Supporting Information

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SI Methods

Plasmids. The full-length RIOK3 coding sequence was amplified from cDNA and fused in frame with an N-terminal HA tag and cloned into the pBabe puro retroviral vector or in a MSCV blasticidin retroviral vector. The S447L mutant was made by amplification of the coding sequence from cDNA derived from the HPAFII cell line which harbored the sequence variant. The K290R and D406N constructs were made by standard site directed mutagenesis protocols. Constructs were sequenced to ensure authenticity. Viral particles produced in 293T cells, filtered, and used for infection in the presence of 5 μg/ml polybrene (Sigma). Cells were selected 2 days later using puromycin (2–2.5 μg/ml). Knockdown was assessed at 48–72 h after selection.

Rac Activation Assay. 293T cells were transfected with 5–10 μg of each expression plasmid using Lipoctamine 2000 (Invitrogen). Approximately 36–48 h after transfection, cells were serum starved in 0.1% serum containing media overnight. Between 1–3 mg of lysate were used per pull-down assay using the supplied GST-Pak RBD and incubated for 1 h, washed three times, and then boiled in sample buffer. Western analysis was performed as described in *Methods*.

Tumorigenicity Assays. Between 4,000–8,000 cells per well were seeded in medium containing 0.4% low-melting agarose on top of bottom agar containing 1% low-melting agarose in regular medium. After 10–14 d, colonies were stained with iodonitrotetrazoliumchloride (Sigma) and counted with positive colonies scored based on comparison of control colony size. Alternatively plates were stained with Alamar blue and read on a plate reader.

Invasion and Migration Assays. The inner portion of the well was scraped clean and then fixed in 10% formalin in PBS and visualized by staining with a 0.1% crystal violet solution. For migration assays, cells were processed by standard crystal violet staining after 7–8 h (overnight for invasion assays) and either direct counting of cells or extraction of crystal violet from the membranes with acetic acid followed by determination of the optical density was performed. For scratch assays, cells were seeded in 6-well plates and allowed to reach confluence. A scratch was made using a sterile p200 pipette tip and photographed 12–16 h later.

aCGH and FISH. The oligonucleotide array contains 43,000 elements representing coding and noncoding human sequences (Human Genome CGH 44B, Agilent Technologies, G4410B). Probes were annotated against build NCBI 35. Well-characterized genes represented by 1+ probes, cancer-relevant genes by 2+ probes. The overall median interval between mapped elements is 43 kb and 24-kb median probe spacing in Refseq genes. Selection criteria for high confidence MCRs included the following: High amplitude, Peak segmentation values >1.0 or <-1.0; Focal change, Width <7Mb and Entrez genes in residence <100. To eliminate events potentially from copy number polymorphism, MCRs with substantial overlap with any of the eight tables in structural variation track on UCSC genome browser (hg17) were removed; MCR inclusion for the final list and boundary definition was subjected to expert visual inspection and review. For FISH, DNA was extracted from BAC clones and labeled by nick-translation mix (Roche Diagnostics). FISH signal evaluation and acquisition were performed manually using filter sets and software developed by Applied Spectral Imaging.

