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Detection of Separase activity using a cleavage sensor in live mouse oocytes

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Detection of Separase activity using a cleavage sensor in live mouse oocytes.

Running head:
Biosensor for Separase activity in mouse oocytes

Abstract:

Separase proteolytically removes Cohesin complexes from sister chromatid arms, which is essential for chromosome segregation. Regulation of Separase activity is essential for proper cell cycle progression and correct chromosome segregation. Onset of Separase activity has not yet been observed in live oocytes.

We describe here a method for detecting Separase activity in mouse oocytes *in vivo*. This method utilizes a previously described cleavage sensor made up of H2B-mCherry fused with Scc1(107-268 aa)-YFP. The cleavage sensor is loaded on the chromosomes through its H2B tag, and the signal from both mCherry and YFP is visible. Upon Separase activation the Scc1 fragment is cleaved and YFP dissociates from the chromosomes. The change in the ratio between mCherry and YFP fluorescence intensity is a readout of Separase activity.

Keywords:
Separase, Cohesin, cleavage sensor, oocyte, chromosome segregation, anaphase, female meiosis, Scc1, live imaging, time lapse.

1. Introduction

During meiosis I, chromosomes are organised as bivalents, where homologous chromosomes are held together via chiasmata and sister chromatids are locked together by a ring complex named Cohesin ***(1, 2)***. At the metaphase to anaphase I transition, Separase proteolytically cleaves Cohesin at the chromosome arms allowing chiasmata resolution and chromosome segregation. In meiosis II, sister chromatids are held together by the remaining Cohesin localized at the centromeres ***(3)***. During the second meiotic division, Separase cleaves centromeric Cohesin leading to sister chromatid segregation.

Regulation of Separase activity is essential for proper meiotic chromosome segregation ***(3, 4)***. Upon translation, Separase is folded with the help of its chaperon and inhibitor Securin ***(5)***. Separase remains in a stable complex with Securin, until Securin is targeted for degradation through APC (Anaphase Promoting Complex) dependent ubiquitination ***(6–8)***. Separase is additionally kept inactive by inhibitory phosphorylation mediated by Cyclin B1-Cdk1 kinase ***(9, 10)***. Cyclin B1 is ubiquitinated in an APC dependent manner at the same time as Securin ***(11, 12)***. Dephosphorylation of Separase and degradation of Securin result in activation of Separase ***(10)***. Separase then cleaves its substrates, the kleisin subunits of Cohesin (Scc1 in mitosis or Rec8 in meiosis) ***(13)***.

Observing and measuring Separase activity during meiosis in mouse oocytes presents a challenge. Since Cohesin complexes containing Rec8 are loaded during embryonic development in low quantities with no turnover ***(14, 15)***, it is

impossible to introduce exogenously expressed fluorescently tagged Cohesin subunits to follow their cleavage without creating a transgenic mouse. In addition, introducing fluorescent tags may inhibit the subunit's ability to integrate into a complex. A different approach for the detection of Separase activity *in vivo* has been developed in mitotic cells ***(16)***, which we have adapted in oocyte meiosis.

We describe a method of detecting Separase activity in mouse oocytes *in vivo.* This method utilizes a cleavage sensor, which is a protein construct made up of H2B-mCherry fused with Scc1(107-268 aa)-YFP. The H2B tag serves to bind the sensor to the chromosomes, the Scc1 fragment is a very efficient Separase substrate, and the ratio of YFP and mCherry fluorescence levels on the chromosomes allows the detection of the cleavage event.

mRNA of the cleavage sensor is injected in mature prophase arrested oocytes and cleavage sensor protein expression begins. After allowing sufficient time for expression, the prophase arrest is lifted, chromosomes condense and the cleavage sensor binds to the chromosomes. Its fluorescence can be followed through live imaging.

When Separase is activated at the metaphase-to-anaphase transition of meiosis I, it cleaves Scc1-YFP with high efficiency leading to YFP dissociation from the chromosomes, visible as a sharp decrease of YFP compared to mCherry fluorescence. This can be clearly observed by live imaging of oocytes (Fig. 1).

The cleavage sensor is not included into the endogenous Cohesin complexes and its expression does not impede cleavage of the endogenous Rec8 and chromosome separation. This sensor may be used in different genetic backgrounds. Moreover, variants of this sensor can be created depending on the aim of the experiments. It allows the observation of Separase activity through time lapse imaging, detection of activation and inactivation of Separase activity as well as its efficiency in different conditions. The cleavage sensor is a powerful and elegant tool useful for observation and measuring Separase activity in mouse oocytes *in vivo*.

In this chapter we describe collection of mature prophase arrested mouse oocytes, microinjection of the cleavage sensor mRNA, time lapse imaging of oocytes during metaphase-to-anaphase I transition and observation and measurement of Separase activity.

