Synthetic-viability genomic screening defines Sae2 function in DNA repair

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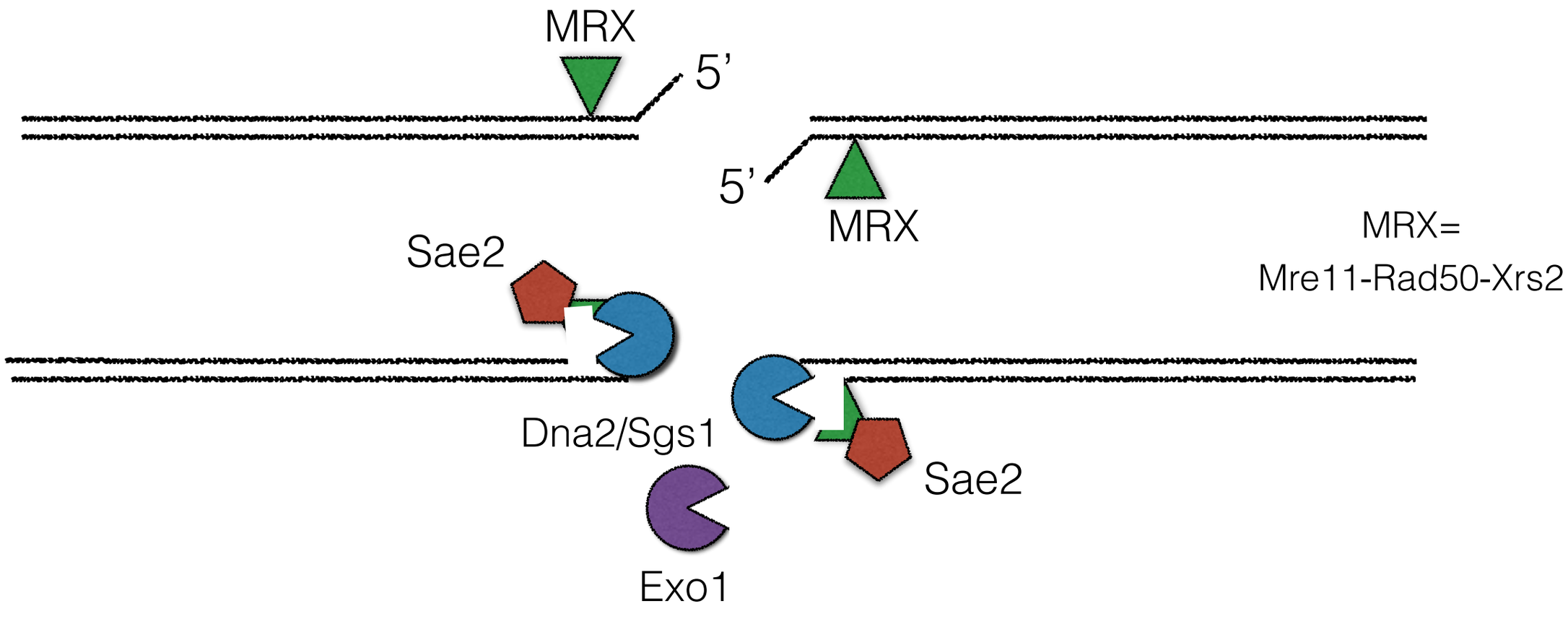
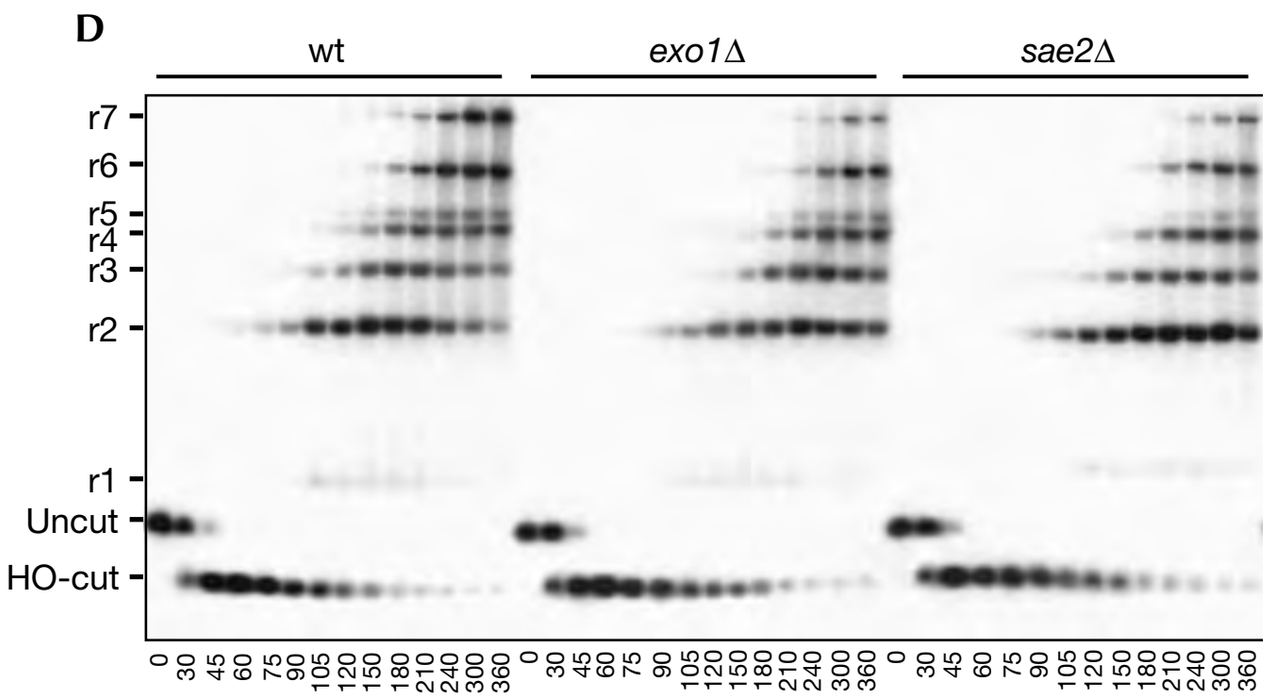
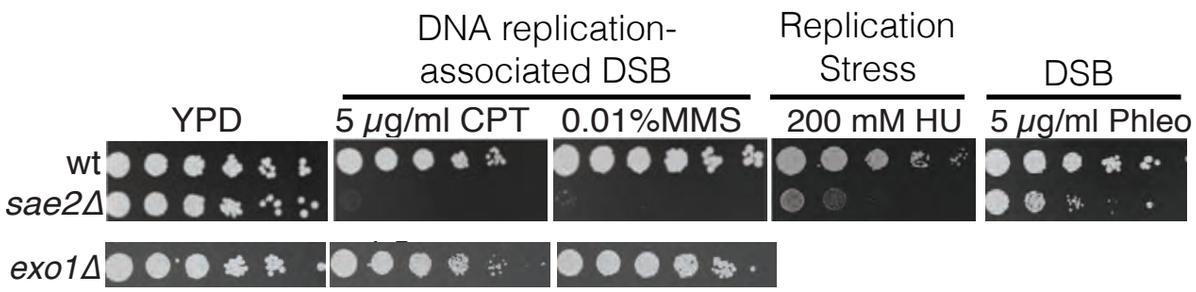
DNA double strand break repair

