GO biological process	-log10 (*P*-value)	Gene name
heat shock-mediated polytene chromosome puffing	7.10	*Heat shock protein 70Aa* (*Hsp70Aa*) *Heat shock protein 70Ab* (*Hsp70Ab*) *Heat shock protein 70Ba* (*Hsp70Ba*) *Heat shock protein 70Bb* (*Hsp70Bb*) *Heat shock protein 67Bc* (*Hsp67Bc*)
defense response to insect	7.50	*Attacin A* (*AttA*) *Cecropin A2* (*CecA2*) *Cecropin A1* (*CecA1*) *Drosocin* (*Dro*) *Attacin B* (*AttB*)
protein refolding	12.67	*Heat shock protein 70Aa* (*Hsp70Aa*) *Heat shock protein 27* (*Hsp27*) *Heat shock protein 70Ab* (*Hsp70Ab*) *Heat shock protein 26* (*Hsp26*) *Heat shock protein 70Ba* (*Hsp70Ba*) *Heat shock protein 70Bb* (*Hsp70Bb*) *lethal(2)essential for life (l(2)efl)* *Heat shock protein 70Bc* (*Hsp70Bc*) *Heat shock protein 23* (*Hsp23*) *Heat shock protein 68 (Hsp68)*
double-strand break repair via nonhomologous end joining	3.90	*Ku80* *DNA ligase 3* (*DNAlig3*) *CG3448*
antibacterial humoral response	8.77	*Diptericin A* (*DptA*) *Attacin A* (*AttA*) *Cecropin A2* (*CecA2*) *Cecropin A1* (*CecA1*) *Metchnikowin* (*Mtk*) *Attacin C* (*AttC*) *Drosocin* (*Dro*) *Attacin B* (*AttB*)

Figure S1. GO enrichment analysis of the differentially expressed genes in the poly(GR) dataset. (Related to Figure 1)

Table showing top 5 enriched clusters and individual genes. GO terms corresponding to biological process (GOTERM_BP_DIRECT) were extracted.

