

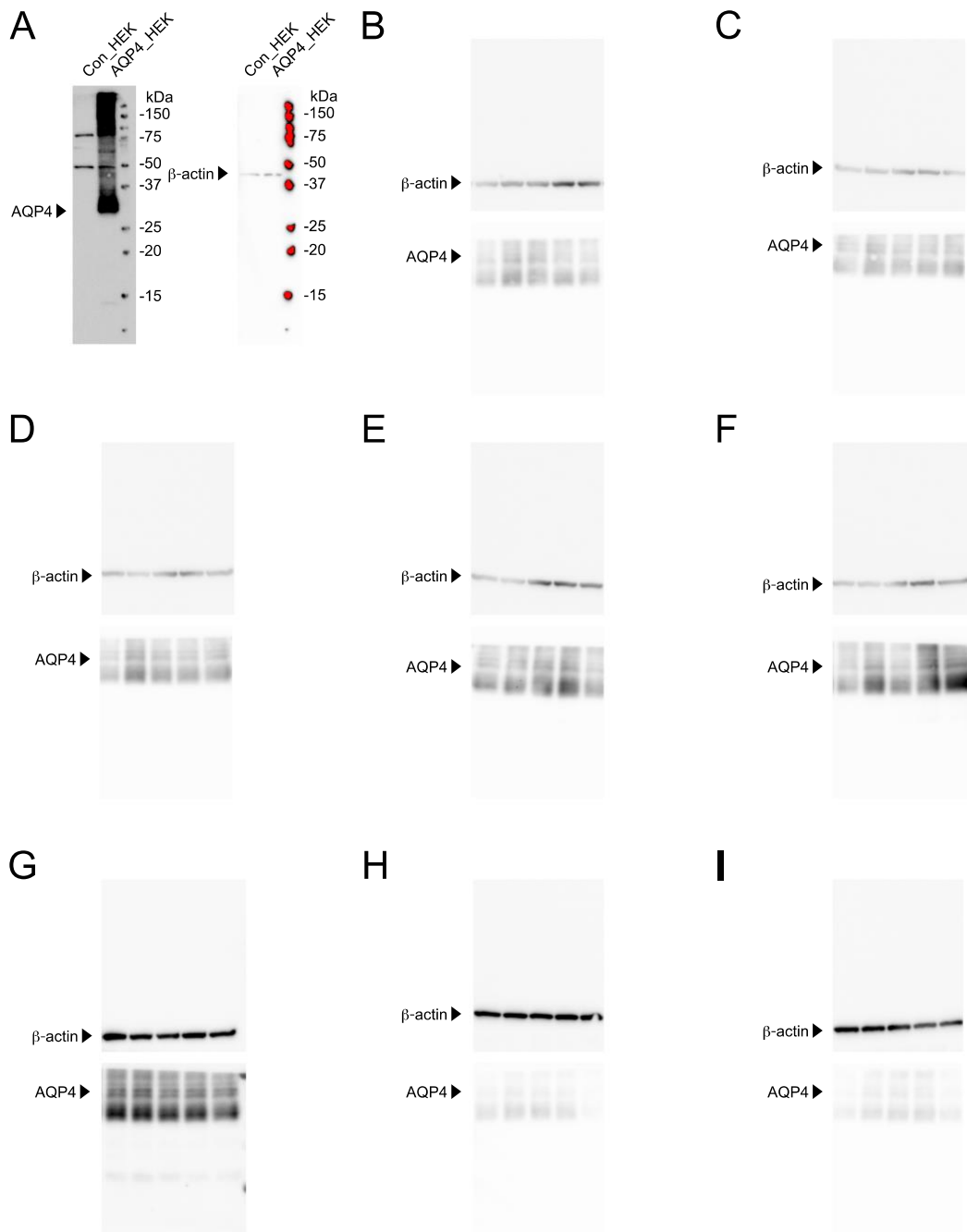
Supplementary Information

Neutrophils induce astrocytic AQP4 expression via IL-1 α and TNF, contributing to cerebral oedema in ischaemic stroke rats

