

Title: Micro RNA analysis of lens capsule in pseudoexfoliation glaucoma and pseudoexfoliation cataract

Supplementary Table 1: Primer sequences along with accession numbers used for qRT-PCR (miRNA) assays

	miRNAs	Accession no.	RT primer Sequence	Primer sequence
1	has-miR-4687-3p	MIMAT0019775	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTGCCTGC	CTGTTGGAGGGGGCAGGC
2	has-miR-6088	MIMAT0023713	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTGCCTCC	GATGAAGCGGGGGG CG
3	hsa-miR-6515-3p	MIMAT0025487	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTCTGGGG	CGCTCTCTTCATCTAC CCC CCAG
4	hsa-miR-638	MIMAT0003308	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTAGGCCG	GGCGGGTGGCGG CCT
5	has-miR-4516	MIMAT0019053	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTGCCCCG	GGGAGAAGGTCGGG GC
6	has-miR-6869-5p	MIMAT0027638	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTGCCGCC	GTGAGTAGTGGCGCG CGG
7	hsa-miR-6749-5p	MIMAT0027398	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTGCTCCC	TCGGGCCTGGGGTTGG
8	has-miR-184	MIMAT0000454	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTTCCT	UGGACGGAGAACUGAUA
9	SNORD 47	NR_002746	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTAACCTC	CGCCAATGATGTAATGATTCTG
10	Universal reverse primer			CAGTGCAGGGTCCGAGGT

Supplementary Table 2: Primer sequences along with accession numbers used for qRT-PCR assays

S. No.	Primer name	Accession No	Sequence 5'-3'
1	PLD1-F	NM_002662.4	AGCATCAGCAGCATTGACAG
	PLD1-R		CCGGTATCAGCATGAGGTCT
2	SP2-F	NM_003110.5	CCGACACCTACTCAGGTCTAC
	SP2-R		TTTGCTGGTCCCACTCAGAT
3	SOX2-F	NM_003106.3	AATGCCTTCATGGTGTGGTC
	SOX2-R		GCTTAGCCTCGTCGATGAAC
4	STARD10-F	NM_006645.2	CTACGACGTCCTACACGACA
	STARD10-R		TGGGACACCTCCAGGAGTAA
5	TP53INP2-F	NM_001329429.1	TGTCGGAGGAGGATGAAGTG
	TP53INP2-R		AACCAGCTCTCGTCCATCAA
6	OSCP1-F	NM_001330493.1	TCATGGACCCAGGAAAGCAT
	OSCP1-R		ACTCTTCTGGCCTGGTTAGC
7	CDK2-F	NM_001290230.1	AACAAGTTGACGGGAGAGGT
	CDK2-R		GAGAGCAGAGGCATCCATGA
8	B-Actin-F	NM_001101	CACCATTGGCAATGAGCGGTTC
	B-Actin-R		AGGTCTTTGCGGATGTCCACGT

