

Fig. S1: Expression of mitotic and endocycle markers in MES cells.

All egg chambers are from normally fed flies. **A)** MES egg chambers show overlapping expression of the N activity reporter line NRE-LacZ (red) and of Hnt (green) and Cut (grey). DAPI (cyan). **B)** The arrowhead indicates Stg-LacZ expression in the MES egg chamber. DAPI (cyan), hnt (red) and Stg-LacZ (green). **C)** DAPI (cyan), Cut (red) and Fzr-LacZ (green). **D)** Arrowheads indicate P-H3, Cyclin B and Fzr-LacZ expressing cells in MES egg chambers. P-H3 (grey), Cyclin B (red) and Fzr-LacZ (green). **E)** Arrowheads indicate P-H3 and Cyclin B expressing cells in MES egg chambers. Cyclin B (grey), NRE-LacZ (red) and P-H3 (green). **F)** Arrowheads indicate EdU incorporation in a few cells from MES egg chambers. EdU (grey), Cut (red) and NRE-LacZ (green). **G)** Cyclin B (red), Fzr-LacZ (green) and P-H3 (grey). Scale bar: 50 μm .

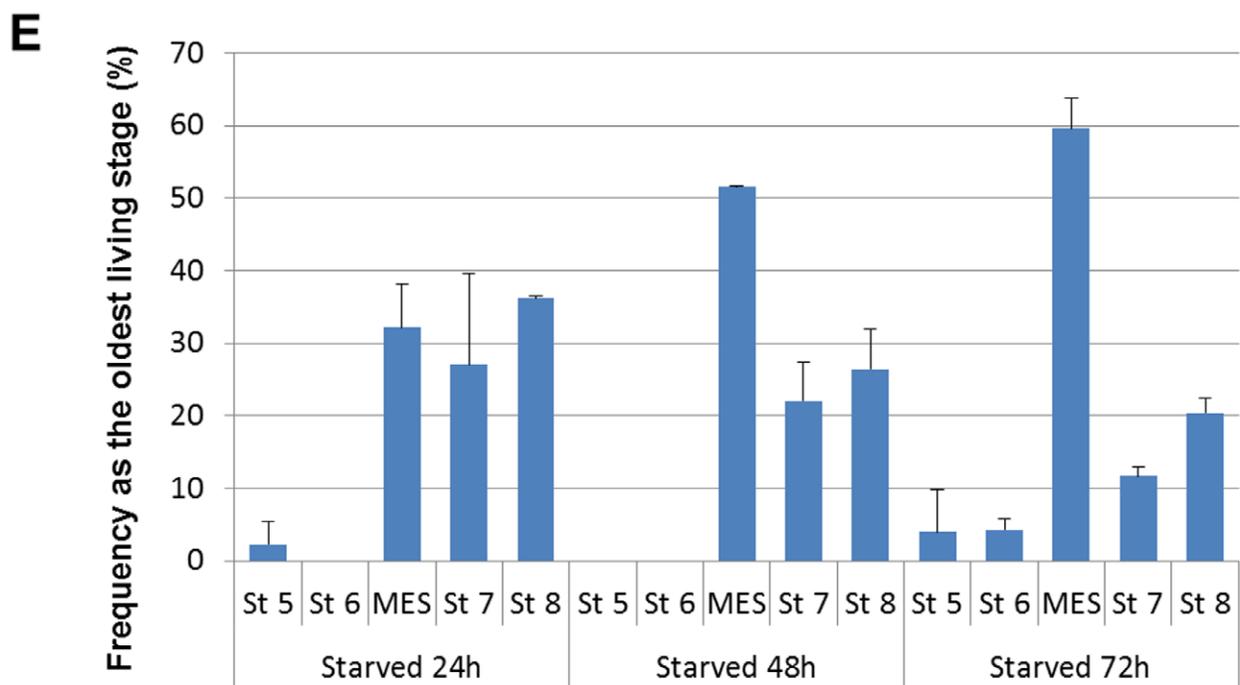
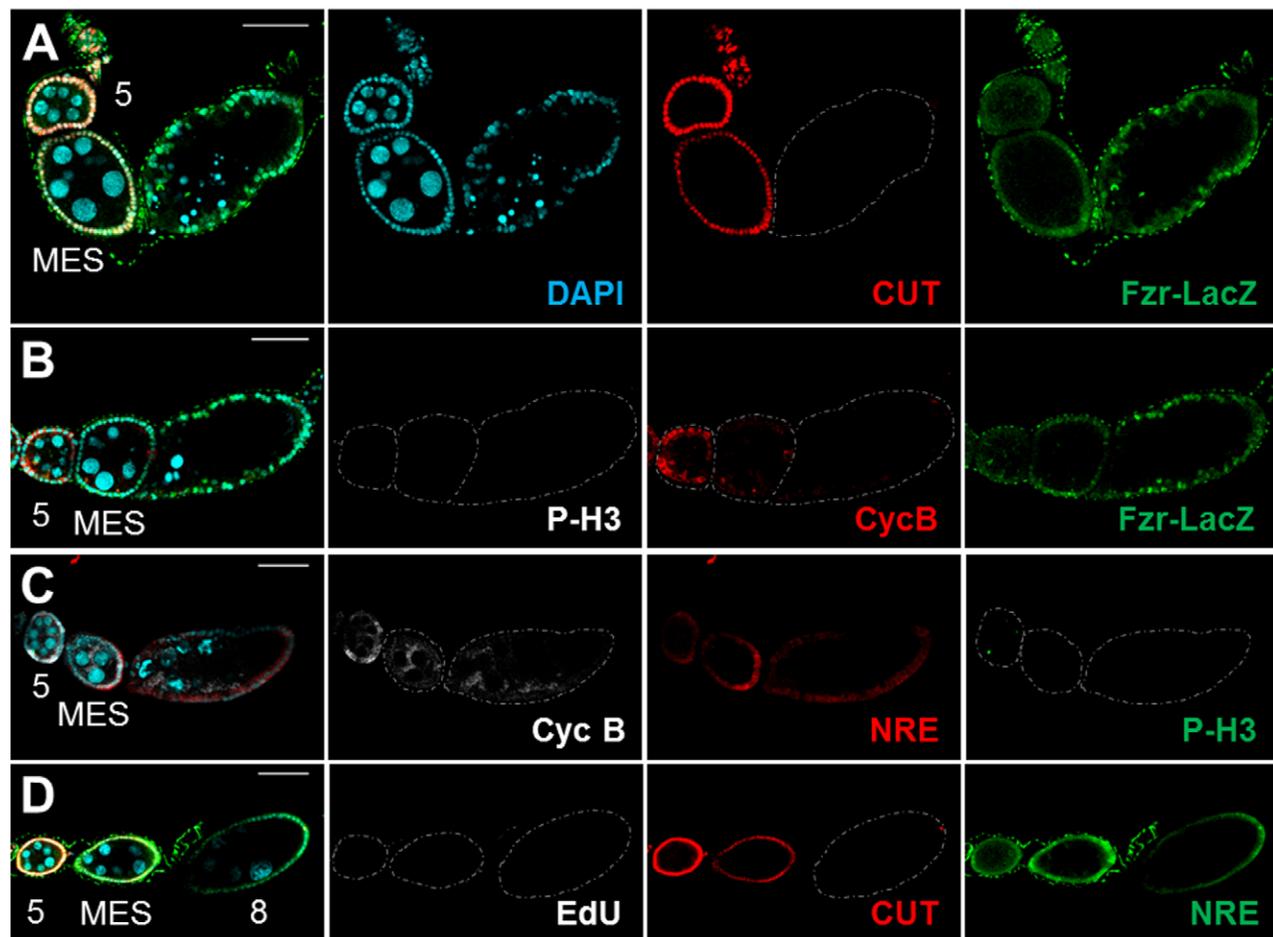


Fig. S2: Starvation-induced paused MES are in the same cell cycle state as MES.

All egg chambers are from flies starved for 24 hrs. **A**) DAPI (cyan), Cut (red) and Fzr-LacZ (green). **B**) P-H3 (grey), Cyclin B (red) and Fzr-LacZ (green). **C**) Cyclin B (grey), NRE-LacZ (red) and P-H3 (green). **D**) EdU (grey), Cut (red) and NRE-LacZ (green). **E**) Distribution of egg chambers occupying the last position before degenerating egg chambers. The results show a progressive increase in paused MES egg chambers over time. Scale bar: 50 μ m.

