SUPPLEMENTARY INFORMATION

microRNA editing in seed region is in synergy with cellular changes in hypoxic conditions.

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Supplementary Figure Legends

Figure S1: Alon-Eisenberg pipeline scheme. Workflow applied to systematically identify editing events in each miRNA-seq dataset originated from each biological duplicate of each experimental condition (GEO reference: GSE47534; miRNA-seq: GSE47602): normoxia, 16h, 32h and 48h hypoxia.

Figure S2: Distribution of modification event location and type. (a) Location and types of modification events in mature miRNAs. **(b)** Percentage of A-to-G modification sites both within and outside miRNA seed regions

Figure S3: Statistically significant miRNA modification events and relative AMLs in all conditions. MSR modification events are marked in bold. Previously documented events are provided with corresponding references: (1), (2), and (3).

Figure S4: A-to-I editing sites occurring in pre-miRNA. Illustration of A-to-I editing sites occurring in pre-miRNA stem-loops. Pre-miRNA stem-loop structures were taken from miRBase database (http://www.mirbase.org/). The red adenosines highlighted in yellow represent the A-to-I editing sites. The bold blue sequences are the A-to-I edited mature miRNAs, while the bold black ones are non-edited mature miRNAs.

Figure S5: A-to-I editing sites neighborhood profiling. (a) A-to-I RNA editing site (at position 3) neighborhood profiling in pre-miRNAs (5' $\rightarrow$ 3'), in sequence Logo format (4). Nucleotides C and U are overrepresented upstream of the edited site (at position 2) while, G is underrepresented and overrepresented, respectively, upstream (at position 2) and downstream of the edited event (at position 4). **(b)** Sequence preference for the bases opposing the A-to-I editing sites in pre-miRNAs (3' $\rightarrow$ 5').

Figure S6: Analysis of the effects of editing in miRNA seed regions (MSRs). Displayed are two possible scenarios: 1) in the first five instances shown above, the editing event leads to the configuration of new human miRNAs, due to the generation of a new human seed sequence; 2) seed sequence modification by the editing event configures the miRNAs as sharing their edited seed sequence with that of another known miRNA.

Figure S7: Target distribution between WT and ED miRNA versions and validation for miRiam predicted miR-27a-3p targeting. c-MET and EGFR expression were assessed by western blot in HeLa cells transfected with miR-27a-3p/ED, miR-27a-3p/WT or negative scramble control (Scr) and harvested after 48 h. Loading control was obtained by using anti-tubulin antibody.

Figure S8: Illustration of binding site prediction for WT and ED (A-to-I editing sites in position 6 of mature miRNA) versions of miR-27a-3p. Binding site predictions on the 3' UTRs of MET and EGFR respectively were obtained by miRiam (5, 6). All coordinates shown in parenthesis are relative to 3' UTR start.

Figure S9: Predicted ability of WT miRNAs and respective A-to-I ED versions to target key genes in VEGF and PI3K/AKT pathways. Genes displayed are predicted to be targeted at least by two of the above WT miRNAs and to no longer be targeted by at least two of their ED versions. In each cell, "WT" represents predicted WT miRNA targeting, while "ED" represents predicted ED miRNA targeting.

Supplementary Table Legend

Table S1: Summary of statistical data on the output obtained from the application of the bioinformatics Alon-Eisenberg pipeline. Statistically significant microRNA modification events for each condition (normoxia, 16, 32, 48 h hypoxia) in each replicate are provided. MSR editing events are marked in bold.

Table S2: Statistics for Figure 4b. Number of differentially expressed targets followed by targets involved in the depicted cellular pathways, considered across 4 groups for each time-point: exclusive targets of the WT miRNA version, exclusive targets of the ED miRNA version, targets shared between WT and ED versions, and DE genes not predicted to be targets of either version. By time-point 48h we can notice a loss of targets for the ED version compared to the WT version, as marked in bold.

Supplementary References

1. Kawahara,Y., Megraw,M., Kreider,E., Iizasa,H., Valente,L., Hatzigeorgiou,A.G. and Nishikura,K. (2008) Frequency and fate of microRNA editing in human brain. **36**, 5270–5280.

2. Alon,S., Mor,E., Vigneault,F., Church,G.M., Locatelli,F., Galeano,F., Gallo,A., Shomron,N. and Eisenberg,E. (2012) Systematic identification of edited microRNAs in the human brain. *Genome Research*, **22**, 1533–1540.

3. Ramaswami,G., Zhang,R., Piskol,R., Keegan,L.P., Deng,P., O'Connell,M.A.A. and Li,J.B. (2013) Identifying RNA editing sites using RNA sequencing data alone. *Nat Meth*, **10**, 128–132.

