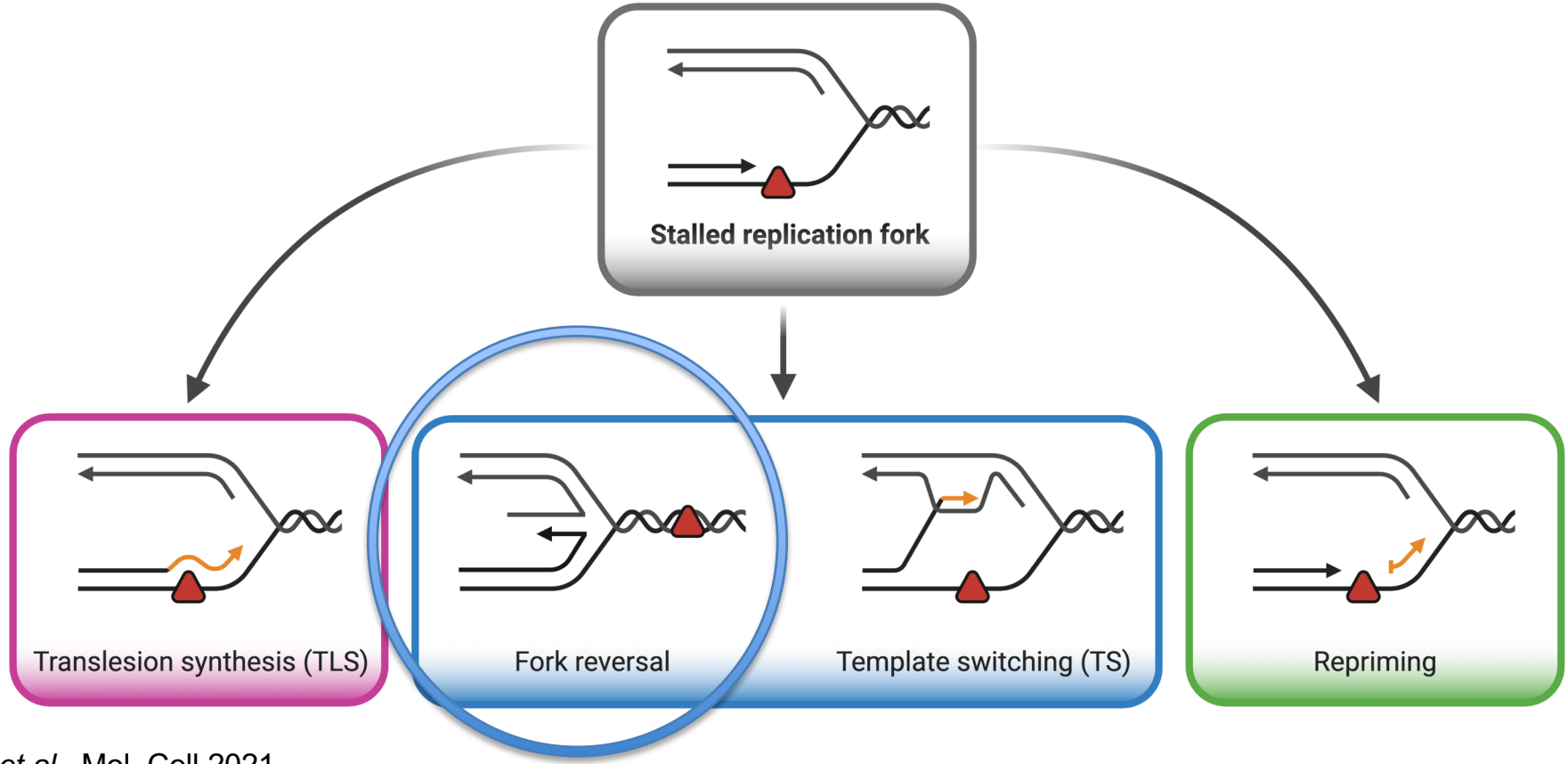
Washington University School of Medicine

Alessandro Vindigni

Linking replication fork dynamics to chemotherapy response

NIH DNA Repair Interest Group
February 15, 2022

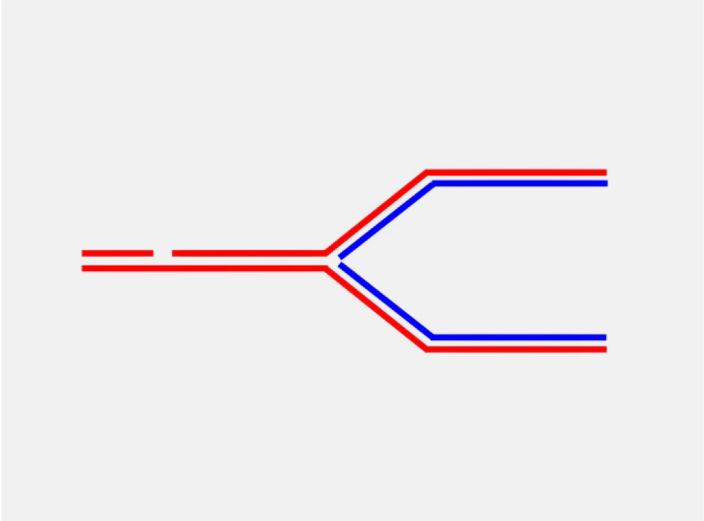
DNA replication stress response mechanisms

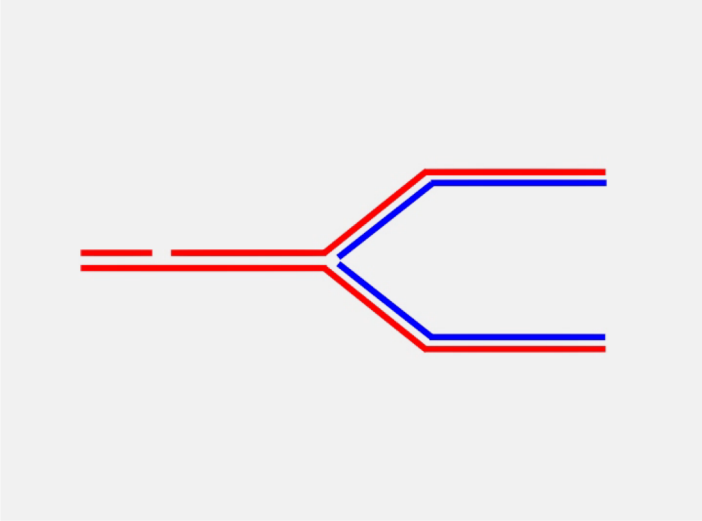


Quinet *et al.*, Mol. Cell 2021

Tirman et al., Crit. Rev. Biochem. Mol. Biol. 2021

Replication fork reversal and restart





Berti *et al.*, Nat. Struct. Mol. Biol. 2013

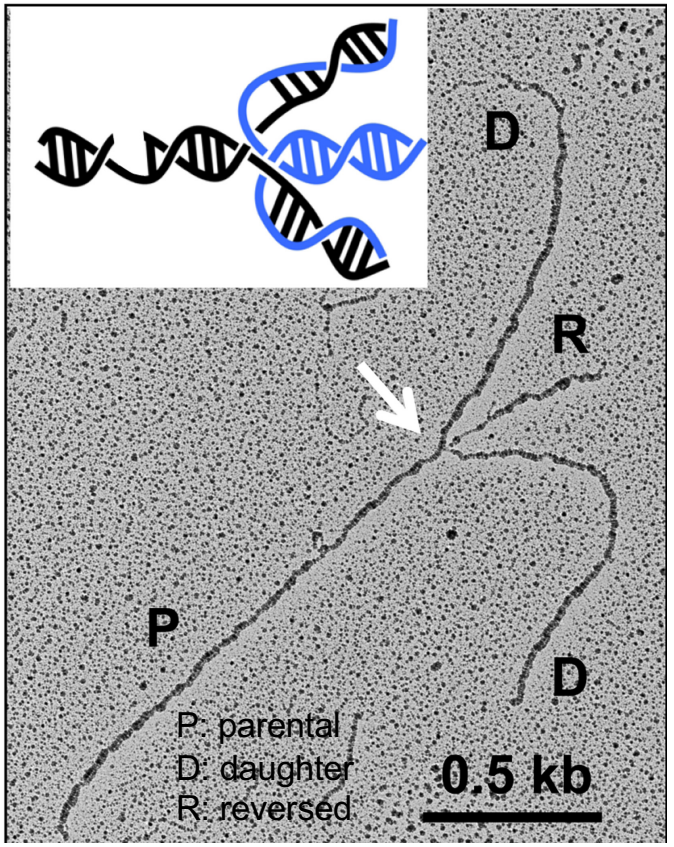
Thangavel *et al.*, J. Cell Biol. 2015

Zellweger *et al.*, J. Cell Biol. 2015

Berti & Vindigni, Nat. Struct. Mol. Biol. 2016

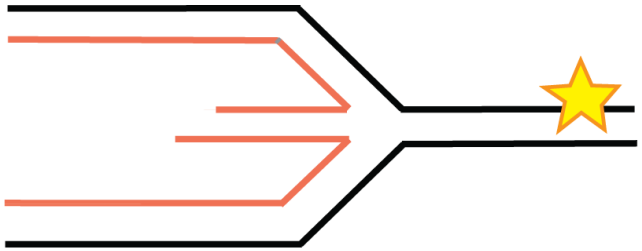
Electron microscopy

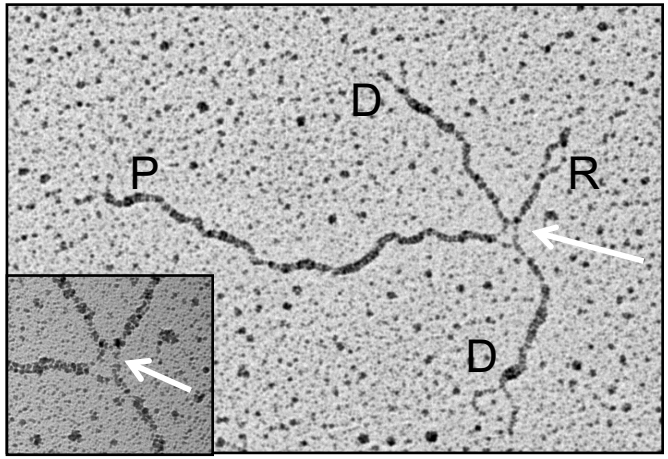
Reversed replication fork

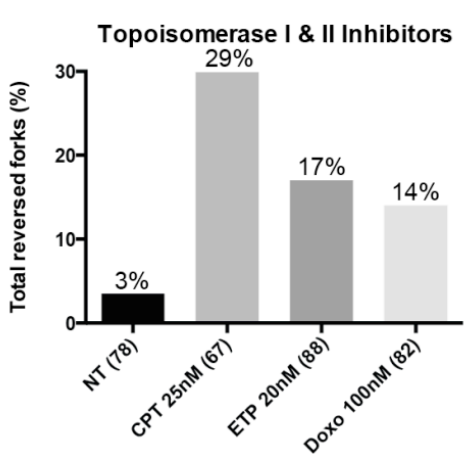


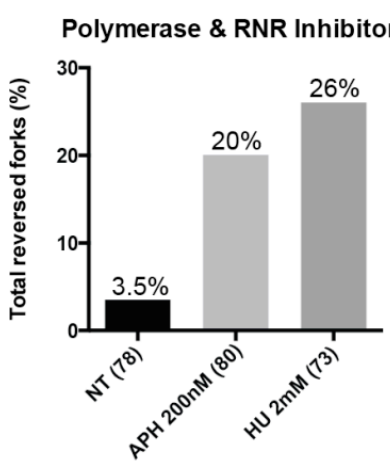
Fork reversal is a general response to drug-induced replication stress

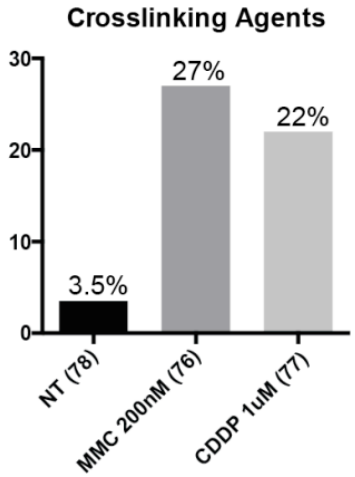
Fork reversal





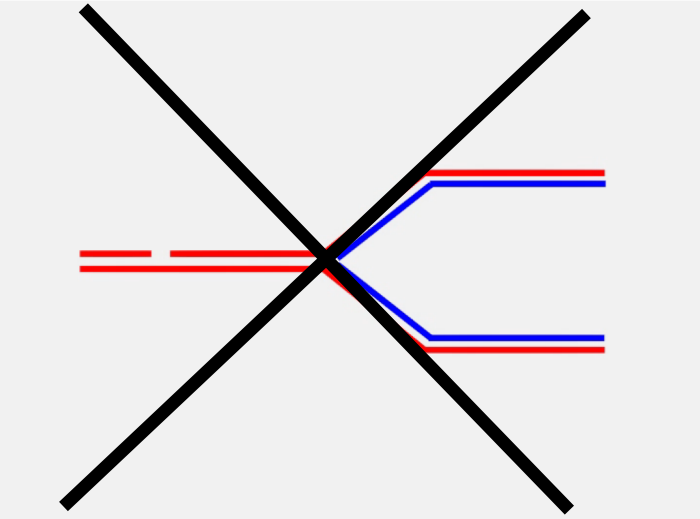


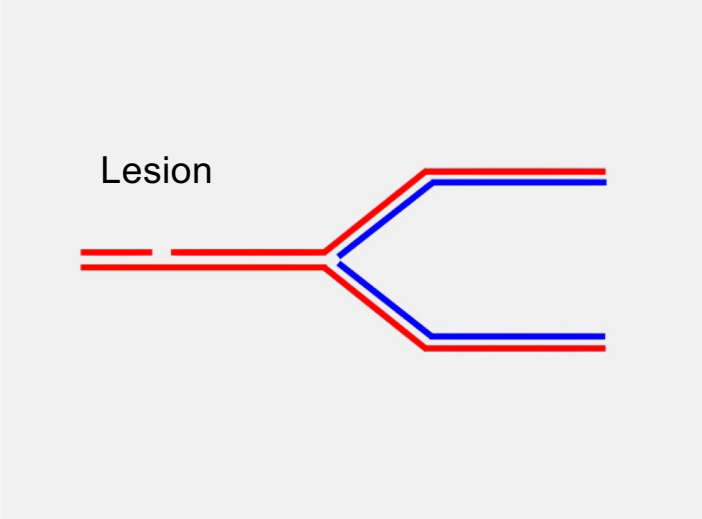




Zellweger *et al.*, J. Cell Biol. 2015

Replication fork reversal and restart





Block replication fork reversal

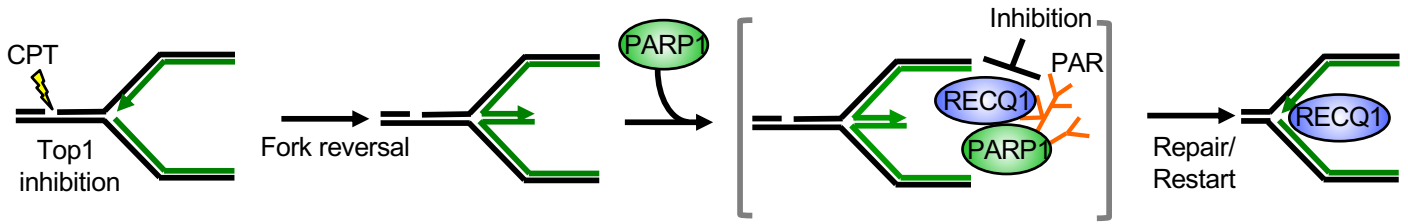
Berti *et al.*, Nat. Struct. Mol. Biol. 2013

Thangavel *et al.*, J. Cell Biol. 2015

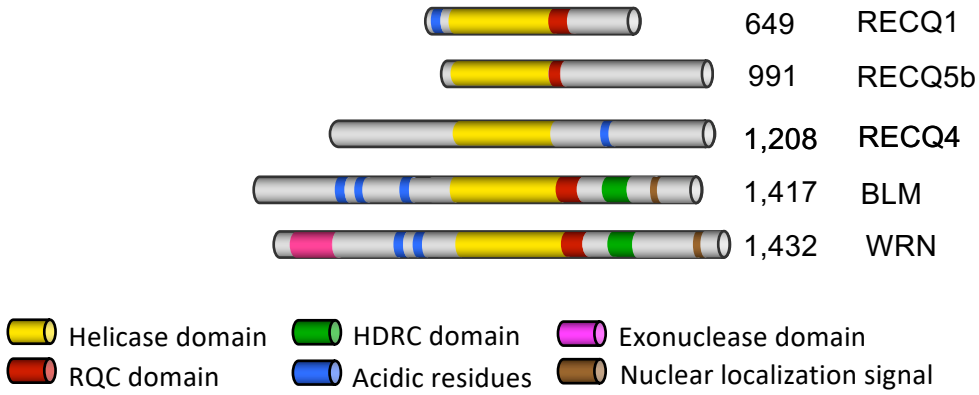
Zellweger *et al.*, J. Cell Biol. 2015

Berti & Vindigni, Nat. Struct. Mol. Biol. 2016

RECQ1 is essential to restart replication forks reversed by DNA topoisomerase I inhibition

