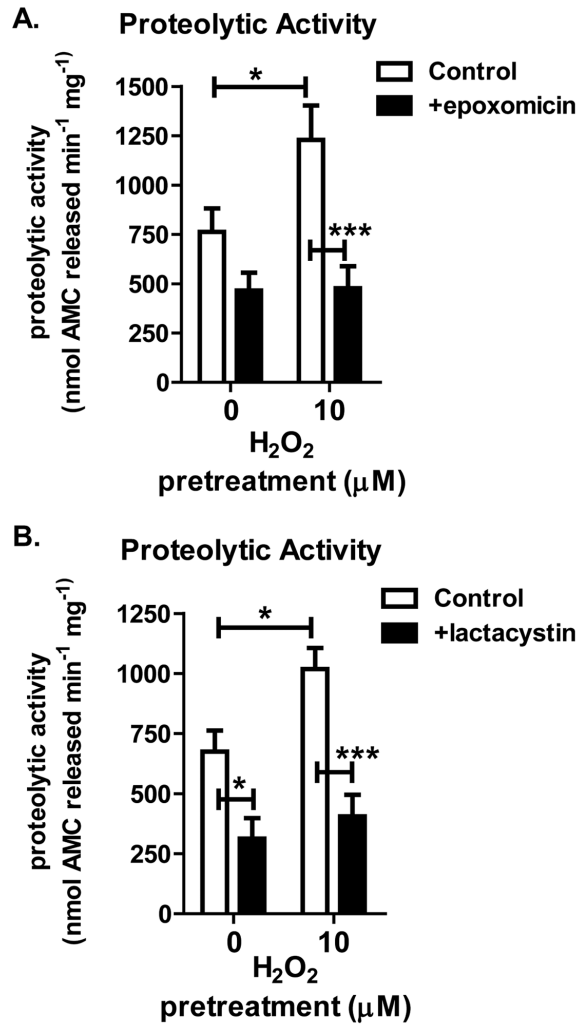
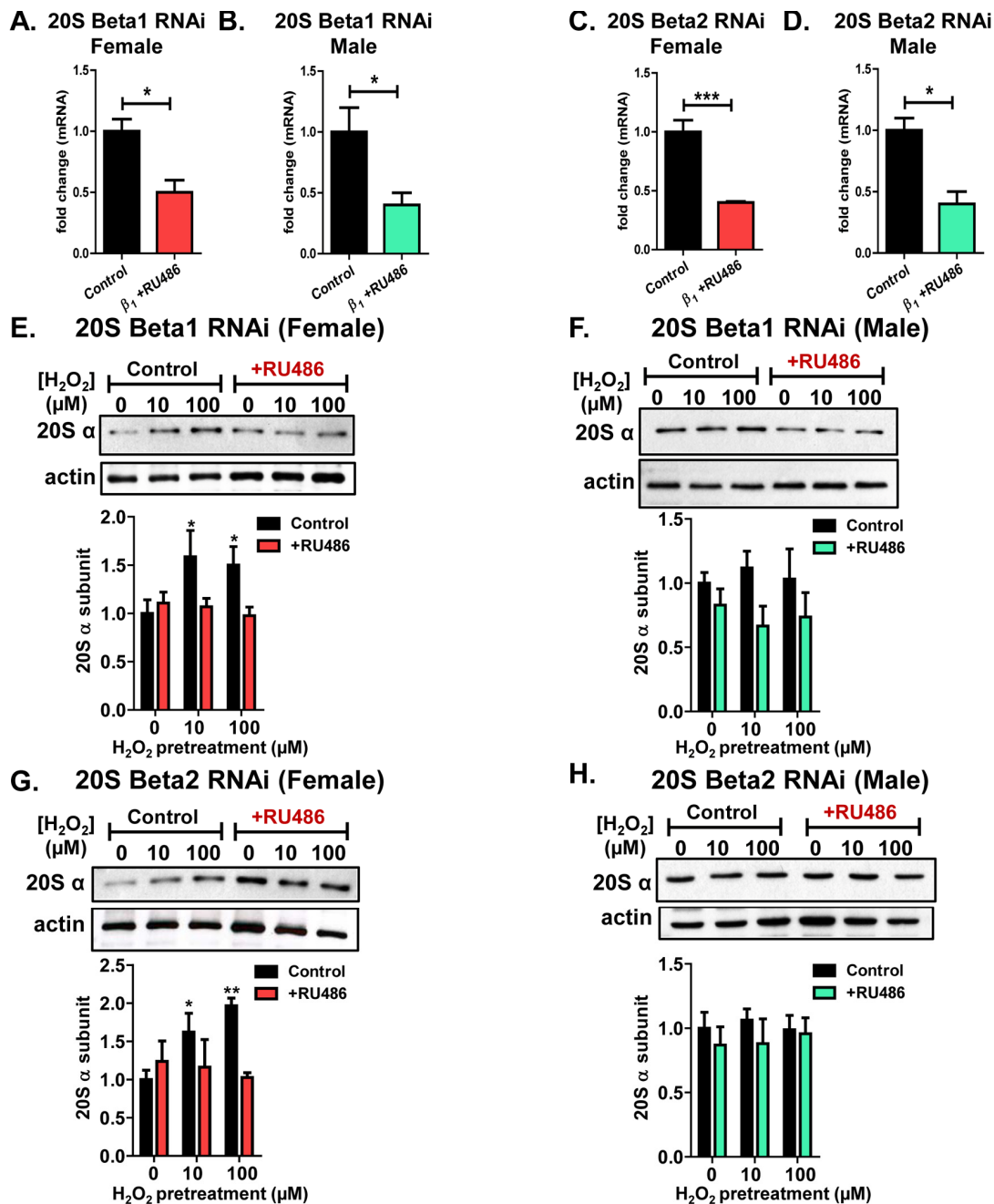


