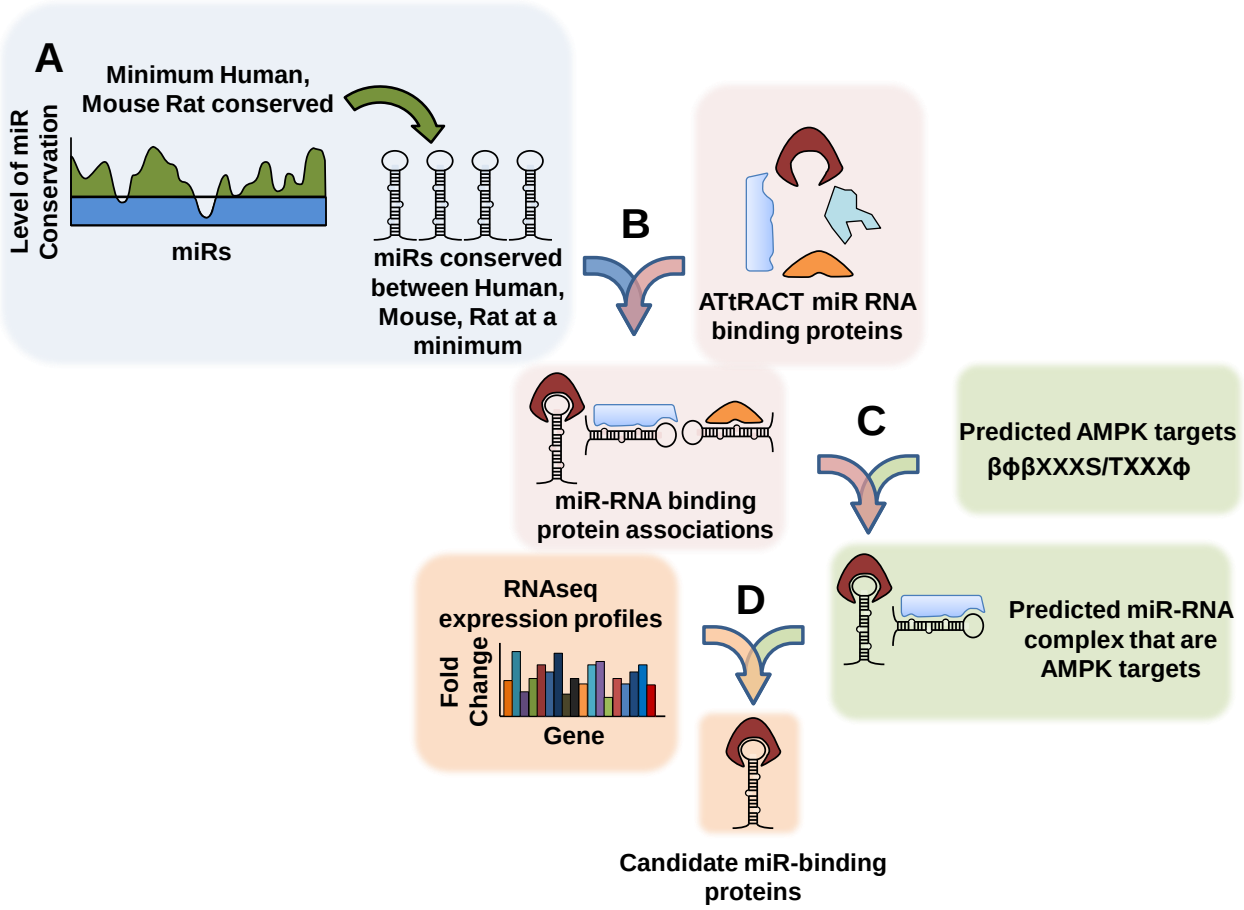

Figure S1. The stepwise bioinformatics approach for the identification of miR binding proteins. (**A**) Initially, miR sequences from miRbase (x-axis) were filtered based on their conservation among 13 different species with those conserved at least between human, mouse, and rat colored in green (y-axis); (**B**) RNA binding sequences specific to RNA binding proteins (represented as colored shapes) from the A daTabase of RNA binding proteins and AssoCiated moTifs (ATtRACT) were matched to miRs from miRbase database; (**C**) RNA binding proteins containing the putative AMPK phosphorylation sequence (14,15) were identified; and (**D**) PS/OS RNAseq data (18) was lastly used to verify the expression level of the AMPK-targeted miR-binding proteins.

