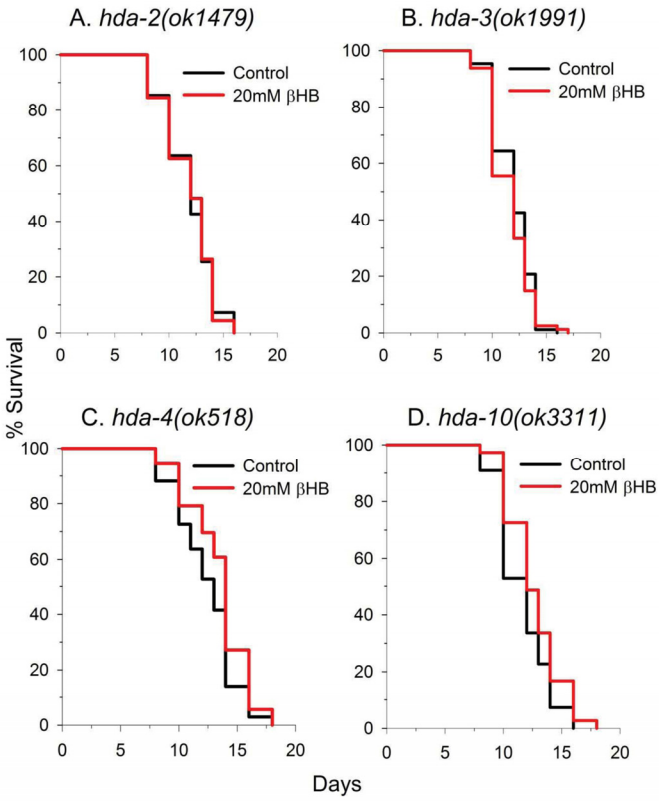
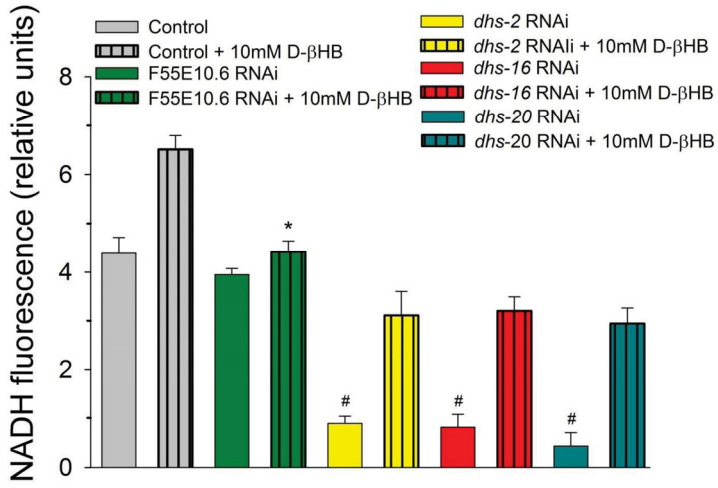
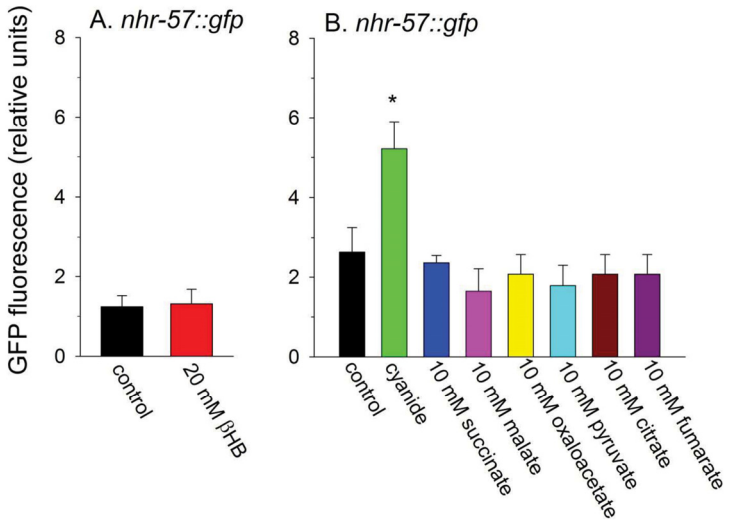
SUPPLEMENTARY DATA