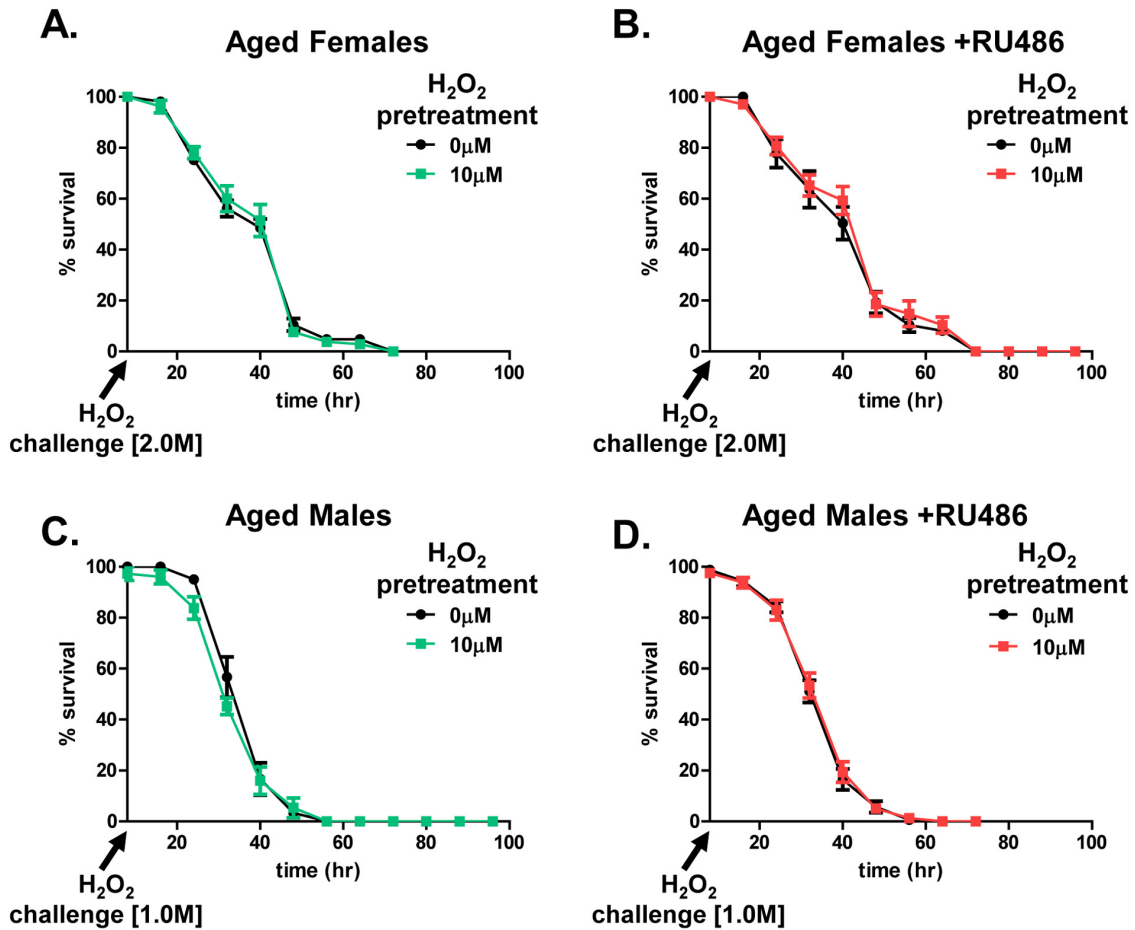
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Inhibition of the 20S proteasome proteolytic activity in young (3 day old) female flies. To test whether the hydrogen peroxide induced proteolytic activity in young female flies is proteasome dependent, three day old female progeny of the Actin-GS-255B strain crossed to the w[1118] strain were either pretreated with 10μM H₂O₂ or not pretreated with H₂O₂. Whole flies were homogenized and lysed in proteolysis buffer and sample lysates were then incubated for 30 minutes either alone (white) “control”, or with proteasome inhibitors (black). (A) epoxomicin or (B) lactacystin, prior to the addition of the Suc-LLVY-AMC proteasome fluoropeptide substrate. Error bars denote standard error of the mean (S.E.M) values. * P < 0.05, and *** P < 0.001, relative to control not treated with inhibitor, using one-way ANOVA.



