



**Figure 5 – figure supplement 2. SMC and female gametophyte identity markers in *katanin*.**

(A) Representative images and quantification of WT (*Ws-4*) and *kat (bot1-7)* ovule primordia expressing *pKNU::nls-YFP* (yellow signal), specifically expressed in the SMC. *kat* ovules show ectopic subepidermal cells with YFP signal, confirming SMC fate acquisition. Related to [Figure 5E](#).

(B) Representative images of WT (*Ws-4*) and *kat (bot1-7)* ovule primordia stained with aniline blue callose dye, and visualized with DIC and epifluorescence microscopy. Callose accumulates specifically in the SMC prior to meiosis (stage 2-IV) and in spore walls of a single tetrad (stage 3-I). In all ovules with callose positive cells (blue signal), staining was observed in a single cell or tetrad, in both wild-

type and *katanin* background. Representative *kat* ovules with ectopic enlarged cells which do not display callose are shown (white arrows). Quantification of the proportion of ovules at stages 2-IV and 3-I displaying or lacking callose accumulation, in WT (Col or *Ws-4*) and *kat* (*lue1* or *bot1-7*), suggesting altered callose deposition in *kat* SMC. Related to [Figure 5F](#).

(C) Representative images (ca 10µm maximum intensity projections of image stacks) and quantification of WT (*Ws-4*) and *kat* (*bot1-7*) ovule primordia expressing *pWOX2:CenH3-GFP* (green signal), labeling centromeres from the functional megaspore stage (FG1) onward. Cells ectopically expressing the marker are detected in *kat* at both early (before centromeres condensation – left panel) and late (after centromeres condensation – middle panel) stages of functional megaspore formation. Ectopic spores were scored based on the presence of a cell wall (white arrows) separating the spores, by contrast to two-nuclei gametophyte stage (right image, shown as an example) where the two nuclei are separated by a vacuole (arrow head). Note that ectopic spores are aligned with the normal, most basal (chalazal) FM, suggesting that they belong to the same tetrad. They also display a similar number of centromeres (5) visible on the projections. Quantification was performed on pooled early and late FG1 stages. Related to [Figure 5D-F](#).

(D) Representative images and quantification of WT (*Ws-4*) and *kat* (*bot1-7*) ovule primordia expressing *pAKV:H2B-GFP* (yellow signal), labeling nucleus from the functional megaspore stage (FG1) to the mature female gametophyte stage (FG7). Spores ectopically expressing the marker are detected in *kat* at significant levels at FG1 stage (left panel and graph below), and non-significantly at stage FG2 (middle panel and graph below). From stage FG4 to stage FG7, *katanin* embryo sacs are often abnormal, however conspicuous expression of *pAKV:H2B-GFP* notably in antipodals cells (arrows, right panel) marked always a single embryo sac in *kat* ovules (noted as “none” ectopic FG in the graph below). Related to [Figure 5D-F](#).

White signal: Renaissance SR2200 cell wall label. Scale bar: 10µm. n: number of ovules scored. Error bar: standard error of the mean. Two-tailed Fischer’s exact test was used to assess differences between genotypes. P values: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ . See also [Figure 5 - Source Data 1](#).