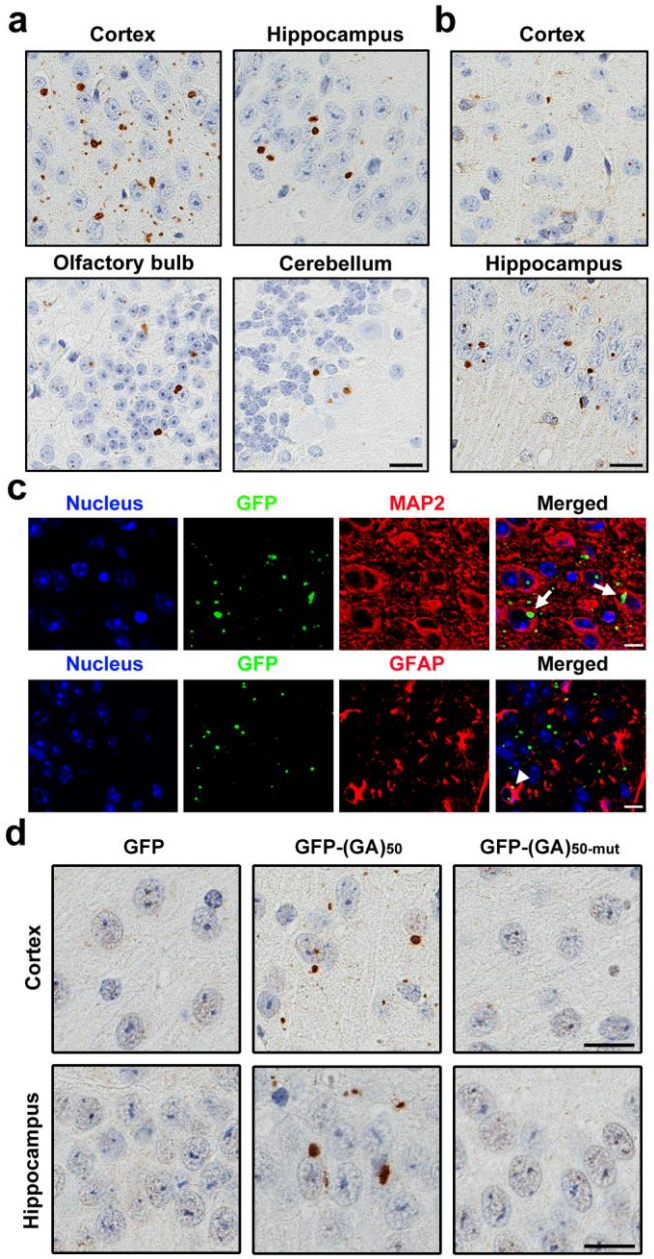
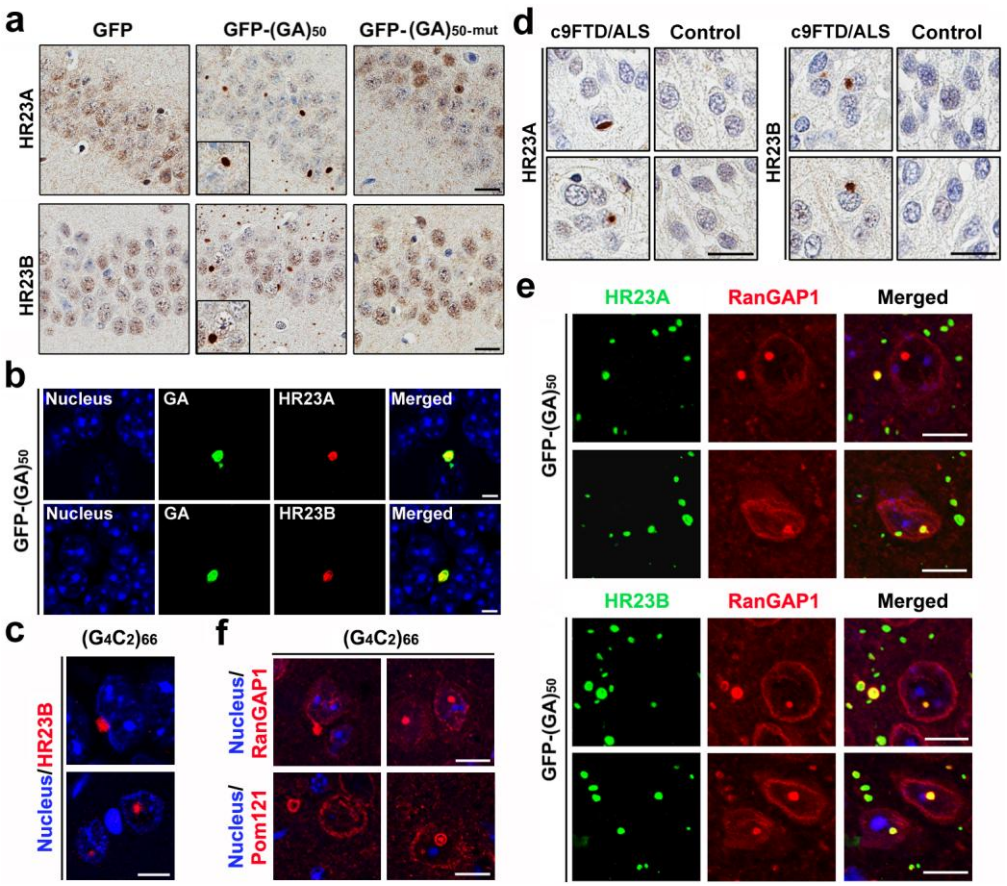


