Roles of Mammalian Mre11 in genomic stability and development

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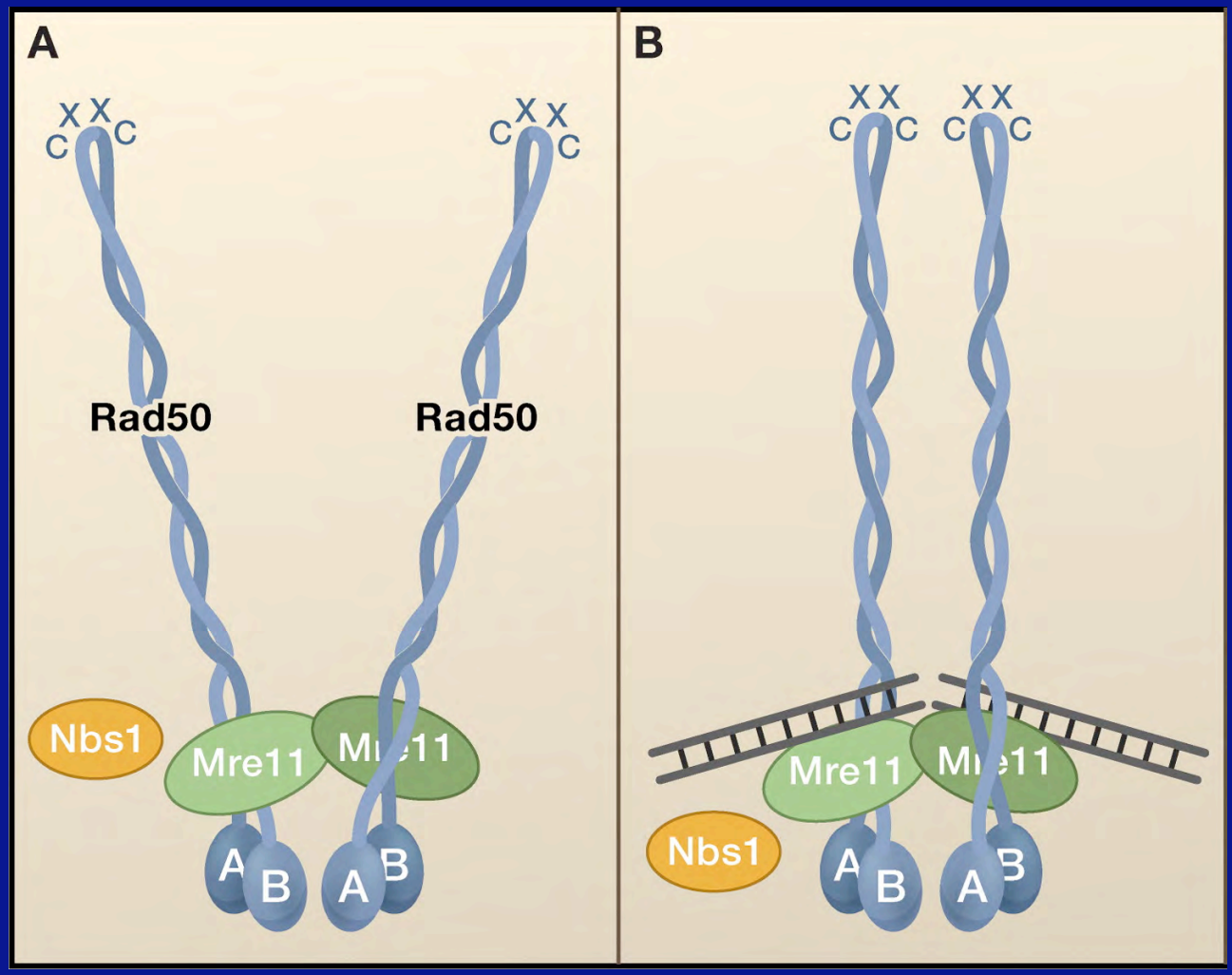
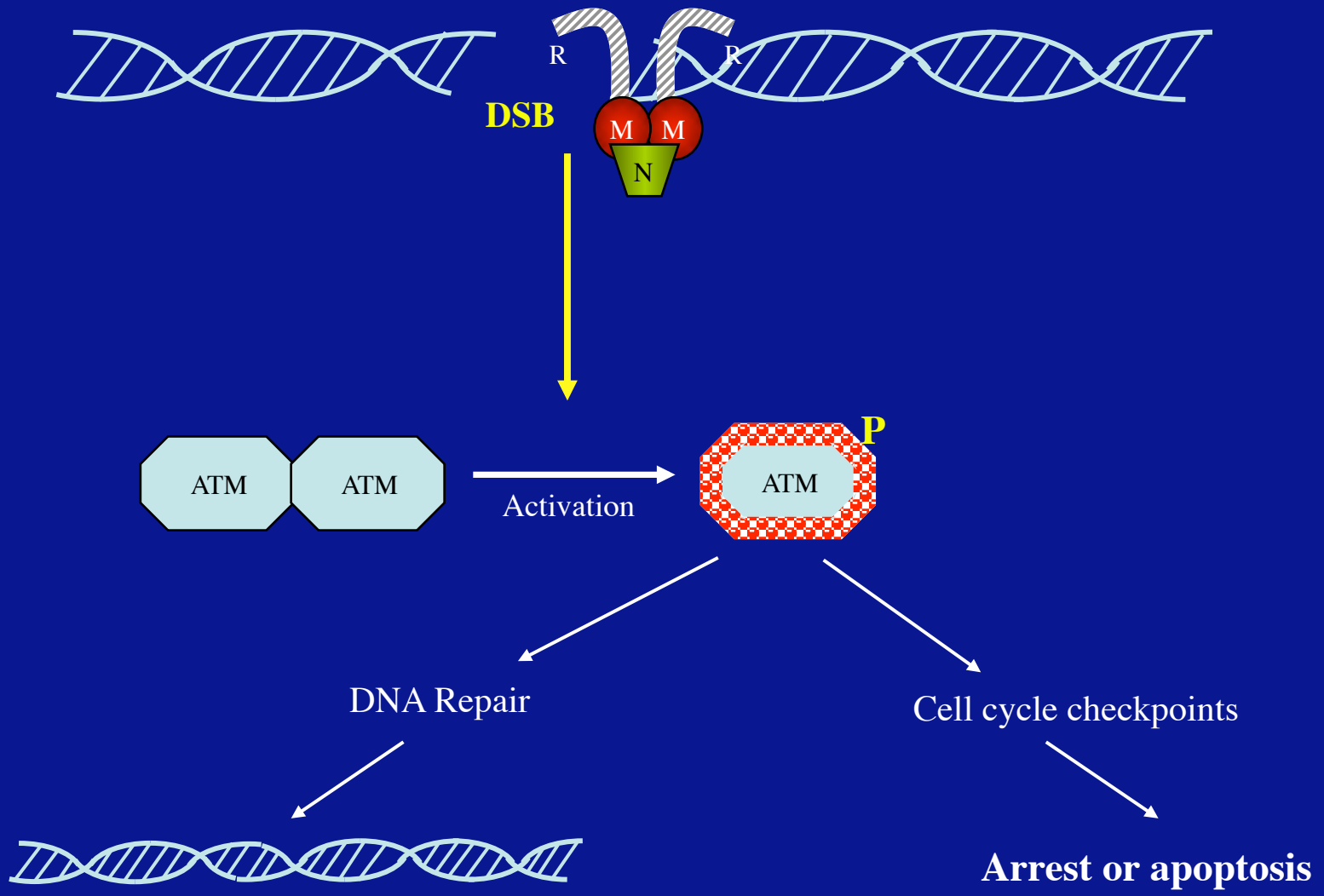
The Mre11/Rad50/NBS complex (MRN)



Kanaar & Wyman, Cell 2008

The MRN complex: a sensor of double strand breaks

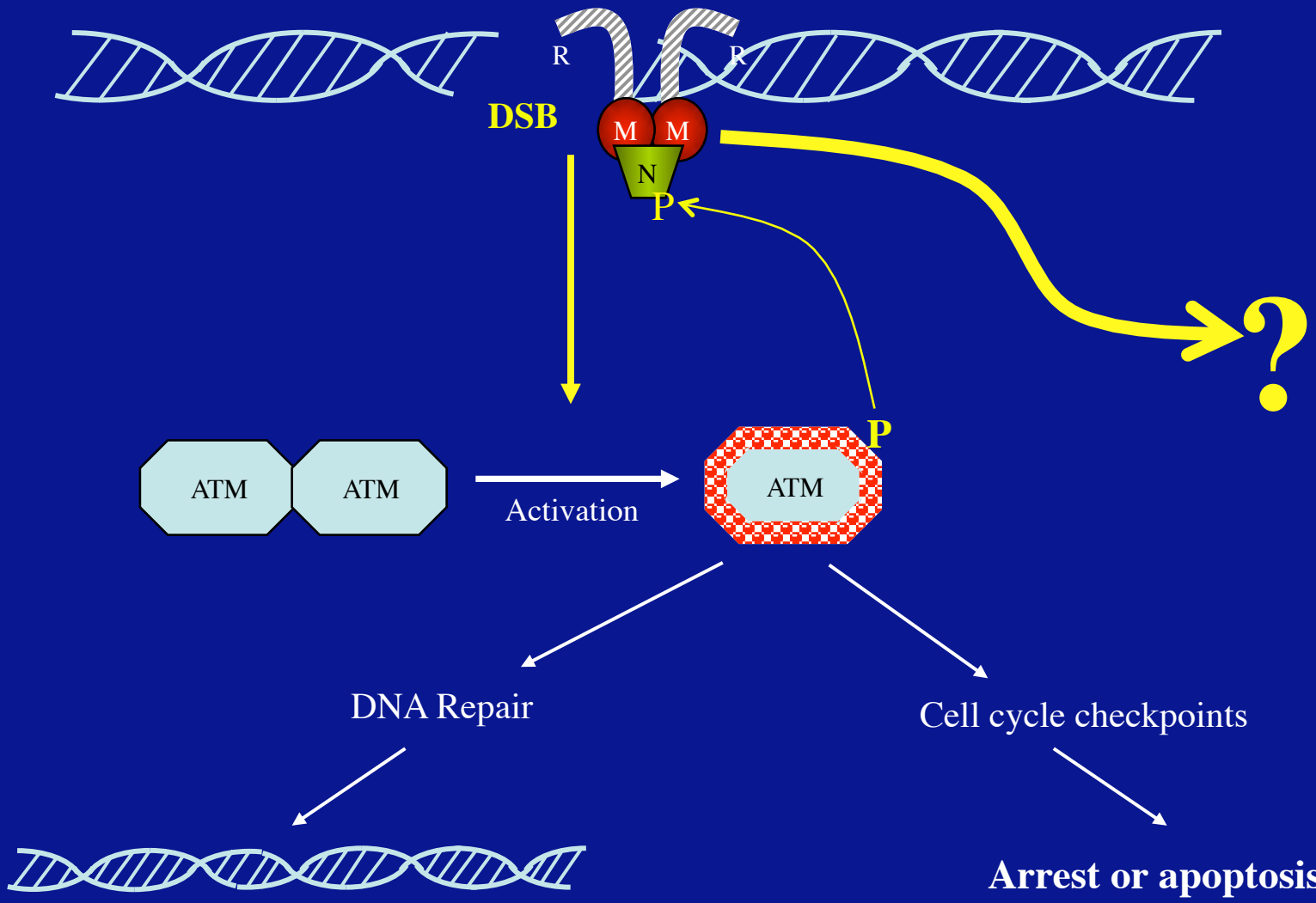


ATM and MRN mutations compared

- Human Syndromes
  - Many AT patients harbor ATM null mutations.
  - All ATLD and NBS patients have hypomorphic Mre11 or NBS1 mutations.

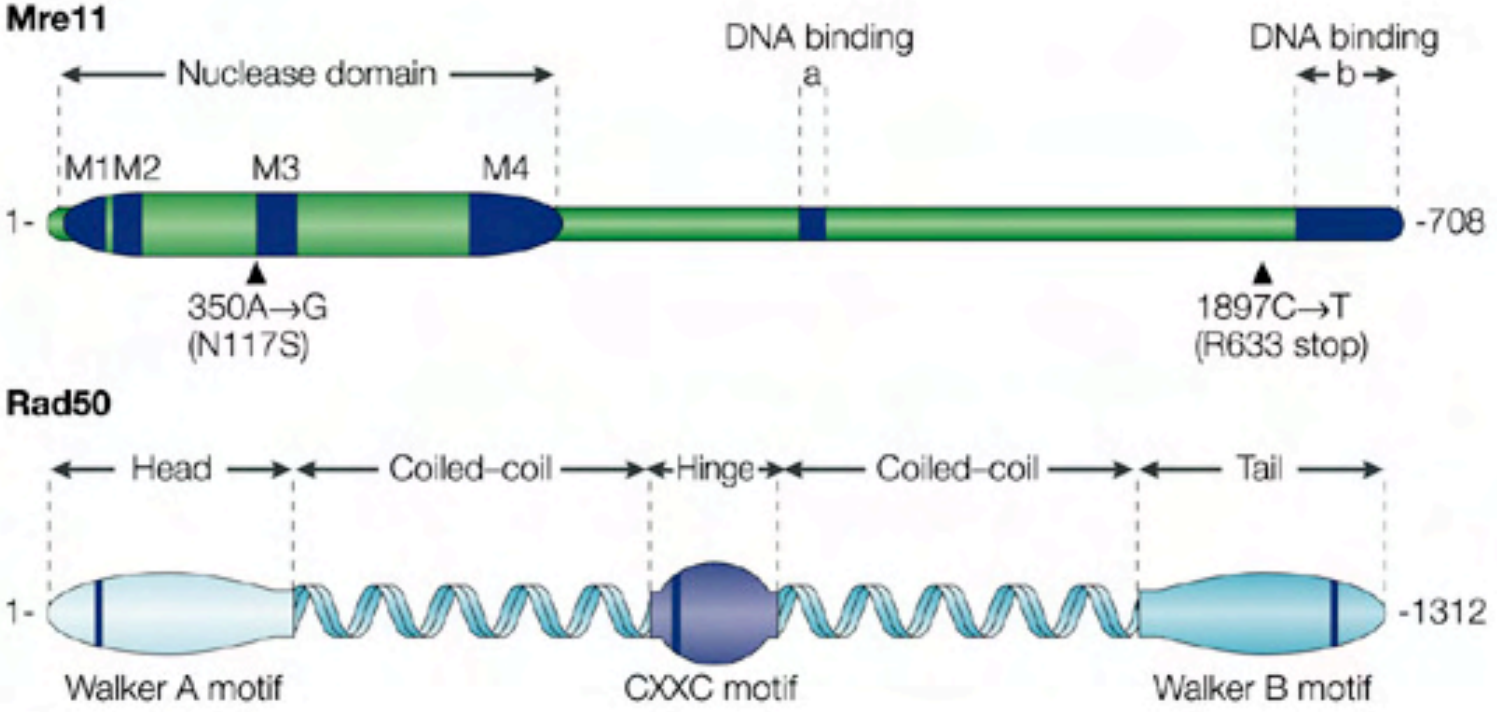
- Mouse knockouts
  - ATM knockout mice are viable.
  - MRN knockouts are very early embryonic lethal (RN).

