**Supplementary data (Materials and Methods)**

# piRNAs in the human retina and retinal pigment epithelium reveal a potential role in intracellular trafficking and oxidative stress

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## Materials and Methods

### 1. Tissue enrichment analysis

The piRNAs annotated in the brain,[15] cardiac progenitors and fibroblasts,[51] ovary,[52] and testis[53] were retrieved from the repetitive sources. To find the common piRNAs between different tissues, the piRNAs expressed in RPE and retina were compared against those expressed in the brain, ovary, testis, and cardiac cells. For piRNAs in cardiac cells, the piRNAs listed in cardiospheres, cardiosphere-derived cells, and cardiac fibroblasts were combined, and the redundant entries were eliminated. A Venn web tool was used to find the common piRNAs expressed in all the tissues.

### 2. Western Blot

After transfection, the cells were lysed using Radio Immunoprecipitation Assay (RIPA) buffer (150 mM NaCl, 0.1 %TritonX-100, 0.5 % sodium deoxycholate, 0.1%SDS, 50 mM Tris, pH 8.0) with protease inhibitors (1 mmol/L dithiothreitol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 1 mg/mL leupeptin, 10 mmol/L p-nitrophenylphosphate, 10mmol/L h-glycerol phosphate) and were sonicated. The lysates were then centrifuged at 10, 000 rpm for 5 min and the proteins in the supernatant were estimated by BCA protein assay reagent (Thermo Scientific, Waltham, USA). 35 μg of protein was resolved using SDS-PAGE gel and was electrotransferred to nitrocellulose membrane (GE healthcare, UK). The blots were then incubated with 5 % blocking solution for 1 h (5 % skimmed milk powder in Tris Buffered Saline) after which they were probed for either HIWI2 (Thermo Scientific, Waltham, USA) or β-actin (Santa Cruz Biotechnology, Dallas, USA) by incubating overnight at 4ºC with 1 in 1,000 dilution of primary

antibody. Imaging of the blots were done using FluorChem C3 (Protein Simple, San Jose, USA) after incubating with the respective secondary antibodies (1 in 10, 000 dilution) for 2 h.

**3. Stem-loop reverse transcription- Real time polymerase chain reaction (RT-qPCR)**

miR182 and piR-hsa-26131 expression in ARPE19 cells was compared by stem-loop quantitative real time PCR (qRT-PCR) in a two-step reaction. Briefly, 100 ng of RNA and 1 µl of 10 pmol/µl stem-loop primer were added and incubated at 80 °C for 5 min and then 50 °C for 5 min following which a master mix containing 2.0 µl 10× RT Buffer, 1.0 µl 10 mM dNTP mix, 1.0 µl 0.1 M DTT, 4.0 µl 25 mM $MgCl_2$, 0.5 µl RNase OUT (40U/µl) and 0.5 µl SuperScript III Reverse Transcriptase (#55066, Invitrogen, 200 U/µl) was added to make a final volume of 20 µl. The cDNA synthesis was carried out at 25 °C for 5 min, followed by 50 °C for 1 h, and finally, 75 °C for 15 min. This was followed by addition of 0.5 µl RNase H (2U/µl) and incubation at 37°C for 20 min.

Stem-loop primers (miR182, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGTGTGAG-3'; piR-hsa-26131, 5'- CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCTCACC-3'; and U6, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAAATATG-3') were designed to specifically reverse transcribe mature miR182, piR-hsa-26131 and U6 snRNA respectively. First-strand cDNA was stored at -20°C until qPCR analysis.

qPCR was then performed on cDNA using SYBR green (#K0221, ThermoFischer) on a ViiA™ 7 Real-Time PCR System (Applied Biosystem) with gene-specific primers: miR182, F, 5'-ACACTCCAGCTGGGTTTGGCAATGGTAG-3'; piR-hsa-26131, F, 5'-ACACTCCAGCTGGGTTTGGCAATGGTAG-3'; U6, F, 5'-

ACACTCCAGCTGGGGTGCTCGCTTCG-3'; and Universal Reverse primer, 5'-TGGTGTCGTGGAGTCGCAATTCAGTTG-3'. The following thermal cycling conditions were used: 50°C for 2 min, 95°C for 10 min, 5 cycles of 95°C for 15 s, 45°C for 30s and 72°C for 15 s followed by 35 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 15 s and melt curve. The cDNA amplicons were run on a 10% native polyacrylamide gel to check for product size.

**4. RNA immunoprecipitation**

ARPE19 cell lysate was prepared in RIPA buffer ((#R0278, Sigma) 150 mM NaCl, 1.0% IGEPAL, CA-360, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris-HCl and freshly added RNase inhibitor (#M03145, BioLabs), protease inhibitor (#5892953001, (cOmplete) Roche Applied Science) and phosphatase inhibitor ( #4906845001, (PhosSTOP) Sigma Aldrich ) cocktail, incubated for 20 min on ice. The lysate was centrifuged at 14000 rpm for 10 min at 4℃ to remove debris. For comparison, $1/10^{th}$ of the protein fraction was aliquoted as Input and total RNA was isolated using TRIzol Reagent (Life Technologies). 5 µg of rabbit polyclonal PIWIL4 antibody (#PA5-31448, Thermo) was added to the remaining protein fraction and kept at 4 °C for 2 hrs on a rotating apparatus followed by overnight incubation with 30 µl of the protein G plus agarose beads (#SC-2002, Santa Cruz) at 4 °C. Next day, beads were washed thrice with 1× RIPA buffer containing 0.2U RNase inhibitor and 1X protease inhibitor, and the enriched RNA was isolated with TRIzol reagent, in accordance with the manufacturer's protocol. 250ng of Input and IP were used for RT-qPCR analysis.