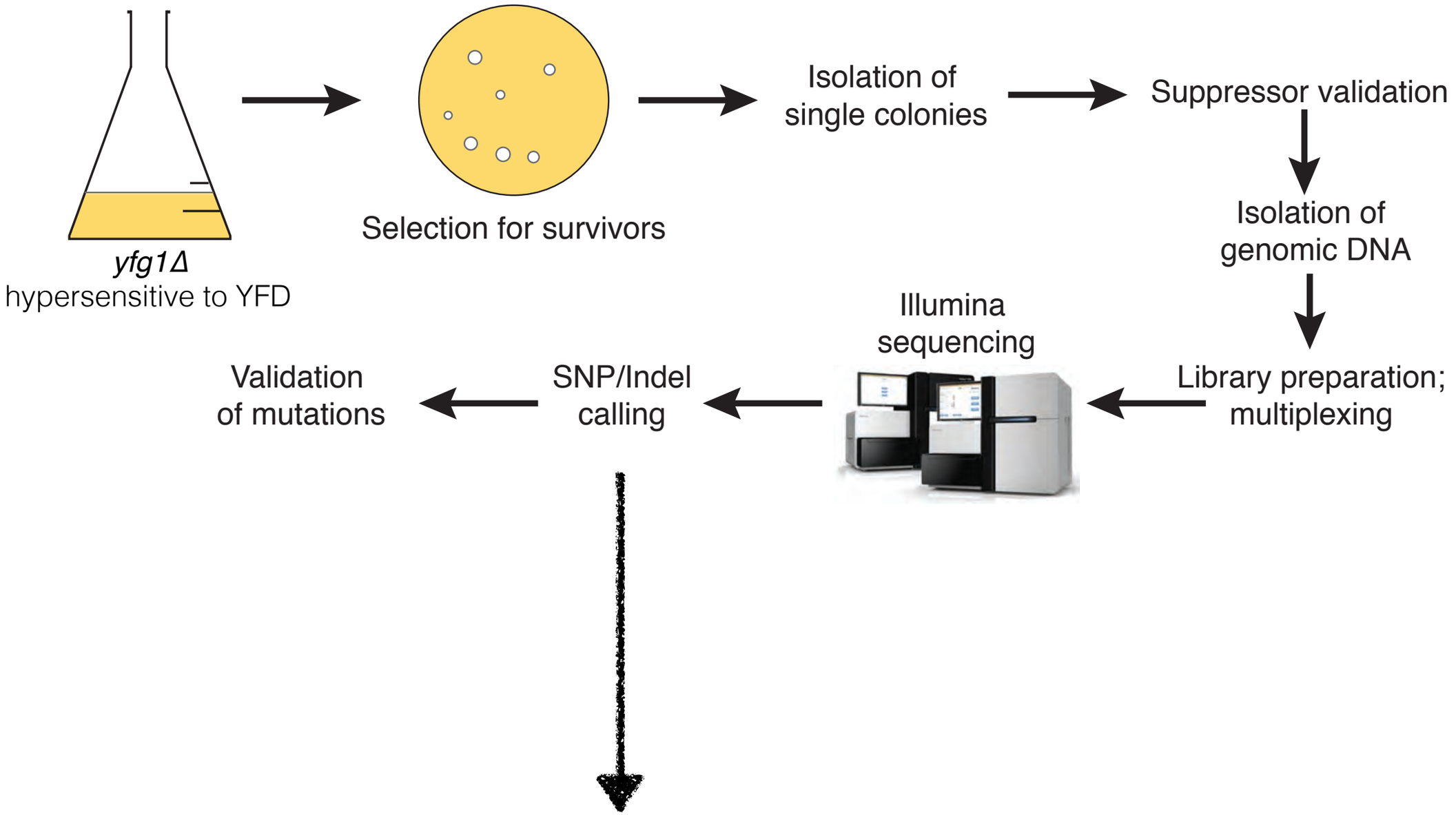




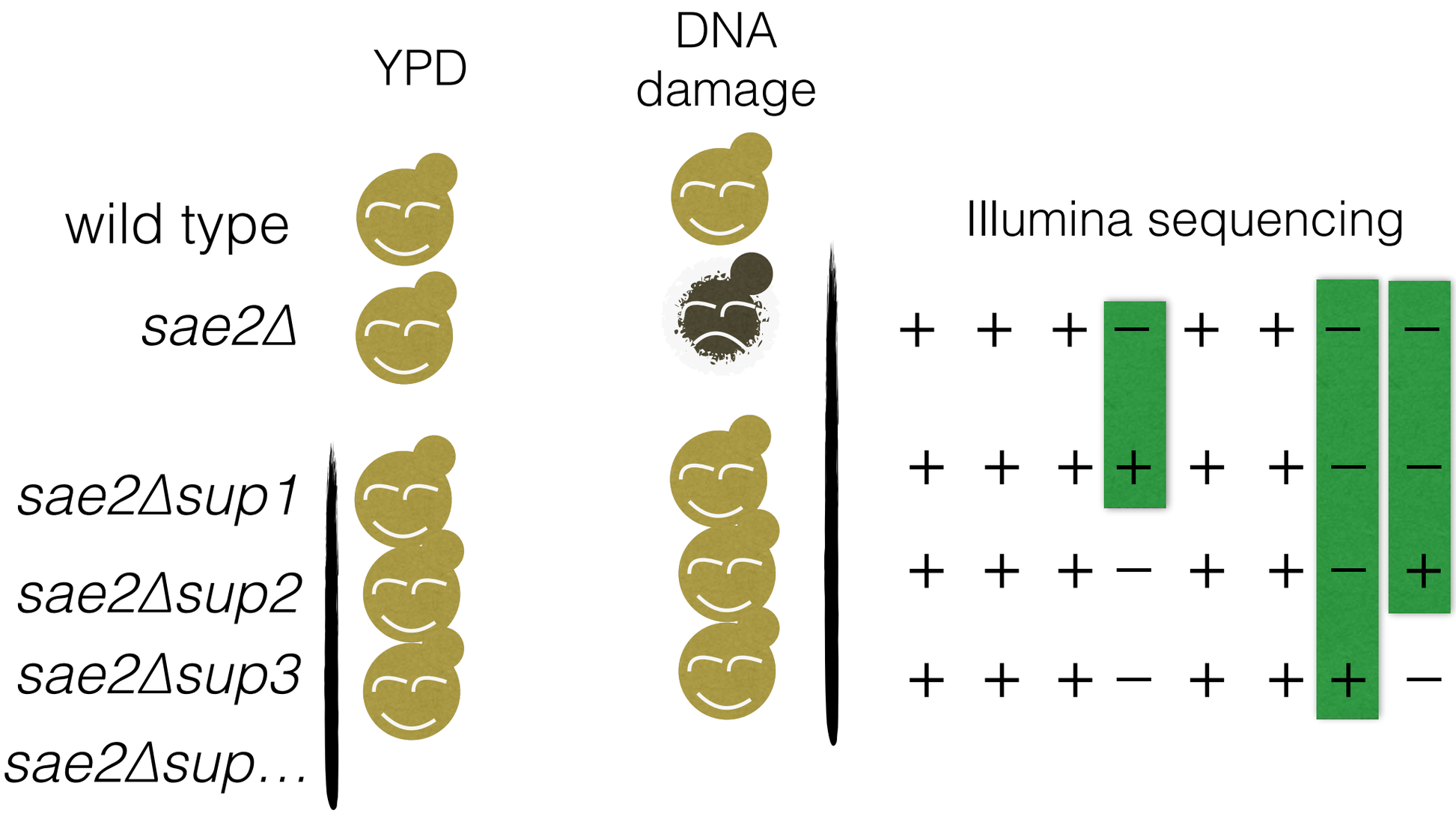
Clerici, 2006



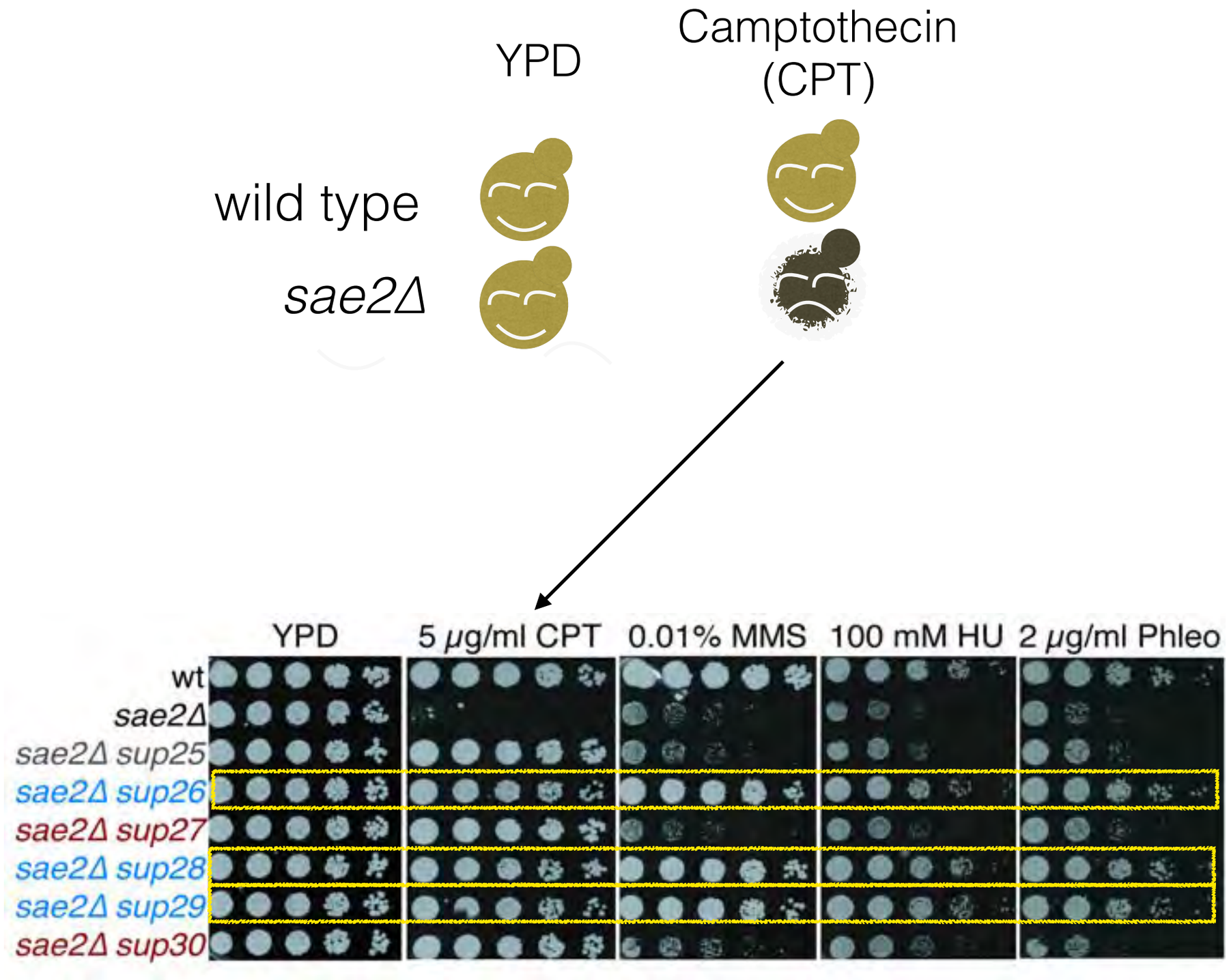
Synthetic viability genomic screening



Syntethic-viability genomic screen: calling mutations



Screening for suppressor of *sae2Δ* on CPT



Suppressors of *sae2Δ* camptothecin sensitivity

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DIFFERENTIAL GENOTYPE
SAMPLE NAME     REF   GENOTYPE
=============================================================================================================================================================

ERS096542  .     TBS1-T833:T>A:3 NSR1-E54A  YGR160W-S142A   DYS1-A321S

ERS096547  .     ALD5-A500:T>A:3 DNF3-E645Q

ERS096551  .     ALD5-A500:T>A:3 DNF3-E645Q
ERS096553  .     ALD5-A500:T>A:3 DNF3-E645Q
ERS096554  .     MRE11-H37R
ERS096556  .