2. Materials

2.1. Collection of oocytes

1. 9-14 week-old mice, strain CD1 Swiss (Janvier labs, France).
2. Sterile-filtered, suitable for mouse embryo culture M2 medium (Sigma) supplemented with penicillin (final concentration 1.678 mM) and streptomycin (final concentration 0.689 mM), and where indicated supplemented with 100 mg/ml dbcAMP (dibutyryl cyclic AMP).
3. Mineral oil suitable for mouse embryo cell culture (Sigma).
4. 60 mm easy grip polystyrene tissue culture dishes (Falcon).
5. Mouth pipette: plastic tubing connected to a mouthpiece. Attach the drawn out glass Pasteur pipette to the tubing and use it for collection and cleaning of oocytes.

6. 25G 16mm BD Microlance hypodermic needle mounted on a 1ml syringe.
7. Dissection scissors and forceps.
8. Incubator set to 38°C.
9. Binocular dissection microscope combined with a heating plate.

2.2. Microinjection of oocytes

1. Glass capillaries: outer diameter 1mm, inner diameter 0.75 mm, length 100 mm (Harvard apparatus).
2. Magnetic puller, for example Narishige PN-30.
3. Microinjection microscope, we use a Nikon Eclipse Ti with Eppendorf TransferMan NK2 micromanipulators and TOKAI HIT Thermoplate.
4. Eppendorf FemtoJet Microinjector connected to the microinjection needle holder.
5. Eppendorf CellTram Oil (or similar) manual microinjector connected to the holding pipette holder.
6. Depression glass slide.
7. Holding pipette: VacuTip microcapillaries, inner diameter 15 µm, outer diameter 100 µm, angle 35°.
8. Microloader tips 20 µl (Eppendorf).
9. Linearized and purified plasmid encoding for the cleavage sensor under T7, T3 or SP6 promoter and allowing synthesis of a poly(A)-tail (Note 1).
10. T7/T3/SP6 mMessage mMachine transcription kit (Invitrogen).
11. Microcentrifuge.
12. RNase-free water.

2.3. Set-up for imaging

1. We use an inverted microscope Zeiss Axiovert 200M, combined with an MS-2000 automated stage (Applied Scientific Instrumentation), a Yokogawa CSU-X1 spinning disc and a nanopositioner MCL Nano-Drive. The spinning disc unit is supplemented with a Quad-Band Dichroic filter 13x15x0.5 DI-T405/488/568/647 for CSU X1. The light sources are 50mW 488nm and 561nm lasers (Roper Scientific). The images are taken with an EMCCD camera, pixel size 16 x 16 µm (Photometrics).
2. Objective: Plan-APOCHROMAT 40x/1.4 Oil DIC (UV) VIS-IR (Zeiss).
3. The microscope is operated with MetaMorph software.
4. The body of the microscope is encased in an incubation chamber heated by a heating unit (PeCon).
5. Heating insert: incubator cover, incubator main body, lens warmer, operated by a touch screen 3-channel temperature controller (Chamlide).
6. Imaging chamber: Chamlide CMB 35mm 1-well magnetic chamber for round coverslips.
7. Round cover glasses, diameter 25mm.
8. Immersion oil with refractive index of 1.518. For example, Cargille Type 37 immersion oil.

3. Methods

3.1. Collection of oocytes

1. Prepare a 60mm polystyrene dish with 6-7 evenly spaced drops of around 50 µl pre-warmed M2 medium supplemented with 100 mg/ml dbcAMP. Cover the drops entirely with mineral oil. Store this dish in a 38°C heated environment (an incubator or a heated surface).
2. Place another 60mm polystyrene dish on a 38°C heated stage of a binocular microscope and put a 500 µl drop of pre-warmed 38°C M2 medium supplemented with dbcAMP in the center. Do not cover it with mineral oil.
3. Sacrifice a non-primed 8-12 week old OF1 mouse (Note 2) by cervical dislocation. Open the peritoneal cavity with a pair of scissors and remove both ovaries.
4. Transfer the ovaries into the prepared 500 µl drop of M2 medium supplemented with dbcAMP. From this step on, all procedures should take place on a 38°C heated surface.
5. Release the mature GV oocytes from the ovaries by piercing the follicles with a syringe-mounted needle. During this procedure, the ovaries can be held down with another needle, or a pair of forceps.
6. Carefully collect the biggest (mature/competent) oocytes. Transfer of the oocytes is done by mouth-assisted aspiration with a glass pipette (Note 3).
7. Transfer all collected oocytes into a drop in the prepared multi-drop dish. Some oocytes have clusters of small follicle cells stuck to them. Separate the oocytes from the follicle cells by a series of aspirations into a glass pipette with a diameter of the opening similar in size to the diameter of the oocyte. Cleaning the oocytes can be performed in other drops of M2 medium in the same dish.
8. Take only the mature GV oocytes and transfer them into an unused drop of M2 medium. Mature GV oocytes are the largest, with a smooth thick zona pellucida and with a germinal vesicle in the center of the oocyte.

