

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'-TTTAAAATCTGGGACGGAAG-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'-AAUCAUGUGCAGUGCCAUAUG-3'
Hsa-miR-96mut	5'-UUUGGCAAUAGCACAUUUUUGCU-3'	5'-AAUCAUGUGCAUUGCCAUAUG-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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3. Betel, D., Koppal, A., Agius, P., Sander, C., and Leslie, C. (2010) Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites, *Genome Biol* 11, R90.
4. Kertesz, M., Iovino, N., Unnerstall, U., Gaul, U., and Segal, E. (2007) The role of site accessibility in microRNA target recognition, *Nat Genet* 39, 1278-1284.
5. Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da Piedade, I., Gunsalus, K. C., Stoffel, M., and Rajewsky, N. (2005) Combinatorial microRNA target predictions, *Nat Genet* 37, 495-500.
6. Wang, X. (2008) miRDB: a microRNA target prediction and functional annotation database with a wiki interface, *Rna* 14, 1012-1017.
7. Wang, X., and El Naqa, I. M. (2008) Prediction of both conserved and nonconserved microRNA targets in animals, *Bioinformatics* 24, 325-332.

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-98/1271	1	1	0	0	0	0	0	0	-0.49	0.80
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-846	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-841	0	0	0	0	1	0	1	0	-0.15	< 0.1
miR-811	0	0	0	0	1	0	0	1	-0.14	< 0.1
miR-823	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-812/1285	0	0	0	0	1	0	1	0	N/A	< 0.1

B

Diana microT v.4

The screenshot shows the Diana microT v.4 web interface. At the top, there is a navigation bar with 'DIANA LAB' and 'DNA Intelligent Analysis'. Below this are tabs for 'HOME', 'SOFTWARE', 'DATABASES', 'MEMBERS', 'PUBLICATIONS', and 'HELP'. A search bar contains the query 'ENSG00000147257' and a threshold is set to '0.45'. The results section displays '7 targets with miRNAs found in genes ENSG00000147257'. A table lists the following targets:

Ensembl Gene Id	miRNA name	miTG score	SNR	Precision	Also Predicted
1 ENSG00000147257 (GPC3)	hsa-miR-96	0.593	6.8	0.8	<input type="checkbox"/> <input type="checkbox"/>
2 ENSG00000147257 (GPC3)	hsa-miR-202	0.503	6.2	0.8	<input type="checkbox"/> <input type="checkbox"/>
3 ENSG00000147257 (GPC3)	hsa-miR-186	0.497	1.7	0.4	<input type="checkbox"/> <input type="checkbox"/>
4 ENSG00000147257 (GPC3)	hsa-miR-1271	0.495	3.0	0.6	<input type="checkbox"/> <input type="checkbox"/>
5 ENSG00000147257 (GPC3)	hsa-miR-182	0.495	5.2	0.8	<input type="checkbox"/> <input type="checkbox"/>
6 ENSG00000147257 (GPC3)	hsa-miR-765	0.470	0.6	0.2	<input type="checkbox"/> <input type="checkbox"/>
7 ENSG00000147257 (GPC3)	hsa-miR-140-3p	0.460	1.5	0.3	<input type="checkbox"/> <input type="checkbox"/>

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C miRanda/mirSVR

hsa-miR-182/GPC3 Alignment

```

3' ucacacucaagaugguaACGGUUu 5' hsa-miR-182
      |||||
233:5' aaaaaaccucaaguuGOCaAa 3' GPC3
  
```

mirSVR score: -0.4984
PhastCons score: 0.6883

Mouseover a miRNA mature name to see the miRNA/GPC3 alignment.

hsa-miR-96/GPC3 Alignment

```

3' ucgUUUU--DACACGADCAAGUUu 5' hsa-miR-96
      |||||
232:5' aaaaaaaCCAUCAAAGUUGGCCAAa 3' GPC3
  
```

mirSVR score: -1.3238
PhastCons score: 0.6883

Mouseover a miRNA mature name to see the miRNA/GPC3 alignment.

