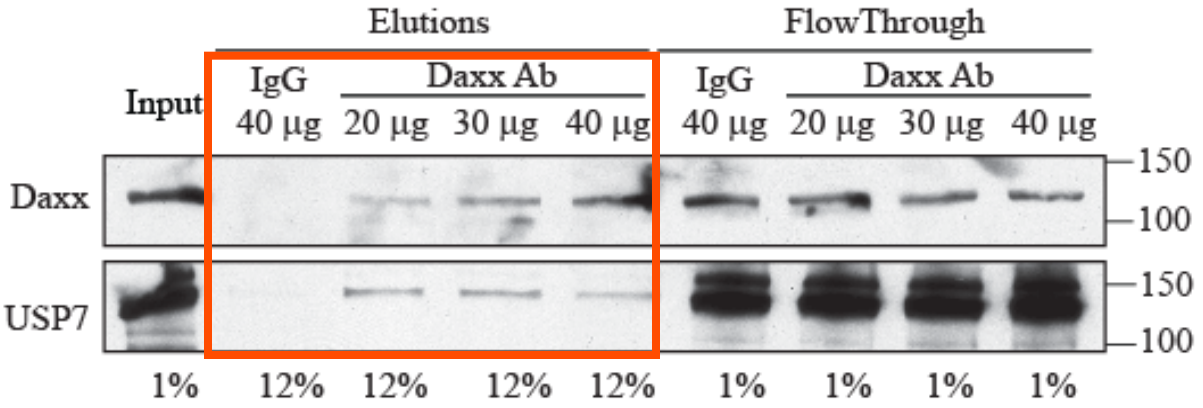
- Цитотоксическая химиотерапия рака молочной железы

- Деубиквитиназа USP7 и резистентность к антимитотической химиотерапии

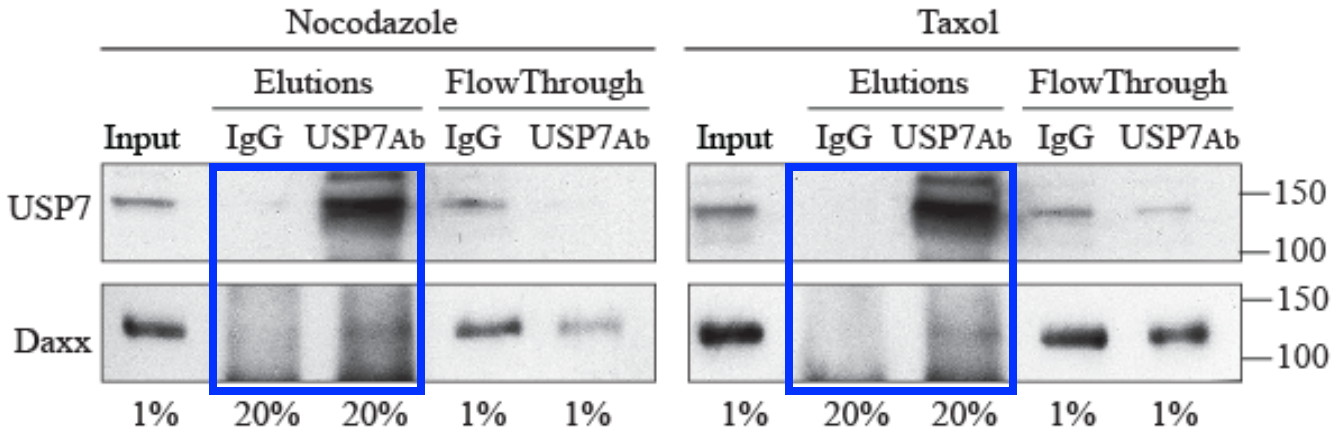
- Новые подходы в преодолении резистентности

Validation of Daxx-USP7 Interaction

Daxx IP:



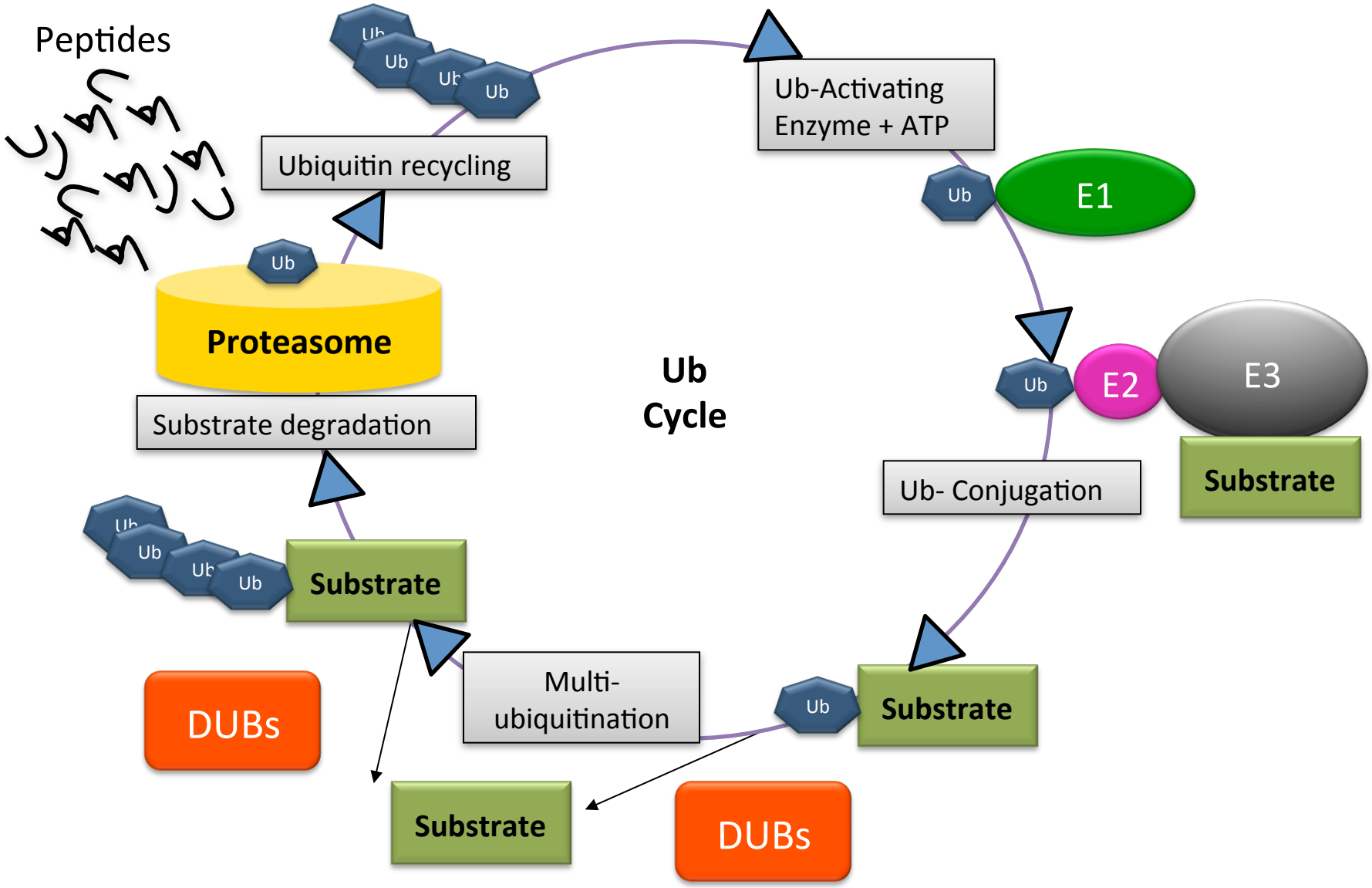
USP7 IP:



Daxx and USP7 interact in mitosis

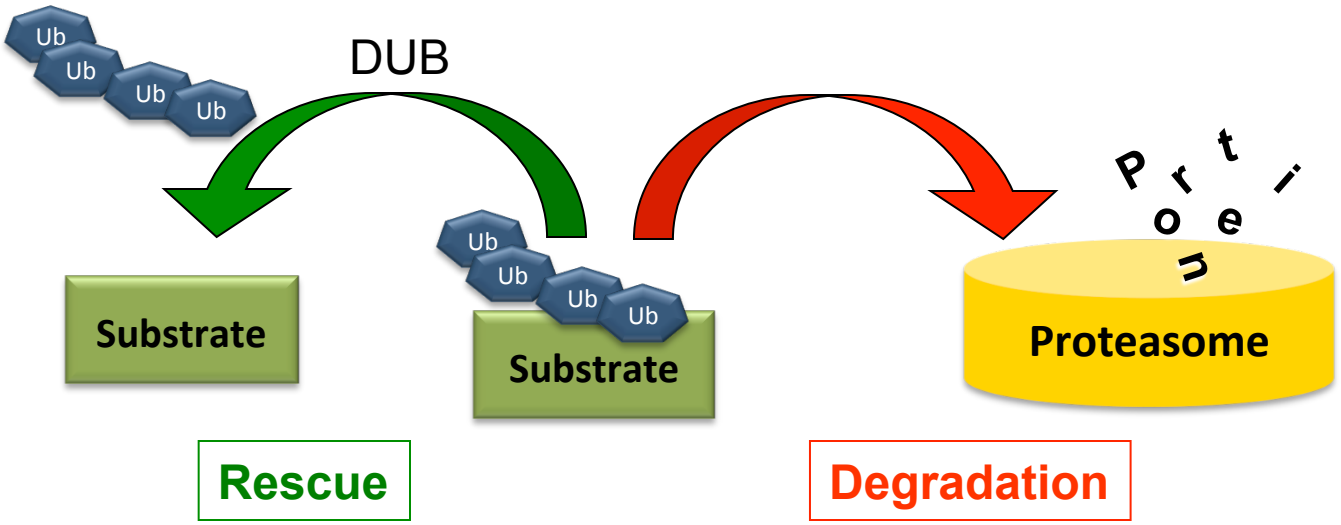
Giovinazzi et Cell Death Differentiation 2013

