WRN regulates pathway choice between classical and alternative non-homologous end joining as it maintains genomic stability

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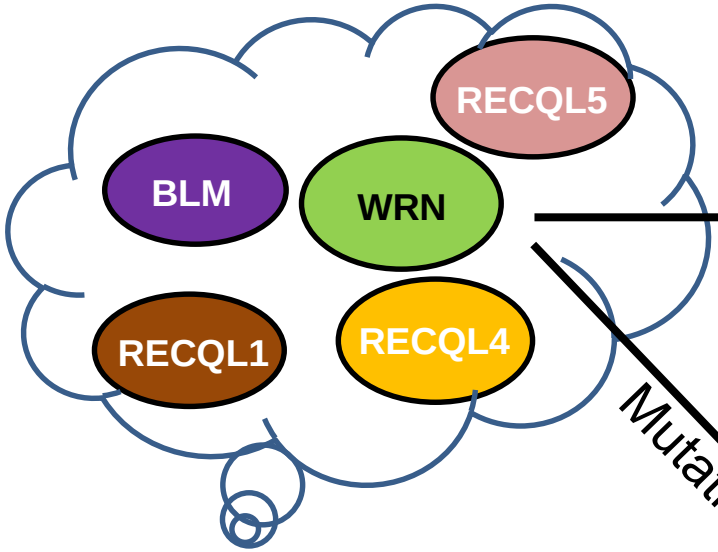
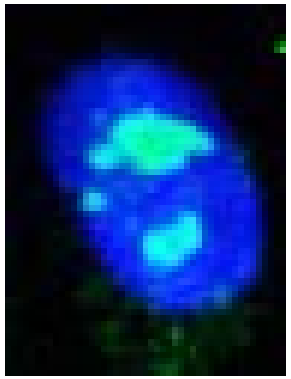
Werner Syndrome Protein (WRN)



Mutations

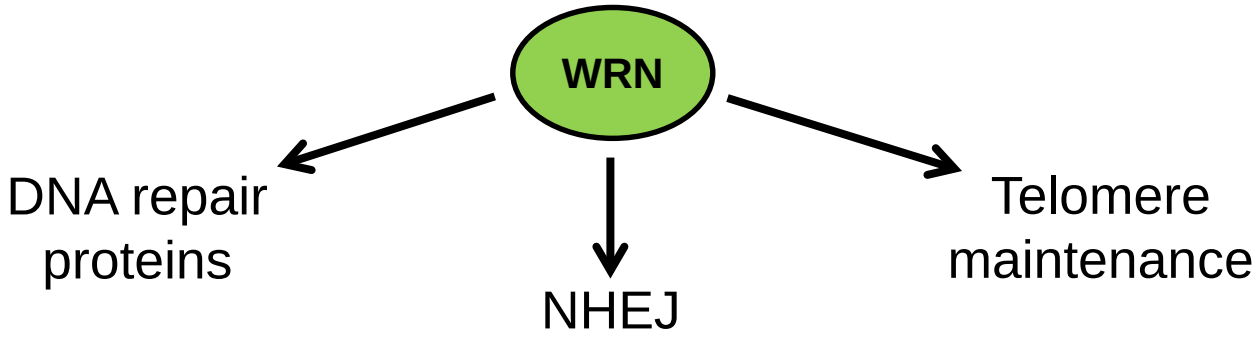
Werner Syndrome



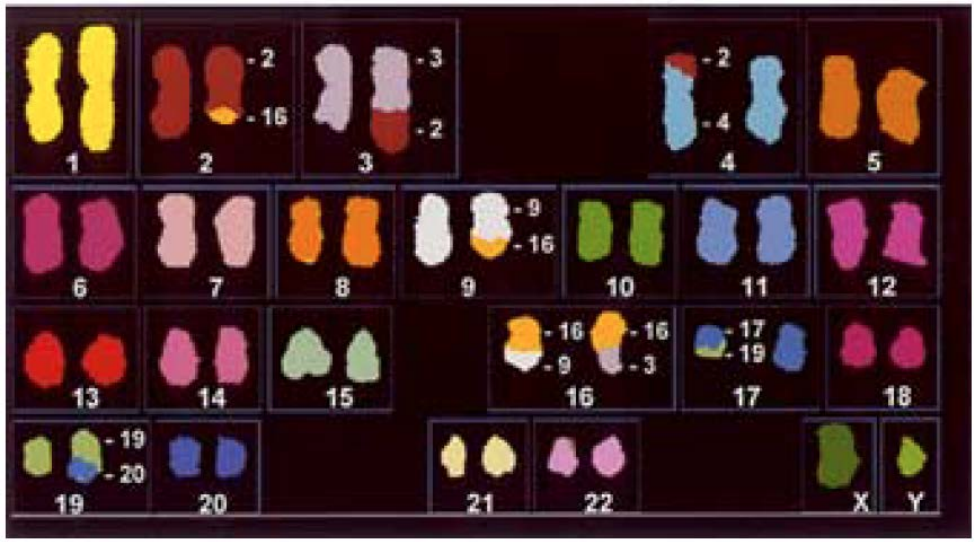
- DNA repair
- DNA replication
- Transcription
- Telomere maintenance
- Senescence
- Cancer

Oshima J. et al, Ageing Research Reviews, 2016

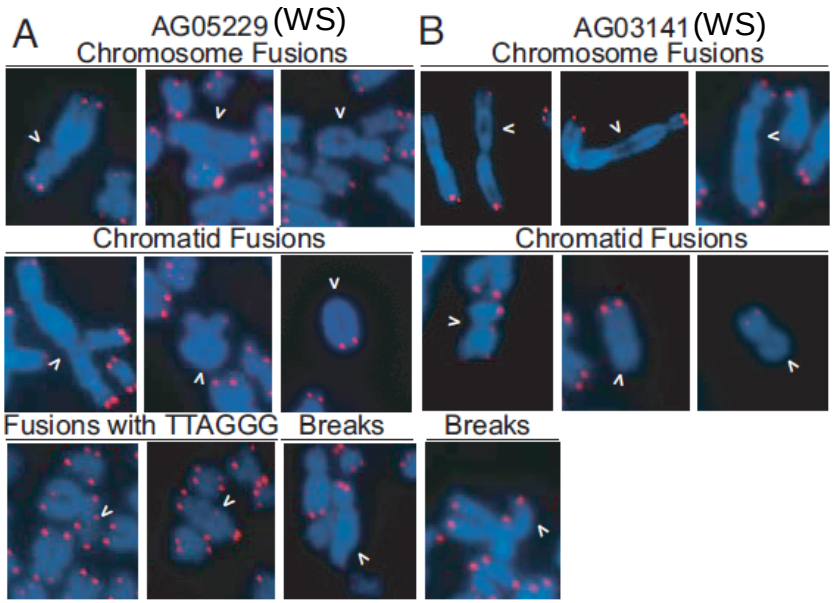
WRN is associated with DNA repair and chromosomal stability



