iScience, Volume *25*

Supplemental information

Co-expression network analysis of human tau-transgenic mice reveals protein modules associated with tau-induced pathologies

Kazuya Tsumagari, Yoshiaki Sato, Aki Shimozawa, Hirofumi Aoyagi, Hideyuki Okano, and Junro Kuromitsu

Figure S1

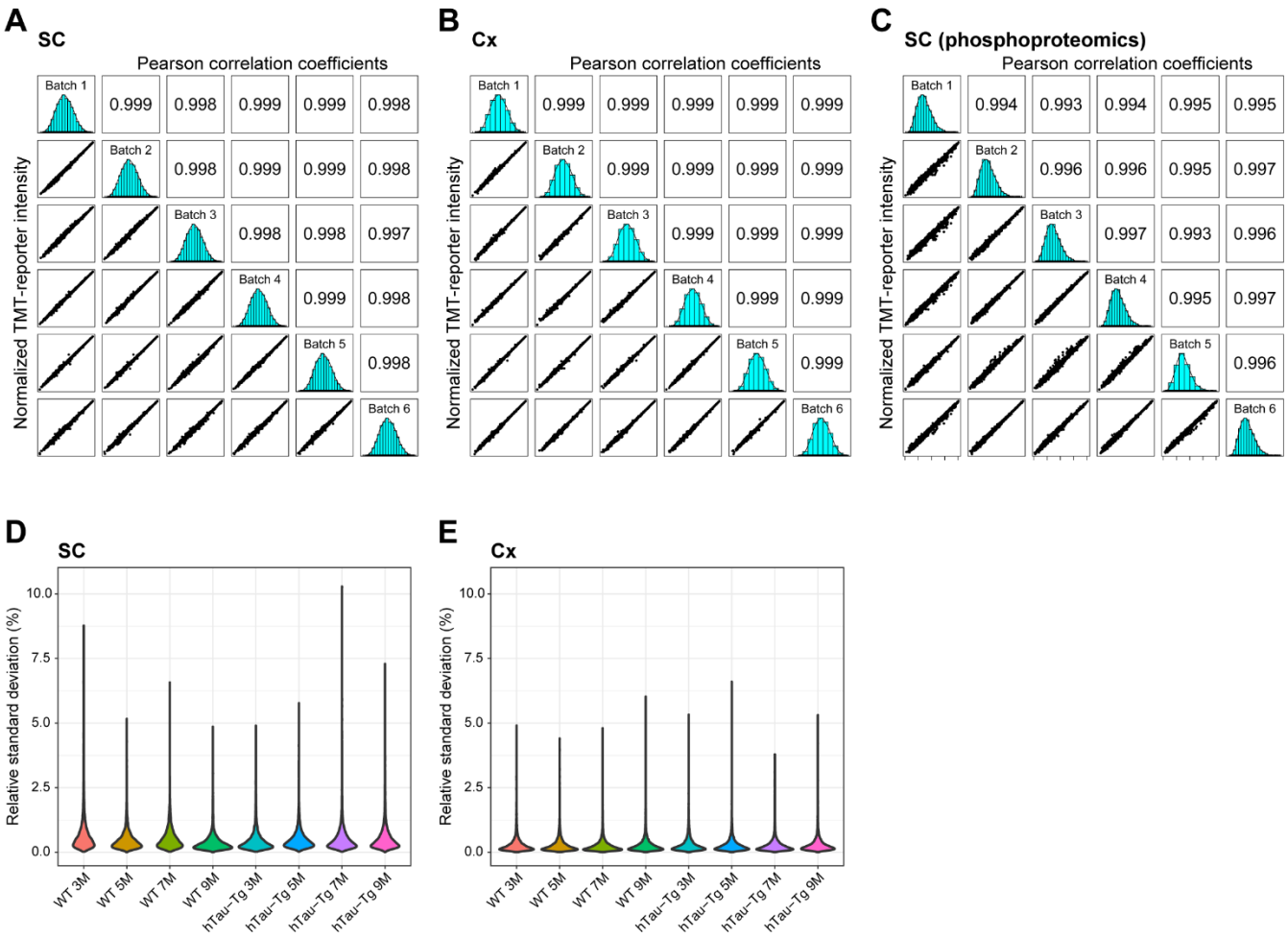

Figure S1. Reproducibility of protein quantification, Related to Figure 1.

