Supplementary Figure Legends

Fig. S1. The effect of FUdR on worm lifespan. While the lifespan of wild-type worms is not affected by the presence of 100 µM FUdR, some strains, such as the mitochondrial mutant *gas-1*, exhibit marked increases in lifespan on plates containing 100 µM FUdR compared to NGM plates (A), while other strains, such as the mitochondrial superoxide deletion mutant *sod-2*, show identical lifespans under both conditions (B).

Fig. S2. *mev-1* worms exhibit decreased lifespan on FUdR plates. *mev-1* encodes subunit C of the succinate dehydrogenase complex of the electron transport chain. Like *gas-1* mutants, *mev-1* worms have decreased mitochondrial function and decreased lifespan on NGM plates. To determine whether FUdR could increase *mev-1* lifespan similar to *gas-1*, we examined the lifespan of *mev-1* worms on plates containing 100 µM, the concentration where *gas-1* worms exhibit the greatest increase in lifespan. In contrast to *gas-1* worms, *mev-1* worms were still short-lived on plates containing 100 µM FUdR. This indicates that the ability of FUdR to convert *gas-1* from short-lived to long-lived does not apply to all mitochondrial mutants.

Fig. S3. Examining the mechanism of lifespan increase by FUdR. There are multiple obvious differences that might account for lifespan increases observed in the presence of FUdR. (A) Since FUdR prevents the development of progeny, it is not necessary to transfer worms every 1-2 days during the initial phases of a lifespan assay. It is possible that FUdR increases lifespan by decreasing the number of times the worms are transferred (i.e. decreasing stress). To investigate this possibility, we examined the lifespan of *gas-1* worms on NGM and 100 µM FUdR plates, in which the worms on FUdR plates were always transferred when the worms on NGM plates were transferred. Under these conditions of equal numbers of transfers, *gas-1* lifespan was still increased on FUdR plates. (B) Since bacteria does not grow as well on plates containing FUdR and bacterial dilution leads to increased lifespan likely by dietary restriction, it is possible that FUdR increases lifespan by inhibiting bacterial growth. To investigate this possibility, we examined *gas-1* lifespan on NGM and 100 µM FUdR plates seeded with heat-killed bacteria to eliminate differences in bacterial growth. Under these conditions, *gas-1* worms grown on FUdR plates still lived longer than *gas-1* worms grown on NGM plates indicating that dietary

restriction by inhibiting bacterial growth does not explain the effect of FUdR on lifespan. (C) Finally, it is possible that FUdR increases lifespan by decreasing reproduction as the germline has been shown to influence lifespan. To test this hypothesis, we examined the lifespan of *glp-1* worms on NGM and 100 µM FUdR plates under conditions where these worms are fertile (20°C) and conditions where these worms are sterile (25°C). We found that FUdR was able to increase *glp-1* lifespan under conditions where the worms are sterile suggesting that the effect of FUdR on reproduction does not account for its effect on lifespan. In support of this conclusion, *gas-1* worms, which show a marked increase in lifespan on FUdR plates, generally produce a very small brood. Error bars indicate SEM. * $p<0.05$.

Fig. S4. Effect of FUdR on development of progeny. FUdR is used the prevent the progeny of experimental worms from reaching adulthood thereby eliminating the need to frequently transfer experimental worms and the possibility of mixing up experimental worms with their offspring. To determine which concentration of FUdR is necessary to prevent the progeny of experimental animals from developing to adulthood, young adult worms were transferred to plates containing different concentrations of FUdR and the development of their progeny was monitored. (A) FUdR exhibits a dose-dependent effect on progeny development. (B) Examination of the furthest developmental stage reached by the progeny of the experimental worms grown on different concentrations of FUdR reveals that worms grown on concentrations of 50 µM FUdR or higher prevents progeny from growing to adulthood. At these concentrations, progeny do not develop past the L1 stage. Since 50 µM FUdR still can have a significant impact on lifespan, we tested whether lower concentrations of FUdR could be used if experimental worms were transferred after the first 3 days of adulthood. (C) If adult worms are transferred to a new plate after 3 days, worms grown on concentrations of 1 µM FUdR or higher do not produce any progeny, while the progeny of worms grown on concentrations up to 0.25 µM FUdR are able to develop to adulthood. (D) Examination of the number of days from the addition of adult worms until their progeny develop to adulthood reveals a delay in development beginning at concentrations of 1 µM FUdR. At concentrations of 1 µM FUdR and higher it is not difficult to distinguish parents from progeny on day 3 of adulthood (indicated by red line). Thus, a lifespan study can be conducted at 1 µM FUdR if the worms are transferred after 3 days of adulthood and they will not produce any progeny on the second plate. Error bars indicate SEM. A = adult, FA = fertile adult.

