

Identification and Analysis of Age-Invariant Proteins during Aging in *Caenorhabditis elegans*

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Research Article

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Abstract

Aging is characterized by a progressive decline in organismal fitness and function and is accompanied by loss of proteostasis, the maintenance of a balanced and functional proteome. Although many proteins change in abundance during aging, a subset remains relatively invariant. The contribution of these age-invariant proteins to proteostasis and aging remains unclear. To identify such proteins, we performed a comparative analysis of age-resolved proteomic datasets from wild-type, short-lived, and long-lived mutant *C. elegans*. Age-invariant proteins were enriched in biosynthetic processes, gene expression, and mRNA metabolism and were predominantly associated with fundamental cellular components. We further analyzed RNA-seq data from wild-type *C. elegans* and identified transcripts that remained invariant throughout the lifespan or after day 5. Eighty-five genes showed invariant abundance at both RNA and protein levels. Our findings identify a conserved set of age-invariant proteins across strains with distinct lifespans and suggest that validated age-invariant genes may serve as reference controls for biochemical experiments and facilitate the identification of age-associated biomarkers.

Introduction

Aging is a complex biological process characterized by a progressive decline in physiological function and an increasing susceptibility to disease. At the cellular level, aging is accompanied by widespread changes in metabolism, gene expression, cellular signaling, and protein homeostasis (Guo et al. 2022). Among the major hallmarks of aging, loss of proteostasis is particularly important because the proper synthesis, folding, localization, and degradation of proteins are essential for maintaining cellular function (Hipp et al. 2019; López-Otín et al. 2023). Proteostasis is maintained by an integrated network of protein quality control (PQC) mechanisms, including molecular chaperones, protein degradation pathways, and regulatory systems that coordinate protein synthesis and turnover (Balch et al. 2008; Hipp et al. 2019). With advancing age, these mechanisms become less effective, increasing the likelihood of protein misfolding, accumulation, and aggregation (Hipp et al. 2014).

Age-associated changes in the proteome have been extensively investigated using transcriptomic and proteomic approaches. These studies have revealed substantial changes in the abundance of proteins and transcripts during different stages of life. Such changes can reflect adaptations to physiological transitions as well as the progressive decline in cellular homeostasis (Narayan et al. 2016; Walther et al. 2017; Son et al. 2019; Koyuncu et al. 2021). However, not all proteins undergo age-dependent changes. A subset of proteins maintains relatively stable abundance throughout life or remains stable after a particular stage of adulthood. The maintenance of these proteins may be important because many are components of essential cellular pathways and complexes that require appropriate stoichiometry and continuous availability. Despite their potential importance, age-invariant proteins have received considerably less attention than proteins whose abundance changes during aging.

Large-scale transcriptomic and proteomic studies have demonstrated that molecular aging is highly dynamic and can differ substantially across tissues and cell types (Schaum et al., 2020; Zhang et al.,

2021). Accordingly, identifying robust biomarkers of aging requires consideration of tissue, cell type, and age-dependent molecular variation (Bao et al., 2023). In contrast to these age-associated changes, recent work has highlighted the value of genes that remain stable throughout the lifespan. González et al. (2025) identified age-invariant genes across 17 mouse tissues and demonstrated that a subset of these genes can serve as reliable reference genes across the lifespan, while also showing that no conventional housekeeping gene is universally stable across aging tissues. Earlier studies similarly demonstrated that the expression stability of commonly used reference genes can vary with age, tissue, and experimental conditions (Zampieri et al., 2010; Uddin et al., 2011), underscoring the need for systematic validation of reference genes in aging studies. Studies of housekeeping genes across tissues have also shown that genes with stable expression are generally associated with fundamental cellular functions (Zeng et al., 2016). Importantly, RNA abundance does not necessarily predict protein abundance uniformly across genes and tissues, highlighting the need to examine stability at both molecular levels (Edfors et al., 2016). Together, these findings suggest that systematically identifying age-invariant genes and proteins may provide both biological insights into conserved cellular functions during aging and practical reference molecules for molecular studies.

The nematode *Caenorhabditis elegans* is an ideal model for investigating the molecular mechanisms underlying aging and is particularly suitable for studying age-associated changes in the proteome. In particular, *daf-2* and *daf-16*, which function in the insulin/insulin-like signaling pathway, have major effects on lifespan (Murphy 2013). Comparing age-related proteomes from wild-type, long-lived, and short-lived animals can therefore help distinguish changes associated with specific lifespan phenotypes. An important physiological transition in *C. elegans* occurs during early adulthood, around day 5–6, when reproductive activity changes substantially (Scharf et al. 2021). Protein and RNA abundance can undergo considerable remodeling during this period. Therefore, examining proteins that remain invariant either throughout adulthood or after this transition may provide insights into components of the proteome that are maintained despite changing physiological states. Furthermore, determining whether protein-level invariance is accompanied by stable RNA abundance can provide insights into the relationship between transcriptional and post-transcriptional regulation during aging.

In this study, we systematically analyzed age-resolved proteomic datasets from wild-type, long-lived *daf-2*, and short-lived *daf-16* *C. elegans* to identify proteins whose abundance remains invariant throughout the lifespan or after day 6. We further analyzed RNA-seq data from wild-type animals to identify age-invariant transcripts and examined the overlap between invariant RNA and protein abundance. Finally, we experimentally validated the expression of selected age-invariant genes during aging. This approach identifies a conserved set of age-invariant proteins and transcripts and provides a framework for investigating their potential contribution to proteome stability, proteostasis, and aging. It may also identify reference genes that can be useful for biochemical and molecular analyses performed across different stages of aging.

