

Supplementary Table 1.

Primer sequences used for real-time quantitative PCR (RT-qPCR)

Primer name	Sequence	Notes	
RalGAPα1	5'-CTCCTACCCAGAGATTCTACCCA-3' (forward)	RT-qPCR	
	5'-CATGTTATGGCAGACACATGAAGGC-3' (reverse)		
RalGAPα2	5'-AGAACGTTTCATCTTCAGGCTCT-3' (forward)		
	5'-AGGGTTGAGTTATTTGCTGCCTA-3' (reverse)		
RalGAPβ	5'-CTCAGTTAAACACGTGCATTGGT-3' (forward)		
	5'-CAACTGAGTAGAAGGCTGTGACT-3' (reverse)		
RalGDS	5'-GAGATGAACCCCAAGAGAG-3' (forward)		
	5'-CTTCTTGGTCTGAGGGTGT-3' (reverse)		
RGL1	5'-CAAGCCCAAACGTGAAGAA-3' (forward)		
	5'-AGCACGTGAATTCTGCTGAC-3' (reverse)		
RGL2	5'-TTCAGTTTATGCCGTGGTGT-3' (forward)		
	5'-CCACGACATTCATTCTGGAG-3' (reverse)		
RGL3	5'-CCTGCAATCTAACCCCATCT-3' (forward)		
	5'-ATCTCCCACTCCTCCTCCT-3' (reverse)		
RALGPS1	5'-AGGGGCCTCTGAGAAGAAAA-3' (forward)		
	5'-GAAATCGGGAACCAGTCTGA-3' (reverse)		
RALGPS2	5'-TTGTGTGGGACACAGCTTTT-3' (forward)		
	5'-AGGCTGCACTCAAATGCTTA-3' (reverse)		
GAPDH	5'-GAAGGTGAAGGTCGGAGTC-3' (forward)		
	5'-GAAGATGGTGATGGGATTC-3' (reverse)		
HRAS	5'-GCAGTCGCGCCTGTGAACGGTG-3' (forward)		PCR for RAS mutation detection
	5'-AATTTACTGTGATCCCATCTGTGCCCGAC-3' (reverse)		
KRAS	5'-GCCATTTCCGACTGGGAGCGAG-3' (forward)		
	5'-AACTCTGGGAATACTGGCACTTAGAGGA-3' (reverse)		
NRAS	5'-TCCCGGCTGTGGTCTAAATCTGTC-3' (forward)		
	5'-GGAAGTCAGGACCAGGGTGTGAGT-3' (reverse)		

Supplementary Table 2.

Antibodies for immunoblotting and immunoprecipitation

Antibody	M.W. (kDa)	Host	polyclonal /monoclonal	Company	Catalogue number
RalA	28	Mouse	monoclonal	BD Transduction Laboratories (San Jose, CA, USA)	610222
RalB	26	Rabbit	polyclonal	Cell Signaling (Danvers, MA, USA)	3523S
Ras	21	Mouse	monoclonal	Millipore (Burlington, MA, USA)	05-516
RhoA	21	Rabbit	polyclonal	Santa Cruz Biotechnology, Inc. (Dallas, TX, USA)	sc-179
Rac1	21	Mouse	monoclonal	Millipore (Burlington, MA, USA)	05-389
cdc42	22	Mouse	monoclonal	BD Transduction Laboratories (San Jose, CA, USA)	610928
GAPDH	38	Mouse	monoclonal	Wako Pure Chemical Industries, Ltd. (Osaka, Japan)	015-25473
RalGAP α 1	260/ 240	Rabbit	polyclonal	Made by ourselves previously (Shirakawa et al. 2009)	/
RalGAP α 2	220	Rabbit	polyclonal	Made by ourselves previously (Shirakawa et al. 2009)	/
RalGAP β	170	Rabbit	polyclonal	Made by ourselves previously (Shirakawa et al. 2009)	/
Horseradish peroxidase-conjugated goat anti-mouse IgG	/	Goat	polyclonal	Jackson ImmunoResearch (West Grove, PA, USA)	115-035-003
Horseradish peroxidase-conjugated goat anti-rabbit IgG	/	Goat	polyclonal	Jackson ImmunoResearch (West Grove, PA, USA)	111-035-144
Easy blot anti-rabbit IgG	/	Goat	polyclonal	Genetex (Irvine, CA, USA)	GTX221666-01
Vimentin	57	Rabbit	monoclonal	Cell Signaling (Danvers, MA, USA)	5741
E-cadherin	135	Rabbit	monoclonal	Cell Signaling (Danvers, MA, USA)	3195

