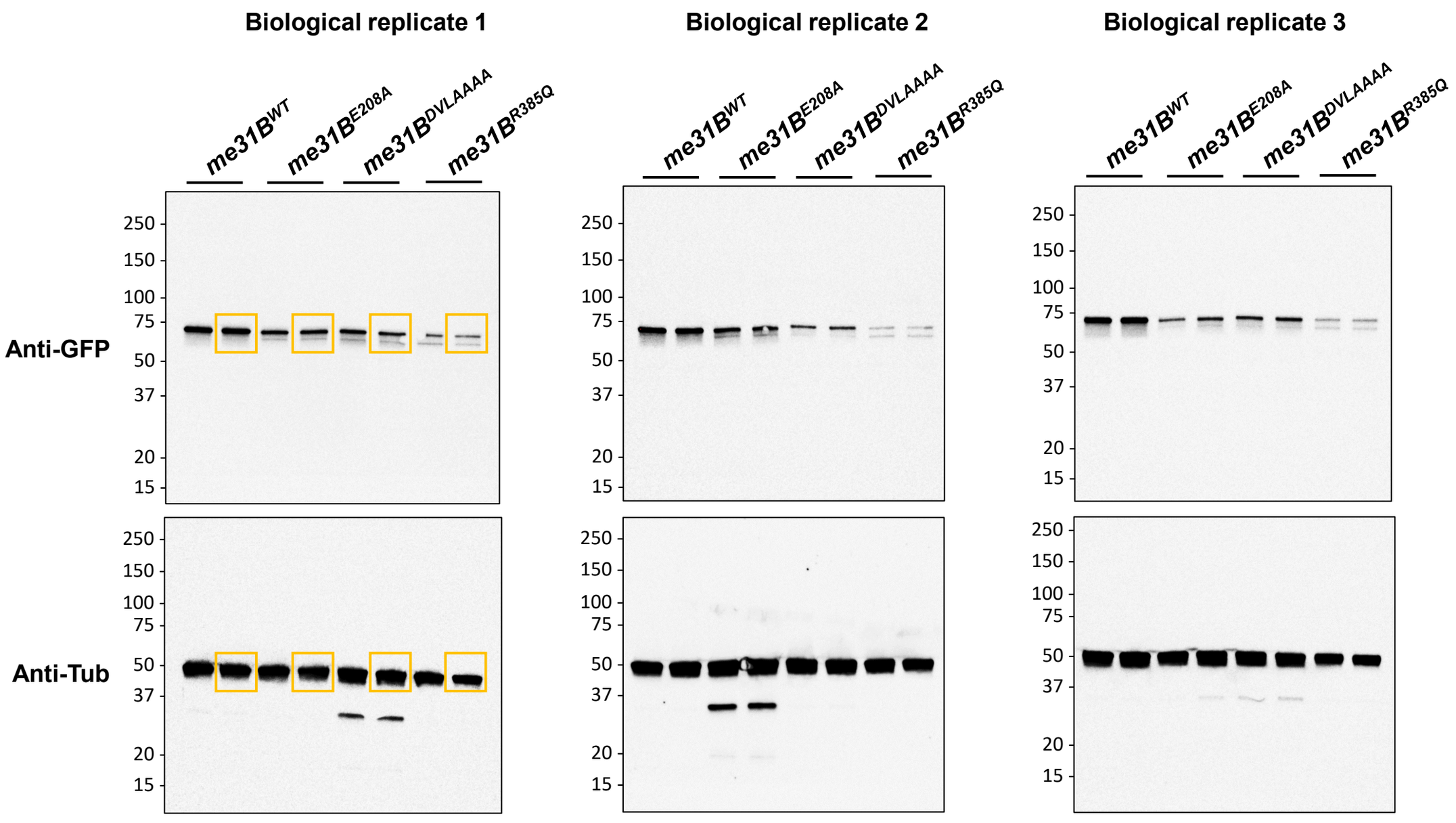
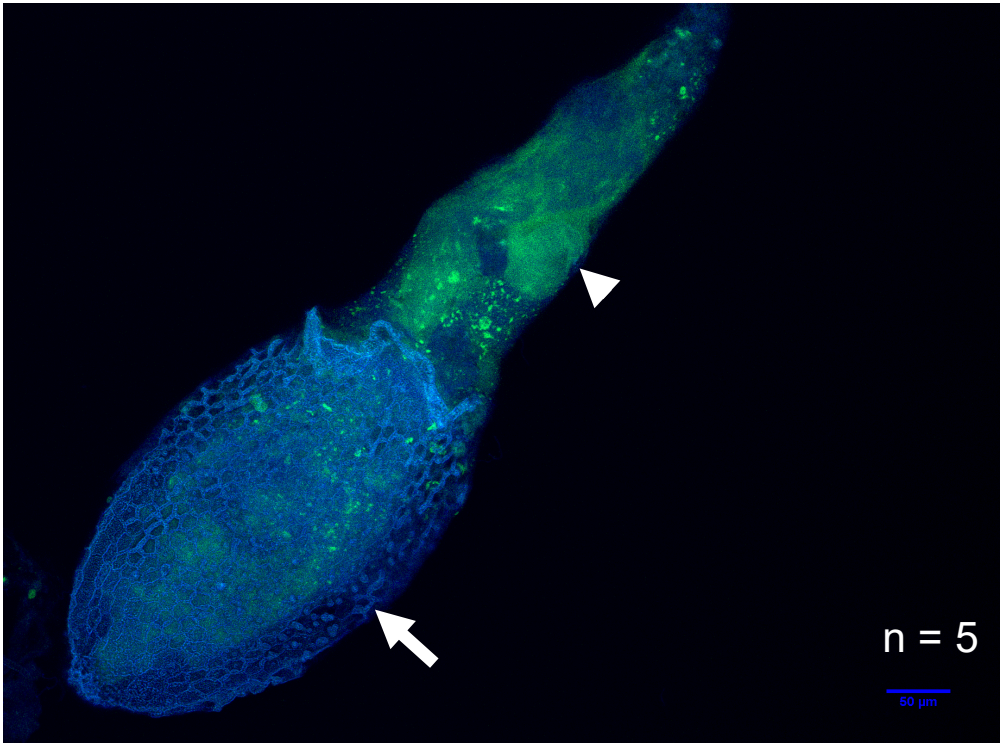
Supplementary Figure 1: Additional and uncropped biological replicate images of main text Figure 3.

