

Long noncoding RNA PPT2-EGFL8 regulates pathological retinal neovascularization in PDR by functioning as a competing endogenous RNA

Running title: LncRNA PPT2-EGFL8 regulates angiogenesis in PDR

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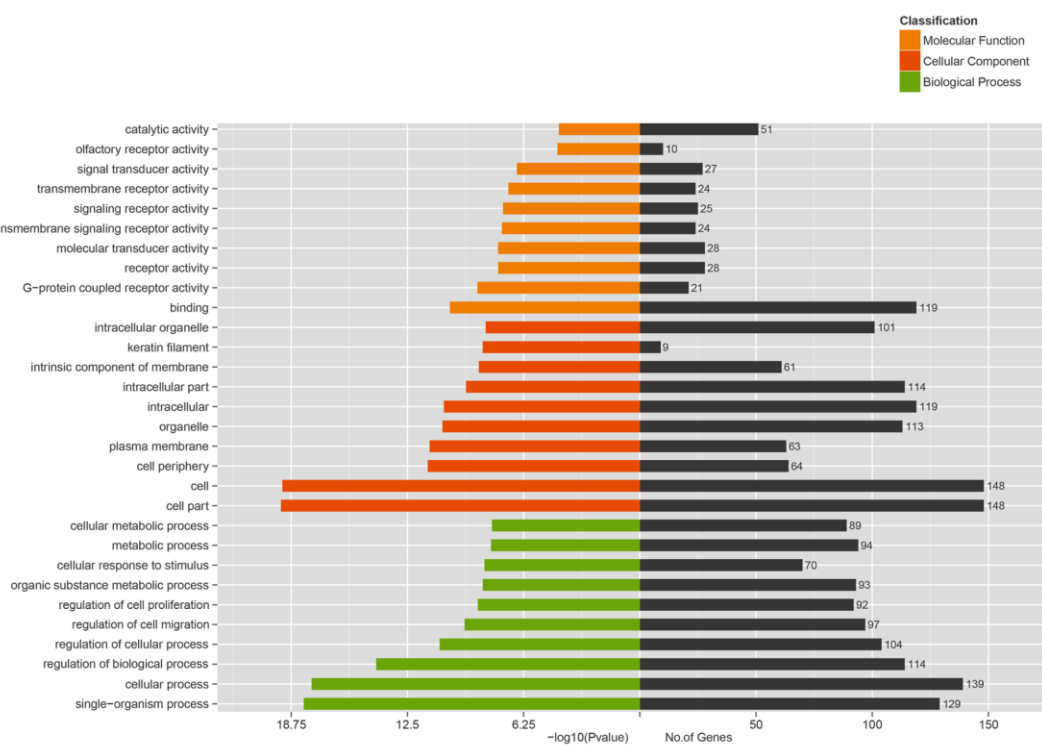
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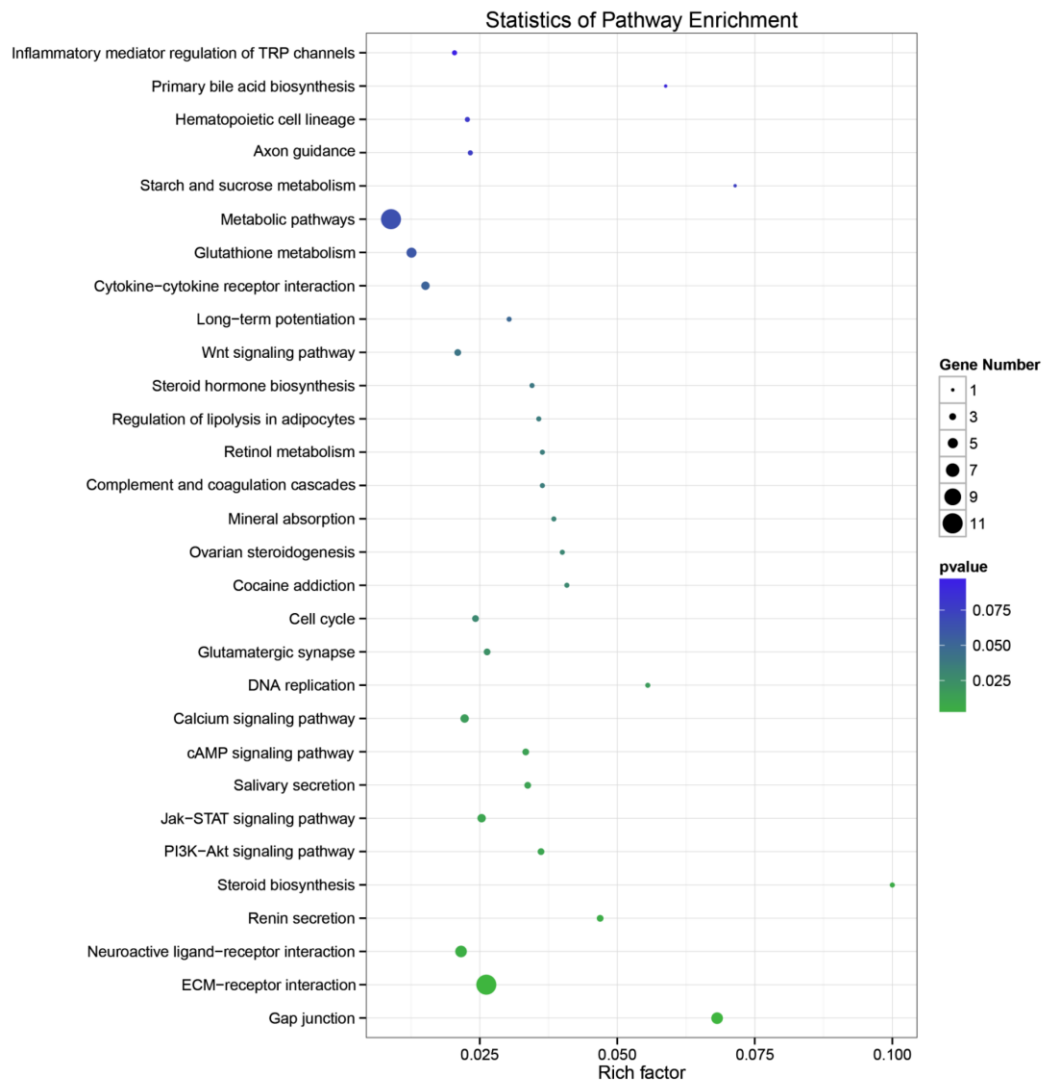
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Supplementary Figures



