Supplemental Methods

RNA library preparation and sequencing

To synchronize the stages of wild-type and *daf-2(e1370)* animals, embryos were obtained by a bleaching method (Hwang et al. 2014) and incubated in S-basal buffer [5.85 g NaCl, 1 g K_2HPO_4, 6 g KH_2PO_4, 1 mL cholesterol (5 mg/mL in ethanol) in 1 L of double-distilled water (ddH_2O)] for 16 h at 20°C with gentle rotation. Animals were placed and cultured on OP50-seeded NGM plates until they reached L4 larval stage at 20°C, unless described otherwise. The animals were then treated with 50 μM 5-fluoro-2′-deoxyuridine (FUDR; SigmaAldrich, MO, USA) to prevent progeny from hatching. The wild-type and *daf-2(e1370)* animals were subsequently harvested with M9 buffer (5 g NaCl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 1 mL 1 M MgSO4 in 1 L ddH_2O) at days 1, 4, 7, and 11 of adulthoods. For experiments with *glp-4(bn2ts)* animals, fertile adult animals cultured at 15°C (a permissive temperature) were bleached for obtaining eggs, and transferred to 25°C (a restrictive temperature) from the embryonic stage to prevent the development of germ line. The synchronized animals that were continuously grown at 25°C were then harvested with M9 buffer at days 1 and 7 of adulthoods. Total RNA was isolated using RNAiso plus (Takara, Shiga, Japan) following the manufacturer's instruction. cDNA library was prepared and paired-end RNA-sequencing was performed by using Illumina NovaSeq 6000 platform (Macrogen Inc., Seoul, South Korea). Three independent biological replicates were used for RNA-seq library preparation and subsequent analysis.

Identification of age-dependently regulated transcripts and terms

Sequenced reads were aligned to the *C. elegans* genome WBcel235 (ce11) and Ensembl transcriptome (release 98) by using STAR (v.2.7.0e) (Dobin et al. 2013). We used cutoffs of minimum total read count per junction for (1) non-canonical motifs, (2) GT/AG and CT/AC motif, (3) GC/AG and CT/GC motif, and (4) AT/AC and GT/AT motif as 3, 1, 1, and 1, respectively, following previously described methods (Dobin et al. 2013). The aligned reads to genes and transcripts were quantified by using RSEM (v.1.3.1) (Li and Dewey 2011). The alignment and quantification were performed by using parameters described in the guidelines of ENCODE long RNA-seq processing pipeline (https://www.encodeproject.org/pipelines/ENCPL002LPE/). Long non-coding RNAs (Akay et al. 2019) were quantified by using featureCounts command in Subread package (v.2.0.0) (Liao et al. 2014). The reads aligned to annotated structural or functional RNA elements were analyzed by using NOISeq (v.2.28.0) (Tarazona et al. 2015). The efficiency of rRNA depletion was variable among our samples (3.6−13.3%), but the difference was not significant among ages or genotypes ($p > 0.25$, pairwise two-tailed Welch's *t*-test). Therefore, rRNAs with random effects were interpreted as noises. To measure the expression levels of introns in individual genes, the exon-intron split analysis was adopted (Gaidatzis et al. 2015). Reads at all known transcript isoforms of a gene were added to obtain the exon level of the gene. The exon level was subtracted from the sum of reads at whole genic regions of the gene and the value represents the intron level of the gene. Subsequently, the exon and the intron levels were compared for a single gene. Expression changes of various regions at different ages were calculated by using DESeq2 (v.1.22.2) (Love et al. 2014). Wald test *p* values were adjusted for multiple testing using the procedure of Benjamini and Hochberg (BH procedure). Differentially

expressed genes and transcript isoforms (fold change > 2 and adjusted *p* value < 0.05) were then identified. Global expression changes of previously published gene sets were represented as normalized enrichment scores (NES) by using GSEA (v.3.0) (Subramanian et al. 2005). Significant gene sets (false discovery rate *q* value < 0.1) were then identified.

