Centrosomes and (asymmetric) cell division in “normal” and pathological conditions

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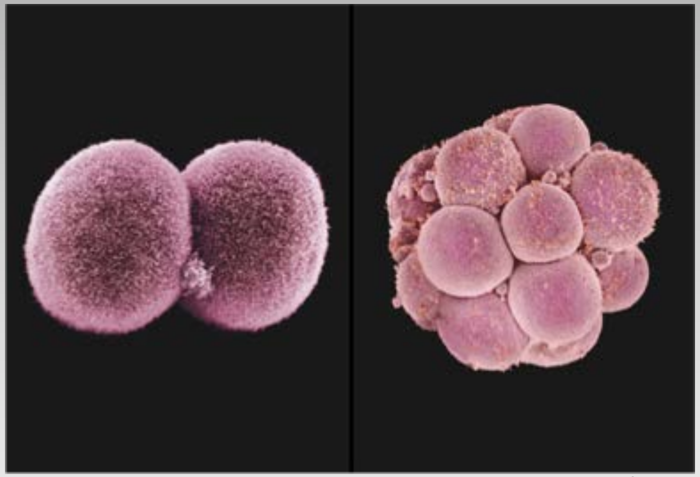
« Cell Fate and plasticity »

October 14, 2022

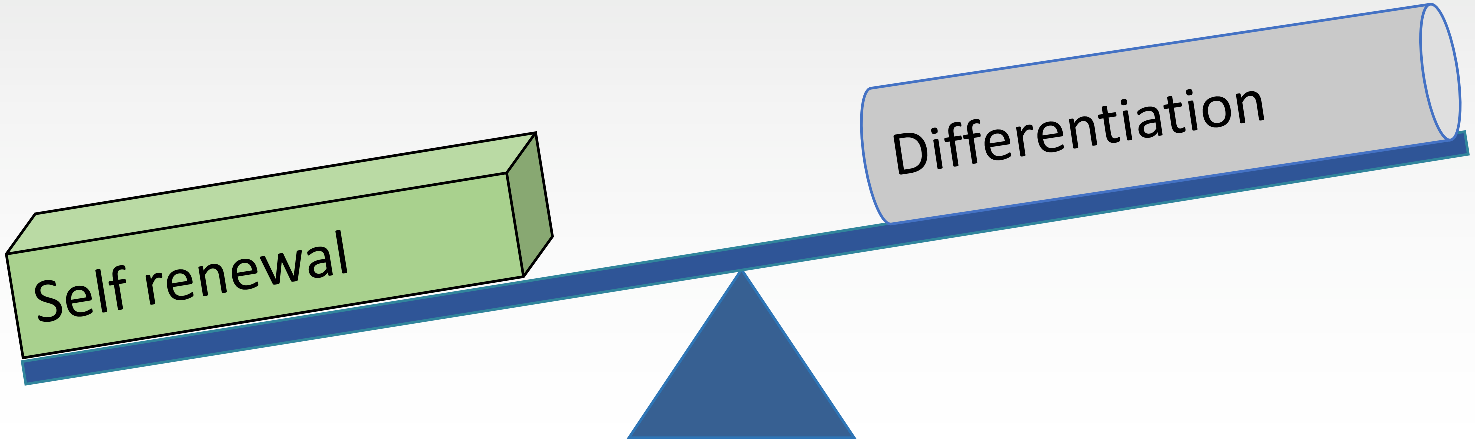
Asymmetric Cell Division (ACD): involved in cell differentiation, tissue morphogenesis and maintenance

Fertilized egg



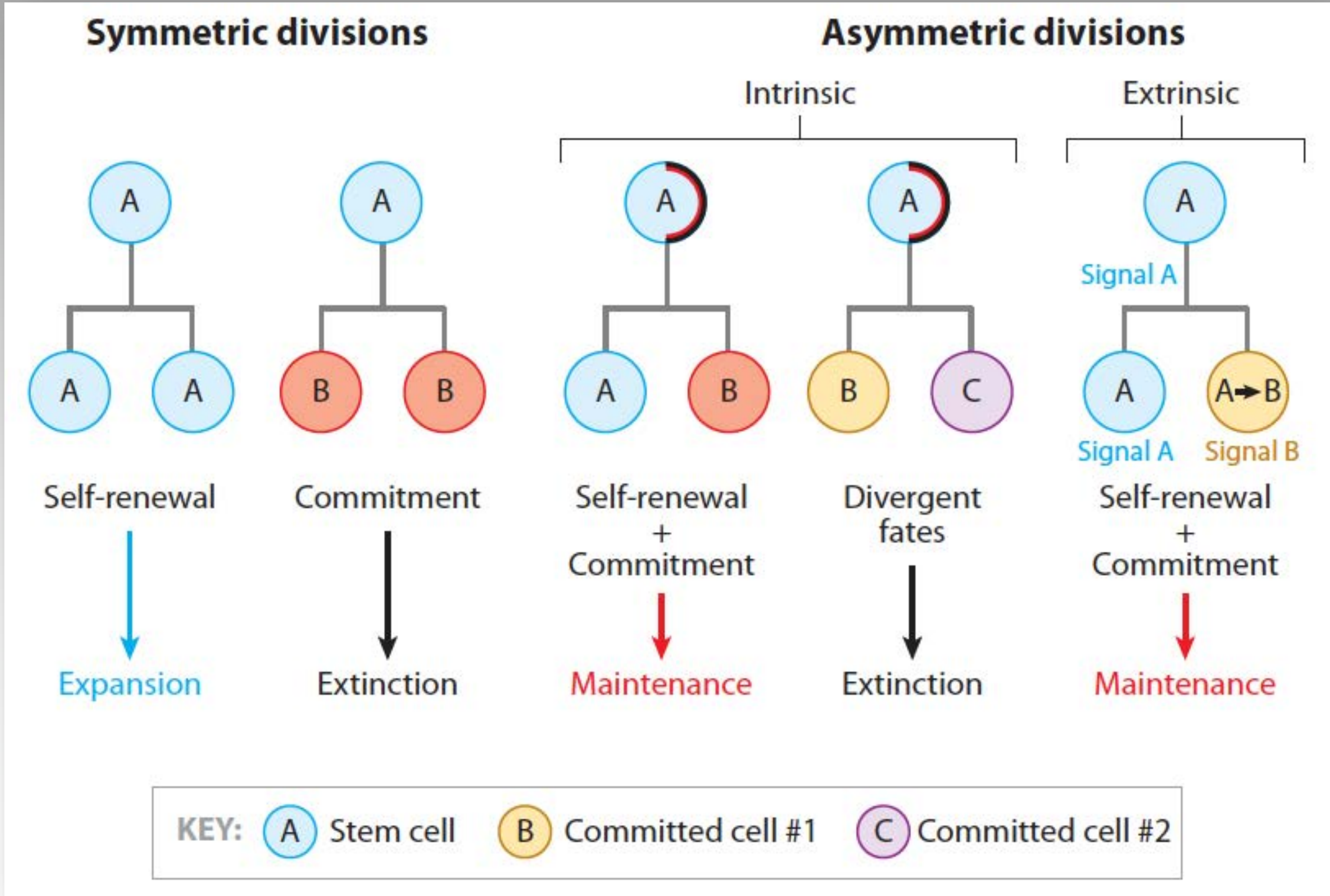
Source: WebMD

Complex organism



Mechanics of asymmetric cell division

Most critical asymmetry for ACD are the one of fate determining factors

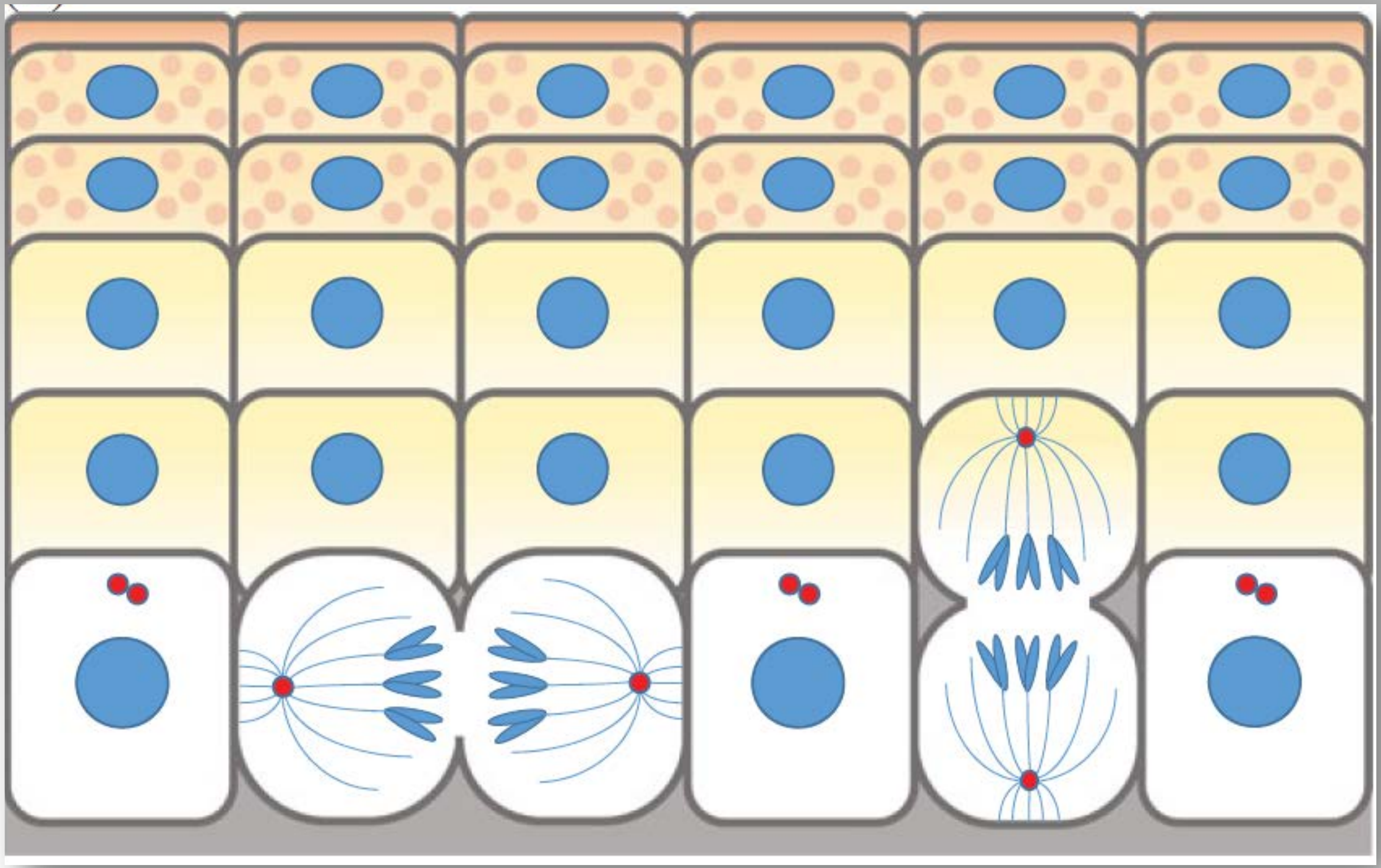


Tajbakhsh *et al*,. Ann. Rev. Cell Dev. Biol. 2009

Mechanics of asymmetric cell division

A second essential aspect is the orientation of cell division plan, thus of the mitotic spindle, in respect to fate determining factors

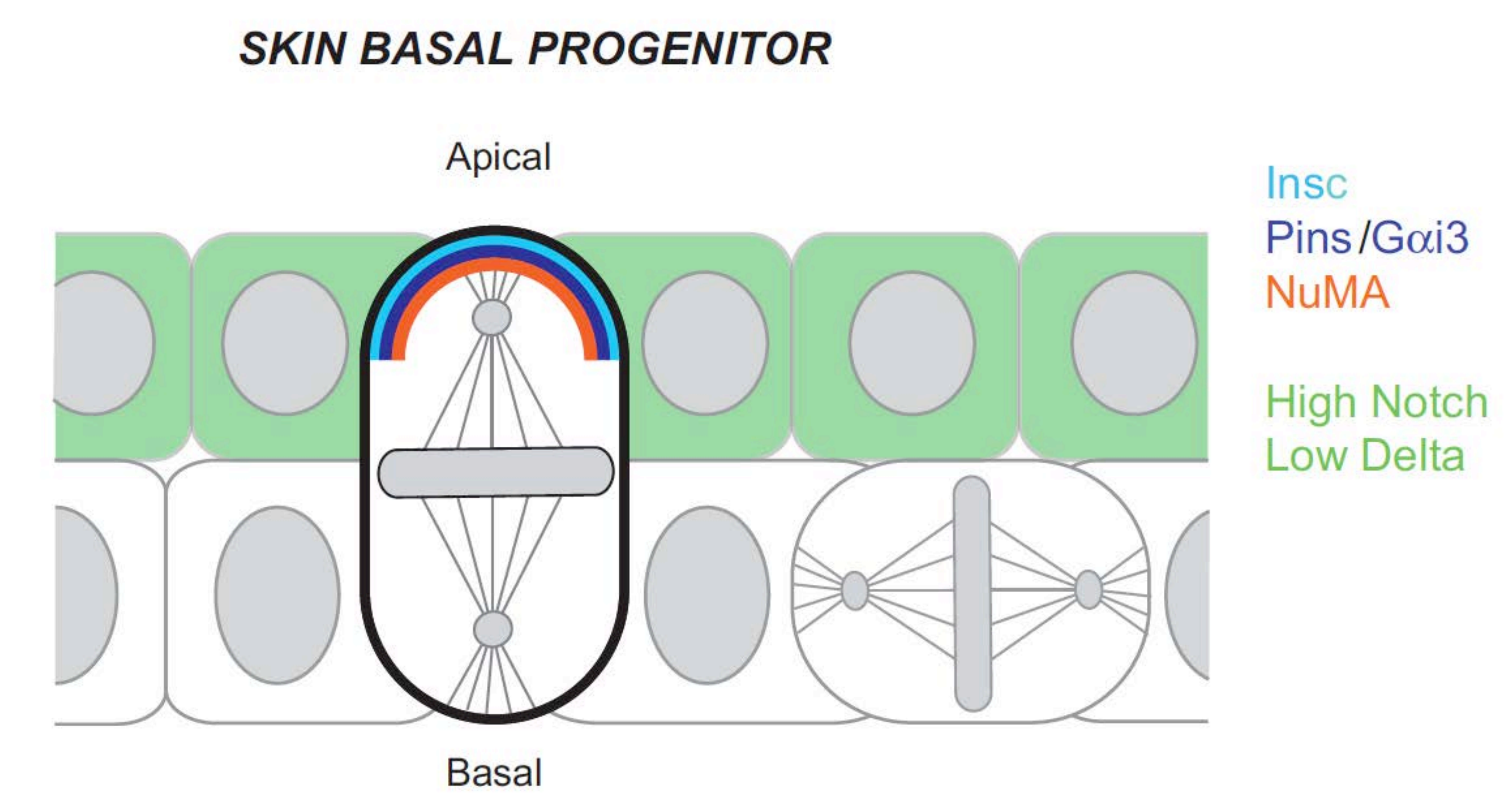
Epidermis



Kulukian *et al,*. Phi. Trans. Roy. Soc. B. 2013

ACD requires proper spindle orientation

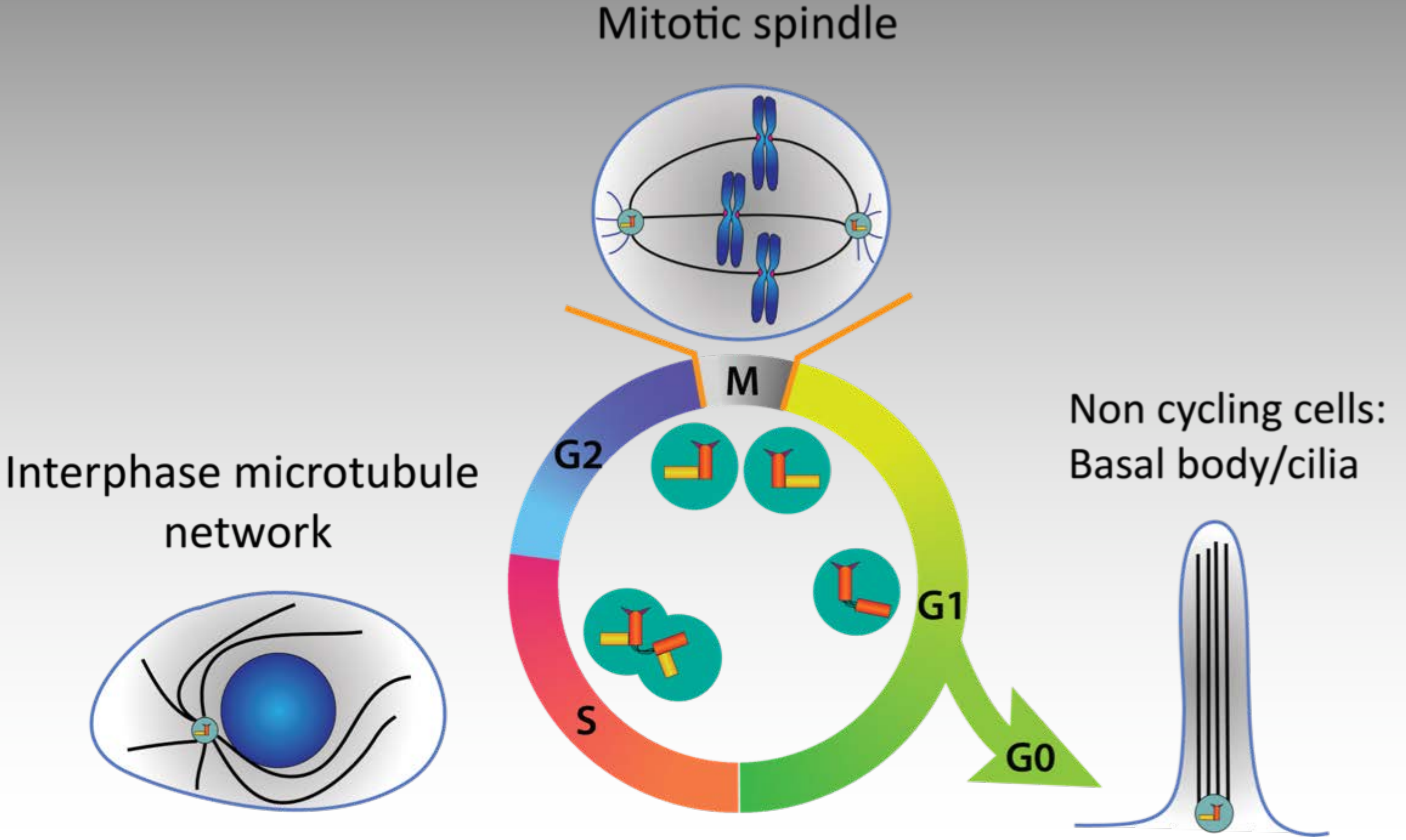
Spindle orientation is mediated by interaction of astral microtubules with cortical protein complexes and cues



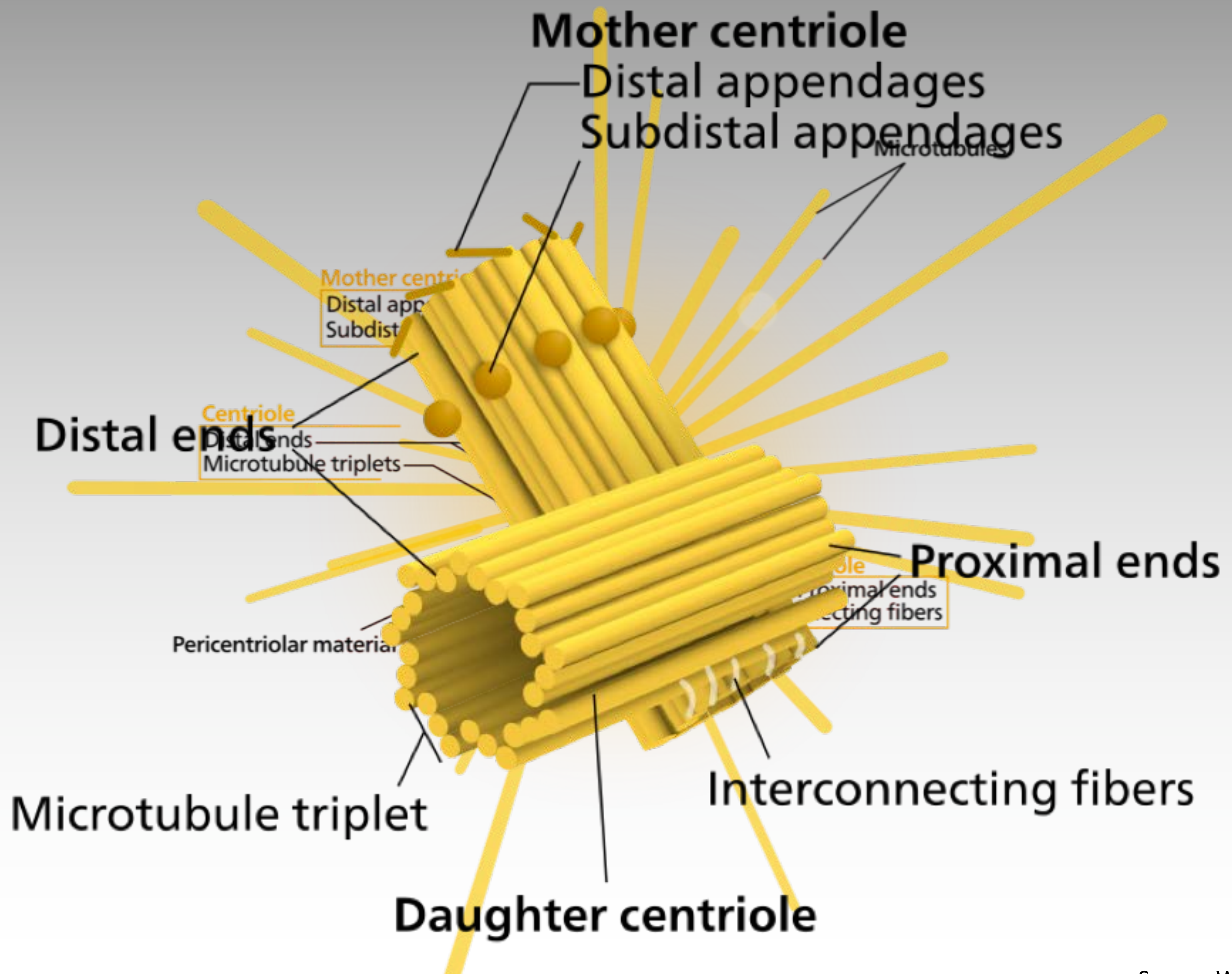
Morin & Bellaïche,. Dev. Cell, 2011

Contribution of Centrosomes to ACD?

