Supplementary information

Royalactin induces copious longevity via increased translation and proteasome activity in *C. elegans*

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Supplementary Methods

Differential proteomics: 2D-DIGE and mass spectrometric analysis

When they were ready for sampling, animals were rinsed from the plates with an isotonic 0.1 M NaCl solution, transferred to a 15 mL tube and pelleted through centrifugation. After washing the pellet twice in 0.1 M NaCl, a sucrose density centrifugation step in 30% w/v sucrose, was performed to separate the living worms from dead animals and bacteria. The live animals were washed trice in 0.1 M NaCl and transferred to 1.5 mL Eppendorf Protein LoBind tubes. A mechanical drill equipped with a Teflon conical pestle was used to homogenize the worms in cold 2D-DIGE lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 1% w/v DTT, 40 mM Tris, supplemented with Roche cOmplete protease inhibitor cocktail tablets) on ice. After centrifugation during 12 min at 16 000 g (4°C) the supernatant was dialyzed overnight (PlusOne Mini-dialysis kit, GE Healthcare, 1 kDa MWCO) at 4°C against MQ. Dialysis removes DTT, among others, which otherwise can negatively affect the CyDye labeling reaction. Protein concentrations were determined using a Qubit protein assay kit (Qubit 2.0 Fluorimeter, Invitrogen), pH was measured and samples were kept at -80°C until further use.

A total of 18 protein extracts of royalactin-treated and non-treated animals (9 per condition) were labelled with CyDye DIGE Fluor minimal dyes and processed with GE Healthcare reagents and equipment. A dye swap was performed to take the possibility of dye bias into account (i.e. a potential difference in fluorescence intensity due to differences in binding affinity between Cy3 and Cy5 for certain proteins). An internal standard, comprised of pooled royalactin and control samples, was labeled with Cy2. Each labeling reaction was performed in parallel for 30 minutes on ice (440 pmol of CyDye per 75 µg of protein), and quenched by adding 10 mM lysine.

CyDye-labeled samples were mixed and actively loaded via cups on non-linear immobilized pH gradient (IPG) strips (pH 3-10, 24 cm) which had been rehydrated overnight. Applied on each strip: 75 µg of the pooled internal standard labeled with Cy2, 75 µg of sample_n labeled with Cy3, 75 µg of sample_{n+1} labeled with Cy5, and 50 µL of 2D-DIGE lysis buffer. Isoelectric focusing took place using the Ettan IPGphor II IEF System until a total of 52000 Vh was reached. Next, IPG strips were twice equilibrated for 15 min in equilibration buffer (30% glycerol, 2% SDS, 6 M urea, 50 mM Tris HCl, pH 8.8) containing subsequently 1% dithiothreitol (DTT) and 4% idoacetamide (IAA). Together with the latter, the tracking dye bromophenol blue was added. The strips were then placed on top of freshly prepared polyacrylamide gels (1.5 mm thick, 12.5%) and the proteins were separated on molecular weight

by SDS-PAGE. Nine analytical gels were ran simultaneously using a vertical Ettan DALTtwelve electrophoresis cassette, until the tracking dye reached the bottom of the gel (30 min at 600V, 30 mA, 10W; followed by 19 h at 600V, 150 mA, 10W; at 13°C). After stopping the second-dimension separation, the gels were scanned using the Ettan DIGE imager at 100 µm resolution with optimized exposure times for each gel and fluorescent channel (to obtain a max intensity in the range of 45000-55000 counts per channel, measured with the ImageQuant TL software, avoiding saturation).

Gel images were merged and analyzed using DeCyder 2D software (version 7.0), to detect protein spots with significantly different abundance between both groups. Settings for intra-gel spot detection in the Differential In-gel Analysis module: estimated number of spots = 10000 and spot exclusion filter = 30000. This excludes spots below 30000 counts (i.e. dust or artefacts) and resulted in ca. 1400 spots per gel, prior to manual verification and spot matching using the Biological Variation Analysis module (according to the manufacturer's instructions). In short, each spot and its boundary were manually checked and matched across gel images using landmarks and re-matching. We matched ca. 850 spots across all nine analytical gels and 27 gel images (i.e. 9 biological replicates per condition).

