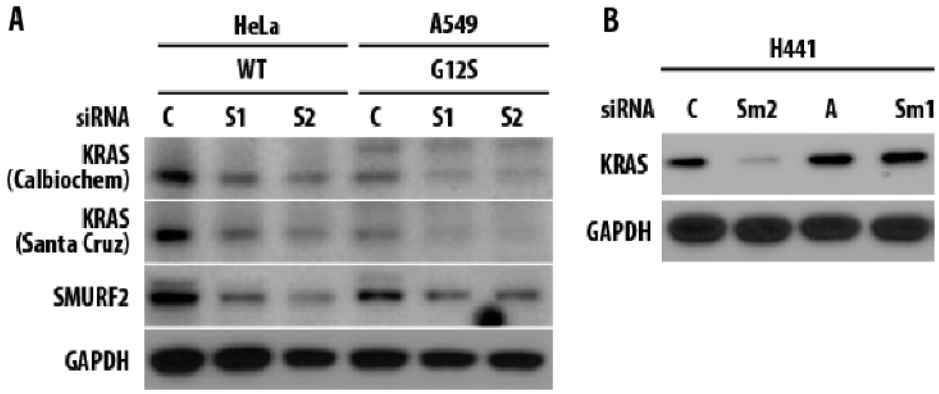

Figure W1. SMURF2 controls mutant KRAS steady-state level. (A) Human cervical carcinoma, HeLa and human lung adenocarcinoma, and A549 cells were treated with two different siRNA targeting different regions of Smurf2 (S1 and S2). Forty-eight hours post-transfection, cell lysates were prepared and subjected to immunoblot analysis using indicated antibodies. We have also used two different KRAS antibodies as indicated in the Materials and Methods section. (B) H441 cells were transfected with either control (C), Smurf2 (Sm2), AIP4 (A), or Smurf1 (Sm1) siRNA. Forty-eight hours post-transfection, cell lysates were subjected to immunoblot analysis using indicated antibodies.

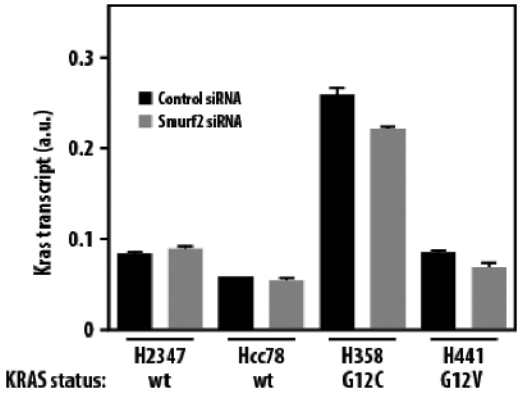

Figure W2. Smurf2 knockdown does not alter Kras transcript level. Kras mRNA levels were quantitated using quantitative reverse transcription–PCR as described in the Materials and Methods section for the indicated cell lines 48 hours post siRNA transfection.

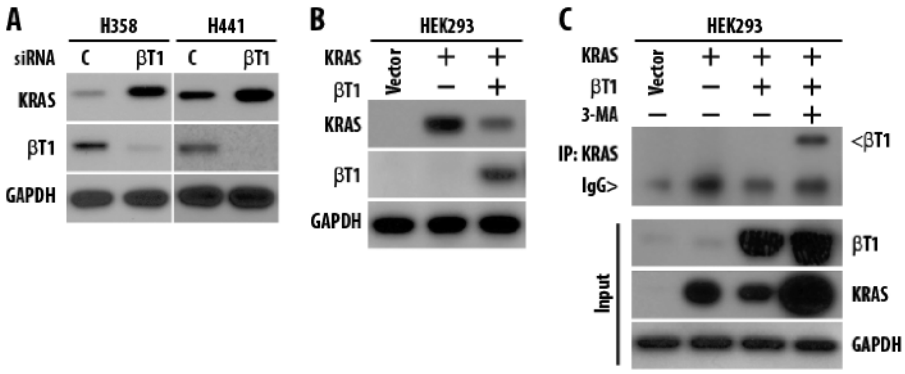

Figure W3. β-TrCP1 degrades KRAS. (A) H358 and H441 cells were transfected with either control (C) or β-TrCP1 (βT1) siRNA, and 48 hours post-transfection, cell lysates were immunoblotted with the indicated antibodies. (B) HEK293 cells were co-transfected with either KRAS alone or along with β-TrCP1 as indicated. Twenty-four hours post-transfection, whole-cell lysates were immunoblotted with the indicated antibodies. (C) HEK293 cells were transfected with either KRAS alone or in combination with β-TrCP1 and where indicated treated with the lysosomal inhibitor 3-MA as described in Figure 2. Cell lysates were subjected to immunoprecipitation with anti-KRAS antibody and immunoblotted with the indicated antibodies.