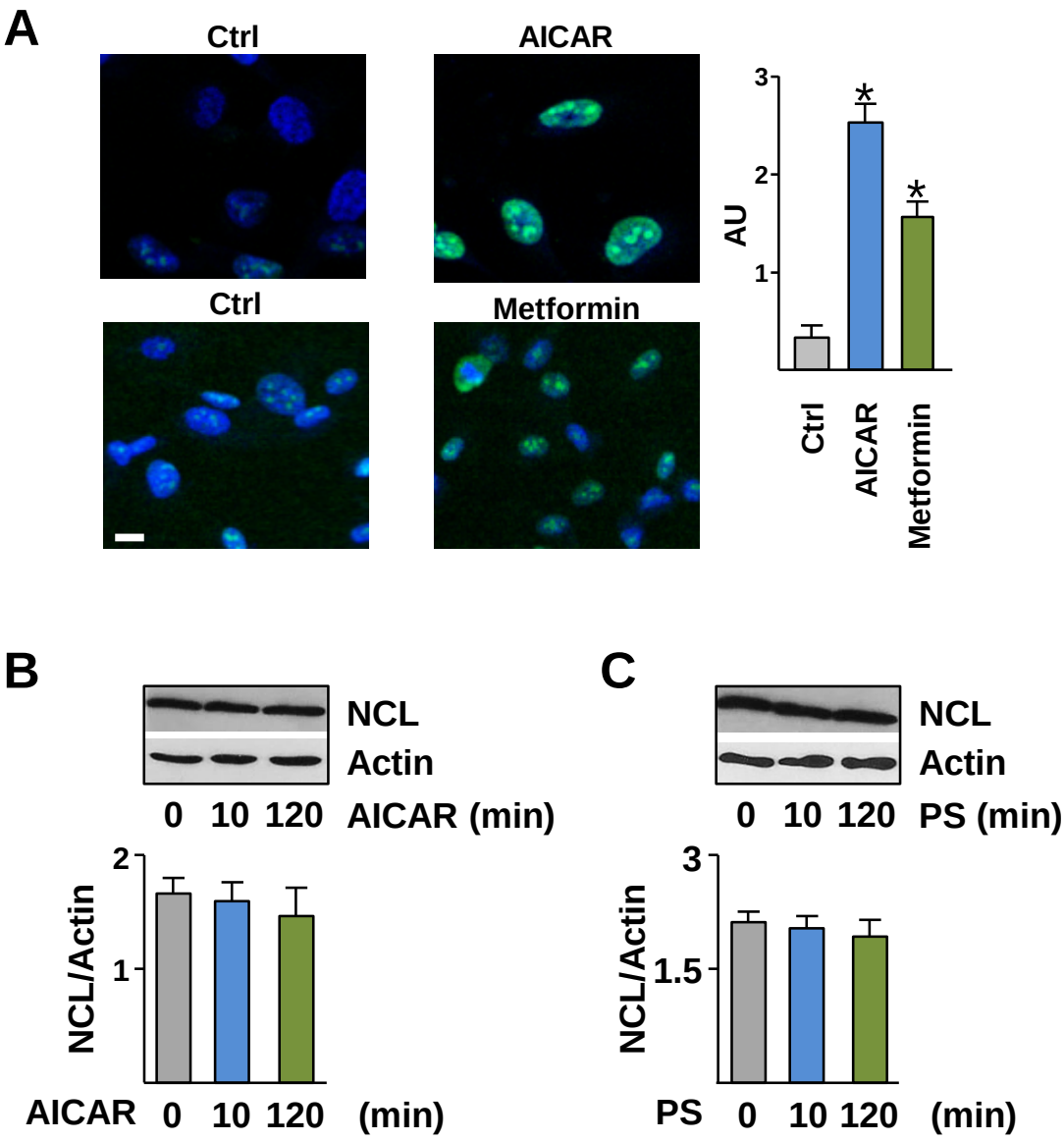

Figure S2. NCL protein level was not changed by AICAR of PS: (A) NCL immunofluorescence (green) and DAPI (blue) staining for HUVECs treated with AICAR (1 mM) or metformin (1 mM) for 10 min. Scale bar indicates 20 μm. **(B-C)** HUVECs were treated with AICAR (B) or subjected to PS (C) with indicated times. Immunoblotting of cell lysates was performed to detect the expression level of NCL. Data are mean ± SEM. n = 3. .

