An age-downregulated ribosomal RpS28 protein variant regulates the muscle proteome

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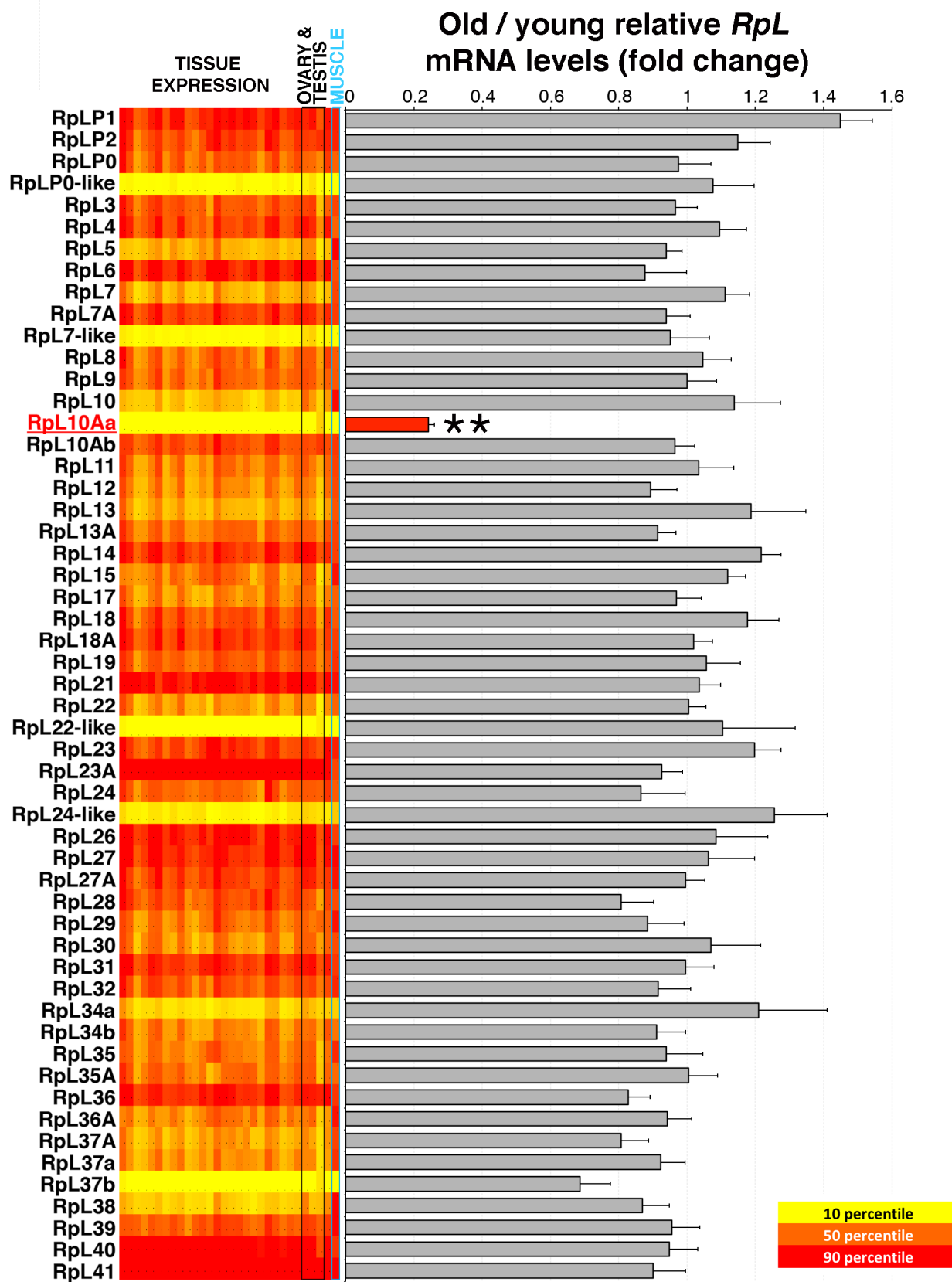**Figures S1-S5 and figure legends**

Legend of Tables S1-S2

TISSUE EXPRESSION

OVARY & TESTIS

MUSCLE

Old / young relative *RpL* mRNA levels (fold change)

