Химиотерапия рака молочной железы: механизмы резистентности и новые подходы в её преодолении

Резистентность к химиотерапии: кто виноват и что делать?

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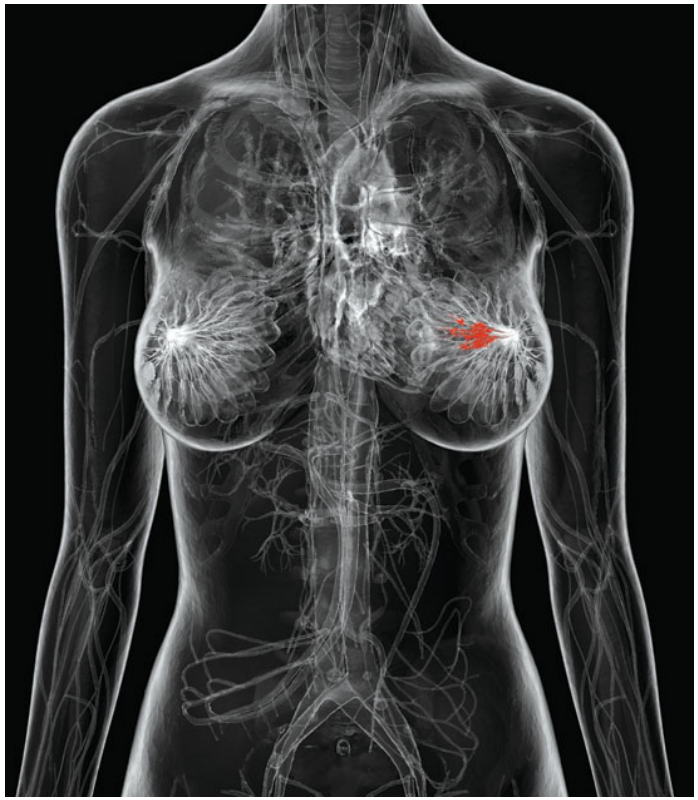
ИНСТИТУТ ЦИТОЛОГИИ
29 апреля 2015

- Цитотоксическая химиотерапия рака молочной железы

- Деубиквитиназа USP7 и резистентность к анти-митотической химиотерапии

- Новые подходы в преодолении резистентности

Breast Cancer



- Breast cancer is the most common cancer in women

2013:

- 1.7 million new cases worldwide
- 232,340 new cases in US
- 39,620 deaths in US
- 1 of 8 US woman is diagnosed during lifespan
- Genetic factors, lifestyle, environment
- 63,000 new cases in Russia

Treatment Options:

- Surgery
- Radiation therapy
- Chemotherapy

Chemotherapy

Targeted therapies

- ER/PR
- HER2 receptor
- PI3K/AKT pathway
- VEGF and angiogenesis

Cytotoxic Drugs

- Cyclophosphamide (Cytoxan)
- Doxorubicin (Adriamycin)
- Antimitotic: Taxol (Paclitaxel)

- Taxol introduced in clinic in 1983
- In 2003 Taxol became a National Historic Chemical Landmark
- Taxanes inhibit depolymerization of microtubules (mitotic poisons)
- Major limitations of these drugs:
  - Toxicity → Novel formulations

Chemotherapy

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  - Toxicity → Novel formulations
  - Resistance
- Resistance rates: 1st line ~50%; 2nd line ~ 70-80%
- In US, more than 60,000 women/year treated with taxanes will not benefit from the therapy
  - Mutations in tubulin gene are rare
  - Derived from defects in mitotic checkpoints

GOALS

- Identify predictive markers for taxane response to allow patients stratification

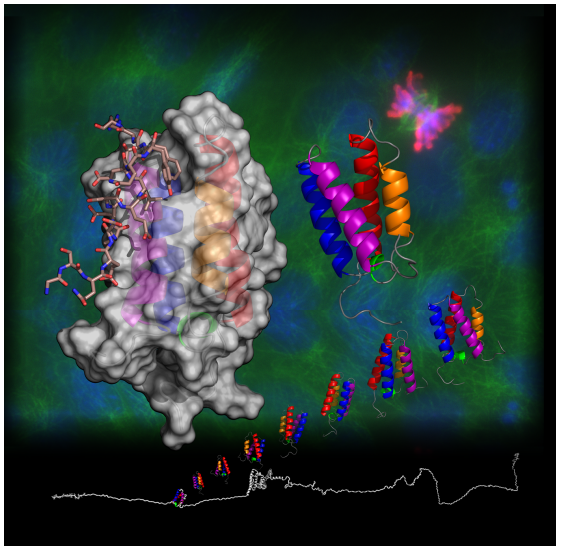
- Understand the mechanism(s) of taxane resistance

Daxx (Death-Domain Associated Protein)

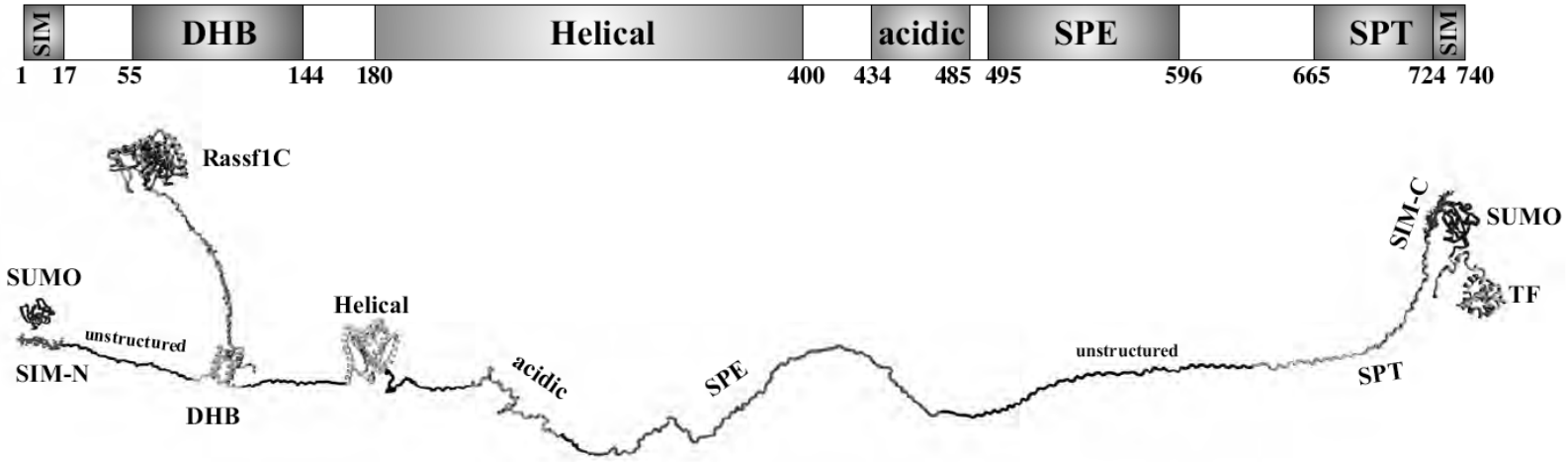
•Daxx is conservative protein essential for embryonic development

•Daxx contains several structural domains and two SUMO-interacting motifs (SIMs)

•Daxx is implicated in apoptosis, transcriptional regulation, mitotic progression

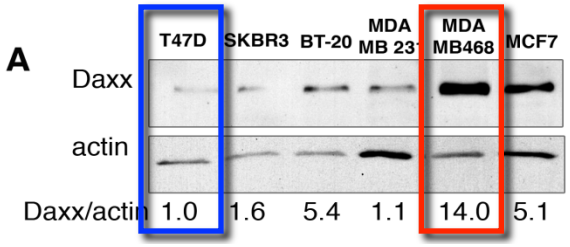


Daxx Helix Bundle (DHB) structure



Escobar-Cabrera et al., *Structure,* 2010

Daxx and Taxane resistance



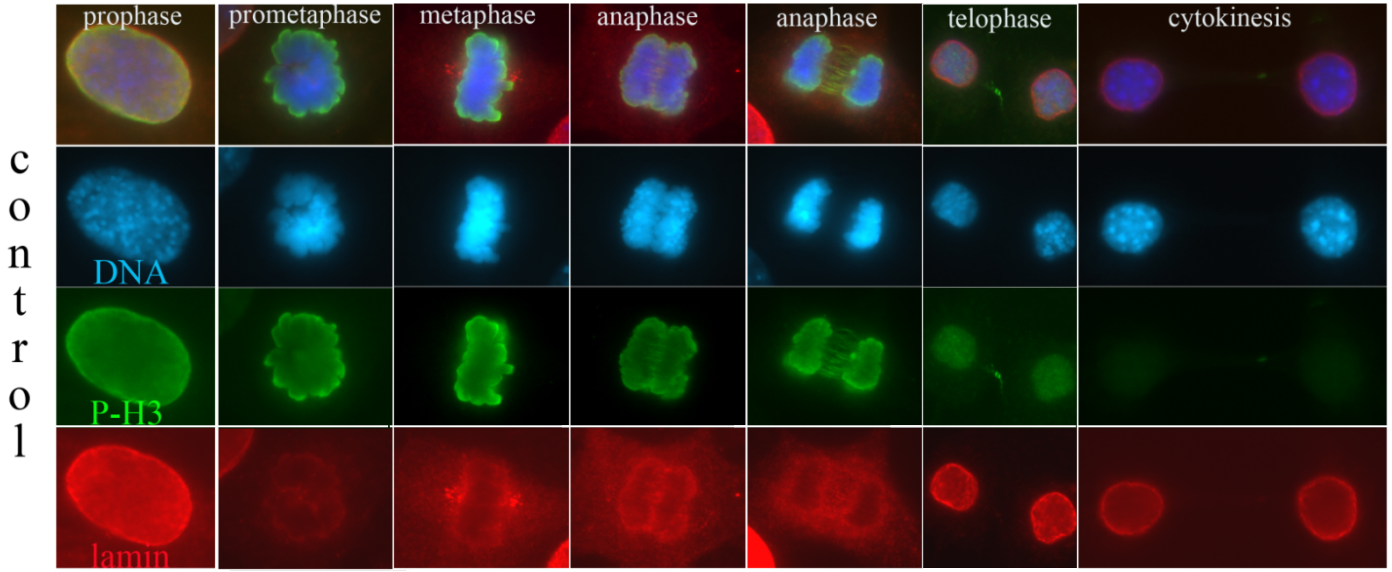
Cory R. Lindsay Ph.D.

- Low level of Daxx expression leads to taxol resistance in breast cancer cell lines

- Confirmed by experimental depletion of Daxxby experimentally depleting Daxx in cancer cell lines (MDA MB-468 and HEp2) and in Daxx-/- MEFs

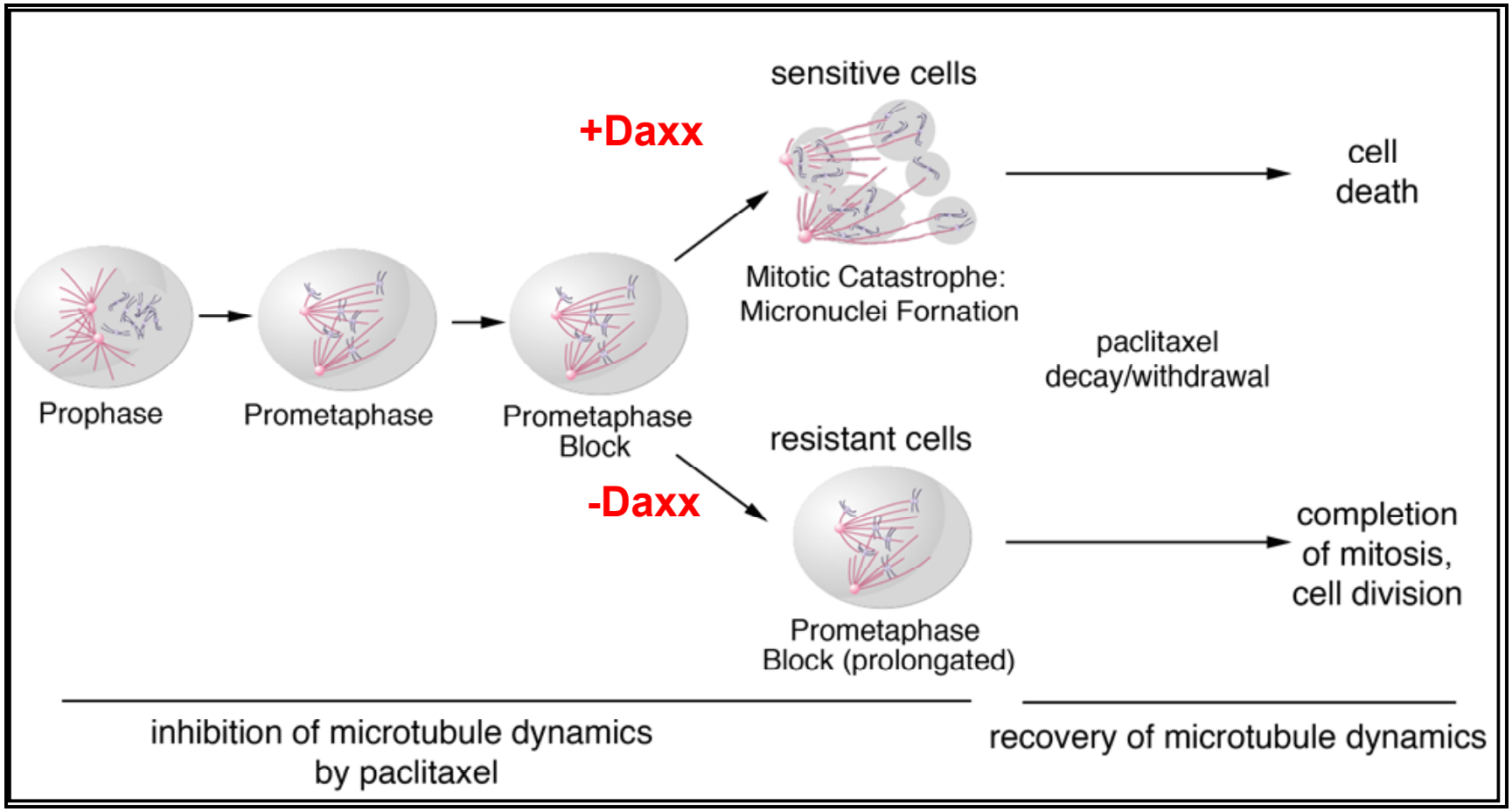
Lindsay, et al. (2007) Cell Cycle 6(10): 1200-1204

Mitotic progression and taxol action



Taxol application inhibits depolymerization of microtubules. Cells are blocked in prometaphase. This block activates micronuclei formation.

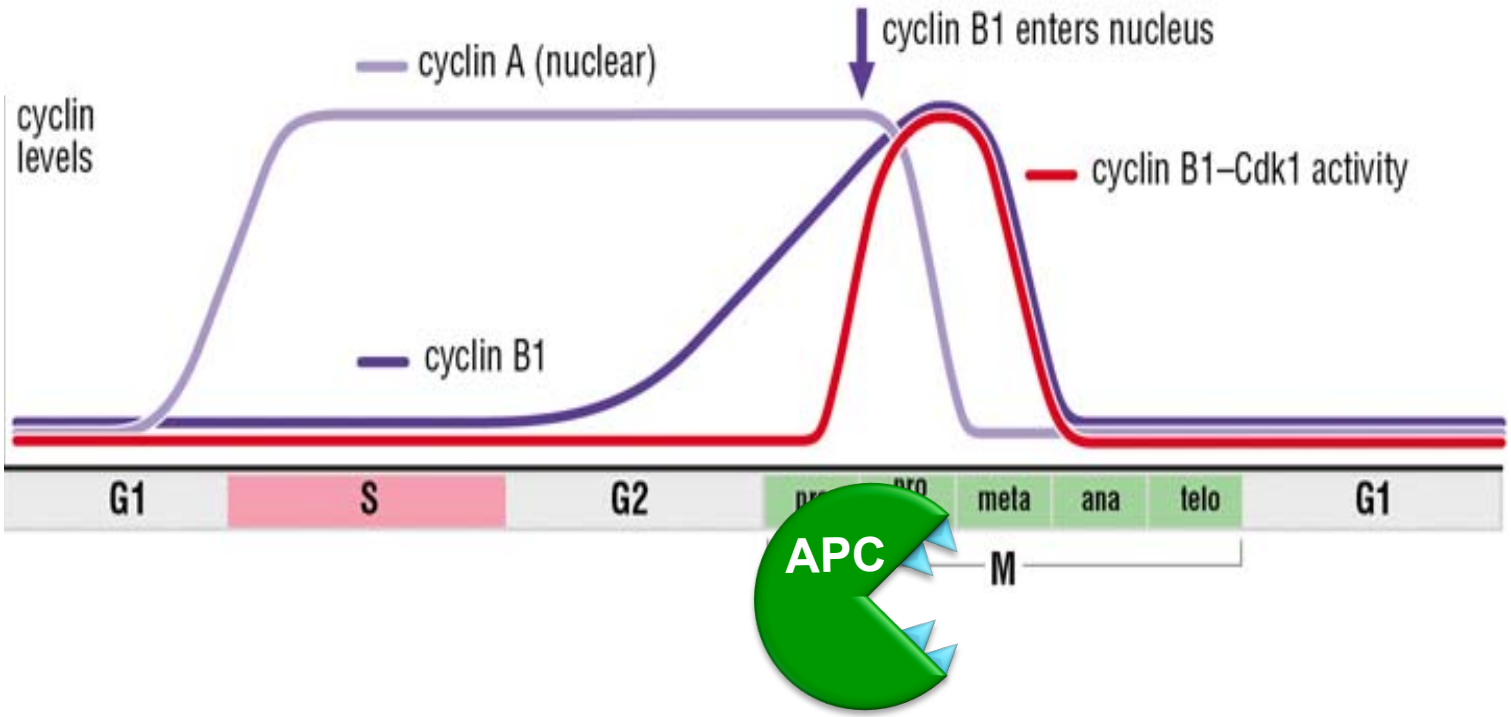
Daxx in Taxane Resistance



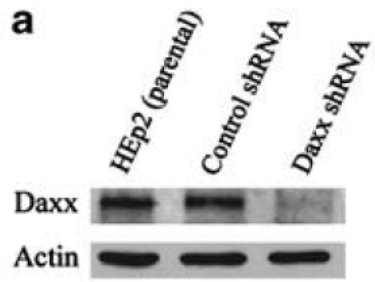
Oncogene. 2012 Jan 5;31(1):13-26.