The MRN complex: a sensor of double strand breaks

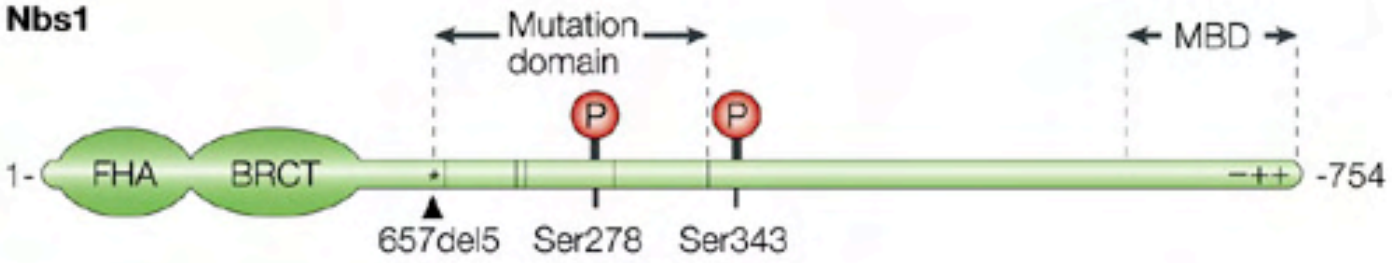


The Mre11/Rad50/NBS complex (MRN)

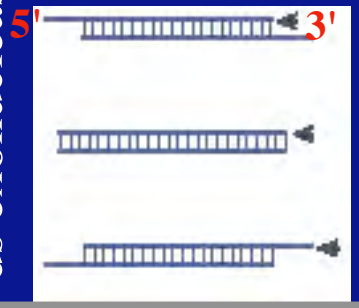
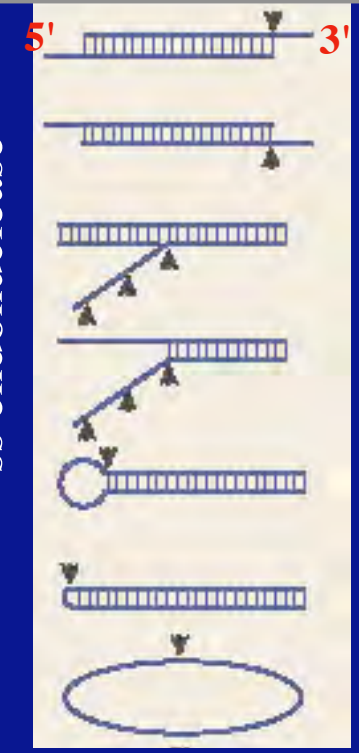
Ataxia telangiectasia like disorder (ATLD)



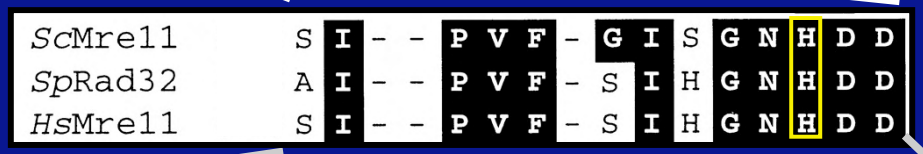
(Nijmegen Breakage Syndrome)



The Mre11 nuclease - in vitro activities

	Substrate		+ Rad50	+ NBS or XRS	In vivo role?
ds exonuclease		3' recessed	+	+	} expose microhomology?
		Blunt	+	+	
		3' overhang	-	ND	Prepare for NHEJ?
ss endonuclease		3' overhang			Prepare for NHEJ?
		5' overhang	-	ND	Prepare for NHEJ?
		3' ssDNA flap	-	ND	} Restore replication fork?
		3' ssDNA branch	+	ND	
		hairpin with loop	+	+	} V(D)J recombination?
		hairpin w/o loop	+	+	
		Closed ssDNA	+	ND	

Generation of a mouse Mre11^{H129N} nuclease deficient allele

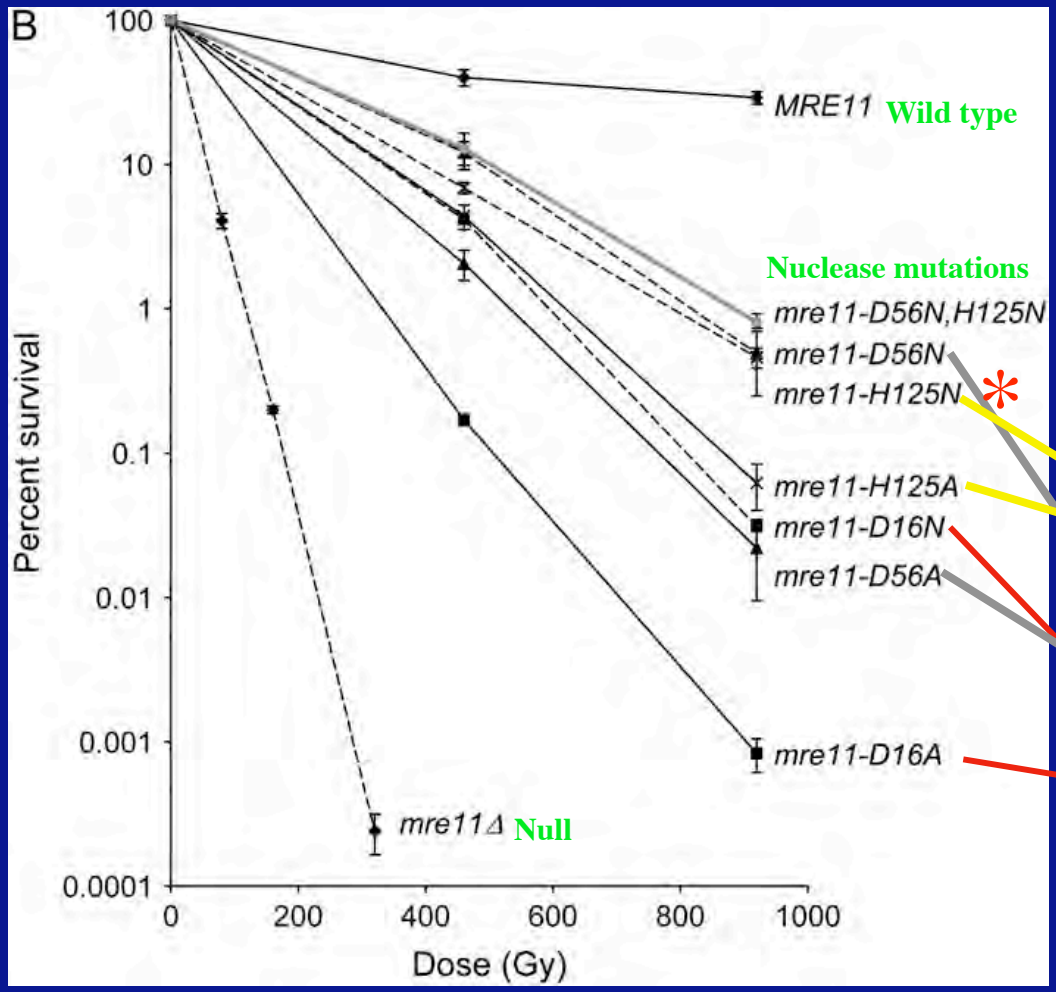


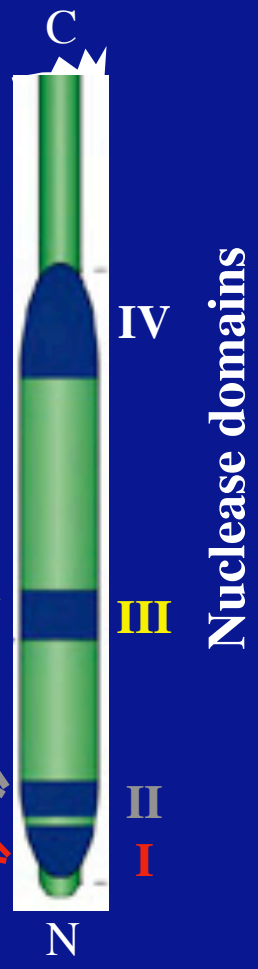
MmMre11 S I - - P V F - S I H G N H D D

CAT
↓
AAT

N = Mre11^{H129N}

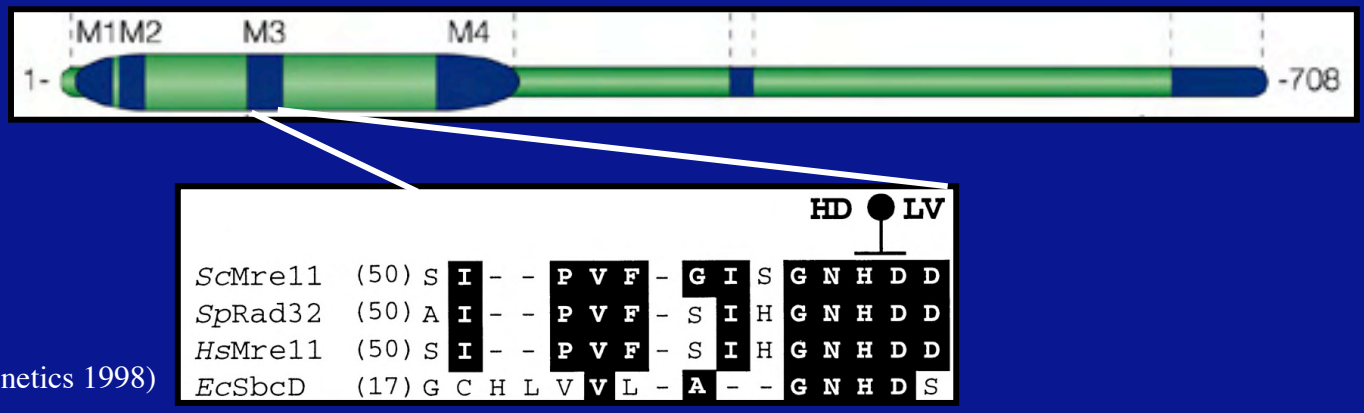
In Saccharomyces cerevisiae, point mutations in the Mre11 nuclease domains confer variable ionizing radiation hypersensitivity



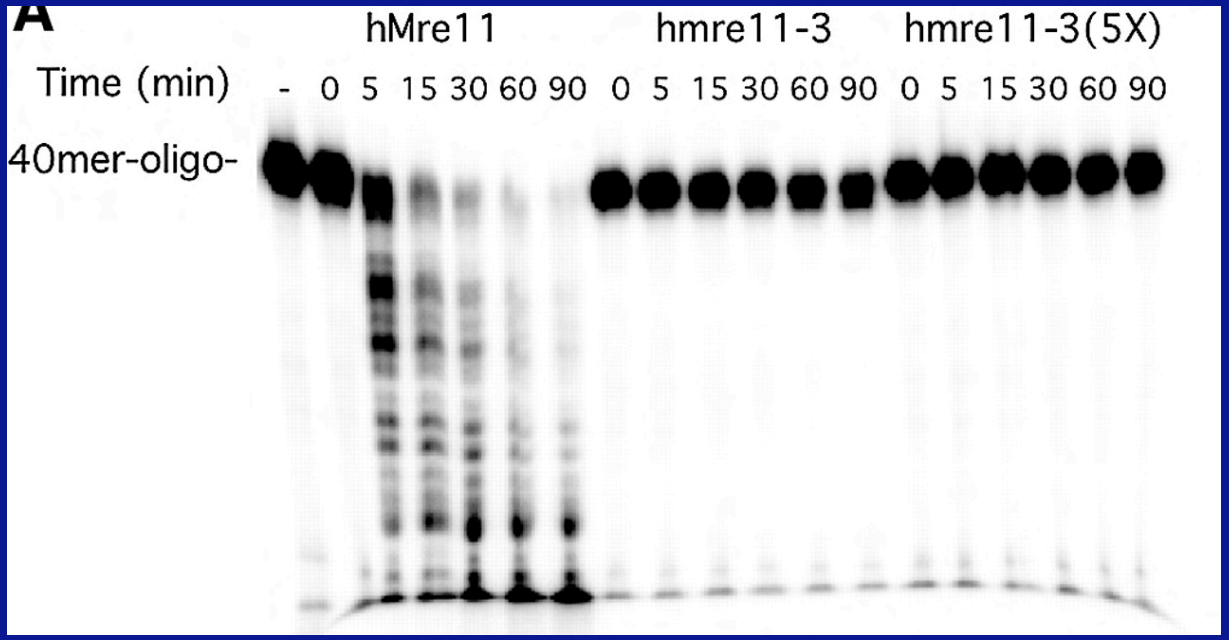


Krogh, Symington, *Genetics*, December 2005

Mutation of human Mre11 nuclease motif III disables exonuclease activity



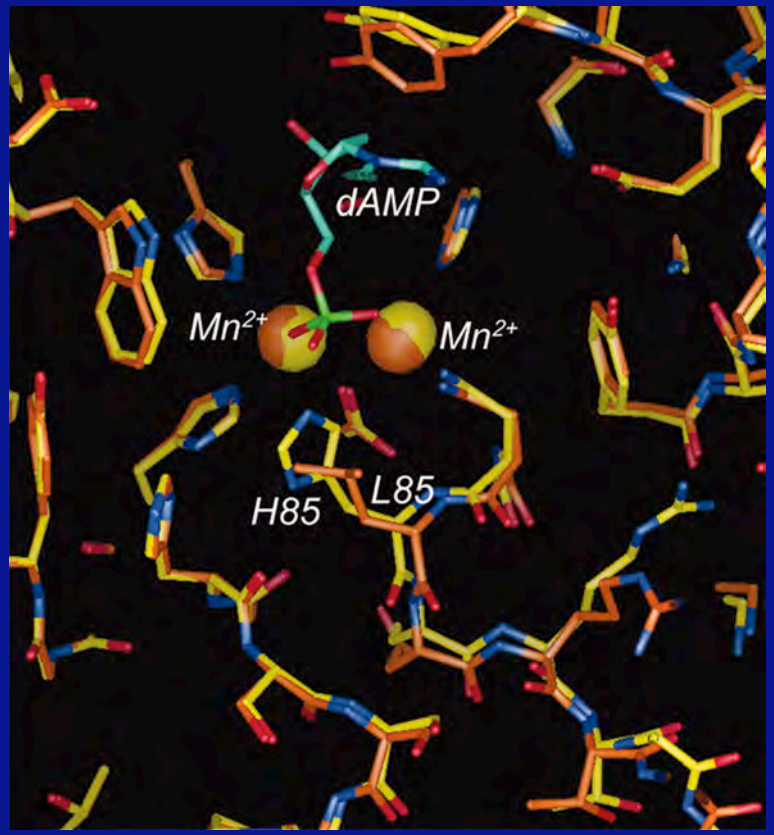
(Bressan et al. Genetics 1998)

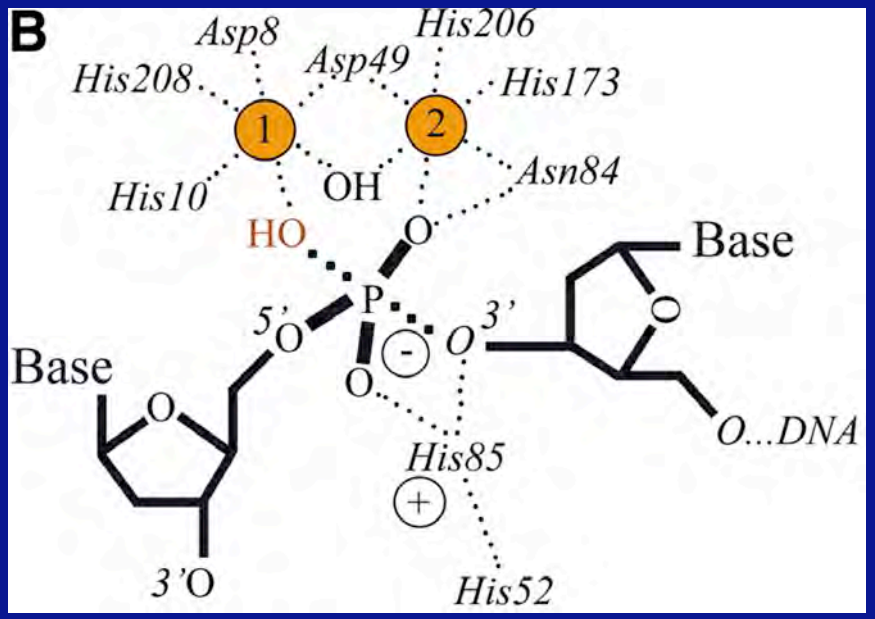


(Arthur et al., NAR 2004)

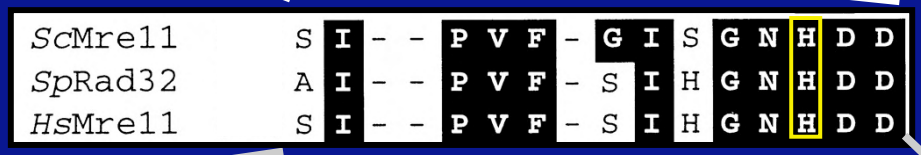
Mutation of Mre11 aa H85 (domain III) *in P. furiosus*

abrogates nuclease activity, no change to crystal structure





Generation of a mouse Mre11^{H129N} nuclease deficient allele



CAT
↓
AAT

N = Mre11^{H129N}

Why H129N?

