, GDC-0941, suppresses AKT phosphorylation in a dose-dependent manner. NCI-H358 and NCI-H1792 *KRAS* mutant cells were treated with the indicated drugs for 3 hours and probed with the indicated antibodies.

(B) Cells were treated with 50 nM trametinib and 250 nM GDC-0941 for 72 hours and analyzed by FACS to quantify annexin V positive cells. Cells were plotted for the amount of apoptosis induced by trametinib with GDC-0941 (x axis) and trametinib with NVP-BGJ398 determined in Fig. 5D and 5E and Supplementary Fig. S8D (Y axis). $p < 0.01$, by linear regression analysis.

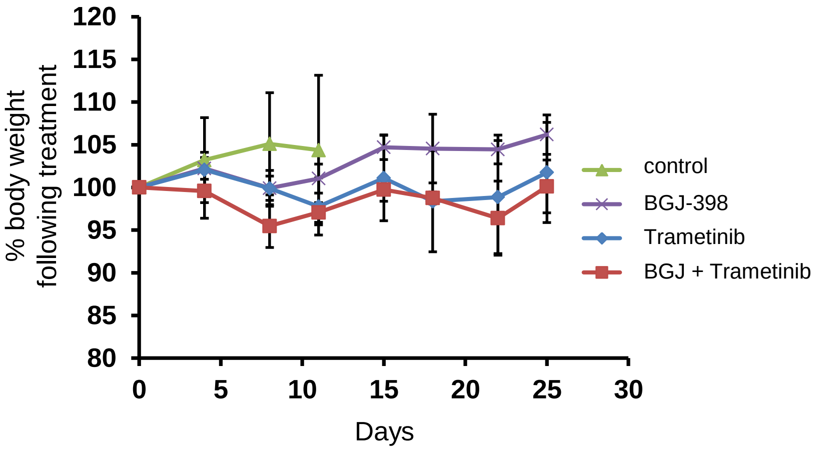


Supplementary Figure S10. Suppression of both MAPK and PI3K signal is not enough to induce cell death in cells resistant to MEK inhibitor with FGFR inhibitor.

(A) Cells were treated with 1uM pan-PI3K inhibitor GDC-0941, 50 nM trametinib, 1uM NVP-BGJ398 or the combination of each drug for 48 hours, and lysates were probed with the indicated antibodies. Independent experiments were performed three times, and a representative result is shown. The combination of these three drugs achieved suppression of both PI3K and MAPK signaling.

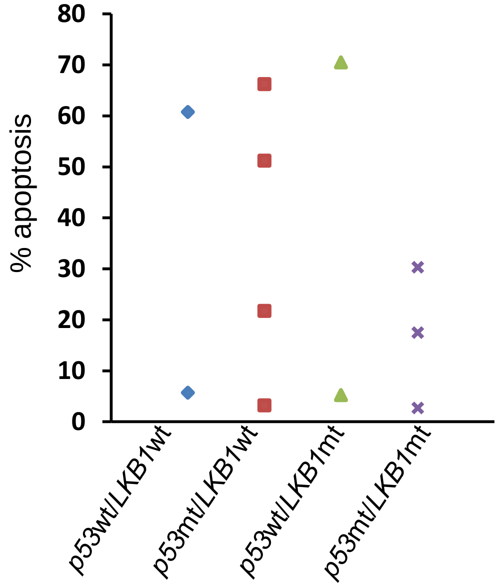
(B, C) Cells were treated with drug and drug combinations as in (A) for 72 hours and analyzed by FACS to quantify annexin V positive cells. The average ± SD of 3 independent experiments is shown.

(D, E) Cells were treated with 50 nM trametinib, 1uM NVP-BGJ398, 100 nM ABT-263 or the combination of each drug for 72 hours and analyzed by FACS to quantify annexin V positive cells. The average ± SD of 3 independent experiments is shown.



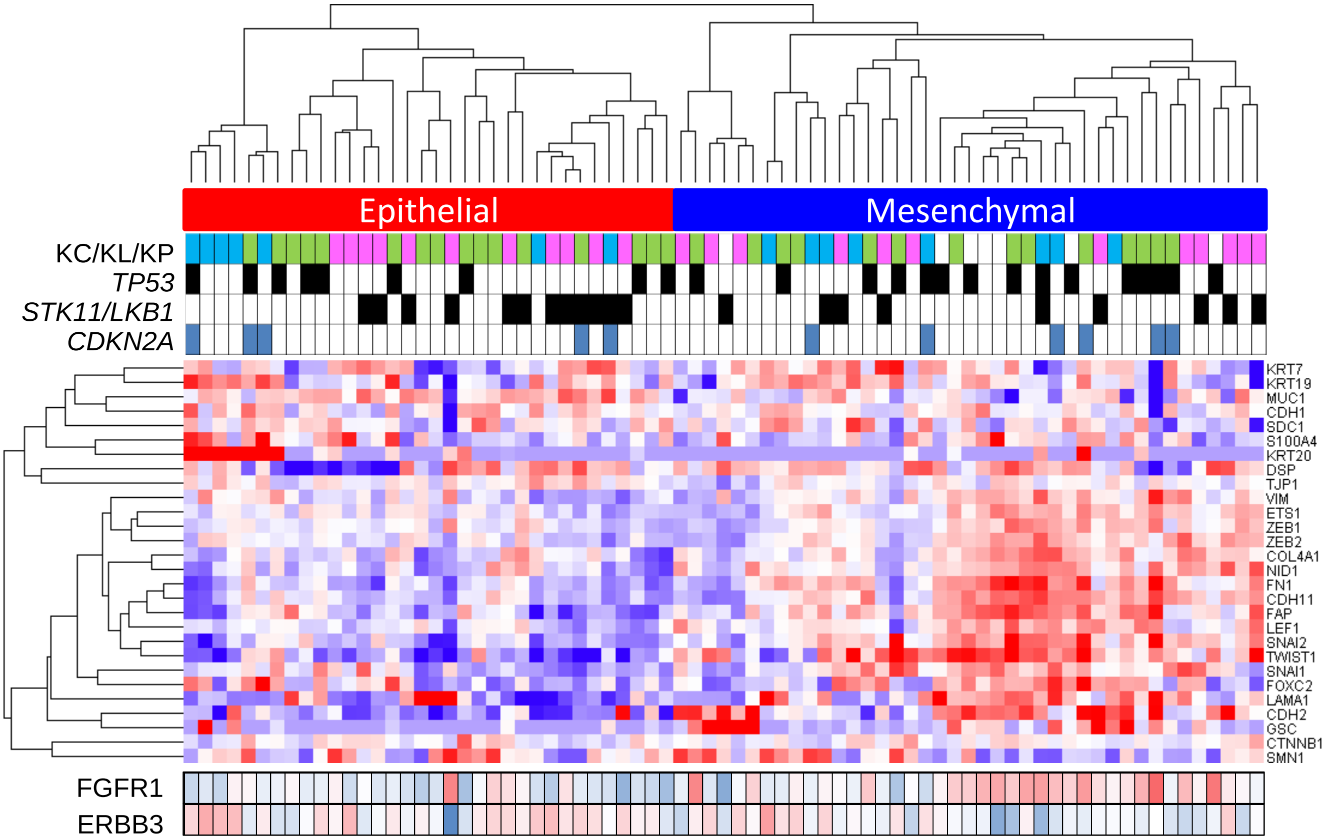
Supplementary Figure S11. Combination of trametinib with NVP-BGJ398 was tolerable in a mouse xenograft model.

Average body weights of LU99 xenograft bearing mice treated with NVP-BGJ398 (15 mg/kg daily), trametinib (0.6 mg/kg daily), or the combination of these drugs are shown relative to starting body weight. Error bars represent SD.