Methods

The first age-related total proteome data of WT and lifespan mutants was taken from Walther et. al 2017 (Walther et al. 2017). The second age-related proteome data of WT worms was taken from Narayan et. al 2016 (Narayan et al. 2016). The age-related RNA sequencing data was extracted from Schiksnis 2024 et al (Schiksnis et al. 2024). All the proteome and RNA sequencing data was processed using Microsoft excel. Proteins and RNA having Log2 values between -0.25 to $+0.25$ were considered as invariant candidates (based on valid values at different days). We took day 6, day 12 and day 17 values normalized to day 1 for proteins while day 5, day 10 and day 15 values normalized to day 1 for RNA.

Venn diagrams used in this study were prepared from interactivenn (Heberle et al. 2015). Go term were prepared using shinyGo 0.85.1 (Ge et al. 2020). Data for tissue specific enrichment were extracted from enrichment analysis from Wormbase (Angeles-Albores et al. 2016, 2018)

Results

1. Age-invariant proteins are maintained throughout the lifespan of wild-type

1. Age-invariant proteins are maintained throughout the lifespan of wild-type *C. elegans*

Protein abundance changes extensively during aging, but a subset of proteins may remain relatively stable. We therefore sought to identify proteins whose abundance remained invariant throughout the lifespan or became invariant after an important developmental transition.

To identify proteins whose abundance remains relatively stable during aging, we analyzed the age-resolved proteome of wild-type *C. elegans* from day 1, day 6, day 12, and day 17. Proteins were classified as invariant when their log2 fold change relative to the reference time point was between -0.25 and $+0.25$, corresponding to approximately 0.84- to 1.19-fold change. At days 6, 12, and 17, 1,410, 1,047, and 908 proteins, respectively, fell within this range, with 398 proteins remaining invariant at all three time points (Fig. 1A, Table-1). Because day 6 represents an important transition in the *C. elegans* lifespan, we also identified proteins that changed between day 1 and day 6 but remained invariant thereafter. Normalizing day 12 and day 17 to day 6 identified 1,935 and 1,451 invariant proteins, respectively, with 1,077 proteins remaining invariant after day 6 (Fig. 1B, Table-2). Thus, a substantial set of proteins maintains stable abundance either throughout adulthood or following the day 6 transition.

Gene ontology analysis revealed that proteins invariant throughout the lifespan were enriched in gene expression, biosynthetic processes, cellular metabolism, primary metabolism, and macromolecule biosynthesis (Fig. 1C). These proteins were predominantly associated with the cytoplasm, protein-containing complexes, ribonucleoprotein complexes, and membrane-bounded organelles (Fig. 1D). A similar enrichment pattern was observed for proteins invariant after day 6, with enrichment in cellular and primary metabolic processes, biosynthesis, gene expression, and translation (Fig. 1E–F). We next examined the abundance and distribution of the invariant proteins using absolute LFQ values. The abundance profiles of invariant proteins were comparable across the analyzed age groups, while their

abundance ranges were distinct from those of proteins showing increased or decreased abundance (Supplementary Fig. 1A–D). Tissue-enrichment analysis showed that the invariant proteins were distributed across multiple tissues, with enrichment in the germline, VB1, and anal depressor muscle (Supplementary Fig. 1E). Additional tissue-enrichment analysis of the invariant proteins identified germline and reproductive tissues among the enriched tissues (Supplementary Fig. 1F–G).

To assess the reproducibility of the invariant protein sets, we compared the invariant proteins identified in our analysis with an independent proteomic dataset from Narayan et al. The invariant protein sets identified throughout adulthood in the two datasets showed an overlap of 117 proteins (Fig. 1J). Similarly, comparison of proteins invariant after adulthood identified 530 common proteins between the Walther et al. and Narayan et al. datasets (Fig. 1K). Within the Narayan et al. dataset, 1,321 proteins were invariant throughout the analyzed age range, while 1,195 proteins were common between proteins invariant throughout age and those invariant after adulthood (Fig. 1H–I). Together, these analyses indicate that age-invariant proteins are reproducibly detected across independent proteomic datasets and are enriched in specific tissues, particularly the germline and reproductive system.

2. Age-invariant RNAs are maintained throughout the lifespan of wild-type

2. Age-invariant RNAs are maintained throughout the lifespan of wild-type *C. elegans*

To determine whether stable protein abundance is accompanied by stable transcript levels, we analyzed RNA-seq data from wild-type *C. elegans* collected at days 5, 10, and 15 and normalized to day 1. Using the same log₂ fold-change range of – 0.25 to + 0.25, we identified 5,377, 4,759, and 5,564 invariant RNAs at days 5, 10, and 15, respectively. Among these, 1,312 RNAs remained invariant at all three time points (Fig. 2A, Table-3). Because the transition around day 5–6 may represent a major change in the physiological state of the animals, we additionally identified RNAs that became invariant after day 5. When day 10 and day 15 RNA levels were normalized to day 5, 5,808 and 4,899 RNAs, respectively, were within the invariant range, with 3,302 RNAs remaining invariant at both time points (Fig. 2B).

The age-invariant RNAs were enriched in macromolecule metabolism, primary metabolism, gene expression, cellular and macromolecule biosynthesis, and nucleic acid and RNA metabolism (Fig. 2C, E). At the cellular component level, these transcripts were predominantly associated with membrane-bounded organelles, the nucleus, cytoplasm, and protein-containing complexes (Fig. 2D, F). Thus, similar to the invariant proteome, the invariant transcriptome is enriched for genes involved in fundamental cellular and metabolic processes. These findings indicate that a substantial subset of transcripts maintains relatively stable abundance during aging, either throughout adulthood or following the day 5 transition.