GPC3 glypican 3

```

                                     miR-519d
                                     miR-93
                                     miR-17
                                     miR-106b
                                     miR-106a
                                     miR-140-3p
                                     miR-211 miR-20b
                                     miR-7  miR-204 miR-20a
1  CUGCCUGGUGCCGACACAUUGUGCCUACAGCACCUGUGGUCUUCUGAUAAAAGGGAACCACUUUCUUAU 75

                                     miR-495  miR-202
76 UUUUUUCUAUUUUUUUUUUUUUUUUAUCCUGUADACCUCUCCAGCAUGAAGUAGAGGACUAACCAUGUGUUAU 150

181 GUUUUCGAAAAUCAAAUGGUAUUCUUUUGGAGGAAGAUACAUUUUAGUGUGUAGCAUAUAGAUAUGUCCUUUUGCAAA 226

                                     miR-182
                                     miR-96
226 GAAAGAAAAAAAACCAUCAAGUUGGCCAAAUUAUUCUCCUAUGUUUGGUCUAGAACAUUGUUAUACCAUUGUCUU 200

                                     miR-1271
301 UCUCUCUCACUCCUCCUUCUUCUUGUUUCUCUUUGCAUGGAUUUUUGAAAAAAAUAUUUUGUCUCAAUA 275

376 A 376
  
```

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

A

GPC3 3'UTR

```

... 240 ..... 250 ..... 260 .....
Hsa ACCAUCAAGUUGGCCAAAUUA -UUCUCCUAUG
Ptr ACCAUCAAGUUGGCCAAAUUAUUUCCUAUG
Mml ACCAUCAAGUUGGCCAAAUUA -UUUCCUAUG
Mmu ACCUUCAAGUUGGCCAAAUUA -UUUUCUACA
Rno AAUUUCAAGUUGGCCAAAUUA -UUUUCUACA
Cpo - - - - CAAGUUGGCCAAAUUA -UUUCCUAUG
Ocu - - - AUCAACUUGGCCAAAUUA -UUUCCUAUG
Eeu - - - - - - - - GUGCCAAAUUA -UUUUCUUAUG
Cfa - - - CUCAAGUUGGCCAAAUUA -UUUCCUAUG
Fca ACACUCAAGUUGGCCAAAUUA -UUUCCUAUG
Eca - - - -UCAAGCUGUGUCAAAAUUA -UUUCCUAUG
Bta - - - -UCAAAUUGGCCAAAUUA -UUUCCUAUG
Dno - - - -AAAGUUGGCCAAAUUA -UUUCCUAUG
Laf - - - -UCAAGUUGGCCAAAUUA -UUUCCUGUG
    
```

miR-96 site

```

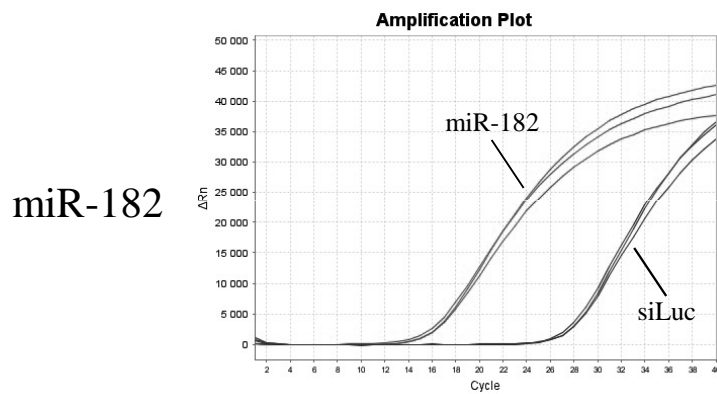
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Ptr ACCAUCAAGUUGGCCAAAUUAUUUCCUAUG
Mml ACCAUCAAGUUGGCCAAAUUA -UUUCCUAUG
Mmu ACCUUCAAGUUGGCCAAAUUA -UUUUCUACA
Rno AAUUUCAAGUUGGCCAAAUUA -UUUUCUACA
Cpo - - - - CAAGUUGGCCAAAUUA -UUUCCUAUG
Ocu - - - AUCAACUUGGCCAAAUUA -UUUCCUAUG
Eeu - - - - - - - - GUGCCAAAUUA -UUUUCUUAUG
Cfa - - - CUCAAGUUGGCCAAAUUA -UUUCCUAUG
Fca ACACUCAAGUUGGCCAAAUUA -UUUCCUAUG
Eca - - - -UCAAGCUGUGUCAAAAUUA -UUUCCUAUG
Bta - - - -UCAAAUUGGCCAAAUUA -UUUCCUAUG
Dno - - - -AAAGUUGGCCAAAUUA -UUUCCUAUG
Laf - - - -UCAAGUUGGCCAAAUUA -UUUCCUGUG
    
```

