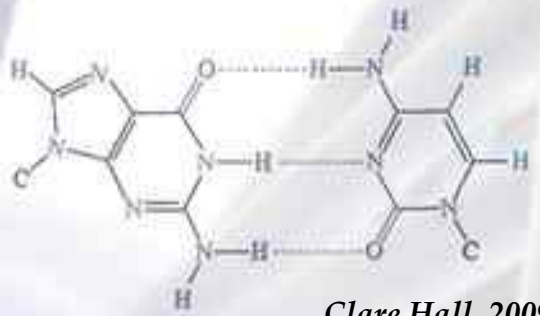
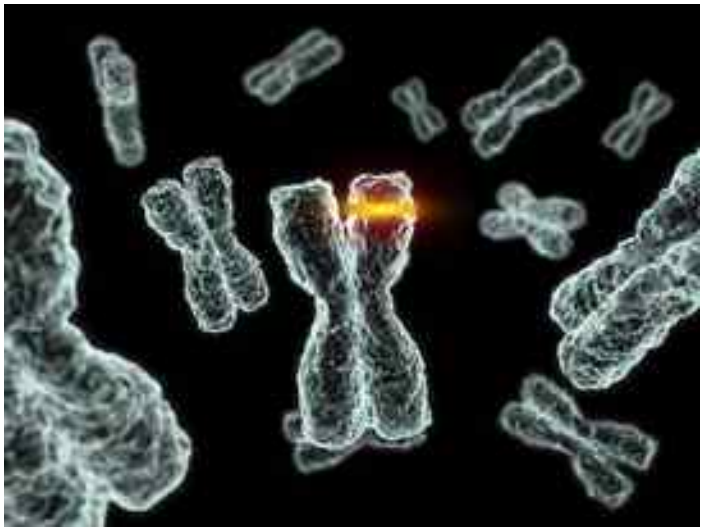
# Dpb11 involvement in DNA damage checkpoint signaling