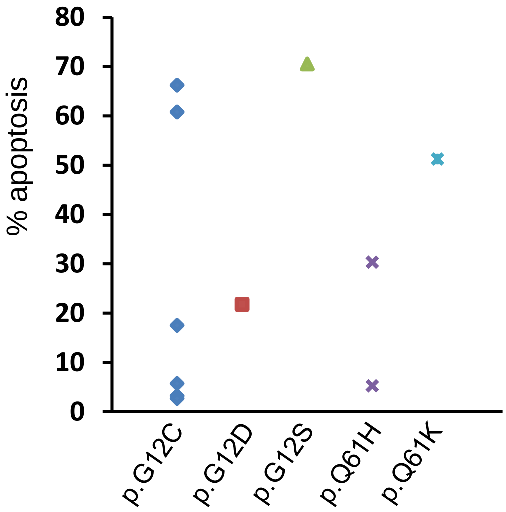
Supplementary Figure S12. No relationship between sensitivity to combination of FGFR inhibitor with trametinib and mutations in *p53* and/or *LKB1* in mesenchymal-like *KRAS* mutant cancers.

Raw data for apoptosis induced by trametinib with NVP-BGJ398 was shown in Fig. 5D, 5E, and Supplementary Fig. S8D.



Supplementary Figure S13. No relationship between EMT and subgroups of *KRAS* mutant lung adenocarcinoma defined by a previous study.

Unsupervised hierarchical clustering of 75 *KRAS* mutant adenocarcinoma extracted from the TCGA dataset was shown using 28 genes listed as EMT related genes by Kalluri and Weinberg (Ref. 35). Major subsets of *KRAS*-mutant lung adenocarcinoma, such as KC (blue), KL (pink), and KP (green), were defined by Skoulidis et al. (Ref. 13) for 68 early-stage *KRAS* mutant lung adenocarcinomas from TCGA dataset (open box; data not available). Co-occurring mutational plot (black) for *TP53*, *STK11/LKB1* and homozygous loss of *CDKN2A* (blue) were obtained from supplementary data in Ref. 34.



Supplementary Figure S14. No relationship between sensitivity to combination of FGFR inhibitor with trametinib and amino acid substitutions in KRAS in mesenchymal-like *KRAS* mutant cancers.

Raw data for apoptosis induced by trametinib with NVP-BGJ398 was shown in Fig. 5D, 5E, and Supplementary Fig. S8D.

Supplementary Table S1. Differentially expressed genes following EMT in the NCI-H358 cell line

Down_100

Rank	ProbeName	UniGeneID	GeneSymbol	Fold Change_H358-TGFβ_vs_H358 (log-based)	GeneName	Ref Seq Accession
1	A_23_P153964	Hs.1735	INHBB	-9.98	inhibin, beta B	NM_002193
2	A_33_P3303810	Hs.519035	LAD1	-7.35	ladinin 1	NM_005558
3	A_23_P214267	Hs.733762	GPR110	-7.30	G protein-coupled receptor 110	NM_153840
4	A_23_P2674	Hs.654610	KRT4	-6.94	keratin 4	NM_002272
5	A_23_P60130	Hs.201083	MAL2	-6.85	mal, T-cell differentiation protein 2 (gene/pseudogene)	NM_052886
6	A_23_P31407	Hs.530009	AGR2	-6.82	anterior gradient 2	NM_006408
7	A_23_P58266	Hs.2962	S100P	-6.69	S100 calcium binding protein P	NM_005980
8	A_23_P139687	Hs.162143	ERP27	-6.62	endoplasmic reticulum protein 27	NM_152321
9	A_23_P393051	Hs.188881	KDF1	-6.60	keratinocyte differentiation factor 1	NM_152365
10	A_23_P140434	Hs.487036	MYO5C	-6.53	myosin VC	NM_018728
11	A_23_P143348	Hs.710157	OVOL2	-6.51	ovo-like zinc finger 2	NM_021220
12	A_23_P205959	Hs.459538	ALDH1A3	-6.33	aldehyde dehydrogenase 1 family, member A3	NM_000693
13	A_24_P365515	Hs.155651	FOXA2	-6.27	forkhead box A2	NM_021784
14	A_32_P206899	Hs.367649	DNAH2	-6.24	dynein, axonemal, heavy chain 2	NM_020877
15	A_33_P3372074	Hs.514477	LLGL2	-6.07	lethal giant larvae homolog 2 (Drosophila)	NM_001031803
16	A_23_P78543	Hs.18894	AP1M2	-5.84	adaptor-related protein complex 1, mu 2 subunit	NM_005498
17	A_33_P3228460	Hs.301350	FXYD3	-5.84	FXYD domain containing ion transport regulator 3	NM_001136007
18	A_32_P218355	Hs.444277	C6orf132	-5.52	chromosome 6 open reading frame 132	NM_001164446
19	A_33_P3256391	Hs.150319	CRB3	-5.52	crumbs family member 3	NM_139161
20	A_23_P149529	Hs.23582	TACSTD2	-5.47	tumor-associated calcium signal transducer 2	NM_002353
21	A_23_P9293	Hs.50382	TJP2	-5.34	tight junction protein 2	NM_004817
22	A_33_P3415191	Hs.216623	ATP8B1	-5.28	ATPase, aminophospholipid transporter, class I, type 8B, member 1	NM_005603
23	A_33_P3422133	Hs.602573	ADAP1	-5.15	ArfGAP with dual PH domains 1	NM_006869
24	A_33_P3215948	Hs.116651	MPZL2	-5.14	myelin protein zero-like 2	NM_005797
25	A_21_P0010596		RNF223	-5.10	ring finger protein 223	
26	A_23_P130027	Hs.670090	EPN3	-5.09	epsin 3	NM_017957
27	A_32_P134968	Hs.417303	SPTB	-5.04	spectrin, beta, erythrocytic	NM_001024858
28	A_23_P349416	Hs.118681	ERBB3	-5.01	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	NM_001982
29	A_23_P161439	Hs.642660	ADIRF	-4.99	adipogenesis regulatory factor	NM_006829
30	A_23_P206359	Hs.461086	CDH1	-4.87	cadherin 1, type 1, E-cadherin (epithelial)	NM_004360
31	A_23_P41344	Hs.115263	EREG	-4.85	epiregulin	NM_001432
32	A_33_P3420545	Hs.13285	KCNV1	-4.81	potassium channel, subfamily V, member 1	NM_014379
33	A_23_P257111	Hs.494496	FBP1	-4.75	fructose-1,6-bisphosphatase 1	NM_000507
34	A_33_P3419190	Hs.270833	AREG	-4.75	amphiregulin	NM_001657
35	A_23_P259071	Hs.270833	AREG	-4.73	amphiregulin	NM_001657
36	A_23_P41804	Hs.240951	NKD2	-4.72	naked cuticle homolog 2 (Drosophila)	NM_033120
37	A_24_P48057	Hs.435730	IRX5	-4.70	iroquois homeobox 5	NM_005853
38	A_23_P160167	Hs.38972	TSPAN1	-4.69	tetraspanin 1	NM_005727
39	A_23_P210210	Hs.468410	EPAS1	-4.65	endothelial PAS domain protein 1	NM_001430
40	A_24_P135748	Hs.745043	GRTP1	-4.64	growth hormone regulated TBC protein 1	NM_024719
41	A_33_P3265855	Hs.102336	PRR5-ARHGAP8	-4.64	PRR5-ARHGAP8 readthrough	NM_181334
42	A_23_P124619	Hs.288998	S100A14	-4.63	S100 calcium binding protein A14	NM_020672
43	A_33_P3229107	Hs.510543	MIR205HG	-4.54	MIR205 host gene (non-protein coding)	NM_001104548
44	A_23_P119943	Hs.438102	IGFBP2	-4.52	insulin-like growth factor binding protein 2, 36kDa	NM_000597
45	A_33_P3366336	Hs.97837	SBK1	-4.49	SH3 domain binding kinase 1	NM_001024401
46	A_33_P3303649	Hs.517586	MB	-4.45	myoglobin	NM_203377
47	A_23_P1962	Hs.17466	RARRES3	-4.44	retinoic acid receptor responder (tazarotene induced) 3	NM_004585
48	A_23_P81158	Hs.654537	ADH1C	-4.41	alcohol dehydrogenase 1C (class I), gamma polypeptide	NM_000669
49	A_33_P3318668	Hs.512469	CYSRT1	-4.41	cysteine-rich tail protein 1	NM_199001
50	A_33_P3228450	Hs.301350	FXYD3	-4.39	FXYD domain containing ion transport regulator 3	NM_001136007

