Phosphorylation of the budding yeast 9-1-1 complex is required for Dpb11 function in the full activation of the DNA damage checkpoint

Fabio Puddu, Porto 2008

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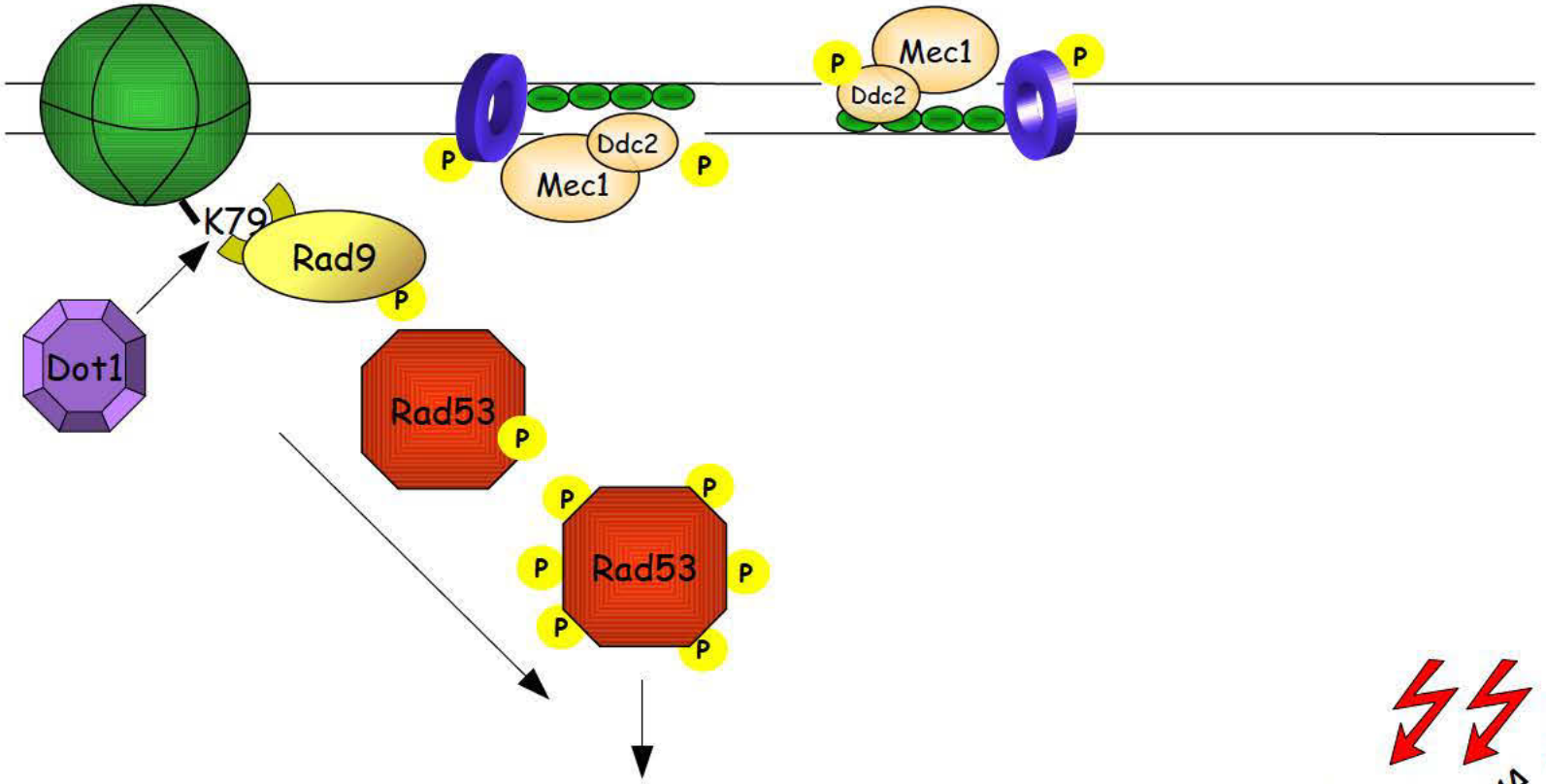


Responses to DNA damage

Cell cycle

DNA repair

Transcription

...

RPA

PCNA-like

Giannattasio, 2005

In M exists an alternative Rad9 recruitment pathway

What are the factors this alternative pathway depends on?

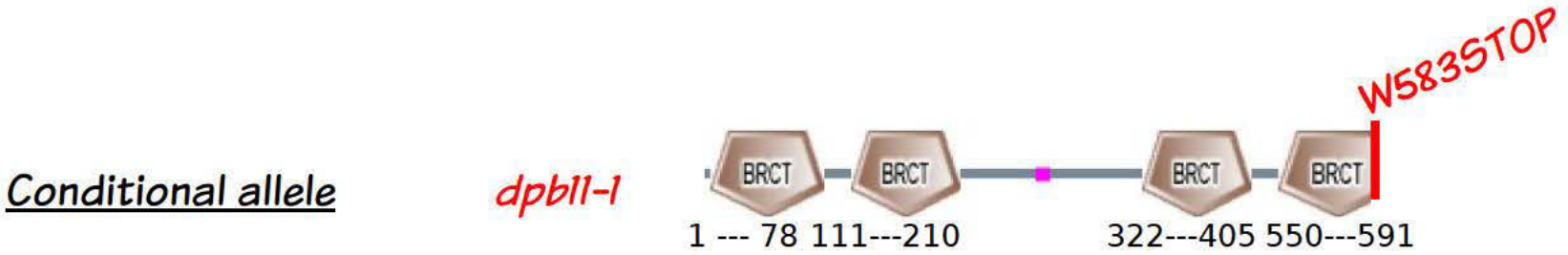
a hint from *Schizosaccharomyces Pombe*...