*Fabio Puddu*



*Clare Hall, 2009*



DNA Damage Response

DNA repair

DNA damage checkpoint

Cell Cycle Arrest

Transcriptional Response


Induction of Apoptosis

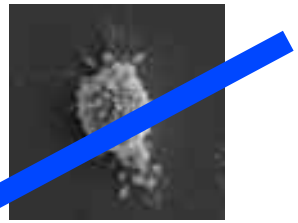
Chromatin Remodelling

Replication Forks Stabilization

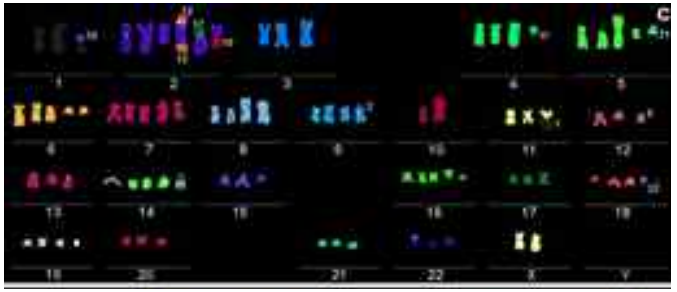
Modulation of DNA Repair Activities


Sensitivity to DNA damaging agents

Skipping Apoptosis



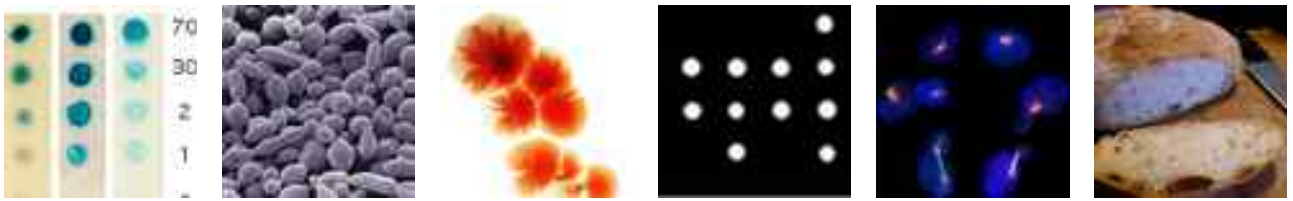
Genomic Instability

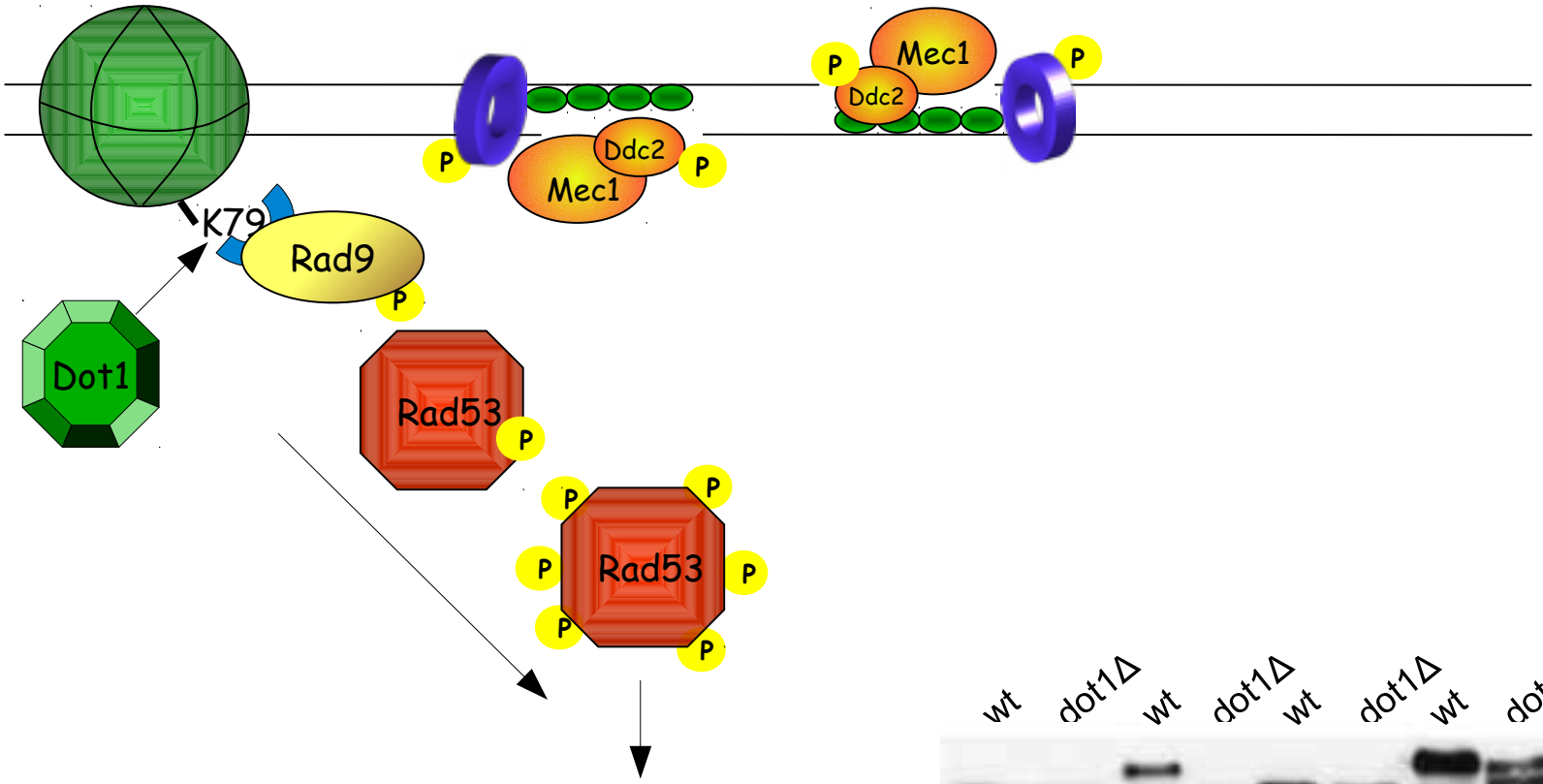


Accumulation of Mutations

Increase in the risk of cancer development

*S.cerevisiae*







RPA

PCNA-like

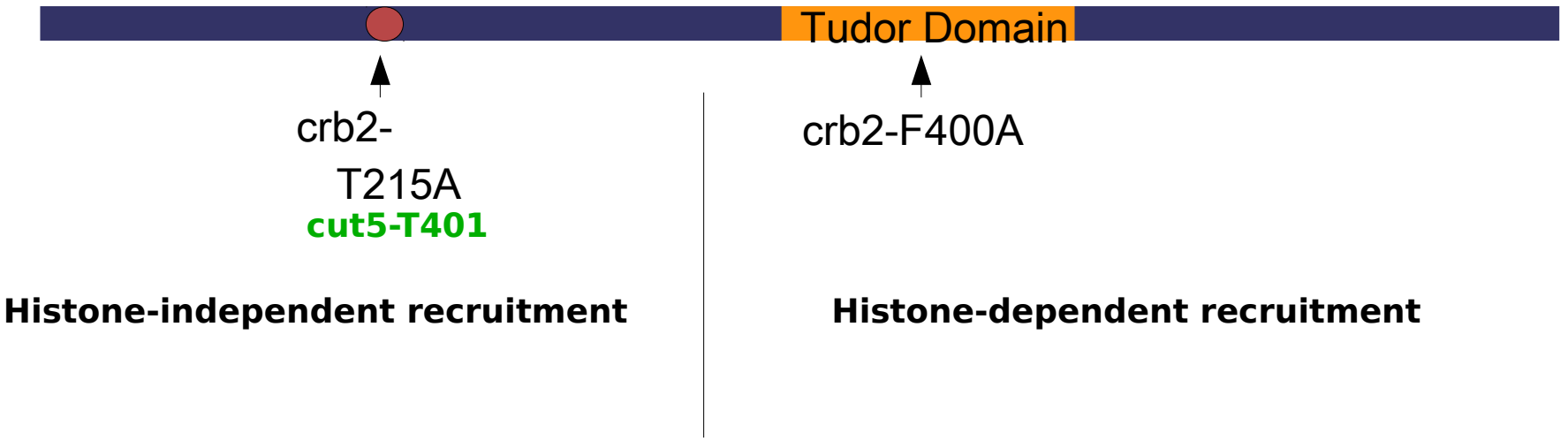
*Giannattasio, 2005*

In M exists an H3-K79 independent Rad9 recruitment pathway

What are the factors this alternative pathway depends on?

a hint from *Schizosaccharomyces pombe...*

Two DNA damage sensitive mutants of Rad9 homolog:




Cut5 is required for histone independent Crb2 recruitment

*Du et al, 2006*

# *S. cerevisiae* Cut5 homolog is Dpb11

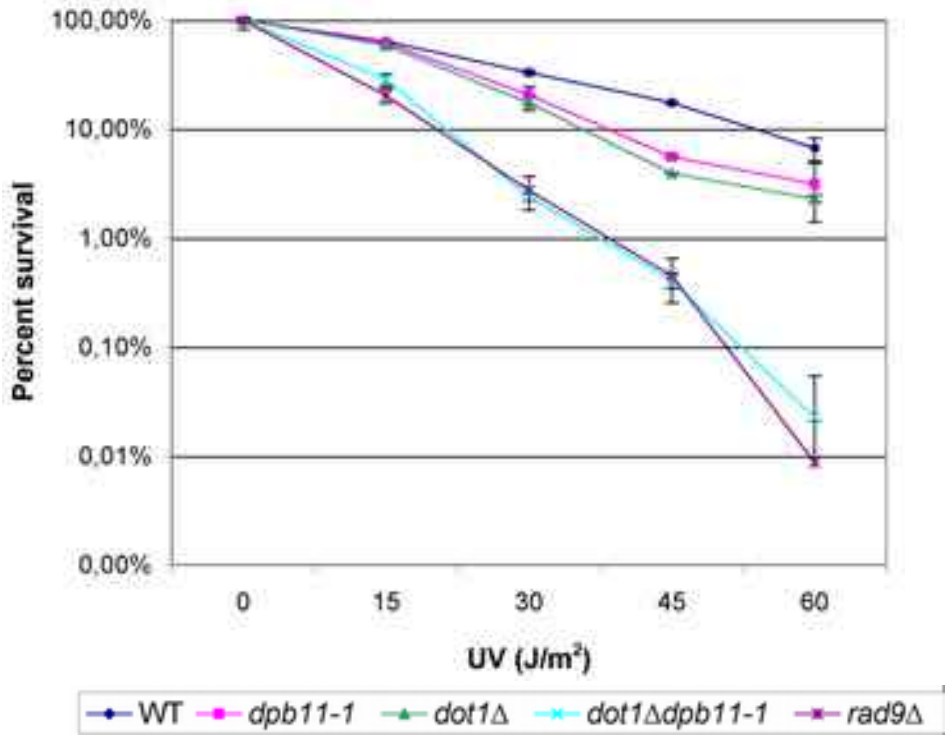

- Isolated as a multicopy suppressor of the mutant *dpb2-1*
- Essential gene
- Protein predicted to contain four BRCT motifs
- Involved in the CDK dependent step of replication initiation
- Involved in replication checkpoint activation
- In vitro stimulation of Mec1(hATR) activity

<u>Conditional allele</u>


- At permissive temperature (25°C):
  mildly sensitive to HU, MMS
- At restrictive temperature (34°C):
  defective in replication checkpoint (elongates spindles in HU)
  Loss in viability

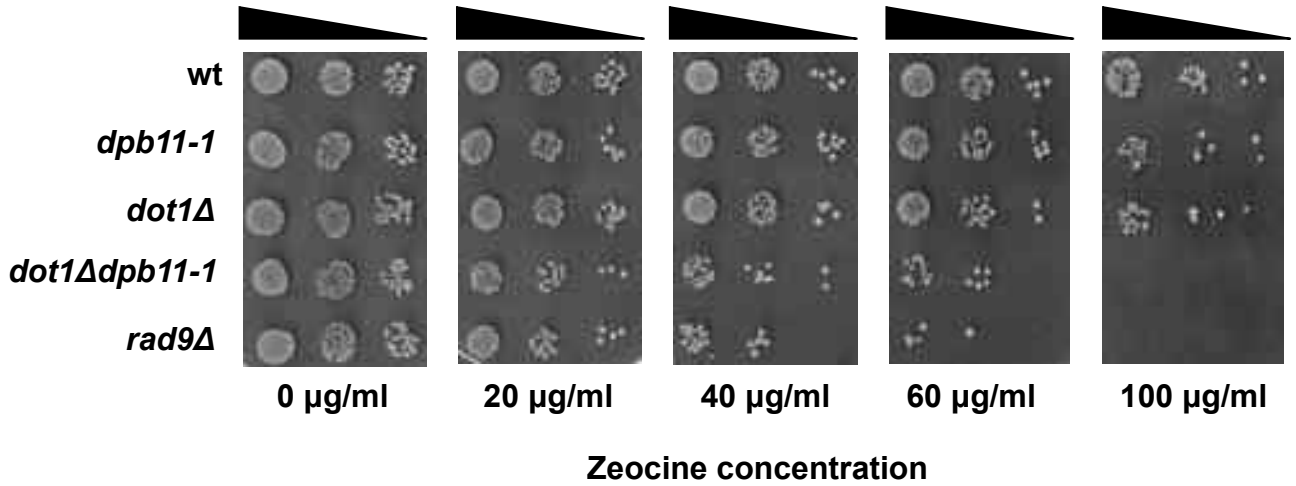
*Araki, 1995; Wang & Elledge, 1999; Zegerman & Diffley, 2007; Mordes, 2008*

