Supplementary Figure Legends

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

(**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 *gcy-31(ok296)* and *gcy-33(ok232)* mutants (**C**), *gcy-35(ok769)* and *gcy-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 *gcy-31* and *gcy-33* mutants vs. controls.

Percentages of animals exhibiting head movement, sinusoidal body posture and active locomotion among wildtype controls (n = 64), *gcy-31(ok296* (n = 41), and *gcy-33(ok232)* mutants (n = 48) at the time point of population median lifespan (Day 17 for *gcy-31* mutants, Day 18 for *gcy-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. *gcy-31(ok296)* (**A**), *gcy-33(ok232)* (**B**), *gcy-35(ok769)* (**C**) or *gcy-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 75th 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 75th 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 75th 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 75th 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 75th 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

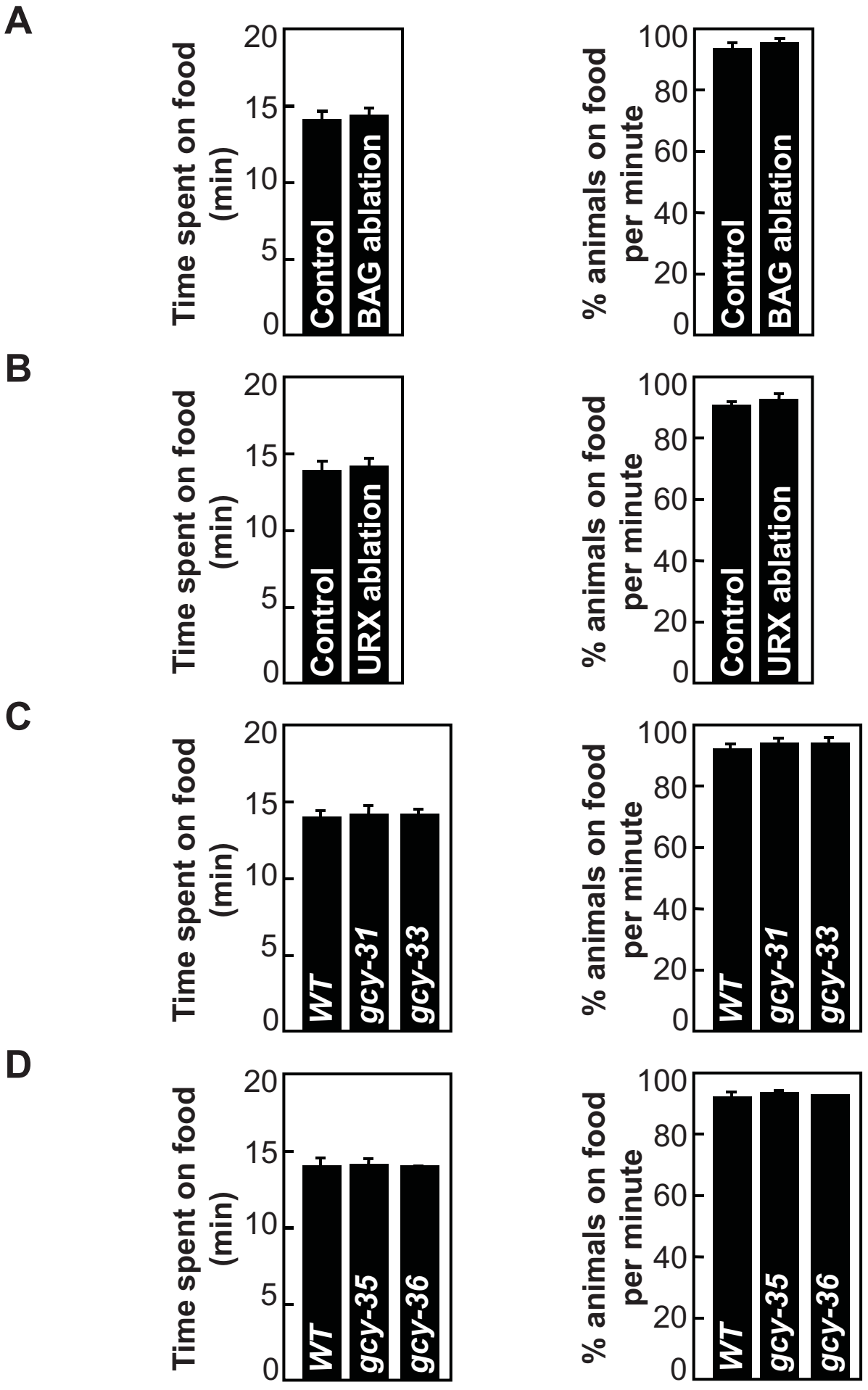

A
Time spent on food (min)
20
15
10
5
0
Control
BAG ablation
% animals on food per minute
100
80
60
40
20
0
Control
BAG ablation
B
Time spent on food (min)
Control
URX ablation
% animals on food per minute
Control
URX ablation
C
Time spent on food (min)
WT
gcy-31
gcy-33
% animals on food per minute
WT
gcy-31
gcy-33
D
Time spent on food (min)
WT
gcy-35
gcy-36
% animals on food per minute
WT
gcy-35
gcy-36

