The schizophrenia-associated variant in *SLC39A8* alters protein glycosylation in the mouse brain

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Supplemental Figures

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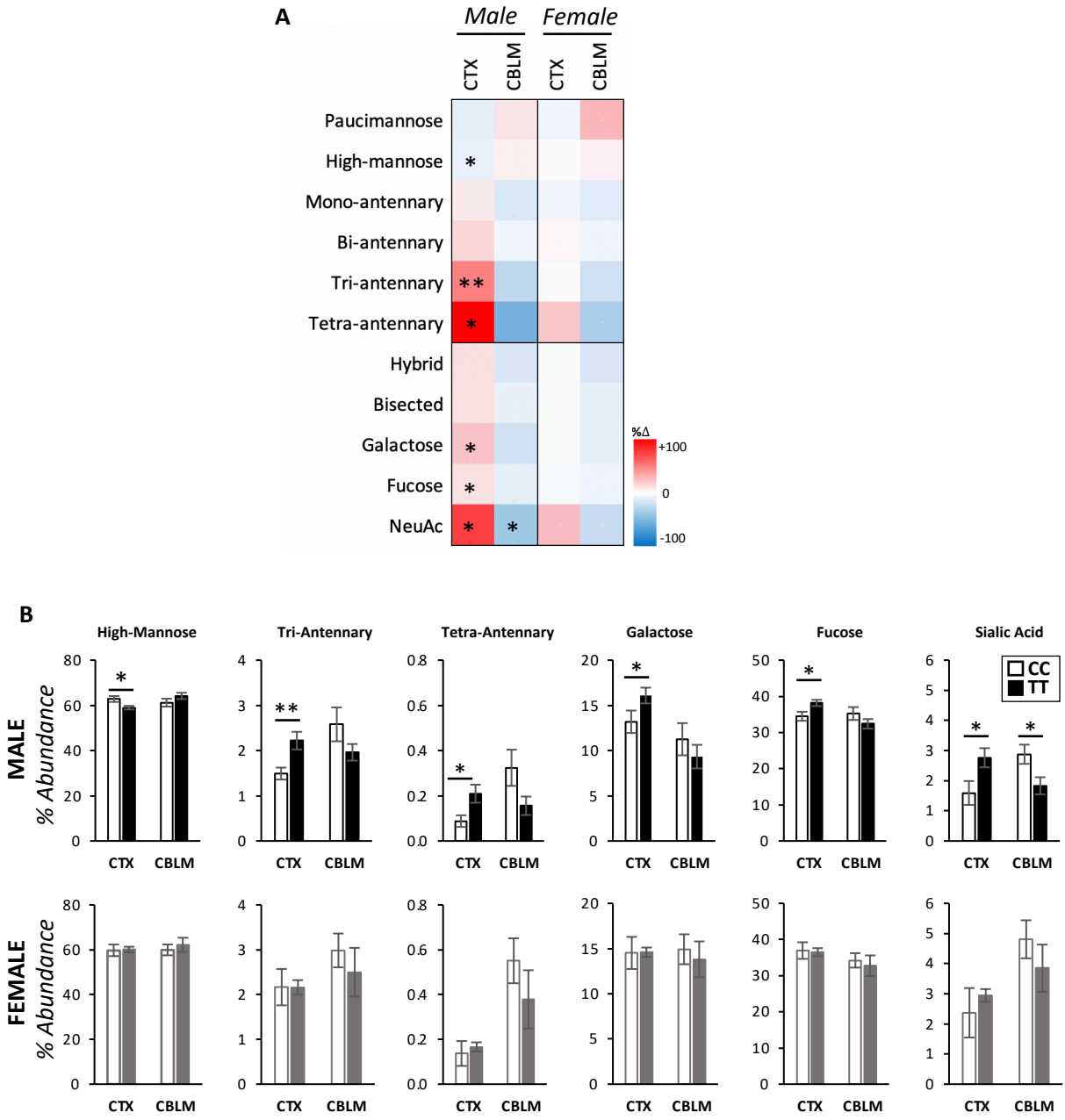

Fig. S1. A391T shows a stronger effect on brain N-glycosylation in males. MALDI-TOF glycomics (A) and categorical analyses (B) of CTX and CBLM N-glycans from female A391T mice showed the same directionality of change as observed in males, but the smaller effect size does not reach statistical significance. Data presented as a heat map of percent change in glycan abundance comparing TT mice to CC controls for A and as mean percent abundance +/- SEM for B. CC=4, TT=4 for each sex. *p*-value *< 0.05, **< 0.01. Related to Fig. 1.

