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Supplementary Materials for

HSB-1/HSF-1 pathway modulates histone H4 in mitochondria to control mtDNA transcription and longevity

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/43/eaaz4452/DC1)

Supplementary Materials

Fig. S1

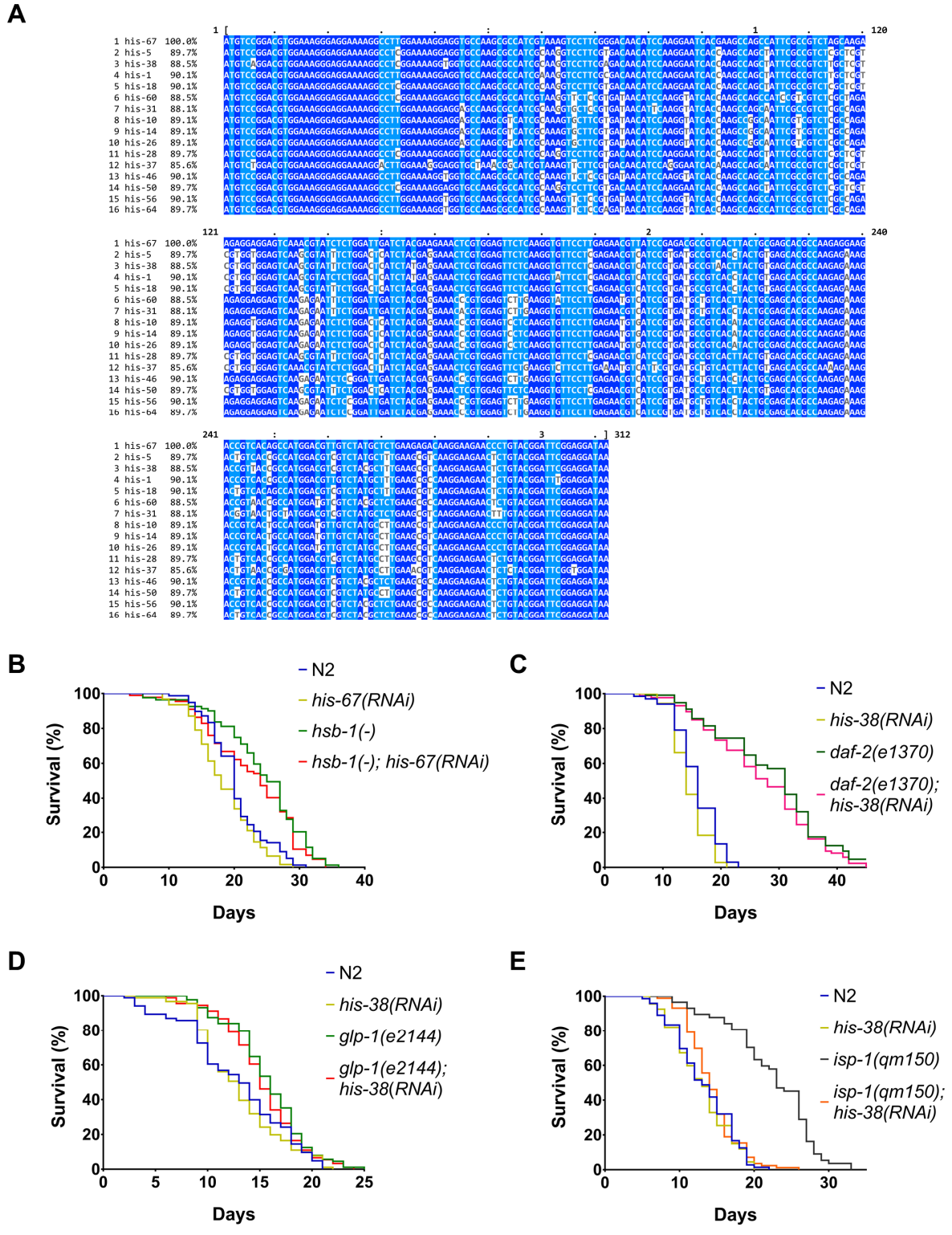

Fig. S1. H4 knockdown suppresses life span extension associated with mitochondrial inhibition. (**A**) Similarity between coding sequences of all 16 H4-coding genes in the *C. elegans* genome. Colored bases indicate sequence identity among genes (dark blue: purines, light blue: pyrimidines). Multiple sequence alignment was performed on Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo). (**B**) Life span analysis of wild-type (N2) and *hsb-1(-)* worms subjected to control or *his-67* RNAi at 20°C. (**C** to **E**) Life span analysis of N2, *daf-2(e1370)*, *glp-1(e2144)* and *isp-1(qm150)* worms subjected to control or *his-38* RNAi at 25°C. (B to E) Statistical data and additional life span replicates are included in table S1.

Fig. S2

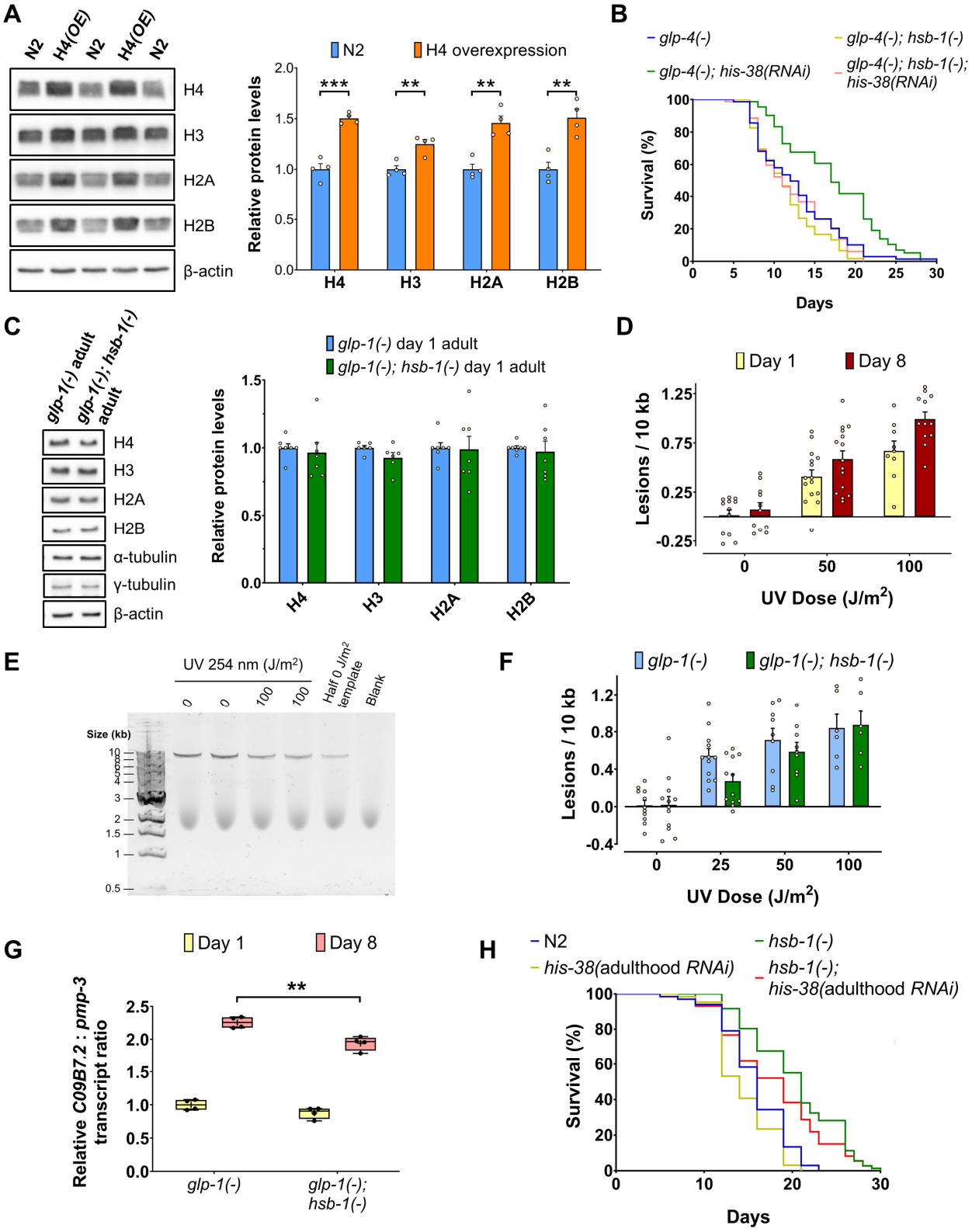
A
N2
H4(OE)
N2
H4(OE)
N2
H4
H3
H2A
H2B
β-actin
N2
H4 overexpression
Relative protein levels
***
**
**
**
H4
H3
H2A
H2B
B
glp-4(-)
glp-4(-); hsb-1(-)
glp-4(-); his-38(RNAi)
glp-4(-); hsb-1(-); his-38(RNAi)
Survival (%)
Days
C
glp-1(-) adult
glp-1(-); hsb-1(-) adult
H4
H3
H2A
H2B
α-tubulin
γ-tubulin
β-actin
glp-1(-) day 1 adult
glp-1(-); hsb-1(-) day 1 adult
Relative protein levels
H4
H3
H2A
H2B
D
Day 1
Day 8
Lesions / 10 kb
UV Dose (J/m2)
E
UV 254 nm (J/m2)
0
0
100
100
Half O J/m2 template
Blank
Size (kb)
F
glp-1(-)
glp-1(-); hsb-1(-)
Lesions / 10 kb
UV Dose (J/m2)
G
Day 1
Day 8
**
Relative C09B7.2 : pmp-3 transcript ratio
glp-1(-)
glp-1(-); hsb-1(-)
H
N2
hsb-1(-)
his-38(adulthood RNAi)
hsb-1(-); his-38(adulthood RNAi)
Survival (%)
Days

