biomedicines

*Article*

# Transcriptome-wide analysis of CXCR5 deficient retinal pigment epithelial (RPE) cells reveals molecular signature of RPE homeostasis

## Supplementary data

**Madhu Sudhana Saddala [1], Anton Lennikov [1], Anthony Mukwaya [2], and Hu Huang [1,*]**

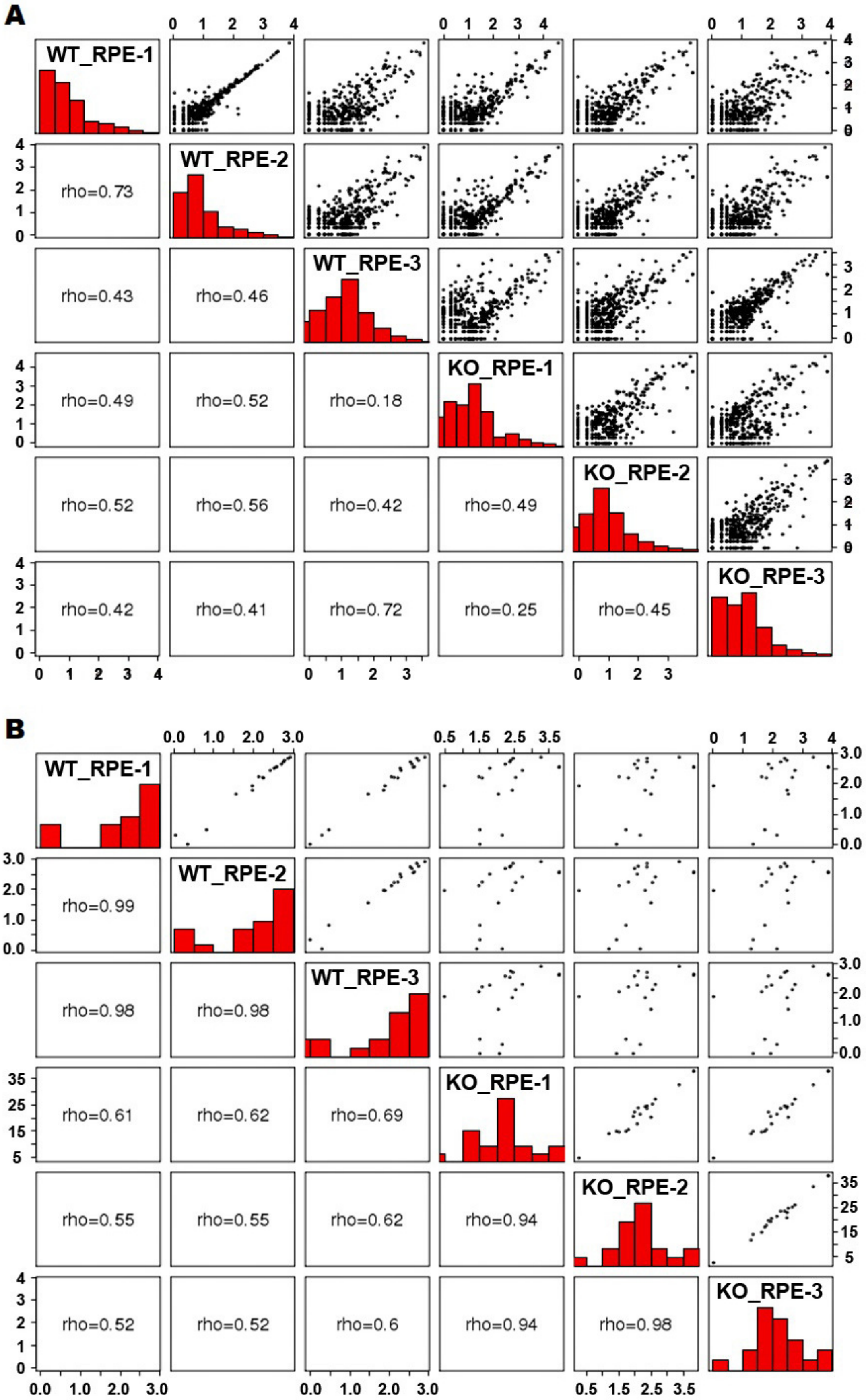
1 Department of Ophthalmology, University of Missouri, Columbia, MO 65212, United States of America; saddalam@missouri.edu (M.S.S.); lennikova@missouri.edu (A.L.)

2 Department of Ophthalmology, Institute for Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, 58183 , Sweden; anthonny.mukwaya@liu.se

* Correspondence:huangh1@missouri.edu; Tel: +1 573-882-9899
# Madhu Sudhana Saddala and Anton Lennikov have contributed equally to this work.

**Keywords:** Age-related macular degeneration; CXCR5; EMT, FoxO; Mitochondria; RNA-Seq, Gene Ontology; KEGG; Retinal pigment epithelium

