Supplementary Information

Supplementary Methods

Cells. RPTC cells were originally from Dr. U. Hopfer at Case Western Reserve University (Cleveland, OH) and cultured as described before[1]. To inhibit mir-489, 200 nM anti-rno-mir-489 LNAs (Exiqon Inc, Woburn, MA) were transfected into RPTC cells by Lipofectamine 2000 following the manufacturer's instruction. To overexpress mir-489 in RPTC cells, 200 nM rno-mir-489 mimics were transfected(Life Technologies, Grand Island, NY). HEK293 cells were originally from ATCC (Manassas, VA) and the HEK HIF-1α knockdown or scramble control cells were generated by transfection of HIF-1α shRNA plasmids or scramble shRNA plasmids from Origene (Rockville, MD) with Lipofectamine 2000 and selected by 2.5 μg/ml puromycin (Clontech Laboratories, Mountain View, CA) for two weeks to obtain cells with stable shRNA expression. The mouse proximal tubular cell line BUMPT was originally obtained from W Lieberthal and JH Shwartz at Boston University[2]. The cells were cultured in DMEM with 10% fetal bovine serum.

Renal ischemia/reperfusion in mice. Male mice of 8-12 week old were subjected to bilateral renal ischemia/reperfusion as described before [3]. Briefly, the mice were anesthetized with 60mg/kg pentobarbital and kept on a homoeothermic blanket. The renal pedicles were clamped with micro-aneurysm clips to induce renal ischemia. After 25 or 30 minutes of renal ischemia, the clips were removed for reperfusion. As control, another group of mice were sham operated without the clamping of renal pedicles.

To inhibit mir-489, C57BL/6 mice were treated with anti-mmu-mir-489 LNA (Exiqon Inc, Woburn, MA) through intravenous injection two days prior to renal ischemia/reperfusion at a dosage of 20mg/kg. Scrambled sequence LNA was used for comparison.

Hypoxic treatment of cells. The cells were seeded in culture dishes to reach the full confluence next day for hypoxic treatment. Before treatment, the cell culture medium was balanced in 1% O2 overnight and then the cells were incubated in the hypoxia balanced medium and kept in hypoxia (1% O2) chamber for 3, 6, 9, 24 or 48 hours. As control, another group of cells was kept in a medium with normal (21%) oxygen.

ATP depletion of RPTC. ATP depletion and recovery was used as the in vitro model of ischemia/reperfusion. Briefly, cells were treated with 10 mM sodium azide in a glucose-free Krebs-Ringer bicarbonate solution for 3 hours to induce ATP depletion. The cells were then returned to full culture medium for 2 hours of recovery to mimic reperfusion, leading to apoptosis [4]. To assess apoptosis, the cells were stained with Hoechst 33342 for morphological examination by phase contrast and fluorescence microscopy as described before[4, 5].

RNA extraction. Kidney cortical and out medulla samples were dissected at animal sacrifice, freshly frozen in liquid N_2 and kept in -80°C till use. Total RNA was extracted from cells or frozen mouse kidney samples with mirVana™ miRNA Isolation Kit (Life Technologies, Grand Island, NY) following the manufacture's instruction.

Real-time PCR of microRNA. 40 nanograms of total RNA were converted to cDNA using TaqMan® MicroRNA Reverse Transcription Kit (Life Technologies, Grand Island, NY). Real-Time PCR was performed using TaqMan® MicroRNA Assays (Life Technologies, Grand Island, NY) following the manufacture's instruction. Small nuclear RNA 202 was used as internal control and the quantification was done using ΔCt values.

Caspase activity measurement. The activity of caspases was measured as described before[4]. Briefly, cells were extracted with a buffer containing 1% Triton X-100. The lysates of 25 μg protein were mixed with enzymatic reactions containing 50 μM DEVD.AFC and incubated for 60 min at 37°C. The fluorescence at excitation 360 nm/emission 530 nm before and after the incubation was monitored by a GENios plate-reader (Tecan US, Research Triangle Park, NC), together with a standard curve of free AFC. The caspase activity was calculated based on the standard curve as the production of nmole free AFC/hour/mg protein.

