# Supplementary materials

**Title:**

**Upregulation of ribosome complexes at the blood-brain barrier in Alzheimer's disease patients**

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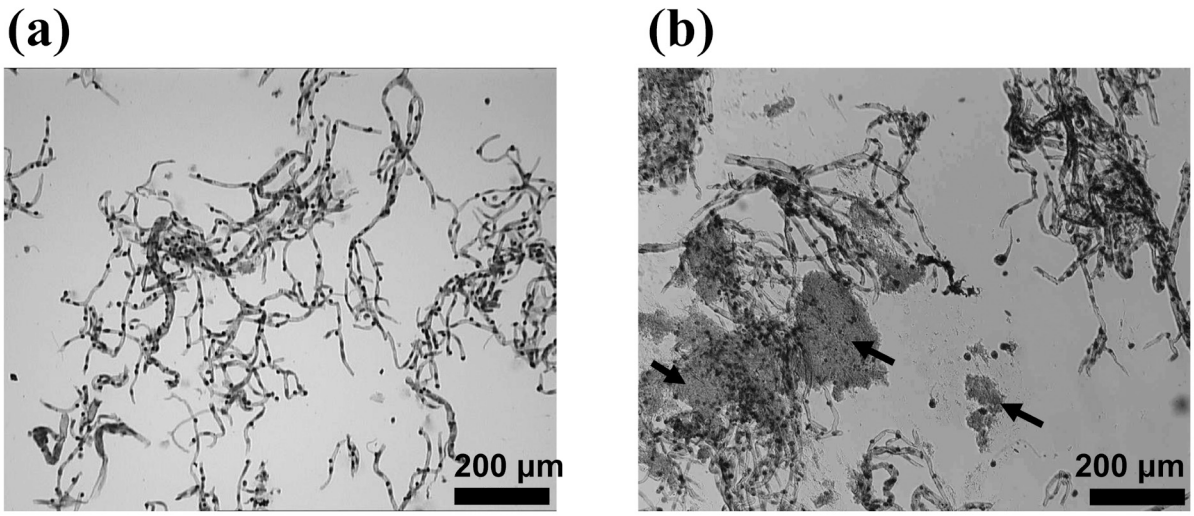
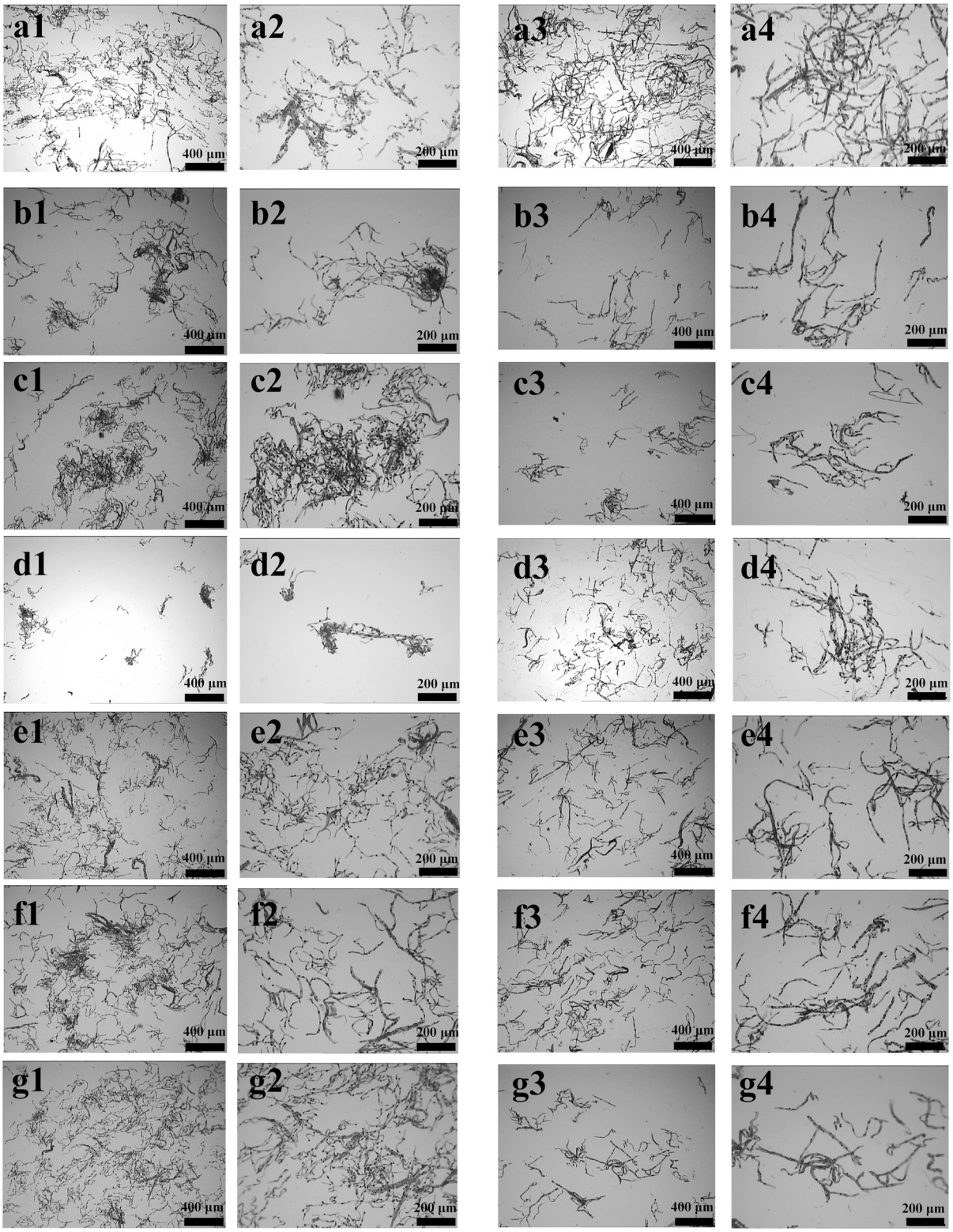
## List of supplementary materials