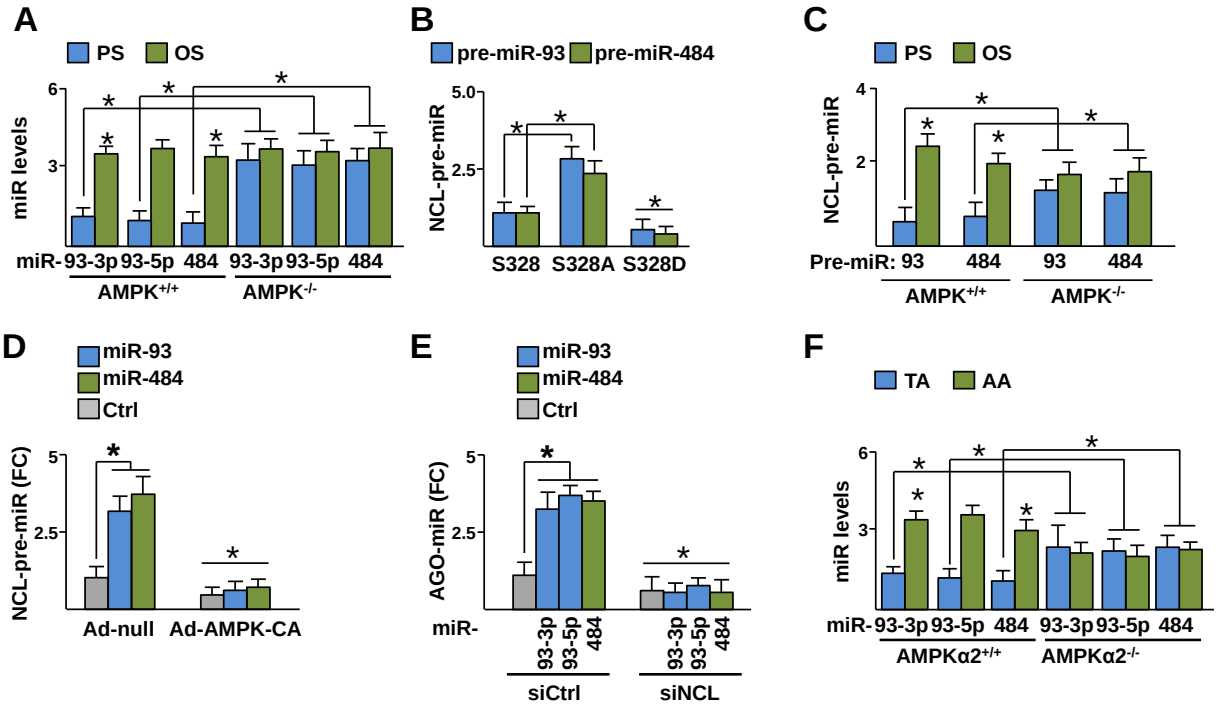

Figure S3. NCL regulates miR-93 and miR-484: (A) AMPK$^{+/+}$ and AMPK$^{-/-}$ MEFs were subjected to PS or OS. The level of miR-93-3p, miR-93-5p, or miR-484 was then measured. **(B)** NCL S328, S328A, or S328D was transfected into HUVECs. Transfected NCL was immunoprecipitated and the level of pre-miR-93 and pre-miR-484 bound to NCL was measured. **(C)** AMPK$^{+/+}$ and AMPK$^{-/-}$ MEFs were subjected to PS or OS. The amount of pre-miR-93 and pre-miR-484 binding to NCL was measured. **(D)** miR-93 or miR-484 was transfected into HUVECs that were then infected with an adenovirus overexpressing activated AMPK (Ad-AMPK-CA) or an empty vector (Ad-null). NCL was immunoprecipitated and the level of pre-miR-93 and pre-miR-484 binding to NCL was measured. **(E)** miR-93 or miR-484 was transfected into HUVECs that were then transfected with control or NCL siRNA. AGO2 was immunoprecipitated and the amount of AGO2-associated miR-93-3p, miR-93-5p, or miR-484 binding was determined. **(F)** The level of miR-93-3p, miR-93-5p, or miR-484 were measured in isolated TA or AA from AMPKα2$^{+/+}$ or AMPKα2$^{-/-}$ mice. * indicates $p<0.05$. Error bars represent mean ± SEM. n = 3.

