Expanded View Figures

Figure EV1. Poly-GA induces cytoplasmic TDP-43 mislocalization.

A, B Immunofluorescence analysis of endogenous TDP-43 in the anterior horn of the spinal cord of GA_{149}-CFP transgenic mice 8–12 months of age (Schludi *et al*, 2017). Single confocal sections are shown in (A). Arrow indicates neuron with cytoplasmic TDP-43 punctae. (B) Manual quantification of neurons with cytoplasmic TDP-43 in the anterior horn. To allow blinded quantification, poly-GA expression was not taken into account. Scatter plot with bar graphs of mean ± SD. Statistical analysis using unpaired *t*-test and Welch's correction (three wild-type and six transgenic animals).

C Immunoblotting of three wild-type and three GA_{149}-CFP transgenic mice spinal cord 8 months of age. Immunoblotting of one wild-type and one GA_{149}-CFP transgenic mouse spinal cord is shown. Proteolytic processing of TDP-43 was not detected in both genotypes.

D Immunofluorescence analysis of endogenous TDP-43 in large ChAT-positive motoneurons in the anterior and posterior horns of the spinal cord of GA_{149}-CFP transgenic mice 8–12 months of age (Schludi *et al*, 2017). Maximum intensity projections are shown. Arrow indicates neurons with cytoplasmic TDP-43 punctae.

