Supplemental

Supplemental Figure Legends

Figure S1. Immunoblots of HeLa cell lysates transfected with Myc-tagged Serpin A1, B1, C1 and I1 expression cDNAs to validate the specificity of serpin antibodies used in this study. All antibodies detect only their cognate recombinant protein and the correct size endogenous protein.

Figure S2. Age-dependent alteration of UBQLN2 and serpin staining in P497S mutant mice. *(A and B)* Immunofluorescence staining of UBQLN2 and Serpin A1, B1, C1, or I1 in the hippocampal region of sagittal brain sections from 8-week-old (A) and 52-week-old (B) P497S animals. Scale bars shown = 100 μm for all images.

Figure S3. Colocalization of serpin and UBQLN2 proteins in different brain regions of P497S animals. Confocal microscopy images of 52-week-old P497S animals similar to Fig 2, but showing the UBQLN2 and Serpin A1, C1, and I1 protein staining in the CA1 (top panel), CA3 (middle panel), and cortex (bottom panel) of the brain. Scale bars shown = 20 μm for all images.

Figure S4. Line scan analysis showing colocalization of serpins with UBQLN2 staining in the dentate gyrus and spinal cord sections of P497S animals. Line scans were performed through representative regions of the magnified images shown in Figs 2 and 3. Plots depict pixel intensity of the individual UBQLN2 (red) and serpin (green) image channels for the lines analyzed in the images shown to the right of each plot. Lines are

drawn through the same region of interest in each image to determine the extent of overlap between the UBQLN2 and serpin staining.

Figure S5. Colocalization of UBQLN2 and serpins proteins in puncta within spinal MN. Confocal microscopy images of 32-week-old P497S animals similar to Fig 3, but showing higher magnification images of MN, identified by ChAT-positive staining, in the SC of age-matched P497S (top panel), WT356 (middle panel), and Non-Tg (bottom panel) animals. Scale bars shown = 20 μm for all images.

Figure S6. Line scans showing the degree of overlap between UBQLN2, serpin, and LC3 staining in P497S and Non-Tg SC sections shown in Fig 4. Plots depict pixel intensity for UBQLN2 (red), serpin (green), and the autophagosome marker LC3 (magenta) for the lines shown in images below each plot. Lines are drawn through the same region of interest containing representative puncta for either the larger, extracellular puncta in P497S animals (left column) or the smaller puncta within motor neurons of P497S and Non-Tg animals (middle and right columns, respectively).

Figure S7. Line scans showing the degree of overlap between UBQLN2, serpins and LAMP1 in P497S and Non-Tg spinal cord sections shown in Fig 5. Similar to Fig S6, but measuring colocalization between UBQLN2 (red), serpins (green), the LAMP1 lysosomal marker (magenta).

Figure S8. The UBQLN2 and serpin double-positive puncta in the SC are more closely juxtaposed with microglia than with astrocytes. Confocal microscopy images of the staining of UBQLN2, Serpin A1 or C1 proteins with either GFAP (top panels) or IBA1 (bottom panels) in the SC of 32-week-old P497S animals. Scale bars shown = 100 μm and 20 μm for 1X and 5X zoom images, respectively.

Figure S9. Comparison of the UBQLN2, Serpin A1 and C1 staining patterns in the dentate gyrus of P497S, WT 356 and Non-Tg animals. Similar to Fig 6, but showing staining for all three genotypes, as indicated. The P497S staining panels are the same as Fig 6. Scale bars shown = 100 μm and 20 μm for 1X and 5X zoom images, respectively.

Figure S10. Comparison of the UBQLN2, Serpin A1 and C1 staining patterns in the SC of P497S, WT 356 and Non-Tg animals. Similar to Fig S8, but showing staining in the SC for all three genotypes, as indicated. The P497S staining panels are the same as Fig 6. Scale bars shown = 100 μm and 20 μm for 1X and 5X zoom images, respectively. Scale bars shown = 100 μm and 20 μm for 1X and 5X zoom images, respectively.

Figure S11. Decreased solubility of UBQLN2 and Serpin A1 in P497S mutant animals. *(A)* Immunoblots of equal portions of low salt (LS), Triton-X100 (TX-100) sarkosyl (SARK) and urea fractions following sequential biochemical extraction of equal weight of cortical brain tissue from 32-week-old Non-Tg, WT356, and P497S mice. The fractions analyzed were obtained by progressive extraction of the tissues with low salt, Triton X-

100, sarkosyl, and urea buffers. The Triton X-100 fraction was not included because of aberrant migration of proteins extracted with the buffer. *(B)* Quantification of penultimate SARK fractions for UBQLN2 and serpin proteins. *(C)* Quantification of ultimate urea fractions for each serpin protein. *(D)* Ubiquitin (UBQTN) immunoblots of urea fractions from 8- and 32-week-old animals. *(E)* Quantification of 32 week urea fractions for UBQLN2 and UBQTN.

Figure S12. Quantification of serpin protein levels in mouse tissues by SDS-PAGE analysis. *(A)* Immunoblots of hippocampus (left) and lumbar SC tissue (right) of 3 WT356, Non-Tg, and P497S animals per genotype analyzed after separation by SDS-PAGE. *(B)* Quantification of immunoblots in A shows a significant decrease in Serpin I1 in the hippocampus and a significant decrease in Serpin C1 in the spinal cord of 32-week-old P497S animals. Other serpins trended toward a decrease in P497S animals though not to a level of significance. As expected, levels of the UBQLN2 transgene in both the hippocampus and spinal cord were significantly increased in P497S animals.

