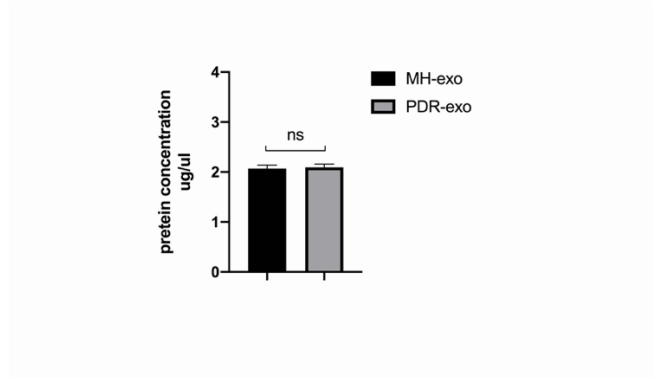


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Supplemental information

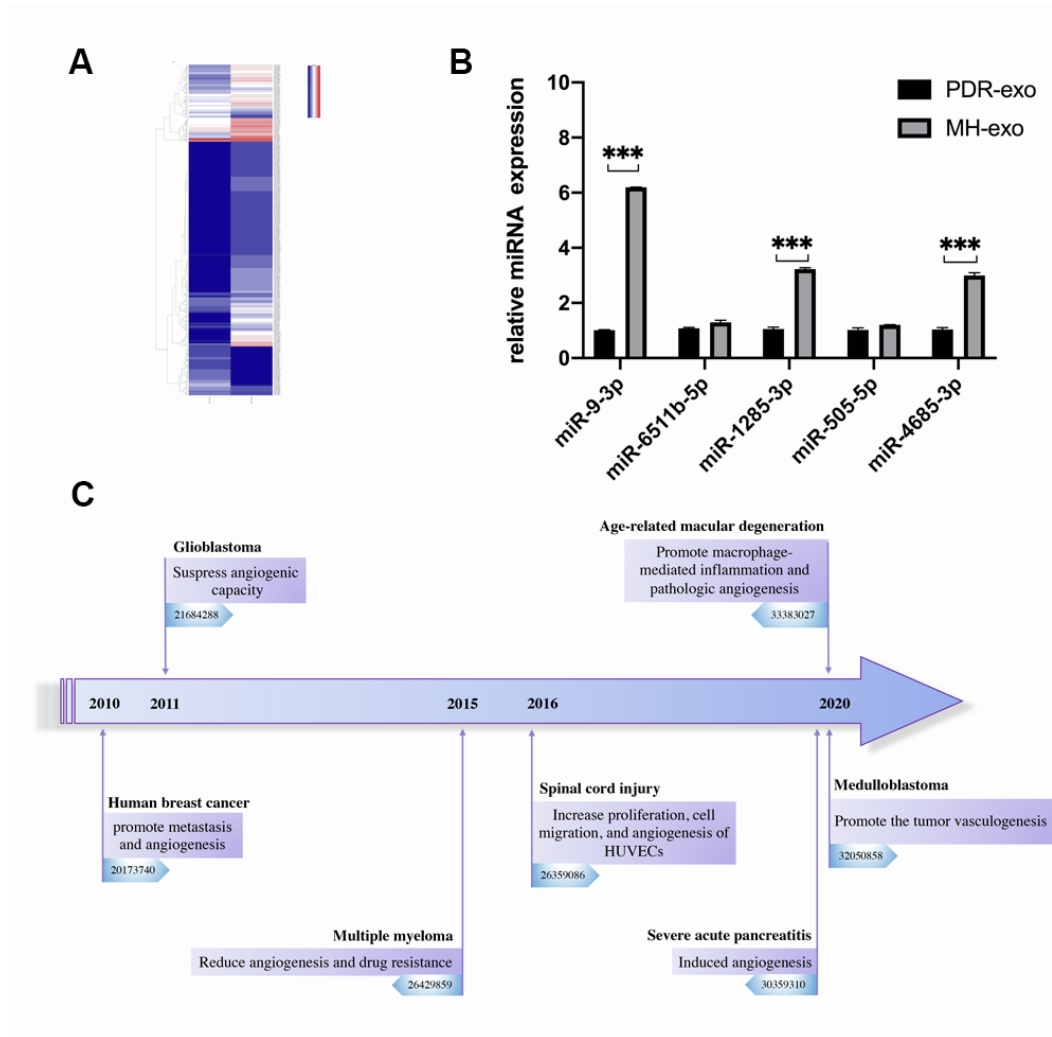
**Müller glia-derived exosomal miR-9-3p promotes
angiogenesis by restricting sphingosine-1-phosphate
receptor S1P₁ in diabetic retinopathy**