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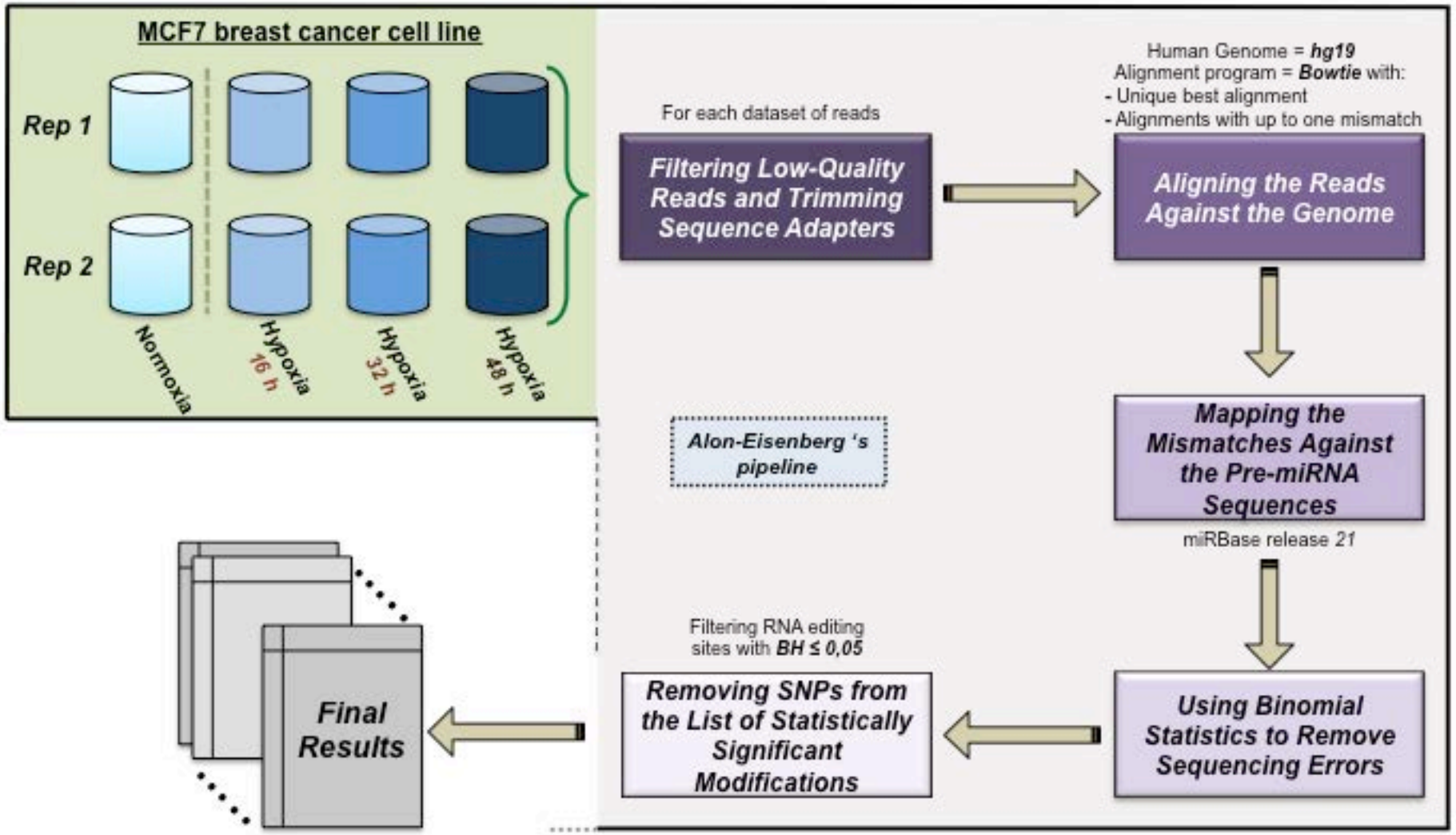
5. Laganà,A., Forte,S., Russo,F., Giugno,R., Pulvirenti,A. and Ferro,A. (2010) Prediction of human targets for viral-encoded microRNAs by thermodynamics and empirical constraints. *J RNAi Gene Silencing*, **6**, 379–385.