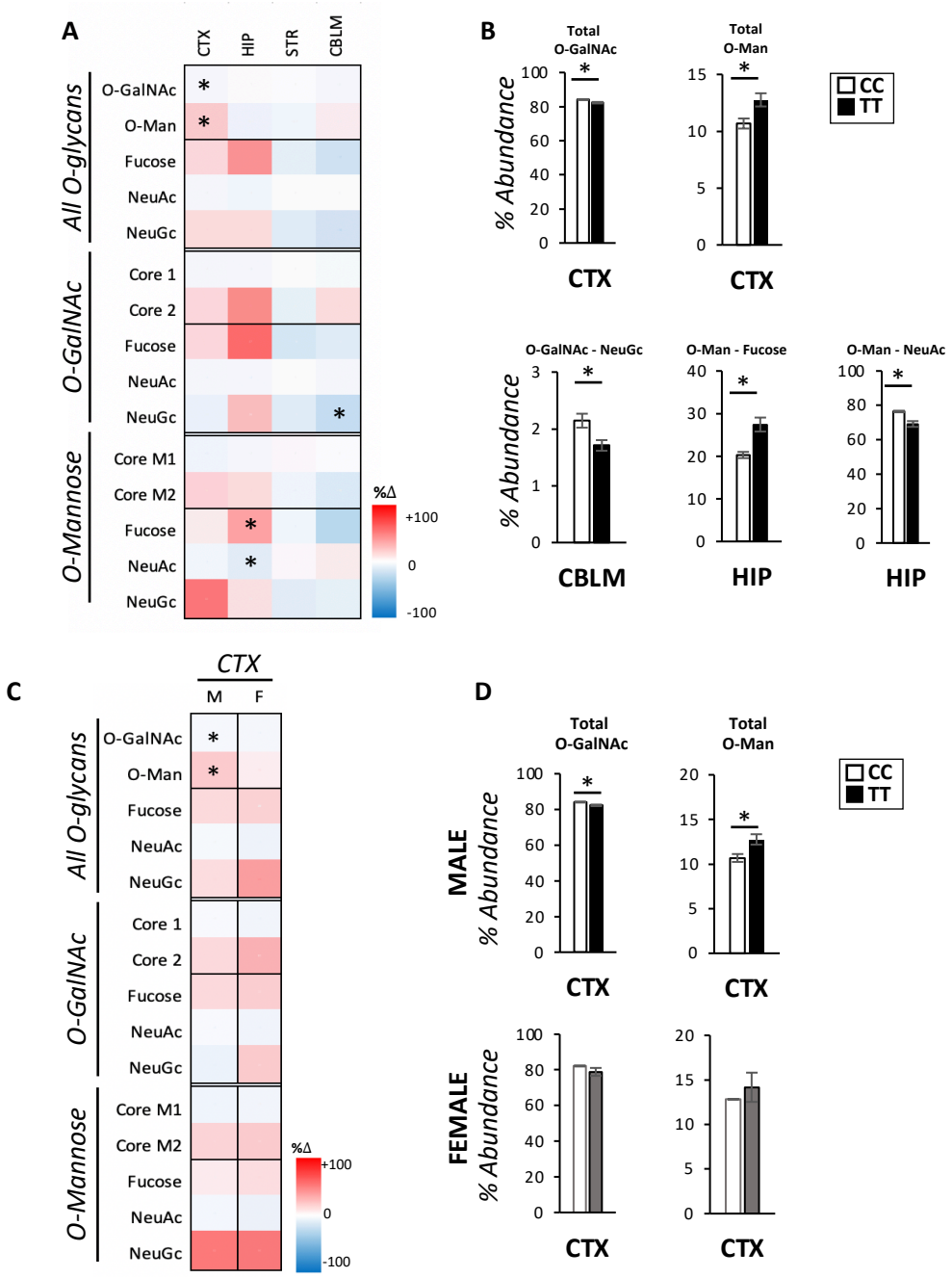

Fig. S2. A391T has a small effect on the relative abundance of protein O-glycans in cortex. A) MALDI-TOF MS analysis of the relative O-glycan abundances from the cortex (CTX), hippocampus (HIP), striatum (STR), and cerebellum (CBLM) of male mice. Data presented as a heat map of percent change in glycan abundance comparing TT mice to CC controls. B) Categorical analysis of MALDI O-glycome data for TT cortex showed a relative decrease in O-GalNAc and increase in O-Man glycans, and a few additional significant differences. CTX CC=4, TT=5; HIP CC=2, TT=4; STR CC=4, TT=6; CBLM CC=4, TT=5 for A and B. C) MALDI-TOF MS analysis of the relative O-glycan abundances from the cortex (CTX) and cerebellum (CBLM) of female mice. D) Categorical analysis of CTX and CBLM O-glycans from female A391T mice showed the same directionality of change as observed in males, but this effect does not reach statistical significance. Data presented as a heat map of percent change in glycan abundance comparing TT mice to CC controls. N = 2 CC, 4 TT for each region and gender. Data presented as mean percent abundance +/- SEM. *p*-value *< 0.05. Related to Fig. 1.