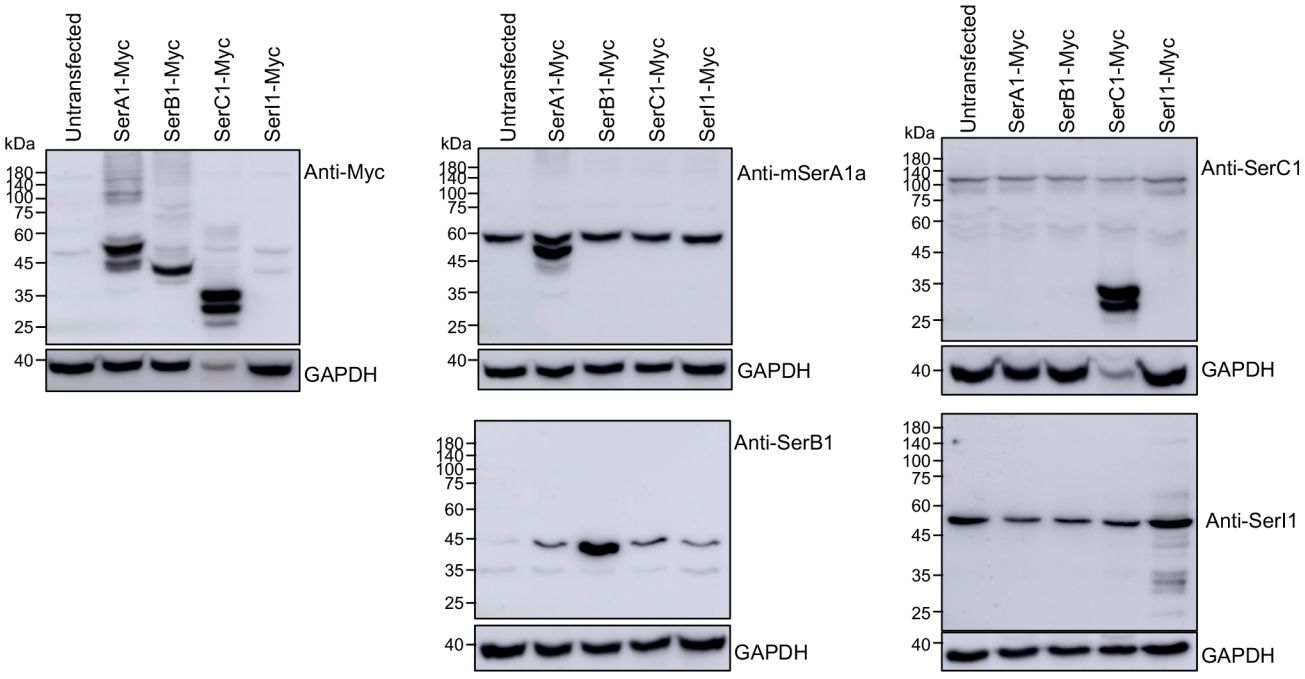
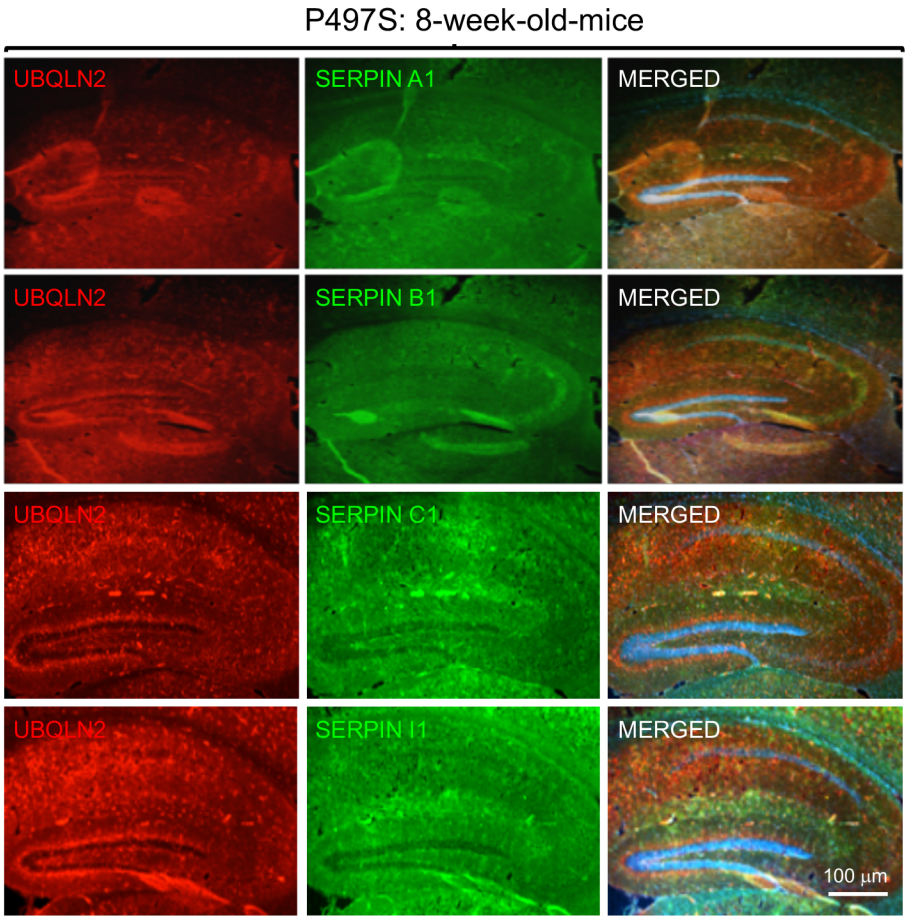

Untransfected
SerA1-Myc
SerB1-Myc
SerC1-Myc
Serl1-Myc
kDa
180
140
100
75
60
45
35
25
40
Anti-Myc
GAPDH
Anti-mSerA1a
Anti-SerB1
Anti-SerC1
Anti-Serl1

Fig S1

A


P497S: 8-week-old-mice
UBQLN2
SERPIN A1
MERGED
UBQLN2
SERPIN B1
MERGED
UBQLN2
SERPIN C1
MERGED
UBQLN2
SERPIN I1
MERGED
100 µm

B

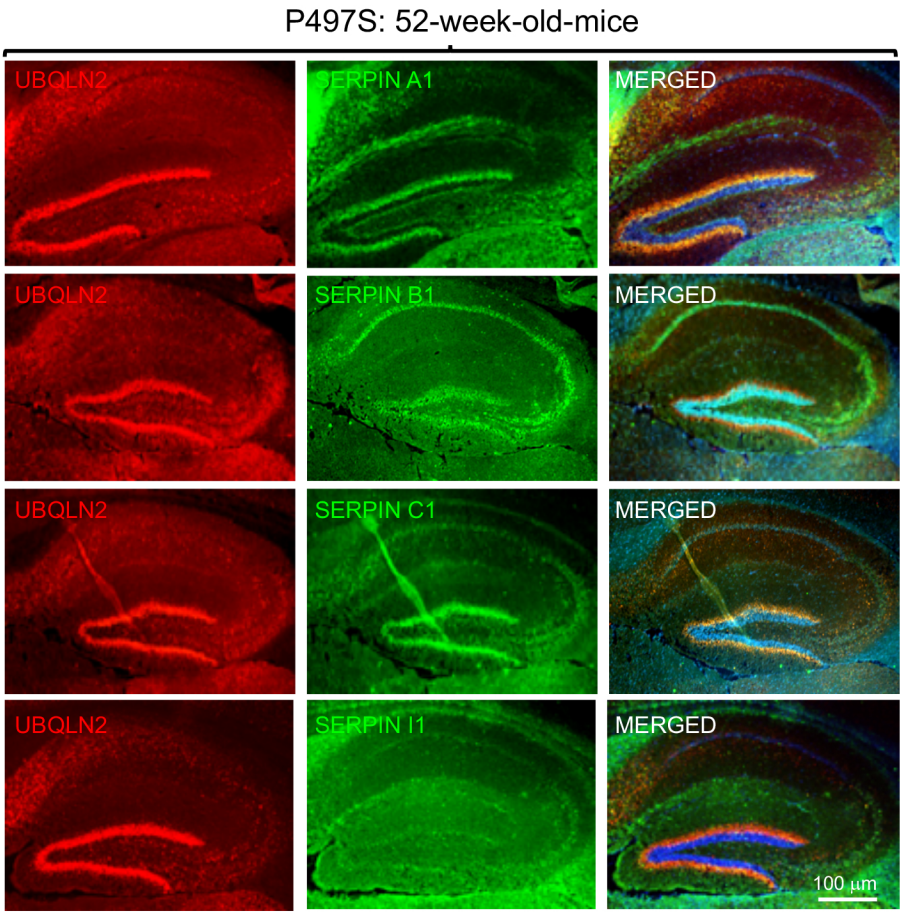

P497S: 52-week-old-mice
UBQLN2
SERPIN A1
MERGED
UBQLN2
SERPIN B1
MERGED
UBQLN2
SERPIN C1
MERGED
UBQLN2
SERPIN I1
MERGED
100 µm

