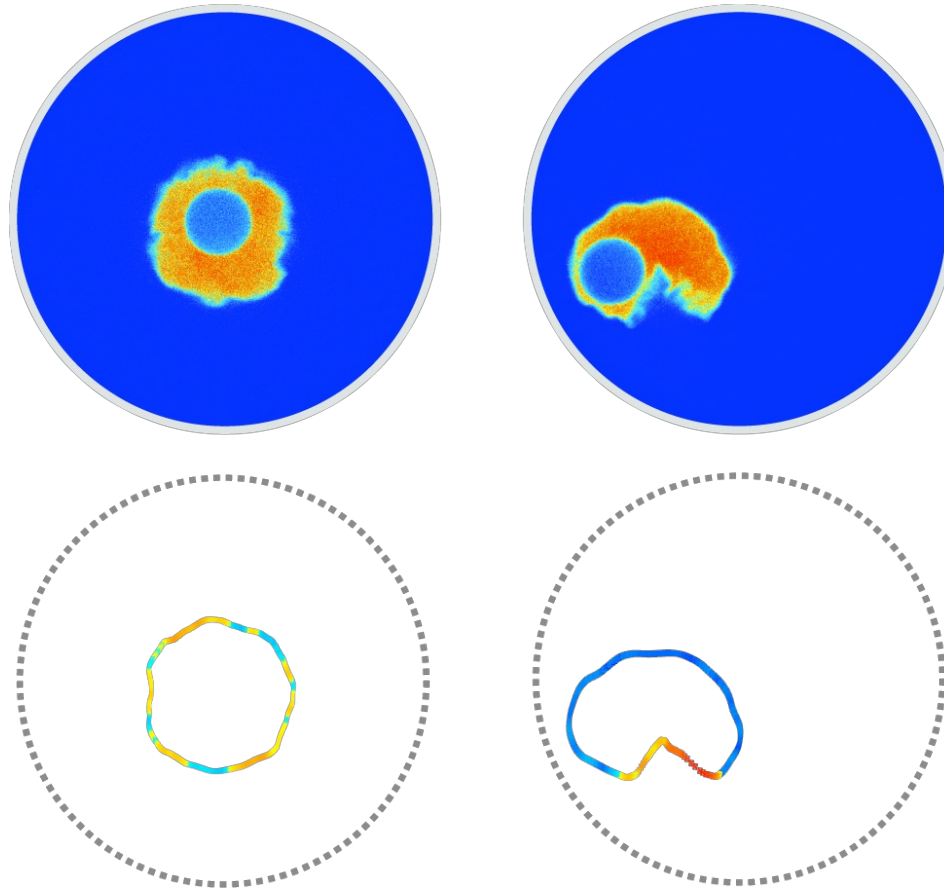


Regulation of the developmental potential of the female gamete by cytoplasmic forces exerted on the nucleus



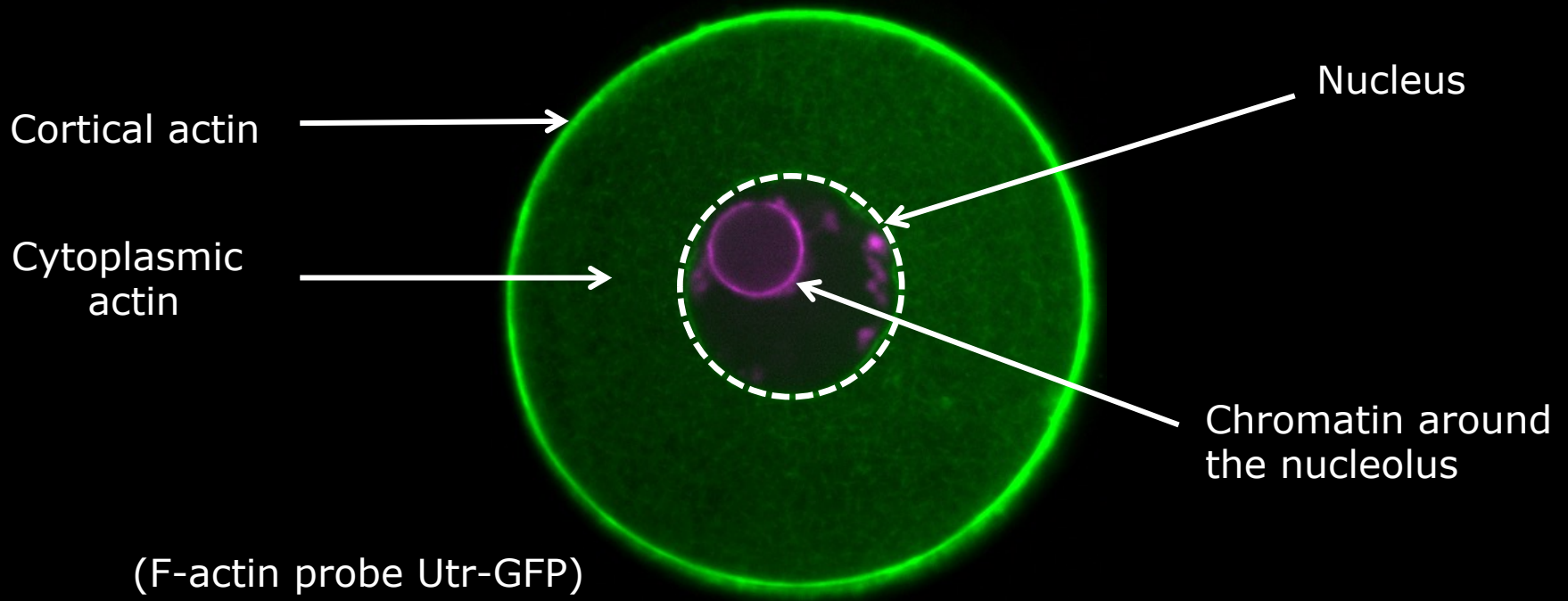
Maria ALMONACID
Verlhac-Terret lab

contact: maria-elsa.almonacid@college-de-france.fr

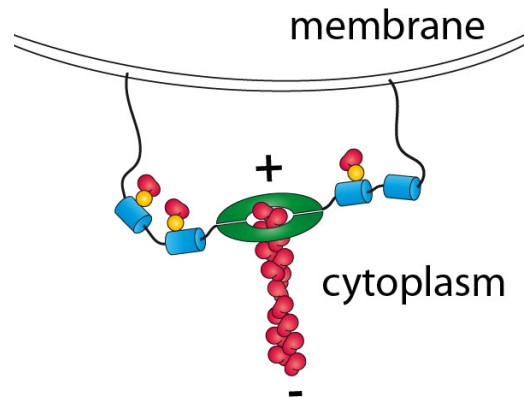
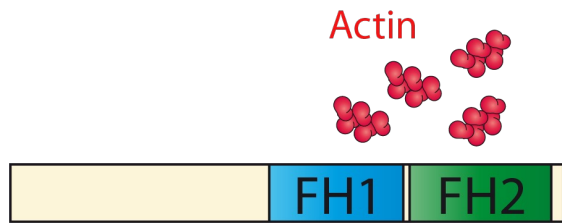
Morphology of the mouse oocyte

1/ No predefined cortical polarity

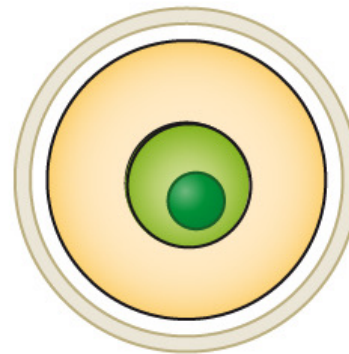
2/ No centrosomes



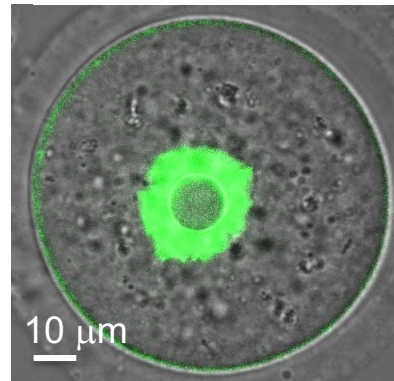
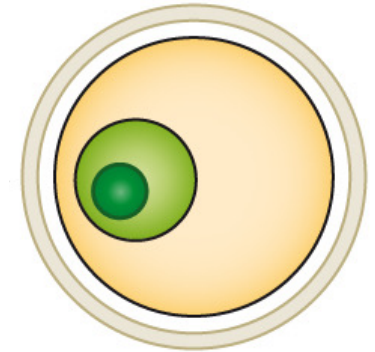
The nucleus is off-centered in oocytes lacking the F-actin nucleator Formin 2



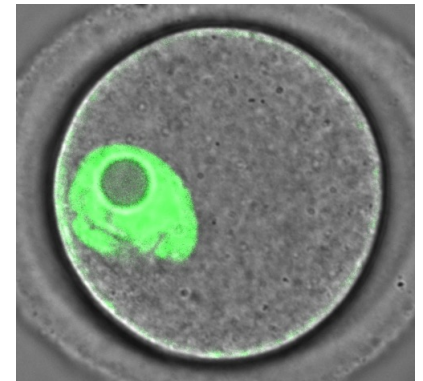
Formin 2 (Fmn2) +/-



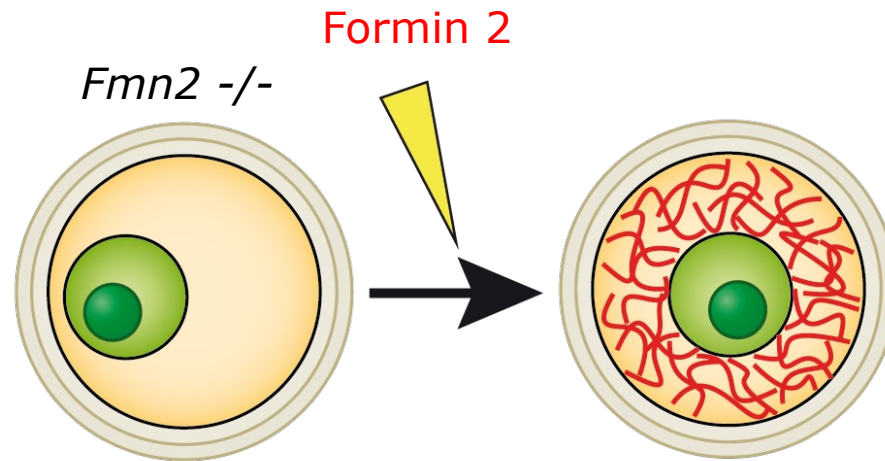
Formin 2 (Fmn2) -/-



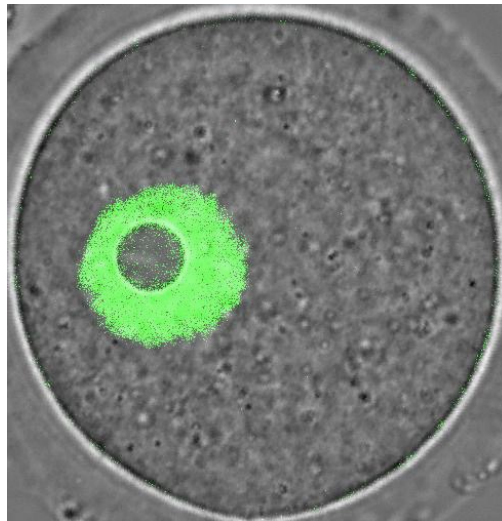
Nucleus



Formin 2 injection in *Fmn2* $-/-$ oocytes triggers nucleus centration



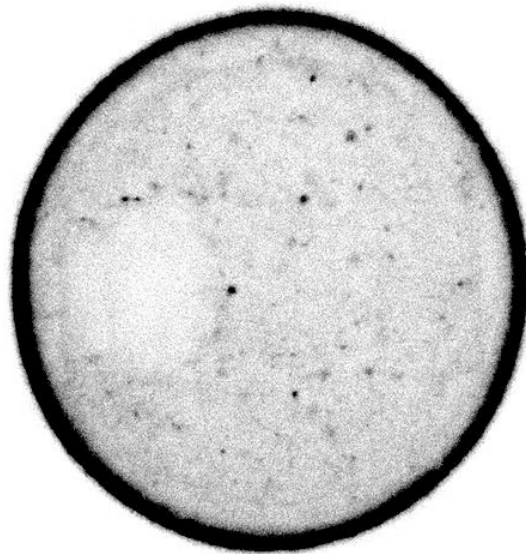
Fmn2 $-/-$ oocyte + Formin 2



Nucleus

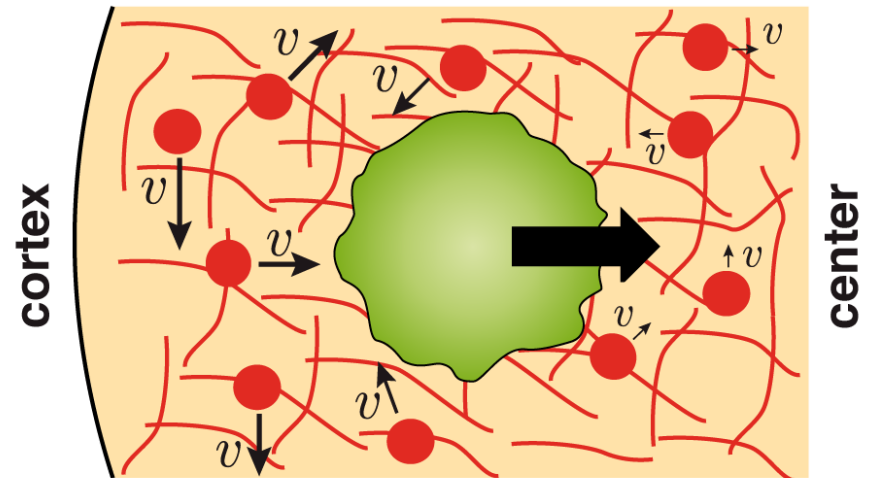
Nucleus centering by the activity of actin-coated vesicles: a thermodynamic model

Fmn2 ^{-/-} + *Fmn2*



F-actin
probe
Utr-GFP

Timepoints
every
500 ms





A purple wedge-shaped diagram pointing to the right, representing a gradient of activity.

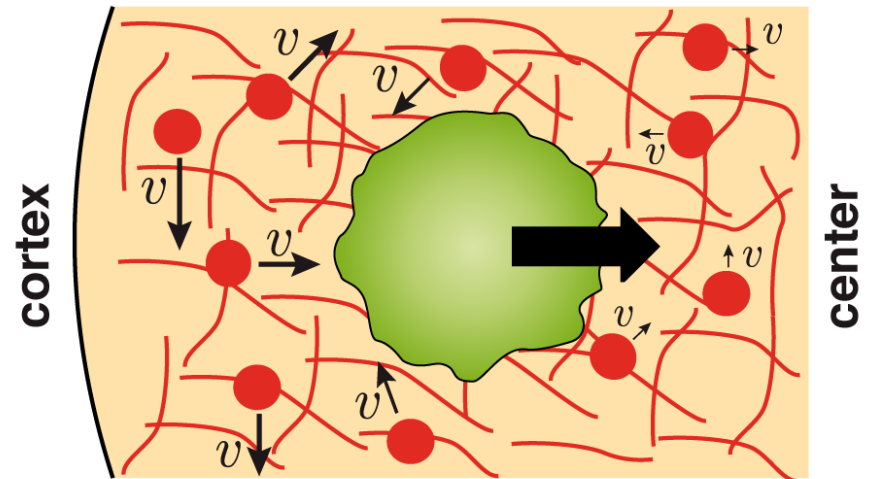
$$\vec{F}_p = -\alpha \nabla \langle v^2 \rangle$$

Nucleus centering by the activity of actin-coated vesicles: a thermodynamic model

Oil droplet injection

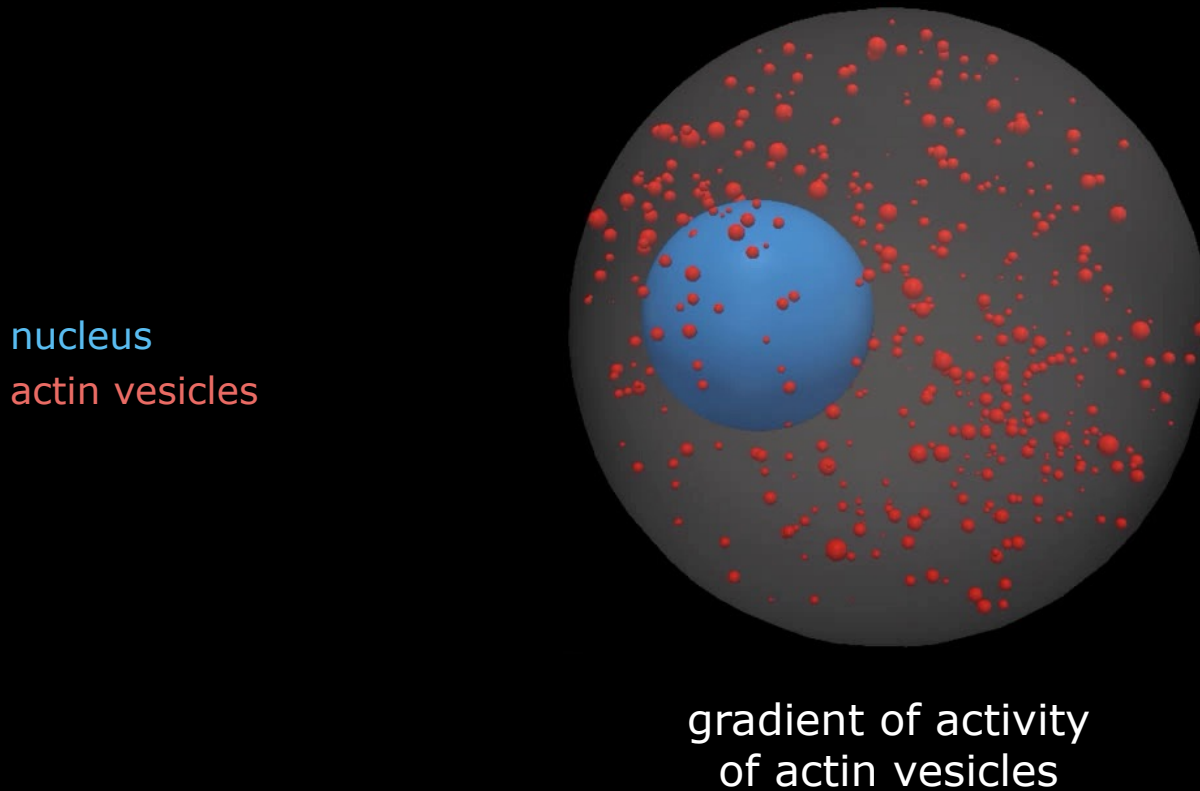


Timepoints every
20 min

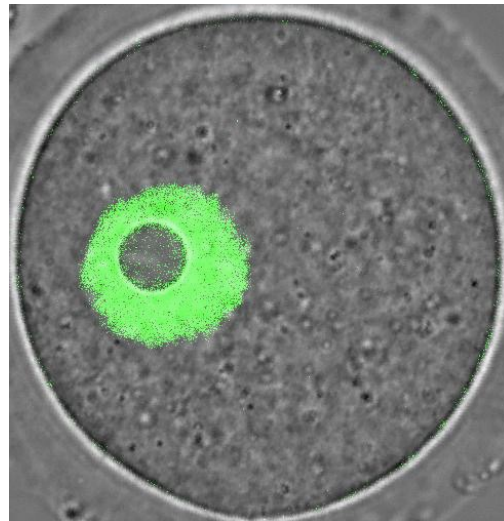
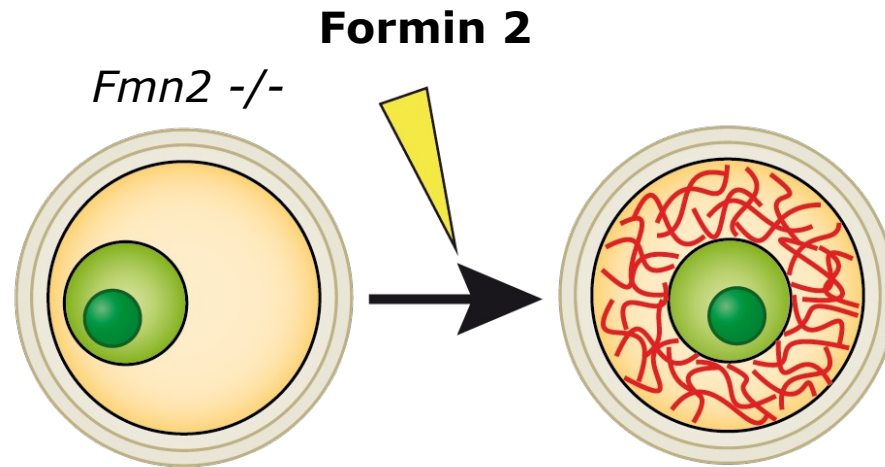



$$\vec{F}_p = -\alpha \nabla \langle v^2 \rangle$$

Virtual experiment:
Simulating the presence or absence of an activity gradient



What is the role of nucleus centering in mouse oocytes?



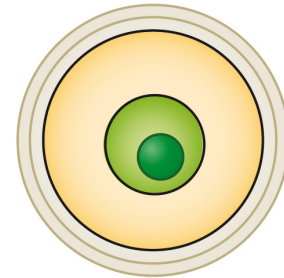
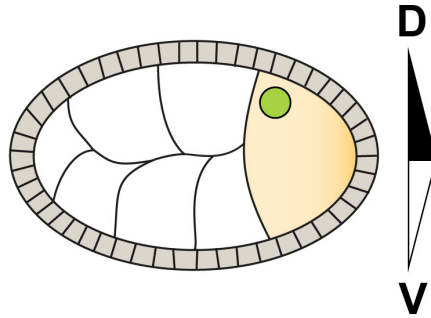
Nucleus

Nucleus position, a morphogen in many oocytes,
except in mammals

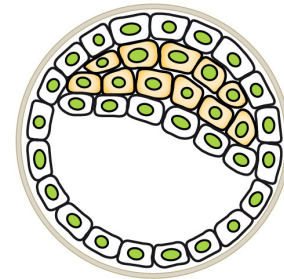
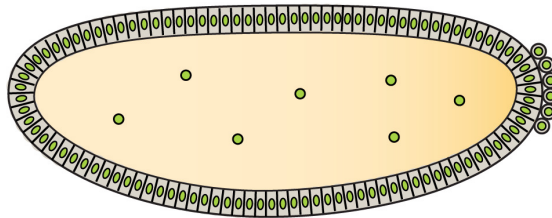
Drosophila, Nematod, *Xenopus*

Mammals

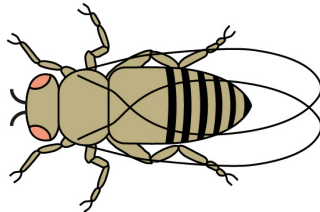
Oocyte



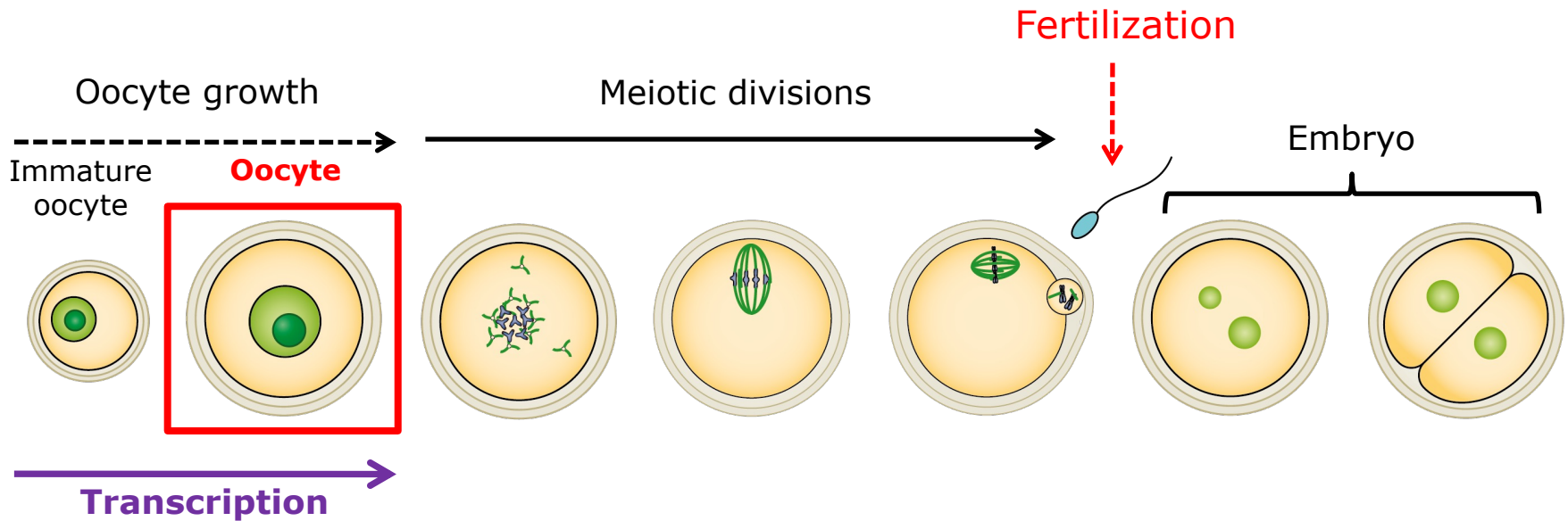
Embryo



Adult

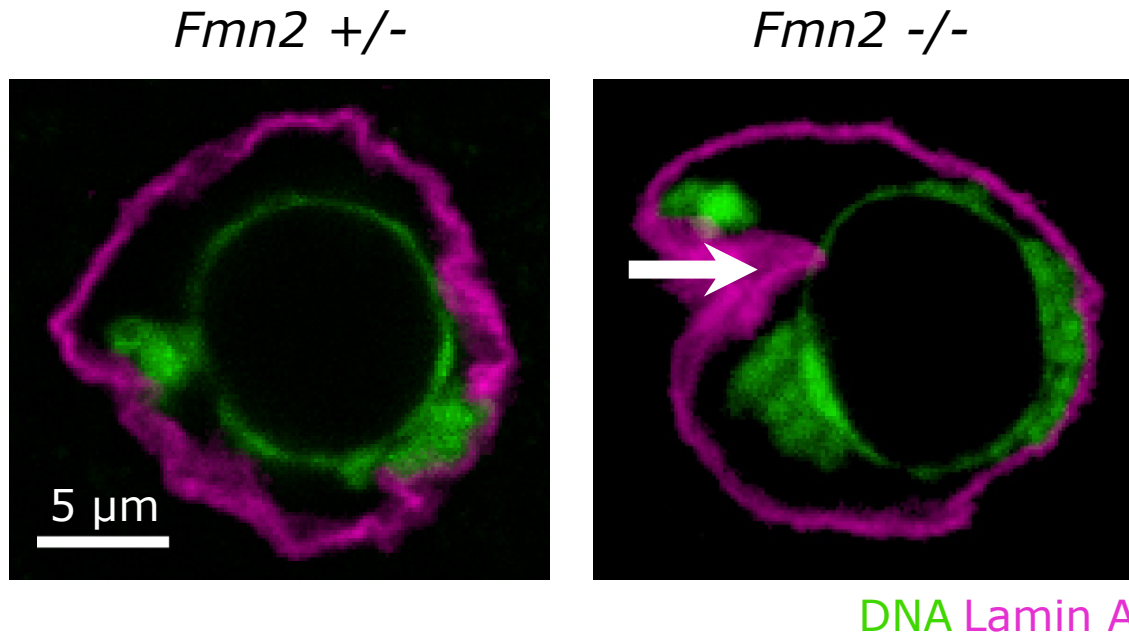


Biological significance of nucleus centering in mammalian oocytes?