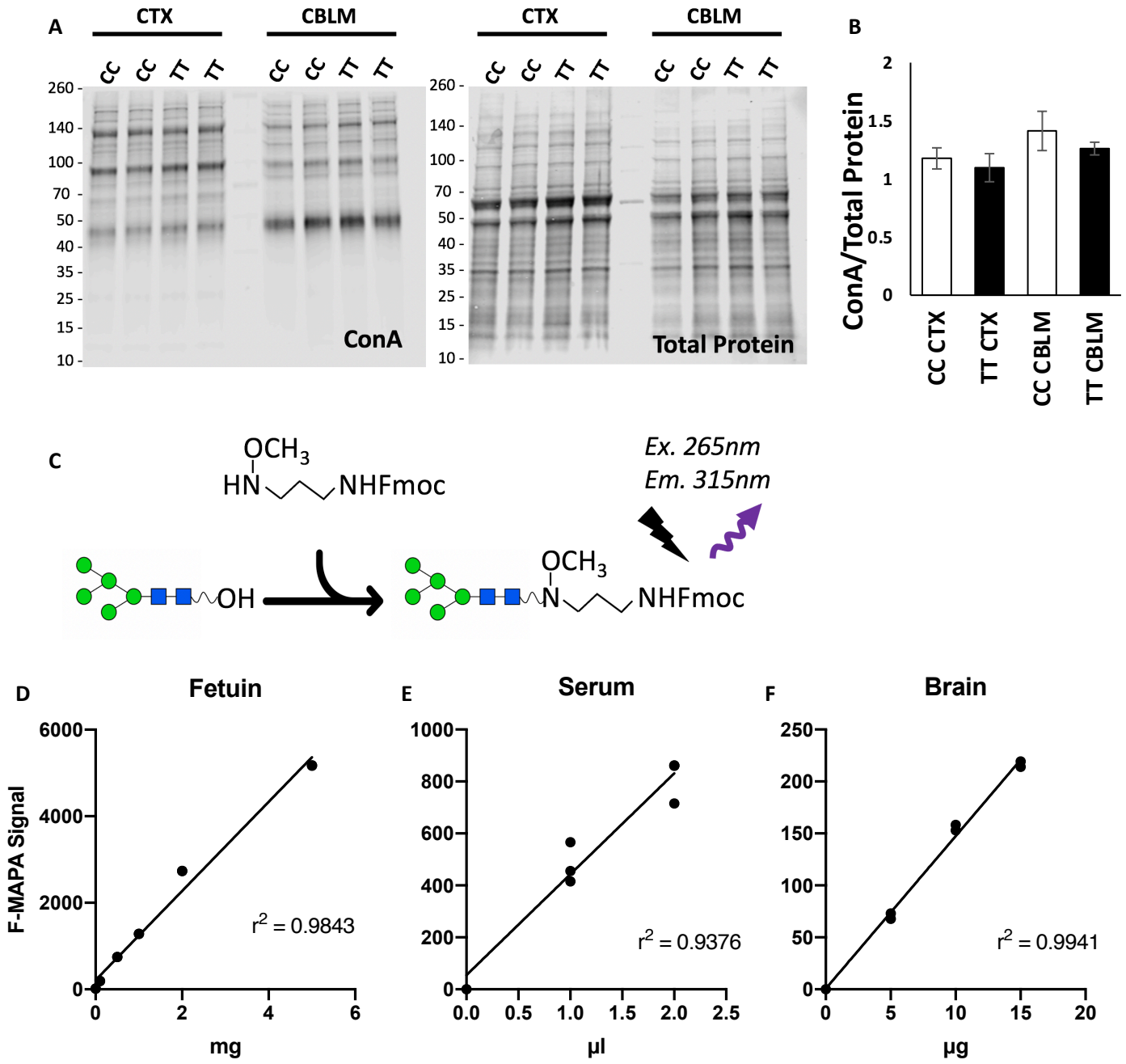

Fig. S3. Quantitative measures of brain glycans. A) Representative lectin western blot analysis of brain lysate using ConA and total protein stain from cortex and cerebellum of male CC and TT mice and a stain for Total Protein. Proteins recognized by ConA are abundant above 15 kD and represent the diverse pool proteins containing N-glycans. B) Quantification of ConA/Total Protein signal from A using the LiCOR Image Studio Software as previously described (Williams, *et al.*, 2021). In brief, glycoproteins are blotted using a biotin-tagged ConA and detected using fluorescently-labelled streptavidin. Quantification was performed by selecting a region of interest in each lane of ConA bound glycoprotein signal, normalizing to the signal of Total Protein stain from the same region. N = 4 per region and genotype. Data shown as mean +/- SEM. *p*-value CC vs TT; cortex = 0.62, cerebellum = 0.45. C) Schematic of F-MAPA fluorescent glycan derivatization assay used in Fig. 1C. Free hydroxyl groups of PNGase F-cleaved N-glycans are labeled in a 1:1 ratio with F-MAPA and quantified using fluorescence (Excitation: 265nm; Emission: 315nm). F-MAPA fluorescence curves using different quantities or concentrations of standards controls of fetuin (D), serum (E), and brain lysate (F), with individual data points and the corresponding r^2 values. Related to Fig. 1.

