

Table S1. Effect of ND75 Disruption on Lifespan, Related to Figure 1

S1A Genotype	Maximum Lifespan (\pms.e.m)	Median Lifespan (\pm s.e.m)	Mean Lifespan (\pm s.e.m)	P value relative to TD-w1118
TD-w1118	73.3 \pm 1.2	61.7 \pm 0.9	60.5 \pm 0.4	
TD-luckd	65.5 \pm 1.5	56.3 \pm 0.3	55.5 \pm 1.0	
TD-vkd	66.3 \pm 1.5	54.0 \pm 0.6	52.6 \pm 0.4	
TDN-w1118	55.7 \pm 1.7	28.3 \pm 1.9	29.3 \pm 0.8	< 0.0001
S1B Genotype				
TD-w1118	75.3 \pm 0.7	61.7 \pm 0.3	60.4 \pm 0.6	
TD-luckd	65.3 \pm 0.7	55.0 \pm 0.6	54.0 \pm 0.5	
TD-vkd	63.3 \pm 0.7	52.0 \pm 1.2	51.0 \pm 0.9	
N-w1118	72.7 \pm 2.4	58.7 \pm 0.7	57.1 \pm 0.4	
TDN-w1118	89.3 \pm 0.7	70.7 \pm 1.8	69.6 \pm 1.2	< 0.0001

Data shown is for 3 independent experiments and $n > 300$ for each genotype; p-values are based on the log rank test. (A) There is a decrease in maximum, median and mean lifespan when ND75 is severely disrupted in muscles. Compare data for TDN-w1118 (i.e where ND75 is disrupted, see text) with TD-w1118. TD-luckd and TD-vkd refer to offspring from TD crossed to luciferase^{mai} or vermillion^{mai} respectively. (B) However, mild ND75 perturbation triggers an increase in maximum, median and mean lifespan (see text for detailed procedure). Compare data for TDN-w1118 with TD-w1118. N-w1118 refers to offspring from a cross between ND75^{mai} and w1118. s.e.m refers to standard error of the mean. See extended experimental procedures for further description of lifespan experiments.

Table S2. Catalase or GTPx-1 Expression Suppresses the Enhanced Longevity Associated with Complex I Perturbation, Related to Figure 2

S2A Genotype	Maximum Lifespan (± s.e.m)	Median Lifespan (± s.e.m)	Mean Lifespan (± s.e.m)	P value relative to TD-w1118
TD-w1118	76.7±4	62.0±1.2	61.7±1.6	
TD-Cat	75.3±2.4	59.7±0.9	61.8±1.5	< 0.05
TDN-w1118	89.7±1.9	71.0±1.5	69.8±0.7	< 0.0001
TDN-Cat	60.7±0.7	51.3±0.7	51.2±0.5	< 0.0001 ^a
Cat-w1118	76.0±3.1	63.0±1.0	62.8±1.3	N.S
S2B Genotype				
TD-w1118	74.0±1.1	61.3±1.3	60.8±1.4	
TD-GTPx-1	74.7±0.7	64.0±1.2	63.0±0.1	0.325 (N.S)
TDN-w1118	90.7±0.7	68.3±0.3	67.8±0.4	< 0.0001
TDN-GTPx-1	61.3±0.7	50.7±1.8	50.0±0.7	< 0.0001 ^b
GTPx-1-w1118	74.7±0.7	64.7±1.8	62.2±1.5	N.S

Data shown is for 3 independent experiments, and for each genotype n > 300; p-values are based on the log rank test. (A) While Catalase overexpression has a slight effect on the lifespan of TD flies, it potently suppresses the enhanced longevity of TDN flies. “a” refers to p value when TDN-Cat is compared to TDN-w1118, TD-w1118, TD-Cat or Cat-w1118. (B) Similarly, GTPx-1 expression abrogates the enhanced longevity of TDN flies. “b” refers to p value when TDN-GTPx-1 is compared to TDN-w1118, TD-w1118, TD-GTPx-1 or GTPx-1-w1118.

Table S3.Synthetic Lethal Genes Identified, Related to Figures 3 to 6

Annotation ID	Gene Name
CG11992	Relish
CG17299	SNF4/AMP-activated protein kinase gamma subunit
CG3051	SNF1A/AMP-activated protein kinase
CG3018	IWR
CG3615	Autophagy-specific gene 9
CG3969	Fak-like tyrosine kinase
CG2151	Thioredoxin reductase-1
CG1633	Thioredoxin peroxidase 1
CG4303	Brahma associated protein 60kD
CG4379	cAMP-dependent protein kinase 1
CG5942	Brahma
CG6033	Downstream of receptor kinase
CG6794	Dorsal-related immunity factor
CG2128	Histone deacetylase 3
CG2078	Myd88
CG14224	Ubiquilin
CG4523	PINK1
CG2615	IkappaB kinase-like 2
CG10272	Grappa
CG17520	Casein kinase II subunit
CG2171	Triose phosphate isomerase
CG1954	Protein C kinase 98E
CG1210	Protein kinase 61C
CG2049	Protein kinase related to protein kinase N
CG17342	Lk6
CG7235	Hsp60C
CG15009	ImpL-2
CG4538	CG4538
CG16910	CG16910

