

## SUPPLEMENTARY MATERIAL

**Supplementary Table 1. List of strains used in this study.**

Strain	genotype	comment
N2E		Wild-type
CF3942	<i>glp-1(e2144ts) III</i>	<i>glp-1(e2144ts)</i> from CF1903 [21], outcrossed 12x to N2E
CF3943	<i>muIs84[Psod-3::gfp]</i>	<i>muIs84</i> from CF1553 [38] outcrossed 12x
CF3949	<i>glp-1(e2144ts) III; muIs84[Psod-3::gfp]</i>	
CF4339	<i>daf-2;(e1370) III; muIs84[Psod-3::gfp]</i>	
CF4054	<i>daf-16(mu86) I</i>	<i>daf-16(mu86)</i> from CF1037 [18], outcrossed 12x to N2E
CF4087	<i>daf-2(e1370) III</i>	<i>daf-16(mu86)</i> from CF1041 [18], outcrossed 12x
CF4096	<i>daf-16(mu86) I; muIs194[Pges-1::ha::gfp::daf-16 + Podr-1::rfp]</i>	<i>muIs194</i> from CF3628: <i>daf-16(mu86) I; muIs194</i>
CF4117	<i>zcls18[Pges-1::gfp(cyt)]</i>	Strain SJ4144 (Ron lab/CGC) outcrossed 6x
CF4164	<i>mbk-1(pk1389) X</i>	<i>mbk-1(pk1389)</i> from EK228 [26] (Kandel lab /CGC) outcrossed 6x
CF4165	<i>glp-1(e2144ts) III; mbk-1(pk1389) X</i>	
CF4166	<i>daf-2(e1370) III; mbk-1(pk1389) X</i>	
CF4167	<i>daf-16(mu86) I; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	<i>muIs145</i> is the integrated version of muEx268 [38]
CF4168	<i>daf-16(mu86) I; glp-1(e2144ts) III; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	
CF4169	<i>daf-16(mu86) I; daf-2(e1370) III; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	
HMT029	<i>daf-16(mu86) I; mbk-1(pk1389) X; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	
HMT030	<i>daf-16(mu86) I; glp-1(e2144ts) III; mbk-1(pk1389) X; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	
HMT031	<i>daf-16(mu86) I; daf-2(e1370) III; mbk-1(pk1389) X; muIs145[Pges-1::gfp::daf-16 + Podr-1::rfp]</i>	
CF4173	<i>hpk-1(pk1393) X</i>	<i>hpk-1(pk1393)</i> from EK273 [26] (Kandel lab/CGC) outcrossed 6x
CF4185	<i>glp-1(e2144ts) III; hpk-1(pk1393) X</i>	
HMT001	<i>daf-2(e1370) III; hpk-1(pk1393) X</i>	Very low progeny, reported to be synthetic lethal [34]
CF4183	<i>hpk-1(pk1393) X; muIs84[Psod-3::gfp]</i>	
HMT002	<i>glp-1(e2144ts) III; hpk-1(pk1393) X; muIs84[Psod-3::gfp]</i>	
CF4184	<i>mbk-1(pk1389) X; muIs84[Psod-3::gfp]</i>	
HMT003	<i>glp-1(e2144ts) III; mbk-1(pk1389) X; muIs84[Psod-3::gfp]</i>	
HMT004	<i>daf-2(e1370) III; mbk-1(pk1389) X; muIs84[Psod-3::gfp]</i>	