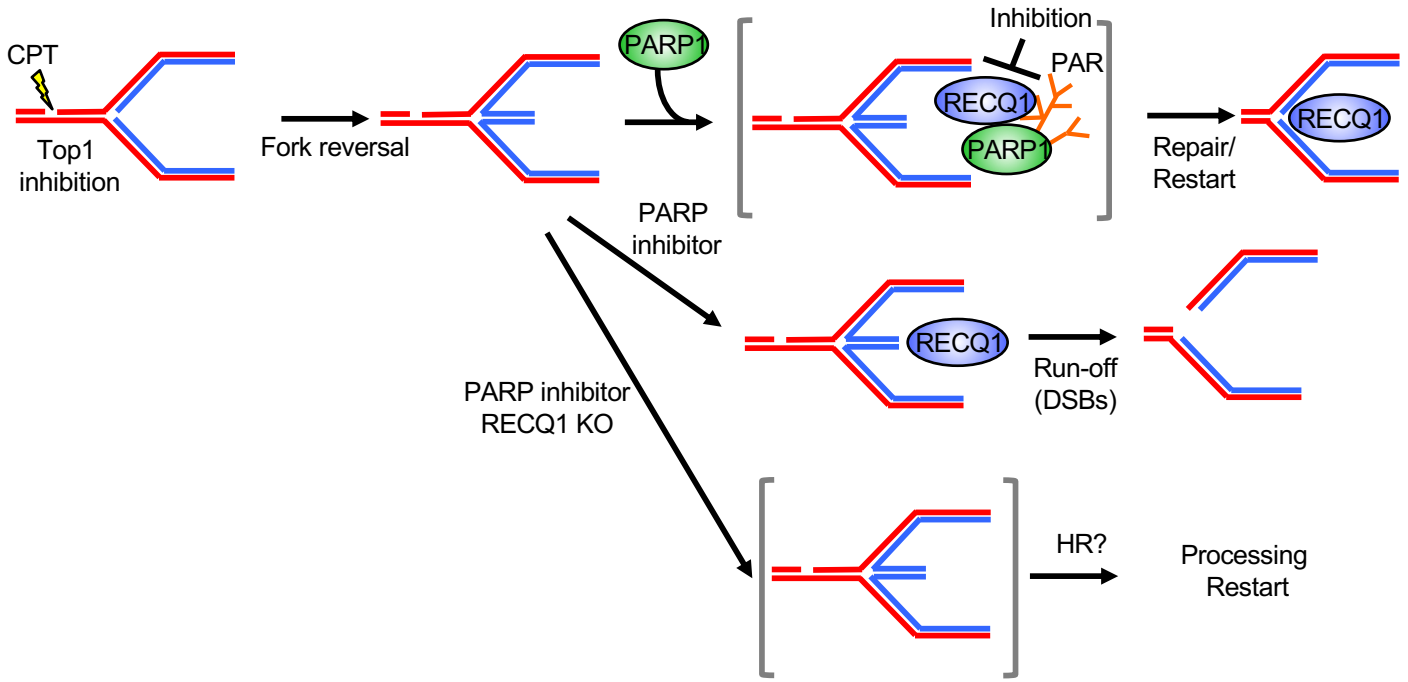


Human RecQ helicase family



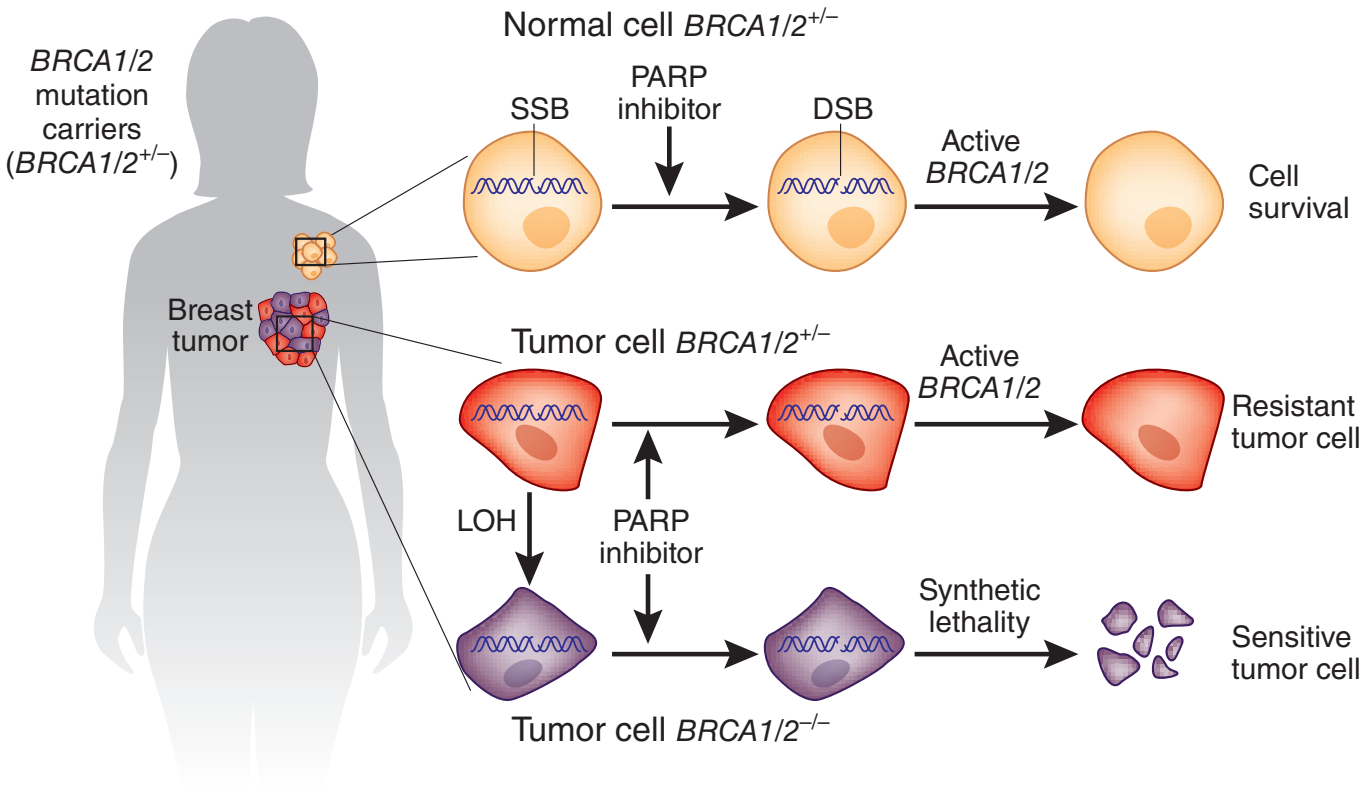
Berti *et al.,* Nat. Struct. Mol. Biol. 2013

RECQ1 is essential to restart replication forks reversed by DNA topoisomerase I inhibition



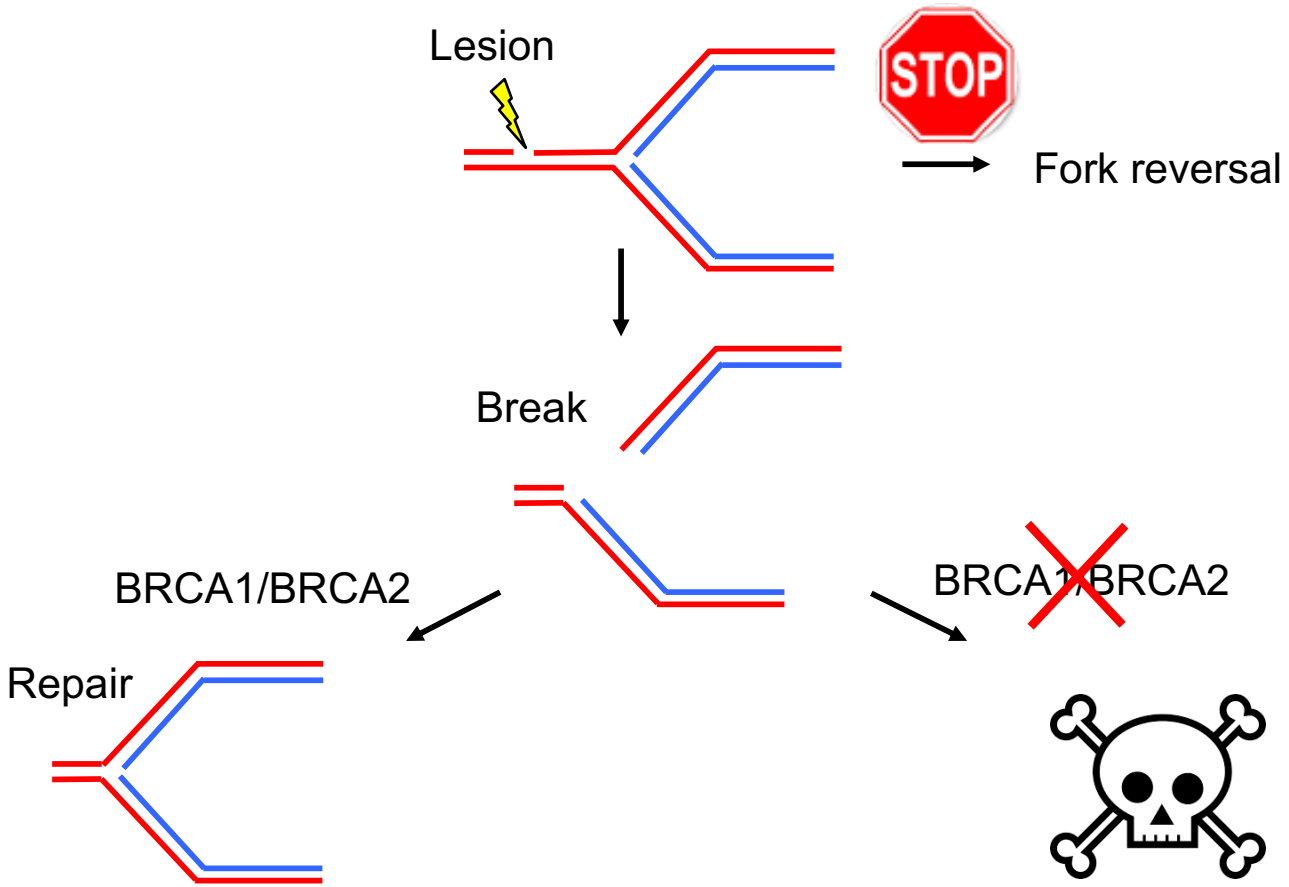
Berti *et al.,* Nat. Struct. Mol. Biol. 2013

Synthetic lethality in tumors from BRCA1 and BRCA2 mutation carriers treated with PARP inhibitors

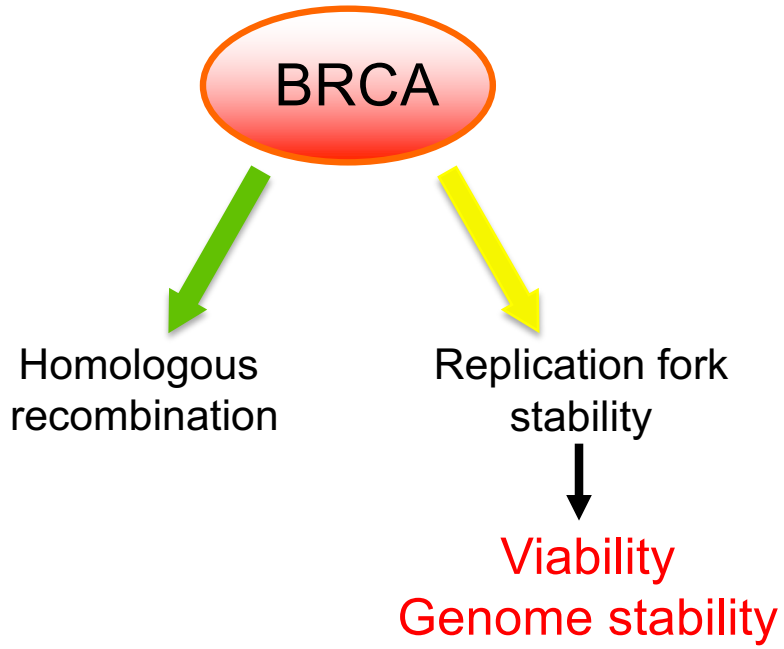


Polyak & Garber, Nat. Medicine 2011

BRCA1 and BRCA2 cancer predisposition genes

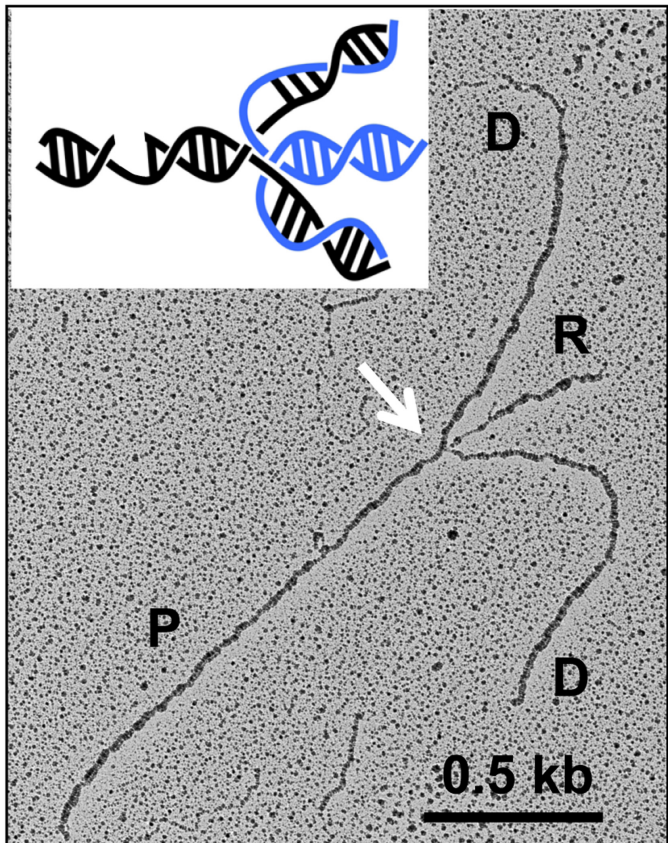


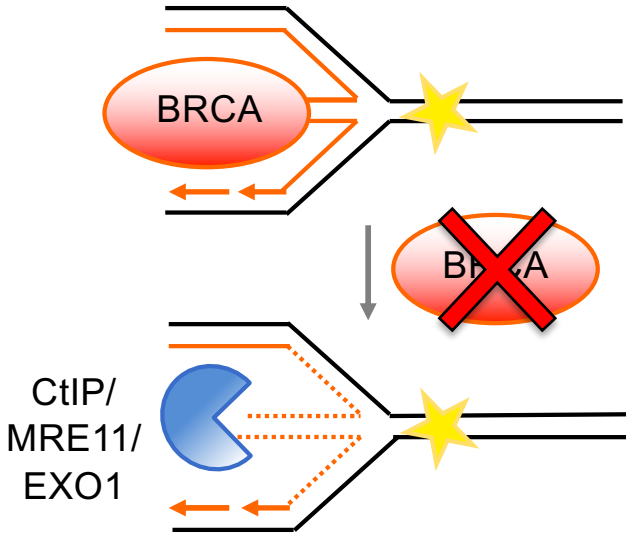
BRCA1 and BRCA2 function in replication fork stability is essential for viability and genome stability