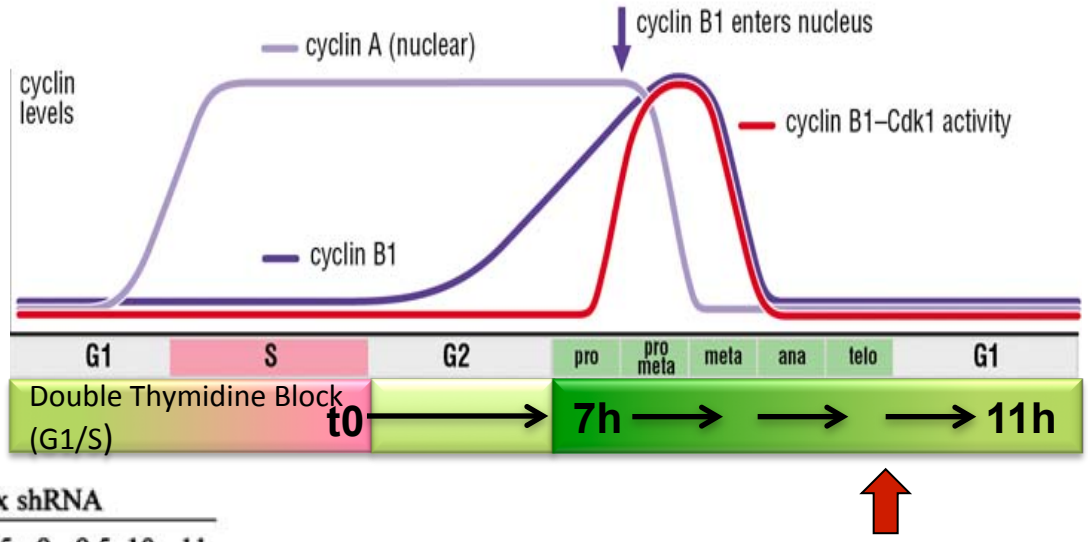
Mitotic Checkpoints, Cyclins and APC

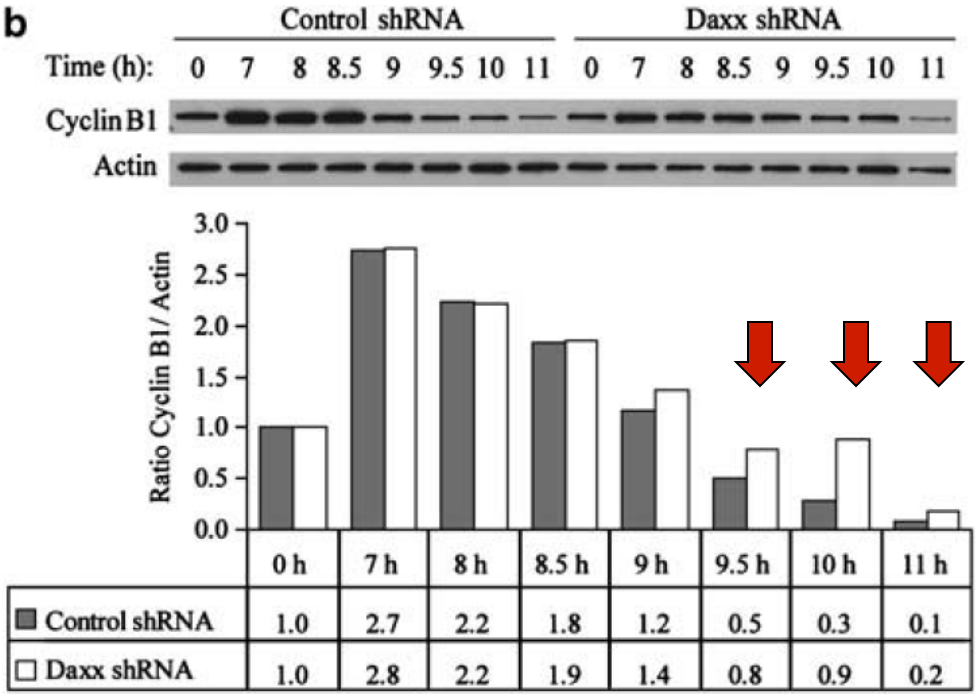
- Cyclin B is required for early Mitotic events
- Accumulates in G2/Prophase
- Cyclin B is degraded at the Methaphase-Anaphase transition by the E3 ubiquitin ligase APC (Anaphase Promoting Complex)



Study of Cyclin B Stability in Mitosis







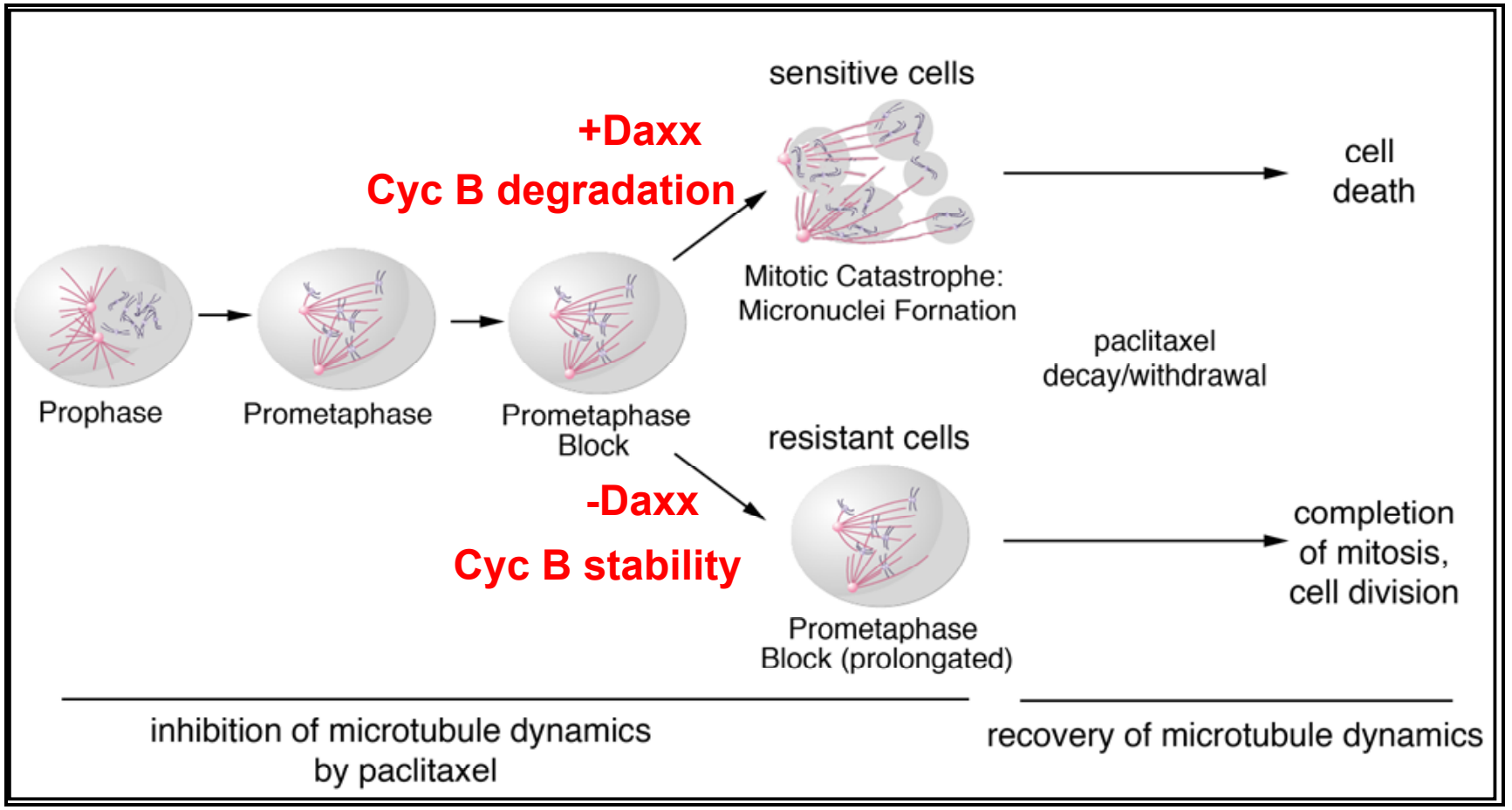
	0 h	7 h	8 h	8.5 h	9 h	9.5 h	10 h	11 h
Control shRNA	1.0	2.7	2.2	1.8	1.2	0.5	0.3	0.1
Daxx shRNA	1.0	2.8	2.2	1.9	1.4	0.8	0.9	0.2

Data is representative of three experiments

- Daxx depletion delays mitotic progression affecting Cyclin B degradation
- These effects are elevated in presence of Taxol
- Confirmed by study of mitotic timing

Giovinazzi et al. 2012, *Oncogene*

Daxx in Taxane Resistance



Oncogene. 2012 Jan 5;31(1):13-26.

Tumor Xenograft Model

4-6 Week Old Nu/Nu Mice

↓

Inject $5X10^6$ control, Daxx Depleted HEp2 cells with matrigel

↓

~100 mm^3 tumor size (day 0)

↓

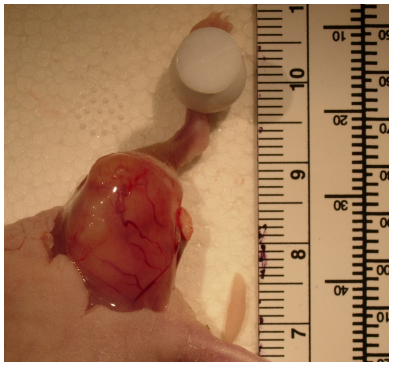
Intraperitoneal administration of Taxol (20 mg/kg) (5X48h intervals (days 1, 3, 5, 7, 9)

↓

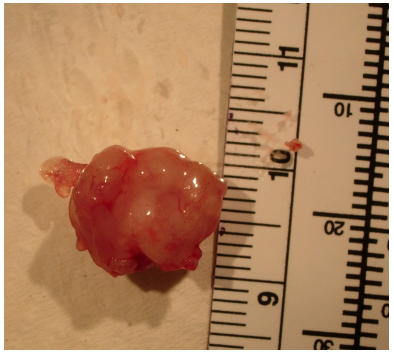
Monitor tumor size every 24h

↓

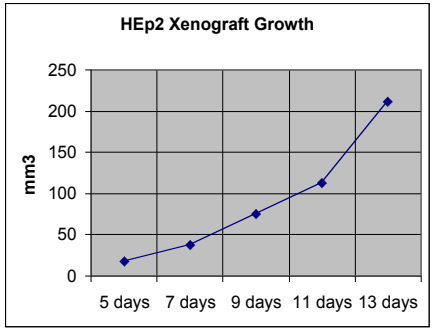
Collect tumors at ~1000 mm^3 or at day 15



Nu/Nu mice are injected on the right dorsal side of the hip/leg



Dissection of HEp2 Xenograft from Nu/Nu mouse

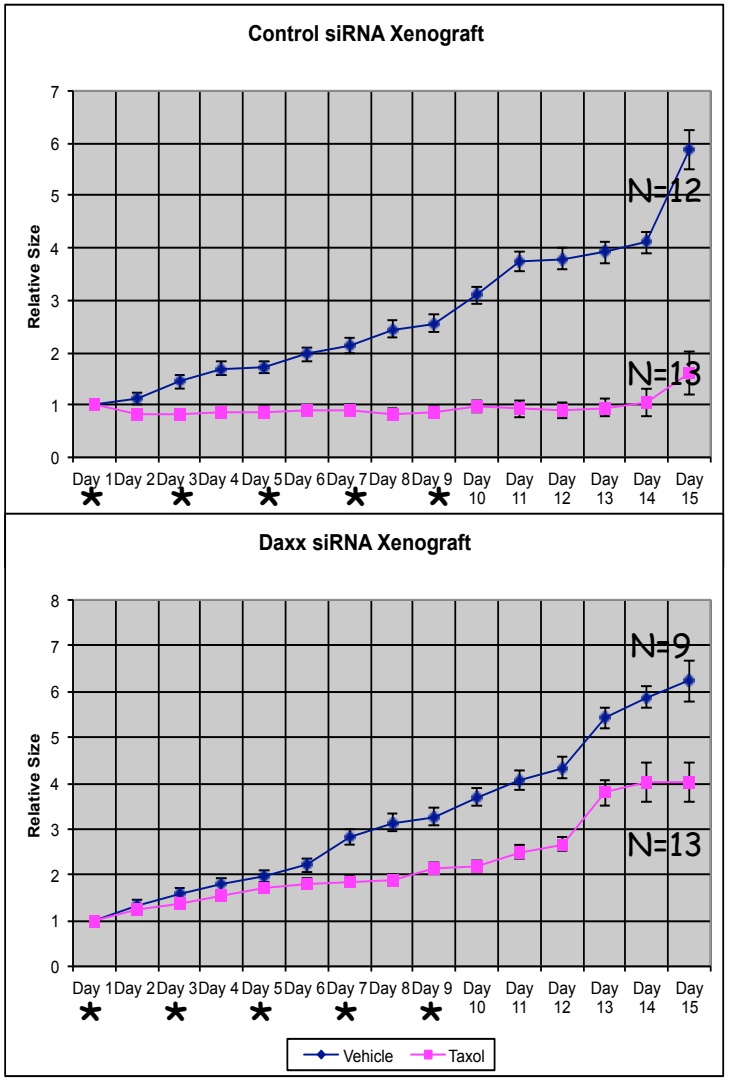


HEp2 cells were injected into Nu/Nu mice. Beginning 7 days later, caliper measurements were taken of tumor volume and recorded every two days subsequently

In vivo Tumor Response to Taxol

Nu/Nu mice s.e. injection of $5X10^6$ cells

At tumor V=150 mm^3, vehicle or taxol (20 mg/kg) are injected by IP every second day

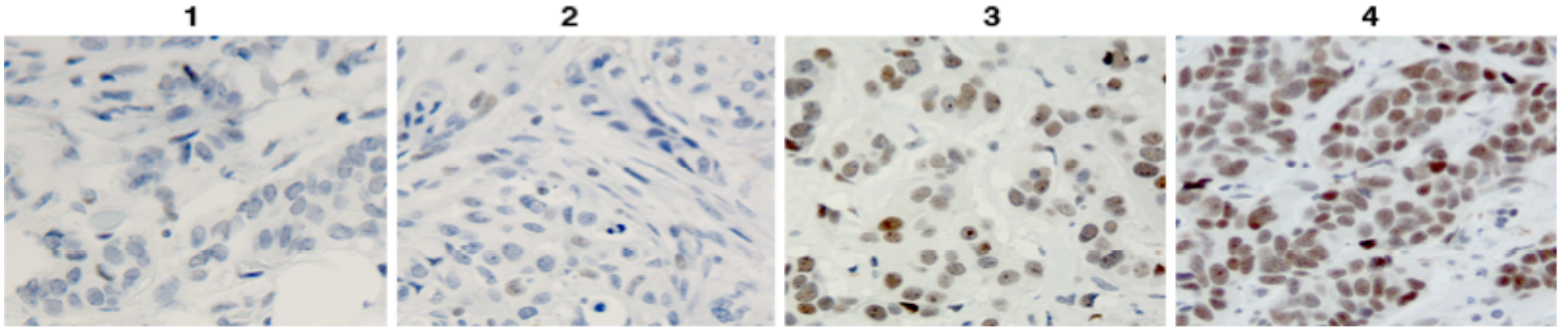


*: Taxol or Vehicle administration

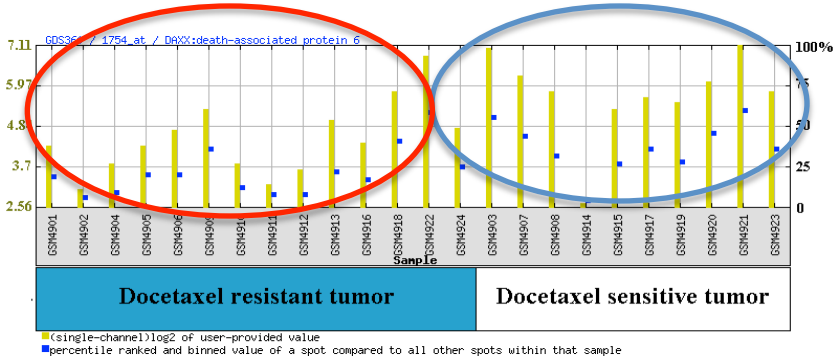
At day 15:	Control siRNA	Daxx siRNA
Relative tumor size (Vehicle)	5.87	6.23
Relative tumor size (Taxol)	1.61	4.02
Residual tumor size(T:V, %)	27.4%	64.5%

Depletion of Daxx reduces taxol response

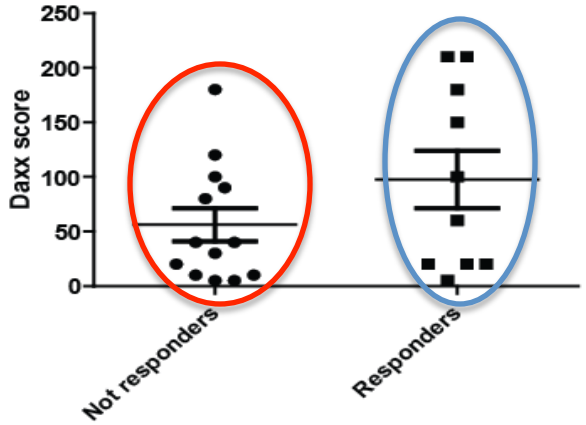
Clinical Relevance



Evaluation of Daxx accumulation in breast ductal carcinoma specimens. Heterogeneity of Daxx in breast malignancies may serve as a basis for differential response in patients to chemotherapy by taxanes.