ERS096557  .     TBS1-T833:T>A:3 DYS1-A321S
ERS096558  .

ERS096561  .
ERS096562  .     YSH1-R353M
ERS096563  .     YSH1-R353M MRE11-H37Y
ERS096565  .     YSH1-R353M MRE11-H37Y
ERS096566  .     MRE11-H37R

ERS096570  .     YSH1-R353M MRE11-H37Y
ERS096571  .     NSR1-E54A  YGR160W-S142A   NSR1-Δ53   YGR160W-Δ141   YSH1-R353M

ERS096574  .     YLR255C-FS@53-58     ALD5-A500:T>A:3 DNF3-E645Q

ERS096577  .     YSH1-R353M MRE11-H37Y

ERS096579  .     YSH1-R353M
ERS096580  .     NSR1-E54A  YGR160W-S142A   NSR1-Δ53   YGR160W-Δ141   YSH1-R353M   MRE11-H37Y

ERS096582  .     YSH1-R353M MRE11-H37Y
ERS096583  .     YSH1-R353M MRE11-H37Y

ERS096585  .     YSH1-R353M MRE11-H37Y
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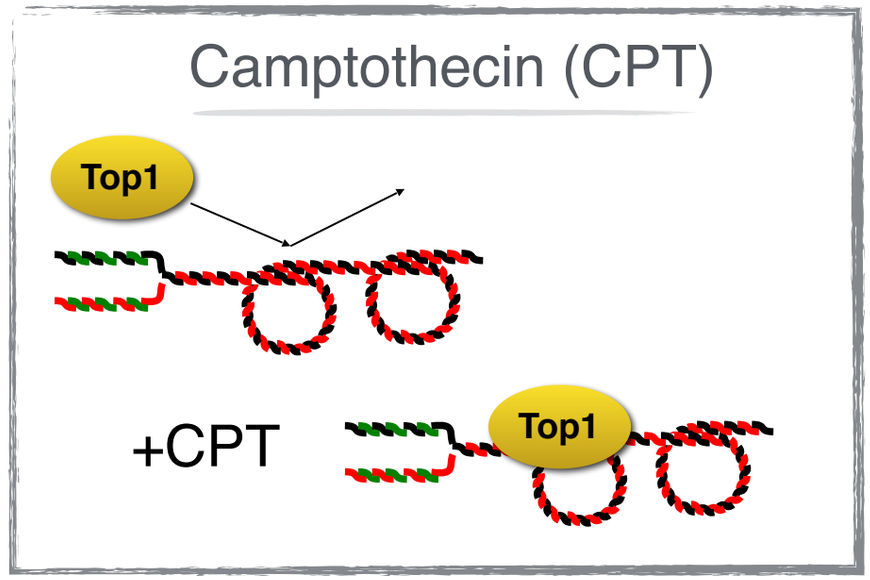
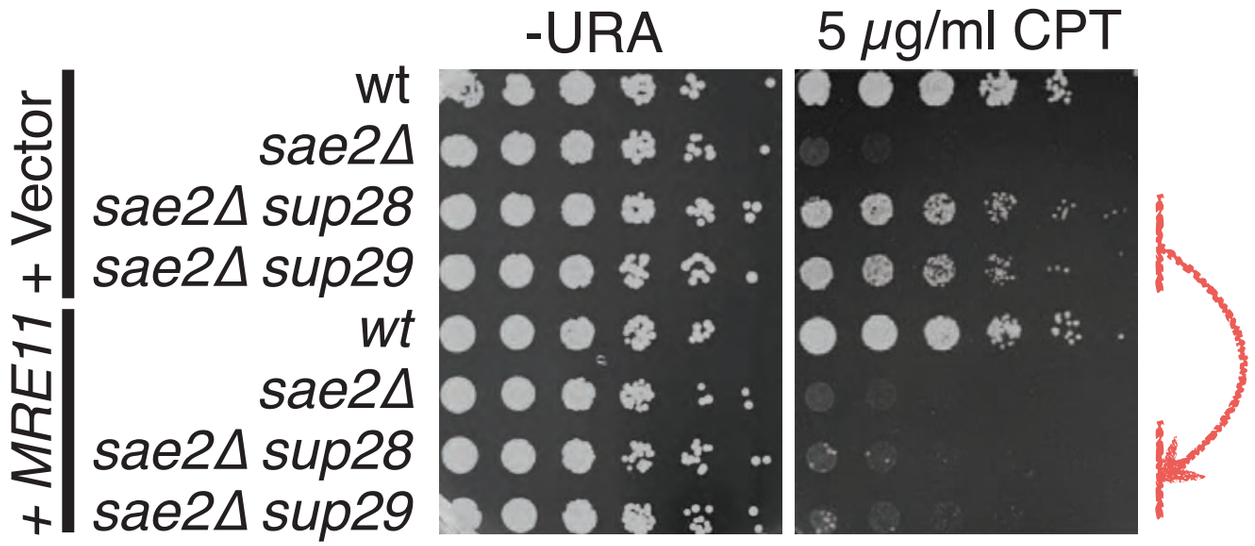
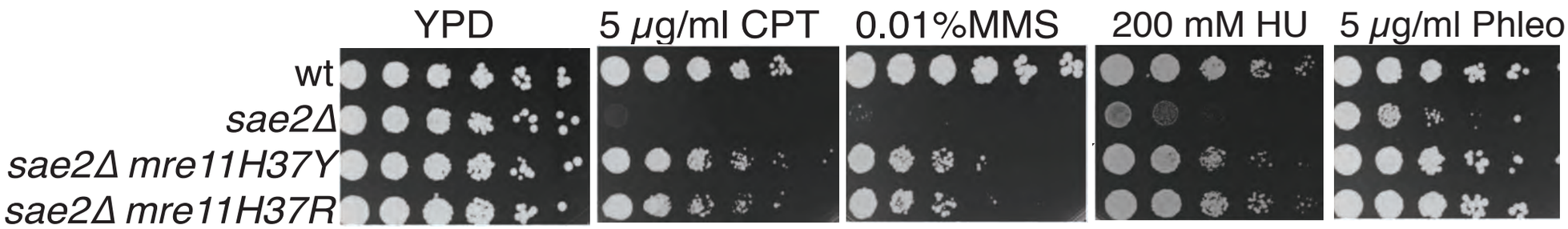
MRE11-H37R

