Recruitment and activation of the ATM kinase in the absence of DNA damage sensors

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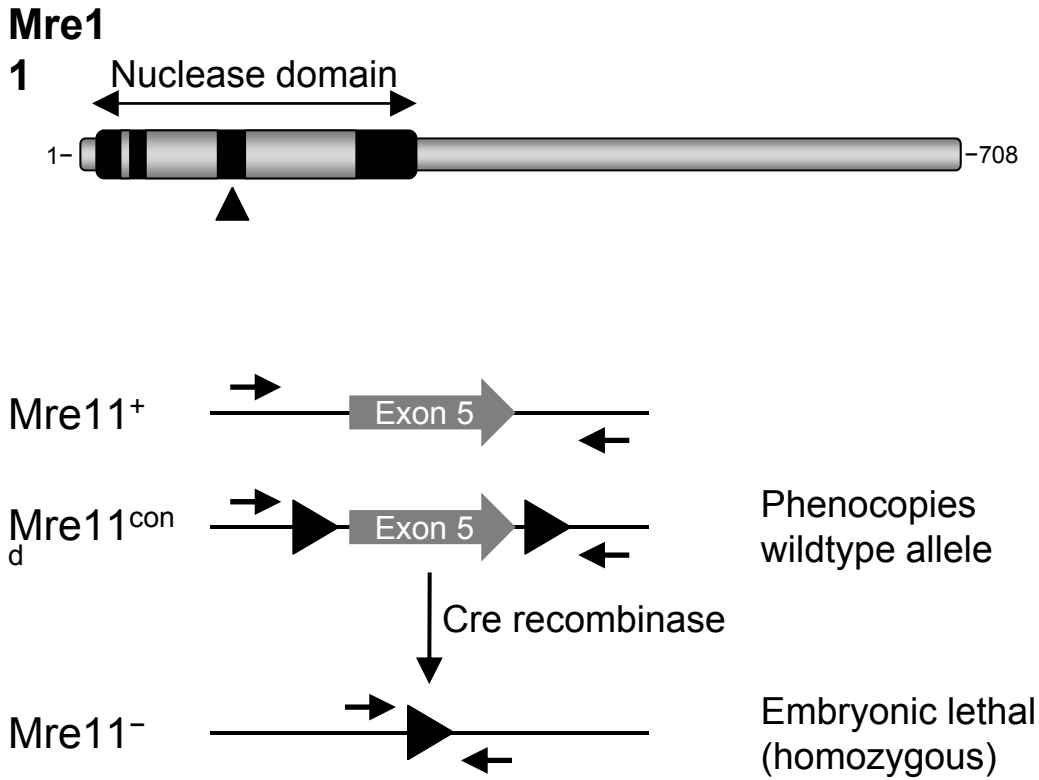
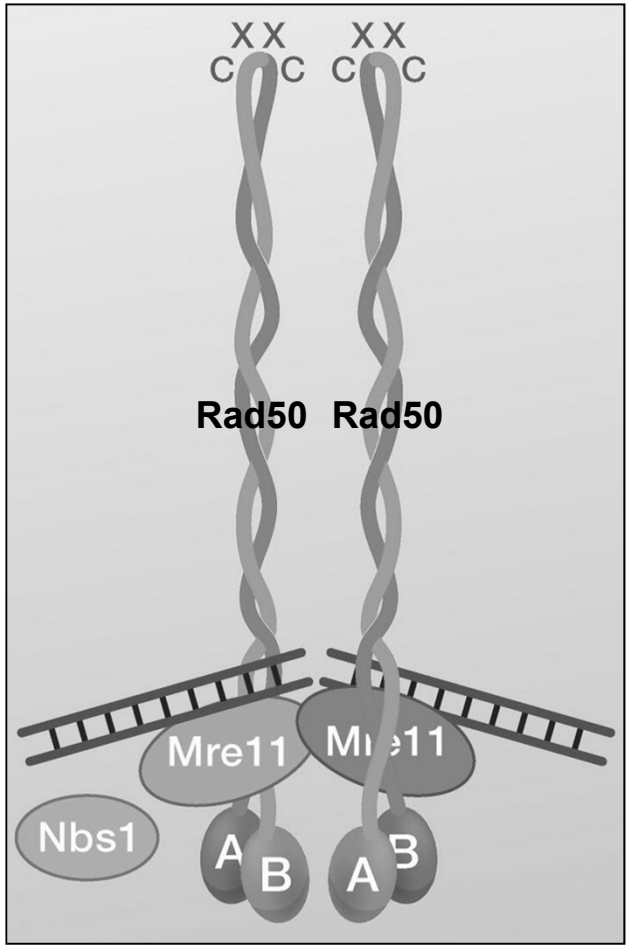
The Mre11/Rad50/NBS1 complex (MRN)





Kanaar and Wyman (2008) Cell 135:14-6

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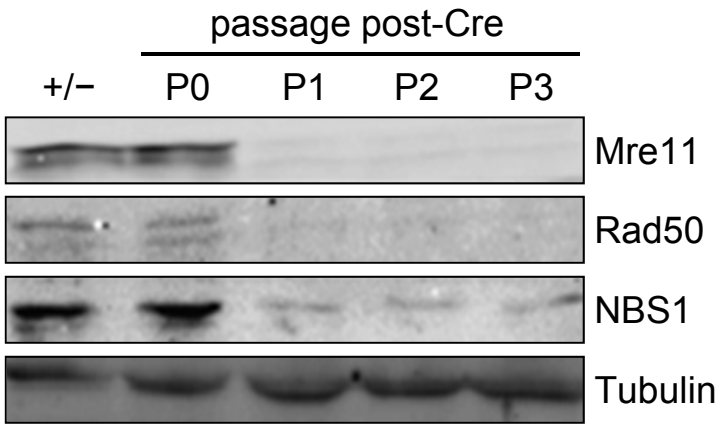
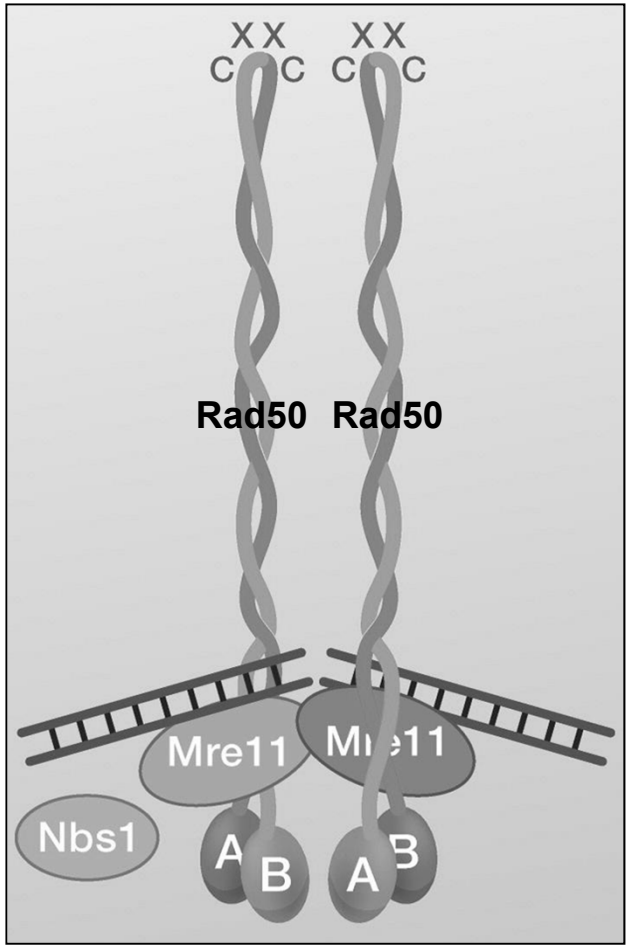
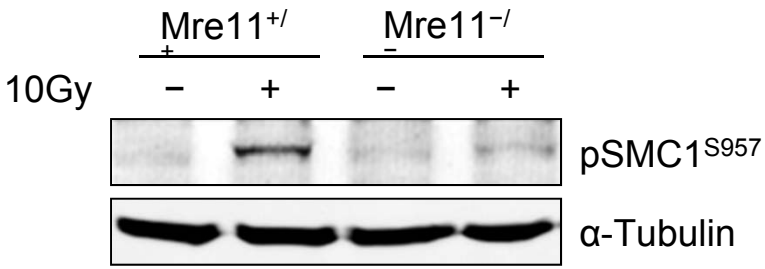


