

1 **Supporting Information**

2 **Experimental Procedures:**

3 **Primer Sequences used in this paper**

Gene Name	Forward Primer	Reverse Primer
<i>rp49</i>	AGTATCTGATGCCCAACATC	ACAATCTCCTTGCGCTTC
<i>Sirt6</i>	TCGGGACACGTTGTCCTCCAC	TCTGGCTTCATCGAAGGAAACAT
<i>Spargel</i>	CATTACCATGGCCAAGAACAAGGTGC	CGGGTGGAGTTTGTGCTGAGTGGC
<i>TFAM</i>	CCAGCATCAGCAACTCC	GGCATCGGACCAGTTCTTAG
<i>Delg</i>	CAAGTTTAAGCGAGAACAATACGCCTG	TACATTCTTTTAGCAGCTGGAGGTG
<i>Cox4</i>	GGCGTTTCACTCCTCTCTG	CTGTTGTTCTCGTAGTCCCCTTG
<i>Eip74EF</i>	GCGAATACTGTCCGCTTTC	GATGTCCTTGGGCACATCCA
<i>Eip75B</i>	GCGGTCCAGAATCAGCAG	GAGGATGTGGAGGAGGATGA
<i>BR-C</i>	AACACCCGGTCATACTGCTG	ATGGCTGTGTGTGTCCTCTG
<i>EcR</i>	ATTTGGTGCTTGTGCCGAAC	AAGGATCATAGCTTCCGGCG
<i>Ultraspiracle</i>	CAGTATCCGCCTAACCATCC	TTCCTCTGCCGCTTGTCTAT
<i>Kr-h1</i>	CCGAATACGACATAACAGCC	CCCATTTCCTGGAATATGT
<i>dilp2</i>	AGCAAGCCTTTGTCCTTCATCTC	ACACCATACTCAGCACCTCGTTG
<i>dilp5</i>	GAGGCACCTTGGGCCTATTC	CATCTGGTGAGATTCGGAGC
<i>Sir2</i>	TATATCCCGGCGAGTTTCAG	GAGGCCGTTGAAAAGGAGCCG
<i>Sirt4</i>	TGTCCAAACCGAATGTTTTGGTTC	GTTCTGGCCAGTACCGCTT
<i>Sirt7</i>	ACGTGGCGCAGTCTTACATAA	TTCGATCTTCCGTCGTGCG
<i>InR</i>	ACGATGTAGAGGGCAGTGTA	TACCACGAAGATAGCGTAGC
<i>GSK</i>	ATATACAGATCTTTTGTGGCAA	AGGAGGAAGTTCTTGGACGA
<i>AKT</i>	TCTCCGTACAGGGAACGACT	TGGACTTGAGCACACGACTC
<i>Raptor</i>	GCGACTGTTTGACAAGCGTT	CGTCCCACTCGTACACAACA
<i>Rheb</i>	CGACGTAATGGGCAAGAAAT	CAAGACAACCGCTCTTCTCC
<i>4eBP</i>	CCAGGAAGGTTGTCATCTCG	GTTGGACGGCGGAGTTTG
<i>GLUT1</i>	GGATTGGCCTCAGGTCTCAC	GCGAAAATGGATACCGCCAC
<i>HexA</i>	GTAACGTATGTGCAGGATCT	ATCATAATGTGCTGCGGTAT
<i>PEPCK</i>	CATTGCGTGGATGAAGTTTG	ATGCCCTCCAGAACACAC
<i>FBPase</i>	TCATCAACATGCTGAAGTCA	CGATGTATTTGCCCTGTTTC
<i>LDH</i>	GTGTGACATCCGTGGTCAAG	CTACGATCCGTGGCATCTTT
<i>PFK</i>	CTGCAGCAGGATGTCTACCA	GTCGATGTTGCGCTTGATCT
<i>PDK1</i>	GGTCGTTACATAGGCGAGGG	AAAGAGAACGCTGGTCCTGG
<i>Tpi</i>	GACTGGAAGAACGTGGTGGT	CGTTGATGATGTCCACGAAC
<i>Mondo</i>	GCGGCGTTACAACATAAAGA	CTCCATGCGCAAAGCTTCAA
<i>HIF1a</i>	CCAAAGGAGAAAAGAAGGAAC	GAATCTTGAGGAAAGCGATG
<i>SREBP</i>	TGGCTTCTACCAAGTGCCAG	CAAGAGCTGTTGCGTTGGAC
<i>Brummer</i>	TCCCGAGTTTCTGTCCAAGT	GCGTCCTTCTGTGCTTCTT
<i>Lipase</i>	GATTTGGGCATAGAGATGTG	CTCATAGGATTACGGAGAG
<i>Trehalase</i>	TATCGGCTTCGGTTATGG	CGGGAGTGTTGAGTTGTTT
<i>ACC</i>	CATTGTTAGTGTGTGGCTGTGA	CTTATCTCTGGCTCTGAACG
<i>DAG</i>	ATCTCCCTTAGCCTGTTTAG	TAACGAAGGTCGCCATAAG
<i>MCAD</i>	CAACCACATTCTGTCTGA	TCTTCTGTTCTTGTACCG