Table S4. Adult-Onset Perturbation of Some Complex I Proteins In Muscles**Increase Lifespan, Related to Figure 3**

S4A Genotype	Maximum Lifespan (± s.e.m)	Median Lifespan (± s.e.m)	Mean Lifespan (± s.e.m)	P value relative to control
TD-verm	55.0±2	47.3±0.9	45.9±1.1	
TD-CG9762	60.0±1.2	56.0±1.2	55.2±0.5	< 0.0001
TD-CG4337	48.3±1.9	41.3±2.9	40.7±1.6	< 0.001 ^a
TD-CG9172	54.0±1.2	52.0±1.2	48.5±0.2	0.6543
TD-CG4217	52.7±1.2	50.0±2.0	47.2±0.8	< 0.05
TD-CG3869	50.0±1.2	40.3±2.8	41.3±1.4	< 0.0001 ^a
S4B Genotype				
TD-wkd	49.3±0.7	44.0±1.2	42.5±0.3	
TD-6343	61.3±0.7	55.3±0.7	54.5±0.3	< 0.0001
TD-8844	54.3±0.9	47.3±0.7	47.4±0.3	< 0.0001

Data shown is for 3 independent experiments, and for each genotype $n > 300$; p-values are based on the log rank test. Complex I proteins that result in increased lifespan are highlighted in red font. (A) Offspring of crosses between TD and various mitochondrial RNAi lines. CG9762 and CG9172 are complex I proteins; and TD-verm refers to offspring from a cross between TD and vermillion^{mai} which serves as an appropriate control for this background. (B) Lifespan results for mitochondrial complex I RNAi lines from the TRiP (HMS) background. CG6343 and CG8844 are complex I proteins; and TD-wkd refers to offspring from a cross between TD and white^{mai} which serves as a suitable control for this background. Note that all three RNAi constructs are inserted in the same location, eliminating the effects of varied insertion sites on lifespan analyses. See knockdown efficacy in Figure S5.

Table S5. ClpX and JNK Components Interact with TDN, Related to Figures 3 and 5

Genotype	Maximum Lifespan (± s.e.m)	Median Lifespan (± s.e.m)	Mean Lifespan (± s.e.m)	P value relative to TD-w1118
TD-w1118	54.3±1.2	46.7±1.8	44.3±0.9	
TDN-w1118	61.0±1.5	54.0±1.2	53.7±0.8	< 0.0001
TD-Clpkd	52.7±0.7	46.7±0.7	45.9±0.4	< 0.05
TDN-Clpkd	48.7±0.7	44.3±0.3	43.3±0.2	< 0.0001(a)
TD-DNbsk	53.3±0.7	46.7±0.7	43.0±0.2	< 0.0001
TDN-DNbsk	50.7±0.7	41.3±0.7	40.2±0.5	< 0.0001(b)
TD-DNjun	55.7±0.3	46.0±1.2	44.4±0.2	0.212 (N.S)
TDN-DNjun	53.3±0.7	44.3±0.3	42.5±0.4	< 0.0001(c)
N-w1118	51.3±0.7	46.7±0.7	44.9±0.8	
Clpkd-w1118	52.7±0.7	45.3±0.7	45.3±0.3	
DNbsk-w1118	52.0±1.2	47.3±0.7	46.5±0.1	
Dnjun-w1118	51.3±0.7	46.7±0.7	45.9±0.4	

Data shown is for 3 independent experiments, and for each genotype n > 300; p-values are based on the log rank test. Clpkd, DNbsk and DNjun refer to knockdown of ClpX, dominant-negative Bsk (i.e JNK) and dominant-negative Jun respectively. “a” refers to when compared to TDN-w1118,TD-w1118 or TD-Clpkd. “b” refers to when compared to TDN-w1118 or TD-w1118; p value is 0.0002 when compared to TD-DNbsk. “c” refers to when compared to TDN-w1118 or TD-w1118; p value is 0.0005 when compared to TD-DNjun.

Table S6. Muscle-Restricted Overexpression of Hsp60 or Hsp60C Increases Lifespan, Related to Figure 4

Genotype	Maximum Lifespan (\pm s.e.m)	Median Lifespan (\pm s.e.m)	Mean Lifespan (\pm s.e.m)	P value relative to Mhc-w1118
Mhc-w1118	83.3 \pm 1.8	75.0 \pm 1.5	71.4 \pm 0.8	
Mhc-60-1	90.0 \pm 1.2	84.0 \pm 1.2	78.5 \pm 0.1	< 0.0001
Mhc-60-2	93.3 \pm 0.7	89.3 \pm 0.7	84.1 \pm 0.1	< 0.0001
Mhc-60C1	90.7 \pm 0.7	87.3 \pm 0.7	82.4 \pm 0.4	< 0.0001
Mhc-60C2	88.7 \pm 1.3	81.3 \pm 0.7	78.1 \pm 0.2	< 0.0001
w1118-60-1	82.7 \pm 0.7	75.0 \pm 1.0	70.1 \pm 0.2	
w1118-60-2	84.0 \pm 1.2	76.0 \pm 2.0	68.9 \pm 0.8	
w1118-60C1	83.3 \pm 1.8	73.3 \pm 1.8	69.7 \pm 1.3	
w1118-60C2	82.7 \pm 0.7	76.7 \pm 0.7	69.9 \pm 0.9	

