Loss of Muscleblind Splicing Factor Shortens *C. elegans* Lifespan by Reducing the Activity of p38 MAPK/PMK-1 and Transcription Factors ATF-7 and Nrf/SKN-1

Olli Matilainen¹, Ana R. S. Ribeiro¹, Jens Verbeeren¹, Murat Cetinbas², Ruslan I. Sadreyev²,
Susana M. D. A. Garcia^{1*}

¹Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland

²Massachusetts General Hospital and Harvard Medical School

*Corresponding Author (susanamaria.garcia@helsinki.fi)

7 **Running title:** Muscleblind and lifespan

8 **Corresponding author:**

9 Susana Maria D. A. Garcia, PhD,

0 Institute of Biotechnology

1 University of Helsinki

2 Biocenter 2, room 4023

3 Viikinkaari 5D,

4 00790 Helsinki, Finland

5 Phone: +358 050 4489545

6 email: susanamaria.garcia@helsinki.fi

7

8 **Key words:** Muscleblind splicing factor, *C. elegans*, lifespan, p38 MAPK, PMK-1 ATF-1 SKN-1,

9 mitochondrial stress

0

1

2

3

4

5

6

7

8

9

0

1

2

3 **Abstract**

4 Muscleblind-like splicing regulators (MBNLs) are alternative splicing factors that have an
 5 important role in developmental processes. Dysfunction of these factors is a key contributor of
 6 different neuromuscular degenerative disorders, including Myotonic Dystrophy type 1 (DM1).
 7 Since DM1 is a multisystemic disease characterized by symptoms resembling accelerated aging,
 8 we asked whether MBNLs regulate cellular processes required to maintain normal lifespan. By
 9 utilizing the model organism *Caenorhabditis elegans*, we found that loss of MBL-1 (the sole
 0 ortholog of mammalian MBNLs), which is known to be required for normal lifespan, shortens
 1 lifespan by decreasing the activity of p38 MAPK/PMK-1 as well as the function of transcription
 2 factors ATF-7 and SKN-1. Furthermore, we show that mitochondrial stress caused by knockdown
 3 of mitochondrial electron transport chain components promotes the longevity of *mb1-1* mutants in
 4 a partially PMK-1-dependent manner. Together, the data establish a mechanism of how DM1-
 5 associated loss of muscleblind affects lifespan. Furthermore, this study suggests that
 6 mitochondrial stress could alleviate symptoms caused by the dysfunction of muscleblind splicing
 7 factor, creating a potential approach to investigate for therapy.

8

9 **Reviewer token for the RNA-seq data (GEO: GSE146801): wvataksittaffcj**

0

1

2

3

4

5

6

7

8

9 Introduction

0 The human Muscleblind-like (MBNL) protein family consists of MBNL1, MBNL2 and
1 MBNL3. These evolutionarily conserved splicing factors have an important role during
2 development as they regulate the alternative exon choice during terminal muscle differentiation
3 (PASCUAL *et al.* 2006). Due to their role as regulators of cellular alternative splicing programs,
4 dysfunction of MBNLs has been found to be implicated in many degenerative diseases such as
5 huntington's disease-like 2, spinocerebellar ataxia type 8 and polyglutamine disorders
6 (FERNANDEZ-COSTA *et al.* 2011). However, MBNL dysfunction is best known for its role in myotonic
7 dystrophies.

8
9 Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic diseases caused by 50-
0 4.000 CUG trinucleotide repeat expansions in 3' untranslated region (UTR) of the myotonic
1 dystrophy protein kinase (*DMPK*) gene and 55-11.000 CCUG repeats in the first intron of
2 the cellular nucleic acid-binding protein (*CNBP*) gene, respectively. DM1 is the most prevalent
3 muscular dystrophy in adults, and its clinical features are more severe compared to DM2 (MEOLA
4 AND CARDANI 2015). At the molecular level, repeat-containing RNAs form imperfect double-
5 stranded structures that sequester, and thereby disrupt the function of MBNLs and several other
6 RNA binding factors (MILLER *et al.* 2000; FARDAEI *et al.* 2001), which leads to splicing defects
7 (RANUM AND COOPER 2006). Importantly, *Mbnl1* and *Mbnl2* knockout mice develop RNA splicing
8 abnormalities that are characteristic of DM1 (KANADIA *et al.* 2003; CHARIZANIS *et al.* 2012;
9 SUENAGA *et al.* 2012), demonstrating that the loss of MBNL splicing factors is a central contributor
0 of DM1 pathogenesis. Since DM1 manifests with phenotypes commonly associated with aging
1 (e.g. cataracts, muscular weakness, atrophy, insulin resistance and metabolic dysfunction), it has
2 also been considered as a disorder of accelerated aging (MATEOS-AIERDI *et al.* 2015). Thus, the
3 function of MBNL splicing factors may play an important regulatory role in aging.

4

MBNLs are well conserved factors across animals. In *Caenorhabditis elegans*, MBL-1, the sole ortholog of mammalian MBNL proteins binds CUG and CCUG repeats (SASAGAWA *et al.* 2009; GARCIA *et al.* 2014), and is required for normal mRNA splicing (NORRIS *et al.* 2017; THOMPSON *et al.* 2019). MBL-1 appears to be required for normal muscle structure and function as well as for proper formation of neuromuscular junction synapses and dendrite morphogenesis (WANG *et al.* 2008; SPILKER *et al.* 2012; ANTONACCI *et al.* 2015), indicating that the loss of MBL-1 recapitulates DM1 patient phenotypes in *C. elegans*. Notably, it has been shown that the loss of MBL-1 shortens *C. elegans* lifespan (WANG *et al.* 2008; SASAGAWA *et al.* 2009), but the mechanism behind this phenotype is not known.

Here we used *C. elegans* to elucidate the mechanism of how loss of MBL-1 shortens lifespan. We found that MBL-1-deficient animals have reduced activity of the conserved p38 mitogen-activated protein kinase (MAPK) (PMK-1 in *C. elegans*) and its downstream transcription factors ATF-7 and SKN-1, which results in shortened lifespan. Furthermore, we demonstrate that mitochondrial stress activates a prolongevity response in *mb1-1* mutants, which is partially p38 MAPK/PMK-1-dependent. These findings establish PMK-1, ATF-7 and SKN-1 as mechanistic links between the developmentally regulated muscleblind splicing factor and lifespan, and establish mitochondria as potential therapeutic targets to treat aging-associated symptoms of DM1.

Materials and methods

C. elegans strains and maintenance

For all experiments *C. elegans* were maintained at 20°C on NGM plates (peptone, P4963, Merck; agar, A4550, Merck; NaCl, 746398, Merck). The N2 (Bristol) strain was used as the wild-type. N2, *tir-1(qd4)* (ZD101), *pmk-1(km25)* (KU25), *gst-4p::gfp* (CL2166) were obtained from the

Caenorhabditis Genetics Center (CGC). *mb1-1(tm1563)* strain was received from National BioResource Project (NBRP), and outcrossed five times with N2. *mb1-1(tm1563);gst-4p::gfp* (GAR132) and *pmk-1(km25);mb1-1(tm1563)* (GAR139) crosses as well as extrachromosomal *mb1-1* overexpressing animals (GAR152: (*iceEx51[mb1-1p(1)::mb1-1-isoA::mCherry]* [*mb1-1p(2)::mb1-1-isoA::mCherry]* [*mb1-1p(1)::mb1-1-isoB::mCherry]* [*mb1-1p(2)::mb1-1-isoB::mCherry]* [*mb1-1p(1)::mb1-1-isoC::mCherry]* [*mb1-1p(2)::mb1-1-isoC::mCherry]*) were created within this study.

***Pseudomonas aeruginosa* assay**

3.5 cm NG plates were seeded with 40 μ L of overnight grown *Pseudomonas aeruginosa* (PA14) suspension and incubated at 37°C for 24 h (TAN *et al.* 1999). 20 μ L of SDS 2% was added to the edges of the plate to prevent the escape of the animals. *C. elegans* were transferred to PA14 plates at L4 stage and incubated at 25°C. Animals were scored daily for survival based on their ability to respond to touch. Animals dehydrated on the wall of the plate were censored from the analysis. For Western blot upon PA14 treatment, Animals were transferred to PA14 plates at L4 stage and collected 24 hours later as day 1 adults.

RNA interference (RNAi)

tir-1, *pmk-1*, *atf-7*, *skn-1* and *cco-1* RNAi clones were taken from either Ahringer or Vidal RNAi library. *daf-16* (#34833) and *daf-2* RNAi (#34834) clones were obtained from Addgene. *mb1-1* RNAi was cloned from *C. elegans* cDNA by using 5'-CTTGAGCTCTTCGACGAAAACAGTAATGCCGC-3' and 5'-CTTCTCGAGCTAGAATGGTGGTGGCTGCATG-3' primers. RNAi was performed using the feeding protocol as described earlier (TIMMONS *et al.* 2001). Lifespan experiments were initiated by letting gravid hermaphrodites (P0 generation) to lay eggs on RNAi/NGM plates, and P1 generation was scored for lifespan. In some lifespan experiments animals were bleached and let

to hatch overnight in M9 before plating P1 generation as L1 larvae to experimental plates. These two alternative ways to initiate lifespan did not affect the conclusions made from the experiments. Since *mbi-1* is expressed mainly in neurons, P0s were put to *mbi-1* RNAi at L4 stage in order to maximize RNAi efficiency in P1 generation, which was scored for lifespan. For qRT-PCR and Western blot animals were bleached and let to hatch overnight in M9 before plating L1 larvae for experimental plates.

Generation of *mbi-1* overexpression strains

GAR152 was generated by microinjection of six different transgenic construct at 15 ng/μl concentration each. These transgenes were expressed as extrachromosomal arrays and express three different isoforms (K02H8.1a; isoform A, K02H8.1b; isoform B, and K02H8.1c, isoform C) fused to mCherry, under two different endogenous promoters; *mbi-1p(1)* and *mbi-1p(2)*. Constructs were made through modification of a plasmid expressing *mbi-1* isoform A fused to mCherry under the *unc-54* promoter (GARCIA *et al.* 2014). PCR-amplified fragments from wild-type *C. elegans* genome, representing two endogenous *mbi-1* promoter regions, replaced the *unc-54* promoter. For promoter 1, *mbi-1p(1)*, primers 5'-TACGCATGCAGGCCCTATATATTCCATCTCAAT-3', containing a *SphI* site, and 5'-TACGGATCCTCTGAAAAGTAGGAAAAGATTGGC, containing a *BamHI* site were used. For promoter two, *mbi-1p(2)*, primers 5'-AACTGCAGGTGCAATGGGCTACTGATCTCC-3' and 5'-CGGGATCCCATTCCGTCACTTGCAAAGAAC-3' were used, containing *PstI* and *BamHI* sites, respectively. Plasmids expressing isoforms B and C were derived from these constructs through PCR using forward primers 5'-CAGCTACAACTGCCGC CT-3' (isoform B) and 5'-GGAGCTGTACCAATGAAGCGAC-3' (isoform C), and reverse primer 5'-CTGATTCACTGCCGCTGCTGTATAAG-3'.

Western blot

Animals were collected at indicated age and frozen in liquid nitrogen. Animals were lysed in protease inhibitor cocktail (abcam, #ab65621) and 3 x phosphatase inhibitor (Thermo Scientific, #78420)-supplemented RIPA buffer (bioWORLD) with micropestle in 1.5 ml Eppendorf tubes. Lysates were resolved on 4–15% precast polyacrylamide gels (Bio-Rad). Phospho-p38 MAPK (used with 1:1000 dilution) was purchased from Cell Signaling Technology (#4511S) and α -tubulin antibody (used with 1:5000 dilution) from Merck (T5168). Western blots were quantified by using Fiji (see S6 Table for Western blot quantifications).