**Supplementary Table 2. List of qPCR primers used in this study.**

<b>primer name</b>	<b>primer sequence 5'--&gt;3'</b>
cdc-42_RT_F	TCA GCG TTG ACG CAG AAG
cdc-42_RT_R	CAT GGA GAC AAG GAA GAC GTT
tba-1_RT_F	TCC ACT GAT CTC TGC TGA CAA
tba-1_RT_R	TGG ATC GCA CTT CAC CAT T
Y45F10D.4_RT_F	AAG CGT CGG AAC AGG AAT C
Y45F10D.4_RT_R	TTT TTC CGT TAT CGT CGA CTC
daf-16_RT_F	TAC GAA TGG ATG GTC CAG AA
daf-16_RT_R	TCG CAT GAA ACG AGA ATGA A
sod-3_RT_F	AAA GGA GCT GAT GGA CAC TAT TAA GC
sod-3_RT_R	AAG TTA TCC AGG GAA CCG AAG TC
aat-1_RT_F	CCC AAA ACG AAA CCT TCC ACT CGC
aat-1_RT_R	TGA AAT TGC TGT GTA GAG AGC CAC
dod-8_RT_F	ACA GGA TGT CTT CAA AAG GAA TAT GG
dod-8_RT_R	TTG CTG GGG TGA TAG CTT GG
gpd-2_RT_F	AAG GCC AAC GCT CAC TTG AA
gpd-2_RT_R	GGT TGA CTC CGA CGA CGA AC
F52H3.5_RT_F	GAA GTT TAC AAA AGC ACT CGA AG
F52H3.5_RT_R	GGT TTA TTT TGA AGT CGG TAT GC
K07B1.4_RT_F	GGT CTT CTT CCA TTC AGA AAA CC
K07B1.4_RT_R	TGT ATG TCT GAT GAA GTG TGT CG
nnt-1_RT_F	CAG TAG AAA CTG CTG ACA TGC TTC
nnt-1_RT_R	GAG CGA TGG GAT ATT GTG CCT GAG
T21D12.9_RT_F	CAT CTA AAT CTA TCA ACT AAT AGA G
T21D12.9_RT_R	GTA GGA CAG GTC CAA AAC TTC CAA G

**Supplementary Table 3. Effect of *mbk-1* loss on *Psod-3::gfp*-expression in wild-type and germline-deficient *C. elegans*. Related to Figure 3C.**

Experiment	Strain	Worm number	Fold-change expression relative to wt			Fold-change expression relative to <i>gfp-1(-)</i>			P-value
			Mean	SD	SEM	Mean	SD	SEM	
#1	wt	24	1.00	0.15	0.03	0.38	0.06	0.01	>0.05
	<i>mbk-1(-)</i>	24	0.91	0.16	0.03	0.34	0.06	0.01	
	<i>gfp-1(-)</i>	22	2.65	1.07	0.23	1.00	0.40	0.09	
	<i>gfp-1(-); mbk-1(-)</i>	9	0.99	0.22	0.07	0.37	0.08	0.03	
#2	wt	24	1.00	0.20	0.04	0.47	0.09	0.02	>0.05
	<i>mbk-1(-)</i>	20	0.82	0.32	0.07	0.39	0.15	0.03	
	<i>gfp-1(-)</i>	15	2.12	0.71	0.18	1.00	0.34	0.09	
	<i>gfp-1(-); mbk-1(-)</i>	10	0.87	0.22	0.07	0.41	0.10	0.03	
#3	wt	9	1.00	0.08	0.03	0.59	0.05	0.02	>0.05
	<i>mbk-1(-)</i>	9	0.83	0.10	0.03	0.49	0.06	0.02	
	<i>gfp-1(-)</i>	8	1.69	0.45	0.16	1.00	0.27	0.09	
	<i>gfp-1(-); mbk-1(-)</i>	9	0.97	0.25	0.08	0.58	0.15	0.05	

The effect of the *mbk-1* loss of function mutation *mbk-1(pk1389)* on the expression of a *Psod-3::gfp* reporter gene (*mul84*) relative to *mbk-1(+)* animals was examined in wild-type and germline-less, *gfp-1(-)* [*gfp-1(e2144ts)*] worms. Fluorescence images were quantified, corrected for background, and fold-changes in reporter gene expression were calculated relative to wild-type and *gfp-1(-)* animals. Statistical significance was determined by two-way ANOVA with Bonferroni post tests. Experiment #3 is shown in Figure 3C.

