Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary data (figures)

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

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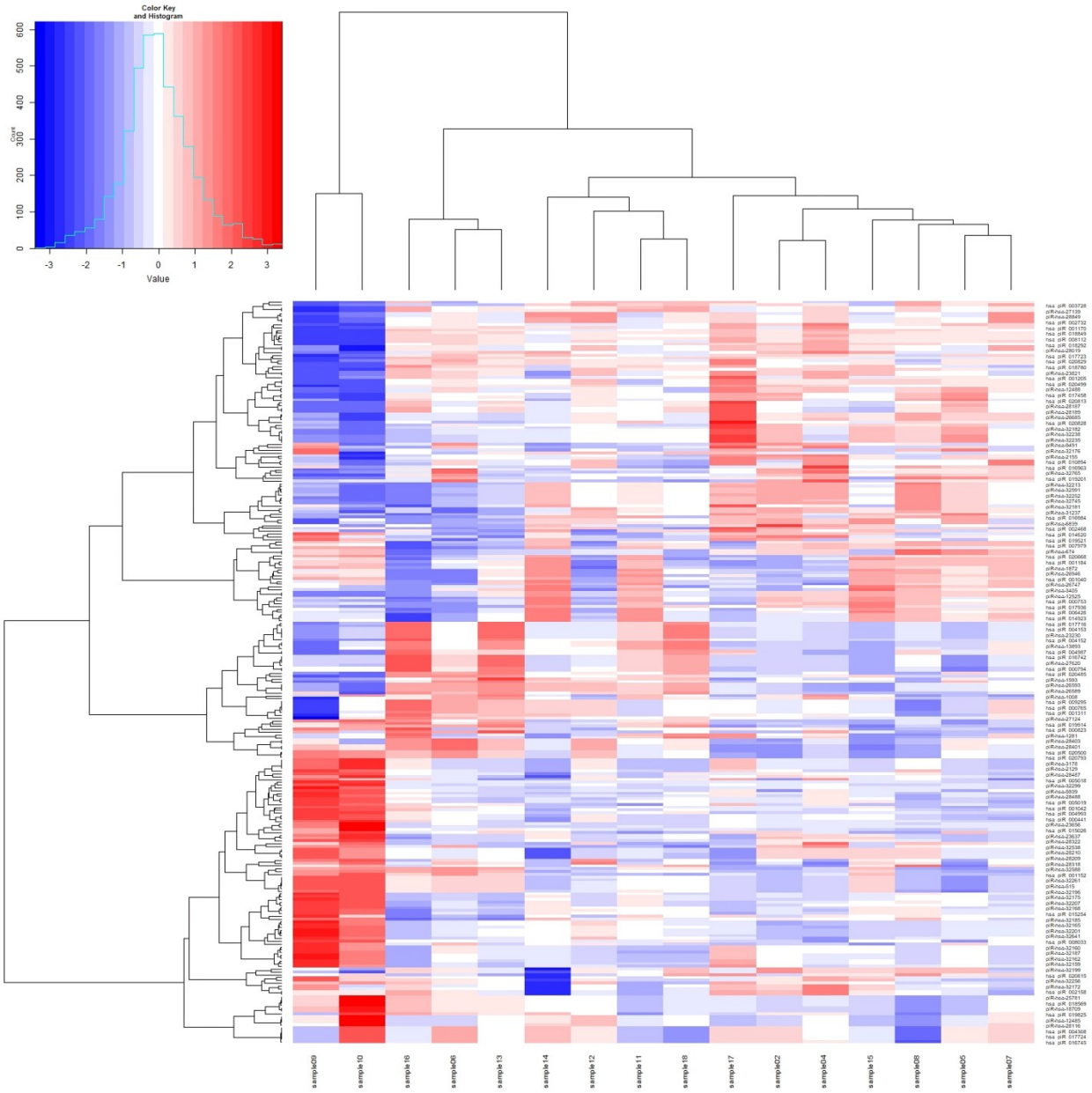
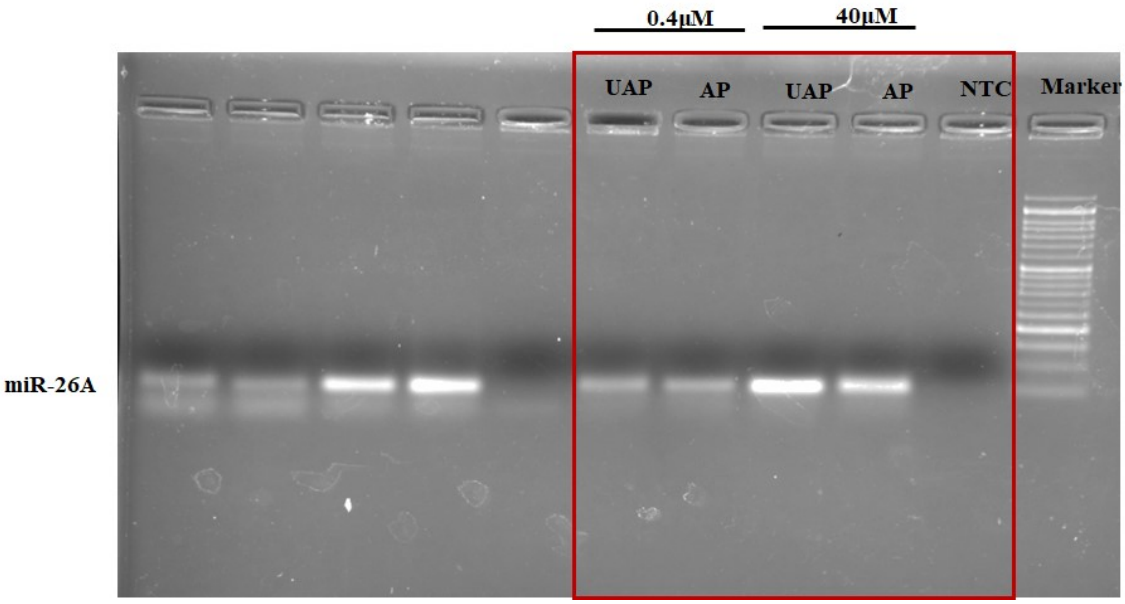
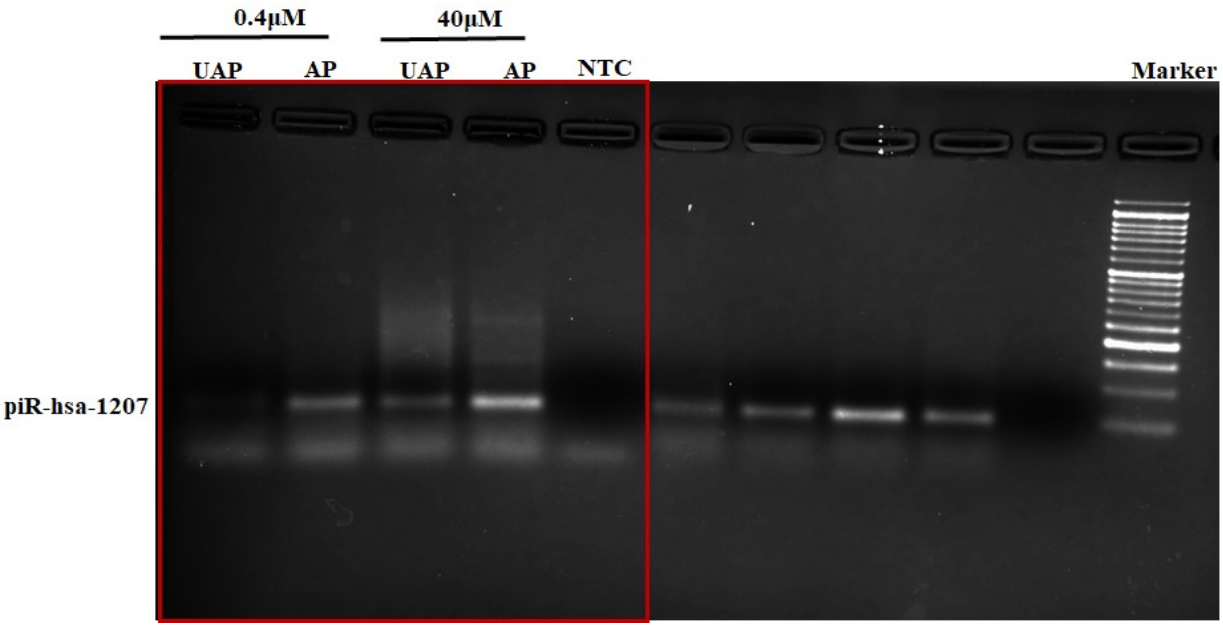


Fig.1 Heatmap based on the expression pattern of piRNAs in retina and RPE across different samples. The Heatmap represents the individual piRNAs in which samples 9 and 10 were of RPE showing a distinct expression pattern compared to the rest of the retinal samples. The red and blue in the Heatmap signify piRNAs' high and low expressions, respectively

