Figures and figure supplements

The GATOR complex regulates an essential response to meiotic double-stranded breaks in *Drosophila*

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Figure 1. Mio prevents the constitutive downregulation of TORC1 activity in response to meiotic DSBs. (A) The GATOR2 complex opposes the activity of the TORC1 inhibitor GATOR1. (B) Representative ovaries from wild type (WT), mio\(^2\), double-mutant mio\(^2\), mei-W68\(^1\) and mei-W68\(^1\) females. Scale bar, 1000 \(\mu\)m. (C) Western blot of p-S6K and total-S6K levels of whole ovaries prepared from WT, mio\(^2\), mio\(^2\), mei-w68\(^1\) and mei-W68\(^1\) mutant females. (D) Quantification of p-S6K levels relative to total S6K. Unpaired student T-test was used to calculate the statistical significance. Error bars represent the standard deviation (SD) for three independent experiments. *p<0.05.

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Figure 1—figure supplement 1. Removing meiotic DSBs partially rescues the low egg production of mio mutants. Five males and five females (WT, mio\(^2\), mio\(^2\), mei-w68\(^1\)) were cultured in cages on grape juice plates and wet yeast. The number of eggs laid in each cage was counted every 24 hr. Data show the number of eggs laid per individual female per day for each genotype. Error bars represent SD from three independent experiments. Unpaired T-student test was used to calculate statistical significance. **p<0.01. ***p<0.001, ****p<0.0001.
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Figure 1—figure supplement 2. TORC1 activity is reduced in spnA/Rad51 mutants. (A) Western blot of p-S6K and total-S6K levels of whole ovaries prepared from WT, spnA1 homozygous and spnA1/spnA093 transheterozygous mutants. Flies were mated and cultured on yeast for 3 days before dissection. (B) ImageJ was used to measure the relative band intensity of all western blots. The graph depicts the fold change of p-S6K/total-S6K of the mutant genotypes compared to wild type. Three independent experiments were performed and the standard deviation between the experiments is indicated in red. Unpaired student T-test was used to calculate statistical significance (****p<0.001).

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Figure 1—figure supplement 3. Blocking the formation of meiotic DSBs fails to increase total TORC1 activity in wild type or nprl3 mutant ovaries as measured by western blot. Whole ovaries dissected from (A) WT, (B) nprl3<sup>1</sup>, (C) nprl3<sup>1</sup>, mei-p22<sup>P22</sup> and (D) mei-p22<sup>P22</sup> homozygous mutants were used for (E) Western blot to assess p-S6K and total-S6K levels. Adults were mated and cultured on yeast for 3 days before the dissections. (F) ImageJ was used to measure the relative band intensity of all western blots. The graph depicts the fold change of p-S6K/total S6K of the mutant genotypes compared to wild type. Six independent experiments were performed and the standard deviation between the experiments is indicated in red. Unpaired T-student test was used to calculate statistical significance.

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Figure 1—figure supplement 4. Mutations in the checkpoint protein *loki* rescues the seh1 ovarian phenotype. Ovaries were dissected and imaged (A) WT (B) *mio*<sup>2</sup> (C) *loki*<sup>P6</sup> and (D) *mio*<sup>2</sup>, *loki*<sup>P6</sup>. (E) Surface area of ovaries from indicated genotypes. Scale bar: 600 μm. Unpaired T-student test was used to calculate statistical significance. ****p<0.0001, n.s.: no significance.