Expression profile for Daxx in breast cancer core biopsies
https://www.oncomine.org



Accumulation of Daxx protein in breast cancer core biopsies

Giovinazzi et al., Oncogene 2012

Taxane response in breast cancer patients has reverse correlation with Daxx expression

Daxx and Taxol Resistance Summary

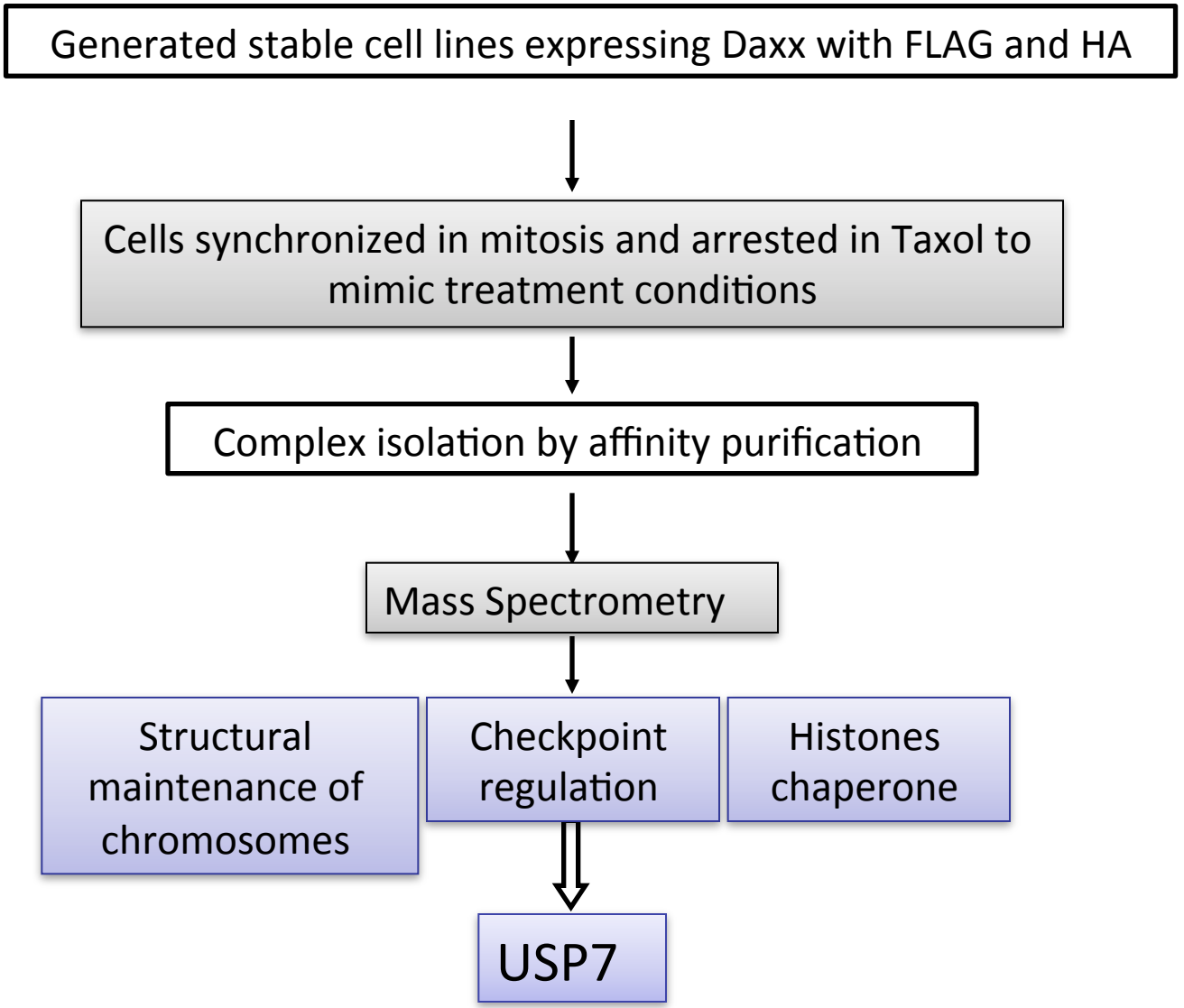
- Daxx protein levels inversely correlate with Taxol response in:
  - Cancer cell lines
  - Xenograft mouse model
  - Breast cancer patients

Daxx can be used as predictive marker for taxane response

- Daxx regulates mitosis:
  - Daxx depletion influences mitotic progression in human cells
    - Timing →Prolonged prometaphase block
    - Cyclin B1 stabilization

Lindsay, et al. 2007, *Cell Cycle*; Giovinazzi et al. 2012, *Oncogene*

Study of Daxx role in mitosis by functional proteomic approach



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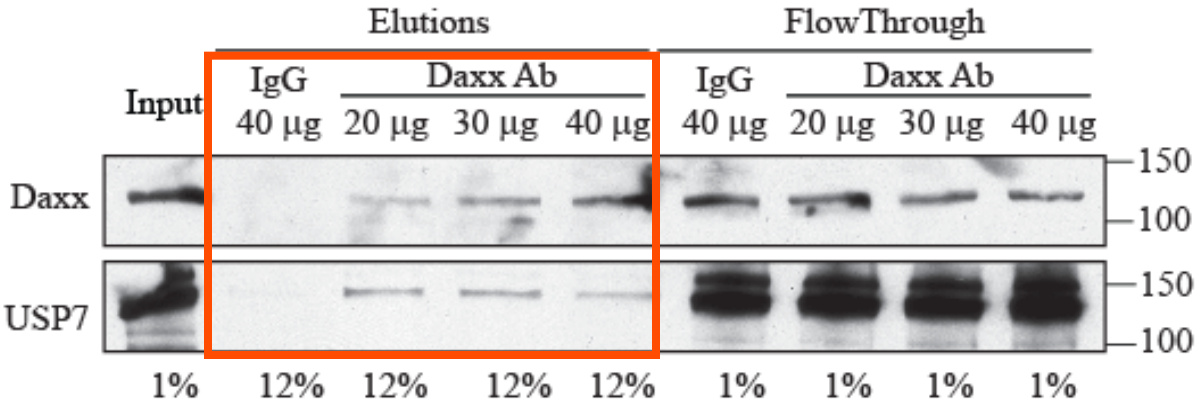
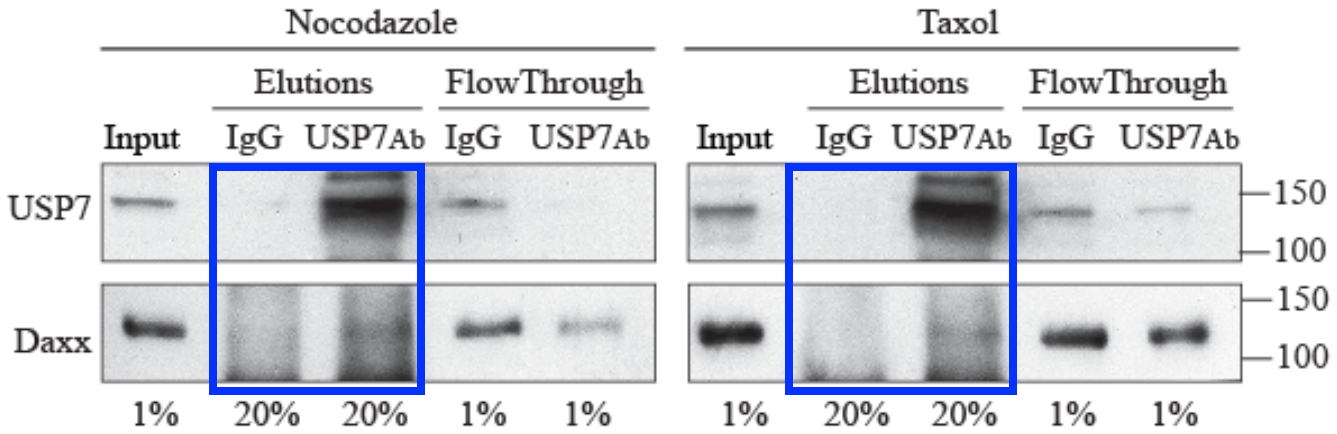
Validation of Daxx-USP7 Interaction

Daxx IP:



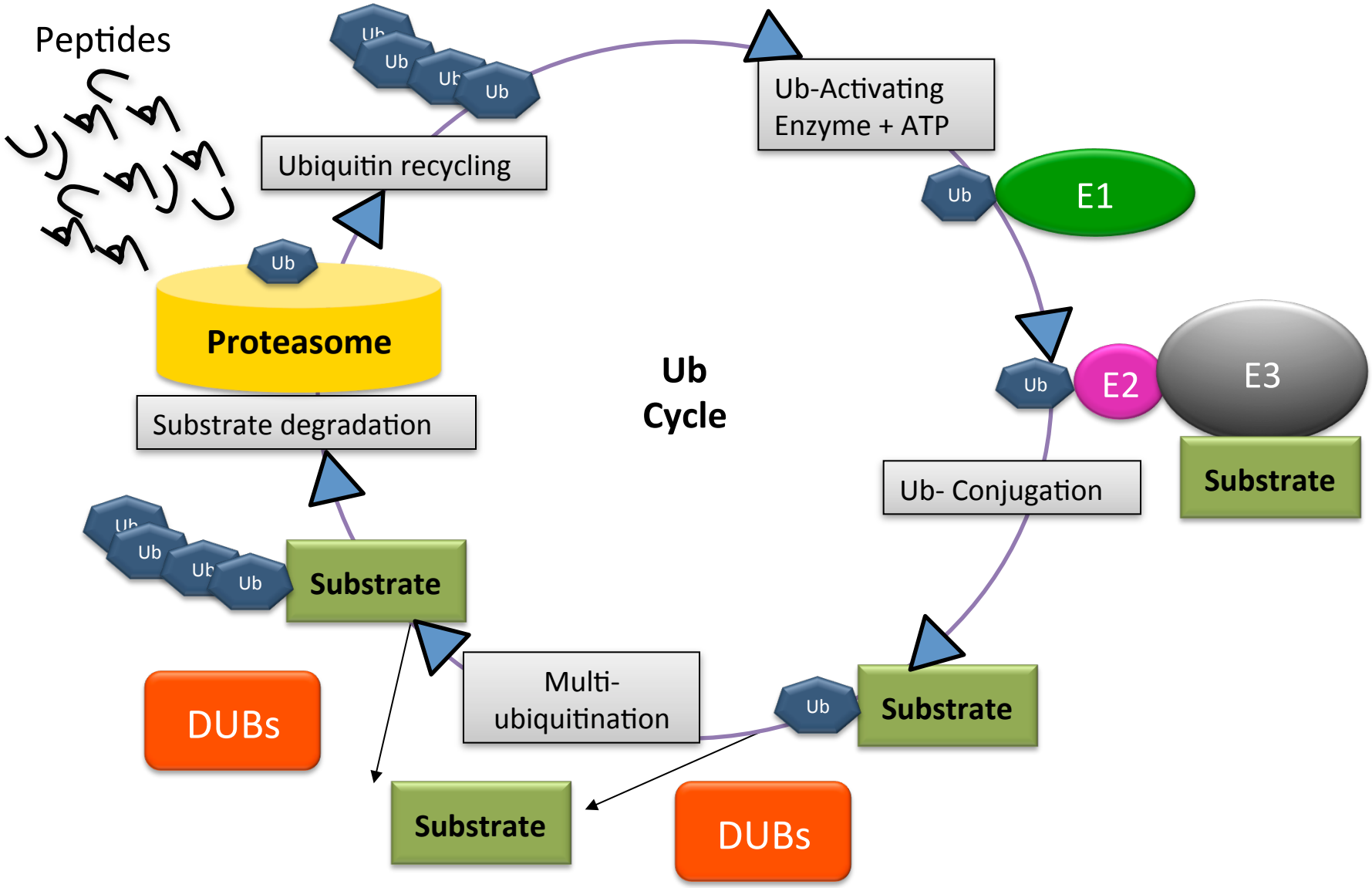
USP7 IP:



Daxx and USP7 interact in mitosis

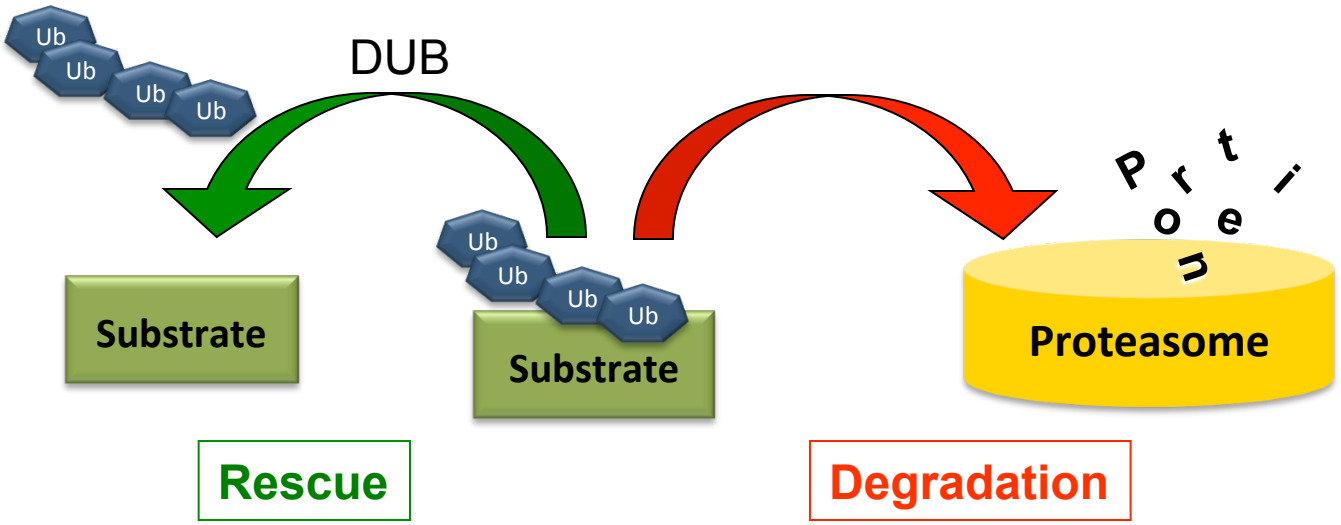
Giovinazzi et Cell Death Differentiation 2013

Ubiquitin dependent degradation pathway

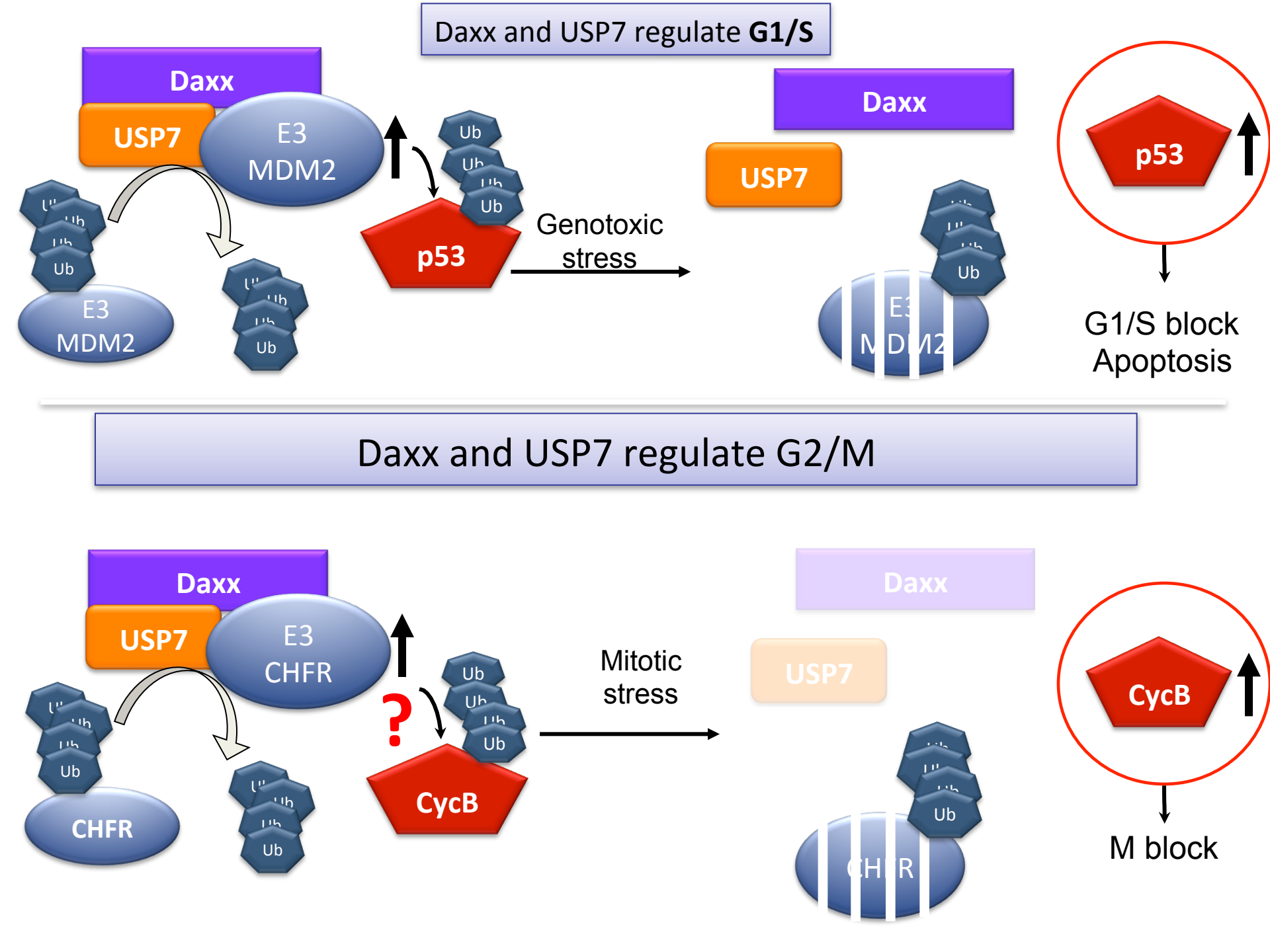


Daxx interacts with USP7 in mitosis

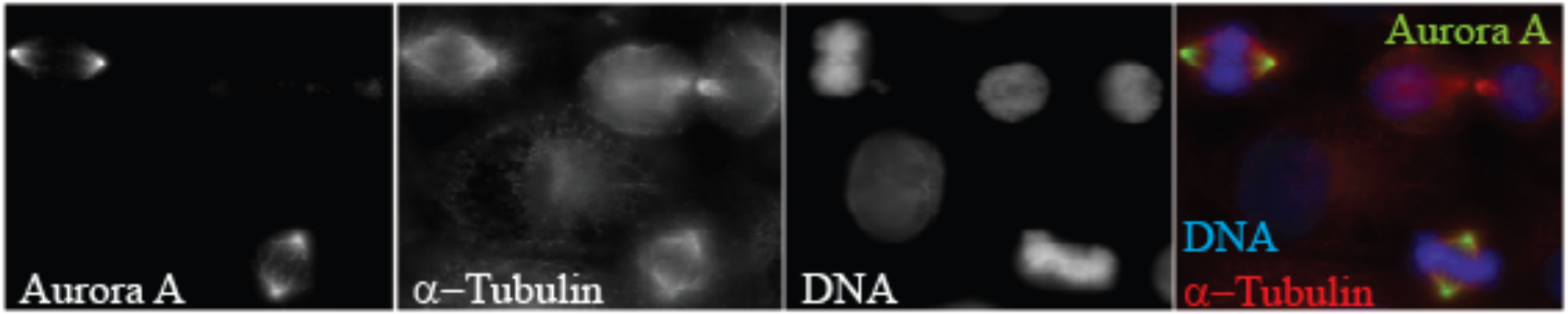
- **Ubiquitin-Specific-Processing Protease 7** (or USP7) is a de-ubiquitylating enzyme or DUB