# *dpb11-1dot1Δ* is hypersensitive to DNA damaging agents



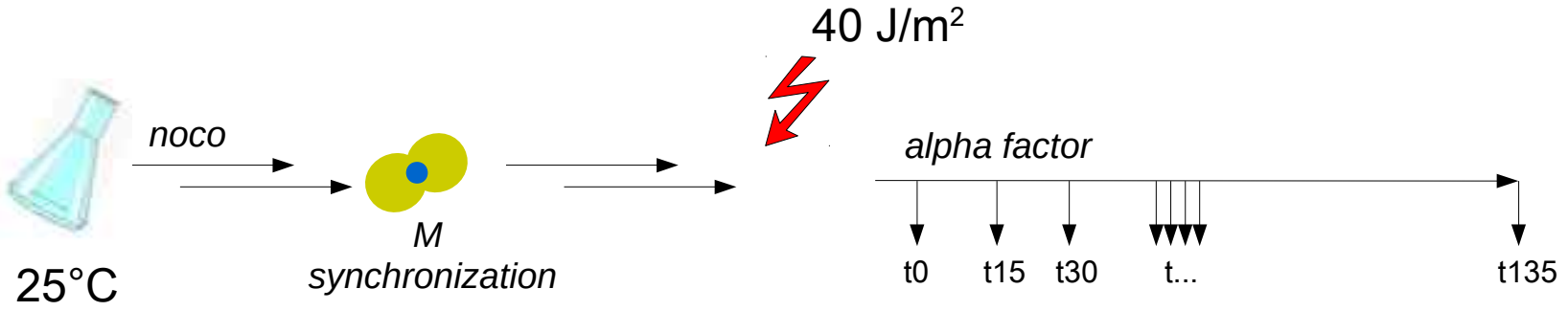

The two mutations display sinergistic effect on DNA damage sensitivity

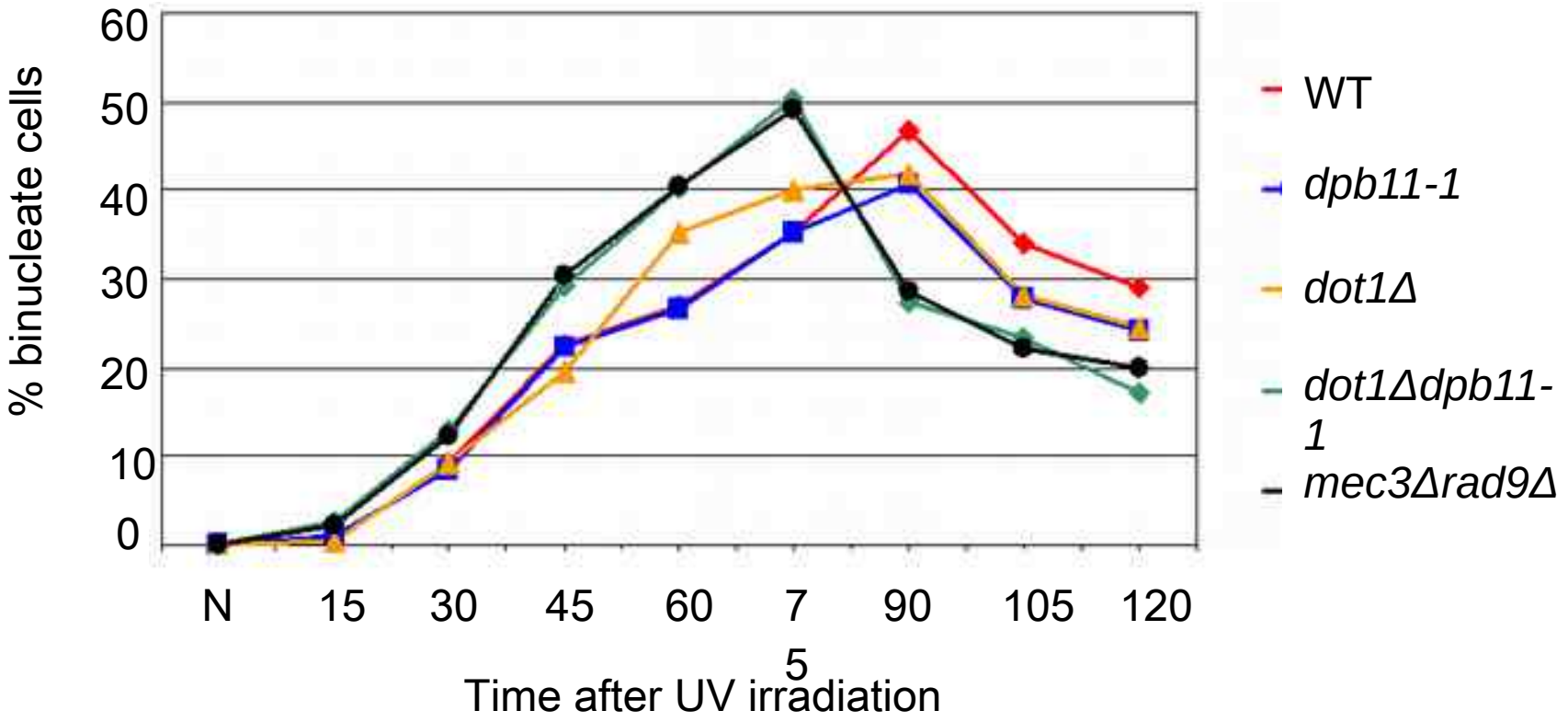
The sensitivity of the double mutant is comparable to that of a *rad9Δ* strain