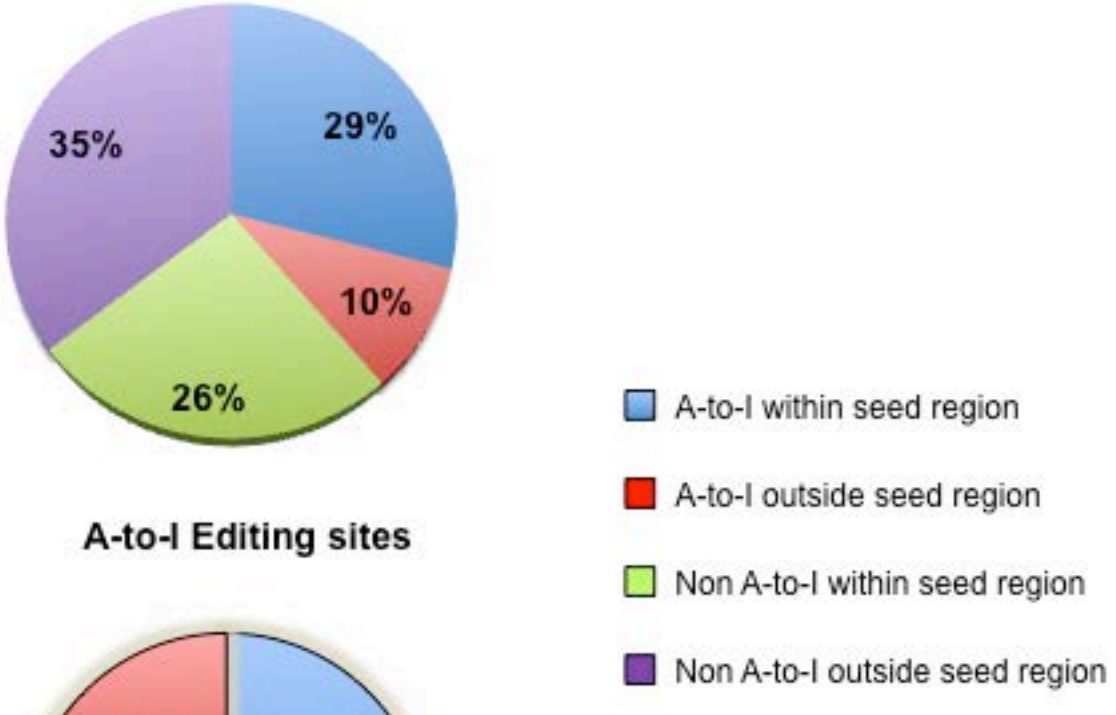
6. Laganà,A., Acunzo,M., Romano,G., Pulvirenti,A., Veneziano,D., Cascione,L., Giugno,R., Gasparini,P., Shasha,D., Ferro,A., *et al.* (2014) miR-Synth: a computational resource for the design of multi-site multi-target synthetic miRNAs. **42**, 5416–5425.

Suppl. Fig. 1

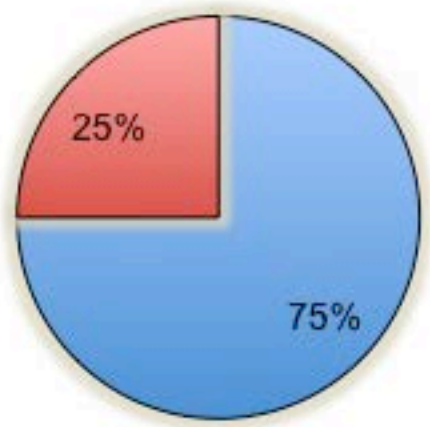

MCF7 breast cancer cell line
Rep 1
Rep 2
Normoxia
Hypoxia 16 h
Hypoxia 32 h
Hypoxia 48 h
For each dataset of reads
Filtering Low-Quality Reads and Trimming Sequence Adapters
Human Genome = hg19
Alignment program = Bowtie with:
- Unique best alignment
- Alignments with up to one mismatch
Aligning the Reads Against the Genome
Mapping the Mismatches Against the Pre-miRNA Sequences
miRBase release 21
Using Binomial Statistics to Remove Sequencing Errors
Filtering RNA editing sites with BH ≤ 0,05
Removing SNPs from the List of Statistically Significant Modifications
Final Results
Alon-Eisenberg 's pipeline