Fig. S2

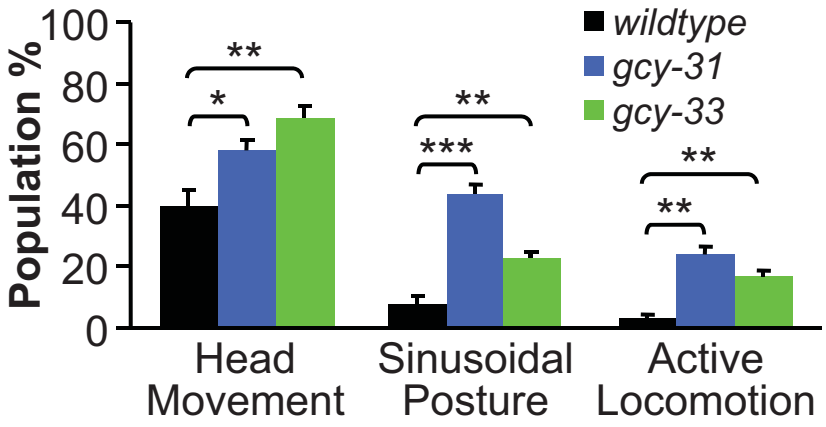
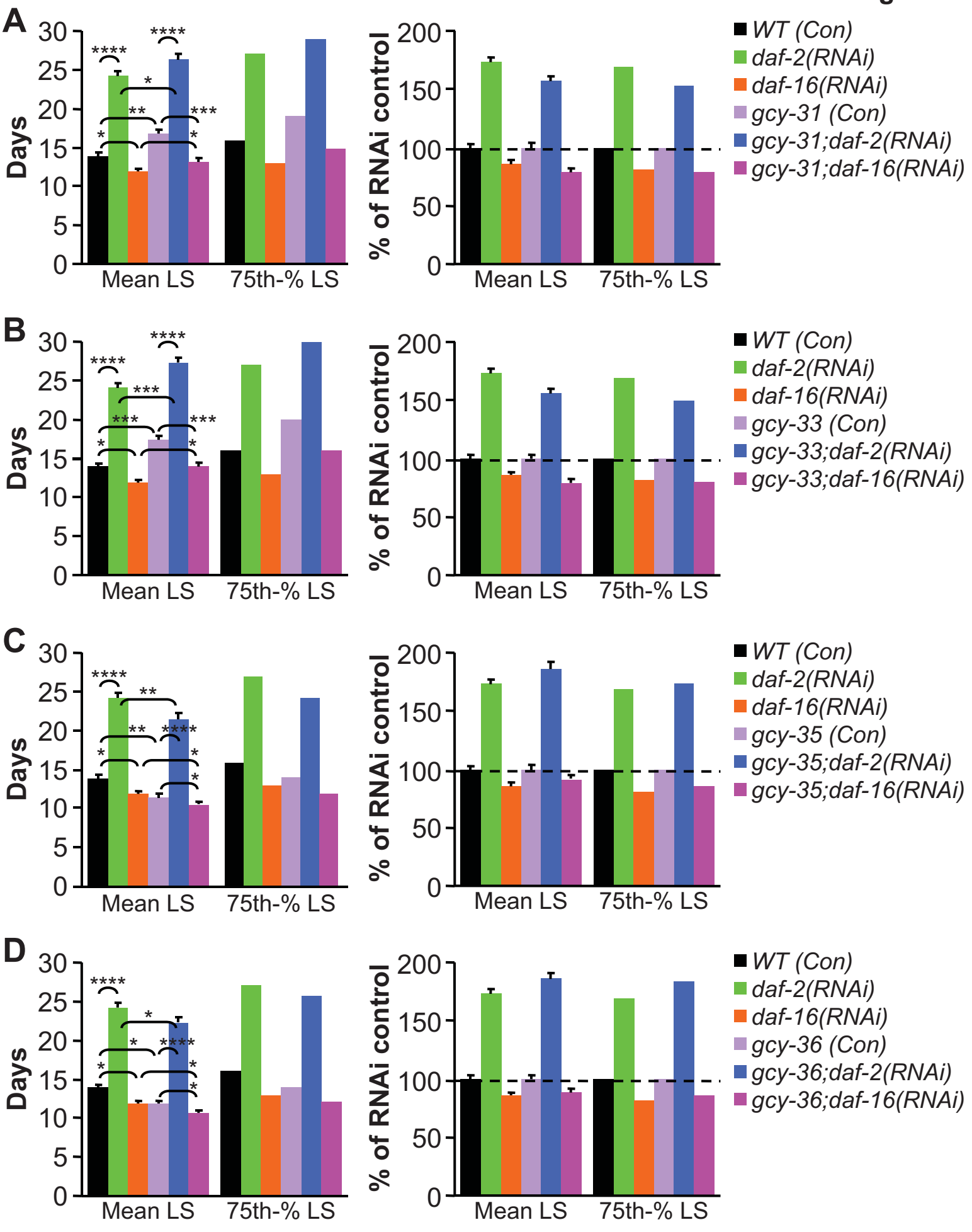

Population %
100
80
60
40
20
0
wildtype
gcy-31
gcy-33
**
*
**
***
**
**
Head Movement
Sinusoidal Posture
Active Locomotion

Fig. S3


A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT (Con)
daf-2(RNAi)
daf-16(RNAi)
gcy-31 (Con)
gcy-31;daf-2(RNAi)
gcy-31;daf-16(RNAi)
gcy-33 (Con)
gcy-33;daf-2(RNAi)
gcy-33;daf-16(RNAi)
gcy-35 (Con)
gcy-35;daf-2(RNAi)
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gcy-36;daf-2(RNAi)
gcy-36;daf-16(RNAi)