Anti-GFP and anti-α tubulin Western blots with *me31B^{WT}*, *me31B^{E208A}* , *me31B^{DVLAAAA}*, *and me31B^{R385Q}* heterozygous fly ovaries. Three biological replicates were performed for each strain. Two technical replicates were run for each biological replicate. The yellow rectangles show the cropped images used in the main text Figure 3.



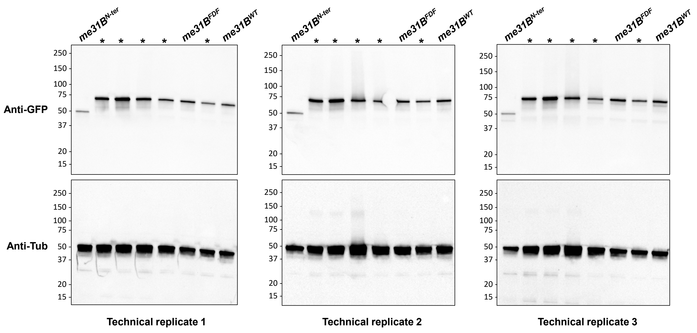
Supplementary Figure 2. *me31B*^{R385Q}/+ mutant has defective egg chamber.



A representative image of an egg chamber from *me31B*^{R385Q}/+ mutant. The blue signals were auto-fluorescent from the developing eggshell, which signifies a late-stage oocyte (likely Stage 13 or 14). The green signals were from Me31B^{R385Q}-GFP. The nurse cell materials that fail to dump were indicated by arrowhead. The developing oocyte (indicated by white arrow) shows severe morphological defects, preventing quantifiable smFISH analysis on its germ plasm RNAs.