Spectral karyotype of Werner syndrome fibroblasts



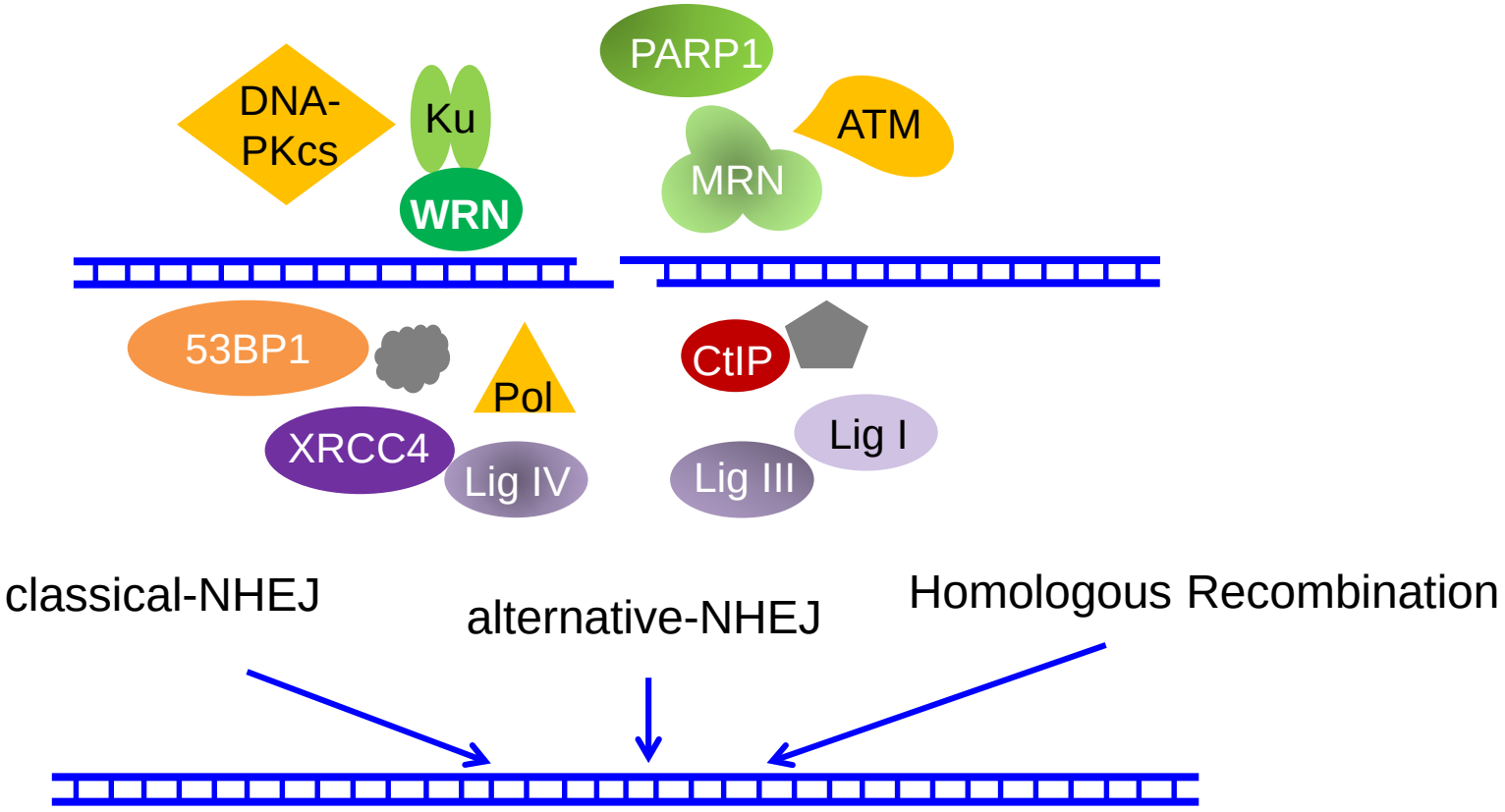
Melcher et al., 2000 .Cytogenet Cell Genet



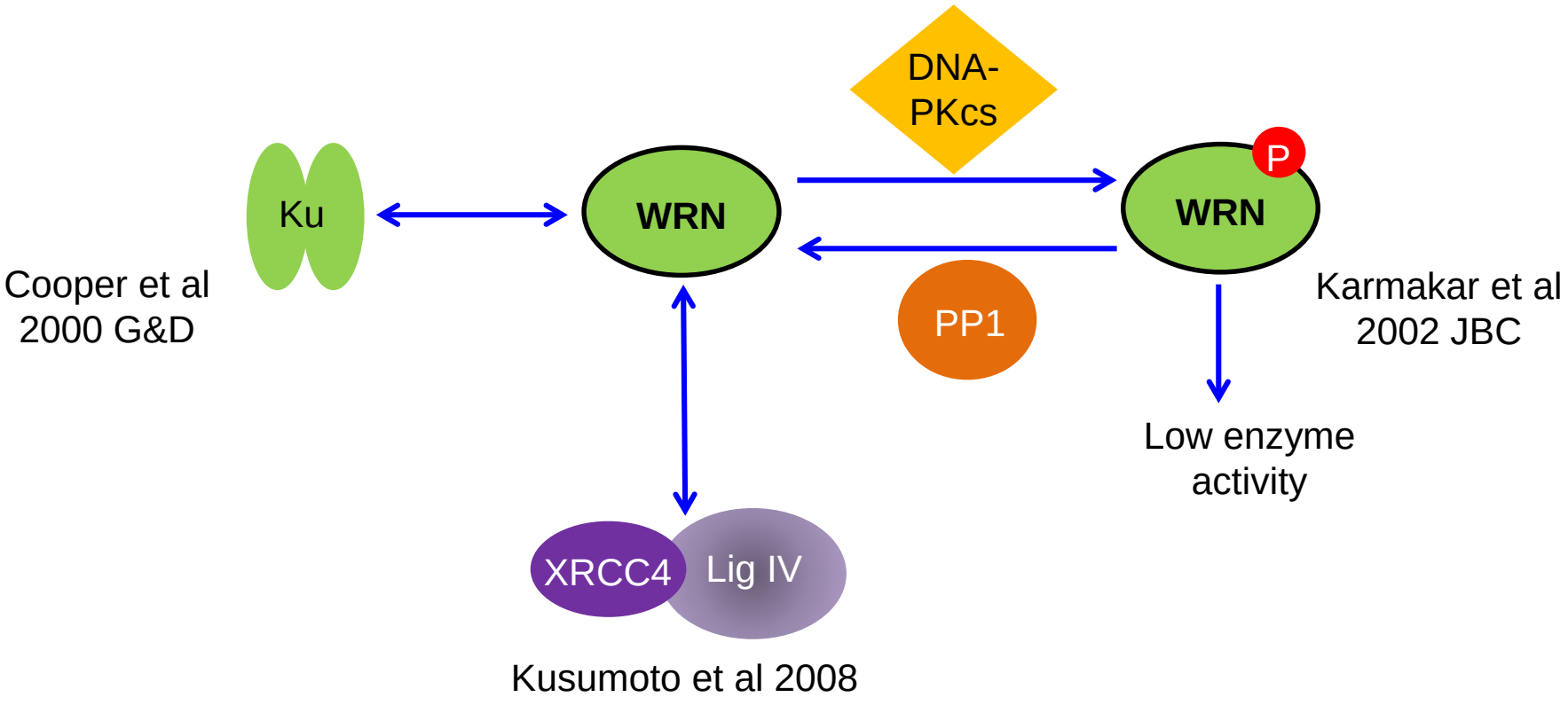
Crabbe et al., 2007 PNAS

Question: Does WRN regulate DSB repair associated with chromosomal aberrations?