51	A_24_P307869	Hs.514477	LLGL2	-4.39	lethal giant larvae homolog 2 (Drosophila)	NM_001015002
52	A_23_P15101	Hs.115838	TMC5	-4.34	transmembrane channel-like 5	NM_024780
53	A_24_P10137	Hs.507866	RGCC	-4.30	regulator of cell cycle	NM_014059
54	A_32_P452655	Hs.462402	LGALS9C	-4.30	lectin, galactoside-binding, soluble, 9C	NM_001040078
55	A_33_P3232504	Hs.512469	CYSRT1	-4.29	cysteine-rich tail protein 1	NM_199001
56	A_23_P202071	Hs.309288	CELF2	-4.28	CUGBP, Elav-like family member 2	NM_001025077
57	A_23_P78980	Hs.69009	B3GNT3	-4.27	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3	NM_014256
58	A_23_P153372	Hs.631617	HSH2D	-4.27	hematopoietic SH2 domain containing	NM_032855
59	A_24_P280846	Hs.532401	GOLT1A	-4.18	golgi transport 1A	NM_198447
60	A_24_P203056	Hs.530970	BCL7A	-4.17	B-cell CLL/lymphoma 7A	NM_020993
61	A_23_P329573	Hs.375957	ITGB2	-4.14	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	NM_000211
62	A_23_P897	Hs.32417	C1orf116	-4.14	chromosome 1 open reading frame 116	NM_023938
63	A_33_P3846653	Hs.527883	KRT19P2	-4.12	keratin 19 pseudogene 2	NR_036685
64	A_23_P79803	Hs.517029	VSTM2L	-4.11	V-set and transmembrane domain containing 2 like	NM_080607
65	A_23_P37127	Hs.163484	FOXA1	-4.10	forkhead box A1	NM_004496
66	A_32_P63562	Hs.659312	MIG7	-4.07	mig-7	
67	A_33_P3274935	Hs.11067	HID1	-4.07	HID1 domain containing	NM_030630
68	A_33_P3365193	Hs.655232	AMY1C	-4.06	amylase, alpha 1C (salivary)	NM_001008219
69	A_23_P127663	Hs.471695	PRRG4	-4.05	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	NM_024081
70	A_23_P256312	Hs.517973	MST1R	-4.05	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	NM_002447
71	A_23_P56978	Hs.51133	PTK6	-4.05	protein tyrosine kinase 6	NM_005975
72	A_24_P329487	Hs.741352	FAM84B	-4.04	family with sequence similarity 84, member B	NM_174911
73	A_33_P3383326	Hs.126667	LPAR1	-4.03	lysophosphatidic acid receptor 1	NM_057159
74	A_23_P207850	Hs.438292	TNS4	-4.02	tensin 4	NM_032865
75	A_23_P115022	Hs.104476	TMEM125	-4.01	transmembrane protein 125	NM_144626
76	A_23_P100220	Hs.592053	ESRP2	-4.01	epithelial splicing regulatory protein 2	NM_024939
77	A_33_P3474319	Hs.352661	SLC37A2	-3.98	solute carrier family 37 (glucose-6-phosphate transporter), member 2	NM_198277
78	A_33_P3329088	Hs.75799	PRSS8	-3.98	protease, serine, 8	NM_002773
79	A_23_P16523	Hs.616962	GDF15	-3.98	growth differentiation factor 15	NM_004864
80	A_23_P74112	Hs.221375	IFNLR1	-3.97	interferon, lambda receptor 1	NM_170743
81	A_23_P64828	Hs.524760	OAS1	-3.95	2'-5'-oligoadenylate synthetase 1, 40/46kDa	NM_002534
82	A_23_P218675	Hs.2719	WFDC2	-3.95	WAP four-disulfide core domain 2	NM_006103
83	A_23_P150379	Hs.116651	MPZL2	-3.95	myelin protein zero-like 2	NM_144765
84	A_23_P30126	Hs.1690	FGFBP1	-3.95	fibroblast growth factor binding protein 1	NM_005130
85	A_24_P66027	Hs.226307	APOBEC3B	-3.94	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	NM_004900
86	A_33_P3249748	Hs.368402	THEM6	-3.93	thioesterase superfamily member 6	NM_016647
87	A_23_P205370	Hs.510327	ASB2	-3.93	ankyrin repeat and SOCS box containing 2	NM_016150
88	A_23_P160881	Hs.123659	SMPDL3B	-3.92	sphingomyelin phosphodiesterase, acid-like 3B	NM_001009568
89	A_33_P3309075	Hs.442657	TBC1D8	-3.88	TBC1 domain family, member 8 (with GRAM domain)	NM_001102426
90	A_23_P201551	Hs.267659	VAV3	-3.87	vav 3 guanine nucleotide exchange factor	NM_006113
91	A_33_P3244274	Hs.512767	RNF208	-3.85	ring finger protein 208	
92	A_23_P216468	Hs.444915	SLC1A1	-3.81	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	NM_004170
93	A_32_P231568	Hs.129136	RASEF	-3.80	RAS and EF-hand domain containing	NM_152573
94	A_24_P411749	Hs.743302	GPR126	-3.78	G protein-coupled receptor 126	NM_020455
95	A_33_P3231670	Hs.512767	RNF208	-3.78	ring finger protein 208	NM_031297
96	A_23_P3221	Hs.511251	SQRDL	-3.74	sulfide quinone reductase-like (yeast)	NM_021199
97	A_24_P105191	Hs.385956	HS6ST2	-3.72	heparan sulfate 6-O-sulfotransferase 2	NM_001077188
98	A_24_P313993	Hs.584744	CAPS	-3.71	calcyphosine	NM_004058
99	A_33_P3275707	Hs.632555	AP1S3	-3.70	adaptor-related protein complex 1, sigma 3 subunit	NM_001039569
100	A_23_P212469	Hs.441145	ENTPD3	-3.69	ectonucleoside triphosphate diphosphohydrolase 3	NM_001248

