Figures and figure supplements

Dynein-mediated transport and membrane trafficking control PAR3 polarised distribution

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Figure 1. Dynamic PAR3 distribution along the oocyte anterior posterior axis. (A) Description of the quantification Fiji Macro. After selecting three points in the oocyte (yellow stars) and delimitation of oocyte perimeter, the macro

Figure 1 continued on next page
Figure 1 continued

allows us to obtain different data about protein repartition in oocytes: the intensity profile of the plasma membrane (magenta); the mean fluorescent intensity and the length of each of the plasma membrane domains are automatically generated (anterior/APM in red; lateral/LPM in green; posterior/PPM in blue) and the mean signal intensity inside the cytoplasm (cyan). (B–E) Distribution of PAR3 between stages 8 and 10 in representative examples. (B) Localisation of PAR3-GFP, expressed in the germline under control of the maternal driver Tub67c-GAL4, from stage 8 to stage 10. The brackets indicate oocytes. (C) Representative intensity profiles of plasma membrane distribution in a single oocyte (APM in red, PPM in blue). Green arrows highlight PAR3 posterior accumulation, and red arrows PAR3 posterior exclusion. (D) Raw quantity of PAR3 in each plasma membrane domain from stage 8 to stage 10 (APM in red, LPM in green; PPM in blue). (E) To avoid size/expression fluctuations of oocytes between the different mutant genotypes, PAR3 distribution has been normalised by the length of the membrane and the total oocyte signal (density/total). In this case, we cannot compare the level between the different stages but only the asymmetrical distribution of PAR3 between the different domains. (F) Evolution of PAR3 asymmetry from stage 8 to stage 10. The asymmetry ratio (APM/PPM of PAR3 density) highlights the increase of PAR3 polarity in oocytes from stage 8 to stage 10. Stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 15; stage 10, n = 14. Mann-Whitney test, NS: not significant; **: p<0.01; ***: p<0.001; ****: p<0.0001. Error bars indicate SEM. The scale bars represent 30 μm in (B) and in all following figures.

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Figure 1—figure supplement 1. Membrane et developmental stage definitions. (A) Egg chamber expressing PAR3-GFP under its own promoter (green/w, P[w+, FRT9-2]18E, f, baz[815.8], P(CaryP, PB[BAC Baz- sfGFP2]attP18]) and nuclear RFP (red) both in somatic and germinal cells. White arrowheads indicate the signal at the APM membrane, white arrow, the double nurse cell membrane, and red arrows, juxtaposition of the oocyte membrane and the apical membrane of follicular cells. (B) Schematic representation of a stage 10 egg chamber. The yellow box highlights the proximity between the oocyte APM and the plasma membrane of the adjacent nurse cells. The green boxes show the juxtaposition between the oocyte LPM or PPM and the apical plasma membrane of the neighbouring follicular cells. (C) Determination of oogenesis stages. Migration of follicular cells (white arrow heads) toward the oocyte was used to determine the egg chamber stage. Stage 9A comprises the oocytes of which follicular cells have migrated until the middle of nurse cell region. Stage 9B includes the oocytes of which the follicular cells have migrated from the middle of nurse cell region to near the oocyte anterior. Then at stage 10, the follicular cells are positioned around the oocyte. The scale bars represent 30 μm.
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Figure 1—figure supplement 2. PAR3 distribution without total signal normalisation. (A) PAR3 density from stage 8 to stage 10. The quantity of PAR3 has been normalised by the membrane length of the domain but not by the total oocyte intensity. Stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 15; stage 10, n = 14. Mann-Whitney test, NS: not significant; *: p<0.05 ; **: p<0.01 ; ****: p<0.0001. Error bars indicate SEM. 
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Figure 1—figure supplement 3. Control experiments of PAR3 quantification process. (A) Localisation of GFP-PAR3 (left) and PAR1-GFP (right) under control of maternal Tub67c-GAL4 in stage 9B egg chambers. Note the weak density of PAR3 and the strong density of PAR1 in the PPM crescent. For Figure 1—figure supplement 3 continued on next page
all egg chambers, we defined the three following points: A and B on both sides of the APM and P on the middle of the PPM. Subsequently, the Fiji Macro automatically segmented the egg chambers tracing three lines (yellow lines). First, it traced the perpendicular line going from the middle of AB to P. It then traced the lines going from A or from B and crossing at 40% of P on the preceding perpendicular line. The crossing between these two last lines and the plasma membrane separates the LPMs from the PPM crescent. The APM comprises the region between points A and B. (B) Validation of PPM crescent identification by the Fiji Macro. The PPM corresponds to the PAR1 (right, white) and the Staufen (left, red) plasma membrane domain. The actin cytoskeleton is stained green. Scale bars indicate 30 μm. (C) Quantification by our Fiji Macro of the signal intensity at the anterior domain coming both from the oocyte APM and the plasma membrane of the two neighbouring nurse cells in the control lines (Osk-GAL4 and Tub67c-GAL4). The proportion of signal arising from oocytes compared to adjacent nurse cells is relatively stable from stage 8 to stage 10. Error bar indicates SEM. (D, D’) Example of distribution of PAR3-GFP (green) expressed with Tub67c-GAL4 driver in a late stage 10 oocyte showing the proximity of the oocyte APM to the adjacent nurse cells. The nurse cell membranes are detached partially (D) or totally (D’) from the oocyte APM. White arrows indicate the oocyte APM and the nurse cell adjacent membrane. DNA is stained with DAPI (blue).