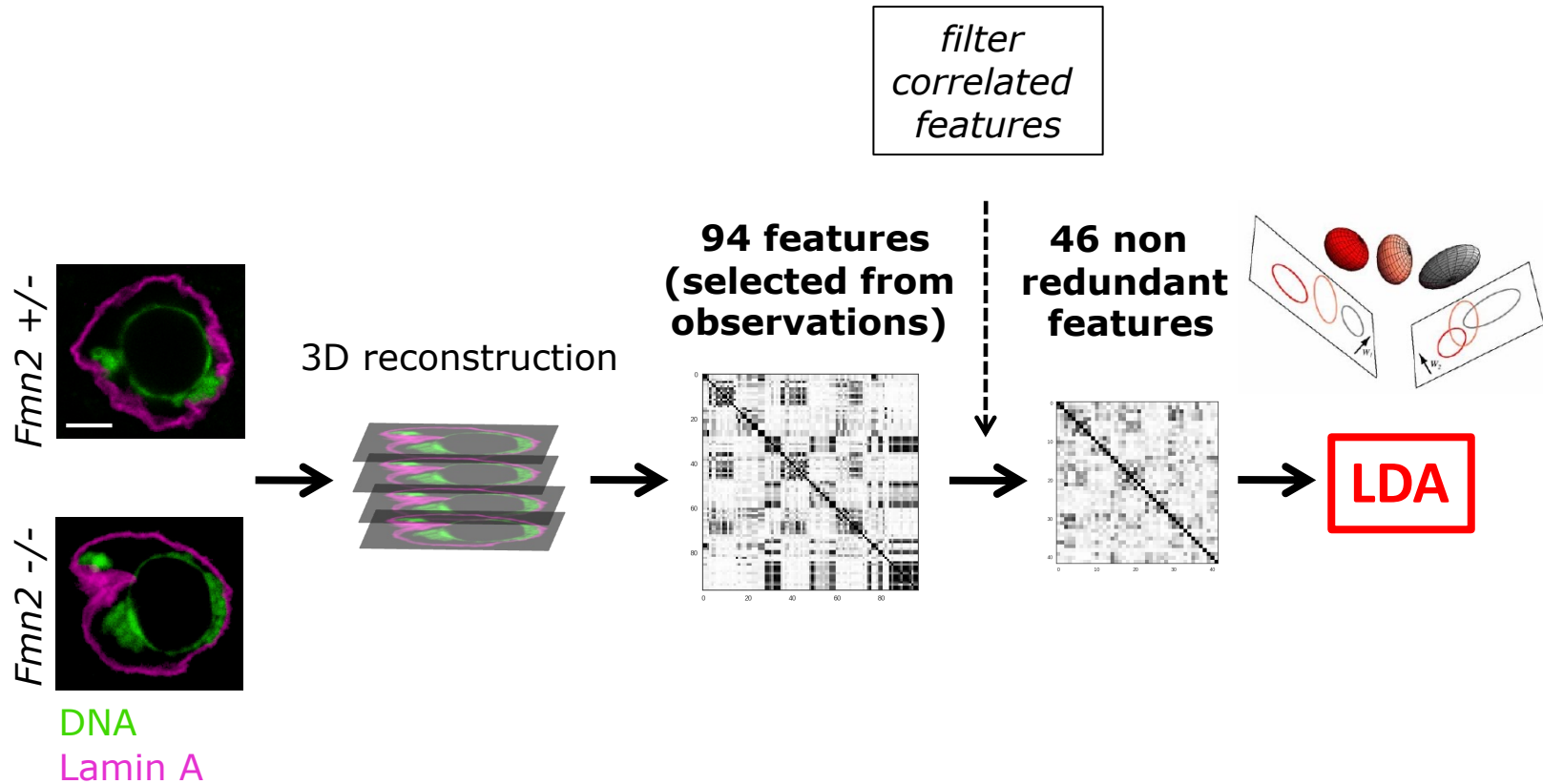


- Correlation with meiotic success
(Brunet & Maro, 2007, Bellone, 2009, Levi, 2013)
- Centering and then excentering?
- Centering and quality of the transcriptome?

Fmn2 ^{-/-} oocytes have altered nuclear architecture

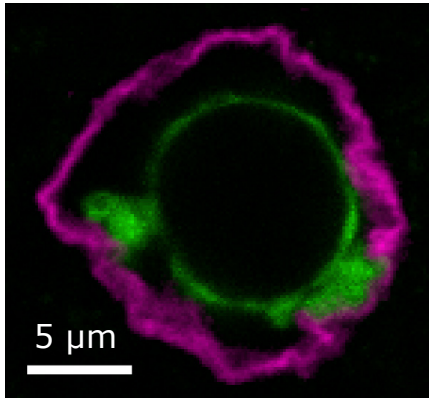


Computational approach for analysis of nuclear architecture

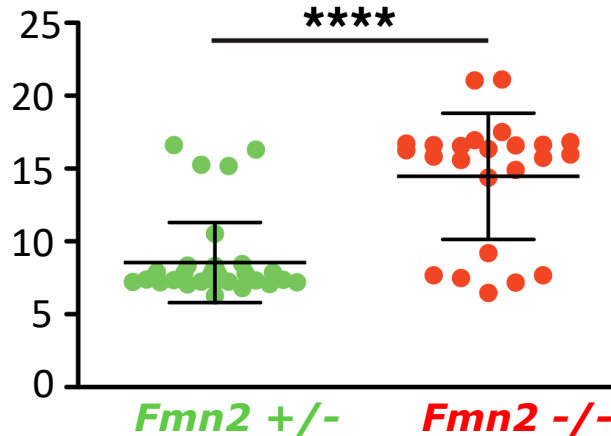
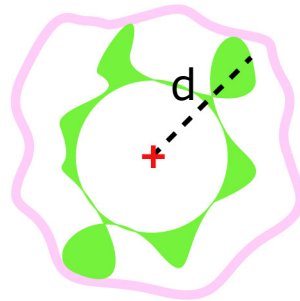


Identification of discriminant features and quantification of *Fmn2* $-/-$ nuclear architecture defects

Fmn2 +/-

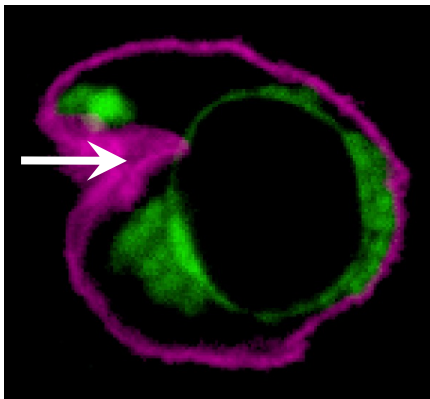


DNA dispersion

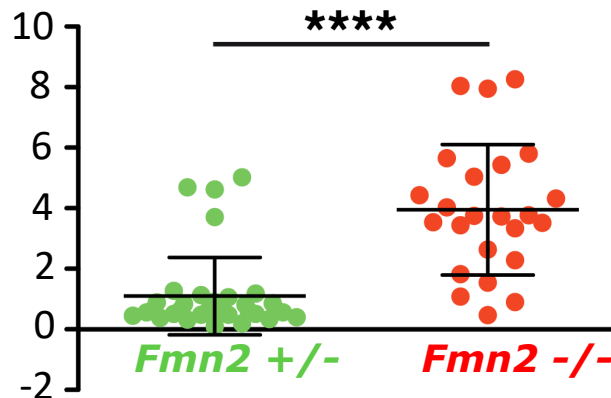
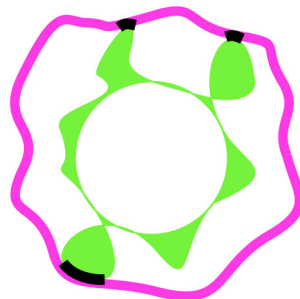


distance *d*
centroid-
outer DNA
surface
(μm)

Fmn2 -/-



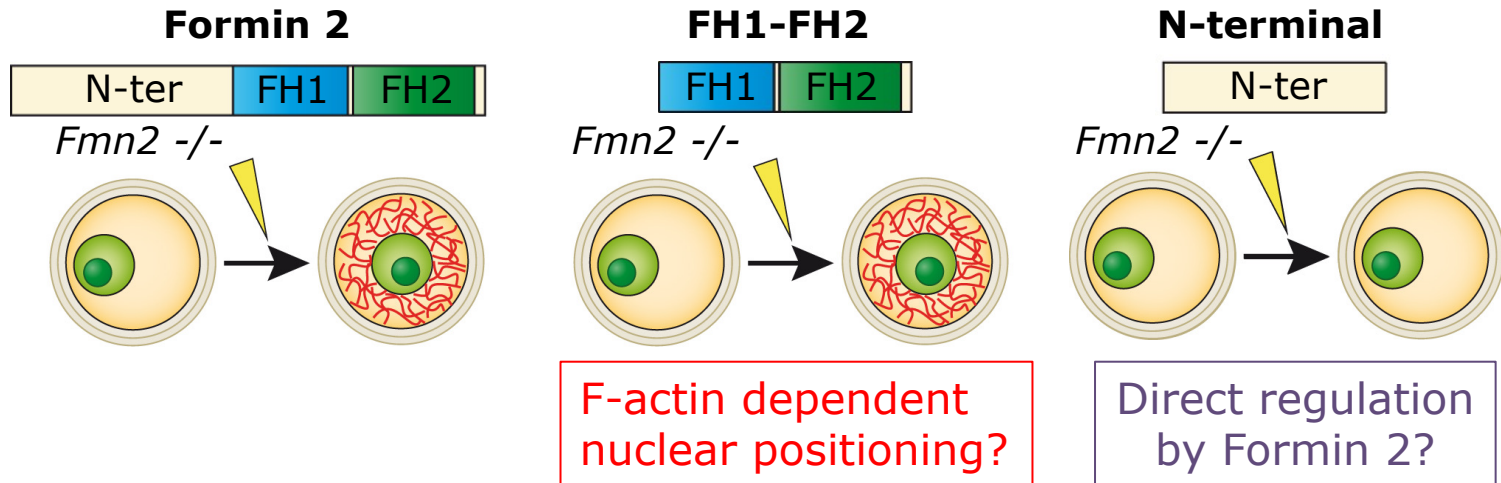
Overlap Lamin A/DNA



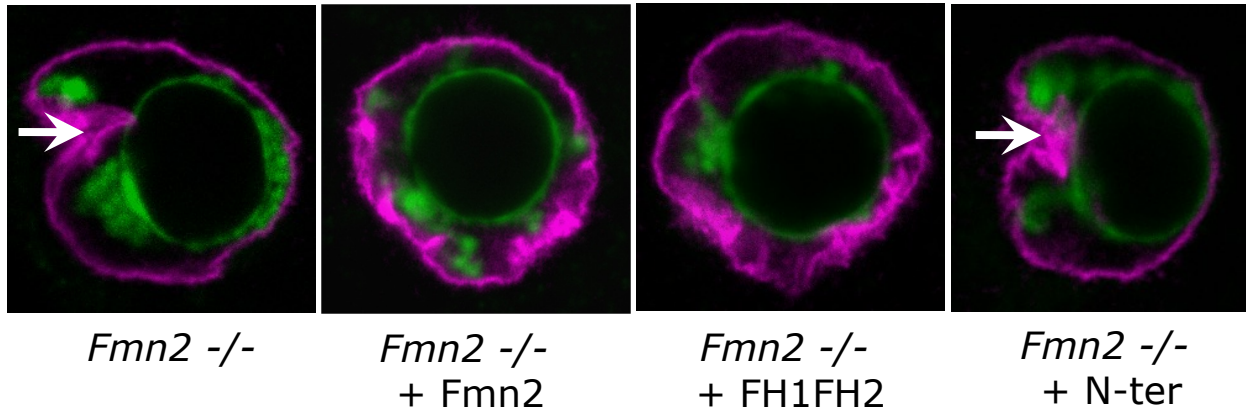
% colocalization
Lamin A/DNA

DNA Lamin A

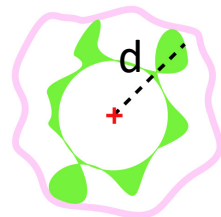
Nucleus positioning and nuclear architecture



Formin 2 rescues nuclear architecture in *Fmn2* $-/-$ oocytes

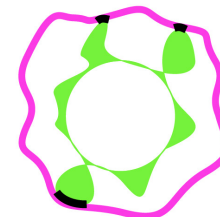


DNA dispersion



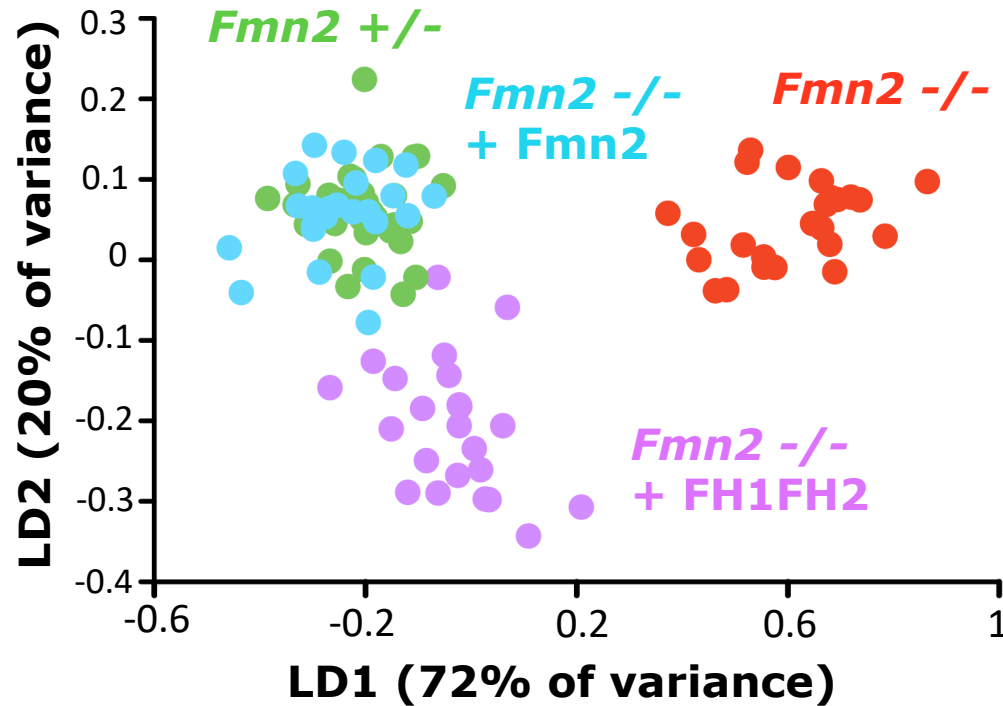
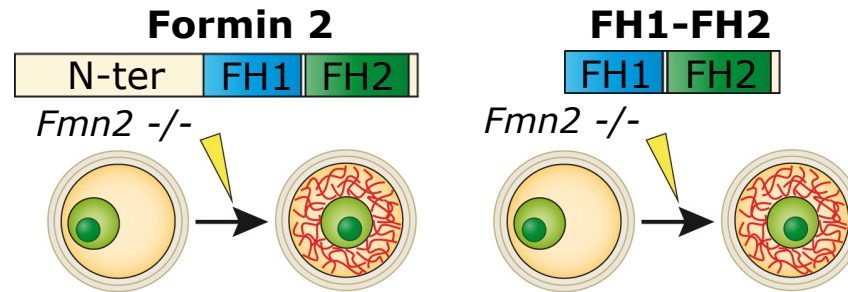
DNA

Overlap Lamin A/DNA



DNA Lamin A

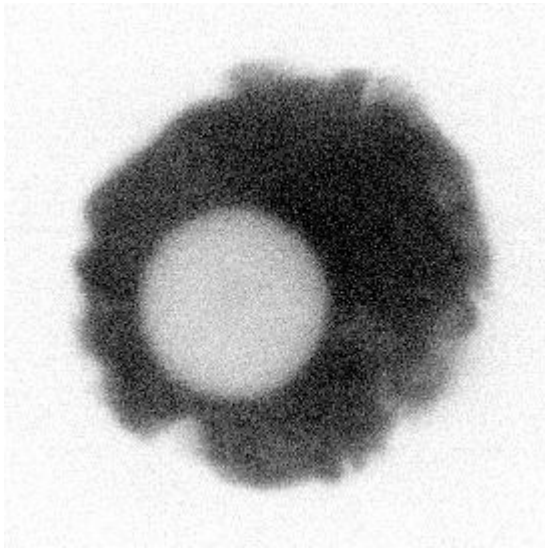
Formin 2 rescues nuclear architecture in *Fmn2* $-/-$ oocytes



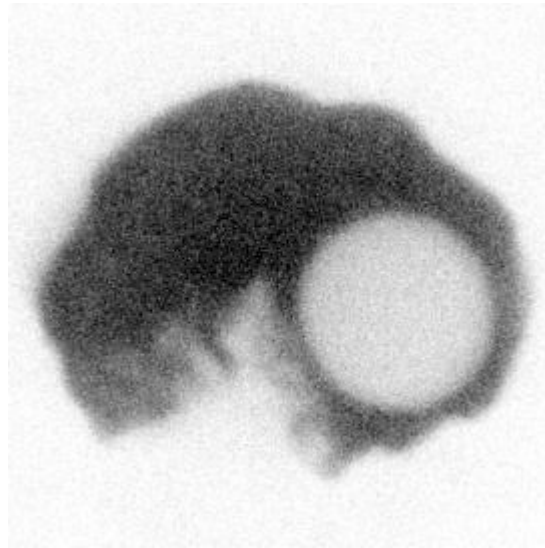
**Linear
Discriminant
Analysis (LDA)
on 46 non
redundant
features**

Actin promotes nuclear envelope fluctuations
and shapes the nucleus

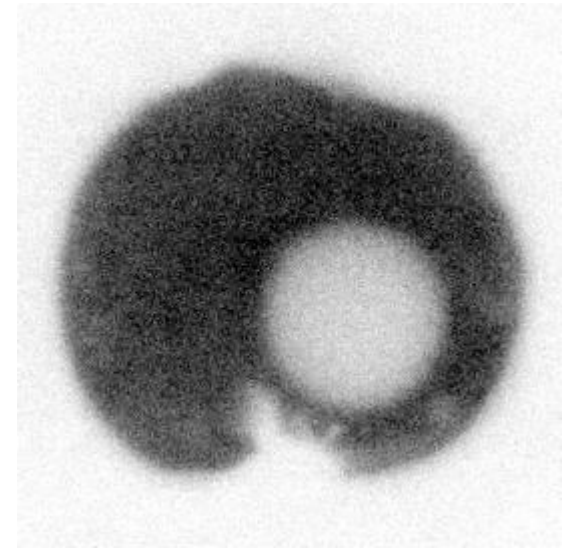
Fmn2 +/-



Fmn2 -/-

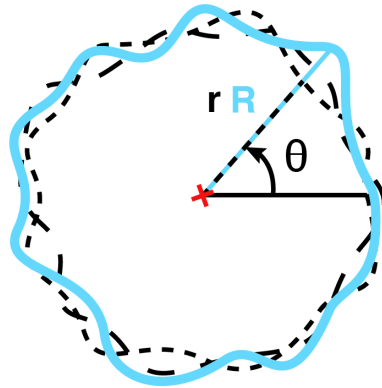


Fmn2 +/-
+ Cytochalasin D



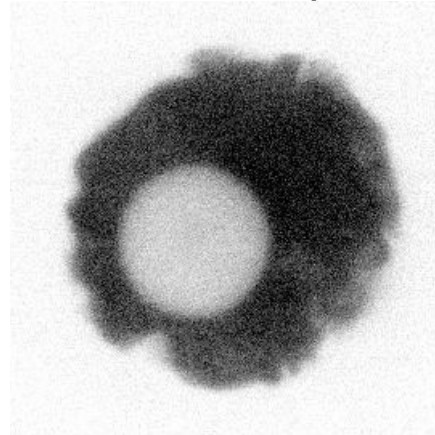
Nuclear probe

Quantitative measurements of nuclear envelope fluctuations

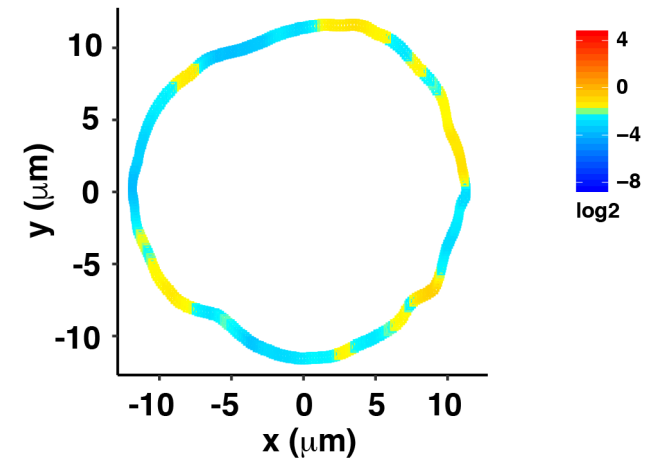


- mean shape over time
- shape at t_1
- - - shape at t_2
- θ rotational angle
- r radius for a given t and θ
- R radius for the mean shape over time for a given t and θ

