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Title:

An oocyte-specific ELAVL2 isoform is a translational repressor ablated from meiotically competent antral oocytes.

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APPENDIX

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MATERIAL AND METHODS

Plasmids

HA-ELAVL2^{oo} plasmid was prepared by amplifying *Elavl2*^{O} cDNA using primers F_Xho_HuB1_SV40 and R_Not_HuB361_SV40 (in the Table A1) and inserting the PCR product between Xba I and Not I sites of pSV40-HA vector (a plasmid map is available on request). NHA-ELAVL2^{oo} plasmid was prepared by inserting an annealed linker carrying the lambda phage N-peptide sequence in the AgeI site upstream of HA tag.

Renila luciferase (RL) reporter is phRL-SV40 plasmid (Promega). RL-3'Ctcf was constructed by inserting PCR-amplified between XbaI and NotI sites of phRL-SV40. Primers F_3UTR_Ctcf_XbaI and R_3UTR_Ctcf_NotI (in the Table A1) were used for amplification 3'UTR *Ctcf* from cDNA of mouse NIH3T3 cells. RL-5BoxB plasmid was previously described by Pillai et al. [1] and was provided by Witold Filipowicz (FMI, Basel, Switzerland). The firefly luciferase (FL) control plasmid is pGL4.10 (Promega).

mCherry and Elavl2^{O}-mCherry plasmids were prepared by replacing HA tag with in SV40-HA and HA-ELAVL2^{oo} vectors by mCherry tag. mCherry cDNA was amplified from mCherry-pYX plasmid (a kind gift from P. Solc).

The pZP3-Elavl2-IR transgenic construct (Figure 4A) was made by inserting an inverted repeat (IR) generated from coding sequence of *Elavl2* (exons 4-7) into the XbaI site of the ZP3 cassette for transgenic RNAi as described previously [2]. The inverted repeat was constructed by ligating PCR products in vitro according to the published protocol [2]. As indicated in Figure 4A a potential polyA signal sequence (AATAAA) in sense arm of the inverted repeat was mutated to AACAAA

using QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene). Preparation of the transgene for microinjection was performed as previously described [2].

All plasmids were validated by sequencing. Primers and oligomers used for plasmid construction are listed in Table A1.

FIGURE LEGENDS

Figure A1

***Elavl2* splicing is generally complex. (A) A scheme of possible *Elavl2* alternative splicing variants in oocytes** and positions of used primers in real-time PCR analyses. The frequency of oocyte-specific *Elavl2* splicing variants was determined from the next generation sequencing data [3]. Mouse *Elavl2* gene is expressed from chromosome 4. (**TSS1-4** – transcription start site 1-4; **P1-3.fwd** – forward primer Promoter 1-3; **UTR.rev** – reverse primer UTR; **common.fwd** – forward primer common for all tested transcription variants; **skipped exon.rev** – reverse primer skipping the second to last exon) **(B) All possible ELAVL2 isoforms translated from several *Elavl2* splicing variants.** Note that the isoform with a start codon ATG1 is almost not present in high-throughput sequencing data from mouse oocytes. **(C) There are three possible ELAVL2 isoforms in oocytes.** They are generated from at least four *Elavl2* transcript variants. Three contain different 5'UTRs spliced to the same exon with the start codon (ATG3) and one has special either 5'UTR and the start codon (ATG2). The shortest oocyte-specific isoform marked in this paper as an ELAVL2^{O} comes from ATG3 and has truncated "hinge" region.

Figure A2

(A) Both HA- and NHA-tagged ELAVL2^{O} proteins predominantly localize in the cytoplasm in transiently transfected HeLa cells. α-ELAVL2 (green) and α-HA (red) antibodies were used for immunofluorescent staining. DNA staining (DAPI) is in blue. Images were taken by a confocal microscope. Scale bar = 10 μm. **(B) Reduced amounts of NHA-ELAVL2^{O} keep showing RL-5BoxB signal repression.** NIH 3T3 cells were co-transfected with constructs

expressing the RL-5BoxB, FL, and tagged ELAVL2^{O} proteins in indicated plasmid amounts. The graph represents relative *Renilla* activity normalized to FL (R.L.A., mean ± s.e.m.).