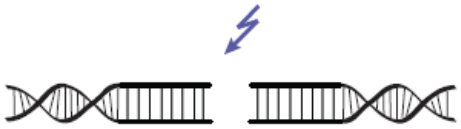
DNA double-strand break (DSB) repair pathway choice



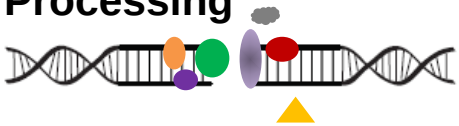
WRN physically and functionally interacts with NHEJ pathway proteins



NHEJ-mediated DNA Double Strand Break (DSB) Repair



NHEJ

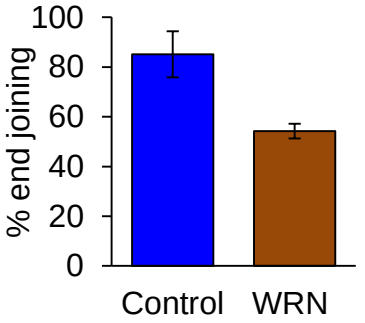
	c-NHEJ	alt-NHEJ
Recognition 	Ku70/Ku80	Ku-independent
End Processing 	**WRN**, Artemis	MRN/CtIP
	Pol X family	PolQ
Ligation 	Ligase IV	Ligase III and Ligase I
Biological relevance:	DSB repair V(D)J recombination Class switch recombination	Chromosomal translocation Class switch recombination Telomere fusions

WRN deficiency inhibits NHEJ and enhance microhomology usage for end-joining

NHEJ assays

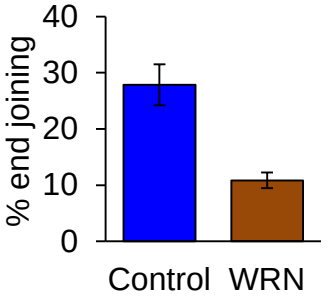
2.67 kb

Cohesive substrate



5.7 kb

Non-cohesive ends



5.7 kb

Junctional sequence

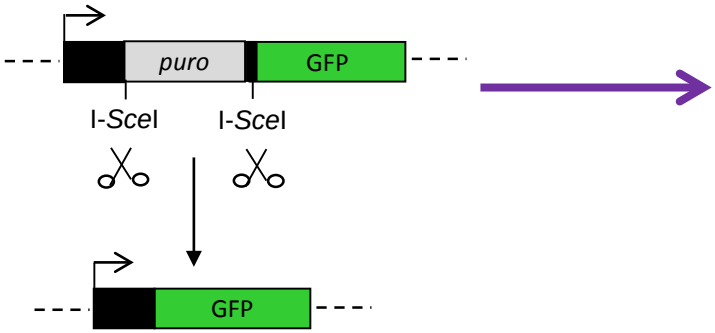
Lymphoblasts

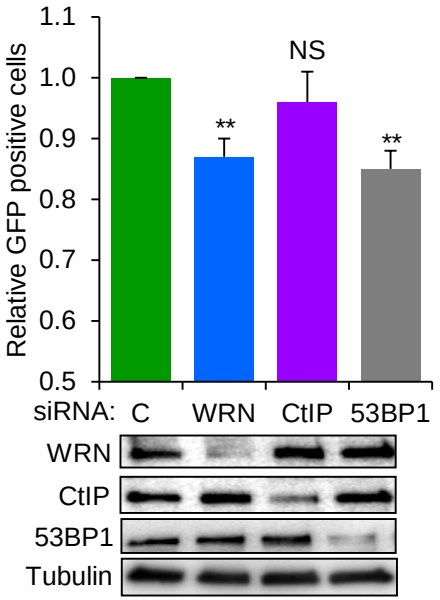
	Normal ($WRN^{+/+}$)	**WS ($WRN^{-/-}$)**
% Microhomology sequences	50.0	70.0
Length of Microhomology	2 – 4 bp	2 – 7 bp

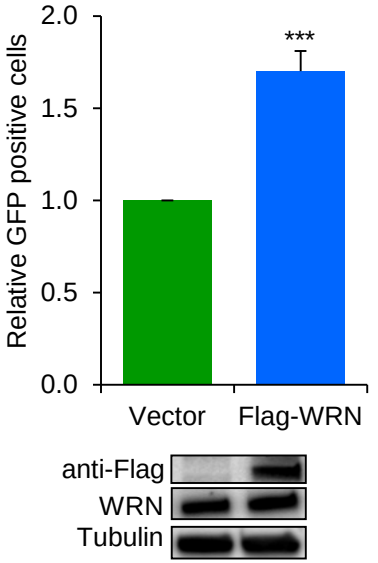
WRN promotes c-NHEJ and inhibits alt-NHEJ

In vivo reporters assays

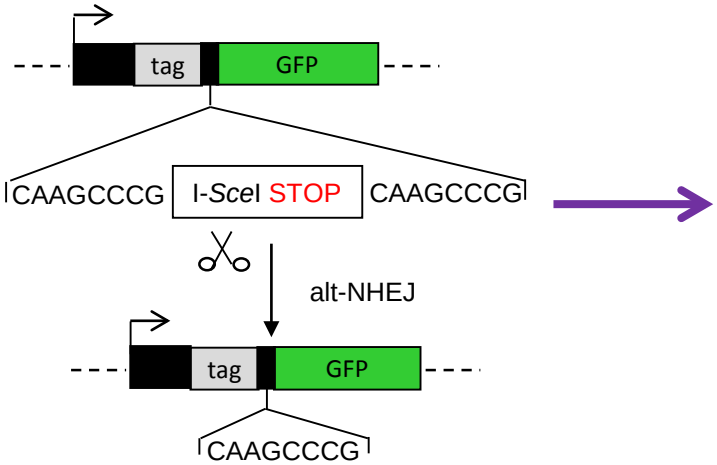
EJ5: NHEJ (c-NHEJ + alt-NHEJ)



