

## Supplemental video legends

Video S1. Fly restrained in Bellymount-PT mount kept on the bench for about 16-hour can lay eggs and stay active.

Video S2. A wider view of a mount kept at room temperature showing multiple females kept on bench for about 16-hour.

Video S3. A fly that survived 16-hour of imaging is still active and eggs deposited during the imaging period are visible in the mount.

Video S4. View through z-stacks across stage 6 egg chamber imaged on the multiphoton microscope for a single time frame. The egg chambers are aligned along the anterior-posterior axis horizontally (Scale bar = 50  $\mu\text{m}$ ) (**Figure 2c**).

Video S5. 3D view of two early egg chambers from **Figure 2d**, The egg chambers are aligned along the anterior-posterior axis horizontally (Scale bar = 10  $\mu\text{m}$ ).

Video S6. 3D reconstruction of two early egg chambers from **Figure 2d**, 3D surface without H2Av-mRFP signal. The egg chambers are aligned along the anterior-posterior axis horizontally (Scale bar = 10  $\mu\text{m}$ ).

Video S7. Stage 10 and 11 staged egg chambers imaged every 10-minute for 16-hour. The egg chambers could be tracked for more than 6-hour (Scale bar = 50  $\mu\text{m}$ ).

Video S8. Stage 10 and 12 staged egg chambers imaged every hour for 16-hour (Scale bar = 50  $\mu\text{m}$ ).

Video S9. Tracking multiple egg chambers for 14-hour through Bellymount-PT. This wider view demonstrates the movement of the egg chambers tracked during long term imaging. The female is aligned along the anterior-posterior axis vertically. (Scale bar = 100  $\mu\text{m}$ ) (**Figure 2g**).

Video S10. Tracking a stage 10 egg chamber through nurse cell dumping and dorsal appendage formation. The egg chamber is aligned along the anterior-posterior axis vertically (Scale bar: d = 100  $\mu\text{m}$  d' = 50  $\mu\text{m}$ ) (**Figure S2d, d'**).

Video S11. Tracking a young pre-vitellogenic egg chamber The egg chamber is aligned along the anterior-posterior axis vertically (Scale bar = 25  $\mu\text{m}$ ) (**Figure 3c**).

Video S12. Tracking egg chamber during active vitellogenic stage. The egg chamber is aligned along the anterior-posterior axis vertically (Scale bar = 25  $\mu\text{m}$ ) (**Figure 3e**).

Video S13. The egg chamber rotation in a stage 8 egg chambers could be visualized. Five follicle cell nuclei per egg chamber were tracked (Scale bar = 25  $\mu\text{m}$ ). These nuclei showed an angular velocity of 28.53 degree/hour.

Video S14. Follicle cells from multiple regions of a stage 12 the egg chamber were tracked during epithelial morphogenesis prior to dorsal appendage formation (Scale bar = 50  $\mu\text{m}$ ).