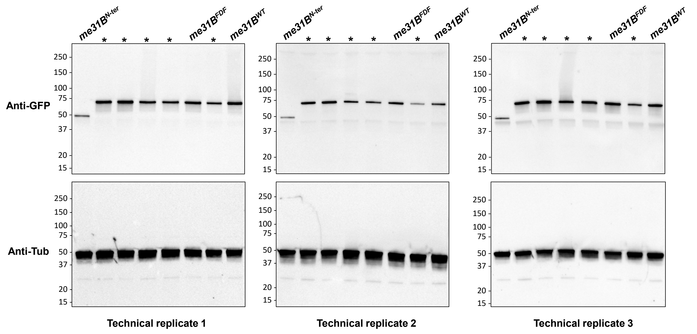
Supplementary Figure 3: Additional and uncropped biological replicate images of main text Figure 6.

Biological replicate 1: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}*, *me31B$^{N\text{-}ter}$* , *me31B$^{C\text{-}ter}$* , *and me31B^{FDF}* ovaries.

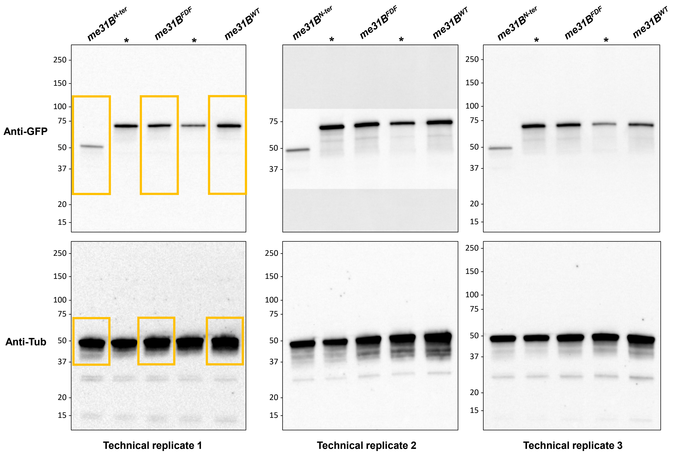


(*Asterisks represent other *me31B* strains not involved in this study. Same below.)

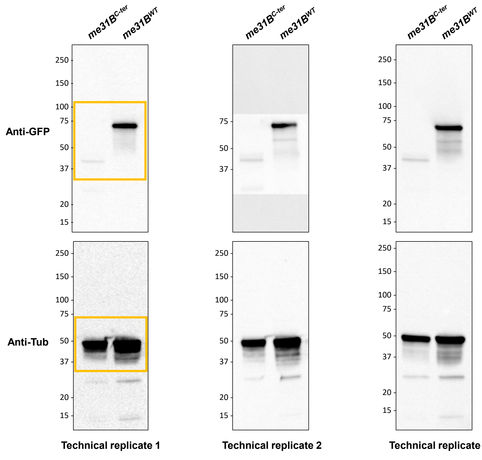
Biological replicate 2: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}*, *me31B$^{N\text{-}ter}$* , *me31B$^{C\text{-}ter}$* , *and me31B^{FDF}* ovaries.



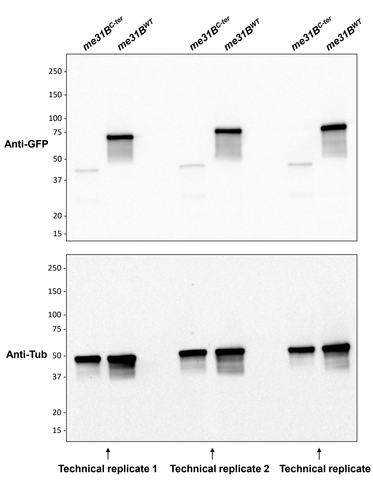
Biological replicate 3: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}*, *me31B$^{N\text{-}ter}$* , *me31B$^{C\text{-}ter}$* , *and me31B^{FDF}* ovaries. The cropped images in main text Figure 5 are highlighted by the yellow squares below.