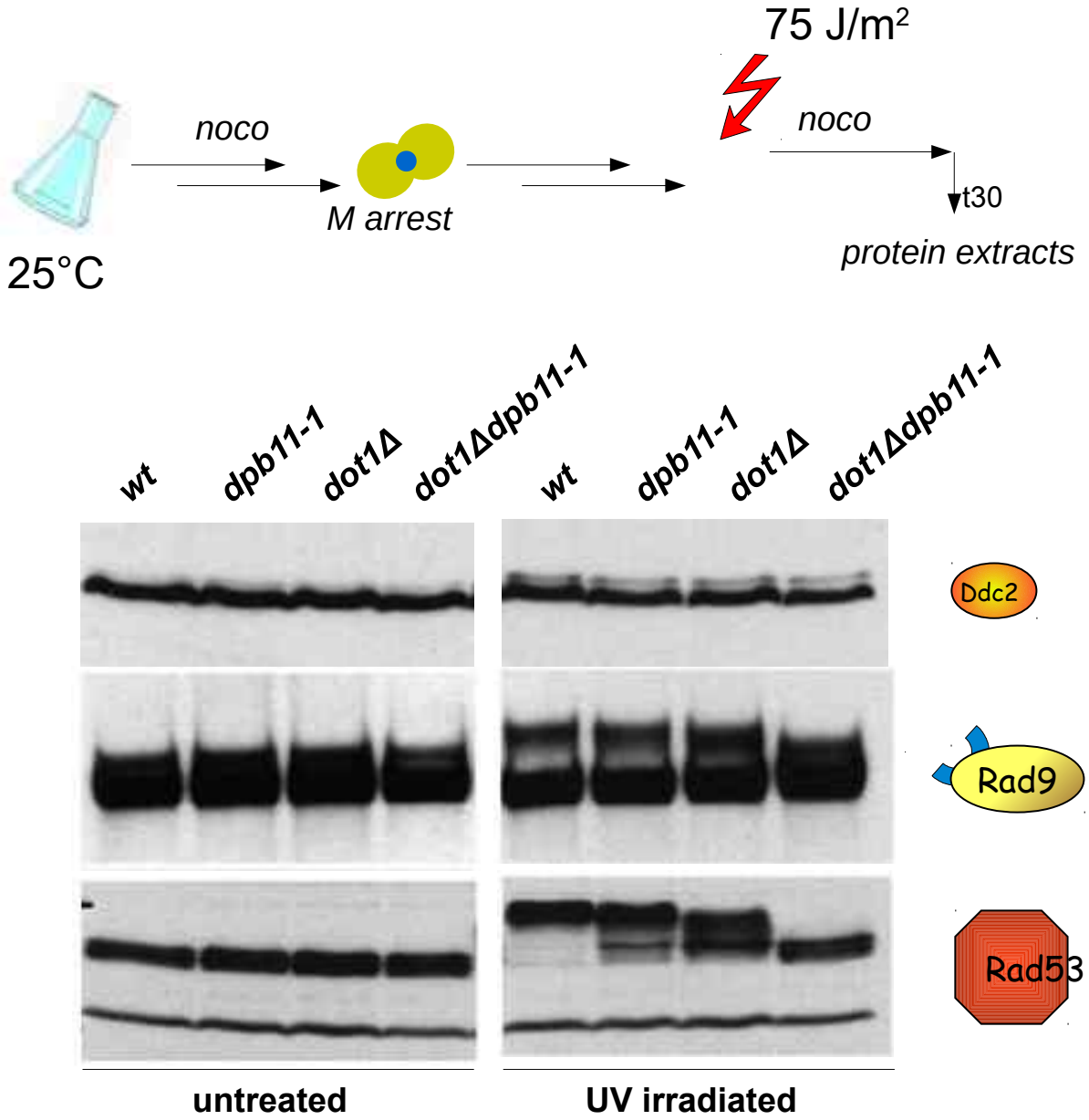

**Is the double mutant able to activate checkpoint?**

# *dpb11-1dot1Δ* does not delay nuclear division after UV irradiation



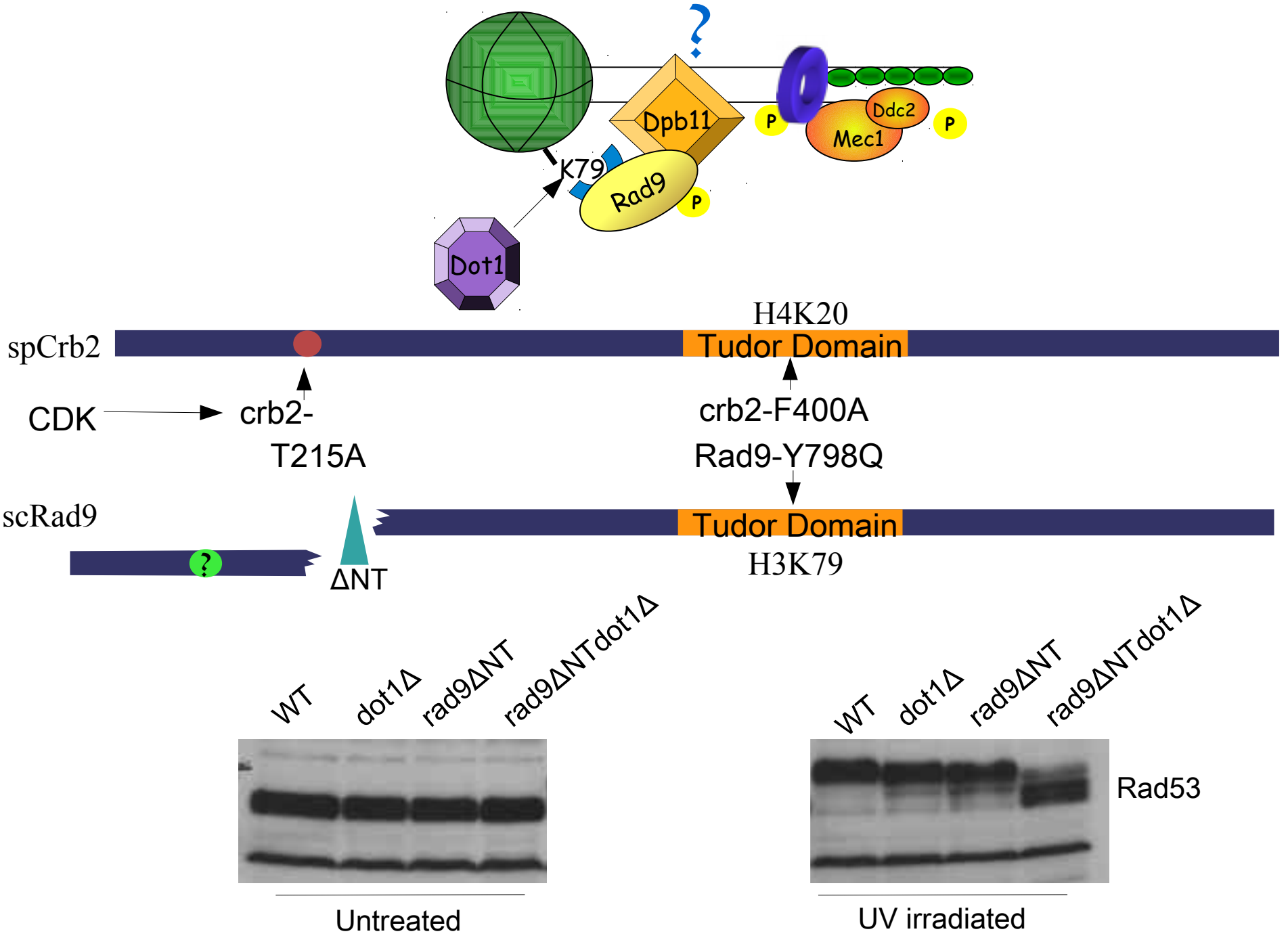



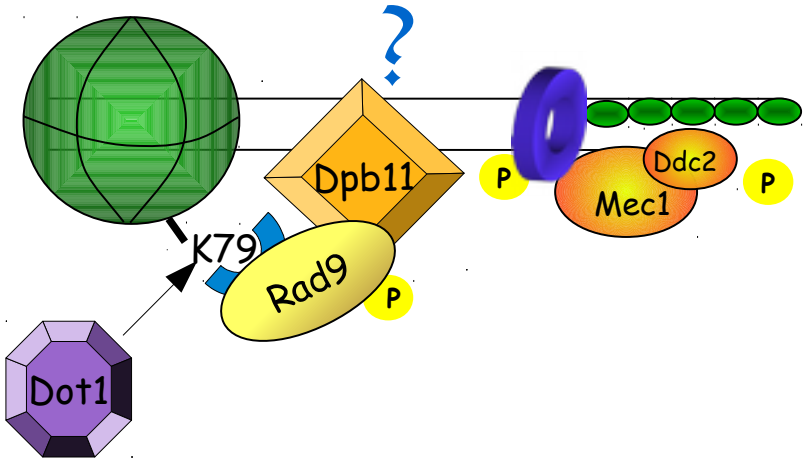
# Dpb11 is required for Rad9 phosphorylation after DNA damage