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Figure 2. Cytoskeleton involvement in PAR3 polarity. (A–D) Role of cytoskeleton on PAR3 distribution. Flies are nourished with latrunculin (cyan) for 48 h, colchicin (yellow) for 24 h, or only yeast paste (control). (A) Representative distribution of PAR3-GFP in stage 9B oocytes. Note the PPM exclusion of PAR3-GFP in the control. (B) PAR3 Posterior exclusion ratio (UA) comparison among stages 9A to 10. Significant differences are marked with asterisks (control: stage 9A, Latrunculin: stage 9A and 10, Colchicin: stage 9B). (C) PAR3 asymmetry ratio (UA) comparison among control, Latrunculin, and Colchicin treatments. Significant differences are marked with asterisks. (D) Cytoplasm repartition comparison among control, Latrunculin, and Colchicin treatments. Significant differences are marked with asterisks.
PAR3 (arrow) or the strong APM accumulation (arrowhead). (B) The PAR3 posterior exclusion ratio (ratio LPM/PPM density) is represented between stage 8 and stage 10 oocytes. Under the value of 1, there is a posterior accumulation of PAR3, and above 1 a posterior exclusion. (C) Antero-posterior asymmetry (ratio APM/PPM density at stage 9B) is strongly affected by colchicin, but not by latrunculin. (D) The two drugs lead to an increase in the cytoplasmic fraction of PAR3 in stage 9B oocytes. Mann-Whitney test, NS: not significant; *p<0.05; ***p<0.001; ****p<0.0001. Error bars indicate SEM. Control (stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 15; stage 10, n = 14); +latrunculin (stage 8, n = 5; stage 9A, n = 12; stage 9B, n = 11; stage 10, n = 9); +colchicin (stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 11; stage 10, n = 6).

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Cytoskeleton involvement in PAR3 distribution. (A) Quantification of PAR3-GFP density between stage 8 and stage 10 upon latrunculin (cyan) and colchicin (yellow) treatment. These data were used to obtain the graph in Figure 2B–D. Error bars indicate SEM. Control (stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 15; stage 10, n = 14); +latrunculin (stage 8, n = 5; stage 9A, n = 12; stage 9B, n = 11; stage 10, n = 9); +colchicin (stage 8, n = 8; stage 9A, n = 9; stage 9B, n = 11; stage 10, n = 6).

