Expanded View Figures

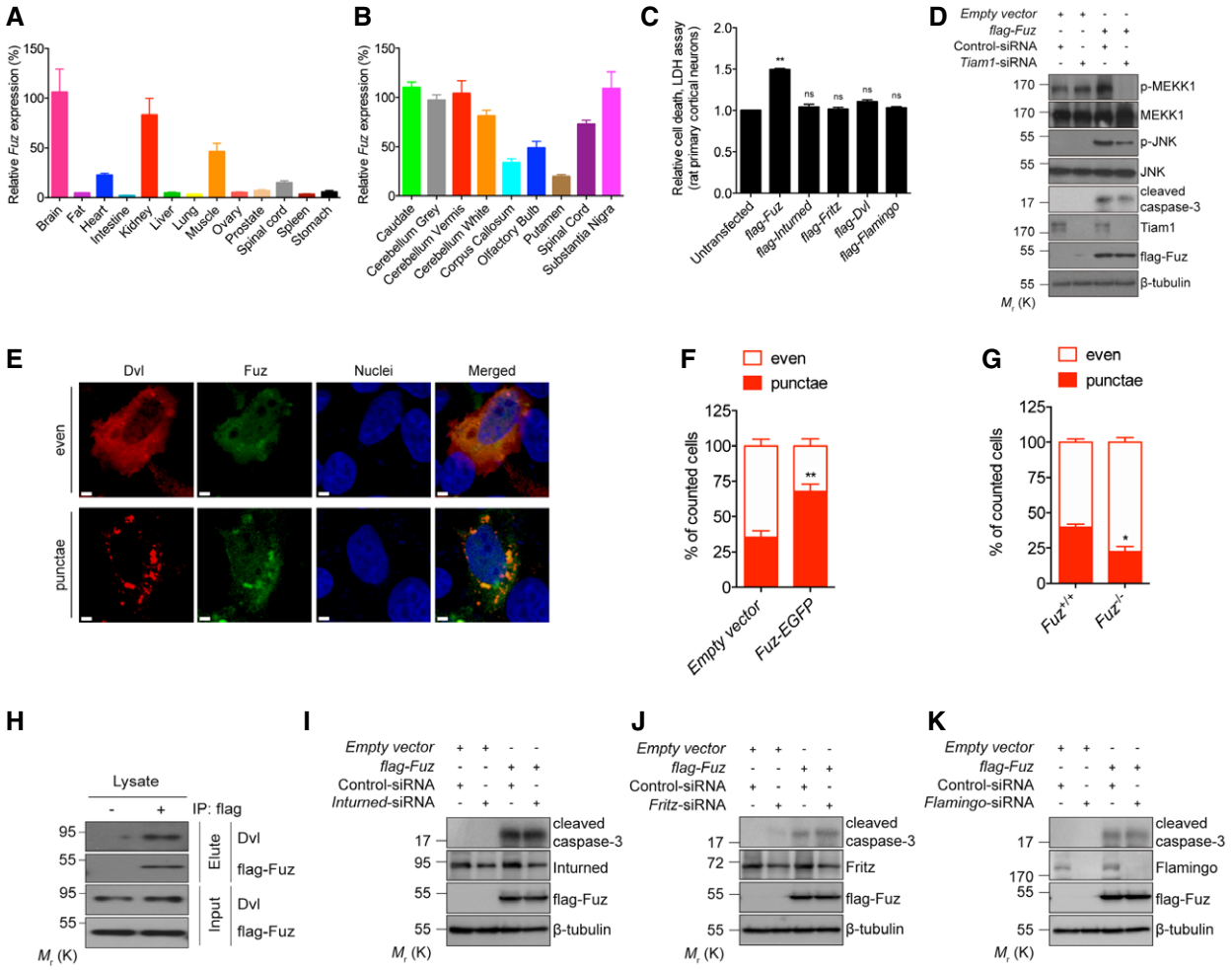

Figure EV1. Data related to Fig 1.

A Differential expression of *Fuz* in normal human tissues. *Fuz* expression in brain from the first experimental trial is defined as "100%", and the relative expression of *Fuz* is normalized to that of brain. Error bars represent s.e.m., $n = 3$.

B Differential expression of *Fuz* in normal human brain regions. *Fuz* expression in caudate from the first experimental trial is defined as "100%", and the relative expression of *Fuz* is normalized to that of caudate. Error bars represent s.e.m., $n = 3$.