Supplementary Figure 1

Neuropathology of poly(GA) inclusions in the brains of GFP-$(GA)_{50}$ mice.

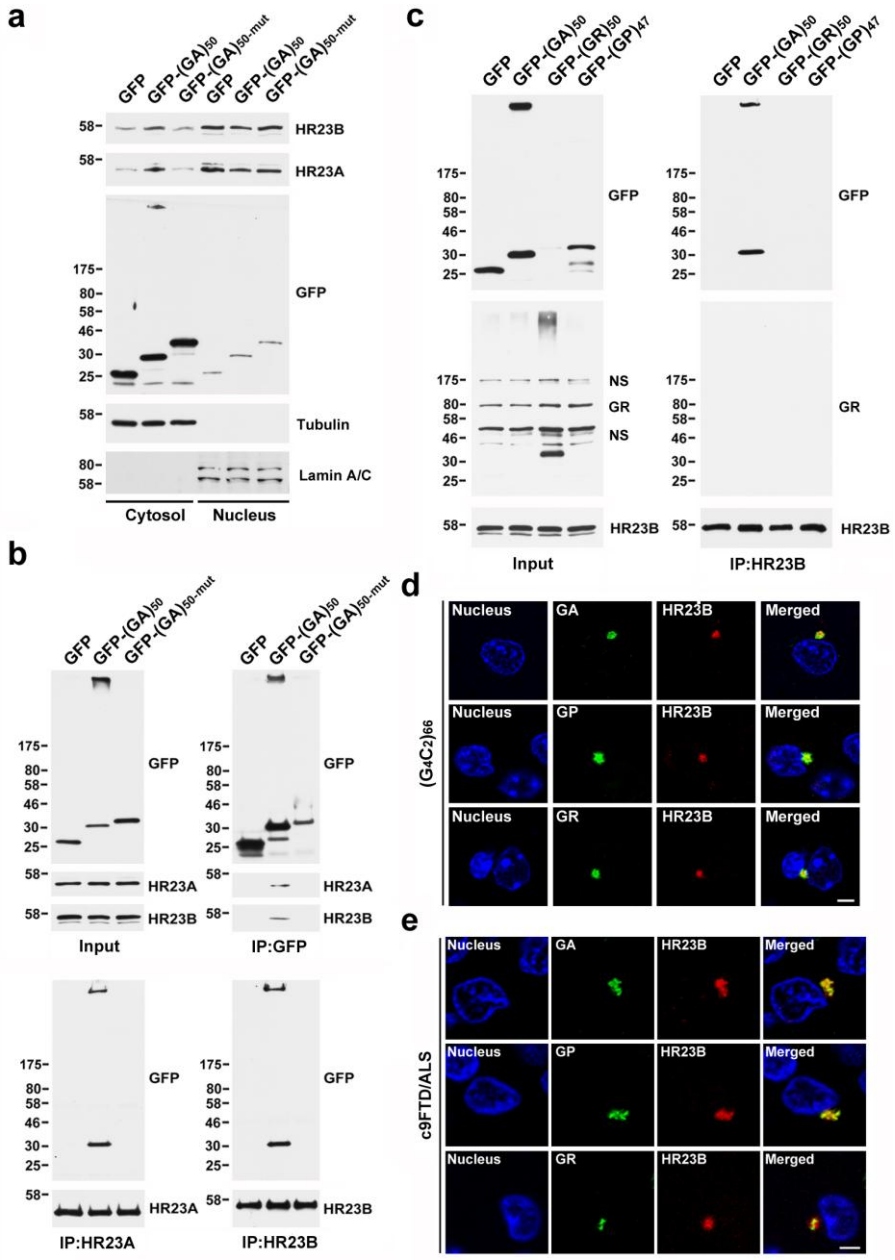
Immunohistochemical analysis in the indicated brain regions of GFP-$(GA)_{50}$ mice with (**a**) anti-GFP antibody or (**b**) anti-GA antibody. Scale bars, 20 μm. (**c**) Double immunofluorescence staining in the cortex of GFP-$(GA)_{50}$ mice for the indicated proteins. The arrows point to MAP2-positive neurons and the arrowhead to an astrocyte. Scale bars, 10 μm. (**d**) Immunohistochemical analysis in the cortex and hippocampus of GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-}mut}$ mice with an anti-ubiquitin antibody. Scale bars, 20 μm.



Supplementary Figure 2

HR23, RanGAP1 and Pom121 form inclusions in the brains of GFP-$(GA)_{50}$ and $(G_4C_2)_{66}$ mice.

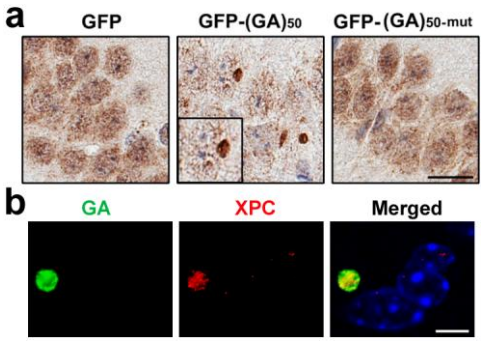
(**a**) Immunohistochemical analysis of HR23A and HR23B proteins in the hippocampus of 6 month-old GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice. Scale bars, 20 μm. (**b**) Double immunofluorescence staining for GFP-$(GA)_{50}$ and HR23A or HR23B in the hippocampus of 6 month-old GFP-$(GA)_{50}$ mice. Scale bars, 5 μm. (**c**) Immunofluorescence staining for HR23B in the cortex of 6 month-old $(G_4C_2)_{66}$ mice. Scale bar, 10 μm. (**d**) Representative images of immunohistochemical analysis of HR23A and HR23B in the hippocampus of c9FTD/ALS subjects (n = 7) or healthy controls (n = 4). Scale bars, 20 μm. (**e**) Double immunofluorescence staining for RanGAP1 and either HR23A or HR23B in the cortex of 6 month-old GFP-$(GA)_{50}$ mice. Scale bars, 10 μm. (f) Immunofluorescence staining for RanGAP1 or Pom121 in the cortex of 6 month-old $(G_4C_2)_{66}$ mice. Scale bars, 10 μm.