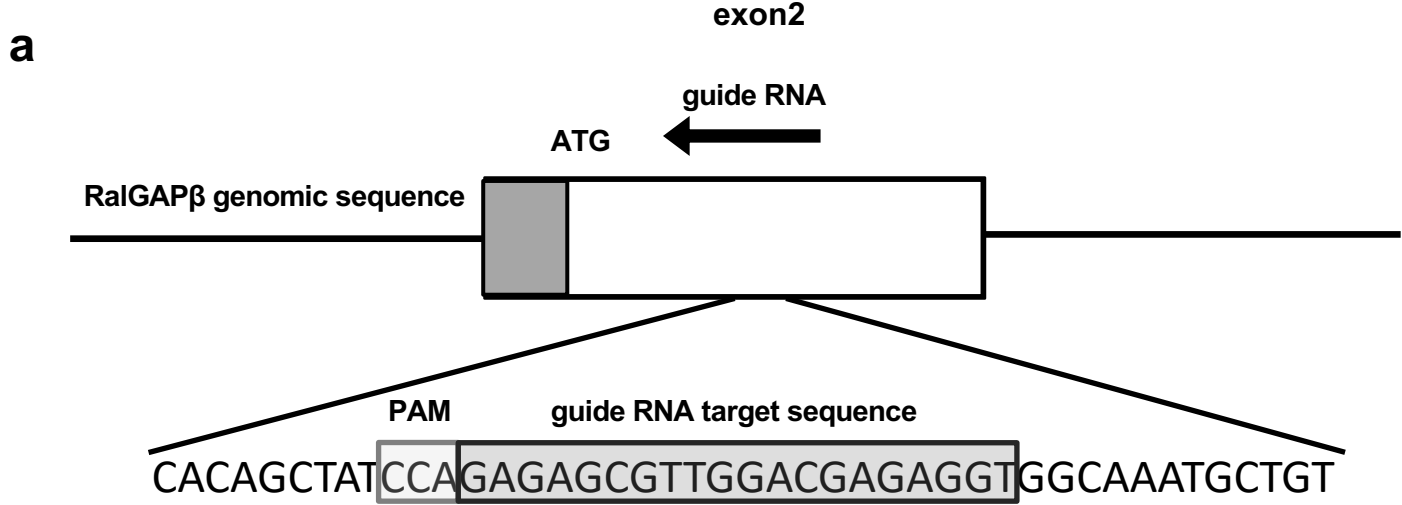
(M.W., molecular weight)

Supplementary Table 3.

DNA oligo sequences for guide RNA cloning, and primer sequence for knock-out screening.

Gene	20-nucleotide guide sequene (5' to 3')	Genomic PCR primers and sequencing primer (5' to 3')
RaIGAPβ	ACCTCTCGTCCAACGCTCTC	Forward: AGAGGCAAGATGTTTGCAGC
		Reverse: TCCAGAGGTAGGGATGAGGC