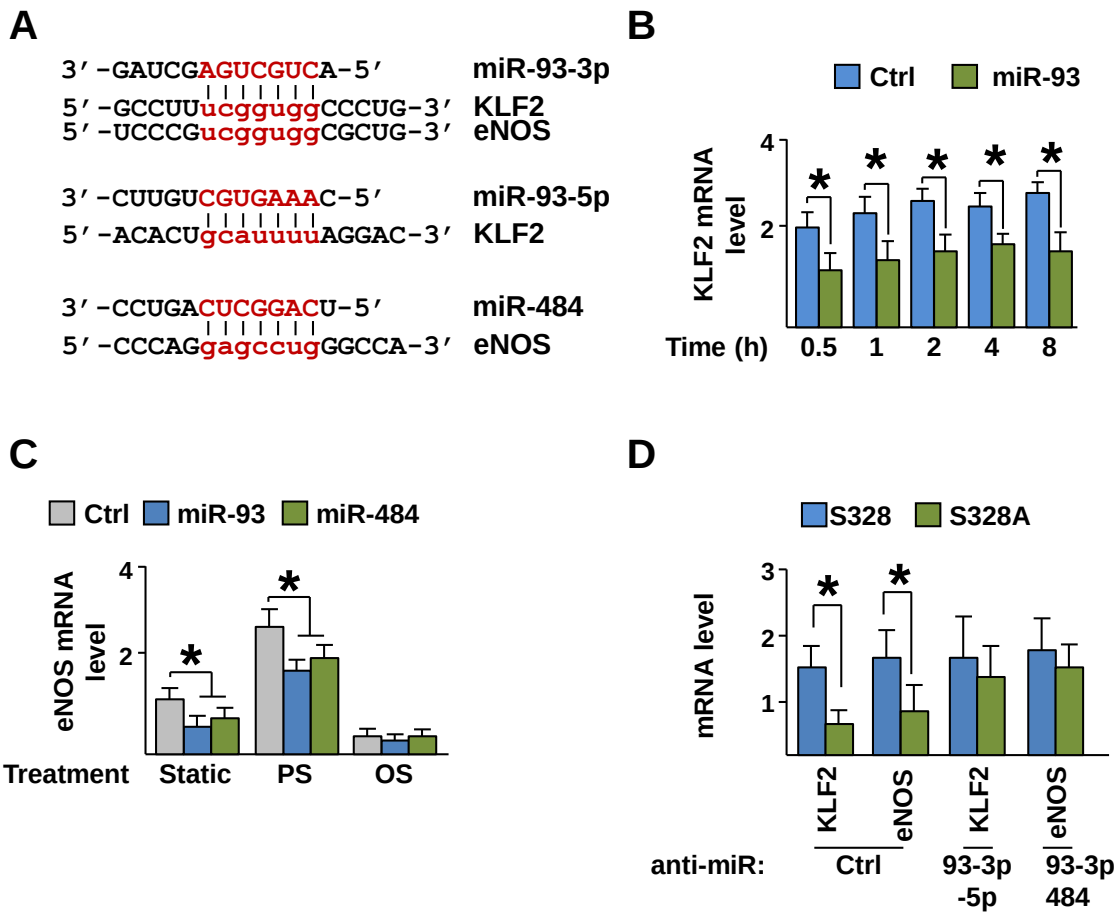

Figure S4. miR-mRNA complementarity. (A) Complementarity between miR-93-3p, miR-93-5p, or miR-484 seed sequences and human KLF2 and eNOS mRNA. (B) miR-93, or scrambled control, were transfected into HUVECs that were then subjected to PS. Following treatment, KLF2 mRNA levels were measured. (C) eNOS mRNA levels in HUVECS transfected with plasmids expressing miR-93, miR-484, or scrambled control, and subjected to PS or OS for 24 hours. (D) HUVECs were transfected with NCL S328 or S328A with or without anti-miR-93-3p, anti-miR-93-5p, or anti-miR-484 and subjected to PS. Following shear, mRNA levels were measured. * indicates $p<0.05$. Error bars represent mean ± SEM. n = 3.

Table S1: Species conservation of NCL binding site

Targeting Factor	Number Species	gene symbol
NCL	23	MIR-93
NCL	15	MIR-484
NCL	9	MIR-206
NCL	9	MIR-887
NCL	5	MIR-598
NCL	4	MIR-2118B
NCL	4	MIR-219-1
NCL	3	MIR-219
NCL	3	MIR-219A-1
NCL	3	MIR-326
NCL	3	MIR-395D
NCL	2	MIR-151B
NCL	2	MIR-199B
NCL	2	MIR-2392
NCL	2	MIR-263B
NCL	2	MIR-30A
NCL	2	MIR-482D
NCL	2	MIR-623
NCL	2	MIR-705
NCL	2	MIR-92B
NCL	2	MIR-H3
NCL	1	LET-7A-3
NCL	1	LET-7B
NCL	1	MIR-1001
NCL	1	MIR-10034-1
NCL	1	MIR-10034-2
NCL	1	MIR-10392
NCL	1	MIR-10462-1
NCL	1	MIR-10462-2
NCL	1	MIR-10547A-1
NCL	1	MIR-10547A-2
NCL	1	MIR-10547A-3
NCL	1	MIR-10693
NCL	1	MIR-1071
NCL	1	MIR-10843
NCL	1	MIR-10984A
NCL	1	MIR-10984B
NCL	1	MIR-10991A
NCL	1	MIR-10991B
NCL	1	MIR-10991D
NCL	1	MIR-10991E
NCL	1	MIR-11019
NCL	1	MIR-11058
NCL	1	MIR-11119