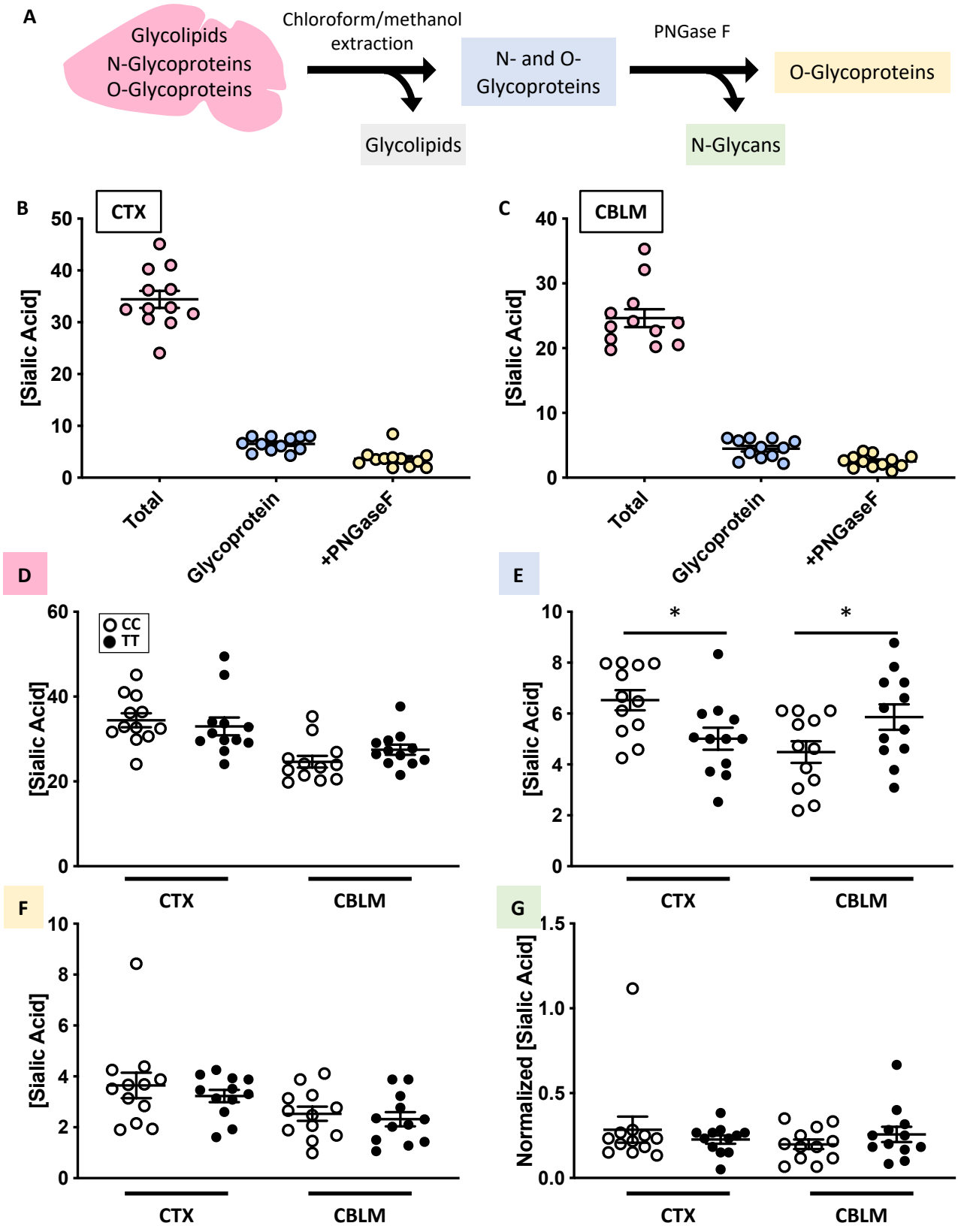

Fig. S4. A391T lowers the absolute quantity of N-glycans in the cortex. A) Workflow for isolation of different glycan fractions from brain homogenate for sialic acid quantification. The concentration of sialic acid in different glycan fractions from wild-type cortex (B) and cerebellum (C) are presented as nmol sialic acid/mg of protein, determined using the NANA kit (Abcam) and normalized to protein concentration using BCA assay (Pierce). In both regions, most sialic acid is contained on glycolipids. Fractions represents crude brain homogenate (Total), N- and O- glycoproteins following glycolipid extraction with methanol/chloroform (Glycoprotein), and O-glycoproteins after PNGase F treatment (+PNGaseF). D) Sialic acid quantification of total lysate (glycolipids and glycoproteins), showing no difference in A391T mice. E) Sialic acid quantification of total glycoproteins (N- and O-), showing a decrease in cortex and increase in cerebellum of A391T mice. F) Sialic acid quantification of O-glycoproteins, showing no difference