Supplementary Material

A Novel Caenorhabditis Elegans Proteinopathy Model Shows Changes in mRNA Translational Frameshifting During Aging

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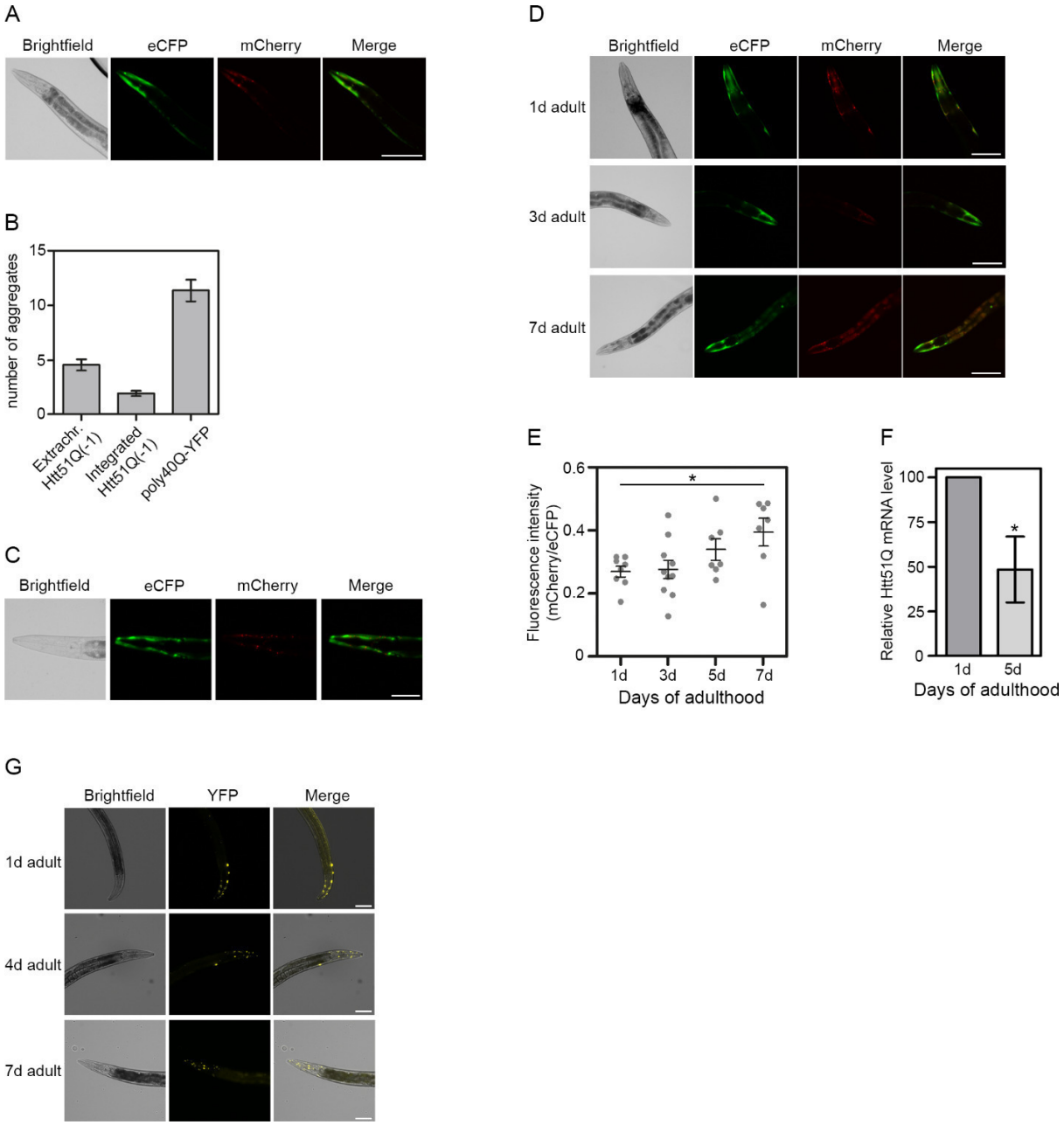
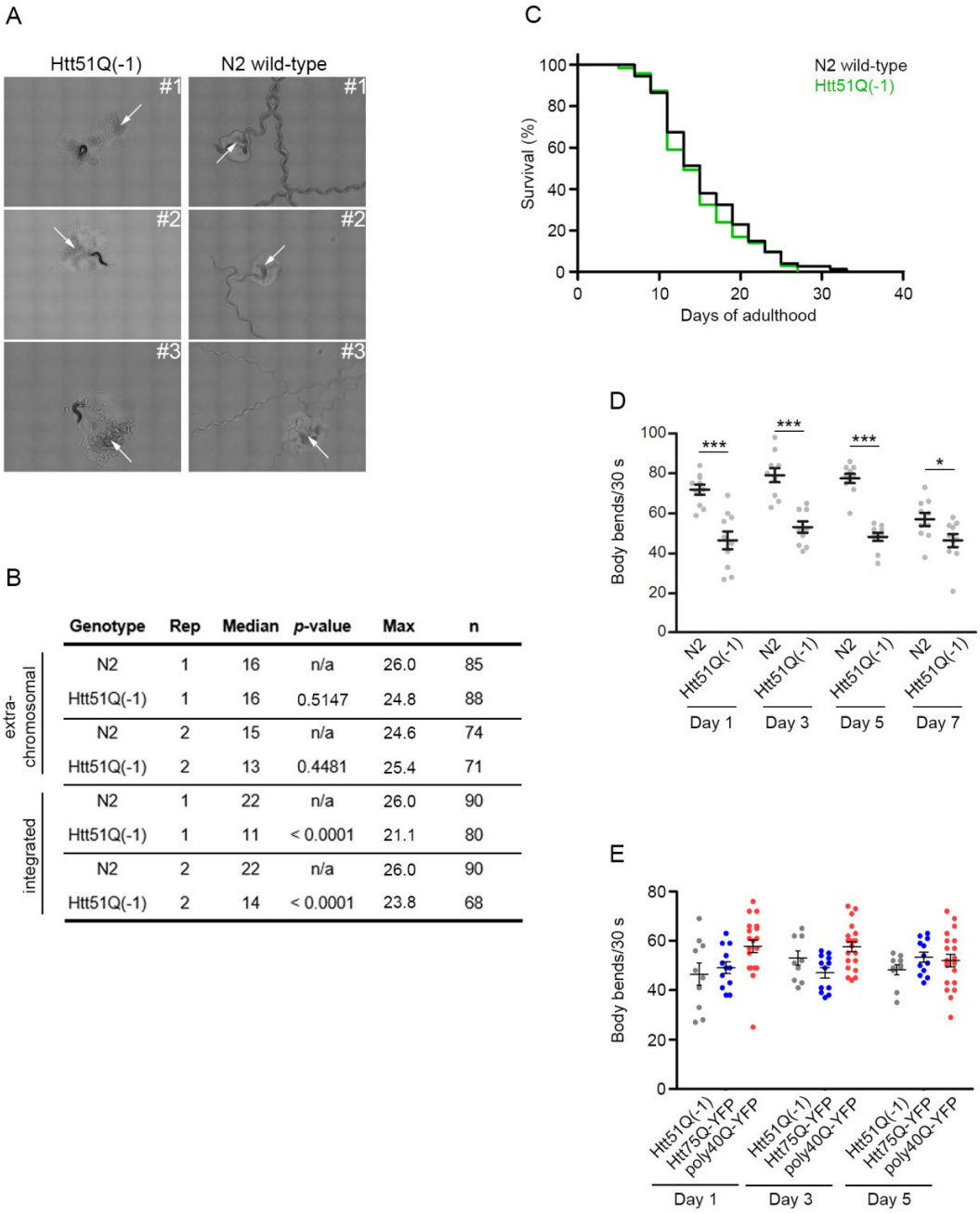


