Replication in the Vicinity of Absolute Blocks to Replication

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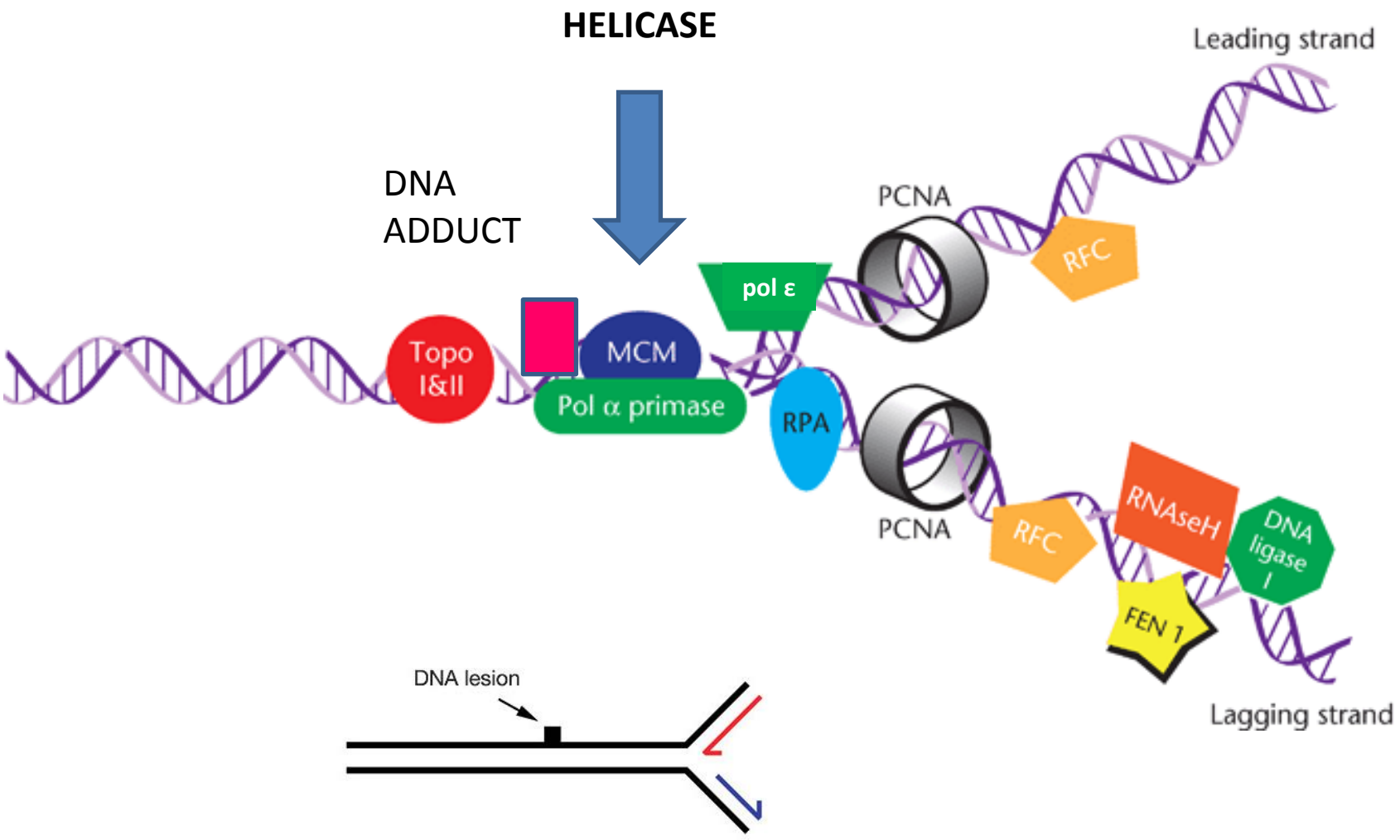
The replication fork is driven by helicases



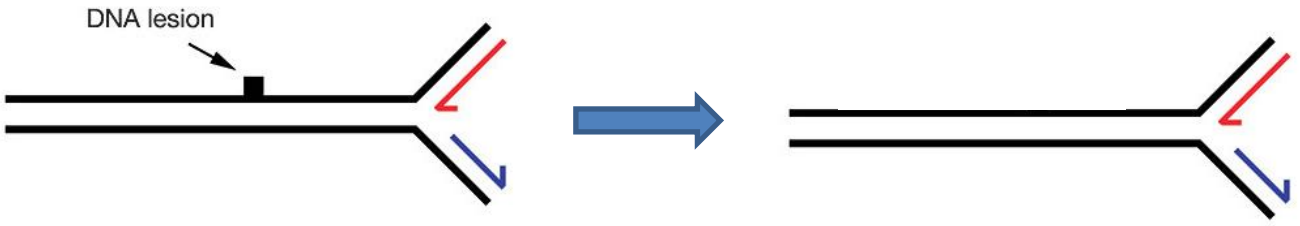
Strategies for responding to replication challenge imposed by DNA adducts

How do cells deal with replication blocks?

1. **Avoid them**

   Remove them before a fork encounter
   Multiple DNA repair pathways

2. **Repair after block**

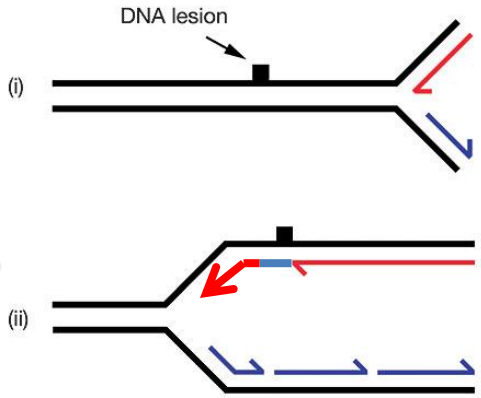


Problem: delay completion of replication
complex genomes with multiple origins
50-100,000

How do cells cope with replication blocks?

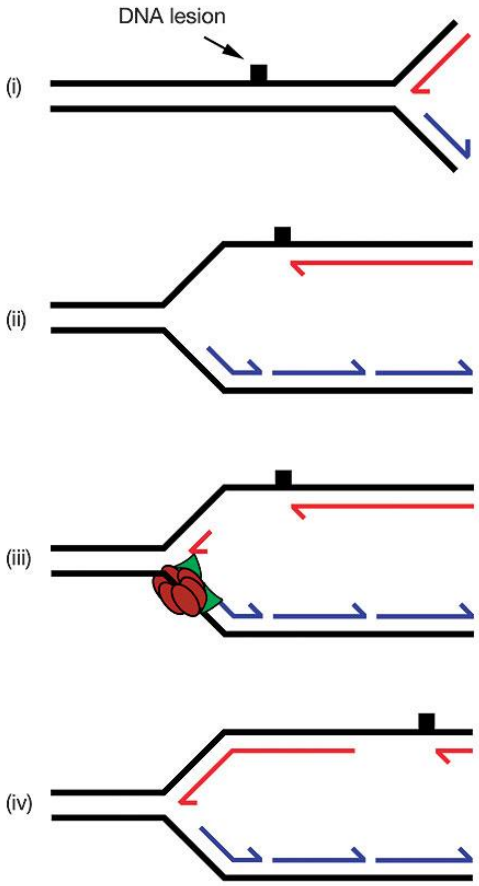
3. **Bypass lesion and continue synthesis**

Unwind
Bypass synthesis



How do cells cope with replication blocks?

4. **Uncouple replication and repair**



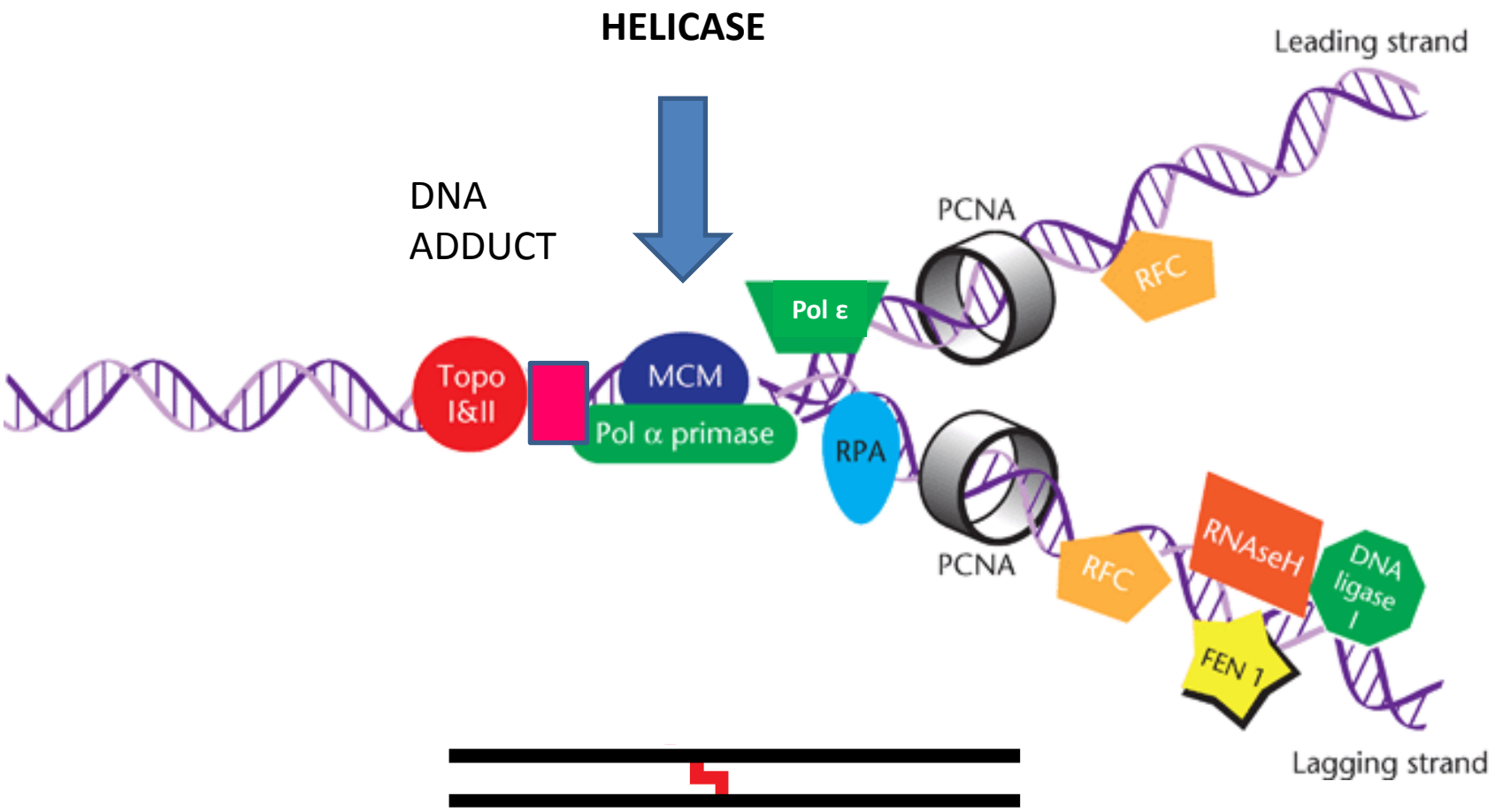
Unwind

Restart synthesis

Post Replication Repair

Rupp, 1968

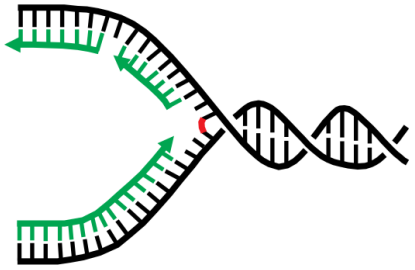
The replication fork is driven by helicases



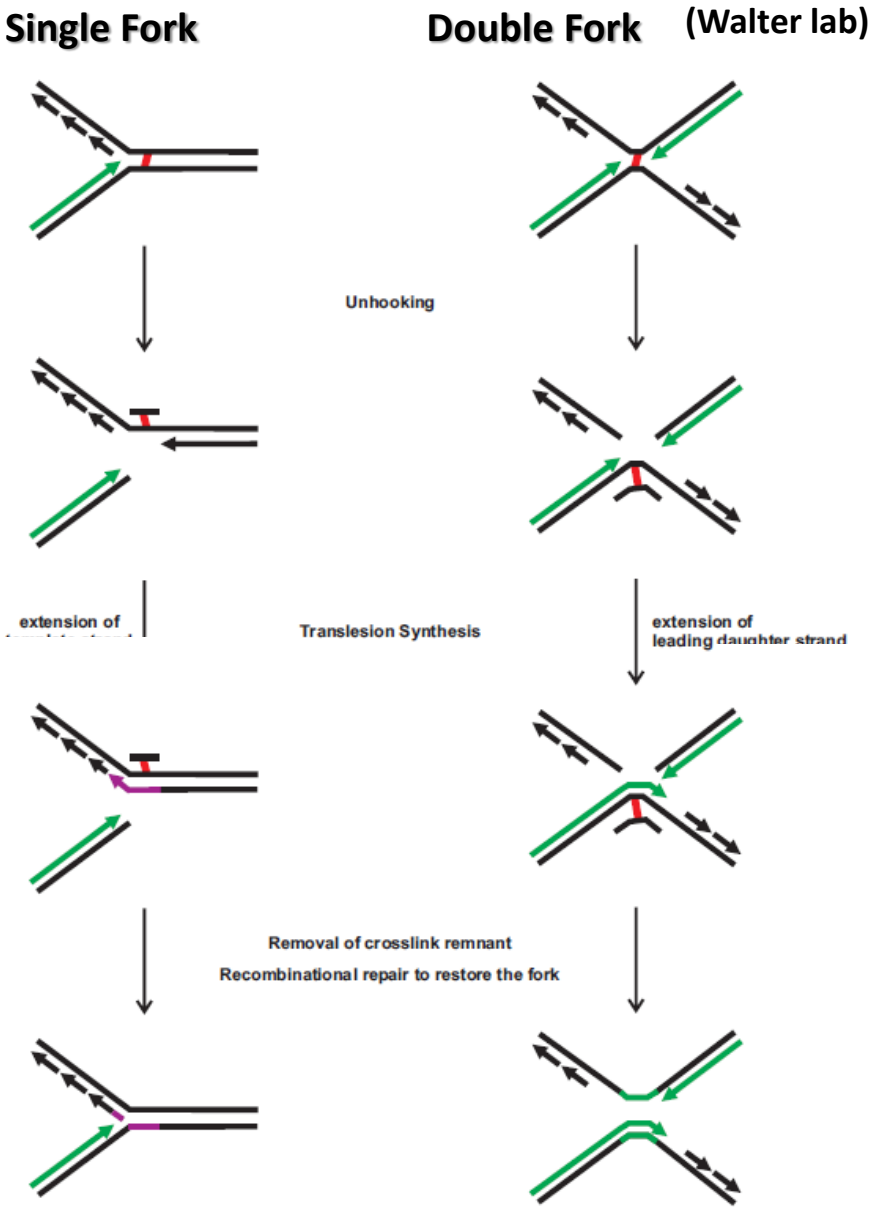
Interstrand crosslinks present a major challenge to the replication apparatus

DNA Interstrand Crosslink (ICL) repair during replication

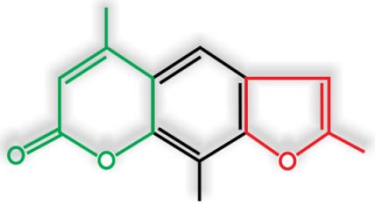
Considered absolute blocks to replication



Do these models describe encounters with genomic ICLs in mammalian cells?



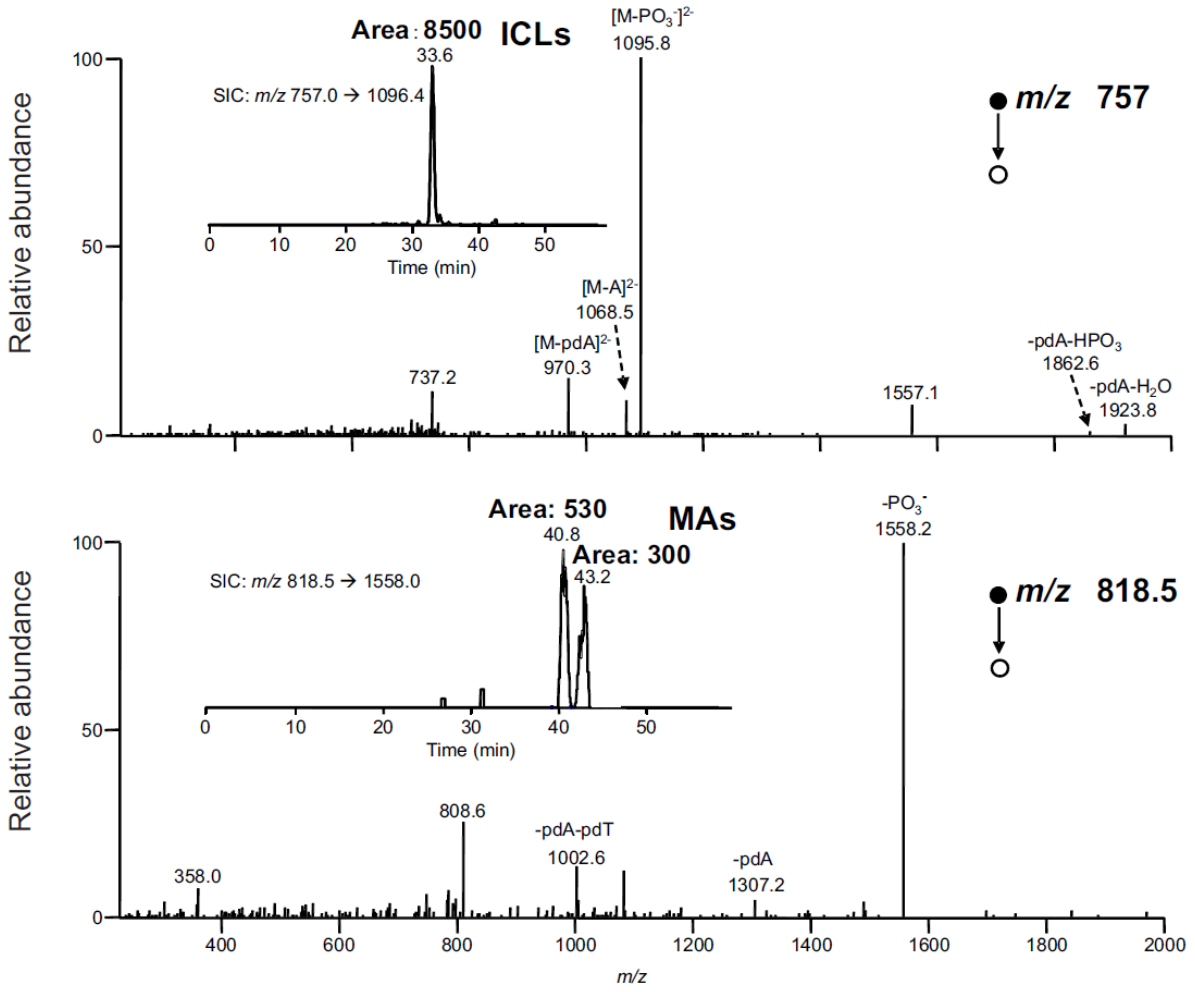
Trimethyl Psoralen forms a high proportion of ICLs