Up_100

Rank	ProbeName	UniGeneID	GeneSymbol	Fold Change_H358-TGFβ_vs_H358 (log-based)	GeneName	Ref Seq Accession
1	A_33_P3283833	Hs.516971	FOXS1	8.27	forkhead box S1	NM_004118
2	A_23_P99661	Hs.35125	ARHGEF40	8.11	Rho guanine nucleotide exchange factor (GEF) 40	NM_018071
3	A_23_P215060	Hs.744213	PODXL	7.24	podocalyxin-like	NM_001018111
4	A_32_P300427	Hs.119286	APCDD1L	6.71	adenomatosis polyposis coli down-regulated 1-like	NM_153360
5	A_33_P3393684	Hs.647718	VSIG8	6.33	V-set and immunoglobulin domain containing 8	NM_001013661
6	A_33_P3382924	Hs.111779	SPARC	6.05	secreted protein, acidic, cysteine-rich (osteonectin)	NM_003118
7	A_24_P709377	Hs.656660	PAX8-AS1	6.04	PAX8 antisense RNA 1	NR_015377
8	A_23_P107775	Hs.590943	TMEM190	6.02	transmembrane protein 190	NM_139172
9	A_33_P3215640	Hs.25391	PI16	5.97	peptidase inhibitor 16	NM_153370
10	A_23_P43197	Hs.65425	CALB1	5.86	calbindin 1, 28kDa	NM_004929
11	A_33_P3268304	Hs.469881	LIMS2	5.86	LIM and senescent cell antigen-like domains 2	NM_001161404
12	A_23_P357104	Hs.412117	ANXA6	5.58	annexin A6	NM_001155
13	A_23_P102681	Hs.280135	-	5.52		
14	A_23_P66637	Hs.463412	SGCA	5.49	sarcoglycan, alpha (50kDa dystrophin-associated glycoprotein)	NM_000023
15	A_23_P111701	Hs.83381	GNG11	5.42	guanine nucleotide binding protein (G protein), gamma 11	NM_004126
16	A_24_P158089	Hs.414795	SERPINE1	5.40	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	NM_000602
17	A_33_P3252286	Hs.114948	CRLF1	5.36	cytokine receptor-like factor 1	NM_004750
18	A_23_P87013	Hs.410977	TAGLN/SIDT2	5.33	transgelin	NM_001001522
19	A_23_P301304	Hs.264887	FGFR1	5.28	fibroblast growth factor receptor 1	NM_023110
20	A_24_P687594	Hs.740619	LIX1L	5.22	Lix1 homolog (mouse)-like	NM_153713
21	A_33_P3243887	Hs.467304	IL11	5.06	interleukin 11	NM_000641
22	A_21_P0012152	Hs.729646	APCDD1L-AS1	4.96	APCDD1L antisense RNA 1 (head to head)	NR_034147
23	A_23_P87011	Hs.410977	TAGLN	4.92	transgelin	NM_001001522
24	A_23_P96383	Hs.15154	SRPX	4.90	sushi-repeat containing protein, X-linked	NM_006307
25	A_21_P0010254		XLOC_013952	4.89		
26	A_23_P7144	Hs.789	CXCL1	4.81	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	NM_001511
27	A_23_P218505	Hs.154704	LHB	4.78	luteinizing hormone beta polypeptide	NM_000894
28	A_19_P00318759	Hs.729646	APCDD1L-AS1	4.78	APCDD1L antisense RNA 1 (head to head)	NR_034147
29	A_24_P395814	Hs.446683	CGB	4.76	chorionic gonadotropin, beta polypeptide	NM_000737
30	A_33_P3331916	Hs.729493	LINC00545	4.76	long intergenic non-protein coding RNA 545	NR_103813
31	A_23_P110791	Hs.586219	CSF1R	4.73	colony stimulating factor 1 receptor	NM_005211
32	A_21_P0012150	Hs.729646	APCDD1L-AS1	4.70	APCDD1L antisense RNA 1 (head to head)	NR_034147
33	A_23_P326319	Hs.401798	C16orf45	4.69	chromosome 16 open reading frame 45	NM_033201
34	A_21_P0014465		LOC101928173	4.68	uncharacterized LOC101928173	XR_245039
35	A_21_P0000129	Hs.507755	DCLK1	4.64	doublecortin-like kinase 1	NM_001195430
36	A_23_P40174	Hs.297413	MMP9	4.62	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	NM_004994
37	A_23_P342744	Hs.740619	LIX1L	4.61	Lix1 homolog (mouse)-like	NM_153713
38	A_32_P234145	Hs.642615	SHC4	4.58	SHC (Src homology 2 domain containing) family, member 4	NM_203349
39	A_33_P3304668	Hs.172928	COL1A1	4.58	collagen, type I, alpha 1	NM_000088
40	A_23_P66525	Hs.462270	HS3ST3A1	4.54	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1	NM_006042
41	A_23_P36562	Hs.505654	ITGA5	4.54	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	NM_002205
42	A_23_P213562	Hs.482562	F2R	4.54	coagulation factor II (thrombin) receptor	NM_001992
43	A_33_P3256510	Hs.591586	KCNK12	4.41	potassium channel, subfamily K, member 12	NM_022055
44	A_23_P122216	Hs.102267	LOX	4.36	lysyl oxidase	NM_002317
45	A_33_P3242923	Hs.325752	LINC00640	4.29	long intergenic non-protein coding RNA 640	NR_038358
46	A_33_P3321432	Hs.567498	FAM198B	4.28	family with sequence similarity 198, member B	NM_016613
47	A_33_P3640690	Hs.124503	ZEB1	4.27	zinc finger E-box binding homeobox 1	NM_001128128
48	A_33_P3276703	Hs.587325	VGF	4.24	VGF nerve growth factor inducible	NM_003378
49	A_33_P3374723	Hs.124503	ZEB1	4.20	zinc finger E-box binding homeobox 1	NM_001128128
50	A_33_P3364741	Hs.7835	MRC2	4.19	mannose receptor, C type 2	NM_006039

51	A_23_P333228	Hs.170388	MARCH4	4.17	membrane-associated ring finger (C3HC4) 4, E3 ubiquitin protein ligase	NM_020814
52	A_23_P160318	Hs.368921	COL16A1	4.16	collagen, type XVI, alpha 1	NM_001856
53	A_21_P0001902		LOC101928173	4.15	uncharacterized LOC101928173	XR_245039
54	A_19_P00801885	Hs.729493	LINC00545	4.15	long intergenic non-protein coding RNA 545	NR_103813
55	A_23_P423331	Hs.163642	NTNG2	4.14	netrin G2	NM_032536
56	A_23_P372834	Hs.76152	AQP1	4.10	aquaporin 1 (Colton blood group)	NM_198098
57	A_33_P3233906	Hs.471783	RAMP1	4.09	receptor (G protein-coupled) activity modifying protein 1	NM_005855
58	A_33_P3357678	Hs.680983	LCTL	4.04	lactase-like	NM_207338
59	A_23_P369994	Hs.507755	DCLK1	4.04	doublecortin-like kinase 1	NM_004734
60	A_19_P00320067	Hs.200644		4.02		
61	A_23_P1029	Hs.389137	MFAP2	4.02	microfibrillar-associated protein 2	NM_017459
62	A_23_P103371	Hs.101807	AZIN2	4.02	antizyme inhibitor 2	NM_052998
63	A_23_P45999	Hs.132753	FBXO2	4.00	F-box protein 2	NM_012168
64	A_23_P380298	Hs.90232	LZTS3	3.94	leucine zipper, putative tumor suppressor family member 3	NM_001282533
65	A_24_P402588	Hs.370549	BCL11A	3.92	B-cell CLL/lymphoma 11A (zinc finger protein)	NM_018014
66	A_24_P88850	Hs.527021	MRAS	3.92	muscle RAS oncogene homolog	NM_012219
67	A_33_P3316786	Hs.129452	DACH1	3.92	dachshund family transcription factor 1	NM_080759
68	A_24_P829183	Hs.535791	FLJ32255	3.91	uncharacterized LOC643977	NR_104643
69	A_23_P50946	Hs.471783	RAMP1	3.91	receptor (G protein-coupled) activity modifying protein 1	NM_005855
70	A_23_P99642	Hs.513147	SLC7A7	3.89	solute carrier family 7 (amino acid transporter light chain, y+L system), member 7	NM_001126106
71	A_33_P3212274	Hs.482562	F2R	3.88	coagulation factor II (thrombin) receptor	
72	A_23_P104471	Hs.178170	DUSP13	3.86	dual specificity phosphatase 13	NM_001007271
73	A_23_P122924	Hs.583348	INHBA	3.84	inhibin, beta A	NM_002192
74	A_23_P386268	Hs.24510	C4orf26	3.84	chromosome 4 open reading frame 26	NM_178497
75	A_21_P0013960	Hs.669210	LOC101930246	3.83	uncharacterized LOC101930246	XR_250239
76	A_33_P3367830	Hs.731454	EFEMP2	3.83	EGF containing fibulin-like extracellular matrix protein 2	NM_016938
77	A_21_P0000645	Hs.729646	APCDD1L-AS1	3.81	APCDD1L antisense RNA 1 (head to head)	NR_034147
78	A_33_P3354464	Hs.65436	LOXL1	3.81	lysyl oxidase-like 1	NM_005576
79	A_32_P75581	Hs.591870	BHLHE22	3.81	basic helix-loop-helix family, member e22	NM_152414
80	A_23_P62081	Hs.156540	SCG5	3.81	secretogranin V (7B2 protein)	NM_003020
81	A_23_P17914	Hs.654800	PNPLA3	3.80	patatin-like phospholipase domain containing 3	NM_025225
82	A_33_P3263432	Hs.158237	ITGA10	3.80	integrin, alpha 10	NM_003637
83	A_21_P0000144	Hs.200101	LOC100505478	3.79	uncharacterized LOC100505478	NM_001199233
84	A_19_P00320759	Hs.535791	FLJ32255	3.78	uncharacterized LOC643977	NR_104643
85	A_33_P3330264	Hs.789	CXCL1	3.75	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	NM_001511
86	A_21_P0001634		LOC102723817	3.75	uncharacterized LOC102723817	XR_425104
87	A_23_P35045	Hs.389374	ARHGAP30	3.75	Rho GTPase activating protein 30	NM_001025598
88	A_23_P384044	Hs.731723	CNIH3	3.74	cornichon family AMPA receptor auxiliary protein 3	NM_152495
89	A_23_P360964	Hs.515490	DACT3	3.72	dishevelled-binding antagonist of beta-catenin 3	NM_145056
90	A_21_P0001085	Hs.200644		3.72		
91	A_23_P129856	Hs.72956	HIC1	3.72	hypermethylated in cancer 1	NM_006497
92	A_24_P11462	Hs.101807	AZIN2	3.70	antizyme inhibitor 2	NM_052998
93	A_23_P58588	Hs.552087	SLIT3	3.69	slit homolog 3 (Drosophila)	NM_003062
94	A_23_P128663	Hs.159492	SACS	3.69	sacsin molecular chaperone	NM_014363
95	A_23_P302005	Hs.732016	STON1	3.65	stonin 1	NM_006873
96	A_23_P100660	Hs.532768	SERPINF1	3.65	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	NM_002615
97	A_19_P00322977	Hs.744176	LINC-PINT	3.63	long intergenic non-protein coding RNA, p53 induced transcript	NR_109854
98	A_21_P0004140	Hs.741936	LOC102477328	3.60	uncharacterized LOC102477328	NR_105007
99	A_33_P3415923	Hs.179838	FMNL3	3.58	formin-like 3	NM_175736
100	A_21_P0001384	Hs.250624	LINC00626	3.55	long intergenic non-protein coding RNA 626	NR_024160