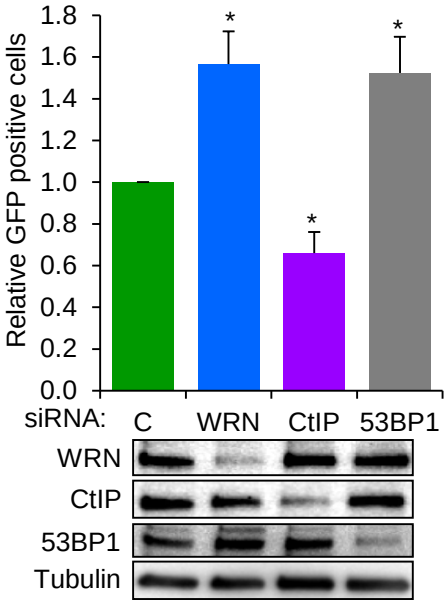


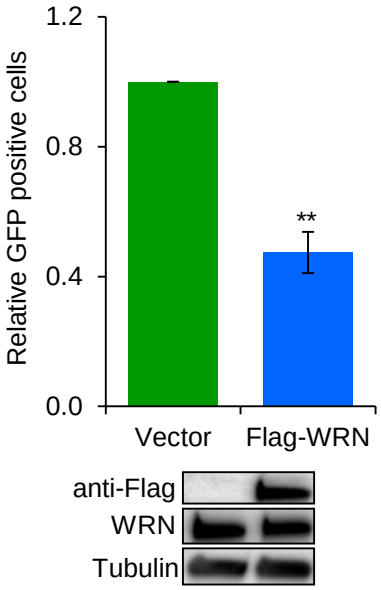


EJ2: alt-NHEJ

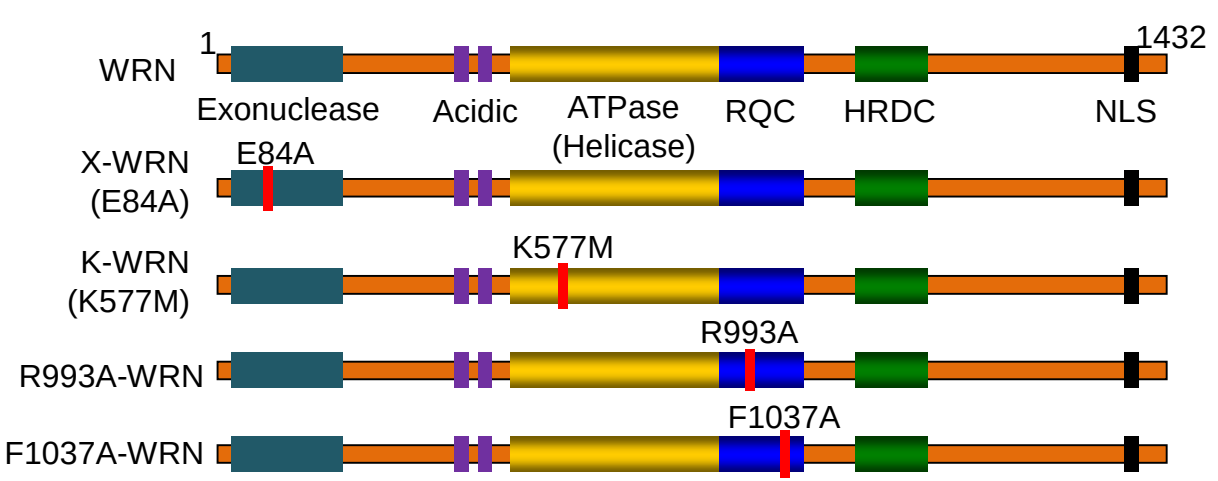


(35 nucleotide deletion, 8 nucleotide microhomology for joining)



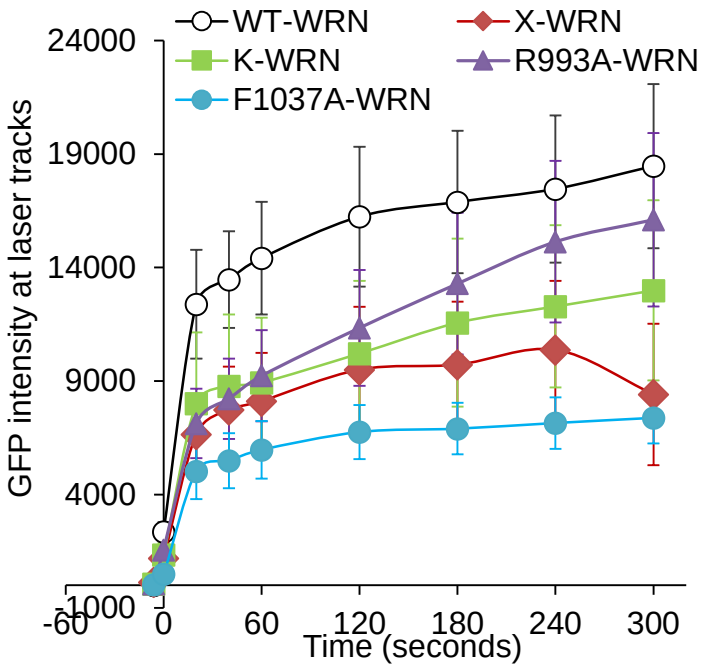


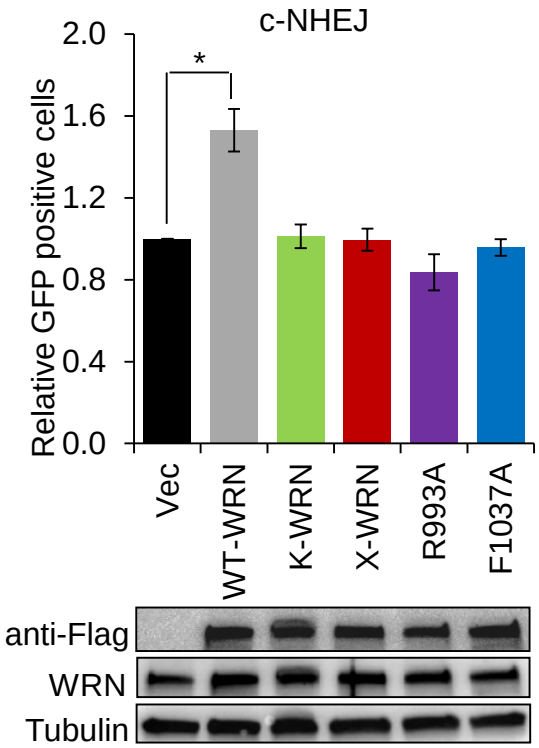
Enzymatic and non-enzymatic functions of WRN in NHEJ

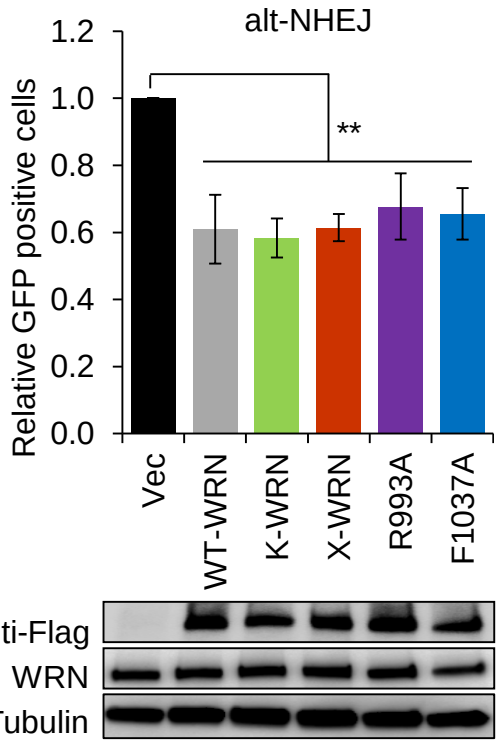


	Helicase	Exonuclease	Annealing
WRN	+++	+++	+++
X-WRN (E84A)	+++	-	+++
K-WRN (K577M)	+	+++	+++
R993A-WRN	+	-	+
F1037A-WRN	+	-	+