BRCA proteins protect reversed forks from nucleolytic degradation

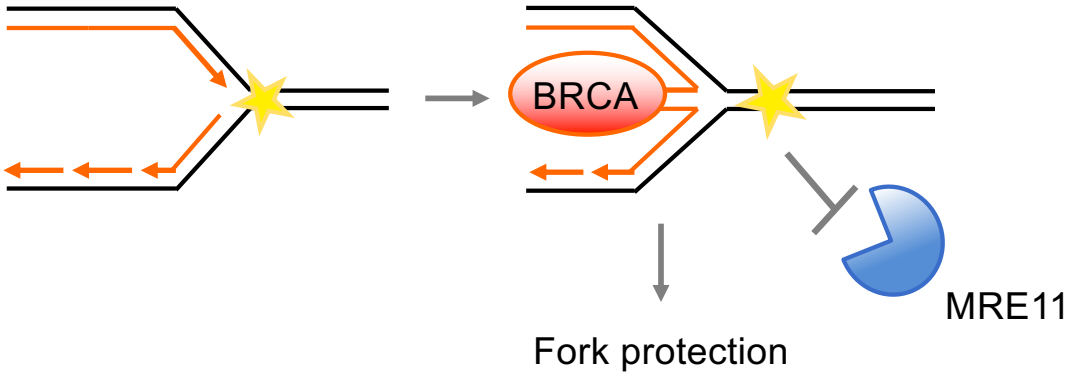
Electron microscopy





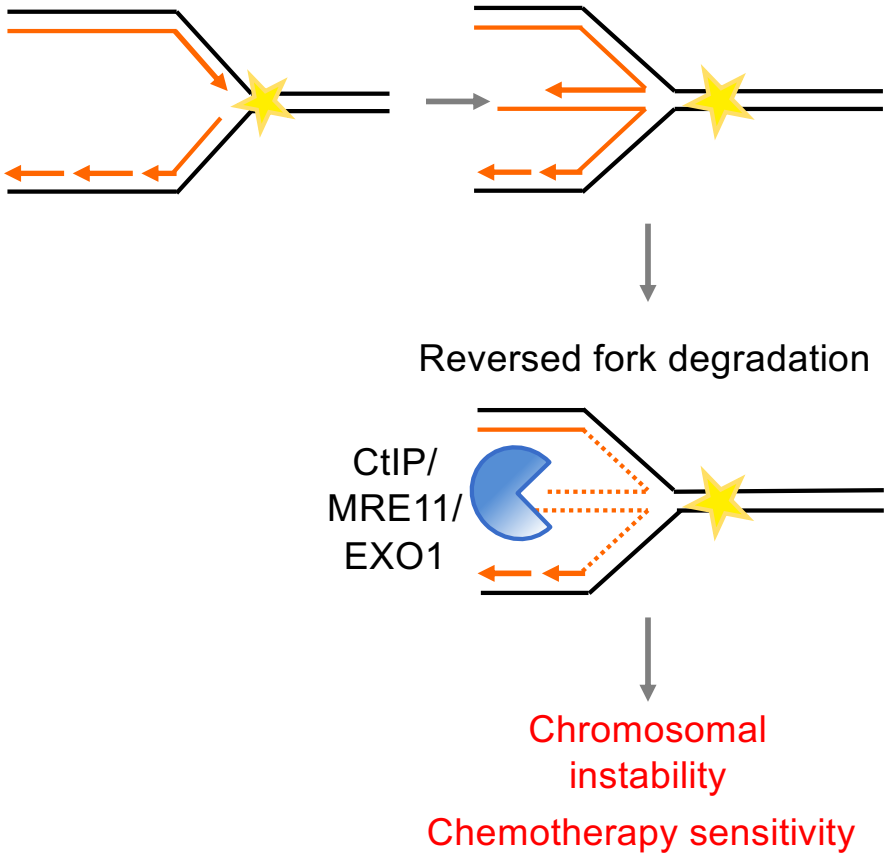
Lemaçon *et al*., Nat. Commun. 2017
Mijic *et al*., Nat. Commun. 2017
Kolinjivadi *et al*., Mol. Cell 2017
Taglialatela *et al*., Mol. Cell 2017

BRCA1/2 protect reversed forks from MRE11-mediated degradation



Hashimoto *et al*., Nat. Struct. Mol. Biol. 2010
Schlacher *et al.*, Cell 2011
Ying *et al*., Cancer Res. 2012
Ray Chaudhuri *et al*., Nature 2016
Lemaçon *et al*., Nat. Commun. 2017
Mijic *et al*., Nat. Commun. 2017
Kolinjivadi *et al*., Mol. Cell 2017
Taglialatela *et al*., Mol. Cell 2017

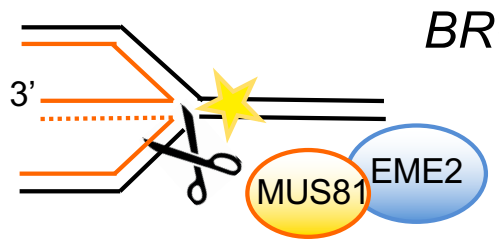
MRE11 is toxic in the absence of BRCA1/2



BRCA-deficient tumors

MUS81-EME2 and POLD3 are required to restart resected forks in BRCA2- but not BRCA1-deficient cells

BRCA2-deficient cells

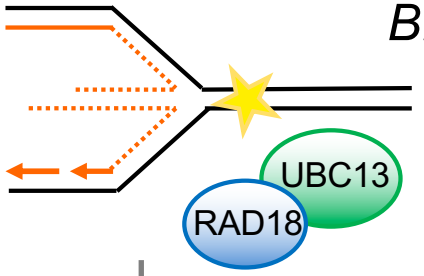


Fork restart

Viability

Lemaçon *et al*., Nat. Commun. 2017

BRCA1-deficient cells

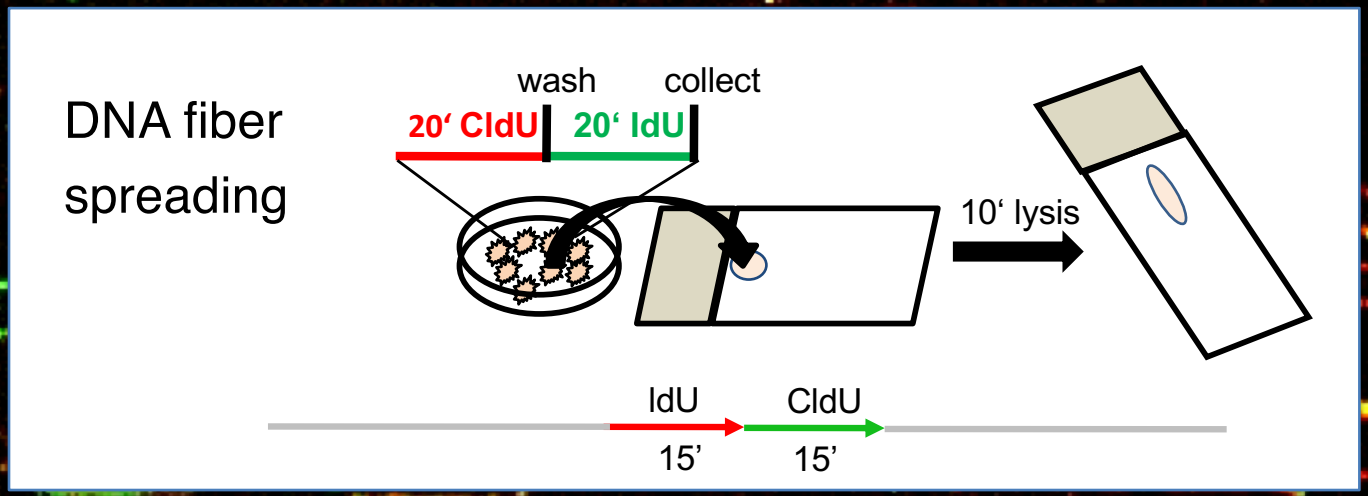


Fork restart

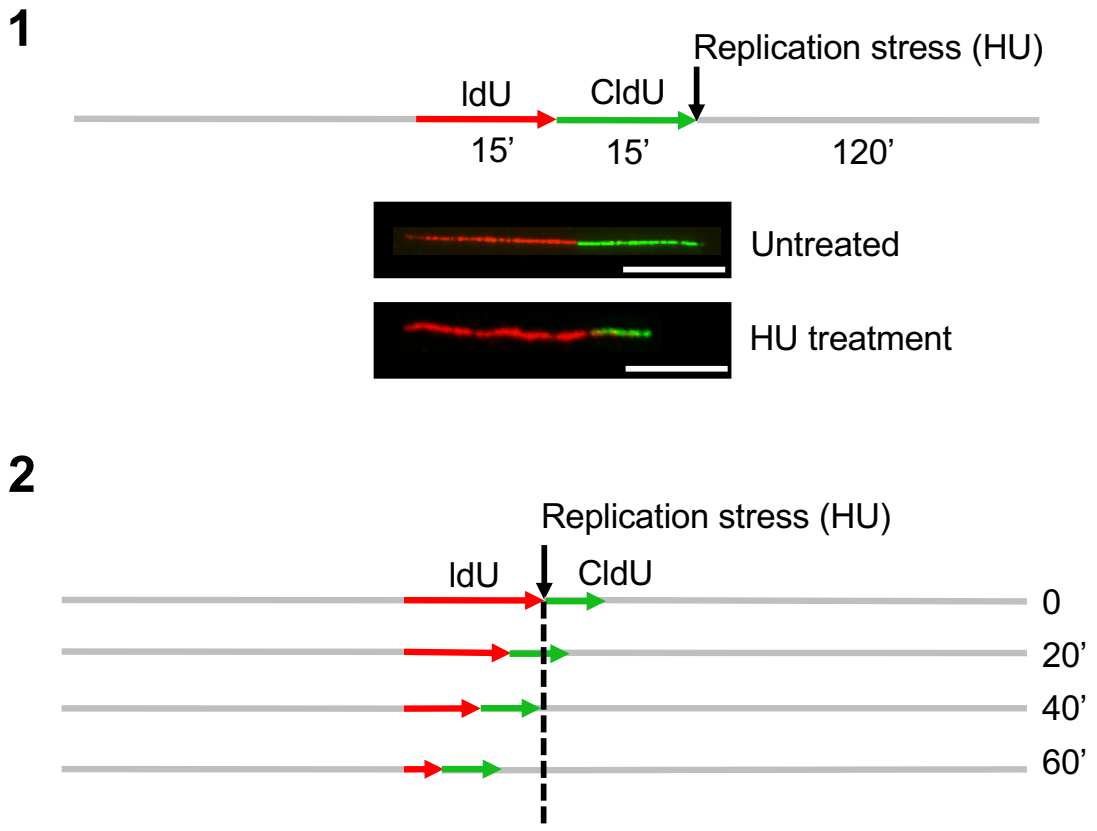
Viability

Cybulla et al., *unpublished*

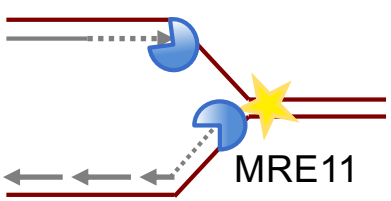
Single-molecule genome-wide DNA fiber analysis

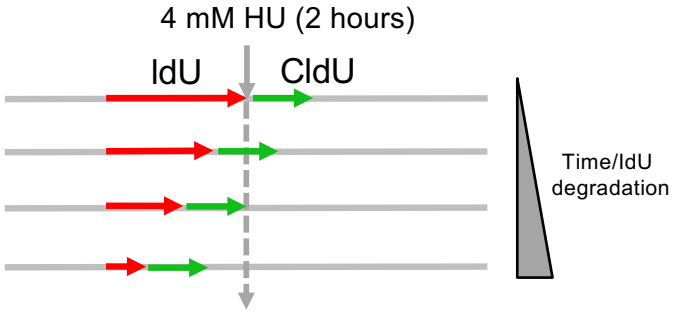


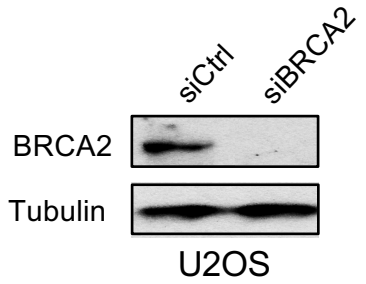
Single-molecule genome-wide DNA fiber analysis in the presence of replication stress