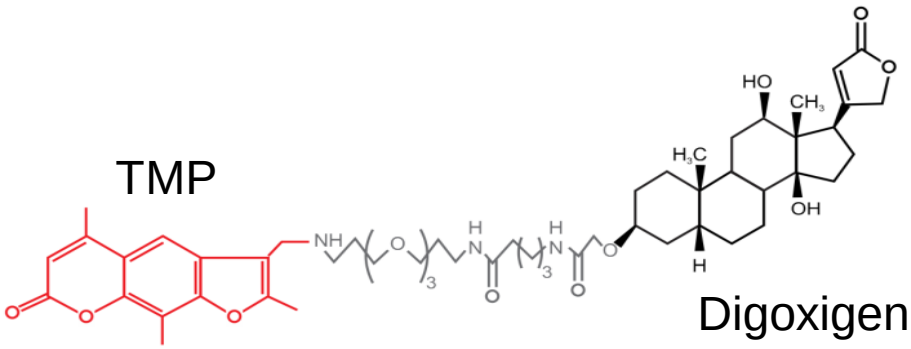
4,5',8-trimethylpsoralen

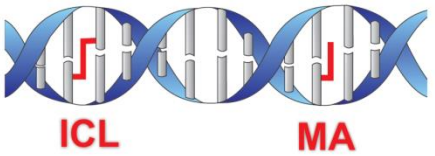
+ UVA

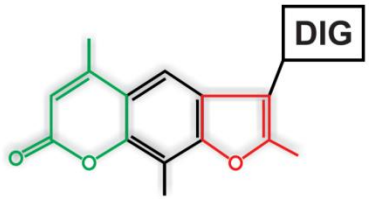
LC/MS/MS



Digoxigenin-tagged TMP

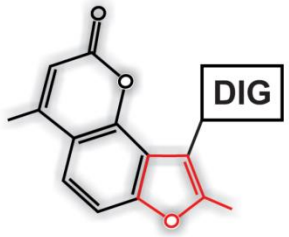






4,5',8-trimethylpsoralen

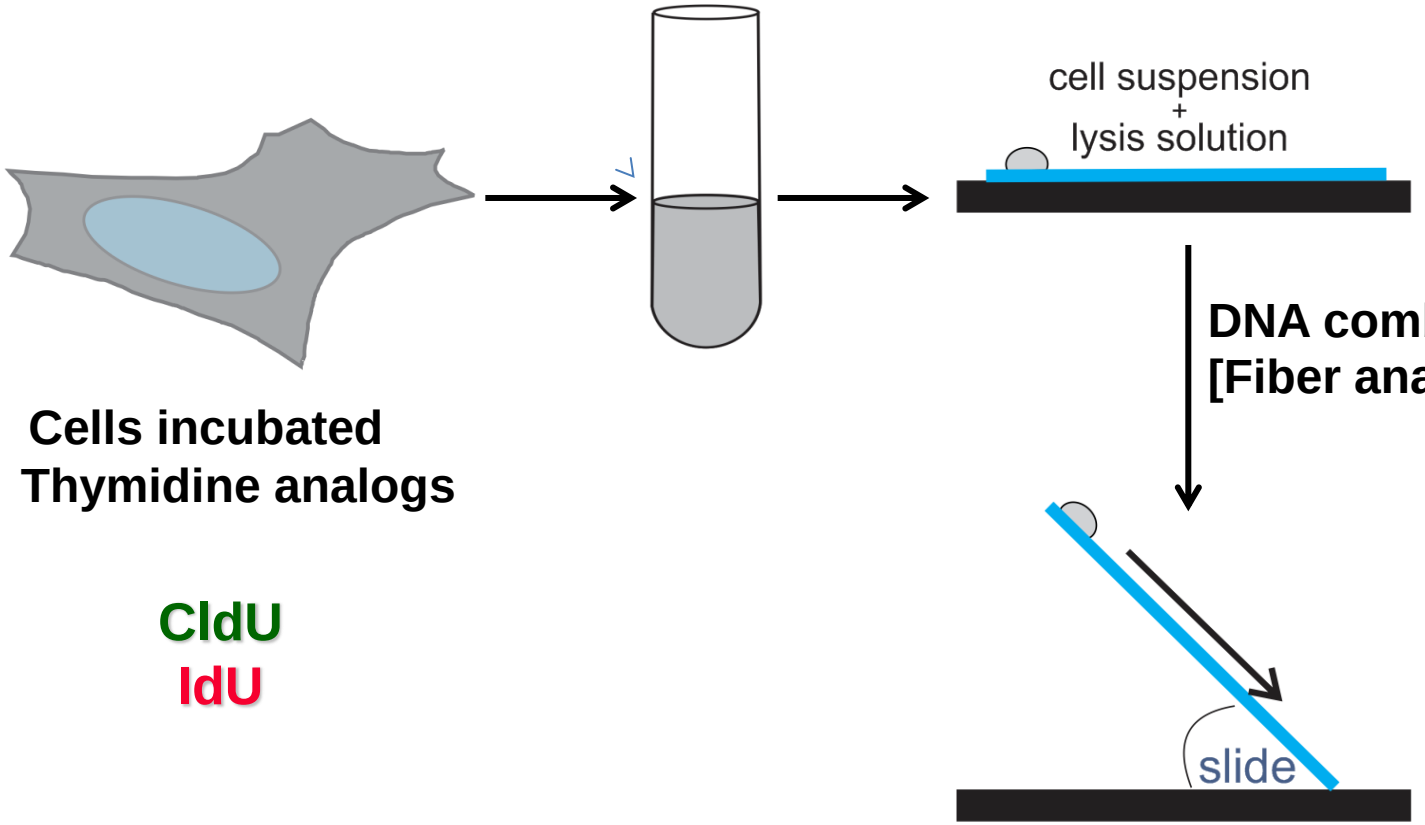
D-TMP **10** **1**



Angelicin

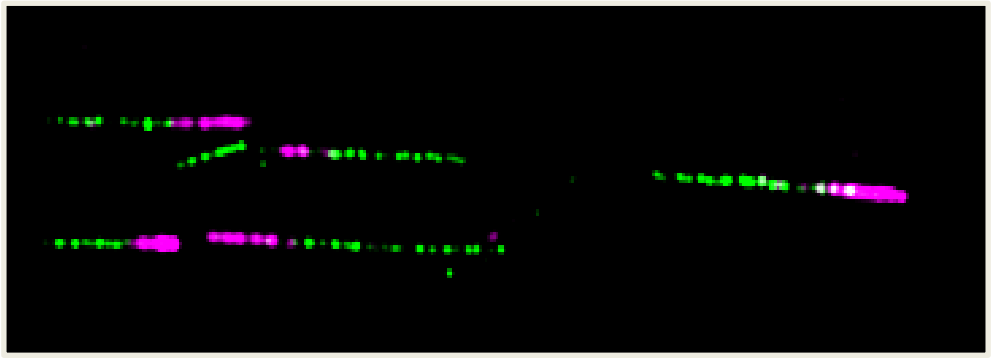
D-Ang **ONLY MAs**

Visualization of replication tracks on DNA Fibers



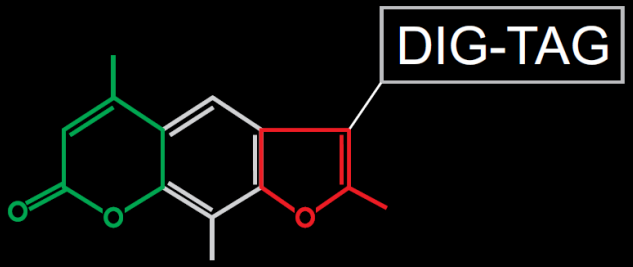
Cells incubated
Thymidine analogs

CldU
IdU



Immunofluorescent detection

Immuno quantum dot detection of Dig-TMP on a DNA fiber

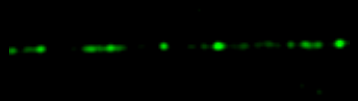


CldU
Overnight

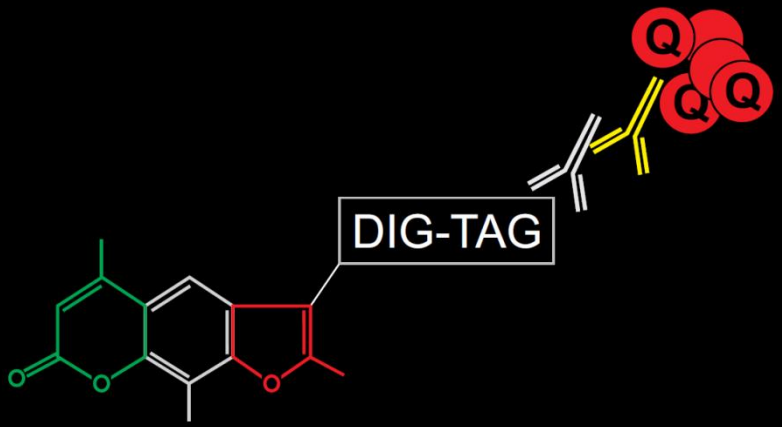
Dig-TMP + UVA

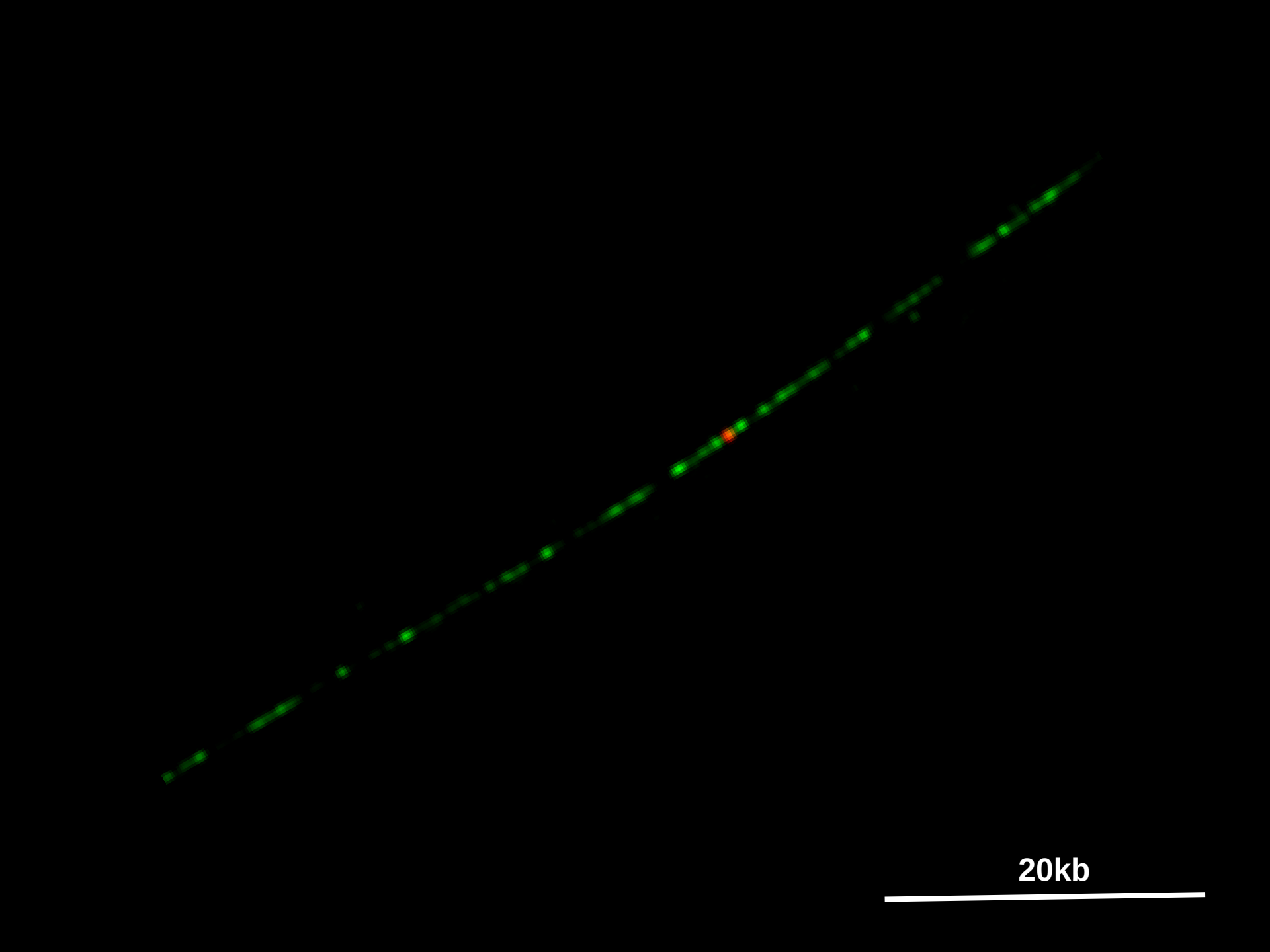
Comb DNA fibers / Detect

- **CldU** immunofluorescence



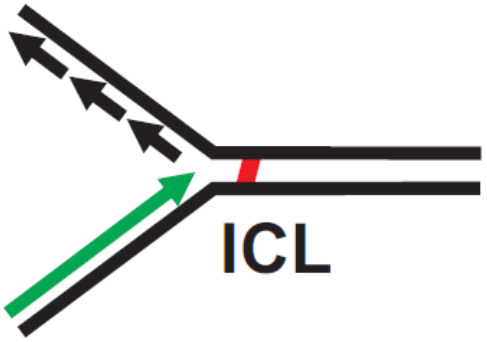
- **Dig-TMP** immunoquantum dot



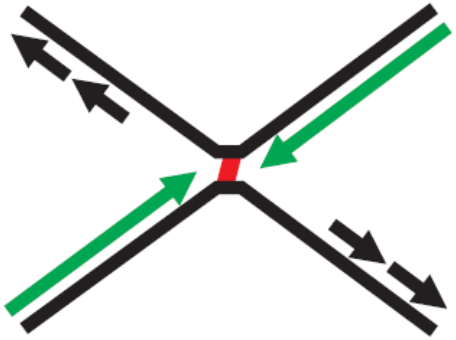

20kb

Possible replication patterns in the vicinity of ICLs

Single Fork

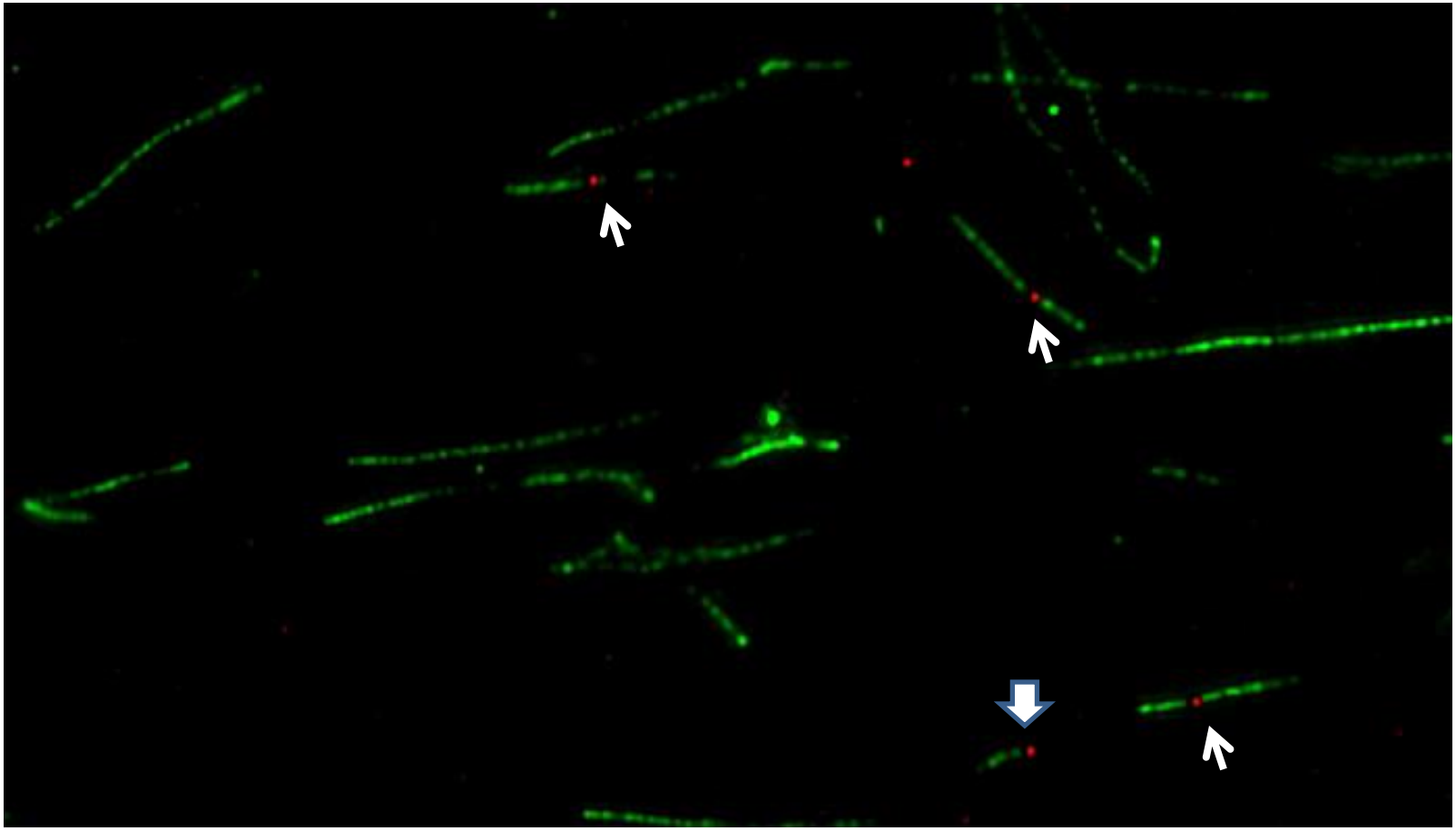


Double Fork



DIG-TMP +UVA ⟶ CldU ⟶ Replication patterns

A minority of replication tracts encounter an adduct Dig-Angelicin



CldU 1 hr

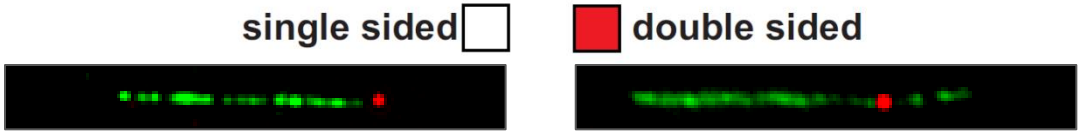
Dig-Ang

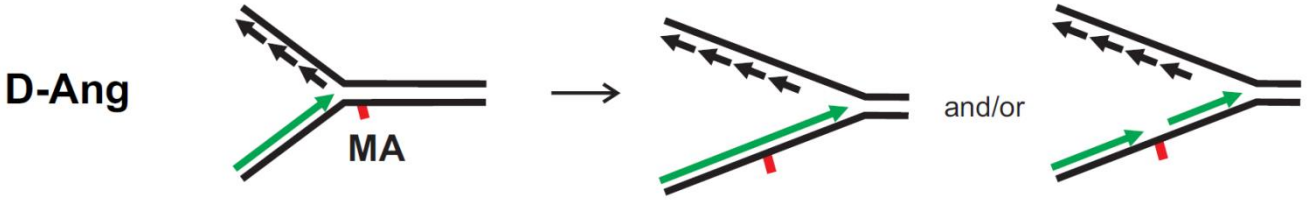
Replication encounters with D-Ang MAs

D-Ang/UVA
or
D-TMP/UVA

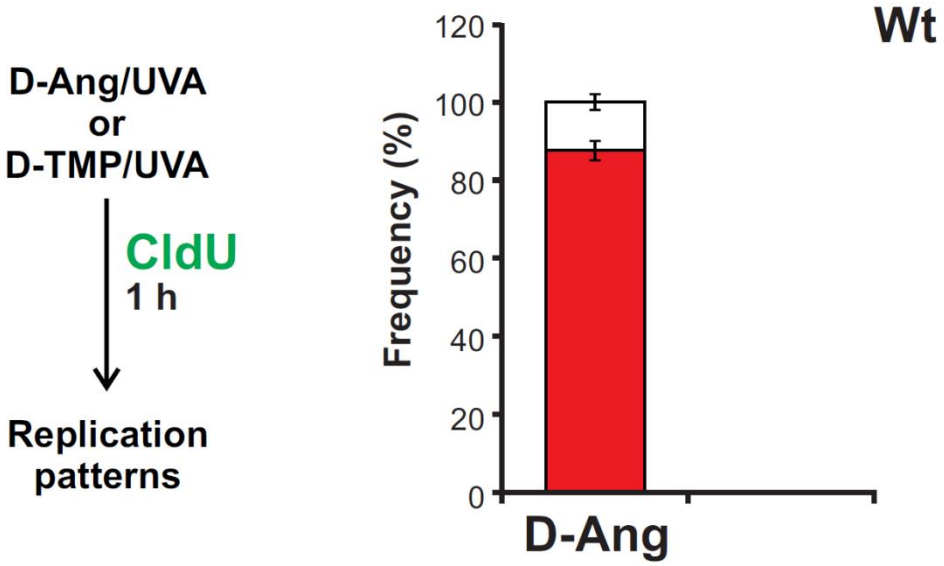
CldU
1 h

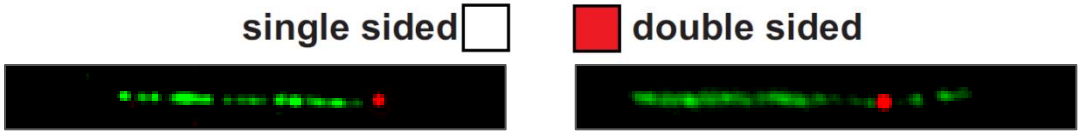
Replication
patterns

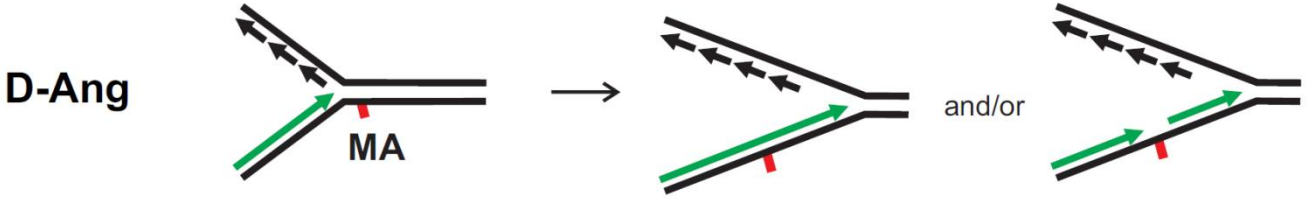




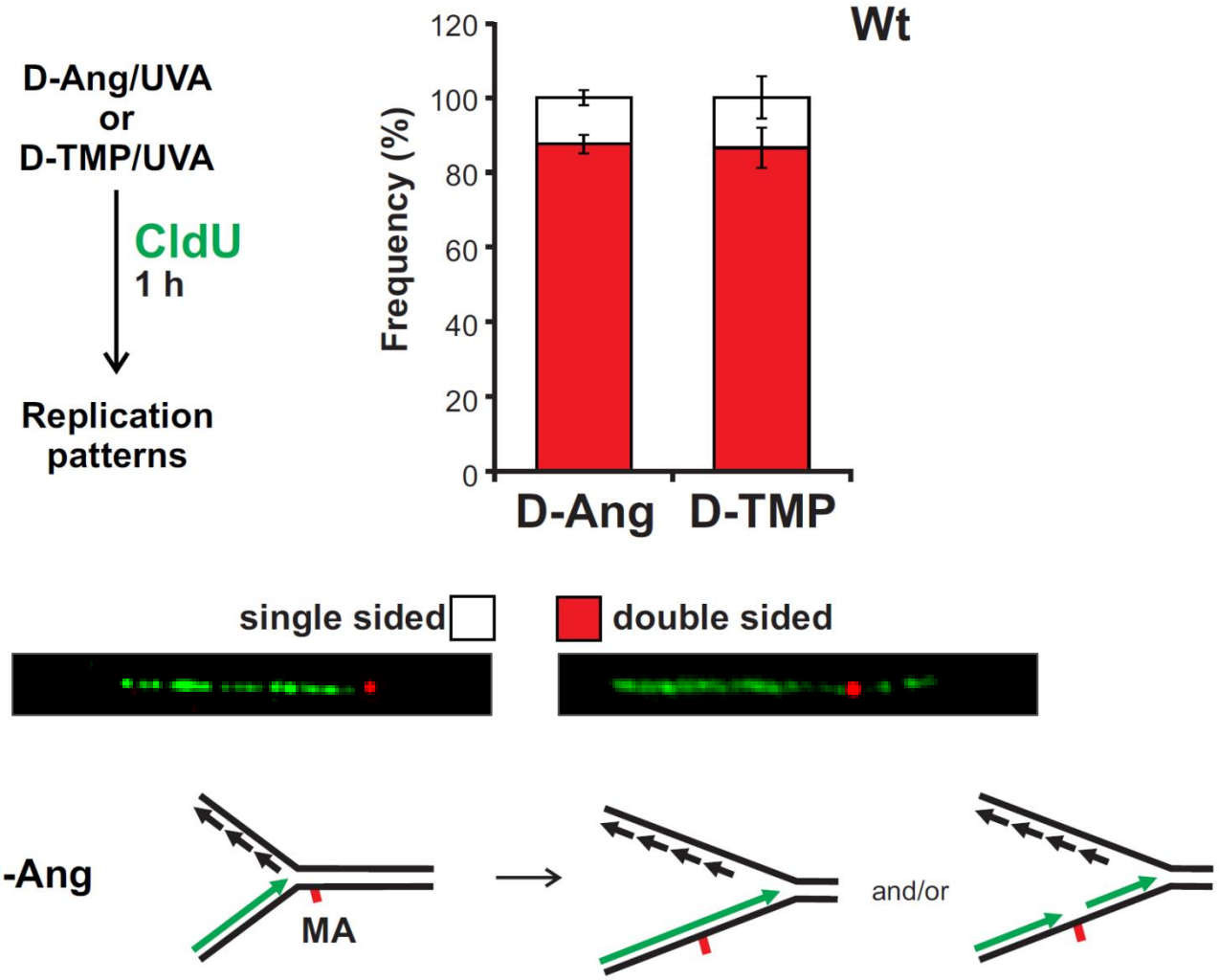
Replication fork encounters with D-Ang MAs