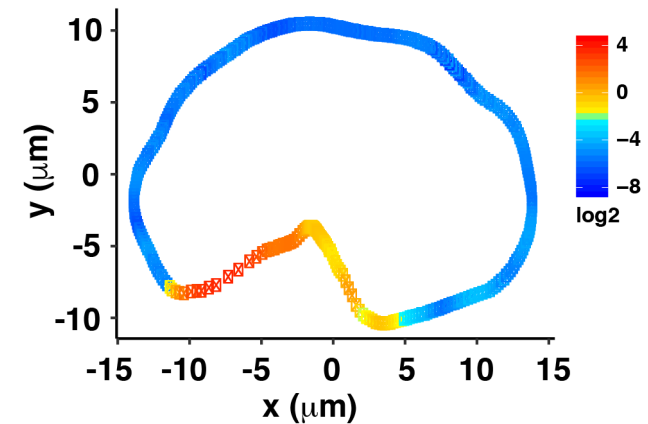
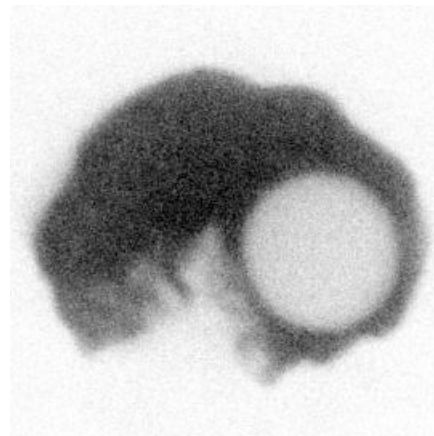
Fmn2 +/-



Mean fluctuations
($r-R$)² over time (μm^2)

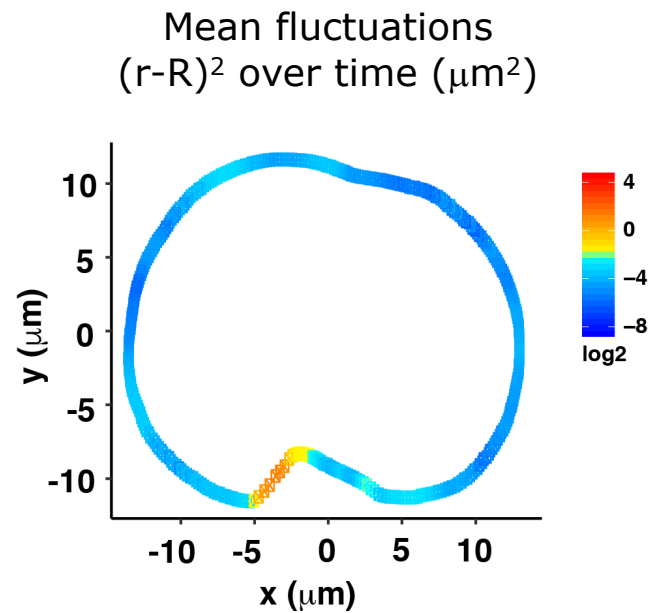
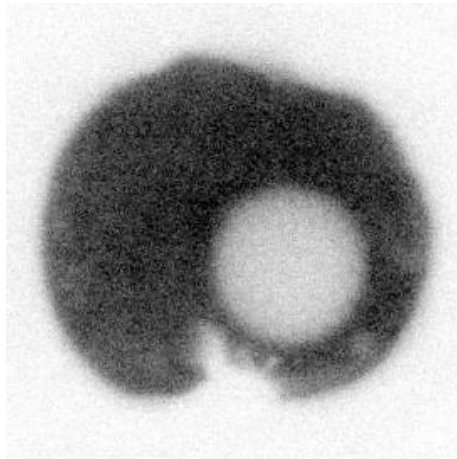


Fmn2 -/-

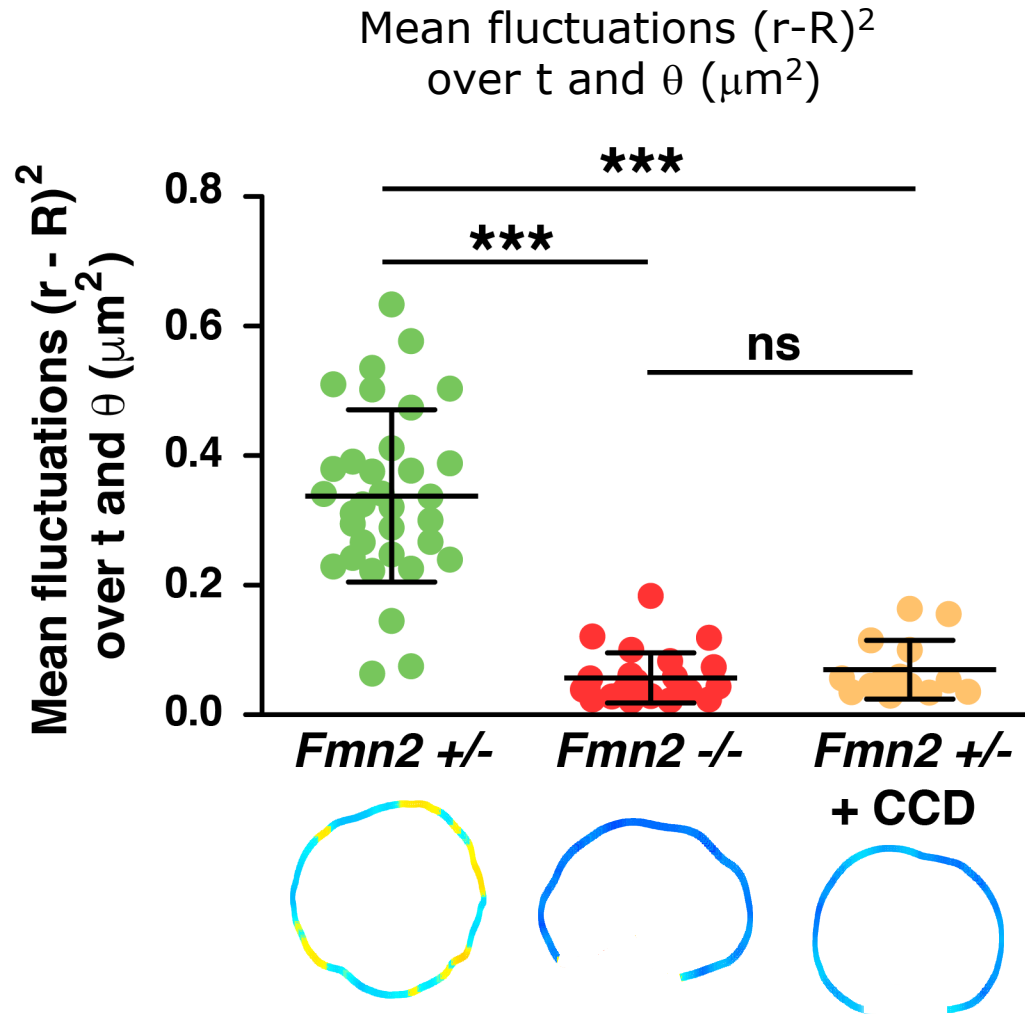


Quantitative measurements of nuclear envelope fluctuations

Fmn2 +/-
+ Cytochalasin D

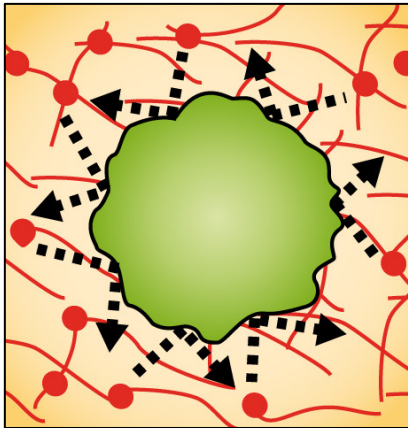


Dynamics of the cytoplasmic actin mesh induces a 6-fold increase in nuclear envelope fluctuations

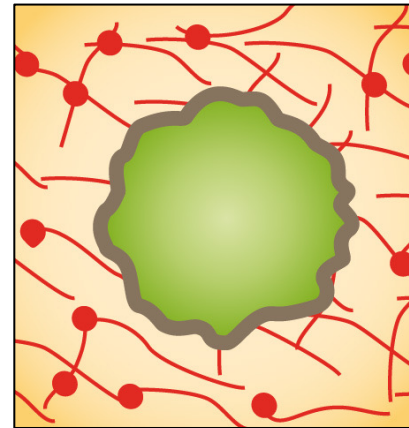


Origin of nuclear envelope fluctuations ?

Activity of the
cytoplasmic actin mesh

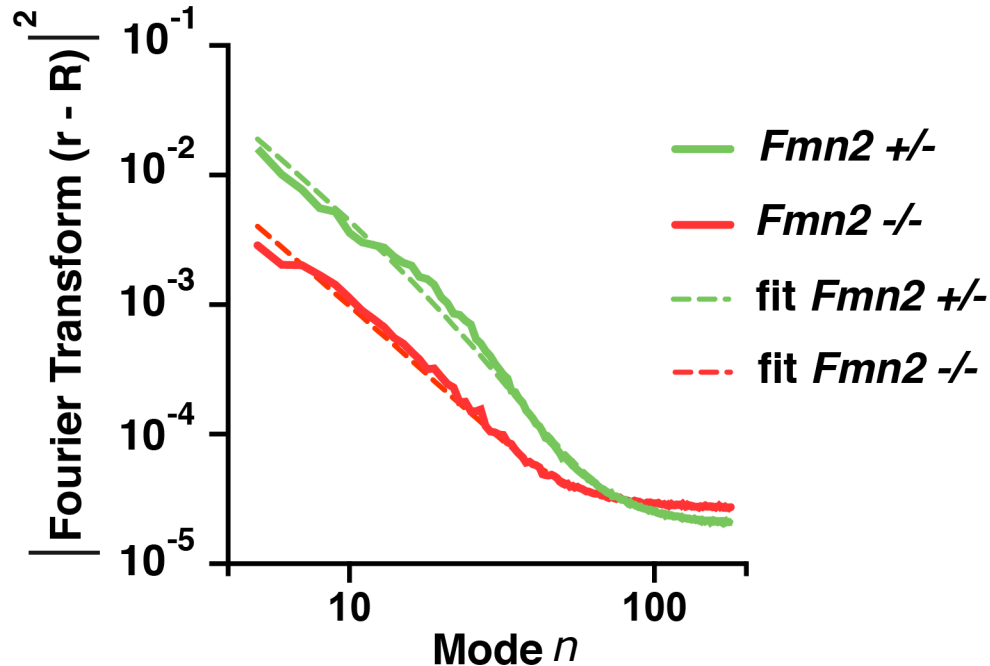


Mechanical properties
of the nuclear envelope



Nuclear envelope fluctuations are generated from the « outside » cytoplasmic activity

Power spectrum
of (r-R) fluctuations

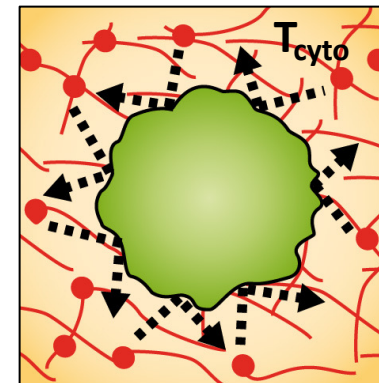


Helfrich model
of membranes

$$\langle |\hat{h}_n|^2 \rangle = \frac{2\pi k_b \mathbf{T}_{\text{cyto}}}{\bar{\sigma} n^2 + \bar{\kappa} n^4}$$

No changes in nuclear
envelope properties

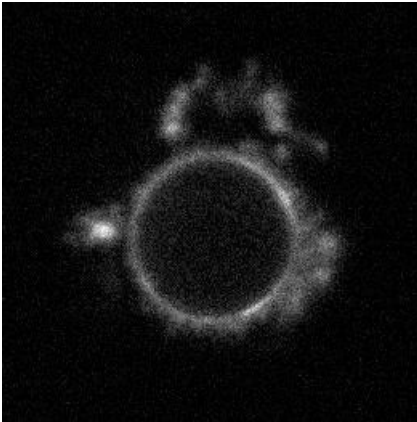
	<i>Fmn2</i> +/-	<i>Fmn2</i> +/- + CCD	<i>Fmn2</i> -/-
$l_c = R\sqrt{\bar{\kappa}/\bar{\sigma}}$	0.4	0.24	0.29
T_{cyto}	1	0.2	0.2



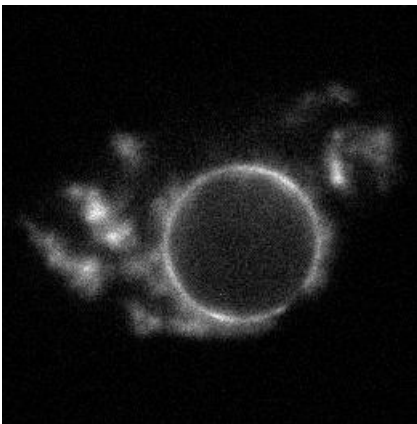
(Raphaël Voituriez)

Nuclear envelope fluctuations correlate with chromatin motion

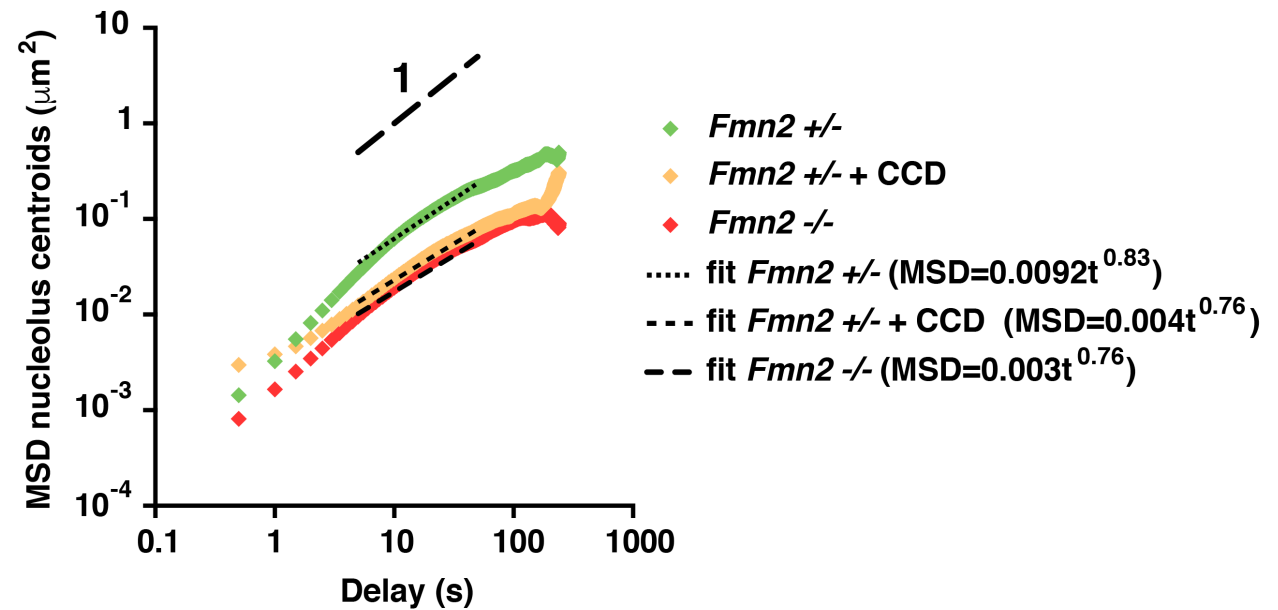
Fmn2 +/-



Fmn2 -/-

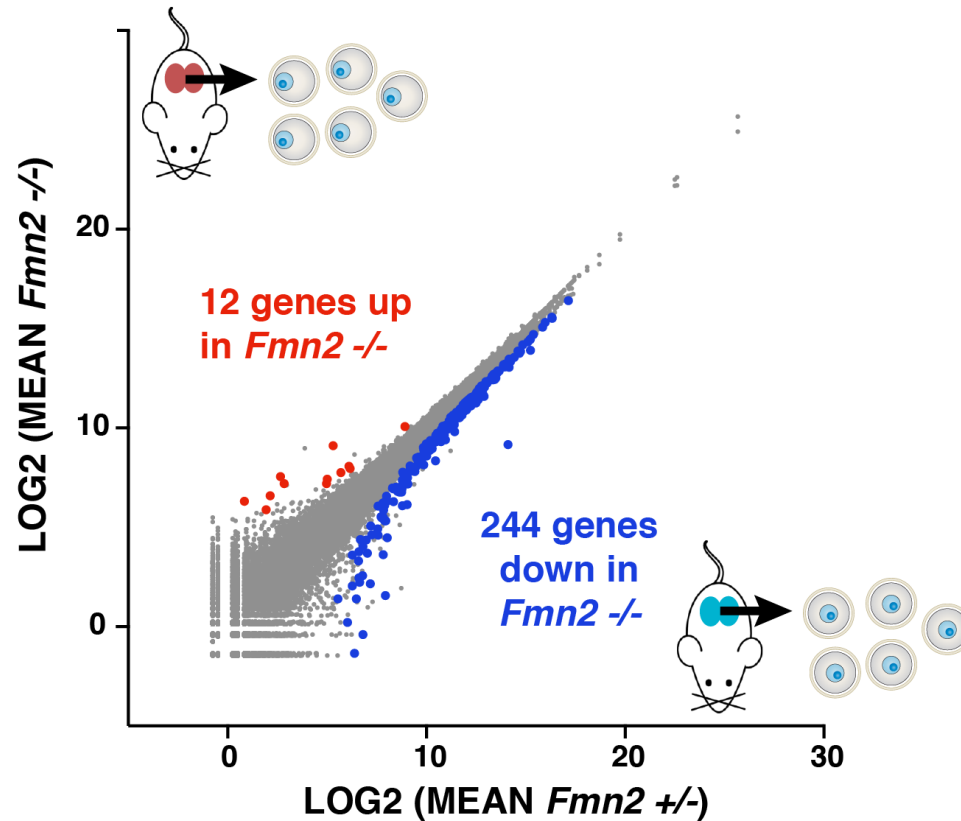


H2B-RFP



Gene expression is modified in *Fmn2* $-/-$ oocytes

**Comparative transcriptomic analysis:
Fmn2 $+/-$ vs *Fmn2* $-/-$ fully-grown oocytes**

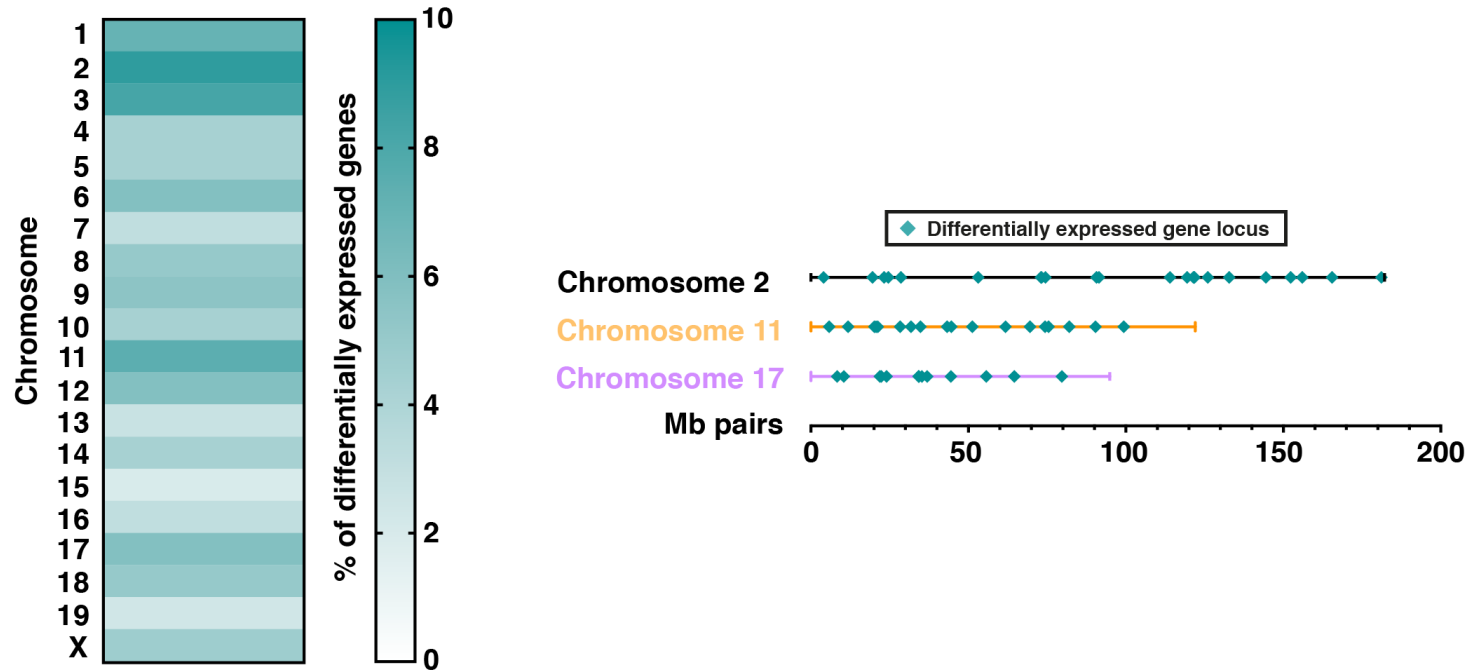


* $p < 0.05$ and
fold change > 2

(Adel Al Jord
with ARTbio)

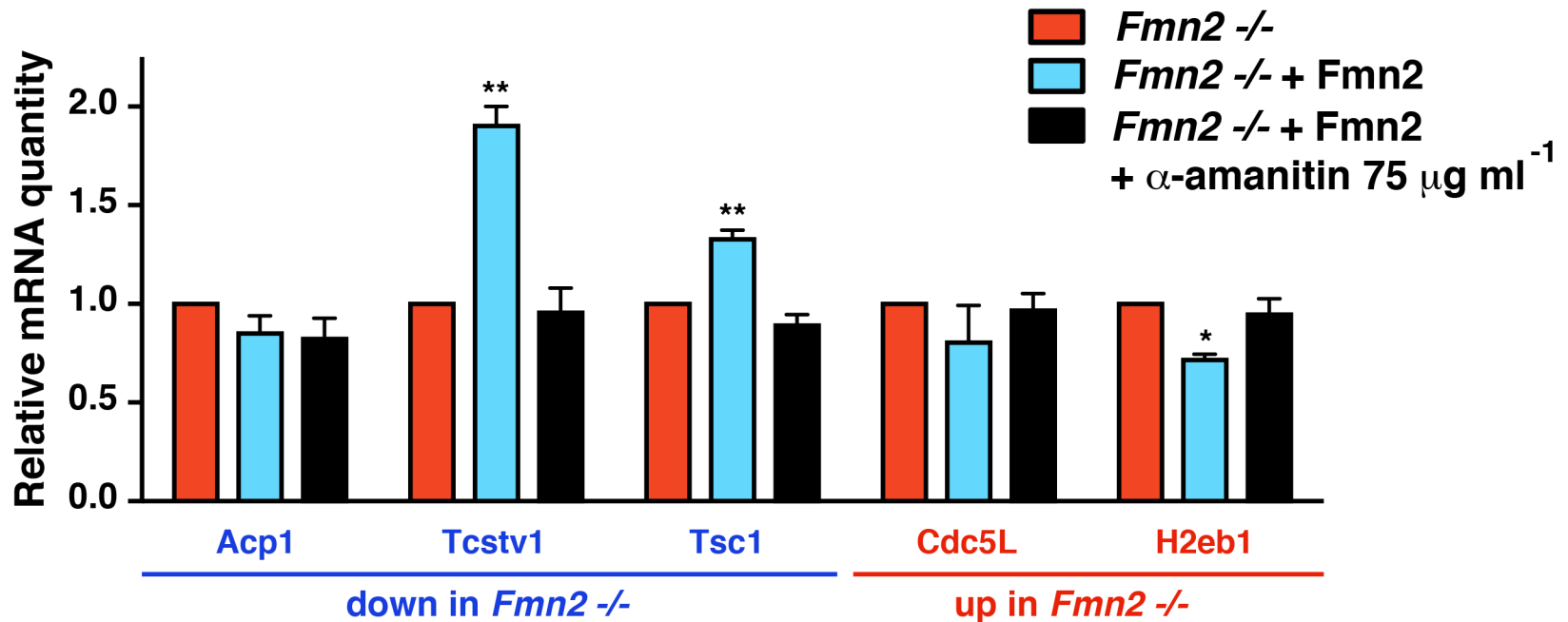
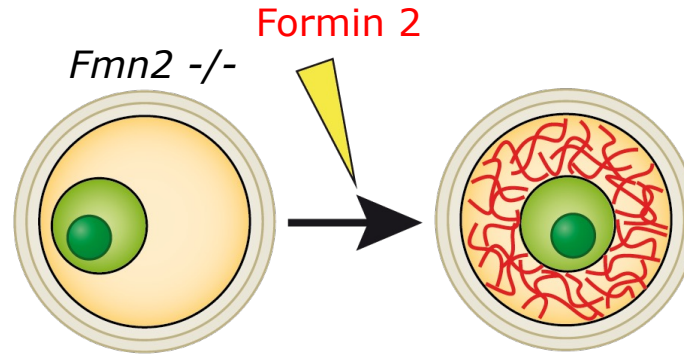
Genome-wide distribution of Formin 2 - regulated genes

Chromosomal positioning
of differentially expressed genes



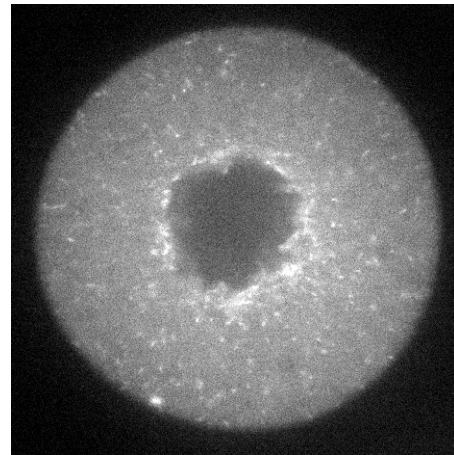
(Adel Al Jord
with ARTbio)

Formin 2 rescues gene expression in *Fmn2* $-/-$ oocytes in a transcription-dependent manner



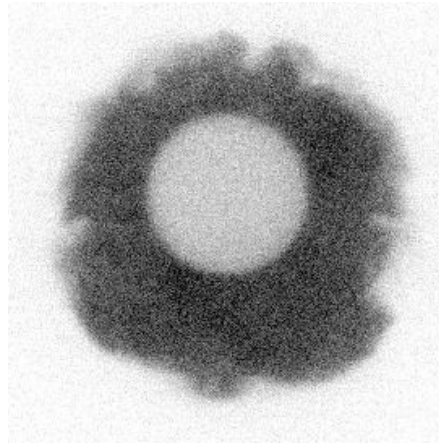
Contribution of MTs ??