in A391T mice. G) Sialic acid quantification of released N-glycans, showing now difference in A391T mice when normalizing for per nmol of N-glycans using F-MAPA. Sialic acid concentration reported as nmol sialic acid/mg of protein for A-F. N = 4 male mice per group, measured in triplicate. Individual data points are shown, with brackets representing group means +/- SEM. *p*-value *< 0.05. Related to Fig. 1.

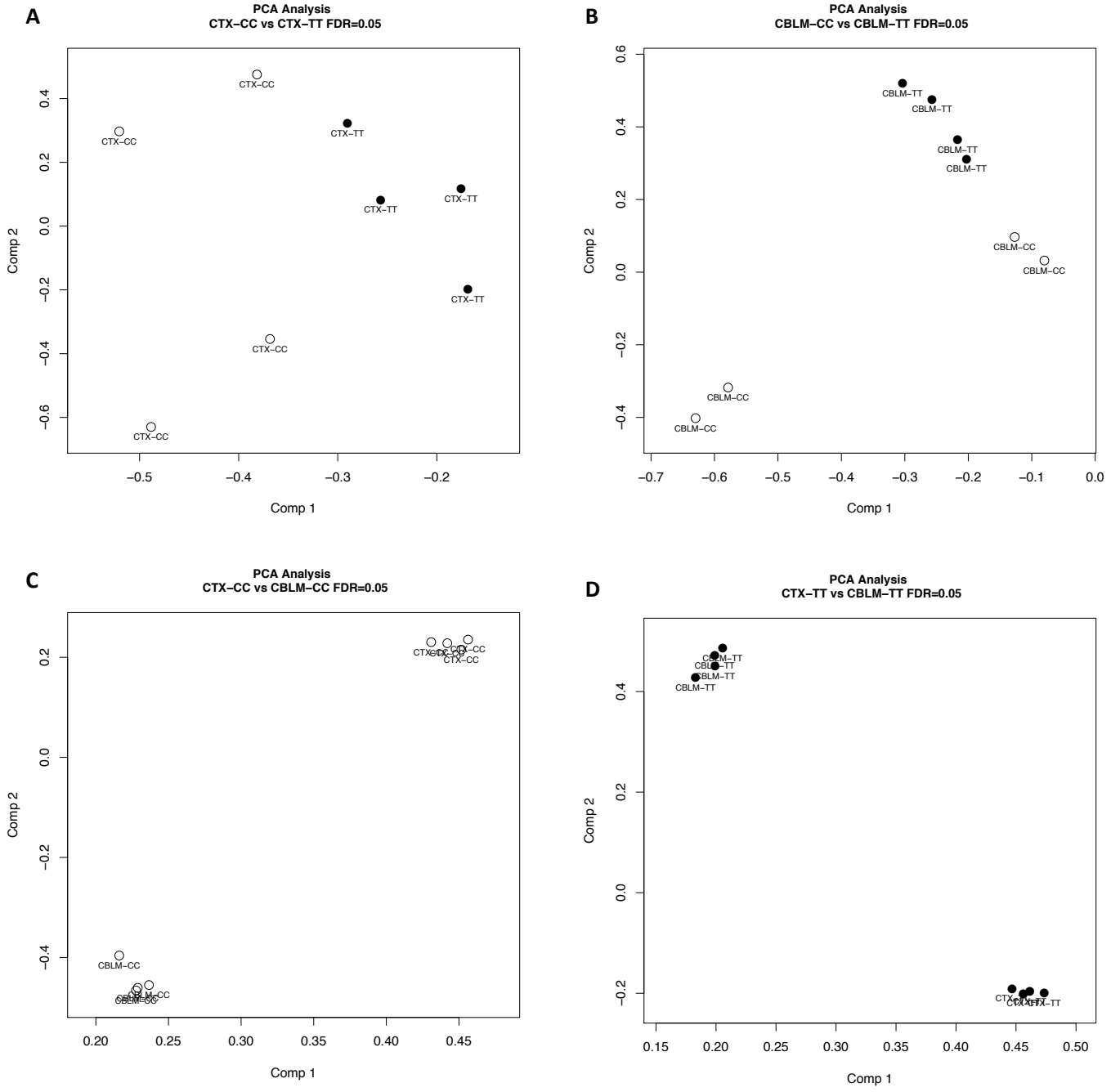

Fig. S5. Gene expression changes are minimal between genotypes but large between regions. PCA plots comparing cortex (A) and cerebellum (B) between CC and TT mice identifies minimal gene expression changes between genotypes. PCA plots of CC (C) and TT (D) mice between cortex and cerebellum shows large gene expression changes, consistent with the known regional differences. E) PCA analysis between all groups highlights the lack of gene expression differences between genotypes in A391T mice, with CC (white dots) and TT (black dots) from cortex and cerebellum shown. N = 4 male mice per group. Related to Fig. 2.