Replication encounters with D-TMP ICLs

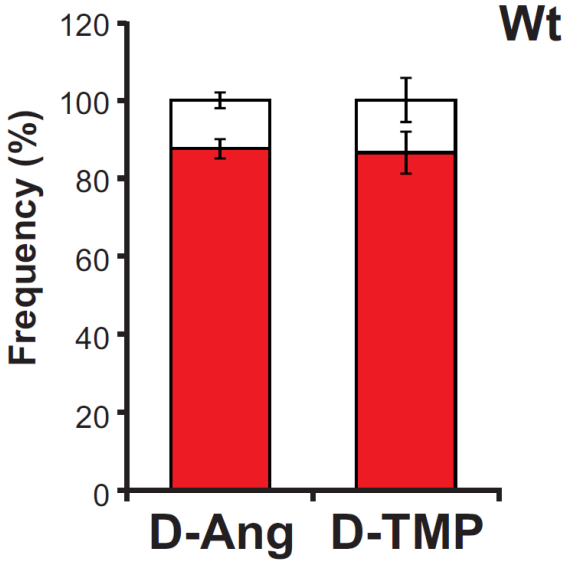


Replication encounters with D-TMP ICLs

D-Ang/UVA
or
D-TMP/UVA

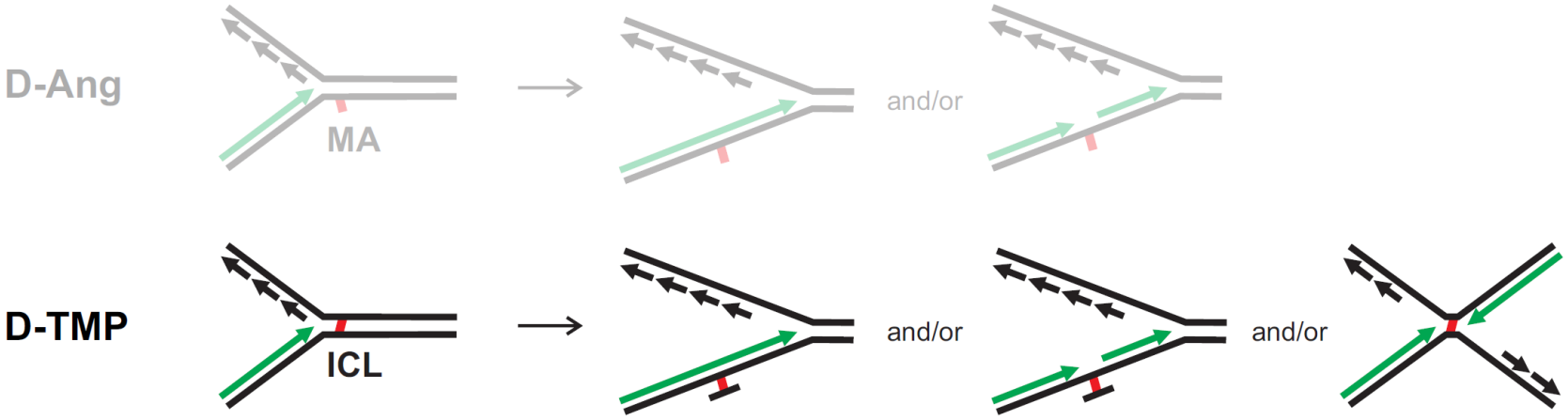
CldU
1 h

Replication
patterns

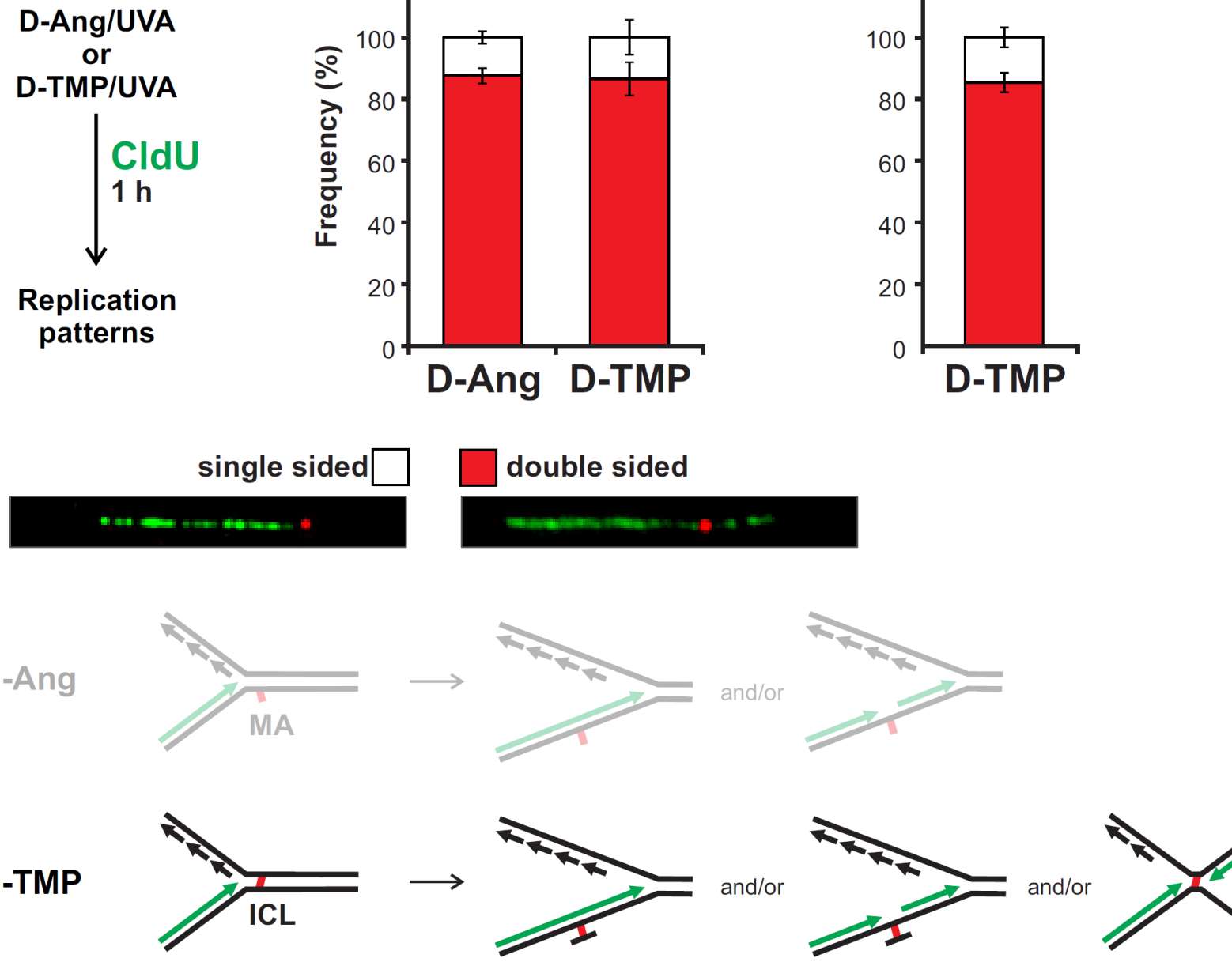


single sided

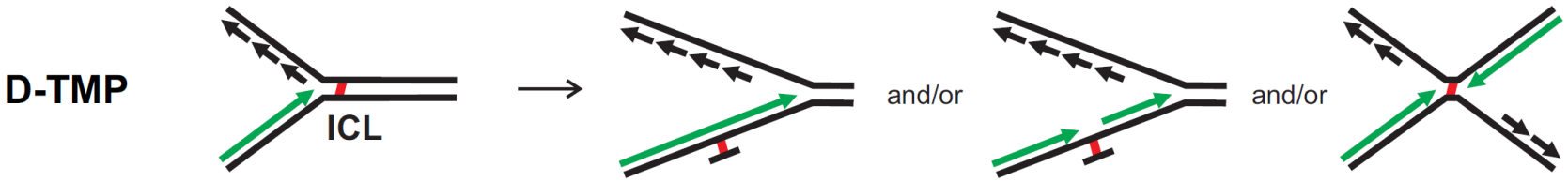
double sided



Double sided events dominate in repair deficient cells

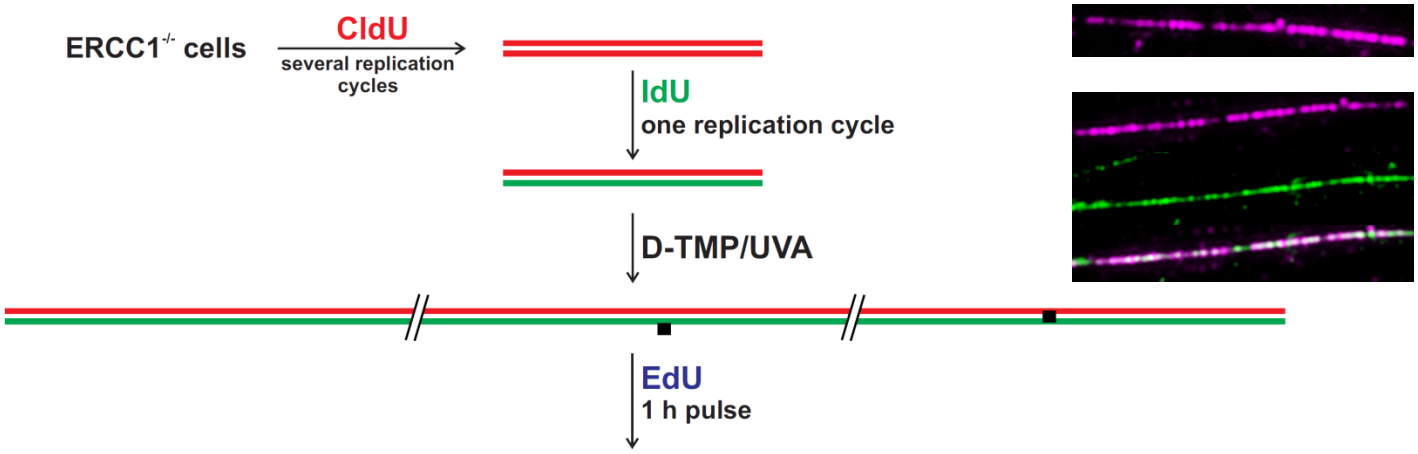


Are the ICLs intact at the time of fork encounters?

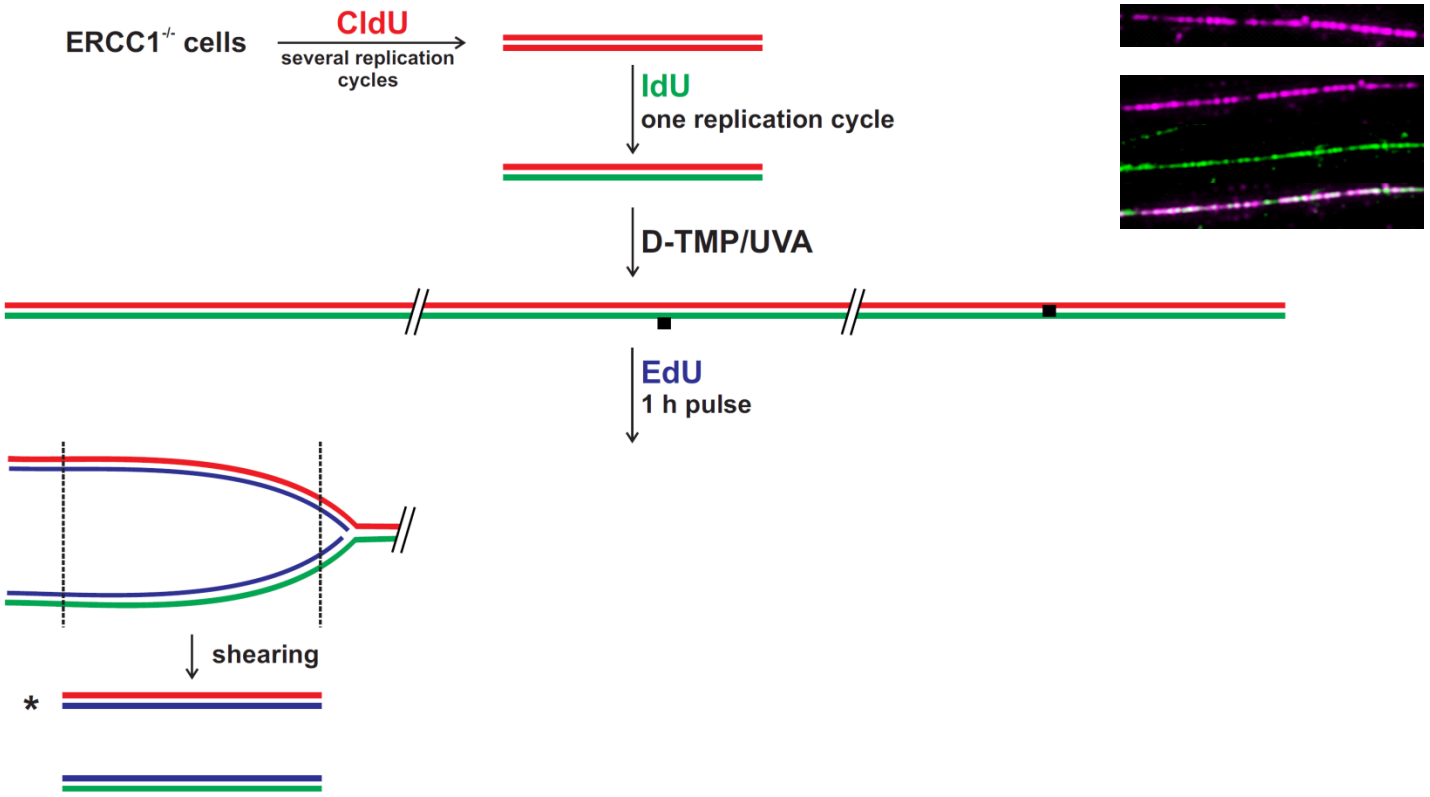


Are parental strands covalently linked at the time of the fork encounter(s)?

Are ICLs intact at the time of fork encounters?



Are ICLs intact at the time of fork encounters?



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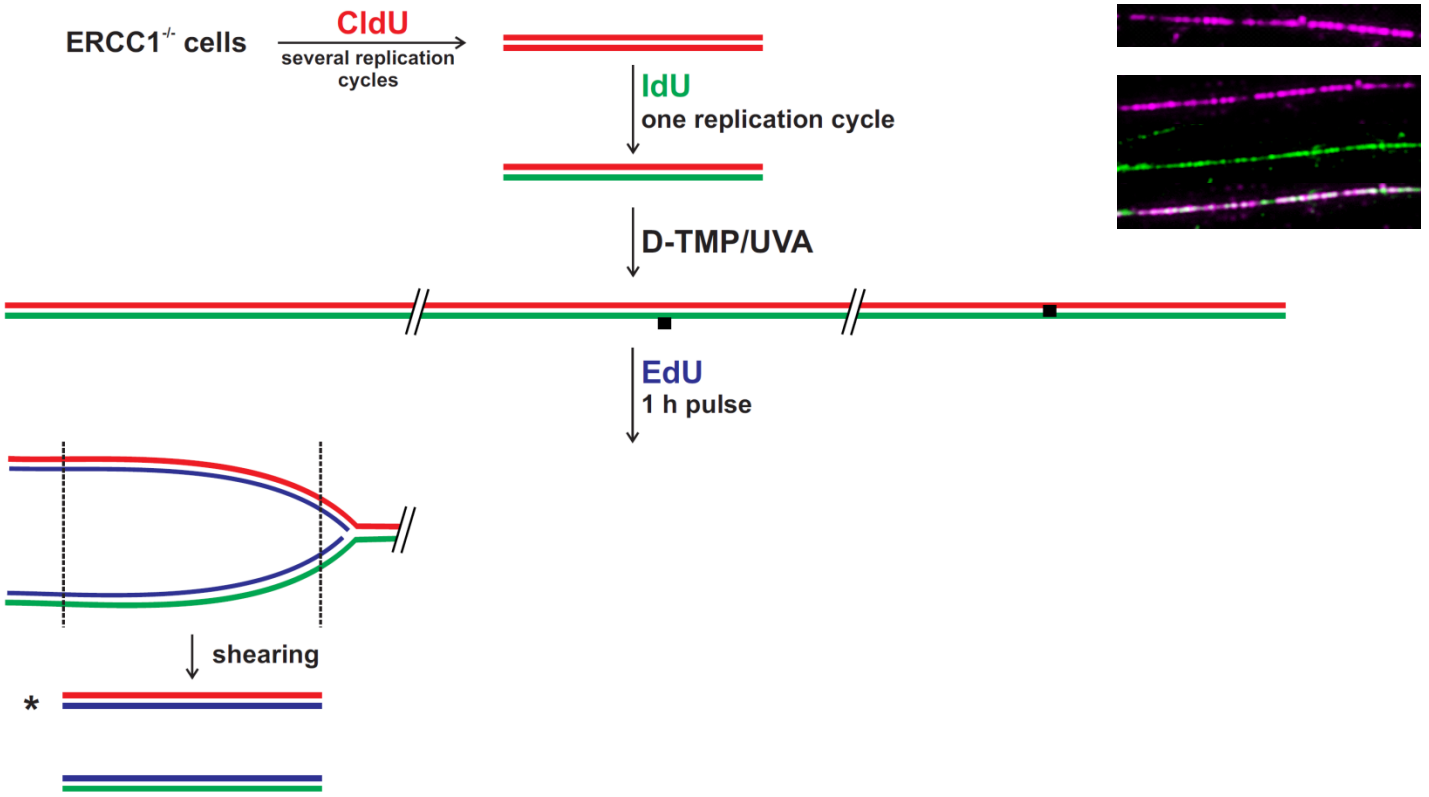

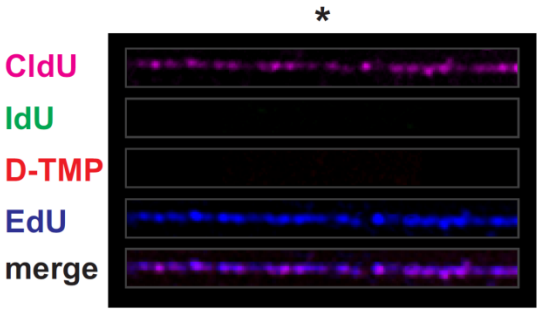

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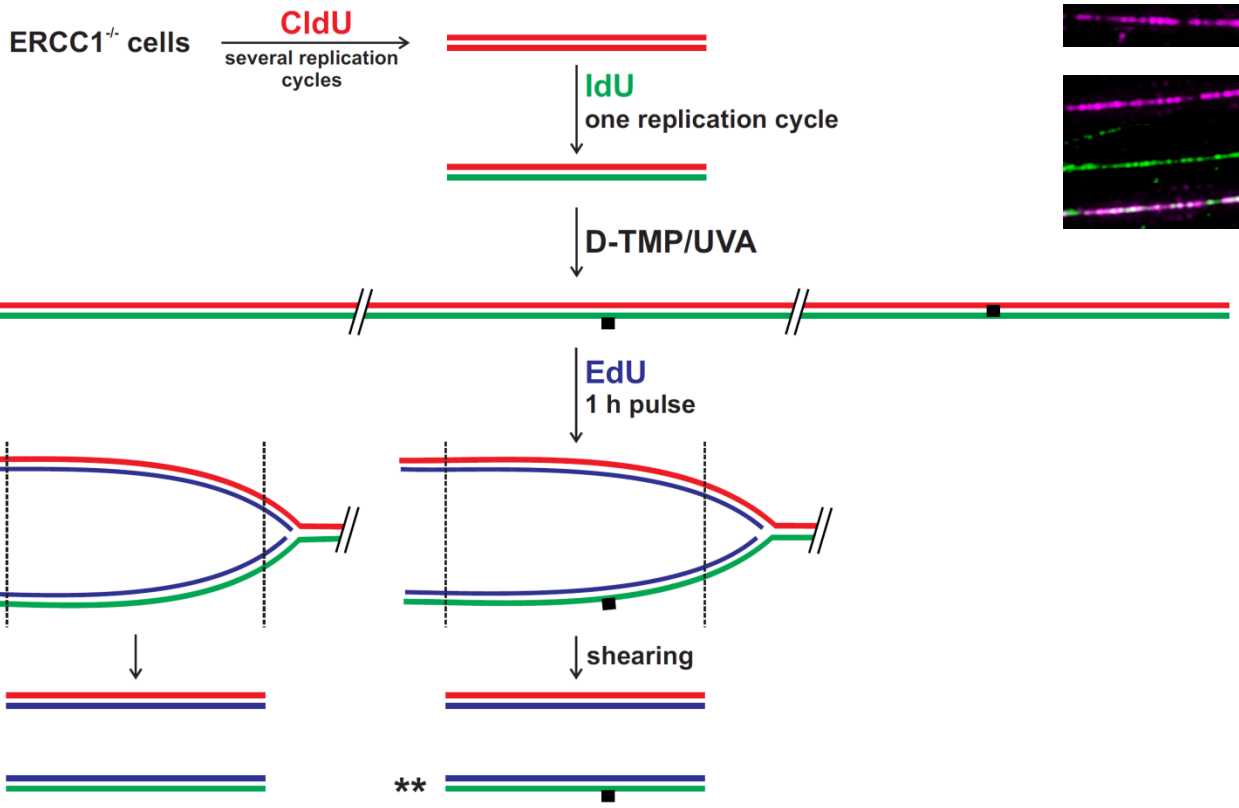