Kidney protein and cell lysate extraction. Kidney samples were collected as described above for RNA extraction. The frozen kidney samples or cultured cells were lysed in SDS lysis buffer (62.5 mM Tris-HCl, pH6.8, 2% SDS, 10% Glycerol and the protein concentration was determined by Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, lL) following the manufacturer's instruction.

Immunoblotting. Equal amount of protein samples or equal volume of IP products from same amount of input protein were loaded for standard reducing SDS-polyacrylamide gel electrophoresis and then transferred to PVDF membrane. After blocking in 5% milk, the blot was exposed to primary antibody in 4°C overnight, followed with horseradish peroxidase (HRP) conjugated secondary antibody incubation. Finally, the blot was incubated with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, lL) and exposed to X-ray film.

Immunohistochemical and immunofluorescence staining. Kidney samples were collected at animal sacrifice and fixed immediately in 4% paraformaldehyde. 4 μm-thick paraffin embedded sections were used for the following immunohistochemical staining. After rehydration, antigen retrieval and blocking, tissue sections were exposed to primary antibodies [anti-HIF-1α from Cayman Chemical (Ann Arbor, MI) and anti-Ki67 from Cell signaling Technology (Danvers, MA)] overnight in 4°C, followed by avidin-biotin blocking and biotin-labeled secondary antibody incubation. After signal amplification by TSA™ Biotin System (PerkinElmer, Waltham, MA), the slides were developed with Vecstatin Elite ABC kit and ImmPACT DAB Peroxidase Substrate (Vector Laboratories, Burlingame, CA).

RPTC cells were fixed in 4% parpaformaldehyde and incubated in blocking buffer containing 2% BSA, 0.2% milk, 2% normal goat serum in PBS with 0.8% Triton X-100. After blocking, the cells were incubated with anti-Ki67 antibody diluted in blocking buffer for

overnight in 4°C, followed by cy3-goat-anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) incubation. The cells were also counter stained with Hoechst to detect the nuclear signal.

Blood urea nitrogen and serum creatinine. Blood samples were collected from tail vein before and daily after ischemia/reperfusion to collect serum by centrifugation after clotting at room temperature. The blood urea nitrogen level was then measured with a kit from Stanbio Laboratory (Boerne, TX) following the manufacturer's instruction. The serum creatinine level was measured with a Jaffe Reaction based kit from STanbio Laboratory (Boerne, TX) following the manufacturer's instruction.

Histology. Paraffin-embedded kidney tissue sections were stained by a standard hematoxylin & eosin staining as described previously. The percentage of injured renal tubules was estimated for each slide in a blinded manner.

TUNEL assay. Paraffin-embedded tissue sections were stained with in situ Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN) following the manufacture's instruction. The slides were mounted with ProLong® Gold Antifade Reagent (Life Technologies, Grand Island, NY). TUNEL positive cells were counted from 10 random images for each specimen from the outer medulla and kidney cortex in a blinded way.

Bioinformatics, data analysis and statistics. The potential HIF-1 binding sites of mir-489 and calcr gene were predicted with online tools JASPAR database (http://jaspar.genereg.net)[6]. The potential mRNA targets were predicted with online tools miRanda (http://www.microrna.org) and Segal Lab of Computational Biology online software (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html). The pathway analysis for the target genes of mir-489 was done by DAVID functional annotation (http://david.abcc.ncifcrf.gov/). Image J 1.46r (http://imagej.nih.gov/ij) was used to count Ki67 positive nuclei in kidneys. Student t-test was used to show the significant difference between two groups ($P<0.05$) and one-way ANOVA analysis was used for multi-group difference analysis. Data were expressed as Mean±SD. NCSS/PASS Dawson Edition for ANOVA analysis and Microsoft Excel 2012 was used for all the other calculations.

References:

1. Woost PG, Orosz DE, Jin W, et al. Immortalization and characterization of proximal tubule cells derived from kidneys of spontaneously hypertensive and normotensive rats. Kidney international 1996; 50: 125-134.

