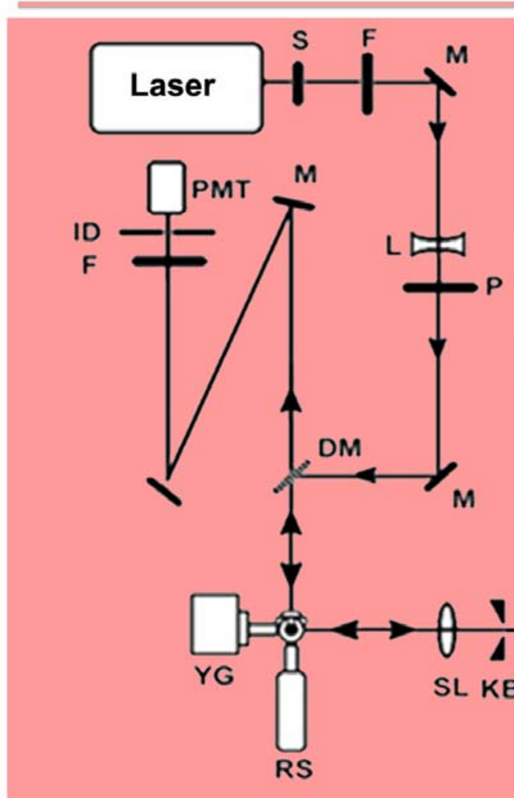
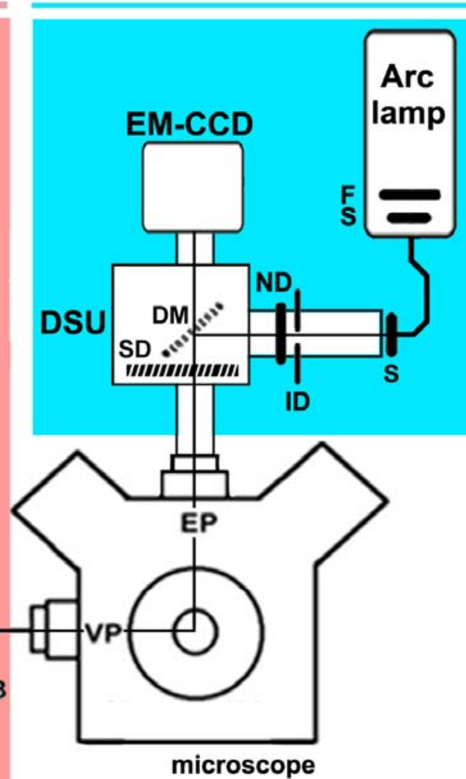
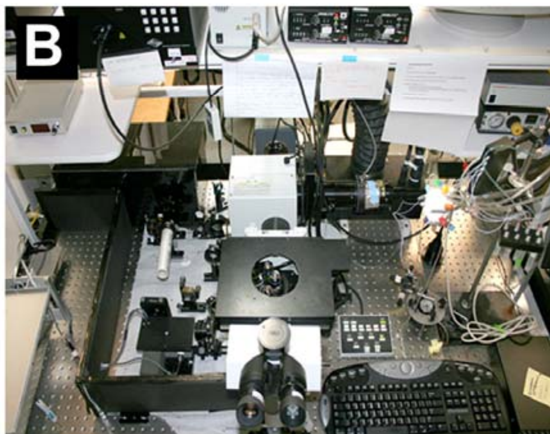
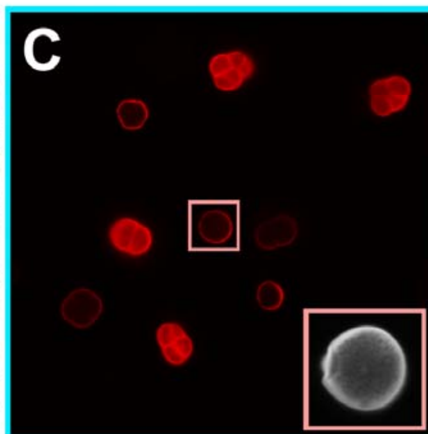
Supplementary Figure 1. Dual confocal imaging system.