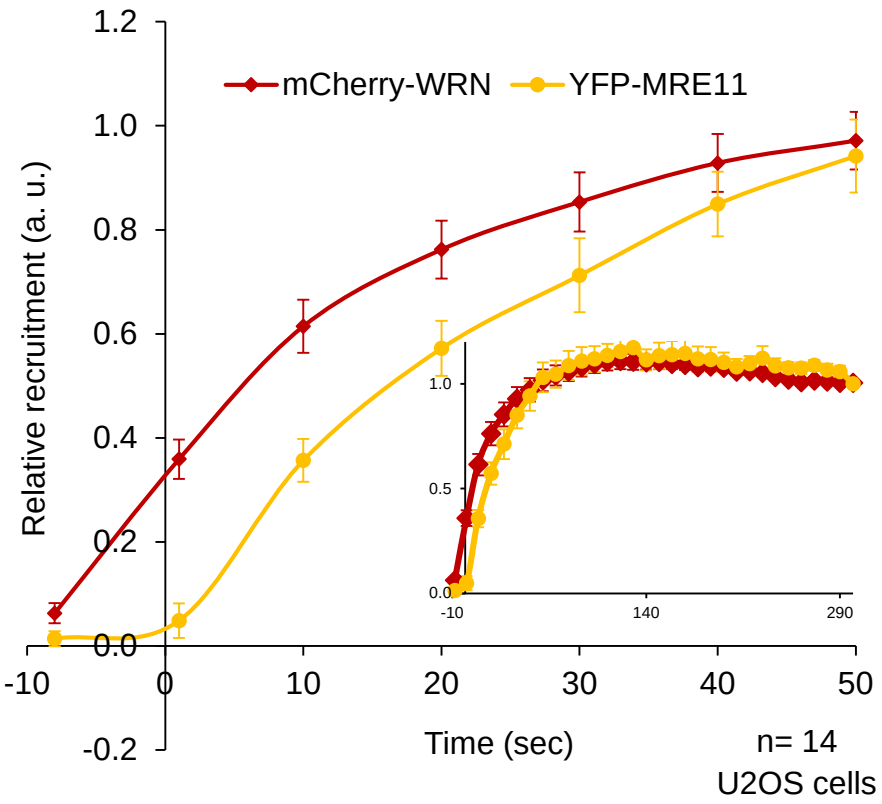
Tadakoro et al., Aging 2010 & unpublished data