Based on the normalized spot intensities, protein abundance ratios between our two groups (i.e. royalactin-treated and untreated) are automatically calculated and compared using a standard Student's t-test. Decyder first normalizes spot intensities (also referred to as spot volumes) between the different gel images against the internal standard, to compensate for differences in system gain (e.g. different laser intensity, different sample amounts, ...)¹. Then, a $\log_{10}$ volume ratio is calculated for each spot, based on the normalized spot volumes². Finally, the $\log_{10}$ volume ratio is converted to the average ratio change (i.e. fold change), which is reported in the results (see ² for more details). The coordinates of spots showing a significant difference ($p < 0.05$) in abundance between royalactin-treated and untreated samples, were saved for later spot picking.

To obtain sufficient protein material to identify the significantly differential spots between groups, preparative SDS-PAGE gels were ran and the spots excised using an automated spot picker. Gels were prepared similarly as described above, with the exception that now Bind-Saline (GE Healthcare) was used to attach the gel to a glass plate. Two IPG strips were passively loaded during rehydration (1 h at 20°C and 19 h at 4°C), with respectively 400 µg and 700 µg of pooled protein sample. First dimension separation took place until a total of 70000 Vh was reached (ca. 24 hours). After the second-dimension separation (Ettan DALTsix cassette), gels were stained for 1 h with Deep Purple total protein stain (GE Healthcare, according to the manufacturer's instructions), scanned (Ettan DIGE imager) and stored in 7.5% acetic acid at 4°C

until spot picking. Decyder was used to match the preparative gel spot pattern to the analytical gel images (excluding spots with area < 2000 or volume < 30000).

Differential spots were excised (i.e. picked) as gel plugs from the preparative gels by an automated Ettan Spot Picker, into a polypropylene microplate well. The corresponding protein spots from both preparative gels were pooled into the same well. Each well was washed thrice with 150 μ L acetonitrile (ACN) during 10 min while shaking, before drying (miVac Quattro Concentrator, Genevac) and storage at -20°C.

Proteins within each gel plug were digested with trypsin, for identification via peptide mass fingerprinting. The masses of tryptic peptides (of an unknown protein) in a given sample are experimentally determined via mass spectrometry, and compared to the theoretical masses of tryptic fragments (of known proteins) in a database. Prior to digestion, gel plugs were thrice subsequently rehydrated (with 150 μ L 0.1 M NH_4HCO_3) and dehydrated (with 150 μ L ACN), while shaking during 15 min and removing the solution after each step. Gel plugs were mixed with 55 μ L of digestion buffer (50 mM NH_4HCO_3 , 50 mM CaCl_2) containing 167 ng of sequencing grade modified trypsin (Promega), kept at 4°C for 2 h 30 min (allowing trypsin to enter the gel plugs) and incubated at 37°C overnight. Next, the supernatant of each digestion well was transferred to a clean LoBind Eppendorf collection tube and 150 μ L of 50 mM NH_4HCO_3 was added per digestion well. After 30 min of gentle shaking at room temperature, the solution was pipetted into the corresponding collection tubes, and 150 μ L of extraction solution (50% ACN, 5% formic acid) was added to each digestion well. After 30 min, the supernatant was also transferred into the corresponding collection tube and the contents were dried in a centrifugal evaporator (Speedvac, Savant SVC100H). Tryptic peptides were subsequently concentrated and desalted using C18 ZipTip pipette tips (Millipore).