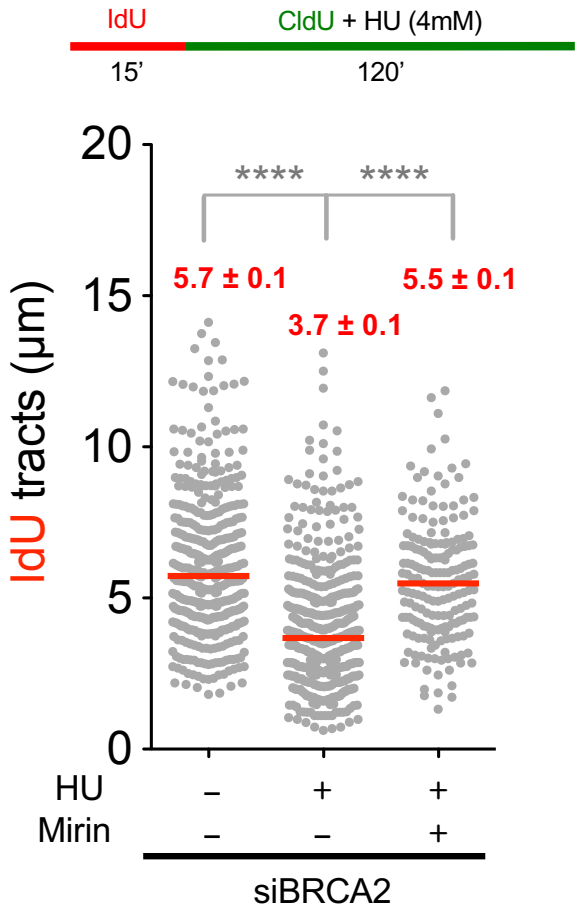


Replication forks are extensively resected by MRE11 in the absence of BRCA2



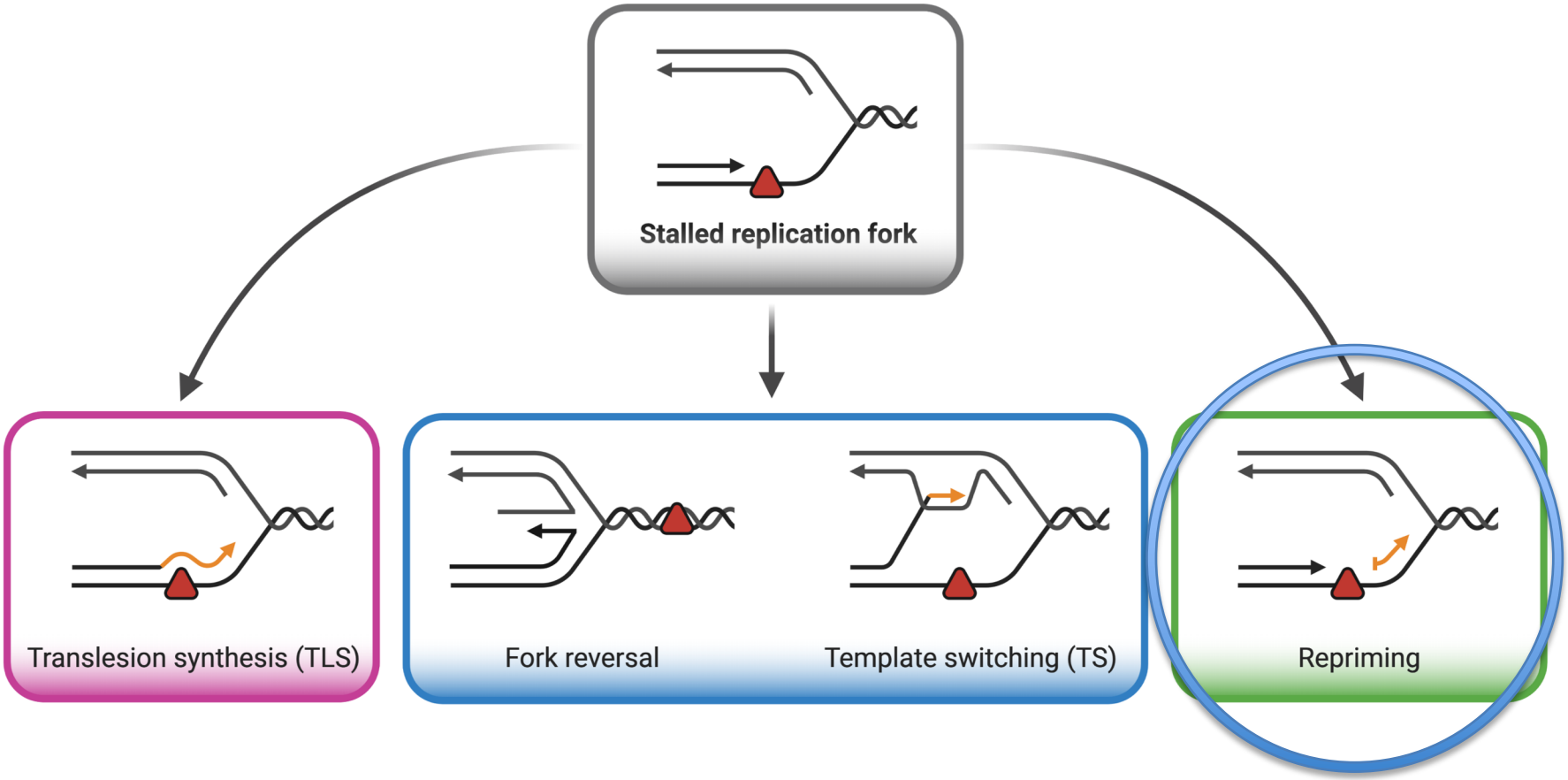




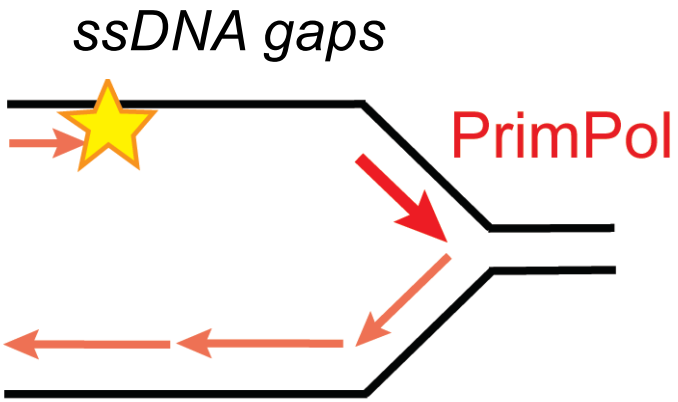


1µm ≈ 2.6 kb

DNA replication stress response mechanisms



Replication fork repriming

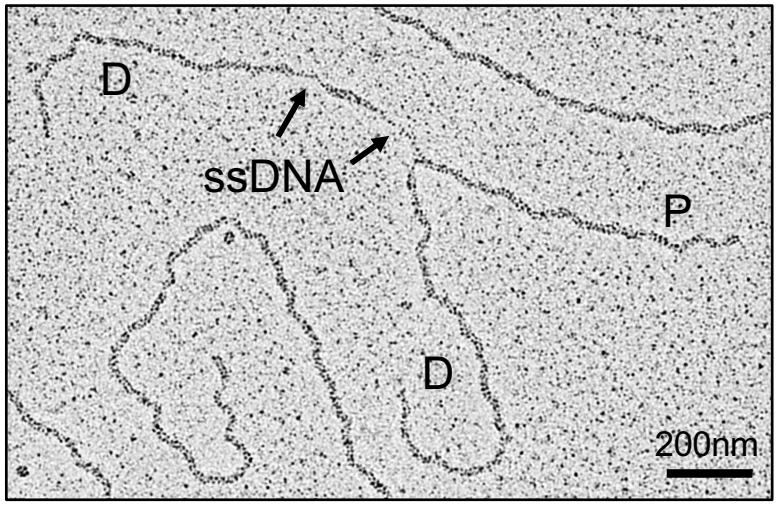


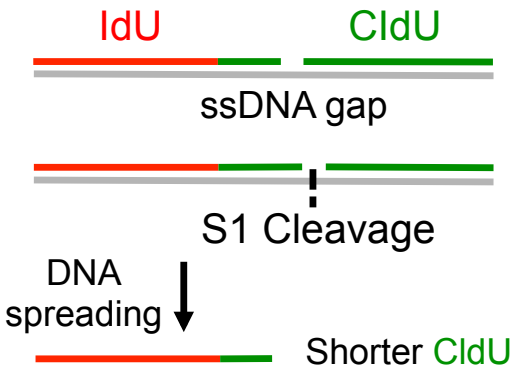
Mourón et al., Nat. Struct. Mol. Biol., 2013

Gárcia-Gómez et al., Mol. Cell 2013

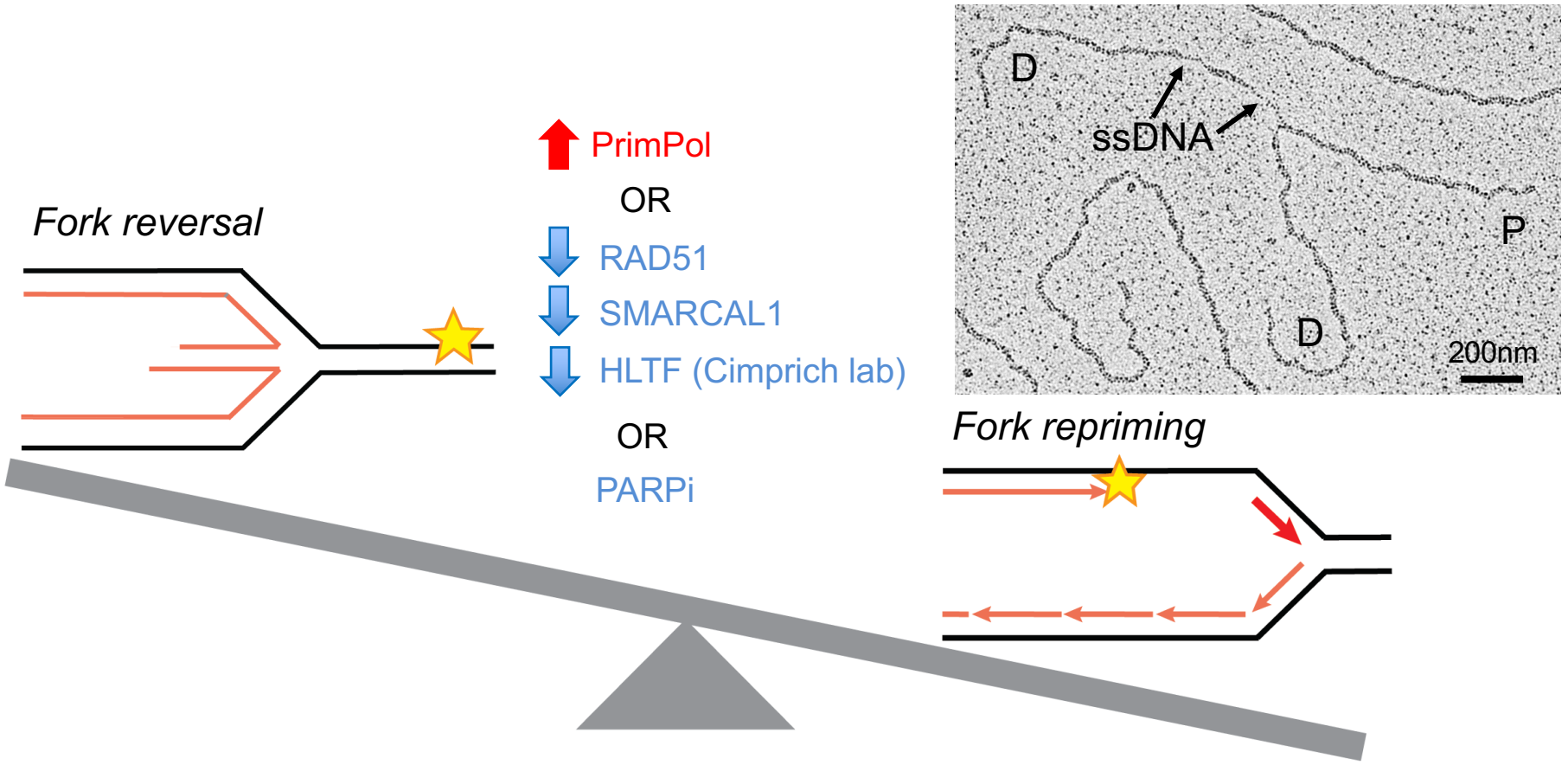
Bianchi et al., Mol. Cell 2013

Wan et al., EMBO Rep. 2013



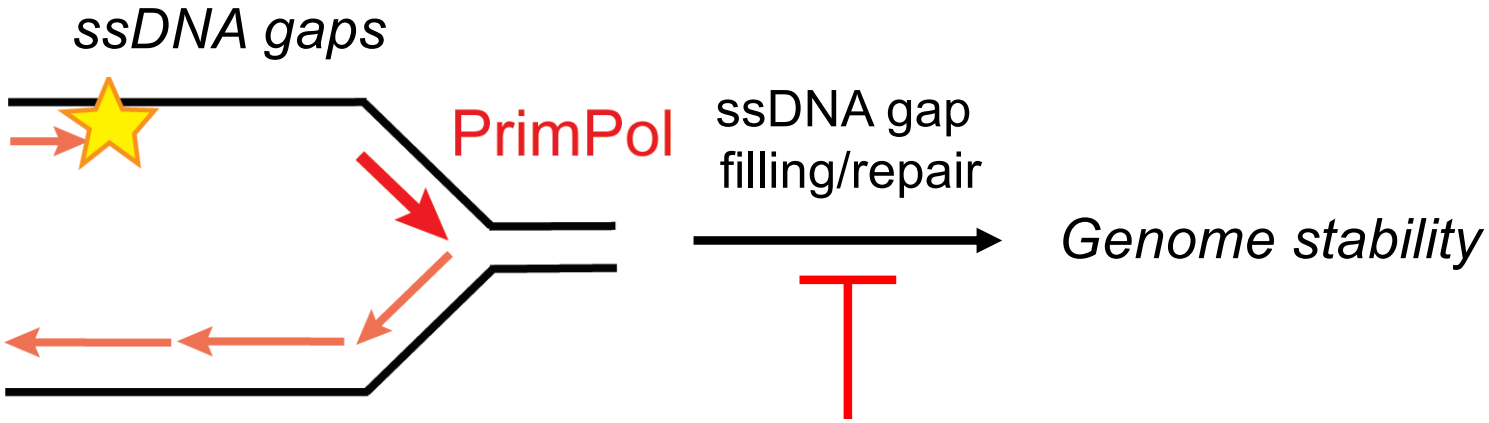


Quinet *et al.,* Methods Enymol. 2020



Quinet *et al.,* Mol. Cell 2020
Bai *et al.*, Mol. Cell 2020
Genois *et al.*, Mol Cell 2020

How are the PRIMPOL-dependent ssDNA gaps filled/repaired?

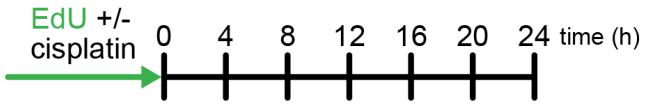


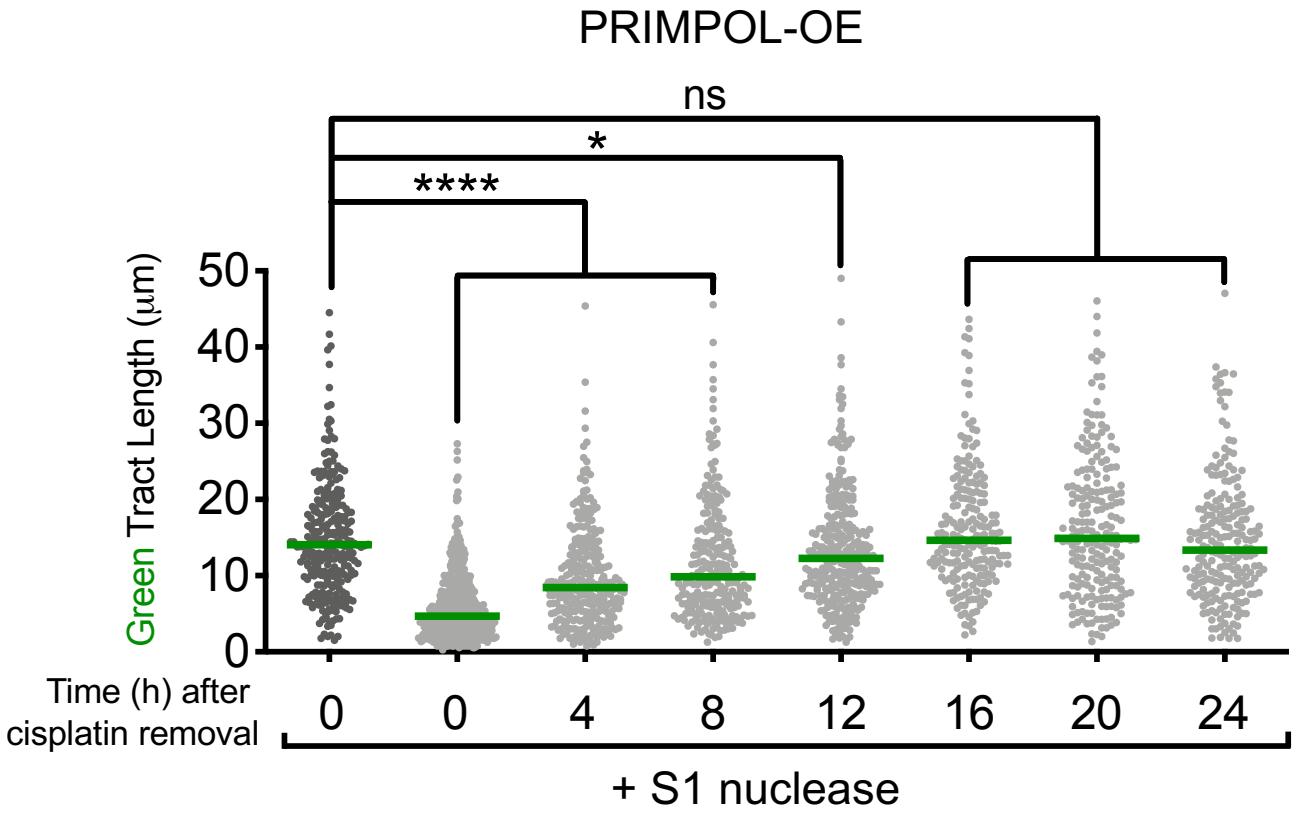
Genome instability
Chemotherapy sensitivity

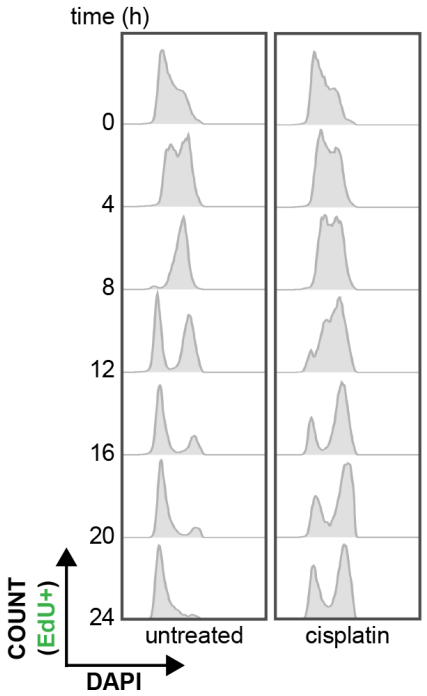
Cong *et al.*, Mol. Cell 2021
Nayak *et al.*, Sci. Adv. 2020
Simoneau *et al.*, Genes Dev. 2021
Taglialatela *et al.*, Mol. Cell 2021

Annabel Quinet *Stephanie Tirman*

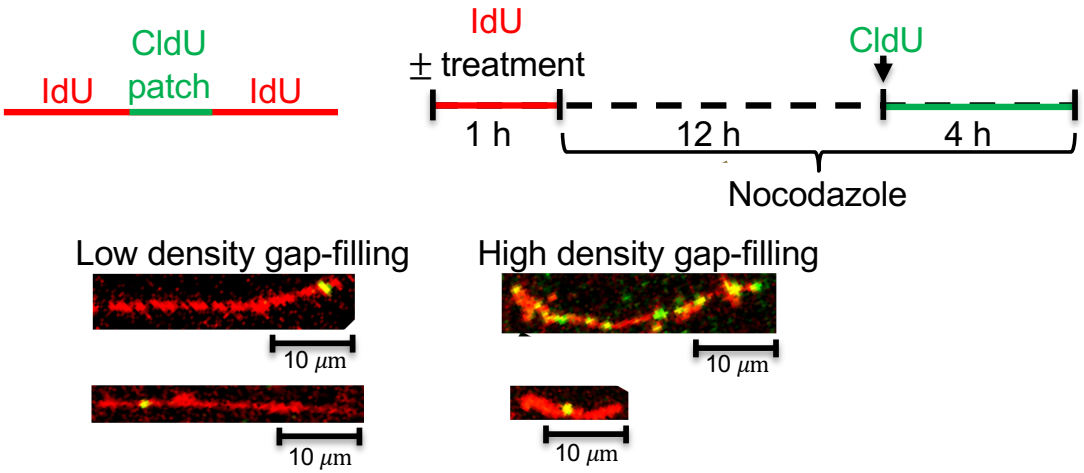
PRIMPOL-dependent ssDNA gap are repaired in late-S/G2 phase



