Polo-like Kinase 1 in genomic instability

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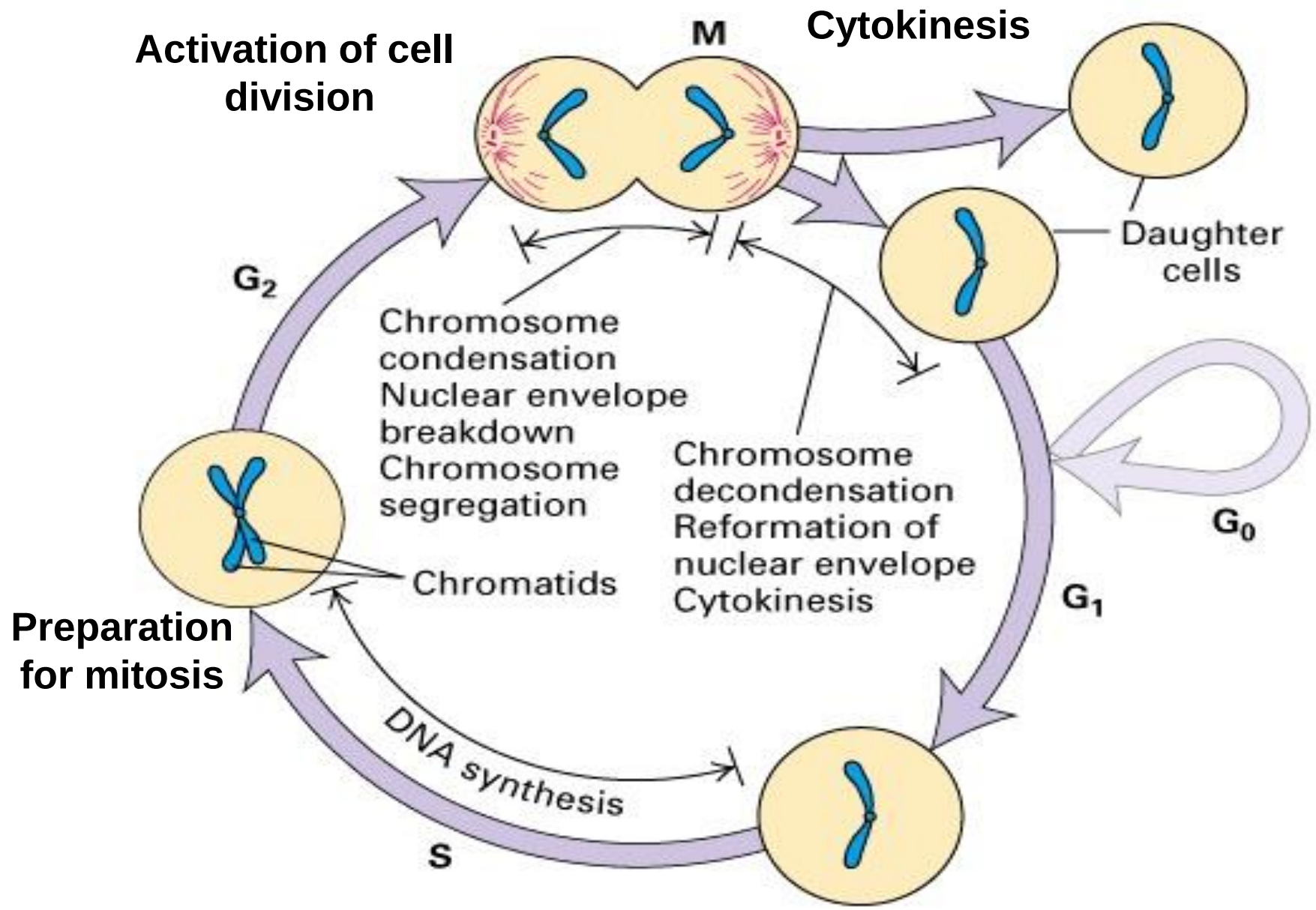
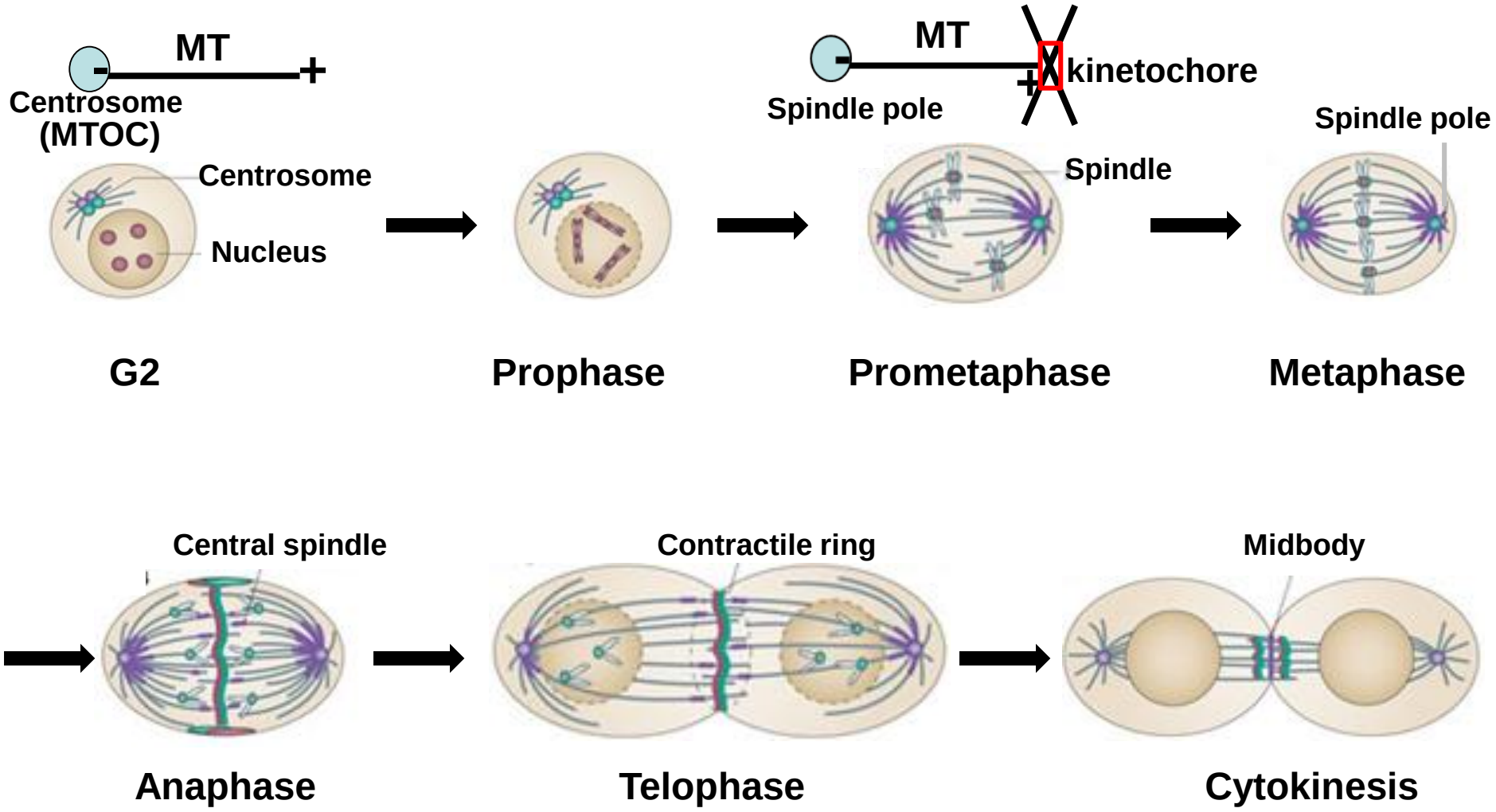
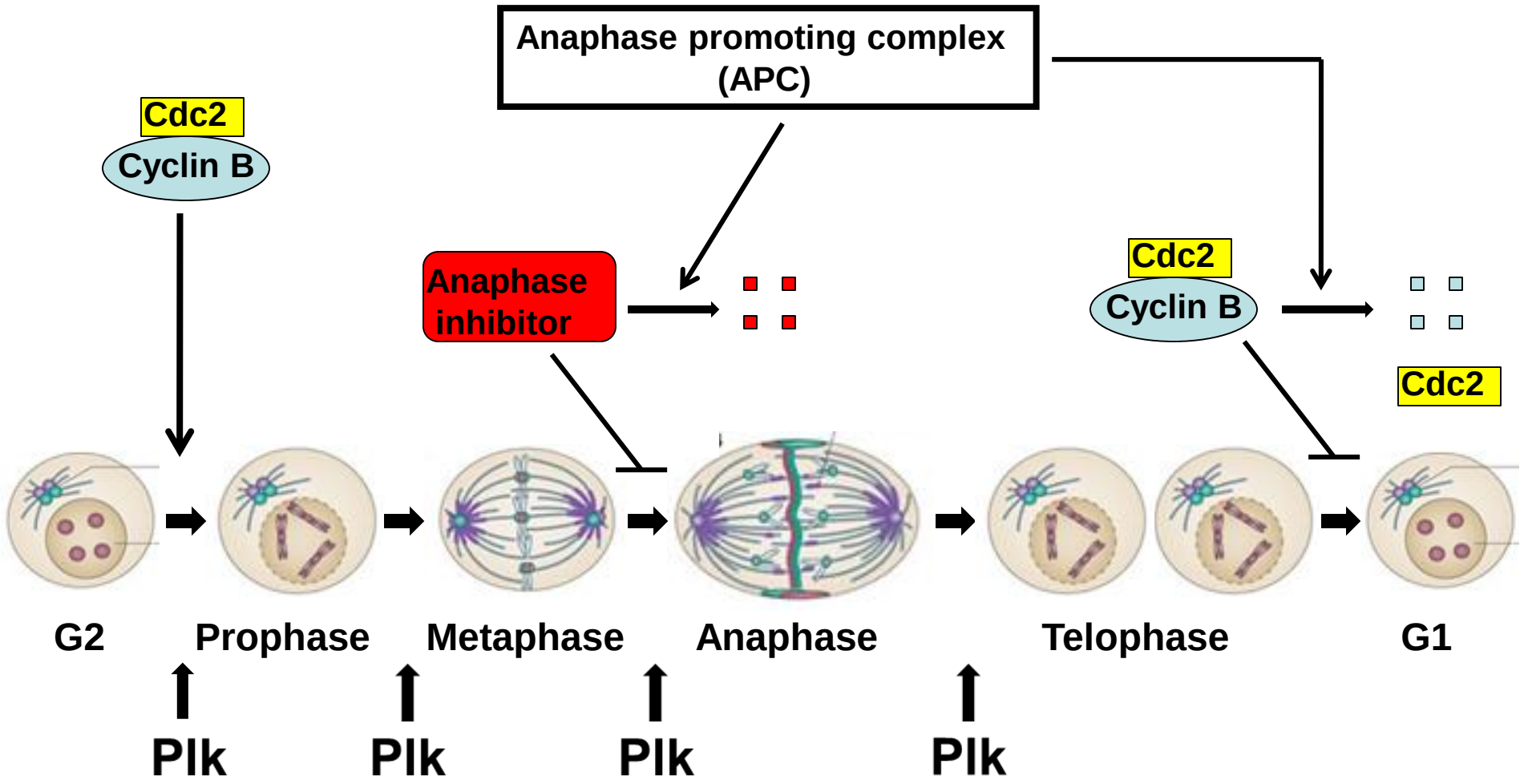
The fate of a single parental chromosome throughout the eukaryotic cell cycle



The stages of mitosis and cytokinesis in an animal cell

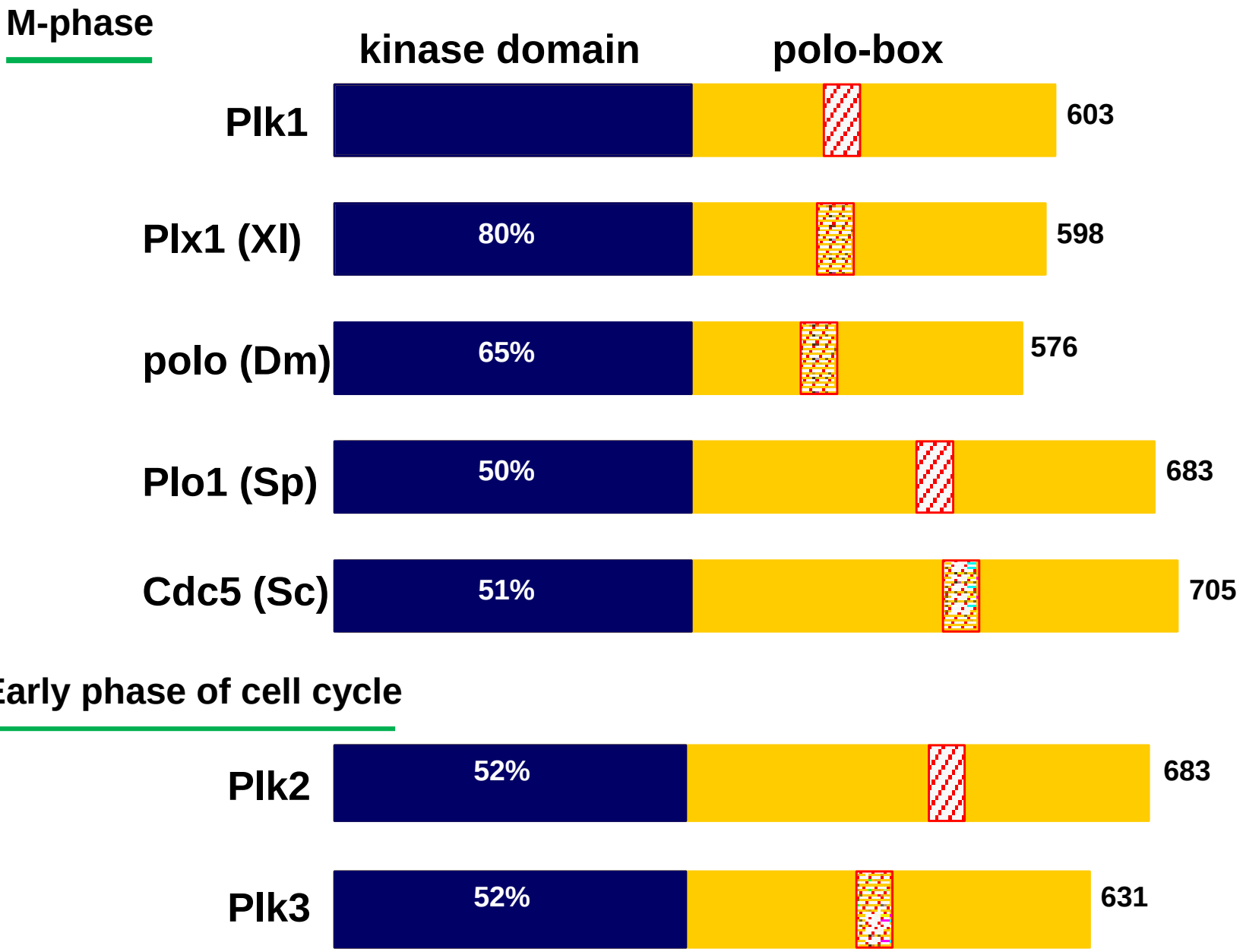


Control of mitotic entry and exit by Cdc2/cyclin B and APC



(Liu & Erikson, PNAS 2002; Liu & Erikson, PNAS 2003; Liu et al, MCB 2005; Liu et al, MCB 2006)

The polo-like kinases have a C-terminal polo-box domain

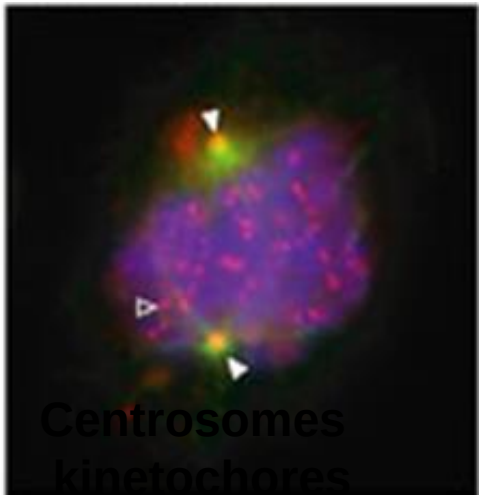


Plk1 localizes to mitotic structures

G2/pro

Prometa

Centrosomes

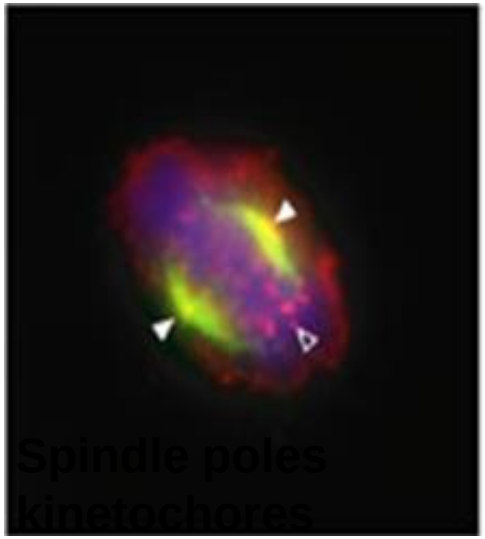


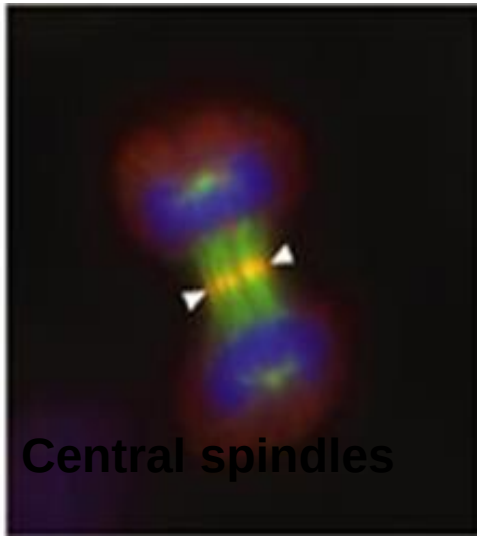
Spindle poles
kinetochores

Meta

Telo

Spindle poles
kinetochores





Midbodies

Plk1 in cancer

---Plk1 overexpression is correlated with cell proliferation and carcinogenesis.

---Plk1 is a new diagnostic marker for cancer.

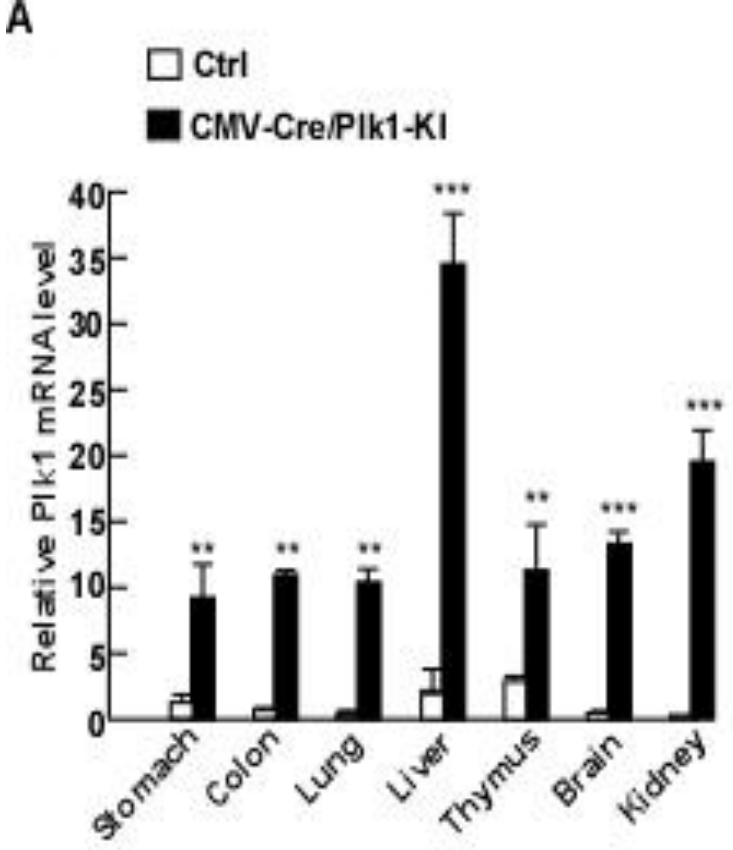
---Plk1 inhibitors are in various clinical trails.

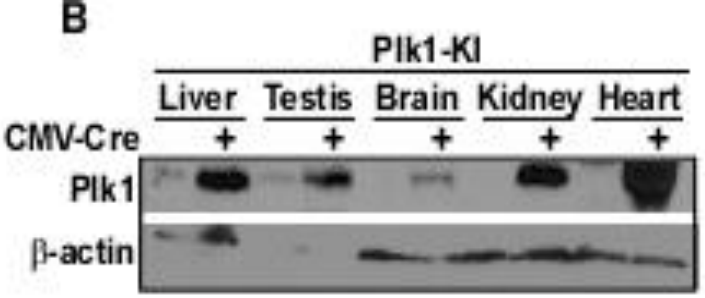
---However, how Plk1 contributes to carcinogenesis is elusive.

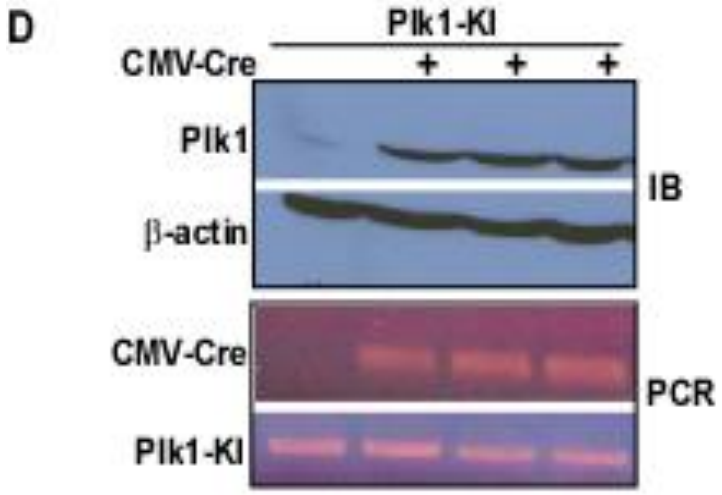
Does Plk1 elevation contribute to cancer progression?