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Figure 2—figure supplement 2. Effect of colchicin on distribution of PAR3 present at an endogenous level. (A) Localisation of PAR3-GFP (Trap line) at a pre-vitellogenic stage egg chamber. We note PAR3 localised at the anterior cortex and also in some small dots in the oocyte cytoplasm (arrowhead). We cannot observe the very weak signal of PAR3 in the cytoplasm at a later stage because of vitellus accumulation. (B–D) Microtubule requirement for endogenous PAR3 distribution. Flies are fed with colchicin (+colchicin) for 48 h or only yeast paste (-colchicin). To enhance the GFP signal, anti-GFP staining was performed. (B,C) Representative distribution of PAR3-GFP in the absence (B) or presence of colchicin (C). Note accumulation of dotted structures (arrowhead) with colchicin. The nuclear staining is not specific for PAR3 and is only linked to the use of anti-GFP antibody. (D)
Figure 2—figure supplement 2 continued

Colchicin leads to an increase in the cytoplasmic fraction of PAR3. Mann-Whitney test, ***, p<0.0002. Control (n = 10) and +colchicin (n = 6). N indicates the oocyte nucleus position. The scale bars represent 30 μm.

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Figure 3. By producing PI(4,5)P₂, SKTL controls PAR3 APM accumulation and PPM exclusion. (A–F) Distribution of PAR3 in response to SKTL activity. PAR3-GFP is expressed in germinal cells at stage 9B under control conditions, or with overexpression (OE) of Myc-SKTL, Myc-SKTLΔNRQ, or in context sktl<sup>2.3</sup>/sktl<sup>D5</sup>. (A–C) Representative distribution of PAR3-GFP in oocytes in these different genetic contexts. (D) Quantification of PAR3 density at each plasma membrane domain in sktl mutant or SKTL overexpressed (OE) contexts in stage 9B oocytes. Error bars indicate SEM. (E) Antero-posterior asymmetry of PAR3 (ratio APM/PPM density) at stage 9B in control or sktl mutant. (F) Quantification of PAR3 posterior exclusion ratio in sktl mutant or SKTL overexpressed contexts at stage 9B. For (D–F): control (stage 9B, n = 10); sktl<sup>2.3</sup>/sktl<sup>D5</sup> (stage 9B, n = 8); Myc-SKTL (stage 9B, n = 8); Myc-SKTL<sup>ΔNRQ</sup> (stage 9B, n = 10). Mann-Whitney test, NS: not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. (G) PAR3-GFP (green, G') expressed in the germline is present occasionally on vesicles containing PI(4,5)P₂ visualised.
Figure 3 continued

with PH$_{PLC}$ RFP (magenta). Arrowheads show the vesicles that are associated with PAR3 and PI(4,5)P2. G’, G” and G’’’ are magnifications of G (white frame). (H) Colocalisation of PAR3 (green, H’), PI(4,5)P2 visualised with PH$_{PLC}$ RFP (red, H’’’), and SKTL visualised with myc tag (white, H’”). Note that coexpression of PAR3 and SKTL increase the cytoplasmic dotted localisation of PAR3. Scale bars indicate 30 μm.

