

SUPPLEMENTARY FIGURES AND TABLES

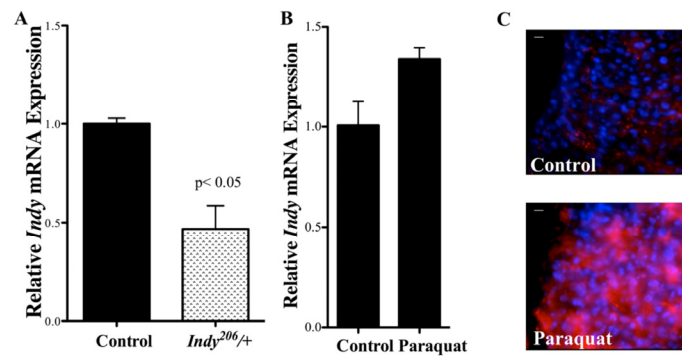


Figure S1. There is an age-related increase in *Indy* mRNA levels. (A) *Indy* mRNA levels in the midgut of male heterozygous *Indy206/+* mutant flies measured by qPCR at 40 days. There is significant reduction of *Indy* mRNA in the male mutant midgut ($p < 0.05$, $n = 3$, 25 guts per replicate). (B) *Indy* mRNA levels in the midgut of *yw* control male flies following overnight exposure to paraquat at 20 days determined by qPCR. There is a paraquat-induced increase in *Indy* mRNA levels in the midgut of control flies ($n = 3$, 25 guts per replicate). (C) Immunofluorescence images of a control female (top) and a female following exposure to 20mM (bottom) at 20 days, viewed with 40x oil immersion objective and stained with anti-INDY antibodies. INDY is increased following exposure to paraquat in the midgut.

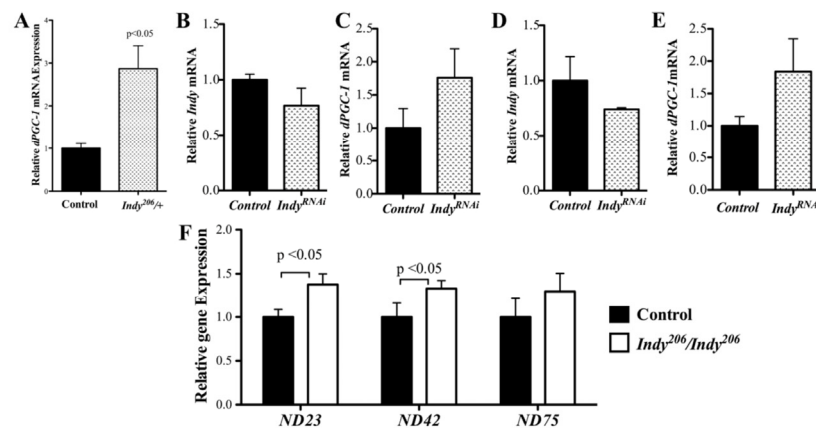


Figure S2. *dPGC-1* mRNA levels increase when *Indy* mRNA levels are reduced. (A) *dPGC-1* mRNA levels in the midgut of heterozygous *Indy206/+* males at 40 days. There is a significant increase in *dPGC-1* mRNA levels in *Indy206/+* male midgut tissue ($p < 0.05$, $n = 3$, 25 guts per replicate). (B) *Indy* mRNA levels in the midgut of female *TIGS2-GS;Indy9981* RNAi lines at 20 days measured by qPCR. (C) *dPGC-1* mRNA levels in the midgut of female *TIGS2-GS;Indy9981* RNAi lines at 20 days measured by qPCR. (D) *Indy* mRNA levels in the midgut of male *TIGS2-GS;Indy9981* RNAi lines at 20 days measured by qPCR. (E) *dPGC-1* mRNA levels in the midgut of male *TIGS2-GS;Indy9981* RNAi lines at 20 days measured by qPCR. *IndyRNAi* flies show a small increase in *dPGC-1* mRNA compared to control flies suggesting that targeted depletion of *Indy* in the midgut increases *dPGC-1* transcription ($n = 3$, 25 guts per replicate). Error Bars represent SEM. (F) Quantification of Complex I genes *ND23*, *ND42* and *ND75* mRNA in the midgut of 2 control and *Indy206/Indy206* mutant flies determined by qPCR. *ND23* and *ND42* mRNA are significantly increased in the *Indy* mutant midgut at 20 days ($p < 0.05$, $n = 3$, 25 guts per replicate).

