

## Description of Additional Supplementary Files

### **Supplementary Data 1: Label-free quantification of mouse brain vessel proteome - reference protein sequences.**

The raw data were searched against a modified, reviewed isoform FASTA database of *Mus musculus*.

Label free quantification (LFQ) required at least 2 unique or razor peptides. Generated LFQ values were log<sub>2</sub> transformed and log<sub>2</sub> ratios were calculated for inter genotype comparison. Corresponding p-values (two-sided unpaired *t*-test) were calculated and are additionally depicted as  $-\log_{10}$  transformed values. *n* = 5 mice / genotype. HtrA1, Ltbp4 and Prss23 are highlighted in orange. The other proteins of interest are highlighted in yellow (see text for explanations).

### **Supplementary Data 2: Analysis of individual tryptic peptides.**

The raw data were searched against a modified, reviewed isoform FASTA database of *Mus musculus* implemented with the sequences of HtrA1-R274Q and HtrA1-D174R-S328A. *n* = 5 mice / genotype.

**Subtab "HtrA1":** Unique peptides matching with the reference sequence of HtrA1 or with the sequences of HtrA1-R274Q or HtrA1-D174R-S328A are listed. Peptides containing Arg174, Arg274 or Gln274 are highlighted in yellow.

**Subtabs "Ltbp4" and "Prss23":** Unique peptides matching with the reference sequences of Ltbp4 or Prss23 are listed. Peptides matching with the aminoterminal region of Ltbp4 (aa 1-143) or with the carboxyterminal region of Prss23 (aa 50-382) are highlighted in yellow.

**Subtab "Other POIs":** Unique peptides matching with the reference sequences of the other proteins of interest (see text for explanations) are listed.

### **Supplementary Data 3: Label-free quantification of mouse brain vessel proteome - custom protein sequences.**

The raw data were searched against a modified, reviewed isoform FASTA database of *Mus musculus*. The canonical HtrA1 sequence was replaced by the Pan-HtrA1 sequence (excluding aa 274); Ltbp4 was replaced with two sequences, which separately depict its N-

and C-terminal parts (aa 1-143 and aa 144-1,666, respectively); Prss23 sequence was shortened (aa 50-382) to only include its C-terminal part.

Label free quantification (LFQ) required at least 2 unique or razor peptides. Generated LFQ values were log<sub>2</sub> transformed and log<sub>2</sub> ratios were calculated for inter genotype comparison. Corresponding p-values (two-sided unpaired *t*-test) were calculated and are additionally depicted as  $-\log_{10}$  transformed values. *n* = 5 mice / genotype. Pan-HtrA1, Ltbp4-Nt and Prss23-Ct are highlighted in orange. The other proteins of interest (including Ltbp4-Ct) are highlighted in yellow (see text for explanations).

**Supplementary Data 4:** Input file for the MD simulations of S328A.

**Supplementary Data 5:** Final frame for the MD simulations of S328A.

**Supplementary Data 6:** Input file for the MD simulations of R166H.

**Supplementary Data 7:** Final frame for the MD simulations of R166H.

**Supplementary Data 8:** Input file for the MD simulations of R274Q.

**Supplementary Data 9:** Final frame for the MD simulations of R274Q.

**Supplementary Data 10:** Input file for the MD simulations of A173T.

**Supplementary Data 11:** Final frame for the MD simulations of A173T.

**Supplementary Data 12:** Input file of the GaMD simulations of R274Q-VDAC2.

**Supplementary Data 13:** Final frame of the GaMD simulations of R274Q-VDAC2.