3.2. Microinjection of oocytes

1. Prepare mRNA coding for the cleavage sensor for microinjection using a mMessage mMachine transcription kit well ahead and freeze down small aliquots for single use. Spin the mRNA solution for at least 30 min at 13 000 rpm at 4°C prior to microinjection. Keep the mRNA solution on ice at all times.
2. Prepare an injection needle from a glass capillary using a magnetic puller (Note 4). Avoid touching both ends of the capillary or the resulting needle.
3. Set up a microinjection microscope: turn on the microinjector, the thermoplate and the light source. Set up the microinjector to Function 0 (Change capillary).
4. Fill the microinjection needle with 1 - 1.6 µl of mRNA solution (Note 5). Attach the microinjection needle to the needle holder. Attach the holding pipette to the holding pipette holder.
5. Form a drop of 3-5 µl of pre-warmed M2 medium supplemented with dbcAMP on a depression glass slide and cover it with a small amount of mineral oil (about 200 µl).
6. Transfer the oocytes into the drop on the prepared depression glass slide. Keep the oocytes grouped together.

7. Put the glass slide with the oocytes on the thermoplate of the microinjection microscope, lower the holding pipette and the microinjection needle into the drop so that their tips appear at the same level as the oocytes (Note 6). Change the microinjection pump setting to Function 4 (Continuous flow) and press "Inject" (Note 7).
8. Bring the tip of the holding pipette close to an un-injected oocyte and apply light suction with the holding pipette pump. The suction force should be strong enough to attach the oocyte to the holding pipette, but light enough so that the oocyte does not change shape or enter into the holding pipette.
9. Bring the tip of the microinjection needle close to the oocyte and adjust the positions of the holding pipette and the microinjection needle (Note 8).
10. Keeping the holding pipette steady bring the tip of the microinjection needle into the oocyte until you see a burst-like change in the oocyte cytoplasm (Note 9). Avoid piercing the nucleus.
11. Quickly withdraw the needle at the same angle (Note 10).
12. Move the holding pipette with the injected oocyte aside and release the suction force (apply negative force) in the holding pipette pump. Keep injected oocytes in a separate group from un-injected oocytes.
13. Repeat steps 8-12 until all oocytes have been injected.
14. Set the microinjector back to function 0. Lift the microinjection needle and the holding pipette from the drop. Transfer the glass slide with the injected oocytes on a heated stage of a binocular microscope. Transfer the oocytes to a clean drop of M2 medium supplemented with dbcAMP. Separate and remove dead oocytes (Note 11).
15. Incubate the injected oocytes at 38°C from 20 min to 4h (Note 12).
16. To release oocytes from the GV block, prepare a 60mm polystyrene dish containing 6-7 evenly spaced drops of M2 medium (without dbcAMP). Cover the drops with mineral oil. Prepare another 60mm polystyrene dish containing 4-5 drops of M2 medium (without dbcAMP) and place on the heated stage of a binocular microscope. Do not cover with mineral oil.
17. Transfer oocytes consecutively through 4 or 5 drops (on the dish without the oil), each time mixing the oocytes in the medium and then allowing them to settle. Change the medium in the pipette completely each time. Transfer all the oocytes into a clean drop of M2 medium in the prepared dish, covered with mineral oil. Incubate from 45min to an hour at 38°C.
18. Check for the germinal vesicle breakdown (GVBD) event - a sign of meiotic resumption and entry into prometaphase I. When this happens, the germinal vesicle in the oocyte disappears. Collect these oocytes and transfer them to an unused drop of M2 medium in the same dish. If not all oocytes had gone through GVBD, check again in 10-15 minutes (Note 13).
19. Incubate for 4-6 hours at 38°C (Note 14). GVBD event serves as a reference point to all subsequent events in the first meiotic division. Anaphase I usually takes place between 7 and 9 hours after GVBD, depending on the mouse strain and culture conditions. OF1 mouse oocytes undergo metaphase-anaphase I transition at GVBD+7-8h.

3.3. Setting up for imaging

1. At least one hour before starting the imaging, pre-warm the imaging platform. This includes switching on a heating chamber or a heating plate and an objective warmer in case of an immersion objective (Note 15). Right before the imaging turn on the whole microscope system.
3. Assemble the imaging chamber with a clean cover glass. Place on a 38°C heated surface or incubate in the 38°C incubator until warm.
3. Form small 2 µl M2 medium (without dbcAMP) drops in the center of the cover glass in the imaging chamber and cover with mineral oil. The number of drops depends on the number of the oocytes and the number of experimental conditions (Note 16).
4. Transfer the injected oocytes into the drops in the imaging chamber, up to 15 per drop, evenly spaced.
5. Apply pre-heated immersion oil without air bubbles on the objective, place the imaging chamber with the oocytes on the heating plate above the objective. Adjust the position of the objective so that the immersion oil is in contact with the bottom of the imaging chamber.

