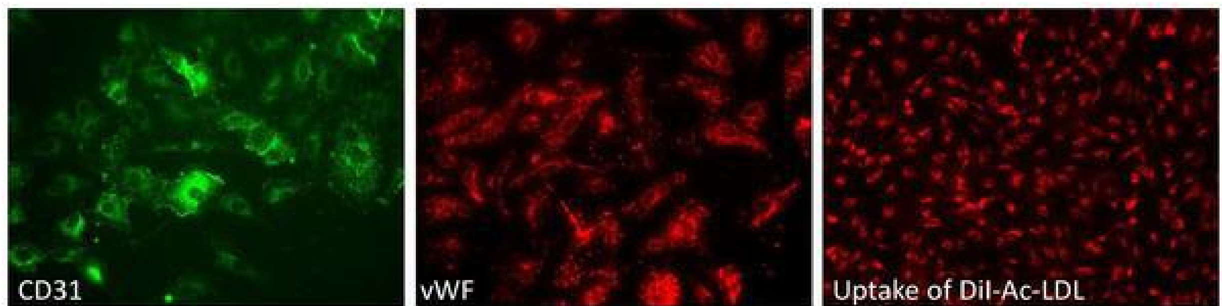
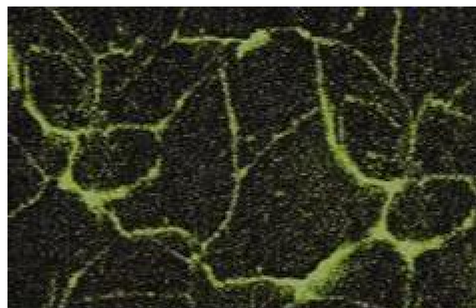
参考文献：

Immunofluorescence Imagery and Characterization in Peer-Reviewed Literature



ACBRI 376 cells express endothelial cell markers including CD31 and von Willebrand Factor. They also take up DiI-Ac-LDL in an endothelial cell functional assay.



ACBRI 376 cells express ZO-1 indicating the formation of tight junctions.