Fig. S4

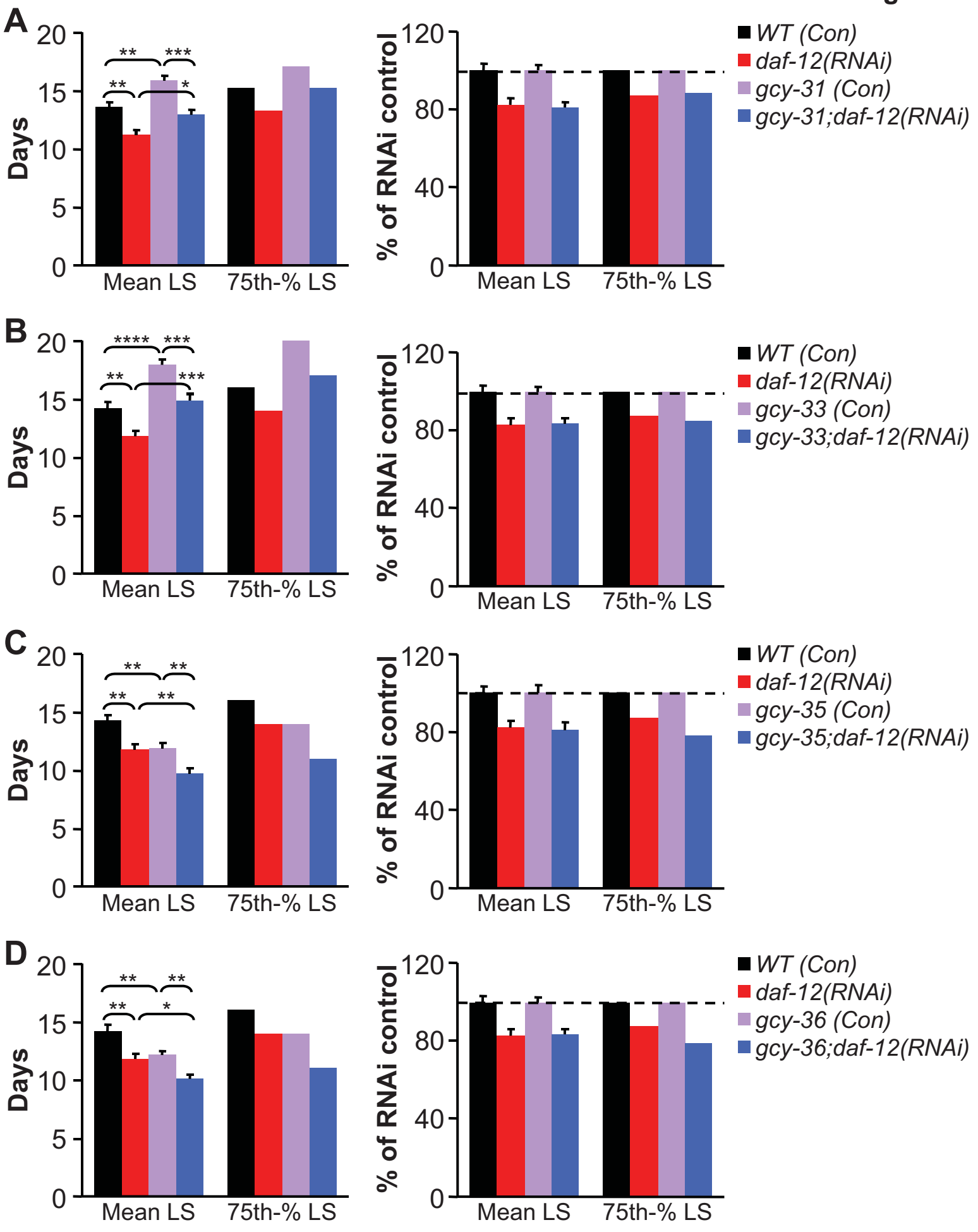

A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT (Con)
daf-12(RNAi)
gcy-31 (Con)
gcy-31;daf-12(RNAi)
gcy-33 (Con)
gcy-33;daf-12(RNAi)
gcy-35 (Con)
gcy-35;daf-12(RNAi)
gcy-36 (Con)
gcy-36;daf-12(RNAi)

