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Supplementary Information for

An antagonistic pleiotropic gene regulates the reproduction and longevity tradeoff

Dou Wu[1,2,3,4], Zi Wang[5,6,7], Jingying Huang[1,2,3,4], Liang Huang[8], Songbo Zhang[1], Ruixue Zhao[1,2,3,4], Wei Li[8], Di Chen[5,6,7], and Guangshuo Ou[1,2,3,4,*].

*Guangshuo Ou
Email: guangshuoou@tsinghua.edu.cn

This PDF file includes:

Other supplementary materials for this manuscript include the following:

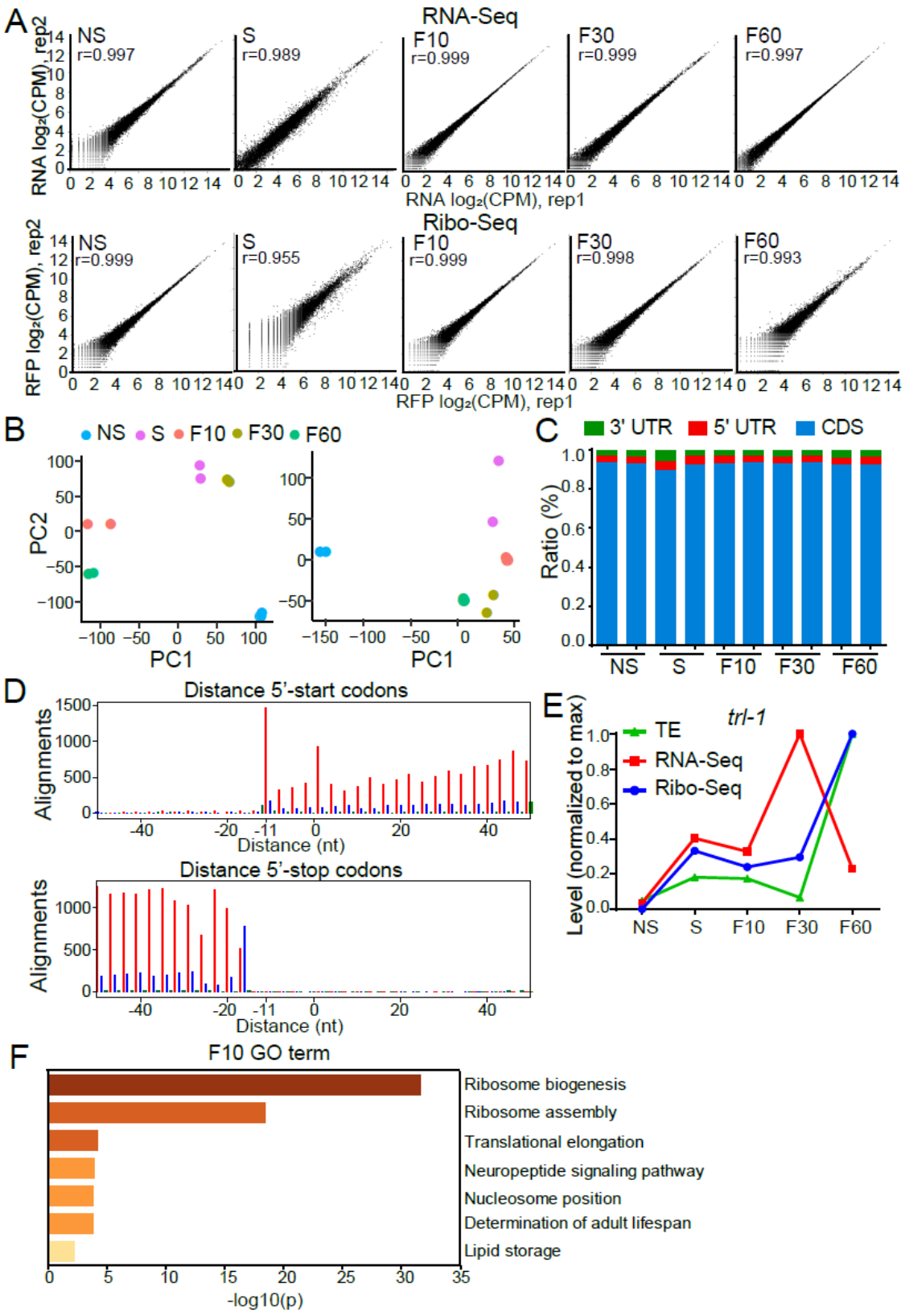

Fig. S1. The quality and reproducibility of RNA-Seq and Ribo-Seq data. (***A***) Correlation between two replicates (rep1/2) of RNA-Seq or Ribo-Seq samples. Data are shown as $\log_2$(CPM) correlation in Ribo-Seq or RNA-Seq for expressed genes with CPM >1. Pearson correlation coefficient r is shown. (***B***) Principal Component Analysis (PCA) in the first two

principal component spaces for RNA-Seq or for Ribo-Seq data over all the time points. (***C***) Read density along 5′ UTR, CDS, and 3′ UTR of total reads from Ribo-Seq. (***D***) Example of reads distribution around the start and stop codons by the RiboCode analysis. (***E***) Transcriptional level (RNA-Seq), translational level (Ribo-Seq), and transcriptional efficiency (TE) are plotted in the *trl-1* locus at different time points. (***F***) Gene enrichment analysis for the upregulated genes in refed 10 min animals compared with starvation conditions.

