Supplemental Table 1.

	N	Lifespan$_{Mean \pm SEM}$[1]	p vs. Gal4[2]	Hazard Ratio[2]
Control	147	47.1±0.7	n/a	n/a
TDP43^{L1}	152	25.1 ±0.4	***	45.7
TDP43^{L2}	141	22.8 ±0.4	***	87.5
UBQLN^{L1}	142	50.7 ±0.8	***	0.6
UBQLN^{L2}	139	48.1 ±0.7	n.s.	0.9
UBQLN^{RNAi}	145	43.2 ±0.9	***	1.5

			p vs.[2]		Hazard Ratio[2]		
	N	Lifespan$_{Mean \pm SEM}$[1]	TDP	UBQLN	Expected[3]	Actual	
TDP43^{L1}/UBQLN^{L1}	144	25.0 ±0.6	n.s.	***	26.7	40.3	‡[4]
TDP43^{L1}/UBQLN^{RNAi}	148	22.0 ±0.5	***	***	67.9	77.3	n.s[4]
TDP43^{L2}/UBQLN^{L2}	143	17.8 ±0.5	***	***	77.0	200.5	‡[4]

1. Mean lifespan and standard error of the mean (SEM) were calculated directly from tabulated survival data, and are measured in days. 2. Hazard ratios and *p* values (derived from chi square tests) were calculated from a Cox regression (see Materials and Methods). ***, $p<0.001$. 3. Expected hazard ratios for double transgenic lines were calculated by multiplying the ratios for the individual component lines. 4. The expected hazard ratio was considered significantly different from the actual hazard ratio (denoted by ‡) if the expected value was outside the 95% confidence interval for the value determined by Cox regression.

Supplemental Figure Legends

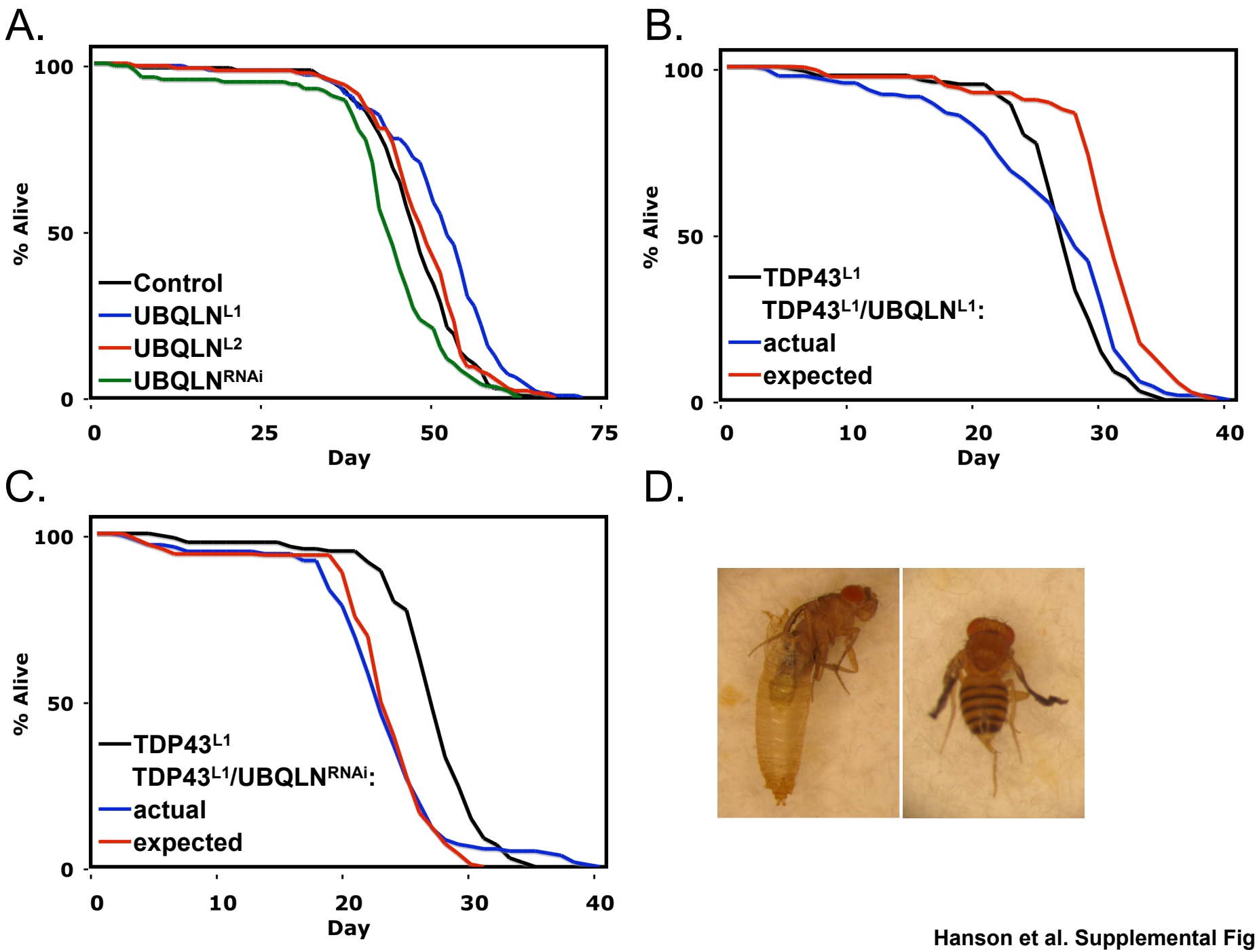
Supplemental Figure 1. Survival curves of flies expressing different combinations of TDP-43 and UBQLN transgenes. A) Survival curves of D42-Gal4 control, D42-UBQLN^{L1}, D42-UBQLN^{L2}, and D42-UBQLN^{RNAi} flies. B) Actual and expected survival curves of D42-TDP43^{L1}/UBQLN^{L1} flies, compared to that for D42-TDP43^{L1} flies. C) Actual and expected survival curves of D42-TDP43^{L1}/UBQLN^{RNAi} flies, compared to that for D42-TDP43^{L1} flies. Expected survival curves were generated by taking the difference between the Gal4 control and UBQLN curves at each coordinate and applying that difference to the TDP43^{L1} curve. See Supplemental Table 1 for statistical analysis. D) Left: Pupal eclosion defect quantified in Fig. 2C. This fly died during the process of eclosion, and part of the body remains stuck in the pupal case. Right: Wing malformation phenotype quantified in Fig. 2D.