The Centrosome: a Multi-functional Organelle

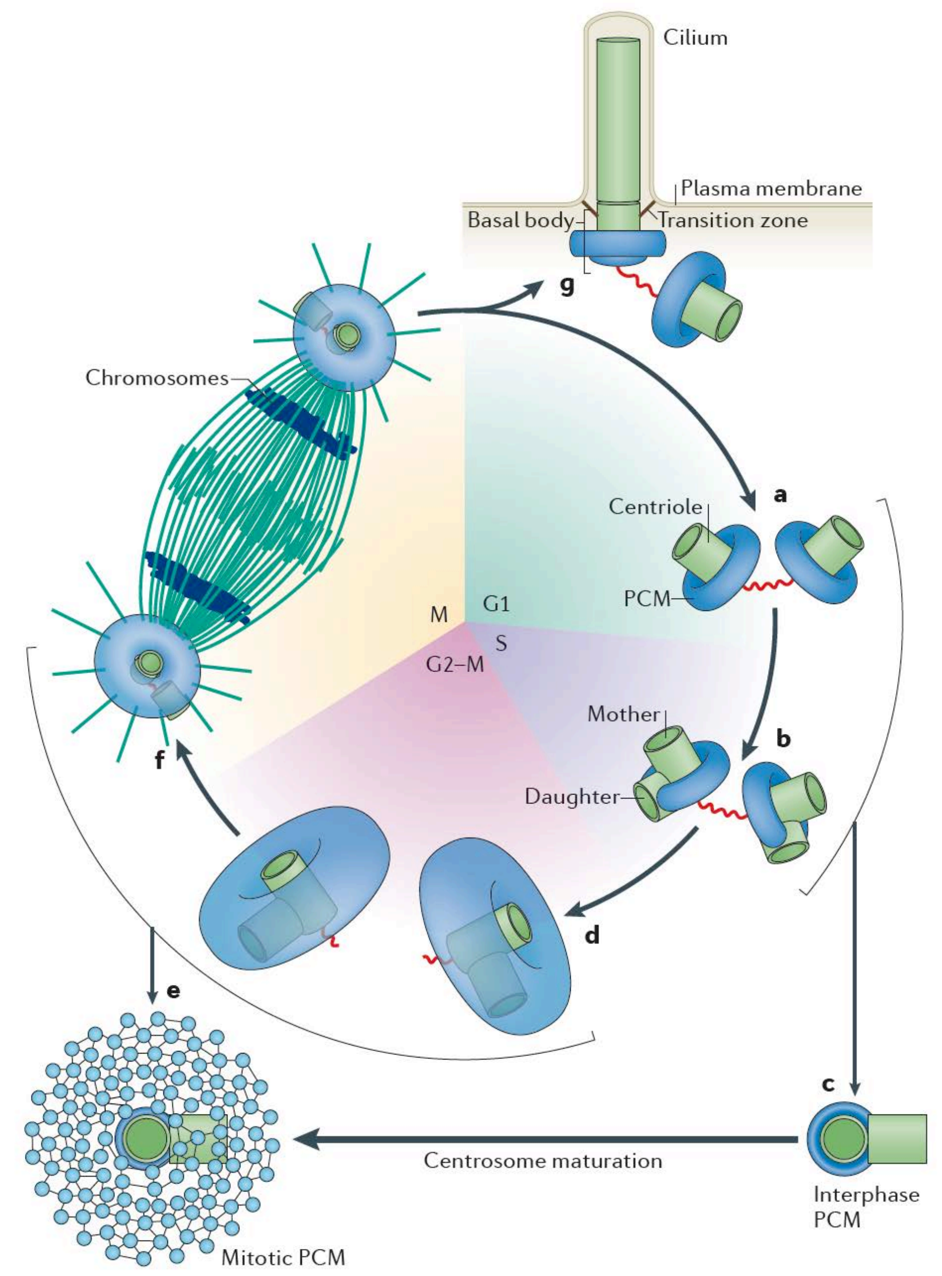


The Centrosome structure



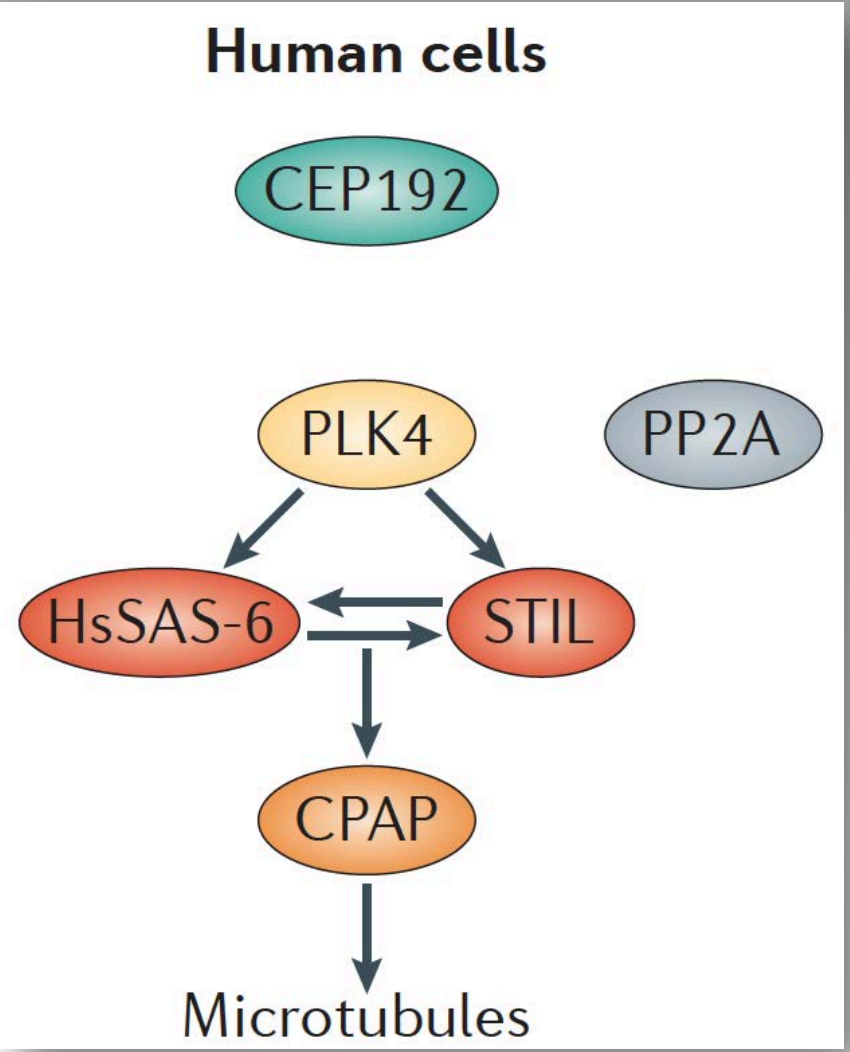
Source:Wikipedia; Kelvinsong

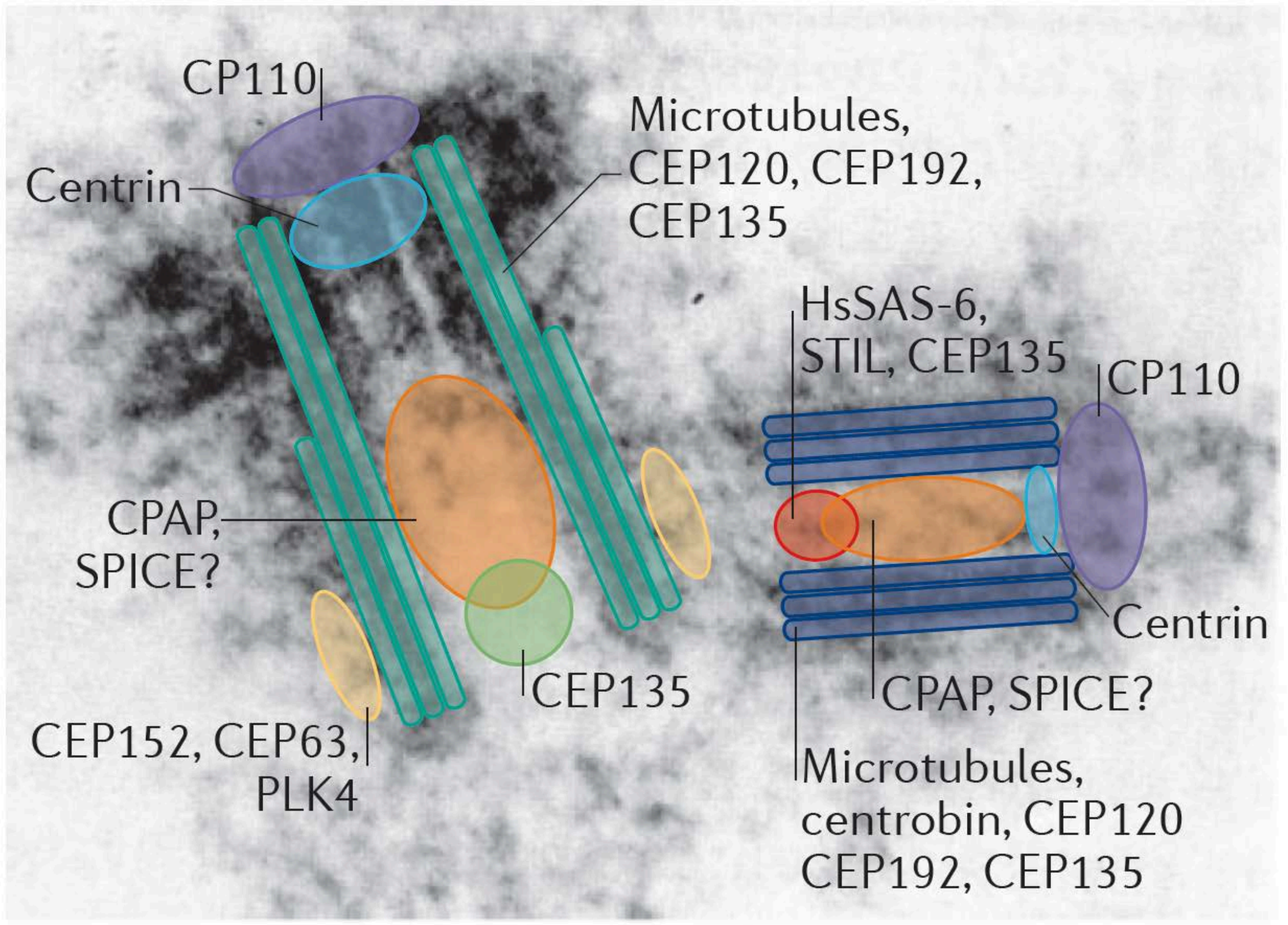
The Centrosome duplication cycle



Conduit *et al*, Nat. Rev. MCB, 2015

The Centrosome duplication regulators



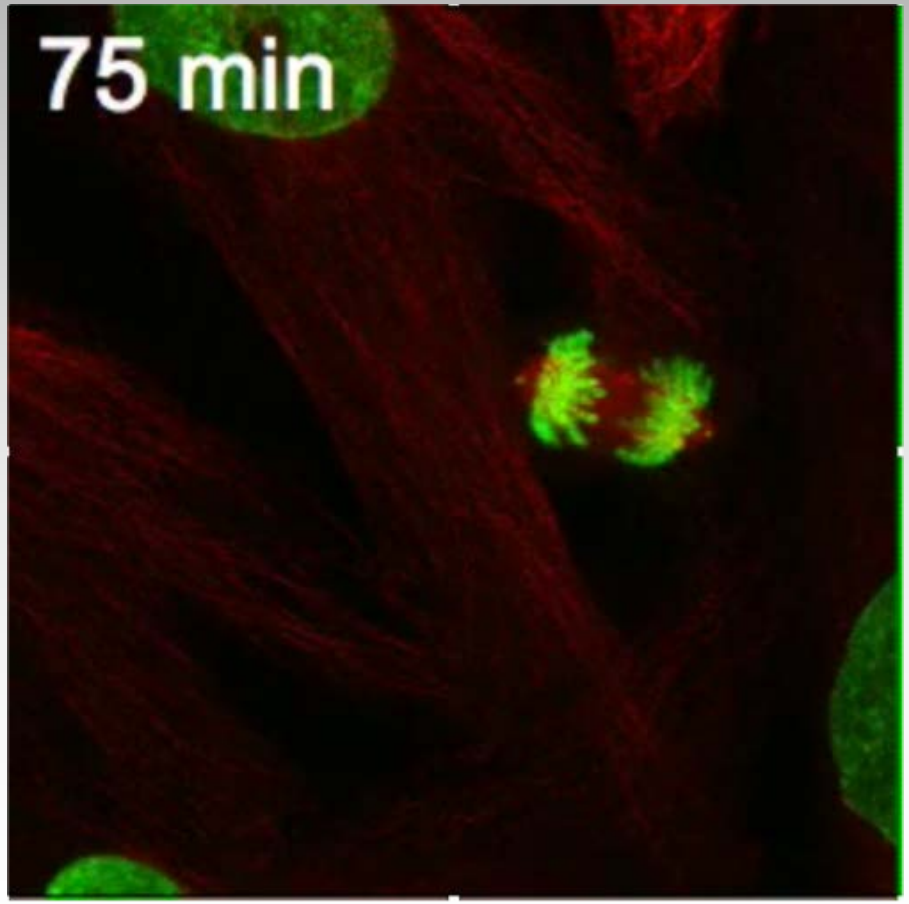


Gönczy *Nat. Rev. MCB.* 2012

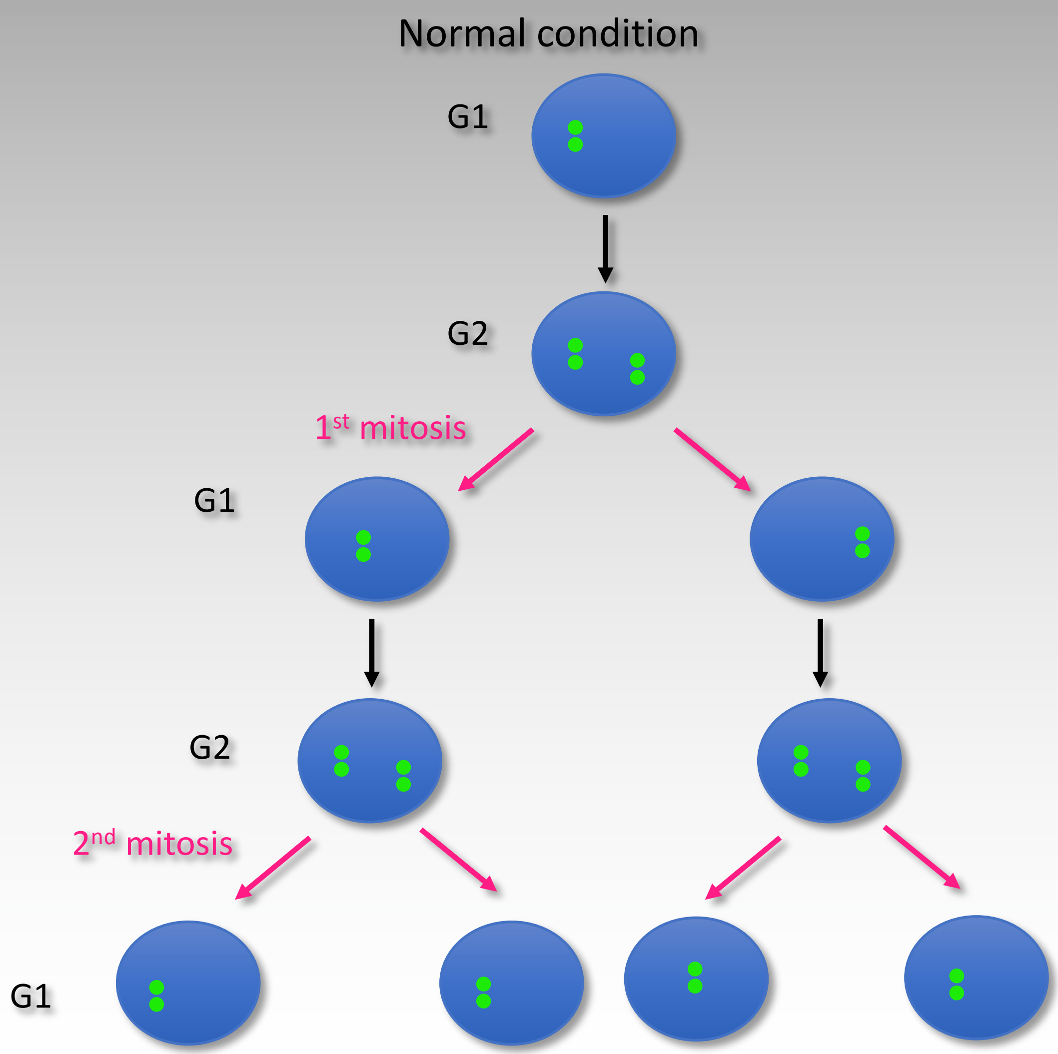
The Centrosome and bipolar spindle assembly

Centrosomes help **focusing spindle poles** and are important for timely and errorless mitosis

Plk4$^{+/+}$: 2 centro.



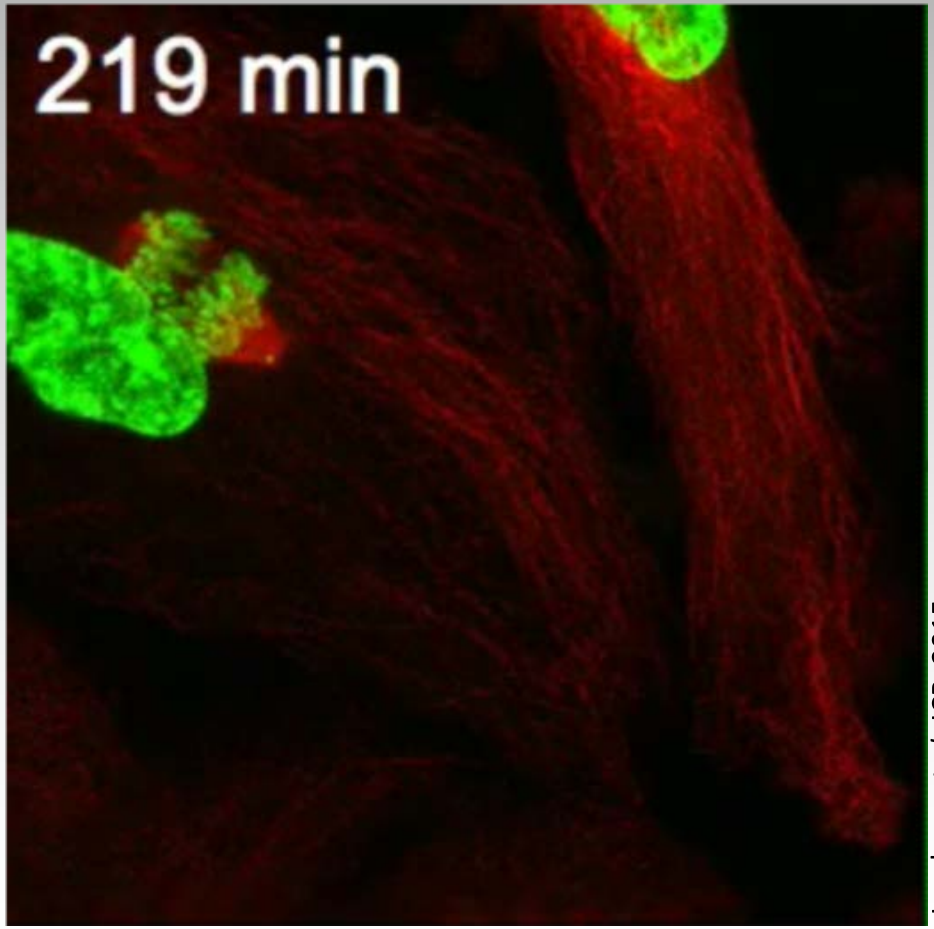
Lambrus *et al*, JCB. 2015



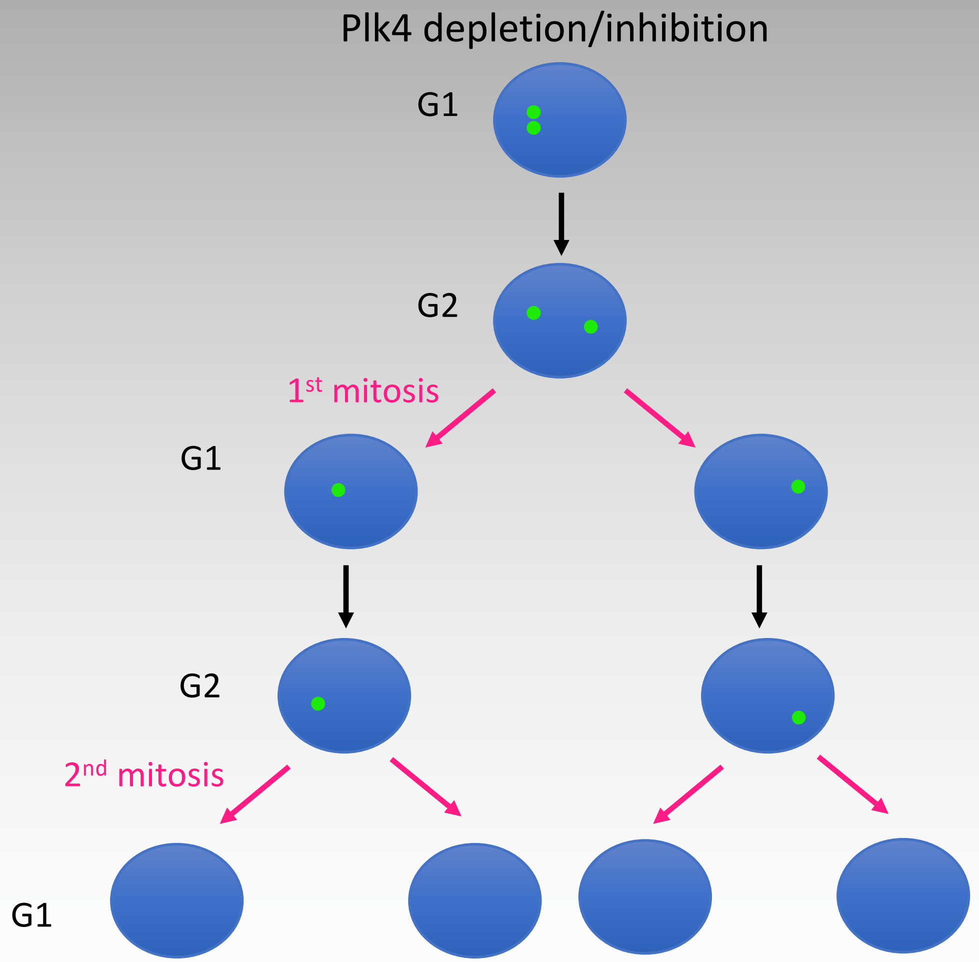
The Centrosome and bipolar spindle assembly

Centrosomes help focusing spindle poles and are important for timely and errorless mitosis

Plk4 $^{-/-}$: 1 centro.