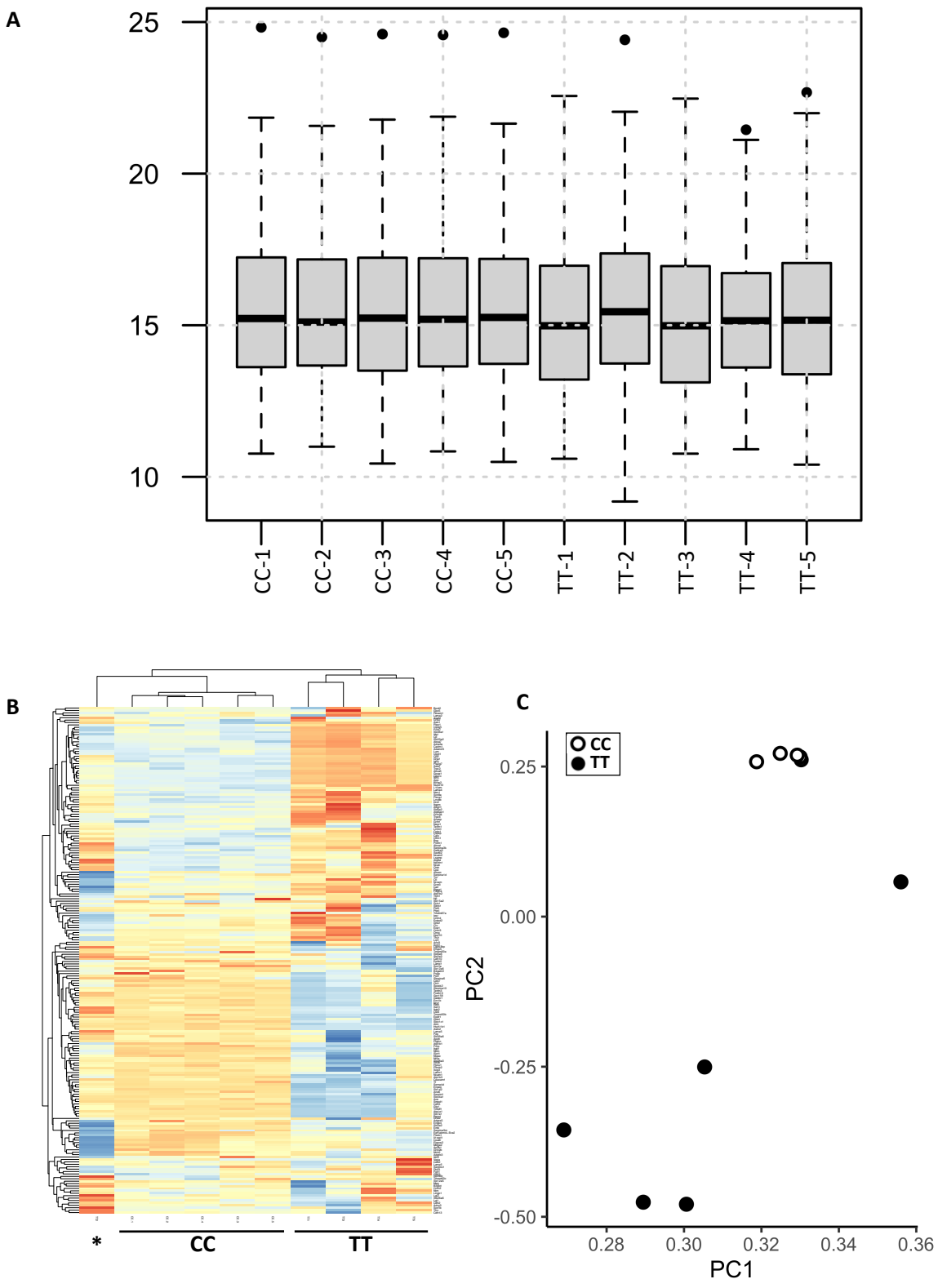

Fig. S6. N-glycoproteomics identified clear group differences between control and A391T cortex. A) Normalization Boxplots for N-glycoproteomic analysis showing the distribution of abundances for each channel incorporating dependence of the variance on the mean intensity and a variance stabilizing data transformation to account for any variation in technical replicates. Overall, wild-type (CC) and A391T (TT) cortex had a similar distribution across replicates validating the statistical significance of the given glycoproteomics results, with only the known outlier TT-2 showing slightly increased variance compared to other samples. B) Clustering heat map showing the N-glycoproteins with altered abundances in A391T cortex. 10 mice are shown in the columns, with the first column representing the one TT outlier (*), followed by 5 CC cortex samples and the remaining 4 TT cortex samples. C) PCA analysis of showing that N-glycoproteins from TT mice (black dots) display a clear group difference in cortex compared to controls (white dots). N = 5 per genotype. Related to Fig. 3.