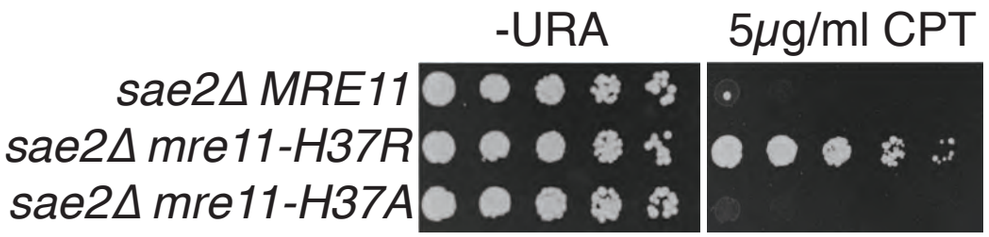
MRE11-H37Y



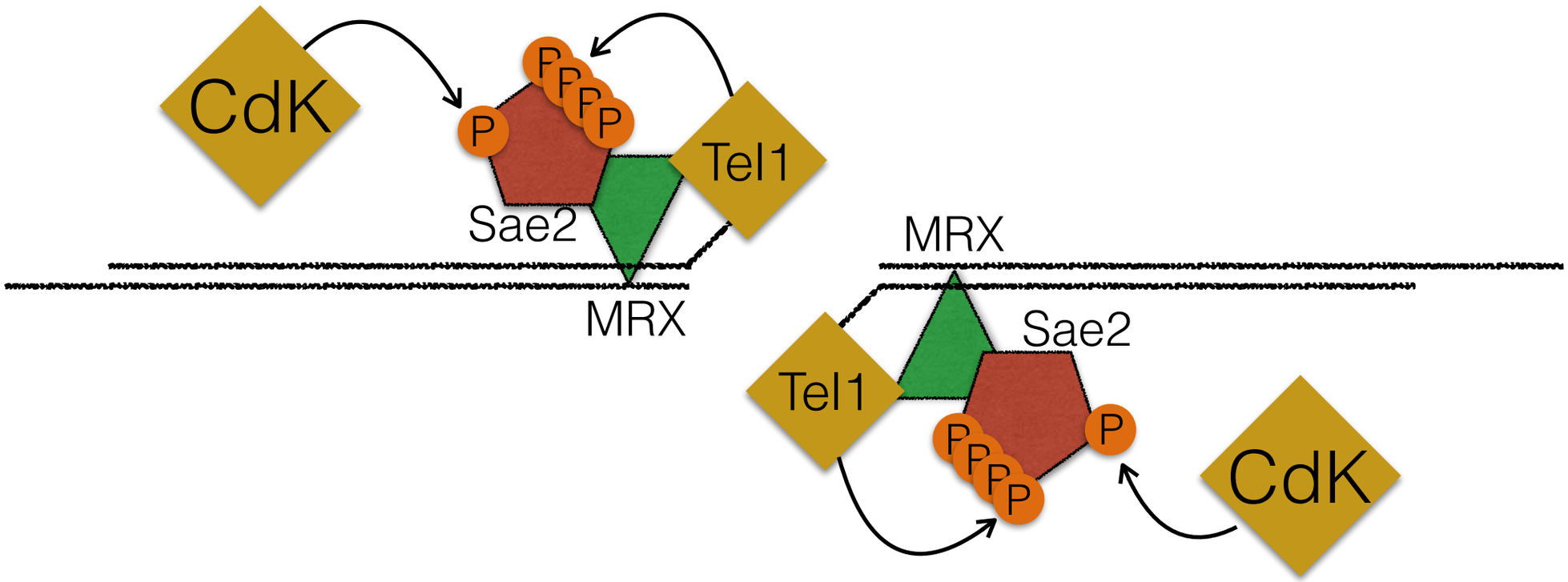
mre11-H37R/Y suppress *sae2Δ* sensitivity to DNA damage

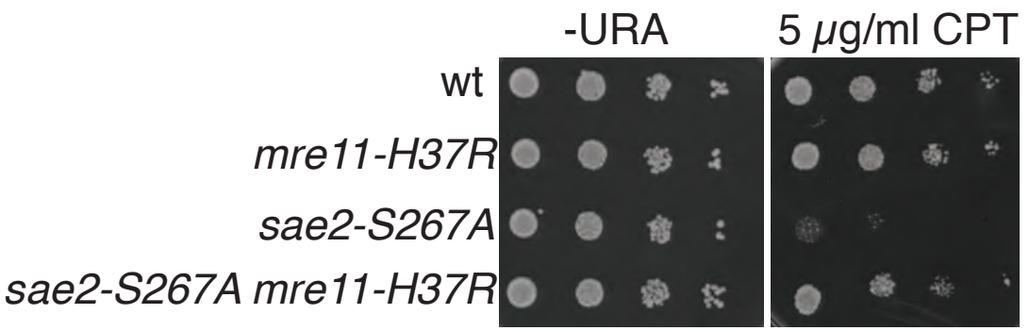


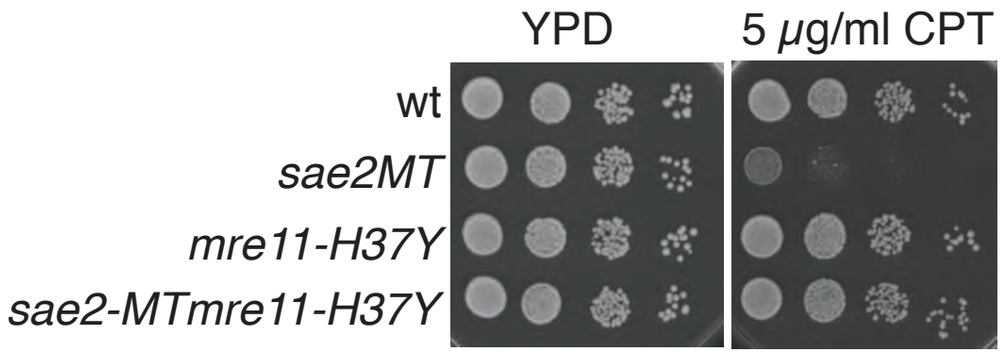




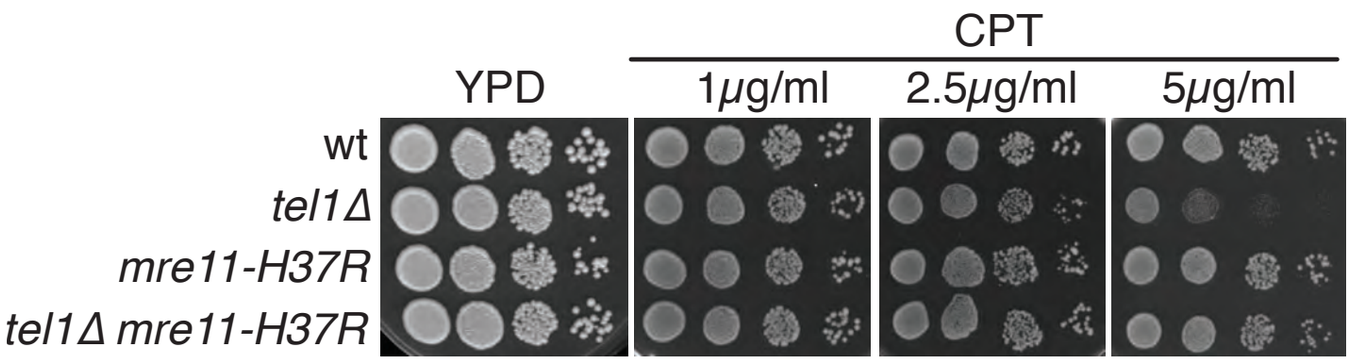
mre11-H37R suppresses the DNA damage sensitivity of *sae2* phosphorylation mutants