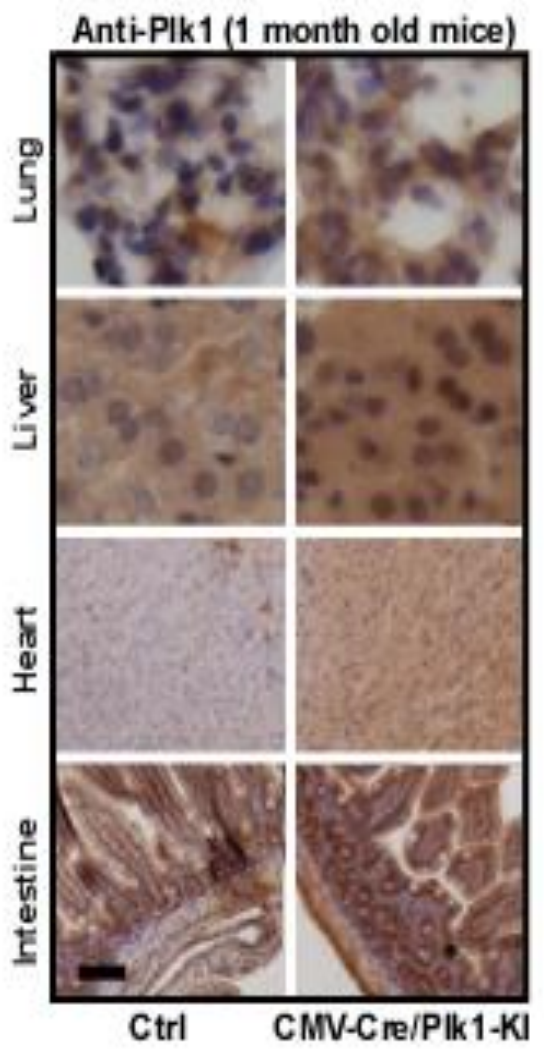
Approach: generation of Plk1-Knock in (KI) mouse line

Plk1 KI mice

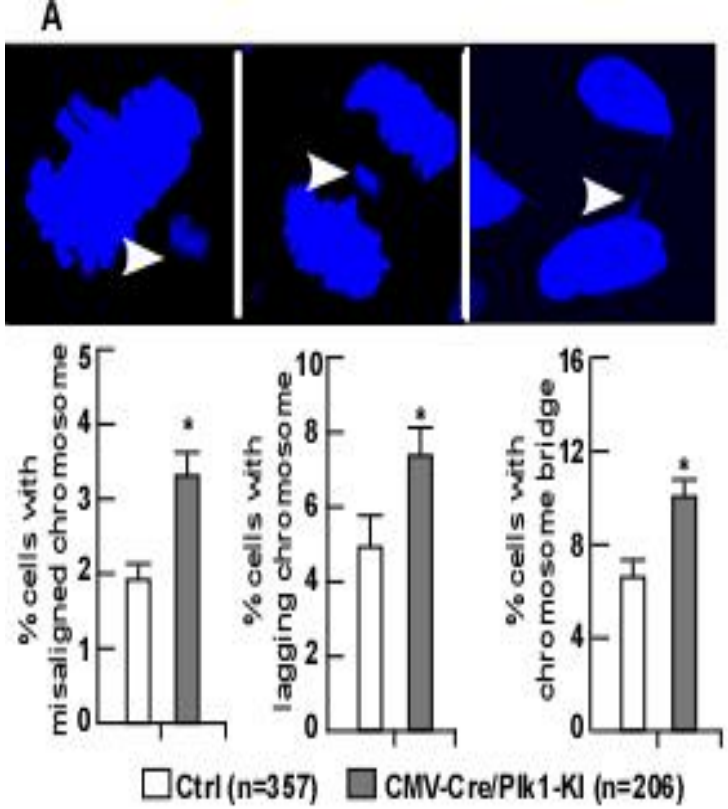

A
Ctrl
CMV-Cre/Plk1-KI
Relative Plk1 mRNA level
40
35
30
25
20
15
10
5
0
**
**
**
***
**
***
***
Stomach
Colon
Lung
Liver
Thymus
Brain
Kidney

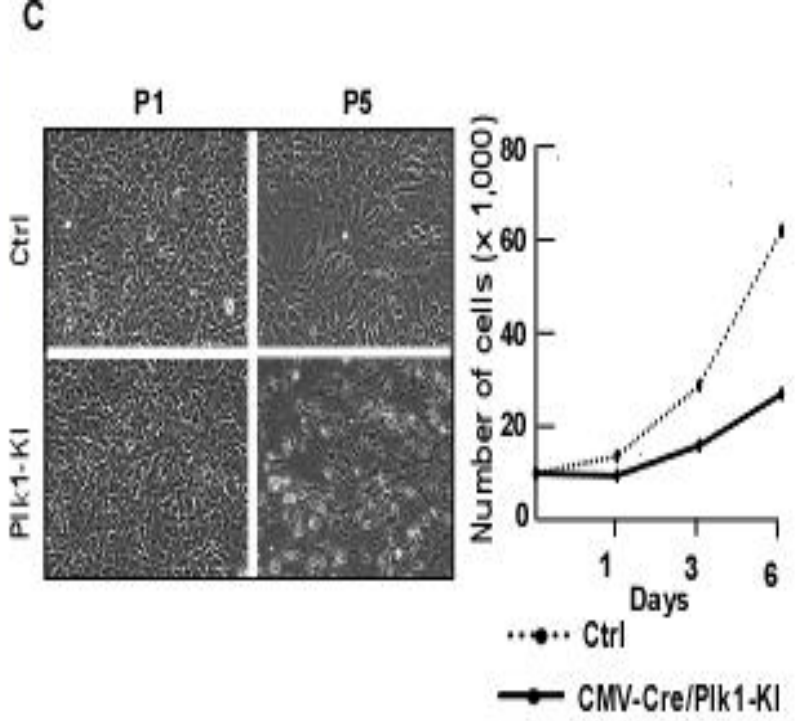

B
Plk1-KI
Liver
Testis
Brain
Kidney
Heart
CMV-Cre
+
+
+
+
+
Plk1
β-actin

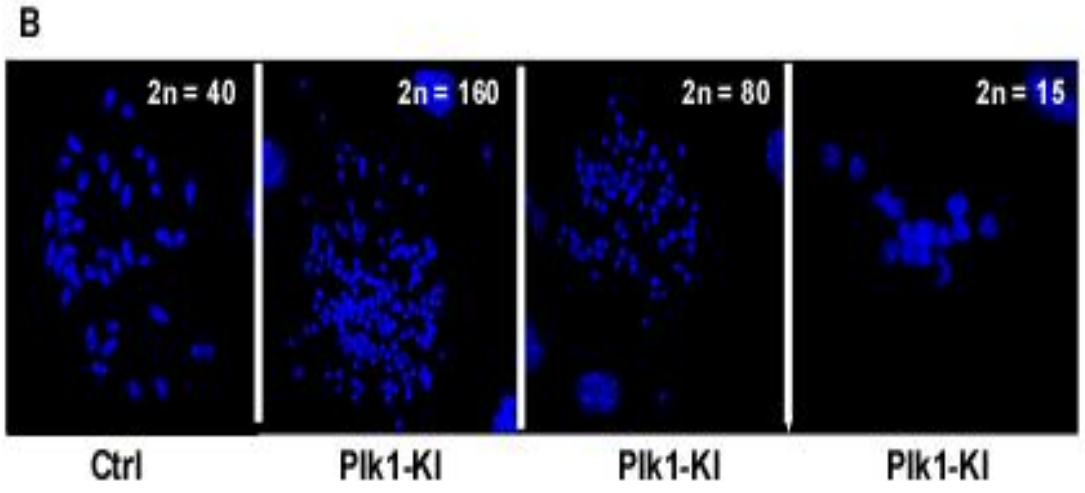

D
Plk1-KI
CMV-Cre
+
+
+
Plk1
β-actin
IB
CMV-Cre
Plk1-KI
PCR

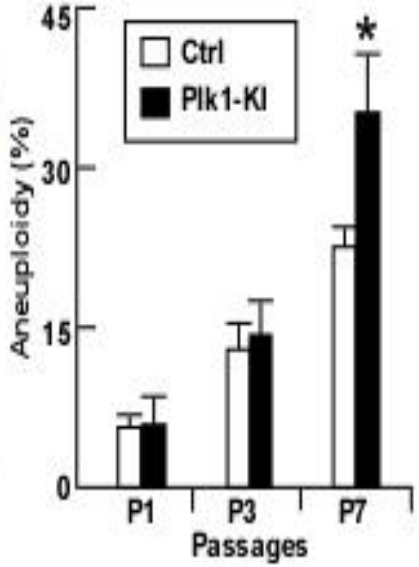

C
Anti-Plk1 (1 month old mice)
Lung
Liver
Heart
Intestine
Ctrl
CMV-Cre/Plk1-KI

Plk1 KI causes aneuploidy in MEFs



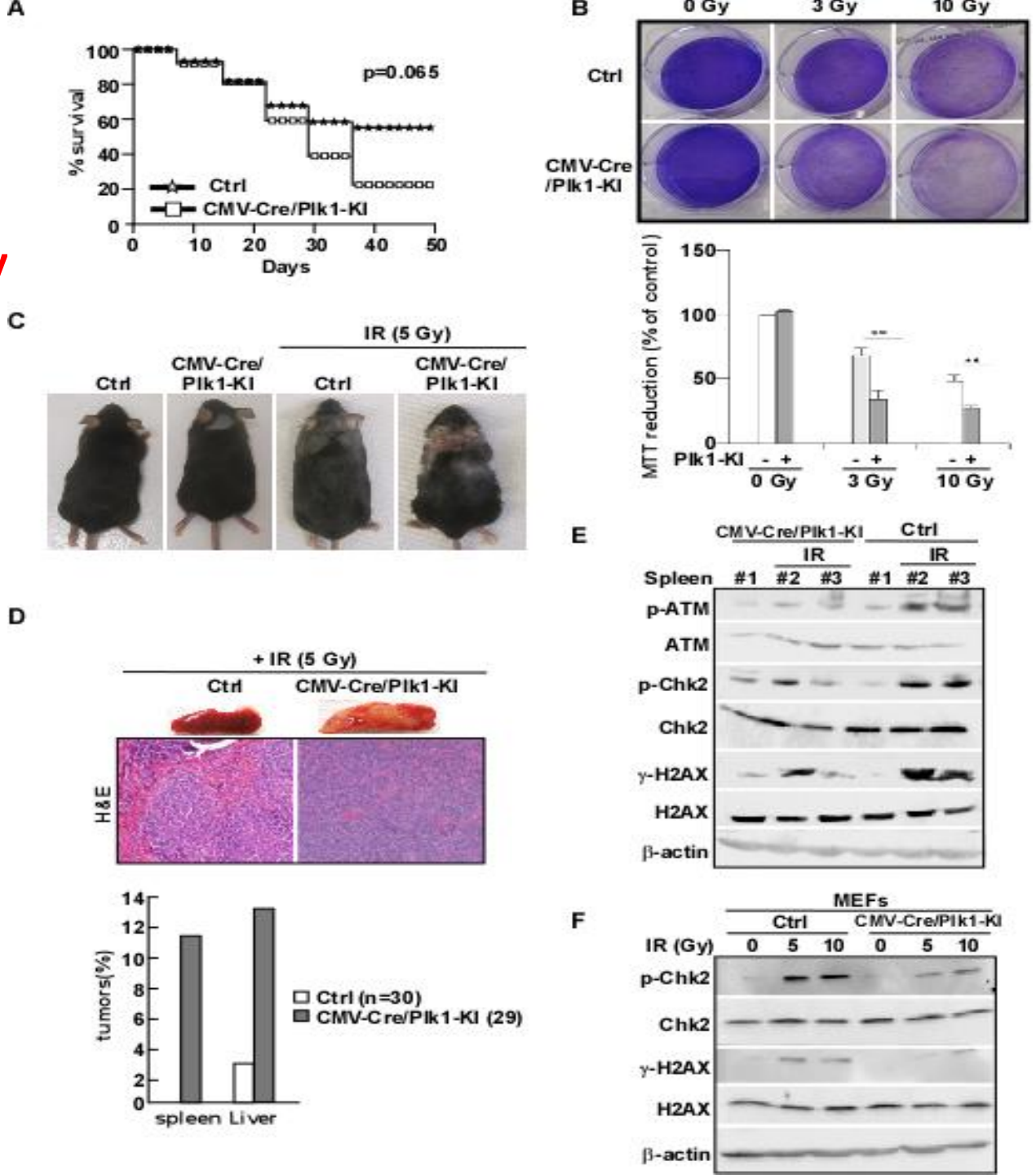






No apparent phenotypes for Plk1-KI mice, but they are hyper-sensitive to IR

DDB
↓
ATM
↓ ↘
Chk2 γH2AX
↓
p53
↓
Cell cycle arrest & DNA repair



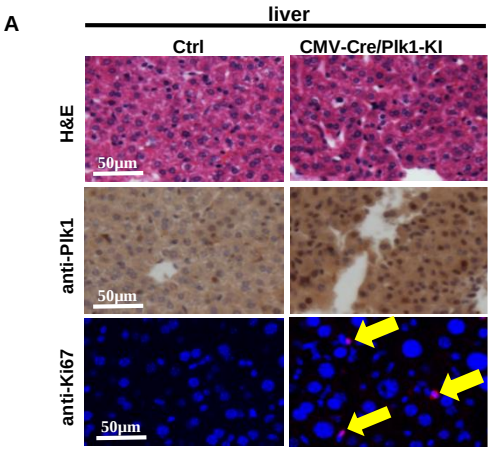
Plk1-KI mice have increased incidence of lymphoma or severe fatty change upon IR

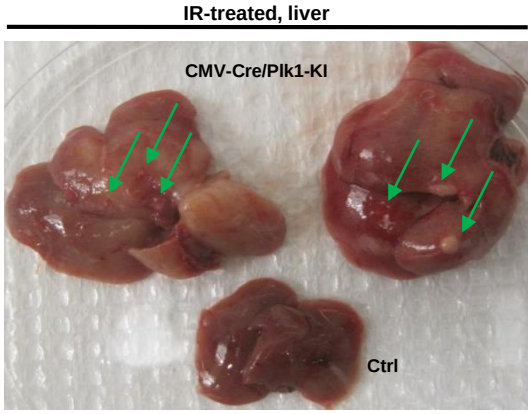
Multifocal lymphoid hyperplasia in perivascular location

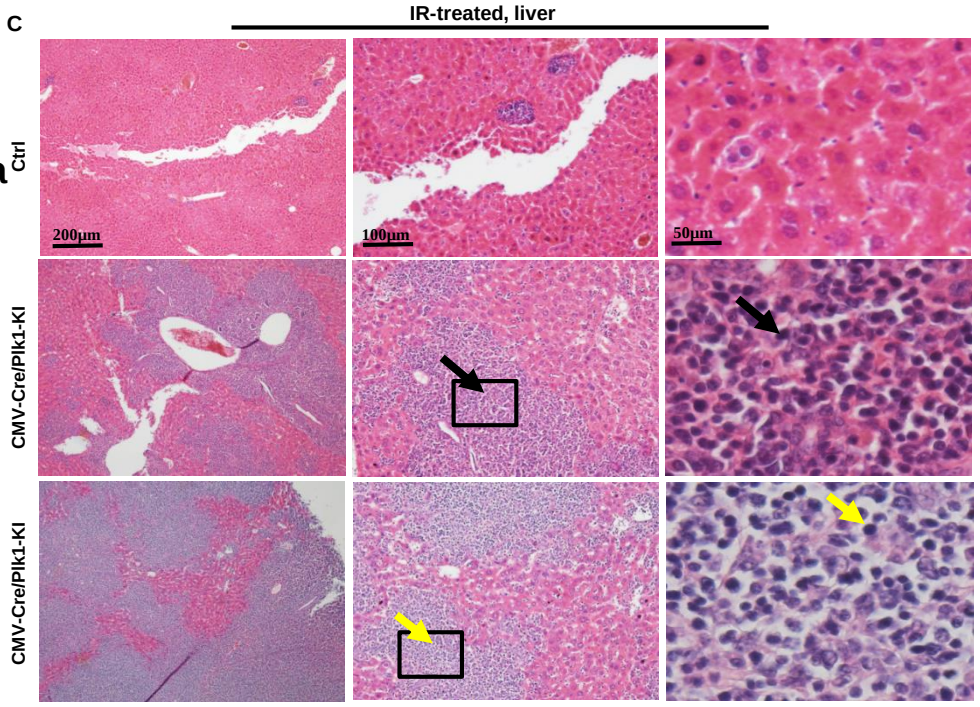
Mild lymphoma

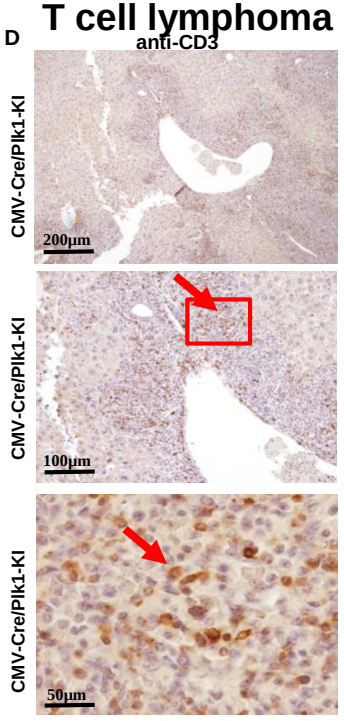
Multiple lymphocytic overgrowth in the parenchyma

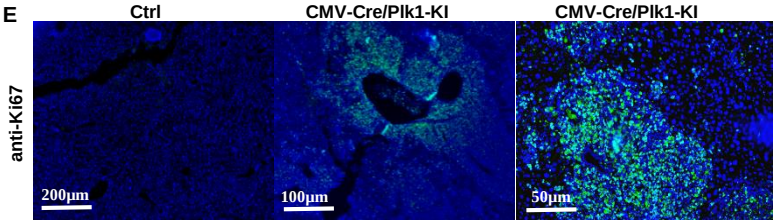
Severe lymphoma

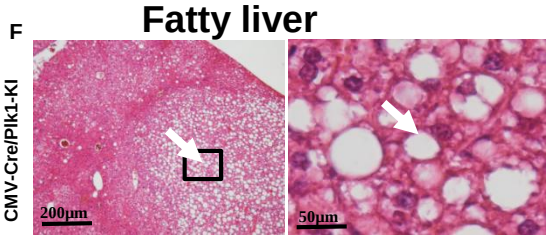












Plk1 KI inhibits expression of DNA damage repair genes

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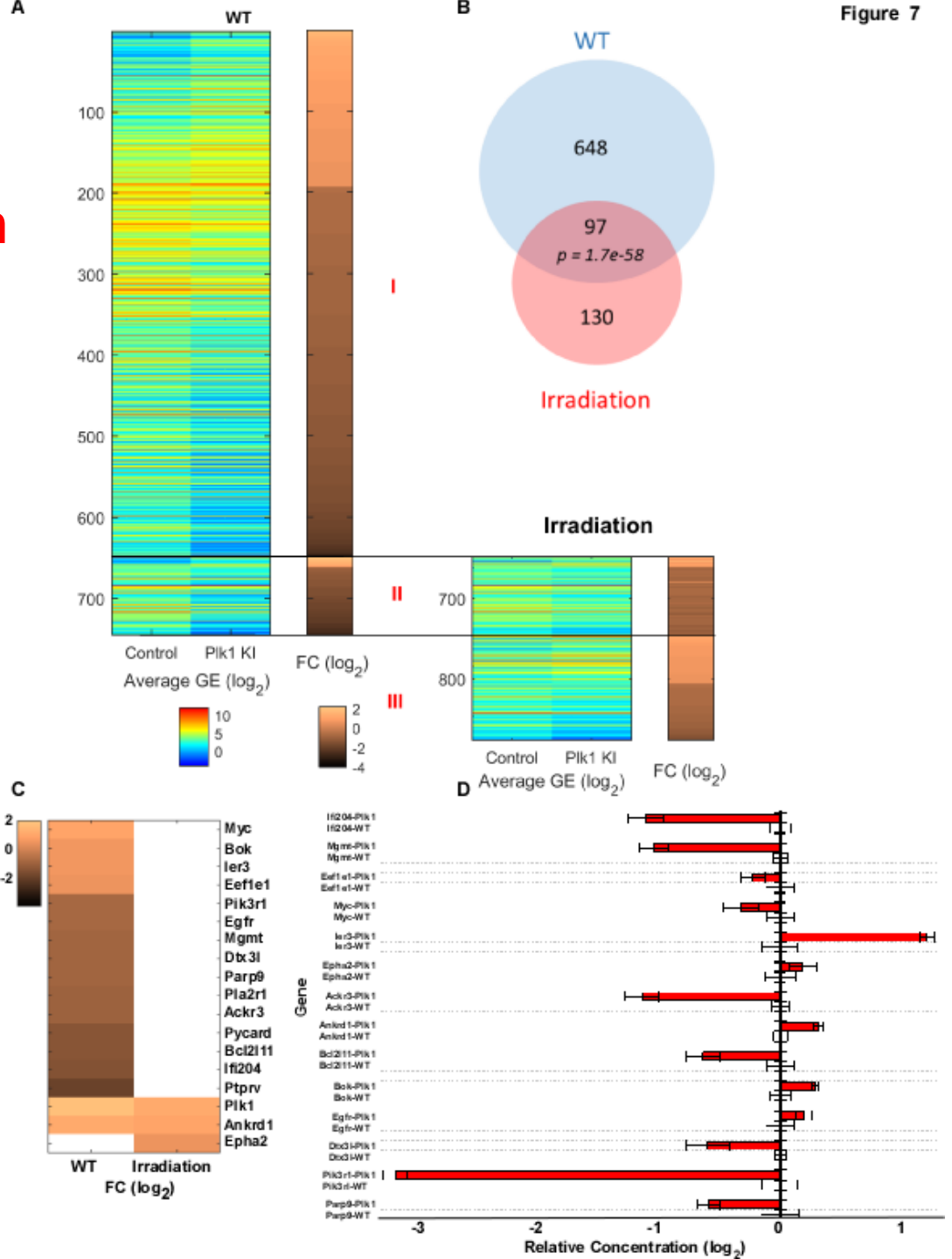
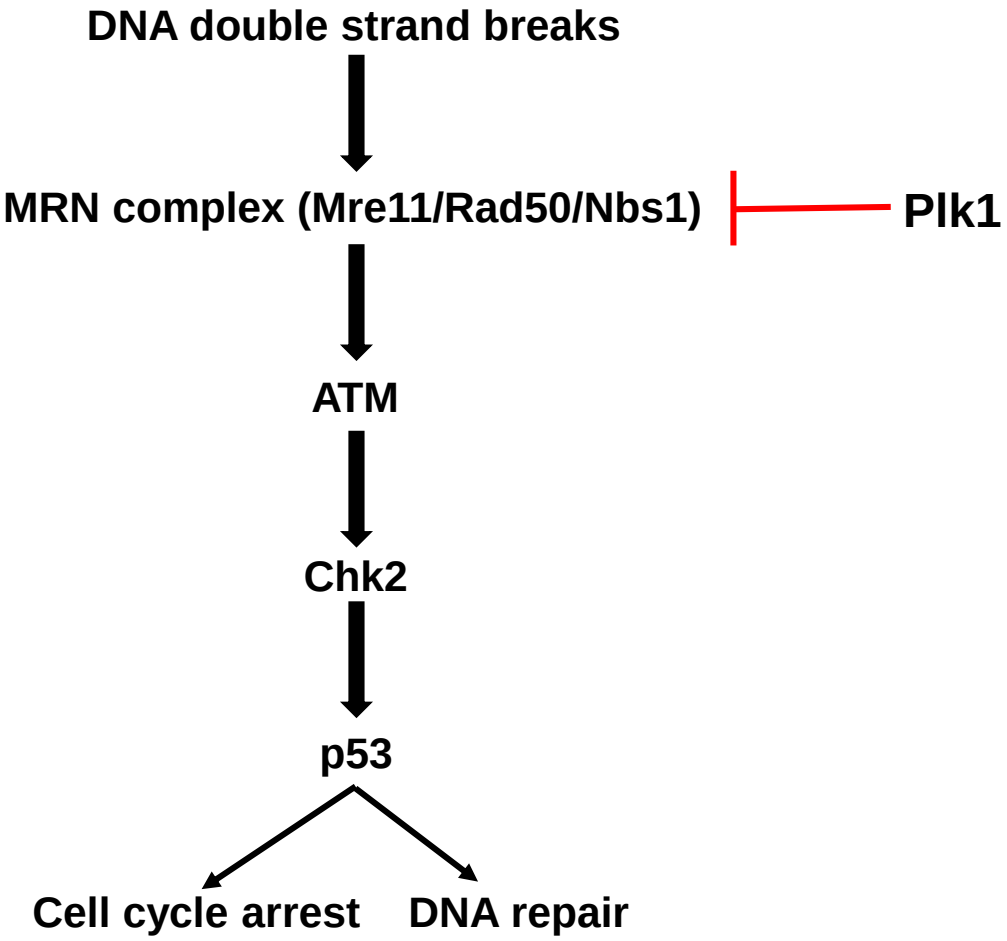
Dr. Jinghui Liu



More specific mechanism?