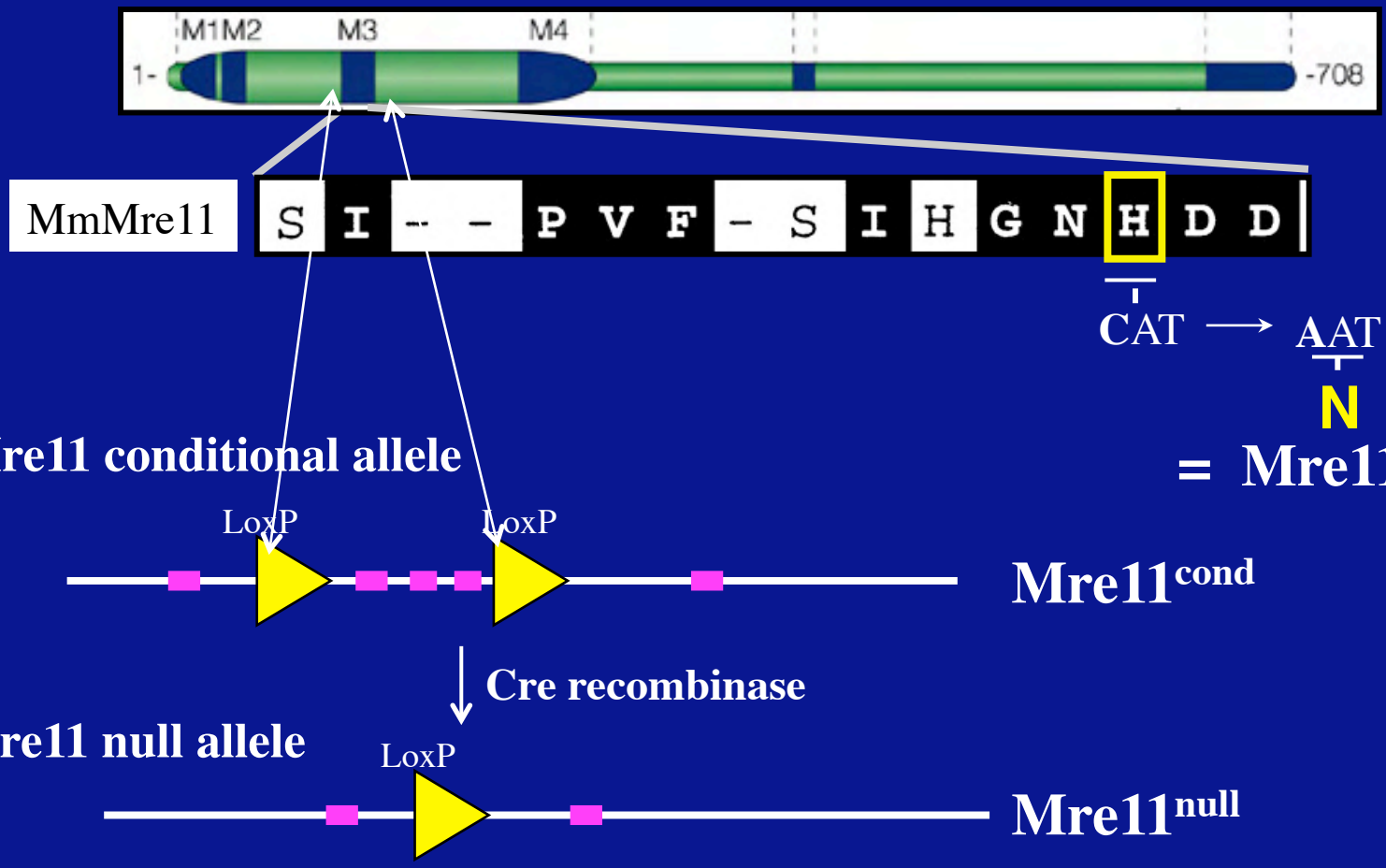
- **100% conserved in evolution.**
- **Histidine^{129} required for transesterification reaction.**
- **Similar mutations abrogate nuclease activity in vitro in yeast and human.**
- **NOT required for structure or protein-protein interactions.**
- **Same mutation in yeast causes mild IR sensitivity, blocked meiosis.**

Mre11$^{H129N/H129N}$ causes lethality early in development: No rescue by p53 deficiency

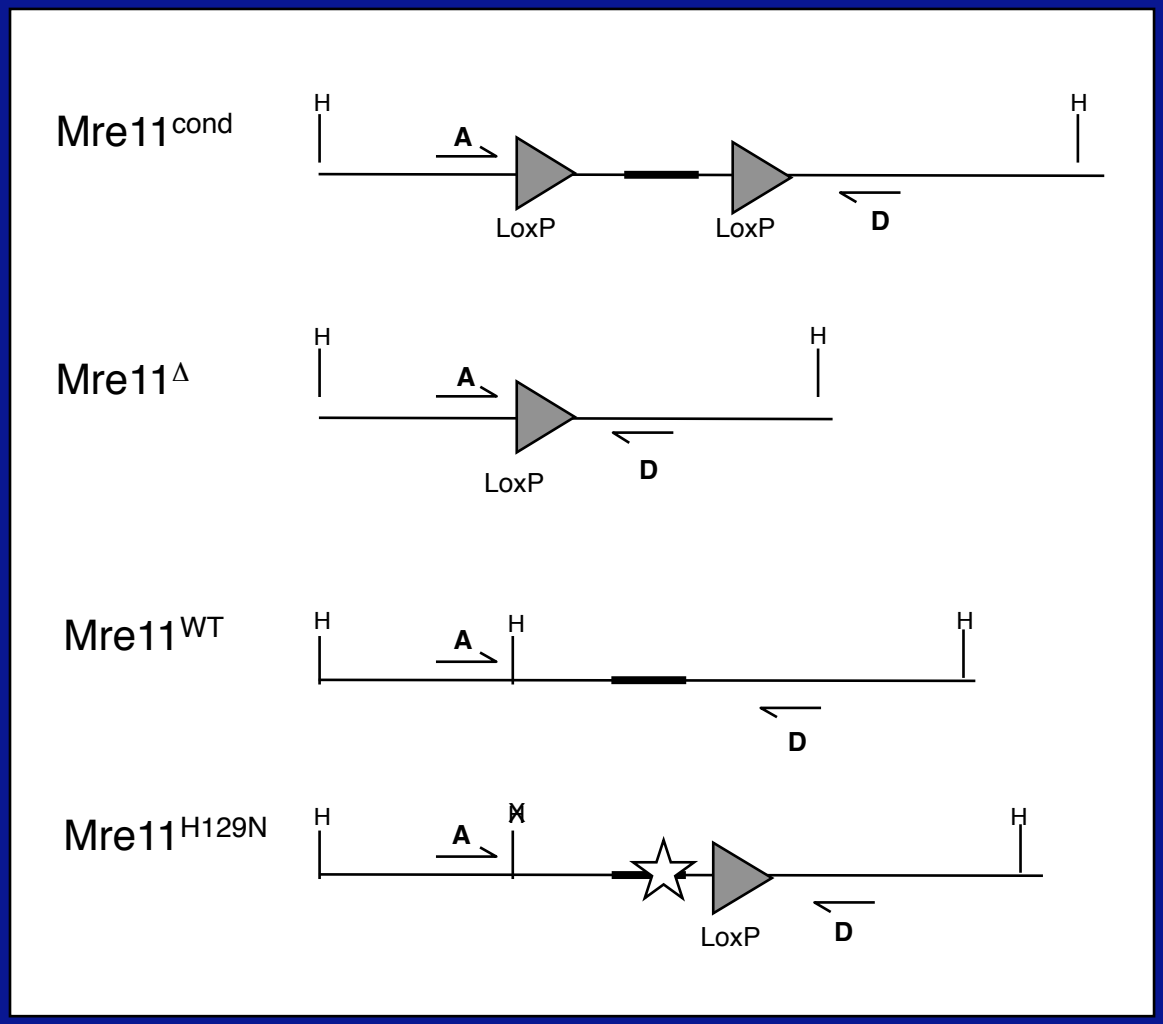
Mre11 genotype	p53 genotype		
LIVE BORN	+/+	+/-	-/-
+ / +	19	24	16
+ / H129	27	37	21
H129N / H129N	0	0	0
DAY E13.5	+/+	+/-	-/-
+ / +	5	13	9
+ / H129	18	24	12
H129N / H129N	0	0	0
DAY E9.5	+/+	+/-	-/-
+ / +	5	13	6
+ / H129	7	14	11
H129N / H129N	2	5	4

Three germline alleles of mouse Mre11

1. Mre11 nuclease deficient allele (H129N)



Four germline mouse alleles of Mre11

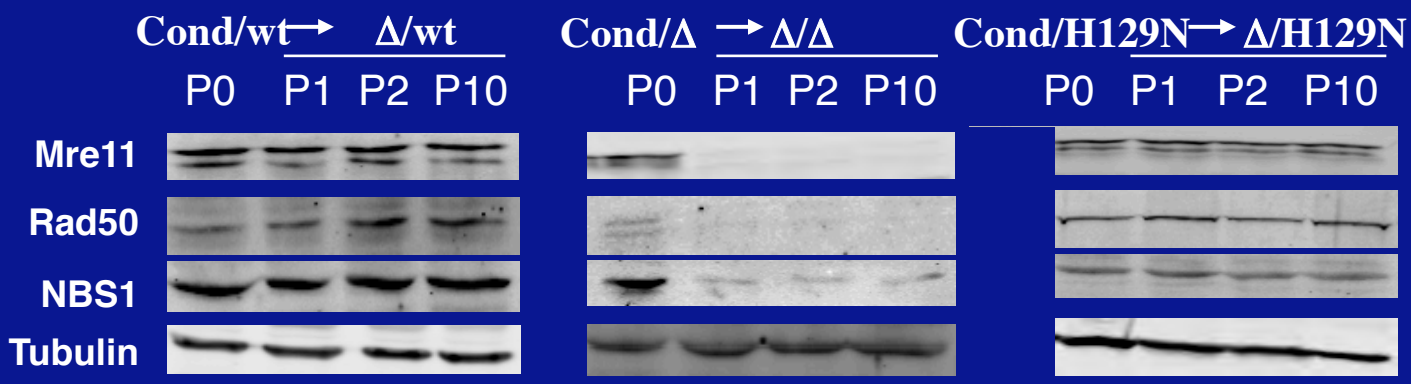


Mre11 cond / Δ

Mre11 cond / WT

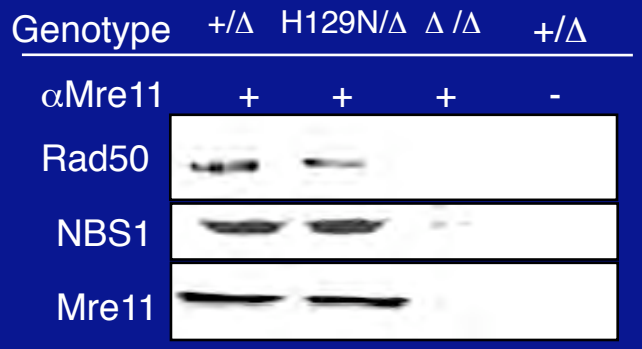
Mre11 cond / H129N

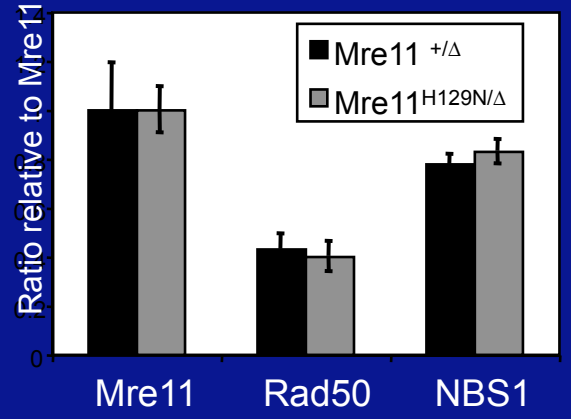
Cre delivered via adenovirus mediates conversion of $Mre11^{Cond}$ to $Mre11^{\Delta}$ in mouse embryonic fibroblasts (MEFs)



Mre11^{H129N} does not disrupt the MRN complex

A. Co-IP of endogenous proteins.



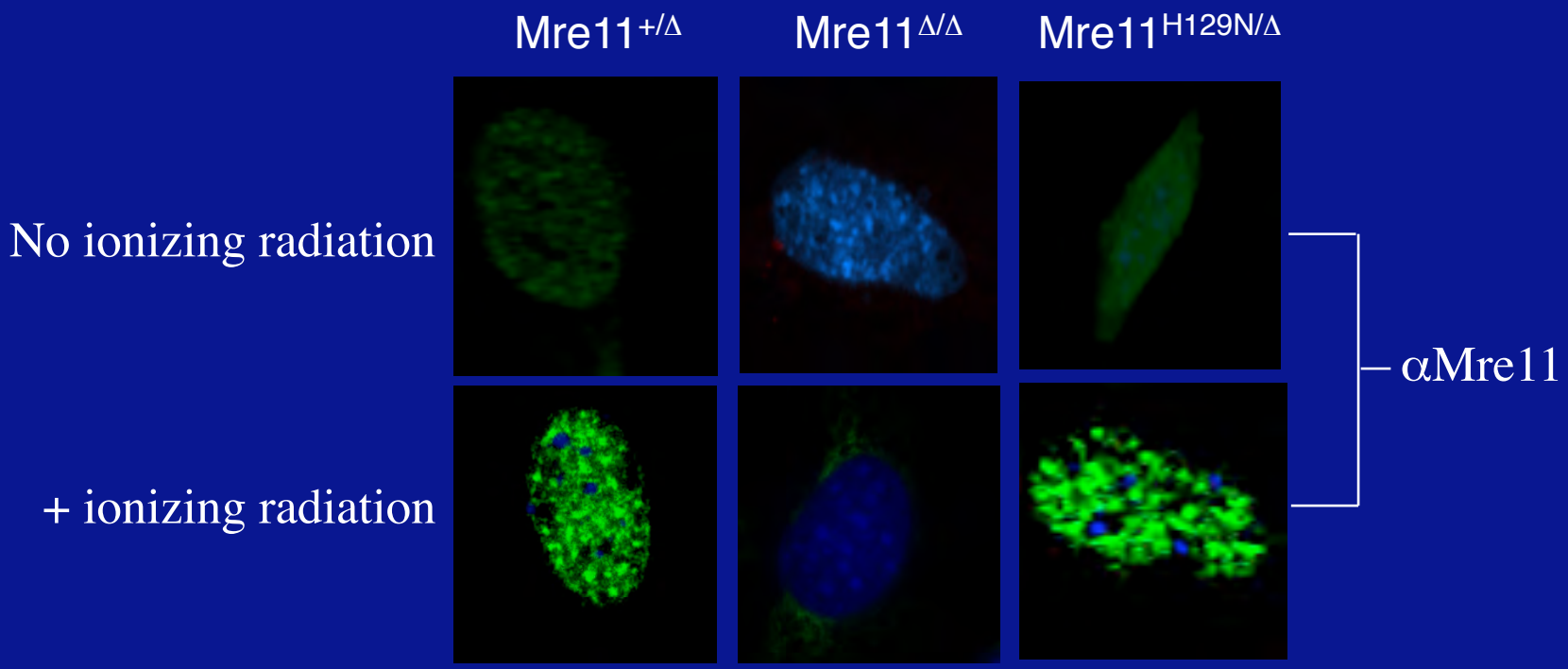


B. Two Hybrid: Mre11^{H129N} homodimerizes.

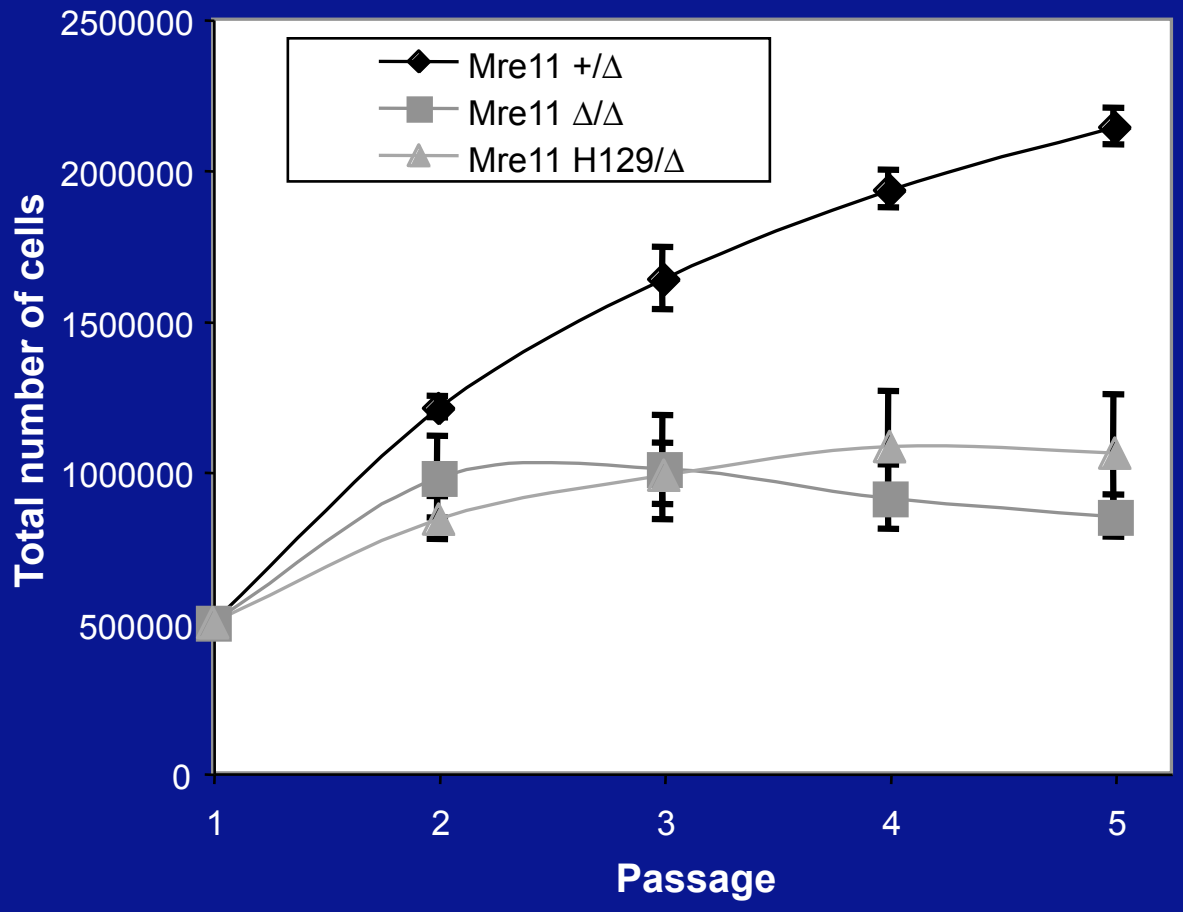
Prey	Bait	5000	500	50 cells
Mre 11	Mre 11			
Mre 11 ^{129N}	Mre 11 ^{129N}			
Mre 11	no insert			
no insert	Mre 11			

