Supplemental Data

Supplemental Figure 1. Ectopic SIAH-2^{WT} expression on 20-Gy radiation- and serum starvation-mediated cell cycle arrest.Ectopic expression of SIAH-2^{WT} is not sufficient to rescue cell cycle arrest induced by lethal radiation (20-Gy) or serum starvation in the A549 cells. (A) Cell cycle analysis. DNA flow cytometric analysis was performed in A549 cells infected with mock, pLenti-GFP or pLenti-SIAH-2^{WT} viruses. At 48 hours post infection, A549 cells were radiated (20Gy) or serum starved for 36 hours and cell cycle profiles were analyzed by flow cytometry. The percentages of cells in G_0-G_1, S and G_2-M phase were calculated using Modfit LT 3.0 computer software and are represented in the histograms. The experiments were repeated two times and similar results were obtained. Representative cell cycle profiles are shown. (B) Immunoblot analysis. Western blot analysis was performed using total cell lysates (20 µg) of pLentiviral infected A549 cells. The results showed that SIAH-2^{WT} is expressed in A549 cells as indicated by FLAG immunoblot. The phospho-ERK activity was examined post infection, radiation and serum starvation and ERK2 expression was used as an internal control.

Supplemental Figure 2: SIAH-2 deficiency reduces ERK signaling, inhibits cell proliferation, induces apoptosis and compromises anchorage-independent tumor growth in soft agar in UMC 11 cells with wild-type RAS.

UMC11 cells were either mock infected or infected with pLenti-GFP, pLenti-SIAH-2^{WT}-GFP or pLenti-SIAH-2^{PD}-GFP viruses. (**A**) Live cell imaging. The GFP and bright field images of untreated and pLentiviral-infected UMC11 cells expressing either GFP, SIAH-2^{WT}-GFP or SIAH-2^{PD}-GFP heterogeneously are shown at 3 days post-infection. The pLentiviral infection rate was close to 95%. (**B**) Western blot analysis. Total cell lysates (15 µg) were collected at day 3 post infection from UMC 11 cells treated with the above lentiviruses. SIAH expression and phospho-ERK activity were examined in the infected UMC 11 cells by Western blot analyses. ERK2 expression level was used as internal loading control. Note that SIAH-2^{PD} expression leads to a

marked reduction in phospho-ERK expression in UMC11 cells. (**C**) Colony formation in soft agar. SIAH deficiency inhibits anchorage-independent tumor growth in soft agar in UMC 11 cells, another lung cancer cell line carrying wild type RAS. Two hundred UMC 11 cells infected with the indicated lentiviruses were plated into a 24-well plate in triplicate with a layer of 0.08% low-melting agarose. At day 14, every colony formed in soft agar was counted. A bar graph representation of the results is shown. Error bars represent 95% confidence intervals. Comparison between groups was performed by Student's *t* test. * statistically significant increase in the percentage of TUNEL-positive cells was observed in SIAH-2 deficient A549 cells ($P < .001$). (**D**) Cell proliferation assays. UMC11 cells were either mock infected or infected with pLenti-shRNA-control or pLenti-shRNA-SIAH-2 #6 viruses. The cell proliferation rates were markedly reduced in UMC11 cells infected with the pLenti-shRNA-SIAH-2 knockdown virus (#6) as compared to control viruses. Error bars represent 95% confidence intervals. Comparison between groups was performed using Student's *t* test. * statistically significant reductions in cell proliferation were observed in SIAH-deficient UMC11 tumors (shRNA-SIAH-2 #6) as compared to either untreated UMC11 cells as a referent group ($P < .001$) or compared to the SIAH-proficient tumors (shRNA control) as a referent group ($P < .001$). (**E**) SIAH-2^{PD}-dependent cytotoxicity in UMC11 cells 3-days post infection.

Supplemental Figure 3: A549 tumor histology

Histology was performed on the large and small A549 tumors resected from nude mice. A549 cells were mock-infected, heterogeneously infected with either eGFP or $SIAH_2^{PD}$-eGFP lentiviruses and injected into nude mice. H&E was used to view the tissue morphology of A549 tumors resected from the nude mice. Human cells are bigger in size and have bigger nuclei than mouse cells. Anti-SIAH mAb (24E6) stains human cells but not the surrounding mouse cells. Anti-vWF antibodies stain both large and small tumors. Immunochemical staining of cell nuclei (**Blue**) and SIAH and vWF (**Brown**) are represented in photo images.