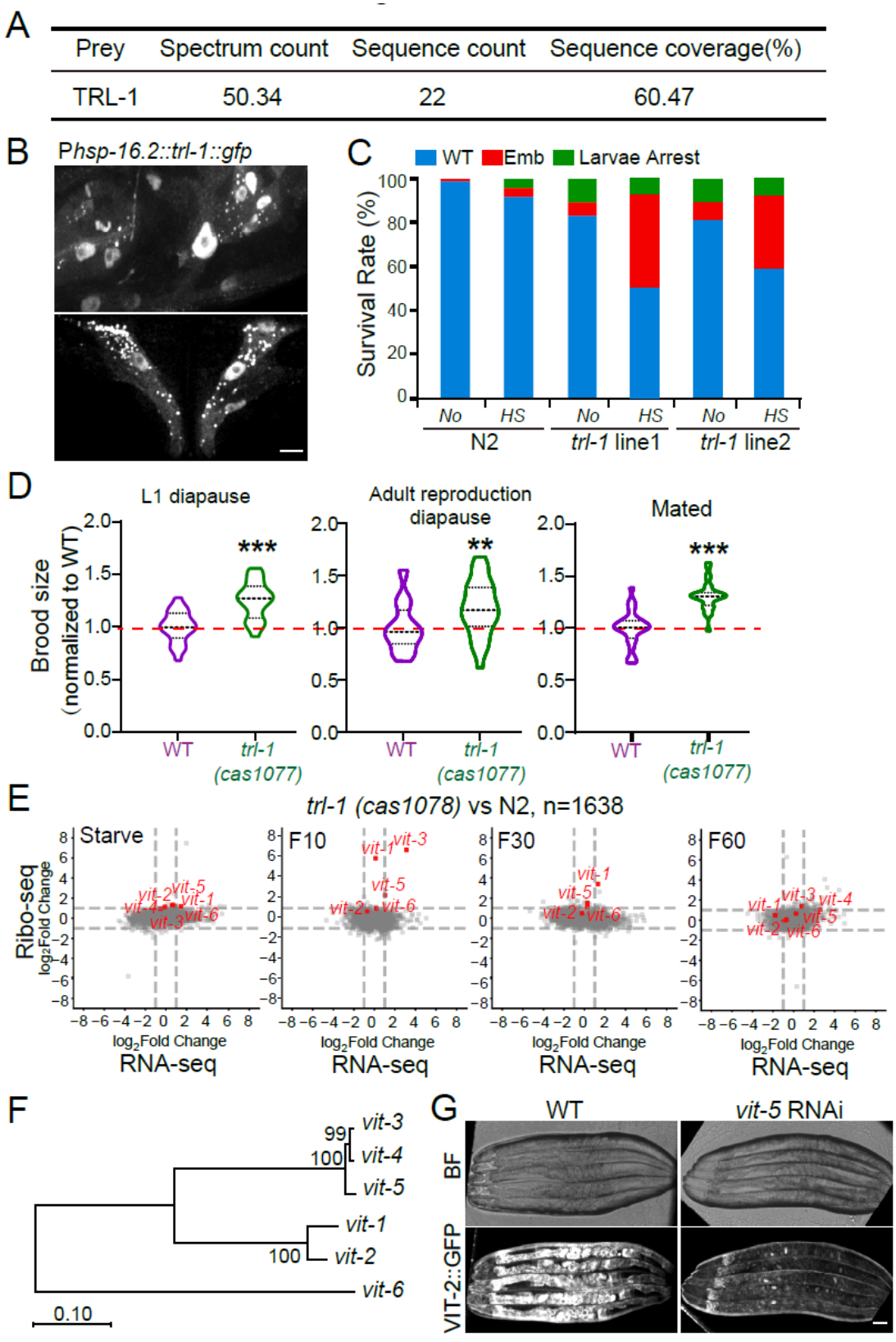

Fig. S2. *trl-1* translates upon refeeding and modulates vitellogenin translation. (***A***) Mass spectrometric results from the starved P*trl-1::trl-1::gfp* animals refed for 60 min. (***B***) Representative images of subcellular localization of TRL-1 under a heat-shock promoter. Scale bar, 5 μm. (***C***) Quantification of embryo survival rates in N2 and heat-shock induced

overexpression of *trl-1* worms. N = 80 – 100 from three generations. (***D***) Left and middle: normalized brood sizes of WT (N2) and *trl-1(cas1077)* animals in L1 diapause and adult reproduction diapause (mean ± SD). Right: normalized brood sizes of WT (N2) and *trl-1(cas1077)* animals mated with well-fed males (mean ± SD). N ⩾ 25, statistical significances were calculated by the Student's t-test, * P < 0.05, ** P < 0.01, and *** P < 0.001. For each worm, the brood size was normalized to the mean value of matched WT controls. (***E***) Scatter plots of the gene expression fold changes of RNA-Seq and Ribo-Seq in *trl-1(cas1078)* worms under starved (S), refed 10 min (F10), 30 min (F30), and 60 min (F60) conditions. Red dots correspond to vitellogenin genes. Gray dots show differentially expressed genes in the two independent *trl-1* deletion alleles, n=1638. (***F***) Schematic representation of the *C. elegans* vitellogenin gene family according to the number of amino acid substituents at each site. The topology and branch length of the tree is generated by the adjacent linking algorithm (Poisson modified distance method, pair deletion selection of gap sites). Numbers at the divergence points represent the percent of the leading confidence level. Scale bar, 0.1. (***G***) Brightfield (BF) and fluorescence images of VIT-2::GFP translational fusion worms under the control or *vit-5* RNAi condition. Scale bar, 5 μm.