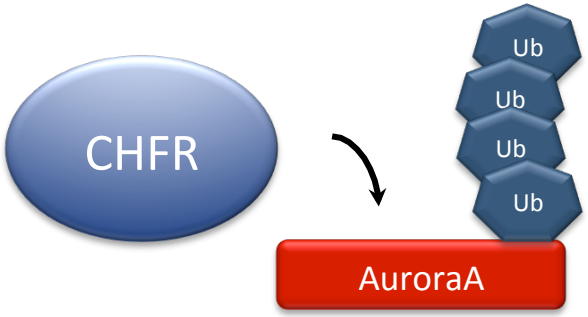
- Its activity leads to stabilization of substrate proteins
- Substrates involved in apoptosis, epigenetic maintenance, DNA stress response
- p53 is one of USP7 substrates


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

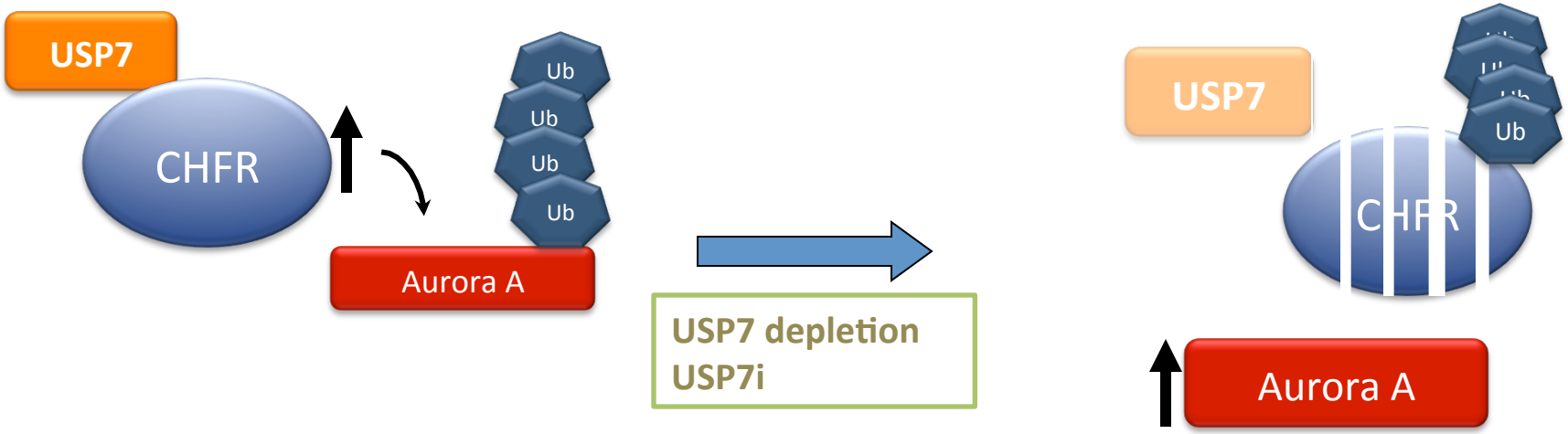
Aurora A kinase: the "Polar Aurora"

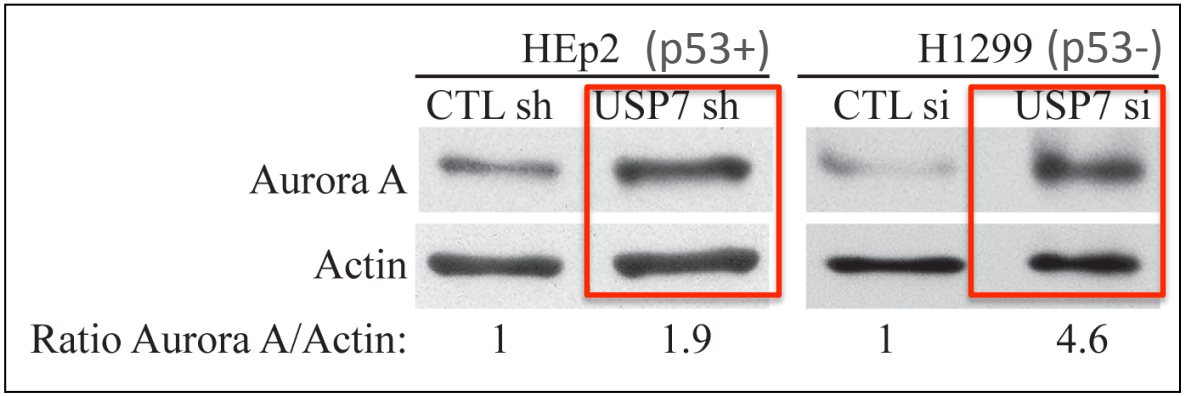


- CHFR regulates stability of Aurora-A
- The primary function of Aurora-A is to promote bipolar spindle assembly
- Aurora-A kinase gene is located in the 20q13 breast cancer amplicon and is over-expressed in breast, colorectal, pancreatic and gastric tumors
- Over-expressed Aurora-A
  - Multipolar-spindles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



Does USP7 affect Aurora A?

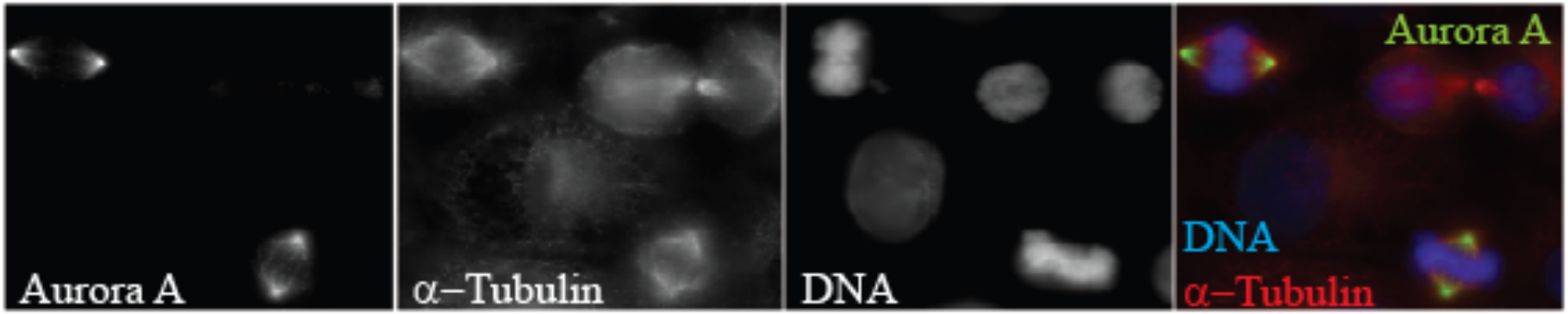




USP7 depleted cells stabilize checkpoint protein Aurora A in p53 positive and negative cells

Giovinazzi et al. *Cell Death Diff 2013*

Aurora A kinase: the “Polar Aurora”



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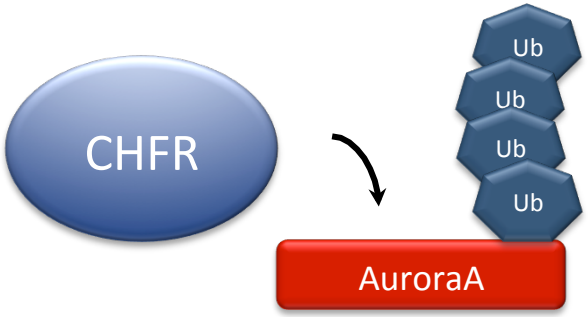
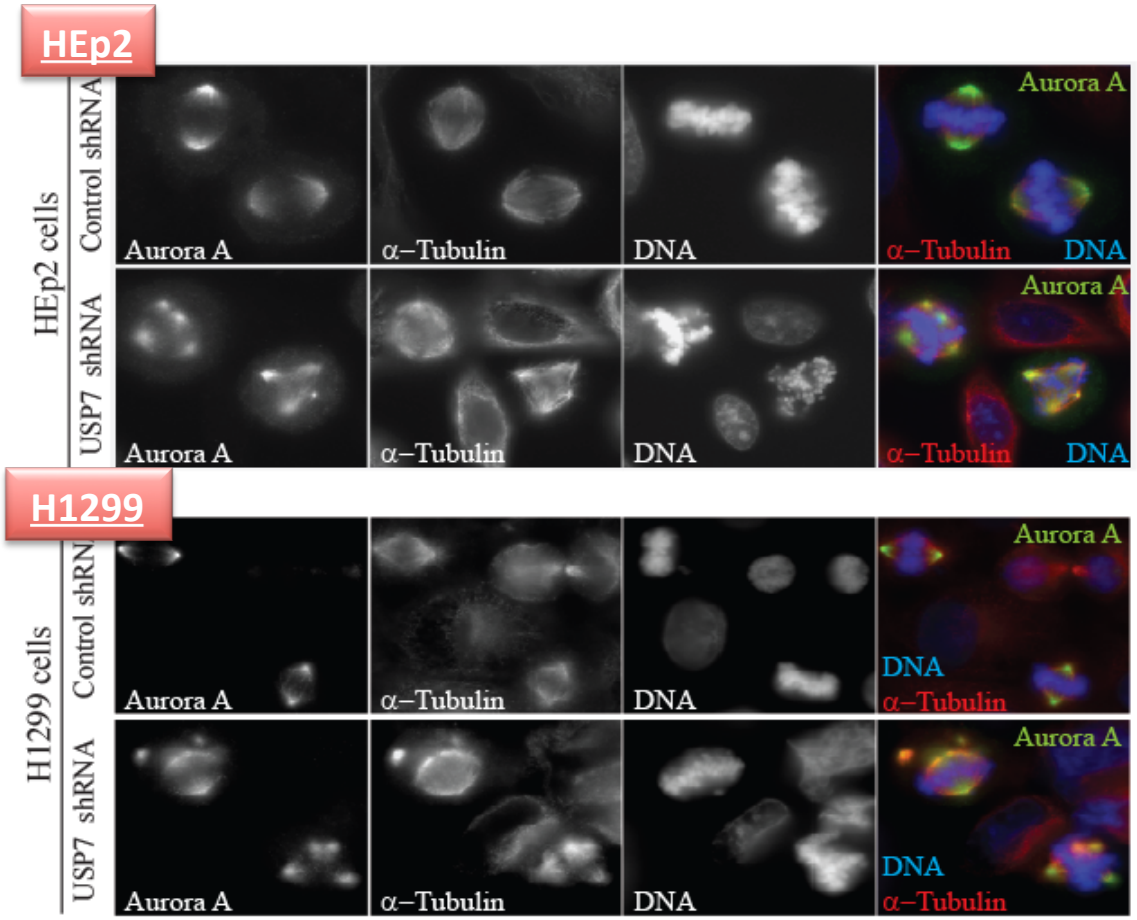
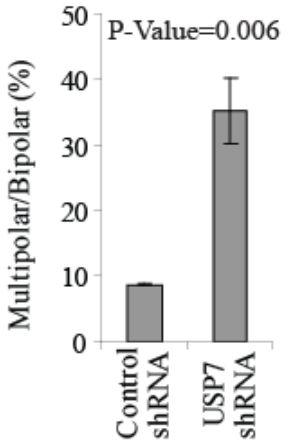
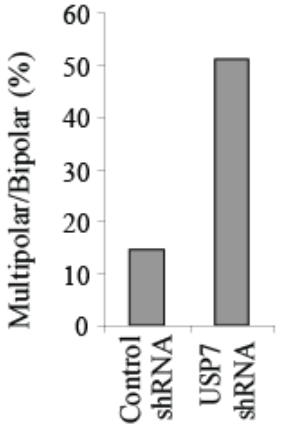


Do USP7 depleted cells accumulate multipolar spindles?

Immunofluorescence staining with **Aurora A kinase** and **α-tubulin**







Multipolar events over the total number of mitosis

<u>Similar results obtained:</u>

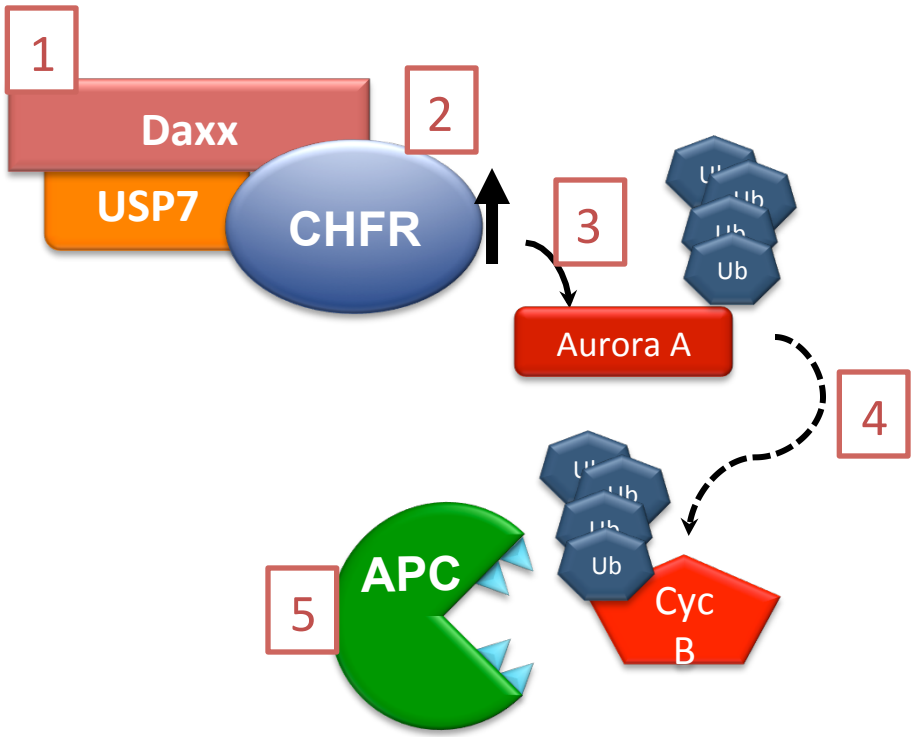
- ✓ Upon Daxx silencing
- ✓ In HCT116 p53+ and p53- cells
- ✓ Non-transformed breast epithelial MCF10A cells

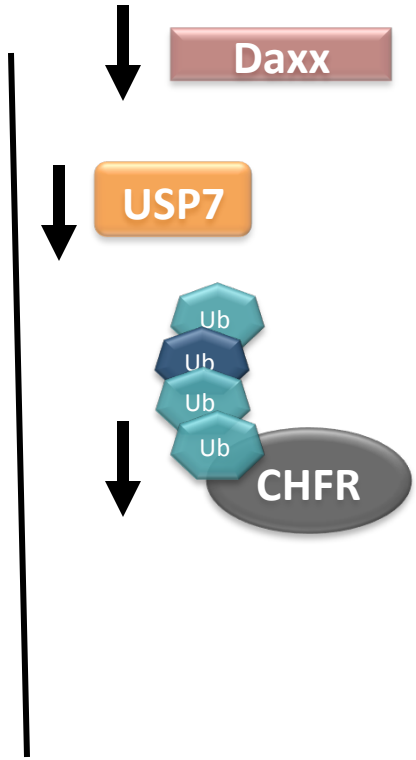
USP7 depleted cells accumulate multipolar spindles

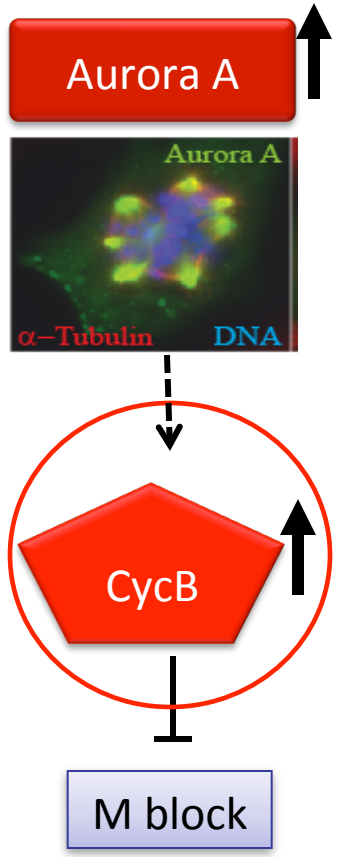
Giovinazzi et al. *Cell Death Diff 2013*