Supplementary Figure 3

HR23 proteins interact with, and are sequestered by, poly(GA) proteins.

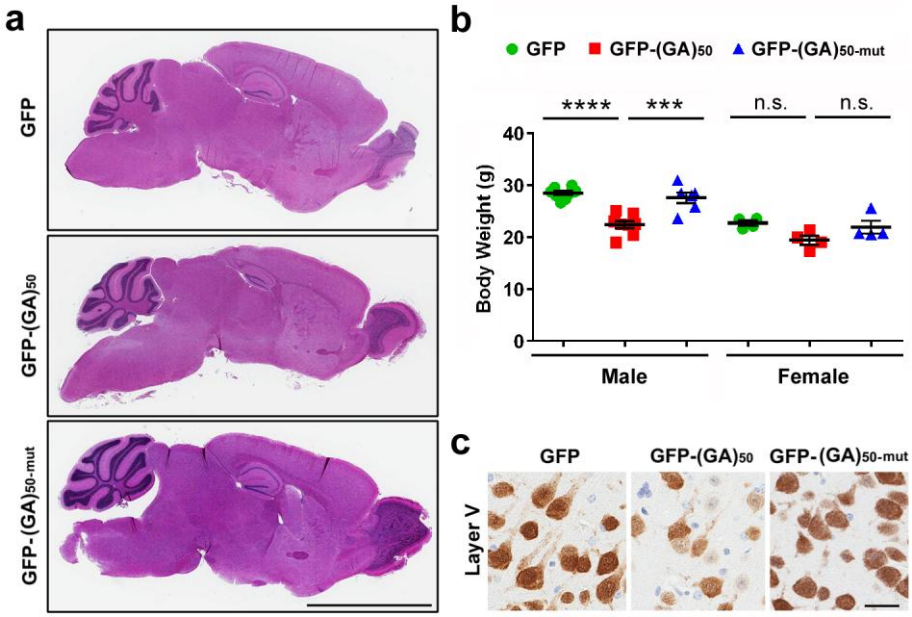
(**a**) Cytoplasmic and nuclear fractions were prepared from HEK293T cells exogenously expressing GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$, followed by immunoblots analysis using the indicated antibodies. Tubulin and Lamin A/C were used as cytoplasmic and nuclear markers, respectively. (**b**) Protein complexes were immunoprecipitated from the indicated input lysates (top left) from HEK293T cells exogenously expressing GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ with antibodies to GFP, HR23A or HR23B, followed by immunoblot analysis using the indicated antibodies. (**c**) Protein complexes were immunoprecipitated from the indicated input lysates (left, top and bottom) from HEK293T cells exogenously expressing GFP, GFP-$(GA)_{50}$, GFP-$(GR)_{50}$, or GFP-$(GP)_{47}$ with an antibody to HR23B, followed by immunoblot analysis using antibodies to GFP and poly(GR). (**d**) Double immunofluorescence staining for HR23B and poly(GA), poly(GP) or poly(GR) in brains of 6 month-old $(G_4C_2)_{66}$ mice. Scale bar, 5 μm. (**e**) Double immunofluorescence staining for HR23B and poly(GA), poly(GP) or poly(GR) in the hippocampus of c9FTD/ALS patients. Scale bar, 5 μm. For **a-c,** full-length immunoblots are presented in **Supplementary Figure 10**.



Supplementary Figure 4

XPC is sequestered into poly(GA) inclusions in the hippocampus of GFP-$(GA)_{50}$ mice.