miR-182 site

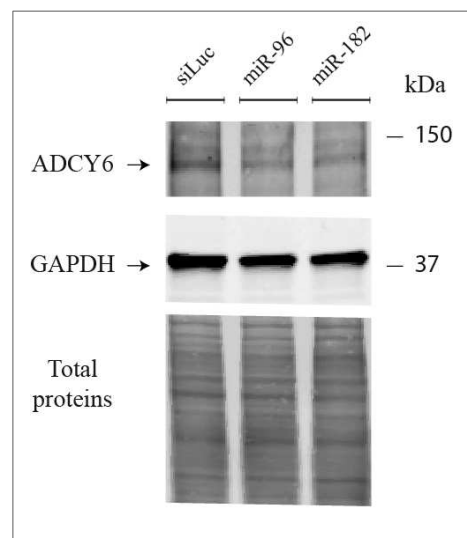
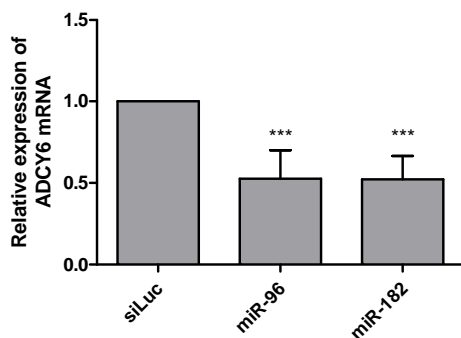
B