NCL	1	MIR-11166
NCL	1	MIR-11423L
NCL	1	MIR-11532
NCL	1	MIR-11556
NCL	1	MIR-12
NCL	1	MIR-12055
NCL	1	MIR-12164
NCL	1	MIR-12295
NCL	1	MIR-128-2
NCL	1	MIR-1337
NCL	1	MIR-135A-2
NCL	1	MIR-1379
NCL	1	MIR-1421M
NCL	1	MIR-1422L
NCL	1	MIR-1486
NCL	1	MIR-1493-1
NCL	1	MIR-1503
NCL	1	MIR-1507B
NCL	1	MIR-1514A
NCL	1	MIR-1515A
NCL	1	MIR-1515B
NCL	1	MIR-1523A
NCL	1	MIR-160B
NCL	1	MIR-166H
NCL	1	MIR-166L
NCL	1	MIR-168
NCL	1	MIR-1706
NCL	1	MIR-171A
NCL	1	MIR-181A
NCL	1	MIR-1851
NCL	1	MIR-1862A
NCL	1	MIR-1868
NCL	1	MIR-193
NCL	1	MIR-193A
NCL	1	MIR-199-3
NCL	1	MIR-1B
NCL	1	MIR-2058
NCL	1	MIR-2071
NCL	1	MIR-2071-1
NCL	1	MIR-2071-2
NCL	1	MIR-210
NCL	1	MIR-2118A
NCL	1	MIR-2118C
NCL	1	MIR-2118N
NCL	1	MIR-2118P
NCL	1	MIR-2118Q
NCL	1	MIR-2120B

NCL	1	MIR-219A
NCL	1	MIR-2235C
NCL	1	MIR-2266
NCL	1	MIR-228
NCL	1	MIR-2573
NCL	1	MIR-2678
NCL	1	MIR-26A
NCL	1	MIR-26A-2
NCL	1	MIR-2731-1
NCL	1	MIR-2731-2
NCL	1	MIR-283
NCL	1	MIR-2867
NCL	1	MIR-2869
NCL	1	MIR-2944C
NCL	1	MIR-296
NCL	1	MIR-29B
NCL	1	MIR-2F
NCL	1	MIR-2H-1
NCL	1	MIR-2H-2
NCL	1	MIR-305
NCL	1	MIR-315
NCL	1	MIR-316
NCL	1	MIR-3167
NCL	1	MIR-3197
NCL	1	MIR-319B
NCL	1	MIR-320D-2
NCL	1	MIR-3244
NCL	1	MIR-33
NCL	1	MIR-3303
NCL	1	MIR-34
NCL	1	MIR-3410
NCL	1	MIR-3422
NCL	1	MIR-3450
NCL	1	MIR-3462
NCL	1	MIR-3470B
NCL	1	MIR-36B
NCL	1	MIR-3709A
NCL	1	MIR-3709B
NCL	1	MIR-3718A
NCL	1	MIR-3718C
NCL	1	MIR-375
NCL	1	MIR-3871
NCL	1	MIR-393
NCL	1	MIR-393A
NCL	1	MIR-393B
NCL	1	MIR-396C
NCL	1	MIR-396J

NCL	1	MIR-396K
NCL	1	MIR-396P
NCL	1	MIR-396T
NCL	1	MIR-397B
NCL	1	MIR-403F
NCL	1	MIR-4076
NCL	1	MIR-4321
NCL	1	MIR-4400
NCL	1	MIR-4588
NCL	1	MIR-46
NCL	1	MIR-4622
NCL	1	MIR-4624
NCL	1	MIR-4674
NCL	1	MIR-4710
NCL	1	MIR-4829C
NCL	1	MIR-4854
NCL	1	MIR-496
NCL	1	MIR-5
NCL	1	MIR-5029
NCL	1	MIR-5100
NCL	1	MIR-5169B
NCL	1	MIR-516A-1
NCL	1	MIR-516A-2
NCL	1	MIR-5289B
NCL	1	MIR-541
NCL	1	MIR-54A
NCL	1	MIR-5510
NCL	1	MIR-5518
NCL	1	MIR-5548
NCL	1	MIR-5608
NCL	1	MIR-5642A
NCL	1	MIR-5642B
NCL	1	MIR-5671A
NCL	1	MIR-5672
NCL	1	MIR-5742
NCL	1	MIR-6066
NCL	1	MIR-611
NCL	1	MIR-6219
NCL	1	MIR-6225
NCL	1	MIR-6238
NCL	1	MIR-6280
NCL	1	MIR-6596
NCL	1	MIR-66
NCL	1	MIR-6664
NCL	1	MIR-6712
NCL	1	MIR-6724-1
NCL	1	MIR-6724-2