Suppl. Fig. 2

a



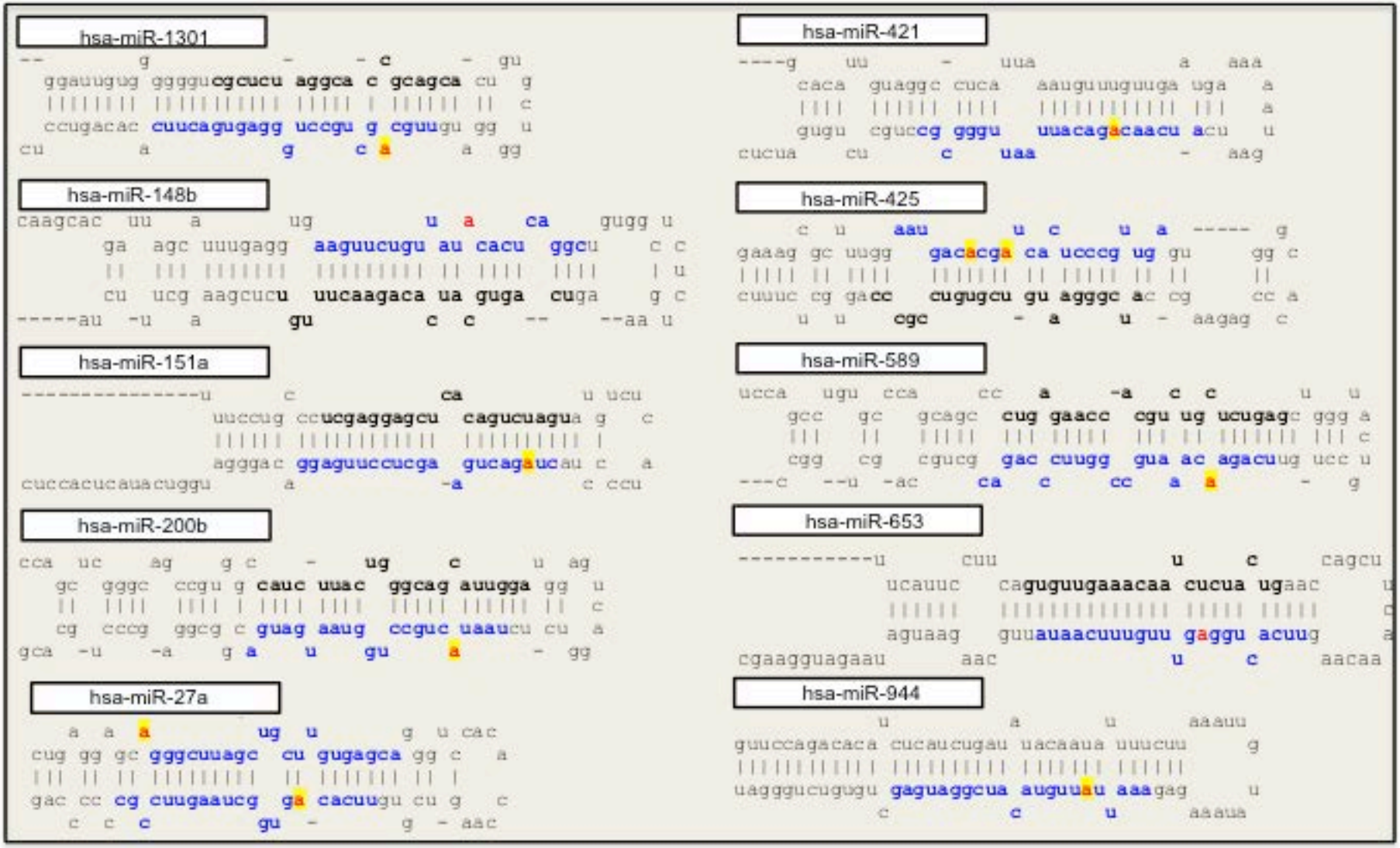
b



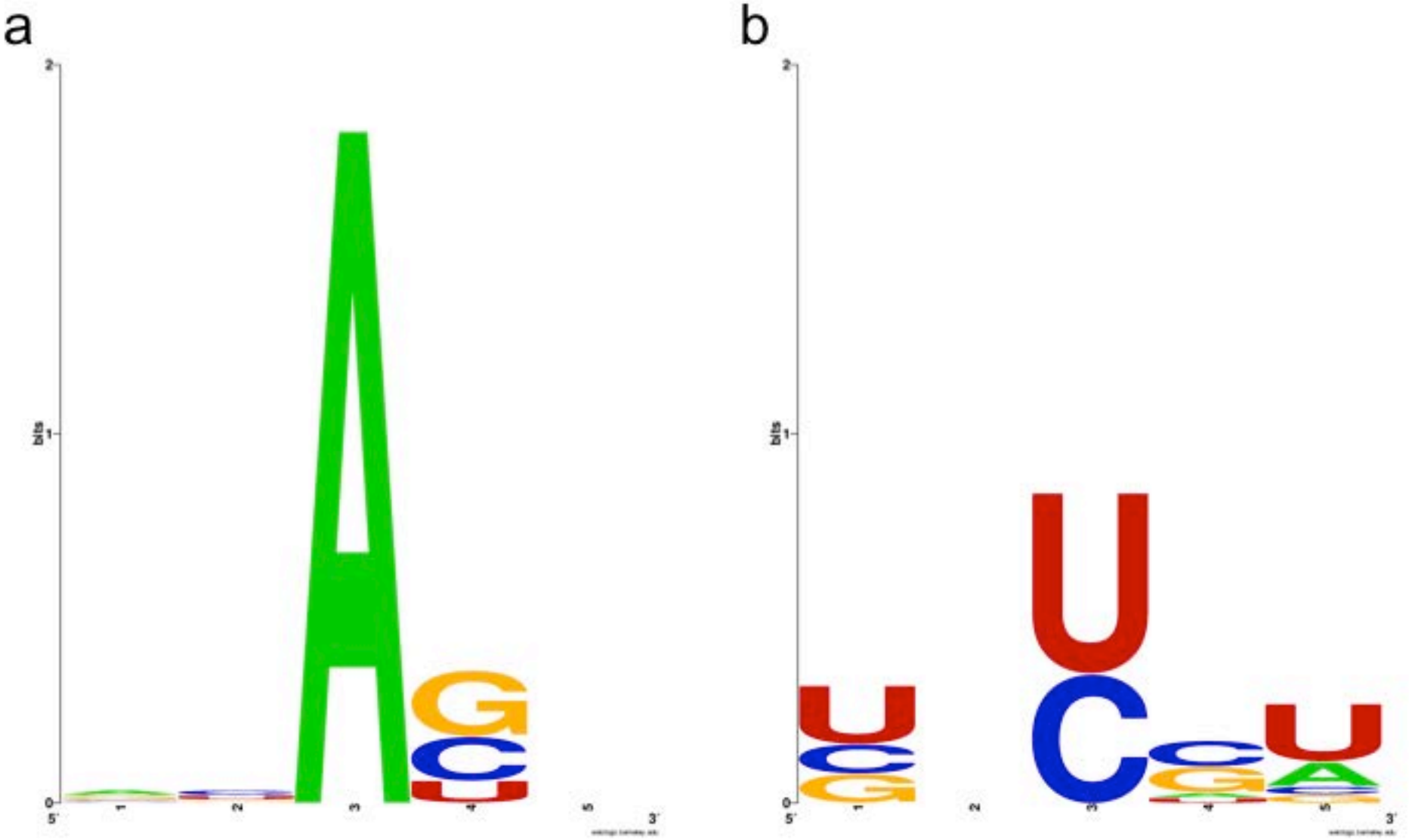
Suppl. Fig. 3

MODIFICATION SITES						AVERAGE MODIFICATION LEVEL				In All Biological Rep.?
ID	miRNA name	Location inside pre-miRNA	Location in mature miRNA	Ref.	Modification type	Normoxia	Hypoxia (16)	Hypoxia (32)	Hypoxia (48)	
1	hsa-mir-191	28	13	-	AC	0,07	0	0	0	No
2	hsa-mir-3168	22	14	-	AC	0	21,88	27,87	8,33	No
3	**hsa-mir-425**	20	**7**	-	**AC**	1,79	0	0	0	Yes
4	hsa-mir-425	23	10	-	AC	7,86	1,48	9,34	5,61	No
5	hsa-mir-96	21	13	-	AC	0,09	0	0	0	No
6	**hsa-mir-1301**	52	**5**	-	**AG**	0	0	0,78	0	No
7	hsa-mir-148b	37	13	-	AG	0	0	0,26	0	No
8	**hsa-mir-151a**	49	**3**	(1, 2)	**AG**	0	0,1	0,21	0,1	No
9	**hsa-mir-200b**	61	**5**	(2)	**AG**	0,39	0,49	0,85	1,27	Yes
10	**hsa-mir-27a**	10	**1**	(1, 2)	**AG**	0,35	0,55	0,97	2,74	Yes
11	**hsa-mir-27a**	56	**6**	(2)	**AG**	0,22	0,20	0,33	0,5	Yes
12	**hsa-mir-421**	54	**7**	(2)	**AG**	4,17	2,78	2,48	3,79	Yes