Cut5/Rad4 is required for histone independent Crb2 recruitment

Du et al, 2006

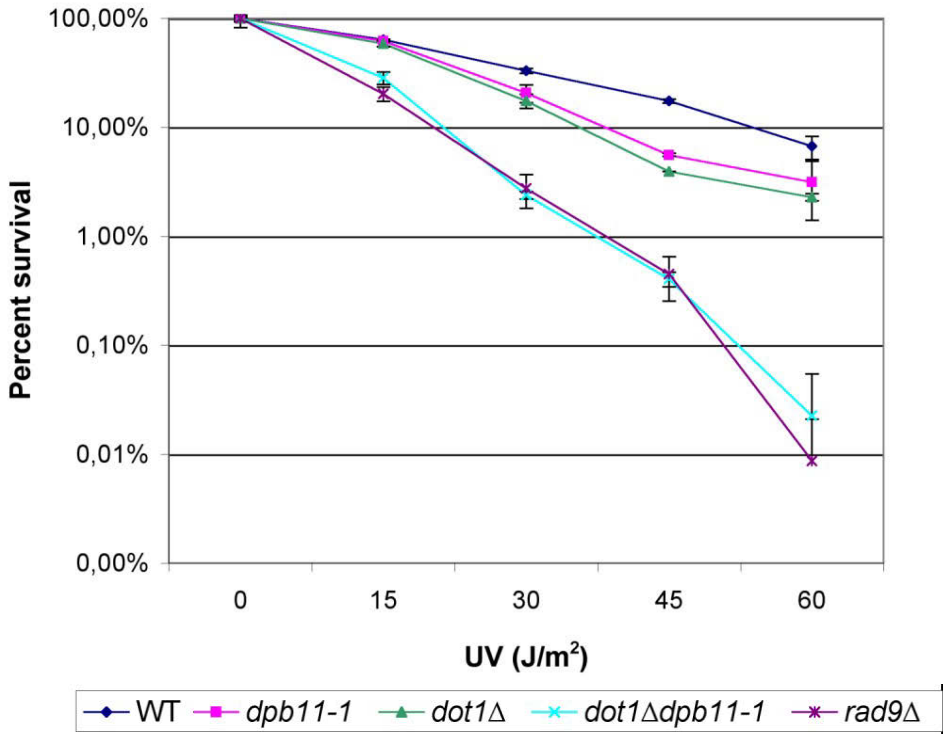
S. cerevisiae Cut5 homolog is Dpb11

- Essential gene
- Protein predicted to contain four BRCT motifs
- Involved in the CDK dependent step of replication initiation
- Involved in replication checkpoint



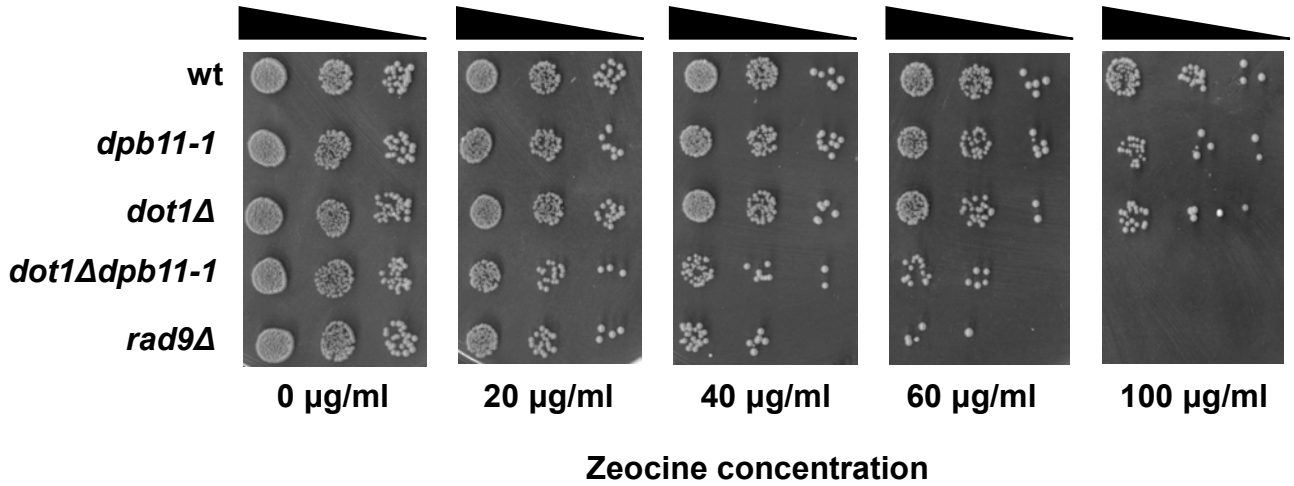
Araki, 1995; Wang & Elledge, 1999; Zegerman & Diffley, 2007