Supplementary Table 3: Targets of has-miR-638 from miRTarBase

ID	Species (miRNA)	Species (Target)	miRNA	Target	Validation methods			Less strong evidence	SUM	NO. OF PAPERS
					Strong evidence					
MIRT035529	Homo sapiens	Homo sapiens	hsa-miR-638	OSCP1	Y	Y	Y	Y	4	1
MIRT053760	Homo sapiens	Homo sapiens	hsa-miR-638	SP2	Y	Y	Y		3	1
MIRT054761	Homo sapiens	Homo sapiens	hsa-miR-638	SOX2	Y	Y	Y		3	2
MIRT731124	Homo sapiens	Homo sapiens	hsa-miR-638	CDK2	Y	Y	Y		3	1
MIRT731281	Homo sapiens	Homo sapiens	hsa-miR-638	STARD10	Y		Y		2	1
MIRT731470	Homo sapiens	Homo sapiens	hsa-miR-638	DACT3	Y	Y	Y		3	1
MIRT732277	Homo sapiens	Homo sapiens	hsa-miR-638	PLD1	Y		Y		2	1
MIRT733639	Homo sapiens	Homo sapiens	hsa-miR-638	TP53INP2	Y	Y	Y		3	1
MIRT734279	Homo sapiens	Homo sapiens	hsa-miR-638	PTEN				Y	3	1
MIRT734281	Homo sapiens	Homo sapiens	hsa-miR-638	TP53				Y	3	1
MIRT367159	Homo sapiens	Homo sapiens	hsa-miR-638	HIST2H4A				Y	1	4
MIRT367182	Homo sapiens	Homo sapiens	hsa-miR-638	HIST2H4B				Y	1	4
MIRT458268	Homo sapiens	Homo sapiens	hsa-miR-638	FUT10				Y	1	1
MIRT463186	Homo sapiens	Homo sapiens	hsa-miR-638	ZNF275				Y	1	2
MIRT470433	Homo sapiens	Homo sapiens	hsa-miR-638	PPP1R15B				Y	1	6
MIRT474089	Homo sapiens	Homo sapiens	hsa-miR-638	LMBR1L				Y	1	1
MIRT479088	Homo sapiens	Homo sapiens	hsa-miR-638	CNNM4				Y	1	1
MIRT490952	Homo sapiens	Homo sapiens	hsa-miR-638	PPM1F				Y	1	2
MIRT495158	Homo sapiens	Homo sapiens	hsa-miR-638	CNGA2				Y	1	2
MIRT495749	Homo sapiens	Homo sapiens	hsa-miR-638	PDE4C				Y	1	2

MIRT525802	Homo sapiens	Homo sapiens	hsa-miR-638	SOD2	Y	1	1
MIRT525890	Homo sapiens	Homo sapiens	hsa-miR-638	BUB1	Y	1	1
MIRT530433	Homo sapiens	Homo sapiens	hsa-miR-638	SULT1B1	Y	1	1
MIRT537079	Homo sapiens	Homo sapiens	hsa-miR-638	GPR137C	Y	1	1
MIRT540852	Homo sapiens	Homo sapiens	hsa-miR-638	NUP155	Y	1	2
MIRT541161	Homo sapiens	Homo sapiens	hsa-miR-638	OTUD7B	Y	1	1
MIRT553929	Homo sapiens	Homo sapiens	hsa-miR-638	STC2	Y	1	2
MIRT566795	Homo sapiens	Homo sapiens	hsa-miR-638	MIER3	Y	1	1
MIRT619283	Homo sapiens	Homo sapiens	hsa-miR-638	CALHM5	Y	1	1
MIRT633581	Homo sapiens	Homo sapiens	hsa-miR-638		Y	1	1
MIRT633602	Homo sapiens	Homo sapiens	hsa-miR-638	HMGN5	Y	1	2
MIRT635614	Homo sapiens	Homo sapiens	hsa-miR-638	ZNF439	Y	1	1
MIRT635894	Homo sapiens	Homo sapiens	hsa-miR-638	SP110	Y	1	1
MIRT636188	Homo sapiens	Homo sapiens	hsa-miR-638	ELOC	Y	1	1
MIRT648740	Homo sapiens	Homo sapiens	hsa-miR-638	FAM46B	Y	1	1
MIRT656751	Homo sapiens	Homo sapiens	hsa-miR-638	LDHA	Y	1	1
MIRT658003	Homo sapiens	Homo sapiens	hsa-miR-638	GALNT10	Y	1	1
MIRT680073	Homo sapiens	Homo sapiens	hsa-miR-638	THAP1	Y	1	1
MIRT685183	Homo sapiens	Homo sapiens	hsa-miR-638	DCTN5	Y	1	1
MIRT687843	Homo sapiens	Homo sapiens	hsa-miR-638	ISCA2	Y	1	1
MIRT698386	Homo sapiens	Homo sapiens	hsa-miR-638	TMED2	Y	1	1
MIRT701342	Homo sapiens	Homo sapiens	hsa-miR-638	NR4A3	Y	1	1
MIRT712723	Homo sapiens	Homo sapiens	hsa-miR-638	NCAPG2	Y	1	1
MIRT714975	Homo sapiens	Homo sapiens	hsa-miR-638	RAB21	Y	1	1

