Supplementary Information

Supplemental Figure Legends:

Supplemental Figure 1. (A) Taqman qPCR analysis of miR-31 expression in RNA isolated from isogenic HCT116 cell lines with oncogenic KRAS (mut/null) or with wild-type KRAS (wt/null). (B) qPCR analysis of *KRAS* expression in the indicated cell lines transfected with siRNA control [-] or siRNA against KRAS [+]. Expression was normalized to □-actin mRNA.

Supplemental Figure 2. Quantitative PCR analysis of *ELK1* and *GAPDH* expression in Panc-1 cells transfected with control siRNAs (siCon) or siRNA targeting GAPDH (si*GAPDH*) or ELK1 (si*ELK1*). Expression was normalized to □-actin mRNA.

Supplemental Figure 3. Comparison of endogenous miR-31 expression in HNPE-KRAS cells to the indicated PDAC cells lines infected with MSCV-empty vector (EV) or MSCV-miR-31 (miR-31). MiR-31 was detected by Taqman qPCR and normalized to U6 snRNA.

Supplemental Figure 4. (A) Growth rates of BxPc3, Capan-1 and MiaPaCa2 cell lines infected with MSCV-empty vector (EV) or MSCV-miR-31 (miR-31) monitored over the indicated time course. Rate of growth measured by MTT assay (BxPc3) or percent confluency (Capan-1 and MiaPaCa2). (B) Western blot analysis of lysates from non-stimulated [-] or FBS stimulated [+] MiaPaCa2 and Capan-1 cell lines stably transfected with MSCV-empty vector (EV) or MSCV-miR-31 (miR-31). pERK and ERK indicate phosphorylated and total ERK. pAKT and AKT indicate phosphorylated and total AKT, respectively.

Supplemental Figure 5. Western blot analysis of RASA1 expression in isogenic HCT116 cells. Tubulin served as a protein loading control.

Supplemental Figure 6. (A) Western blot analysis of lysates from non-stimulated [-] or FBS stimulated [+] MiaPaCa2 and Capan-1 cell lines transfected with control siRNA (siCon) or siRNA

targeting *RASA1* (si*RASA1*). pERK and ERK indicate phosphorylated and total ERK. pAKT and AKT indicate phosphorylated and total AKT, respectively. (B) Western blot analysis of flag-RASA1 expression in Panc-1 cells transfected with CMV-empty vector (EV) or CMV-flag-RASA1 (*RASA1*). Tubulin served as a protein loading control.

Supplemental Tables:

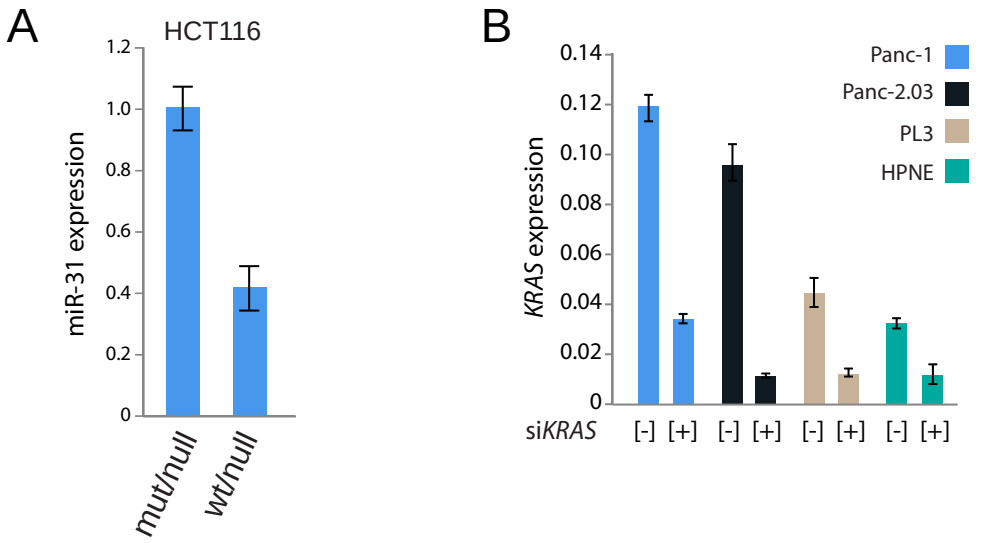
Supplemental Table 1: Primer sequences. A list of all primers used in the study and their respective applications.

