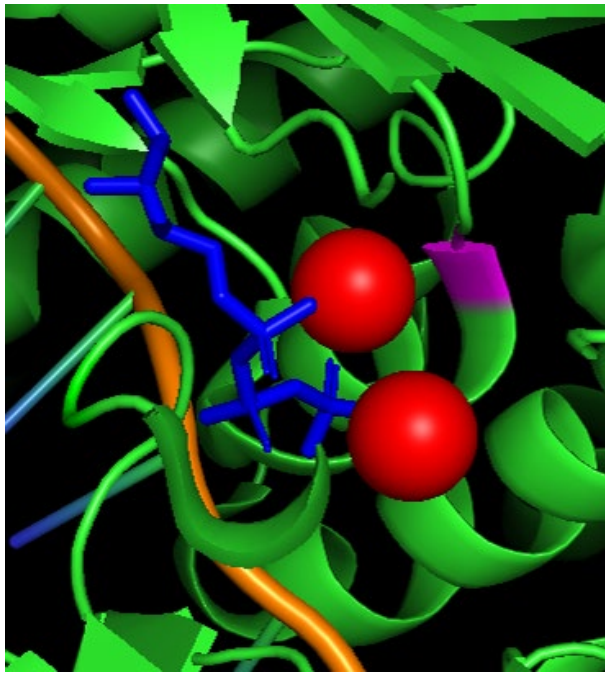
Cancer-associated RAD51 S296L mutant is defective in D-loop formation and leads to genome instability

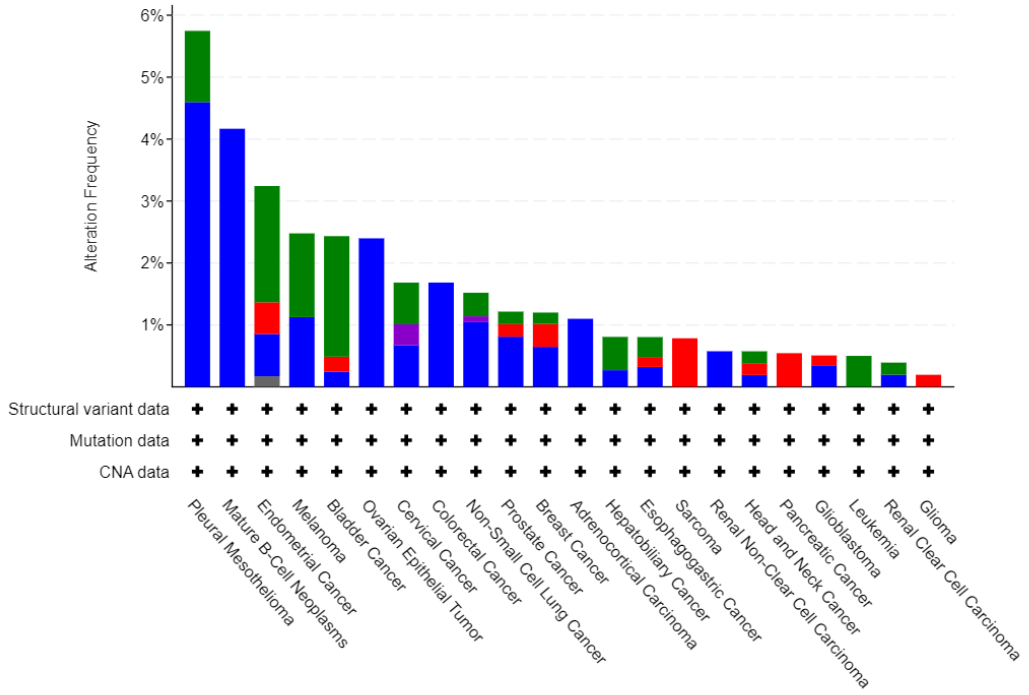
Felicity Feiser

24/2/2025



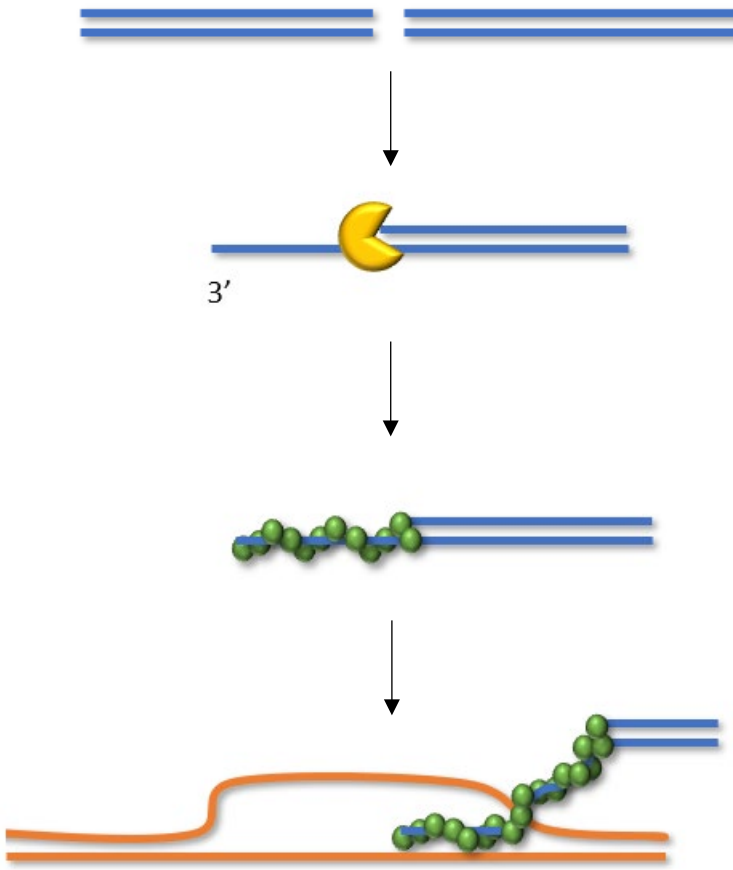
LABORATORY OF RECOMBINATION AND DNA REPAIR