MIRT718086	Homo sapiens	Homo sapiens	hsa-miR-638	ABHD12	Y	1	1
MIRT720058	Homo sapiens	Homo sapiens	hsa-miR-638	CD4	Y	1	1
MIRT722107	Homo sapiens	Homo sapiens	hsa-miR-638	RPS6KA1	Y	1	1
MIRT723712	Homo sapiens	Homo sapiens	hsa-miR-638	PEAK1	Y	1	1
MIRT752709	Homo sapiens	Homo sapiens	hsa-miR-638	GPAT4	Y	1	1
MIRT752710	Homo sapiens	Homo sapiens	hsa-miR-638	HOXB6	Y	1	1
MIRT752711	Homo sapiens	Homo sapiens	hsa-miR-638	INAFM1	Y	1	1
MIRT776149	Homo sapiens	Homo sapiens	hsa-miR-638	BLOC1S4	Y	1	1
MIRT776150	Homo sapiens	Homo sapiens	hsa-miR-638	BMPR1A	Y	1	1
MIRT776151	Homo sapiens	Homo sapiens	hsa-miR-638	MFGE8	Y	1	1

Supplementary Table 4: TarBase v.8 for has-miR-638

Interactions: 53, Experiments: 16 (low: 13, high: 3, unknown: 0) Cell lines: 7, Tissues: 5, Publications: 4

Gene name	miRNA name	Experiments	Publications	Cell lines	Tissues	Pred. Score
		throughput				
TCERG1L	hsa-miR-638	low: 1 high: 0	1	1	1	0.897
SOX2	hsa-miR-638	low: 5 high: 0	1	2	1	0.648
HNRNPA0	hsa-miR-638	low: 0 high: 1	1	1	1	0.623
PNKD	hsa-miR-638	low: 0 high: 1	1	1	1	0.622
NIPBL	hsa-miR-638	low: 0 high: 1	1	1	1	0.594
LAMC1	hsa-miR-638	low: 0 high: 1	1	1	1	0.585
PATL1	hsa-miR-638	low: 0 high: 1	1	1	1	0.578
PDE4DIP	hsa-miR-638	low: 0 high: 2	1	2	1	0.565
GYS1	hsa-miR-638	low: 0 high: 1	1	1	1	0.551
VCP	hsa-miR-638	low: 0 high: 1	1	1	1	0.518
ZBED4	hsa-miR-638	low: 0 high: 1	1	1	1	0.497
SIX4	hsa-miR-638	low: 0 high: 1	1	1	1	0.479
NAMPT	hsa-miR-638	low: 0 high: 1	1	1	1	0.469
CFL1	hsa-miR-638	low: 0 high: 1	1	1	1	0.46
SP2	hsa-miR-638	low: 6 high: 0	1	2	2	-
TJP1	hsa-miR-638	low: 1 high: 0	1	1	1	-
CDH1	hsa-miR-638	low: 1 high: 0	1	1	1	-
VIM	hsa-miR-638	low: 1 high: 0	1	1	1	-
PLXDC2	hsa-miR-638	low: 1 high: 0	1	1	1	-
WDR47	hsa-miR-638	low: 1 high: 0	1	1	1	-