A-B. Reproducibility of protein quantification among six batches of LC/MS/MS measurements. Pearson correlation coefficients were calculated for identical samples, the pooled samples (TMT-131N), and are shown for spinal cord (SC) and cortex (Cx). The matrix (6 × 6) correlation plots revealed the measurements were highly reproducible (Pearson correlation coefficients are close to 1).

C. Reproducibility of phosphorylation site quantification among six batches of LC/MS/MS measurements. Pearson correlation coefficients were calculated for identical samples, the pooled samples (TMT-131N). The matrix (6 × 6) correlation plots revealed the measurements were highly reproducible (Pearson correlation coefficients are close to 1).

D-E. Reproducibility of biological replicates. Relative standard deviation (RSD) of each protein under the conditions investigated is shown for SC and Cx, respectively.

Figure S2

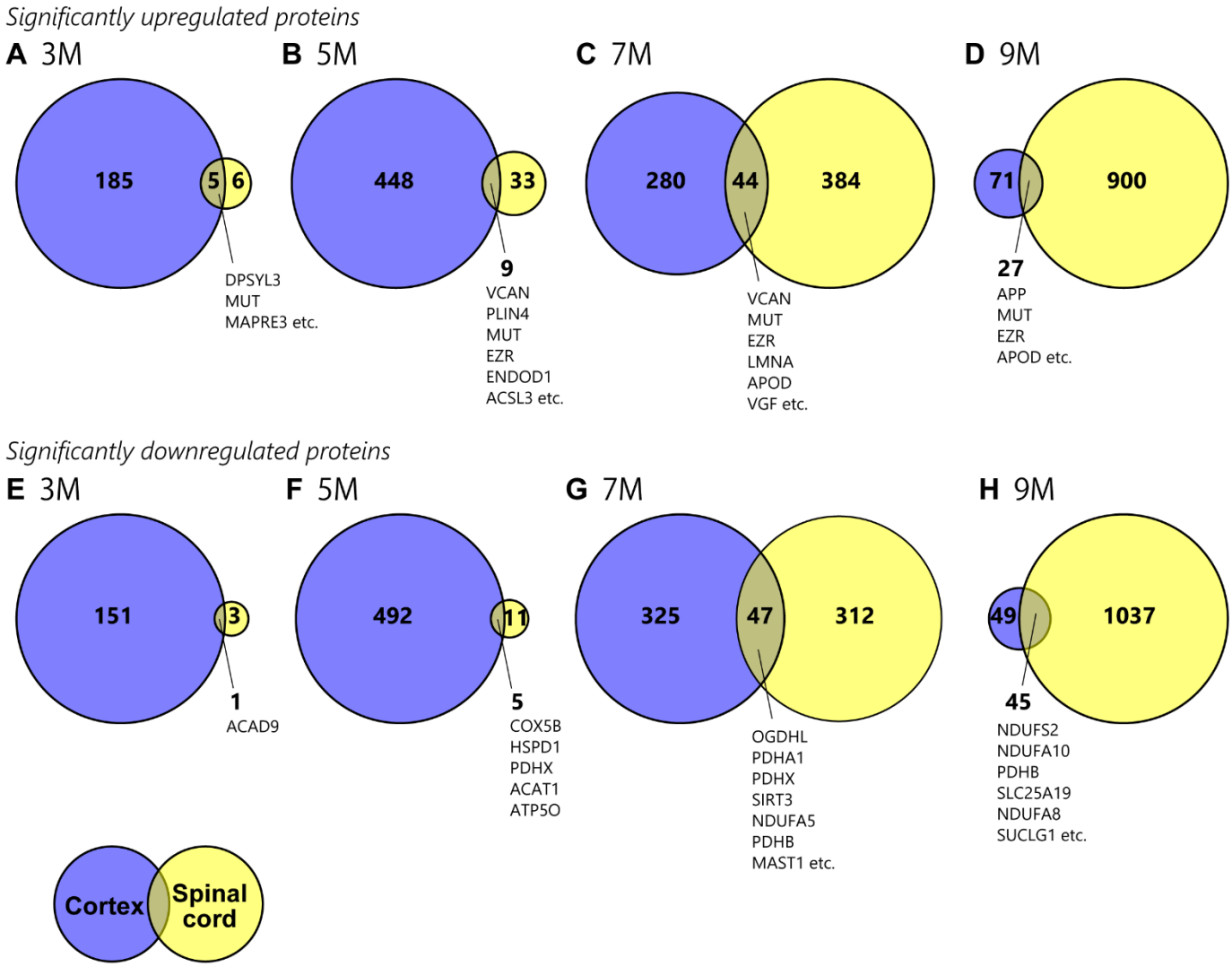

Figure S2. Overlap of significantly regulated proteins between spinal cord and cortex, Related to Figure 2.