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Figure 2. Suppressing the production of meiotic DSBs increases p4E-BP staining in the female germline of mio mutants. (A) Schematic representation of the Drosophila germarium. (A’A”) pseudo-color representation of p-4E-BP staining, arrowhead denotes a region one ovarian cyst with high p-4E-BP levels. Ovaries from (B) wild type (C) mio2, (D) mei-W681, (E) mio2; mei-W681, (F) spnA1, (G) thor2 females stained for C(3)G (cyan) to mark the synaptonemal complex, p-4E-BP (white), Vasa (magenta) to highlight the germline cytoplasm and DNA (Blue). (B) In wild-type ovarian cysts, p-4E-BP staining begins to increase in region 2b (arrow). (C) In mio mutant ovarian cysts, p-4E-BP levels remain low in region 2b and region 3. (D,E) mio, mei-W68 double mutants have p-4E-BP expression levels similar to mei-W68 single mutants. (F) spnA mutants, which fail to repair meiotic DNA breaks, have low levels of p-4E-BP staining (G) thor2/4E-BP null mutants serve as a negative control. (H) p-4E-BP intensity measurement of region 2b data (B)-(G). Scale bar: 7 μm. Unpaired T-student test was used to calculate statistical significance. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
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Figure 3. GATOR1 influences the steady state number and persistence of DSBs in early oocytes. Ovaries from (A) wild type, (B) nprl2\(^1\), (C) nprl3\(^1\)/Df, (D) iml\(^1\)/Df, (E) nanos-GAL4; UAS-Nprl3; nprl3\(^1\)/Df, (F) mei-P22\(^{222}\), nprl3\(^1\), and (G) spnA\(^1\)/spnA\(^{093}\) females were stained for C(3)G (green, A’–G’) and g-H2Av (red, A–G). C(3)G marks the synaptonemal complex (SC) and is used to mark oocytes and follow meiotic progression. γ-H2Av marks DSBs. Scale bars, 10 μm. In wild type oocytes, meiotic DSBs are induced in region 2a and repaired by region 3 (arrow). In GATOR1 mutants, DSBs persist in region three oocytes. In nanos-GAL4; UAS-Nprl3; nprl3\(^1\)/Df oocytes, DSBs are repaired by region 3. mei-P22\(^{222}\), nprl3\(^1\) mutants have no DSBs. (H) Ovaries from wild type, nprl2\(^1\), nprl3\(^1\)/Df, iml\(^1\)/Df, spnA\(^1\)/spnA\(^{093}\), nprl2\(^1\); spnA\(^1\)/spnA\(^{093}\), mei-P22\(^{222}\), nprl3\(^1\) and nanos-GAL4; UAS-Nprl3; nprl3\(^1\)/Df flies were stained for C(3)G (green) and γ-H2Av (red). Representative immunofluorescent microphotographs of the γ-H2Av foci in region 2a oocyte are shown. (I) Percentage of region three oocytes with γ-H2Av foci. (J and K) Quantification of γ-H2Av foci in region 2a oocytes. Unpaired T-student test was used to calculate the Figure 3 continued on next page.
statistical significance. Error bars represent SD from at least three independent experiments. **p<0.01, ****p<0.0001, ns: no significance. Note that the three GATOR1 mutants, nprl3<sup>1</sup>, nprl2<sup>1</sup> and iml1<sup>1</sup> are null alleles (Cai et al., 2016; Wei et al., 2016). 

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Figure 3—figure supplement 1. *nprl2* and *nprl3* mutant females are semi-sterile. *y, w (WT), nprl2* and *nprl3*/Df females were cultured on standard media with wet yeast for two days. (A) Eggs laid per female per day were counted starting from the third day for the following four days, and the average egg laid each day were calculated. (B) Eggs were cultured for three additional days after eggs were laid to determine eggs hatched. Error bars represent standard deviation from at least three independent groups. Unpaired T-student test was used to calculate the statistical significance.

****p<0.0001.

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Figure 4. Reducing the dose of S6K rescues the meiotic DSB phenotype in iml1 knockdowns. (A) γ-H2Av foci in region 2a oocytes in the indicated genotypes. (B) Quantification of γ-H2Av foci in region 2a oocytes in the indicated genotypes. Note that meiotic DSBs were increased in nanos-GAL4; iml1RNAi females. Moreover, removing a single copy of S6K in the nanos-GAL4; iml1RNAi background reduced the steady state number of meiotic DSBs in nanos-GAL4; iml1RNAi, S6K^{l-1}/+ females. Unpaired T-student test was used to calculate statistical significance. *p<0.05, ****p<0.0001, n.s.: no significance. (B).
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Figure 5. GATOR1 prevents p53 hyperactivation in Drosophila early ovarian cysts. Ovaries from (A) p53R-GFP, (B) nprl21; p53R-GFP, (C) p53R-GFP; nprl31/Df, (D) p53R-GFP; iml1/Df and (E) p53R-GFP; mei-P22p22, nprl31 were stained for GFP (green) and 1B1 (red). Germarial regions are defined by 1B1 staining. In wild-type ovaries the p53-GFP reporter is briefly activated in region 2 (indicated by arrow). Note the low level of GFP staining. In contrast, in GATOR1 mutants, p53R-GFP is robustly activated with strong GFP signal often persisting into germarial region three and beyond. Additionally, in GATOR1 mutant germaria, p53R-GFP is frequently activated in germline stem cell (GSC) and daughter cystoblasts (CB). In mei-P22p22, nprl31 double mutant germaria, the hyperactivation of p53R-GFP is rescued in region 2a ovarian cysts. However, p53-GFP activation in GSC and CB is retained in the double mutants (asterisk) indicating that in these cells the activation of p53 is not contingent on the presence of meiotic DSBs. Scale bars, 10 μm. (F) Percentage of germaria with sustained p53R-GFP signal in region 3. (G) Percentage of germaria with high p53R-GFP signal in region 2. (H) Percentage of germaria with p53R-GFP expression in GSC and CB. Unpaired student T-test was used to calculate the statistical significance. Error bars represent SD from at least three independent experiments. **p<0.01, ****p<0.0001.