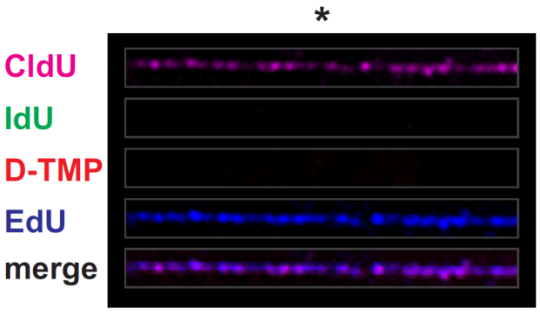

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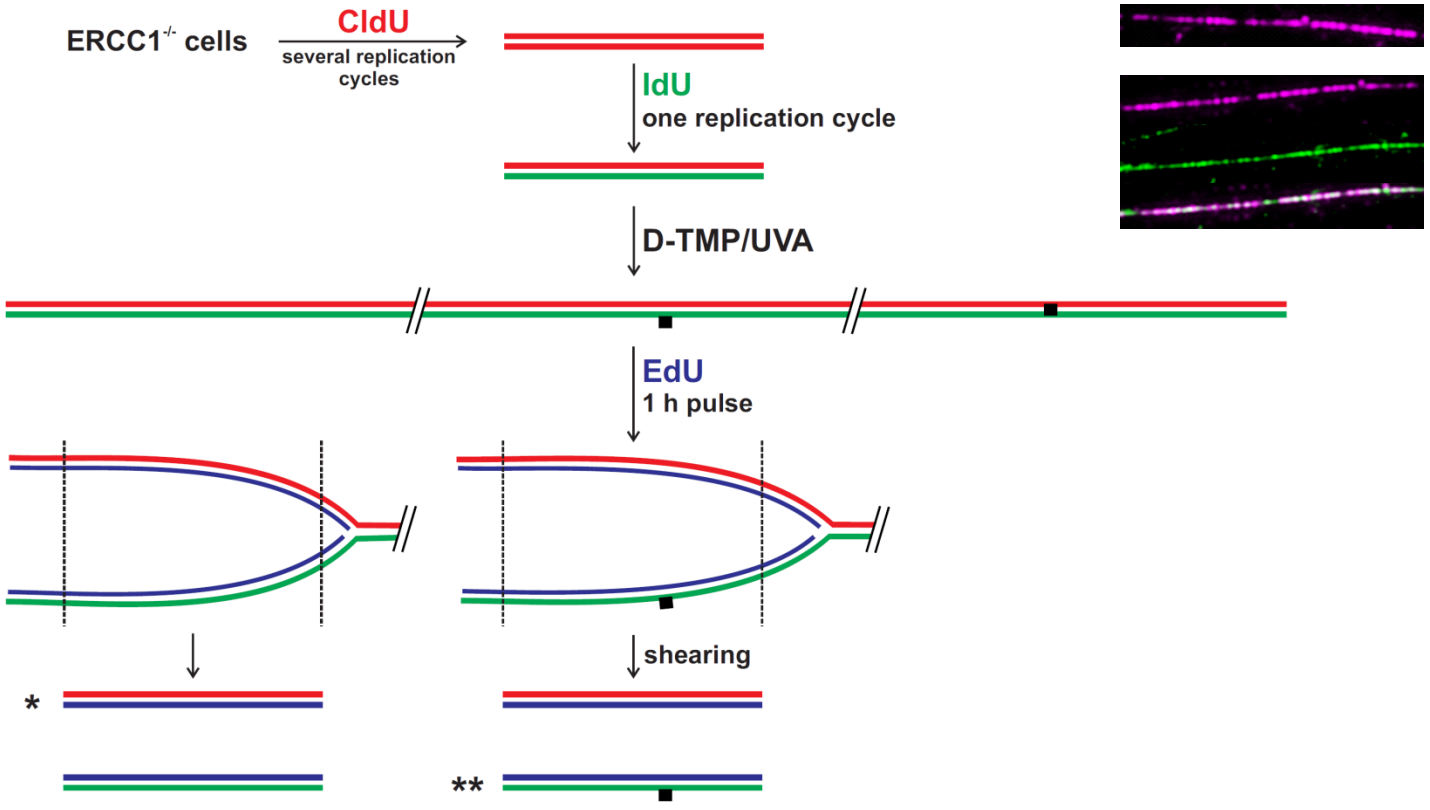

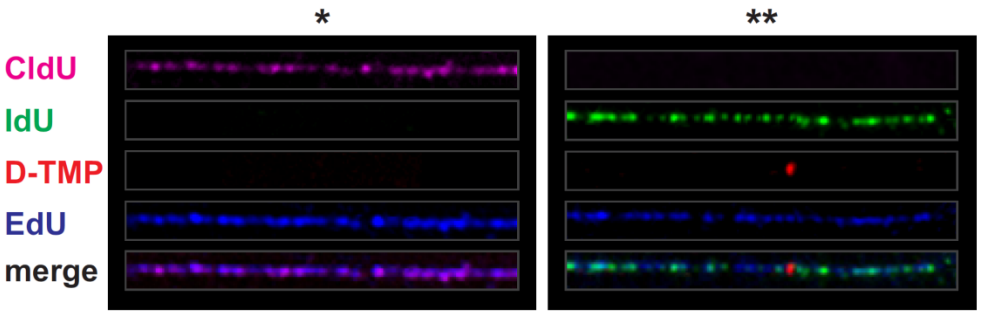

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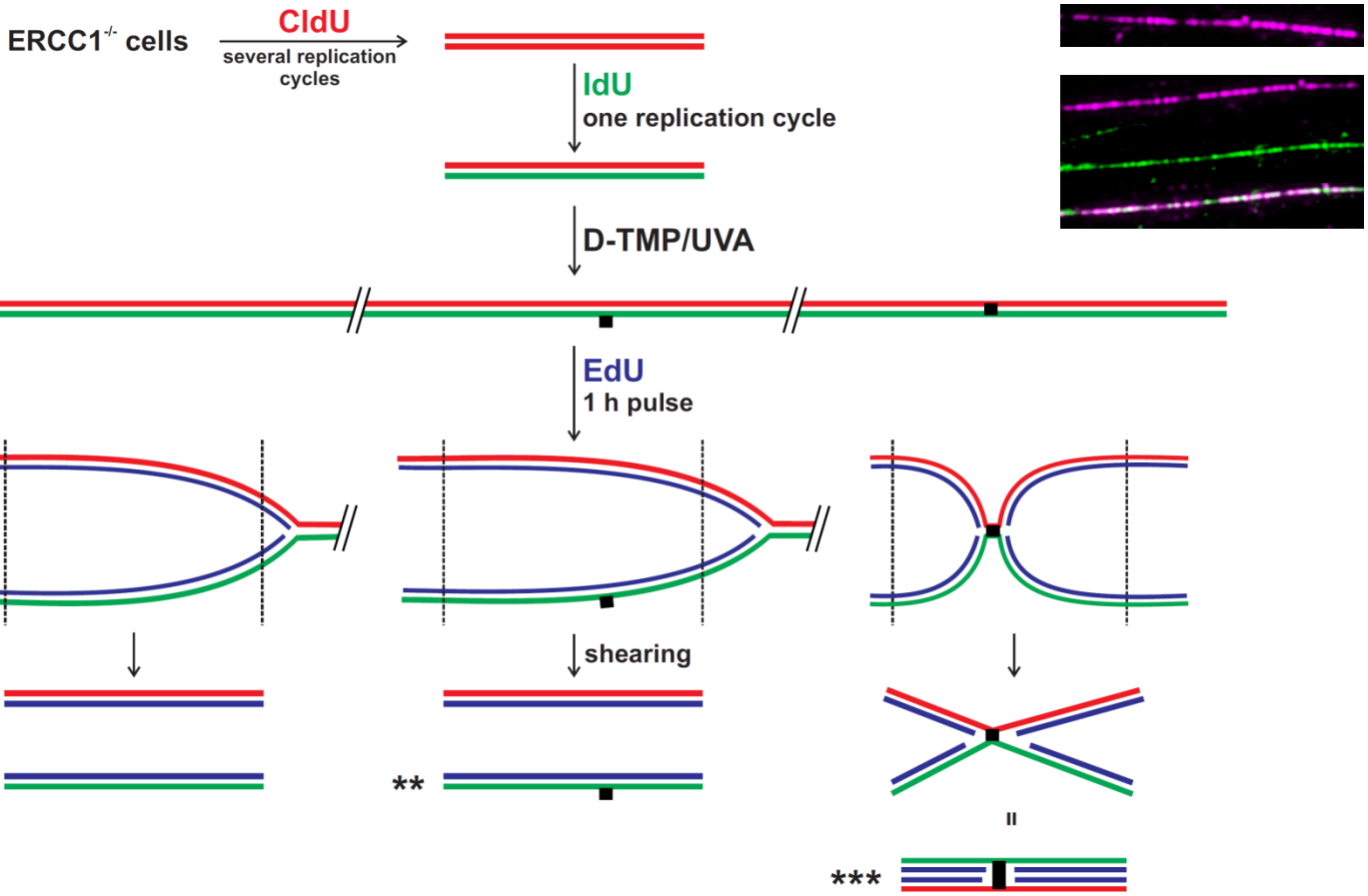
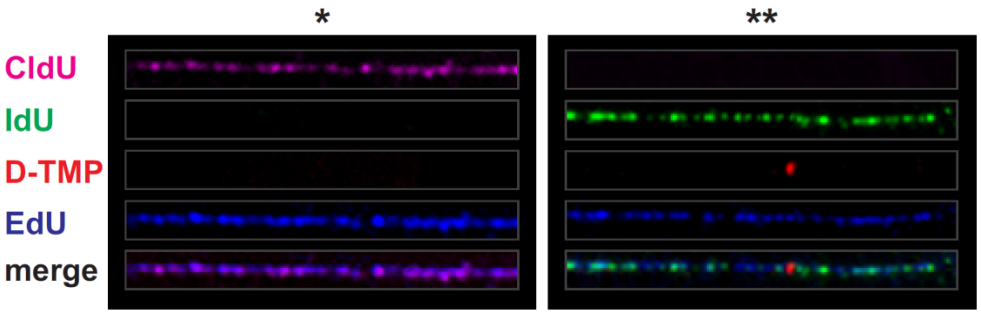
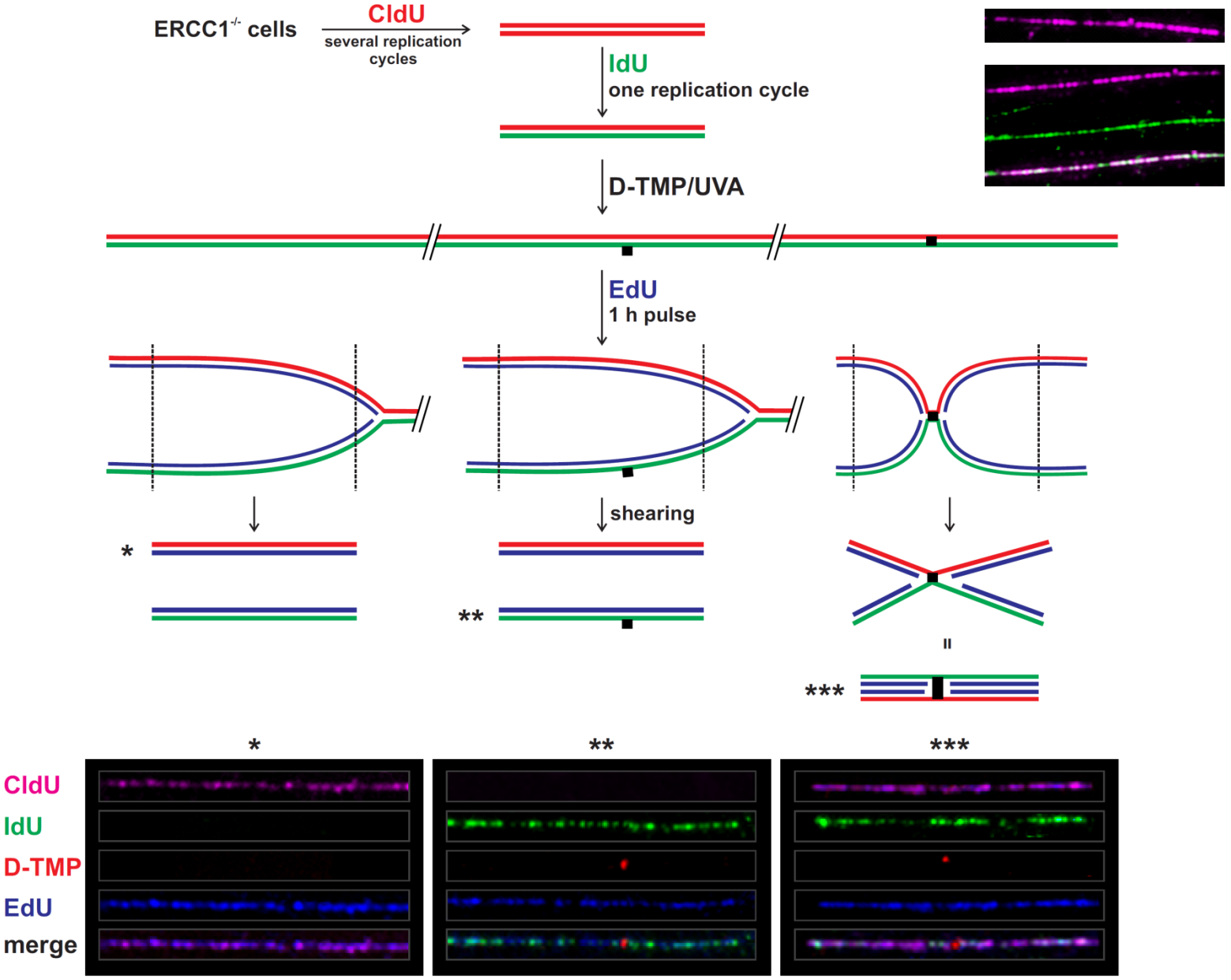




Are ICLs intact at the time of fork encounters?



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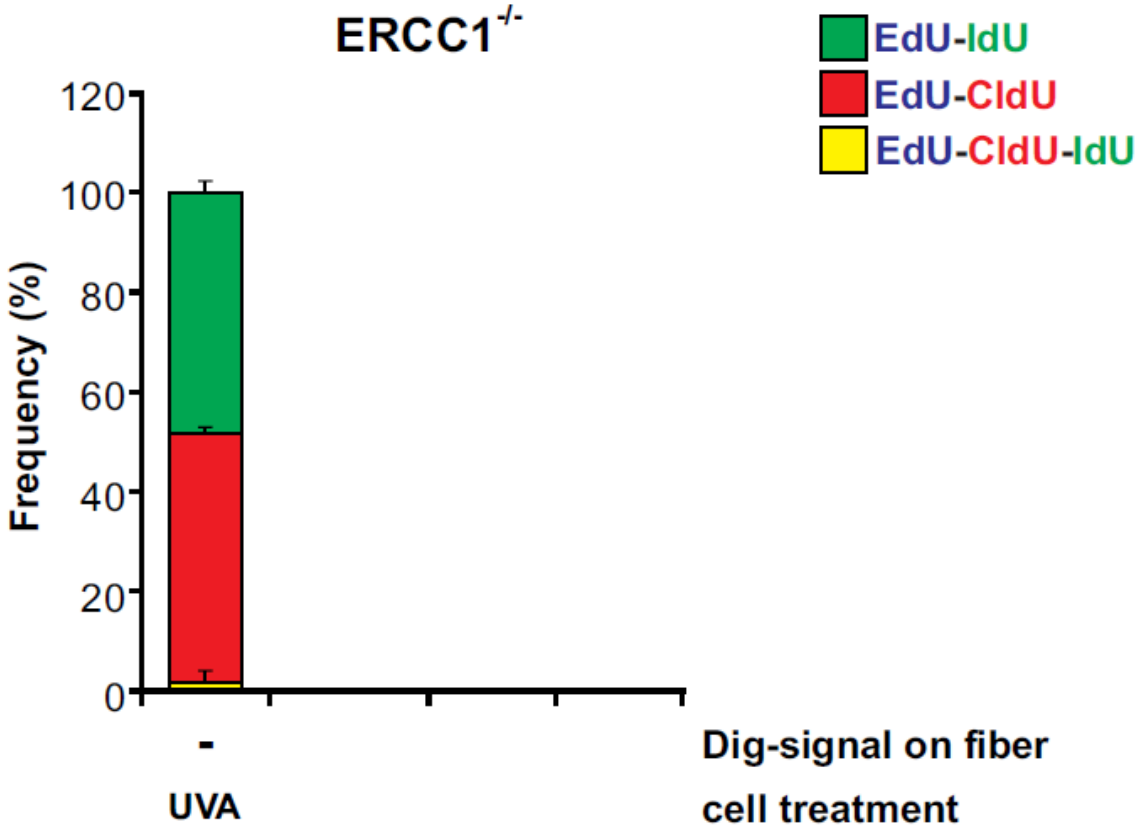

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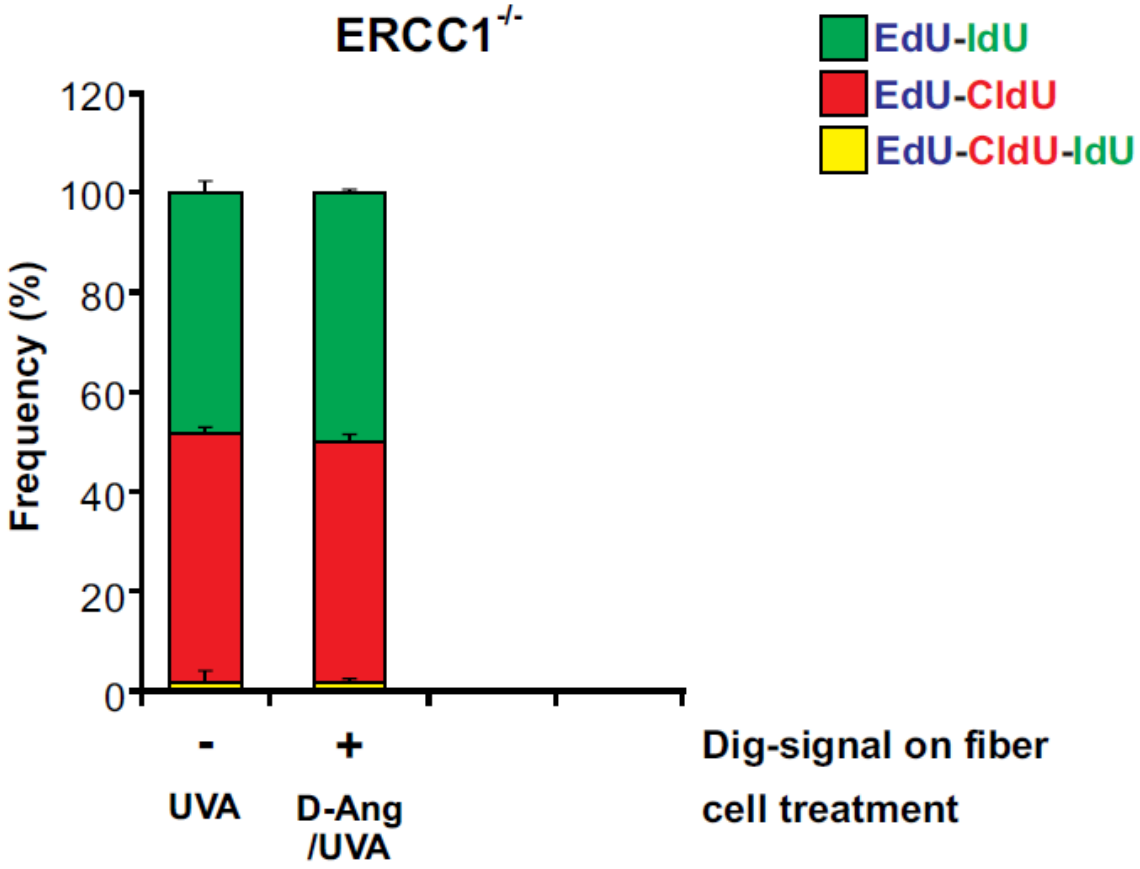

