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Supporting Information

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Astrocyte-Derived Extracellular Vesicular miR-143-3p Dampens Autophagic Degradation of Endothelial Adhesion Molecules and Promotes Neutrophil Transendothelial Migration after Acute Brain Injury

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Supplementary Materials for

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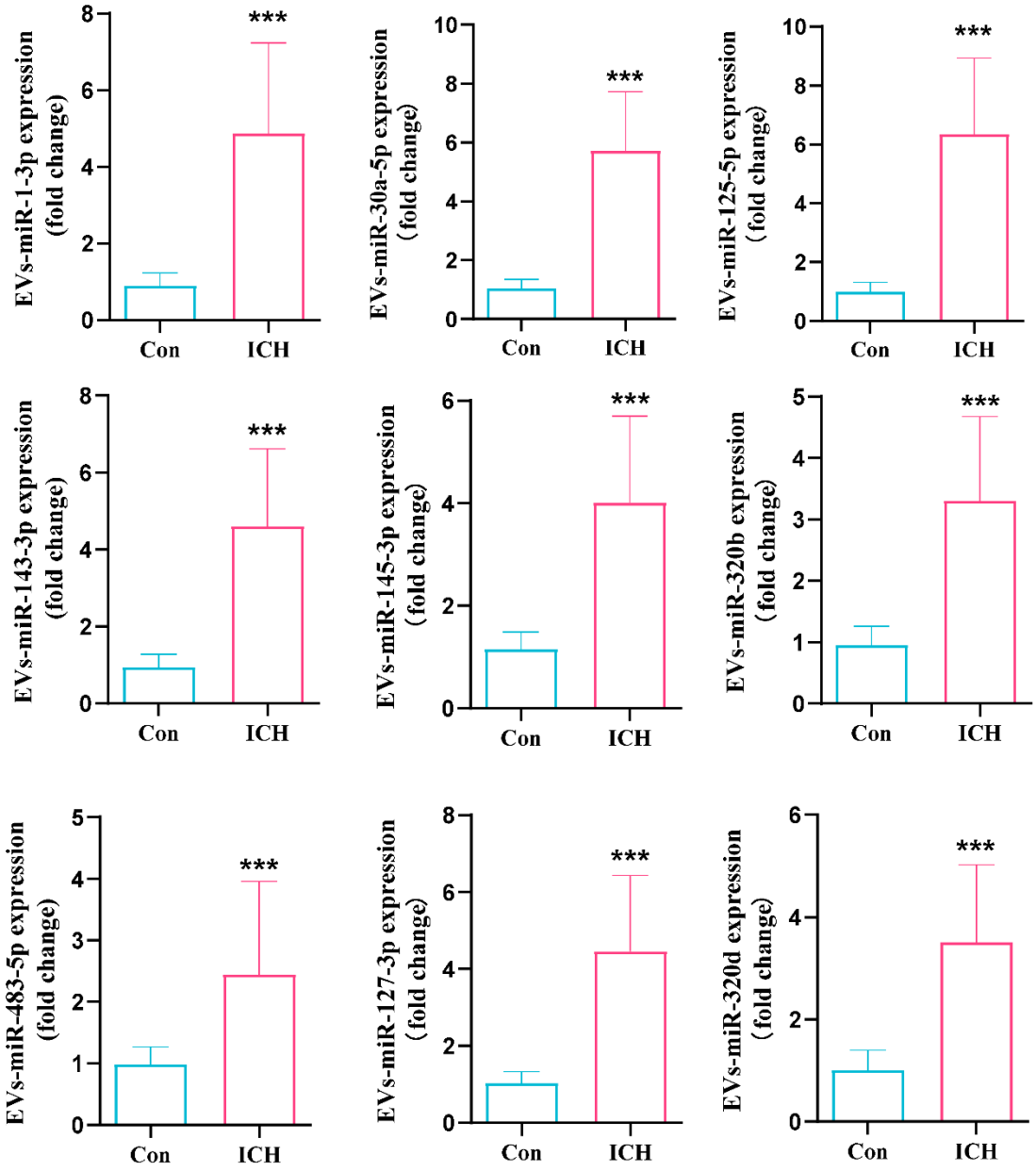

Figure S1. RT-qPCR analysis verify the differently expressed EVs-miRNAs between ICH patients and healthy controls (ICH = 76, control = 45; Student' s t-test). Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.