Fig S2

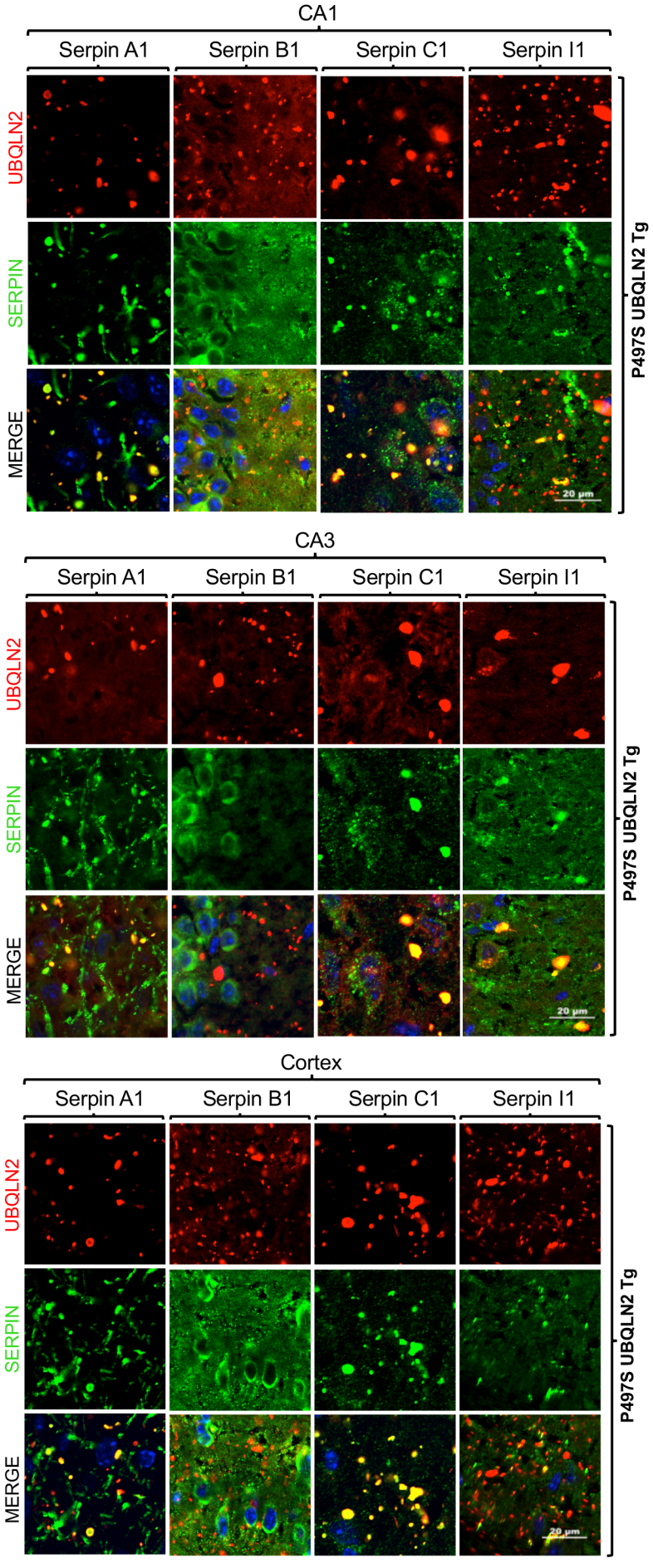

CA1
Serpin A1
Serpin B1
Serpin C1
Serpin I1
UBQLN2
SERPIN
MERGE
P497S UBQLN2 Tg
20 μm
CA3
Serpin A1
Serpin B1
Serpin C1
Serpin I1
UBQLN2
SERPIN
MERGE
P497S UBQLN2 Tg
20 μm
Cortex
Serpin A1
Serpin B1
Serpin C1
Serpin I1
UBQLN2
SERPIN
MERGE
P497S UBQLN2 Tg
20 μm