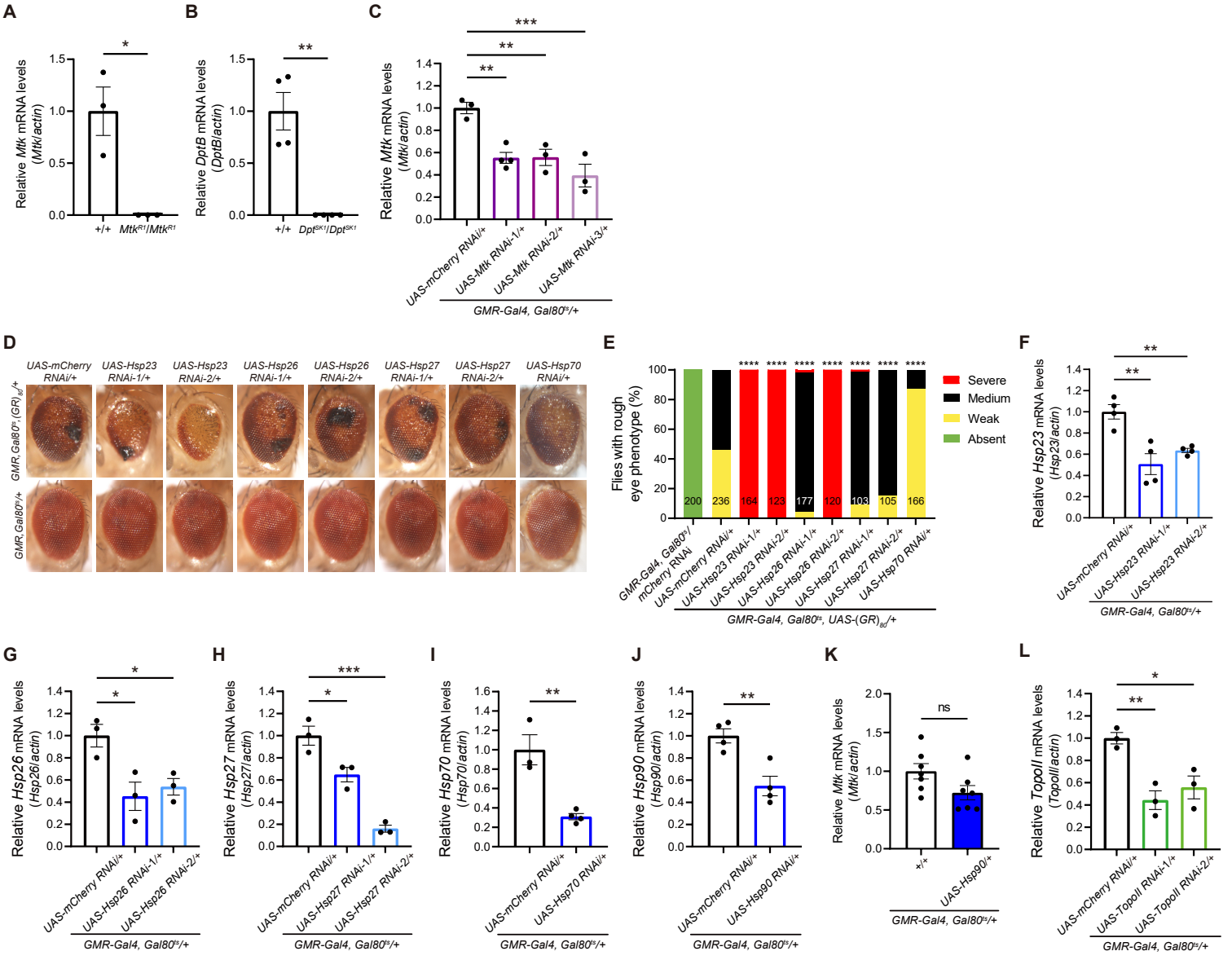
A
B
C
Relative Mtk mRNA levels (Mtk/actin)
Relative DptB mRNA levels (DptB/actin)
+/+ MtkR1/MtkR1
+/+ DptSK1/DptSK1
UAS-mCherry RNAi/+
UAS-Mtk RNAi-1/+
UAS-Mtk RNAi-2/+
UAS-Mtk RNAi-3/+
GMR-Gal4, Gal80ts/+
D
UAS-mCherry RNAi/+
UAS-Hsp23 RNAi-1/+
UAS-Hsp23 RNAi-2/+
UAS-Hsp26 RNAi-1/+
UAS-Hsp26 RNAi-2/+
UAS-Hsp27 RNAi-1/+
UAS-Hsp27 RNAi-2/+
UAS-Hsp70 RNAi/+
GMR,Gal80ts,(GR)sd/+
GMR,Gal80ts/+
E
Flies with rough eye phenotype (%)
Severe
Medium
Weak
Absent
200
236
164
123
177
120
103
105
166
GMR-Gal4, Gal80ts, UAS-(GR)sd/+
F
Relative Hsp23 mRNA levels (Hsp23/actin)
G
Relative Hsp26 mRNA levels (Hsp26/actin)
H
Relative Hsp27 mRNA levels (Hsp27/actin)
I
Relative Hsp70 mRNA levels (Hsp70/actin)
J
Relative Hsp90 mRNA levels (Hsp90/actin)
K
Relative Mtk mRNA levels (Mtk/actin)
ns
UAS-Hsp90/+
L
Relative TopoII mRNA levels (TopoII/actin)
UAS-TopoII RNAi-1/+
UAS-TopoII RNAi-2/+

Figure S2. Suppression of heat shock proteins exacerbates degenerative eye phenotypes in poly(GR)-expressing flies (Related to Figure 2)

(A and B) Loss-of-function of *Mtk* and *DptB* mutant alleles is validated by real-time quantitative PCR (RT-qPCR). One-way ANOVA with Tukey post-hoc test for multiple comparisons and two-tailed Student-*t* test for two groups.

(C) Knockdown efficiency of *Mtk* RNAi lines is confirmed by RT-qPCR. One-way ANOVA with Tukey post-hoc test for multiple comparisons and two-tailed Student-*t* test for two groups.

(D) Representative adult external eye phenotypes in flies of different genotypes showing enhancement of poly(GR) toxicity by reduction of activity of Hsp23, Hsp26, Hsp27; conversely, Hsp70 reduction in activity showed reduced poly(GR) toxicity.

(E) Quantification of the knockdown effects of Hsp23, Hsp26, Hsp27 and Hsp70 on the eye degeneration phenotype caused by poly(GR). The number of flies of each genotype is presented in each column. The *P* value was determined by chi-square test.

(F-J) Knockdown efficiency of Hsp23, Hsp26, Hsp27, Hsp70, and Hsp90 RNA lines were determined by RT-qPCR. One-way ANOVA with Tukey post-hoc test for multiple comparisons and two-tailed Student-t test for two groups.

(K) Mtk mRNA expression levels were in Hsp90 overexpression flies. Two-tailed Student-t test.