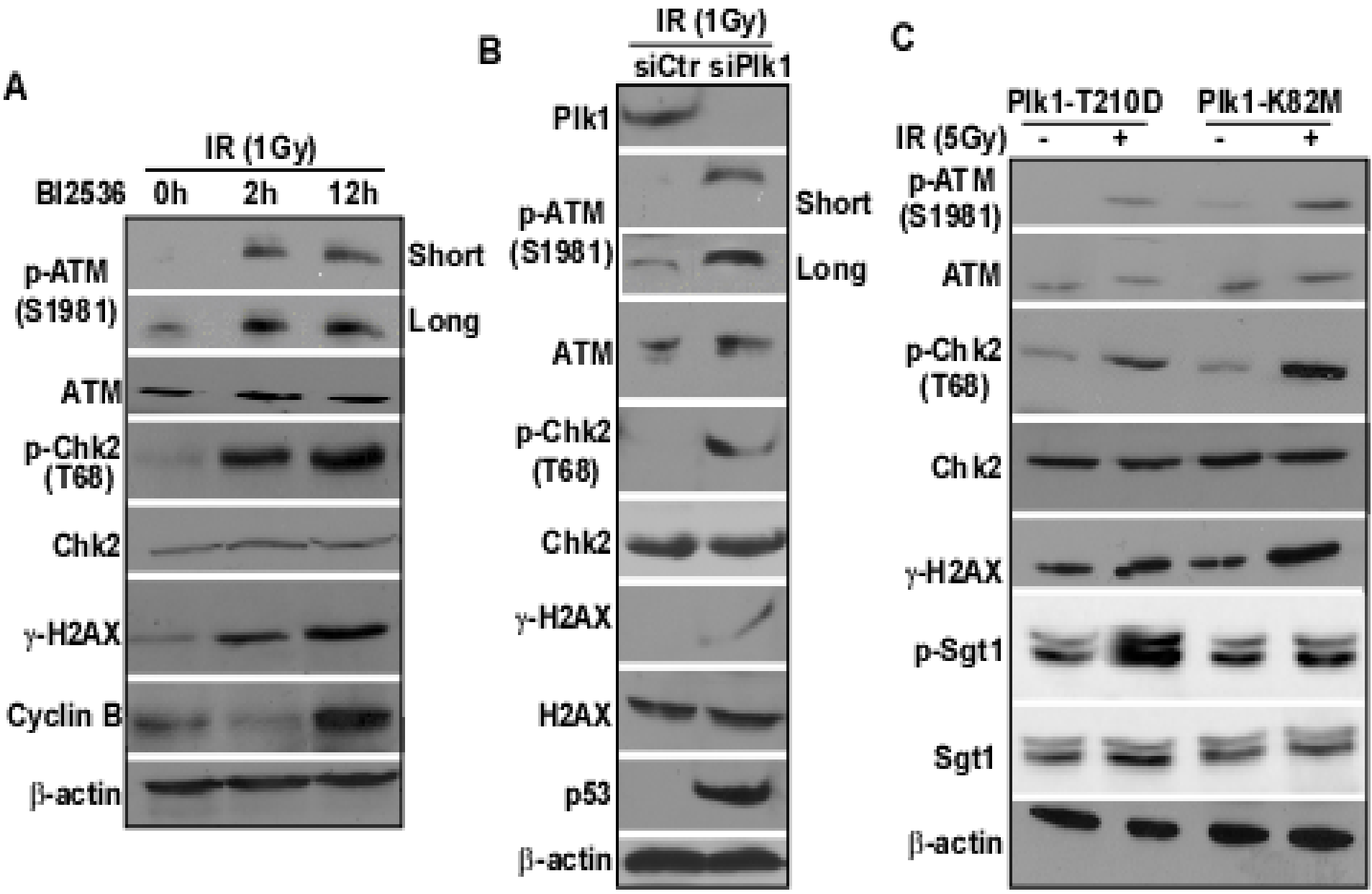
A hypothesis to be tested

Plk1 elevation leads to

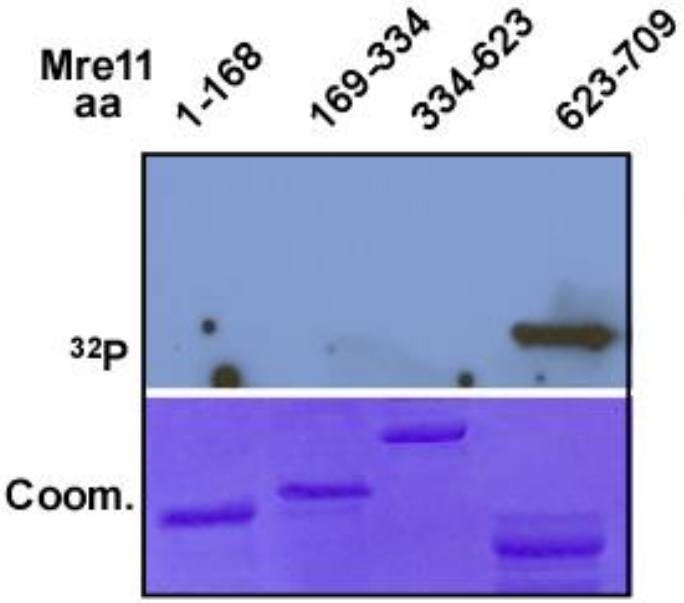
1) premature termination of DNA damage checkpoint and

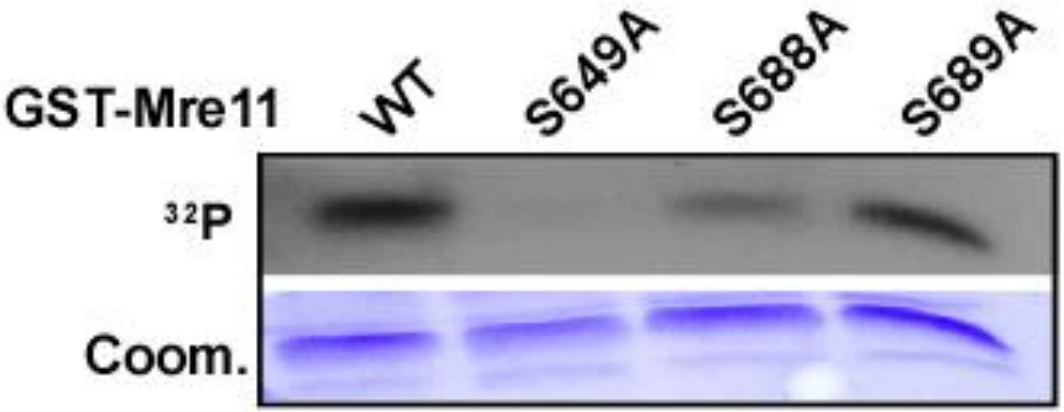
2) reduced DNA repair

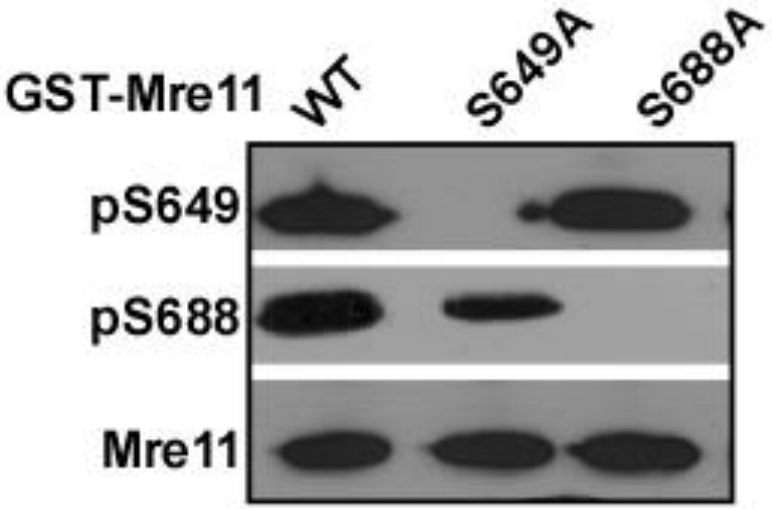
Plk1-associated activity antagonizes DNA damage checkpoint

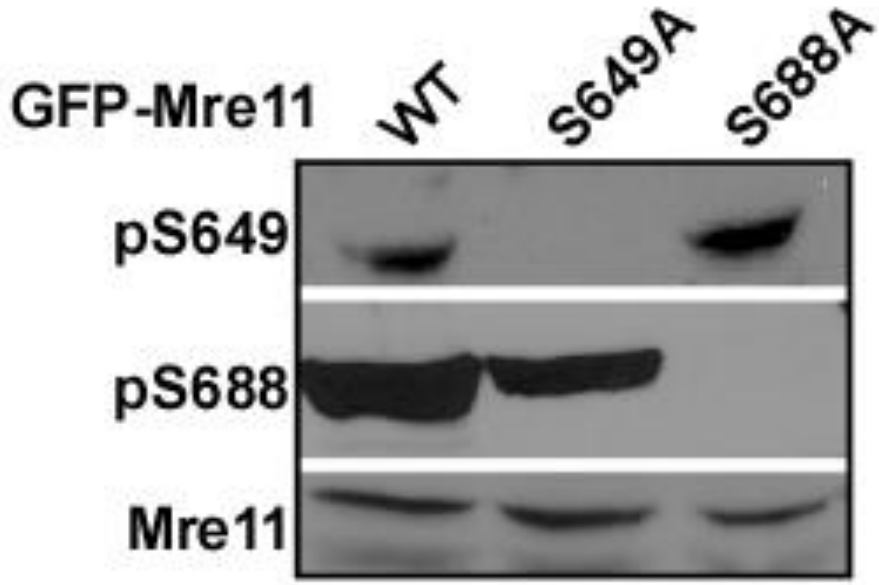


Plk1 phosphorylates Mre11 at S649 and S688 in vitro

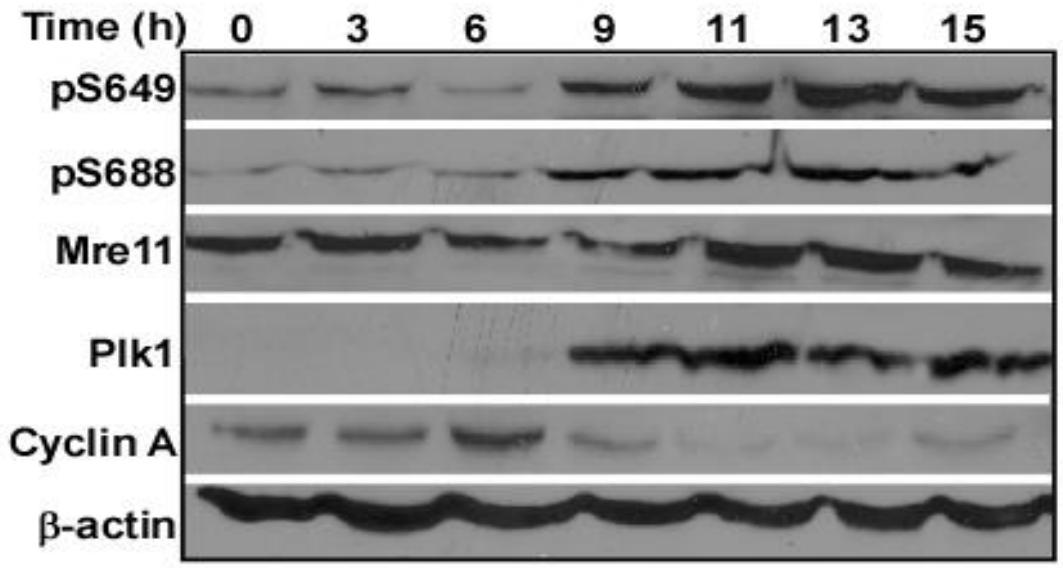


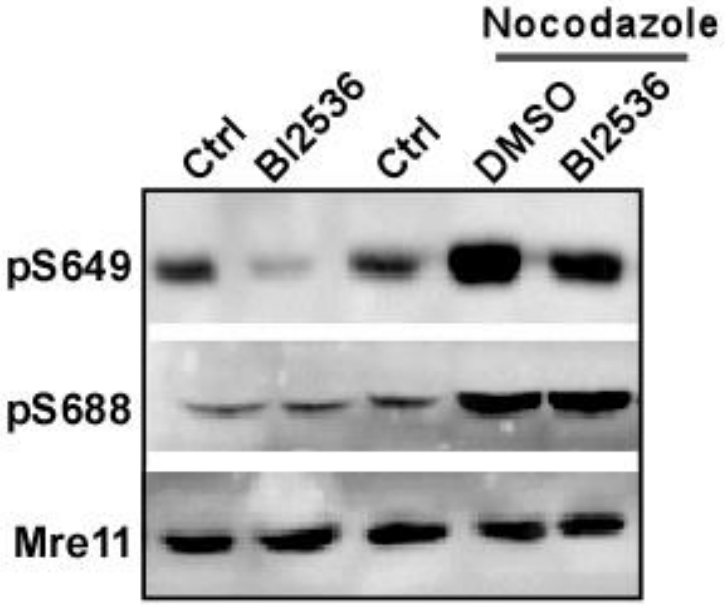


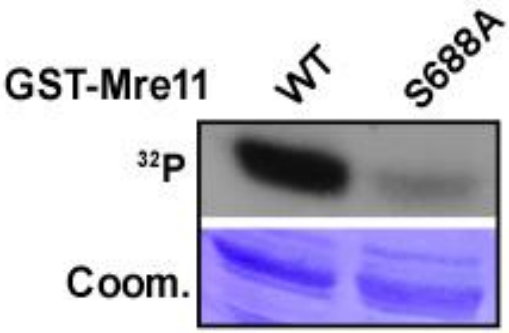


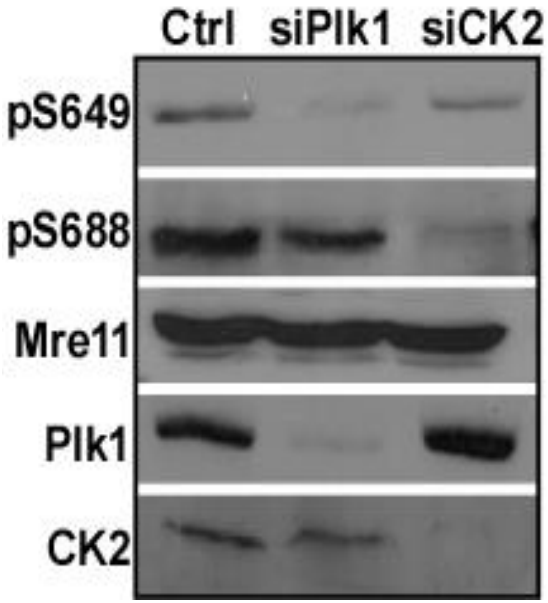


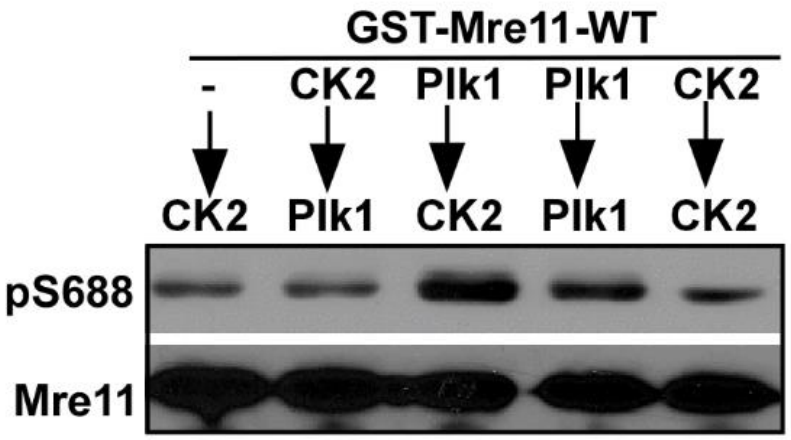
In vivo, Plk1 phosphorylates Mre11-S649 and CK2 targets Mre11-S688





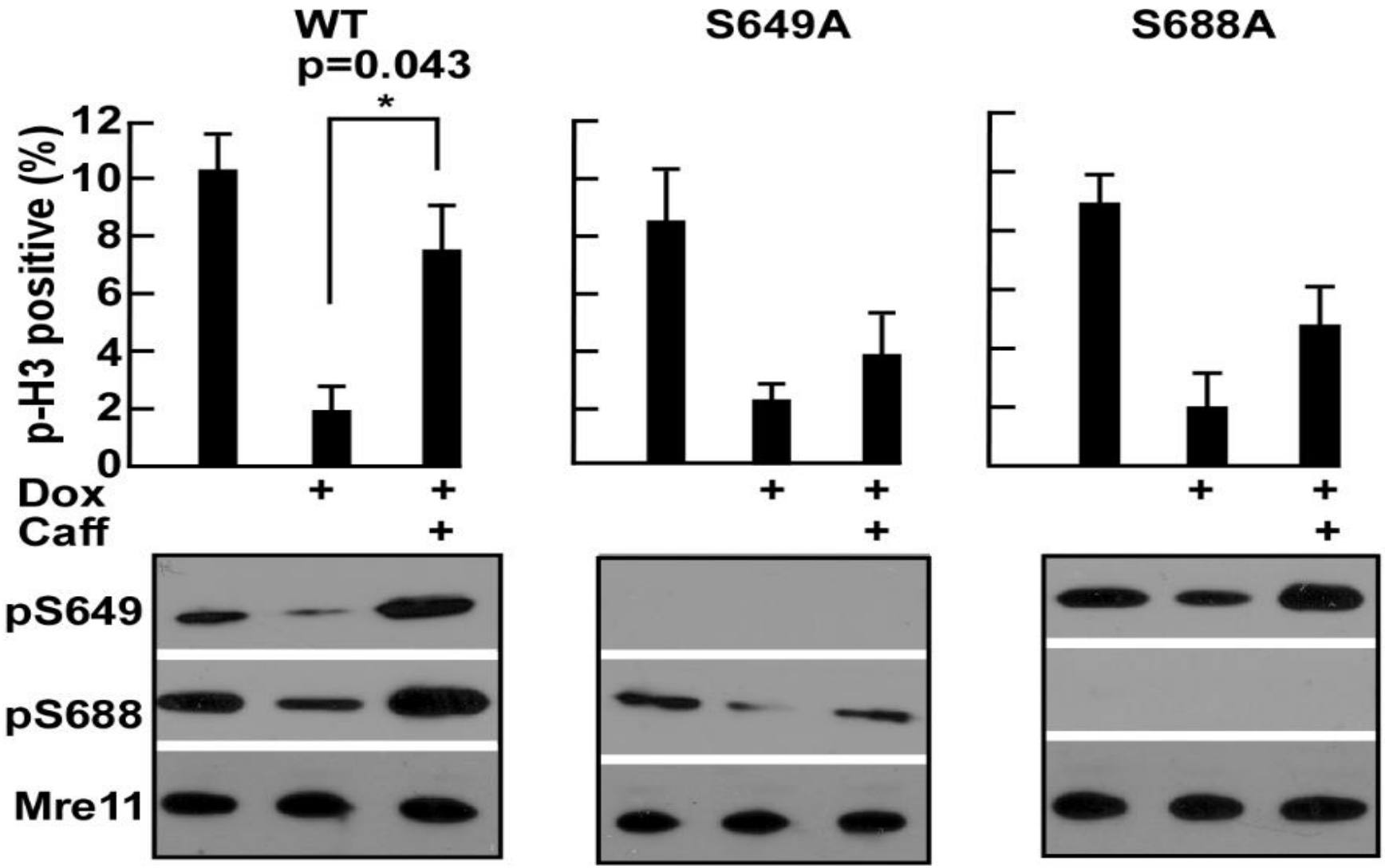






Mre11-S649/S688 phosphorylation is required for G2 DNA damage checkpoint recovery

1st T block ⟶ 8h rel. ⟶ 2nd T block ⟶ 7h rel. ⟶ Dox for 1h ⟶ caffeine for 3h ⟶ p-H3 IF



Phosphorylation of Mre11 at S649/S688 inhibits its binding to DNA

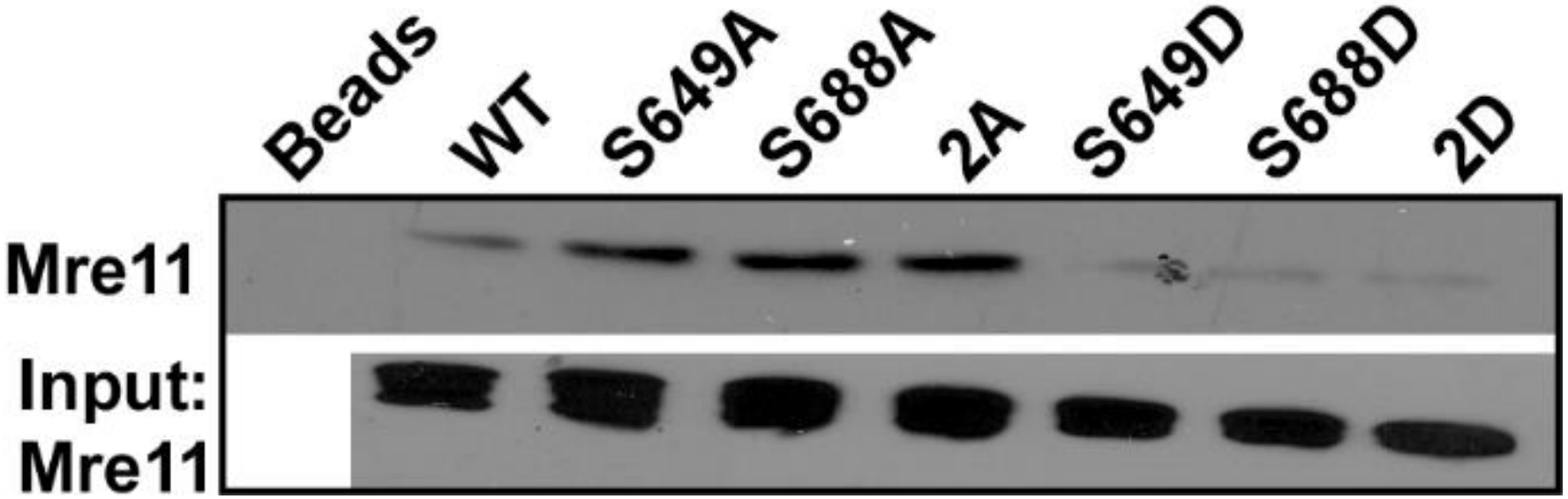
Xenopus egg extract + Purified Mre11 proteins + Biotin-tagged dsDNA-bound avidin beads