Fig S3

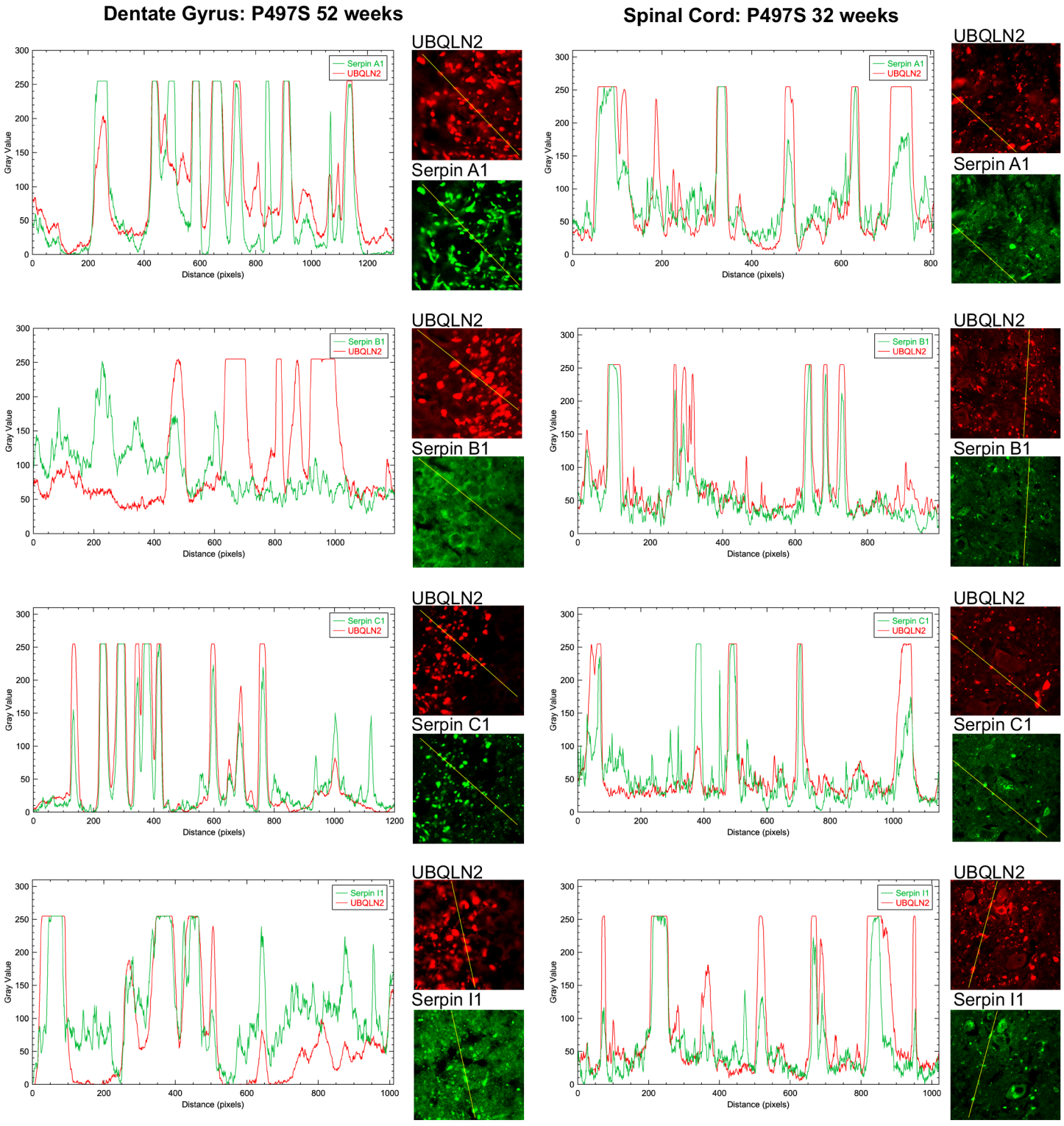

Dentate Gyrus: P497S 52 weeks
Spinal Cord: P497S 32 weeks
Serpin A1
UBQLN2
Serpin B1
Serpin C1
Serpin I1
Gray Value
Distance (pixels)

Fig S4

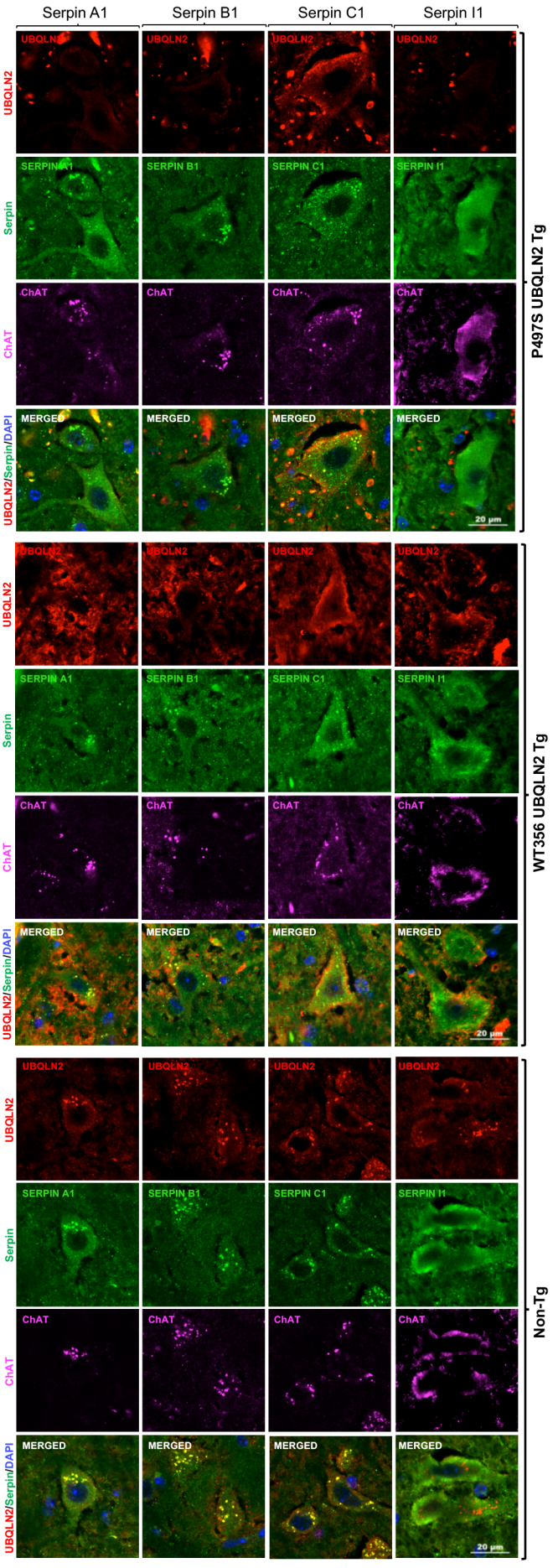
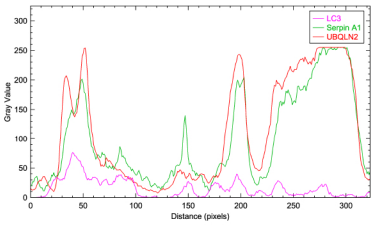
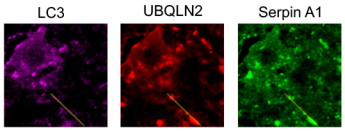
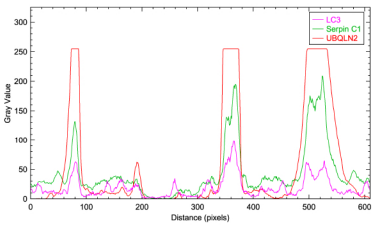

Serpin A1
Serpin B1
Serpin C1
Serpin I1
UBQLN2
Serpin
ChAT
UBQLN2/Serpin/DAPI
MERGED
20 μm
P497S UBQLN2 Tg
WT356 UBQLN2 Tg
Non-Tg

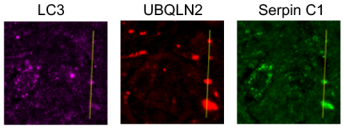
Fig S5

Spinal Cord: P497S 32 weeks (outside MN)

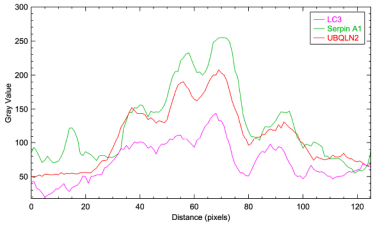


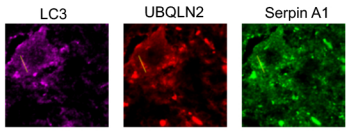


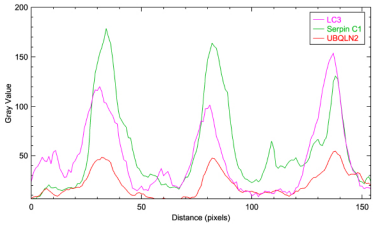


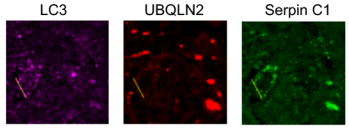


Spinal Cord: P497S 32 weeks (inside MN)

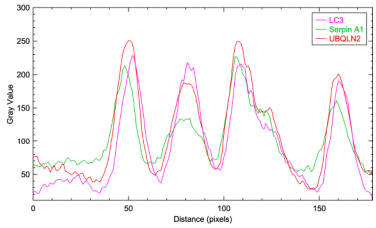


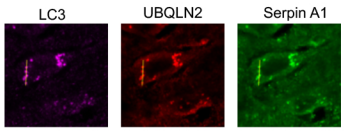


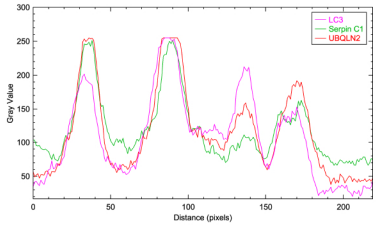




Spinal Cord: Non-Tg 32 weeks (inside MN)







