# Expanded View Figures

Figure EV1.   TDP1/gkt mutation alleviated neurodegeneration and muscle atrophy in DM2 flies.

(A) Quantification of the average counts per day in flies across different genotypes from 15-days-old to the 25-days-old. Five biological replicates. Data are mean ± SD. Two-tailed, unpaired *t* test. (B, C) The average counts of per 30 min in flies across different genotypes from 15-days-old to the 25-days-old. Fifteen animals per sample. (C) Quantification from five biological replicates. Data are mean ± SD. Two-tailed, unpaired t-test. ****$P < 0.0001$ $(CCTG)_{720}$ versus $(CCTG)_{720} + gkt\ mut$. (D, E) Representative images of paraffin-embedded adult thoraces showing IFMs of flies at 1, 7, 15-days-old. Scale bars 100 μm. (E) Quantification at least ten independent thoraces of each sample/experiment, 3 experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. **$P = 0.084$ 1-day-old versus 7-days-old $(CCTG)_{720}$, ***$P = 0.00042$ 7-days-old versus 15-days-old $(CCTG)_{720}$. (F–H) Representative images of paraffin-embedded adult thoraces showing IFMs of flies at 15-days-old. Black arrows, holes and fragmentations. Scale bars 100 μm. (G, H) Quantification at least ten independent thoraces of each sample/experiment, three experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. ****$P < 0.0001$ $(CCTG)_{16}$ versus $(CCTG)_{720}$, ****$P < 0.0001$ $(CCTG)_{720}$ versus $(CCTG)_{720} + gkt\ mut$. (I) Quantification of climbing index across different genotypes at 15-days-old from five biological replicates. Data are mean ± SD. Two-tailed, unpaired *t* test. ****$P < 0.0001$ $(CCTG)_{16}$ versus $(CCTG)_{720}$, ****$P < 0.0001$ $(CCTG)_{720}$ versus $(CCTG)_{720} + gkt\ RI\ 1$, ****$P < 0.0001$ $(CCTG)_{720}$ versus $(CCTG)_{720} + gkt\ RI\ 2$. (J, K) Representative images of the compound eyes across different genotypes at 15-days-old. Top, LM images. Middle and Bottom, SEM images. Scale bars 20 μm. (K) Quantification at least sixty independent eyes of each genotype/experiment, three experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. ****$P < 0.0001$.
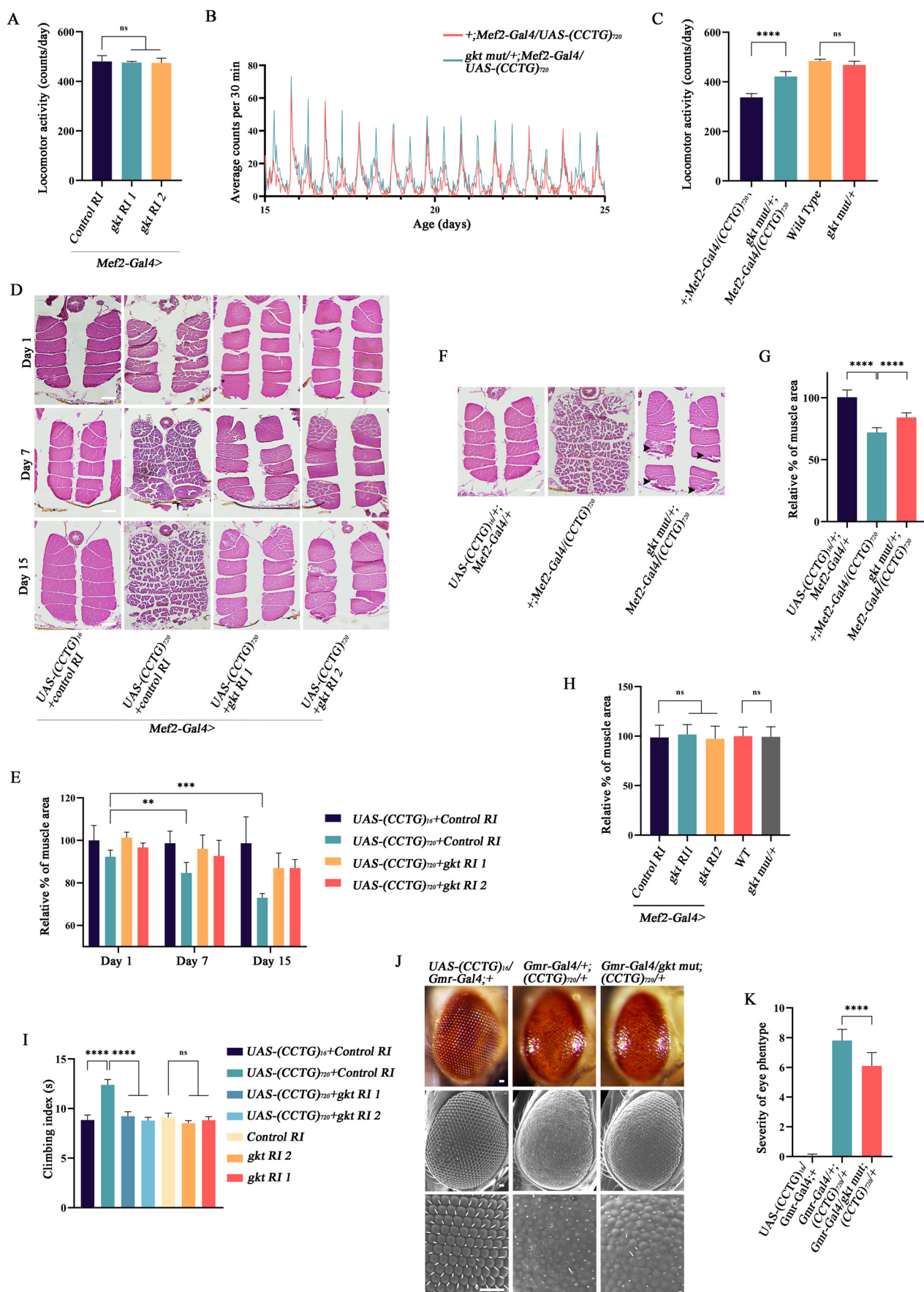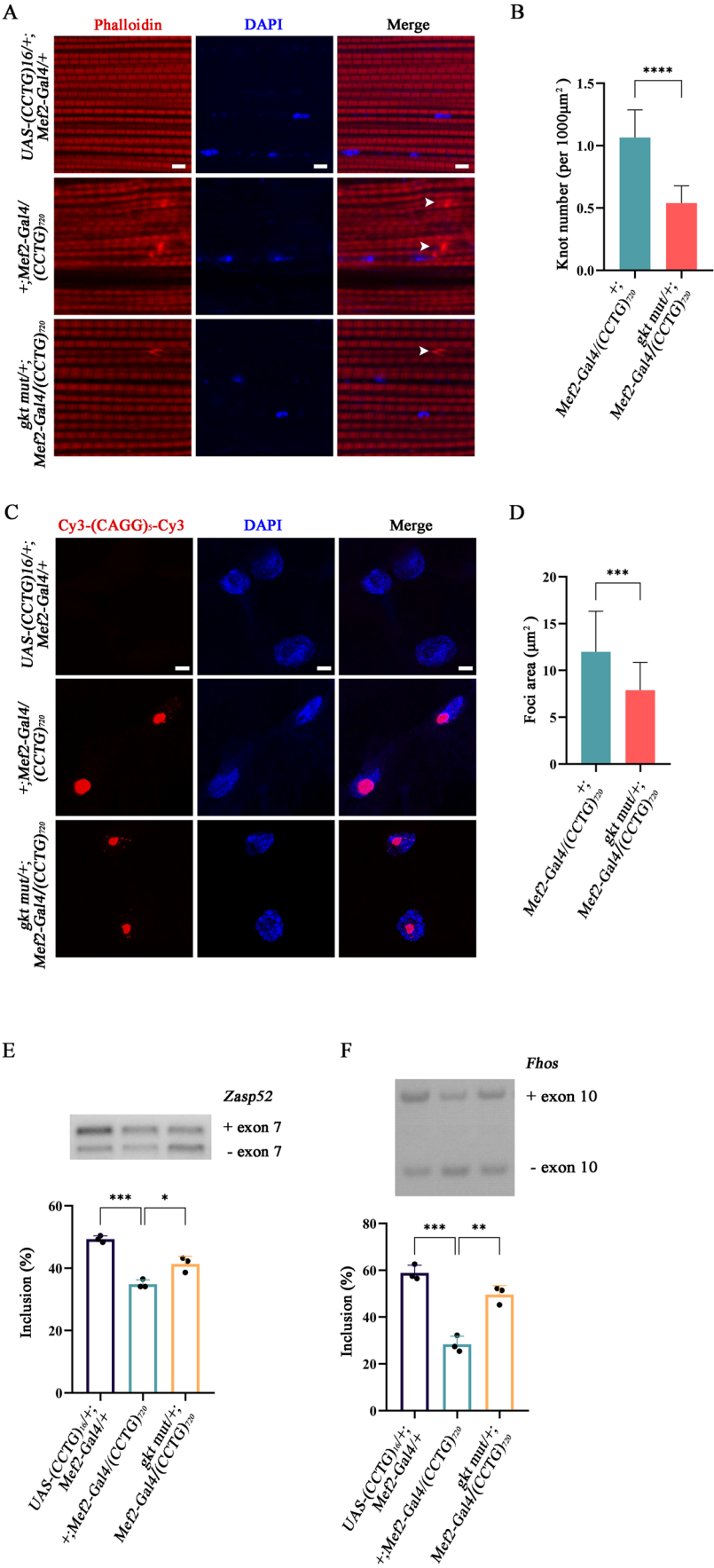
◄ **Figure EV2. TDP1/gkt mutation rescues molecular pathology of DM2.**