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

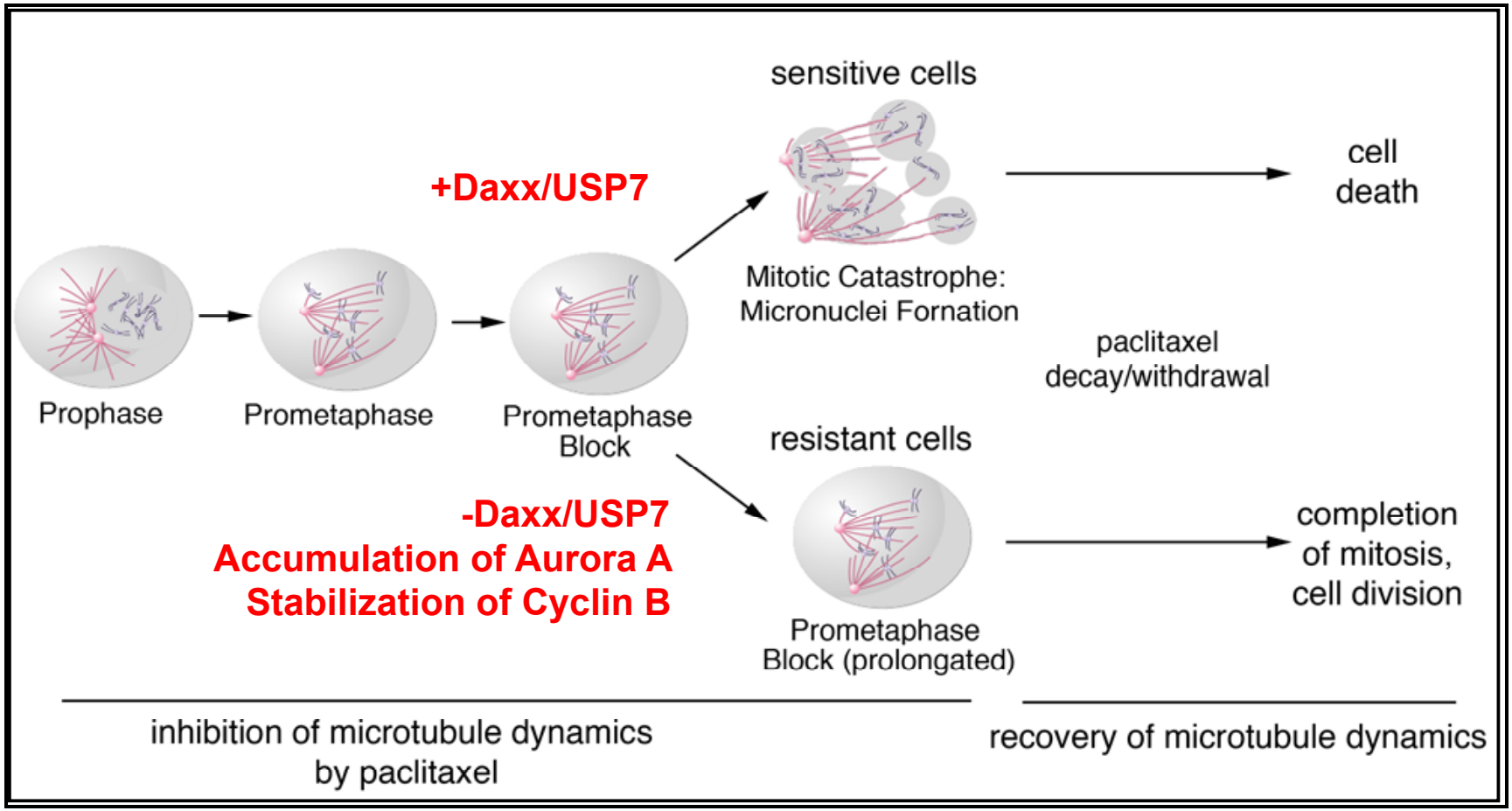






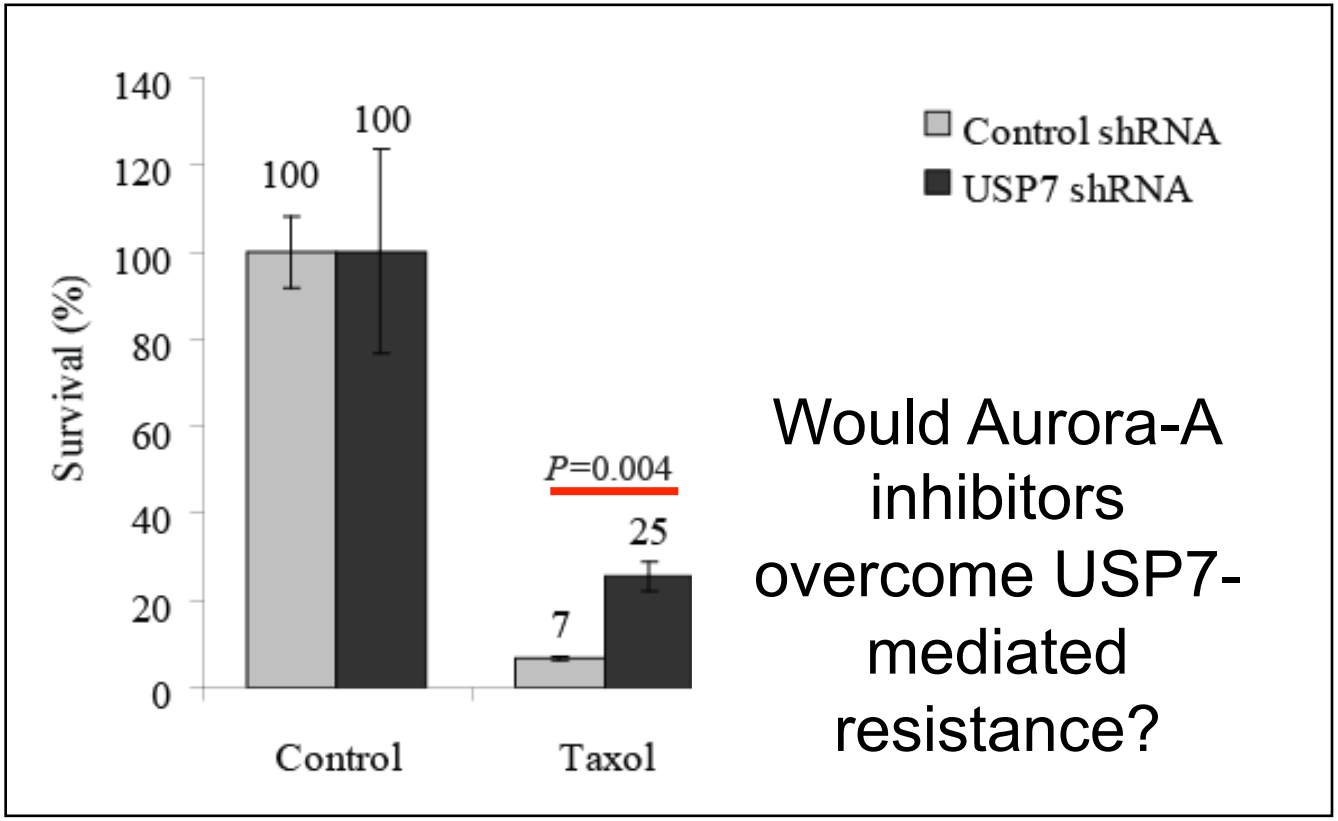
Working model

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



Does USP7 regulate response to taxanes?

Colony formation assay



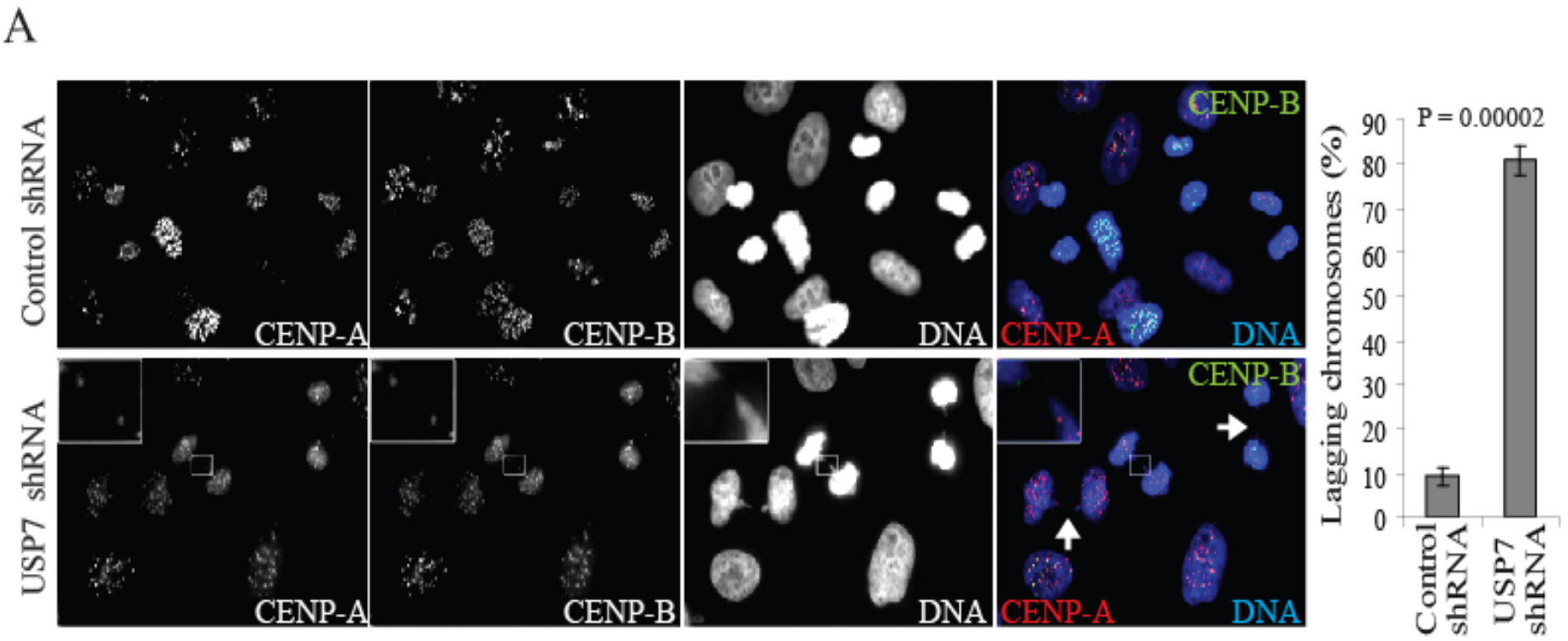
MLN8054: Selective small molecule inhibitor of Aurora-A

1. USP7 Silencing Increases Cellular Resistance to Taxol
2. Aurora-A inhibitor combined with Taxol **partially** rescues USP7-mediated resistance
3. Additional, Aurora-A independent mechanism?

Giovinazzi et al. *Cell Death Diff 2013*

Analysis of Anaphases

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

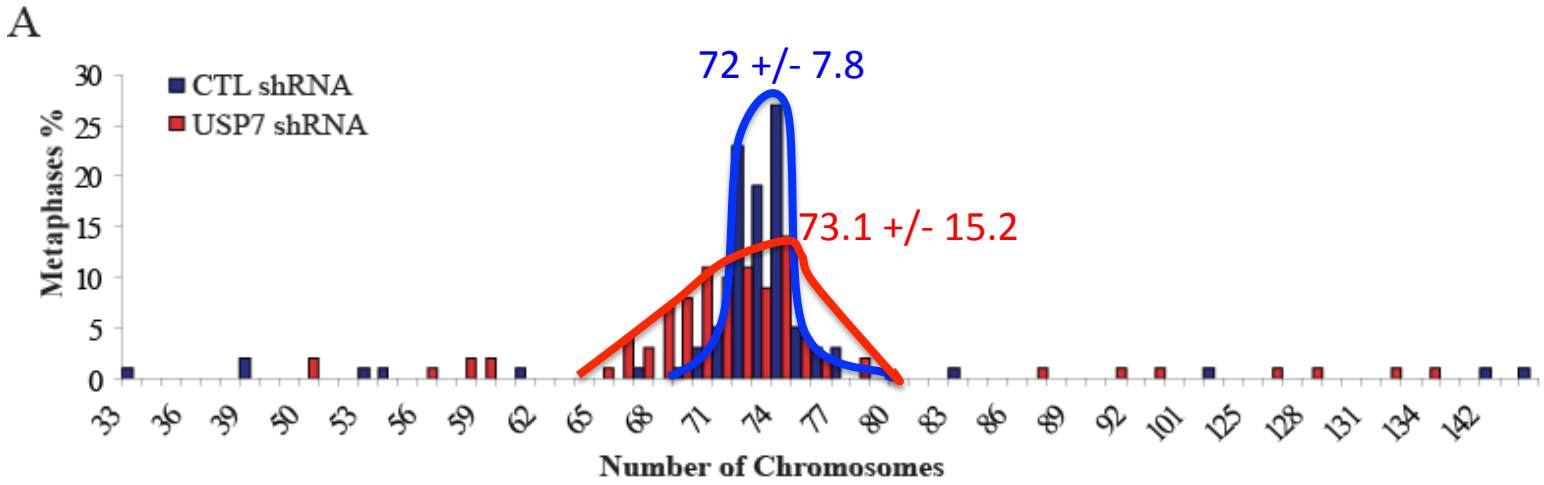


- USP7 depleted cells accumulate lagging chromosomes in anaphases
- The increase in number of lagging chromosomes explains the high MN scores documented upon USP7 depletion

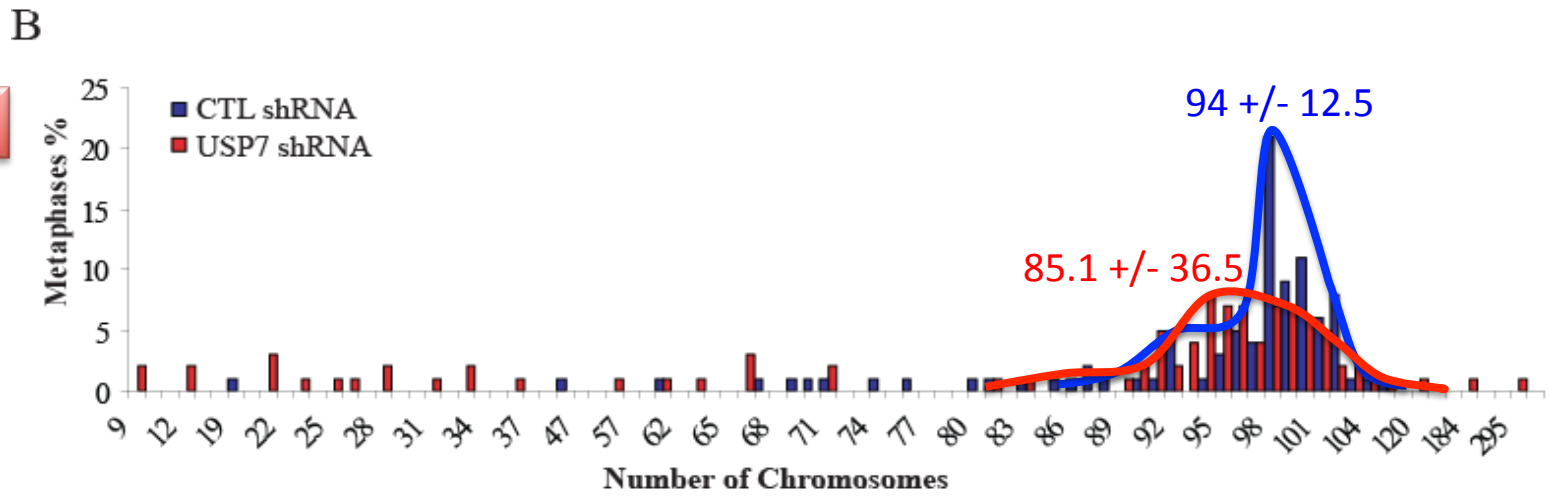
Giovinazzi et al., *Oncotarget* 2014

Karyotype in USP7-depleted cells

HEp2



H1299

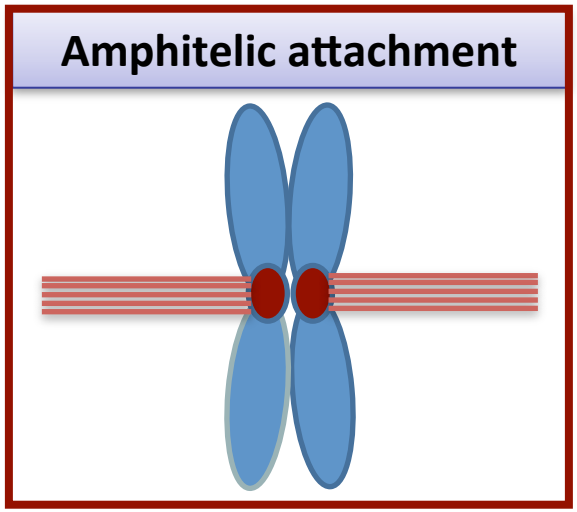


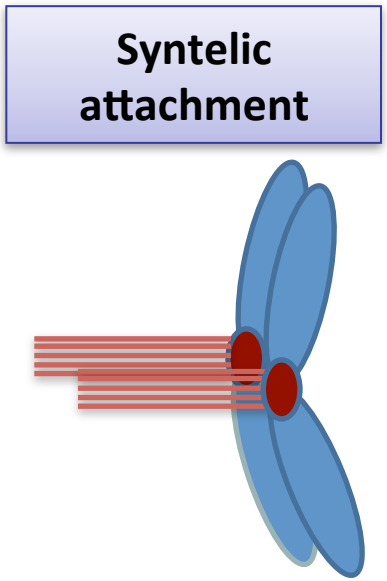
USP7 depletion increases aneuploidy:

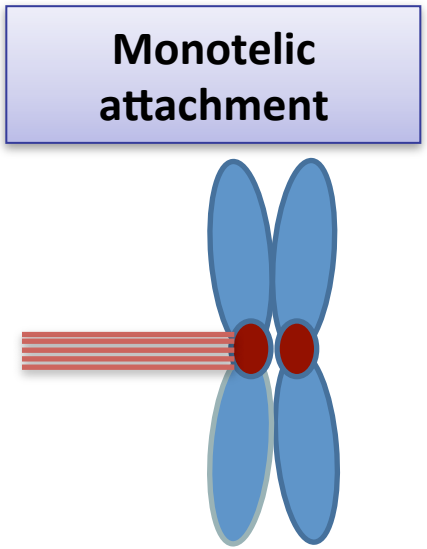
- In cancer cells (HEp2 and H1299)
- Non-tumorigenic breast epithelial cells MCF-10A

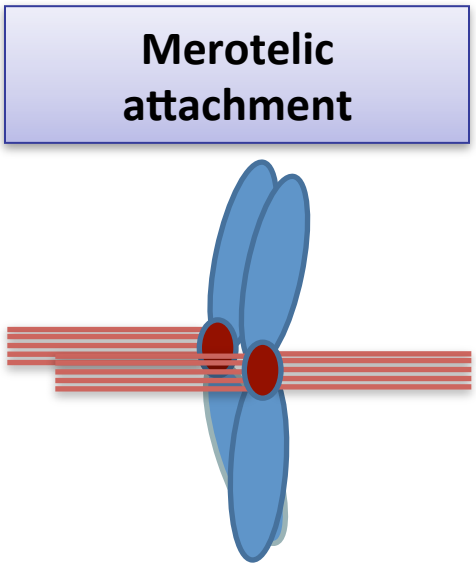
Giovinazzi et al., *Oncotarget* 2014

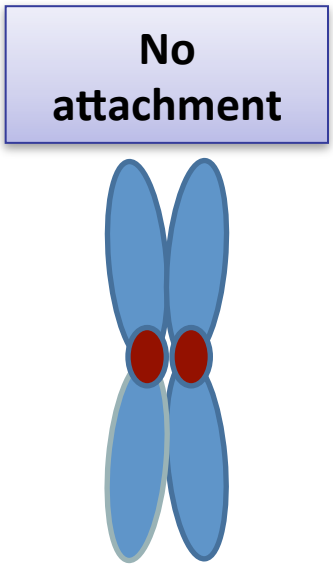
Genomic Instability Arises from the Inability to Correct Errors