Table W1. List of Smurf2 shRNA.

Smurf2 shRNA	Sequences
shRNA#1	cagttaatccggaacattt
shRNA#2	gcccgagactctttaccat
shRNA#3	gtcacaacgacatagaaat
shRNA#4	ctgtgtttcatggacattata
shRNA#5	ctgacagtactctgtgcaa
shRNA#6	agcgagacctggttcagaa
shRNA#7	tggaagaatccagtatcta
shRNA#8	tggaagcgattaatgataa

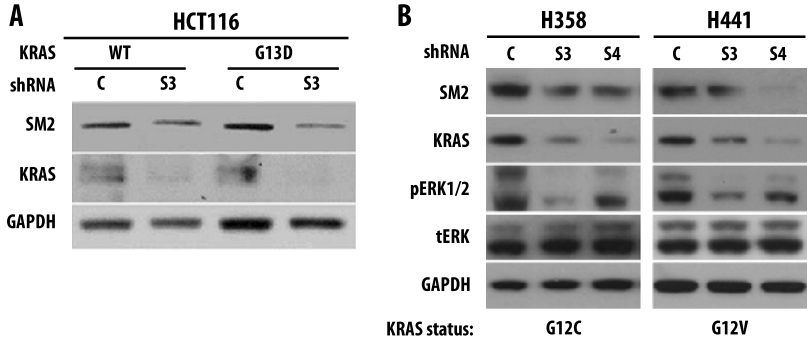

Figure W4. Lentivirus shRNA-mediated knockdown of Smurf2 reduces KRAS steady-state levels. (A) Human isogenic colorectal cell line HCT-116 harboring either wild-type or G13D Kras allele were transduced with either control or Smurf2 (S3) shRNA. Forty-eight hours post transduction, cell lysates were prepared and subjected to immunoblot analysis using indicated antibodies. (B) Human lung adenocarcinoma cell lines, H358 and H441, were transduced with two different Smurf2 shRNA (S3 and S4), and immunoblot analysis was performed using indicated antibodies.

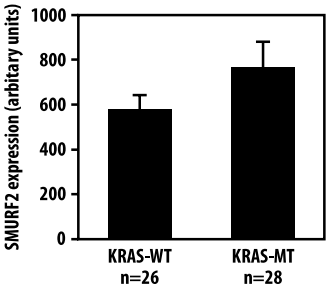

Figure W5. Smurf2 mRNA expression is relatively higher in patients with mutant Kras-containing lung adenocarcinoma. Smurf2 mRNA expression levels were plotted with respect to Kras mutation status ($n = 26$, wild-type Kras; $n = 28$, mutant Kras) confirmed among our patients with lung adenocarcinoma.

A

Inverse Correlation of gene expression between Smurf2 and Btrc in lung cancer.

Type	Sample size	r value	*p* value
Lung adenocarcinoma	595	-0.41	<0.0001
SCC	130	-0.31	0.0003
Lung cancer cell lines	79	-0.27	0.02
NSCLC	183	-0.42	<0.0001

B

Inverse Correlation of gene expression between Kras and Btrc in lung cancer.

Type	Sample size	r value	*p* value
Lung adenocarcinoma	595	-0.52	<0.0001
SCC	130	-0.16	0.07
Lung cancer cell lines	79	-0.25	0.03
NSCLC	183	-0.65	<0.0001

Figure W6. β-TrCP1 gene expression is inversely correlated to both Smurf2 and Kras mRNA expression in patients with lung adenocarcinoma. (A) Smurf2 and β-TrCP1 (Btrc) gene expression obtained from a large cohort of gene expression microarray data showing inverse correlation among lung adenocarcinoma patients ($r = -0.41$, $n = 594$, $P < .0001$), squamous cell cancer ($r = -0.31$, $n = 130$, $P = .0003$), and lung cancer cell lines ($r = -0.27$, $n = 79$, $P = .02$). (B) Similar analysis between Kras and β-TrCP1 (Btrc) gene expression also shows inverse correlation among lung adenocarcinoma patients ($r = -0.52$, $n = 594$, $P < .0001$), squamous cell cancer ($r = -0.16$, $n = 130$, $P = .07$), and lung cancer cell lines ($r = -0.25$, $n = 79$, $P = .03$).

Figure W7. Partial sequence alignment between human ubiquitin-conjugating enzyme UBCH5 and UBCH7 showing ^{61}PF^{62} and ^{95}PA^{96} areas; * indicates amino acid identity in the area represented.