Fig. S2. HSB-1 inhibition partially attenuates genomic instability in somatic tissues. (**A**) Representative immunoblots for core histones in wild-type (N2) and H4 overexpression strain (*left*). Densitometric quantification of histone: β-actin ratio relative to N2 worms (*right*). Mean ± SEM for four biological replicates. ** $p < 0.01$, *** $p < 0.001$ compared to corresponding wild-type transcript levels in two-tailed *t*-test. (**B**) Life span analysis of *glp-4(-)* and *glp-4(-); hsb-1(-)* worms subjected to control or *his-38* RNAi. (**C**) Representative immunoblots for core histones in day 1 adult *glp-1(-)* and *glp-1(-); hsb-1(-)* worms (*left*). Densitometric quantification of histone: β-actin ratio relative to *glp-1(-)* worms (*right*). Mean ± SEM for ≥ 6 biological replicates. (**D**) UV-induced DNA lesions in day 1 and day 8 old *glp-1(-)* worms. Mean ± SEM for ≥ 9 biological replicates, each of n = 6 worms. UV: $p < 0.0001$, age: $p < 0.01$ in two-way ANOVA. (**E**) Representative agarose gel electrophoresis for 9.3 kb fragment of the *unc-2* gene amplified from genomic DNA of *glp-1(-)* worms that were exposed to 0 or 100 J/m^2 UV irradiation on day 1 of adulthood. 'Half 0 J/m^2 template': PCR with half the amount of template DNA as for the 0 J/m^2 reaction to confirm the quantitative nature of PCR amplification. 'Blank': PCR with no template DNA. (**F**) UV-induced DNA lesions in day 1 old *glp-1(-)* and *glp-1(-); hsb-1(-)* worms. Mean ± SEM for ≥ 6 biological replicates, each of n = 6 worms. UV: $p < 0.02$, genotype: $p < 0.05$ in two-way ANOVA for intermediate doses. (**G**) Relative *C09B7.2* transcript levels in day 1 and day 8 old *glp-1(-)* and *glp-1(-); hsb-1(-)* worms. Central line, box limits, '+' and whiskers indicate median, interquartile range, mean and data range, respectively, for four biological replicates. Age: $p < 0.0001$, genotype: $p < 0.001$, interaction: $p = 0.05$ in two-way ANOVA. ** $p < 0.01$ in Sidak's multiple comparisons test. (**H**) Life span analysis of N2 and *hsb-1(-)* worms subjected to control or *his-38* RNAi only during adulthood. (B and H) Statistical data and additional life span replicates are included in table S1.

Fig. S3

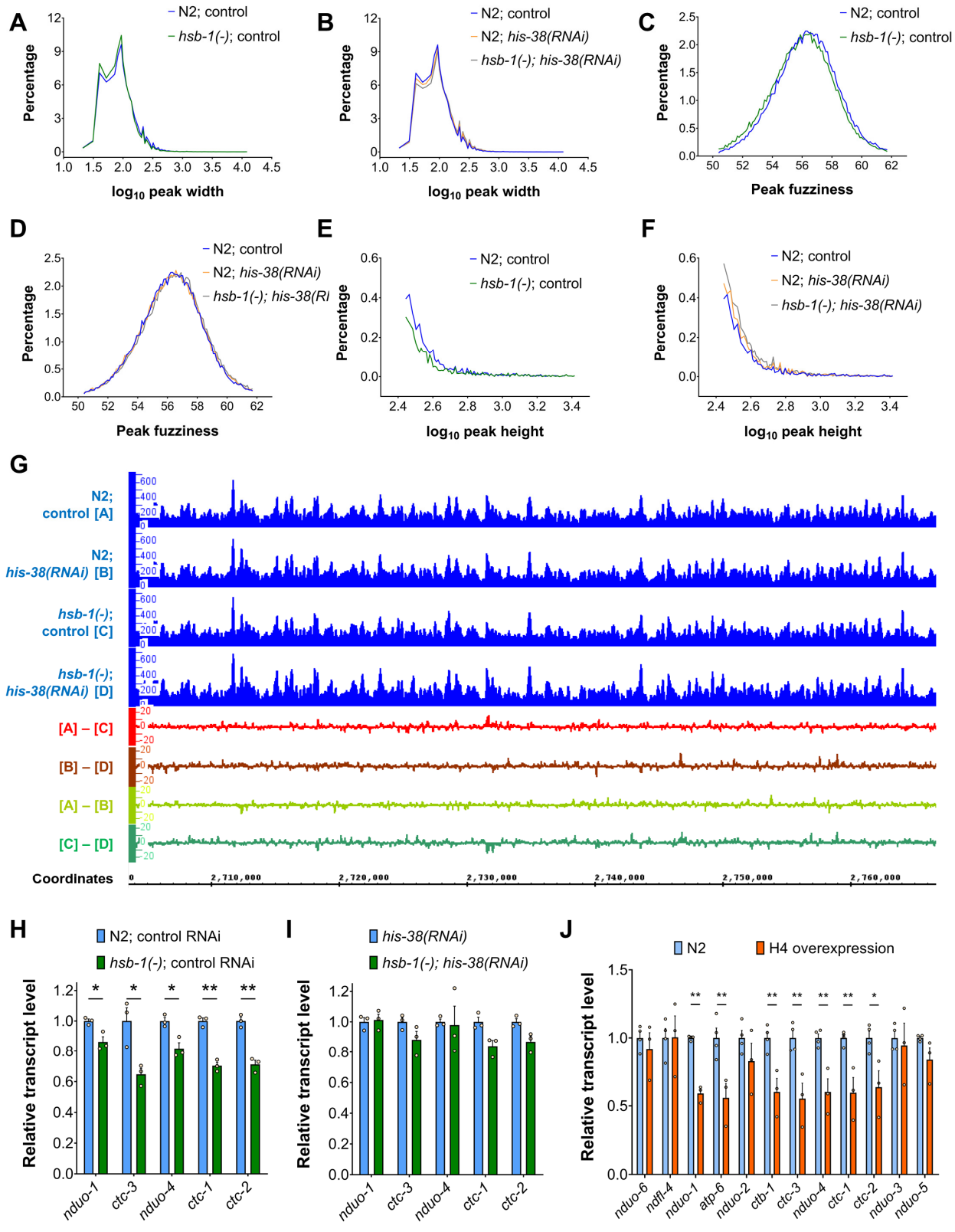
A
N2; control
hsb-1(-); control
Percentage
log10 peak width
B
N2; control
N2; his-38(RNAi)
hsb-1(-); his-38(RNAi)
Percentage
log10 peak width
C
N2; control
hsb-1(-); control
Percentage
Peak fuzziness
D
N2; control
N2; his-38(RNAi)
hsb-1(-); his-38(RI
Percentage
Peak fuzziness
E
N2; control
hsb-1(-); control
Percentage
log10 peak height
F
N2; control
N2; his-38(RNAi)
hsb-1(-); his-38(RNAi)
Percentage
log10 peak height
G
N2; control [A]
N2; his-38(RNAi) [B]
hsb-1(-); control [C]
hsb-1(-); his-38(RNAi) [D]
[A] – [C]
[B] – [D]
[A] – [B]
[C] – [D]
Coordinates
0
2,710,000
2,720,000
2,730,000
2,740,000
2,750,000
2,760,000
H
N2; control RNAi
hsb-1(-); control RNAi
Relative transcript level
nduo-1
ctc-3
nduo-4
ctc-1
ctc-2
I
his-38(RNAi)
hsb-1(-); his-38(RNAi)
Relative transcript level
nduo-1
ctc-3
nduo-4
ctc-1
ctc-2
J
N2
H4 overexpression
Relative transcript level
nduo-6
ndfl-4
nduo-1
atp-6
nduo-2
ctb-1
ctc-3
nduo-4
ctc-1
ctc-2
nduo-3
nduo-5