Supplementary Figure 1. The histogram of the category of enriched GO

terms for the down-regulated genes.

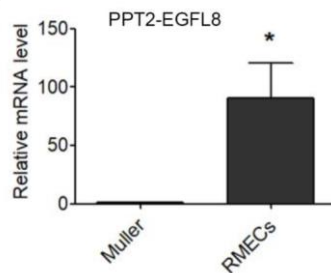


Supplementary Figure 2. The histogram of KEGG enrichment analysis associated to down-regulated lncRNAs.

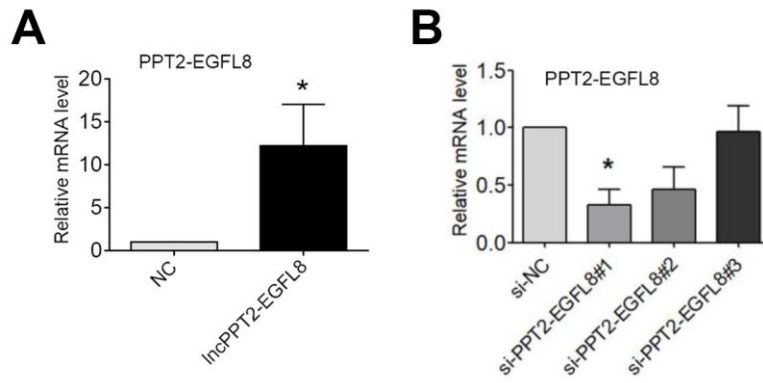
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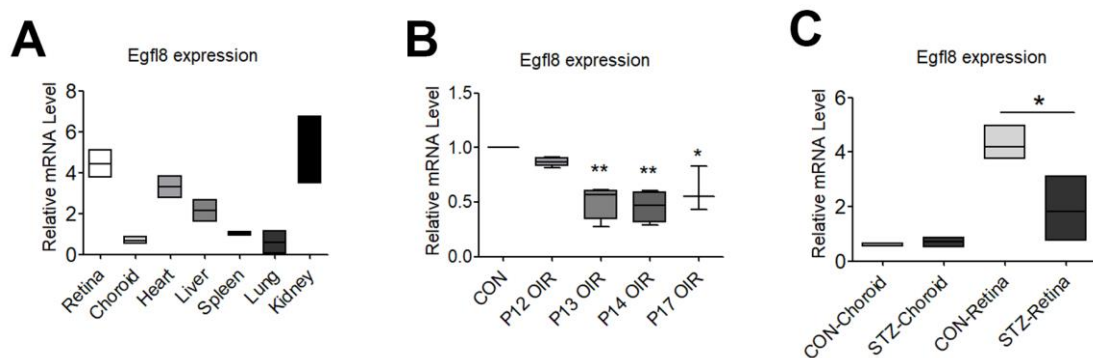
Supplementary Figure 3. PPT2-EGFL8 sequence (2728 nt).