Yu Liu, Qin Yang, Haixin Fu, Jingfan Wang, Songtao Yuan, Xinsheng Li, Ping Xie, Zizhong Hu, and Qinghuai Liu



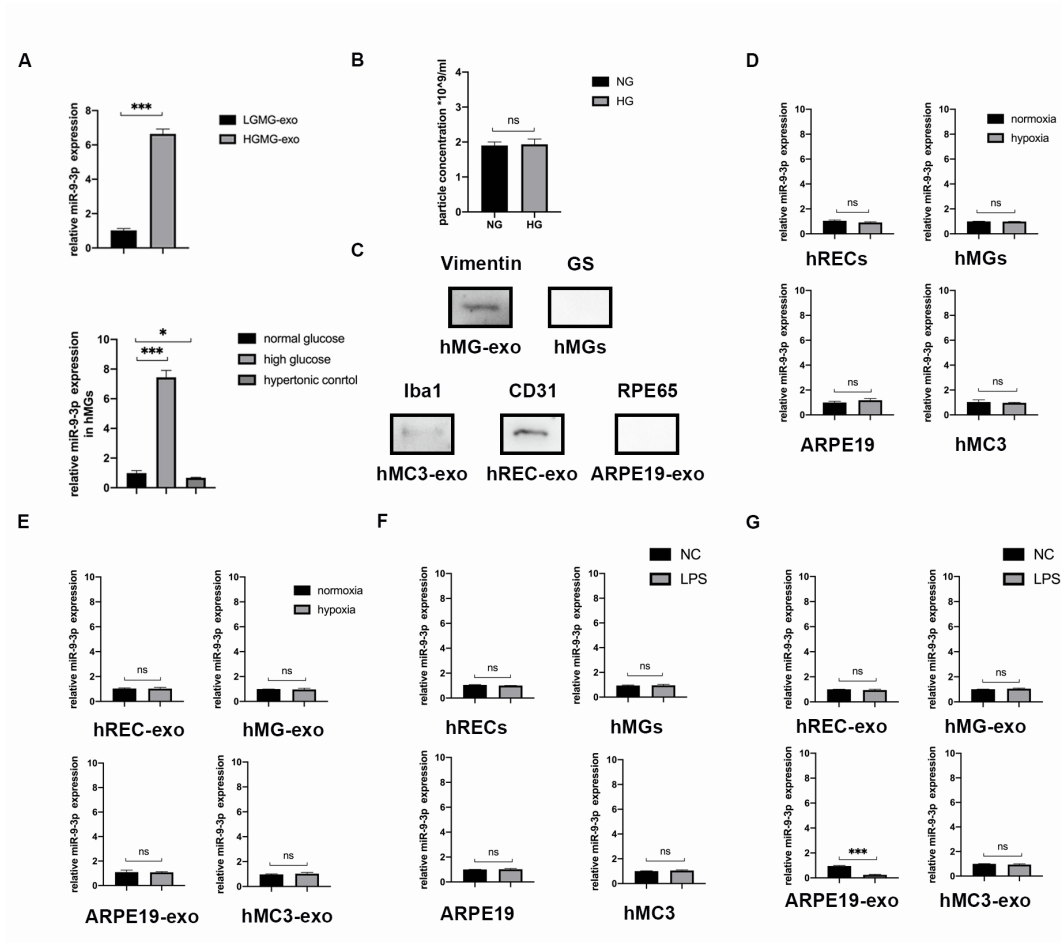
Supplementary Figure 1 Quantitation of protein level of MH-exo and PDR-exo

BCA results determined that nonsignificant difference was found in concentration of proteins level of MH-exo and PDR-exo ($p>0.05$).



Supplementary Figure 2 Exosomal miR-9-3p was markedly upregulated in the vitreous humor of PDR