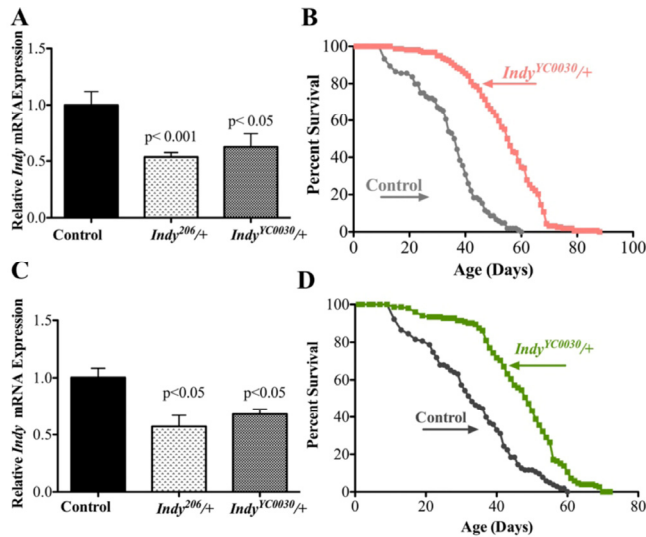
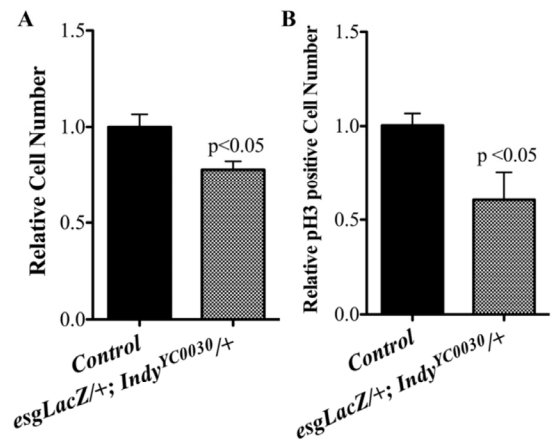


Figure S3. Characterization of *IndyYCO030/+* heterozygous mutant flies. (A) *Indy* mRNA levels in the midgut of *yw* control, *Indy206/+* and *IndyYCO030/+* female flies aged 20 days determined by qPCR. There is a significant ($p < 0.001$, $p < 0.05$, $n = 3$, 25 guts per replicate) decrease in *Indy* mRNA levels in the midgut of *Indy206/+* and *IndyYCO030/+*. Error Bars represent SEM. (B) Life-span curves of control (gray) and *IndyYCO030/+* females (pink). A 58.3% increase in median survival was observed in *IndyYCO030/+* females. (C) Endogenous *Indy* mRNA levels in the midgut of control, *Indy206/+* and *IndyYCO030/+* male flies aged 20 days determined by qPCR. There is a significant ($p < 0.05$, $n = 3$, 25 guts per replicate) decrease in *Indy* mRNA levels in the midgut of *Indy206/+* and *IndyYCO030/+*. Error Bars represent SEM. (D) Life-span curves of control (gray) and *IndyYCO030/+* males (green). A 46.8% increase in median survival was observed in *IndyYCO030/+* males.

Figure S4. Reduced *INDY* modulates intestinal homeostasis. (A) Quantification of *esg* positive cells in the midgut of control (*esgLacZ/+*) and *Indy* mutant (*esgLacZ;IndyYCO030/+*) male flies at 40 days. There are reduced ISC/EBs in the midgut of *Indy* mutant flies ($p < 0.05$, $n > 15$). (B) Quantification of pH3-positive cells in the midgut of control (*esgLacZ/+*) and *Indy* mutant (*esgLacZ;IndyYCO030/+*) male flies at 40 days. There are reduced dividing cells in the midgut of *Indy* mutant flies ($p < 0.05$, $n > 15$). Error bars represent SEM.



SFigure 4

Figure S5. *Indy* and *dPGC-1* longevity pathways overlap. (A) Relative *Indy* mRNA levels measured by qPCR. *Indy* is significantly decreased in *esgGal4;Indy206/UAS-dPGC-1* females and (B) males at 20 days compared to control ($p < 0.001$, $p < 0.05$, $n = 3$, 25 guts per replicate). (C) Relative *dPGC-1* mRNA levels in the midguts of females (C) and males (D) measured by qPCR. *dPGC-1* is significantly increased in *esgGal4;UAS-dPGC-1*, *Indy206;UAS-dPGC-1* and *esgGal4;Indy206/UAS-dPGC-1* and significantly decreased in *Indy206/dPGC-1KG08646*, compared to control *esgGal4/+* and *UAS-dPGC-1/+* flies ($p < 0.05$, $p < 0.001$, $n = 3$, 25 guts per replicate) but not between groups. Error bars represent SEM.

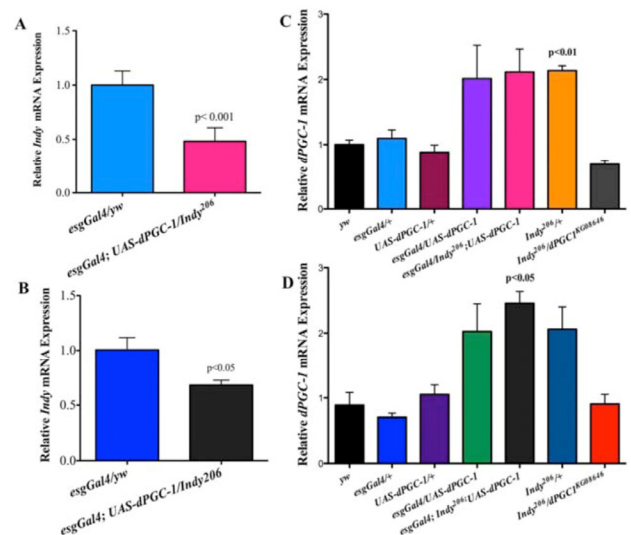


Table S1: *Indy* mutant flies are more resistant to paraquat stress.