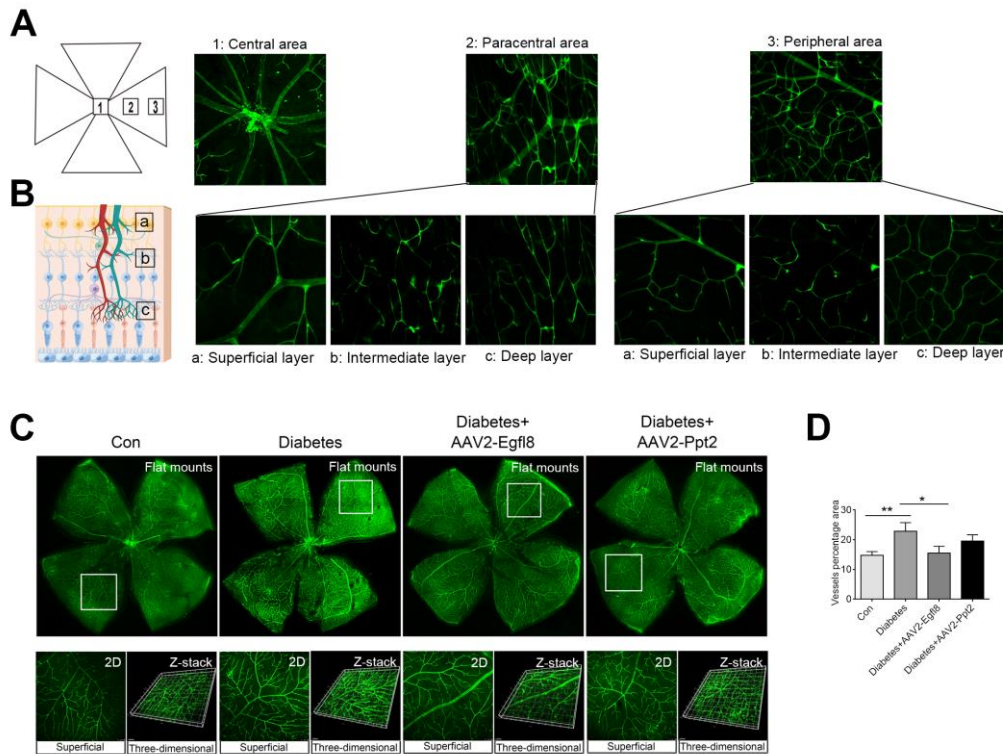
Supplementary Figure 4. Relative mRNA expression of PPT2-EGFL8 expression in hRMECs and Muller cells; n = 3. The results are presented as means \pm SEM; *p<0.05 for each pair of groups indicated.



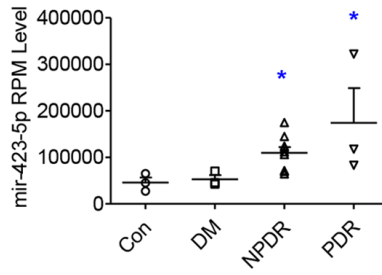
Supplementary Figure 5. (A, B) Relative mRNA expression of PPT2-EGFL8 expression in PPT2-EGFL8 overexpressed (A) and siRNA transfected (B) hRMECs; n = 3. The results are presented as means \pm SEM; *p<0.05 for each pair of groups indicated.