(A, B) Representative IF images of thoracic muscle fibers across different genotypes at 15-days-old. White arrows, action blobs. Scale bars 5 μm. (B) Quantification at least ten animals of each genotype. Data are mean ± SD. Two-tailed, unpaired *t* test. ****$P <$ 0.0001. (C, D) Representative Confocal images of CCUG-repeat foci with $(CAGG)_5$ probe across different genotypes at body-wall muscles of third instar larvae. Scale bars 5 μm. (D) Quantification at least ten animals of each genotype/experiment, three experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. ***$P =$ 0.0003. (E) Representative gel image of endogenous *Zasp52* exon 7 with RT-PCR in flies at 15-days-old. Tissue from ten animals per sample/experiments, 5 experiments. Lower panel, quantification of *Zasp52* exon 7 inclusion from three biological replicates. Data are mean ± SD. Two-tailed, unpaired *t* test. *$P =$ 0.0162, ***$P =$ 0.0001. (F) Representative gel image of endogenous *Fhos* exon 10 with RT-PCR in flies at 15-days-old. Tissue from ten animals per sample/experiment, 5 experiments. Lower panel, quantification of *Fhos* exon 10 inclusion from three biological replicates. Data are mean ± SD. Two-tailed, unpaired *t* test. **$P =$ 0.021,***$P =$ 0.0004.