Supplementary figures 1 to 7

Supplementary tables 1 and 2 (Uploaded as Excel file)

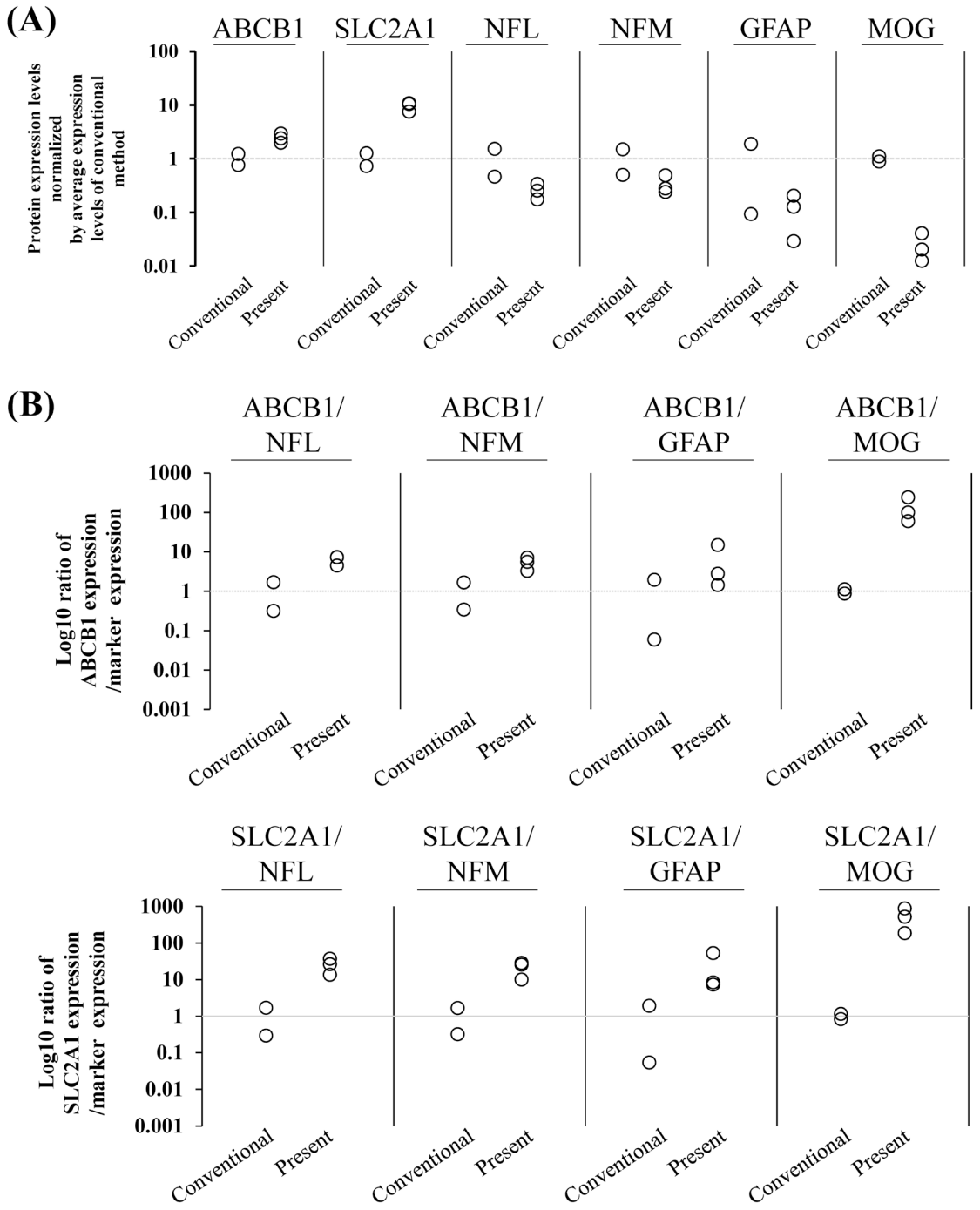



**Supplementary figure 1 Photographs of human brain capillaries isolated by highly purified isolation method (a) and conventional standard method (b)**
The brain capillaries were isolated as described in materials and methods. Arrows indicate the contamination tissue other than brain capillaries. The scale bar, 200 µm.