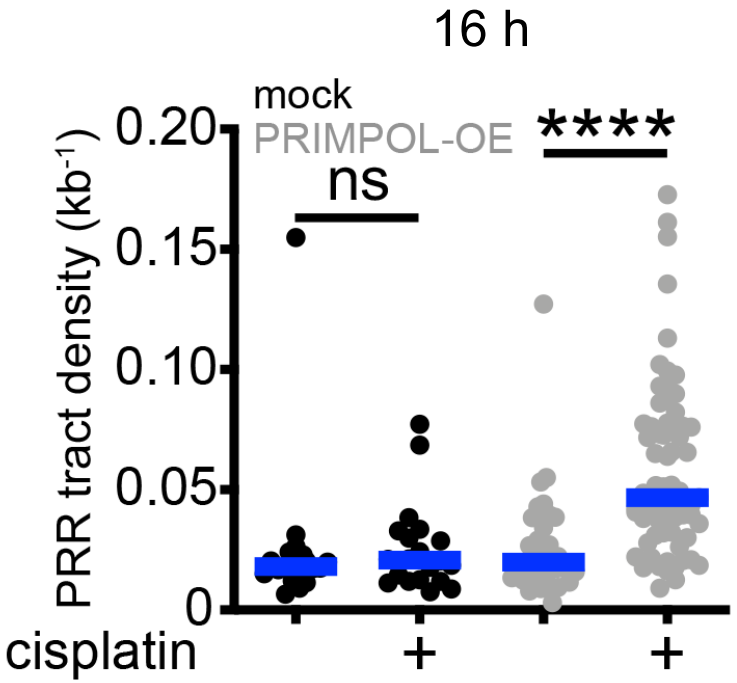




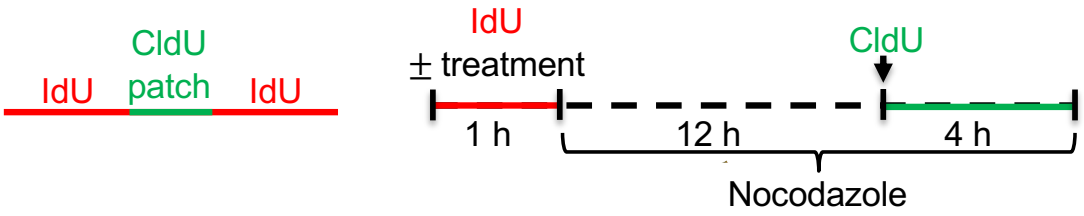
ssDNA gaps repair in G2 in PRIMPOL overexpressing cells

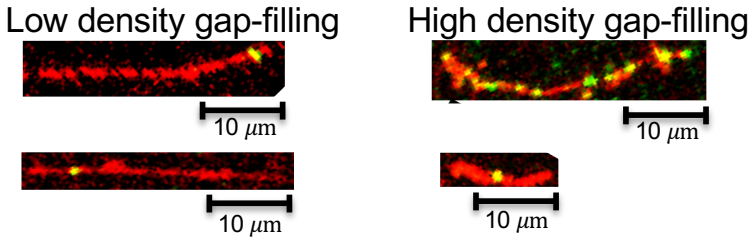


PRIMPOL-OE

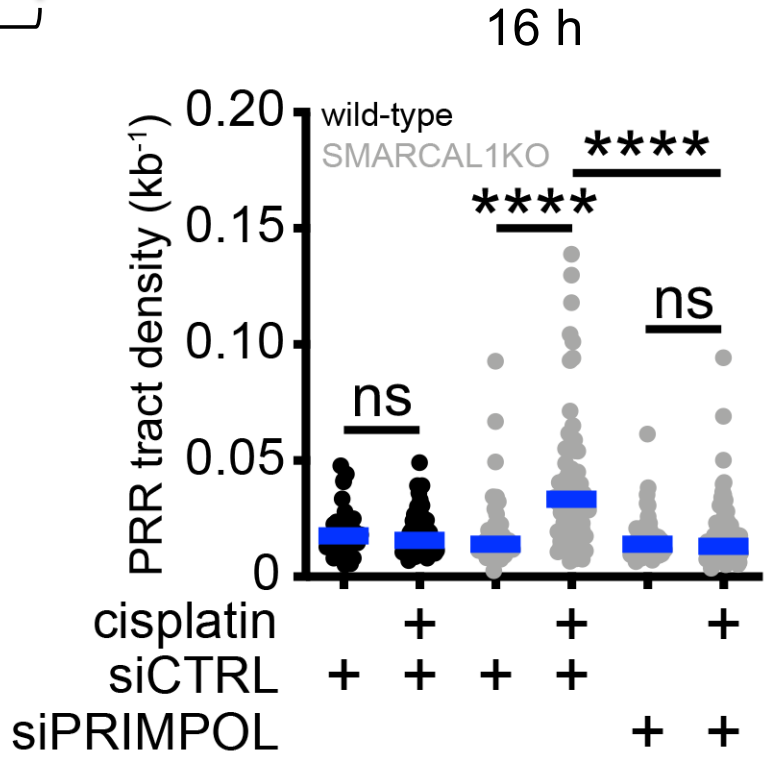


ssDNA gap repair in G2 in SMARCAL1KO cells

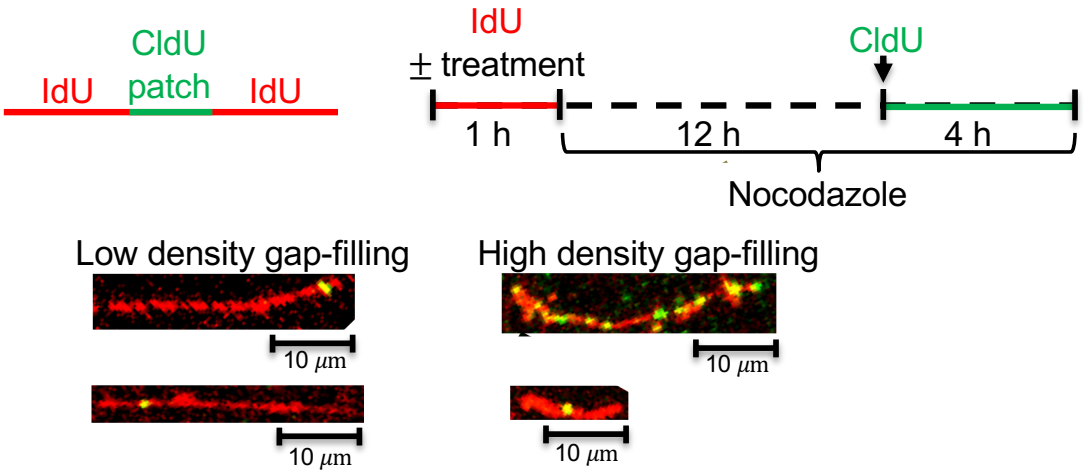




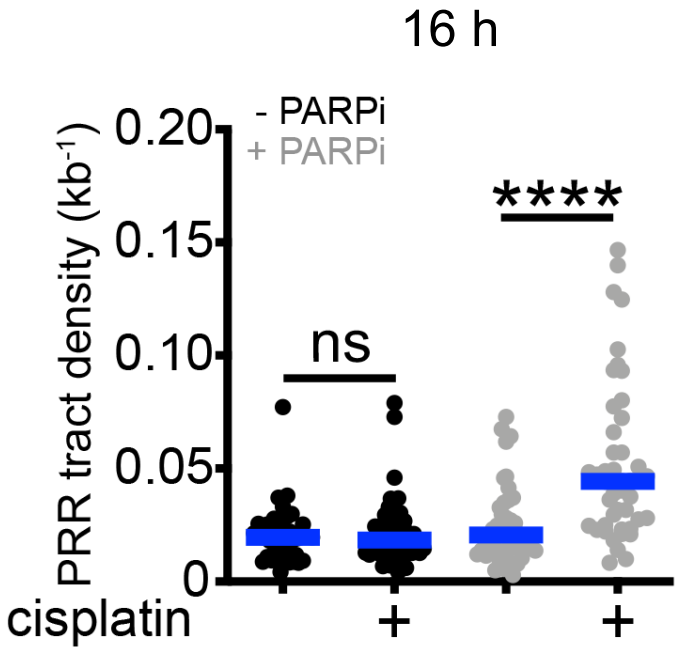
SMARCAL1KO



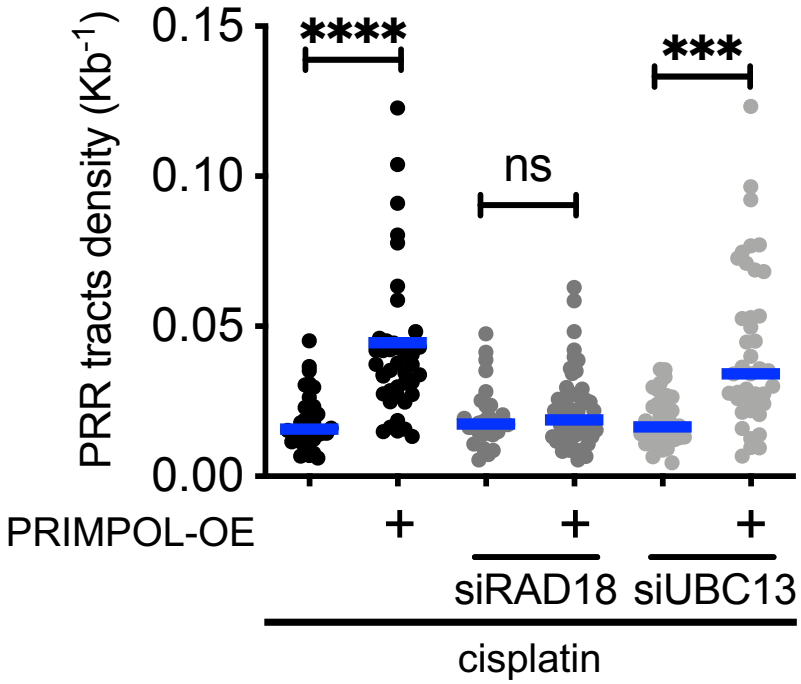
ssDNA gap repair in G2 in PARP inhibited cells

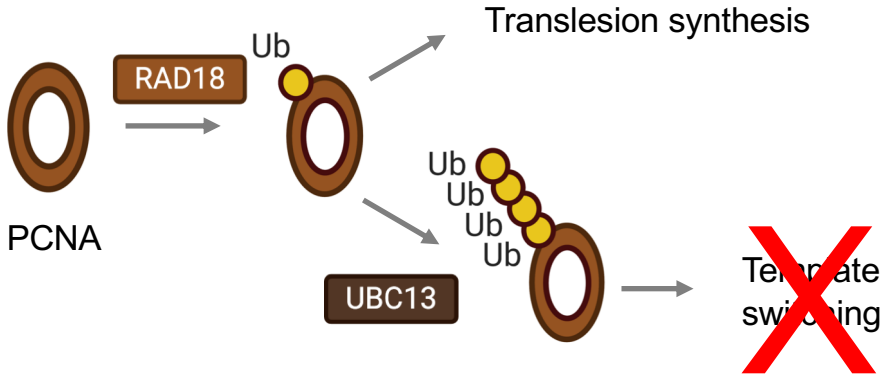


PARP inhibition (Olaparib)

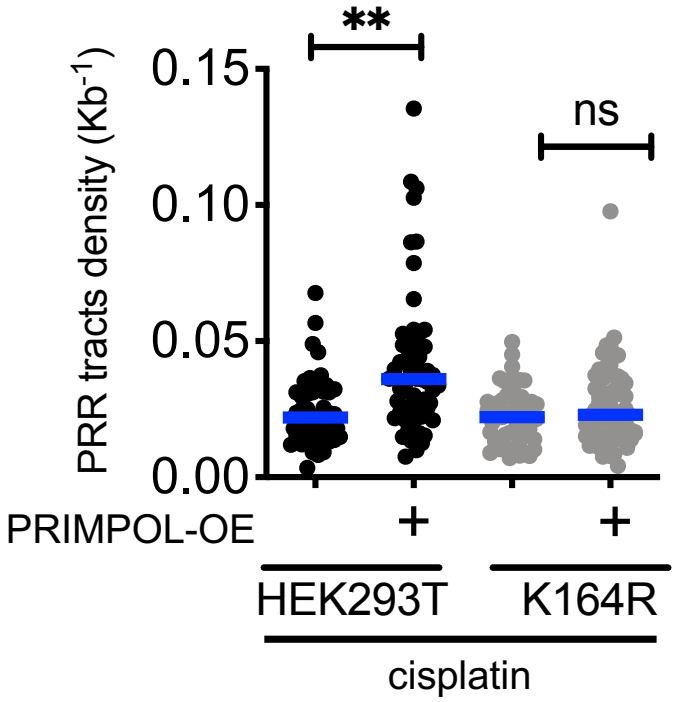


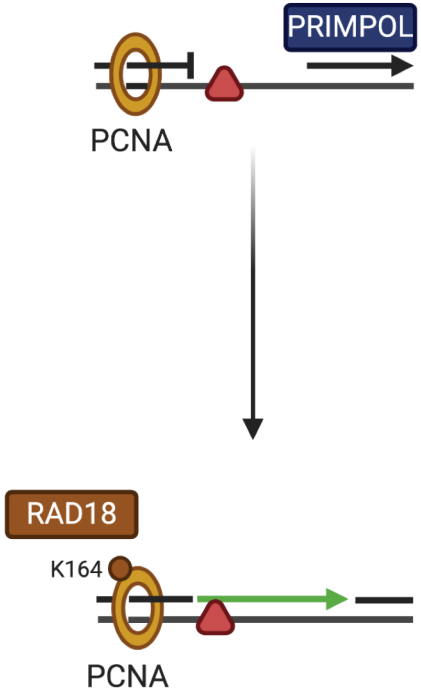
RAD18 is required for ssDNA gap filling in G2



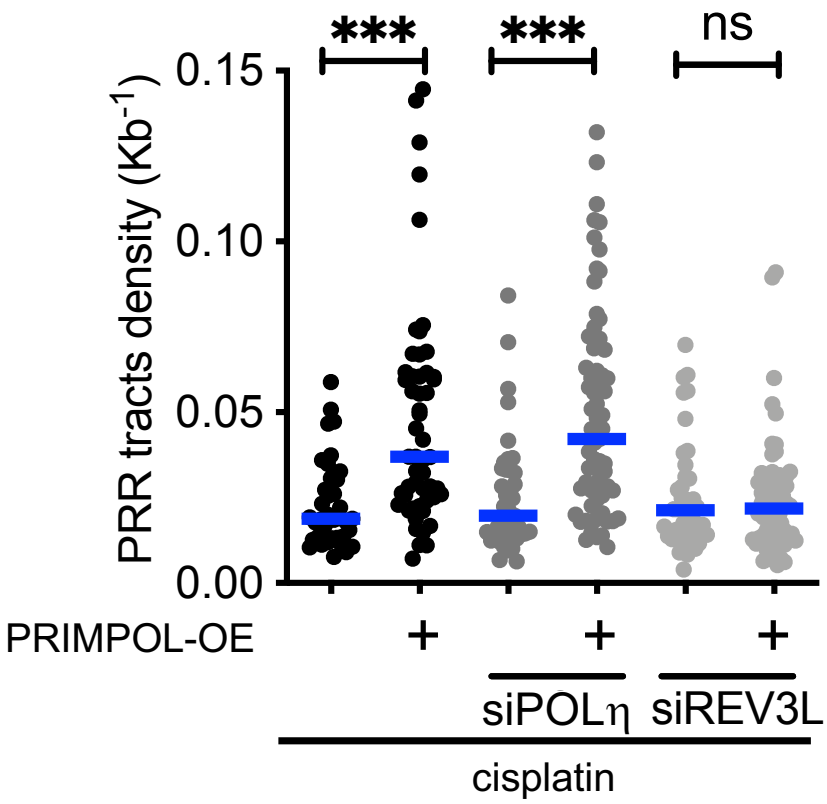


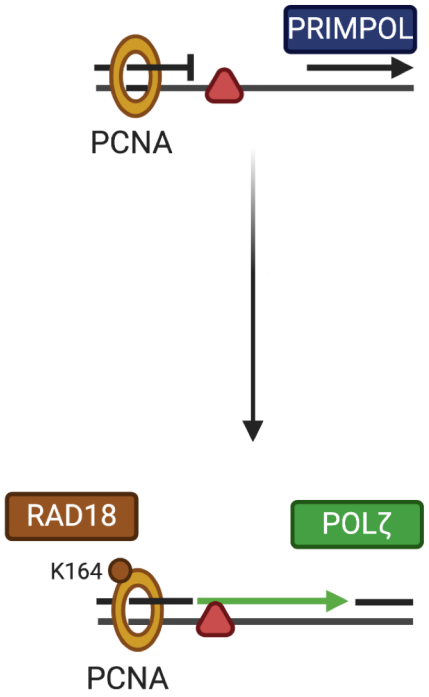
PCNA ubiquitination is required for ssDNA gap filling in G2



