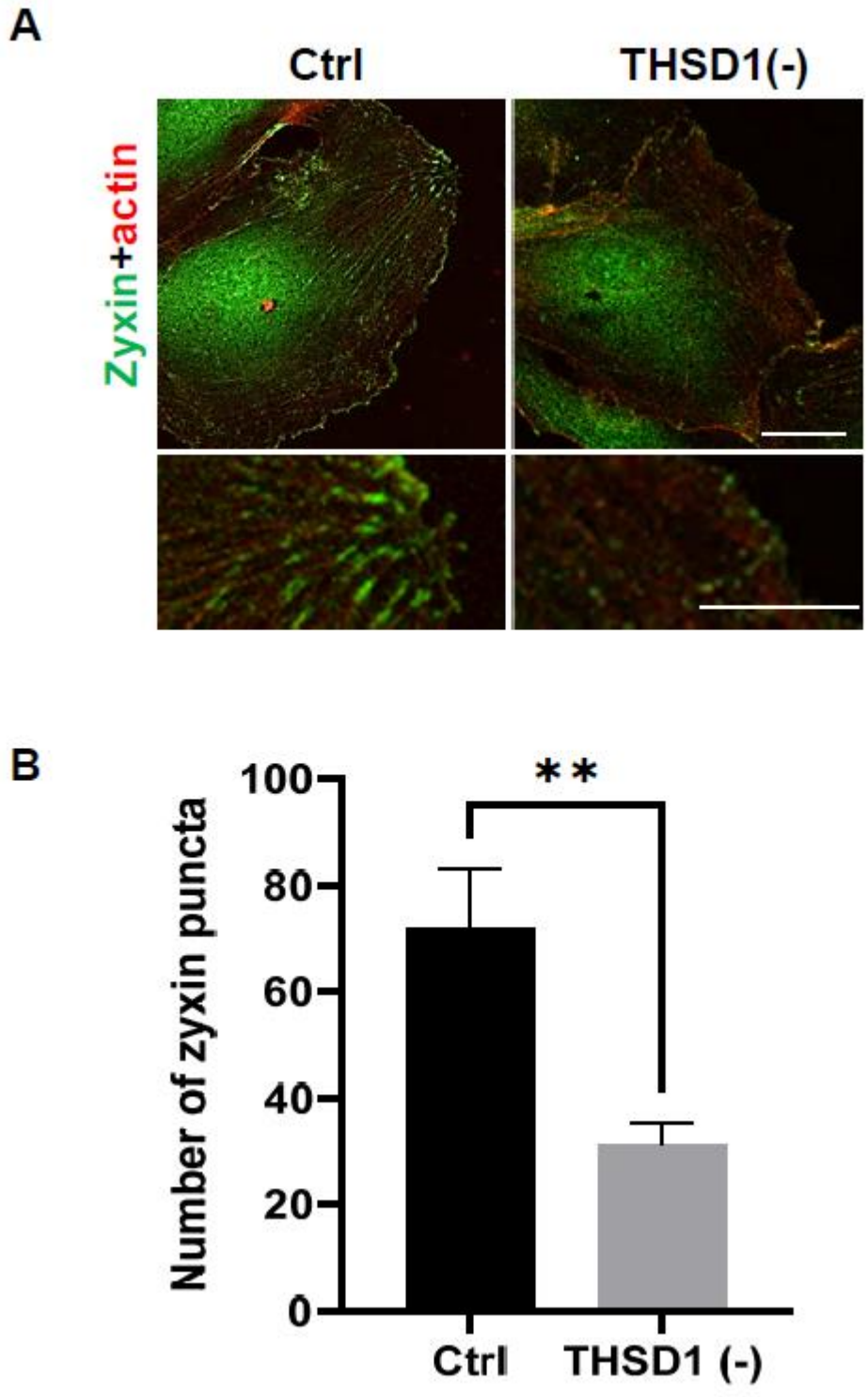

Figure S1. Inactivation of THSD1 reduces the number of mature focal adhesions. (**A**) The subcellular distribution of mature focal adhesions in both control and THSD1-deficient HBMECs was visualized through immunostaining against zyxin (in green) and actin (in red). Enlarged images from selected regions are displayed within white dashed boxes. (**B**) The quantification of focal adhesions involved counting them across at least 12 distinct fields using a 20X objective and subsequent analysis via a Student's t-test. **$p < 0.01$. Scale bar: 10 μm.