Figure A3

(A) *Elavl2*^{O} transcript variant expression in three TG mouse lines. Real-time PCR data represent *Elavl2* expression normalized to *Hprt1* (mean ± s.e.m., n = 3). *Elavl2* expression was set to 1 in oocytes isolated from WT animals. **(B) *Elavl2* knock-down in three transgenic mouse lines.** ELAVL2 is successfully down-regulated only in one transgenic mouse line – tg1629 as shown on depicted Western blot. Tubulin was used as a loading control.

REFERENCES:

1. Pillai RS, Artus CG, Filipowicz W. Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. RNA 2004; 10:1518-25.
2. Svoboda P, Stein P. RNAi experiments in mouse oocytes and early embryos. Cold Spring Harb Protoc 2009; 2009:pdb top56.
3. Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet 2011; 43:811-4.

Figure A1

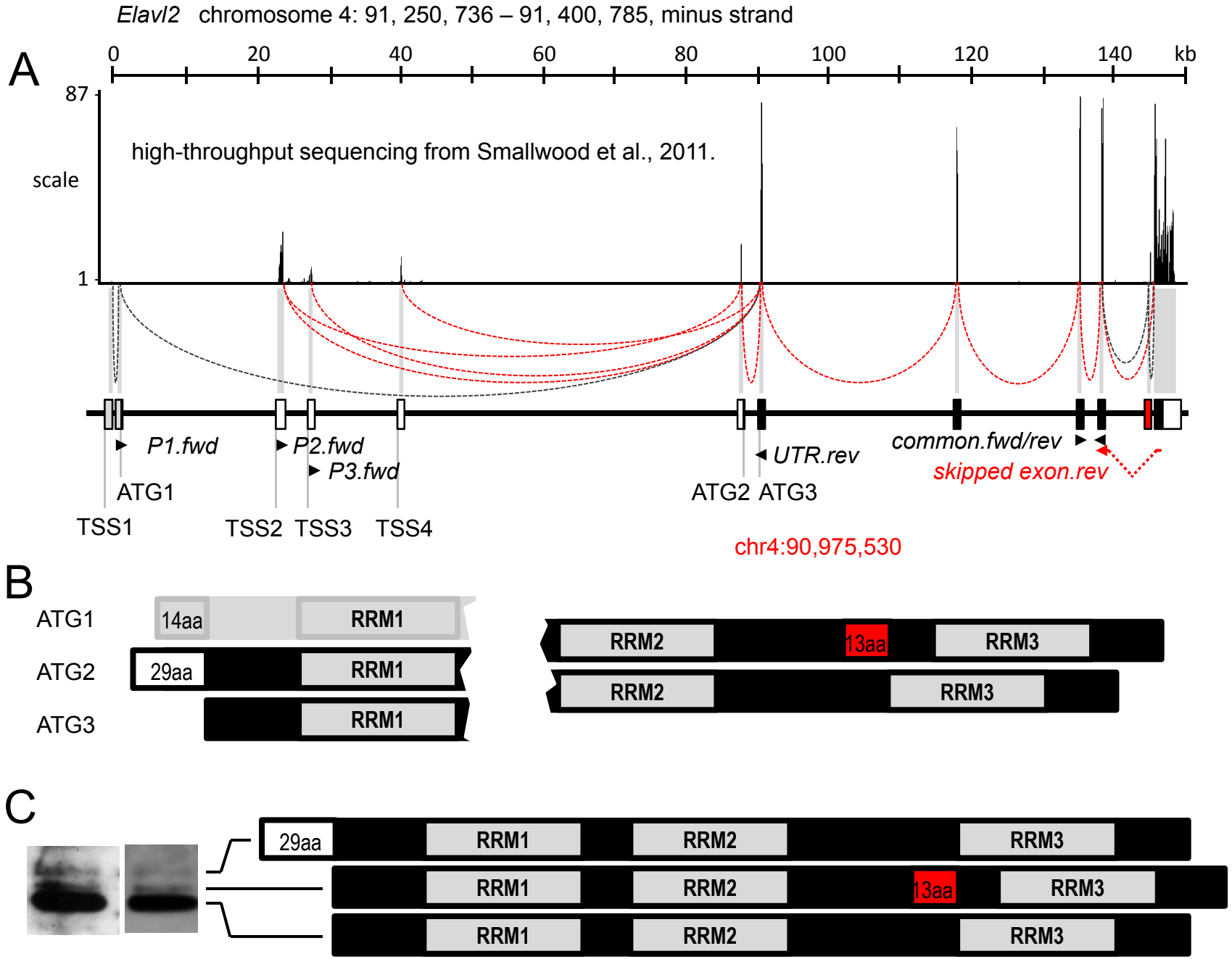

Elavl2 chromosome 4: 91, 250, 736 – 91, 400, 785, minus strand
A
0
20
40
60
80
100
120
140
kb
87
scale
1
high-throughput sequencing from Smallwood et al., 2011.
P1.fwd
P2.fwd
P3.fwd
UTR.rev
common.fwd/rev
skipped exon.rev
ATG1
ATG2
ATG3
TSS1
TSS2
TSS3
TSS4
chr4:90,975,530
B
ATG1
ATG2
ATG3
14aa
29aa
RRM1
RRM2
RRM3
13aa
C
29aa
RRM1
RRM2
RRM3
13aa