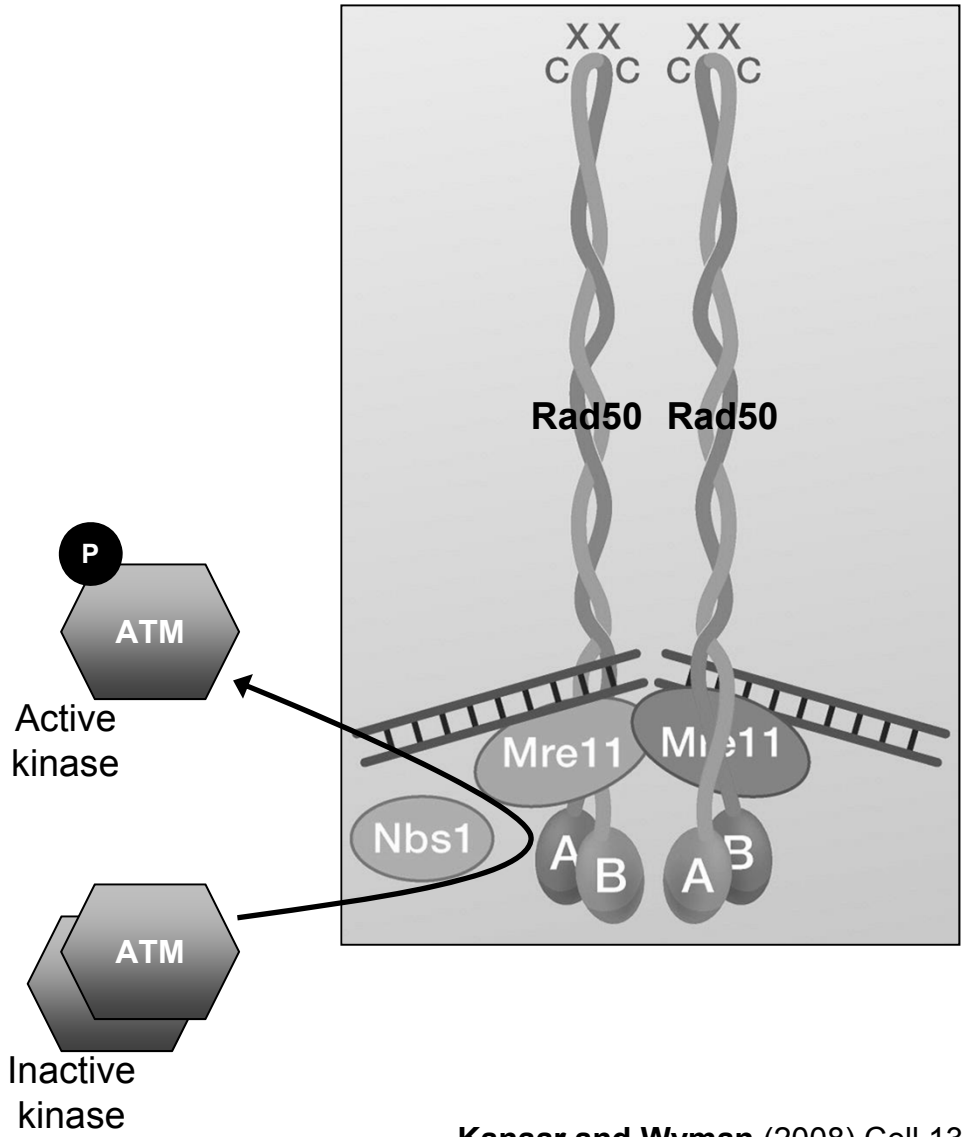
Mre11$^{cond/-}$ to Mre11$^{-/-}$



Kanaar and Wyman (2008) Cell 135:14-6

Activation of the ATM kinase is disrupted in Mre11-deficient cells

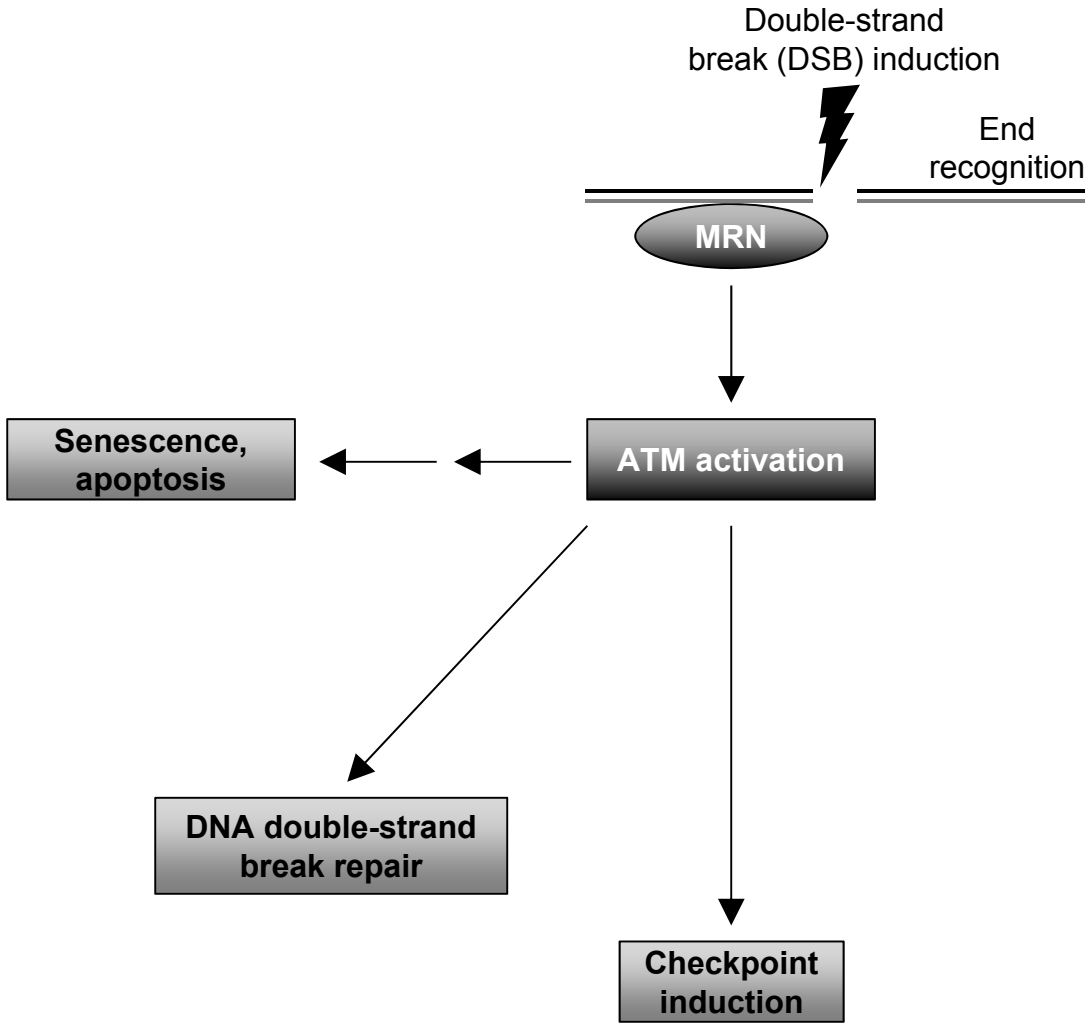




Buis *et al*. (2008) Cell 135:85-96

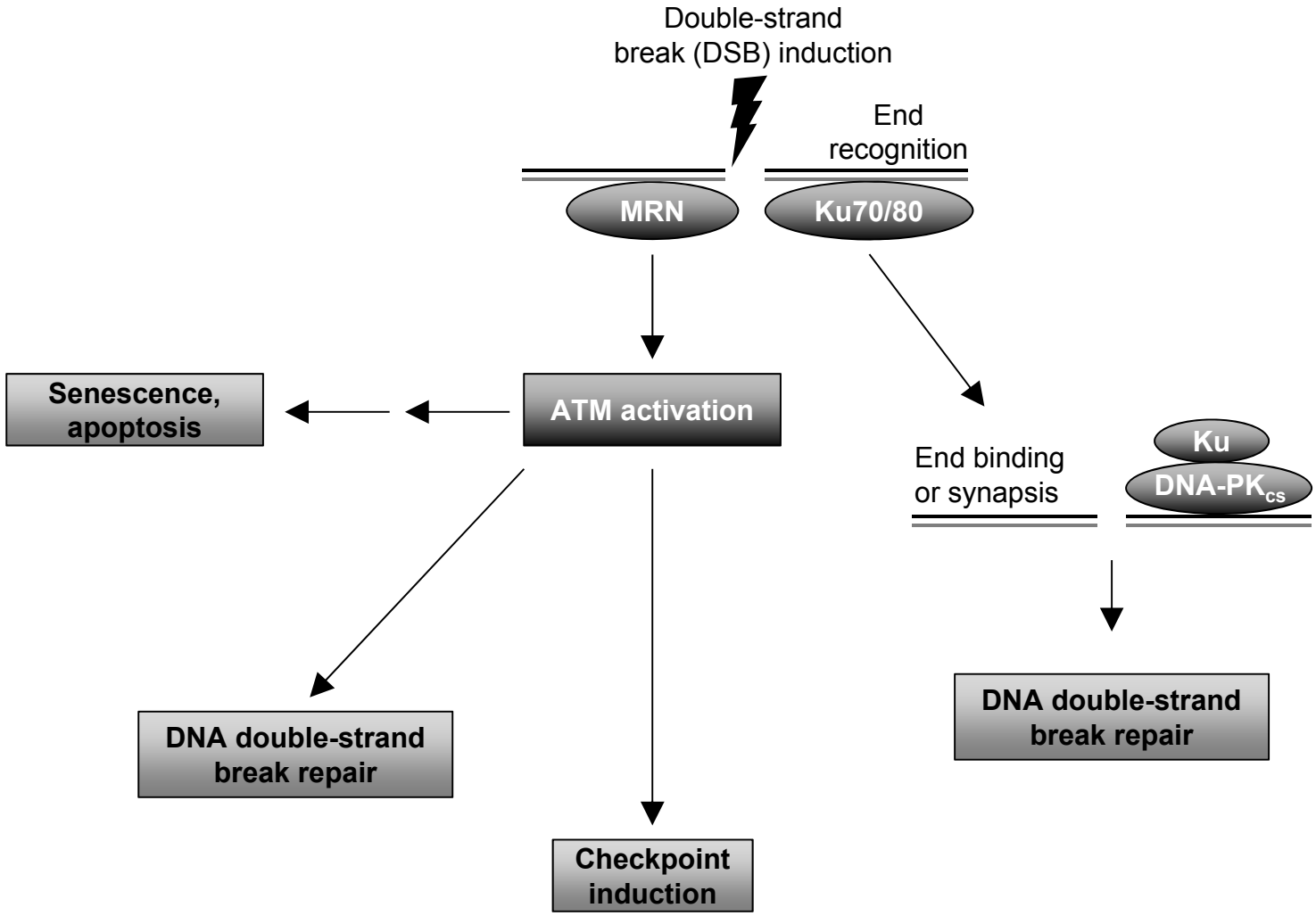
Kanaar and Wyman (2008) Cell 135:14-6