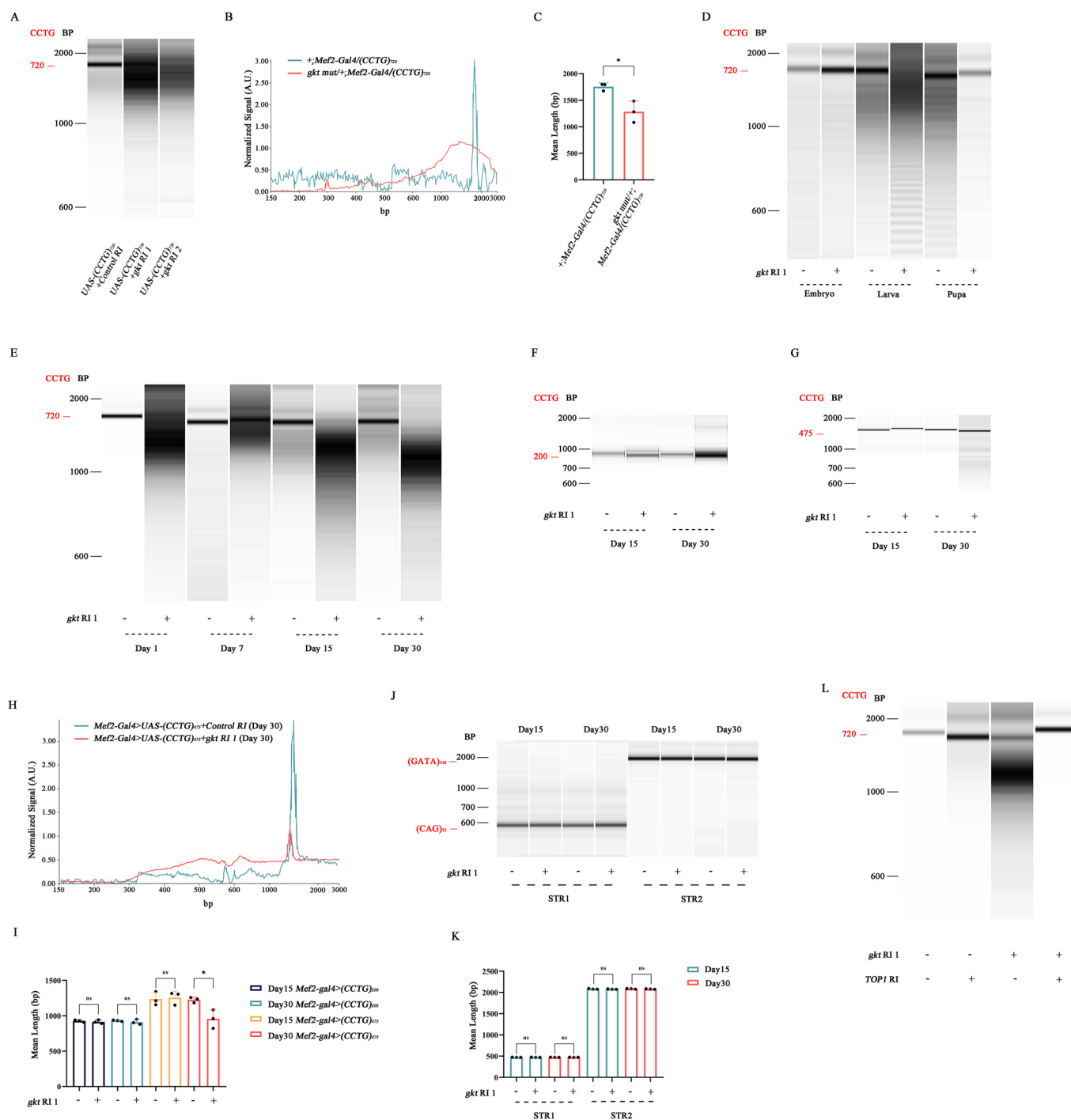
Figure EV3. TDP1/gkt loss of function induce large CCTG repeats contraction but not affect stability of endogenous STR loci.
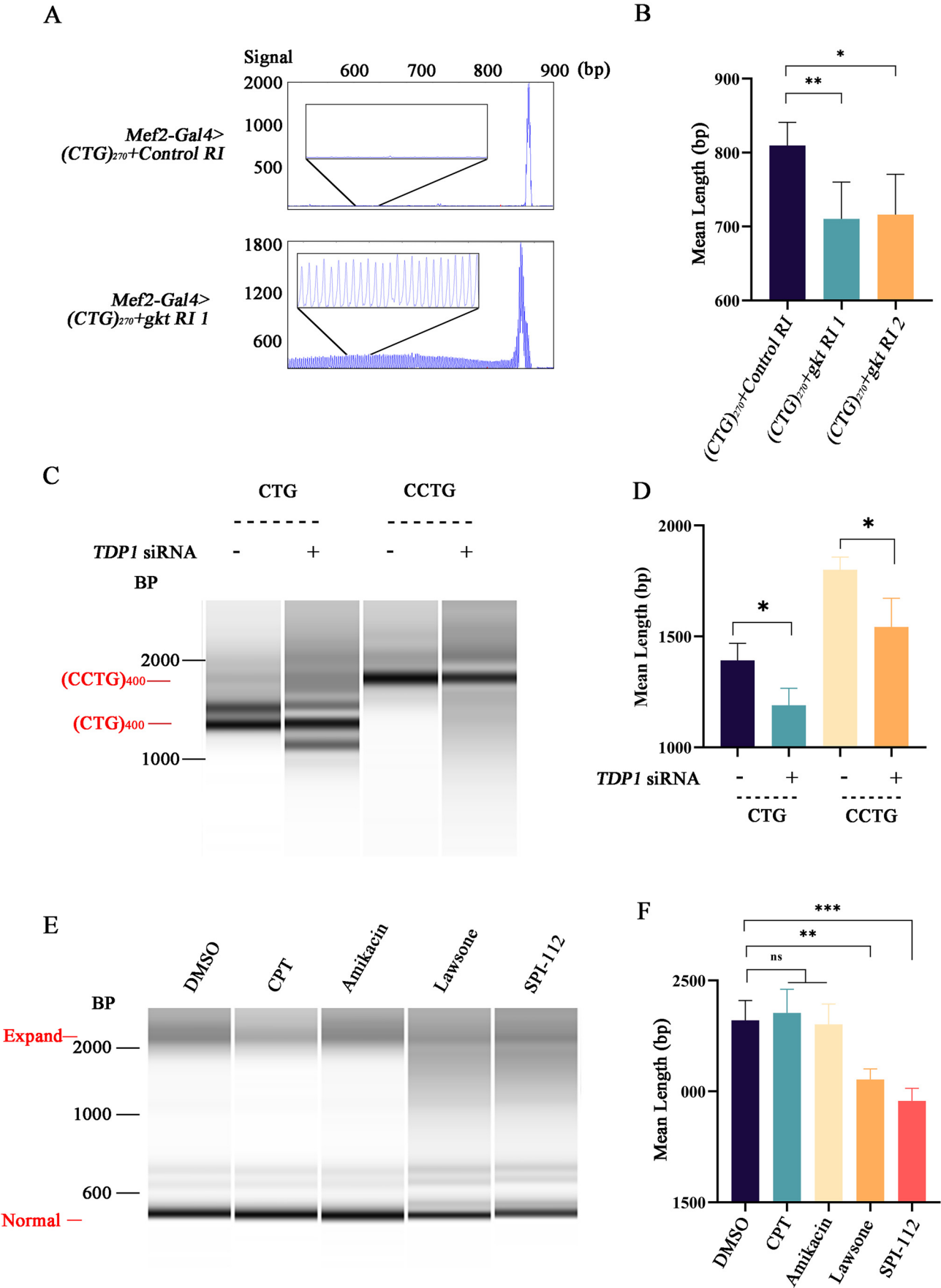
(A) Representative gel image of PCR across different genotypes at 15-days-old from bioanalyzer. Tissue from three animals per sample. (B, C) Bioanalyzer quantitation of CCTG repeat lengths across different genotypes at 15-days-old. Tissue from three animals per sample. (B) Quantification of mean length of CCTG repeats from three biological replicates. Data are mean ± SD. Two-tailed, unpaired t test. *P = 0.0182. (D) Representative gel image of PCR across different genotypes at embryo, larva, and pupa. Embryo from ten animals per sample. Larva and pupa from five animals per sample. (E) Representative gel image of PCR across *Mef2-Gal4 > UAS-(CCTG)_{720} + Control RI* and *Mef2-Gal4 > UAS-(CCTG)_{720} + gkt RI 1* at 1, 7, 15, 30-days-old. Tissue from three animals per sample. (F) Representative gel image of PCR across *Mef2-Gal4 > UAS-(CCTG)_{200} + Control RI* and *Mef2-Gal4 > UAS-(CCTG)_{200} + gkt RI 1* at 15-days-old and 30-days-old. Tissue from three animals per sample. (G) Representative gel image of PCR across *Mef2-Gal4 > UAS-(CCTG)_{475} + Control RI* and *Mef2-Gal4 > UAS-(CCTG)_{475} + gkt RI 1* at 15-days-old and 30-days-old. Tissue from three animals per sample. (H) Bioanalyzer quantitation of CCTG repeat lengths across *Mef2-Gal4 > UAS-(CCTG)_{475} + Control RI* and *Mef2-Gal4 > UAS-(CCTG)_{475} + gkt RI 1* at 30-days-old. Tissue from three animals per sample. (I) Quantification of mean length of CCTG repeats from three biological replicates. Data are mean ± SD. Two-tailed, unpaired t test. *P = 0.0263. (J, K) Representative gel image of STR1 and STR2 PCR across *Mef2-Gal4 > UAS-(CCTG)_{720} + Control RI* and *Mef2-Gal4 > UAS-(CCTG)_{720} + gkt RI 1* at 15-days-old and 30-days-old. Tissue from three animals per sample. (K) Quantification of mean length of GATA and CAG repeats from three biological replicates. Two-tailed, unpaired t test. (L) Representative gel image of across different genotype at 15-days-old. Tissue from three animals per sample.
