Supplementary Figure Legends

**Figure S1.** Time spent on food of BAG or URX neuron-ablated animals or gc mutation.

(A–D) Average time on food per animal in 15-min duration (A–D left) and average percentages of animals on food recorded every minute of 15-min observation (A–D right) for ablation models including BAG-ablated vs. control animals (A) and URX-ablated vs. control animals (B), and for genetic models of gc-31(ok296) and gc-33(ok232) mutants (C), gc-35(ok769) and gc-36(db66) mutants (D), and their shared controls (C and D). n = 30 per group; data shown are one representative assay from three independent assays. Error bars represent mean ± s.e.m.

**Figure S2.** Assessment of aging phenotypes for gc-31 and gc-33 mutants vs. controls.

Percentages of animals exhibiting head movement, sinusoidal body posture and active locomotion among wildtype controls (n = 64), gc-31(ok296) (n = 41), and gc-33(ok232) mutants (n = 48) at the time point of population median lifespan (Day 17 for gc-31 mutants, Day 18 for gc-33 mutants, and Day 14 for wildtype controls). *P < 0.05, **P < 0.01, ***P < 0.001. Error bars represent mean ± s.e.m.

**Figure S3.** GCYs modulate lifespan independently of DAF-2/DAF-16 signaling.

(A–D) N2 wildtype (WT) strain vs. gc-31(ok296) (A), gc-33(ok232) (B), gc-35(ok769) (C) or gc-36(db66) (D) mutants treated with daf-2(RNAi), daf-16(RNAi) or control RNAi (Con). Data show mean lifespan and 75th percentile lifespan (A–D left) and percentage of lifespan change relative to corresponding control RNAi (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (Con), daf-2(RNAi) and daf-
16(RNAi) were compared to individual gcy mutants. \( *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 \), \( n = 24–45 \) per group. Error bars represent mean ± s.e.m.

**Figure S4.** GCYs modulate lifespan independently of DAF-12 signaling.

(A–D) N2 wildtype (WT) strain vs. gcy-31(ok296) (A), gcy-33(ok232) (B), gcy-35(ok769) (C) or gcy-36(db66) (D) mutants treated with daf-12(RNAi) or control RNAi (Con). Data show mean lifespan and 75th percentile lifespan (A–D left) and percentage of lifespan change relative to corresponding control RNAi (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (Con) and daf-12(RNAi) were compared to individual gcy mutants. \( *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 \), \( n = 25–43 \) per group. Error bars represent mean ± s.e.m.

**Figure S5.** GCYs modulate lifespan independently of germline signaling.

(A–D) N2 wildtype (WT) strain vs. gcy-31(ok296) (A), gcy-33(ok232) (B), gcy-35(ok769) (C) or gcy-36(db66) (D) mutants were treated with glp-1(RNAi) or control RNAi (Con). Data show mean lifespan and 75th percentile lifespan (A–D left) and percentage of lifespan change relative to corresponding control RNAi (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (Con) and glp-1(RNAi) were compared to individual gcy mutants. \( **P < 0.01, ***P < 0.001 \), \( n = 34–48 \) per group, and statistics for panels A&C are shown in the figure. Error bars represent mean ± s.e.m.

**Figure S6.** GCYs modulate lifespan independently of sensory perception.
(A–D) N2 wildtype (WT) strain vs. gcy-31(ok296) (A), gcy-33( ok232) (B), gcy-35(ok769) (C) or gcy-36(db66) (D) mutants were treated with osm-3(RNAi), odr-7(RNAi) or control RNAi (Con). Data show mean lifespan and 75th percentile lifespan (A–D left) and percentage of lifespan change relative to corresponding control RNAi (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (Con), osm-3(RNAi) and odr-7(RNAi) were compared to individual gcy mutants. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 24–46 per group. Error bars represent mean ± s.e.m.

**Figure S7.** GCYs modulate lifespan independently of dietary restriction.

(A–D) N2 wildtype (WT) strain vs. gcy-31(ok296) (A), gcy-33( ok232) (B), gcy-35(ok769) (C) or gcy-36(db66) (D) mutants were subjected to ad libitum feeding or dietary restriction. Data show mean lifespan and 75th percentile lifespan (A–D left), and percentage of lifespan change relative to corresponding dietary control (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (ad libitum) and WT (DR) were compared to individual gcy mutants. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 24–38 per group. Error bars represent mean ± s.e.m.

**Figure S8.** GCYs modulate lifespan independently of egl-9 knockdown.

(A–D) N2 wildtype (WT) strain vs. gcy-31(ok296) (A), gcy-33( ok232) (B), gcy-35(ok769) (C) or gcy-36(db66) (D) mutants were treated with egl-9(RNAi) or control RNAi (Con). Data show mean lifespan and 75th percentile lifespan (A–D left) and percentage of lifespan change relative to corresponding control RNAi (A–D right) of indicated animals. All experiments in (A–D) were performed at the same time, and the same groups of WT (Con) and egl-9(RNAi) were compared
to individual gcy mutants. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, n = 27–46 per
group, and statistics for panels A&C are shown in the figure. Error bars represent mean ± s.e.m.

**Figure S9.** Effects of different levels of environmental O$_2$ on *C. elegans* lifespan.

(A, B) Survival curves (A) and mean lifespan and 75$^{\text{th}}$ percentile lifespan (B) of wildtype *C.
elegans* under 4% O$_2$ (n = 51), 8% O$_2$ (n = 50), 12% O$_2$ (n = 57), 21% O$_2$ (n = 65), and 40% O$_2$
(n = 75). *$P < 0.05$, ****$P < 0.0001$, statistics for curve comparisons are shown in the figure.
Error bars represent mean ± s.e.m.