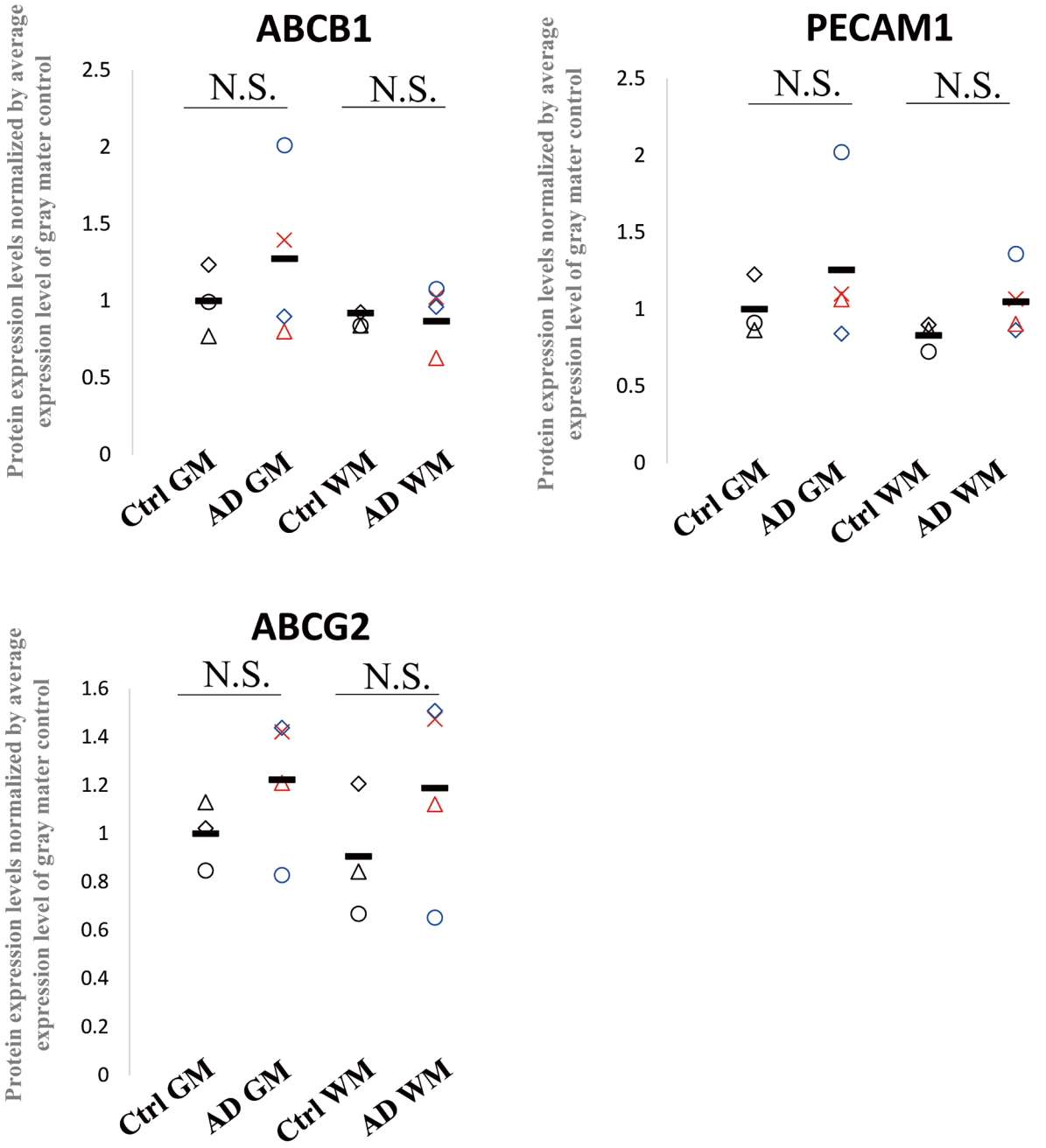

**Supplementary figure 2 Photographs of highly purified brain capillaries used for the present SWATH study**

All the brain capillaries used for the SWATH analysis in the present study were isolated by highly purified isolation method as described in materials and methods. Photographs a, b, c, d, e, f and g represent the brain capillaries of control 1, 2, 3, AD 1, 2, 3 and 4, respectively. Photographs 1 and 2 represent the brain capillaries of gray matters with different magnification. Photographs 3 and 4 represent the brain capillaries of white matters with different magnification.