E, F Automated analysis of cytoplasmic mislocalization of TDP-43 in frontal cortex of *C9orf72* FTLD patients. Representative raw image and the resulting CellProfiler mask (see Materials and Methods for details). Poly-GA-positive neurons were significantly more likely to have detectable cytoplasmic TDP-43 than neighboring poly-GA-negative neurons (paired *t*-test $t(7) = 5.58$, partial $\eta^2 = 0.816$, mean ± SD).

Data information: $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bars: 50 μm.
Source data are available online for this figure.

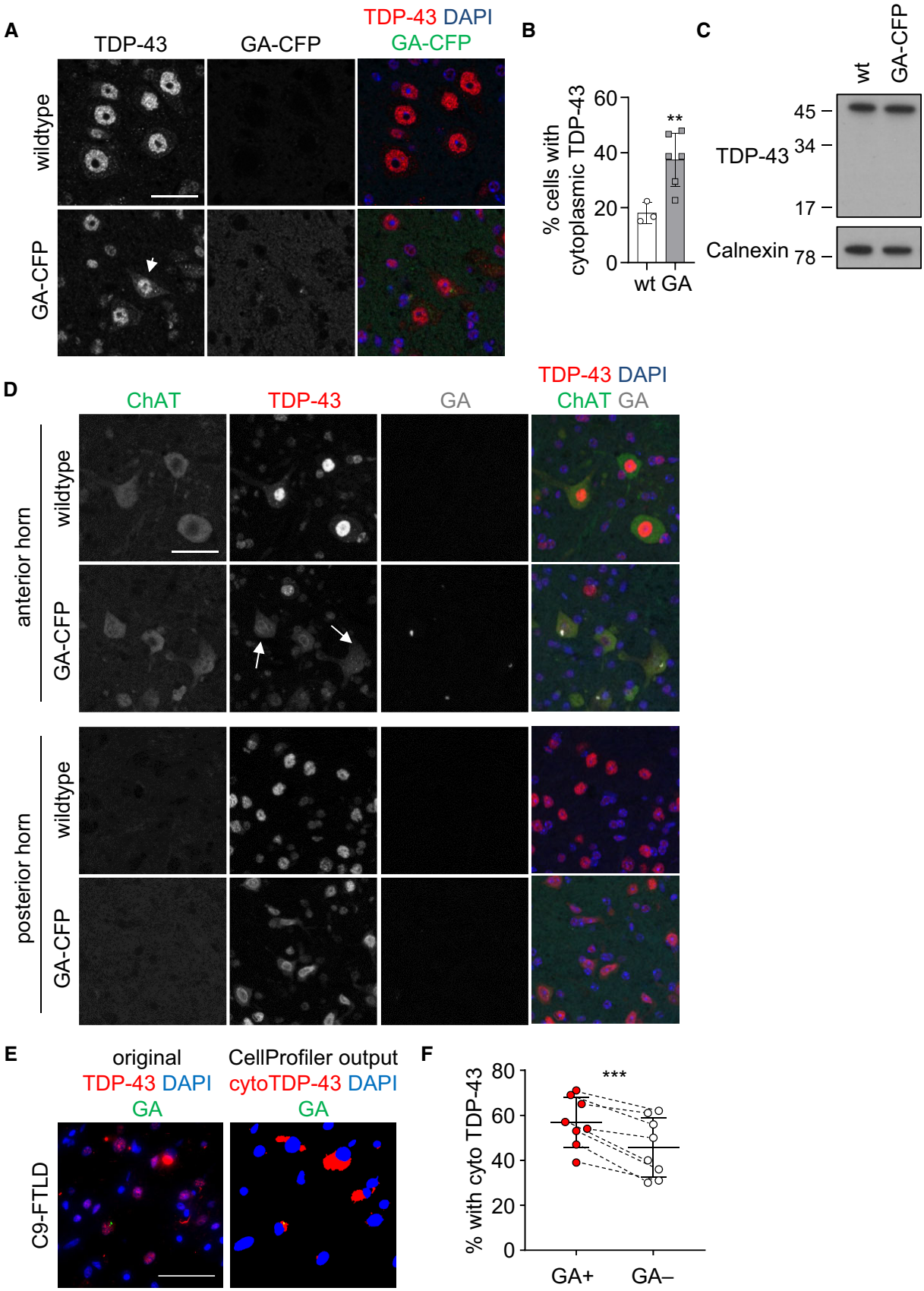
A
TDP-43
GA-CFP
TDP-43 DAPI
GA-CFP
wildtype
GA-CFP
B
% cells with cytoplasmic TDP-43
60
40
20
0
**
wt GA
C
wt
GA-CFP
45
34
17
TDP-43
Calnexin
78
D
ChAT
TDP-43
GA
TDP-43 DAPI
ChAT GA
anterior horn
wildtype
GA-CFP
posterior horn
wildtype
GA-CFP
E
original
TDP-43 DAPI
GA
CellProfiler output
cytoTDP-43 DAPI
GA
C9-FTLD
F
% with cyto TDP-43
80
60
40
20
0
***
GA+
GA–