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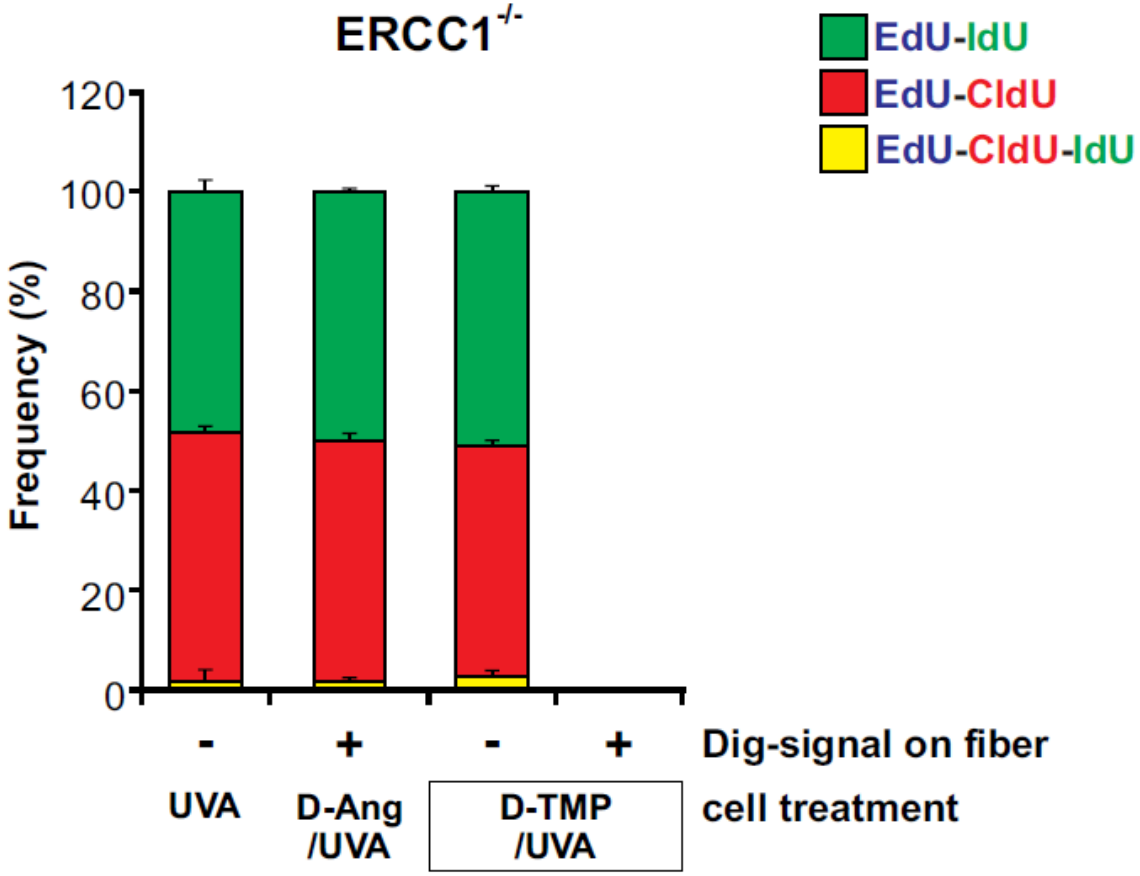

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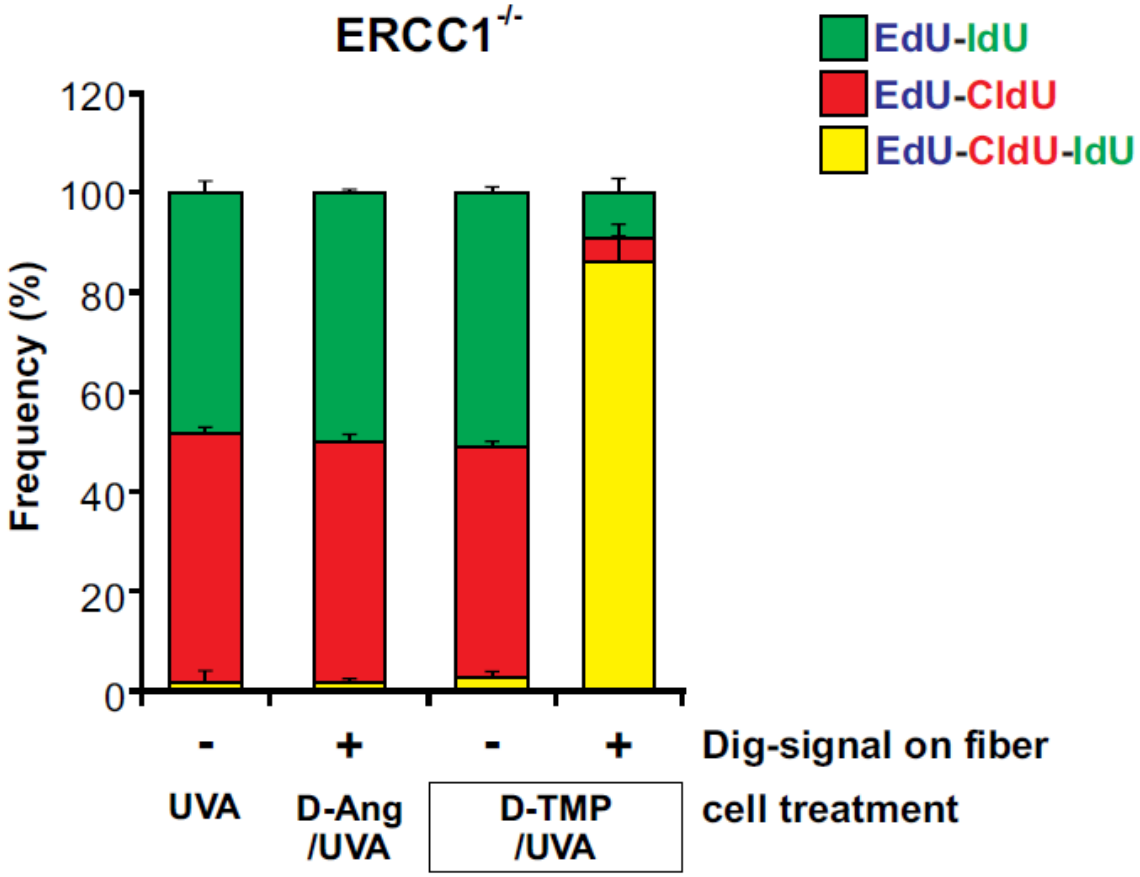
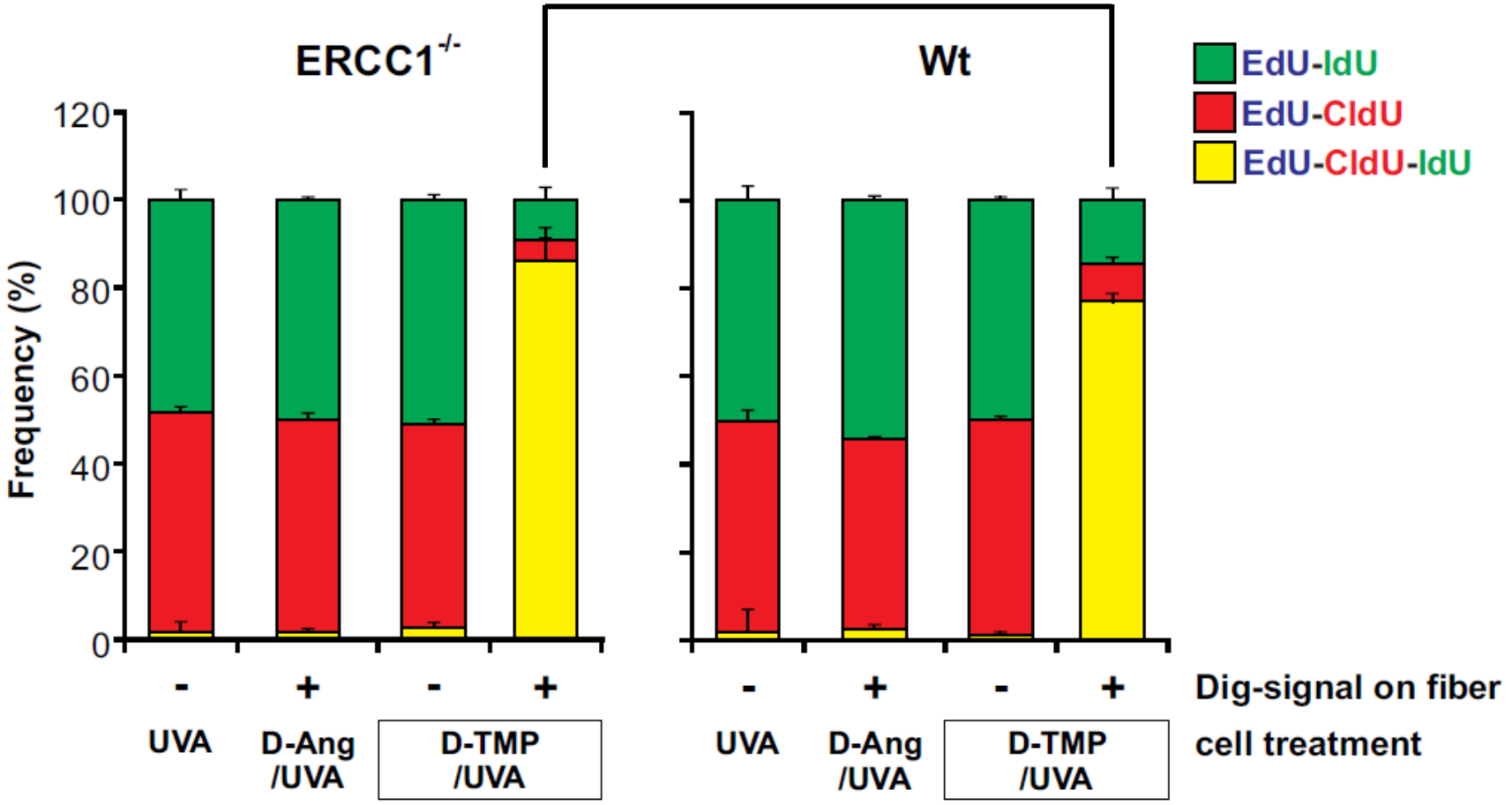
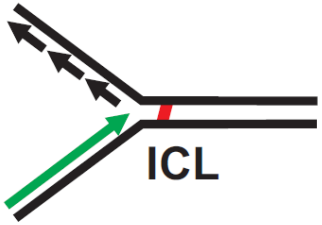


Most Dig-TMP adducts are intact ICLs

Parental strands are crosslinked at the time of fork encounter(s)



The timing of DTMP/UVA ICL unhooking



XPD -/- cells

XPD$^{-/-}$ cells

CldU
several replication cycles

IdU
one replication cycle

How long it takes for %BRG to decline?

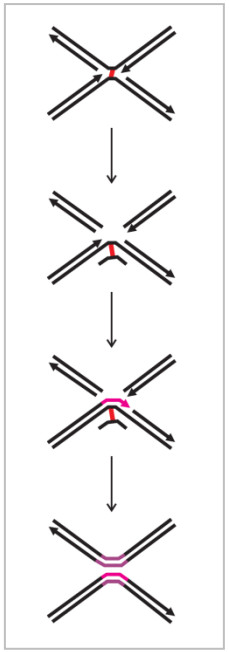
D-TMP/UVA

EdU
1 h pulse

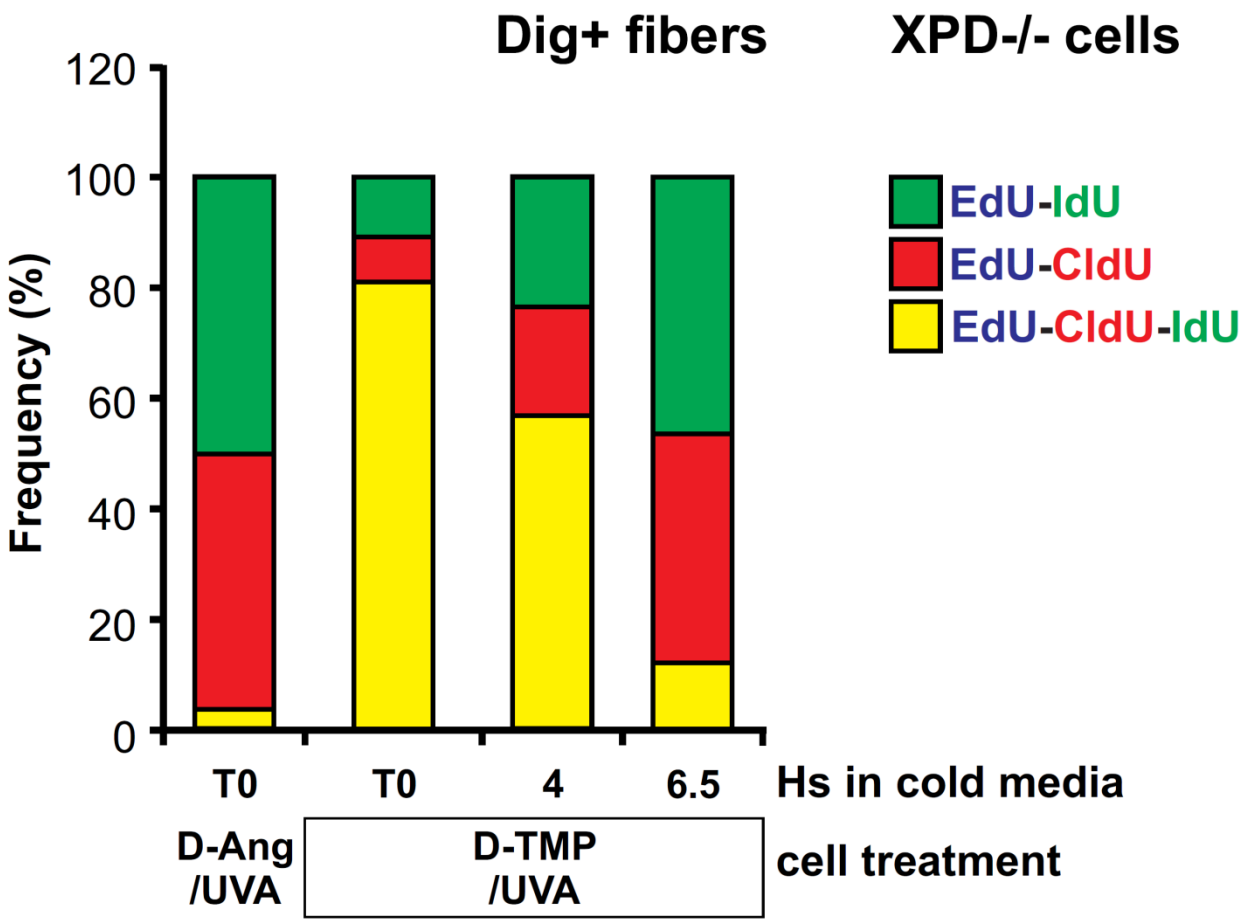
T0

cold chase

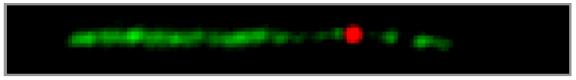
sample at different timepoints
How long does it take for %BRG to decline?

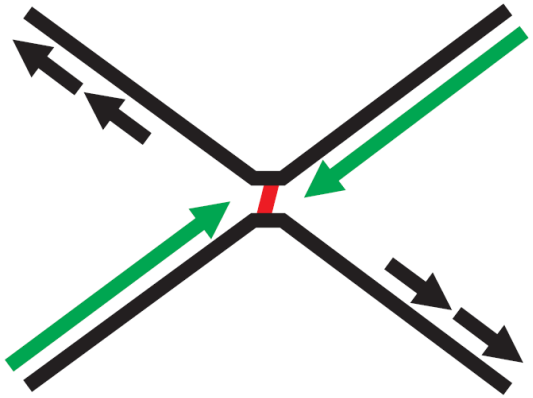


Unhooking of DIG-TMP ICLs at the fork takes >6 hours

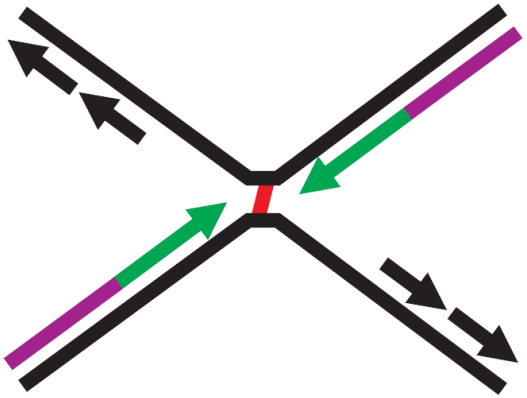


Are double sided patterns the result of dual fork stalling at an ICL?

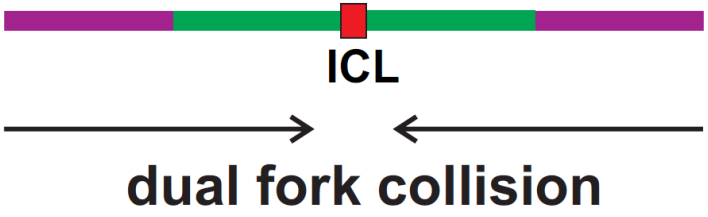




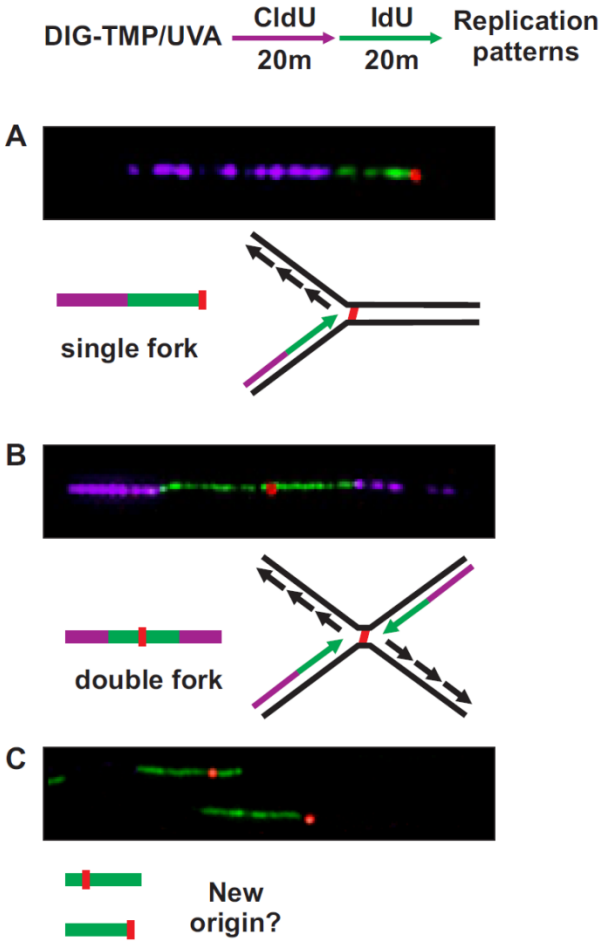
Two sequential pulses to visualize the direction of the replication fork