PRKAR1A	hsa-miR-638	low: 0 high: 2	2	2	2 -
DPP9	hsa-miR-638	low: 0 high: 2	1	2	1 -
TADA2B	hsa-miR-638	low: 0 high: 1	1	1	1 -
PRPF6	hsa-miR-638	low: 0 high: 1	1	1	1 -
SETD5	hsa-miR-638	low: 0 high: 1	1	1	1 -
HSPA14	hsa-miR-638	low: 0 high: 1	1	1	1 -
USP54	hsa-miR-638	low: 0 high: 1	1	1	1 -
KIF1B	hsa-miR-638	low: 0 high: 1	1	1	1 -
BARX2	hsa-miR-638	low: 0 high: 1	1	1	1 -
RASA2	hsa-miR-638	low: 0 high: 1	1	1	1 -
RPS4X	hsa-miR-638	low: 0 high: 1	1	1	1 -
ATP5F1	hsa-miR-638	low: 0 high: 1	1	1	1 -
ATF4	hsa-miR-638	low: 0 high: 1	1	1	1 -
NDUFB4	hsa-miR-638	low: 0 high: 1	1	1	1 -
PRPS2	hsa-miR-638	low: 0 high: 1	1	1	1 -
GRB10	hsa-miR-638	low: 0 high: 1	1	1	1 -
EIF4G1	hsa-miR-638	low: 0 high: 1	1	1	1 -
PLEC	hsa-miR-638	low: 0 high: 1	1	1	1 -
RBM4	hsa-miR-638	low: 0 high: 1	1	1	1 -
KLF6	hsa-miR-638	low: 0 high: 1	1	1	1 -
SMC1A	hsa-miR-638	low: 0 high: 1	1	1	1 -
ADNP	hsa-miR-638	low: 0 high: 1	1	1	1 -
NDE1	hsa-miR-638	low: 0 high: 1	1	1	1 -
PANK3	hsa-miR-638	low: 0 high: 1	1	1	1 -

REPS1	hsa-miR-638	low: 0 high: 1	1	1	1	-
RNF19A	hsa-miR-638	low: 0 high: 1	1	1	1	-
RCOR1	hsa-miR-638	low: 0 high: 1	1	1	1	-
SNRNP48	hsa-miR-638	low: 0 high: 1	1	1	1	-
SIPA1L1	hsa-miR-638	low: 0 high: 1	1	1	1	-
BRPF3	hsa-miR-638	low: 0 high: 1	1	1	1	-
MAZ	hsa-miR-638	low: 0 high: 1	1	1	1	-
ANAPC13	hsa-miR-638	low: 0 high: 1	1	1	1	-
MGAT4A	hsa-miR-638	low: 0 high: 1	1	1	1	-

Supplementary Table 5: Validated miRNA-target interactions of has-miR-638 from miRWalk 2.0

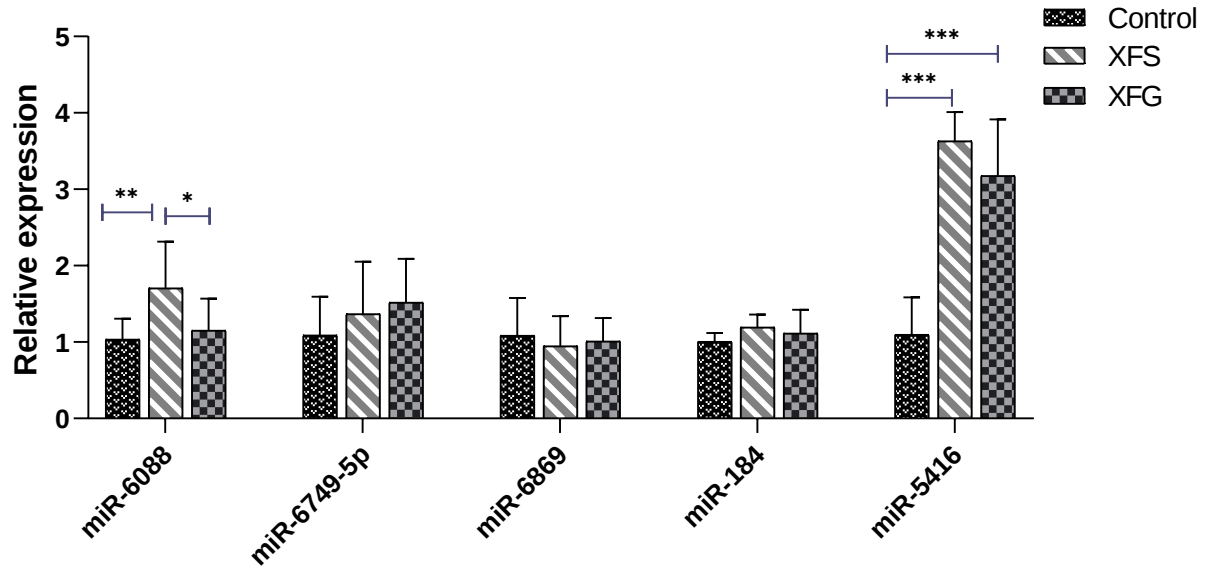
miRNA	MIMATID	Gene	EntrezID	PMID
hsa-miR-638	MIMAT0003308	CNGA2	1260	22291592
hsa-miR-638	MIMAT0003308	GPR137C	283554	22012620
hsa-miR-638	MIMAT0003308	HIST2H4B	554313	23592263
hsa-miR-638	MIMAT0003308	PDE4C	5143	22291592
hsa-miR-638	MIMAT0003308	NCAPG2	54892	19536157
hsa-miR-638	MIMAT0003308	OSCP1	127700	23554459
hsa-miR-638	MIMAT0003308	FUT10	84750	23592263
hsa-miR-638	MIMAT0003308	CNGA2	1260	23708386
hsa-miR-638	MIMAT0003308	RAB21	23011	19536157
hsa-miR-638	MIMAT0003308	HIST2H4A	8370	23446348
hsa-miR-638	MIMAT0003308	ZNF275	10838	23592263
hsa-miR-638	MIMAT0003308	SP2	6668	24623314