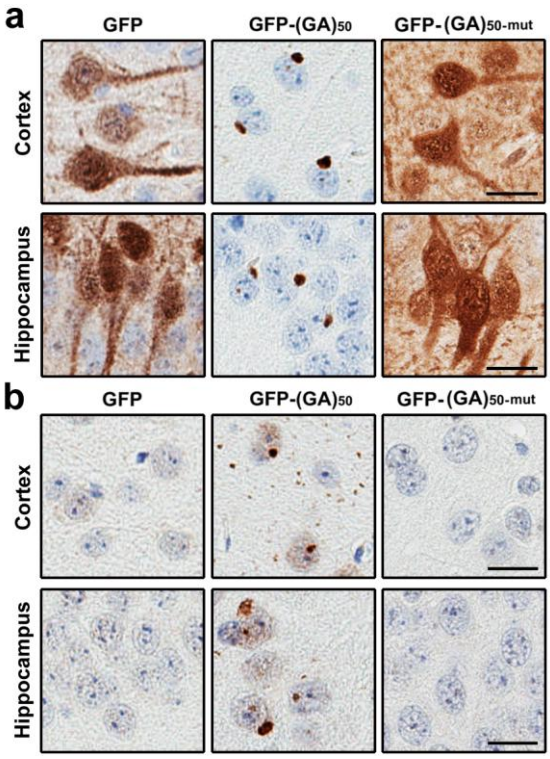
(**a**) Immunohistochemical analysis of XPC in the hippocampus of GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice. Scale bar, 20 μm. (**b**) Double immunofluorescence staining of XPC and poly(GA) proteins in the hippocampus of GFP-$(GA)_{50}$ mice. Scale bar, 5 μm.



Supplementary Figure 5

Analysis of brain morphology, body weight and motor cortex neurons in GFP-$(GA)_{50}$ mice.

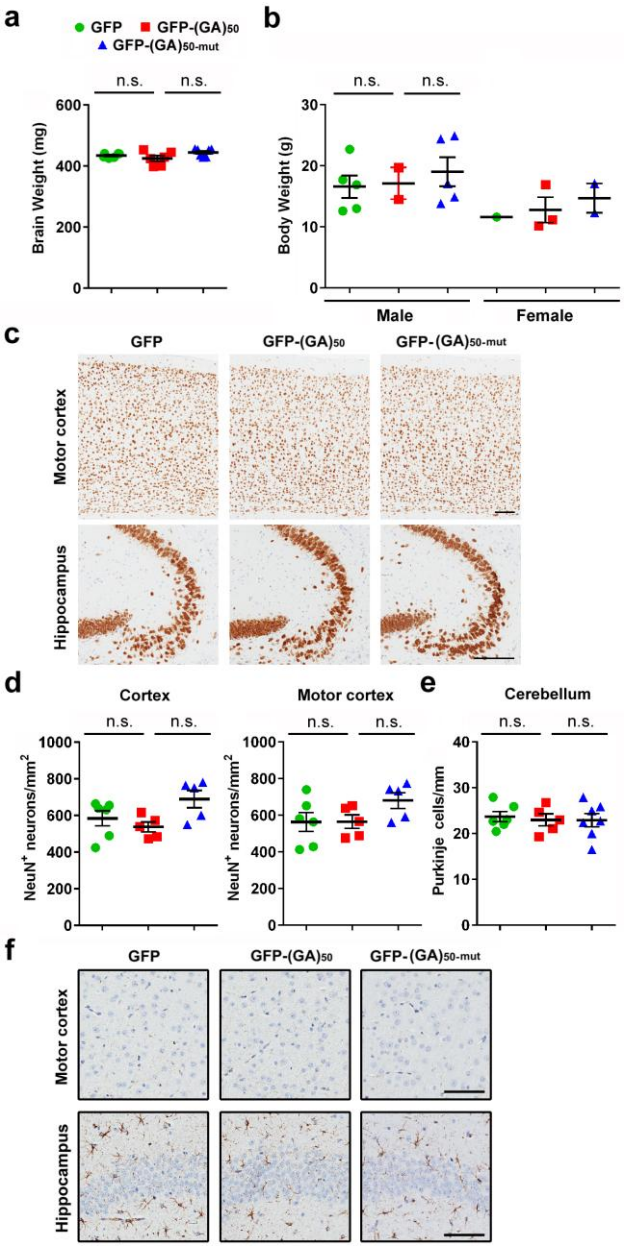
(**a**) Gross morphological analysis with hematoxylin and eosin staining of brains from 6 month-old GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice. Scale bar, 5 mm. (**b**) The mean body weight of 6 month-old male and female GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice, using 6–8 male mice or 4 female mice per group. Data are presented as mean ± s.e.m. Male mice: $P < 0.0001$, as analyzed by one-way ANOVA; ****$P < 0.0001$ and ***$P = 0.0002$, Tukey's *post-hoc* analysis. Female mice: $P = 0.0724$, one-way ANOVA. n.s., not significant. (**c**) Immunohistochemical analysis of NeuN in layer V of the motor cortex of GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice. Scale bar, 30 μm.