↓

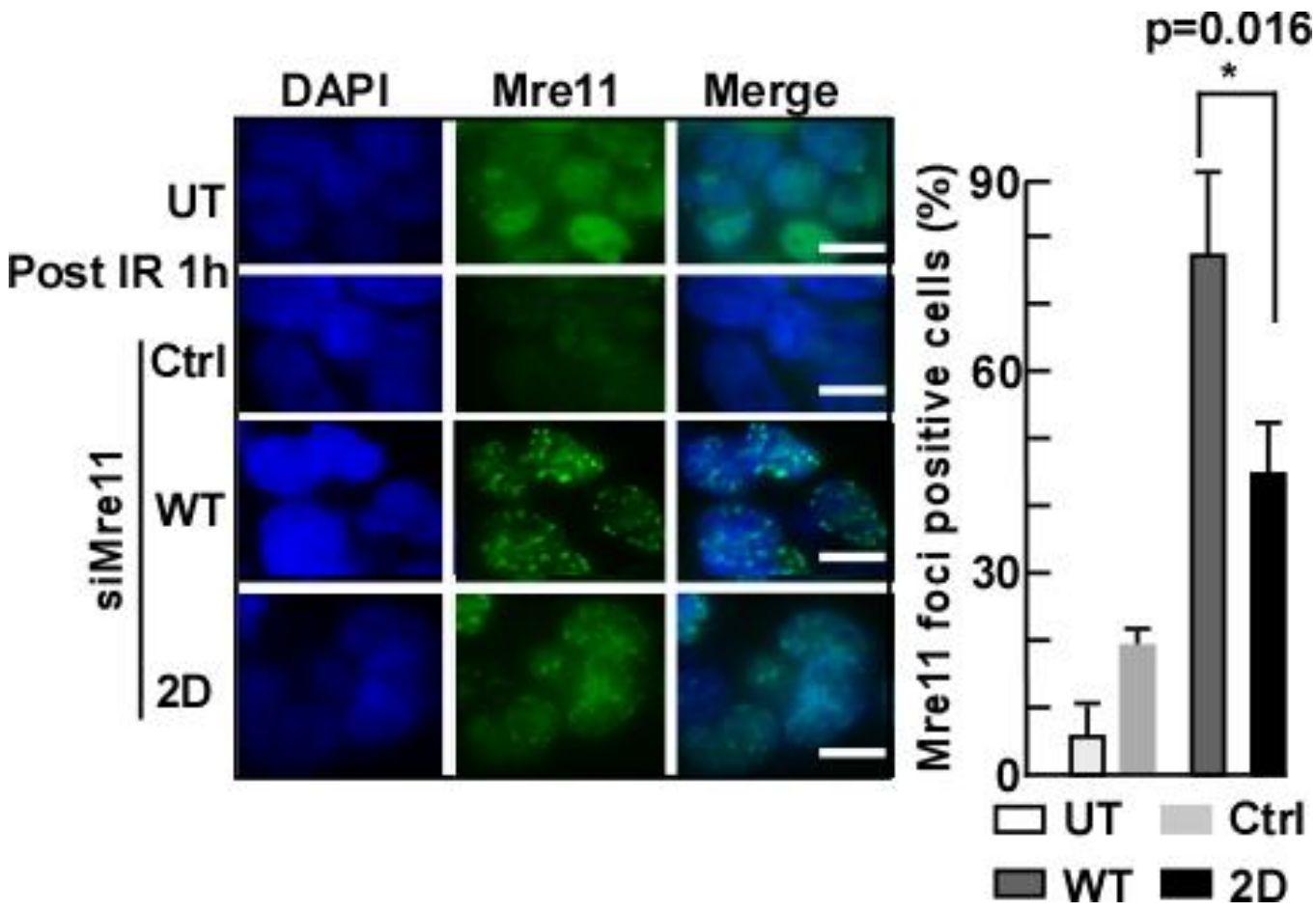
Pellets

↓

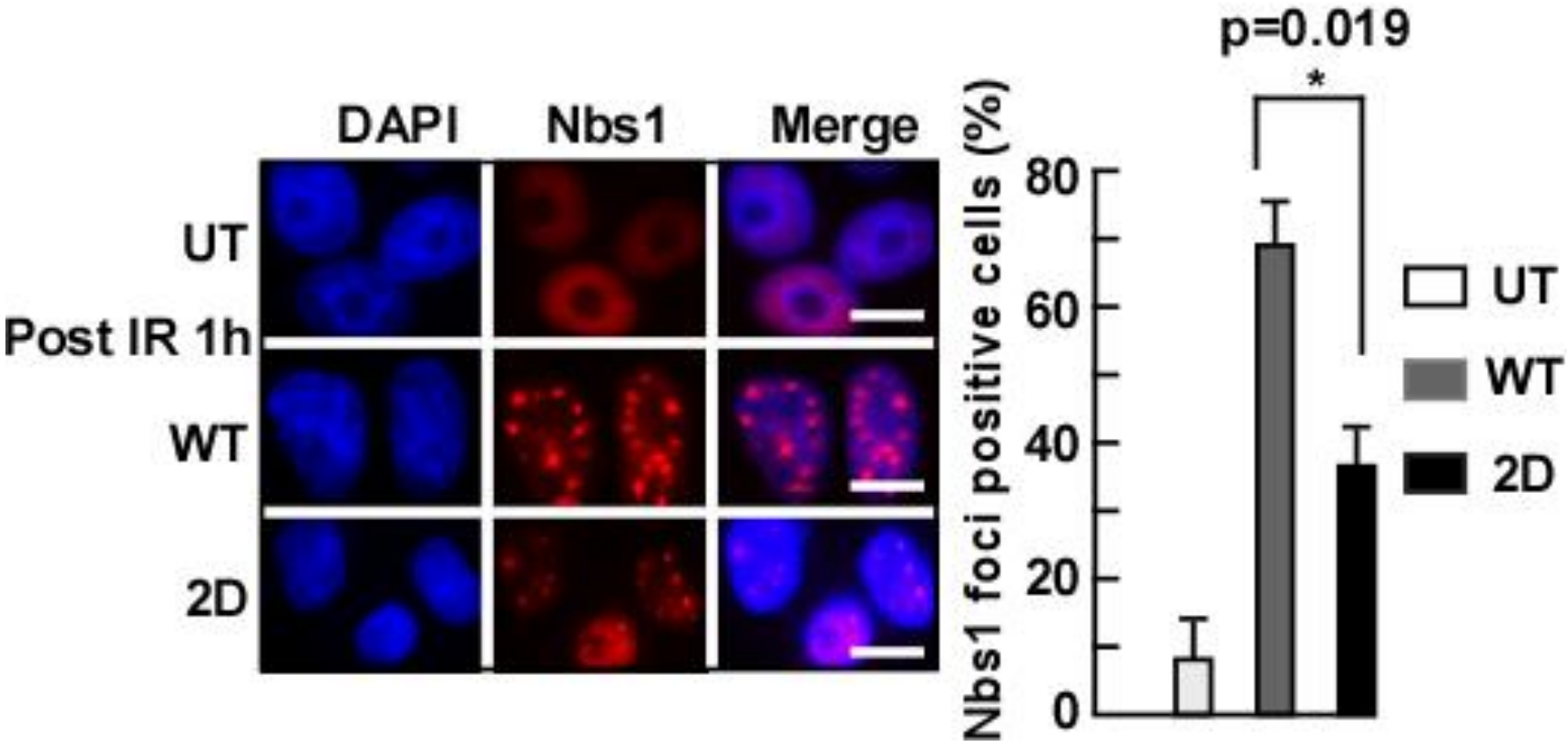
Mre11 IB



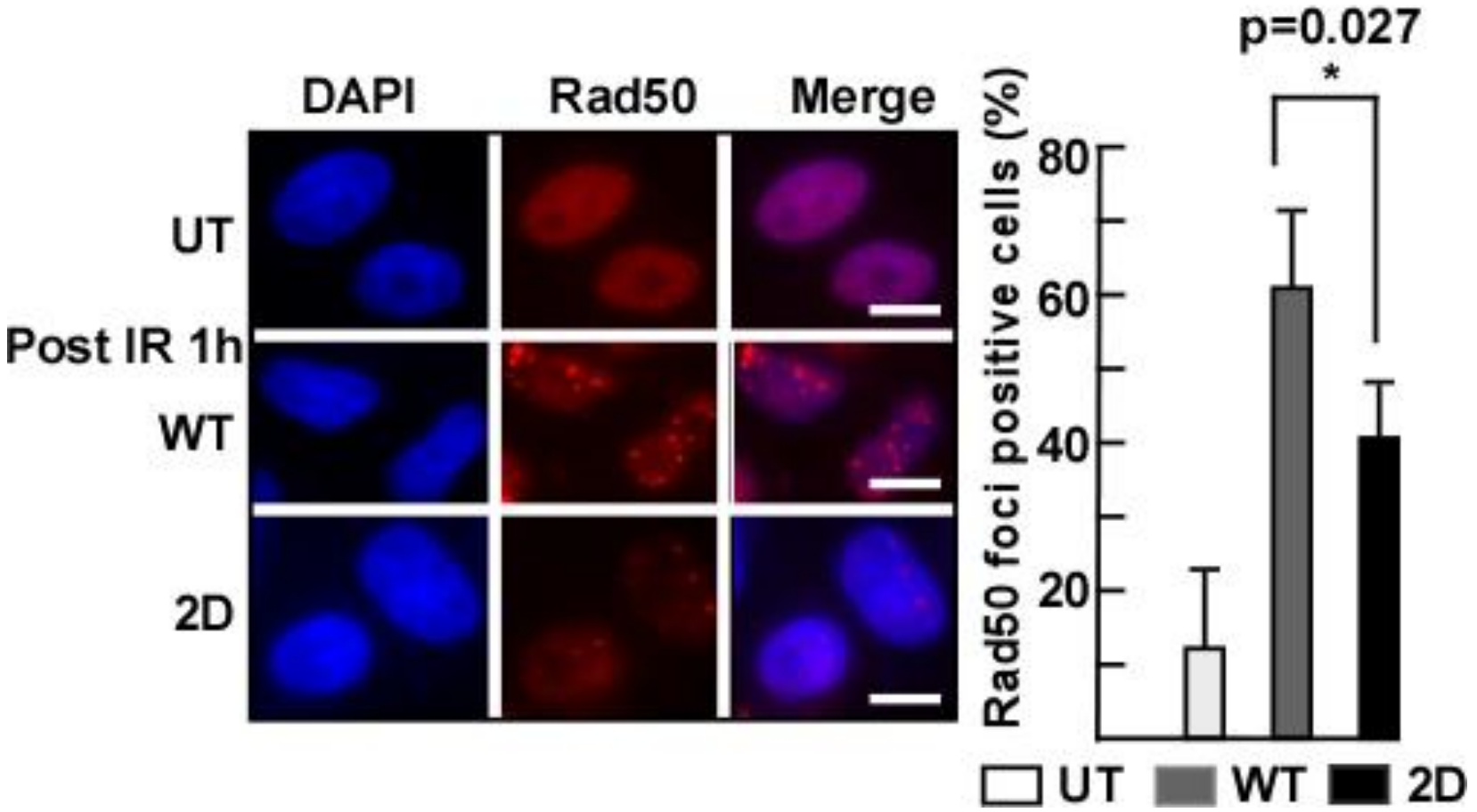
Phosphorylation of Mre11 at S649/S688 abolishes its foci formation



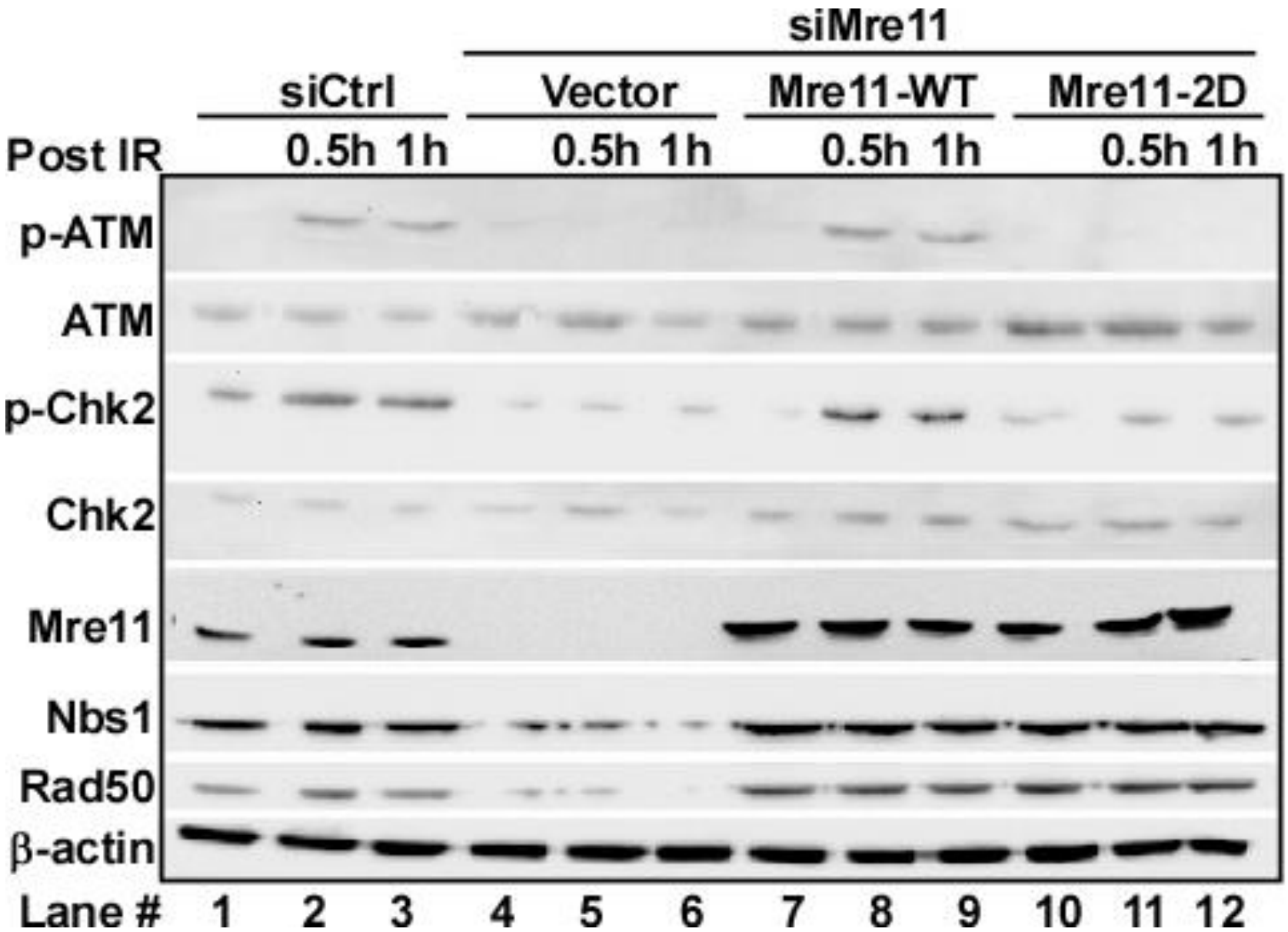
Phosphorylation of Mre11 at S649/S688 abolishes Nbs1 foci formation



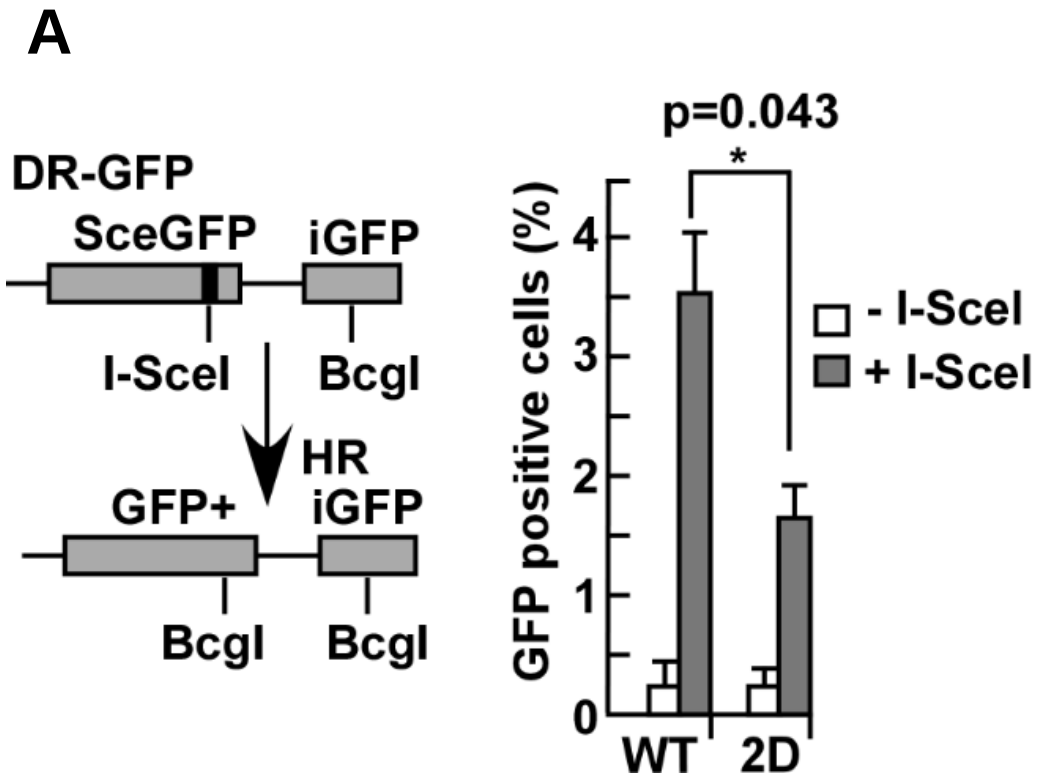
Phosphorylation of Mre11 at S649/S688 abolishes Rad50 foci formation

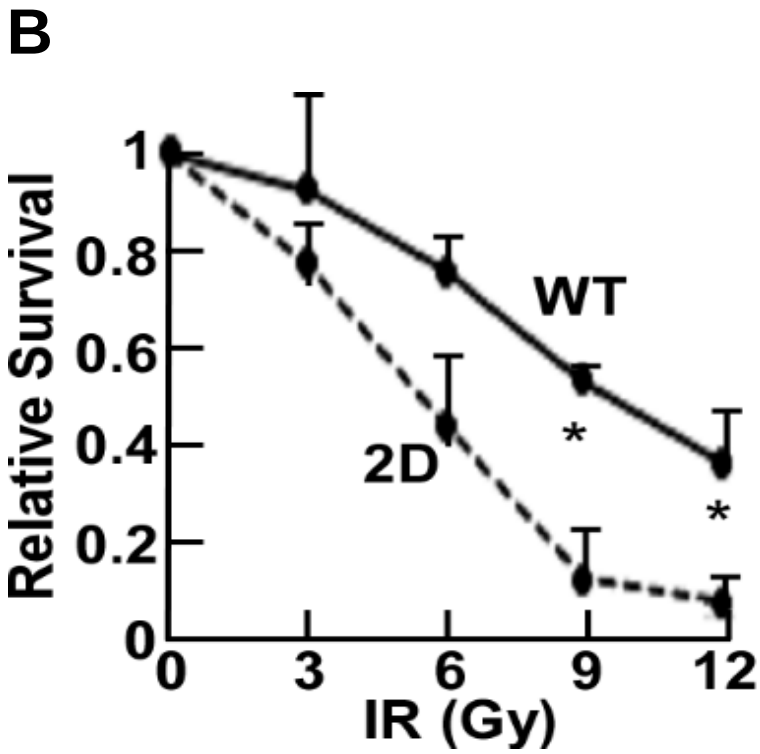


Phosphorylation of Mre11 at S649/S688 inhibits DNA damage checkpoint



Mre11-S649/S688 phosphorylation inhibits DNA repair





Conclusions

Plk1 phosphorylation of Mre11 leads to

1) **premature termination of DNA damage checkpoint**

2) **reduced DNA repair**

Dr. Zhiguo Li

Publications: Li et al., *Cancer Research*, 2017, 77, 3169.

Li et al., *JBC*, 2017, 292, 17461.

MDC1

- **MEDIATOR OF DNA DAMAGE CHECKPOINT**

DNA damage

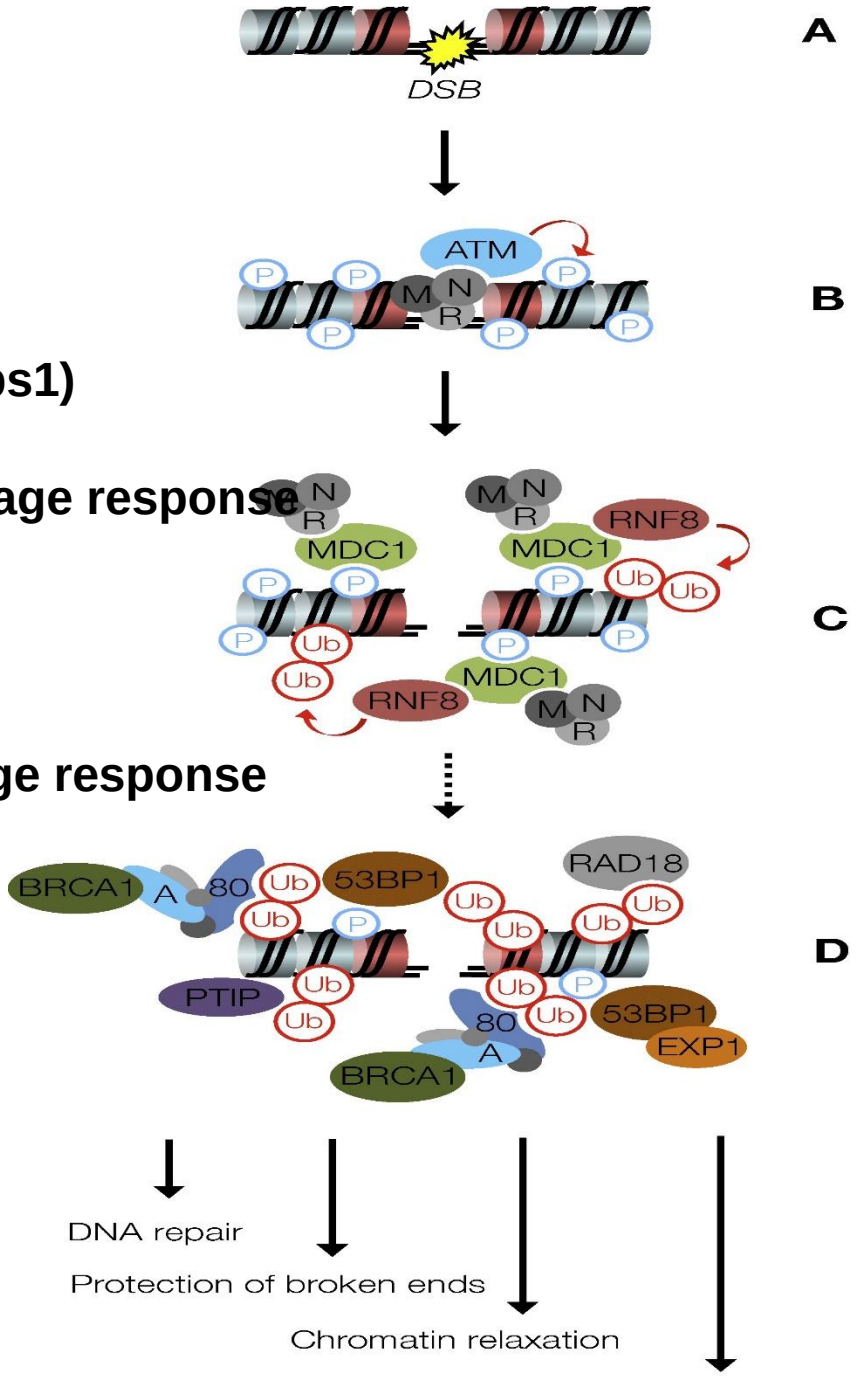
MRN (Mre11/Rad50/Nbs1)

Initiation of damage response

Plk1

MDC1

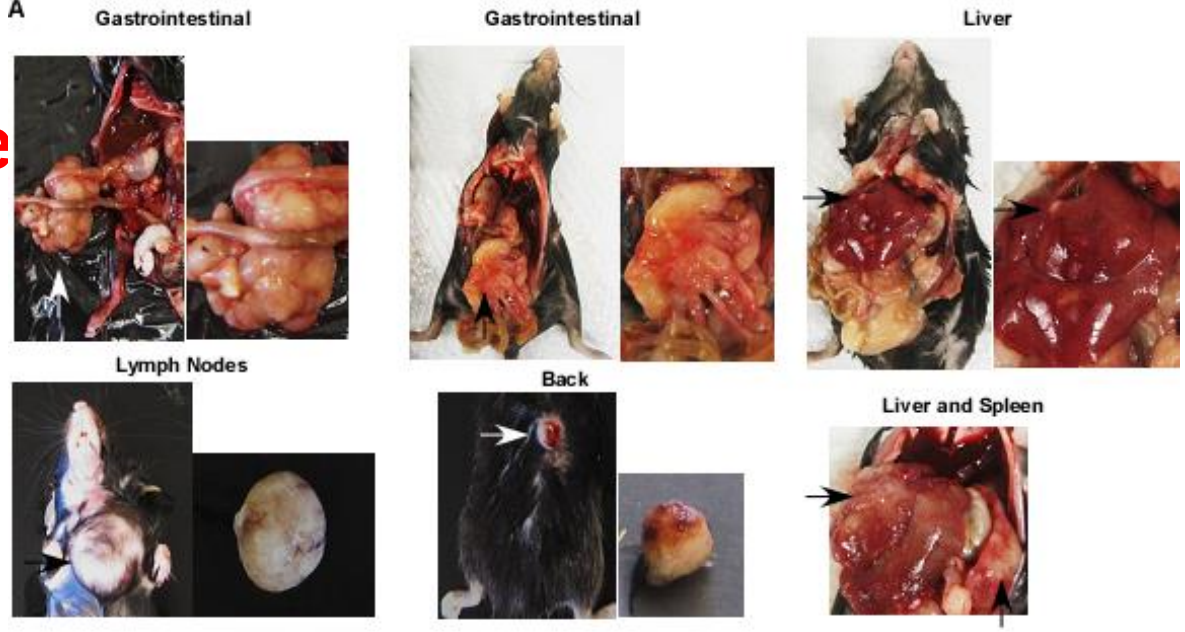
Amplification of damage response



Bekker-Jensen and Mailand, DNA repair, 2010, 9, 1219

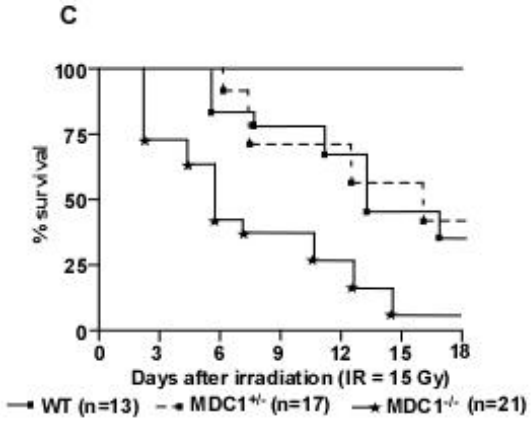
MDC1 KO phenotype

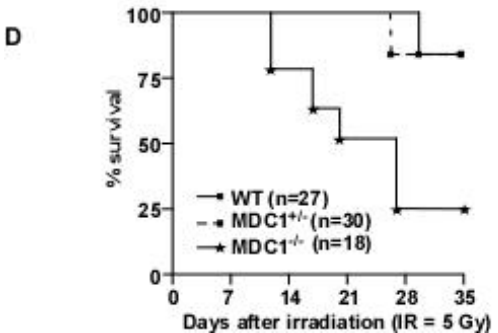
MDC1 heterozygous mice are cancer prone by a DDR-independent pathway

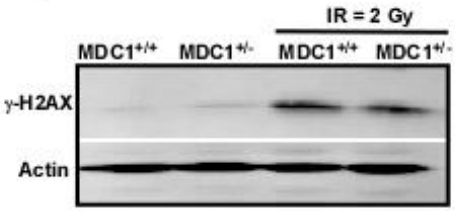


B

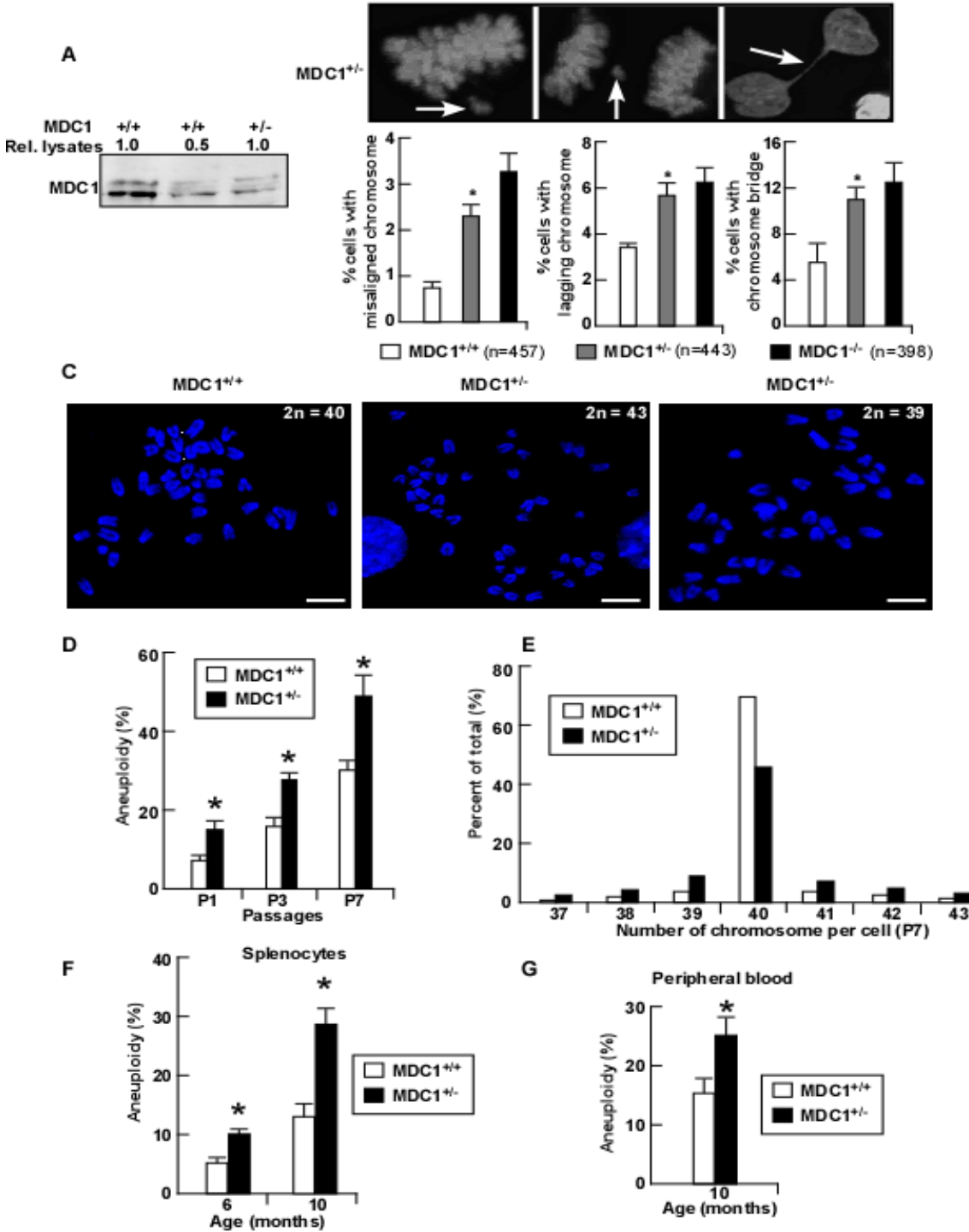
Organs \ Genotypes	WT	MDC1$^{+/-}$
Number mice	26	43
Liver	1	3
Spleen		1
Lymph Nodes		2
Gastrointestinal	1	2
Back		1