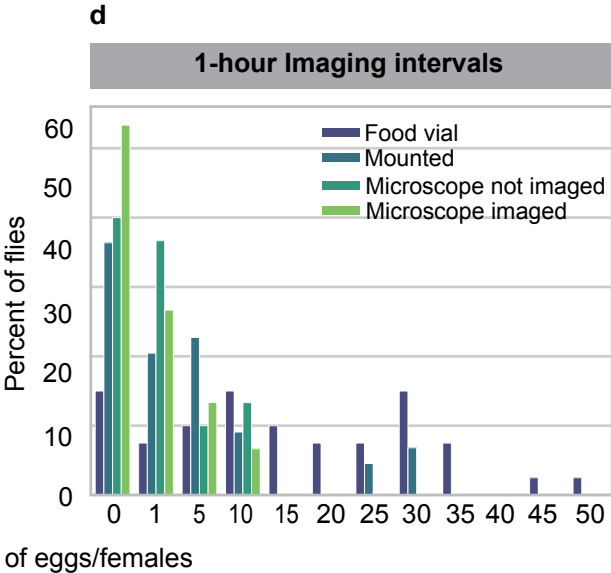
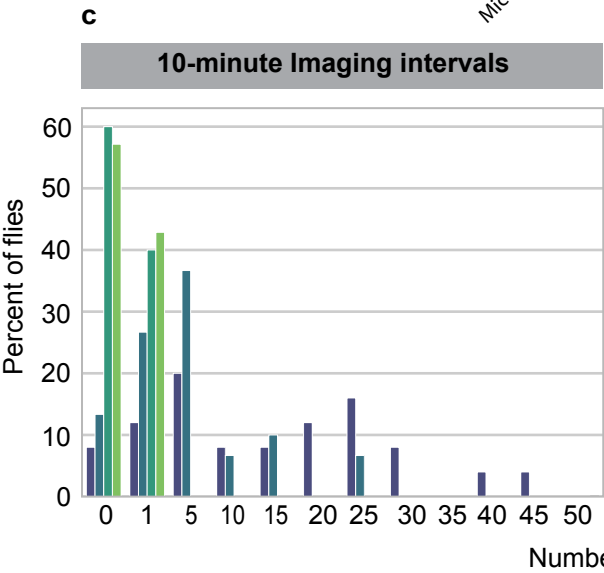
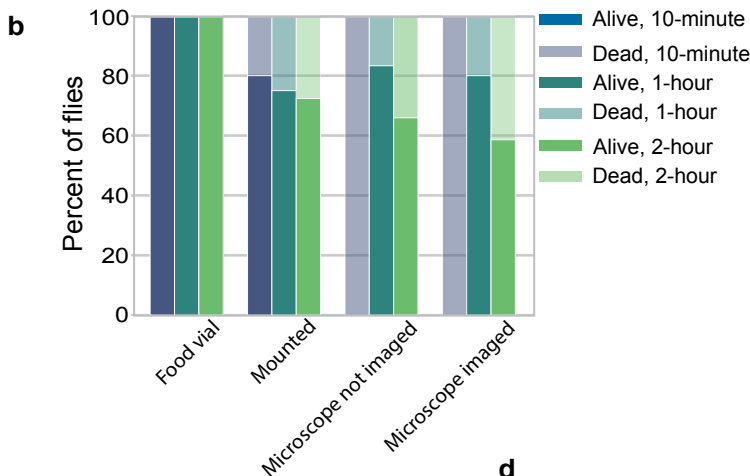
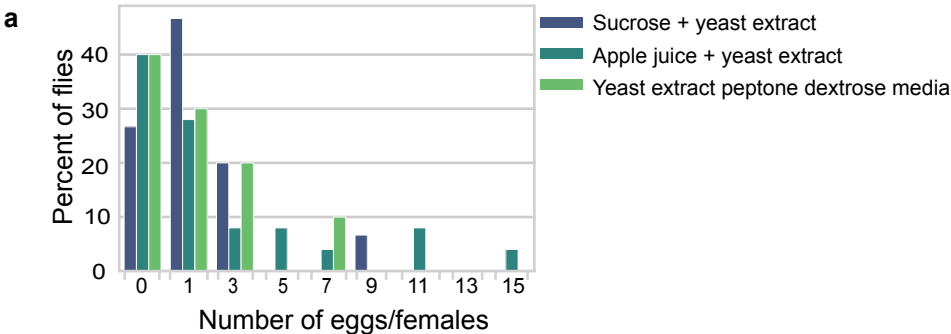
Video S15. Yp1-sfGFP uptake by a stage 8 egg chamber (Scale bar = 25  $\mu\text{m}$ ) (**Figure 4a**).

Video S16. Early stages of nurse cell dumping and transfer of H2Av-mRFP (Scale bar = 50 $\mu\text{m}$ ) (**Figure 5a**).

Video S17. Late-stage nurse cell dumping and transfer of H2Av-mRFP (Scale bar = 50  $\mu\text{m}$ ) (**Figure 5c**).