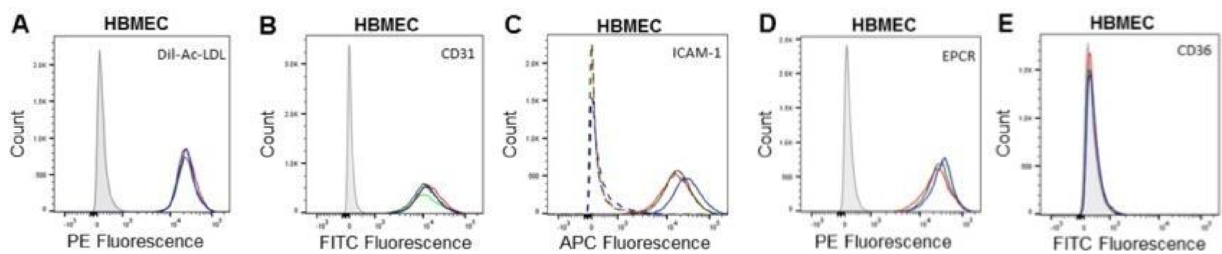


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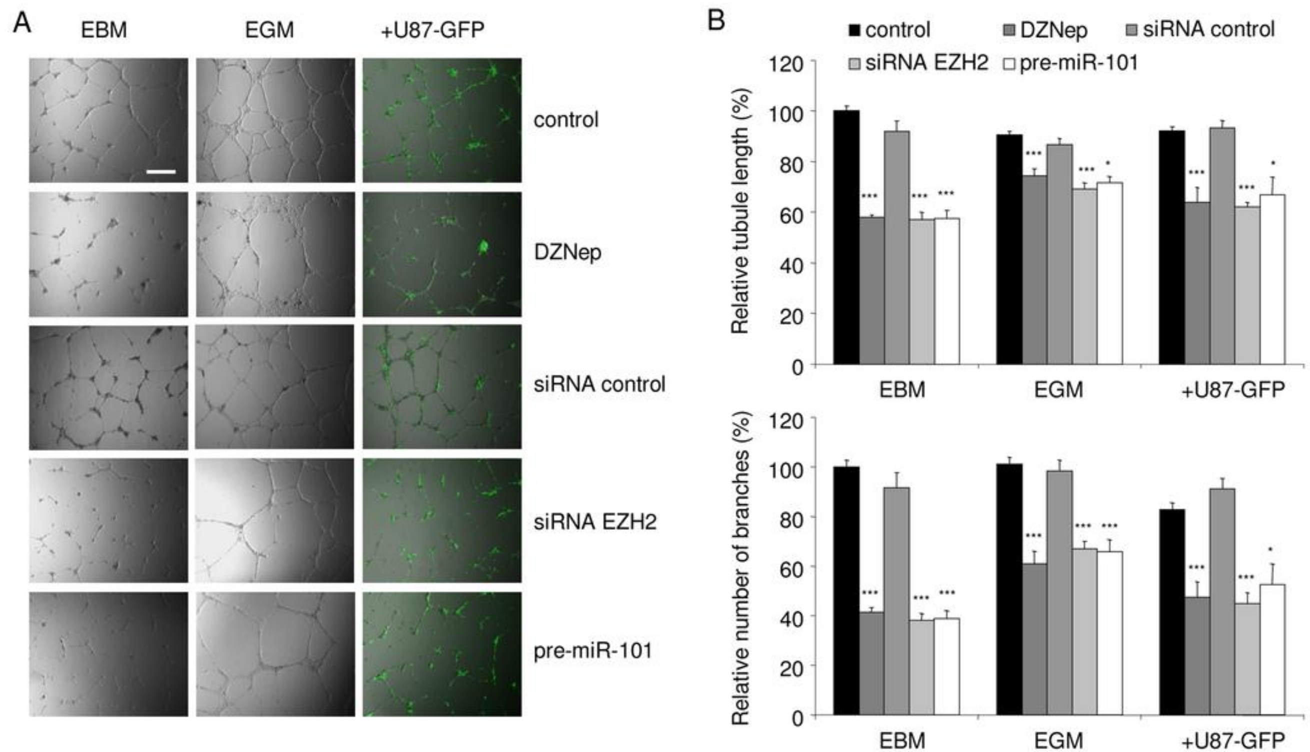
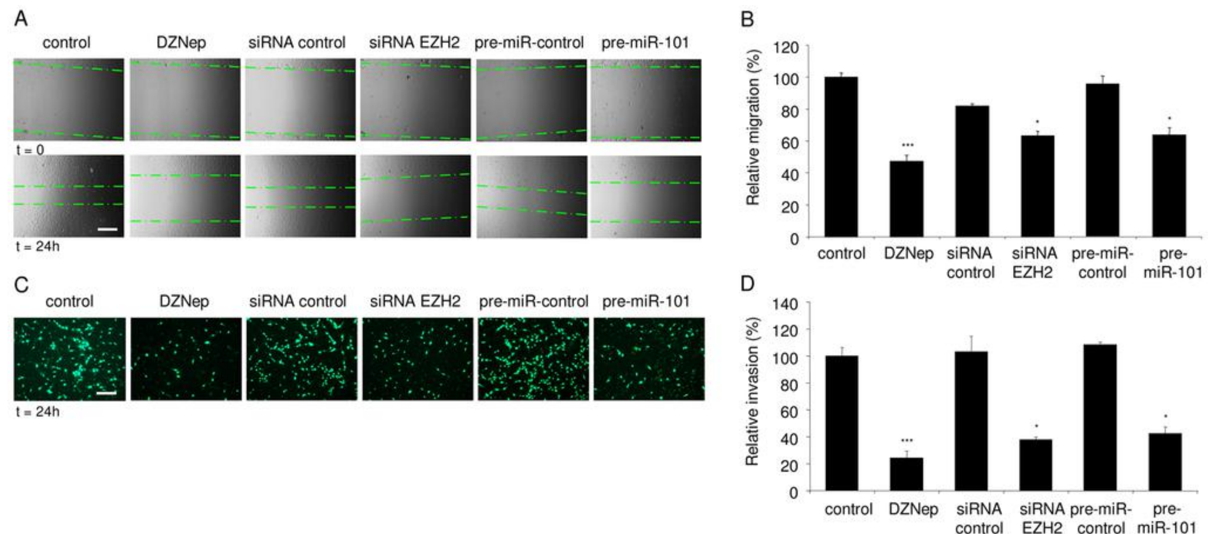
Figure S1. Characterisation of **HBMEC (Cell Systems ACBRI 376)** at number of passages, as measured by flow cytometry. Unlabelled cells are depicted in grey. Blue = passage 5, light green = passage 7 (only in B), red = passage 8, dark green = passage 9. A, Uptake of DiI-labelled acetylated low density lipoprotein. B, Levels of CD31 expression. C, Upregulation of ICAM-1 by 10ng/ml TNF; dotted lines represent unstimulated labelled cells and the continuous lines represent the TNF-stimulated cells. D, Levels of EPCR expression on TNF-stimulated HBMEC. E, Levels of CD36 expression on TNF-stimulated HBMEC.