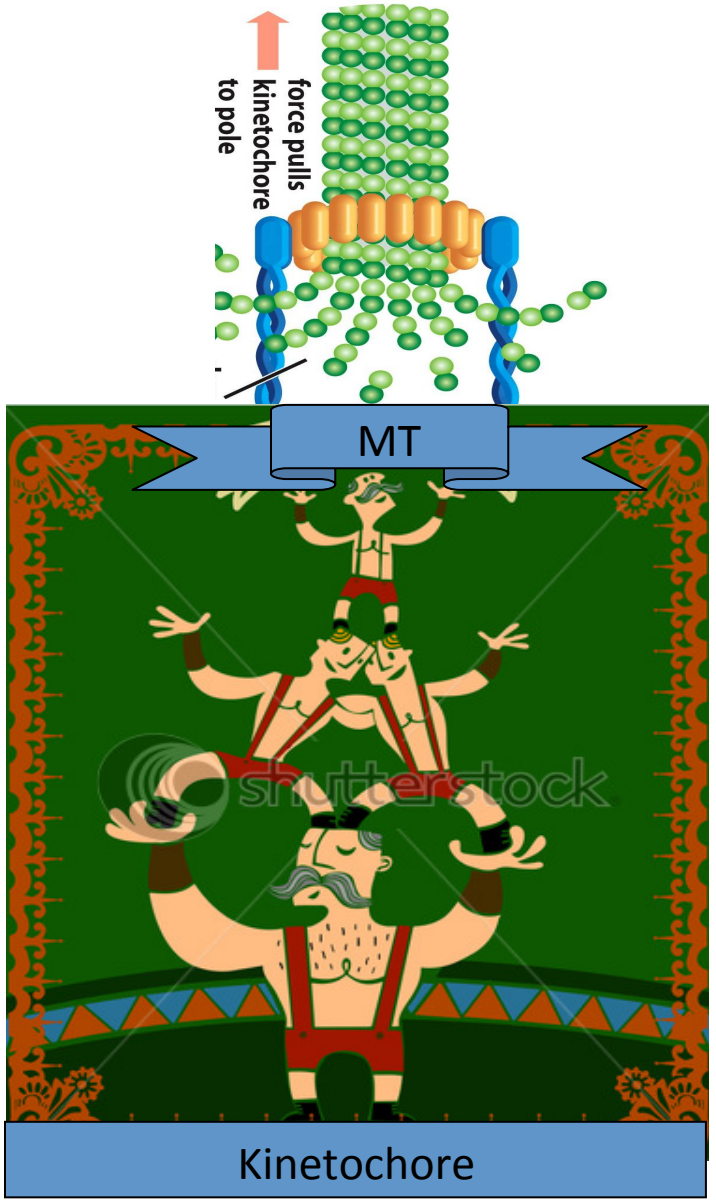




SAC sensors and signal transducer

Functions:

- Essential for establishment of MT-K
- Essential for correction of faulty attachments
- Produce a ‘Wait’ signal until satisfactory MT-K are achieved



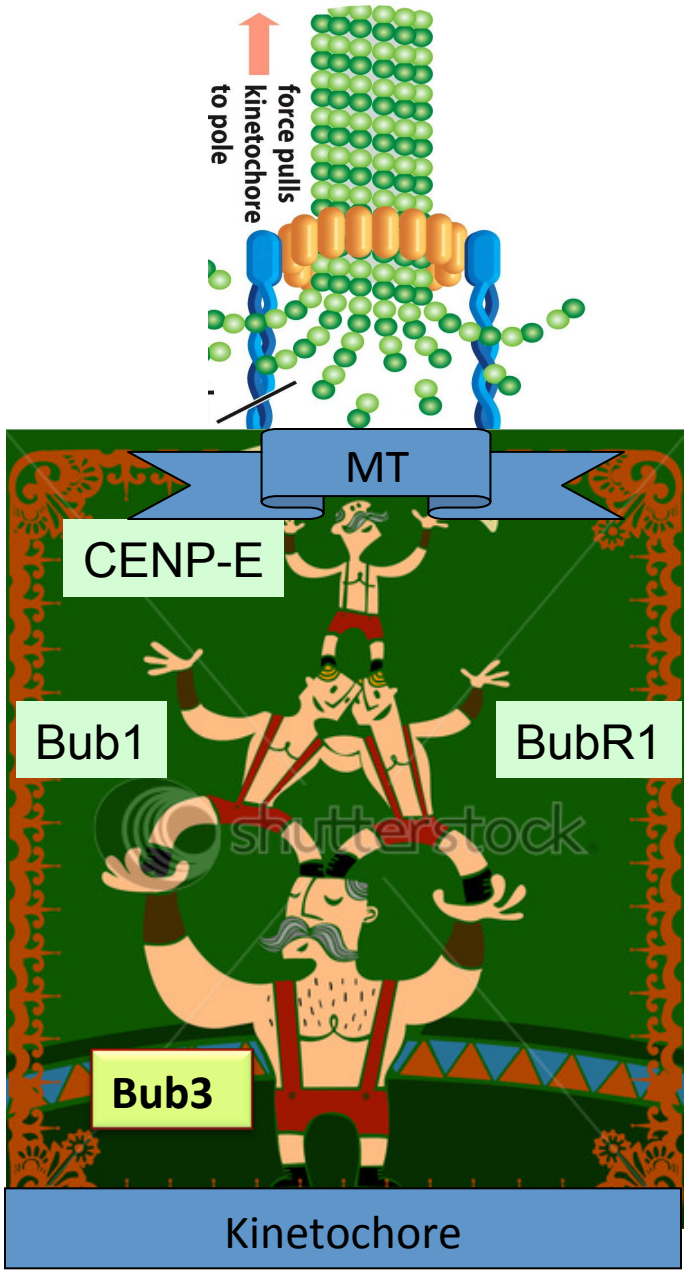
Defining the Human Deubiquitinating Enzyme Interaction Landscape

Mathew E. Sowa[1,§], Eric J. Bennett[1,§], Steven P. Gygi[2], and J. Wade Harper[1,*]

Bait	COMMON
USP7	AKAP1 BRCC3 BRE BUB3 C10orf119 C1orf164 CCDC6 CRKL DNMT1 DOCK7 EIF4B FBXO38 GMPS GTF2I HLTF HSPC142 KIAA0157 KIF3A MCM4 MCM6 NUP98 PPIL4 SCML2 SRP68 TRIP12 USP14 USP19

"Interactome" analysis of DUB interaction partners

Bub3



Bub3 functions:

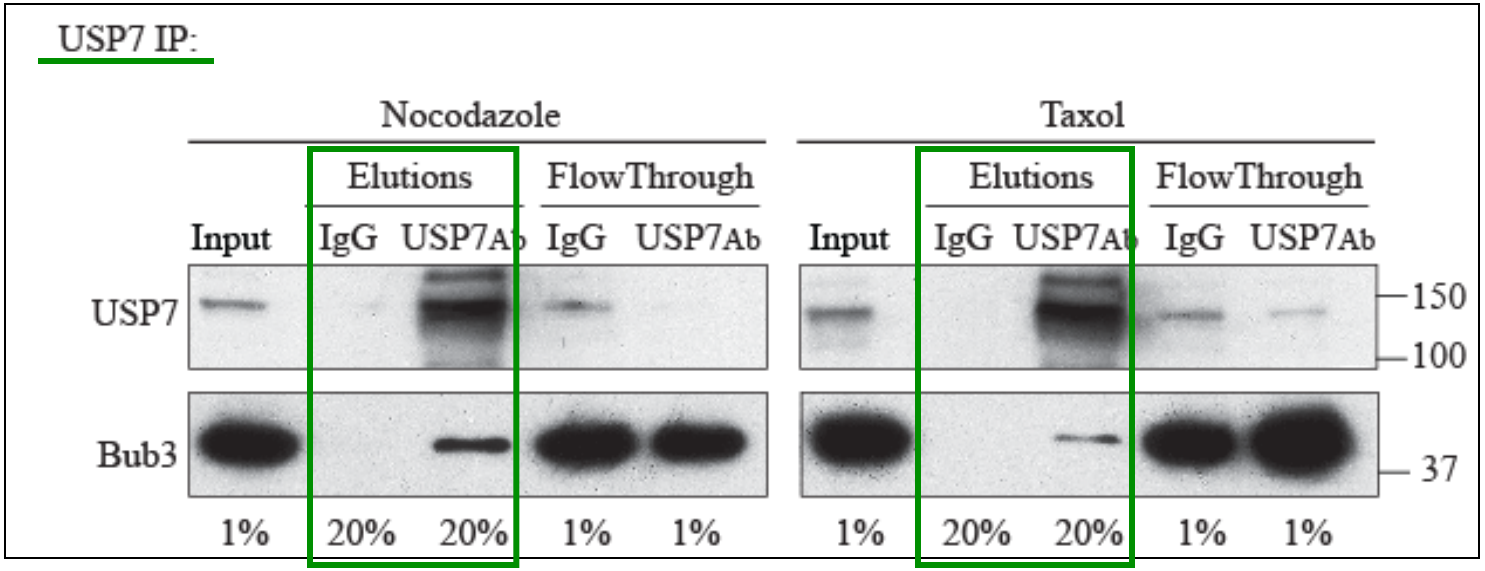
- To recruit other SAC components, thus is essential for MT-K
- Produce a ‘Wait’ signal until satisfactory MT-K are

If lacking:

- Increase in faulty attachments
- Lagging chormosomes

USP7 and Bub3: Interaction

Immunoprecipitation experiments of endogenous USP7 in HEp2 cells synchronized in mitosis by nocodazole (left) or Taxol (right) exposure. Numbers below the blots represent the sample percentile loaded.

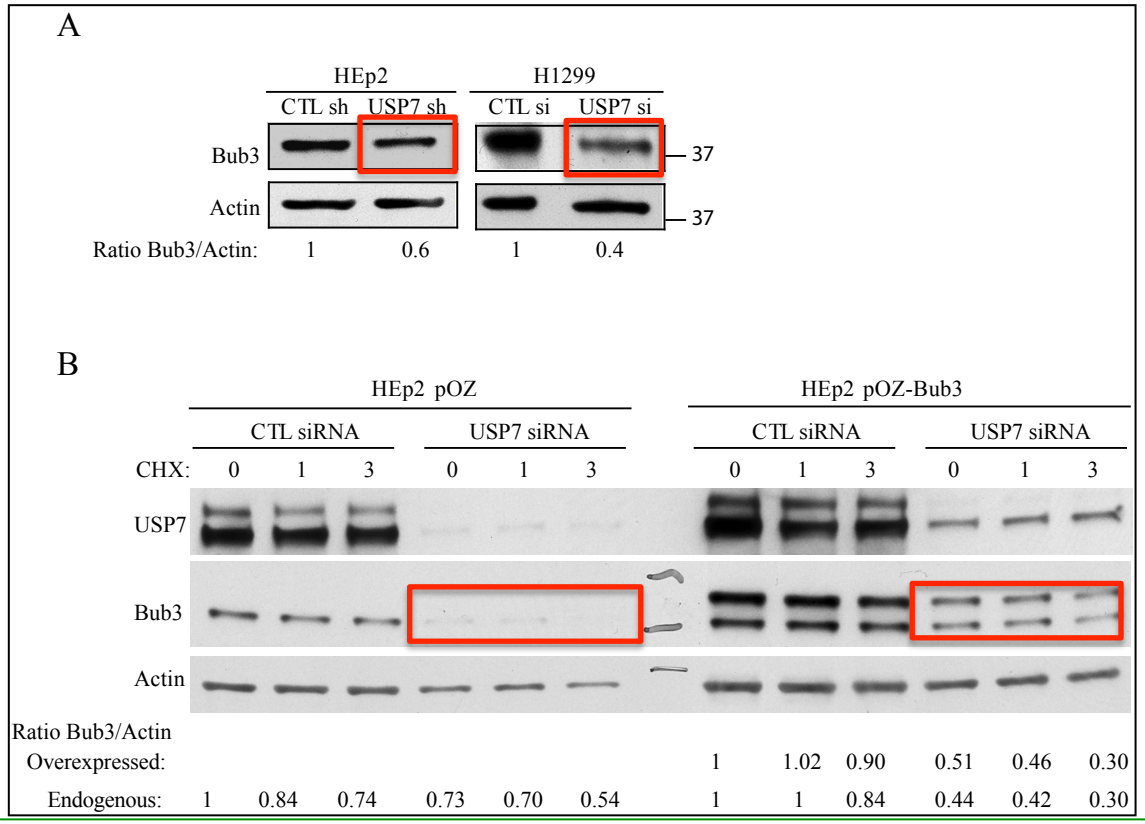


USP7 and Bub3 interact in mitosis

Giovinazzi et al., *Oncotarget* 2014

USP7 and Bub3: Protein Stability

Analysis of Bub3 protein levels in control- and USP7 depleted HEp2 and H1299 cells. Numbers below the blot represent relative quantification of Bub3 signal over actin. CHX: cycloheximide.



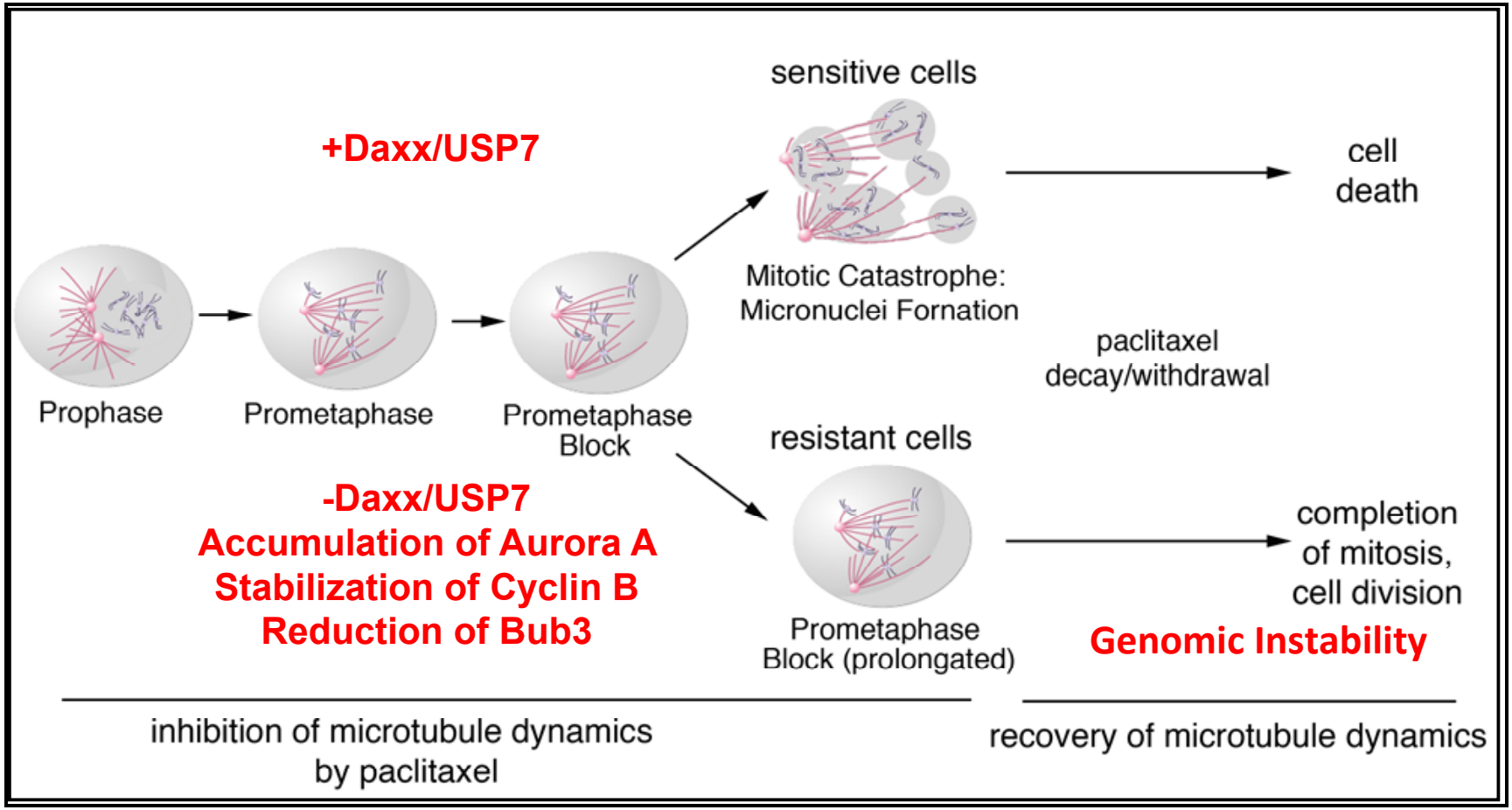
Levels of Bub3 are decreased in cells with depleted USP7