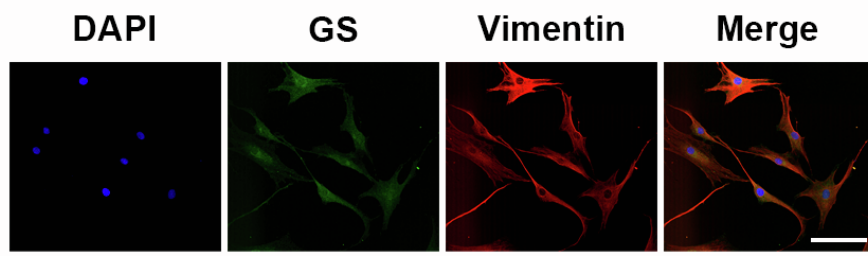
(A) Heat map indicated that 82 miRNAs were up-regulated and 352 miRNAs were downregulated in the PDR-exo compared with the MH-exo. (B) RT-qPCR validated the expression level of top 5 upregulated exosomal miRNAs in the vitreous humor of PDR compared to MH patients. *** $p < 0.001$, $n = 3$. (C) Timeline highlighting the advances in miR-9-3p. From being reported to promote angiogenesis in human breast cancer in 2010, there has been advances on the physical characteristics and biofunction of miR-9-3p in multiple diseases.



Supplementary Figure 3 Expression pattern of miR-9-3p in multiple retinal cell types

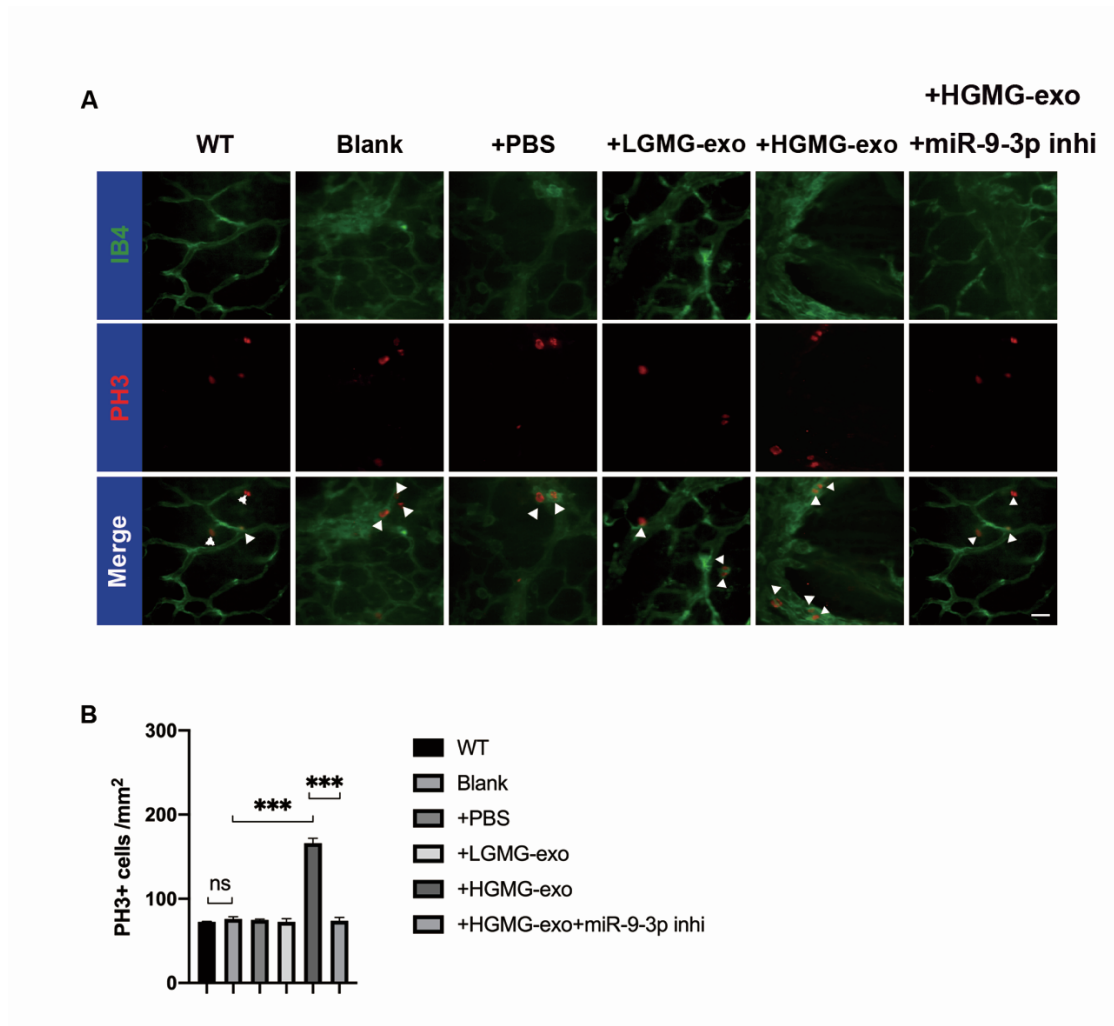
(A) Upper panel: RT-qPCR results validated that the expression level of miR-9-3p was higher in HGMG-exo than in LGMG-exo. Lower panel: Hyperosmolar status induced by 5.5 mM glucose and 24.5 mM mannitol did not alter the expression level of miR-9-3p in hMGs. (B) Nanoparticle tracking analysis confirmed that there was no difference between the particle concentration of vesicles secreted from hMGs under low (5.5mM) and high glucose (30mM). (C) Specific cell marker in cell derived exosomes as detected by western blot. Vimentin and Glutamine synthesis in müller cell derived exosomes, Iba1 in human microglial hMC3 cell derived exosomes, CD31 in endothelial cell in hRECs derived exosomes, and RPE65 in ARPE19 derived exosomes was detected. (D)-

