1/2012	**72yF presented at the MSKCC with new dx SCC left lateral tongue clinically T4aN1M0 (AJCC staging).** **Social history: never smoker; no alcohol history, Cantonese-speaking** **Med History: HTN, peptic ulcer disease**
1/18/2012 -4/2/2012	Due to rapid tumor growth was referred for induction chemo carboplatin (AUC 2) + paclitaxel (90 mg/m^2) given together on a 3 weeks on/1 week off cycle, for 3 cycles. She experienced initial transient response followed by rebound progression
4/27/2012	Total glossectomy, mandibulotomy, bilateral neck dissections. Pathology: squamous cell carcinoma, moderately differentiated, T4aN2bM0 4.5-cm tumor invading skeletal muscle, 2 left neck nodes positive with extranodal extension identified. **This is the sample referred to as Cetux^{Sen}**
6/19/2012 -8/9/2012	Radiation therapy (maximum total dose 6804 cGy) concurrent with weekly cisplatin (30 mg/m^2 weekly × 6 weeks)
8/23/2012 imaging	Recurrent primary tumor and b/l lung mets
8/27/2012	Began cetuximab 500 mg/m^2 q2W, and continues on treatment 10+ months with near complete response (9/30/2013 CT scans were "best response")
1/7/2014 imaging	Lesion in right upper lobe of lung (2.1 cm) is clearly increased compared with prior CT chest of September 2013, when it was subcentimeter in size. This was an isolated progressing lesion.
2/5/2014	Right lung core biopsy: + SCC
2/25/2014	Right upper lung lobectomy. + SCC. Tumor size 4.2 × 2.9 × 1.7 cm. **This is the sample referred to as Cetux ^{Res}.** Due to surgery, there was brief interruption in cetuximab dosing. After the 2/10/2014 dose, the next cetuximab dose was on 3/10/2014, at which time cetuximab 500 mg/m2 q2W resumed
5/27/2014 imaging	Isolated progression of a single lesion, with new hypermetabolic nodule in upper pole of right kidney
6/18/2014	Biopsy of right kidney mass: + SCC. There was insufficient material on this sample to support correlative tests
7/28/2014	Compared with PET/CT of 5/27/2014, multiple new bilateral pulmonary mets and increase in mass of right kidney mass
8/4/2014	Begins clinical trial of cetuximab 500 mg/m^2 weekly + HER3 inhibitor (LJM716), but experiences rapid disease progression. Last dose on study was 9/2/2014 Patient received additional palliative chemotherapy off protocol, but experienced further progression of disease
10/8/2014	Died

Figure S1

A

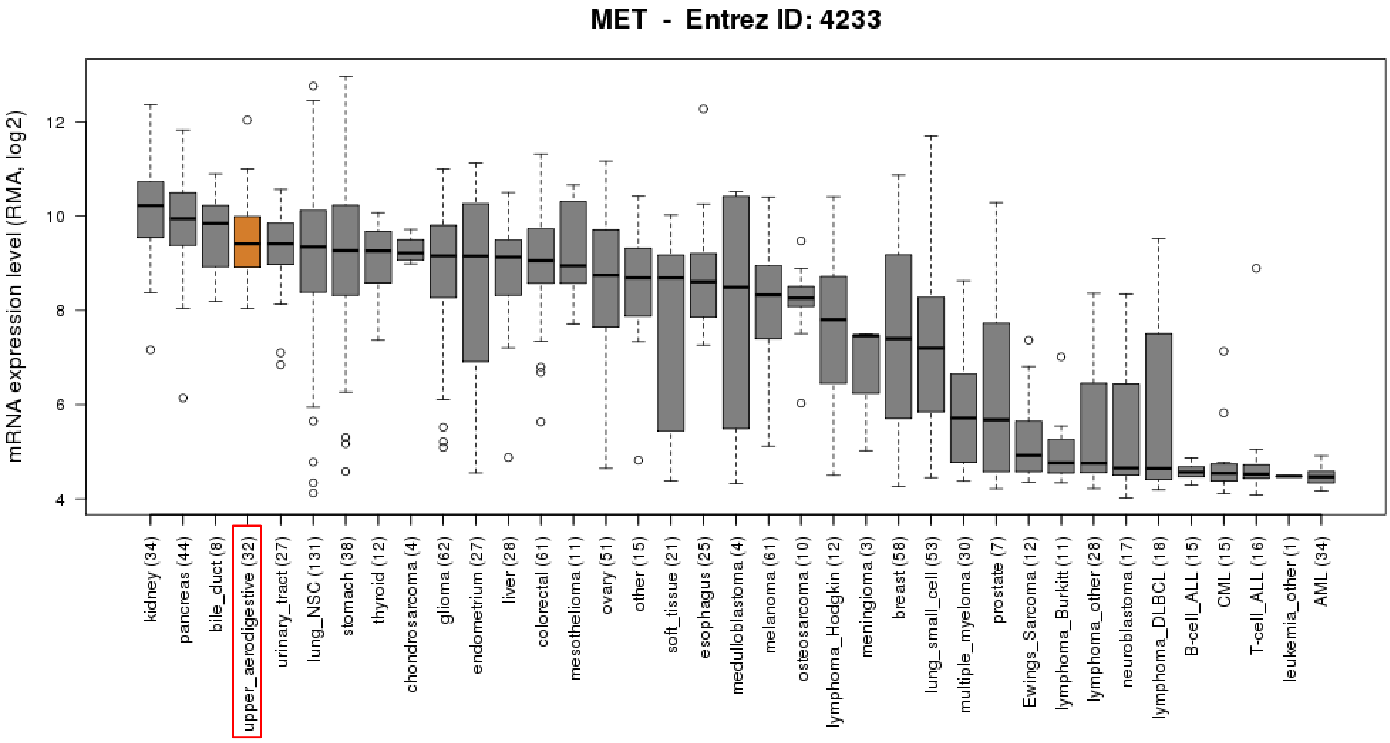
	Gene	Base Mean	Log2 Fold Change	p-adj
1	SFTPB	26760.90	11.28	>E-295
2	SFTPA2	20034.24	11.26	>E-295
3	KRT16	32990.98	-3.48	>E-295
4	**MET**	**7907.71**	**3.21**	**4.71E-294**
5	LTF	4624.83	8.42	1.10E-279
6	SLC34A2	7936.38	9.91	4.52E-257
7	ABCA3	2014.43	6.35	7.13E-243
8	ROS1	2382.55	7.11	1.15E-235
9	SFTPA1	14317.45	11.41	1.88E-232
10	CA12	4068.92	-2.99	3.86E-229
11	SBSN	2839.90	-2.89	1.7E-192
12	EGFR	14898.22	-2.37	1.4E-191
13	S100A2	21689.51	-2.39	6E-187
14	CAV1	6879.32	2.49	7.4E-187
15	CLU	2014.20	3.29	3.2E-184
16	COMP	1446.65	-3.43	2E-177
17	NAPSA	2196.67	7.95	3E-170
18	KRT6A	58497.21	-2.30	1.2E-166
19	MUC1	1436.96	3.43	3.3E-164
20	KRT6B	8580.45	-2.78	1.6E-157
21	DMBT1	3860.40	9.61	5.6E-155
22	SUMF2	4567.64	-2.42	1.3E-152
23	CALML3	8081.78	-2.66	1.3E-145
24	FAM46C	2174.29	2.69	8E-141
25	KRT14	80923.04	-2.04	1.4E-139

