Supporting Information

Wang et al. 10.1073/pnas.0904115106

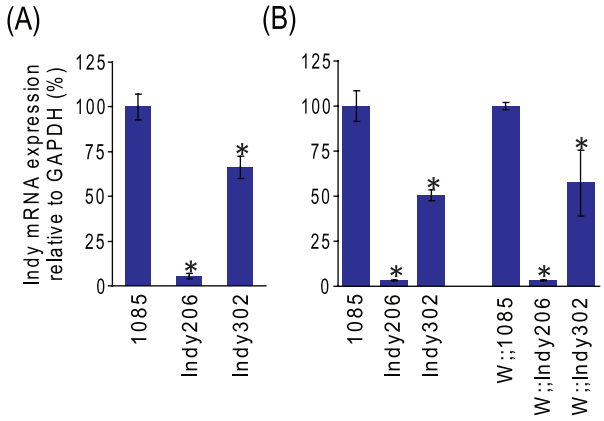

Fig. S1. *Indy* mutants show transcriptional defects. Real-time PCR detection of *Indy* mRNA expression levels in (*A*) original Canton-S derived *Indy* 206, *Indy* 302, and 1085 control flies (Rogina et al., 2000), and in (*B*) flies provided by (Toivonen et al., 2007). *Indy206* and *Indy302* homozygous flies show significant transcriptional defects before and after backcrossing into *white* Dahomey (W). Data are presented as mean ± SD. Experiments were done in triplicate, and more than 100 flies in each group were measured. The * indicates $P < 0.05$ by *t* test.

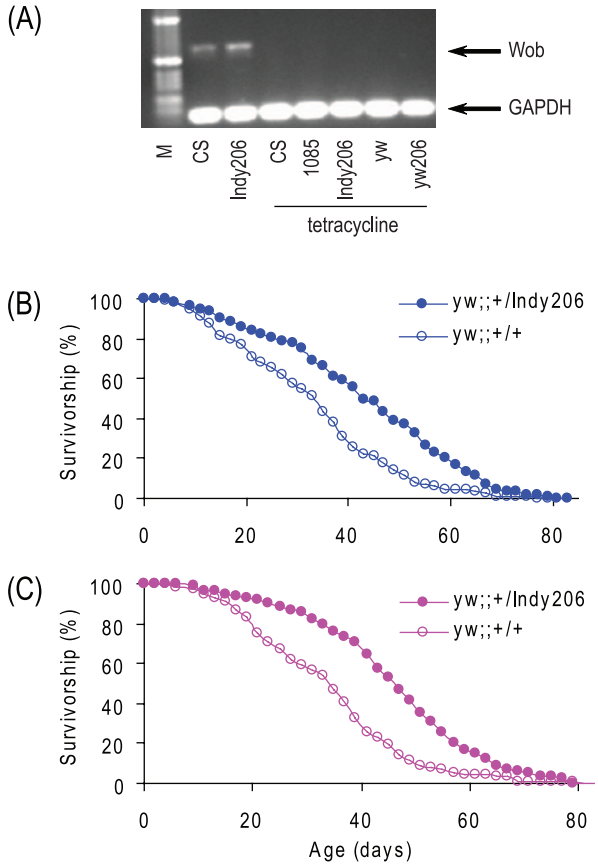

Fig. S2. *Wolbachia* infection does not affect *Indy* life span extension in *yw* inbred background. (*A*) PCR confirmation shows *Wolbachia* infection is removed after tetracycline (Tet) treatment. *Wolbachia* free *Indy206* heterozygous (*yw;;+/*206) male (*B*) and female (*C*) flies both display a longer life span compared with their *yw* control (*yw;;+/+*) flies in 1.5 N food condition (29% and 34% median life span extension in male and female, respectively, $P < 0.0001$).

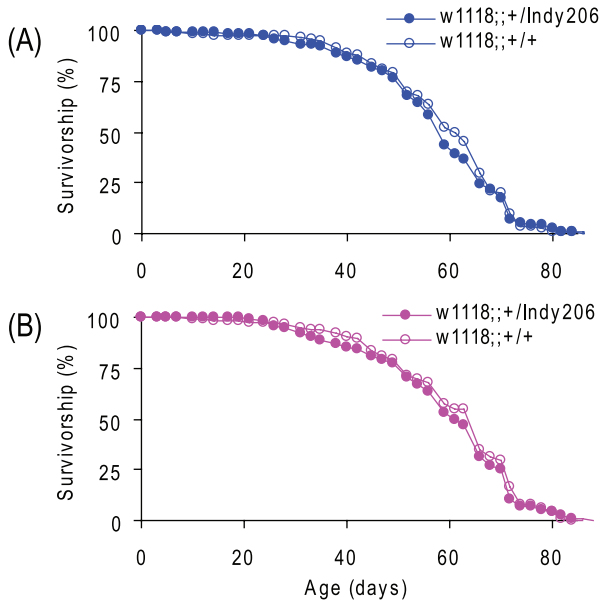

Fig. S3. *Indy206* heterozygous male (*A*) and female (*B*) flies in original Canton-S background (CS/*Indy206*) show 26% and 11% ($P < 0.0001$) life span extension when compared with control flies (CS/1085). Life spans on 1.5 N food.