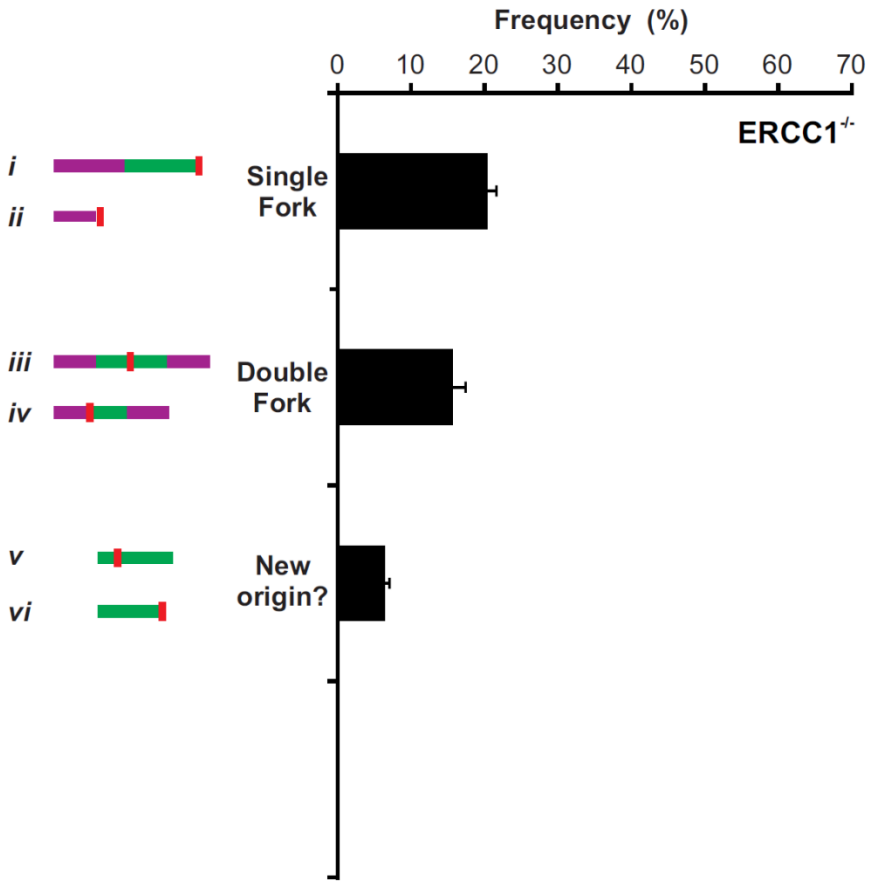


DIG-TMP/UVA → CldU 20m → IdU 20m → Replication patterns

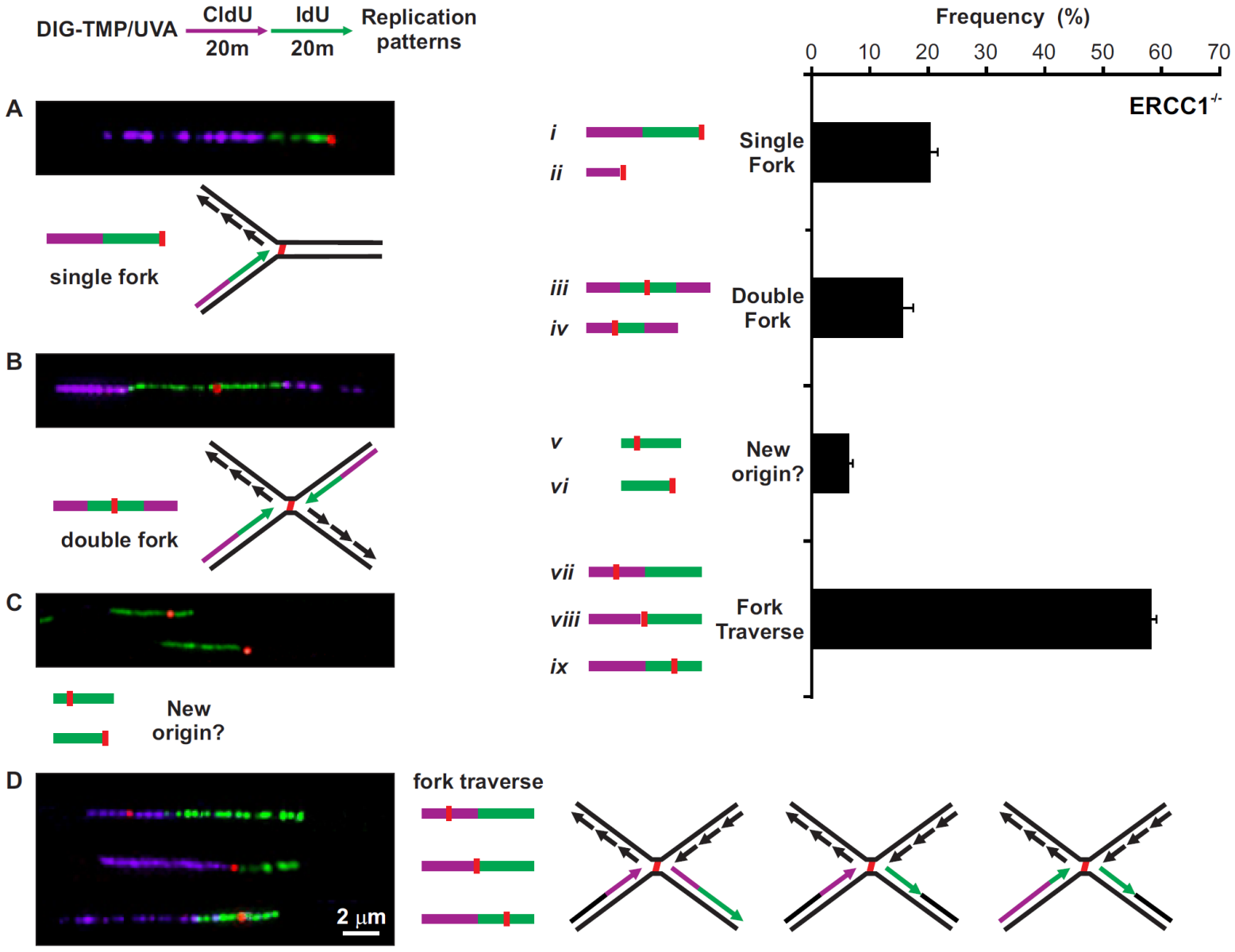


Replication in the vicinity of ICLs

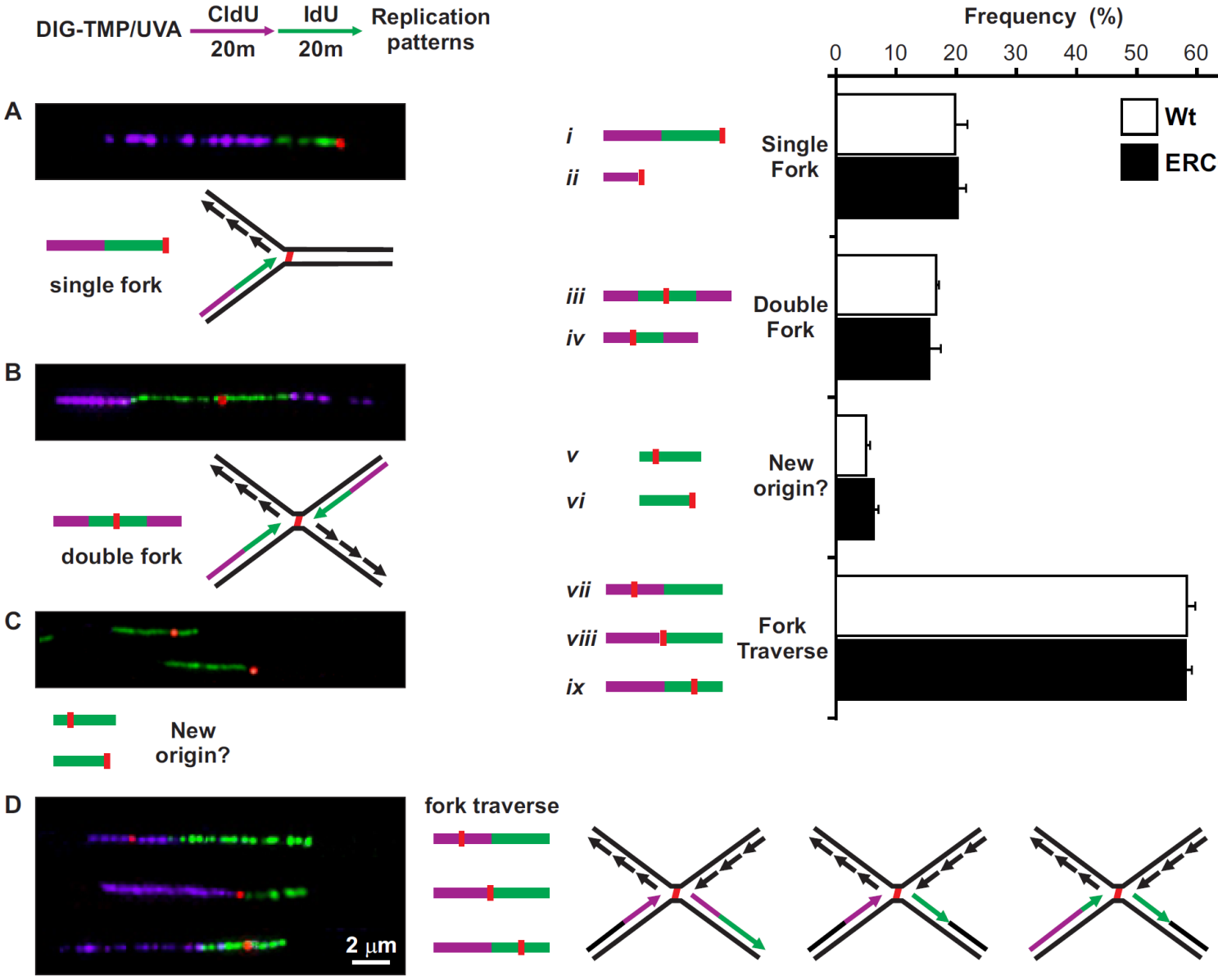




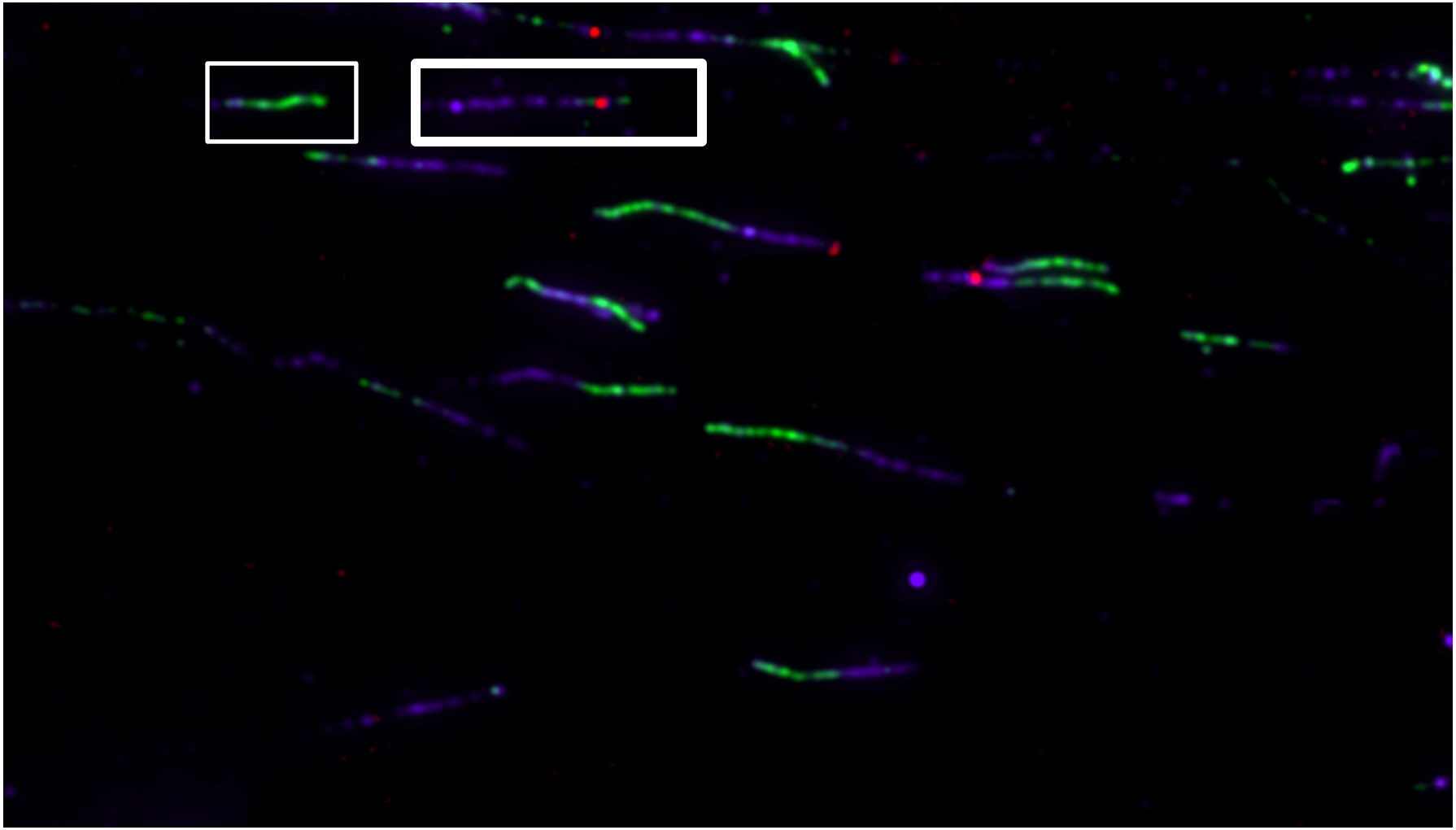
Replication in the vicinity of ICLs



Equivalent results in repair proficient cells

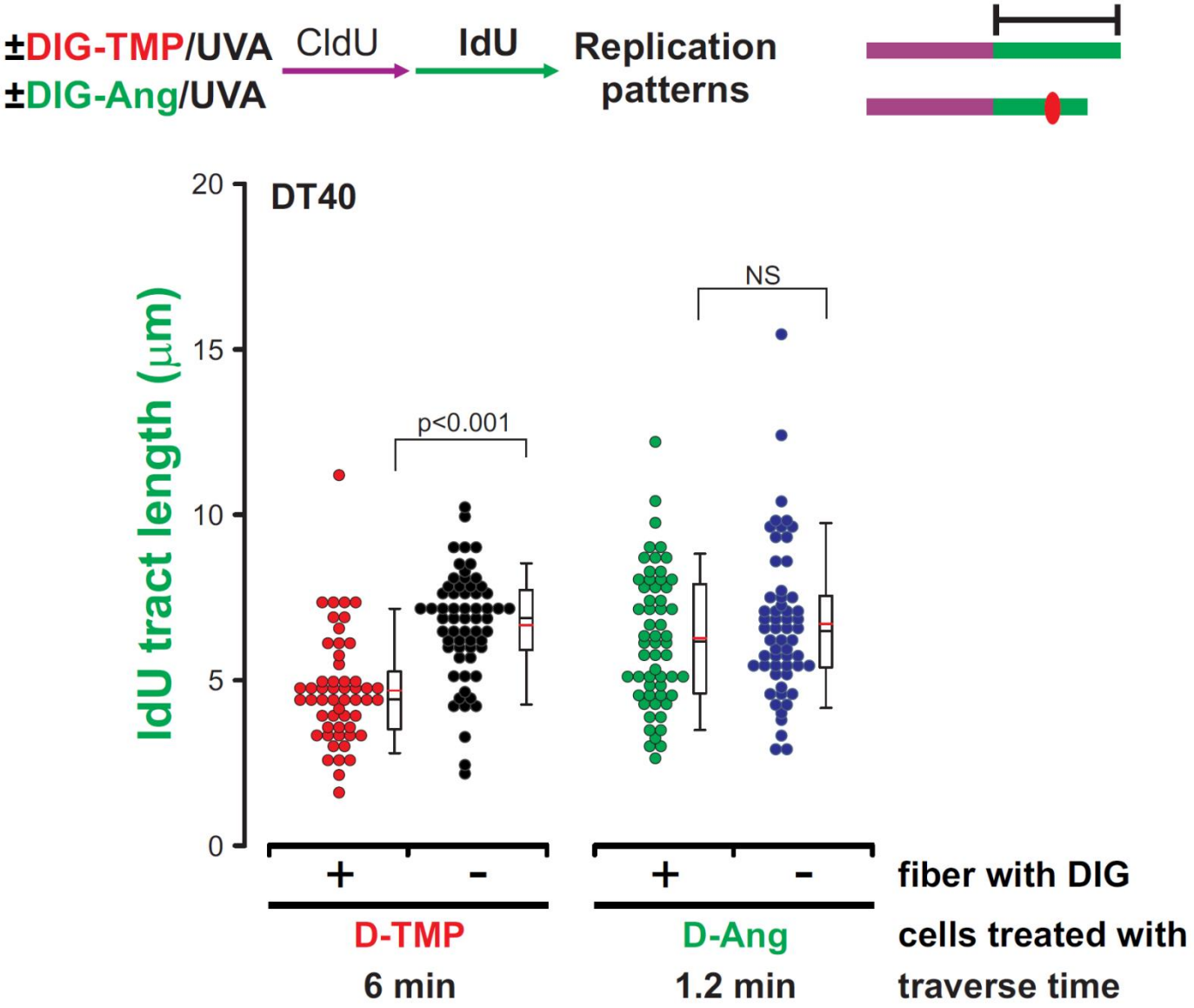


What is the time cost of traverse?



Dig-pso/UVA Double pulse

Duration of traverse



What drives replication traverse of ICLs?