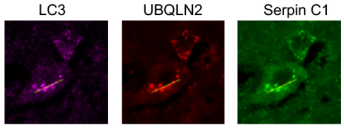


Fig S6

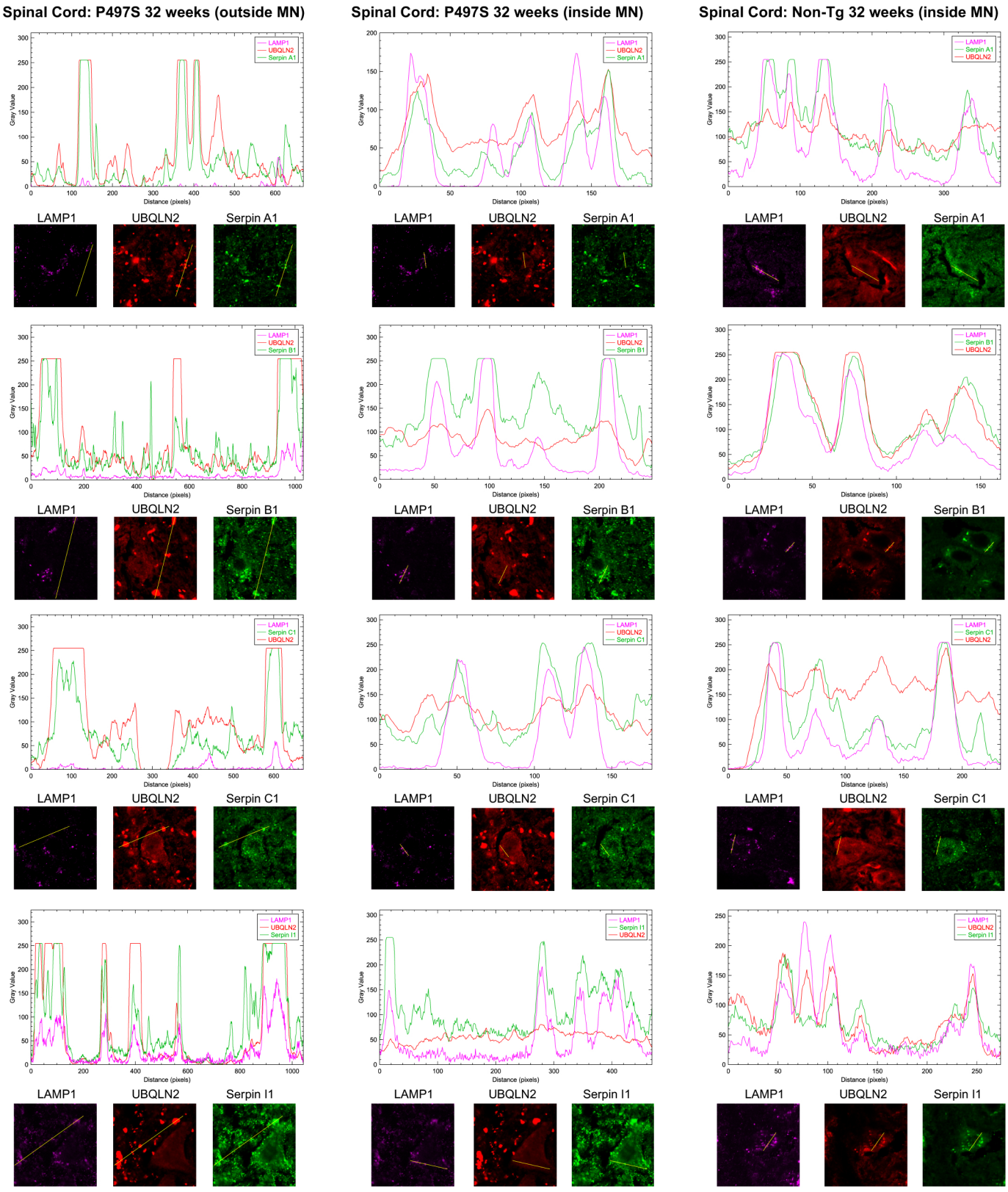

Spinal Cord: P497S 32 weeks (outside MN)
Spinal Cord: P497S 32 weeks (inside MN)
Spinal Cord: Non-Tg 32 weeks (inside MN)
LAMP1
UBQLN2
Serpin A1
Serpin B1
Serpin C1
Serpin I1
Gray Value
Distance (pixels)