Ubiquitin dependent degradation pathway



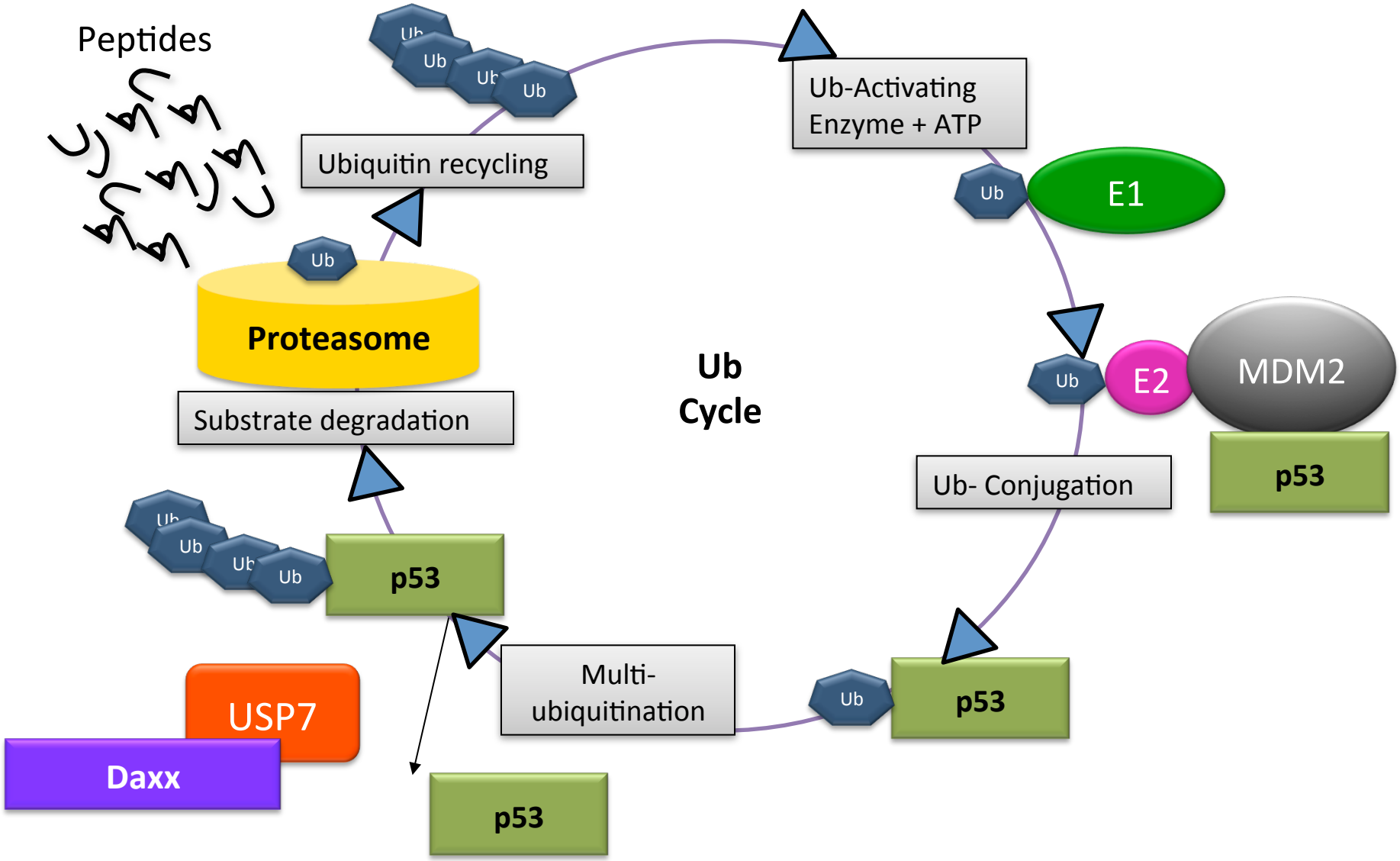
Daxx interacts with USP7 in mitosis

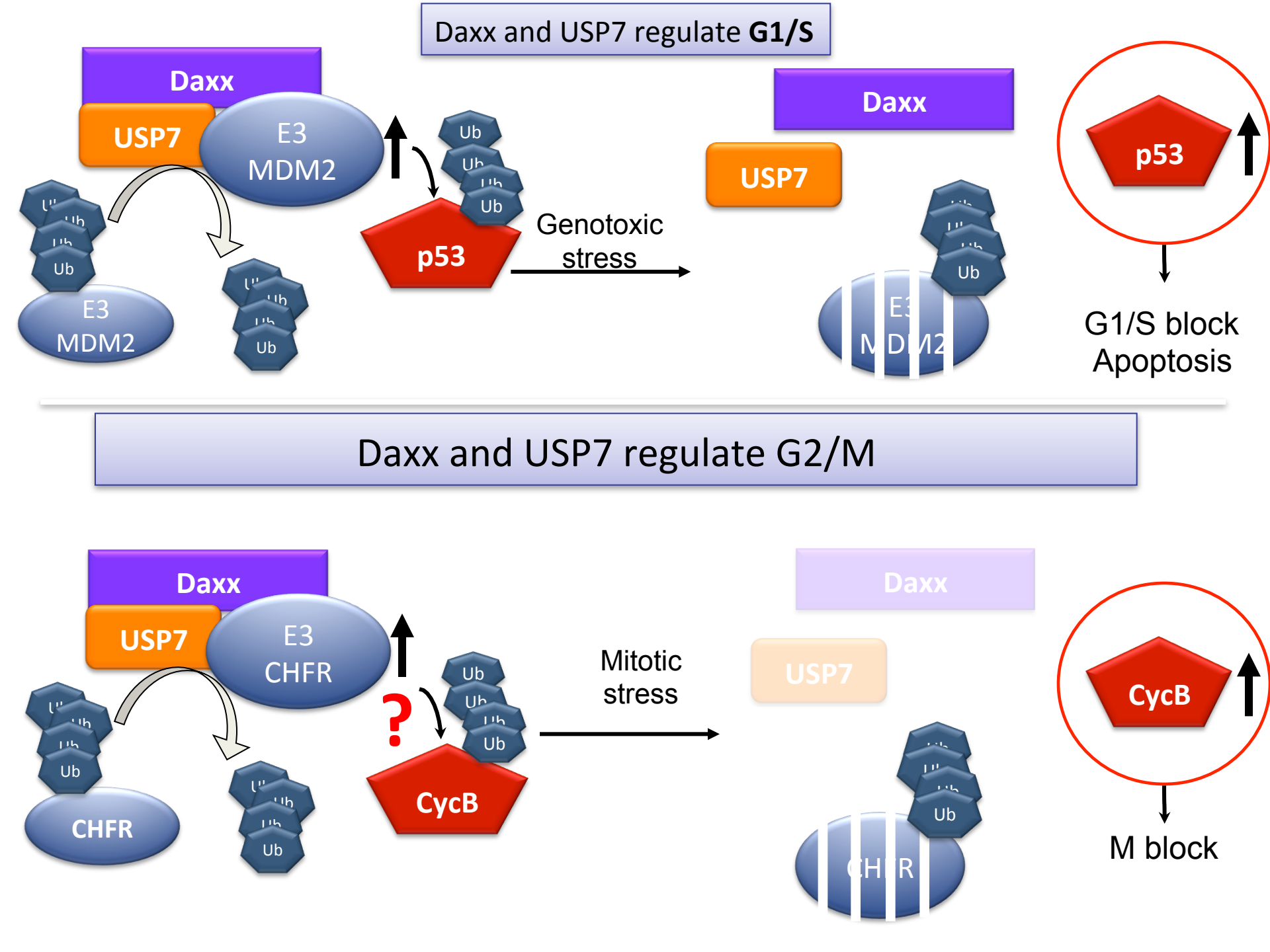
- **Ubiquitin-Specific-Processing Protease 7** (or USP7) is a de-ubiquitylating enzyme or DUB



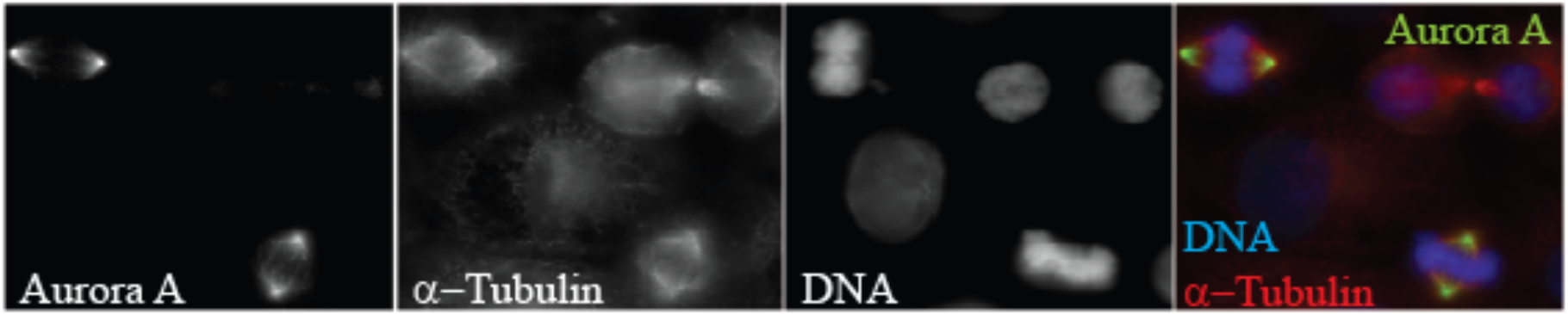
- Its activity leads to stabilization of substrate proteins
- Substrates involved in apoptosis, epigenetic maintenance, DNA stress response
- p53 is one of USP7 substrates