hsa-miR-638	MIMAT0003308	ABHD12	26090	19536157
hsa-miR-638	MIMAT0003308	HIST2H4B	554313	23446348
hsa-miR-638	MIMAT0003308	PPP1R15B	84919	23592263
hsa-miR-638	MIMAT0003308	SOX2	6657	24885288
hsa-miR-638	MIMAT0003308	CD4	920	19536157
hsa-miR-638	MIMAT0003308	PPP1R15B	84919	23446348
hsa-miR-638	MIMAT0003308	LMBR1L	55716	23592263
hsa-miR-638	MIMAT0003308	RPS6KA1	6195	19536157
hsa-miR-638	MIMAT0003308	HMGN5	79366	23313552
hsa-miR-638	MIMAT0003308	CNNM4	26504	23592263
hsa-miR-638	MIMAT0003308	PEAK1	79834	19536157
hsa-miR-638	MIMAT0003308	DCTN5	84516	23313552
hsa-miR-638	MIMAT0003308	PPM1F	9647	23592263
hsa-miR-638	MIMAT0003308	HIST2H4A	8370	20371350
hsa-miR-638	MIMAT0003308	ISCA2	122961	23313552
hsa-miR-638	MIMAT0003308	FAM26E	254228	23824327
hsa-miR-638	MIMAT0003308	HMGN5	79366	23824327
hsa-miR-638	MIMAT0003308	HIST2H4B	554313	20371350
hsa-miR-638	MIMAT0003308	TMED2	10959	23313552
hsa-miR-638	MIMAT0003308	ZNF439	90594	23824327
hsa-miR-638	MIMAT0003308	PPP1R15B	84919	20371350
hsa-miR-638	MIMAT0003308	NR4A3	8013	23313552
hsa-miR-638	MIMAT0003308	SP110	3431	23824327
hsa-miR-638	MIMAT0003308	PDE4C	5143	20371350