Fig. S3. MNase-accessibility of most genomic regions is unaltered in *hsb-1(-)* worms. (**A** to **F**) Frequency distribution of nucleosome peak width, peak fuzziness and peak height in genome-wide sequencing data of MNase-digested chromatin from wild-type (N2) and *hsb-1(-)* worms subjected to control or *his-38* RNAi. Statistical data are included in table S2. (**G**) MNase-Seq data for a representative genomic region in N2 and *hsb-1(-)* worms subjected to control or *his-38* RNAi. Difference between groups, such as (A – B), indicates occupancy difference at each base pair between the two conditions. The region shown corresponds to genomic location X: 2,703,479 – 2,766,712 that spans *unc-2*, *igcm-3* and several ncRNA genes, a typical example of a genomic region at which MNase-accessibility is not significantly affected by either *hsb-1(-)* mutation or *his-38* RNAi. (**H** and **I**) Relative transcript levels of selected mitochondrial genes in N2 and *hsb-1(-)* worms subjected to control or *his-38* RNAi. Mean ± SEM for three biological replicates. * $p < 0.05$, ** $p < 0.01$ in two-tailed *t*-test. (**J**) Relative transcript levels of mitochondrial protein-coding genes in N2 and H4 overexpression strain. Mean ± SEM for ≥ 3 biological replicates. * $p < 0.05$, ** $p < 0.01$ in two-tailed *t*-test.

Fig. S4

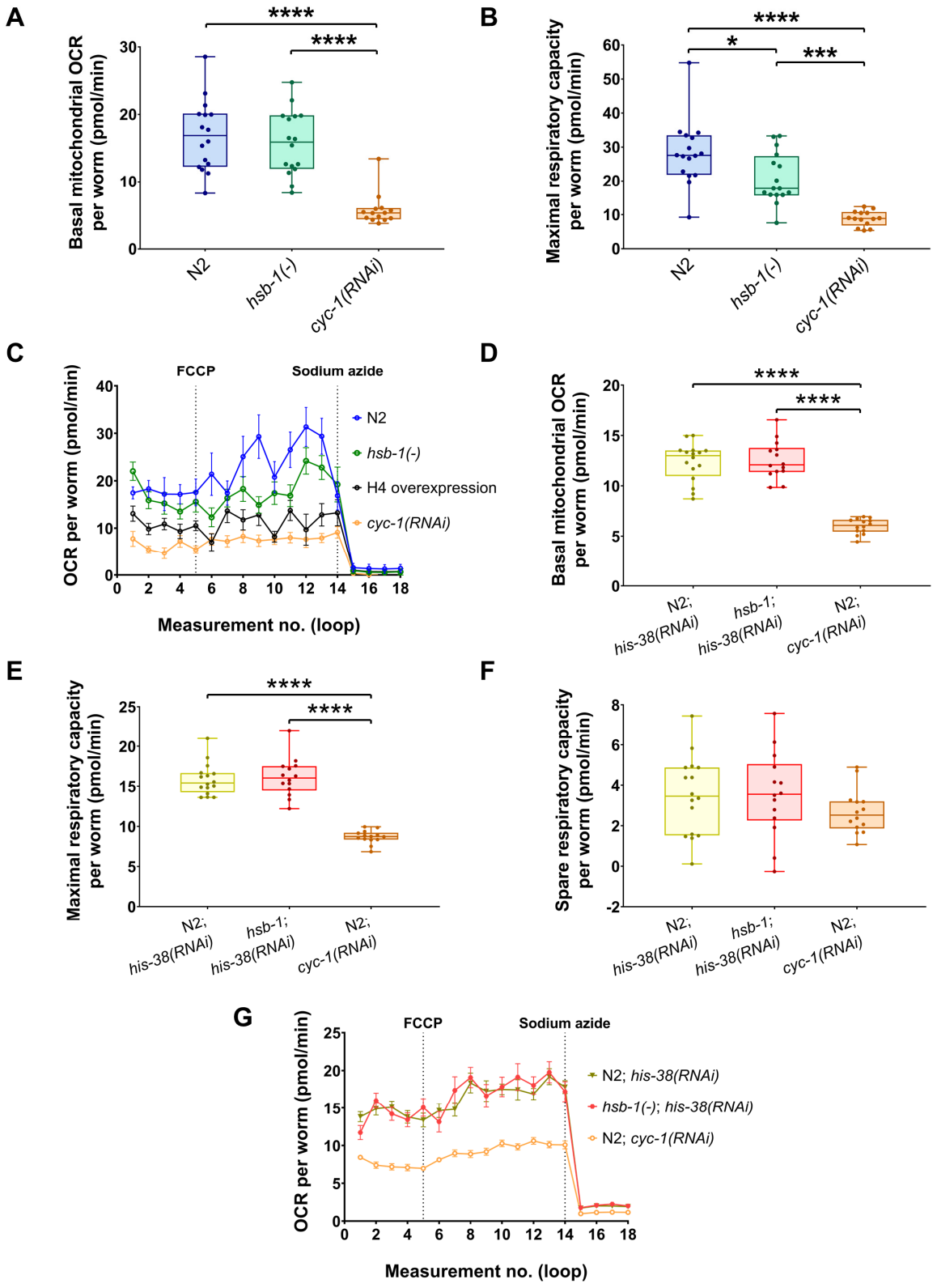
A
Basal mitochondrial OCR per worm (pmol/min)
****
****
N2
hsb-1(-)
cyc-1(RNAi)
B
Maximal respiratory capacity per worm (pmol/min)
****
*
***
N2
hsb-1(-)
cyc-1(RNAi)
C
OCR per worm (pmol/min)
FCCP
Sodium azide
N2
hsb-1(-)
H4 overexpression
cyc-1(RNAi)
Measurement no. (loop)
D
Basal mitochondrial OCR per worm (pmol/min)
****
****
N2; his-38(RNAi)
hsb-1; his-38(RNAi)
N2; cyc-1(RNAi)
E
Maximal respiratory capacity per worm (pmol/min)
****
****
N2; his-38(RNAi)
hsb-1; his-38(RNAi)
N2; cyc-1(RNAi)
F
Spare respiratory capacity per worm (pmol/min)
N2; his-38(RNAi)
hsb-1; his-38(RNAi)
N2; cyc-1(RNAi)
G
OCR per worm (pmol/min)
FCCP
Sodium azide
N2; his-38(RNAi)
hsb-1(-); his-38(RNAi)
N2; cyc-1(RNAi)
Measurement no. (loop)

Fig. S4. Reduced maximal respiratory capacity in *hsb-1(-)* worms is H4-dependent. (**A** and **B**) Basal mitochondrial OCR and maximal respiratory capacity per worm in wild-type (N2), *hsb-1(-)* and *cyc-1(RNAi)* worms; corrected for non-mitochondrial oxygen consumption. Central line, box limits and whiskers indicate median, interquartile range and data range, respectively, for ≥ 14 biological replicates, each of n = 10–20 worms. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ in Tukey's multiple comparisons test performed after one-way ANOVA. (**C**) Raw values for OCR per worm in N2, *hsb-1(-)*, H4 overexpression strain and *cyc-1(RNAi)* worms. Mean ± SEM for ≥ 14 biological replicates, each of n = 10–20 worms. (**D** to **F**) Basal mitochondrial OCR, maximal respiratory capacity and spare respiratory capacity per worm in N2; *his-38(RNAi)*, *hsb-1(-); his-38(RNAi)* and N2; *cyc-1(RNAi)* worms; corrected for non-mitochondrial oxygen consumption. Central line, box limits and whiskers indicate median, interquartile range and data range, respectively, for ≥ 14 biological replicates, each of n = 10–20 worms. **** $p < 0.0001$ in Tukey's multiple comparisons test performed after one-way ANOVA. (**G**) Raw values for OCR per worm in N2; *his-38(RNAi)*, *hsb-1(-); his-38(RNAi)* and N2; *cyc-1(RNAi)* worms. Mean ± SEM for ≥ 14 biological replicates, each of n = 10–20 worms. (C and G) Measurement points (loops) 2–5 represent basal OCR. FCCP is a mitochondrial electron transport chain (ETC) accelerator that induces maximal uncontrolled OCR, while OCR measurements post-sodium azide injection correspond to non-mitochondrial respiration.