C Inturned, Fritz, Dvl and Flamingo did not trigger neuronal cell death when overexpressed, respectively, in rat primary cortical neurons. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using one-way ANOVA followed by *post hoc* Tukey's test. ns represents no significant difference. $^{**}P < 0.01$.

D Knockdown of *Tiam1* expression suppressed the MAPK-caspase pathway activation induced by Fuz overexpression in HEK293 cells. $n = 3$.

E, F (E) HEK293 cells transfected with 0.2 μg *flag-Dvl* displayed both "even" and "punctae" staining patterns. "even" indicates cells with evenly distributed Dvl, with occasional small Dvl dots. "punctae" indicates cells with large Dvl aggregates. Nuclei were stained with Hoechst 33342 (blue). Scale bars: 2 μm. Overexpression of Fuz (green) promoted Dvl (red) to form "punctae", and Fuz protein colocalized with these "punctae". (F) is the quantification of (E). Error bars represent s.e.m., $n = 3$. For every control or experimental group, at least 120 cells were counted in each replicate. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{**}P < 0.01$.

G Knockout of *Fuz* reduced the percentage of cells with Dvl "punctae". The parental HEK293 cells were used as control. Error bars represent s.e.m., $n = 3$. For every control or experimental group, at least 120 cells were counted in each replicate. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$.

H Exogenous Fuz protein interacted with endogenous Dvl protein in HEK293 cells. "+" denotes the immunoprecipitation was performed using anti-flag antibody, "−" denotes no antibody control. $n = 3$.

I–K Knockdown of *Inturned* (I), *Fritz* (J) or *Flamingo* (K) did not suppress Fuz-induced caspase-3 cleavage in HEK293 cells. $n = 3$.

Data information: Beta-tubulin was used as loading control. *n* represents the number of biological replicates. Only representative images and blots are shown.
Source data are available online for this figure.