0   0.2   0.4   0.6   0.8   1   1.2   1.4   1.6

RpLP1
RpLP2
RpLP0
RpLP0-like
RpL3
RpL4
RpL5
RpL6
RpL7
RpL7A
RpL7-like
RpL8
RpL9
RpL10
RpL10Aa ✶ ✶
RpL10Ab
RpL11
RpL12
RpL13
RpL13A
RpL14
RpL15
RpL17
RpL18
RpL18A
RpL19
RpL21
RpL22
RpL22-like
RpL23
RpL23A
RpL24
RpL24-like
RpL26
RpL27
RpL27A
RpL28
RpL29
RpL30
RpL31
RpL32
RpL34a
RpL34b
RpL35
RpL35A
RpL36
RpL36A
RpL37A
RpL37a
RpL37b
RpL38
RpL39
RpL40
RpL41

10 percentile
50 percentile
90 percentile

2

Figure S1. Age-associated changes in components of the large subunit of the ribosome ribosome. Old versus young fold change in *RpL* gene expression reveals a significant decrease in *RpL10Aa* gene expression (n=4; *p<0.05). ModEncode tissue expression profile is reported for a panel of *Drosophila* tissues and developmental stages, including gene expression in the germline (ovary and testis; black box), and RNAseq data for skeletal muscle (blue box). Low (yellow) to high (red) expression is indicated.

ModEncode expression data (left to right): L3 wandering larval imaginal discs; L3 larval CNS; P8 pupal CNS; virgin female head at 1, 4, and 20 days; mated female head at 1, 4, and 20 days of age; mated male head at 1, 4, and 20 days of age; L3 wandering larval salivary gland; white prepupa salivary gland; L3 wandering larval digestive system; digestive system at 1, 4, and 20 days of age; L3 wandering larval fat body; white prepupa fat body; P8 pupal fat body; L3 wandering larval carcass; carcass at 1, 4, and 20 days of age; virgin female ovary at 4 days of age; mated female ovary at 4 days of age; mated male testis at 4 days of age; mated male accessory gland at 4 days of age; RNAseq of skeletal muscle (1-week-old males).

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RpS28-like MPQPVASKARVIKILNRIGARGILTEVRVQLVDQPKMQFMRTVKGPVRLGDIVDFEDTELTWQSSSRSNSNFEDIC
    RpS28a MDKP-QYARVVEILGRTGSQGQCTQVRVEFLGDQSRQIIRNVKGPVRVGDILSLLETEREEARRLR----------
    RpS28b MDKPVVWARVMKVLGRTGSQGQCTQVKVEFLGEQNRQIIRNVKGPVREGDILTLLESEREEARRLR----------
           * :*     ***:::*.* *::*  *:*:*:::.: . *::*.****** ***: : ::*    :
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Figure S2. Alignment of the amino acid sequences of *Drosophila* RpS28-like, RpS28a, and RpS28b reveals extensive sequence similarity, in particular between RpS28a and RpS28b (*amino acid identity, :amino acid similarity).
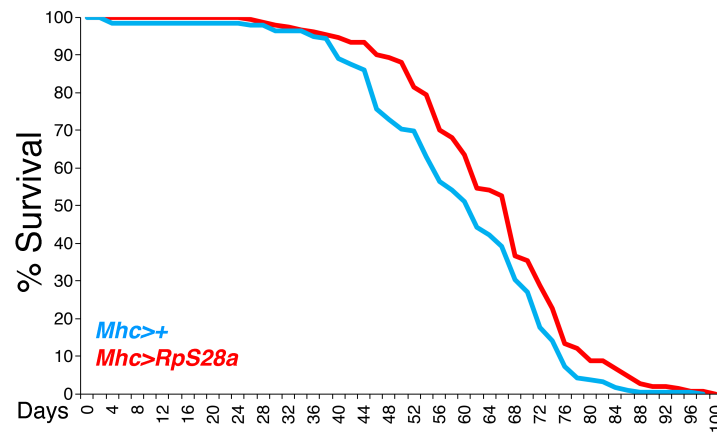
Figure S3. Additional lifespan experiment showing lifespan extension by RpS28a. *RpS28a* overexpression in skeletal muscle with the UAS/Gal4 system and the *Mhc-Gal4* driver results in lifespan extension, compared with isogenic controls ($p<0.01$, Log-rank test; control: *Mhc>+,* n=111; RpS28a[OE]: *Mhc>RpS28a*, n=79).
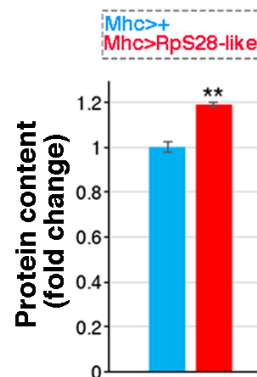

Figure S4. *RpS28-like* overexpression (OE) increases overall protein content in skeletal muscle when compared to isogenic controls (**p<0.01; control: *Mhc>+,* n=4; RpS28-like[OE]: *Mhc>RpS28-like*, n=3).