The ATM kinase controls DSB responses



adapted from **Stracker and Petrini** (2011) Nat Rev Mol Cell Bio 12:90-103

Ku DSB sensor recruits/activates the kinase DNA-PKcs



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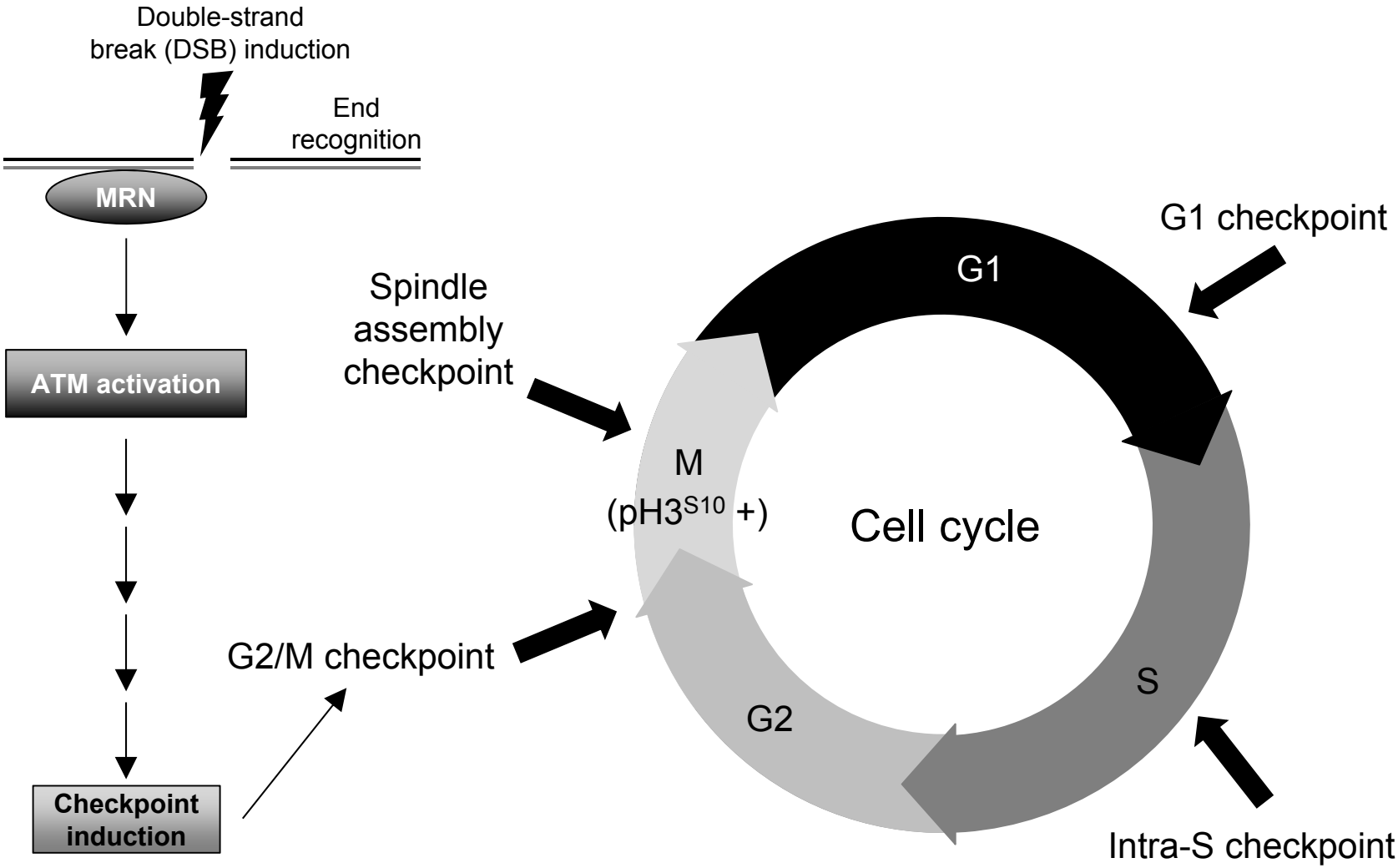
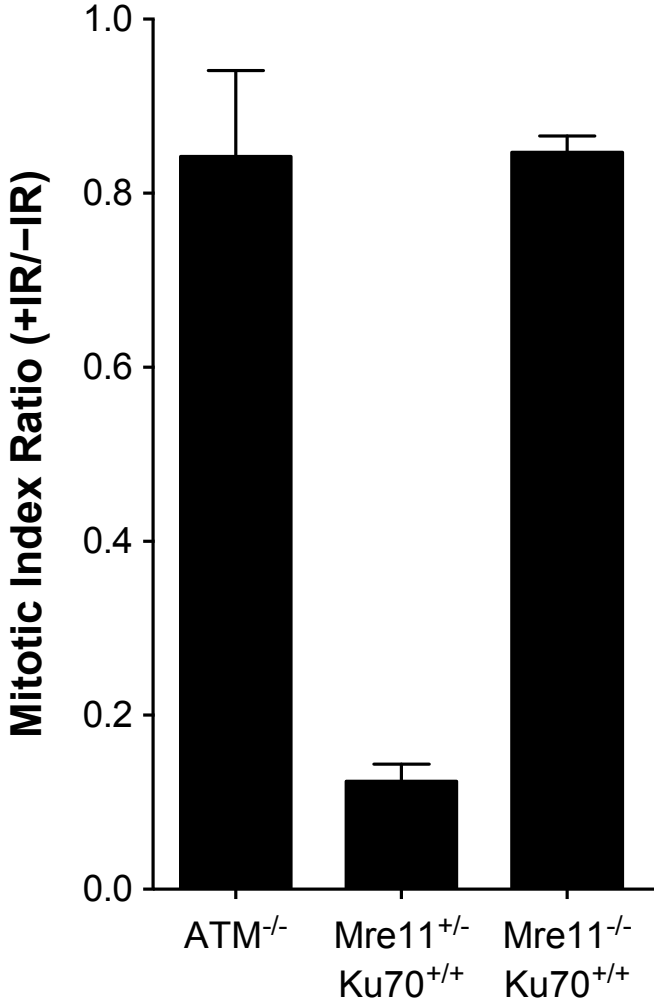
MRN and ATM control the G2/M cell cycle checkpoint