Kana Sugimoto, Chihpin Yang, Miharuo Ono, Mai Shirazaki, Ryuichi Katada, Hiroshi Matsumoto

Protein name	Gene name	Forward	Reverse
AQP4	<i>Aqp4</i>	GAATCCAGCTCGATCCTTTG	CTTCCTTTAGGCGACGTTTG
CXCL1	<i>Cxcl1</i>	AGACAGTGGCAGGGATTAC	ACTTGGGGACACCTTTAGC
CXCL2	<i>Cxcl2</i>	AGGGTACAGGGTTGTTGTG	TTTGGACGATCCTCTGAACC
CXCL3	<i>Cxcl3</i>	ACCTTCAGGGACTGTTGTGG	GGAGCTTGAGGGTTGAGACA
CXCL6	<i>Cxcl6 (Cxcl5)</i>	GTTCACTGCCACAGCATC	GGAGCTTCTGGGTCAAGACA
CXCL7	<i>Ppbp</i>	CCTTGCAATTGGAAACCTG	GGCACACTTCCTTTCCATTG
CXCR2	<i>Cxcr2</i>	CCAAGCTGATCAAGGAGACC	GGAGGTGTTGCTGAAGAAG
E-selectin	<i>Sele</i>	TGCCAAGAACAGGAATACCC	CTCCAGGATTTGAGGAACA
GAPDH	<i>Gapdh</i>	ATGTATCCGTTGTGGATCTGAC	CCTGCTTACCACCTTCTTG
Iba1	<i>Aif1</i>	GTCCTTGAAGCGAATGCTGG	CATTCTCAAGATGGCAGATC
ICAM-1	<i>Icam1</i>	CAGACCCTGGAGATGGAGAA	CCTGGGGGAAGTACTGTTCA
ICAM-2	<i>Icam2</i>	CCACAGAGGCTTGGAGAGTC	CCTGAGCTGGAGGCTCATAC
IL-1 α	<i>Il1a</i>	CGTCAAGCAGGAGTTCATCA	TTCTCCCTGAGCACTCACAA
IL-1 β	<i>Il1b</i>	CACCTTCTTTTCCTTCATCTGT	GTCGTTGCTTGTCTCTCCTGTA
Ly6C	<i>Ly6c</i>	TTTTTCCTGGCCCTACTGTG	GATTGGAAGGCAGAGATTGC
P-selectin	<i>Selp</i>	TAATCCCCCGCAGTGTAAG	AGGTTGGCAATGGTTCACTC
TNF	<i>Tnf</i>	CCCAGACCCTCACACTCAGAT	TTGTCCCTTGAAGAGAACCTG
VCAM-1	<i>Vcam1</i>	TGACATCTCCCCTGGATCTC	CTCCAGTTTCCTTCGCTGAC

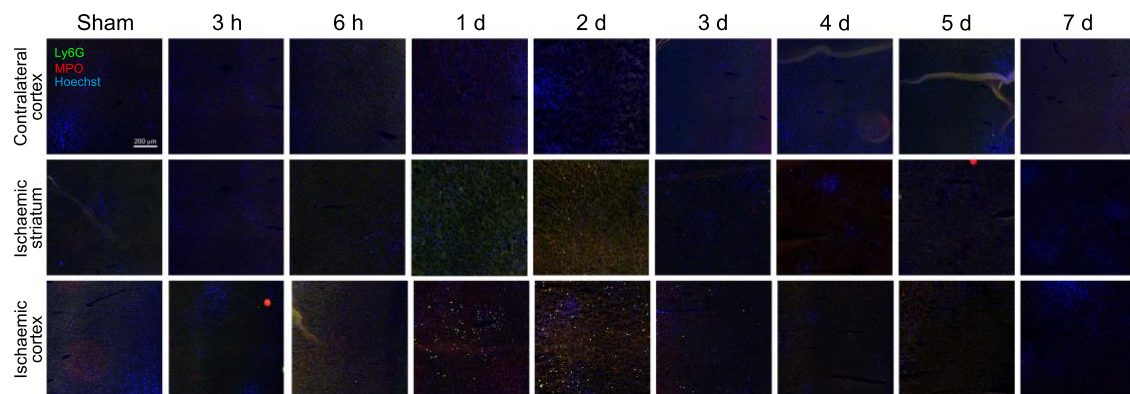
Supplementary Table S1. Primers used for quantitative real-time polymerase chain reaction