Fig S7

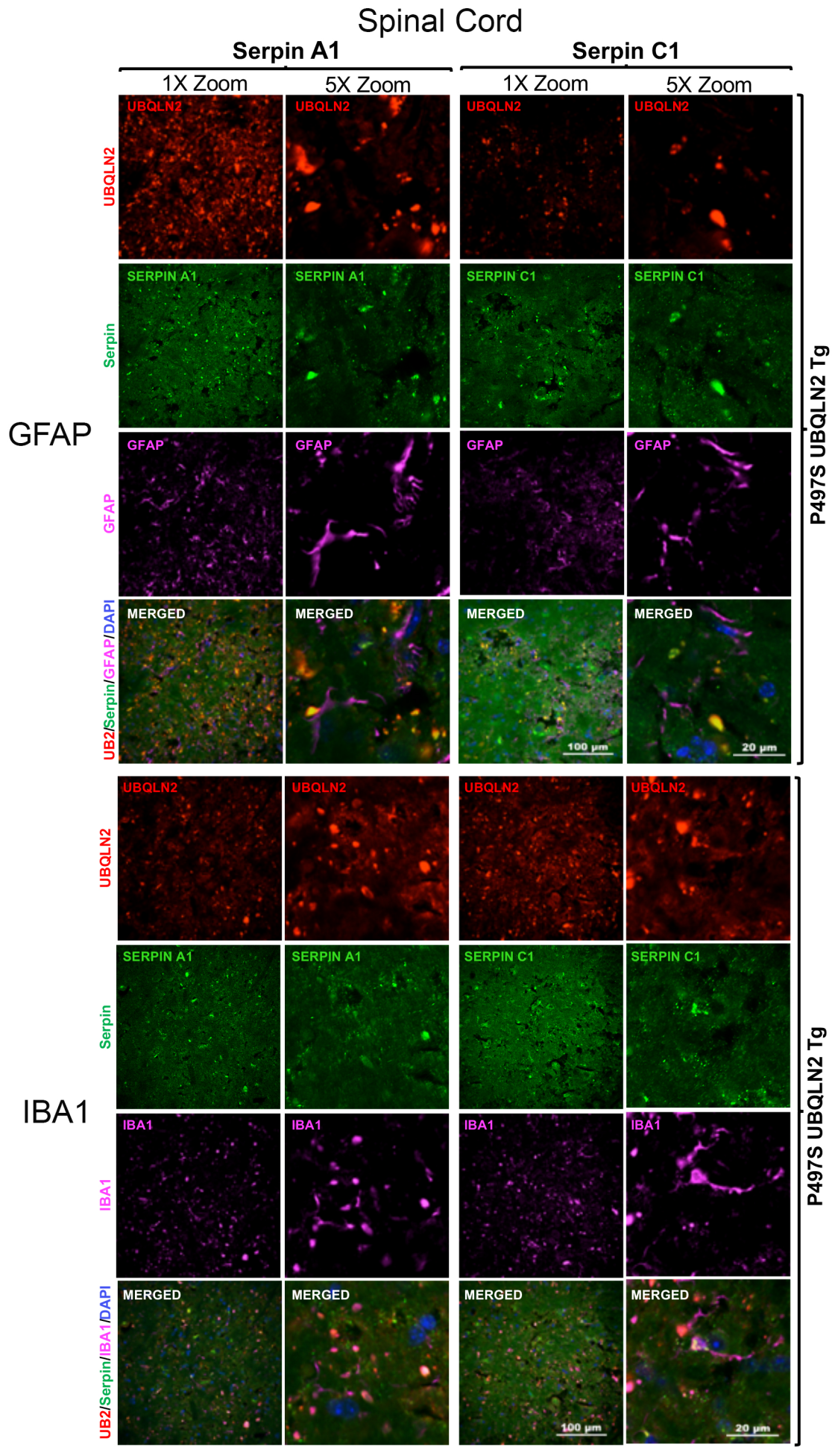

Spinal Cord
Serpin A1
Serpin C1
1X Zoom
5X Zoom
1X Zoom
5X Zoom
UBQLN2
UBQLN2
UBQLN2
UBQLN2
UBQLN2
SERPIN A1
SERPIN A1
SERPIN C1
SERPIN C1
Serpin
GFAP
GFAP
GFAP
GFAP
GFAP
MERGED
MERGED
MERGED
MERGED
UB2/Serpin/GFAP/DAPI
100 µm
20 µm
P497S UBQLN2 Tg
GFAP
UBQLN2
UBQLN2
UBQLN2
UBQLN2
UBQLN2
SERPIN A1
SERPIN A1
SERPIN C1
SERPIN C1
Serpin
IBA1
IBA1
IBA1
IBA1
IBA1
MERGED
MERGED
MERGED
MERGED
UB2/Serpin/IBA1/DAPI
100 µm
20 µm
P497S UBQLN2 Tg
IBA1

Fig S8

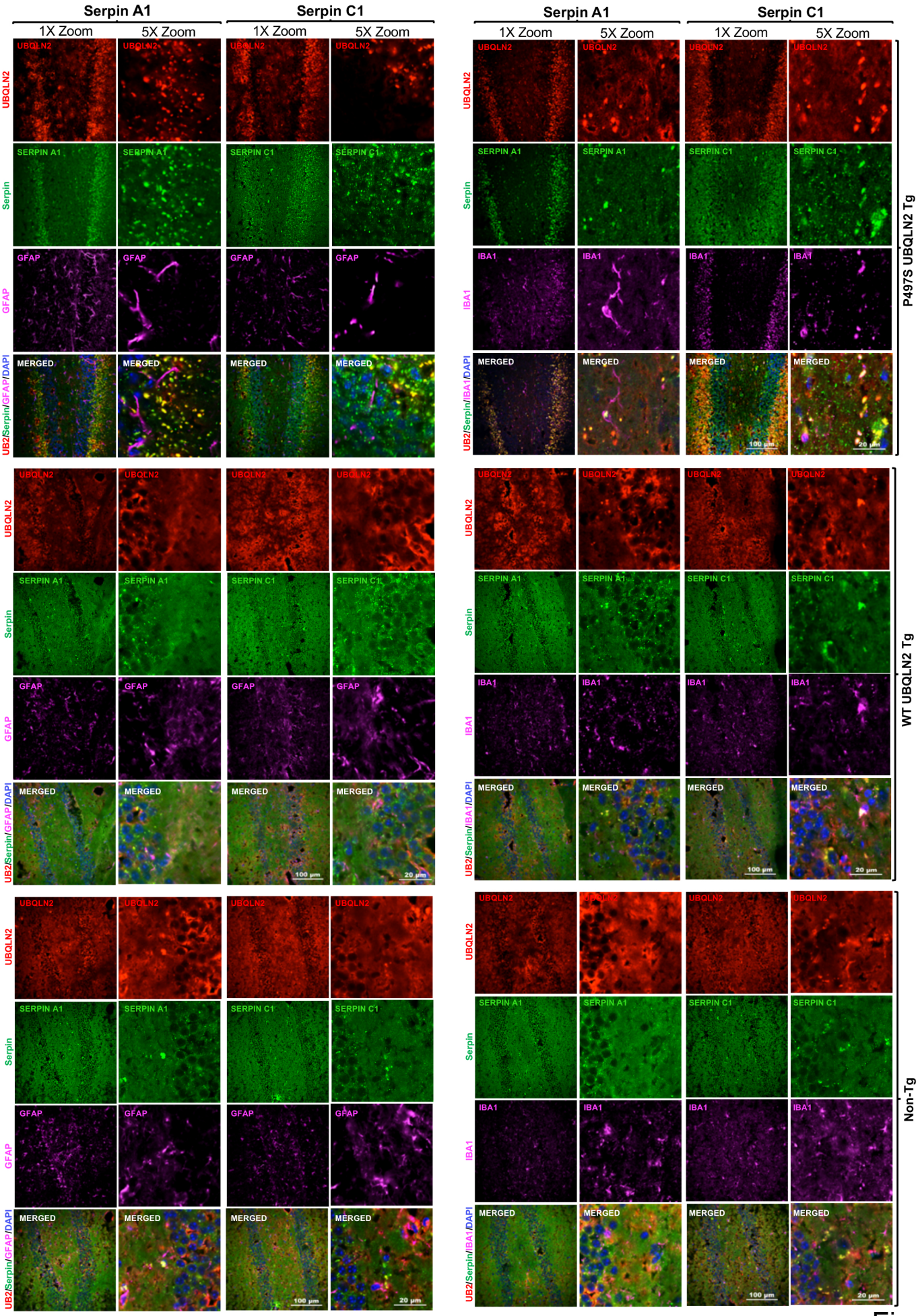

Serpin A1
Serpin C1
1X Zoom
5X Zoom
UBQLN2
Serpin
GFAP
IBA1
MERGED
UB2/Serpin/GFAP/DAPI
UB2/Serpin/IBA1/DAPI
P497S UBQLN2 Tg
WT UBQLN2 Tg
Non-Tg
100 µm
20 µm