(L) Knockdown efficiency of TopoII RNAi lines were confirmed by RT-qPCR. One-way ANOVA.

with Tukey post-hoc test for multiple comparisons and two-tailed Student-t test for two groups

All data values are mean ± s.e.m. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

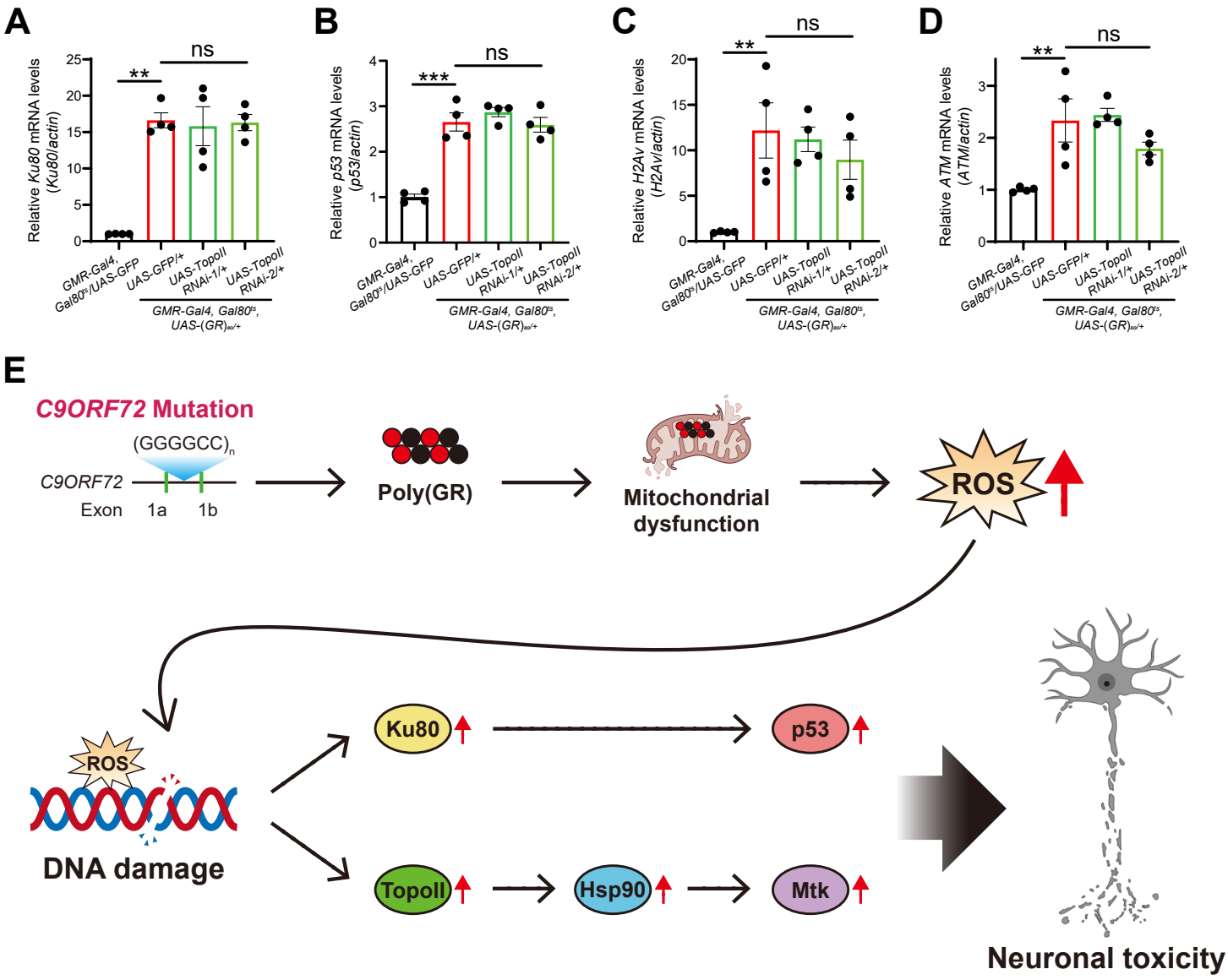

Figure S3. The suppressor effect of TopoII in poly(GR)-expressing flies is mediated by a pathway parallel to the DNA damage repair pathway (Related to Figure 3)

(A-D) Relative levels of the DNA damage repair pathway genes *Ku80* (A), *p53* (B), *H2Av* (C), and *ATM* (D) determined by RT-qPCR. All data values are mean ± s.e.m. of four independent experiments. One-way ANOVA with Tukey post-hoc test for multiple comparisons.

(E) Schematic of the role of TopoII-Hsp90-Mtk axis in *C9ORF72*-ALS/FTD. The TopoII-Hsp90-Mtk axis is a novel pathway parallel to Ku80-p53-Caspase 3 pathway in *C9ORF72-ALS*/FTD pathogenesis.