For the commonly quantified proteins in spinal cord and cortex, overlaps of significantly upregulated proteins (**A-D**) and downregulated proteins (**E-H**) are shown by Venn diagrams.

Figure S3

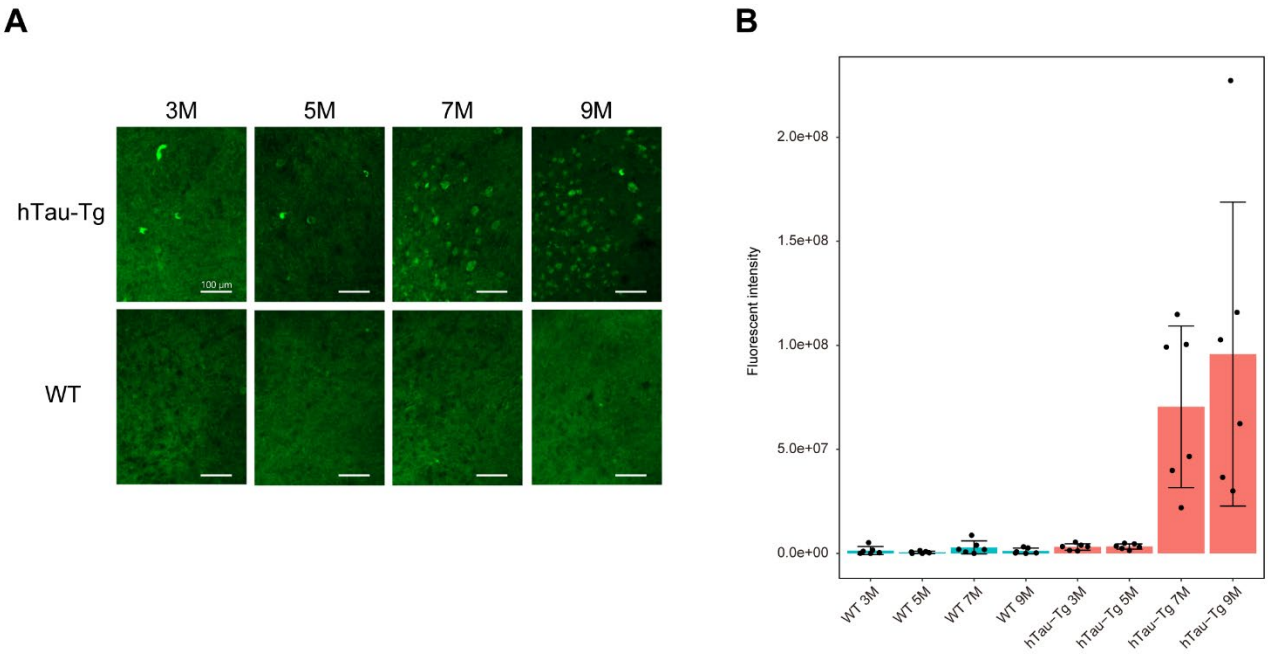

Figure S3. Quantification of filamentous tau inclusions in spinal cord, Related to Figure 3.
Filamentous tau inclusions were stained by 1-fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene (FSB) (**A**) and quantified by fluorescence microscopy (**B**). Error bars represents standard deviations.