Data shown is for 3 independent experiments, and for each genotype n > 300; p-values are based on the log rank test. Two independent insertions of Hsp60 (i.e 60-1 and 60-2) and Hsp60C (i.e 60C1 and 60C2) were used.

Table S7. Muscle Secretion of ImpL2 influences Lifespan, Related to Figure 6

S7A Genotype	Maximum Lifespan (± s.e.m)	Median Lifespan (± s.e.m)	Mean Lifespan (± s.e.m)	P value relative to Control
TD-w1118 (control)	54.7±0.7	44.3±2.0	44.7±0.6	
TDN-w1118	62.0±1.2	56.7±0.7	54.9±0.4	< 0.0001
TD-vermkd	53.3±0.7	45.3±0.7	42.9±0.2	N.S
TDN-vermkd	59.3±0.7	54.7±0.7	53.3±0.7	
TD-ImpL2kd	53.3±0.7	47.3±0.7	43.3±0.1	
TDN-ImpL2kd	44.7±1.3	41.0±0.6	39.5±0.5	< 0.0001 ^a
ImpL2kd-w1118	50.7±0.7	44.7±1.8	43.9±0.8	
TD-ImpL2kd-2	49.3±0.7	43.3±0.7	42.2±0.8	
TDN-ImpL2kd-2	44.0±1.2	38.7±1.3	38.2±0.7	< 0.0001 ^a
ImpL2kd-2-w1118	49.3±0.7	44.7±0.7	42.3±0.7	
S7B Genotype				
Mhc-w1118 (control)	83.3±1.7	69.0±2.6	67.1±1.5	
Mhc-ImpL2	91.0±0.6	79.3±2.9	76.1±1.7	< 0.0001
w1118-ImpL2	79.3±2.9	69.0±3.2	65.7±1.8	
S7C Genotype				
TD-w1118 (control)	55.3±0.7	48.0±1.2	47.2±0.2	
TD-ImpL2	62.7±0.7	54.3±0.3	53.2±0.2	< 0.0001
w1118-ImpL2	53.3±0.7	45.3±0.7	45.7±0.3	

Data shown is for 3 independent experiments, and for each genotype n > 300; p-values are based on the log rank test. (A) Knockdown of ImpL2 (i.e ImpL2kd) in TDN muscles suppresses the extended lifespan. “a” refers to p value when compared to TDN-w1118.

As in all TD crosses, lifespan experiments were performed at 27C. ImpL2kd-2 is another RNAi line for ImpL2 (due to likelihood of a very busy graph, data for ImpL2kd-2 is not shown in Figure 6E) (B) As in all Mhc-Gal4 experiments, lifespan assays were performed at 25C. Mhc-Gal4-driven overexpression of ImpL2 increases lifespan (C) TD-Gal4-driven overexpression of ImpL2 also increases lifespan. Note that lifespan at 27C is shorter than at 25C.

Table S8. Disruption of Atg6, Atg8a or Atg12 Suppresses the Enhanced Longevity of TDN Flies, Related to Figure 7

	Maximum Lifespan (± s.e.m)	Median Lifespan (± s.e.m)	Mean Lifespan (± s.e.m)	P value relative to TDN-wkd
TD-wkd	49.3±1.3	42.7±0.7	41.9±0.3	
TDN-wkd	57.3±0.7	52.7±2.4	51.0±1.5	
TD-Atg6kd	50.0±2.0	43.3±0.7	42.1±0.9	
TDN-Atg6kd	45.3±0.7	40.7±0.7	39.9±0.9	< 0.0001
TD-Atg8akd	48.7±1.3	43.3±1.8	41.4±0.9	
TDN-Atg8akd	43.3±0.7	40.0±1.2	38.5±0.4	< 0.0001
TD-Atg12kd	48.7±1.3	41.3±0.7	41.2±0.2	
TDN-Atg12kd	44.0±1.2	37.3±1.8	37.1±0.9	< 0.0001

Data shown is for 3 independent experiments, and for each genotype $n > 300$; p-values are based on the log-rank test. Atg6kd, Atg8akd and Atg12kd refer to knockdown of Atg6, Atg8a and Atg12 respectively. TD-wkd refers to offspring from a cross between TD and white^{RNAi} which serves as a suitable control for this background. Note that all four RNAi constructs (the three Atg^{RNAi} lines and the white^{RNAi} line) are inserted in the same location, eliminating the effects of varied insertion sites on lifespan analyses. See knockdown efficacy in Figure 7.