RNA sequencing

N2 and *mb1-1(tm1563)* strains were synchronized by bleaching and plated as L1 larvae on RNAi plates seeded with HT115 bacteria carrying empty vector (control vector for RNAi). To prevent the hatching of the progeny, animals were transferred to plates containing 10 μ M of 5-fluorouracil (Sigma) at the L4 stage. Animals were collected at day 2 of adulthood (three biological replicates for both strains) and frozen in liquid nitrogen. Total RNA was extracted with TRIzol Reagent (Ambion) and assessed for degradation using Agilent 2100 Bioanalyzer. Illumina Truseq stranded polyA-mRNA library was prepared and sequenced for 86 cycles at the DNA Sequencing and Genomics laboratory (Institute of Biotechnology, University of Helsinki). The 6 samples were multiplexed and sequenced on one lane of Illumina NextSeq 500, yielding circa 18–20 million reads per sample. Sequencing reads were mapped to the *Caenorhabditis elegans* reference transcriptome (WBcel235 assembly) using STAR (DOBIN *et al.* 2013). Read counts over transcripts were calculated using HTSeq (ANDERS *et al.* 2015). For differential expression analysis we used the EdgeR method (ROBINSON *et al.* 2010) and classified genes as differentially expressed based on the cutoffs of 2-fold change in expression value and false discovery rates (FDR) below 0.05.

Lifespan analysis

C. elegans lifespan experiments were done at 20°C. At L4 larval stage animals were transferred to plates containing 10 µM of 5-Fluorouracil (Sigma) in order to prevent progeny production. Animals that had exploded vulva or that crawled off the plate were censored. Animals were counted as dead if there was no movement after poking with a platinum wire. Lifespans were checked every 1-3 days. For lifespan data, mean lifespan ± standard error (s.e.) is reported (see S1-S2 Tables).

Quantitative RT-PCR (qRT-PCR)

Animals were collected at indicated age and frozen in liquid nitrogen. TRIzol Reagent (Ambion) was used to extract RNA. cDNA synthesis was done with QuantiTect Reverse Transcription Kit (Qiagen) and qRT-PCR reactions were run with the SYBR Green reagent (Roche) using Lightcycler 480 (Roche). qRT-PCR data was normalized to the expression of *cdc-42* and *pmp-3*. qRT-PCR oligos used in this study are provided in S8 Table. qRT-PCR experiments were performed with three biological replicates (see S7 Table for raw qRT-PCR data), and repeated at least twice with similar results. Statistical significances were analyzed by using Student's t-test or two-way ANOVA.

Splicing assay

Animals were collected at indicated age and frozen in liquid nitrogen. TRIzol Reagent (Ambion) was used to extract RNA. cDNA synthesis was done with QuantiTect Reverse Transcription Kit (Qiagen). Splicing of *unc-43* and *unc-104* mRNAs was analyzed by running PCR products in agarose gels. Oligos used for PCR are provided in S9 Table. Splicing assays were performed with three biological replicates. Agarose gels were quantified by using Fiji. Statistical significances were analyzed by using two-way ANOVA.

Fluorescent imaging and oxidative stress assay

gst-4p::GFP reporter strains (wild-type and *mb1-1(tm1563)* background) were grown on EV RNAi plates and imaged as day 1 adults with a standard stereomicroscope with fluorescent light source (Zeiss). For the oxidative stress, 5 % H₂O₂ (in H₂O, H₂O used as a control) was pipetted to EV plates containing 1 day adult *gst-4p::gfp* reporter animals. Worms were washed after 20 minutes, and let to recover on EV plates for 5 hours before imaging. GFP fluorescence was quantified by using Fiji (see S7 Table for GFP quantifications).

Statistical analysis

Statistical analyses for qRT-PCR data was carried out in GraphPad Prism or Excel, and the data represents the mean of 3 biological replicates $\pm$ SD. Statistical analyses for Western blot data, splicing assays (agarose gels) and GFP fluorescence were carried out in GraphPad Prism. Statistical details can be found in the figures and figure legends. Statistical analyses for lifespan experiments were carried out in R by using the Cox-proportional hazard regression. Statistical details for the lifespan data can be found in S1 and S2 Tables.

Data and reagent availability

Strains and plasmids are available upon request. The Gene Expression Omnibus (GEO) accession number for the RNA-seq data originating from this study is GSE146801.

Results

Loss of MBL-1 leads to shortened lifespan and reduced expression of p38 MAPK/PMK-1-regulated genes

To examine how the loss of MBL-1 affects *C. elegans* lifespan, we utilized a strain carrying the *mb1-1(tm1563)* allele (513 bp deletion) that eliminates an exon shared among all *mb1-1* isoforms and creates a putative null allele. This mutant has been shown to shorten lifespan (SASAGAWA *et*

1 *al.* 2009). We repeated this experiment, performing lifespan assays on two *E. coli* strains, HT115
2 and OP50, which are both widely used in *C. elegans* experiments. We found that *mbi-1(tm1563)*
3 mutant animals have significantly shorter lifespan on both bacterial strains compared to wild-type
4 *C. elegans* (N2) (Fig 1A and S1-S2 Tables). Interestingly, loss of MBL-1 has a more drastic
5 decrease in lifespan on HT115 compared to OP50. One potential explanation is that the
6 microbiome could modulate lifespan partly through MBL-1. As many experiments in this study
7 take advantage of RNAi, all following experiments (with the exception of the *Pseudomonas*
8 *aeruginosa* resistance assay) were performed using HT115 bacteria. After confirming the earlier
9 finding that *mbi-1(tm1563)* mutants have shortened lifespan (SASAGAWA *et al.* 2009) (Fig 1A and
0 S1-S2 Tables), we asked whether knockdown of *mbi-1* produces a similar phenotype. Notably, it
1 has been shown earlier that *mbi-1* RNAi shortens lifespan (WANG *et al.* 2008). We also found that
2 *mbi-1* RNAi shortens N2 lifespan (Fig 1B and S1-S2 Tables), thus further demonstrating that
3 disruptions to MBL-1 function affects lifespan. Interestingly, also simultaneous overexpression of
4 three mCherry-tagged *mbi-1* isoforms (Fig S1A-S1B) under endogenous *mbi-1* promoters
5 shortens lifespan compared to N2 (Fig 1C and S1-S2 Tables), indicating that an optimal level of
6 this developmentally-regulated splicing factor is required for normal lifespan. Next, we asked
7 whether MBL-1 activity decreases with aging. For this purpose, we scanned the *unc-43* and *unc-*
8 *104* genes, known targets of MBL-1-mediated splicing (NORRIS *et al.* 2017), for age-related
9 changes in their alternative splicing patterns (Fig S2A). PCR analysis revealed that MBL-1 is
0 required for exon exclusion events in both *unc-43* and *unc-104* (Fig S2B-S2E, S3 Table). Day 4
1 adult N2 shows enrichment of longer *unc-43* isoform, which is observed with *mbi-1(tm1563)*
2 mutants (Fig S2B-S2C, S3 Table), whereas *unc-104* splicing shows a similar enrichment already
3 in day 2 adult N2 (Fig S2D-S2E, S3 Table). These data suggest that MBL-1 activity decreases
4 upon aging.

5

6 Since the reduced activity of MBNLs is a hallmark of DM1, we focused on the mechanism by
7 which the loss of MBL-1 shortens lifespan. As an initial step, we performed RNA-seq of day 2
8 adult (day 5 from hatch) N2 and *mbi-1(tm1563)* mutants. Our analysis revealed that 135 genes
9 are up- and 235 downregulated (at least 2-fold change in expression, FDR < 0.05) in *mbi-*
0 *1(tm1563)* mutants compared to N2 (S4 Table). In order to identify aging-associated signaling
1 pathways linking the loss of MBL-1 with shortened lifespan, we utilized the WormExp database,
2 which collates nearly all published *C. elegans* expression data sets from public databases (YANG
3 *et al.* 2016). We found that genes differentially regulated in *mbi-1(tm1563)* mutants are modulated
4 by factors such as PQM-1, ELT-2, DAF-16 and PMK-1 (Fig 1D-1E and S5 Table). Interestingly,
5 PMK-1, which encodes the conserved p38 mitogen-activated protein kinase (p38 MAPK), was
6 the only factor associated exclusively with downregulated genes (Fig 1E and S5 Table). PMK-1-
7 regulated genes, whose expression is reduced in day 15 vs. day 6 old *C. elegans* (YOUNGMAN *et*
8 *al.* 2011), are also enriched among the downregulated genes in *mbi-1(tm1563)* mutants (Fig 1E
9 and S5 Table). Due to this enrichment, we focused on the link between p38 MAPK/PMK-1 and
0 MBL-1 to regulate lifespan.

Fig. 1 The loss of MBL-1 shortens lifespan and reduces the expression of p38 MAPK/PMK-1 target genes.

(A) *mbl-1(tm1563)* mutants have shortened lifespan compared to wild-type (N2) on both HT115 ($p < 0.01$) and OP50 ($p < 0.01$) *E. coli*. (B) *mbl-1* RNAi shortens lifespan compared to empty vector (EV, control RNAi)-treated N2 ($p < 0.01$). (C) Strains expressing extrachromosomal mCherry-tagged MBL-1 have short lifespan compared to N2. See S1-S2 Tables for lifespan statistics. (D) The most significant terms from WormExp output for upregulated genes (135) in day 2 adult *mbl-1(tm1563)* mutants compared to N2. (E) The most significant terms from WormExp output for downregulated genes (235) in day 2 adult *mbl-1(tm1563)* mutants compared to N2. In (D) and (E) numbers next to bars represent number of genes associated with the particular WormExp term. See S4 Table and S5 Table for differentially expressed genes between N2 and *mbl-1(tm1563)* mutants and WormExp statistics, respectively.

5 **Loss of MBL-1 does not impair innate immune response**

6 When subjecting up- and downregulated genes to gene ontology (GO) analysis by using the
7 DAVID database (HUANG DA *et al.* 2009), we found that innate immune response-related GO
8 terms are enriched among genes upregulated in *mbi-1(tm1563)* mutants (Fig 2A). This is
9 interesting since the WormExp analysis indicated that PMK-1, a central factor of innate immunity-
0 regulating p38 MAPK signaling (KIM *et al.* 2002), has reduced activity in *mbi-1(tm1563)* mutants
1 (Fig 1E, S5 Table). On the other hand, WormBase GO enrichment analysis of 34 PMK-1-
2 regulated genes whose expression is decreased in *mbi-1(tm1563)* mutants (Fig 1E, S5 Table)
3 reveals that only 6 genes are associated with innate immunity (S5 Table). These data indicate
4 that the loss of MBL-1 does not impair the expression of PMK-1-regulated innate immunity genes.
5 Nevertheless, we asked whether the loss of MBL-1 affects pathogen resistance, and for this
6 experiment we investigated the survival of *mbi-1(tm1563)* mutants on pathogenic *Pseudomonas*
7 *aeruginosa* (PA14) in a slow-killing assay. In this assay we cultured PA14 on normal NGM plates
8 where the killing occurs over the course of several days (TAN *et al.* 1999). *mbi-1(tm1563)* mutants
9 were found to have similar survival on PA14 when compared to N2. This is in contrast to *pmk-*
0 *1(km25)* mutants, which are extremely susceptible to PA14 (Fig 2B and S1-S2 Tables).
1 Furthermore, *mbi-1(tm1563)* mutants show significantly elevated levels of phosphorylated p38
2 MAPK/PMK-1 upon PA14 infection (Fig 2C-2D, Fig S3 and S6 Table). The data demonstrate that
3 MBL-1 dysfunction does not impair the ability to mount a p38 MAPK/PMK-1-mediated innate
4 immune response upon infection.