NCL	1	MIR-6724-3
NCL	1	MIR-6724-4
NCL	1	MIR-6790
NCL	1	MIR-7144
NCL	1	MIR-7471
NCL	1	MIR-750
NCL	1	MIR-7504E
NCL	1	MIR-767
NCL	1	MIR-7769
NCL	1	MIR-7771
NCL	1	MIR-7816
NCL	1	MIR-786
NCL	1	MIR-7911C-1
NCL	1	MIR-7911C-2
NCL	1	MIR-8032A
NCL	1	MIR-8032B
NCL	1	MIR-8032C
NCL	1	MIR-8032D
NCL	1	MIR-8032E
NCL	1	MIR-8032F
NCL	1	MIR-8032G
NCL	1	MIR-8128
NCL	1	MIR-8232
NCL	1	MIR-828
NCL	1	MIR-8348
NCL	1	MIR-8448
NCL	1	MIR-8473
NCL	1	MIR-8521A-1
NCL	1	MIR-8521A-2
NCL	1	MIR-8521A-3
NCL	1	MIR-8521A-4
NCL	1	MIR-8521A-5
NCL	1	MIR-8521B-1
NCL	1	MIR-8521B-10
NCL	1	MIR-8521B-11
NCL	1	MIR-8521B-2
NCL	1	MIR-8521B-3
NCL	1	MIR-8521B-4
NCL	1	MIR-8521B-5
NCL	1	MIR-8521B-6
NCL	1	MIR-8521B-7
NCL	1	MIR-8521B-8
NCL	1	MIR-8521B-9
NCL	1	MIR-8558B
NCL	1	MIR-858
NCL	1	MIR-858B
NCL	1	MIR-8624B

NCL	1	MIR-8670A
NCL	1	MIR-8789
NCL	1	MIR-8823
NCL	1	MIR-8905
NCL	1	MIR-8908D-3
NCL	1	MIR-8979
NCL	1	MIR-9052-1
NCL	1	MIR-9052-2
NCL	1	MIR-9123
NCL	1	MIR-9157
NCL	1	MIR-9198B-1
NCL	1	MIR-9198B-2
NCL	1	MIR-9198B-4
NCL	1	MIR-9214-1
NCL	1	MIR-9214-2
NCL	1	MIR-93A-2
NCL	1	MIR-9483B
NCL	1	MIR-9514
NCL	1	MIR-979
NCL	1	MIR-9828-1
NCL	1	MIR-9828-2
NCL	1	MIR-9828-3
NCL	1	MIR-9873
NCL	1	MIR-9879
NCL	1	MIR-993A-2
NCL	1	MIR-B20
NCL	1	MIR-D8
NCL	1	MIR-H7
NCL	1	MIR-M59-2
NCL	1	MIR-M7
NCL	1	MIR-RO6-1
NCL	1	MIR-RR1-4

Table S2: Predicted NCL regulated miRs

gene symbol	OS/PS logFC	P.Value	adj.P.Val	Targeting Factor	Gene_ID	Identified_sequence
LET-7E	-0.643713639	0.010541	0.7365227	NCL	ENSG00000115053	GguA
MIR-106B	0.435911316	0.001722	0.3488662	NCL	ENSG00000115053	AguG
MIR-107	0.287554387	0.044707	0.9769808	NCL	ENSG00000115053	AguG
MIR-133B	-0.287709587	0.021087	0.8942505	NCL	ENSG00000115053	GguC
MIR-155	0.775289378	0.034111	0.9764284	NCL	ENSG00000115053	UguU
MIR-187	1.158116187	0.001828	0.3488662	NCL	ENSG00000115053	GguC
MIR-23B	-0.837009406	0.0002	0.0869341	NCL	ENSG00000115053	GguG
MIR-25	0.460191782	0.032049	0.9682536	NCL	ENSG00000115053	AguG
MIR-27B	-0.675261639	0.006756	0.6873233	NCL	ENSG00000115053	GguG
MIR-30A	-0.253294968	0.006175	0.6873233	NCL	ENSG00000115053	UguA
MIR-363	-0.206360979	0.048283	0.9769808	NCL	ENSG00000115053	UguU
MIR-377	-0.188781741	0.018958	0.8942505	NCL	ENSG00000115053	GguU
MIR-452	0.621560242	0.018745	0.8942505	NCL	ENSG00000115053	UguU
MIR-505	-0.689303148	0.027655	0.9682536	NCL	ENSG00000115053	AguG
MIR-582	0.558866512	0.024411	0.963822	NCL	ENSG00000115053	UguG
MIR-877	0.398529873	0.006766	0.6873233	NCL	ENSG00000115053	guA
MIR-92A-1	0.90399659	0.001563	0.3488662	NCL	ENSG00000115053	GguU

