Supplementary Information

Supplementary Table 1 : Primers used in Real time quantitative PCR amplifications.

Target	DNA template	Forward primer	Reverse primer
18s ribosomal RNA	cDNA	5’- GCCATGCATGTCTGAGTACG-3’	5’- CCGTCGGCATGTATTAGCTC-3’
FOXO1 mRNA	cDNA	5’-AAGAGCGTGCCCTACTTCAA-3’	5’-CTGTTGTTGTCCATGGATGC-3’
ADCY6 mRNA	cDNA	5’-AGGCAAACAATGAGGGTGTC-3’	5’-CGCTCCTCGCTGATAATCTC-3’
AGO1 mRNA	cDNA	5’-ACAAGTCCACCCGTTTCAAG-3’	5’-ATCCCAGGCTGGTAGTCCTT-3’
AGO2 mRNA	cDNA	5’-TGTGTACCGAGTGGGATTCA-3’	5’-TTTGAAATCTGGGACGGAAG-3’
AGO3 mRNA	cDNA	5’-AGTGAACAGGGAGGTGGTTG-3’	5’-AGTGGATTGGCGGTGTAAAG-3’
AGO4 mRNA	cDNA	5’-GCAAATCCACCATGATACCC-3’	5’-GCGGTAGGCATTTGAAACAT-3’
GPC3 mRNA	cDNA	5’-AACTCCGAAGGACAACGAGA-3’	5’-GCACCAGGAAGAAGAAGCAC-3’

Supplementary Table 2 : Sequence of synthetic double-stranded small RNAs

miRNA	Guide strand	Passenger strand
Hsa-miR-96 8mer	5’-UUUGGCAC-3’	5’-GUGCCAAA-3’
Hsa-miR-182	5'-UUUGGCAAUGGUAGAACUCACACU-3'	5’-UGGUUCUAGACUUGCCAACUA-3’
Hsa-miR-182mut	5'-UUUGGCACUGGUAGAACUCACACU-3'	5’-UGGUUCUAGACGUGCCAACUA-3’
Hsa-miR-96	5'-UUUGGCACUAGCACAUUUUUGCU-3'	5'-AAUCAUGUGCAGUGCCAAUAUG-3'
Hsa-miR-96mut	5’-UUUGGCAAUAGCACAUUUUUGCU-3’	5’-AAUCAUGUGCAUUGCCAAUAUG-3’

Supplementary Figure Legends:

Supplementary Figure S1: miRNA:GPC3 predictions using Targetscan (Panel A) (*1*), Diana microT v.4 (Panel B) (*2*), miRanda/mirSVR (Panel C) (*3*), PITA (Panel D) (*4*), Pictar (Panel E) (*5*) and miRDB (Panel F) (*6, 7*) algorithms.

Supplementary Figure S2: Panel A: Location and ribonucleic sequence of miR-96/182 site in the GPC3 3'UTR and conservation amongst species (Targetscan predictions; (*1*)). The predicted site recognized by each miRNA 5'-end is shown in a white box. Panel B: Table summarizing the relative expression of miR-96 and miR-182 in HuH7 and SNU398 cells. Panel C: Amplification plots (in triplicate) of miR-182 using reverse-transcribed RNA from HuH7 cells transfected with the small RNA control siLuc or miR-182 as shown. Panel D: Relative expression of ADCY6 mRNA (left panel; ANOVA: $p < 0.0001$; n=3) and protein (right panel; representative of two independent experiments) measured in HuH7 cells transfected with the small RNA control siLuc, miR-96 or miR-182 as shown. Left panel:.

Supplementary Figure S3: Panel A: eGFP-GPC3-expressing SNU398 cells were transfected with the indicated small RNAs. Three days later, eGFP protein expression was analyzed by FACS following the FunREG pipeline (see Figure 2B; ANOVA: $p < 0.0001$; n=4). Panel B: eGFP-GPC3-expressing HuH7 cells were transfected with the indicated concentrations of miR-96. Three days later, the amounts of eGFP protein was analyzed as described in Panel A (n=3). Panels C to F: Relative expression of the corresponding Argonaute mRNAs measured in HuH7 cells transfected with the indicated small RNAs (ANOVA as indicated, n=3). *: $p < 0.05$; ***: $p < 0.001$.

Supplementary Figure S4: Relative expression of FOXO1 mRNA measured in HuH7 cells transfected with the indicated small RNA (ANOVA: $p < 0.0001$; n=3). ***: $p < 0.001$.