Supplementary Figure S2. Loss of the 20S proteasome abrogates the adaptive response. (A-H) Progeny of the Actin-GS-255B strain crossed to the β_1 or β_2 RNAi strains were incubated for 5 days in the absence or presence of RU486, to block the transcription/translation-dependent adaptive increase in proteasome expression following H₂O₂ pretreatment. RNAi conditions blocked increased proteasome expression, without depressing basal protein levels. At least 50% decrease in mRNA in RNAi strains, and within proteasome western blots and activity, blockage of the adaptive increase. (A,C) In the presence of RU486 (pink), females show 50% decrease in mRNA for both beta subunits. (B,D) Similarly, in the presence of RU486 (blue), males show at least 50% reduction in the amount of mRNA. (E-H) Following incubation, in the presence or absence of RU486, flies were either pretreated with adaptive doses of H₂O₂ [10 μM or 100 μM] or used as controls, before whole flies were collected for analysis of protein expression. (E,G) In the absence of RU486 (black), pretreated females show a robust increase in the 20S α expression, which was blocked in RU486-fed females (pink). (F,H) Irrespective of the absence or presence of RU486, males showed no change in 20S α expression. Western blots were performed in triplicate, normalized to Actin-HRP. Error bars denote standard error of the mean (S.E.M) values. * P < 0.05, ** P < 0.01, *** P < 0.001, relative to the control using one-way ANOVA.