<i>FAS</i>	CGGAGAAGAGTTACATCCTG	CAATCACCACCTTTACGC
<i>Cpt1</i>	AGGAACTGCAGCCTATCATGG	GGAGTTGCTTTGCCCTTCAG
<i>SCD1</i>	TTTCTTCGCCAAGATCGGCT	CAATCTCCTCCTGGGCTGG
<i>DGAT</i>	ACTGCTCTGCATTGGAGGTC	ATGTCCTTCGCCTTCGTTGT
<i>Thiolase</i>	GAAGAACAGCCAGAATATCG	CACGATGTAGTCGATGAGC
<i>MnSOD</i>	AGGCCAAGTCGAAGAGCGA	ACGGTCAGCGTGGTCAGCTC
<i>Catalase</i>	GACGATGTACATTCAGGTCA	CACCTCAGCAAAGTAGTTCT
<i>Thioredoxin1</i>	GGTGCGTTCTATTGTGCT	ACGGTGTCGTAAACATCCT
<i>GSTD2q</i>	TACGGCAAGGATGACTATCT	CAGGAAGGTGTCGAGAAAT

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23 **Supplementary Figures and Legends:**

24 **Supplementary Figure 1:**

25 (A) Protein sequence alignment of *SIRT6* from *Drosophila*, mouse and human (uniprot.org)
26 using ClustalW, indicating conserved active site residues, NAD⁺ binding and catalytic domain.
27 (B) Schematic representation of chromosomal position indicating site of CRISPR mediated
28 deletion of *Sirt6* and insertion of knock-in cassette containing STOP codons and RFP. (C)
29 Genome sequence pre- and post CRISPR mediation deletion-insertion, as indicated. Primers
30 used for confirmation are highlighted in yellow. (D) PCR based confirmation of STOP-RFP
31 cassette insertion in *Sirt6*^{-/-bck-L1/L2} flies. L1 and L2 indicate two independently generated fly
32 lines (male-M and female-F) were confirmed for *Sirt6* deletion. (E) Quantitative PCR showing
33 absence of *Sirt6* mRNA in CRISPR mutants of *Sirt6* relative to *w*¹¹¹⁸ controls. (F) Larval weights
34 at 24, 48 and 72 hours post synchronised egg laying of *w*¹¹¹⁸ and *Sirt6*^{-/-bck-L1} flies (N=3, n=30-
35 40 per genotype). (G) Quantitative PCR to confirm over-expression of *Sirt6* mRNA from *Sirt6*^{OE}
36 transgenic flies (*actingal4>Sirt6*^{OE}) relative to control (*actingal4>w*¹¹¹⁸). (H) Larval body size at
37 24, 48 and 72 hours post synchronised egg laying in *actingal4>w*¹¹¹⁸ and transgenic *Sirt6* over-
38 expressing flies. (I) Percentage pupation in control and *Sirt6* over-expressing transgenic flies
39 (N=3, n=150-200). (J) Body weight in control and *Sirt6* over-expressing transgenic flies (N=3,
40 n=20-25 per genotype). (K-L) Relative wing dimensions (K) and wing aspect ratio (L) in control
41 and *Sirt6* over-expressing transgenic flies (N=3, n=20-25). (M) Quantitative PCR showing *UAS*-
42 *Sirt6* (*Sirt6*^{OE}) mediated rescue of *Sirt6* mRNA expression in *Sirt6*^{-/-bck-1} flies. Relative changes
43 in *Sirt6* expression are computed with respect to control *UAS-Sirt6* (*Sirt6*^{OE}) flies. All data
44 presented is mean ± s.e.m. (N=3, n=3). Asterisk depicts p values (*p<0.05, **p<0.01 and
45 ***p<0.001) as observed by Student's t-test.