Supplementary Figure 6

Poly(GA) inclusions in 4- to 6-week-old GFP-$(GA)_{50}$ mice.

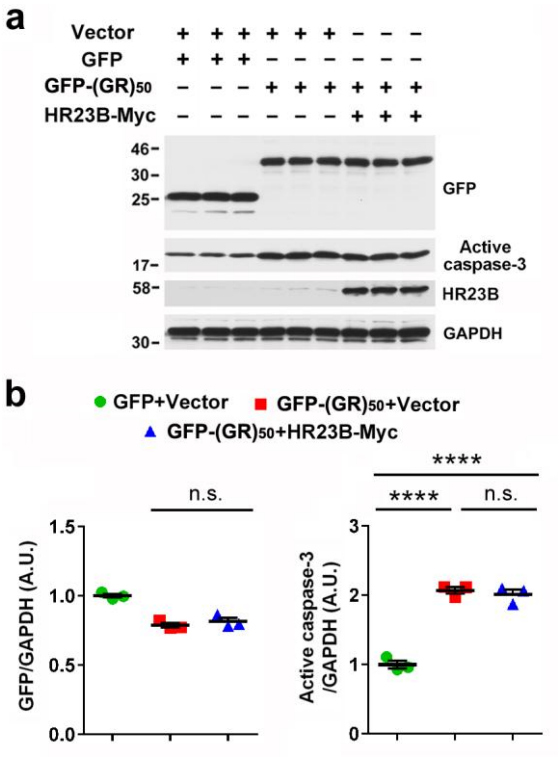
Immunohistochemical analysis of cortex and hippocampus of GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-}mut}$ mice with (**a**) anti-GFP antibody or (**b**) anti-ubiquitin antibody. Scale bars, 20 μm.



Supplementary Figure 7

No signs of neurodegeneration were observed in 4- to 6-week-old GFP-$(GA)_{50}$ mice.