Figure EV1.

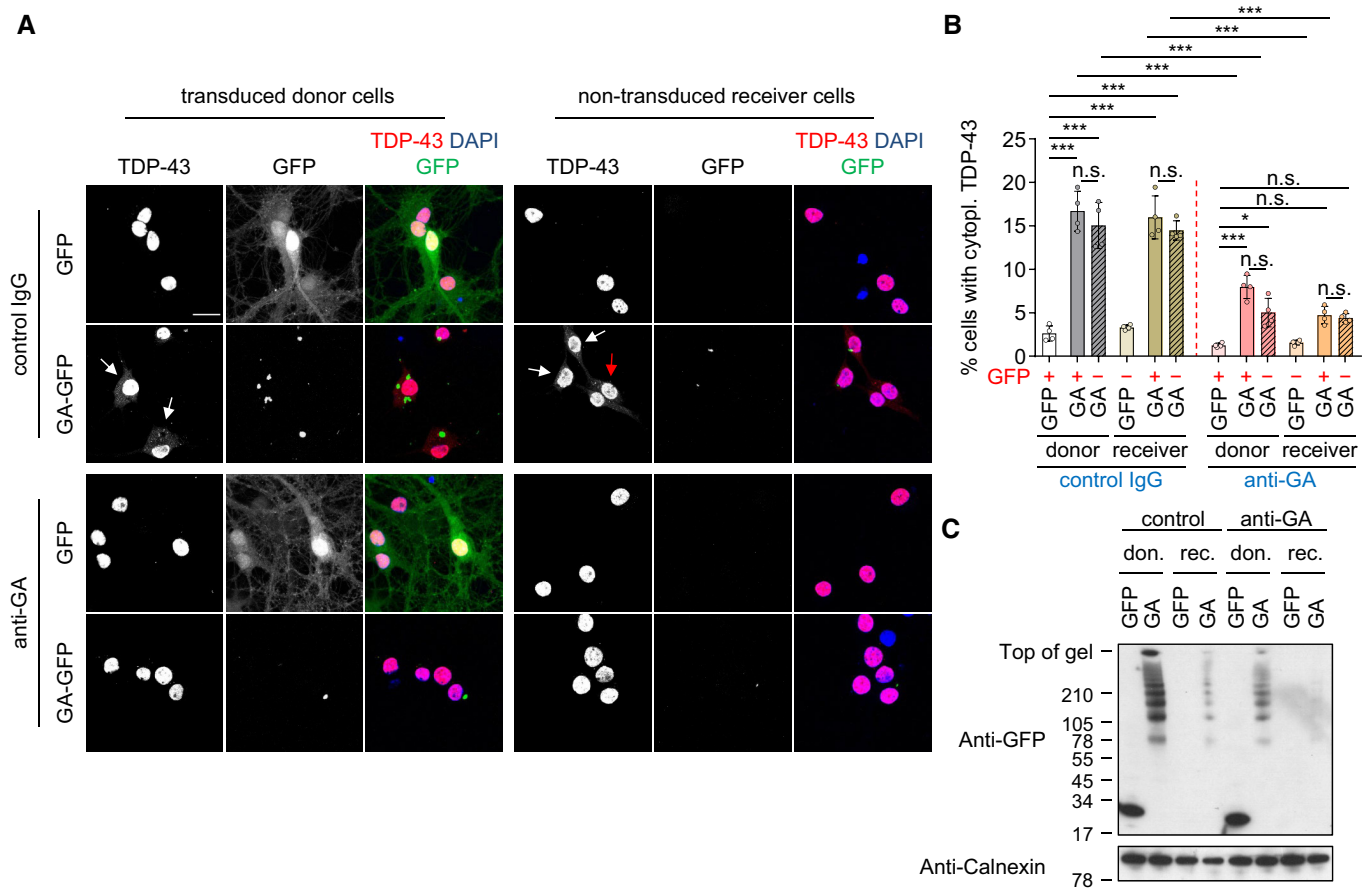

Figure EV2. Anti-GA antibodies block the non-cell-autonomous effects of poly-GA on TDP-43 in a co-culture assay.

Primary hippocampal neurons were transduced with GFP or GA_{175}-GFP (DIV4 + 4) and treated with IgG control and anti-GA (5F2) antibody.

A Confocal imaging revealed that anti-GA antibody treatment reduces Poly-GA-induced cytoplasmic mislocalization of TDP-43 in hippocampal neurons. White and red arrows show cells with cytoplasmic TDP-43 in GFP-positive and GFP-negative cells, respectively. Scale bar denotes 20 μm.

B Automated quantification of cells with cytoplasmic TDP-43 in GFP or GA_{175}-GFP-transduced cells. Cells with and without GFP signal were analyzed separately (indicated by +/−). As in Fig 1C, GFP-negative donor and GFP-positive receiver cells were excluded due to high transduction and low transmission rate of GFP. $n = 4$ biological replicates. Scatter plot with bar graphs of mean ± SD. One-way ANOVA with Tukey's multiple comparisons test. *$P < 0.05$, and ***$P < 0.001$.

C Immunoblotting shows reduced poly-GA expression upon anti-GA antibody treatment.

Source data are available online for this figure.