Fig. S5

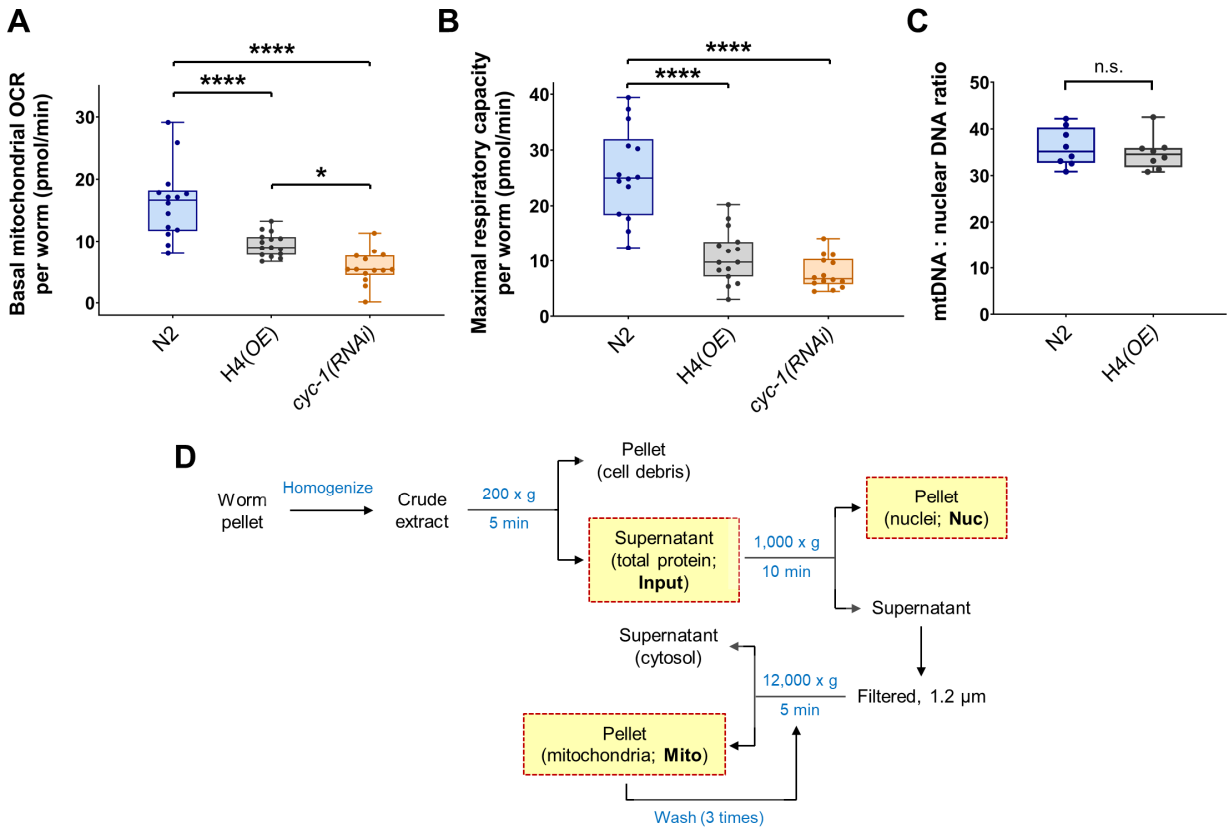
A
Basal mitochondrial OCR per worm (pmol/min)
****
****
*
N2
H4(OE)
cyc-1(RNAi)
B
Maximal respiratory capacity per worm (pmol/min)
****
****
N2
H4(OE)
cyc-1(RNAi)
C
mtDNA : nuclear DNA ratio
n.s.
N2
H4(OE)
D
Worm pellet
Homogenize
Crude extract
200 x g
5 min
Pellet (cell debris)
Supernatant (total protein; Input)
1,000 x g
10 min
Pellet (nuclei; Nuc)
Supernatant
Filtered, 1.2 µm
12,000 x g
5 min
Supernatant (cytosol)
Pellet (mitochondria; Mito)
Wash (3 times)
E
Input
Mito
H2A
H2B
H3
Fibrillarin
F
N2
H4(OE)
H4
NDUFS3
Isolated mitochondria

Fig. S5. H4 overexpressing worms have reduced mitochondrial respiratory capacity and elevated level of histone H4 in their mitochondria. (**A** and **B**) Basal mitochondrial OCR and maximal respiratory capacity in wild-type (N2), H4 overexpression strain and *cyc-1(RNAi)* worms; corrected for non-mitochondrial oxygen consumption. Central line, box limits and whiskers indicate median, interquartile range and data range, respectively, for ≥ 14 biological replicates, each of n = 10–20 worms. * $p < 0.05$, **** $p < 0.0001$ in Tukey's multiple comparisons test performed after one-way ANOVA. (**C**) Mitochondrial DNA copy number relative to nuclear DNA in N2 and H4 overexpression strain. Central line, box limits and whiskers indicate median, interquartile range and data range, respectively, for eight biological replicates, each of n = 8 worms. $p = 0.54$ for comparison between the two genotypes in two-tailed t-test. (**D**) Schematic representation for differential centrifugation steps used to obtain cellular fractions enriched with nuclei or intact mitochondria. The supernatant obtained after pelleting nuclei was passed through a filter of pore size 1.2 µm to remove any nuclei contamination prior to pelleting down mitochondria (see Materials and Methods for details). (**E**) Representative immunoblots for histones H2A, H2B and H3 and nuclear protein FIB-1 (fibrillarin homolog) in mitochondrial fraction (Mito) obtained from wild-type worms. (**F**) Representative immunoblots for histone H4 and mitochondrial protein NUO-2 (NDUFS3 homolog) in mitochondria isolated from N2 and H4 overexpressing worms.

Fig. S6

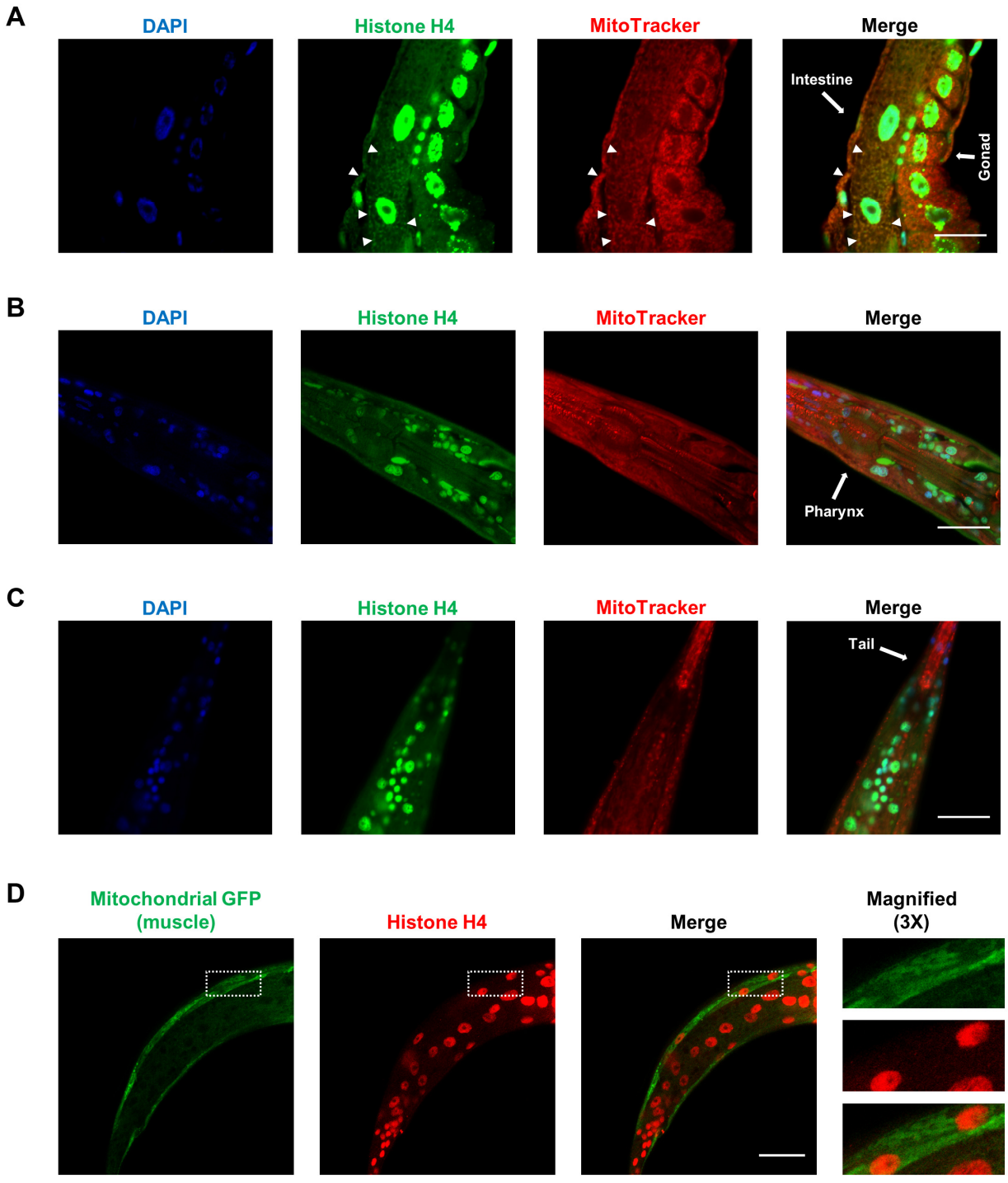
A
DAPI
Histone H4
MitoTracker
Merge
Intestine
Gonad
B
DAPI
Histone H4
MitoTracker
Merge
Pharynx
C
DAPI
Histone H4
MitoTracker
Merge
Tail
D
Mitochondrial GFP (muscle)
Histone H4
Merge
Magnified (3X)

