

## Supplemental Figure 1

### A.

<b>Let-7a</b>	<b>UUGAUUGUUG-GAUGAUGGAGU</b>	<b>Let-7b</b>	<b>UUGGUGUGUUG-GAUGAUGGAGU</b>
<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>	<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>
<b>Let-7c</b>	<b>UUGGUAUGUUG-GAUGAUGGAGU</b>	<b>Let-7d</b>	<b>UUGAUACG--UUG-GAUGAUGGAGA</b>
<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>	<b>Ago1 3'UTR</b>	<b>TTGAAGGCAGAACGCTGTTACCTCA</b>
<b>Let-7e</b>	<b>UUGAUUGUUGGAGGAUGGAGU</b>	<b>Let-7f</b>	<b>UUGAUUGUU-AGAUGAUGGAGU</b>
<b>Ago1 3'UTR</b>	<b>TCCT-TAT-TCCTCCTACCTCC</b>	<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>
<b>Let-7g</b>	<b>UUGACAUGUU-UGAUGAUGGAGU</b>	<b>Let-7i</b>	<b>UUGUCGUGUU-UGAUGAUGGAGU</b>
<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>	<b>Ago1 3'UTR</b>	<b>GAAGGCAGAACGCTGTTACCTCA</b>
<b>miR-103</b>	<b>AGUAUCGGGACAU----GUUACGACGA</b>	<b>miR-107</b>	<b>ACUAUCGGGACAU----GUUACGACGA</b>
<b>Ago1 3'UTR</b>	<b>TGCCTTCCCTGTAACGGTAATGCTGCA</b>	<b>Ago1 3'UTR</b>	<b>TGCCTTCCCTGTAACGGTAATGCTGCA</b>

### B. Let-7 target site

```

Hsa  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG 115
Ptr  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Mml  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Cpo  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Sar  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGGAGGTGGGGTTAAGGG--GAGTGTAGG
Eeu  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Cfa  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---AGGGTAGGGAG--GAGTGTAGG
Fca  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---AGGGTAGGGAG--GAGTGTAGG
Eca  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---GGAGT---GGGGTAGGGAG--GAGTGTAGG
Bta  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Dno  TGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---GGTGGGGTAGGGAGGAAATGTAGG
Laf  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Ete  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---AGGGTAGGGAG--GAGTGTAGG
Mmu  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