The Mre11^{H129N} protein can form IR induced foci

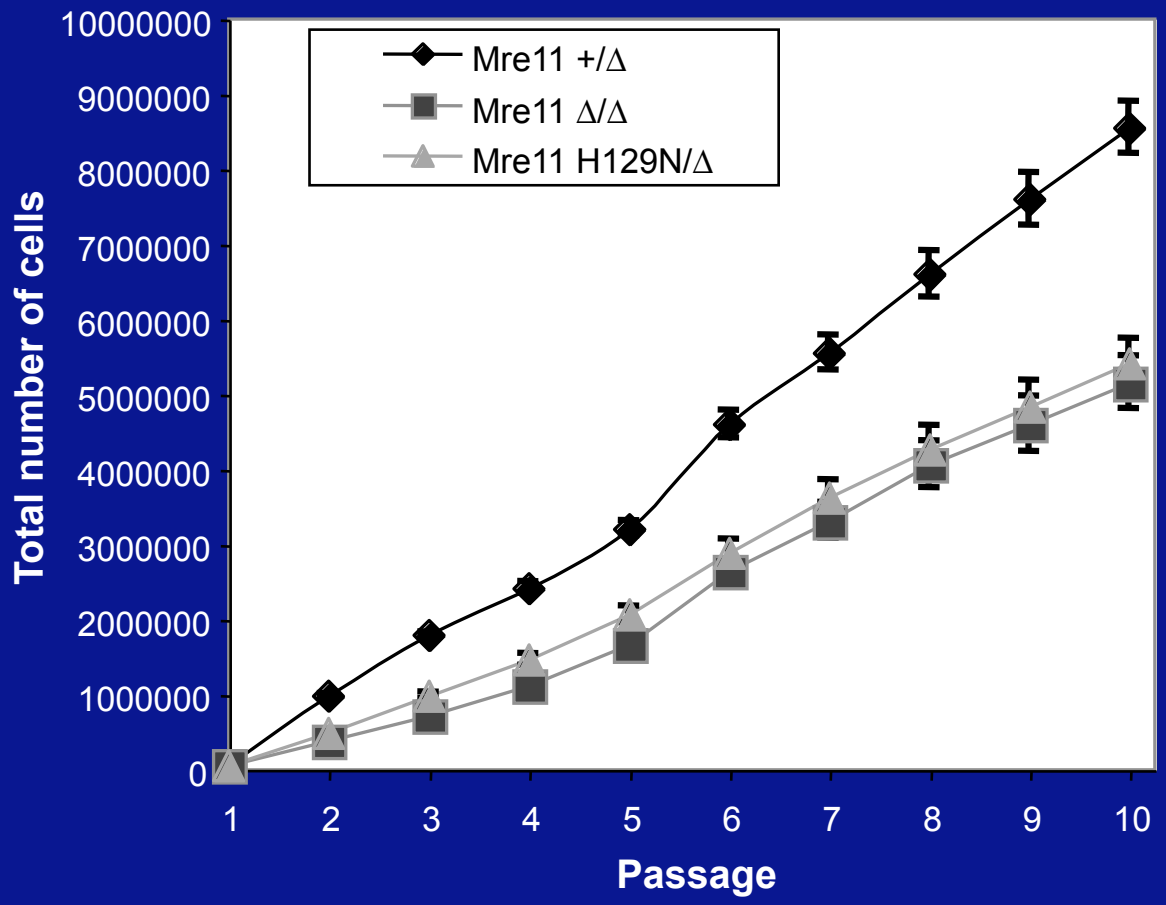
Implication: Nuclease deficiency does not impair localization to DSBs



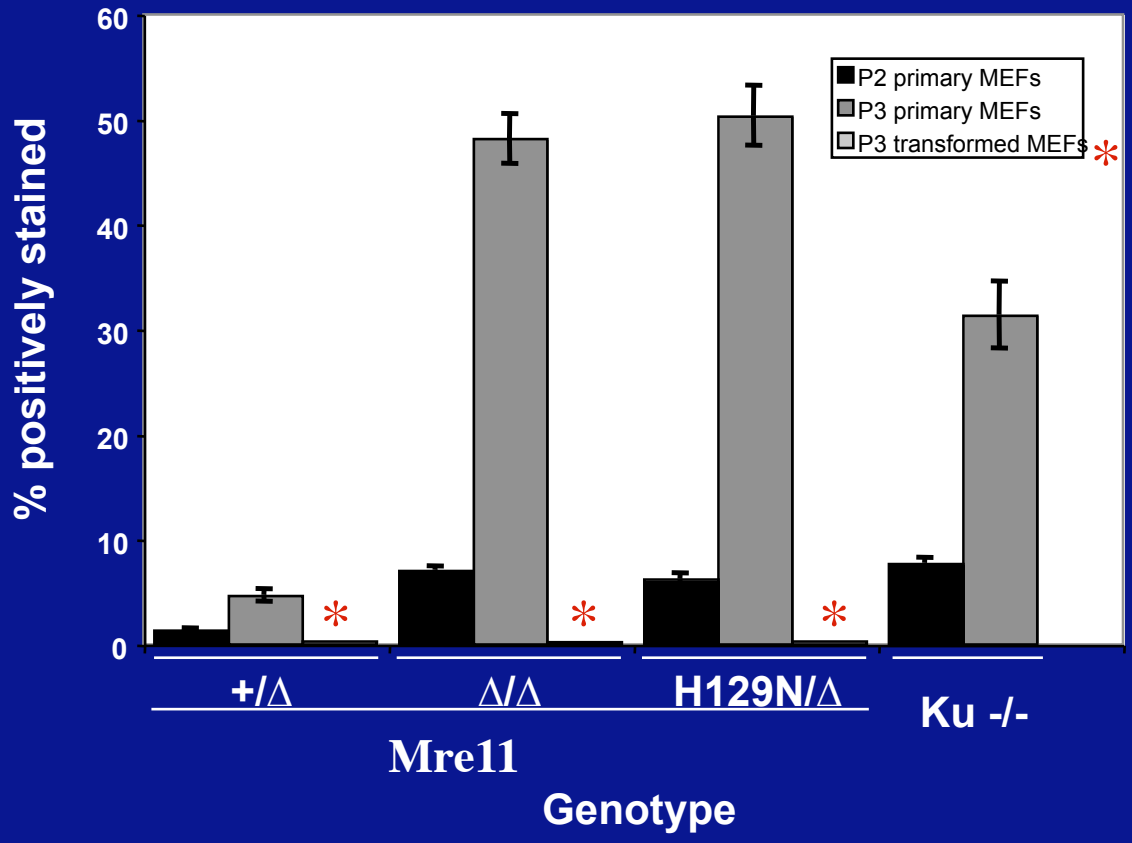
Severe proliferation defect in primary MEFs conferred by MRN loss or Mre11 nuclease deficiency



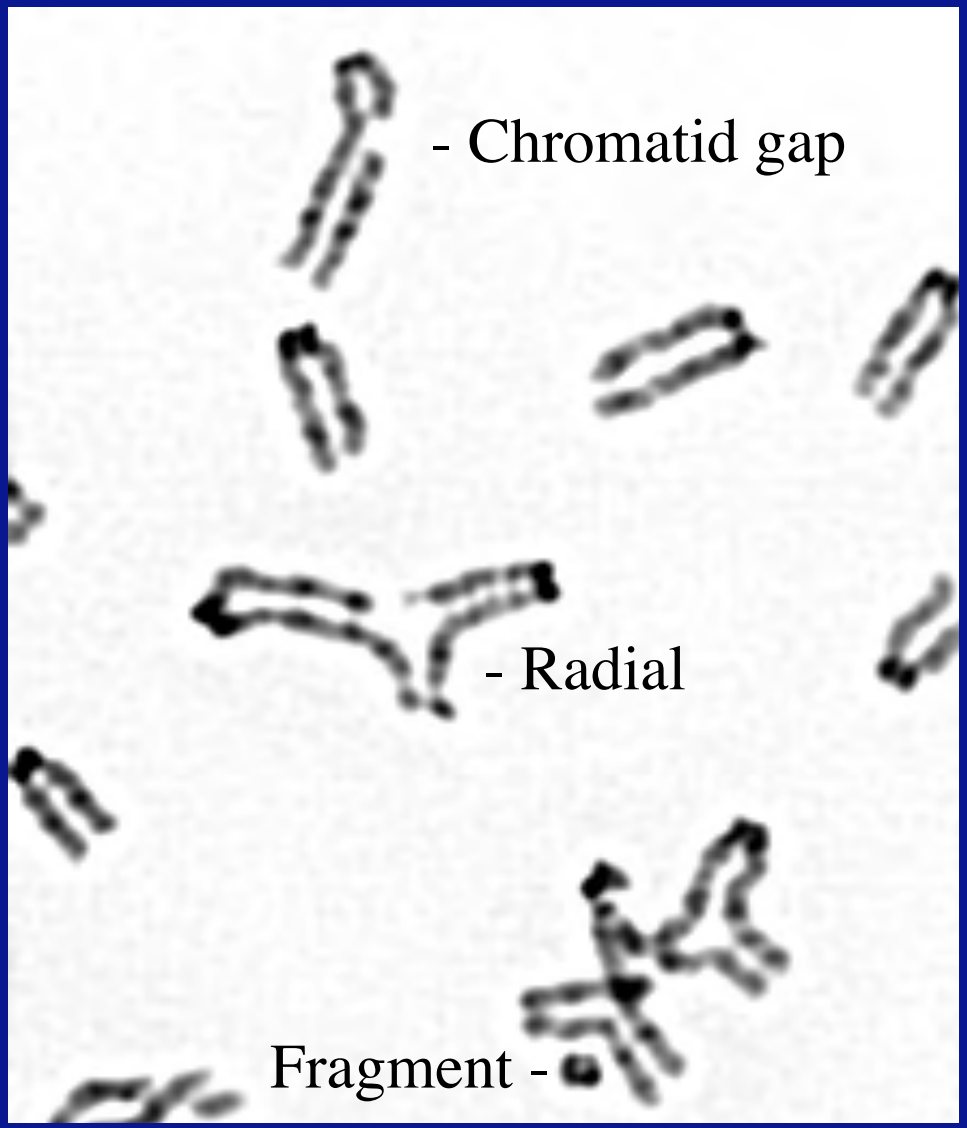
Significant rescue of proliferation defects by large T antigen immortalization



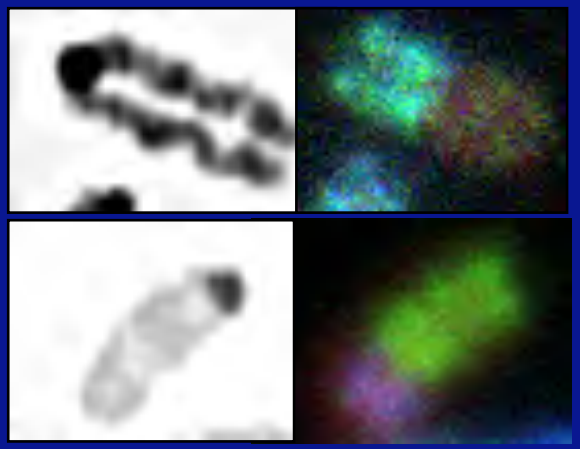
Proliferation defects are associated with premature senescence (β-gal staining)



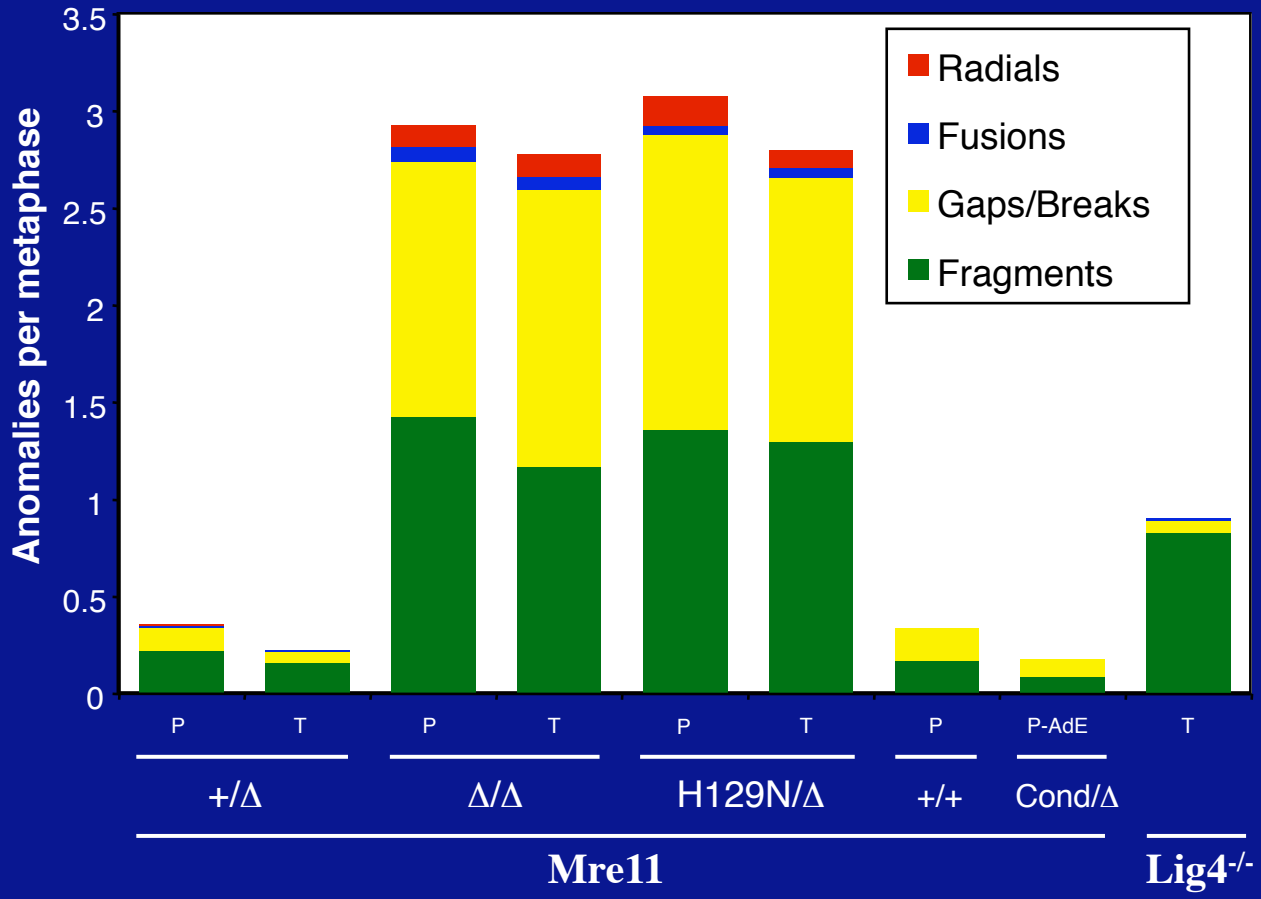
Dramatic chromosome instability in Mre11 null MEFs



Translocations (SKY)