Fig. S6. Extranuclear histone H4 foci co-localize with mitochondria in intestinal tissue, but not in muscle tissue. (**A** to **C**) H4 immunofluorescence and labeling of mitochondria in different tissues of *hsb-1(-)* worms. White arrowheads: Co-localization of extranuclear histone foci with MitoTracker staining in intestine of worms. No obviously detectable co-localization of histone foci with MitoTracker staining was observed in other tissues. Scale bars: 20 µM. (**D**) H4 immunofluorescence in worms expressing mitochondrially-localized GFP in muscle tissue [*myo-3p::gfp*(mitochondrial)]. Histone H4 immunostaining does not overlap with muscle mitochondria. Scale bar: 20 µM. Region outlined in white box is magnified (3X) and shown on the right.

Fig. S7

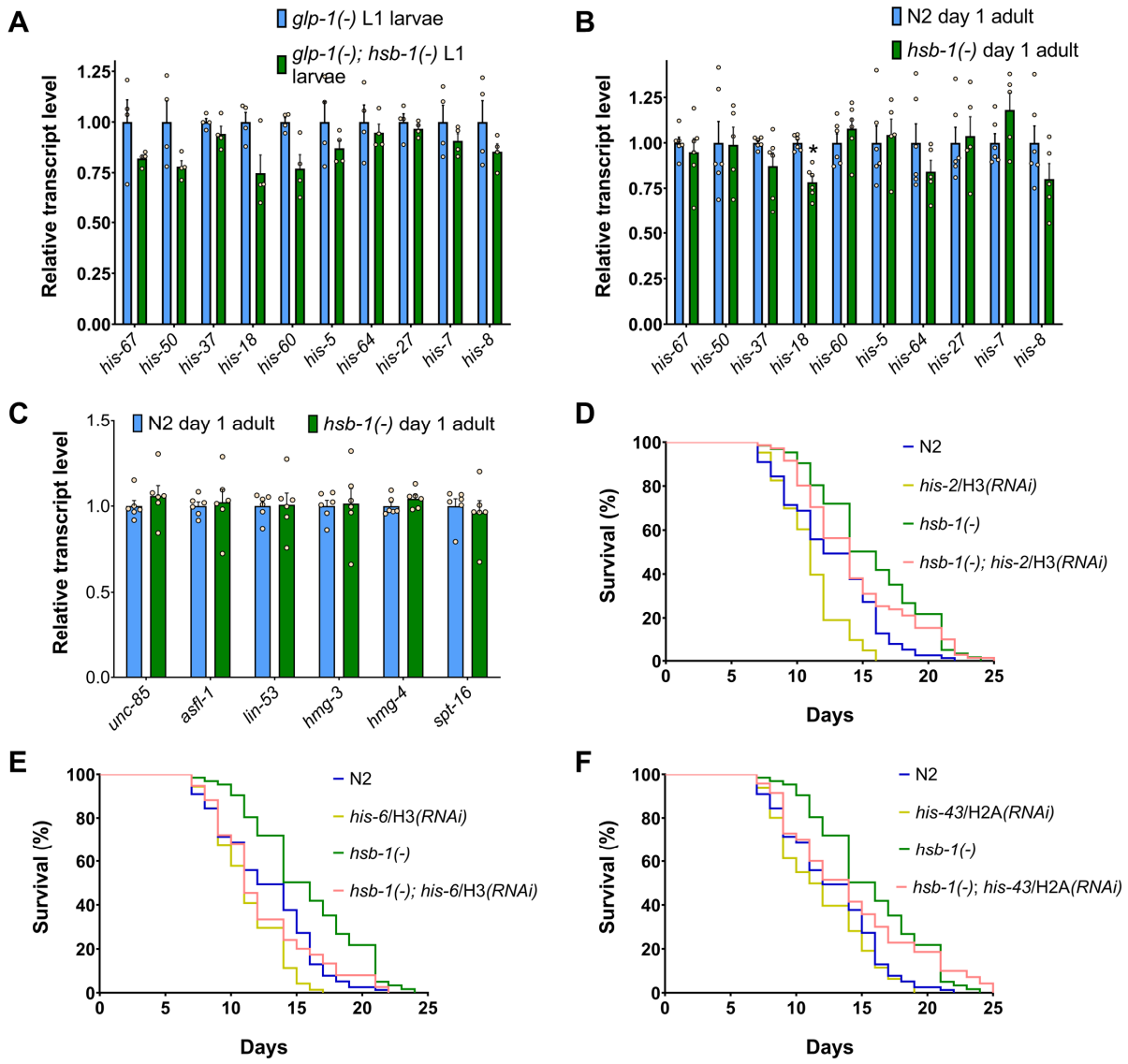
A
glp-1(-) L1 larvae
glp-1(-); hsb-1(-) L1 larvae
Relative transcript level
his-67
his-50
his-37
his-18
his-60
his-5
his-64
his-27
his-7
his-8
B
N2 day 1 adult
hsb-1(-) day 1 adult
Relative transcript level
*
C
N2 day 1 adult
hsb-1(-) day 1 adult
Relative transcript level
unc-85
asfl-1
lin-53
hmg-3
hmg-4
spt-16
D
N2
his-2/H3(RNAi)
hsb-1(-)
hsb-1(-); his-2/H3(RNAi)
Survival (%)
Days
E
N2
his-6/H3(RNAi)
hsb-1(-)
hsb-1(-); his-6/H3(RNAi)
Survival (%)
Days
F
N2
his-43/H2A(RNAi)
hsb-1(-)
hsb-1(-); his-43/H2A(RNAi)
Survival (%)
Days

Fig S7. *hsb-1(-)* worms do not show increased transcription of H4-coding genes. (**A**) Relative transcript levels of different core histone genes in *glp-1(-)* and *glp-1(-); hsb-1(-)* L1 larval worms. Mean ± SEM for four biological replicates. No statistically significant difference was detected between the two genotypes in two-tailed *t*-tests. (**B**) Relative transcript levels of different core histone genes in day 1 adult wild-type (N2) and *hsb-1(-)* worms. Mean ± SEM for ≥ 5 biological replicates. * $p < 0.05$ compared to corresponding wild-type transcript levels in two-tailed *t*-test. (**C**) Relative transcript levels of histone chaperone genes in day 1 adult N2 and *hsb-1(-)* worms. Mean ± SEM for six biological replicates. No statistically significant difference was detected between the two genotypes in two-tailed *t*-tests. (**D** to **F**) Life span analysis of N2 and *hsb-1(-)* worms subjected to control, *his-2* (H3), *his-6* (H3) or *his-43* (H2A) RNAi. Statistical data are included in table S1.