Microtubules dampen actin-driven nuclear envelope fluctuations

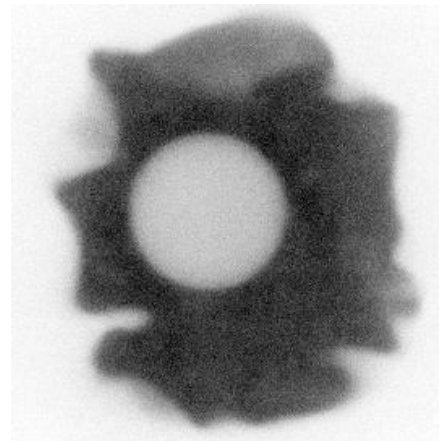


EB3-GFP

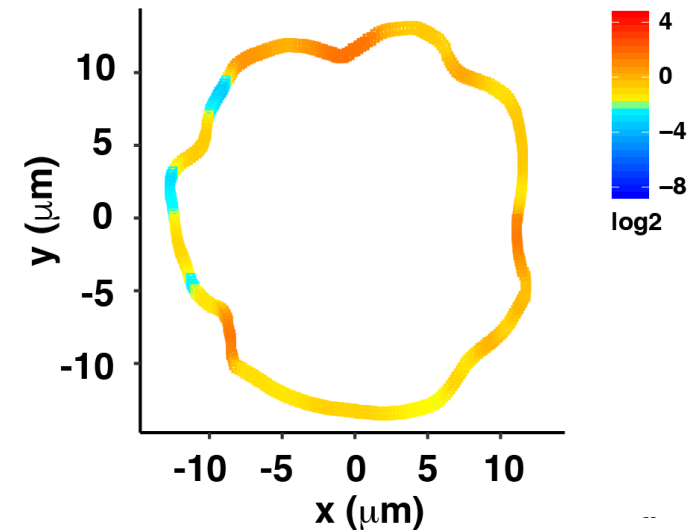
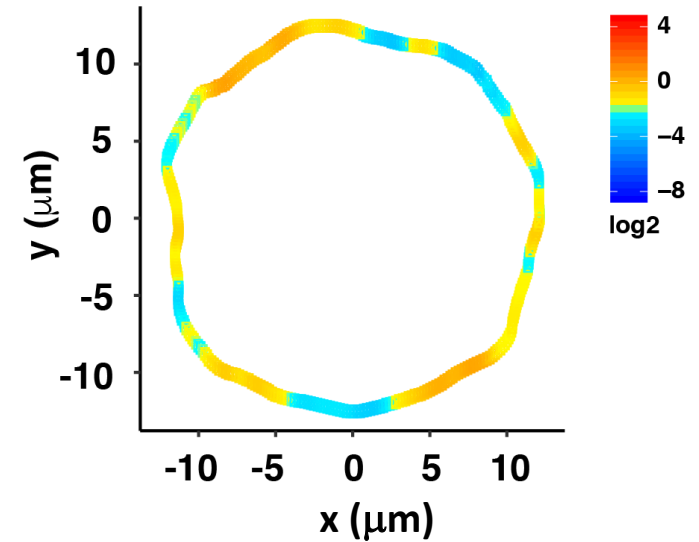
Fmn2 +/-



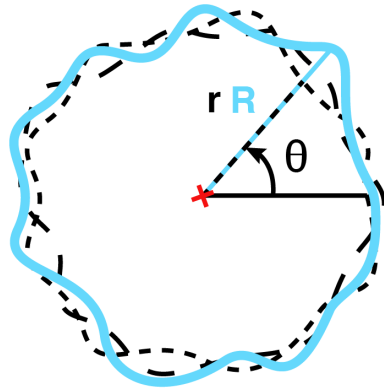
Fmn2 +/- + NZ



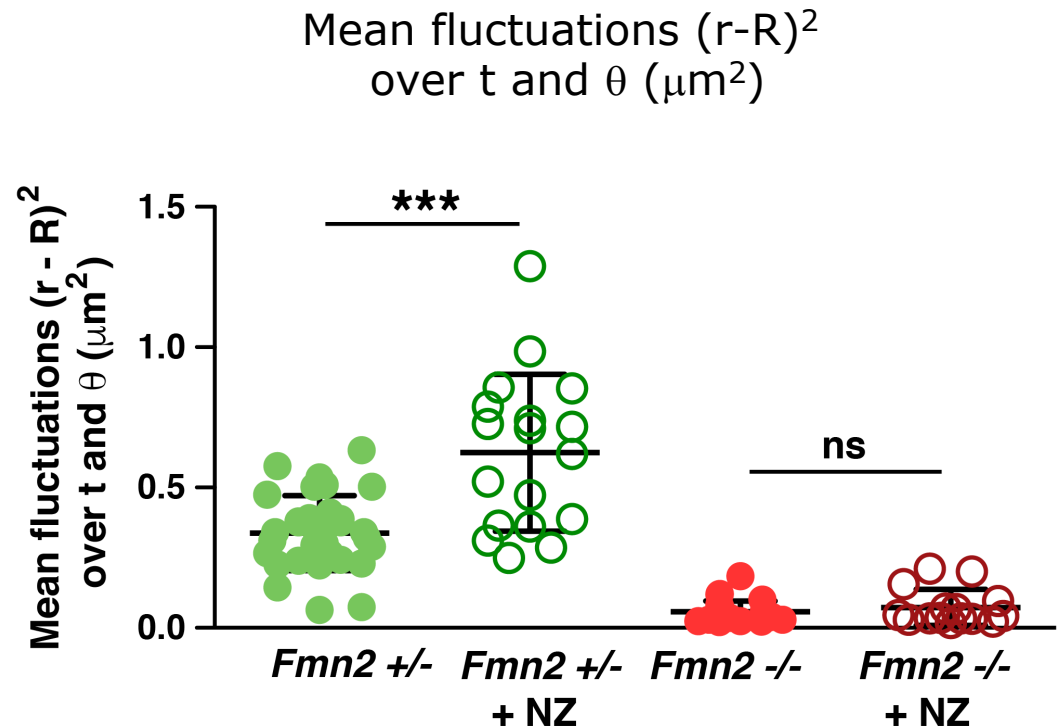
Mean fluctuations
($r-R$)² over time (μm^2)



Microtubules dampen actin-driven nuclear envelope fluctuations

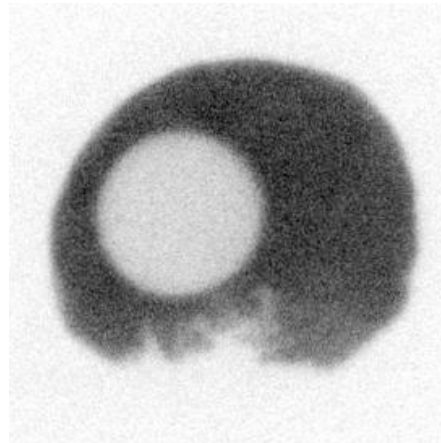


- mean shape over time
- shape at t_1
- - - shape at t_2
- θ rotational angle
- r radius for a given t and θ
- R radius for the mean shape over time for a given t and θ

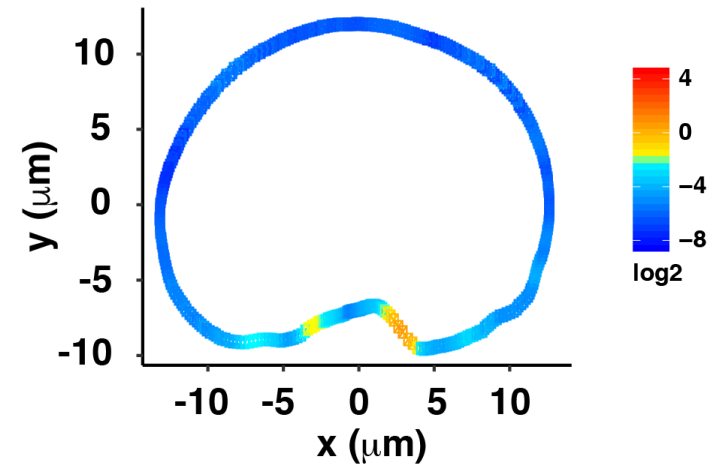


The invagination contains a major MTOC

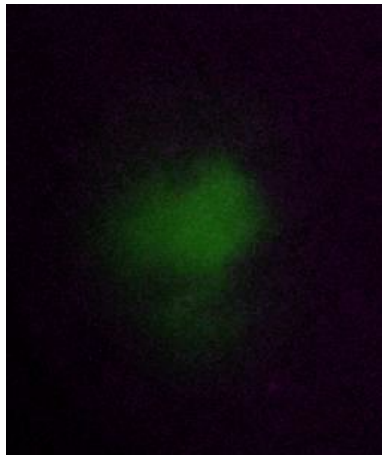
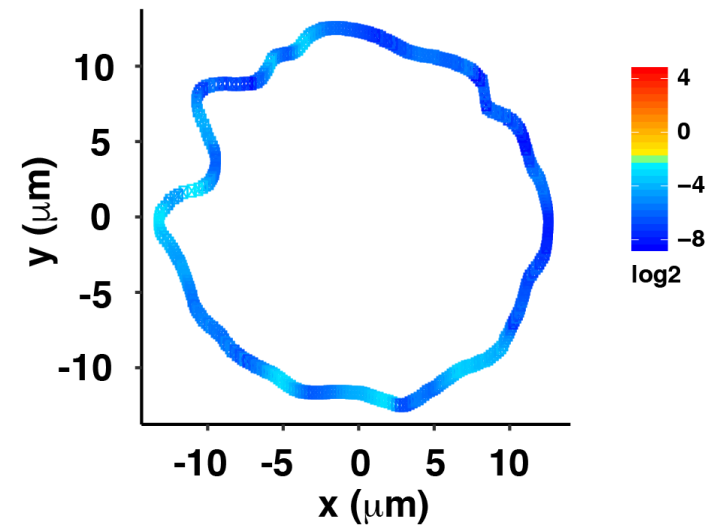
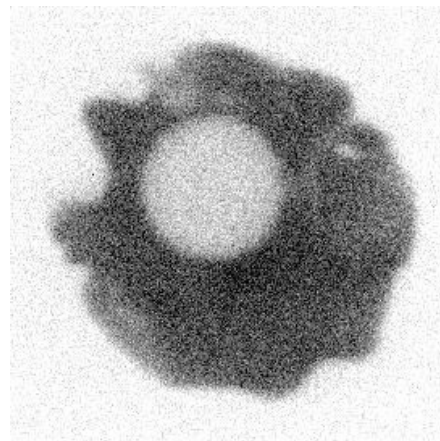
Fmn2 -/-



Mean fluctuations
($r-R$)² over time (μm^2)

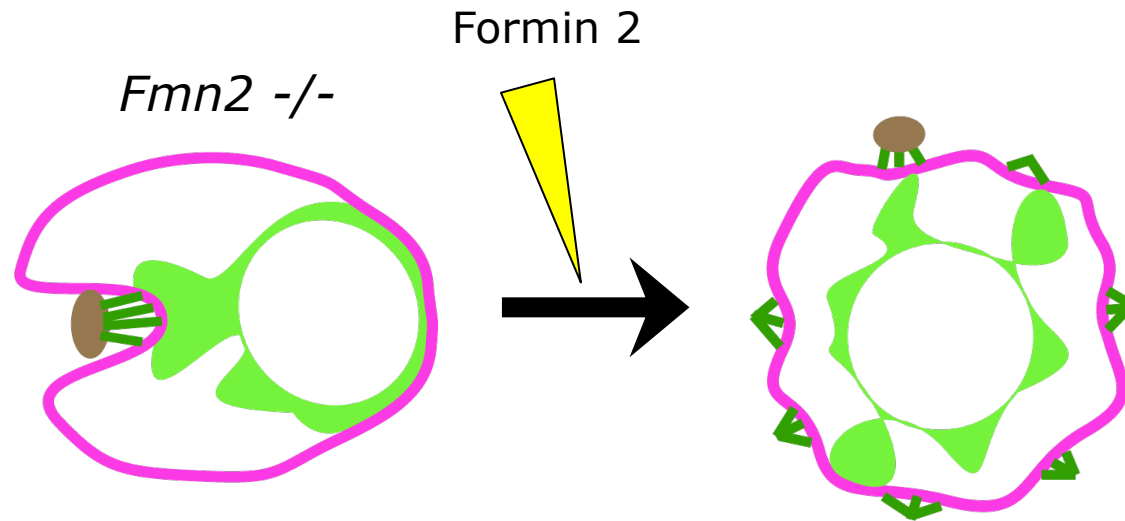


Fmn2 -/- + NZ



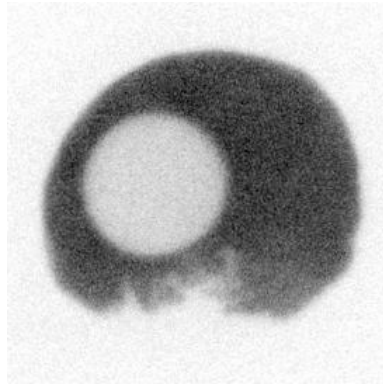
Nuclear probe
Plk4

F-actin promotes the homogeneous distribution of MTs around the nuclear envelope

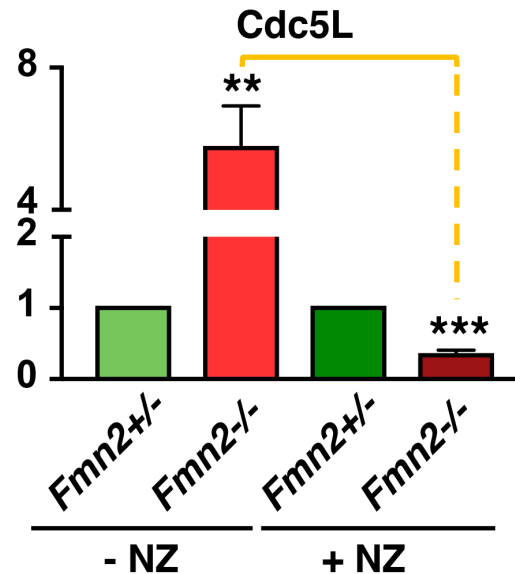
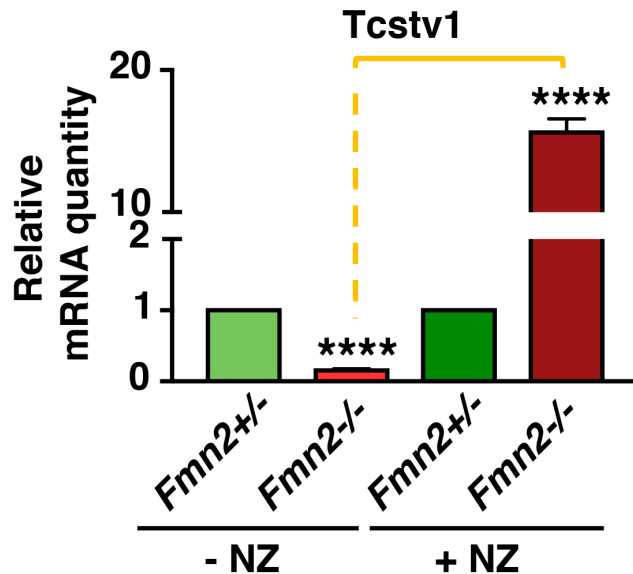
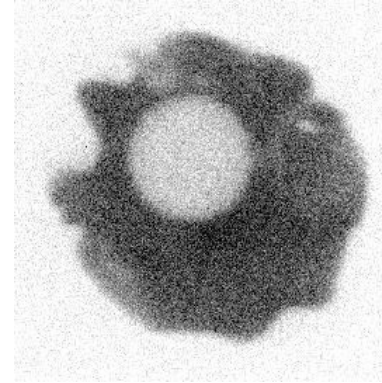


Nuclear fluctuations but also nuclear shape regulate gene expression in mouse oocytes

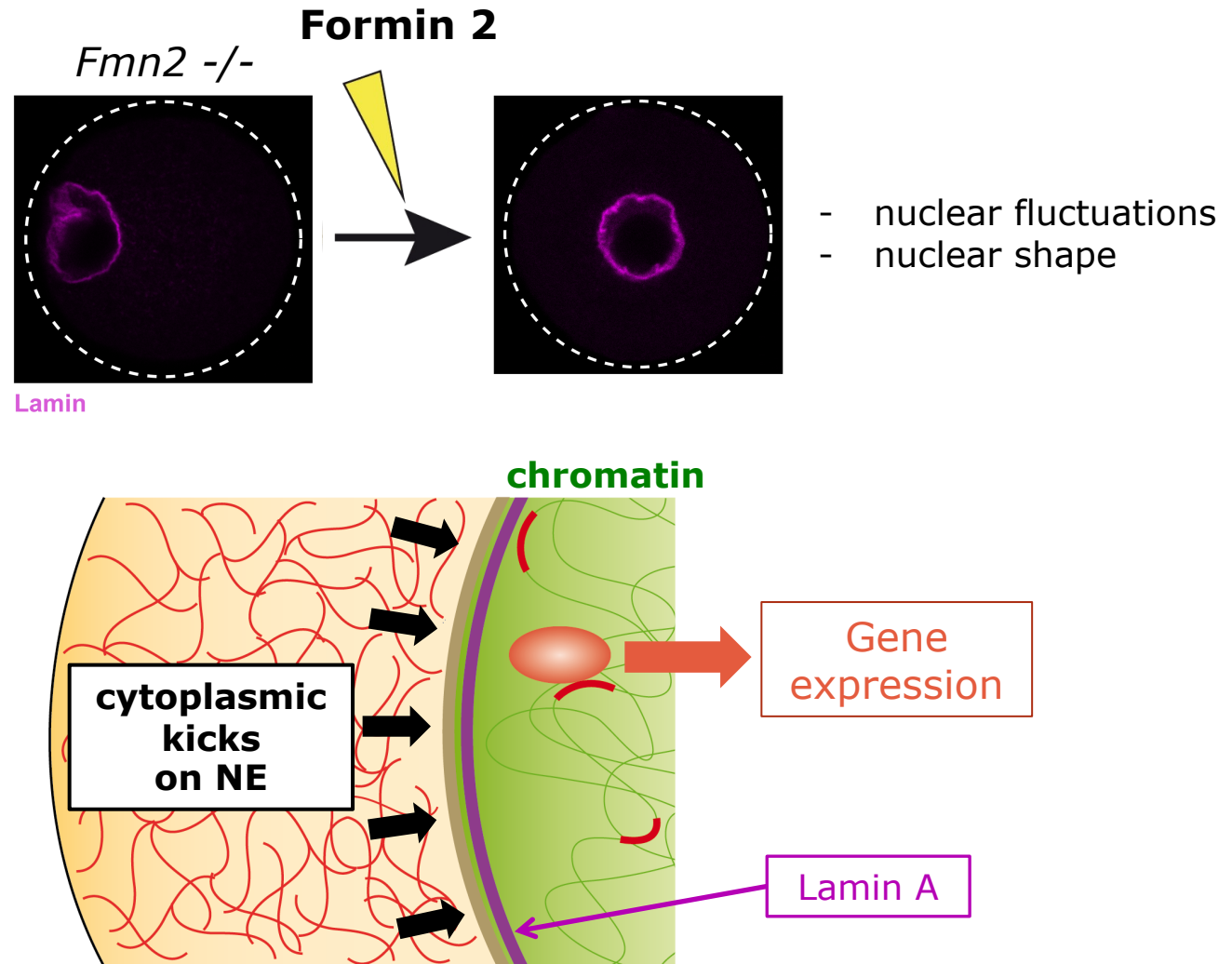
Fmn2 ^{-/-}



Fmn2 ^{-/-} + NZ

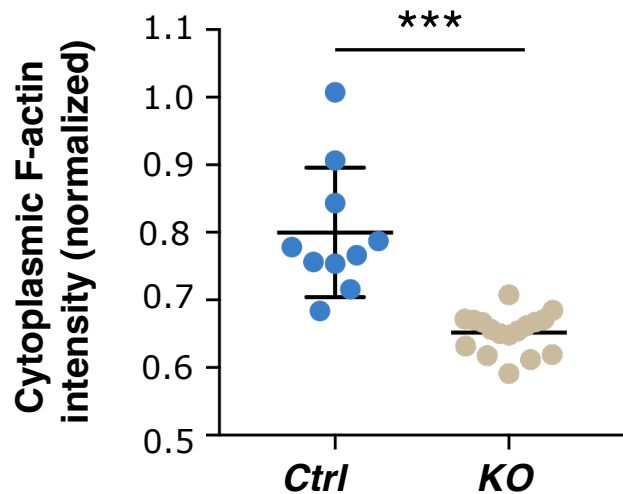
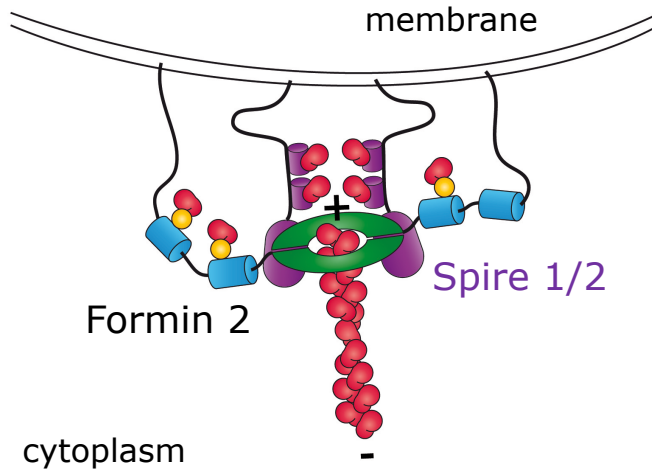


A mechano-transduction model for the regulation of oocyte gene expression

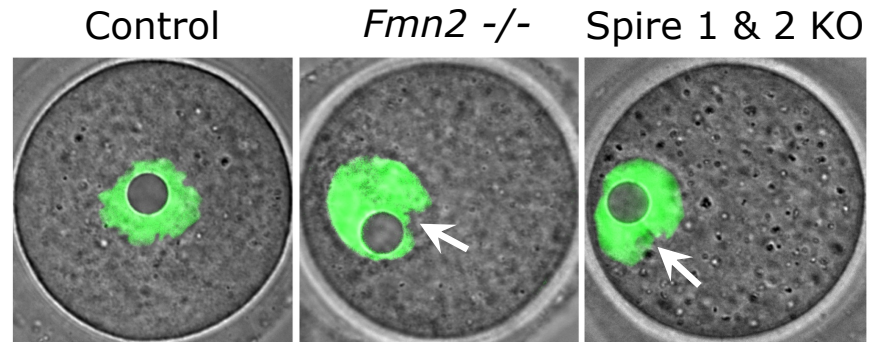


Going deeper into our mechano-transduction model
for the regulation of gene expression