**Supplementary Table 4. Effect of *hpk-1* loss on *Psod-3::gfp*-expression in wild-type and germline-deficient *C. elegans*. Related to Supplementary Figure S2A.**

Experiment	Strain	Worm number	Fold-change expression relative to wt			Fold-change expression relative to <i>gfp-1(-)</i>			P-value
			Mean	SD	SEM	Mean	SD	SEM	
#1	wt	24	1.00	0.15	0.03	0.38	0.06	0.01	<0.01
	<i>hpk-1(-)</i>	23	1.65	0.26	0.05	0.62	0.10	0.02	
	<i>gfp-1(-)</i>	22	2.65	1.07	0.23	1.00	0.40	0.09	
	<i>gfp-1(-); hpk-1(-)</i>	22	2.60	0.59	0.13	0.98	0.22	0.05	
#2	wt	24	1.00	0.20	0.04	0.47	0.09	0.02	>0.05
	<i>hpk-1(-)</i>	14	1.27	0.43	0.12	0.60	0.21	0.05	
	<i>gfp-1(-)</i>	15	2.12	0.71	0.18	1.00	0.34	0.09	
	<i>gfp-1(-); hpk-1(-)</i>	3	1.71	0.65	0.37	0.81	0.31	0.18	
#3	wt	9	1.00	0.08	0.03	0.59	0.05	0.02	>0.05
	<i>hpk-1(-)</i>	25	1.14	0.20	0.04	0.67	0.12	0.02	
	<i>gfp-1(-)</i>	8	1.69	0.45	0.16	1.00	0.27	0.09	
	<i>gfp-1(-); hpk-1(-)</i>	17	1.30	0.14	0.03	0.77	0.08	0.02	

The effect of the *hpk-1* loss of function mutation *hpk-1(pk1393)* on the expression of a *Psod-3::gfp* reporter gene (*mul84*) relative to *hpk-1(+)* animals was examined in wild-type and germline-less, *gfp-1(-)* [*gfp-1(e2144ts)*] worms. Fluorescence images were quantified, corrected for background, and fold-changes in reporter gene expression were calculated relative to wild-type and *gfp-1(-)* animals. Statistical significance was determined by two-way ANOVA with Bonferroni post tests. Experiment #3 is shown in Supplementary Figure S2A. Note: In Experiment #3, 3 images were taken for *hpk-1(-)* and 2 images for *gfp-1; hpk-1(-)*.

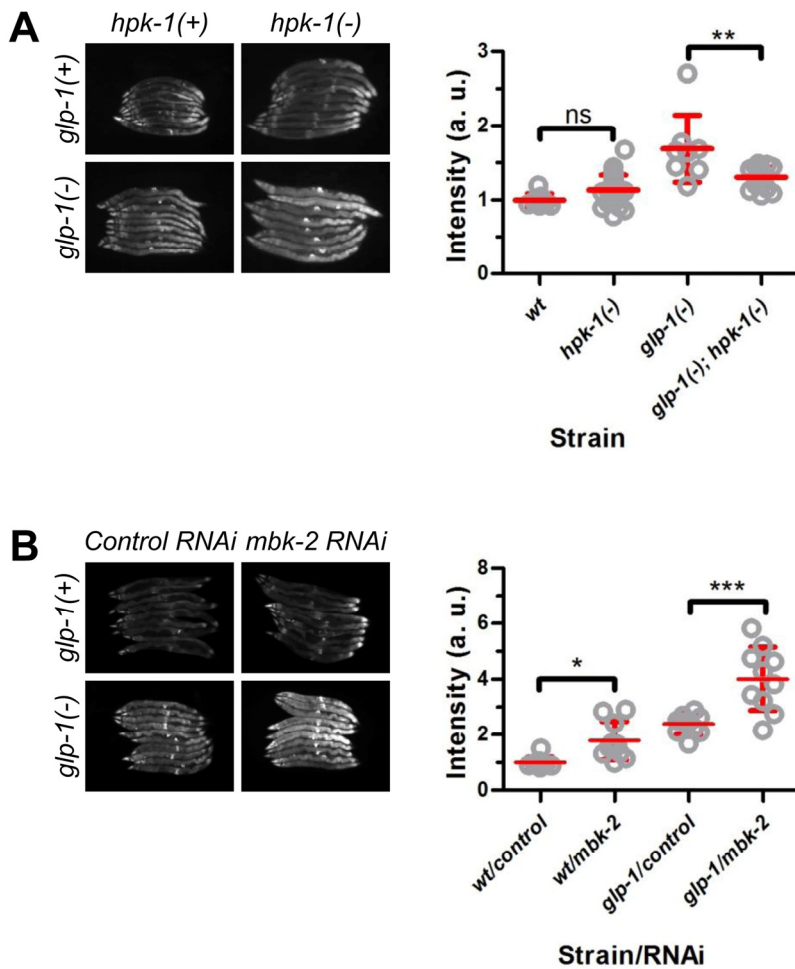
**Supplementary Table 5. Effect of *mbk-2* knockdown on *Psod-3::gfp*-expression in wild-type and germline-deficient *C. elegans*. Related to Supplementary Figure S2B.**