Supplementary Table S2. No significant alterations in the expression of FGF ligands and receptors after trametinib treatment in NCI-H1792 cells.

ProbeName	UniGeneID	Gene Symbol	GeneName	Ref Seq Accession	Fold change (H1792 trametinib) vs.(H1792 no Trx)	Regulation (H1792 Tramtinib) vs.(H1792 no Trx)
A_23_P372923	Hs.264887	FGFR1	fibroblast growth factor receptor 1	NM_001174066	1.102	down
A_23_P301304	Hs.264887	FGFR1	fibroblast growth factor receptor 1	NM_023110	1.077	up
A_23_P303145	Hs.533683	FGFR2	fibroblast growth factor receptor 2		1.430	down
A_23_P202334	Hs.533683	FGFR2	fibroblast growth factor receptor 2	NM_022970	1.063	up
A_24_P206624	Hs.533683	FGFR2	fibroblast growth factor receptor 2	NM_022970	1.220	down
A_23_P500501	Hs.1420	FGFR3	fibroblast growth factor receptor 3	NM_000142	2.178	up
A_23_P213336	Hs.483635	FGF1	fibroblast growth factor 1 (acidic)	NM_000800	1.206	up
A_33_P3242136	Hs.483635	FGF1	fibroblast growth factor 1 (acidic)	NM_001257206	1.035	up
A_33_P3379886	Hs.284244	FGF2	fibroblast growth factor 2 (basic)	NM_002006	1.423	up
A_33_P3419696	Hs.284244	FGF2	fibroblast growth factor 2 (basic)	NM_002006	1.427	up
A_33_P3380797	Hs.37092	FGF3	fibroblast growth factor 3	NM_005247	1.093	up
A_24_P355720	Hs.1755	FGF4	fibroblast growth factor 4	NM_002007	1.664	down
A_23_P212800	Hs.37055	FGF5	fibroblast growth factor 5	NM_004464	1.594	down
A_33_P3411907	Hs.37055	FGF5	fibroblast growth factor 5	NM_033143	1.939	down
A_32_P196021	Hs.567268	FGF7	fibroblast growth factor 7	NM_002009	1.203	down
A_33_P3257027	Hs.567268	FGF7	fibroblast growth factor 7	NM_002009	1.232	up
A_23_P46829	Hs.57710	FGF8	fibroblast growth factor 8 (androgen-induced)	NM_033163	1.060	down
A_33_P3348714	Hs.111	FGF9	fibroblast growth factor 9	NM_002010	1.204	down
A_33_P3348719	Hs.111	FGF9	fibroblast growth factor 9	NM_002010	1.457	down
A_23_P377750	Hs.664499	FGF10	fibroblast growth factor 10	NM_004465	1.222	down
A_24_P334300	Hs.390250	FGF12	fibroblast growth factor 12	NM_004113	1.178	down
A_23_P217319	Hs.6540	FGF13	fibroblast growth factor 13	NM_004114	1.207	down
A_23_P88033	Hs.508616	FGF14	fibroblast growth factor 14	NM_175929	1.956	down
A_23_P429363	Hs.248192	FGF17	fibroblast growth factor 17	NM_003867	1.225	down
A_23_P427587	Hs.249200	FGF19	fibroblast growth factor 19	NM_005117	1.209	down
A_23_P395404	Hs.199905	FGF20	fibroblast growth factor 20	NM_019851	1.215	down
A_33_P3400248	Hs.199905	FGF20	fibroblast growth factor 20	NM_019851	2.047	up
A_23_P101564	Hs.283015	FGF21	fibroblast growth factor 21	NM_019113	1.117	down
A_23_P117095	Hs.287370	FGF23	fibroblast growth factor 23	NM_020638	1.207	down

Supplementary Table S3. Differentially expressed genes by microarray analysis between control and trametinib-treated NCI-H1792 cells.