**Why this pathway is present only in M cells?**

# Dpb11 is required for Rad9 phosphorylation after DNA damage

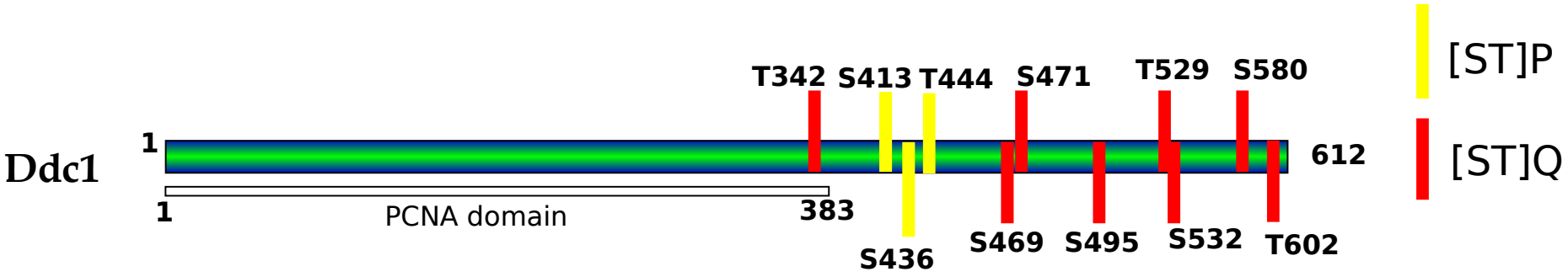


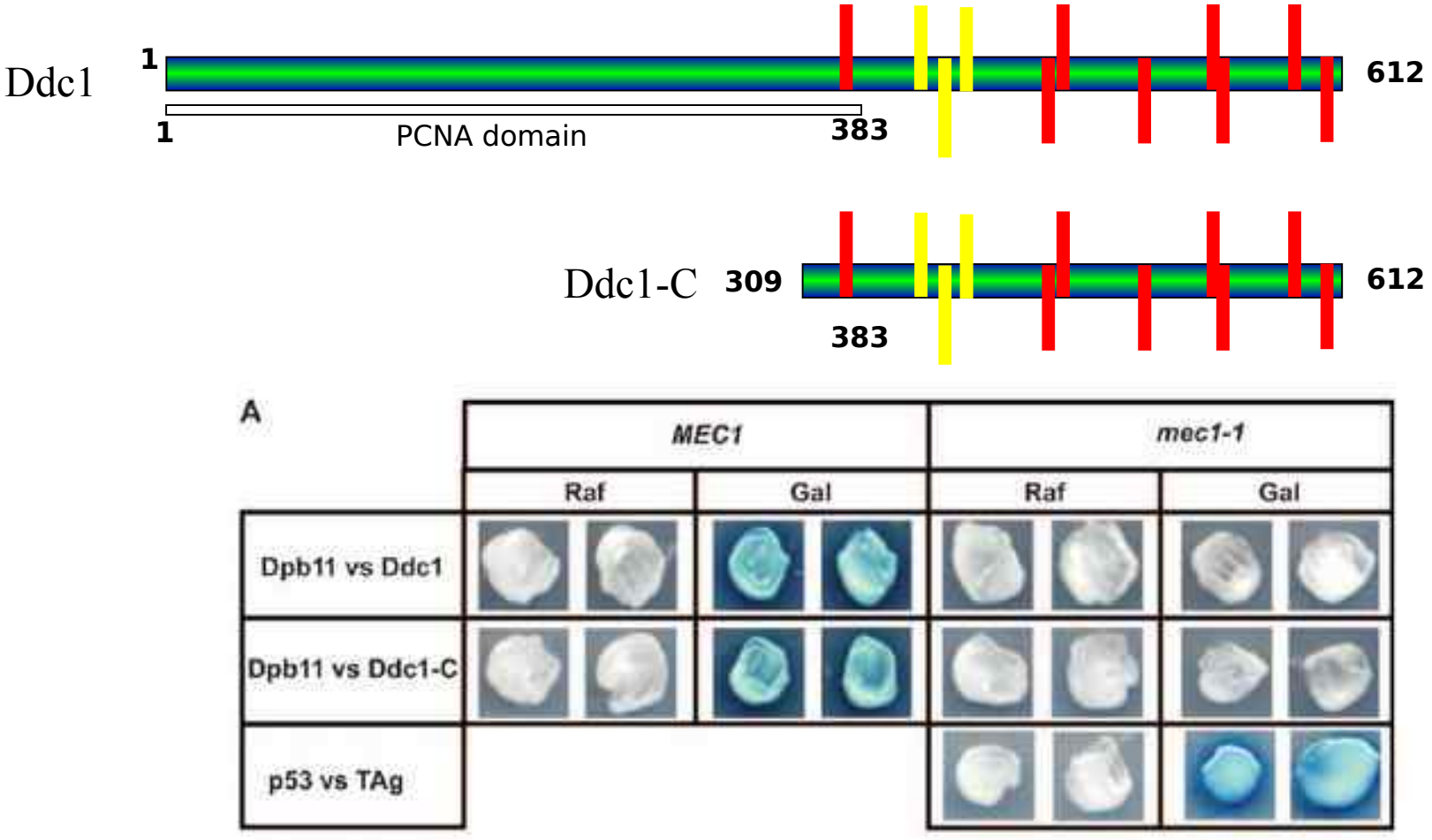



*DPB11* and *DDC1* interacts by TH

*dpb11-1* mutant abolish this interaction

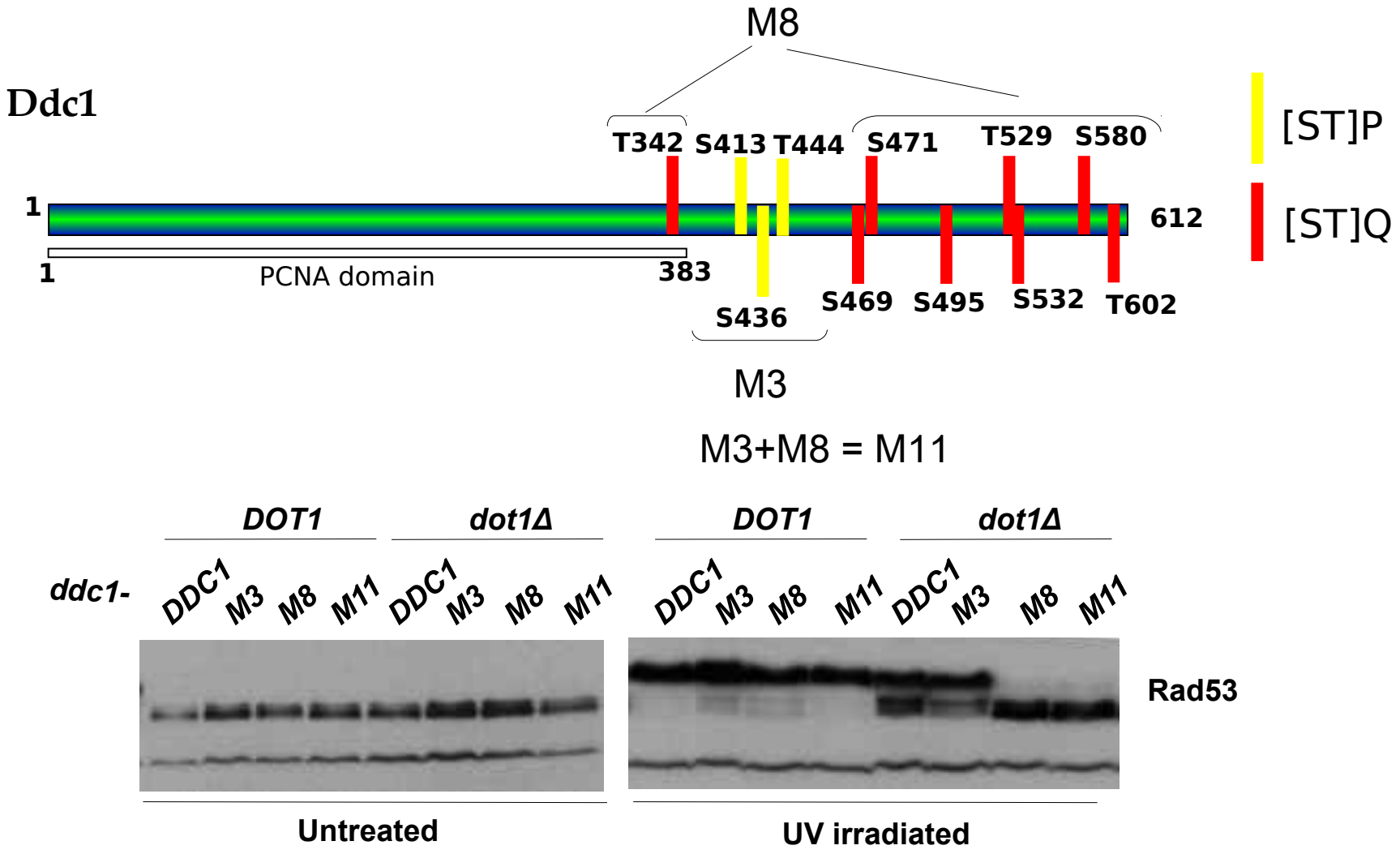
*(Wang & Elledge, 2002)*






Dpb11-Ddc1 interaction requires the presence of an intact Mec1 kinase

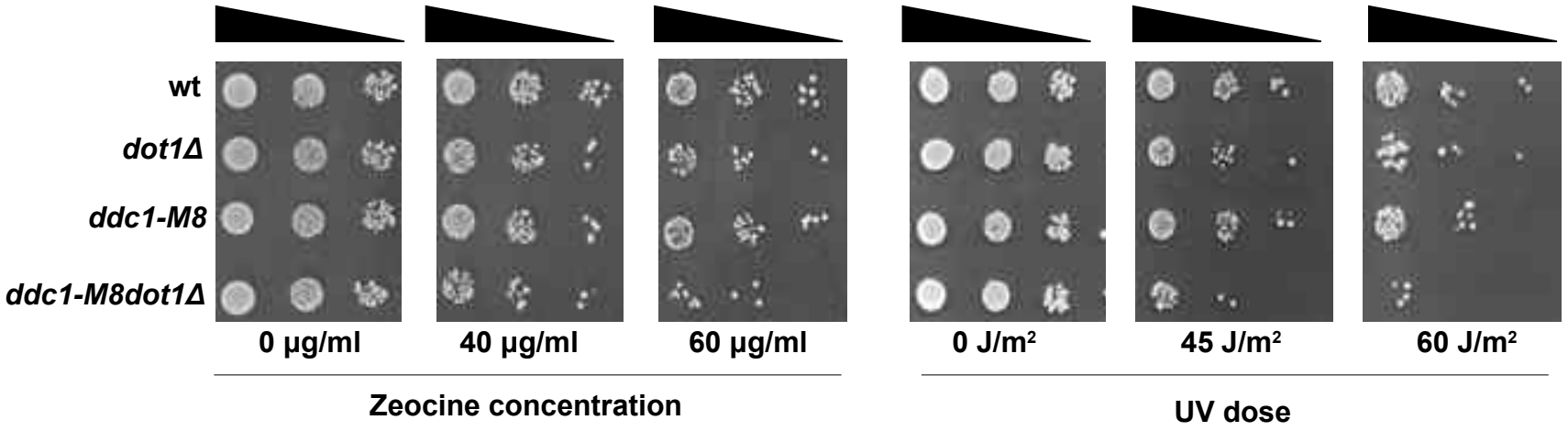
# *dot1Δddc1-M8* mimics the *dot1Δdpb11-1* mutant