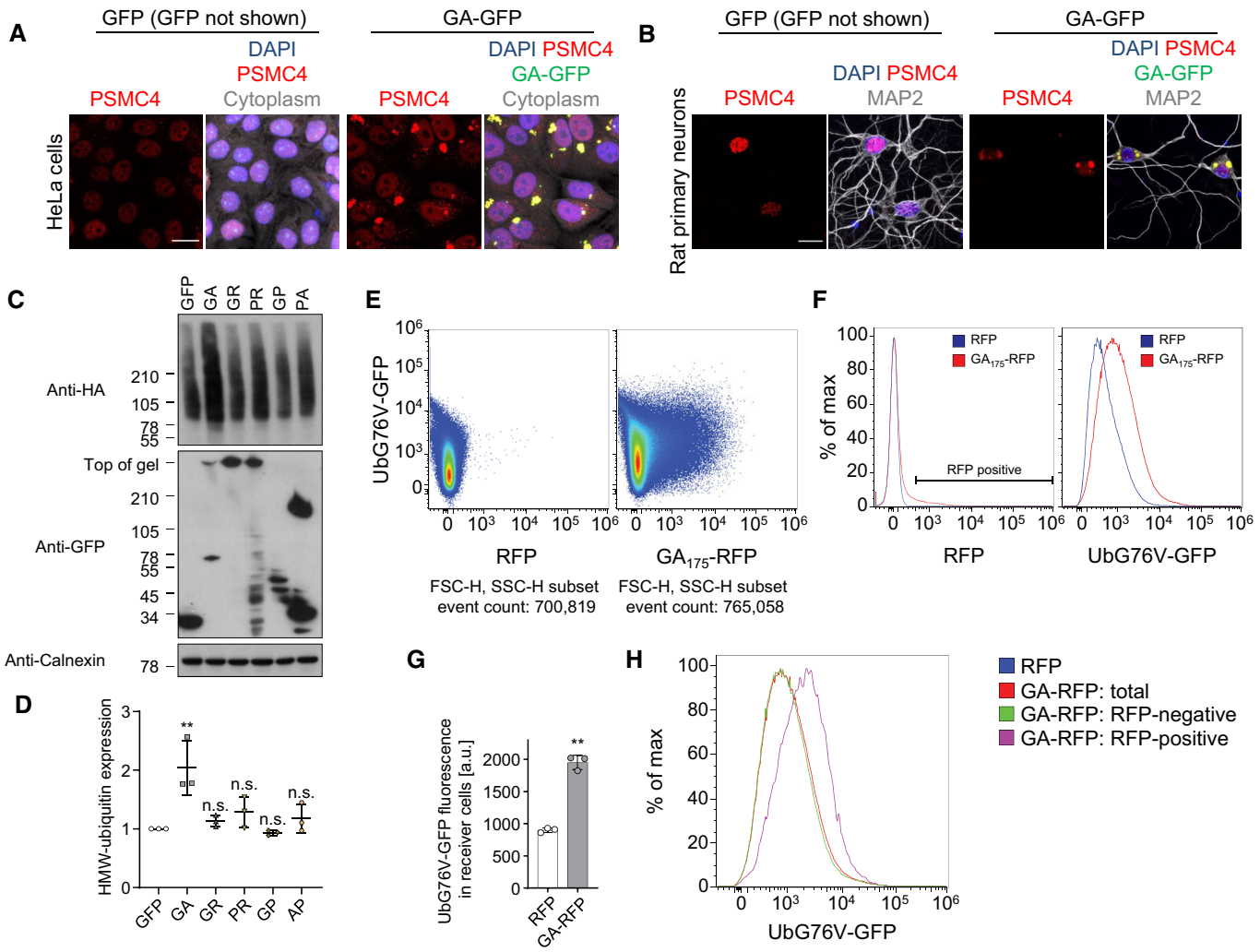

Figure EV3. Poly-GA inclusions sequester the proteasome.

A, B Immunofluorescence of the proteasome subunit PSMC4 and poly-GA inclusions in GA_{175}-GFP-transfected HeLa cells and GA_{175}-GFP-transduced rat primary neurons. To confirm cell viability, the cytoplasm of HeLa cells was stained with HCS CellMask™ Deep Red Stain and neuronal dendrites were labeled with MAP2. Scale bar denotes 20 μm.

C, D Immunoblots of HeLa cells that were co-transfected with HA-ubiquitin and GFP, GA_{175}-GFP, GFP-GR_{149}, PR_{175}-GFP, GFP-GP_{47}, and PA_{175}-GFP and incubated for 48 h and analyzed by densitometry. Scatter plot, mean ± SD. One-way ANOVA with Tukey's multiple comparisons test. $^{**}P < 0.01$.

E–H Flow cytometry analysis of non-cell-autonomous proteasome inhibition using HEK293 Ub_{G76V}-GFP reporter cells co-cultured for 48 h with GA_{175}-RFP- or RFP-transfected cells. (E) Two-color scatter plots, presented as pseudo-color density plots, with compensated RFP fluorescence plotted on the *x*-axis and compensated GFP fluorescence on the *y*-axis. A representative experiment out of three independent repeats is shown. (F) Comparisons of the corresponding histograms for compensated RFP and Ub_{G76V}-GFP fluorescence from one representative experiment that shows specific transmission of GA_{175}-RFP associated with accumulation of Ub_{G76V}-GFP in cells co-cultured with GA_{175}-RFP. (G) Accumulation of Ub_{G76V}-GFP signal in non-transfected receiver cells that were co-incubated for 48 h with GA_{175}-RFP-transfected donor cells compared with RFP control. $n = 3$ biological replicates. Scatter plot with bar graphs of mean ± SD. Unpaired two-tailed *t*-test with Welch's correction. $^{**}P < 0.01$. (H) Histograms for Ub_{G76V}-GFP intensity showing separate analysis of RFP-positive and RFP-negative receiver cells for the GA_{175}-RFP condition. RFP gating as indicated in (F).