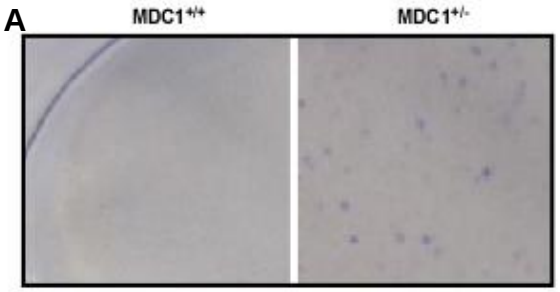


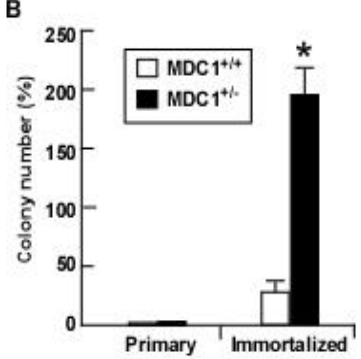
MDC1 reduction causes aneuploidy



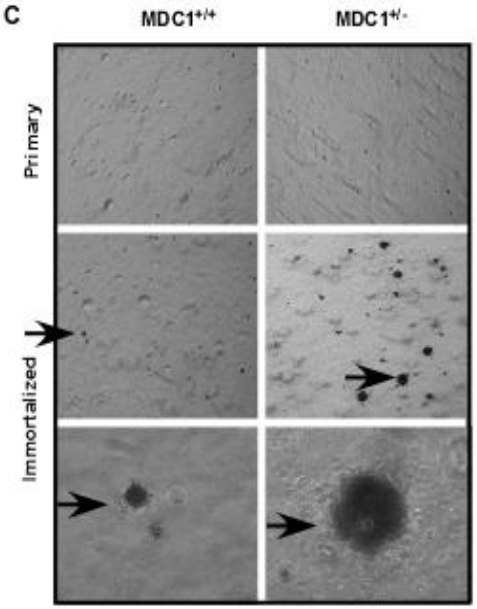
MDC1 heterozygosity sensitizes transformation of immortalized MEFs

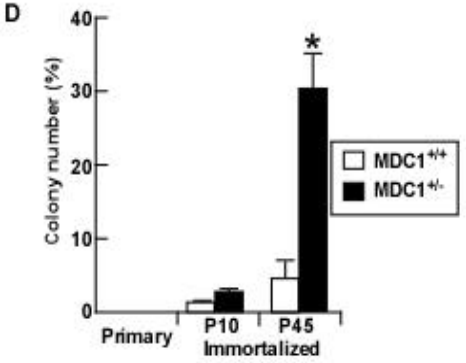
Foci formation in regular dish



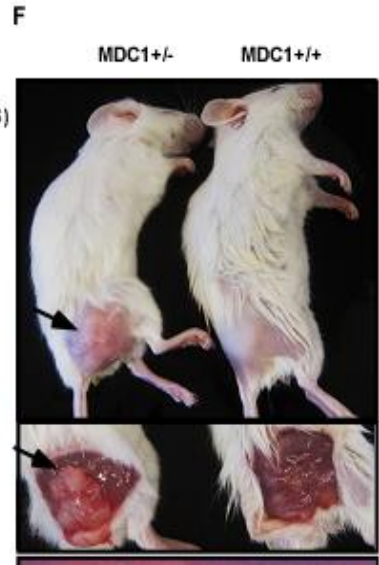


Soft agar assay



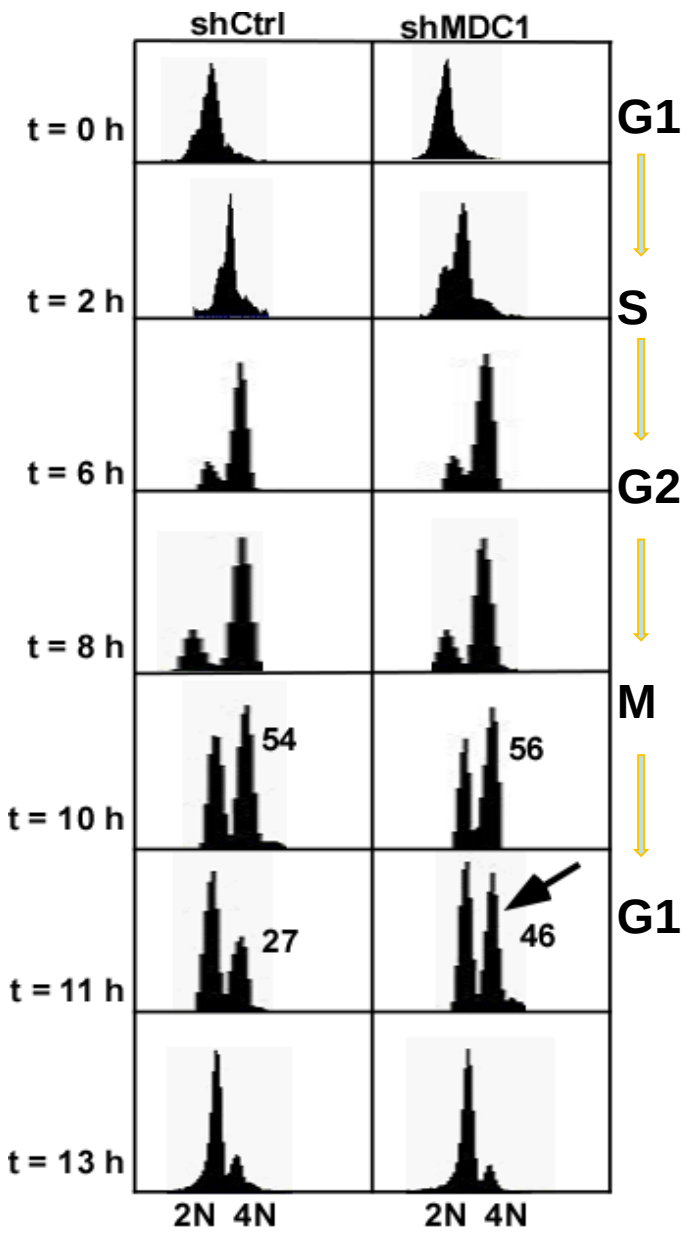


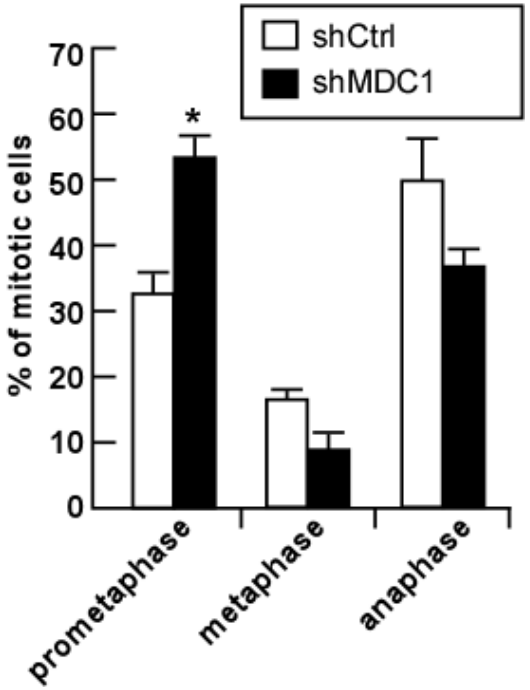
To immortalize, MEFs were transfected with SV40 large T antigen

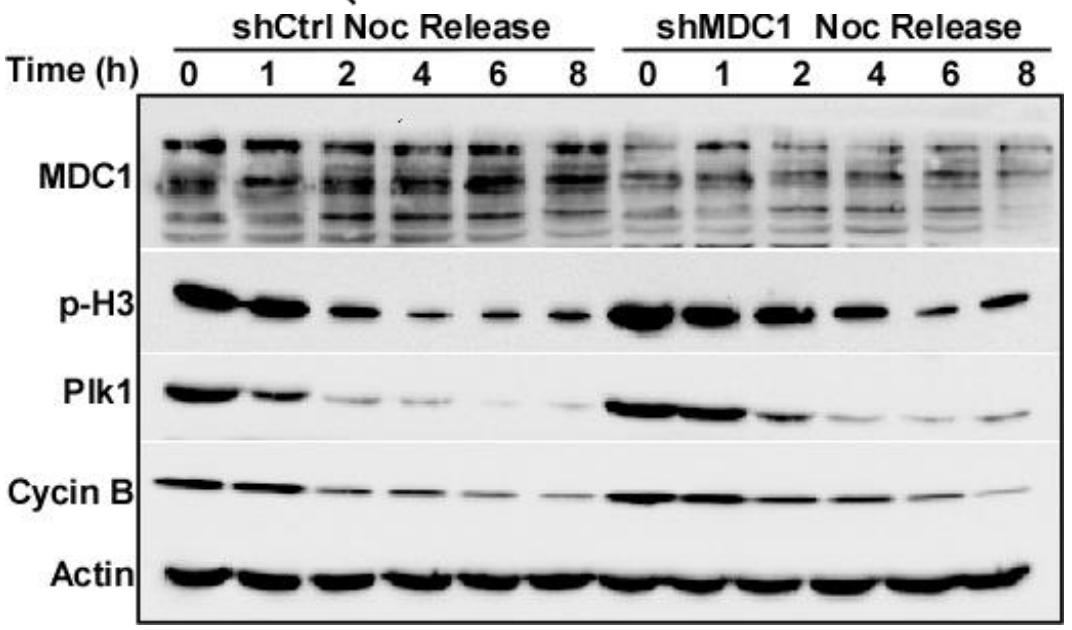


MDC1 is required for mitotic progression

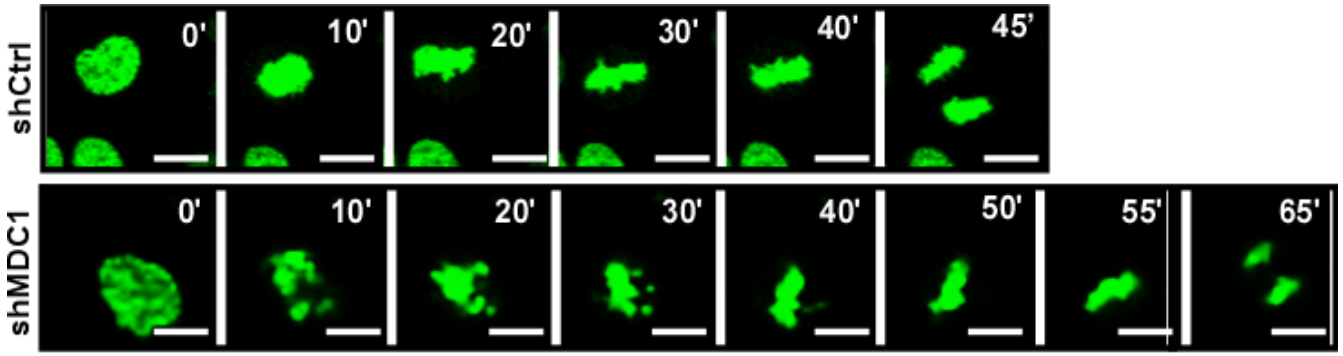
Double Thymidine Block--- Release

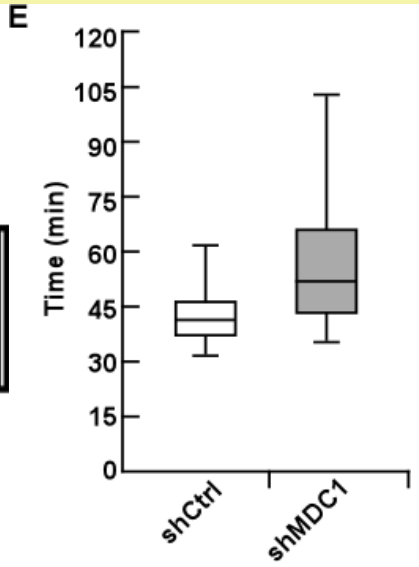




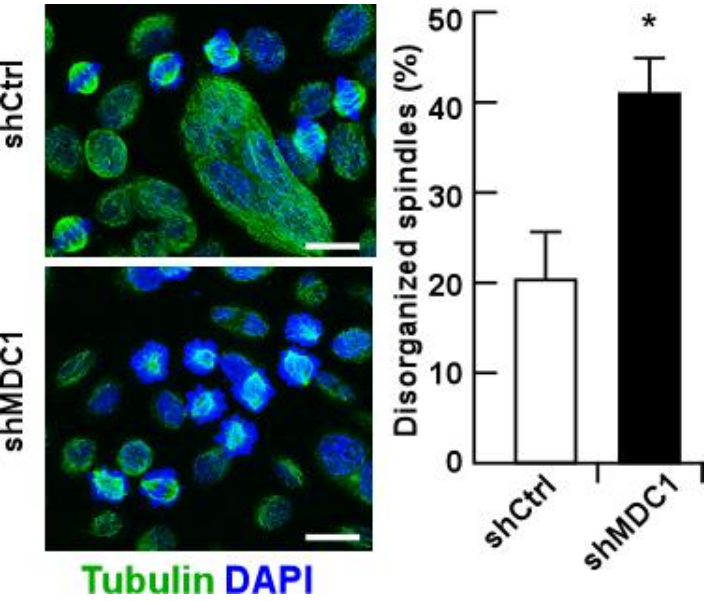


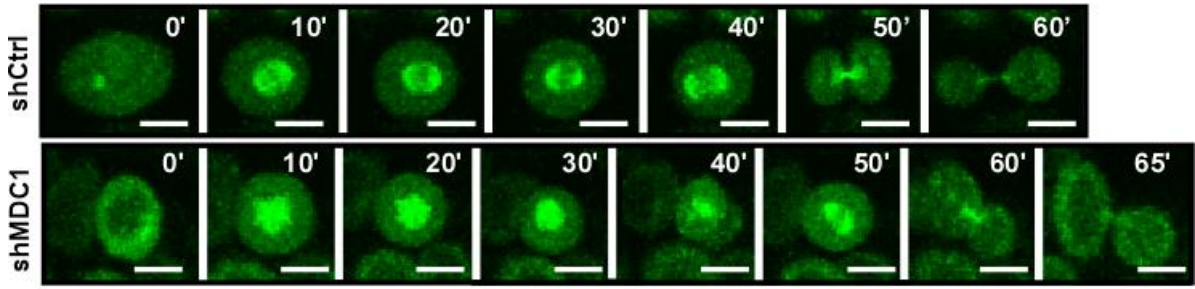
MDC1 is required for timely progression of mitosis