Genomic instability: loss of MRN versus Mre11 nuclease



Radial

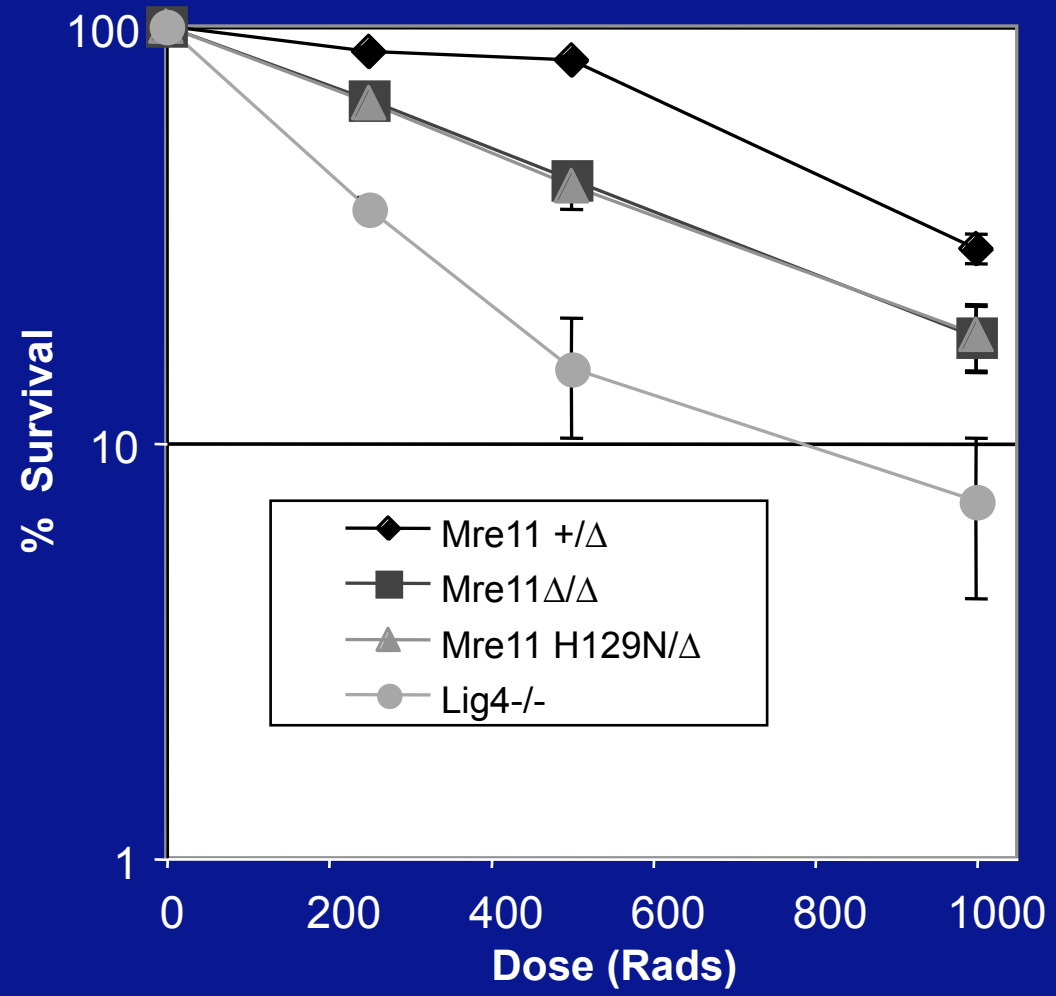
Fusion

Gap /break

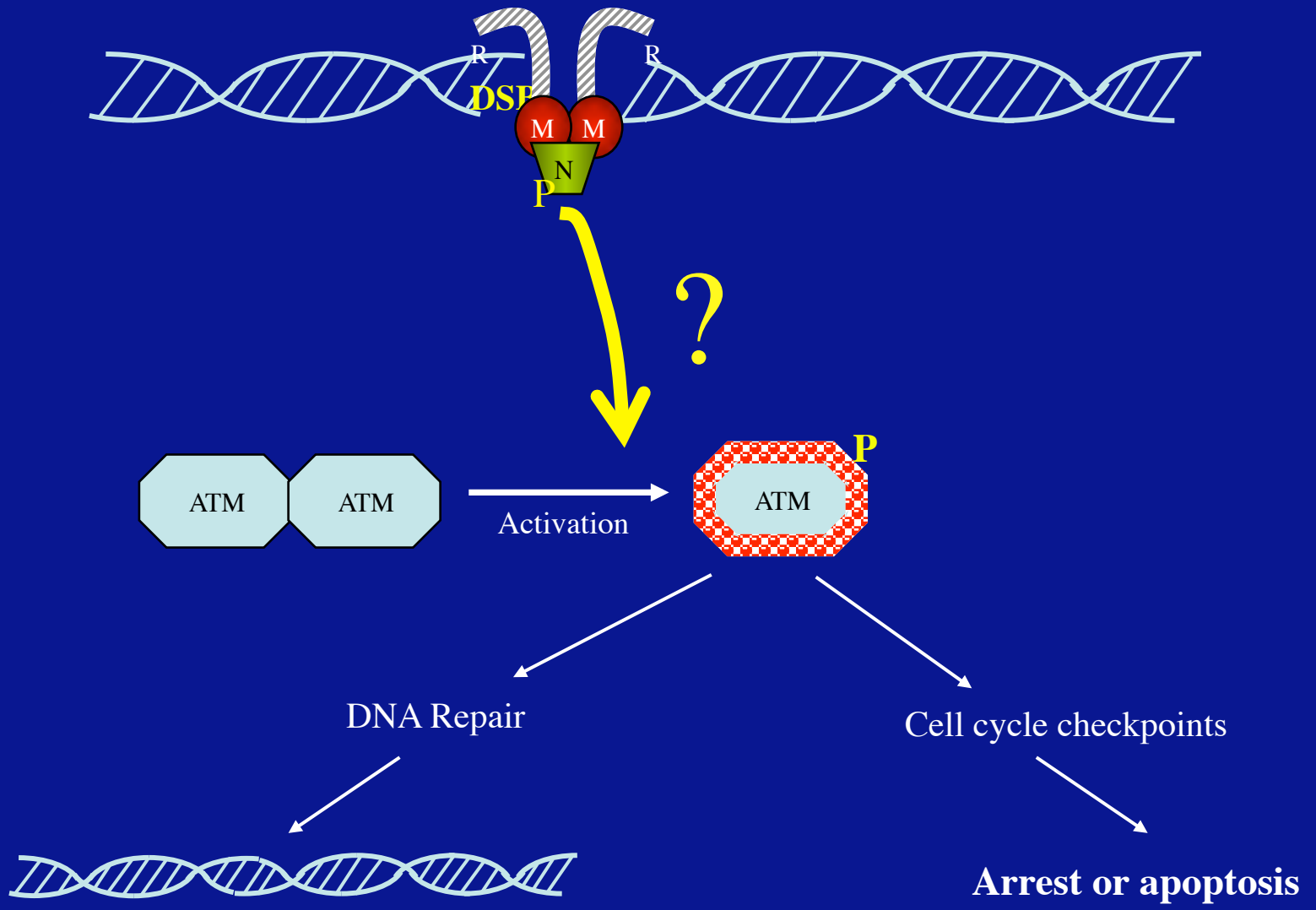
Fragment

- Mre11 nuclease deficiency phenocopies loss of entire MRN complex.
- Genomic instability not impacted by cellular transformation.
- Genomic instability conferred by Mre11 and Lig4 deficiencies are distinct;
  - Mre11 deficiencies more severe and feature gaps, radials, fusions.

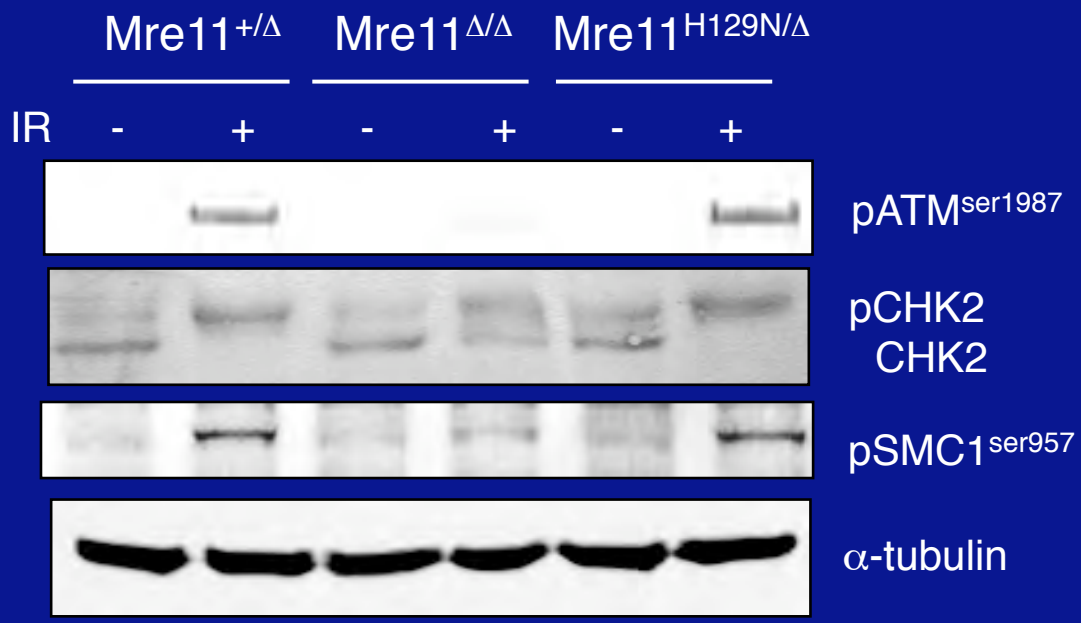
Mre11$^{\Delta/\Delta}$ and Mre11$^{H129N/\Delta}$ confer equivalent hypersensitivity to IR



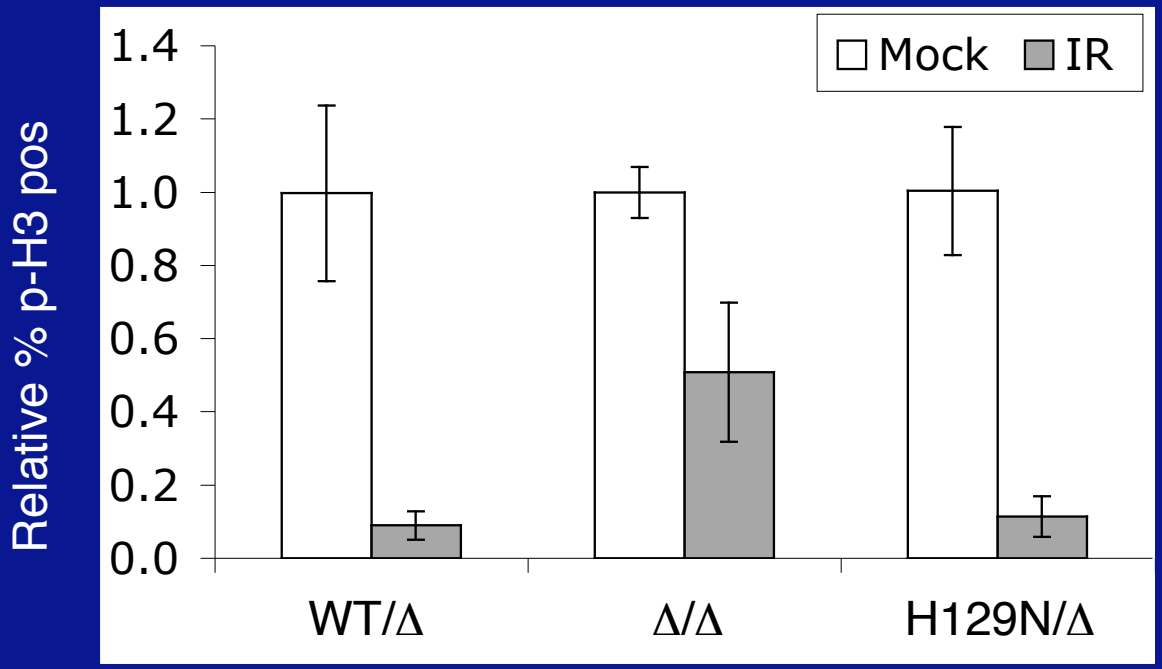
Does the nuclease deficient $M^{H129N}RN$ complex activate ATM?



Mre11 nuclease deficiency does not impact ATM activation induced by ionizing radiation



Intact G2/M checkpoint in Mre11 nuclease deficiency
(staining for phospho-histone H3)



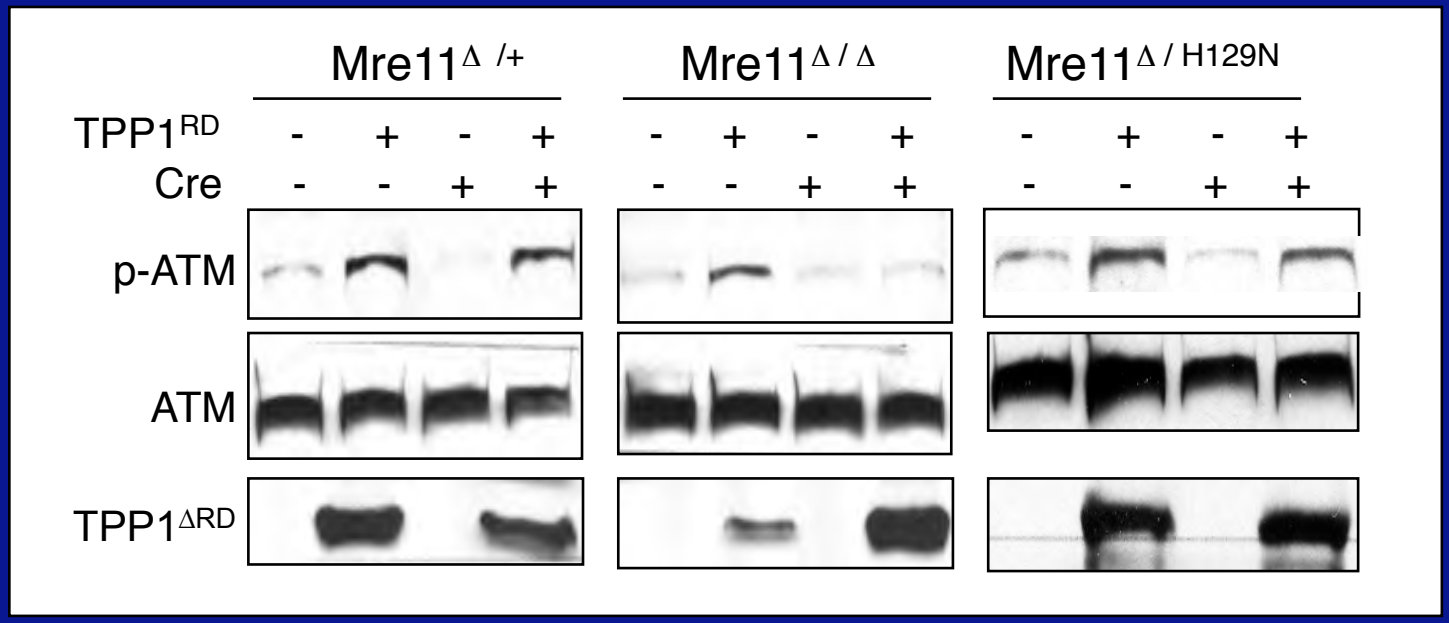
Nuclease activities of mammalian Mre11 provide an essential function distinct from MRN control of IR induced ATM activation

Next question:

Do our observations hold in the absence of ionizing radiation?