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Figure 4. SKTL-dependent PAR3 posterior exclusion bypasses regulation by PAR1. (A–C) Representative distribution of PAR3-GFP in stage 9B oocyte in a control situation (A), in RNAi PAR1 context (B), or when PAR3 phosphorylation sites by PAR1 are mutated (C). In the control genotype, PAR3 is excluded from PPM (arrow), unlike the other two genotypes. N indicates the oocyte nucleus position. The scale bars represent 30 μm. (D) PAR3 posterior exclusion in response to PAR1 at stage 9B. In germinal cells, PAR3AA-GFP, a mutant form, non phosphorylatable by PAR1 or PAR3-GFP is expressed with nothing, with par1, or with mCherry knock-down contexts. The posterior exclusion ratios in stage 9B oocytes are represented. PAR3 (stage 9B, n = 15); PAR3-AA (stage 9B, n = 6); PAR3 RNAi mCherry (stage 9B, n = 10); PAR3 RNAi PAR1 (stage 9B, n = 10). (E) SKTL effect on PAR3 posterior exclusion is observed in combination with a PAR1 activity decrease (in green) or with the PAR3-AA non phosphorylatable form (in red). PAR3, RNAi PAR1, RNAi mCherry (stage 9B, n = 10); PAR3, RNAi PAR1, mycSKTL (stage 9B, n = 11) PAR3 RNAi PAR1 (stage 9B, n = 10); PAR3, RNAi mCherry, mycSKTL (stage 9B, n = 10); PAR3-AA, RNAi mCherry (stage 9B, n = 10); PAR3-AA, mycSKTL (stage 9B, n = 10). Mann-Whitney test, NS: not significant; *p<0.05 ; **p<0.01 ; ***p<0.001 ; ****p<0.0001.
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Figure 4—figure supplement 1. Validation of RNAi PAR1 efficiency. Flies Tub67c-GAL4 or Tub67c-GAL4; UASp RNAi par1 were put on fly cages to lay eggs on agar plates. The plates were collected every hour to produce the embryonic extracts revealed after western blot. PAR1 was revealed with a rabbit anti-PAR1 antibody.

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Figure 5. PAR3 asymmetry depends on RAB5. (A) PAR3-GFP (A'), (A''') green) expressed in germline is present occasionally in RAB5-positive early endosomes (A'''), (A''') magenta). A', A'' and A''' are magnifications of A (white frame). Arrowheads show the vesicles that are associated with PAR3 and RAB5. (B–F) PAR3 distribution in response to RAB5 activity impairment. PAR3-GFP is expressed in germinial cells at stage 9B in RAB5 RNAi, RAB5DN(S43N) or in mCherry knockdown (control) contexts. (B–D) Representative images of PAR3 distribution under control conditions (B), in RAB5 RNAi (C), or in RAB5DN(S43N) (D). Scale bars indicate 30 μm. (E) Quantification of PAR3 density at each plasma membrane domain. Error bars indicate SEM. (F) Quantification of PAR3 posterior exclusion in different RAB5 mutant contexts. Control (stage 9B, n = 10); RAB5 RNAi (stage 9B, n = 12); RAB5DN(S43N) (stage 9B, n = 6). Mann-Whitney test, ns: not significant; *p<0.05 ; **p<0.01.

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Figure 5—figure supplement 1. Validation of RAB5DN effect on endocytosis. Endocytosis uptake of FM4-64 dye was performed on control (A, Tub67c-GAL4) or on RAB5DN(S34N) expressing egg chambers (B, Tub67c-GAL4; UASp RAB5DN). In panel (A), the dye is internalised in dotted structures all around the plasma membrane with a higher rate at the posterior. In panel (B), there is a strong decrease of FM4-64 internalisation, indicating that endocytosis is altered by RAB5DN(S34N) expression. Note that FM4-64 accumulates at the APM, and there are no clear explanations for this.

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Figure 6. Role of RAB11 on PAR3 asymmetrical localisation. (A) Colocalisation of PAR3 (PAR3-GFP maternally expressed) with vesicular trafficking markers. To quantify the colocalisation, we measured the Pearson’s coefficient on stage 9B oocytes and results are presented in the box plot. As a control, we used the value of colocalisation obtained with the same images but after rotation of one by 90 degrees. The stars represent the p-value with the control. For HRS, KDEL, and SYX16: n = 7; for PH-PLC: n = 8; for RAB5: n = 10; for RAB11: n = 13. (B) PAR3-GFP (B’), green) expressed in germline is present occasionally in RAB11-positive recycling endosomes (B”), (B”’), magenta). B’, B”, and B”’ are magnifications of B (white frame). Arrowheads show the vesicles associated with PAR3 and RAB11. The arrow points to the vesicle associated only with PAR3. (C,D) RAB11 effect on asymmetrical distribution of PAR3. The antero-posterior asymmetry (C) and the posterior exclusion (D) of PAR3 at stage 9B have been evaluated in rab11P2148 germline clones. Control (stage 9B, n = 10); rab11P2148 (stage 9B, n = 10). Mann-Whitney test, NS: not significant; *p<0.05; ***p<0.001; ****p<0.0001. Error bars indicate SEM. (E,F) Representative images of PAR3 distribution in rab11P2148 clones. The rab11P2148 mutant cells are indicated by the absence of nuclear RFP staining (nls-RFP). (E) The heterozygote egg chamber (rab11P2148/+ ) is used as a control. (F) rab11P2148 mutant oocyte. Note that PAR3 is always excluded from PPM (arrow). Scale bars indicate 30 μm.