Supplementary Figure 1



b

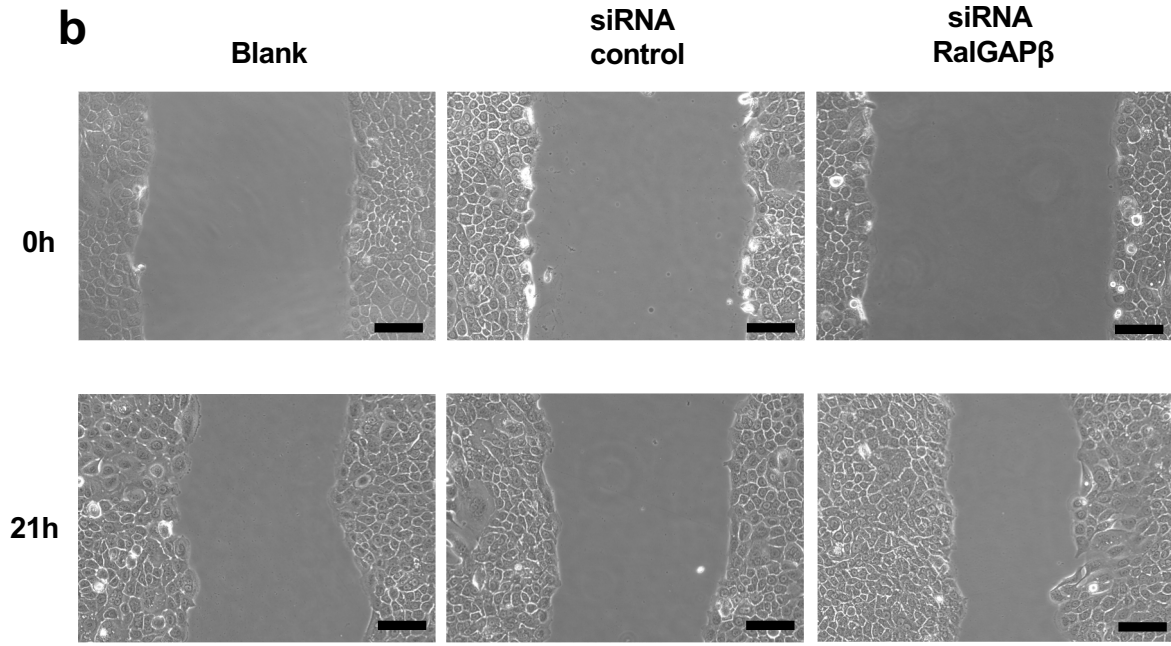
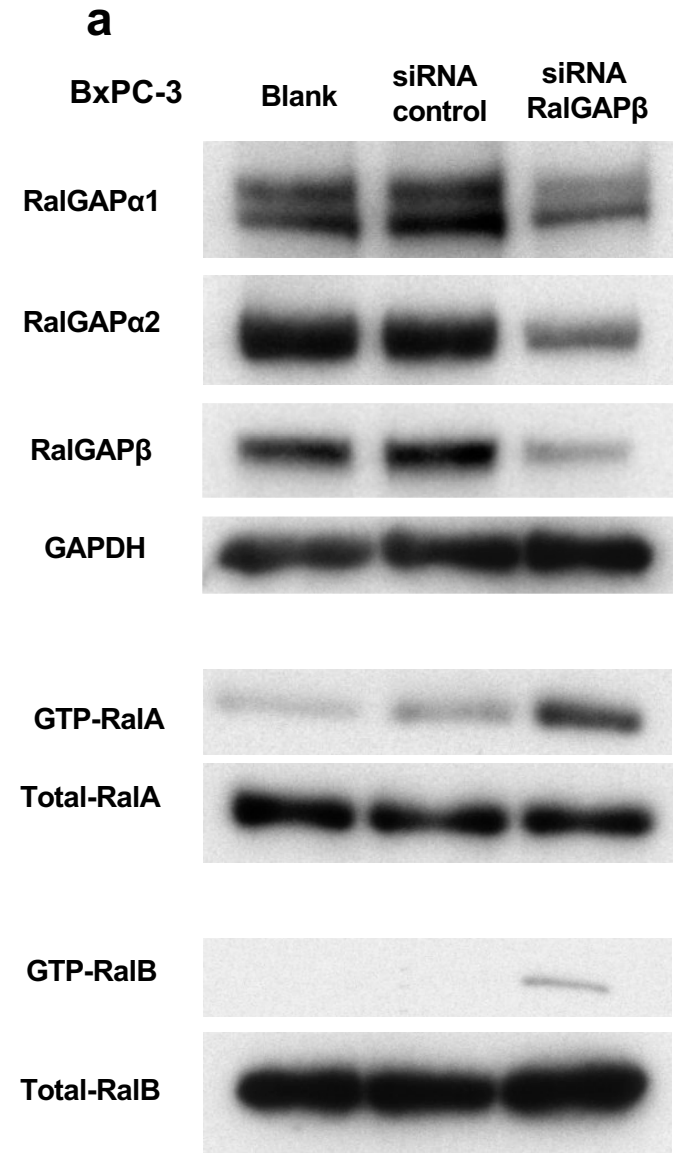
<PANC-1 RaIGAP β KO>

No	Allele1	Allele2
1	2 base deletion	1 base insertion
2	4 base deletion	1 base insertion
3	2 base deletion	1 base deletion

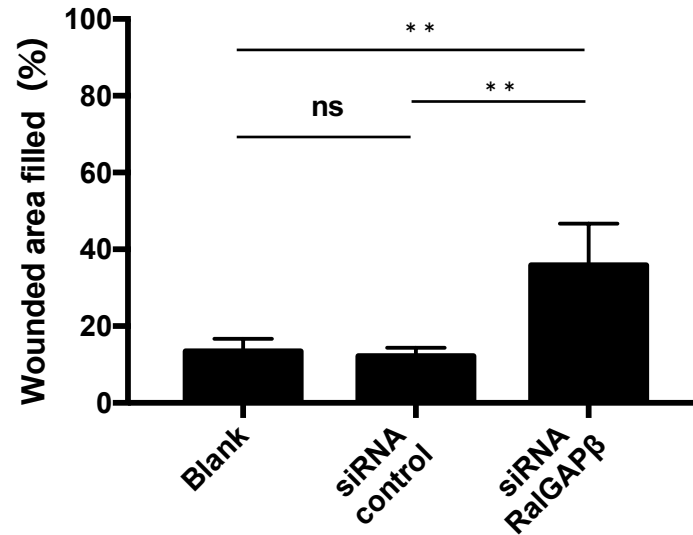
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No	Allele1	Allele2
1	1 base insertion	1 base insertion
2	1 base deletion	1 base insertion
3	2 base insertion	2 base insertion