46 **Supplementary Figure 2:**

47 (A) Relative change in *Sirt6* mRNA expression (black dashed line) and NAD⁺ levels (green) in
48 *w*¹¹¹⁸ larvae during development, as indicated. (B) Relative expression of genes in *Sirt6*^{-/-bck-L1}
49 larvae compared to *w*¹¹¹⁸ (black dashed line) during the time course of larval development.
50 Asterisk depicts comparison with *w*¹¹¹⁸ at 24 hours and hashtags depict comparison of the
51 *Sirt6*^{-/-bck-L1} to *w*¹¹¹⁸, at the respective time points, as indicated. All data presented is mean ±
52 s.e.m. (N=3, n=3). (C) Relative change in expression of *dilp2* and *dilp5* in transgenic rescue of

53 Sirt6 larvae at 24, 48 and 72 hours post egg laying (N=3, n=3 with 10-20 larvae per n). Asterisk
54 and hashtags depict p values (*, #p<0.05, **, ##p<0.01 and ***, ###p<0.001) as observed by
55 Student's t-test and Two-way ANOVA, as applicable.

56 **Supplementary Figure 3:**

57 (A) Starvation survival in 3-5 day old *w¹¹¹⁸* and *Sirt6^{-/-bck-L1/L2}* flies (N=3, n=6 with 10 flies per
58 n). (B) Oxidative stress survival on 20mM Paraquat in *w¹¹¹⁸* (control) and *Sirt6^{-/-bck-L1}* flies (N=3,
59 n=6 with 10 flies per n). (C) Relative mRNA levels of genes involved in mitochondrial
60 biogenesis *w¹¹¹⁸* and *Sirt6^{-/-bck-L1/L2}* flies (N=3, n=3 with 8 flies per n). (D) Representative blots
61 and quantification (right) for change in levels of mitochondrial proteins in *w¹¹¹⁸* and *Sirt6^{-/-bck-}*
62 *L1/L2* flies (N=3, n=3 with 8 flies per n). (E) Mitochondrial DNA content (normalised to nuclear
63 DNA) in 35-37 day old *w¹¹¹⁸* (control) and *Sirt6^{-/-bck-L1/L2}* flies (N=2, n=3 with 30 flies per n). (F)
64 Mitochondrial DNA content (normalised to nuclear DNA) in transgenic rescue of *Sirt6* in 35-
65 37 days old flies. (G) Average number of eggs laid per fly over a period of 20 days from a
66 heterogeneous cross, parents as indicated (N=5, n=10). All data presented is mean ± s.e.m.
67 Asterisk depict p values (*, #p<0.05, **, ##p<0.01 and ***, ###p<0.001) as observed by Student's
68 t-test and Two-way ANOVA, as applicable.