A

Genotype	Life span (days)		Changes in mean life span[c]	n[d]	*p*[e]
	Mean[a]	Max[b]			
WT[f]	20.13,18.57	29,29	/	115,61	/
trl-1 (cas1077)[f]	16.07,14.66	25,23	-20%,-21%,	92,71	< 0.0001,< 0.0001
trl-1 (cas1078)[f]	16.09,15.03	27,25	-20%,-19%,	94,67	< 0.0001,< 0.0001
WT[g]	19.46,16.74	29,27	/	114,47	/
trl-1 (cas1077)[g]	15.65,14.71	25,25	-20%,-12%	111,70	< 0.0001,0.0101
trl-1 (cas1078)[g]	16.07,14.32	23,25	-17%,-14%	105,62	< 0.0001,0.0067
WT[h]	18.63,18.59,18.48	29,29,27	/	108,111,107	/
trl-1 (cas1077)[h]	15.50,15.31,15.22	27,27,25	-17%,-18%,-18%	109,103,102	< 0.0001,< 0.0001,< 0.0001
WT[i]	18.66,18.20,18.68	29,27,29	/	105,113,117	/
trl-1 (cas1077)[i]	15.70,15.42,15.97	27,25,25	-16%,-15%,-15%	123,113,115	< 0.0001,< 0.0001,< 0.0001
daf-2(e1370)	43.32,44.73,43.95	69,69,69	/	50,95,110	/
daf-2(e1370); trl-1(cas1077)	40.40,44.73,43.85	67,67,71	-7%,0%,0%	100,89,89	0.2069,0.7944,0.9383
eat-2(ad465)	26.93,28.42,25.86	45,49,43	/	89,90,112	/
eat-2(ad465); trl-1(cas1077)	25.45,28.73,26.96	47,47,41	-5%,1%,4%	85,81,102	0.4373,0.7090,0.2483
rsks-1(ok1255)	23.53,21.32,20.91	39,37,35	/	76,63,70	/
rsks-1(ok1255); trl-1(cas1077)	21.09,20.55,20.87	37,39,35	-10%,-4%,0%	67,62,61	0.0558,0.6991,0.9212
glp-1(e2144)	31.76,28.08,32.53	47,53,51	/	95,83,132	/
glp-1(e2144); trl-1(cas1077)	25.09,22.71,24.47	37,37,41	-21%,-19%,-25%	90,104,120	< 0.0001,< 0.0001,< 0.0001
control RNAi[j]	20.24,19.70,19.33	29,27,29	/	71,74,90	/
trl-1 RNAi[j]	17.20,16.67,16.83	25,25,27	-15%,-15%,-13%	82,72,92	< 0.0001,< 0.0001,< 0.0001
non-tg control[k]	16.89,16.67,16.67	25,27,27	/	76,85,85	/
trl-1 tg[k]	17.60,17.85,17.41	27,27,29	4%,7%,4%	73,73,79	0.2172,0.0588,0.2284
non-tg control[m]	16.81,16.85,16.18	27,25,25	/	73,67,73	/
trl-1 tg[m]	16.97,16.97,16.25	25,25,27	1%,1%,0%	70,61,72	0.7854,0.8478,0.7460
non-tg control HS[m]	17.88,17.84,17.23	29,29,31	/	57,55,53	/
trl-1 tg HS[m]	19.34,19.14,19.47	33,33,33	8%,7%,13%	53,57,60	0.0851,0.1328,0.0220

[a] average life span, [b] maximum life span, [c] percentage of mean life span changes compared to control animals (N2 or single mutants), [d] numbers of animals scored, [e] log-rank test, [f] regulalar conditions, [g] post-dauer condition, [h] L1 conditions, [i] L1 diapause conditions, [j] intestine-specific RNAi, strain: DCL907[*rde-1(mkc36)* V; kbIs7(P*nhx-2::rde-1*+rol-6)], [k] intestine-specific overexpression, strain: GOU4378[*casEX6133*(P*ges-1::trl-1*+rol-6)], [m] heat shock promoter induced overexpression, strain: GOU4738[*casEX6132*(P*hsp-16.2::trl-1*+rol-6)], non-tg control:non-roller siblings.

Fig. S3. *trl-1* deletions shorten lifespan and impair germline deficiency-induced longevity. (***A***) The lifespan of *trl-1* mutant worms compared with the WT (N2) under the post-dauer, regular adult stages, L1 stages, and L1 diapause conditions, the lifespan of *trl-1* double mutants compared with the corresponding single mutants [*rsks-1(ok1255)*, *daf-2(e1370)*, *eat-2(ad465),* and *glp-1(e2144)*] under ample food conditions, lifespan of intestine-specific RNAi and overexpression and lifespan of heat-shock induced overexpression of TRL-1 at the L4 larval stage compared with non-tg control (non-roller siblings). Post-dauer and L1 diapause stage

worms were refed with food, and adulthood day 1 is as t = 1 for lifespan analyses. The mean and max lifespan and the number of nematodes counted under both conditions are shown. Mean lifespan is generated by nonparametric methods. P values were calculated using the Log-rank (Mantel-Cox) method.