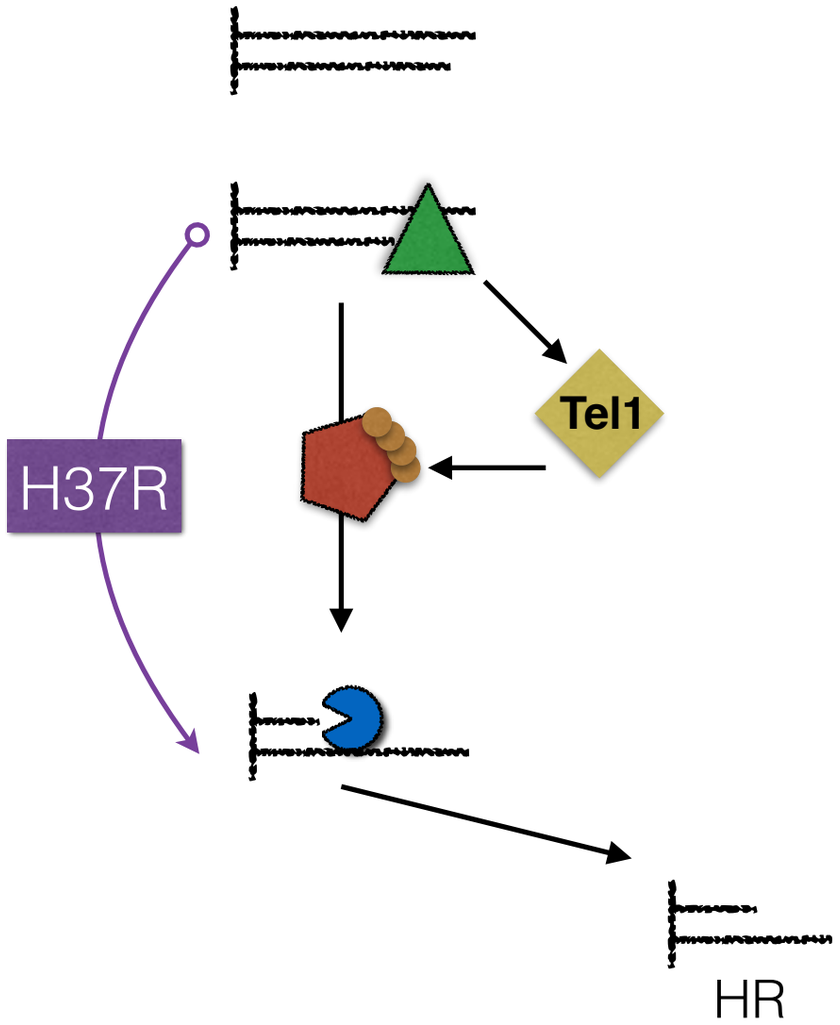






mre11-H37R suppresses the weak CPT sensitivity of *tel1Δ*





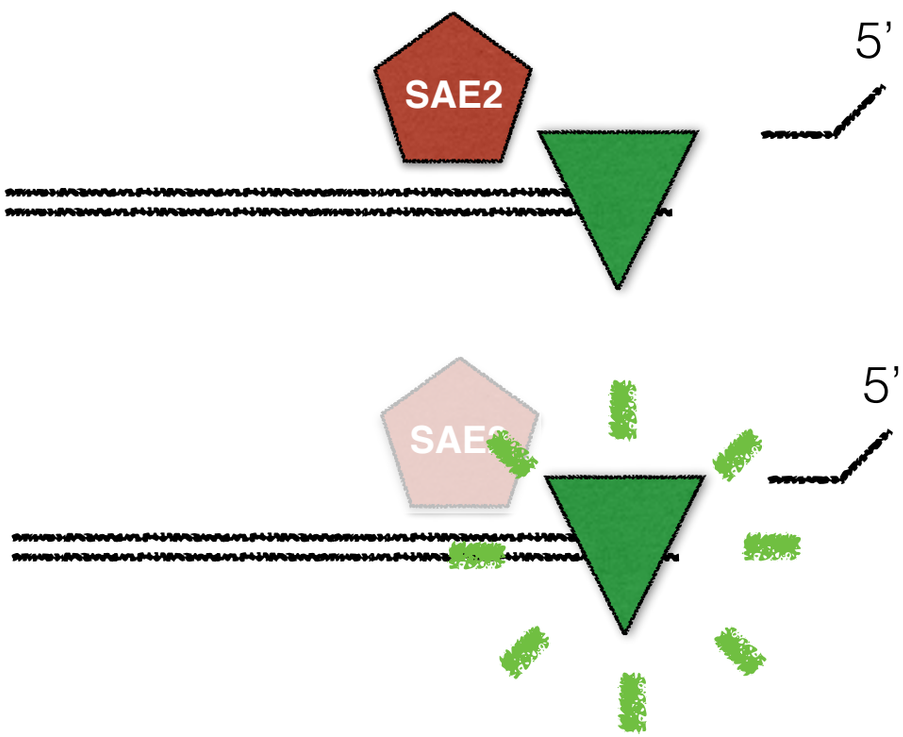
MRX

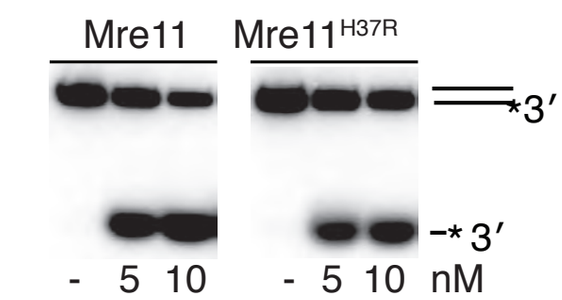
Sae2

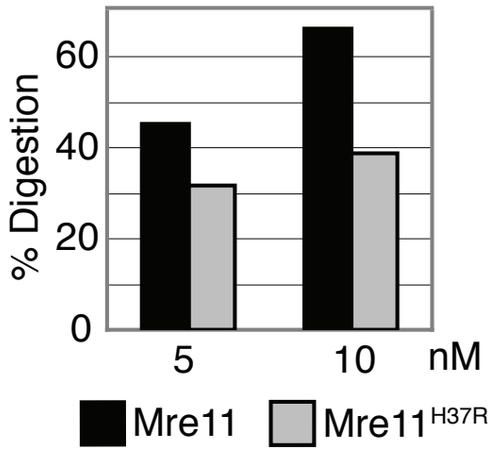
Dna2/Sgs1

Exo1

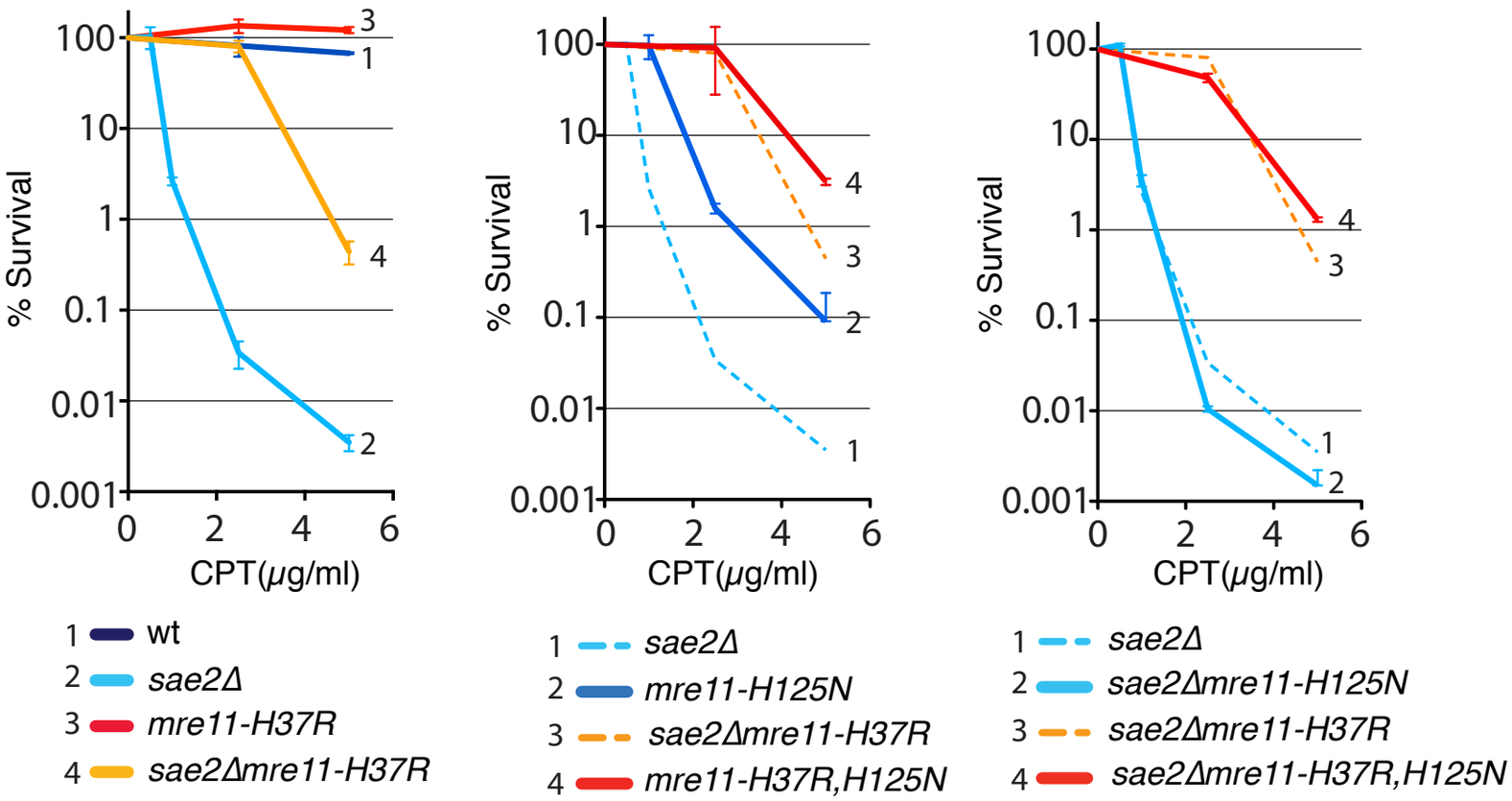
mre11-H37R does not increase the nuclease activity of Mre11