RAD51 in Cancer



cBioPortal, Pan-cancer studies

RAD51 Functions in DSBR and Replication

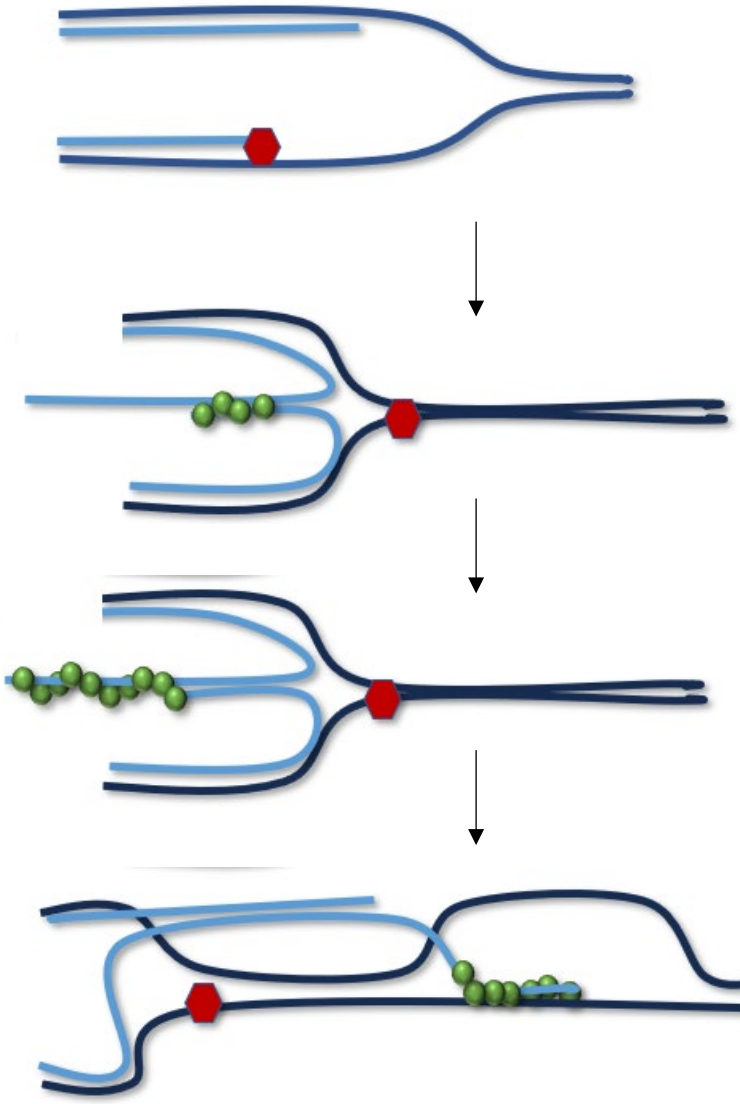


DSB

End resection by MRE11 (and other nucleases)