Supplementary Fig. S1. Raw images of western blotting

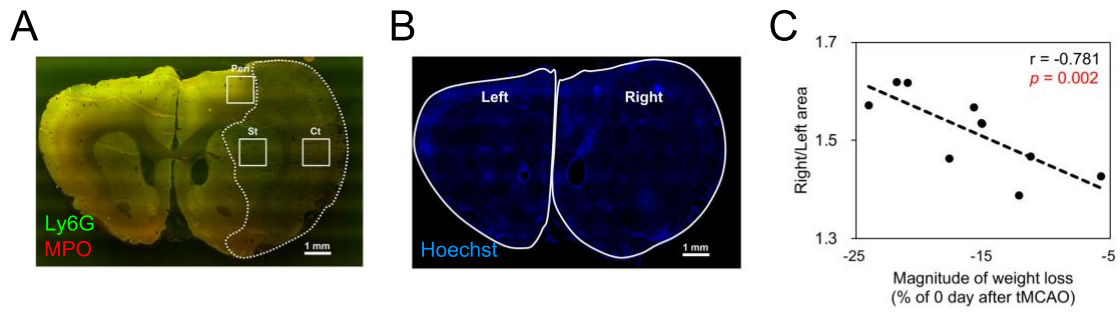
(A) Western blot analysis showing the protein levels of AQP4 in AQP4_HEK using AQP4 antibody

(Alomone). (B–I) Raw images of AQP4 and β -actin used for western blot analysis in Fig. 6B.



Supplementary Fig. S2. Images of Ly6G and MPO fluorescence immunostaining in ischaemic brain after transient middle cerebral artery occlusion (tMCAO)

Images of Ly6G (green) and MPO (red) fluorescence immunostaining in the ischaemic brain from 3 h to 7 d after tMCAO. Hoechst 33258 fluorescence (blue) indicates the nuclei.