A



B



C

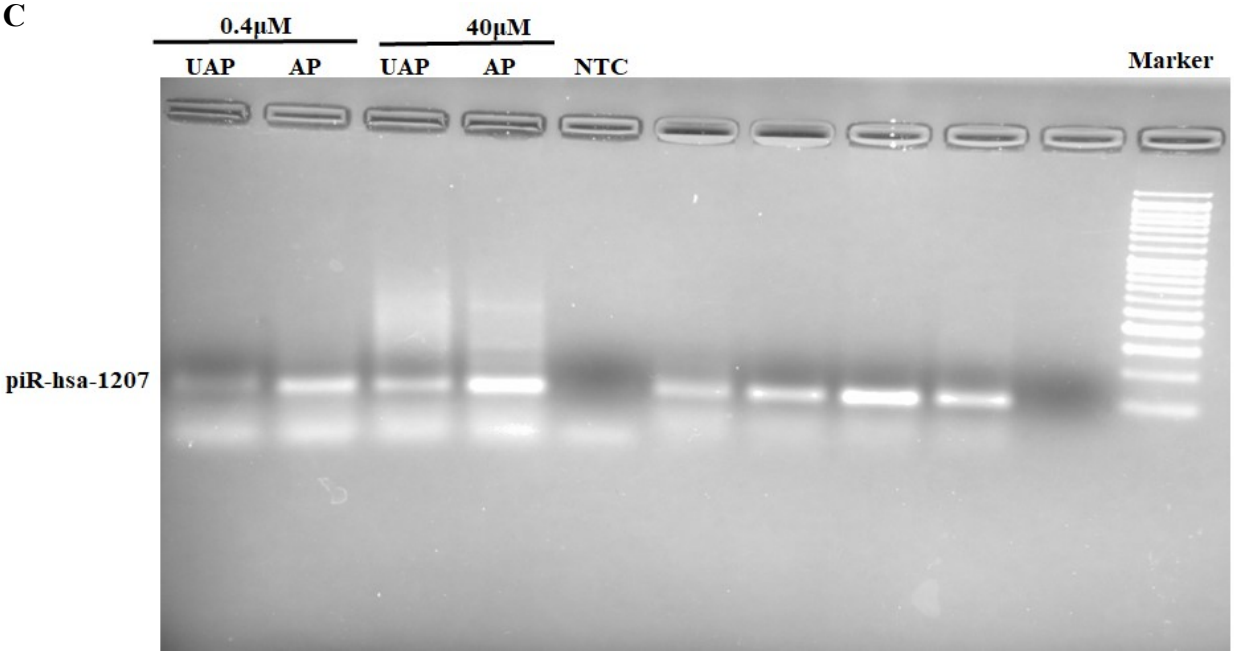


Fig.S2 RTL-P analysis confirms the 3′-terminal 2′-O-methylation of piRNAs. A. Amplification of miR-26A with anchored and unanchored primers, B. Amplification of piRNA with anchored and unanchored primer C. Amplification of piRNA with anchored and unanchored primer at high exposure (RTL-P – Reverse Transcription at Low dNTP concentration followed by PCR; UAP – Unanchored primer; AP – Anchored primer, NTC- no template control), Boxed lanes are kept in the final fig.2B

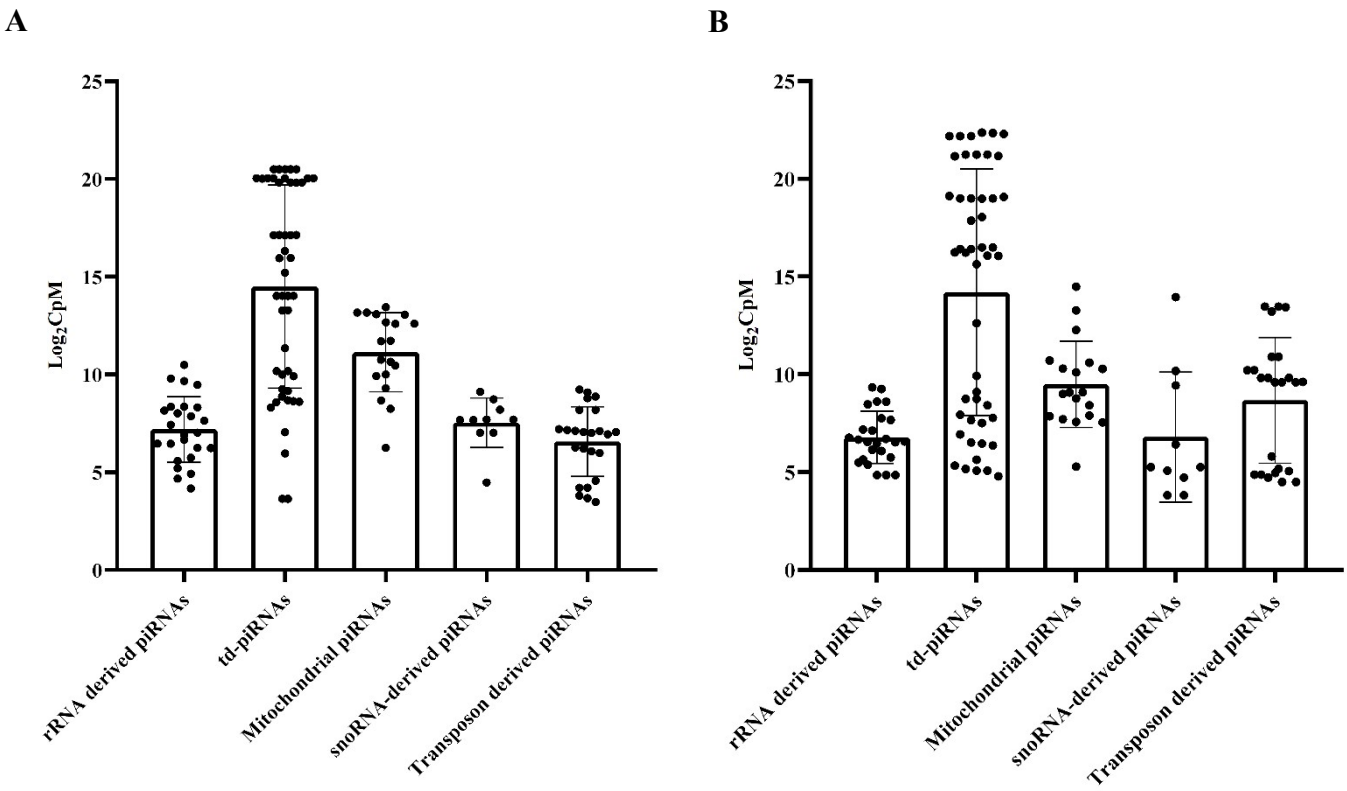


Fig.S3 The replicate data plots represent the distribution in terms of expression of piRNAs derived from repeat elements across human retina and RPE samples. A. Distribution across human retinal samples (n=14) B. Distribution across human RPE samples (n=2).

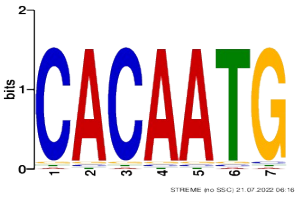
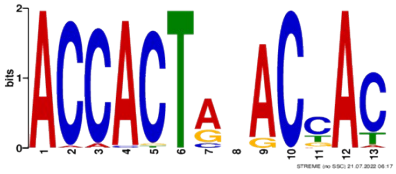
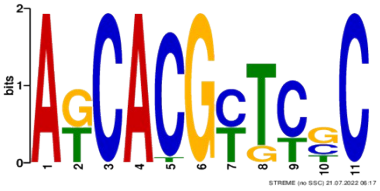
MOTIF	LOGO	E-VALUE	P-VALUE	SITES
1. CACAATG		1.2e-001	3.0e-002	30, 11.5%
2. ACCACTANACCAC		2.4e-001	6.0e-002	62, 23.8%
3. AKCACGYTCSC		2.0e+000	5.0e-001	23, 8.8%
4. CTCATCAKYG		2.0e+000	5.0e-001	20, 7.7%

Fig.S4 Enriched motifs among the piRNAs. - Logos with the corresponding E-values and P-values represent the motifs identified by **STREME**.