Figure S4

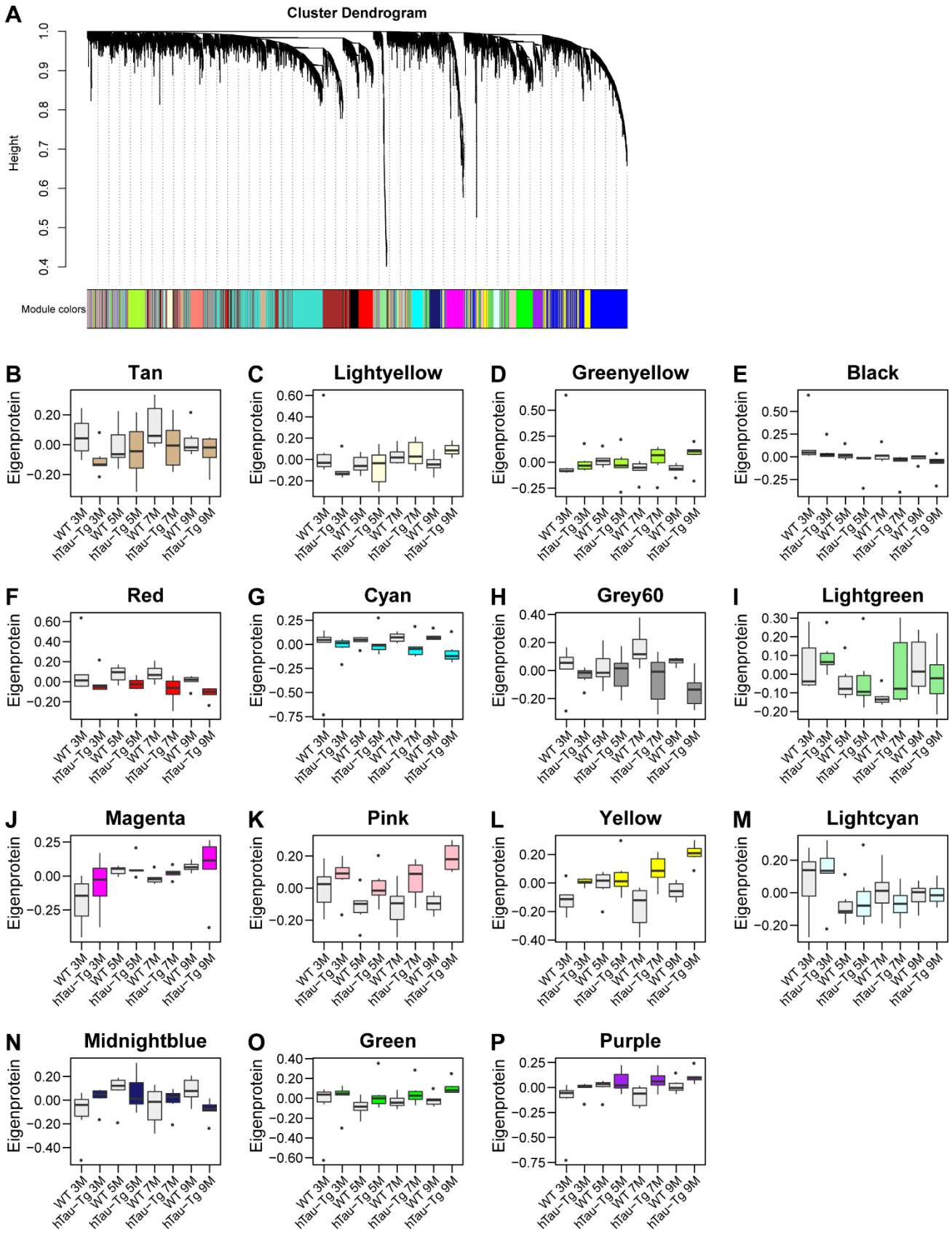

Figure S4. Protein dendrogram and module eigenproteins of each module, Related to Figure 3.

A. WGCNA cluster dendrogram utilized to define modules by the “hybrid” branch cutting method.

B-P. Module eigenprotein values, which are defined as the first principal component of a given module and serve as representatives. The eigenprotein values of the Blue, Turquoise, Brown, and Salmon

modules are shown in Figure 3E-H. The top and bottom of the whisker indicate the largest value no further than 1.5 * IQR and the smallest value at most 1.5 * IQR, respectively, where IQR is the inter-quartile range, or the distance between the first and third quartiles. Data beyond the end of the whiskers are plotted individually.