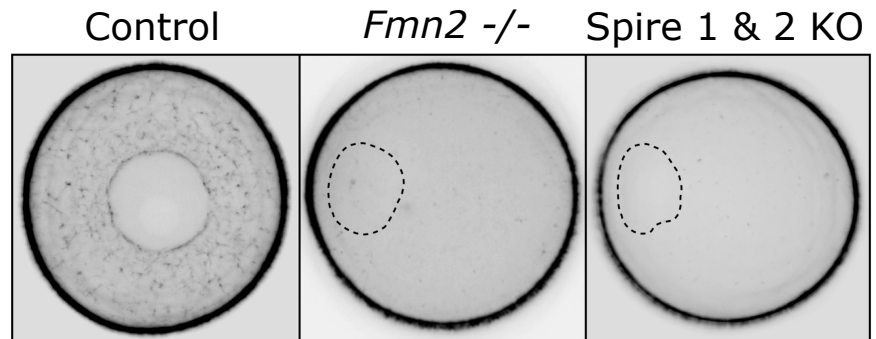
Development of Spire 1 & 2 double conditional KO in oocytes



Spire 1 & 2 KO
recapitulates *Fmn2* $-/-$ phenotype

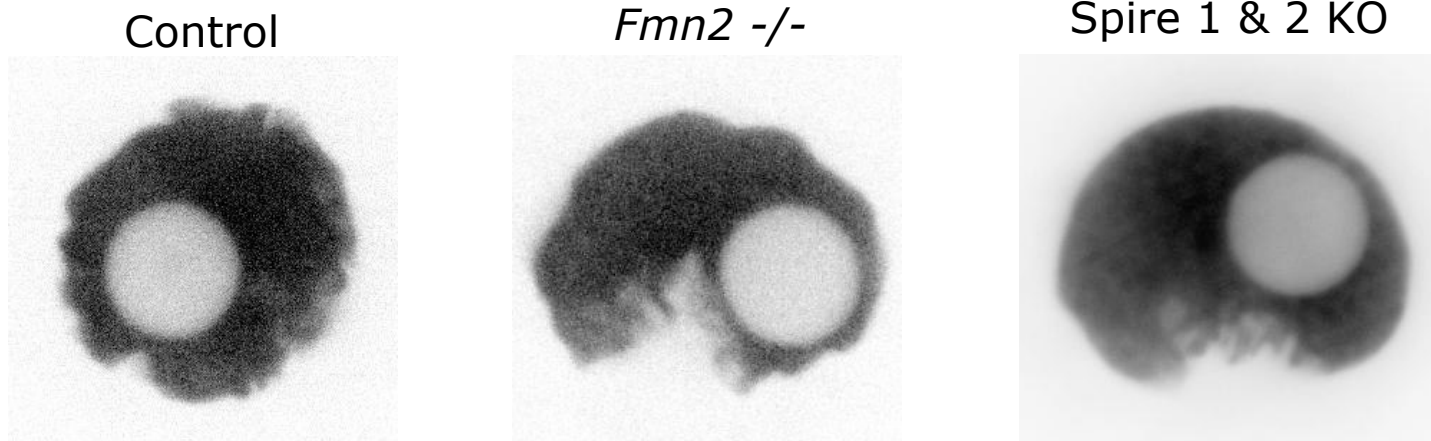


Nucleus

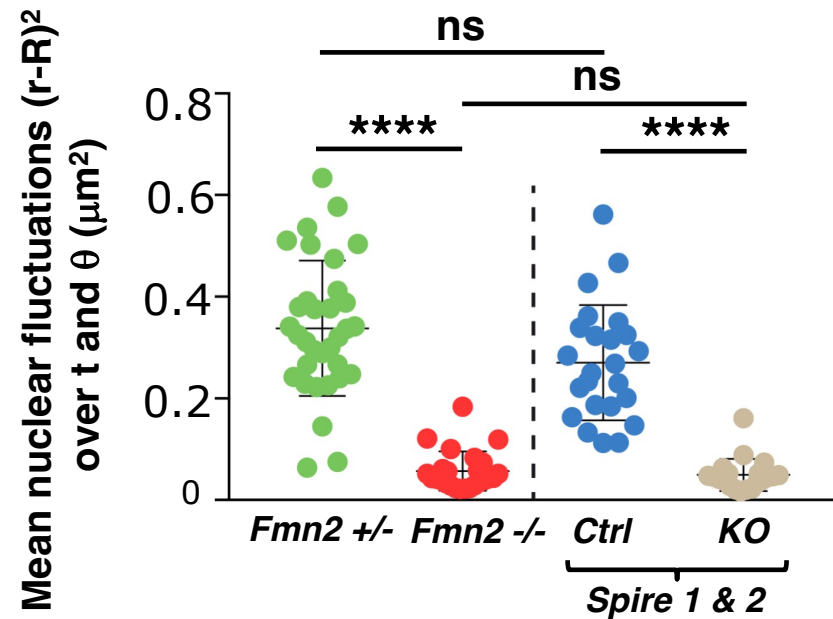
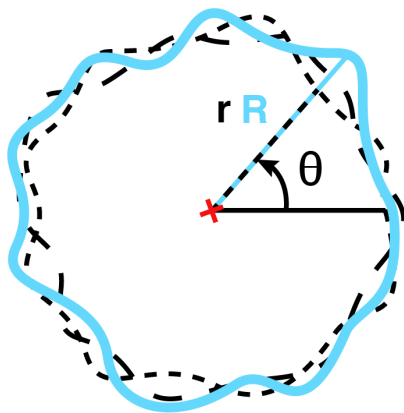


F-actin probe Utr-GFP

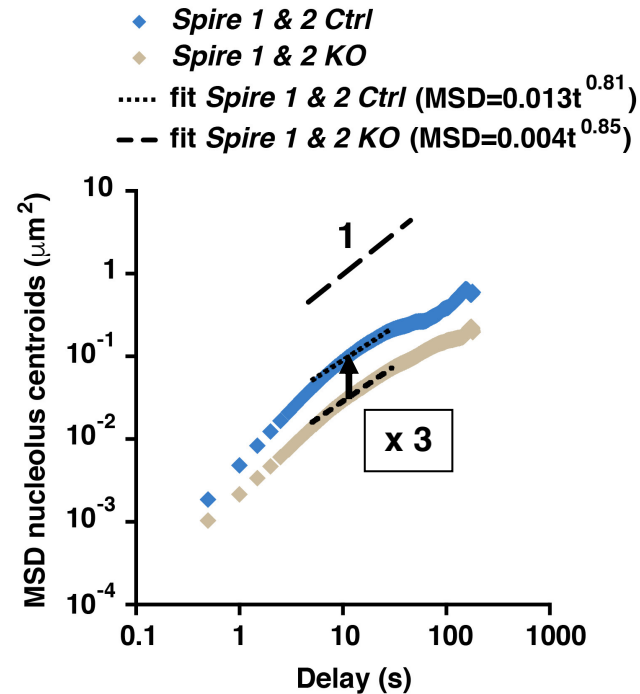
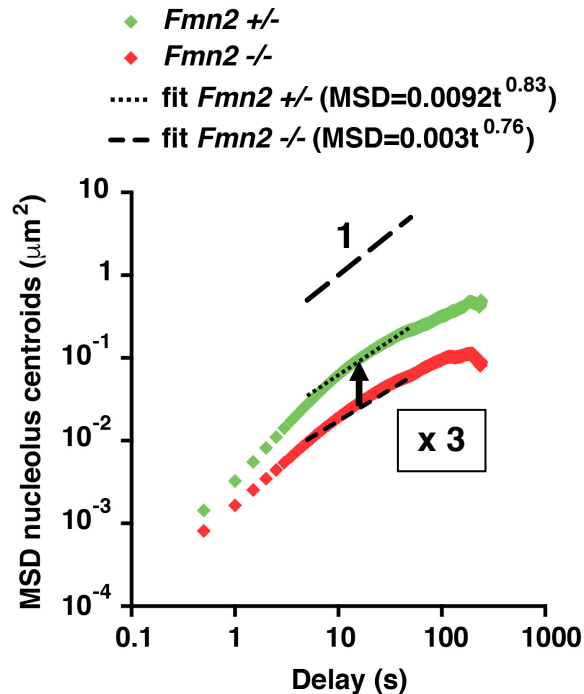
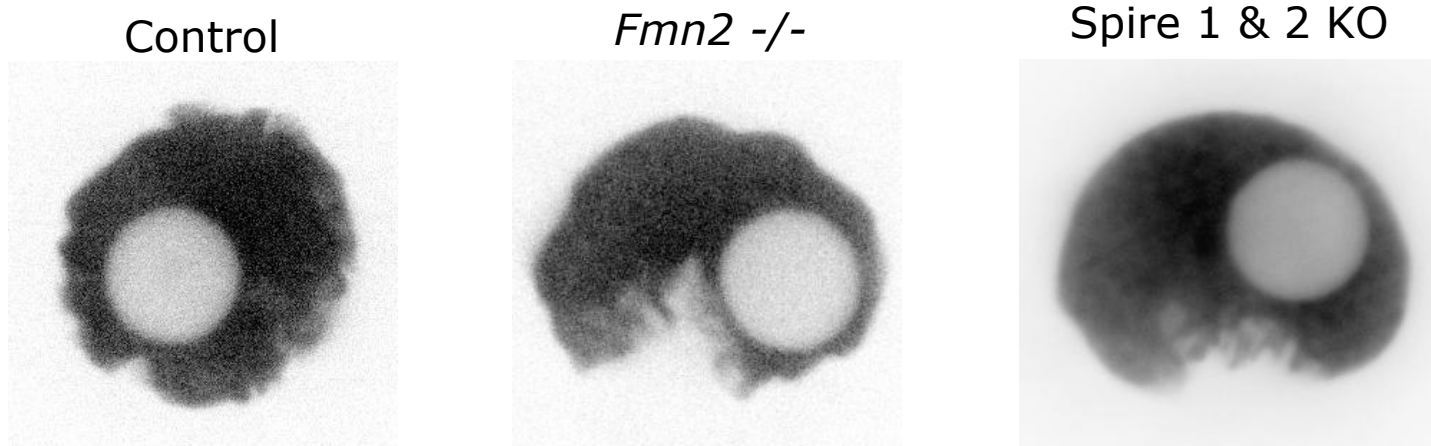
Nuclear fluctuations in Spire 1 & 2 KO oocytes are impacted in the same range as in *Fmn2* $-/-$



Nuclear probe

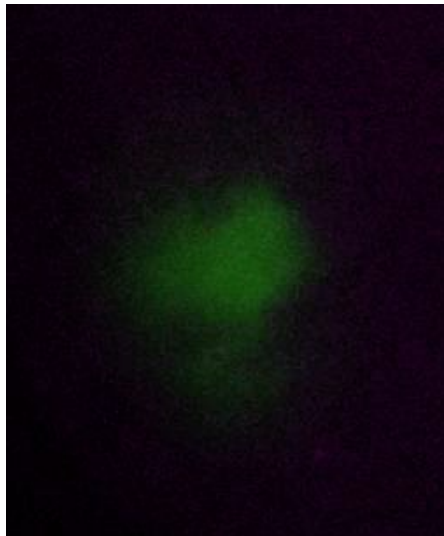


Chromatin motion in Spire 1 & 2 KO oocytes is impacted in the same range as in *Fmn2* $-/-$

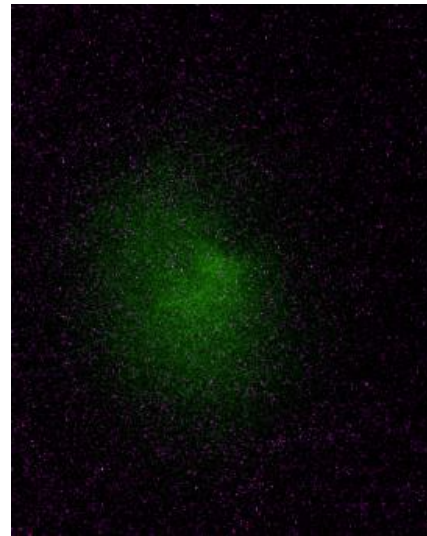


Spire 1 & 2 KO nuclei also harbor a large MTOC
at the invagination

Fmn2 -/-

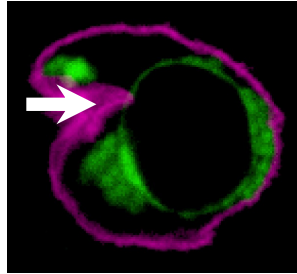


Spire 1 & 2 KO



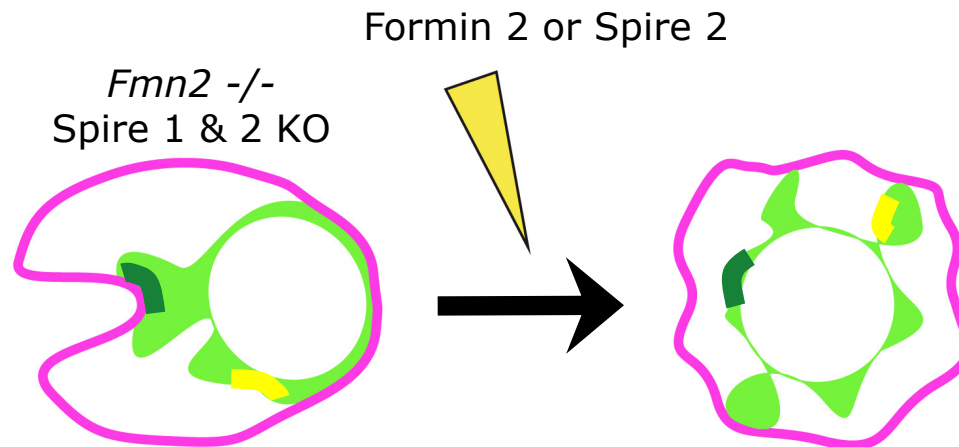
Spire 1 & 2 KO recapitulate very well
the phenotype of *Fmn2* $-/-$ oocytes
and are a good model
for nuclear mechano-transduction

What are the are the relative contributions of nuclear fluctuations and nuclear shape to our model?



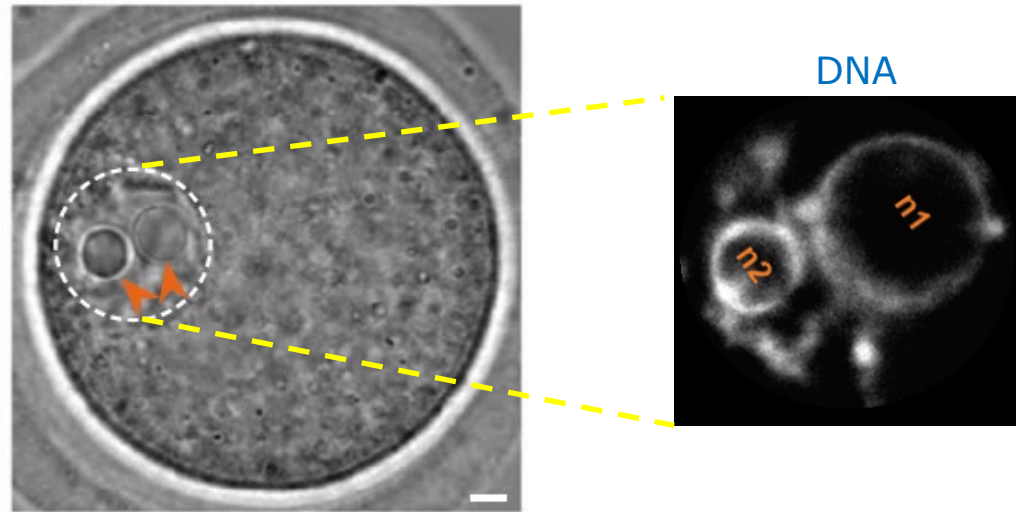
Effect of the invagination?

- Compare genes deregulated in *Fmn2* $-/-$ and Spire 1 & 2 oocytes
- Refine the list of genes sensitive to mechano-transduction
- Follow loci in fixed (DNA FISH) and live oocytes during nucleus re-positioning



Mechano-transduction and nuclear bodies

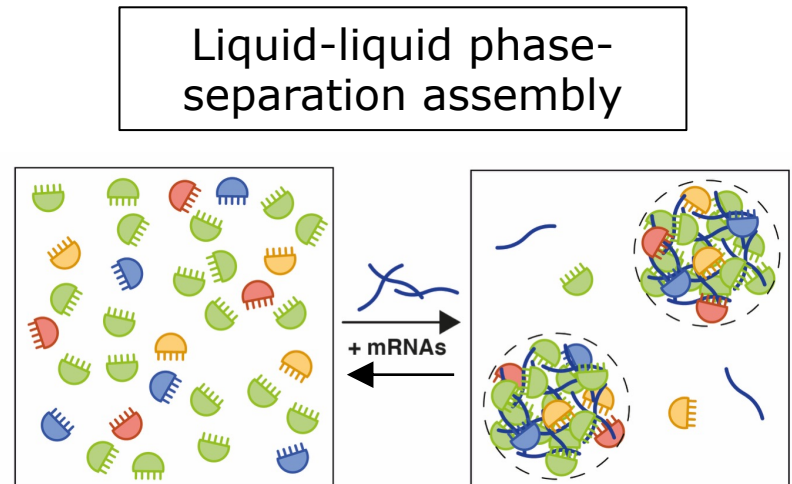
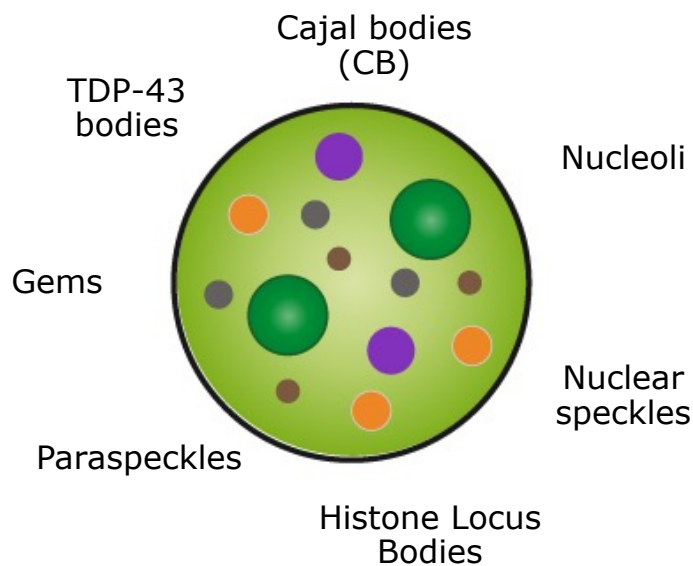
Fmn2 ^{-/-} oocytes often display more than one nucleolus



Nucleoli number	<i>Fmn2</i> +/- (94 oocytes)	<i>Fmn2</i> -/- (110 oocytes)
1	91,5% +/- 9.3	80% +/- 14.9
>1	8.51 +/- 9.3	20% +/- 14.9

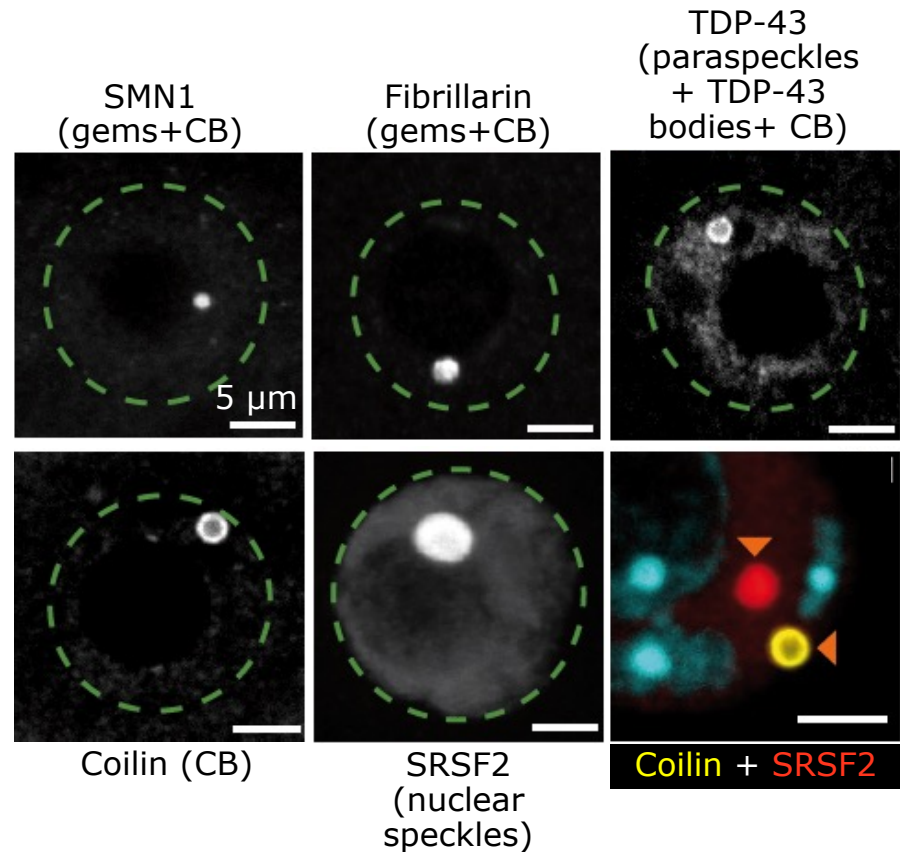
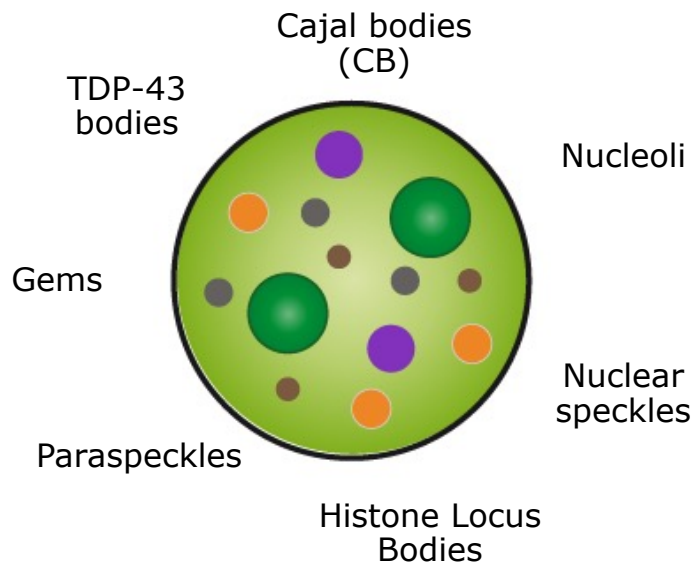
*P=0.02

Nucleoli and other nuclear bodies are liquid-like droplet organelles involved in mRNA processing

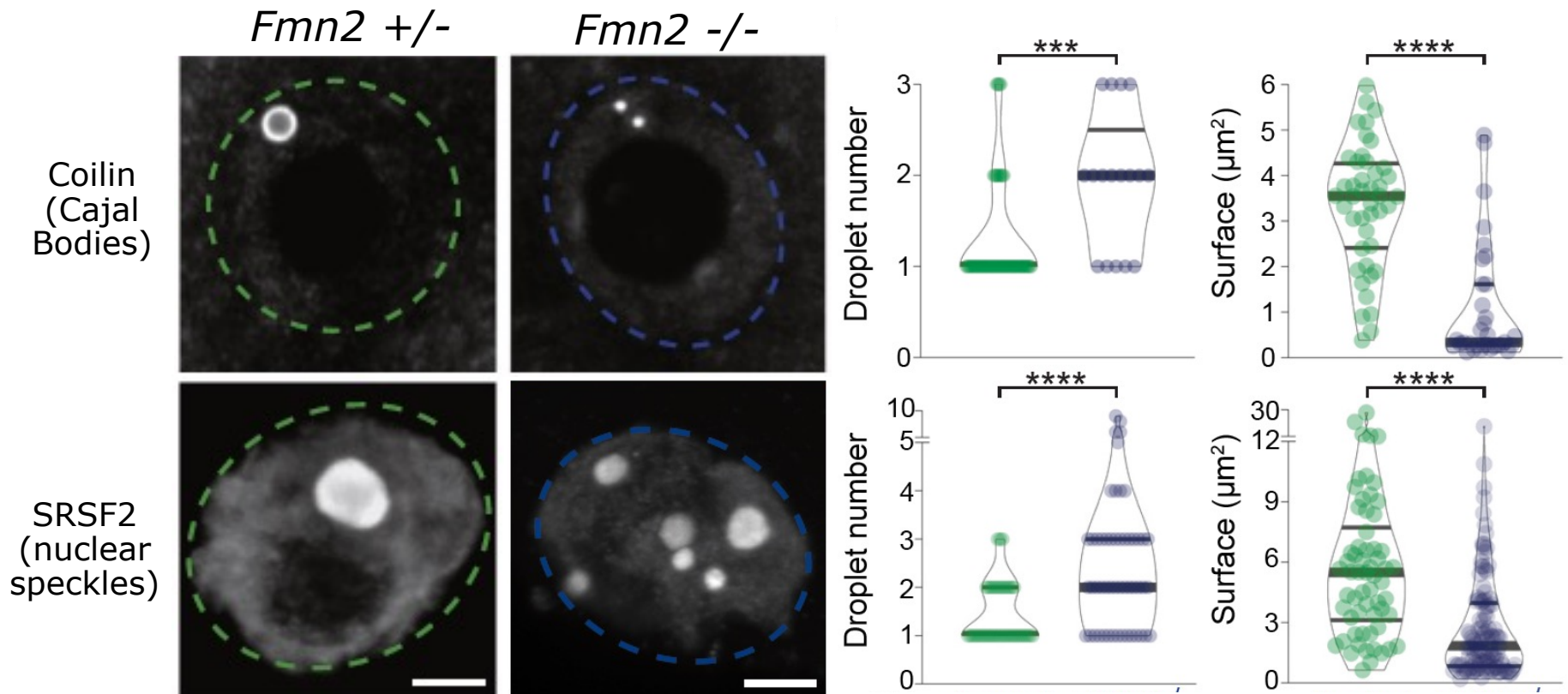


**Factories
that boost
enzymatic
reactions**

An immunofluorescence screen for nuclear bodies in mouse oocytes



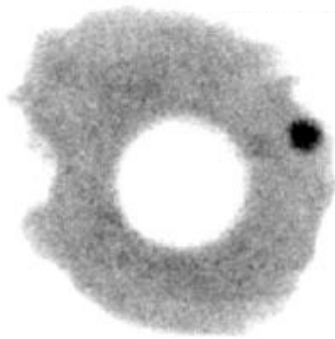
More numerous nuclear droplets in *Fmn2* $-/-$ oocytes