All data values are mean ± s.e.m. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

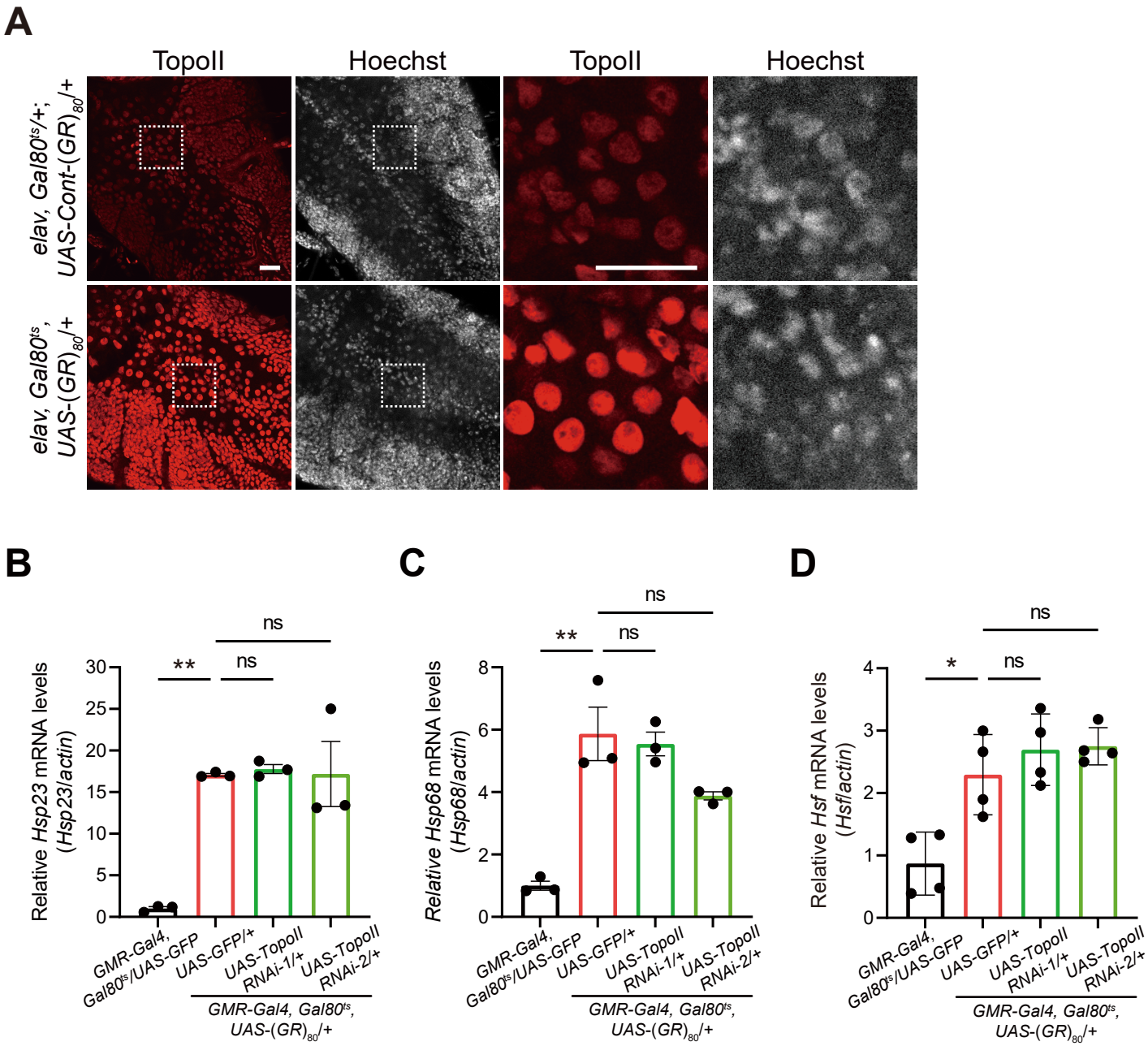

Figure S4. Poly(GR) Increases TopoII expression and TopoII doesn't affect mRNA levels of *Hsp23*, *Hsp68* and *Hsf* in *Drosophila* (Related to Figure 4)

(A) Confocal images of third instar larval ventral nerve cords stained with anti-TopoII antibody (red) and Hoechst (white) showing that poly(GR) expression increases TopoII level in the *Drosophila* brain. Right panel shows expanded view of dotted white box in the left panel. More than five larval brains from each group of the indicated genotype were examined. Scale bar: 25 μm.

(B-D) Relative levels of the Hsp genes Hsp23 (B), Hsp68 (C) and Hsf (D) determined by RT-qPCR. All data values are mean ± s.e.m. of three independent experiments, one-way ANOVA with Tukey post-hoc test for multiple comparisons. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns, not significant.