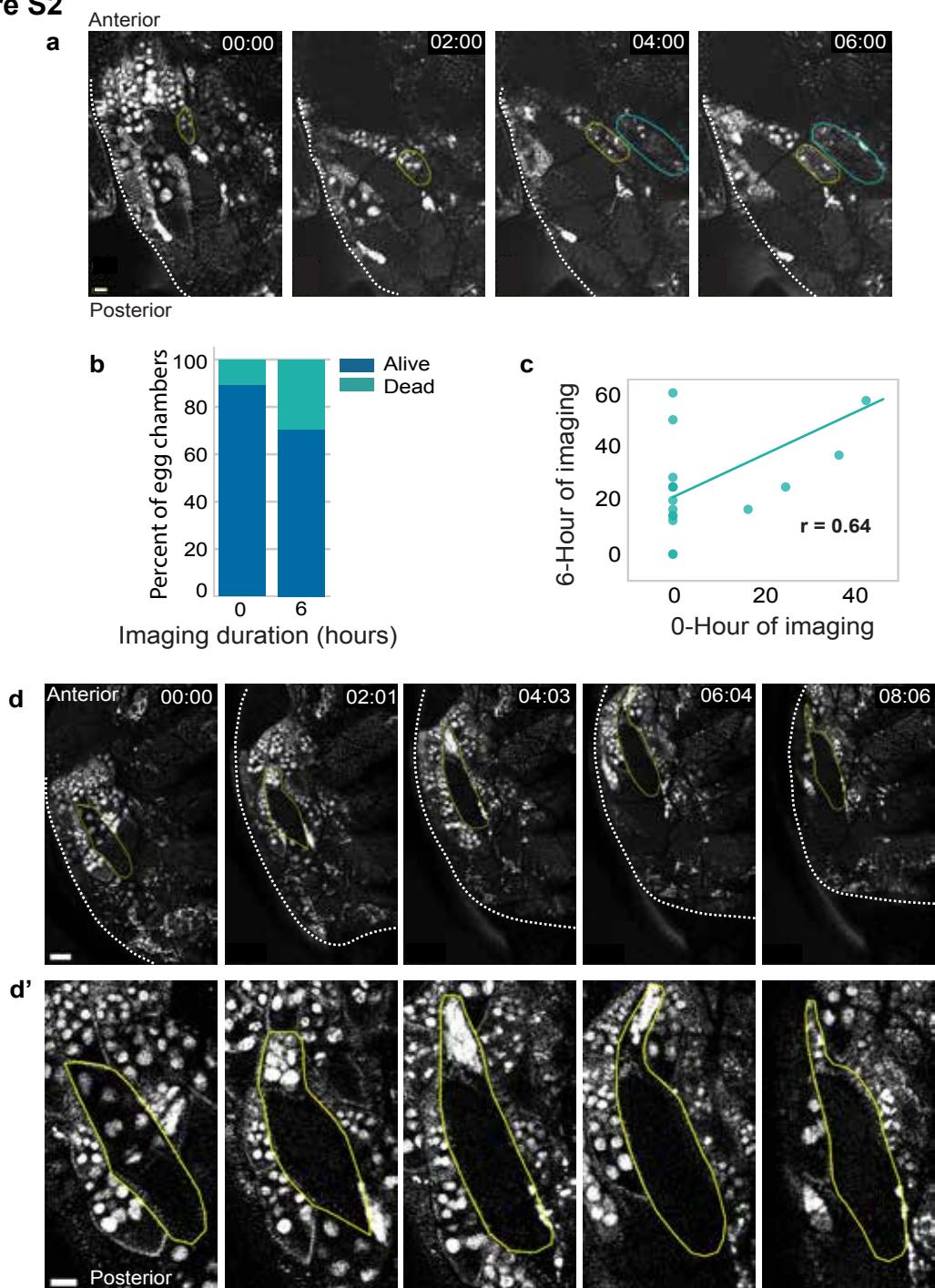
Videos S18-S19. Additional examples of nurse cell dumping and transfer of H2Av-mRFP (Scale bar = 50  $\mu\text{m}$ ).