Figure S1

Extrachromosomal expression of Htt51Q(-1) frameshift reporter. (A) Representative fluorescence images of one day-old nematodes expressing Htt51Q(-1) in the body wall muscle cells. For better visualization, the eCFP fluorescence is depicted in green. Scale bar, 100 µm. (B) Quantification of the hyper-fluorescent foci representing Htt51Q(-1) aggregates considering the head regions only. Data are means ±SEM (n = 24 for extrachromosomally expressing and n = 10 for animals with genome-integrated expression). For comparison, *C. elegans* model strain AM141 expressing solely polyQ stretch (poly40Q-YFP) is included (n=11). (C) Representative fluorescence images of synchronized nematodes at larval L4 stage. Scale bar, 50 µm. (D) Representative fluorescence images of synchronized nematodes imaged at

different ages. Starting at day 5, autofluorescence in the intestine becomes visible. Scale bar, 100 µm. Note that the mCherry channel was disproportionally enhanced compared to the blue CFP channel for better visualization in panels A, C and D. The quantification however in panel E was performed with non-enhanced images. (E) Quantification of the ratio between mCherry and CFP fluorescence (panel D; n = 7-11 animals). Fluorescence was integrated over the head area since the expression was more prominent in the head and only this region was considered in the quantifications. *, $p < 0.05$; ***, $p < 0.001$, Student's *t*-test. See also Table S2. (F) qPCR analysis of adult nematodes with extrachromosomal Htt51Q(-1) expression. *Htt51Q(-1)* mRNA was normalized to *act-2*. Expression in day 1 adults was set to 100%. Data are means ± SEM (n = 3). * $p < 0.05$, Student's *t*-test. (G) Representative fluorescence images of synchronized nematodes expressing Htt75Q-YFP (strain AM881) at different ages. Scale bar, 75 µm.



B

	Genotype	Rep	Median	*p*-value	Max	n
extra-chromosomal	N2	1	16	n/a	26.0	85
	Htt51Q(-1)	1	16	0.5147	24.8	88
	N2	2	15	n/a	24.6	74
	Htt51Q(-1)	2	13	0.4481	25.4	71
integrated	N2	1	22	n/a	26.0	90
	Htt51Q(-1)	1	11	< 0.0001	21.1	80
	N2	2	22	n/a	26.0	90
	Htt51Q(-1)	2	14	< 0.0001	23.8	68

Figure S2

Phenotypic characterization of nematodes expressing Htt51Q(-1) frameshift reporter. (A) Visualization of traces left by three 1d-old individuals of N2 wild-type or animals with genome-integrated Htt51Q(-1) 60 minutes after placing them onto a fresh NGM plate (arrow marks the start position). Using "Tile Scan" mode of the microscope (Leica DMi8), an area of 6x6 cm was scanned and compiled to one single image. (B) Details of the life span assay of wild-type N2 and nematodes expressing Htt51Q(-1) extrachromosomally or integrated in the genome. Median, median life span; n, number of animals that remained after censoring (the starting test population was 100 animals); max, the maximal life span

calculated by averaging the life span of the 10 longest lived nematodes. *p* values were calculated from log-rank test compared to N2 control. (C) Life span assay of wild-type N2 and nematodes expressing Htt51Q(-1) extra-chromosomally. Kaplan-Meier survival curves were compared using log-rank test. Median lifespan of 14.5 days for extra-chromosomally expressing Htt51Q(-1) vs. 15.5 days for N2. See also panel B. (D) Motility assay of synchronized animals with extrachromosomal expression of Htt51Q(-1) and compared to N2 wild-type on different days of adulthood. Thrashing rate was analyzed by counting body bends for each individual animal. Each dot represents one single nematode, the horizontal line indicates the mean thrashing rate (± SEM, n = 10 animals for each strain and time point). Data among one time-point were normalized to the body bends of N2 wild-type. ***$p < 0.001$, *$p < 0.5$ (Student‘s t-test). (E) Motility assay of synchronized animals with extrachromosomal expression of Htt51Q(-1) (see also panel D), Htt75Q-YFP (AM881) or poly40Q-YFP (AM141). Thrashing rate was analyzed by counting body bends for each individual animal. Each dot represents one single nematode, the horizontal line indicates the mean thrashing rate (± SEM, n = 10 for Htt51Q(-1), n = 12 for Htt75Q-YFP, n = 20 for poly40Q-YFP animals for each time point).