2. Sinha D, Wang Z, Price VR, et al. Chemical anoxia of tubular cells induces activation of c-Src and its translocation to the zonula adherens. American journal of physiology Renal physiology 2003; 284: F488-497.

3. Wei Q, Dong Z. Mouse model of ischemic acute kidney injury: technical notes and tricks. American journal of physiology Renal physiology 2012; 303: F1487-1494.

4. Wei Q, Wang J, Wang MH, et al. Inhibition of apoptosis by Zn2+ in renal tubular cells following ATP depletion. American journal of physiology Renal physiology 2004; 287: F492-500.

5. Brooks C, Wei Q, Cho SG, et al. Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. The Journal of clinical investigation 2009; 119: 1275-1285.

6. Mathelier A, Zhao X, Zhang AW, et al. JASPAR 2014: an extensively expanded and updated open-access database of transcription factor binding profiles. Nucleic acids research 2014; 42: D142-147.

Supplementary Tables

Supplementary Table 1: mRNAs of coding genes recruited to RISC by mir-489. HEK293 cells were co-transfected with Flag-Ago-2 and mir-489 mimic (489 group) or negative control RNA oligos (NC group). The lysates were subjected to immunoprecipitation with anti-Flag-Ago-2. The RNAs in the immunoprecipitates were extracted for RNA-seq analysis. mir-489 mimics induced significant (>2 fold) increases in 905 RNAs associated with Ago-2 RISC, including 417 mRNAs of coding genes listed in this table.