3. A subset of genes maintains invariant RNA and protein abundance during aging

We next asked whether the age-invariant RNA and protein datasets identified independently contained common genes. Among the invariant RNAs identified at days 5, 10, and 15, 181, 139, and 147 encoded proteins that were also classified as invariant in the corresponding proteomic analysis. Conversely, 246, 188, and 168 invariant proteins identified at days 6, 12, and 17, respectively, corresponded to RNAs classified as invariant in the RNA-seq dataset (Fig. 3A–B, Table-4). Across the two datasets, 85 genes showed invariant abundance at both the RNA and protein levels across the lifespan, using the predefined log2 fold-change range of -0.25 to $+0.25$.

Functional enrichment analysis of these 85 common candidates revealed enrichment in biosynthetic processes, gene expression, mRNA metabolism, and RNA processing (Fig. 3C). At the cellular component level, these genes were primarily associated with the cytoplasm, membrane-bounded organelles, spliceosomal complexes, ribonucleoprotein complexes, and other protein-containing complexes (Fig. 3D). The enrichment of these candidates in processes related to gene expression and RNA metabolism suggests that stable regulation of these cellular functions may be maintained at both the transcript and protein levels during aging. Thus, although RNA and protein abundances are globally remodeled during aging, a defined subset of genes maintains relatively stable abundance at both molecular levels.

4. Age-invariant proteins are conserved in long- and short-lived mutants

4. Age-invariant proteins are conserved in long- and short-lived *C. elegans* mutants

To determine whether age-invariant proteins are specific to normal aging or are conserved across different lifespan trajectories, we analyzed age-resolved proteomes from the long-lived *daf-2* and short-lived *daf-16* mutants. In *daf-2* mutants, 1,367, 1,057, and 895 proteins were invariant at days 6, 12, and 17, respectively, with 426 proteins remaining invariant throughout the lifespan (Fig. 4A, Table-5). To identify proteins that became stable after day 6, day 12 and day 17 were normalized to day 6, yielding 1,933 and 1,453 invariant proteins, respectively, of which 1,168 were invariant at both time points (Fig. 4B, Table-6). In the short-lived *daf-16* mutants, 1,235, 955, and 814 proteins were invariant at days 6, 12, and 17, respectively, with 296 proteins invariant throughout the lifespan (Fig. 4E, Table-7). When normalized to day 6, 1,818 and 1,307 proteins were invariant at days 12 and 17, respectively, with 994 proteins remaining invariant after day 6 (Fig. 4F, Table-8).

The invariant proteins in both mutants showed broadly similar functional enrichment patterns to those observed in wild-type animals. In *daf-2* mutants, invariant proteins were enriched in cellular and primary metabolic processes, biosynthesis, translation, gene expression, and macromolecule metabolism, and were predominantly associated with the cytoplasm, protein-containing complexes, membrane-bounded organelles, ribonucleoprotein complexes, and mitochondria (Fig. 4C–D). Similarly, invariant proteins in *daf-16* mutants were enriched in cellular and primary metabolism, biosynthesis, protein metabolism, translation, and gene expression and were associated mainly with the cytoplasm, membrane-bounded

organelles, protein-containing complexes, and mitochondria (Fig. 4G–H). The abundance, aggregation, and physicochemical characteristics of invariant proteins in both mutants showed distributions broadly comparable to those observed in wild-type animals (Supplementary Fig. 2). Together, these findings indicate that a substantial invariant proteome is maintained despite major differences in lifespan and that these proteins are predominantly associated with fundamental cellular functions.

5. A set of age-invariant proteins is shared across wild-type and lifespan mutants

Having identified age-invariant proteins in wild-type (WT), *daf-2*, and *daf-16* animals, we next determined whether a common set of proteins was maintained across these distinct lifespan backgrounds. Among the proteins invariant throughout the lifespan, 398, 426, and 296 were identified in WT, *daf-2*, and *daf-16* animals, respectively, with 34 proteins common to all three strains (Fig. 5A). These 34 proteins were enriched in intracellular protein transport, translation, protein transport, and establishment of protein localization and were predominantly associated with the cytoplasm, vesicle coats, membrane coats, and coated membranes (Fig. 5C–D). The common set included proteins involved in diverse cellular functions, including protein targeting to the endoplasmic reticulum (*F38A1.8*), translation and aminoacyl-tRNA synthesis (*eef-1g*, *eef-1B.2*, *fars-2*, and *ears-1*), glycolysis and metabolism (*aldo-1*, *pfk-1.1*, and *mccc-1*), mitochondrial function (*mtx-2*, *sco-1*, *atp-3*, and *Y69A2AR.18*), and vesicular trafficking and membrane organization (*agef-1*, *copb-2*, *sar-1*, *rab-1*, *vps-36*, and *epn-1*) (Table 9). Several of these proteins also have reported RNAi phenotypes involving development, reproduction, apoptosis, lifespan, metabolism, and stress or pathogen responses, indicating that the conserved invariant set includes proteins with diverse and essential cellular functions.