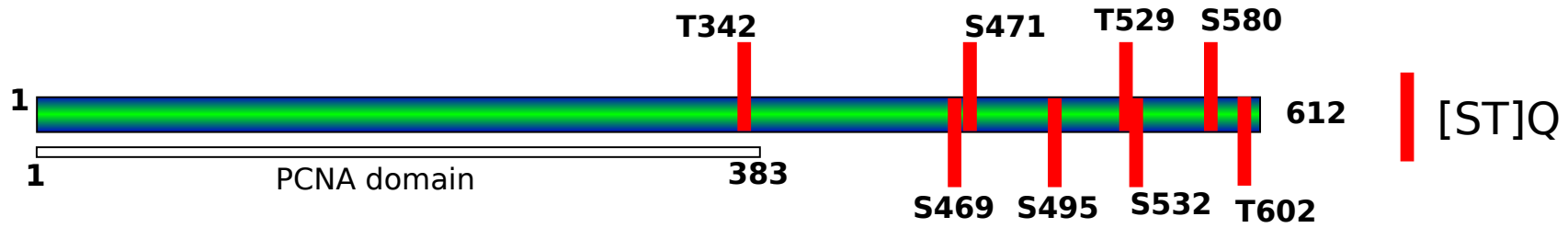



Mec1 consensus sites on Ddc1 are required for Dot1 independent checkpoint activation...

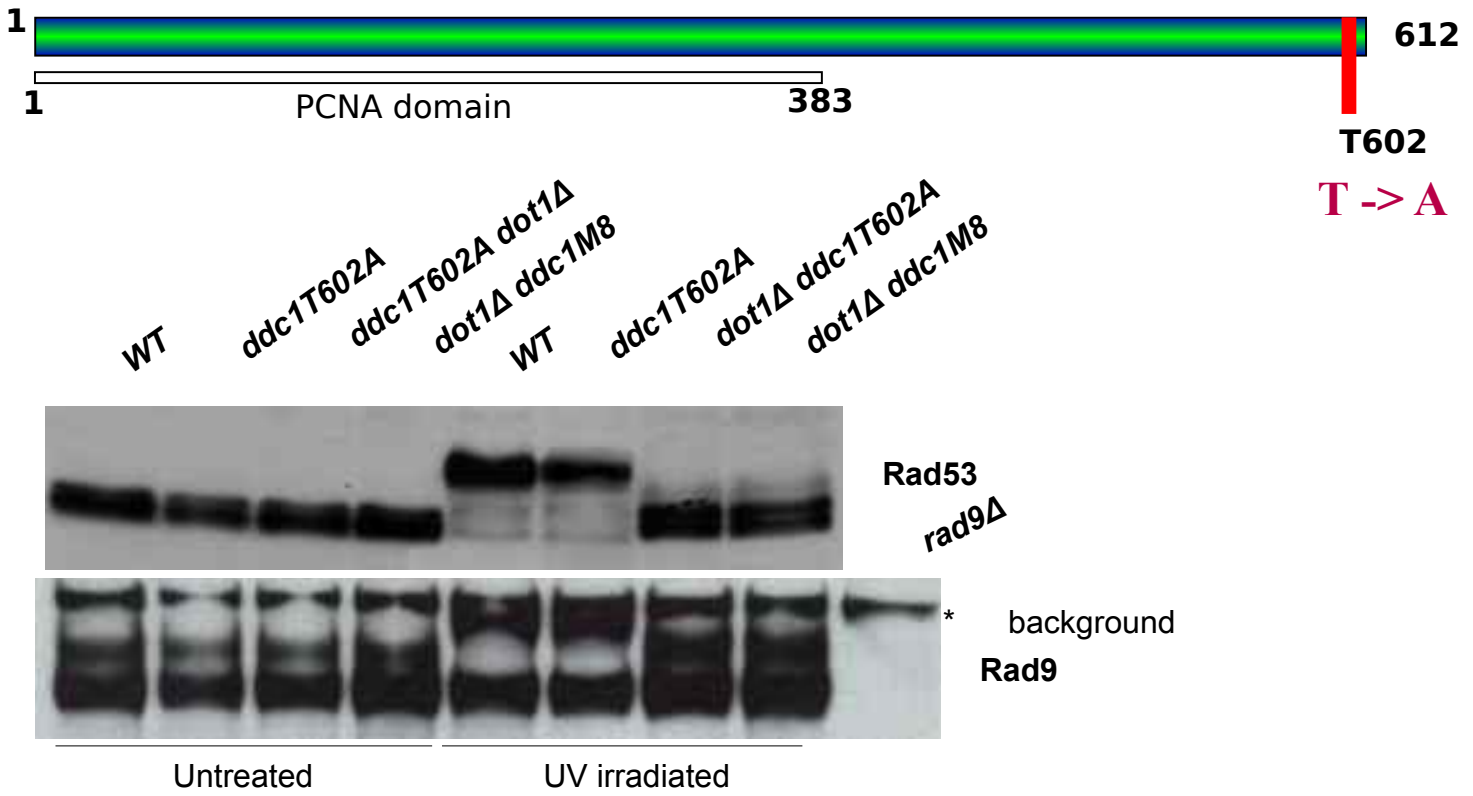
# *dot1Δddc1-M8* mimics the *dot1Δdpb11-1* mutant

...and increase the sensitivity to DNA damaging agents of a *dot1Δ* strain



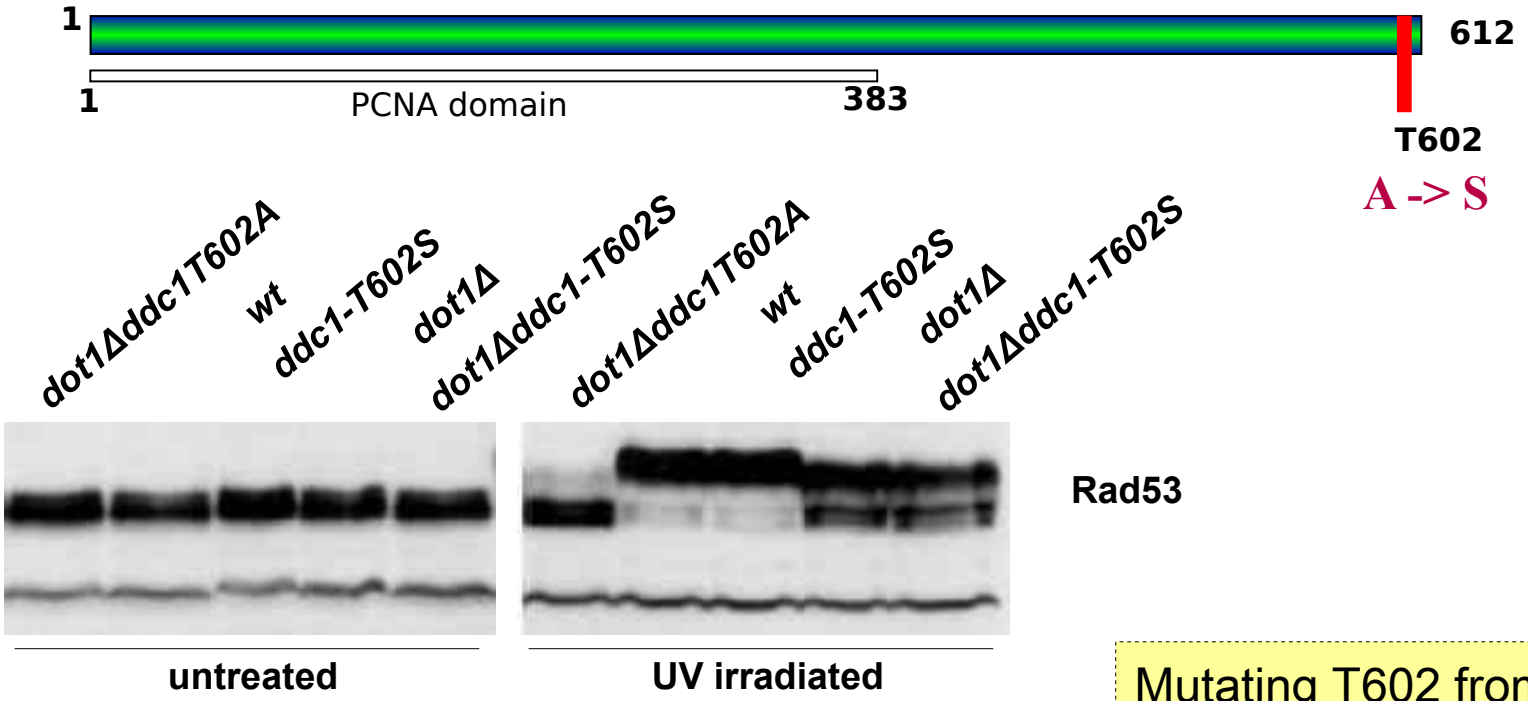
# *dot1Δddc1-T602A* mimics the *dot1Δdpb11-1* mutant