Supplementary Material and Methods

Strains. The following strains were used in these studies: wild-type(N2), *gas-1(fc21)*: subunit of mitochondrial complex I, *sod-2(ok1030)*: primary superoxide dismutase in mitochondrial matrix, *mev-1(kn-1)*: succinate dehydrogenase complex II subunit C, *glp-1(e2141)*: N-glycosylated transmembrane protein. Strains were maintained at 20°C on OP50 bacteria.

Lifespan. Lifespan studies were completed on NGM plates containing concentrations of FUdR ranging from 0 to 100 µM. FUdR was prepared as a 1 M solution and frozen at -80°C until use. FUdR was added to NGM media just prior to pouring plates. Unless indicated lifespan assays were performed at 20°C. To test lifespan on heat-killed bacteria, OP50 bacteria was heated to 70°C for 1 hour and then concentrated by centrifugation and removal of supernatant. For *glp-1* lifespan assays under conditions of sterility, worms were grown at 25°C until adulthood and then shifted to 20°C for the completion of the lifespan assay.

Development. To examine the effect of FUdR on the development of progeny, young adult worms (parents) were transferred to plates containing 0 to 100 µM FUdR (first plate). The developmental stage of the progeny was monitored daily for 10 days. Fertile adults are those who produce L1 worms. In some experiments, the parents were transferred to a second plate after three days on the first plate. The development of progeny on the second plate was monitored daily.

Statistical Analysis. Differences between multiple conditions were compared by ANOVA. Individual comparisons between two conditions were assessed using a student's t-test. Kaplan-Meier survival curves were compared using the Mandel-Cox Long-Rank test.

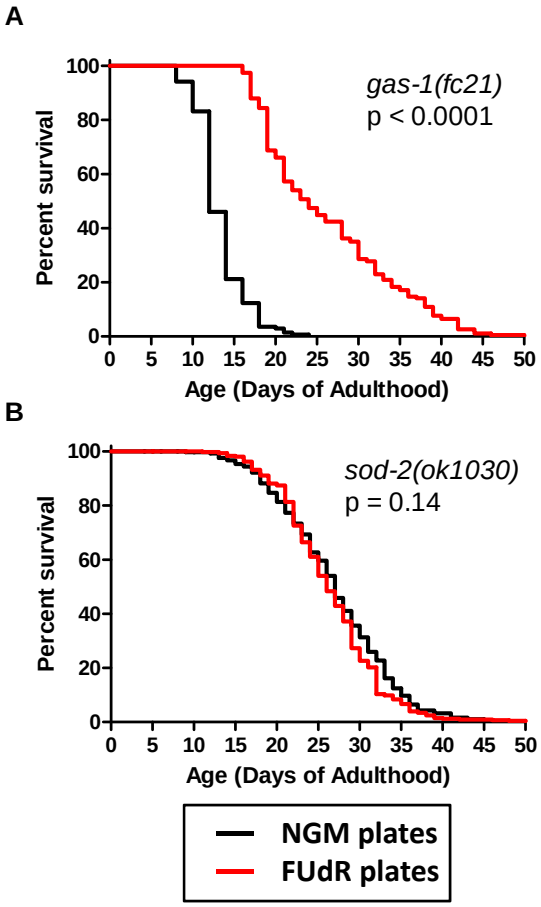
A
Percent survival
100
80
60
40
20
0
0 5 10 15 20 25 30 35 40 45 50
Age (Days of Adulthood)
gas-1(fc21)
p < 0.0001
B
Percent survival
100
80
60
40
20
0
0 5 10 15 20 25 30 35 40 45 50
Age (Days of Adulthood)
sod-2(ok1030)
p = 0.14
NGM plates
FUdR plates

Fig. S1.