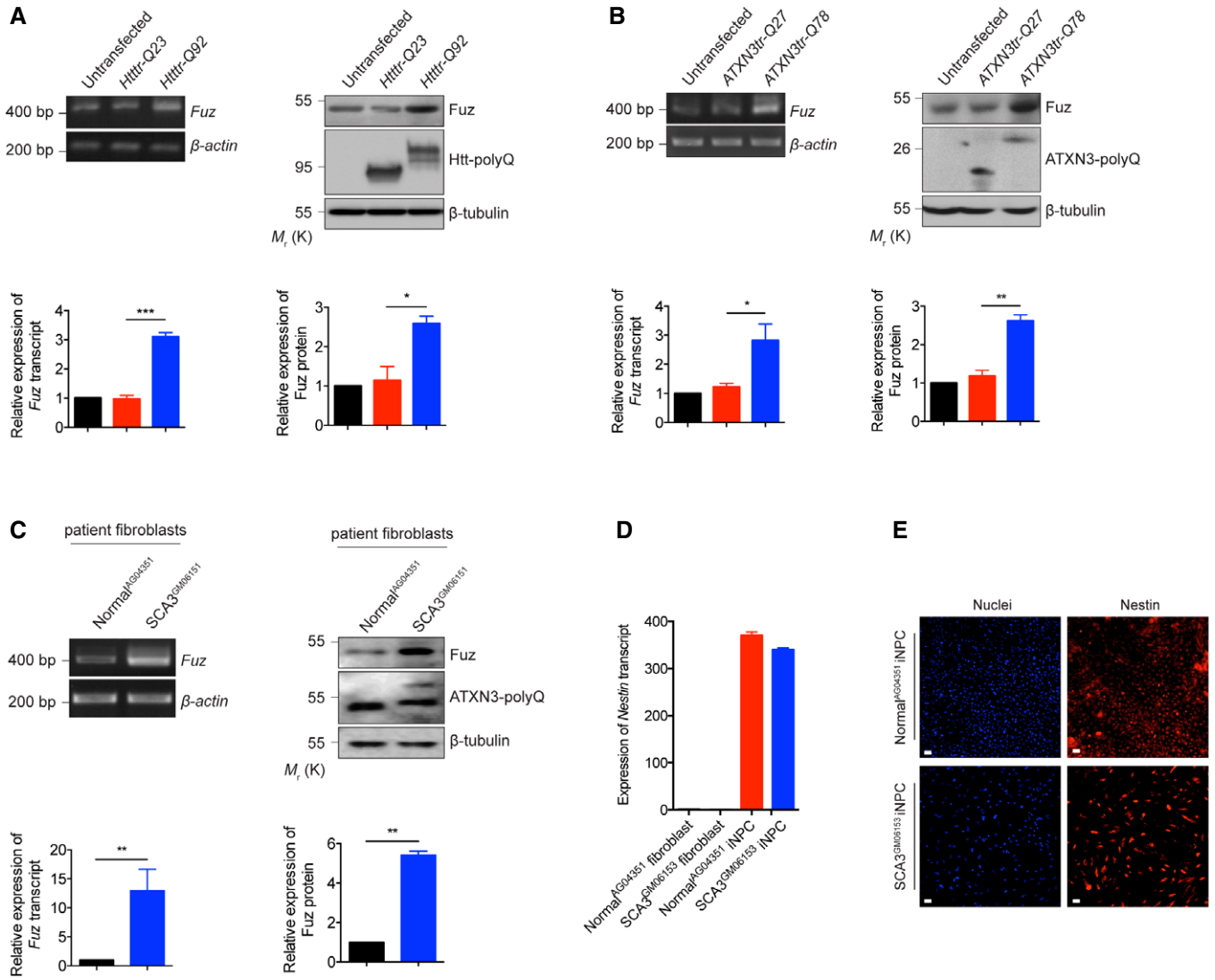

Figure EV2. Data related to Fig 2.

A Both *Fuz* transcript and Fuz protein levels were upregulated in the Htttr-Q92-expressing HEK293 cells. "Htttr" indicates truncated Huntingtin (Htt), disease protein of Huntington's disease (HD). Lower panel shows the quantification of *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$, $^{***}P < 0.001$.

B Overexpression of expanded ATXN3tr-Q78 protein increased the levels of expression of *Fuz* transcript and Fuz protein in HEK293 cells. "ATXN3tr" indicates truncated ataxin-3 (ATXN3), disease protein of spinocerebellar ataxia type 3 (SCA3). Lower panel shows the quantification of *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$.

C The expression levels of *Fuz* transcript and Fuz protein were induced in SCA3 patient fibroblasts. Lower panel shows the quantification of *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{**}P < 0.01$.

D Quantitative RT–PCR was used to determine the expression level of induced neural progenitor cells (iNPCs) specific marker *Nestin* in fibroblasts and iNPCs. Expression of *Nestin* was robustly induced in both normal and SCA3 iNPCs. Error bars represent s.e.m., $n = 3$.

E Immunostaining of iNPCs using anti-Nestin (red) antibody, and nuclei were stained with Hoechst 33342 (blue). Both normal and SCA3 iNPCs showed the expression of Nestin protein. Scale bars: 100 μm. $n = 3$.

Data information: *Beta-actin* or beta-tubulin was used as loading control. *n* represents the number of biological replicates. Only representative images, gels and blots are shown.
Source data are available online for this figure.

