The most abundant maternal lncRNA *Sirena1* acts post-transcriptionally and impacts mitochondrial distribution

Sravya Ganesh[1], Filip Horvat[1,2], David Drutovic[3], Michaela Efenberkova[1], Dominik Pinkas[1], Anna Jindrova[3], Josef Pasulka[1], Rajan Iyyappan[3], Radek Malik[1], Andrej Susor[3], Kristian Vlahovicek[2], Petr Solc[3], Petr Svoboda[1]

SUPPLEMENTARY MATERIAL

[1] Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

[2] University of Zagreb, Zagreb, Croatia

[3] Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Libechov, Czech Republic

Corresponding author: Petr Svoboda (svobodap@img.cas.cz)

Keywords: lncRNA, oocyte, maternal, siRNA, RNAi, cytoplasmic polyadenylation, mitochondria

Supplementary Figures:

Supplementary Figure S1

Transcriptional control of *Sirena1*. Shown is *Sirena1* expression in microarray analysis of newborn ovary transcriptomes lacking *Lhx8* (GSE7774), *Nobox* (GSE7775), and *Figla* (GSE5558) (13,14). *Figla* mutant profiling was on a non-commercial spotted microarray platform VMSR Mus 20K while the other two knockouts were profiled on MOE430 Affymetrix platform. Black points represent signals of microarray probes for selected genes, red and blue points represent significantly upregulated and downregulated genes, respectively. *Sirena1* was present on arrays in two probes/probe sets, the reason why they show different levels of downregulation is unclear.

Supplementary Figure S2

Additional *Sirena1*$^{-/-}$ analysis data. (A) *Sirena1* deletion identified in the founder animal. (B) Meiotic maturation of wild type (upper panel) and *Sirena1*$^{-/-}$ (lower panel) oocytes as assessed by live-cell imaging. Oocytes were microinjected with histone H2B-mCherry and stained with SiR-tubulin. Representative still images of maximum z-projections are shown. Time in hh:mm is relative to GVBD. Scale bar, 10 µm. Dashed circles indicate oocyte edges. (C) Frequency of the first polar body extrusion in wild type and *Sirena1*$^{-/-}$ oocytes. (D) Quantification of the percentage of oocytes as in (A) with chromosome misalignment. (E) Quantification of the percentage of oocytes as in (A) with chromosome segregation errors. Data in C-E are expressed as means, error bars represent exact confidence intervals.

Supplementary Figure S3

Additional *Sirena1*$^{-/-}$ analysis data. (A) The transcriptional landscape of the *Sirena1* locus in wild type and *Sirena1*$^{-/-}$ oocytes. Shown is UCSC snapshot where maximum displayed expression level was set to 250 CPM. (B) Transcripts from *Sirena1* locus in *Sirena1*$^{-/-}$ oocytes. Shown is a UCSC snapshot of transcription in Sirena1 locus in *Sirena1*$^{-/-}$ replicate1 dataset. Dashed horizontal line depicts the maximum CPM value of 2.4.

Supplementary Tables

Supplementary Table S1 Primers and oligonucleotides

Supplementary Table S2 High-throughput datasets

Supplementary Table S3 ORF analysis

Supplementary Table S4 *Elob* pseudogenes

Supplementary Table S5 Differentially expressed genes in *Sirena1* knock-out

Supplementary Files:

Supplementary File 1 – meiotic maturation of wild type oocytes movie

Video of the maximum intensity z-stack projection from confocal time-lapse imaging of wild type oocytes microinjected with histone H2B-mCherry (cyan) and stained with SiR-tubulin (red). Time in hh:mm relative to GVBD. Bars, 10 µm. Brightfield is one confocal section selected from the area of the nucleus and the 1st polar body.

Supplementary File 2 – meiotic maturation of *Sirena1*$^{-/-}$ oocytes movie

Video of the maximum intensity z-stack projection from confocal time-lapse imaging of *Sirena1*$^{-/-}$ oocytes microinjected with histone H2B-mCherry (cyan) and stained with SiR-tubulin (red). Time in hh:mm relative to GVBD. Bars, 10 µm. Brightfield is one confocal section selected from the area of the nucleus and the 1st polar body.