**Figure S1**



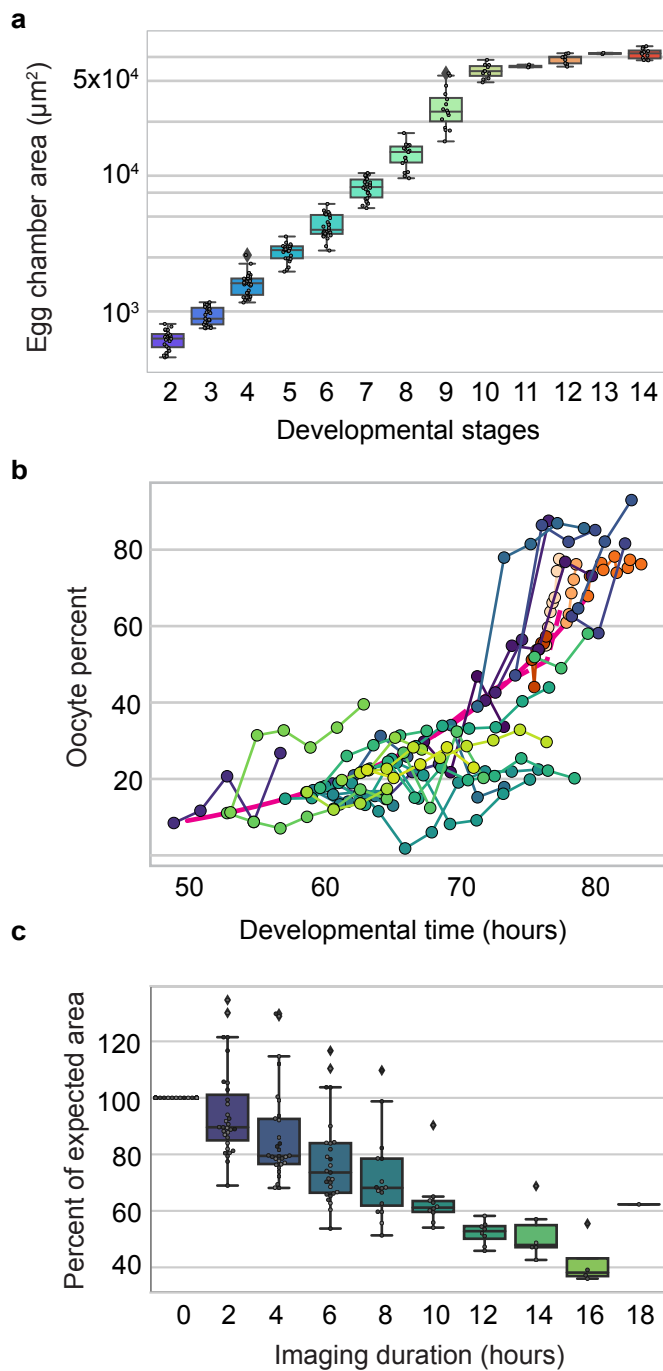
**Figure S1.** Liquid diet and CO<sub>2</sub> regime on fly health during imaging. (a) Different liquid foods were tested for their ability to support oogenesis during long term imaging. We tested combination of glucose and protein diet in a liquid media to ensure the flies are hydrated and well fed. We used 10% sucrose and yeast extract (n = 15), apple juice extract with yeast extract (n = 25) and yeast extract peptone dextrose media (YPD) (n = 10). We added a few grains of active yeast into each solution. Though we did not observe any significant difference in the average eggs laid by females fed with these diets ( $p < 0.05$ ) maximal egg production occurred on apple juice with yeast extract. (b) Influence of imaging frequency on survival during long-term imaging. Increasing the imaging frequency to every hour compared to 2-hour did not increase the mortality. However, further increase in frequency to every 10-minute for 16-hour lead to 100% mortality. (c) 10-minute imaging interval adversely affected fecundity (n = 7). Females laid an average of  $0.43 \pm 0.2$  eggs (SEM) during the imaging duration. (d) Though imaging every hour did not increase the mortality, but it did reduce fecundity compared to 2-hour intervals (Fig. 2B) (n = 15). Females imaged every hour laid on average of  $1 \pm 0.43$  eggs (SEM) during the 16-hour of imaging.

**Figure S2**



**Figure S2.** Tracking egg chambers over time. (a) A representative image of initial (t0) and 6-hour (t3) time frames of an overnight Bellymount-PT imaging to show egg chamber degeneration at the start of the imaging session and 6-hour into the imaging duration. Two egg chambers are tracked are mark with blue and yellow outline. (b) Percent of degenerating egg chamber at 0 (n = 130) and 6-hour (n = 129) from 19 females with 2-hour imaging intervals (Scale bar = 50  $\mu$ m). (c) Positive correlation between the survival status of the egg chambers at the 0-hour and 6-hour ( $r = 0.6362$ ). (d) A wider view depicting the movement of egg chambers during the imaging intervals (Scale bar = 100  $\mu$ m). (d') Cropped images from **Figure S2d** with a view of a tracked egg chamber (Scale bar = 50  $\mu$ m).