Figure A2

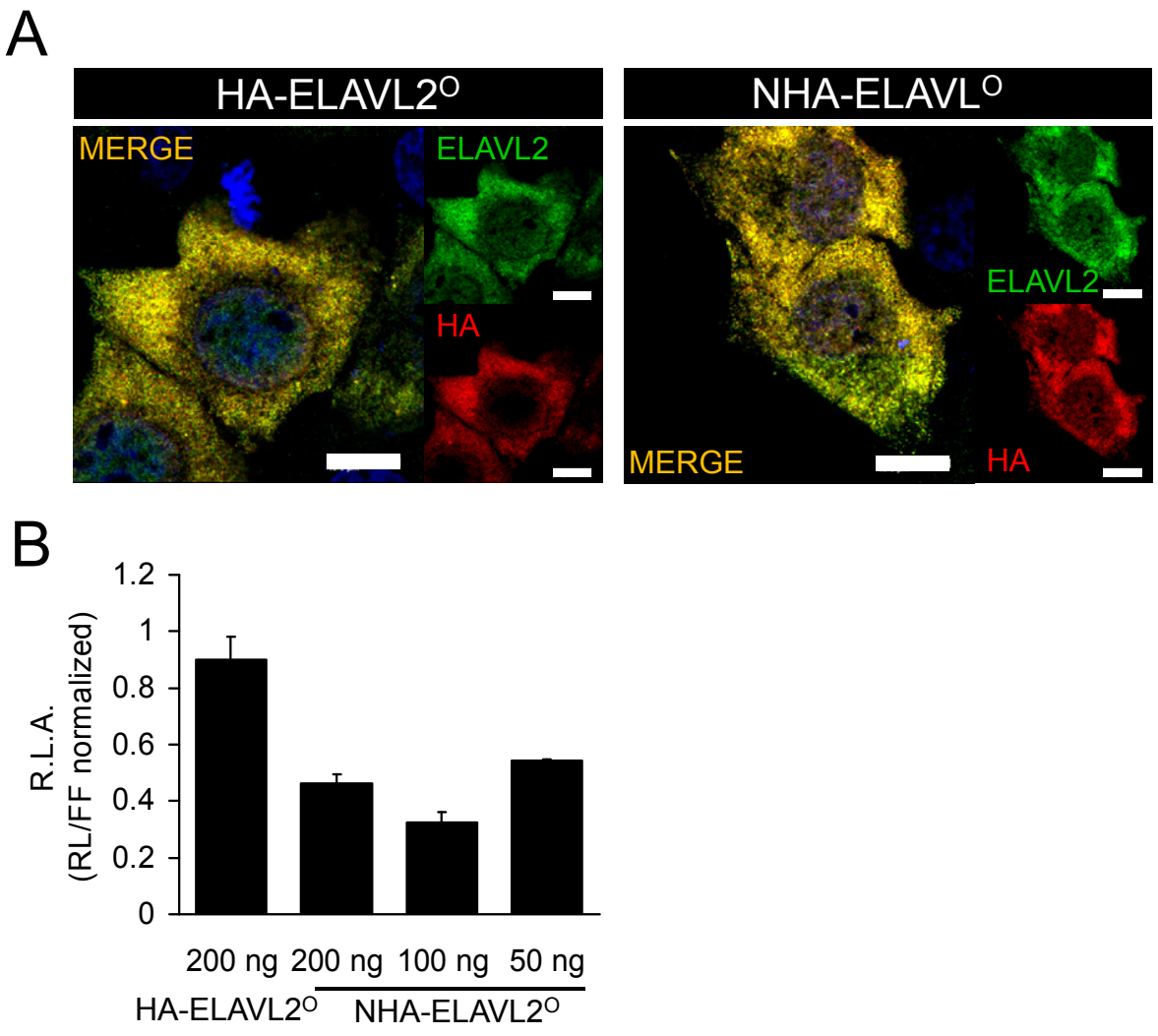
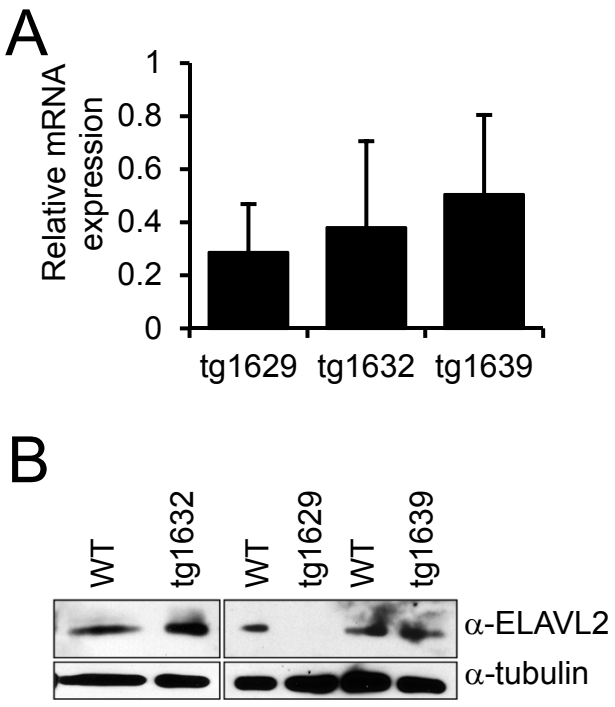

A
HA-ELAVL2O
NHA-ELAVLO
MERGE
ELAVL2
HA
ELAVL2
MERGE
HA
B
R.L.A.
(RL/FF normalized)
1.2
1
0.8
0.6
0.4
0.2
0
200 ng
200 ng
100 ng
50 ng
HA-ELAVL2O
NHA-ELAVL2O