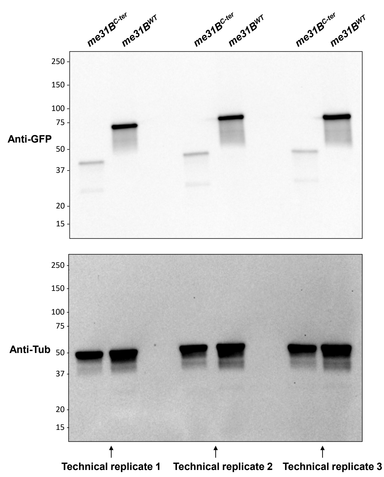
Biological replicate 1: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}* and *me31B$^{C\text{-}ter}$* ovaries. The cropped images in main text Figure 5 are highlighted by the yellow squares below.



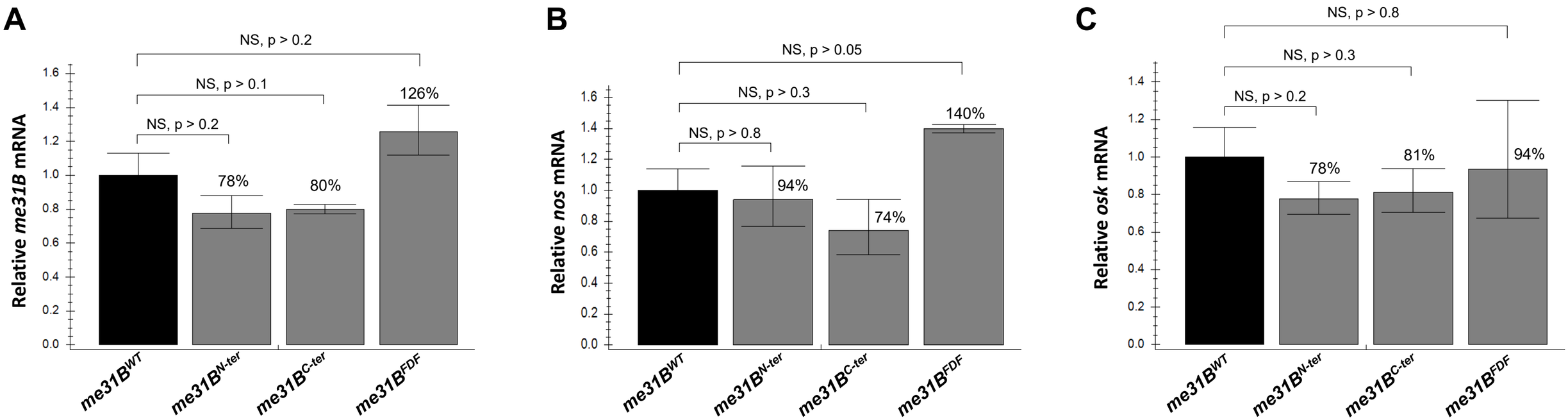
Biological replicate 2: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}* and *me31B$^{C\text{-}ter}$* ovaries.



Biological replicate 3: Anti-GFP and anti-α Tubulin Western blots with *me31B^{WT}* and *me31B$^{C\text{-}ter}$* ovaries.

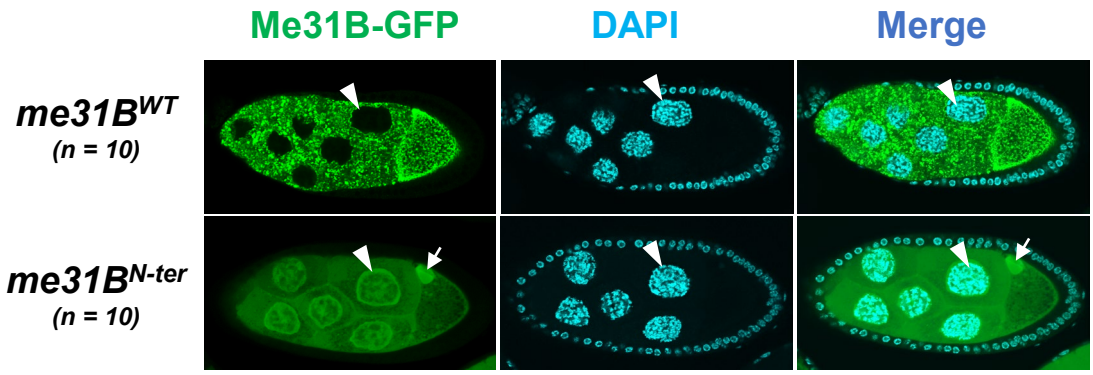


Supplementary Figure 4. RT-PCR analysis of *me31B*, *nos*, and *osk* mRNAs in the *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutants.