## Supplementary Figures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WT_RPE-2
WT_RPE-3
KO_RPE-1
KO_RPE-2
KO_RPE-3
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rho=0.46
rho=0.49
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0 1 2 3 4
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WT_RPE-1
WT_RPE-2
WT_RPE-3
KO_RPE-1
KO_RPE-2
KO_RPE-3
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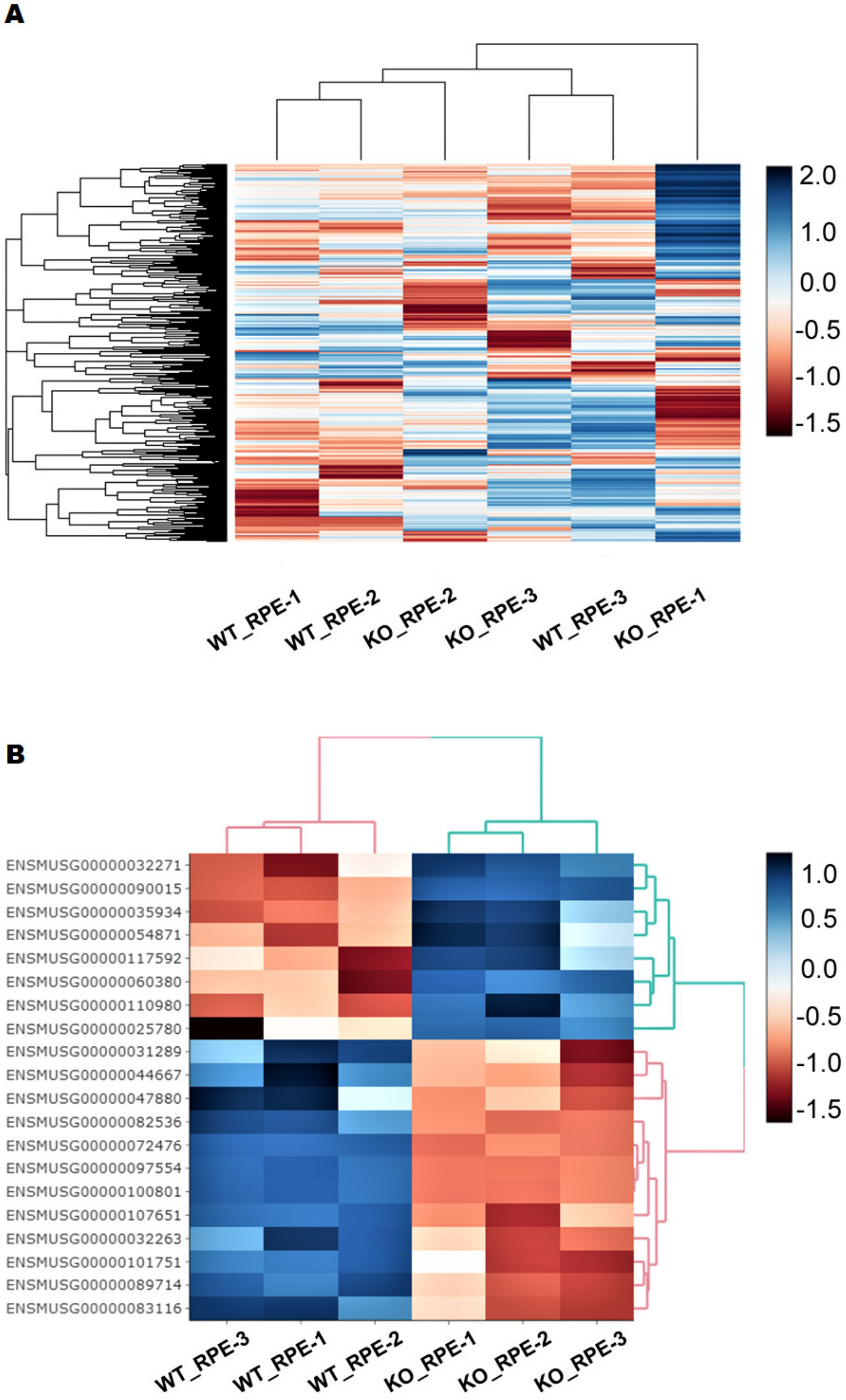
0.5 1.5 2.5 3.5
5 15 25 35
0 1 2 3 4

**Figure S1**: Quantitative profiling of mouse RPE tissue between control and CXCR5 ko groups. Pearson correlation coefficient plot of the log2 ratio between two groups (A) before and (B) after normalization. Potential relationships or correlations amongst the different data attributes is to leverage a pair-wise correlation matrix in control and CXCR5 knockout groups.


A
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-0.5
-1.0
-1.5
WT_RPE-1
WT_RPE-2
KO_RPE-2
KO_RPE-3
WT_RPE-3
KO_RPE-1
B
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ENSMUSG00000090015
ENSMUSG00000035934
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ENSMUSG00000117592
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ENSMUSG00000083116
1.0
0.5
0.0
-0.5
-1.0
-1.5
WT_RPE-3
WT_RPE-1
WT_RPE-2
KO_RPE-1
KO_RPE-2
KO_RPE-3

**Figure S2**: Potential relationships or correlations amongst the different data attributes is to leverage a pair-wise correlation matrix in control and CXCR5 knockout groups. (A) heatmap of total differentially expressed genes (B) The cluster of main heatmap represented twenty differentially expressed genes. The color code for the scored data is shown above the heat map. The red color denoted with a down-regulated genes and blue color denoted with up-regulated genes.

