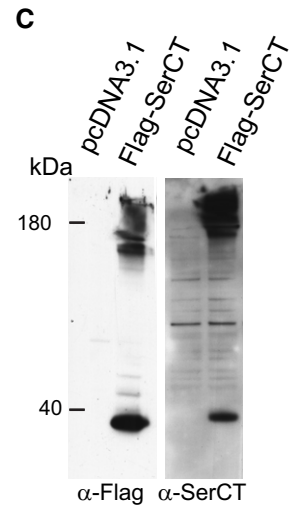
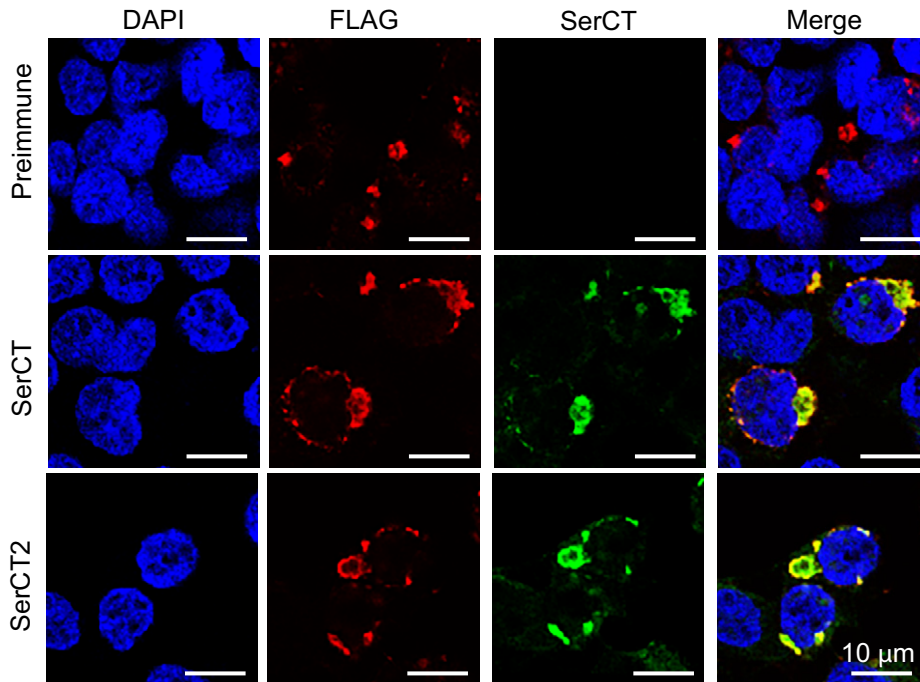
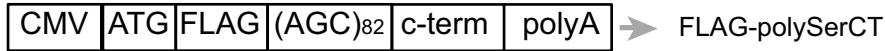


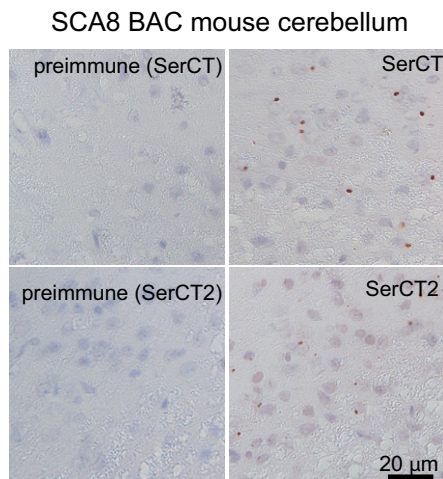
## Expanded View Figures

**A** **(Ser)<sub>n</sub> SSSKARFSNMKDPG**  
**SQGIGNRASANRVNLSVEA**  
**GSQKRQSECKDK\***

**B** Flag-SerCT



**D**



**E**

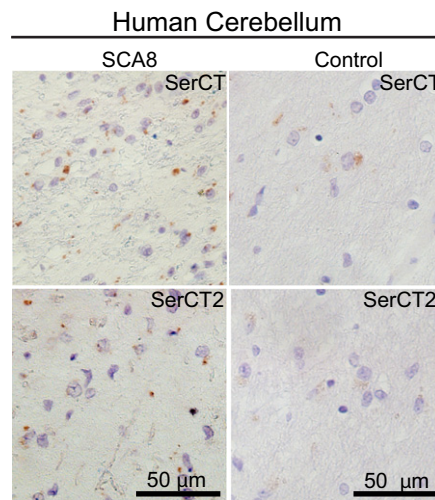
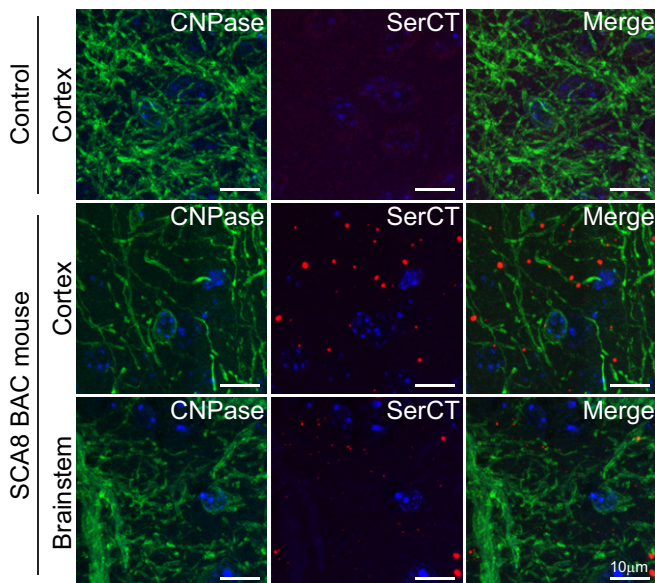