Lambrus *et al*, JCB. 2015



Non transformed human cells w/o centrosomes stop proliferating

Wong *et al*, Science. 2015; Meitinger et al, JCB, 2016, Lambrus et al, 2016

Centrosome and ACD: case studies

- **In Cell/tissues with normal homeostasis:**

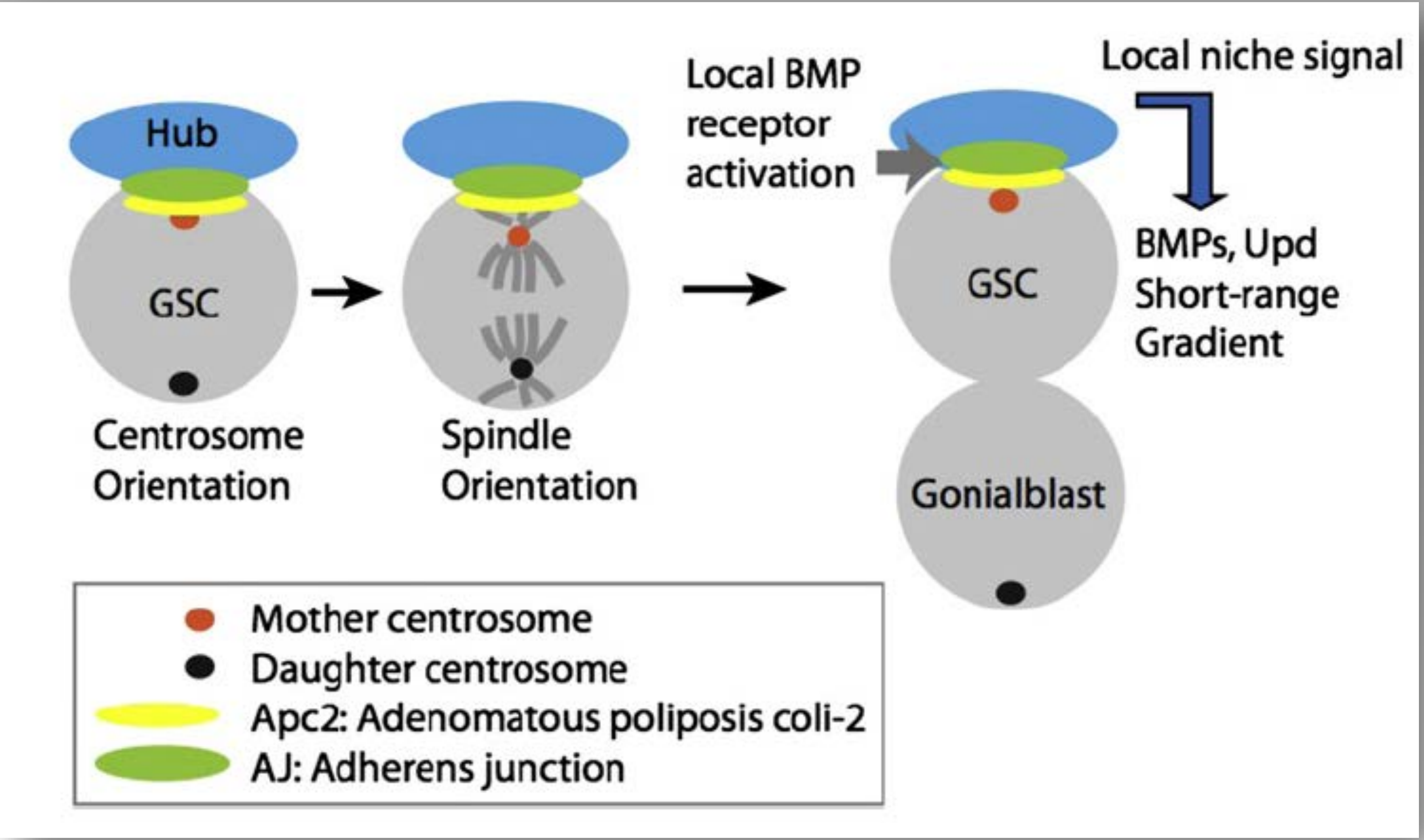
  a- Drosophila neuroblasts
  b- C. elegans first embryonic division
  c- Mice neocortex development

- **In Cell/tissues with perturbed homeostasis**

  a- Centrosome amplification and cancer in Fly
  b- Centrosome amplification, microcephaly and cancer in mice
  c- Contribution of IFT proteins to centrosome clustering

Centrosome and germ cells differentiation

Drosophila male Germline Stem Cells (GSC) ACD:

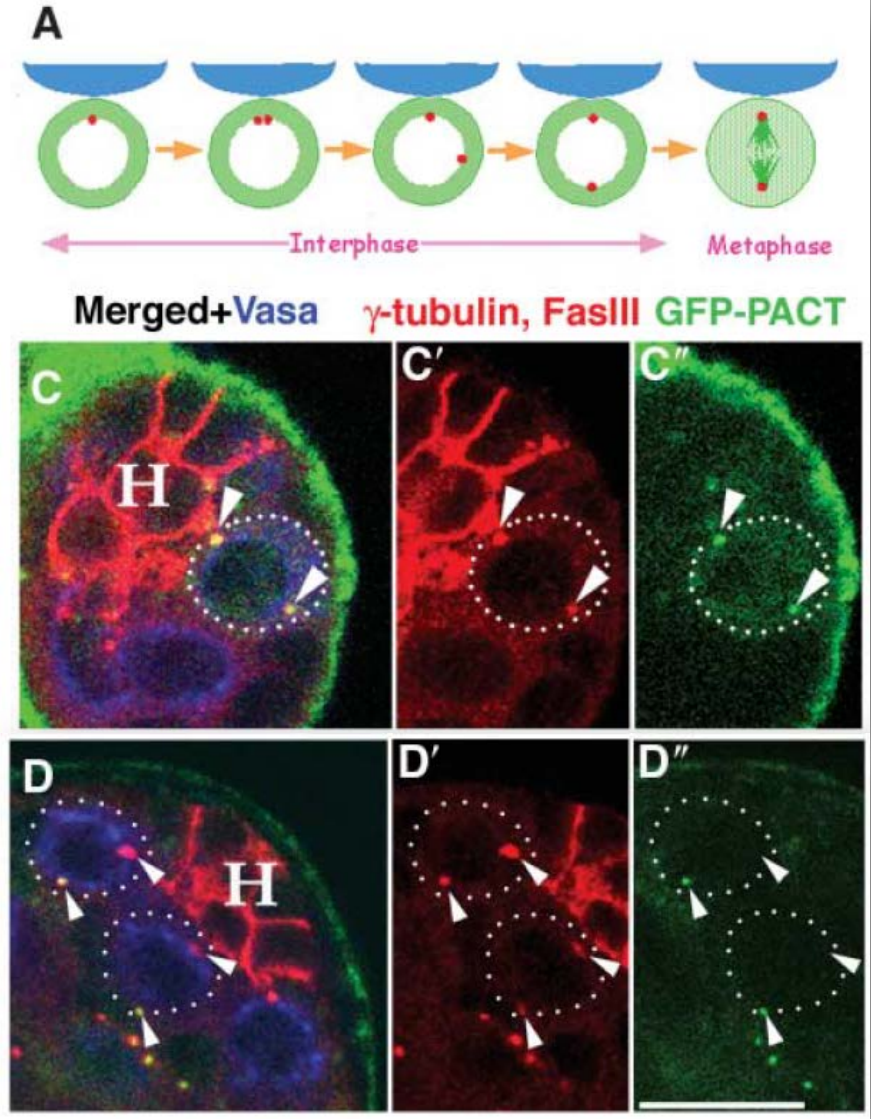


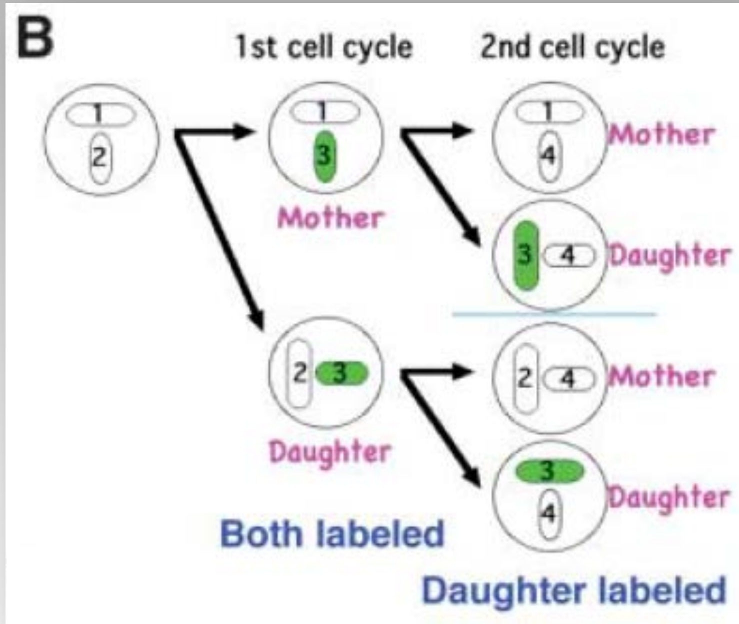
Inaba & Yamashita Cell Stem Cell. 2012

Determined by extrinsic clues and directed by spindle orientation.

Centrosomes and germ cells differentiation

What will determine spindle orientation in Drosophila Germ Stem Cells (GSC)?

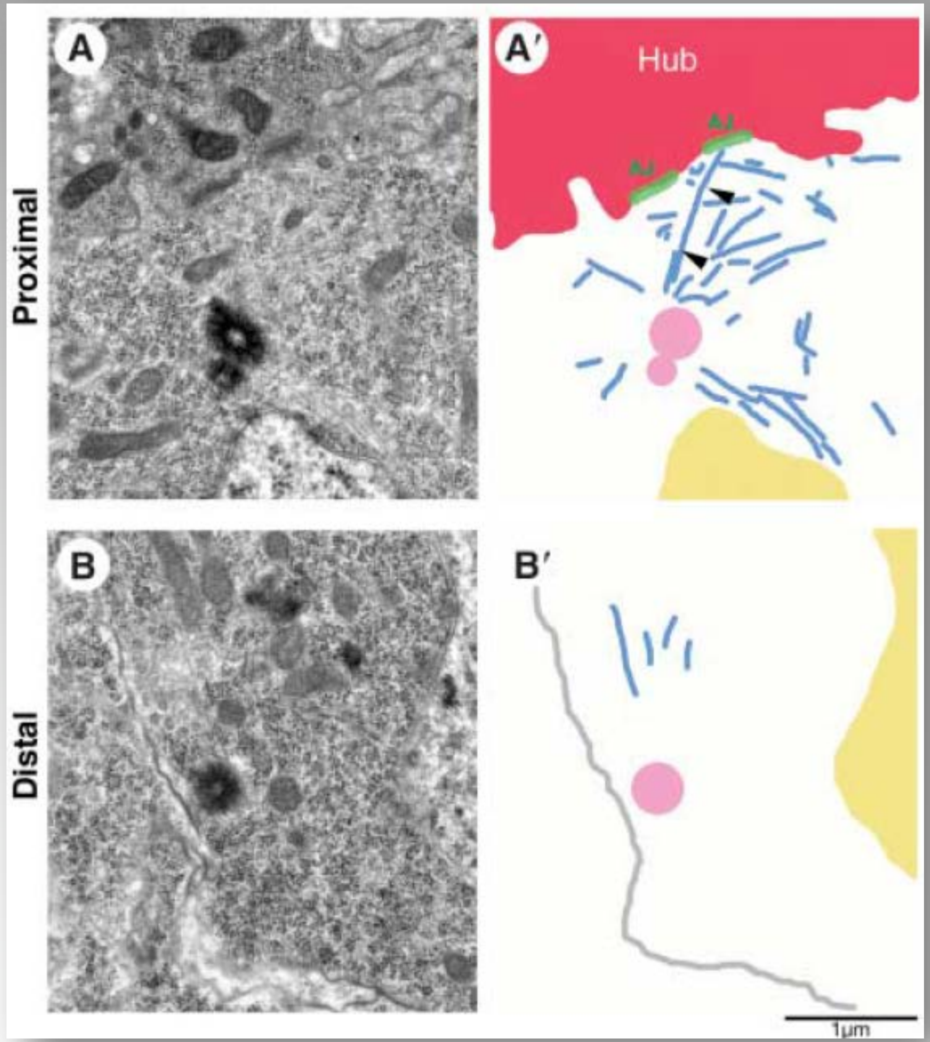




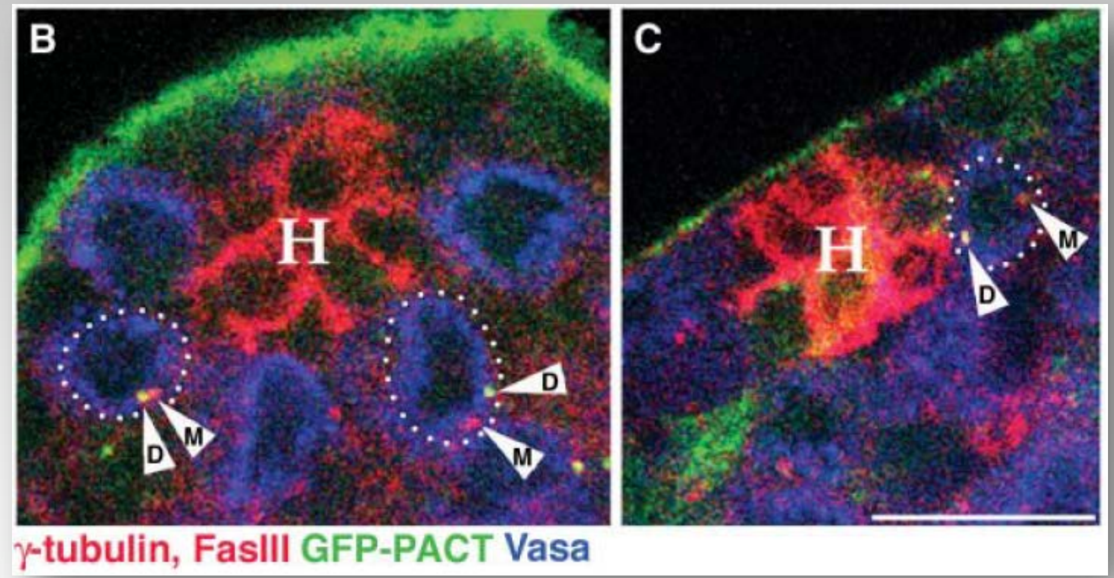
Yamashita *et al*, Science. 2007

Centrosomes and germ cells differentiation

Centrosomes age influences MTs aster formation and centrosome attachment.



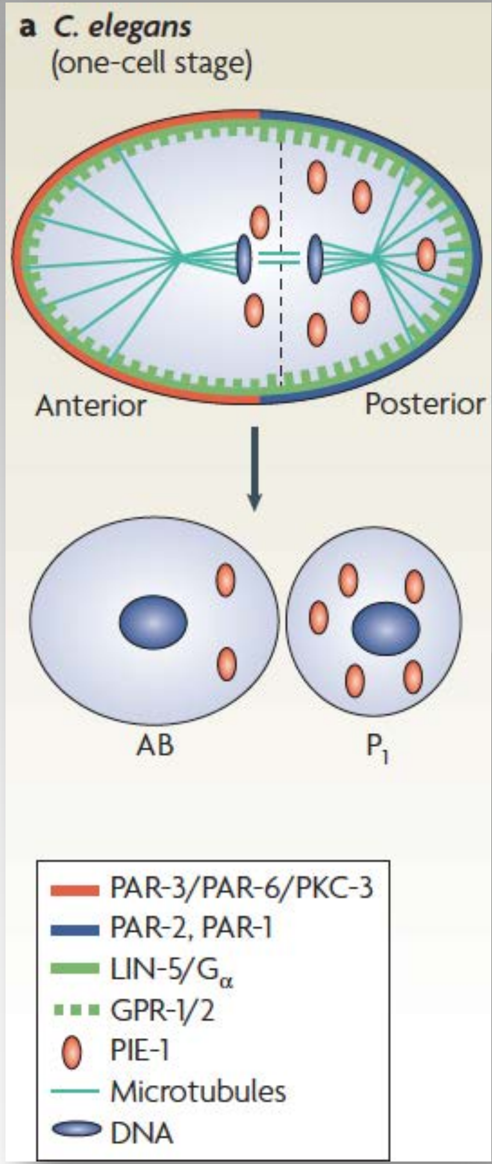
Yamashita *et al*, Science. 2007

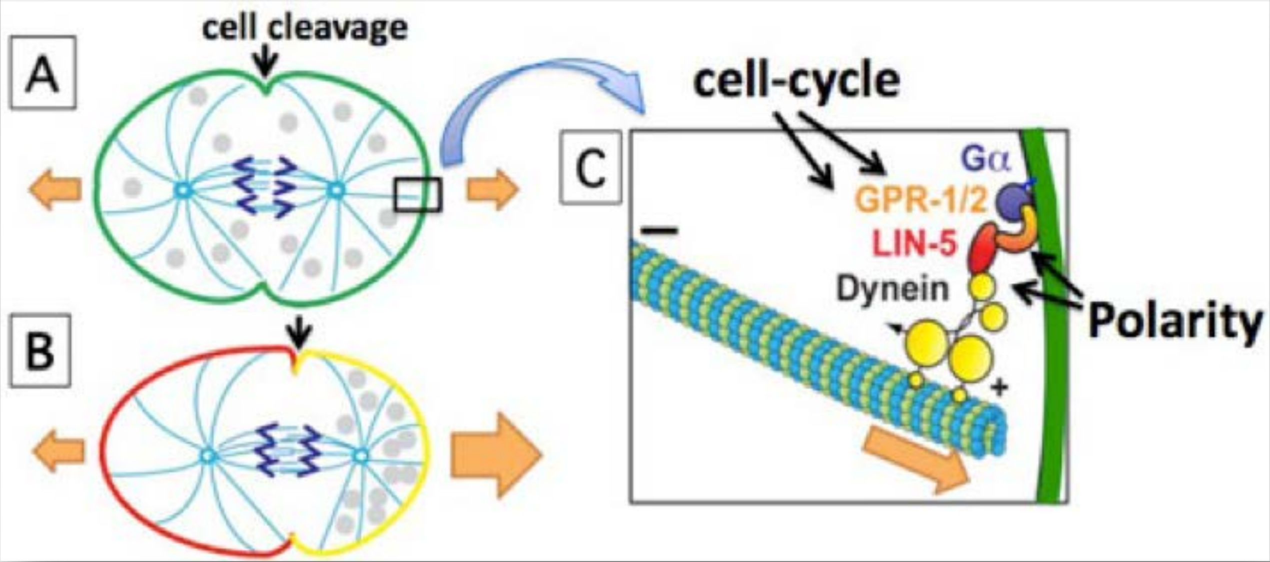


Centrosomes asymmetry is an integral part of ACD.

Centrosomes and C. elegans development

One cell C. elegans embryo requires symmetry breaking to establish polarity

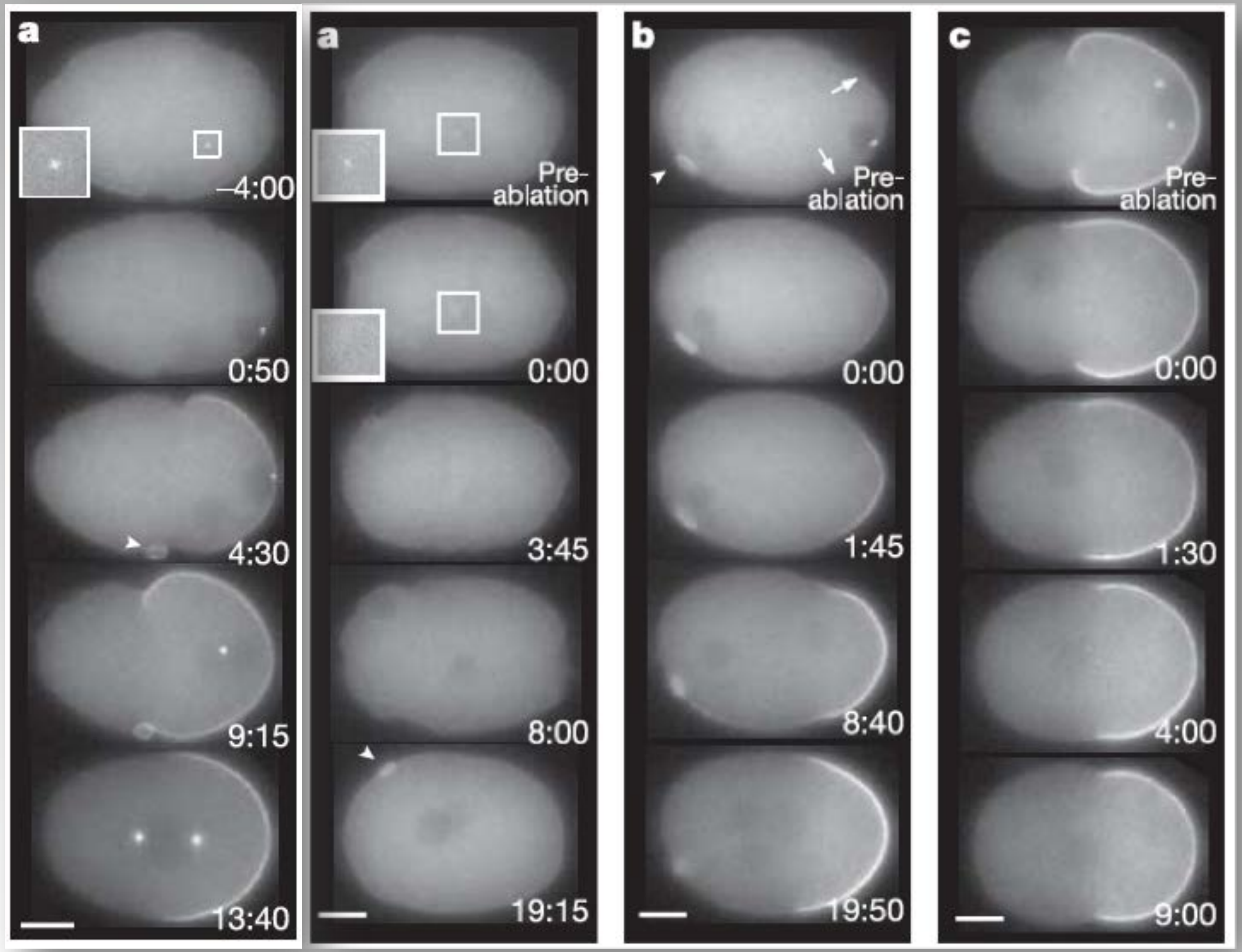




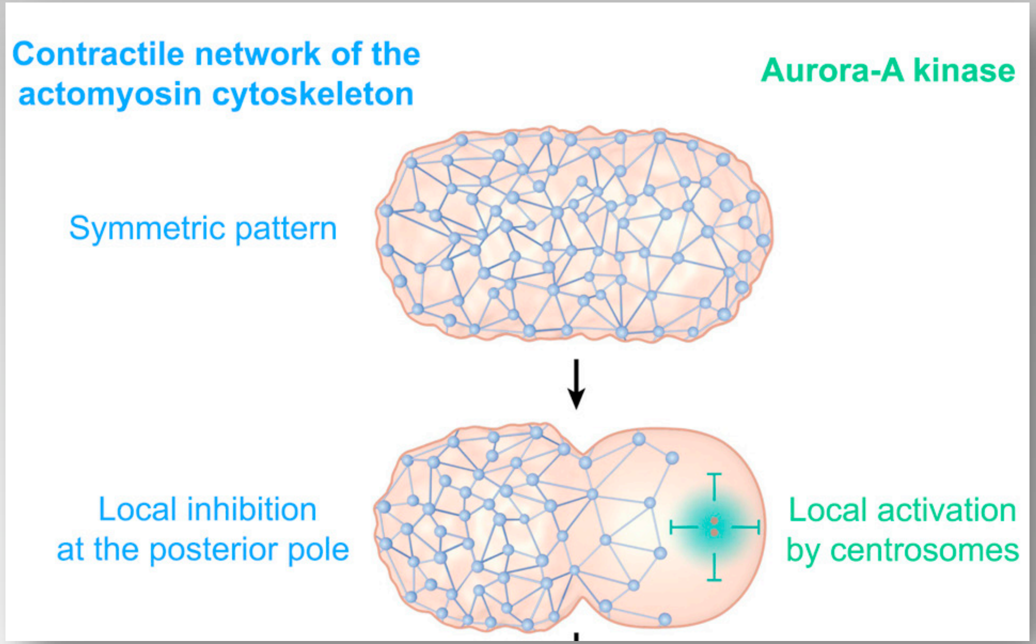
Gönczy, Nat. Rev. Mol. Cell. Bio. 2008

Centrosomes and C. elegans development

Centrosomes contribute to polarity in *C. elegans* embryo through AIR-1 kinase activity