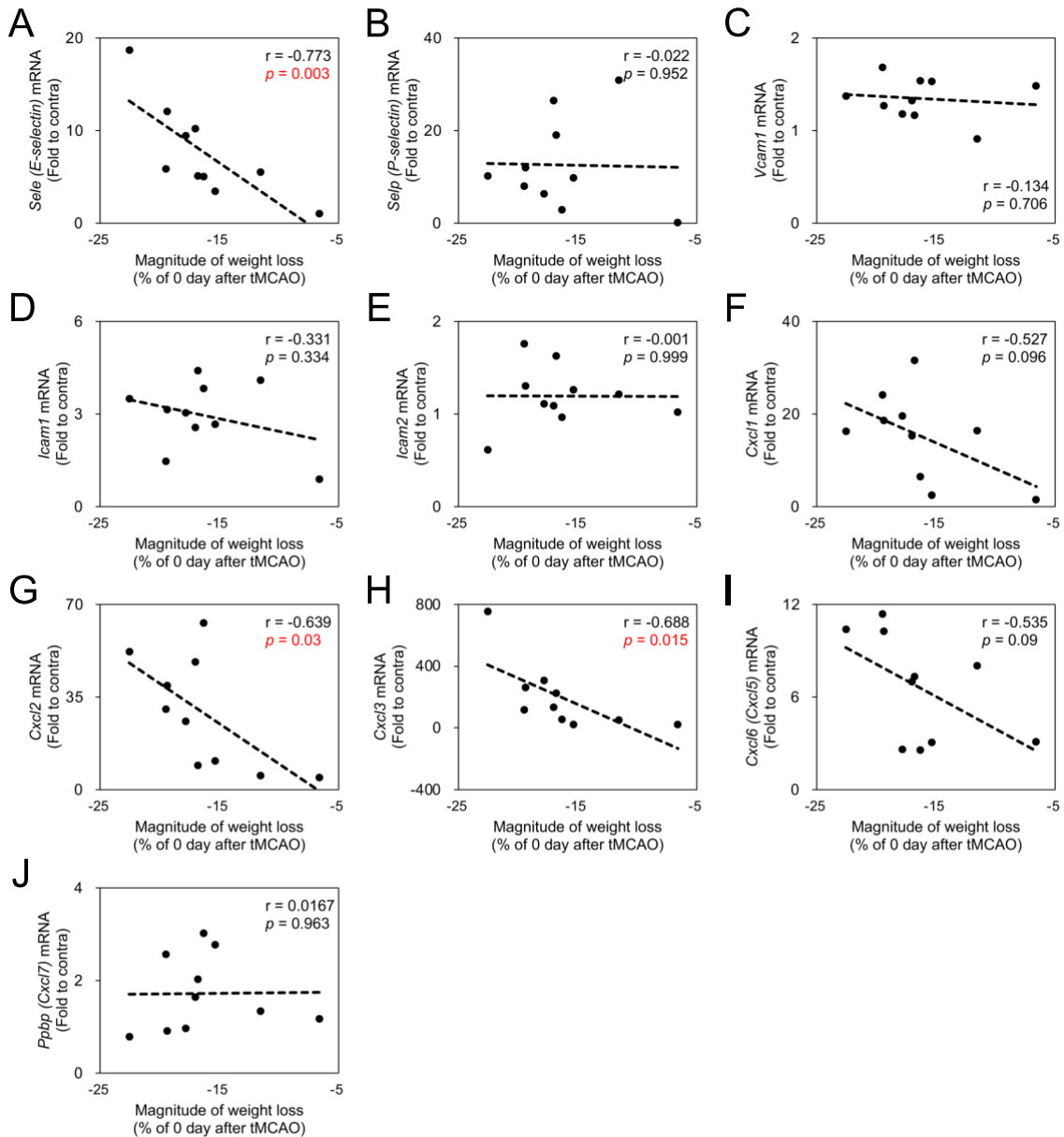


Supplementary Fig. S3. Images of Ly6G, MPO, and Hoechst fluorescence immunostaining in ischaemic brain after transient middle cerebral artery occlusion (tMCAO)

(A) Image of Ly6G (green) and MPO (red) fluorescent immunostaining in the ischaemic brain at 2 days after tMCAO, showing the peri-infarct tissue (Peri), ischaemic striatum (St), and ischaemic cortex (Ct). (B)

Image of Hoechst 33258 (blue) fluorescent immunostaining in the ischaemic brain at 2 days after tMCAO.

(C) Correlation between the magnitude of weight loss and the area ratio of the right and left hemispheres at 2 days after tMCAO ($n = 10$).



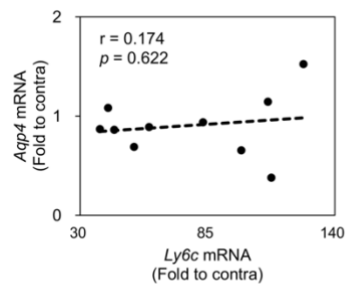
Supplementary Fig. S4. Correlation between neutrophil trafficking-related factors and illness severity after

transient middle cerebral artery occlusion (tMCAO)

Correlation between the magnitude of weight loss and *Sele* (*E-selectin*) (A), *Selp* (*P-selectin*) (B), *Vcam1*

(C), *Icam1* (D), *Icam2* (E), *Cxcl1* (F), *Cxcl2* (G), *Cxcl3* (H), *Cxcl6* (*Cxcl5*) (I), or *Pbp* (*Cxcl7*) (J) mRNA

expression in the peri-infarct tissue at 2 d after tMCAO (n = 10).



Supplementary Fig. S5. Correlation between neutrophils and *Aqp4* mRNA expression level after transient middle cerebral artery occlusion (tMCAO)

Correlation between the *Ly6c* and *Aqp4* mRNA expression in the ischemic core at 2 d after tMCAO (n = 10).