Figure EV1

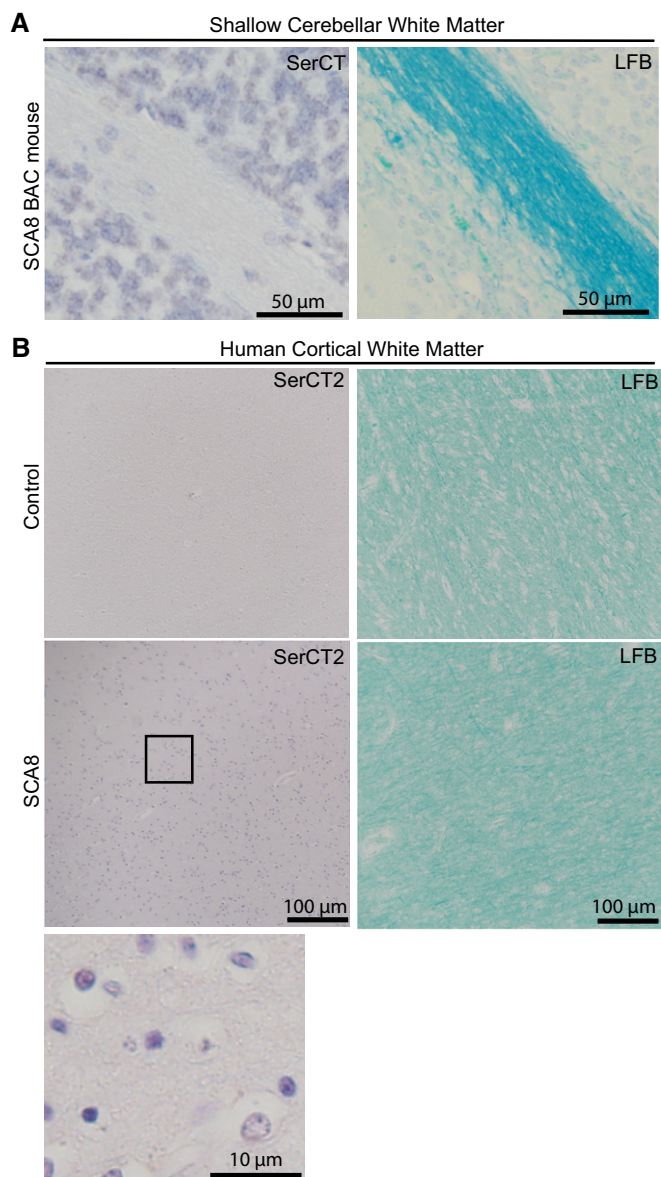
**Figure EV1. Validation of rabbit polyclonal  $\alpha$ -SerCT and  $\alpha$ -SerCT2 antibodies.**

- A Amino acid sequence of predicted polySer RAN protein with the unique C terminus. Peptide sequences used to generate rabbit polyclonal antibodies are underlined.
- B Schematic diagram of FLAG-SerCT construct expressing an ATG-initiated N-terminal FLAG-tagged polySer expansion protein followed by its endogenous C-terminal sequence. Co-localization of immunofluorescence (IF) staining using  $\alpha$ -FLAG (red) and  $\alpha$ -SerCT and  $\alpha$ -SerCT2 (green) in HEK293T cells transfected with FLAG-SerCT but not preimmune serum.
- C Immunoblots showing detection of recombinant polySer protein using  $\alpha$ -FLAG (left) and  $\alpha$ -SerCT (right) in the lysates of HEK293T cells transfected with FLAG-SerCT (second lanes) but not pcDNA3.1 (first lanes).
- D Immunohistochemistry of SCA8 mouse brain using  $\alpha$ -SerCT and  $\alpha$ -SerCT2 (left panels) antibodies shows similar punctate aggregates. Aggregates are not detected with respective preimmune sera (right panels).
- E Immunohistochemistry using both  $\alpha$ -SerCT and  $\alpha$ -SerCT2 detect similar aggregates in SCA8 human autopsy tissue but not control cerebellum.