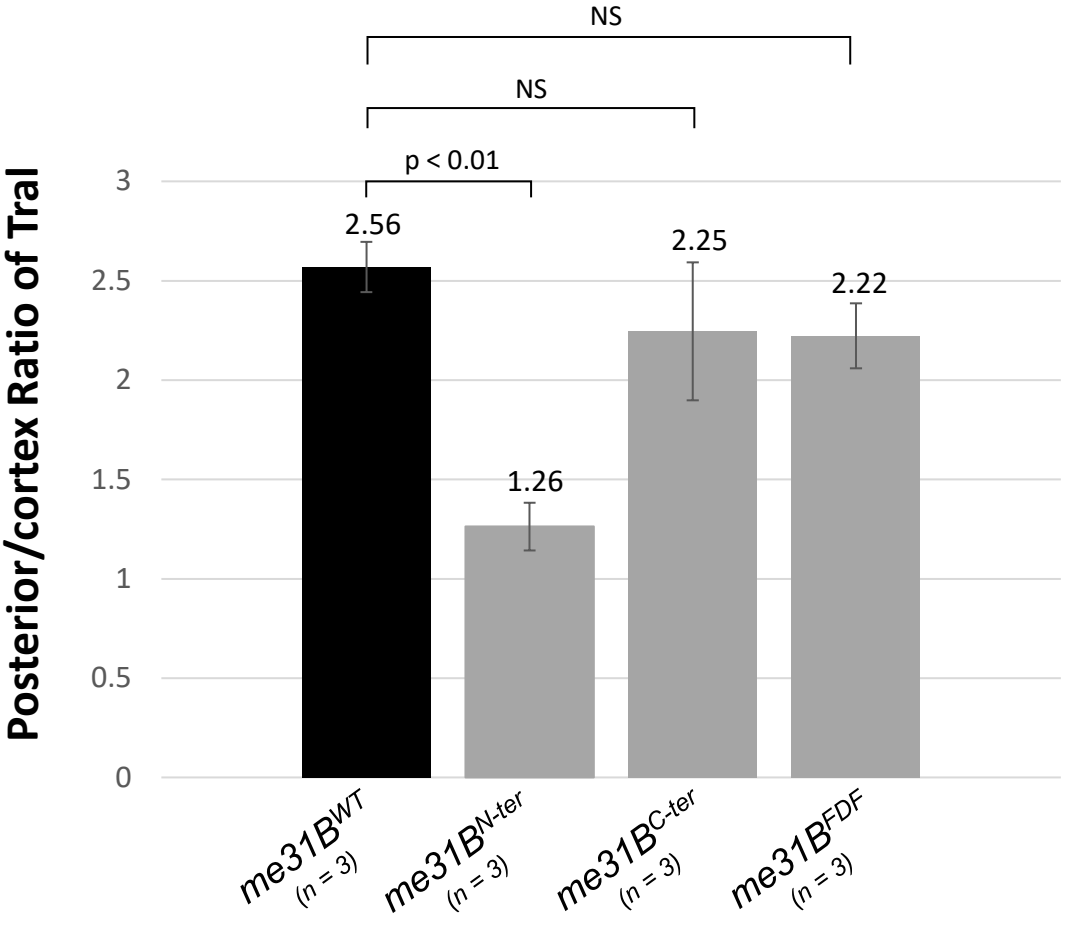
(A) For *me31B* mRNA, the *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutants showed a level of 78%, 80%, and 126% of that in the *me31B*^{WT} control, respectively. The level changes were not significant. **(B)** For *nos* mRNA, the *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutants showed a level of 94%, 74%, and 140% of that in the *me31B*^{WT} control, respectively. The level changes were not significant. **(C)** For *osk* mRNA, the *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutants showed a level of 78%, 81%, and 94% of that in the *me31B*^{WT} control, respectively. The level changes were not significant. NS, not significant. Error bar represents the standard error of the mean. Three biological replicates (each with three technical replicates) were performed for each strain.