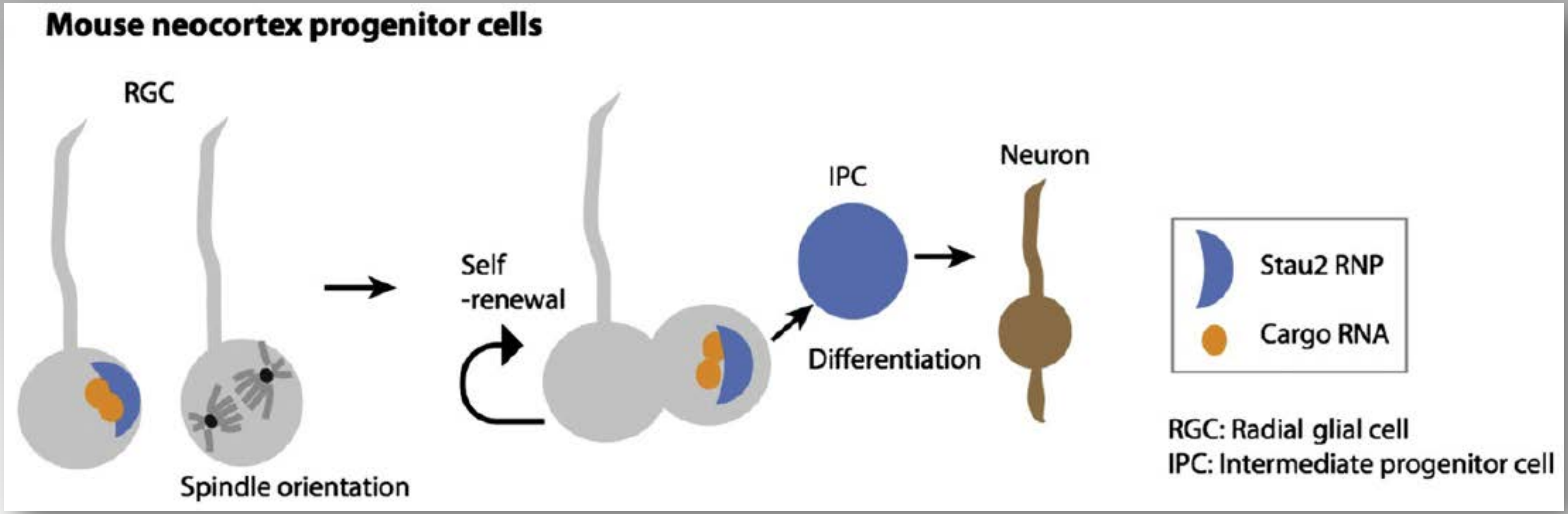
Cowan & Hyman. Nature 2004



Zhao *et al*, Dev. Cell 2019

Centrosome and mice neocortex development

Asymmetric centrosome segregation in the neocortex:

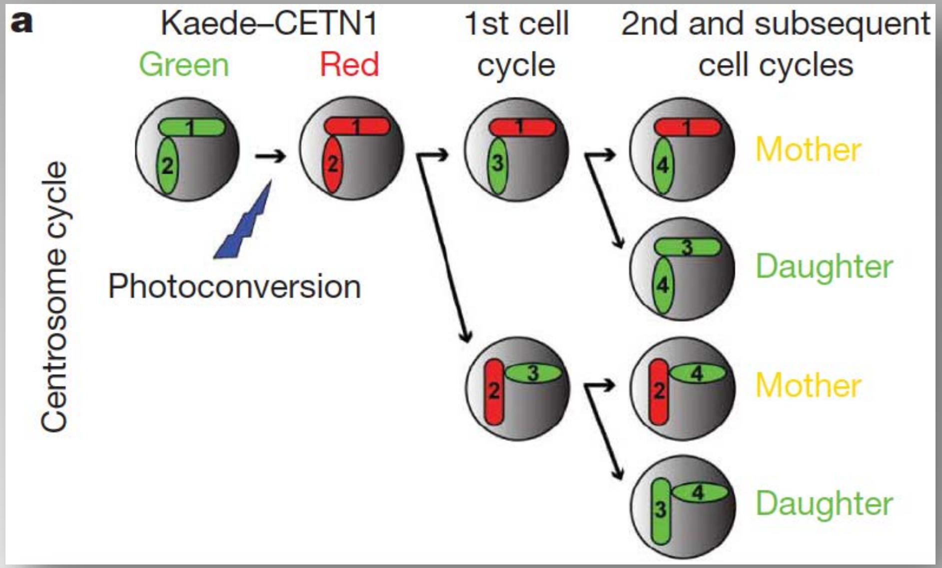


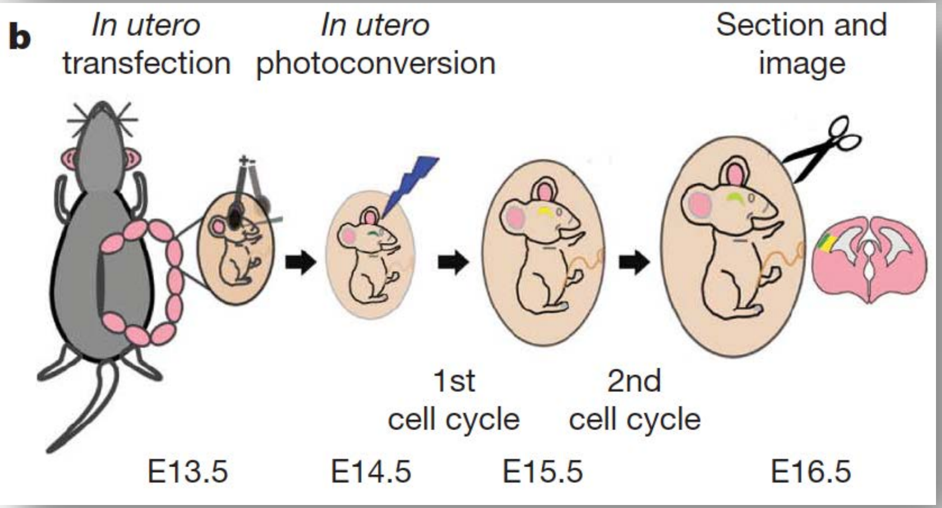
Inaba & Yamashita Cell Stem Cell. 2012

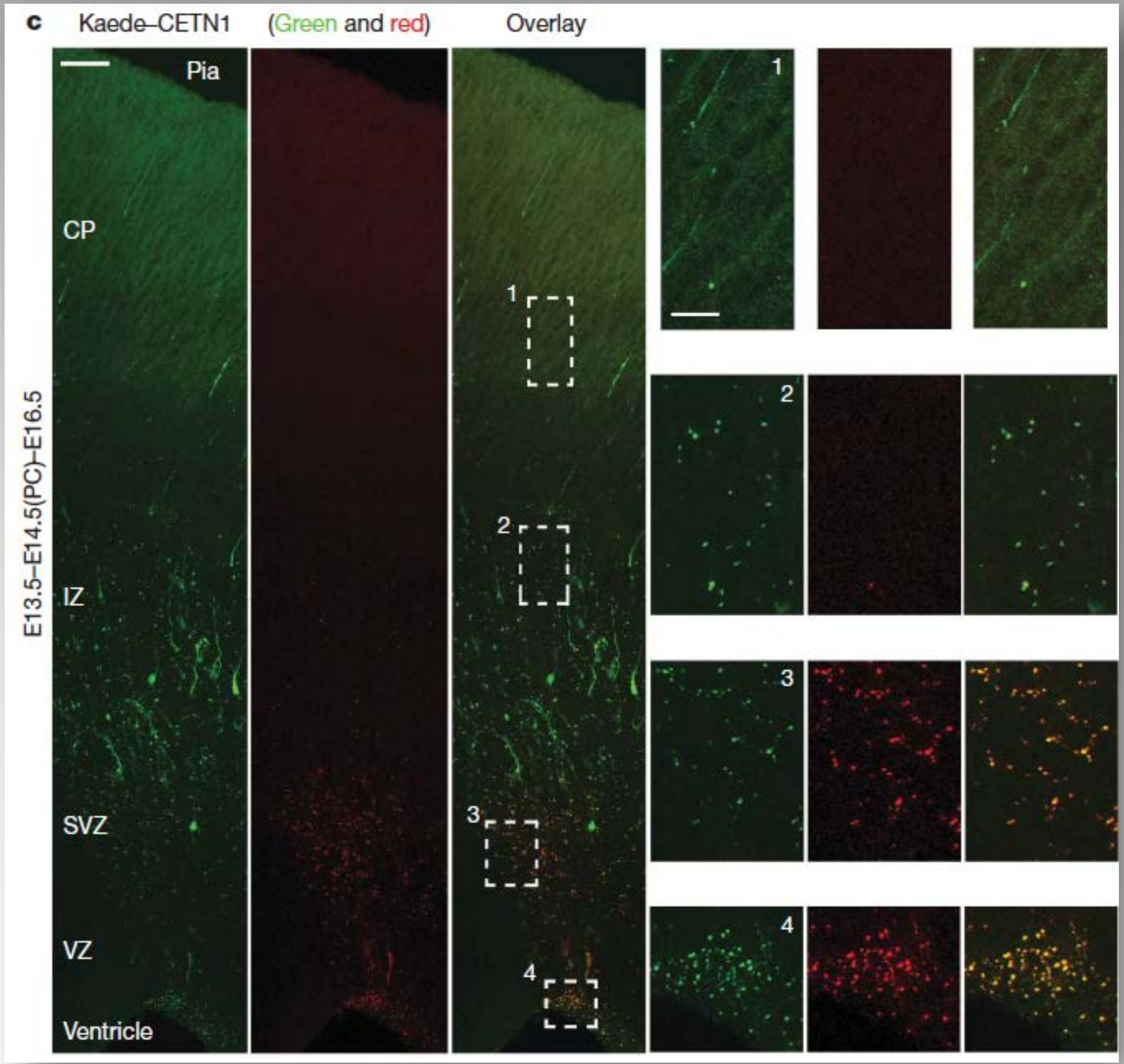
Is required for maintenance of radial glial progenitors pool and differentiation of differentiated mature neurons

Centrosome and mice neocortex development

Asymmetric centrosomes segregation is necessary for mice neocortex development



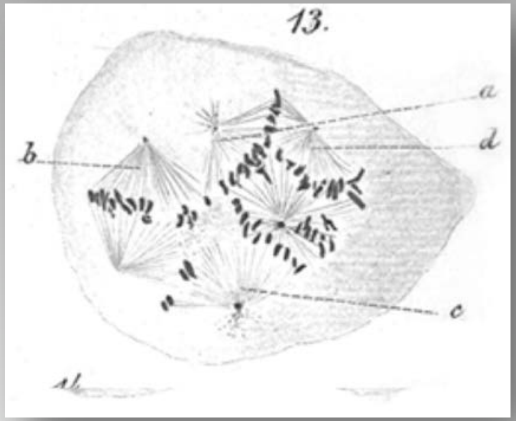




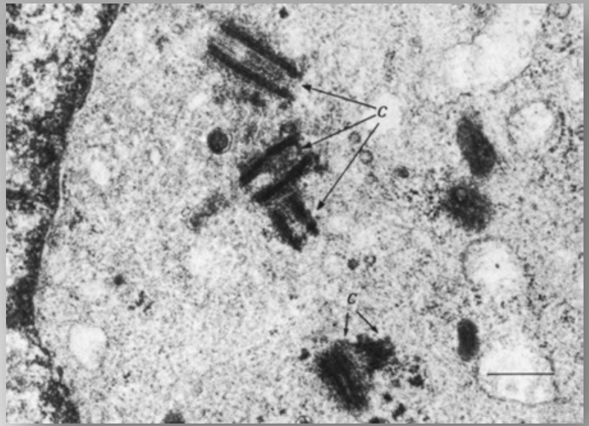
Wang et al, Nature, 2009

Centrosomes abnormalities and diseases

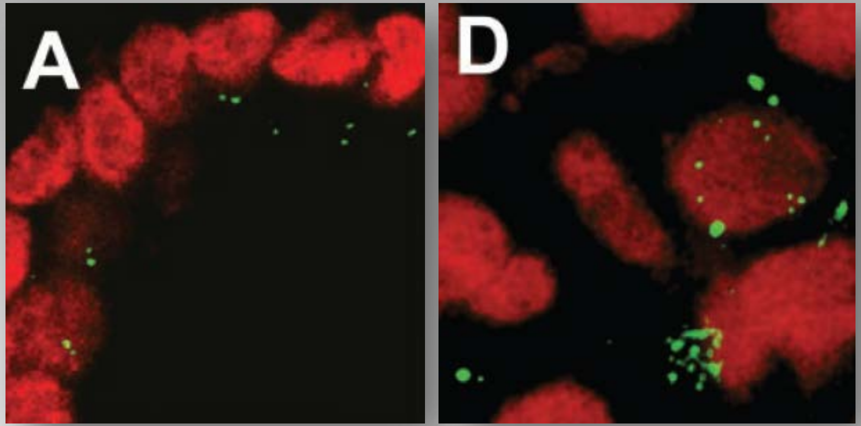
Abnormal centrosome number is extremely rare in normal tissue but very frequent in tumors (>90 % in solid tumors).



Hansemann, 1890

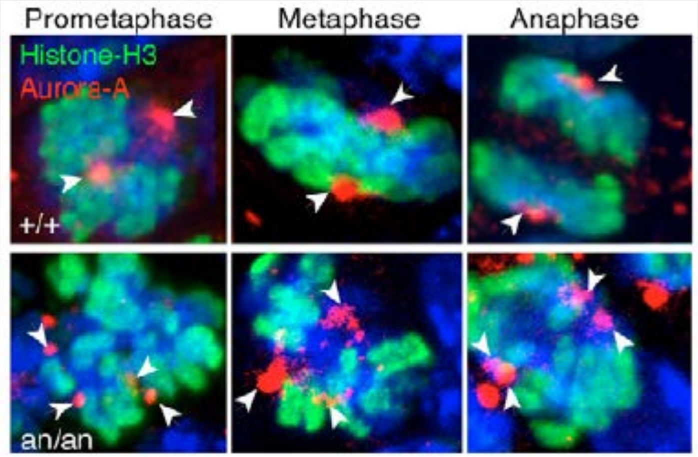


Sharp et al, J. Cell Sci., 1981

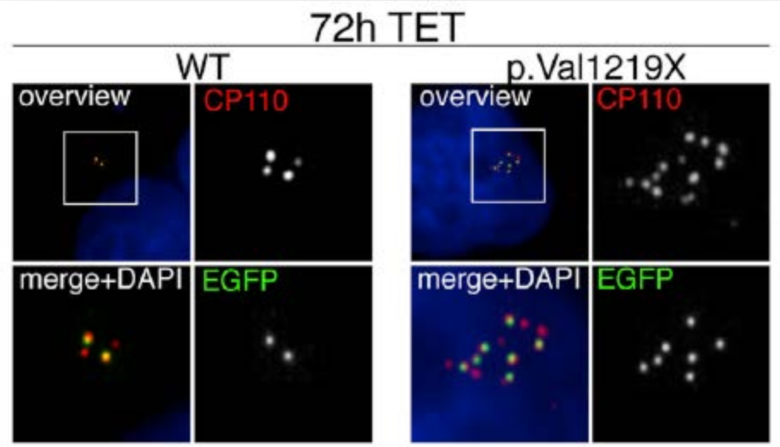


Lingle et al, PNAS, 2002

It is also observed in brain developmental defects



Lizarraga et al, Development, 2010

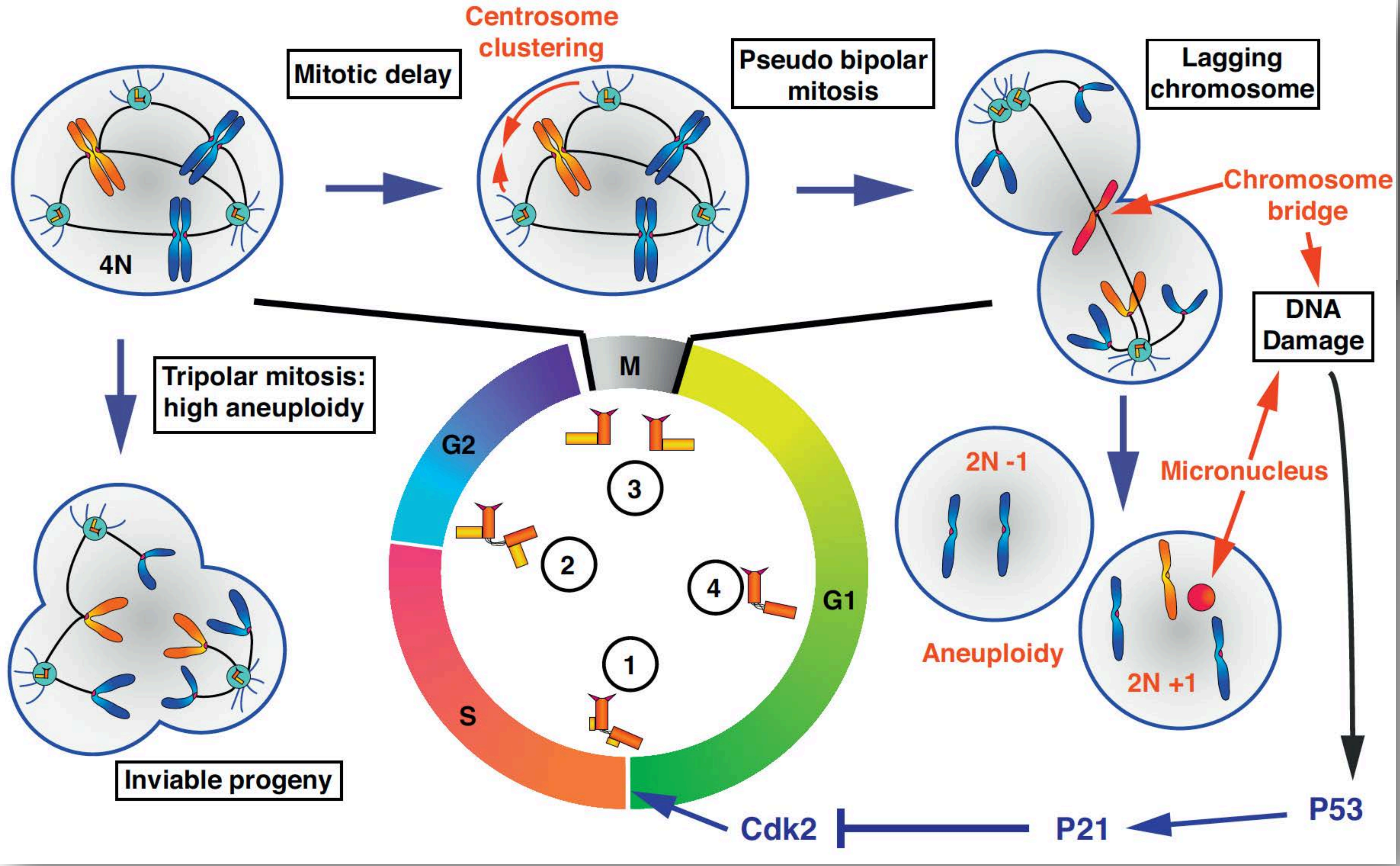


Arquint & Nigg , Current Biology, 2014

Does centrosome abnormalities account for ACD defects and disease formation?

Perturbation of centrosome homeostasis

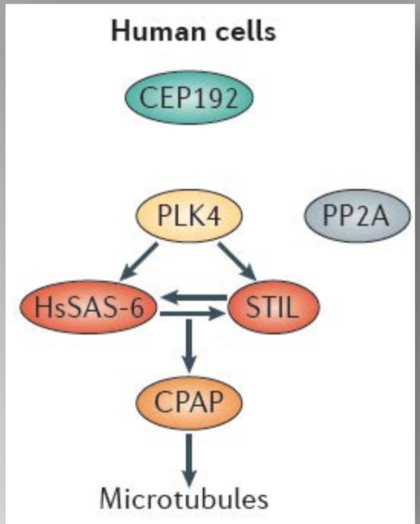
Extra centrosome generate asymmetry during cell division



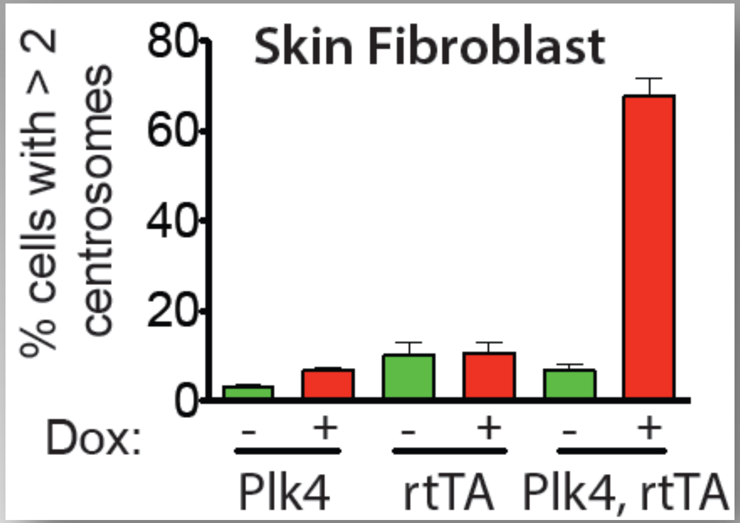
Vitre & Cleveland, Cur. Op. Cell Bio., 2012

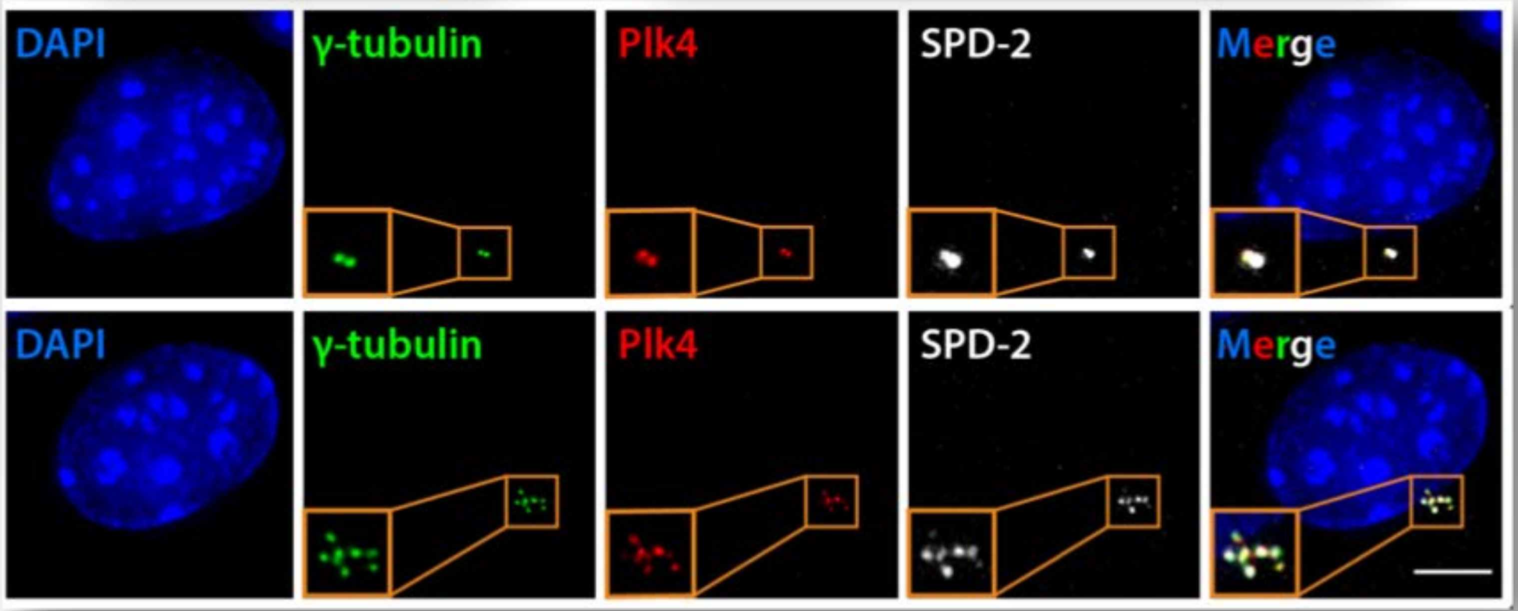
Assess the consequences of centrosome amplification