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

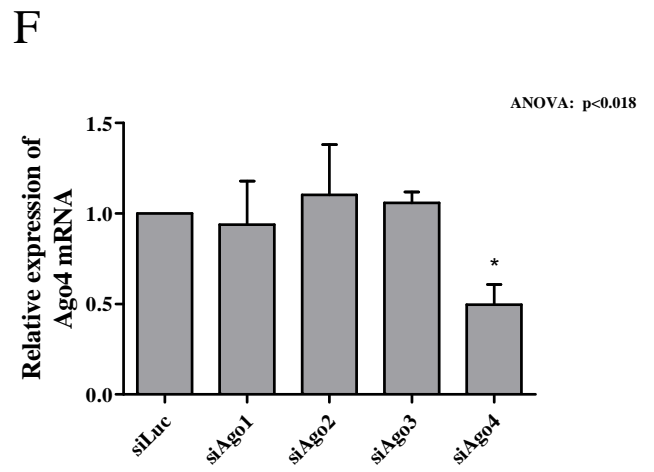
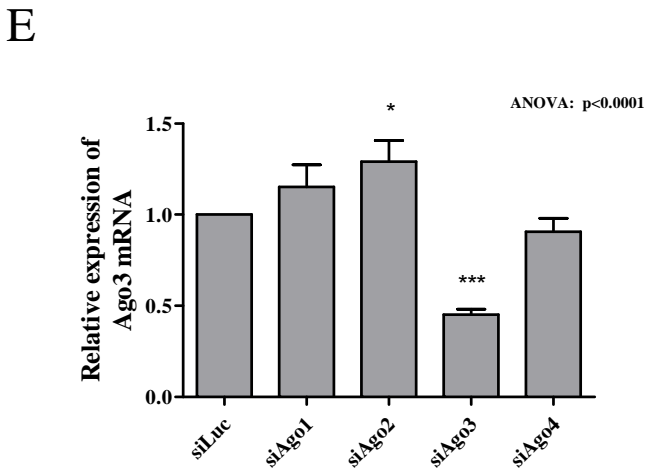
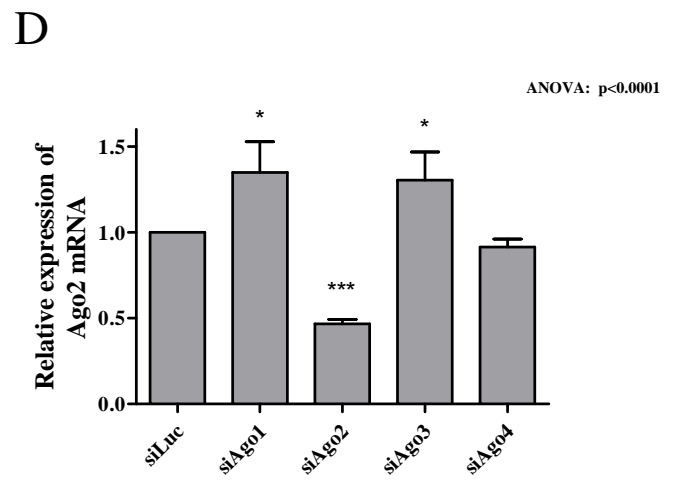
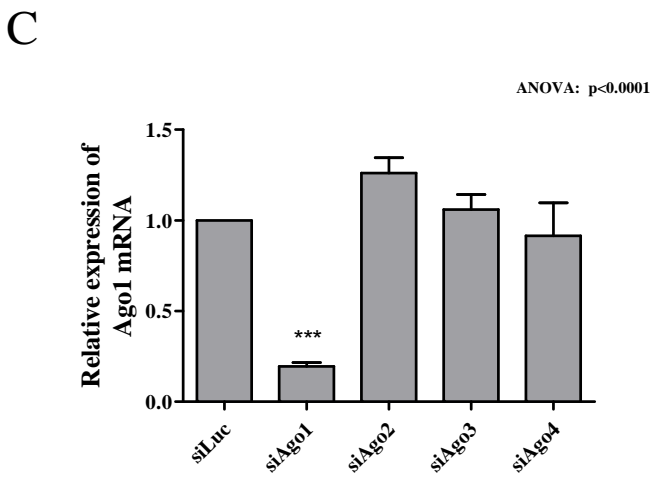
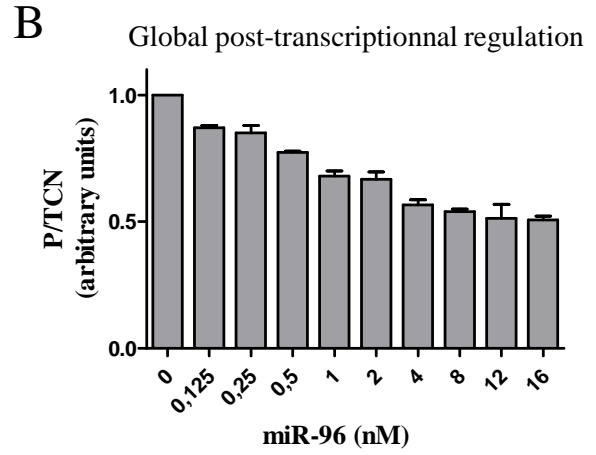
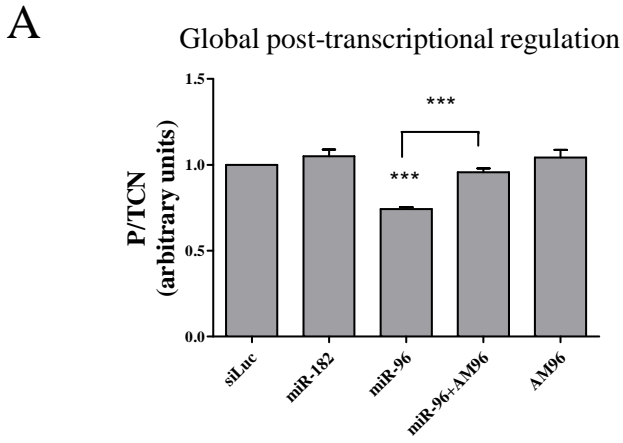
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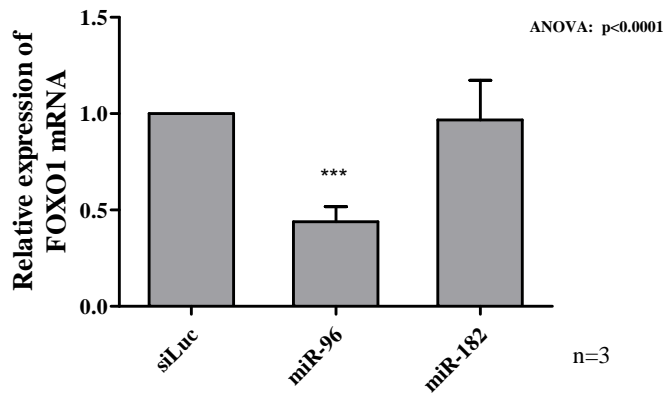
D



Jalvy S et al, Supplementary Figure S2



Jalvy S et al, Supplementary Figure S3



Jalvy S et al, Supplementary Figure S4