adapted from **Stracker and Petrini** (2011) Nat Rev Mol Cell Bio 12:90-103

Loss of the MRN complex impairs the G2/M checkpoint



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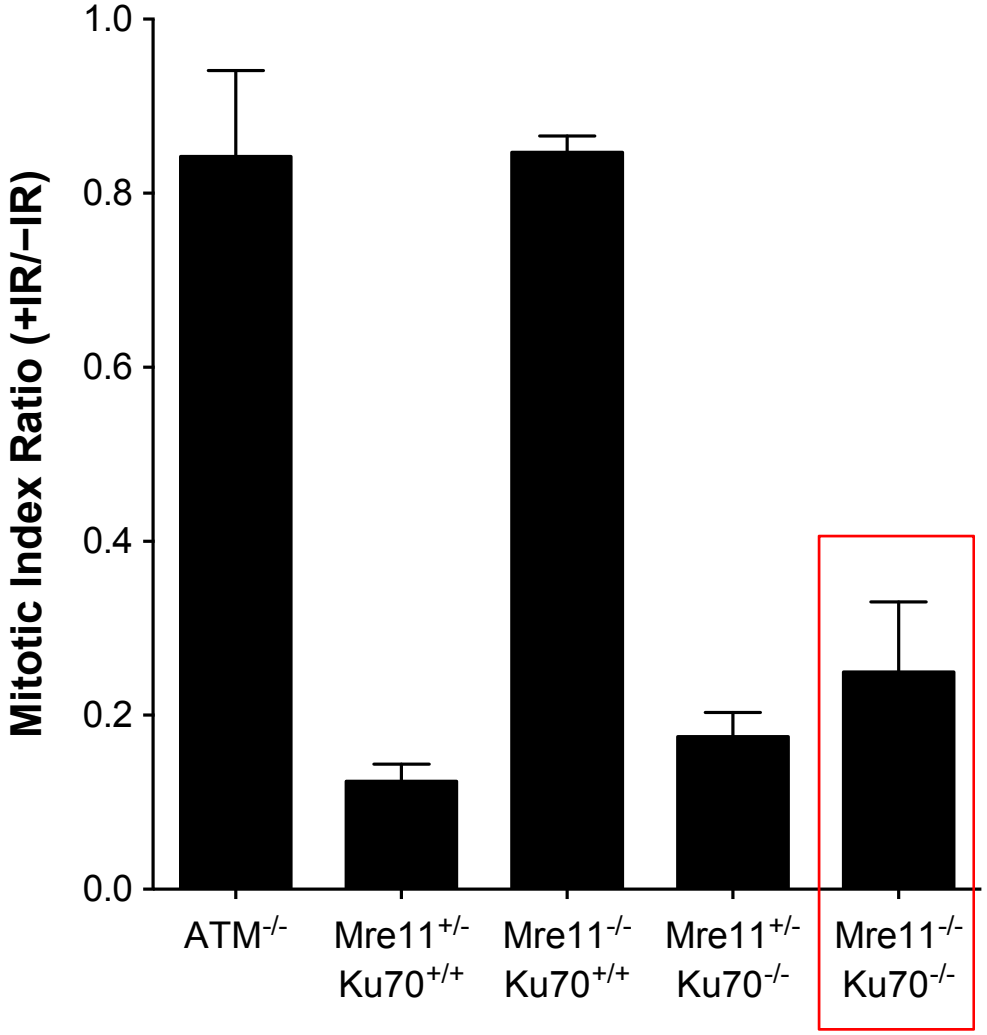
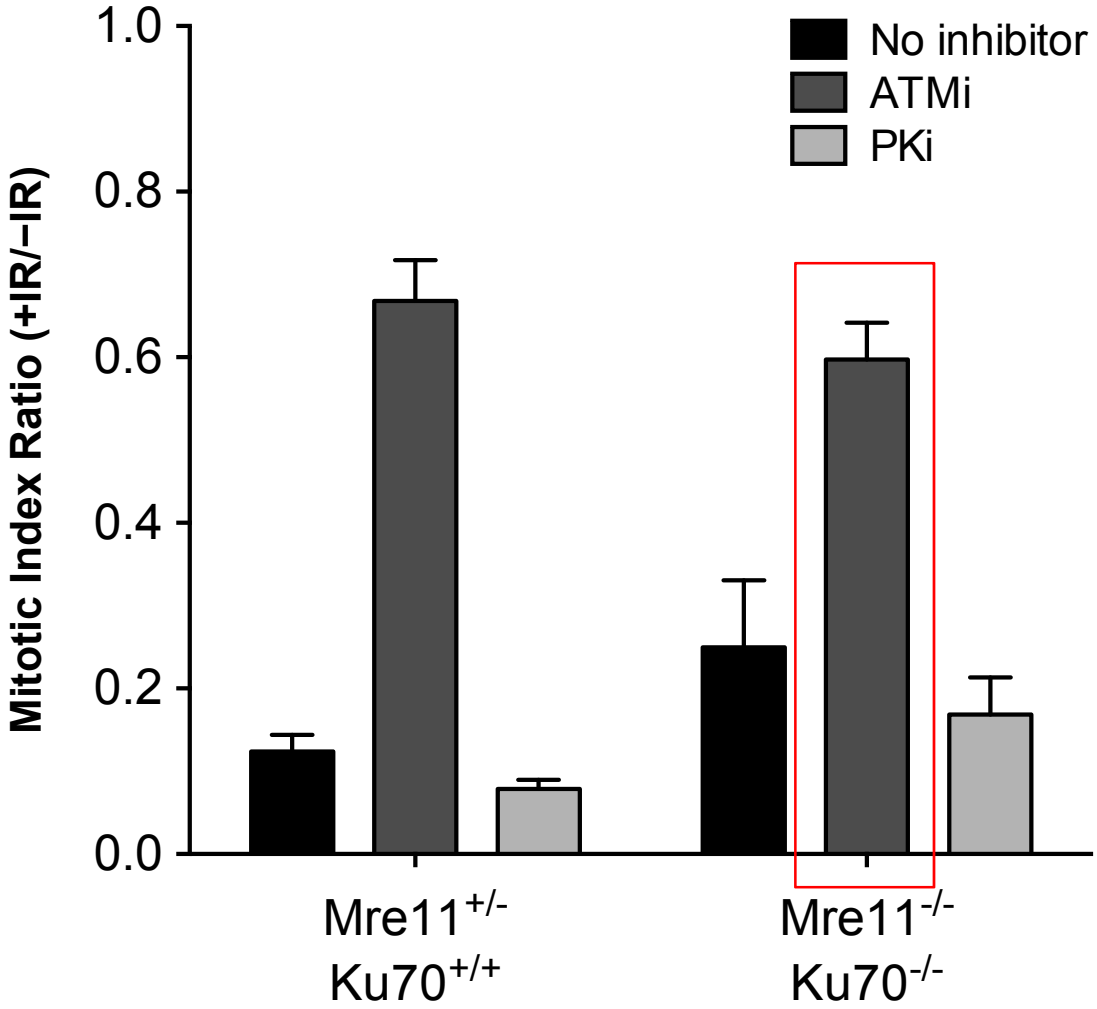
The defective G2/M checkpoint in MRN-deficient cells is rescued by Ku deficiency

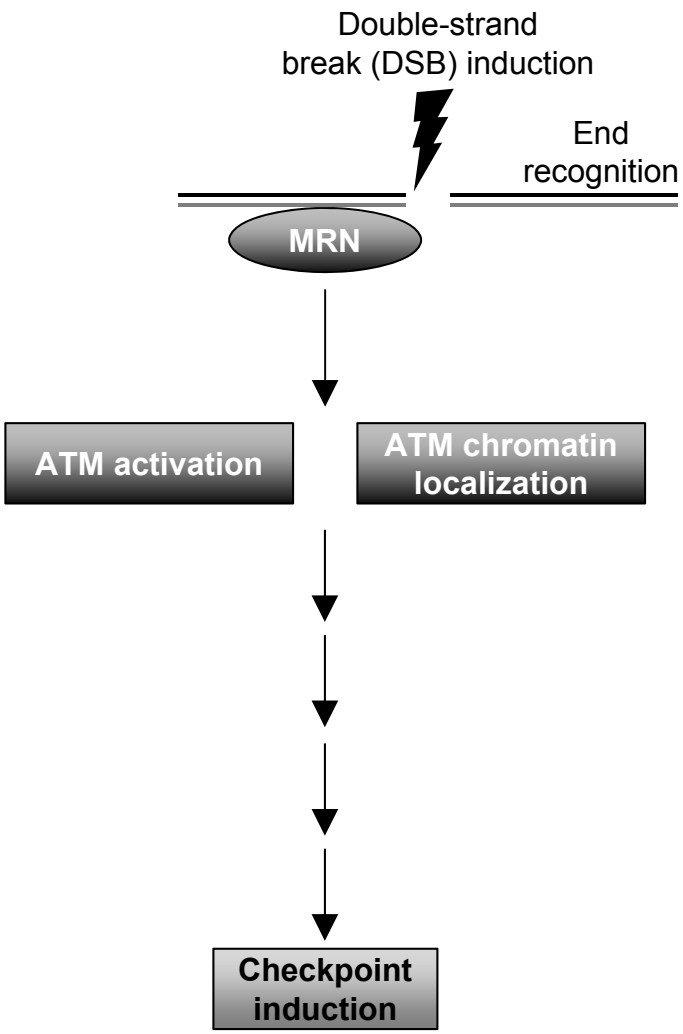


The rescued G2/M checkpoint in MRN/Ku-deficient cells is ATM-dependent

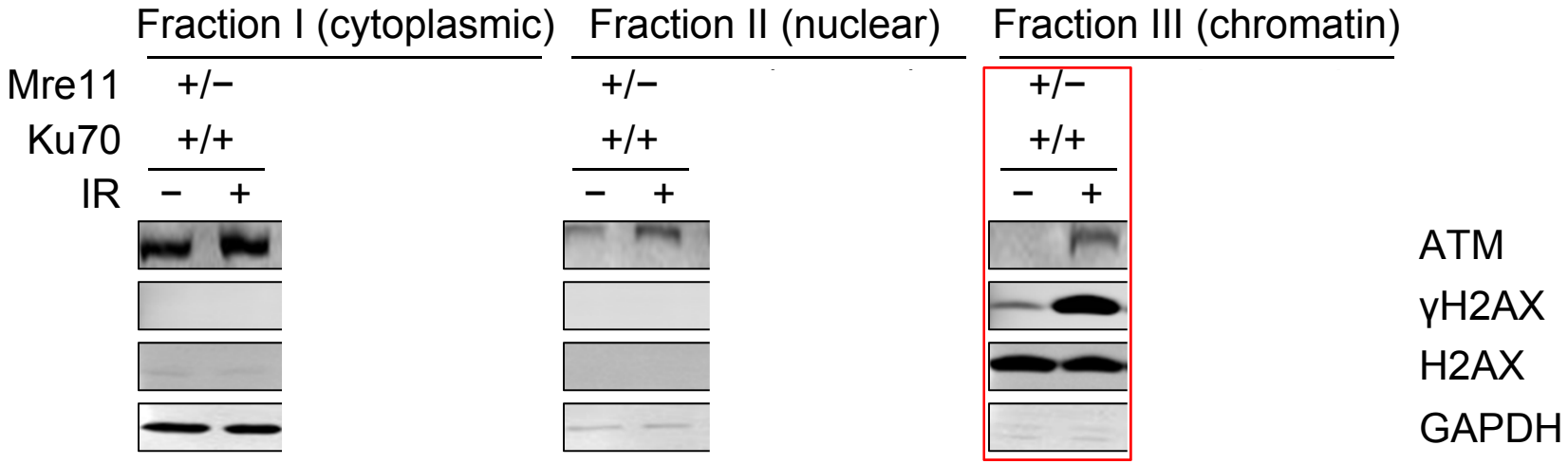


PKi = DNA-PK inhibitor (NU7026)
ATMi or Ai = ATM inhibitor (KU55933)