Figure A3


A
Relative mRNA expression
1
0.8
0.6
0.4
0.2
0
tg1629
tg1632
tg1639
B
WT
tg1632
WT
tg1629
WT
tg1639
α-ELAVL2
α-tubulin

name	sequence	product length (bp)	note
pZP3-Elavl2-IR transgenic construction			
Elavl_2_long.fwd	CTATGGATCCTGGAAACACAACTGTCTAATGGGC	520	Long arm IR
Elavl_2_short.fwd	CCCAAGGATCCGGTCCAACCACTGTAAACAACAA	460	Short arm of IR
Elavl_2.rev	TACCTTCTAGAAAACCCTACACCCCTTGATATGC		
Elavl_2_mutg.fwd	GAGACTTCAAACCAAAACAACAAAAGTTTCCTATGCTCGCCC		
Elavl_2_mutg.rev	GGGCGAGCATAGGAAACTTTTGTTGTTTTGGTTTGAAGTCTC		Mutation of a potential polyA signal sequence
pZP3-Elavl2-IR (inverted repeat) detection			
ZP3-EGFP_GT_fwd	CGGGATCACTCTCGGCAT	557	
ZP3-ELAVL2-IR_GT_rev	CACAACTGTCTAATGGGCCAAC		
Plasmid constraction			
Fw_HuB_cDNA2	ATGGAAACACAACTGTCTAATG	1044	Elavl2^{O} cDNA from oocytes for TOPO-II-TA cloning
Rv_HuB_cDNA	TTAGGCTTTGTGCGTTTTGTTTG		
F_Xho_HuB1_SV40	GCACTCGAGATGGAAACACAACTGTC	1066	
R_Not_HuB361_SV40	TAAGCGGCCGCAATTAGGCTTTGTGCG		Inserted in SV40-HA between XbaI and NotI
s_N-peptid_AgeI	CCGGTATGGACGCACAAACACGACGACGTGAGCGTCGCGCTG AGAAACAAGCTCAATGGAAAGCTGCAAACCCACCGCTCGAGA	90	Oligomers for N-peptid insertion
as_N-peptid_AgeI	CCGGTCTCGAGCGGTGGGTTTGCAGCTTTCCATTGAGCTTGTT TCTCAGCGCGACGCTCACGTCGTCGTGTTTGTGCGTCCATA		
F_mCHERRY_AgeI	ATTACCGGTACCATGGTGAGCAAGGGCGAGGAG	1431	Exchange of HA tag for mCHERRY in SV40-HA vector
R_mCHERRY_EcoRI	ATTGAATTCCTTGTACAGCTCGTCCATGCCG		
F_3UTR_Ctcf_XbaI	TTTTCTAGAGCAACAGCCATCATTCAGGTCG	1500	Inserted in phRL-SV40 between XbaI and NotI
R_3UTR_Ctcf_NotI	TTTAGCGGCCGCGCCTGTTAATCCGTTATGATTTATTAG		
qPCR primers			
Fw_HuBall_2	TCTTGTCGACCAGGTCACTG	106	Elavl2 common primers
Rv_HuBall_2	AGGTTTCTGGCCATTTAGGC		
UPL_Fw_HuB1	GCAATATGAGGTTGCTGTGC	108	*Elavl2 P1*
UPL_Rv_HuB1	CAGTTGTTGTTTACAGTGGTTGG		
F_ELAVL2_v3	GTTCCGTCGTGTTCCAGTC	129	Elavl2 P2
R_ELAVL2_v3	TTCCATGGCAGCAATTACCT		
F_ELAVL2_v4	GCAGCTTCTTGCTCATCCTT	97	Elavl2 P3
R_ELAVL2_v4	TGGCAGCAATTACCTGCTTT		
Fw_HuB3	TCTTGTCGACCAGGTCACTG	260	Elavl2 skipped exom
Rv_HuB3	TTGGAGAAAACCTACTAAAACGC		
Hprt1_QPCR_Fwd	GTCCCAGCGTCGTGATTAG	224	Houskeeping gene
Hprt1_QPCR_Rev	CAGCAGGTCAGCAAAGAAC		
RL_Fwd	CAGATTGTCCGCAACTACAACGCC	165	No intron spanning
RL_Rev	CTTACCCATTTCATCTGGAGCGTC		
FL_Fwd	GCTACAAACGCTCTCATCGACAAG	90	No intron spanning
FL_Rev	GTATTTGATCAGGCTCTTCAGCCG		