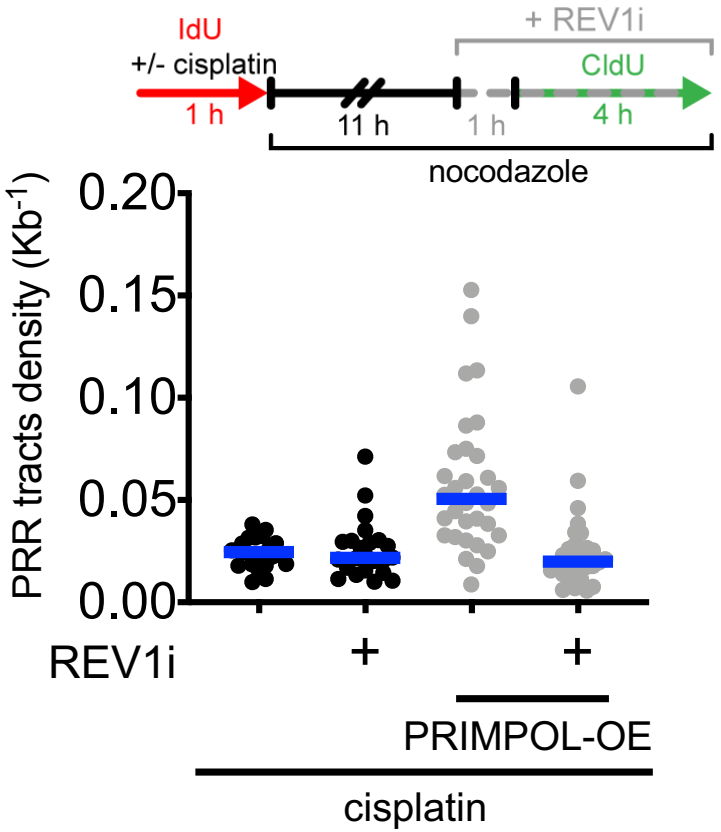


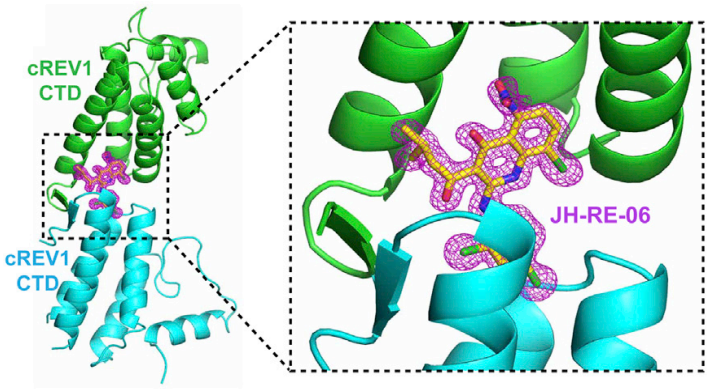
REV3L (POLζ) is required for ssDNA gap filling



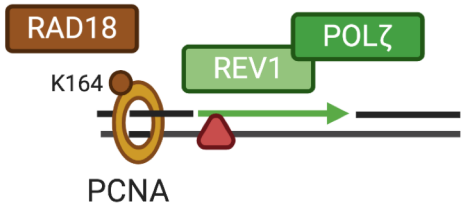


REV1 is required for ssDNA gap filling

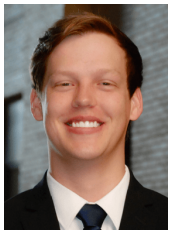




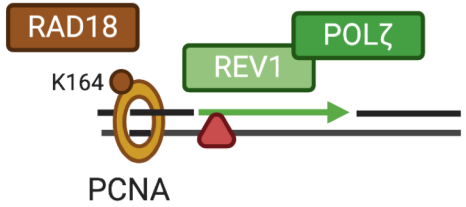
Wojtaszek *et al.*, Cell 2019

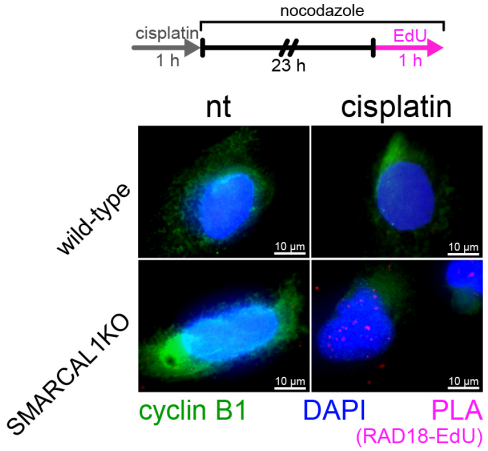


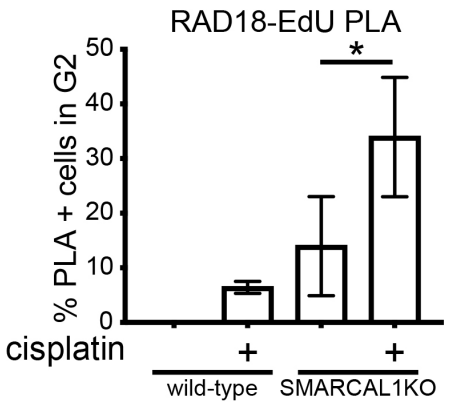
RAD18 and REV1 are recruited to EdU foci in G2 during gap filling

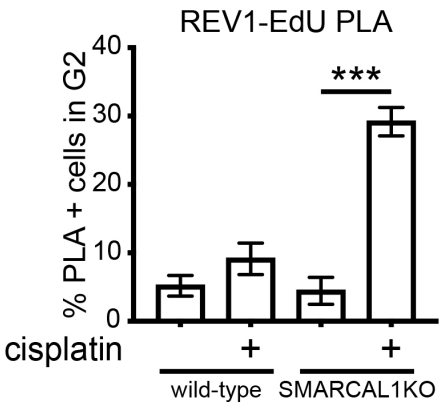


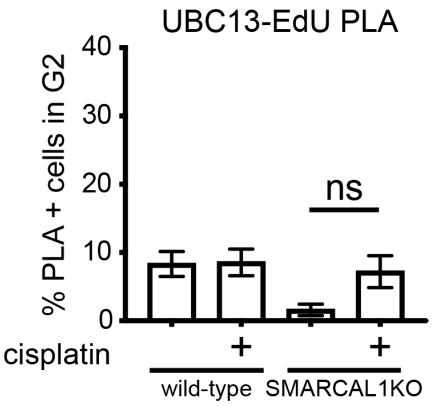
Matthew Wood



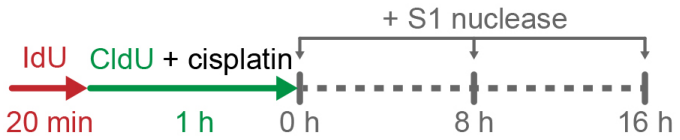


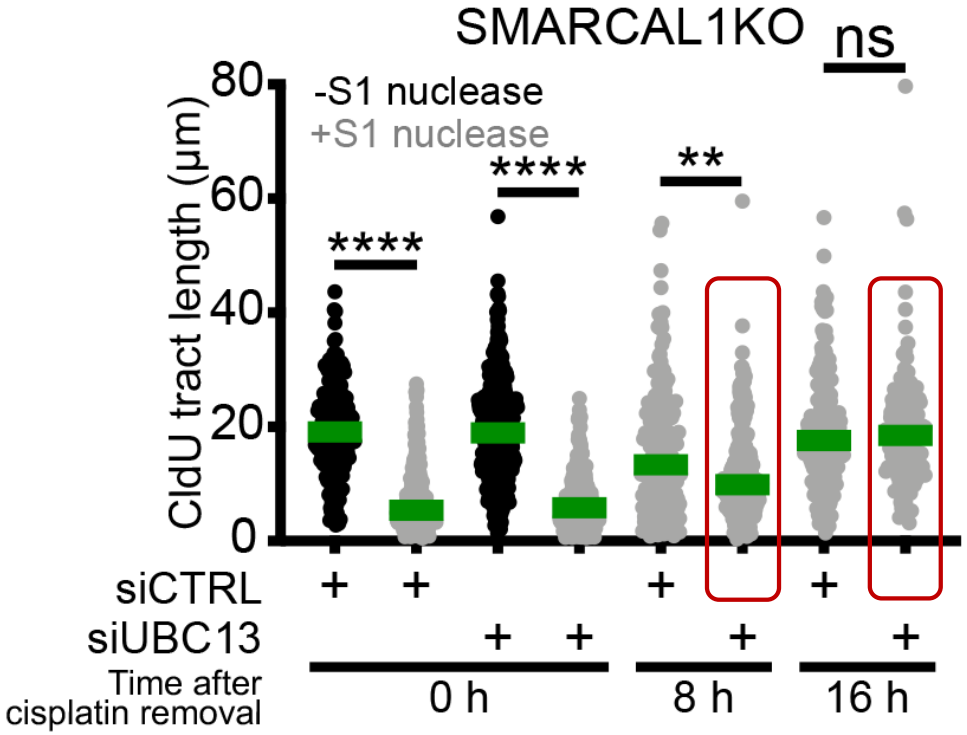


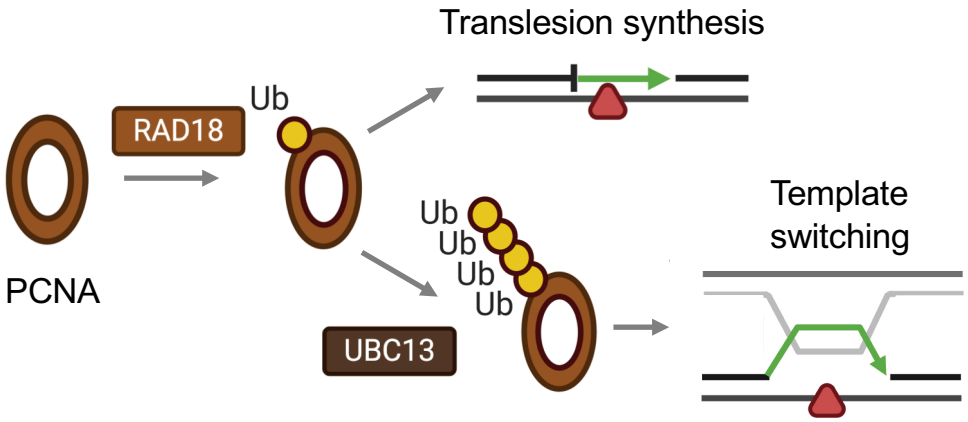




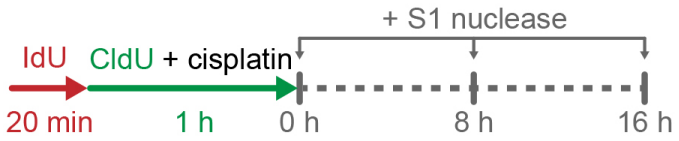
UBC13 mediates gap filling in S but not in G2