Fig 2. *mbi-1(tm1563)* mutants have innate immunity-associated gene expression signature, but normal response to *Pseudomonas aeruginosa*

(A) Gene Ontology (GO) Terms (biological process) enriched among genes that are up- and downregulated in day 2 adult *mbi-1(tm1563)* mutants compared to N2 in RNA-seq (GSE146801). Numbers next to bars represent number of genes associated with the particular GO term. See S4 Table for differentially expressed genes between N2 and *mbi-1(tm1563)* mutants. (B) *mbi-1(tm1563)* mutants have similar survival on pathogenic *Pseudomonas aeruginosa* (PA14) compared to N2, but enhanced survival compared to *pmk-1(km25)* mutants. See S1-S2 Tables for lifespan statistics. (C) Both N2 and *mbi-1(tm1563)* mutants have significantly elevated level of phosphorylated p38 MAPK upon PA14 infection. Animals were transferred to PA14 at L4 stage and collected 24 hours later (day 1 adults). (D) Quantified levels of phosphorylated p38 MAPK upon exposure to PA14. Bars represent the level of tubulin-normalized phosphorylated p38 MAPK relative to N2 EV with error bars indicating mean $\pm$ s.d. of three biological replicates (* $p < 0.05$, two-way ANOVA with Tukey's test). See Fig S3 for Western blot repeats and S6 Table for Western blot quantifications.

***mb1-1(tm1563)* mutants have reduced level of activated p38 MAPK/PMK-1, which contributes to their short lifespan**

Since our RNA-seq data suggested that *mb1-1(tm1563)* mutants exhibit decreased PMK-1 activity (Fig 1E, S5 Table), we analyzed whether the level of phosphorylated p38 MAPK/PMK-1, which is traditionally used to assess the activity of this kinase, is altered in these mutants. Western blot analysis showed that day 2 old *mb1-1(tm1563)* mutants have reduced level of phosphorylated PMK-1 (Fig 3A-3B, Fig S3-S4 and S6 Table), thus demonstrating that basal p38 MAPK/PMK-1 activity is decreased upon disrupted MBL-1 function. *mb1-1* RNAi does not significantly affect PMK-1 phosphorylation (Fig 3C-3D, Fig S3 and S6 Table), which could be due to the insufficient knockdown efficiency. However, the quantified mean of phosphorylated PMK-1 level from three independent experiments (Fig 3C) indicates that also *mb1-1* knockdown modulates PMK-1 phosphorylation. Since MBNL1 is known to also regulate mRNA stability (MASUDA *et al.* 2012), we asked whether mRNA levels of *C. elegans* p38 MAPK pathway genes are altered in *mb1-1(tm1563)* mutants. We found that at the L4 larval stage the expression of *tir-1* is upregulated in *mb1-1(tm1563)* mutants, whereas the expression of *nsy-1*, *sek-1* and *pmk-1* is unchanged (Fig 3E, S7 Table). In day 2 adult animals only the expression of *nsy-1* is decreased (Fig 3F, S7 Table). As a conclusion, expression changes in p38 MAPK pathway genes are unlikely to explain the reduced p38 MAPK/PMK-1 activity in *mb1-1(tm1563)* mutants.

To determine whether the decreased p38 MAPK/PMK-1 activity contributes to the short lifespan of *mb1-1(tm1563)* mutants, we studied how these mutants are affected by the knockdown of *pmk-1*. RNAi of *pmk-1* reduces N2 lifespan, but does not affect the lifespan of *mb1-1(tm1563)* mutants (Fig 3G and S1-S2 Tables). Next we tested how RNAi of other components of the p38 MAPK pathway (Fig S5A) affect *mb1-1(tm1563)* mutant lifespan. We found that RNAi of *tir-1* shortens N2 lifespan but does not affect the lifespan of *mb1-1(tm1563)* mutants (Fig S5B, S1-S2 Tables). However, RNAi of *nsy-1* and *sek-1* does not affect N2 lifespan, whereas *sek-1* RNAi shortens

mbi-1(tm1563) mutant lifespan (Fig S5C, S1-S2 Tables). These data indicate that the dysfunction of p38 MAPK/PMK-1 contributes to the short lifespan of *mbi-1(tm1563)* mutants independently of the upstream p38 MAPK signaling pathway.

Fig 3. Decreased p38 MAPK/PMK-1 activation at non-pathogenic conditions shortens *mbi-1(tm1563)* mutant lifespan

(A) Day 2 adult *mbi-1(tm1563)* mutants have reduced level of phosphorylated p38 MAPK compared to N2. Shown are technical replicates of one biological replicate. (B) Quantified level of phosphorylated p38 MAPK in day 2 adult N2 and *mbi-1(tm1563)* mutants. (C) *mbi-1* RNAi does not significantly reduce the level of phosphorylated p38 MAPK in day 1 adult N2. (D) Quantified levels of phosphorylated p38 MAPK upon *mbi-1* RNAi in day 1 adult N2. In (B) and (D) bars represent the level of tubulin-normalized phosphorylated p38 MAPK relative to EV-treated N2 with error bars indicating mean $\pm$ s.d. of (B) seven and (D) three biological replicates (**p < 0.01,

unpaired Student's *t*-test). See Fig S3-S4 for Western blot repeats and S6 Table for Western blot quantifications. (E) The expression of p38 MAPK pathway components in L4 larvae N2 and *mbi-1(tm1563)* mutants. (F) The expression of p38 MAPK pathway components in day 2 adult N2 and *mbi-1(tm1563)* mutants. In (E) and (F) bars represent mRNA levels relative to N2 EV with error bars indicating mean $\pm$ s.d. of three biological replicates, each with three technical replicates (**p* < 0.05, ***p* < 0.01, unpaired Student's *t*-test). See S7 Table for raw qRT-PCR data. (G) *pmk-1* RNAi causes reduction in N2 lifespan (*p* < 0.01) but does not affect the lifespan of *mbi-1(tm1563)* mutants (*p* = 0.605). See S1-S2 Tables for lifespan statistics.

Reduced activity of ATF-7 and SKN-1 transcription factors modulate *mbi-1(tm1563)* mutant lifespan

In mammals, p38 MAPK signaling modulates cellular processes such as inflammation, development, cell differentiation and senescence by controlling the activity of multiple transcriptional regulators (ZARUBIN AND HAN 2005). Therefore, it is likely that reduced activity of p38 MAPK/PMK-1 kinase shortens the lifespan of *mbi-1(tm1563)* mutants by affecting the function of transcription factors. To this end, we investigated how downregulation of known PMK-1-regulated transcriptional regulators affect the lifespan of *mbi-1(tm1563)* mutants. Knockdown of *atf-7*, which encodes a transcription factor phosphorylated by PMK-1 in innate immune response (SHIVERS *et al.* 2010), induces larger decrease in N2 lifespan (14 %) than in *mbi-1(tm1563)* mutant lifespan (5 %) (Fig 4A and S1-S2 Tables). This finding is further supported by Cox proportional hazard regression analysis, which shows that *atf-7* RNAi increases hazard ratio (HR) more in N2 background than in *mbi-1(tm1563)* background (HR compared to N2 EV: N2 *atf-7* RNAi 2.290, *mbi-1(tm1563)* EV 3.728, *mbi-1(tm1563)* *atf-7* RNAi 4.742; HR compared to *mbi-1(tm1563)* EV: *mbi-1(tm1563)* *atf-7* RNAi 1.272). Since PMK-1 has been shown to phosphorylate and activate also SKN-1 (INOUE *et al.* 2005), we asked whether the aberrant function of this mammalian Nrf ortholog is linked with the shortened lifespan of *mbi-1(tm1563)* mutants. Similarly

to *atf-7* knockdown, *skn-1* RNAi causes 1.8 % decrease in *mbi-1(tm1563)* mutant lifespan, whereas it shortens N2 lifespan by 14 % (HR compared to N2 EV: N2 *skn-1* RNAi 3.465, *mbi-1(tm1563)* EV 4.857, *mbi-1(tm1563) skn-1* RNAi 6.397; HR compared to *mbi-1(tm1563)* EV: *mbi-1(tm1563) skn-1* RNAi 1.317) (Fig 4B and S1-S2 Tables). To study further whether the loss of MBL-1 modulates SKN-1 function, we crossed *mbi-1(tm1563)* mutants with the transcriptional reporter of *gst-4* (*gst-4p::gfp*), which is a well-known target of SKN-1 (TULLET *et al.* 2008). The *mbi-1(tm1563)* mutants were found to have impaired induction of *gst-4p::gfp* expression upon oxidative stress (5 % H₂O₂ for 20 minutes, imaging after five hours recovery) (Fig 4C-4D, S7 Table), thus further demonstrating that SKN-1 activity is decreased in *mbi-1(tm1563)* mutants. Despite the requirement of MBL-1 on *gst-4* expression upon H₂O₂ treatment, MBL-1 expression pattern is not responsive to oxidative stress (Fig S1B).

The expression of *gst-4* is regulated also by DAF-16 (TULLET *et al.* 2008), the sole FOXO ortholog in *C. elegans*. DAF-16 is regulated by insulin/IGF-1-like signaling, which is a well-known pathway affecting aging (KENYON 2010). In addition to *gst-4*, our RNA-seq revealed that the expression of a sub-set of DAF-16/FOXO target genes is altered in *mbi-1(tm1563)* mutants (Fig 1D-1E and S4 Table). To further examine this, we analyzed mRNA levels of a few DAF-16 target genes by qRT-PCR in *mbi-1(tm1563)* mutants. We found that the expression of *sod-3* and *mtl-1*, which is promoted by DAF-16 (MURPHY *et al.* 2003), is downregulated in *mbi-1(tm1563)* mutants, whereas the expression of *ins-7*, which is suppressed by DAF-16 (MURPHY *et al.* 2003), is upregulated upon the loss of MBL-1 (Fig 4E). Despite these changes in DAF-16 target gene expression, longevity assays showed that *daf-16* RNAi shortens N2 and *mbi-1(tm1563)* mutant lifespan by 6.8 % and 9 %, respectively (Fig 4F and S1-S2 Tables), consistent with the notion that the loss of MBL-1 shortens lifespan independently of DAF-16. Additionally, RNAi of *daf-2*, which leads to increased longevity in an DAF-16-dependent manner (KENYON *et al.* 1993), results in a similar increase in both N2 and *mbi-1(tm1563)* mutant lifespan (31.9 % and 32.7 %, respectively) (Fig

4G and S1-S2 Tables). Taken together, these data indicate that loss of MBL-1 leads to shortened lifespan by reducing the activity of p38 MAPK/PMK-1, which in turn decreases the activity of ATF-7 and SKN-1 transcription factors. Furthermore, although the loss of MBL-1 causes differential expression of some DAF-16 target genes, the altered DAF-16 function does not contribute to the short lifespan of *mbi-1(tm1563)* mutants.

Fig 4. The short lifespan of *mbi-1(tm1563)* mutants is due to the dysfunction of ATF-7 and SKN-1 transcription factors.

(A) *att-7* RNAi shortens both N2 and *mbi-1(tm1563)* mutant lifespan ($p < 0.01$, $p = 0.0291$, respectively). (B) *skn-1* RNAi shortens both N2 and *mbi-1(tm1563)* mutant lifespan ($p < 0.01$, $p = 0.0151$, respectively). (C) *mbi-1(tm1563)* mutants crossed with the transcriptional *gst-4p::GFP* reporter strain (CL2166) show reduced fluorescence upon acute oxidative stress. Scale bar, 100 μ m. (D) Quantification of *gst-4p::GFP* reporter strain fluorescence. Quantification was done

in groups of six animals, $n = 30$ animals for each condition. Bars represent GFP fluorescence relative to wild-type (wt) Ctrl with error bars indicating mean $\pm$ s.d. of five replicates ($*p < 0.05$, one-way ANOVA with Tukey's test). (E) Expression of DAF-16 regulated genes in N2 and *mbi-1(tm1563)* mutants. Animals were grown on EV and collected for qRT-PCR analysis at day 2 adult stage. Bars represent mRNA levels relative to N2 with error bars indicating mean $\pm$ s.d. of three biological replicates, each with three technical replicates ($**p < 0.01$, unpaired Student's *t*-test). See S7 Table for raw qRT-PCR data. (F) *daf-16* RNAi shortens both N2 ($p < 0.01$) and *mbi-1(tm1563)* mutant ($p < 0.01$) lifespan. (G) *daf-2* RNAi increases both N2 ($p < 0.01$) and *mbi-1(tm1563)* mutant ($p < 0.01$) lifespans. See S1-S2 Tables for lifespan statistics.