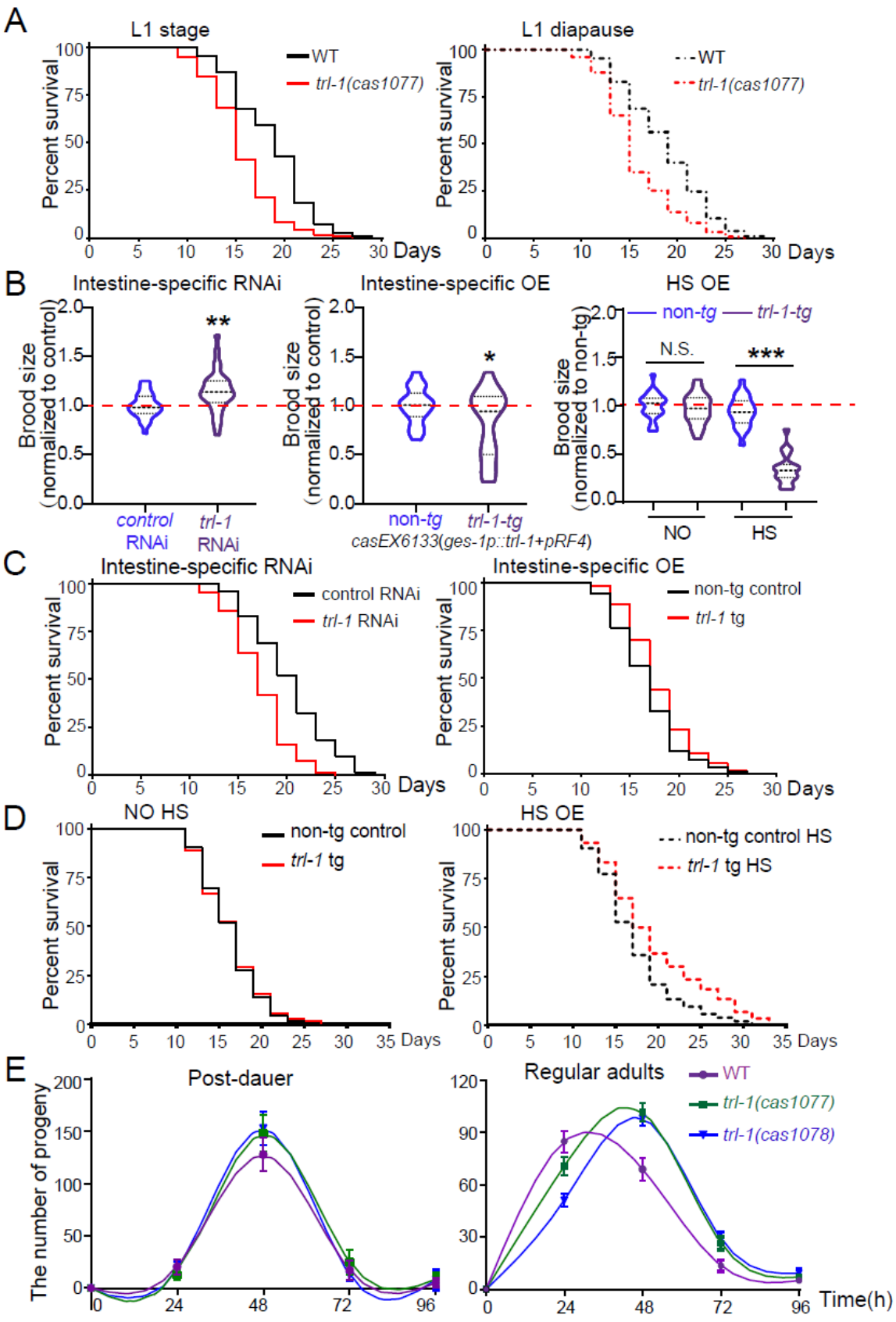

Fig. S4. *trl-1* functions in the intestine to regulate longevity. (***A***) The lifespan of *trl-1* mutant worms compared with the WT (N2) under the L1 stage (left) and the L1 diapause stage (right). L1 diapause worms were refed with food, and the adulthood day 1 is as t = 1 in lifespan analyses.

Survival plots are generated by nonparametric methods. The results are consistent in three replicates. (***B***) Left and middle: normalized brood size of intestine-specific RNAi and overexpression (mean ± SD). The strains used for analysis are DCL907[*rde-1(mkc36*) V; kbIs7(P*nhx-2::rde-1+rol-6*)] and GOU4378[*casEX6133*(P*ges-1::trl-1+rol-6*)], separately. Right: normalized brood size of heat-shock induced overexpression of TRL-1 at the L4 larval stage (mean ± SD). The strain used for analysis is GOU4738[*casEX6132*(P*hsp-16.2::trl-1+rol-6*)]. N ⩾ 25, statistical significances were calculated by the Student's t-test, * P < 0.05, ** P < 0.01, and *** P < 0.001. For each worm, the brood size was normalized to the mean value of matched WT controls. Non-tg: non-roller siblings, *trl-1* tg: roller siblings. (***C***) Lifespan of intestine-specific RNAi (left) and overexpression (right). Survival plots are generated by nonparametric methods. The results are consistent in three replicates. Non-tg: non-roller siblings, *trl-1* tg: roller siblings. (***D***) Lifespan of heat-shock induced overexpression of TRL-1 at the L4 larval stage compared with non-tg control (non-roller siblings). Survival plots are generated by nonparametric methods. The results are consistent in three replicates. Non-tg: non-roller siblings, *trl-1* tg: roller siblings. (***E***) Time course analysis of egg-laying rate of WT(N2), *trl-1(cas1077)* and *trl-1(cas1078)* at the post-dauer(left) and regular adult stages(right) (mean ± SD). N ≥ 17 animals, post-dauer stage worms were refed with food, and adulthood day 1 is as t = 0 for the brood size analysis, and the number of progeny was measured at 24 h intervals.

