

Assessment of circulating extracellular vesicles from calorie restricted mice and humans in ischaemic injury models

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Supplemental Materials

Supplemental Table 1: List of qPCR primers used for macrophage polarisation analysis

Supplemental Table 2: Highest expressed miRNAs detected in human serum EV samples

Supplemental Table 3: Increased/decreased miRNAs in human serum EV samples following water-only fasting

Supplemental Table 4: Predicted gene targets of differentially expressed miRNAs

Supplemental Figure 1: SDS-PAGE of serum proteins

Supplemental Figure 2: SDS-PAGE of EV proteins

Supplemental Figure 3: Kidney cell (HK-2, NRK-52E) line hypoxia and toxic injury models

Supplemental Figure 4: miRNA quality control data

Gene	ID	Species	Forward primer	Reverse primer
<i>Gapdh</i>	14433	mouse	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCCAG
<i>Il10</i>	16153	mouse	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
<i>Il1b</i>	16176	mouse	TTCAGGCAGGCAGTATCACTC	GAAGGTCCACGGGAAAGACAC
<i>Nlrp3</i>	216799	mouse	ATTACCCGCCCGAGAAAGG	CATGAGTGTGGCTAGATCCAAG
<i>Il6</i>	16193	mouse	TCTATACCACTTCACAAGTCGGA	GAATTGCCATTGCACAACCTCTTT
<i>Tnfa</i>	21926	mouse	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
<i>Arg1</i>	11846	mouse	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
<i>Irf4</i>	16364	mouse	CTTTGAGGAATTGGTCGAGAGG	GAGAGCCATAAGGTGCTGTCA
<i>Nos2</i>	18126	mouse	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTTCGATGTCAC
<i>Retnla</i>	57262	mouse	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA

Supplemental Table S1. Sequences of primers used for assessment of mouse macrophage polarisation.

miRNA ID	CT (average)
hsa-miR-451a	21.87066083
hsa-miR-223-3p	22.85588139
hsa-miR-16-5p	23.29236142
hsa-miR-21-5p	25.06708715
hsa-miR-19b-3p	25.08065023
hsa-miR-92a-3p	25.13799492
hsa-miR-142-3p	25.2191205
hsa-miR-23a-3p	25.2985377
hsa-miR-19a-3p	25.36102776
hsa-miR-486-5p	25.89541101
hsa-miR-126-3p	26.00600847
hsa-miR-24-3p	26.2453557
hsa-miR-20a-5p	26.30825085
hsa-miR-26a-5p	26.62005947
hsa-miR-25-3p	26.76693549
hsa-miR-27a-3p	26.88601213
hsa-miR-15b-5p	26.91025456
hsa-miR-103a-3p	27.12654937
hsa-let-7a-5p	27.13418923
hsa-miR-15a-5p	27.13717905

Supplemental Table S2. List of top 20 highest expressed miRNAs across serum EV samples, sorted from highest to lowest expressed. miRNAs are shown if they were detected in all samples.

Up/down-regulated miRNAs

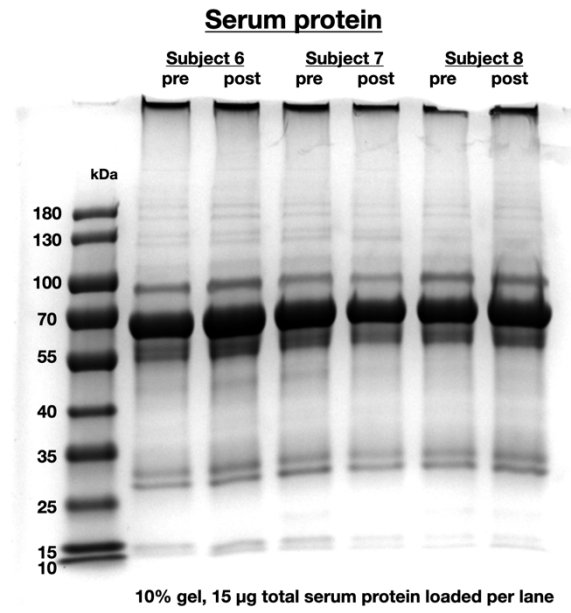
miRNA ID	log2fc	<i>p</i> value	Avg. CT
hsa-miR-622	8.68	0.003283	27.90
hsa-miR-487b-3p	2.99	0.032831	31.57
hsa-miR-378a-3p	2.29	0.043125	29.19
miRNA ID	Fold	<i>p</i> value	Avg. CT
hsa-miR-760	-6.64	0.002342	29.80
hsa-miR-98-5p	-3.05	0.002332	31.54
hsa-miR-31-5p	-2.32	0.012526	34.02
hsa-miR-744-3p	-1.09	0.037690	30.22
hsa-miR-485-3p	-1.29	0.048495	33.00

Supplemental Table S3. List of differentially-expressed miRNAs between post- and pre-fasting EV samples. The average cycle threshold (Avg. CT), significance (p-value) and log₂-fold change (log₂fc) are shown.