Supplemental Figure 2. TDP-43 toxicity in HeLa cells and coaggreation with UBQLN in S2 cells. A) Toxicity of TDP-43 in HeLa cells. HeLa cells were transfected with the indicated combinations of HA-TDP-43, Myc-UBQLN, and Myc-UBA, and 48 hours later cell viability was measured by MTT assay. Data shown are the average of three experiments each normalized to the vector only condition. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. p values were calculated using student's t-test. B) Localization of TDP-43 and UBQLN in Drosophila S2 cells. S2 cells were transfected with UAS-TDP-43 and/or UAS-UBQLN plasmids, along with the PMT-Gal4 vector. Cells were immunostained using α-TDP-43 and α-UBQLN antibodies 48 hours after induction. C) Western blot analysis of TDP-43 and UBQLN in S2 cells. Lysates from S2 cells were transfected as in (B) with UAS-UBQLN (Lane 1), UAS-TDP-43 (Lane 2), or both (Lane 3). The lower amount of all three species in Lane 3 is probably due to the reduced transfection efficiency using 3 as compared to 2 plasmids.

Supplemental Experimental Procedures

HeLa cell culture and MTT assay—HeLa cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. For MTT assay, Fugene 6 Transfection Reagent (Roche) was used to transfect HeLa cells with pCMV-HA-TDP-43, pCMV-Myc-UBQLN, and/or pCMV-Myc-UBA in a 96 well plate. Forty-eight hours later, cell viability was detected by the MTT assay (Sigma) using the manufacturer's conditions. The absorbance at 595 nm was measured using an ELISA plate reader. Data were then normalized to the vector-only control for each experiment.