Fig. S5

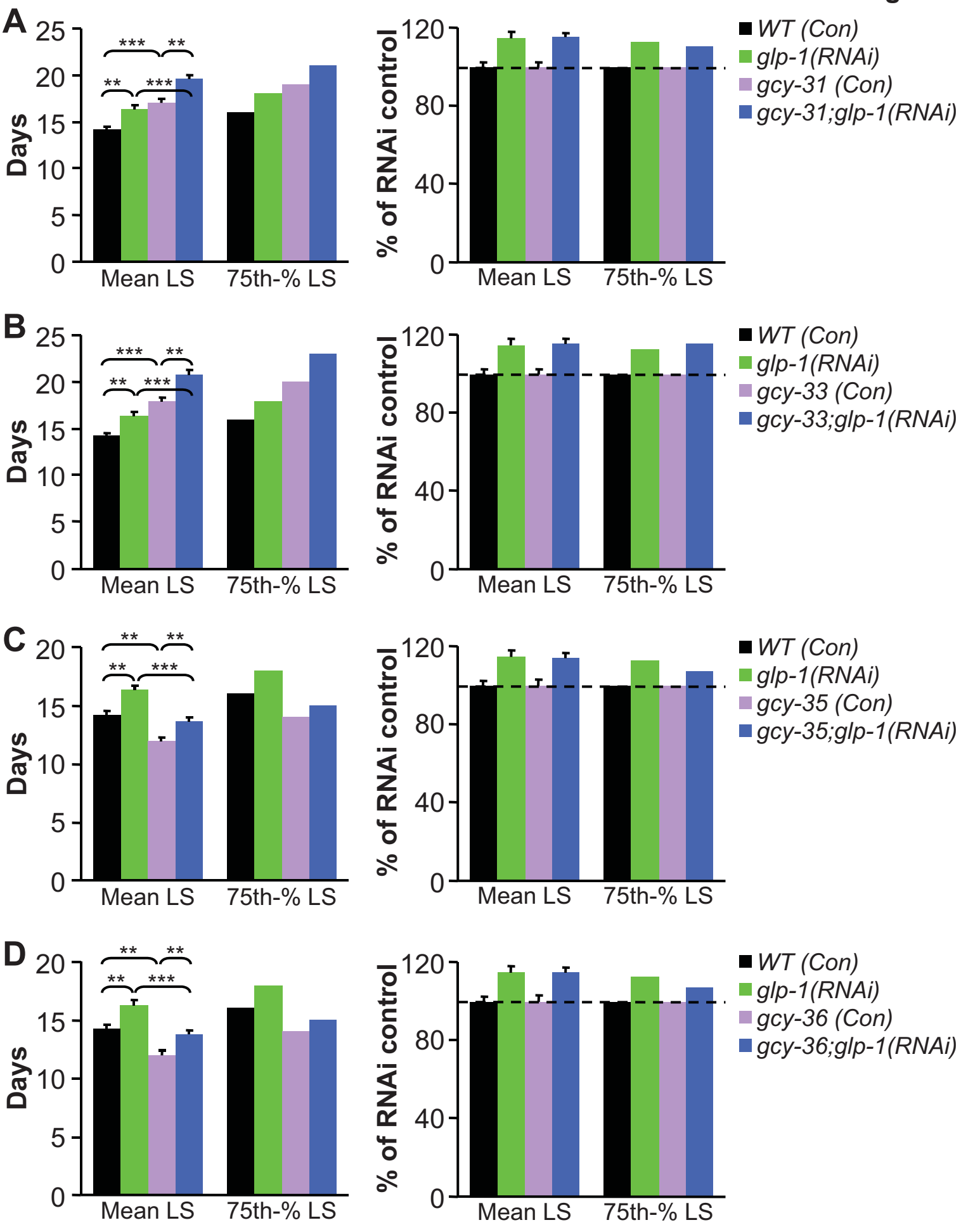

A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT (Con)
glp-1(RNAi)
gcy-31 (Con)
gcy-31;glp-1(RNAi)
gcy-33 (Con)
gcy-33;glp-1(RNAi)
gcy-35 (Con)
gcy-35;glp-1(RNAi)
gcy-36 (Con)
gcy-36;glp-1(RNAi)