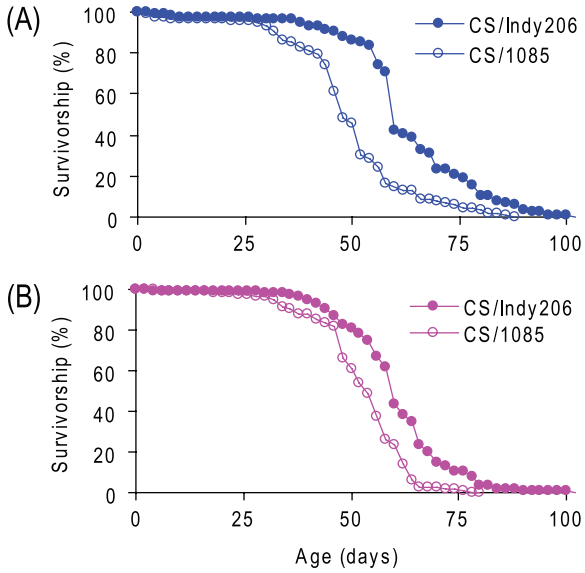

Fig. S4. *Indy206* heterozygous male (*A*) and female (*B*) flies in *w1118* inbred background (*w1118*;;+/*Indy206*) show no life span extension when compared with control flies (*w1118*;;+/+). Life spans on 1.5 N food.

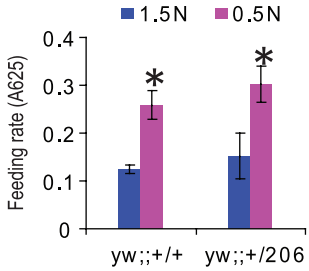

Fig. S5. Feeding rates of *Indy206* heterozygous (+/206) and *yw* control (+/+) flies over a 24-h period on 1.5 N (blue) or 0.5 N (red) foods with addition of 0.5% FD & C no. 1 blue food dye. The * indicates $P < 0.05$ by T-test.

Table S1. The effects of different food conditions on the lifespan (LS) of male control (+/+), *Indy206* heterozygous (+/206) and *Indy206* homozygous (206/206) flies

Food	Genotype	Mean LS	Mean LS extension (%)	Median LS	Median LS extension (%)	Max LS	Max LS extension (%)	χ^2	*P*	Fly No.
1.5N	+/+	33	—	35	—	62	—	—	—	243
	+/206	43	30	45	28.6	68	9.7	22.1	<0.0001	213
	206/206	36	9.1	40	14.3	63	1.6	0.9	0.0043	246
1.0N	+/+	37	—	43	—	62	—	—	—	221
	+/206	44	18.9	49	14.0	68	9.7	17.4	<0.0001	214
	206/206	40	8.1	44	2.3	60	−3.2	0.8	0.35	243
0.5N	+/+	43	—	48	—	65	—	—	—	188
	+/206	47	9.3	50	4.2	68	4.6	4.6	0.03	241
	206/206	40	−7.0	44	−8.3	58	−10.8	13.6	0.0002	237

Mean, median and maximum lifespan, percent change of lifespan comparing to controls, chi-square in each food condition, and *P* values were derived from survivorship curves. Maximum lifespan was calculated as the median lifespan of the longest surviving 10% of the population.

Table S2. Oligonucleotide primer sequences

Primer	Sequence	Size, bp
GAPDH-F	5′- GACGAAATCAAGGCTAAGGTCG −3′	109
GAPDH-R	5′- AATGGGTGTCGCTGAAGAAGTC −3′	
Indy-F	5′-CTGCCCAACTCTGTCCTCTTACTG-3′	150
Indy-R	5′-CAGGATCAGGTACAGAGGATGGAT-3′	
dilp2-F	5′-AGCAAGCCTTTGTCCTTCATCTC-3′	118
dilp2-R	5′-ACACCATACTCAGCACCTCGTTG-3′	
dilp3-F	5′-TGTGTGTATGGCTTCAACGCAATG −3′	106
dilp3-R	5′-CACTCAACAGTCTTTCCAGCAGGG-3′	
dilp5-F	5′-GAGGCACCTTGGGCCTATTC-3′	70
dilp5-R	5′-CATGTGGTGAGATTCGGAGCTA-3′	
Wolbachia-F	5′-TGGTCCAATAAGTGATGAAGAAAC-3′	590–632
Wolbachia-R	5′-AAAAATTAAACGCTACTCCA-3′	