Supplemental Table 2: ConSite analysis of transcription factor binding elements in the miR-31 promoter. Shown is a list of TF binding elements within the miR-31 proximal promoter. Transcription factors were analyzed for known involvement in KRAS-MAPK pathway.

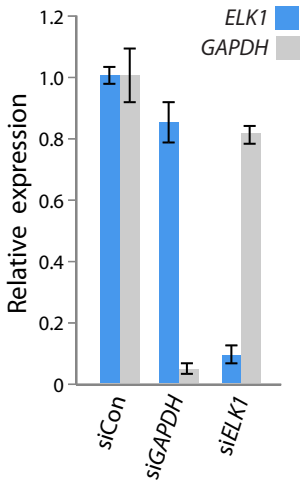
Supplemental Table 3: Analysis of potential miR-31 target genes. Shown are the 18 target genes from the intersection of 4 prediction algorithms and the representative number of conserved and non-conserved miR-31 binding sites (from Targetscan).

Supplemental Table 4: Literature analysis of miR-31 invasion-migration publications.

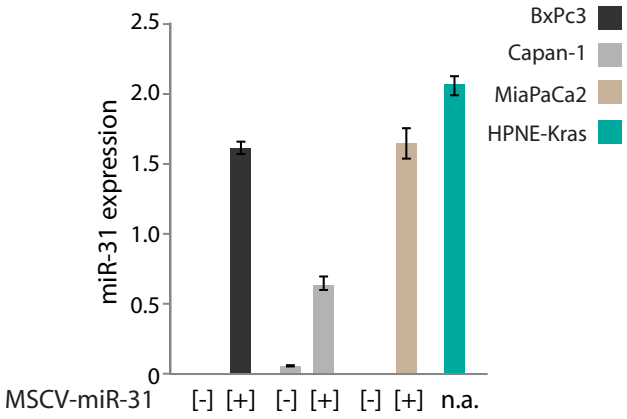
Supplemental Figure 1



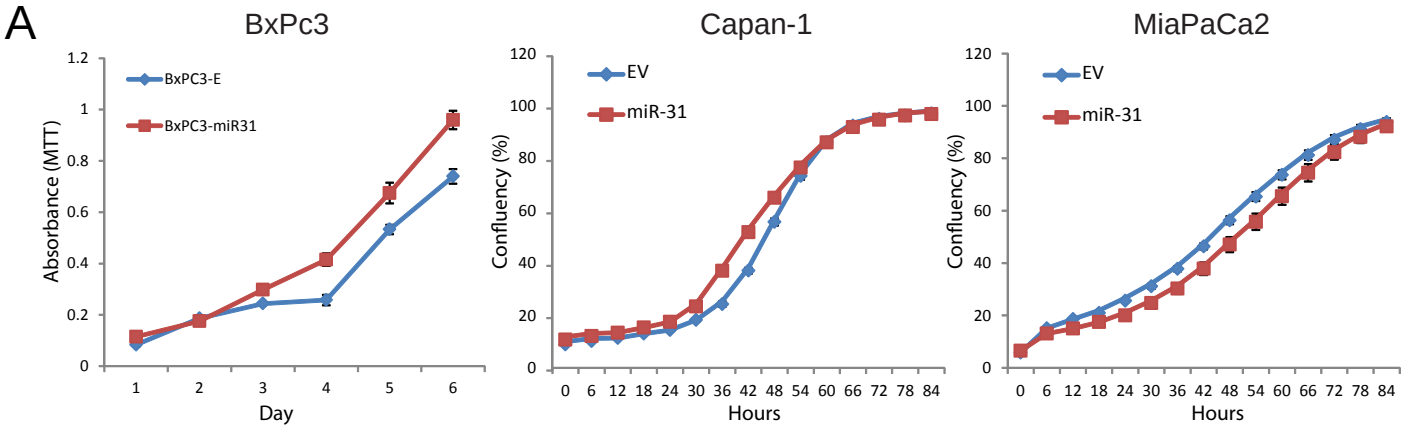
Supplemental Figure 2



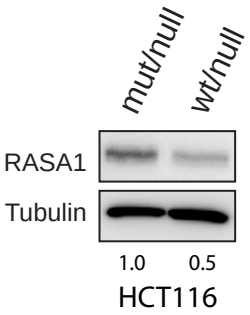
Supplemental Figure 3



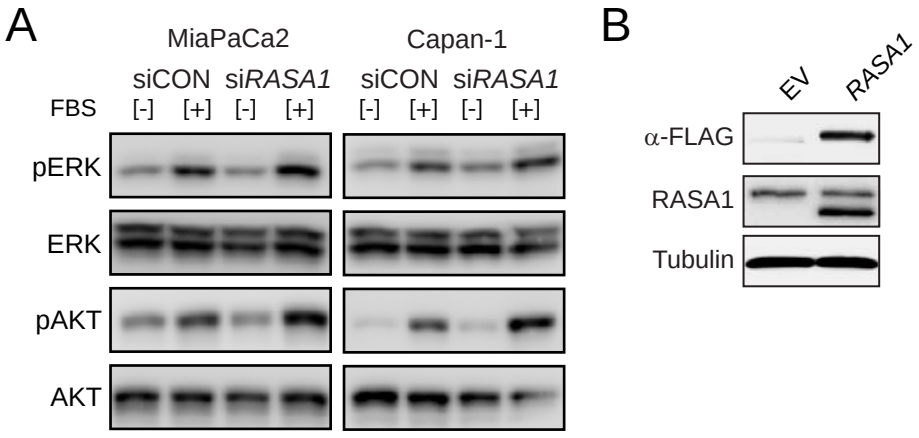
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Table 1: Primer sequences

Application	Target	FW / RV
QPCR	B-actin	AGGCACCAGGGCGTGAT
		GCCCACATAGGAATCCTTCTGAC
	ELK1	AATTCAAGCTGGTGGATGC
		TAACAGACACCTCTGGCTG
	GAPDH	AATCCCATCACCATCTTCCA
		TGGACTCCACGACGTACTCA.