3.4. Time-lapse imaging

1. Using MetaMorph interface, set up the acquisitions as follows:
2. Create a folder where the files from the specific time lapse experiment will be stored.
3. Find and save the positions of all the oocytes in the imaging chamber.
4. Use these channels: 491nm for YFP and 561nm for RFP fluorescence. Adjust the laser power and the exposition time of 491nm and 561nm channels to as low as possible (Note 17).
5. Set time lapse: acquisitions 10-20 min for 8-10 hours depending on when the first metaphase-anaphase transition is expected.
6. Z stacks: 11 slices with a 3 µm interval. Adjust the position of the saved point so that Z stacks would cover the whole volume of the chromosome mass.
7. Start the acquisition (see Note 18).

3.5. Quantification and analysis with ImageJ

1. Transfer the files to the analysis station. Using ImageJ assemble raw images into Z-projection stacks by individual oocyte and wavelength. You can do this manually or by using a macro MetaMorph Raw into Stacks (available upon request).
2. Open the stacks for 491 nm and 561 nm channels for the first oocyte.
3. Select a square region of interest around the chromosome mass in one of the images at the first time point and measure the fluorescence intensity within this region with the ImageJ function.
4. Select the window with the image of the same oocyte taken on another channel and restore the selected region of interest through “Image - Selection - Restore Selection” function of ImageJ. Measure the fluorescence intensity of this region, too (Note 19).

5. Return to the image taken with the first channel, switch to the next time point, adjust the region of interest so that it covers the moved chromosome mass and repeat the measurement on both channels.
6. Repeat the measurement steps until the chromosomes segregate, then measure only the chromosome mass that stays in the oocyte until the end of the time lapse.
7. Measure the intensities of oocyte cytoplasm fluorescence in both channels (background fluorescence) in a similar manner.
8. Subtract background fluorescence from the chromosome fluorescence in both channels respectively for each time point.
9. Normalize the fluorescence intensities to the first time point for both channels individually. On a line chart the fluorescence value will drop in both channels upon chromosome segregation (Fig. 2A,B left panels), indicative of chromosome segregation.
10. Calculate the ratio between YFP and RFP fluorescence intensities for each time point.
11. Build a line chart of the ratio changes over time. Mark the time point when the chromosomes segregate, i.e. are first seen as 2 masses. The dip in the YFP/mCherry fluorescence ratio represents the time of Separase activation (Fig. 2B, right panel) (see Note 20).
12. Repeat steps 2-11 for all oocytes.

Notes

1. Any plasmid suitable for *in vitro* transcription would serve. It has to contain a transcription start (usually T7, T3 or SP6 promoter), a Kozak sequence, the sensor cDNA (H2B, mCherry, Scc1 107-268 aa, YFP as a single reading frame) and a polyadenylation signal sequence (can be replaced by a string of around 30 adenosines in the coding strand). Include a unique restriction enzyme cut site after the poly(A) signal for plasmid linearization.
2. One 8-10 weeks old mouse of this strain usually harbours around 50 mature GV oocytes.
3. The collection pipette can be produced from a glass Pasteur pipette. Heat up a thin section of the pipette over a flame until the glass becomes malleable and rapidly pull on both ends of the pipette. Break off the extended thin portion at 5-7 cm and check the diameter of the opening. The diameter of the pipette opening should not be smaller than the diameter of the oocyte. It's better to prepare pipettes of 2 sizes, one around 2-3 diameters of the oocyte for oocyte collection, and another with the diameter very close to the diameter of the oocyte for cleaning.
4. To determine the puller settings, follow the manufacturer's instructions. The settings and the resulting needle will have to be adjusted for individual conditions in the lab.
5. Use a P-10 automatic pipette with Eppendorf Microloader tips to reach inside the microinjection needle from the wide end. In case air bubbles appear after loading the needle with mRNA solution, tap lightly on the side of the needle. mRNA can also be loaded with a regular 10 µl tip by placing the drop of the mRNA solution on top of the wider end of the microinjection needle. The solution will travel to the working end of the needle by capillary forces within 5-10 minutes.

