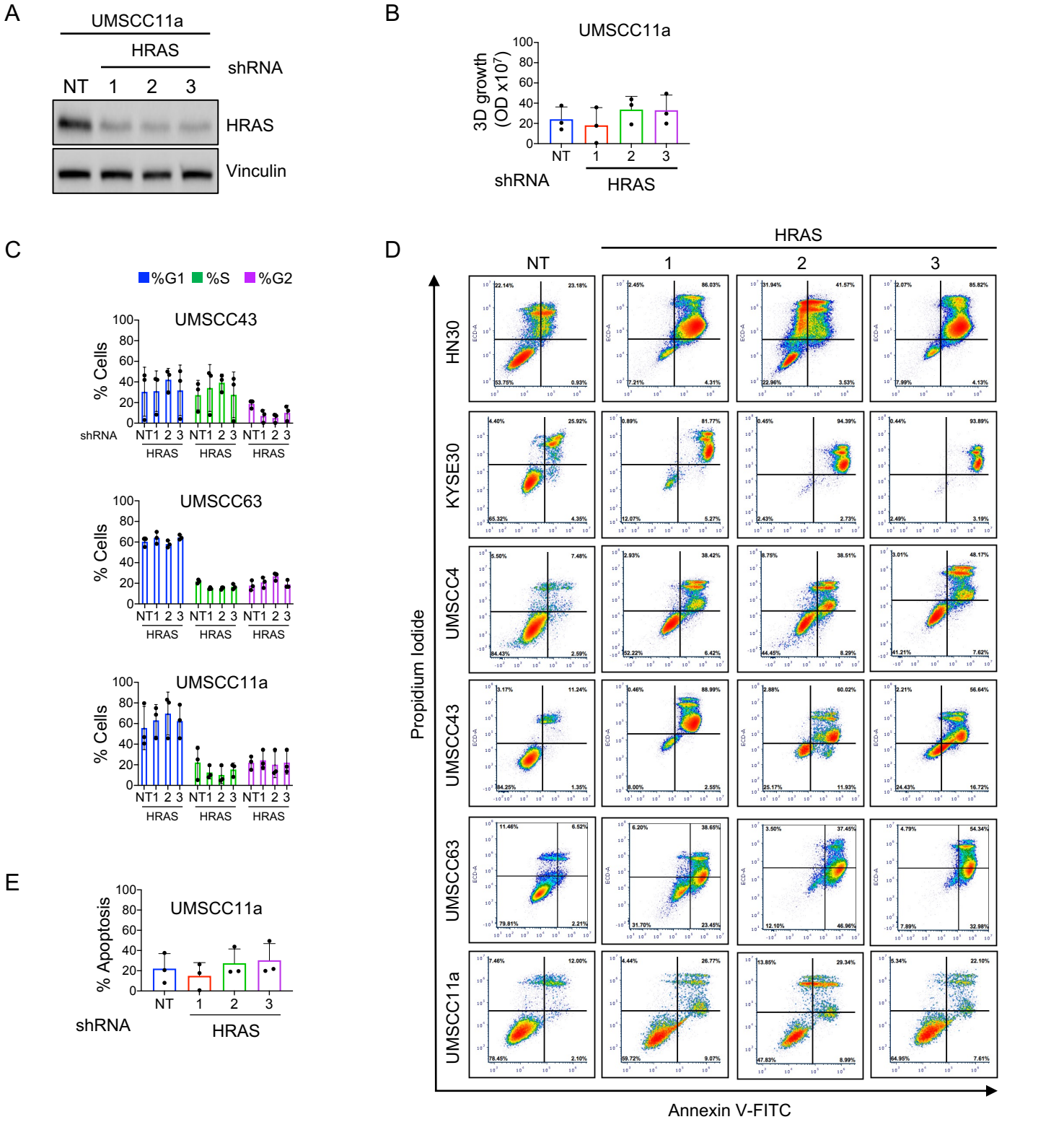


Supplementary Figure 1: Consequences of HRAS knockdown in an HRAS WT HNSCC cell line
UMSCC11a is HRAS WT; all other cell lines shown are HRAS mutant. Data are representative (A, D) or the average +/- SD (B, C, E) of at least 3 independent experiments.