Figure S5

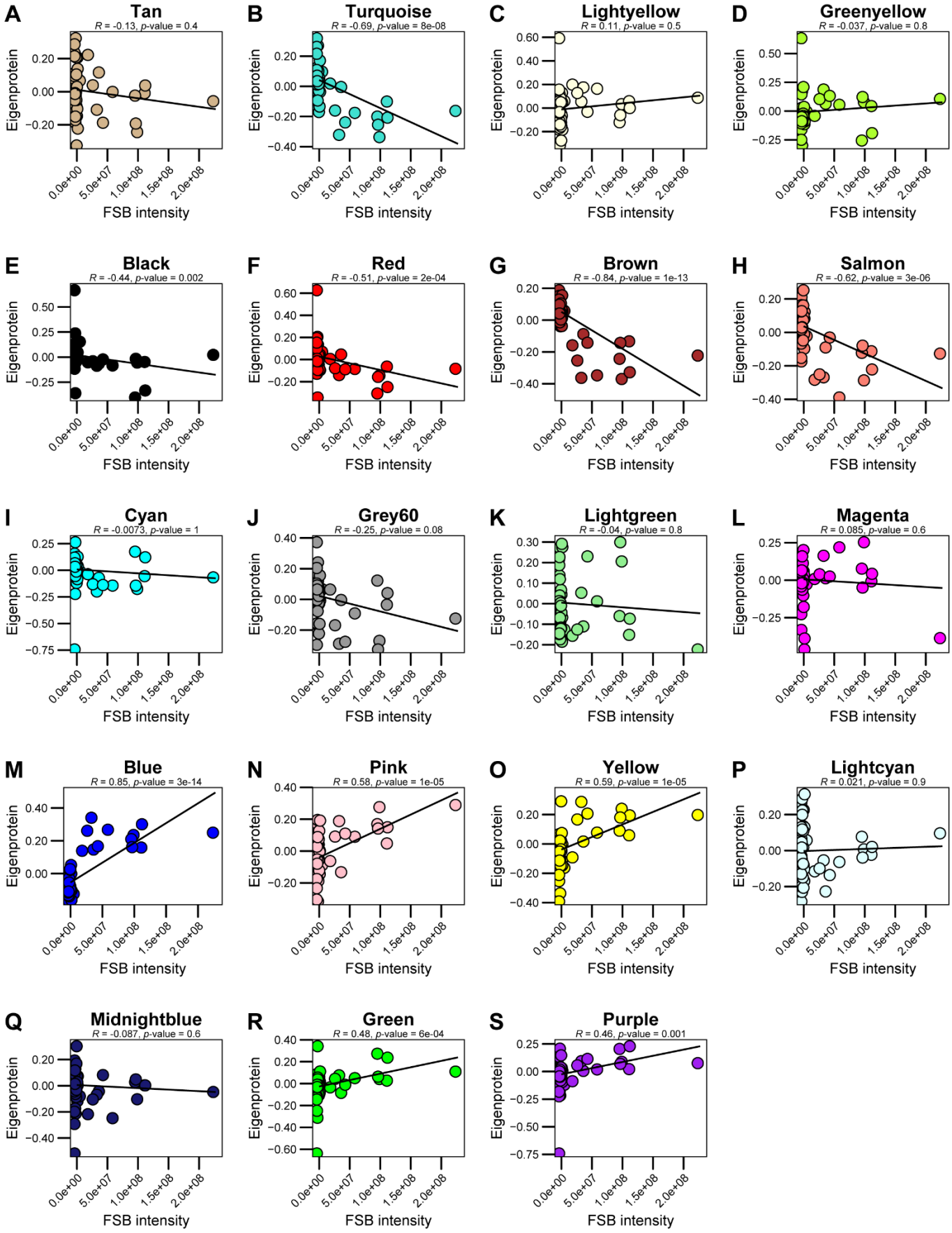

Figure S5. Correlation between each module and tau accumulation, Related to Figure 3.

Pearson correlations between module eigenproteins and the abundance of tau estimated by FSB-staining (Figure S3) are plotted.