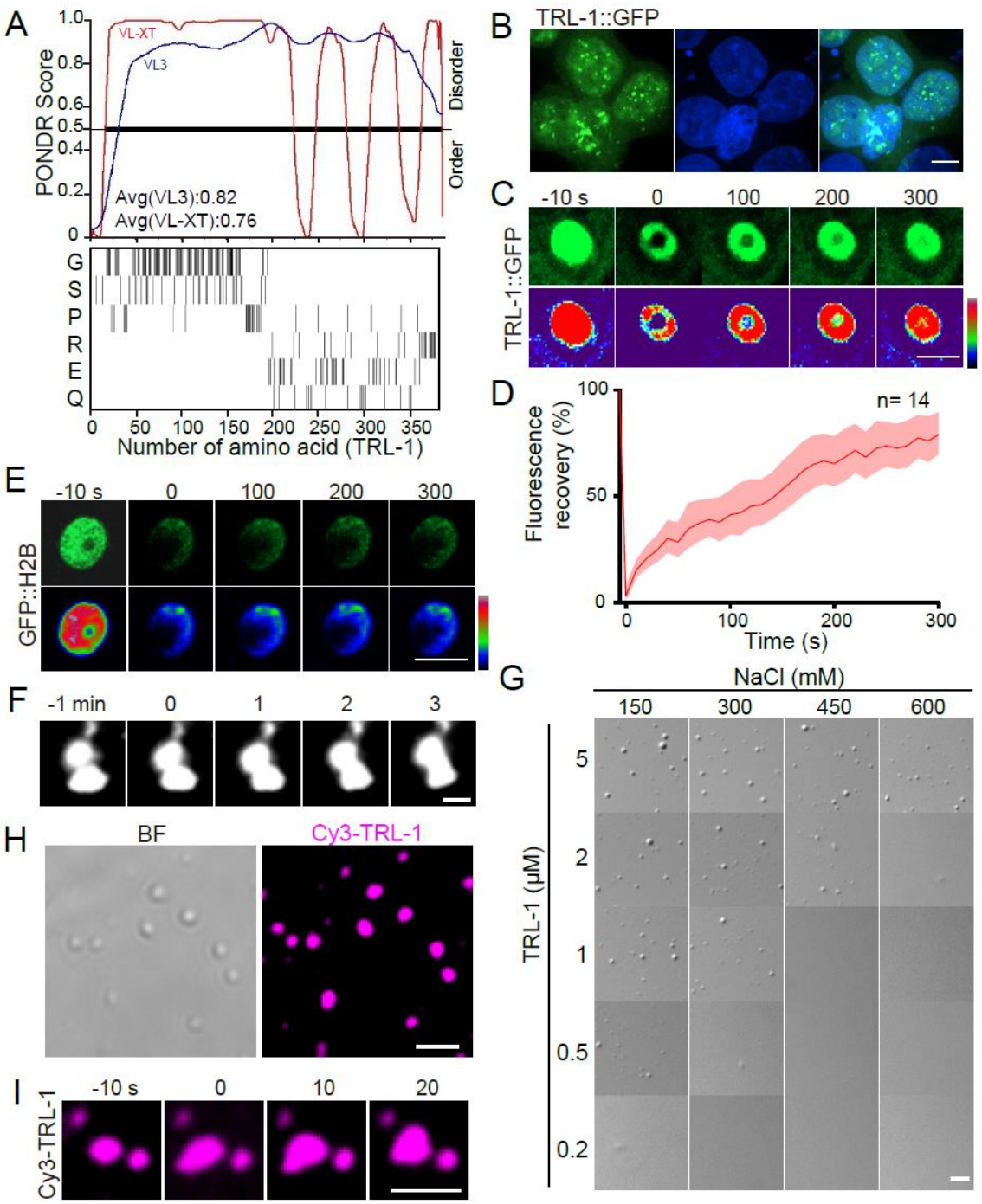

Fig. S5. TRL-1 undergoes liquid-liquid phase separation. (***A***) Prediction of the intrinsically disordered region (IDR) in TRL-1 using PONDR (top) and the distributions of representative amino acids (G: glycine; S: serine; P: proline; R: arginine; E: glutamic acid; Q: glutamine) (bottom). (***B***) Representative images of subcellular localization of TRL-1::GFP in HEK293T cells, green: TRL-1 proteins, blue: DAPI. Scale bar, 5 μm. (***C***) FRAP analysis of the recovery of the TRL-1::GFP fluorescent signal in HEK293T cells. Scale bar, 5 μm. (***D***) Quantitative analysis of FRAP data in HEK293T cells. Data are mean ± 95% confidence interval (CI) from independent animals, n =14 cells. (***E***) FRAP analysis of the recovery of the GFP::H2B fluorescent signal in *C. elegans*. Scale bar, 5 μm. (***F***) Droplet fusion of TRL-1::GFP puncta in HEK293T cells. Scale bar, 2 μm. (***G***) DIC images of the TRL-1 droplet formation in the buffers

containing different NaCl concentrations. Scale bar, 2 μm. (**H**) Brightfield (BF) and fluorescence images of TRL-1 droplets. Scale bar, 2 μm. (**I**) The Cy3-labeled TRL-1 droplets formed and grew larger within 30 seconds. Scale bar, 2 μm.