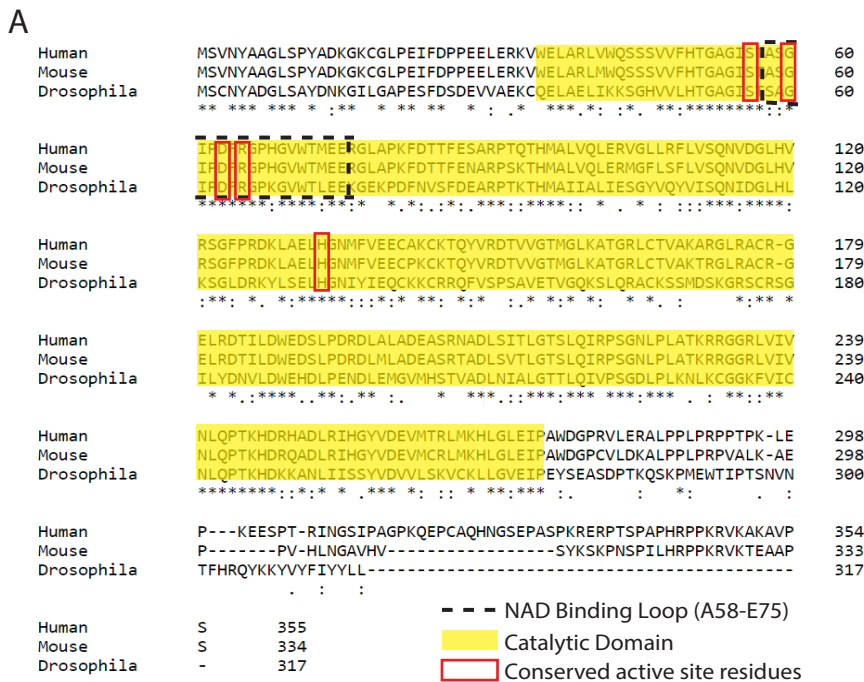
69 **Supplementary Figure 4:**

70 (A-B) Weight gain and pupation onset (marked in black) in *w¹¹¹⁸* larvae reared on differential
71 concentration of yeast (A) and glucose (B) diets, as indicated (N=3, n=20-25). (C) Weight gain
72 and pupation onset (marked in black) in *w¹¹¹⁸* and *Sirt6^{-/-bck-L1}* larvae reared on differential
73 concentration of glucose diets, as indicated (N=3, n=20-25). (D) Body weight measurement in
74 *w¹¹¹⁸* and *Sirt6^{-/-bck-L1}* 3-5 day old flies (N=3, n=20-25 per genotype), reared on differential
75 concentration of glucose diets, as indicated. (E) Median survival under starvation in *w¹¹¹⁸* and
76 *Sirt6^{-/-bck-L1}* flies reared on differential concentrations of yeast and glucose diets, from three
77 independent experiments (N=3, n=8 with 10 flies per n). (F) Median survival under oxidative
78 stress in *w¹¹¹⁸* and *Sirt6^{-/-bck-L1}* flies reared on differential concentrations of yeast and glucose
79 diets, from three independent experiments (N=3, n=8 with 10 flies per n). All data presented
80 is mean ± s.e.m. Student's t-test and Two-way ANOVA were used to analyse statistical
81 significance of the data (*p<0.05, **p<0.01 and ***p<0.001).

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83 **Supplementary Figure 5:**

84 (A-B) Life spans of w^{1118} (A) and $Sirt6^{-/-bck-1}$ (B) flies on normal diet, which were reared on
85 differential yeast diets during larval development (n = 10 with 10 flies per n). (C-D) Life spans
86 of w^{1118} (C) and $Sirt6^{-/-bck-1}$ (D) flies on normal diet, which were reared on differential glucose
87 diets during larval development (n = 10 with 10 flies per n). (E) Median life spans of w^{1118} and
88 $Sirt6^{-/-bck-1}$ reared on differential yeast and glucose diets (N=3, n = 10 with 10 flies per n). Log-
89 rank (Mantel-Cox) test was used to plot survival curves and statistical analysis. Asterisks
90 indicate comparison with w^{1118} grown on ND and hashtag indicates comparison among w^{1118}
91 and $Sirt6^{-/-bck-1}$ for the particular diet. All data presented is mean \pm s.e.m. Two-way ANOVA
92 was used to analyse statistical significance of the data (*, #p<0.05, **, ##p<0.01 and ***,
93 ###p<0.001).