RAD51 forms ATP-dependent filament

D-loop facilitates copying of DNA



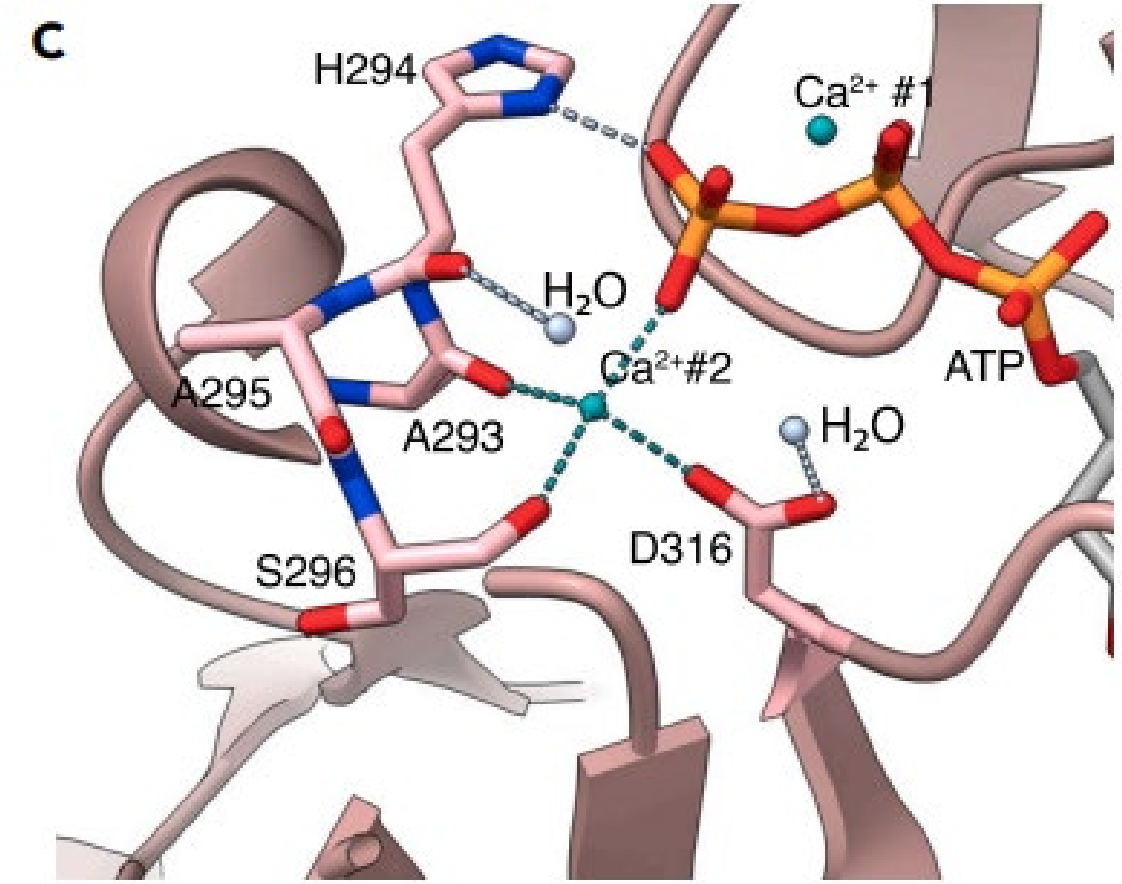
Replication fork stalls due to lesion

RAD51-mediated fork reversal

RAD51 protects fork

D-loop facilitates fork restart

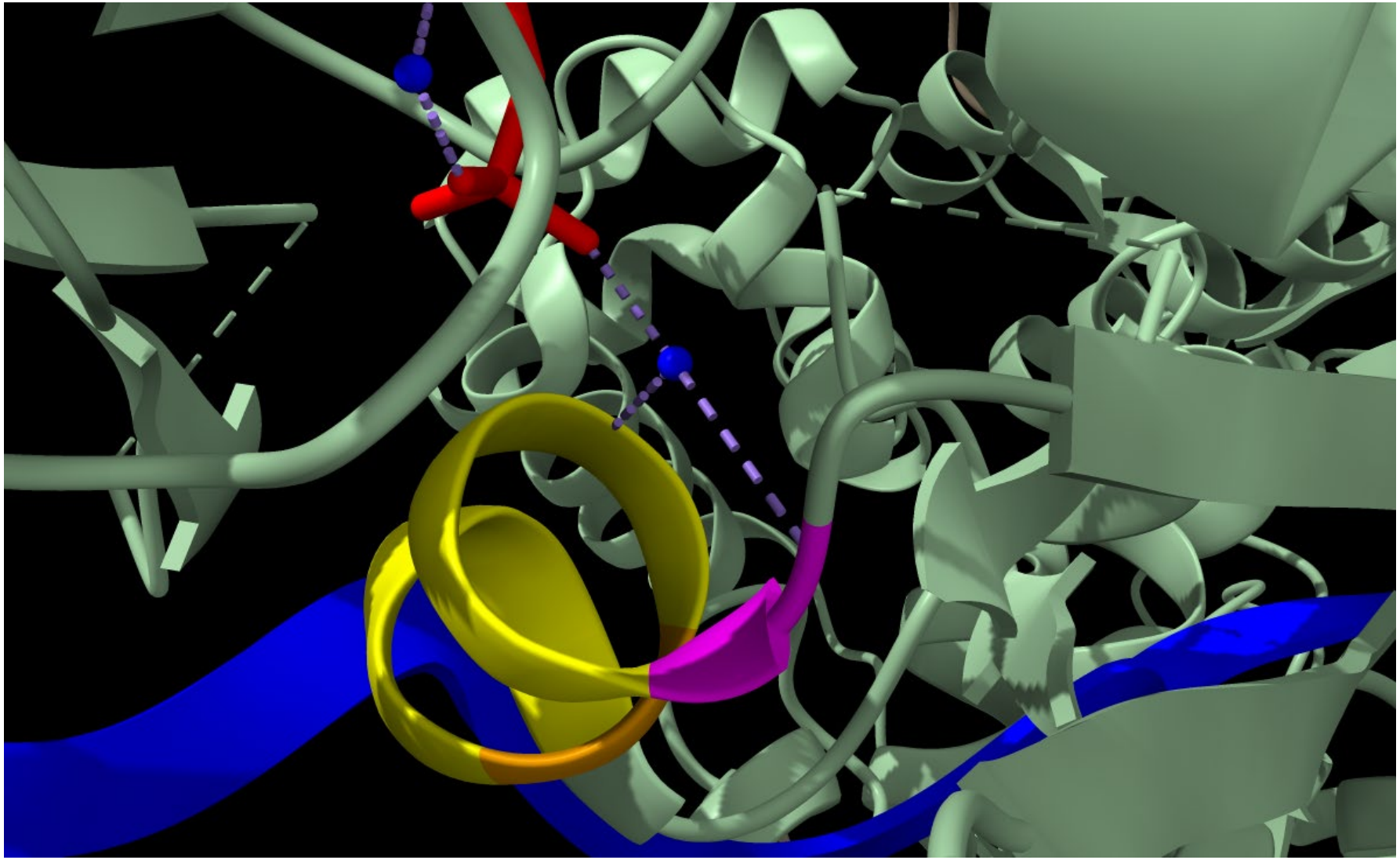
RAD51 S296L – Reported Mutation in Calcium Coordinating Residue