Supplementary Figure S3. RU486 by itself does not affect H₂O₂ adaptation or resistance in aged (60 day old) male or female flies. (A-D) Female and male progeny of the Actin-GS-255B strain were crossed to the w[1118] strain aged in the absence or presence of RU486, continually, for 60 days and then used as controls, or given a 10 μM H₂O₂ pretreatment, prior to exposure to a 2.0M H₂O₂ challenge (females) or a 1.0M H₂O₂ challenge. (A) 60 day old females raised in the absence of RU486. (B) 60 day old females raised in the presence of RU486. (C) 60 day old males raised in the absence of RU486. (D) 60 day old males raised in the presence of RU486. Blue line indicates males and females, raised in the absence of RU486, pretreated with 10 μM H₂O₂. Pink line indicates males and females, raised in the present of RU486, pretreated with 10 μM H₂O₂. Statistical summary is located in Supplementary Table S5.

Supplementary Table S1. Hydrogen peroxide adaptation in 3 day and 60 day old males and females statistical summary. Two cohorts of 3 day old and 60 day old females and males either not pretreated with H2O2 or pretreated [10µM] H2O2. Crosses are in order males x virgin females.

Female (Cohort 1)

| Genotype | Age (day) | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-----------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| w[1118] x Actin-GS-255B | 3 | 0µM | 69 | 82 (4) | 84 | 104 | | | |
| w[1118] x Actin-GS-255B | 3 | 10µM | 81 | 94 (4) | 95 | 108 | 9.42 | 7.69 | 0.0009 |
| w[1118] x Actin-GS-255B | 60 | 0µM | 97 | 49 (16) | 48 | 73 | | | |
| w[1118] x Actin-GS-255B | 60 | 10µM | 106 | 49 (16) | 48 | 73 | -0.778 | 0 | 0.7997 |

Female (Cohort 2)

| Genotype | Age (day) | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-----------|-------------------------------|-----|-----------|--------|-----|----------|------------|----------|
| w[1118] x Actin-GS-255B | 3 | 0µM | 58 | 53 (16) | 55 | 80 | | | |
| w[1118] x Actin-GS-255B | 3 | 10µM | 69 | 66 (18) | 67 | 88 | 15.6 | 20.0 | 3.82E-12 |
| w[1118] x Actin-GS-255B | 60 | 0µM | 107 | 48 (16) | 48 | 70 | | | |
| w[1118] x Actin-GS-255B | 60 | 10µM | 90 | 51 (15) | 52 | 73 | 3.42 | 4.76 | 0.3131 |

Male (Cohort 1)

| Genotype | Age (day) | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-----------|-------------------------------|----|-----------|--------|-----|----------|------------|--------|
| w[1118] x Actin-GS-255B | 3 | 0µM | 73 | 58 (13) | 60 | 71 | | | |
| w[1118] x Actin-GS-255B | 3 | 10µM | 79 | 58 (13) | 60 | 71 | 0.584 | 0.000 | 0.7838 |
| w[1118] x Actin-GS-255B | 60 | 0µM | 88 | 33 (14) | 34 | 40 | | | |
| w[1118] x Actin-GS-255B | 60 | 10µM | 93 | 33 (14) | 34 | 40 | -0.406 | 0 | 0.9166 |

Male (Cohort 2)

| Genotype | Age (day) | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-----------|-------------------------------|----|-----------|--------|-----|----------|------------|--------|
| w[1118] x Actin-GS-255B | 3 | 0µM | 57 | 57 (15) | 61 | 76 | | | |
| w[1118] x Actin-GS-255B | 3 | 10µM | 69 | 56 (15) | 60 | 74 | -0.562 | 0 | 0.7609 |
| w[1118] x Actin-GS-255B | 60 | 0µM | 79 | 29.9 (16) | 28 | 37 | | | |
| w[1118] x Actin-GS-255B | 60 | 10µM | 80 | 29.5 (16) | 28 | 37 | -0.778 | 0 | 0.7997 |

Supplementary Table S2. Hydrogen peroxide adaptation in Beta1 and Beta2 RNAi strains. Two cohorts of females and males propagated in the absence or presence of RU486. Flies were either not pretreated with H₂O₂ or pretreated [10μM or 100μM] H₂O₂. Crosses are in order males x virgin females.