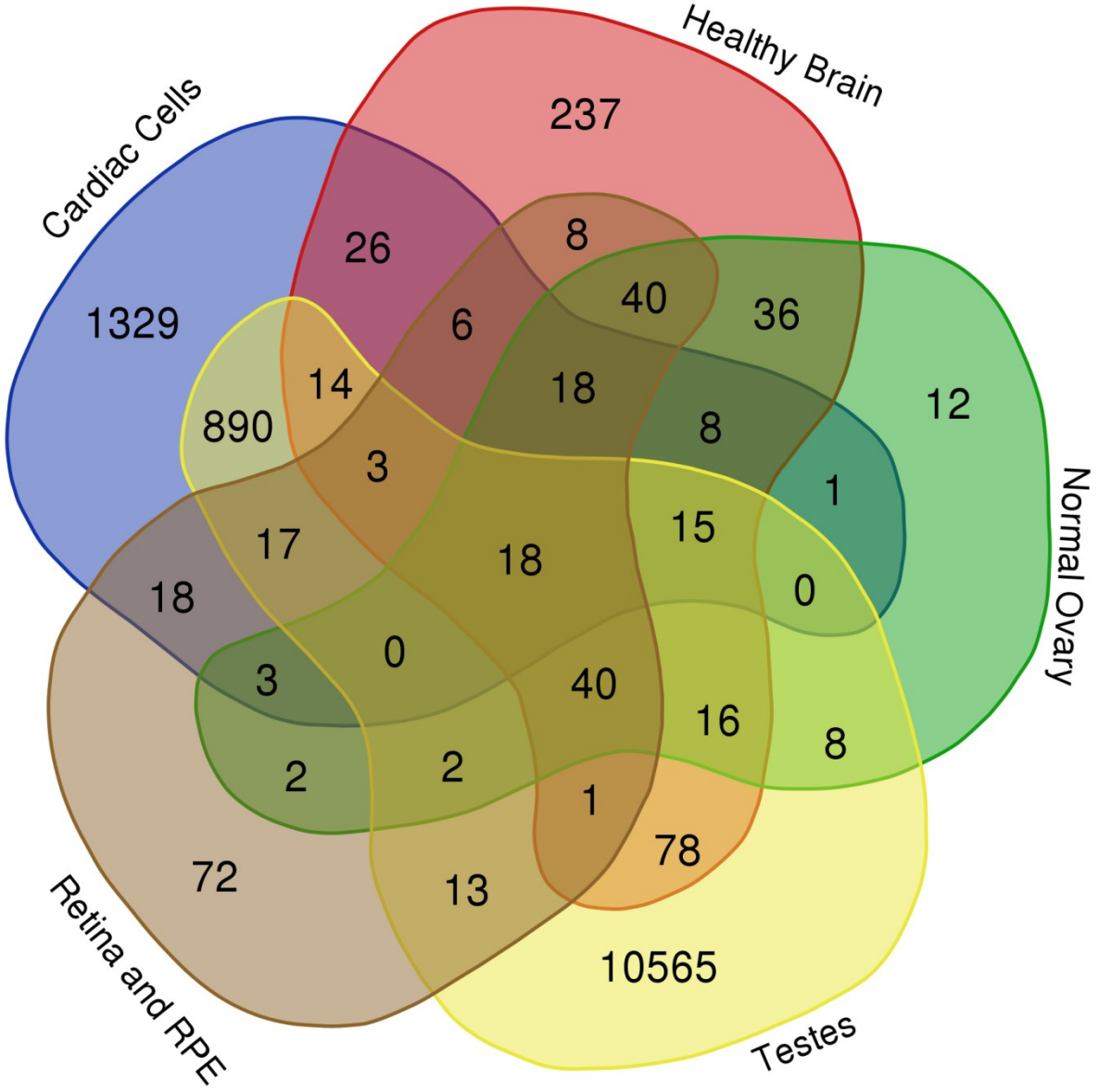


Fig.S5 Venn diagram of commonly expressed piRNAs between human retina and RPE and other tissues such as brain, cardiac cells, ovary and testis

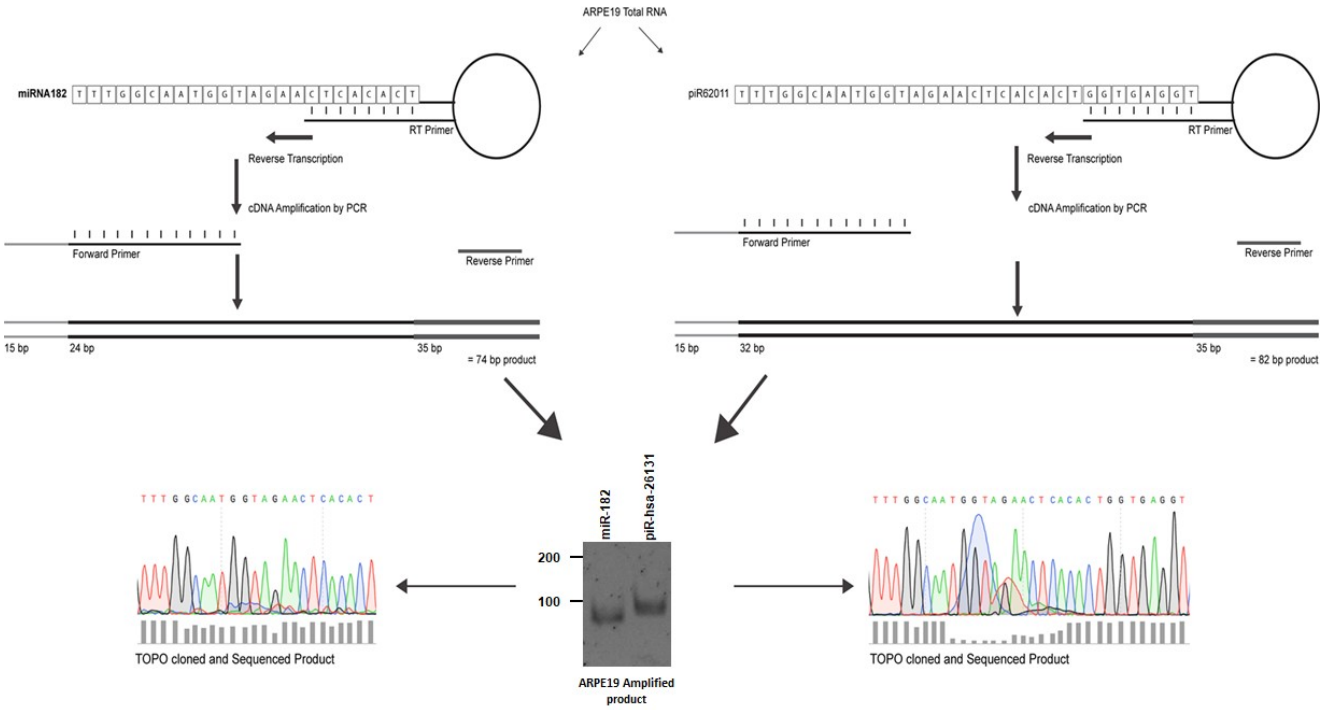


Fig.S6 Amplification of miR-182 and piR-hsa-26131 from ARPE-19 Total cellular RNA.

Total RNA was isolated from ARPE-19 cells using TRIzol extraction and subjected to reverse transcription using specific primers for either miR-182 or piR-hsa-26131. This was followed by cDNA amplification using the same forward and reverse primers. The amplified product was verified by running on a 12% polyacrylamide gel and by TOPO-cloning plus Sanger sequencing.

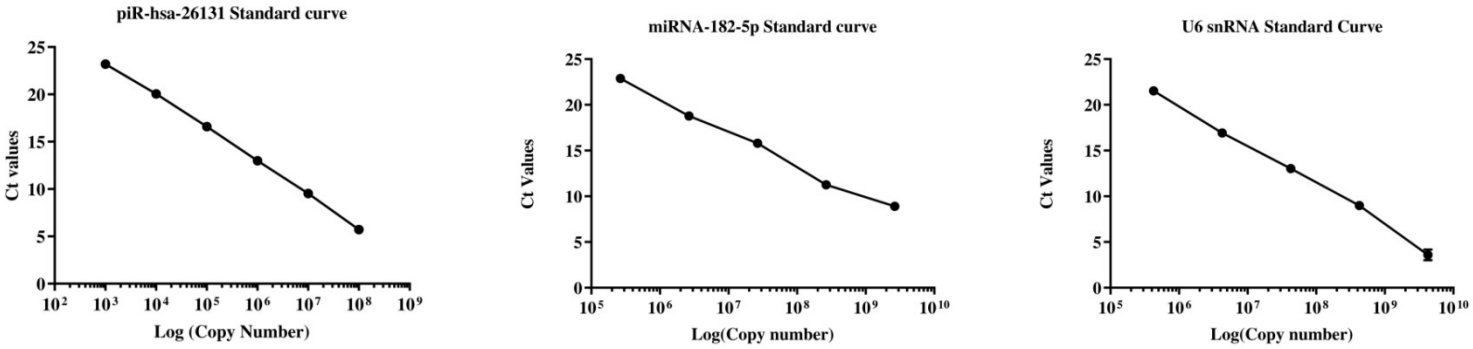


Fig.S7 Standard Curves for piR-hsa-26131, miR-182 and U6. TOPO cloned plasmids from the PCR products were used to draw standard curves for the different small RNAs amplified. These were then used to extrapolate copy numbers from Ct values for ARPE-19 cells.