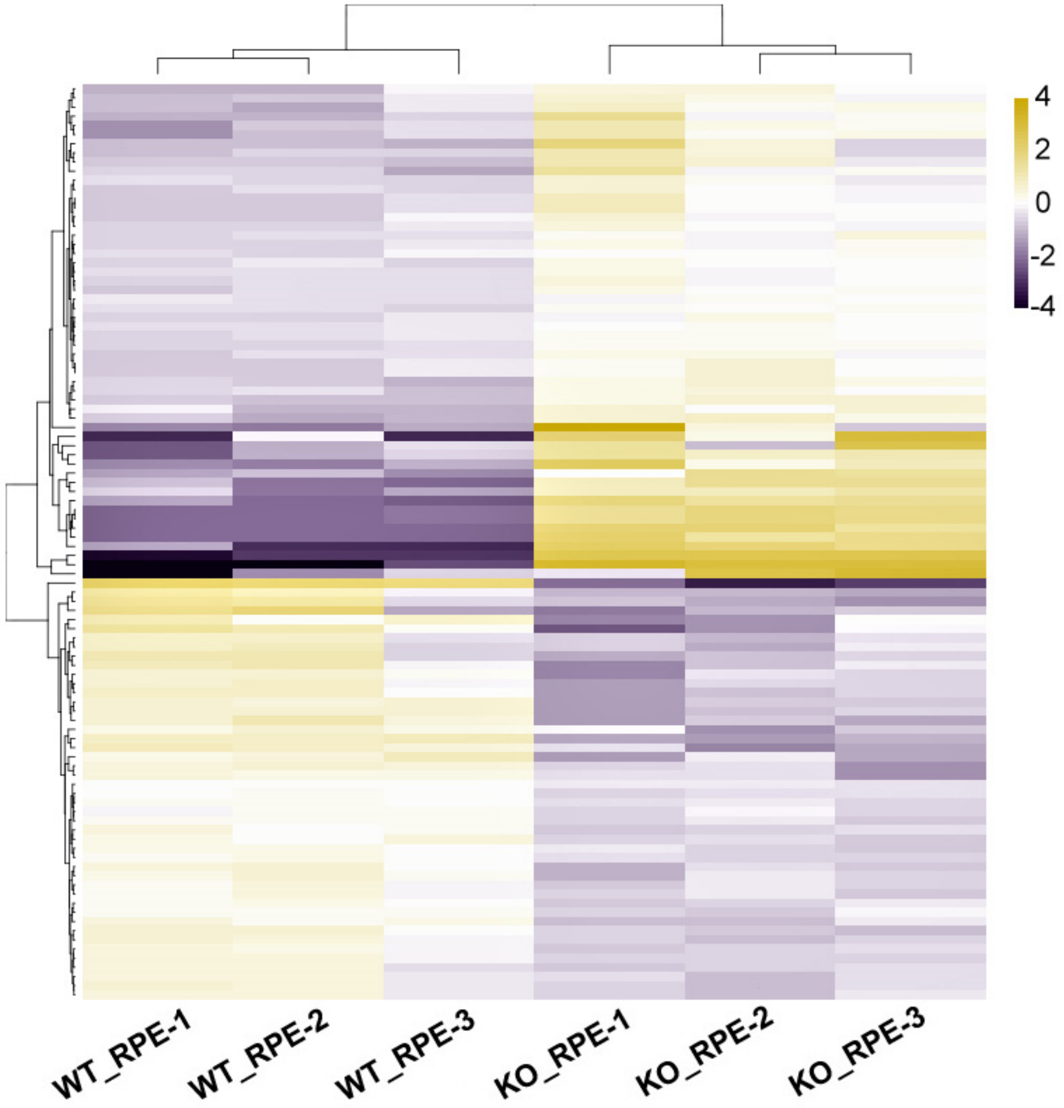
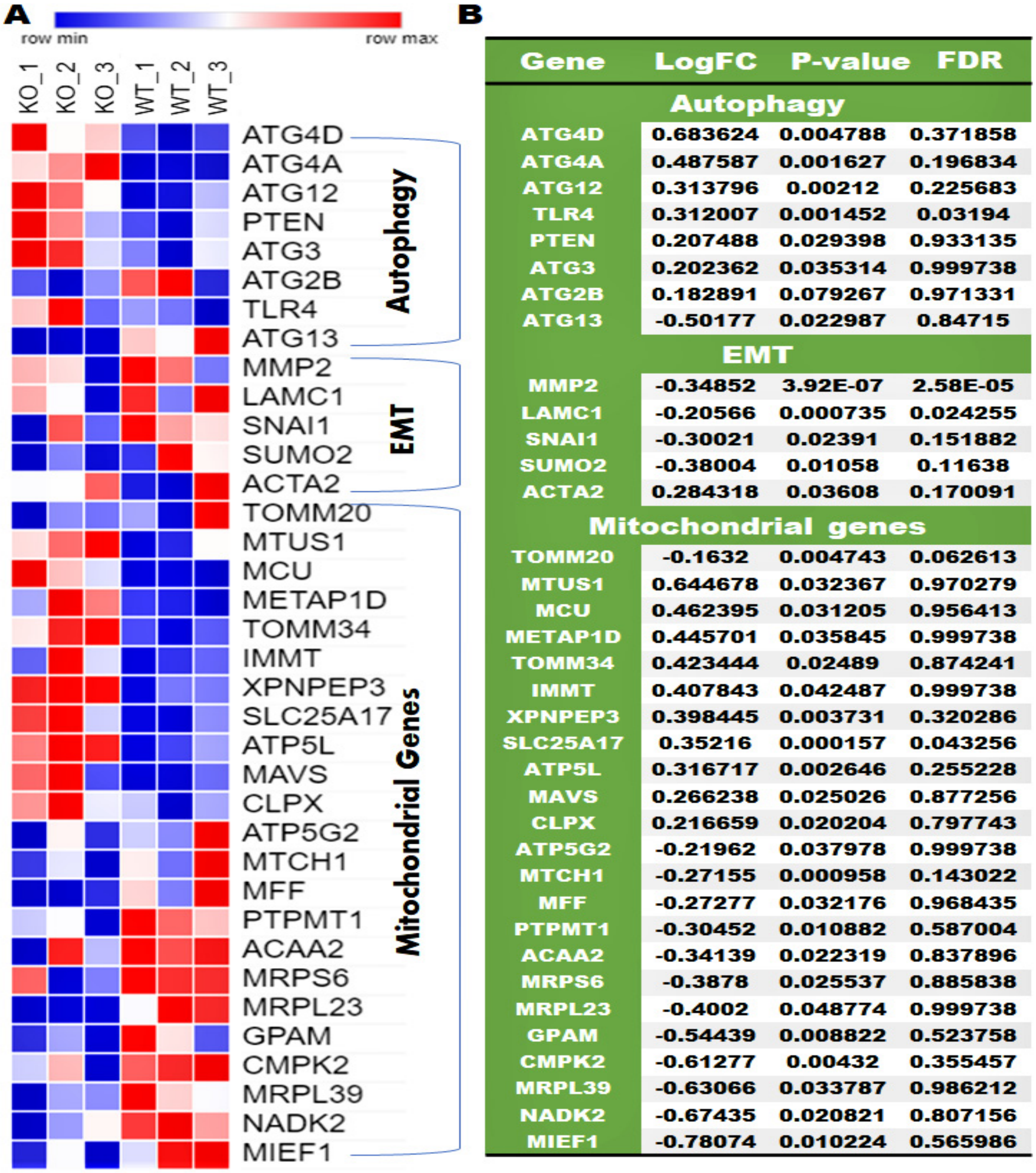