Plk4 overexpression to generate extra centrosomes



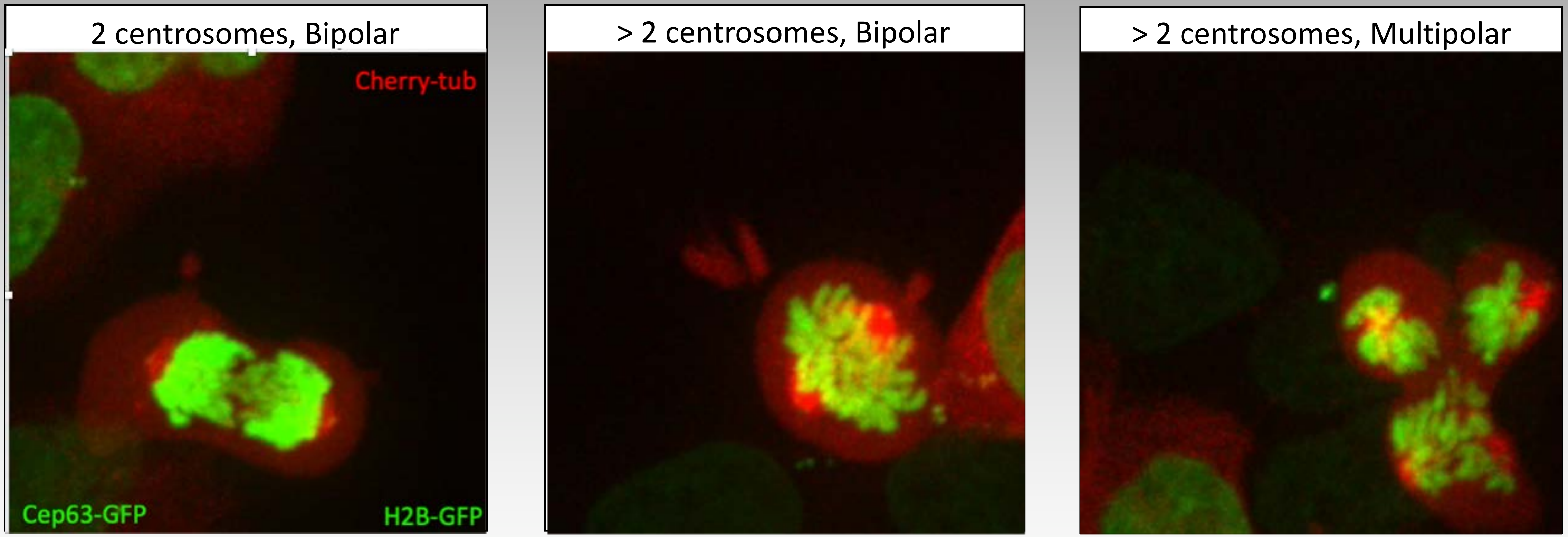
Gönczy *Nat. Rev. Mol. Cell Biol.* 2012





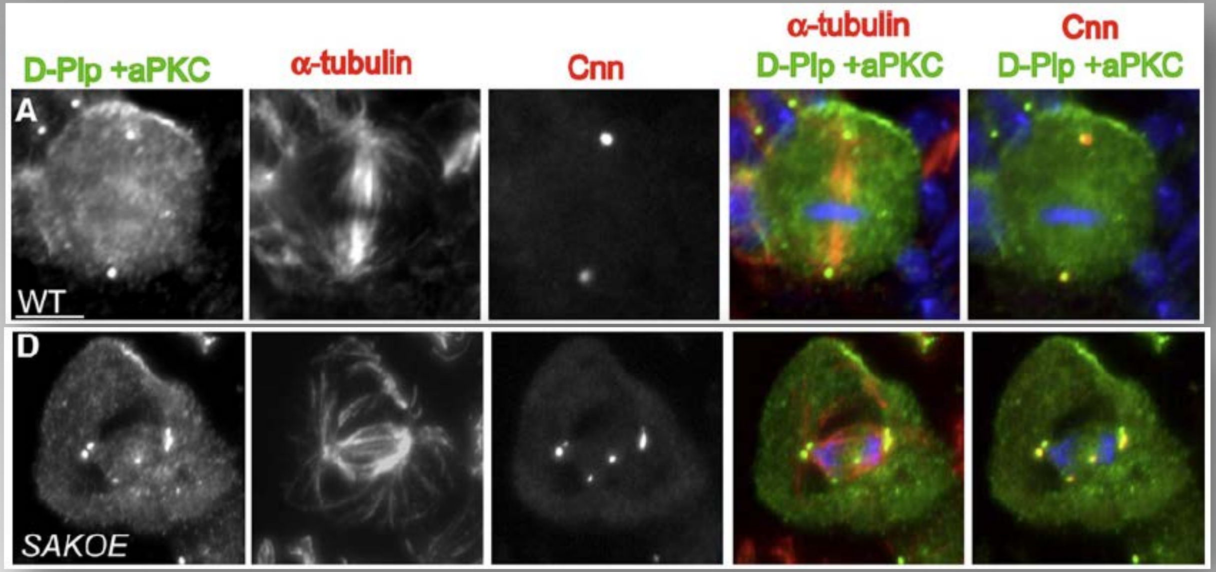
Assess the consequences of centrosome amplification

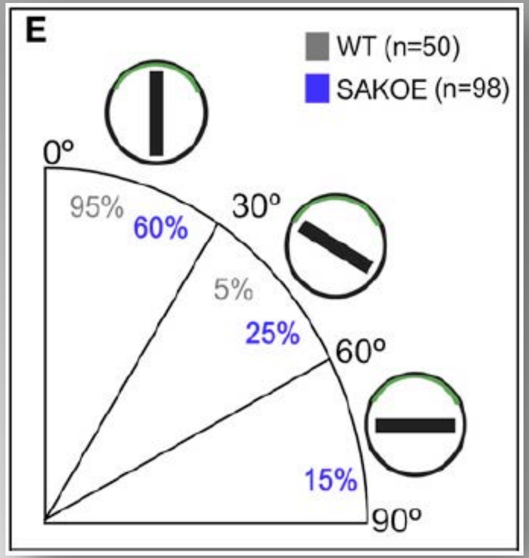
Extra centrosomes give rise to chromosome segregation errors and aneuploidy.



Extra centrosome and drosophila neuroblasts

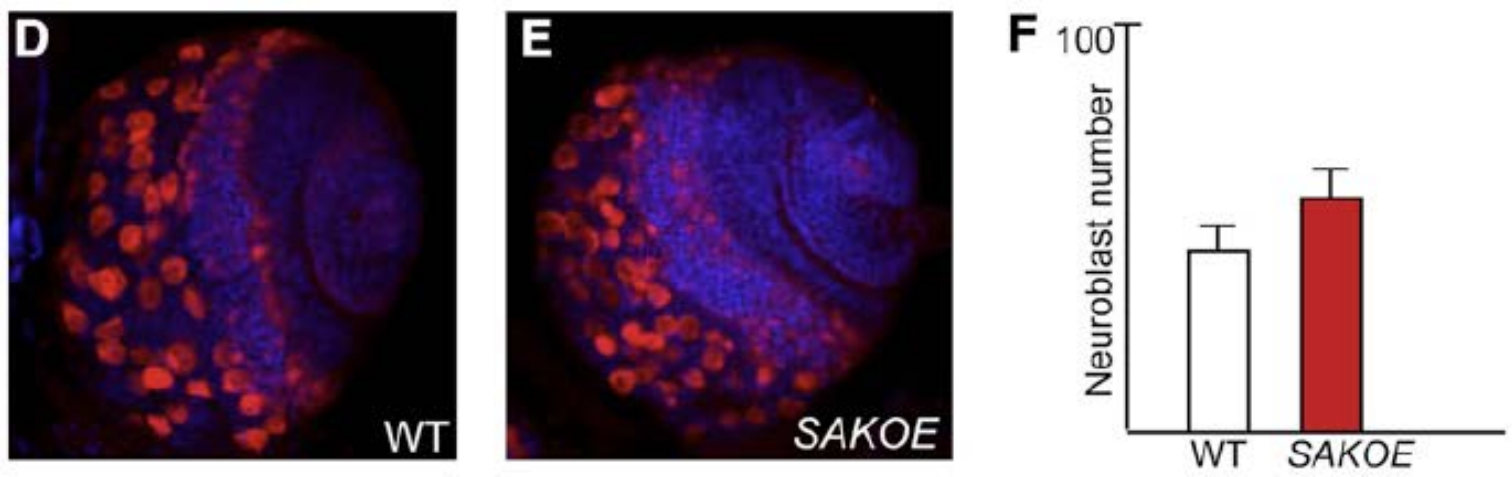
Extra centrosomes perturb ACD in fly neuroblasts





Basto et al, *Cell.* 2008

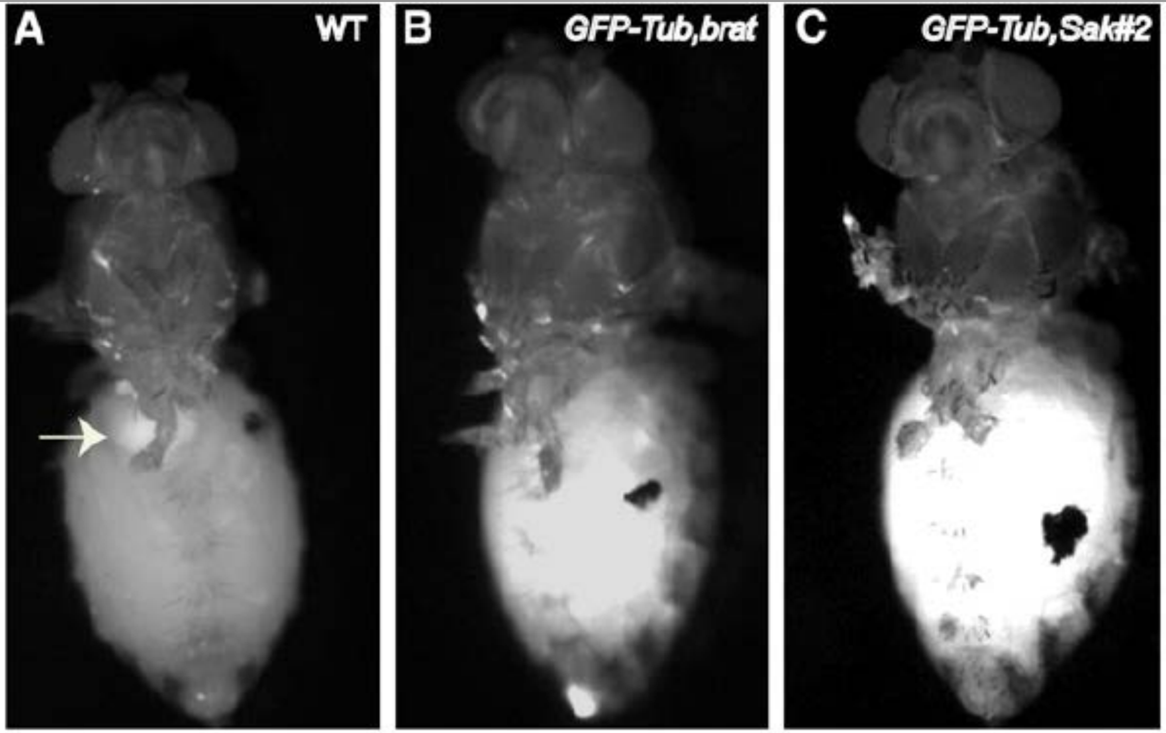
Resulting in abnormal proliferation of the neuroblasts

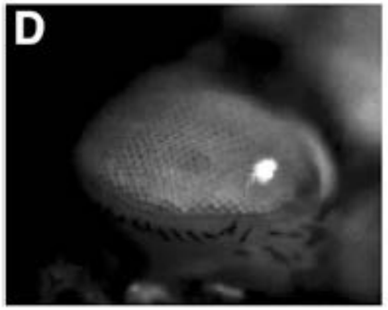


Basto et al, *Cell.* 2008

Extra centrosome can drive cancer in drosophila

Brain transplantation leads to tumor development



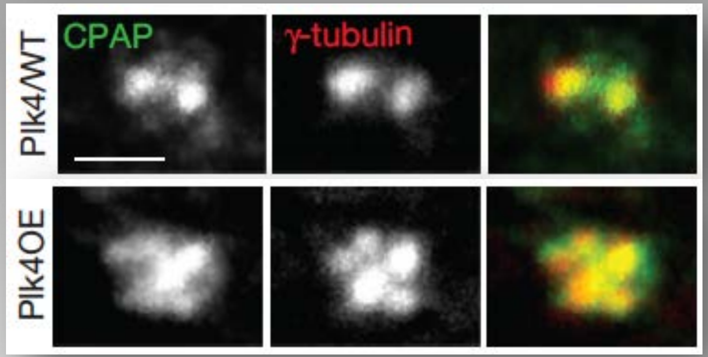


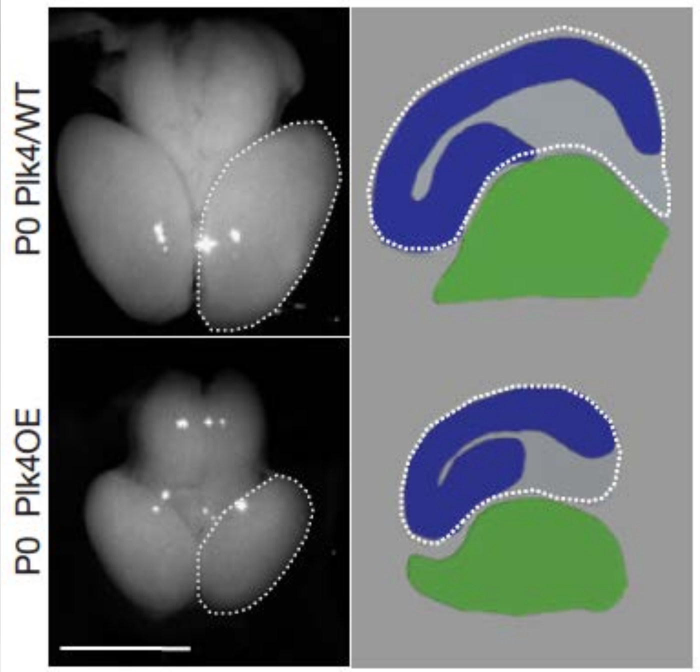
E	GFP-Tub WT	GFP-Tub *brat*	GFP-Tub *Sak#2*	GFP-Tub *Sak#1*
n° of tumors	0	18	15	12
% tumor formation	----	36%	14%	20%
Hosts injected	90	50	104	60

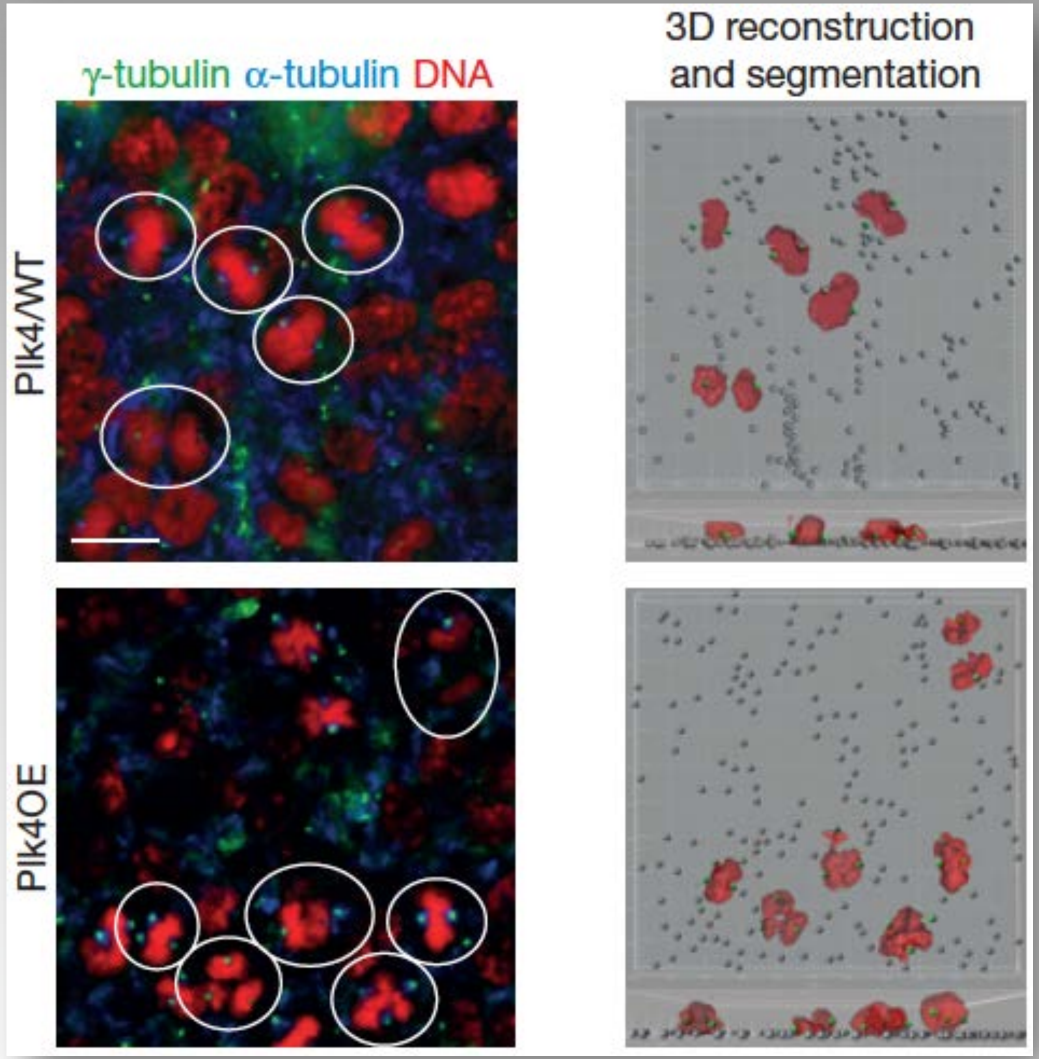
Basto et al, *Cell.* 2008

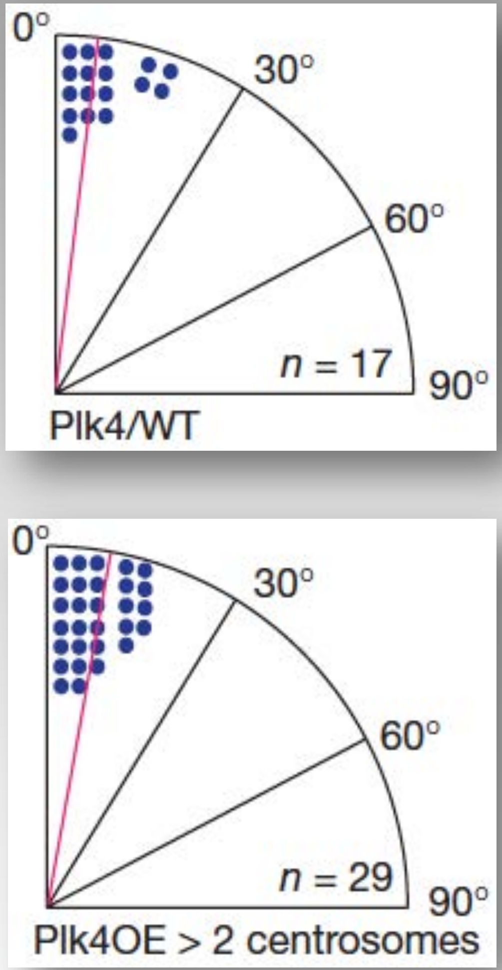
Extra centrosome and brain development in mice

Extra centrosomes lead to microcephaly but not through spindle miss-orientation





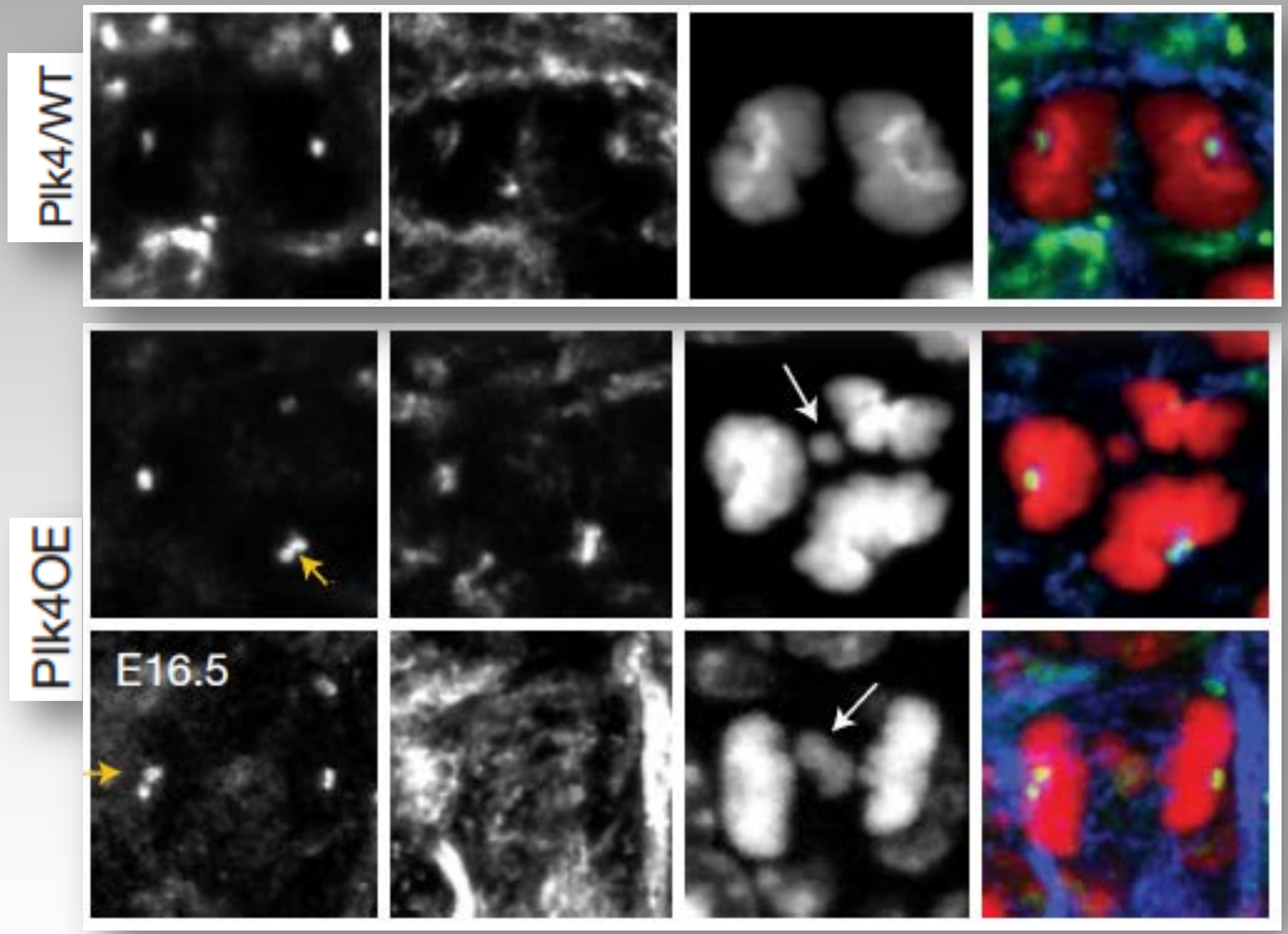




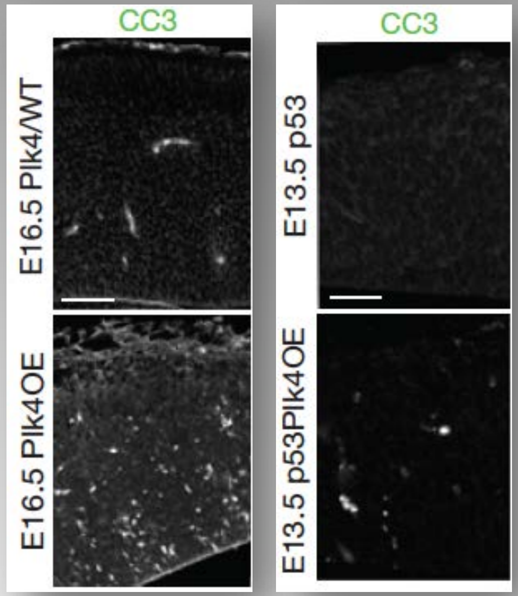
Marthiens et al, *NCB.* 2013

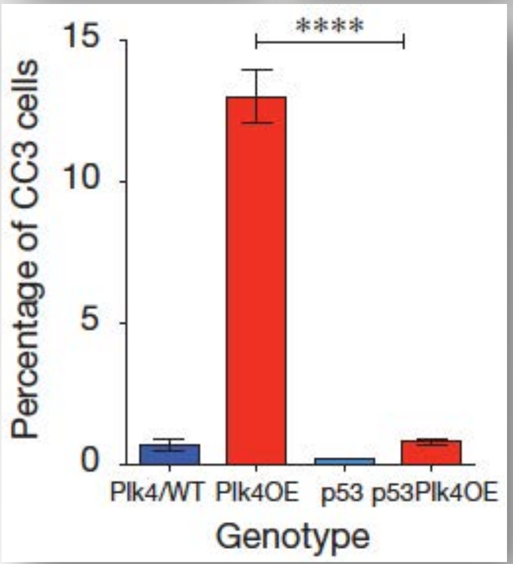
Extra centrosome and brain development in mice

Extra centrosomes generate aneuploidy, cell cycle arrest and apoptosis through p53 activation.