Fig. S6

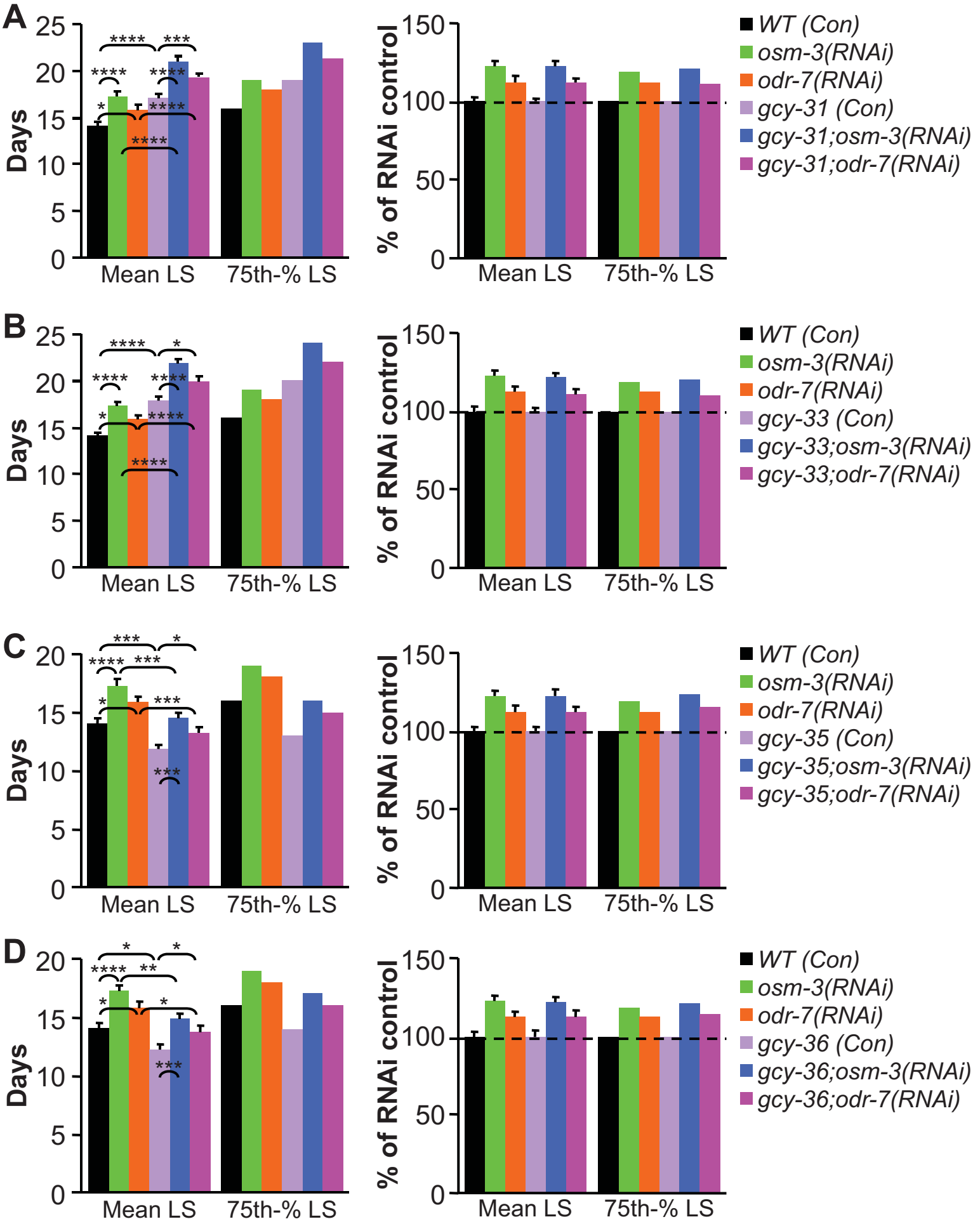

A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT (Con)
osm-3(RNAi)
odr-7(RNAi)
gcy-31 (Con)
gcy-31;osm-3(RNAi)
gcy-31;odr-7(RNAi)
gcy-33 (Con)
gcy-33;osm-3(RNAi)
gcy-33;odr-7(RNAi)
gcy-35 (Con)
gcy-35;osm-3(RNAi)
gcy-35;odr-7(RNAi)
gcy-36 (Con)
gcy-36;osm-3(RNAi)
gcy-36;odr-7(RNAi)

Fig. S7

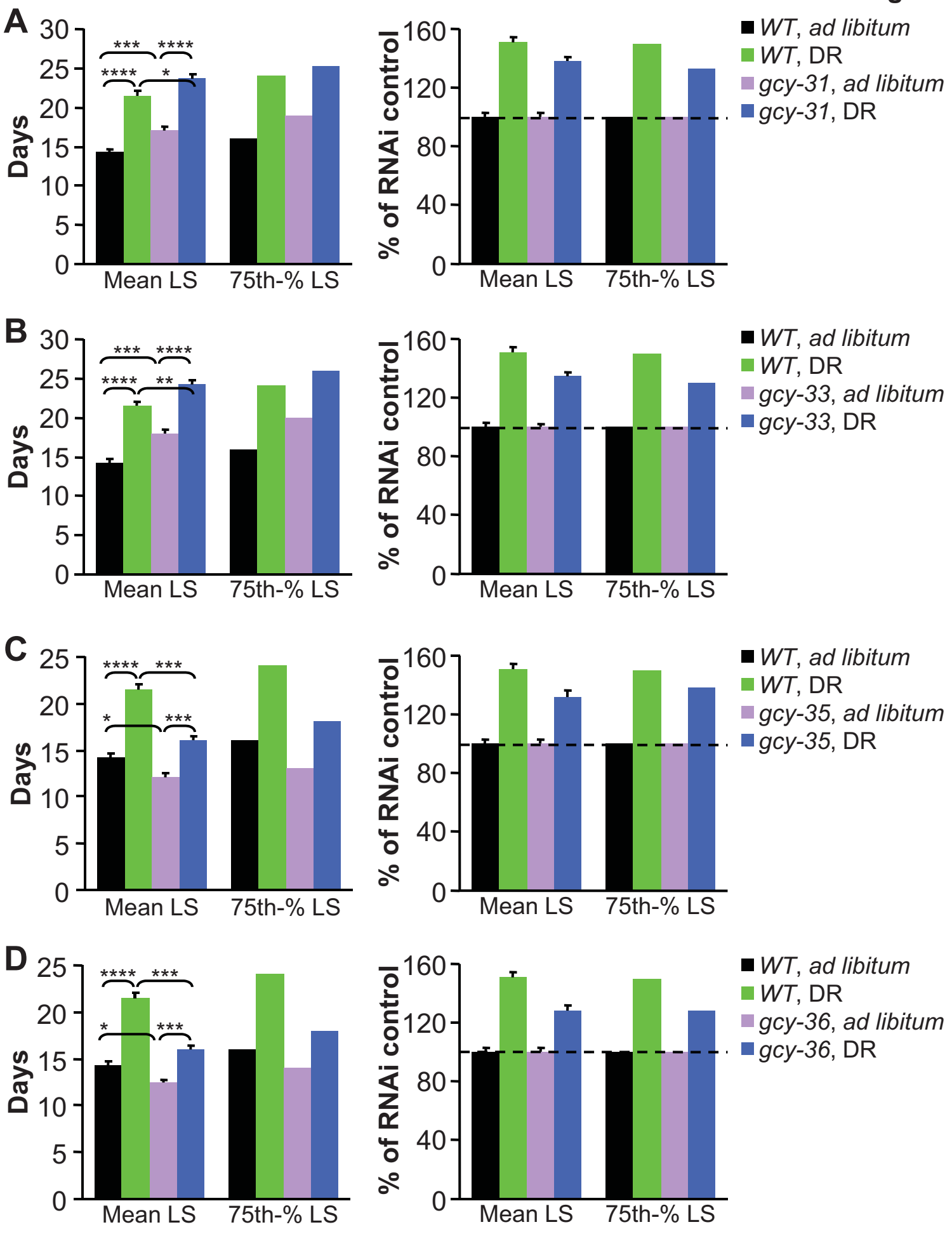

A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT, ad libitum
WT, DR
gcy-31, ad libitum
gcy-31, DR
gcy-33, ad libitum
gcy-33, DR
gcy-35, ad libitum
gcy-35, DR
gcy-36, ad libitum
gcy-36, DR