- The RAD51 S296L mutation has been identified in Head & Neck Squamous Cell Carcinoma patient.
- S296, along with A293 and D316, have been reported to coordinate a previously undescribed second Calcium ion located at the y phosphate of ATP

Appleby et al, 2023

RAD51 S296L Links ATP/Ca^{2+} and the DNA Binding Loop 2



Purple – S296
Yellow – Portion of L2 loop
Orange – I292

S296 backbone interacts with Ca2+ ion associated with y phosphate of ATP

S296 side chain interacts with I292 in L2 loop. Yellow portion of L2 loop forms structure incompatible with ssDNA binding in presence of ADP

Hypothesis:

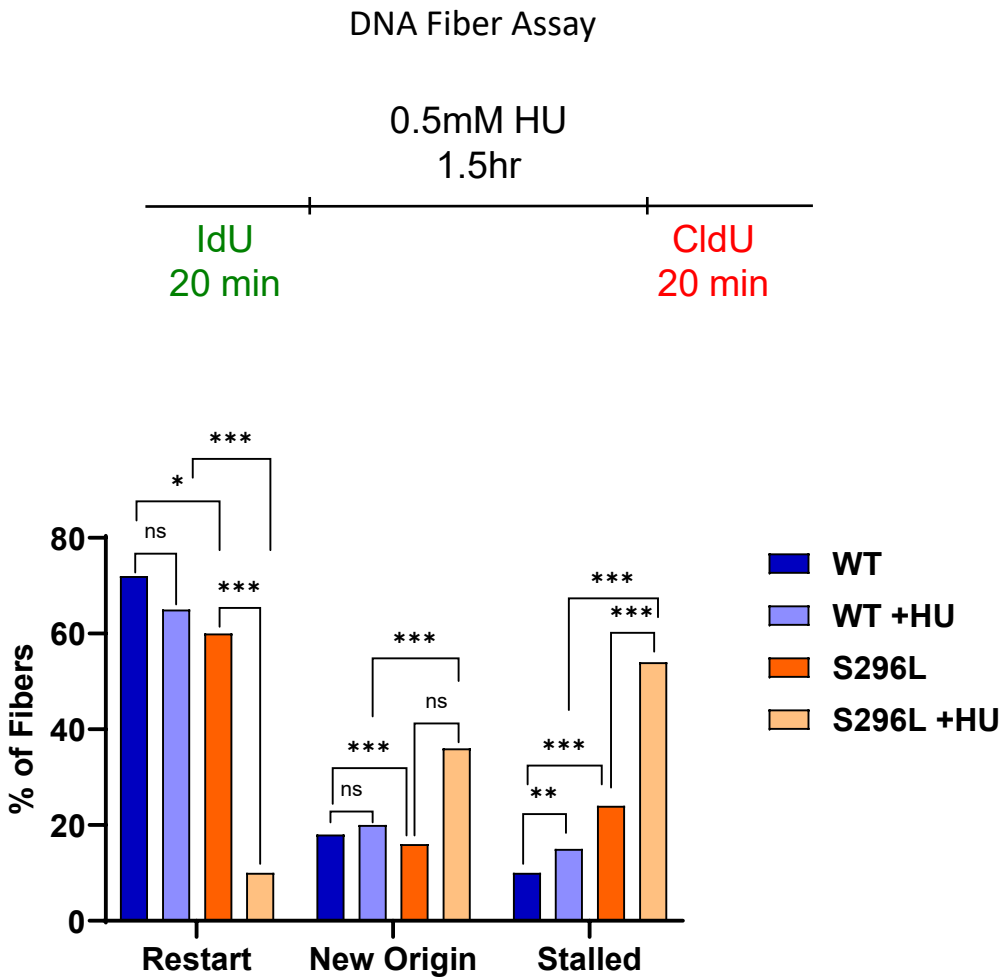
The serine to lysine mutation at RAD51 S296 leads to defective coordination between ATP hydrolysis state and ssDNA binding. This mutation causes defects in replication and double-strand break repair, increasing the likelihood of genomic instability and contributing to cancer development

Project Aims:

- Determine the biochemical defects that are caused by the S296L mutation

- Clarify effects of mutation on cell survival, replication stress and chromosomal aberrations