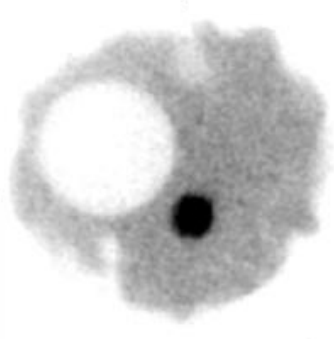
Cytoplasmic forces promote dynamics of liquid-like nuclear droplets

Time (mm:ss) 00:00

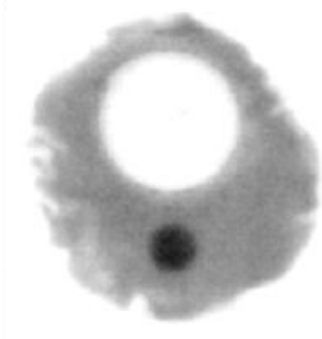
Fmn2 +/-



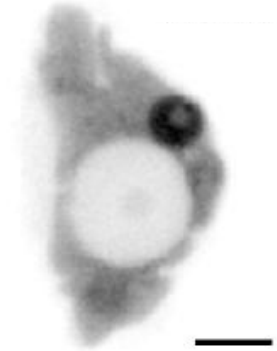
Fmn2 +/- +NZ



Fmn2 +/- + Taxol



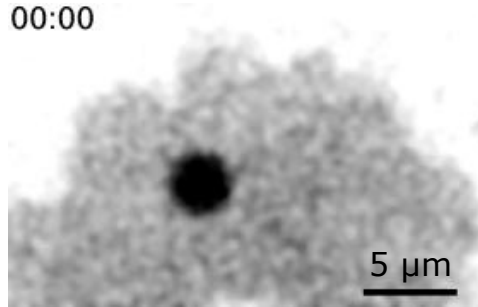
Fmn2 -/-



Cytoplasmic forces promote fusion of liquid-like nuclear droplets

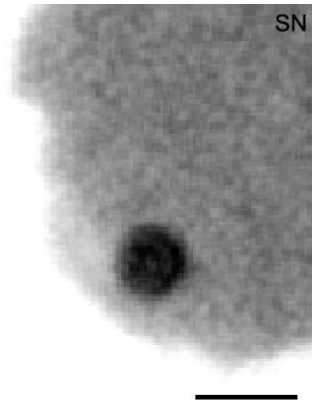
Fusion of two nuclear droplets

Time (mm:ss) 00:00

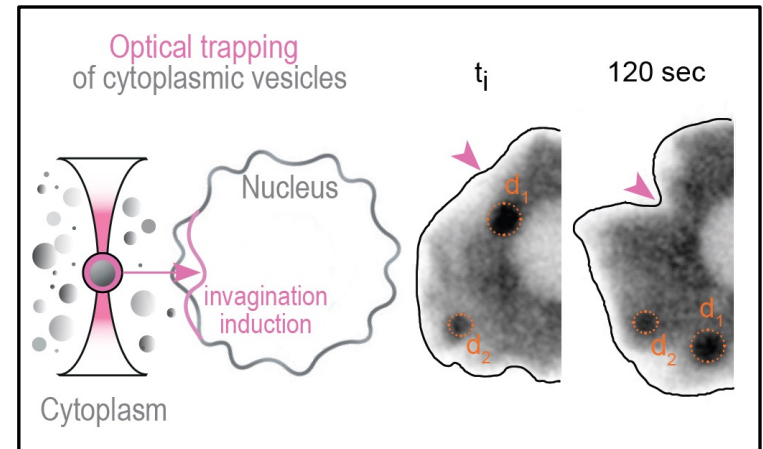


SRSF2-GFP

Nuclear envelope kick against droplet

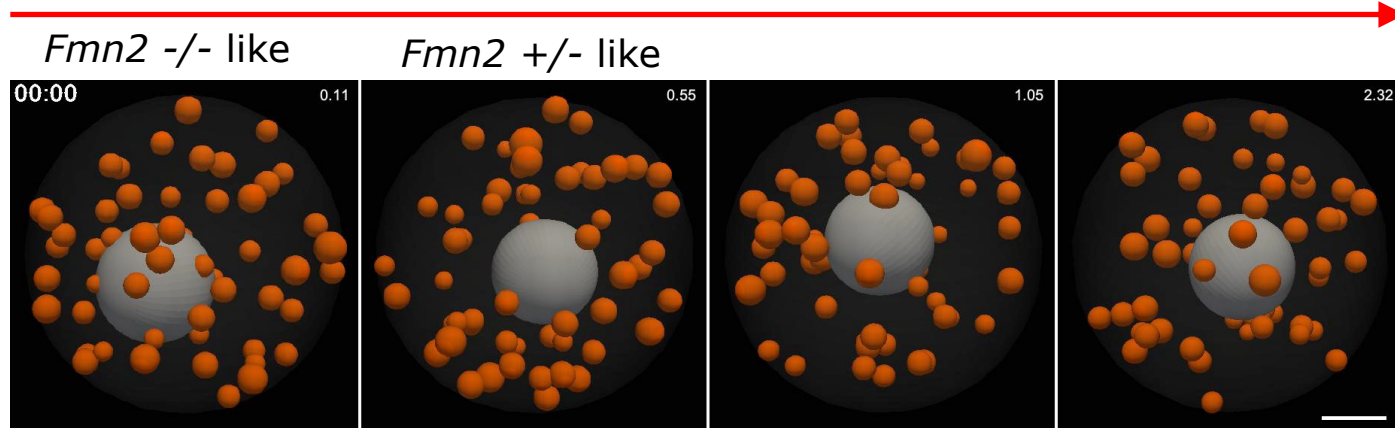


Kicking against a nuclear droplet using optical tweezers

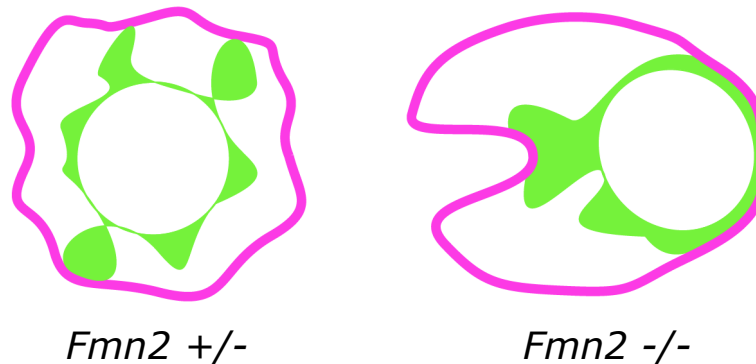


Virtual experiment: Simulating the fusion of liquid-like droplets in the nucleus

increasing cytoplasmic activity

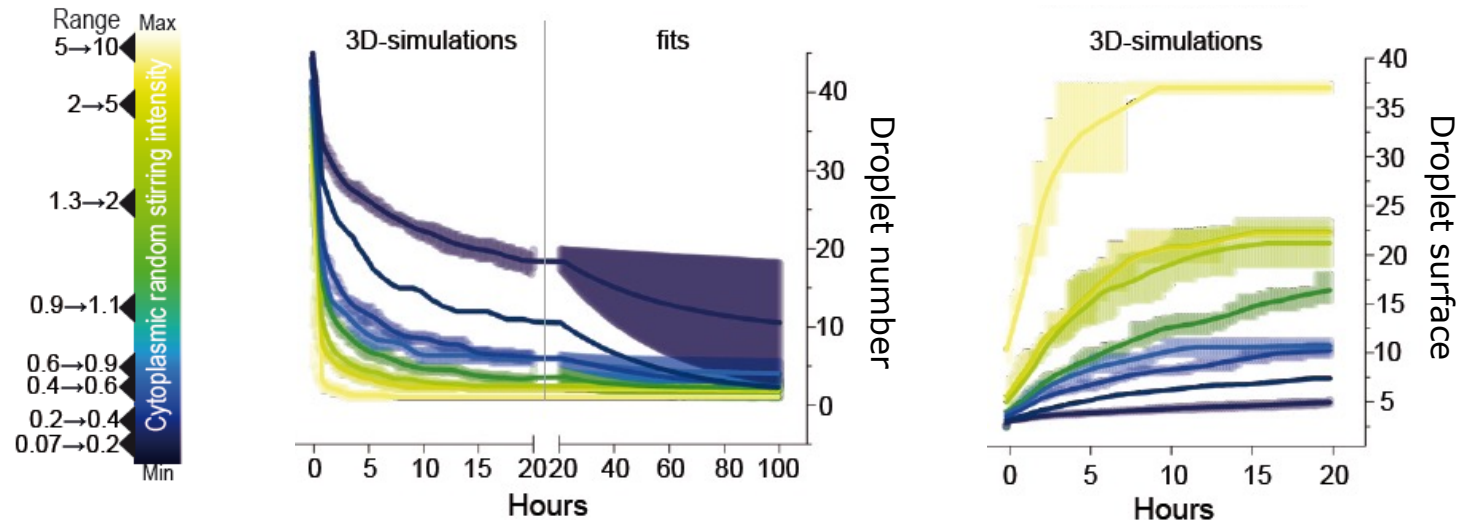
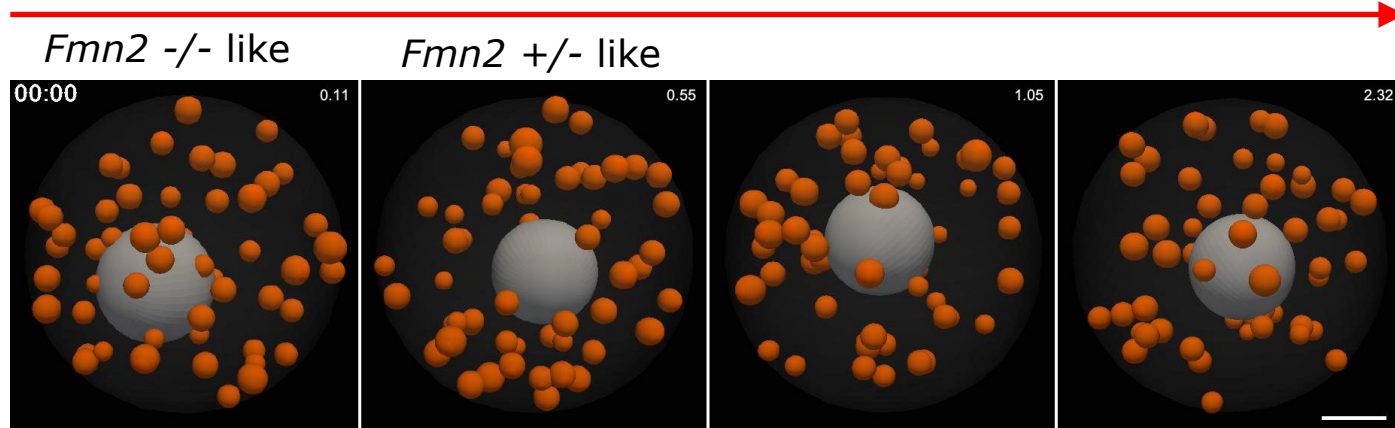


Important parameter: chromatin spatial hindrance



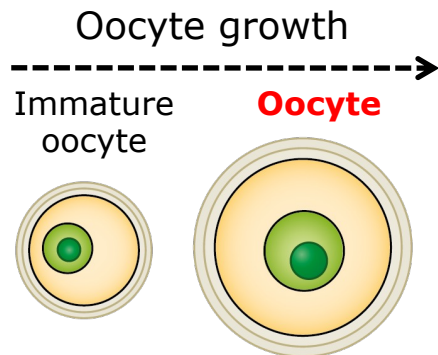
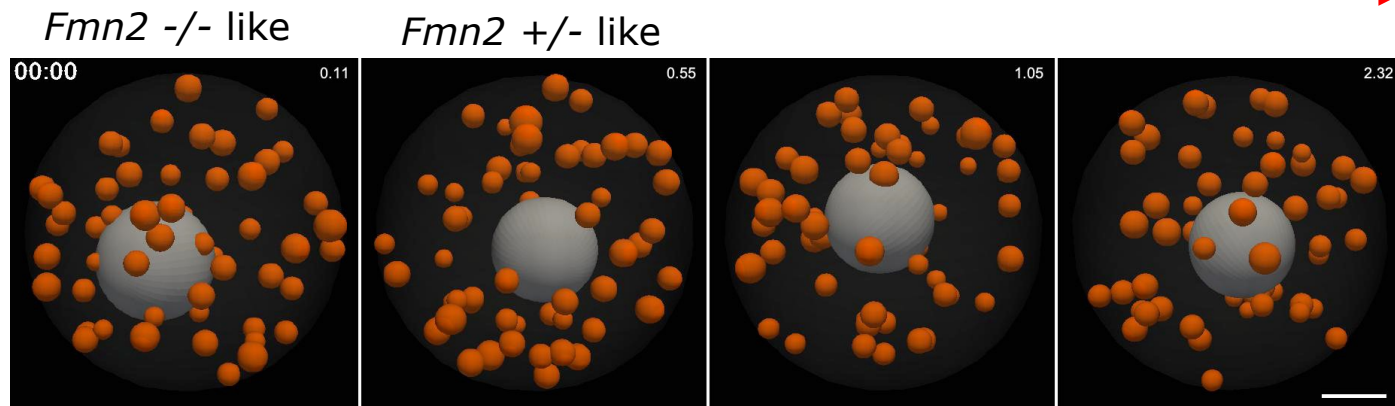
Virtual experiment: Simulating the fusion of liquid-like droplets in the nucleus

increasing cytoplasmic activity



Virtual experiment: Simulating the fusion of liquid-like droplets in the nucleus

increasing cytoplasmic activity →



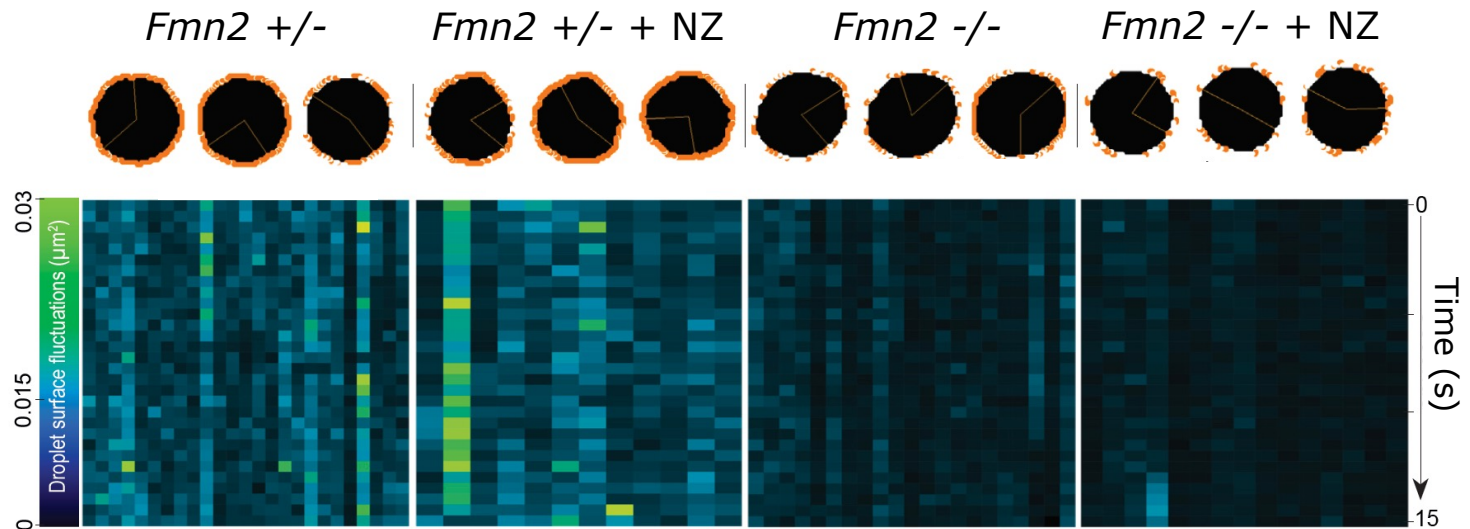
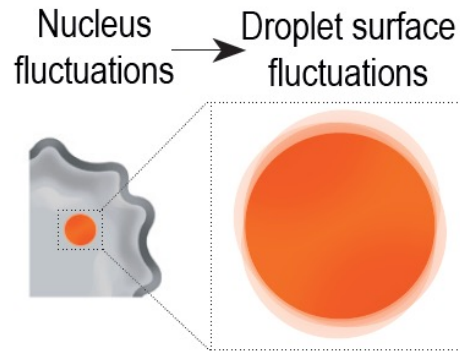
Simulating oocyte growth:

Time to reach a stage resembling end of growth

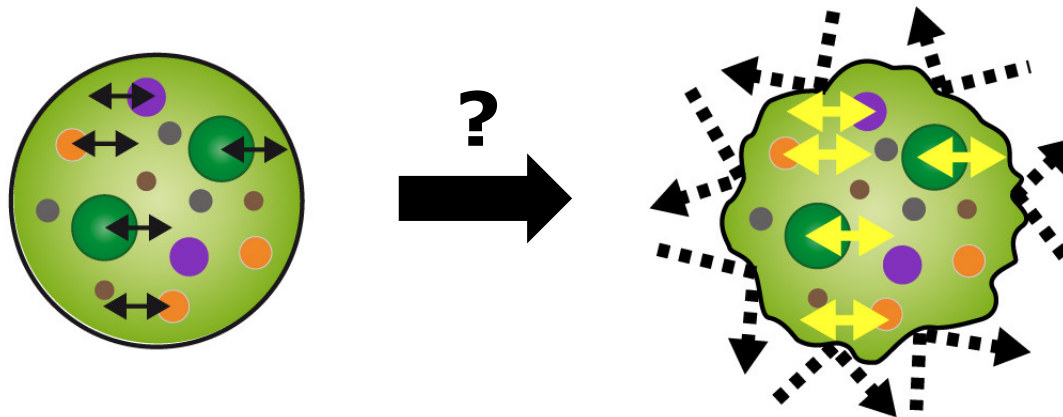
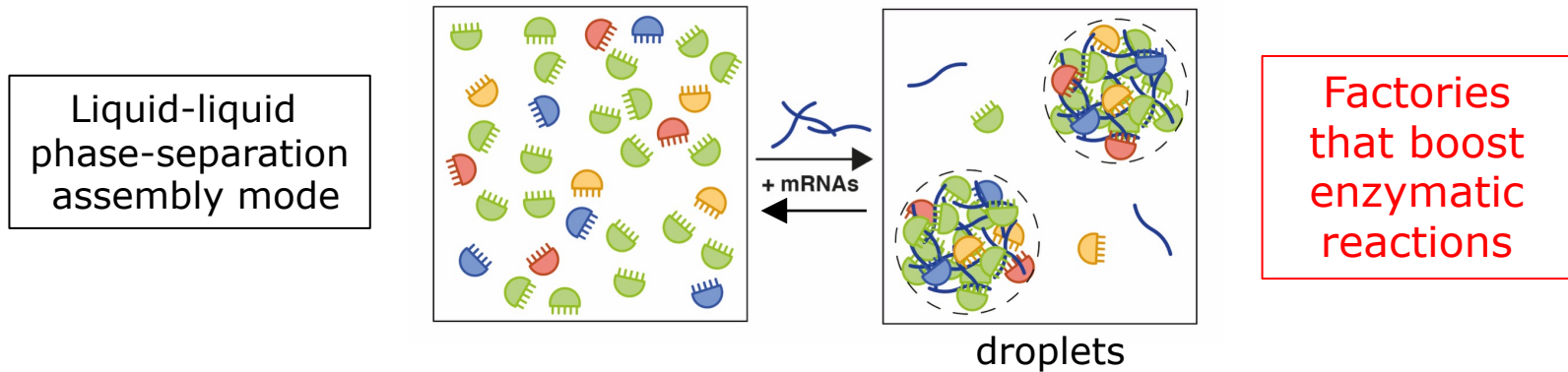
Fmn2 +/- like scenario: 15 h

Fmn2 -/- like scenario : 60 h

Cytoplasmic forces are transmitted onto nuclear droplets

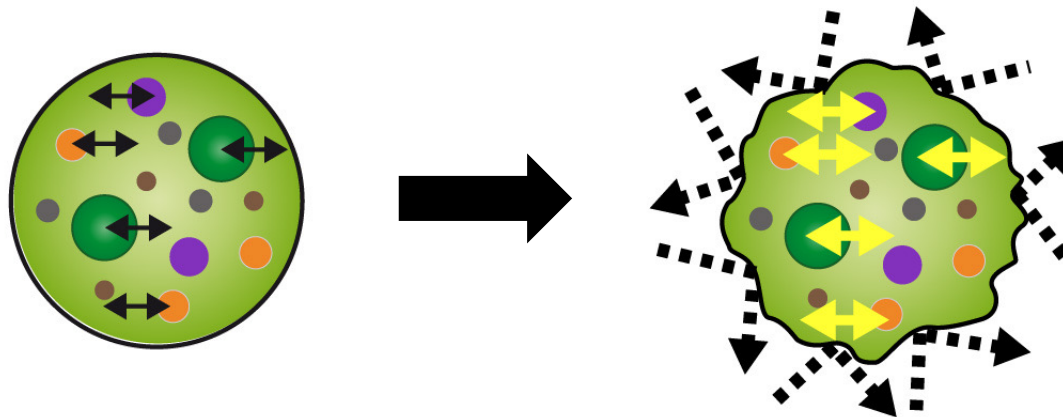
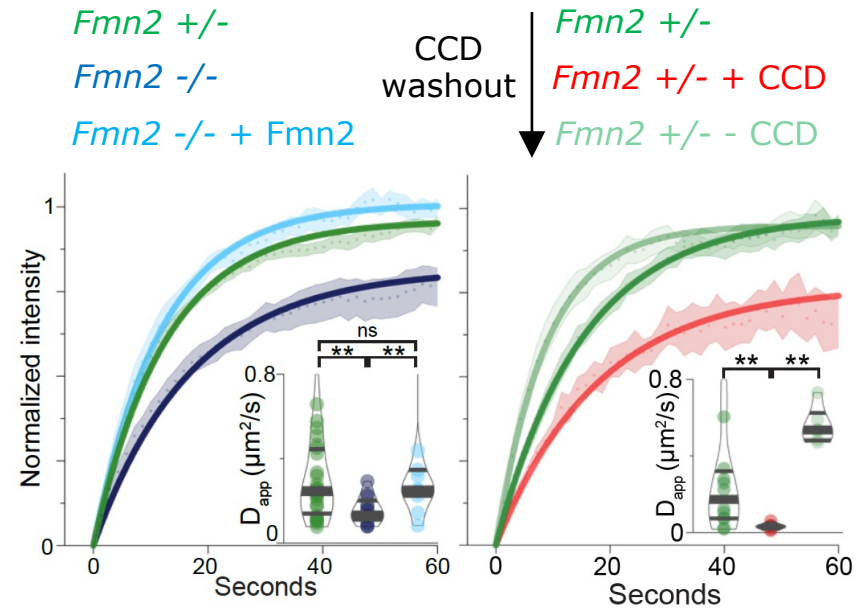
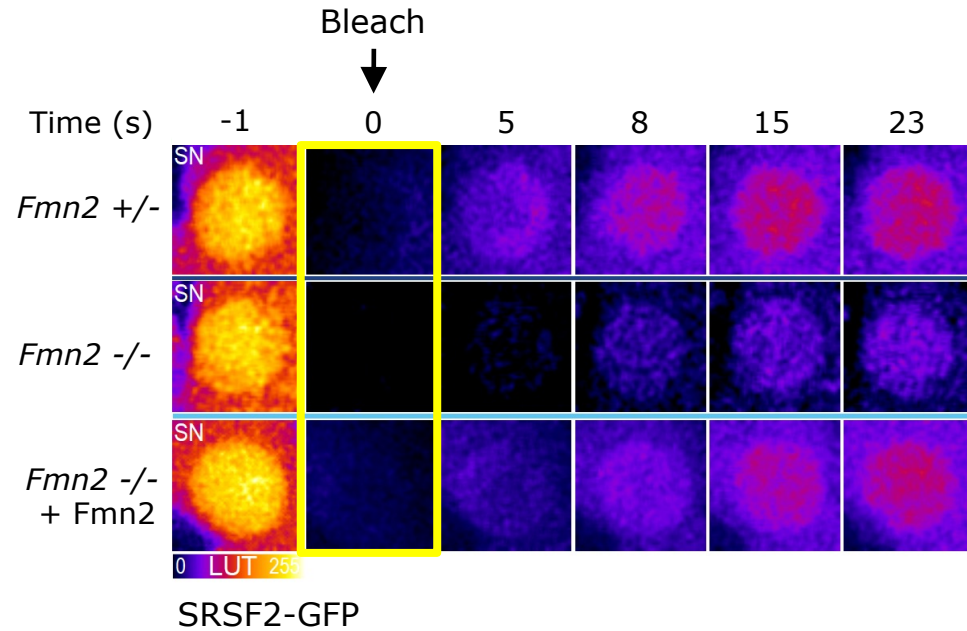


Do cytoplasmic forces increase molecular kinetics inside droplets?