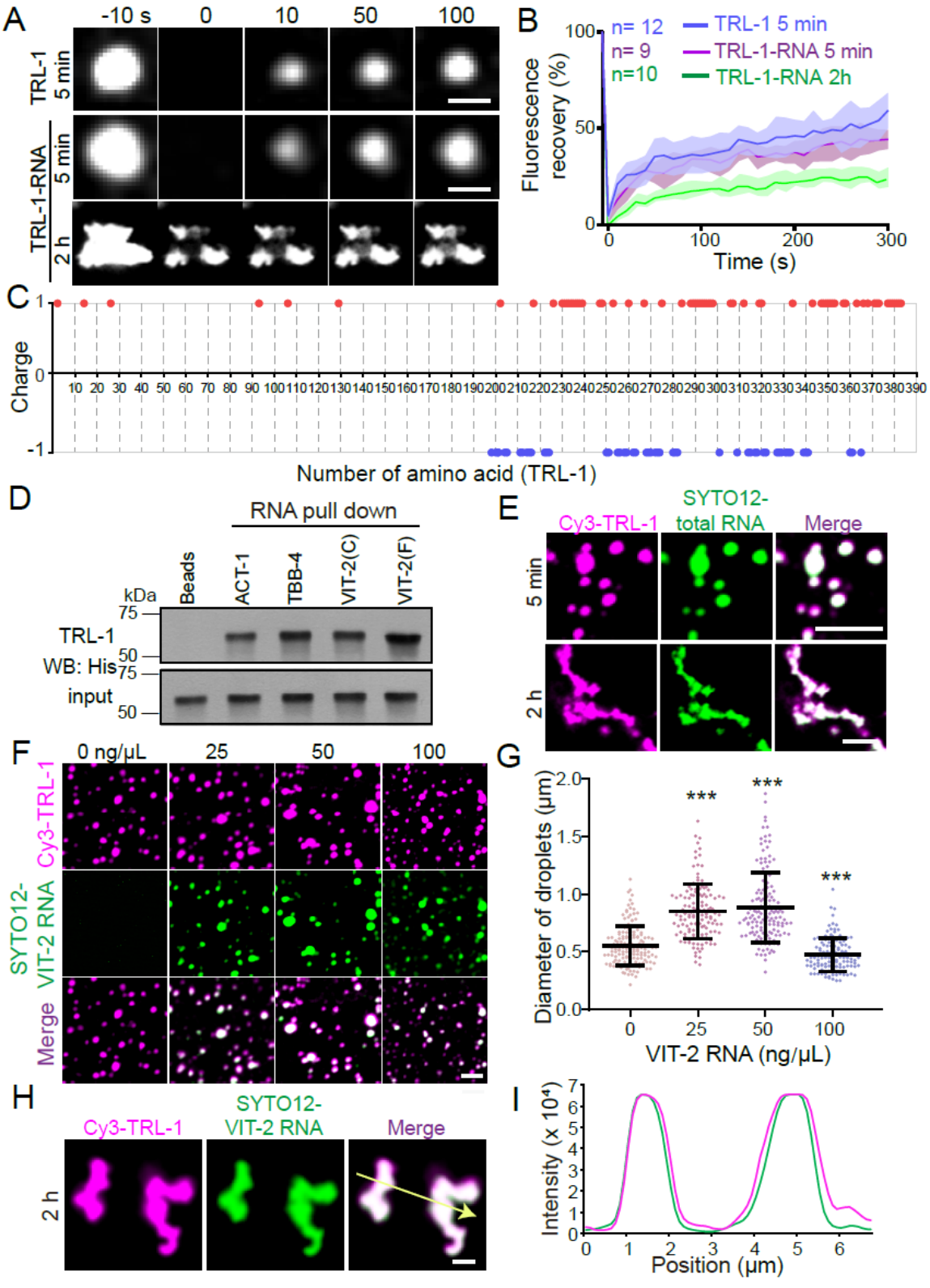

Fig. S6. TRL-1 protein interacts with vitellogenin RNA. (***A***) FRAP analysis of the recovery of the Cy3-TRL-1 fluorescent signal with or without RNA. Scale bar, 2 μm. (***B***) Quantitative analysis of FRAP data in TRL-1 droplets. Data are mean ± 95% confidence interval (CI) from independent animals, n = 9-12 droplets. (***C***) The charge of TRL-1 protein. Red dots represent positive charge, and blue dots represent negative charge. (***D***) Western blotting analysis of the

interactions between the fragmented *act-1* RNA, *tbb-4* RNA and the fragmented *vit-2* RNA molecules with the TRL-1 protein. (***E***) Representative images of the co-localization of TRL-1 proteins (magenta) and *C. elegans* total RNA (green) in droplets and the irregular shape of TRL-1-RNA droplets at two hours. Scale bar, 2 µm. (***F***) Representative images of TRL-1 droplets in the presence of 0-100 ng/µl of *vit-2* RNA. Scale bar, 2 µm. (***G***) Quantifications of TRL-1 droplets diameter (mean ± SD) in the presence of 0-100 ng/µl of *vit-2* RNA from five independent fields (n = 100-150 droplets). The Student's t-test calculated statistical significances, *** $P < 0.001$. (***H***) Representative images of the co-localization of TRL-1 proteins (magenta) and *vit-2* RNA (green) in the irregular shape of TRL-1-RNA droplets at two hours. Scale bar, 2 µm. (***I***) Quantification of TRL-1 and *vit-2* RNA fluorescence intensity along a line drawn from (H). Magenta line: Cy3-TRL-1, green line: *vit-2* RNA.

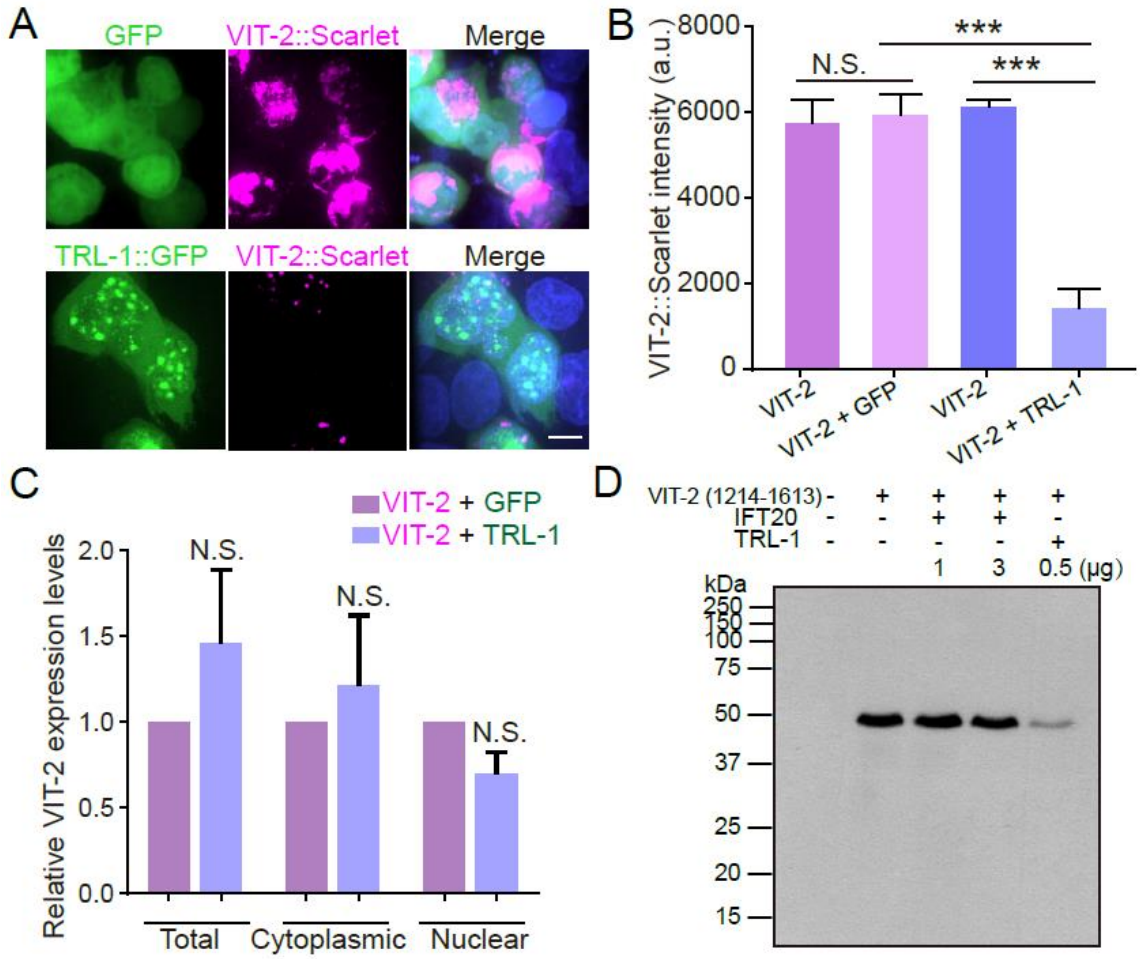

Fig. S7. TRL-1 inhibits vitellogenin RNA translation. (***A***) Representative images of VIT-2-Scalet fluorescence with or without TRL-1::GFP. Scale bar, 5 μm. (***B***) Quantifications of VIT-2-Scalet fluorescence intensity (mean ± SD) in the presence of TRL-1 or not (n = 100-150 cells). Statistical significances were calculated by the Student's t-test, *** P < 0.001, N.S., not significant. (***C***) qPCR quantification of the *vit-2* and the *gfp* (represent *gfp* and *trl-1::gfp*) transcripts (mean ± SD) in *trl-1::gfp* and *vit-2* co-transfected HEK293T cells or *gfp* and *vit-2* co-transfected cells. Statistical significances were calculated by the Student's t-test, N.S., not significant. (***D***) Western blot analysis of the VIT-2 translation levels in the presence of IFT20 or TRL-1.