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**Figure 6—figure supplement 1.** PAR3 and vesicular compartments. (A–C”) Characterisation of the components of PAR3-GFP vesicular structures. PAR3-GFP (green, (A, B, C) is expressed in the germline under control of the maternal driver Tub67c-GAL4. By immunostaining, we can observe that PAR3-GFP cytoplasmic vesicular structures do not colocalise with HRS (magenta, A’), a late endosome marker, KDEL (magenta, B’), an endoplasmic reticulum marker, or SYNTAXIN 16 (magenta C’), a TRANS Golgi marker. The scale bars represent 30 μm.

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Figure 6—figure supplement 2. PAR3 colocalises with PI(4,5)P2 and RAB11 in front of the ring canal. (A, B) Localisation of PAR3-GFP near a ring canal between a nurse cell (NC) plasma membrane and the oocyte (Oo) APM. PAR3-GFP is expressed in the germline under the control of the maternal driver Tub67c-GAL4. In the NC, besides being at the plasma membrane, PAR3 can be accumulated in front of the ring canal. In this case, PAR3 is present in vesicular structures that are RAB11-positive (A) and PI(4,5)P2-positive (B). The scale bars represent 10 μm.

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Figure 7. Dynein regulates PAR3 asymmetry. (A–C) Distribution of PAR3 in response to dynein activity decrease. PAR3-GFP is expressed in germinal cells at stage 9B in control (osk-GAL4; UASp PAR3-GFP) or dhc64 knockdown contexts (osk-GAL4; UASp RNAi dhc64; UASp PAR3-GFP). (A) Representative distribution of PAR3 in oocyte (note the dotted accumulation of PAR3 under the plasma membrane in the insert). The scale bars represent 30 μm. (B) Quantification of PAR3 density at each plasma membrane domain. (C) Quantification of PAR3 posterior exclusion (ratio LPM/PPM) in dynein mutant context. For (A–C), control (stage 9B, n = 10); RNAi dhc64c (stage 9B, n = 10). For (B, C), Mann-Whitney tests; NS: not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Error bars indicate SEM. (D) Co-immunoprecipitation (IP) of PAR3 by DLIC-GFP in ovarian extracts. Ovaries were dissected from Pubi-DLIC-GFP flies or control Pubi-PDI-GFP flies. The ovarian extract was incubated on magnetic beads coupled with antibody anti-GFP. PAR3 was revealed by anti-PAR3 antibody. (E) SKTL interacts with both PAR3 and DLIC. CoIP of PAR3-HA or DLIC with GFP-SKTL in Figure 7 continued on next page
Figure 7 continued

S2 cell extracts. Cells were transfected with GFP-SKTL or GFP-SNAP (as control) and with PAR3-HA. The cell extracts were incubated on magnetic beads with anti-GFP antibody. PAR3 was revealed by anti-HA antibody and DLIC by anti-DLIC antibody.