dpb11-1dot1Δ is hypersensitive to DNA damaging agents

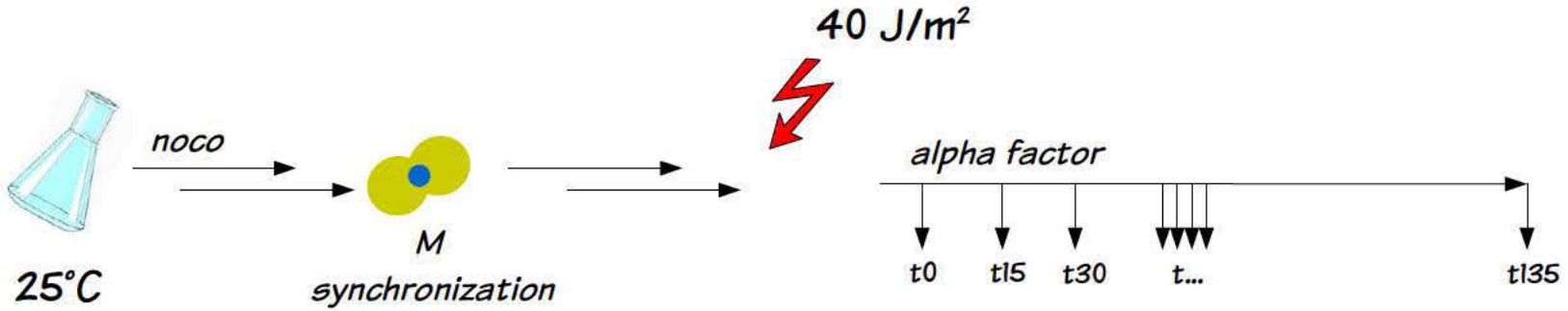


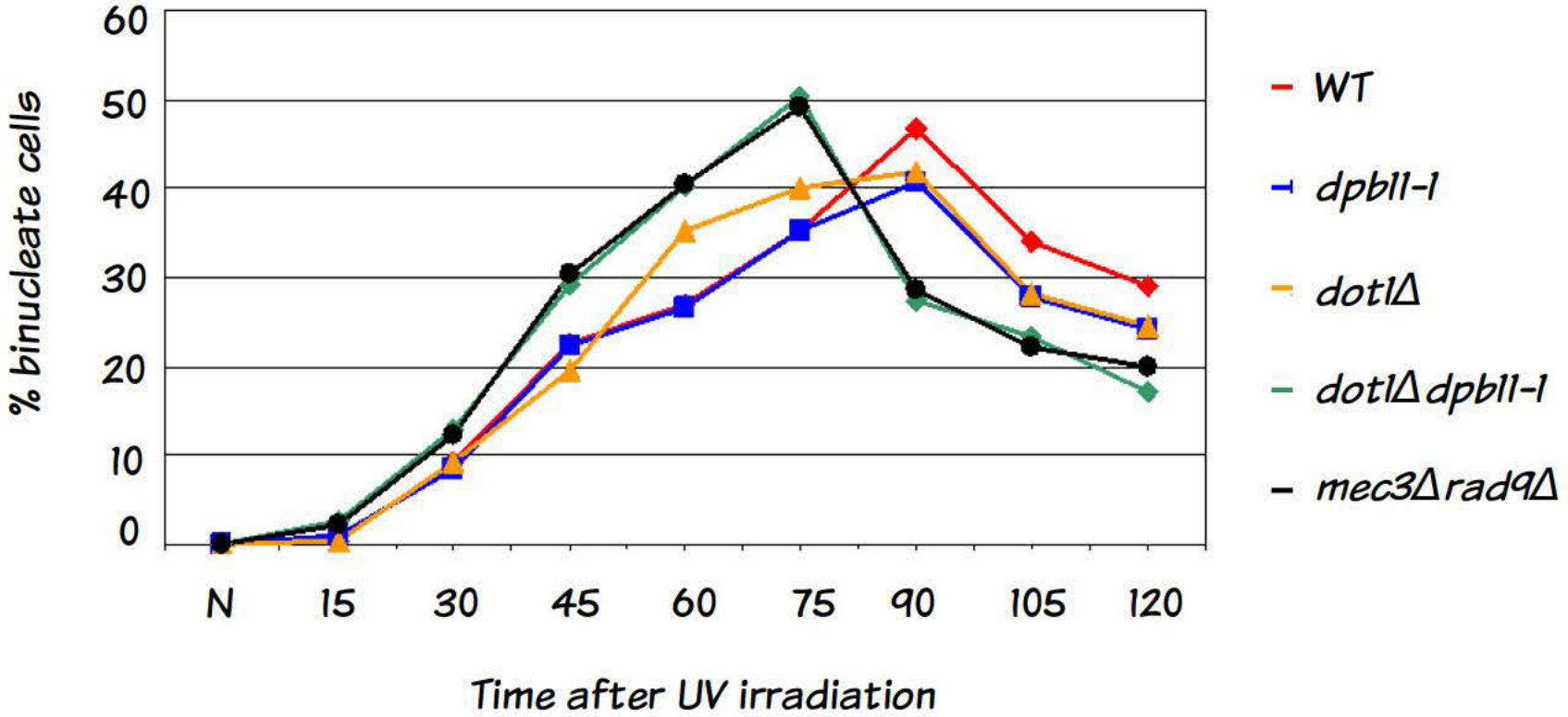
The two mutations display sinergistic effect on DNA damage sensitivity

