

Supplementary Material

Exosomal miR-320e alleviates cognitive impairment and depression in cerebral small-vessel disease through Wnt2-targeted inhibition of the Wnt/ β -catenin pathway

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1.1 Primer sequences

Gene	5'-3'
Wnt2	F:TCTCGGTGGAATCTGGCTCTGG R:CGGAAGTGGTGCTGGCATTCTG
FZD2	F:CGCTTCTTCCTGTGCTCCATGTAC R:ATCACGCTCGCCCAGAACTTG
Axin2	F:GCCTGCCACCAAGACCTACATAAG R:GGAGATAGCCACACACGACCTTTAG
GSK3 β	F:GGCTGTGTGTTGGCTGAATTGTTG R:ATGTCCTGCTCCTGGTGAGTCC
β -Catenin	F:GAGTGCTGAAGGTGCTATCTGTCTG R:GATGGCAGGCTCAGTGATGTCTTC

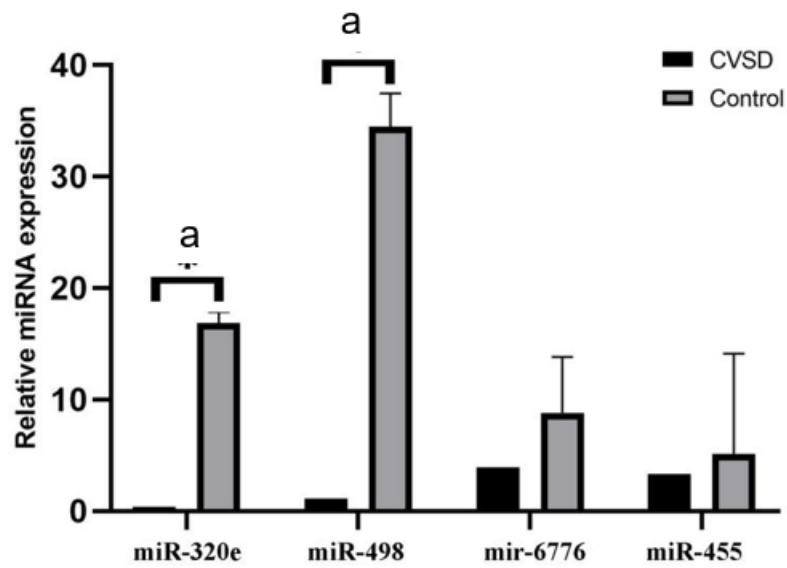
ACTB	F:TCGTGCGTGACATTAAGGAGAAGC
	R:GGCGTACAGGTCTTTGCGGATG

1.2 Comparison of general clinical data between groups

	Control (n = 40)	CVSD (n = 80)	T value	P value
Age (year, mean $\pm$ SD)	65.91 $\pm$ 10.13	66.47 $\pm$ 10.52	0.312	0.756
Male, n (%)	36 (54.5)	38 (57.6)		0.726
BMI, kg/m ² (mean $\pm$ SD)	25.76 $\pm$ 3.10	25.35 $\pm$ 3.92		0.574
Hypertension, n (%)	21 (31.8)	26 (39.4)		0.589
Diabetes, n (%)	7 (10.6)	9 (13.6)		0.594
TC, mmol/L, (mean $\pm$ SD)	4.45 $\pm$ 1.0	4.37 $\pm$ 1.1	0.457	0.648
Smoking, n (%)	9 (13.6)	11 (16.7)		0.627
Drinking, n (%)	7 (10.6)	15 (27.7)		0.108
TG, mmol/L (mean $\pm$ SD)	1.36 $\pm$ 0.96	1.66 $\pm$ 1.33		0.138
HDL, mmol/l (mean $\pm$ SD)	1.28 $\pm$ 0.38	1.28 $\pm$ 0.30		0.953
LDL, mmol/L (mean $\pm$ SD)	2.53 $\pm$ 0.80	2.58 $\pm$ 0.82		0.721
BUN, mmol/L (mean $\pm$ SD)	5.68 $\pm$ 2.60	5.36 $\pm$ 1.20		0.358
Cr, μ mol/L (mean $\pm$ SD)	55.90 $\pm$ 21.03	58.93 $\pm$ 26.32		0.469
Uric acid, μ mol/L (mean $\pm$ SD)	289.25 $\pm$ 75.59	314.40 $\pm$ 92.71		0.091