Fig. S8

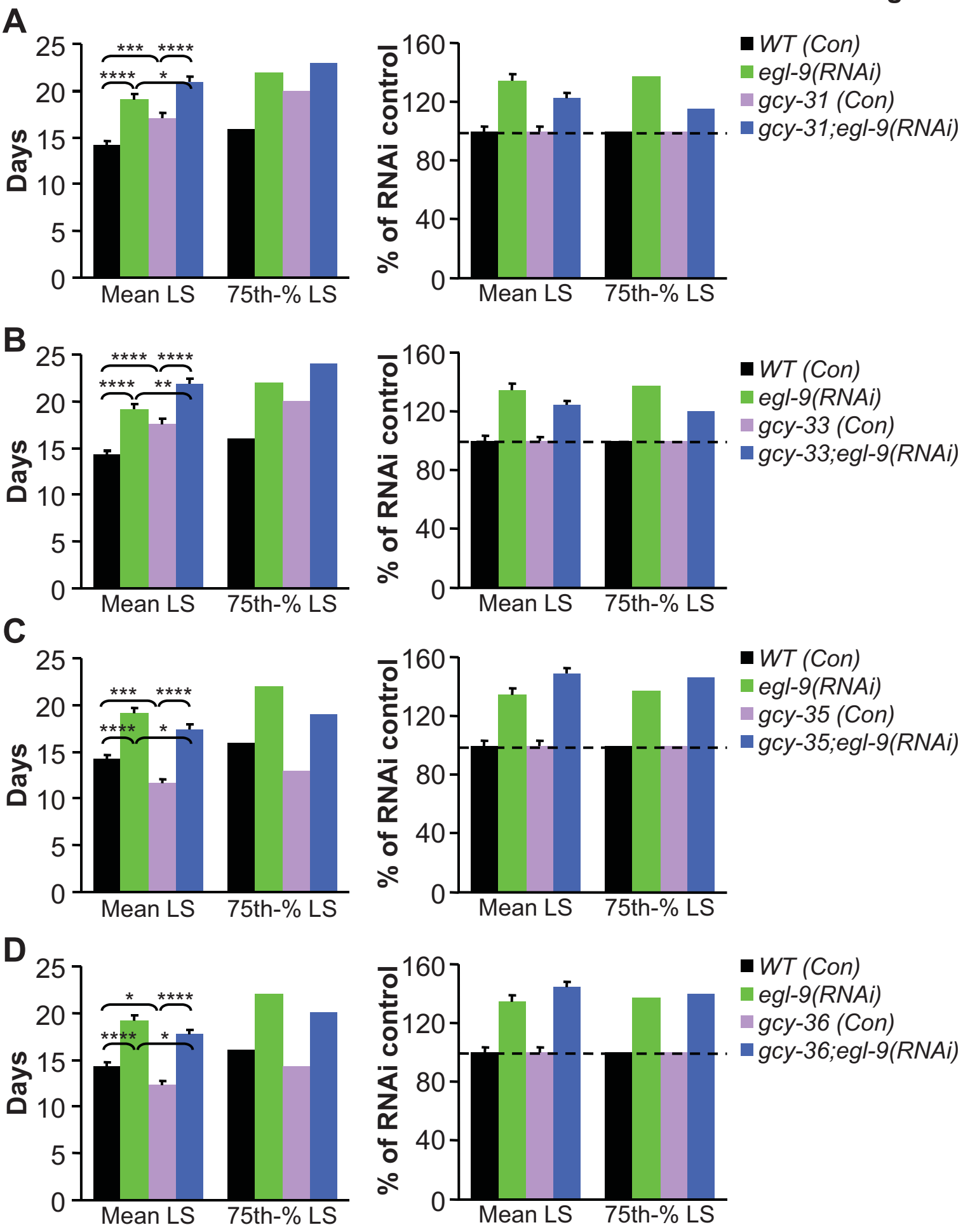

A
B
C
D
Days
% of RNAi control
Mean LS
75th-% LS
WT (Con)
egl-9(RNAi)
gcy-31 (Con)
gcy-31;egl-9(RNAi)
gcy-33 (Con)
gcy-33;egl-9(RNAi)
gcy-35 (Con)
gcy-35;egl-9(RNAi)
gcy-36 (Con)
gcy-36;egl-9(RNAi)