flip-out:UAS-GFP

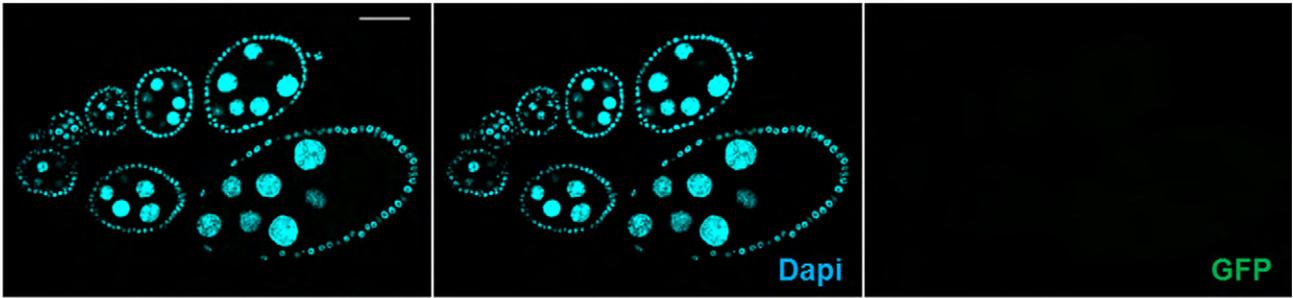


Fig. S3: Re-feeding triggers the entry into the endocycle.
The line used to generate flip-out clones (in Fig. 3) does not show leakiness.

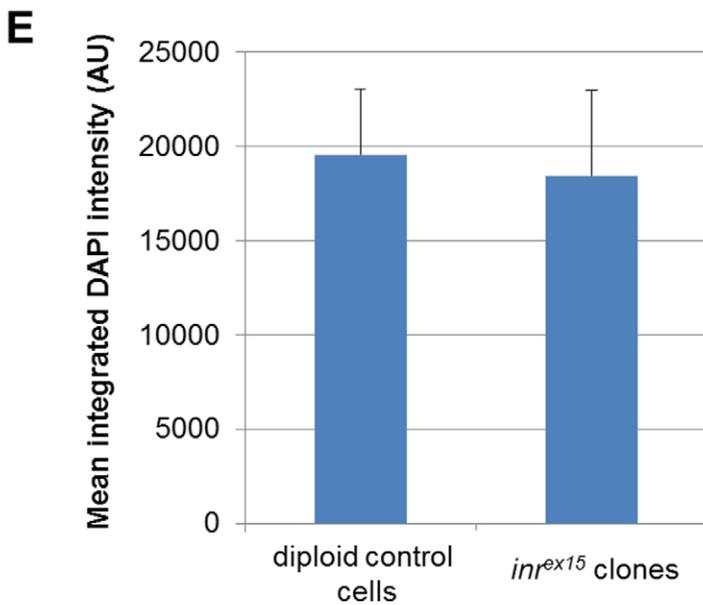
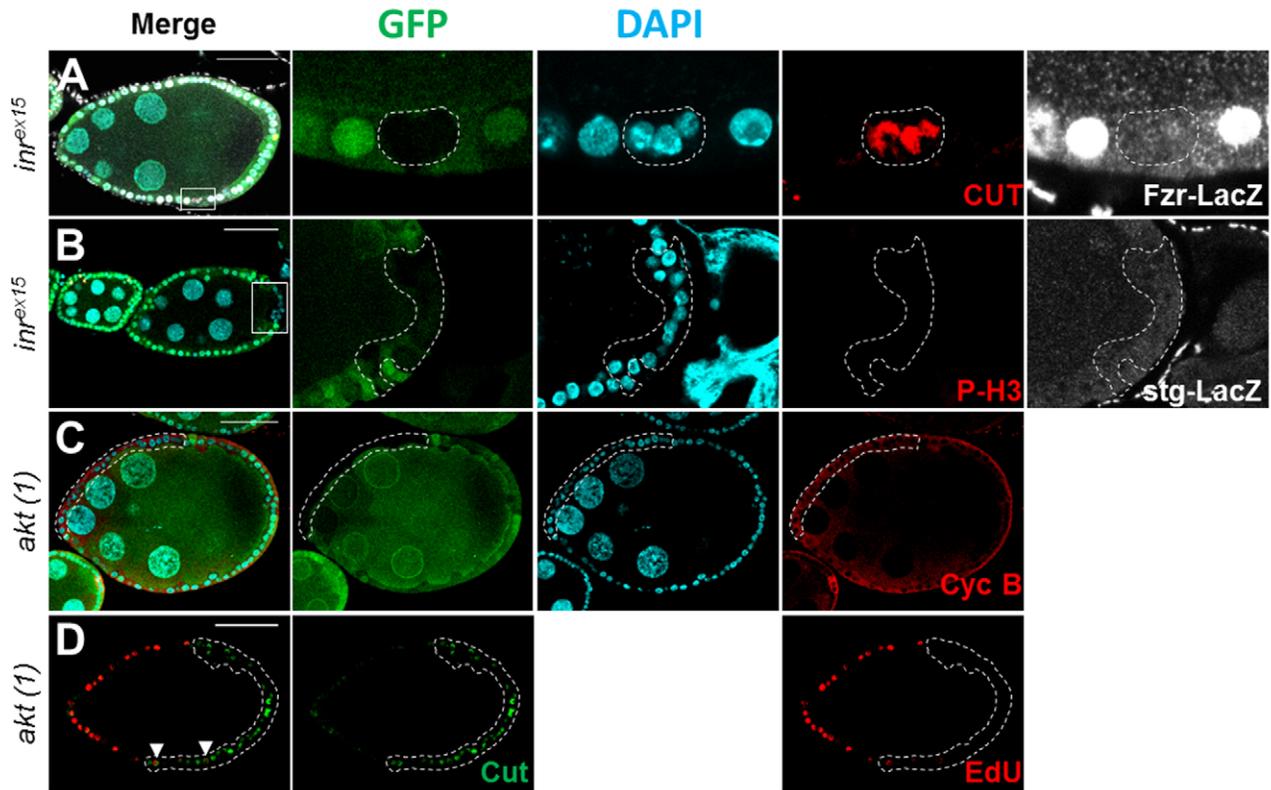