**Figure S3**: Significantly hundred differentially expressed genes were represented as a heat map (cluster of Suppl. Figure 2A), yellow color shows up-regulated and purple color shows down-regulated genes.



| Gene | LogFC | P-value | FDR |
|---|---|---|---|
| | **Autophagy** | | |
| ATG4D | 0.683624 | 0.004788 | 0.371858 |
| ATG4A | 0.487587 | 0.001627 | 0.196834 |
| ATG12 | 0.313796 | 0.00212 | 0.225683 |
| TLR4 | 0.312007 | 0.001452 | 0.03194 |
| PTEN | 0.207488 | 0.029398 | 0.933135 |
| ATG3 | 0.202362 | 0.035314 | 0.999738 |
| ATG2B | 0.182891 | 0.079267 | 0.971331 |
| ATG13 | -0.50177 | 0.022987 | 0.84715 |
| | **EMT** | | |
| MMP2 | -0.34852 | 3.92E-07 | 2.58E-05 |
| LAMC1 | -0.20566 | 0.000735 | 0.024255 |
| SNAI1 | -0.30021 | 0.02391 | 0.151882 |
| SUMO2 | -0.38004 | 0.01058 | 0.11638 |
| ACTA2 | 0.284318 | 0.03608 | 0.170091 |
| | **Mitochondrial genes** | | |
| TOMM20 | -0.1632 | 0.004743 | 0.062613 |
| MTUS1 | 0.644678 | 0.032367 | 0.970279 |
| MCU | 0.462395 | 0.031205 | 0.956413 |
| METAP1D | 0.445701 | 0.035845 | 0.999738 |
| TOMM34 | 0.423444 | 0.02489 | 0.874241 |
| IMMT | 0.407843 | 0.042487 | 0.999738 |
| XPNPEP3 | 0.398445 | 0.003731 | 0.320286 |
| SLC25A17 | 0.35216 | 0.000157 | 0.043256 |
| ATP5L | 0.316717 | 0.002646 | 0.255228 |
| MAVS | 0.266238 | 0.025026 | 0.877256 |
| CLPX | 0.216659 | 0.020204 | 0.797743 |
| ATP5G2 | -0.21962 | 0.037978 | 0.999738 |
| MTCH1 | -0.27155 | 0.000958 | 0.143022 |
| MFF | -0.27277 | 0.032176 | 0.968435 |
| PTPMT1 | -0.30452 | 0.010882 | 0.587004 |
| ACAA2 | -0.34139 | 0.022319 | 0.837896 |
| MRPS6 | -0.3878 | 0.025537 | 0.885838 |
| MRPL23 | -0.4002 | 0.048774 | 0.999738 |
| GPAM | -0.54439 | 0.008822 | 0.523758 |
| CMPK2 | -0.61277 | 0.00432 | 0.355457 |
| MRPL39 | -0.63066 | 0.033787 | 0.986212 |
| NADK2 | -0.67435 | 0.020821 | 0.807156 |
| MIEF1 | -0.78074 | 0.010224 | 0.565986 |

**Figure S4**: The DEGs involved in (**A**) autophagy, Epithelial-Mesenchymal Transition (EMT) and mitochondrial pathways (heatmap), (**B**) genes, logFC, p-value and FDR values. The heatmap designates red color as up-regulated and blue color as down-regulated genes in CXCR5-deficient RPE cells relative to WT control.