13	**hsa-mir-425**	20	**7**	-	**AG**	0	1,02	0	0	No
14	hsa-mir-425	23	10	-	AG	0	2,94	0	0	No
15	**hsa-mir-589**	66	**6**	(2, 3)	**AG**	10,24	14,9	19	15,31	Yes
16	hsa-mir-653	59	**9**	-	AG	0	0	0,77	0	No
17	**hsa-mir-944**	59	**6**	-	**AG**	0,96	0	0	1,02	No
18	**hsa-mir-23a**	47	**3**	-	**CA**	0	2,38	0	0	No
19	**hsa-mir-425**	21	**8**	-	**CU**	1,48	0	1,14	0	No
20	hsa-mir-425	25	12	-	CU	0	0,94	0	0	No
21	**hsa-mir-342**	20	**2**	-	**GA**	0	0	0,25	0,41	No
22	hsa-mir-425	22	9	-	GA	3,83	4,64	1,72	0	No
23	**hsa-let-7e**	11	**4**	-	**GU**	0,07	0	0,08	0,21	No
24	hsa-let-7e	18	11	-	GU	0,20	0,32	0,22	0,40	Yes
25	**hsa-let-7i**	9	**4**	-	**GU**	0	0	0,2	0,34	Yes
26	**hsa-mir-203a**	65	**1**	-	**GU**	0,23	0,13	0,38	0,23	No
27	hsa-mir-425	22	9	-	GU	0	0	0	4,11	Yes
28	**hsa-mir-98**	26	**5**	-	**GU**	0,25	0	0,34	0,48	Yes
29	hsa-mir-425	24	11		UA	0	1,15	0	0	No
30	hsa-mir-425	24	11	-	UC	0	0,94	1,35	0,72	No
31	hsa-let-7a-1	14	9	(2)	UG	0	0,83	0	0	No