Figure S6

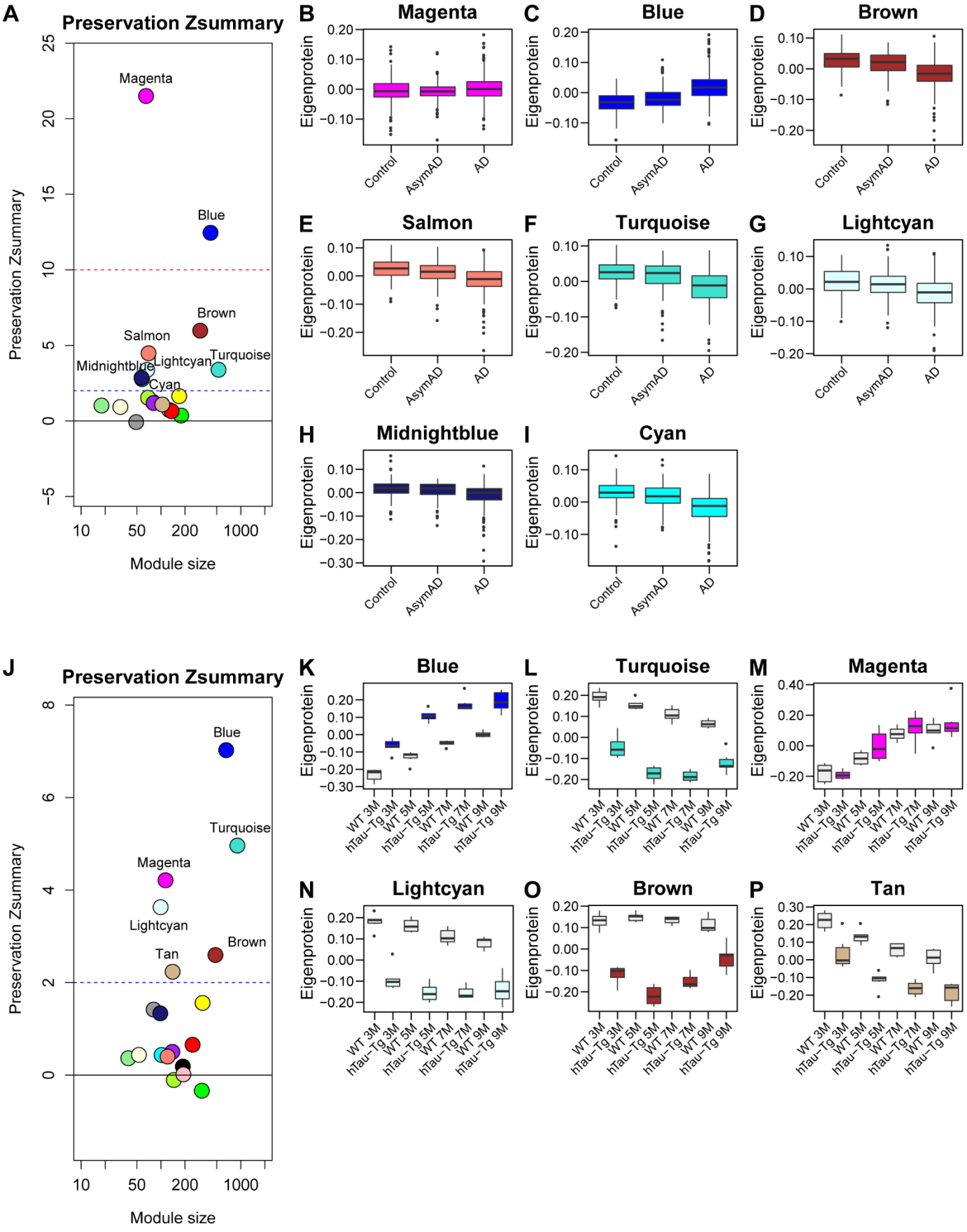

Figure S6. Module preservation analysis for cortex of hTau-Tg and human AD postmortem brains, Related to Figure 3.

A. Module preservation analysis examining whether the protein co-expression networks detected in the spinal cord proteomes were preserved in human AD brains. The proteome dataset of dorsolateral prefrontal cortex (DLPFC) was derived from cohorts consisting of 91 controls, 98 asymptomatic AD patients, 230 AD patients (in total 419 tissues, 3,274 proteins). The dashed blue line indicates a Zsummary score of 2, above which module preservation was considered significant, and the dashed red line indicates a Zsummary score of 10, above which module preservation was considered highly significant.

B-I. For the significantly preserved modules in **A**, synthetic module eigenprotein values were computed based on the expression in the DLPFC of AD brains. The top and bottom of the whisker indicate the largest value no further than 1.5 * IQR and the smallest value at most 1.5 * IQR, respectively, where IQR is the inter-quartile range, or the distance between the first and third quartiles. Data beyond the end of the whiskers are plotted individually.