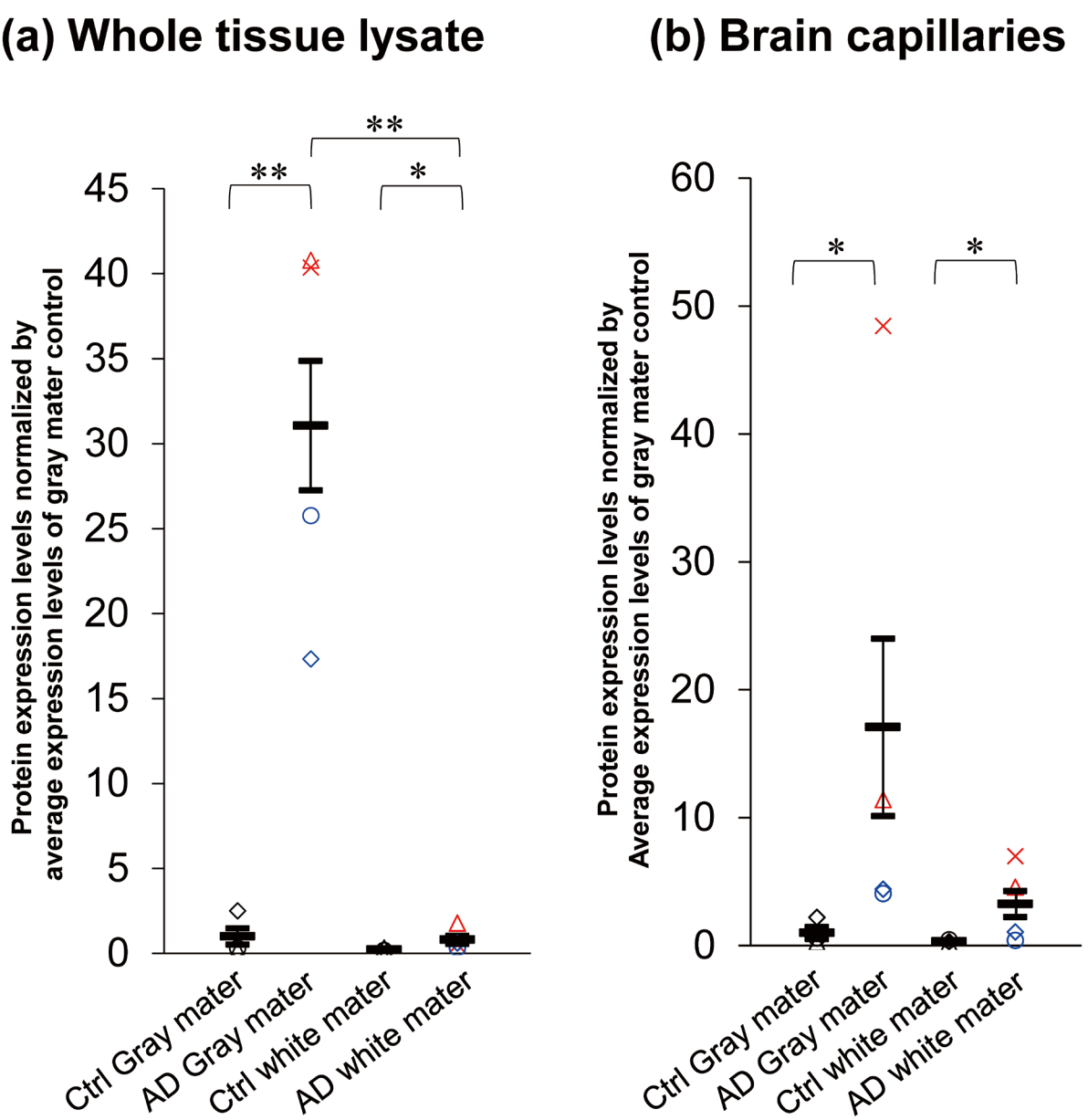

**Supplementary figure 3 The purity of cerebral endothelial cells and contamination by astrocytes, neurons and oligodendrocytes in the brain capillaries isolated by present (highly purified) and conventional isolation methods**

The brain capillaries were isolated from normal human brain tissues by using the present (highly purified) and conventional isolation methods which are described in the Materials and Methods section (Triplicate for present method; Duplicate for conventional method). Two endothelial markers (ABCB1 and SLC2A1), two neuronal markers (NFL and NFM), an astrocyte marker (GFAP), and an oligodendrocyte marker (MOG) were quantified by SWATH analysis. **A,** the protein amounts of markers were relatively compared between conventional and present methods, after the normalization by average protein amount in conventional method. **B,** the protein amounts of endothelial markers were divided by those of non-endothelial markers, and relatively compared between conventional and present methods, after the normalization by average value in conventional method.