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A

B

C

Details

UPREGULATED	ALL	P-value	E-value	Unerased E-value
8 / 99	23 / 4794	1.1e-007	5.0e-003	5.0e-003

Enriched Matching Words

Word	UPREGULATED	ALL	P-value	E-value
GTGAGGAG	5 / 99	14 / 4794	2.8e-005	1.2e+000
GTGAGAAG	3 / 99	9 / 4794	1.5e-003	6.8e+001

Details

UPREGULATED	ALL	P-value	E-value	Unerased E-value
28 / 99	459 / 4794	1.7e-007	5.1e-003	5.1e-003

Enriched Matching Words

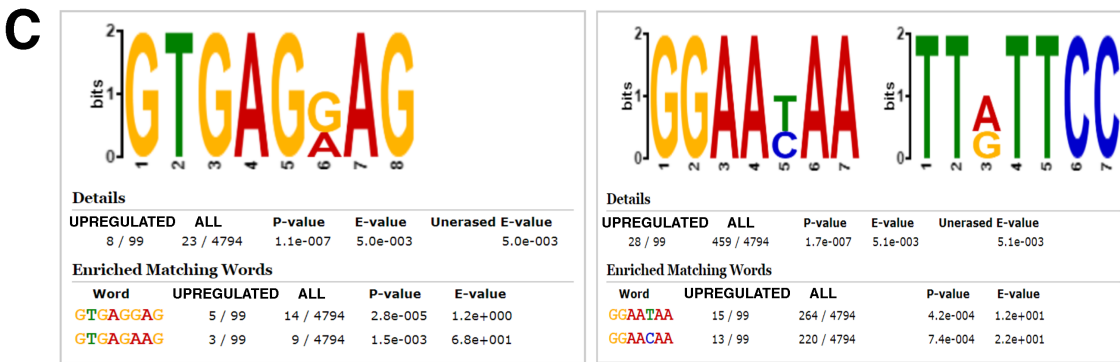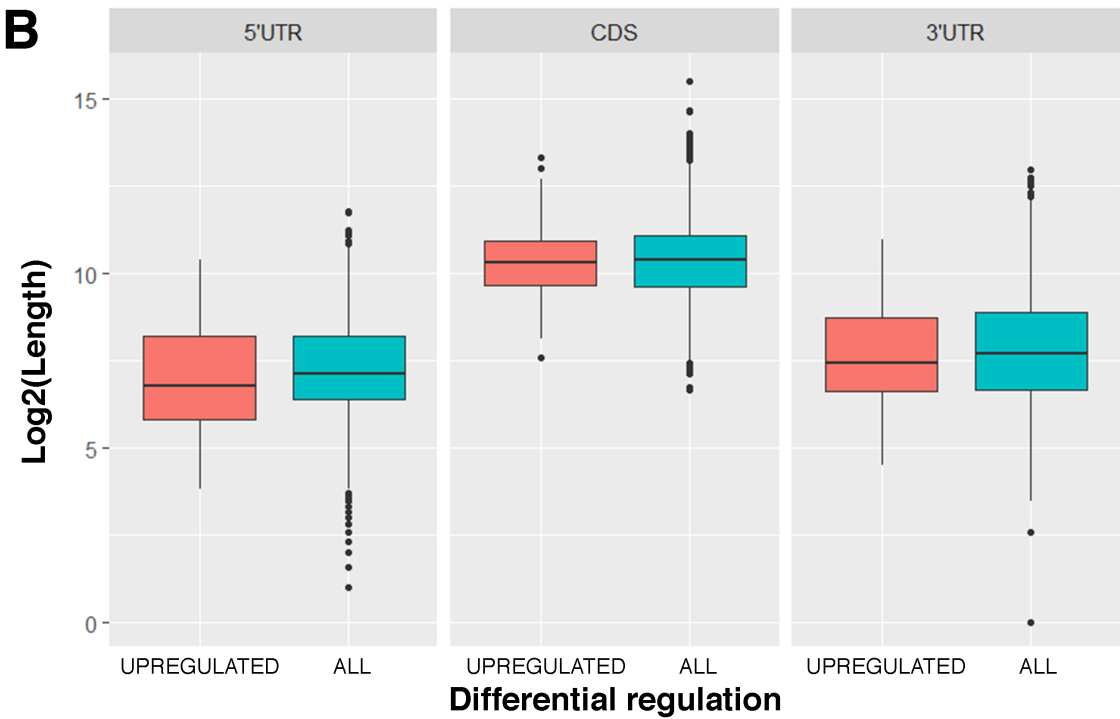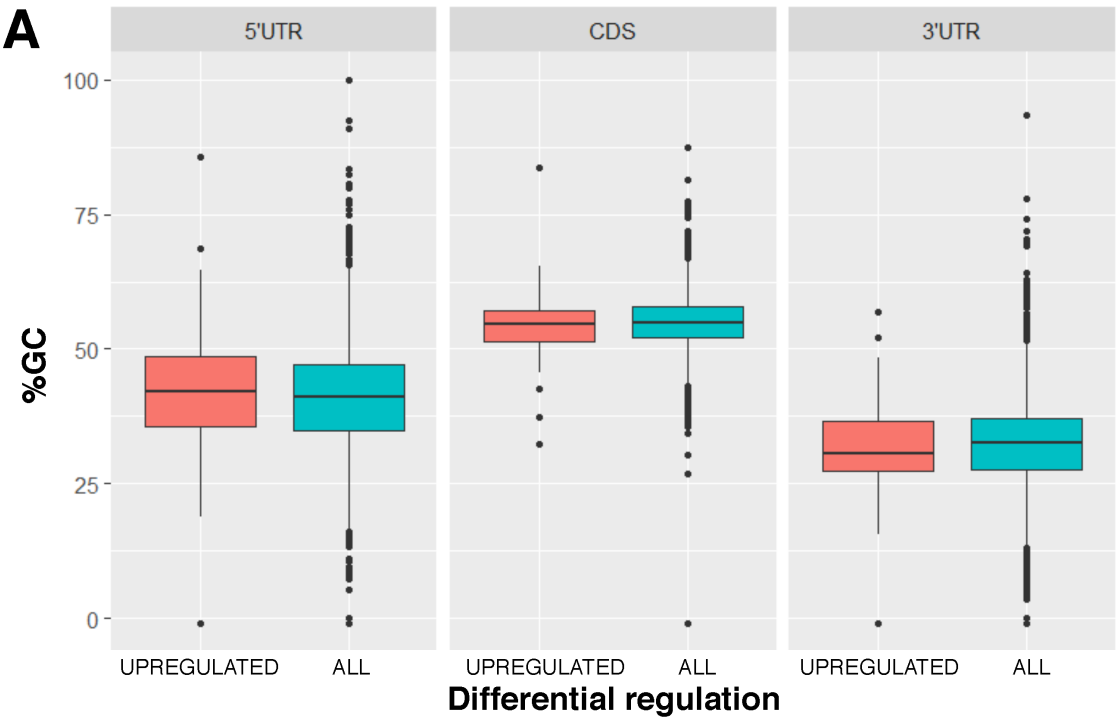| Word	UPREGULATED	ALL	P-value	E-value
GGAATAA	15 / 99	264 / 4794	4.2e-004	1.2e+001
GGAACAA	13 / 99	220 / 4794	7.4e-004	2.2e+001

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Figure S5. A) Analysis of % GC content and B) length of 5'UTRs, coding sequences (CDS), and 3'UTRs from mRNAs corresponding to 99 upregulated proteins with >20% change and p<0.05 (UPREGULATED), compared to 4794 detected proteins (ALL). No significant difference is detected between upregulated proteins versus all detected proteins.

C) Motif analysis with DREME reveals motifs that are significantly enriched in the 3'UTRs of mRNAs corresponding to upregulated proteins versus all proteins (GTGAGRAG: 17.2-fold motif enrichment, i.e. 5/99 versus 14/4794; GGAAYAA: 2.7-fold motif enrichment, i.e. 15/99 versus 264/4794; p-value is indicated).

No significant enrichment of motifs is detected in the 5'UTRs of mRNAs corresponding to upregulated proteins.

Table S1. TMT mass spectrometry results of muscles with RpS28a overexpression versus isogenic controls.

Table S2. Other primary data.