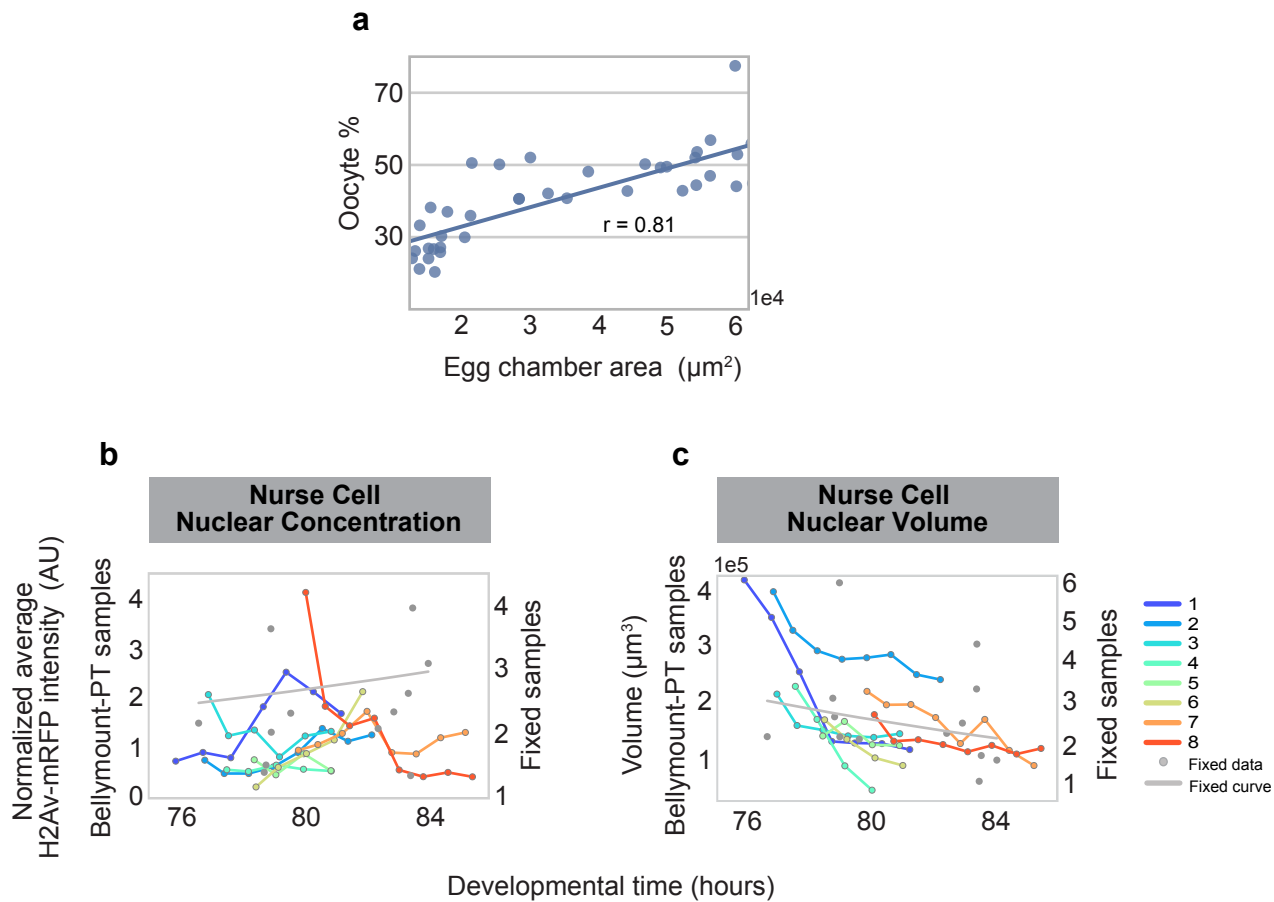
**Figure S3**



**Figure S3. Validating the reliability of Bellymount-PT for growth measurements.** (a) The midsection area of fixed egg chambers plotted across the developmental stages. Fixed egg chambers were staged based on milestones of oogenesis as per ((King 1970) see methods). The measured sizes combined with the frequency of each stage, total number of egg chambers per ovariole, and the number of eggs laid by individual females in 24-hour were used to construct a standard growth curve (solid black line in **Figure 3a**) for the fly line that we used for live growth analysis (w; Mat- $\alpha$ -tub67-gal4/CyO; Mat- $\alpha$ -tub15-gal4, H2Av-mRFP/TM3). Y-axis is in log scale. (b) The oocyte percent from the overnight Bellymount-PT imaged egg chambers were fit to a standard curve generated in **Figure 4c**. Magenta dotted line represents the fixed samples used to generate the curve and solid magenta line represents the curve. In this plot we included samples from 2-hour imaging intervals and samples from 10-minute imaging intervals to test the effect of imaging frequency on the egg chamber growth. Samples from 2-hour imaging intervals are in blue-green shades and 10-minute imaging is depicted in shades of orange. (c) Box plots of the percent of expected area each egg chamber had achieved by the given hour of imaging for flies imaged once every 2-hour. Egg chamber growth is slowed in the imaged females compared to the expected values derived from fixed samples (**Figure 3b**) (n = 31, egg chambers stages 4-8).