Rno  AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGG---TGGGGTAGGGAG--GAGTGTAGG
*****

```

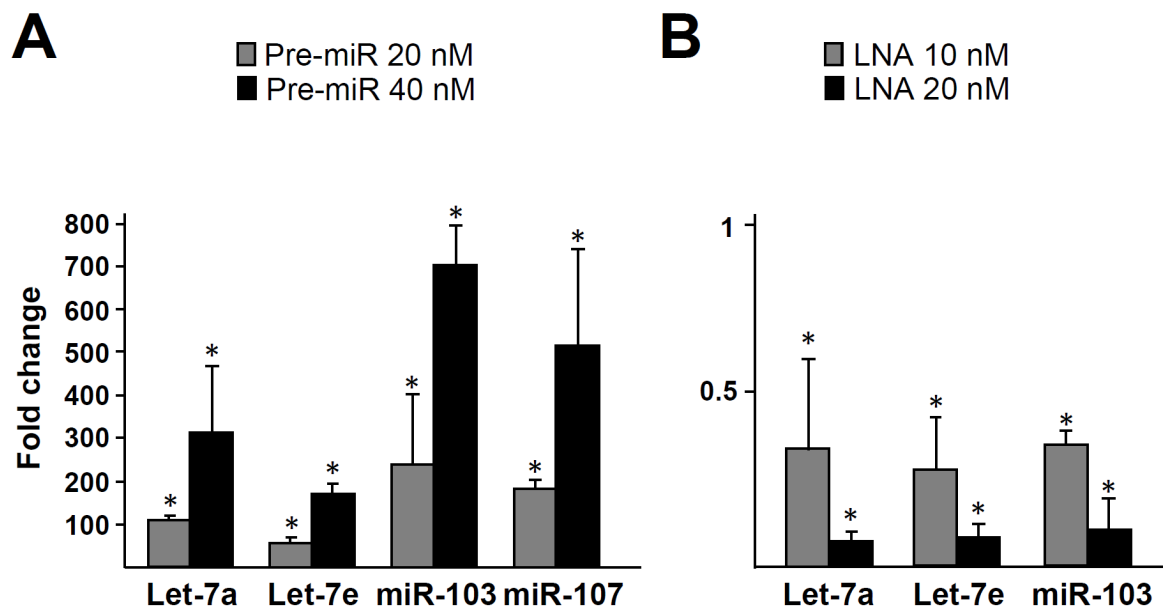
### C. miR-103/107 target site

```

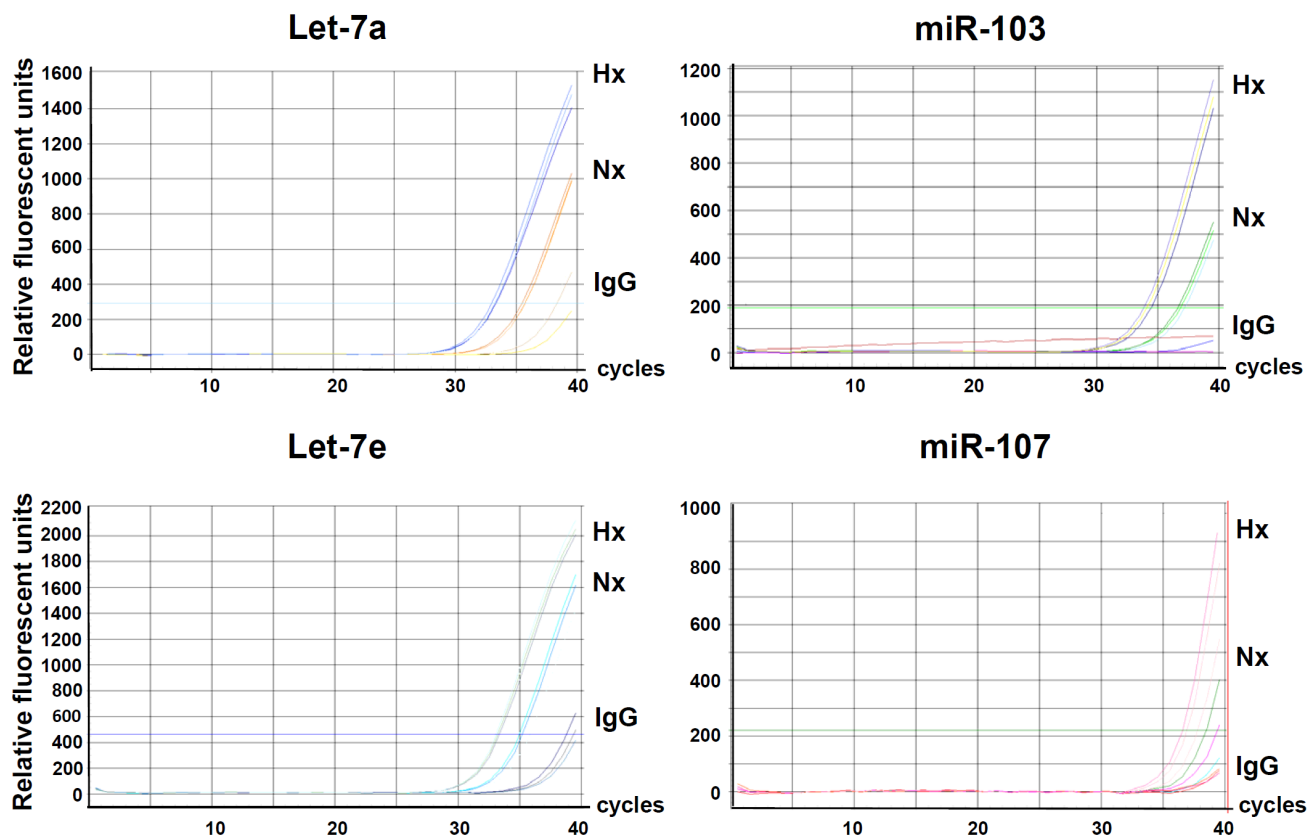
Hsa  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GAATGGGGTGGGGTGGGAGAGGAGGTAGATGGGGAAG---AAATACCCAGAC 881
Ptr  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GAATGGGGTGGGGTGGGAGAGGAGGTAGATGGGGAAG---AAATACCCAGAC
Mml  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GAATGGGGTGGGGTGGGAGAGGAGGTAGATGGGGAAG---AAATACCCAGAC
Cpo  ACCAGTGCCTTCCCTGTAATGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--AGTTGGGGTGGGAGTGGGAGAGGAGGTAGATGGGGAAG---AAATATCCCAAC
Sar  ATCAGTGCCTTCCCTGTAATGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GAGTGGGGTGGGG--GGGCTGGG-----AGAGG---AGTTAGATAGGA-
Eeu  ACCAGTGCCTTCCCTGTAATGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGTGGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Cfa  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGATAGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Fca  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGATAGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Eca  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGATAGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATATCCCTAAC
Bta  ACCAGTGCCTTCCCTGTAATGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTTGGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Dno  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGATAGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Laf  ACCAGTGCCTTCCCTGTAATGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGATAGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Ete  ACCAGTGCCTTCCCTGTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTTTGGG--GGGACTGGGTGGGGTGGGAGAGGAGGTAGGTTGGGGAAG---AAATACCCCTAAC
Mmu  ACCAGTGCCTTCCC-GTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTCTGGG--GGGTGGGGTGGGGTGGGAGAGGAGGAGGAGGGAGGGCAGAAATAGCCCAATG
Rno  ACCAGTGCCTTCCC-GTAACGGTAATGCTGCGAAGTGAACCTTTTGTACCTTTCTCTGGG--GGATGGGGTGGGGTGGGAGAGGAGGTAGGAGGGAGGGACGAGCTATCCCAATG
*****