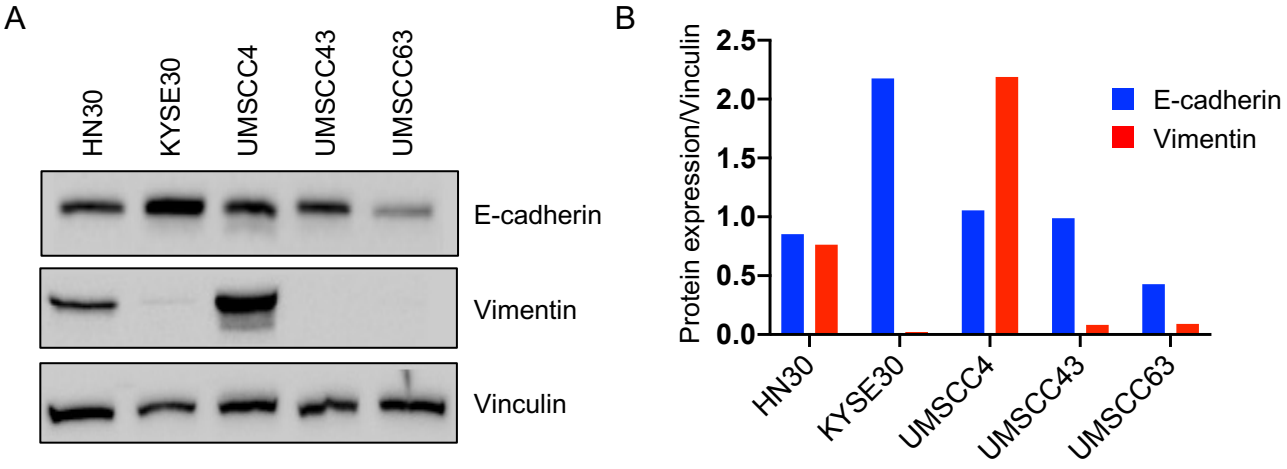
A) Western blot showing knockdown of HRAS protein in HRAS WT HNSCC line UMSCC11a. Cells were transduced with anti-HRAS shRNA and selected in puromycin for 72 hr.

B) Quantification of 3D colonies formed following HRAS knockdown. UMSCC11a cells treated as in panel A were grown in Matrigel for 2 weeks, then stained with alamarBlue.

C) Cell cycle distribution following HRAS knockdown, treated as in panel A. Only UMSCC11a cells are HRAS WT. Cells were stained with propidium iodide and subjected to flow cytometry. Related to Fig 1E-F.

D) Distribution of apoptotic cells following HRAS knockdown. Cells were stained with Annexin V-FITC and propidium iodide and apoptotic cells assessed by flow cytometry. Related to Fig 1G.

E) Quantification of apoptosis in UMSCC11a cells; representative example in panel D.



Supplementary Figure 2: HRAS mutant HNSCC cell lines exhibit heterogenous expression of EMT markers