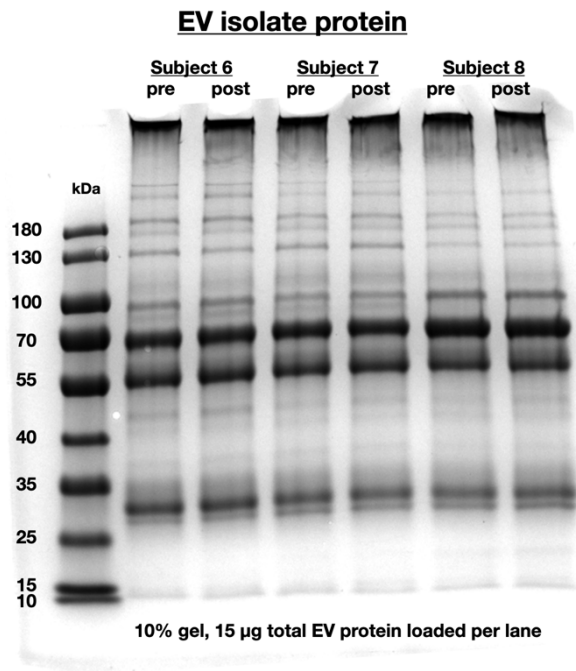
Predicted genes regulated by decreased miRNAs			
Gene Symbol	# of miRNA Targeting this	Total # of Target Sites Identified in	Range of Strength Scores
GRIN2D	1	3	-0.12 -- -0.07
MNT	2	2	-0.18 -- -0.14
MPHOSPH8	2	2	-0.12 -- -0.07
NUFIP2	2	2	-0.10 -- -0.10
PAPOLA	2	2	-0.32 -- -0.23
SPOPL	2	2	-0.22 -- -0.16
TLK2	2	2	-0.25 -- -0.21

Predicted genes regulated by increased miRNAs			
Gene Symbol	# of miRNA Targeting this	Total # of Target Sites Identified in	Range of Strength Scores
IGF2BP1	2	6	-0.25 -- -0.01
HMG2A	1	6	-0.38 -- -0.10
ARID3B	1	5	-0.24 -- -0.04
PURB	3	4	-0.29 -- -0.01
CLCN5	2	4	-0.29 -- -0.03
LIN28B	1	4	-0.43 -- -0.14
ANKFY1	3	3	-0.12 -- -0.00
DAGLA	3	3	-0.48 -- -0.20
GATAD2B	3	3	-0.08 -- 0.03
PHC3	3	3	-0.39 -- -0.09
SNN	3	3	-0.31 -- -0.09
SNX1	3	3	-0.16 -- -0.05
STX3	3	3	-0.41 -- -0.13