Mitochondrial stress rescues the short lifespan of *mbi-1(tm1563)* mutants

Next, we asked whether targeting the known aging mechanisms could modulate p38 MAPK activity, and thereby, rescue the short lifespan of *mbi-1(tm1563)* mutants. Interestingly, it has been shown that inhibition of mitochondrial respiratory chain complex I activates the PMK-1/ATF-7 signaling pathway (CHIKKA *et al.* 2016). Moreover, mitochondrial chaperone HSP-60 binds and stabilizes SEK-1 MAPK (see Fig S5A for *C. elegans* p38 MAPK pathway), thus promoting PMK-1 activation (JEONG *et al.* 2017). Due to these reports, we asked whether mitochondrial function could extend *mbi-1(tm1563)* mutant lifespan.

To study a role for mitochondrial stress on the *mbi-1(tm1563)* mutant lifespan, we used RNAi of mitochondrial respiratory chain complex IV subunit *cox-5B* (also known as *cco-1*), which is known to extend lifespan (DILLIN *et al.* 2002). As *cox-5B* RNAi also promotes innate immunity (MATILAINEN *et al.* 2017), we hypothesized that it leads to activation of p38 MAPK/PMK-1 kinase. Strikingly, we found that *cox-5B* RNAi leads to a significantly larger ($p < 0.01$, Cox proportional hazard regression analysis) lifespan extension in *mbi-1(tm1563)* mutants (+31 %) compared to N2 animals (+15.3 %) (HR compared to N2 EV: N2 *cox-5B* RNAi 0.42, *mbi-1(tm1563)* EV 3.426, *mbi-*

1 (*tm1563*) *cox-5B* RNAi 0.302; HR compared to *mb1-1(tm1563)* EV: *mb1-1(tm1563)* *cox-5B* RNAi
 2 0.088) (Fig 5A and S1-S2 Tables). To test whether *cox-5B* RNAi affects the p38 MAPK pathway,
 3 we performed Western blot to analyze p38 MAPK/PMK-1 phosphorylation. We found that *cox-5B*
 4 knockdown does not increase the level of phosphorylated p38 MAPK/PMK-1 in L3 larvae (Fig 5B-
 5 5C, Fig S4, S6 Table). At L4 larval stage *cox-5B* RNAi increases the level of phosphorylated p38
 6 MAPK/PMK-1 in both N2 and *mb1-1(tm1563)* mutants in all individual experiments (Fig 5B, Fig
 7 S4, S6 Table), but due to the variation between experiments, the increase is not statistically
 8 significant (Fig 5D). Also in day 2 adults the effect of *cox-5B* RNAi on p38 MAPK/PMK-1
 9 phosphorylation varies between experiments, and the increase in p38 MAPK/PMK-1
 0 phosphorylation is not statistically significant (Fig 5E-5F, Fig S4, S6 Table). However, in day 2
 1 adult *mb1-1(tm1563)* mutants *cox-5B* RNAi increases the level of phosphorylated p38
 2 MAPK/PMK-1 in four out of five independent experiments (Fig S4, S6 Table), thus indicating that
 3 similar to L4 larvae, *cox-5B* RNAi may modulate the activation p38 MAPK/PMK-1 in day 2 adults.

Fig 5. *cox-5B* RNAi-mediated mitochondrial stress modulates the level of phosphorylated p38 MAPK/PMK-1 and leads to increase in *mb1-1(tm1563)* mutant lifespan.

(A) *cox-5B* RNAi-treated *mb1-1(tm1563)* mutants have longer lifespan than *cox-5B* RNAi-treated N2 animals ($p < 0.01$). (B) Western blot of phosphorylated p38 MAPK/PMK-1 in L3 and L4 larvae

0 N2 and *mb1-1(tm1563)* mutants upon *cox-5B* RNAi. (C-D) Quantified levels of phosphorylated
 1 p38 MAPK upon *cox-5B* RNAi in (C) L3 and (D) L4 larvae N2 and *mb1-1(tm1563)* mutants. (E)
 2 Western blot of phosphorylated p38 MAPK/PMK-1 in day 2 adult N2 and *mb1-1(tm1563)* mutants
 3 upon *cox-5B* RNAi. (F) Quantified levels of phosphorylated p38 MAPK upon *cox-5B* RNAi in day
 4 2 adult N2 and *mb1-1(tm1563)* mutants. In (C), (D) and (F) bars represent the level of tubulin-
 5 normalized phosphorylated p38 MAPK relative to EV-treated N2 with error bars indicating mean
 6 $\pm$ s.d. of (C-D) three and (F) five biological replicates (two-way ANOVA with Tukey's test). See
 7 Fig S4 for Western blot repeats and S6 Table for Western blot quantifications.

8
 9 Next we asked how *cox-5B* RNAi-mediated mitochondrial stress affects the expression of PMK-
 0 1-regulated genes (TROEMEL *et al.* 2006; YOUNGMAN *et al.* 2011). Importantly, the majority of PMK-
 1 1 target genes have decreased expression in *mb1-1(tm1563)* mutants (Fig 6A). The
 2 downregulated genes include C17H12.8 and K08D8.5 (Fig 6A), which are also regulated by ATF-
 3 7 (SHIVERS *et al.* 2010), providing further evidence for ATF-7 dysfunction in *mb1-1(tm1563)*
 4 mutants. Although less efficiently than *mb1-1(tm1563)* mutation, also *mb1-1* RNAi leads to
 5 decreased expression of a sub-set of PMK-1 target genes (Fig 6B). We found that *cox-5B*
 6 knockdown increases the expression of *mul-1*, *catp-3* and *amt-1* in both N2 and *mb1-1(tm1563)*
 7 mutants (Fig 6C). To examine whether the upregulation of these genes is dependent on PMK-1,
 8 we compared their expression between *mb1-1(tm1563)* mutants and *pmk-1(km25);mb1-1(tm1563)*
 9 double mutants. Loss of PMK-1 blunts the *cox-5B* RNAi-mediated induction of these genes (Fig
 0 6D). The data further demonstrate that *cox-5B* knockdown modulates p38 MAPK/PMK-1 activity.

Fig 6. Loss of MBL-1 leads to altered *cox-5B* RNAi-mediated mitochondrial stress modulates the expression of p38 MAPK/PMK-1 target genes

(A) The expression of PMK-1-regulated genes in L4 larvae N2 and *mbi-1(tm1563)* mutants. (B) The expression of PMK-1-regulated genes in *mbi-1* RNAi-treated L4 larvae N2. In (A) and (B) bars represent mRNA levels relative to N2 EV with error bars indicating mean $\pm$ s.d. of three biological replicates, each with three technical replicates (* $p < 0.05$, ** $p < 0.01$, unpaired Student's *t*-test). (C) The expression of PMK-1 regulated genes in L4 larvae N2 and *mbi-1(tm1563)* mutants upon *cox-5B* RNAi. The EV bars are from the same experiment presented in (A). (D) The expression of selected PMK-1 targets in L4 larvae *mbi-1(tm1563)* mutants and *pmk-1(km25);mbi-1(tm1563)* double mutants. In (C) and (D) bars represent mRNA levels relative to (C) N2 EV or (D) *mbi-1(tm1563)* EV with error bars indicating mean $\pm$ s.d. of three biological replicates, each with three technical replicates (* $p < 0.05$, ** $p < 0.01$, two-way ANOVA with Tukey's test). See S7 Table for raw qRT-PCR data.

5

6 ***mbl-1(tm1563)* mutants require p38 MAPK/PMK-1 for maximal lifespan extension upon**
7 **mitochondrial stress**

8 Although mitochondrial stress induces innate immune response (PELLEGRINO *et al.* 2014; CHIKKA
9 *et al.* 2016; JEONG *et al.* 2017; MATILAINEN *et al.* 2017), p38 MAPK/PMK-1 has not been associated
0 with mitochondria-mediated longevity. We asked whether PMK-1 is required for the increased
1 *mbl-1(tm1563)* mutant lifespan upon *cox-5B* RNAi. Although *cox-5B* RNAi extends the lifespan of
2 all tested strains (Fig 7A-7B and S1-S2 Tables), *pmk-1(km25);mbl-1(tm1563)* double mutation
3 blocks the maximal lifespan extension observed with *cox-5B* RNAi-treated *mbl-1(tm1563)*
4 mutants (Fig 7A-7B and S1-S2 Tables). Importantly, *mbl-1(tm1563)* mutants and *pmk-*
5 *1(km25);mbl-1(tm1563)* double mutants have similar lifespan on control RNAi (EV) (Fig 7A-7B
6 and S1-S2 Tables). In addition to *cox-5B* RNAi, we also investigated whether PMK-1 is required
7 for the lifespan increase of *mbl-1(tm1563)* mutants upon RNAi of mitochondrial respiratory chain
8 complex I subunit *nduf-6*, which has been shown to increase p38 MAPK/PMK-1 phosphorylation
9 (CHIKKA *et al.* 2016). Although on *nduf-6* RNAi *mbl-1(tm1563)* mutant lifespan is not increased
0 beyond *nduf-6* RNAi-treated N2 animals, *nduf-6* knockdown leads to 30.6 % increase in *mbl-*
1 *1(tm1563)* mutant lifespan (HR compared to *mbl-1(tm1563)* EV: 0.089), whereas N2 lifespan is
2 increased by 15.7 % (HR compared to N2 EV: 0.507) (Fig S5D-S5E and S1-S2 Tables).
3 Furthermore, similarly as with *cox-5B* RNAi, *pmk-1(km25);mbl-1(tm1563)* double mutation blocks
4 the maximal lifespan extension observed with *nduf-6* RNAi-treated *mbl-1(tm1563)* mutants (Fig
5 S5D-S5E and S1-S2 Tables). Altogether, these data demonstrate that mitochondrial stress
6 modulates the activity of p38 MAPK/PMK-1, thereby resulting in extended lifespan.

Fig 7. *cox-5B* RNAi-mediated mitochondrial stress results in PMK-1-dependent increase in *mbl-1(tm1563)* mutant lifespan.

(A-B) *cox-5B* RNAi-treated *mbl-1(tm1563)* mutants have extended longevity compared to *cox-5B* RNAi-treated N2 ($p < 0.01$), *pmk-1(km25)* ($p < 0.01$) and *pmk-1(km25);mbl-1(tm1563)* ($p < 0.01$) mutants. (B) shows the sub-set of lifespan curves presented in (A). See S1-S2 Tables for lifespan statistics. (C) Model based on the data presented here.

Discussion

The data presented here support the hypothesis that loss of MBL-1 disrupts the mechanism maintaining basal p38 MAPK/PMK-1 activity in normal conditions, which leads to reduced activity of transcription factors ATF-7 and SKN-1 and shortened lifespan (Fig 7C). Since the decline in basal p38 MAPK/PMK-1 activity is an aging-associated process (YOUNGMAN *et al.* 2011), it can be hypothesized that the aberrant p38 MAPK signaling contributes to the premature aging-phenotype observed with MBNL deficiency in DM1 patients. Although *mbl-1(tm1563)* mutants have a decreased level of activated p38 MAPK/PMK-1 at non-pathogenic condition, they do not show increased susceptibility to pathogens (Fig 2B and S1-S2 Tables). This demonstrates that p38 MAPK/PMK-1 regulates lifespan independently of its role in innate immunity. In this context, chronic stress caused by aging differs from acute stress caused by pathogen infection. Therefore, we propose that moderate reduction in p38 MAPK/PMK-1 activity shortens *mbl-1(tm1563)* mutant

lifespan, but it retains sufficient activity to respond to acute pathogenic bacterial stress. On the other hand, upregulation of innate immunity-associated genes in *mbi-1(tm1563)* mutants (Fig 2A) suggests that other pathogen surveillance-mechanisms may compensate the reduced p38 MAPK/PMK-1 activity, thus ensuring the normal survival of *mbi-1(tm1563)* mutants on PA14 (Fig 2B).