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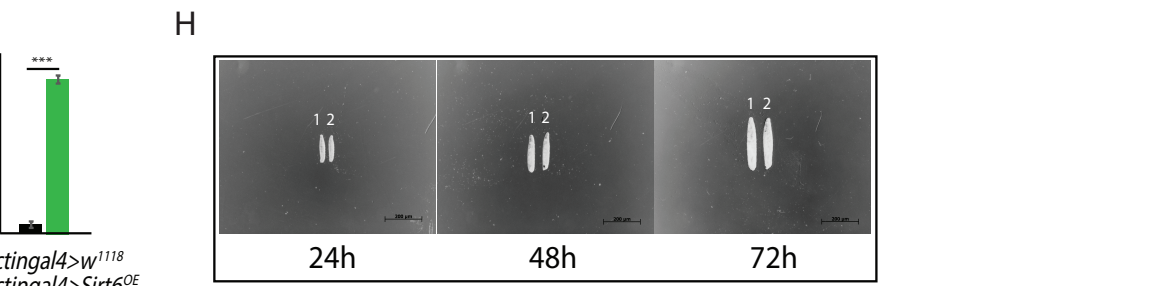
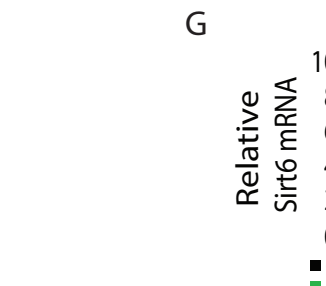
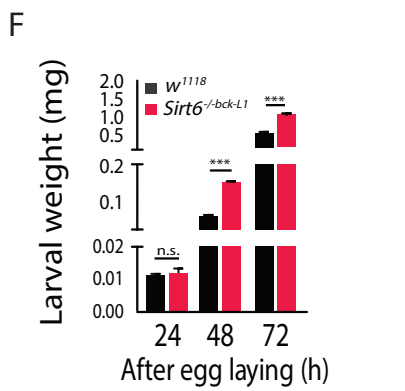
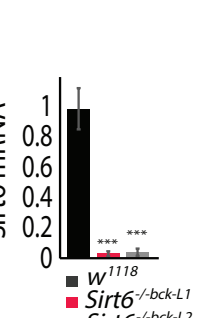
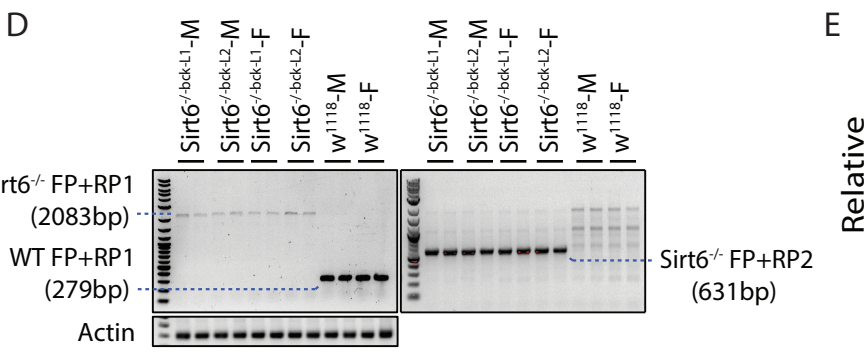
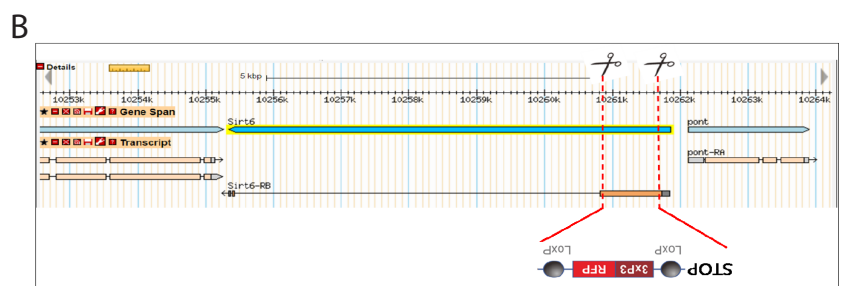
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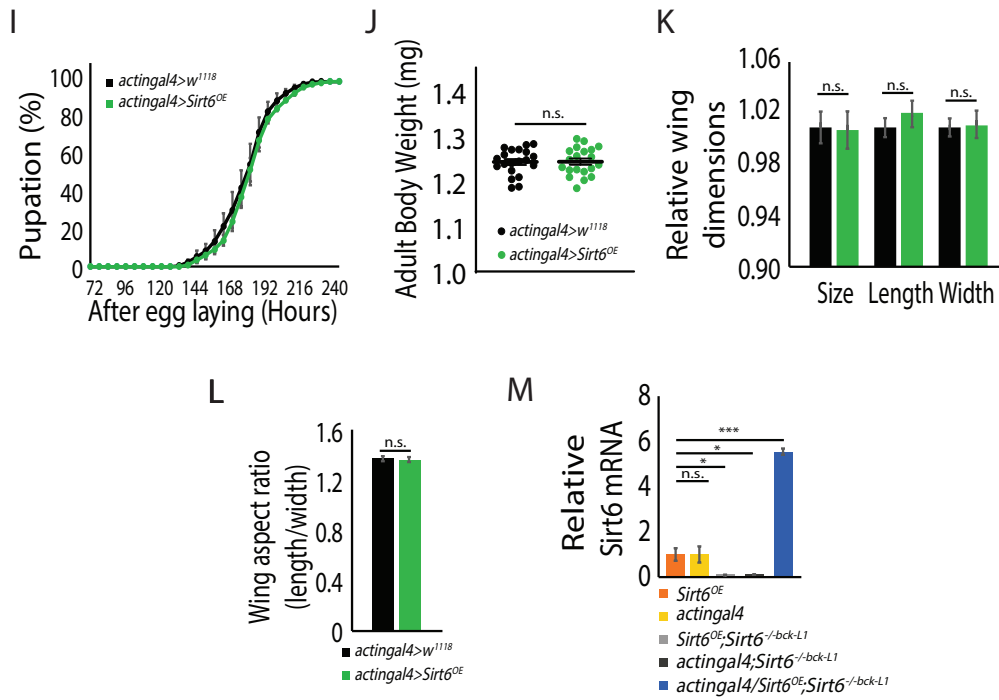
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STOP Codons
 LoXP Sites
 3x P3 Promoter
 RFP
 Tubulin 3'UTR