B

Name	Size	Enrichment Score	Normalized Enrichment Score	Normalized p-val	FDR q-val	FWER p-val
CHR14Q32	129	0.89197	2.200935	>0.001	>0.001	>0.001
CHR2P11	53	0.885296	1.947813	>0.001	>0.001	>0.001
CHR22Q11	108	0.78945	1.908233	>0.001	4.47E-04	0.001
CHR7Q31	**50**	**0.877718**	**1.895376**	**>0.001**	**3.35E-04**	**0.001**
CHR5P13	35	0.819092	1.658656	0.011905	0.16508	0.487
CHR2Q24	24	0.812358	1.641653	0.015748	0.175098	0.575
CHR18Q11	24	0.817887	1.578883	0.02681	0.325985	0.848

Figure S2

A



Cell Line	mRNA expression level (RMA, Log2)
SNU1076	9.8722
HSC4	8.1258
CAL33	9.9910
HSC2	8.6362
FADU	8.7896

B

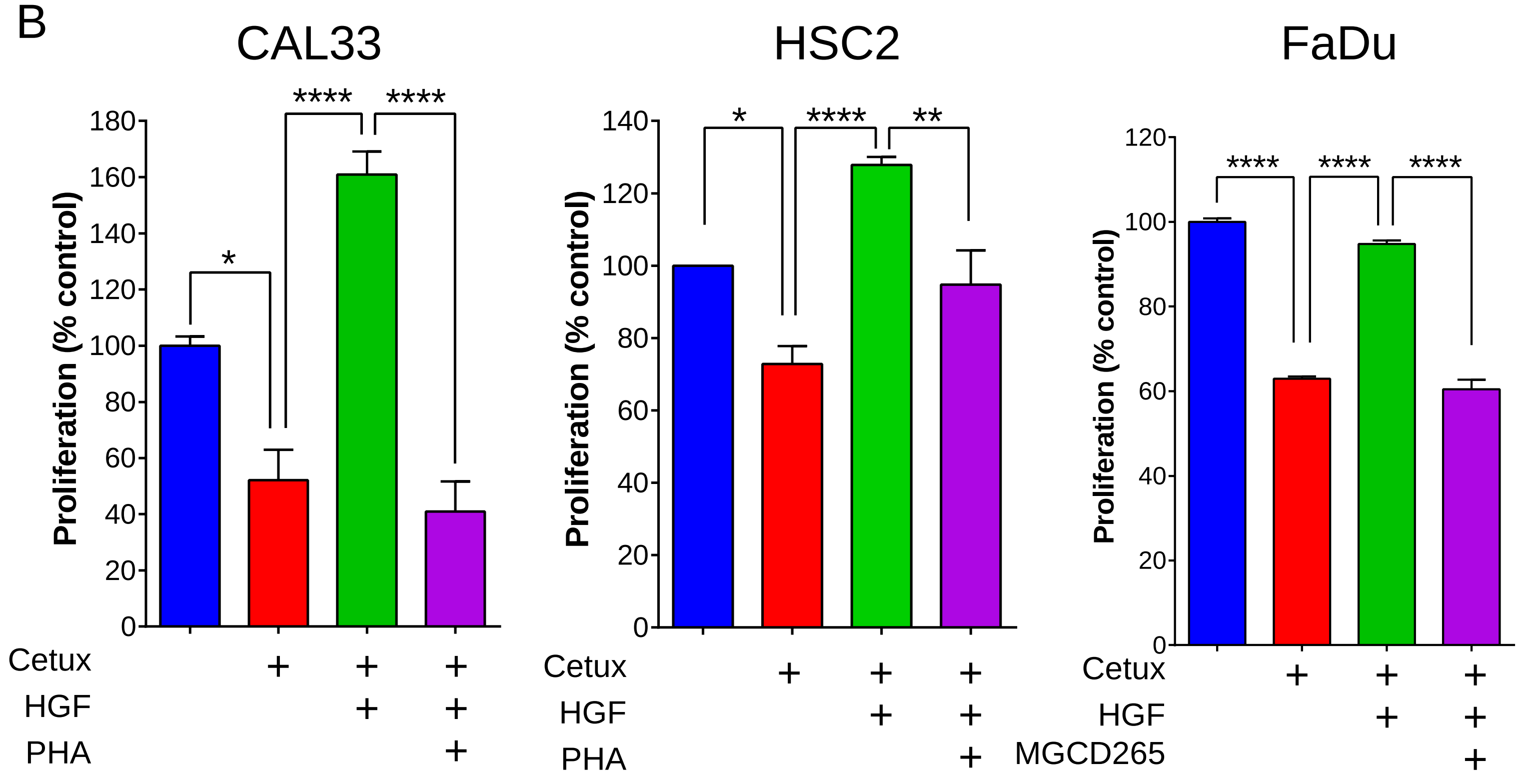


Figure S3