Supplementary Figure 5. Me31B$^{N\text{-}ter}$-GFP localize to nurse cell nuclei



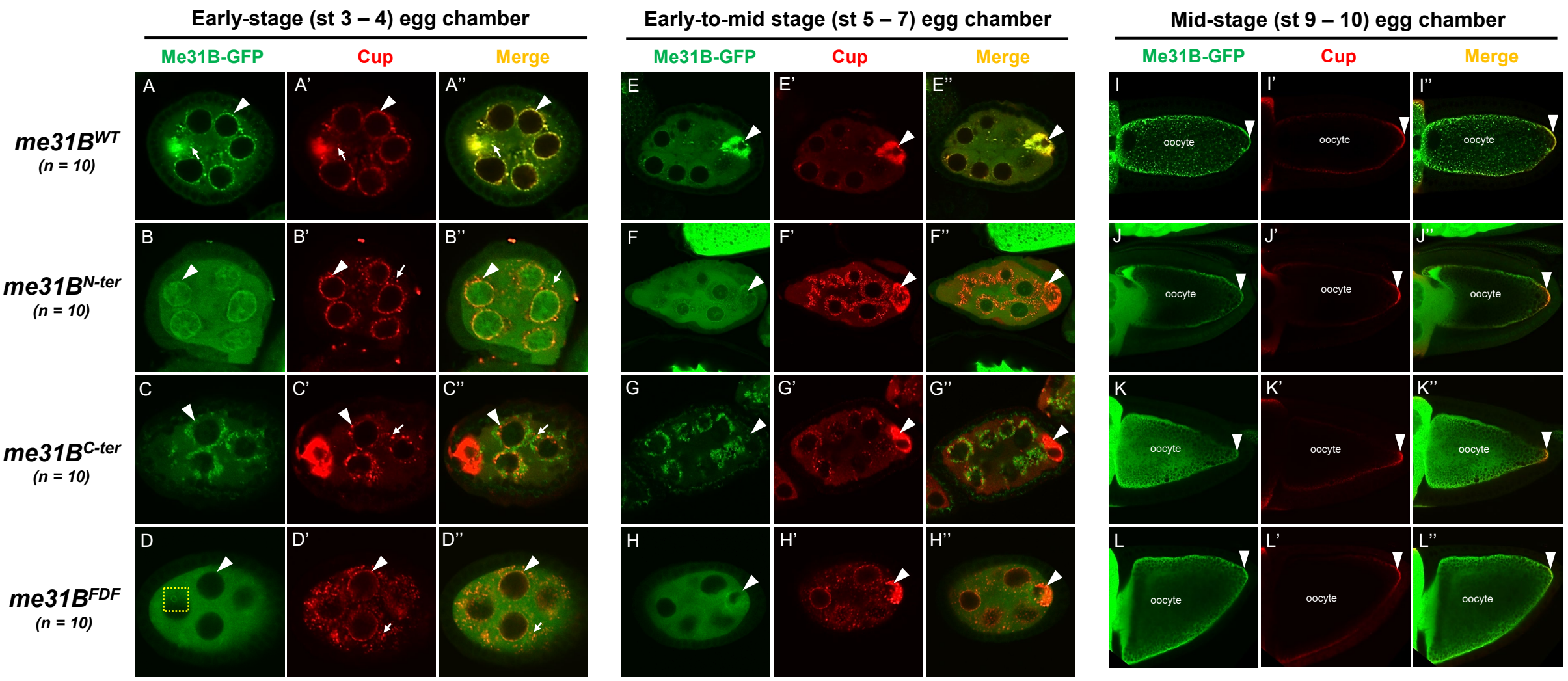
Me31B$^{N\text{-}ter}$-GFP (green channel) proteins are present in the nuclei of nurse cells (indicated by arrow heads) in the *me31B$^{N\text{-}ter}$* strain, while Me31B^{WT}-GFP proteins are found only in the cytoplasm of nurse cells and oocytes. Nucleus DNA are stained by DNA stain DAPI. Nurse cell nuclei are indicated by arrow heads. Interestingly, Me31B$^{N\text{-}ter}$-GFP proteins are also found as a small accumulation in the dorsal-anterior corner of the developing oocyte (indicated by arrows), but this structure does not seem to contain DAPI-stained DNA.