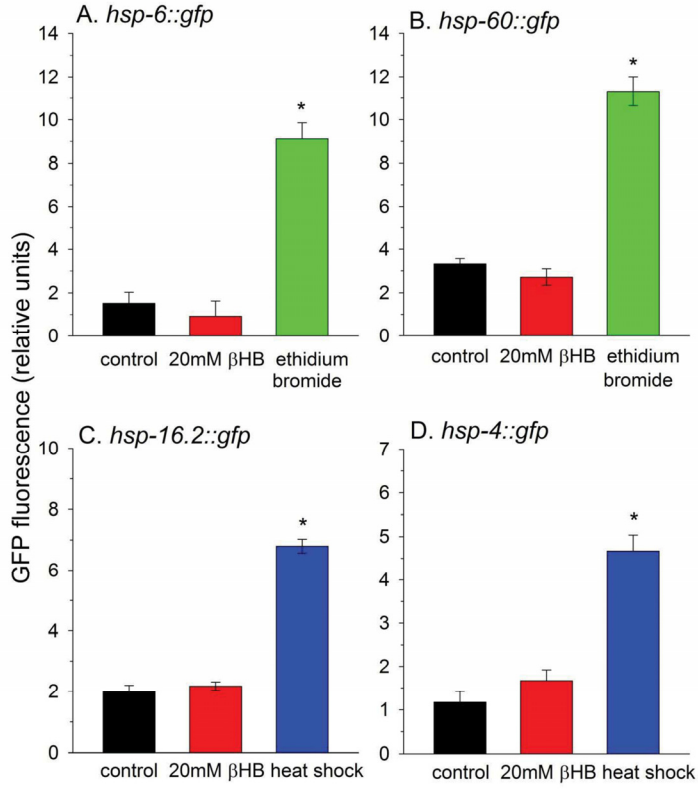
Supplementary Figure 1. The effect of βHB on lifespan in histone deacetylase mutants. βHB addition did not extend the lifespan of **(A)** hda-2(ok1479) mutant worms or **(B)** hda-3(ok1991) mutant worms. βHB addition extended the lifespan of **(C)** hda-4(ok518) mutant worms (log-rank p =0.001) and **(D)** hda-10(ok3311) mutant worms (log-rank p =0.002).



Supplementary Figure 2. L-βHB dehydrogenase activity in worm extracts grown in the absence or presence of 10 mM D-βHB. Knockdown of F55E10.6 almost completely prevented the increased L-βHB dehydrogenase activity induced by culturing with D-βHB (* $p < 0.05$ vs. (Control + 10mM D-βHB)). Knockdown of *dhs-2*, *dhs-16*, or *dhs-20* decreased endogenous L-βHB dehydrogenase activity in the extracts (# $p < 0.05$ vs. Control).



Supplementary Figure 3. βHB, pyruvate, or TCA cycle metabolites do not activate HIF-1 transcriptional activity. The effect of **(A)** βHB, **(B)** pyruvate, or TCA cycle metabolites on GFP fluorescence in nhr-57::gfp worms. 20 M potassium cyanide was used as a positive control. (* p < 0.05).



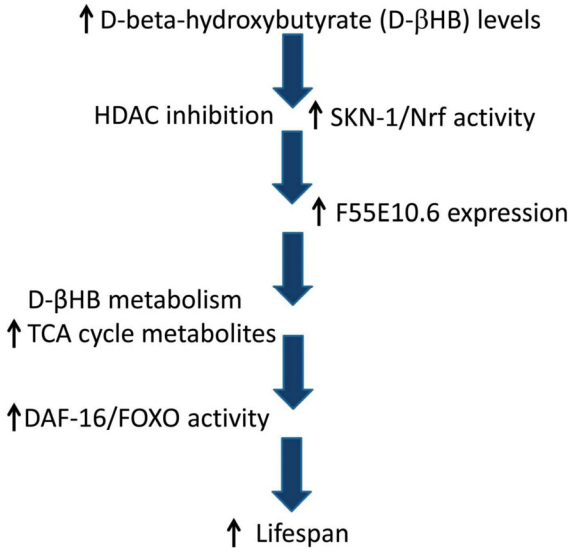
Supplementary Figure 4. βHB addition does not induce expression of several heat shock proteins. The effect of βHB addition on the fluorescence of **(A)** *hsp-6::gfp*, **(B)** *hsp-60::gfp*, **(C)** *hsp-16.2p::GFP*, or **(D)** *hsp-4::GFP* worms. For **(A)** and **(B)** 50 _g/ml ethidium bromide treatment for 2 days was used as a positive control. For **(C)** and **(D)** heat shock at 35°C for 2 hours was used as a positive control. (* *p* < 0.05).