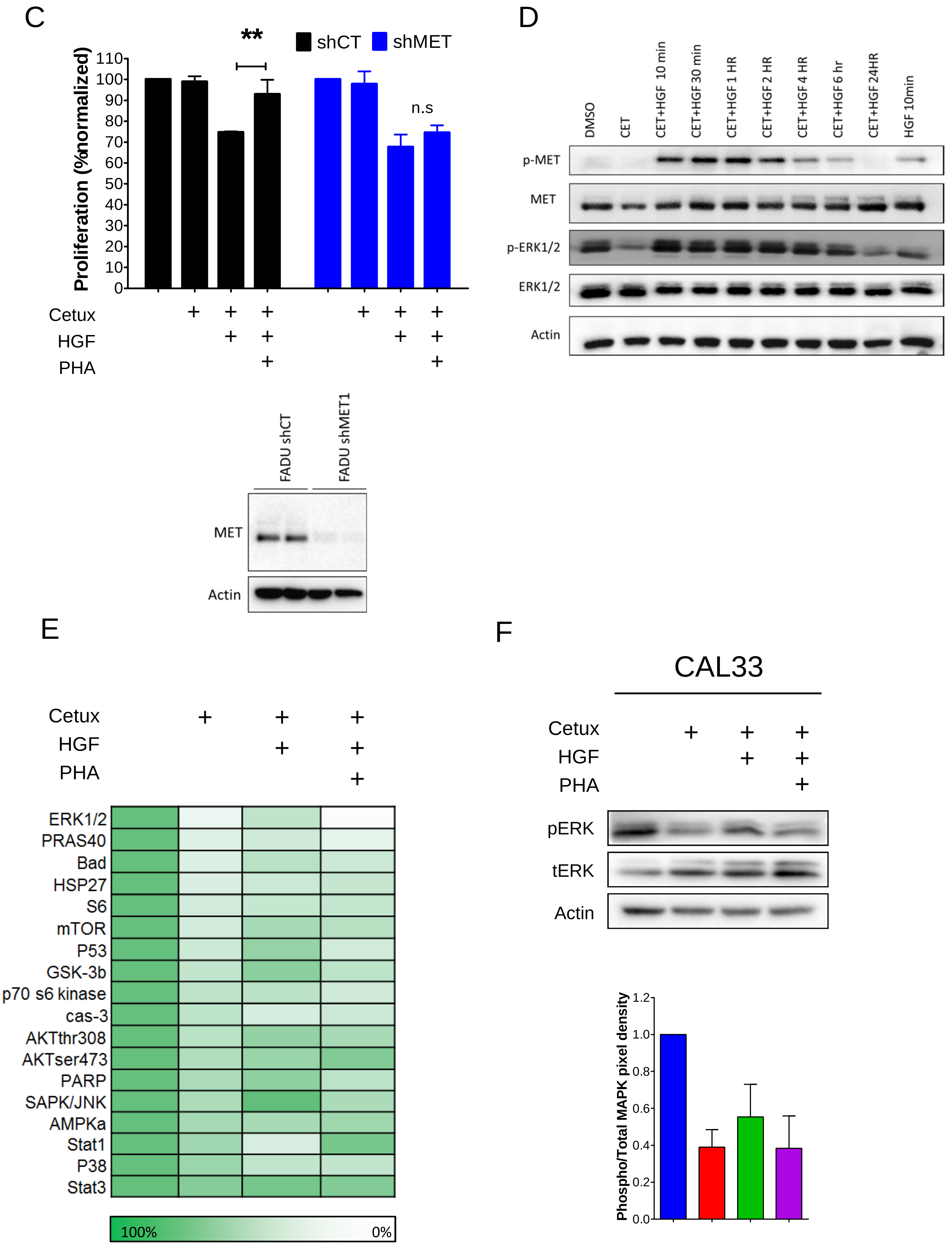

C
**
shCT
shMET
n.s
Proliferation (%normalized)
110
100
90
80
70
60
50
40
30
20
10
0
Cetux + + + + + +
HGF + + + +
PHA + +
FADU shCT
FADU shMET1
MET
Actin
D
DMSO
CET
CET+HGF 10 min
CET+HGF 30 min
CET+HGF 1 HR
CET+HGF 2 HR
CET+HGF 4 HR
CET+HGF 6 hr
CET+HGF 24HR
HGF 10min
p-MET
MET
p-ERK1/2
ERK1/2
Actin
E
Cetux + + +
HGF + +
PHA +
ERK1/2
PRAS40
Bad
HSP27
S6
mTOR
P53
GSK-3b
p70 s6 kinase
cas-3
AKTthr308
AKTser473
PARP
SAPK/JNK
AMPKa
Stat1
P38
Stat3
100%
0%
F
CAL33
Cetux + + +
HGF + +
PHA +
pERK
tERK
Actin
Phospho/Total MAPK pixel density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Cetux + + +
HGF + +
PHA +