MRN-dependent recruitment of ATM to chromatin

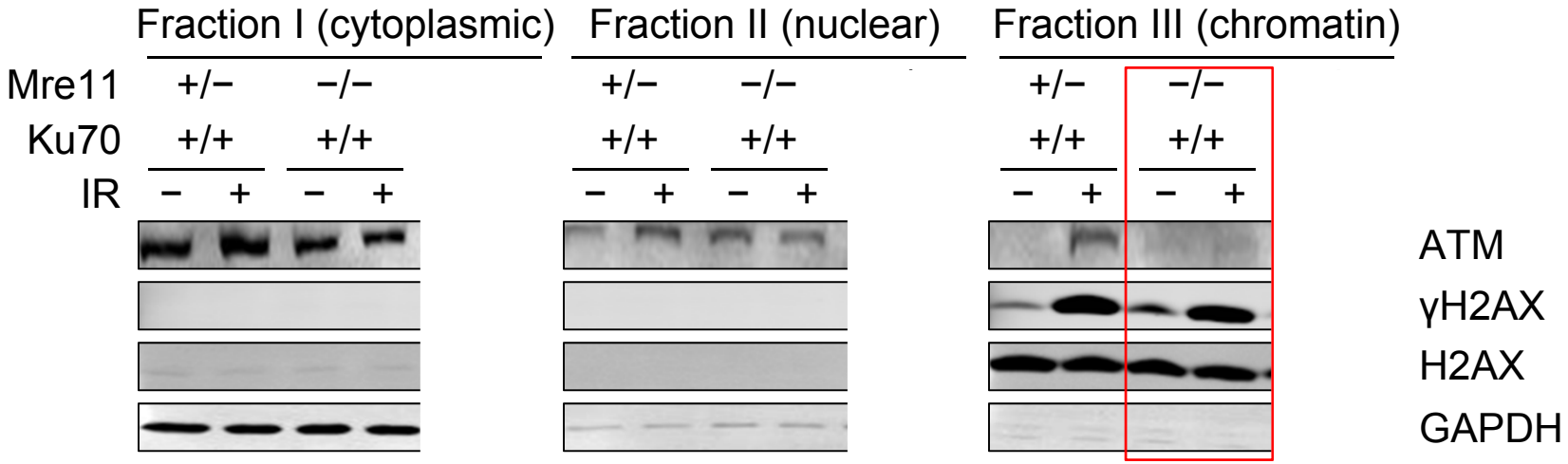


ATM localizes to chromatin following DNA damage



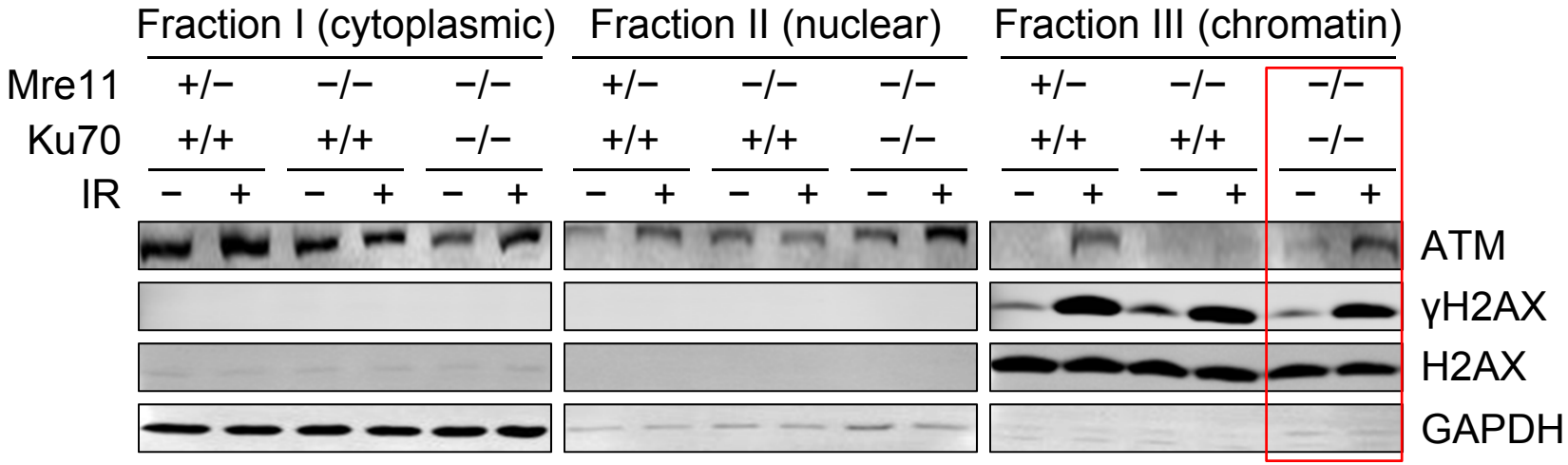
Andegeko *et al.* (2001) J Biol Chem 246:38224-30

ATM cannot localize to chromatin in the absence of MRN

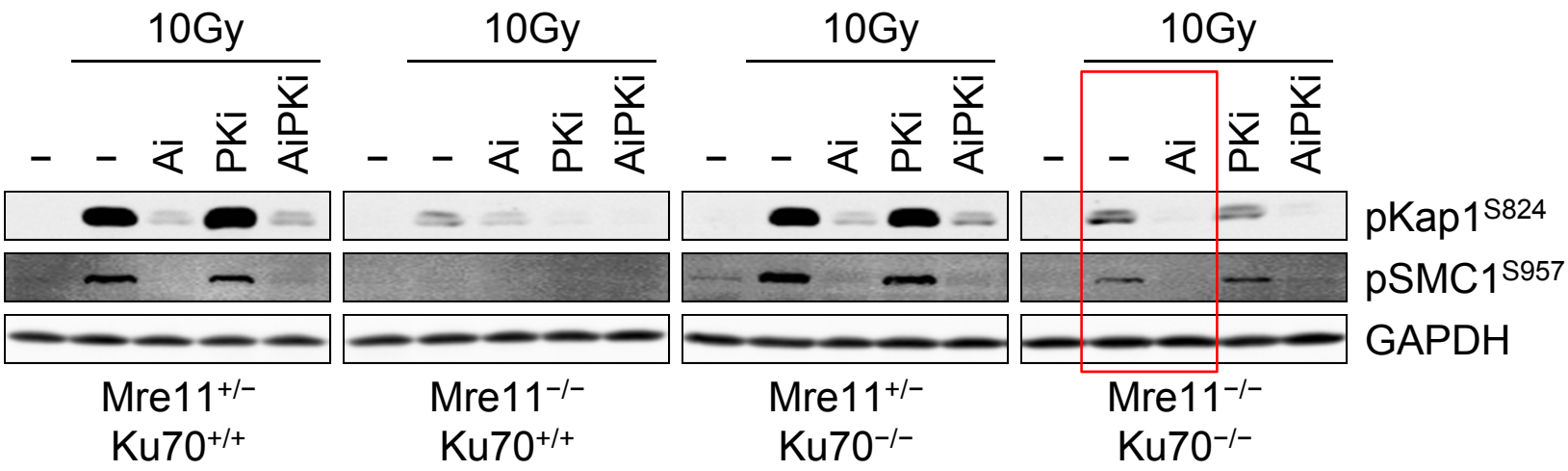


Uziel *et al.* (2003) EMBO J 22:5612-21

Ku removal restores damage-induced ATM chromatin recruitment in the absence of the MRN complex



ATM-dependent nuclear phosphorylation events are rescued by loss of Ku in MRN deficiency



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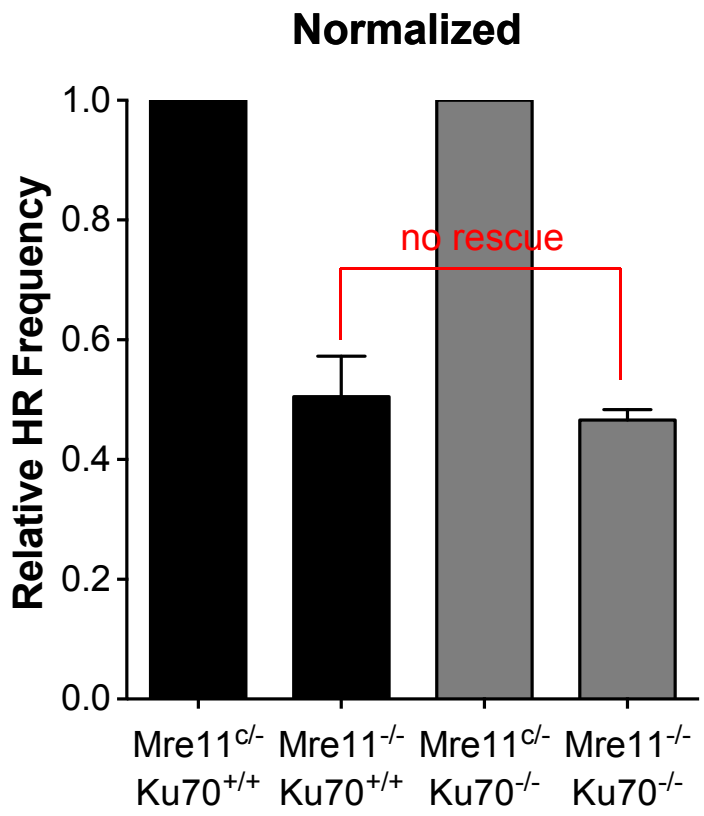
Ku deficiency does not rescue MRN-dependent homologous recombination (DR-GFP)