The masses of tryptic peptides were quantified using a Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific) at the SyBioMa facility (KU Leuven, Belgium). Samples were dissolved in 5% ACN, 0.1% formic acid and injected. Both MS and MS/MS peak lists were obtained. Proteins were identified by performing a MS/MS ion search using an in-house Mascot Server (version 2.2, Matrix Science). Both the manually curated Swiss-Prot and the more extensive NCBI databases were searched for *C. elegans* proteins. Settings: Peptide mass tolerance = $\pm$ 0.02 Da; MS/MS fragment mass tolerance = $\pm$ 0.02 Da; Fixed modifications = Carbaminomethyl (C); Variable modifications = Oxidation (M); Mass values = monoisotopic; Max mixed cleavages = 1; Instrument = ESI-FTICR. The peptide mass tolerance and/or MS/MS fragment mass tolerance was lowered to $\pm$ 0.01 Da in case no significant identification was obtained with said settings. Note that this merely influenced the final Mascot protein score for the initial top hit, and never resulted in identification of a different protein. Identifications were

considered significant at a Mascot E-value < 0.05 (i.e. Mascot protein score > 64 at the aforementioned standard search settings).

C. elegans lifespan assay details

To perform RNAi by feeding against *eef-2*, NGM_{RNAi} plates were seeded with the corresponding *E. coli* HT115(DE3) clone from the Ahringer RNAi feeding library³. Control plates were seeded with a HT115(DE3) clone containing the empty pL440 RNAi vector. RNAi plasmid sequences were verified via Sanger sequencing (LGC Genomics, Berlin). NGM_{RNAi} refers to NGM supplemented with 50 µg mL⁻¹ ampicillin and 50 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), the latter needed to induce dsRNA production. Wild-type (WT) animals were grown on control RNAi plates from the L1 stage (after hypochlorite treatment) until the onset of adulthood. Subsequently, RNAi was initiated by transferring the young adult animals to *eef-2* RNAi plates. RNAi effectivity was evaluated by in parallel subjecting other animals to *eef-2* RNAi from the L1 stage onwards, which resulted in a stark growth defect.

Lifespan of WT and PS1839 animals, both untreated and treated with cycloheximide (CHX), was monitored in parallel to lifespan of *eef-2* RNAi animals. At least 100 animals were used per condition (Supplementary Dataset 2), spread across 4 separate plates. NGM_{CHX} was prepared by adding 1 µM of sterile filtered (0.45 µm) CHX to liquid NGM_{RNAi} agar at ±50°C immediately prior to pouring the plates. An additional 1 µM of CHX was applied to the plates approximately 30 min before transferring the animals. Animals were treated with CHX starting from the late L4 stage, until the end of the experiment. CHX treatment of *C. elegans* is comparable to ⁴, although our treatment was started slightly earlier (in the late L4 stage versus the adult stage).

Supplementary Discussion and detailed results

Detailed breakdown of the differential proteomics data

We found 47 proteins to be differentially abundant in adult royalactin-fed compared to untreated animals, in all nine biological replicates. Out of 47 spots with differential abundances, 41 were identified via mass spectrometry. Ultimately, 35 unique proteins were identified, out of which 21 were upregulated and 14 were downregulated. The complete list of differentially

regulated proteins including their putative biological functions, fold changes and Gene Ontology enrichment analysis is presented in Supplementary Dataset 1.

Various proteins that are upregulated after royalactin treatment of wild-type (WT) *C. elegans*, are associated with protein translation, ribosomal function, cellular detoxification, muscle maintenance or possess a protein binding and folding (e.g. chaperone) function (Fig. 1, Supplementary Table 1, Appendix S1). We found upregulation of two *C. elegans* mRNA translation elongation factors (EEF-1G and EEF-2), the 40S ribosomal protein SA (RPS-0) and two chaperonins (CCT-6 and CCT-8) that assist folding of nascent proteins in the cytosol with the help of ATP⁵. Also upregulated was the catalytic β subunit of mitochondrial ATP synthase (ATP-2), which is required for ATP production⁶.