Supplementary references

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6. Wang, X. (2008) miRDB: a microRNA target prediction and functional annotation database with a wiki interface, *Rna 14*, 1012-1017.
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A

Targetscan

Human GPC3 3' UTR miRNA Table

Table sorted by total context score [Sort table by aggregate P_{CT}]

miRNA families broadly conserved among vertebrates

miRNA	conserved sites				poorly conserved sites				Total Context score	Aggregate P_{CT}
	Total	8mer	7mer-m8	7mer-1A	Total	8mer	7mer-m8	7mer-1A		
miR-96/1271	1	1	0	0	0	0	0	0	-0.49	0.60
miR-204/211	1	1	0	0	0	0	0	0	-0.41	0.15
miR-140/140-5p/876-3p	0	0	0	0	1	0	1	0	-0.24	< 0.1
miR-182	0	0	0	0	1	0	0	1	-0.20	0.51
miR-15/16/195/424/497	0	0	0	0	1	0	0	1	-0.18	< 0.1
miR-503	0	0	0	0	1	0	0	1	-0.18	< 0.1
miR-7/7ab	0	0	0	0	1	0	1	0	-0.14	0.14

miRNA families conserved only among mammals

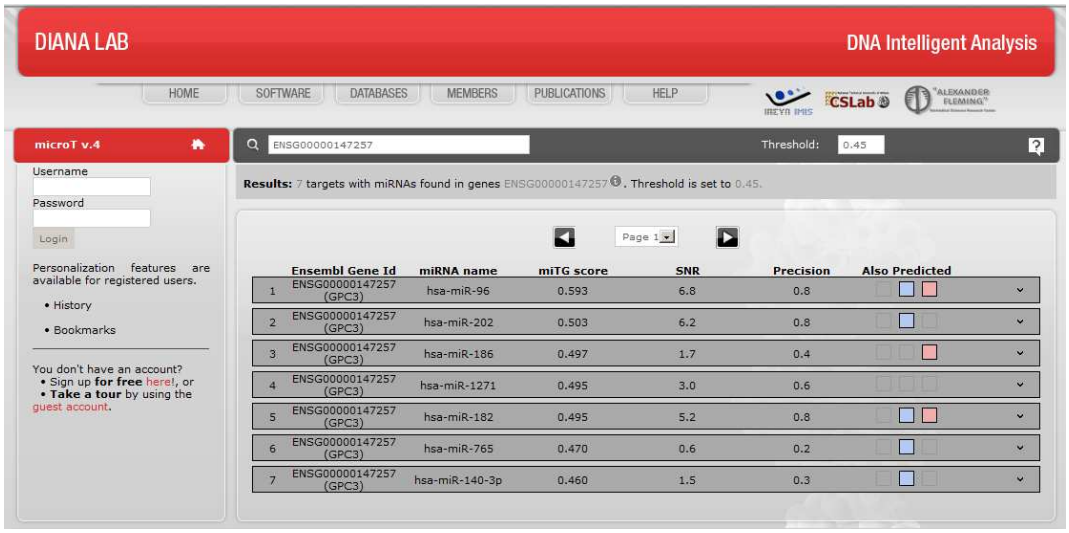
miRNA	conserved sites				poorly conserved sites				Total Context score	Aggregate P_{CT}
	Total	8mer	7mer-m8	7mer-1A	Total	8mer	7mer-m8	7mer-1A		
miR-202/202-3p	0	0	0	0	1	0	1	0	-0.21	< 0.1
miR-495/1192	0	0	0	0	1	0	0	1	-0.15	< 0.1

Poorly conserved miRNA Families

miRNA	conserved sites				poorly conserved sites				Total Context score	Aggregate P_{CT}
	Total	8mer	7mer-m8	7mer-1A	Total	8mer	7mer-m8	7mer-1A		
miR-765	1	1	0	0	0	0	0	0	-0.37	< 0.1
miR-518a-5p/527	0	0	0	0	2	0	1	1	-0.36	< 0.1
miR-570	0	0	0	0	1	0	1	0	-0.27	< 0.1
miR-450b/450b-5p	0	0	0	0	1	0	1	0	-0.25	< 0.1
miR-1321	0	0	0	0	1	0	1	0	-0.23	< 0.1
miR-607	0	0	0	0	1	0	0	1	-0.23	< 0.1
miR-220c	0	0	0	0	1	0	1	0	-0.20	< 0.1
miR-1305	0	0	0	0	1	0	0	1	-0.19	< 0.1
miR-646	0	0	0	0	1	0	0	1	-0.17	< 0.1
miR-151	0	0	0	0	1	0	0	1	-0.16	< 0.1
miR-641	0	0	0	0	1	0	1	0	-0.15	< 0.1
miR-611	0	0	0	0	1	0	0	1	-0.14	< 0.1
miR-623	0	0	0	0	1	0	0	1	-0.12	< 0.1
miR-140-3p	0	0	0	0	1	0	1	0	-0.11	< 0.1
miR-612/1285	0	0	0	0	1	0	1	0	N/A	< 0.1