Figure S1

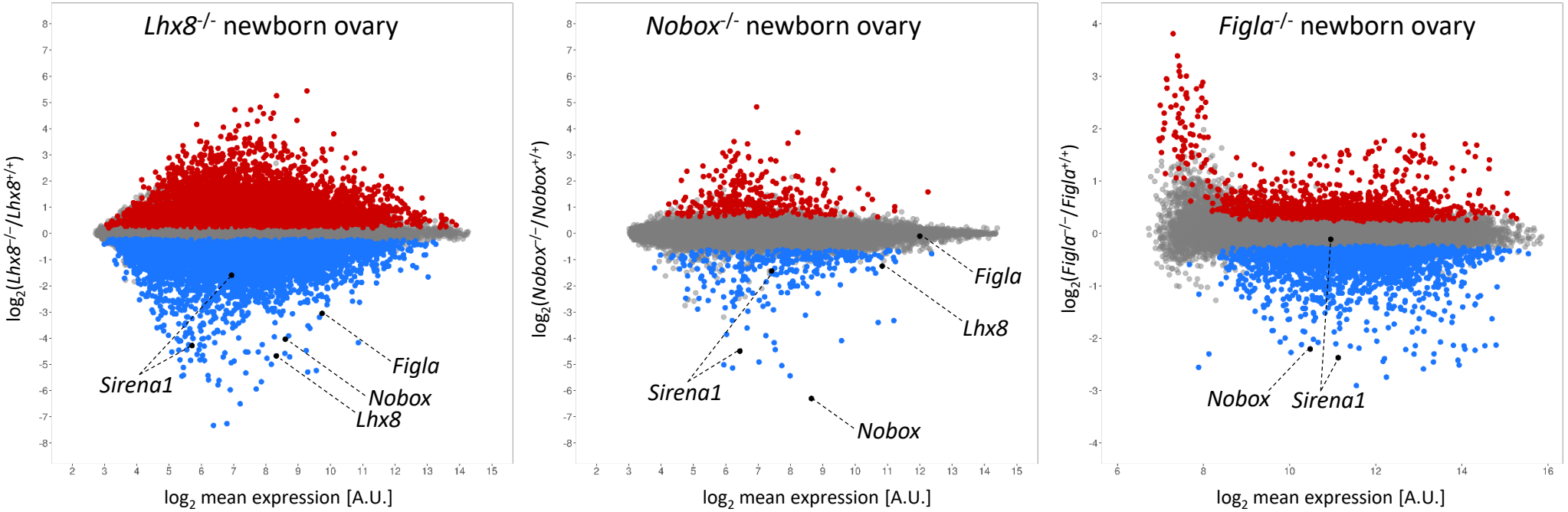

Lhx8-/- newborn ovary
log2(Lhx8−/−/Lhx8+/+)
log2 mean expression [A.U.]
Sirena1
Figla
Nobox
Lhx8
Nobox-/- newborn ovary
log2(Nobox−/−/Nobox+/+)
log2 mean expression [A.U.]
Figla
Lhx8
Sirena1
Nobox
Figla-/- newborn ovary
log2(Figla−/−/Figla+/+)
log2 mean expression [A.U.]
Nobox
Sirena1