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**Figure 5—figure supplement 1.** The GATOR1 complex acts cell autonomously in the female germ line. Germaria from (A) HS-FLP; p53RE; arm-LacZ, FRT80B/nprl3^1, FRT 80B female and (B) HS-FLP; p53RE; arm-LacZ, FRT80B/iml1^1, FRT 80B females stained for GFP (green) and lacZ (red). The nprl3^1 and iml1^1 homozygous clones are identified by the absence of anti-lacZ staining. Scale bar 10 μm.

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Figure 6. The TORC1 inhibitor TSC1 promotes genomic stability in early oocytes. Ovaries from (A) pS3R-GFP; MTD >mCherry RNAi and (B) pS3R-GFP; MTD >Tsc1 RNAi flies were stained for GFP (green) and 1B1 (red). In the mCherry RNAi (control) ovaries the pS3-GFP expression is very low. In contrast, the Tsc1 RNAi ovaries have sustained GFP signal that persists into germinal region 3. Scale bars, 10 μm. (C) Percentage of germaria with strong pS3R-GFP signal in region 2. (D) Percentage of germaria with sustained pS3R-GFP signal in region 3. The γ-H2Av foci were determined in MTD >mCherry RNAi and MTD >Tsc1 RNAi ovaries. (E) Percentage of region three oocytes with γ-H2Av foci. (F) Quantification of γ-H2Av foci per oocyte in region 2a. Unpaired student T-test was used to calculate the statistical significance Error bars represent SD from at least three independent experiments. *p<0.05, ****p<0.0001.

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Figure 7. Nprl3 promotes DNA repair in somatically derived follicle cells. Egg chambers from fed (A) WT and (B) nprl3<sup>1</sup>/Df females exposed to 10 Gy γ-irradiation. Ovaries were dissected at 0 hr (no irradiation) (A, B), 1 hr (A', B') and 6 hr post-irradiation (A'', B'') and stained with antibodies against γ-H2Av (dsDNA breaks, Red), C(3)G (cyan) and the DNA dye DAPI (blue). (C) Quantification of γ-H2Av positive follicle cells from the indicated time points and genotypes. Note that an increased percentage of nprl3/Df follicle cells contain γ-H2Av foci 6 hr post irradiation relative to controls. Scale bar: 7 μm. Arrows denote γ-H2Av positive follicle cells.

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Figure 8. GATOR1 opposes retrotransposon expression in parallel to p53. (A) Quantitative RT-PCR analysis of expression levels for retrotransposons in wild type, nprl2<sup>1</sup>, nprl3<sup>1</sup>/Df, nanos-GAL4, UAS-Nprl3, nprl3<sup>1</sup>/Df, spnA<sup>1</sup>/spnA<sup>093</sup> and spnA<sup>1</sup>/spnA<sup>1</sup> ovaries. (B) Quantitative RT-PCR analysis of expression levels for transposons in wild type, nprl3<sup>1</sup> and mei-P22<sup>222</sup>, nprl3<sup>1</sup> ovaries. (C) Quantitative RT-PCR analysis of expression levels for the transposons in wild type, p53<sup>5A-1-4</sup>, nprl3<sup>1</sup>/Df and nprl3<sup>1</sup>/Df, p53<sup>5A-1-4</sup> ovaries. Rp49 is used for normalization. Fold expression levels are relative to wild type. Error bars represent SD of three independent experiments.

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Figure 8—figure supplement 1. Quantitative RT-PCR analysis of retrotransposon transcript levels in Tsc1 knockdowns. RT-PCR was used to identify (A) Tsc1 and (B) retrotransposons transcript levels in MTD > Tsc1 RNAi ovaries. Rp49 was used for normalization. Fold changes are relative to MTD > mCherry RNAi (control). Standard deviations were calculated from three biological replicates.

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Figure 9. A working model for the role of the GATOR complex in the response to meiotic DSBs. (A) After ovarian cysts enter meiosis, meiotic DSBs function to activate and/or maintain a GATOR1/TSC dependent pathway to ensure low TORC1 activity in early prophase of meiosis I. Low TORC1 activity promotes the timely repair of meiotic DSBs. Currently, whether meiotic DSBs directly activate the GATOR1/TSC pathway or an alternative pathway that works in concert with, or in parallel to, GATOR1/TSC is not known. (B) Subsequently, the GATOR2 component Mio is required to attenuate the activity of the GATOR1/TSC pathway, thus allowing for increased TORC1 activity and the growth and development of the oocyte in later stages of oogenesis.

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