Table S3: Taiwan patient characteristics

Characteristic	Healthy control (n=10)	CAD patient (n= 51)
Gender (male)	8 (80.0%)	38 (74.5%)
Age (years)	47.7 ± 18.4	66.4 ± 12.0
CAD	0	51
DM	0	22 (43.1%)
Total cholesterol	161.7 ± 17.2	159.5 ± 36.4
HDL	51.3 ± 5.5	40.9 ± 13.0
LDL	106.1 ± 31.67	87.8 ± 27.6
TG	87.7 ± 41.4	145.3 ± 80.9

Table S4: Xi'an patient characteristics

Characteristic	Healthy control (n=35)	CAD patient (n= 25)
Gender (male)	14 (40%)	17 (68%)
Age (years)	40.69 ± 11.69	59.6 ± 8.74
CAD	0	25
DM (glucose)	4.48 ± 0.51	4.66 ± 0.87
Total cholesterol	4.33 ± 0.59	3.54 ± 0.74
HDL	1.30 ± 0.26	0.95 ± 0.19
LDL	2.62 ± 0.54	2.04 ± 0.64
TG	1.25 ± 0.75	1.48 ± 0.72

Table S5: miRNA primers

Company	Catalog number	target	Species
Qiagen	MP00003717	pre-miR-93	Human
Qiagen	MS00003346	miR-93-5p	Human
Qiagen	MS00010815	miR-93-3p	Human
Qiagen	MP00002338	pre-miR-484	Human
Qiagen	MS00004277	miR-484	Human
Qiagen	MP00006867	pre-miR-93	Mouse
Qiagen	MS00001449	miR-93-5p	Mouse
Qiagen	MS00012894	miR-93-3p	Mouse
Qiagen	MS00002499	miR-484	Mouse
Thermofisher scientific	4441114 Assay ID: AR322R7	pre-miR-484	Mouse

Table S6: mRNA primers

Target	Direction	Sequence	Species
eNOS	Forward	GGTACATGAGCACTGAGATCG	Human
eNOS	Reverse	ACGATGGTGACTTTGGCTAG	Human
KLF2	Forward	CAAGACCTACACCAAGAGTTCG	Human
KLF2	Reverse	GTGCCGTTTCATGTGCAG	Human
eNOS	Forward	AAGTCCTCACCGCCTTTTC	Mouse
eNOS	Reverse	TGACACAATCCCTCTTTCCG	Mouse
KLF2	Forward	CCAAGAGCTCGCACCTAAAG	Mouse
KLF2	Reverse	GTGGCACTGAAAGGGTCTGT	Mouse

Materials and Methods

AMPK Kinase Assay

AMPK kinase assays were performed a buffer containing 50 mM HEPES, 0.375 mM AMP, 0.375 mM ATP, 9 mM $MgCl_2$, 1 µCi (γ-^{32}P)ATP, and 2 mg of target proteins with or without 11 pM AMPK and the reaction was performed at 37 ^{o}C for 1 hr. Reaction mixtures were then resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), stained with Coomassie blue, and radioactivity detected with autoradiography. When peptides were analyzed, the reaction conditions were the same as above except that the samples were spotted on Whatman filter paper. Following several washes in 1% phosphoric acid, the level of ^{32}P was quantified using a Beckman LS 6500 scintillation counter.

Isoelectric focusing

Cells were lysed in a solution containing 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB 3-10, 10 mM DTT, 0.2% (w/v) Bio-Lytes, and 0.001% bromophenol blue. The protein lysates were loaded into Immobilized pH gradient strips (IPG) and isoelectric focusing was conducted at 8,000 V for 35,000 volt-hours in a Protean IEF cell. Samples were then equilibrated in 6 M urea, 2% SDS, 0.375 M Tris-HCL, pH 6.8, 20% glycerol, and 130 mM DTT and then resolved on an SDS-PAGE gel prior to transferring to a PVDF membrane. Immunoblotting was then conducted.