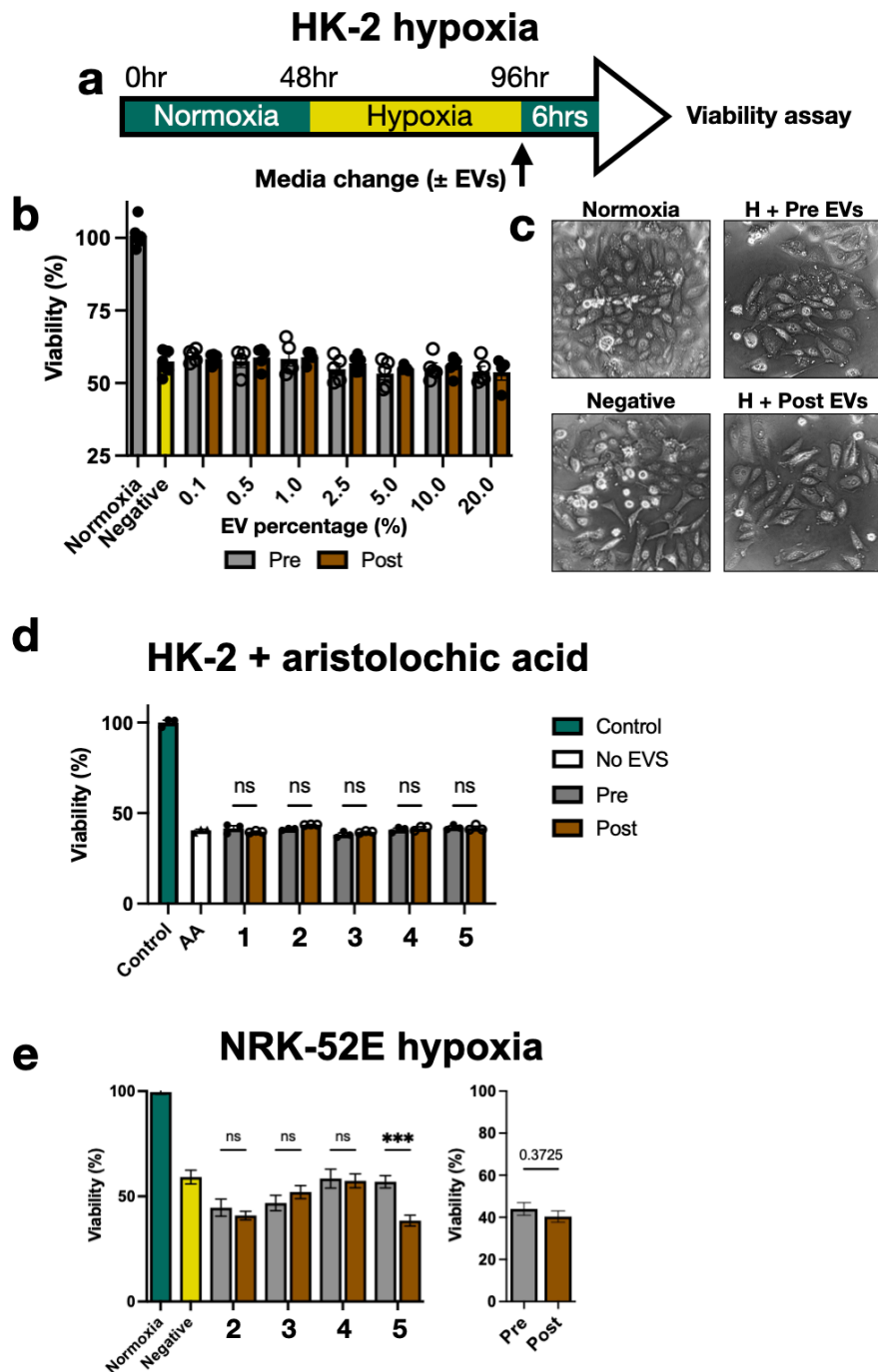
Supplemental Table S4. Predicted gene targets of differentially-expressed miRNAs. The table on the left shows predicted genes targeted by decreased (post-fasting vs. pre-fasting) miRNAs, and the table on the right shows genes targeted by increased miRNAs. Data were provided by Qiagen Geneglobe online analysis tool.



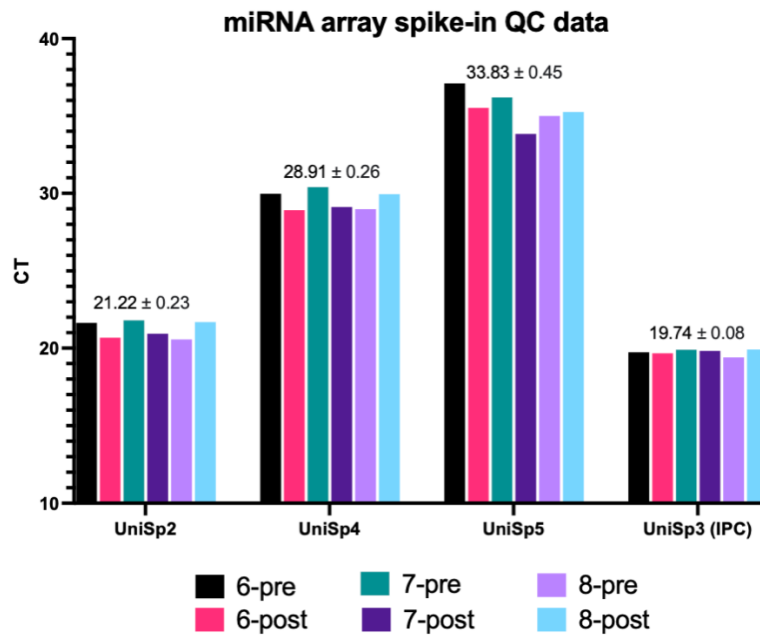
Supplemental Figure S1. SDS-PAGE gel showing serum protein extracts run on a 10 % polyacrylamide gel. Each lane was loaded with 15 μ g total protein.



Supplemental Figure S2. SDS-PAGE gel showing EV protein extracts run on a 10 % polyacrylamide gel. Each lane was loaded with 15 µg total protein.



Supplemental Figure 3. **a.** Timeline of HK-2 hypoxia experiments. **b.** Cell viability results. **c.** cell morphology. **d.** Cell viability in toxic injury model using 30 μ g/ml aristolochic acid. **e.** Cell viability in NRK-52E hypoxia model. Nested *t*-test of all samples is shown on the right with the P value indicated. ns = not significant



Supplemental Figure 4. Cycle threshold (CT) values for spike-in UniSp2, UniSp4 and UniSp5 across six samples. These spike-ins represent high, medium and low expressed miRNAs respectively. The mean inter-plate calibration (UniSp3 IPC) samples are also shown. Numbers above the graph indicate mean CT values.