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

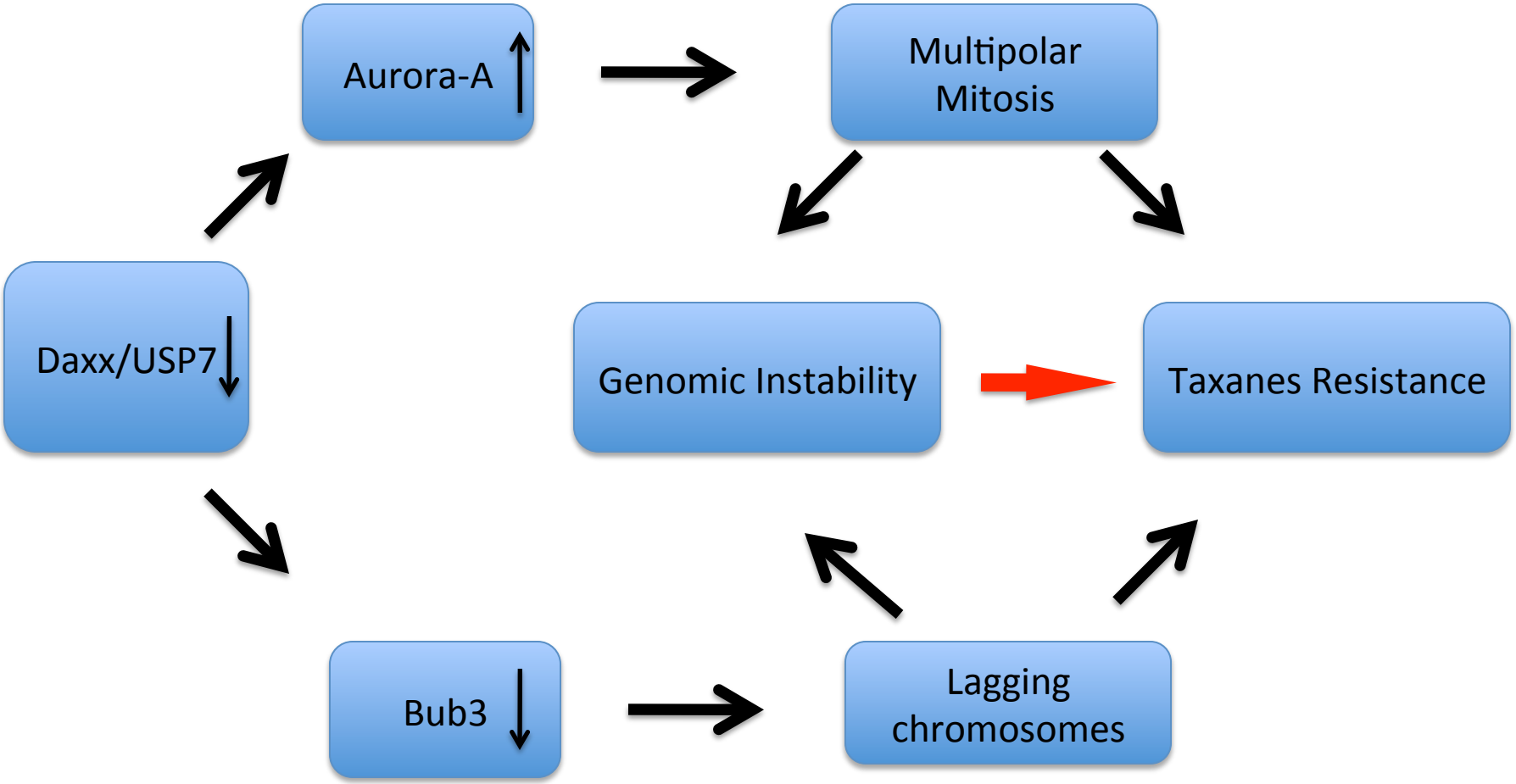
Giovinazzi et al., *Oncotarget* 2014

Working model

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



USP7/Daxx in taxane resistance



Hypothesis: Genomic Instability 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

- Цитотоксическая химиотерапия рака молочной железы

- Деубиквитиназа USP7 и резистентность к антимитотической химиотерапии

- Новые подходы в преодолении резистентности

Representative mitotic targets and inhibitors in clinical or preclinical studies

Target	Activity	Inhibitors	Clinical phase	Tumor types
Clinical stage				
Aurora A	Bipolar spindle formation	MLN-8237, MLN-8054	I–II	Melanoma, hematopoietic malignancies, ovarian, breast and prostate carcinoma
Aurora B	Chromosome alignment and mitotic checkpoint	GSK1070916A, AZD1152	I	Advanced solid tumors and acute myeloid leukemia
Cdk1	Mitotic entry and progression	P276-00, EM-1421	I–II	Hematopoietic malignancies, breast cancer, melanoma
CENP-E	Spindle dynamics and mitotic checkpoint	GSK923295A	I	Acute lymphoblastic leukemia
Eg5	Spindle dynamics	Ispinesib, AZD4877, ARRY-520	I–II	Hematopoietic malignancies, bladder cancer and advanced solid tumors
Plk1	Bipolar spindle formation	GSK461364, TKM-080301, NMS-1286937, BI6727, ON01910	I–II	Advanced or metastatic solid tumors and hematopoietic malignancies
Preclinical stage				
APC/C (Cdc20)	Mitotic E3-ubiquitin ligase	TAME	None	
Mps1	Mitotic checkpoint	NMS-P715, reversine, Mps1 IN-1	None	

Manchado E, et al. Cell Death Differ. 2012 19(3):369-77

Targeting mitosis for cancer therapy

- Recent studies of mitosis regulation opened new directions in cancer therapy.
- Targeting mitotic exit has been proposed as a better therapeutic strategy than targeting spindle assembly and spindle regulators.
- Targeting regulators of mitotic entry, progression and exit have demonstrated efficacy in preclinical models but limited activity in clinical trials.

Outstanding Question:

- Why new mitotic inhibitors have demonstrated limited activity *in vivo* in comparison with classical microtubule-targeting drugs as Taxol?

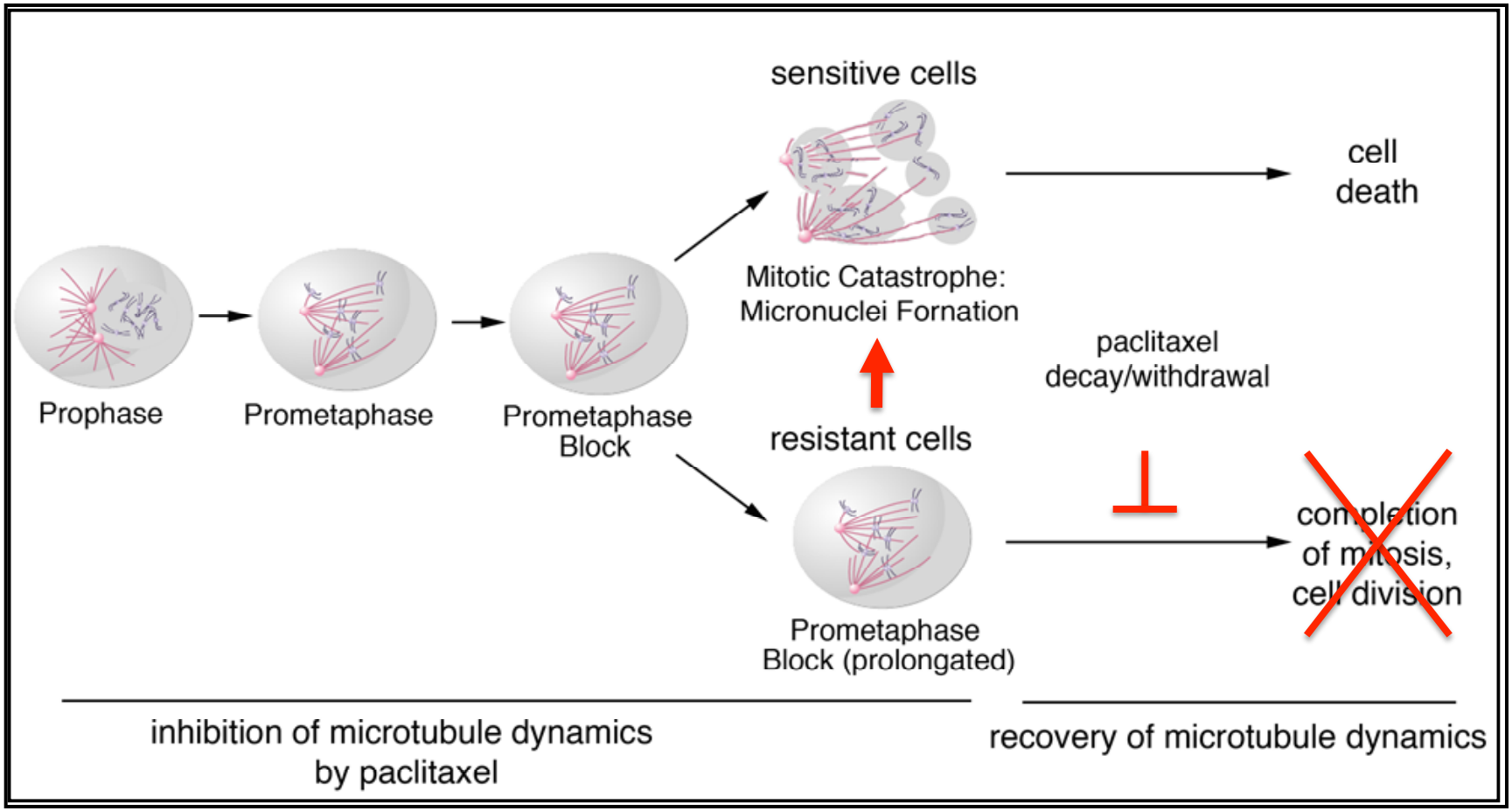
Directions of Study:

- We need to identify and characterize specific biomarkers for a better definition of patients that may benefit from therapeutic strategies targeting mitosis.
- We need to understand the molecular mechanisms of mitotic cell death. Can modulation of these cell death pathways synergize with current antimitotic drugs?

Modified from: Manchado E, et al. Cell Death Differ. 2012 19(3):369-77

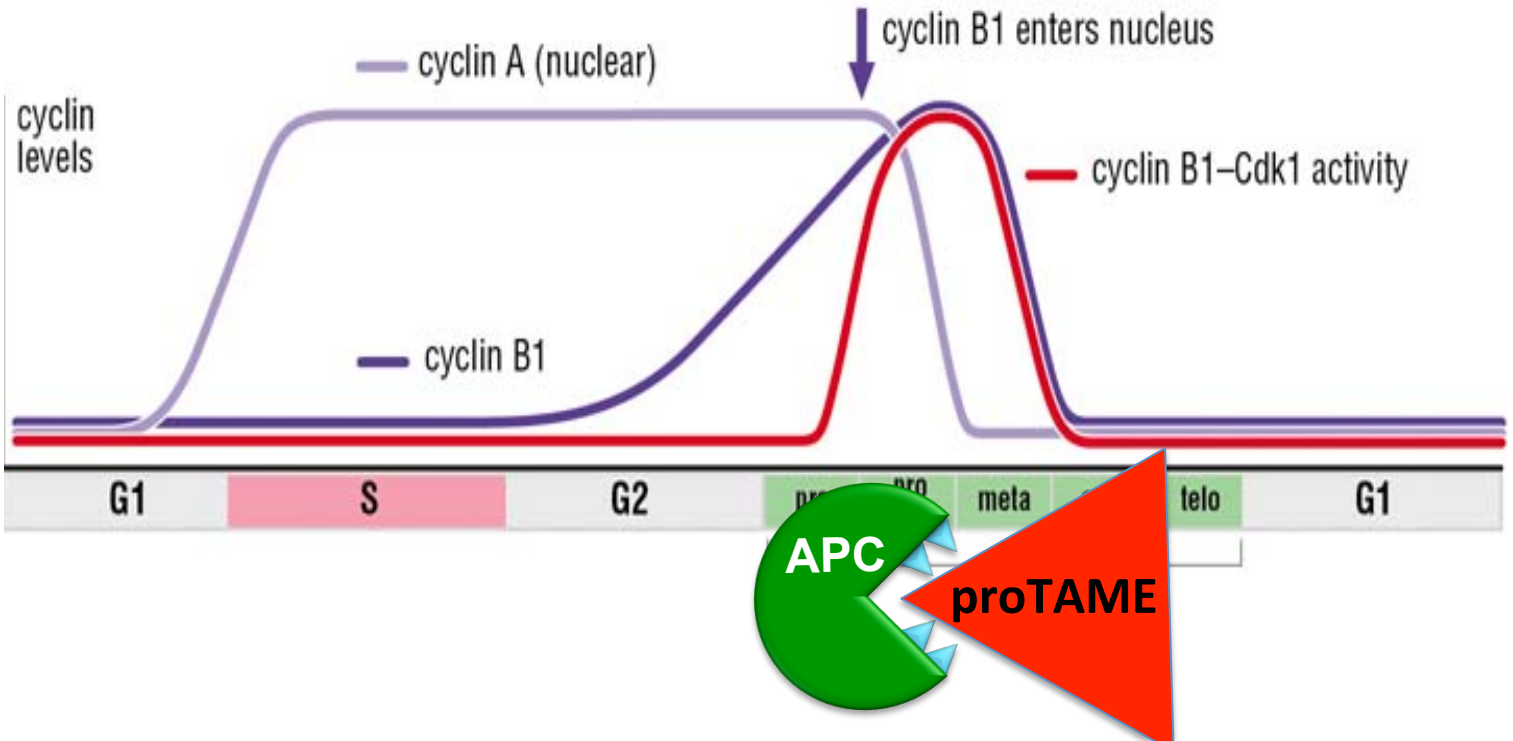
Резистентность к химиотерапии:
кто виноват и что делать?

Mitotic Poisons in Chemotherapy: “Should I Stay (in Mitosis) or Should I Go”?



Mitotic Checkpoints, Cyclins and APC

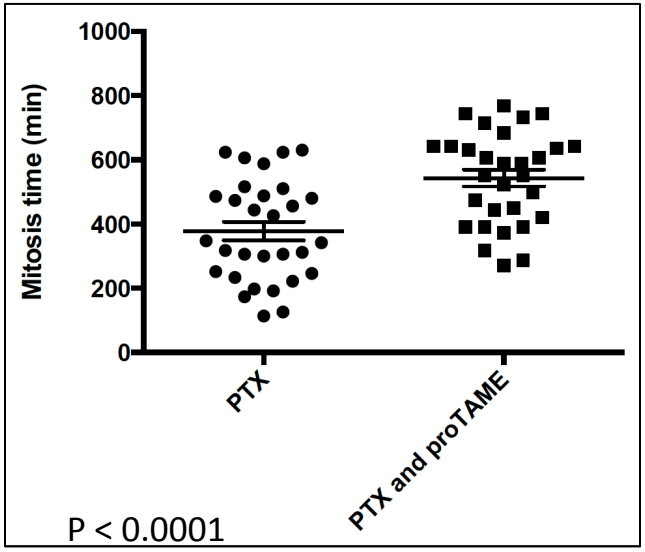
- Cyclin B is required for early Mitotic events
- Accumulates in G2/Prophase
- Cyclin B is degraded at the Methaphase-Anaphase transition by the E3 ubiquitin ligase APC (Anaphase Promoting Complex)



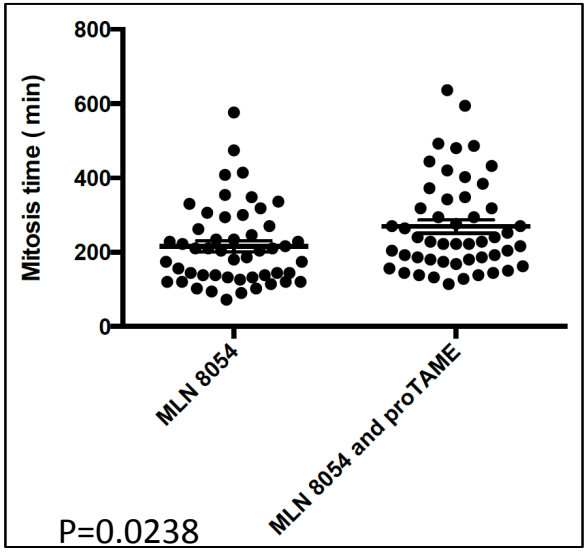
Mitotic timing of cells treated with Taxol, Aurora A inhibitor MLN8054 and APC/C inhibitor proTAME

Time lapse analysis of mitotic progression in HEp2-H2B-GFP cells. Cells were treated with Taxol (PTX, 10nM, 12h) Aurora A inhibitor (MLN 8054, 4mkM, 8h); proTAME (12mkM) was added at the beginning of movie. 30 mitoses analyzed for PTX and PTX+proTAME; 50 mitoses analyzed for MLN8054 and MLN8054+proTAME