Table S1. Statistical data for life span experiments.

Strain	Mean life span ± SEM (days)	75% dead (days)	n	*p* value	
Life span experiments at 25°C					
N2; control (i)	11.83 ± 0.41	14	55/72	—	a
N2; *his-67(RNAi)* (i)	11.05 ± 0.34	13	65/72	0.0956[a]	b
hsb-1(cg116); control (i)	14.26 ± 0.60	18	50/72	0.0001[a]	c
hsb-1(cg116); his-67(RNAi) (i)	11.64 ± 0.50	16	51/72	0.8419[a], 0.0964[b], 0.0005[c]	d
N2; control (xv)	11.57 ± 0.41	14	70/90	—	a
N2; *his-67(RNAi)* (ii)	10.87 ± 0.35	13	79/90	0.1443[a]	b
hsb-1(cg116); control (xi)	14.58 ± 0.46	18	71/100	< 0.0001[a]	c
hsb-1(cg116); his-67(RNAi) (ii)	11.54 ± 0.46	15	78/90	0.7566[a], 0.0857[b], 0.0001[c]	d
N2; control (ii)	12.59 ± 0.50	15	51/72	—	a
N2; *his-5(RNAi)* (i)	10.72 ± 0.36	12	68/72	0.0008[a]	b
hsb-1(cg116); control (ii)	15.88 ± 0.59	20	50/72	0.0001[a]	c
hsb-1(cg116); his-5(RNAi) (i)	11.07 ± 0.36	12	69/72	0.0101[a], 0.4150[b], < 0.0001[c]	d
N2; control (iii)	12.52 ± 0.42	15	61/72	—	a
N2; *his-38(RNAi)* (i)	11.13 ± 0.39	13	64/72	0.0146[a]	b
hsb-1(cg116); control (iii)	16.38 ± 0.54	20	55/72	< 0.0001[a]	c
hsb-1(cg116); his-38(RNAi) (i)	11.72 ± 0.35	13	64/72	0.0774[a], 0.4358[b], < 0.0001[c]	d
N2; control (iv)	11.78 ± 0.30	14	69/84	—	a
N2; *his-38(RNAi)* (ii)	11.87 ± 0.32	14	68/84	0.8386[a]	b
hsb-1(cg116); control (iv)	13.47 ± 0.42	16	69/84	0.0004[a]	c
hsb-1(cg116); his-38(RNAi) (ii)	10.20 ± 0.30	12	75/84	0.0004[a], 0.0005[b], < 0.0001[c]	d
N2; control (v)	13.44 ± 0.49	17	68/90	—	a
N2; *his-38(RNAi)* (iii)	13.05 ± 0.48	17	59/90	0.4376[a]	b
hsb-1(cg116); control (v)	17.56 ± 0.74	22	57/90	< 0.0001[a]	c
hsb-1(cg116); his-38(RNAi) (iii)	12.15 ± 0.44	14	66/90	0.0809[a], 0.2303[b], < 0.0001[c]	d
N2; control (vi)	13.22 ± 0.48	17	72/90	—	a
N2; *his-38(RNAi)* (iv)	12.95 ± 0.48	17	67/90	0.6626[a]	b
sur-5p::hsf-1(OE); control (i)	17.26 ± 0.65	21	85/90	< 0.0001[a]	c
sur-5p::hsf-1(OE); his-38(RNAi) (i)	12.99 ± 0.37	15	83/90	0.3456[a], 0.6651[b], < 0.0001[c]	d

N2; control (vii)	13.51 ± 0.49	16	65/90	—	a
N2; *his-38(RNAi)* (v)	11.14 ± 0.32	13	74/90	< 0.0001[a]	b
sur-5p::hsf-1(OE); control (ii)	19.99 ± 0.64	25	82/90	< 0.0001[a]	c
sur-5p::hsf-1(OE); his-38(RNAi) (ii)	14.85 ± 0.54	17	85/90	0.0542[a], < 0.0001[b], < 0.0001[c]	d
N2; control (vi)	13.22 ± 0.48	17	72/90	—	a
N2; *his-38(RNAi)* (iv)	12.95 ± 0.48	17	67/90	0.6626[a]	b
daf-2(e1370); control (i)*	31.51 ± 1.87	39	37/90	< 0.0001[a]	c
daf-2(e1370); his-38(RNAi) (i)*	30.04 ± 0.91	35	89/90	< 0.0001[a], < 0.0001[b], 0.2169[c]	d
N2; control (viii)	15.84 ± 0.45	19	67/90	—	a
N2; *his-38(RNAi)* (vi)	14.61 ± 0.34	16	71/90	0.0077[a]	b
daf-2(e1370); control (ii)*	28.64 ± 1.14	35	51/150	< 0.0001[a]	c
daf-2(e1370); his-38(RNAi) (ii)*	27.04 ± 1.00	33	86/90	< 0.0001[a], < 0.0001[b], 0.2205[c]	d
N2; control (ix)	12.36 ± 0.39	15	68/84	—	a
N2; *his-38(RNAi)* (vii)	11.88 ± 0.31	14	74/84	0.1942[a]	b
glp-1(e2144); control (i)	14.68 ± 0.42	17	71/72	< 0.0001[a]	c
glp-1(e2144); his-38(RNAi) (i)	13.88 ± 0.48	17	72/72	0.002[a], 0.0001[b], 0.5421[c]	d
N2; control (x)	12.75 ± 0.55	17	83/91	—	a
N2; *his-38(RNAi)* (viii)	12.95 ± 0.42	15	91/91	0.8499[a]	b
glp-1(e2144); control (ii)	15.76 ± 0.39	18	88/91	0.0025[a]	c
glp-1(e2144); his-38(RNAi) (ii)	15.24 ± 0.38	18	91/91	0.0202[a], 0.0015[b], 0.2934[c]	d
N2; control (vi)	13.22 ± 0.48	17	72/90	—	a
N2; *his-38(RNAi)* (iv)	12.95 ± 0.48	17	67/90	0.6626[a]	b
isp-1(qm150); control (i)	22.46 ± 0.77	27	56/90	< 0.0001[a]	c
isp-1(qm150); his-38(RNAi) (i)	14.35 ± 0.36	16	85/90	0.2856[a], 0.1004[b], < 0.0001[c]	d
N2; control (vii)	13.51 ± 0.49	16	65/90	—	a
N2; *his-38(RNAi)* (v)	11.14 ± 0.32	13	74/90	< 0.0001[a]	b
isp-1(qm150); control (ii)	23.15 ± 0.59	26	62/108	< 0.0001[a]	c
isp-1(qm150); his-38(RNAi) (ii)	14.54 ± 0.48	16	86/90	0.1713[a], < 0.0001[b], < 0.0001[c]	d
N2 (xi)	13.06 ± 0.39	15	70/84	—	a
hsb-1(cg116) (vii)	14.46 ± 0.49	18	66/84	0.0093[a]	b
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE) (i)	14.98 ± 0.53	19	69/84	0.0001[a], 0.4037[b]	c