	KRAS	CATGGACTGTGTCCCCACG
		TGACTAACCAATGCATGACAACACT
	MIR31HG	CCGAGTAGGAGGACAGAAGC
		GAGGCGGTGTTCCGTGAG
	RASA1	GGCGTTCTTCTGCTATCGTT
		TGGACCAGAATACGAGGAGG
ChiP amplicon	miR-31-promoter up	CTCCATACTGCAAACCAGCA
		GTGAGAAGGCAGTCCAGGAA
	miR-31-promoter down	AAGTAAACGACAGCTAGGAATCA
		GGCAGTCATATGGTTTGAGATTT
	miR-31 promoter TSS	GGAGCTTCTCGCAGTCAGAG
		ACTTCCGCTGTTCAATTTGC
Cloning	miR-31 pre-miRNA	AGCCTCGAGTACCCACAAACCTCCTGTGC
		AGCCTCGAGTGTGGCTAGCATGGAGTGAA
	RASA1 3'UTR	CTAGTCTAGACTGCATGGATTCAGCATGTC
		CTAGTCTAGATTCGTTTTGTGAAAGGTGGTC
	Mutageneis UTR	CACTTCAGTTTAATGTCTCCTTTGCTGTAGGCTAAAAATAGCACACTTTTCCACATTCC
		GGAATGTGGAAAAGTGTGCTATTTTTAGCCTACAGCAAAGGAGACATTAAACTGAAGTG
	HG-2 promoter	GCGGAGATCTGGTGTCGACTGCCGTGTG
		GCGGCTAGCTGGGAGTTCATAAATTCAACCTG
	HG-3 promoter	GCGGAGATCTGGTGTCGACTGCCGTGTG
		GCGGCTAGCGAGAGAGGCCACTCCAGATG
	HG-4 promoter	GCGGAGATCTTATCCTCAACCCTCCGTGTC
		GCGGCTAGCTGGGAGTTCATAAATTCAACCTG
	HG-7 promoter	GCGGAGATCTGGTGTCGACTGCCGTGTG
		GCGGCTAGCCTTCCCTCCCCCTCCTTT
	HG-9 promoter	GCGGAGATCTAAGAGGCGCCTGGACG
		GCGGCTAGCGAGAGAGGCCACTCCAGATG

Supplemental Table 2: Consite analysis of transcription factor binding elements in the miR-31 promoter.