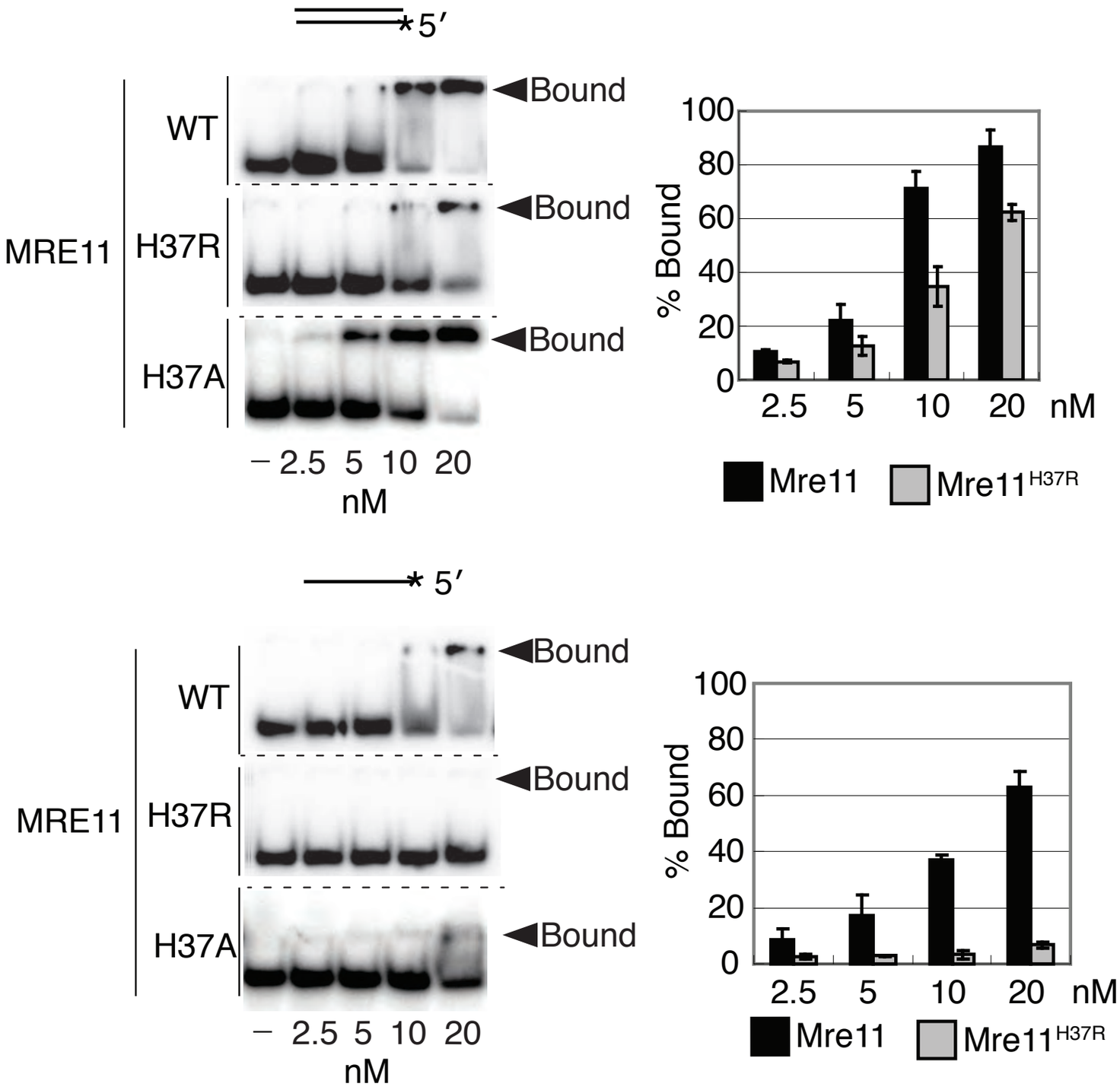


mre11-H37R suppresses the DNA damage sensitivity of *mre11-nd* mutants



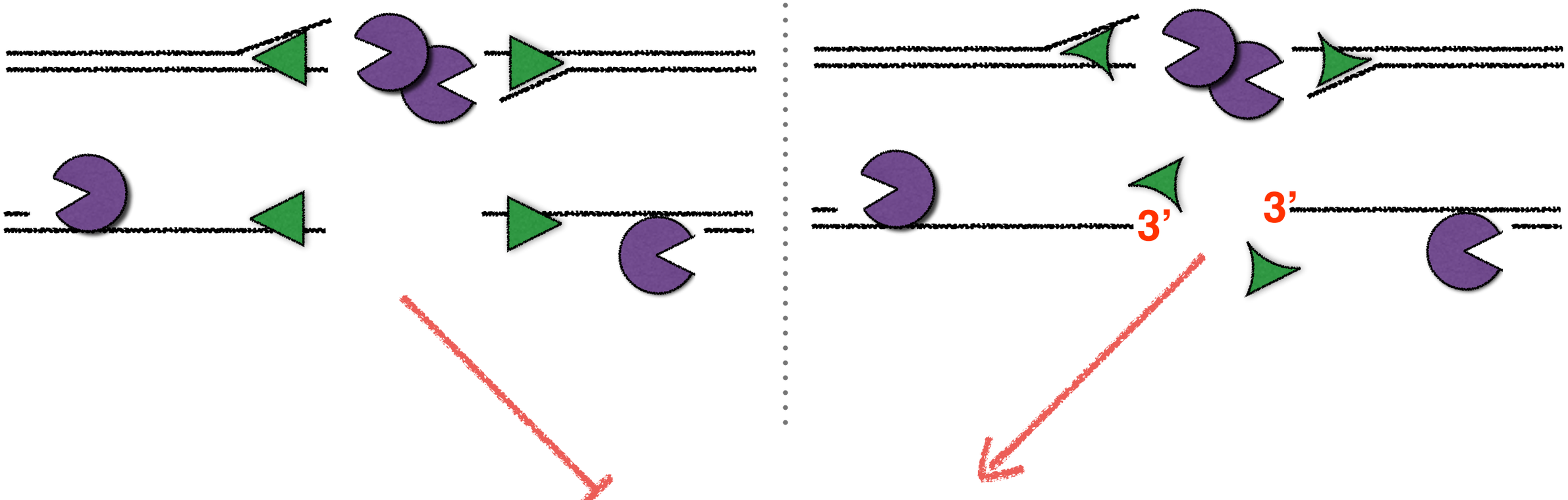
- *mre11-H37R* does not bypass all the functions of Sae2
- *mre11-H37R* suppresses the lack of Mre11 nuclease activity
- The suppression does not require the nuclease activity of Mre11