Figure S3

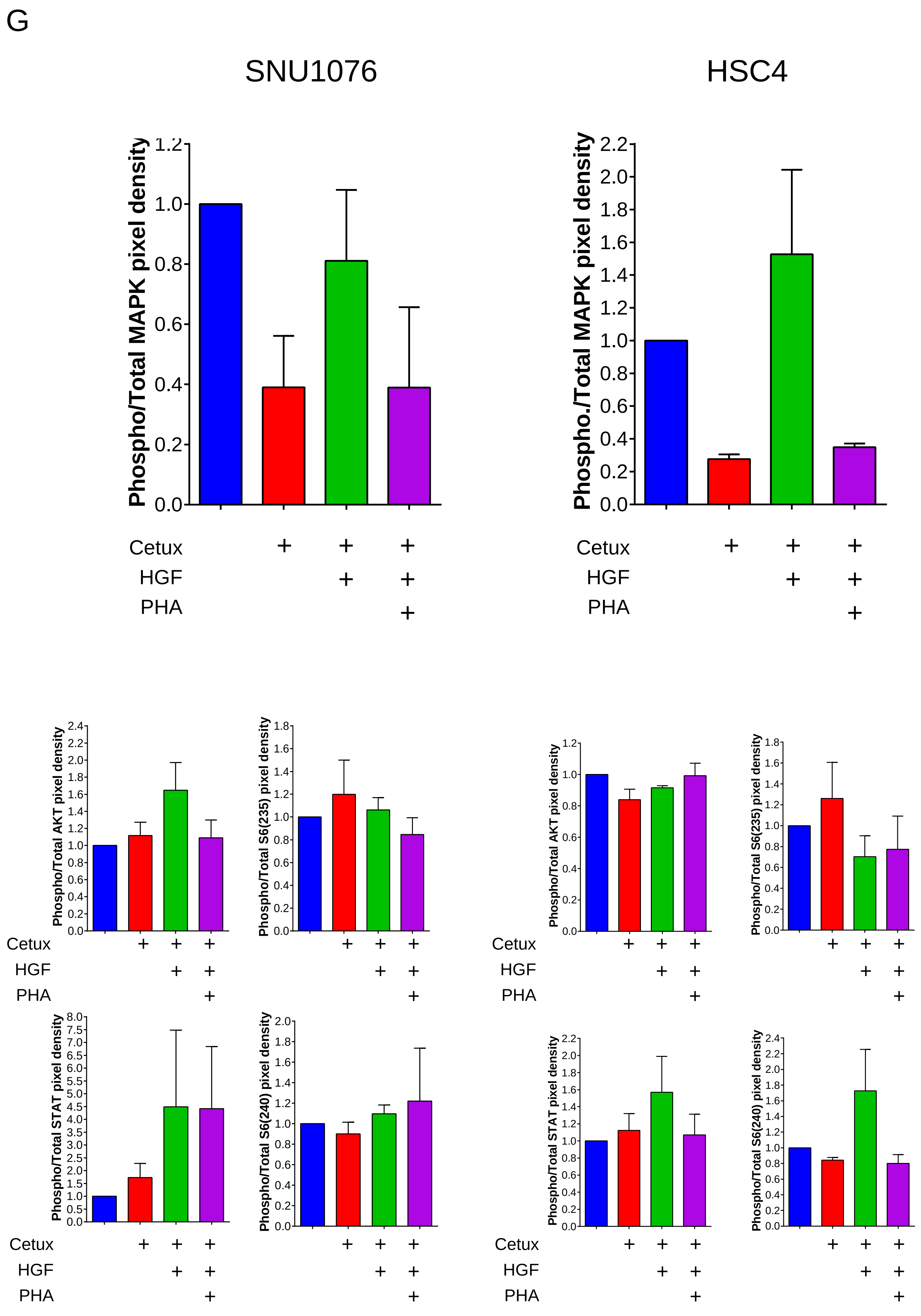

G
SNU1076
HSC4
Phospho/Total MAPK pixel density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Phospho./Total MAPK pixel density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Cetux + + +
HGF + +
PHA +
Phospho/Total AKT pixel density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Phospho/Total S6(235) pixel density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Phospho/Total STAT pixel density
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
Phospho/Total S6(240) pixel density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4