Ubiquitin dependent degradation of p53

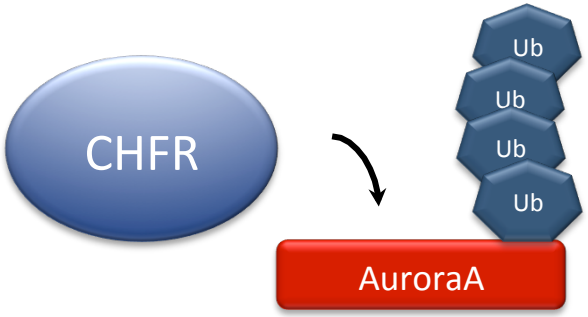



Daxx and USP7 regulate G1/S
Daxx
USP7
E3
MDM2
Ub
p53
Genotoxic
stress
Daxx
USP7
E3
MDM2
p53
G1/S block
Apoptosis
Daxx and USP7 regulate G2/M
Daxx
USP7
E3
CHFR
?
CycB
CHFR
Mitotic
stress
Daxx
USP7
CycB
M block

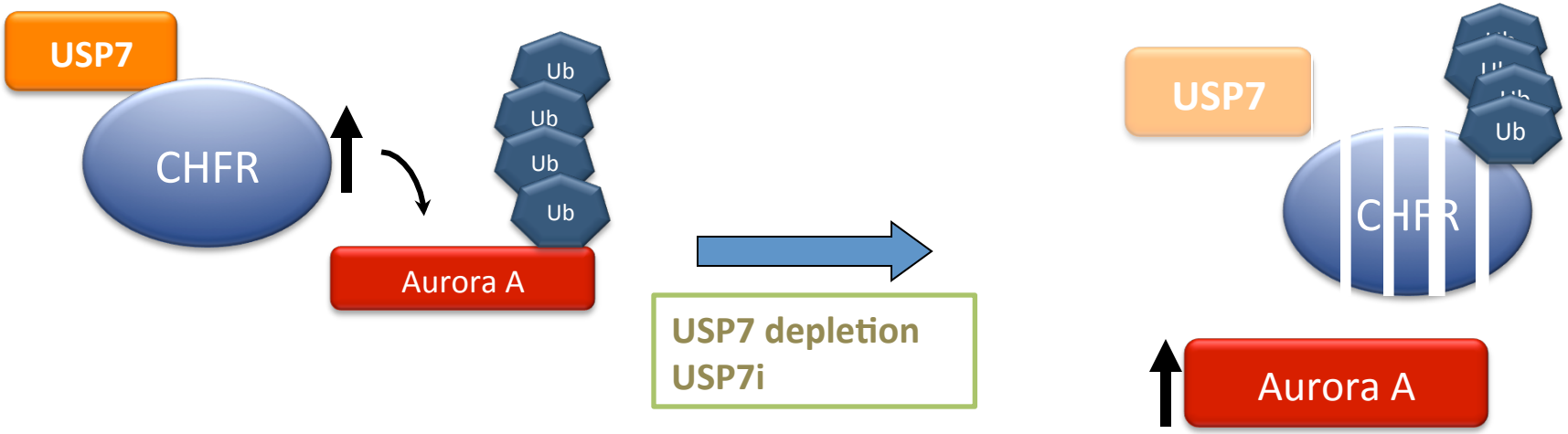
Aurora A kinase: the "Polar Aurora"

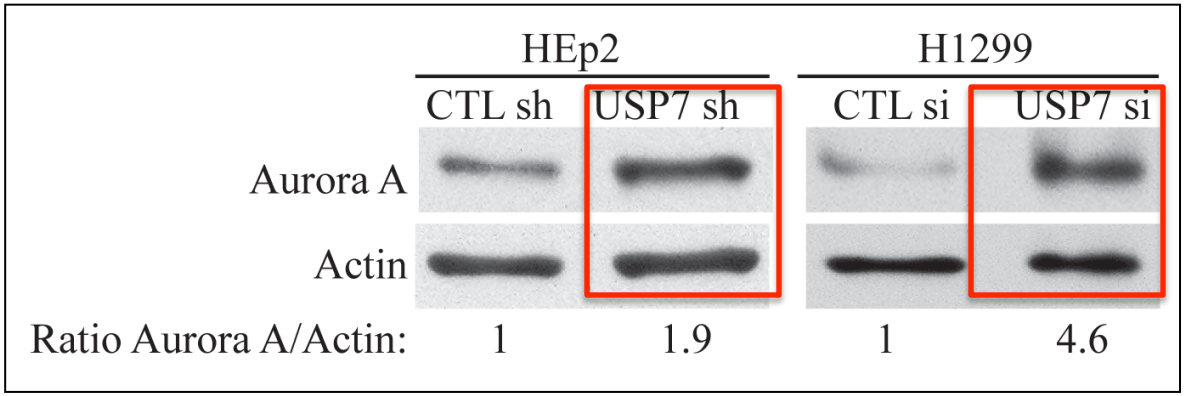


- CHFR regulates stability of Aurora-A
- The primary function of Aurora-A is to promote bipolar spindle assembly
- Aurora-A kinase gene is located in the 20q13 breast cancer amplicon and is over-expressed in breast, colorectal, pancreatic and gastric tumors
- Over-expressed Aurora-A
  - Multipolar-spindles formation
  - Induces resistance to Taxanes
- Selective Aurora-A inhibitors are in clinical trials



Does USP7 affect Aurora A?

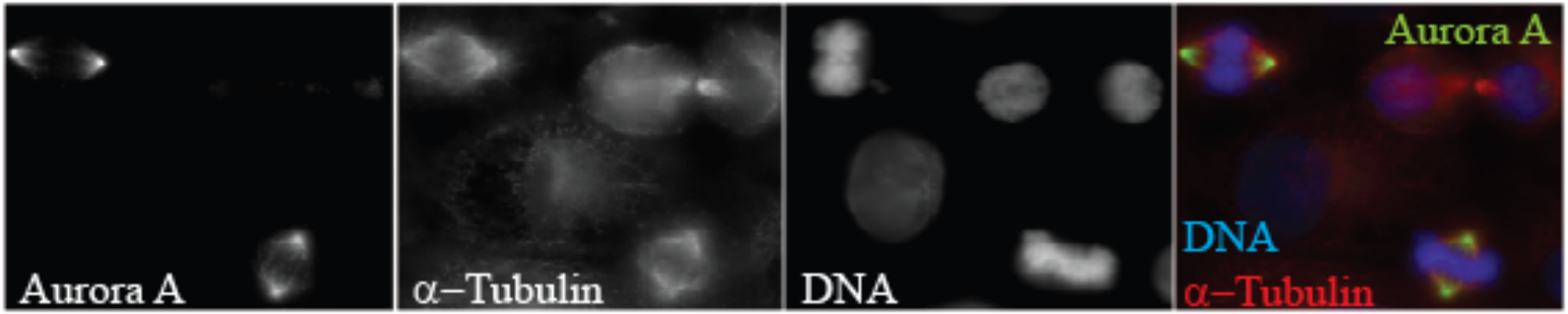




USP7 depleted cells stabilize checkpoint protein Aurora A

Giovinazzi et al. *Cell Death Diff 2013*

Aurora A kinase: the “Polar Aurora”



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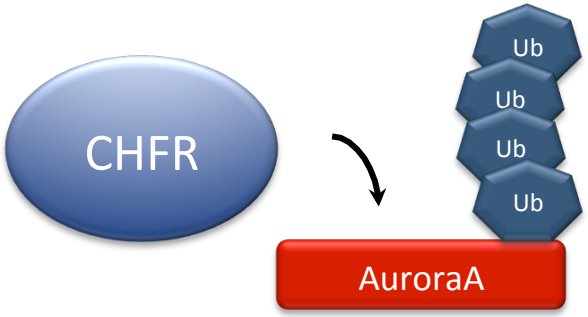
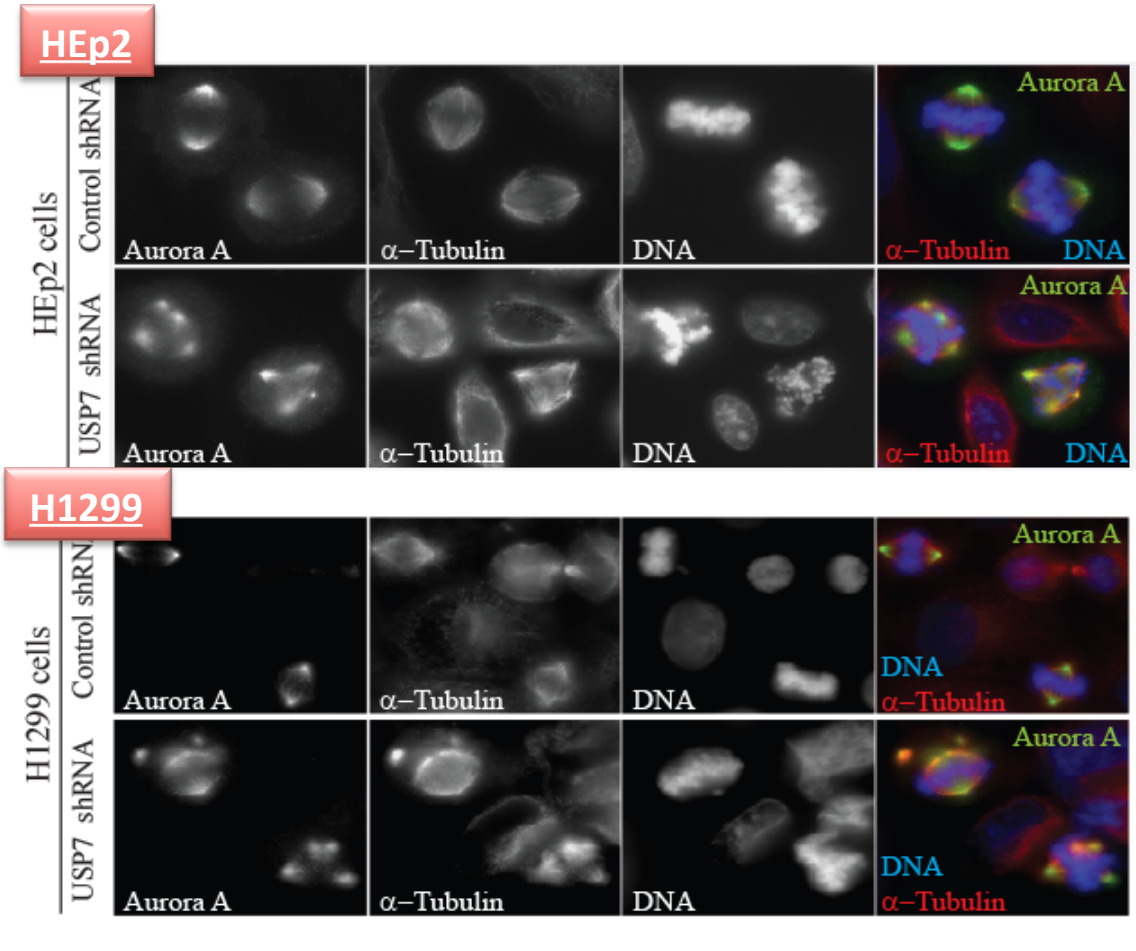
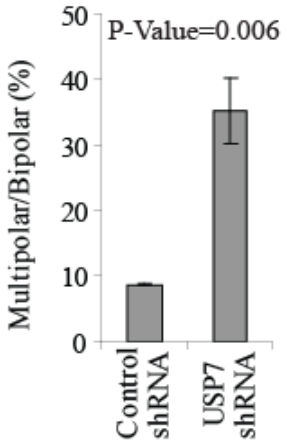
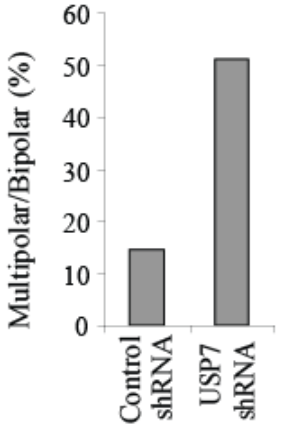


