### Supplemental Figures, Table and Movie

### Figure S1. Misoriented GSCs are not permanently arrested.

- A. BrdU labeling outcome after 5 days of feeding (at day 10 of age). Almost all GSCs (and other germ cells) were labeled.
- **B.** BrdU dilution during the chase period. Labeling completely disappeared 5 days later. Data are expressed as mean  $\pm$  SD.

### Figure S2. Increase in frequency of LacZ<sup>+</sup> GSCs with age.

Mean (%)  $\pm$  SD. Number of the GSCs counted for each time point is shown at the top of the graph.

# Figure S3. Examples of LacZ- (left) and LacZ+ (right) dedifferentiation.

Flies were irradiated with 2000 or 3000 rad and analyzed 24 hours later. Remnant of Ring canal (arrowhead) and disintegrating fusome (arrow) are indicated. Red; LacZ and Adducine. Green; Anillin. Blue; Vasa. Bar; 10 $\mu$ m. Hub (\*).

Table S1. Centrosome misorientation frequency and GSC number in older flies.

Strain <sup>1</sup> ; age	Centrosome	GSC number	% survival at the day of
	misorientation %	/testis <sup>2</sup>	sampling
	(n=GSCs counted)	(n=testes counted)	-
<i>yw</i> ; 42 days +YP <sup>3</sup>	35.5% (n=332)	6.54±2.2 (n=59)	9.1% (~40/440)
yw; 42 days	39.1% (n=64)	5.7±2.42 (n=13)	3.1% (~10/320)
LacZ <sup>4</sup> ; 40 days	42.3% (n=234)	6.36±1.89 (n=69)	42.9% (~180/420)
LacZ⁴; 50 days	45.1% (n=417)	6.11±1.5 (n=81)	31.0% (~100/320)

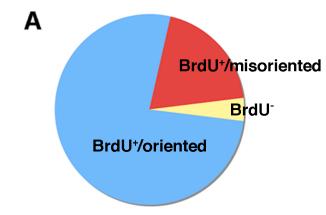
<sup>1.</sup> Strains used; yw, as used in Figure 1, 2 and 3. LacZ, as used in Figure 5 and 6

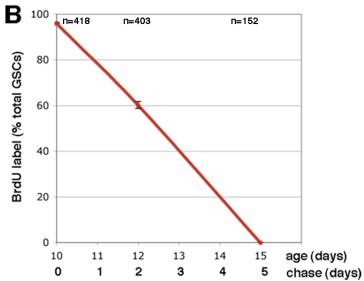
- 2. GSC number ± SD / testis
- 3. +YP; yeast paste was supplemented for better survival.
- 4. Centrosome misorientation and GSC number of LacZ strain were comparable to those of *yw* at day 0 (9.0% misorientation (n=311 GSCs) and 9.3±1.5 GSCs/testis (n=44 testes)).

# Movie S1. Time-lapse live imaging of centrosome behavior in a misoriented GSC.

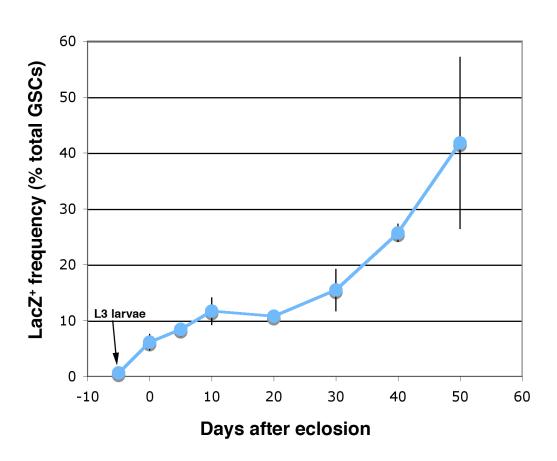
Centrosome movement was tracked by Sas-6-mCherry. Hub (\*) and two centrosomes (red and blue arrowhead) in an originally misoriented GSC (surrounded by yellow dotted line) are marked in 5 frames during the movie. The GSC entered mitosis right after centrosome re-orientation occurred. One second corresponds to 6 min in a real time.

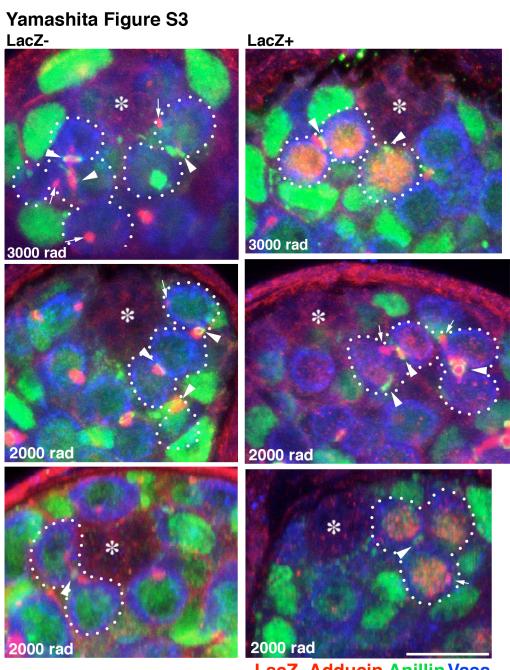
## Yamashita Figure S1





# Yamashita Figure S2





LacZ, Adducin Anillin Vasa