Figure S3

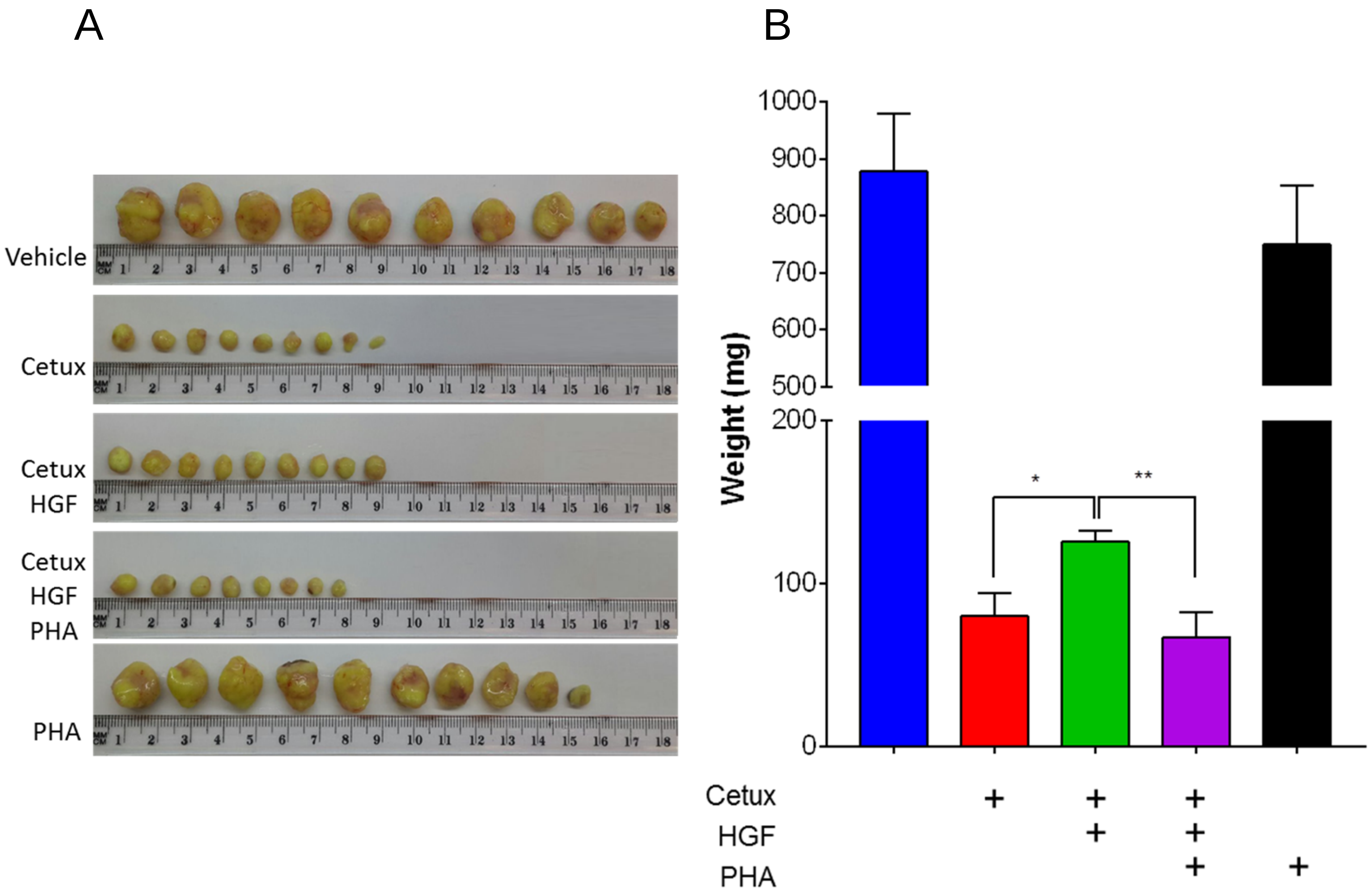
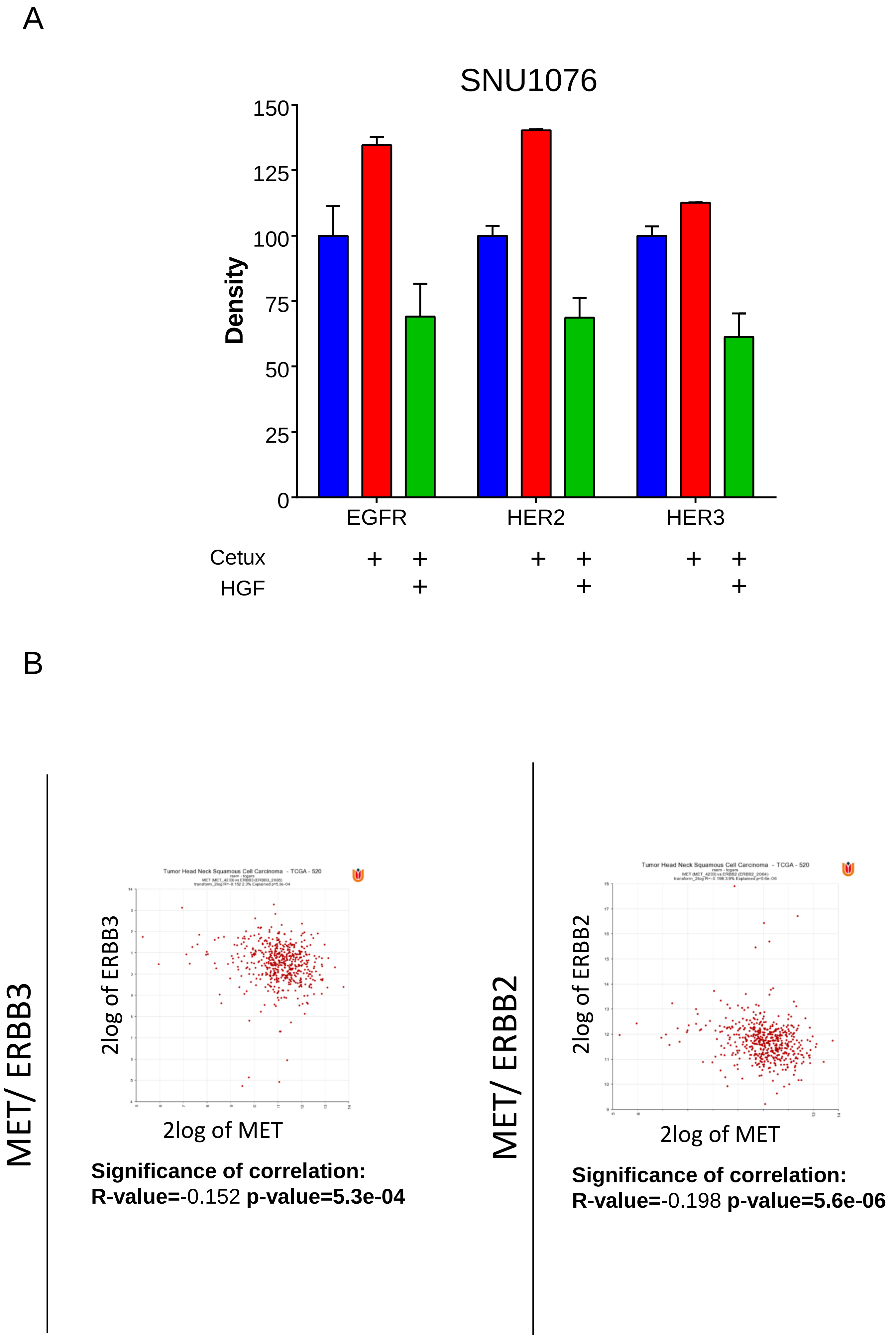

A
Vehicle
Cetux
Cetux
HGF
Cetux
HGF
PHA
PHA
B
Weight (mg)
1000
900
800
700
600
500
200
100
0
*
**
Cetux
HGF
PHA
+
+
+
+
+
+
+