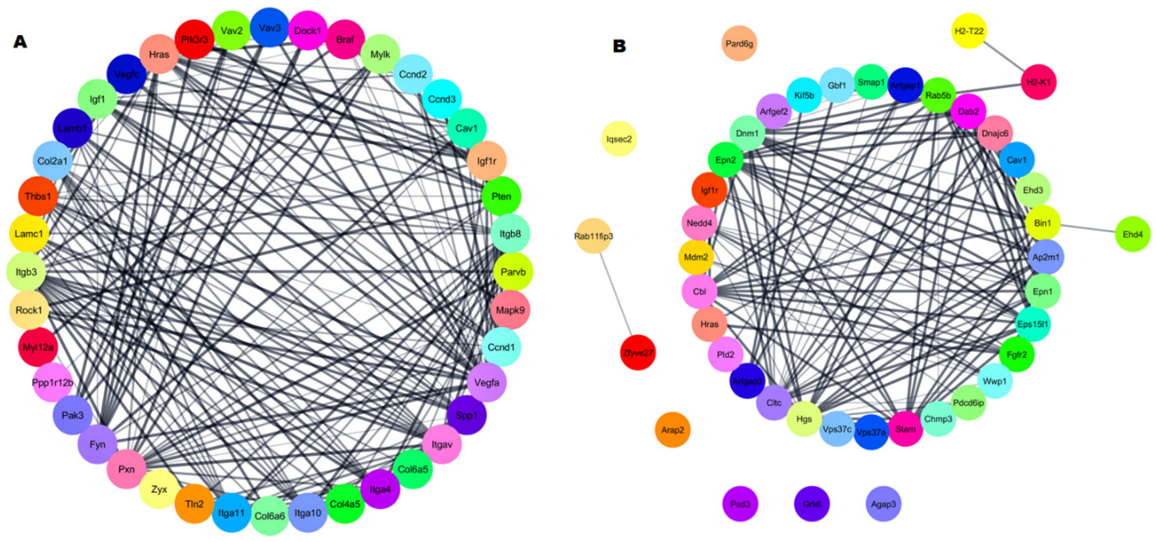


**Figure S5**: Gene-gene interaction network analysis. **(A)** Focal adhesion pathway genes. **(B)** Endocytosis pathway genes.

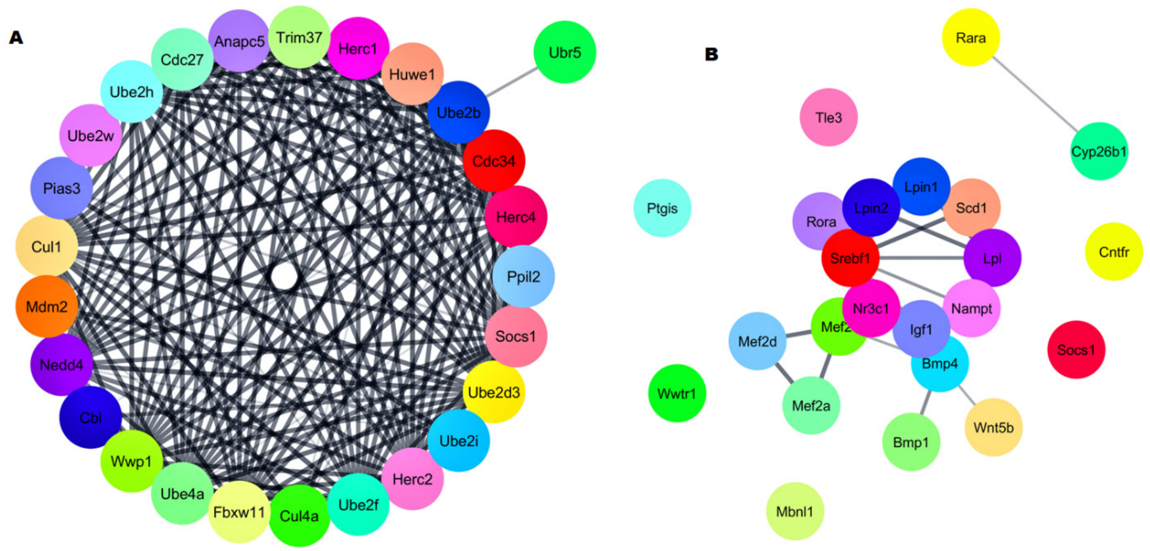


**Figure S6**: Gene-gene interaction network analysis. (**A)** Ubiquitin pathway genes. (**B)** Adipogenesis pathway genes.