Importantly, the role of p38 MAPK/PMK-1 as a regulator of lifespan under non-pathogenic conditions is controversial, as it has been shown that *pmk-1(km25)* mutants have either normal lifespan (KIM *et al.* 2002; TROEMEL *et al.* 2006; WU *et al.* 2019) or reduced lifespan (PUJOL *et al.* 2008; PARK *et al.* 2018; ZHOU *et al.* 2019). Since PMK-1 is required for response to biotic stimulus, one possible explanation for differences between laboratories could be due to bacterial growth conditions, which in turn may be affected by brand of peptone and agar used for plate media. Interestingly, although we found that both *pmk-1(km25)* mutants and *pmk-1* RNAi-treated animals have shortened lifespan, the hazard ratio is bigger for *pmk-1* RNAi-treated animals in Fig 3G (HR compared to N2 EV: 5.062) than for *pmk-1(km25)* mutants in Fig 7A (HR compared to N2 EV: 2.406) and in Fig S5D (HR compared to N2 EV: 2.769). Although conclusions should not be made by comparing independent lifespan experiments, these data suggest that acute loss of PMK-1 could be more detrimental relative to chronic PMK-1 depletion. To hypothesize further, it is possible that *pmk-1(km25)* mutants have activated stress responses, which in turn can lead to inconsistencies in lifespan experiments in different laboratory settings.

Although demonstrating that the MBL-1 alternative splicing factor is required to maintain the basal p38 MAPK/PMK-1 activity and normal lifespan, this study leaves an open question about the molecular mechanism behind this phenotype. Interestingly, in addition to alternative splicing, MBNL1 regulates also mRNA decay and mRNA localization (MASUDA *et al.* 2012; WANG *et al.* 2012). Hence, it is possible that MBL-1 affects p38 MAPK/PMK-1 activity through a combination

of these mechanisms. Interestingly, p38 MAPK can also be activated by mechanical stimuli (NGUYEN *et al.* 2000; ZHANG *et al.* 2000; SAWADA *et al.* 2001; WANG *et al.* 2005; LEWTHWAITE *et al.* 2006; CHAUDHURI AND SMITH 2008; DOLINAY *et al.* 2008; HOFFMAN *et al.* 2017). Since *mbi-1* mutants display improper formation of neuromuscular junction synapses and altered locomotion (SPILKER *et al.* 2012), one intriguing hypothesis is that dysfunctional neuromuscular communication and the consequent abnormal muscle function leads to lower level of mechanical stimuli, and thus to decreased basal p38 MAPK/PMK-1 activity. In this context, one interesting experiment would be to address whether electrical stimulation promote basal p38 MAPK/PMK-1 activity and increase in lifespan in *mbi-1* mutants.

In addition to the model that MBL-1 promotes normal lifespan by maintaining the basal p38 MAPK/PMK-1 signaling (Fig 7C), our data show that mitochondrial stress promotes the longevity of *mbi-1(tm1563)* mutants in a partially PMK-1-dependent manner (Fig 7A-7B, Fig S5D-S5E and S1-S2 Tables). Although Western blot and qRT-PCR data (Fig 5B-5F, Fig S4, Fig 6C-D) indicate that *cox-5B* knockdown modulates the activity of p38 MAPK/PMK-1, it must be taken into account that p38 MAPK can also be activated by acetylation (PILLAI *et al.* 2011), raising the possibility that mitochondrial stress promotes p38 MAPK/PMK-1 function through different mechanisms. Importantly, overactive p38 MAPK signaling is toxic and shortens lifespan (CHEESMAN *et al.* 2016), and therefore, it can be assumed that p38 MAPK/PMK-1 is only moderately activated during non-infectious conditions such as mitochondrial stress. Since p38 MAPK/PMK-1 is only partially required for the increased *mbi-1(tm1563)* mutant lifespan upon mitochondrial stress (Fig 7A-7B, Fig S5D-S5E and S1-S2 Tables), there are also other mechanisms involved. For example, it is possible that the loss of MBL-1 disrupts mitochondrial function, and therefore, mitochondrial perturbation-activated stress responses may restore the homeostasis in this organelle, thus leading to strikingly increased lifespan.

Taken together, these data elucidate an important mechanism of how the loss of DM1-associated muscleblind splicing factor affects lifespan. In terms of quality of life, it has been proposed that targeting aging may be a more efficient approach compared to targeting individual diseases (KAEBERLEIN *et al.* 2015). Therefore, since DM1 is a multisystemic disease with similarities to aging (MATEOS-AIERDI *et al.* 2015; MEINKE *et al.* 2018), this study suggests that targeting the aging process could provide a powerful complementary therapeutic approach for this severe disorder.

Acknowledgements

The Garcia laboratory is supported by the Academy of Finland Project Funding (309173) and by the Institute of Biotechnology, University of Helsinki. O.M. is supported by the University of Helsinki three-year grant. A.R.S.R. was supported by the INOV Contacto-program. R.I.S. is supported by NIH NIDDK P30DK040561. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). We also thank the National BioResource Project (NBRP) for sharing the *mbl-1(tm1563)* strain, the laboratory of Dr. Carina Holmberg for sharing reagents, and Garcia lab members and Dr. Brendan Battersby for comments on the manuscript.

Literature cited

- Anders, S., P. T. Pyl and W. Huber, 2015 HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31: 166-169.
- Antonacci, S., D. Forand, M. Wolf, C. Tyus, J. Barney *et al.*, 2015 Conserved RNA-binding proteins required for dendrite morphogenesis in *Caenorhabditis elegans* sensory neurons. *G3 (Bethesda)* 5: 639-653.
- Charizanis, K., K. Y. Lee, R. Batra, M. Goodwin, C. Zhang *et al.*, 2012 Muscleblind-like 2-mediated alternative splicing in the developing brain and dysregulation in myotonic dystrophy. *Neuron* 75: 437-450.
- Chaudhuri, S., and P. G. Smith, 2008 Cyclic strain-induced HSP27 phosphorylation modulates actin filaments in airway smooth muscle cells. *Am J Respir Cell Mol Biol* 39: 270-278.
- Cheesman, H. K., R. L. Feinbaum, J. Thekkiniath, R. H. Downen, A. L. Conery *et al.*, 2016 Aberrant Activation of p38 MAP Kinase-Dependent Innate Immune Responses Is Toxic to *Caenorhabditis elegans*. *G3 (Bethesda)* 6: 541-549.
- Chikka, M. R., C. Anbalagan, K. Dvorak, K. Dombeck and V. Prahlad, 2016 The Mitochondria-Regulated Immune Pathway Activated in the *C. elegans* Intestine Is Neuroprotective. *Cell Rep* 16: 2399-2414.

- 3 Dillin, A., A. L. Hsu, N. Arantes-Oliveira, J. Lehrer-Graiwer, H. Hsin *et al.*, 2002 Rates of behavior
4 and aging specified by mitochondrial function during development. *Science* 298: 2398-
5 2401.
- 6 Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski *et al.*, 2013 STAR: ultrafast
7 universal RNA-seq aligner. *Bioinformatics* 29: 15-21.
- 8 Dolinay, T., W. Wu, N. Kaminski, E. Ifedigbo, A. M. Kaynar *et al.*, 2008 Mitogen-activated protein
9 kinases regulate susceptibility to ventilator-induced lung injury. *PLoS One* 3: e1601.
- 0 Fardaei, M., K. Larkin, J. D. Brook and M. G. Hamshire, 2001 In vivo co-localisation of MBNL
1 protein with DMPK expanded-repeat transcripts. *Nucleic Acids Res* 29: 2766-2771.
- 2 Fernandez-Costa, J. M., M. B. Llamusi, A. Garcia-Lopez and R. Artero, 2011 Alternative splicing
3 regulation by Muscleblind proteins: from development to disease. *Biol Rev Camb Philos*
4 *Soc* 86: 947-958.
- 5 Garcia, S. M., Y. Tabach, G. F. Lourenco, M. Armakola and G. Ruvkun, 2014 Identification of
6 genes in toxicity pathways of trinucleotide-repeat RNA in *C. elegans*. *Nat Struct Mol Biol*
7 21: 712-720.
- 8 Hoffman, L., C. C. Jensen, M. Yoshigi and M. Beckerle, 2017 Mechanical signals activate p38
9 MAPK pathway-dependent reinforcement of actin via mechanosensitive HspB1. *Mol Biol*
0 *Cell* 28: 2661-2675.
- 1 Huang da, W., B. T. Sherman and R. A. Lempicki, 2009 Systematic and integrative analysis of
2 large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44-57.
- 3 Inoue, H., N. Hisamoto, J. H. An, R. P. Oliveira, E. Nishida *et al.*, 2005 The *C. elegans* p38 MAPK
4 pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress
5 response. *Genes Dev* 19: 2278-2283.
- 6 Jeong, D. E., D. Lee, S. Y. Hwang, Y. Lee, J. E. Lee *et al.*, 2017 Mitochondrial chaperone HSP-
7 60 regulates anti-bacterial immunity via p38 MAP kinase signaling. *EMBO J* 36: 1046-
8 1065.
- 9 Kaeberlein, M., P. S. Rabinovitch and G. M. Martin, 2015 Healthy aging: The ultimate preventative
0 medicine. *Science* 350: 1191-1193.
- 1 Kanadia, R. N., K. A. Johnstone, A. Mankodi, C. Lungu, C. A. Thornton *et al.*, 2003 A muscleblind
2 knockout model for myotonic dystrophy. *Science* 302: 1978-1980.
- 3 Kenyon, C., J. Chang, E. Gensch, A. Rudner and R. Tabtiang, 1993 A *C. elegans* mutant that
4 lives twice as long as wild type. *Nature* 366: 461-464.
- 5 Kenyon, C. J., 2010 The genetics of ageing. *Nature* 464: 504-512.
- 6 Kim, D. H., R. Feinbaum, G. Alloing, F. E. Emerson, D. A. Garsin *et al.*, 2002 A conserved p38
7 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* 297: 623-626.
- 8 Lewthwaite, J. C., E. R. Bastow, K. J. Lamb, J. Blenis, C. P. Wheeler-Jones *et al.*, 2006 A specific
9 mechanomodulatory role for p38 MAPK in embryonic joint articular surface cell MEK-ERK
0 pathway regulation. *J Biol Chem* 281: 11011-11018.
- 1 Masuda, A., H. S. Andersen, T. K. Doktor, T. Okamoto, M. Ito *et al.*, 2012 CUGBP1 and MBNL1
2 preferentially bind to 3' UTRs and facilitate mRNA decay. *Sci Rep* 2: 209.
- 3 Mateos-Aierdi, A. J., M. Goicoechea, A. Aiastui, R. Fernandez-Torron, M. Garcia-Puga *et al.*,
4 2015 Muscle wasting in myotonic dystrophies: a model of premature aging. *Front Aging*
5 *Neurosci* 7: 125.
- 6 Matilainen, O., M. S. B. Sleiman, P. M. Quiros, S. Garcia and J. Auwerx, 2017 The chromatin
7 remodeling factor ISW-1 integrates organismal responses against nuclear and
8 mitochondrial stress. *Nat Commun* 8: 1818.
- 9 Meinke, P., S. Hintze, S. Limmer and B. Schoser, 2018 Myotonic Dystrophy-A Progeroid
0 Disease? *Front Neurol* 9: 601.
- 1 Meola, G., and R. Cardani, 2015 Myotonic dystrophies: An update on clinical aspects, genetic,
2 pathology, and molecular pathomechanisms. *Biochim Biophys Acta* 1852: 594-606.