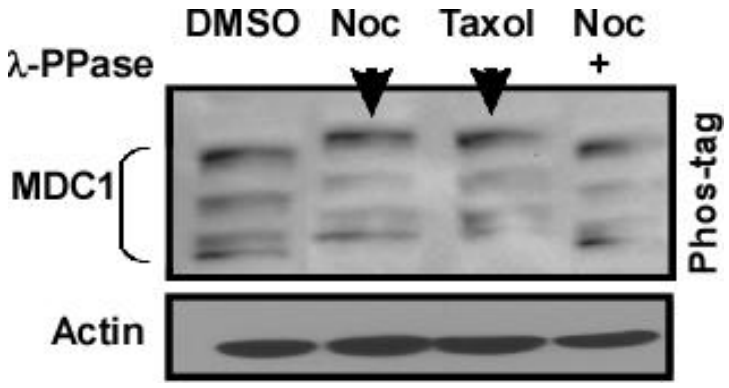
HeLa expressing GFP-H2B

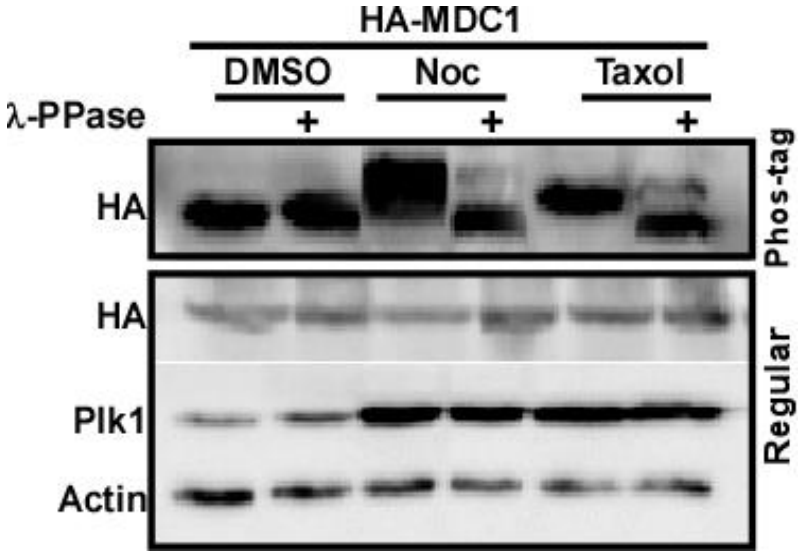


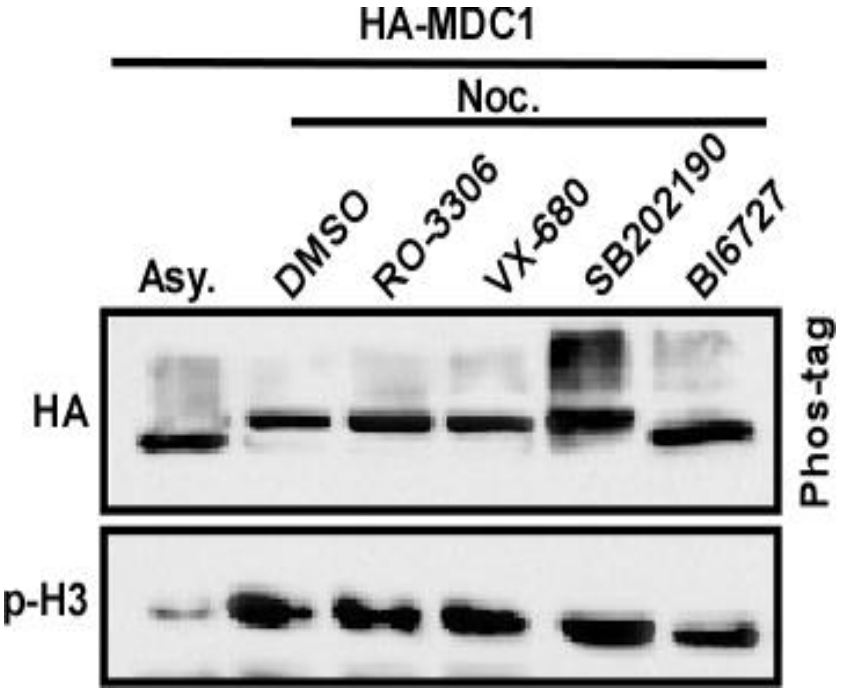


HeLa expressing GFP-α-tubulin

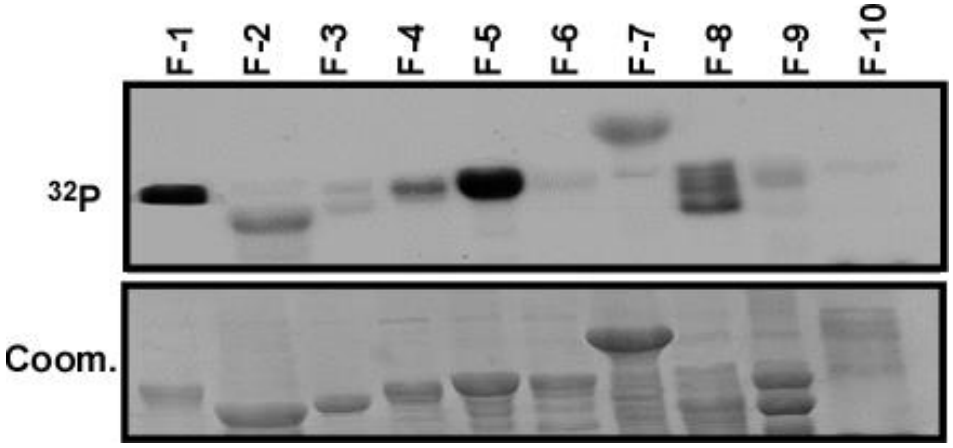
MDC1 is phosphorylated in mitosis

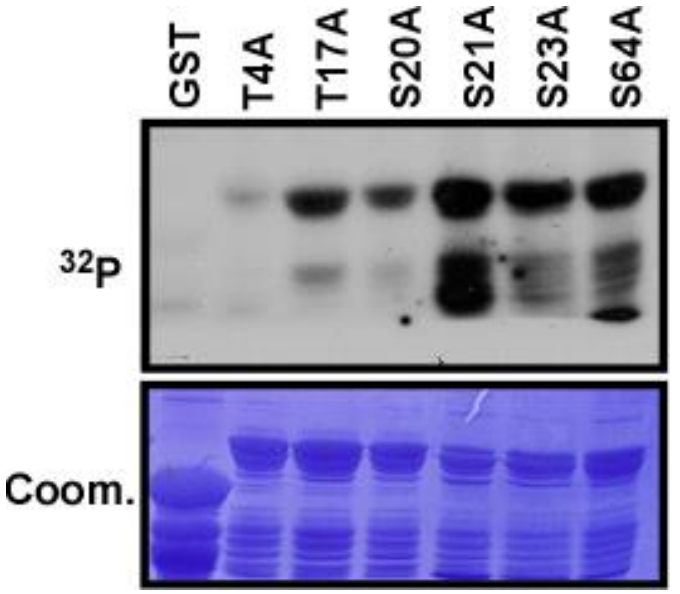


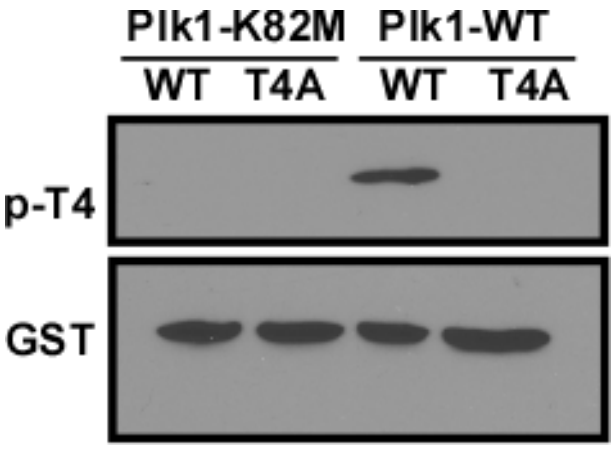


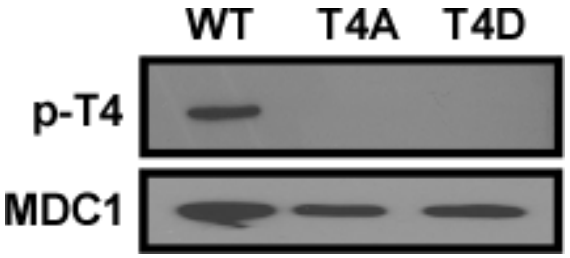


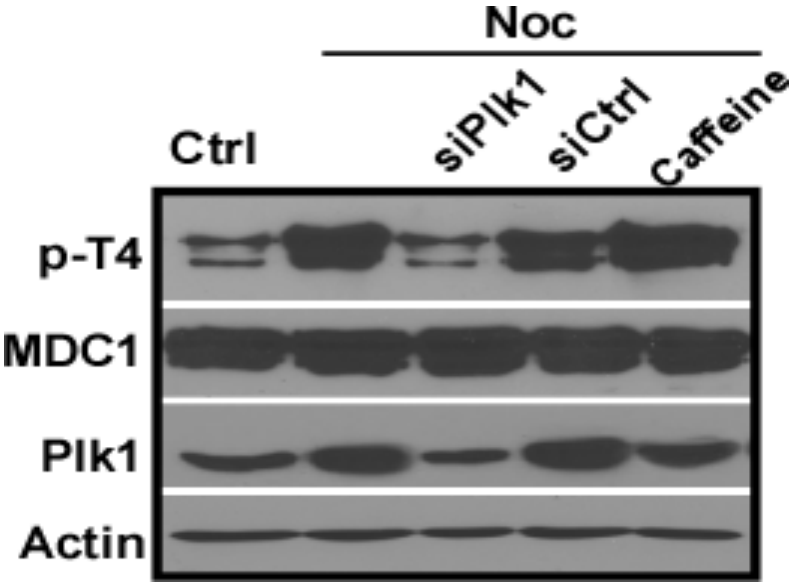
Plk1 phosphorylates MDC1 at T4



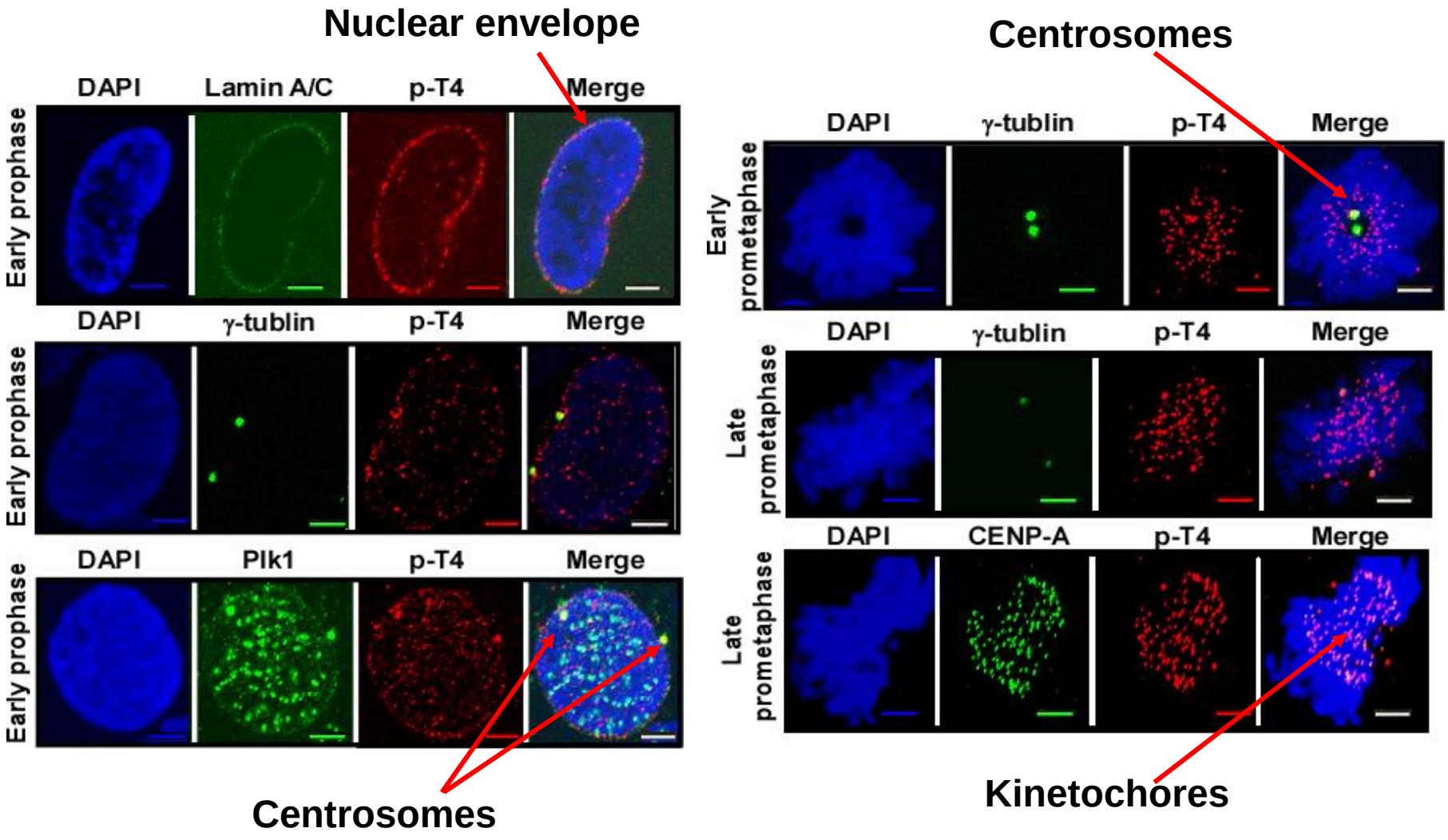




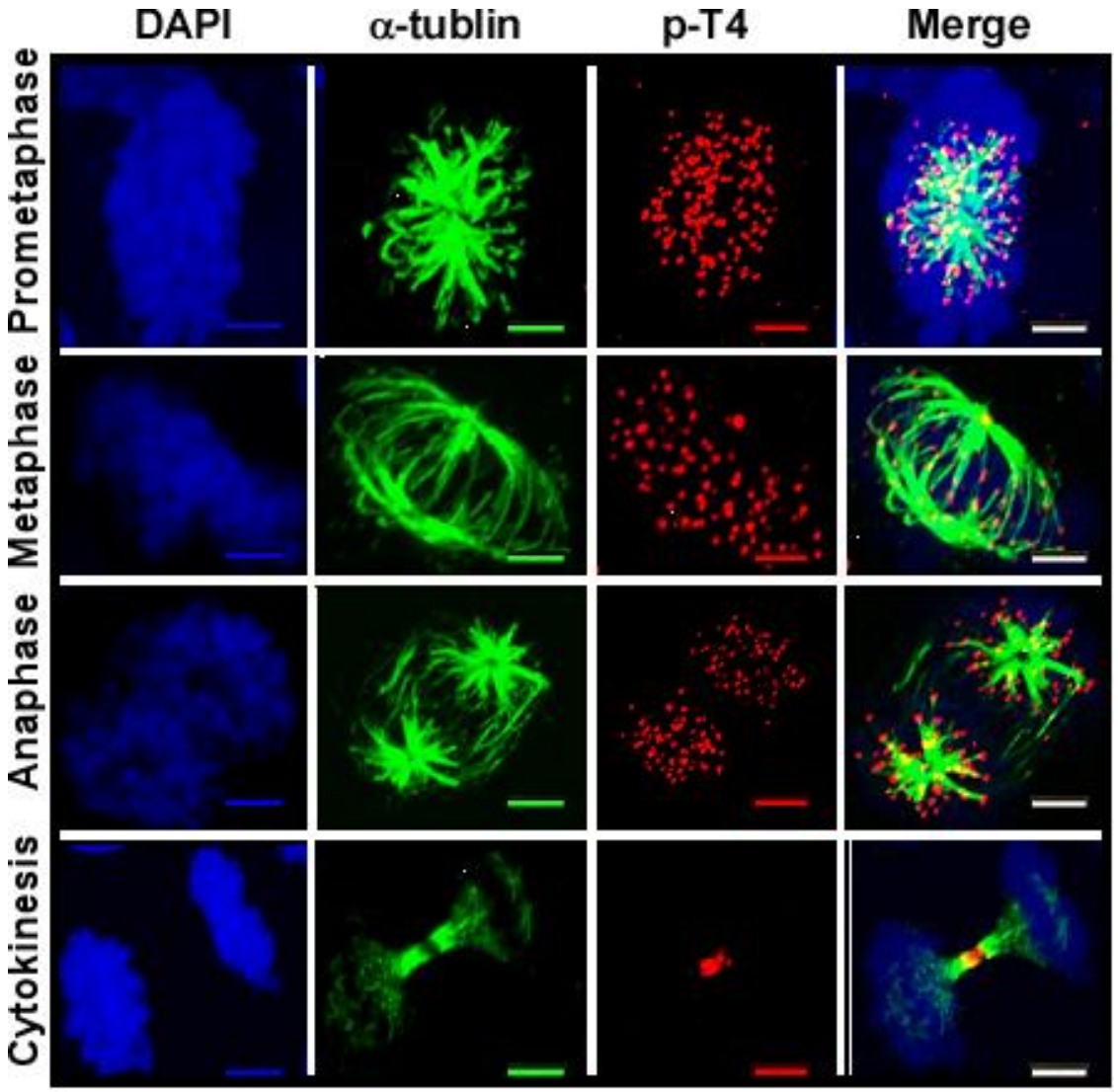




p-T4-MDC1 localization in prophase and prometaphase



p-T4-MDC1 localization in mitosis

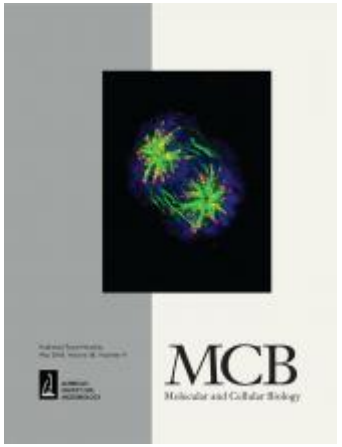


Kinetochores

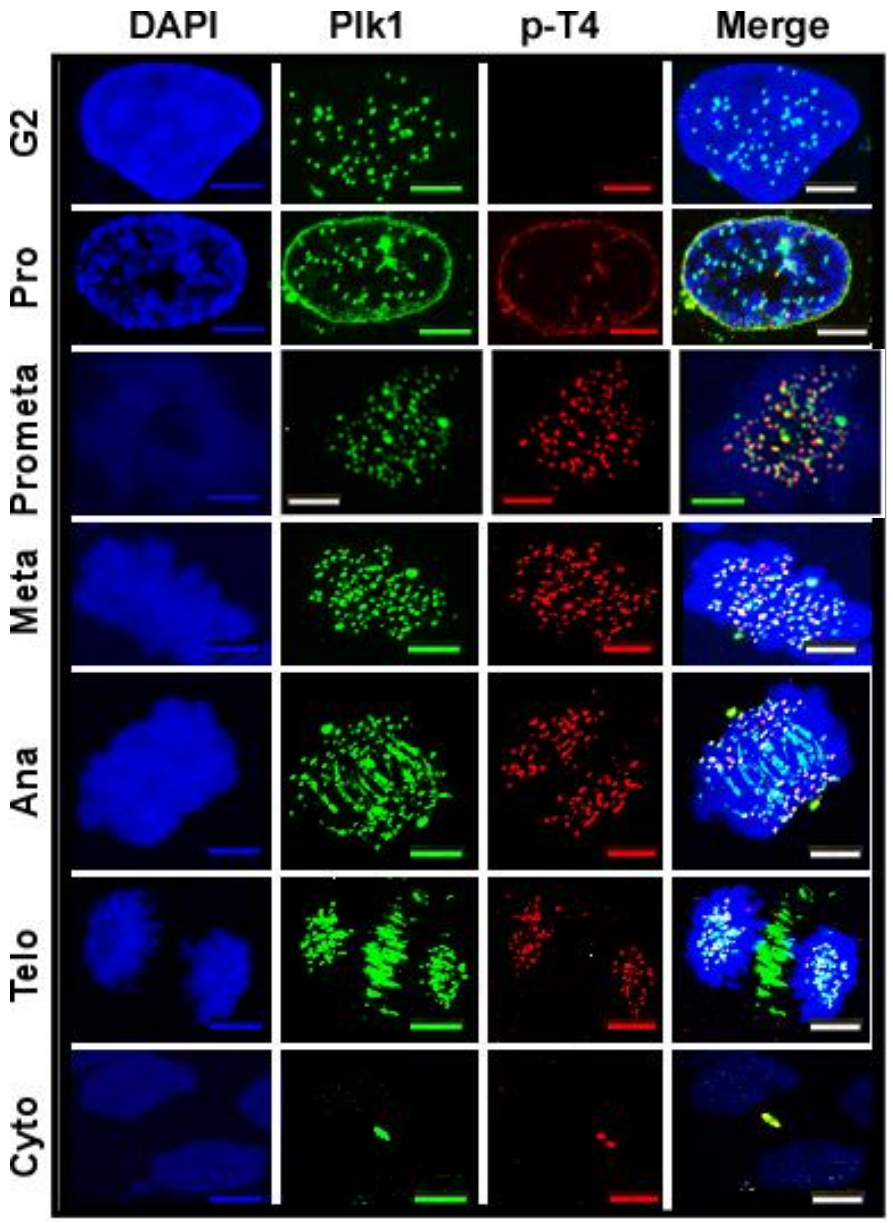
Kinetochores

Microtubule (+) ends

Midbody



p-T4-MDC1 localization in mitosis



Nuclear envelope

Kinetochores

Kinetochores

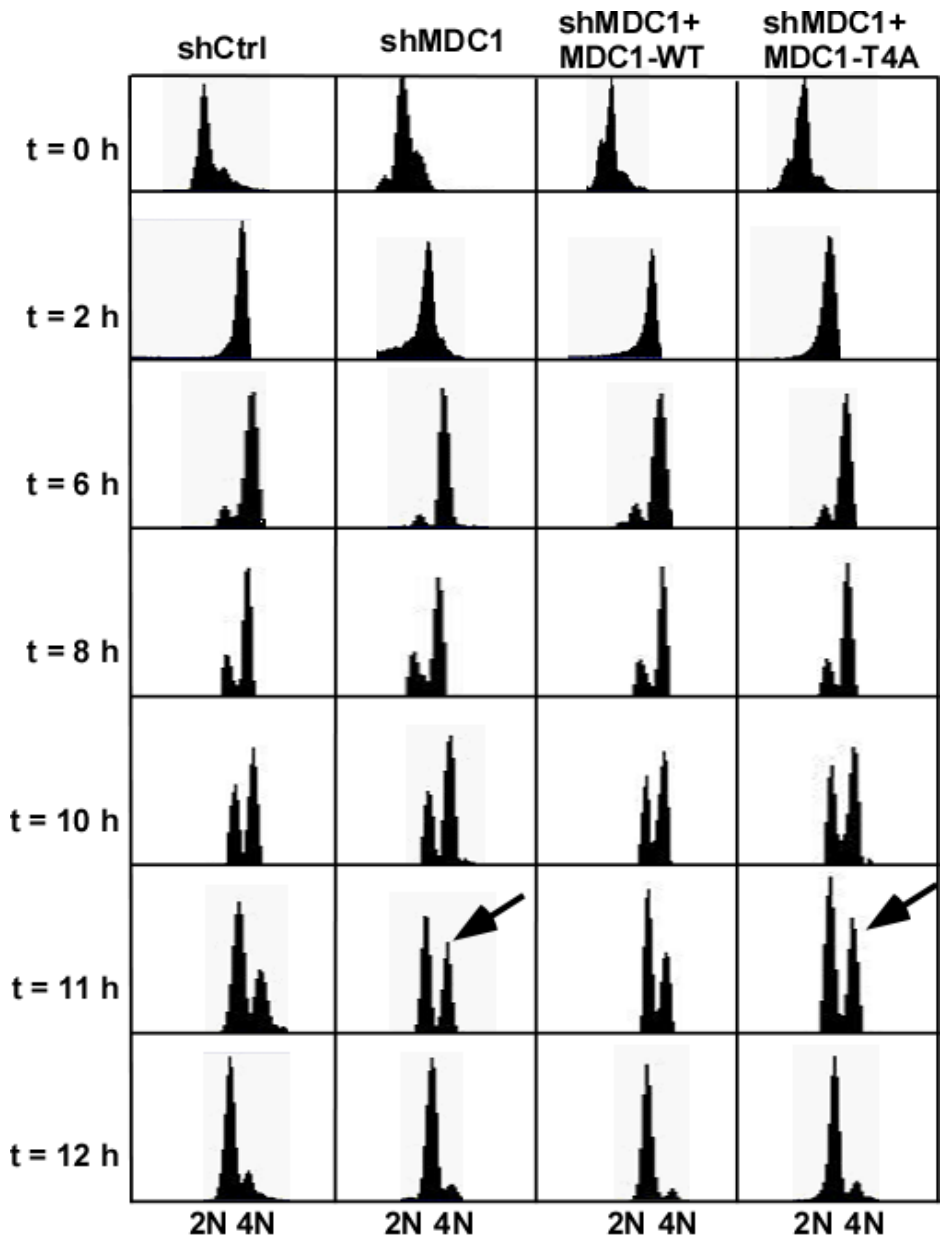
Kinetochores

Midzone

Midbody

Plk1 phosphorylation of MDC1-T4 is required for mitotic progression

Double Thymidine Block --- release



G1

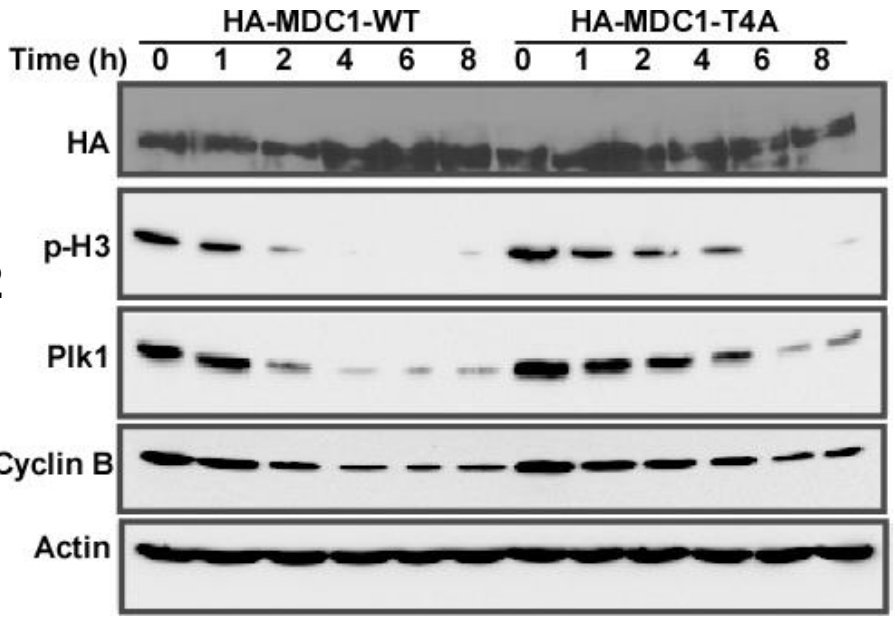
S

G2

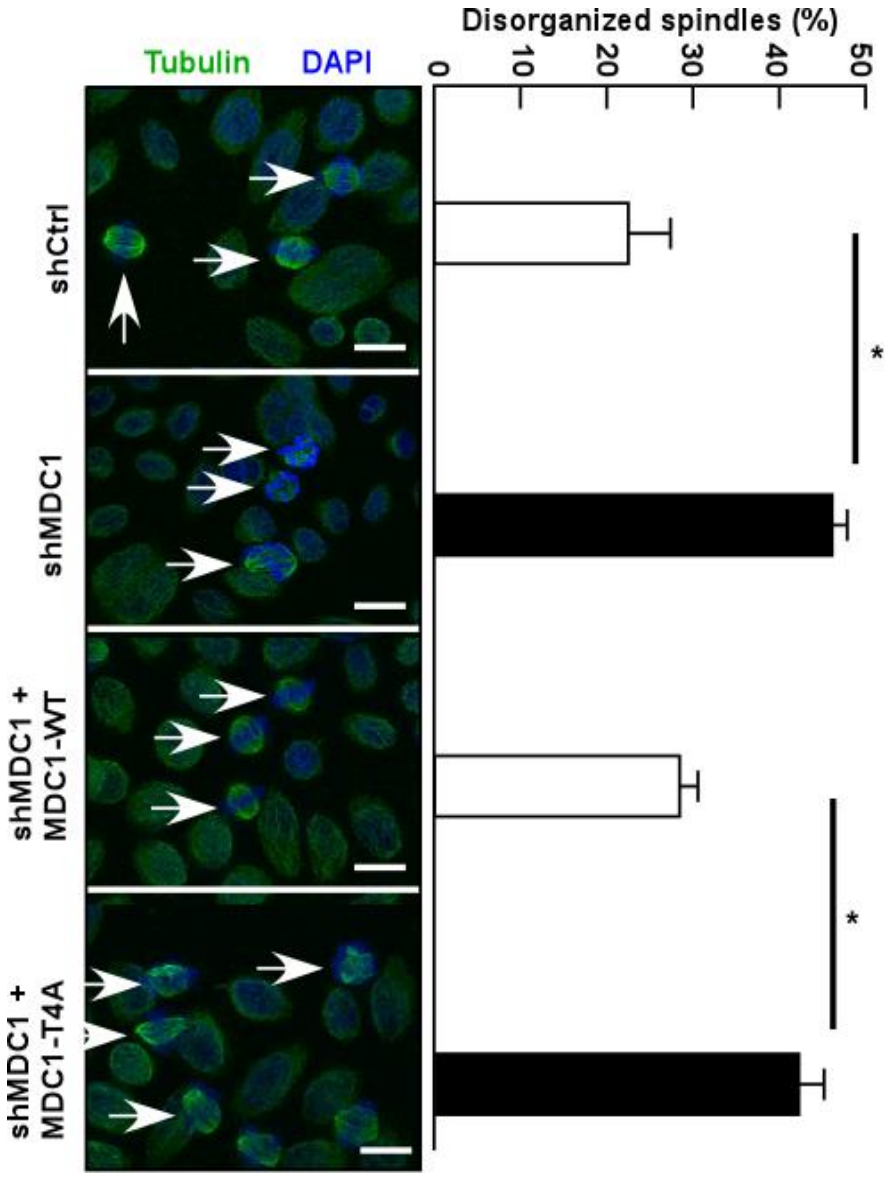
M

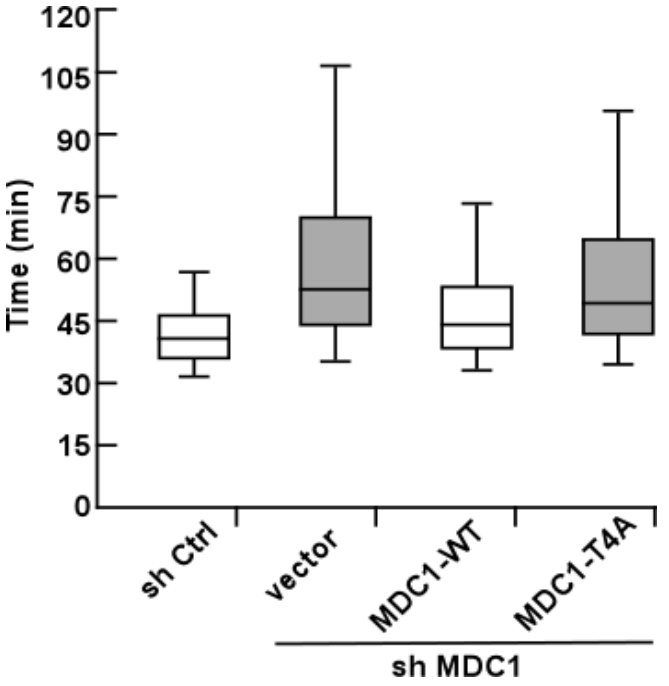
G1

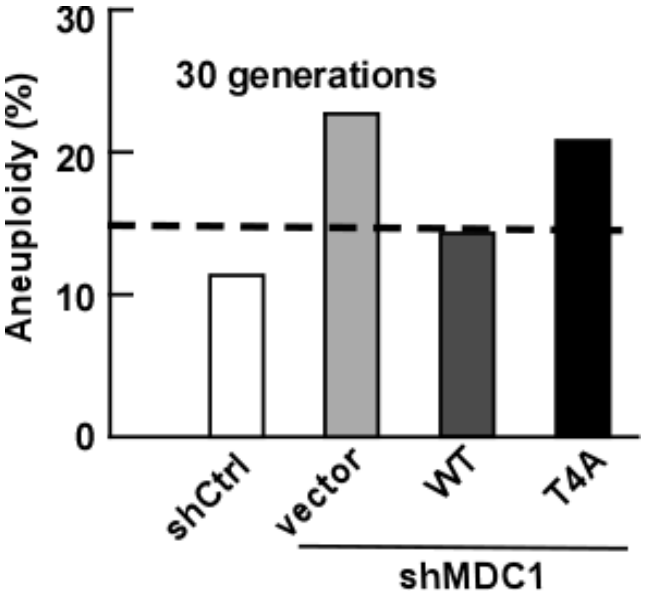
Noc release



Plk1 phosphorylation of MDC1-T4 is required for mitotic progression







Summary

--MDC1 has a function independent of DNA damage response

--MDC1 is required for mitosis

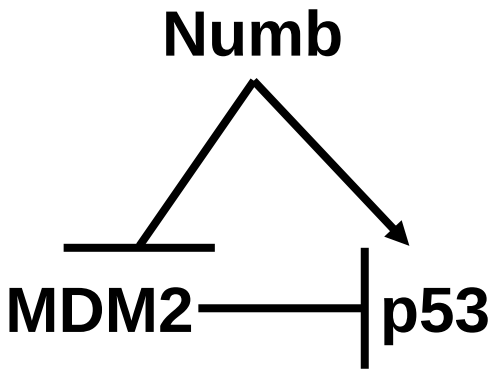
--MDC1 is phosphorylated by Plk1 at T4

--Plk1 phosphorylation of MDC1-T4 is required for timely progression through mitosis

Publications: Li et al., *Mol Cell Biol*, 2017, 37, e00595.