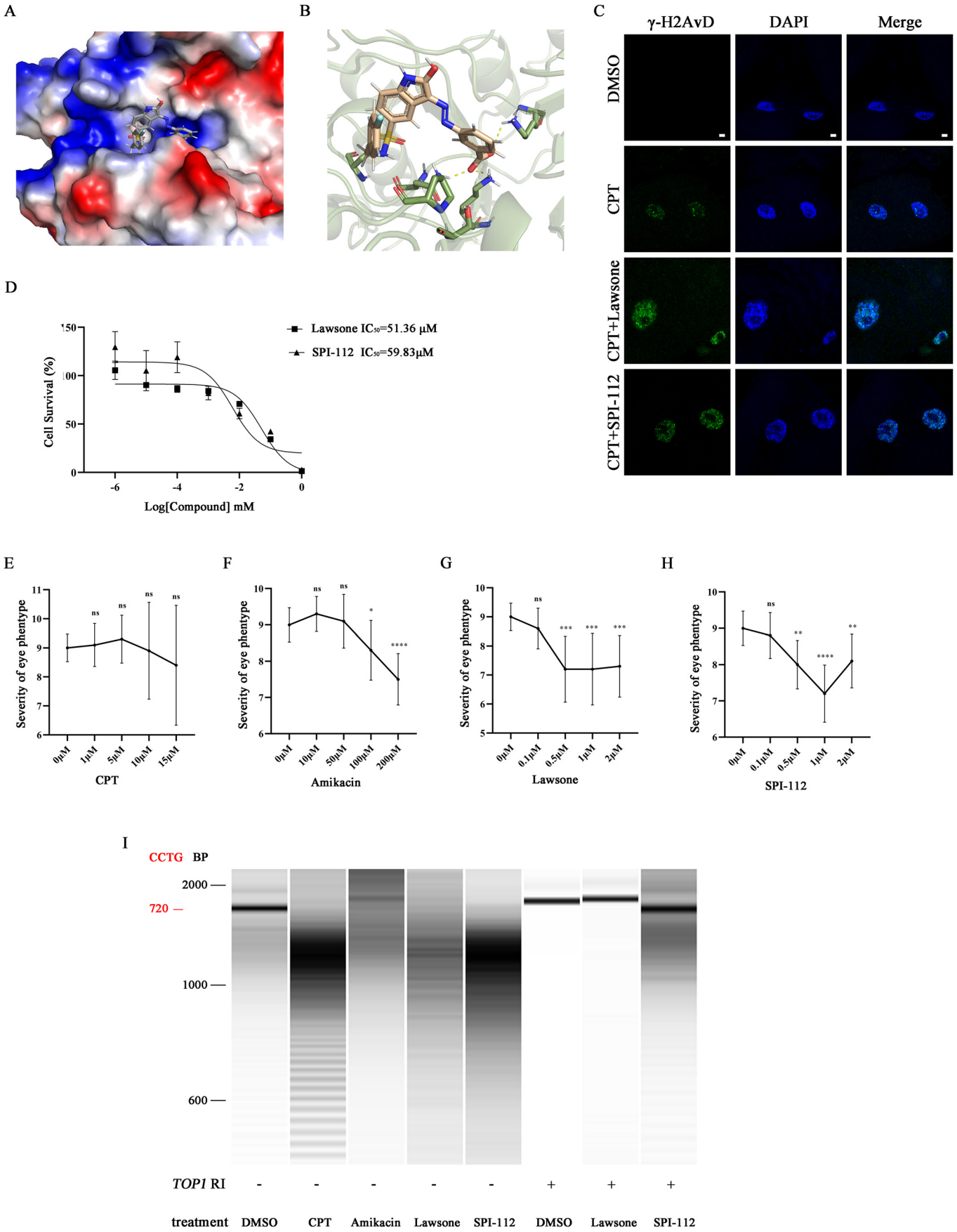
◀ **Figure EV4. Small molecules predicted to bind TDP1 and cytotoxicity.**

(A) Surface electrostatic potentials of predicted crystal structure of human TDP1/SPI-112 complex. Red depicts a positive partial charge on the surface, blue depicts negative partial charge and gray shows neutral/lipophilic areas. (B) Docking model of Lawsone bind to TDP1 and SPI-112. Dotted yellow line: hydrogen bond, Dotted green line: Pi bond. Blue: N atom, Red: O atom, White: H atom, Green: protein skeleton, Gold: compound skeleton. (C) Representative IF images of body-wall muscles of third instar larvae from WT flies. Scale bars 5 μm. (D) Dose-survival analysis of Lawsone and SPI-112. The HEK293 cells were treated with purified TDP1 enzyme 0, 0.001, 0.01, 0.1, 1, 10, 100, 1000 μM for 72 h. Data are mean ± SD from three biological replicates. IC_{50} values were calculated using log(inhibitor) compared with normalized survival rate (variable slope), using the program Prism, and are given in graphs. (E) Quantitative analysis of compound eye phenotypic severity of *Gmr-Gal4 > UAS-(CCTG)$_{720}$* after treatment with 0, 1, 5, 10, 15 μM CPT, at least twenty independent eyes of each sample/experiment, 3 experiments. Flies treated with 20 μM CPT were lethal. Data are mean ± SD. Two-tailed, unpaired *t* test. (F) Quantitative analysis of compound eye phenotypic severity of *Gmr-Gal4 > UAS-(CCTG)$_{720}$* after treatment with 0, 10, 50, 100, 200 μM Amikacin, at least twenty independent eyes of each sample/experiment, 3 experiments. Flies treated with 400 μM Amikacin were lethal. Data are mean ± SD. Two-tailed, unpaired *t* test. *$P = 0.0314$, ****$P < 0.0001$. (G) Quantitative analysis of compound eye phenotypic severity of *Gmr-Gal4 > UAS-(CCTG)$_{720}$* after treatment with 0, 0.1, 