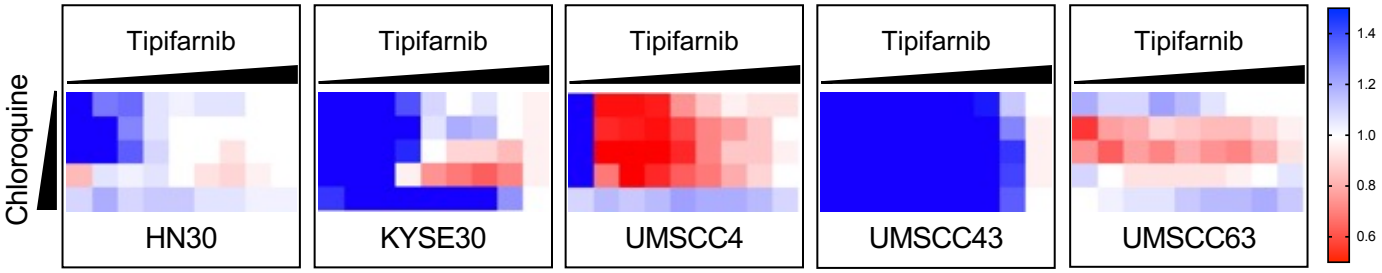
A) Western blot showing basal expression of E-cadherin and vimentin. Early passage HRAS mutant HNSCC cells were lysed and immunoblotted as indicated.

B) Densitometric quantification of data in panel (A). Expression was normalized to that of vinculin loading control.

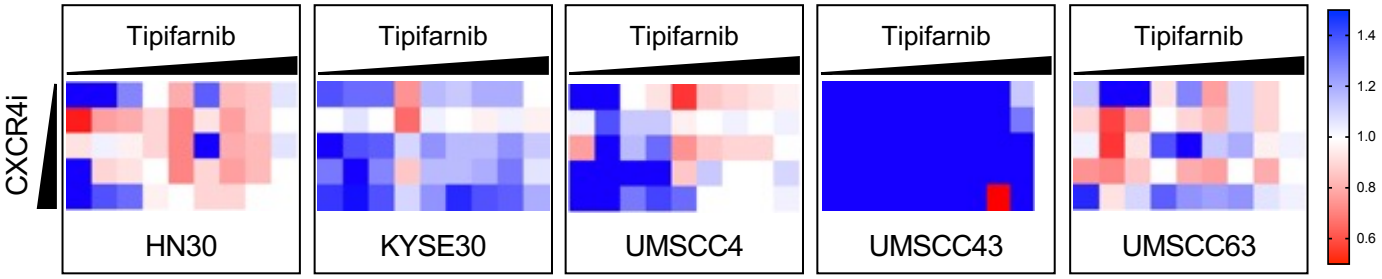
A

KEGG Pathway Analysis (Top 10% of hits)			
Tipifarnib (2 weeks)		Tipifarnib (4 weeks)	
Term description	Observed gene count	Term description	Observed gene count
Metabolic pathways	25	Pathways in cancer	17
PI3K-AKT signaling pathway	17	Insulin signaling pathway	14
Pathways in cancer	17	MAPK signaling pathway	14
Human papillomavirus infection	16	Thermogenesis	12
Neuroactive ligand-receptor interaction	13	PI3K-AKT signaling pathway	12
AMPK signaling pathway	12	Transcriptional misregulation in cancer	11
Insulin signaling pathway	11	Chemokine signaling pathway	11
Viral carcinogenesis	11	HTLV-I infection	11
cAMP signaling pathway	11	Human papillomavirus infection	11
MAPK signaling pathway	11	NF-kappa B signaling pathway	10