Female (Cohort 1)

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|---------|
| Beta1 RNAi x Actin-GS-255B | No | 0μM | 96 | 65 (14) | 66 | 79 | | | |
| | | 10μM | 104 | 73 (17) | 77 | 90 | 7.762 | 12.17 | 7.9E-06 |
| | | 100μM | 102 | 75 (13) | 76 | 90 | 9.541 | 10.79 | 6.3E-06 |
| Beta1 RNAi x Actin-GS-255B | Yes | 0μM | 142 | 29 (23) | 29 | 80 | | | |
| | | 10μM | 132 | 28 (22) | 29 | 80 | -1.000 | 0.000 | 0.623 |
| | | 100μM | 131 | 28 (23) | 29 | 80 | -1.425 | 0.000 | 0.561 |
| Beta2 RNAi x Actin-GS-255B | No | 0μM | 99 | 66 (13) | 67 | 77 | | | |
| | | 10μM | 101 | 75 (13) | 75 | 104 | 9.974 | 8.919 | 2.2E-06 |
| | | 100μM | 102 | 75 (12) | 75 | 103 | 10.17 | 9.091 | 2.0E-06 |
| Beta2 RNAi x Actin-GS-255B | Yes | 0μM | 101 | 56 (15) | 58 | 65 | | | |
| | | 10μM | 100 | 55 (16) | 58 | 65 | -1.131 | 0.000 | 0.595 |
| | | 100μM | 101 | 56 (16) | 58 | 65 | 0.895 | 0.000 | 0.537 |

Female (Cohort 2)

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|---------|
| Beta1 RNAi x Actin-GS-255B | No | 0μM | 107 | 54 (16) | 59 | 70 | | | |
| | | 10μM | 144 | 70 (18) | 76 | 92 | 13.97 | 14.66 | 2.3E-07 |
| | | 100μM | 148 | 70 (17) | 77 | 92 | 14.01 | 15.76 | 3.1E-07 |
| Beta1 RNAi x Actin-GS-255B | Yes | 0μM | 141 | 61 (22) | 62 | 93 | | | |
| | | 10μM | 120 | 60 (23) | 62 | 93 | -4.448 | 0.000 | 0.079 |
| | | 100μM | 119 | 59 (22) | 62 | 93 | -2.954 | 0.000 | 0.368 |
| Beta2 RNAi x Actin-GS-255B | No | 0μM | 142 | 68 (15) | 69 | 88 | | | |
| | | 10μM | 156 | 76 (15) | 79 | 106 | 7.795 | 9.110 | 1.5E-05 |
| | | 100μM | 164 | 77 (15) | 81 | 105 | 6.688 | 9.091 | 4.2E-05 |
| Beta2 RNAi x Actin-GS-255B | Yes | 0μM | 80 | 55 (11) | 59 | 68 | | | |
| | | 10μM | 79 | 55 (12) | 59 | 68 | 0.314 | 0.000 | 0.7830 |
| | | 100μM | 79 | 55 (12) | 59 | 68 | 0.175 | 0.000 | 0.7998 |

Male (Cohort 1)

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| Beta1 RNAi x Actin-GS-255B | No | 0μM | 91 | 49 (19) | 50 | 70 | | | |
| | | 10μM | 89 | 46 (19) | 50 | 69 | -0.613 | 0.000 | 0.619 |
| | | 100μM | 88 | 46 (19) | 50 | 69 | -0.620 | 0.000 | 0.608 |
| Beta1 RNAi x Actin-GS-255B | Yes | 0μM | 78 | 45 (20) | 46 | 67 | | | |
| | | 10μM | 80 | 44 (21) | 45 | 64 | -0.278 | -0.616 | 0.336 |
| | | 100μM | 80 | 44 (22) | 45 | 64 | -0.323 | -0.281 | 0.878 |
| Beta2 RNAi x Actin-GS-255B | No | 0μM | 80 | 71 (13) | 70 | 79 | | | |
| | | 10μM | 79 | 70 (12) | 70 | 79 | -0.765 | 0.000 | 0.7295 |
| | | 100μM | 79 | 70 (13) | 70 | 79 | -0.813 | 0.000 | 0.6968 |
| Beta2 RNAi x Actin-GS-255B | Yes | 0μM | 100 | 53 (16) | 58 | 70 | | | |
| | | 10μM | 101 | 48 (16) | 49 | 68 | -1.984 | -10.12 | 0.5909 |
| | | 100μM | 100 | 47 (17) | 47 | 68 | -2.978 | -11.11 | 0.2803 |