Down_100

Rank	UniGeneID	GeneSymbol	Fold Change_H1792 trametinib_vs_H1972 no treatment (log-based)	GeneName	raw (H1792 trametinib)	raw (H1792 no Trx)	normalized (H1792 trametinib)	normalized (H1792 no Trx)
1	Hs.326035	EGR1	-4.95	early growth response 1	24	739	-4.625	0.325
2	Hs.444959	ACOX2	-4.06	acyl-CoA oxidase 2, branched chain	18	294	-4.062	0.000
3	Hs.657859	XLOC_014512	-3.73		19	248	-0.508	3.219
4	Hs.298654	DUSP6	-3.71	dual specificity phosphatase 6	251	3260	-3.454	0.253
5			-3.35		161	1631	-0.070	3.280
6	Hs.657859		-3.34		27	275	-0.242	3.102
7	Hs.434878	TNFRSF6B	-3.22	tumor necrosis factor receptor superfamily, member 6b, decoy	401	3718	1.370	4.591
8	Hs.695934	COL13A1	-3.22	collagen, type XIII, alpha 1	74	681	-2.391	0.828
9	Hs.434878	TNFRSF6B	-3.09	tumor necrosis factor receptor superfamily, member 6b, decoy	115	975	0.999	4.086
10	Hs.602085	PHLDA1	-2.92	pleckstrin homology-like domain, family A, member 1	609	4598	-3.004	-0.081
11	Hs.491582	PLAT	-2.90	plasminogen activator, tissue	1800	13395	-1.276	1.627
12	Hs.534987	LOC100506457	-2.90	uncharacterized LOC100506457	19	138	-0.869	2.030
13	Hs.159226	HAS2	-2.85	hyaluronan synthase 2	33	240	0.386	3.237
14	Hs.689698	SNORA51	-2.85	small nucleolar RNA, H/ACA box 51	47	339	-0.922	1.925
15	Hs.49265	HHIP	-2.84	hedgehog interacting protein	145	1036	0.395	3.237
16	Hs.155651	FOXA2	-2.81	forkhead box A2	27	188	-2.145	0.667
17	Hs.434059	ETV4	-2.69	ets variant 4	197	1261	-1.349	1.339
18	Hs.170388	MARCH4	-2.69	membrane-associated ring finger (C3HC4) 4, E3 ubiquitin protein ligase	72	459	-1.466	1.221
19	Hs.517617	MAFF	-2.67	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F	120	756	-1.146	1.523
20	Hs.689718	SNORA30	-2.66	small nucleolar RNA, H/ACA box 30	33	208	-0.507	2.151
21	Hs.689635	SCARNA8	-2.65	small Cajal body-specific RNA 8	74	462	0.000	2.653
22	Hs.507991	HHIP	-2.63	hedgehog interacting protein	80	492	0.000	2.634
23	Hs.144725	GRAMD1B	-2.48	GRAM domain containing 1B	28	153	0.543	3.024
24	Hs.101307	SLC14A1	-2.47	solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	37	203	1.601	4.068
25	Hs.737705		-2.44		28	153	0.989	3.431
26	Hs.66	IL1RL1	-2.43	interleukin 1 receptor-like 1	23	122	0.782	3.210
27	Hs.153952	NT5E	-2.40	5'-nucleotidase, ecto (CD73)	218	1141	-3.701	-1.302
28	Hs.386726	RGS4	-2.40	regulator of G-protein signaling 4	44	229	0.000	2.396
29	Hs.382212		-2.39		3899	20252	2.708	5.093
30	Hs.659987		-2.38		40	207	-1.676	0.701
31	Hs.658237	SNORA28	-2.34	small nucleolar RNA, H/ACA box 28	70	353	-0.886	1.453
32	Hs.449320		-2.33		1772	8870	1.976	4.307
33	Hs.380089	EPHB6	-2.32	EPH receptor B6	712	3527	3.419	5.735
34	Hs.498571	LINC00707	-2.30	long intergenic non-protein coding RNA 707	41	204	1.647	3.950
35	Hs.550772	LINC01204	-2.26	long intergenic non-protein coding RNA 1204	27	131	-2.421	-0.157
36	Hs.507348	HS3ST1	-2.25	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	45	215	-3.684	-1.432
37	Hs.624	CXCL8	-2.21	chemokine (C-X-C motif) ligand 8	145	667	-0.218	1.992
38	Hs.689694	SCARNA1	-2.14	small Cajal body-specific RNA 1	41	179	-1.591	0.551
39	Hs.270833	AREG	-2.13	amphiregulin	41	179	-2.865	-0.739
40	Hs.270833	AREG	-2.12	amphiregulin	102	441	-2.870	-0.746
41	Hs.647132	NKX6-3	-2.12	NK6 homeobox 3	24	102	0.000	2.118
42	Hs.744120	TRIL	-2.11	TLR4 interactor with leucine-rich repeats	34	144	1.747	3.856
43	Hs.9914	FST	-2.06	follistatin	272	1131	0.795	2.856
44	Hs.689719	SNORA27	-2.06	small nucleolar RNA, H/ACA box 27	135	556	-1.677	0.379
45	Hs.732289	CREG2	-2.04	cellular repressor of E1A-stimulated genes 2	52	212	0.437	2.482
46		XLOC_009788	-2.03		29	116	-2.595	-0.566
47	Hs.22634	ETV1	-2.02	ets variant 1	63	255	-1.160	0.857
48	Hs.197143	CMKLR1	-2.02	chemokine-like receptor 1	171	689	2.727	4.743
49	Hs.689724	SNORA1	-2.01	small nucleolar RNA, H/ACA box 1	115	460	-0.232	1.779
50	Hs.22634	ETV1	-2.00	ets variant 1	54	213	-1.046	0.949

51	Hs.270	CYTIP	-1.99	cytohesin 1 interacting protein	22	85	0.619	2.604
52	Hs.642615	SHC4	-1.96	SHC (Src homology 2 domain containing) family, member 4	44	170	-0.991	0.967
53	Hs.9613	ANGPTL4	-1.94	angiopoietin-like 4	826	3160	0.000	1.944
54	Hs.153952	NT5E	-1.94	5'-nucleotidase, ecto (CD73)	107	407	-2.630	-0.695
55	Hs.25318	RAB27B	-1.93	RAB27B, member RAS oncogene family	103	391	-0.340	1.595
56	Hs.177164	VSIG1	-1.93	V-set and immunoglobulin domain containing 1	21	80	-3.072	-1.138
57	Hs.323308	SPRY4	-1.93	sprouty homolog 4 (Drosophila)	86	327	-1.717	0.214
58	Hs.633254	C14orf23	-1.92	chromosome 14 open reading frame 23	79	300	2.383	4.308
59	Hs.459538	ALDH1A3	-1.92	aldehyde dehydrogenase 1 family, member A3	8816	33239	-0.187	1.736
60	Hs.641525	LOC100289473	-1.92	cytoskeleton associated protein 2-like pseudogene	30	112	0.312	2.230
61	Hs.279746	TRPV2	-1.92	transient receptor potential cation channel, subfamily V, member 2	35	130	-0.398	1.519
62	Hs.125474	LPXN	-1.91	leupaxin	1311	4907	1.801	3.714
63	Hs.22634	ETV1	-1.91	ets variant 1	335	1249	-1.128	0.782
64	Hs.710069	LOC100130938	-1.90	uncharacterized LOC100130938	46	170	-2.663	-0.764
65	Hs.731723	CNIH3	-1.89	cornichon family AMPA receptor auxiliary protein 3	859	3164	0.501	2.391
66	Hs.567047	LOC101928413	-1.89	uncharacterized LOC101928413	32	116	-1.981	-0.094
67	Hs.710069	LOC100130938	-1.88	uncharacterized LOC100130938	107	391	-2.639	-0.754
68	Hs.693446	SNORA38B	-1.88	small nucleolar RNA, H/ACA box 38B	38	138	-0.503	1.378
69	Hs.740645	CHD6	-1.88	chromodomain helicase DNA binding protein 6	24	88	-1.027	0.854
70		XLOC_012847	-1.86		118	425	0.000	1.858
71	Hs.451802		-1.85		19	70	1.241	3.088
72	Hs.23735	KCNF1	-1.84	potassium voltage-gated channel, subfamily F, member 1	29	103	-0.905	0.936
73	Hs.129673	SNORA67	-1.83	small nucleolar RNA, H/ACA box 67	1818	6437	0.000	1.833
74	Hs.162963	ANTXR2	-1.83	anthrax toxin receptor 2	62	221	-1.029	0.803
75	Hs.710069	LOC100130938	-1.83	uncharacterized LOC100130938	77	270	-2.679	-0.853
76	Hs.719042	EMP1	-1.81	epithelial membrane protein 1	11257	39294	-0.581	1.231
77	Hs.149545		-1.80		67	232	0.063	1.866
78	Hs.498571	LINC00707	-1.80	long intergenic non-protein coding RNA 707	249	865	3.225	5.028
79	Hs.689720	SNORA23	-1.79	small nucleolar RNA, H/ACA box 23	124	427	-0.516	1.278
80	Hs.745521	SNORA6	-1.79	small nucleolar RNA, H/ACA box 6	272	936	-0.074	1.720
81	Hs.25318	RAB27B	-1.78	RAB27B, member RAS oncogene family	423	1447	-0.163	1.619
82	Hs.558645	ANKRD30BP2	-1.78	ankyrin repeat domain 30B pseudogene 2	26	88	-1.556	0.223
83		SCARNA14	-1.77	small Cajal body-specific RNA 14	193	655	0.235	2.009
84	Hs.633254	C14orf23	-1.76	chromosome 14 open reading frame 23	266	898	3.216	4.978
85	Hs.149545	LOC100507420	-1.76	uncharacterized LOC100507420	260	877	0.112	1.872
86	Hs.668122	LOC100506178	-1.75	uncharacterized LOC100506178	29	96	-0.831	0.919
87	Hs.162963	ANTXR2	-1.75	anthrax toxin receptor 2	205	684	-1.270	0.479
88	Hs.633254	C14orf23	-1.74	chromosome 14 open reading frame 23	500	1665	3.307	5.051
89	Hs.98288	AQPEP	-1.74	laeverin	149	493	2.093	3.829
90	Hs.684893	SNORA74A	-1.72	small nucleolar RNA, H/ACA box 74A	29	93	-0.438	1.280
91			-1.71		670	2176	-2.247	-0.539
92	Hs.434828	HES7	-1.70	hes family bHLH transcription factor 7	20	66	-0.964	0.739
93	Hs.98288	AQPEP	-1.70	laeverin	1139	3669	3.266	4.962
94	Hs.713717	FUT8-AS1	-1.70	FUT8 antisense RNA 1	124	401	-1.934	-0.238
95	Hs.632352	ANKRD20A5P	-1.69	ankyrin repeat domain 20 family, member A5, pseudogene	19	62	-0.148	1.547
96	Hs.523848	MYEOV	-1.69	myeloma overexpressed	1713	5507	-0.746	0.947
97	Hs.693283	SCARNA18	-1.69	small Cajal body-specific RNA 18	515	1651	-0.808	0.880
98	Hs.567236	SHROOM2	-1.69	shroom family member 2	490	1568	1.051	2.738
99	Hs.668122	LOC100506178	-1.67	uncharacterized LOC100506178	35	110	-1.301	0.370
100	Hs.505924	HMGA2	-1.66	high mobility group AT-hook 2	280	879	-1.657	0.000

