Expanded View Figures

Figure EV1. Expanded G4C2 repeats are translated into polyGA.

A Scheme and sequence of the human *C9ORF72* sense transcript with 80 G4C2 repeats fused to the eGFP in the three possible frames and cloned into the pcDNA3.1 plasmid.

B, C Fluorescence (B) and RT–qPCR (C) GFP analyses of HEK293 cells transfected for 24 h with 80 G4C2 repeats embedded in the human sense *C9ORF72* sequence and fused in all three possible frames with the GFP deleted of its ATG.

D, E GFP expression (D) and immunoblotting (E) analysis of HEK293 cells transfected for 24 h with constructs expressing either polyGA, polyGP, or polyGR expressed under an artificial ATG start codon and fused to the GFP.

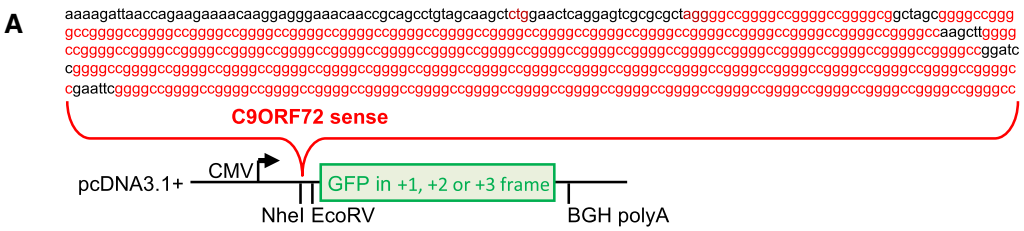
F Immunoblotting against the GFP or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with either a wild-type or a mutant (CTG into CTT) construct containing 80 G4C2 repeats embedded in sense *C9ORF72* fused to the GFP in the GA frame.

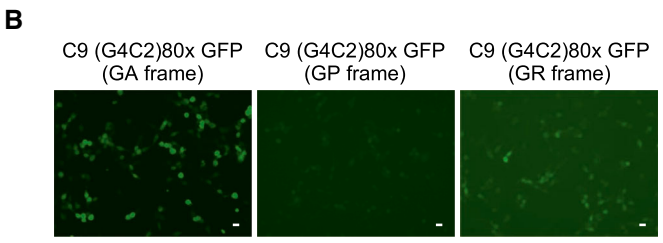
G Immunoblotting against the GFP or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with either a wild-type or a mutant (CTG into ATG) construct containing 80 G4C2 repeats embedded in sense *C9ORF72* fused to the GFP in the GA frame.

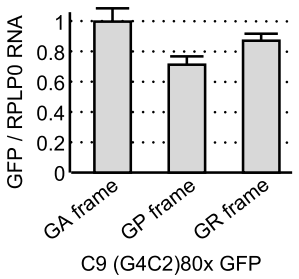
H RT–qPCR analysis of GFP expression of HEK293 cells transfected for 24 h with either wild-type or mutant (CTG into CTT or ATG) constructs containing 80 G4C2 repeats embedded in sense *C9ORF72* fused to the GFP in the GA frame.

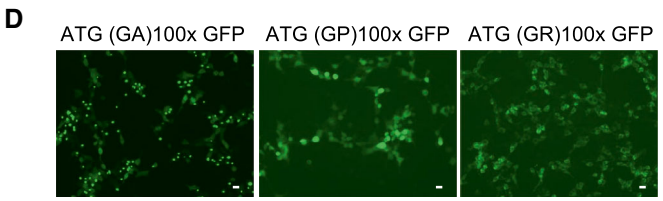
I Immunoblotting against the HA-tag or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with 20 G4C2 repeats embedded in the human sense *C9ORF72* sequence fused to a HA-tag in the GA frame and treated or not with MG132 and/or bafilomycin A1 for 15 h.