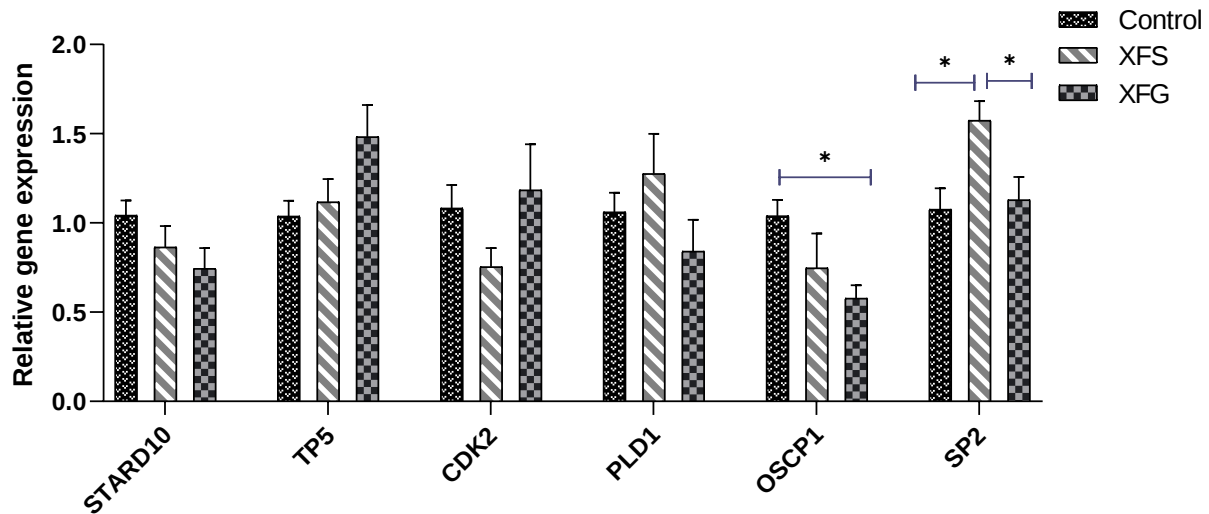
hsa-miR-638	MIMAT0003308	ZNF275	10838	21572407
hsa-miR-638	MIMAT0003308	TCEB1	6921	23824327
hsa-miR-638	MIMAT0003308	STC2	8614	20371350
hsa-miR-638	MIMAT0003308	PPP1R15B	84919	21572407
hsa-miR-638	MIMAT0003308	FAM46B	115572	23824327
hsa-miR-638	MIMAT0003308	MIER3	166968	20371350
hsa-miR-638	MIMAT0003308	NUP155	9631	21572407
hsa-miR-638	MIMAT0003308	LDHA	3939	23824327
hsa-miR-638	MIMAT0003308	SOD2	6648	22012620
hsa-miR-638	MIMAT0003308	OTUD7B	56957	21572407
hsa-miR-638	MIMAT0003308	GALNT10	55568	23824327
hsa-miR-638	MIMAT0003308	BUB1	699	22012620
hsa-miR-638	MIMAT0003308	STC2	8614	21572407
hsa-miR-638	MIMAT0003308	THAP1	55145	23824327
hsa-miR-638	MIMAT0003308	SULT1B1	27284	22012620
hsa-miR-638	MIMAT0003308	HIST2H4A	8370	23592263

Validation of miRNA microarray profile by q-PCR

Supplementary Figure 1: Quantitative real-time PCR validation of miRNA expression profile results in anterior lens capsule.



Supplementary Figure 2: Quantitative real-time PCR validation of gene expression in anterior lens capsule.