(**A**) Simplified optical schematic of the dual confocal system. The resonant mirror, video-rate confocal system (pink), described in detail previously (Callamaras and Parker, 1999), is built around the video-port (VP) of an Olympus automated IX81 microscope. The spinning-disc confocal assembly (blue) was interfaced to the epifluorescence port (EP) and comprises (i) an arc lamp illumination system with output ranges suitable for flash photolysis (either a rapid wavelength switching system permitting ratiometric excitation imaging (Lambda DG-4, Sutter Instruments), or more standard epifluorescence hardware (X-Cite 120, EXFO) have been used successfully); (ii) a spinning disc confocal unit (Olympus Disk Scanning Unit, DSU), and (iii) an electron multiplication CCD camera (Hamamatsu, C9100-02, EM-CCD). S = shutters (Uniblitz, VMM-T1 driver), M = mirrors, DM = dichroic mirrors, YG = *y*-galvanometer mirror, RS = resonant scanner mirror, L = plano-concave lens, P = polarizer, F = filter or filter wheel, SL = scan lens, KB = knife-blade, ID = iris diaphragm, ND = neutral density filters, PMT = photomultiplier tube (Hamamatsu, H7422-40). (**B**) Photograph of the dual confocal system. (**C**) Images of the same sample (mixed pollen grain slide, Carolina Biological Supply) captured to show spinning disc illumination (RFP cube, blue box, 200µm) centered around video-rate scan area (λ_{EX}=488nm, greyscale image in pink box). *Inset*, higher magnification view of indicated pollen grain. Scalebar = 10µm.