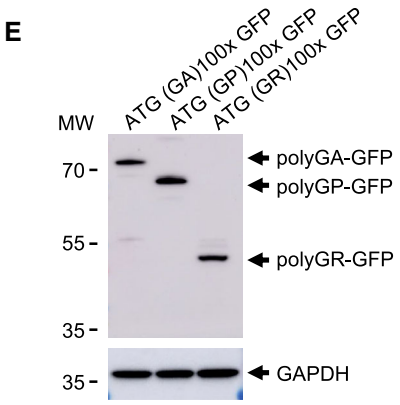
J RT–qPCR analysis of GFP expression of HEK293 cells transfected for 24 h with either wild-type or mutant (AGG into CGG) constructs containing 80 G4C2 repeats embedded in sense *C9ORF72* fused to the GFP in the GR frame.

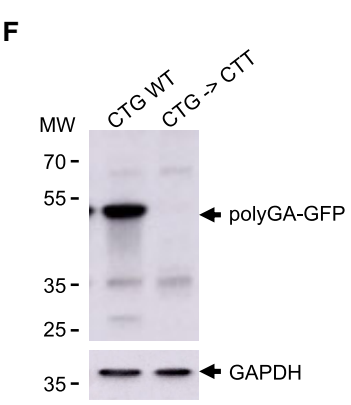


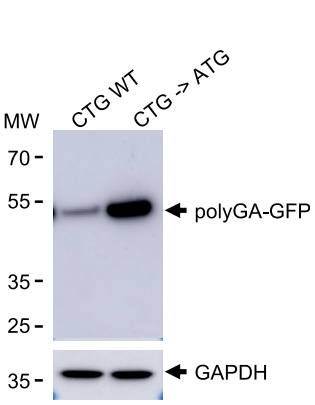


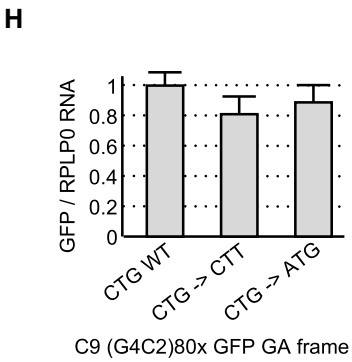


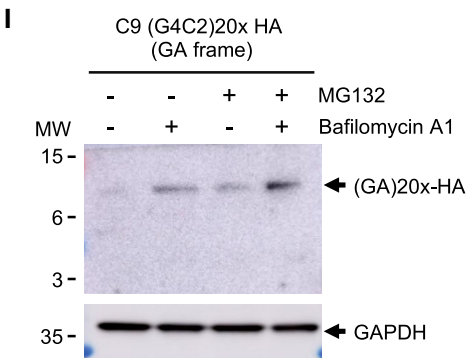












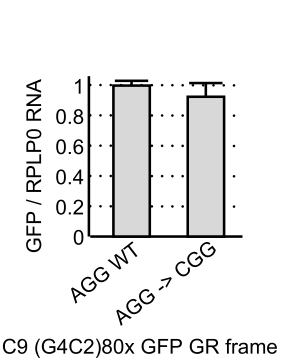


Figure EV1.

Figure EV2. Expanded C4G2 repeats are translated into polyPG.

A, B Fluorescence (A) and RT–qPCR (B) GFP analyses of HEK293 cells transfected for 24 h with 100 C4G2 repeats embedded in the human antisense *C9ORF72* sequence and fused in all three possible frames with the GFP deleted of its ATG.

C, D GFP expression (C) and immunoblotting (D) analysis of HEK293 cells transfected for 24 h with constructs expressing either polyPA or polyPR expressed under an artificial ATG start codon and fused to the GFP. Fluorescence of ATG-polyGP from Fig EV1D is shown as control.

E Immunoblotting against HA or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with either a wild-type or a mutant (ΔATG) construct containing 100 C4G2 repeats embedded in the antisense *C9ORF72* sequence fused to a HA-tag in the PG frame.

F RT–qPCR GFP expression analysis of HEK293 cells transfected for 24 h with either a wild-type or a mutant (ΔATG) construct containing 100 C4G2 repeats embedded in the antisense *C9ORF72* sequence fused to the GFP in the PG frame.

G Immunoblotting against the HA or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with either a wild-type or a Kozak consensus mutant construct containing 100 C4G2 repeats embedded in antisense *C9ORF72* fused to the HA-tag in the PG frame.

H Immunoblotting against the HA-tag or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with 10 C4G2 repeats embedded in the human antisense *C9ORF72* sequence fused to a HA-tag in the PG frame and treated or not with MG132 and/or bafilomycin A1 for 15 h.