B



C

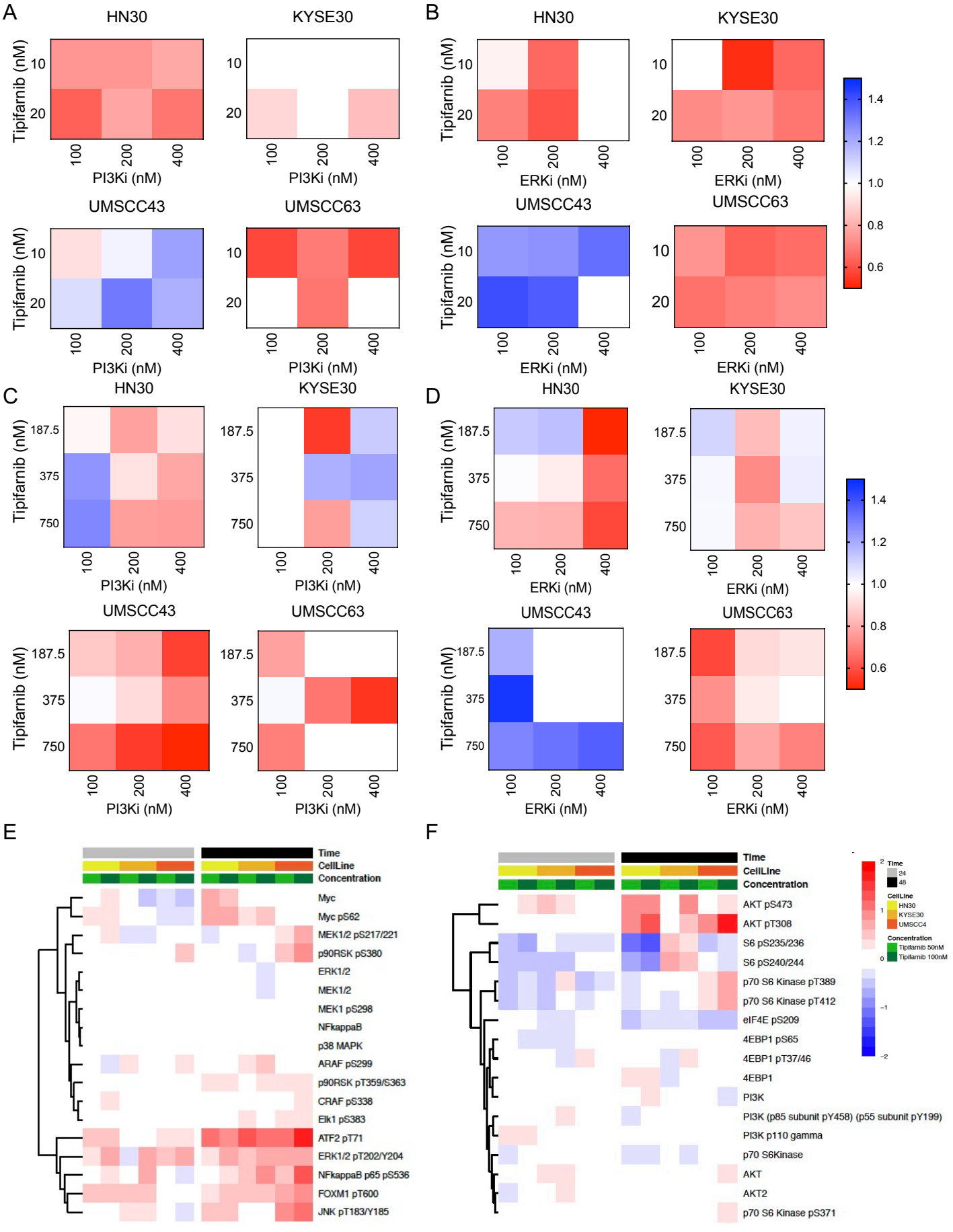


D

GI_{50} (M)		
Cell Line	ERKi (M)	PI3Ki (M)
HN30	1.99E-06	6.83E-06
KYSE30	1.70E-07	6.08E-06
UMSCC4	1.53E-05	1.68E-04
UMSCC43	7.62E-07	2.17E-07
UMSCC63	4.89E-07	3.86E-06