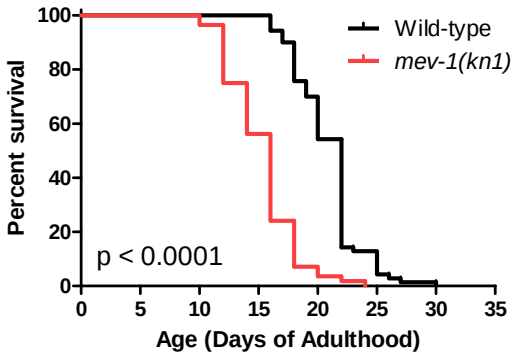
Wild-type
mev-1(kn1)
Percent survival
100
80
60
40
20
0
p < 0.0001
0
5
10
15
20
25
30
35
Age (Days of Adulthood)

Fig. S2.

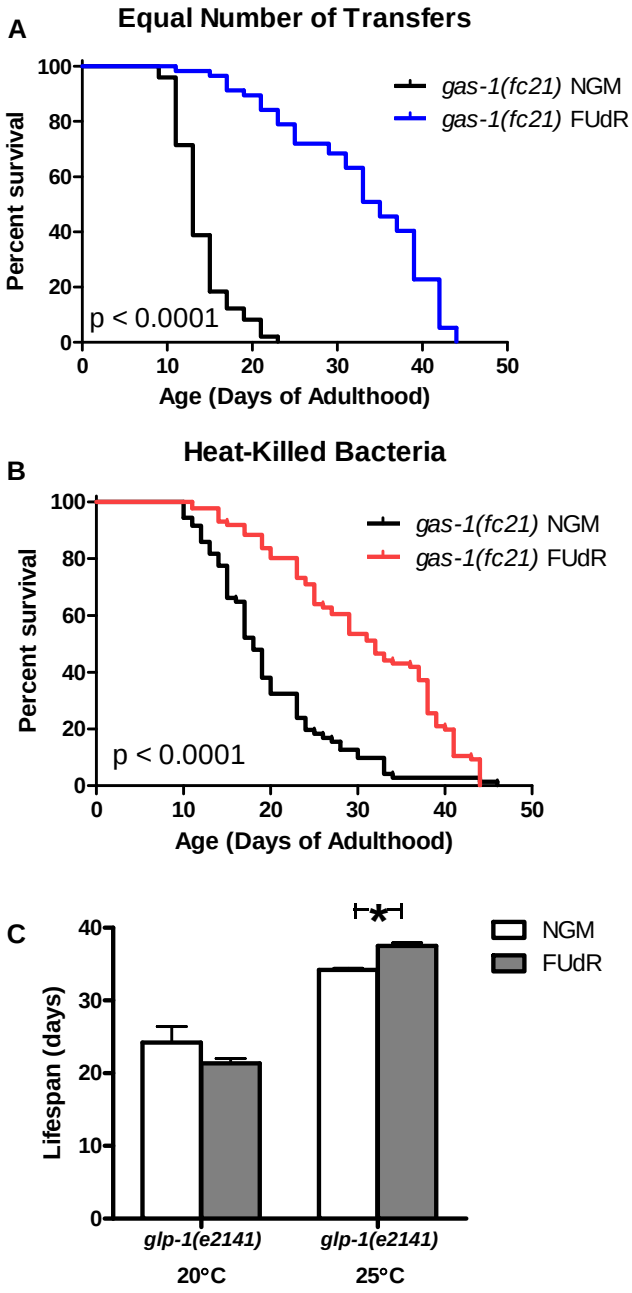
A
Equal Number of Transfers
Percent survival
100
80
60
40
20
0
p < 0.0001
0
10
20
30
40
50
Age (Days of Adulthood)
gas-1(fc21) NGM
gas-1(fc21) FUdR
B
Heat-Killed Bacteria
Percent survival
100
80
60
40
20
0
p < 0.0001
0
10
20
30
40
50
Age (Days of Adulthood)
gas-1(fc21) NGM
gas-1(fc21) FUdR
C
Lifespan (days)
40
30
20
10
0
*
NGM
FUdR
glp-1(e2141)
20°C
glp-1(e2141)
25°C

Fig. S3.