Figure S2

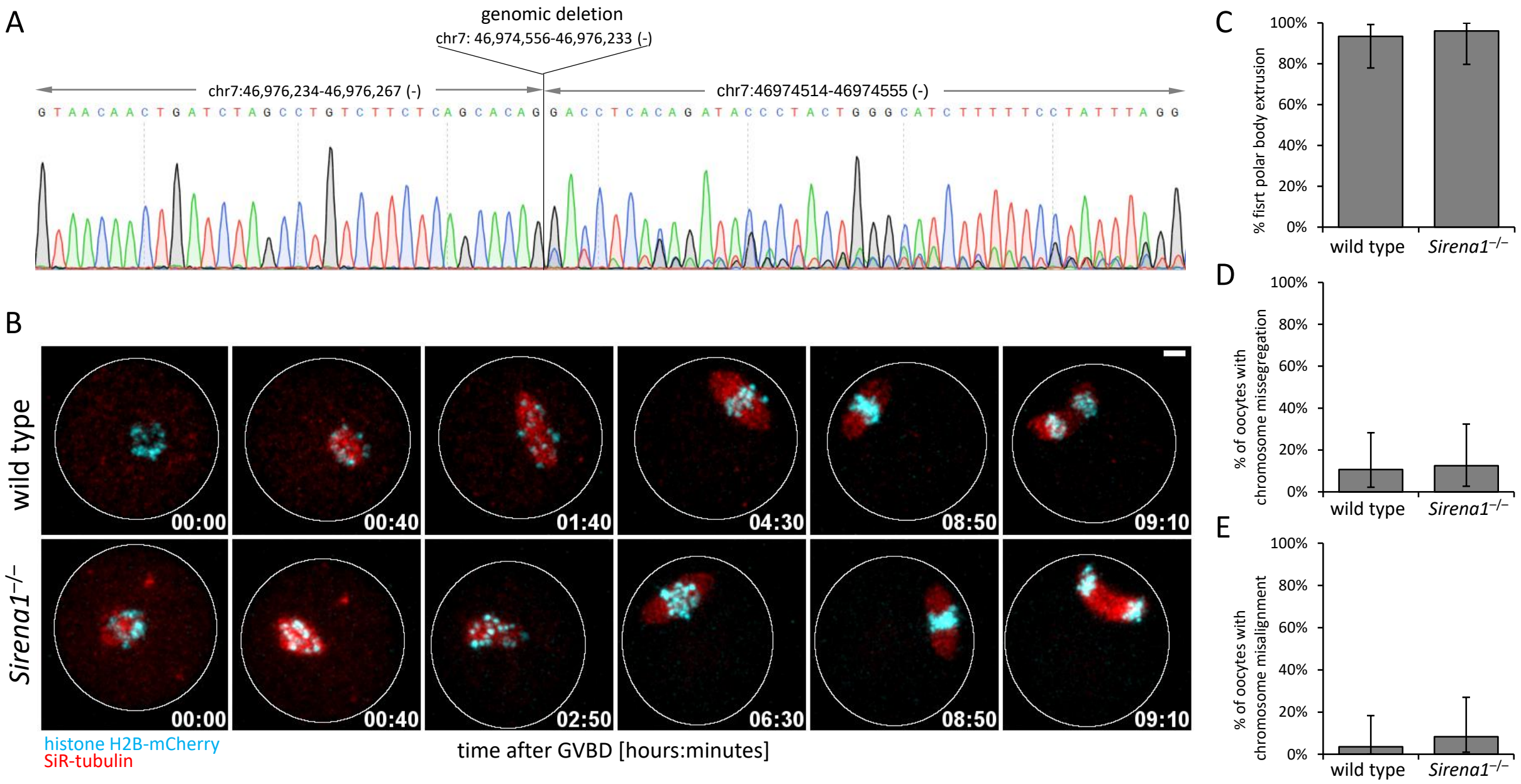
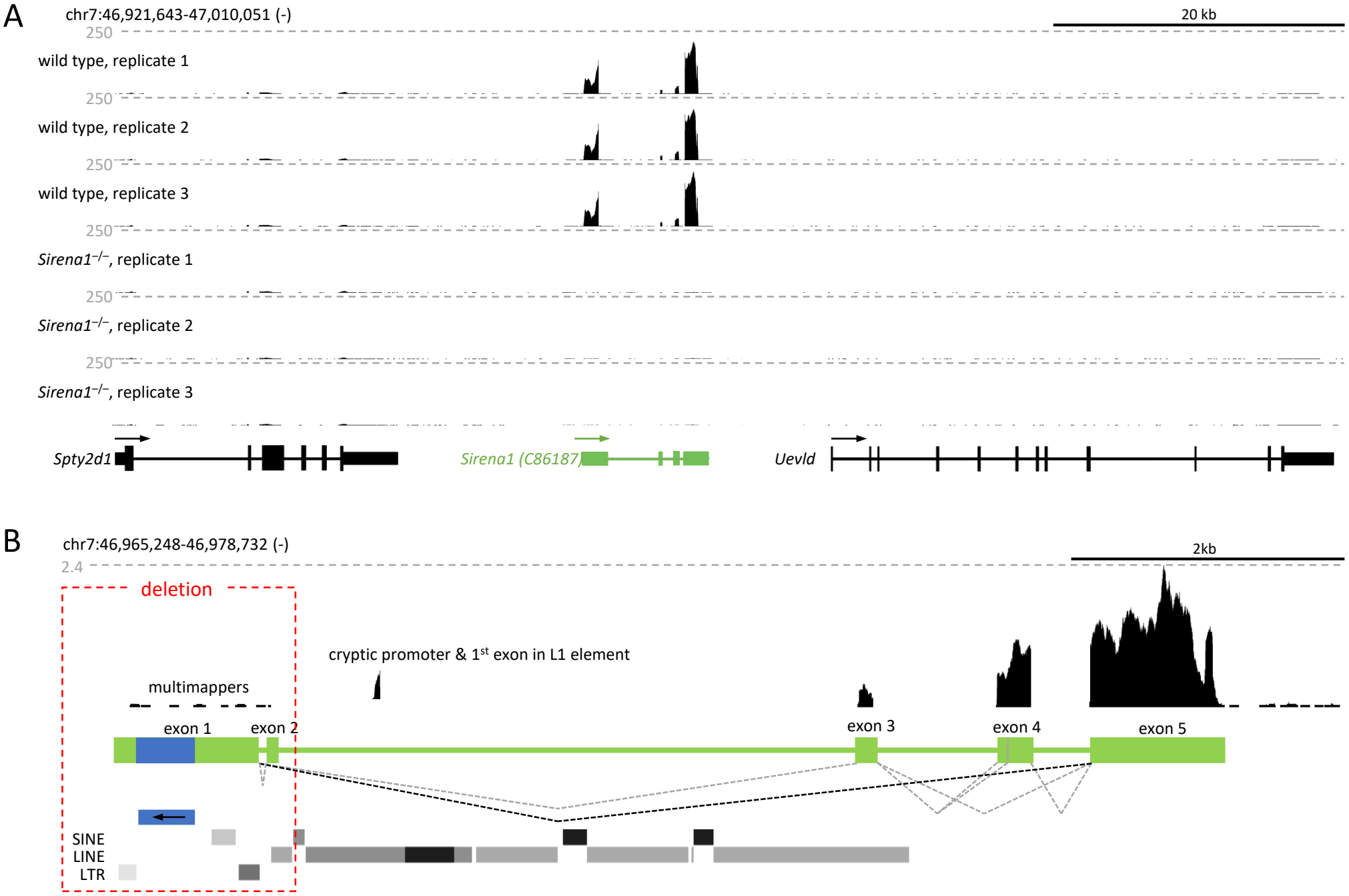