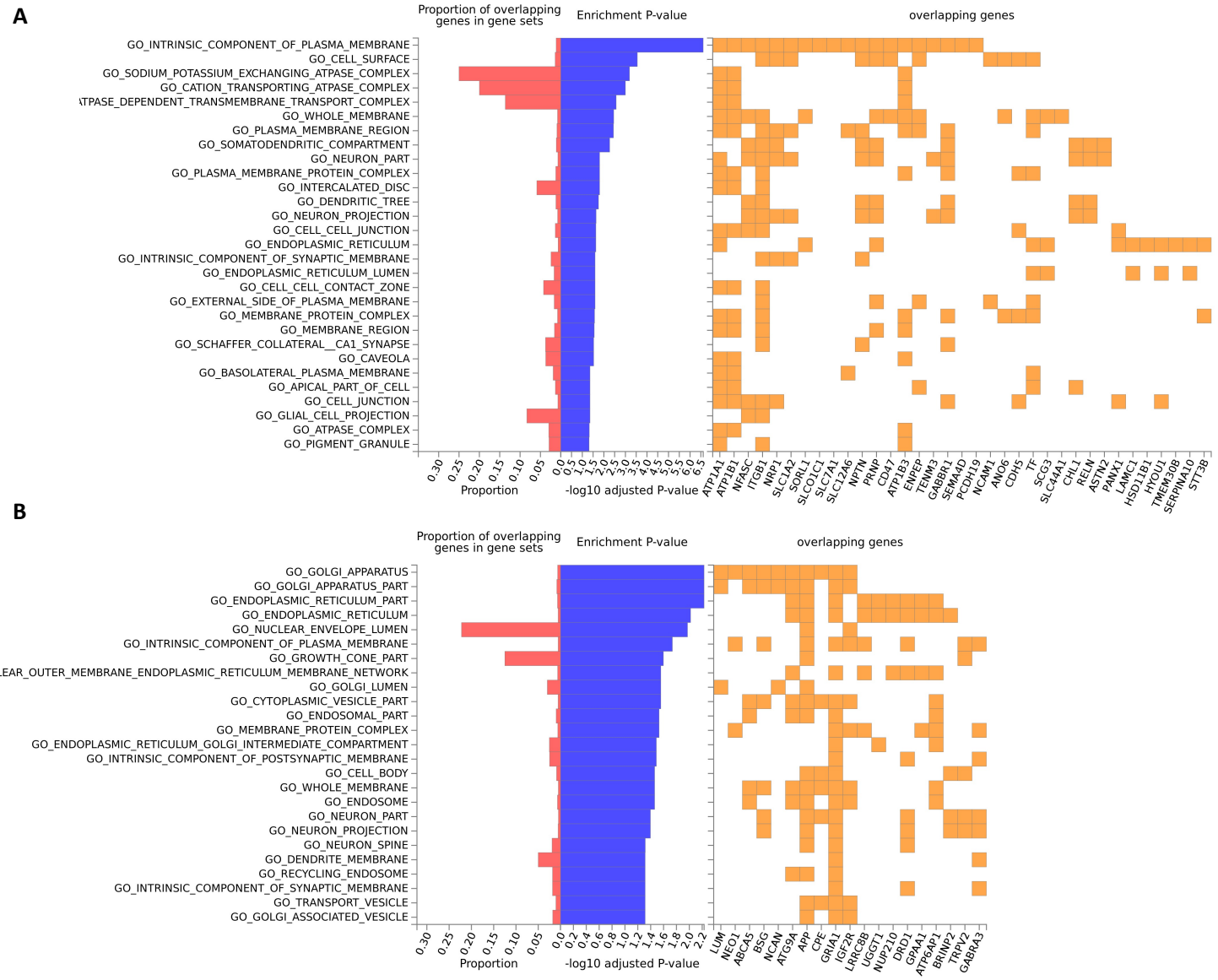

Fig. S7. Decreased and increased N-glycoproteins in A391T cortex are enriched in unique cellular components. A) GO analysis of N-glycoproteins decreased in A391T cortex shows an enrichment of proteins in the plasma membrane and involved in ion transport. B) GO analysis N-glycoproteins increased in A391T cortex shows an enrichment of proteins in the ER and Golgi apparatus. Adjusted p-values <0.05 (-log10) were calculated using the FUMA GENE2FUNC platform. Related to Fig. 3.