Do USP7 depleted cells accumulate multipolar spindles?

Immunofluorescence staining with polar Aurora A kinase and α-tubulin







Multipolar events over the total number of mitosis

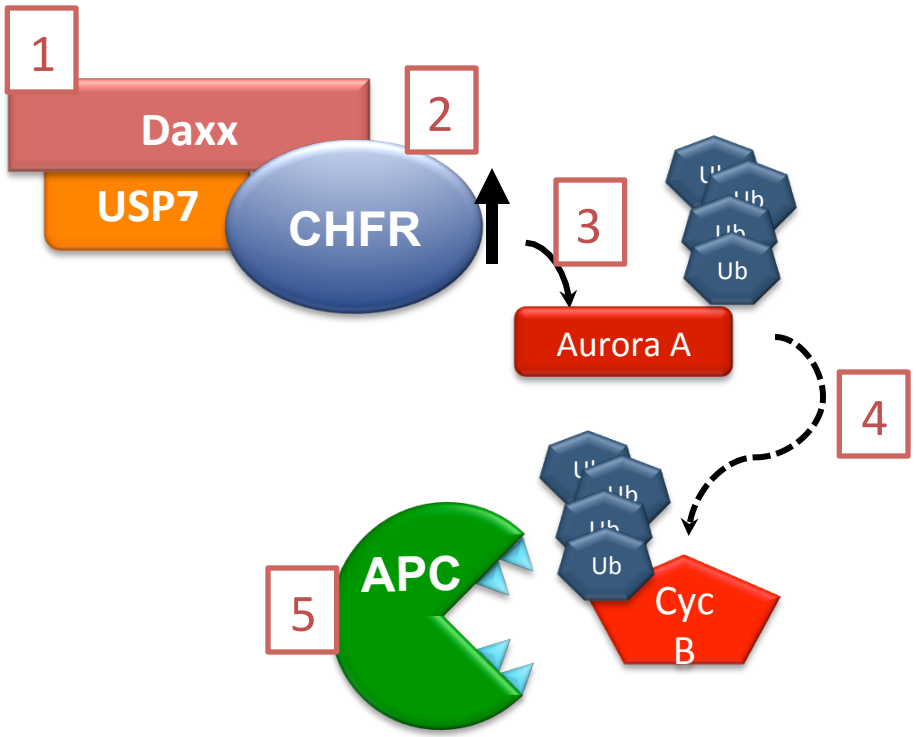
100+ mitosis *per* experiment cunted

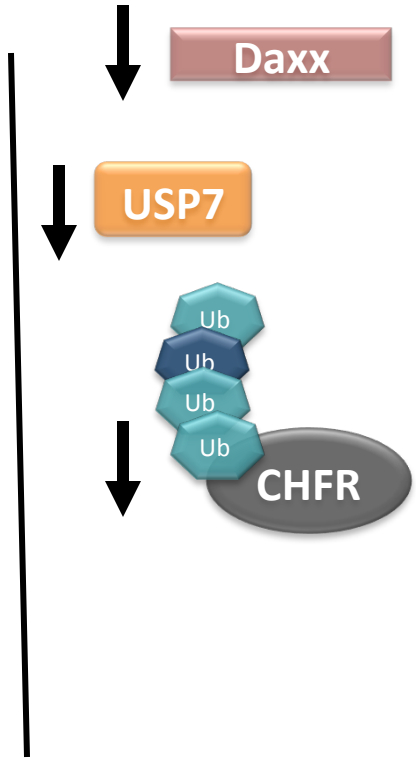
USP7 depleted cells accumulate multipolar spindles

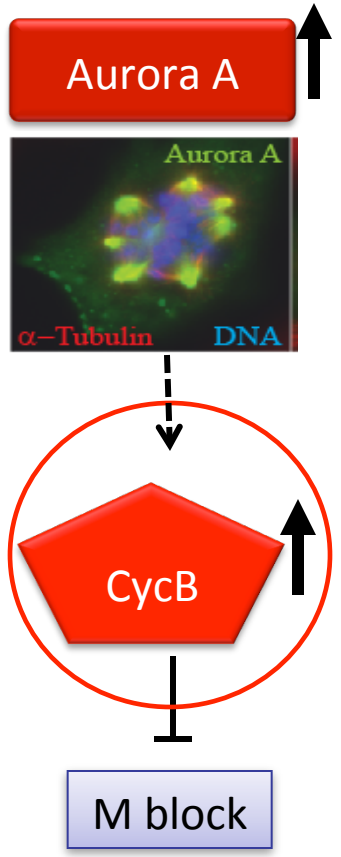
Giovinazzi et al. *Cell Death Diff 2013*

How Daxx regulates mitosis?

1. By interacting and activating USP7
2. USP7 stabilizes E3 ligase CHFR
3. CHFR controls levels of Aurora A
4. Aurora A ensures timely degradation of cyclin B
5. Cyclin B is degraded by APC