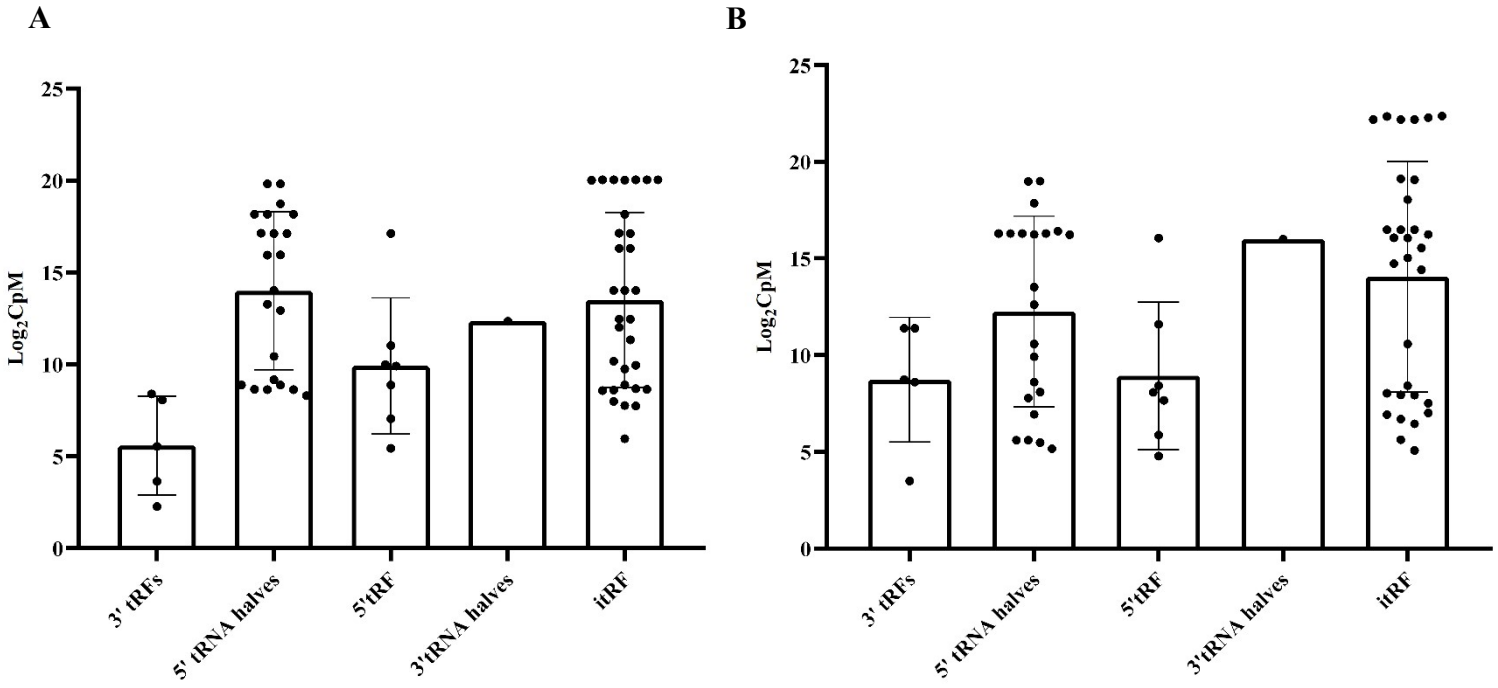


Fig.S8 The replicate data plots represent the distribution in terms of expression of piRNAs derived from tRNAs across human retina and RPE samples. A. Distribution across human retinal samples (n=14) B. Distribution across human RPE samples (n=2).

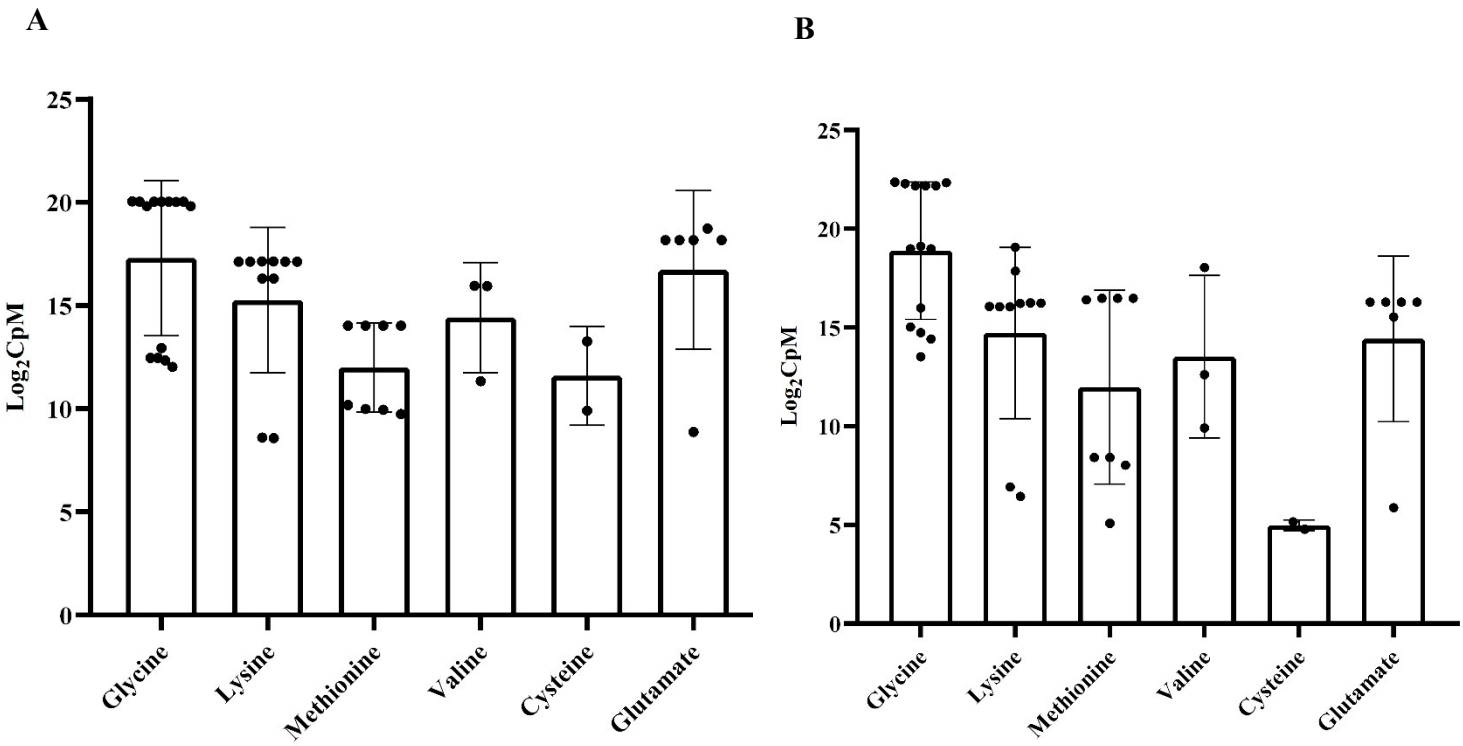


Fig.S9 The replicate data plots represent the distribution in terms of expression of td-piRNAs with respect to their amino acid counterpart containing tRNAs across human retina and RPE samples. A. Distribution across human retinal samples (n=14) B. Distribution across human RPE samples (n=2).

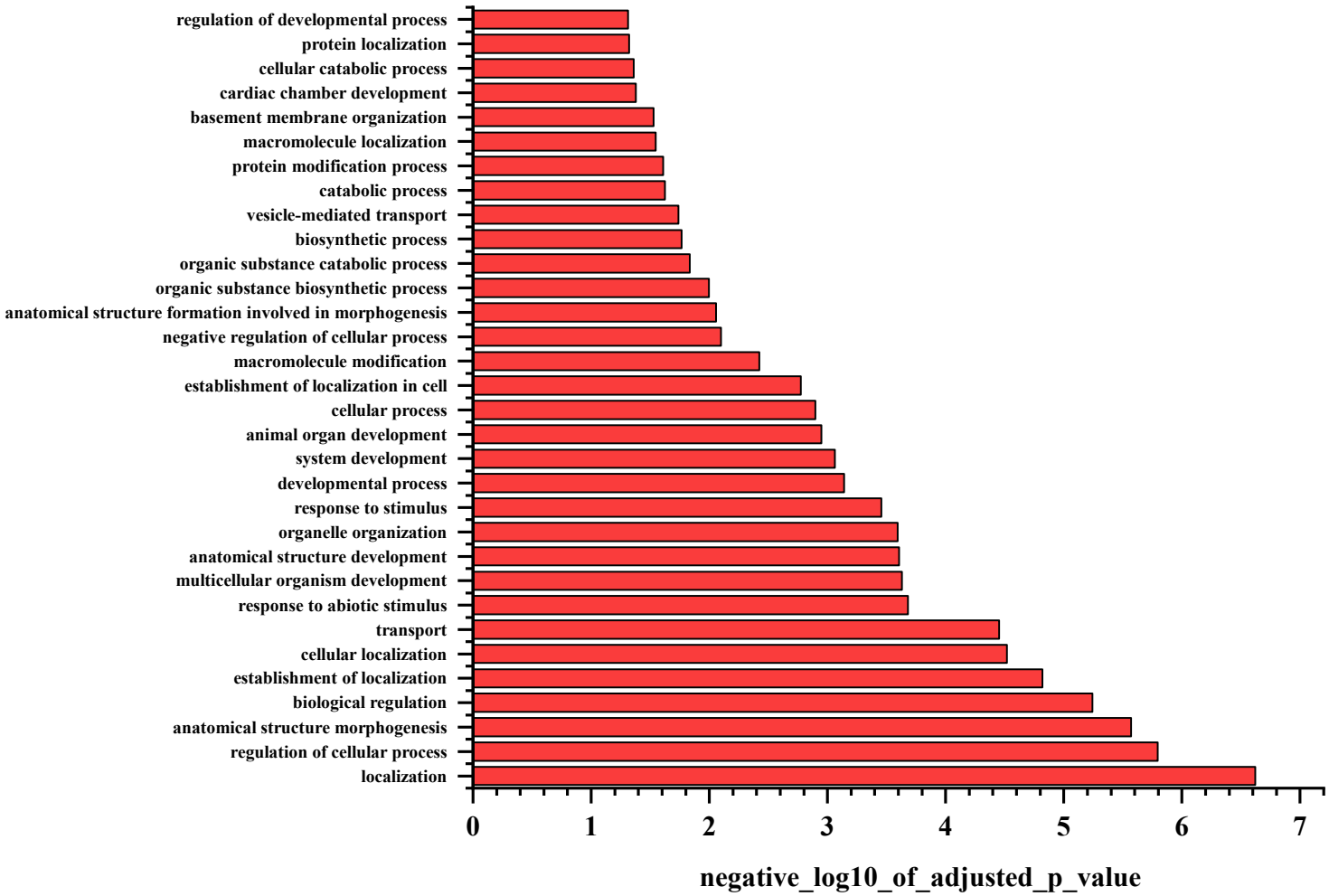


Fig.S10 Functional enrichment analysis of predicted target genes using g: profiler representing the GO terms with statistically significant negative log10 of adjusted p-values