Analysis of RNA-processing events in age-dependently regulated transcript isoforms

Unannotated transcripts were detected and all detected transcripts were quantified by using Cufflinks (v.2.2.1) (Trapnell et al. 2010) based on the reads aligned to the Ensembl transcriptome by using STAR (v.2.7.0e) (Dobin et al. 2013). Difference in transcript level and relative isoform fraction at different ages were calculated by using IsoformSwitchAnalyzeR (v.1.10.0) (Vitting-Seerup and Sandelin 2019). *p* values were adjusted for multiple testing using the BH procedure. Transcripts that originated from possible polymerase run-on fragments and genomic repeat elements were excluded. In subsequent analysis of RNA-processing events, genes with more than two transcript isoforms were considered. Gene expression contribution to variance in isoform level was calculated based on R-squared values of simple linear regression to predict isoform levels at different ages. Differential transcript isoforms (isoform fraction > 0.1 and adjusted *p* value < 0.1) were identified. Enrichment of each RNA-processing event in age-dependently upregulated isoforms relative to age-dependently downregulated ones was calculated by using IsoformSwitchAnalyzeR (v.1.10.0). Sequence composition and strength of regions surrounding certain splice sites were visualized and calculated by using WebLogo (v.

3.7.4) (Crooks et al. 2004) and MaxEntScan (Yeo and Burge 2004), respectively.

Analysis of transcripts associated with physiological and chronological aging

To dissect the difference between chronological and physiological ages at transcript levels, transcripts were selected if their levels were upregulated or downregulated (absolute fold change > 2 and BH-adjusted *p* value < 0.05) in aged animals by using a simple regression model with genotypes and ages. The transcripts were chosen if their normalized levels of transcript per million (TPM) were affected by ages (*p* value < 0.05) in the two-way ANOVA. Transcripts associated with physiological aging were defined if their normalized levels were affected by either genotypes or the interaction between genotypes and ages (*p* value < 0.05). Transcripts associated with temporal shift were defined if their normalized levels were affected by genotypes (*p* value < 0.05), but not by the interaction (*p* value > 0.1). In contrast, transcripts associated with slope change were defined if their normalized levels were affected by the interaction (*p* value < 0.05), but not by genotypes (*p* value > 0.1). Transcripts associated with chronological aging were defined if their normalized levels were independent of genotypes (*p* value > 0.1), and the interaction between genotypes and ages (*p* value > 0.1). Age-dependently regulated gene sets were selected if their expression was upregulated or downregulated both in wild-type and *daf-2* mutant animals. Among them, the gene sets were selected if their expression was significantly changed (absolute NES > 0.5 and *q* value < 0.1) during aging in either wild-type or *daf-2* mutant animals. Gene sets for physiological aging were determined as follows. First, genes associated with temporal shift were defined if the difference of NES between wild-type and *daf-2* mutant animals was greater than 0.2

at least at one age, and if the range of the differences was within ±0.2 in all the tested ages. Second, the association of gene sets with slope change was determined if the difference of NES slope between wild-type and *daf-2* mutant animals was greater than 0.5. Gene sets associated with chronological aging were defined if the difference of NES between wild-type and *daf-2* mutant animals was smaller than 0.2 at all the tested ages. Among genes that encode components annotated as “RNA processing”, genes whose expression was significantly downregulated in wild-type animals but not in *daf-2* mutants (difference of likelihood-ratio test statistics between wild-type and *daf-2* mutant animals > 2 and *p* value of likelihood-ratio test > 0.1 in *daf-2* mutants) were selected for lifespan assays. For the analysis of RNA-processing events, significance levels at different ages were separately compared. For the analysis of A3 in genes that were enriched in various systems, expressed genes enriched in the male-specific system were not included because of irrelevance, as our RNA-seq data were generated using hermaphrodites. Isoform fraction and junction usage of particular transcripts were visualized by using Gviz (v.1.34.1) (Hahne and Ivanek 2016). R (v.4.0.2, http://www.r-project.org) was used for plotting all the data in this study. The published sequencing data used in this study are available from NCBI-SRA (https://www.ncbi.nlm.nih.gov/sra) and Gene Expression Omnibus.