```

Supplemental Figure 2

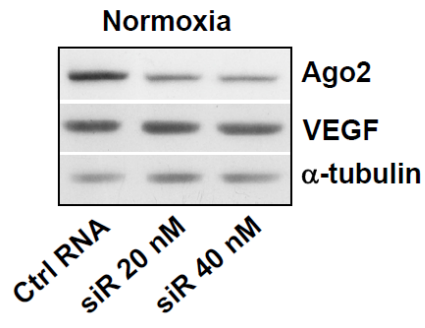


Supplemental Figure 3

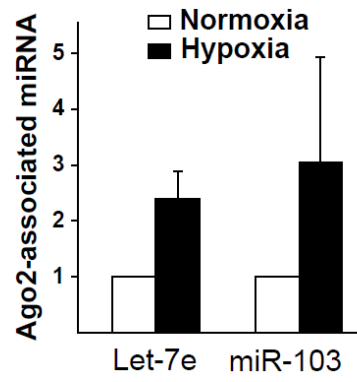


Supplemental Figure 4

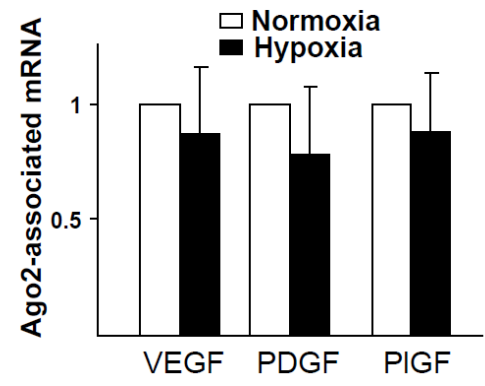
**A**



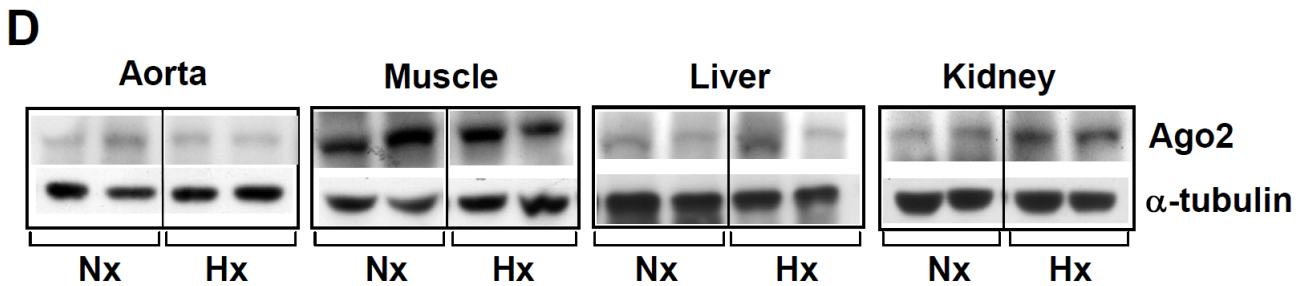
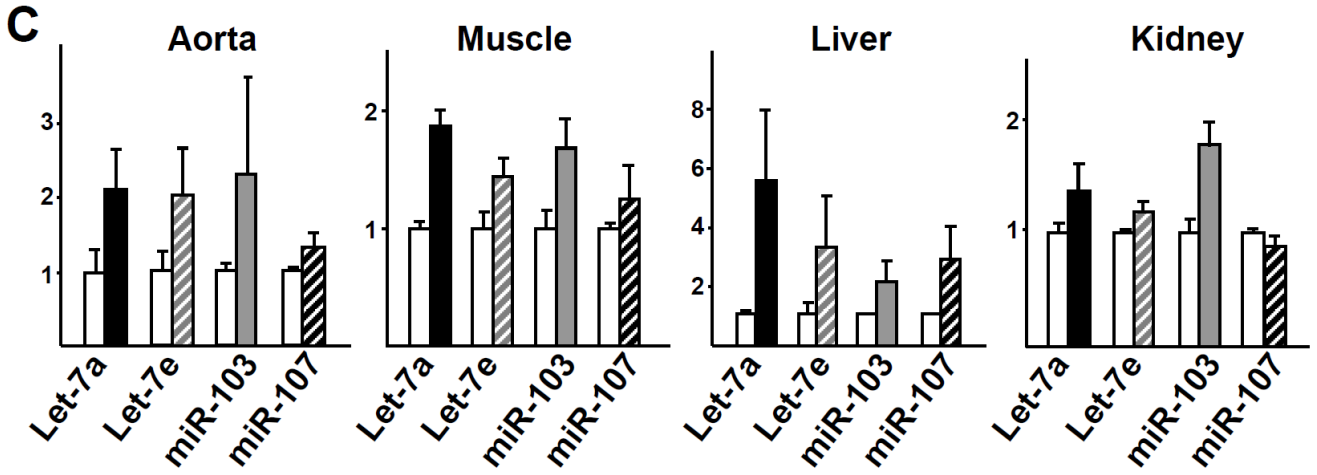
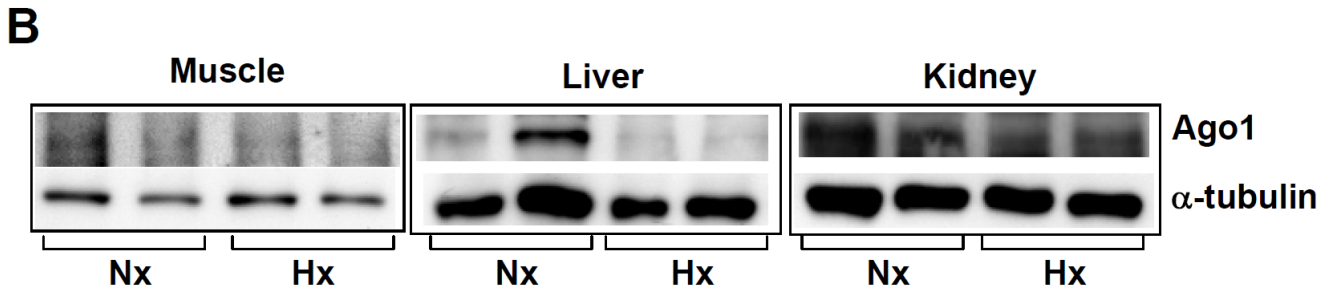
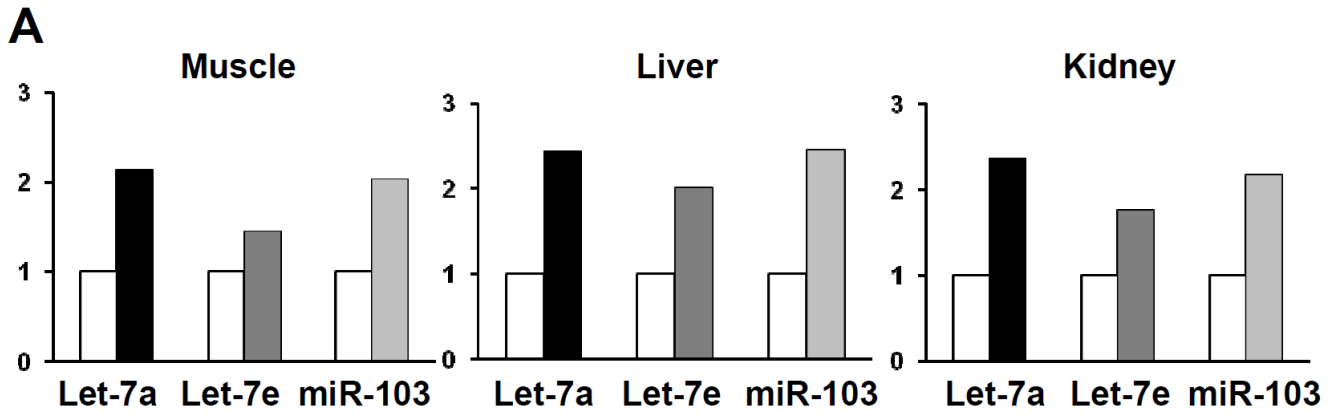
**B**