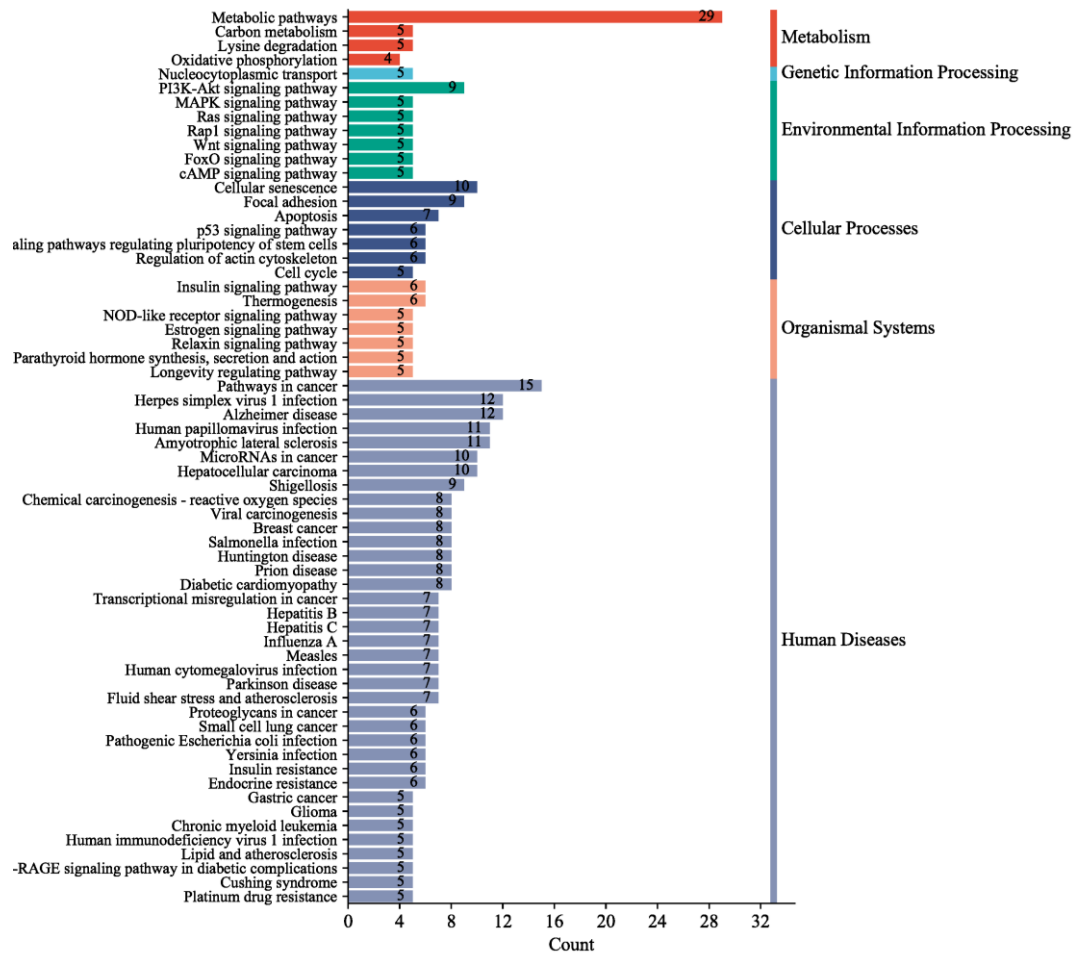
CVSD: Cerebral small vessel disease; BMI: Body mass index; TC: T cells; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; BUN: Blood urea nitrogen; CR: Creatinine.

