Figure S1. Hyperactivity precedes cognitive and motor deficits in (G₄C₂)₁₄₉−mice at 3 months of age. a) Significant increase in distance traveled in (G₄C₂)₁₄₉ mice in open field analysis indicative to hyperactivity. b) Hanging wire test revealed no difference in the number of falls in (G₄C₂)₁₄₉ mice relative to age-matched (G₄C₂)₂ mice. c) Contextual fear conditioning demonstrated no difference in freezing between (G₄C₂)₂ and (G₄C₂)₁₄₉ mice at 3 months of age. Data represent the mean ± SEM. ****p<0.0001 as analyzed by unpaired two-tailed t tests.
Figure S2. (G₄C₂)₁₄₉–mice exhibit gliosis and neurodegeneration at 6 months of age. a-b) Representative images of immunohistochemical analysis of GFAP (a) and NeuN (b) in the cortex of (G₄C₂)₂ and (G₄C₂)₁₄₉ mice. Scale bar represents 150μm.
Figure S3. Sense and antisense RNA foci observed in the CNS of (G₄C₂)₁₄₉−mice. a-b) Sense (a) and antisense (b) were observed in the hippocampus, Purkinje layer of the cerebellum, thalamus, and anterior horn of the spinal cord. c-d) Quantitative analysis of the number of sense (c) or antisense (d) RNA foci present in foci-bearing cells in the motor cortex of (G₄C₂)₁₄₉−mice at 3, 6, and 12 months of age (n=6 per age group). Scale bar represents 5 μm.
Figure S4. Sense DPR pathology detected throughout the CNS in (G4C2)149–mice. a-c) Representative images of immunohistochemical analysis of poly(GA), poly(GP), and poly(GR) in the hippocampus (a), Purkinje layer of the cerebellum (b), and ventral horn of the spinal cord (c). Scale bar represents 20µm.
Figure S5. Antisense DPR pathology detected in both (G\textsubscript{4}C\textsubscript{2})\textsubscript{66} and (G\textsubscript{4}C\textsubscript{2})\textsubscript{149}–mice. Representative images of immunohistochemical analysis and quantitation of poly(PR) and poly(PA) inclusions in the cortex of (G\textsubscript{4}C\textsubscript{2})\textsubscript{66} and (G\textsubscript{4}C\textsubscript{2})\textsubscript{149}–mice at 6 months of age. Data represent the mean ± SEM. **p<0.01 and ***p<0.001 as analyzed by unpaired two-tailed t tests. Scale bar represents 20µm.
Figure S6. Sense and antisense DPR pathology is not detected in control (G₄C₂)₂–mice. a-b) Immunohistochemical analysis of poly(GA, poly(GP), poly(GR), poly(PA), and poly(PR) demonstrates absence of pathology in control (G₄C₂)₂ mice at all ages evaluated. Scale bar represents 20 μm.
Figure S7. Hippocampal pTDP-43 (pS409/410) pathology detected in (G₄C₂)_{149}–mice. a-b) Representative images of immunohistochemical analysis of pTDP-43 in the hippocampus of (G₄C₂)₂ (a) and (G₄C₂)_{149} mice (b) at 3, 6, and 12 months of age (inclusions indicated by black arrowheads). Scale bar represents 20μm.
Figure S8. Deposition of stress granule-associated proteins in \((G_4C_2)_{149}\)-mice. a) Representative images of immunohistochemical analysis of G3BP1, eIF3\(\eta\), and ataxin-2 in the hippocampus of \((G_4C_2)_2\) and \((G_4C_2)_{149}\) mice at 12 months of age. b) Representative immunofluorescent images depicting colocalization between pTDP-43 (green) and TIA-1 (red) in \((G_4C_2)_{149}\) mice, with control \((G_4C_2)_2\) mice characterized by the absence of pTDP-43 pathology and TIA-1 exhibiting a normal nuclear distribution. Nuclei are labeled with DAPI. Scale bars represent 20\(\mu\)m (a) and 5\(\mu\)m (b).