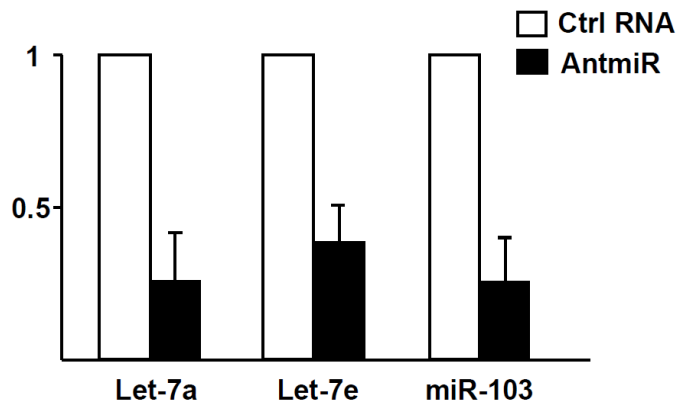
**C**



Supplemental Figure 5

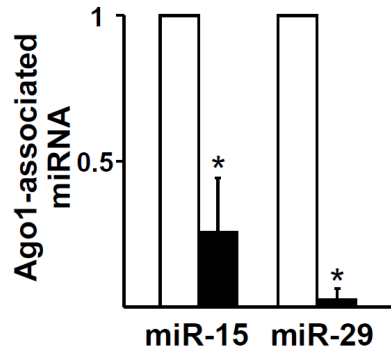


Supplemental Figure 6

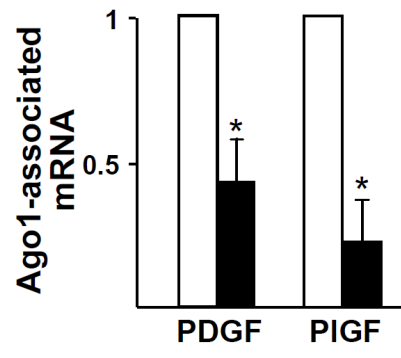


Supplemental Figure 7

**A**

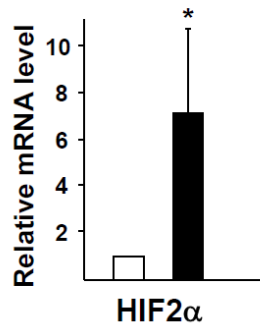


**B**

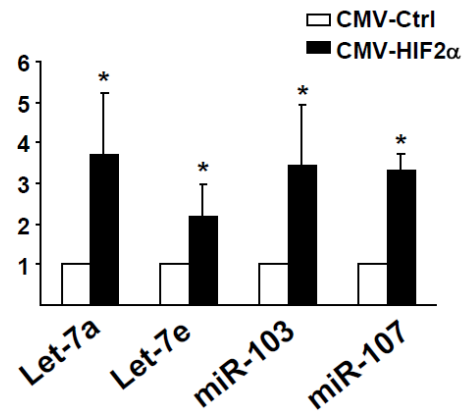


Supplemental Figure 8

**A**



**B**



**C**

miRNA	TFBS	Sequence
Let-7a-1	-378 (-)	gcgCACGTgt
Let-7a-2	-3540 (-)	acgCACGTac
Let-7a-3	-359 (-)	cagCACGTgc
Let-7b	-359 (-)	cagCACGTgc
Let-7d	-378 (-)	gcgCACGTgt
Let-7f-1	-378 (-)	gcgCACGTgt
Let-7g	-394 (+)	agACGTGctc
Let-7i	-1672 (+)	ccACGTGcaa
miR-103-1	-1315 (-)	atgCACGTtt
miR-103-2	-2190 (+)	caACGTGctg



**Supplemental Table 1. Deep Sequencing of miRNA Profile in Normoxia and Hypoxia HUVECs**

<b>miRNA</b>	<b>Reads in Nx</b>	<b>Reads in Hx</b>	<b>Fold Change</b>
hsa-let-7a	277	2614	6.96
hsa-let-7b	146	900	4.54
hsa-let-7c	205	1089	3.92
hsa-let-7d	252	1032	3.02
hsa-let-7e	52	655	9.29
hsa-let-7f	984	5820	4.36
hsa-let-7g	171	1476	6.36
hsa-let-7i	142	488	2.53
hsa-miR-101	307	199	0.48
hsa-miR-103	334	3743	8.26
hsa-miR-106b	22	18	0.60
hsa-miR-107	336	3789	8.31
hsa-miR-139-5p	9	11	0.90
hsa-miR-146a	27	70	1.91
hsa-miR-148b	12	38	2.33
hsa-miR-151-3p	13	90	5.10
hsa-miR-151-5p	7	104	10.95
hsa-miR-152	3	19	4.67
hsa-miR-15a	14	16	0.84
hsa-miR-15b	29	28	0.71
hsa-miR-16	18	174	7.13
hsa-miR-17	17	27	1.17
hsa-miR-185	6	20	2.46
hsa-miR-186	1	14	10.32
hsa-miR-191	11	16	1.07
hsa-miR-192	55	85	1.14
hsa-miR-193a-3p	11	37	2.48
hsa-miR-196b	10	23	1.70
hsa-miR-199a-3p	68	220	2.39
hsa-miR-199b-3p	68	220	2.39
hsa-miR-19b	22	21	0.70
hsa-miR-20a	25	11	0.32
hsa-miR-21	2828	1508	0.39
hsa-miR-216a	16	18	0.83
hsa-miR-217	50	113	1.67
hsa-miR-22	85	202	1.75
hsa-miR-221	737	2881	2.88
hsa-miR-222	386	593	1.13

<b>miRNA</b>	<b>Reads in Nx</b>	<b>Reads in Hx</b>	<b>Fold Change</b>
hsa-miR-23a	489	149	0.22
hsa-miR-23b	19	6	0.23
hsa-miR-24	54	96	1.31
hsa-miR-26a	24		
hsa-miR-27a	1197	1698	1.05
hsa-miR-27b	118	129	0.81
hsa-miR-28-3p	5	43	6.34
hsa-miR-28-5p	7	10	1.05
hsa-miR-299-3p	30	12	0.29
hsa-miR-30c	42	9	0.16
hsa-miR-31	6	62	7.62
hsa-miR-320	11	101	6.77
hsa-miR-323-3p	10	8	0.59
hsa-miR-340	76	50	0.49
hsa-miR-34a	15	29	1.43
hsa-miR-369-3p	4	12	2.21
hsa-miR-376c	9	21	1.72
hsa-miR-378	119	304	1.88
hsa-miR-379	256	221	0.64
hsa-miR-381	12	1	0.06
hsa-miR-382	1	18	13.27
hsa-miR-410	6	10	1.23
hsa-miR-411	88	40	0.34
hsa-miR-423-3p	12	17	1.04
hsa-miR-423-5p	19	42	1.63
hsa-miR-432	4	13	2.40
hsa-miR-455-3p	6	24	2.95
hsa-miR-495	7	16	1.69
hsa-miR-497	16	22	1.01
hsa-miR-503	10	24	1.77
hsa-miR-532-5p	7	51	5.37
hsa-miR-574-3p	38	52	1.01
hsa-miR-584	53	56	0.78
hsa-miR-652	7	25	2.63
hsa-miR-877	1	12	8.85
hsa-miR-886-3p	8	25	2.30
hsa-miR-886-5p	11	114	7.64
hsa-miR-92b	8	12	1.11
hsa-miR-98	17	56	2.43