Immunofluorescence

Four percent paraformaldehyde was added to the culture medium to a final concentration of 0.75%. After a 10 min incubation, cells were washed three times with phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) and incubated in blocking buffer (3% BSA in PBS) for 1 h. After blocking, cells were incubated in primary antibody diluted in blocking buffer overnight, stained with DAPI, and microphotographs made with a Leica SP5 inverted confocal microscope.

Co-Immunoprecipitation

After washing three times with PBS, cells were lysed in FA lysis buffer (50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, Halt protease inhibitors). Cell lysates were incubated in primary antibody bound protein A conjugated sepharose beads overnight. Following several washes with FA lysis buffer, remaining proteins were resolved with SDS-PAGE followed by immunoblotting using standard protocols.

qPCR Methods

RNA was purified using TRIzol reagent from Life Technologies (cat # 10296-028) according to the manufacturer's instructions. Two µg RNA was converted to cDNA using Promega reverse transcriptase according to the manufacturer's instructions (cat # M1701). Messenger RNA was then quantified via qPCR using cyber green qPCR master mix purchased from Bio Rad. Results were calculated using the Δ-Δ ct method. Primers used are listed in SI Appendix, Table S5 and S6.

Immunoblotting

Following transfer, PVDF membranes were blocked for 1 h in blocking buffer (5% milk in TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20)). Membranes were then washed three times with TBST and incubated with primary antibody diluted in blocking buffer overnight and followed by the secondary antibody. After washing three times in TBST, membranes were incubated with chemiluminescent Horseradish peroxidase (HRP) substrate and immunoblots were developed.

RNA Immunoprecipitation

RNA immunoprecipitations were conducted according to protocols available from ABcam. Following treatment, cells were fixed in 0.75% formaldehyde followed by glycine to a

final concentration of 125 mM. Following fixation, cells were scraped from the surface in RIPA buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 0.5 mM DTT, 0.5% NP40, 100 U/ml RNAase inhibitor). Resulting cell lysates were incubated overnight in primary antibody conjugated protein A beads at 4^{o}C. Following incubation beads were washed three times in RIPA buffer and RNA was isolated with TRIzol reagent according to the manufacturer's instructions.

NO bioavailability

NO bioavailability was quantified with the use of the Nitric Oxide colorimetric assay kit (ABcam cat# ab65328) according to the manufacturer's instructions.

Transfection

Transfections were conducted as previously described (33). The following miRNA inhibitors were used from ThermoFisher scientific: Anti-miR-93-5p (Cat# AM10951), Anti-miR-93-3p (Cat# AM12787), Anti-miR-484 (Cat# AM10379). The following siRNAs were used from Qiagen: AMPKα1 (product# 1027416), AMPKα2 (product# 1027416), NCL (product# 1027416).

Bioinformatics

All bioinformatics analyses were conducted in R programming language supported by packages installed from the Bioconductor and Comprehensive R Archive Network (CRAN) repositories. miR93 and miR484 overexpression GEO dataset GSE86497 and GSE66844 were used in the analysis. Read alignments to GRCh38 were conducted with Hisat2 read alignment software followed by Fold change and statistical calculations with EdgeR via the systempipeR Bioconductor package.

Study Population

Fifty-two patients with CAD were included in the initial study cohort (discovery cohort). Forty-five healthy controls without CAD or inflammatory diseases were defined as the control group. The

validation cohort includes 32 patients with CAD and 31 controls. The exclusion criteria include a known history of leukopenia, thrombocytopenia, hepatic or renal dysfunction and inflammatory or malignant disease. The research was approved by the institutional ethics committee of Taipei Veterans General Hospital (discovery cohort) and Xi'an Jiaotong University Medical School (validation cohort). The study protocol was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistics

Data are presented as mean ± SEM. Comparisons between 2 groups involved Student t test. Comparisons between 3 or more groups involved one-way ANOVA followed by Bonferroni post-hoc test for equal sample sizes or Turkey-Kramer test for unequal sample sizes. Correlations were determined by Spearman correlation analysis after determining the non-normal distribution of data. Statistical analyses involved use of SPSS for Windows, v18.0 (SPSS, Inc.; Chicago, IL, USA) and GraphPad Prism v5.01 (GraphPad Software, San Diego, CA, USA). Two-tailed $p < 0.05$ was considered statistically significant.