Experiment	Strain/RNAi	Worm number	Fold-change expression relative to wt			Fold-change expression relative to <i>glp-1</i>			P-value
			Mean	SD	SEM	Mean	SD	SEM	
#1	wt/control	7	1.00	0.12	0.04	0.47	0.06	0.02	>0.05
	wt/ <i>mbk-2</i>	10	1.35	0.27	0.08	0.64	0.13	0.04	
	<i>glp-1(-)</i> /control	8	2.12	0.76	0.27	1.00	0.36	0.13	<0.01
	<i>glp-1(-)</i> / <i>mbk-2</i>	10	2.90	0.65	0.21	1.37	0.31	0.10	
#2	wt/control	9	1.00	0.16	0.05	0.51	0.08	0.03	<0.001
	wt/ <i>mbk-2</i>	9	1.78	0.22	0.07	0.91	0.11	0.04	
	<i>glp-1(-)</i> /control	10	1.96	0.51	0.16	1.00	0.26	0.08	<0.001
	<i>glp-1(-)</i> / <i>mbk-2</i>	10	5.10	0.57	0.18	2.60	0.29	0.09	
#3	wt/control	10	1.00	0.09	0.03	0.58	0.05	0.02	>0.05
	wt/ <i>mbk-2</i>	10	1.22	0.19	0.06	0.71	0.11	0.04	
	<i>glp-1(-)</i> /control	10	1.72	0.30	0.10	1.00	0.18	0.06	<0.01
	<i>glp-1(-)</i> / <i>mbk-2</i>	10	2.30	0.69	0.22	1.34	0.40	0.13	
#4	wt/control	16	1.00	0.06	0.01	0.60	0.03	0.01	>0.05
	wt/ <i>mbk-2</i>	11	1.06	0.07	0.02	0.64	0.04	0.01	
	<i>glp-1(-)</i> /control	20	1.65	0.29	0.06	1.00	0.17	0.04	<0.01
	<i>glp-1(-)</i> / <i>mbk-2</i>	8	2.02	0.53	0.19	1.22	0.32	0.11	
#5	wt/control	10	1.00	0.19	0.06	0.42	0.08	0.03	<0.05
	wt/ <i>mbk-2</i>	10	1.79	0.69	0.22	0.75	0.29	0.09	
	<i>glp-1(-)</i> /control	10	2.39	0.35	0.11	1.00	0.15	0.05	<0.001
	<i>glp-1(-)</i> / <i>mbk-2</i>	10	4.01	1.14	0.36	1.68	0.48	0.15	