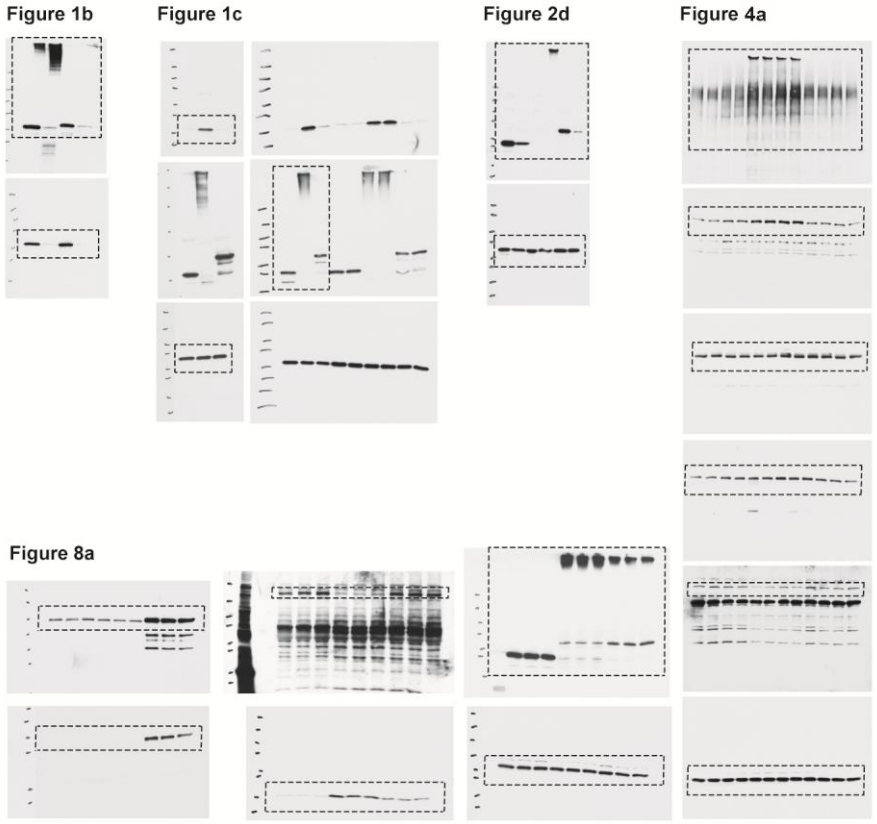
(**a**) Graph showing the mean brain weight of mice expressing GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ (n = 5–7 per group). (**b**) The mean body weight of male and female GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-mut}}$ mice using 2–5 male mice or 1–3 female mice. (**c**) Representative images of NeuN-labeled cells in the motor cortex and hippocampus of GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ mice. Scale bars, 200 μm. (**d**) Quantification of the number of NeuN-positive cells in the cortex (left) or motor cortex (right) of GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ mice (n = 5–7 per group). (**e**) Quantification of the number of Purkinje cells in the cerebellum of GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ mice (n = 5–7 per group). (**f**) Representative images of GFAP staining to identify reactive astrocytes in the motor cortex and hippocampus of GFP, GFP-$(GA)_{50}$ or GFP-$(GA)_{50\text{-mut}}$ mice. Scale bars, 100 μm. Data are presented as mean ± s.e.m., and analyzed by one-way ANOVA; $P = 0.0988$ (**a**), $P = 0.7026$ (**b**), $P = 0.0563$ (**d**, Cortex), $P = 0.1609$ (**d**, Motor cortex) and $P = 0.9042$ (**e**). n.s., not significant.



Supplementary Figure 8

Exogenous HR23B does not decrease poly(GR) levels nor attenuate poly(GR)-induced neurotoxicity.