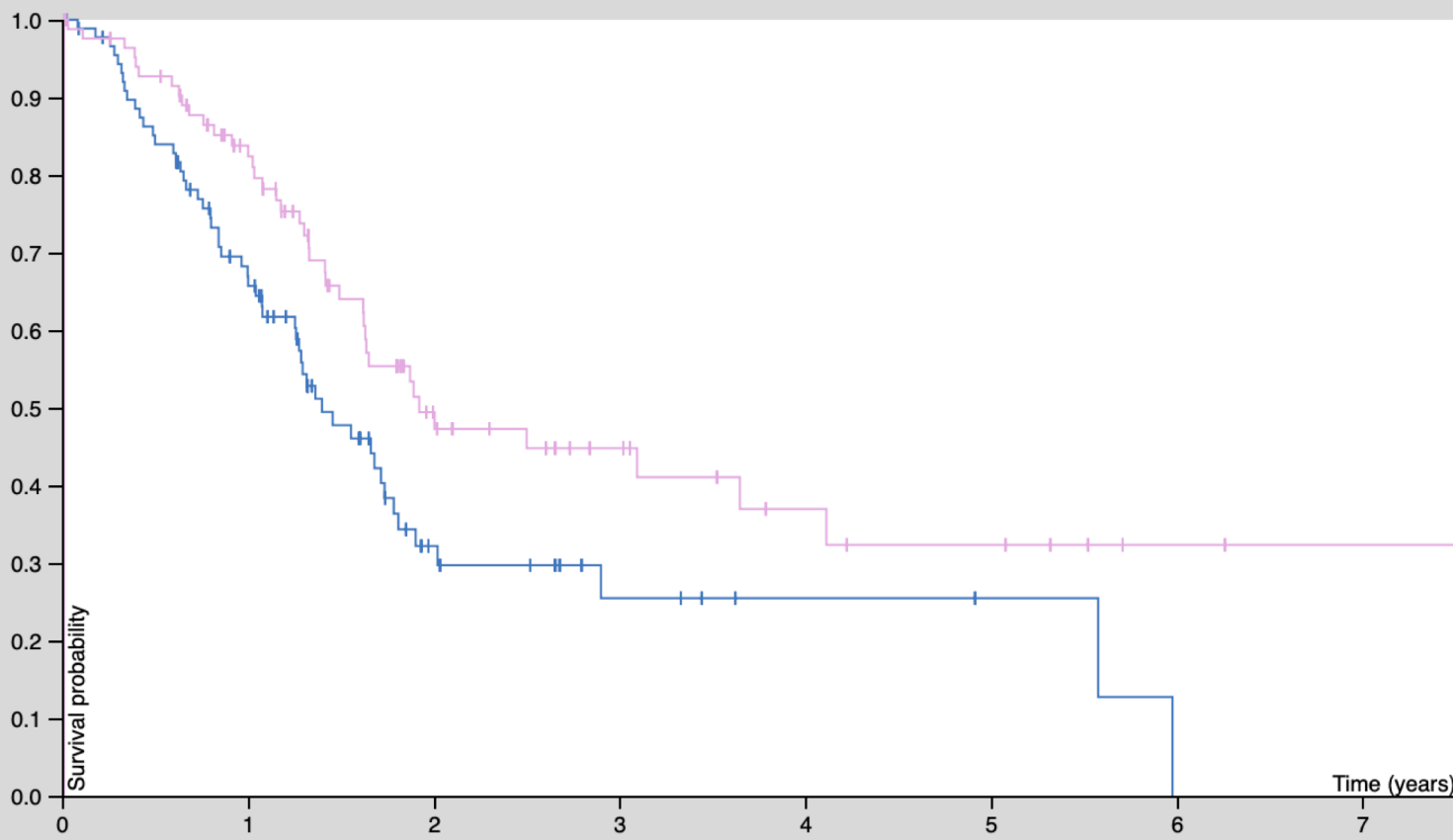
Supplementary Figure 2



c



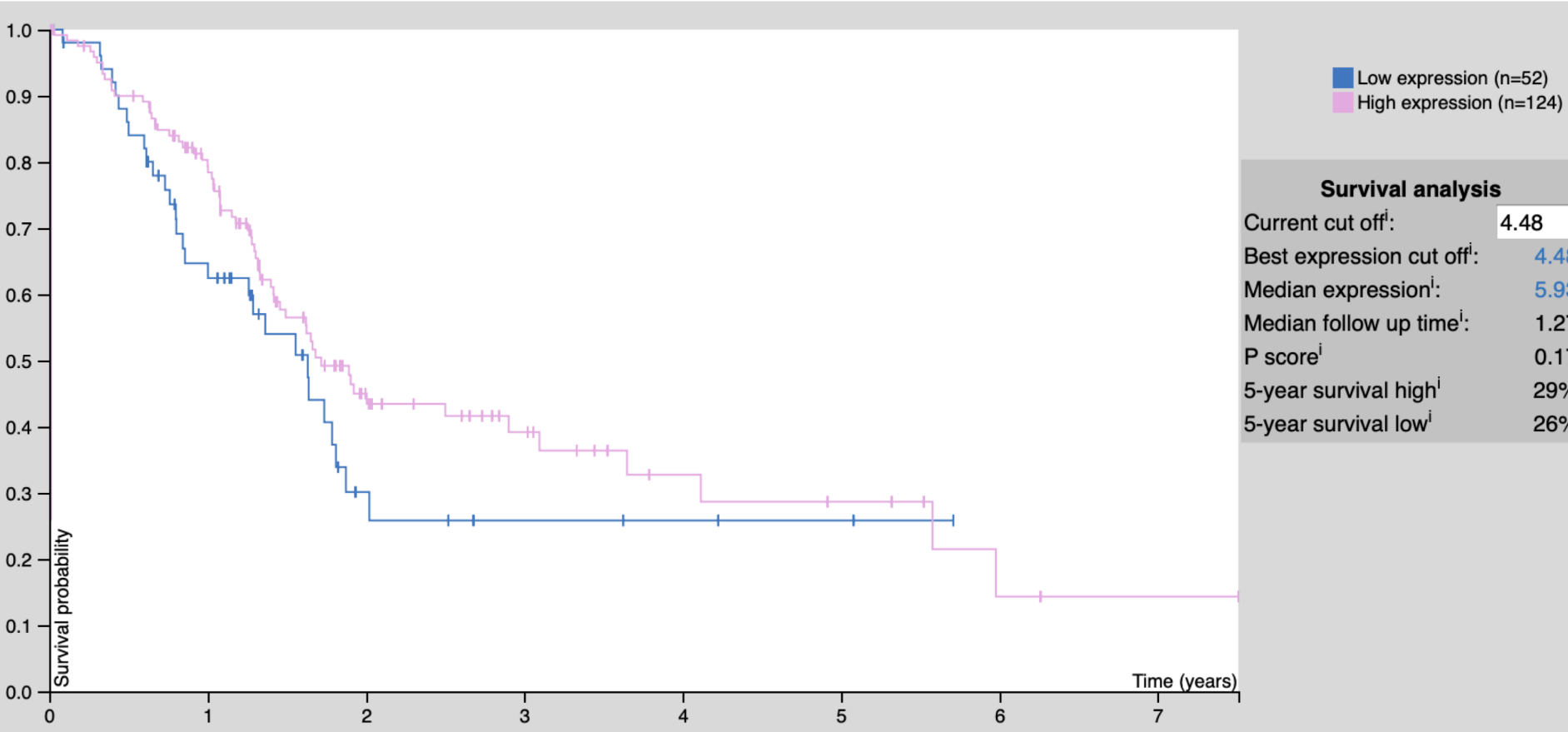
The cancer genome atlas (TCGA) mRNA expression level of RalGAP α 1 in pancreatic cancer



■ Low expression (n=91)
■ High expression (n=85)

Survival analysis	
Current cut off ⁱ :	1.21
Best expression cut off ⁱ :	1.21
Median expression ⁱ :	1.19
Median follow up time ⁱ :	1.27
P score ⁱ :	0.012
5-year survival high ⁱ :	32%
5-year survival low ⁱ :	25%

The cancer genome atlas (TCGA) mRNA expression level of RalGAP α 2 in pancreatic cancer



The cancer genome atlas (TCGA) mRNA expression level of RalGAP β in pancreatic cancer