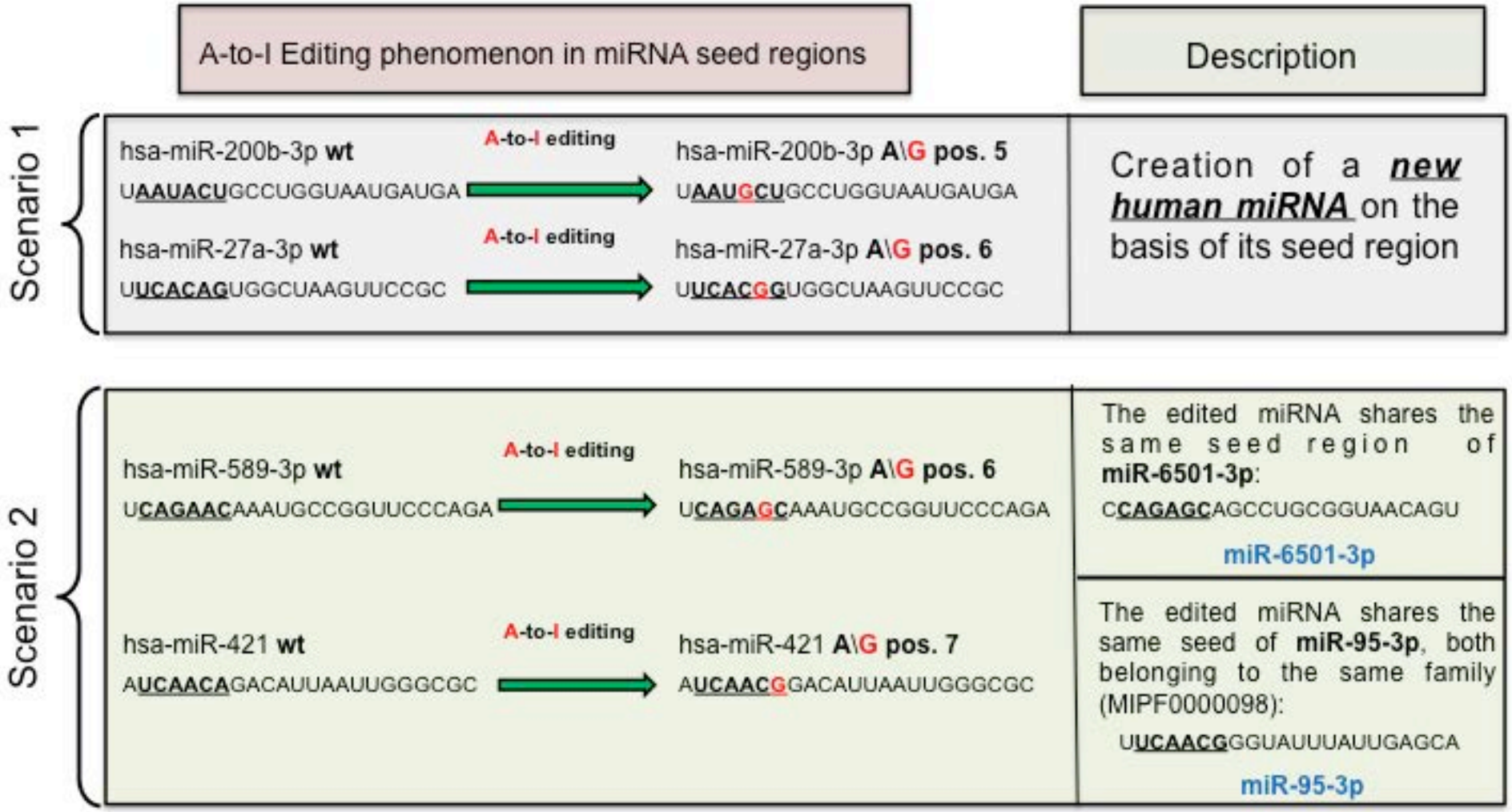
Suppl. Fig. 4


hsa-miR-1301
hsa-miR-148b
hsa-miR-151a
hsa-miR-200b
hsa-miR-27a
hsa-miR-421
hsa-miR-425
hsa-miR-589
hsa-miR-653
hsa-miR-944