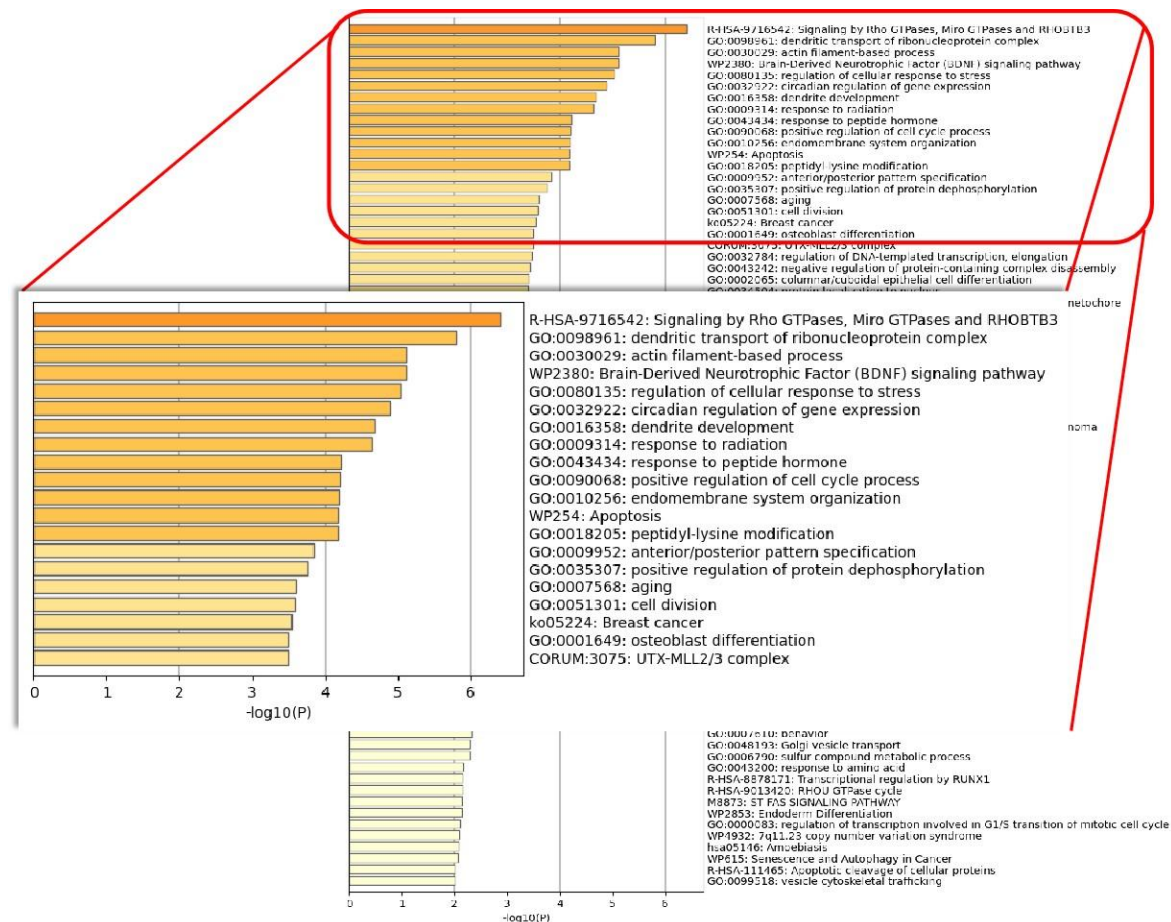
1.3 Validation of differential expression of plasma exosomal target miRNAs



Supplementary Figure 1 PCR results of differentially expressed miRNAs. ^a $P < 0.01$. PCR: Polymerase chain reaction.