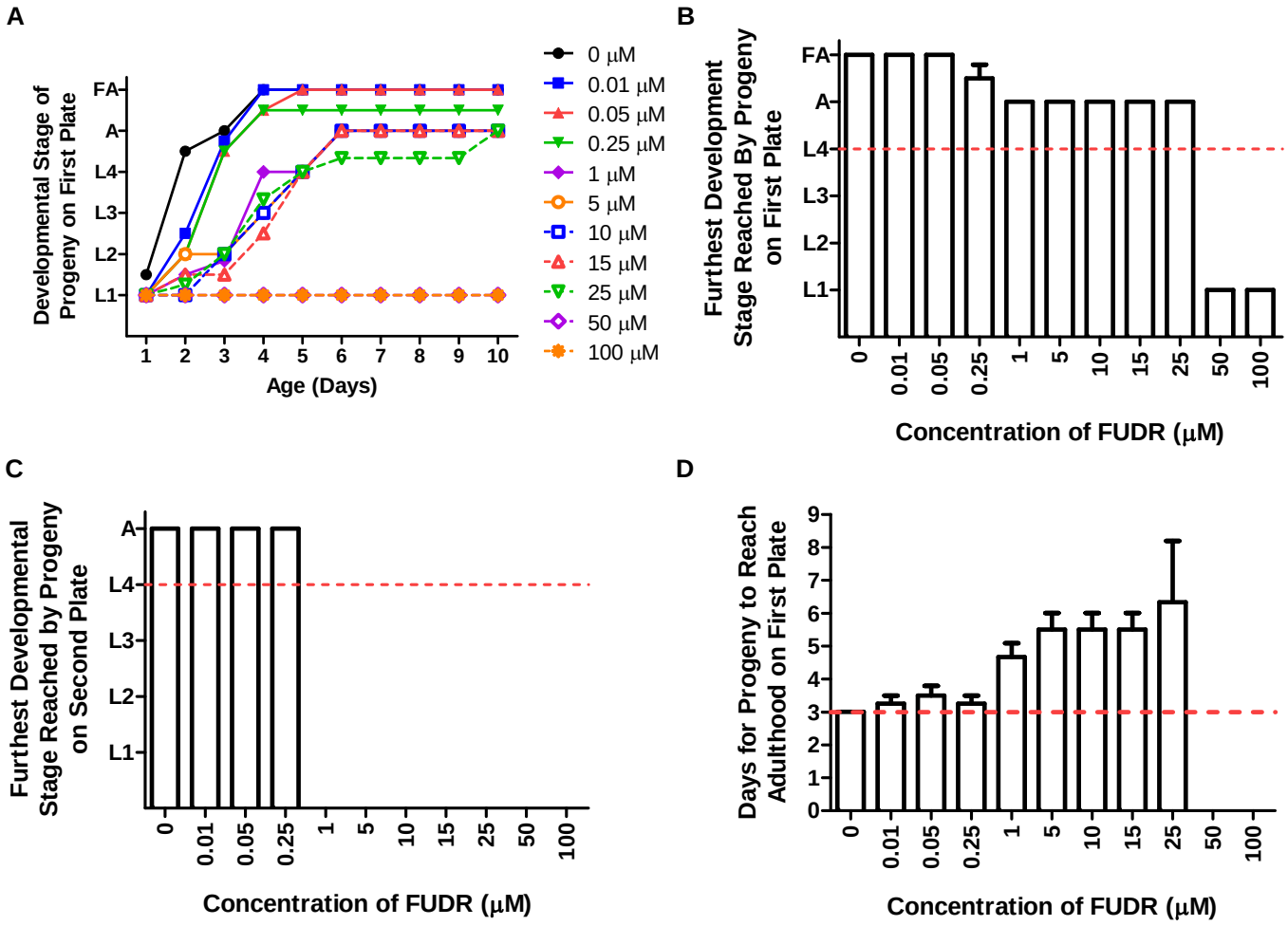
A
Developmental Stage of Progeny on First Plate
FA
A
L4
L3
L2
L1
1 2 3 4 5 6 7 8 9 10
Age (Days)
0 µM
0.01 µM
0.05 µM
0.25 µM
1 µM
5 µM
10 µM
15 µM
25 µM
50 µM
100 µM
B
Furthest Development Stage Reached By Progeny on First Plate
FA
A
L4
L3
L2
L1
0 0.01 0.05 0.25 1 5 10 15 25 50 100
Concentration of FUDR (µM)
C
Furthest Developmental Stage Reached by Progeny on Second Plate
A
L4
L3
L2
L1
0 0.01 0.05 0.25 1 5 10 15 25 50 100
Concentration of FUDR (µM)
D
Days for Progeny to Reach Adulthood on First Plate
0 1 2 3 4 5 6 7 8 9
0 0.01 0.05 0.25 1 5 10 15 25 50 100
Concentration of FUDR (µM)

Fig. S4.