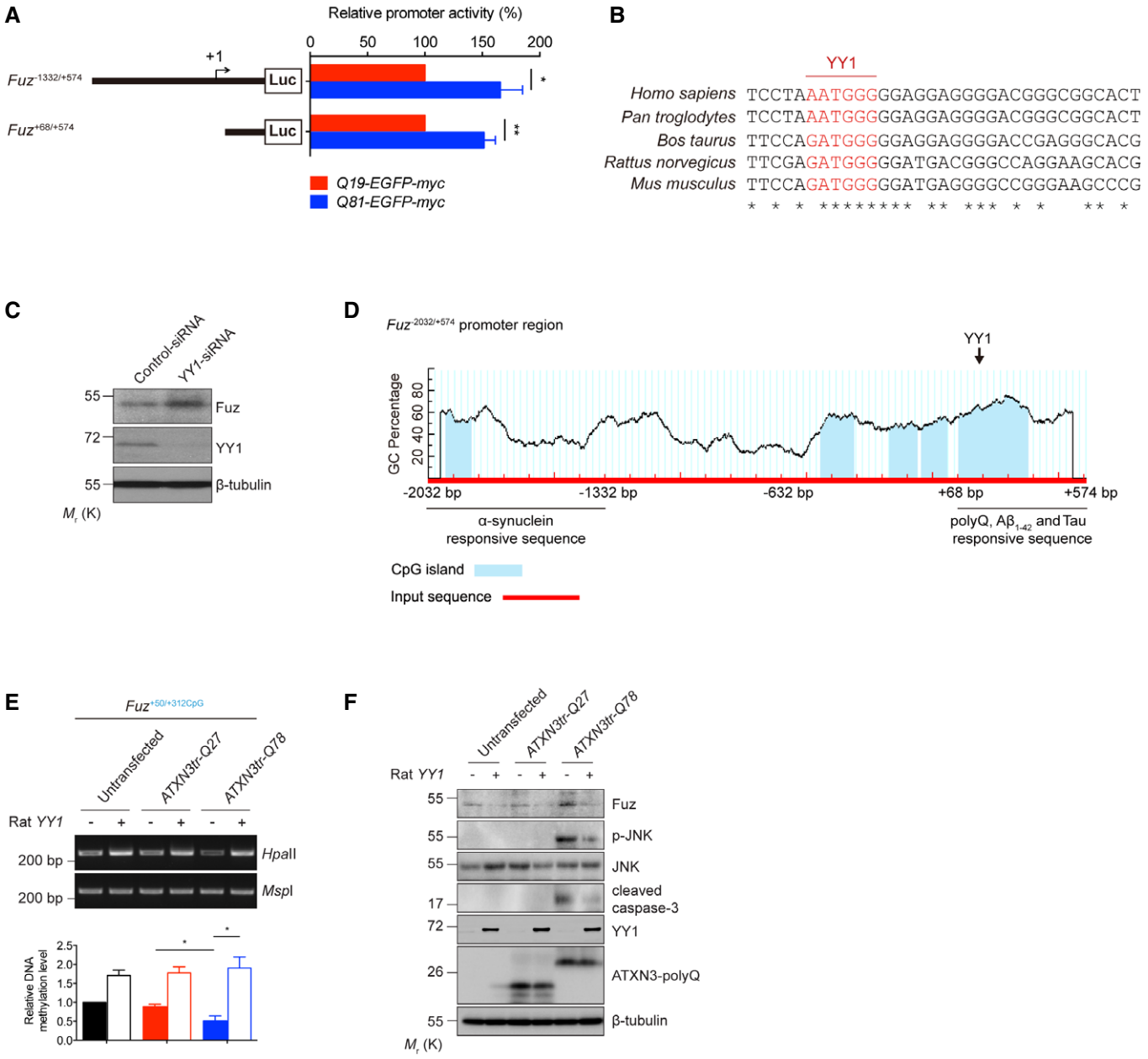

Figure EV3. Data related to Figs 4 and 5.

A Luciferase assay was performed to examine human *Fuz* promoter activity in Q19-EGFP-myc or Q81-EGFP-myc-expressing HEK293 cells. Two expanded polyQ-responsive regions, $Fuz^{-1332/+574}$ and $Fuz^{+68/+574}$, were identified. Error bars represent s.e.m., $n = 5$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$.

B Inter-species sequence comparison of the human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), cow (*Bos taurus*), rat (*Rattus norvegicus*) and mouse (*Mus musculus*) *Fuz* promoter regions. Nucleotides highlighted in red indicate putative YY1 binding sites. "*" indicates the identical nucleotides among the different species.

C Knockdown of *YY1* expression increased the protein expression of Fuz in HEK293 cells. $n = 3$.

D A diagram illustrates the location of five putative CpG islands (blue) within the human $Fuz^{-2032/+574}$ promoter region. A putative YY1 binding site was uncovered within $Fuz^{+117/+347CpG}$. This diagram also summarizes the *Fuz* promoter sequences that are responsive to the different disease proteins and peptide. $Fuz^{-2032/-1332}$ responds to α-synuclein overexpression. $Fuz^{+68/+574}$ responds to expanded polyQ overexpression, $A\beta_{1-42}$ peptide treatment and Tau overexpression.

E *Hpa*II DNA methylation assay was performed to determine the methylation status of $Fuz^{+50/+312CpG}$ in rat primary cortical neurons. The ATXN3tr-Q78-induced hypomethylation in *Fuz* promoter was rescued by rat YY1 overexpression. Lower panel shows the quantification of DNA methylation level of $Fuz^{+50/+312CpG}$ relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using one-way ANOVA followed by *post hoc* Tukey's test. $^{*}P < 0.05$.