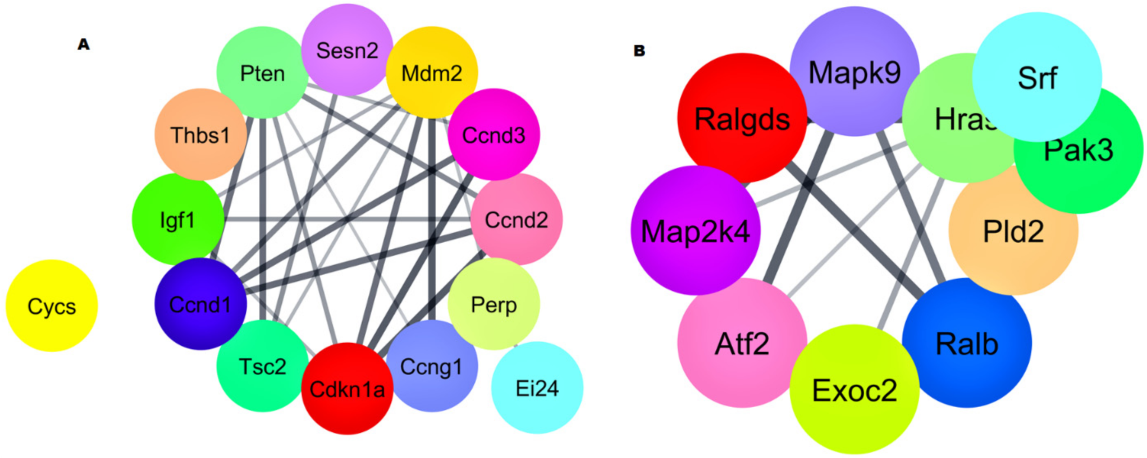


**Figure S7**: Gene-gene interaction network analysis. (**A**) p53 pathway genes. (**B)** Ras pathway genes.

# Supplementary Tables

**Table S1**: Trimmomatic for trimming Illumina FASTQ data and removing sequence adapters.

| **Samples** | **Trimmomatic trimming reads** | | **Drop of reads** | **Survival percentage of reads (%)** |
|---|---|---|---|---|
| | **Before** | **After** | | |
| RPEWT_1_L1_1 | 29276152 | 28197164 | 1078988 | 96.32 |
| RPEWT_1_L1_2 | 29276152 | 28197164 | 1078988 | 96.32 |
| RPEWT_2_L2_1 | 26792021 | 25764597 | 1027424 | 96.17 |
| RPEWT_2_L2_2 | 26792021 | 25764597 | 1027424 | 96.17 |
| RPEWT_3_L2_1 | 29958156 | 28923170 | 1034986 | 96.55 |
| RPEWT_3_L2_2 | 29958156 | 28923170 | 1034986 | 96.55 |
| RPECXCR5_1_L2_1 | 30697606 | 29617540 | 1080066 | 96.49 |
| RPECXCR5_1_L2_2 | 30697606 | 29617540 | 1080066 | 96.49 |
| RPECXCR5_2_L2_1 | 31530713 | 30295204 | 1235509 | 96.08 |
| RPECXCR5_2_L2_2 | 31530713 | 30295204 | 1235509 | 96.08 |
| RPECXCR5_3_L2_1 | 27742490 | 26649285 | 1093205 | 96.06 |
| RPECXCR5_3_L2_2 | 27742490 | 26649285 | 1093205 | 96.06 |

**Table S2**: List of Focal adhesion pathway genes with logFC, p-value and FDR value.

| Gene Symbol | Gene Name | logFC | P-value | FDR |
|---|---|---|---|---|
| Braf | Braf transforming gene | 0.52977 | 0.028286 | 0.924117 |
| Cav1 | Caveolin 1, caveolae protein | 0.79122 | 0.009721 | 0.554537 |
| Ccnd1 | Cyclin D1 | 0.819543 | 0.001353 | 0.178882 |
| Ccnd2 | Cyclin D2 | 0.550356 | 0.003991 | 0.334988 |
| Ccnd3 | Cyclin D3 | -0.70571 | 0.014237 | 0.672932 |
| Col2a1 | Collagen, type II, alpha 1 | -0.60353 | 0.012355 | 0.627982 |
| Col4a5 | Collagen, type IV, alpha 5 | -0.85519 | 0.001471 | 0.187435 |
| Col6a5 | Collagen, type VI, alpha 5 | -0.58554 | 0.022181 | 0.835681 |
| Col6a6 | Collagen, type VI, alpha 6 | -1.03427 | 0.000611 | 0.108132 |
| Dock1 | Dedicator of cytokinesis 1 | -0.72792 | 0.00884 | 0.523758 |
| Fyn | Fyn proto-oncogene | -0.3561 | 0.006077 | 0.427738 |
| Hras | Harvey rat sarcoma virus oncogene | 0.319113 | 0.041055 | 0.999738 |
| Igf1 | Insulin-like growth factor 1 | 0.582664 | 0.011964 | 0.618509 |
| Igf1r | Insulin-like growth factor I receptor | 0.839659 | 0.001664 | 0.199427 |
| Itga10 | integrin, alpha 10 | 0.722545 | 0.005989 | 0.424382 |
| Itga11 | Integrin alpha 11 | -0.52376 | 0.040259 | 0.999738 |
| Itga4 | Integrin alpha 4 | 0.669187 | 0.021709 | 0.828055 |
| Itgav | Integrin alpha V | 0.17317 | 0.02295 | 0.84715 |
| Itgb3 | Integrin beta 3 | -0.42099 | 0.032828 | 0.976771 |
| Itgb8 | Integrin beta 8 | 0.758588 | 0.001698 | 0.200273 |
| Lamb1 | Laminin B1 | -0.43077 | 4.18E-05 | 0.015966 |
| Lamc1 | Laminin, gamma 1 | -0.19129 | 0.031532 | 0.958818 |
| Mapk9 | Mitogen-activated protein kinase 9 | -0.63709 | 0.037388 | 0.999738 |
| Myl12a | Myosin, light chain 12A, regulatory, non-sarcomeric | 0.487208 | 0.002373 | 0.238982 |
| Mylk | Myosin, light polypeptide kinase | -2.17604 | 3.16E-25 | 5.51E-21 |
| Pak3 | p21 (RAC1) activated kinase 3 | 0.694405 | 0.000394 | 0.082617 |
| Parvb | Parvin, beta | -0.59728 | 0.028586 | 0.927543 |
| Pik3r3 | Phosphoinositide-3-kinase regulatory subunit 3 | 0.655702 | 0.005241 | 0.393197 |
| Ppp1r12b | Protein phosphatase 1, regulatory subunit 12B | 0.990421 | 0.000248 | 0.059475 |
| Pten | Phosphatase and tensin homolog | 0.207488 | 0.029398 | 0.933135 |
| Pxn | Paxillin | -0.48393 | 0.006902 | 0.46301 |
| Rock1 | Rho-associated coiled-coil containing protein kinase 1 | 0.403765 | 0.009553 | 0.548533 |

| | | | | |
|---|---|---|---|---|
| Spp1 | Secreted phosphoprotein 1 | 0.640272 | 0.003407 | 0.3029 |
| Thbs1 | Thrombospondin 1 | 0.62293 | 0.000441 | 0.086938 |
| Tln2 | Talin 2 | -5.05373 | 1.78E-70 | 9.33E-66 |
| Vav2 | Vav 2 oncogene | 0.560233 | 0.037862 | 0.999738 |
| Vav3 | Vav 3 oncogene | -0.83643 | 0.006246 | 0.436735 |
| Vegfa | Vascular endothelial growth factor A | -0.50133 | 8.23E-05 | 0.027091 |
| Vegfc | Vascular endothelial growth factor C | 0.251123 | 0.000599 | 0.106649 |
| Zyx | Zyxin | 0.331246 | 0.005829 | 0.420451 |

[1] The positive values indicate up-regulated genes, and negative values indicate down-regulated genes in CXCR5-deficient RPE cells relative to WT controls.