Lifespan assays

Lifespan assays were conducted at 20°C using adult *C. elegans* on NGM plates containing 50 μg/mL ampicillin (USB, Cleveland, OH, USA) and 1 mM isopropyl-β-D-thiogalactoside (IPTG; Gold Biotechnology, St. Louis, MO, USA) seeded with *E. coli*

strain HT115 that expresses double-stranded RNA targeting a specific gene (RNAi bacteria), as described previously (Park et al. 2020). For whole-life RNAi lifespan assays, gravid day 1 adult animals were allowed to lay eggs on RNAi bacteria-seeded plates and the progeny were grown to reach a pre-fertile adult stage. The pre-fertile adult animals were transferred to new RNAi bacteria-seeded plates containing 5 μM FUDR to prevent progeny from hatching. For adult-only RNAi lifespan assays, gravid day 1 adult animals were allowed to lay eggs on HT115 containing empty RNAi plasmid (L4440; control RNAi bacteria) and the progeny were grown to reach L4 larval stage. The L4-stage animals were then transferred to new plates seeded with RNAi bacteria targeting a specific gene. On the next day, pre-fertile or gravid adults were transferred to new RNAi bacteria-seeded plates containing FUDR. Two biological replicates of experiments were performed by two independent researchers for all the lifespan assays. Lifespan assays were performed double-blindly; the researchers were unaware of which genes were targeted by RNAi clones during the experiments. Animals that did not respond to gentle touch with a platinum wire were considered as dead. Animals that crawled off the plates, displayed internal hatching or vulval rupture were censored but included in the statistical analysis. Statistical analysis of the lifespan data was conducted using online application of the survival analysis 2 (http://sbi.postech.ac.kr/oasis2) (Han et al. 2016). *p* values were calculated using the log-rank (Mantel-Cox) test.

Quantitative reverse transcription PCR

Stage-synchronized animals were obtained by using an embryo-bleaching method (Stiernagle 2006). Animals were cultured until reaching a pre-fertile adult stage, and

harvested with M9 buffer containing 0.01% polyethylene glycol 4000 (PEG 4000, Tokyo Chemical Industry, Tokyo, Japan), washed three times, and stored at −80°C before RNA isolation. For whole-life RNAi treatments, synchronized embryos were placed on RNAi bacteria-seeded plates and cultured until reaching a pre-fertile adult stage. For adult-only RNAi experiments, synchronized embryos were placed on control RNAi bacteria-seeded plates and transferred to plates seeded with each of indicated RNAi bacteria at L4 larval stage. Animals were harvested 24 h after the transfer. Total RNA was isolated by using RNAiso Plus (Takara, Shiga, Japan) following the manufacturer's instruction. cDNA was synthesized from 1 μg of isolated total RNA by using ImProm-II Reverse Transcriptase (Promega, Madison, WI, USA) with random hexamer primers (25 ng/μL; Cosmo Genetech, South Korea). Quantitative reverse transcription PCR was performed with *Power* SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) using StepOne real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Comparative C_T method was used to calculate the relative quantity of each target. *ama-1* was used as an endogenous control for normalization. *p* values were calculated by two-tailed Student's *t* test.

Reverse transcription PCR

One microgram of total RNA from each sample was used for reverse transcription (RT) experiments. For A3 isoforms, gene-specific primers (10 μM) were used in a PCR reaction with 5 μL cDNA from an RT reaction. The PCR reaction conditions for *gale-1* and *unc-61* were annealing temperatures 62°C and 55°C, and the elongation time 15 sec with 23 and 25 cycles, respectively. For *let-49*, annealing temperature

60°C and the elongation time 15 sec with 30 cycles were optimal PCR conditions, but nonspecific bands were detected (Supplemental Fig. S15 D, E). Therefore, *let-49* was not further characterized for the RT-PCR analysis of A3. Primer sequences are available in Supplemental Table S4. Ten microliter of the product was run on a 12% TBE (Tris-Boric acid-EDTA)-urea gel [8 M urea, 7.5 mL 40% acrylamide (19:1), 2.5 mL 10x TBE (890 mM Tris base, 890 mM boric acid, 20 mM EDTA (10x, pH 8.3)), 100 µL 20% ammonium persulfate, 25 µL TEMED] in 1x TBE buffer at 200 V for 90 min. After staining with SYBR® Green II (Invitrogen, Carlsbad, CA, USA, S9305) in 1x TBE for 40 min, the gels were visualized using ChemiDoc XRS+ system (Bio-Rad, Contra Costa County, CA, USA), and analyzed by using Image Lab software (Bio-Rad). To estimate changes in A3 ratio, calculation for the quantity of transcripts that used the distal 3′ splice sites (β) and transcripts that used the proximal 3′ splice sites (α) was adopted as below.

$$\text{Isoform fraction of A3 isoform} = \beta / (\alpha + \beta)$$

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234