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

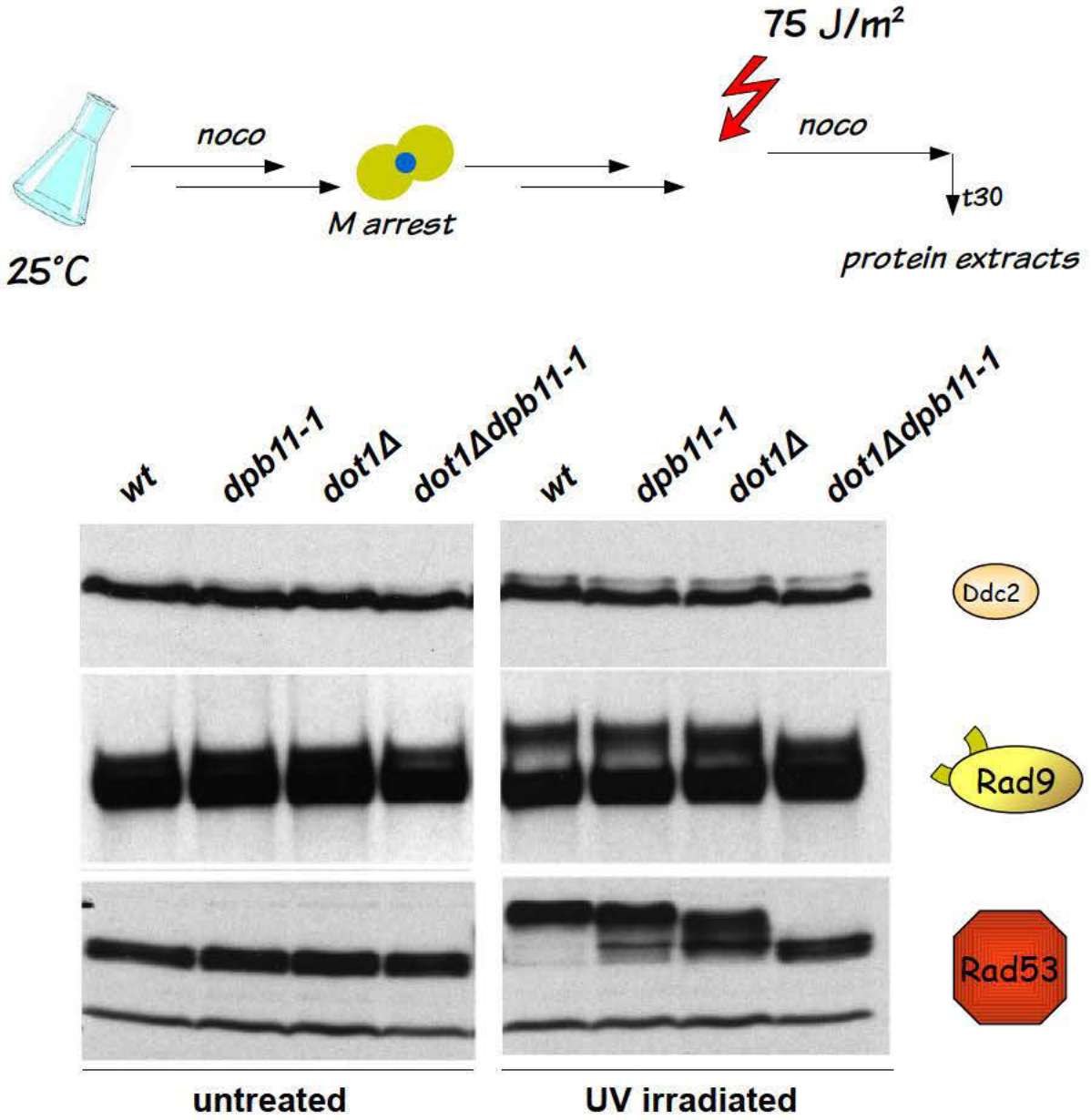


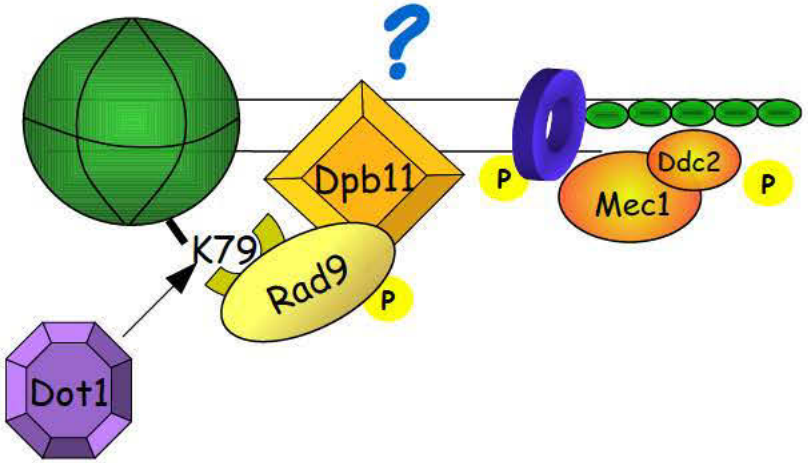
dpb11-1dot1Δ does not delay nuclear division after UV irradiation