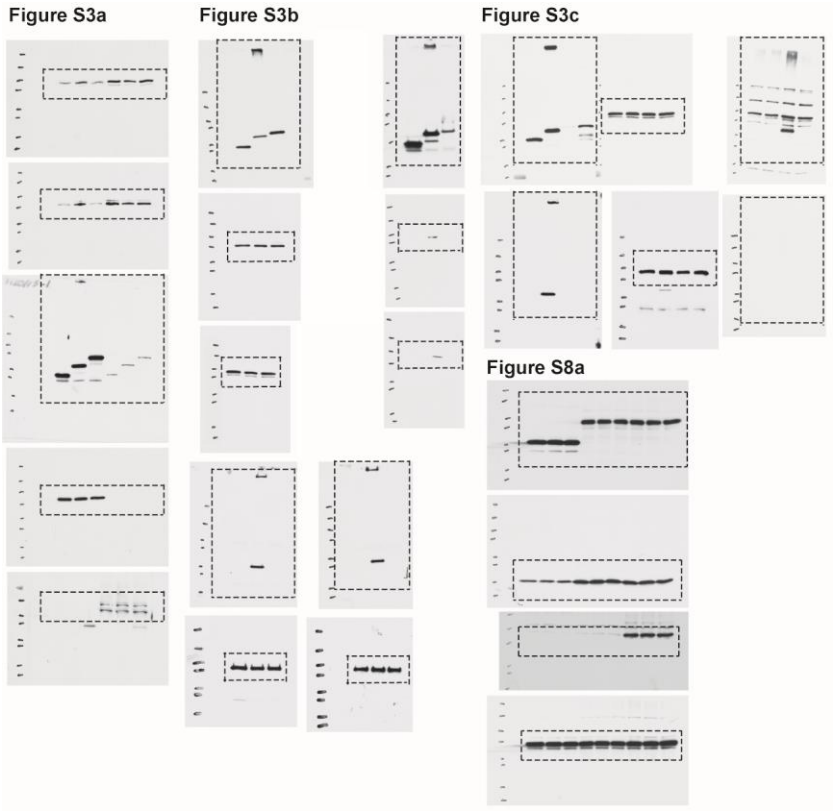
(**a**) Immunoblot and (**b**) densitometric analysis of immunoblots for the indicated proteins to determine their levels of expression in primary neurons transduced to express GFP-$(GR)_{50}$ or GFP in the presence or absence of exogenous Myc-tagged HR23B. Data are presented as mean ± s.e.m. from 3 separate experiments. In **b**, left: $P = 0.0005$, one-way ANOVA; $P = 0.6252$ (GFP-$(GR)_{50}$+Vector vs. GFP-$(GR)_{50}$+HR23B-Myc), Tukey's *post-hoc* analysis. Right: $P < 0.0001$, one-way ANOVA; ****$P < 0.0001$ and $P = 0.7798$ (GFP-$(GR)_{50}$+Vector vs. GFP-$(GR)_{50}$+HR23B-Myc), Tukey's *post-hoc* analysis. n.s., not significant. For **a**, full-length immunoblots are presented in **Supplementary Figure 10**.



Supplementary Figure 9

Full-length immunoblots for main figures.

The region delineated by the box on each blot is the image shown in the corresponding figure.



Supplementary Figure 10

Full-length immunoblots for supplementary figures.

The region delineated by the box on each blot is the image shown in the corresponding figure.

Supplementary Table 1. Regional distribution of GFP, GFP-$(GA)_{50}$ and GFP-$(GA)_{50\text{-}mut}$ in the mouse brain

Brain region	GFP	GFP-$(GA)_{50}$	GFP-$(GA)_{50\text{-}mut}$
Cortex	+++	+++	+++
Hippocampus	++/+++	++/+++	++/+++
Thalamus	+	+	+
Olfactory bulb	+/++	+	+
Cerebellum	+	+	+

+: mild, ++: moderate, +++: severe

Supplementary Table 2. Regional distribution of HR23A/B, RanGAP1 and Pom121 pathology in the brains of GFP-$(GA)_{50}$ mice

Brain region	HR23A/B	RanGAP1	Pom121
Cortex	+++	+++	++
Hippocampus	++/+++	++/+++	+
Thalamus	+	+	+
Olfactory bulb	+	+	+
Cerebellum	+	+	+

+: mild, ++: moderate, +++: severe

Supplementary Table 3. Summary of human subjects

Case #	Path Dx	Age	Gender	*C9ORF72* mutation
1	FTLD	73	Male	Yes
2	FTLD-MND	68	Male	Yes
3	FTLD	74	Male	Yes
4	FTLD	72	Male	Yes
5	FTLD-MND	55	Female	Yes
6	ALS	68	Female	Yes
7	ALS	68	Female	Yes
8	Normal	66	Male	No
9	Normal	74	Male	No
10	Normal	62	Female	No
11	Normal	60	Female	No

ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; MND, motor neuron disease