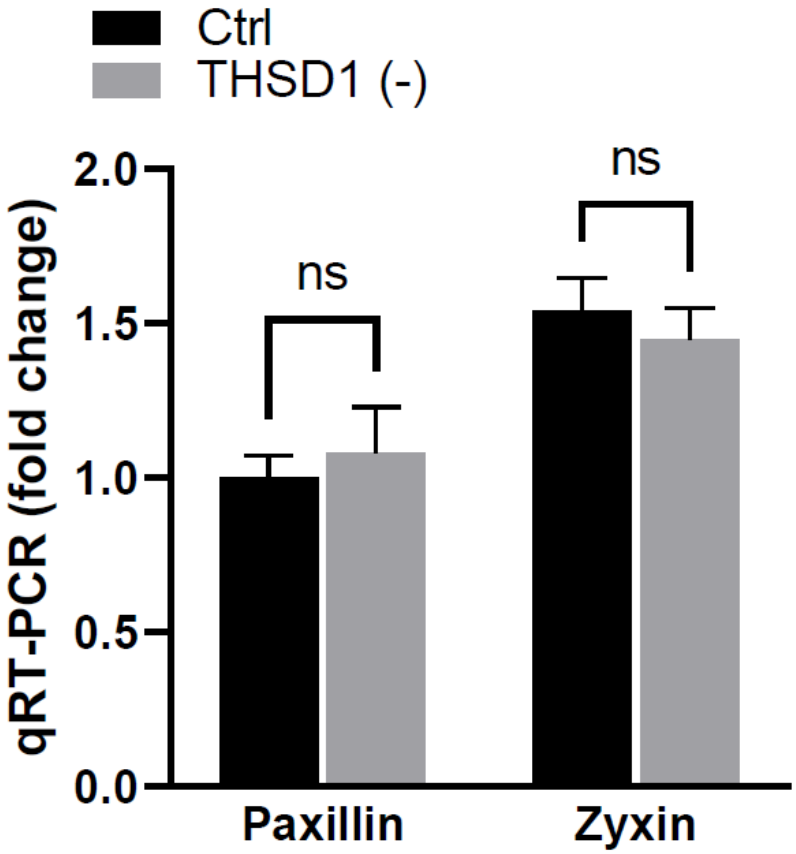

Figure S2. Inactivation of THSD1 has no effects on the mRNA level of paxillin or zyxin. Total mRNA was extracted from both control and THSD1-deficient HBMECs, followed by reverse transcriptase reactions. The mRNA expression levels of paxillin and zyxin were determined through quantitative RT-PCR and subsequently normalized to GAPDH. The fold change was calculated by comparing the mRNA levels in each sample to those in control cells. The statistical comparison was conducted through two-way ANOVA, followed by Bonferroni correction. ns: not significant.