Fig. S9

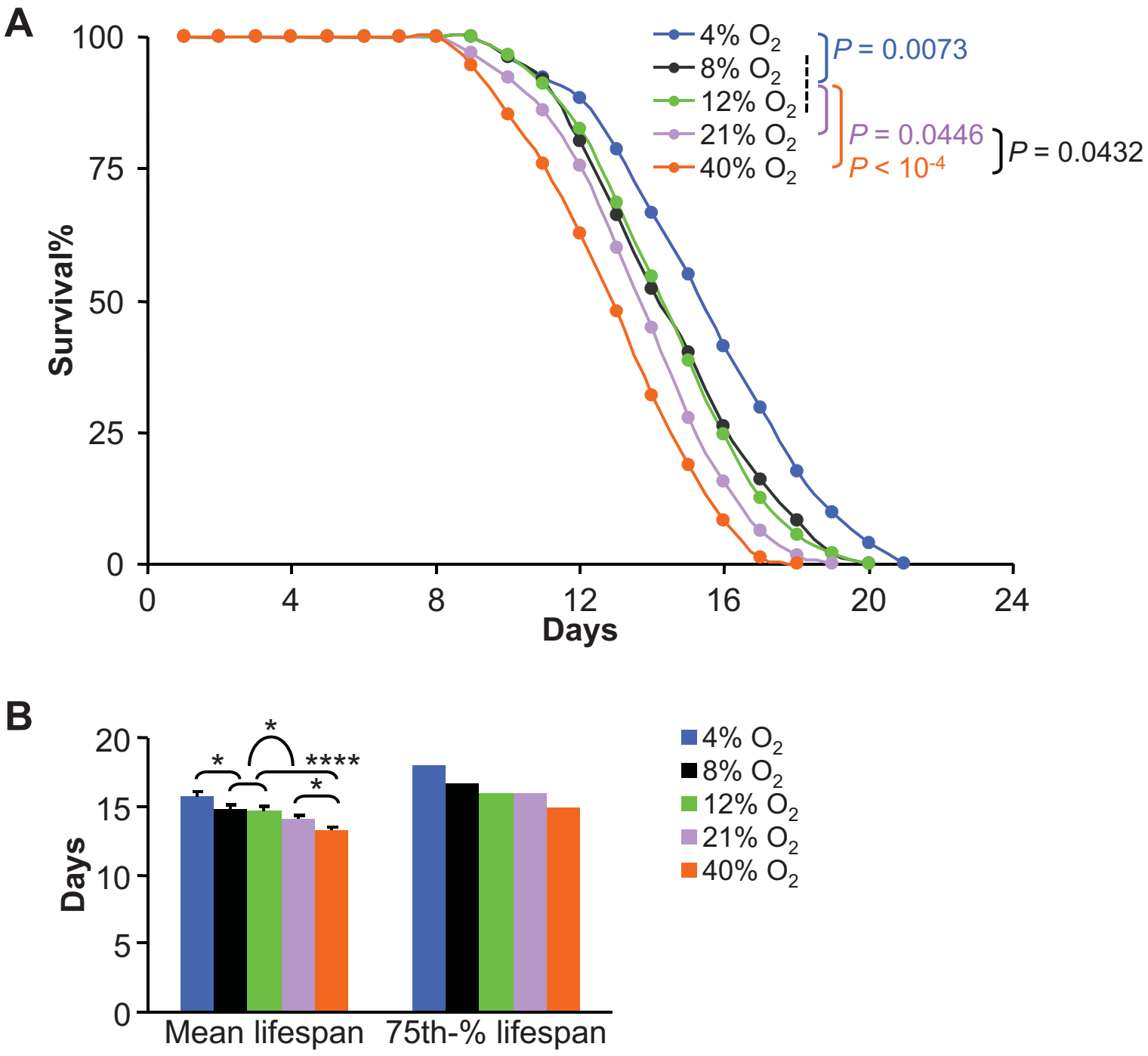
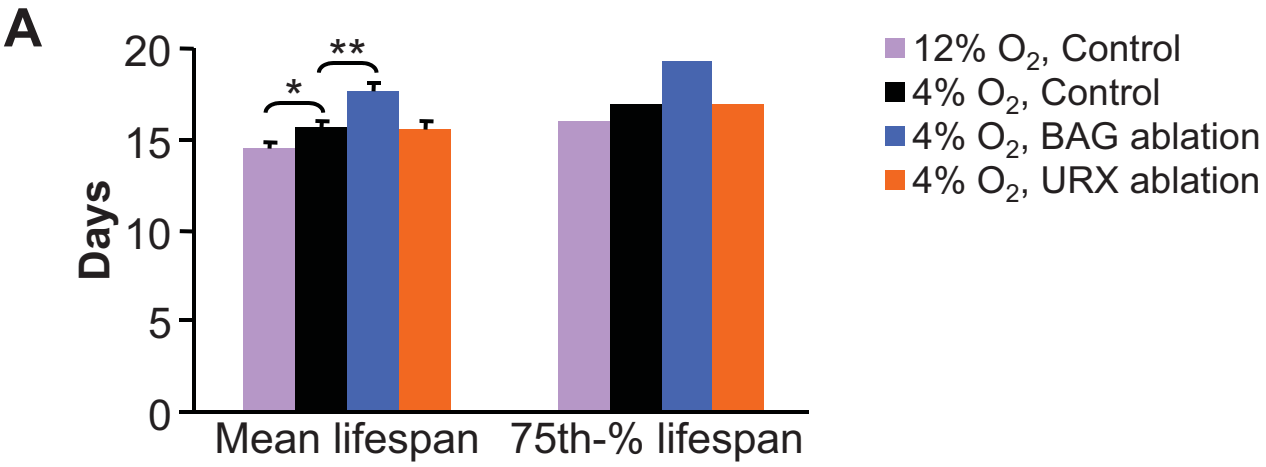
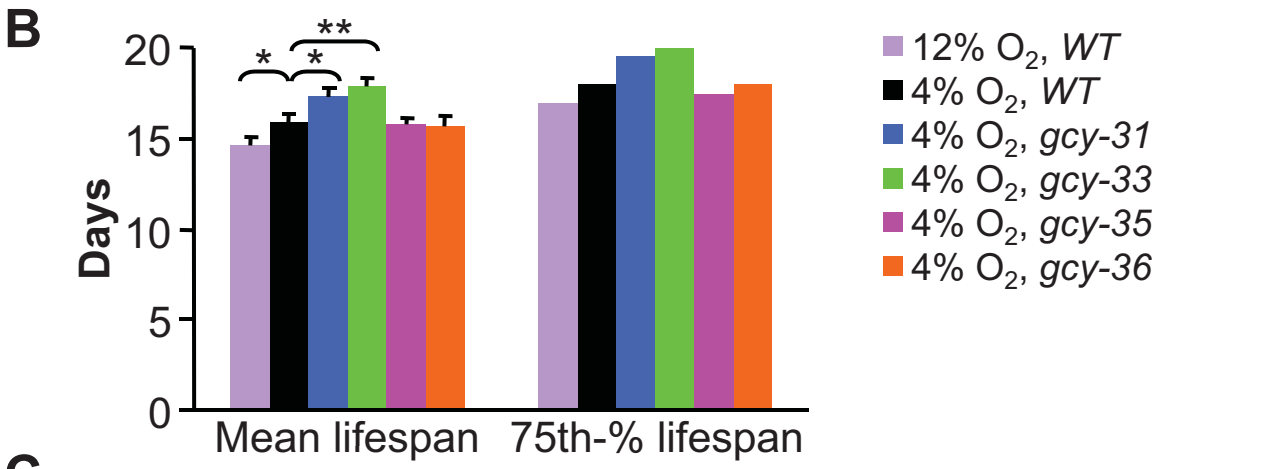
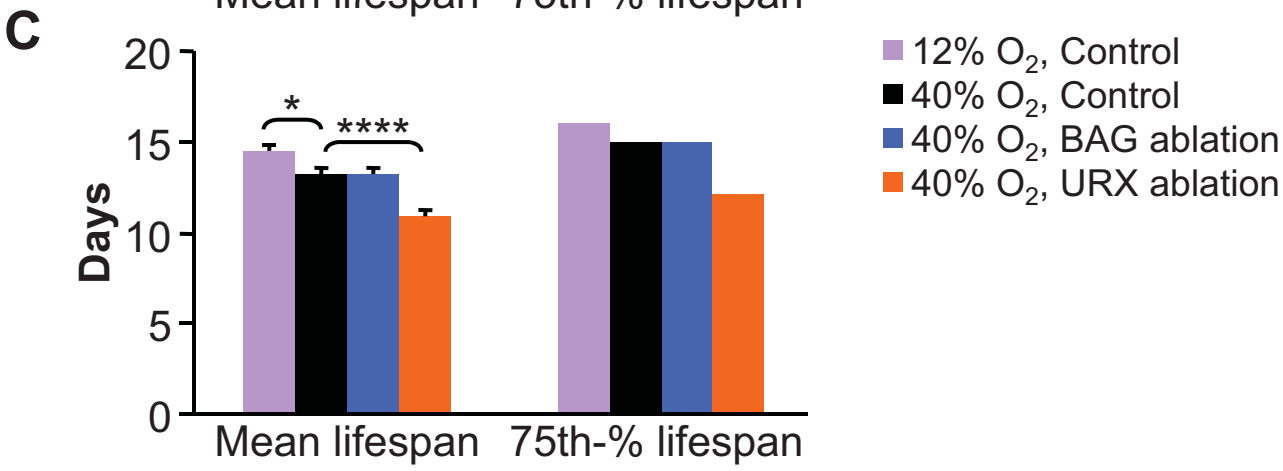
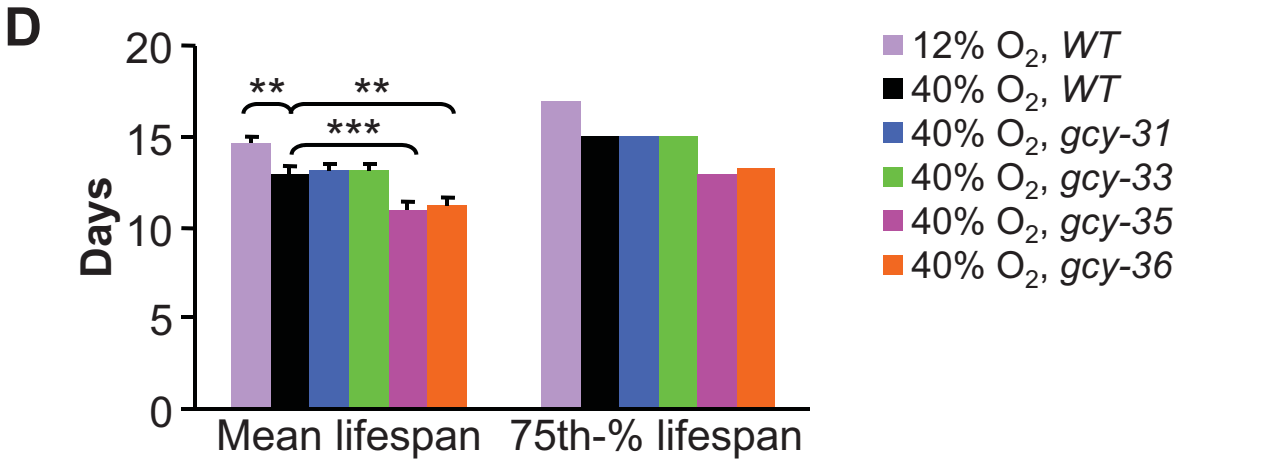

A
100
75
50
25
0
Survival%
0
4
8
12
16
20
24
Days
4% O2
8% O2
12% O2
21% O2
40% O2
P = 0.0073
P = 0.0446
P < 10-4
P = 0.0432
B
20
15
10
5
0
Days
*
*
****
*
Mean lifespan
75th-% lifespan
4% O2
8% O2
12% O2
21% O2
40% O2

Fig. S10


A
20
15
10
5
0
Days
*
**
Mean lifespan
75th-% lifespan
12% O2, Control
4% O2, Control
4% O2, BAG ablation
4% O2, URX ablation


B
20
15
10
5
0
Days
*
*
**
Mean lifespan
75th-% lifespan
12% O2, WT
4% O2, WT
4% O2, gcy-31
4% O2, gcy-33
4% O2, gcy-35
4% O2, gcy-36


C
20
15
10
5
0
Days
*
****
Mean lifespan
75th-% lifespan
12% O2, Control
40% O2, Control
40% O2, BAG ablation
40% O2, URX ablation


D
20
15
10
5
0
Days
**
**
***
Mean lifespan
75th-% lifespan
12% O2, WT
40% O2, WT
40% O2, gcy-31
40% O2, gcy-33
40% O2, gcy-35
40% O2, gcy-36