We next examined proteins that remained invariant after day 6. Of the 1,077, 1,168, and 994 proteins identified in WT, *daf-2*, and *daf-16* animals, respectively, 223 proteins were common to all three strains (Fig. 5B). These shared proteins were enriched in translation, primary and cellular metabolic processes, protein transport, and localization and were mainly associated with the cytoplasm, cytosol, protein-containing complexes, and ribonucleoprotein complexes (Fig. 5E–F). Thus, although the number of invariant proteins differed among the three lifespan backgrounds, a common set of proteins was maintained across all strains. The functional diversity of the 34 proteins invariant throughout the lifespan, together with their reported phenotypes, suggests that these proteins participate in fundamental cellular processes that may require stable abundance despite differences in lifespan. However, whether their invariant abundance directly contributes to lifespan regulation or proteostasis remains to be experimentally determined.

Discussion

Aging is accompanied by extensive remodeling of the proteome, with changes in protein abundance reflecting alterations in metabolism, gene expression, protein synthesis, and protein quality control.

While age-dependent changes in protein abundance have been widely studied, relatively little attention has been given to proteins that remain stable during aging. In this study, we analyzed age-resolved proteomic datasets from wild-type, long-lived *daf-2*, and short-lived *daf-16* *C. elegans* to identify proteins that remain invariant throughout life or become invariant after day 6. We also analyzed RNA-seq data to determine whether invariant protein abundance is accompanied by stable transcript levels. Together, these analyses identified a set of proteins and transcripts that remain relatively stable across different stages of aging and lifespan backgrounds.

In wild-type animals, 398 proteins remained invariant throughout the analyzed lifespan, whereas 1,077 proteins were invariant after day 6. These proteins were predominantly enriched in gene expression, biosynthetic, metabolic, and translation-related processes and were mainly associated with the cytoplasm, protein-containing complexes, and ribonucleoprotein complexes. The enrichment of these proteins in fundamental cellular processes suggests that maintaining stable abundance of core cellular components may be important for cellular function during aging. The invariant proteins were also enriched in the germline and reproductive tissues, consistent with the importance of these tissues during early adulthood. Interestingly, although their overall abundance remained relatively stable, these proteins showed increased aggregation with age. This observation suggests that stable protein abundance does not necessarily indicate preservation of protein quality or function during aging.

Analysis of the transcriptome identified 1,312 RNAs that remained invariant throughout the lifespan and 3,302 RNAs that were invariant after day 5. Similar to the invariant proteome, these transcripts were enriched in metabolic processes, biosynthesis, gene expression, and nucleic acid metabolism. However, only 85 genes were invariant at both RNA and protein levels. The relatively limited overlap suggests that maintenance of protein abundance during aging is not necessarily determined by stable transcript abundance and may involve post-transcriptional regulation, translation, or protein turnover. Nevertheless, the 85 common genes represent a particularly robust group of age-invariant factors that remain stable at both molecular levels and may be useful for further functional investigation.

The analysis of lifespan mutants revealed a broadly conserved invariant proteome despite differences in lifespan. The number of proteins invariant throughout the lifespan was 426 in long-lived *daf-2* mutants, 398 in wild-type animals, and 296 in short-lived *daf-16* mutants. Although the numbers differed, the invariant proteins in all three strains showed similar enrichment in metabolic processes, biosynthesis, translation, gene expression, and protein-containing complexes. Furthermore, 34 proteins were invariant throughout the lifespan in all three strains, while 223 proteins were shared when proteins invariant after day 6 were considered. Comparison with an independent proteomic dataset further identified common invariant proteins, supporting the reproducibility of this observation. Together, these findings suggest that age-invariant proteins constitute a conserved component of the proteome that is maintained across different lifespan trajectories. Overall, our analysis suggests that age-invariant proteins are broadly similar across wild-type, long-lived, and short-lived strains and are primarily associated with fundamental cellular functions. Their stable abundance, despite increasing aggregation with age, highlights the distinction between maintaining protein abundance and maintaining protein quality during aging.

Finally, the experimentally validated age-invariant genes may have practical value as reference genes for biochemical and molecular experiments performed across different ages, provided their stability is independently validated in the specific experimental system. Because these genes remain relatively stable rather than changing with age, they may also provide a useful reference framework for evaluating age-associated molecular changes, rather than serving as conventional biomarkers of aging. Thus, age-invariant genes could potentially facilitate the identification and interpretation of genuine age-dependent changes in gene and protein abundance.

Declarations

Supplementary Information The online version contains supplementary material available at

Author contributions Deepanshu Verma: Methodology, Data curation, Formal analysis, Writing. Prasad Kasturi: Conceptualization, Writing-review and editing, Funding acquisition, Supervision.

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Data availability No datasets were generated during the current study.

Competing interests The authors declare no competing interests.

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Tables

Tables 1 to 9 are available in the Supplementary Files section.