6. After the tip of the holding pipette is lowered into the medium, use the holding pipette pump to take in some medium in the holding pipette and equilibrate the suction so that it doesn't take any medium in or out. Also, sometimes the tip of the microinjection needle can be sealed during pulling. Test this by pressing "Clean" button on the microinjector for a second. If the tip of the needle is not sealed, an air bubble should exit. If the tip is sealed, it can be broken off by tapping the tip of the needle against the holding pipette.
7. The force of the flow required for a successful injection depends on the diameter of the needle. At the beginning of the injections set the force to 150 hPa and adjust accordingly to the injection outcome.
8. In absence of an anti-vibration table, if the holding pipette tremors slightly adjust it so it touches lightly the glass slide
The microscope should be focused on the middle of the opening of the holding pipette, which is also the centre of the oocyte attached to the pipette. The microinjection needle should be adjusted so that only the very end of the needle is in focus.
9. Sometimes the tip of the needle is not well positioned against the centre of the oocyte and upon injection, it can slide between the zona pellucida and the oocyte membrane. If this happens, a bubble appears, created by the constant flow of mRNA from the needle. Withdraw the needle, readjust its position and try again.
10. It is very easy to rupture the membrane of the oocyte. Always withdraw the needle at the same angle as the injection path. Do not inject too much: the area of the cytoplasmic burst upon injection should not exceed the diameter of the nucleus. Make sure the tip of the injection needle is thin enough and is clean. Sometimes the material from the cytoplasm can stick to the tip of the needle. This greatly increases the probability of the membrane rupture of the oocytes injected after that. Clean the needle by rubbing its sides against the sides of the holding pipette. Exchange the needle if needed.
Sometimes mRNA solution can contain a small amount of precipitates, which can clog the injection needle. It is visible in the injection microscope as a mass inside the needle. Sometimes it can be cleared by applying extra pressure from the microinjector (press "Clean" several times). If possible (depending on the length of the injection needle and its diameter), try to break off the end of the injection pipette by bumping it softly onto the holding pipette. If the clog does not exit the needle, exchange the needle.
The concentration of the mRNA solution can also influence the clogging. If the injection is very difficult, try diluting the mRNA solution with RNAse-free water and repeat the microcentrifugation step.
11. Dead oocytes have a more granulated cytoplasm and are slightly less transparent than intact oocytes. If the membrane is ruptured, a slow movement of cytoplasm can be visible. Sometimes the membrane remains intact but the oocytes die anyway. Don't be disappointed if most oocytes die in the beginning!.
12. The incubation time depends on the expression of the target protein. Longer incubation tends to increase the expression levels.
13. The time of meiosis resumption after release from dbcAMP depends on the mouse strain, source of the medium and the time of incubation in the arrested state (in dbcAMP-supplemented M2). Variations between 45 and 80 min are normal. To keep the oocyte culture synchronised do not collect any oocytes that went through GVBD after 15-20 minutes after the first batch.

14. Due to oocyte sensitivity to phototoxicity, it is best not to expose oocytes to the laser light before GVBD+4h in this assay.
15. Setting up the right temperature conditions is the key to successful imaging. Oocyte culture is very sensitive to temperature changes and even 1°C variation interferes with meiotic progression. The best way to adjust the temperature is by experimental testing. The temperature inside of the drop in the imaging chamber should be around 38°C. This can be measured initially using a thermometer with a wire temperature probe. Then try incubating the oocytes overnight in the heated microscope in the imaging chamber. If the conditions are optimal, the oocytes should be alive and have extruded polar bodies (PB) at GVBD+7h. If the oocytes are still in metaphase I, but visually unaffected, the temperature is probably too low. If the oocytes are in metaphase I, but look unhealthy, i.e. with very granulated cytoplasm, uneven surface, full of bubbles, or even dead, the temperature may be too high. Another reason for failures in meiotic progression can be the composition of the imaging chamber (certain materials are toxic for the oocytes) or the source of the medium and mineral oil.
To keep the microscope temperature stable, we keep the heating chamber around the microscope at a constant temperature at all times.
16. We place 15 oocytes per drop, evenly spaced. Time-lapse imaging with the current set up (described later) is optimal for up to 30 oocytes. If there is no difference in oocyte treatment conditions, 2 drops of medium is enough. If the conditions are different (i.e. drug treatments, injection of different constructs), use separate drops of medium to place oocytes from different conditions. In case of drug treatments, it is essential to make sure that the drug is not lipophilic as it may affect the untreated oocytes through diffusion in the mineral oil. If the drug is lipophilic, also add it to the oil in the matching final concentration so there is an equilibrium maintaining the correct concentration in the medium.
17. Ooocytes are very sensitive to phototoxicity. Try using a very low laser power and exposition times, where chromosomes are still visible. If there are still effects of phototoxicity (chromosome missegregations, lagging chromosomes), reduce the number of the oocytes per time-lapse experiment.
18. As acquisitions take place for 8-10 hours, it's advisable to schedule the time lapse imaging overnight.
19. It is important that the region of interest has the exact same location in images from both channels at the same time point. To simplify the measuring routine you can use the Sensor Measurement Plugin (available upon request).
20. The rapid decrease of the YFP/mCherry ratio upon chromosome segregation represents the cleavage of the sensor by Separase (Fig. 2B). Note that despite the drop in both mCherry and YFP fluorescence intensity in the negative control H2B-mCherry-YFP (Fig. 2A, left panel), the YFP/mCherry ratio remains unchanged during the chromosome segregation event (Fig. 2A, right panel). As Scc1 is a very good Separase substrate, it is cleaved almost completely (Fig. 2B). Often the area of the chromosomes after sensor cleavage has lower fluorescence intensity than the rest of the cytoplasm; this is why the curve dips below zero. After the cleavage and a rapid decrease in YFP/mCherry ratio, it slowly grows back up. We believe this behaviour represents replacement of the cleaved sensor molecules with the intact ones still found in the cytoplasm (or newly synthesized molecules of the sensor). We believe that the time between the start of sensor cleavage (the dip of the ratio curve) and the reaccumulation of uncleaved sensor (end of the dip)

represents the time window of Separase activity. The efficiency of the cleavage can be estimated by the speed of the cleavage and whether or not most of the sensor molecules were cleaved during the time where Separase was active.