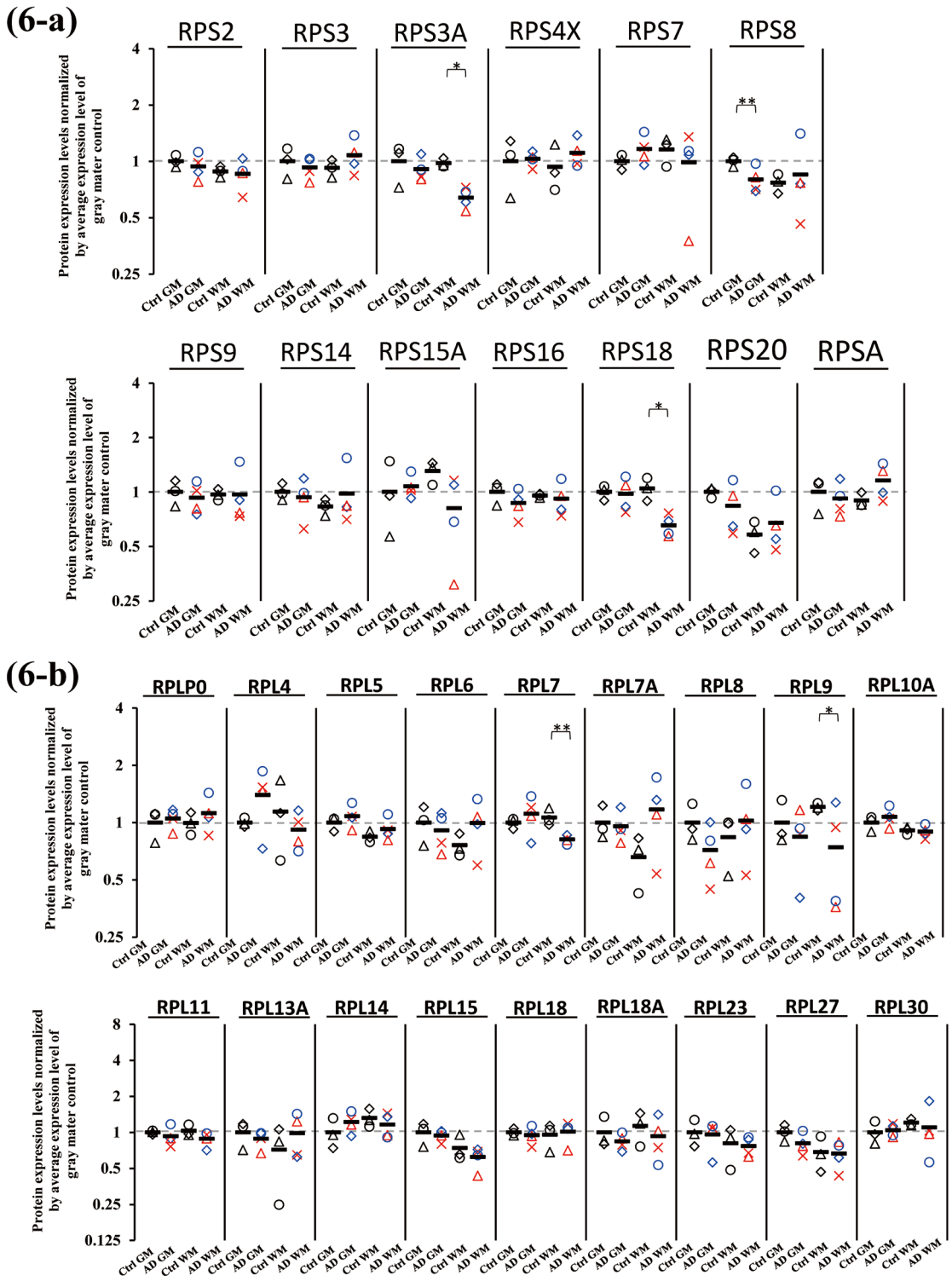

**Supplementary figure 4 Comparison of protein expression levels between AD and control donors for cerebral endothelial cell markers in isolated brain capillaries**

Brain capillaries were isolated from gray (neocortex) and white (corona radiata) matter of four AD and three control donors. Tryptic digests were produced and subjected to SWATH analysis. The protein expression levels were normalized by the average protein expression levels in gray matter capillaries of control donors. N.S., not significantly different between AD and control groups (BH-adjusted p value > 0.05). Ctrl GM, gray matter capillaries in control donors; AD GM, gray matter capillaries in AD donors; Ctrl WM, white matter capillaries in control donors; AD WM, white matter capillaries in AD donors. Circle, control 1 or AD 1; Triangle, control 2 or AD 2; Diamond, control 3 or AD 3; Cross, AD 4. Bar, average value in each group. For AD patients, blue plots are male, and red plots are female. ABCB1, PECAM1 and ABCG2 are marker proteins which are specifically expressed in the capillary endothelial cells in brain.