mre11-H37R impairs binding of Mre11 to ssDNA



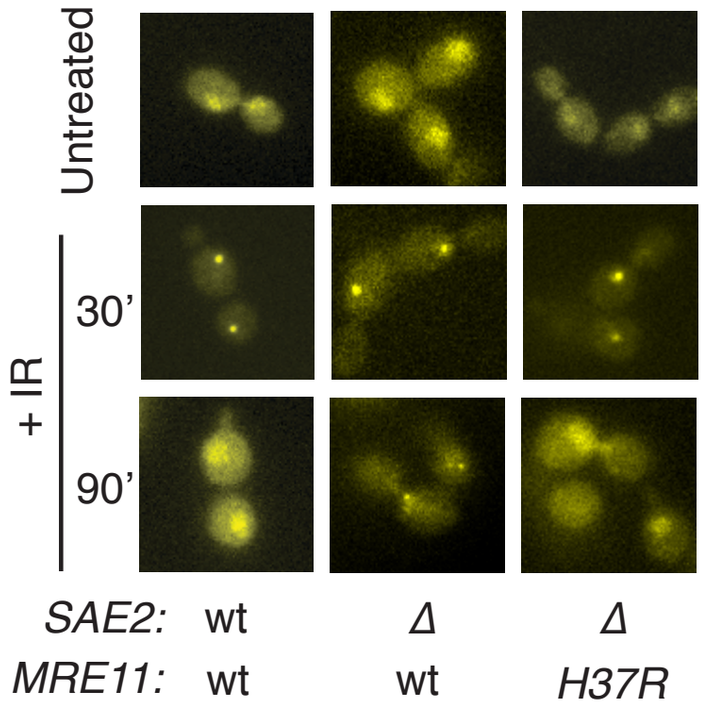
sae2Δ

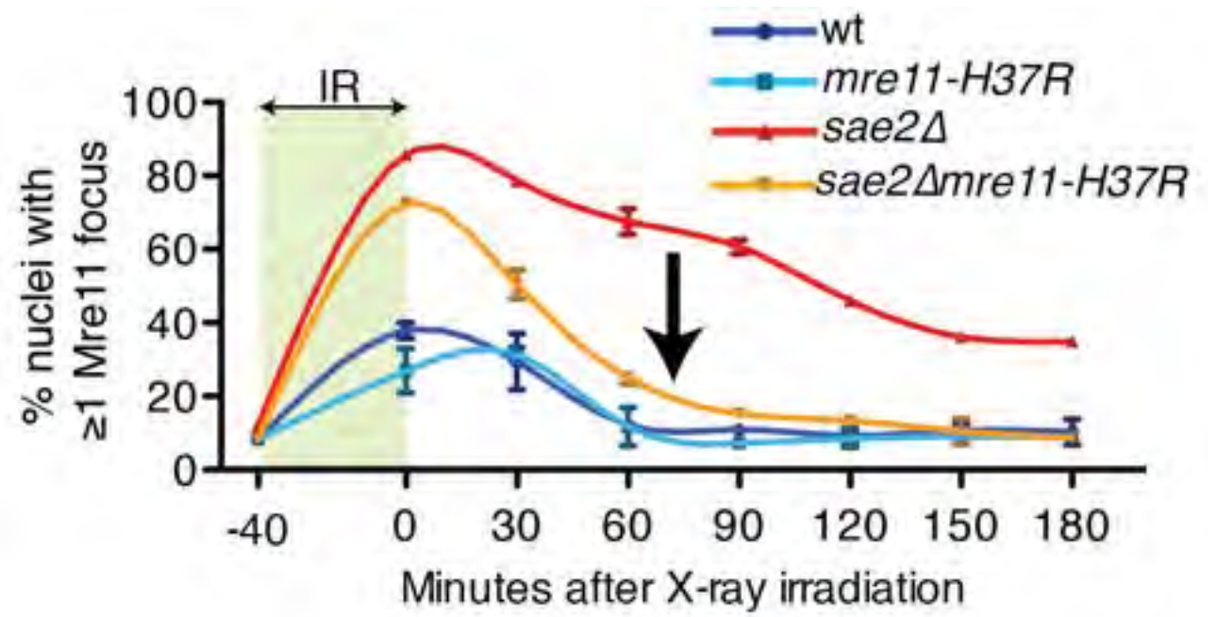
sae2Δ
mre11-H37R



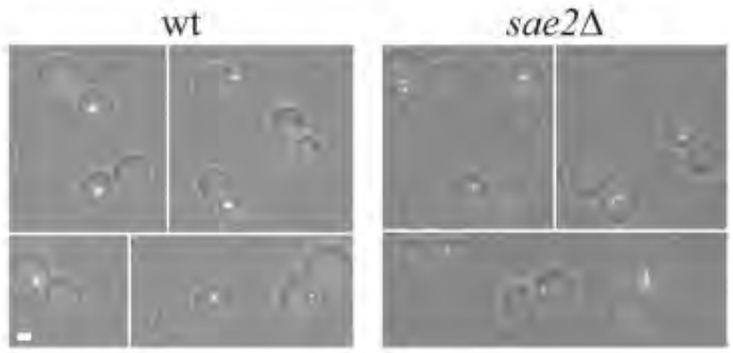
Homologous
Recombination

mre11-H37R rescues the delayed release of Mre11 from DNA damage sites

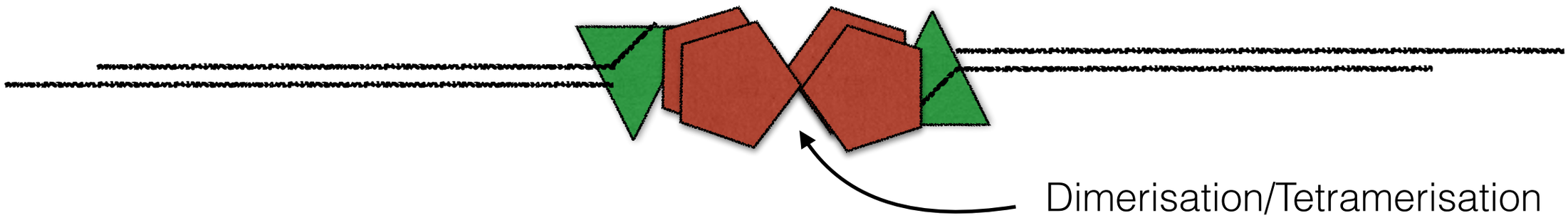


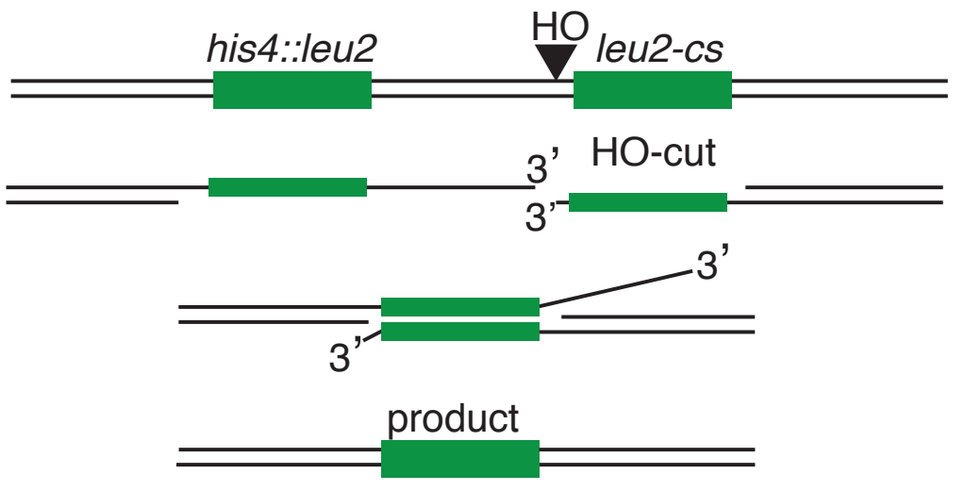


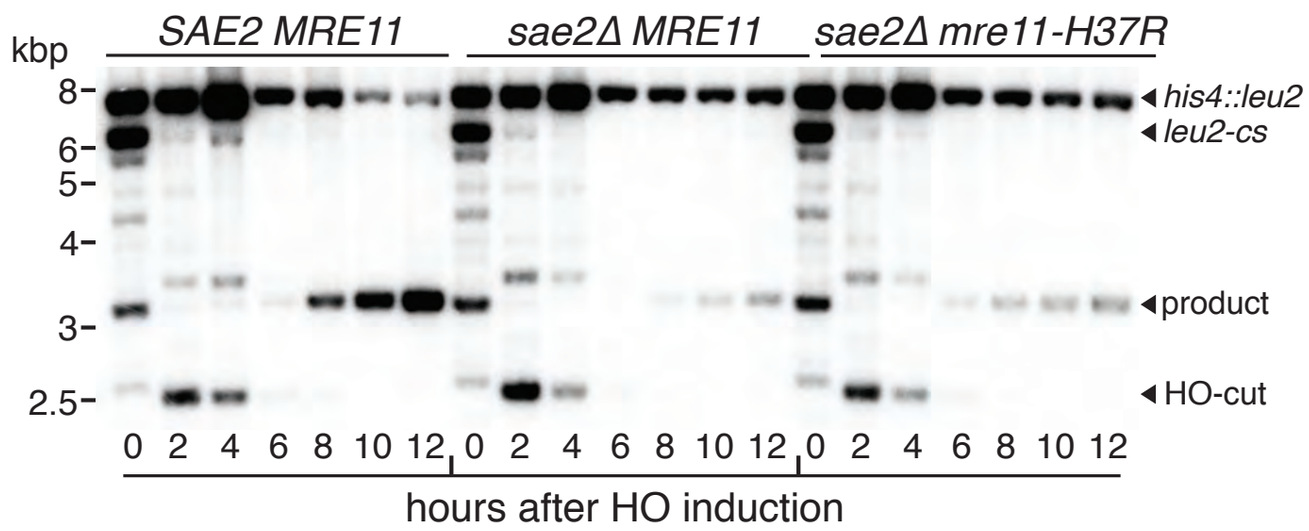
mre11-H37R does not suppress the end-bridging function of Sae2