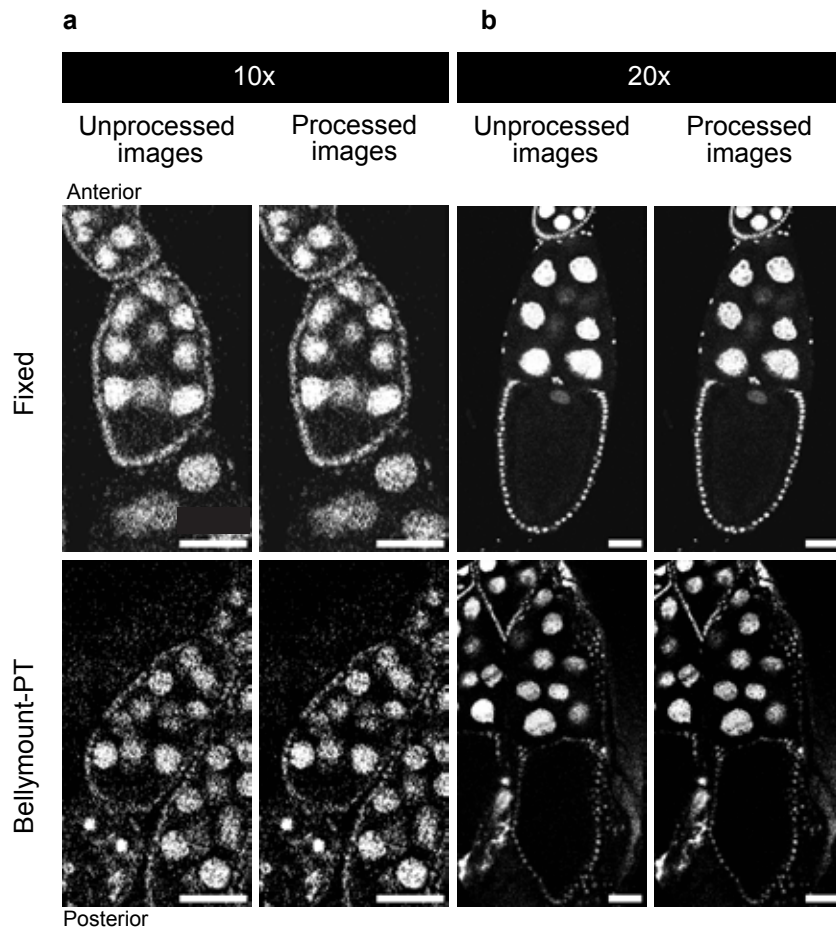


**Figure S4**



**Figure S4. Oocyte area as proxy for staging advanced egg chambers while assessing maternal transfer.** (a) Correlation plot of the oocyte percent against the overall egg chamber area ( $n = 44$ ). We used whole egg chamber size to measure the growth rates (**Figure 3**). However, we relied on oocyte percent to stage the events that occur during vitellogenesis and nurse cell dumping. These two measures correlate well (**Figure 4, 5**). (b) Normalized average pixel intensity (concentration) of H2Av-mRFP in the nurse cell nucleus remains relatively constant, or slightly increasing during early nurse cell dumping. (c) Conversely, the total nurse cell nuclear volume falls steadily throughout dumping. This results in the steady decrease in total H2Av-mRFP signal observed in **Figure 5e**.

**Figure S5**



**Figure S5. Comparison of unprocessed and processed images from fixed and Bellymout-PT samples for presentation in figures.** We used a low degree of filtering to reduce noise. Note that the images used for analysis were not processed. We applied brightness and contrast adjustments, background subtraction, gaussian filter and median filter. (a) Images captured on 10x objective (Scale bar = 50  $\mu\text{m}$ ) while (b) from a 20x objective (Scale bar = 50  $\mu\text{m}$ ).

## References

King RC. 1970. Ovarian Development in *Drosophila melanogaster*. New York, NY: Academic Press.