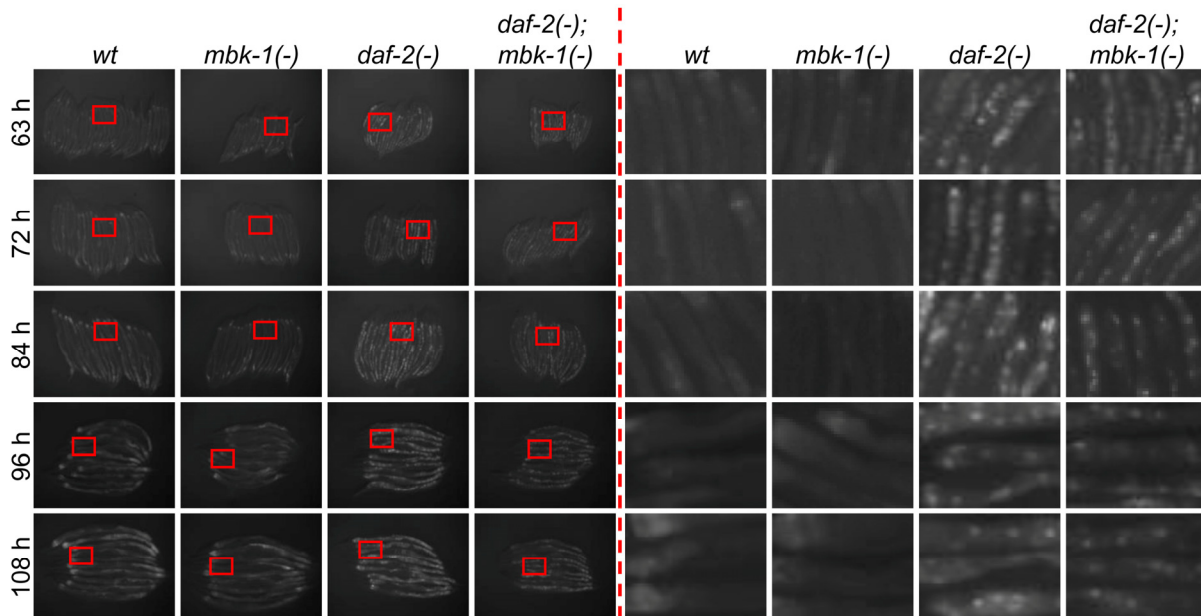
The effect of *mbk-2* knockdown on the expression of a *Psod-3::gfp* reporter gene (*muls84*) relative to control-RNAi (vector L4440) treated animals was examined in wild-type and germline-less, *glp-1(-)* [*glp-1(e2144ts)*] worms. Fluorescence images were quantified, corrected for background, and fold-changes in reporter gene expression were calculated relative to wild-type and *glp-1(-)* animals. Statistical significance was determined by two-way ANOVA with Bonferroni post tests. Experiment #5 is shown in Supplementary Figure S2B.