**Figure S10.** GCYs modulate lifespan independently of and counteractively with gas conditions.

(A) Mean lifespan and 75$^{\text{th}}$ percentile lifespan of *C. elegans* following laser ablation of BAG
neurons (n = 28) or URX neurons (n = 29) under 4% O$_2$ vs. ablation controls under 4% O$_2$ (n =
26) and 12% O$_2$ (n = 38), respectively. (B) Mean lifespan and 75$^{\text{th}}$ percentile lifespan of gcy-31
mutants (n = 31), gcy-33 mutants (n = 33), gcy-35 mutants (n = 43) and gcy-36 mutants (n = 27)
under 4% O$_2$ vs. wildtype controls under 4% O$_2$ (n = 38) and 12% O$_2$ (n = 43), respectively. (C)
Mean lifespan and 75$^{\text{th}}$ percentile lifespan of *C. elegans* following laser ablation of BAG neurons
(n = 30) or URX neurons (n = 44) under 40% O$_2$ vs. ablation controls under 40% O$_2$ (n = 33) and
12% O$_2$ (n = 28), respectively. (D) Mean lifespan and 75$^{\text{th}}$ percentile lifespan of gcy-31 mutants
(n = 42), gcy-33 mutants (n = 45), gcy-35 mutants (n = 38) and gcy-36 mutants (n = 32) under
40% O$_2$ vs. wildtype controls under 40% O$_2$ (n = 35) and 12% O$_2$ (n = 45), respectively. *$P <$
0.05, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Error bars represent mean ± s.e.m.
Supplementary Movie Captions

Movie S1: Locomotory behavior of wildtype animals at population median lifespan.

Movie S2: Locomotory behavior of gcy-31(ok296) mutants at population median lifespan.

Movie S3: Locomotory behavior of gcy-33(ok232) mutants at population median lifespan.
Fig. S1

A

B

C

D

Time spent on food (min)

% animals on food per minute

Time spent on food (min)

% animals on food per minute

Time spent on food (min)

% animals on food per minute

Time spent on food (min)

% animals on food per minute
**Fig. S2**

- **wildtype**
- **gcy-31**
- **gcy-33**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Population %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Movement</td>
<td><strong>60</strong></td>
</tr>
<tr>
<td>Sinusoidal Posture</td>
<td><strong>45</strong></td>
</tr>
<tr>
<td>Active Locomotion</td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>
Fig. S3

A

B

C

D

WT (Con)  
*daf-2(RNAi)*  
*daf-16(RNAi)*  
gcy-31 (Con)  
gcy-31;daf-2(RNAi)  
gcy-31;daf-16(RNAi)

Mean LS  
75th-% LS

% of RNAi control

Days

WT (Con)  
*daf-2(RNAi)*  
*daf-16(RNAi)*  
gcy-33 (Con)  
gcy-33;daf-2(RNAi)  
gcy-33;daf-16(RNAi)

Mean LS  
75th-% LS

% of RNAi control

Days

WT (Con)  
*daf-2(RNAi)*  
*daf-16(RNAi)*  
gcy-35 (Con)  
gcy-35;daf-2(RNAi)  
gcy-35;daf-16(RNAi)

Mean LS  
75th-% LS

% of RNAi control

Days

WT (Con)  
*daf-2(RNAi)*  
*daf-16(RNAi)*  
gcy-36 (Con)  
gcy-36;daf-2(RNAi)  
gcy-36;daf-16(RNAi)

Mean LS  
75th-% LS

% of RNAi control

Days
Fig. S9

A

![Survival vs. Days Graph]

- Survival% vs. Days graph with different oxygen concentrations (4%, 8%, 12%, 21%, 40% O₂).
- Survival rates decrease as oxygen concentration increases.
- Statistical significance indicated with P-values: $P = 0.0073$, $P = 0.0446$, $P < 10^{-4}$, $P = 0.0432$.

B

![Bar Chart]

- Bar chart showing mean lifespan and 75th-% lifespan for different oxygen concentrations.
-Significance indicated with asterisks: * indicates $P < 0.05$. 

---

Mean lifespan and 75th-% lifespan for different oxygen concentrations:

- 4% O₂: 20 days mean, 15 days 75th-%
- 8% O₂: 15 days mean, 10 days 75th-%
- 12% O₂: 10 days mean, 5 days 75th-%
- 21% O₂: 7.5 days mean, 3 days 75th-%
- 40% O₂: 5 days mean, 3 days 75th-%
Fig. S10

A

[Graph showing mean lifespan and 75th-% lifespan for 12% O₂, Control, 4% O₂, Control, 4% O₂, BAG ablation, and 4% O₂, URX ablation.]

B

[Graph showing mean lifespan and 75th-% lifespan for 12% O₂, WT, 4% O₂, WT, 4% O₂, gcy-31, 4% O₂, gcy-33, 4% O₂, gcy-35, and 4% O₂, gcy-36.]

C

[Graph showing mean lifespan and 75th-% lifespan for 12% O₂, Control, 40% O₂, Control, 40% O₂, BAG ablation, and 40% O₂, URX ablation.]

D

[Graph showing mean lifespan and 75th-% lifespan for 12% O₂, WT, 40% O₂, WT, 40% O₂, gcy-31, 40% O₂, gcy-33, 40% O₂, gcy-35, and 40% O₂, gcy-36.]