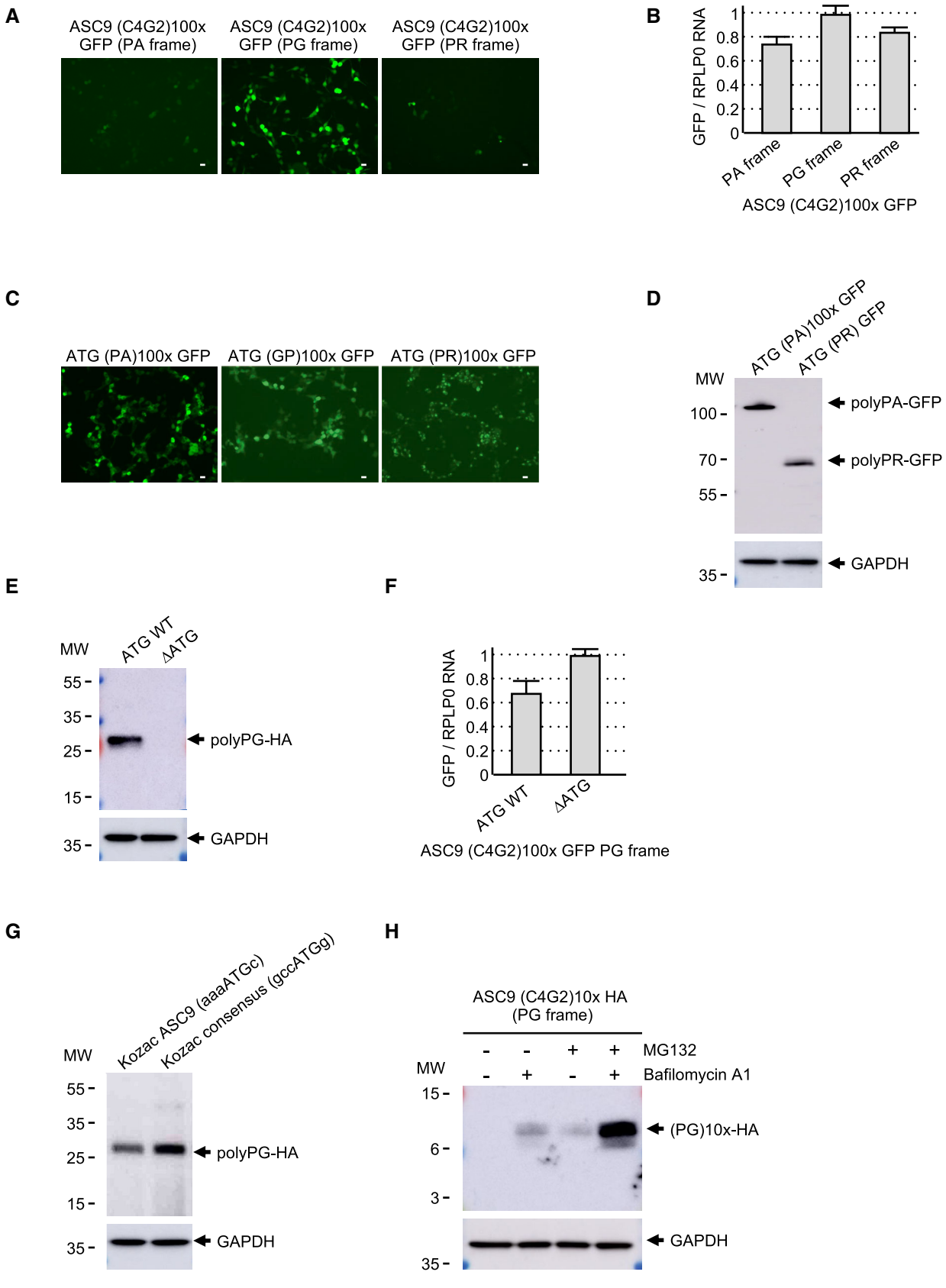

A
ASC9 (C4G2)100x GFP (PA frame)
ASC9 (C4G2)100x GFP (PG frame)
ASC9 (C4G2)100x GFP (PR frame)
B
GFP / RPLP0 RNA
1
0.8
0.6
0.4
0.2
0
PA frame
PG frame
PR frame
ASC9 (C4G2)100x GFP
C
ATG (PA)100x GFP
ATG (GP)100x GFP
ATG (PR)100x GFP
D
ATG (PA)100x GFP
ATG (PR) GFP
MW
100
70
55
35
polyPA-GFP
polyPR-GFP
GAPDH
E
MW
ATG WT
ΔATG
55
35
25
15
35
polyPG-HA
GAPDH
F
GFP / RPLP0 RNA
1
0.8
0.6
0.4
0.2
0
ATG WT
ΔATG
ASC9 (C4G2)100x GFP PG frame
G
MW
Kozac ASC9 (aaaATGc)
Kozac consensus (gccATGg)
55
35
25
15
35
polyPG-HA
GAPDH
H
ASC9 (C4G2)10x HA (PG frame)
MW
- - + + MG132
- + - + Bafilomycin A1
15
6
3
35
(PG)10x-HA
GAPDH

Figure EV2.

Figure EV3. Decreased expression of C9ORF72 synergizes DPR toxicity.

A Immunoblotting against the HA-tag or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with 80 G4C2 repeats embedded in the human sense *C9ORF72* sequence fused to a HA-tag in the GA frame and treated or not with MG132 and/or bafilomycin A1.

B As in (A) but with cells transfected with 100 C4G2 repeats embedded in the human antisense *C9ORF72* sequence fused to a HA-tag in the PG frame.

C, D Left panel, representative images of immunofluorescence labeling of endogenous P62/SQSTM1 (C) or ubiquitin (D) and the GFP in GT1-7 neuronal cells co-transfected for 24 h with either a control siRNA or a siRNA targeting *C9orf72* mRNA and a construct containing 80 G4C2 repeats embedded in sense *C9ORF72* fused to the GFP in the GA frame. Right panel, quantification of the percent of co-localization of P62 or ubiquitin with polyGA aggregates.

E Immunofluorescence labeling of M6PR in GT1-7 neuronal cells transfected for 24 h with either a control siRNA or a siRNA targeting *C9orf72* mRNA.

F Immunofluorescence labeling of EEA1 in GT1-7 neuronal cells transfected for 24 h with either a control siRNA or a siRNA targeting *C9orf72* mRNA.

Data information: Error bars indicate s.e.m. Student's *t*-test, ***$P < 0.001$. $n = 3$ independent transfection. Scale bars, 10 μm. Nuclei were counterstained with DAPI.