**Table S3**: List of Endocytosis pathway genes with logFC, p-value and FDR value.

| **Gene Symbol** | **Gene Name** | **logFC** | **P-value** | **FDR** |
|---|---|---|---|---|
| Agap3 | ArfGAP with GTPase domain, ankyrin repeat and PH domain 3 | 0.472514 | 0.03436 | 0.993067 |
| Ap2m1 | Adaptor-related protein complex 2, mu 1 subunit | 0.235135 | 0.019347 | 0.788484 |
| Arap2 | ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2 | 0.539452 | 0.012804 | 0.637988 |
| Arfgap1 | ADP-ribosylation factor GTPase activating protein 1 | 0.598167 | 0.020056 | 0.796902 |
| Arfgap3 | ADP-ribosylation factor GTPase activating protein 3 | -0.22345 | 0.042507 | 0.999738 |
| Arfgef2 | ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited) | -0.70729 | 1.33E-05 | 0.006275 |
| Bin1 | Bridging integrator 1 | -0.41634 | 0.002254 | 0.231417 |
| Cav1 | Caveolin 1, caveolae protein | 0.79122 | 0.009721 | 0.554537 |
| Cbl | Casitas B-lineage lymphoma | -1.19727 | 3.91E-08 | 4.99E-05 |
| Chmp3 | Charged multivesicular body protein 3 | 0.70947 | 0.018809 | 0.77501 |
| Cltc | Clathrin, heavy polypeptide (Hc) | 0.628175 | 0.031378 | 0.958818 |
| Dab2 | Disabled 2, mitogen-responsive phosphoprotein | 0.394081 | 0.007187 | 0.473408 |
| Dnajc6 | DnaJ heat shock protein family (Hsp40) member C6 | 0.571836 | 0.013292 | 0.647561 |
| Dnm1 | Dynamin 1 | -0.77705 | 0.009459 | 0.544977 |
| Ehd3 | EH-domain containing 3 | 0.601715 | 0.002116 | 0.225683 |
| Ehd4 | EH-domain containing 4 | 0.294066 | 0.025567 | 0.886143 |
| Epn1 | Epsin 1 | -0.33443 | 0.049664 | 0.999738 |
| Epn2 | Epsin 2 | 0.649684 | 0.033892 | 0.986212 |
| Eps15l1 | Epidermal growth factor receptor pathway | 0.84856 | 0.004189 | 0.347096 |

| | | | | |
|---|---|---|---|---|
| | substrate 15-like 1 | | | |
| Fgfr2 | Fibroblast growth factor receptor 2 | 0.641456 | 0.033897 | 0.986212 |
| Gbf1 | Golgi-specific brefeldin A-resistance factor 1 | 0.289734 | 0.030258 | 0.946044 |
| Grk6 | G protein-coupled receptor kinase 6 | 1.137069 | 5.11E-05 | 0.018864 |
| H2-K1 | Histocompatibility 2, K1, K region | -0.45265 | 0.013726 | 0.66025 |
| H2-T22 | Histocompatibility 2, T region locus 22 | -0.38692 | 0.016101 | 0.720927 |
| Hgs | HGF-regulated tyrosine kinase substrate | 0.330257 | 0.002811 | 0.265882 |
| Hras | Harvey rat sarcoma virus oncogene | 0.319113 | 0.041055 | 0.999738 |
| Igf1r | Insulin-like growth factor I receptor | 0.839659 | 0.001664 | 0.199427 |
| Iqsec2 | IQ motif and Sec7 domain 2 | -0.61004 | 0.047881 | 0.999738 |
| Kif5b | Kinesin family member 5B | 0.240581 | 0.005744 | 0.418374 |
| Mdm2 | Transformed mouse 3T3 cell double minute 2 | 0.707868 | 0.002289 | 0.234591 |
| Nedd4 | Neural precursor cell expressed, developmentally down-regulated 4 | 0.464203 | 0.046913 | 0.999738 |
| Pard6g | Par-6 family cell polarity regulator gamma | 0.318767 | 0.009863 | 0.559003 |
| Pdcd6ip | Programmed cell death 6 interacting protein | 0.612938 | 0.000449 | 0.087793 |
| Pld2 | Phospholipase D2 | 0.705743 | 0.020714 | 0.806734 |
| Psd3 | Pleckstrin and Sec7 domain containing 3 | 0.618409 | 0.027502 | 0.912732 |
| Rab11fip3 | RAB11 family interacting protein 3 (class II) | 0.880577 | 0.000249 | 0.059475 |
| Rab5b | RAB5B, member RAS oncogene family | -0.41939 | 0.015551 | 0.708775 |
| Smap1 | Small ArfGAP 1 | -0.6663 | 0.028345 | 0.924519 |
| Stam | Signal transducing adaptor molecule (SH3 domain and ITAM motif) 1 | 0.65634 | 0.023574 | 0.856219 |
| Vps37a | Vacuolar protein sorting 37A | 0.241828 | 0.016802 | 0.732061 |
| Vps37c | Vacuolar protein sorting 37C | -0.68304 | 0.010018 | 0.562388 |
| Wwp1 | WW domain containing E3 ubiquitin protein ligase 1 | 0.488816 | 0.035938 | 0.999738 |
| Zfyve27 | Zinc finger, FYVE domain containing 27 | 0.212258 | 0.047278 | 0.999738 |

[1] The positive values indicate up-regulated genes, and negative values indicate down-regulated genes in CXCR5-deficient RPE cells relative to WT controls.