Male (Cohort 2)

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| Beta1 RNAi x Actin-GS-255B | No | 0μM | 81 | 50 (15) | 48 | 65 | | | |
| | | 10μM | 81 | 50 (16) | 48 | 67 | -0.247 | 0.000 | 0.965 |
| | | 100μM | 77 | 48 (15) | 48 | 63 | -1.744 | 0.000 | 0.606 |
| Beta1 RNAi x Actin-GS-255B | Yes | 0μM | 78 | 43 (22) | 47 | 64 | | | |
| | | 10μM | 80 | 42 (21) | 46 | 64 | -0.920 | -0.837 | 0.900 |
| | | 100μM | 80 | 40 (22) | 46 | 60 | -2.444 | -0.824 | 0.530 |
| Beta2 RNAi x Actin-GS-255B | No | 0μM | 100 | 52 (15) | 57 | 70 | | | |
| | | 10μM | 119 | 52 (15) | 56 | 70 | -0.846 | 0.152 | 0.7444 |
| | | 100μM | 119 | 53 (16) | 58 | 70 | 0.171 | 0.334 | 0.9139 |
| Beta2 RNAi x Actin-GS-255B | Yes | 0μM | 81 | 50 (16) | 49 | 65 | | | |
| | | 10μM | 82 | 50 (16) | 49 | 66 | -0.420 | 0.000 | 0.9824 |
| | | 100μM | 80 | 53 (16) | 57 | 66 | 1.547 | 7.127 | 0.4374 |

Supplementary Table S3. Lifespan statistical summary. Two cohorts of male and female progeny were transferred every other day onto fresh food and dead flies were recorded. Crosses in order: males x virgin females.

Cohort 1

| Genotype | Sex | RU486 | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------------------|-----|-------|-----|-----------|--------|-----|----------|------------|----------|
| w[1118] x Actin-GS-255B | F | No | 92 | 66 (24) | 64 | 92 | | | |
| | | Yes | 99 | 67 (24) | 66 | 92 | 3.08 | 5.55 | 0.0778 |
| w[1118] x Actin-GS-255B | M | No | 116 | 76 (23) | 76 | 82 | | | |
| | | Yes | 123 | 75 (23) | 74 | 82 | -2.11 | -5.55 | 0.5618 |
| β ₁ RNAi x Actin-GS-255B | F | No | 88 | 35 (24) | 33 | 50 | | | |
| | | Yes | 94 | 9.8(13) | 10 | 11 | -46.1 | -43.7 | 3.11E-13 |
| β ₁ RNAi x Actin-GS-255B | M | No | 111 | 84 (20) | 85 | 99 | | | |
| | | Yes | 113 | 34 (21) | 33 | 42 | -74.5 | -78.6 | 0.0000 |
| B ₂ RNAi x Actin-GS-255B | F | No | 93 | 85 (12) | 84 | 96 | | | |
| | | Yes | 93 | 32 (20) | 30 | 44 | -51.1 | -53.3 | 0.0000 |
| β ₂ RNAi x Actin-GS-255B | M | No | 112 | 70 (23) | 68 | 88 | | | |
| | | Yes | 122 | 32 (10) | 32 | 42 | -67.9 | -67.5 | 0.0000 |
| CnC RNAi x Actin-GS-255B | F | No | 102 | 67 (25) | 68 | 89 | | | |
| | | Yes | 82 | 58 (21) | 57 | 74 | -20.1 | -23.3 | 2.30E-10 |
| CnC RNAi x Actin-GS-255B | M | No | 110 | 57 (21) | 63 | 82 | | | |
| | | Yes | 99 | 49 (18) | 48 | 68 | -16.4 | -19.3 | 4.23E-07 |
| Keap1 RNAi x Actin-GS-255B | F | No | 97 | 61 (19) | 61 | 80 | | | |
| | | Yes | 83 | 75 (21) | 75 | 92 | 14.3 | 16.3 | 0.0002 |
| Keap1 RNAi x Actin-GS-255B | M | No | 115 | 62 (21) | 60 | 74 | | | |
| | | Yes | 119 | 65 (23) | 62 | 78 | 5.86 | 3.25 | 0.0390 |