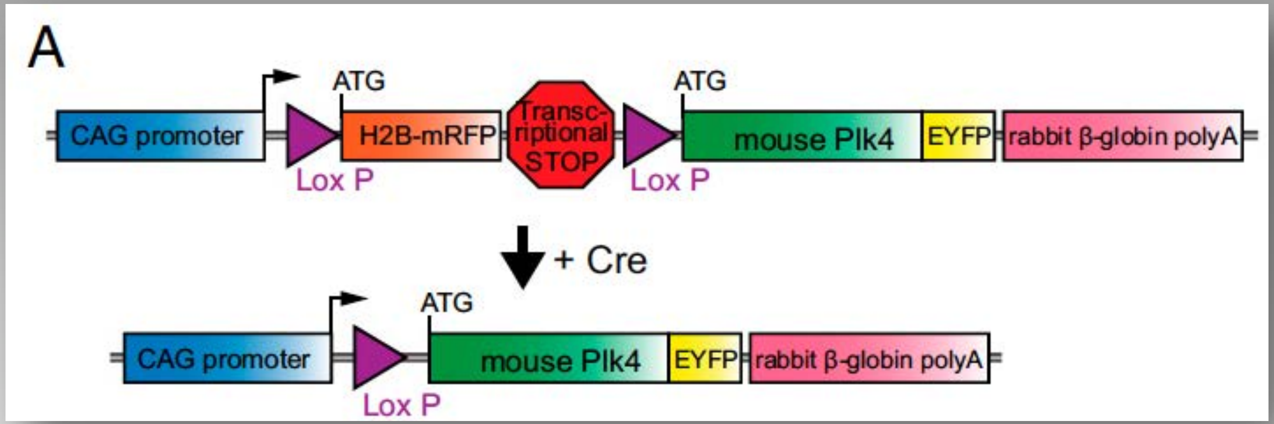
Marthiens et al, *NCB.* 2013

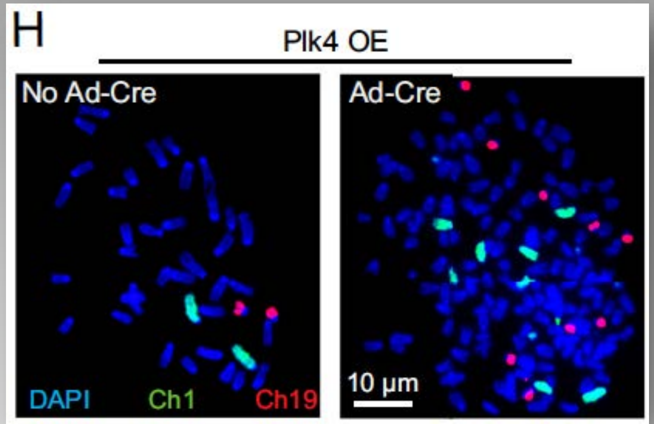


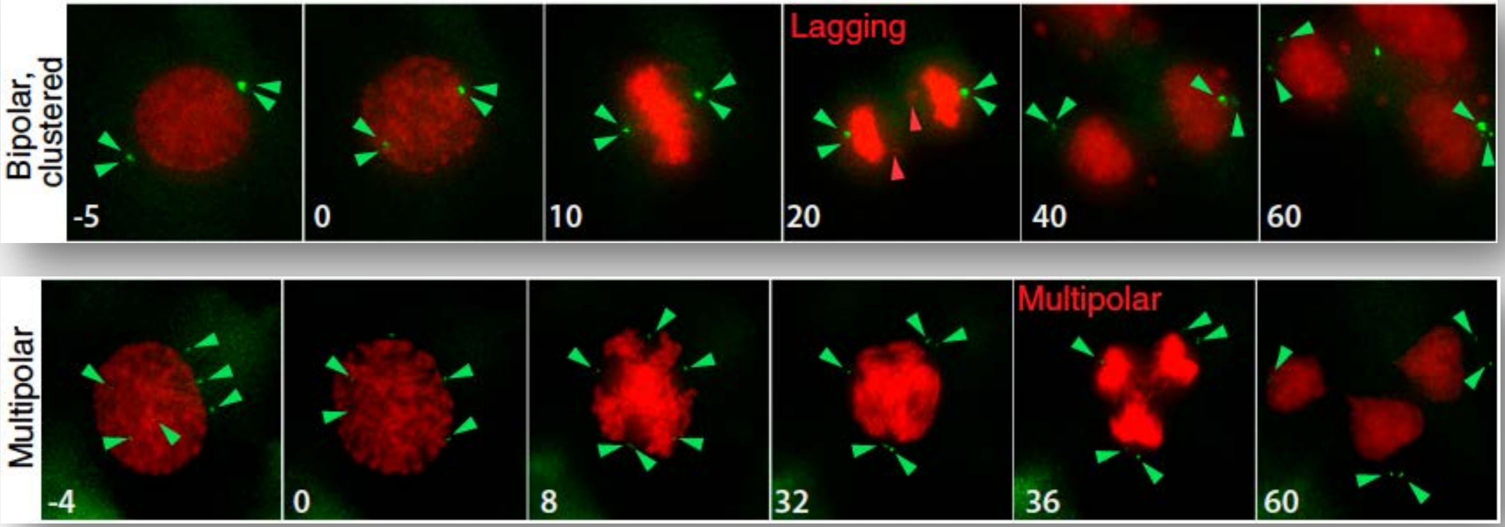


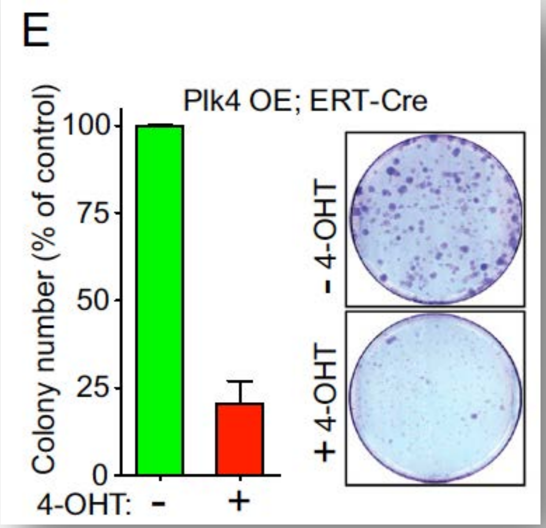
Extra centrosome and cancer in mice

Cells with extra centrosomes stop proliferating





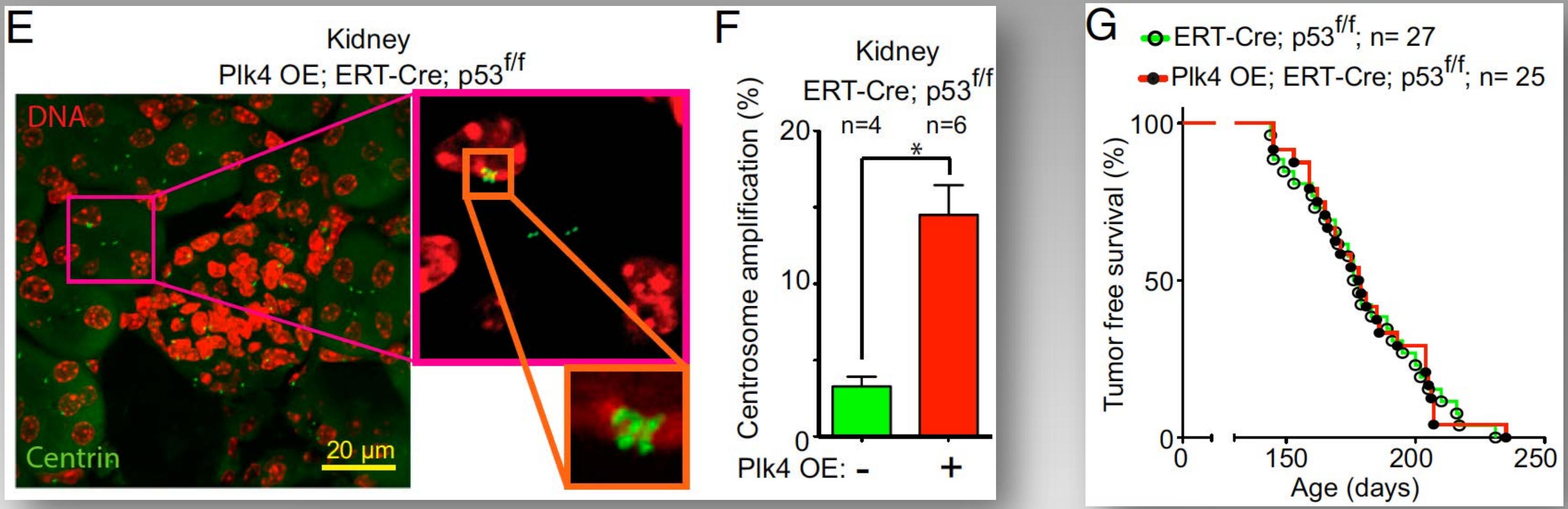




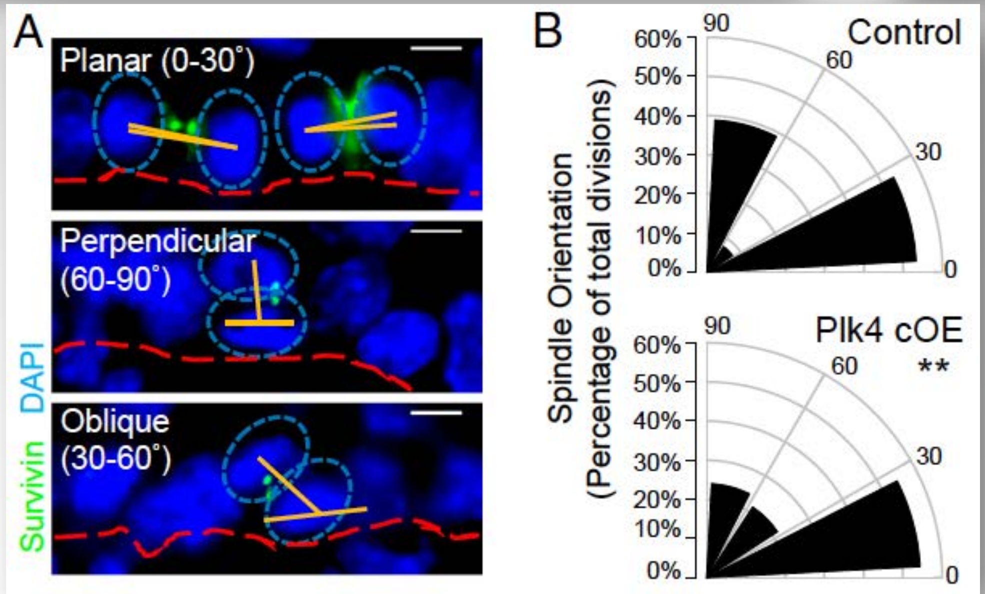
Vitre et al, *PNAS.* 2015

Extra centrosome and cancer in mice

Centrosome amplification can drive tumor development depending on context:



Vitre et al PNAS. 2015



Kulukian et al, PNAS. 2015

Extra centrosome and cancer in mice

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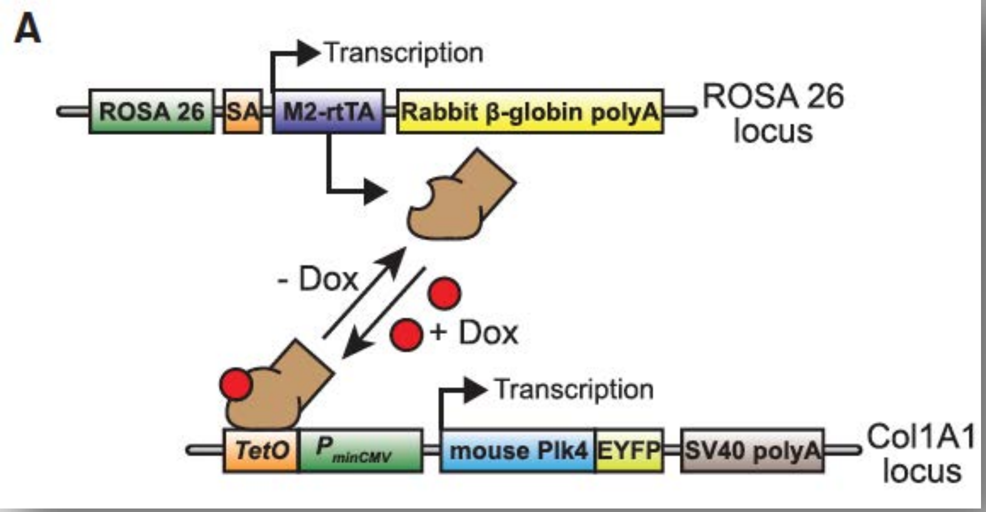
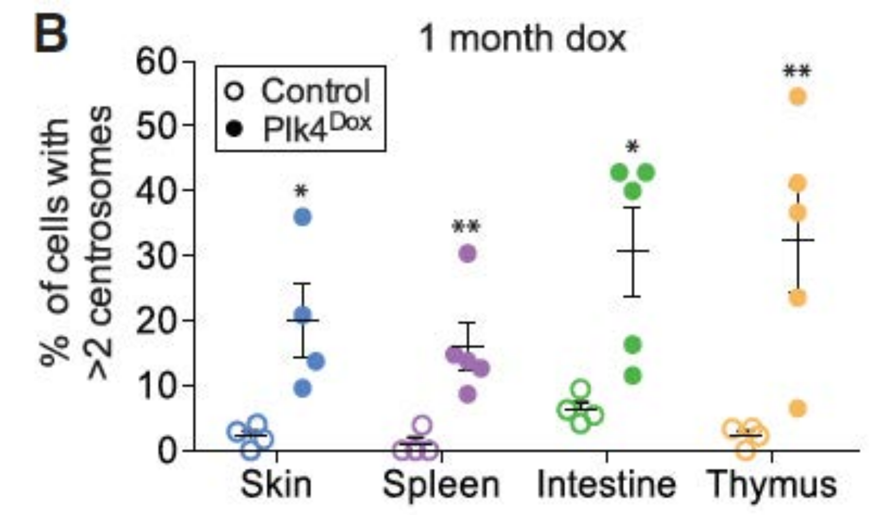
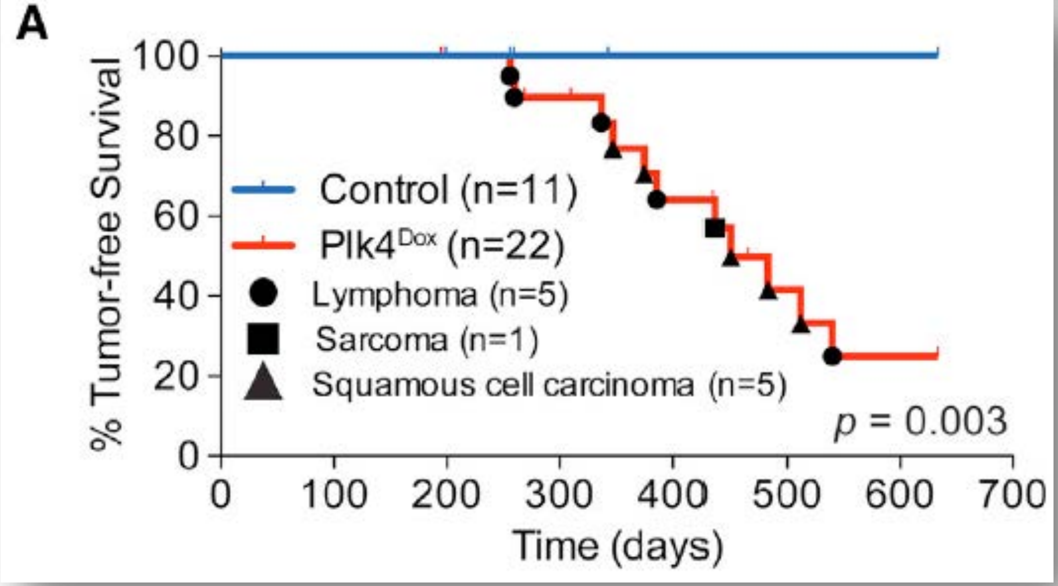
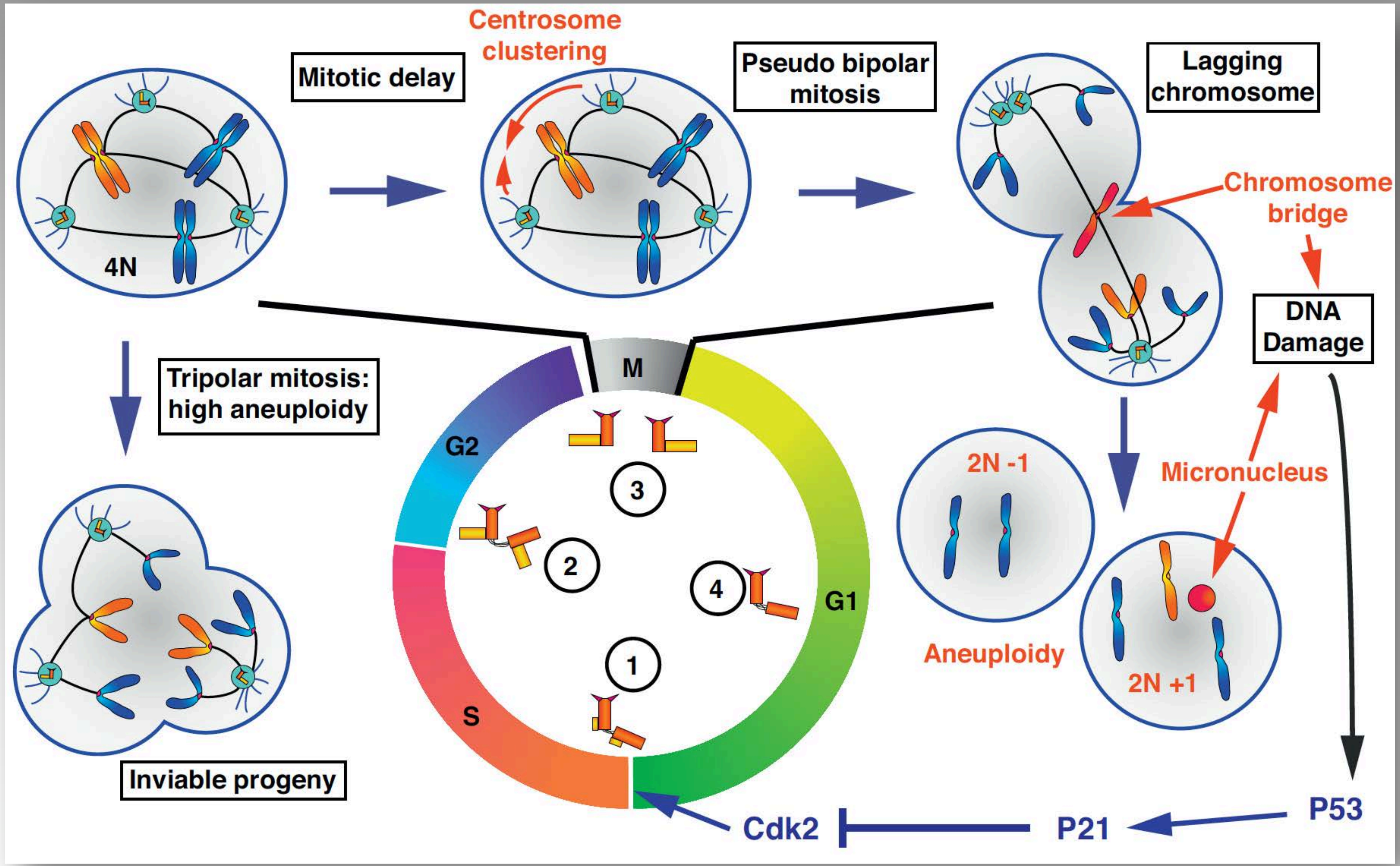






Levine et al, *Dev Cell.* 2017

Perturbation of centrosome homeostasis



Preventing clustering could be used as a strategy to selectively kill cancer cells

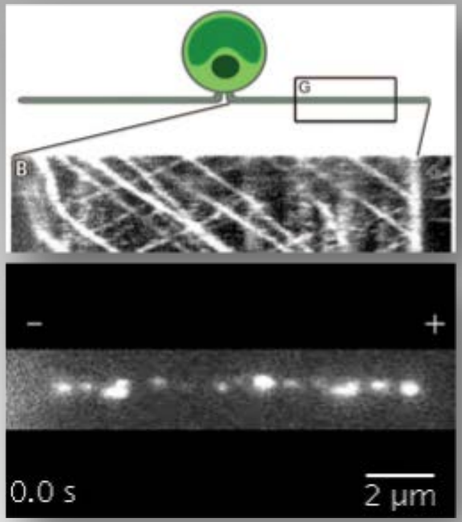
IFT machinery is required for efficient centrosome clustering in mitosis.