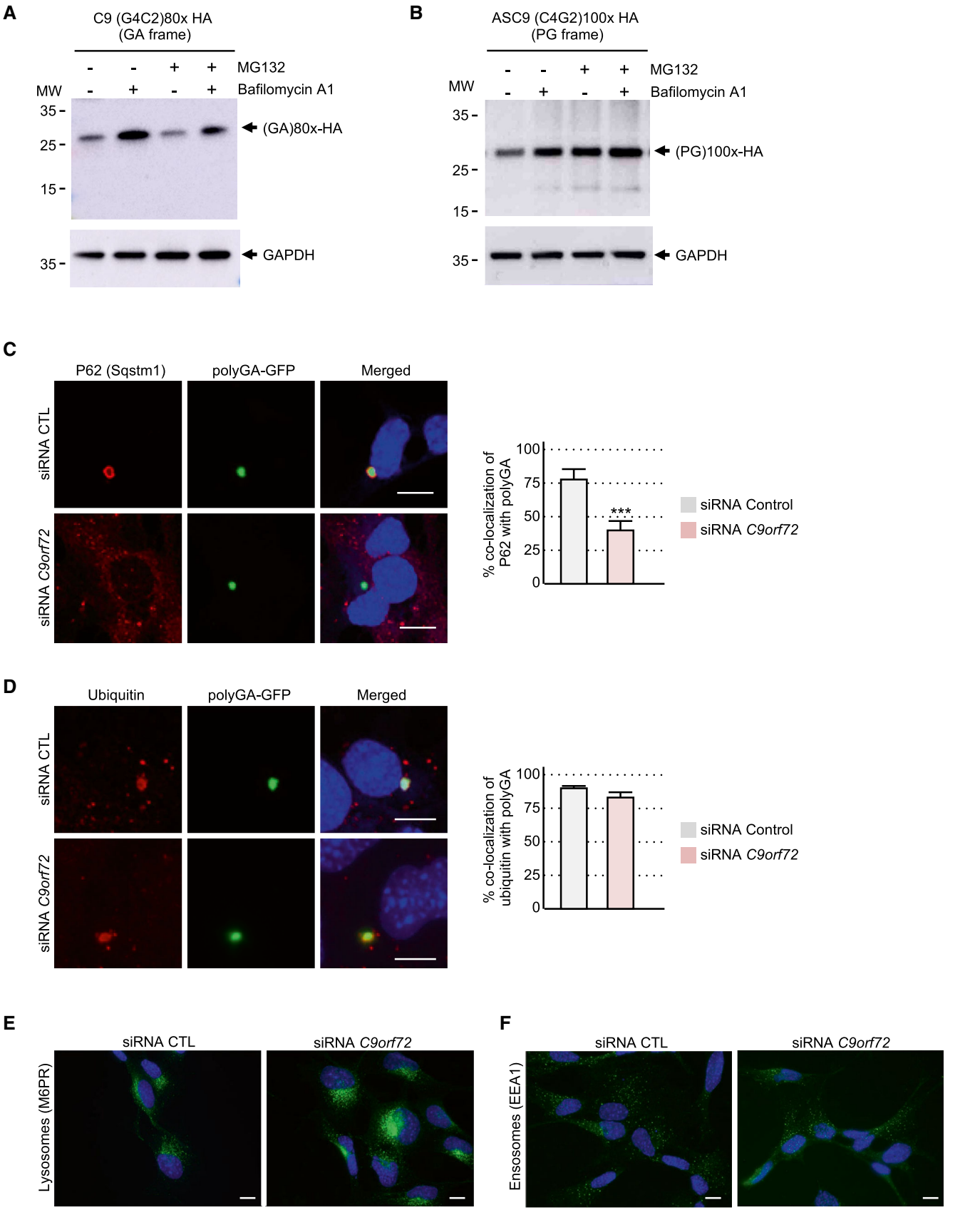


Figure EV3.

Figure EV4. Promethazine reduces DPR protein accumulation and toxicity.

A Left panel, immunoblotting against the GFP or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with a construct expressing under an artificial ATG start codon 100 GA repeats fused to the GFP (ATG (GA)100× GFP) and treated 15 h with 10 μM of the indicated drug. Right panel, quantification of polyGA expression relative to the GAPDH.

B Immunoblotting against the GFP or the GAPDH of proteins extracted from HEK293 cells transfected for 24 h with ATG (GA)100× GFP and treated 15 h with 1, 3, or 10 μM of the indicated drug.

C Left panel, GFP fluorescence of HEK293 cells transfected for 24 h with ATG (GA)100× GFP and treated with 10 μM of the indicated compound. Scale bars, 10 μm. Nuclei were counterstained with DAPI. Right panel, quantification of polyGA aggregates.

D Cell viability (TO-PRO-3 FACS staining) of GT1-7 neuronal cells treated with 1, 3, or 10 μM of promethazine and co-transfected for 24 h with ATG (GA)100× GFP and either a control siRNA or a siRNA targeting *C9orf72* mRNA.

Data information: Error bars indicate s.e.m. Student's *t*-test, $^{**}P < 0.01$, and $^{***}P < 0.001$. $n = 5$ independent transfection.