B

Diana microT v.4



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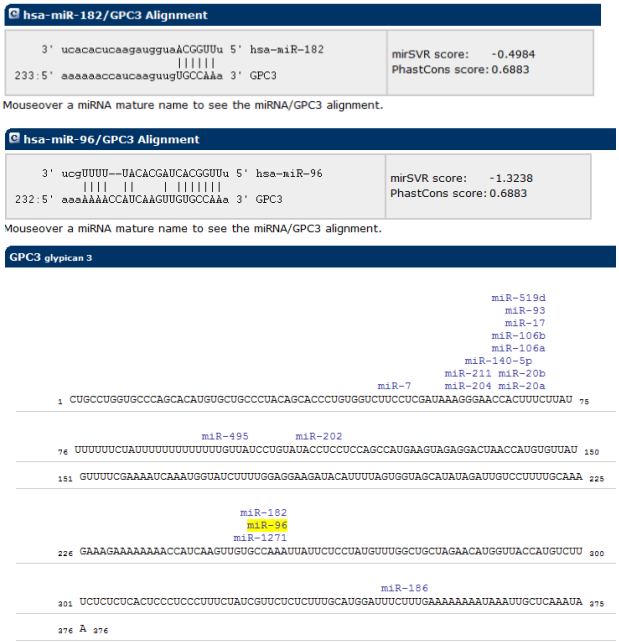
C miRanda/mirSVR



D PITA

Predicted Sites

Gene	microRNA	Position	Seed	dGduplex	dGopen	ddG
Human_GPC3_3UTR	hsa-miR-765	112	7:0:0	-18.5	-2.27	-16.22
Human_GPC3_3UTR	hsa-miR-1321	313	7:0:0	-17.8	-3.20	-14.59
Human_GPC3_3UTR	hsa-miR-518a-5p	334	7:0:0	-14.9	-2.53	-12.36
Human_GPC3_3UTR	hsa-miR-527	334	7:0:0	-14.9	-2.53	-12.36
Human_GPC3_3UTR	hsa-miR-202	107	8:0:0	-15.6	-5.77	-9.82
Human_GPC3_3UTR	hsa-miR-96	249	7:0:0	-15.2	-7.57	-7.62
Human_GPC3_3UTR	hsa-miR-220c	37	7:0:0	-22.2	-15.36	-6.83
Human_GPC3_3UTR	hsa-miR-204	56	7:0:0	-17.54	-11.32	-6.21
Human_GPC3_3UTR	hsa-miR-211	56	7:0:0	-17.54	-11.32	-6.21
Human_GPC3_3UTR	hsa-miR-140-3p	38	8:0:0	-21.17	-15.34	-5.82
Human_GPC3_3UTR	hsa-miR-140-5p	62	7:0:0	-14.62	-9.06	-5.55
Human_GPC3_3UTR	hsa-miR-450b-5p	219	7:0:0	-11.71	-6.26	-5.44
Human_GPC3_3UTR	hsa-miR-1271	249	7:0:0	-12.65	-7.57	-5.07
Human_GPC3_3UTR	hsa-miR-570	150	7:0:0	-13.7	-9.41	-4.28
Human_GPC3_3UTR	hsa-miR-641	294	7:0:0	-13.3	-9.70	-3.59
Human_GPC3_3UTR	hsa-miR-7	44	7:0:0	-17.6	-14.48	-3.11

E Pictar

Predicted miRNAs	Pictar Score
Hsa-miR-369-3p	8.7245
Hsa-miR-182	5.3604
Hsa-miR-96	4.2667
Hsa-miR-186	1.4920