Figures

Figure 1

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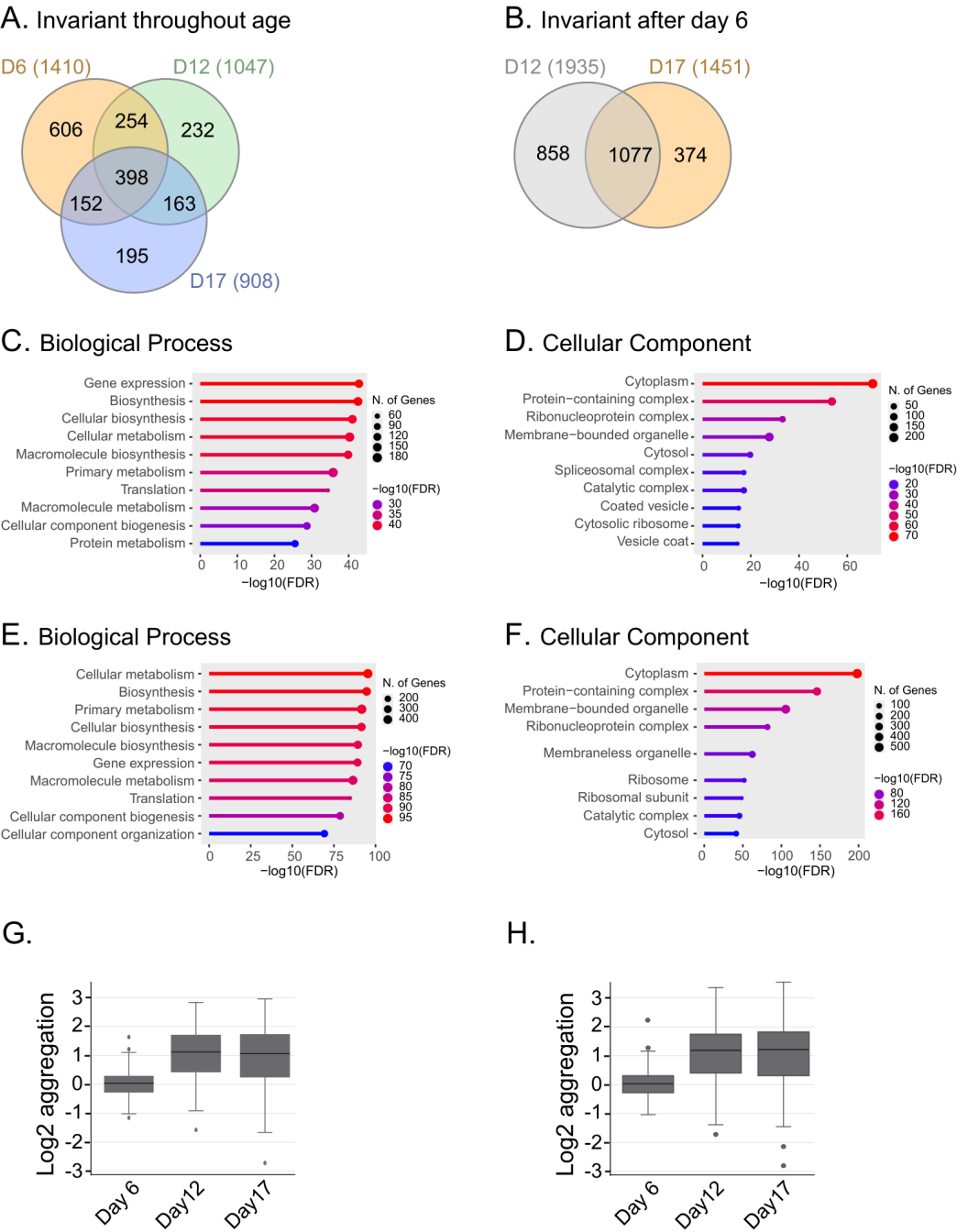


Figure 1

Age-invariant proteins across the lifespan of wild-type *C. elegans*.

(A). Venn diagram showing proteins with invariant abundance at days 6, 12, and 17 relative to day 1. A total of 398 proteins were invariant at all three time points. (B) Venn diagram showing proteins with invariant abundance at days 12 and 17 relative to day 6; 1,077 proteins were invariant at both time

points. (C–D) Gene ontology enrichment analysis of proteins invariant throughout the lifespan showing enriched biological processes and cellular components, respectively. (E–F) Gene ontology enrichment analysis of proteins invariant after day 6 showing enriched biological processes and cellular components, respectively. (G–H) Distribution of protein aggregation at days 6, 12, and 17 for proteins invariant throughout the lifespan (G) and proteins invariant after day 6 (H). Biological process and cellular component enrichment are shown as $-\log_{10}(\text{FDR})$, with point size indicating the number of genes.