Fig. S4: Loss of function for IIS induces paused MES cells with intermediate cell cycle phenotype.

(A-D) Cross-section of *dinr* (A,B) and *dakt* (C,D) loss-of-function clones generated in stage 8 egg chambers. Mutant follicle cells are outlined and marked by the absence of GFP (green) or by Cut expression. Mutant clones are negative for *fzr-lacZ* (grey) (A), *stg-lacZ* (grey) and P-H3 (red) (B), CycB (red) (C). D) Some rare mutant cells show weak EdU (red) staining (arrow-head) and express Cut (green). E) Histogram showing the integrated DAPI intensity from control diploid cells or from stage 7-9 egg chambers of *inr^{ex15}* mutant cells. The results show that they are not statistically different. $N \geq 50$, $P^{N.S.} = 0.166$. Scale bar: 50 μ m.

UAS: hfoxo^{3a}-TM; UAS: cut^{RNAi}

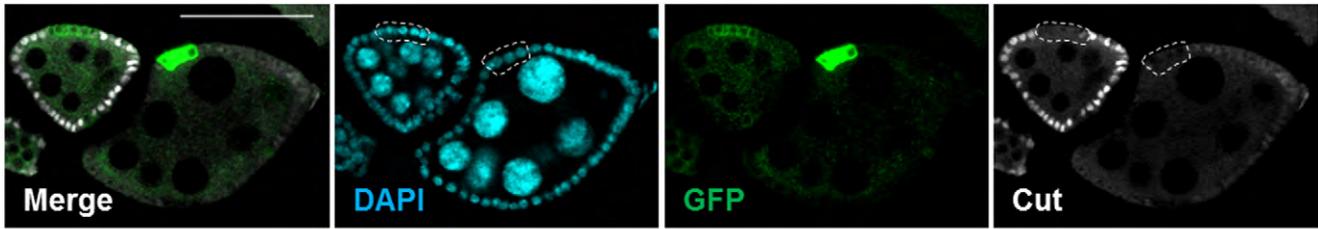


Fig. S5: Efficiency of *cut* RNAi silencing.

Cut expression in flip-out clones from stage 5 and 7 egg chambers expressing *cut* RNAi in a *foxo3A* (activating) background (*act<<gal4-UAS:GFP; UAS-hfoxo3a-TM; UAS-cut^{RNAi}*). DAPI (cyan), GFP (green), Cut (grey). Scale bar: 50 μ m.

Table S1: Determination of MES stage duration

	<i>n</i>	Frequency (%)	Duration (hrs)
Stage 5	230	48.5	5
MES stage	95	20	2

MES duration was estimated by its relative frequency compared with stage 5 within each ovariole (3 independent experiments, $n > 100$ in each experiment). Results show that MES are observed in 20% of ovarioles, compared with 48.5 % for stage 5 egg chambers (25°C; NRE>GFP/CyO genetic background). Therefore, the duration for MES is estimated at 2 hours, well consistent with the transient nature of MES.