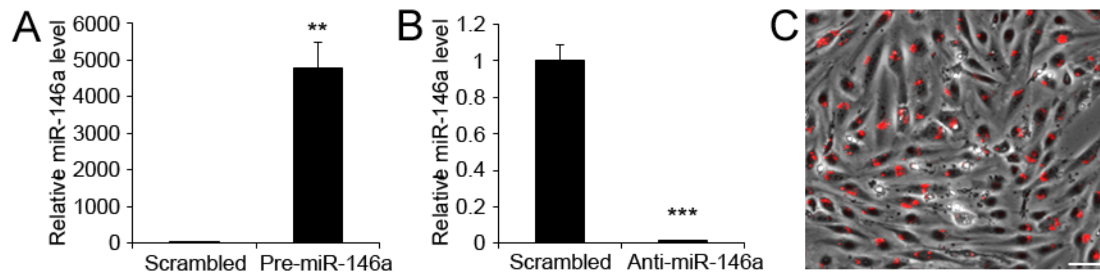
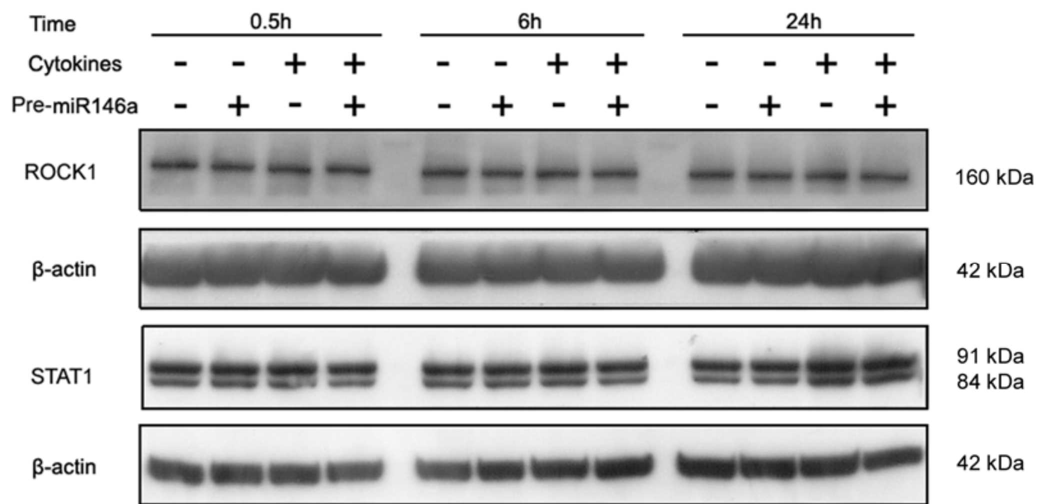