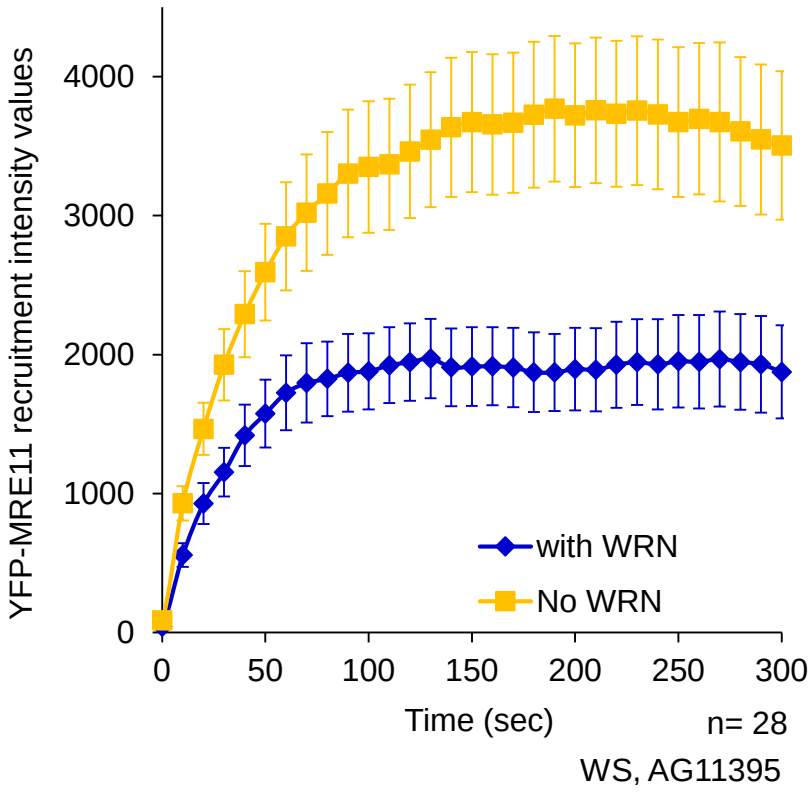




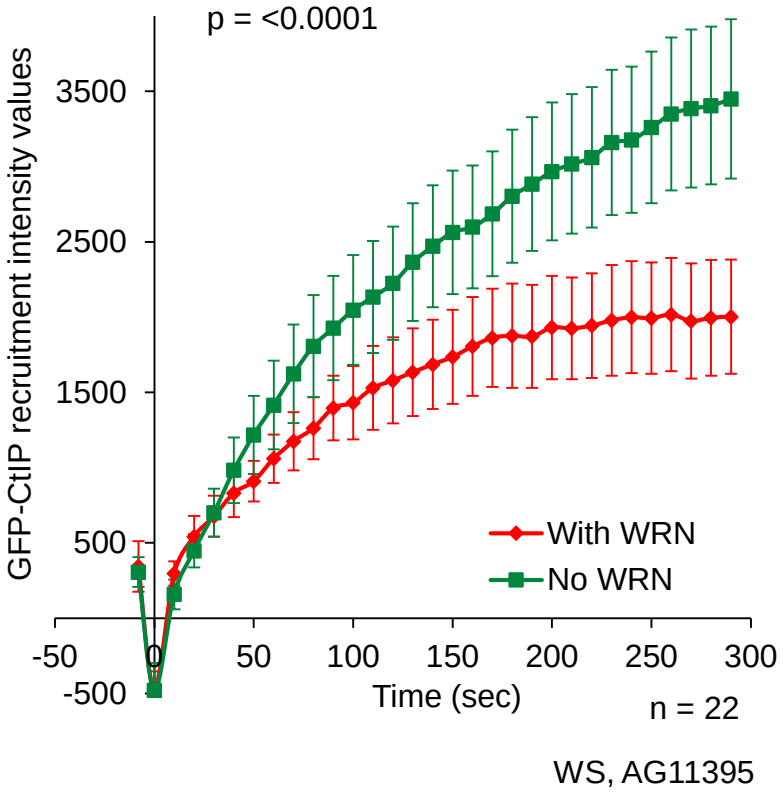


WRN suppresses the recruitment of MRE11 to DSBs

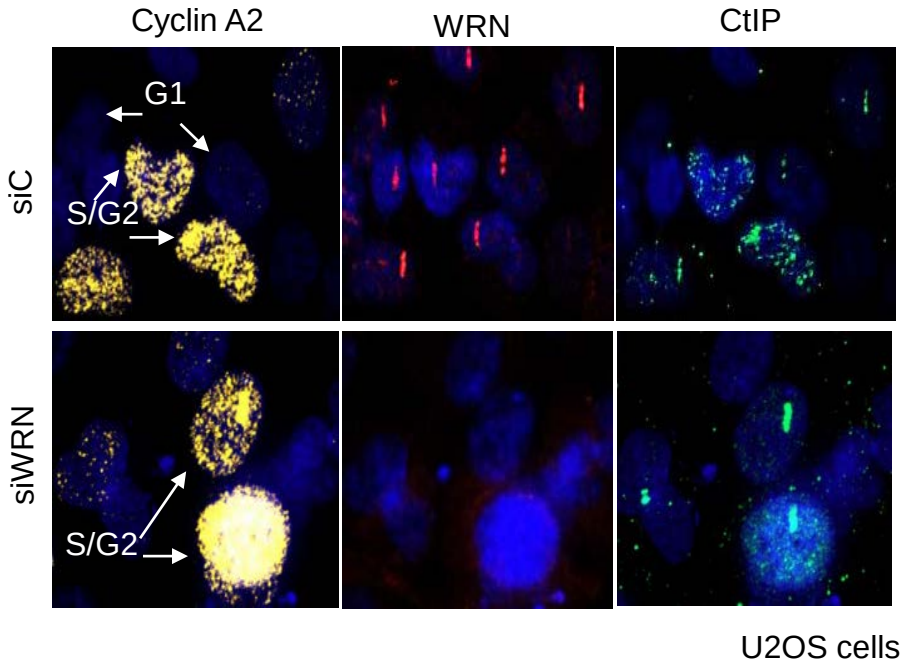




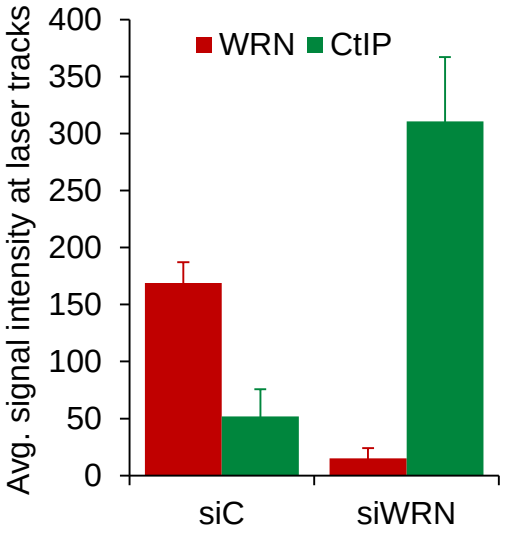
WRN suppresses the recruitment of CtIP to DSBs