Figure S4



Significance of correlation:
R-value=-0.152 **p-value=5.3e-04**

Significance of correlation:
R-value=-0.198 **p-value=5.6e-06**

Figure S5

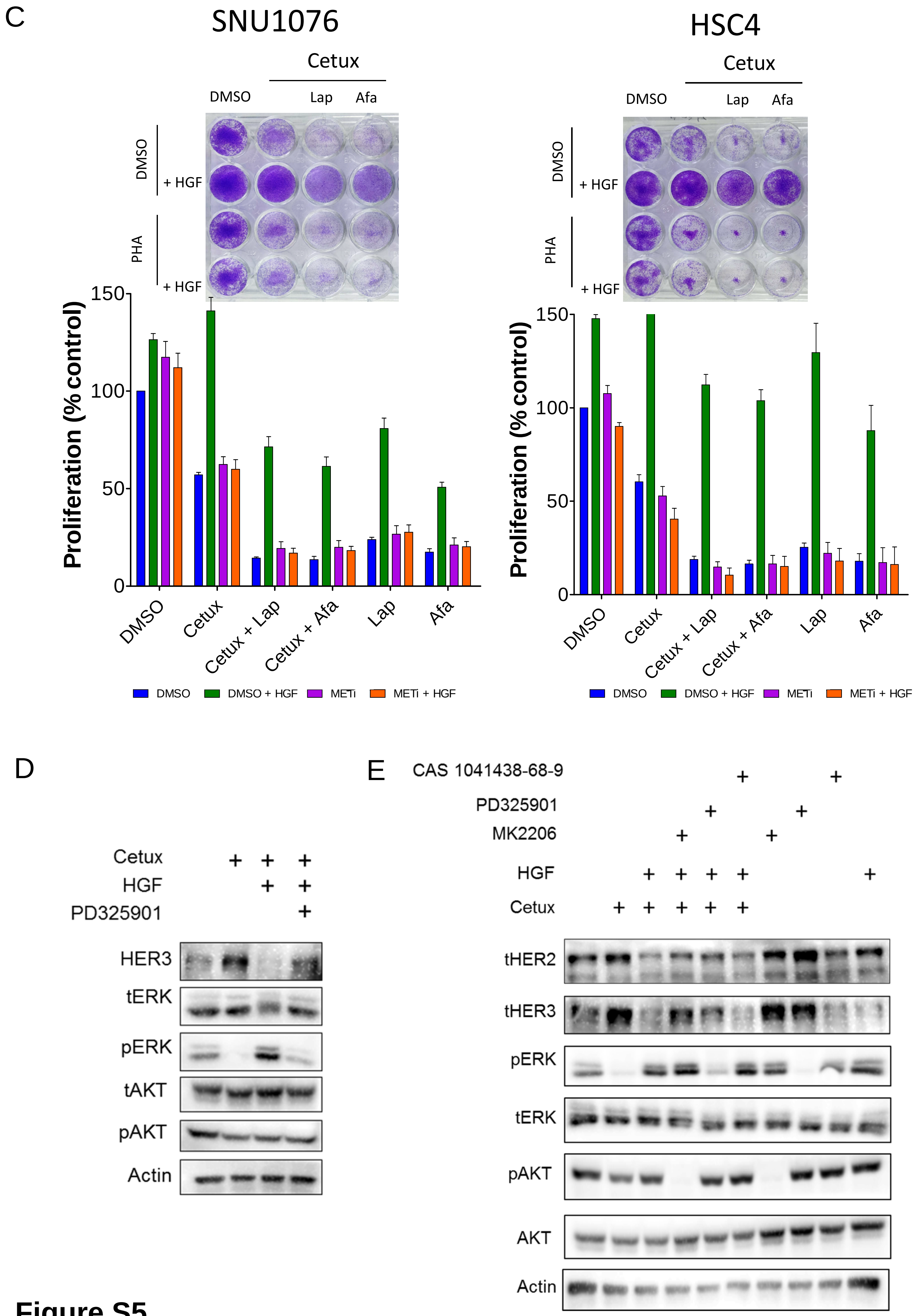

C
SNU1076
Cetux
DMSO
Lap
Afa
DMSO
+ HGF
PHA
+ HGF
Proliferation (% control)
150
100
50
0
DMSO
Cetux
Cetux + Lap
Cetux + Afa
Lap
Afa
DMSO
DMSO + HGF
METi
METi + HGF
HSC4
Cetux
DMSO
Lap
Afa
DMSO
+ HGF
PHA
+ HGF
Proliferation (% control)
150
100
50
0
DMSO
Cetux
Cetux + Lap
Cetux + Afa
Lap
Afa
DMSO
DMSO + HGF
METi
METi + HGF
D
Cetux + + +
HGF + +
PD325901 +
HER3
tERK
pERK
tAKT
pAKT
Actin
E
CAS 1041438-68-9 + +
PD325901 + +
MK2206 + +
HGF + + + + +
Cetux + + + + +
tHER2
tHER3
pERK
tERK
pAKT
AKT
Actin

