Expanded View Figures

A RNA-seq data Rpp30^{18.2}/white virgin ovaries:

<table>
<thead>
<tr>
<th>Gene identity</th>
<th>log2FoldChange</th>
<th>pValue</th>
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<tbody>
<tr>
<td>FBgn0022246 (Rpp30)</td>
<td>-1.163357556</td>
<td>0.000508915</td>
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B Germine clones Rpp30^{18.2}

C Somatic clones Rpp30^{18.2}

D Rpp30^{18.2}; ubiRpp30GFP

Figure EV1. Rpp30 protein expression is affected in Rpp30^{18.2} mutants and PCNA loss in Rpp30^{18.2} ovaries is restored when overexpressing ubiRpp30GFP (related to Figs 1 and 3).

A Normalized fold change and P-value between Rpp30^{18.2} mutant ovaries and white virgins issue from RNA-seq transcriptome analysis showing a downregulation of Rpp30 transcripts in Rpp30^{18.2} mutant ovaries.

B, C flp/FRT clones mutant for Rpp30^{18.2} specifically in the germ line (B) or follicular cells (C) are detected by the absence of RFP or GFP, respectively. Magnifications show Rpp30 absence in the nucleus of mutant cells. Perinuclear Rpp30 foci are indicated by white arrows in a wild-type nurse cell (B). Scale bar, 10 µm.

D Rpp30^{18.2} homozygous ovaries overexpressing ubiRpp30GFP were dissected, fixed, and stained for PCNA (red). Rpp30GFP is in green and DAPI is in blue. Scale bar, 10 µm.
Figure EV2. Size of ovaries used in this work (related to Figs 4–6). Ovaries of different genotypes used for small RNA sequencing, RNA sequencing, and ChIP analysis. The left panel of heterozygous Rpp30^{18.2}/CyO ovaries has been included again in this figure (also present in Fig 1A) in order to better compare the different ovaries used altogether. Scale bar, 100 μm.
Figure EV3. piRNAs are rescued in double mutants Rpp30, chk2 (related to Fig 5).

A Nuage-specific factors (Vasa, Tejas, Kumo, and Krimper) and Rhino and Piwi protein localizations are shown in wild-type and germ line mutant clones for Rpp30^{18.2}.

B piRNA-specific unique mappers from different piRNA clusters are shown in Rpp30^{18.2} homozygous ovaries versus Rpp30^{18.2}, mnk^{p6} double-mutant ovaries. The scales are indicated on the left. Red, sense. Blue, antisense.

Source data are available online for this figure.
**Figure EV4.** *Rpp30, chk2* double mutant characterization (related to Figs 3, 4, and 6).

A, B RNA extracted from heterozygous *Rpp3018*/CyO, homozygous *Rpp3018* and double mutant *Rpp3018,mnk1* ovaries was used to study piRNA clusters 1 and 2 expression levels (A) and transposable element expression (B) by RT-qPCR.

C About 100 ovaries from white and *Rpp3018*, *mnk1* were used for ChIP experiments with H3K9me3 antibody. The corrected percentage of input normalized to control genes is shown as the ratio between the double mutant and the control, reflecting a rescue of methylation profiles in different regions of the two principal piRNA clusters (1 and 2) and in some TEs (Het-A, I-ele, and 412) in double-mutant ovaries.

D Double-mutant ovaries *Rpp3018*, *mnk1* were fixed and stained for PCNA. Example of one rescued and one non-rescued egg chamber are shown. Scale bar, 10 μm.
Figure EV5. Chk2 activation by spnA mutation does not recapitulate Rpp30 phenotype (related to Figs 3, 5, and 6).

A RNA extracted from heterozygous or homozygous spnA\(^{1}\) and spnE\(^{1}\) flies was used to study transposable element expression by RT–qPCR. The fold change of TEs expression comparing homozygous versus respective heterozygous ovaries is shown.

B Ovaries from homozygous spnA\(^{1}\) and spnE\(^{1}\) flies were fixed and stained for Aub (left) or PCNA (right). Scale bar, 10 \(\mu\)m.