N2; control (xv)	11.57 ± 0.41	14	70/90	—	a
N2; *his-67(RNAi)* (ii)	10.87 ± 0.35	13	79/90	0.1443[a]	b
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); control (iv)	16.16 ± 0.34	18	87/90	< 0.0001[a]	c
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); his-67(RNAi) (i)	14.09 ± 0.40	17	90/90	0.0001[a], < 0.0001[b], 0.0046[c]	d
N2; control (ix)	12.36 ± 0.39	15	68/84	—	a
N2; *his-38(RNAi)* (vii)	11.88 ± 0.31	14	74/84	0.1942[a]	b
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); control (ii)	15.52 ± 0.34	17	75/84	< 0.0001[a]	c
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); his-38(RNAi) (i)	13.10 ± 0.34	15	79/84	0.2235[a], 0.011[b], < 0.0001[c]	d
glp-1(e2144); control (i)	14.68 ± 0.42	17	71/72	—	a
glp-1(e2144); his-38(RNAi) (i)	13.88 ± 0.48	17	72/72	0.5421[a]	b
glp-1(e2144); hsb-1(cg116); control (i)	17.49 ± 0.65	22	58/72	< 0.0001[a]	c
glp-1(e2144); hsb-1(cg116); his-38(RNAi) (i)	14.35 ± 0.42	17	71/72	0.55[a], 0.6578[b], < 0.0001[c]	d
glp-1(e2144); control (iii)	15.77 ± 0.43	18	84/90	—	a
glp-1(e2144); his-38(RNAi) (iii)	14.62 ± 0.34	17	85/90	0.0032[a]	b
glp-1(e2144); hsb-1(cg116); control (ii)	20.83 ± 0.43	24	89/90	< 0.0001[a]	c
glp-1(e2144); hsb-1(cg116); his-38(RNAi) (ii)	16.10 ± 0.42	20	90/90	0.7401[a], 0.0013[b], < 0.0001[c]	d
glp-4(bn2); control (i)	12.23 ± 0.56	17	66/90	—	a
glp-4(bn2); his-38(RNAi) (i)	11.55 ± 0.48	14	63/90	0.22[a]	b
glp-4(bn2); hsb-1(cg116); control (i)	17.25 ± 0.76	22	58/90	< 0.0001[a]	c
glp-4(bn2); hsb-1(cg116); his-38(RNAi) (i)	12.72 ± 0.62	17	69/90	0.6043[a], 0.0898[b], < 0.0001[c]	d
glp-4(bn2); his-38(RNAi) (ii)	11.62 ± 0.42	14	66/90	—	a
glp-4(bn2); hsb-1(cg116); his-38(RNAi) (ii)	12.35 ± 0.51	17	65/90	0.20[a]	b
glp-1(e2144); control (i)	14.68 ± 0.42	17	71/72	—	a
*glp-1(e2144); his-38(*adulthood *RNAi)* (i)	15.60 ± 0.48	19	72/72	0.0581[a]	b

glp-1(e2144); hsb-1(cg116); control (i)	17.49 ± 0.65	22	58/72	< 0.0001[a]	c
*glp-1(e2144); hsb-1(cg116); his-38(*adulthood *RNAi)* (i)	17.14 ± 0.65	21	72/72	< 0.0001[a], 0.0018[b], 0.8655[c]	d
glp-1(e2144); control (iii)	15.77 ± 0.43	18	84/90	—	a
glp-1(e2144); *his-38(*adulthood *RNAi)* (ii)	15.17 ± 0.43	18	90/90	0.5274[a]	b
glp-1(e2144); hsb-1(cg116); control (ii)	20.83 ± 0.43	24	89/90	< 0.0001[a]	c
*glp-1(e2144); hsb-1(cg116); his-38(*adulthood *RNAi)* (ii)	20.80 ± 0.40	23	89/90	< 0.0001[a], < 0.0001[b], 0.6236[c]	d
N2; control (viii)	15.84 ± 0.45	19	67/90	—	a
N2; *his-38(*adulthood *RNAi)* (i)	14.47 ± 0.40	16	64/90	0.0126[a]	b
hsb-1(cg116); control (vi)	20.33 ± 0.60	26	71/90	< 0.0001[a]	c
*hsb-1(cg116); his-38(*adulthood *RNAi)* (i)	18.03 ± 0.65	22	73/90	0.0008[a], < 0.0001[b], 0.0594[c]	d
N2; control (ii)	12.59 ± 0.50	15	51/72	—	a
N2; *his-67(*adulthood *RNAi)* (i)	13.04 ± 0.39	15	54/72	0.7155[a]	b
hsb-1(cg116); control (ii)	15.88 ± 0.59	20	50/72	0.0001[a]	c
*hsb-1(cg116); his-67(*adulthood *RNAi)* (i)	14.58 ± 0.41	17	60/72	0.0165[a], 0.001[b], 0.0197[c]	d
N2; control (xii)	13.08 ± 0.39	15	65/90	—	a
N2; *ubl-5(RNAi)* (i)	12.61 ± 0.38	15	56/90	0.2935[a]	b
hsb-1(cg116); control (viii)	17.13 ± 0.78	22	58/100	< 0.0001[a]	c
hsb-1(cg116); ubl-5(RNAi) (i)	14.59 ± 0.57	18	56/90	0.012[a], 0.0012[b], 0.0009[c]	d
N2; control (xiii)	12.65 ± 0.39	15	77/100	—	a
N2; *ubl-5(RNAi)* (ii)	13.05 ± 0.40	15	76/100	0.5189[a]	b
hsb-1(cg116); control (ix)	16.04 ± 0.53	20	73/100	< 0.0001[a]	c
hsb-1(cg116); ubl-5(RNAi) (ii)	14.39 ± 0.39	18	70/90	0.0058[a], 0.0289[b], 0.0012[c]	d
N2; control (xiii)	12.65 ± 0.39	15	77/100	—	a
N2; *ubl-5(RNAi)* (ii)	13.05 ± 0.40	15	76/100	0.5189[a]	b
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); control (iii)	16.07 ± 0.38	18	90/100	< 0.0001[a]	c
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); ubl-5(RNAi) (i)	14.55 ± 0.28	16	88/100	0.0065[a], 0.0378[b], < 0.0001[c]	d
N2; control (xv)	11.57 ± 0.41	14	70/90	—	a
N2; *ubl-5(RNAi)* (iii)	11.87 ± 0.37	14	76/90	0.7908[a]	b

sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); control (iv)	16.16 ± 0.34	18	87/90	< 0.0001[a]	c
sur-5p::his-67(OE); sur-5p::his-50(OE); sur-5p::his-37(OE); ubl-5(RNAi) (ii)	13.88 ± 0.29	15	76/90	0.0025[a], 0.0029[b], < 0.0001[c]	d
rde-1(ne219); ges-1p::rde-1; control (i)	13.83 ± 0.40	16	82/96	—	a
rde-1(ne219); ges-1p::rde-1; his-38(RNAi) (i)	13.28 ± 0.35	16	82/97	0.1716[a]	b
hsb-1(cg116); rde-1(ne219); ges-1p::rde-1; control (i)	16.62 ± 0.53	20	112/119	< 0.0001[a]	c
hsb-1(cg116); rde-1(ne219); ges-1p::rde-1; his-38(RNAi) (i)	12.36 ± 0.39	14	106/120	0.0496[a], 0.3281[b], < 0.0001[c]	d
rde-1(ne219); ges-1p::rde-1; control (ii)	15.32 ± 0.42	18	59/72	—	a
rde-1(ne219); ges-1p::rde-1; his-38(RNAi) (ii)	14.43 ± 0.35	16	55/68	0.0458[a]	b
hsb-1(cg116); rde-1(ne219); ges-1p::rde-1; control (ii)	17.42 ± 0.83	21	42/72	0.0003[a]	c
hsb-1(cg116); rde-1(ne219); ges-1p::rde-1; his-38(RNAi) (ii)	14.46 ± 0.80	18	33/70	0.9176[a], 0.2844[b], 0.0033[c]	d
N2; control (xiv)	12.68 ± 0.42	16	77/90	—	a
N2; *his-2(RNAi)* (i)	11.00 ± 0.30	12	63/90	0.0002[a]	b
hsb-1(cg116); control (x)	15.63 ± 0.53	19	60/90	< 0.0001[a]	c
hsb-1(cg116); his-2(RNAi) (i)	14.37 ± 0.51	17	71/90	0.0137[a], < 0.0001[b], 0.2527[c]	d
N2; control (xiv)	12.68 ± 0.42	16	77/90	—	a
N2; *his-6(RNAi)* (i)	11.21 ± 0.31	14	71/90	0.0004[a]	b
hsb-1(cg116); control (x)	15.63 ± 0.53	19	60/90	< 0.0001[a]	c
hsb-1(cg116); his-6(RNAi) (i)	12.37 ± 0.45	14	75/90	0.8743[a], 0.0163[b], 0.0001[c]	d
N2; control (xiv)	12.68 ± 0.42	16	77/90	—	a
N2; *his-43(RNAi)* (i)	11.89 ± 0.40	15	79/90	0.2187[a]	b
hsb-1(cg116); control (x)	15.63 ± 0.53	19	60/90	< 0.0001[a]	c
hsb-1(cg116); his-43(RNAi) (i)	14.15 ± 0.62	17	70/90	0.0121[a], 0.0006[b], 0.4155[c]	d
Life span experiments at 20°C					
N2; control (i)	15.36 ± 0.47	17	56/72	—	a
N2; *his-67(RNAi)* (i)	14.62 ± 0.35	17	65/84	0.0576[a]	b
hsb-1(cg116); control (i)	19.48 ± 0.69	24	72/84	< 0.0001[a]	c

hsb-1(cg116); his-67(RNAi) (i)	18.50 ± 0.42	22	78/84	< 0.0001[a], < 0.0001[b], 0.0035[c]	d
N2; control (ii)	20.26 ± 0.49	22	78/91	—	a
N2; *his-67(RNAi)* (ii)	18.44 ± 0.59	22	62/91	0.0443[a]	b
hsb-1(cg116); control (ii)	24.39 ± 0.72	29	79/91	< 0.0001[a]	c
hsb-1(cg116); his-67(RNAi) (ii)	22.72 ± 0.73	29	87/91	0.0001[a], < 0.0001[b], 0.2298[c]	d
N2; control (ii)	20.26 ± 0.49	22	78/91	—	a
N2; *his-38(RNAi)* (i)	16.48 ± 0.50	20	81/91	< 0.0001[a]	b
hsb-1(cg116); control (ii)	24.39 ± 0.72	29	79/91	< 0.0001[a]	c
hsb-1(cg116); his-38(RNAi) (i)	21.13 ± 0.70	27	79/91	0.0351[a], < 0.0001[b], 0.0002[c]	d