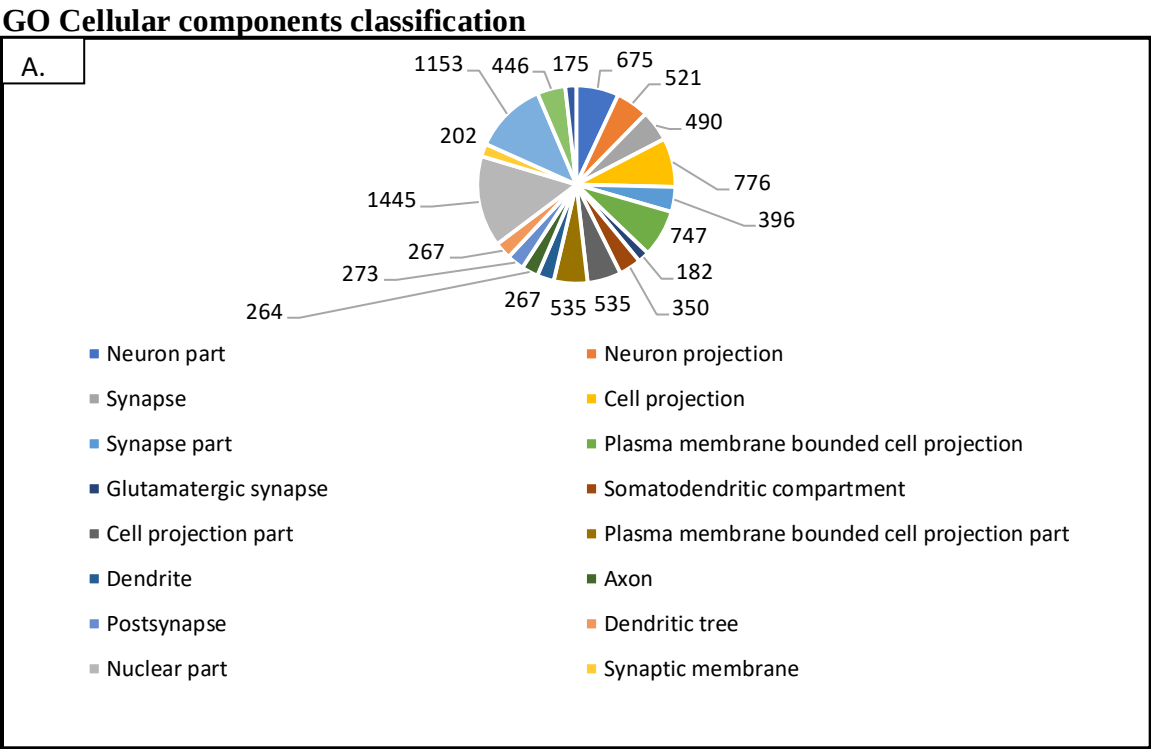


PLD1= Phospholipase D1; OSCP1= Organic solute carrier partner1; CDK2= Cyclin dependent kinase2; TP53INP2= Tumor protein53-induced nuclear protein 2; STARD10= StAR related lipid transfer domain containing 10; SP2=Sp2 Transcription factor

Bioinformatic analysis of target genes

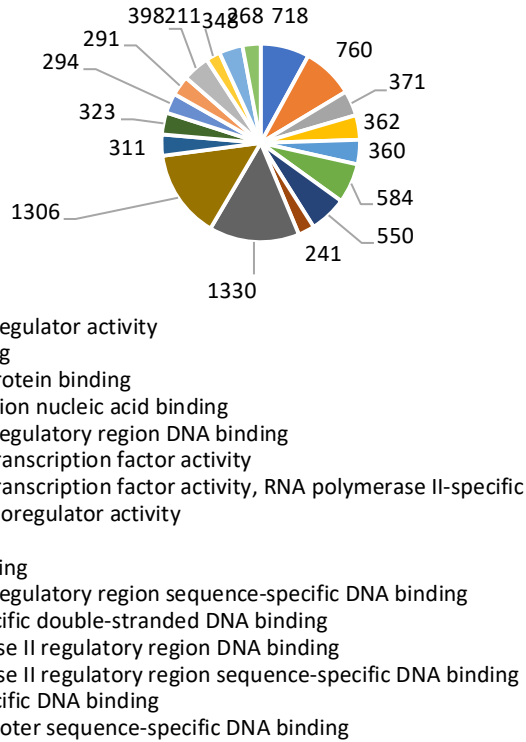
The miRanda algorithm was used for finding genomic targets for miRNAs. For each miRNA, target genes were selected on the basis of sequence complementarity, free energies of RNA-miRNA duplexes, and conservation of target sites in related genomes. To understand the potential functions of significantly differentially expressed miRNA in XFS and XFG, GO enrichment analysis was performed (using Kegg color pathway https://www.genome.jp/kegg/tool/map_pathway2.html.) for the genes regulated by miRNAs in XFG groups. Molecular function, biological processes and cellular components were studied in detail (Supplementary Figure3). In cellular component category, GO terms were related to neuron part, neuron projection, synapse, cell projection and synapse part. Most of the top molecular functional activities were transcription regulator activity, enzyme binding, cytoskeletal protein binding, regulatory region nucleic acid binding and transcription regulatory region DNA binding. Biological processes terms were enriched with nervous system development, anatomical structure morphogenesis, neurogenesis, cell development and generation of neurons. KEGG pathway enrichment analysis showed role in microRNAs in cancer, MAPK signaling pathway, signaling pathways regulating pluripotency of stem cells, pathways in cancer and focal adhesion (Supplementary Figure 3).