The role of MRN may not be as simple as direct recruitment and allosteric activation of ATM

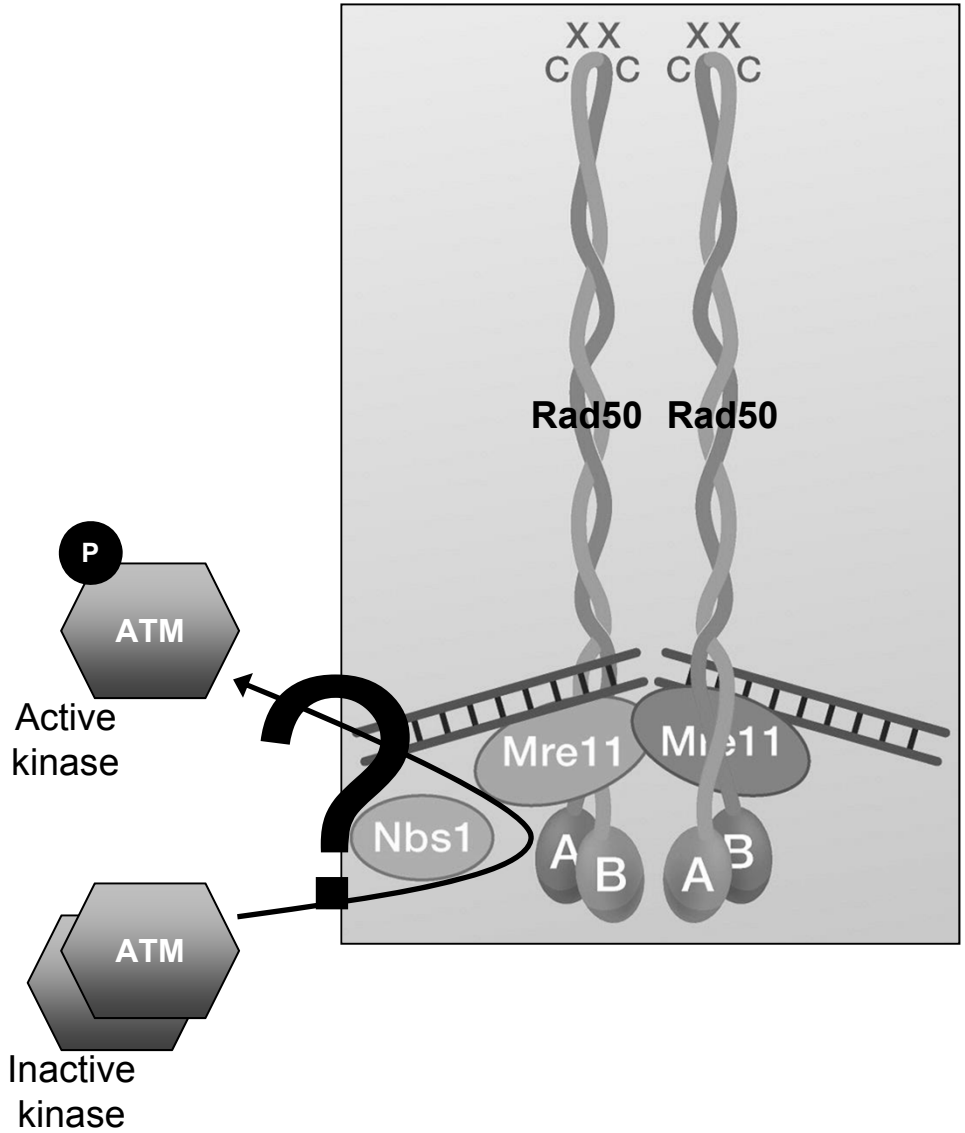
In the absence of MRN:

Ku removal restores:

- ATM chromatin localization
- ATM kinase activation
- The ATM-dependent G2/M cell cycle checkpoint

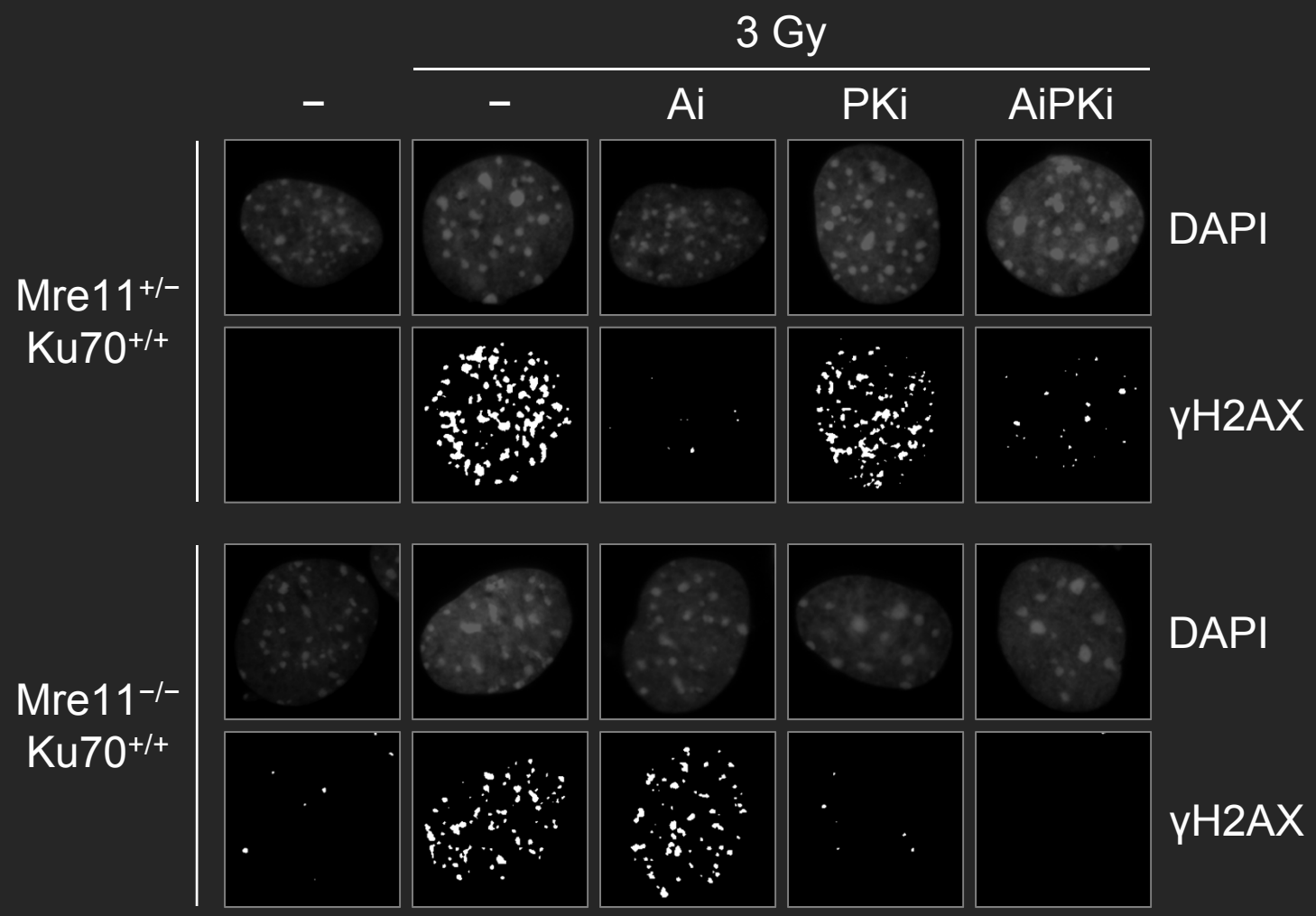
Ku removal does not rescue:

- MRN-dependent HR



Kanaar and Wyman (2008) Cell 135:14-6

DNA-PKcs can efficiently phosphorylate H2AX in the absence of MRN



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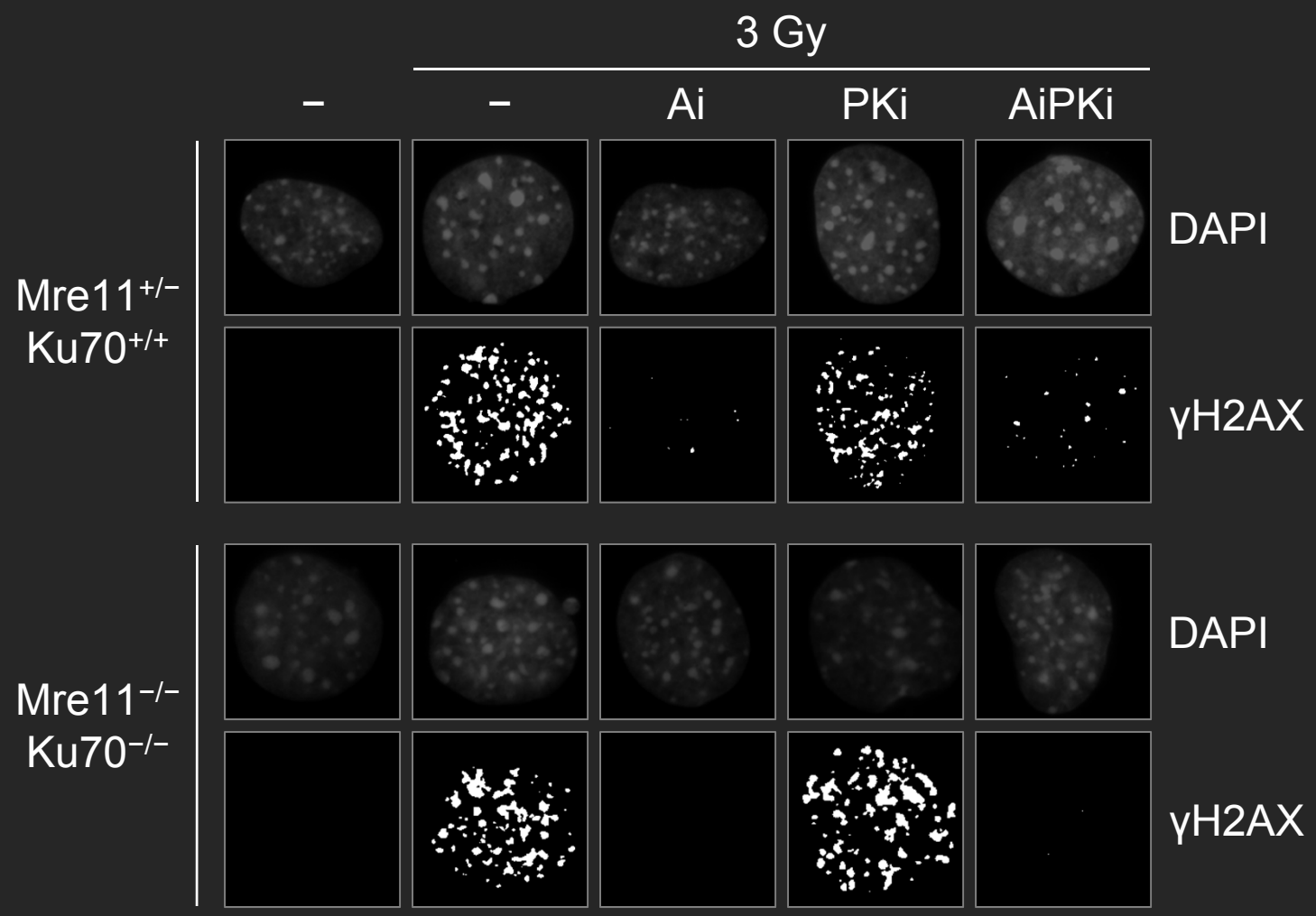
γH2AX foci in the absence of Mre11 and Ku are ATM-dependent and indistinguishable from controls



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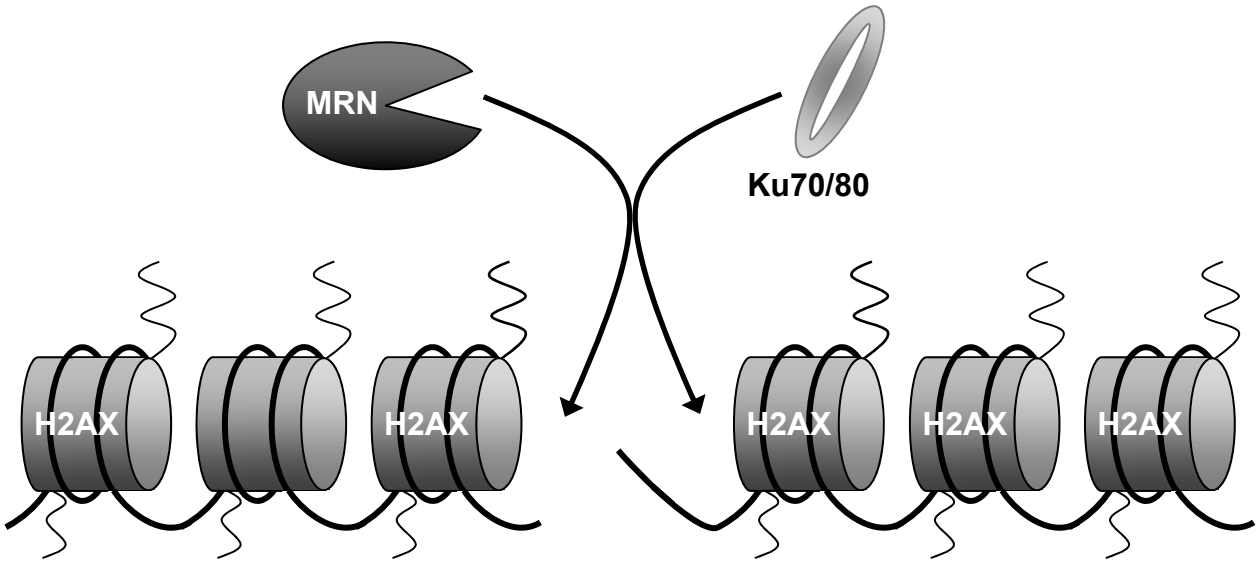
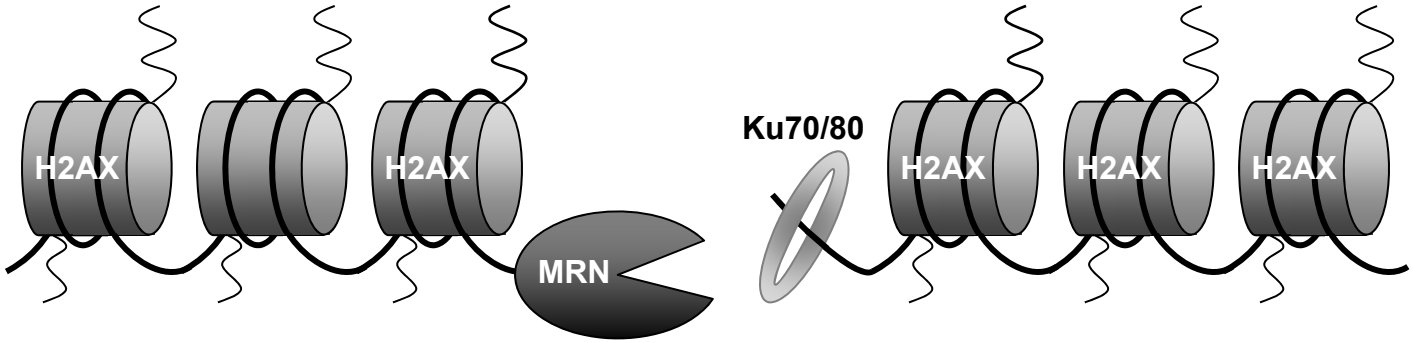
Antagonism between Mre11 and Ku at some level?

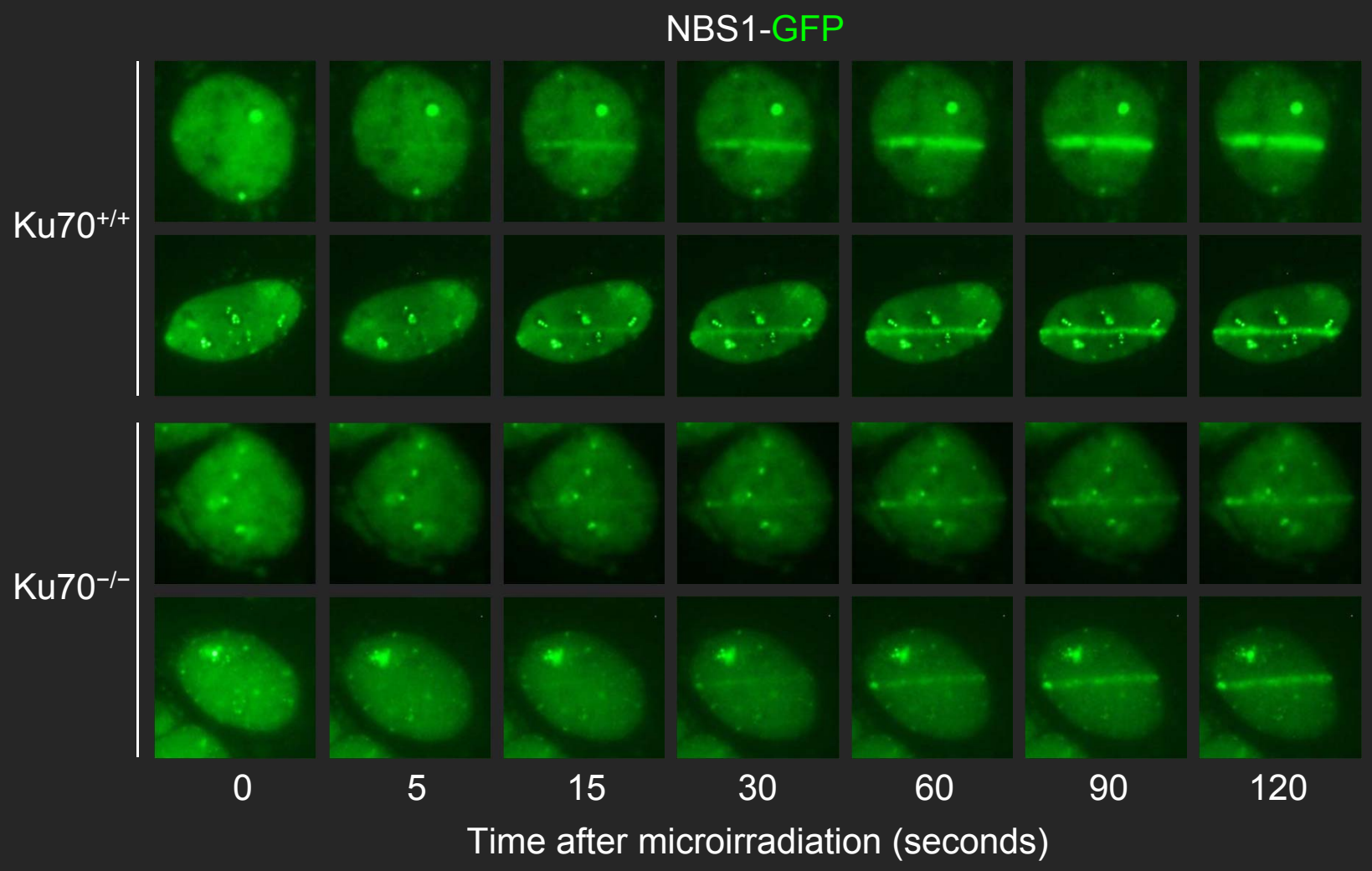
Competition for DNA ends?