ABCC8
ABI3BP
AC006207.1
AC010487.1
AC010724.2
AC068775.1
AC093768.1
AC107977.1
AC126603.1
AC135983.2
ACCS
ACE2
ACPP
ACSS3
ACTA1
ADC
ADORA1
AGAP2
AGBL2
AKAP6
AKR1C1
AL359853.2
AL662890.2
AL669831.1
ALDH1A1
ALDH1L2
ALG10B
ANKK1
ANKRD30B
ANKRD53
ANO4
AP000974.1
AP003068.23
APLN
APOBEC3A
APOL1
AQP4
ARHGAP24
ASB12,MTMR8
ASB9
ATAD3C
ATP7A
ATP8B3
AZU1
BDNF
BEND5
BEST4
BMP3
BMP8A
BMPER
BTC
C10orf125
C11orf1
C14orf182
C17orf50
C17orf87
C19orf35
C1orf101
C1orf187
C1orf204,CCDC19,VSIG8
C1orf88
C20orf132
C20orf196
C20orf94
C2orf77
C3orf15
C5AR1
C5orf49
C6orf163
C6orf52
C7orf51
C7orf58,HMGN1P18
C8orf84
C9orf133,TIGD1L2
C9orf43
C9orf96
CAMK4
CASC1
CATSPERG
CBLN1
CCDC114
CCDC144A
CCDC148
CCDC81
CCHCR1
CCR4
CD200R1
CD27,TAPBPL
CD3G
CD70
CD79A
CDH13
CDH26
CELF3
CELF4
CERS4
CFP
CHRDL1
CHSY3
CLDN9
CLEC18B
CLIC2
CNFN
CNPY1
CNTD1
COL7A1
COL8A1
CPLX2
CPLX4
CRABP1
CRB2
CREG2,RFX8
CRX
CSMD2
CT45A1
CT45A2
CTC-241N9.1
CTF1
CUX2
CXCL10
CXCL2
CYGB
CYLC2
CYP2C18,CYP2C19
CYP2D6,CYP2D7P1
CYP2E1
DACT2
DACT3
DDX11L9
DECR1
DEGS2
DKK1
DLGAP3
DMC1
DNAH10
DNAH6
DNAI1
DNAJC3
DPEP1
DSC1
DSG3
DUX4L2
DUX4L3
DUX4L9
DYNC1I1
EFCAB4B
EGF
ELF3
ELFN1
EMX1
ENG
ENHO
EPHA6
ESPN
ESR1
F12
FAM132A
FAM150A
FAM151A
FAM157A
FAM198A
FAM19A2
FAM27C
FAM57B
FAM66E
FAM72C
FAM78B
FBXL7
FCGR2A
FIBCD1
FOXD4L1
FOXH1
FOXJ1
FRMD3
FRMPD2
FUT6
GABBR2
GABRP
GAD2
GAL3ST2
GBP3
GBP4
GFPT2
GJC2
GLP2R
GLRA2
GLT1D1
GOLGA6C
GOLGA8G
GPR113
GPR158
GPR85
GPR88
GPRIN2
GRHL3
GRIN2A
GRIP2
GRM2
GSTM2
GSTM3P1
GSTM3P1
HBA1
HDAC9
HES2
HFE
HHIP
HIST1H2AM,HIST1H3J
HIST1H2BC
HIST1H2BG
HIST1H2BJ
HIST1H4K
HNF4G
HPCA
HPD
HR
HSFX2
HYAL1
IDUA
IGSF11
IKZF1
IKZF3
IL17F
IL3RA
IL7R
IQUB
IRAK3
KCNH3
KCNIP2
KCP
KIAA0825
KIAA1324
KIAA1683
KIAA2022
KIF25
KLHDC1
KLHL30
KLK4
KREMEN2
LACC1
LAIR1
LANCL3
LCT
LDHC
LEKR1,RP11-6F2.7
LIMS3L
LMOD3
LPAR6
LPP
LRP1B
LRRC2
LRRC28
LRRC39
LRRN4CL
LY6G6C
LY6K
LYNX1
LYPD5
LZTS1
MAB21L1
MACROD1
MAPK15
MASP2
METTL20
MFNG
MGMT
MMP10
MOBP
MS4A4E
MUC3A
MYBPHL
MYLK4
NAP1L6
NAV3
NBPF15
NECAB2
NEK10
NFAM1
NLRP11
NMNAT3
NPM1P21
NSUN7
NWD1
OLIG1
OPRL1
PABPC3
PARP1
PCDH11Y
PCSK2
PDE4C
PDZD2
PEX5L
PFKFB4
PGBD2
PI16
PKD1L2
PLA2G4A
PLAC8L1
PLEKHA2
PLGLA
PLXNB3,SRPK3
PNMA6B
PNMA6D
PNPLA7
POU2F2
POU3F1
PPM1N
PPP1R3F
PRDM16
PROC
PRRX1
PSG4
PSMB9
PSORS1C1
RAB33A
RAB40A
RAB6C
RAD9B
RASAL1
RASIP1
RGS17
RGSL1
RNF224,SLC34A3
ROM1
RP11-108K14.4
RP11-357H14.4
RP11-382J12.1
RP11-383H13.1
RP11-45B20.3
RP11-631M21.2
RP11-683L23.1
RP11-849H4.2
RPL3L
RPS6KA6
RRAD
RRH
RSPH9
RSPO1
S100A1
S100A16
S100A2
SAMD12
SCARA5
SCG5
SCNN1A
SCNN1G
SECTM1
SEMA4A
SEPT12
SEPT14
SERPINE3
SERPINF1
SERPINI2,WDR49
SHC4
SHISA7
SHQ1
SLC15A1
SLC15A2
SLC16A12
SLC16A3
SLC16A7
SLC17A8
SLC1A2
SLC22A1
SLC25A34
SLC26A1
SLC2A9
SLC44A5
SLC4A8
SLC6A16
SLITRK3
SNTB2
SORCS1
SPDEF
SPEF1
SPG20OS
SPRY3
SRCRB4D
SRGAP2P1
SSTR2
STAP2
STC1
STK31
STK32B
STXBP5L
SYT12
SYT13
SYT6
T
TCP11L2
TDRD6
TG
TMC4
TMEM107
TMEM132B
TMEM146
TMPRSS9
TNS4
TPH2,TRHDE
TRIM10
TSPAN2
TTC23L
UBA7
UCP1
UGT3A1
UPK1A
VAMP1
VPREB3
VSIG10L
VTN
WFIKKN1
WNK3
YPEL4
ZC3H12B
ZC3H12D
ZNF560
ZNF717
ZNF807

Supplementary Table 2: RNAs of non-coding genes and unnamed genes recruited to RISC by mir-489. HEK293 cells were co-transfected with Flag-Ago-2 and mir-489 mimic (489 group) or negative control RNA oligos (NC group). The lysates were subjected to immunoprecipitation with anti-Flag-Ago-2. The RNAs in the immunoprecipitates were extracted for RNA-seq analysis. mir-489 mimic induced significant (>2 fold) increases in 905 RNAs associated with Ago-2 RISC, including 387 RNAs of non-coding genes and unnamed genes listed in this table.