**Supplemental Table 2. Putative HIF1 $\alpha$  binding sites in the HRM promoter regions.**

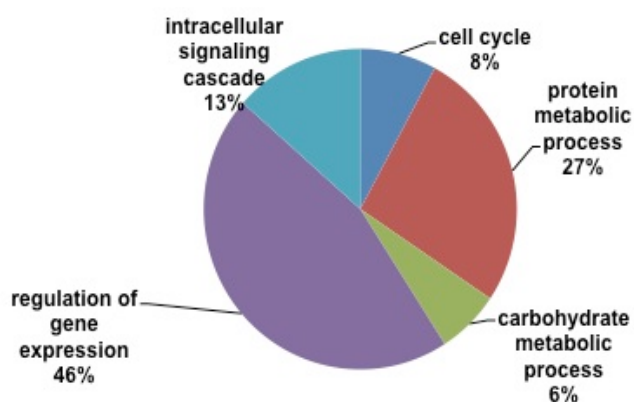
<b>miRNA</b>	<b>Chromosome</b>	<b>Human sequence</b>	<b>Mouse sequence</b>	<b>Conservation Score</b>
Let-7a-1	9	GTCACGTGGCCG	GTCACGTGGCCT	0.315
Let-7d				
Let-7f-1	11	AAAACGTGGCAC	ATGGTGTAGCAT	0.079
Let-7a-2				
Let-7b	22	GGAACGTGGTCT	CAA---TGCTCT	0.574
Let-7a-3				
Let-7c	21	CAAACGTGTGTA	CGAACGTGTGCA	0.996
Let-7g	3	AGGACGTGGCTG	AGGACGTGGCTG	0.147
miR-103-1	5	GTCACGTGGGCG	GTCACGTGGGCG	0.793
miR-103-2	20	ACTACGTGAGTG	GTTTCCTAAGTA	0.049
miR-107	10	GTAACGTGAATG	-TAGCATGGGTG	0.915

Supplemental Table 3. Summary of Putative HRM Targets

A.

miRNA	Let-7s	miR-103/107
Total targets	169	148
Intracellular targets	95	80
Extracellular targets	74	42

B.



C.

Let-7s	<i>EIF2C1</i> (Eukaryotic translation initiation factor 2C 1) (Argonaute-1) <i>HOXA1</i> (Homeobox protein A1) (Hox-1F) <i>DAPK1</i> (Death-associated protein kinase 1) (DAP kinase 1) <i>ACSS2</i> (Acetyl-CoA synthetase)
miR-103/107	<i>EIF2C1</i> (Eukaryotic translation initiation factor 2C 1) (Argonaute-1) <i>TAF5</i> (transcription initiation factor TFIID subunit 5). <i>AATF</i> (Apoptosis-antagonizing transcription factor)

**Supplemental Table 4. Correlation among HRM, Ago1, VEGF, and CD31 expression in HCC tissue array.**

<b>Correlation</b>	<b>Sample size</b>	<b>Spearman's rho</b>	<b><i>p</i> value</b>
Let-7e vs Ago1	176	-0.243	0.001
miR-103 vs Ago1	176	-0.247	0.001
Ago1 vs VEGF	173	-0.187	0.014
Ago1 vs CD31	163	-0.127	0.106

**Supplemental Table 5. Genes involved in glycolysis that are decreased in Ago1-miRISC under hypoxia.**

<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
LDHB	lactate dehydrogenase B	-5.103
GPI	glucose-6-phosphate isomerase	-4.735
LDHA	lactate dehydrogenase A	-4.627
HK2	hexokinase 2	-4.420
OGDH	oxoglutarate (alpha-ketoglutarate) dehydrogenase	-4.354
PGK1	phosphoglycerate kinase 1	-4.263
LDHAL6B	lactate dehydrogenase A-like 6B	-4.210
NCOR1	nuclear receptor corepressor 1	-4.209
PGAM1	phosphoglycerate mutase 1 (brain)	-4.185
PGK2	phosphoglycerate kinase 2	-4.167
ADPGK	ADP-dependent glucokinase	-4.110
PGAM4	phosphoglycerate mutase family member 4	-4.053
DLAT	dihydrolipoamide S-acetyltransferase	-4.044
ECD	ecdysoneless homolog (Drosophila)	-3.891
ARNT	aryl hydrocarbon receptor nuclear translocator	-3.807
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	-3.784
HK3	hexokinase 3 (white cell)	-3.637
PGAM2	phosphoglycerate mutase 2 (muscle)	-3.635
ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	-3.620
TPI1	triosephosphate isomerase 1	-3.617
DHTKD1	dehydrogenase E1 and transketolase domain containing 1	-3.591
INSR	insulin receptor	-3.468
MLXIPL	MLX interacting protein-like	-3.456
PDHA1	pyruvate dehydrogenase (lipoamide) alpha 1	-3.448
HDAC4	histone deacetylase 4	-3.442
HK1	hexokinase 1	-3.350
OGDHL	oxoglutarate dehydrogenase-like	-3.275
PDHB	pyruvate dehydrogenase (lipoamide) beta	-3.182
PPARA	peroxisome proliferator-activated receptor alpha	-3.158
LDHC	lactate dehydrogenase C	-2.759
ENO4	enolase family member 4	-2.706

**Supplemental Table 6. Genes involved in angiogenesis that are decreased in Ago1-miRISC under hypoxia.**

<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
VEGFA	vascular endothelial growth factor A	-4.793
VEGFB	vascular endothelial growth factor B	-3.499
VEGFC	vascular endothelial growth factor C	-4.201
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)	-3.371
PDGFA	platelet-derived growth factor alpha polypeptide	-4.311
PDGFB	platelet-derived growth factor beta polypeptide	-4.648
PDGFC	platelet derived growth factor C	-3.207
PDGFD	platelet derived growth factor D	-2.861
PIGF	placental growth factor	-4.750
FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	-4.527
KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	-5.028
FLT3	fms-related tyrosine kinase 3	-2.667
ANGPT1	angiopoietin 1	-2.771
ANGPT2	angiopoietin 2	-4.373
TIE1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1	-4.995
NCL	nucleolin	-4.985
FOXS1	forkhead box S1	-4.933
ADAM15	ADAM metallopeptidase domain 15	-4.931
HSPG2	heparan sulfate proteoglycan 2	-4.928
VEZF1	vascular endothelial zinc finger 1	-4.916
EPHB4	EPH receptor B4	-4.742
GPI	glucose-6-phosphate isomerase	-4.735
GPX1	glutathione peroxidase 1	-4.731
STAB1	stabilin 1	-4.691
VASH1	vasohibin 1	-4.657
HDAC7	histone deacetylase 7	-4.566
NOTCH4	notch 4	-4.565
EDN1	endothelin 1	-4.513
JAG1	jagged 1	-4.510
SRF	serum response factor (c-fos serum response element-binding transcription factor)	-4.458