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Figure 7—figure supplement 1. Control experiments of PAR3 antibody specificity and charge control of immunopurification of GFP-SKTL. (A) Role of dynein on PAR3 cytoplasmic accumulation (quantification of PAR3 proportion in the cytoplasm related to the whole oocyte intensity). Control (stage 9b, n = 10); RNAi dhc64c (stage 9b, n = 10). Mann-Whitney test, ***p<0.001. Error bars indicate SEM. (B) PAR3 association with PI(4,5)P2 vesicles is not affected by colchicin. Flies are nourished with colchicin for 24 h. PAR3-GFP (B', green) still colocalises with PI (4,5)P2 visualised with PH<sub>PLC</sub> RFP (B'', B''', magenta). B', B'', and B''' are magnifications of B (white frame). The scale bars represent 30 µm.

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Figure 7—figure supplement 2. Validation of PAR3 detection by antibody. By western blot with anti-PAR3 antibody, we revealed numerous bands corresponding to several isoforms of PAR3 and to post-transcriptionally modified forms. We hypothesised that the higher band (>250 kD) corresponds to an oligomer form of PAR3. (A) To confirm that this higher band is PAR3, we tried to decrease its intensity by PAR3 RNAi. We immunoprecipitated PAR3-GFP (Tub67c-GAL4, UASp PAR3-GFP) in embryonic extracts expressing or not PAR3 RNAi. We revealed PAR3 using anti-PAR3 antibody or GFP antibody. We used tubulin as loading control. (B) We ran the same experiment with a strain expressing PAR3-GFP at an endogenous level (Bac PAR3-GFP). PAR3-GFP was immunoprecipitated by GFP antibody in ovarian or embryonic extracts. In this case, we also revealed a PAR3 band of high molecular weight, which disappeared with PAR3 RNAi. These results confirm that the high molecular weight band revealed by anti-PAR3 antibody is a form of PAR3, perhaps a dimer form, in accordance to its weight. (C) Visualisation with Coomassie blue staining of the total proteins in Figure 7E-input samples.

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Figure 8. PAR3 recovery after APM photobleaching. (A) PAR3-GFP expressed in ovarian follicle (Tub67c-GAL4; UASp PAR3-GFP) is photobleached in all the nurse cells and at the APM (yellow area, A1). The fluorescence recovery was followed for around 1400 s (A2). (B) PAR3 quantity in each domain Figure 8 continued on next page
Figure 8 continued

(anterior and posterior domains) was quantified using the same method as previously for three ovarian follicles and raised to 0 after bleaching. It can be seen that, after photobleaching, PAR3 accumulates progressively at the anterior while it is excluded from the posterior. (C) PAR3 quantity of each zone before FRAP was normalised to 1, and recovery of fluorescence was observed. (D) The same experiment as in (A) was performed on ovarian follicle incubated with colcemid. The quantification is shown in graphs in (C) and (D). In (B) and (C), the error bar represents SEM. The scale bars represent 20 μm.