Gender	Genotype	N	Median Lifespan (% Change)	X ²	p	Maximal Lifespan (% Change)
F	<i>yw</i>	95	15.1	-	-	21
F	<i>Indy²⁰⁶/+</i>	85	19.8 (31.3)	62.2	p< 0.0001	26.8 (27.6)
F	<i>Indy²⁰⁶/Indy²⁰⁶</i>	85	17.1 (13.2)	24.3	p< 0.0001	24.4 (16.1)
M	<i>yw</i>	80	13.5	-	-	19.9
M	<i>Indy²⁰⁶/+</i>	80	19.4 (43.7)	25.9	p< 0.0001	24.4 (22.6)
M	<i>Indy²⁰⁶/Indy²⁰⁶</i>	70	16.1 (19.2)	1.81	p=0.1787	22.1 (11.05)

The median and maximal lifespan of female (F) and male (M) *yellow-white* (*yw*) control, heterozygous (*Indy²⁰⁶/+*) and homozygous (*Indy²⁰⁶/Indy²⁰⁶*) *Indy* mutant flies in *yw* background. N: number of flies in each lifespan. Median and Maximal Lifespans are in hours. Long-rank analyses were performed using the JMP 10 program.

Table S2. Heterozygous *Indy^{YC0030}/+* mutant flies have increased longevity compared to *yw* genetic control flies.

Gender	Genotype	N	Median Lifespan (% Change)	X ²	p	Maximal Lifespan (% Change)
F	<i>yw</i>	160	34.1	-	-	53.5
F	<i>Indy^{YC0030}/+</i>	180	54 (58.3)	148.5	p< 0.0001	72 (34.5)
M	<i>yw</i>	145	32.9	-	-	55.6
M	<i>Indy^{YC0030}/+</i>	160	46.8 (42.2)	61.2	p<0.0001	64.8 (16.4)

The median and maximal lifespan of female (F) and male (M) *yellow-white* (*yw*) control and heterozygous *Indy* (*Indy^{YC0030}/+*) mutant flies in *yw* background. N: number of flies in each lifespan. Median and maximal lifespans are in days. Long-rank analyses were performed using the JMP 10 program.

Table S4: *dPGC-1* is required for *Indy* mutant longevity extension

Gender	Genotype	N	Median Lifespan (% Change)	X ²	p	Maximal Lifespan (% Change)
F	<i>UAS-dPGC-1/+</i>	150	51.5	-	-	73.3
F	<i>Indy²⁰⁶/UAS-dPGC-1</i>	173	63.1 (22.5)	58.48	p< 0.001	95.9 (30.8)
F	<i>Indy²⁰⁶/UAS-dPGC-1^{KG08646}</i>	188	51.8 (0.58)	5.19	p=0.022	70.1 (-4.3)
M	<i>UAS-dPGC-1/+</i>	145	48.5	-	-	71.3
M	<i>Indy²⁰⁶;UAS-dPGC-1</i>	173	59.9 (23.5)	84.21	p<0.0001	92.9 (30.2)
M	<i>Indy²⁰⁶/UAS-dPGC-1^{KG08646}</i>	205	49.2 (1.4)	1.21	p=0.27	66.7 (-6.5)

The median and maximal lifespan of female (F) and male (M) control *UAS-dPGC-1/+*: heterozygous flies with the *UAS-dPGC-1* construct in *yellow white* background. *Indy²⁰⁶/UAS-dPGC-1* flies have *UAS-dPGC-1* construct in *Indy²⁰⁶* mutant background. *Indy²⁰⁶/UAS-dPGC-1^{KG08646}* flies were generated by crossing *Indy²⁰⁶* flies to *dPGC-1^{KG08646}* flies (hypomorph for the *dPGC-1*). All values are compared to either male or female *UAS-dPGC-1/+* control groups to determine the percent increase in median and maximal lifespan. *N*: number of flies used in the experiment. Median and maximal lifespan are in days. Data are censored for 0-9 days. Long-rank analyses were performed using the JMP 10 program.

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The median and maximal lifespan of female (F) and male (M) control *UAS-dPGC-1/+*: heterozygous flies with the *UAS-dPGC-1* construct in *yellow white* background. *Indy²⁰⁶/UAS-dPGC-1* flies have *UAS-dPGC-1* construct in *Indy²⁰⁶* mutant background. *Indy²⁰⁶/UAS-dPGC-1^{KG08646}* flies were generated by crossing *Indy²⁰⁶* flies to *dPGC-1^{KG08646}* flies (hypomorph for the *dPGC-1*). All values are compared to either male or female *UAS-dPGC-1/+* control groups to determine the percent increase in median and maximal lifespan. *N*: number of flies used in the experiment. Median and maximal lifespan are in days. Data are censored for 0-9 days. Long-rank analyses were performed using the JMP 10 program.