Up_100

Rank	UniGeneID	GeneSymbol	Fold Change_H1792 trametinib_vs_H1972 no treatment (log-based)	GeneName	raw (H1792 trametinib)	raw (H1792 no Trx)	normalized (H1792 trametinib)	normalized (H1792 no Trx)
1	Hs.465929	CNN1	5.578	calponin 1, basic, smooth muscle	2495	52	4.496	-1.082
2	Hs.516105	ACTG2	4.401	actin, gamma 2, smooth muscle, enteric	1259	59	3.791	-0.610
3	Hs.591104	BMF	4.194	Bcl2 modifying factor	982	53	1.513	-2.682
4	Hs.410977	TAGLN	4.191	transgelin	1083	59	2.026	-2.165
5	Hs.410977	TAGLN	3.787	transgelin	1771	128	1.872	-1.916
6	Hs.219140	NPPB	3.500	natriuretic peptide B	354	31	3.994	0.493
7	Hs.529860	LINC00963	3.351	long intergenic non-protein coding RNA 963	6945	677	2.514	-0.838
8	Hs.943	IL32	3.288	interleukin 32	2311	235	2.574	-0.714
9	Hs.104672	FILIP1L	3.238	filamin A interacting protein 1-like	724	76	2.847	-0.390
10	Hs.943	IL32	3.137	interleukin 32	1207	136	2.282	-0.855
11	Hs.349204	SOST	3.102	sclerostin	872	101	3.102	0.000
12	Hs.513463	NUPR1	3.083	nuclear protein, transcriptional regulator, 1	1523	179	3.803	0.720
13	Hs.463652	LOC645638	3.079	WDNM1-like pseudogene	597	70	2.776	-0.303
14	Hs.469881	LIMS2	3.004	LIM and senescent cell antigen-like domains 2	233	29	1.337	-1.667
15	Hs.64746	CLIC3	2.957	chloride intracellular channel 3	475	61	-0.522	-3.479
16	Hs.334629	SLN	2.923	sarcolipin	222	29	1.995	-0.928
17	Hs.744068	ANXA8L1	2.842	annexin A8-like 1	709	98	0.000	-2.842
18	Hs.591645	COL4A4	2.813	collagen, type IV, alpha 4	212	30	2.001	-0.812
19	Hs.463652	LOC645638	2.810	WDNM1-like pseudogene	404	57	2.743	-0.067
20	Hs.644105	CD24	2.744	CD24 molecule	1866	277	-0.833	-3.576
21	Hs.529571	RBP1	2.728	retinol binding protein 1, cellular	795	119	0.000	-2.728
22	Hs.162016	SULF2	2.721	sulfatase 2	784	118	4.327	1.606
23	Hs.40098	GREM1	2.718	gremlin 1, DAN family BMP antagonist	2974	449	4.544	1.825
24	Hs.40098	GREM1	2.695	gremlin 1, DAN family BMP antagonist	832	128	5.232	2.537
25	Hs.655519	SYNPO2	2.655	synaptopodin 2	180	28	1.521	-1.134
26	Hs.587558	NCF2	2.640	neutrophil cytosolic factor 2	769	123	1.163	-1.478
27	Hs.471751	ACKR3	2.614	atypical chemokine receptor 3	10537	1712	2.614	0.000
28	Hs.448497	PODNL1	2.601	podocan-like 1	315	52	2.940	0.340
29	Hs.112242	C15orf48	2.600	chromosome 15 open reading frame 48	4080	669	0.000	-2.600
30	Hs.516971	FOXS1	2.543	forkhead box S1	1393	238	1.172	-1.370
31	Hs.120949	CD36	2.315	CD36 molecule (thrombospondin receptor)	371	74	3.677	1.362
32	Hs.514451	SSTR2	2.310	somatostatin receptor 2	234	47	3.629	1.318
33	Hs.459088	CEMIP	2.263	cell migration inducing protein, hyaluronan binding	519	107	0.945	-1.318
34	Hs.592145	WISP2	2.213	WNT1 inducible signaling pathway protein 2	1297	278	3.028	0.815
35	Hs.666723	C1QTNF1-AS1	2.171	C1QTNF1 antisense RNA 1	257	57	2.701	0.530
36	Hs.224698	PROC	2.151	protein C (inactivator of coagulation factors Va and VIIIa)	505	113	1.201	-0.950
37	Hs.531251	TRAF1	2.144	TNF receptor-associated factor 1	193	43	0.951	-1.193
38	Hs.75535	MYL2	2.138	myosin, light chain 2, regulatory, cardiac, slow	126	29	0.923	-1.215
39	Hs.647971	PDE5A	2.126	phosphodiesterase 5A, cGMP-specific	3568	813	2.126	0.000
40	Hs.513147	SLC7A7	2.091	solute carrier family 7 (amino acid transporter light chain, y+L system), member 7	167	39	1.052	-1.039
41	Hs.567498	FAM198B	2.086	family with sequence similarity 198, member B	1955	458	1.003	-1.083
42	Hs.529053	C3	2.047	complement component 3	199	48	1.009	-1.038
43	Hs.266616	ZNF608	2.029	zinc finger protein 608	154	37	0.390	-1.639
44	Hs.365706	MGP	2.011	matrix Gla protein	9199	2268	3.950	1.939
45	Hs.365706	MGP	2.000	matrix Gla protein	4592	1142	3.834	1.834
46	Hs.504687	MYL9	2.000	myosin, light chain 9, regulatory	6356	1580	2.095	0.095
47	Hs.591127	RASGRP1	1.994	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	236	59	2.110	0.116
48	Hs.172928	COL1A1	1.994	collagen, type I, alpha 1	40626	10143	2.700	0.707
49	Hs.69771	CFB	1.993	complement factor B	200	50	2.017	0.024
50	Hs.201398	C1QTNF1	1.965	C1q and tumor necrosis factor related protein 1	497	127	4.137	2.173