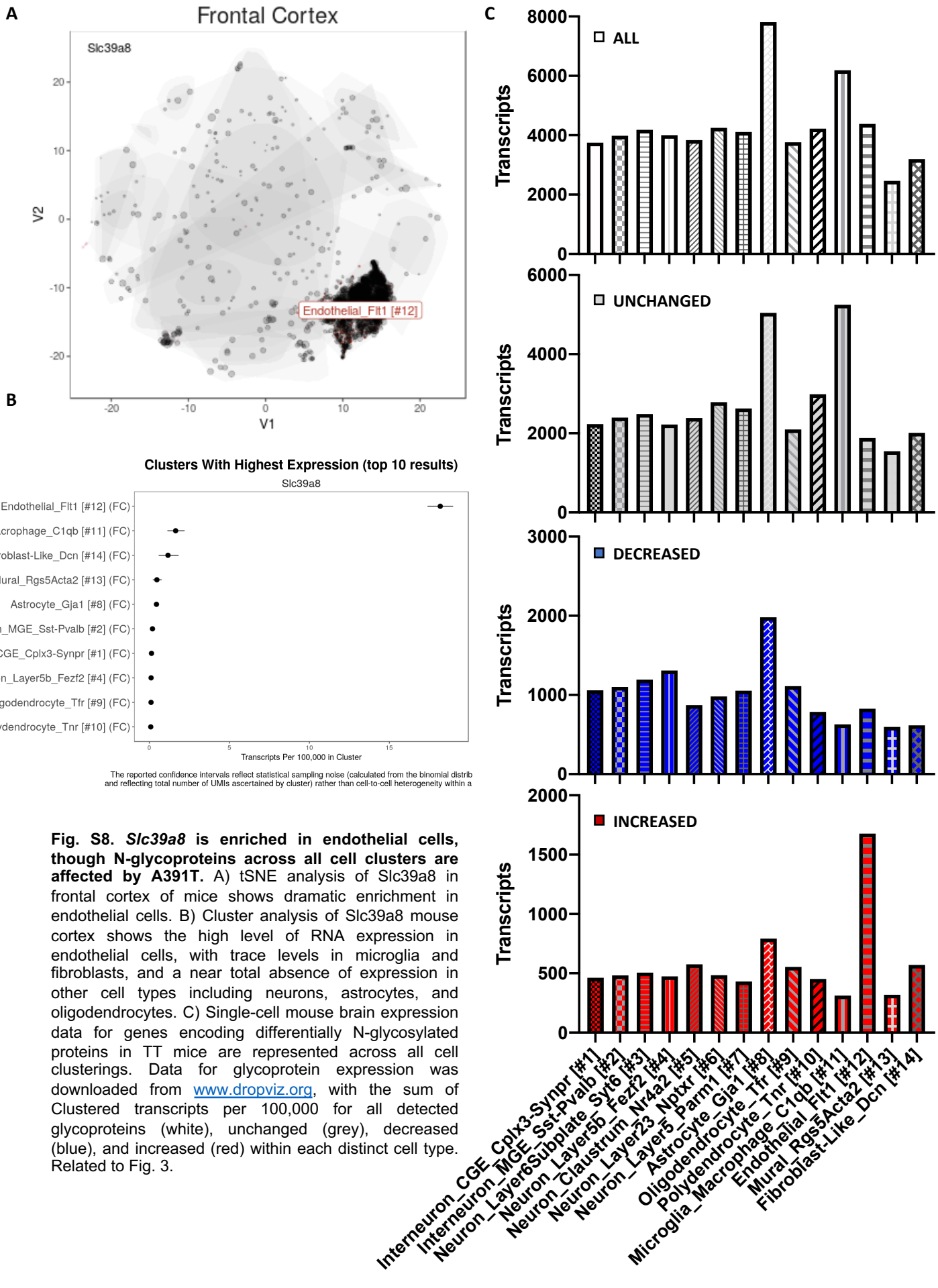

Fig. S8. *Slc39a8* is enriched in endothelial cells, though N-glycoproteins across all cell clusters are affected by A391T. A) tSNE analysis of Slc39a8 in frontal cortex of mice shows dramatic enrichment in endothelial cells. B) Cluster analysis of Slc39a8 mouse cortex shows the high level of RNA expression in endothelial cells, with trace levels in microglia and fibroblasts, and a near total absence of expression in other cell types including neurons, astrocytes, and oligodendrocytes. C) Single-cell mouse brain expression data for genes encoding differentially N-glycosylated proteins in TT mice are represented across all cell clusterings. Data for glycoprotein expression was downloaded from www.dropviz.org, with the sum of Clustered transcripts per 100,000 for all detected glycoproteins (white), unchanged (grey), decreased (blue), and increased (red) within each distinct cell type. Related to Fig. 3.