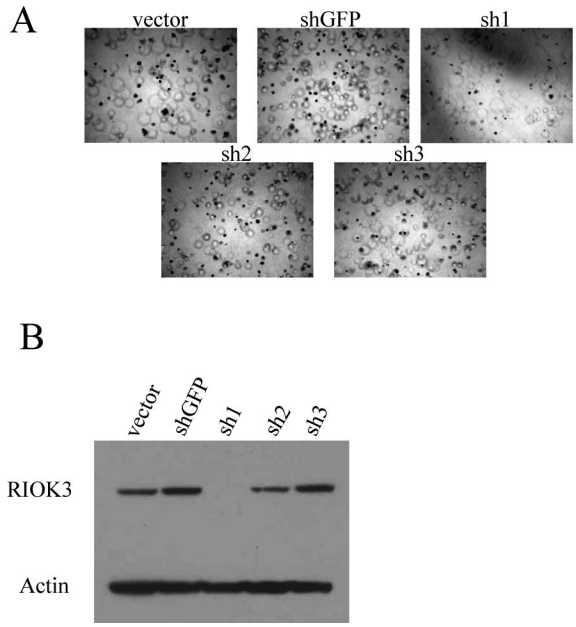

Fig. S1. Knockdown of RIOK3 in PDAC cells causes decreased anchorage independence. (*A*) 8988T cells were transduced with various shRNAs for RIOK3 and controls. These were seeded into semisolid agar and resulting colonies allowed to form. Note the significant reduction of colonies in sh1 cells, correlating with knockdown. (*B*) Western blot of lines from *A*. Significant knockdown in the sh1 line correlates with decreased colony formation. Lower band is an actin loading control.

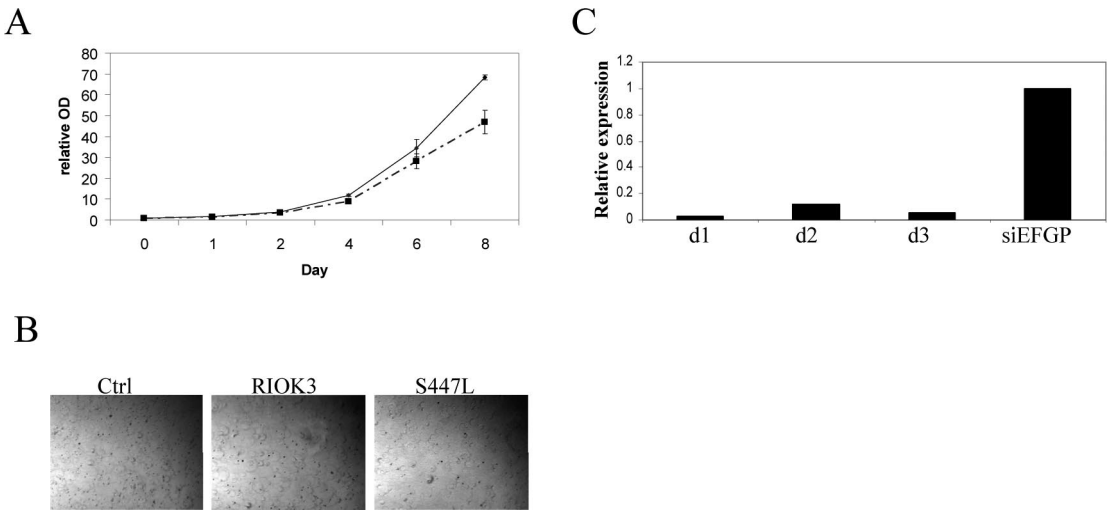

Fig. S2. Functional analysis of RIOK3 and PAK4. (*A*) Growth curves of human pancreatic ductal cells either expressing control vector (top curve) or RIOK3 (lower curve), showing no significant differences in proliferation over eight days. Data are plotted as relative to day 0. Cell number is estimated by amount of crystal violet staining as determined by relative OD from extracted dye. (*B*) Representative photographs of stable RIOK3 or S447L INK4A/ARF null MEFs seeded in soft agar. Notice no colonies of significant size are present. (*C*) RTqPCR showing >90% knockdown of PAK4 expression in 8988T cells with three siRNA duplexes, compared to an siEGFP control.