Reprinted with permission from Smits and Mir et al. PLoS ONE (2011) 6:e16282. Figure 5. Inhibition of EZH2 reduces endothelial tubule formation *in vitro*. (A) **HBMVECs (ACBRI 376)** were cultured on Matrigel coated plates in EBM, EGM, or EBM supplemented with U87-GFP cells. Scale bar =450 µm. Inhibition of EZH2 in HBMVECs, either by transfection with pre-miR-101, EZH2 siRNA, or treatment with DZNep significantly reduced tubule formation as compared to control cells. Tubule formation was assessed as tubule length and branching. (B) Quantitation of tubule length and branching

using ImageJ software. (n=3) Error bars indicate s.d. *$p<0.05$, ***$p<0.001$, t test.



Reprinted with permission from Smits and Mir et al. PLoS ONE (2011) 6:e16282. Figure 6. Inhibition of EZH2 reduces endothelial migration and invasion *in vitro*. (A) **HBMVEC (ACBRI 376)** monolayer cultures were scratched. Images were acquired directly after scratching (t=0) and 24 h later (t=24). The migration front is indicated by the dashed lines. Scale bar =450 μm. Inhibition of EZH2 in HBMVECs, either by transfection with pre-miR-101, EZH2 siRNA or treatment with DZNep significantly reduced migration as compared to control. (B) Quantitation of endothelial cell migration into the scratched area using ImageJ software. (C and D) HBMVECs were transfected with pre-miR-101, EZH2 siRNA, non-related control molecules, or treated with DZNep, incubated on

a Transwell system and subsequently analyzed for invasion capability. EZH2 inhibition, either through pre-miR-101, EZH2 siRNA or DZNep, significantly decreased invasion as shown by Hoechst staining. Scale bar =225 μm. (n=3) Error bars indicate s.d. *p<0.05, ***p<0.001, t test.