ATM activation induced by dysfunctional telomeres depends on the MRN complex, but not Mre11 nuclease activity

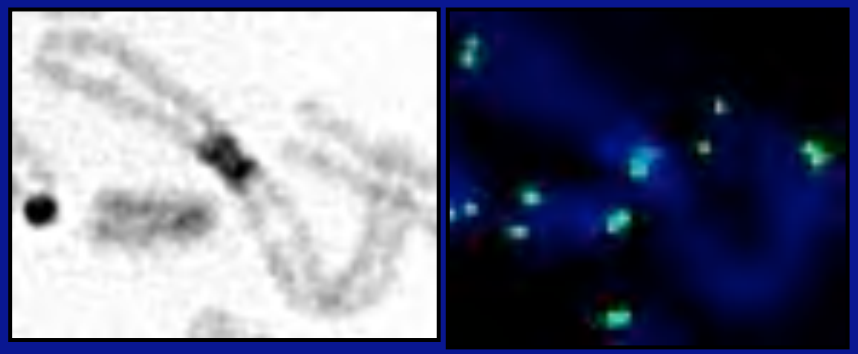
(transfection of dominant negative TPP1 allele)



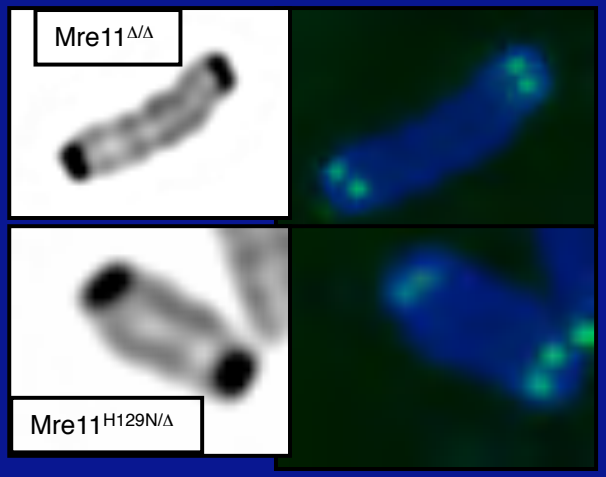
Fusions in Mre11 deficiencies do not involve telomeres

- MRN is not a Shelterin component -

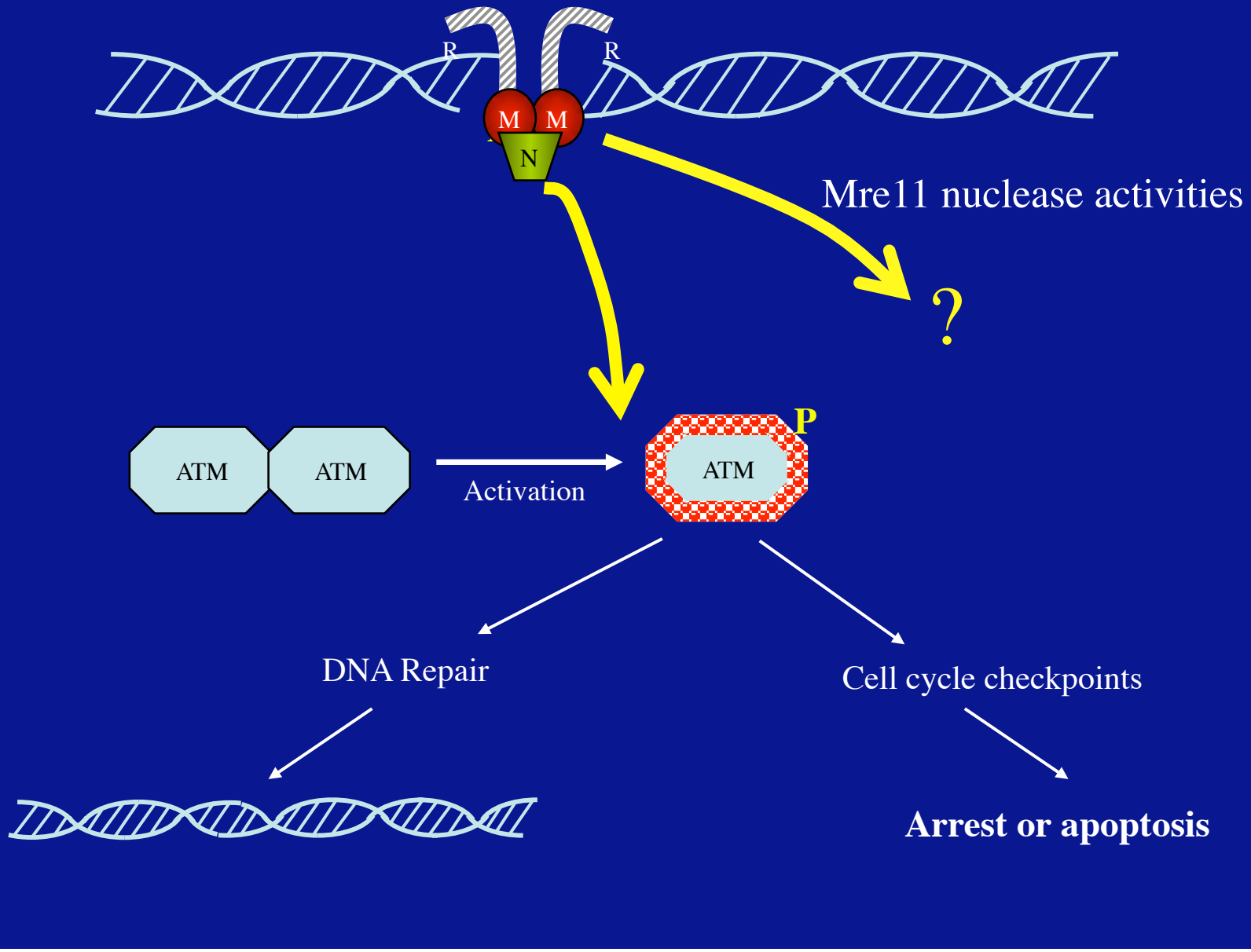
TPP1$^{acd/acd}$



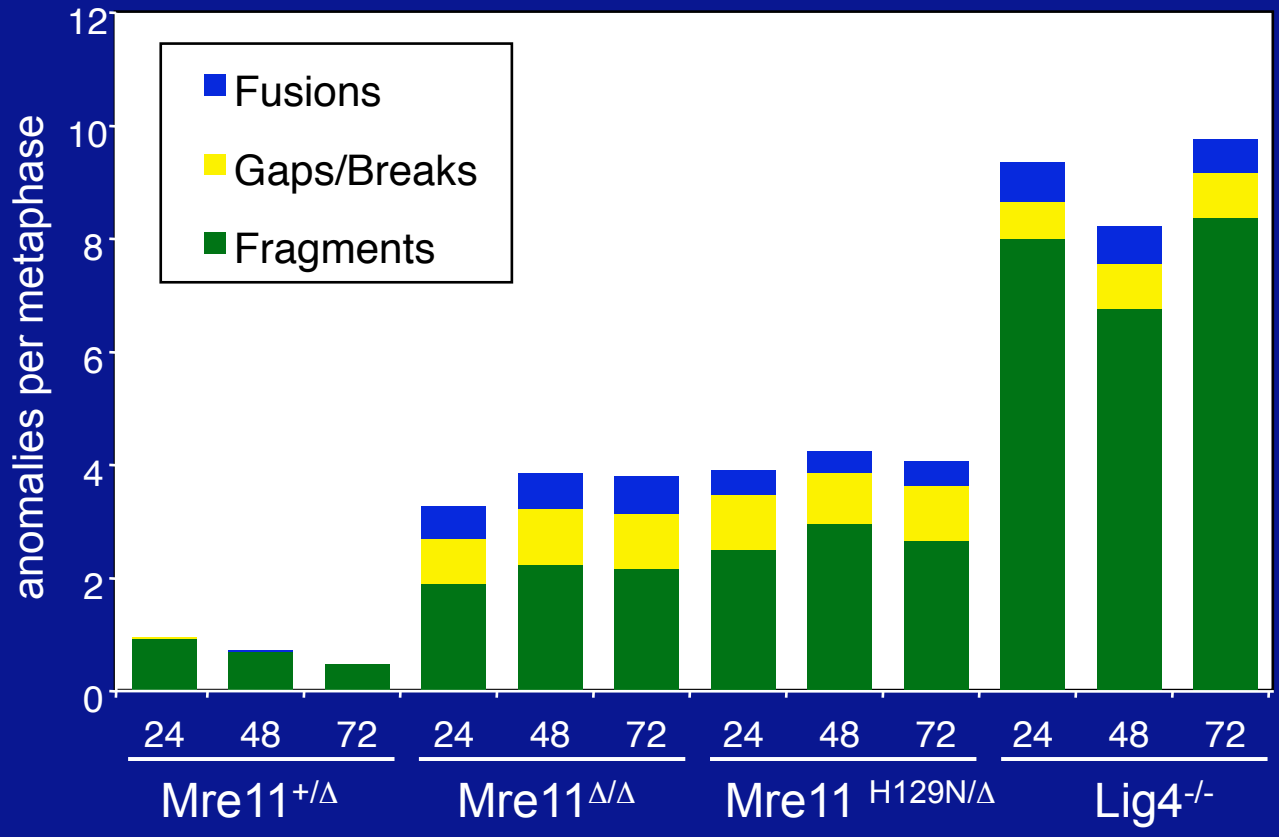
Mre11$^{\Delta/\Delta}$ or Mre11$^{H129N/\Delta}$



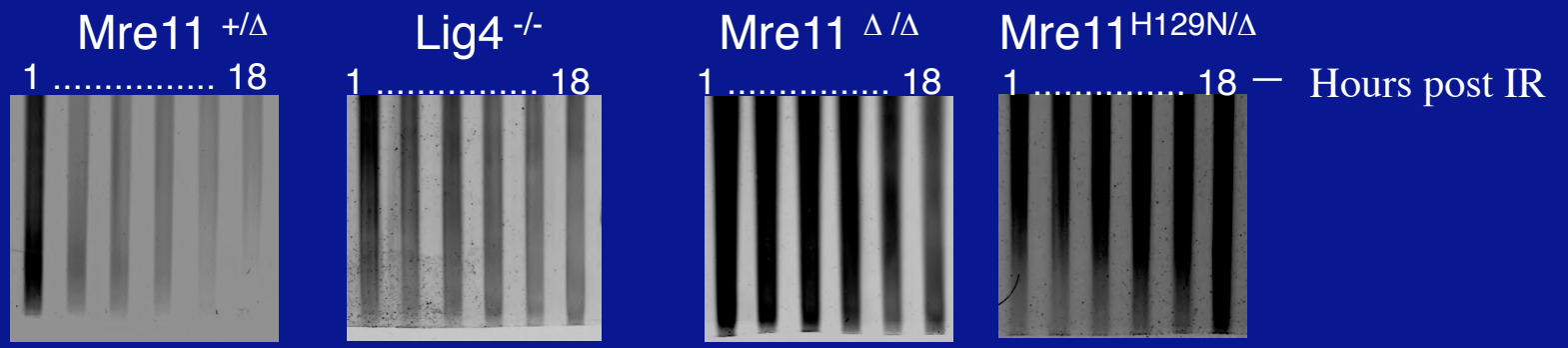
What are the nuclease activities of Mre11 doing?

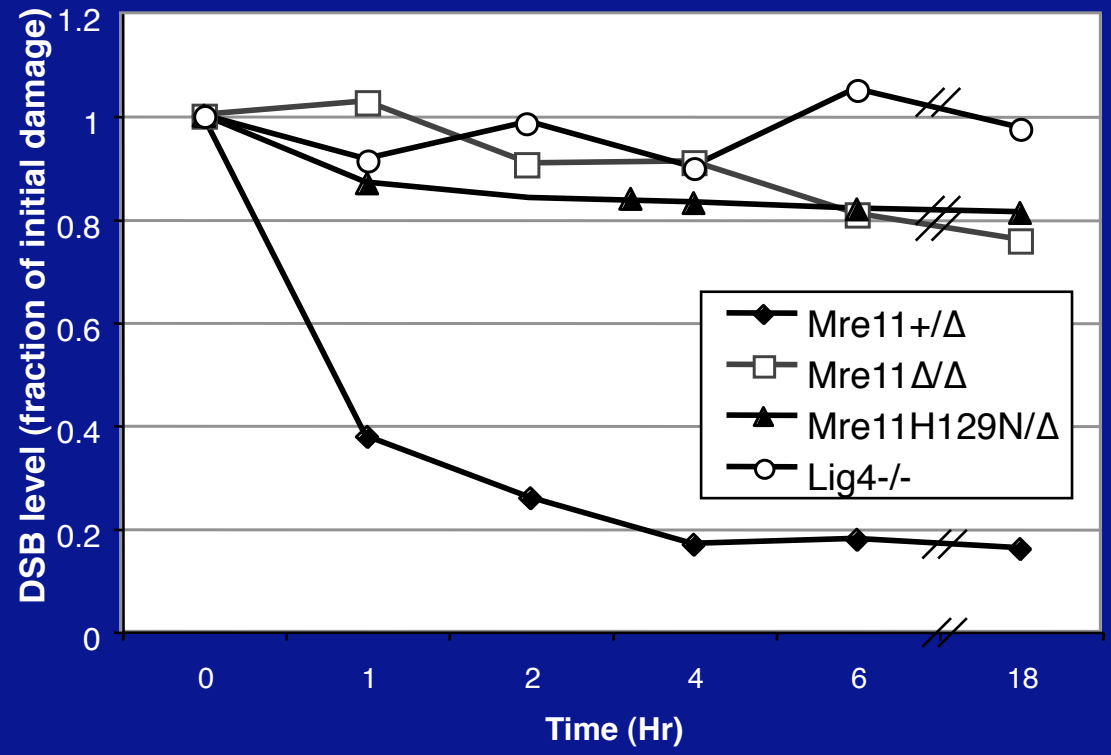


Persistence of chromosome anomalies after IR
- suggesting a severe DSB repair defect? -

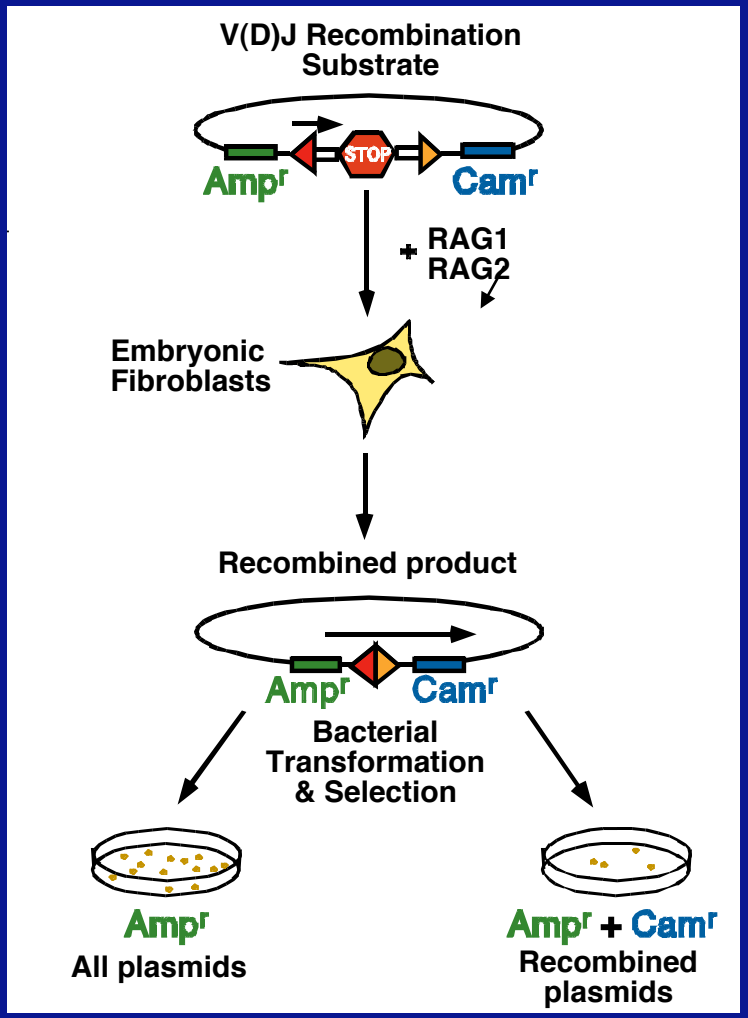


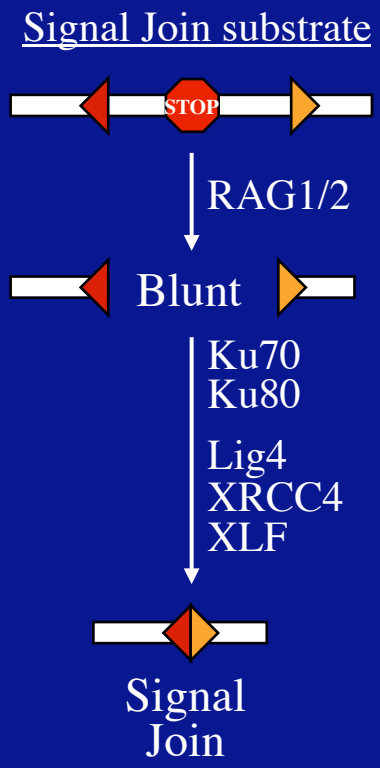
Pulsed field gel DNA repair assay; Absence of MRN or Mre11 nuclease activities cause defective double strand break repair

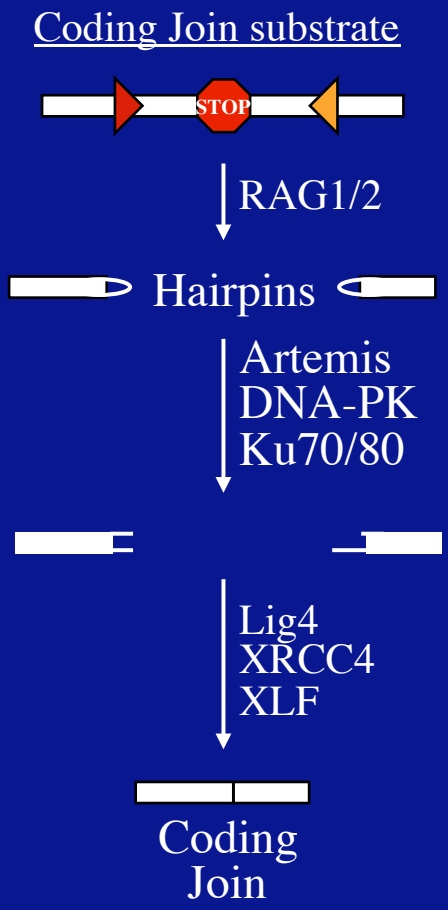




Does mammalian MRN function in NHEJ?
V(D)J recombination - transient transfection assay