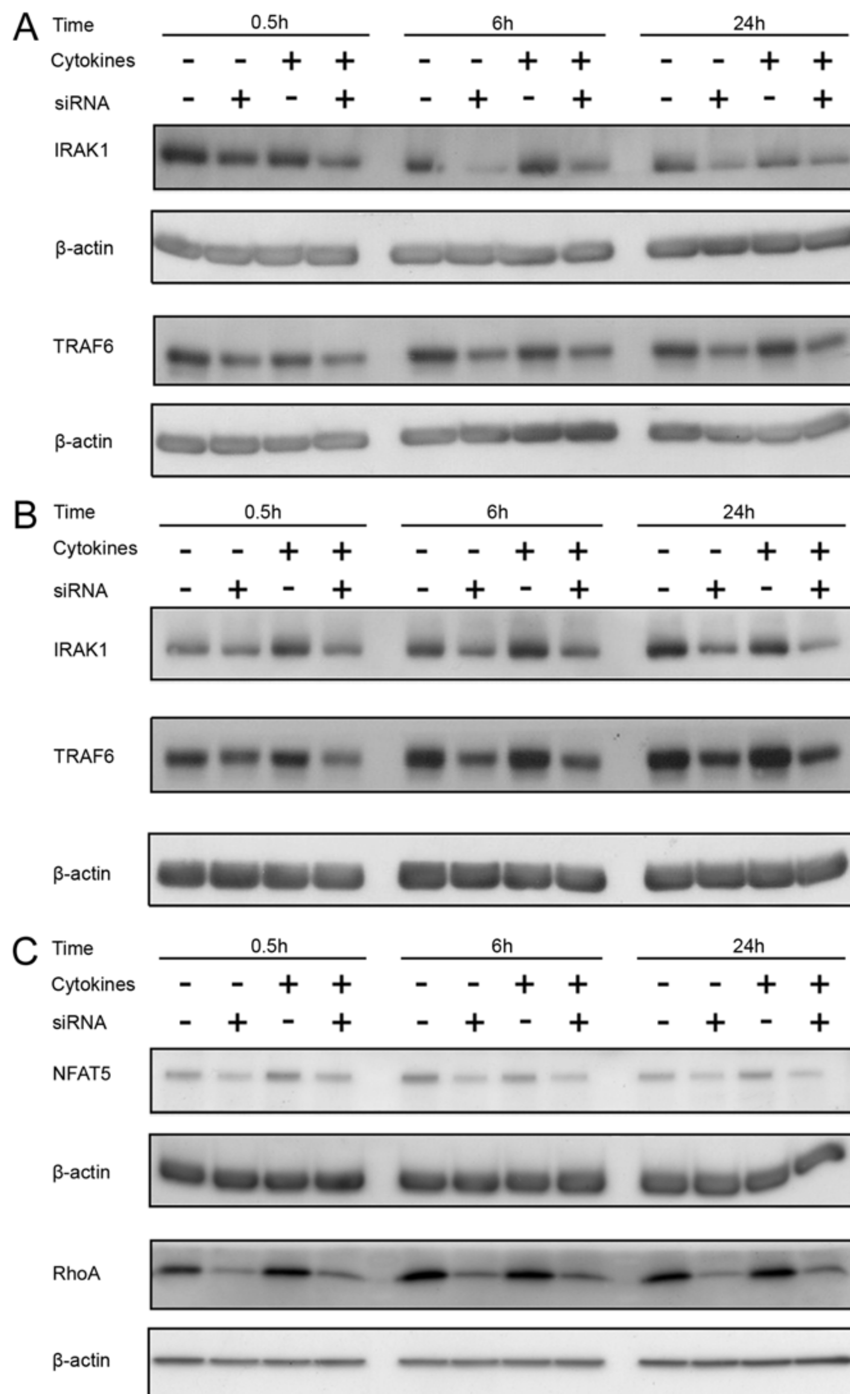
Supplementary Figures and Figure Legends