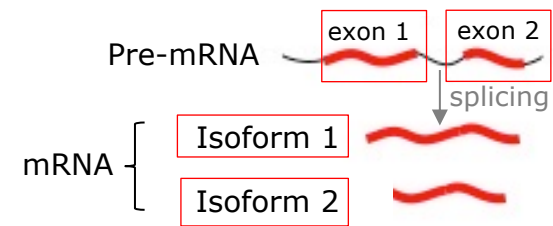
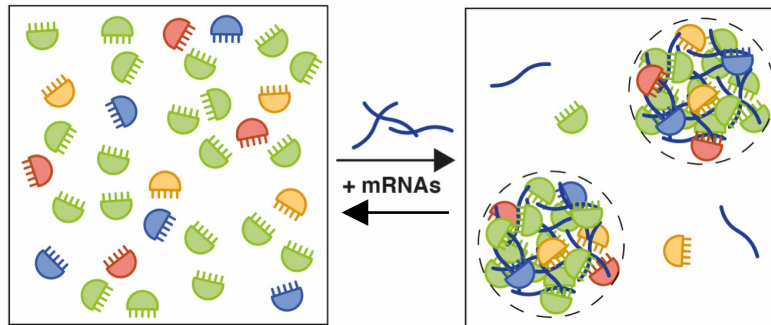
Cytoplasmic forces increase molecular kinetics inside droplets

FRAP experiment



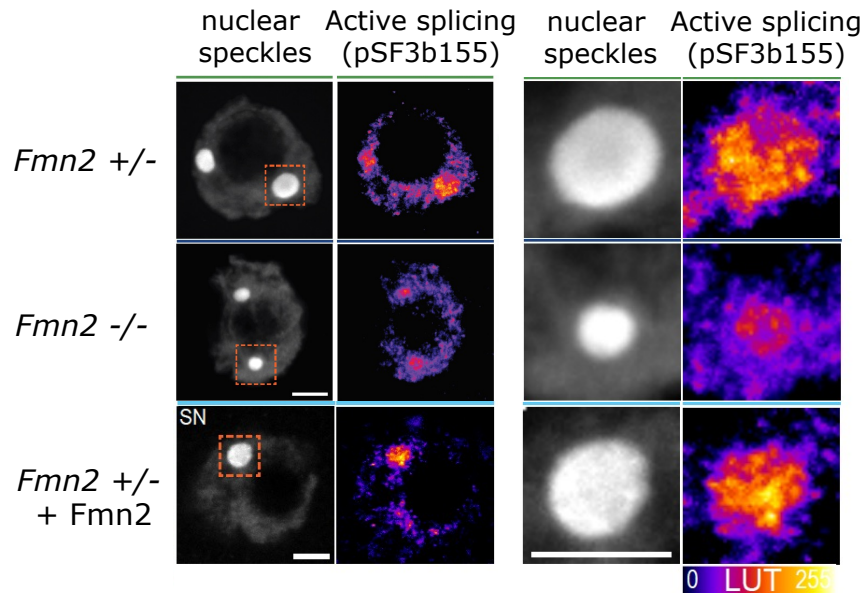
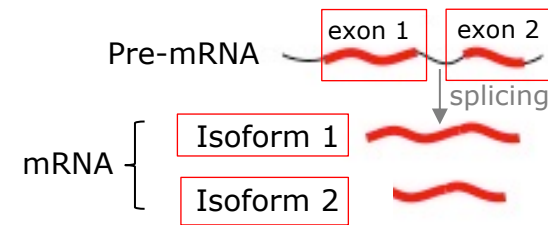
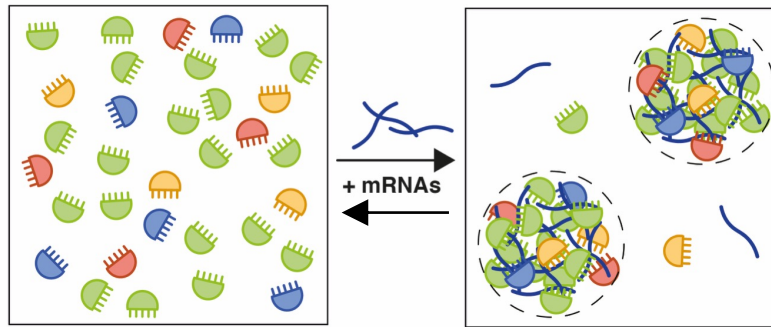
Cytoplasmic forces on the nucleus not only modulate the quantity, but also the quality of mRNAs

*Nuclear speckles are splicing factories:
does increase of molecular kinetics inside droplets regulate splicing?*

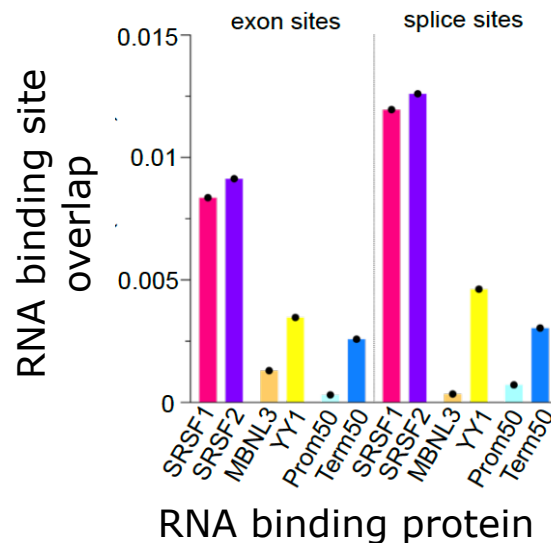
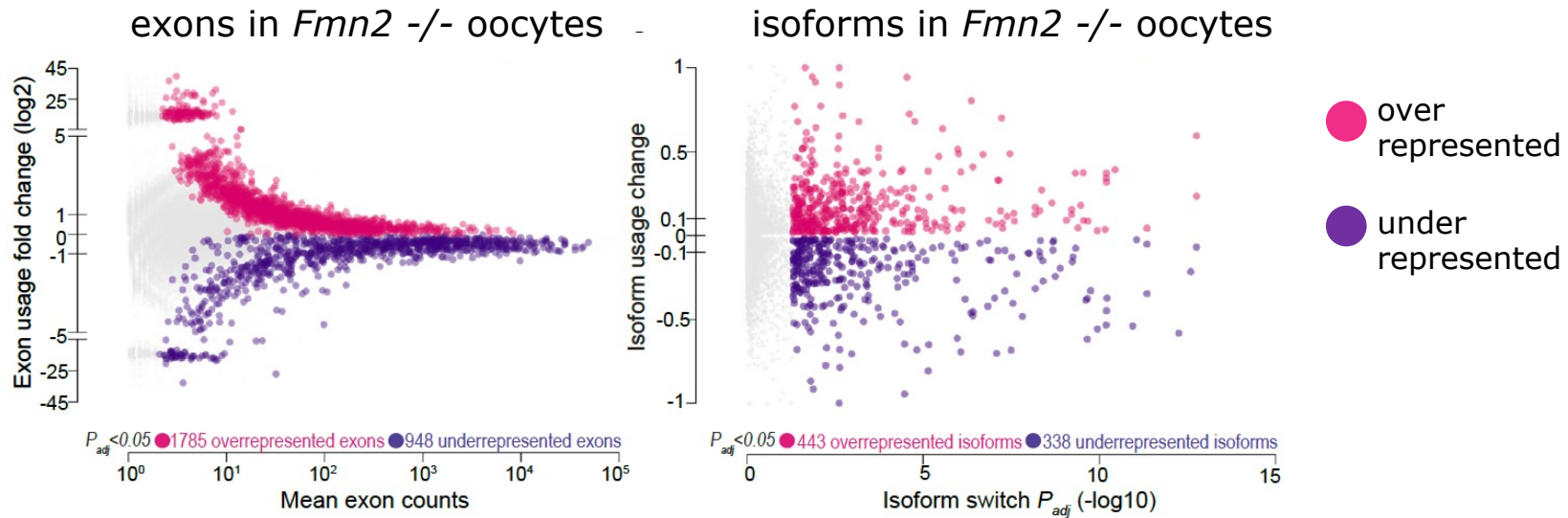


Cytoplasmic forces on the nucleus not only modulate the quantity, but also the quality of mRNAs

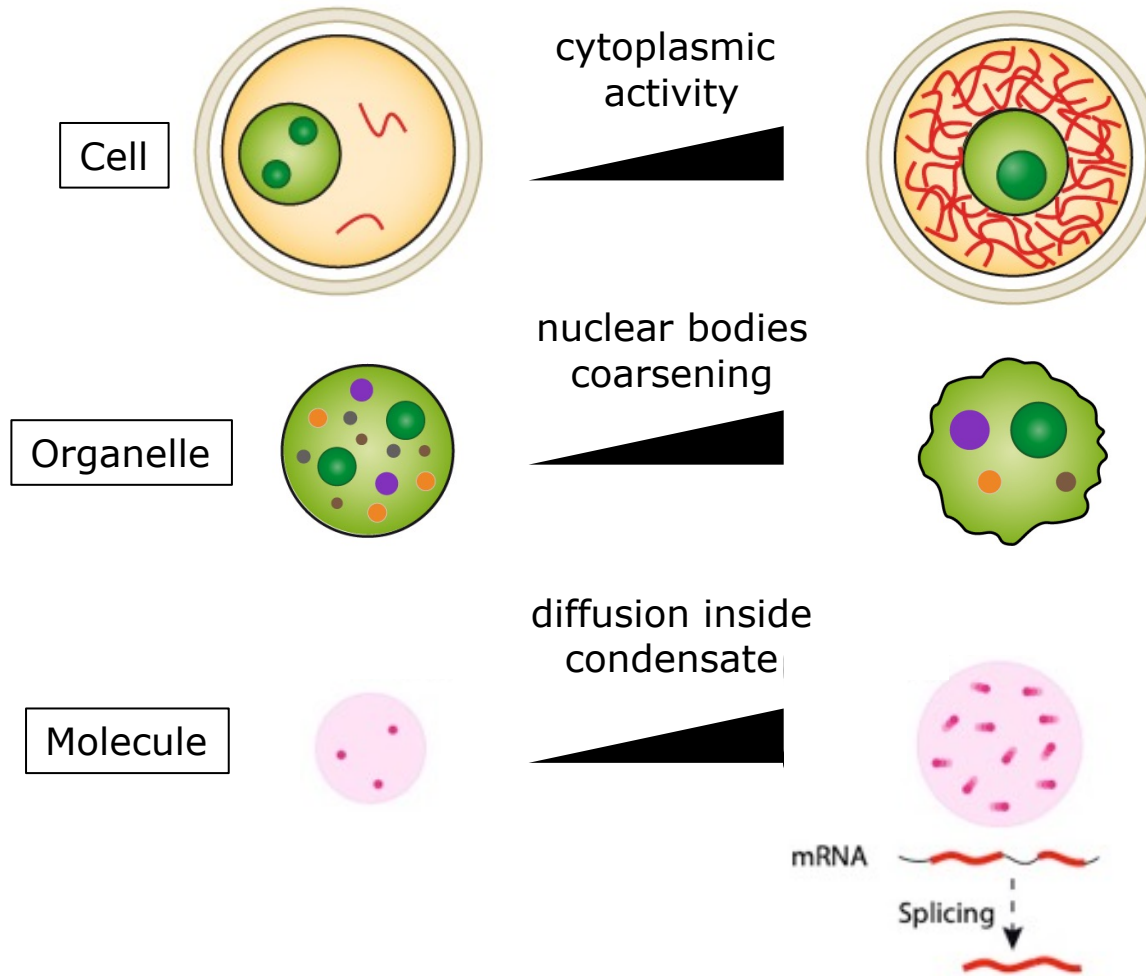
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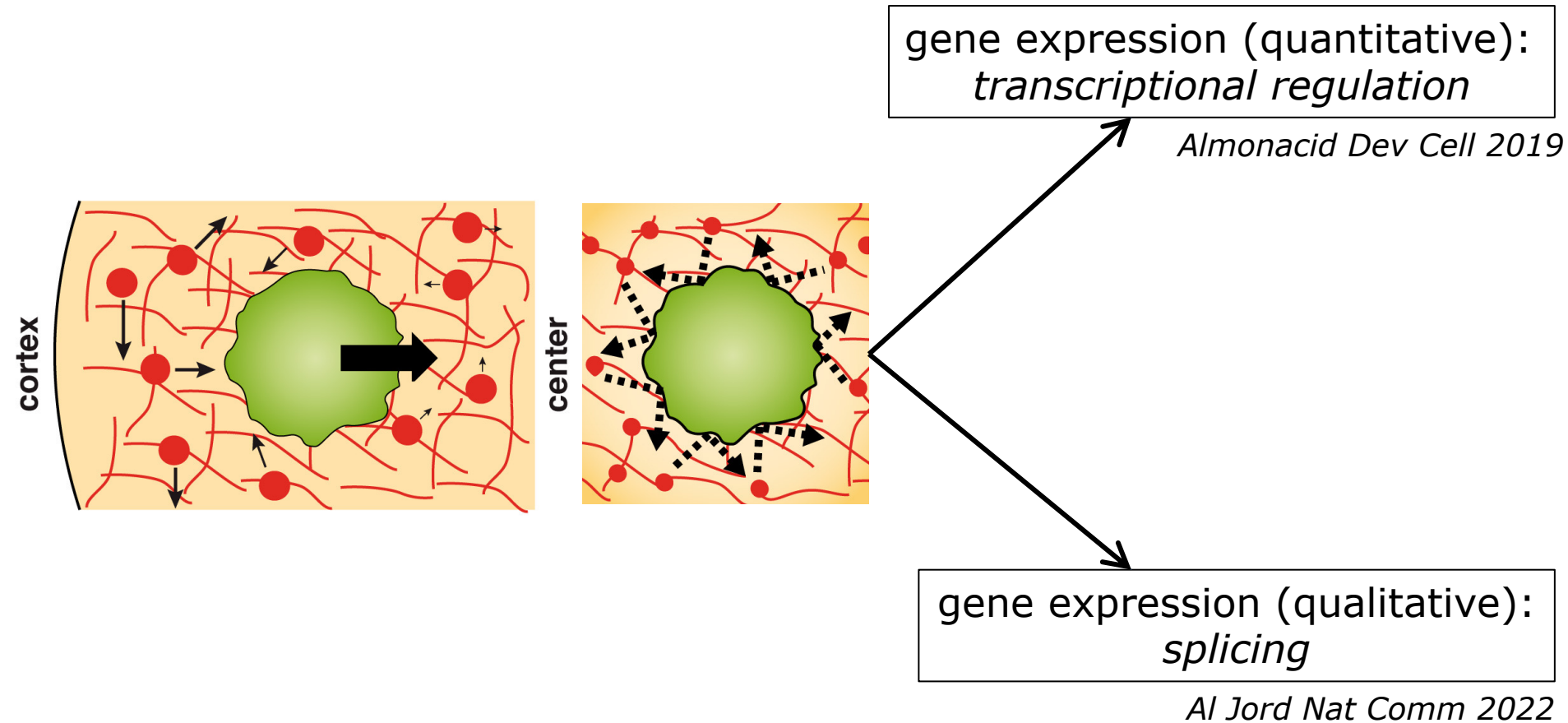
Cytoplasmic forces on the nucleus not only modulate the quantity, but also the quality of mRNAs



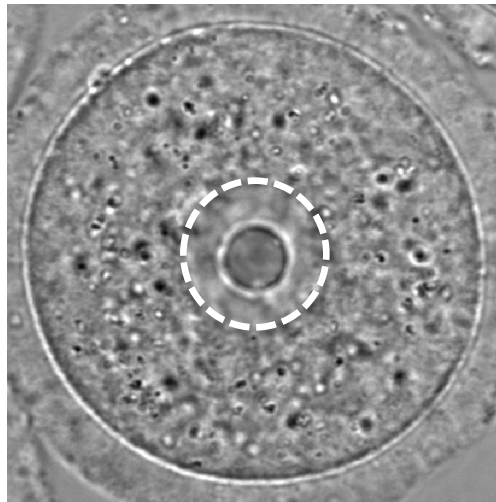
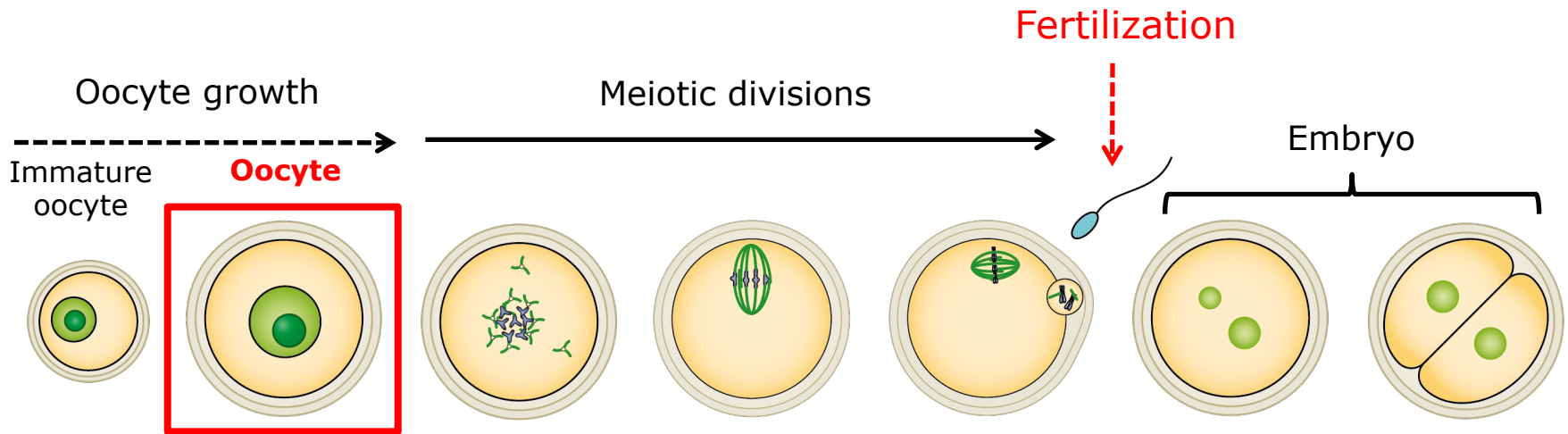
A cascade of active forces crossing the scales



Oocyte nucleus centering as a marker of developmental potential



Oocyte nucleus centering as a marker of developmental potential





COLLÈGE
DE FRANCE
—1530—

contact: maria-elsa.almonacid@college-de-france.fr

CIRB/Collège de France, Paris

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Elvira Nikalayevich (Post-doc)

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Marie-Hélène Verlhac (DRCE2, CNRS)

Adel Al Jord (former Post-doc, now PI at CRG)

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CIRB imaging facility « Orion »