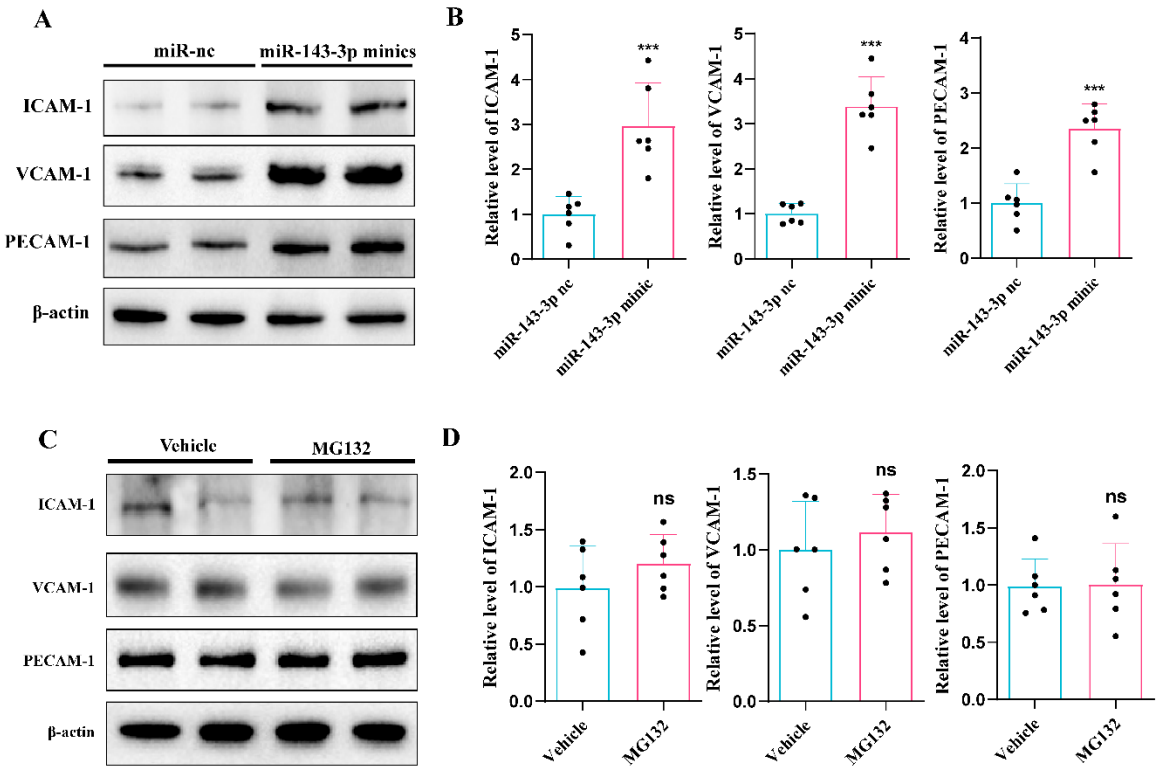

Figure S2. Detection of the levels of VCAM-1, ICAM-1 and PECAM-1 in BMECs. (A-B) Western blot assay of the expression of CAMs including VCAM-1, ICAM-1 and PECAM-1 in mouse bEnd.3 cells transfected with miR-143-3p minics or miR-nc (n =6 per group, Student' s t-test). (C-D) Detection of the levels of VCAM-1, ICAM-1, and PECAM-1 in hCMEC/D3 cells after the treatment of the proteasome inhibitor MG132 (n =6 per group, Student' s t-test). Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.