- 3 Miller, J. W., C. R. Urbinati, P. Teng-Umuay, M. G. Stenberg, B. J. Byrne *et al.*, 2000 Recruitment
4 of human muscleblind proteins to (CUG)(n) expansions associated with myotonic
5 dystrophy. *EMBO J* 19: 4439-4448.
- 6 Murphy, C. T., S. A. McCarroll, C. I. Bargmann, A. Fraser, R. S. Kamath *et al.*, 2003 Genes that
7 act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature*
8 424: 277-283.
- 9 Nguyen, H. T., R. M. Adam, S. H. Bride, J. M. Park, C. A. Peters *et al.*, 2000 Cyclic stretch
0 activates p38 SAPK2-, ErbB2-, and AT1-dependent signaling in bladder smooth muscle
1 cells. *Am J Physiol Cell Physiol* 279: C1155-1167.
- 2 Norris, A. D., X. Gracida and J. A. Calarco, 2017 CRISPR-mediated genetic interaction profiling
3 identifies RNA binding proteins controlling metazoan fitness. *Elife* 6.
- 4 Park, M. R., S. Ryu, B. E. Maburutse, N. S. Oh, S. H. Kim *et al.*, 2018 Probiotic *Lactobacillus*
5 *fermentum* strain JDFM216 stimulates the longevity and immune response of
6 *Caenorhabditis elegans* through a nuclear hormone receptor. *Sci Rep* 8: 7441.
- 7 Pascual, M., M. Vicente, L. Monferrer and R. Artero, 2006 The Muscleblind family of proteins: an
8 emerging class of regulators of developmentally programmed alternative splicing.
9 *Differentiation* 74: 65-80.
- 0 Pellegrino, M. W., A. M. Nargund, N. V. Kirienko, R. Gillis, C. J. Fiorese *et al.*, 2014 Mitochondrial
1 UPR-regulated innate immunity provides resistance to pathogen infection. *Nature* 516:
2 414-417.
- 3 Pillai, V. B., N. R. Sundaresan, S. A. Samant, D. Wolfgeher, C. M. Trivedi *et al.*, 2011 Acetylation
4 of a conserved lysine residue in the ATP binding pocket of p38 augments its kinase activity
5 during hypertrophy of cardiomyocytes. *Mol Cell Biol* 31: 2349-2363.
- 6 Pujol, N., S. Cypowyj, K. Ziegler, A. Millet, A. Astrain *et al.*, 2008 Distinct innate immune
7 responses to infection and wounding in the *C. elegans* epidermis. *Curr Biol* 18: 481-489.
- 8 Ranum, L. P., and T. A. Cooper, 2006 RNA-mediated neuromuscular disorders. *Annu Rev*
9 *Neurosci* 29: 259-277.
- 0 Robinson, M. D., D. J. McCarthy and G. K. Smyth, 2010 edgeR: a Bioconductor package for
1 differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139-
2 140.
- 3 Sasagawa, N., E. Ohno, Y. Kino, Y. Watanabe and S. Ishiura, 2009 Identification of
4 *Caenorhabditis elegans* K02H8.1 (CeMBL), a functional ortholog of mammalian MBNL
5 proteins. *J Neurosci Res* 87: 1090-1097.
- 6 Sawada, Y., K. Nakamura, K. Doi, K. Takeda, K. Tobiume *et al.*, 2001 Rap1 is involved in cell
7 stretching modulation of p38 but not ERK or JNK MAP kinase. *J Cell Sci* 114: 1221-1227.
- 8 Shivers, R. P., D. J. Pagano, T. Kooistra, C. E. Richardson, K. C. Reddy *et al.*, 2010
9 Phosphorylation of the conserved transcription factor ATF-7 by PMK-1 p38 MAPK
0 regulates innate immunity in *Caenorhabditis elegans*. *PLoS Genet* 6: e1000892.
- 1 Spilker, K. A., G. J. Wang, M. S. Tugizova and K. Shen, 2012 *Caenorhabditis elegans* Muscleblind
2 homolog mbl-1 functions in neurons to regulate synapse formation. *Neural Dev* 7: 7.
- 3 Suenaga, K., K. Y. Lee, M. Nakamori, Y. Tatsumi, M. P. Takahashi *et al.*, 2012 Muscleblind-like
4 1 knockout mice reveal novel splicing defects in the myotonic dystrophy brain. *PLoS One*
5 7: e33218.
- 6 Tan, M. W., S. Mahajan-Miklos and F. M. Ausubel, 1999 Killing of *Caenorhabditis elegans* by
7 *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc Natl*
8 *Acad Sci U S A* 96: 715-720.
- 9 Thompson, M., R. Bixby, R. Dalton, A. Vandenburg, J. A. Calarco *et al.*, 2019 Splicing in a single
0 neuron is coordinately controlled by RNA binding proteins and transcription factors. *Elife*
1 8.
- 2 Timmons, L., D. L. Court and A. Fire, 2001 Ingestion of bacterially expressed dsRNAs can
3 produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* 263:
4 103-112.

- 5 Troemel, E. R., S. W. Chu, V. Reinke, S. S. Lee, F. M. Ausubel *et al.*, 2006 p38 MAPK regulates
6 expression of immune response genes and contributes to longevity in *C. elegans*. PLoS
7 Genet 2: e183.
- 8 Tullet, J. M., M. Hertweck, J. H. An, J. Baker, J. Y. Hwang *et al.*, 2008 Direct inhibition of the
9 longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132: 1025-
0 1038.
- 1 Wang, E. T., N. A. Cody, S. Jog, M. Biancolella, T. T. Wang *et al.*, 2012 Transcriptome-wide
2 regulation of pre-mRNA splicing and mRNA localization by muscleblind proteins. Cell 150:
3 710-724.
- 4 Wang, J., J. Fan, C. Laschinger, P. D. Arora, A. Kapus *et al.*, 2005 Smooth muscle actin
5 determines mechanical force-induced p38 activation. J Biol Chem 280: 7273-7284.
- 6 Wang, L. C., W. T. Hung, H. Pan, K. Y. Chen, Y. C. Wu *et al.*, 2008 Growth-dependent effect of
7 muscleblind knockdown on *Caenorhabditis elegans*. Biochem Biophys Res Commun 366:
8 705-709.
- 9 Wu, Z., M. Isik, N. Moroz, M. J. Steinbaugh, P. Zhang *et al.*, 2019 Dietary Restriction Extends
0 Lifespan through Metabolic Regulation of Innate Immunity. Cell Metab 29: 1192-1205
1 e1198.
- 2 Yang, W., K. Dierking and H. Schulenburg, 2016 WormExp: a web-based application for a
3 *Caenorhabditis elegans*-specific gene expression enrichment analysis. Bioinformatics 32:
4 943-945.
- 5 Youngman, M. J., Z. N. Rogers and D. H. Kim, 2011 A decline in p38 MAPK signaling underlies
6 immunosenescence in *Caenorhabditis elegans*. PLoS Genet 7: e1002082.
- 7 Zarubin, T., and J. Han, 2005 Activation and signaling of the p38 MAP kinase pathway. Cell Res
8 15: 11-18.
- 9 Zhang, D., V. Gaussion, G. E. Taffet, N. S. Belaguli, M. Yamada *et al.*, 2000 TAK1 is activated in
0 the myocardium after pressure overload and is sufficient to provoke heart failure in
1 transgenic mice. Nat Med 6: 556-563.
- 2 Zhou, L., B. He, J. Deng, S. Pang and H. Tang, 2019 Histone acetylation promotes long-lasting
3 defense responses and longevity following early life heat stress. PLoS Genet 15:
4 e1008122.
- 5
- 6

7 Supplemental material

Day 1 adult animals expressing extrachromosomal *mbl-1p::mbl-1::mCherry*

S1 Fig. Overexpression of mCherry-tagged MBL-1. (A) Screenshot from WormBase presenting *mbl-1* gene. Arrows indicate isoforms expressed in *mbl-1* OE lines. (B) Day 1 adult animals expressing *mbl-1p::mbl-1::mCherry* under normal conditions (Ctrl, EV) and after acute oxidative stress (5 % H₂O₂ for 20 minutes, image taken after five hours recovery on EV). Abbreviations: n, neuron; v, vulva; s, spermatheca; i, intestine. Scale bar, 100 μ m.

S2 Fig. MBL-1 activity decreases upon aging. (A) Screenshot from WormBase presenting *unc-43* and *unc-104* genes. Arrows indicate exons whose exclusion were examined upon aging. (B-C) PCR-mediated splicing assay of *unc-43* and (D-E) *unc-104* genes. (B) and (D) show representative agarose gels and (C) and (E) agarose gel quantifications. In (C) and (E) bars represent the intensity of shorter PCR product (exon excluded) relative to longer PCR product (exon included) with error bars indicating mean $\pm$ s.d. of three biological replicates (** $p < 0.01$, two-way ANOVA with Tukey's test). See S3 Table for agarose gel quantifications.

3

4

5

S3 Fig. Western blot repeats for figures 2C, 3A and 3C. See S6 Table for Western blot quantifications.

S4 Fig. Western blot repeats for figures 3A, 5B and 5E. See S6 Table for Western blot quantifications.

Fig S5. Lifespan effects upon knockdown of p38 MAPK pathway components *tir-1*, *nsy-1* and *sek-1* and mitochondrial electron transport chain subunit *nduf-6*. (A) *C. elegans* p38 MAPK pathway. (B) *tir-1* RNAi causes reduction in N2 lifespan, but does not affect the lifespan of *mb1-1(tm1563)* mutants. (C) *nsy-1* and *sek-1* RNAi does not affect the lifespan of N2, whereas *sek-1* RNAi shortens the lifespan of *mb1-1(tm1563)* mutants. (D-E) *nduf-6* RNAi-treated *mb1-1(tm1563)* mutants have extended longevity compared to *nduf-6* RNAi-treated *pmk-1(km25);mb1-1(tm1563)* mutants. (D) shows the sub-set of lifespan curves presented in (E). See S1-S2 Tables for lifespan statistics.

S1 Table. Summary of *C. elegans* lifespan experiments.

Genotype, RNAi and treatment	mean lifespan ± SE (days)	variation compared to control (%)	P-values against control	N (trials)
------------------------------	---------------------------------	-----------------------------------	--------------------------	------------

Figure 1A				
N2, HT115	20 ± 0.27			166 (2)
<i>mbl-1(tm1563)</i>, HT115	16.4 ± 0.16	- 18	<10 ⁻³	169 (2)
N2, OP50	17.3 ± 0.28	- 13.5	<10 ⁻³	165 (2)
<i>mbl-1(tm1563)</i>, OP50	15.2 ± 0.16	- 24 § - 12.1	<10 ⁻³ § <10 ⁻³	167 (2)
Figure 1B				
N2, EV RNAi	20.4 ± 0.34			152 (2)
N2, <i>mbl-1</i> RNAi	16.0 ± 0.18	- 21.6	<10 ⁻³	152 (2)
Figure 1C				
N2, EV RNAi	20.3 ± 0.35			166 (2)
<i>mbl-1</i> OE line 1, EV RNAi	16.7 ± 0.29	- 17.7	<10 ⁻³	159 (2)
<i>mbl-1</i> OE line 2, EV RNAi	16.4 ± 0.31	- 19.2	<10 ⁻³	162 (2)
Figure 2B				
N2, PA14	2.2 ± 0.03			175 (2)
<i>mbl-1(tm1563)</i>, PA14	2.3 ± 0.03	+ 4	0.511	162 (2)
<i>pmk-1(km25)</i>, PA14	1.4 ± 0.03	- 36	<10 ⁻³	181 (2)
Figure 3G				
N2, EV RNAi	21.2 ± 0.3			170 (2)
N2, <i>pmk-1</i> RNAi	17.3 ± 0.18	- 18.4	<10 ⁻³	168 (2)
<i>mbl-1(tm1563)</i>, EV RNAi	15.8 ± 0.33	- 25.5	<10 ⁻³	152 (2)
<i>mbl-1(tm1563)</i>, <i>pmk-1</i> RNAi	16.9 ± 0.2	- 20 # + 6.5	<10 ⁻³ # 0.605	158 (2)