Source data are available online for this figure.

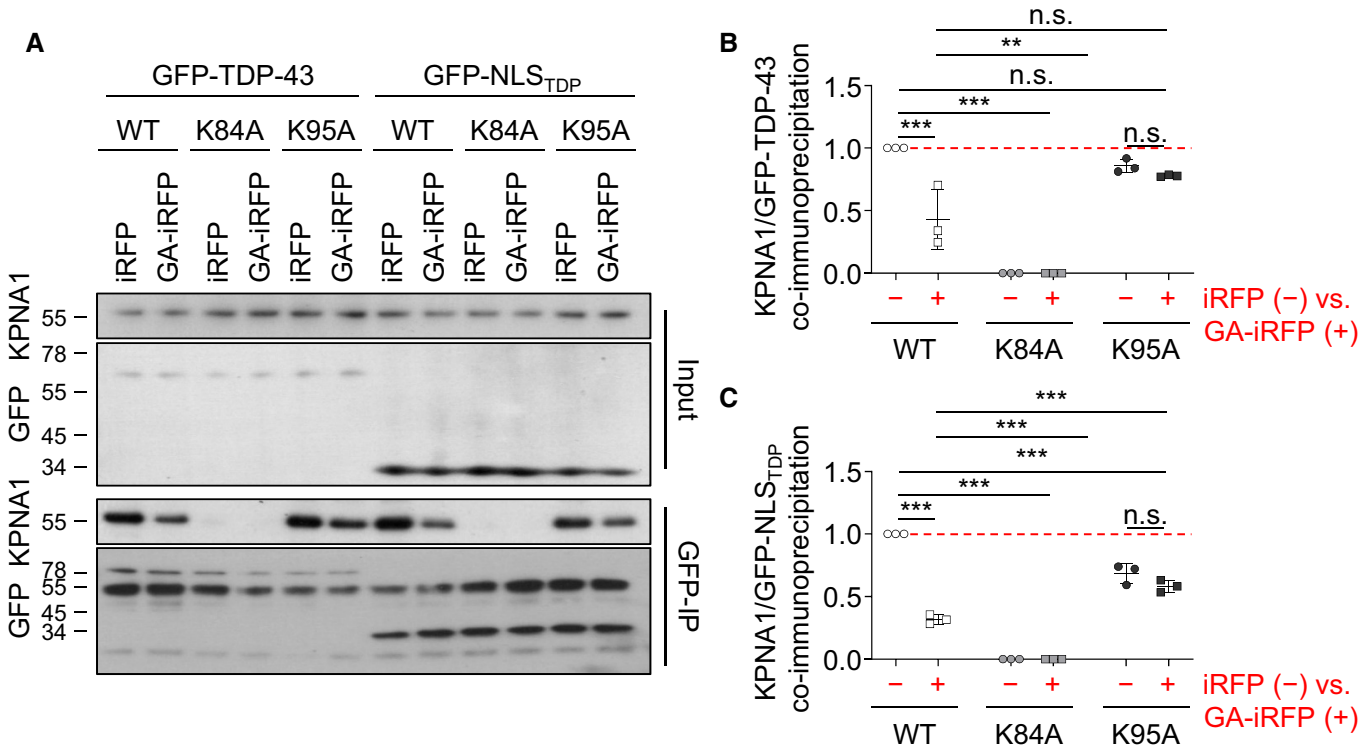

Figure EV4. Poly-GA reduces KPNA1 binding of full-length TDP-43.

A HeLa cells were co-transfected with either full-length GFP-TDP-43 (wild type, K84A, K95A) or GFP-NLS$_{TDP}$ (wild type, K84A, K95A) as well as iRFP or GA$_{175}$-iRFP and incubated for 48 h. Lysates were immunoprecipitated with anti-GFP and immunoblotted with an anti-importin-α5/KPNA1 antibody to detect binding of the nuclear import receptor. Protein expression in the input is also shown.

B, C Quantification of KPNA1 levels normalized to total GFP-TDP-43 and GFP-NLS$_{TDP}$ reporter levels in anti-GFP immunoprecipitates. n = 3 biological replicates. Scatter plot with mean ± SD. One-way ANOVA with Tukey's multiple comparisons test. $^{**}P < 0.01$, $^{***}P < 0.001$. Red dashed line indicates the control's expression level.