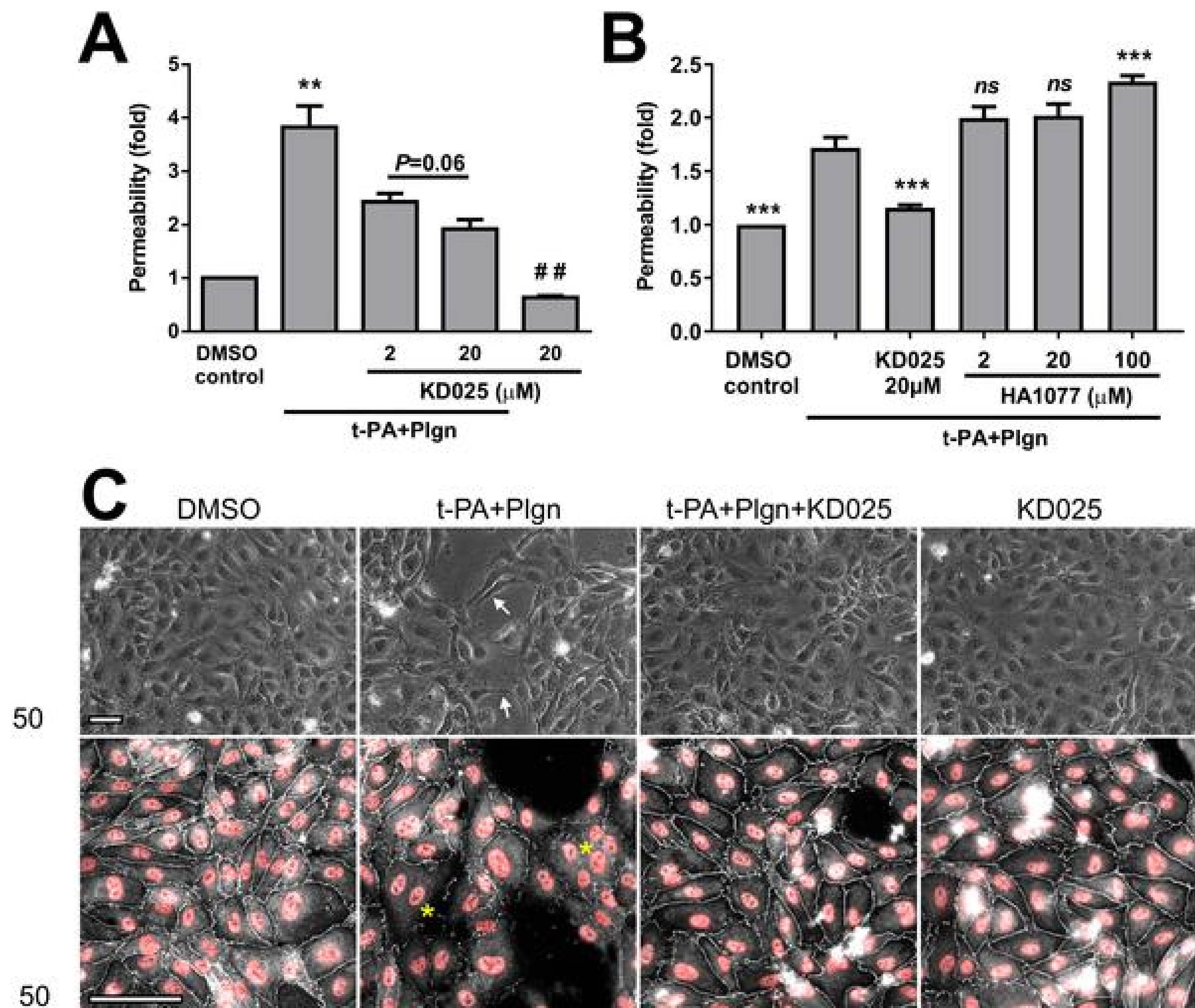


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Figure 5. Only KD025, but not fasudil, protects **brain endothelial cells (ACBRI 376)** from rt-PA and plasmin. **(A)** Small molecule KD025 concentration-dependently blocks permeability changes in primary human brain microvascular endothelial cells (**ACBRI 376 BECs**; cultured without astrocytes) treated for 6 h with DMSO as control or with rt-PA (25nM) and human plasminogen (plgn; 100nM), with or without KD025 (2 and 20μM). n = 4. **(B)** Comparison of selective ROCK-2 inhibition by KD025 (20μM) *versus* non-selective ROCK inhibition by fasudil (HA1077; 2, 20 and 100μM) against rt-PA+plasminogen (25nM+100nM, respectively) in BECs, as assessed 6 h post stimulation. HA1077 displays no protective capacity in BECs. n = 3. Bars represent mean±SEM. ***P*<0.01 compared to all other groups, ****P*<0.001 compared to t-PA+Plgn by one-way ANOVA with Tukey's post hoc analysis. ## *P*<0.01 compared to DMSO control and *P* = 0.06 by two-tailed paired t-test. **(C)** Representative phase-contrast micrographs (top panels) and double immunofluorescence images of zonula occludens 1 (ZO-1, representing tight junctions (TJs); white) and nuclei (Hoechst; red) (bottom panels) of **ACBRI 376 BECs** stimulated for 6 h with DMSO

(control) or with rt-PA+plasminogen (25nM+100nM, respectively), in the presence or absence of KD025 (20μM). KD025 attenuates morphological changes (arrows) and preserves TJs in endothelial cells. Asterisks depict areas with degraded TJs. Scale bars = 200μm