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Figure legends

Figure 1. Live imaging of Separase activity in mouse oocyte
At 5h30 mark (metaphase I), the intact cleavage sensor (H2b-mCherry-Scc1-YFP) is observed at the chromosomes. Once Separase is activated at anaphase I onset (8h30 mark), it cleaves Scc1. This leads to a sharp decrease of YFP, leaving only the signal mCherry on the chromosomes.

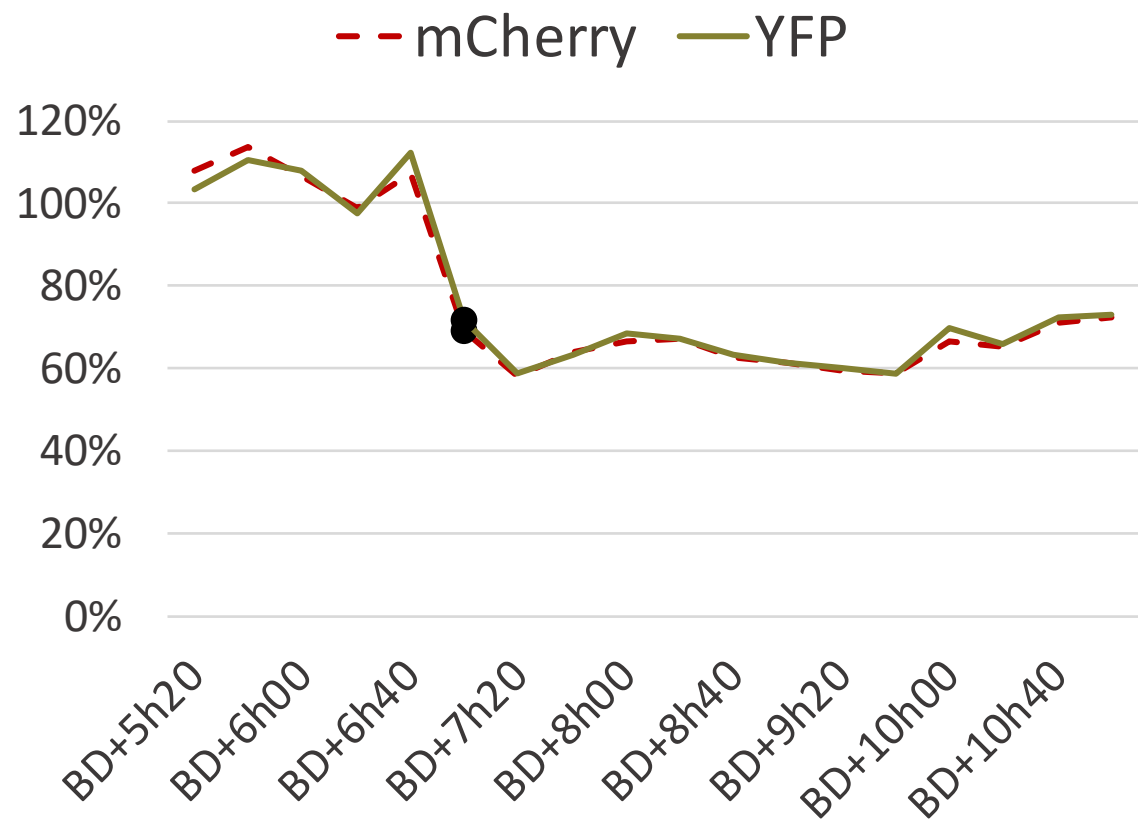
Figure 2. Quantification of YFP and mCherry fluorescence
A) Quantification of YFP and mCherry fluorescence of the negative control (H2B-mCherry-YFP) measured on the chromosomes by live imaging of a mouse oocyte during metaphase to anaphase I transition (left) and the ratio of normalized YFP/mCherry fluorescence intensity by live imaging of a mouse oocyte during metaphase to anaphase I transition. Black circle: chromosome segregation time point. **B)** Quantification of YFP and mCherry fluorescence of the cleavage sensor (H2B-mCherry-Scc1(107-268aa)-YFP), measured on the chromosomes by live imaging of a mouse oocyte during metaphase to anaphase I transition (left) and the ratio of normalized YFP/mCherry fluorescence intensity by live imaging of a mouse oocyte during metaphase to anaphase I transition. Black circle: chromosome segregation time point.