Table S1. *C. elegans* Strains in this study.

Strain Name	Genotype	Method
N2	*wild-type*	N.A.
GOU4735	*trl-1(cas1077) V.*	Microinjection
GOU4736	*trl-1(cas1078) V.*	Microinjection
GOU4737	*CasEX6131*[P*hsp-16.2::trl-1::gfp*].	Microinjection
GOU4738	*CasEX6132*[P*hsp-16.2::trl-1::gfp*].	Microinjection
BCN9071	*crg9070[vit-2::gfp]) X.*	*Caenorhabditis Genetics Center*
GOU3826	*trl-1(cas1077) V; crg9070[vit-2::gfp]) X.*	Genetic cross
GOU3829	*trl-1(cas1078) V; crg9070[vit-2::gfp]) X.*	Genetic cross
GOU4739	*trl-1(cas1077) V; CasEX6131[Ptrl-1::trl-1::gfp].*	Genetic cross
GOU4740	*trl-1(cas1078) V; CasEX6131[Ptrl-1::trl-1::gfp].*	Genetic cross
GE68	*glp-1(e2144) III.*	*Caenorhabditis Genetics Center*
GOU4761	*trl-1(cas1077) V; glp-1(e2144) III.*	Genetic cross
DA465	*eat-2(ad465) II.*	*Caenorhabditis Genetics Center*
GOU4762	*trl-1(cas1077) V; eat-2(ad465) II.*	Genetic cross
CB1370	*daf-2(e1370) III.*	*Caenorhabditis Genetics Center*
GOU4763	*trl-1(cas1077) V; daf-2(e1370) III.*	Genetic cross
RB1206	*rsks-1(ok1255) III.*	*Caenorhabditis Genetics Center*
GOU4764	*trl-1(cas1077) V; rsks-1(ok1255) III.*	Genetic cross
DCL907	*rde-1(mkc36) V; kbIs7[Pnhx-2::rde-1+rol-6]*	N.A.
GOU4378	*casEX6133[Pges-1::trl-1+rol-6]*	Microinjection

Table S2. Plasmids and Primers in this study.

Plasmid Name	Forward Primer	Reverse Primer	Notes
pDD162-P*eft-3*::Cas9 + P*U6*::*trl-1 sg1*	CTAATCCACAGTGTCATTGCGTTTTAGAGCTAGAAATAGCA	ATGACATGGCTTCTTGCCCTCAAGACATCTCGCAATAGG	PCR from pDD162-P*eft-3::Cas9+PU6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6*::*trl-1 sg2*	TACTCAGAAGGGTCTTCGTCGTTTTAGAGCTAGAAATAGCA	GCAATGACACTGTGGATTAGCAAGACATCTCGCAATAGGAG	PCR from pDD162-P*eft-3::Cas9+PU6::Empty sgRNA*
pDONR-P*trl-1::trl-1*	GTACCGGTAGAAAAACATGAGTCGTTGTTTTATGT	GAAGAGTAATTGGACCCACCAGATGATCCTCCTCC	*trl-1* coding sequence was amplified from N2 genome and inserted into pDONR plasmid via In-Fusion Advantage PCR Cloning Kit.
pDONR-P*trl-1::trl-1::gfp*	GGAGGAGGATCATCTGGTGGGCTGGAAGTGGTAGC	AAGAGTAATTGGACCTATTTGTATAGTTCATCCA	*gfp* coding sequencing was amplified and cloned into pDONR-P*trl-1::trl-1* via In-Fusion Advantage PCR Cloning Kit.
pDONR-P*hsp-16.2::trl-1::gfp*	GTACCGGTAGAAAAACAGATCCAGTGAGTTCGTCC	GAAGACAGTCTTCATGATTATAGTTTGAAGATTTC	*hsp-16.2* promoter sequencing was amplified and cloned into pDONR-P*trl-1::trl-1::gfp* to modify promoter via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*trl-1* template for Knock-in	GTACCGGTAGAAAAACATGAGTCGTTGTTTTATG	GAAGAGTAATTGGACCTACCACCAGATGATCCTCC	*trl-1* genomic fragment was amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit
pPD95.77-*gfp::trl-1* template for GFP Knock-in	GAAGACTTTCTCACGATGAGTAAAGGAGAAGAACT	TAGAAGAAGACAGTCTTCATACCGCTACCACTTCC	*gfp* coding sequencing was amplified and cloned into pPD95.77-*trl-1* genomic template for GFP Knock-in via In-Fusion Advantage PCR Cloning Kit
pCMV::GFP::Strep II	CGCGGCCGCTCTAGAATGGTGAGCAAGGGCGAGGAGCTGT	CCAACCCATGGATCCCTTGTACAGCTCGTCCATGCC	*gfp* coding sequencing was amplified and cloned into pCMV::Strep II via In-Fusion Advantage PCR Cloning Kit.
pCMV::GFP::TRL-1::Strep II	GACGAGCTGTACAAGGCTGGAAGTGGTAGCGGTATGAAGA	CCAACCCATGGATCCACCGCTACCACTTCCAGCCC	*trl-1* coding sequence was amplified from N2 genome and inserted into pCMV::GFP::Strep II plasmid via In-Fusion Advantage PCR Cloning Kit.