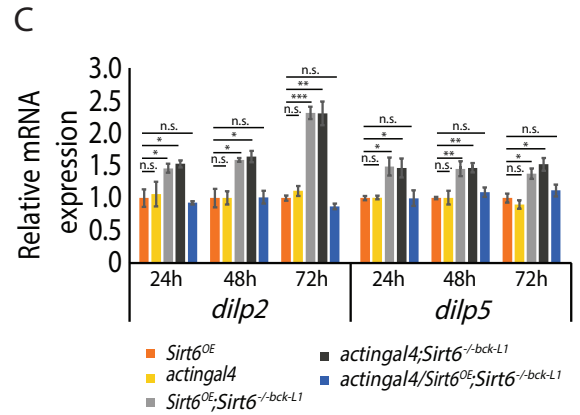
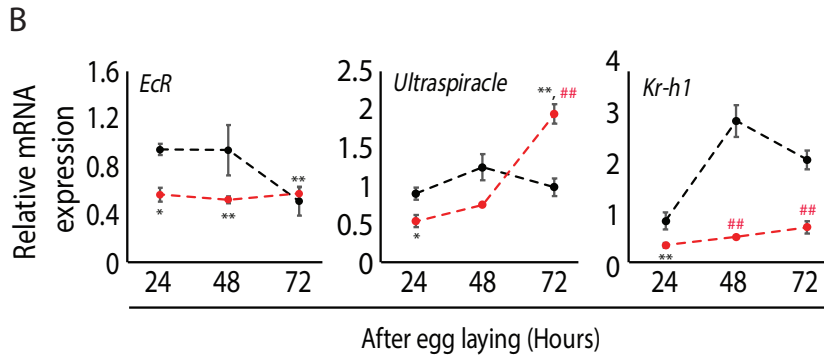
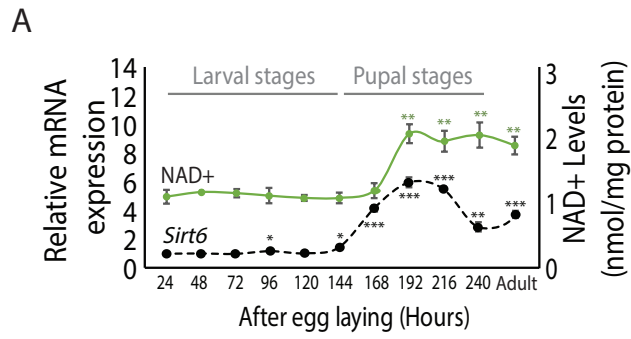