F Overexpression of rat YY1 suppressed the ATXN3tr-Q78-mediated Fuz induction, JNK phosphorylation and caspase-3 cleavage in rat primary cortical neurons. $n = 3$.

Data information: Beta-tubulin was used as loading control. *n* represents the number of biological replicates. Only representative gels and blots are shown.
Source data are available online for this figure.

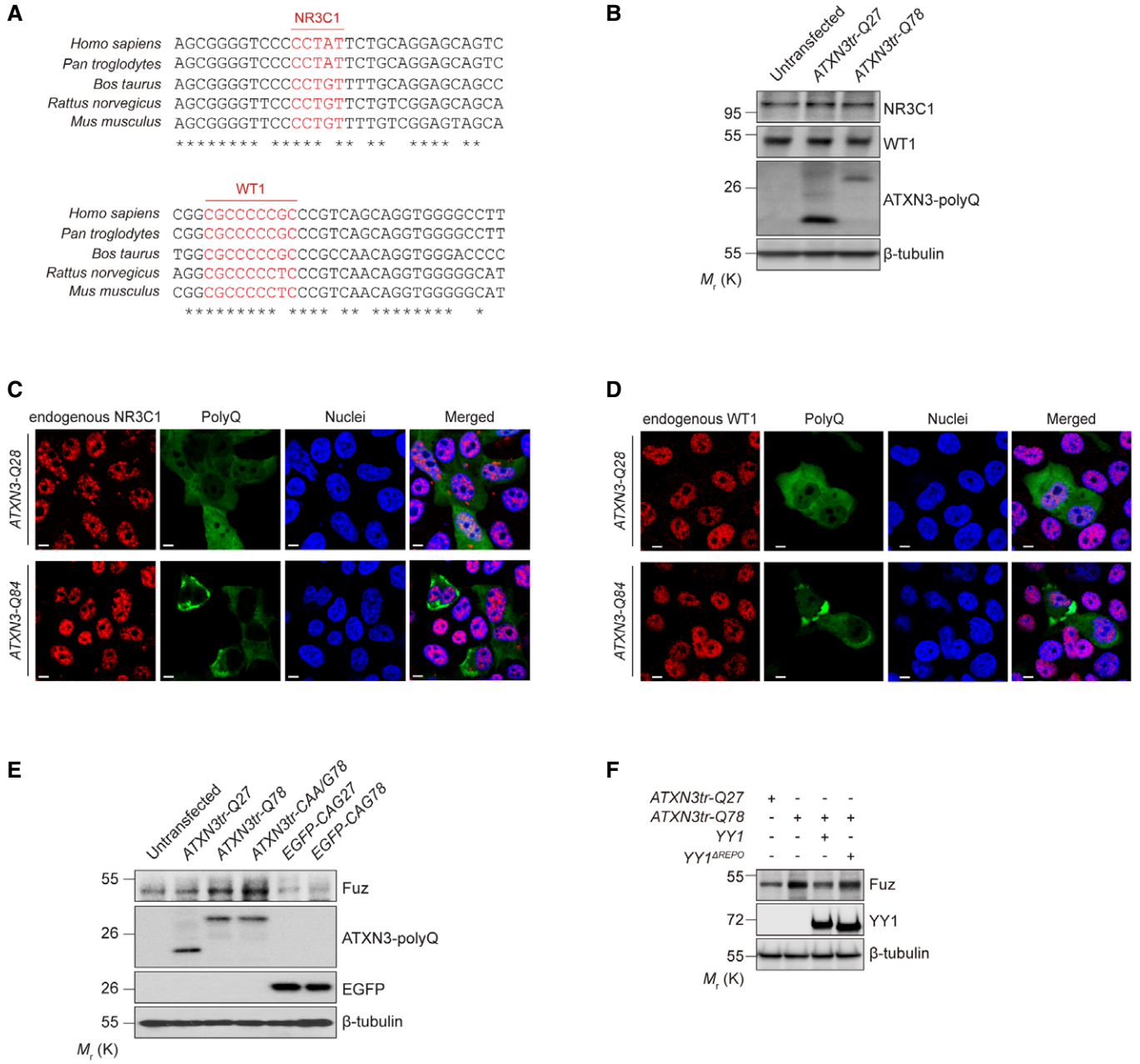

Figure EV4. Data related to Fig 6.