While the bulk of the changes we noted were different to those in most other lifespan extending condition, we did encounter some general similarities as well (Supplementary Table 1). Similar to earlier proteomic reports on dietary and genetic interventions that extend *C. elegans* lifespan, we observed upregulation of proteins involved in cellular detoxification and xenobiotic metabolism (i.e. ALH-1, C30G12.2, F20G2.2, GST-4; Supplementary Table 1; Table 1) and downregulation of yolk proteins (i.e. VIT-2, Supplementary Dataset 1) after royalactin treatment. Yolk protein abundance is consistently lowered in long-lived *C. elegans* adults⁷⁻¹¹ and this reduction might have a causal role in lifespan extension. Sustained yolk production in post-reproductive WT animals may lead to mortality due to excessive and uncontrolled intestinal accumulation – a process which can be inhibited by downregulating *vit* genes¹². Alternatively, inhibiting yolk production may lead to drastic changes in *C. elegans*' lipid metabolism, concomitant nuclear receptor signalling and activation of autophagy and lysosomal lipolysis – processes which can both modulate ageing¹³.

Likewise, upregulation of detoxifying glutathione S-transferase (GST) enzymes is a common feature of long-lived *C. elegans*^{8,10,11,14-19} and is linked to longevity not only in worms but across species²⁰. We observed robust upregulation of glutathione S-transferase 4 (GST-4) both at the protein (Supplementary Dataset 1) and transcriptional level²¹, the latter independent of the SKN-1/Nrf transcription factor which normally activates *gst-4* transcription during reduced insulin/insulin-like growth factor signalling, germline loss and DR (amongst others)^{22,23}.

Detailed exposition of stress assay results

Dithiotreitol (DTT) is a reducing agent that prevents disulfide bond formation, leading to protein aggregation in the cytosol and the endoplasmic reticulum (ER)^{24,25}. Mean survival was significantly improved both in day one (+13.1%, $p_{t-test} = 0.034$) and day three (+8.4%, $p_{t-test} = 0.026$) royalactin-treated adults subjected to 5 mM DTT for 24h (Fig. 5A, Supplementary Dataset 5). Indicative of lowered ER stress levels in royalactin-treated animals, we detected

lower expression of the *hsp-4p::gfp* transgene reporter both under basal and DTT-stressed (incubation in 5 mM DTT for 2 h) conditions (Fig. 5D). HSP-4 is the *C. elegans* homolog of BiP/GRP78, a major ER lumen chaperone whose expression increases under ER stress and is part of the ER unfolded protein response (UPR^{ER})^{26–28}. Attenuated *hsp-4* transcription is indicative of a lower ER stress burden (*i.e.* fewer HSP-4 chaperones are needed to maintain ER function).

Acute heat stress causes denaturation, aggregation and misfolding of proteins in multiple intracellular compartments²⁹. When heat-induced perturbations of proteostasis are not sufficiently countered, they lead to necrotic cells and organismal death^{30,31}. Exposure to 35°C is sufficient to kill an entire *C. elegans* population within hours, whereas the ability to withstand higher temperatures is tightly linked to the ability of cells to avoid and correct heat-induced protein damage^{31–33}.

Survival under acute heat stress was improved in young adult (+13.8% mean survival, $p_{\log\text{-rank}} = 5.32\text{E-}9$, >212 animals per condition) and day 6 adult (+15.1% mean survival, $p_{\log\text{-rank}} = 0.0035$, 108 animals per condition) worms grown in the presence of royalactin (Fig. 5B, Supplementary Dataset 5).

HSP-16.2 is a small heat-shock protein induced after heat and other stressors, acting as a molecular chaperone in the cytosol to avoid protein aggregation^{34–36}. Transcriptional levels of *hsp-16.2* are often inversely correlated with organismal lifespan and are indicative of cytosolic stress^{17,33,34,37–40}. In long-lived royalactin-treated animals, the induction of *hsp-16.2p::gfp* fluorescence due to heat-shock is attenuated under stress conditions (2h at 35°C; Fig. 5E). Taken together with enhanced thermotolerance, this reduction in *hsp-16.2* transcription suggests that royalactin treatment activates alternative defensive mechanisms to cope with heat stress.