Figure 4A				
N2, EV RNAi	22.9 ± 0.42			156 (2)
N2, <i>atf-7</i> RNAi	19.7 ± 0.34	- 14	<10 ⁻³	161 (2)
<i>mbl-1(tm1563)</i>, EV RNAi	18.1 ± 0.25	- 21	<10 ⁻³	162 (2)
<i>mbl-1(tm1563)</i>, <i>atf-7</i> RNAi	17.2 ± 0.24	- 24.9 # - 5	<10 ⁻³ # 0.0291	168 (2)
Figure 4B				
N2, EV RNAi	21.4 ± 0.41			148 (2)
N2, <i>skn-1</i> RNAi	18.4 ± 0.22	- 14	<10 ⁻³	158 (2)
<i>mbl-1(tm1563)</i>, EV RNAi	16.8 ± 0.3	- 21.5	<10 ⁻³	158 (2)
<i>mbl-1(tm1563)</i>, <i>skn-1</i> RNAi	16.5 ± 0.23	- 22.9 # - 1.8	<10 ⁻³ # 0.0151	161 (2)
Figure 4F				
N2, EV RNAi	22.1 ± 0.35			156 (2)
N2, <i>daf-16</i> RNAi	20.6 ± 0.33	- 6.8	<10 ⁻³	162 (2)
<i>mbl-1(tm1563)</i>, EV RNAi	17.8 ± 0.27	- 19.5	<10 ⁻³	165 (2)
<i>mbl-1(tm1563)</i>, <i>daf-16</i> RNAi	16.2 ± 0.24	- 26.7 # - 9	<10 ⁻³ # <10 ⁻³	166 (2)
Figure 4G				
N2, EV RNAi	23.5 ± 0.58		<10 ⁻³	75 (1)
N2, <i>daf-2</i> RNAi	34.5 ± 0.5	+ 31.9	<10 ⁻³	79 (1)
<i>mbl-1(tm1563)</i>, EV RNAi	18.1 ± 0.39	- 23	<10 ⁻³	81 (1)

<i>mb1-1(tm1563), daf-2</i>		+ 12.6	<10 ⁻³	
RNAi	26.9 ± 0.6	# + 32.7	# <10 ⁻³	85 (1)
Figure 5A				
N2, EV RNAi	21.6 ± 0.32			193 (2)
N2, <i>cox-5B</i> RNAi	25.5 ± 0.29	+ 15.3	<10 ⁻³	200 (2)
<i>mb1-1(tm1563), EV</i>	18.0 ± 0.23	- 16.7	<10 ⁻³	181 (2)
RNAi				
<i>mb1-1(tm1563), cox-5B</i>		+ 17.2	<10 ⁻³	
RNAi	26.1 ± 0.4	# + 31	# <10 ⁻³	185 (2)
		¶ + 2.3	¶ <10 ⁻³	
Figure 7A-7B				
N2, EV RNAi	22.7 ± 0.39			150 (2)
N2, <i>cox5B</i> RNAi	29.2 ± 0.38	+ 22.2	<10 ⁻³	
		@ - 9	@ <10 ⁻³	153 (2)
<i>mb1-1(tm1563), EV</i>	17.6 ± 0.24	- 22.5	<10 ⁻³	142 (2)
RNAi				
<i>mb1-1(tm1563), cox5B</i>		+ 29.3	<10 ⁻³	
RNAi	32.1 ± 0.35	# + 45.2	# <10 ⁻³	149 (2)
<i>pmk-1(km25), EV RNAi</i>	19.1 ± 0.36	- 15.9	<10 ⁻³	149 (2)
<i>pmk-1(km25), cox-5B</i>		+ 12.7	<10 ⁻³	
RNAi	26 ± 0.41	@ -19	@ <10 ⁻³	152 (2)
		* + 26.5	* <10 ⁻³	
<i>pmk-1(km25);mb1-1(tm1563), EV RNAi</i>	17.7 ± 0.28	- 22	<10 ⁻³	145 (2)
<i>pmk-1(km25);mb1-1(tm1563), cox-5B</i>		+ 20.4	<10 ⁻³	
RNAi	28.5 ± 0.42	@ - 11.2	@ <10 ⁻³	150 (2)
		& + 37.9	& <10 ⁻³	

Supplemental figure 5B				
N2, EV RNAi	22.3 ± 0.45			148 (2)
N2, <i>tir-1</i> RNAi	18.8 ± 0.35	- 15.7	<10 ⁻³	153 (2)
<i>mbl-1(tm1563)</i>, EV RNAi	17.1 ± 0.26	- 23.3	<10 ⁻³	175 (2)
<i>mbl-1(tm1563)</i>, <i>tir-1</i> RNAi	16.8 ± 0.27	- 24.7 # - 1.8	<10 ⁻³ # 0.345	159 (2)
Supplemental figure 5C				
N2, EV RNAi	21.6 ± 0.61			81
N2, <i>nsy-1</i> RNAi	23.2 ± 0.56	+ 6.9	0.057	82
N2, <i>sek-1</i> RNAi	22 ± 0.5	+ 1.8	0.428	83
<i>mbl-1(tm1563)</i>, EV RNAi	17.4 ± 0.43	- 19.4	<10 ⁻³	78
<i>mbl-1(tm1563)</i>, <i>nsy-1</i> RNAi	17 ± 0.43	- 21.3 # - 2.3	<10 ⁻³ # 0.562	87
<i>mbl-1(tm1563)</i>, <i>sek-1</i> RNAi	16.1 ± 0.43	- 25.5 # - 7.5	<10 ⁻³ # 0.034	79
Supplemental figure 5D-5E				
N2, EV RNAi	20.4 ± 0.48			70
N2, <i>nduf-6</i> RNAi	24.2 ± 0.33	+ 15.7 @ + 4.1	<10 ⁻³ @ 0.07	85
<i>mbl-1(tm1563)</i>, EV RNAi	16.1 ± 0.22	- 21.1	<10 ⁻³	77
<i>mbl-1(tm1563)</i>, <i>nduf-6</i> RNAi	23.2 ± 0.38	+ 12.1 # + 30.6	0.017	79
<i>pmk-1(km25)</i>, EV RNAi	18.1 ± 0.37	- 11.3	<10 ⁻³	85

		0	0.3918	
<i>pmk-1(km25), nduf-6</i>	20.4 ± 0.49	* + 11.3	* <10 ⁻³	83
RNAi		@ - 12.1	@ <10 ⁻³	
<i>pmk-1(km25);mbl-1(tm1563), EV RNAi</i>	16.4 ± 0.2	- 19.6	<10 ⁻³	78
<i>pmk-1(km25);mbl-1(tm1563), nduf-6</i>	20.5 ± 0.38	+ 0.5	0.0326	
RNAi		& + 20	& <10 ⁻³	80
		@ - 11.6	@ <10 ⁻³	

§ Compared to N2, OP50 treatment

Compared to *mbl-1(tm1563)*, EV RNAi treatment

¶ Compared to N2, *cox-5B* RNAi treatment

@ Compared to *mbl-1(tm1563)*, *cox-5B* or *nduf-6* RNAi treatment

* Compared to *pmk-1(km25)*, EV treatment

& Compared to *pmk-1(km25);mbl-1(tm1563)*, EV treatment

S2 Table. Individual replicates of *C. elegans* lifespan experiments.

Genotype, RNAi and treatment	mean lifespan ± SE (days)	variation compared to control (%)	P-values against control	N
Figure 1A				
N2, HT115, rep1	19.8 ± 0.31			86
N2, OP50, rep1	16.4 ± 0.33	- 17.2	<10 ⁻³	87
<i>mbl-1(tm1563)</i>, HT115, rep1	16.5 ± 0.18	- 16.7	<10 ⁻³	86
<i>mbl-1(tm1563)</i>, OP50, rep1	15 ± 0.22	- 24.2 § - 8.5	<10 ⁻³ § <10 ⁻³	84
N2, HT115, rep2	19.9 ± 0.41			80
N2, OP50, rep2	18.2 ± 0.42	- 8.5	<10 ⁻³	78

<i>mbi-1(tm1563)</i>, HT115, rep2	16.3 ± 0.27	- 18.1	<10 ⁻³	83
<i>mbi-1(tm1563)</i>, OP50, rep2	15.3 ± 0.22	- 23.1 § - 6.1	<10 ⁻³ § <10 ⁻³	83
Figure 1B				
N2, EV RNAi, rep1	20.5 ± 0.48			76
N2, <i>mbi-1</i> RNAi, rep1	16.0 ± 0.28	- 22	<10 ⁻³	75
N2, EV RNAi, rep2	20.1 ± 0.45			76
N2, <i>mbi-1</i> RNAi, rep2	16.0 ± 0.24	- 20.4	<10 ⁻³	77
Figure 1C				
N2, EV RNAi, rep1	21.1 ± 0.47			88
<i>mbi-1</i> OE line 1, EV RNAi, rep1	17.8 ± 0.40	- 15.6	<10 ⁻³	83
<i>mbi-1</i> OE line 2, EV RNAi, rep1	17.3 ± 0.43	- 18	<10 ⁻³	85
N2, EV RNAi, rep2	19.4 ± 0.5			78
<i>mbi-1</i> OE line 1, EV RNAi, rep2	15.4 ± 0.37	- 20.6	<10 ⁻³	76
<i>mbi-1</i> OE line 2, EV RNAi, rep2	15.3 ± 0.41	- 21.1	<10 ⁻³	77
Figure 2B				
N2, PA14, rep1	2.2 ± 0.04			89
<i>mbi-1(tm1563)</i>, PA14, rep1	2.2 ± 0.04	0	0.665	84
<i>pmk-1(km25)</i>, PA14, rep1	1.4 ± 0.05	- 36	<10 ⁻³	92
N2, PA14, rep2	2.2 ± 0.04			86

<i>mbi-1(tm1563)</i>, PA14, rep2	2.2 ± 0.04	0	0.619	78
<i>pmk-1(km25)</i>, PA14, rep2	1.4 ± 0.07	- 36	<10 ⁻³	89
Figure 3G				
N2, EV RNAi, rep1	19.3 ± 0.28			86
N2, <i>pmk-1</i> RNAi, rep1	17.3 ± 0.19	- 10.4	<10 ⁻³	88
<i>mbi-1(tm1563)</i>, EV RNAi, rep1	15.8 ± 0.22	- 18.1	<10 ⁻³	63
<i>mbi-1(tm1563)</i>, <i>pmk-1</i> RNAi, rep1	16.1 ± 0.16	- 16.6 # + 1.9	<10 ⁻³ # 0.89	81
N2, EV RNAi, rep2	21.2 ± 0.37			84
N2, <i>pmk-1</i> RNAi, rep2	17.4 ± 0.29	- 17.9	<10 ⁻³	80
<i>mbi-1(tm1563)</i>, EV RNAi, rep2	15.5 ± 0.57	- 26.9	<10 ⁻³	89
<i>mbi-1(tm1563)</i>, <i>pmk-1</i> RNAi, rep2	17.6 ± 0.34	- 17 # + 11.9	<10 ⁻³ # 0.297	77
Figure 4A				
N2, EV RNAi, rep1	20.8 ± 0.42			73
N2, <i>atf-7</i> RNAi, rep1	18.7 ± 0.35	- 10.1	<10 ⁻³	78
<i>mbi-1(tm1563)</i>, EV RNAi, rep1	17.8 ± 0.27	- 14.4	<10 ⁻³	75
<i>mbi-1(tm1563)</i>, <i>atf-7</i> RNAi, rep1	17.0 ± 0.28	- 18.3 # - 4.5	<10 ⁻³ # 0.17	82
N2, EV RNAi, rep2	23.3 ± 0.59			83
N2, <i>atf-7</i> RNAi, rep2	20.5 ± 0.55	- 12	<10 ⁻³	83
<i>mbi-1(tm1563)</i>, EV RNAi, rep2	18.4 ± 0.4	- 21	<10 ⁻³	87