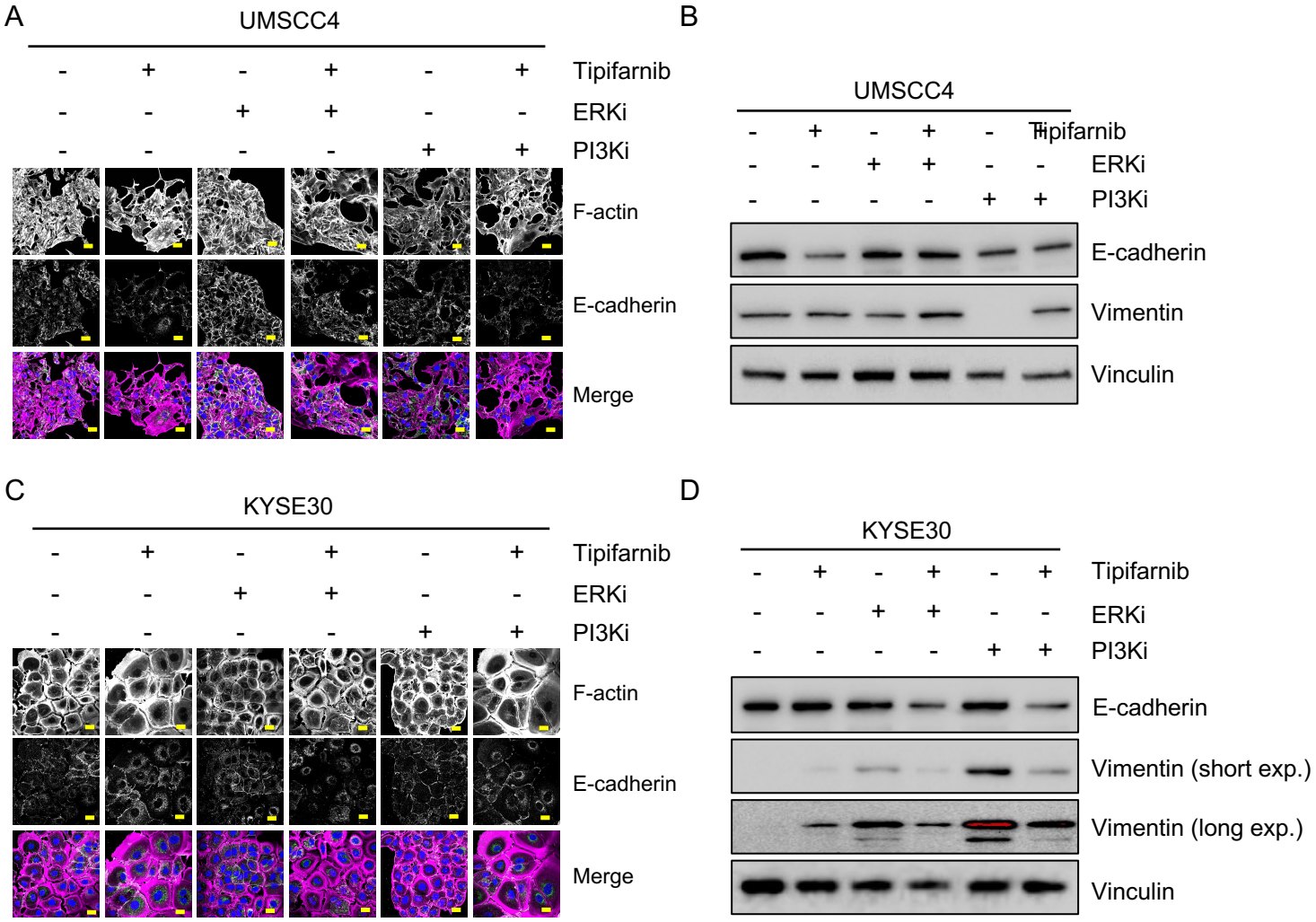
Supplementary Figure 3: KEGG pathway analysis of top hits for tipifarnib sensitizers

A) Top 10 pathways enriched after tipifarnib treatment for 2 or 4 weeks. The top 10% of hits from each dataset from CRISPR-Cas9 loss-of-function screens in Figure 5 were entered into db-String.
B) Bliss scores quantifying synergy between tipifarnib (1.5 nM - 10 μM) and autophagy inhibitor chloroquine (0.78 - 12.5 μM). Cells were treated for 5 days.
C) Bliss scores quantifying synergy between tipifarnib (1.5 nM - 10 μM) and CXCR4 inhibitor WZ811 (100 - 1600 nM).
D) GI_{50} values for PI3K-alpha inhibitor alpelisib and ERK1/2 inhibitor SCH772984 across the panel of cell lines.