pCMV::Scarlet::Strep II	CGCGGCCGCTCTAGAATGGTCAGCAAGGGAGAGGCAGTT	TCCAACCCATGGATCCCTTGTAGAGCTCGTCCATTCCT	*Scarlet* coding sequencing was amplified and cloned into PCMV::Strep II via In-Fusion Advantage PCR Cloning Kit.
pCMV::VIT-2::Scarlet::Strep II	CGCGGCCGCTCTAGAATGAGGTCGATCATCATCGCCT	ACCGCTACCACTTCCAGCATAAGCGACGCAGGCGGTTG	*vit-2* coding sequence was amplified from cDNA and inserted into pCMV::Scarlet::Strep II plasmid via In-Fusion Advantage PCR Cloning Kit.
pT7CFE::3x FLAG for *in vitro* translation	ACCCATATGGGATCCGACTACAAAGACCATGACGG	CAGCTTAATTAAGATCTTGTCGTCATCGTCTTTGT	*flag* coding sequencing was amplified and cloned into pT7CFE via In-Fusion Advantage PCR Cloning Kit.
pT7CFE::3x FLAG::VIT-2(1-265) for *in vitro* translation	GACGATGACGACAAGAGGTCGATCATCATCGCCTC	CAGCTTAATTAAGATTCCGTTGACGTTGACAGTGT	VIT-2(1-265) was amplified and cloned into pT7CFE::3x FLAG for *in vitro* translation via In-Fusion Advantage PCR Cloning Kit.
pT7CFE::3x FLAG::VIT-2(1214-1613) for *in vitro* translation	GACGATGACGACAAGGCTGAGTACAAGCTCACCCG	CAGCTTAATTAAGATATAAGCGACGCAGGCGGTTG	VIT-2(1214-1613) was amplified and cloned into pT7CFE::3x FLAG for *in vitro* translation via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled full length *vit-2* RNA transcription	TAATACGACTCACTATAGGACCATGAGGTCGATCATCATCGCCT	TCAAGCGTAGTCTGGGACGTCGTATGGGTAATAAGCGACGCAGGCGGTTGGGA	*vit-2* coding sequence was amplified from cDNA and inserted into pXT7-MCS plasmid via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *vit-2* fragment A transcription	TAATACGACTCACTATAGGA	TCCGTTGACGTTGACAGTGT	PCR *vit-2* fragment A from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *vit-2* fragment B transcription	TAATACGACTCACTATAGGAAGAAGTAATGAAGACTGAA	TGGGAGTGGTTGGCGCTTAT	PCR *vit-2* fragment B from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *vit-2*	TAATACGACTCACTATAGGGTCCGCAAGGAAGCCATTGA	AGAAGACGGCGGATTTCGCT	PCR *vit-2* fragment C from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.

fragment C transcription			
pXT7-MCS::VIT-2 for biotin labeled *vit-2* fragment D transcription	TAATACGACTCACTATAGGCAACCAAGAGCTCGAGTTCG	AGTTTCAAGGAGATGACGGA	PCR *vit-2* fragment D from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *vit-2* fragment E transcription	TAATACGACTCACTATAGGTGAGGCCCCACGTGACTACA	CAGTCTTCTTGGAGAGAACA	PCR *vit-2* fragment E from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *vit-2* fragment F transcription	TAATACGACTCACTATAGGAAAAGAATTCTGAAGAGATG	TCAAGCGTAGTCTGGGACGT	PCR *vit-2* fragment F from pXT7-MCS::VIT-2 for *in vitro* transcription via In-Fusion Advantage PCR Cloning Kit.
pET-Duet-6xhis::TRL-1	CACAAGCCAGGATCCATGAAGACTGTCTTCTTCTA	TGCGGCCGCAAGCTTCCACCAGATGATCCTCCTCC	*trl-1* coding sequence was amplified from cDNA and cloned into pET-Duet for protein purification via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::ACT-1 for biotin labeled *act-1* fragment transcription	ACTCACTATAGGACCATCCTTACCCTCAAGTACCC	GACGTCGTATGGGTAATGGGGCAAGAGCGGTGATT	*act-1* coding sequence was amplified from cDNA and inserted into pXT7-MCS plasmid via In-Fusion Advantage PCR Cloning Kit.
pXT7-MCS::VIT-2 for biotin labeled *tbb-4* fragment transcription	ACTCACTATAGGACCATGCGTGAAATTGTTCATA	GACGTCGTATGGGTATGACGTTGTTTGGAATCCAT	*tbb-4* coding sequence was amplified from cDNA and inserted into pXT7-MCS plasmid via In-Fusion Advantage PCR Cloning Kit.
pDONR-P*ges-1::trl-1*	GTACCGGTAGAAAAACGTGAGGCGTGTCTGATAAG	GAAGACAGTCTTCATCTGAATTCAAAGATAAGATATG	*ges-1* promoter sequencing was amplified and cloned into pDONR-P*trl-1::trl-1* to modify promoter via In-Fusion Advantage PCR Cloning Kit.

Movie S1 (separate file). Recovery of the TRL-1::GFP fluorescence in *C. elegans* after FRAP.

Movie S2 (separate file). Recovery of the TRL-1::GFP fluorescence in HEK293T after FRAP.

Movie S3 (separate file). Recovery of the GFP::H_2B fluorescence in *C. elegans* after FRAP.

Movie S4 (separate file). Droplet fusion of TRL-1::GFP puncta in *C. elegans*.

Movie S5 (separate file). Droplet fusion of TRL-1::GFP puncta in HEK293T cells.

Movie S6 (separate file). The formation and growth of Cy3-labeled TRL-1 droplets.

Movie S7 (separate file). Recovery of the Cy3-TRL-1 fluorescence without RNA after FRAP.

Movie S8 (separate file). Recovery of the Cy3-TRL-1 fluorescence with 50 ng/μl RNA after FRAP.

Movie S9 (separate file). Recovery of the Cy3-TRL-1 fluorescence incubated with 50 ng/μl RNA for 2h after FRAP.

Dataset S1 (separate file). Annotated ORFs and non-canonical ORFs annotated from Ribo-Seq by RiboCode.

Dataset S2 (separate file). Markedly altered novel ORFs upon refeeding.

Dataset S3 (separate file). Transcriptional and translational changes and translational efficiency.

Dataset S4 (separate file). Mass spectrum data.

Dataset S5 (separate file). Transcriptional and translational differentially expressed genes in *trl-1(cas1077)* and *trl-1(cas1078)* mutants compared with wide-type.

Dataset S6 (separate file). Candidates in the human genome.