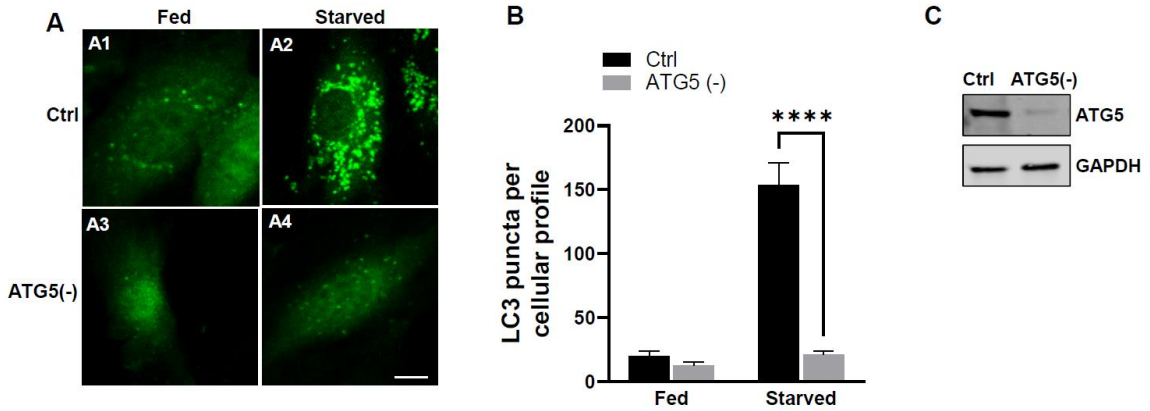

Figure S3. Evaluation of GFP-LC3 reporter in human brain microvascular endothelial cells. (**A**) Representative images showed control or ATG5-deficient HBMECs expressing the GFP-LC3 reporter under nutrient-rich conditions

(Fed) or following starvation treatment (Starved) for 2 hours. (**B**) Quantification involved counting the number of GFP-LC3 puncta within cells across at least 9 different areas using a 20X objective and analyzing the data through two-way ANOVA, followed by Bonferroni correction. (****p < 0.0001) (**C**) Knockdown efficiency of ATG5 was verified by Western blotting, with GAPDH serving as a loading control. Scale bar: 10 μm.

