**Supplementary Figure legends**

**Extended Data Figure 1. Related to Figure 1. RU486 induces BNIP3 expression in the brain of *elavGS>UAS-BNIP3* flies.**

(a) Immunostaining of brains from 10-day-old *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward, showing BNIP3 expression level (green channel, anti-HA) and nuclear DNA (blue channel, stained with DAPI). Scale bar is 50 ㎛.

(b) Quantification of BNIP3 expression level in brain as shown in (a). n > 7 flies. \*\*\**p*<0.001; unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Figure 2. Related to Figure 1. Neuronal BNIP3 induction prevents loss of neurons in aged brains.**

(a) TOPRO staining of nuclei in brains from young (10-day-old) and aged (30-day-old) *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward.

(b) Quantification of nuclei in brain as shown in (a). n = 5-7 flies. \**p*<0.05; unpaired *t* test.

**Extended Data Figure 3. Related to Figure 1. RU486 treatment in control flies has no effect on mitochondria homeostasis in aged brains.**

(a) Immunostaining of brains from young (10-day-old) and aged (30-day-old) *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward, showing mitochondria morphology (red channel, anti-ATP5a) and nuclear DNA (blue channel, stained with DAPI). Scale bar is 5 ㎛.

(b) Quantification of mitochondria area in brain as shown in (a). n = 8 flies. \*\*\**p*<0.001, non-significant (n.s.); unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent Min to Max.

**Extended Data Figure 4. Related to Figure 2. Neuronal specific BNIP3 induction reduces ATG8 levels in aged brains.**

(a) Immunostaining of brains from young (10-day-old) and aged (30-day-old) *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward, showing Atg8a levels (red channel, anti-Atg8a). Scale bar is 5 ㎛.

(b) Quantification of Atg8a levels in brain as shown in (a). n = 5-7 flies. \**p*<0.05; unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Figure 5. Related to Figures 2 and 3. Midlife neuronal induction of BNIP3 induces mitophagy and extends lifespan.**

(a and b) mito-QC of brains from 37-day-old (a) and 44-day-old (b) flies. Genotypes analyzed were *elavGS>UAS-mito-QC,UAS-lacZ*, as a control, and *elavGS>UAS-mito-QC,UAS-BNIP3* flies with RU486-mediated transgene induction from day 30 to day 37 or from day 30 to day 44. Images shown of merged GFP and mCherry along with punctate mCherry-only foci (from merged images where GFP has been quenched; mitolysosomes). Scale bar is 5 ㎛.

(c and d) Quantification of mitolysosomes in brains as shown in (a) and (b) at day 37 (c) and day 44 (d). n = 7-11 flies. \**p*<0.05; \*\**p*<0.01; unpaired *t* test.

(e) Survival curves of *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 30 onward. The shaded area indicates the duration of Atg1RNAi and BNIP3 induction. \*\*\**p*<0.001; log-rank test; n > 147 flies. RU486 was provided in the media at a concentration of 25 ㎍/㎖.

**Extended Data Figure 6. Related to Figure 2. RU486 induces BNIP3 expression in the brain of *elavGS>UAS-Atg1RNAi,UAS-BNIP3*****flies.**

(a) Immunostaining of brains from 10-day-old *elavGS>UAS-Atg1RNAi,UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward, showing BNIP3 expression level (green channel, anti-HA) and nuclear DNA (blue channel, stained with DAPI). Scale bar is 50 ㎛.

(b) Quantification of BNIP3 expression level in brain as shown in (a). n = 5 flies. \*\*\**p*<0.001; unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Figure 7. Related to Figure 3. RU486 treatment in control flies has no effect on lifespan and healthspan.**

(a) Survival curve of *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward. non-significant (n.s.);log-rank test. n > 218.

(b) Con-Ex feeding assay of 10-day-old *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward. n = 6 vials of 10 flies per condition. non-significant (n.s.); unpaired *t* test.

(c) Climbing index as a measure of endurance of 30-day-old *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward. n = 8 biological replicates with 100 flies per replicate. non-significant (n.s.); unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Figure 8. Related to Figure 3. Neuronal-specific BNIP3 induction does not alter food consumption or fecundity.**

(a) Con-Ex feeding assay of 10-day-old *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward. n = 6 vials of 10 flies per condition. non-significant (n.s.); unpaired *t* test.

(b) Fecundity of 37-day-old *elavGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward. n =5-6. non-significant (n.s.); unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Figure 9. Related to Figure 3. Ubiquitous, gut-, or muscle-specific BNIP3 induction shortens lifespan.**

(a) Survival curves of *daGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward. \*\*\**p*<0.001, non-significant; log-rank test; n > 198 flies.

(b) Survival curves of *5966GS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward. \*\*\**p*<0.001; log-rank test; n > 194 flies.

(c) Survival curves of *Act88FGS>UAS-BNIP3* flies with or without RU486-mediated transgene induction from day 5 onward. \*\*\**p*<0.001; log-rank test; n > 229 flies.

**Extended Data Figure 10. Related to Figure 4. RU486 treatment in control flies has no effect on mitochondria homeostasis in aged muscle.**

(a) Immunostaining of indirect flight muscles from young (10-day-old) and aged (30-day-old) *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward, showing mitochondria morphology (red channel, anti-ATP5a), muscles (magenta channel, stained with phalloidin/F-Actin), and nuclear DNA (blue channel, stained with DAPI). Scale bar is 10 ㎛.

(b) Quantification of mitochondria area in muscle as shown in (a). n = 8 flies. \*\*\**p*<0.001, non-significant (n.s.); unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent Min to Max.

**Extended Data Figure 11. Related to Figure 5. RU486 treatment in control flies has no effect on mitochondria homeostasis in the aged gut.**

(a) Immunostaining of guts from young (10-day-old) and aged (30-day-old) *elavGS>UAS-GFP* flies with or without RU486-mediated transgene induction from day 5 onward, showing mitochondria morphology (red channel, anti-ATP5a) and nuclear DNA (blue channel, stained with DAPI). Scale bar is 5 ㎛.

(b) Quantification of mitochondria area in gut as shown in (A). n = 8 flies. \*\**p*<0.01, non-significant (n.s.); unpaired *t* test.

RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent Min to Max.

**Extended Data Figure 12. Related to Figure 5. Neuronal-specific BNIP3 induction does not alter microbial dynamics in the gut.**

Bacterial levels assayed by qPCR of 16S rRNA gene in surface sterilized, *elavGS>UAS-BNIP3* flies with or without 5 μg/ml RU486 treatment from day 4 onwards (post-eclosion). n.s.; not significant, unpaired *t*-test; n = 3 replicates of ten flies per timepoint. RU486 was provided in the media at a concentration of 5 ㎍/㎖. Error bars represent SEM.

**Extended Data Table 1. Related to Figure 3. Neuronal-specific BNIP3 induction extends lifespan.**