51	Hs.654775	DNM3	1.963	dynamin 3	181	46	-1.648	-3.611
52	Hs.591683	DCLK2	1.963	doublecortin-like kinase 2	2778	708	3.971	2.008
53	Hs.124940	RND1	1.933	Rho family GTPase 1	236	61	2.603	0.670
54	Hs.660996	USP30-AS1	1.931	USP30 antisense RNA 1	108	28	1.800	-0.132
55	Hs.143811	ODAM	1.928	odontogenic, ameloblast asssociated	137	36	2.951	1.024
56	Hs.137007	KLHDC7B	1.924	kelch domain containing 7B	182	48	3.872	1.948
57	Hs.156540	SCG5	1.910	secretogranin V (7B2 protein)	424	112	0.926	-0.984
58	Hs.558865	CES1	1.870	carboxylesterase 1	660	180	4.692	2.821
59	Hs.518921	NPNT	1.867	nephronectin	999	272	1.950	0.082
60	Hs.224698	PROC	1.844	protein C (inactivator of coagulation factors Va and VIIIa)	1056	293	0.808	-1.036
61		LOC102723834	1.833	uncharacterized LOC102723834	562	157	2.168	0.335
62	Hs.86447	TNFRSF9	1.799	tumor necrosis factor receptor superfamily, member 9	315	90	1.507	-0.292
63	Hs.558865	CES1	1.794	carboxylesterase 1	6293	1804	5.857	4.063
64	Hs.1027	RRAD	1.793	Ras-related associated with diabetes	4794	1376	1.793	0.000
65	Hs.634554		1.786		324	93	2.103	0.317
66	Hs.592128	CDRT1	1.786	CMT1A duplicated region transcript 1	106	31	0.886	-0.900
67	Hs.44278	RAB17	1.745	RAB17, member RAS oncogene family	107	32	-2.352	-4.096
68	Hs.200644		1.740		103	31	0.610	-1.130
69	Hs.532768	SERPINF1	1.735	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	156	47	0.739	-0.997
70	Hs.146186	SIM2	1.731	single-minded family bHLH transcription factor 2	87	26	2.771	1.040
71	Hs.519024	IGFN1	1.726	immunoglobulin-like and fibronectin type III domain containing 1	205	62	2.429	0.703
72	Hs.591683	DCLK2	1.708	doublecortin-like kinase 2	176	54	2.688	0.980
73	Hs.442619	MSC	1.690	musculin	4112	1267	5.998	4.308
74	Hs.631804	CATSPER3	1.690	cation channel, sperm associated 3	125	38	0.642	-1.048
75		XLOC_004874	1.689		83	26	0.711	-0.979
76	Hs.48950	DACT1	1.682	dishevelled-binding antagonist of beta-catenin 1	888	275	3.747	2.065
77	Hs.659104	RNF150	1.671	ring finger protein 150	124	39	0.502	-1.169
78	Hs.435228	SYNPO	1.664	synaptopodin	4663	1464	2.801	1.137
79	Hs.513779	CRISPLD2	1.641	cysteine-rich secretory protein LCCL domain containing 2	134	43	1.641	0.000
80	Hs.558865	CES1	1.633	carboxylesterase 1	93	30	3.244	1.611
81	Hs.741750	IL4I1	1.616	interleukin 4 induced 1	296	96	0.000	-1.616
82	Hs.502876	RHOB	1.589	ras homolog family member B	931	308	0.162	-1.428
83	Hs.41707	HSPB3	1.575	heat shock 27kDa protein 3	26449	8825	5.909	4.334
84	Hs.525626	PACS2	1.551	phosphofurin acidic cluster sorting protein 2	200	68	-1.103	-2.653
85	Hs.36053	NREP	1.537	neuronal regeneration related protein	276	95	0.000	-1.537
86	Hs.458355	C1S	1.533	complement component 1, s subcomponent	17317	5948	4.593	3.060
87	Hs.696346	SYT15	1.524	synaptotagmin XV	159	55	0.139	-1.384
88	Hs.555934	SPINK4	1.516	serine peptidase inhibitor, Kazal type 4	130	45	1.675	0.159
89	Hs.458355	C1S	1.505	complement component 1, s subcomponent	544	191	3.744	2.239
90	Hs.275775	SEPP1	1.501	selenoprotein P, plasma, 1	1951	685	2.423	0.922
91	Hs.406475	LUM	1.495	lumican	6970	2458	1.658	0.163
92	Hs.200644		1.470		731	262	0.401	-1.069
93	Hs.744937	RABGAP1L	1.457	RAB GTPase activating protein 1-like	450	163	0.649	-0.808
94	Hs.364739	LOC102724927	1.454	uncharacterized LOC102724927	120	43	-0.079	-1.532
95	Hs.502876	RHOB	1.453	ras homolog family member B	1582	574	0.181	-1.272
96	Hs.58877	HMCN1	1.452	hemicentin 1	1818	661	1.962	0.509
97	Hs.93675	C10orf10	1.452	chromosome 10 open reading frame 10	445	162	1.270	-0.182
98	Hs.516036	TRIM54	1.452	tripartite motif containing 54	174	63	0.384	-1.067
99	Hs.99004	MURC	1.441	muscle-related coiled-coil protein	1293	473	2.255	0.814
100	Hs.2681	GAST	1.440	gastrin	63	23	1.132	-0.307

Supplementary Table S4. Mutational status of cell lines used in this study

	KRAS	CDKN2A	CTNNB1	PIK3CA	PTEN	RB1	STK11	TP53	NF1	NF2
Epithelial										
NCI-H358	p.G12C	WT	p.T75A	WT	WT	WT	WT	WT	WT	WT
NCI-H1573	p.G12A	WT	WT	WT	WT	WT	p.S216F	p.R248L	WT	WT
SW900	p.G12V	WT	WT	WT	WT	WT	WT	p.Q167*	WT	WT
LU65	p.G12C	p.G135G	WT	WT	WT	p.S82*	c.583_600del93	p.E11Q, p.T125T	p.H310H	WT
RERF-LC-AD2	p.G12V	WT	WT	WT	WT	WT	WT	p.A159V	WT	WT
Mesenchymal										
A549	p.G12S	del	WT	WT	WT	WT	p.Q37*	WT	WT	WT
Calu-1	p.G12C	WT	WT	WT	WT	WT	WT	del	WT	WT
Calu-6	p.Q61K	del	WT	WT	WT	WT	WT	p.R196*	WT	WT
HCC2108	p.Q61H	WT	WT	WT	WT	WT	p.E223K	p.G154V	WT	WT
LU99	p.G12C	del	WT	p.T1025A	WT	WT	WT	WT	WT	WT
NCI-H1792	p.G12C	p.W110*	WT	WT	WT	WT	WT	c.672+1G>A in intron	WT	WT
NCI-H2030	p.G12C	WT	WT	WT	WT	WT	p.E317*	p.G262V	WT	WT
NCI-H23	p.G12C	WT	WT	WT	WT	WT	p.W332*	p.M246I	WT	WT
NCI-H460	p.Q61H	del	WT	p.E545K	WT	WT	p.Q37*	WT	WT	WT
SK-LU-1	p.G12D	del	WT	WT	WT	WT	WT	p.H193R	WT	WT
SW1573	p.G12C	del	p.S33F	p.K111E	WT	WT	WT	WT	WT	WT

Supplementary Table S5: Details of the antibodies

	Company	Catalogue #
FGFR1	Cell signaling	9740
phospho-EGFR (Tyr1068)	Cell signaling	3777
EGFR	R&D systems	AF231
phospho-HER2 (Tyr1221/1222)	Cell signaling	2243
HER2	Cell signaling	4290
phospho-HER3 (Tyr1289)	Cell signaling	4791
HER3	Cell signaling	12708
phospho-HER4(Tyr1284)	Cell signaling	4757
HER4	Cell signaling	4795
IGFR	Cell signaling	9750
phospho-IRS1 (Ser612)	Cell signaling	3203
IRS1	Merck Millipore	05-1085
phospho-MET (Tyr1234/1235)	Cell signaling	3077
MET	Cell signaling	3127
phospho-PDGFRα (Tyr754)	Cell signaling	2992
PDGFRα	Cell signaling	5241
phospho-FRS2α (Tyr436)	Cell signaling	3861
FRS2	Santa Cruz Biotechnology	sc-8318
phospho-AXL(Tyr702)	Cell signaling	5724
AXL	Cell signaling	8661
E-cadherin	Cell signaling	3195
Vimentin	Cell signaling	5741

phospho-AKT (Ser473)	Cell signaling	4060
AKT	Cell signaling	9272
phospho-ERK	R&D systems	AF1018
ERK	R&D systems	AF1576
phospho-S6 (Ser235/236)	Cell signaling	4858
S6	Cell signaling	2217
Cleaved PARP (Asp214)	Cell signaling	5625
β-actin	Cell signaling	4970
ZEB1	Cell signaling	3396
SPRY4	Santa Cruz Biotechnology	sc-30051
p85	Merck Millipore	ABS-233
DUSP6	Abcam	ab76310