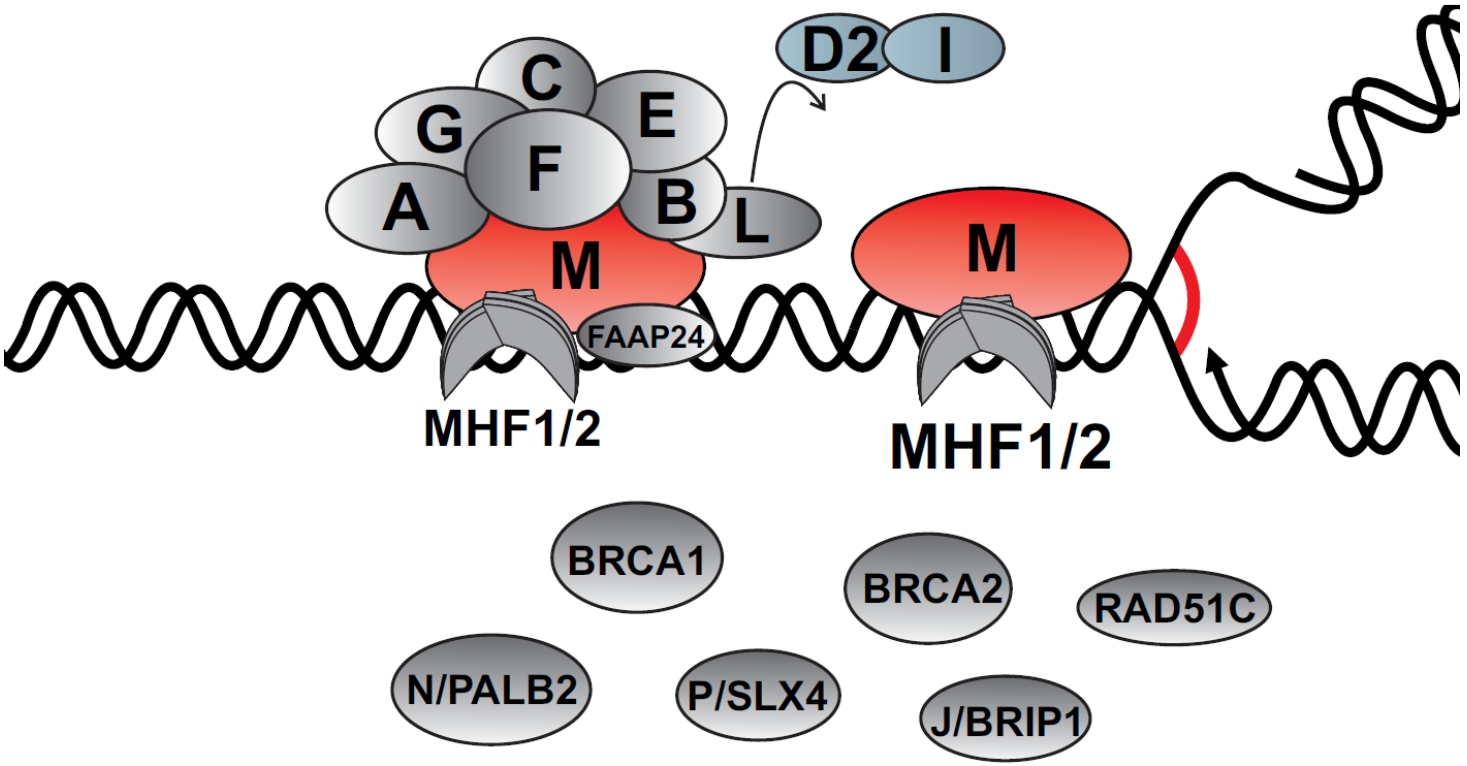
ICLs are absolute blocks to HELICASES

DNA TRANSLOCASES can move along DNA without unwinding

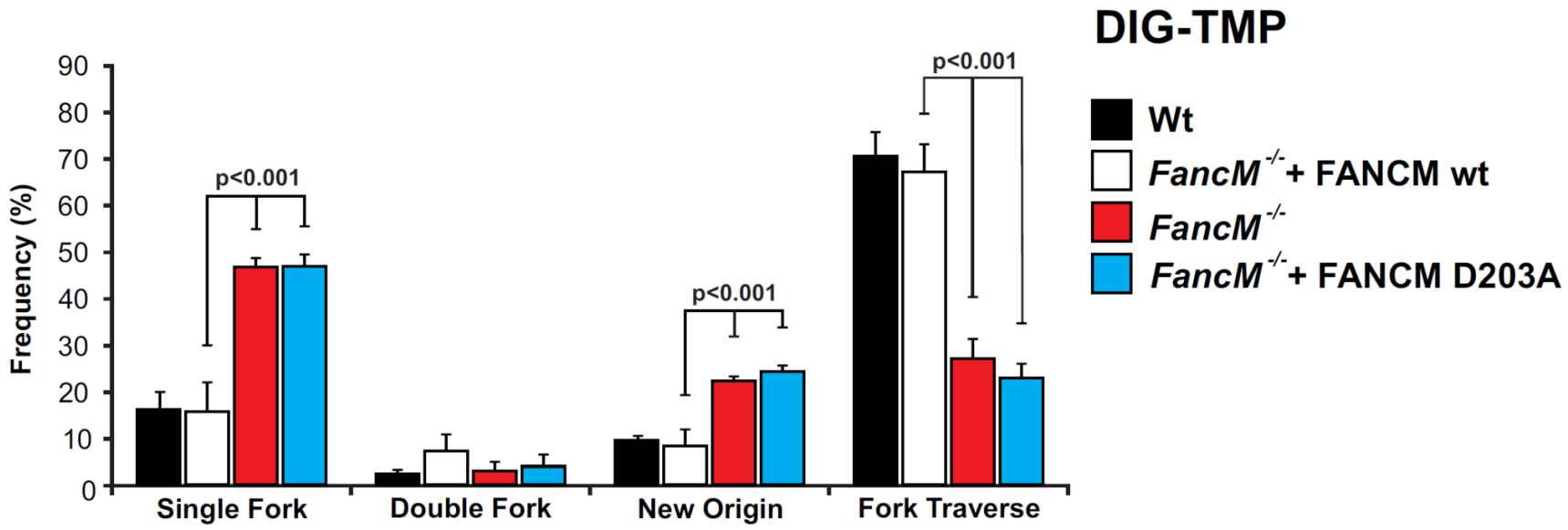
FANCM

translocase activity

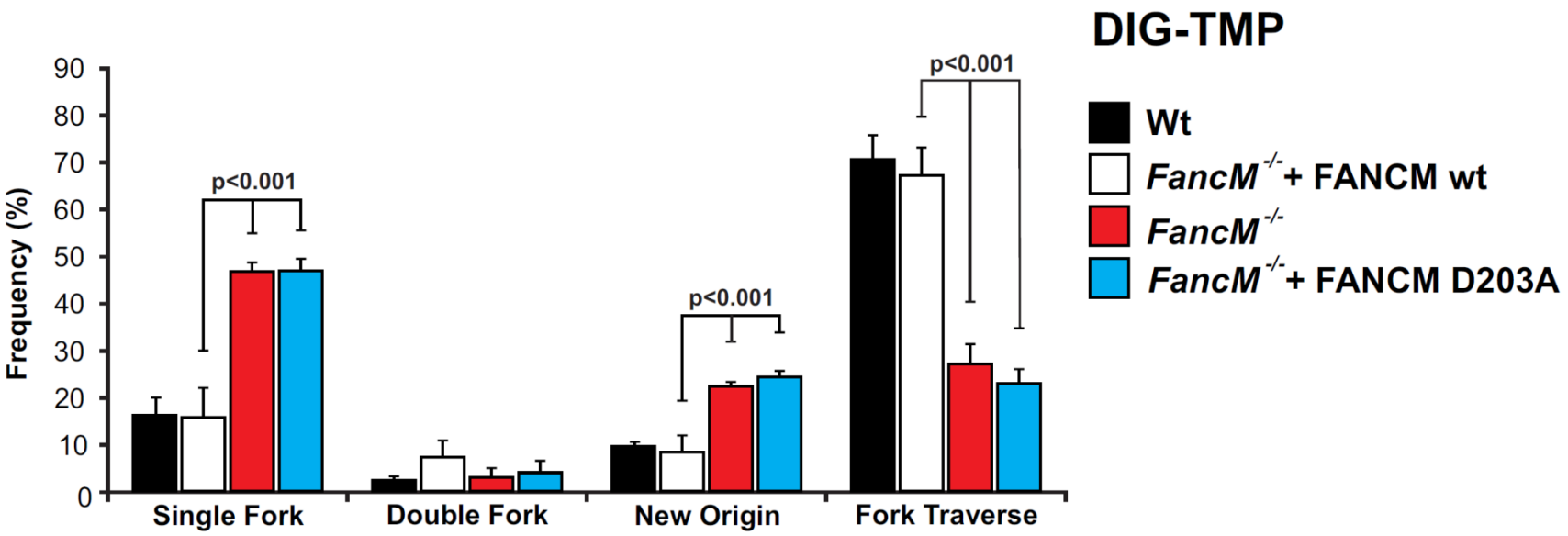
recruited to ICLs only in S phase



Influence of FANCM translocase activity on traverse

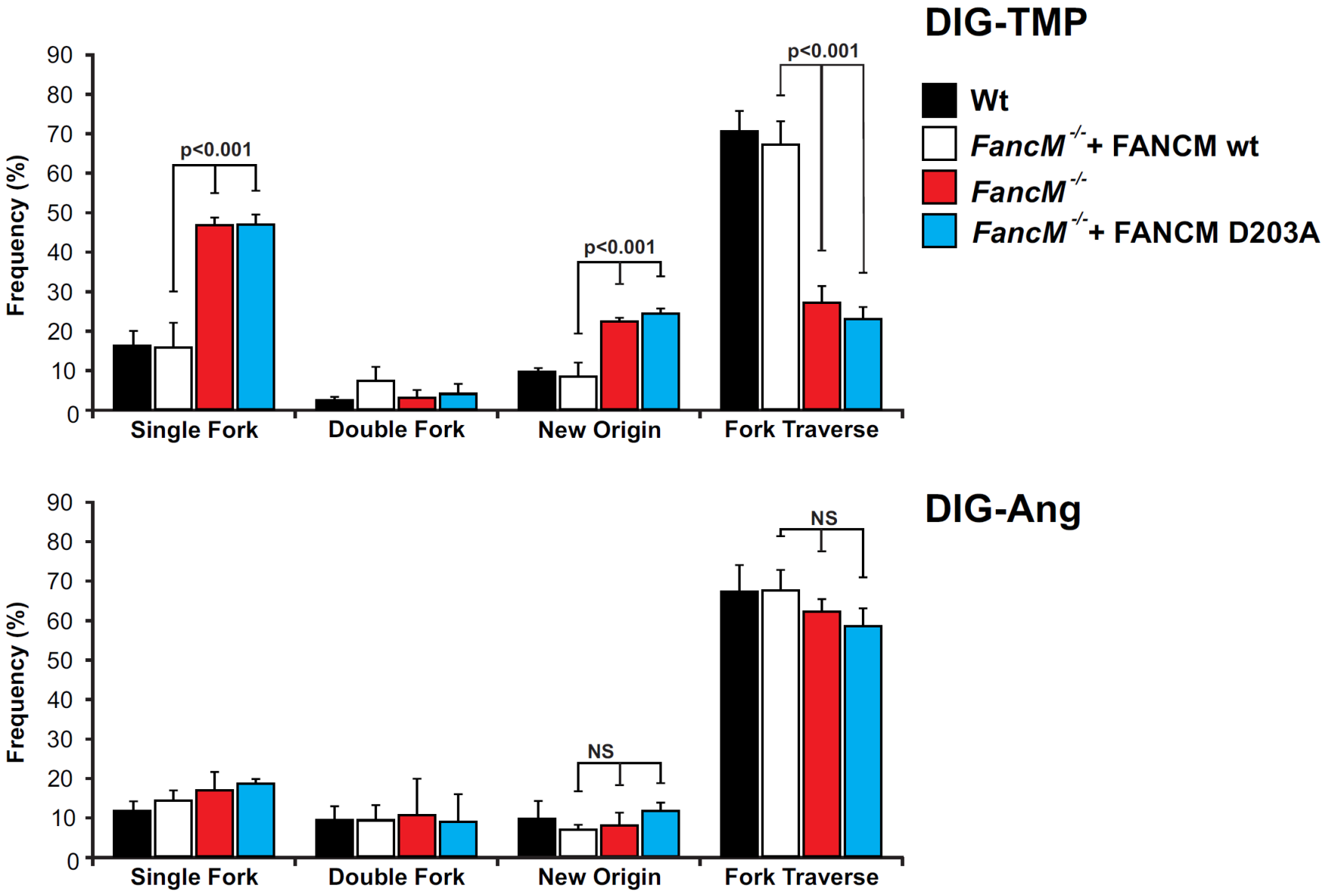


Influence of FANCM translocase activity on traverse

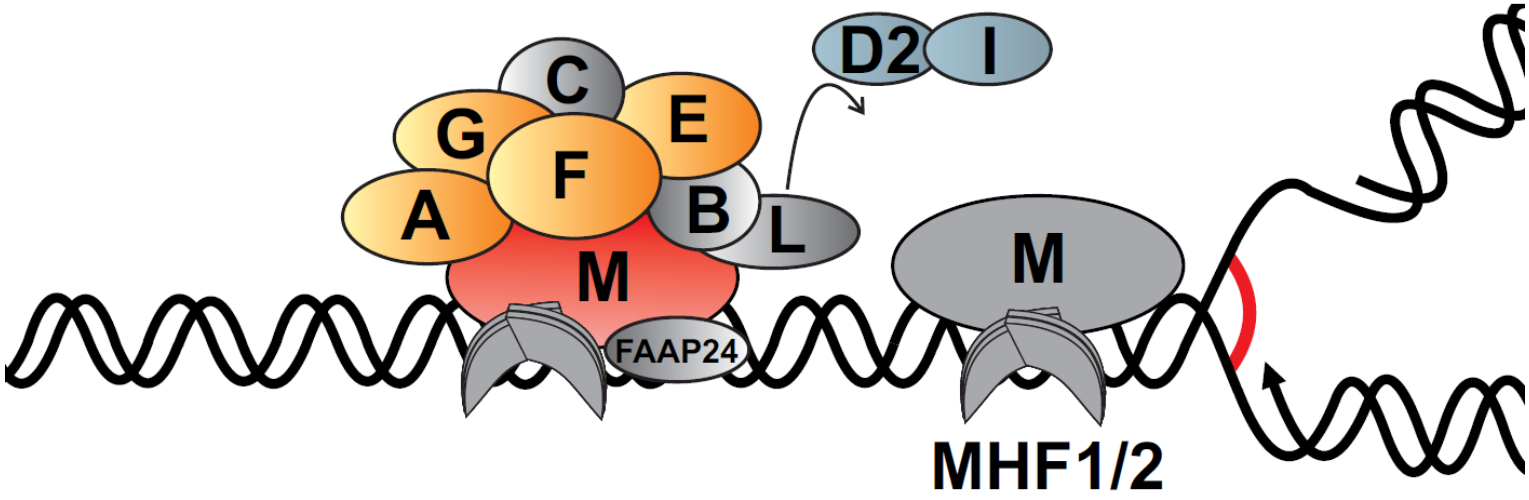


FancM protein is important for traverse of ICLs

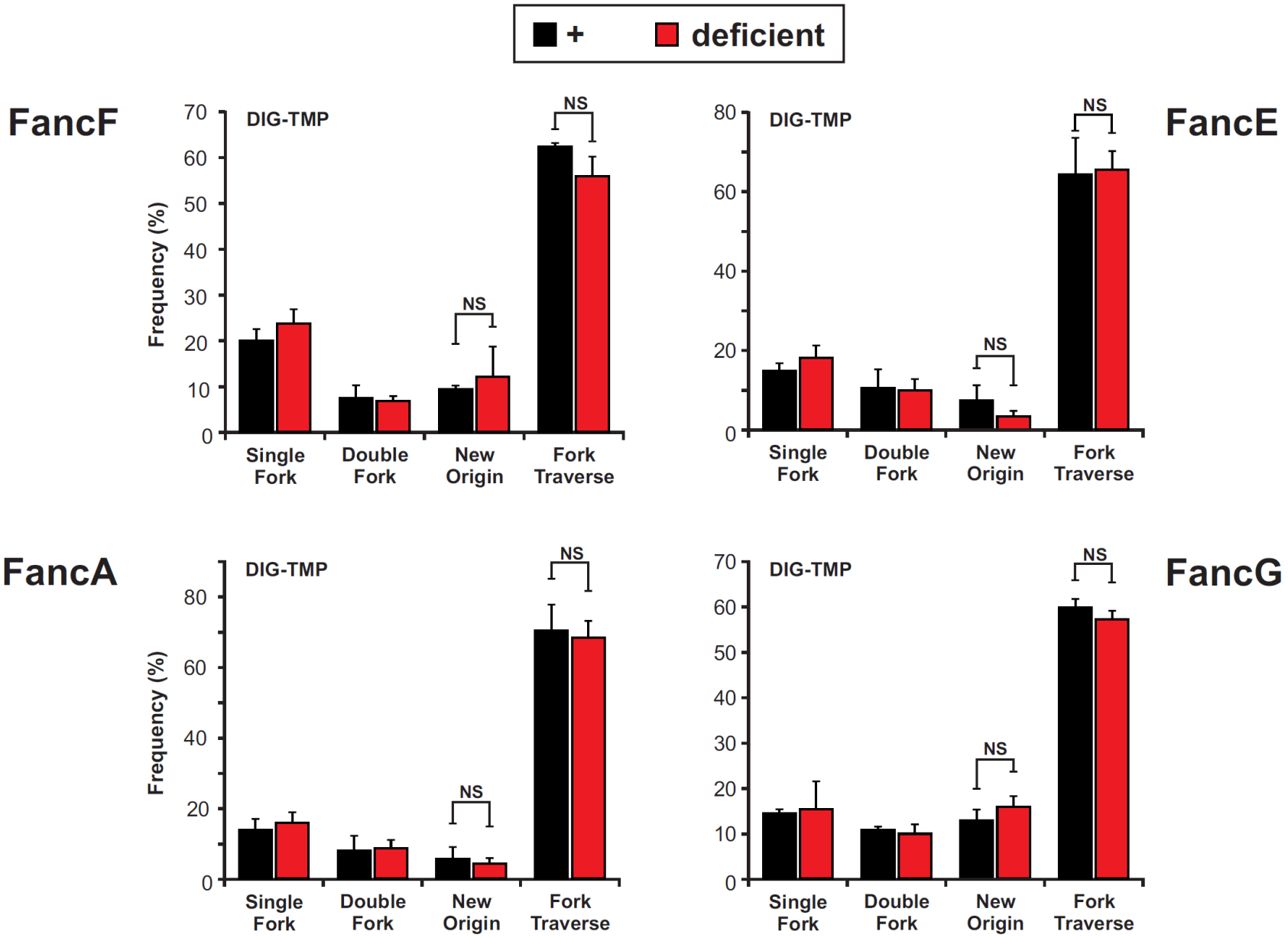
Replication traverse of ICLs, but not MAs, is promoted by FANCM translocase activity



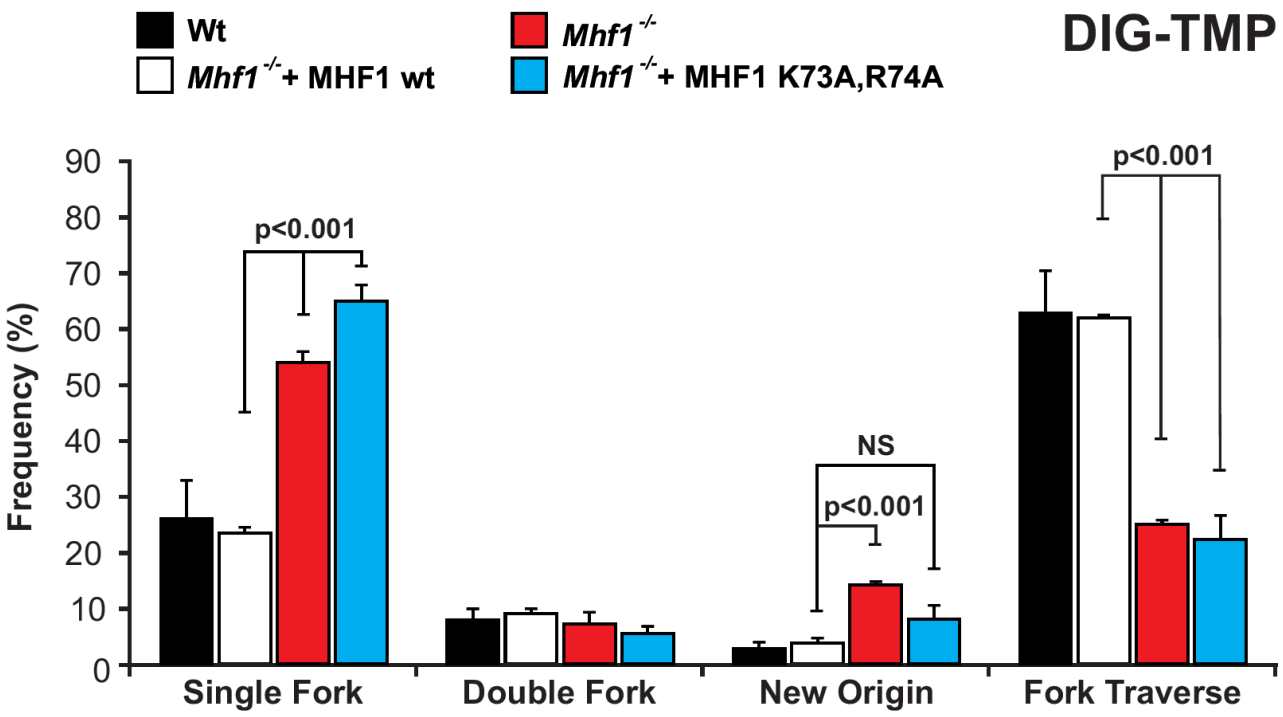
Are the FA core proteins required for replication traverse?

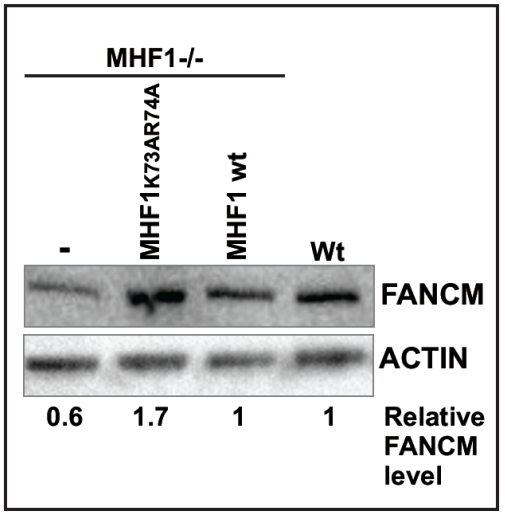


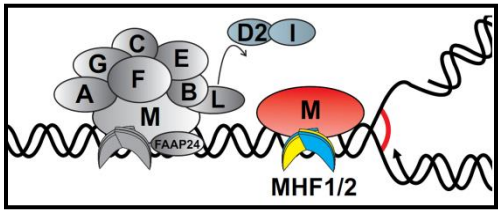
Deficiency in FA core proteins does not influence the frequency of replication patterns



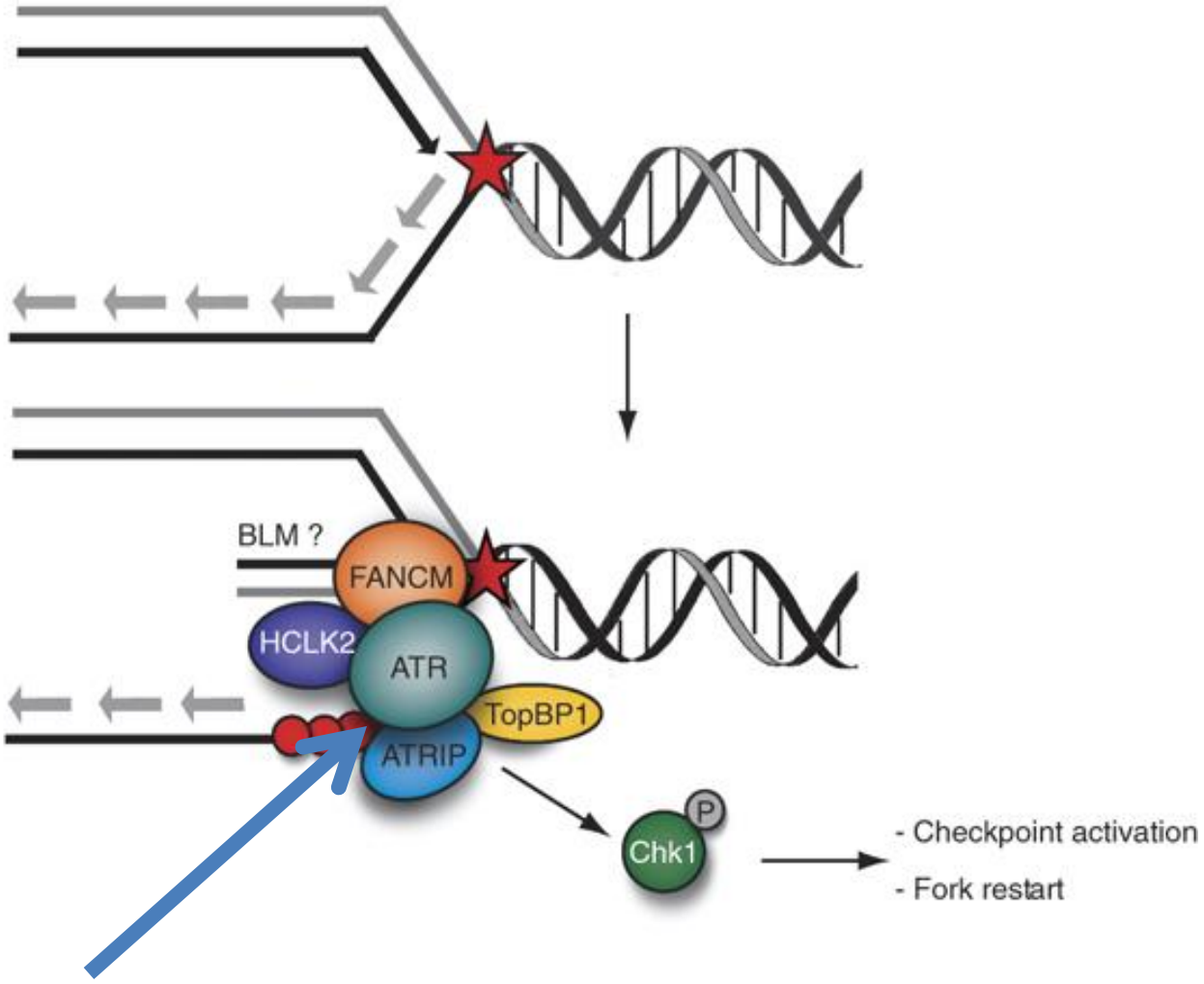
Replication fork traverse of ICLs is mediated by FANCM in the context of the FANCM-MHF complex