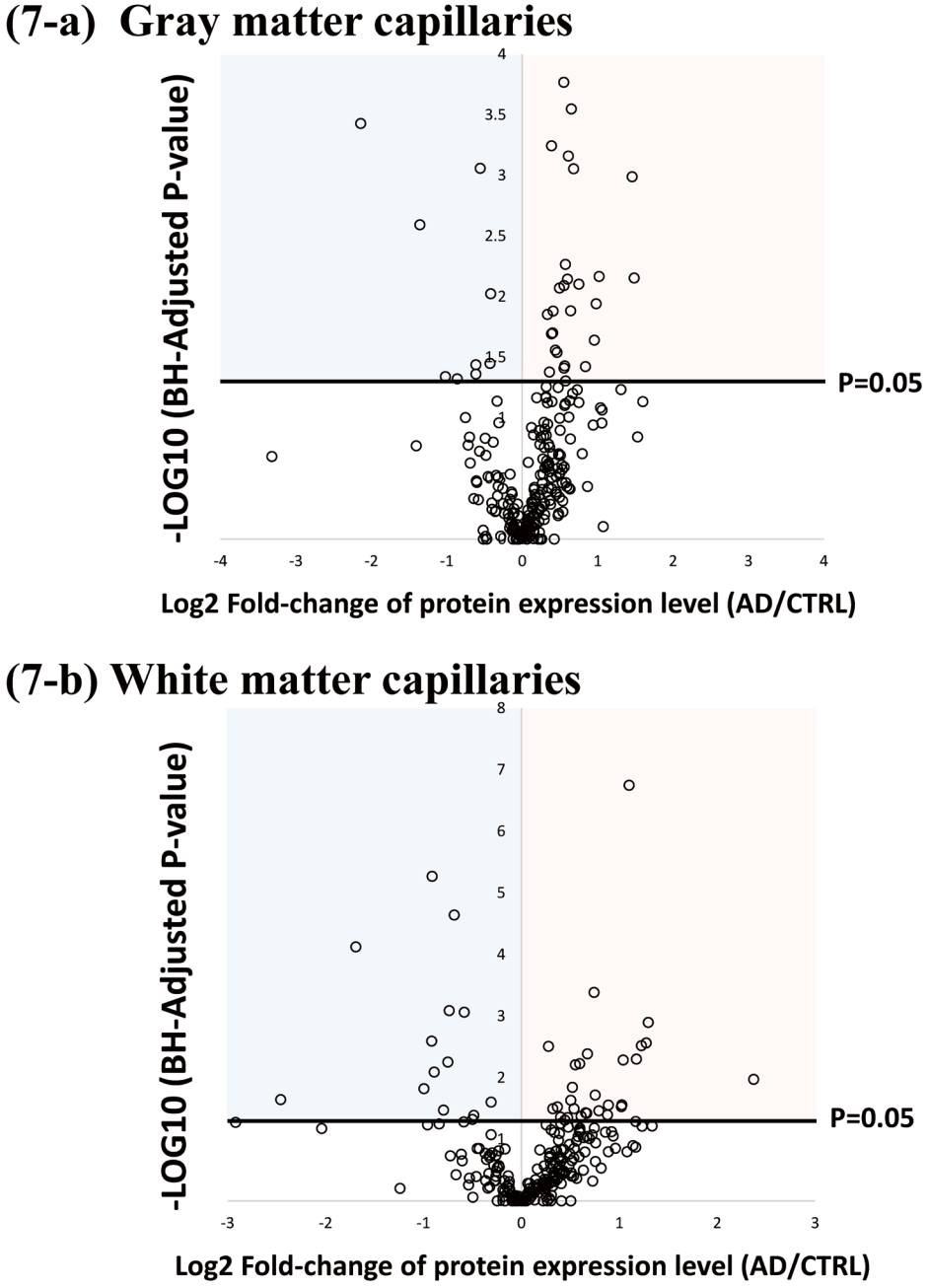

**Supplementary figure 5 Total accumulation of Aβ and APP in whole tissue lysate (a) and the capillary fraction (b) from gray and white matters**

Whole tissue lysate and brain capillaries were prepared from gray (neocortex) and white (corona radiata) matters of four AD and three control donors. Tryptic digests were produced and subjected to SWATH analysis. A total levels of Aβ and APP were quantified by the peak area of tryptic peptide "LVFFAEDVGSNK" which is a shared peptide in Aβ and APP. The levels were normalized by the average levels in gray matters of control donors. BH-adjusted p value < 0.05 (*), and < 0.0001 (**), significantly different between two groups. Circle, control 1 or AD 1; Triangle, control 2 or AD 2; Diamond, control 3 or AD 3; Cross, AD 4. Bar, average ± SEM in each group. For AD patients, blue plots are male, and red plots are female.




**Supplementary figure 6 Protein expression levels of ribosomal proteins in the parenchyma of cerebral gray and white matter in AD patients**

Whole tissue lysate was prepared from gray (neocortex) and white (corona radiata) matters of four AD and three control donors. Tryptic digests were produced and subjected to SWATH analysis. The protein expression levels were normalized by the average protein expression levels in gray matter of control donors. BH-adjusted p value < 0.05 (*), and < 0.01 (**), significantly different between AD and control groups. 6-a, Small ribosomal subunit proteins (40S ribosomal protein); 6-b, Large ribosomal subunit proteins (60S ribosomal protein). Ctrl GM, gray matter in control donors; AD GM, gray matter in AD donors; Ctrl WM, white matter in control donors; AD WM, white matter in AD donors. Circle, control 1 or AD 1; Triangle, control 2 or AD 2; Diamond, control 3 or AD 3; Cross, AD 4. Bar, average value in each group. For AD patients, blue plots are male, and red plots are female.




**Supplementary figure 7 Up-regulated expression of N-glycosylated proteins in AD brain capillaries**

Using the Uniprot database, N-glycosylated proteins were searched. Based on the results of this search, N-glycosylated proteins were extracted from the SWATH data for capillary samples of gray (7-a) and white (7-b) matters. The fold change of protein expression level (AD/control) and BH-adjusted p value were calculated for individual proteins. The plots of colored zones represent the proteins that showed significant differences between AD and control groups (BH-adjusted p value < 0.05). Red zone, significantly up-regulated proteins in AD group; Blue zone, significantly down-regulated proteins in AD group.