Supplementary Figure 6. (A, B, C) Relative mRNA expression of PPT2-EGFL8 expression in mice; n = 6. The results are presented as means \pm SEM; *p<0.05 and **p<0.01 for each pair of groups indicated.



Supplementary Figure 7. (A, B) A schematic presentation of the positions analyzed on the retinal blood vessel density in horizontal and vertical direction. (C) IB4 staining of whole-mount retinae (upper), the superficial retinal layer of peripheral-area retina (Down Left) and the 3-dimensional (3D) image (Down Right) in control, STZ-induced diabetes, AAV2-Egfl8-treated and AAV2-Ppt2-treated mice. (D) The vessels percentage area of IB4 staining were determined (n = 6). The results are presented as means \pm SEM; * $p < 0.05$ and ** $p < 0.01$ for each pair of groups indicated.



Supplementary Figure 8. The expression profile of miR-423-5p detected by the next-generation sequencing in the serum of healthy individuals or individuals with diabetes mellitus without diabetic retinopathy, NPDR and PDR. The results are presented as means \pm SEM; * $p < 0.05$ for each pair of groups indicated.

Supplementary Tables

Supplementary Table 1. siRNA target sequence

	siRNA target sequence
si-PPT2-EGFL8	GGGTGAGCTCTTCTCAGGA
si-PPARD	ACCCTTCAGTGATATCATT

Supplementary Table 2. Sequences of microRNA mimics and the negative control.

Name	Sequences
hsa-mir-423-5p	5'-UGA GGG GCA GAG AGC GAG ACU UU-3'
mimics	5'-AGU CUC GCU CUC UGC CCC UCA UU-3'
hsa-mir-6827-5p	5'-UGGGAGCCAUGAGGGUCUGUGC-3'
mimics	5'-ACAGACCCUCAUGGCUCCCAUU-3'
hsa-mir-6929-5p	5'-UGGGCUGCUGAGAAGGGGCA-3'
mimics	5'-CCCCUUCUCAGCAGCCCAUU-3'
Negative control	5'-UUCUCCGAACGUGUCACGUTT-3' 5'-ACGUGACACGUUCGGAGAATT-3'

Supplementary Table 3. Antibodies sources

Antibodies	Source	Host	Cat #
PPARD	Abcam	Rabbit	ab178866
ANGPTL4	Abcam	Rabbit	ab206420
VEGFA	Abcam	Mouse	ab1316
GFAP	Sigma-Aldrich	Mouse	G3893
IBA1	Cell Signaling	Rabbit	17198
β -actin	Sigma-Aldrich	Rabbit	A5316

Supplementary Table 4. Primer sequences and conditions for conventional RT-PCR

Genes	Forward Primer (5'-3')	Reverse Primer (5'-3')
ENSG00000232633.3	GTACACCTTGGGCTGG TAGG	GCTGTCCGACTTCAGCTTC T
TEN1-CDK3	AGGAAACAGCAGCCTA GCAA	TGAGCCAATCCCAAAGTAG G
has-PPT2-EGFL8	CCAGCTTCTTTGGTTT CTATGATG CAGGTACAAGGGTTCC	CTCAGCAGAAGCAGCTCAG ACT CTCCATACTGGCCCATCTG
mus-PPT2	GAGA AAGCAGACAGCGAAGA	T CACCCAGTCTTTCCTCCAG
mus-EGFL8	GGAG	T
miR-423-5p	CAGTGCGTGTCGTGGA GT	GCCCTGAGGGGCAGAGAG C
miR-6827-5p	CGTGGGAGCCATGAG GGT	AGTGCAGGGTCCGAGGTAT T
miR-6829-5p	GCGTGGGCTGCTGAG AA	AGTGCAGGGTCCGAGGTAT T
PPARD	ACTGAGTTCGCCAAGA GCATC	ACGCCATACTTGAGAAGGG TAA
ANGPTL4	GGCTCAGTGGACTTCA ACCG	CCGTGATGCTATGCACCTT CT
VEGFA	ACAGGTACAGGGATGA GGACAC	AAGCAGGTGAGAGTAAGCG AAG
GAPDH	ATCACCATCTTCCAGG AGCG	CGCCTGCTTCACCACCTTC TTG
U6	TGCGGGTGCTCGCTTC GGCAGC	CCAGTGCAGGGTCCGAGG T