AB019441.29
AC004074.3
AC004878.5
AC005000.1,RP1-241P17.4
AC005017.1
AC005224.2
AC005251.3
AC005329.6
AC005488.13
AC005534.8
AC005550.6
AC005696.1
AC005822.1
AC005840.1
AC005971.3
AC006050.3
AC007000.12
AC007255.8
AC007621.1
AC008103.5,AC008132.13
AC008132.14,GGT3P
AC009061.1
AC009108.1
AC009487.6
AC010096.1
AC011357.1
AC011747.7
AC012358.4
AC012531.17
AC017076.4
AC018738.2
AC019322.2
AC021224.1
AC022210.2
AC022431.1
AC022916.1
AC023490.1,AC023490.2
AC024937.4
AC024937.6
AC026150.6
AC026271.5
AC027612.1
AC034105.1
AC044860.1
AC046143.3
AC069292.6
AC073135.1
AC073135.2
AC074289.1
AC079250.1
AC079776.7
AC092155.1
AC092171.2,AC093620.5
AC092651.1
AC093106.7
AC093901.1
AC097523.1
AC100793.1,HMGN2P42
AC103702.1
AC104233.1
AC104843.3
AC107977.3
AC114737.6
AC114755.7
AC116165.1
AC120114.2
AC126365.7
AC129778.3
AC131280.1,GOLGA8DP
AC134878.1
AC135995.1,RP11-152F13.3
AC139452.2
AD000091.1
AL049757.3
AL109755.1
AL121936.3
AL162731.2
AL163052.1
AL390877.1
AL391994.1
AL445670.1
AL671277.1
AL671710.1
AL672183.2
AL772307.1
ANKRD20A7P
ANKRD20A8P
AP000253.1
AP000330.8
AP000487.5
AP000525.8
AP006216.11
AP006621.8
ASMTL-AS1
BMPR1APS2
BX004987.3,BX004987.5
BX088645.3,RP11-133G22.1
BX255925.2
BX571672.2
C21orf34
C6orf112
C6orf26-AS1,C6orf27
C8orf77
CELP
CICP5
CICP7
CSNK2A1P
CTA-211A9.5,MIAT
CTA-384D8.20
CTA-984G1.5
CTAGE7P
CTB-12O2.1
CTC-205M6.2
CTC-338M12.7
CTC-426B10.1
CTD-2035E11.3
CTD-2062F14.3
CTD-2192J16.15
CTD-2194L12.1
CTD-2287O16.1
CTD-2518G19.1
CTD-2555K7.2
DGKZP1
DPY19L2P4
DSTNP1
EAF1-AS1
EEF1A1P11
EEF1A1P24
EEF1A1P33
EEF1A1P8
EEF1B2P3
EIF3FP3
EIF3LP2
EMX2OS
ESPNP,RP1-163M9.6
FAM108A3P
FAM108A5P
FAM108A7P
FAM115D
FAM90A17
FAM90A25P
FTH1P10
FTH1P12
FTH1P2
GOLGA8E
GS1-184P14.2
HCG25
HNRNPA1P12
HNRNPA1P8
HNRNPA3P1
HNRNPKP2
HS6ST1P1
HSPB1P2
IMPDH1P6
ISCA1P1
KB-1269D1.8
KRT18P34
KRT8P3
LL22NC03-2H8.4
LPAL2
MEMO1P1
MIR22
MIR221
MIR302B,RP11-148B6.1
MTND1P23
NAV2-AS2
NCRNA00087
NCRNA00119,RP11-39E4.1
NCRNA00158
NCRNA00202
NCRNA00246A
NCRNA00306
NKX2-1-AS1
OR7E125P
PARP4P2
PGM5P1
PGM5P2
PHKA1P1
PMS2P1
PPP1R2P3
PROSP
PSMC1P5
RALGAPA1P
RANP1
RNase_MRP
RNPS1P1
RNU1-1
RNU1-3
RNU1-5
RNU1-6
RP1-102E24.6
RP11-106M3.1
RP11-109L13.1
RP11-112J1.1
RP11-1134I14.8
RP11-1148L6.5
RP11-118B22.2
RP11-119H12.2
RP11-121L10.3
RP11-1236K1.8
RP11-123K19.1
RP11-124N14.4
RP11-133M24.1
RP11-134G8.5,RP11-134G8.6
RP11-135F9.1
RP11-14C22.4