Mutant has Defects in Replication and DSBR



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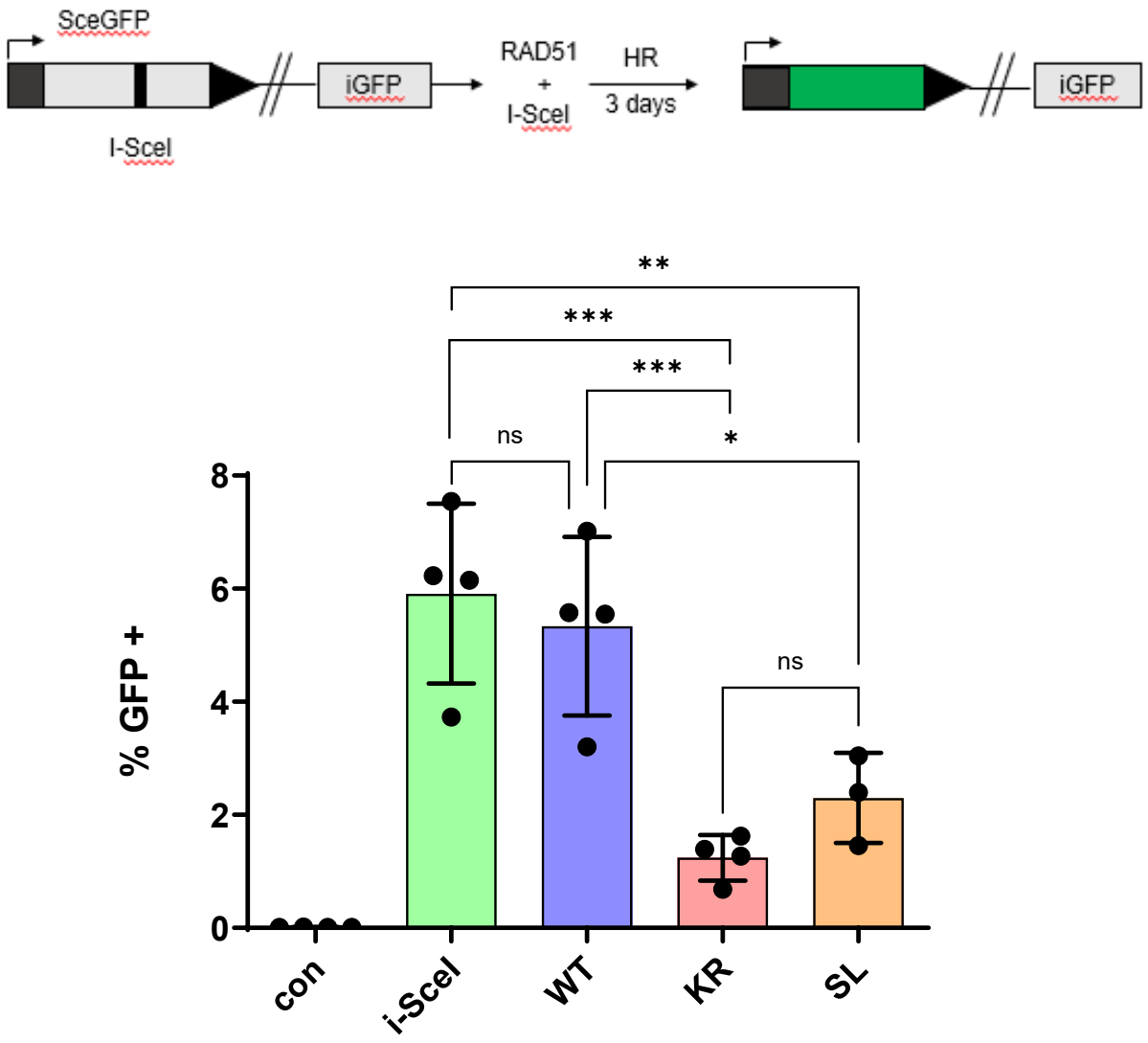
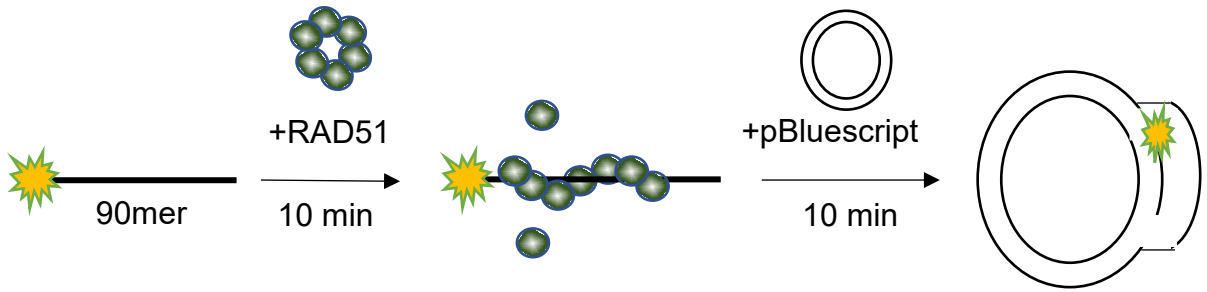
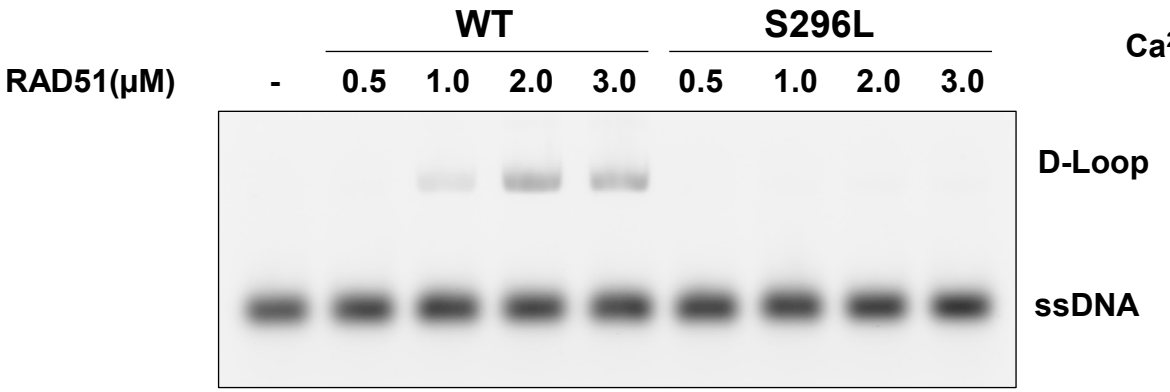
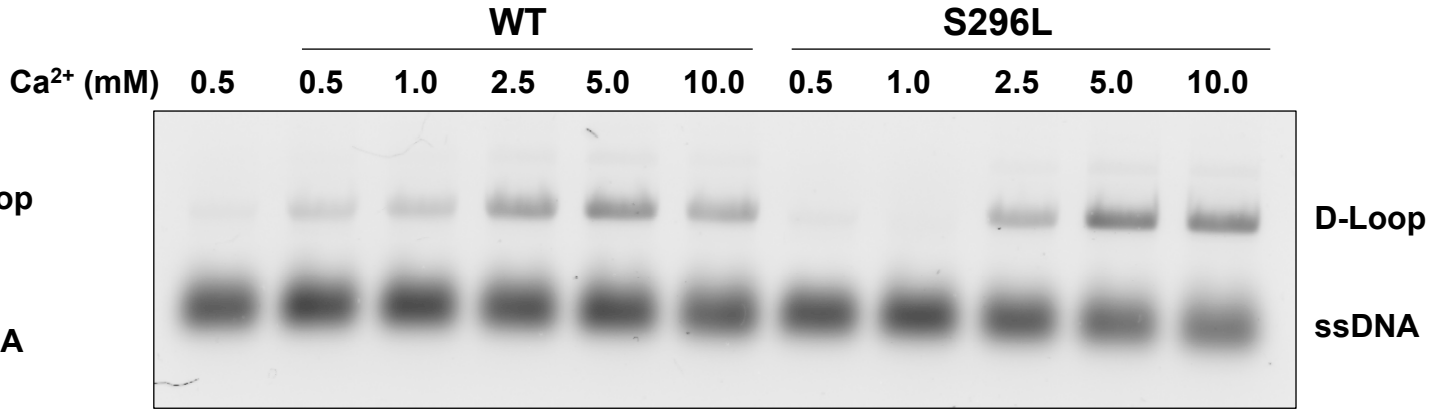