0.5, 1, 2 μM Lawsone, at least twenty independent eyes of each sample/experiment, 3 experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. ***$P = 0.0002$ 0 μM versus 0.5 μM, ***$P = 0.0004$ 0 μM versus 1 μM, ***$P = 0.0002$ 0 μM versus 2 μM. (H) Quantitative analysis of compound eye phenotypic severity of *Gmr-Gal4 > UAS-(CCTG)$_{720}$* after treatment with 0, 0.1, 0.5, 1, 2 μM SPI-112, at least twenty independent eyes of each sample/experiment, 3 experiments. Data are mean ± SD. Two-tailed, unpaired *t* test. **$P = 0.0011$ 0 μM versus 0.5 μM, ****$P < 0.0001$ 0 μM versus 1 μM, **$P = 0.0044$ 0 μM versus 2 μM. (I) Representative gel image of across different genotype with treatment of different compounds at 15-days-old. Tissue from three animals per sample.

A

B

C

D

E

F

◀ **Figure EV5.   TDP1/gkt inhibition induce CTG/CCTG repeats contraction in other repeat expansion models.**

(A, B) Representative capillary electropherograms across different genotypes at 60-days-old. Tissue from three animals. (B) Quantification of mean length of CTG repeats from five biological replicates. Data are mean ± SD. Two-tailed, unpaired t test. *P = 0. 0103, **P = 0. 0054. (C, D) Representative gel image of PCR across different genotypes Hek293 cell line following 7 days of treatment from bioanalyzer. (D) Quantification of mean length of CTG/CCTG repeats from three biological replicates. Data are mean ± SD. Two-tailed, unpaired t test. *P = 0.0324 (CTG)$_{400}$ cell line, *P = 0.0341 (CCTG)$_{400}$ cell line. (E, F) Representative gel image of DM1 patient-derived lymphocytes following 15 days treatment of different compounds from bioanalyzer. (F) Quantification of (E) from three biological replicates. Data are mean ± SD. One-way ANOVA. **P = 0.0082 DMSO versus Lawsone, ***P = 0.001 DMSO versus SPI-112.