A Inter-species sequence comparison of the human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), cow (*Bos taurus*), rat (*Rattus norvegicus*) and mouse (*Mus musculus*) *Fuz* promoter regions. Nucleotides highlighted in red indicate putative NR3C1 and WT1 binding sites. "*" indicates the identical nucleotides among the different species.

B Endogenous soluble NR3C1 and WT1 protein levels were not altered in ATXN3tr-Q78-expressing HEK293 cells. $n = 3$.

C, D Both the NR3C1 (C) and WT1 (D) proteins showed nuclear localization in both ATXN3-Q28-and ATXN3-Q84-expressing cells. NR3C1 (C) and WT1 (D) were not detected in protein aggregate in ATXN3-Q84-expressing cells. Cell nuclei (blue) were stained with Hoechst 33342. Scale bars: 5 μm. $n = 3$.

E Overexpression of either ATXN3tr-Q78 or ATXN3tr-CAA/G78 induced the expression of Fuz protein in HEK293 cells. Such induction was not observed in cells transfected with *EGFP-CAG78* expresses only the expanded CAG transcript but not polyQ protein. $n = 3$.

F $YY1^{\Delta REPO}$ was less effective than full-length YY1 in suppressing Fuz induction in ATXN3tr-Q78-expressing cells. $n = 3$.

Data information: Beta-tubulin was used as loading control. *n* represents the number of biological replicates. Only representative images and blots are shown.
Source data are available online for this figure.