Figure 2 Verma and Kasturi

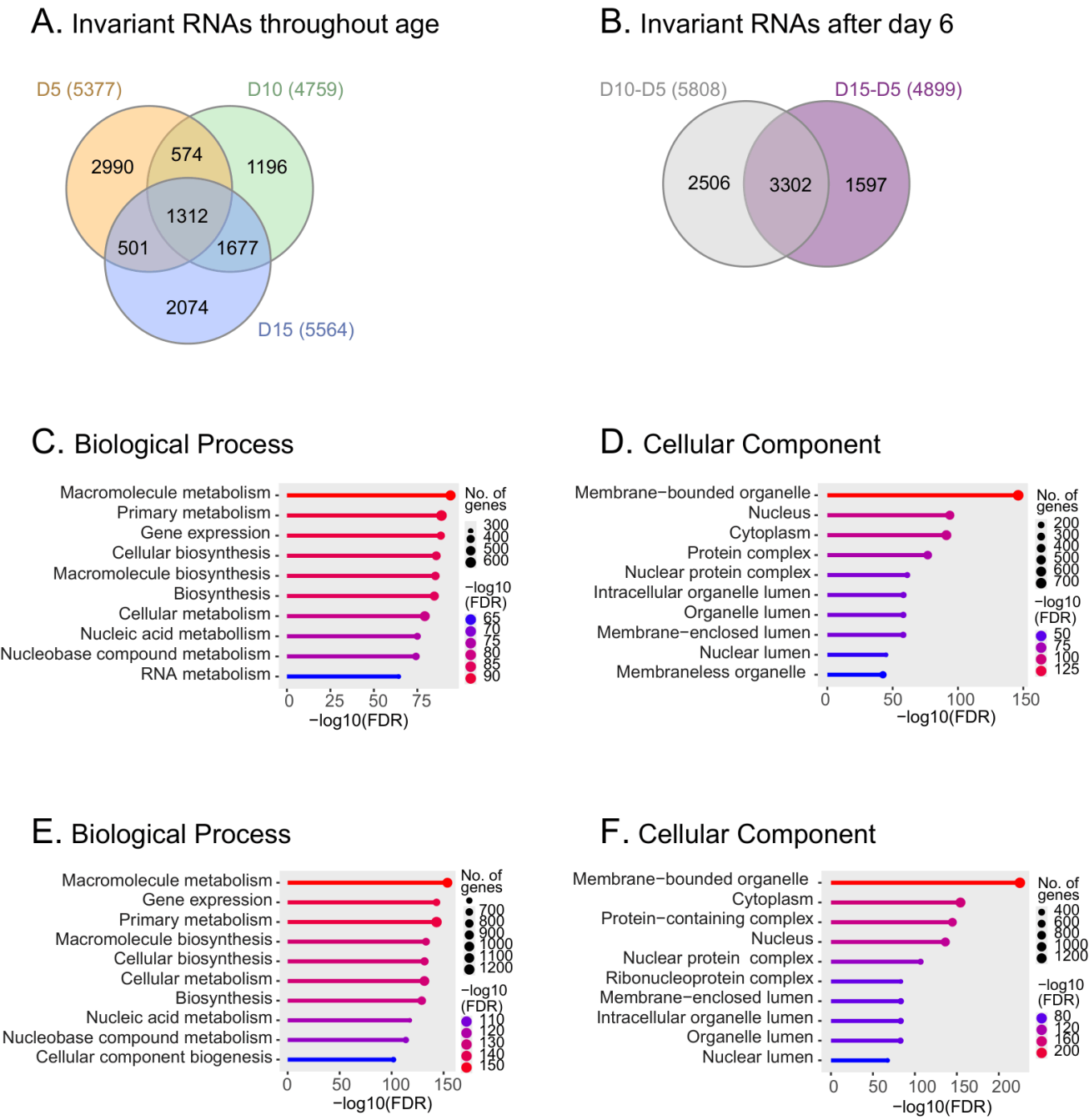


Figure 2

Age-invariant RNAs in wild-type *C. elegans*

(A) Venn diagram showing RNAs with invariant abundance at days 5, 10, and 15 relative to day 1. A total of 1,312 RNAs were invariant at all three time points. (B) Venn diagram showing RNAs with invariant abundance at days 10 and 15 relative to day 5; 3,302 RNAs were invariant at both time points. (C–D) Gene ontology enrichment analysis of RNAs invariant throughout the lifespan showing enriched biological processes and cellular components, respectively. (E–F) Gene ontology enrichment analysis of RNAs invariant after day 5 showing enriched biological processes and cellular components, respectively. Enrichment is represented as $-\log_{10}(\text{FDR})$, with point size indicating the number of genes.