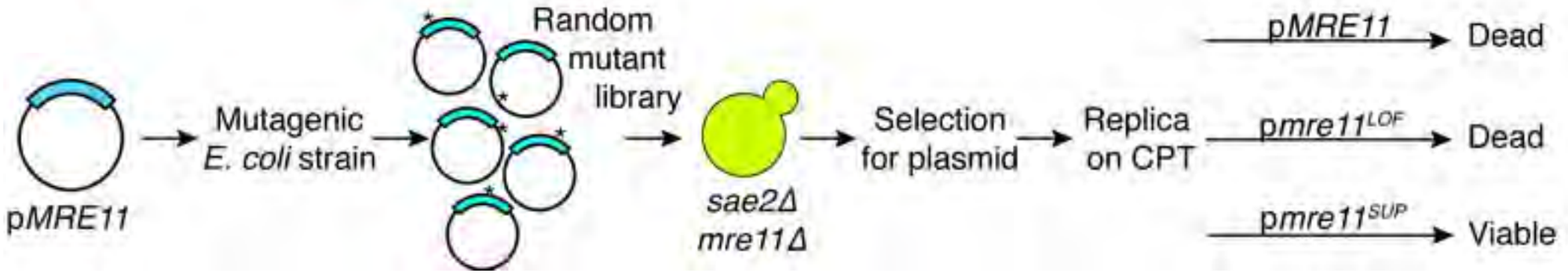
Clerici, 2006

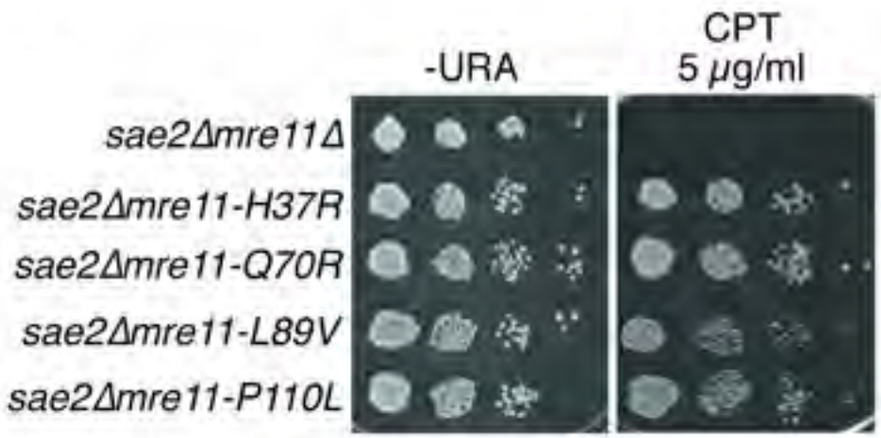


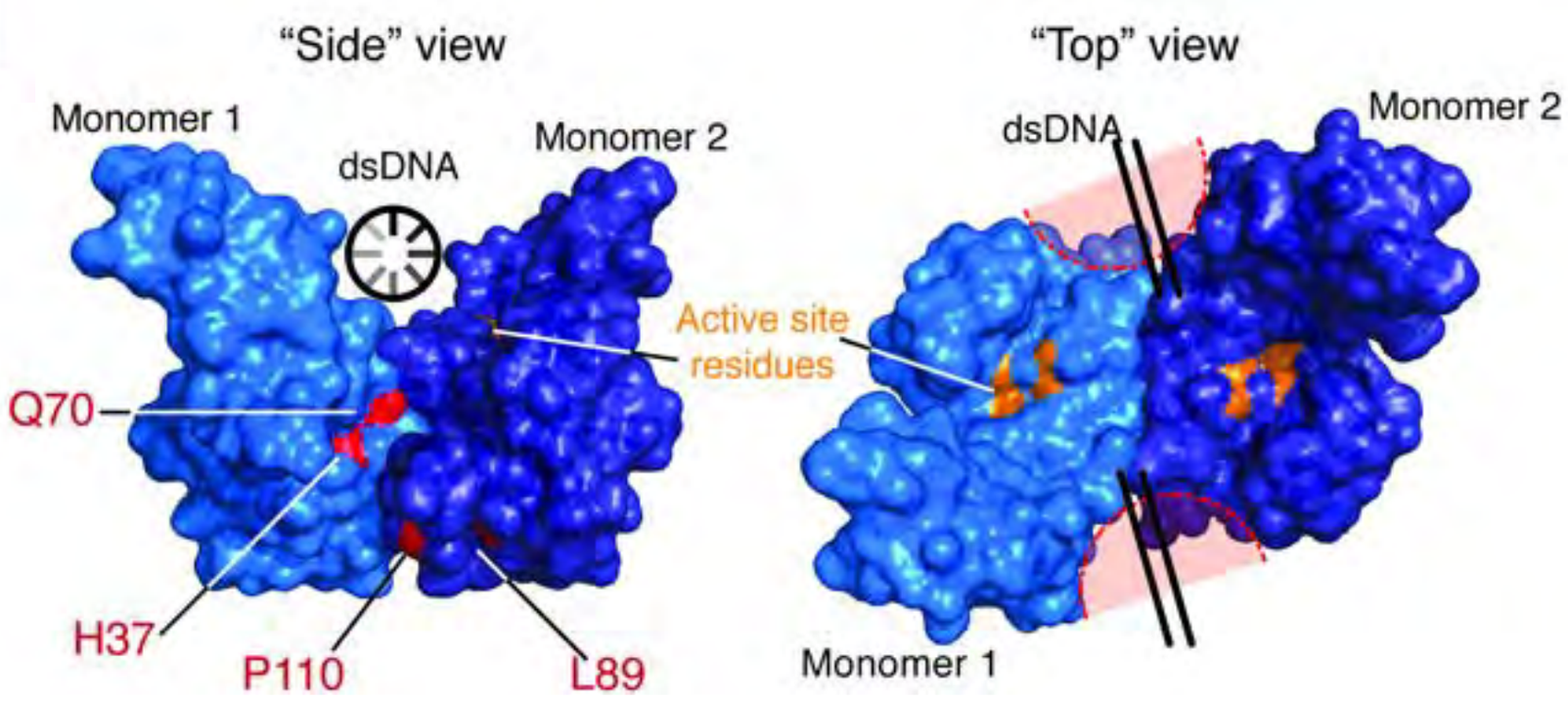




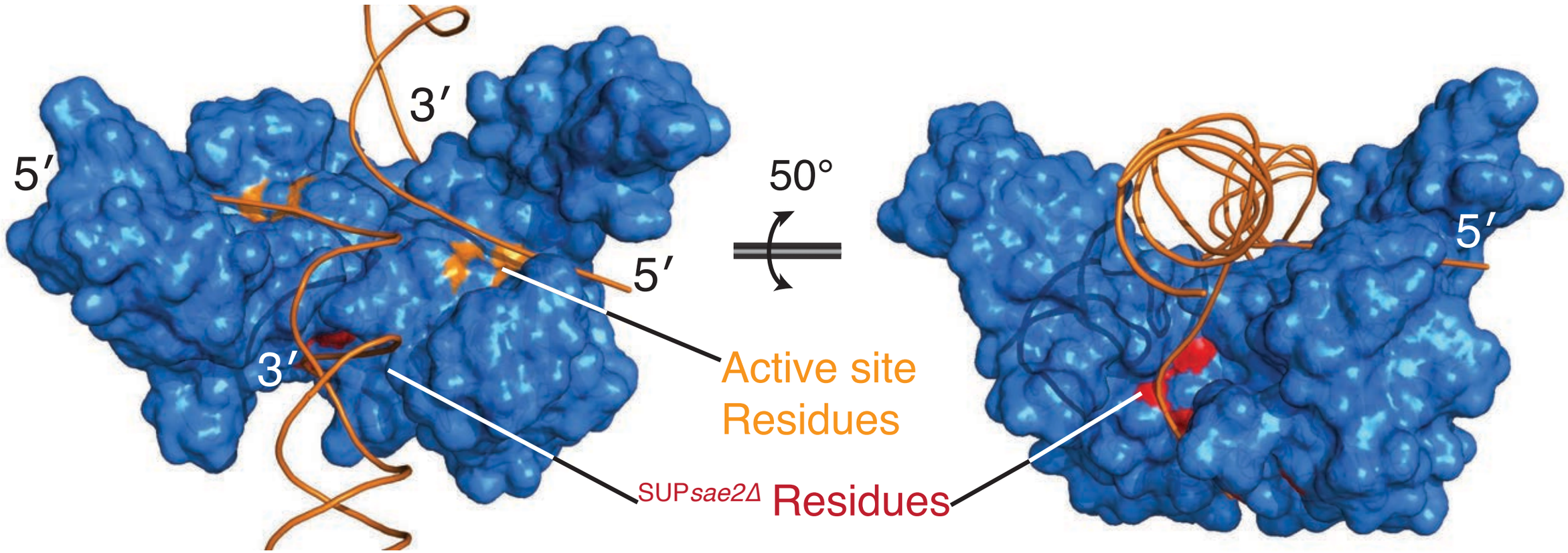
Additional mutations in *MRE11* suppress *sae2Δ*







Additional mutations in *MRE11* suppress *sae2Δ* phenotypes



Summary

The essential function of Sae2 is not to promote 5’ DNA resection at DSB ends

The essential function of Sae2 is to clear the 3’ end from Mre11

The synthetic viability genomic screening approach is a quick and powerful tool to understand protein function

Acknowledgements

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mre11-H37R suppresses the weak CPT sensitivity of *tel1Δ*