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Figure 9. IKKε regulates PAR3 microtubule unloading and APM accumulation. (A–C) Distribution of PAR3 in response to IKKε knockdown. (A) IKKε knockdown affects the localisation of PAR3 and leads to an accumulation of circular actin clumps (ACs) enriched in PAR3. (B) Quantification of PAR3.
density at each plasma membrane domain from stage 9A to stage 10 oocytes in WT or IKKε knockdown contexts (Tub67c-GAL4; UASp RNAi ikkε; UASp PAR3-GFP). (C) Quantification of PAR3-GFP distribution in the cytoplasm related to the whole oocyte intensity at stage 9B in WT or in IKKε knockdown contexts. For (B, C), Control (stage 9A, n = 9; stage 9B, n = 15; stage 10, n = 14), RNAi IKKε (stage 9A, n = 15; stage 9B, n = 10; stage 10, n = 9). (C) Mann-Whitney test. Error bars indicate SEM. **** indicates p<0.0001. (D, E) Actin clumps (D', E', green) contain RAB11 (magenta, D''), but no RAB5 (E'', red) is around these actin clumps. Actin is visualised after staining with phalloidin. (D'-D'') and (E'-E''), are magnifications of D and E (white frame). (F, G) In an IKKε knockdown oocyte, ACs contain PI(4,5)P2, visualised with PHPLC GFP probe, (F) and Myc-SKTL, visualised with Myc tag (G). (H, I) PI(4,5)P2 or SKTL are involved in formation of the ACs. ACs are reduced in SKTLDNQ context (H) and disappear in sktl2.3/sktlD5 context (I). (J, K) MTs are necessary for actin ring formation. Flies were nourished with colchicin for 24 h (K) or only yeast paste (J). Actin (green) is visualised after staining with phalloidin and the nuclear membranes after staining with WGA (red). The oocyte nucleus position is indicated by an ‘N’ in (G), (J), and (K). DOI: https://doi.org/10.7554/eLife.40212.043
Figure 9—figure supplement 1. Effect of IKKe knockdown. (A) In an IKKe knockdown oocyte, ACs (A, A’ green) are accumulated around the nucleus (red). The oocyte nucleus position is indicated by an ‘N’ in A. (A’) is a magnification of the oocyte presented in (A) and represents five Z-plans (13.5 μm of depth). (B–D) ACs (B’, C’, D’ green) contain NUF (B’”, red), aPKC (C’”, red) but not SYX16 (D’”, red). Actin is visualised after staining with phalloidin. (B”–B’’’) and (C”–C’’’) are magnifications of B, C, and D (white frame).

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Figure 9—figure supplement 2. Alteration of microtubule network in IKKe knockdown. (A) Dynein is trapped in ACs. Dynein is visualised thanks to DLIC-GFP transgene (green) and actin (red) is visualised after staining with phalloidin. (B–C) Effect of IKKe on MT minus ends. MTs minus ends are visualised with the transgene UASp Nod-LacZ after staining with anti-β-galactosidase. In a wild-type oocyte, MT minus ends are localised around the nucleus and surround the nucleus at the plasma membrane. (B1) and (B2) are two independent examples of this staining. In an IKKe knockdown oocyte, MT minus ends are isotropically localised at the plasma membrane, around the nucleus (C1) and in the clumps around the nucleus (C2). (C1) and (C2) are two independent examples of this staining. ‘N’ indicates the oocyte nucleus position.

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Figure 10. Speculative model of PAR3 localisation regulation. (A) In Drosophila oocytes, PAR3 asymmetrical localisation proceeds in at least two steps. The first step (C) occurs at the posterior plasma membrane and leads to the PAR3 exclusion starting from stage 9B. This step implicates PAR3 phosphorylation by PAR1 (C2) and RAB5-dependent endocytosis (C1). PI(4,5)P2, SKTL, and its product PI(4,5)P2 (orange) are crucial for this process, as well as the actin cytoskeleton (blue gradient). As PI(4,5)P2 is critical for the endocytosis onset but also for actin cytoskeleton regulation, the link between all these protagonists remains hypothetical. Furthermore, as the polarised localisation of PAR1 depends on actin, we cannot exclude a role for PAR1 on endocytosis directly or indirectly by PAR3 phosphorylation. The second step (B) takes place at the anterior cortex where PAR3 has to be strongly enriched. Dynein-dependent transport brings PAR3 to the MT minus ends likely with recycling endosomal cargo (RE). Then PAR3 cargo would be released from the dynein through an IKK-pathway-dependent process. However, how PAR3 reaches the cortex is unknown. PI(4,5)P2 is also important for the PAR3 endosomal sorting and we can speculate that an association with vesicles is required for anterior cortex targeting. Finally, while we have shown that the posterior fraction of PAR3 provides, through MTs, the anterior pool of PAR3, the neo-synthesized fraction of PAR3 from the nurse cell could also contribute to the anterior pool. As the MT network presents only a slight bias of minus end directed transport in the oocyte, it is thus possible that PAR3 is transported step by step along the lateral cortex before reaching the anterior membrane.

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