Dpb11 is required for Rad9 phosphorylation after DNA damage

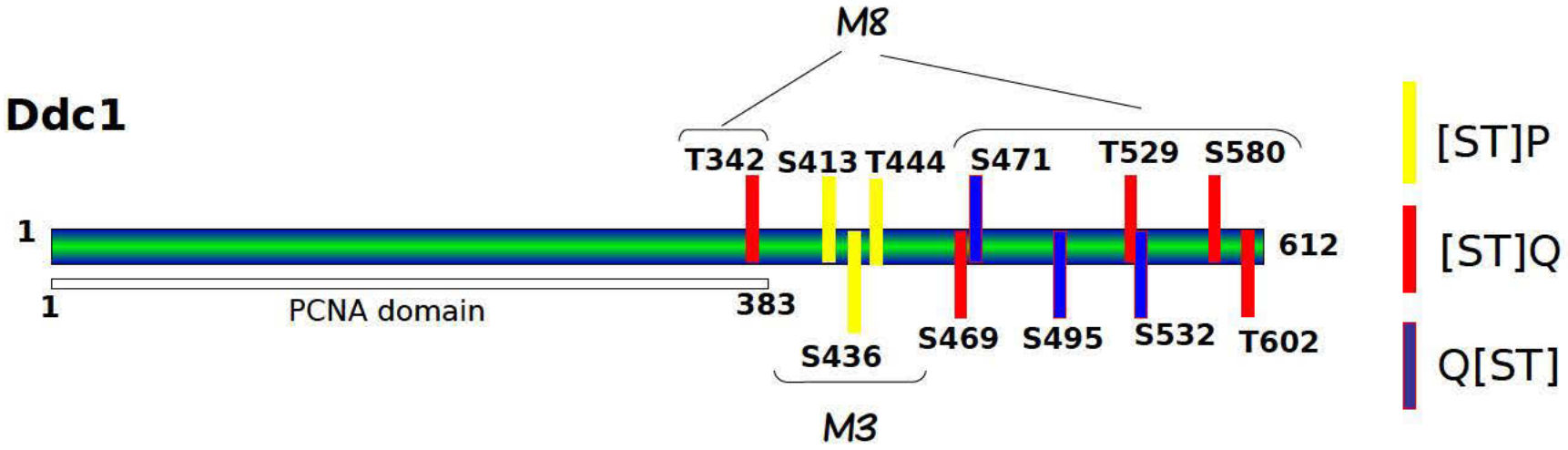




DPB11 and DDC1 interacts by TH

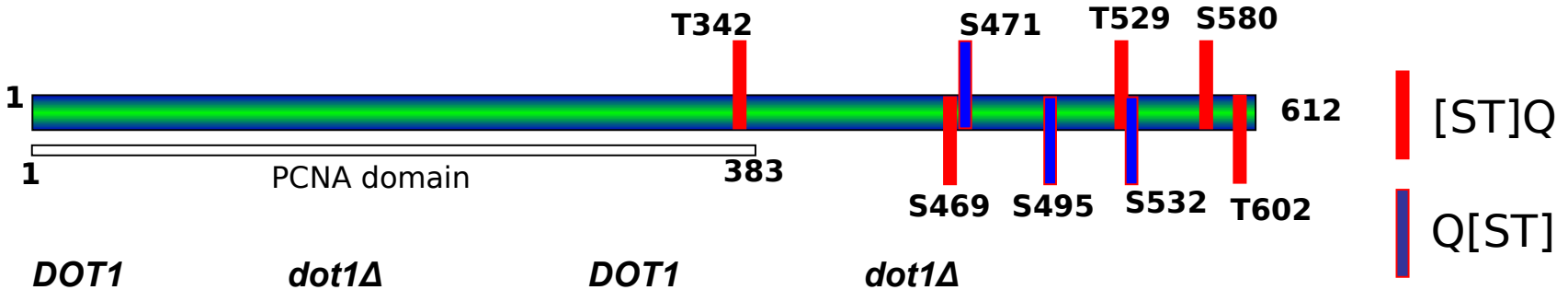
dpb11-1 mutant abolish this interaction

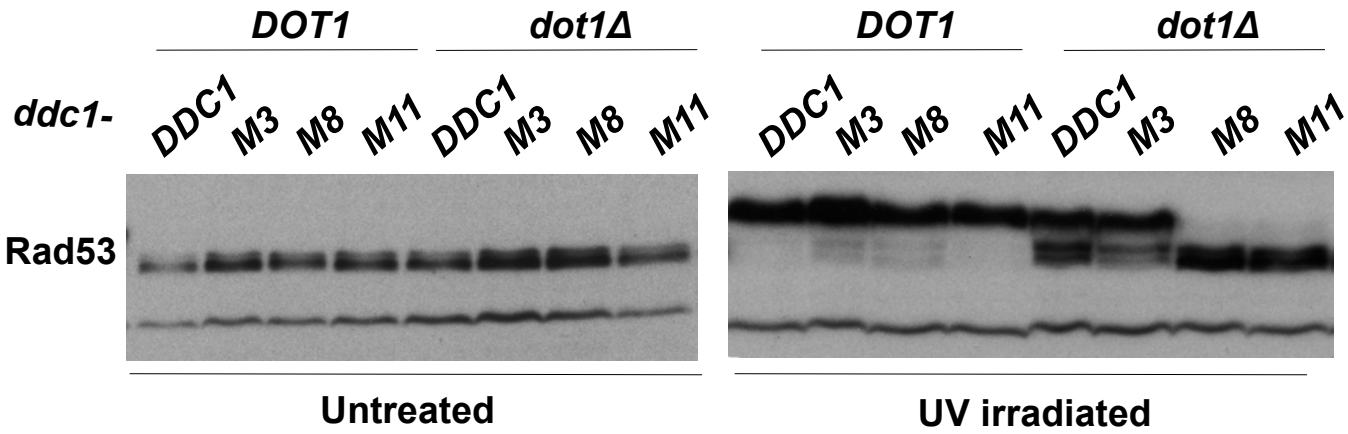
(Wang & Elledge, 2002)



M3+M8 = M11

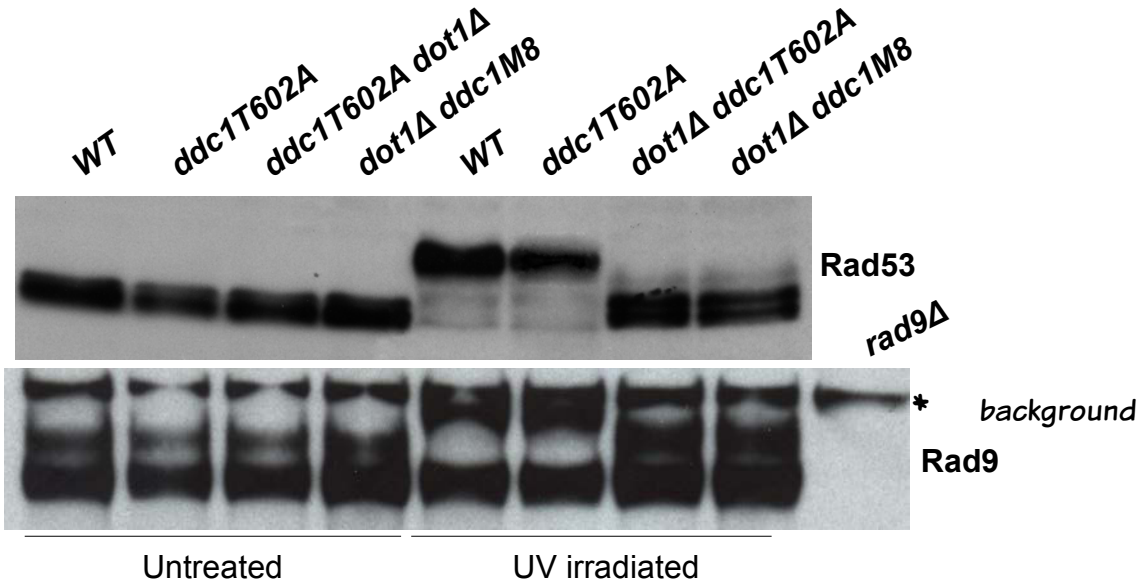
dot1Δddc1-M8 mutant mimics the *dot1Δdpb11-1* mutant





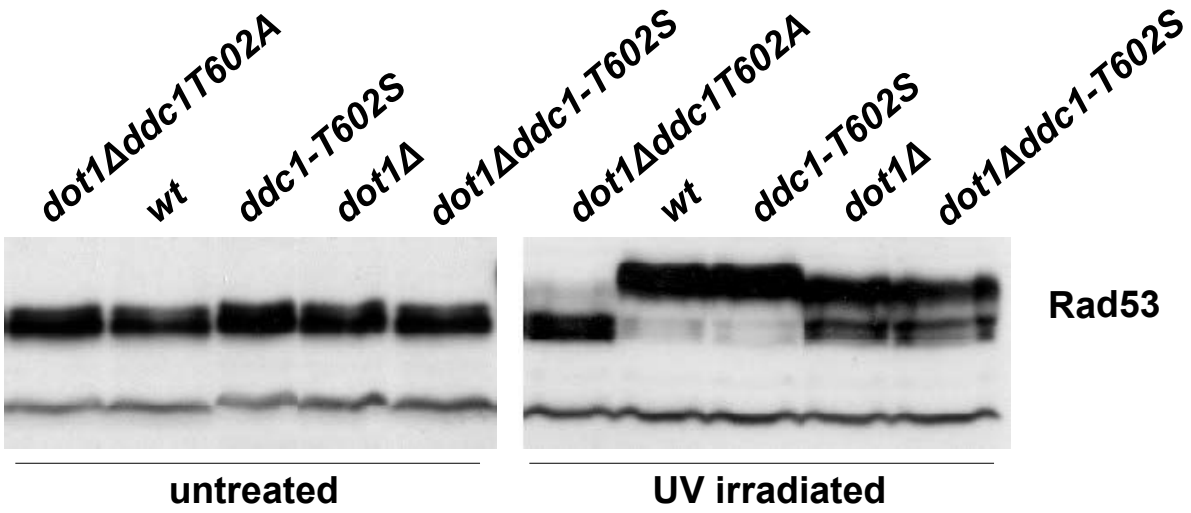
Mecl consensus sites on Ddcl are requiredfor Dotl independent checkpoint activation...