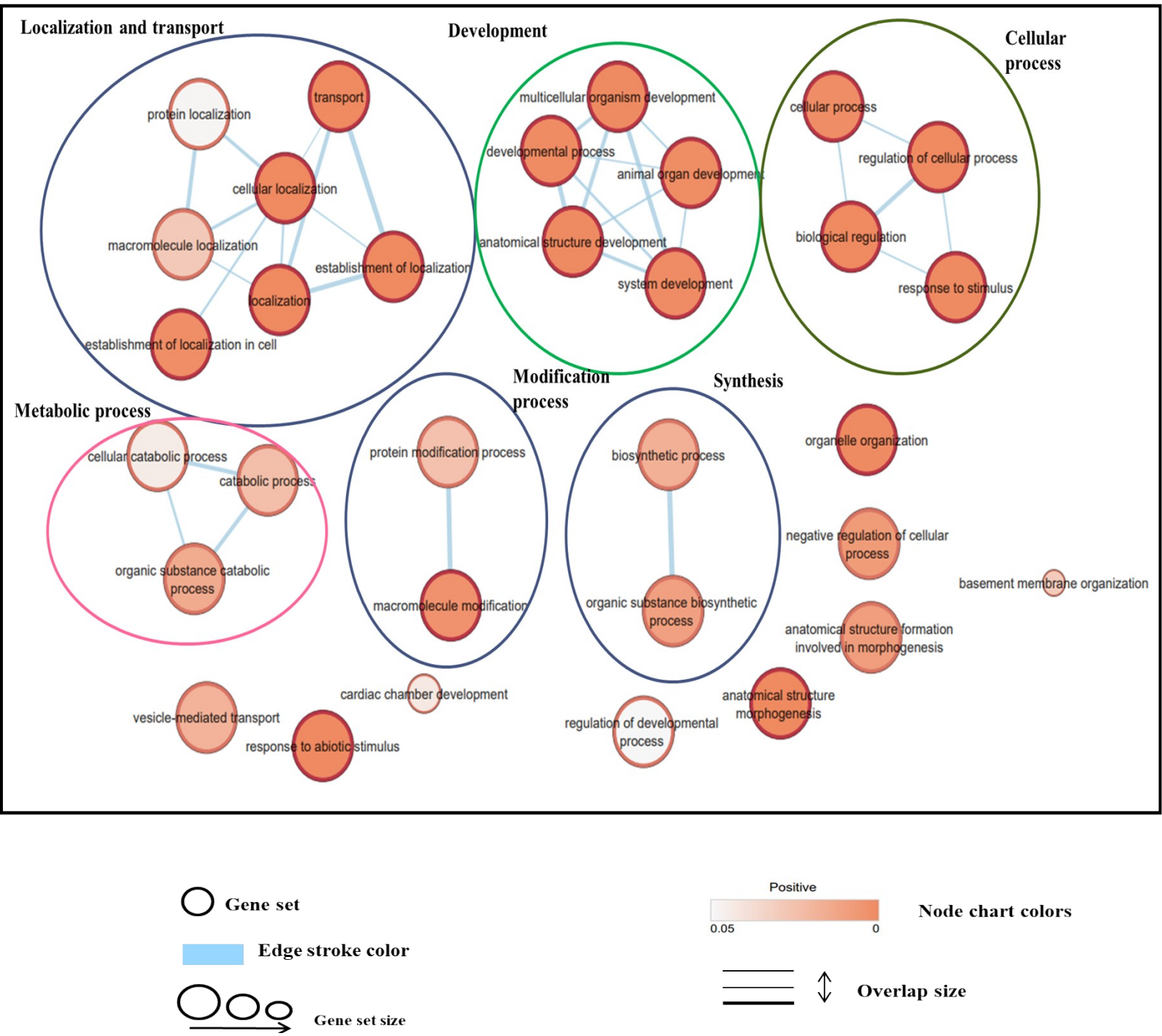
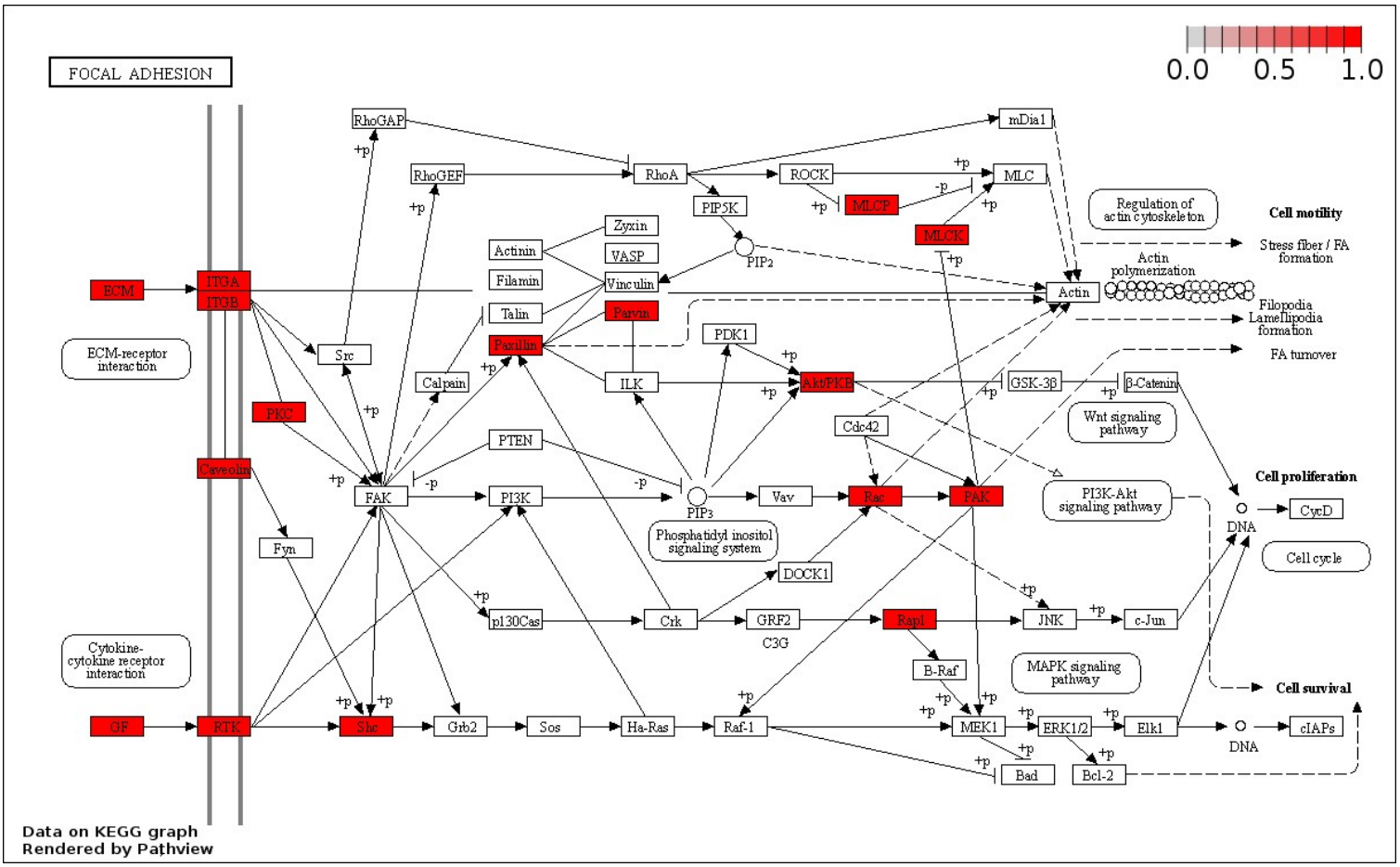