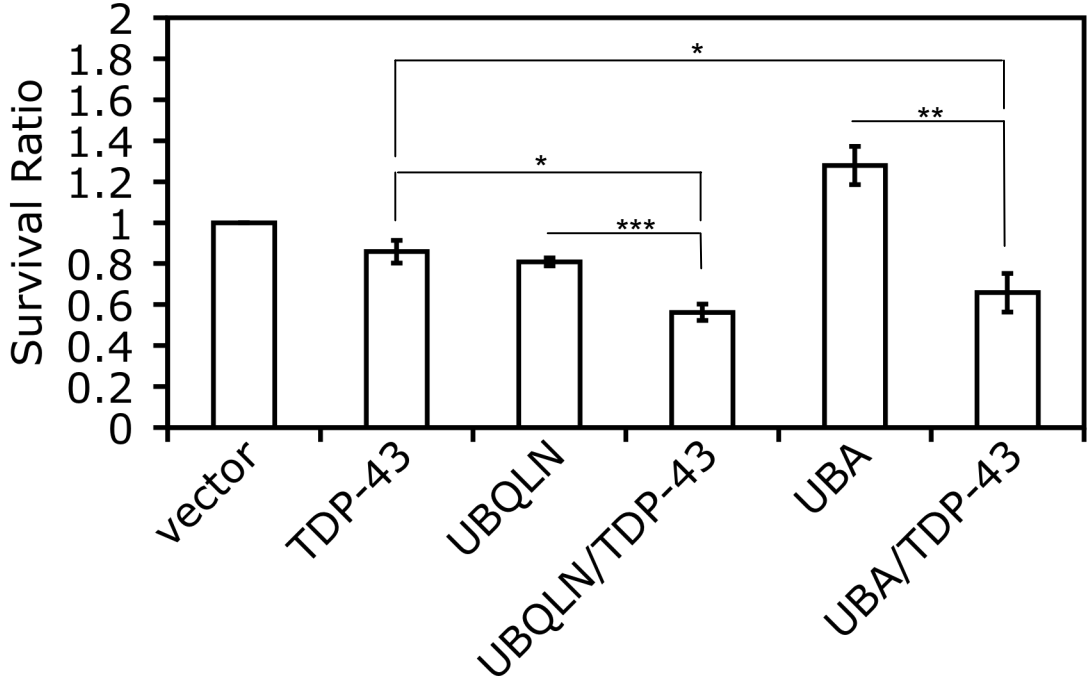
S2 cell culture and microscopy—S2 cells were maintained in Schneider's S2 Cell Medium (Gibco) with 10% heat-inactivated fetal bovine serum (FBS). For S2 immunocytochemistry, Effectene Transfection Reagent (Qiagen) was used to transfect cells with pUAST-TDP-43 and/or pUAST-UBQLN and the copper-sensitive PMT-Gal4 vector. One day after transfection, cells were treated with 500 μM copper sulfate to induce protein expression. Two days later cells were fixed and immunostained using standard methods. Antibody concentrations used were: α-TDP-43 (1:500), α-UBQLN (1:250), α-mouse-Alexa 568 (1:500), and α-rabbit-Alexa 488 (1:500).



Hanson et al. Supplemental Fig. 1

Hanson et al. Supplemental Fig. 2

A.



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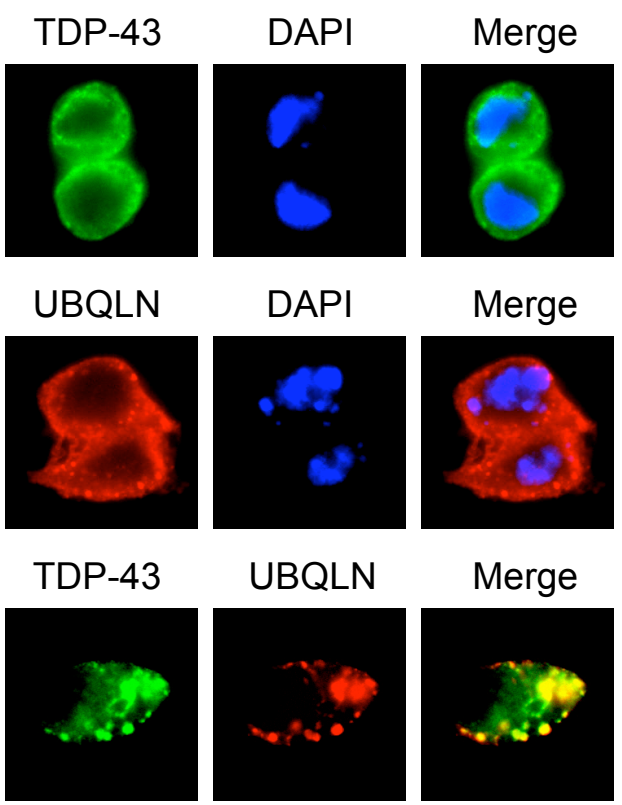

C.