Centrosome, Cilia and Pathologies Team, CRBM

IFT machinery basics

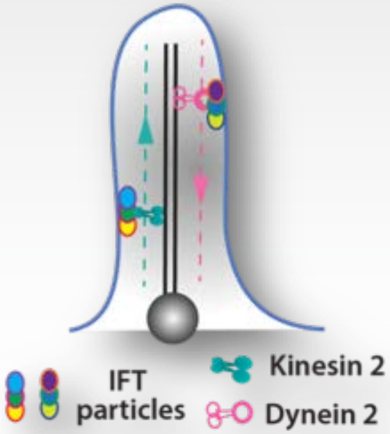
IFT is central to cilia/flagella formation and function.

Intraflagellar transport

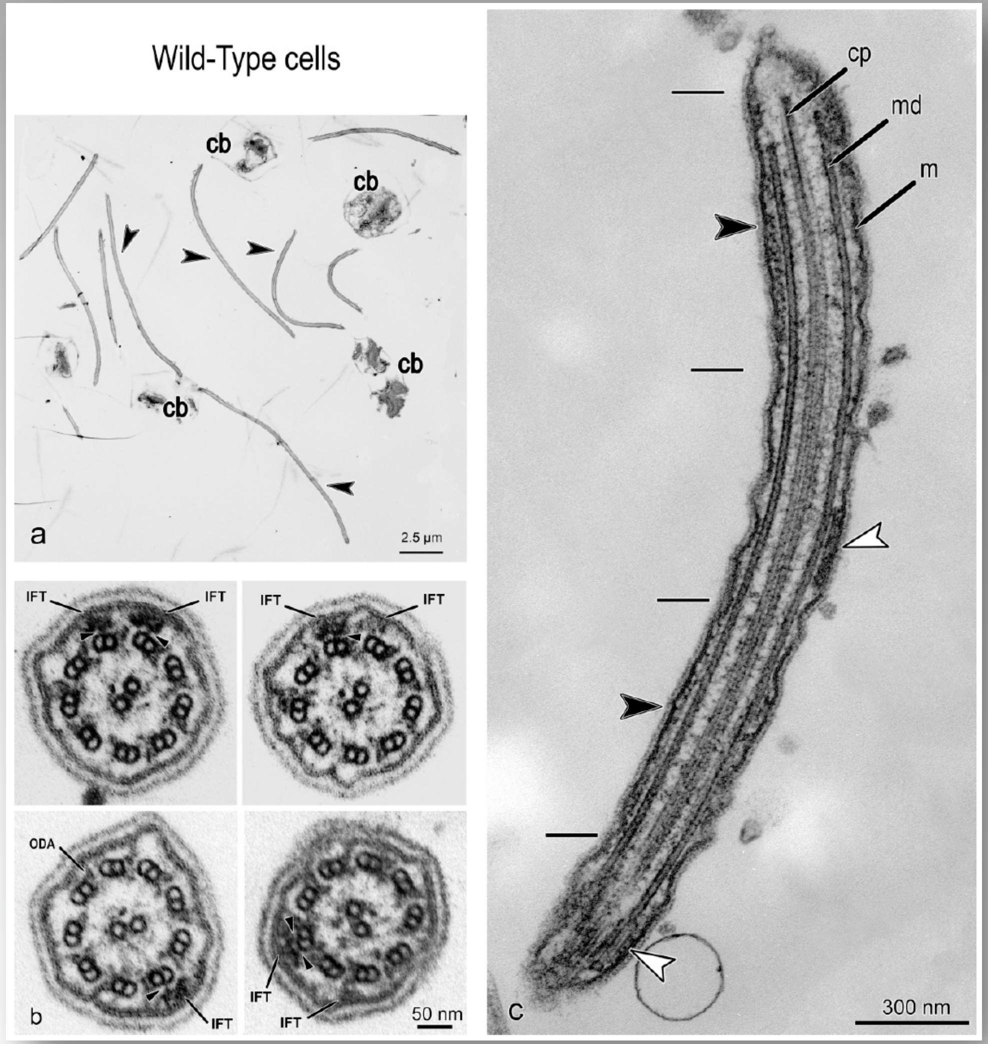


Stepanek et al, Science, 2016

Transport along axoneme MTs

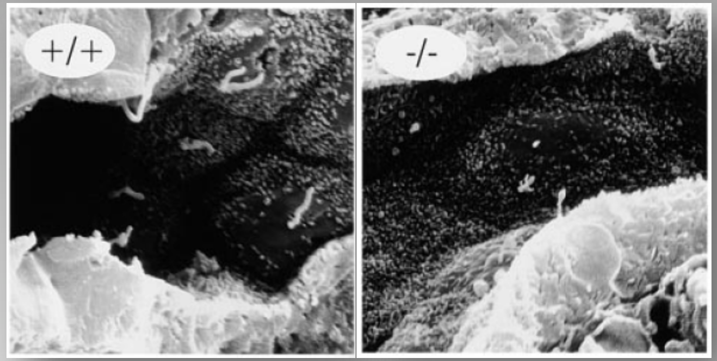


IFT trains



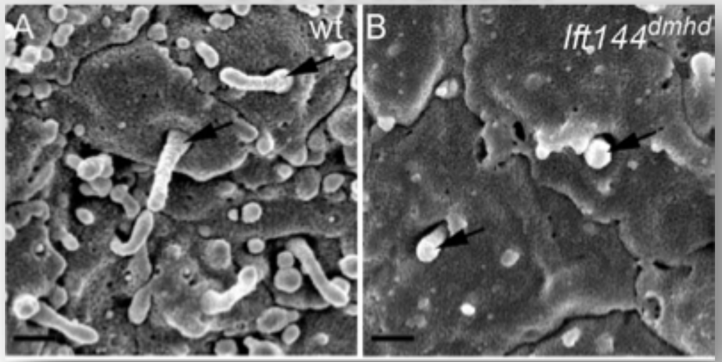
Pigino et al, JCB, 2009

IFT88 mutation



Pazour et al, JCB, 2000

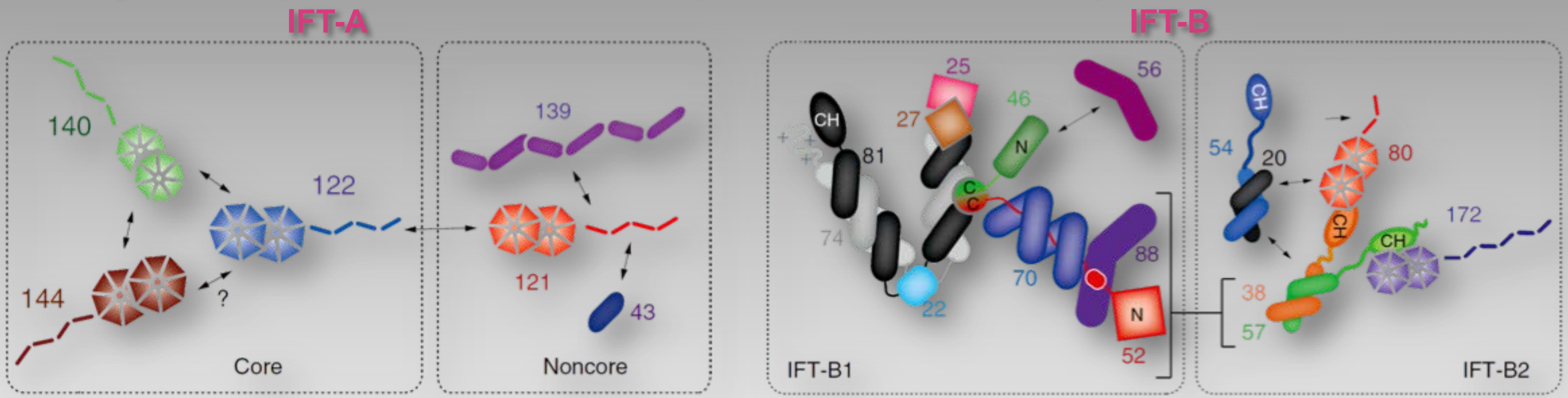
IFT144 mutation



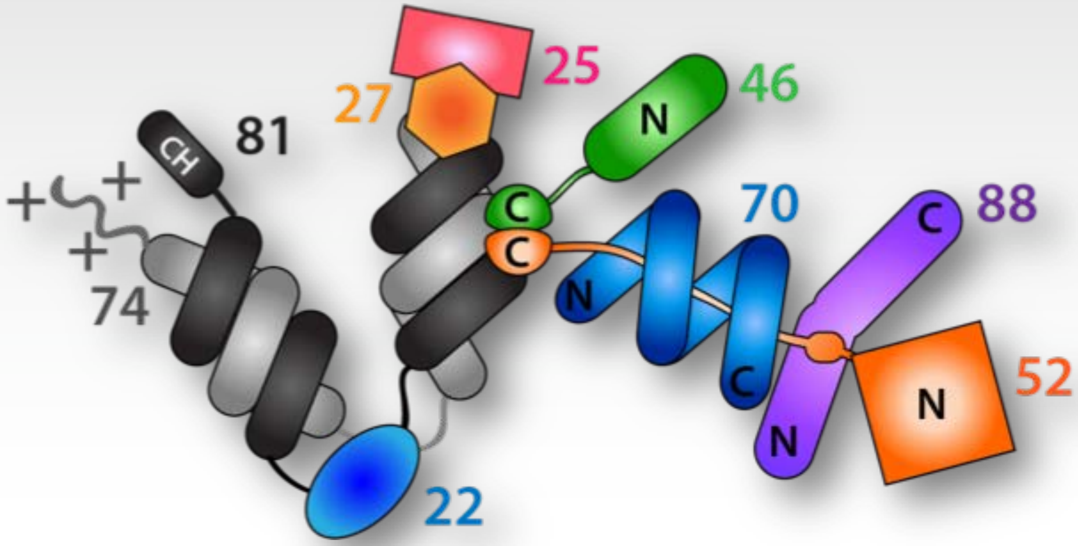
Liem et al, JCB, 2012

IFT machinery basics

IFT machinery is composed of two subcomplexes made of multiple proteins.



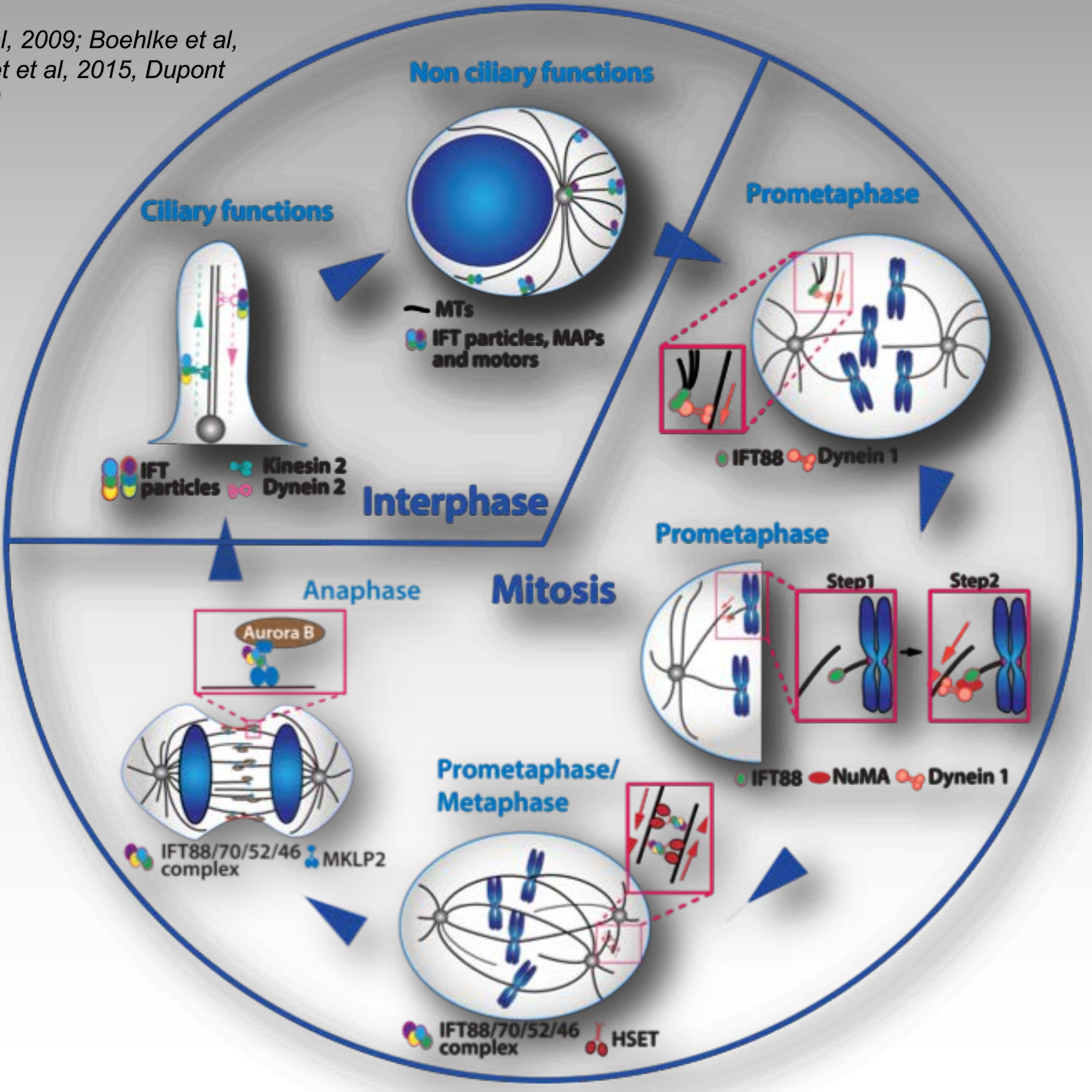
Taschner & Lorentzen, CSH perspectives Biol, 2016



Non ciliary functions of IFT proteins

IFT proteins contributes to intracellular transport and cytoskeleton organization outside cilia.

Finetti et al, 2009; Boehlke et al, 2015; Bizet et al, 2015, Dupont et al, 2019

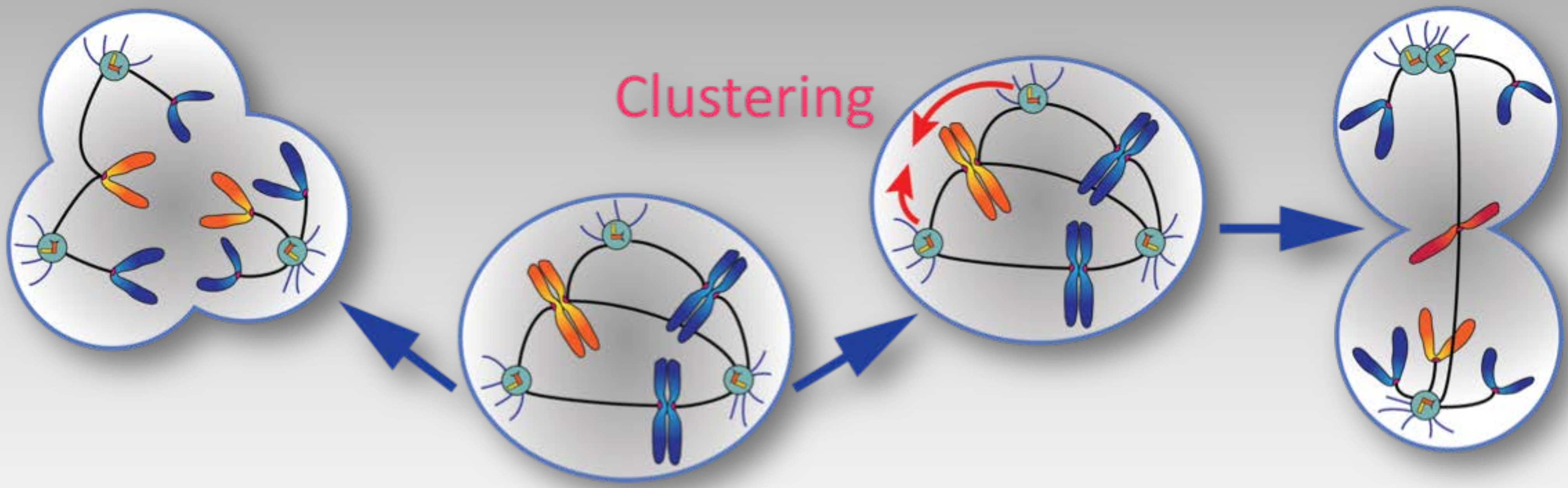


Delaval et al, 2011; Taulet, Douanier et al, 2019; Vitre at al, 2020; Taulet et al, 2017

Centrosome amplification and centrosome Clustering

Multipolar mitosis:
High aneuploidy, cell death

Pseudo-bipolar mitosis:
Low aneuploidy, cell survival



Quintyne *et al,* Science, 2005; Kwon *et al,* G&D, 2008; Ganem *et al,* Nature 2009

Cancer cells are particularly dependent on centrosome clustering for survival

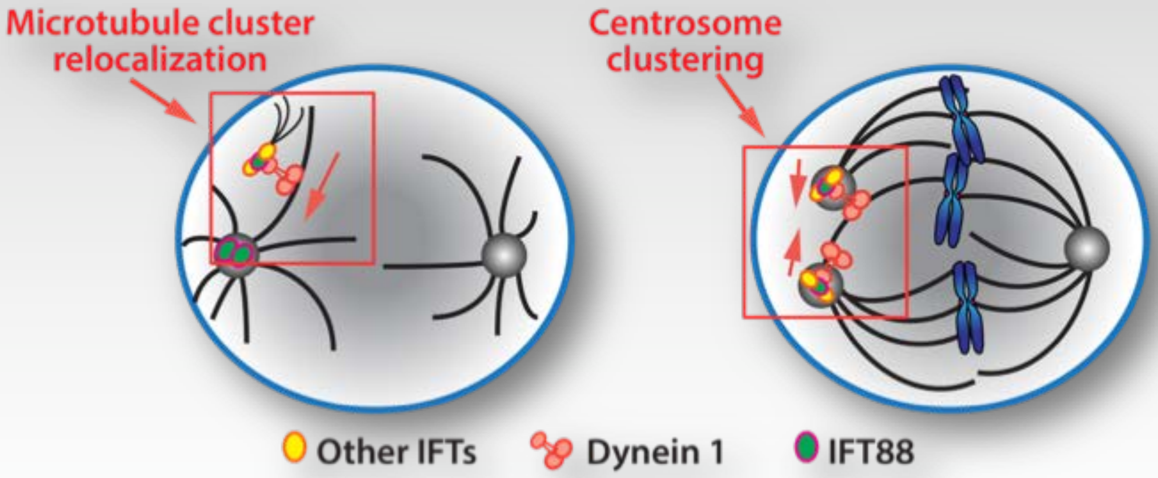
Molecular Mechanisms of centrosome clustering

- Genome wide siRNA screening identify key players involved in clustering (Kwon et al, G&D, 2008; Leber et al, Science Trans. Med, 2010):

1. Actin / MT cytoskeleton proteins; 2. Molecular motors; 3. Mitotic checkpoint

- IFT-B proteins interact with mitotic motors involved in clustering (Dynein, Mklp2) and modulate their activity (Mklp2)
Delaval *et al*, 2011; Taulet *et al*, 2017.

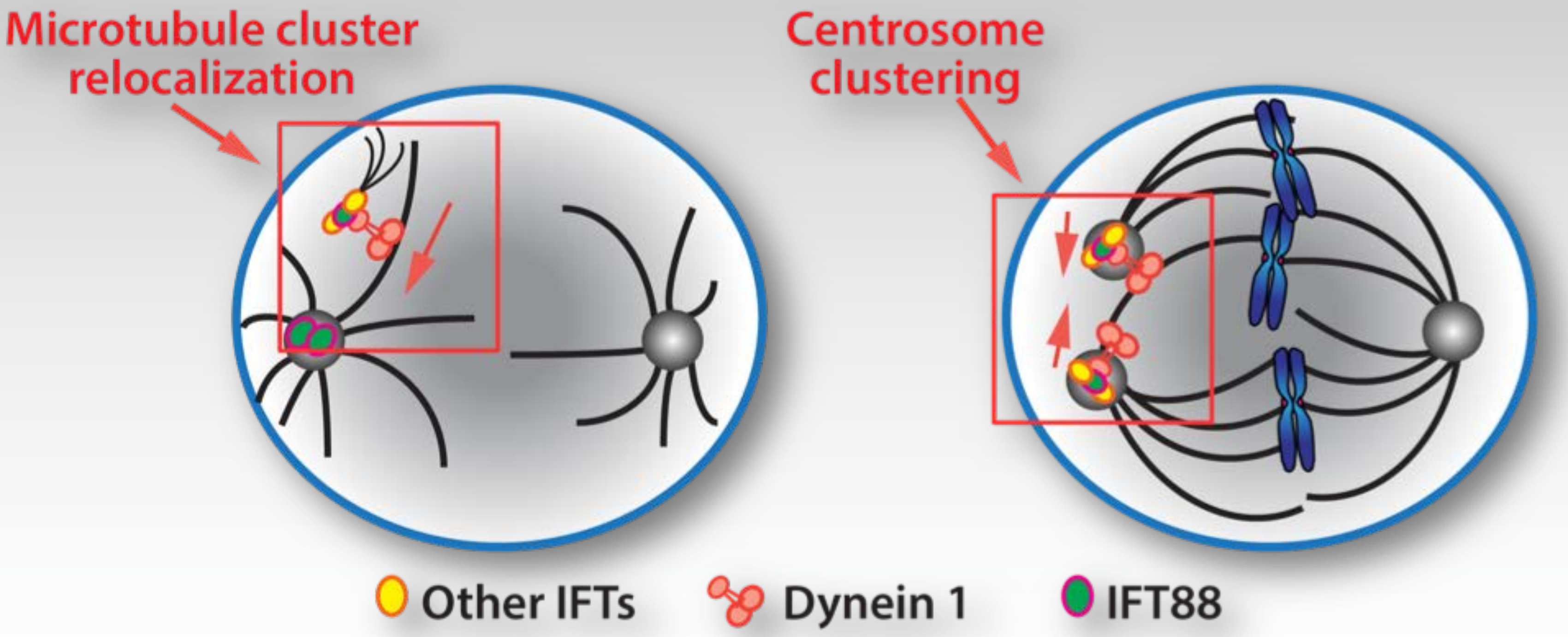
- Additional interactions identified in proteomics screening: Mklp1/IFT27 Dynein/IFT20 HSET/IFT70



Could IFTs contribute to centrosome clustering?