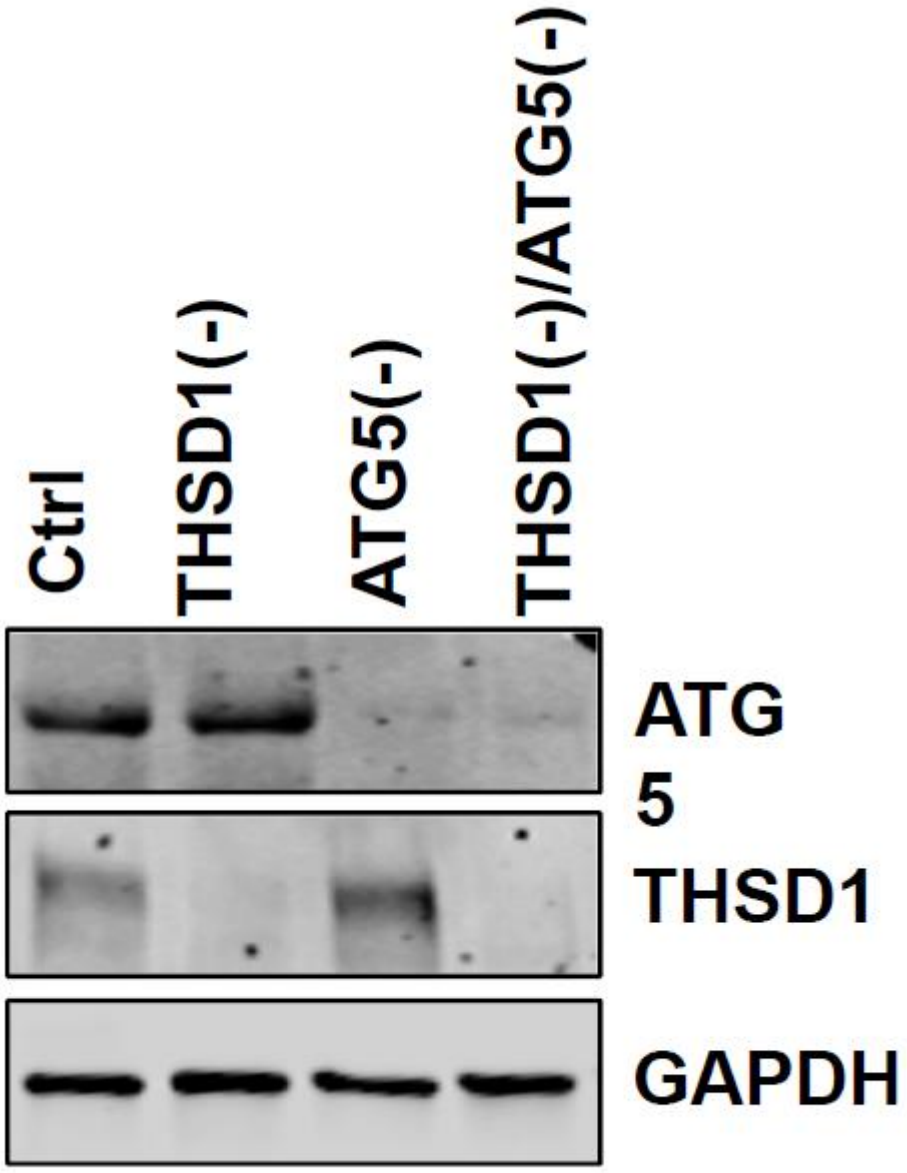

Figure S4. Knockdown efficiency of ATG5 or THSD1 was confirmed by Western blot. The efficacy of ATG5, THSD1, or their combined knockdown was validated through Western blotting using whole cell lysates from HBMECs treated with the designated siRNAs for 48 hours. GAPDH was utilized as a loading control.

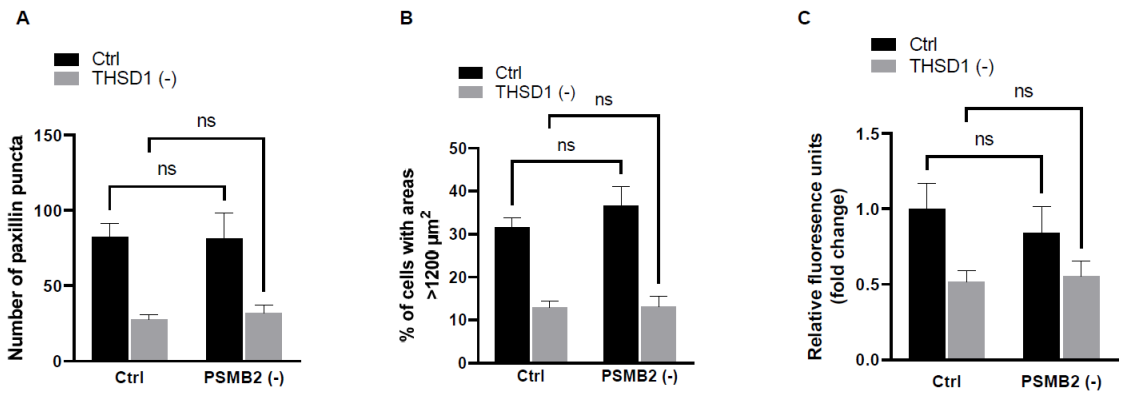

Figure S5. Inactivation of PSMB2 has no effects on focal adhesion number, cell spreading, and attachment in HBMECs**.** HBMECs subjected to siRNA treatment targeting *thsd1* (10 nM, THSD1 (-)), *psmb2* (2 nM, PSMB2 (-)), or both were examined for focal adhesion (FA) stability, cell spreading, and adhesion ability, respectively. (**A**) The total number of FAs was enumerated based on immunostaining against paxillin. (**B**) The percentage of cells with surface areas exceeding 1200 nm^2 was calculated, as described in the cell spreading assay. (**C**) Cell adhesion ability in THSD1 or PSMB2-deficient cells was quantified by measuring relative fluorescence units using CyQuant dye at a wavelength of 480/520 nm.