Source data are available online for this figure.

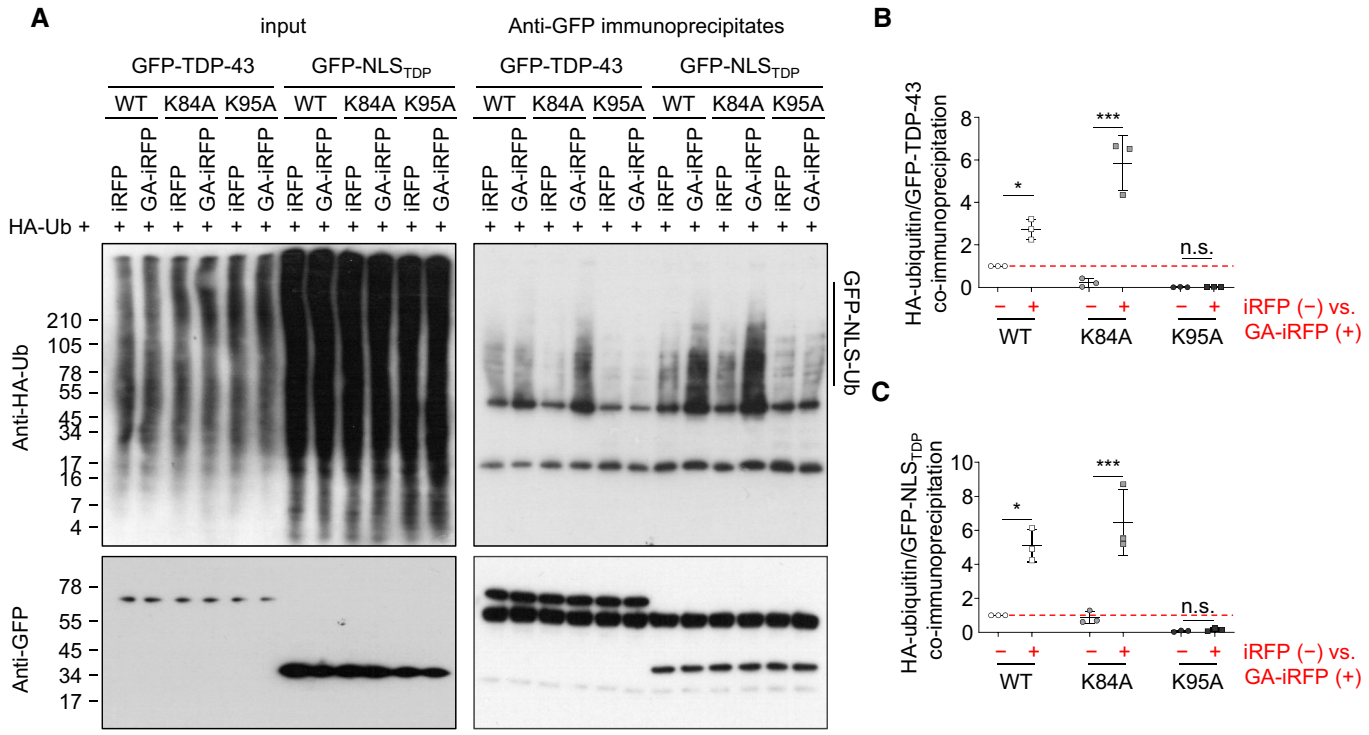

Figure EV5. Poly-GA induces poly-ubiquitination of TDP-43 at lysine 95.

A–C HeLa cells were co-transfected with either full-length GFP-TDP-43 (wild type, K84A, K95A) or GFP-NLS$_{TDP}$ (wild type, K84A, K95A) as well as HA-ubiquitin and iRFP or GA$_{175}$-iRFP, and incubated for 48 h. Lysates were immunoprecipitated with anti-GFP antibody. Immunoblotting of input (left panels) and anti-GFP immunoprecipitates (right panels) to show TDP-43 bait levels and poly-ubiquitination. (B, C) Quantification of HA-ubiquitin levels normalized to total GFP-TDP-43 and GFP-NLS$_{TDP}$ reporter levels in anti-GFP immunoprecipitates. n = 3 biological replicates. Scatter plot, mean ± SD. One-way ANOVA with Tukey's multiple comparisons test. *$P < 0.05$, and ***$P < 0.001$. Red dashed line indicates the control's expression level.

Source data are available online for this figure.