<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
EPHA2	EPH receptor A2	-4.434
NRP1	neuropilin 1	-4.434
RBM15	RNA binding motif protein 15	-4.432
ENG	endoglin	-4.411
HOXA5	homeobox A5	-4.399
S1PR1	sphingosine-1-phosphate receptor 1	-4.395
UTS2R	urotensin 2 receptor	-4.391
HAND1	heart and neural crest derivatives expressed 1	-4.388
RUNX1	runt-related transcription factor 1	-4.374
PLXND1	plexin D1	-4.323
EPAS1	endothelial PAS domain protein 1	-4.285
MMP14	matrix metalloproteinase 14 (membrane-inserted)	-4.283
HMOX1	heme oxygenase (decycling) 1	-4.281
ACVRL1	activin A receptor type II-like 1	-4.259
GATA6	GATA binding protein 6	-4.255
ANPEP	alanyl (membrane) aminopeptidase	-4.202
MTDH	metadherin	-4.199
HDAC3	histone deacetylase 3	-4.192
VAV2	vav 2 guanine nucleotide exchange factor	-4.186
ANXA2	annexin A2	-4.152
FOXO4	forkhead box O4	-4.150
NOTCH1	notch 1	-4.147
SFRP1	secreted frizzled-related protein 1	-4.133
FGFR1	fibroblast growth factor receptor 1	-4.116
ARHGAP24	Rho GTPase activating protein 24	-4.109
ANXA2P2	annexin A2 pseudogene 2	-4.096
MFGE8	milk fat globule-EGF factor 8 protein	-4.083
SHB	Src homology 2 domain containing adaptor protein B	-4.043
HAND2	heart and neural crest derivatives expressed 2	-4.042
AMOT	angiomin	-4.034
PML	promyelocytic leukemia	-4.029
HOXA7	homeobox A7	-3.994
RBPJ	recombination signal binding protein for immunoglobulin kappa J region	-3.984
MAP3K7	mitogen-activated protein kinase kinase kinase 7	-3.938
CX3CL1	chemokine (C-X3-C motif) ligand 1	-3.932
CCL24	chemokine (C-C motif) ligand 24	-3.931



<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
PROK1	prokineticin 1	-3.907
FZD6	frizzled family receptor 6	-3.905
ARHGAP22	Rho GTPase activating protein 22	-3.904
HDAC5	histone deacetylase 5	-3.869
MAPK7	mitogen-activated protein kinase 7	-3.855
WARS	tryptophanyl-tRNA synthetase	-3.835
APOLD1	apolipoprotein L domain containing 1	-3.819
TBX4	T-box 4	-3.819
NF1	neurofibromin 1	-3.810
SLIT2	slit homolog 2 (Drosophila)	-3.808
HDAC9	histone deacetylase 9	-3.806
FZD5	frizzled family receptor 5	-3.790
NOS3	nitric oxide synthase 3 (endothelial cell)	-3.782
EREG	epiregulin	-3.750
SPHK1	sphingosine kinase 1	-3.739
CXCL17	chemokine (C-X-C motif) ligand 17	-3.737
ZC3H12A	zinc finger CCCH-type containing 12A	-3.712
CTGF	connective tissue growth factor	-3.708
KLF5	Kruppel-like factor 5 (intestinal)	-3.708
FGF18	fibroblast growth factor 18	-3.689
ADAM8	ADAM metallopeptidase domain 8	-3.668
C3	complement component 3	-3.666
FGF10	fibroblast growth factor 10	-3.626
GATA4	GATA binding protein 4	-3.616
GHRL	ghrelin/obestatin prepropeptide	-3.613
BMP4	bone morphogenetic protein 4	-3.601
MMP19	matrix metallopeptidase 19	-3.592
ATPIF1	ATPase inhibitory factor 1	-3.586
NUS1	nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae)	-3.584
SOX17	SRY (sex determining region Y)-box 17	-3.567
IL17F	interleukin 17F	-3.566
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	-3.565
GREM1	gremlin 1	-3.554
SH2D2A	SH2 domain containing 2A	-3.540
DLL4	delta-like 4 (Drosophila)	-3.535
ANGPTL4	angiopoietin-like 4	-3.534

<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
PTEN	phosphatase and tensin homolog	-3.509
ADORA2B	adenosine A2b receptor	-3.502
PLXDC1	plexin domain containing 1	-3.494
C5AR1	complement component 5a receptor 1	-3.488
HOXB3	homeobox B3	-3.455
ANXA3	annexin A3	-3.453
ECM1	extracellular matrix protein 1	-3.450
AIMP1	aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	-3.434
AQP1	aquaporin 1 (Colton blood group)	-3.430
FGF2	fibroblast growth factor 2 (basic)	-3.426
NPR1	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	-3.425
FGFR2	fibroblast growth factor receptor 2	-3.420
CCL11	chemokine (C-C motif) ligand 11	-3.414
TBXA2R	thromboxane A2 receptor	-3.407
EFNA1	ephrin-A1	-3.393
IHH	Indian hedgehog	-3.338
AGGF1	angiogenic factor with G patch and FHA domains 1	-3.334
ERAP1	endoplasmic reticulum aminopeptidase 1	-3.312
FN1	fibronectin 1	-3.304
ANGPT4	angiopoietin 4	-3.298
CCBE1	collagen and calcium binding EGF domains 1	-3.290
HOXA3	homeobox A3	-3.290
CSPG4	chondroitin sulfate proteoglycan 4	-3.286
KLK3	kallikrein-related peptidase 3	-3.273
HS6ST1	heparan sulfate 6-O-sulfotransferase 1	-3.238
C3AR1	complement component 3a receptor 1	-3.230
VASH2	vasohibin 2	-3.202
LIF	leukemia inhibitory factor (cholinergic differentiation factor)	-3.181
TSPAN12	tetraspanin 12	-3.156
PROK2	prokineticin 2	-3.112
PTK2B	PTK2B protein tyrosine kinase 2 beta	-3.107
OVOL2	ovo-like 2 (Drosophila)	-3.103
ANGPTL6	angiopoietin-like 6	-3.093
THBS4	Thrombospondin 4	-3.090
FGF6	fibroblast growth factor 6	-3.080

<b>Gene Symbol</b>	<b>Gene Name</b>	<b>Expression Difference</b>
MEOX2	mesenchyme homeobox 2	-3.066
MEIS1	Meis homeobox 1	-3.054
BMPER	BMP binding endothelial regulator	-3.037
NPPB	natriuretic peptide B	-3.034
STAB2	stabilin 2	-3.005
SCG2	secretogranin II	-2.989
S100A7	S100 calcium binding protein A7	-2.946
SFRP2	secreted frizzled-related protein 2	-2.941
SHH	sonic hedgehog	-2.901
PDCD10	programmed cell death 10	-2.879
VHL	von Hippel-Lindau tumor suppressor	-2.866
NOX1	NADPH oxidase 1	-2.827
PF4	platelet factor 4	-2.773
C6	complement component 6	-2.735
APOH	apolipoprotein H (beta-2-glycoprotein I)	-2.717
IL8	interleukin 8	-2.609
IL18	interleukin 18 (interferon-gamma-inducing factor)	-2.533

## Supplemental Figure/Table Legends

**Supplemental Figure 1. (A) Sequence alignment between HRMs and 3'UTR of Ago1. (B) Cross-species alignment of HRM target sequences in Ago1 3'UTR.** Alignment of Ago1 3'UTR from various species using UCSC genome browser. Target sites are in the colored region (seed sequences in red). Asterisks (\*) indicate conserved sites cross-species.