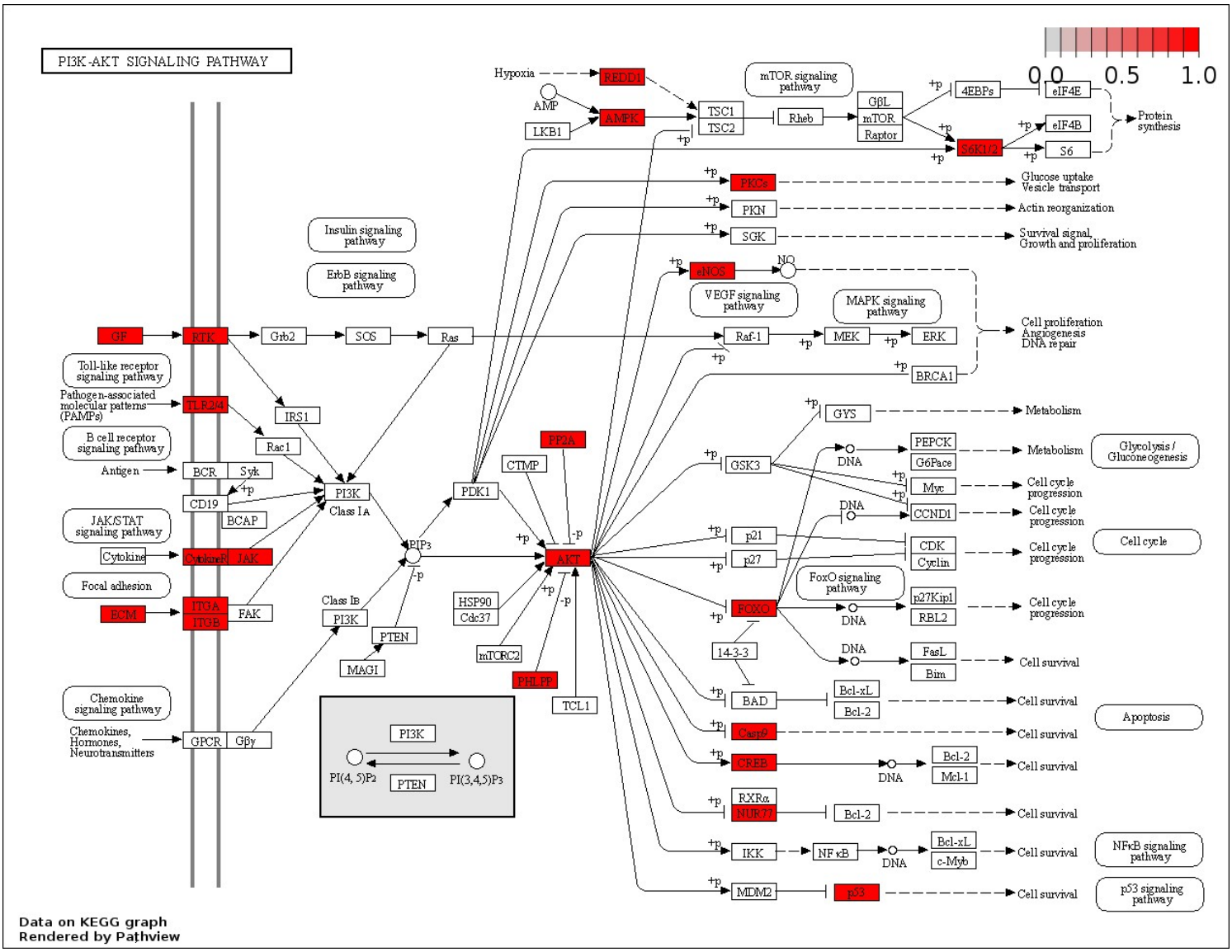
Fig.S11 The visualization of the enriched pathways among the piRNA target genes with p-value<0.05, q-value<0.05 and jaccard index of 0.50 using EnrichmentMap in Cytoscape

A



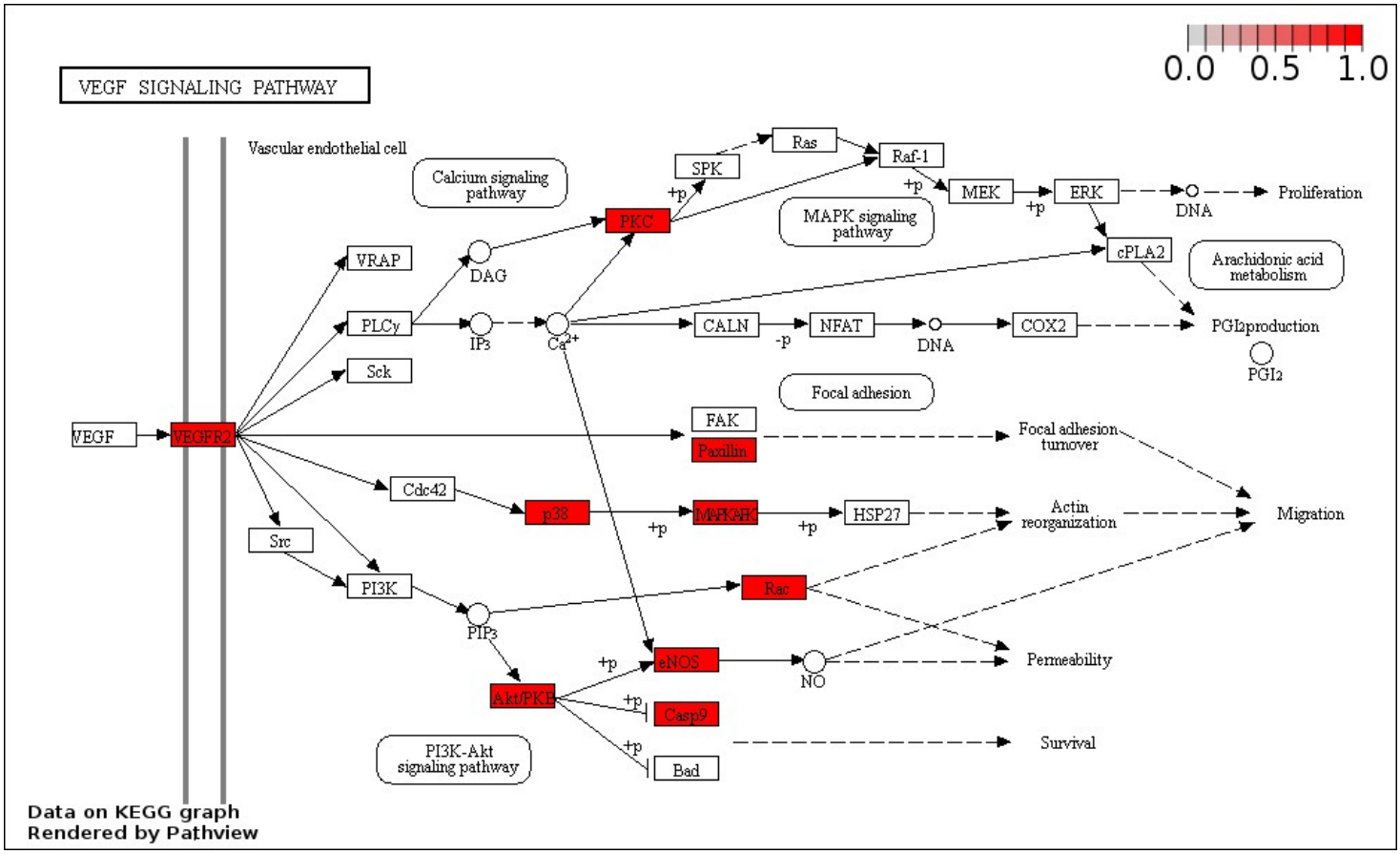
Supplementary Fig.S12 KEGG view: (A) Focal adhesion pathway using piRNA target genes

B



Supplementary Fig.S12 KEGG view: (B) PI3K-Akt Signaling pathway using piRNA target genes

C



Supplementary Fig.S12 KEGG view: (C) VEGF Signaling pathway using piRNA target genes