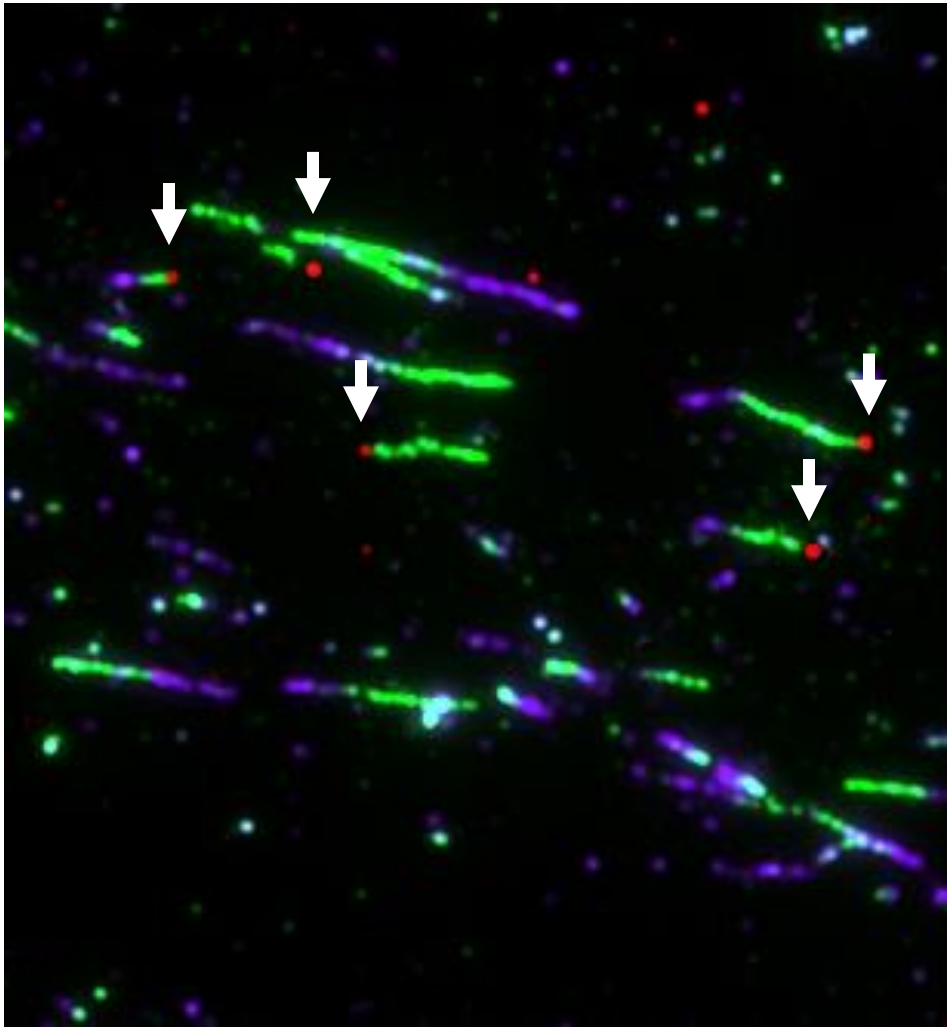




ATR/ATRIP at replication impediments

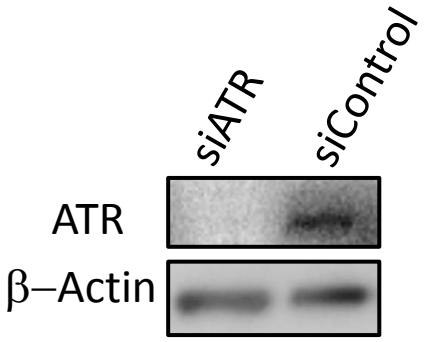


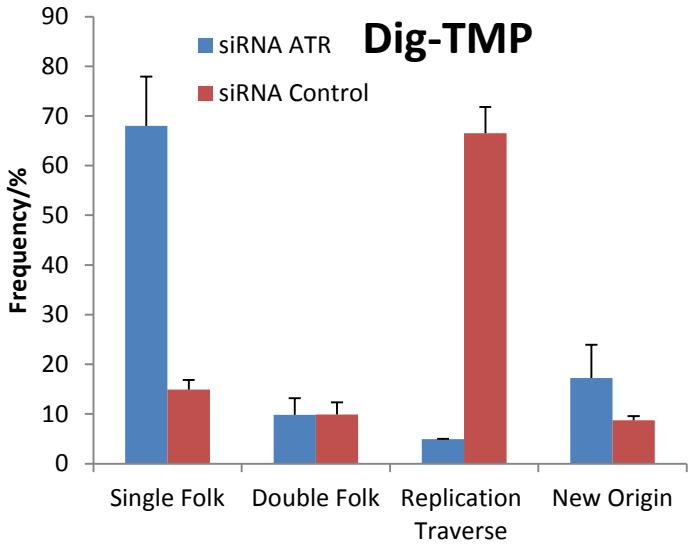
Replication patterns in cells deficient for ATR

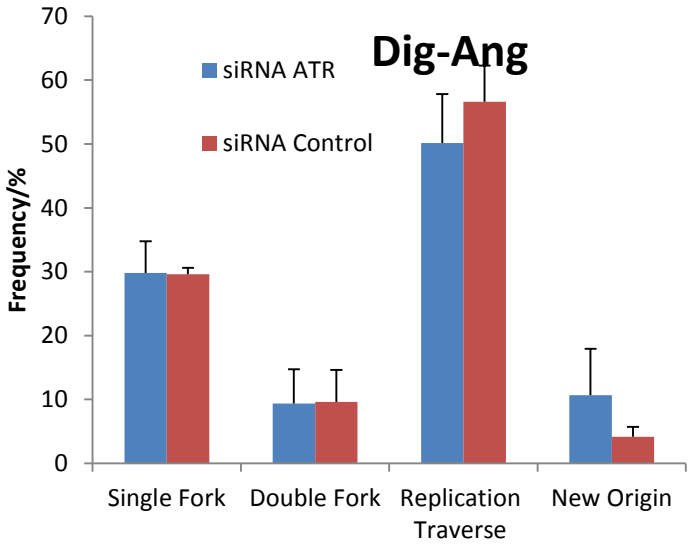


Dominated by single sided patterns

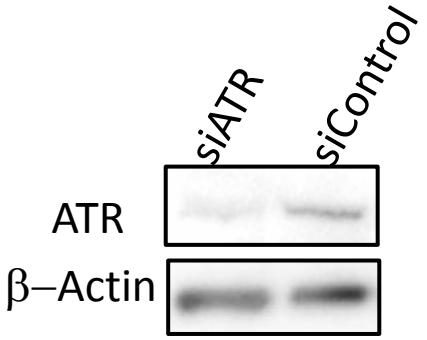
ATR is required for Replication Traverse of ICLs, not MAs

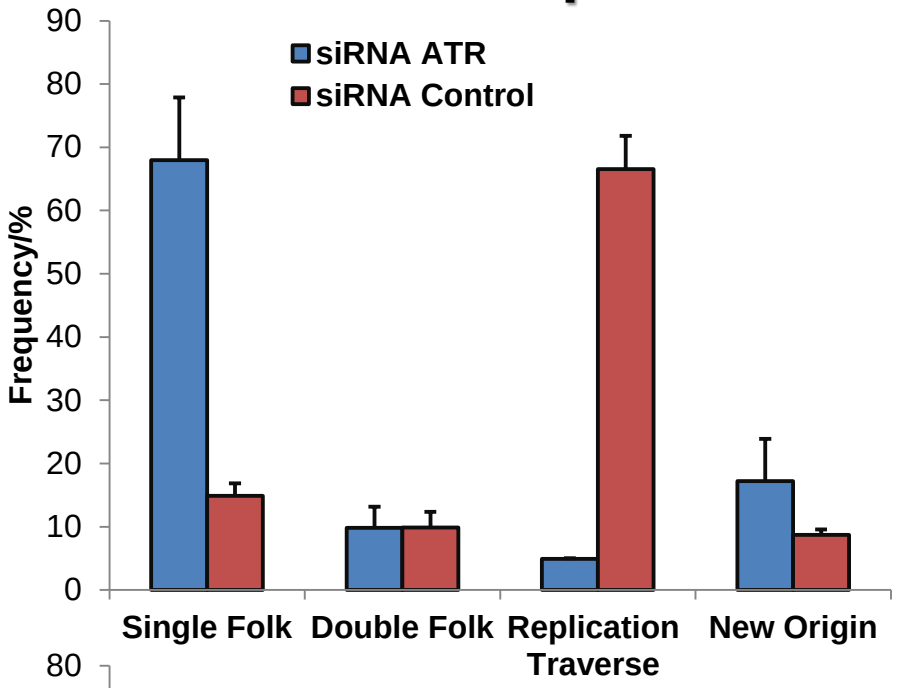


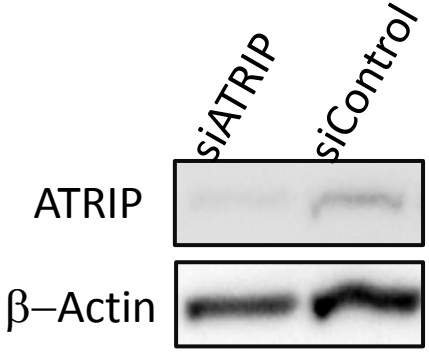


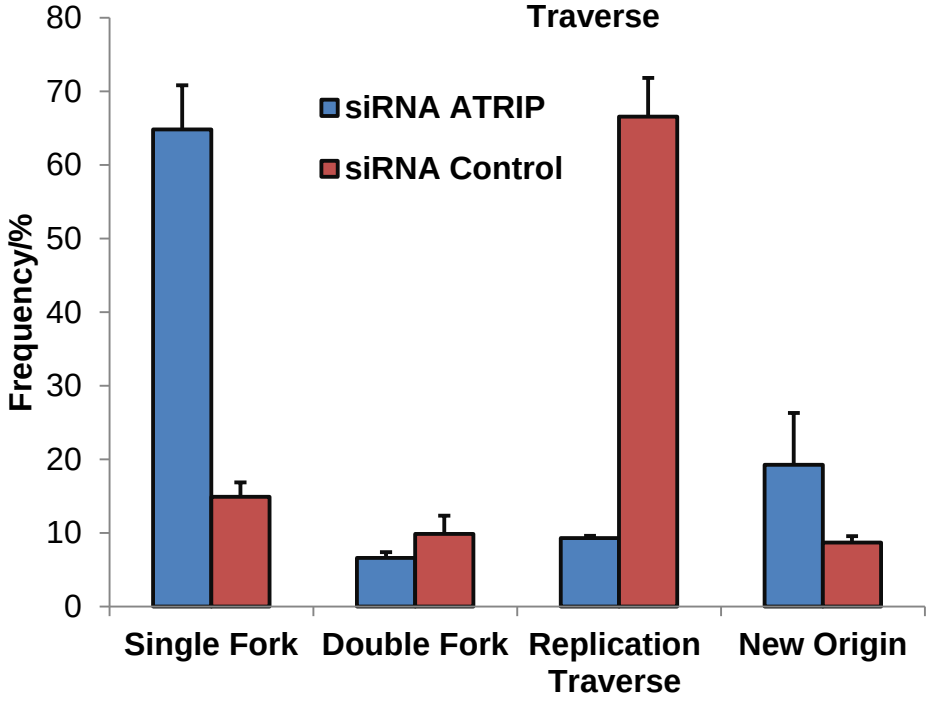


ATR/ATRIP is essential for replication traverse of ICL

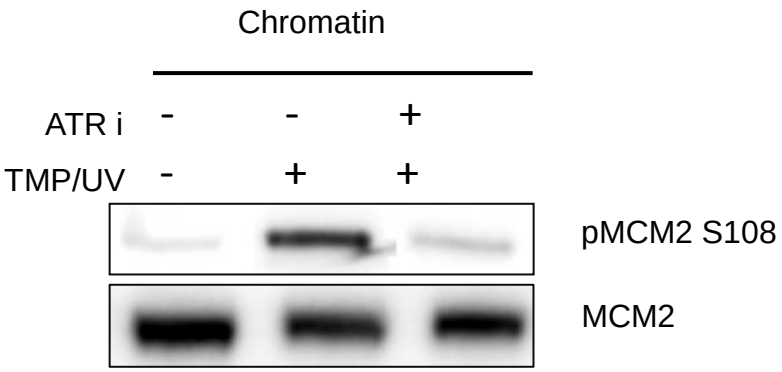






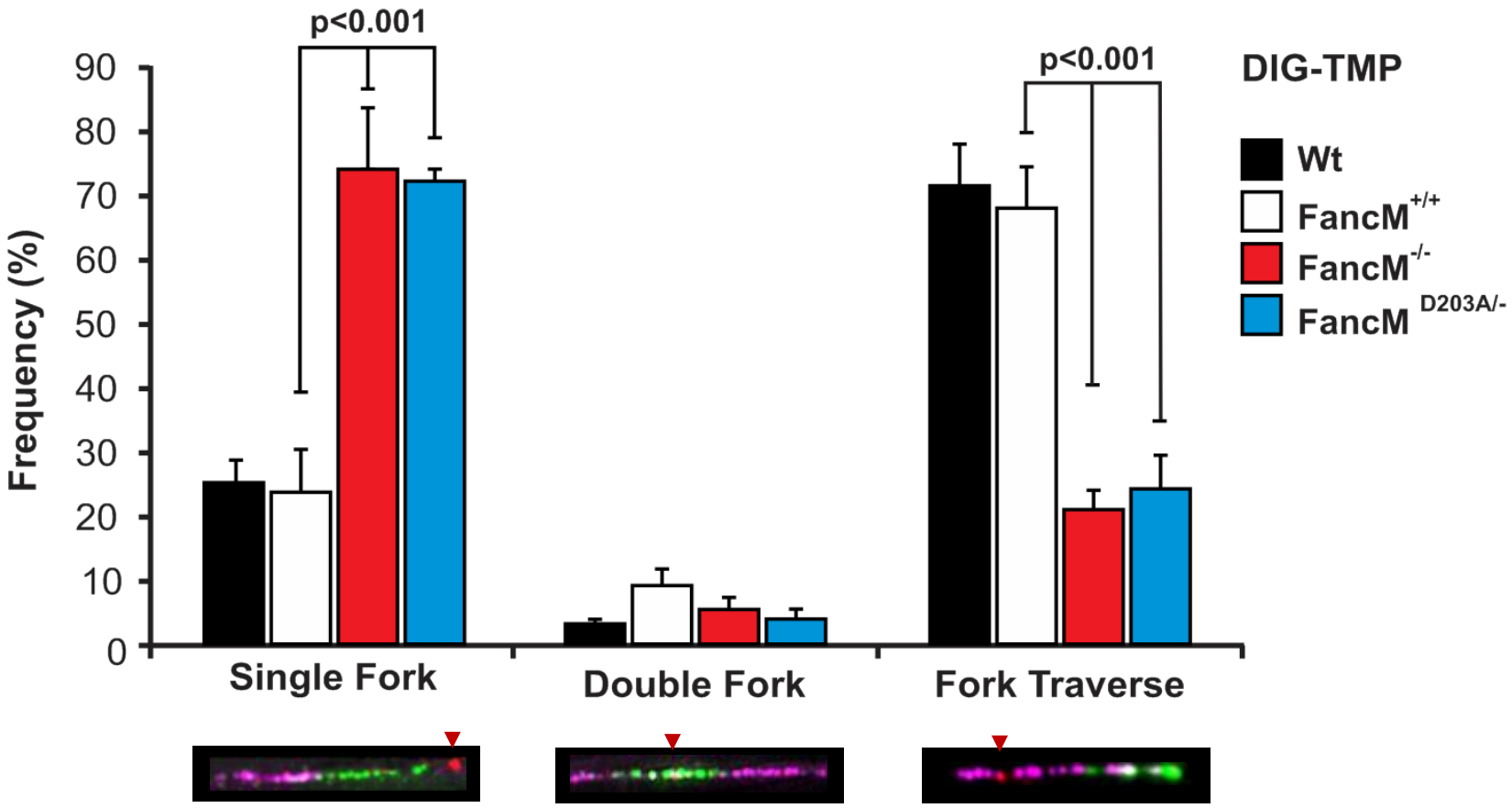


MCM-2 is phosphorylated by ATR in response to psoralen/UVA



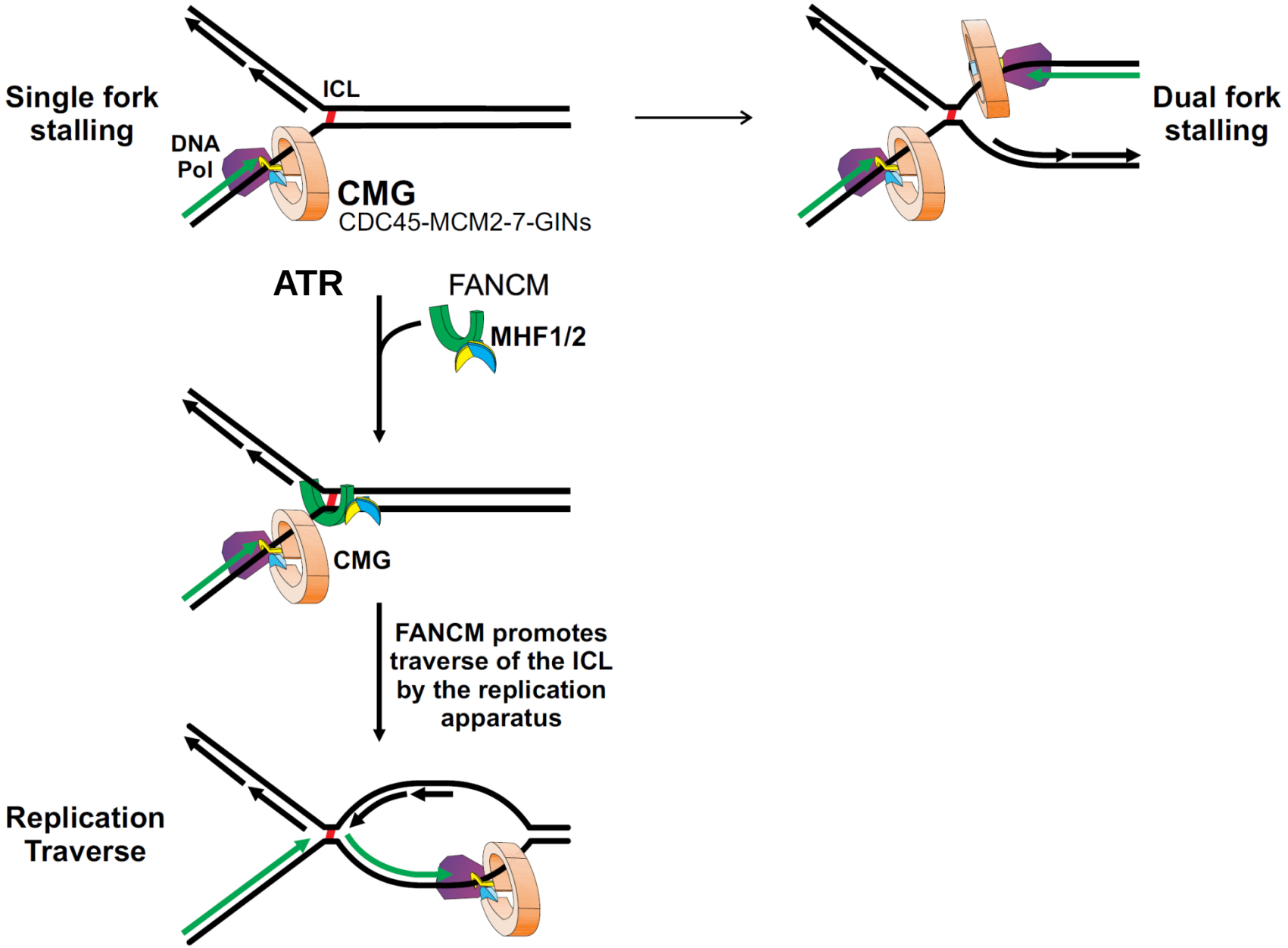
A kinase resistant FANCM mutant = a FANCM null

FANCM$^{(S1045A)}$

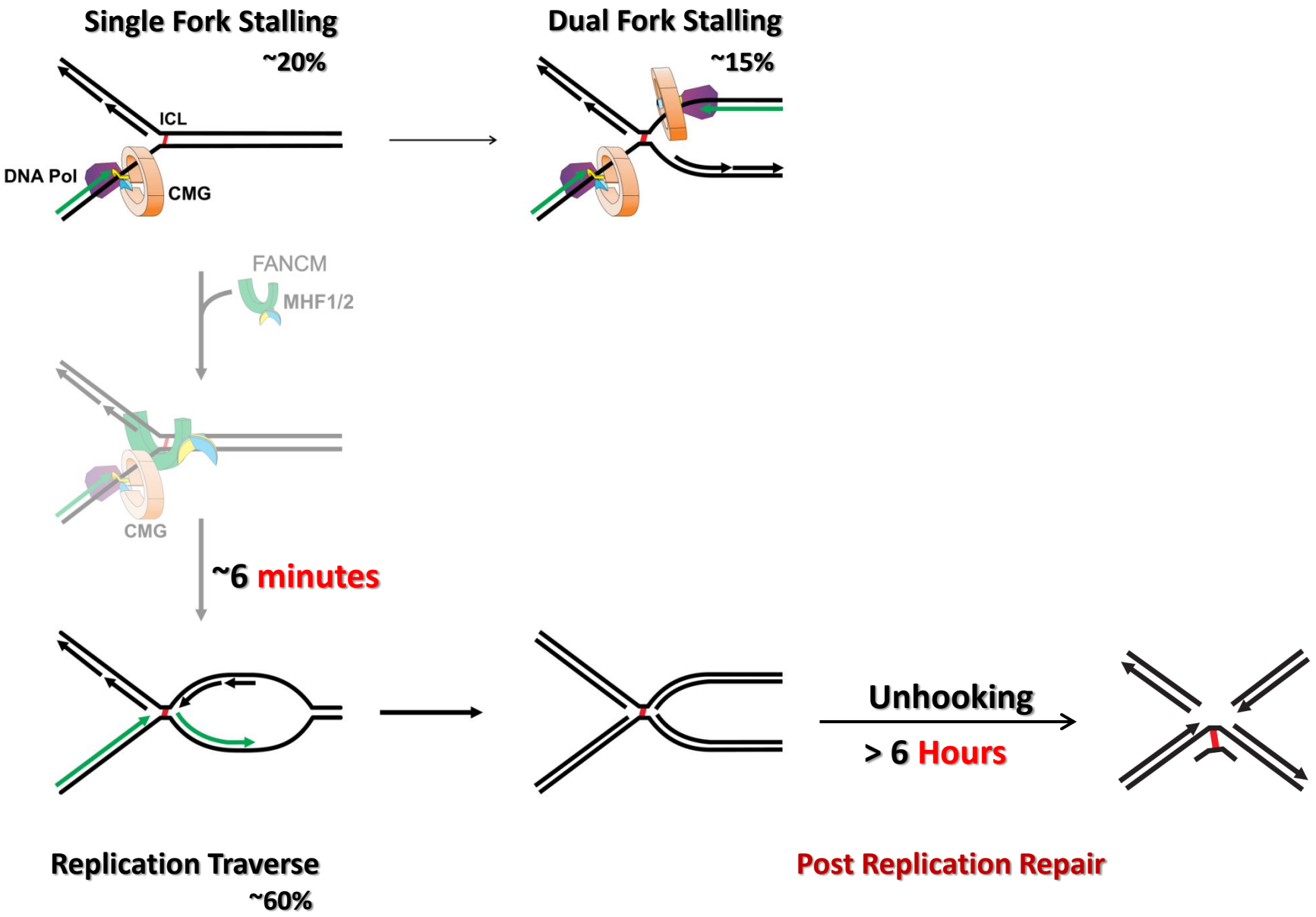


FANCM D203A: Translocase mutant

Replication Fork encounters with an ICL



Replication restart is much faster than repair



The Replication Imperative:

Complete replication! Repair later