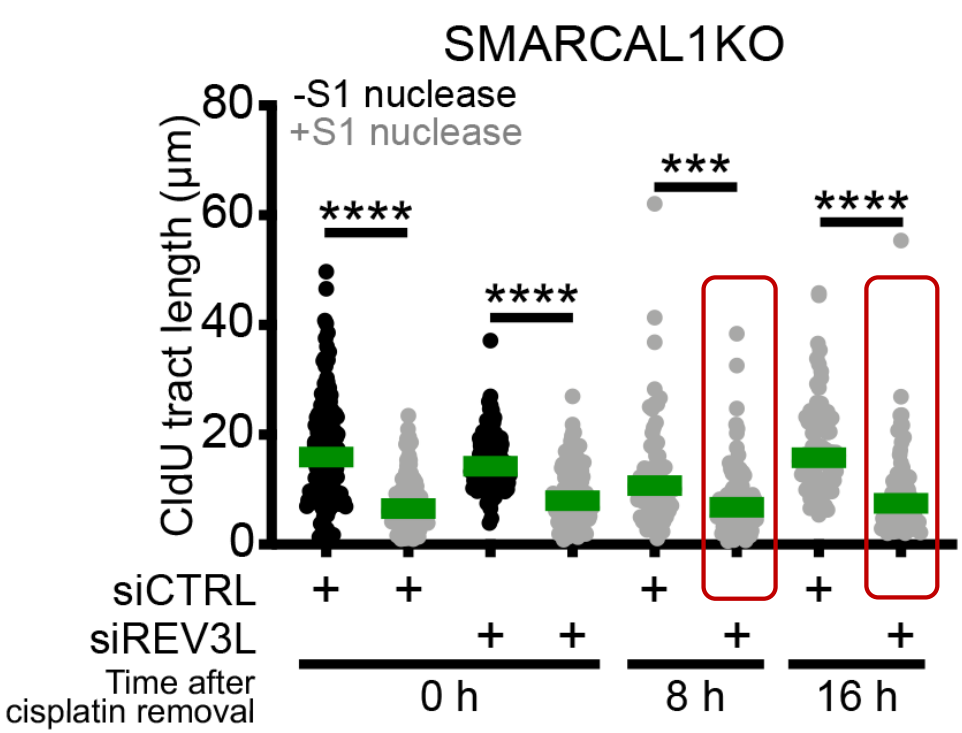


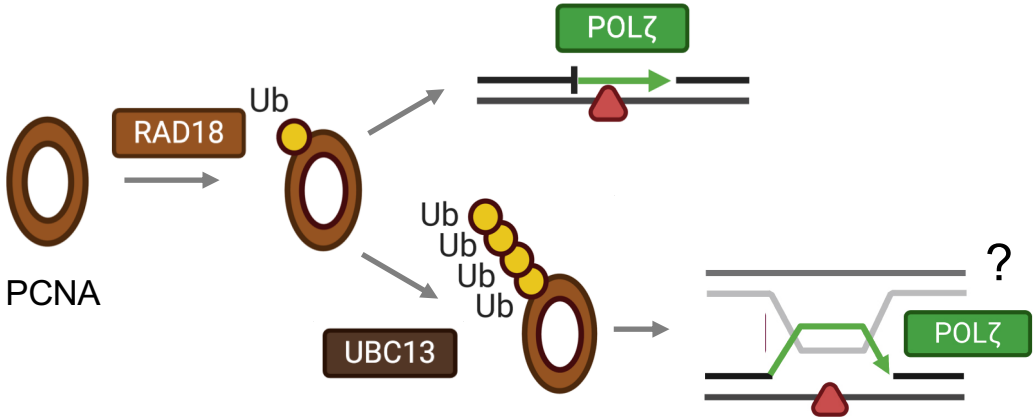




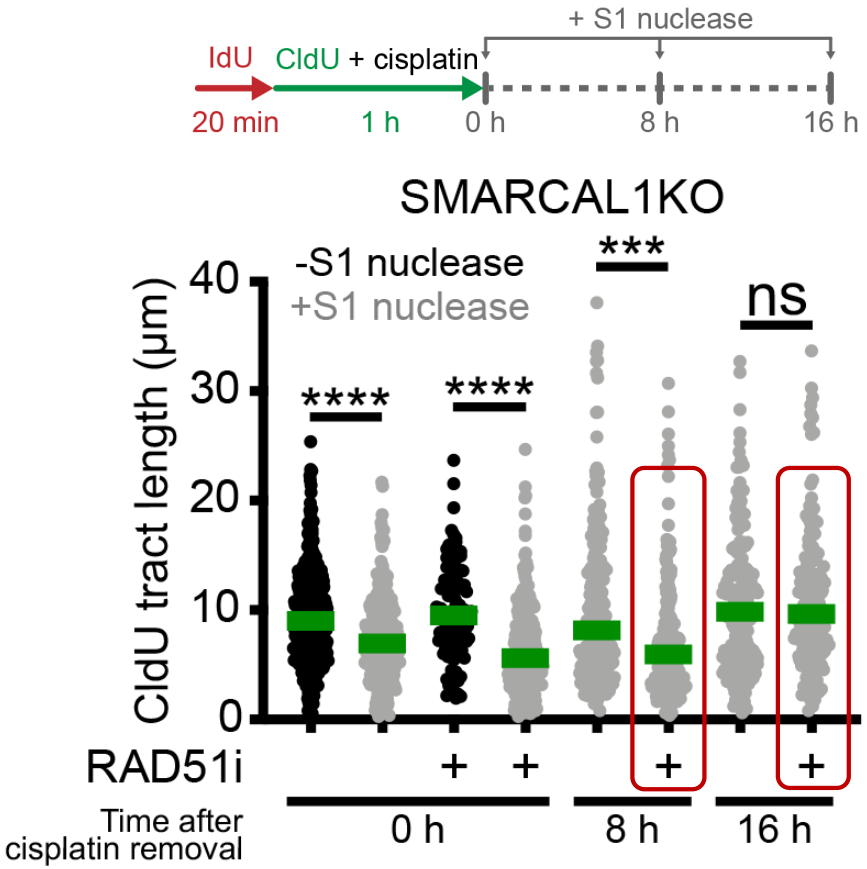
POLζ mediates gap filling in S and G2

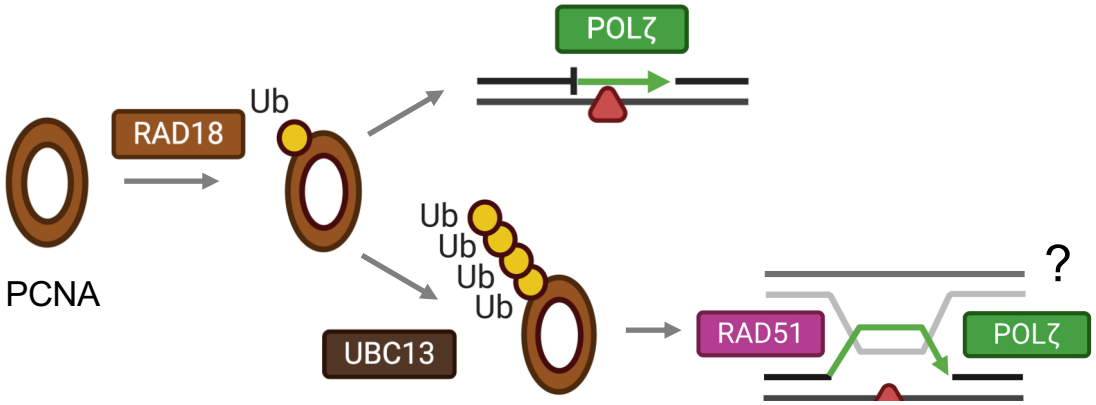




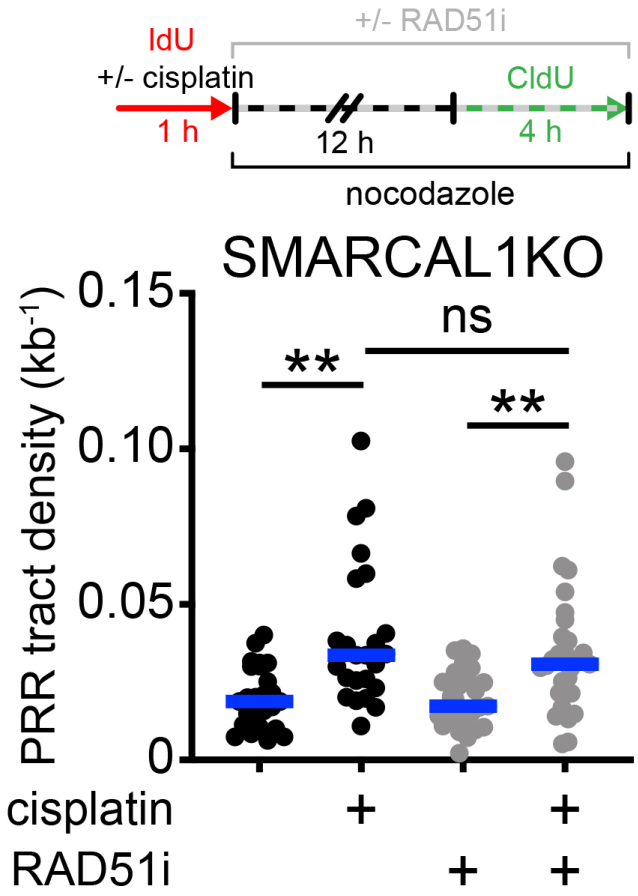


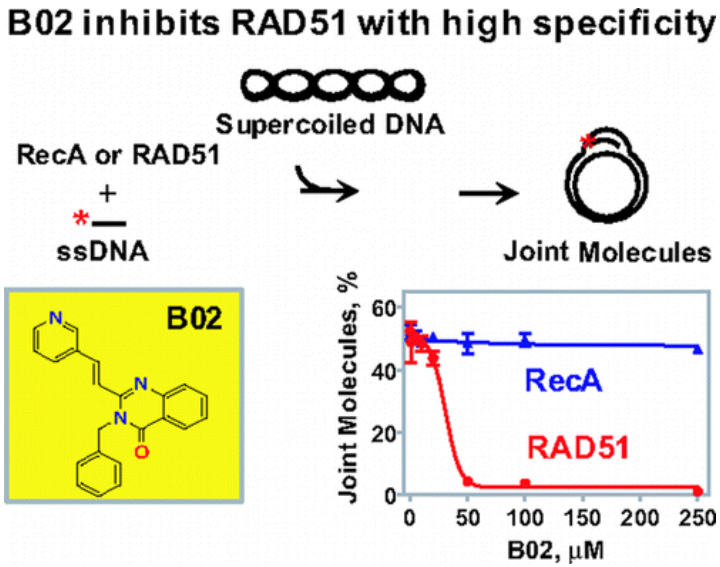
RAD51 mediates gap filling in S but not in G2





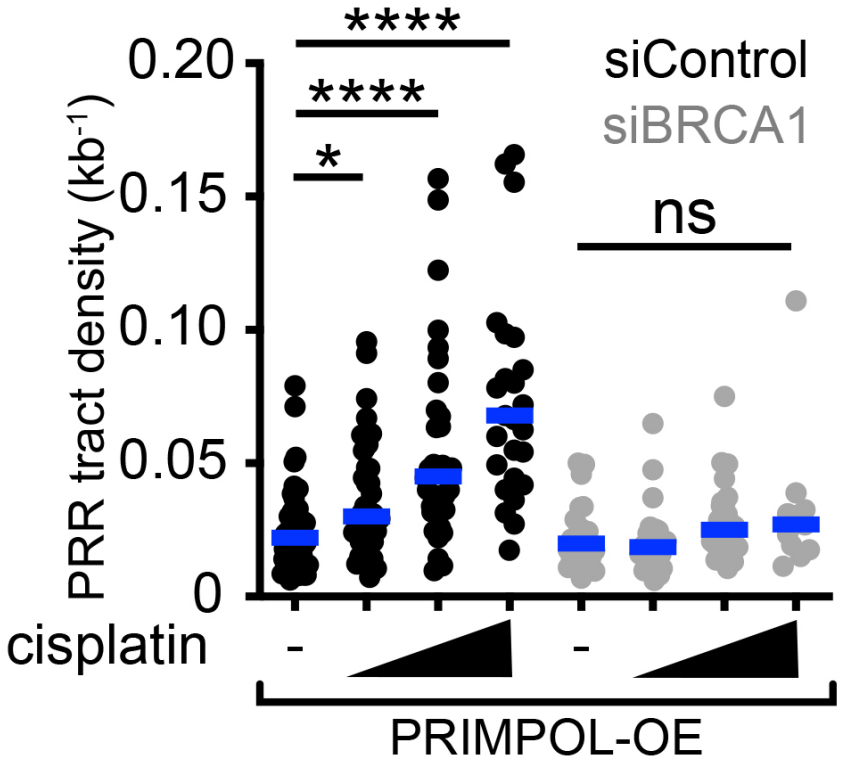
RAD51 does not mediate gap filling in G2

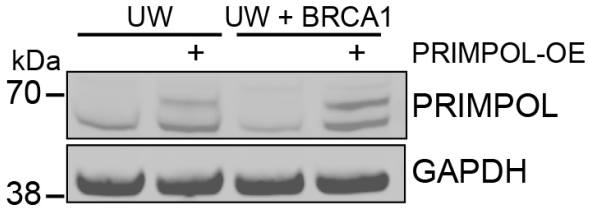


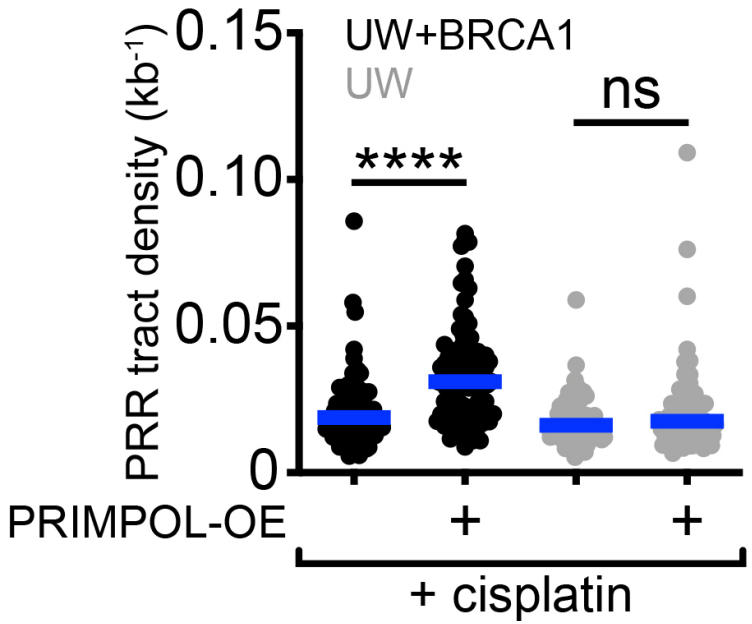


Huang *et al.,* ACS Chem Biol 2011

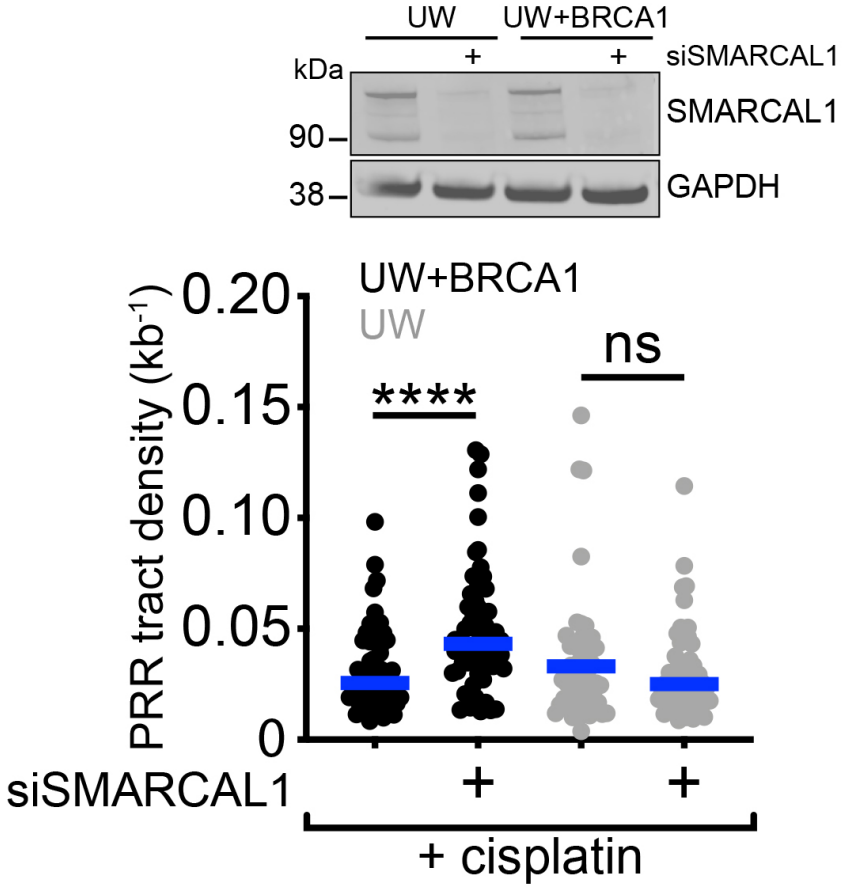
BRCA1 is required for ssDNA gap filling in PRIMOL-OE cells



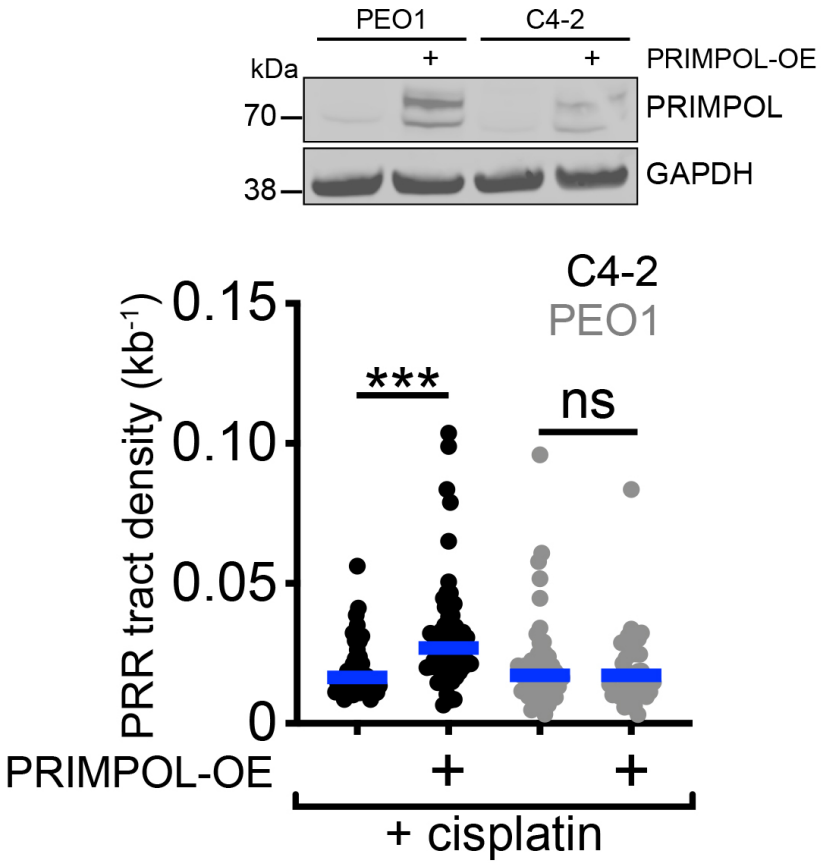




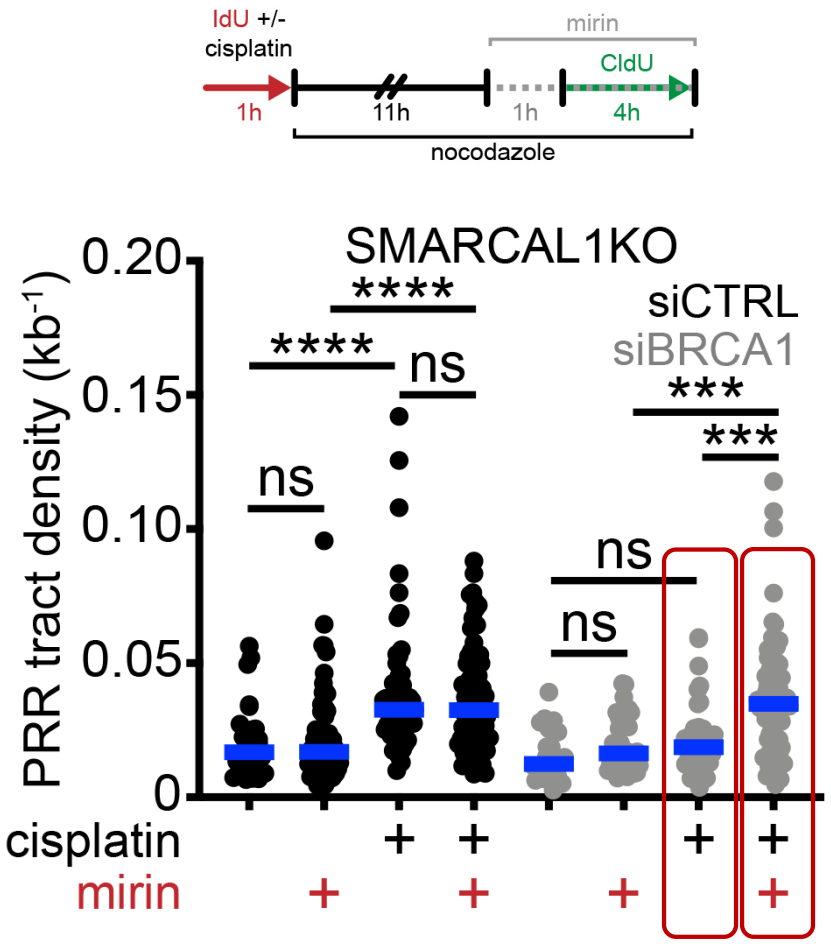
BRCA1 is required for ssDNA gap filling in SMARCAL1 depleted cells