**Table S1: Frequency distribution and developmental duration of individual chambers from stage 2-14, and the total cumulative duration of oogenesis to each stage.**

<b>Stages</b>	<b>Egg chambers size (<math>\mu\text{m}^2</math>)</b>	<b>Egg chambers</b>	<b>Frequency</b>	<b>Duration /stage (hours)</b>	<b>Total cumulative duration of oogenesis (hours)</b>
2	621.92 $\pm$ 20.94	23	9.8291	8.4854	8.4854
3	931.01 $\pm$ 26.22	29	12.3932	10.6990	19.1845
4	1601.33 $\pm$ 56.44	31	13.2479	11.4369	30.6214
5	2746.23 $\pm$ 82.6	23	9.8291	8.4854	39.1068
6	4251.8 $\pm$ 145.94	31	13.2479	11.4369	50.5437
7	8206.29 $\pm$ 283.44	26	11.1111	9.5922	60.1359
8	14315.84 $\pm$ 694.04	18	7.6923	6.6408	66.7767
9	32791.42 $\pm$ 2871.94	16	6.8376	5.9029	72.6796
10	59036.84 $\pm$ 2081.46	11	4.7009	4.0583	76.7379
11	63994.96 $\pm$ 929.76	3	1.2821	1.1068	77.8447
12	72549.9 $\pm$ 1917.56	9	3.8462	3.3204	81.1650
13	79614.48 $\pm$ 920.01	2	0.8547	0.7379	81.9029
14	79410.55 $\pm$ 1951.13	12	5.1282	4.4272	86.3301

**Table S2: Information related to microscopy and image processing for all images in this paper**

Figure/ Video	Microscope	Objective	Fluorophores, laser	Pixel size, pixel time:	Imaging interval	Background subtraction, Gaussian blur, median blur
<b>1f</b>	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27.	H2Av-mRFP 561 nm, 1%	0.171 x 0.171 x 3 $\mu\text{m}$ 0.32 $\mu\text{s}$	Fixed samples	50 pixel radius, 0.5 pixel radius, 1 pixel radius, images were compiled from multiple females.
<b>1g</b>	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27.	H2Av-mRFP 561 nm, 0.3%	0.171 x 0.171 x 3 $\mu\text{m}$ 0.32 $\mu\text{s}$	Single image live fly	
<b>2c, d</b>	Olympus FVMPE-RV Multiphoton system	25x XLPLN25xWMP2, NA 1.05 531	Moesin-GFP, 920 nm H2Av-mRFP, 1040 nm	0.497 x 0.497 x 3 $\mu\text{m}$ 0.012 s	Bellymount-PT sample acquired at a single time frame	50 pixel radius, 0.5 pixel radius, 1 pixel radius, e in e' were processing in Arivis during 3D reconstructions (see methods).
<b>2f</b>	LSM980 Airyscan MPLX CO-8Y	Fluar 10x/0.50 M27 Air objective	H2Av-mRFP 561 nm, 0.3%	0.274 x 0.274 x 5 $\mu\text{m}$ 0.34 $\mu\text{s}$	Fixed sample	50 pixel radius, 0.5 pixel radius, 1 pixel radius. Same egg chamber from an individual female imaged over time is represented in the panels.
<b>2g, S2d, d'</b>					Bellymount-PT samples imaged every 2-hour once	
<b>S2a</b>	LSM980 Airyscan MPLX CO-8Y	Fluar 10x/0.50 M27 Air objective	H2Av-mRFP 561 nm, 0.3%	0.274 x 0.274 x 5 $\mu\text{m}$ 0.34 $\mu\text{s}$	Bellymount-PT samples imaged every 2-hour once	50 pixel radius, 0.5 pixels, 1 pixel, Same egg chamber from an individual female imaged over time is represented in the panels.
<b>3c, e</b>			H2Av-mRFP			