Taxol

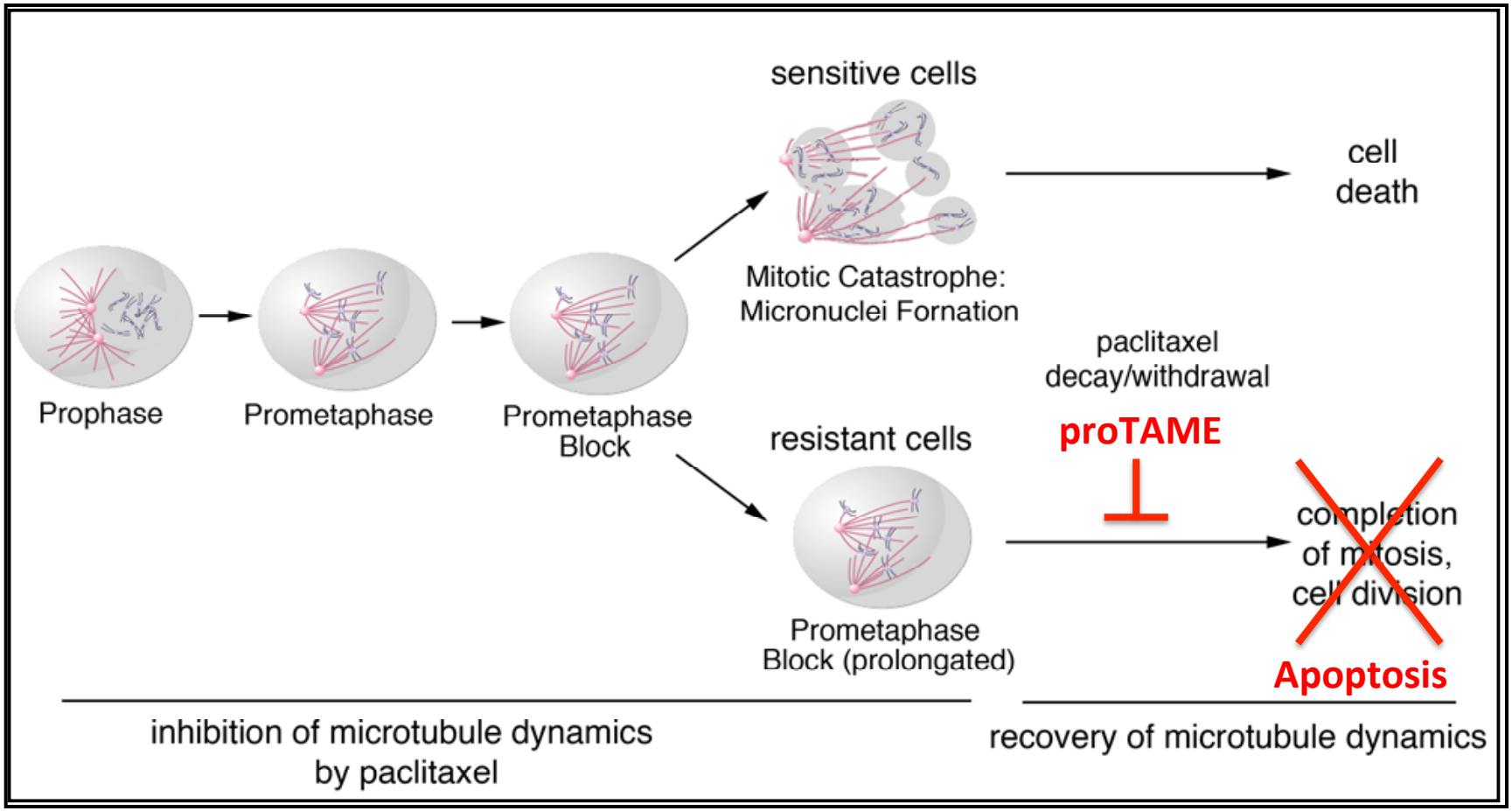


Aurora A inhibitor

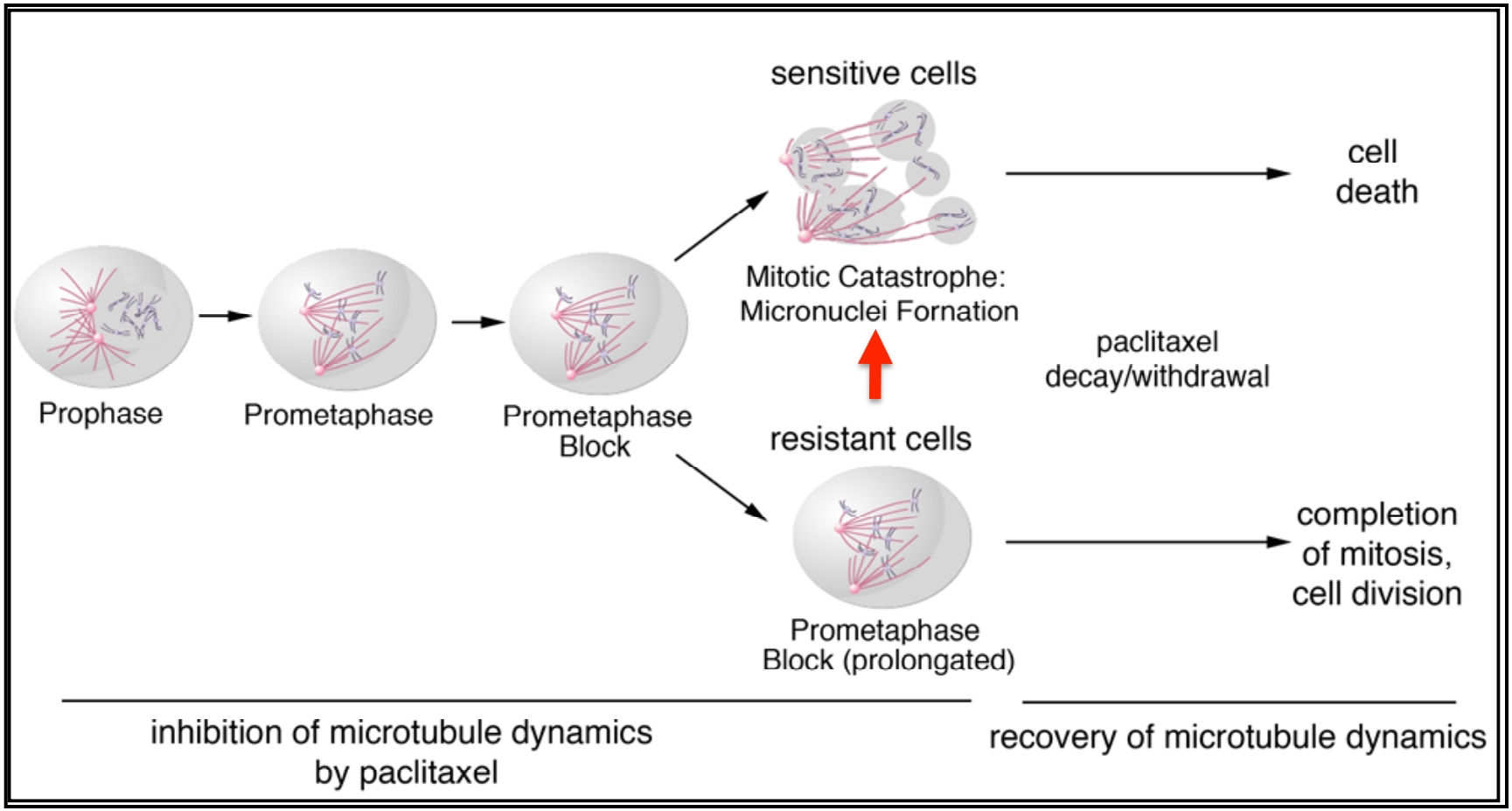


Giovinazzi et al., *Cell Cycle* 2013

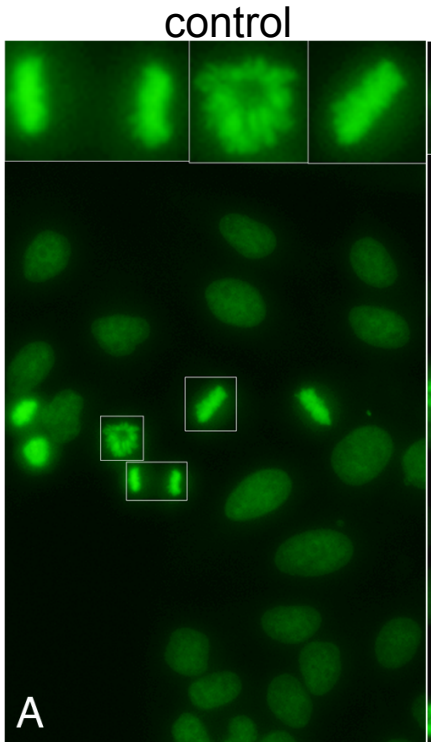
Mitotic Poisons in Chemotherapy: “Should I Stay…



Mitotic Poisons in Chemotherapy: "…or Should I Go"?



…Or Should I go?



DNA staining of HEp2 cells

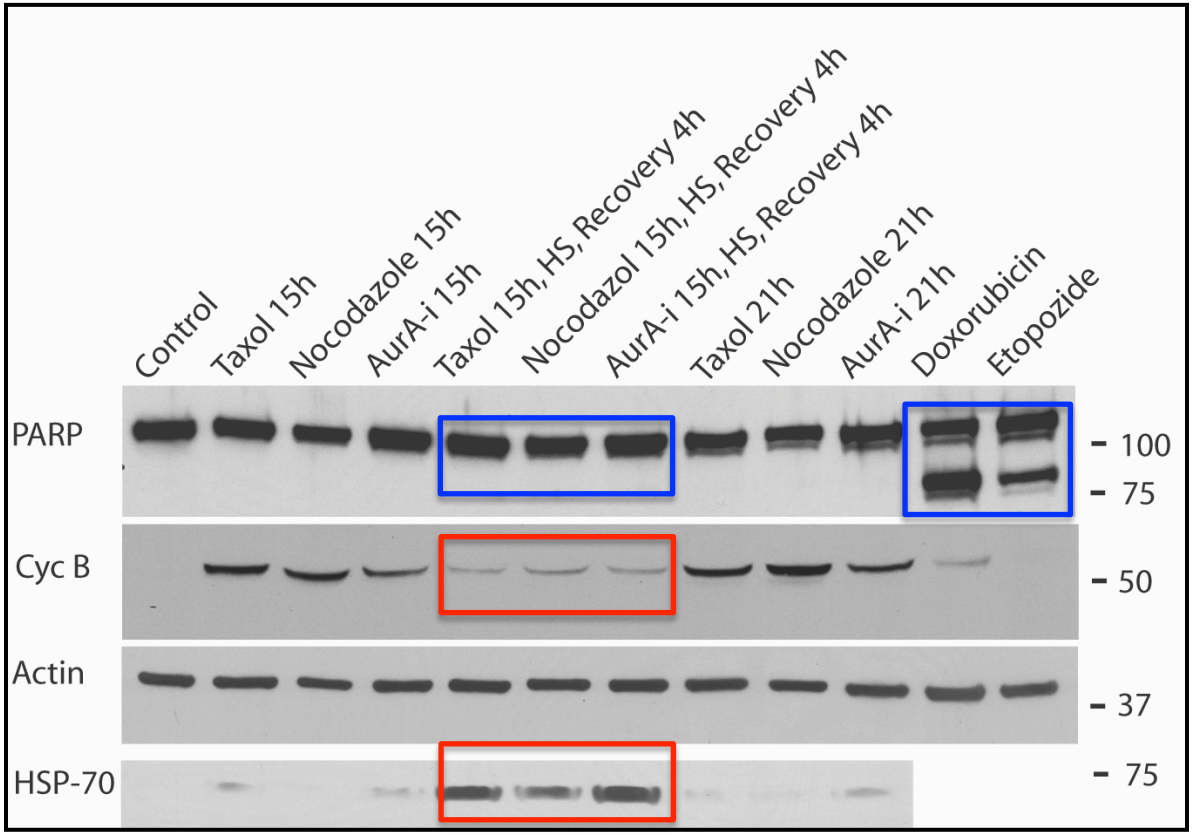
- Control: all stages of mitosis were detected (in the magnified boxes a pro-methaphase, a metaphase and anaphase are represented).
- 18 h of PTX treatment: majority of cells were arrested in prometa-methaphase.
- PTX treated cells + HS = massive mitotic catastrophe.

Heat shock forces mitotic catastrophe in PTX arrested cells!

Giovinazzi et al., *Cell Cycle* 2013

HS induces degradation of Cycline B in cells treated by PTX (Taxol), Nocodazole, Aurora-A inhibitor

Analysis of PARP cleavage (apoptotic marker) and Cyclin B stability in HEp2 cells treated for a total time of 21h with **PTX, Nocodazole (Noc) or MLN8054**. After 15h of treatment some samples were exposed to 2h HS and then returned to 37ºC for 4h (R4h). Doxorubicin and etoposide were used as positive controls of PARP cleavage (apoptosis induction)

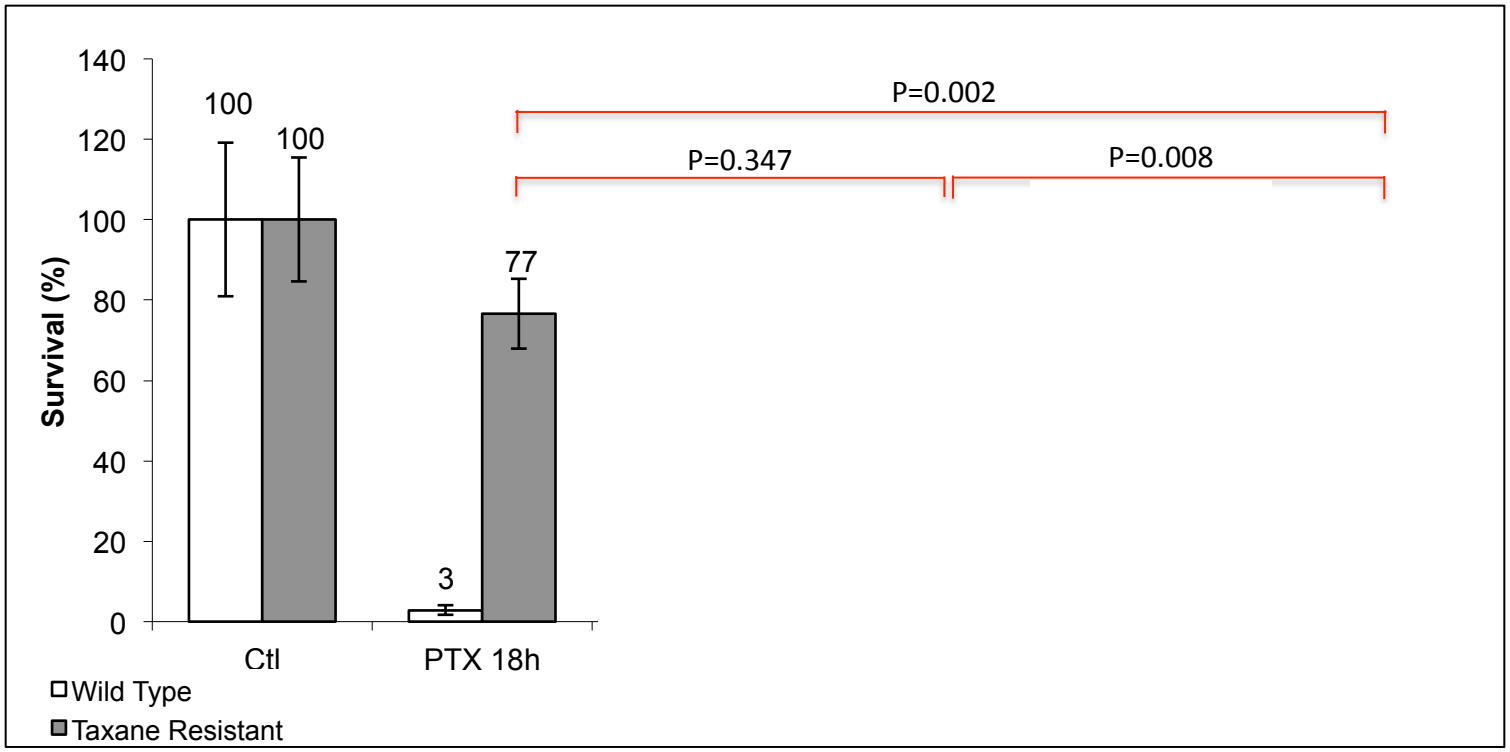


-degradation of cycline B after HS in cells blocked in mitosis by PTX, Noc and AurAi
-no induction of apoptosis

Giovinazzi et al., *Cell Cycle* 2013

Survival assay of MCF7 cells, wild type (WT) and a PTX resistant (TR)

Cells treated with PTX for a total time of 18 h with or without proTAME and 1h of HS (SD±3).



- PTX followed by HS or treatment with APC/C inhibitor proTAME significantly increases PTX cytoxicity in PTX resistant cells
- HS elevates cytotoxisity better than proTAME (“to go” better than “to stay”)

Giovinazzi et al., *Cell Cycle* 2013

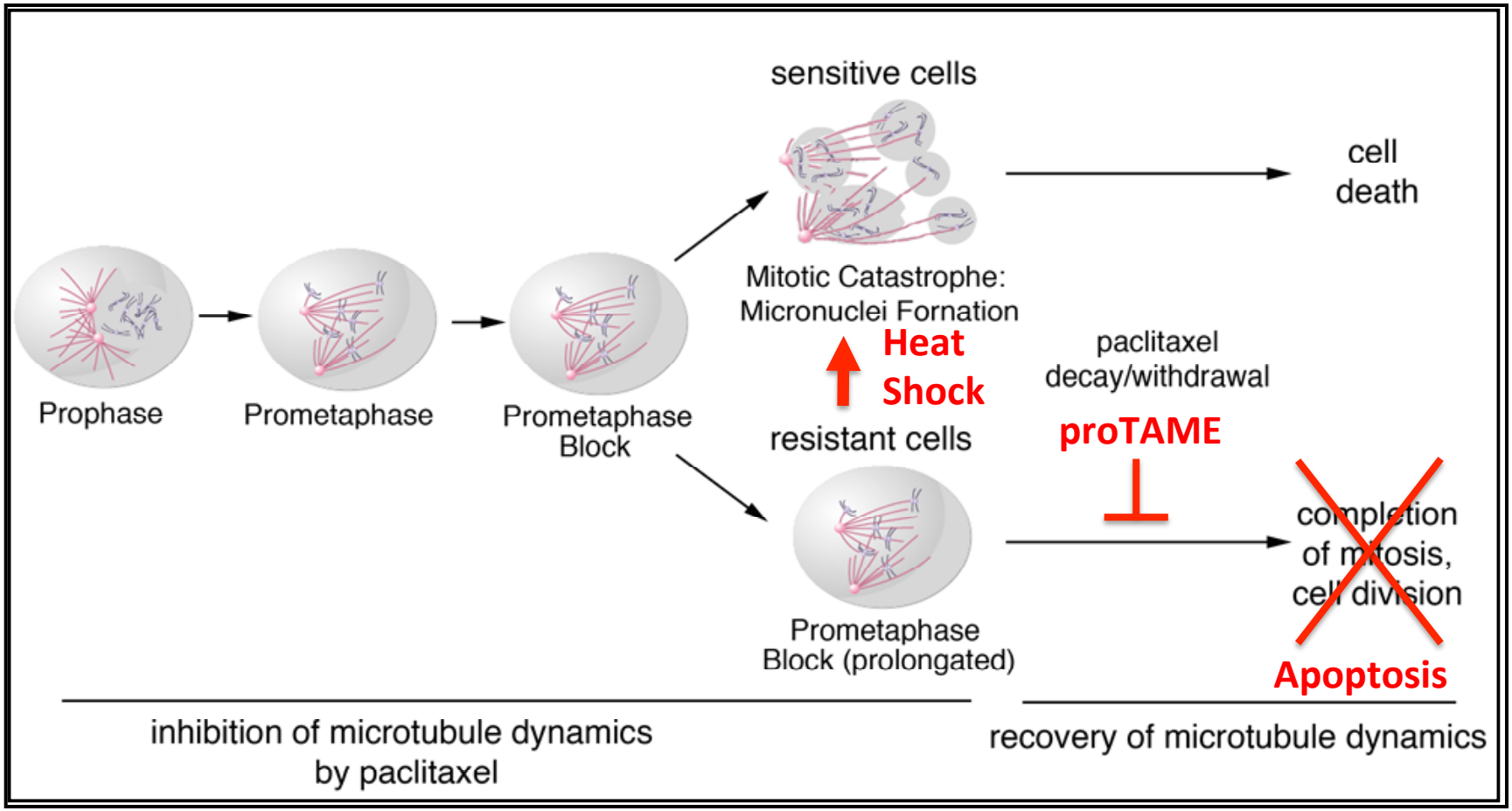
Conclusions

- proTAME, a newly developed APC/C inhibitor blocks mitotic exit of cells treated with antimitotic drugs as paclitaxel (PTX) and Aurora A inhibitor and activates apoptosis.

- Heat Shock (HS) forces mitotic exit of cells blocked in mitosis with antimitotic drugs.

- PTX treatment followed by HS or by treatment with proTAME significantly increases cell death of both taxanes sensitive and resistant cells.

Mitotic Poisons in Chemotherapy: “Should I Stay (in Mitosis) or Should I Go”?



Резистентность к химиотерапии:
кто виноват и что делать?

-Новые маркеры резистентности:
Daxx, USP7, Aurora-A, CHFR, Bub3 для выбора типа терапии

-Поиск новых сочетанных терапевтических подходов при лечении

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

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Фонд «Династия»