A
HN30
KYSE30
UMSCC43
UMSCC63
Tipifarnib (nM)
10
20
100
200
400
PI3Ki (nM)
B
ERKi (nM)
1.4
1.2
1.0
0.8
0.6
C
187.5
375
750
D
E
Time
CellLine
Concentration
Myc
Myc pS62
MEK1/2 pS217/221
p90RSK pS380
ERK1/2
MEK1/2
MEK1 pS298
NFkappaB
p38 MAPK
ARAF pS299
p90RSK pT359/S363
CRAF pS338
Elk1 pS383
ATF2 pT71
ERK1/2 pT202/Y204
NFkappaB p65 pS536
FOXM1 pT600
JNK pT183/Y185
F
AKT pS473
AKT pT308
S6 pS235/236
S6 pS240/244
p70 S6 Kinase pT389
p70 S6 Kinase pT412
eIF4E pS209
4EBP1 pS65
4EBP1 pT37/46
4EBP1
PI3K
PI3K (p85 subunit pY458) (p55 subunit pY199)
PI3K p110 gamma
p70 S6Kinase
AKT
AKT2
p70 S6 Kinase pS371
24
48
HN30
KYSE30
UMSCC4
Tipifarnib 50nM
Tipifarnib 100nM

Supplementary Figure 4: Combinations of tipifarnib with PI3K-alpha inhibitor or ERK1/2 inhibitor are synergistic

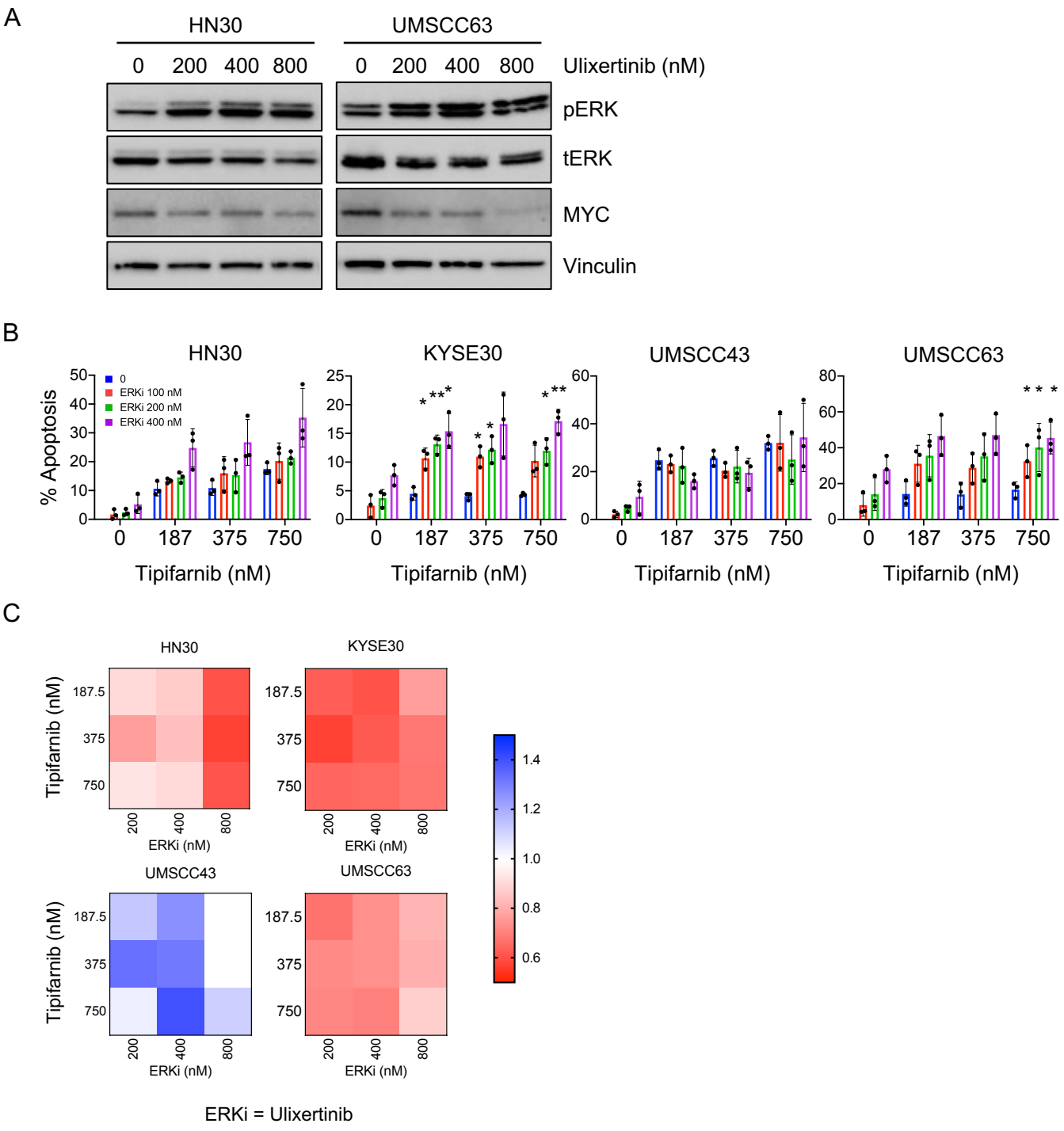
A-D) Calculation of Bliss scores for apoptosis assays to determine synergy of combinations between low (A, B) or high dose (C, D) tipifarnib and indicated doses of inhibitors of ERK1/2 or PI3K-alpha. Data are from cells treated and analyzed in Fig 6A-D.
E) Heatmap showing changes in activation of the MAPK signaling pathway and key ERK substrates. RPPA analysis was performed on HRAS mutant cell lines treated with tipifarnib for 24 or 48 h, as shown in Fig 3B.
F) Heatmap showing changes in activation of the PI3K-AKT-mTOR signaling pathway. RPPA analysis was performed on HRAS mutant cell lines treated with tipifarnib for 24 or 48 h, as shown in Fig 3B.