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



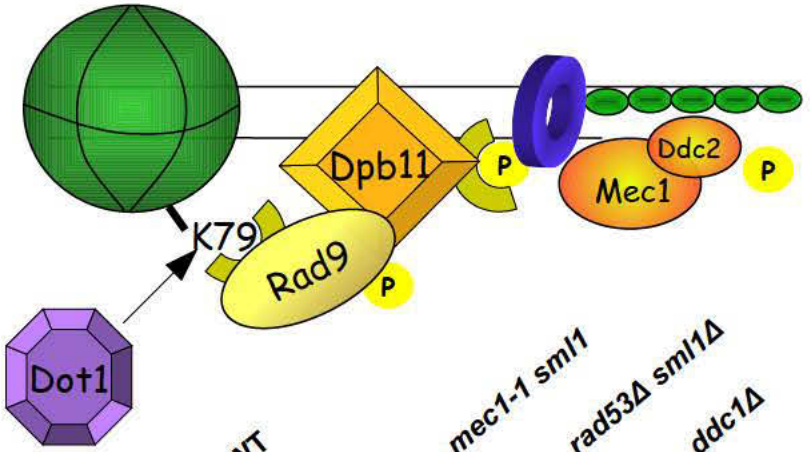
Ddc1 T602 phosphorylation is required for Rad9 function in the absence of Dot1

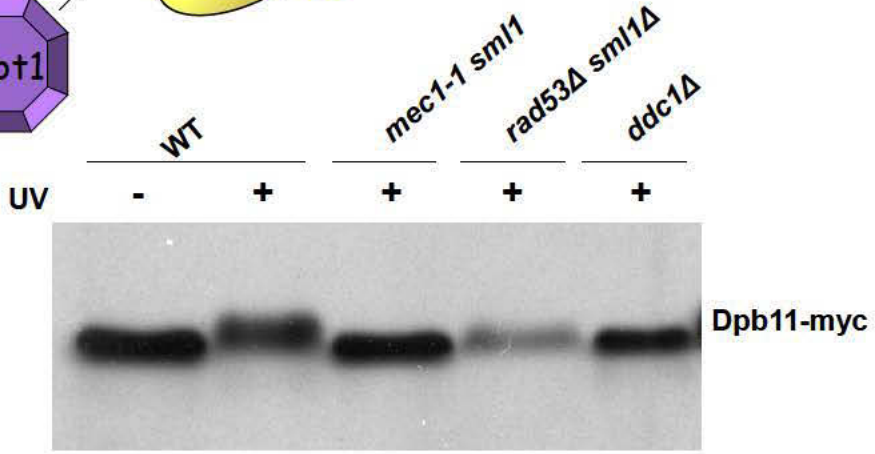
Is this an effect of the lack of phosphorylation or the mutation change the conformation of the protein?