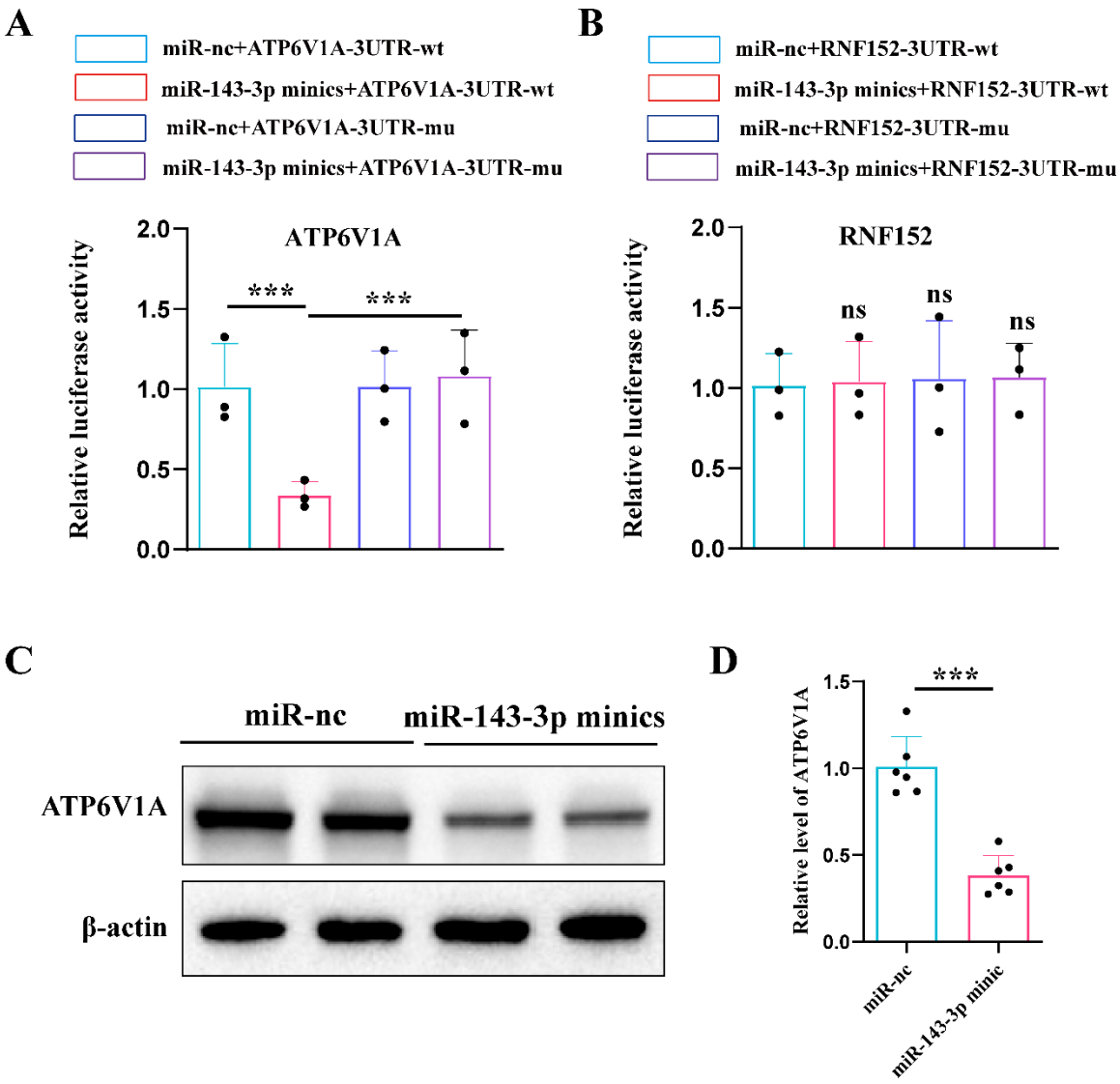

Figure S3. The regulation of miR-143-3p on ATP6V1A. (A) Dual-luciferase analysis in HEK293T cells co-transferred with miR-143-3p mimic and the wild-type or mutated ATP6V1A-3' UTR (n =5 per group, one-way ANOVA). (B) Dual-luciferase analysis in HEK293T cells co-transferred with miR-143-3p mimic and the wild-type or mutated RNF152-3' UTR (n =5 per group, one-way ANOVA). (C-D) Western blot analysis of the protein level of ATP6V1A in