Fig S9

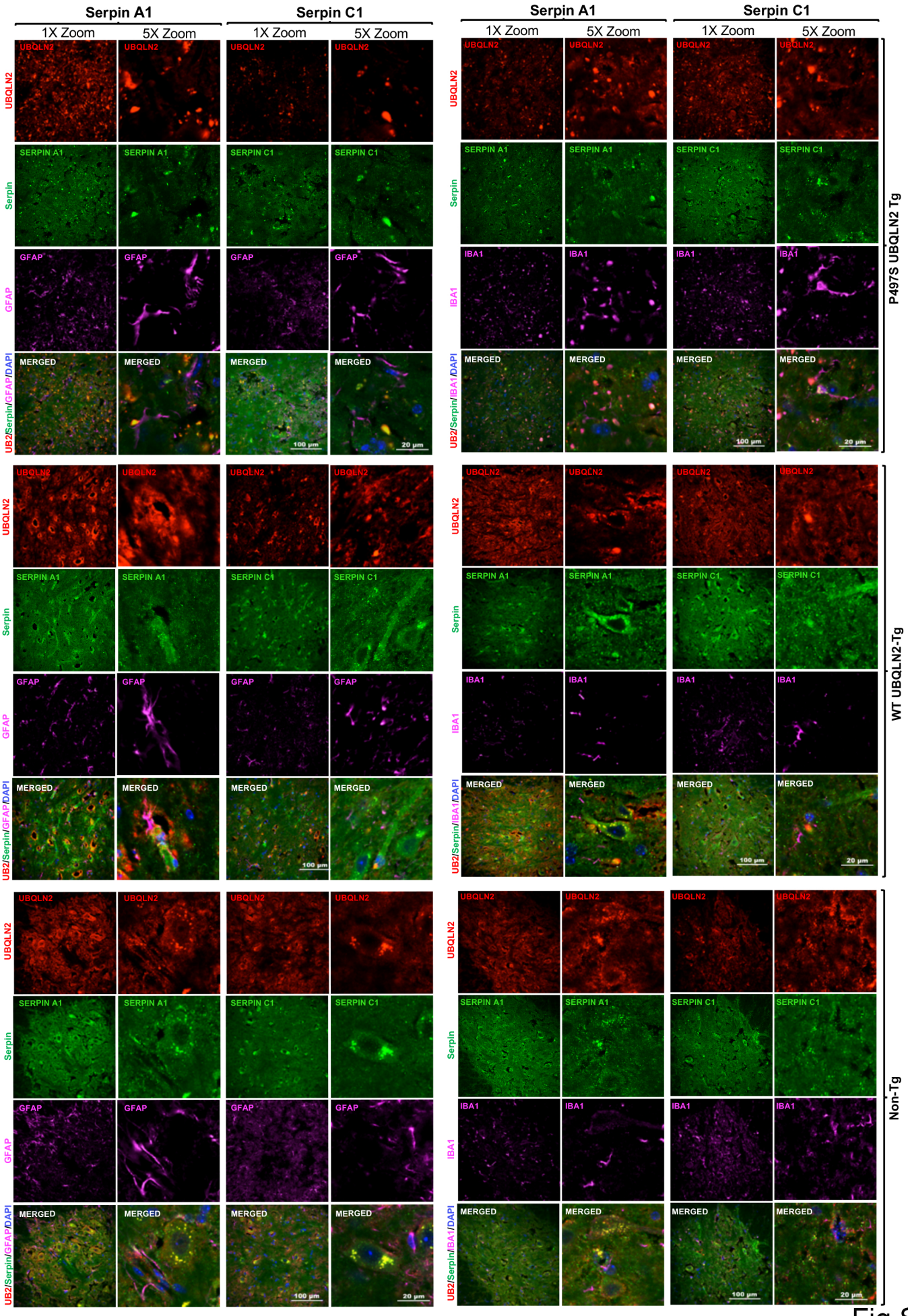

Serpin A1
Serpin C1
1X Zoom
5X Zoom
UBQLN2
Serpin
GFAP
IBA1
UB2/Serpin/GFAP/DAPI
UB2/Serpin/IBA1/DAPI
MERGED
SERPIN A1
SERPIN C1
100 µm
20 µm
P497S UBQLN2 Tg
WT UBQLN2-Tg
Non-Tg