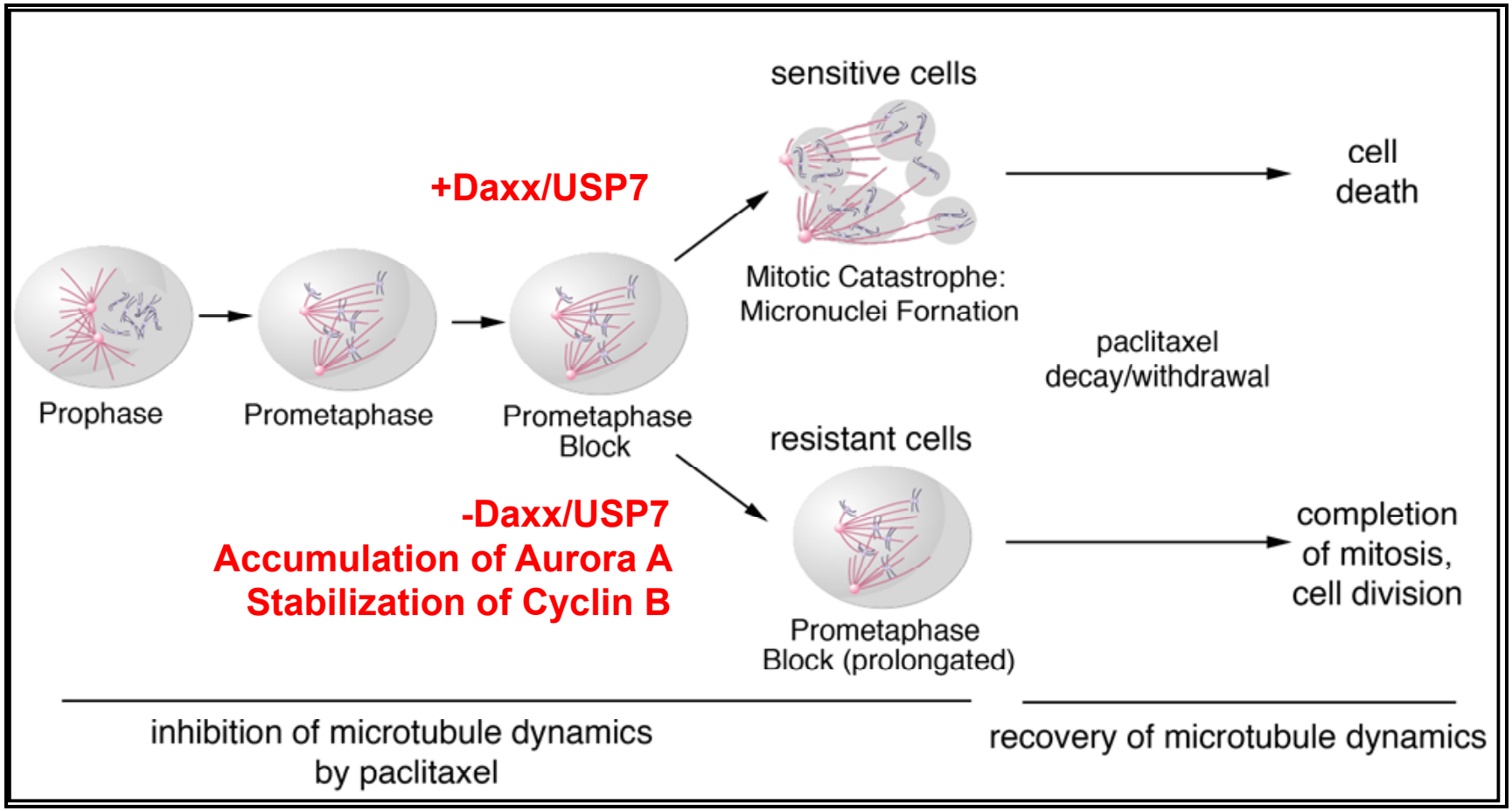




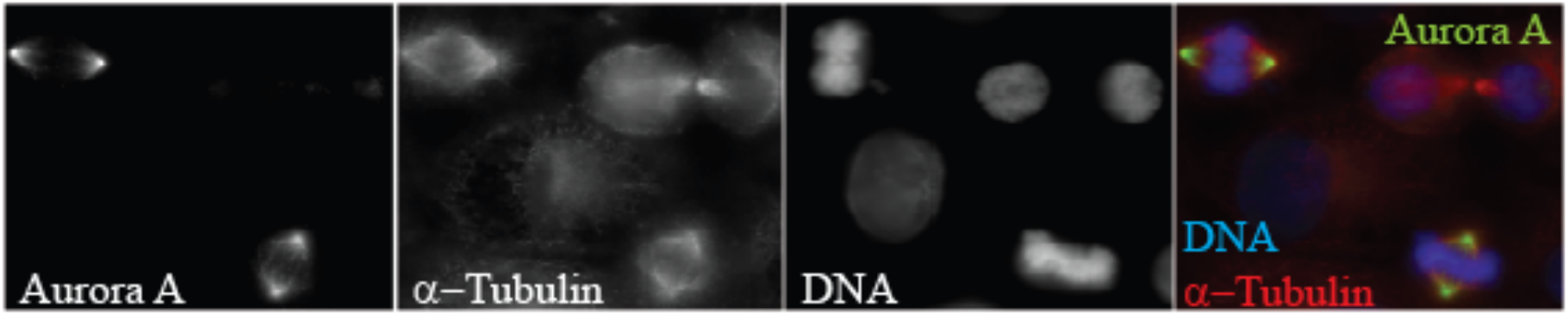


Working model

Loss of Daxx/USP7 induces Taxanes resistance by regulating mitotic checkpoint proteins



Aurora A kinase: the "Polar Aurora"



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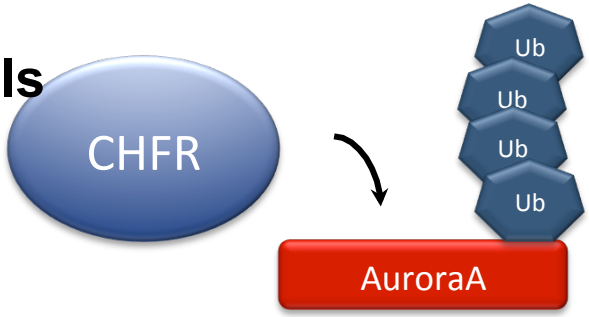
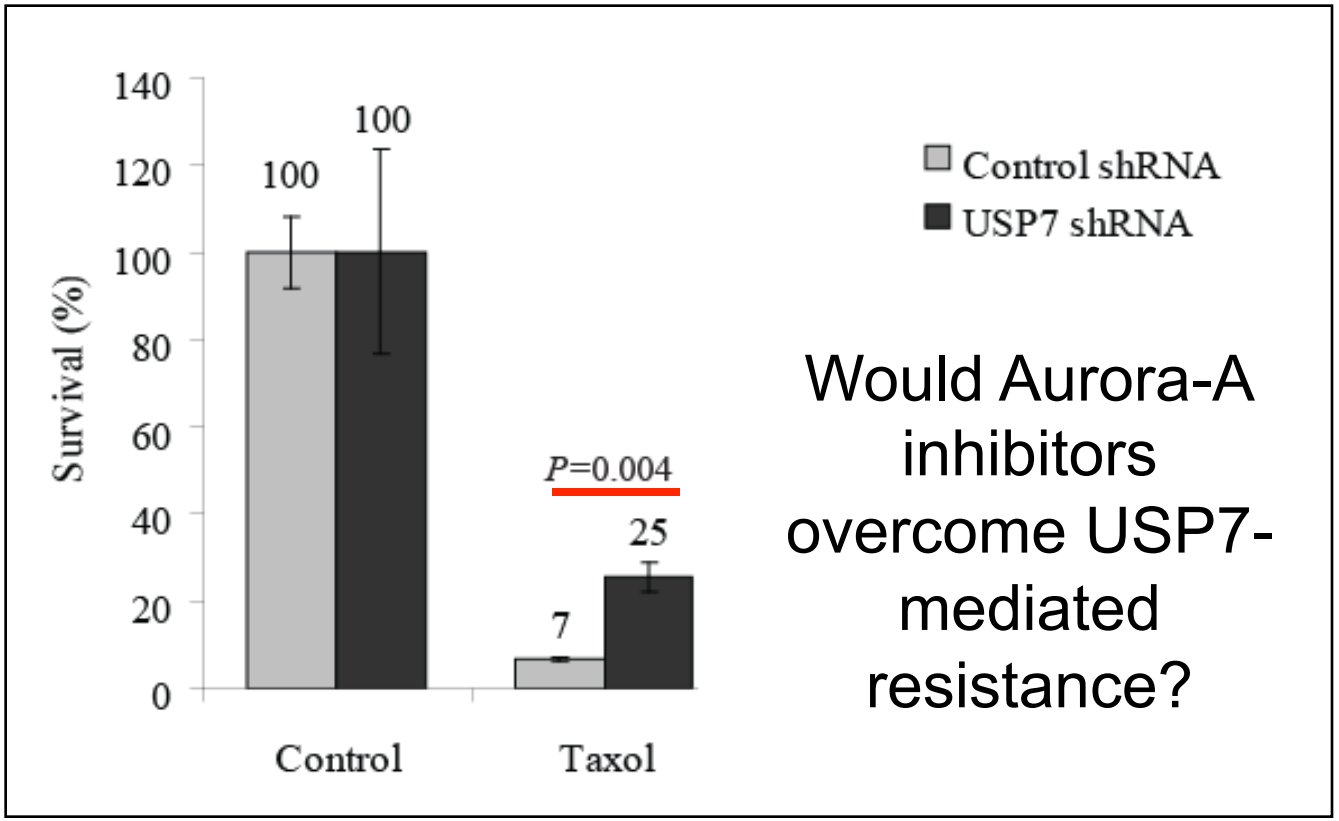
- Aurora-A is regulated by CHFR



Does USP7 regulate response to taxanes?

Colony formation assay



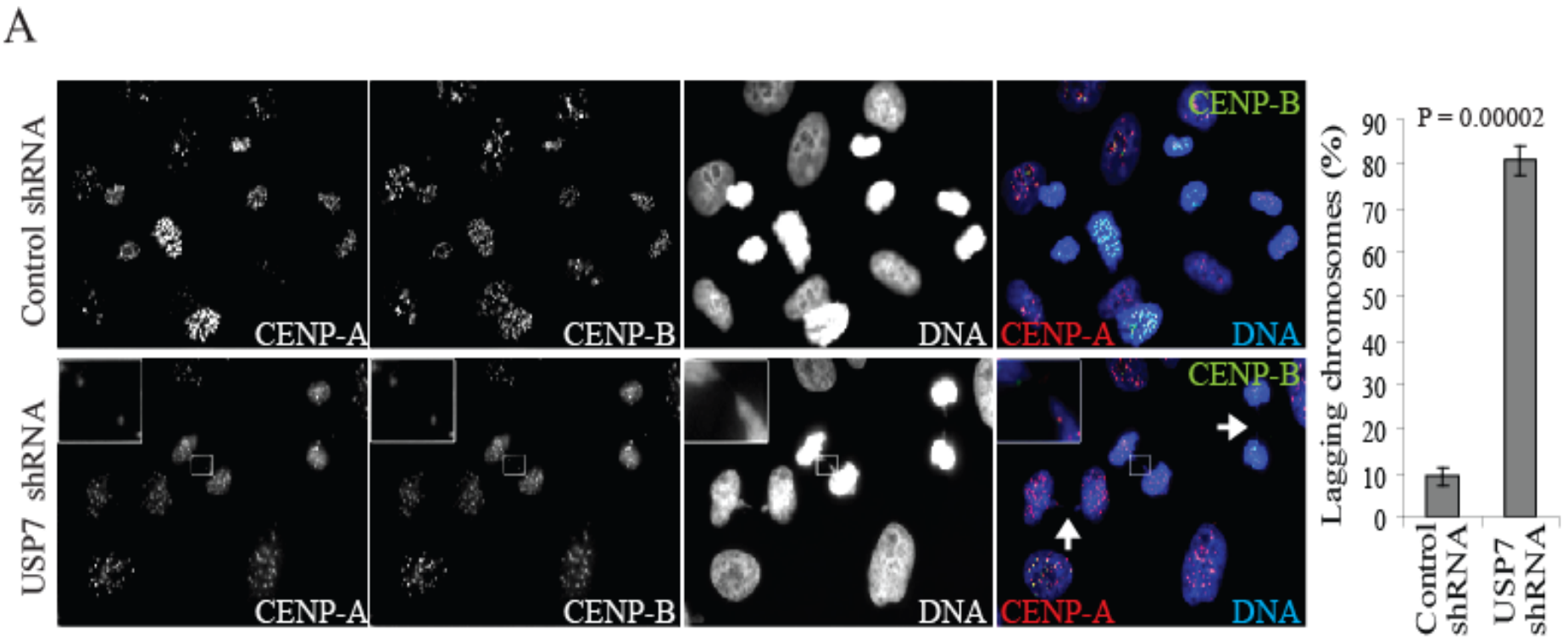
MLN8054: Selective small molecule inhibitor of Aurora-A