Supplementary Figure 6. Tral enrichment in the posterior (germ plasm area) of mid-stage eggs



The Tral posterior/cortex enrichment ratio (see Materials and Methods) in the *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutants are 1.26 (-50.8%, $p < 0.01$), 2.25 (-12.6%, $p > 0.4$), and 2.22 (-13.5%, $p > 0.1$), respectively, lower than the ratio of 2.56 in the *me31B*^{WT} control. Error bar represents the standard error of the mean.

Supplementary Figure 7. *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} mutations caused distinct Me31B subcellular localization phenotypes without affecting that of Cup



Mutant Me31B proteins in *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, *me31B*^{FDF} strain show altered localization in developing egg chambers. In early-stage egg chambers (left panel, A through D''), mutant Me31B-GFP proteins (green channel) in *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, and *me31B*^{FDF} strains are much more diffused in the nurse cell and oocytes, in contrast to the aggregated status of Me31B^{WT} in RNP granules like nuage granules and P-bodies. And none of the three mutant proteins overlap with partner protein Cup. Unlike Me31B mutant proteins, Cup (Red channel) localization to the RNPs are not affected in the three mutants. Interestingly, Me31B$^{N\text{-}ter}$-GFP proteins are present in the nuclei of nurse cells. Me31B$^{C\text{-}ter}$-GFP proteins form fewer numbers and smaller size granules, and the granules wrapped around the nurse cell nuclei like “thick clouds”. The Me31B$^{C\text{-}ter}$ granules do not associate with Cup-marked granules. Nurse cell perinuclear regions (nuage) are indicated by arrowheads. P-body granules marked by Cup are indicated by arrows. Note that Me31B^{FDF}-GFP proteins were found in ring-like structures that appear to be ring canals (an example is highlighted by the yellow dashed square in D), structures that connect the cytoplasm between nurse cells and oocytes and allow for intracellular transportations. In early-to-mid stage egg chambers (middle panel, E through H''), mutant Me31B-GFP proteins (green channel) in *me31B*$^{N\text{-}ter}$ and *me31B*$^{C\text{-}ter}$ strains do not enrich in the developing oocytes like that in the *me31B*^{WT} control. Me31B^{FDF}-GFP protein’s enrichment in the developing oocytes is much weaker than that in the control. Cup (red channel)’s enrichment in the oocytes is not affected in the three mutants. Developing oocytes are indicated by arrowheads. In mid-stage egg chambers (right panel, I through L''), mutant Me31B-GFP proteins of *me31B*$^{N\text{-}ter}$, *me31B*$^{C\text{-}ter}$, *and me31B*^{FDF} strains localize to the cortex and the germplasm area at the posterior of the oocytes, like the control. However, all three mutant Me31B proteins appear slightly more diffused than the aggregated Me31B^{WT}-GFP proteins in the above areas. The germplasm areas are indicated by arrowheads. Cup protein (Red channel)’s localization to the cortex and germplasm was not affected in the three mutants. For better visualization, the green fluorescence signals of the Me31B$^{N\text{-}ter}$-GFP and Me31B$^{C\text{-}ter}$-GFP proteins were tuned up relative to other images because of the lower expression levels of the proteins in the egg chambers.

Supplementary Table 1. The helicase mutants' embryos do not hatch and show morphological defects.