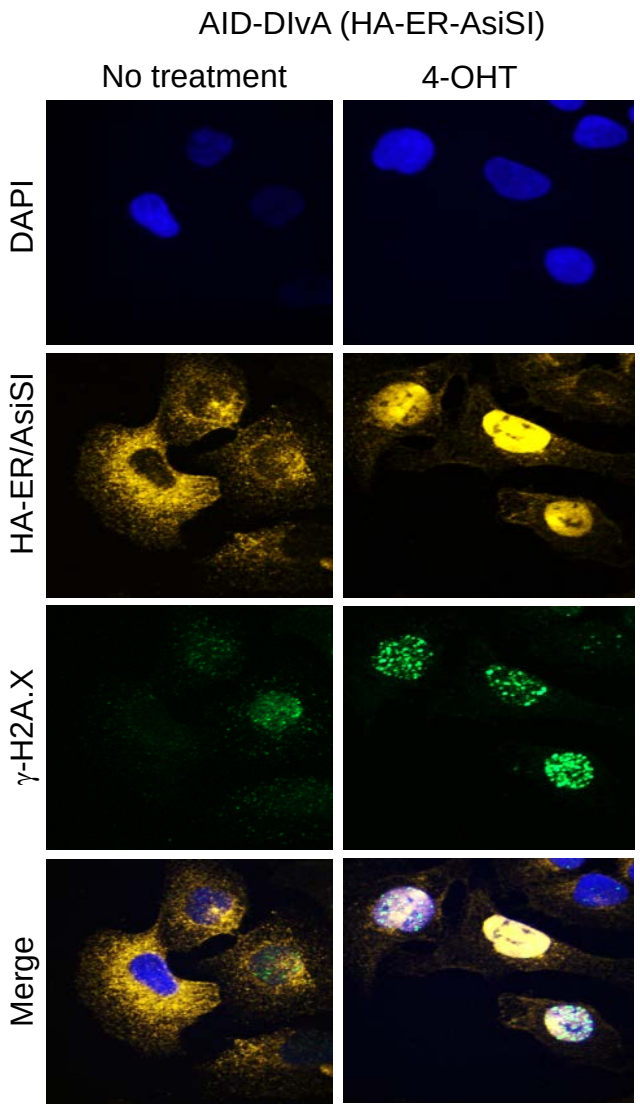
WS, AG11395

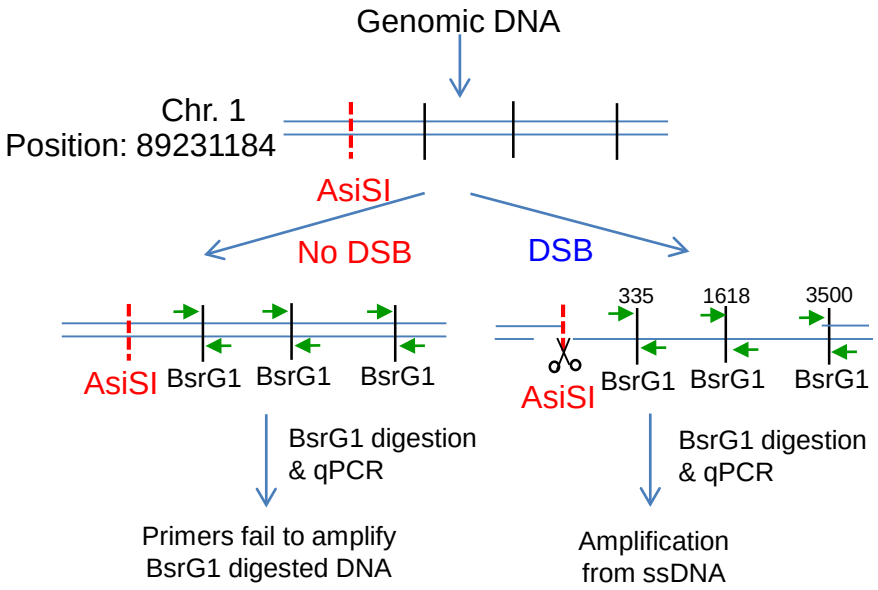


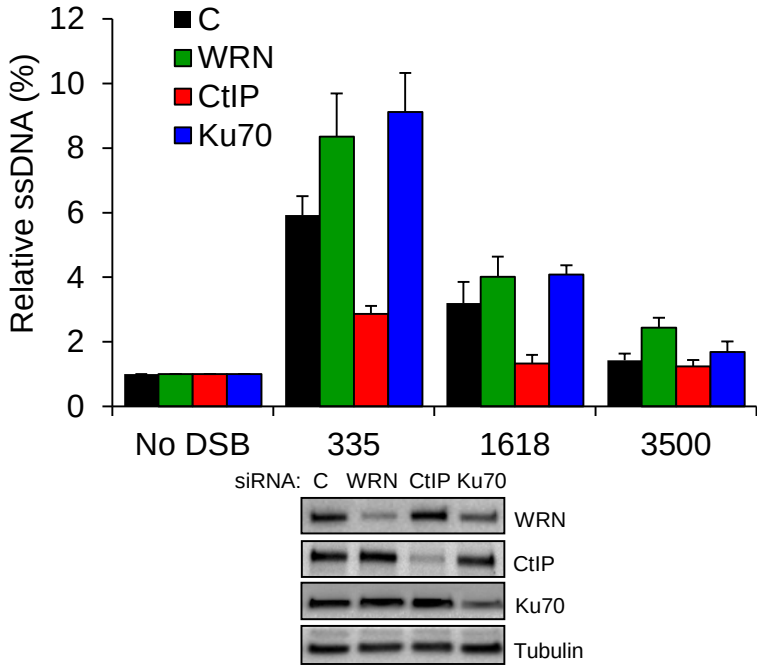
U2OS cells



WRN deficiency increases resection at AsiSI induced DSBs







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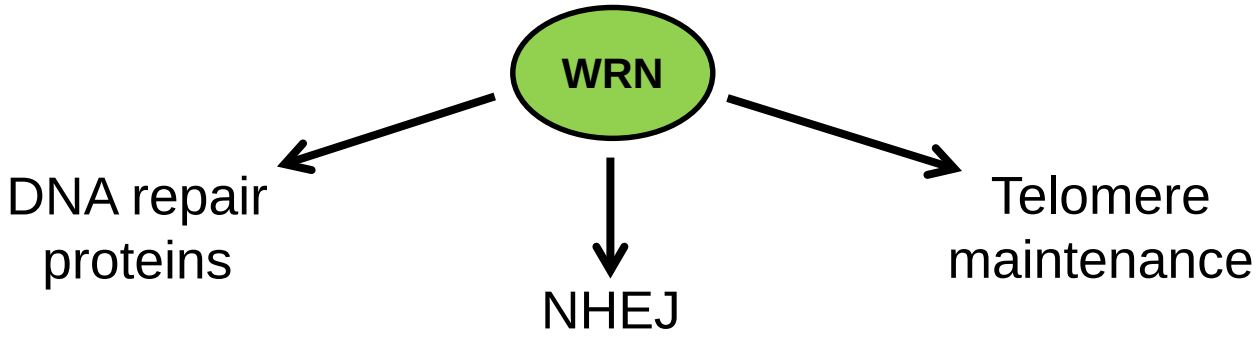

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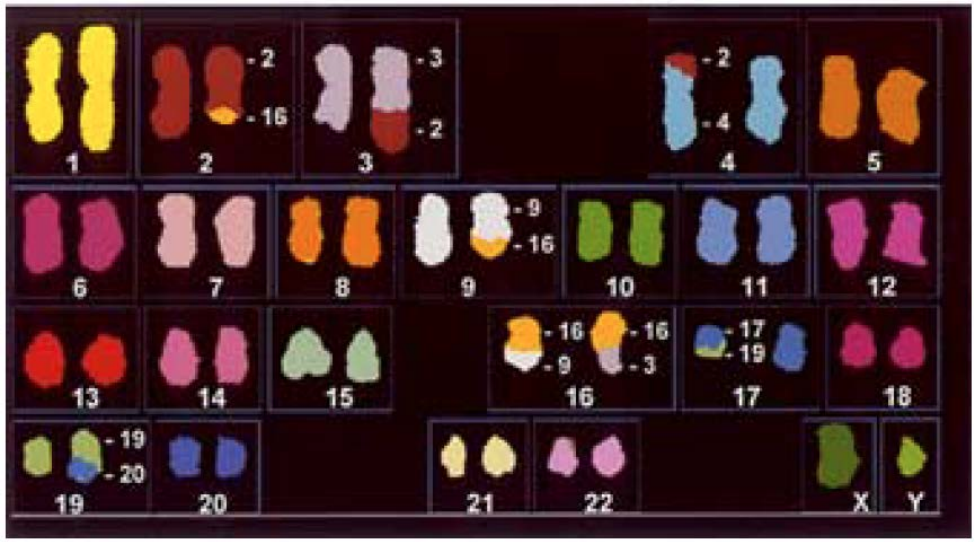