Transcription factor	Sequence	From	To	Score	Strand	Known Pathway
AML-1	TTTGCGGGT	168	176	8.12	+	u
AP2alpha	GCCTGGACG	18	26	6.203	+	u
AP2alpha	CCCTTGGGC	58	66	8.599	-	
AP2alpha	GCCCGGACG	65	73	6.793	+	
AP2alpha	GCTTTTGGC	149	157	6.288	-	
AP2alpha	GCCCCGTGG	345	353	7.587	+	
E2F	TTTGGCAC	152	159	8.832	+	ErbB/HER
Elk-1	GGCCCGGACG	64	73	8.131	+	MAPK/P38/TCF/SRF
Elk-1	ACAGCGGAAG	273	282	7.333	+	
FREAC-3	TACGCTCG	142	149	5.723	-	WNT
FREAC-3	GCACAGTA	156	163	6.501	+	
FREAC-3	TACTCTCC	243	250	6.986	-	
FREAC-3	CGGAAGTA	277	284	6.624	+	
FREAC-3	TACTTCCT	283	290	6.624	-	
GATA-2	TATCA	337	341	5.778	-	u
GATA-2	CATCT	388	392	5.559	-	
GATA-2	TATCC	409	413	6.651	-	
GATA-3	CTATCA	336	341	8.092	-	u
GATA-3	CCATCT	387	392	5.612	-	
GATA-3	CTCTCT	401	406	5.286	-	
GATA-3	CTATCC	408	413	6.215	-	
Max	GGCCCCGTGG	344	353	8.798	+	P38
Max	CCCCGTGGTA	346	355	8.251	-	
Myc-Max	GGCCCCGTGGT	344	354	9.956	+	
MZF_1-4	GGGGGA	2	7	8.252	+	u
MZF_1-4	TGGGGG	123	128	6.469	+	
MZF_1-4	GGGGGA	124	129	8.252	+	
MZF_1-4	TCCTCA	411	416	6.469	-	
MZF_5-13	TGTGGGGGAA	121	130	7.301	+	u
SAP-1	AGCGGAAGT	275	283	10.27	+	MAPK/P38/TCF/SRF
SPI-1	GGGAAG	8	13	8.579	+	MAPK
SPI-1	CGGAAT	113	118	5.504	+	
SPI-1	GGGAAG	126	131	8.579	+	
SPI-1	CTTCTC	182	187	5.496	-	
SPI-1	CGGAAG	277	282	8.711	+	
SPI-1	CTTCCT	285	290	8.184	-	
SPI-B	AGCGGAA	275	281	9.933	+	
Thing1-E47	TAACTGGCAT	356	365	8.885	+	WNT
Thing1-E47	CATCTGGAGT	388	397	9.404	+	
USF	CCCCGTG	346	352	7.859	-	u
Yin-Yang	TCCATC	386	391	7.389	+	u

Notes:

u = unknown

Supplemental Table 3: Analysis of potential miR-31 target genes

GENE	Gene name	Conserved sites	Poorly conserved sites
		total	total
AKAP7	A kinase (PRKA) anchor protein 7	0	3
CAMK2D	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	1	0
DCBLD2	discoidin, CUB and LCCL domain containing 2	1	1
HIAT1	hippocampus abundant transcript 1	1	0
HIF1AN	hypoxia-inducible factor 1, alpha subunit inhibitor	1	3
KHDRBS3	KH domain containing, RNA binding, signal transduction associated 3	1	0
NUMB	numb homolog (Drosophila)	1	0
PEX5	peroxisomal biogenesis factor 5	1	1
RASA1	RAS p21 protein activator (GTPase activating protein) 1	1	0
RGS4	regulator of G-protein signaling 4	1	2
RHOBTB1	Rho-related BTB domain containing 1	1	1
RSBN1	round spermatid basic protein 1	2	3
SH2D1A	SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome)	2	0
SLC1A2	solute carrier family 1 (glial high affinity glutamate transporter), member 2	2	2
SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	1	1
STX12	syntaxin 12	1	0
TESK2	testis-specific kinase 2	1	1
TFRC	transferrin receptor (p90, CD71)	1	0