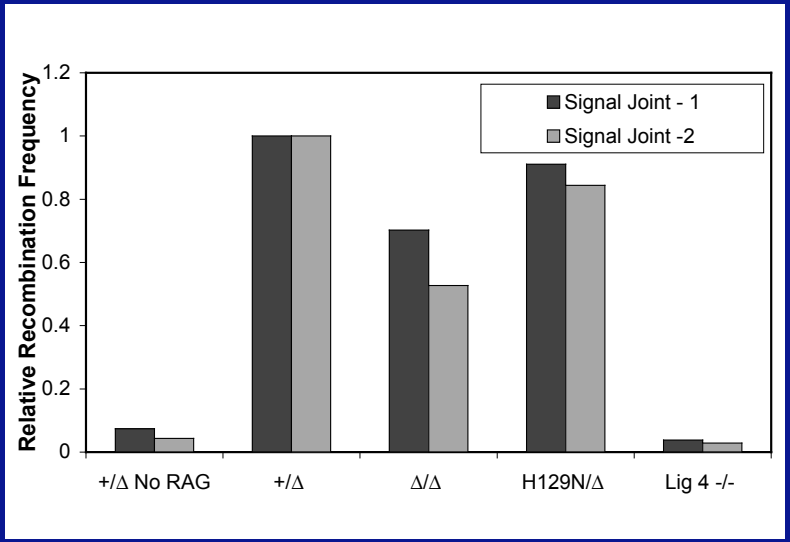




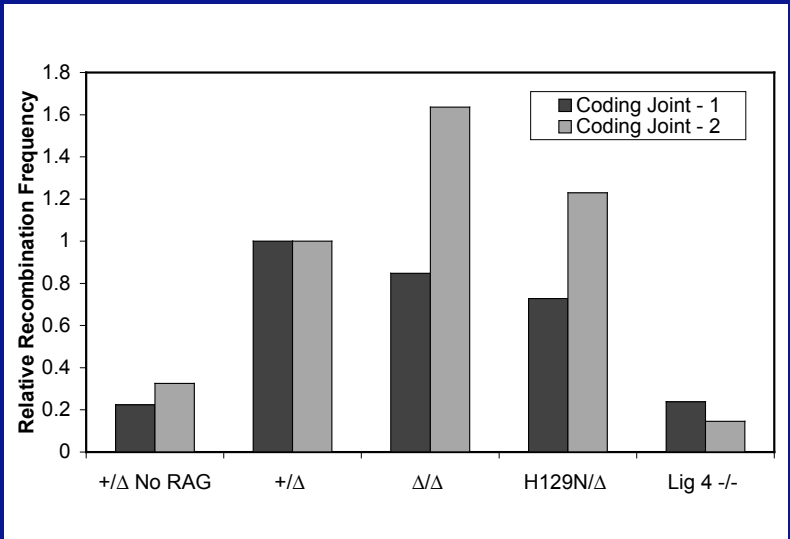
Mre11 and non-homologous end joining;

No impact of Mre11 deficiency on plasmid based V(D)J recombination

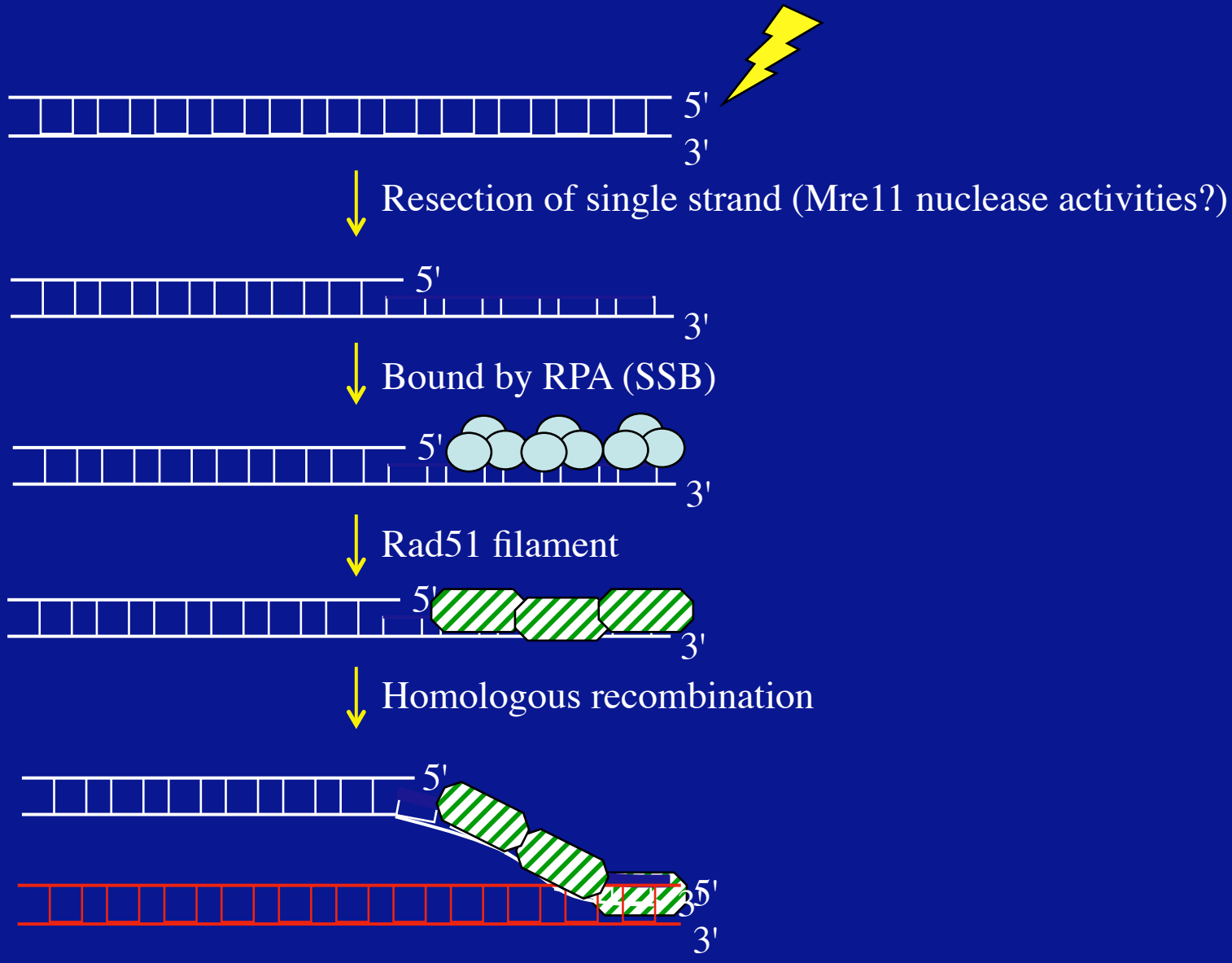
Signal joins - blunt ends



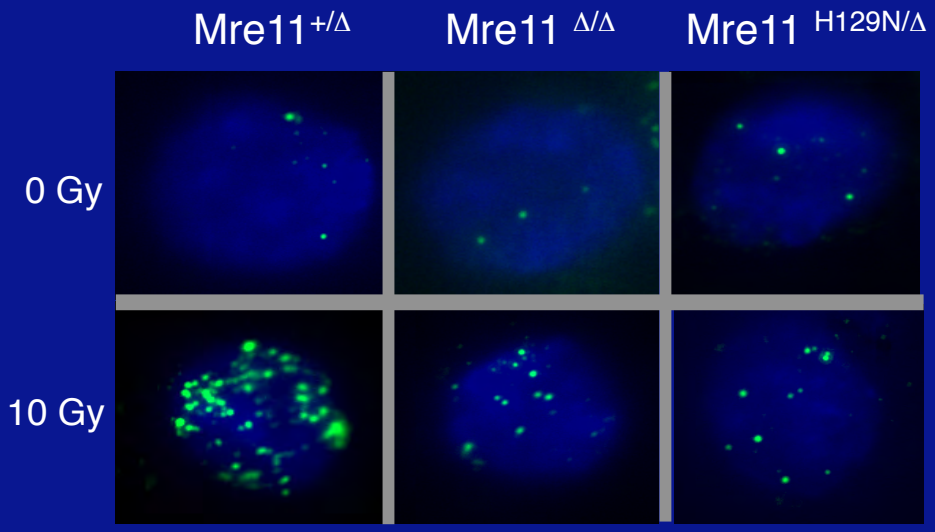
Coding joins - hairpin ends

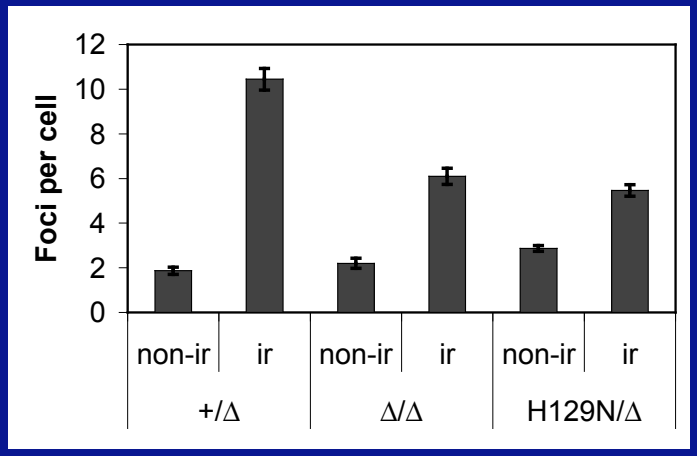


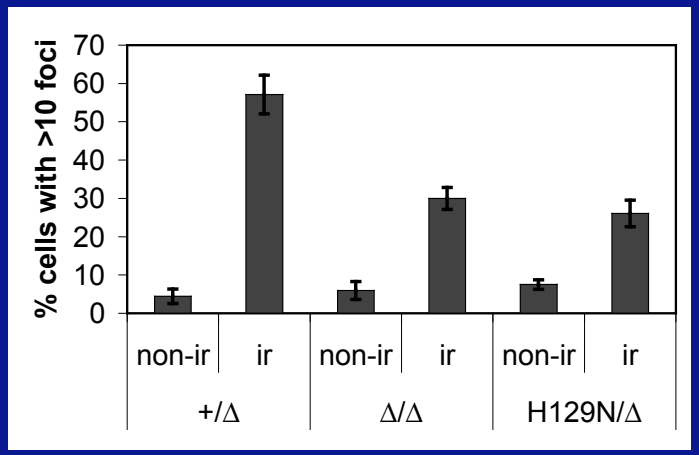
Mre11 nuclease activities and homologous recombination?



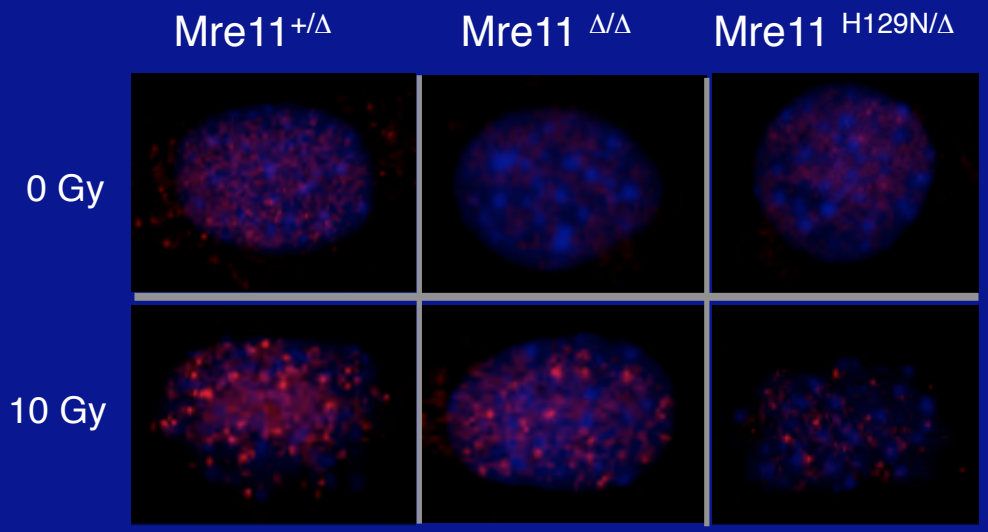
Reduced RPA focus formation in MRN or Mre11 nuclease deficiency