Figure 3

Verma and Kasturi

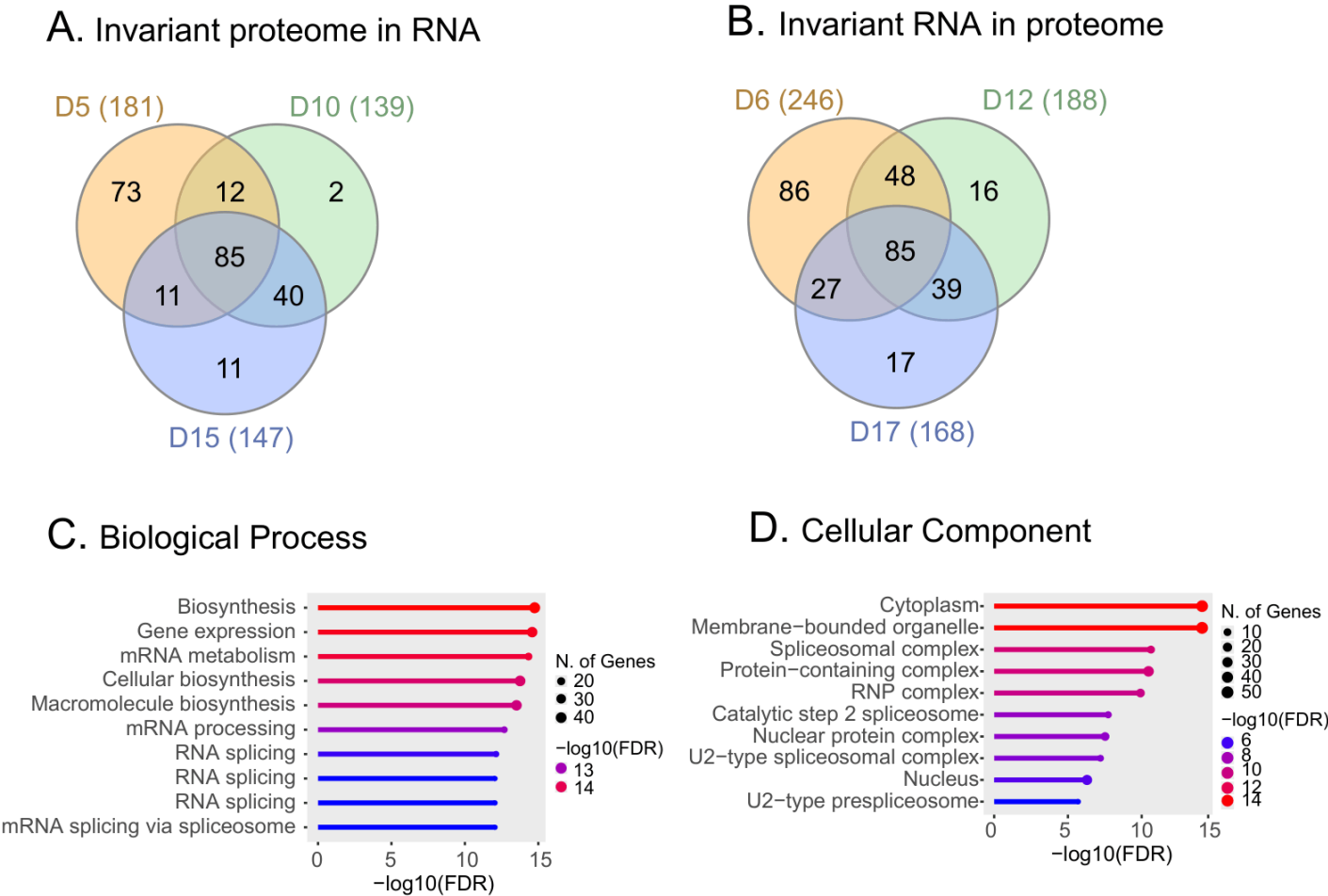


Figure 3

Common invariant RNAs and proteins

(A) Venn diagram showing the overlap between invariant proteins identified in the proteome and the corresponding invariant RNAs at days 5, 10, and 15. (B) Venn diagram showing the overlap between invariant RNAs and the corresponding proteins at days 6, 12, and 17. Eighty-five genes were common to the invariant RNA and protein datasets. (C) Gene ontology enrichment analysis of the common invariant genes showing enriched biological processes. (D) Enriched cellular components associated with the common invariant genes. Enrichment is represented as $-\log_{10}(\text{FDR})$, with point size indicating the number of genes.

Figure 4

Verma and Kasturi

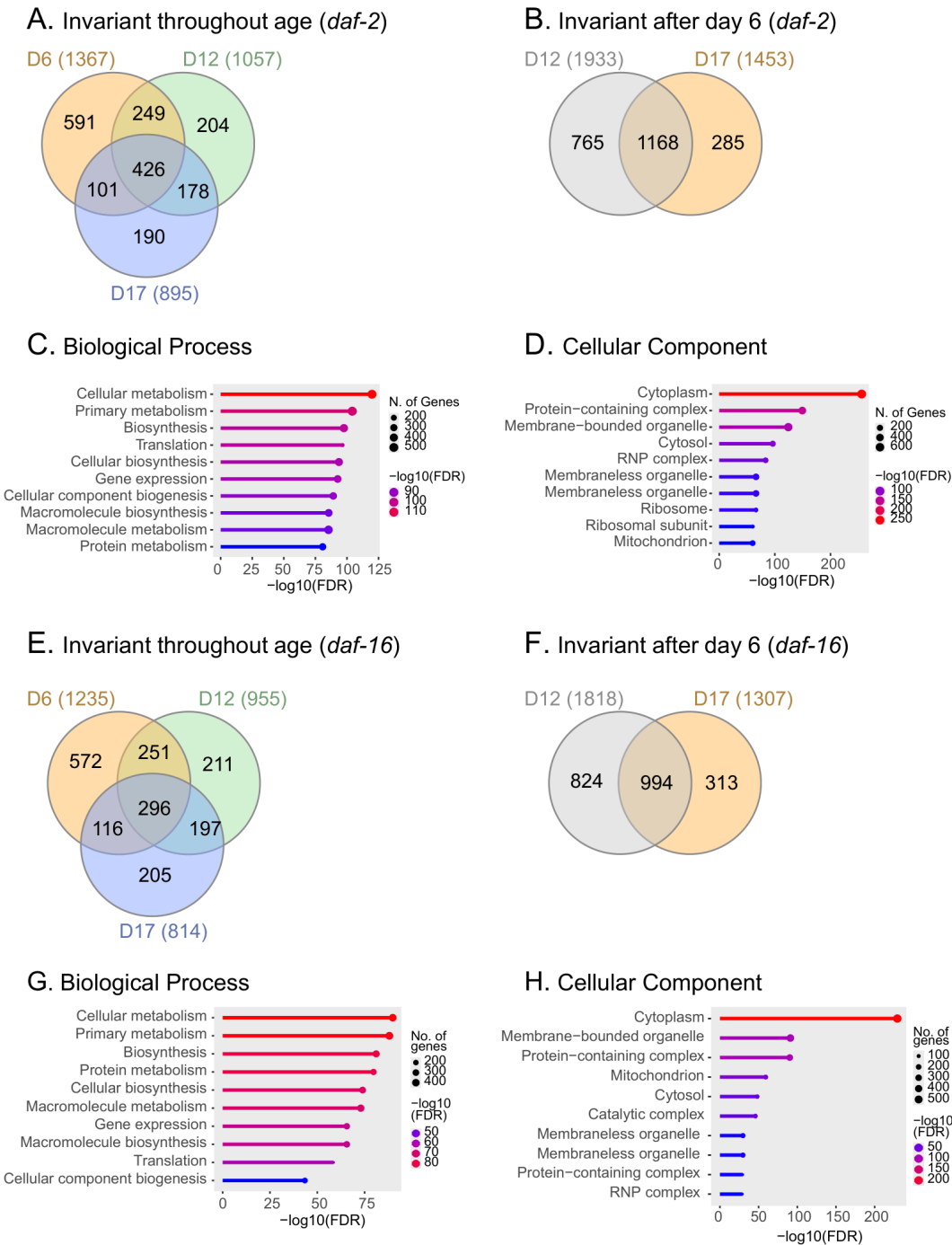
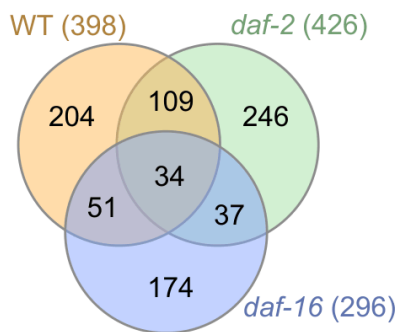