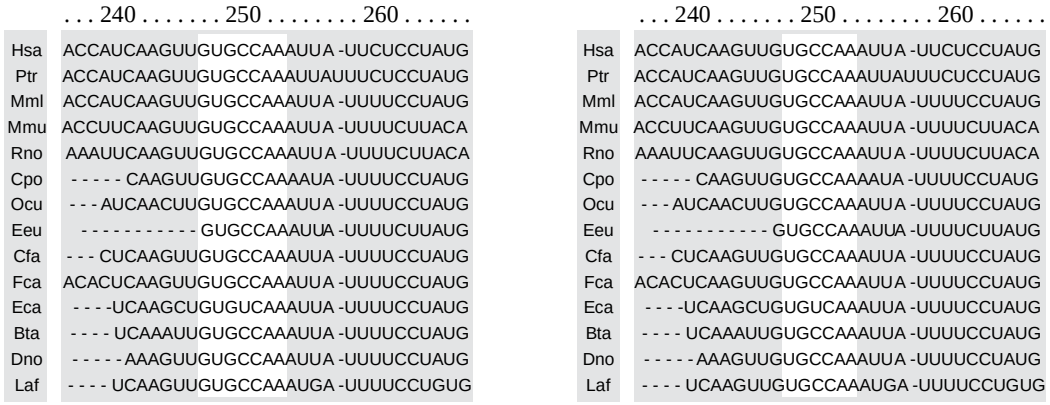
F miRDB

Target Detail	Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
Details	1	69	hsa-miR-1271	GPC3	glypican 3
Details	2	69	hsa-miR-450b-5p	GPC3	glypican 3
Details	3	67	hsa-miR-96	GPC3	glypican 3
Details	4	59	hsa-miR-570	GPC3	glypican 3

Jalvy S et al, Supplementary Figure S1

A

GPC3 3'UTR

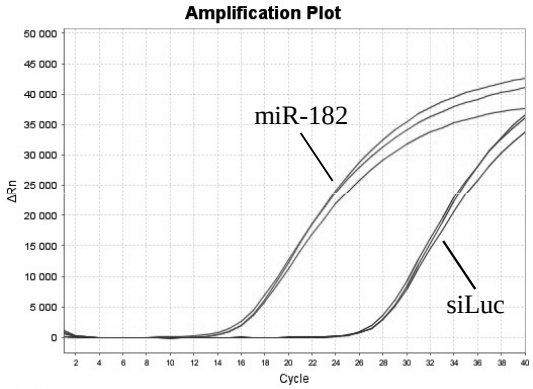


B

Cells	RNA 18s CT	miR-182 CT	miR-96 CT	ΔCT miR-182	ΔCT miR-96
HuH7	6,7	27,7	33,7	-21	-27
SNU398	6,4	26,3	32,1	-19,9	-25,7

C

miR-182



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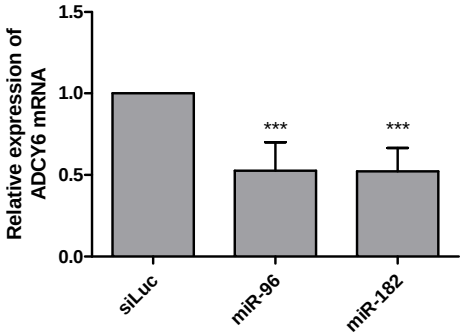

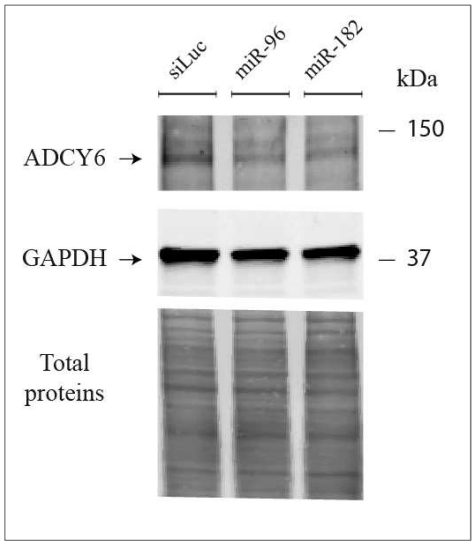

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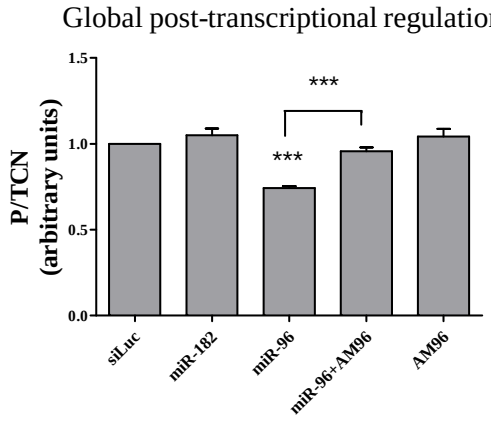
A