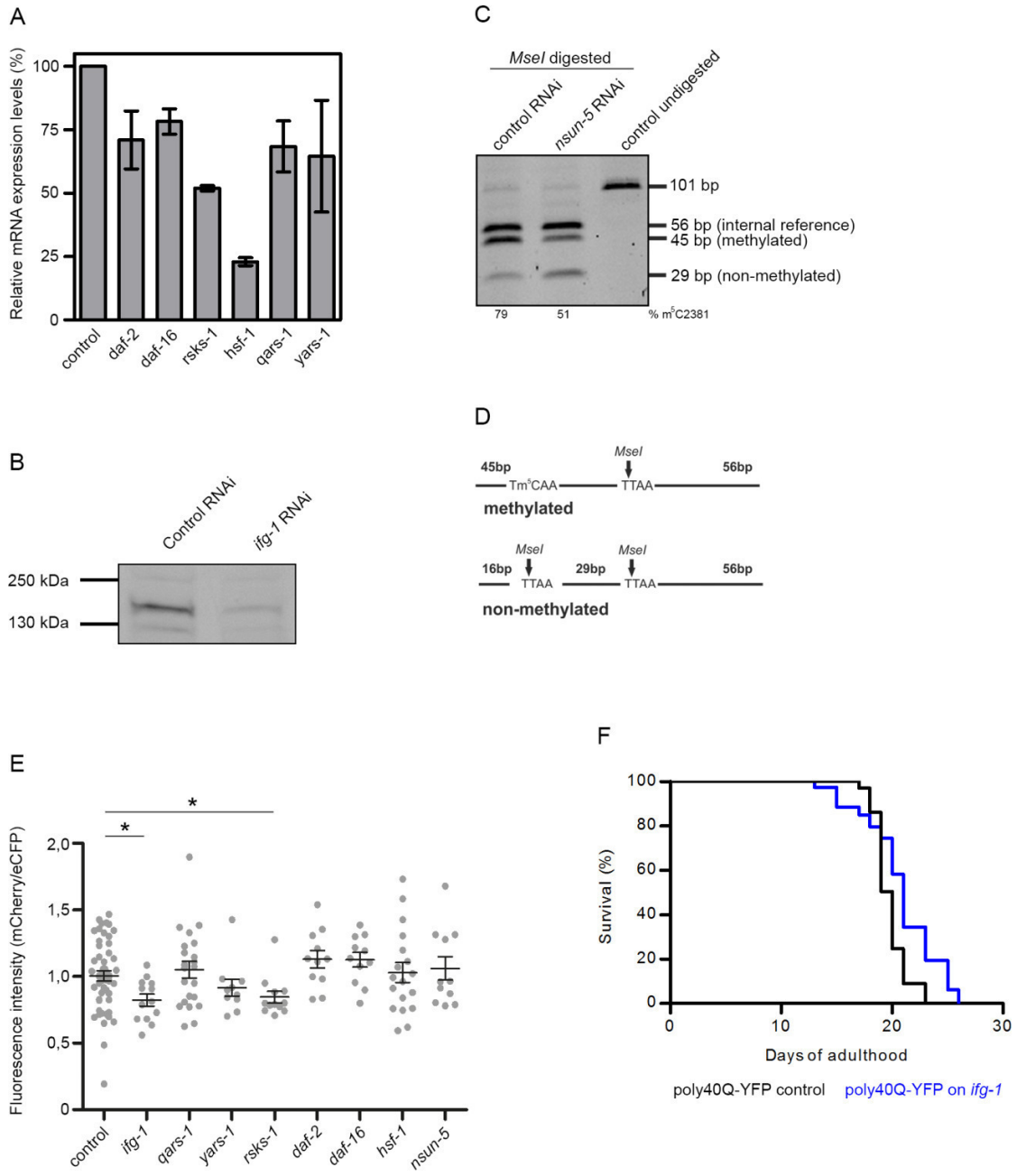


Figure S3
Validation of the RNAi knockdown in nematodes expressing Htt51Q(-1). (A) qRT-PCR analysis of different genes following RNAi knockdown. mRNA levels were normalized to *act-2*. Data are further compared to the control RNAi experiment with empty L4440 vector (control) and presented as means ± SD (n = 2). (B) Expression of IFG-1 following RNAi knockdown compared to the control RNAi/empty L4440 vector (control RNAi) monitored by immunoblot. 45 animals were loaded per lane. (C) Analysis of methylation activity in nematodes expressing the integrated frameshift Htt51Q(-1) grown on control or *nsun-5* RNAi, respectively. Knockdown of *nsun-5* results in approx. 30% decrease of methylation