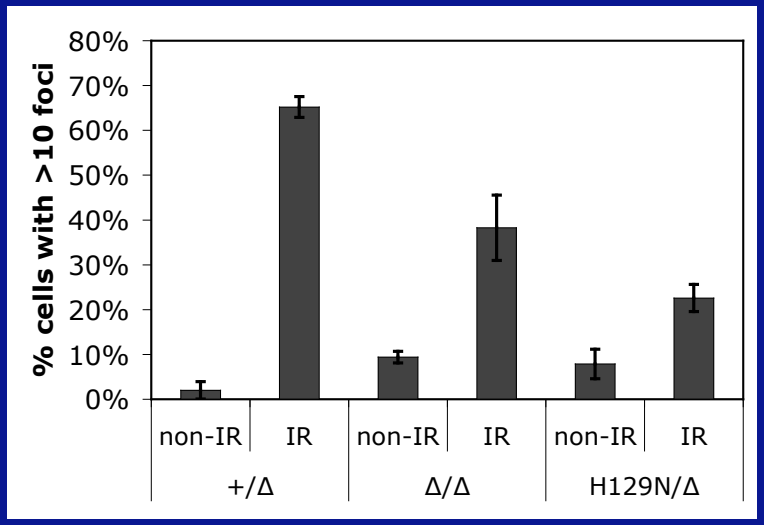




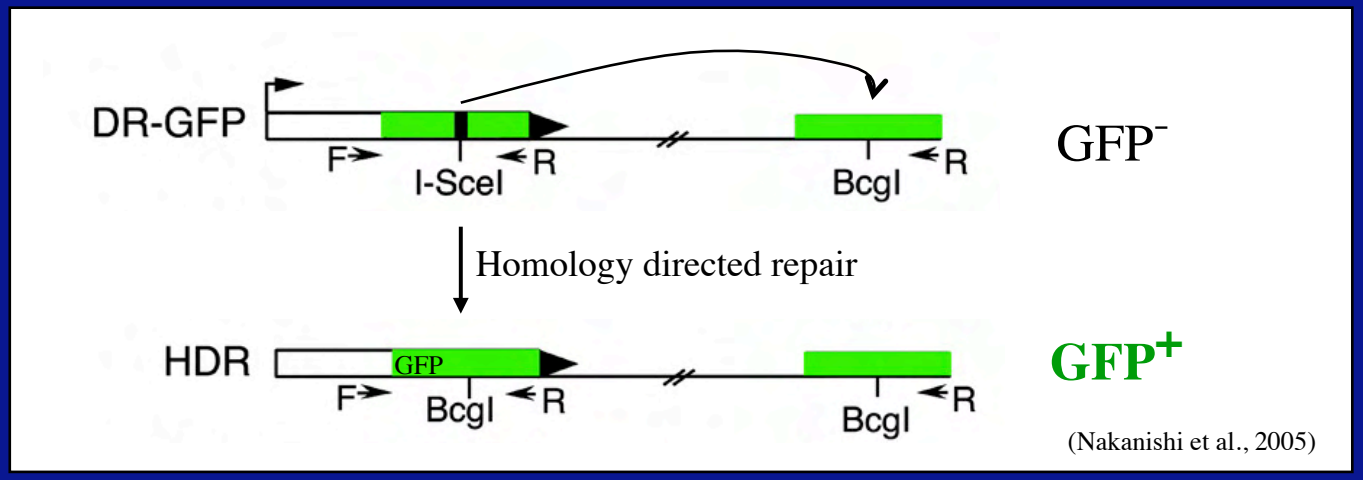


Reduced Rad51 focus formation in MRN or Mre11 nuclease deficiency



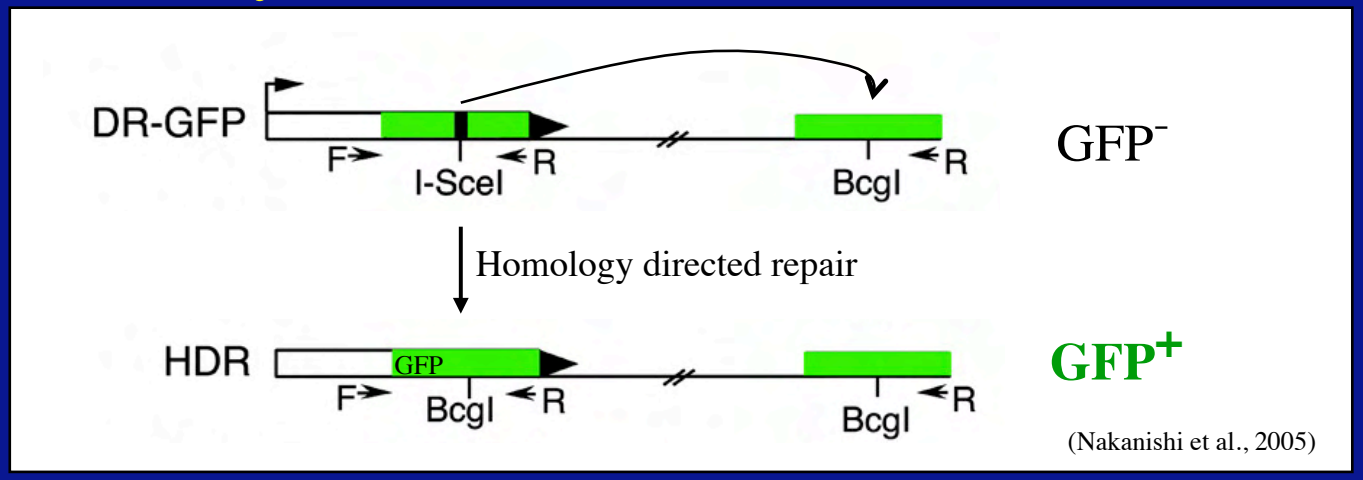


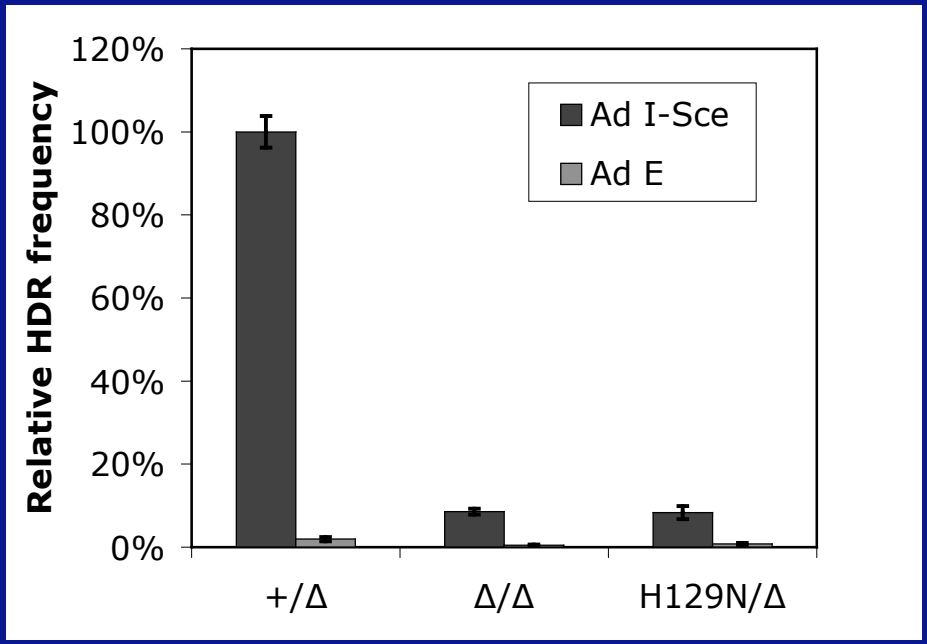
Direct assessment of homology directed repair



(Nakanishi et al., 2005)

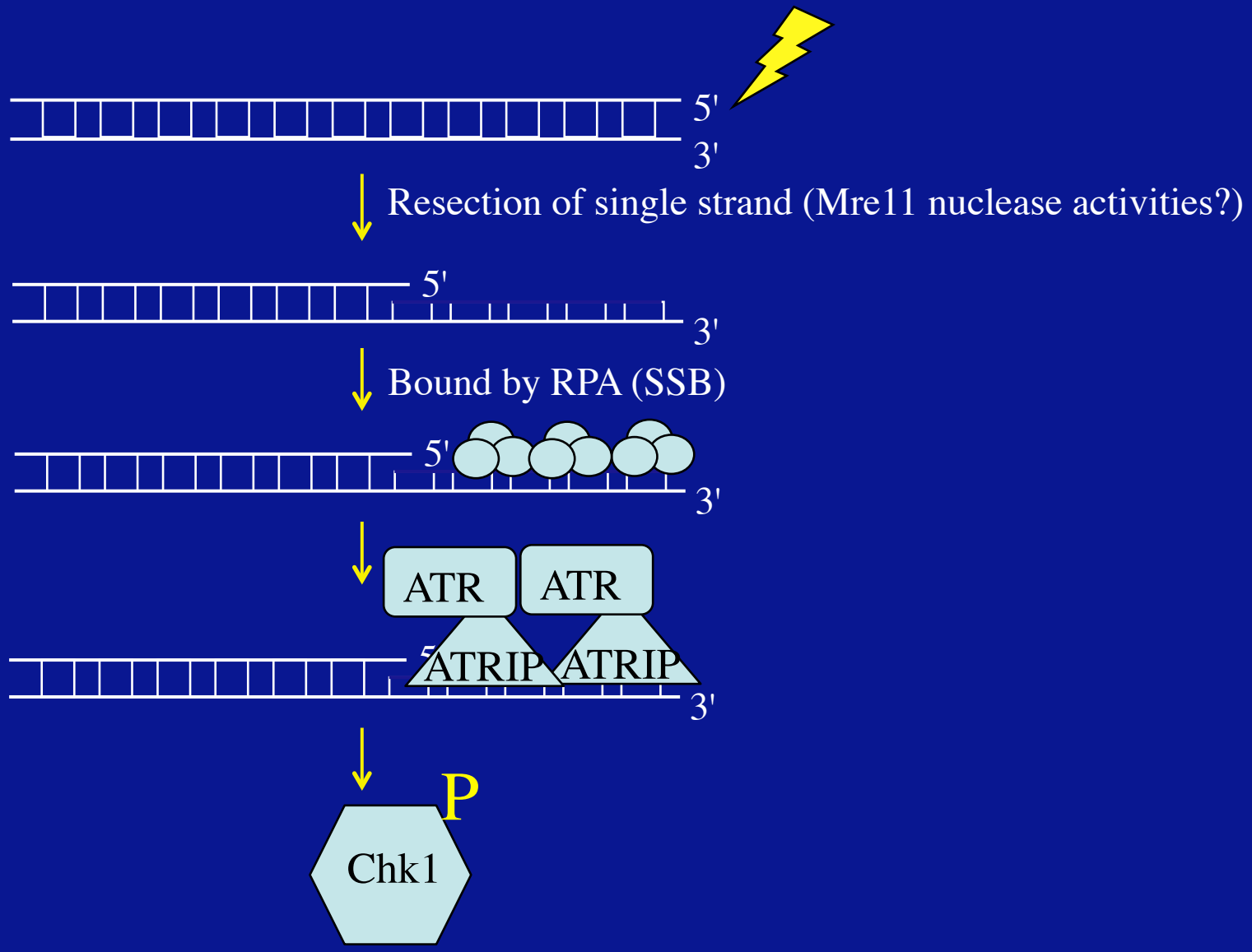
Dramatic reduction in homology directed repair conferred by deficiency of MRN or of Mre11 nuclease activities





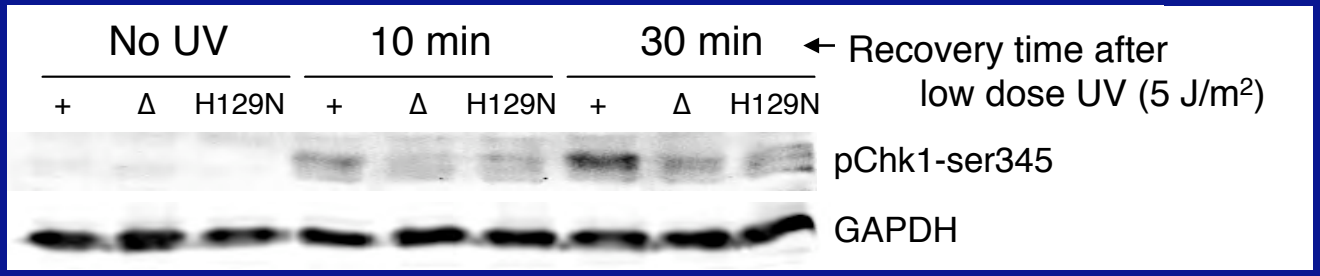
- Average of three independent DR -GFP lines for each genotype.
- +/Δ control set to 100%.
- Mre11$^{\Delta/\Delta}$ and Mre11$^{H129N/\Delta}$ are each <10% of control.

Mre11 nuclease activities and ATR activation?

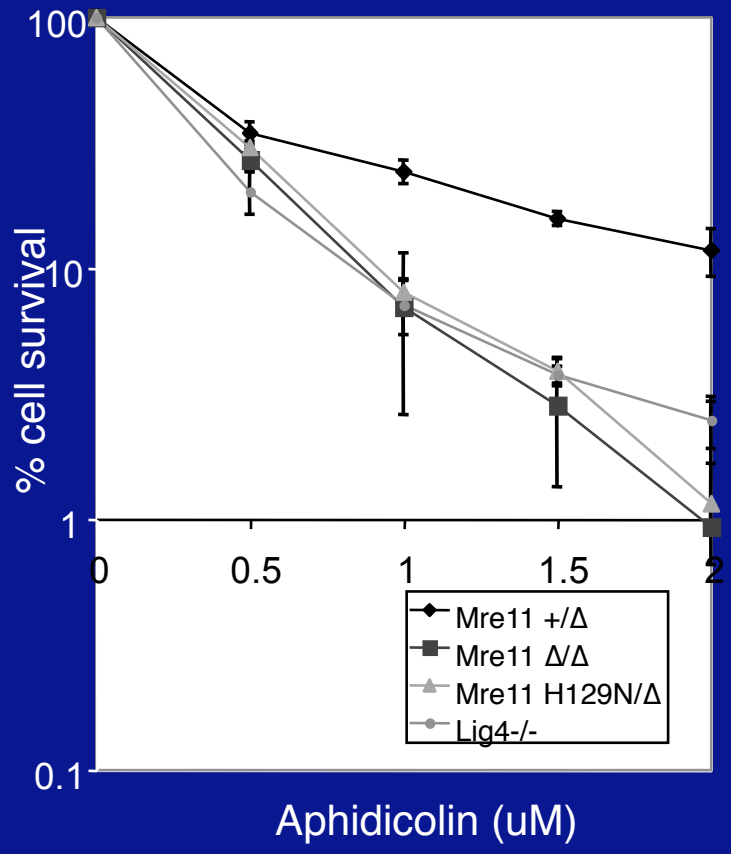


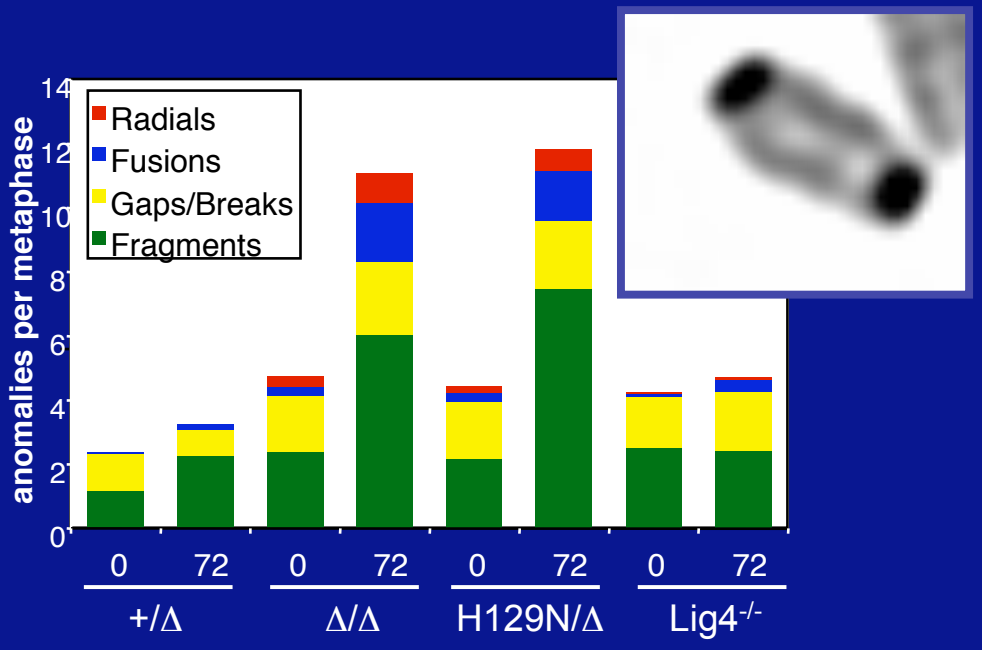
UV induced Chk1^{ser345} phosphorylation depends on Mre11 nuclease activities;

implication - role in ATR activation



The nuclease activity of Mre11 is required during replication stress





Hours after 24 hr exposure (1 uM)

Dicentrics in 25 metaphases			
4	46	39	9

Summary

In mammals, the nuclease activities of Mre11 are;

essential in early mammalian development.

fundamentally important for maintaining genomic stability, and resistance to IR and replication stress (aphidicolin).

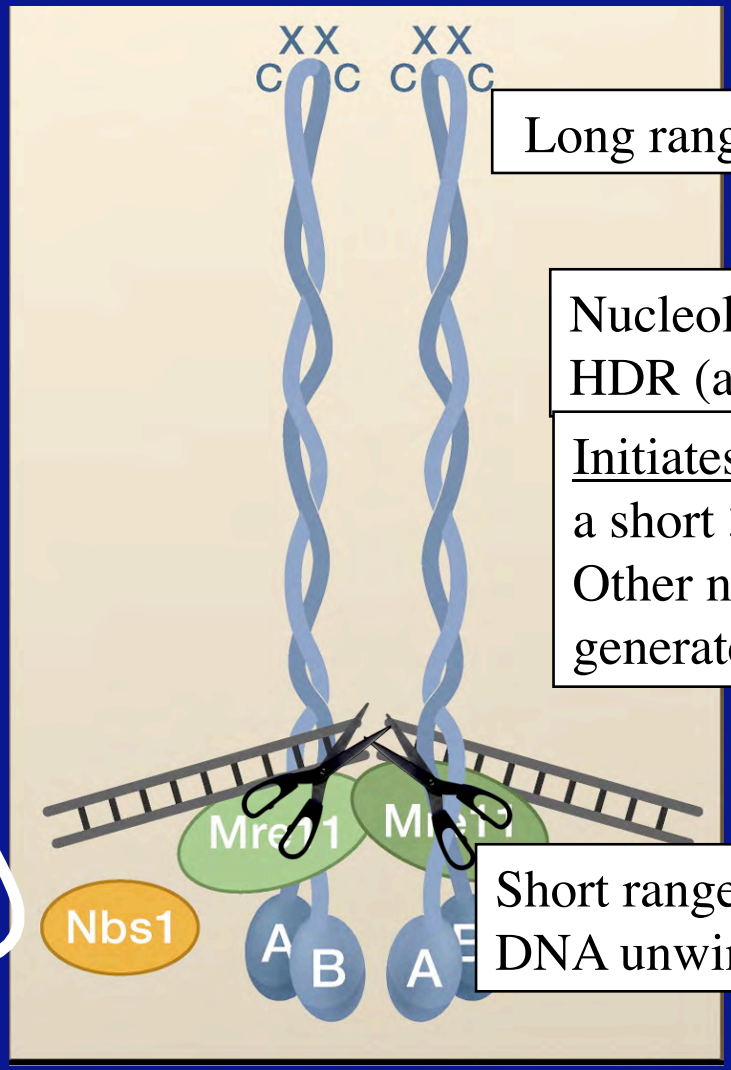
play an important role in DSB repair via homologous recombination, likely during resection.

contribute to ATR activation (likely through ss DNA)

The essential role(s) is distinct from MRN control of ATM activation.

ATP dependant unwinding of single strands by Rad50 is separable from ATP dependant resection by Mre11.

Mre11 Nuclease Activities and ATM control are separable functions of the MRN complex



Long range interactions (Rad50)

Nucleolytic processing by Mre11 – HDR (and some ATR activation).

Initiates HDR by generating a short 3' overhang – Other nucleases and helicases generate long 3' overhang.

Short range interactions (Mre11 dimer). DNA unwinding (Rad50).

substrates

Active kinase

P-ATM

Inactive kinase

ATM

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