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

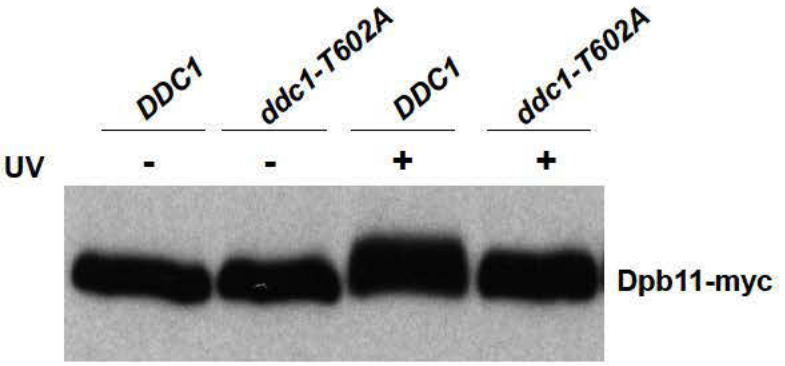
Damage induced Dpb11 phosphorylation



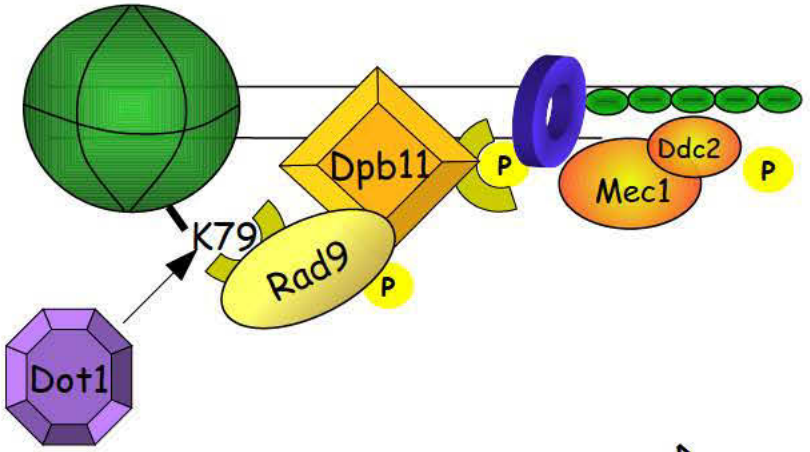


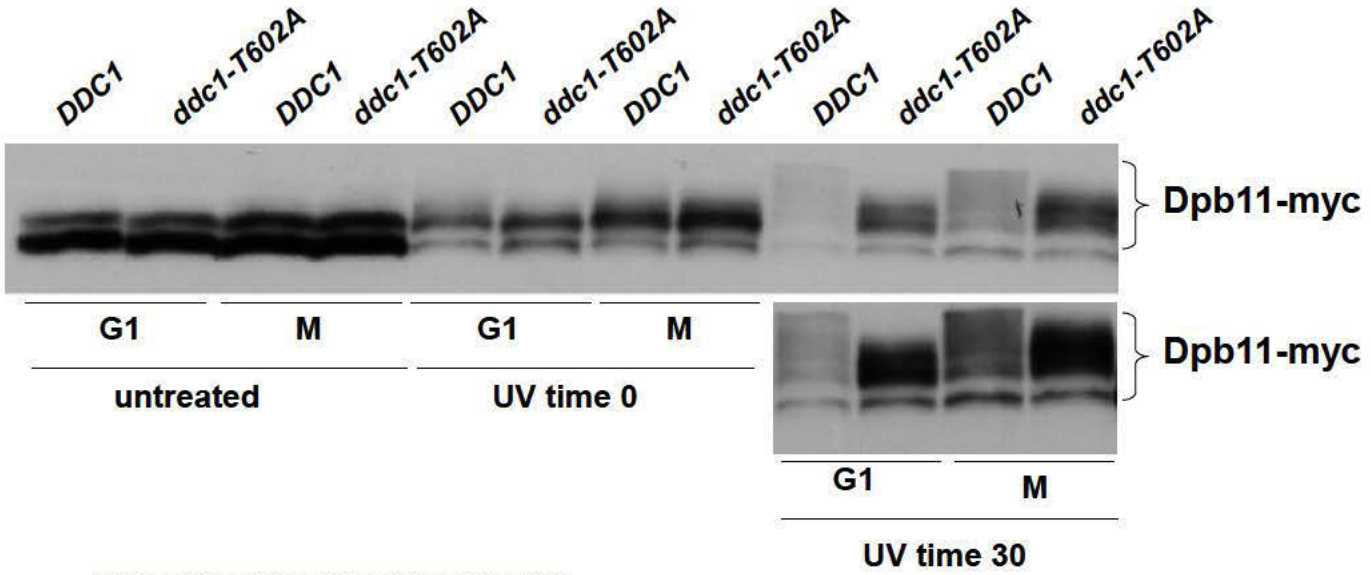
If ddc1-T602 is required for Dpb11 recruitment, then...