To further test enhanced resilience to proteostatic insults after royalactin treatment, we examined *C. elegans* expressing polyglutamine (polyQ) repeats. Proteins containing overly expanded polyQ regions cause a number of neurodegenerative disorders, including Huntington's disease, by aggregating and impairing essential cellular processes such as mitochondrial function and protein turnover^{41,42}. We used a transgenic strain expressing 40 glutamine repeats coupled to a fluorescent tag (*Q40::YFP*) in the body wall muscle cells⁴³. As the worms age, polyQ inclusions form that greatly inhibit the animals motility and general health⁴³. The fluorescent reporter strain can be used as a 'proteostasis' or 'protein-folding' sensor: animals with more robust proteostasis generally display attenuated polyQ aggregation (and *vice versa*)^{44,45}. We observed a slight decrease in polyQ aggregates abundance from the L4 stage onwards in royalactin-treated animals (Fig. 5G), with the strongest effect around the late L4 stage (-22.3%, $p_{t\text{-test}} = 0.0061$). The difference with untreated control animals narrows at later time points and loses statistical significance. However, a slight delay in the onset of polyQ aggregation is

observed. This is in agreement with the upregulation of CCT chaperonins [Supplementary Fig. 1, Supplementary Dataset 1; shown to reduce polyQ aggregation both in *C. elegans* and yeast⁴⁶], enhanced clearance of damaged proteins via the proteasome (Fig. 3) and enhanced survival after DTT stress (another form of proteotoxic challenge – Fig. 5A) in royalactin-treated animals.

Paraquat is an intracellular superoxide-radical-generating compound which causes extensive mitochondrial oxidative damage and, at relatively high concentrations, mortality in *C. elegans*^{47–49}. When exposed to 8 mM paraquat, *skn-1(-)*, *eor-1(-)* and *gst-4(-)* mutants displayed significantly lowered survival compared to the WT control (Fig. 5C): -12.6% ($p_{\log\text{-rank}} = 0.0022$) for *skn-1(-)*; -18.8% ($p_{\log\text{-rank}} = 6.64\text{E-}5$) for *eor-1(-)*; -11.6% ($p_{\log\text{-rank}} = 6.35\text{E-}4$) for *gst-4(-)*; as expected from these genes' roles' in oxidative stress resistance. In the presence of royalactin, survival on paraquat was slightly but significantly increased in WT (+7.7%, $p_{\log\text{-rank}} = 0.048$) and *skn-1(-)* animals (+12.4%; $p_{\log\text{-rank}} = 0.0117$), while the effect was diminished and remained insignificant when knocking out *eor-1* (+1.8% survival; $p_{\log\text{-rank}} = 0.718$) or *gst-4* (+4.2%; $p_{\log\text{-rank}} = 0.215$) (Fig. 6C). Although the effect of royalactin on WT survival during paraquat stress was rather limited, this observation is in line with a dependency on EOR-1, but not on SKN-1, for enhanced resistance to H₂O₂-induced oxidative stress after royalactin treatment [as described in ²¹]. Expression of *hsp-6p::gfp*, a mitochondrial oxidative stress sensor^{28,50}, was decreased by 25.7% after royalactin-feeding compared to untreated animals ($p_{t\text{-test}} = 0.0002$, Fig. 5F). The positive control condition (1h incubation in 20 mM H₂O₂) showed an expected increase in mean fluorescence intensity (+35.9%, $p_{t\text{-test}} = 0.0056$).