(E) The miR-9-3p expression in four cell types or cell derived exosomes under hypoxia environment (1.5% O_2). (F)-(G) Under inflammation condition, the miR-9-3p expression was significantly downregulated in ARPE19 derived exosomes and maintain the same in the other three cell types. *** $p < 0.001$.



Supplementary Figure 4 Validation of primarily cultured mMGs at passage 1

Immunocytochemistry confirmed that expressions of vimentin and glutamine synthetase (GS) in MG. Scale bar = 20 μm .



Supplementary Figure 5 PH3 immunofluorescence is underpowered to detect endothelial proliferation in OIR model

(A)-(B) Compared with normal control, no significant increase was observed in OIR-blank or OIR-PBS group using PH3 immunofluorescence ($p > 0.05$)

Supplementary Table 1 Vitreous specimens were harvested for exosomal sequencing from 10 PDR patients and 15 sex-age matched MH patients

	Sex	Age (Years)	Volume of collected vitreous humor	Operation type
PDR1	Male	58	2ml	pars plana vitrectomy
PDR2	Female	37	2ml	pars plana vitrectomy
PDR3	Female	48	2ml	pars plana vitrectomy
PDR4	Male	51	2ml	pars plana vitrectomy
PDR5	Female	61	2ml	pars plana vitrectomy
PDR6	Male	58	2ml	pars plana vitrectomy
PDR7	Male	62	2ml	pars plana vitrectomy
PDR8	Female	61	2ml	pars plana vitrectomy
PDR9	Male	59	2ml	pars plana vitrectomy
PDR10	Female	63	2ml	pars plana vitrectomy
MH1	Male	51	2ml	pars plana vitrectomy
MH2	Female	63	2ml	pars plana vitrectomy
MH3	Female	64	2ml	pars plana vitrectomy
MH4	Male	65	2ml	pars plana vitrectomy
MH5	Male	61	2ml	pars plana vitrectomy
MH6	Female	57	2ml	pars plana vitrectomy
MH7	Male	46	2ml	pars plana vitrectomy
MH8	Male	61	2ml	pars plana vitrectomy
MH9	Female	55	2ml	pars plana vitrectomy
MH10	Male	51	2ml	pars plana vitrectomy
MH11	Male	61	2ml	pars plana vitrectomy
MH12	Male	60	2ml	pars plana vitrectomy
MH13	Female	49	2ml	pars plana vitrectomy
MH14	Male	60	2ml	pars plana vitrectomy
MH15	Female	62	2ml	pars plana vitrectomy

Supplementary Table 2 Clinic features of 65 PDR patients and 63 sex-age matched

MH patients.

Characteristic	Number	
	PDR	MH
Age (Years)		
45-49	10	11
50-54	18	18
55-59	22	20
60-65	15	14
Gender		
Male	32	33
Female	33	30

Supplementary Table 3 Total exosomal protein collected from PDR and MH patients

Characteristics	Replica 1		Replica 2		Replica 3	
	PDR	MH	PDR	MH	PDR	MH
Number	21	21	21	21	23	21
Volume (ml)	42	42	42	42	46	42
Total exosomal protein (ug)	60.0	59.7	61.9	60.8	67.2	60.6

Supplementary Table 4 RNA sequence of miR-9-3p mimic, inhibitor, mimic nc and inhibitor nc

Oligonucleotide	Forward	Rerverse
miR-9-3p mimic	AUAAAGCUAGAUACCGAAA GU	ACUUUCGGUUAUCUAGCUU UAU
miR-9-3p inhibitor	ACUUUCGGUUAUCUAGCUUU AU	CAGUACUUUUGUGUAGUAC AAA
Mimic-nc	UUUGUACUACACAAAAGUAC UG	
Inhi-nc	CAGUACUUUUGUGUAGUACA AA	