Dpb11 is phosphorylated in a Mec1 and Ddc1 dependent manner



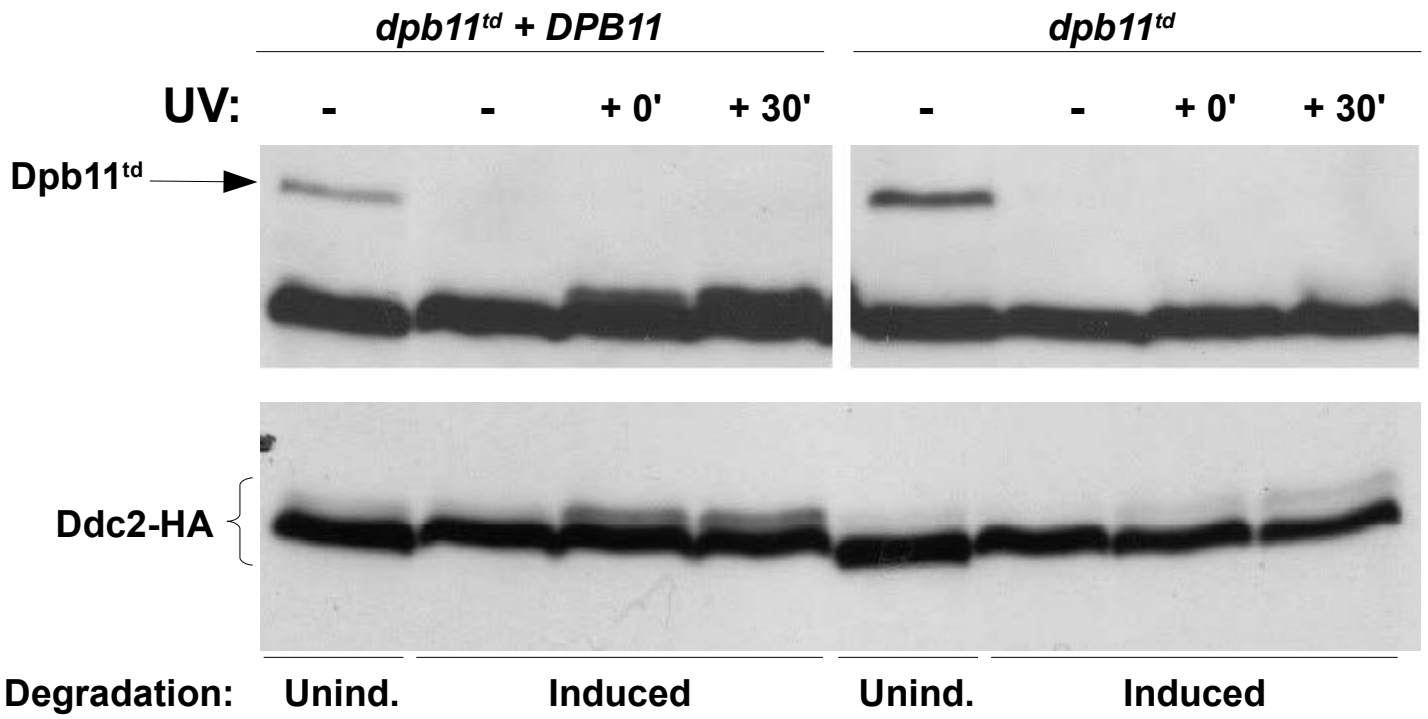
Damage induced Dpb11 phosphorylation



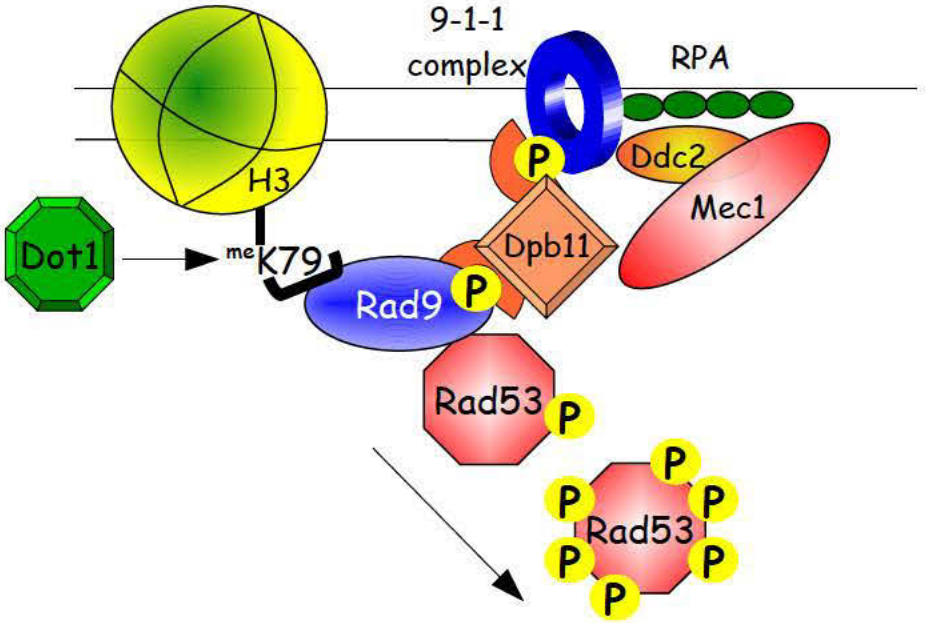


...and also Ddc1 T602 is required for this modification

dpb11-1 is not defective in Mec1 kinase activation, but...



...in the absence of Dpb11 Ddc2 phosphorylation is greatly reduced


9-1-1
complex
RPA
H3
Ddc2
Mec1
Dot1
me K79
Dpb11
Rad9
Rad53
Rad53

Acknowledgments

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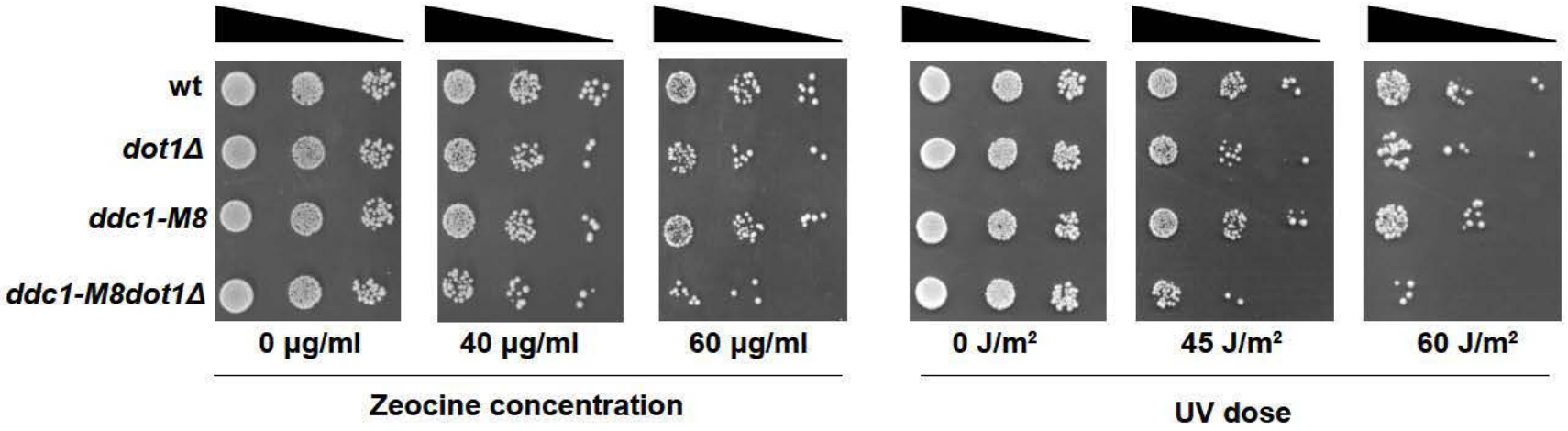
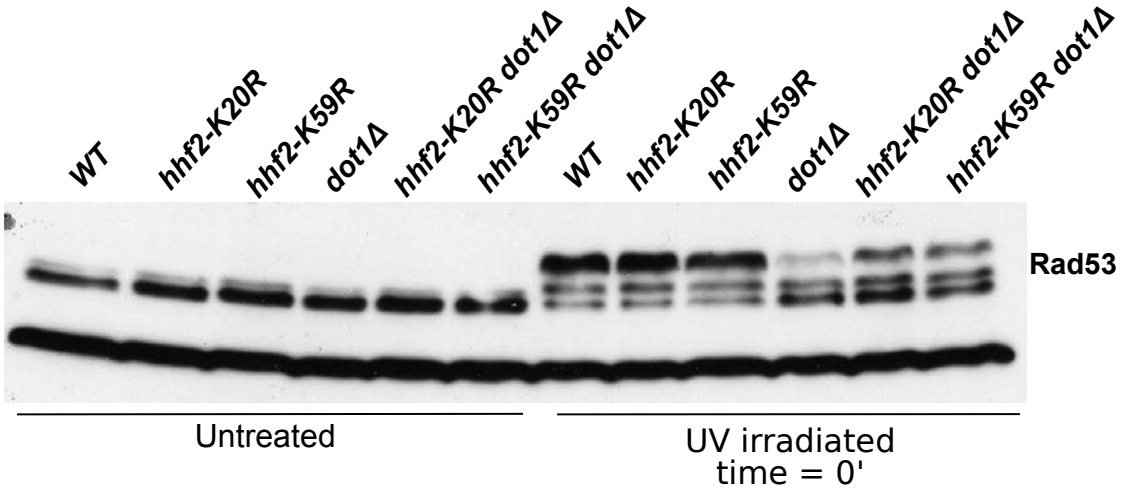
dot1Δddc1-M8 mutant mimics the *dot1Δdpb11-1* mutant

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



How is Rad9 recruited in absence of histone H3 methylation?



We looked for something else...

Dpb11 exerts its function also in G1/S Rad53 phosphorylation

I G1 dot1Δ strain:

- *defective in Rad53 phosphorylation (only small level of pRad53 at later time points)*
- *G1/S checkpoint defective*

Giannattasio, 2005