hCMEC/D3 cells transferred with the miR-nc or miR-143-3p mimic (n=6 per group, Student' s t-test). Data are presented as means ± SD. *p < 0.05,

**p < 0.01, ***p < 0.001.

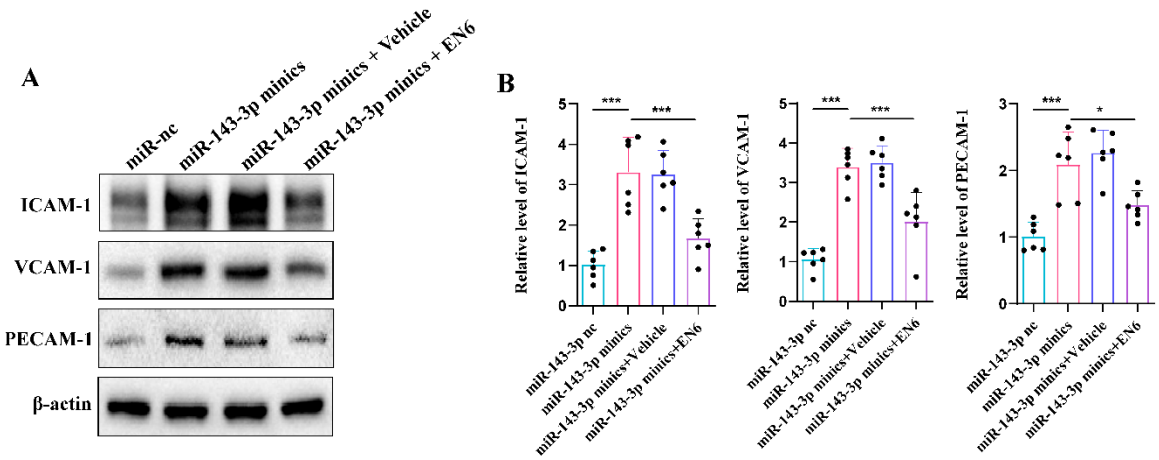

Figure S4. The effects of EN6 treatment on the protein level of VCAM-1, ICAM-1 and PECAM-1 in bEnd.3 cells transferred with miR-nc or miR-143-3p-minics (n =6 per group, one-way ANOVA). Data are presented as means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