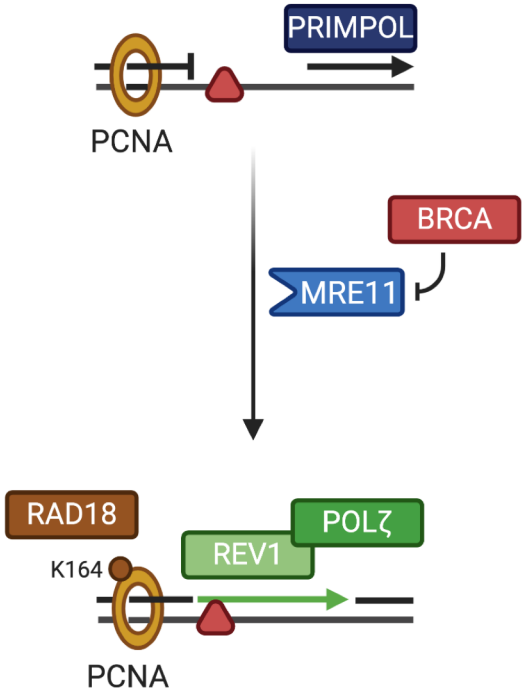


BRCA2 is required for ssDNA gap filling in PRIMOL-OE cells

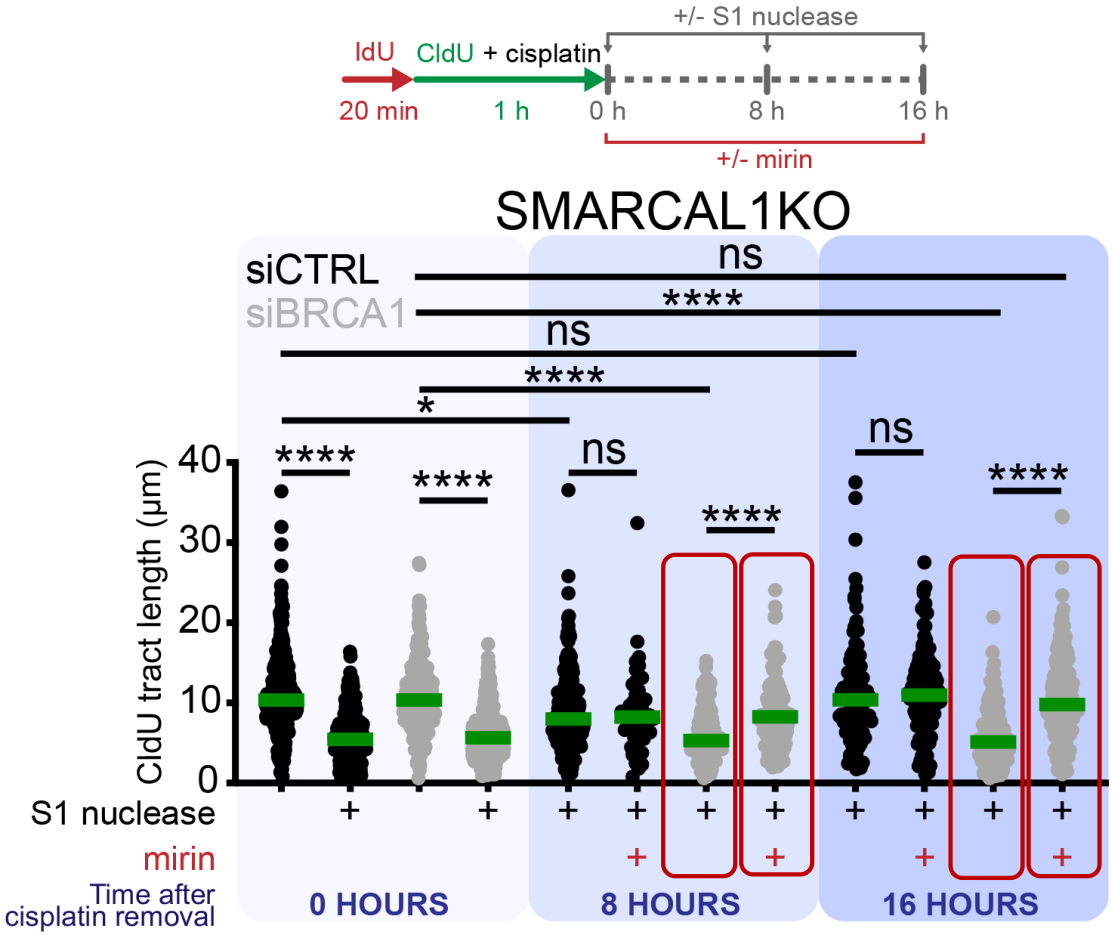


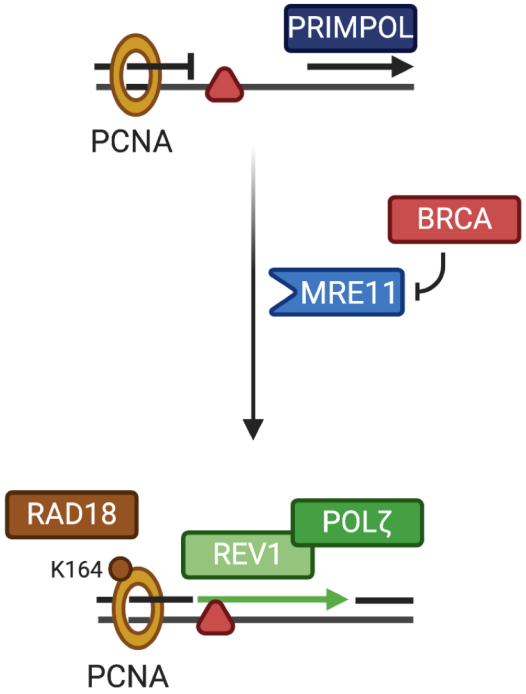
BRCA1 promotes gap filling by limiting MRE11 activity



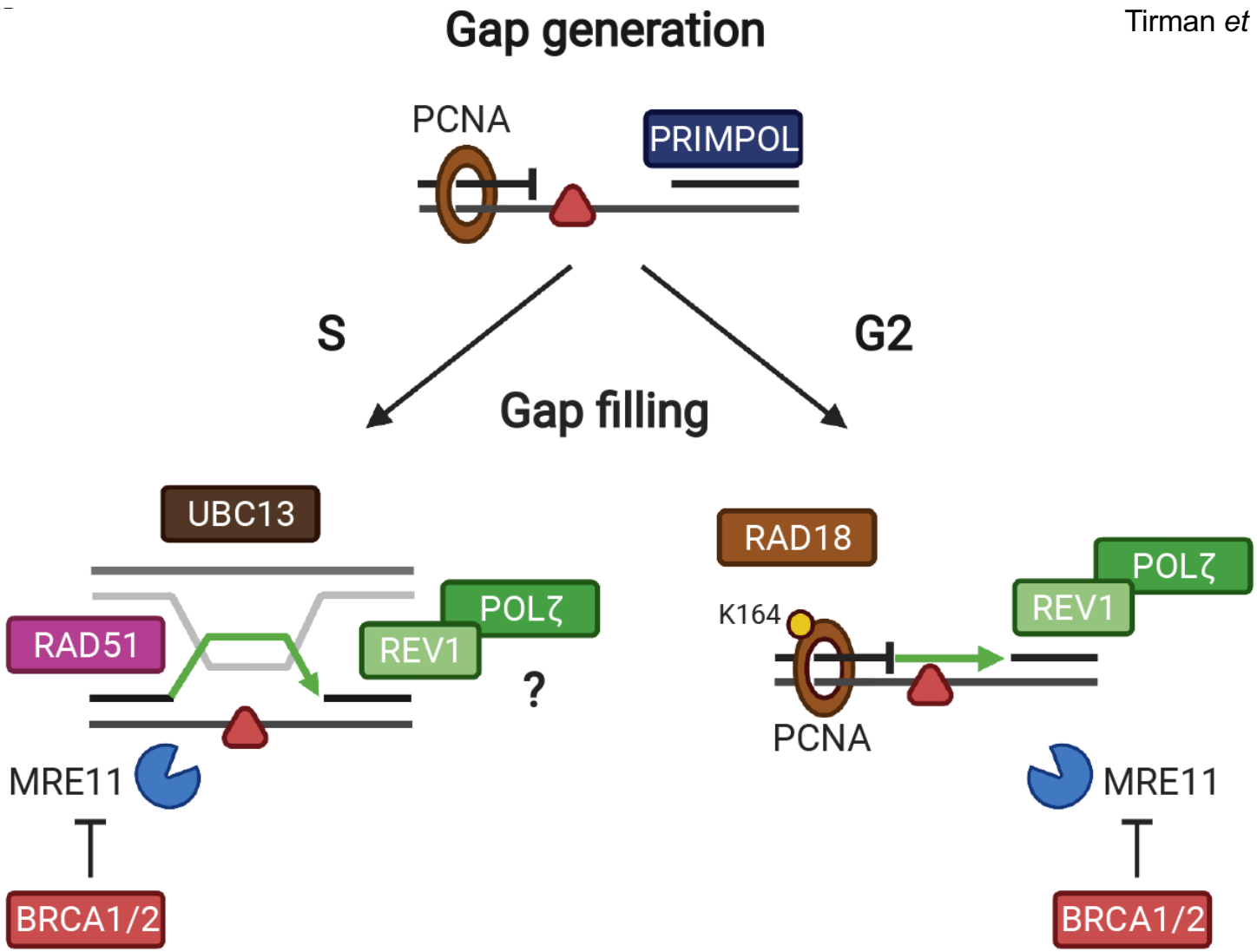


BRCA1 promotes gap filling by limiting MRE11 activity

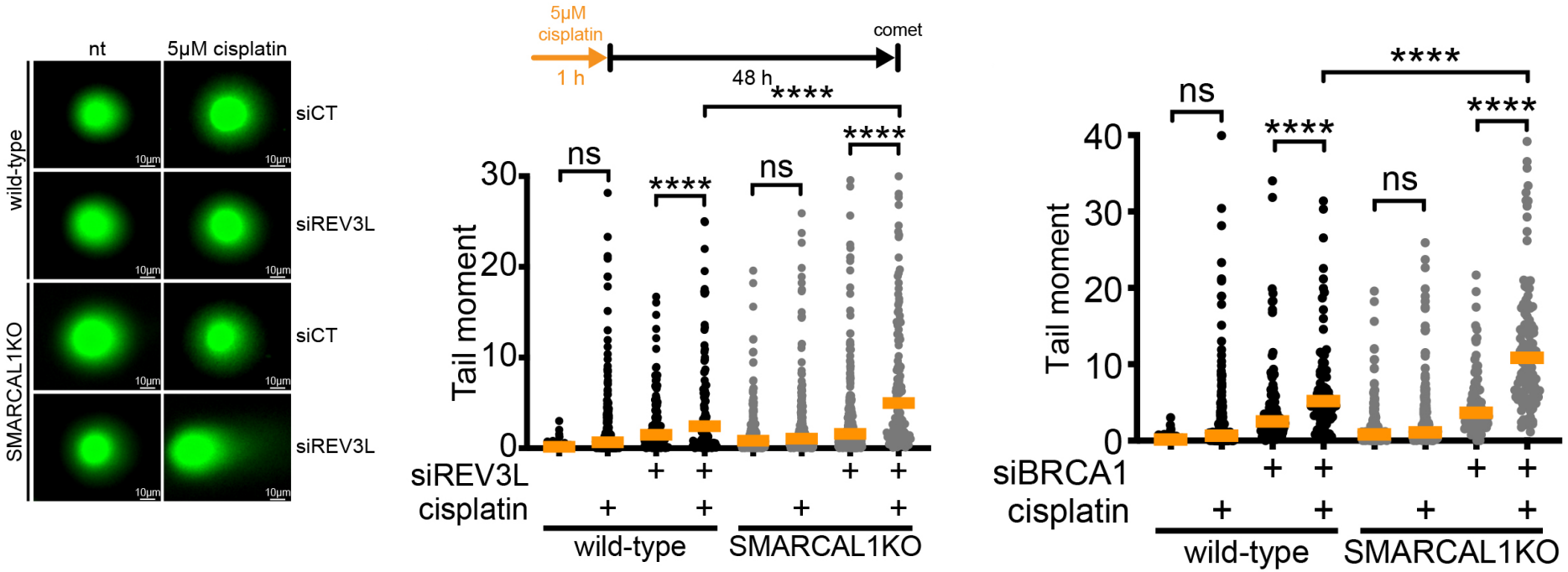




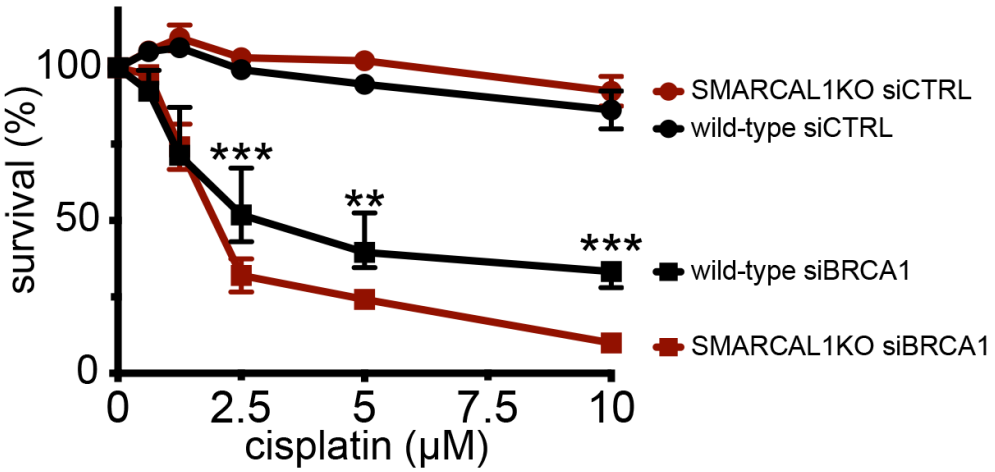
Tirman *et al.,* Mol. Cell 2021

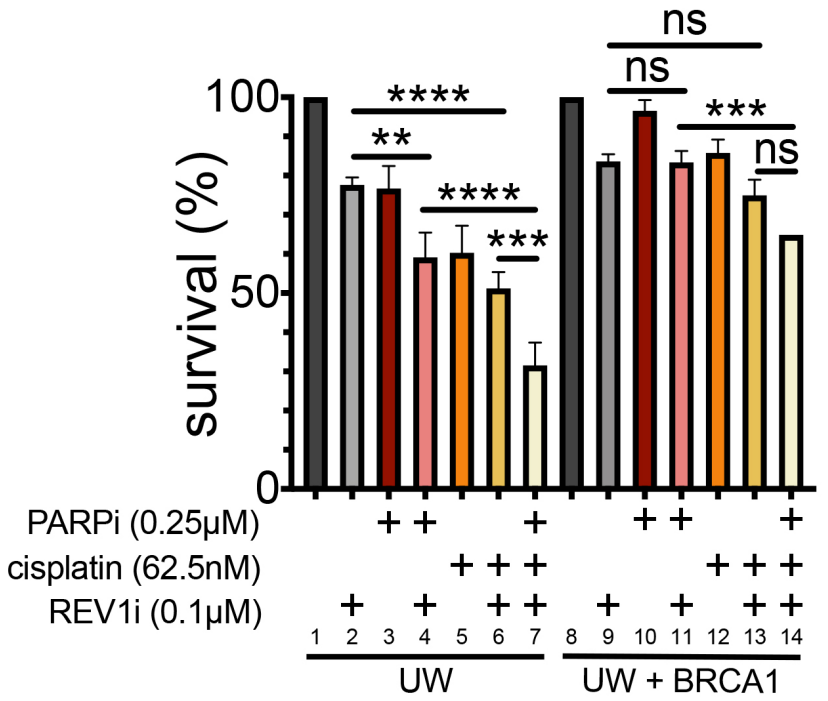


Impaired gap filling promotes DSB accumulation



Preventing ssDNA gap filling leads to increased cisplatin and PARPi sensitivity





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