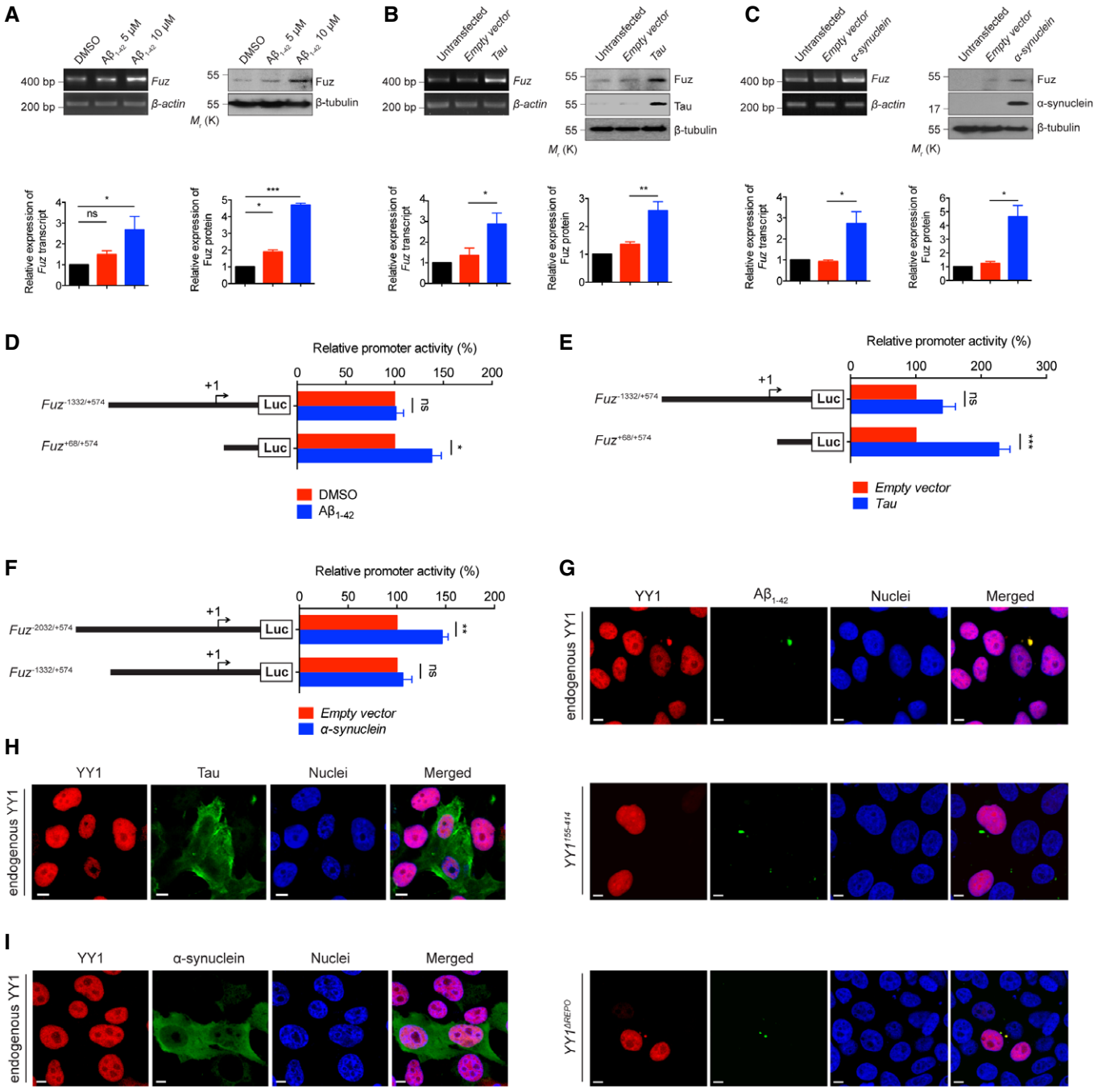

Figure EV5.

Figure EV5. Data related to Fig 7.

A HEK293 cells treated with $A\beta_{1-42}$ peptide showed induction of *Fuz*/Fuz at both mRNA and protein levels. Lower panel shows the quantification of *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using one-way ANOVA followed by *post hoc* Tukey's test. ns represents no significant difference. $^{*}P < 0.05$, $^{***}P < 0.001$.

B HEK293 cells transfected with *Tau* caused an increase in the expression levels of both *Fuz* transcript and Fuz protein. Lower panel shows the quantification of *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$. $^{**}P < 0.01$.

C The mRNA and protein expression of *Fuz*/Fuz levels were elevated in α-synuclein-expressing HEK293 cells. Lower panel shows the quantification of relative *Fuz* transcript and protein expression relative to controls. Error bars represent s.e.m., $n = 3$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. $^{*}P < 0.05$.

D Luciferase assay was performed to examine human *Fuz* promoter activity in $A\beta_{1-42}$-treated HEK293 cells. The activity of $Fuz^{+68/+574}$, but not $Fuz^{-1332/+574}$ was upregulated in $A\beta_{1-42}$-treated cells. Error bars represent s.e.m., $n = 5$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. ns represents no significant difference. $^{*}P < 0.05$.

E Luciferase assay was performed to examine human *Fuz* promoter activity in Tau-expressing HEK293 cells. Overexpression of Tau induced the transcriptional activity of $Fuz^{+68/+574}$. Error bars represent s.e.m., $n = 5$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. ns represents no significant difference. $^{***}P < 0.001$.

F Luciferase assay was performed to examine human *Fuz* promoter activity in α-synuclein-expressing HEK293 cells. The activity of $Fuz^{-2032/+574}$, but not $Fuz^{-1332/+574}$ was upregulated in α-synuclein-expressing cells. Error bars represent s.e.m., $n = 5$. Statistical analysis was performed using two-tailed unpaired Student's *t*-test. ns represents no significant difference. $^{**}P < 0.01$.

G YY1 protein was sequestered to the $A\beta_{1-42}$ aggregates (green) in HEK293 cells. Endogenous YY1 (red) was stained with anti-YY1 antibody. $YY1^{\Delta REPO}$, but not $YY1^{155-414}$ protein (red), was sequestered to the $A\beta_{1-42}$ aggregates (green) in HEK293 cells. Cell nuclei (blue) were stained with Hoechst 33342. Scale bars: 5 μm. $n = 3$.

H YY1 protein was not sequestered to the Tau aggregates (green) in HEK293 cells. Endogenous YY1 (red) was stained with anti-YY1 antibody. Cell nuclei (blue) were stained with Hoechst 33342. Scale bars: 5 μm. $n = 3$.

I The nuclear localization of YY1 was not changed in α-synuclein-expressing HEK293 cells. Endogenous YY1 (red) was stained with anti-YY1 antibody. Cell nuclei (blue) were stained with Hoechst 33342. Scale bars: 5 μm. $n = 3$.

Data information: *beta-actin* or beta-tubulin was used as loading control. *n* represents the number of biological replicates. Only representative images, gels and blots are shown.
Source data are available online for this figure.