Supplementary figure 1

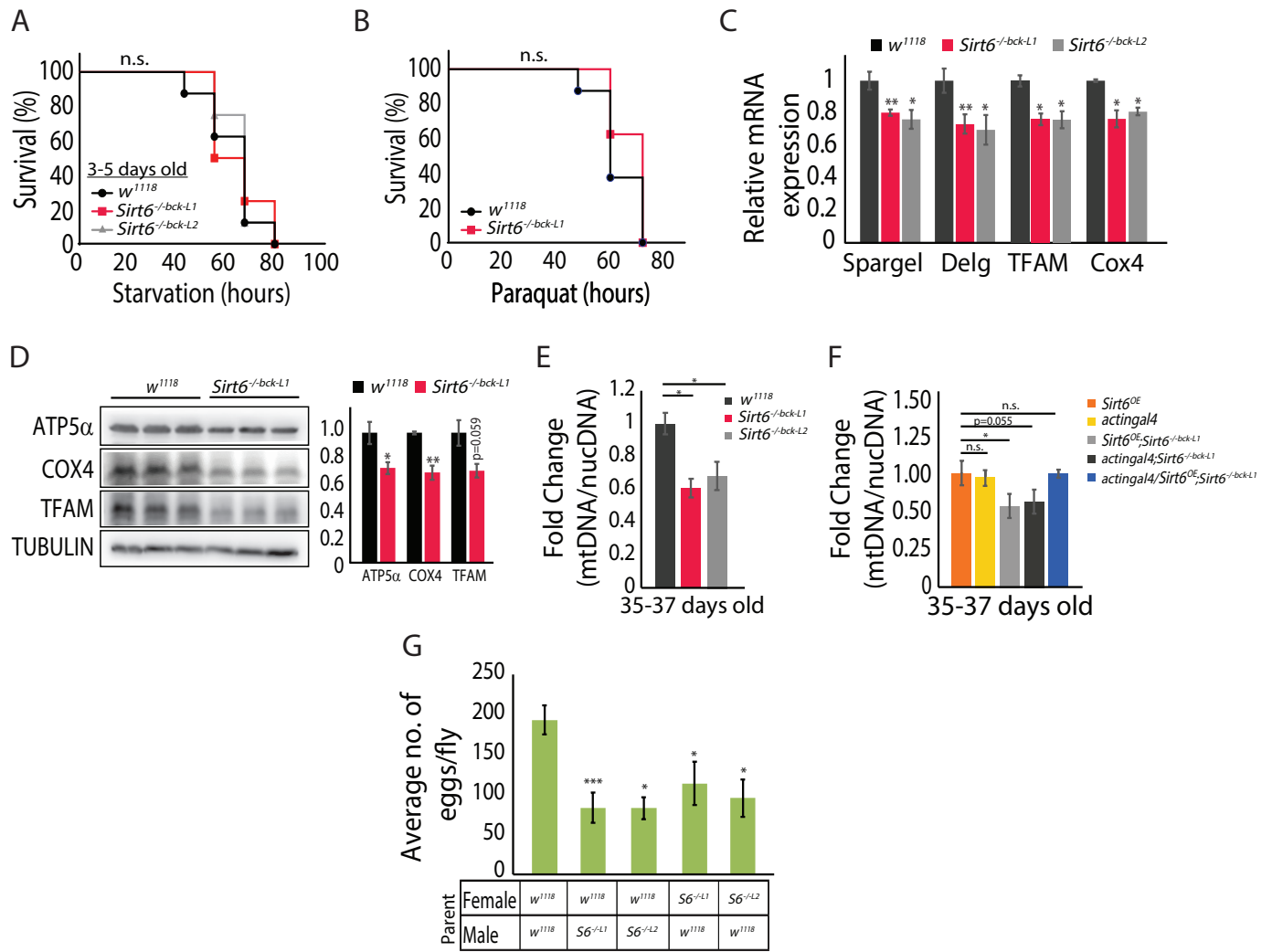
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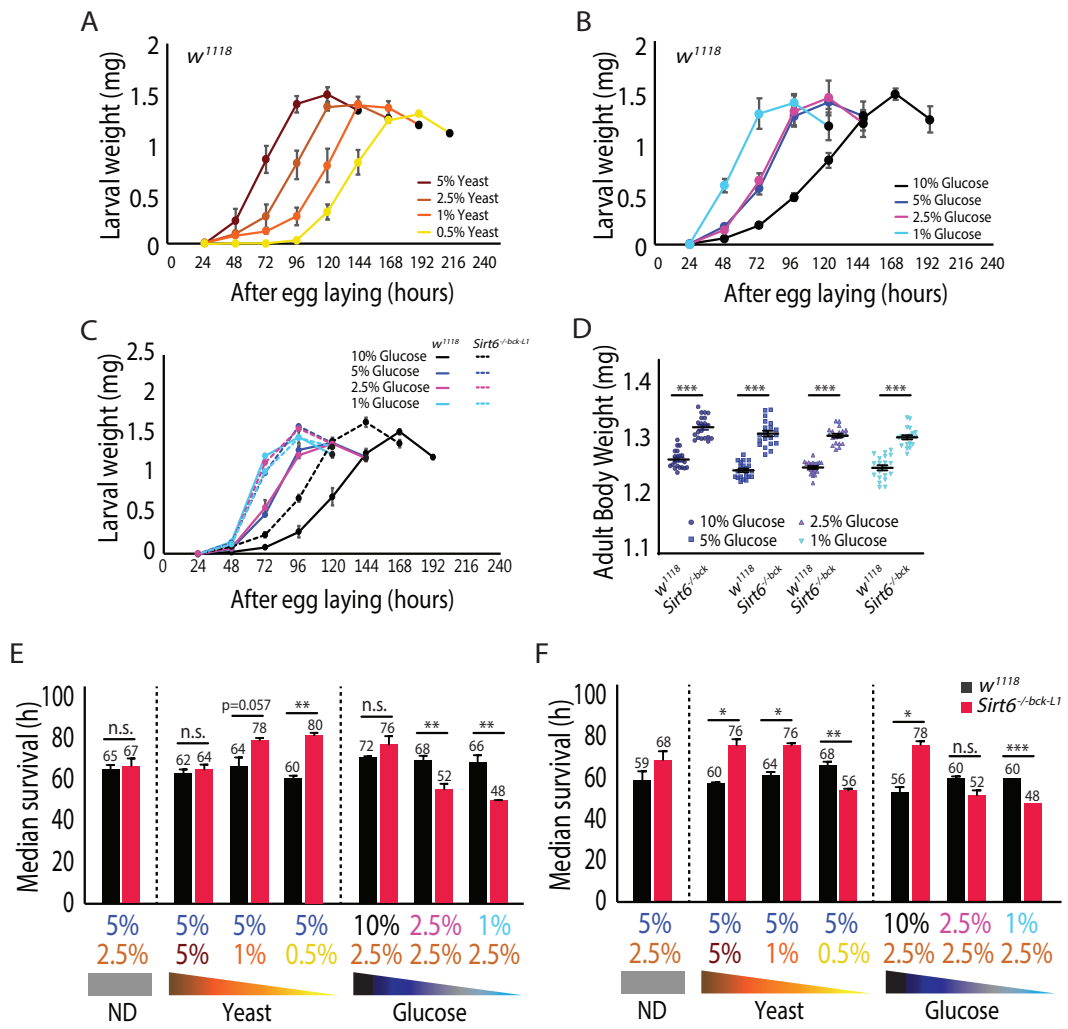
Supplementary figure 1