Supplemental Table 4: Literature analysis of miR-31 invasion-migration publications

Publication:	Cancer type:	miR-31 expression:	Invasion migration phenotype:	Validated miR-31 target(s):	Functional studies:	Reference:
1	Ovarian (Serous epithelial)	dn	decreased	AF4/FMR2	PCMI	Ibrahim et al.,J Ovarian Res., 2015
2	Cervical	up	enhanced	n/a	PMI	Zheng et al., Arch Gynecol Obstet. 2015
3	Esophageal	dn	decreased	n/a	PMI	Koumangoye et al., Mol Cancer 2015
4	Breast	dn	decreased	n/a	MI	Ben-chetrit et al., Sci Signal 2015
5	(Chinese)					
6	Cutaneous squamous cell carcinoma	up	increased	n/a	CIM	Wang et al., PLoS One 2014
7	Cervical	up	increased	ARID1A	PCMI	Wang et al., Gynecol Oncol 2014
8	Breast	dn	decreased	EMSY	PMI	Vire etal., Mol Cell 2014
9	Colon	n/a	increased	HIF1A/FIH1	PMI	Chen etal., 2014
10	Colon	up	increased	SATB2	PMI	Yang et al., PLoS One 2013
11	Lung adenocarcinoma	up	increased	n/a	PMI	Meng et al., 2013
12	Breast	dn	decreased	PKC epsilon	A	Korner et al., J Biol Chem, 2013
13	C.A.F. (not included)					
14	Melanoma	dn	decreased	SRC, MET, MAP3K14, RAB27A, EZH2	n/a	Asangani et al., Oncotarget 2012
15	Prostate	dn	decreased	examined putative targets only	PMI	Fuse et al., J Hun Genet. 2012
16	Cutaneous squamous cell carcinoma	up	*	*focused on miR-125b		Xu et al., J Biol Chem 2012
17	Oral leukoplakia	up (miR-31*)	increased	FGF3	PCMIA	Xiao et al., PLoS One 2012
18	Ovarian	n/a	increased	TIAM1	PMI	Li et al., Oncol Rep 2012
19	Pancreatic	on/off	both	n/a	PMI	Laurila et al., Genes Chromosomes Cancer 2012
20	Oral	dn	*	*focused on miR-10b		Lu et al., Cancer Prev Res (Phila) 2012
21	Glioma	dn	decreased	n/a	MI	Hua et al., Oncol Rep 2012
22	Esophageal (squamous cell carcinoma)	up	increased	EMP1, KSR2, RGS4	CMI	Zhang et al., Clin Sci (Lond) 2011
23	Colon	n/a	decreased	n/a	CMI	Wang et al., BMC Cancer 2010
24	C.A.F. (not included)					
25	Colon	up	increased	TIAM1	MI	Cottonham et al., J Biol Chem 2010
26	Mesothelioma	dn	decreased	PPP6C	PCMI	Ivanov et al., J Biol Chem 2010
27	Kaposi's sarcoma	up	increased	n/a	MI	Tsai et al., J Virol 2009

Notes:

n/a	Not analyzed
P	Proliferation growth assay
C	Clonegenicity/colony assay
M	Migration
I	Invasion
A	Apoptosis

Supplemental Materials:

Antibodies used: Antibodies were obtained from Cell Signaling Technologies (AKT: 9272; pAKT:4060; ERK: 9102; pERK: 9101; GAPDH: 2118; RHO: 2117), Santa Cruz (ELK1: sc-22804X; RASA1: sc-063; Tubulin: sc-69969) and Sigma (Flag-M2: F3165).