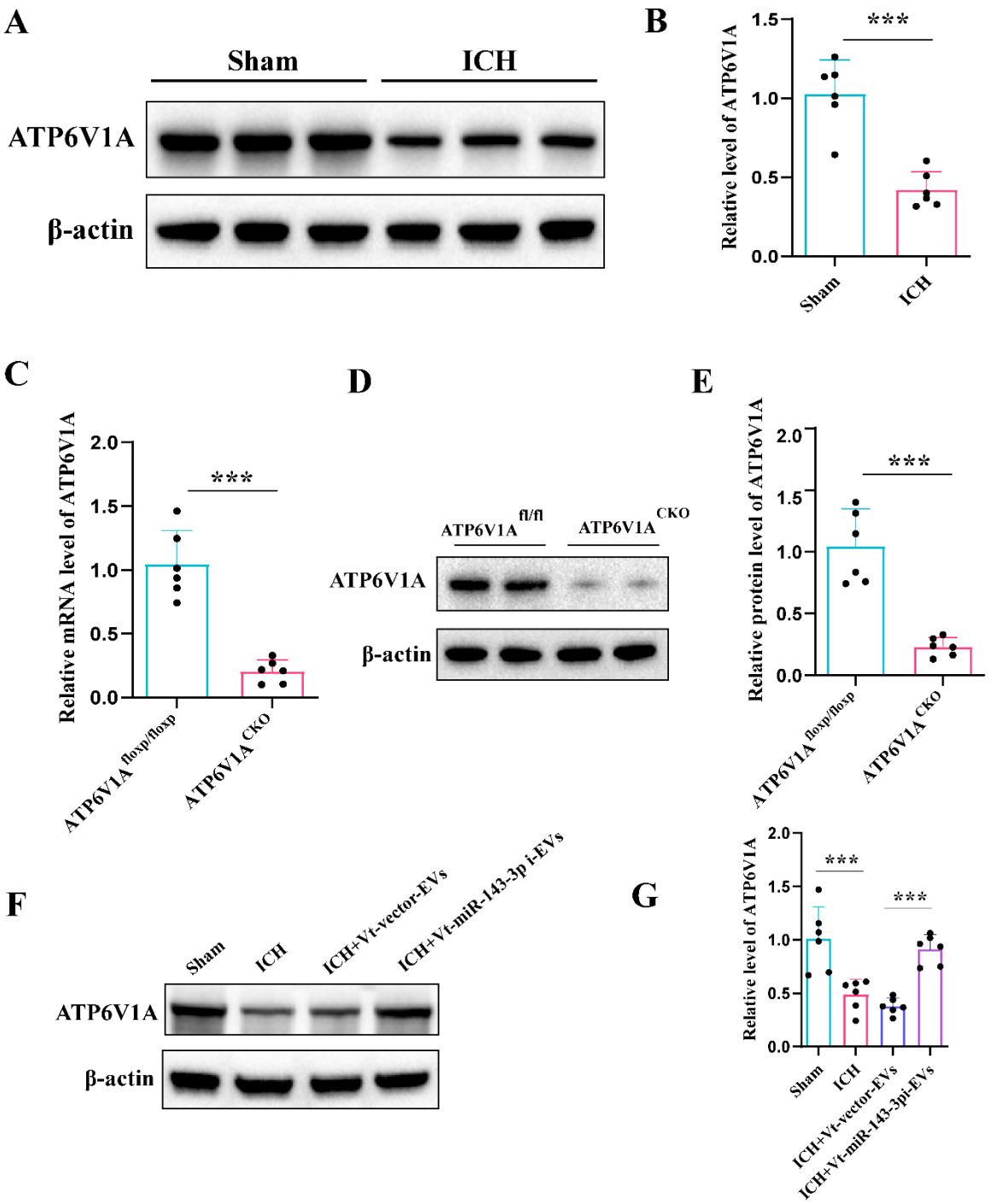

Figure S5. Detection of the level of ATP6V1A in isolated BMECs. (A-B) Western blot assay of the expression of ATP6V1A in isolated BMECs in mice with or without ICH (n =6 per group, Student' s t-test). (C) PCR analysis of the mRNA level of ATP6V1A in isolated BMECs in ATP6V1A^{CKO} mice (n =6 per group, Student' s t-test). (D-E) WB

analysis of the protein level of ATP6V1A in isolated BMECs in ATP6V1A^{CKO} mice (n =6 per group, Student' s t-test). (F-G) Western blot assay of the expression of ATP6V1A in isolated BMECs in ICH mice with or without Vt-miR-143-3pi-EVs treatment (n =6 per group, one-way ANOVA). Data are presented as means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