Supplementary Table 1. Cell proliferation rates of SIAH-2-proficient versus SIAH-2-deficient lung epithelial cells*

Cell line, treatment	Day 5 post infection			Day 6 post infection			Day 7 post infection		
	Mean no. of cells, $\times 10^3$ (95% CI)	*P*	*P*	Mean no. of cells, $\times 10^3$ (95% CI)	*P*	*P*	Mean no. of cells, $\times 10^3$ (95% CI)	*P*	*P*
BEAS									
Untreated	152 (116 to 188)	Referent	.72	284 (140 to 428)	Referent	.15	710 (476 to 945)	Referent	.042
Non-target shRNA	159 (93 to 225)	.72	Referent	213 (119 to 308)	.15	Referent	488 (263 to 713)	.042	Referent
shRNA SIAH-2 #6	145 (108 to 183)	.61	.49	195 (145 to 245)	.06	.49	577 (183 to 972)	.26	.45
Untreated	23 (12 to 34)	Referent	.052	66 (57 to 74)	Referent	<.001	54 (35 to 72)	Referent	0.081
GFP lentivirus	34 (27 to 40)	.052	Referent	24 (9 to 39)	<.001	Referent	38 (28 to 49)	.081	Referent
SIAH-2^{PD}-GFP lentivirus	18 (4 to 33)	.478	.028	33 (31 to 35)	<.001	.153	40 (34 to 46)	.084	.731
BZR									
Untreated	152 (116 to 188)	Referent	.72	439 (389 to 489)	Referent	.01	771 (646 to 896)	Referent	.002
Non-target shRNA	159 (93 to 225)	.72	Referent	370 (300 to 420)	.01	Referent	528 (475 to 580)	.002	Referent
shRNA SIAH-2 #6	145 (108 to 183)	.62	.49	57 (22 to 92)	<.001	<.001	45 (30 to 60)	<.001	<.001
Untreated	150 (97 to 202)	Referent	.039	118 (97 to 140)	Referent	.43	172 (135 to 209)	Referent	.222
GFP lentivirus	89 (44 to 133)	.039	Referent	96 (82 to 110)	0.43	Referent	159 (137 to 169)	.222	Referent
SIAH-2^{PD}-GFP lentivirus	80 (52 to 127)	.033	.971	63 (46 to 81)	0.001	.004	67 (29 to 85)	.001	.001
A549									
Untreated	444 (194 to 694)	Referent	.04	1001 (252 to 1749)	Referent	.69	2301 (1087 to 3514)	Referent	0.33
Non-target shRNA	752 (404 to 1100)	.04	Referent	1107 (304 to 1910)	.69	Referent	1883 (798 to 2969)	.33	Referent
shRNA SIAH-2 #6	14 (3 to 26)	.002	.001	8 (0 to 25)	.005	.004	19 (0 to 47)	.001	.002
Untreated	320 (265 to 375)	Referent	.945	476 (362 to 589)	Referent	.006	605 (424 to 785)	Referent	.688
GFP lentivirus	322 (246 to 398)	0.945	Referent	657 (587 to 727)	0.006	Referent	578 (559 to 596)	.688	Referent
SIAH-2^{PD}-GFP lentivirus	57 (22 to 92)	<.001	<.001	544 (33 to 75)	<.001	<.001	101 (89 to 112)	<.001	<.001
H727									
Untreated	308 (174 to 442)	Referent	.21	544 (182 to 906)	Referent	.55	1220 (528 to 1920)	Referent	.07
Non-target shRNA	372 (243 to 501)	.21	Referent	624 (253 to 1010)	.55	Referent	784 (438 to 1130)	.07	Referent
shRNA SIAH-2 #6	16 (3 to 36)	.001	.001	13 (8 to 19)	.003	.002	8 (0 to 25)	.002	.001
Untreated	268 (206 to 330)	Referent	.901	337 (296 to 377)	Referent	.159	443 (418 to 469)	Referent	.005
GFP lentivirus	271 (228 to 315)	.901	Referent	299 (254 to 353)	.159	Referent	365 (314 to 416)	.005	Referent
SIAH-2^{PD}-GFP lentivirus	126 (79 to 173)	.001	.001	104 (71 to 138)	<.001	<.001	130 (82 to 177)	<.001	<.001

A panel of human normal lung epithelial cells, BEAS-2B, lung tumor cells, BZR, and the lung cancer cells A549 and H727 were mock infected or infected with pLenti-GFP, pLenti-SIAH-2^{PD}-GFP, pLenti-shRNA-control, or pLenti-shRNA-SIAH-2 #6 viruses using an infection ratio (viruses:cells) of 20:1. Cell proliferation assay was performed for each cell line under each infection condition in which 10000 infected cells were plated into each well of a total of 72 wells under each infection condition in triplicates. The total number of cells per well was manually counted over a 7–8-day period post infection. CI = confidence interval; shRNA = short hairpin RNA; GFP = green fluorescent protein; PD = protease deficient. *P* values were generated using the Unpaired two-sided Student *t*-test.