Supplementary Figure 5: Combination of tipifarnib with ERKi and PI3Ki alters the distribution and expression of E-cadherin and vimentin

A, C) Immunofluorescence images showing expression and distribution of F-actin and E-cadherin in UMSCC4 and KYSE30 cells, respectively, in response to combinations of tipifarnib with ERKi or PI3Ki. Cells were treated as in Fig 6E, F. In the merge image, F-actin is shown in magenta, E-cadherin in green and the nucleus in blue. Scale bar = 20 mm.

B, D) Western blots showing expression of E-cadherin and vimentin in UMSCC4 and KYSE30 in response to combinations of tipifarnib with ERKi or PI3Ki. Lysates taken from same cells as in (A, C).



Supplementary Figure 6: Combination of FTI tipifarnib with clinical candidate ERKi causes apoptosis in HRAS mutant HNSCC

A) Western blot showing target inhibition of MAPK pathway by ERK1/2 inhibitor ulixertinib/BVD-523. Cells were treated for 48 h then blotted for pERK and MYC. Although pERK has rebounded at this time point, as expected with this ATP-competitive inhibitor, ERK substrate MYC is still reduced indicating continued pathway inhibition.

B) Quantification of apoptosis. Cells were treated with the indicated doses of tipifarnib and ulixertinib for five days. Annexin-FITC positive cells were quantified and normalized to vehicle control. Data represent the average +/- SD of three independent replicates; values above zero are shown. P value: combination versus tipifarnib alone. *P<0.05, **P<0.01, ***P<0.001

C) Bliss scores quantifying synergy between tipifarnib and ERK1/2 inhibitor, calculated from apoptosis data in (B).