DAF-16	242	TIETTTKAQLEKSRRGAKKRIKERALMGSLH	S	TL-NGNSIAG	S	IQTI	SHDL	YDDDD	SMQGA	300																																					
mFOXO1	254	-MDNNSKFAKSRGRAAKKKASLQSGQEGPGD	S	P	GSQF	SKWPAS	P	GS	HSND	303																																					
hFOXO1	257	-MDNNSKFAKSRGRAAKKKASLQSGQEGAGD	S	P	GSQF	SKWPAS	P	GS	HSND	306																																					
hFOXO3	254	-MDNSNKYTKSRGRAAKKKAALQTAPESADD	S	P	-SQL	SKWPG	S	P	TSRSSD	302																																					
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DAF-16	301	FDNVPSFRPRTQSNLSIPGSSSRV	S	P	AIGSD	IYDDLE	-----	FPSW	VE	345																																					
mFOXO1	304	FD-NWSTFRPRTSSNASTI--SGRL	S	P	IMTE	QDD--	LGDG--	DVHSL	VYPPSAAK	351																																					
hFOXO1	307	FD-NWSTFRPRTSSNASTI--SGRL	S	P	IMTE	QDD--	LGE	GD--	DVHSMVYPPSAAK	354																																					
hFOXO3	303	LD-AWTDFRSRTNSNASTV--SGRL	S	P	IMASTE	LDEVQDD	DAPLSP	MLYSS	SASLSPSVS	359																																					
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DAF-16	346	-----SVP	AI	-----	PSD	IVDR	T	DQ	-----	MRIDATTHIGGVQIKQE	377																																				
mFOXO1	352	--MASTLPSLSEISNPE-----	N	MENLLD	NLNLLS	S	P	TSLT	TVSTQ	S	P	GS	M	MQ	T	P	400																														
hFOXO1	355	--MASTLPSLSEISNPE-----	N	MENLLD	NLNLLS	S	P	TSLT	TVSTQ	S	P	GS	M	MQ	T	P	403																														
hFOXO3	360	KPCTVELPRLTDMAGTMNLDGLTENLMDDLLDNIT	-----	LPP	SQ	S	P	T	GG	M	Q	R	S	S	412																																
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DAF-16	378	SKPIK---TEPIA	P	P	P	SY	HELNS	VRG	S	CAQ	N	P	L	L	R	N	P	I	V	P	S	T	N	F	K	P	M	P	L	G	A	Y	G	N	Y	Q	N	G	G	434							
mFOXO1	401	CYSFAPPNTSLNS	S	P	S	P	NY	S	K--	Y	T	Y	Q	S	S	M	S	P	L	P	Q	M	P	M	Q	T	L	Q	D	S	K	S	-----	Y	G	L	N	Q	Y	N	453						
hFOXO1	404	CYSFAPPNTSLNS	S	P	S	P	NY	Q	--	Y	T	Y	Q	S	S	M	S	P	L	P	Q	M	P	I	Q	T	L	Q	D	N	K	S	-----	Y	G	G	M	S	Q	Y	N	456					
hFOXO3	413	SFPYTTKGSGLG	S	P	T	S	S	F	N	--	T	V	F	G	P	S	S	L	N	S	L	R	Q	S	P	M	Q	T	I	Q	E	N	K	P	A	----	T	F	S	S	M	S	H	Y	G	466	
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**Supplementary Figure 1. NLK-sites in FOXO-proteins.** ClustalΩ alignment of full-length DAF-16 with murine and human FOXO1 and human FOXO3. Only the part covering the 8 NLK-sites reported in murine FOXO1 is shown [6]. The Ser/Thr-residues phosphorylated by NLK are highlighted in blue, the obligatory Pro immediately following an NLK-phosphorylated Ser/Thr is highlighted in yellow. The only SP-site in this region that is conserved between DAF-16 and murine/human FOXO1s is Ser326/Ser326/Ser329. Note: NLK-phosphorylation of individual residues has been reported to be weak [6].



**Supplementary Figure 2. Effect of DYRK-family kinases HPK-1 and MBK-2 on *Psod-3::gfp* expression.** Accompanies Figure 3. (A) The *hpk-1* loss of function mutation *hpk-1(pk1393)* decreases *Psod-3::gfp*-expression in germline-deficient *glp-1(-)* [*glp-1(e2144ts)*], but not in wild-type animals (representative experiment shown, n=5). (B) Depletion of *mbk-2* by RNAi increases *Psod-3::gfp*-expression in *glp-1(-)*, and –to a lesser extent– in wild-type background. RNAi treatment was initiated at the L1 stage (representative experiment shown, n=3). Error bars indicate standard deviations. Statistical significance of fluorescence intensity differences was determined by two-way ANOVA with Bonferroni post tests. All experiments in (A) and (B) were performed on day-2 adult worms. Images were taken at 100x magnification.



**Supplementary Figure 3. Loss of *mbk-1* does not affect DAF-16 subcellular localization in *daf-2* mutant *C. elegans*.** Accompanies Figure 4. The effect of the *mbk-1* loss of function mutation *mbk-1(pk1389)* on subcellular localization of an intestine-specific GFP::*DAF-16* protein (encoded by transgene *muls145[Pges-1::gfp::daf-16]*) was determined at the times indicated in wild-type and *daf-2(-)* [*daf-2(e1370)*] animals. Images on the left were taken at 100x magnification, images on the right are 6.5x magnifications of the areas boxed in red.