J. Module preservation analysis examining whether the protein co-expression networks detected in the spinal cord proteomes of hTau-Tg were preserved in the cortex proteomes of hTau-Tg. The dashed blue line indicates a Zsummary score of 2, above which module preservation was considered significant.

K-P. For the significantly preserved modules in **J**, synthetic module eigenprotein values were computed based on the expression in the cortex of hTau-Tg. The top and bottom of the whisker indicate the largest value no further than 1.5 * IQR and the smallest value at most 1.5 * IQR, respectively, where IQR is the inter-quartile range, or the distance between the first and third quartiles. Data beyond the end of the whiskers are plotted individually.

Figure S7

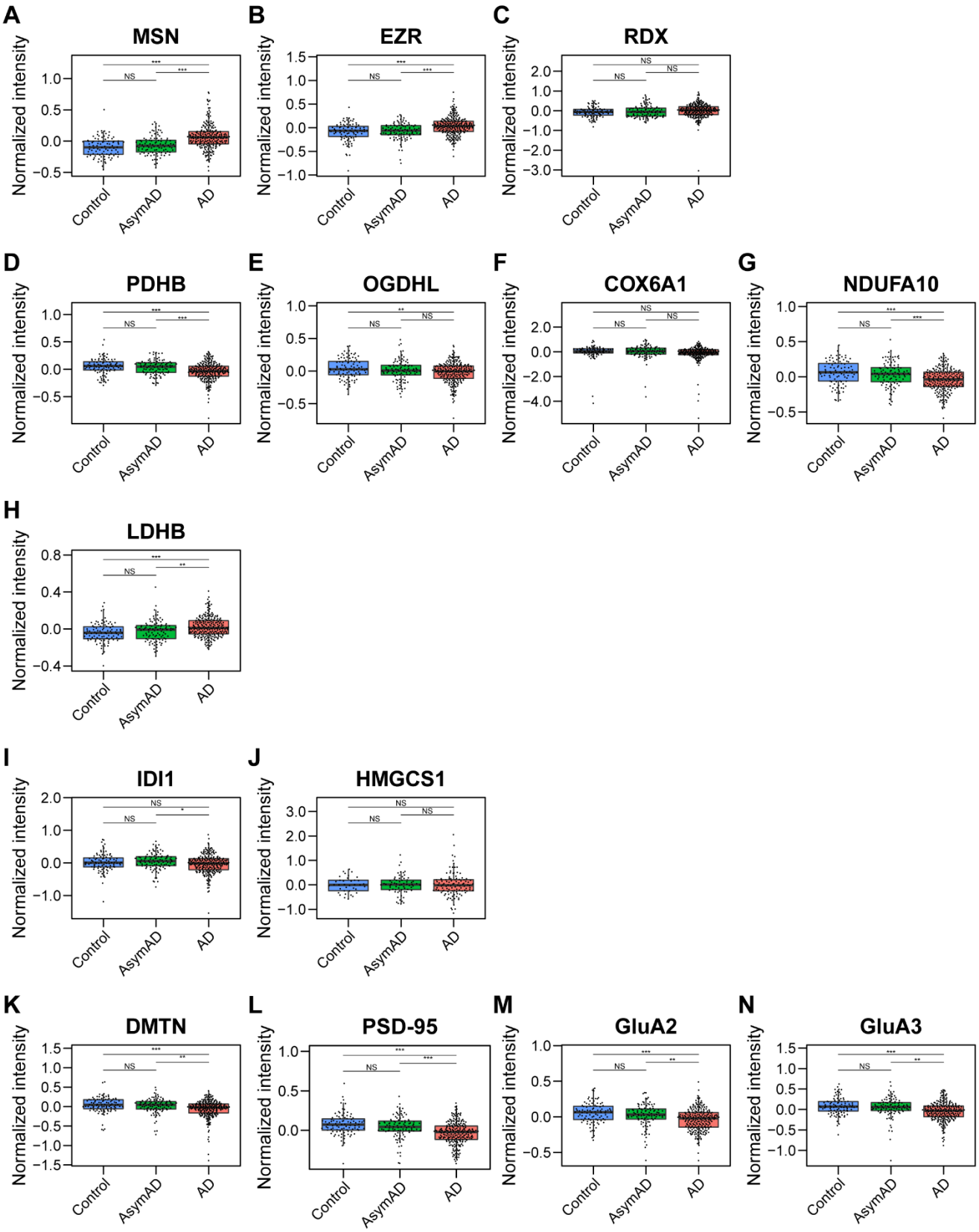

Figure S7. Protein expressions in the AD brain datasets, Related to Figure 4.