Mutating T602 to alanine completely abolishes Ddc1 function in the histone independent Rad9 recruitment pathway

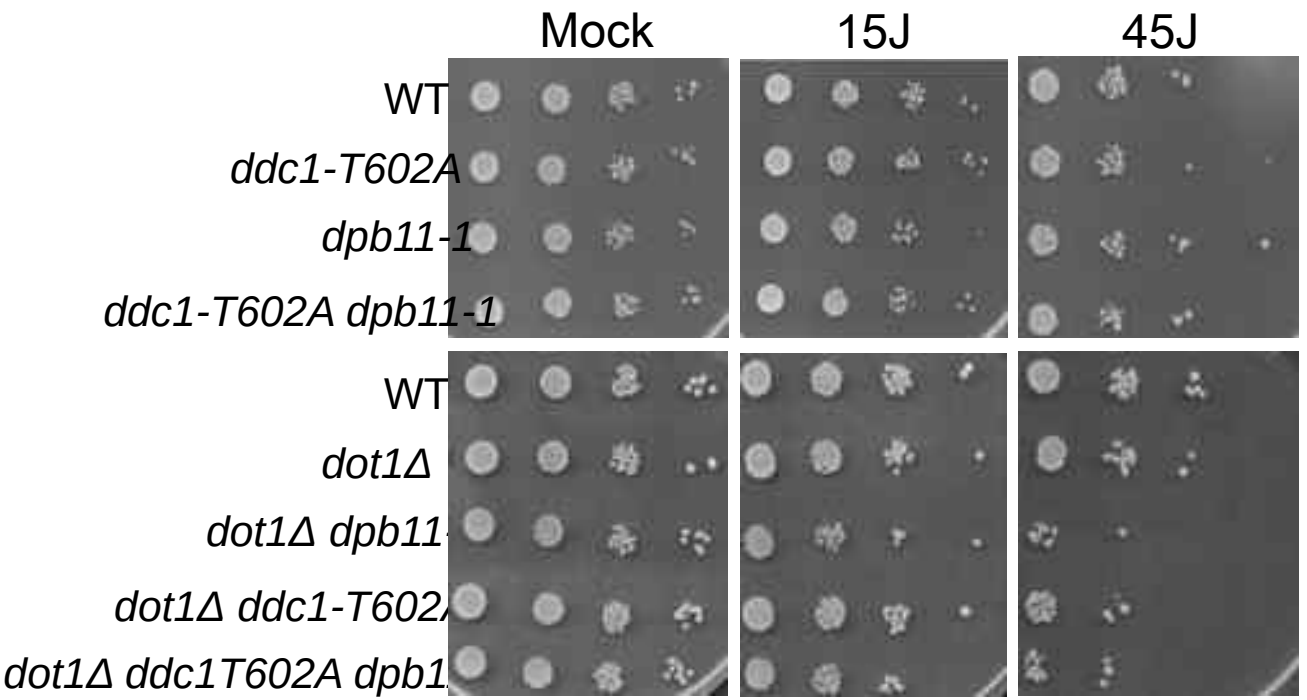
# Ddc1 T602 phosphorylation is required for Rad9 function in the absence of Dot1

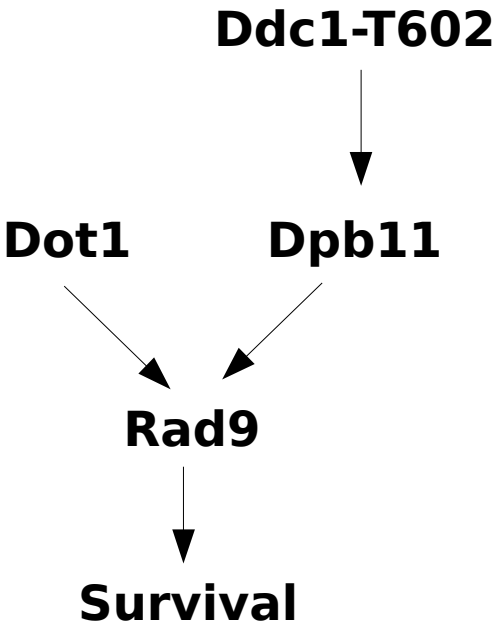
Is this an effect of the lack of phosphorylation
or the mutation changes
the conformation of the protein?



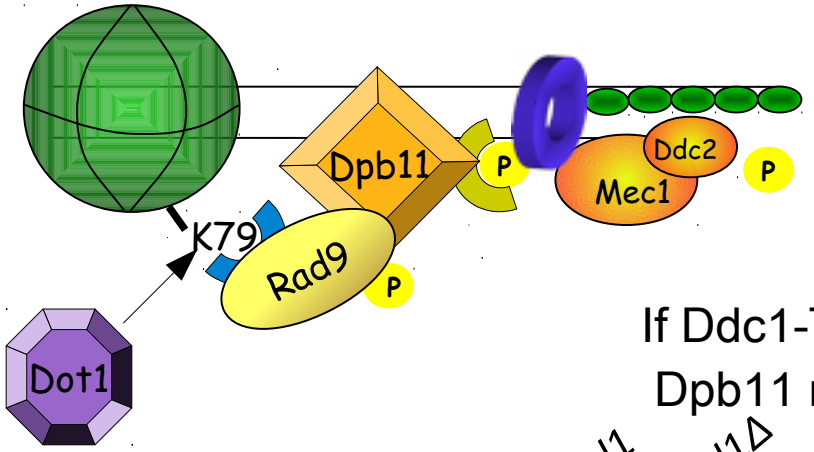

Mutating T602 from alanine back to a different Mec1 phosphorylable residue (S) totally rescues the checkpoint defect