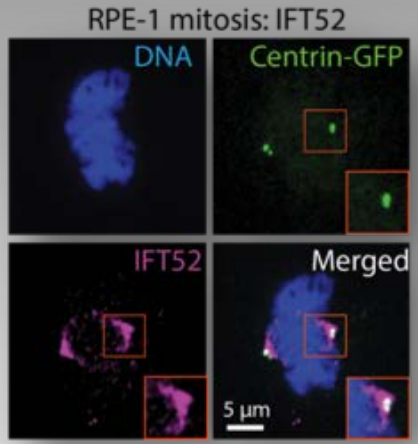
IFT proteins and centrosome clustering

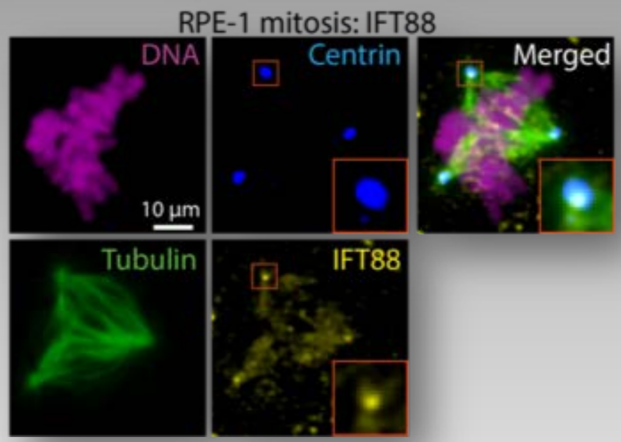
- Does IFT machinery proteins contribute to centrosome clustering?
- Is this effect mediated through mitotic motors?
- Does cancer cells relies on IFTs for clustering and survival?

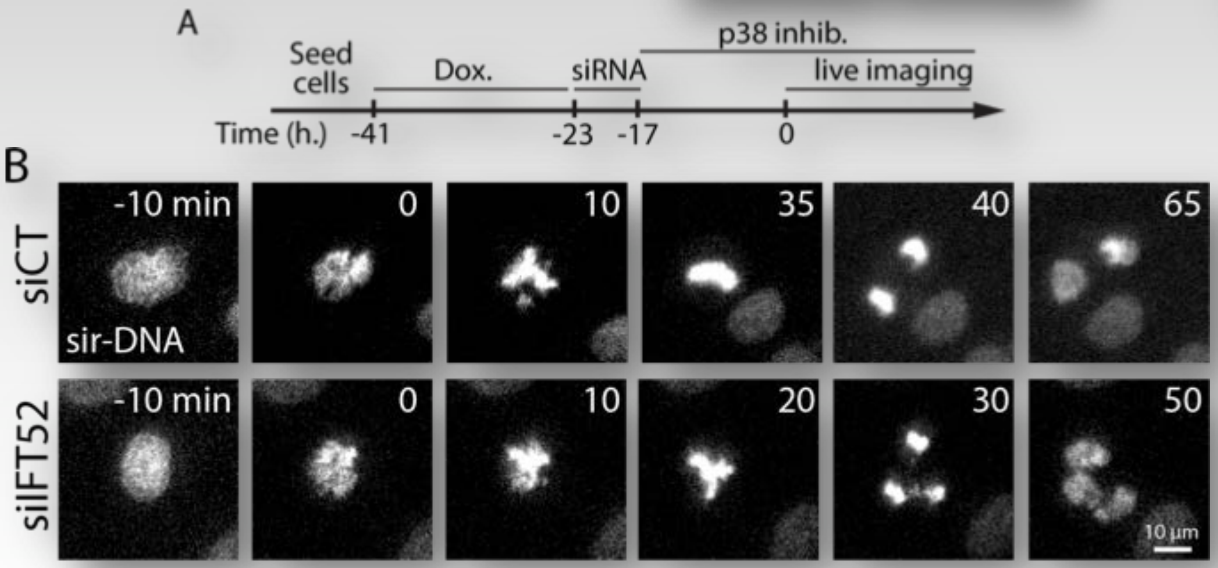


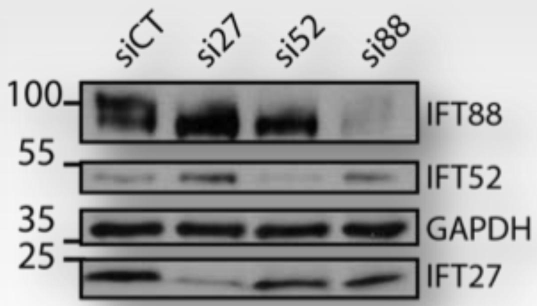
Effects of various IFT depletion on cells with extra centrosomes

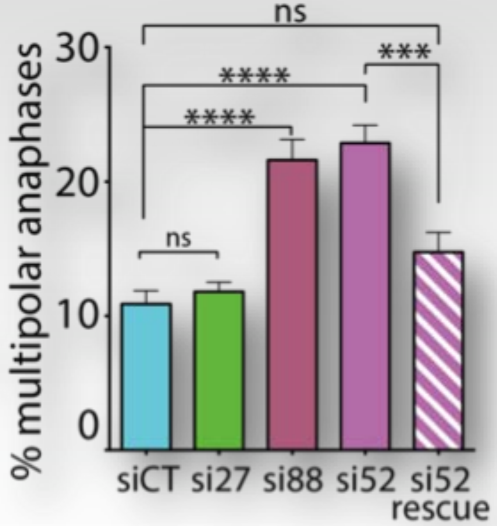
Centrosome amplification is induced in RPE-1 using Doxycycline inducible Plk4 overexpression (>90% cells with centrosome amplification)







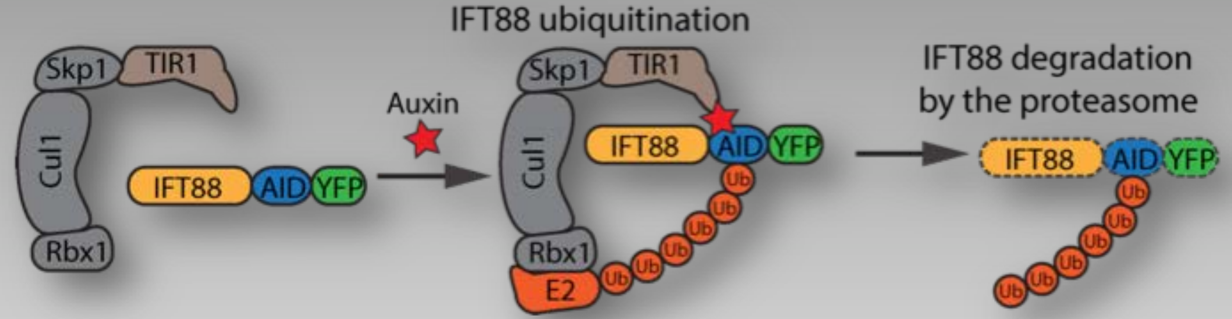


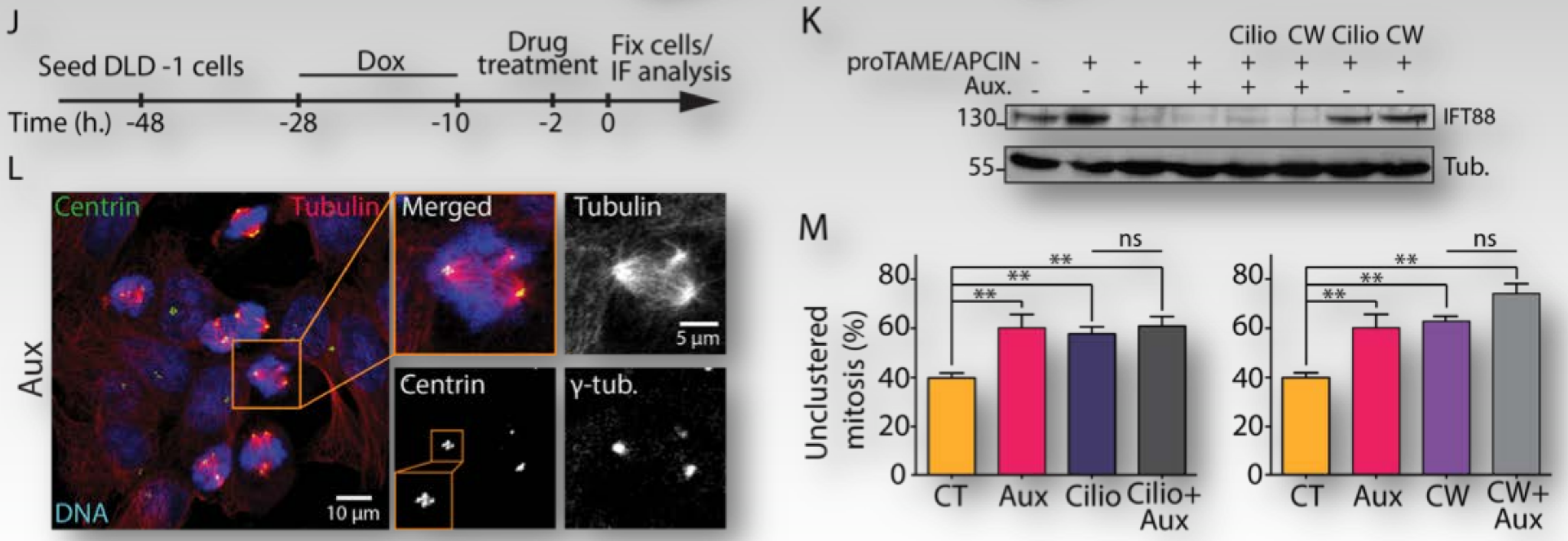


IFTs depletion impairs clustering in cells with extra centrosomes

Analysis of IFT effects on clustering in association with mitotic motors

Combination of acute, auxin induce, degradation of IFT88 in mitosis with small molecules motor inhibition in DLD-1 cells

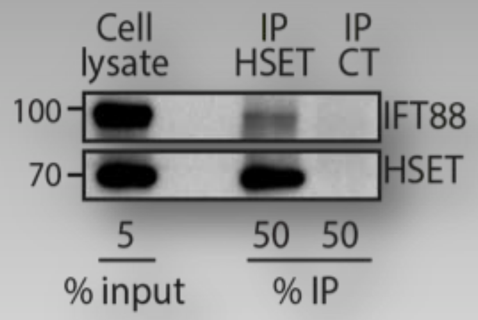


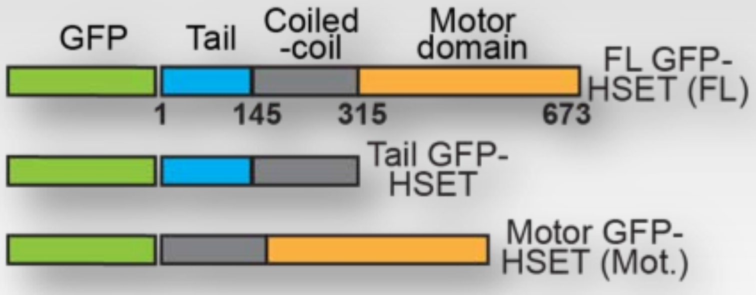


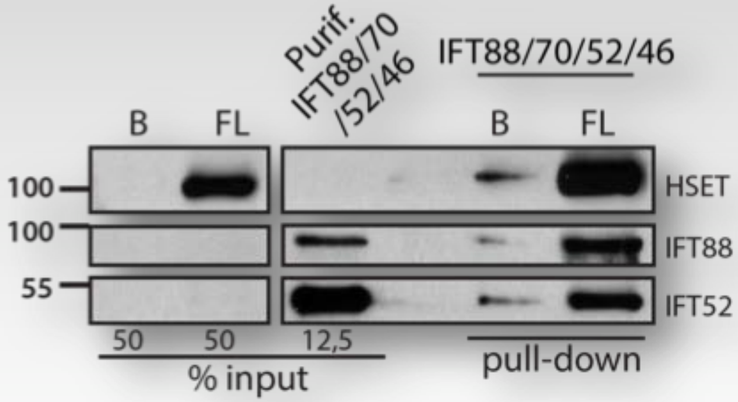
IFT88 activity on centrosome clustering could be mediated through HSET and dynein motors

Analysis of IFTs / HSET interactions

Using purified proteins to assess IFT/HSET interaction in cells and *in vitro*



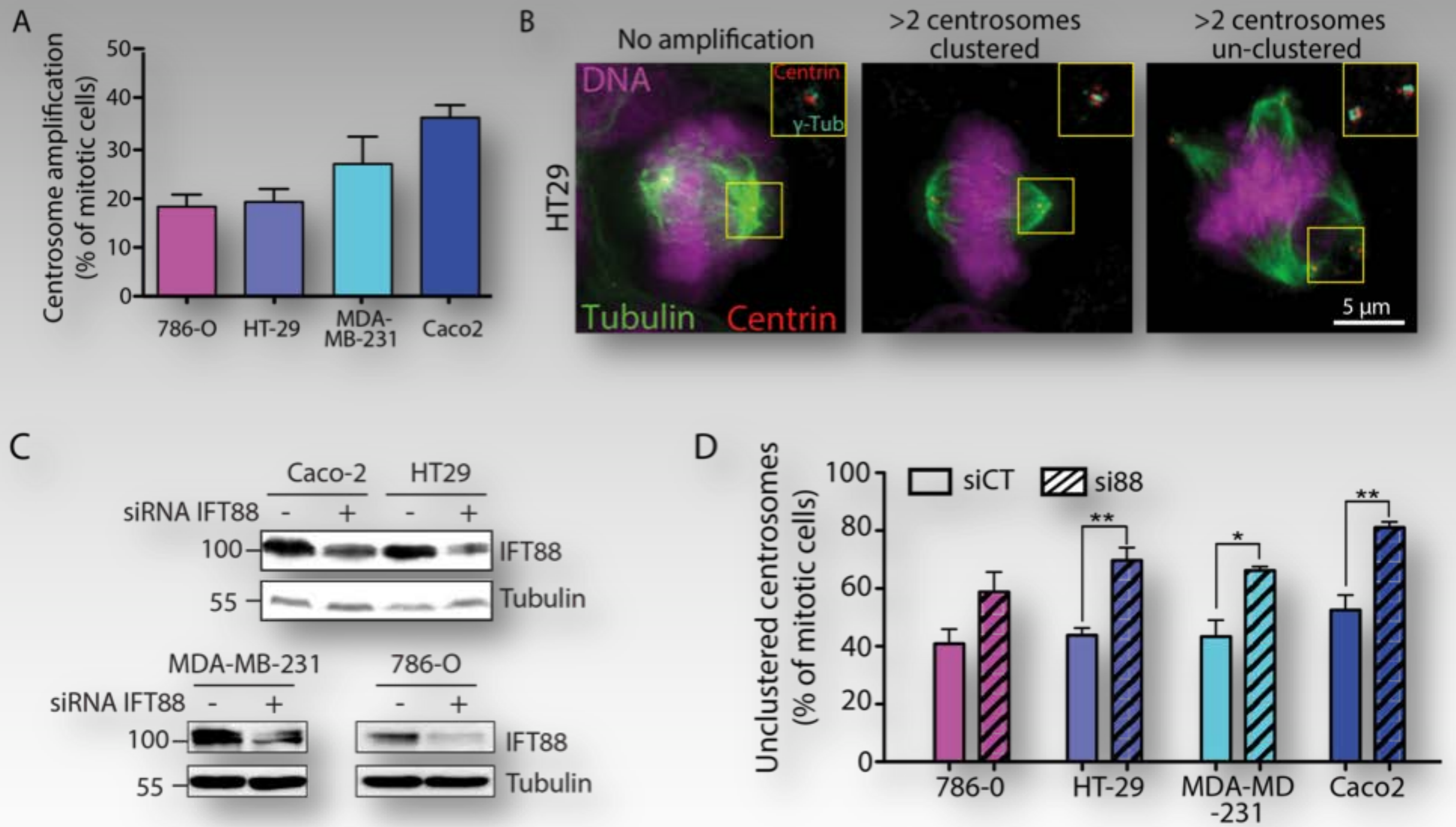




IFT-B proteins interact with HSET *in cellulo* and *in vitro*

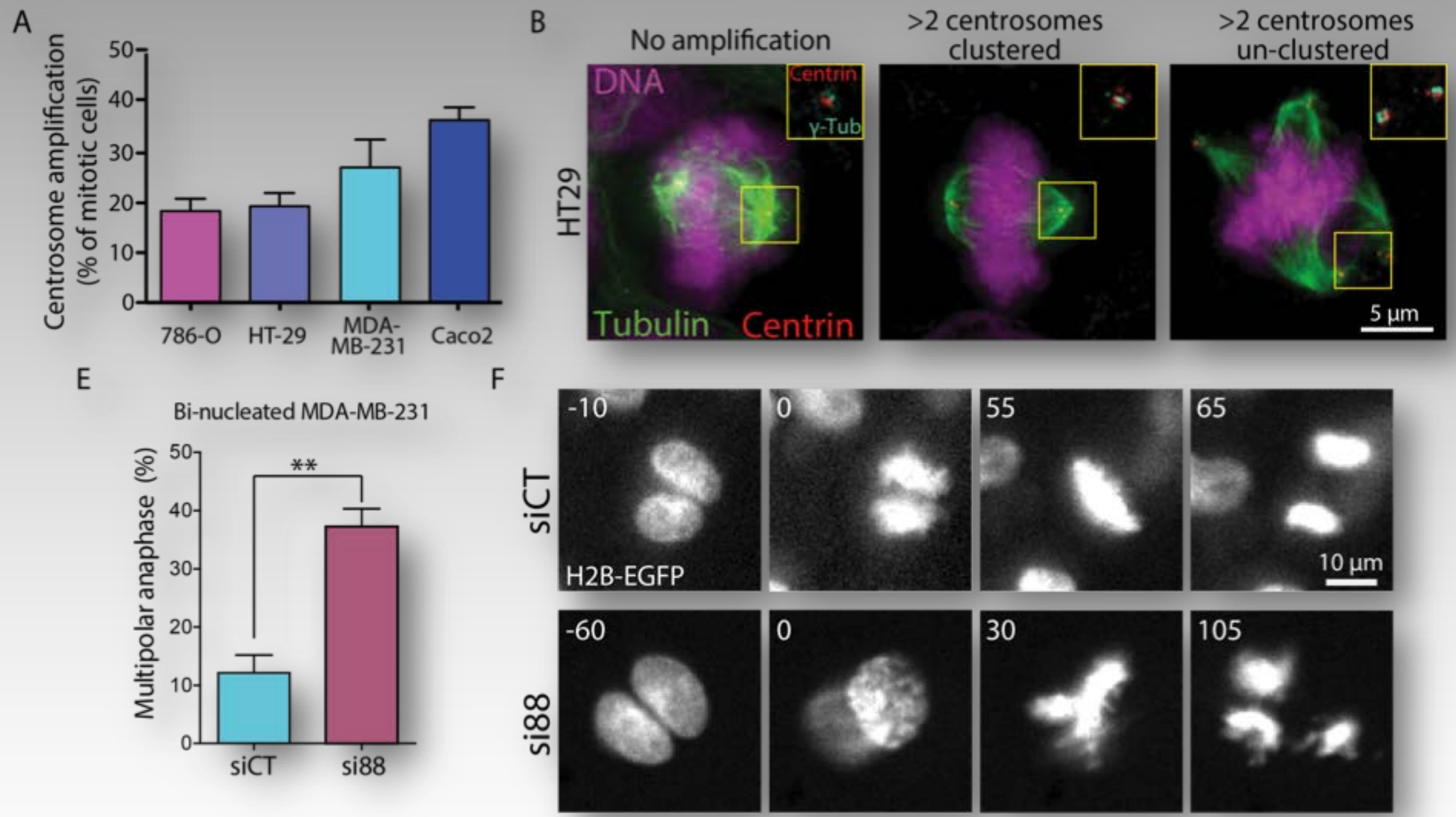
Effects of IFT88 depletion cancer cell lines naturally harboring extra centrosomes

Centrosome clustering is analyzed in cancer cell lines following IFT88 depletion.



Effects of IFT88 depletion cancer cell lines naturally harboring extra centrosomes

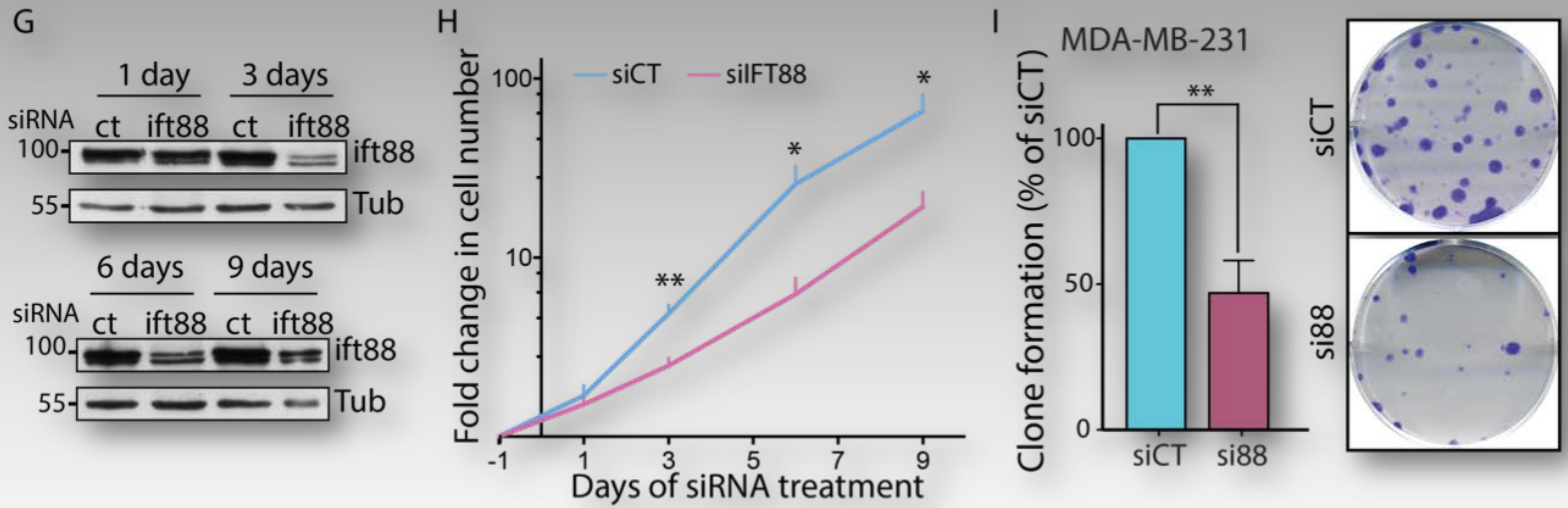
Centrosome clustering is analyzed in cancer cell lines following IFT88 depletion.



IFT88 depletion in cancer cell lines with natural centrosome amplification perturbs centrosomes clustering

Effects of IFT88 depletion cancer cell lines naturally harboring extra centrosomes

Effects of IFT88 depletion on cancer cells proliferative and clonogenic capabilities



Vitre *et al*, EMBO Reports, 2020

IFT88 depletion in cancer cell lines with natural centrosome amplification results in reduce proliferation and clonogenic capabilities

Take Home message

- ACD is necessary for tissue development and homeostasis

- ACD polarity axis is mechanically determined by centrosome positioning and mitotic spindle axis

- Centrosome can directly contribute to ACD by breaking the symmetry of cell division

- Extra centrosomes are common in cancer and microcephaly

- Forcing cells to asymmetric division can be a strategy to impair proliferative disorder such as cancer

- Targeting IFT88 could be use as a strategy to force multipolar division of cancer cells with extra centrosomes

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