Adult ‘mean life span ± SEM’ in days. ‘75% dead’ refers to 75th percentile, age at which surviving proportion of the population reaches 0.25. ‘n’ indicates number of observed deaths relative to total number of animals on day 1 of the experiment. The difference refers to animals that were censored at different time points due to bagging, exploding, crawling off plates or being accidentally killed. Statistical analysis was performed using Mantel-Cox log-rank test to obtain *p* values. Conditions for which life span analysis was performed more than once, their descriptions are followed by a Roman numeral in parentheses to identify independent biological replicates. * *daf-2(e1370)* animals, being dauer-constitutive at 25°C, were transferred from 20°C to 25°C on day 1 of adulthood.

Table S2. Statistical data for frequency distributions of genome-wide nucleosomal parameters determined from MNase-Seq analysis.

Sample 1	Sample 2	Mean (sample 1)	Mean (sample 2)	SD (sample 1)	SD (sample 2)	*D* value	Bonferroni *p* value
Nucleosome peak width							
N2; control	N2; *his-38(i)*	136.30	142.22	303.56	304.11	0.0267	$< 1.4 \times 10^{-15}$
N2; control	*hsb-1(-);* control	136.30	128.54	303.56	299.39	0.0427	$< 1.4 \times 10^{-15}$
N2; control	*hsb-1(-); his-38(i)*	136.30	144.88	303.56	286.61	0.0528	$< 1.4 \times 10^{-15}$
N2; *his-38(i)*	*hsb-1(-);* control	142.22	128.54	304.11	299.39	0.0689	$< 1.4 \times 10^{-15}$
N2; *his-38(i)*	*hsb-1(-); his-38(i)*	142.22	144.88	304.11	286.61	0.0269	$< 1.4 \times 10^{-15}$
hsb-1(-); control	*hsb-1(-); his-38(i)*	128.54	144.88	299.39	286.61	0.0954	$< 1.4 \times 10^{-15}$
Nucleosome peak fuzziness							
N2; control	N2; *his-38(i)*	56.28	56.26	2.08	2.06	0.0069	0.27414
N2; control	*hsb-1(-);* control	56.28	56.01	2.08	2.11	0.0498	$< 1.4 \times 10^{-15}$
N2; control	*hsb-1(-); his-38(i)*	56.28	56.38	2.08	2.09	0.0215	$< 1.4 \times 10^{-15}$
N2; *his-38(i)*	*hsb-1(-);* control	56.26	56.01	2.06	2.11	0.0471	$< 1.4 \times 10^{-15}$
N2; *his-38(i)*	*hsb-1(-); his-38(i)*	56.26	56.38	2.06	2.09	0.0259	$< 1.4 \times 10^{-15}$
hsb-1(-); control	*hsb-1(-); his-38(i)*	56.01	56.38	2.11	2.09	0.0708	$< 1.4 \times 10^{-15}$
Nucleosome peak height							
N2; control	N2; *his-38(i)*	541.91	508.42	442.76	389.89	0.0294	0.27996
N2; control	*hsb-1(-);* control	541.91	587.17	442.76	487.94	0.0579	0.00013
N2; control	*hsb-1(-); his-38(i)*	541.91	503.10	442.76	387.76	0.0388	0.01165
N2; *his-38(i)*	*hsb-1(-);* control	508.42	587.17	389.89	487.94	0.0734	3.89×10^{-8}
N2; *his-38(i)*	*hsb-1(-); his-38(i)*	508.42	503.10	389.89	387.76	0.0156	1

hsb-1(-); control	*hsb-1(-); his-38(i)*	587.17	503.10	487.94	387.76	0.0845	1.63×10^{-11}

Statistical analysis was performed for comparison between genome-wide frequency distributions of nucleosome peak width, peak fuzziness and peak height across conditions. '*D* value' refers to *D* statistic determined from Kolmogorov-Smirnov test. *p* values are reported after Bonferroni's correction for multiple comparisons. '*his-38(i)*' indicates *his-38(RNAi)*.

Table S3. Genomic regions with large-scale changes in MNase-Seq signal intensity associated with HSB-1 inhibition or H4 RNAi.

Genomic location	Brief description of peak location	Occupancy difference
Gain of peak intensity in hsb-1(-) worms compared to N2 in control RNAi		
mtDNA: 40 – 13,790	Entire stretch of mitochondrial DNA	2,529.87
IV: 11,068,570 – 11,086,730	Contains pseudogene *Y5F2A.3*	1,972.07
I: 15,032,020 – 15,072,410	rRNA gene repeats and ncRNA genes near right arm telomere of chromosome I	1,280.87
V: 8,713,960 – 8,727,030	Region between genes *ZC178.2* and *glr-5*	617.03
Loss of peak intensity in hsb-1(-) worms compared to N2 in control RNAi		
I: 13,947,370 – 14,015,560	Includes genes *Y71A12B.18*, *Y71A12B.12* and four ncRNA genes	896.39
IV: 14,919,710 – 14,940,680	Possible background deletion close to *hsb-1(cg116)* deletion	689.88
IV: 12,557,590 – 12,594,200	*hsb-1(cg116)* deletion	607.49
IV: 3,567,570 – 3,629,160	Region between genes *unc-17* and *ZC416.2*	514.43
Gain of peak intensity in hsb-1(-) worms compared to N2 in his-38 RNAi		
mtDNA: 40 – 13,780	Entire stretch of mitochondrial DNA	2,126.29
IV: 11,068,560 – 11,086,710	Contains pseudogene *Y5F2A.3*	1,918.40
I: 15,031,930 – 15,072,410	rRNA gene repeats and ncRNA genes near right arm telomere of chromosome I	1,587.67
V: 8,713,970 – 8,720,440	Region between genes *ZC178.2* and *glr-5*	554.26
V: 18,695,560 – 18,792,030	Pseudogene-rich region on chromosome V	502.44
Loss of peak intensity in hsb-1(-) worms compared to N2 in his-38 RNAi		
IV: 14,919,770 – 14,939,020	Possible background deletion close to *hsb-1(cg116)* deletion	722.74
IV: 12,556,970 – 12,584,290	*hsb-1(cg116)* deletion	689.88
I: 13,946,630 – 14,015,560	Includes genes *Y71A12B.18*, *Y71A12B.12* and four ncRNA genes	566.36
IV: 3,626,530 – 3,629,160	Region between genes *unc-17* and *ZC416.2*	556.38
Loss of peak intensity in hsb-1(-) worms subjected to his-38 RNAi compared to control RNAi		
X: 15,788,520 – 15,838,140	Region between gene *Y7A5A.7* and pseudogene *Y7A5A.11*	725.67
mtDNA: 40 – 13,790	Entire stretch of mitochondrial DNA	609.78

Occupancy difference was determined using Dregion function of DANPOS2 (see Materials and Methods). Genomic regions with occupancy difference values ≥ 500 are reported. No region

passed the threshold value of ≥ 500 for reduction in peak intensity after *his-38(RNAi)* in wild-type N2 strain. Also, no region showed an increase in peak intensity above the threshold value of ≥ 500 after *his-38(RNAi)* in either N2 or *hsb-1(-)* strain. Data for all identified genomic regions are included in Data file S1.

Data file S1. Genomic regions with altered MNase-Seq signal intensity due to HSB-1 inhibition or H4 RNAi (available as separate file).

Data file S2. List of primer sequences used for PCR experiments.