**Table S4**: List of mTOR signaling pathway genes with logFC, p-value, and FDR values.

| Gene Symbol | Gene Name | logFC | P-value | FDR |
|---|---|---|---|---|
| Akt1s1 | AKT1 substrate 1 (proline-rich) | -0.79272 | 0.000912 | 0.140096 |
| Atp6v1b2 | ATPase, H+ transporting, lysosomal V1 subunit B2 | 0.150992 | 0.040314 | 0.999738 |
| Atp6v1h | ATPase, H+ transporting, lysosomal V1 subunit H | 0.290488 | 0.013095 | 0.642713 |
| Braf | Braf transforming gene | 0.52977 | 0.028286 | 0.924117 |
| Clip1 | CAP-GLY domain containing linker protein 1 | 0.415577 | 0.023969 | 0.860121 |
| Depdc5 | DEP domain containing 5 | -0.63296 | 0.007948 | 0.499113 |
| Eif4e2 | Eukaryotic translation initiation factor 4E member 2 | 0.339331 | 0.017923 | 0.756945 |
| Fnip1 | Folliculin interacting protein 1 | 0.600433 | 0.042345 | 0.999738 |
| Fzd6 | Frizzled class receptor 6 | 0.871764 | 0.002598 | 0.253124 |
| Grb10 | Growth factor receptor bound protein 10 | -0.69377 | 0.004018 | 0.336169 |
| Hras | Harvey rat sarcoma virus oncogene | 0.319113 | 0.041055 | 0.999738 |
| Igf1 | Insulin-like growth factor 1 | 0.582664 | 0.011964 | 0.618509 |
| Igf1r | Insulin-like growth factor I receptor | 0.839659 | 0.001664 | 0.199427 |
| Insr | Insulin receptor | -0.50178 | 0.0276 | 0.914738 |
| Lpin1 | Lipin 1 | 0.842541 | 0.002497 | 0.246194 |
| Mapkap1 | Mitogen-activated protein kinase associated protein 1 | 0.465516 | 0.023405 | 0.853275 |
| Mios | Meiosis regulator for oocyte development | -0.75748 | 0.00268 | 0.25751 |
| Nprl3 | Nitrogen permease regulator-like 3 | 0.411513 | 0.011277 | 0.595943 |
| Nras | Neuroblastoma ras oncogene | -0.98599 | 0.000281 | 0.064468 |
| Pik3r3 | Phosphoinositide-3-kinase regulatory subunit 3 | 0.655702 | 0.005241 | 0.393197 |
| Pten | Phosphatase and tensin homolog | 0.207488 | 0.029398 | 0.933135 |
| Sesn2 | Sestrin 2 | 0.211571 | 0.03607 | 0.999738 |
| Sgk1 | Serum/glucocorticoid regulated kinase 1 | 0.331703 | 3.24E-05 | 0.012944 |
| Slc3a2 | Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 | 0.616334 | 0.043362 | 0.999738 |
| Tsc1 | tuberous sclerosis 1 | 0.727053 | 0.003532 | 0.307612 |
| Tsc2 | Tuberous sclerosis 2 | -0.65896 | 0.029703 | 0.937812 |
| Wnt11 | Wingless-type MMTV integration site family, member 11 | -0.81981 | 0.001098 | 0.156251 |
| Wnt5a | Wingless-type MMTV integration site family, member 5A | -0.70649 | 0.000767 | 0.126041 |
| Wnt5b | Wingless-type MMTV integration site family, member 5B | -0.57215 | 0.022638 | 0.841847 |

[1] The positive values indicate up-regulated genes, and negative values indicate down-regulated genes in CXCR5-deficient RPE cells relative to WT controls.

**Table S5**: List of FoxO signaling pathway genes with logFC, p-value and FDR values.

| Gene Symbol | Gene Name | logFC[1] | P-value | FDR |
|---|---|---|---|---|
| Mapk9 | Mitogen-activated protein kinase 9 | -0.63709 | 0.037388 | 0.999738 |
| Pten | Phosphatase and tensin homolog | 0.207488 | 0.029398 | 0.933135 |
| Hras | Harvey rat sarcoma virus oncogene | 0.319113 | 0.041055 | 0.999738 |
| Ccnd2 | Cyclin D2 | 0.550356 | 0.003991 | 0.334988 |
| Braf | Braf transforming gene | 0.52977 | 0.028286 | 0.924117 |
| Igf1 | Insulin-like growth factor | 0.582664 | 0.011964 | 0.618509 |
| Ccnd1 | Cyclin D1 | 0.819543 | 0.001353 | 0.178882 |
| Pik3r3 | Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55) | 0.655702 | 0.005241 | 0.393197 |
| Igf1r | Insulin-like growth factor I receptor | 0.839659 | 0.001664 | 0.199427 |
| Nras | Neuroblastoma ras oncogene | -0.98599 | 0.000281 | 0.064468 |
| Cdkn1a | Cyclin-dependent kinase inhibitor 1A (P21) | 0.600435 | 0.000799 | 0.129915 |
| Mdm2 | Transformed mouse 3T3 cell double minute 2 | 0.707868 | 0.002289 | 0.234591 |
| Bcl2l11 | BCL2-like 11 (apoptosis facilitator) | -0.85382 | 0.001046 | 0.152302 |
| Insr | Insulin receptor | -0.50178 | 0.0276 | 0.914738 |
| Sgk1 | Serum/glucocorticoid regulated kinase 1 | 0.331703 | 3.24E-05 | 0.012944 |
| Crebbp | CREB binding protein | 0.289525 | 0.015087 | 0.698369 |
| Usp7 | Ubiquitin specific peptidase 7 | 0.50216 | 0.001425 | 0.184667 |
| Atg12 | Autophagy related 12 | 0.313796 | 0.00212 | 0.225683 |

[1] The positive values indicate up-regulated genes, and negative values indicate down-regulated genes in CXCR5-deficient RPE cells relative to WT controls.

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