Cohort 2

| Genotype | Sex | RU486 | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------------------|-----|-------|-----|-----------|--------|-----|----------|------------|----------|
| w[1118] x Actin-GS-255B | F | No | 95 | 72 (21) | 70 | 90 | | | |
| | | Yes | 96 | 72 (21) | 70 | 92 | 0.58 | 0.00 | 0.1875 |
| w[1118] x Actin-GS-255B | M | No | 95 | 76 (18) | 76 | 80 | | | |
| | | Yes | 88 | 75 (18) | 72 | 78 | -2.59 | -7.69 | 0.2469 |
| β ₁ RNAi x Actin-GS-255B | F | No | 110 | 11 (19) | 10 | 12 | | | |
| | | Yes | 99 | 41 (21) | 42 | 49 | -45.3 | -44.2 | 1.51E-12 |
| β ₁ RNAi x Actin-GS-255B | M | No | 105 | 78 (21) | 76 | 98 | | | |
| | | Yes | 112 | 37 (19) | 39 | 42 | -49.3 | -46.7 | 4.09E-12 |
| B ₂ RNAi x Actin-GS-255B | F | No | 96 | 89 (19) | 87 | 100 | | | |
| | | Yes | 94 | 29 (12) | 29 | 43 | -72.2 | -70.0 | 0.0000 |
| β ₂ RNAi x Actin-GS-255B | M | No | 101 | 75 (20) | 74 | 80 | | | |
| | | Yes | 103 | 34 (22) | 33 | 40 | -53.2 | -50.0 | 0.0000 |
| CnC RNAi x Actin-GS-255B | F | No | 89 | 63 (21) | 62 | 83 | | | |
| | | Yes | 94 | 55 (18) | 53 | 70 | -19.1 | -20.1 | 6.24E-10 |
| CnC RNAi x Actin-GS-255B | M | No | 112 | 63 (21) | 62 | 83 | | | |
| | | Yes | 119 | 56 (17) | 56 | 77 | -13.4 | -14.1 | 0.0004 |
| Keap1 RNAi x Actin-GS-255B | F | No | 101 | 70 (19) | 70 | 80 | | | |
| | | Yes | 96 | 76 (20) | 76 | 88 | 10.7 | 6.67 | 0.0013 |
| Keap1 RNAi x Actin-GS-255B | M | No | 123 | 61 (21) | 61 | 71 | | | |
| | | Yes | 112 | 64 (22) | 65 | 79 | 3.78 | 4.25 | 0.0312 |

Supplementary Table S4. Hydrogen peroxide adaptation following loss of Keap1 in aged females and males. Flies were aged to 60 days either in the absence or presence of RU486. Crosses in order: males x virgin females.