**Supplemental Figure 2. Effects of pre-miRs and LNAs in miRNA level.** HUVECs were transfected with pre-miRs (A) or LNAs (B) as indicated. miRNA level in ECs transfected with control RNAs was set as 1. Data represent mean  $\pm$  SD from 3 independent experiments. \*  $p < 0.05$  compared to control RNA group.

**Supplemental Figure 3. Examples of amplification charts from miRISC-associated miRNA qPCR.** Ago1 was immunoprecipitated from normoxic or hypoxic HUVECs and the associated miRNAs were detected by qPCR. Signals of normoxia (Nx) or hypoxia (Hx) were plotted in the amplification chart. IgG was used as an isotype control.

**Supplemental Figure 4. HRMs are associated with Ago2 in hypoxic ECs.** (A) HUVECs were transfected with control RNA or Ago2 siRNA and kept under normoxia for 72 hr. Respective proteins were detected by Western Blotting. (B,C) Ago2 was immunoprecipitated from normoxic or hypoxic HUVECs. qPCR analysis of associated miRNA (B) and mRNA (C).

**Supplemental Figure 5. (A,B) Effect of 72-hr, 8% O<sub>2</sub> hypoxia on HRMs and Ago1 levels.** C57BL6 mice were subjected to hypoxia (Hx, 8% O<sub>2</sub>) or normoxia (Nx) for 3 days. Let-7s and miR-103/107 were detected by qPCR in multi-organs/tissues (A) and Ago1 by Western blotting analysis (B). Data represent 2 mice per group. **(C,D) Effect of 7-day, 10% O<sub>2</sub> hypoxia on HRMs and Ago2 levels.**

miRNAs (C) and Ago2 (D) were detected in multiple organs from animals subjected to hypoxia (Hx, 10% O<sub>2</sub>) or normoxia (Nx) for 7 days. Data were obtained from 4-6 mice per group. The level of various molecules in normoxia group was set to 1. Open bars: levels under normoxia; filled bars: levels under hypoxia.

**Supplemental Figure 6. Effect of antagomiR in miRNA abundance in Matrigel plugs from hypoxic mice.** Matrigel mixed with control RNA or antagomiRs against Let-7a, Let-7e, and miR-103 were subcutaneously injected into the dorsal surface of C57BL6 mice. After subjection to 10% O<sub>2</sub> for 5 days, Matrigel plugs were harvested, homogenized in Trizol, and RNA was extracted. Levels of specific miRNA were detected by qPCR. Results presented were from 3 animals per group.

**Supplemental Figure 7. Hypoxia alters Ago1-associated miRNAs and mRNAs.** Ago1 was immunoprecipitated from HUVECs subjected to normoxia (open bar) or hypoxia (close bar). Ago1-associated miRNA (A) or mRNAs (B) as indicated were detected by qPCR.

**Supplemental Figure 8. HIF2 $\alpha$  Induction of HRMs.** (A, B) HUVECs were transfected with pCMV parental vector or pCMV-HIF2 $\alpha$  (3  $\mu$ g per 10<sup>6</sup> cells) for 36 hr. mRNA levels were determined by qPCR. The level of HIF2 $\alpha$  was relative to that of  $\beta$ -actin (A), and that of HRMs was relative to U6 (B). Data are mean  $\pm$  SD of 3 independent experiments. (C) Putative HIF2 $\alpha$  binding sites in the respective promoters of HRMs.

**Supplemental Table 1. miRNA profile in normoxia (Nx) and hypoxia (Hx) in HUVECs.** The abundance of miRNAs was demonstrated by their reads in the libraries derived from normoxic or hypoxic cells, and then fold change under hypoxia relative to normoxic controls was determined. miRNAs of reads more than 10 were included.

**Supplemental Table 2. Putative HIF1 $\alpha$  binding sites in the HRM promoter regions.** Putative HIF1 $\alpha$  binding sites in human and mouse genomes were predicted by TransFac and position-weight matrix approach. The conservation score was calculated by PhastCons in the UCSC Genome Browser.

**Supplemental Table 3 Summary of putative HRM targets.** A) The number of potential miRNA targets commonly predicted from 3 algorithms. B) The classification of genes involved in various cellular functions based on gene ontology (GO) terms. C) Examples of putative target genes of Let-7s and miR-103/107. Note that Ago1 is a common target of HRMs.

**Supplemental Table 4. Correlation among HRM, Ago1, VEGF, and CD31 levels in HCC tissue array.** Spearman's non-parametric correlation test was used to assess the correlation between various molecules. The negative values of Spearman's rho in conjunction with  $p$  values  $<0.05$  indicate significant inverse correlations.

**Supplemental Table 5, 6. Genes involved in glycolysis (Table 5) and angiogenesis (Table 6) that are decreased in Ago1-miRISC under hypoxia.** Ago1 was immunoprecipitated from a pool of 8 batches of normoxic or hypoxic HUVEC lysates. mRNA transcripts associated in the immunoprecipitates were measured by using Affymetrix human Gene 1.0 ST array. The difference of Ago1-associated mRNA in hypoxia relative to normoxia was presented as log<sub>2</sub> of the fold change. The cut-off was set to be -2.5. Genes were categorized in Tables 5 and 6 by their GO terms.