1. USP7 Silencing Increases Cellular Resistance to Taxol
2. Aurora-A inhibitor combined with Taxol **partially** rescues USP7-mediated resistance

Giovinazzi et al. *Cell Death Diff 2013*

Analysis of Anaphases

Immunofluorescence staining of HEp2 CTL shRNA or USP7 shRNA with centromeric markers CENP-A and CENP-B

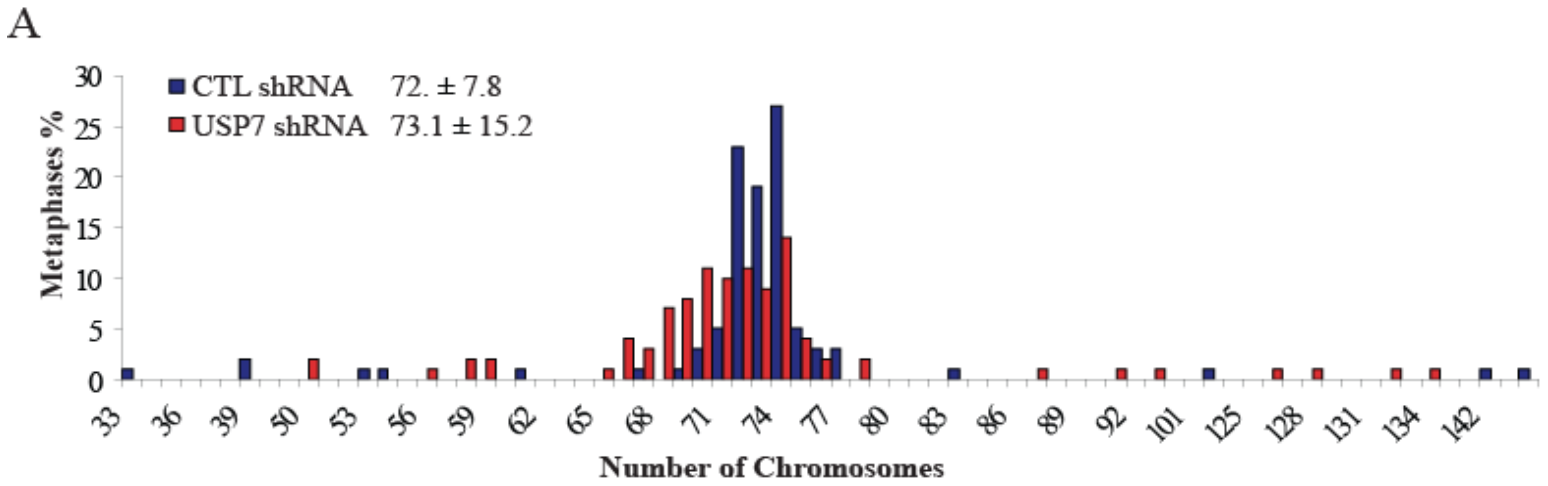


- USP7 depleted cells accumulate lagging chromosomes in anaphases
- The increase in number of lagging chromosomes explains the high MN scores documented upon USP7 depletion

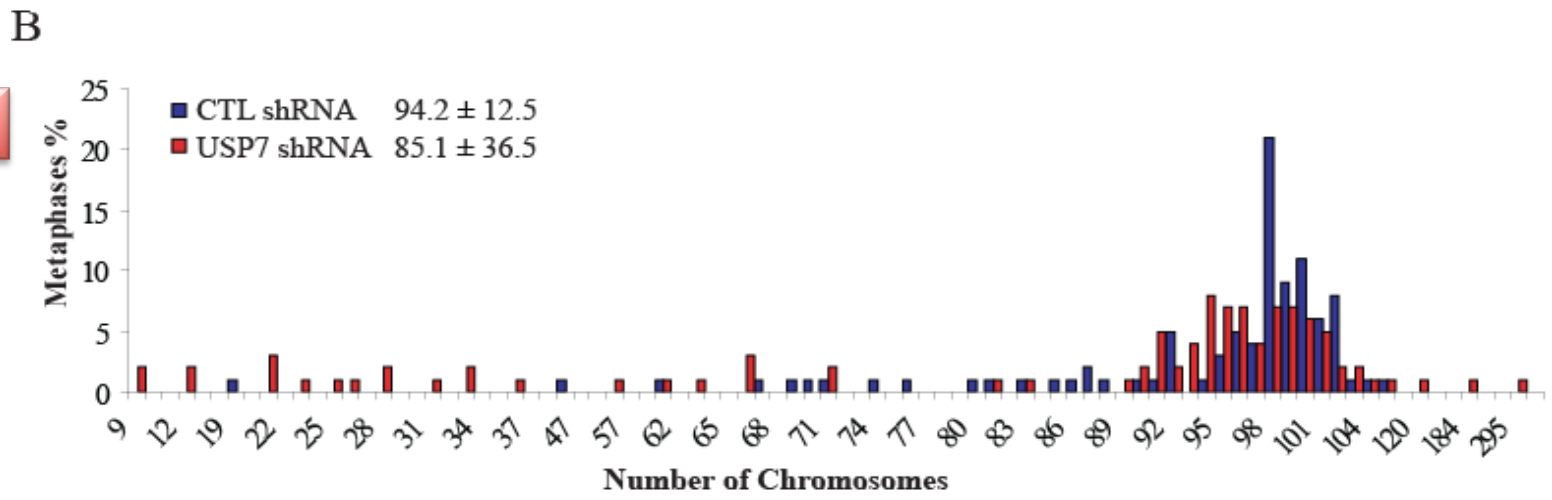
Giovinazzi et al., *under review*

Karyotype in USP7-depleted cells

HEp2



H1299

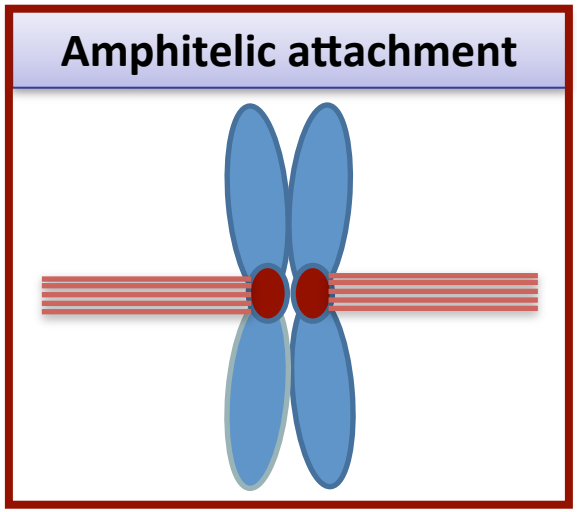


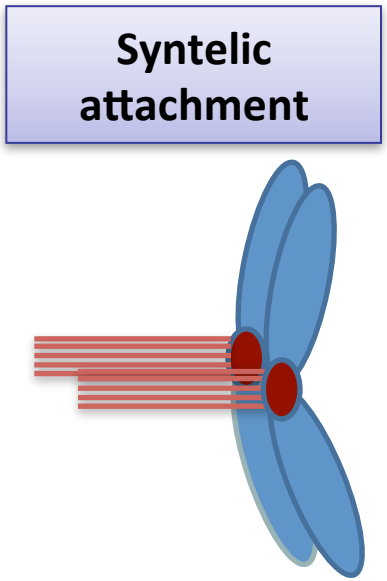
USP7 depletion increases aneuploidy:

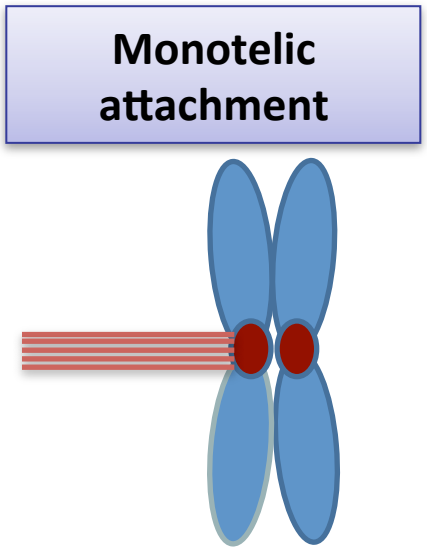
- In cancer cells (HEp2 and H1299)
- Non-tumorigenic breast epithelial cells MCF-10A

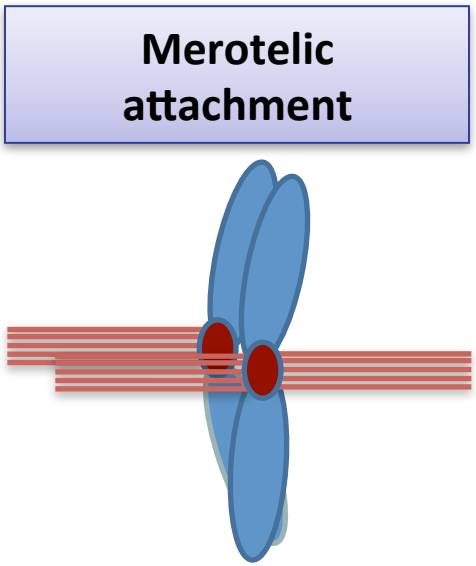
Giovinazzi et al., *under review*

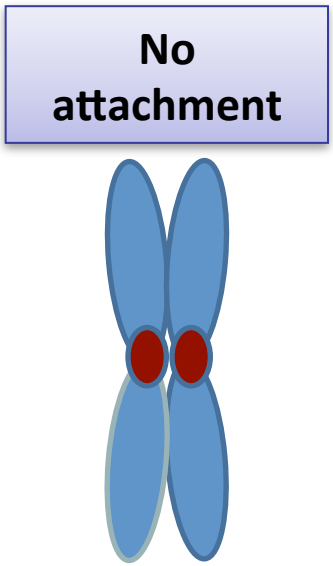
Genomic Instability Arises from the Inability to Correct Errors