Generally, expression of heat-shock chaperones is induced to counter unwanted proteomic changes (*e.g.* unfolded or misfolded proteins) resulting from oxidation, heat and other stressors²⁸. The overall downregulation of heat-shock promoter-driven GFP fluorescence in royalactin-treated animals (Fig. 5D-F) is indicative of reduced cellular stress, and suggests that during royalactin feeding other mechanisms counter unwanted proteomic changes. This is in line with enhanced resilience to various acute stressors in these animals, boosted UPS-mediated protein degradation (Fig. 3) and the upregulation of cellular and xenobiotic detoxification enzymes (*i.e.* ALH-1, C07D8.6, C30G12.2, F20G2.2, GST-4; Supplementary Table 1).

Supplementary Figures and Tables

Supplementary Fig. 1

Annotated protein-protein interaction network of differential proteins. Line color indicates the type of interaction evidence. Associations between the nodes in the network are intended to be specific and meaningful, i.e. proteins jointly contribute to a shared biological function, although this does not necessarily mean they are physically binding each other⁵¹. Visualized with STRING 10.0 and manually annotated based GO and KEGG pathway analysis.

Supplementary Fig. 2

***C. elegans* body size is significantly increased after royalactin treatment in day 2 adults.**
Error bars indicate SEM; ** $p_{t-test} < 0.01$; * $p_{t-test} < 0.05$. For clarity, the y-axis does not start at zero.

Supplementary Fig. 3

Individual fluorescence channel intensity readouts in the *in vivo* proteasome assay, illustrating a decline in absolute GFP levels from 65 hours onwards. Corresponds to the data shown in Fig. 3B. Logarithmic y-axis. Error bars indicate SEM. Used strain: PP608.

Supplementary Table 1

Royalactin			Other lifespan-extending interventions					
Protein name	Protein ID	Abundance change	Tyrosol	Reduced IIS	Dietary restriction	Tasco	Metformin	Germline depletion
Glutathione S-transferase 4	GST-4	↑						
Triosephosphate isomerase	TPI-1	↑		a, b				
F20G2.2 dehydrogenase	F20G2.2	↑		a				
Aldehyde reductase	C07D8.6	↑		a, b				
40S ribosomal protein SA	RPS-0	↑		a				
C30G12.2 dehydrogenase	C30G12.2	↑		b				
Probable elongation factor 1-gamma	EEF-1G	↑		b				
T-complex protein 1 theta	CCT-8	↑						
Isocitrate/isopropylmalate dehydrogenase	IDHG-1	↑		a				
Elongation factor 2	EEF-2	↑		a, b				
Isocitrate dehydrogenase	IDH-1	↑						
Aldehyde dehydrogenase 2	ALH-1	↑		a				
T-complex protein 1 zeta	CCT-6	↑						
Phosphoenolpyruvate carboxykinase	PCK-1	↑		b				
Intermediate filament protein IFB-2	IFB-2	↑		a				
ATP synthase subunit beta	ATP-2	↑						
Cofactor-independent phosphoglycerate mutase	IPGM-1	↑						
Succinyl-CoA ligase subunit beta	SUCA-1	↑						
Probable enoyl-CoA hydratase	ECH-6	↓						
Glutamate dehydrogenase	GDH-1	↓		a				
Heat shock 70 kDa protein A	HSP-1	↓		a				
Dihydrolipoyllysine-residue acetyltransferase component	DLAT-1	↓		a				
Protein kinase C substrate ortholog	ZK1307.8	↓						
Vitellogenin 2	VIT-2	↓		b				
V-type proton ATPase catalytic subunit A	VHA-13	↓						
Glucose-6-phosphate 1-dehydrogenase	GSPD-1	↓		a				
Methylmalonate-semialdehyde dehydr.	ALH-8	↓		a				
Abundance changes in the same direction as royalactin			75% (3/4)	53% (9/17)	43% (3/7)	67% (2/3)	45% (5/11)	50% (2/4)