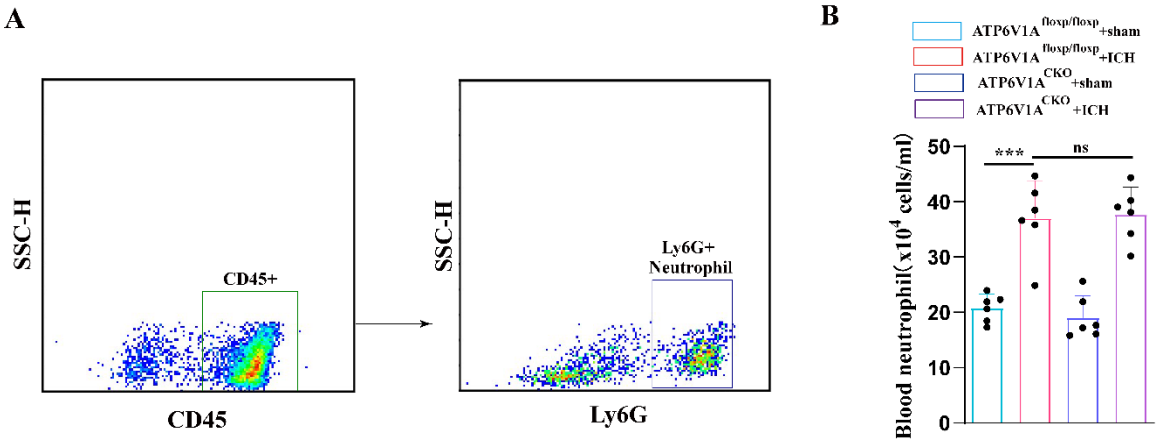

Figure S6. Flow cytometric analysis of the cell numbers of Ly6G+ neutrophils in blood at day 1 post ICH in mice (n =6 per group, one-way ANOVA). Data are presented as means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

S-table1. Characteristics of patients with TBI and multivariate logistic regression analysis

Variables	Study population (n=113)	Good outcome (n=67)	Poor outcome (n=46)	Crude OR (95% CI)	Crude P value	Adjusted OR (95% CI)	Adjusted P value
Demographics (age, mean ± SD; male, %)							
Age (y)	48.97(13.36)	47.88(20.41)	50.56(18.46)	0.98-1.02	0.473		
male	76(67.2)	44(65.6)	32(69.5)	0.37-1.87	0.664		
Clinical characteristics							
GCS	12.20 (2.33)	13.23(1.41)	10.69(2.59)	0.43-0.69	<0.001	0.015-0.748	0.024
Tracheotomy n(%)	37(32.74)	2(2.99)	35 (76.09)	21.69-492.93	<0.001	3.82-1.18E+13	0.032
Brain midline shift (mm)	7.39(4.06)	5.63(3.68)	9.96(3.15)	1.22-1.59	<0.05	1.33-64.02	0.024
Laboratory test (mean ± SD)							
WBC (10^9/L)	11.02(5.35)	10.27(4.71)	12.11(6.01)	0.99-1.14	0.075		
AST/ALT	1.43(0.58)	1.28(0.51)	1.65(0.61)	1.53-7.38	0.20		
BG (mmol/L)	8.43(3.51)	7.09(2.58)	10.38(3.8)	1.19-1.65	<0.001	1.30-36.34	0.023
APTT (s)	26.50(7.34)	25.33(6.34)	28.21(8.36)	0.99-1.12	0.58		
FDP (mg/L)	15.47 (20.89)	12.20(20.18)	20.23(21.22)	1.00-1.04	0.58		
miR-143-3p	1.40(0.54)	1.19(0.40)	1.70(0.59)	3.50-29.37	<0.001	4.14-2.52E+6	0.017

Abbreviations: BP, blood pressure; GCS, Glasgow Coma Scale; WBC: white blood cells; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; BG blood glucose; APTT, activated partial thromboplastin time; FDP, fibrin degradation products;