Figure S5

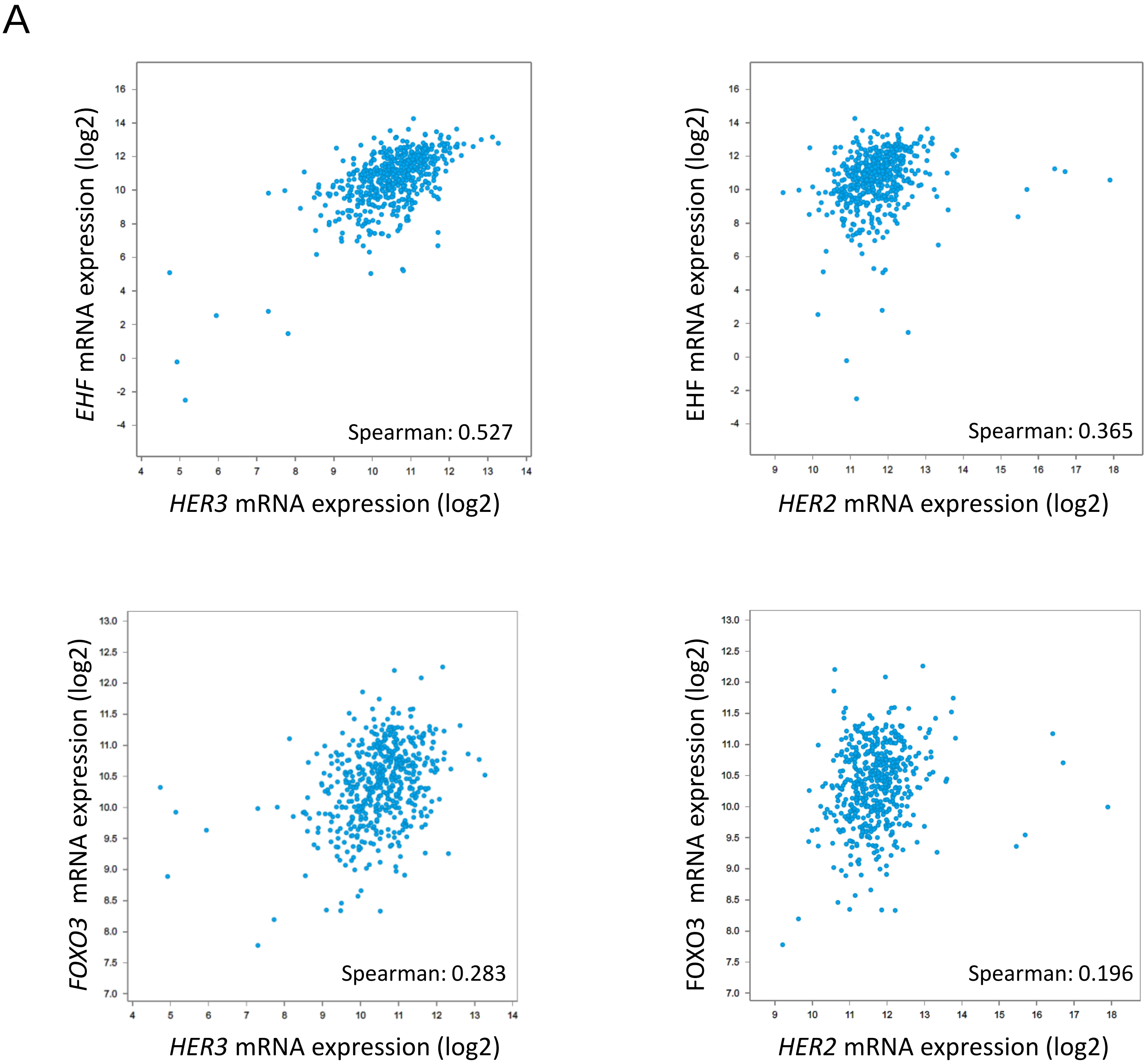

A
EHF mRNA expression (log2)
HER3 mRNA expression (log2)
Spearman: 0.527
16
14
12
10
8
6
4
2
0
-2
-4
4
5
6
7
8
9
10
11
12
13
14
EHF mRNA expression (log2)
HER2 mRNA expression (log2)
Spearman: 0.365
9
10
11
12
13
14
15
16
17
18
FOXO3 mRNA expression (log2)
HER3 mRNA expression (log2)
Spearman: 0.283
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
FOXO3 mRNA expression (log2)
HER2 mRNA expression (log2)
Spearman: 0.196