Strains	Total embryos	Number of embryos with patterning defects (%)	Number of embryos with normal appearance (%)	Number of embryos that developed to larva or later stages (%)
w^{1118}	304	0 (0%)	304 (100%)	265 (87.2%)
me31B^{WT}/+	336	0 (0%)	336 (100%)	325 (96.7%)
me31B^{E208A}/+	541	417 (78%)	124 (22%)	0 (0%)
me31B^{DVLAAAA}/+	100	33 (33%)	67 (67%)	0 (0%)
me31B^{R385Q}/+	17	17 (100%)	0 (0%)	0 (0%)

Supplementary Table 2. Fertility assay daily data log for the *me31B[N-ter]* , *me31B[C-ter]* , and *me31B[FDF]* mutants.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Bio replicate 1	*me31B[WT]* eggs laid	3	35	12	3	6	12	8	4	5	5
	me31B[WT] progenies hatched	2	30	9	2	6	10	7	4	4	4
	me31B[N-ter] eggs laid	58	19	9	1	6	5	0	0	0	0
	me31B[N-ter] progenies hatched	37	13	8	1	6	5	0	0	0	0
	me31B[C-ter] eggs laid	10	7	1	0	1	0	0	0	0	0
	me31B[C-ter] progenies hatched	0	0	0	0	0	0	0	0	0	0
	me31B[FDF] eggs laid	57	26	12	1	2	0	0	0	0	0
	me31B[FDF] progenies hatched	56	26	12	1	2	0	0	0	0	0
Bio replicate 2	*me31B[WT]* eggs laid	78	22	8	9	7	7	1	0	0	0
	me31B[WT] progenies hatched	76	22	8	9	4	7	0	0	0	0
	me31B[N-ter] eggs laid	30	8	8	3	5	5	2	0	0	0
	me31B[N-ter] progenies hatched	27	8	7	2	5	5	2	0	0	0
	me31B[C-ter] eggs laid	1	3	10	0	1	0	0	0	0	0
	me31B[C-ter] progenies hatched	0	0	0	0	0	0	0	0	0	0
	me31B[FDF] eggs laid	31	13	8	0	0	0	0	0	0	0
	me31B[FDF] progenies hatched	27	13	8	0	0	0	0	0	0	0
Bio replicate 3	*me31B[WT]* eggs laid	48	18	16	5	5	10	8	0	0	0
	me31B[WT] progenies hatched	46	18	14	5	5	10	6	0	0	0
	me31B[N-ter] eggs laid	18	16	2	3	6	7	9	6	7	1
	me31B[N-ter] progenies hatched	10	11	1	3	6	5	8	1	1	0
	me31B[C-ter] eggs laid	29	7	0	0	0	0	0	0	0	0
	me31B[C-ter] progenies hatched	0	0	0	0	0	0	0	0	0	0
	me31B[FDF] eggs laid	30	15	9	10	2	2	5	5	2	0
	me31B[FDF] progenies hatched	30	15	9	10	2	2	5	5	2	0
Bio replicate 4	*me31B[WT]* eggs laid	25	13	10	0	8	18	9	5	6	5
	me31B[WT] progenies hatched	24	13	10	0	8	18	9	4	6	5

	me31B[N-ter] eggs laid	22	7	7	11	4	0	0	0	0	0
	me31B[N-ter] progenies hatched	18	4	4	1	0	0	0	0	0	0
	me31B[C-ter] eggs laid	13	16	4	11	0	0	0	0	0	0
	me31B[C-ter] progenies hatched	0	0	0	0	0	0	0	0	0	0
	me31B[FDF] eggs laid	fly dead or lost during transfer*									
	me31B[FDF] progenies hatched	fly dead or lost during transfer*									
Bio replicate 5	*me31B[WT]* eggs laid	47	24	18	18	6	8	8	10	5	6
	me31B[WT] progenies hatched	45	20	13	13	5	6	6	8	3	4
	me31B[N-ter] eggs laid	9	6	9	6	4	6	5	2	3	3
	me31B[N-ter] progenies hatched	6	5	7	5	3	3	4	0	1	0
	me31B[C-ter] eggs laid	fly dead or lost during transfer*									
	me31B[C-ter] progenies hatched	fly dead or lost during transfer*									
	me31B[FDF] eggs laid	fly dead or lost during transfer*									
	me31B[FDF] progenies hatched	fly dead or lost during transfer*									

*Experiments that could not be completed because of fly death or lost during transfer were excluded from the final analysis