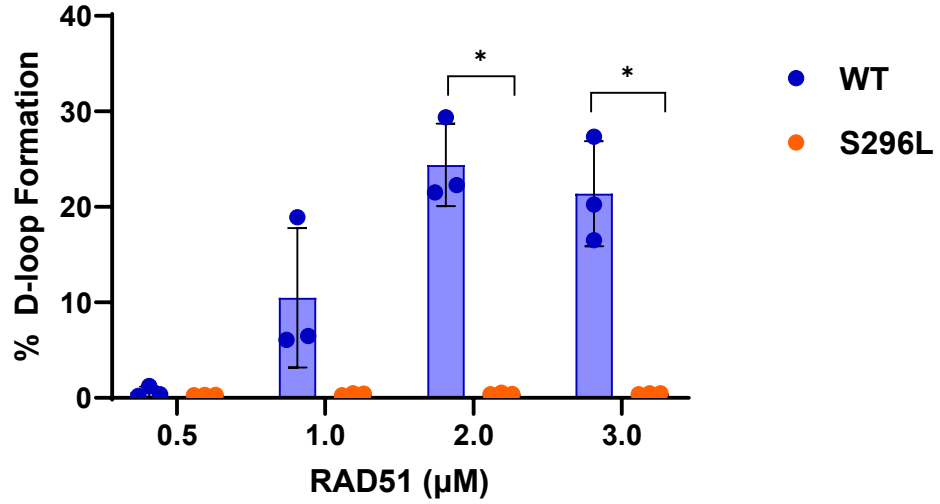
Fedor Nikulenkov

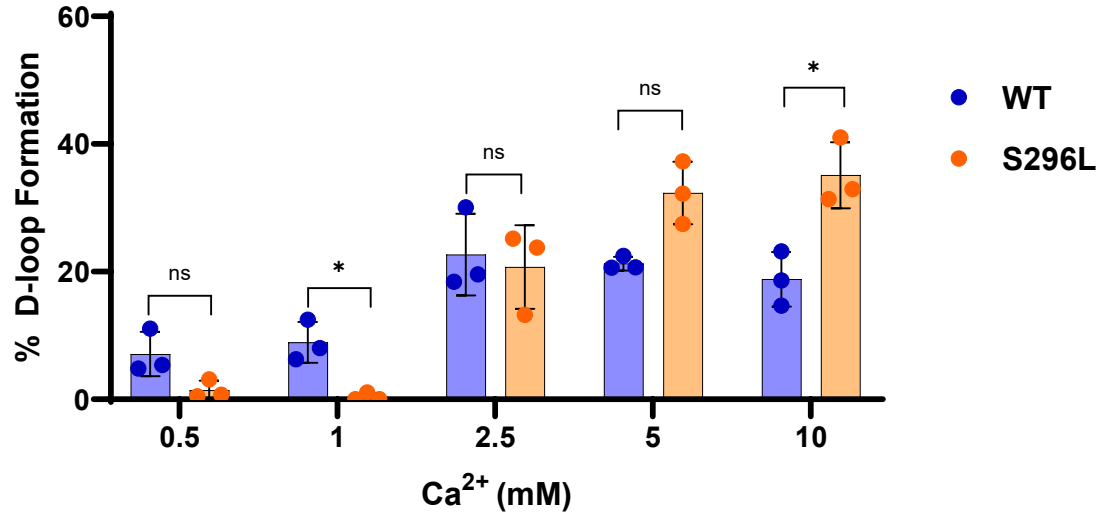
Mutant has Ca^{2+} Dependent D-Loop Defect