Comparison of proteome changes induced by royalactin and other lifespan-extending interventions in *C. elegans*. Both gel-based^{10,11,33} and gel-free^{7,8,16,18} proteomic studies were

compared. Similar and opposite changes in protein abundance are marked in green and red, respectively. Grey means that no protein abundance data is available in the published dataset (e.g. not measured or not given by the authors). Proteomic changes due to tyrosol (250 μ M)¹¹, metformin (25 mM)¹⁰, Tasco (300 μ g mL⁻¹)³³, DR (bacterial dilution)⁸ and the mutants *glp-1(e2141)* (i.e. germline-depletion)¹⁶ and *daf-2(e1370)* (i.e. rIIS)^{7,8} were compared to changes due to royalactin (1.5 μ g mL⁻¹). For rIIS, the results of ^aDepuydt *et al.* (2013) and ^bDong *et al.* (2007) were combined.

Legends for Supplementary Datasets 1 to 5

Supplementary Dataset 1 (separate .xlsx file) legend

Differentially expressed proteins in royalactin-treated *C. elegans* at day three of adulthood.

(A) All proteins identified via mass spectrometry (MS) are listed, including identical identifications in separate spots. Fold change is the average ratio change between the two groups, calculated by DeCyder. Matched peaks represents the number of experimental masses (observed via MS) that could be matched to theoretical masses (of the identified protein). Coverage (%) is the proportion of amino acids that is covered in the identified protein by experimentally observed peptides. Sample ID corresponds to the physical sample location after spot picking. **(B)** Gene Ontology (GO) and KEGG pathway enrichment analysis, performed with STRING 10.0. Non-significant hits ($p_{FDR} > 0.05$) were omitted. **(C)** Phenotype, tissue and GO enrichment analysis via Wormbase.

Supplementary Dataset 2 (separate .xlsx file) legend

Detailed overview of mean and median royalactin-mediated lifespan extension of *C. elegans* under certain conditions.

This dataset lists all of our lifespan data (depicted in Fig. 1). “N” is the number of tested animals, “SEM” the standard error of the mean, ^{N.S.} $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ^a[Each percentual change of a Control condition indicates the change in MLS of that strain compared to WT Control. Each percentual change of an experimental condition (*i.e.* Royalactin, CHX or Royalactin + CHX) indicates the change in MLS of that treatment, compared to untreated (Control) animals of the same worm strain. Significant differences ($p < 0.05$) in survival of the latter are highlighted in yellow.]

Supplementary Dataset 3 (separate .xlsx file) legend

Extended polysome profiling data. (A)

For each biological replicate plotted on Fig. 2, the determined areas under the curve (of the corresponding A_{254 nm} polysome profile) and initial A_{260 nm} values (prior to loading the sample on sucrose gradients) are given. **(B)** Representative absorbance profiles: each data point corresponds to the absorbance in a single microtiter well before (right y-axis) and after (left y-axis) normalization. The black shapes indicate the estimated position of free ribosomes, and ribosomes bound to one or more mRNAs (*i.e.* monosomes and polysomes) in the sucrose gradient.

Supplementary Dataset 4 (separate .xlsx file) legend

Extended reproductive data. Data corresponding to Fig. 4. **(A)** The time interval between transfers is indicated, as well as the average calculated reproductive rate (amount of progeny day⁻¹) and the average amount of observed progeny. **(B)** The raw data and calculations to derive the reproductive rate are indicated for each individual animal. No significant differences between royalactin-fed and control animals were detected. See Material and Methods for more information.

Supplementary Dataset 5 (separate .xlsx file) legend

Extended stress assay data. **(A)** Survival of royalactin-fed and control animals subjected to reductive stress (5 mM DTT). **(B)** Survival of royalactin-fed and control animals subjected to heat stress (35°C). **(C)** Average amount of polyQ aggregates (*Q40::YFP*) per royalactin-fed and control animal. **(D)** Survival of royalactin-fed and control animals subjected to paraquat-induced oxidative stress (8 mM paraquat). Significant differences ($p < 0.05$) between royalactin-fed and control animals are highlighted in yellow. Significant differences ($p < 0.05$) between different *C. elegans* strains are highlighted in blue. Data corresponding to Fig. 5.

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