<i>mb1-1(tm1563), atf-7</i>		- 25.3	<10 ⁻³	
RNAi, rep2	17.4 ± 0.38	# - 5.4	# 0.137	86
Figure 4B				
N2, EV RNAi, rep1	21.8 ± 0.51			73
N2, <i>skn-1</i> RNAi, rep1	18.9 ± 0.4	- 13.3	<10 ⁻³	75
<i>mb1-1(tm1563), EV RNAi, rep1</i>	16.8 ± 0.31	- 22.9	<10 ⁻³	72
<i>mb1-1(tm1563), skn-1</i>		- 23.4	<10 ⁻³	
RNAi, rep1	16.7 ± 0.21	# - 0.6	# 0.314	81
N2, EV RNAi, rep2	20 ± 0.55			75
N2, <i>skn-1</i> RNAi, rep2	18 ± 0.21	- 10	<10 ⁻³	83
<i>mb1-1(tm1563), EV RNAi, rep2</i>	16.6 ± 0.5	- 17	<10 ⁻³	86
<i>mb1-1(tm1563), skn-1</i>		- 18.5	<10 ⁻³	
RNAi, rep2	16.3 ± 0.4	# - 1.8	# 0.0137	80
Figure 4F				
N2, EV RNAi, rep1	22.7 ± 0.48			75
N2, <i>daf-16</i> RNAi, rep1	21.6 ± 0.48	- 4.8	<10 ⁻³	78
<i>mb1-1(tm1563), EV RNAi, rep1</i>	18.1 ± 0.39	- 20.3	<10 ⁻³	81
<i>mb1-1(tm1563), daf-16</i>		- 24.7	<10 ⁻³	
RNAi, rep1	17.1 ± 0.28	# - 5.5	# <10 ⁻³	83
N2, EV RNAi, rep2	21.4 ± 0.47			81
N2, <i>daf-16</i> RNAi, rep2	19.6 ± 0.42	- 8.4	<10 ⁻³	84
<i>mb1-1(tm1563), EV RNAi, rep2</i>	17.4 ± 0.38	- 18.7	<10 ⁻³	84

<i>mb1-1(tm1563), daf-16</i>		- 28	<10 ⁻³	
RNAi, rep2	15.4 ± 0.36	# - 11.5	# <10 ⁻³	83
Figure 4G				
N2, EV RNAi	23.5 ± 0.58		<10 ⁻³	75
N2, <i>daf-2</i> RNAi	34.5 ± 0.5	+ 31.9	<10 ⁻³	79
<i>mb1-1(tm1563), EV RNAi</i>	18.1 ± 0.39	- 23	<10 ⁻³	81
<i>mb1-1(tm1563), daf-2</i>		+ 12.6	<10 ⁻³	
RNAi	26.9 ± 0.6	# + 32.7	# <10 ⁻³	85
Figure 5A				
N2, EV RNAi, rep1	21.6 ± 0.53			81
N2, <i>cox-5B</i> RNAi, rep1	26.1 ± 0.48	+ 17.2	<10 ⁻³	85
<i>mb1-1(tm1563), EV RNAi, rep1</i>	16.3 ± 0.26	- 24.5	<10 ⁻³	78
<i>mb1-1(tm1563), cox-5B</i>		+ 19.1	<10 ⁻³	
RNAi, rep1	26.7 ± 0.68	# + 39	# <10 ⁻³	80
		¶ + 2.2	¶ 0.021	
N2, EV RNAi, rep2	21.5 ± 0.39			112
N2, <i>cox-5B</i> RNAi, rep2	25 ± 0.34	+ 14	<10 ⁻³	115
<i>mb1-1(tm1563), EV RNAi, rep2</i>	19.2 ± 0.29	- 10.7	<10 ⁻³	103
<i>mb1-1(tm1563), cox-5B</i>		+ 16	<10 ⁻³	
RNAi, rep2	25.6 ± 0.45	# + 25	# <10 ⁻³	105
		¶ + 2.3	¶ 0.037	
Figure 7A-7B				
N2, EV RNAi, rep1	22.5 ± 0.6			72
N2, <i>cox5B</i> RNAi, rep1	30.6 ± 0.5	+ 26.5	<10 ⁻³	80

		@ - 6.9	@ 0.001	
<i>mbi-1(tm1563)</i>, EV RNAi, rep1	18.1 ± 0.33	- 19.6	<10 ⁻³	73
<i>mbi-1(tm1563)</i>, <i>cox5B</i> RNAi, rep1	32.9 ± 0.44	+ 31.6 # + 45	<10 ⁻³ # <10 ⁻³	75
<i>pmk-1(km25)</i>, EV RNAi, rep1	18.8 ± 0.53	- 16.4	<10 ⁻³	79
<i>pmk-1(km25)</i>, <i>cox-5B</i> RNAi, rep1	27.1 ± 0.53	+ 17 @ - 17.6 * + 30.6	<10 ⁻³ @ <10 ⁻³ * <10 ⁻³	75
<i>pmk-1(km25);mbi-1(tm1563)</i>, EV RNAi, rep1	18 ± 0.38	- 20	<10 ⁻³	74
<i>pmk-1(km25);mbi-1(tm1563)</i>, <i>cox-5B</i> RNAi, rep1	28.2 ± 0.68	+ 20.2 @ - 14.3 & + 36.2	<10 ⁻³ @ <10 ⁻³ & <10 ⁻³	74
N2, EV RNAi, rep2	22.9 ± 0.49			78
N2, <i>cox5B</i> RNAi, rep2	27.3 ± 0.44	+ 16.1 @ - 9	<10 ⁻³ @ <10 ⁻³	73
<i>mbi-1(tm1563)</i>, EV RNAi, rep2	17.1 ± 0.34	- 25.3	<10 ⁻³	69
<i>mbi-1(tm1563)</i>, <i>cox5B</i> RNAi, rep2	30 ± 0.39	+ 23.7 # + 43	<10 ⁻³ # <10 ⁻³	74
<i>pmk-1(km25)</i>, EV RNAi, rep2	19.4 ± 0.5	- 15.3	<10 ⁻³	70
<i>pmk-1(km25)</i>, <i>cox-5B</i> RNAi, rep2	24.8 ± 0.55	+ 7.6 @ - 17.3 * + 21.8	<10 ⁻³ @ <10 ⁻³ * <10 ⁻³	77

<i>pmk-1(km25);mbl-1(tm1563)</i>, EV RNAi	17.4 ± 0.4	- 24	<10 ⁻³	71
<i>pmk-1(km25);mbl-1(tm1563)</i>, <i>cox-5B</i> RNAi, rep2	28.3 ± 0.43	+ 19.1 @ - 5.6 & + 38.5	<10 ⁻³ @ <10 ⁻³ & <10 ⁻³	76
Supplemental figure 5B				
N2, EV RNAi, rep1	21.3 ± 0.67			65
N2, <i>tir-1</i> RNAi, rep1	18.2 ± 0.52	- 14.6	<10 ⁻³	72
<i>mbl-1(tm1563)</i>, EV RNAi, rep1	15.9 ± 0.28	- 25.4	<10 ⁻³	88
<i>mbl-1(tm1563)</i>, <i>tir-1</i> RNAi, rep1	15.9 ± 0.37	- 25.4 # 0	<10 ⁻³ # 0.12	80
N2, EV RNAi, rep2	22.3 ± 0.48		<10 ⁻³	83
N2, <i>tir-1</i> RNAi, rep2	19.4 ± 0.46	- 13	<10 ⁻³	81
<i>mbl-1(tm1563)</i>, EV RNAi, rep2	18.4 ± 0.38	- 17.5	<10 ⁻³	87
<i>mbl-1(tm1563)</i>, <i>tir-1</i> RNAi, rep2	17.7 ± 0.35	- 20.6 # - 3.8	<10 ⁻³ # 0.613	79
Supplemental figure 5C				
N2, EV RNAi	21.6 ± 0.61			81
N2, <i>nsy-1</i> RNAi	23.2 ± 0.56		0.057	82
N2, <i>sek-1</i> RNAi	22 ± 0.5		0.428	83
<i>mbl-1(tm1563)</i>, EV RNAi	17.4 ± 0.43		<10 ⁻³	78
<i>mbl-1(tm1563)</i>, <i>nsy-1</i> RNAi	17 ± 0.43		<10 ⁻³ # 0.562	87
<i>mbl-1(tm1563)</i>, <i>sek-1</i> RNAi	16.1 ± 0.43		<10 ⁻³ # 0.034	79

Supplemental figure 5D-5E				
N2, EV RNAi	20.4 ± 0.48			70
N2, <i>nduf-6</i> RNAi	24.2 ± 0.33	+ 15.7 @ + 4.1	<10 ⁻³ @ 0.07	85
<i>mb1-1(tm1563)</i>, EV RNAi	16.1 ± 0.22	- 21.1	<10 ⁻³	77
<i>mb1-1(tm1563)</i>, <i>nduf-6</i> RNAi	23.2 ± 0.38	+ 12.1 # + 30.6	0.017	79
<i>pmk-1(km25)</i>, EV RNAi	18.1 ± 0.37	- 11.3	<10 ⁻³	85
<i>pmk-1(km25)</i>, <i>nduf-6</i> RNAi	20.4 ± 0.49	0 * + 11.3 @ - 12.1	0.3918 * <10 ⁻³ @ <10 ⁻³	83
<i>pmk-1(km25);mb1-1(tm1563)</i>, EV RNAi	16.4 ± 0.2	- 19.6	<10 ⁻³	78
<i>pmk-1(km25);mb1-1(tm1563)</i>, <i>nduf-6</i> RNAi	20.5 ± 0.38	+ 0.5 & + 20 @ - 11.6	0.0326 & <10 ⁻³ @ <10 ⁻³	80

§ Compared to N2, OP50 treatment

Compared to *mb1-1(tm1563)*, EV RNAi treatment

¶ Compared to N2, *cox-5B* RNAi treatment

@ Compared to *mb1-1(tm1563)*, *cox-5B* RNAi treatment

* Compared to *pmk-1(km25)*, EV treatment

& Compared to *pmk-1(km25);mb1-1(tm1563)*, EV treatment

S3 Table. Agarose gel quantifications. Supplemental Excel file.

S4 Table. List of up- and downregulated genes in day 2 adult *mb1-1(tm1563)* mutants. Supplemental Excel file.

S5 Table. WormExp output for up- and downregulated genes in day 2 adult *mb1-1(tm1563)* mutants. Supplemental Excel file.

S6 Table. Western blot quantifications. Supplemental Excel file.

S7 Table. Raw qRT-PCR and *gst-4p::gfp* imaging data. Supplemental Excel file.

S8 Table. Oligonucleotide sequences used for qRT-PCR in this study.

Gene	Forward (5' → 3')	Reverse (5' → 3')
<i>cdc-42</i>	CTGCTGGACAGGAAGATTACG	CTCGGACATTCTCGAATGAAG
<i>pmp-3</i>	GTTCCCGTGTTTCATCACTCAT	ACACCGTCGAGAAGCTGTAGA
<i>tir-1</i>	TGGAGTCAACCGGTCTCTTC	AGAGCATTGCAACTTCCACG
<i>nsy-1</i>	CACCAGAGCCAATCGTTTCC	TTGAGCACCACATGAAGCAC
<i>sek-1</i>	ATGGAGCAATGTTTCGCGAA	GACCGACAGAGCCATTTTCC
<i>pmk-1</i>	TGGATTGGCACGTCAAACCTG	TGCAAGAATACACCCAACTGAC
<i>C17H12.8</i>	CACTGTGCGATTGCTCACTCC	TGTTGAGCTTGTGAAAGTGGA
<i>mul-1</i>	GGCTCAAACCTACGTTGCTCA	GACGAATCCTTGTCATGGTGG
<i>irg-4</i>	GAACCCATTAGCAATCTACCGT	GTTGAGCTTGTGACAGTGGG
<i>K08D8.5</i>	TCCGGGAAGTCGAATGAACA	CTGGAATTGAGAAGCCGACG
<i>C29F3.7</i>	CAAGACTGTGACGGCATTGT	GTTTTAGTGACCCCGCCATC
<i>catp-3</i>	CGACGTTGGAGTTGCTATGG	G TTCAGGCCACATGTGAGTC
<i>T25C12.3</i>	ACGCTGCTGATGAGACATCT	AGATCATTTGGCCCGTCTGA
<i>grd-3</i>	ACATGCCCATCACTCAAGGA	GCCTGAACGATTGGTCCAAG
<i>amt-1</i>	CGGGTATTGGGCTATCGGAT	CCGGAAACAATAGTAGCGGC

S9 Table. Oligonucleotide sequences used for splicing assay in this study.

Gene	Forward (5' → 3')	Reverse (5' → 3')
<i>unc-43</i>	GACCACCATCGACAATCAAGG	CAACAACGTCTGAGTCACCC

unc-104 TGCCACCTGATCTATTGCCA AACGAATGCTCTTCCAACCA

2

3

4