Figure S6

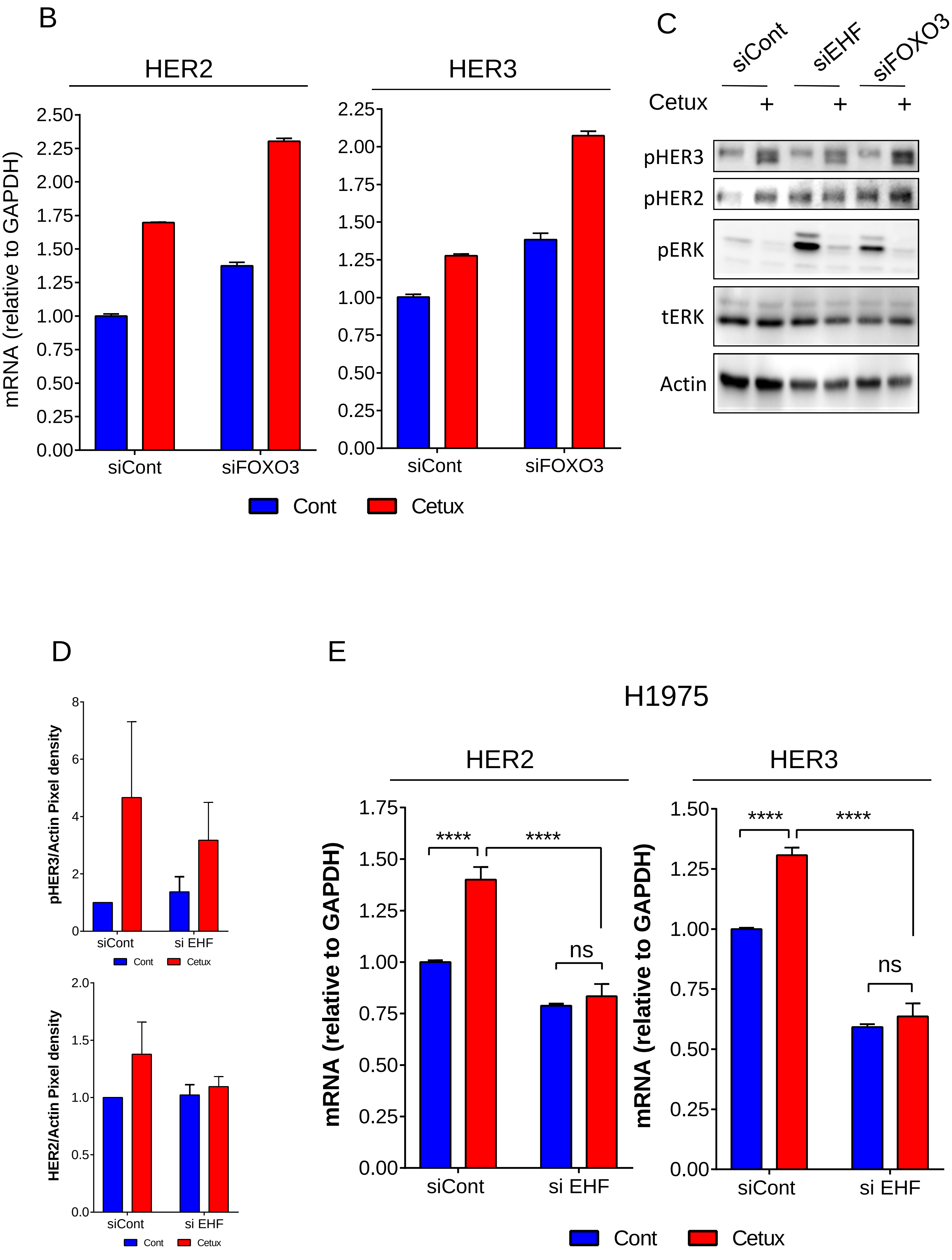

B
HER2
HER3
mRNA (relative to GAPDH)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
siCont
siFOXO3
Cont
Cetux
C
siCont
siEHF
siFOXO3
Cetux
+
+
+
pHER3
pHER2
pERK
tERK
Actin
D
pHER3/Actin Pixel density
8
6
4
2
0
siCont
si EHF
Cont
Cetux
HER2/Actin Pixel density
2.0
1.5
1.0
0.5
0.0
E
H1975
HER2
HER3
mRNA (relative to GAPDH)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
****
****
ns
siCont
si EHF
Cont
Cetux

Figure S6

Figure S1:

Detailed summary of the history of the disease and treatment in the case report

Figure S2:

RNAseq gene expression analysis **(A)** and Locus analysis **(B)** of genes expressed differentially between the Cetux^{Res} and the Cetux^{Sen} tumors.

Figure S3:

(A) MET expression in the upper aerodigestive cell lines compared to other tumor cell lines extracted from the Cancer Cell Line Encyclopedia (CCLE), and MET expression level (RMA, log2) in the HNSCC cell lines used in this study **(B)** 5-day proliferation assay testing cetuximab (12.5ug/ml) efficacy in CAL33 and HSC2 and the FaDu HNSCC cell lines, with and without rHGF (50ng/ml) and the MET inhibitors PHA-665752 (1uM) or MGCD265 (1uM). Statistical significance was calculated using one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001). **(C)** 5-day proliferation assay testing cetuximab (12.5ug/ml) efficacy in FaDu shCont and FaDu shMET with and without rHGF (50ng/ml), low panel - Western blot of MET **(D)** Western blot for the indicated protein levels following 24h treatment of cetuximab (12.5ug/ml), with different duration of rHGF (50ng/ml) stimulation **(E)** Protein expression (Protein Array -Pathscan) of the SNU1076 HNSCC cell line treated with cetuximab (12.5ug/ml) for 24 hours with and without rHGF (50ng/ml) and the MET inhibitor PHA-665752 (1uM). Results are presented according to the cetuximab inhibition level. **(F)** Western blot for the indicated protein levels following 24h treatment of cetuximab (12.5ug/ml), with and without rHGF (50ng/ml) and the MET inhibitor PHA-665752 (1uM), in the CAL33 HNSCC cell lines and densitometry of phosphorylated ERK normalized to total ERK. Statistical significance was calculated using an unpaired t-test with Welch's correction (*p<0.05, **p<0.01)**.** **(G)** Densitometry of phosphorylated ERK normalized to total ERK Statistical significance was calculated using one way ANOVA test (*p<0.05, **p<0.01)

Figure S4:

(A) Excised tumors and the mice after 25-day treatments. **(B)** Tumor weight (mg) at the end point (day 25). Statistical significance was calculated using one-way ANOVA (*p<0.05, **p<0.01).

Figure S5:

(A) Quantification of the Proteome Profiler™ Antibody Arrays (R&D systems) pixel density for the indicated phospho-RTK in the SNU1076 HNSCC cell line following 24h treatment of cetuximab (12.5ug/ml), with and without rHGF (50ng/ml). **(B)** Dotplot analysis of mRNA expression levels of MET and ERBB2/ERBB3obtained from the TCGA dataset of HNSCC. **(C)** 5-day proliferation assay testing cetuximab (12.5ug/ml) efficacy in SNU1076 and HSC4 HNSCC cell lines, HER2/3 blockers afatininb (500 nM) and lapatinib (500 nM) with and without rHGF (50ng/ml) and the MET inhibitors PHA-665752 (1uM) . Representative experiment of two, each experiment was performed in biological triplicate. **(D)** Western blot for the indicated protein levels following 24h treatment of cetuximab (12.5ug/ml), with and without rHGF (50ng/ml) and the MEK1/2 inhibitor PD-0325901 (25nM), in the HSC4 HNSCC cell line. **(E)** Western blot for the indicated protein levels following 24h treatment of cetuximab (12.5ug/ml), with and without rHGF (50ng/ml) and the MEK1/2 inhibitor PD-0325901 (25 nM), AKT inhibitor MK22016 (1 uM), and STAT3 inhibitor CAS 1041438-68-9 (25nM) in the SNU1076 HNSCC cell line

Figure S6:

(A) Correlation between mRNA expression level (log2) of HER2 or HER3 with EHF (top panels) or Foxo3 (bottom panels), in the HNSCC datasets of TCGA. Blue dots indicate that neither of the genes is mutated. **(B)** mRNA levels of HER2 and HER3 following 24h treatment of cetuximab (12.5ug/ml), with either siCT or siFOXO3 in SNU1076 HNSCC. **(C)** Western blot for the indicated protein levels following 24h treatment of cetuximab (12.5ug/ml), with either siCT, siEHF, or siFoxo3 in SNU1076 HNSCC. **(D)** Densitometry of ERBBs normalized to total actin. **(E)** mRNA levels of HER2 and HER3 following 24h treatment of cetuximab (12.5ug/ml), with either siCont or siEHF in H1975 lung cancer cell line.