	LSM980 Airyscan MPLX CO-8Y	Fluar 10x/0.50 M27 Air objective	561 nm, 0.3%	0.274 x 0.274 x 5 μm 0.34 μs	Bellymount-PT samples imaged every 2-hour once	50 pixel radius, 0.5 pixels, 1pixel. Same egg chamber from an individual female imaged over time is represented in the panels.
4a	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27.	Yp1-sfGFP 488nm, 0.2% H2Av-Endo- mCherry 561 nm. 0.3%	0.149 x 0.149 x 4 μm 0.56 μs	Bellymount-PT samples imaged every hour	50 pixel radius, 0.5 pixels, 1pixel. Same egg chamber from an individual female imaged over time is represented in panels.
5a	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27.	H2Av-mRFP: 561 nm, 0.3%	0.171 x 0.171 x 3 μm 0.32 μs	Bellymount-PT samples imaged every ~10- minute	50 pixel radius, 0.5 pixels, 1pixel, Same egg chamber from an individual female imaged over time is represented in panels.
5c						
Video S1	Digital USB- pluggable 60x- 250x microscope	NA	Bright field	NA	Acquired manually	NA
Video S2						
Video S3						
Video S4	Olympus FVMPE-RV Multiphoton system	Same image presented in <b>Figure. 2c</b> is shown through the z sections, image is oriented anterior-posterior axis from left to right.				
Video S5		Two egg chambers represented in <b>Figure 2d</b> were 3D reconstructed using H2Av-mRFP signal for germline cell and H2Av-mRFP signal of the follicle cells was used for the surface reconstruction of the whole egg chambers using Arivis 4D vision program. 2D images of the reconstructions are shown in <b>Figure. 2e</b> and <b>e'</b>				
Video S6						

<b>Video S7</b>	LSM980 Airyscan MPLX CO-8Y	Images acquired every 10-minute to test the egg chamber health in response to increased exposure to anesthesia.				
<b>Video S8</b>	LSM980 Airyscan MPLX CO-8Y	Images acquired every 1-hour to test the egg chamber health in response to increased exposure to anesthesia.				
<b>Video S9</b>	LSM980 Airyscan MPLX CO-8Y	Wider view of egg chambers in the imaging field of view represented in <b>Figure 2g</b> . The movie processed as described for <b>Figure 2g</b> . In the video and the figure the egg chambers are oriented along anterior-posterior axis vertically.				
<b>Video S10</b>		Midsection of an individual egg chamber starting at stage 10 at the beginning of the imaging. The movie was processed as described for <b>Figure S2d, d'</b> and selected time frames are represented in the figure, while the movie was captured for 10-hour.				
<b>Video S11</b>	LSM980 Airyscan MPLX CO-8Y	Midsection of a previtellogenic egg chamber starting at stage 7 at the beginning of the imaging. The movie was processed as described for <b>Figure 3c</b> and selected time frames are represented in the figure, while the movie was captured for 8-hour.				
<b>Video S12</b>		Midsection of a vitellogenic egg chamber starting at stage 8 at the beginning of the imaging. The movie was processed as described for <b>Figure 3e</b> and selected time frames are represented in the figure, while the movie was captured for 12-hour.				
<b>Video S13</b>	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27	H2Av-mRFP: 561 nm, 0.3%	0.171 x 0.171 x 3 μm 0.32 μs	Bellymount-PT samples imaged every ~10- minute for about 3-hour	100 pixel radius, 2 pixel radius, 2 pixel radius



<b>Video S14</b>	LSM980 Airyscan MPLX CO-8Y	Plan-Apochromat 20x, 0.8M27	H2Av-mRFP, 561 nm, 0.3%	0.171 x 0.171 x 3 $\mu\text{m}$ 0.32 $\mu\text{s}$	Bellymount-PT samples imaged every ~10- minute for about 5-hour	100 pixel radius, 2 pixel radius, 2 pixel radius.
<b>Video S15</b>	LSM980 Airyscan MPLX CO-8Y	Midsection of stage 8 egg chamber at initial stages of yolk uptake capture for 4-hour, the image was processed as described in <b>Figure 4a</b> . Images were acquired every 1-hour.				
<b>Video S16</b>	LSM980 Airyscan MPLX CO-8Y	In <b>Figure 5a</b> the images were acquired every 10-minute to capture histone loading. Defined ROI were used to measure H2Av-mRFP fluorescent intensities every fifth time frame from the raw files, image in the movie was process as described for <b>Figure 5a</b> .				
<b>Video S17</b>	LSM980 Airyscan MPLX CO-8Y	In <b>Figure 5c</b> the images were acquired every 10-minute to capture histone loading. Defined ROI were used to measure H2Av-mRFP fluorescent intensities every fifth time frame from the raw files, image in the movie was process as described for <b>Figure 5c</b> .				
<b>Video S18, S19</b>	LSM980 Airyscan MPLX CO-8Y	These two movies are additional examples of histone loading into the oocyte. Defined ROI were used to measure H2Av-mRFP fluorescent intensities every fifth time frame from the raw files similar to <b>Video S17</b> .				