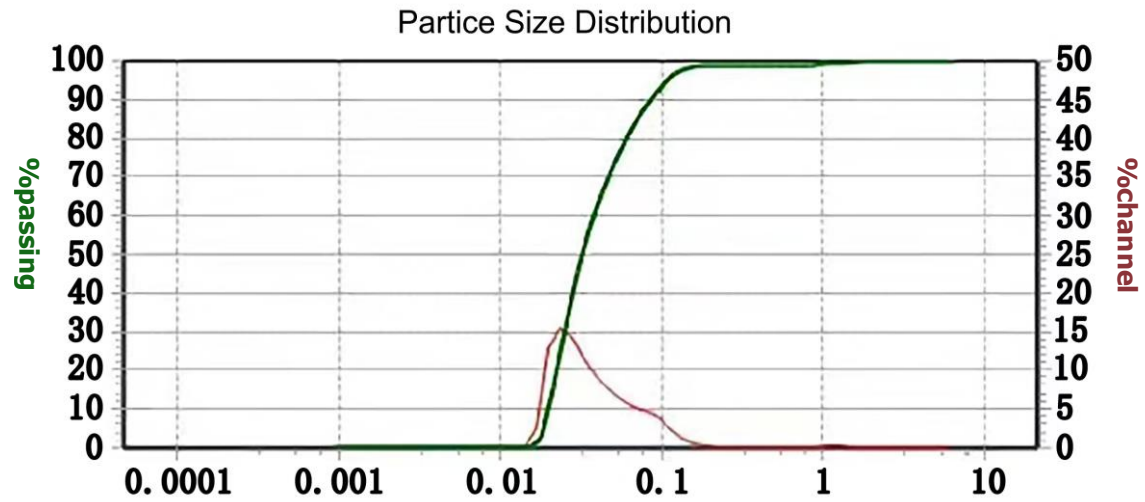
1.4 Bioinformatics analysis of miRNA-320e



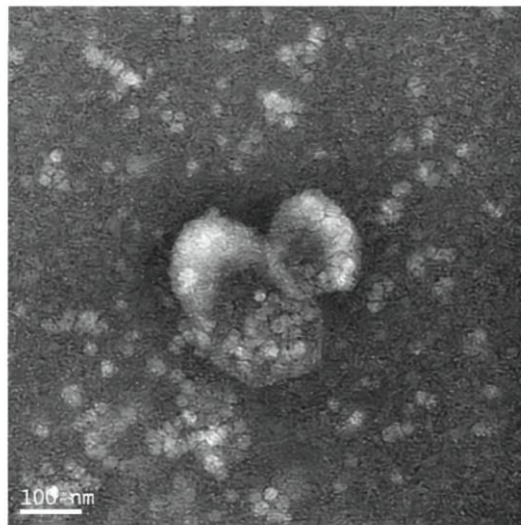


1.5 Identification of exosomes in plasma from CVSD patients

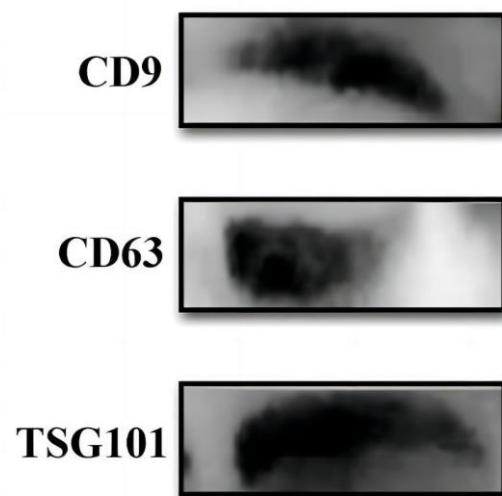
A



B



C



Supplementary Figure 2 miR-320e could bind to Wnt2 to inhibit the Wnt/ β -catenin pathway. A and B: The morphology of exosomes was observed by transmission electron microscopy. The shape and size of exosomes are shown above. The exosomes were round or oval in shape, and the particle size diagram also showed that the vesicle diameter was between 30 nm and 150 nm; C: We used western blotting to identify exosome membrane marker proteins, and the marker proteins selected were CD9, CD63, and TSG101.