RP11-14N7.2
RP11-15J10.4
RP11-169K16.7
RP11-16F15.1
RP11-175B9.3
RP11-177B4.1
RP11-186N15.3
RP11-18H7.1
RP11-190J1.7
RP11-192I3.2
RP11-204J18.2,RP11-204J18.3
RP11-213G2.1
RP11-215A21.2
RP11-229P13.2
RP1-122K4.3
RP11-241F15.2
RP11-248E9.6
RP11-255B23.1
RP11-258F22.1
RP11-25K19.1
RP11-261C10.5
RP11-263K19.4
RP11-266F12.1
RP11-278J6.1
RP11-280F2.2
RP11-296A18.3
RP11-305M3.2
RP11-308D13.2
RP11-309L24.6
RP11-313D6.3
RP11-318M2.2
RP11-319G6.1
RP11-31F19.1
RP11-328M4.2
RP11-343H5.4
RP11-343L14.2
RP11-348F1.2
RP11-349N19.2
RP11-350E12.4
RP11-354M20.3
RP11-363N22.2
RP11-364L4.1
RP11-374M1.2
RP11-381O7.5
RP11-384J4.2
RP11-38O14.1
RP11-392P7.1
RP11-393B14.1,SNORA63
RP11-393H6.2
RP11-397E7.3
RP11-398A8.3
RP11-398K22.3
RP11-399D6.2
RP11-3B12.1
RP11-401F24.4
RP11-404P21.2
RP11-410L14.2
RP11-426A6.8
RP11-429J17.2
RP11-434C1.1
RP11-436H11.1
RP11-439L18.3
RP11-44M6.3
RP11-44N21.2
RP11-460E7.5
RP11-460H18.1
RP11-460N11.3
RP11-46F15.2
RP11-471J12.1
RP11-475I24.4
RP11-480D4.3
RP11-481K16.2
RP11-486O13.4
RP11-488C13.6
RP11-490G8.1
RP11-493P1.2
RP11-495P10.1
RP11-495P10.7,RP11-495P10.8
RP11-497H16.8
RP1-14N1.2
RP11-506M13.3
RP11-512M8.3
RP11-513G11.1
RP11-51O6.1
RP11-543P15.1
RP11-553P9.1
RP11-567I13.1
RP11-576D8.2
RP11-578F5.1
RP11-579D7.4
RP11-582J16.3
RP11-600F24.1
RP11-603B24.2
RP11-624L12.1
RP11-627K11.1
RP11-632K5.5
RP11-641D5.1
RP11-666A1.1
RP11-668N23.1
RP11-673E1.3
RP11-680G24.3
RP11-687M24.3
RP11-702A23.1
RP11-713P17.3
RP11-721G13.1
RP11-761N21.2
RP11-76N22.2
RP11-785H5.1
RP11-791G16.4
RP11-807H7.1
RP11-809C9.1
RP11-80H5.7
RP11-834C11.5
RP11-84A19.2
RP11-854K16.1
RP11-875O11.1
RP11-87H9.2
RP11-88H10.1
RP11-904M10.1
RP11-92K2.2
RP11-93K22.13
RP11-94I2.4
RP11-950C14.3
RP1-203P18.1
RP1-238O23.5
RP1-283E3.4,RP1-283E3.8
RP13-178D16.2
RP13-33H18.1
RP13-36G14.2
RP13-507I23.1
RP13-507I23.2
RP1-93H18.3
RP1-99E18.2
RP3-334F4.1
RP3-342P20.2
RP3-375P9.2
RP3-406P24.3
RP3-417G15.1
RP3-423B22.5
RP3-449M8.6
RP3-510D11.1,RP3-510D11.2
RP4-560B9.1
RP4-560B9.2
RP4-592A1.2
RP4-594I10.2
RP4-655C5.1
RP4-756H11.3
RP4-775C13.1
RP5-1024G6.5
RP5-1071N3.1
RP5-1073O3.7
RP5-1074L1.1
RP5-983L19.2
RP6-74O6.3
RPL13AP25
RPL23AP53
RPL35P5
RPL3P9
RPL7AP11
RPS2P4
RPS2P7
RPS4XP16
RPS7P1
RPS7P11
RPS9P1
RUNDC2B
SNORA11
SNORA31
SNORD60
SOX21-AS1
SUMO2P1
TMEM185AL
TP53TG1
TPM3P6
U52111.14
UPF3AP1
XXbac-BPG55C20.1
YBX1P10
YWHAZP5
ZC3H11B
ZNF890P