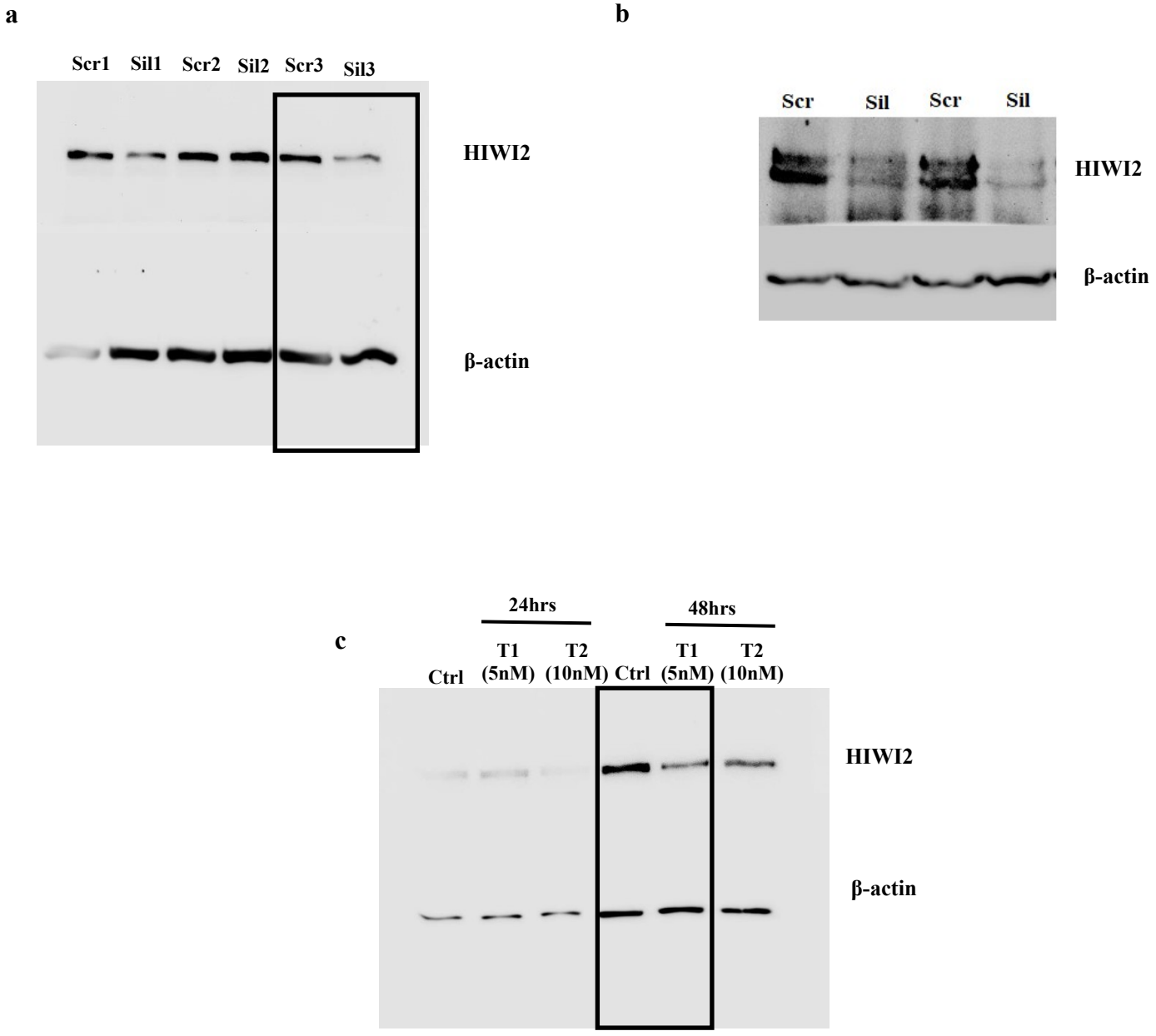
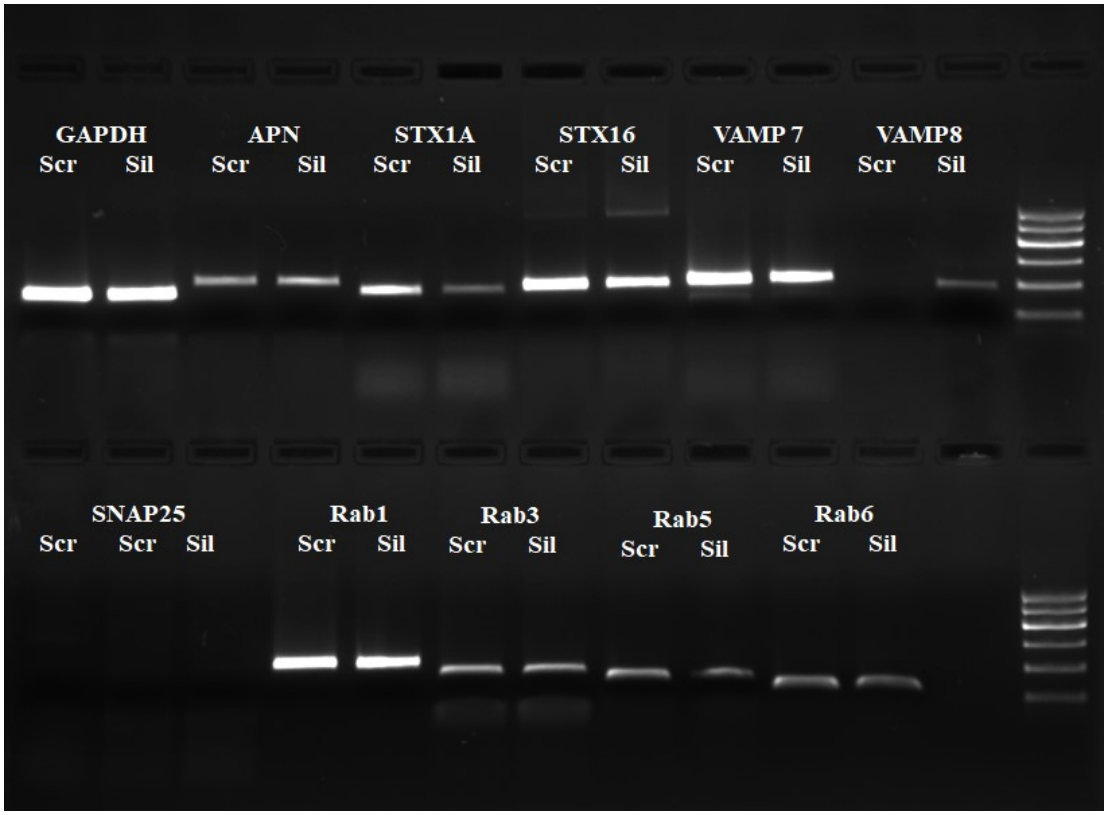


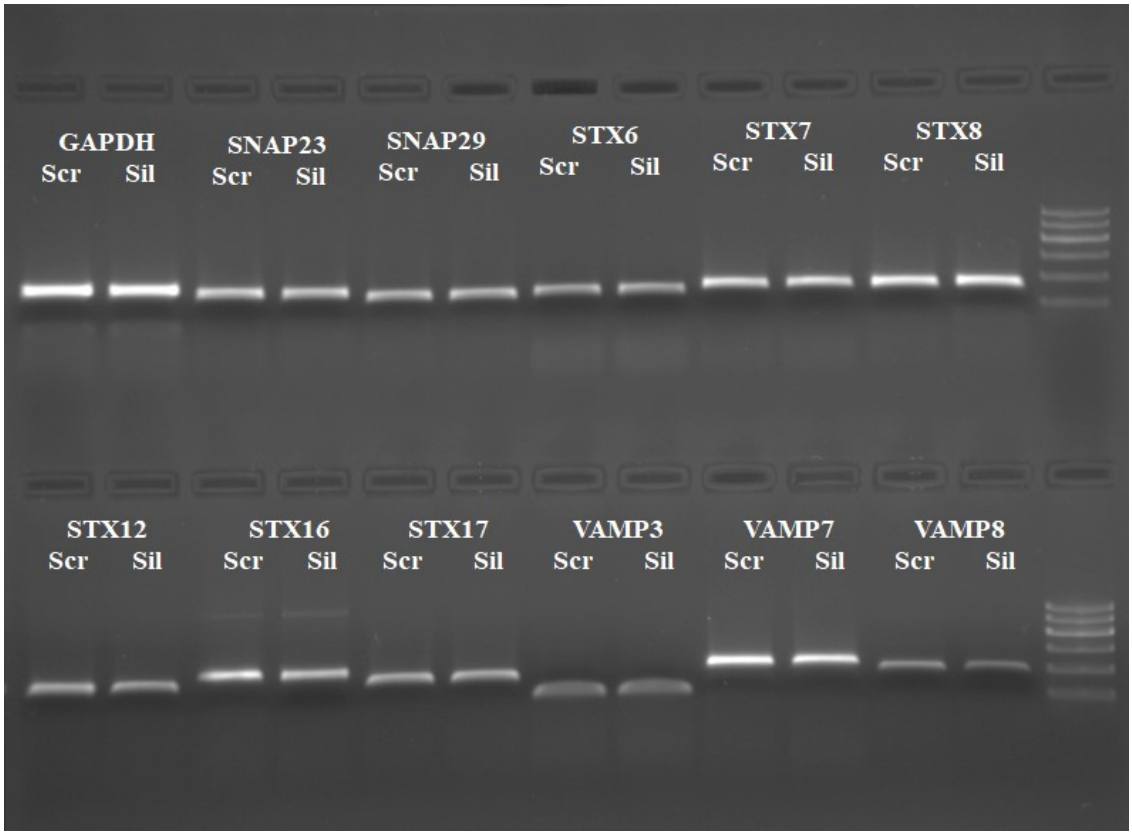
Fig.S13 HIWI2 silencing in ARPE19 cell line and Y79 cell line. (a) HIWI2 silencing was done in ARPE19 cell line and three representative experiments were shown [probed with anti-rabbit-PIWIL4 (Pierce)], boxed lanes are kept in **Fig.8A**, (b) HIWI2 silencing was repeated in ARPE19 cell line for the reproducibility of the results [probed with anti-mouse-PIWIL4 (Santa Cruz) which always detected 2 bands, but the knockdown was evident], (c)

HIWI2 silencing was done in Y79 cell line at different time points and concentration, boxed lanes are kept in **Fig.4A**. Scr: Scramble, Sil: Silencing, T: Treatment