Table S1. High confidence MCRs

Cytobands	Position, bp		Width, bp	Peak (Log_2)	Recurrence		No. of Genes	Targets
	Start	End			Total	Tumors		
Amplification								
1p13.1–1p12	116,578,180	119,327,033	2,748,853	1.24	1	1	27	
7q11.21–7q11.22	62,277,763	66,674,506	4,396,743	1.99	1	1	69	SBDS
7q21.3–7q22.1	96,682,860	100,133,837	3,450,977	2.62	3	1	86	
8p11.23–8p11.21	39,295,686	42,137,387	2,841,701	1.54	1	0	18	
8q24.13–8q24.21	124,210,606	130,926,595	6,715,989	2.14	5	0	42	MYC
9p13.3–9p13.2	35,602,907	36,620,296	1,017,389	2.35	2	1	32	
12q12–12q13.11	43,854,614	45,756,535	1,901,921	2.15	2	1	10	
12q24.31–12q24.32	120,795,122	127,205,234	6,410,112	1.46	1	0	64	BCL7A
17q12–17q21.2	34,881,044	37,255,292	2,374,248	2.53	6	1	86	ERBB2,RARA
17q23.2–17q23.3	57,378,553	59,088,916	1,710,363	3.81	1	0	20	
18q11.2	17,574,014	19,999,081	2,425,067	2.49	4	2	18	
19q12	34,522,574	35,707,747	1,185,173	3.19	3	2	10	
19q13.2	44,142,487	44,603,715	461,228	1.49	4	1	17	
19q13.2	44,721,427	46,436,894	1,715,468	2.51	2	1	56	AKT2
20p11.22–20p11.21	22,015,808	24,171,157	2,155,349	1.90	3	1	31	
Deletion								
1p36.13	16,040,284	16,439,902	399,618	−1.69	2	1	14	
2p21	44,051,319	44,170,341	119,022	−1.46	1	1	1	
4q35.1–4q35.2	187,071,600	187,989,101	917,501	−1.51	2	0	11	
5q12.3	64,303,384	64,805,843	502,459	−1.20	2	0	3	
5q34	165,451,579	167,200,035	1,748,456	−1.95	1	0	3	
6p21.32	32,435,603	32,821,066	385,463	−1.35	2	0	10	
6q23.1–6q23.2	131,354,338	131,522,922	168,584	−1.24	4	0	1	
6q25.3	155,868,335	157,478,353	1,610,018	−4.42	5	0	3	
7q31.1	109,897,403	110,325,325	427,922	−1.41	1	0	3	
8p22–8p21.3	17,845,554	19,297,165	1,451,611	−1.65	7	0	11	PCM1
9p24.3–9p24.2	720,061	3,718,474	2,998,413	−5.21	7	0	16	
9p22.1–9p21.3	19,716,229	20,300,541	584,312	−3.72	5	0	1	
9p21.3	21,795,300	22,889,614	1,094,314	−5.60	10	1	6	CDKN2A
9q21.2	77,373,718	77,863,757	490,039	−1.84	3	1	2	
9q22.31	92,816,518	93,140,559	324,041	−1.89	2	1	5	
9q32–9q33.1	114,473,507	114,894,977	421,470	−2.23	2	0	5	
10p13–10p12.31	13,254,674	18,874,858	5,620,184	−3.01	3	0	36	
11p15.3	11,862,668	12,186,068	323,400	−2.76	2	0	4	
16q12.1–16q12.2	47,965,378	51,807,641	3,842,263	−1.30	2	0	27	CYLD
16q23.1	76,981,706	77,573,238	591,533	−1.28	3	0	2	
18q21.1	46,480,734	47,708,308	1,227,574	−6.47	7	0	8	SMAD4
18q21.2	49,190,910	50,063,290	872,380	−6.25	7	0	4	
18q23	73,464,159	74,719,405	1,255,246	−2.85	5	1	10	
21q22.3	41,725,601	42,564,265	838,664	−1.77	3	0	13	

Table S2. Downstream kinases in the Rho family

sym	chr	start	end
PAK1	11	76710708	76862581
PAK2	3	197951312	198043756
PAK3	X	110226256	110350816
PAK4	19	44308260	44363829
MAP3K9	14	70264605	70345641
MAP3K10	19	45389491	45413314
MAP3K11	11	65121802	65138296
KIAA1804	1	231530137	231586374
ROCK1	18	16787533	16944869
ROCK2	2	11239229	11402162