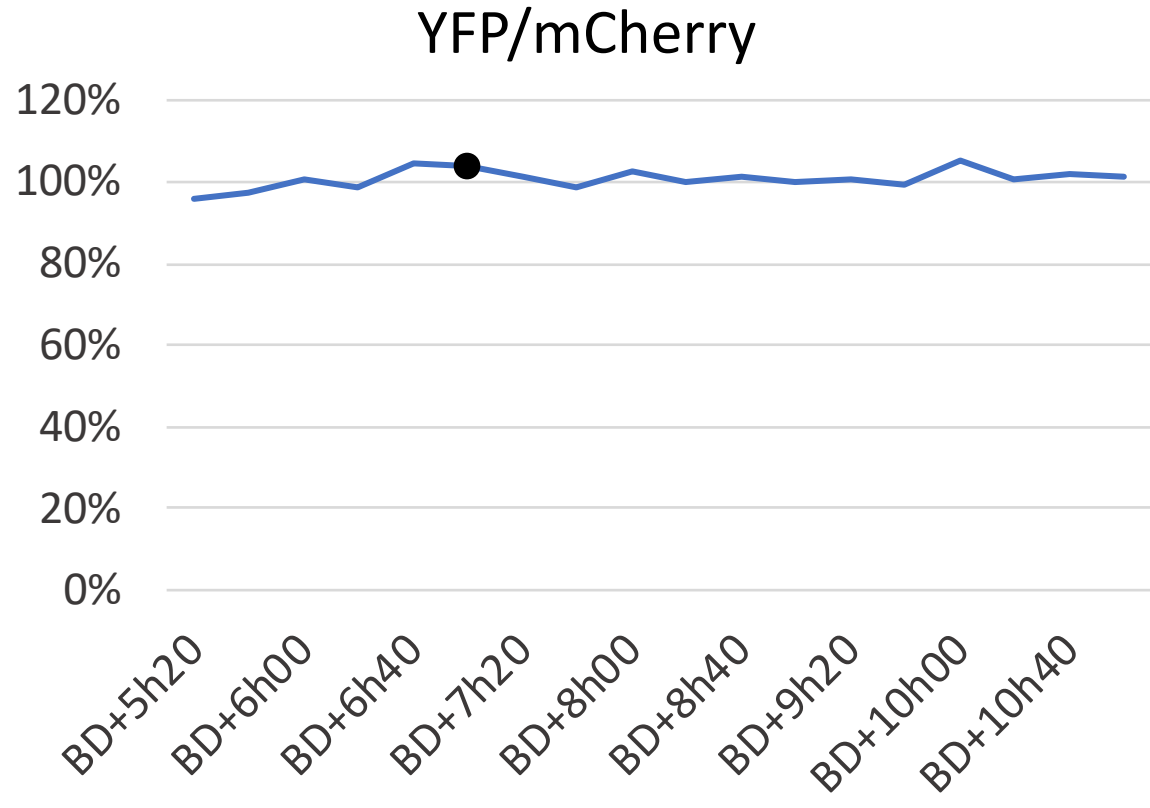
5h30 6h30 7h30 8h30 9h30 10h30 11h30

mCherry

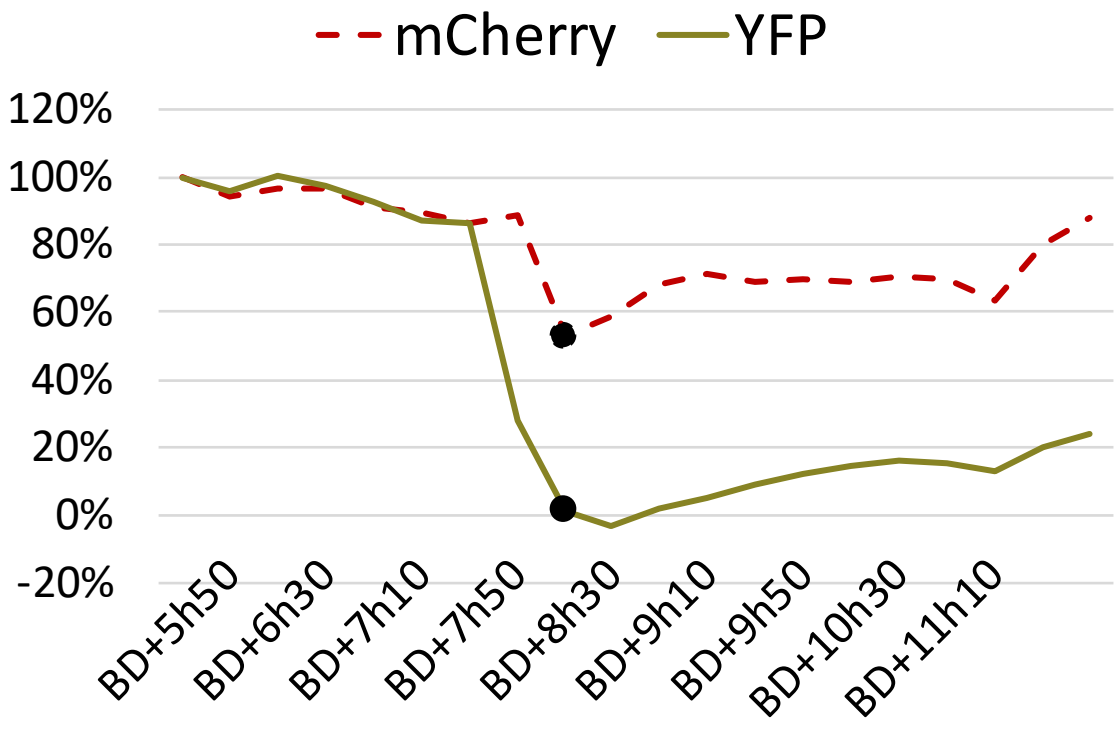
YFP

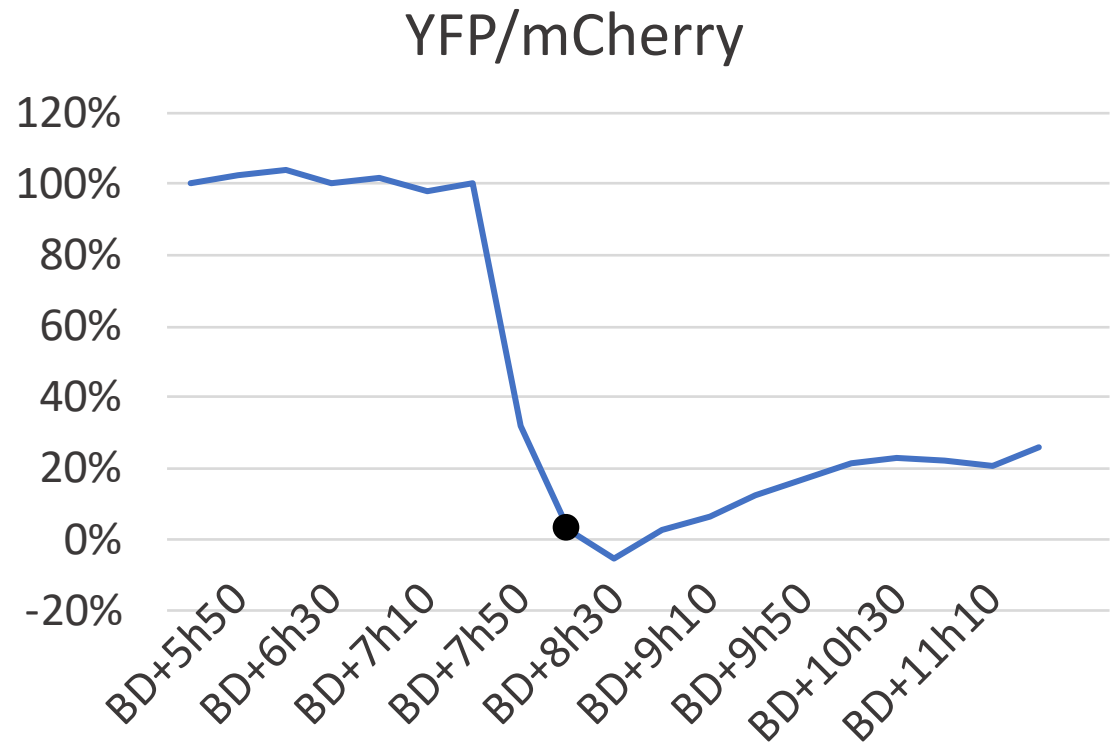
A)


mCherry
YFP
120%
100%
80%
60%
40%
20%
0%
BD+5h20
BD+6h00
BD+6h40
BD+7h20
BD+8h00
BD+8h40
BD+9h20
BD+10h00
BD+10h40


YFP/mCherry
120%
100%
80%
60%
40%
20%
0%
BD+5h20
BD+6h00
BD+6h40
BD+7h20
BD+8h00
BD+8h40
BD+9h20
BD+10h00
BD+10h40

B)


mCherry
YFP
120%
100%
80%
60%
40%
20%
0%
-20%
BD+5h50
BD+6h30
BD+7h10
BD+7h50
BD+8h30
BD+9h10
BD+9h50
BD+10h30
BD+11h10


YFP/mCherry
120%
100%
80%
60%
40%
20%
0%
-20%
BD+5h50
BD+6h30
BD+7h10
BD+7h50
BD+8h30
BD+9h10
BD+9h50
BD+10h30
BD+11h10