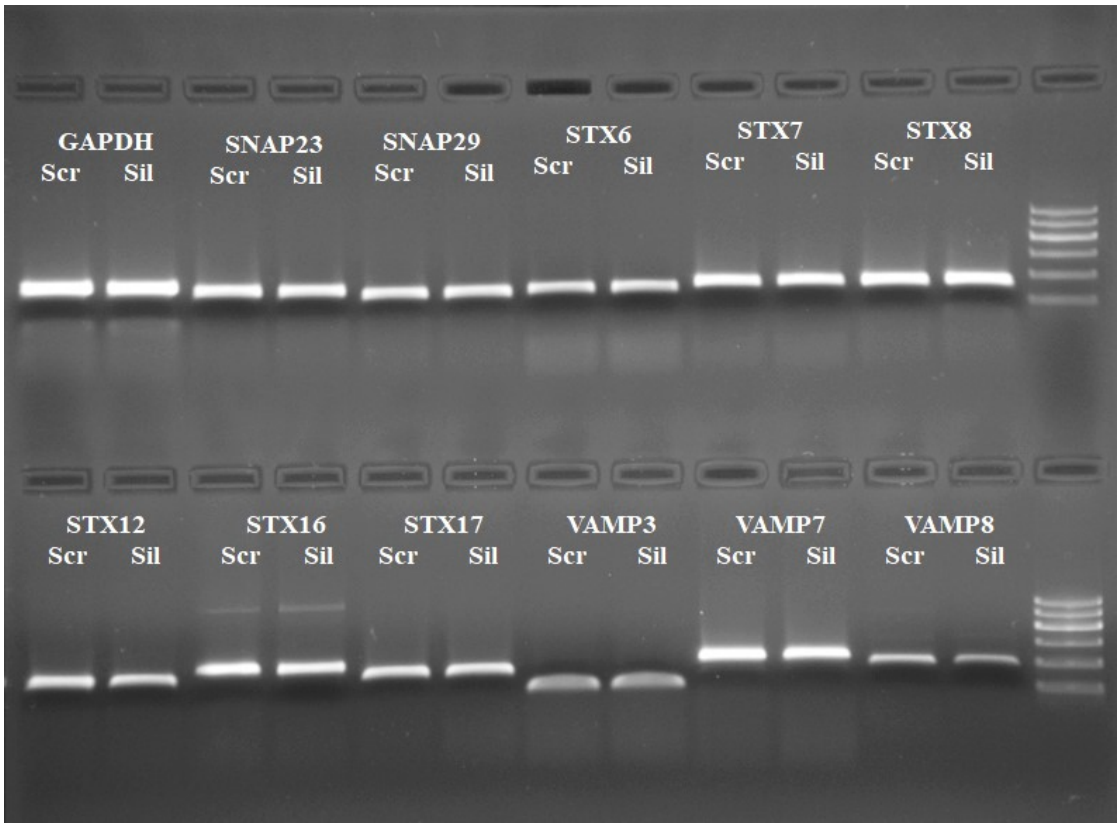
a



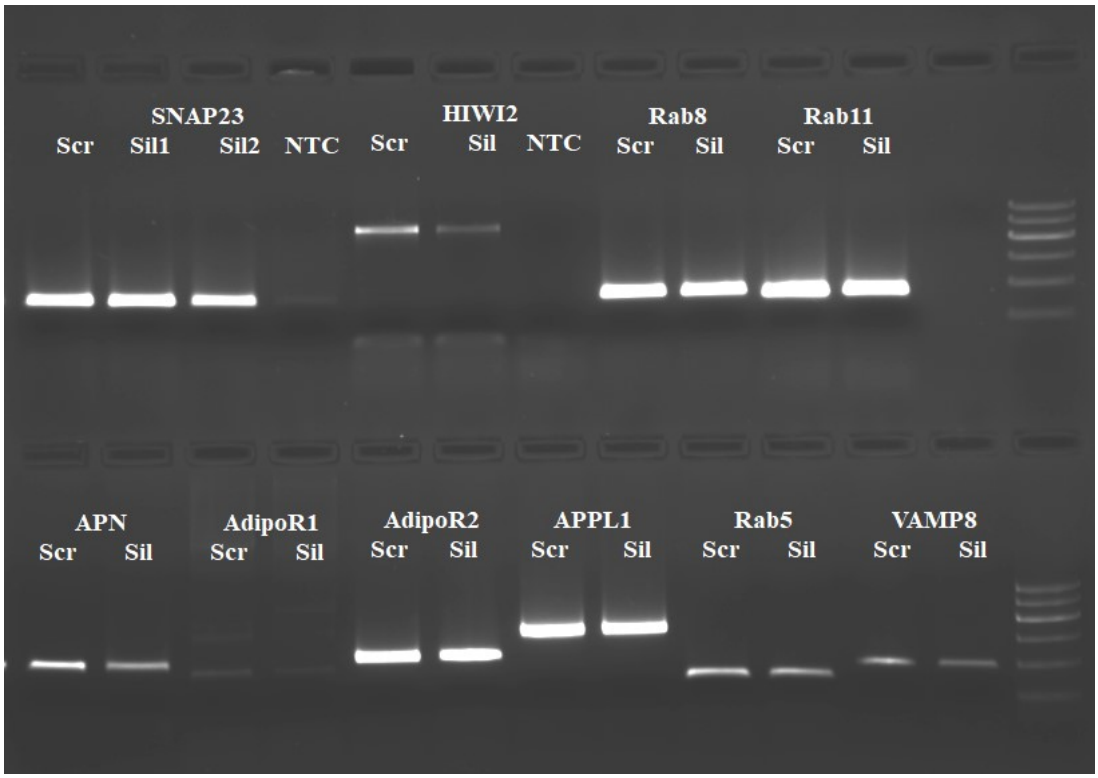
b


GAPDH
Scr Sil
SNAP23
Scr Sil
SNAP29
Scr Sil
STX6
Scr Sil
STX7
Scr Sil
STX8
Scr Sil
STX12
Scr Sil
STX16
Scr Sil
STX17
Scr Sil
VAMP3
Scr Sil
VAMP7
Scr Sil
VAMP8
Scr Sil

b.1


GAPDH
Scr Sil
SNAP23
Scr Sil
SNAP29
Scr Sil
STX6
Scr Sil
STX7
Scr Sil
STX8
Scr Sil
STX12
Scr Sil
STX16
Scr Sil
STX17
Scr Sil
VAMP3
Scr Sil
VAMP7
Scr Sil
VAMP8
Scr Sil

c


SNAP23
Scr
Sil1
Sil2
NTC
HIWI2
Scr
Sil
NTC
Rab8
Scr
Sil
Rab11
Scr
Sil
APN
Scr
Sil
AdipoR1
Scr
Sil
AdipoR2
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Sil
Rab5
Scr
Sil
VAMP8
Scr
Sil

d

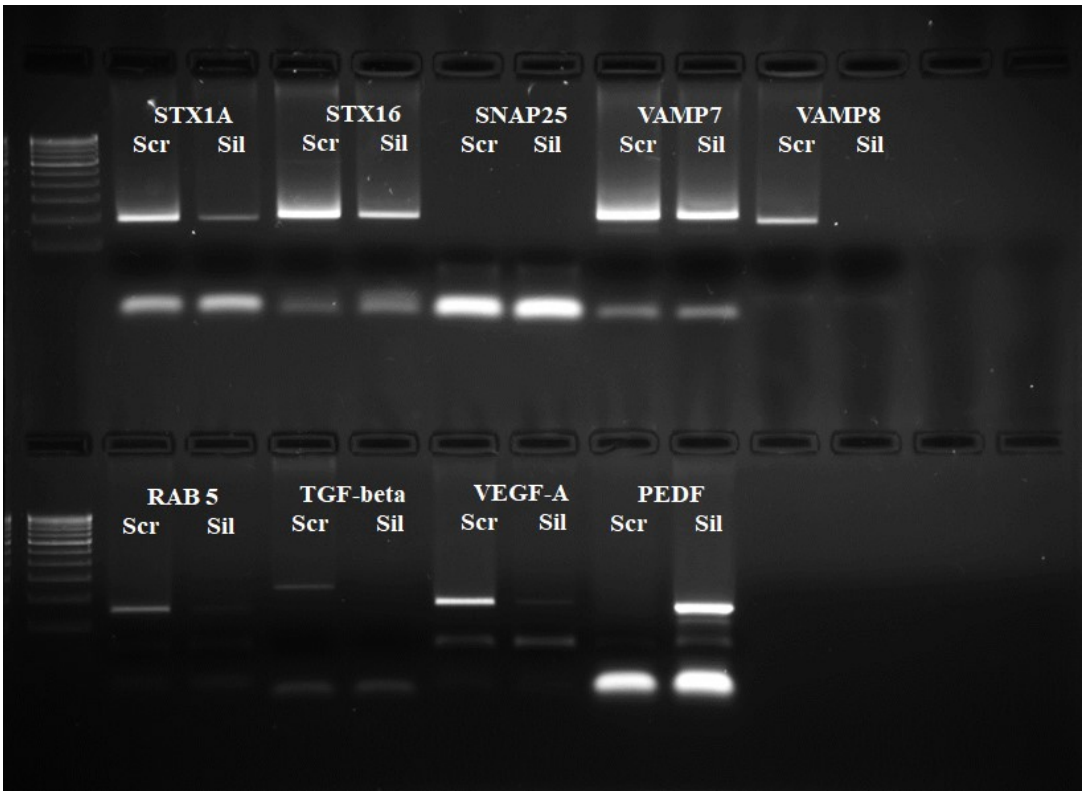

Fig. S14 – Raw agarose gel images showing the expression of SNAREs and Rabs in Si-Control and Si-HIWI2 ARPE19 cells that are presented in Fig.7C. GAPDH was used as the loading control. The SNARE expression in HIWI2 silenced samples were processed from the following images.

a. STX1A, Rab5, and GAPDH
b. VAMP7*, VAMP8, and, STX6
b.1. High contrast image of "b"
c. Rab8
d. STX16

*genes that are marked with asterisk are in higher contrast in **Fig.8C**.
Scr-Scramble, Sil or Si-Silenced, NTC - No template control.