Supplementary Table 3: Functional analysis of mir-489 target genes. The 127 genes identified as mir-489 targets by RISC immunoprecipitation/RNA-seq and bioinformatics were analyzed by The Database for Annotation, Visualization and Integrated Discovery (DAVID) for functional classification.

STRESS RESPONSE	CELL COMMUNICATION	MULTICELLULAR ORGANISMAL DEVELOPMENT	CELL DIFFERENTION	CELL JUNCTION	TRANSPORT
HDAC9	SLC1A2	HDAC9	HDAC9	SHC4	PEX5L
PSG4	HFE	TRIM10	TRIM10	PDZD2	AKAP6
ATP7A	SSTR2	CELF4	CELF4	SNTB2	ATP7A
NFAM1	BMP3	MOBP	ATP7A	ARHGAP24	ANO4
SLC1A2	GRIN2A	ATP7A	NFAM1	GABRP	CLIC2
HFE	GABBR2	NFAM1	HHIP	GRIN2A	SLC1A2
SCARA5	STC1	SLC1A2	TSPAN2	GABBR2	HFE
DNAJC3	BDNF	CRABP1	EMX	GAD2	CRABP1
SCNN1G	CXCL10	SPRY3	ARHGAP24	LPP	SCARA5
MASP2	GAD2	IKZF3	BMP3	DSG3	SLC25A34
GRIN2A	TRHDE	HHIP	GRIN2A	GLRA2	SLC16A7
PARP1		TSPAN2	BMP8A	SLC17A8	SLC15A2
GRHL3		EMX1	PRDM16		VAMP1
PROC		PRRX1	CRX		SCNN1G
CXCL2		ARHGAP24	BDNF		SCG5
BDNF		BMP3	ELF3		GABRP
ELF3		GRIN2A	CHRDL1		GRIN2A
CXCL10		GRHL3	CYLC2		SLC15A1
		BMP8A	IKZF1		SLC2A9
		PRDM16			SLC22A1
		AQP4			STXBP5L
		STC1			SLC16A12
		CRX			BDNF
		BDNF			SLC4A8
		ELF3			CXCL10
		CXCL10			GLRA2
		PCSK2			SLC17A8
		CHRDL1			
		GLRA2			
		CYCL2			
		IKZF1			

Supplementary Figure 1 renal ischemia/reperfusion injury in PT-HIF-1 (+/+) and PT-HIF-1(-/-) mice. PT-HIF-1(+/+) and PT-HIF-1(-/-) mice were subjected to 30 minutes of bilateral kidney ischemia followed by 12 hours or 48 hours of reperfusion. Sham-operated mice were used for normal control. (A) Blood urea nitrogen. (B) Serum creatinine. 3-9 mice were used in each of the conditions.