Dr. Zhiguo Li

The Numb/p53 pathway



-- Numb is an inhibitor of Notch signaling

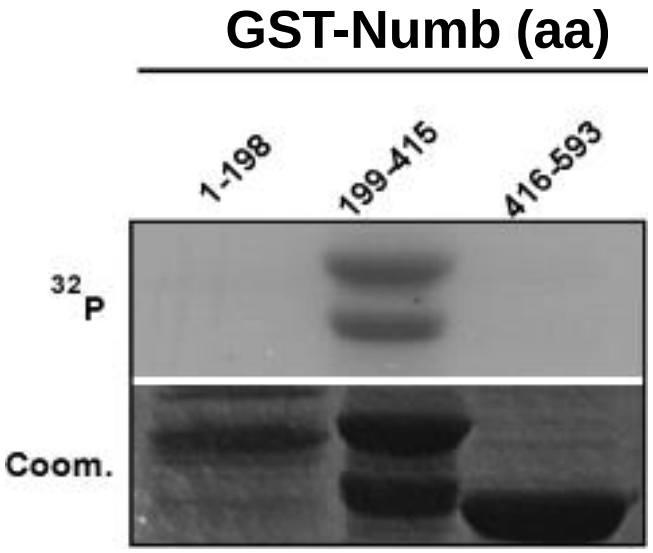
-- Numb is involved in the cell-fate decisions of a number of cell lineages

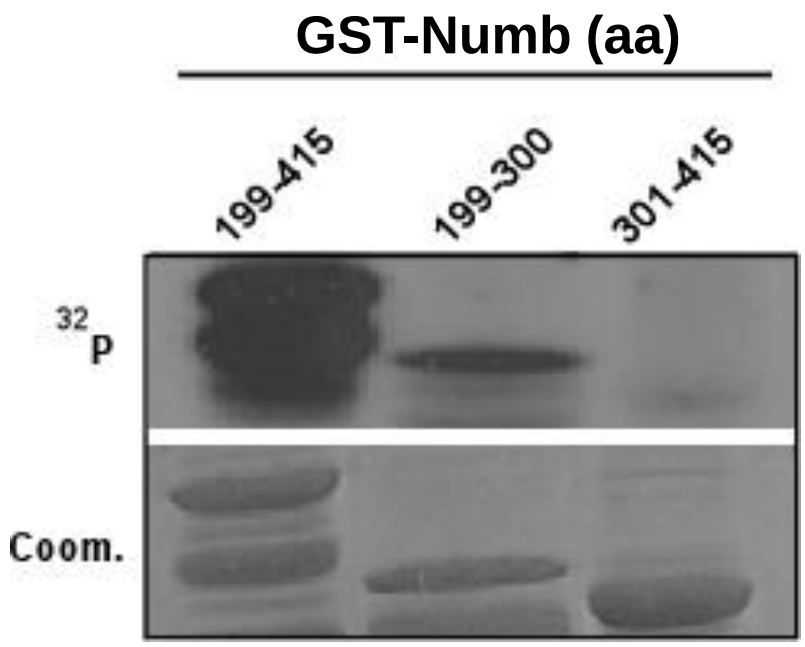
-- Numb, MDM2 and p53 form a trimeric complex

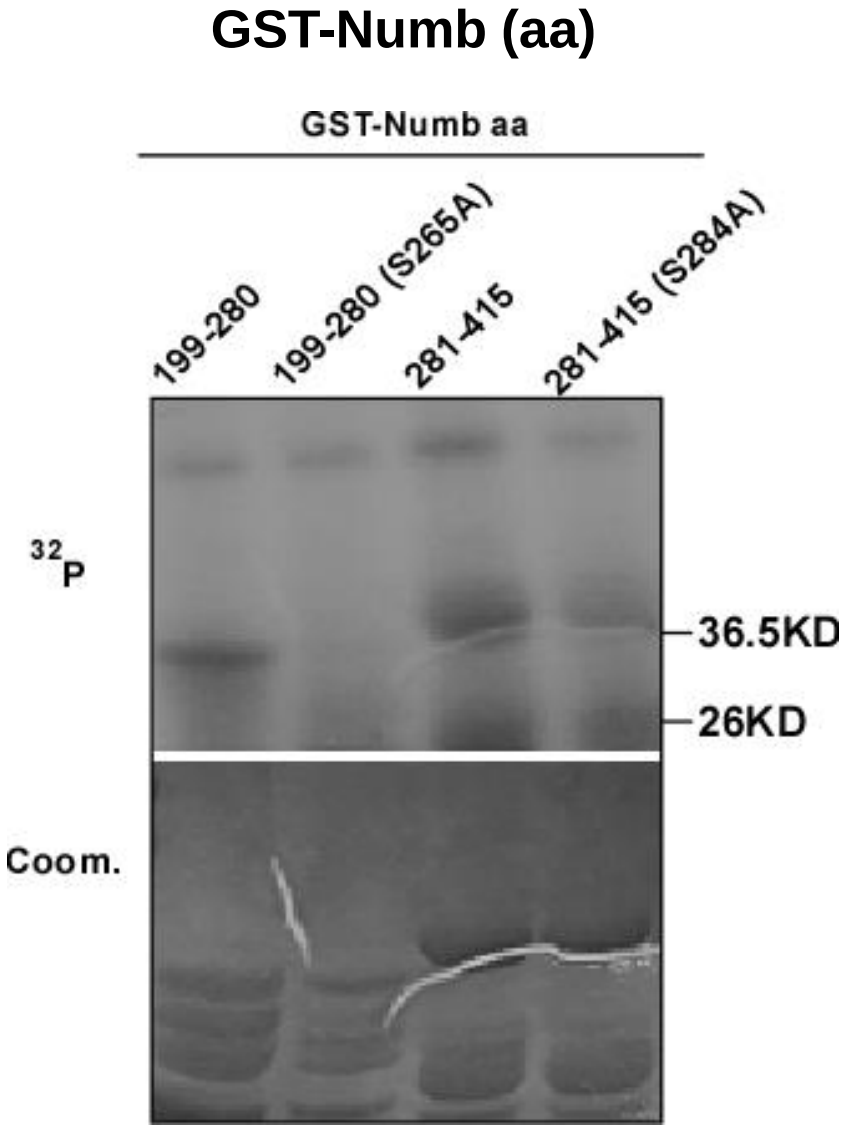
-- Numb stabilizes p53 by inhibiting the E3 ubiquitin ligase activity of MDM2

Colaluca, *Nature* 2008, 451, 76-80.

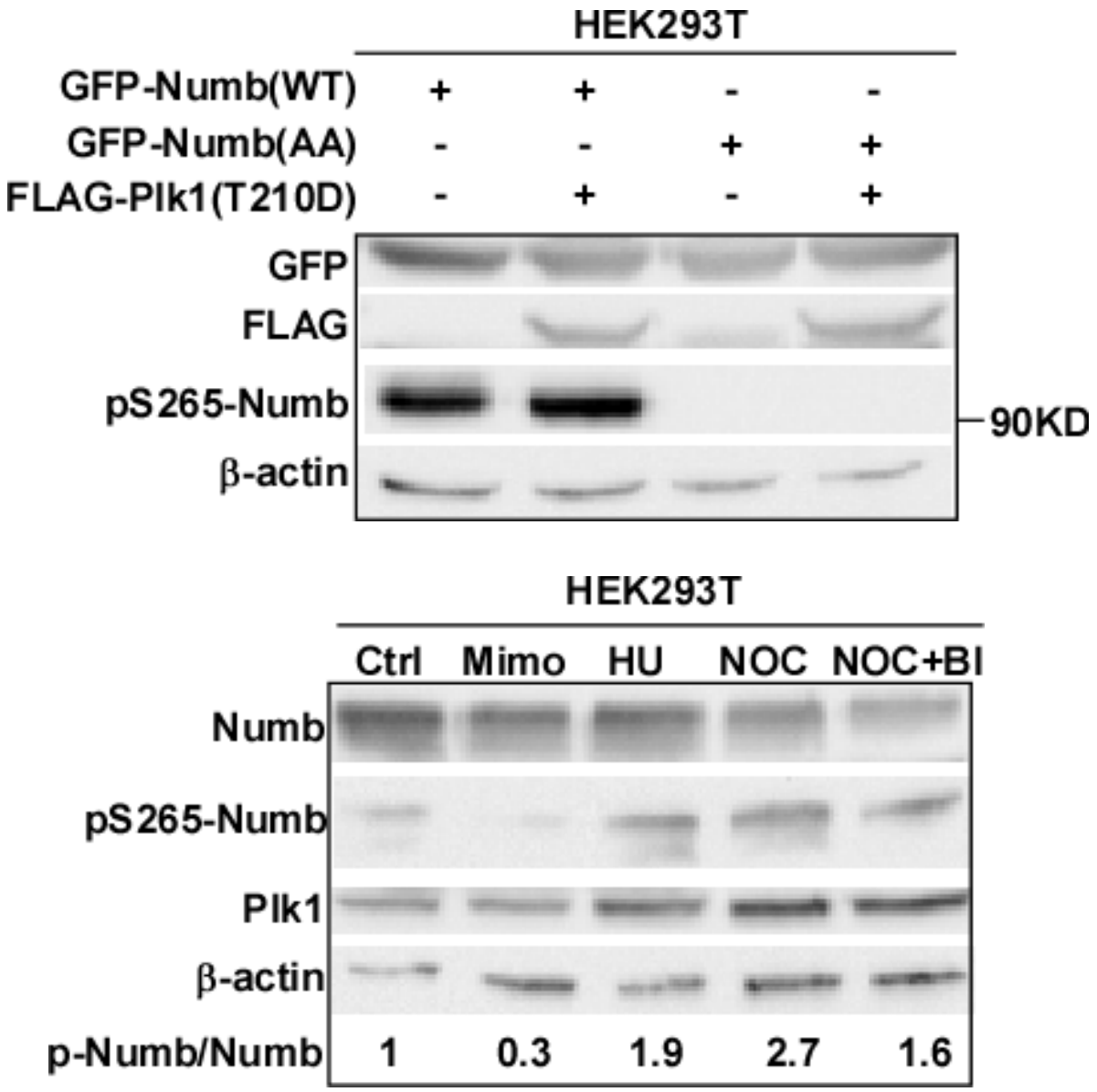
Plk1 phosphorylates Numb at S265 and S284 *in vitro*



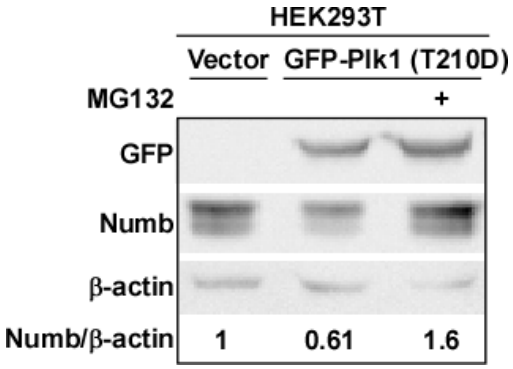


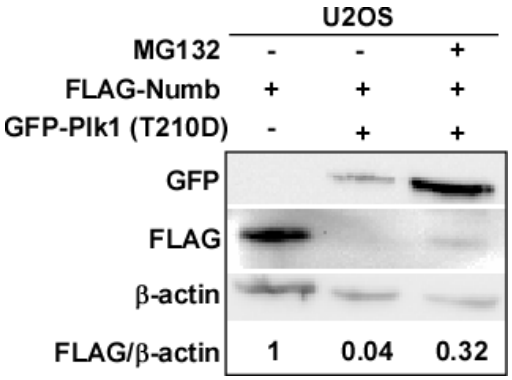


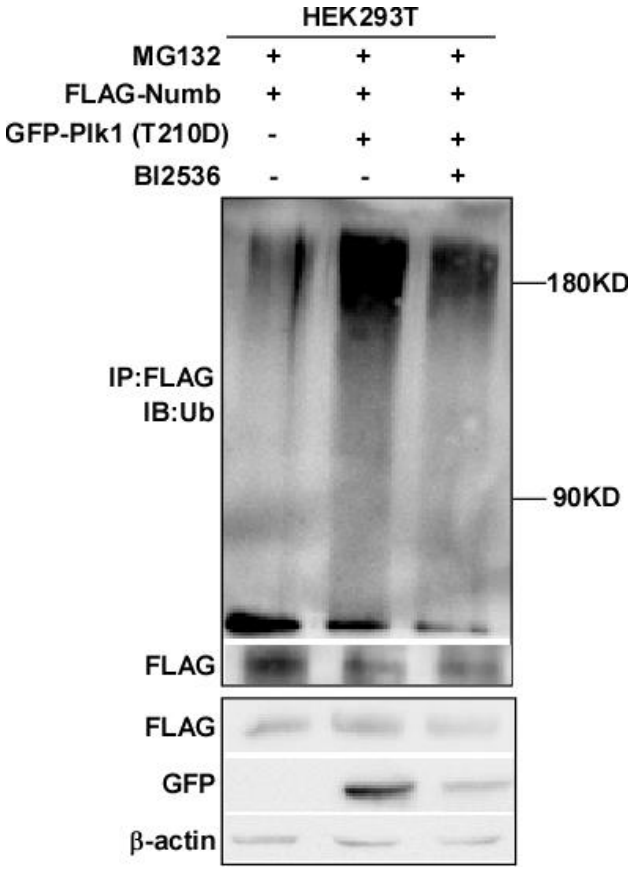
Numb-S265 is phosphorylated *in vivo* in a Plk1 dependent manner

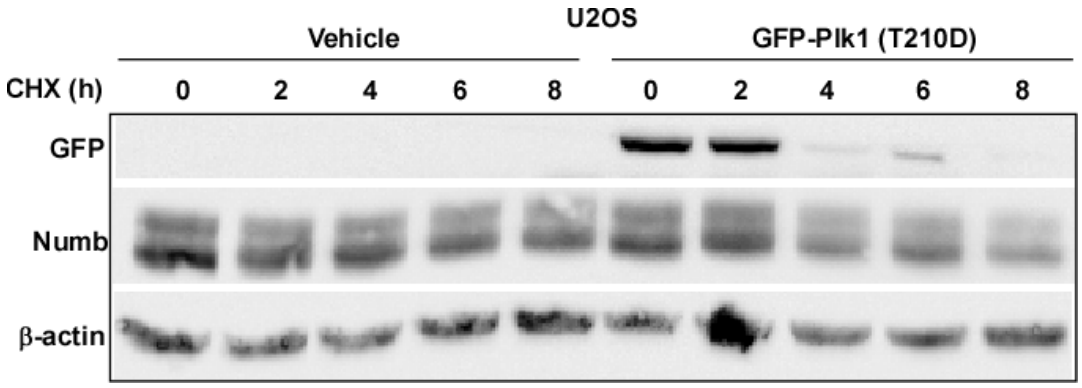


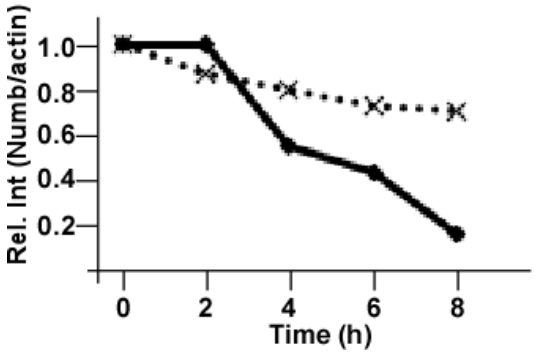
Numb degradation is enhanced by Plk1-associated kinase activity

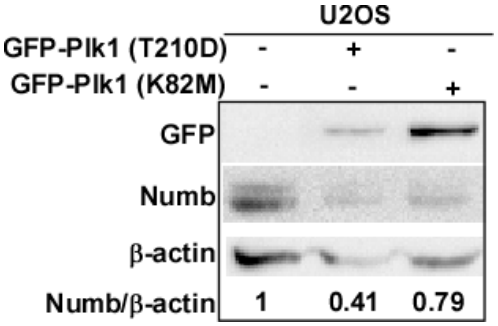




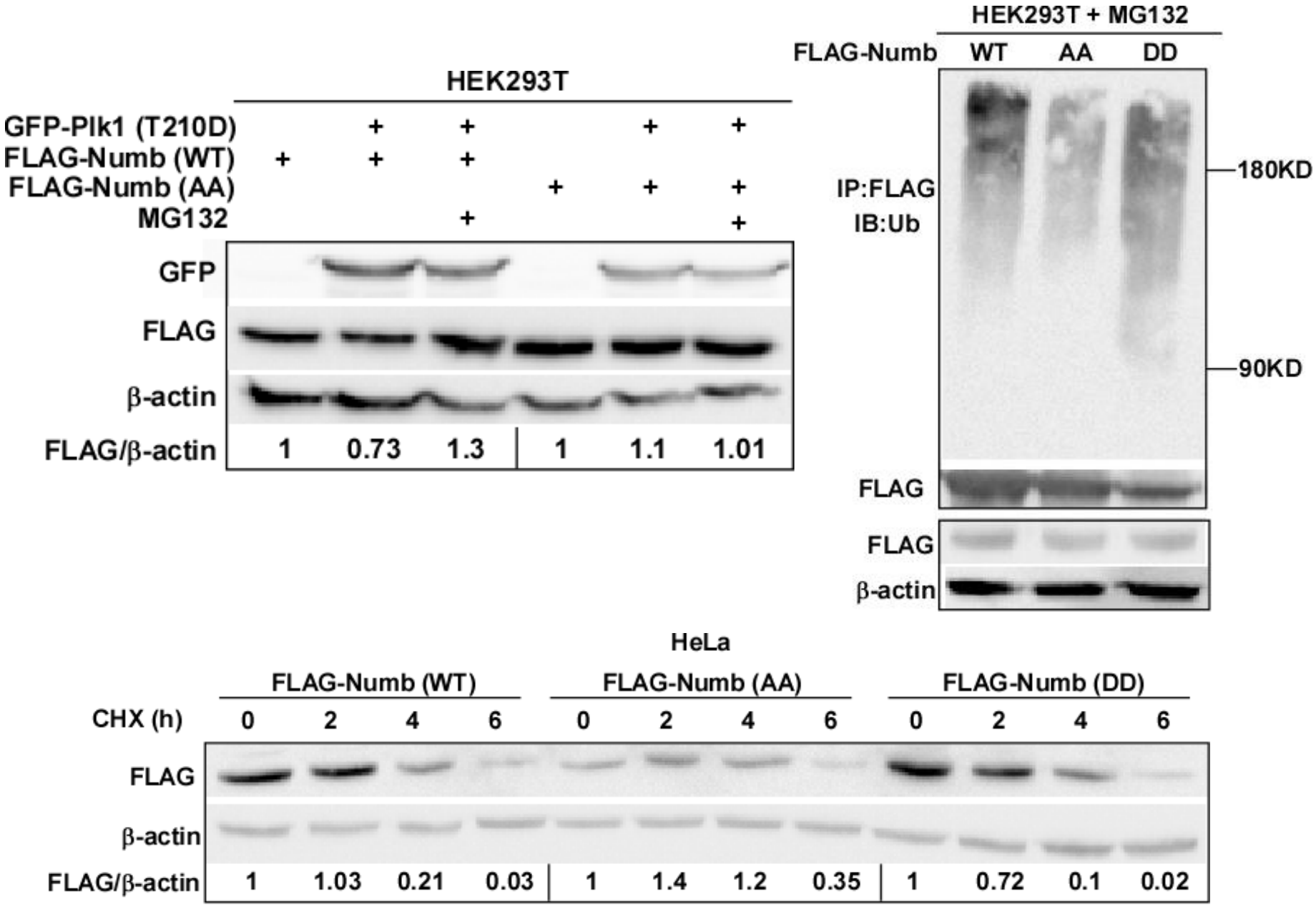




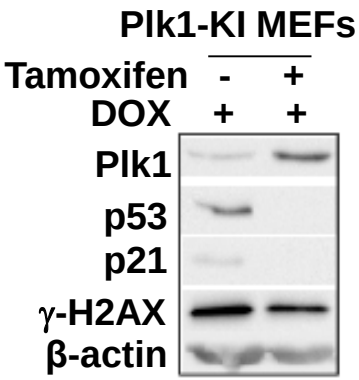




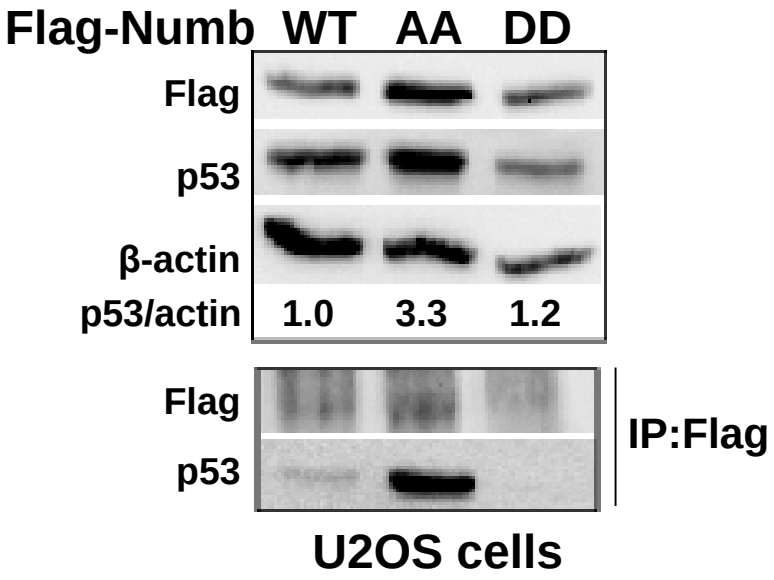
Plk1 phosphorylation of Numb results in its proteasome degradation