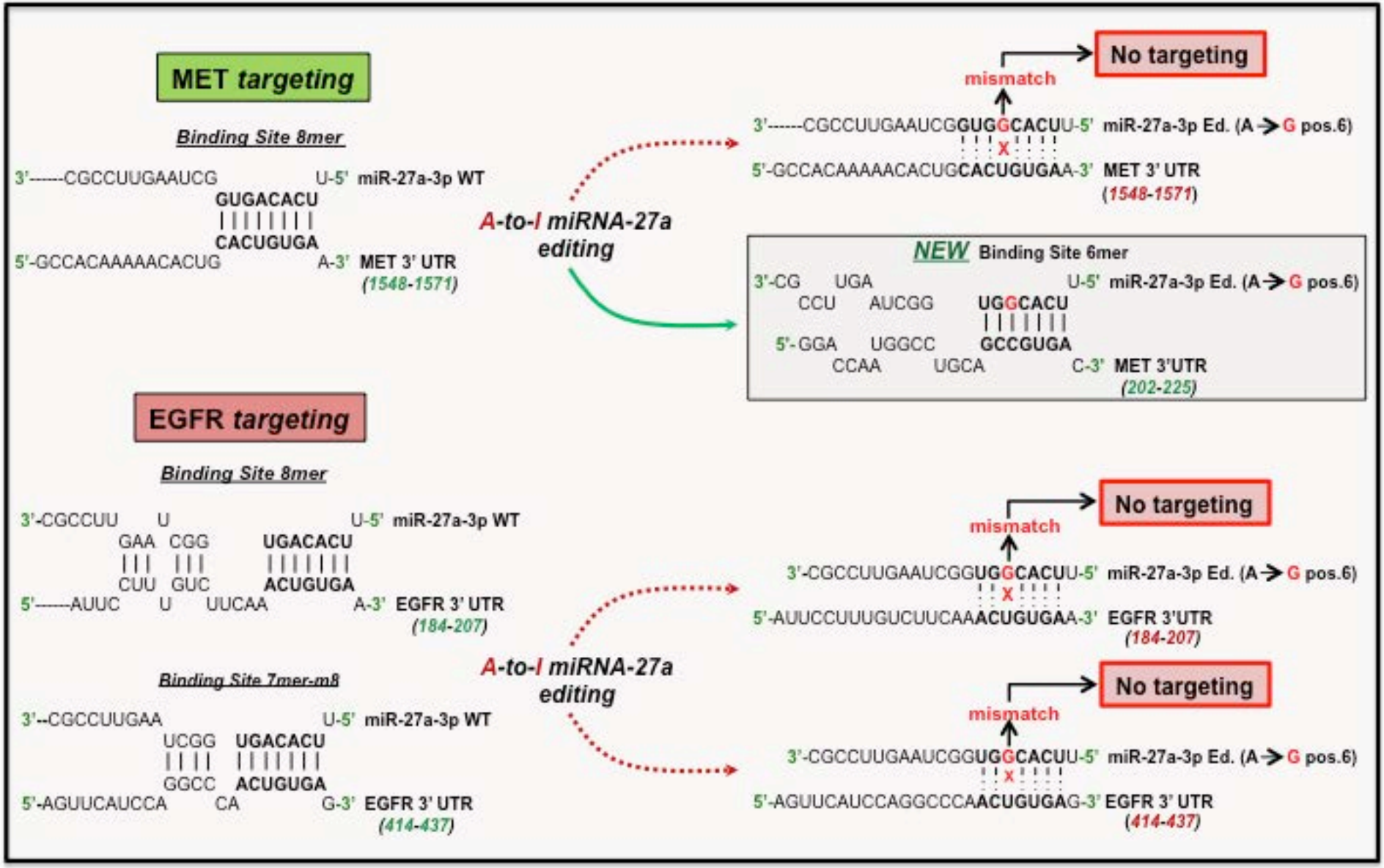
Suppl. Fig. 5



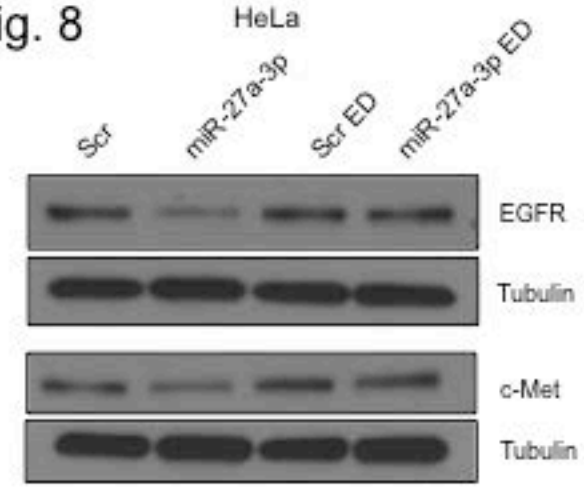
Suppl. Fig. 6


A-to-I Editing phenomenon in miRNA seed regions
Description
Scenario 1
hsa-miR-200b-3p wt
UAAUACUGCCUGGUAAUGAUGA
A-to-I editing
hsa-miR-200b-3p A\G pos. 5
UAAUGCUGCCUGGUAAUGAUGA
hsa-miR-27a-3p wt
UUCACAGUGGCUAAGUUCCGC
A-to-I editing
hsa-miR-27a-3p A\G pos. 6
UUCACGGUGGCUAAGUUCCGC
Creation of a new human miRNA on the basis of its seed region
Scenario 2
hsa-miR-589-3p wt
UCAGAACAAAUGCCGGUUCCCAGA
A-to-I editing
hsa-miR-589-3p A\G pos. 6
UCAGAGCAAAUGCCGGUUCCCAGA
The edited miRNA shares the same seed region of miR-6501-3p:
CCAGAGCAGCCUGCGGUAACAGU
miR-6501-3p
hsa-miR-421 wt
AUCAACAGACAUUAAUUGGGCGC
A-to-I editing
hsa-miR-421 A\G pos. 7
AUCAACGGACAUUAAUUGGGCGC
The edited miRNA shares the same seed of miR-95-3p, both belonging to the same family (MIPF0000098):
UUCAACGGGUAUUUAUUGAGCA
miR-95-3p

Suppl. Fig. 7


MET targeting
Binding Site 8mer
3'------CGCCUUGAAUCG U-5' miR-27a-3p WT
GUGACACU
CACUGUGA
5'-GCCACAAAAACACUG A-3' MET 3' UTR
(1548-1571)
A-to-I miRNA-27a editing
No targeting
mismatch
3'------CGCCUUGAAUCGGUGGCACUU-5' miR-27a-3p Ed. (A→G pos.6)
5'-GCCACAAAAACACUGCACUGUGAA-3' MET 3' UTR
(1548-1571)
NEW Binding Site 6mer
3'-CG UGA U-5' miR-27a-3p Ed. (A→G pos.6)
CCU AUCGG UGGCACU
5'-GGA UGGCC GCCGUGA