Figure 4

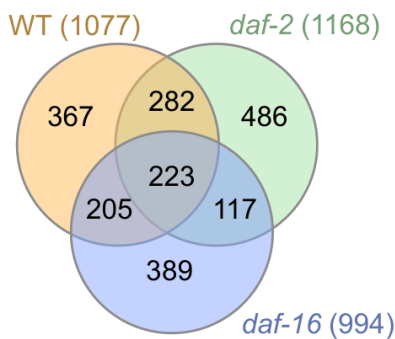
Age-invariant proteins in lifespan mutants

(A) Venn diagram showing proteins invariant throughout the lifespan in long-lived *daf-2* mutants at days 6, 12, and 17; 426 proteins were invariant at all three time points. (B) Proteins invariant after day 6 in *daf-2* mutants; 1,168 proteins were invariant at days 12 and 17 relative to day 6. (C–D) Gene ontology enrichment analysis of proteins invariant throughout the lifespan in *daf-2* mutants showing enriched biological processes and cellular components, respectively. (E) Venn diagram showing proteins invariant throughout the lifespan in short-lived *daf-16* mutants; 296 proteins were invariant at days 6, 12, and 17. (F) Proteins invariant after day 6 in *daf-16* mutants; 994 proteins were invariant at days 12 and 17 relative to day 6. (G–H) Gene ontology enrichment analysis of proteins invariant throughout the lifespan in *daf-16* mutants showing enriched biological processes and cellular components, respectively. Enrichment is represented as $-\log_{10}(\text{FDR})$, with point size indicating the number of genes.

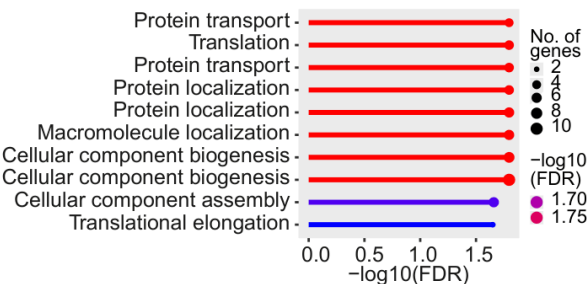
A. Invariant throughout age



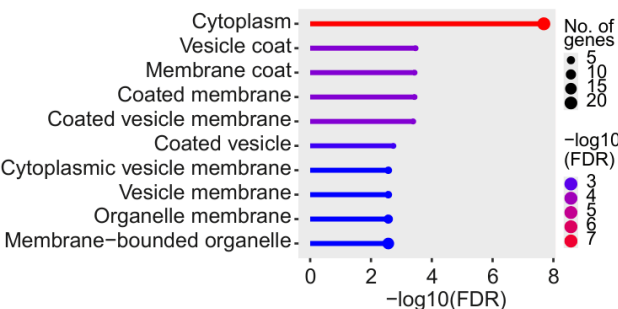
B. Invariant after day 6



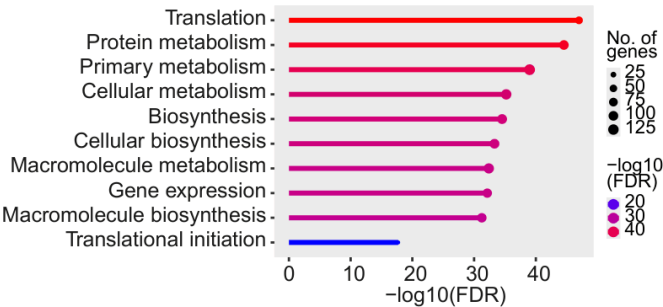
C. Biological Process



D. Cellular Component



E. Biological Process



F. Cellular Component

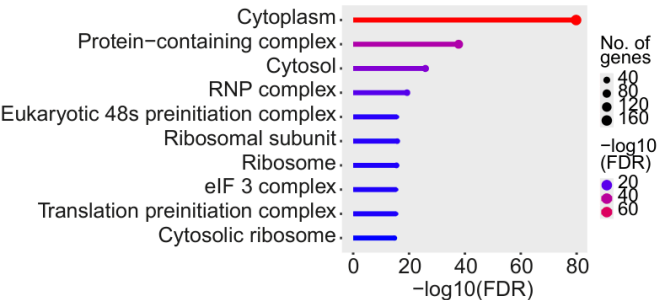


Figure 5

Common invariant proteins across strains

(A) Venn diagram showing proteins invariant throughout the lifespan in wild-type (WT), *daf-2*, and *daf-16* animals. Thirty-four proteins were common to all three strains. (B) Venn diagram showing proteins invariant after day 6 in WT, *daf-2*, and *daf-16* animals. A total of 223 proteins were common to all three

strains. (C) Gene ontology enrichment analysis of the 34 proteins invariant throughout the lifespan showing enriched biological processes. (D) Enriched cellular components associated with these proteins. (E) Gene ontology enrichment analysis of the 223 proteins invariant after day 6 showing enriched biological processes. (F) Enriched cellular components associated with these proteins.

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