Female

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| Keap1 RNAi x Actin-GS-255B | No | 0μM | 112 | 34 (18) | 36 | 46 | | | |
| | | 10μM | 118 | 35 (17) | 36 | 46 | 1.67 | 0.000 | 0.5437 |
| Keap1 RNAi x Actin-GS-255B | Yes | 0μM | 107 | 49 (20) | 54 | 62 | | | |
| | | 10μM | 91 | 50 (18) | 54 | 62 | 3.79 | 0.000 | 0.5821 |
| Keap1 RNAi x Actin-GS-255B | No | 0μM | 185 | 25 (18) | 26 | 50 | | | |
| | | 10μM | 196 | 26 (19) | 26 | 50 | 1.35 | 0.000 | 0.5558 |
| Keap1 RNAi x Actin-GS-255B | Yes | 0μM | 108 | 40 (19) | 39 | 70 | | | |
| | | 10μM | 106 | 42 (19) | 44 | 70 | 4.77 | 4.28 | 0.1413 |

Male

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|----------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| Keap1 RNAi x Actin-GS-255B | No | 0μM | 118 | 24 (12) | 28 | 46 | | | |
| | | 10μM | 123 | 26 (12) | 28 | 46 | 2.76 | 2.55 | 0.4611 |
| Keap1 RNAi x Actin-GS-255B | Yes | 0μM | 88 | 52 (18) | 54 | 70 | | | |
| | | 10μM | 102 | 54 (19) | 56 | 70 | -2.63 | -2.50 | 0.4396 |
| Keap1 RNAi x Actin-GS-255B | No | 0μM | 132 | 27 (11) | 26 | 48 | | | |
| | | 10μM | 123 | 27 (14) | 28 | 49 | -0.640 | 0.000 | 0.8549 |
| Keap1 RNAi x Actin-GS-255B | Yes | 0μM | 119 | 56 (21) | 57 | 72 | | | |
| | | 10μM | 117 | 55 (21) | 57 | 72 | -0.726 | 0.000 | 0.7931 |

Supplementary Table S5. Hydrogen peroxide adaptation in the presence or absence of RU486.

Flies were aged to 60 days either in the absence or presence of RU486. Crosses in order: males x virgin females.

Female

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-------|-------------------------------|-----|-----------|--------|-----|----------|------------|--------|
| w[1118] x Actin-GS-255B | No | 0μM | 78 | 42 (18) | 42 | 49 | | | |
| | | 10μM | 79 | 42 (18) | 42 | 49 | 1.65 | 0.000 | 0.4889 |
| w[1118] x Actin-GS-255B | Yes | 0μM | 86 | 44 (21) | 44 | 52 | | | |
| | | 10μM | 76 | 46 (21) | 46 | 52 | 3.50 | 1.50 | 0.1181 |
| w[1118] x Actin-GS-255B | No | 0μM | 101 | 41 (20) | 40 | 46 | | | |
| | | 10μM | 99 | 41 (20) | 43 | 46 | 0.310 | 5.88 | 0.9281 |
| w[1118] x Actin-GS-255B | Yes | 0μM | 103 | 40 (20) | 43 | 66 | | | |
| | | 10μM | 100 | 41 (20) | 43 | 66 | 0.622 | 0.000 | 0.7242 |

Male

| Genotype | RU486 | H ₂ O ₂ | N | Mean (SD) | Median | 90% | Δ Mean % | Δ Median % | (p) |
|-------------------------|-------|-------------------------------|----|-----------|--------|-----|----------|------------|--------|
| w[1118] x Actin-GS-255B | No | 0μM | 61 | 35 (21) | 34 | 40 | | | |
| | | 10μM | 68 | 33 (22) | 34 | 40 | -4.01 | 0.000 | 0.2806 |
| w[1118] x Actin-GS-255B | Yes | 0μM | 64 | 37 (18) | 37 | 42 | | | |
| | | 10μM | 74 | 36 (19) | 36 | 41 | -2.21 | -1.11 | 0.2148 |
| w[1118] x Actin-GS-255B | No | 0μM | 79 | 30 (20) | 30 | 46 | | | |
| | | 10μM | 80 | 32 (20) | 30 | 46 | 2.68 | 0.000 | 0.4316 |
| w[1118] x Actin-GS-255B | Yes | 0μM | 66 | 32 (14) | 34 | 44 | | | |
| | | 10μM | 78 | 32 (14) | 34 | 44 | 0.083 | 0.000 | 0.9676 |