A
genomic deletion
chr7: 46,974,556-46,976,233 (-)
chr7:46,976,234-46,976,267 (-)
chr7:46974514-46974555 (-)
GTAACAACTGATCTAGCCTGTCTTCTCAGCACAGGACCTCACAGATACCCTACTGGGCATCTTTTTCCTATTTAGG
B
wild type
Sirena1−/−
00:00
00:40
01:40
04:30
08:50
09:10
00:00
00:40
02:50
06:30
08:50
09:10
histone H2B-mCherry
SiR-tubulin
time after GVBD [hours:minutes]
C
% fisrt polar body extrusion
100%
80%
60%
40%
20%
0%
wild type
Sirena1−/−
D
% of oocytes with chromosome missegregation
100%
80%
60%
40%
20%
0%
wild type
Sirena1−/−
E
% of oocytes with chromosome misalignment
100%
80%
60%
40%
20%
0%
wild type
Sirena1−/−

Figure S3


A
chr7:46,921,643-47,010,051 (-)
20 kb
250
wild type, replicate 1
wild type, replicate 2
wild type, replicate 3
Sirena1−/−, replicate 1
Sirena1−/−, replicate 2
Sirena1−/−, replicate 3
Spty2d1
Sirena1 (C86187)
Uevld
B
chr7:46,965,248-46,978,732 (-)
2kb
2.4
deletion
cryptic promoter & 1st exon in L1 element
multimappers
exon 1
exon 2
exon 3
exon 4
exon 5
SINE
LINE
LTR