Supplementary Figure 1. Over-expression or know-down of miR-146a in brain endothelium. (**A**) Over-expression of miR-146a in hCMEC/D3 cells via transfection with Pre-miR-146a using siPORT. (**B**) Knock-down of miR-146a in hCMEC/D3 cells via transfection with Anti-miR-146a using lipefectamine 2000. Expression of miR-146a was analysed using Taqman quantitative RT-PCR and normalized to the small nuclear RNA U6B, expressed as relative level to scrambled control. Data represent mean ± s.e.m. n = 3, $**P < 0.01$, $***P < 0.001$ via Student *t* test. (**C**) Photomicrograph demonstrating transfection efficiency via Cy3 tagged scrambled Pre-miR. Scale bar, 50 µm.

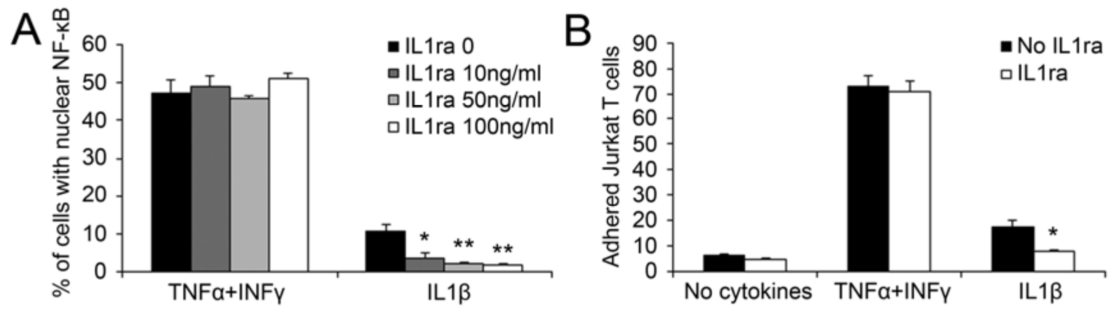


Supplementary Figure 2. Two known targets ROCK1 and STAT1 are not affected by miR-146a in brain endothelial cell. Western blot analysis show that over-expression of miR-146a via transfection with Pre-miR-146a did not repress the expression of ROCK1 and STAT1 in hCMEC/D3 cells either unstimulated or stimulated with cytokines (1 ng/ml TNFα/INFγ) for 0.5, 6 and 24 h.



Supplementary Figure 3. Small interference RNAs knock-down the expression of IRAK, TRAF6, NFAT5 and RhoA in brain endothelium. (**A**) Western blot analysis show that siIRAK1 or siTRAF6 repressed the expression of IRAK1 and TRAF6 respectively in hCMEC/D3 cells either unstimulated or stimulated with cytokines for 0.5, 6 and 24 h. (**B**) Western blot analysis show that co-transfection of siIRAK1 and siTRAF6 in hCMEC/D3 cells decreased simultaneously the expression of IRAK1 and

TRAF6 in cells untreated or treated with 1 ng/ml TNFα/INFγ for 0.5, 6 and 24 h. (**C**) Western blot analysis show that siNFAT5 or siRhoA suppressed the expression of NFAT5 and RhoA respectively in hCMEC/D3 cells either non-stimulated or cytokine-stimulated for 0.5, 6 and 24 h. siRNA -, siControl; siRNA +, siIRAK1, or siTRAF6, or siIRAK1 + siTRAF6, or siNFAT5, or siRhoA.



Supplementary Figure 4. IL1 receptor antagonist does not inhibit TNFα and INFγ induced NFκB activation and leukocyte adhesion. (**A**), hCMEC/D3 cells were treated with 0-100 ng/ml IL1 receptor antagonist (IL1ra) for 30 min before further treatment with 1 ng/ml TNFα/INFγ or 200 pg/ml IL1β for 24 h, subject to immunocytochemistry of NFκB p65. Percentage of cells with nuclear NFκB was analysed. Data represent mean ± s.e.m. n = 3, *$P < 0.05$, **$P < 0.01$ compared with that treated with IL1β but untreated with IL1ra via Student t test. (**B**), hCMEC/D3 cells in 6-channel slides were treated with 50 ng/ml IL1ra for 30 min, and left untreated or treated with 1 ng/ml TNFα/INFγ or IL1β for 24 h, exposed to Jurkat T cells for 5 min at 0.5 dyn/cm^2. Data represent mean ± s.e.m. n = 4, *$P < 0.05$ compare with that treated with IL1β but untreated with IL1ra via Student *t* test.

Supplementary Video Legends:

miR146a modulates Jurkat T cell adhesion to cytokine-stimulated hCMEC/D3 cells. hCMEC/D3 cells in 6-channel slides were transfected with scrambled Pre-miR **(Video. A and B)**, or Pre-miR-146a **(Video. C)**, and left untreated **(Video. A)** or treated with 1 ng/ml TNFα and IFNγ **(Video. B and C)** for 24 h. hCMEC/D3 cells were then exposed to 5-chloromethylfluorescein diacetate (CMFDA)-labelled Jurkat T cells for 5 min at 0.5 dyn/cm^2. Note that Pre-miR-146a markedly decreased Jurkat T cells adherent to cytokine-activated brain endothelium. Images were recorded at one frame (640 x 480 μm^2/field) per second with an inverted time-lapse fluorescence microscope (Olympus IX70, Tokyo, Japan) by using Image Pro Plus software (Media Cybernetics Inc., Bethesda, USA).