Tristan Piolot

Philippe Mailly

Center for Computational Biology, IBENS

Auguste Genovesio

Institut Curie

Feng-Ching Tsai



GenomiqueENS facility

ART-Bio Bioinformatics facility

Christophe Antoniewski

Sorbonne Université

Raphaël Voituriez

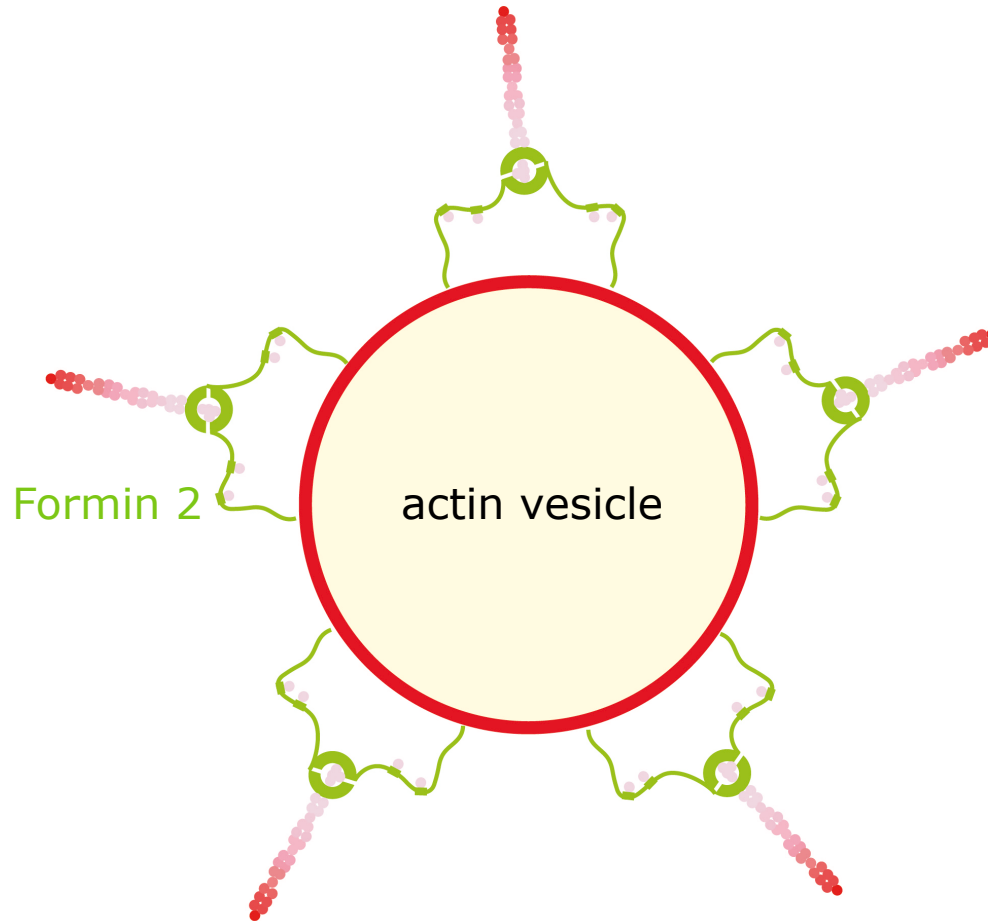
Christophe Klein

Weizmann Institute

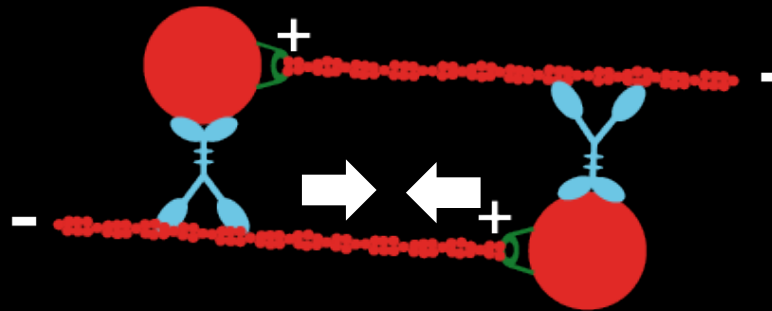
Nir Gov



Formin 2 nucleates filaments on actin vesicles

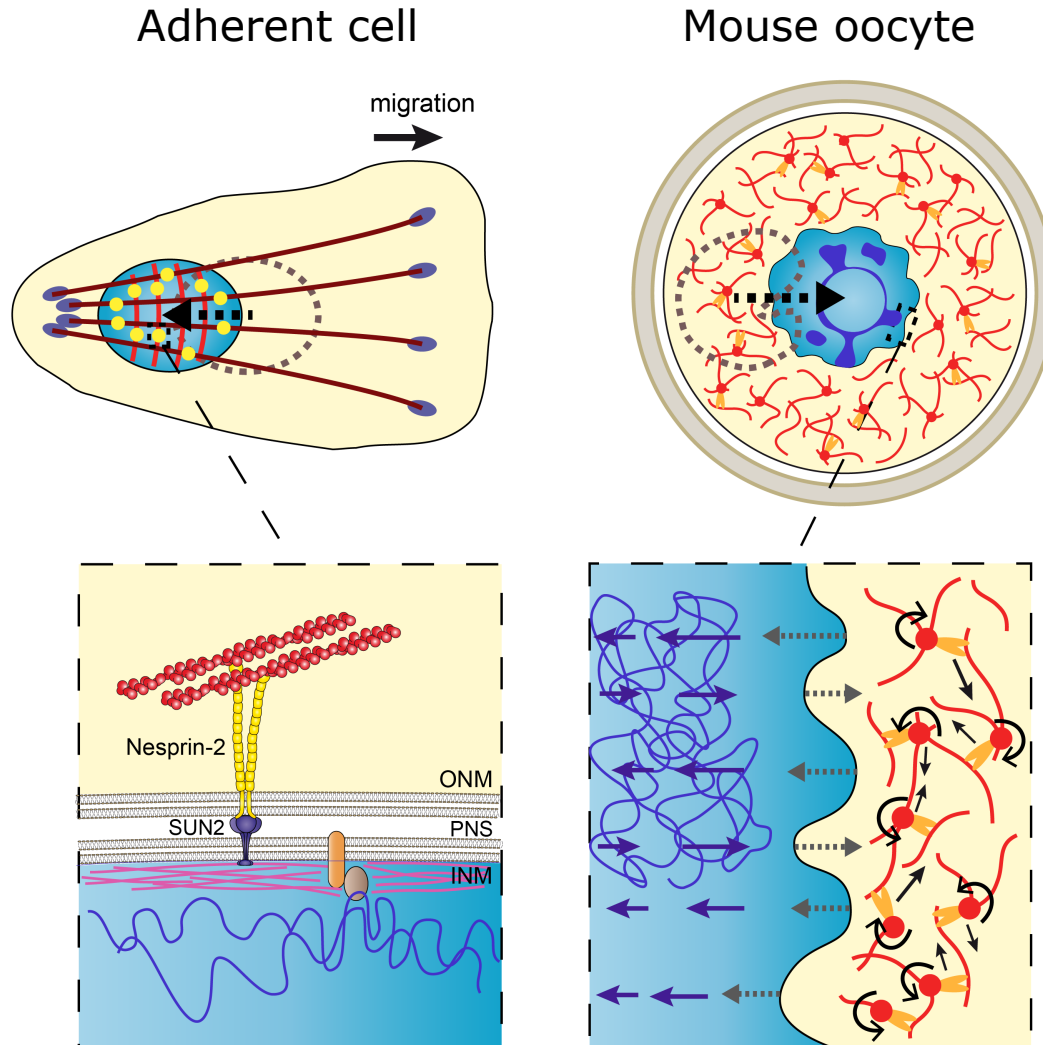


Myosin Vb drives the F-actin mesh dynamics

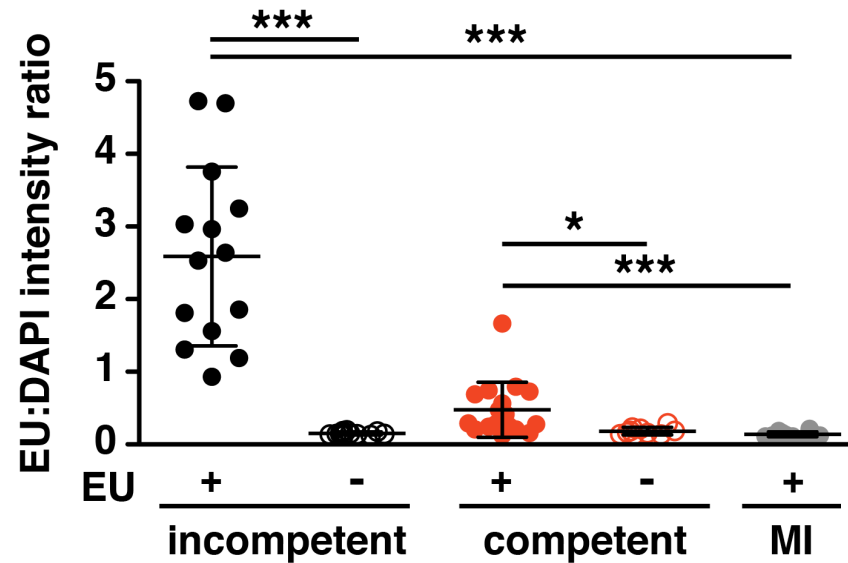
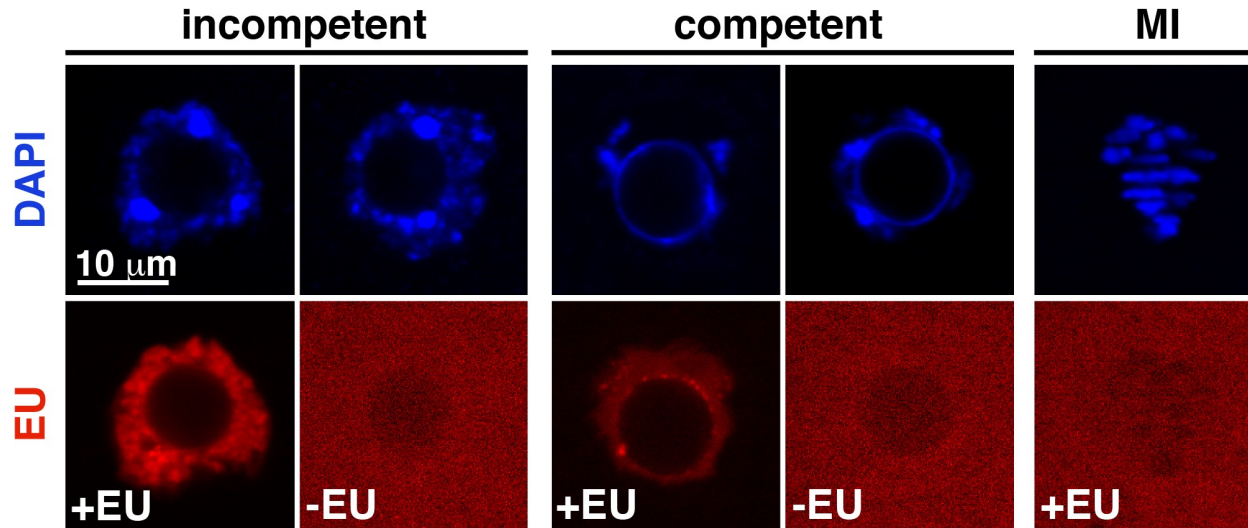


-  actin filaments
-  actin vesicle
-  Formin 2
-  Myosin Vb

A original model of nuclear mechano-transduction



Residual transcription at the end of oocyte growth

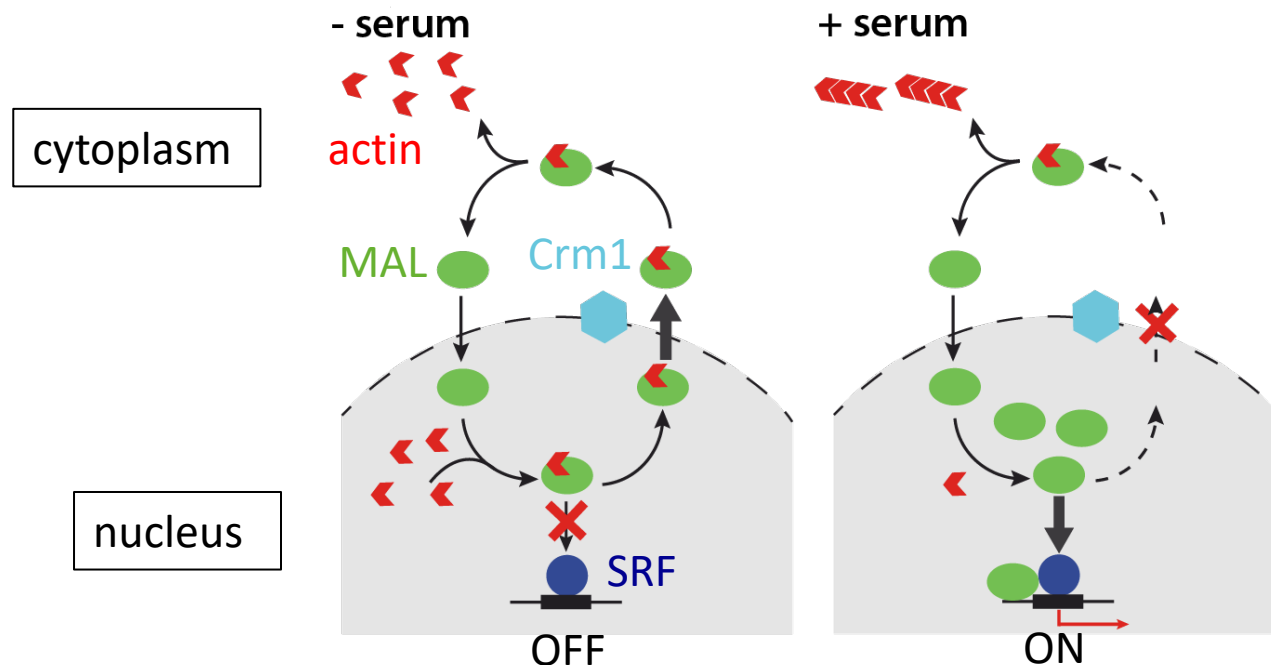


Actin-regulated pathways of gene expression

→ Actin together with RNA Pol II in transcriptional complexes (Hofmann et al, 2004, Kukalev et al, 2005, Obrdlik et al, 2008)

→ Actin in the MAL-SRF inducible system:

➡ crosstalk cytoplasm-nucleus



A nuclear actin probe to address actin-regulated pathways of gene expression



RPEL1: actin-monomer binding domain of MAL
(Belin et al, 2013)

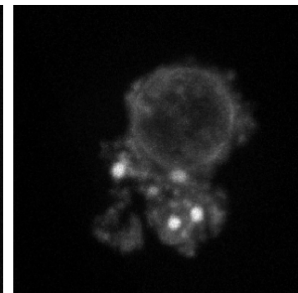
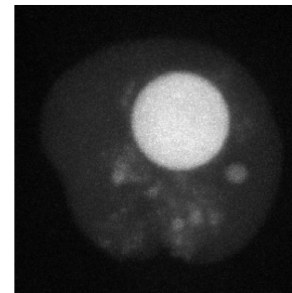
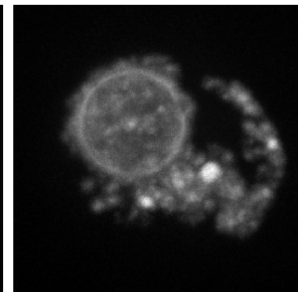
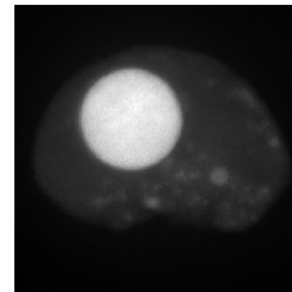
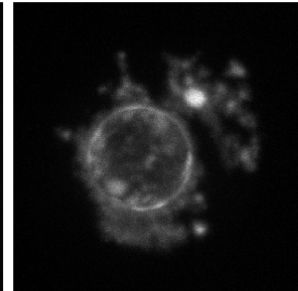
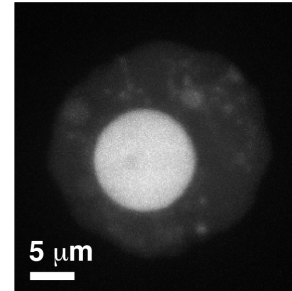
Fmn2 +/-

Fmn2 -/-

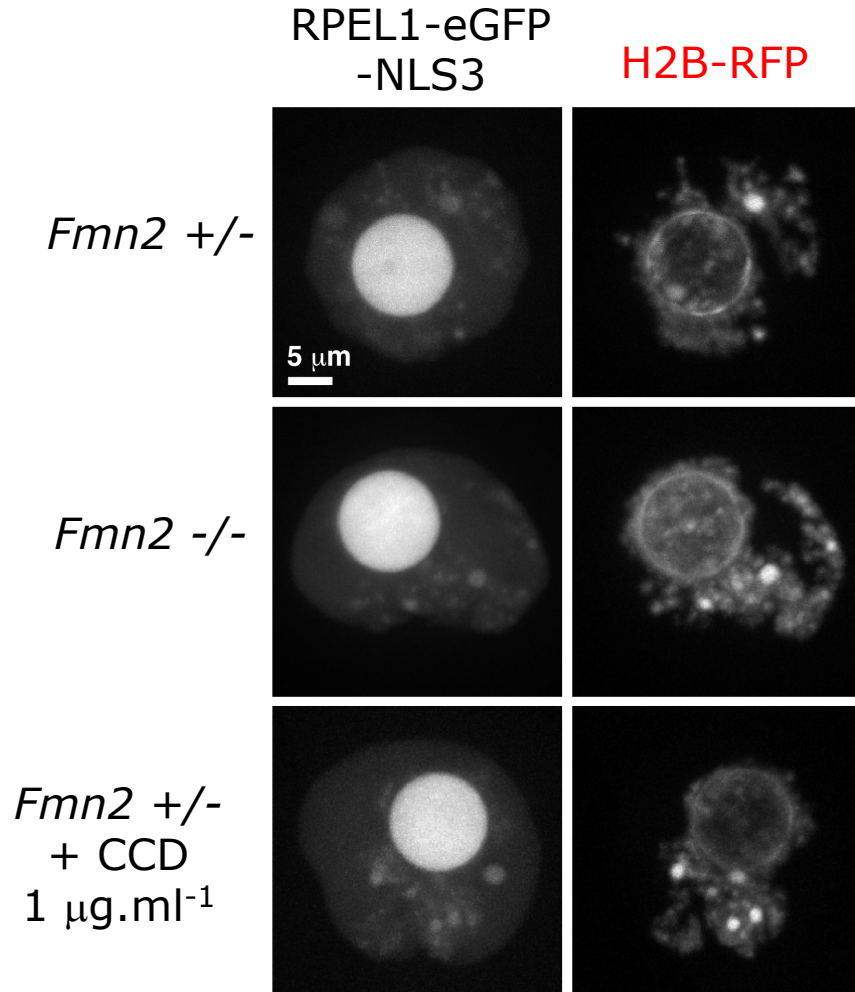
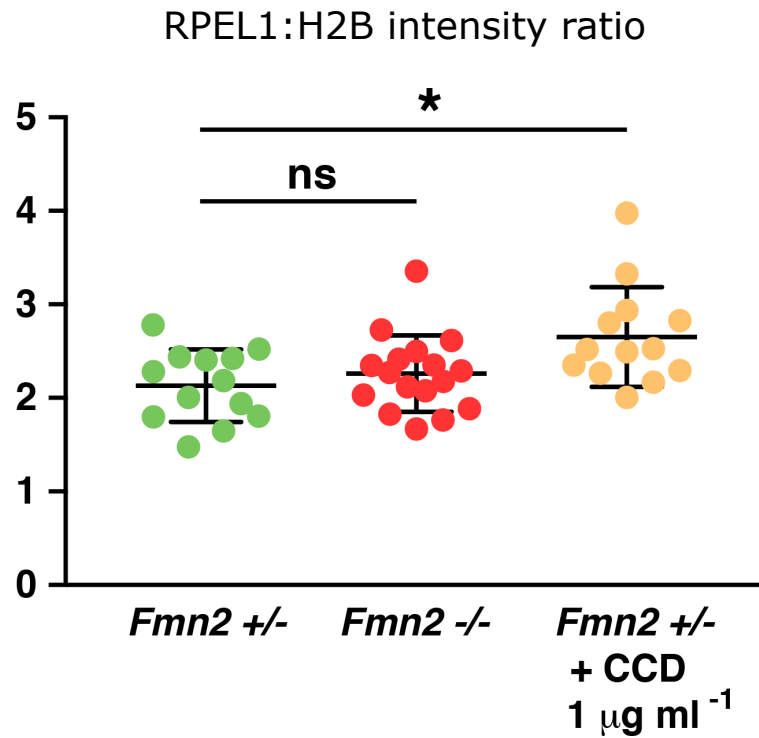
Fmn2 +/-
+ CCD
1 $\mu\text{g}.\text{ml}^{-1}$

RPEL1-eGFP
-NLS3

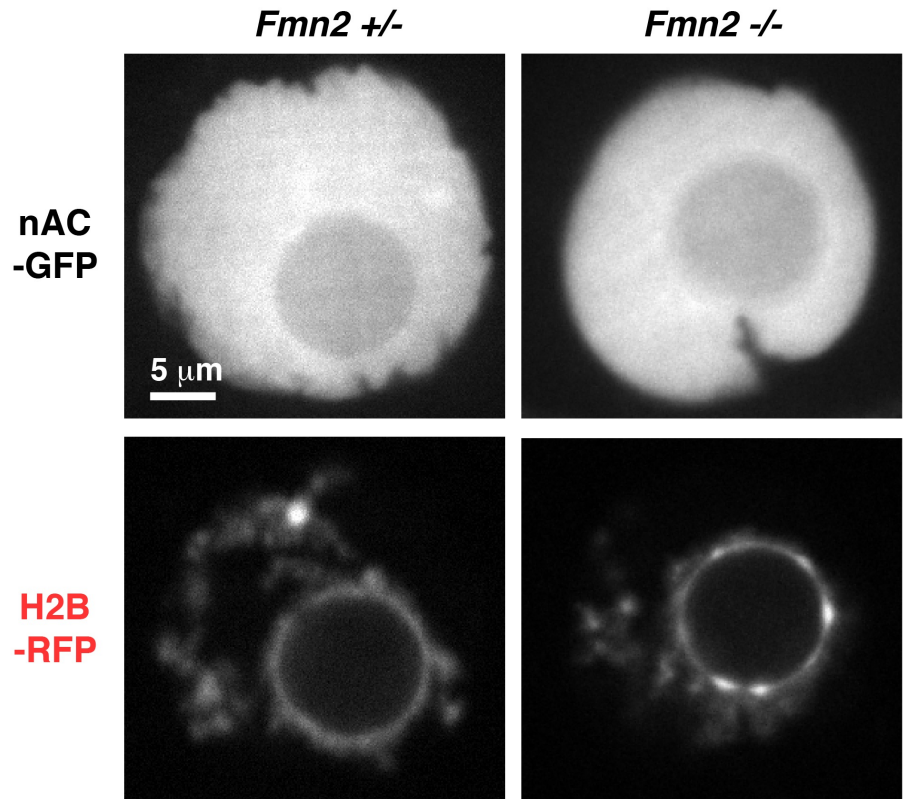
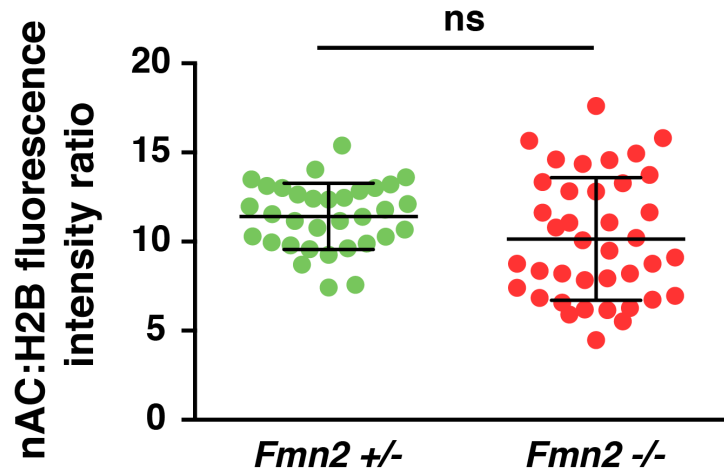
H2B-RFP



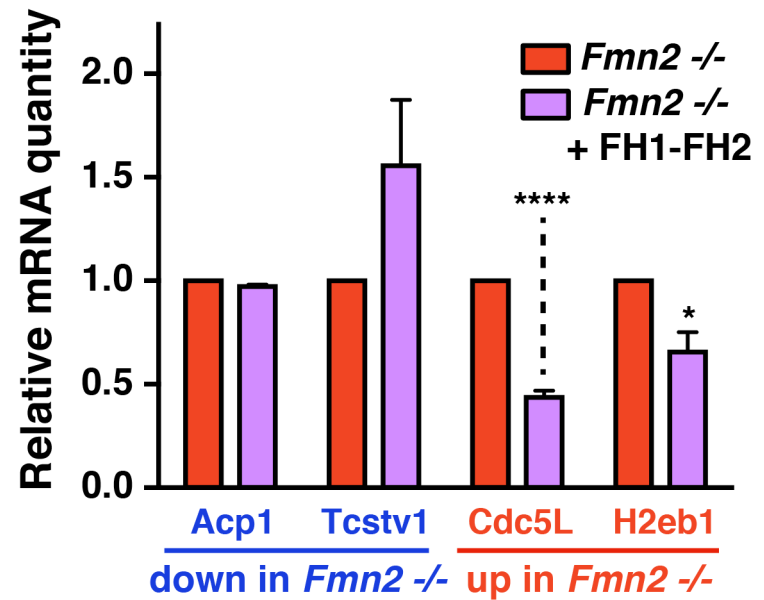
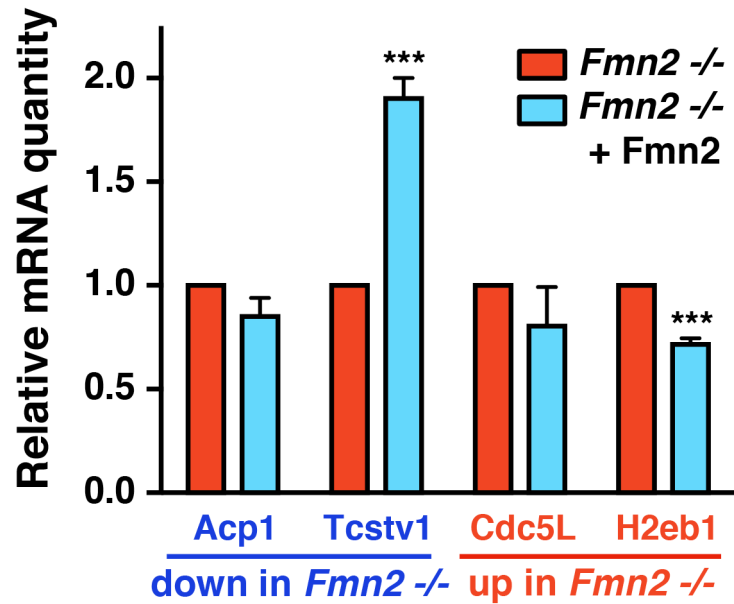
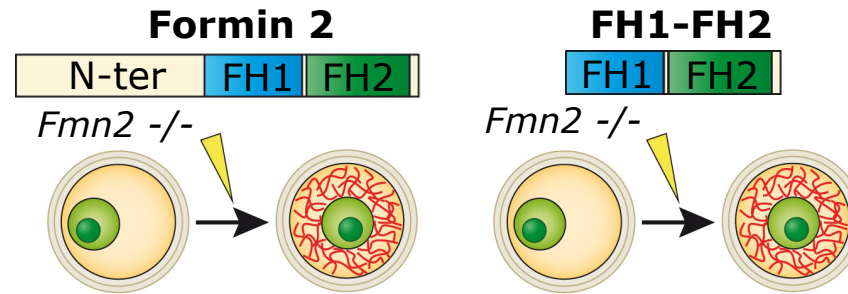
A nuclear actin probe to address actin-regulated pathways of gene expression



A nuclear actin probe to address actin-regulated pathways of gene expression



Formin 2 rescues gene expression in *Fmn2* $-/-$ oocytes



Formin 2 rescues gene expression in *Fmn2* $-/-$ oocytes