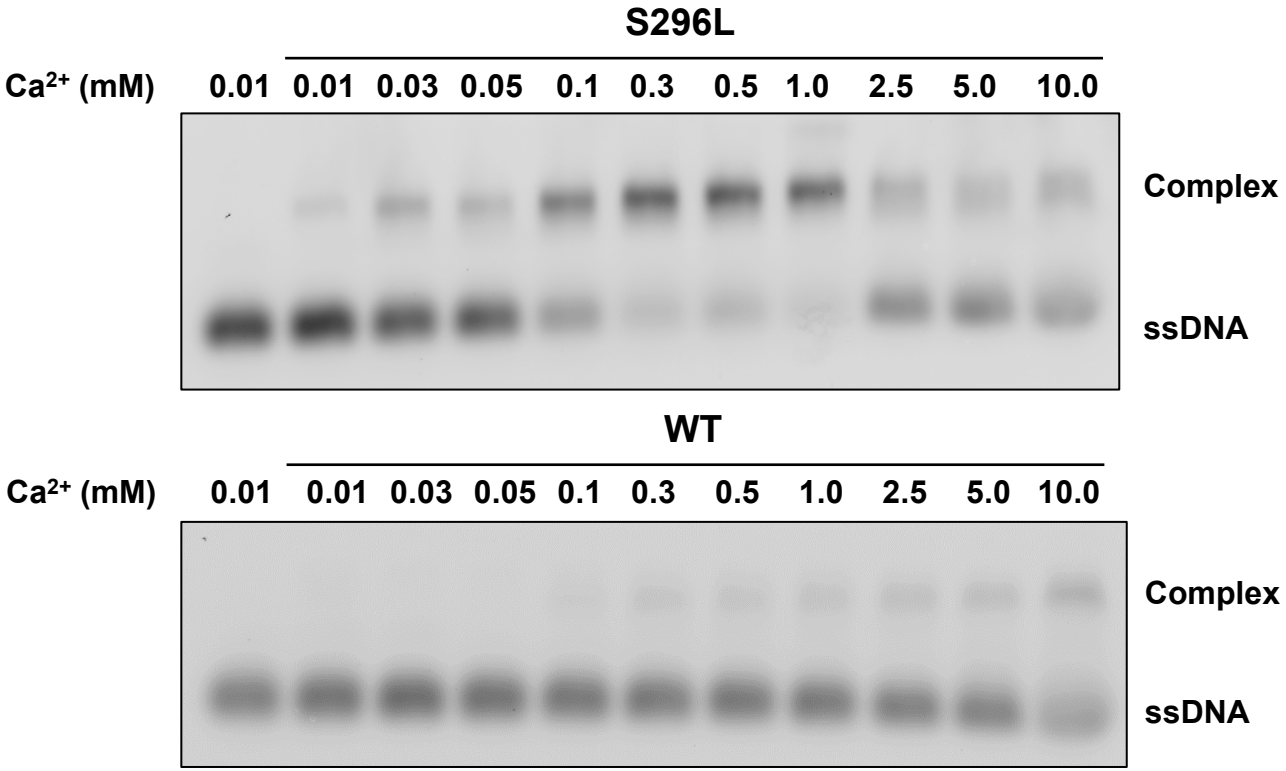


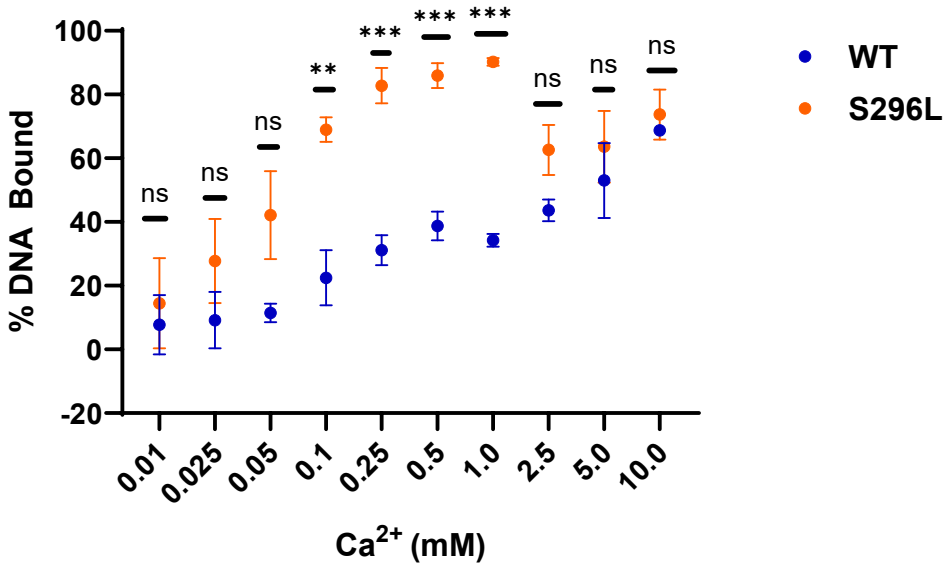




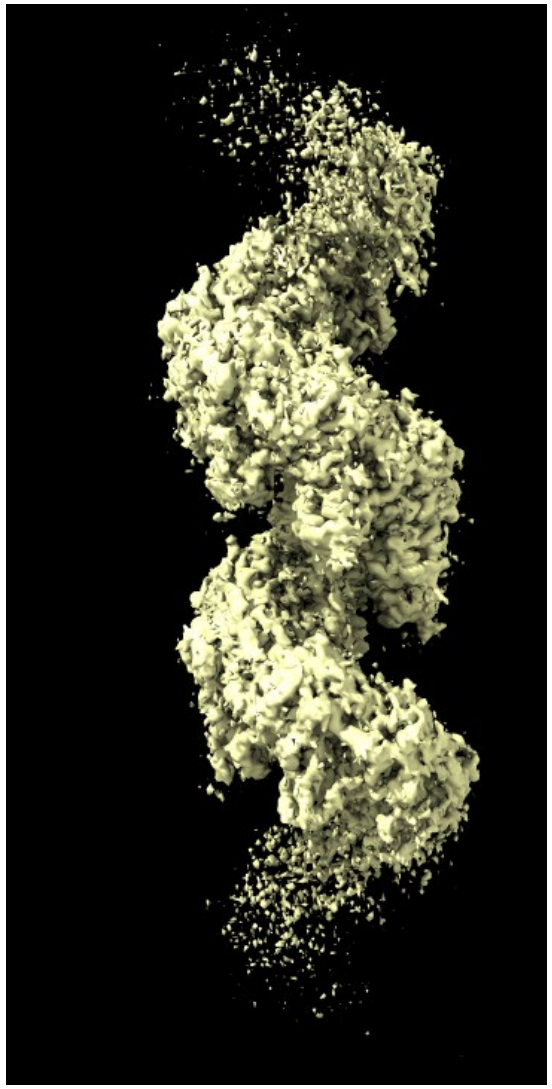