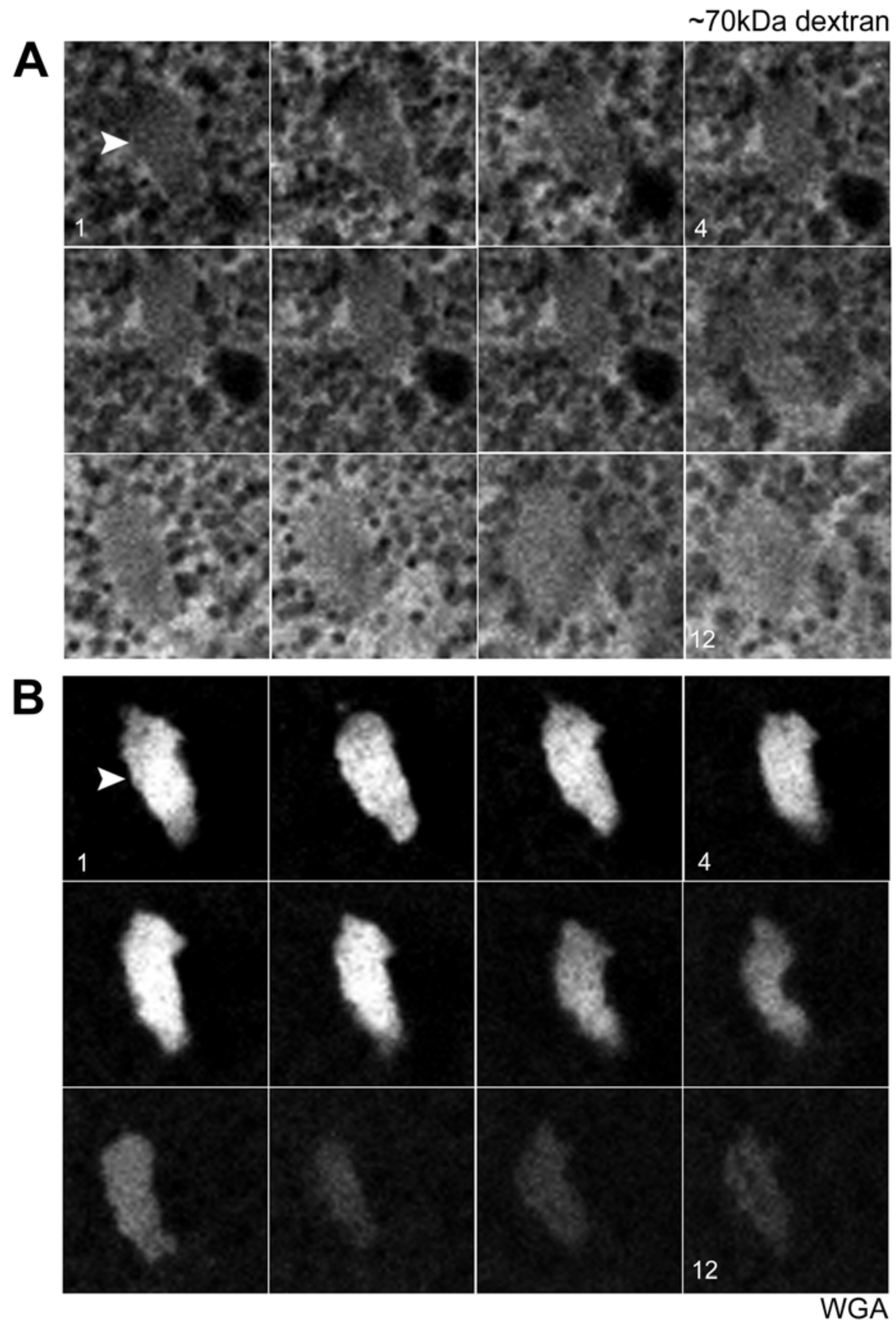




Supplementary Figure 1

Supplementary Figure 2. Changes in AL organization during maturation.

Corresponding image series encompassing ~120 mins, showing (**A**) Distribution of ~70kDa dextran and (**B**) fluorescently-tagged WGA around an AL at 10 minute intervals (images 1 -12) during progesterone-evoked oocyte maturation. Scalebar =10µm. Images are representative of those used to generate data shown in Figure 6C.


~70kDa dextran
A
1
4
12
B
1
4
12
WGA

Supplementary Movies

Supplementary Movie 1 (SM1). ***AL dynamics in the resting oocyte.***
Timelapse movie of AL dynamics (stained with rhodamine red conjugated WGA, 200μm by 200μm field of view) in the vegetal hemisphere of a stage VI oocyte recorded over ~4.5 hrs (images captured every 10 mins) .

Supplementary Movie 2 (SM2). ***ER dynamics during maturation***
Thresholded timelapse movie (~14hrs, images captured every 10mins) of ER morphology (DsRed2-ER) during progesterone-evoked maturation. Images from this timelapse are depicted in Figure 1.

Supplementary Movie 3 (SM3). ***NPC dynamics during oocyte maturation.***
Timelapse movie (~14hrs, images captured every 10mins) depicting NPC dynamics (stained with AlexaFluor488®-WGA) during progesterone-evoked maturation. Images from this timelapse, as well as the corresponding ER morphology in the same cell, are depicted in Figure 2A.

Supplementary Movie 4 (SM4). ***Heterogeneous NPC dissociation from AL.***
Movie collation of lateral ('*xy*') confocal images (taken ~7-15 mins apart) showing several AL spanning the phase of NPC dissociation ('phase 2', Figure 1C). For ease of visualization, ER morphology (DsRed2-ER) is shown in grey and NPC (AlexaFluor488®-WGA) distribution is shown in pink. Individual image frames are shown in Figure 2B.

Supplementary Movie 5 (SM5). ***Fluorescently-labeled vesicles during AL disassembly.***
Higher resolution images of NPC dynamics (duration, ~4hrs) during the phase of NPC disassembly frequently revealed a 'starburst' type appearance of vesicles emanating from AL during remodeling.

Supplementary Movie 6 (SM6). ***AL dynamics during maturation with jasplakinolide.***
Thresholded timelapse movie (~6.5hrs, images captured every 10mins) of ER morphology (DsRed2-ER) during progesterone-evoked maturation in cells incubated with jasplakinolide. A lamellogram from this timelapse movie is shown in Figure 3A.

Supplementary Movie 7 (SM7). ***Initiation of Ca^{2+} waves from AL***
Simultaneous projection of ER morphology (greyscale, left) and fluo-4 fluorescence (pseudocolor, right) showing the initiation and propagation of a Ca^{2+} wave from an AL in response to photorelease of IP_3 (the scoring phenotype for measurements reported in Figures 4A & B).

Supplementary Movie 8 (SM8). ***Dynamics of GFP-vimentin in a Xenopus oocyte***
Dynamics of GFP-vimentin (field of view, 130μm by 130μm, 500ms per frame) within the *Xenopus* oocyte periphery. Similar data was processed in Figure 5C.