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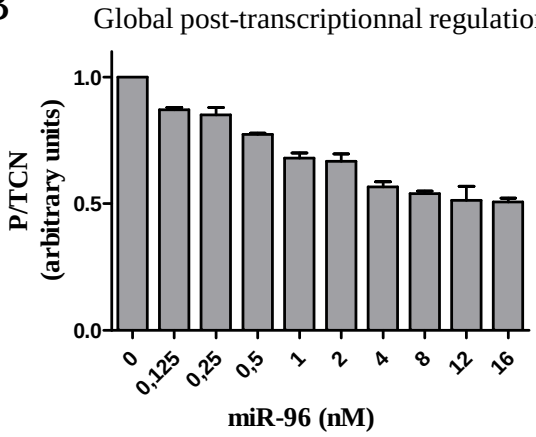

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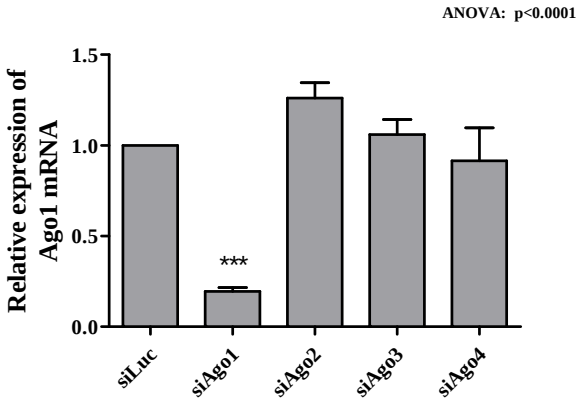

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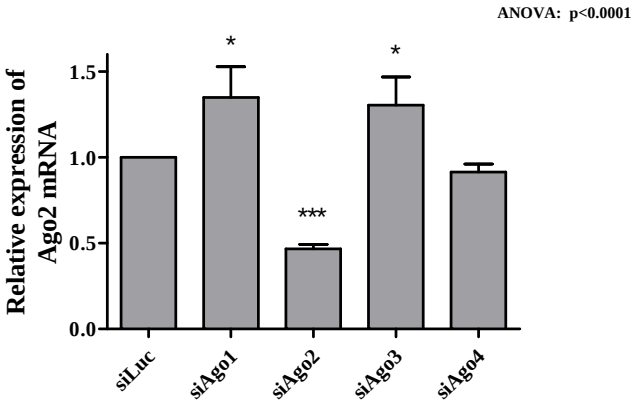

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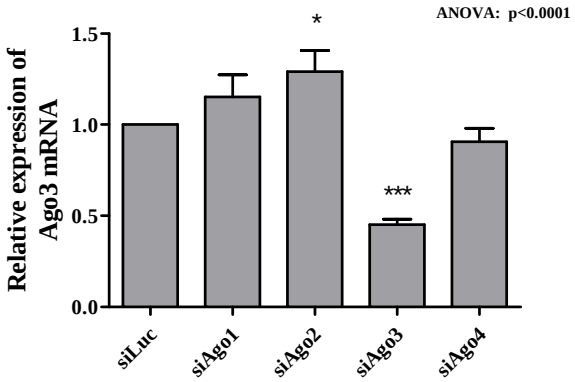

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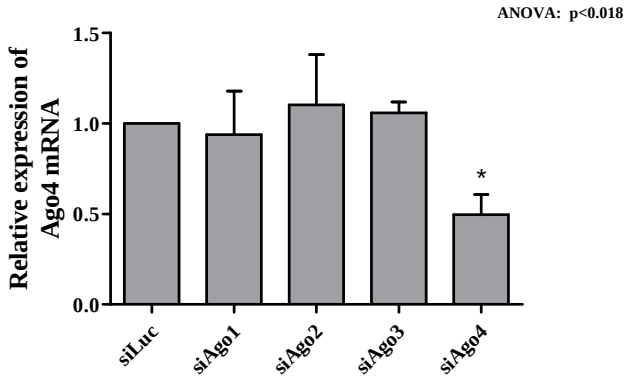

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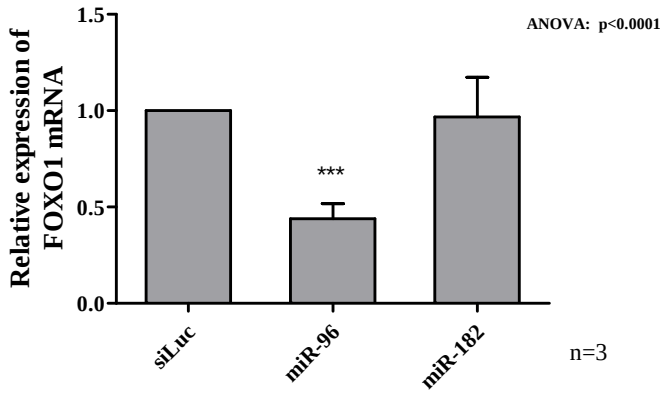

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