# *ddc1-T602A* and *dpb11-1* are epistatic either in a WT background and a *dot1Δ*



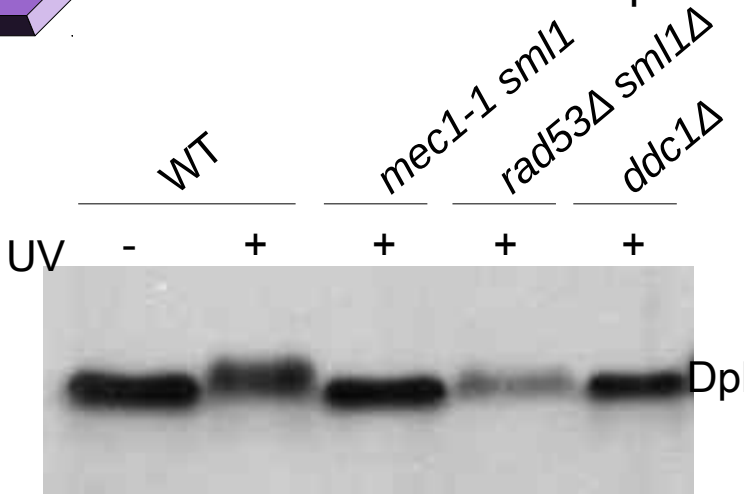



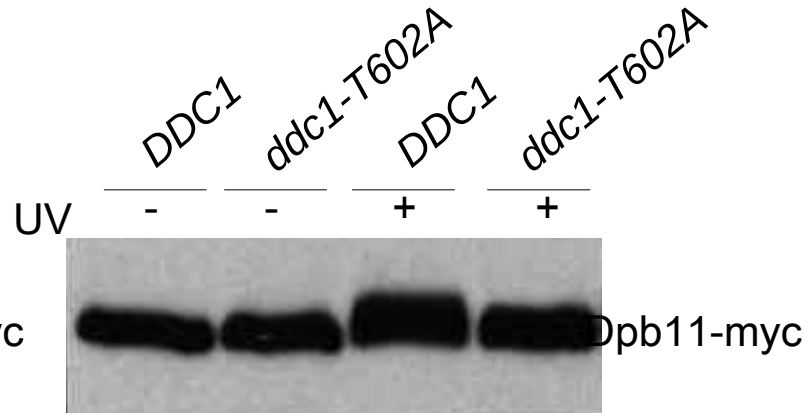
# Damage induced Dpb11 phosphorylation





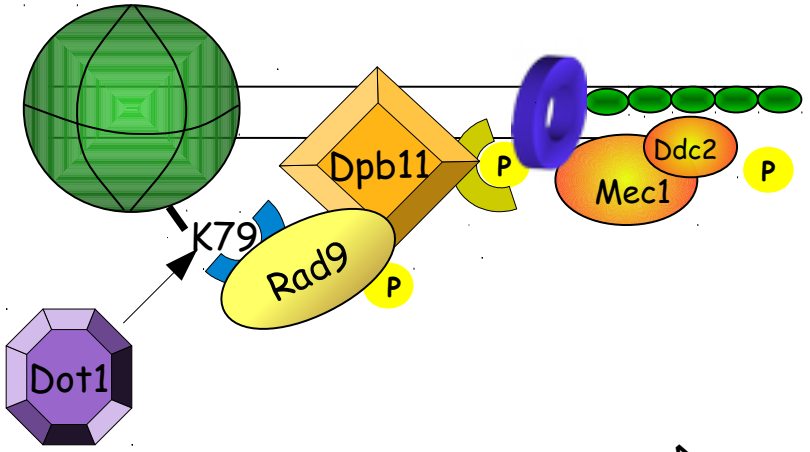
If Ddc1-T602 is required for Dpb11 recruitment, then...



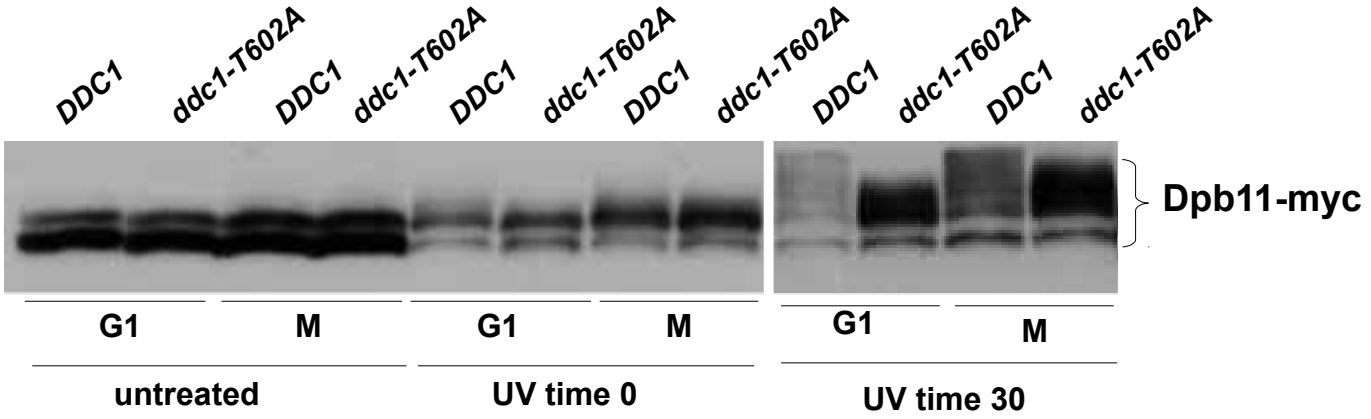




Dpb11 is phosphorylated in a Mec1 and Ddc1 dependent manner

# Damage induced Dpb11 phosphorylation

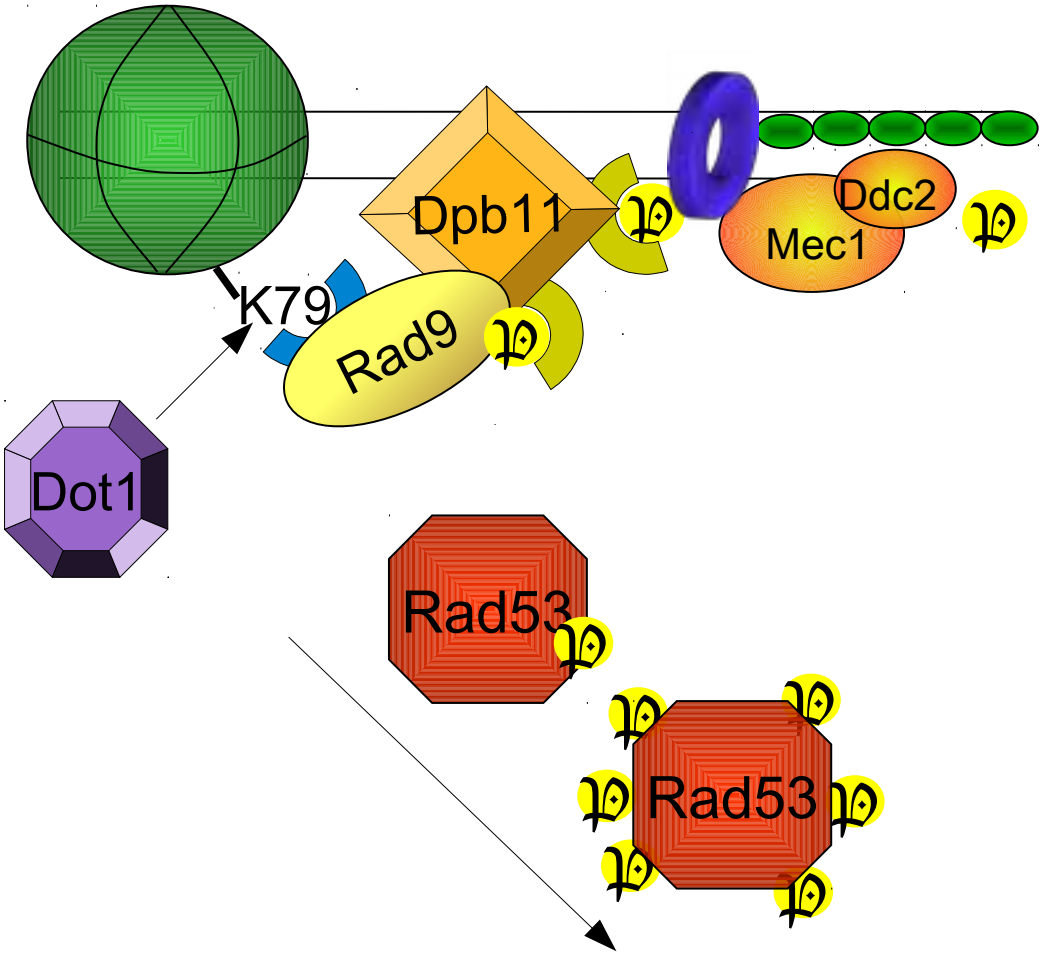







...and also Ddc1 T602
is required for
this modification

# A model for Dpb11 role after UV damage



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**Thank You**

DSBB

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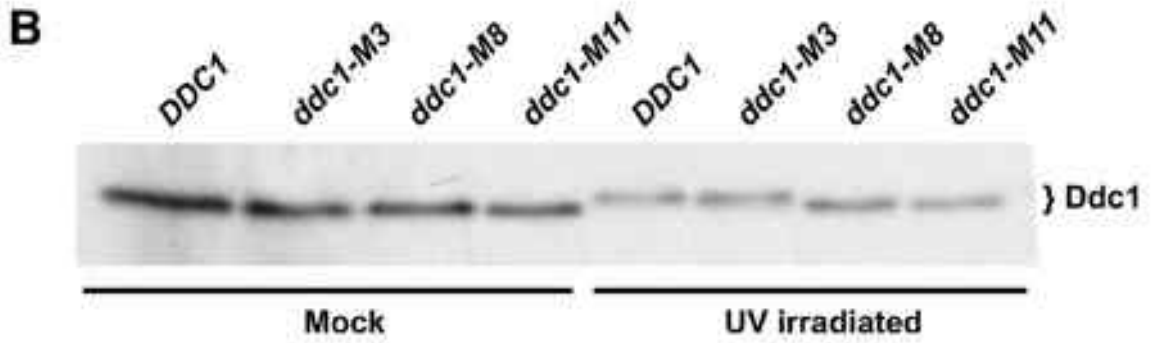
Università degli Studi di Milano

# *ddc1-M8* mutation abrogates damage-dependent Ddc1 phosphorylation



# Dpb11 and Dot1 partecipates in Rad9 recruitment to chromatin