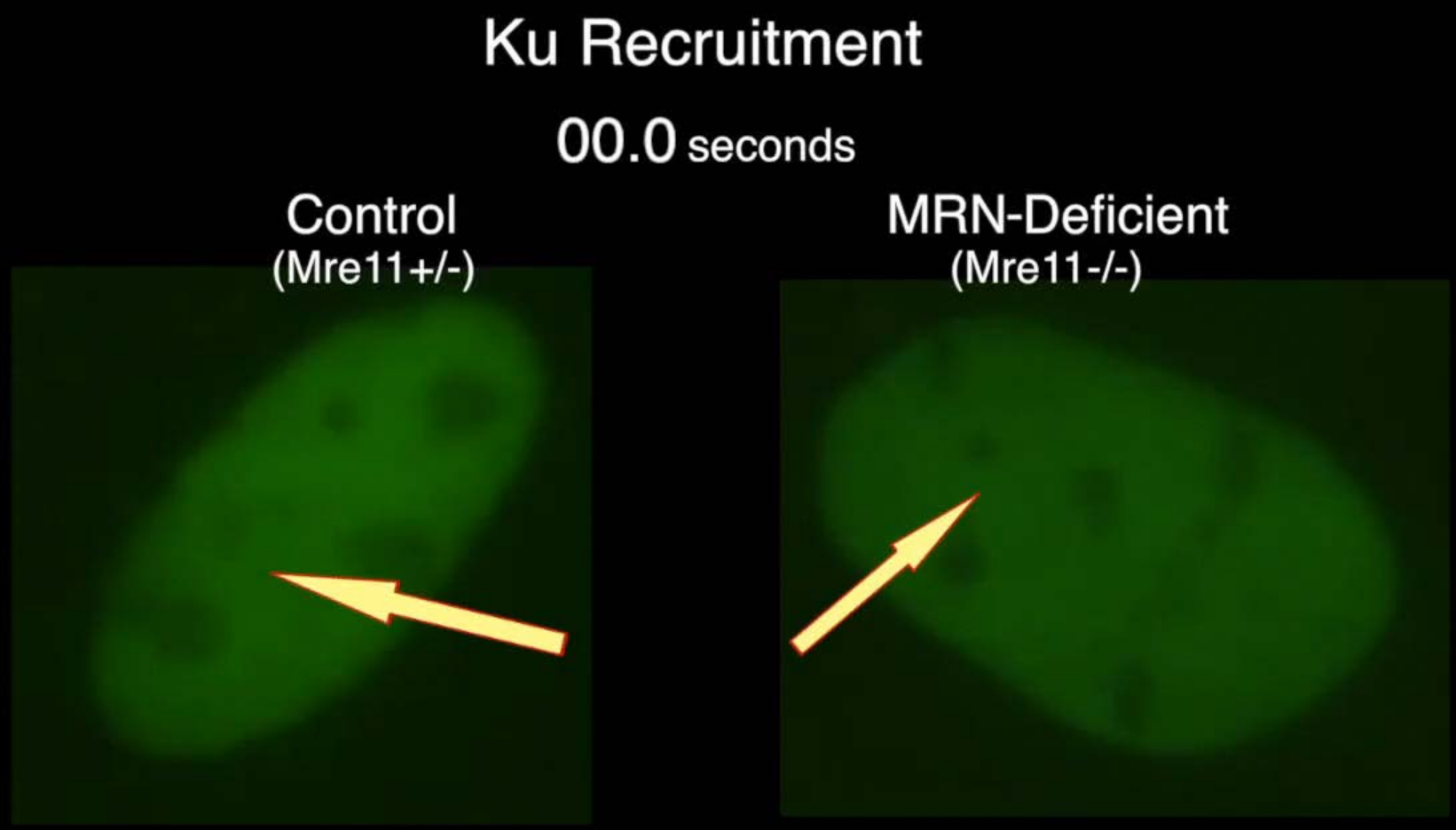
Opposing functions after both sensors are recruited?



MRN recruitment is not altered by loss of Ku



Ku recruitment is rapid and unaltered by loss of MRN



GFP-Ku70/80

Summary

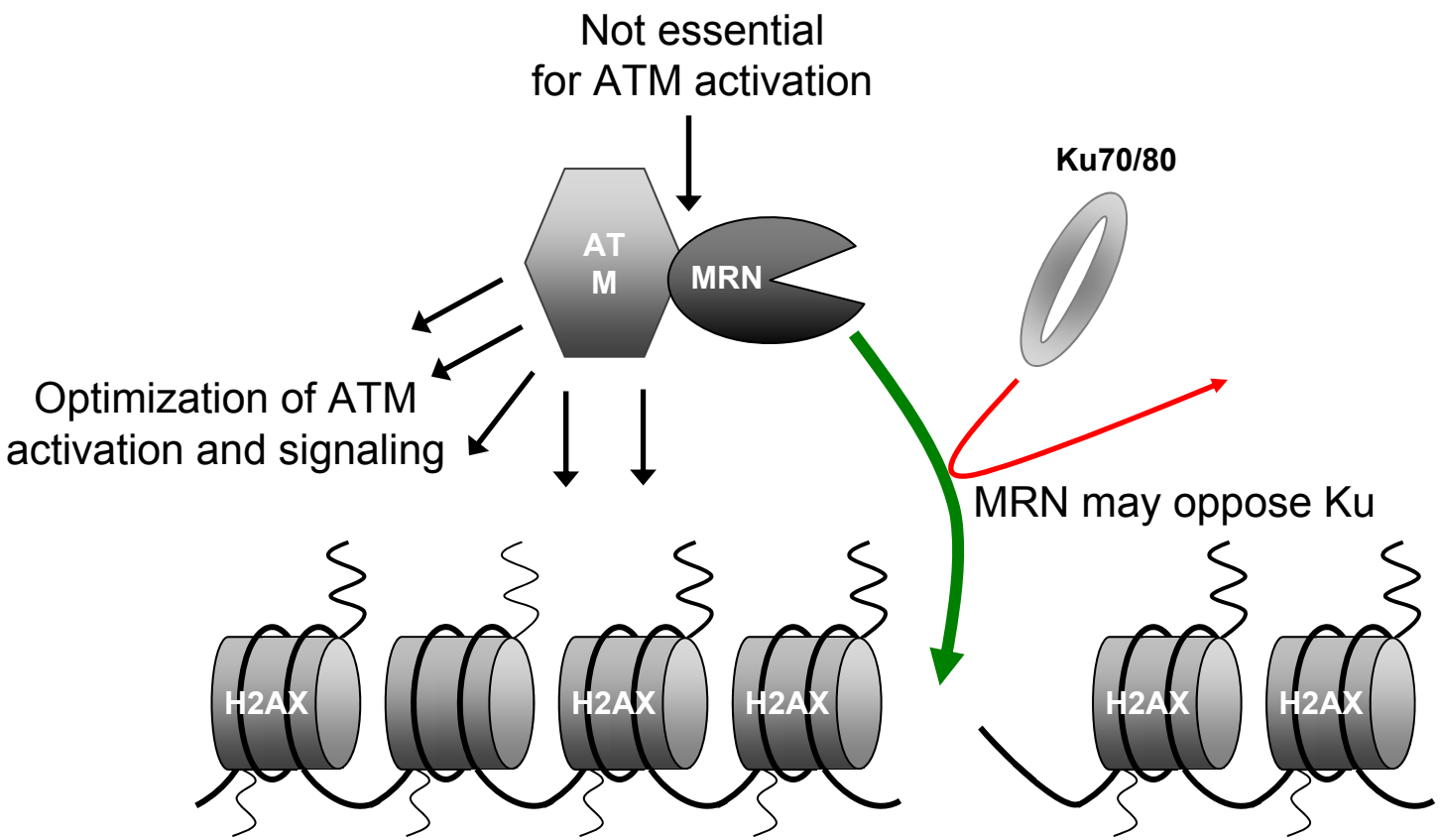
In the absence of MRN, Ku removal restores:

- ATM chromatin localization
- ATM kinase activation
- The ATM-dependent G2/M cell cycle checkpoint

In the absence of MRN, Ku removal does not restore:

- MRN-dependent homologous recombination

Implications



Acknowledgements

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Elizabeth Spehalski PhD
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Yu Lab (Univ. of Michigan)

Xiaochun Yu PhD
Chao Liu PhD
Lin-Yu (Charles) Lu PhD

Our Neighbors

Sekiguchi Lab
Canman Lab