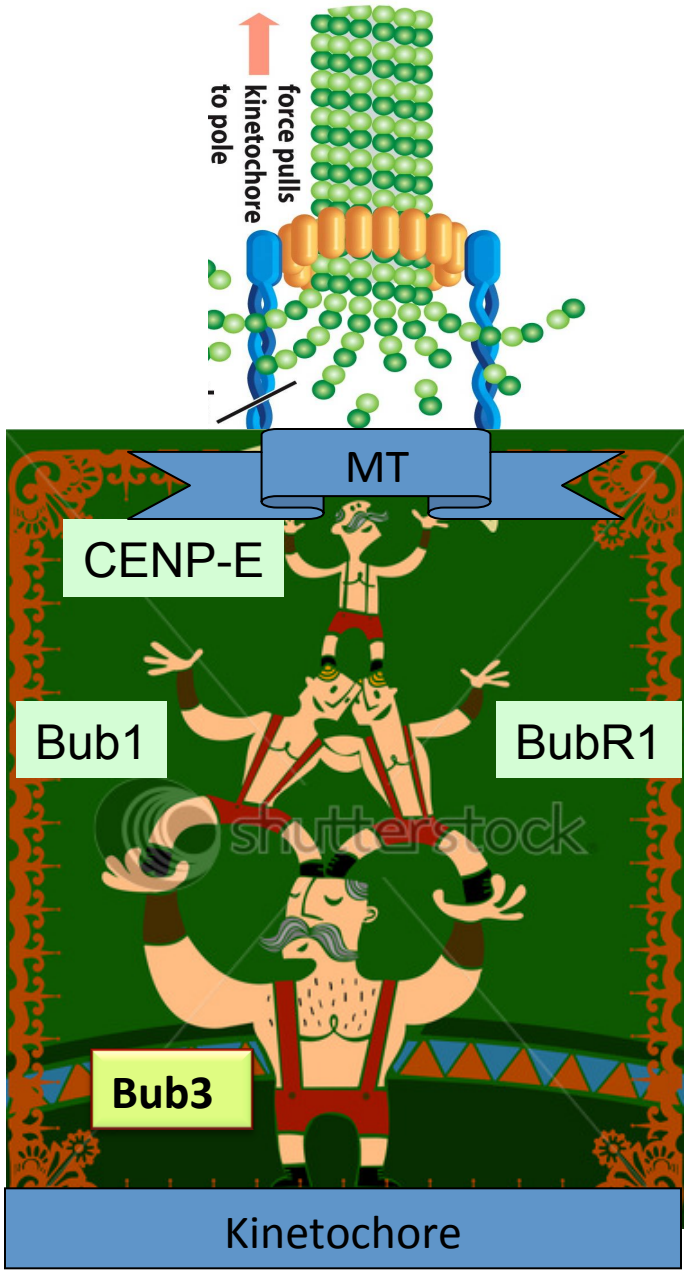








Bub3



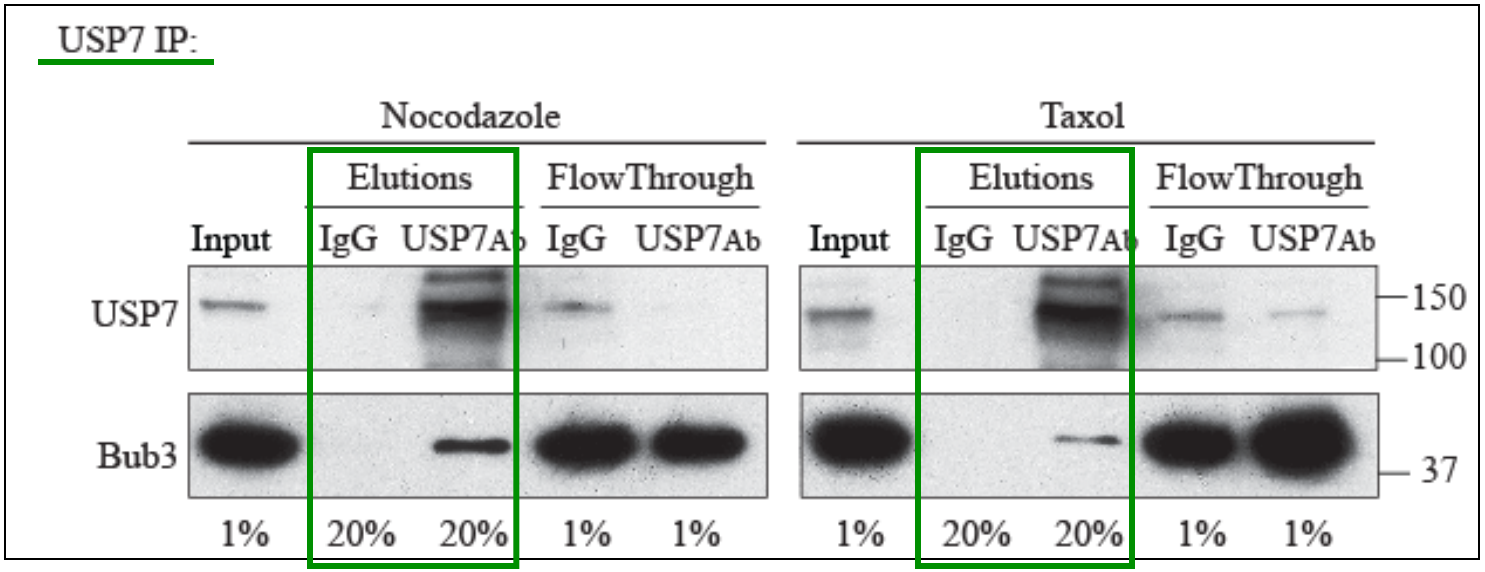
Bub3 functions:

- To recruit other SAC components, thus is essential for MT-K
- Produce a ‘Wait’ signal until satisfactory MT-K are

If lacking:

- Increase in faulty attachments
- Lagging chormosomes

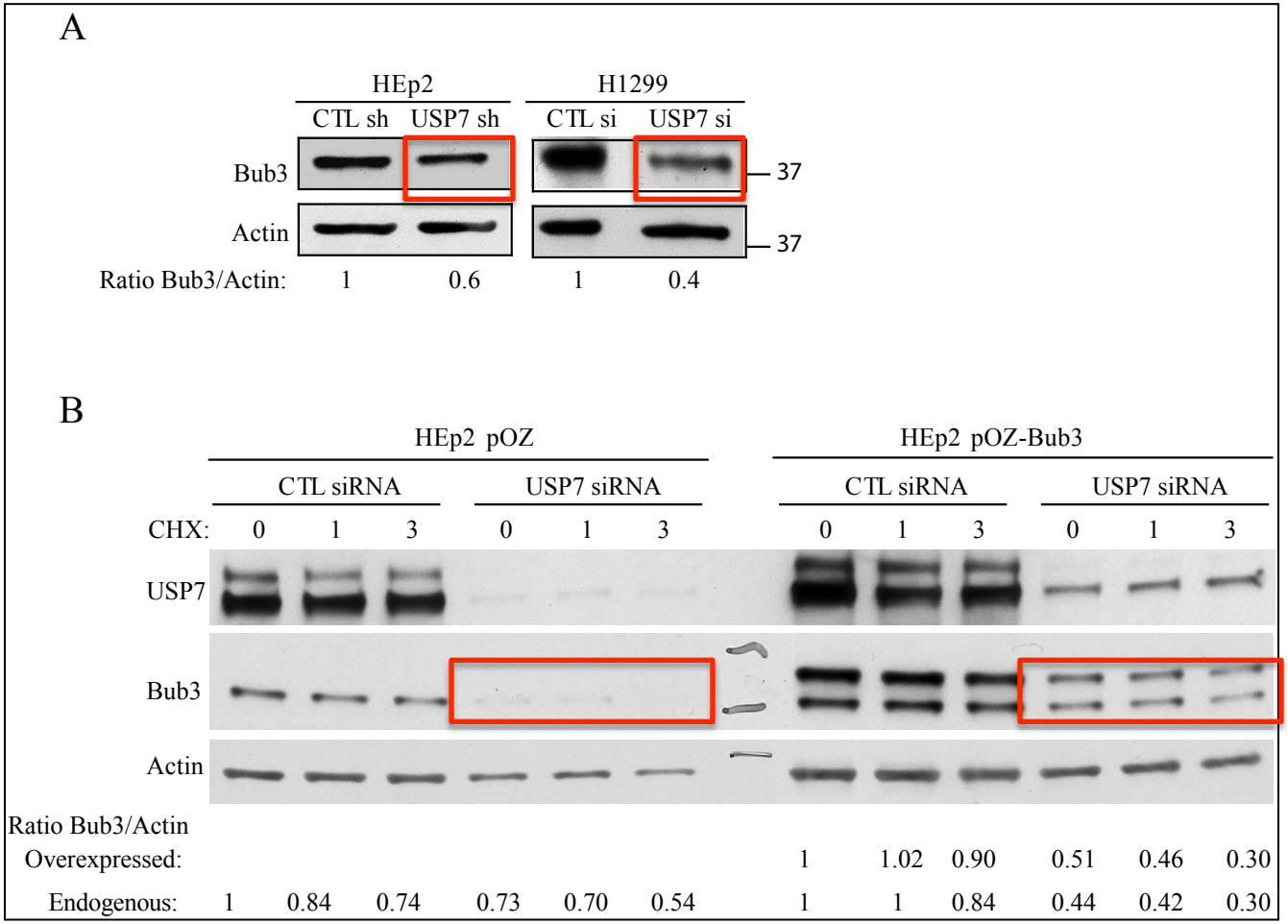
USP7 and Bub3



USP7 and Bub3 interact in mitosis

Giovinazzi et al., *under review*

USP7 and Bub3



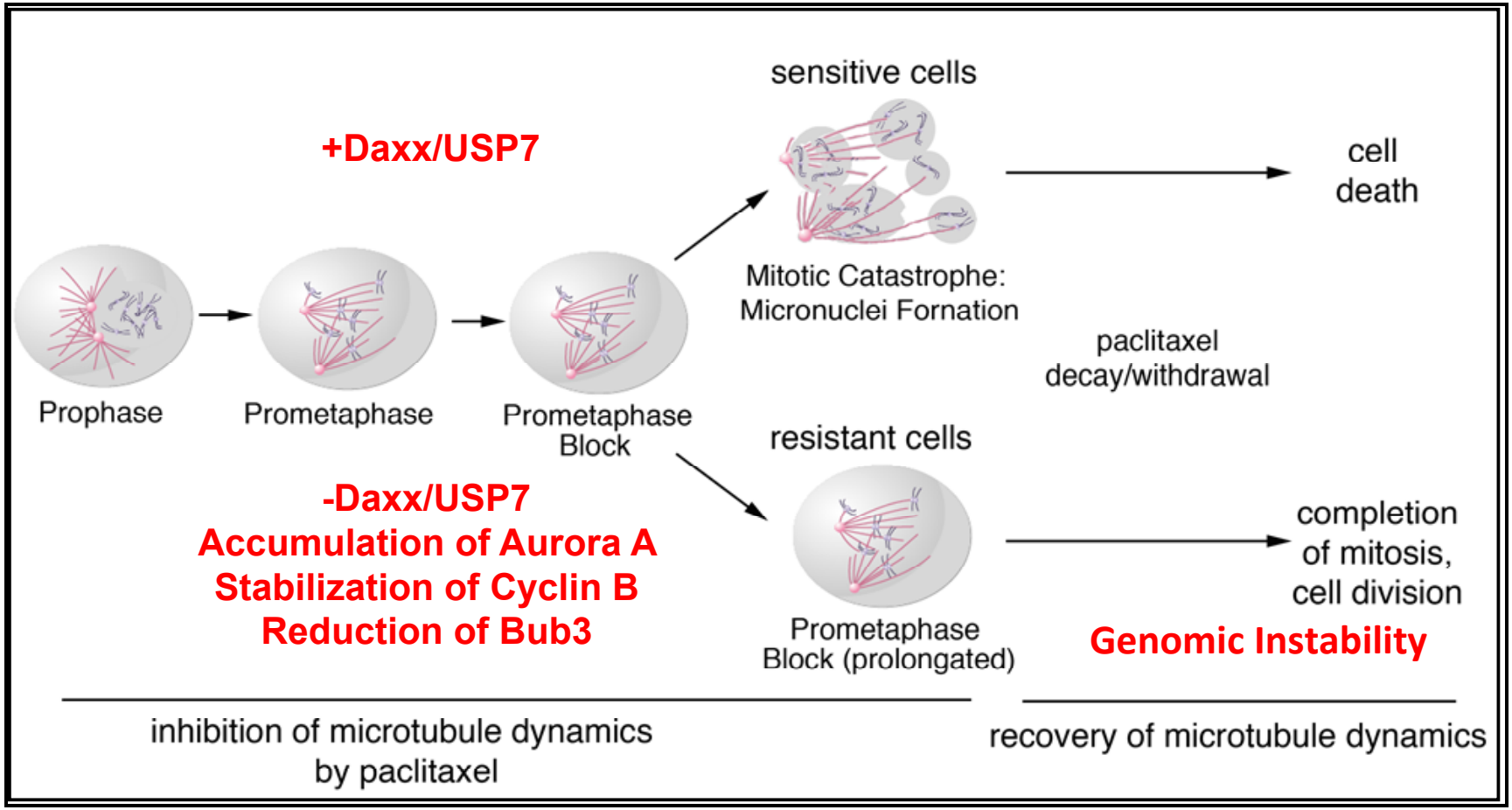
Levels of Bub3 are decreased in cells with depleted USP7

Reduction of Bub3 in cells with silenced USP7 explains the lagging chromosomes and genomic instability

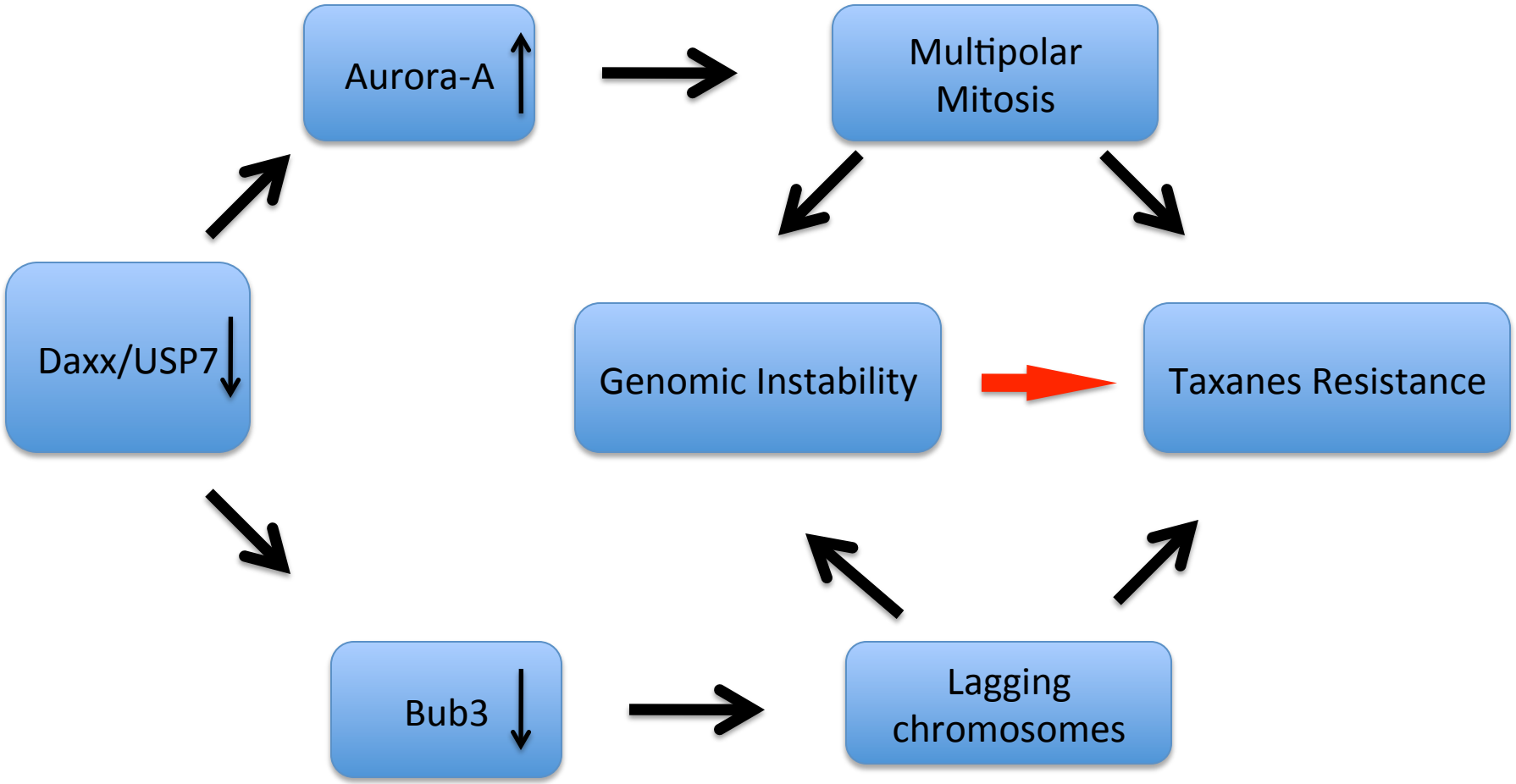
Giovinazzi et al., *under review*

Working model

Hypothesis: Loss of Daxx/USP7 induces Taxanes resistance by regulating mitotic checkpoint proteins



USP7/Daxx in taxane resistance



Hypothesis: Genomic Instability (including one induced by Daxx/USP7 inactivation) activates Spindle Assembly Checkpoint that blocks cells in mitosis and thus increases resistance to taxanes and other antimitotic drugs.

Clinical applications

Do's

- Use Daxx and USP7 as predictive markers of taxane response
- Use Aurora-A Inhibitors + taxanes

Don'ts

- Do not use USP7i in combination with taxane treatment (regardless p53 cellular status)
- Do not use DNA damage agents before taxanes

Acknowledgements

Lab members:

Dr. Cory Lindsay

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Dhruv Bellapu

Christian Reintgen

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Dr. Colland F., Hybrigenics, Paris, France (USP7 inhibitors)

Dr. Everett R., University of Glasgow, Scotland, UK (USP7 constructs)

Collaborators:

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Dr. Han, Moffitt Cancer Center

Dr. Reinhold W.C. Genomics & Bioinformatics, NCI, Bethesda, MD

Dr. Roberto Zori, Division of Genetics-Department of Pediatrics, UF

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Dr. Roux K.J. Sanford Children's Health Research Center, Sioux Falls, SD

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