CCAA UGCA C-3' MET 3'UTR
(202-225)
EGFR targeting
Binding Site 8mer
3'-CGCCUU U U-5' miR-27a-3p WT
GAA CGG UGACACU
CUU GUC ACUGUGA
5'------AUUC U UUCAA A-3' EGFR 3' UTR
(184-207)
Binding Site 7mer-m8
3'--CGCCUUGAA U-5' miR-27a-3p WT
UCGG UGACACU
GGCC ACUGUGA
5'-AGUUCAUCCA CA G-3' EGFR 3' UTR
(414-437)
A-to-I miRNA-27a editing
No targeting
mismatch
3'-CGCCUUGAAUCGGUGGCACUU-5' miR-27a-3p Ed. (A→G pos.6)
5'-AUUCCUUUGUCUUCAAACUGUGAA-3' EGFR 3'UTR
(184-207)
No targeting
mismatch
3'-CGCCUUGAAUCGGUGGCACUU-5' miR-27a-3p Ed. (A→G pos.6)
5'-AGUUCAUCCAGGCCCAACUGUGAG-3' EGFR 3' UTR
(414-437)

Suppl. Fig. 8


HeLa
Scr
miR-27a-3p
Scr ED
miR-27a-3p ED
EGFR
Tubulin
c-Met
Tubulin

Suppl. Fig. 9

miR-200b-3p	miR-27a-3p	miR-421	miR-589-3p	Genes	
WT/ED	WT/-	-/-	WT/-	AKT2	*VEGF*
WT/-	WT/-	WT/-	WT/ED	ARNT	
-/-	WT/-	WT/-	-/-	EIF1AX	
WT/ED	WT/-	WT/-	WT/-	EIF2S1	
WT/-	-/-	WT/-	WT/ED	EIF2S2	
-/-	WT/-	WT/-	WT/ED	EIF2S3	
WT/ED	WT/-	WT/-	-/-	KRAS	
WT/-	WT/-	WT/-	WT/ED	NRAS	
WT/ED	WT/-	WT/-	WT/-	RAF1	
WT/ED	WT/-	-/-	WT/-	AKT2	*PI3K/Akt*
WT/ED	WT/-	WT/ED	WT/-	CCND1	
WT/-	WT/-	-/-	WT/-	HSP90AA1	
WT/ED	WT/-	WT/-	-/-	KRAS	
WT/-	WT/-	WT/-	WT/ED	MCL1	
WT/-	-/-	WT/-	-/-	PTGS2	
WT/-	-/-	WT/-	-/-	RAF1	
WT/-	WT/-	-/-	WT/-	SHC1	
WT/-	WT/-	WT/-	WT/-	YWHAB	
WT/-	WT/-	WT/-	WT/-	YWHAG	

WT and ED miRNAs target

miRNA WT target

WT and ED miRNAs not targeting