Fig S10

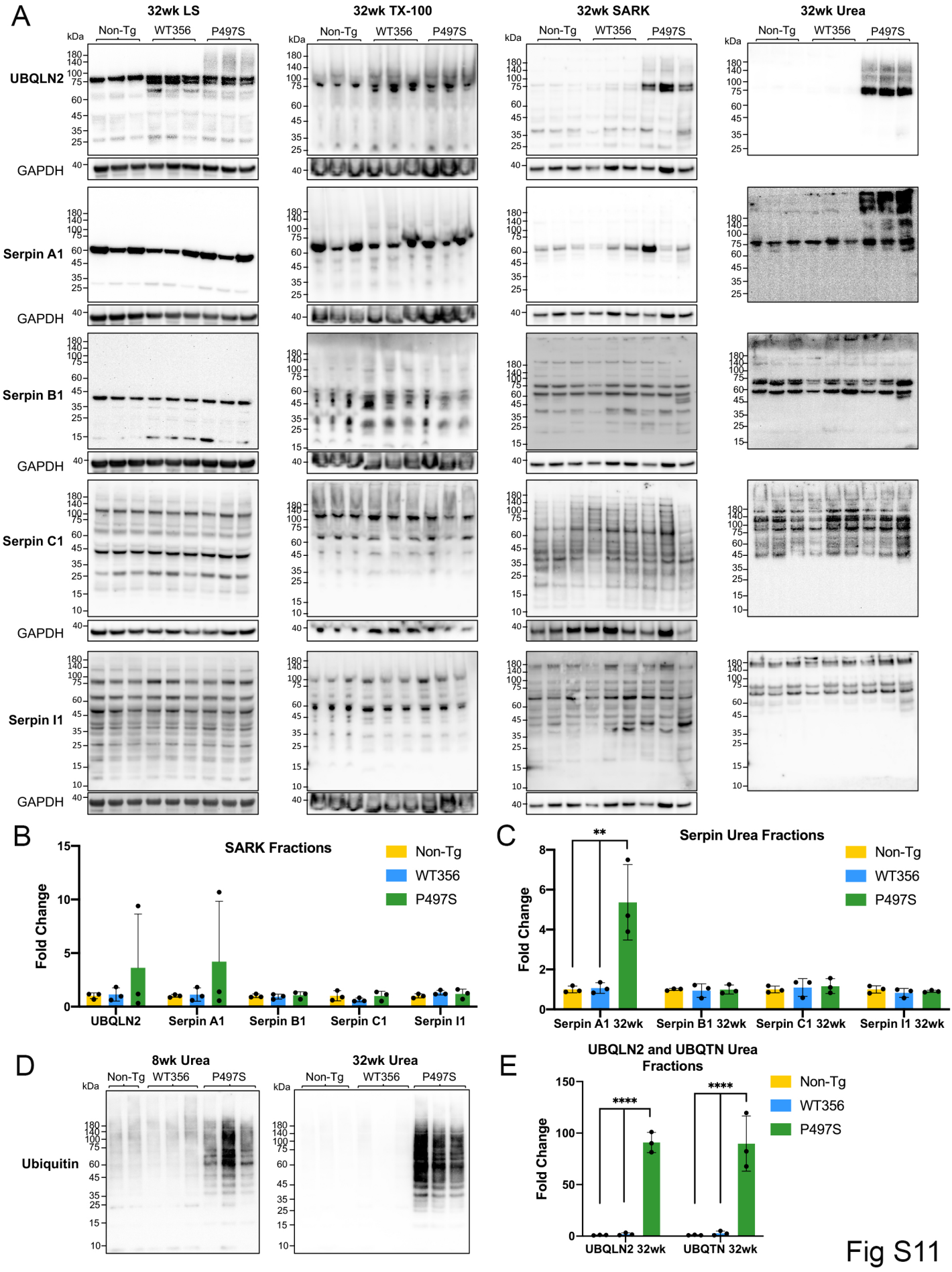
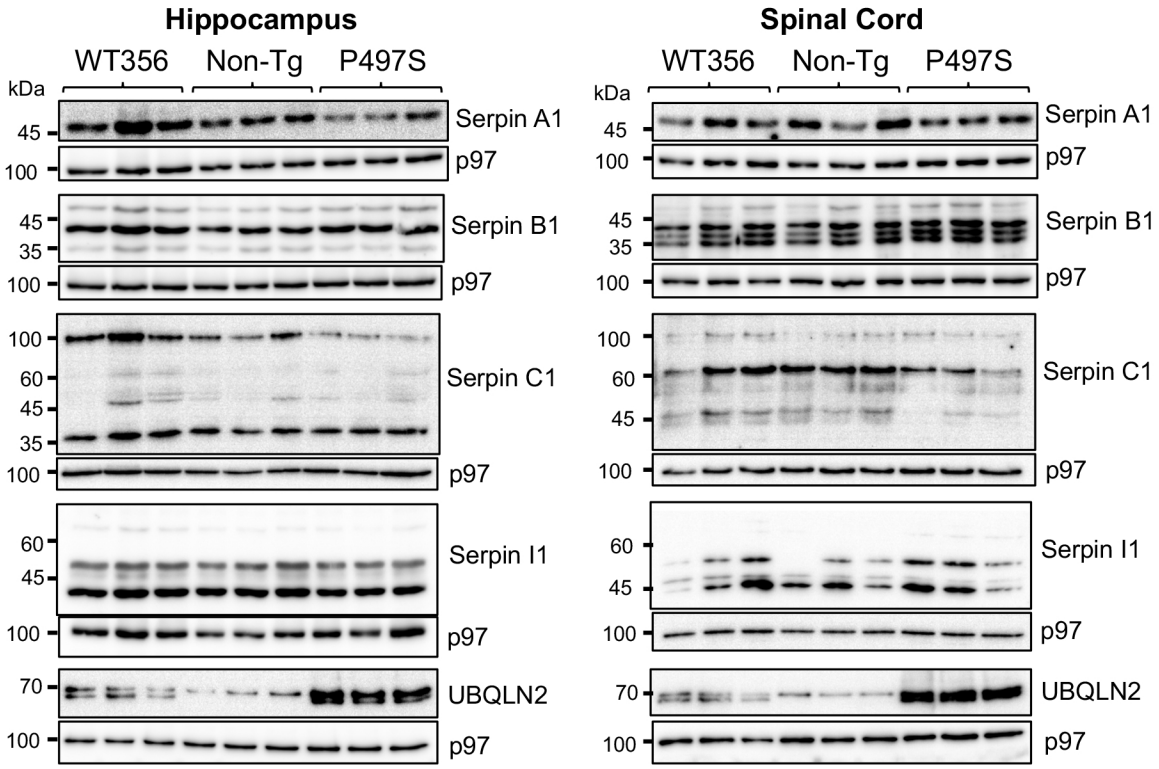

A
32wk LS
32wk TX-100
32wk SARK
32wk Urea
Non-Tg
WT356
P497S
kDa
UBQLN2
GAPDH
Serpin A1
Serpin B1
Serpin C1
Serpin I1
B
SARK Fractions
Non-Tg
WT356
P497S
Fold Change
UBQLN2
Serpin A1
Serpin B1
Serpin C1
Serpin I1
C
Serpin Urea Fractions
**
Serpin A1 32wk
Serpin B1 32wk
Serpin C1 32wk
Serpin I1 32wk
D
8wk Urea
32wk Urea
Ubiquitin
E
UBQLN2 and UBQTN Urea Fractions
****
UBQLN2 32wk
UBQTN 32wk

Fig S11

A


Hippocampus
WT356
Non-Tg
P497S
kDa
45
100
Serpin A1
p97
45
35
100
Serpin B1
p97
100
60
45
35
100
Serpin C1
p97
60
45
100
Serpin I1
p97
70
100
UBQLN2
p97
Spinal Cord
WT356
Non-Tg
P497S
kDa
45
100
Serpin A1
p97
45
35
100
Serpin B1
p97
100
60
45
100
Serpin C1
p97
60
45
100
Serpin I1
p97
70
100
UBQLN2
p97

B

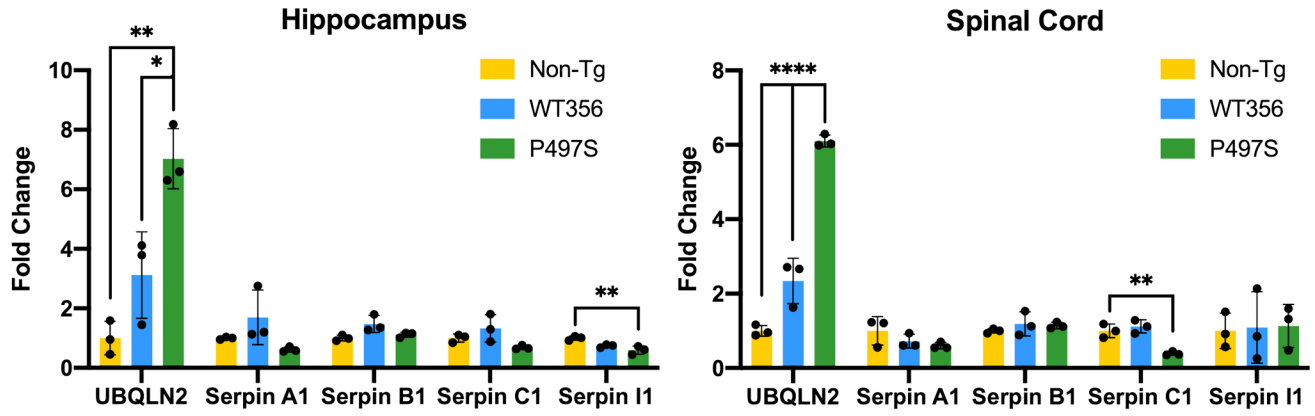

Hippocampus
Non-Tg
WT356
P497S
Fold Change
10
8
6
4
2
0
**
*
**
UBQLN2
Serpin A1
Serpin B1
Serpin C1
Serpin I1
Spinal Cord
Non-Tg
WT356
P497S
Fold Change
8
6
4
2
0
****
**
UBQLN2
Serpin A1
Serpin B1
Serpin C1
Serpin I1

Fig S12