Supplementary Figure 3: Gene Ontology (GO) analysis of miRNA target genes in pseudoexfoliation glaucoma according to A. Cellular component, B. Molecular function, C. Biological processes and D. Kegg pathway enrichment classification.



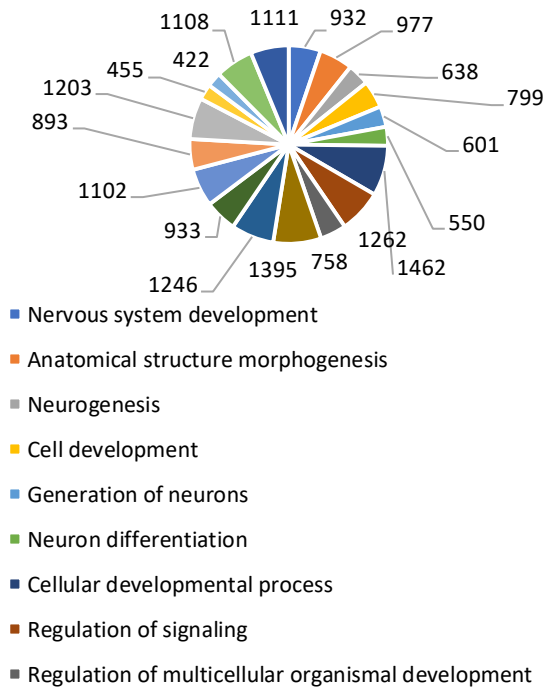
GO Molecular Function Classification

B.



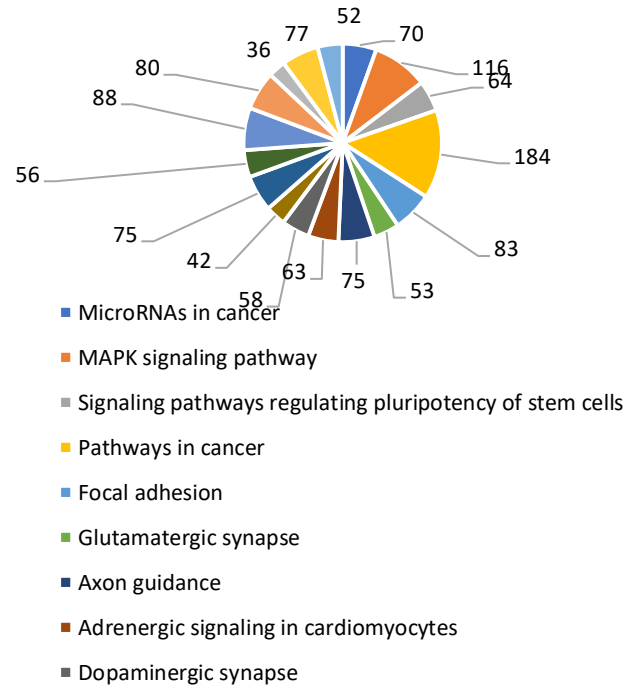
GO Biological Process classification

C.



Kegg Pathway enrichment classification

D.



All the categories were significantly enriched (FDR <0.05).

FDR=false discovery rate; XFS= Pseudoexfoliation syndrome; XFG= Pseudoexfoliation Glaucoma.