Source data are available online for this figure.

**Figure EV2. PolySer aggregates show perinuclear localization in CNPase positive regions.**

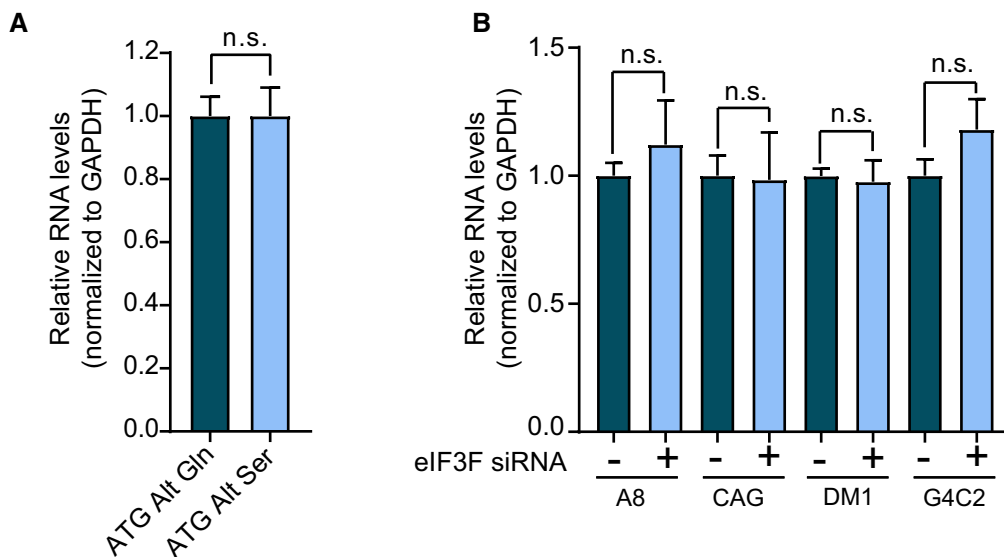
Immunofluorescence of CNPase, polySer (SerCT), and DAPI in non-transgenic (top) and SCA8 BAC cortex (middle), along with SCA8 BAC brainstem (bottom).



**Figure EV3. No polySer aggregates in unaffected white matter regions of SCA8 BAC mouse cerebellum and SCA8 human autopsy brains.**

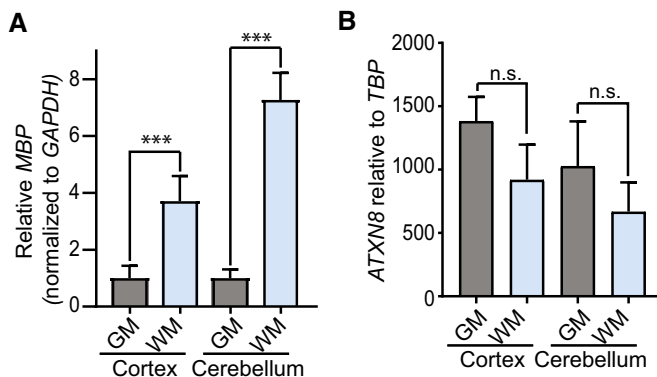
A White matter integrity visualized by luxol fast blue (LFB) staining (right) and polySer aggregates detected by  $\alpha$ -SerCT antibody (left).

B White matter integrity visualized by luxol fast blue (LFB) staining (right) and polySer aggregates detected by  $\alpha$ -SerCT2 antibody (left) in control (top) and SCA8 (bottom and enlarged panel) human autopsy tissue.



**Figure EV4. eIF3F knockdown does not affect minigene RNA expression levels.**

A Bar graph showing relative minigene expression measured by qRT-PCR in cells transfected with the codon replacement constructs expressing polyGln (dark blue) or polySer (light blue;  $n = 5$ , n.s. no significance; mean  $\pm$  SEM; unpaired  $t$ -test).  
 B Bar graph showing relative minigene expression measured by qRT-PCR in cells co-transfected with the various repeat expansion and control (dark blue) or eIF3F (light blue) targeting siRNA ( $n = 5$ ; n.s. no significance; mean  $\pm$  SEM; unpaired  $t$ -test).



**Figure EV5. MBP is enriched in white matter regions and AXTN8 shows no regional specific expression in SCA8 BAC mouse brain.**

A qRT-PCR of MBP in cerebellar white and gray matter regions shows increased expression of MBP in cerebellar white matter compared to cerebellar gray matter ( $n = 3$ ;  $***P < 0.001$ ; mean  $\pm$  SEM; unpaired  $t$ -test).  
 B qRT-PCR of AXTN8 in cortical and cerebellar white and gray matter shows no difference in AXTN8 RNA expression levels ( $n = 3$ ; n.s. no significance; mean  $\pm$  SEM; unpaired  $t$ -test).