The expressions of proteins of interest in the AD brain dataset acquired by Johnson *et al*. (2020) are shown. **A-C**, proteins in the Blue/neuroinflammatory response module; **D-H**, proteins in the Turquoise/mitochondrial module; **I-J**, proteins in the Brown/cholesterol biosynthesis module; **K-N**, proteins in the Salmon/postsynaptic density module and postsynaptic-density-related proteins. Welch's t-test was performed (91 controls, 98

asymptomatic AD patients, and 230 AD patients). The top and bottom of the whisker indicate the largest value no further than 1.5 * IQR and the smallest value at most 1.5 * IQR, respectively, where IQR is the inter-quartile range, or the distance between the first and third quartiles. Data beyond the end of the whiskers are plotted individually. *, *q*-value <0.05; **, *q*-value <0.01; ***, *q*-value <0.001.

Figure S8

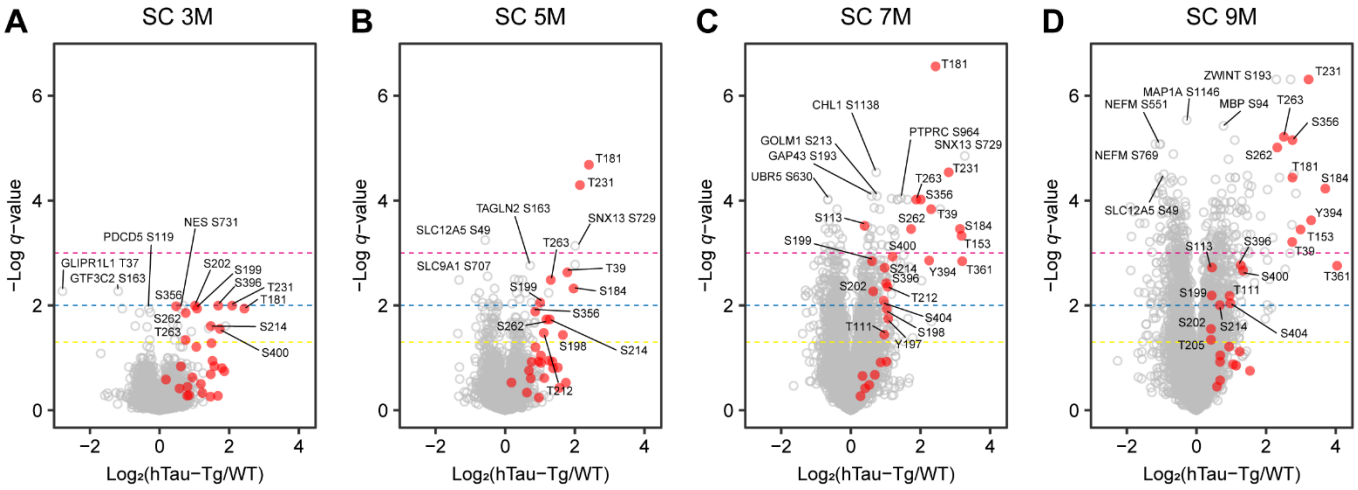

Figure S8. Volcano plots comparing the phosphoproteomes of the spinal cord of WT and hTau-Tg at various ages, Related to Figure 6.

Volcano plots comparing the intensities of phosphorylation sites in the spinal cord (SC) of WT and hTau-Tg at different ages. Welch's t-tests were performed to identify significantly changed phosphorylation sites (*N*=6). The dashed yellow, blue, and magenta lines indicate *q*-value = 0.05, 0.01, and 0.001, respectively. Tau phosphorylation sites are highlighted in red, and sites with significantly changed tau phosphorylation are labeled with the positions in the 2N4R form. Selected significantly changed phosphorylation sites are labeled with the protein names and positions.