detected with *MseI* restriction enzyme. (D) Scheme of the *MseI* restriction pattern of NSUN-5-methylated and non-methylated rRNA. (E) Quantification of the ratio between mCherry and CFP fluorescence (n = 8-50 animals) of nematodes expressing Htt51Q(-1) extrachromosomally. Fluorescence was integrated over the head area and only this region was considered in the quantifications. *, $p < 0.05$; Student's *t*-test. (F) Life span assay of poly40Q-YFP on RNAi for *ifg-1* and control L4440 respectively. Kaplan-Meier survival curves were compared using log-rank test. Median life span of 21 days for *ifg-1* vs. 20 days for control. Survival was enhanced for poly40Q-YFP grown on *ifg-1* ($p < 0.0001$).

Table S1

Fluorescence intensities of *C. elegans* at different age carrying the genome-integrated frameshift reporter. The head of the animals was only used for quantification. Fluorescence intensities of both eCFP and mCherry were normalized to the background fluorescence.

Age	Worm #	eCFP	mCherry	mCherry/CFP
1d of adulthood	1	4.774	0.830	0.174
	2	4.532	0.662	0.146
	3	3.620	0.839	0.232
	4	6.194	1.777	0.287
	5	17.758	1.850	0.104
	6	20.593	2.048	0.099
	7	15.516	1.791	0.115
	8	26.940	2.557	0.095
	9	17.021	1.915	0.113
3d of adulthood	1	13.845	1.839	0.133
	2	23.248	2.836	0.122
	3	24.273	2.672	0.110
	4	12.367	2.632	0.213
	5	5.816	1.884	0.324
	6	6.385	2.403	0.376
	7	6.468	2.783	0.430
	8	8.102	3.317	0.409
	9	19.039	4.053	0.213
	10	21.357	3.801	0.178
	11	11.378	2.833	0.249
5d of adulthood	1	2.757	3.665	1.329
	2	5.096	1.294	0.254
	3	4.429	2.367	0.534
	4	5.338	3.362	0.630
	5	3.561	2.375	0.667
	6	5.952	3.700	0.622
	7	5.462	3.203	0.586
	8	5.658	3.125	0.552
7d of adulthood	1	1.779	2.899	1.630
	2	4.313	3.654	0.847
	3	3.828	3.514	0.918
	4	2.650	2.789	1.052
	5	3.332	4.067	1.221
	6	4.941	3.745	0.758
	7	3.342	3.959	1.185

Table S2

Fluorescence intensities of *C. elegans* at different age carrying the frameshift reporter extrachromosomally. The head of the animals was only used for quantification. Fluorescence intensities of both eCFP and mCherry were normalized to the background fluorescence.

Age	Worm #	eCFP	mCherry	mCherry/eCFP
1d of adulthood	1	50.884	8.840	0.174
	2	37.572	11.861	0.316
	3	39.903	12.610	0.316
	4	44.937	10.572	0.235
	5	57.901	17.533	0.303
	6	32.214	7.943	0.247
	7	46.559	13.562	0.291
	8	43.799	12.093	0.276
3d of adultood	1	40.094	10.121	0.252
	2	38.11	17.055	0.448
	3	34.295	7.247	0.211
	4	28.536	5.564	0.195
	5	36.897	14.265	0.387
	6	24.087	7.115	0.295
	7	46.08	5.906	0.128
	8	29.591	7.561	0.256
	9	31.577	8.463	0.268
	10	34.542	11.086	0.321
5d of adulthood	1	38.371	11.129	0.290
	2	33.229	16.615	0.500
	3	29.115	11.465	0.394
	4	35.03	9.692	0.277
	5	50.46	14.783	0.293
	6	37.726	14.224	0.377
	7	25.752	6.247	0.243
7d of adulthood	1	33.555	15.767	0.470
	2	44.149	21.437	0.486
	3	34.408	13.991	0.407
	4	21.676	9.387	0.433
	5	23.507	11.362	0.483
	6	61.47	19.605	0.319
	7	75.158	12.308	0.164

Table S2

Fluorescence intensities of *C. elegans* at different age carrying the genome-integrated frameshift reporter. The head of the animals was only used for quantification. Fluorescence intensities of both eCFP and mCherry were normalized to the background fluorescence.

Age	Worm #	eCFP	mCherry	mCherry/CFP
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