Supplementary Table 1. Lifespan data

Strain	RNAi	Treatment	% of untreated mean lifespan	% of N2 mean lifespan	*n*	# of worms	*p*-value
N2		2 mM βHB	105	105	2	224	0.024
N2		10 mM βHB	113	113	2	201	<0.001
N2		20 mM βHB	126	126	6	586	<0.001
N2		50 mM βHB	81	81	1	100	<0.001
N2		100 mM βHB	69	69	1	100	<0.001
N2		6 mM valproic acid	93	93	2	118	<0.001
N2		6 mM valproic acid + 20 mM βHB	84	84	2	126	<0.001
N2		5 mM butyrate	110	110	2	124	<0.001
N2		5 mM butyrate + 20 mM βHB	95	95	2	117	0.023
N2		1 mM NAC + 20 mM βHB	117	117	2	146	<0.001
N2		1 mM NAC	109	109	2	132	0.001
N2		50 mM glucose	70	70	4	336	<0.001
N2		50 mM glucose + 20 mM βHB	79	79	4	362	<0.001[a]
N2	F55E10.6 RNAi			107	3	375	0.008
N2	F55E10.6 RNAi	20 mM βHB	100		3	403	0.527
N2	*skn-1* RNAi			76	2	145	<0.001
N2	*skn-1* RNAi	20 mM βHB	103		2	156	0.557
N2	*cbp-1* RNAi			44	2	118	<0.001
N2	*cbp-1* RNAi	20 mM βHB	101		2	112	0.803
N2	*hda-1* RNAi			99	2	153	0.425
N2	*hda-1* RNAi	20 mM βHB	108		2	159	0.002
N2	*hda-2* RNAi			112	2	396	<0.001
N2	*hda-2* RNAi	20 mM βHB	96		2	362	0.018
N2	*hda-3* RNAi			111	2	316	<0.001
N2	*hda-3* RNAi	20 mM βHB	102		2	380	0.648
hda-2(ok1479)				69	1	110	<0.001
hda-2(ok1479)		20 mM βHB	100		1	91	0.98
hda-3(ok1991)				69	1	87	<0.001
hda-3(ok1991)		20 mM βHB	97		1	81	0.29
hda-4(ok518)				72	1	135	<0.001
hda-4(ok518)		20 mM βHB	108		1	125	0.001
hda-10(ok3311)				67	1	110	<0.001
hda-10(ok3311)		20 mM βHB	109		1	113	0.002
daf-16(mgDf50)				83	2	295	<0.001
daf-16(mgDf50)		20 mM βHB	99		2	270	0.361
aak-2(gt33)				88	2	208	<0.001
aak-2(gt33)		20 mM βHB	100		2	184	0.793
sir-2.1(ok434)				84	2	210	<0.001
sir-2.1(ok434)		20 mM βHB	98		2	265	0.113
eat-2(ad1116)				146	2	114	<0.001
eat-2(ad1116)		20 mM βHB	95		2	157	0.114

gas-1(fc21)				69	2	119	<0.001
gas-1(fc21)		20 mM βHB	111		2	120	<0.001
mev-1(kn1)				70	2	273	<0.001
mev-1(kn1)		20 mM βHB	127		2	269	<0.001
rsks-1(ok1255)				106	3	362	<0.001
rsks-1(ok1255)		20 mM βHB	105		3	337	0.002
gcn-2(ok871)				88	3	479	<0.001
gcn-2(ok871)		20 mM βHB	108		3	428	<0.001
CL6049 *(16°C) snb-1::TDP-43*				57	1	123	<0.001
CL6049 *(16°C) snb-1::TDP-43*		20 mM βHB	99		1	114	0.413
CL6049 *(16°C) snb-1::TDP-43*		30 mm βHB	105		1	117	0.006
CL6049 *snb-1::TDP-43*				61	1	90	<0.001
CL6049 *snb-1::TDP-43*		50 mM βHB	101		1	93	0.951
CL6049 *snb-1::TDP-43*		100 mM βHB	103		1	53	0.791

[a]Compared to 50 mM glucose treated worms or untreated worms.



Supplementary Figure 5. One possible mechanism through which βHB may extend lifespan in *C. elegans.*