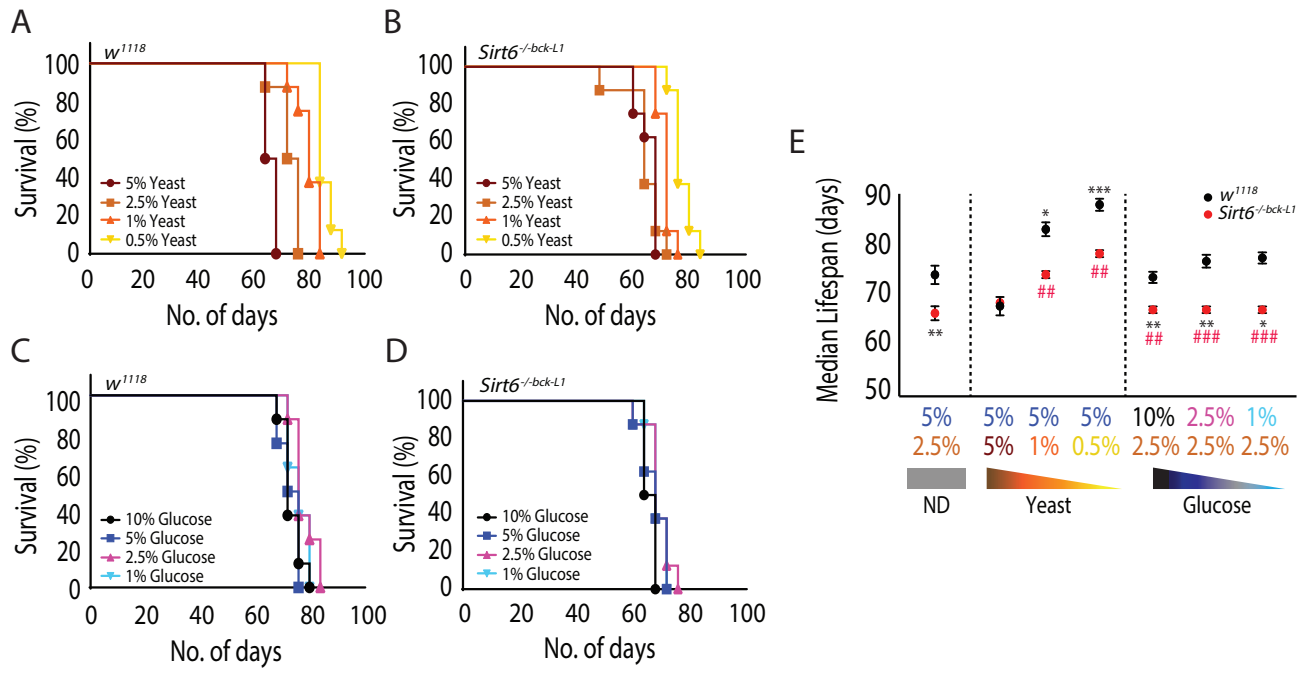
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary Figure 5