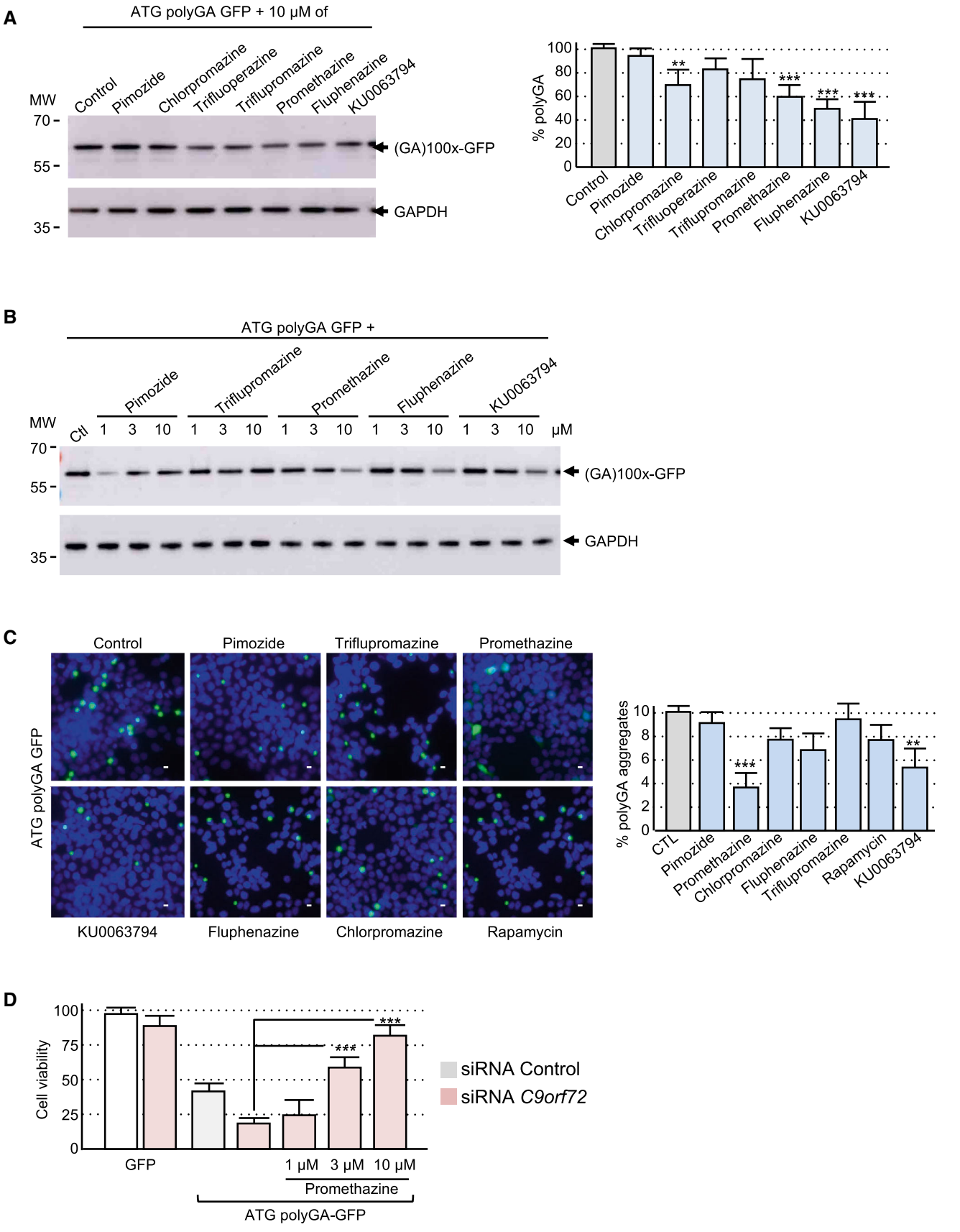

A
ATG polyGA GFP + 10 μM of
MW
Control
Pimozide
Chlorpromazine
Trifluoperazine
Triflupromazine
Promethazine
Fluphenazine
KU0063794
70
55
35
(GA)100x-GFP
GAPDH
% polyGA
100
80
60
40
20
0
**
***
***
***
B
ATG polyGA GFP +
Pimozide
Triflupromazine
Promethazine
Fluphenazine
KU0063794
MW
Ctl
1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 μM
70
55
35
(GA)100x-GFP
GAPDH
C
Control
Pimozide
Triflupromazine
Promethazine
ATG polyGA GFP
KU0063794
Fluphenazine
Chlorpromazine
Rapamycin
% polyGA aggregates
10
8
6
4
2
0
***
**
CTL
Pimozide
Promethazine
Chlorpromazine
Fluphenazine
Triflupromazine
Rapamycin
KU0063794
D
Cell viability
100
75
50
25
0
***
***
GFP
1 μM 3 μM 10 μM
Promethazine
ATG polyGA-GFP
siRNA Control
siRNA C9orf72

Figure EV4.