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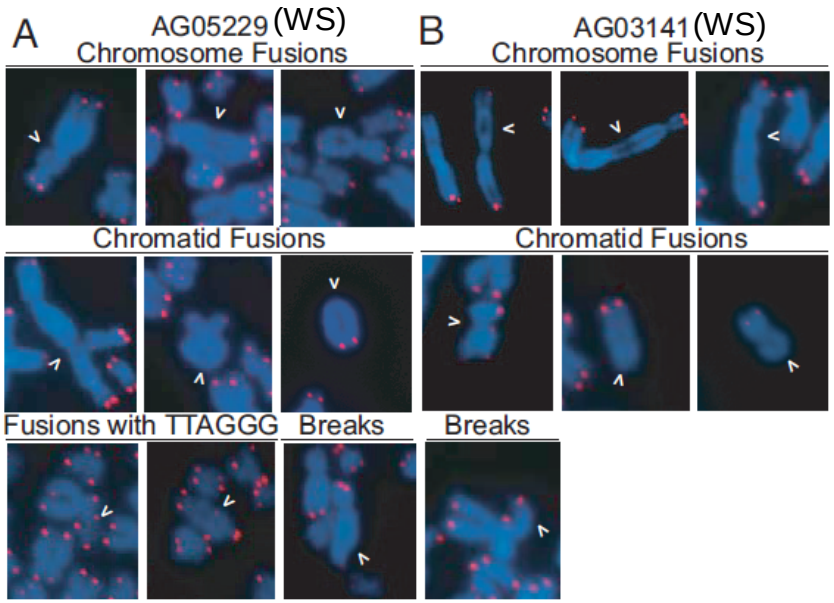
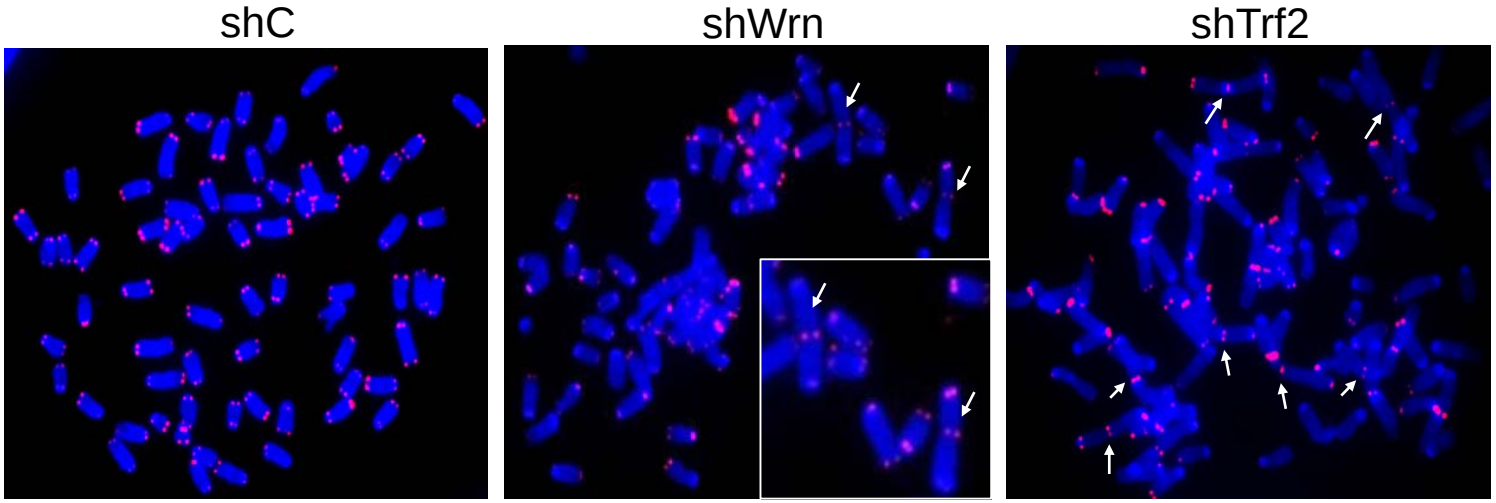
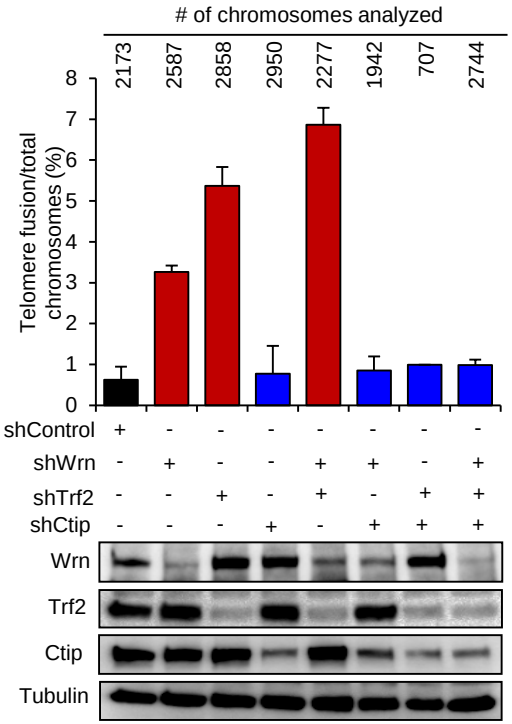


Crabbe et al., 2007 PNAS

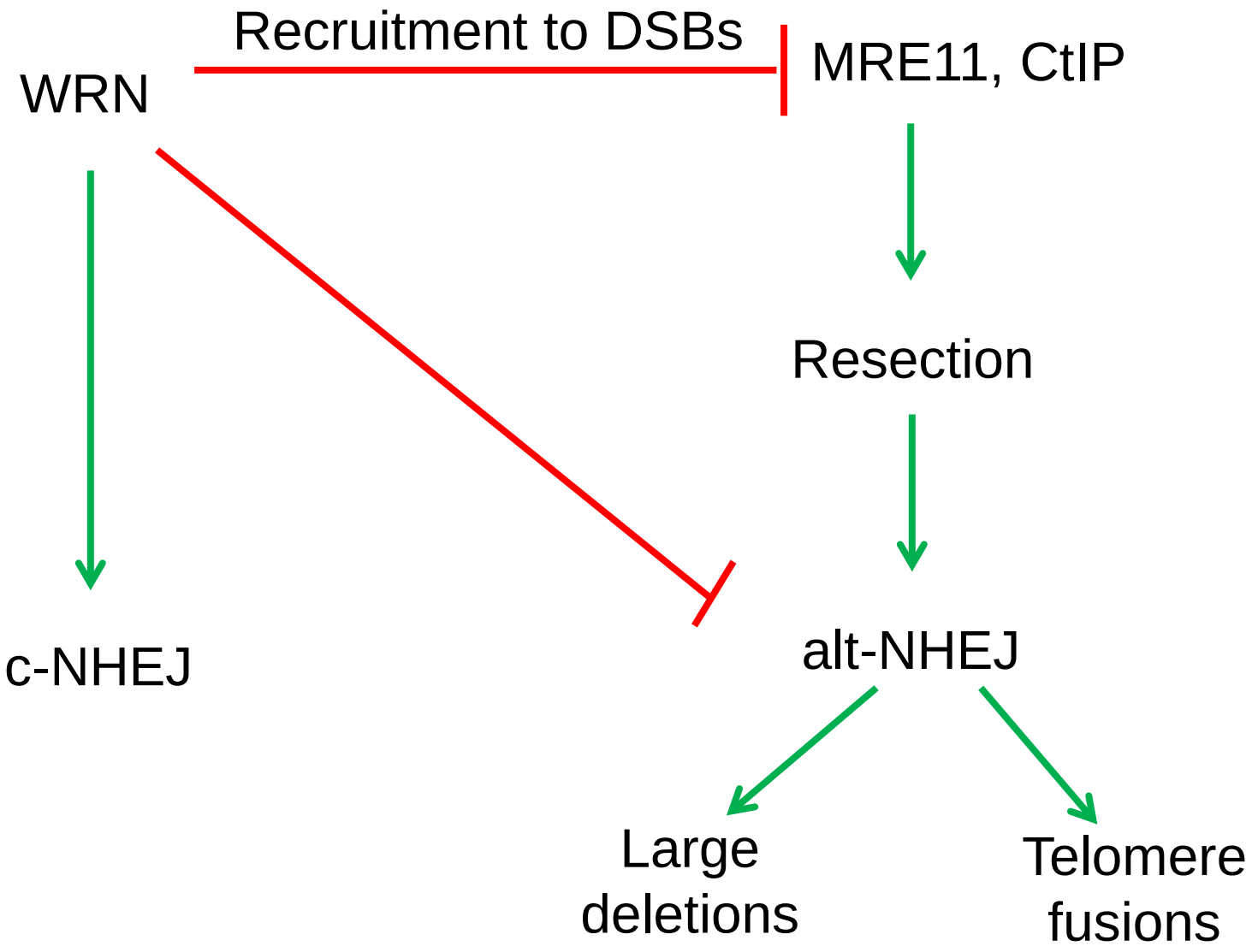
Question: Does increased alt-NHEJ activity induces telomere fusions in the absence of WRN?

Ctip is required for telomere fusions in WRN-depleted MEFs





WRN regulates pathway choice between c-NHEJ and alt-NHEJ



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