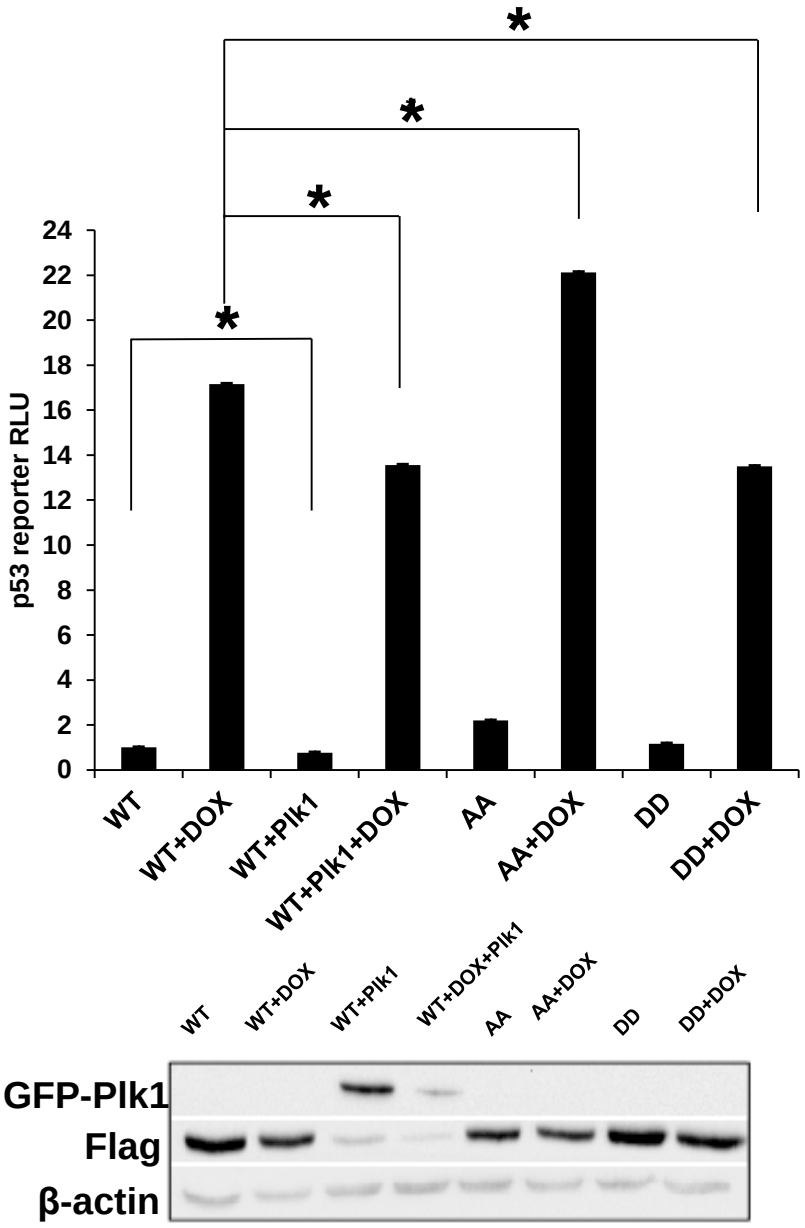


Plk1 negatively regulates the Numb/p53 pathway

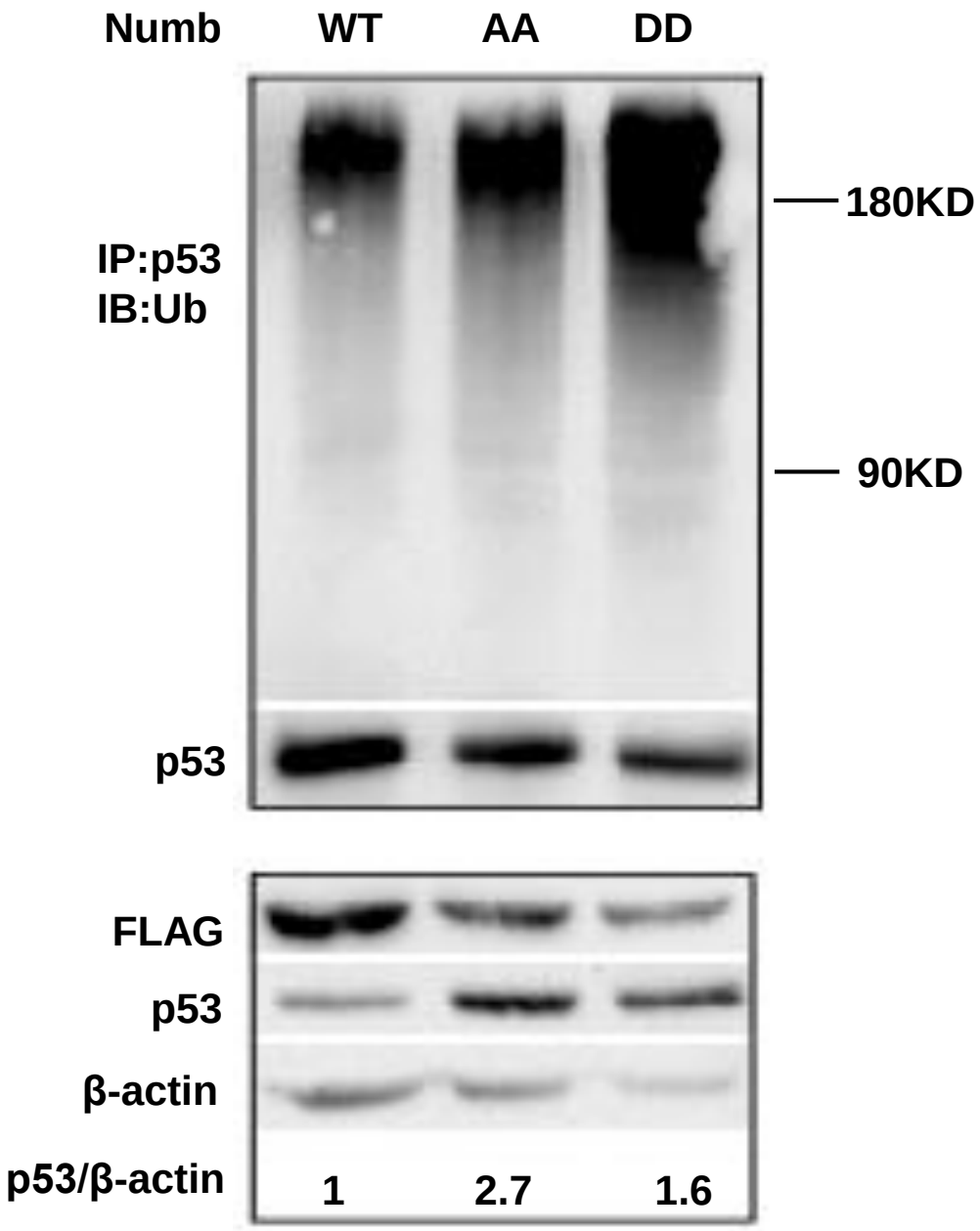


A higher binding affinity between p53 and Numb-AA

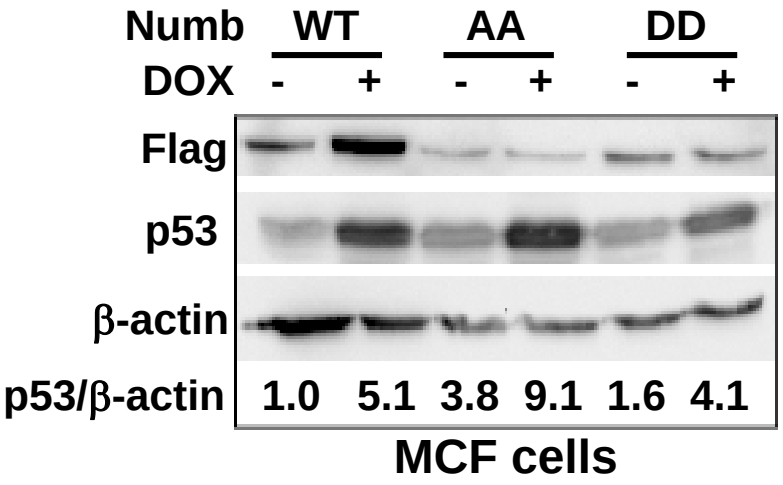




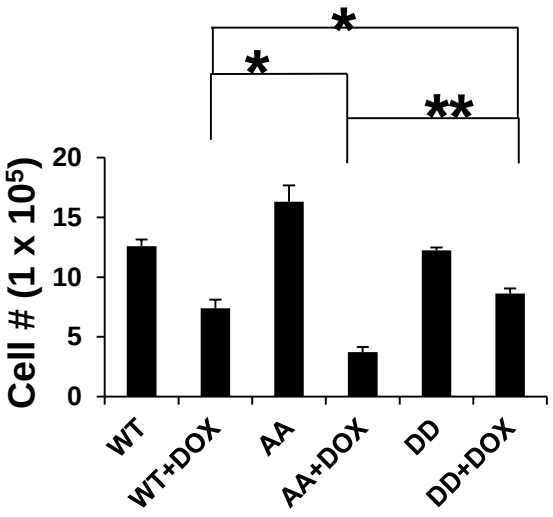
Plk1 phosphorylation of Numb contributes to p53 degradation

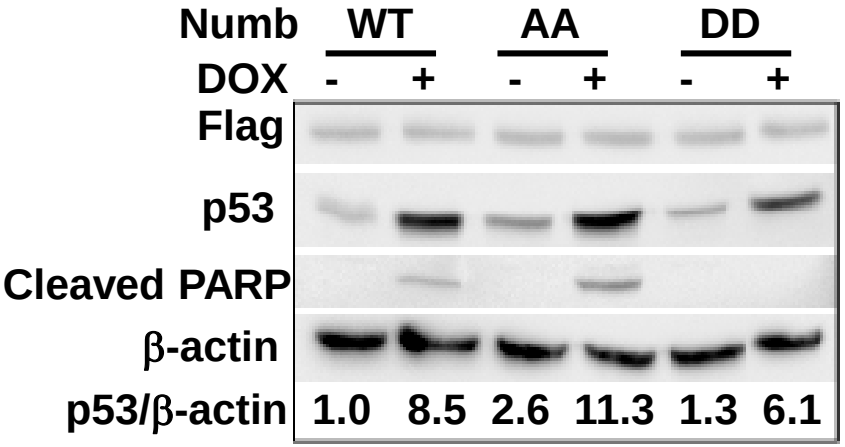


Cells expressing Numb-S265A/S284A are more sensitive to doxorubicin

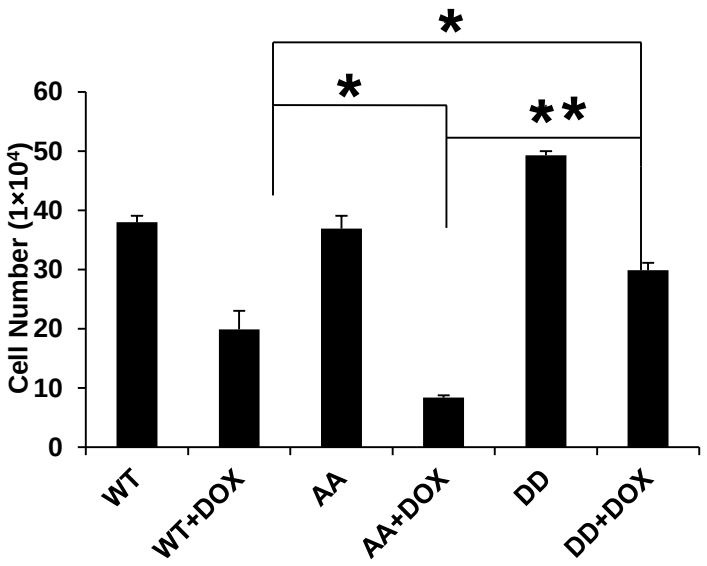


MCF cells

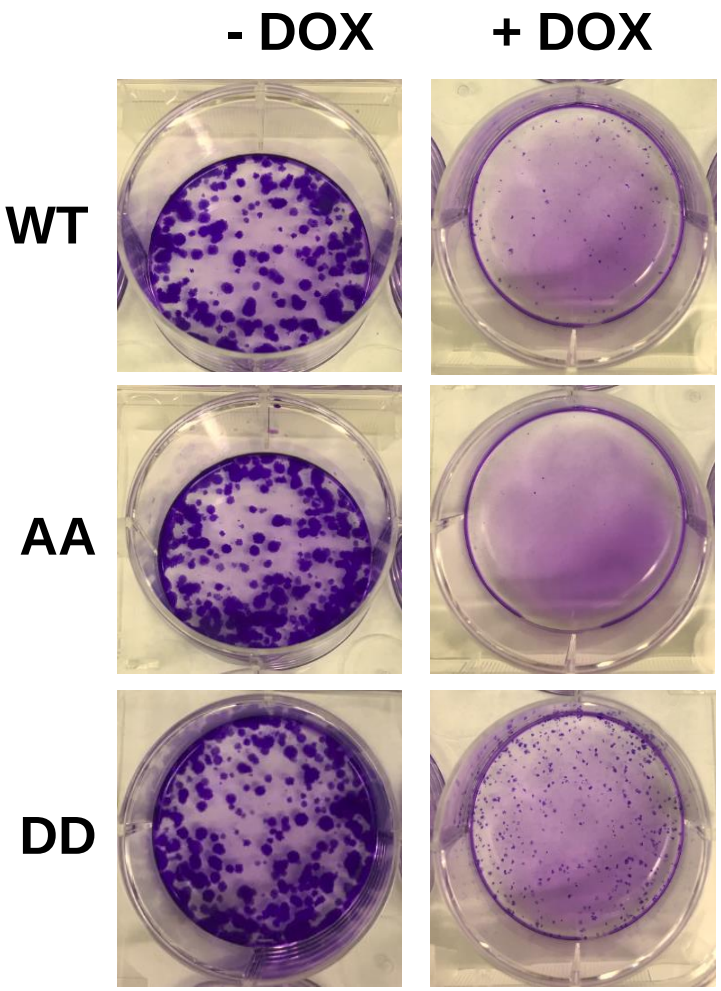


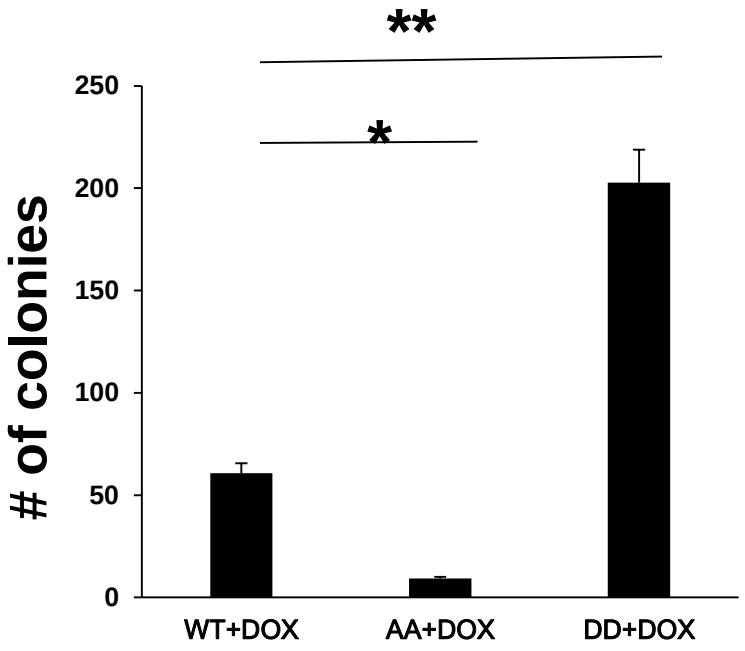


U2OS cells

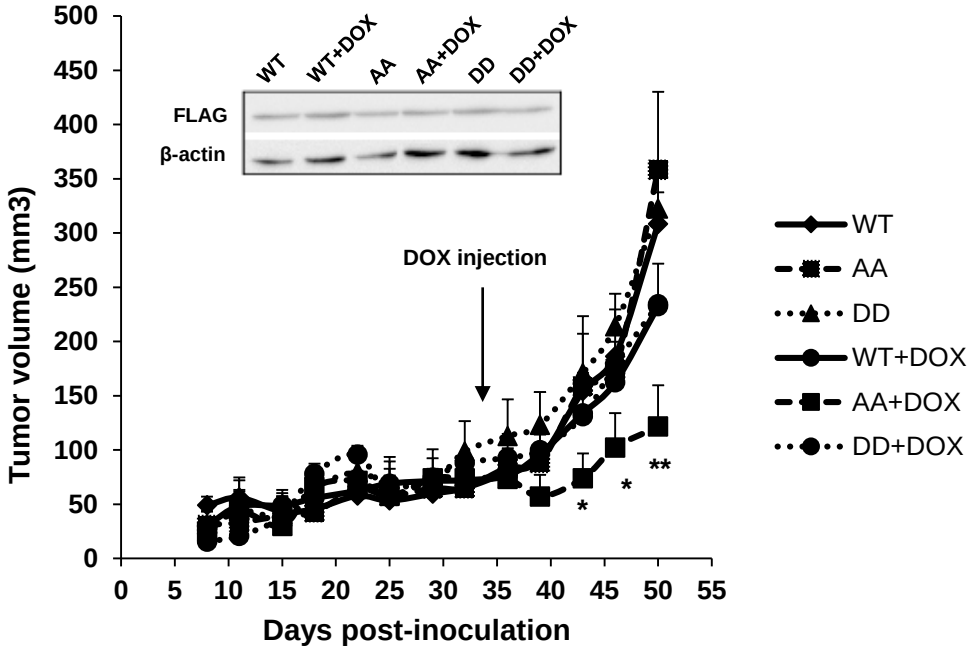


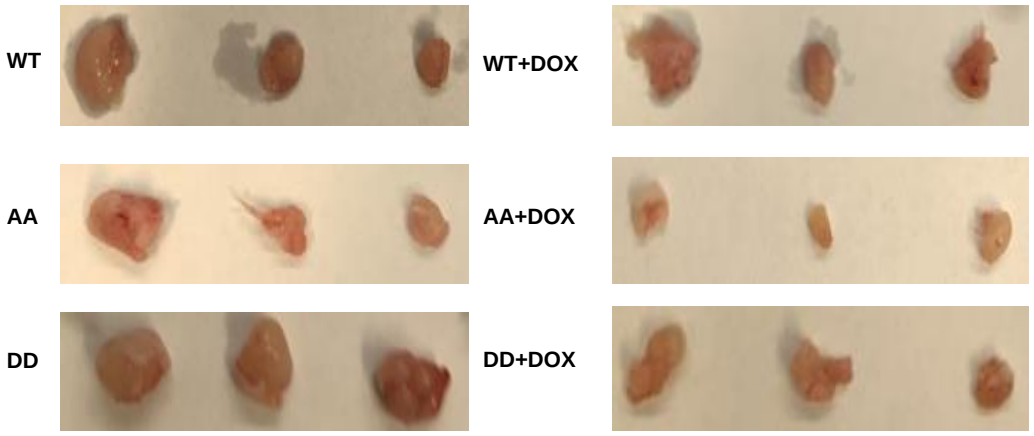
Cells expressing Numb-S265A/S284A are more sensitive to doxorubicin

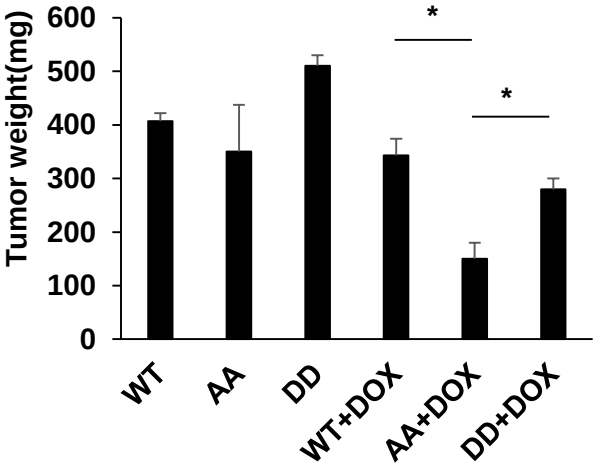




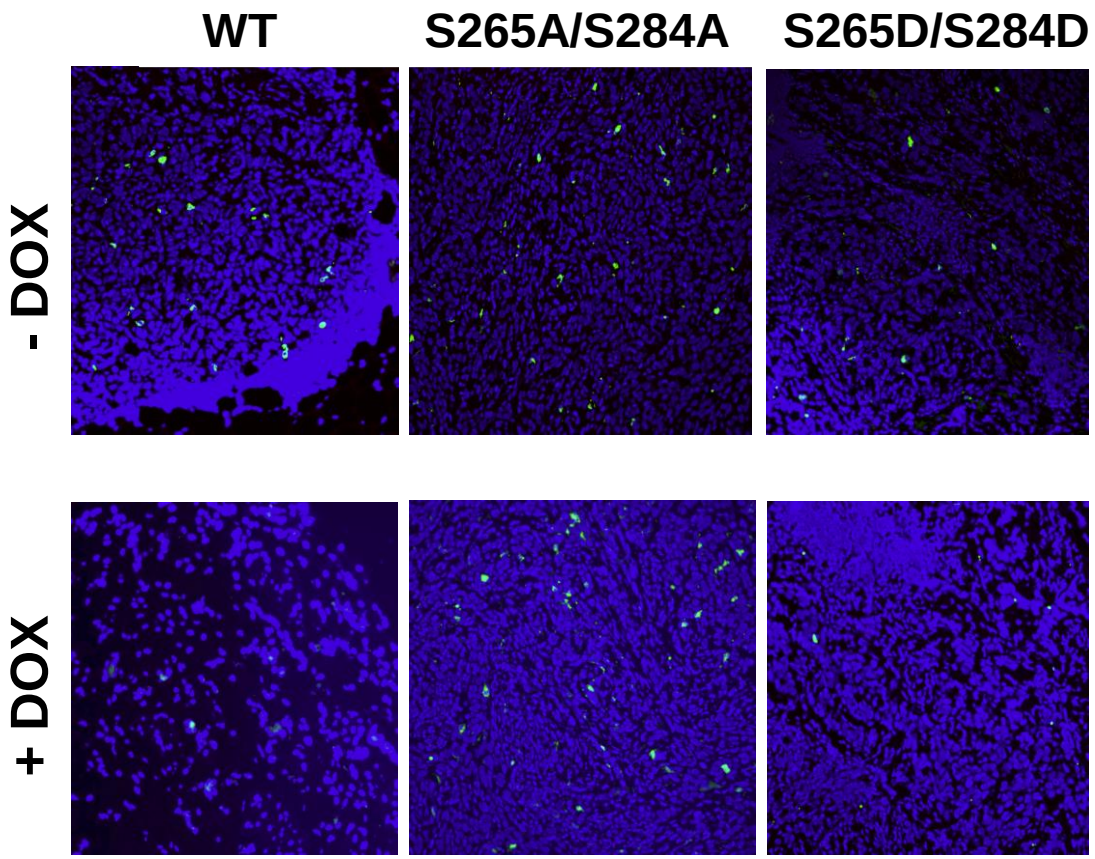
Tumors carrying Numb-S265A/S284A are more sensitive to chemotherapy



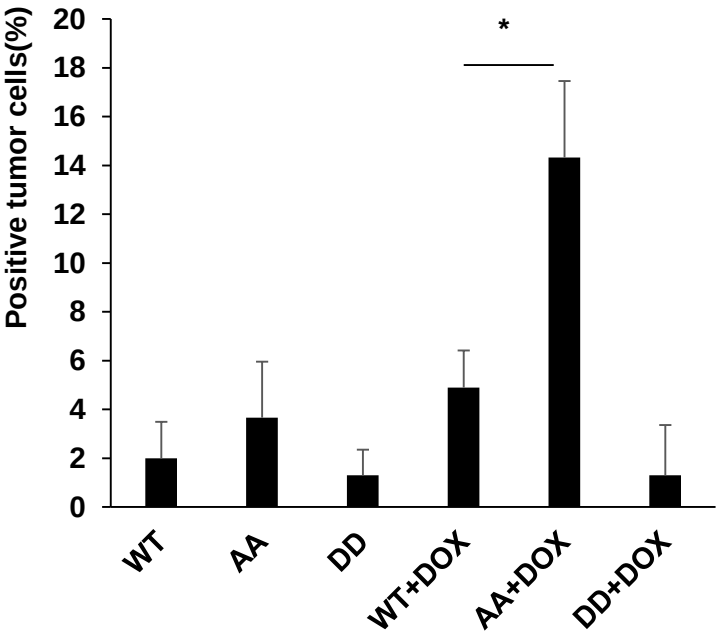




Tumors carrying Numb-S265A/S284A are more sensitive to chemotherapy



Cleaved Caspase 3 IHC

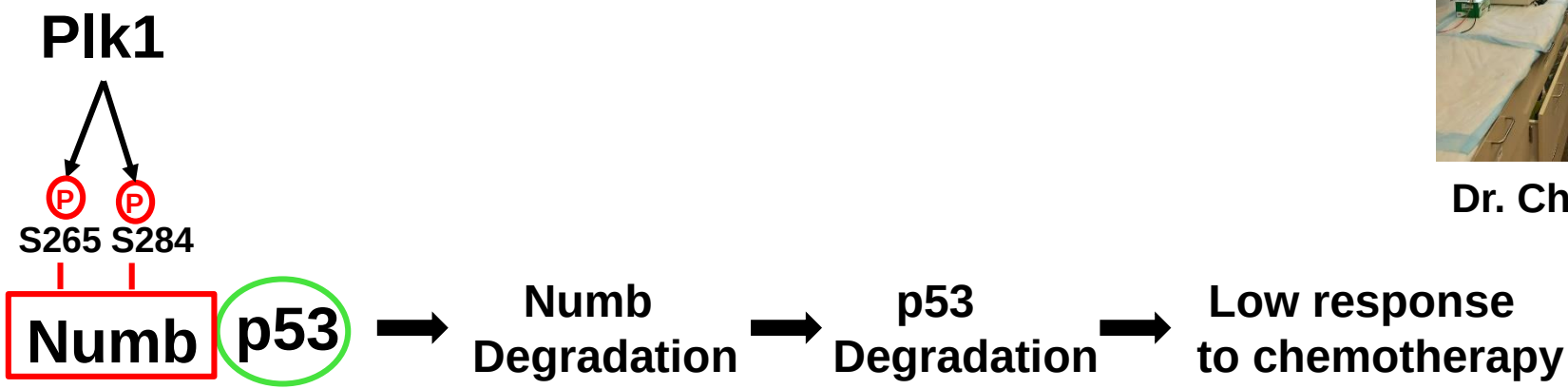


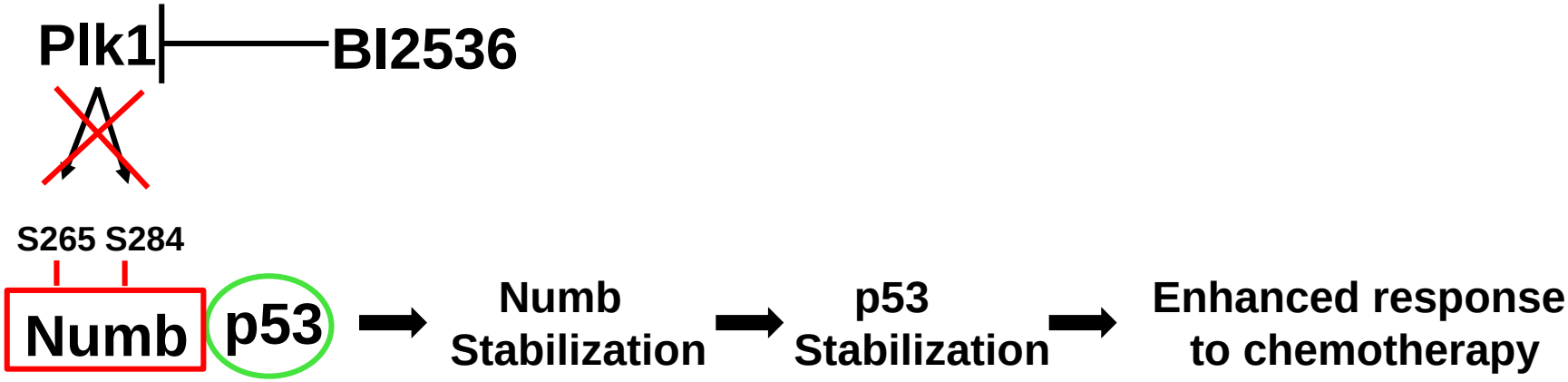
Working model

Publications: Shao et al., *Oncogene* 2018, 37, 810.

Dr. Chen Shao

Tumors carrying a high level of Plk1 and WT p53





Acknowledgements

Funding: NIH