## Supplemental Materials and Methods

### Oligo Sequences for Construction of Small RNA Libraries

5' tags:

Normoxia: 5'-GUUCAGAGUUCUACAGUCCGACGAUCCG-3'

Hypoxia: 5'-GUUCAGAGUUCUACAGUCCGACGAUCCU-3'

3' adaptor: 5'-pUCGUAUGCCGUCUUCUGCUUGidT-3'

### Northern Blotting, Taqman miRNA Quantitative PCR (qPCR), and RT-qPCR

Total RNA was fractionated and transferred to nylon membranes, UV-crosslinked, and hybridized with <sup>32</sup>P-labeled oligos complementary to mature miRNAs. The results were visualized by exposure to x-ray films. Taqman miRNA qPCR followed the manufacturer's protocols (Applied Biosystems). To quantitate mRNAs, total RNAs underwent RT and real-time qPCR with iQ SYBR Green (Bio-Rad). Fold changes were calculated by the  $\Delta\Delta C_t$  method. The sequences of primer sets used in mRNA qPCRs are listed below:

Human Ago1 forward: 5'-ATTGTGAGCTGGCGAATGCT-3'

Human Ago1 reverse: 5'-CAGGCGGTGAGAAGAAGGAG-3'

Human Ago2 forward: 5'-CTTCAAGCCTCCACCTAGACC-3'

Human Ago2 reverse: 5'-CATGTGTTCCACGATTTCCCT-3'

Human Ago3 forward: 5'-CCTTCTACAAAGCACAACCTGT-3'

Human Ago3 reverse: 5'-CCACAATGAGTCACTTCAACC-3'

Human VEGF-A forward: 5'-CGCAGCTACTGCCATCCAAT-3'

Human VEGF-A reverse: 5'-GTGAGGTTTGATCCGCATAAT-3'

Human PDGF-B forward: 5'-GATCCGCTCCTTTGATGATC-3'

Human PDGF-B reverse: 5'-GTCTCACACTTGCATGCCAG-3'

Human PIGF forward: 5'-CAGAGGTGGAAGTGGTACCCTTCC-3'

Human PIGF reverse: 5'-CGGATCTTTAGGAGCTGCATGGTGAC-3'

Human HIF2 $\alpha$  forward: 5'-CGGAGGTGTTCTATGAGCTGG-3'

Human HIF2 $\alpha$  reverse: 5'-AGCTTGTGTGTTTCGCAGGAA-3'

Human  $\beta$ -actin forward: 5'-GCACCACACCTTCTACAATG-3'

Human  $\beta$ -actin reverse: 5'-ATCACGATGCCAGTGGTAC-3'

### **Cell Culture, Adenovirus Infection, and Transient Transfection**

HUVECs, HEK-293, and bovine aortic endothelial cells (BAECs) were cultured by standard protocols. The normoxic cells were kept at 37°C ventilated with 5% CO<sub>2</sub> and atmospheric 21% oxygen. The hypoxic cells were maintained in an incubator infused with 2% oxygen, 5% CO<sub>2</sub>, and 93% nitrogen. HIF1 $\alpha$  was overexpressed in HUVECs with use of an adenoviral vector (Ad-HIF1 $\alpha$ , Cell Biolab). Pre-miR, anti-miRs (Ambion), LNAs (Exiqon), Ago1 or Ago2 siRNA (Qiagen), or DNA plasmids (for BAECs) were transfected into cells with use of Lipofectamine 2000 (Invitrogen).

To overexpress HA, HA-Ago1, or HA-HIF2 $\alpha$  in HUVECs, cells were transfected with Cytfect-HUVEC Transfection Kit (Cell Applications), following manufacturer's protocol. Specifically, cells were cultured to 70-80% confluency in a 6-well plate. Per well, 3  $\mu$ g of DNA plasmid were mixed with 2.5  $\mu$ l of CF2 and 2.5  $\mu$ l of PE reagents and incubated at 37°C for 25 min. The mixture was then added to HUVECs and kept for 1 hr, before replacement with fresh EC culture medium. The transfection was allowed for 18 to 24 hr before the cells were subjected to subsequent experiments.

### ***In vitro* EC Tube Formation Assay**



HUVECs were seeded at 30,000 cells/well in 24-well plates pre-coated with Matrigel (BD Biosciences) and subjected to normoxia or hypoxia for 16 hr. The images were captured using a digital camera connected to a stereoscope.

### **AntagomiRs Used in Animal Study**

Single-stranded RNA with chemical modifications were synthesized and HPLC-purified by Dharmacon, Inc. The sequences and modifications of antagomiRs used in this study are as follows:

AntagomiR-let-7a:

5'mA(\*)mC(\*)mUmCmCmAmUmCmAmUmCmCmAmAmCmAmUmAmU(\*)mC(\*)mA(\*)mA(\*) 3' Chl

AntagomiR-let-7e:

5'mA(\*)mC(\*)mUmCmCmAmUmCmCmUmCmCmAmAmCmAmUmAmU(\*)mC(\*)mA(\*)mA(\*)3' Chl

AntagomiR-103:

5'mU(\*)mC(\*)mGmUmCmGmUmAmACmAmUmGmUmCmCmCmGmAmU(\*)mA(\*)mC(\*)mU(\*) 3' Chl

Control antagomiR:

5'mA(\*)mA(\*)mGmGmCmAmAmGmCmUmGmAmCmCmCmUmGmAmA(\*)mG(\*)mU(\*)mU(\*)3' Chl

The lower case “m” represents 2'-OMe-modified nucleotides; “(\*)” represents a phosphorothioate linkage; “Chl” represents cholesterol linked through a hydroxyprolinol linkage.

### **Hematoxylin and Eosin Staining (HE staining)**

HE staining of matrigel plugs involved use of an autostainer (Leica Autostainer CV5030). Paraffin sections of each sample were heated to 60°C and treated with xylene to remove paraffin, followed by rehydration of ethanol solutions from 99.99% to 75%. After hematoxylin treatment, each sample was immersed in NH<sub>3(aq)</sub> for 20 sec followed by eosin staining. Specimens were then gradually dehydrated with increasing ethanol gradient and subsequent xylene treatment. All specimens were then sealed for further morphology examinations.

### **Immunohistochemical Staining of Matrigel Plugs**

Paraffin-embedded matrigel sections were deparaffinized as described above before antigen retrieval with target retrieval solution (pH 6.1) (DAKO) at 121°C for 10 min. Samples were then blocked with 3% H<sub>2</sub>O<sub>2</sub> to eliminate endogeneous peroxidase activities, then 3% normal goat serum in PBS to reduce endogeneous biotin activities. For IHC staining, samples were incubated with rabbit anti-vWF antibody (Sigma-Aldrich, HPA001815, dilution 1:250) or CD31 antibody (Santa Cruz, SC01506, dilution 1:100) overnight at 4°C, then after three washes with PBS, anti-rabbit IgG secondary antibody (dilution 1:200), then avidin biotin complex (Vector Laboratories Inc.). A DAB peroxidase substrate kit (Vector Laboratories Inc., SK-4100) was used to visualize the staining. Samples underwent counterstaining by use of an autostainer (Leica Autostainer CV5030). All samples were viewed and photographed under an Olympus microscope.