Altered ssDNA Binding

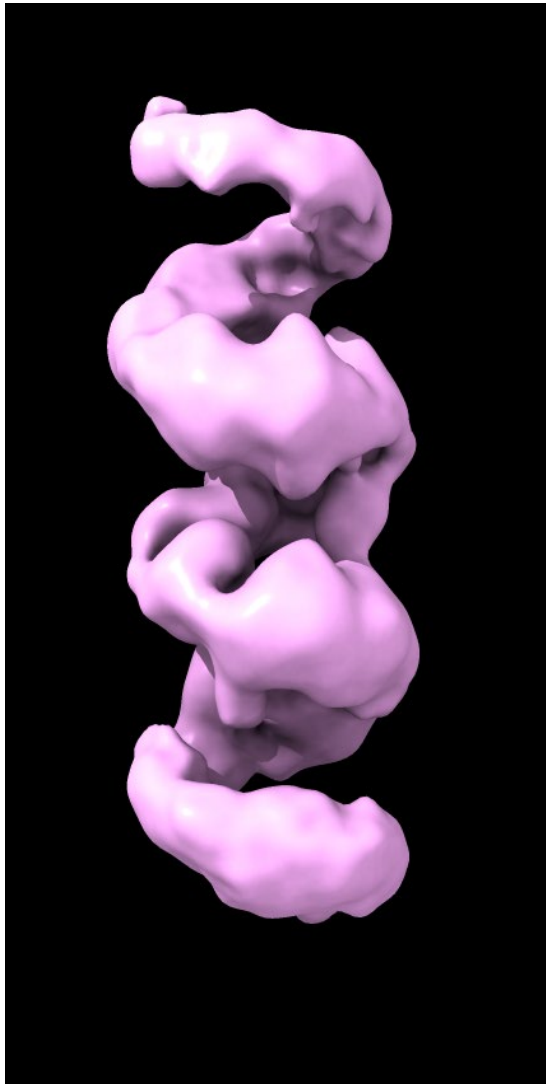




The S296L Filament is more Flexible, Condensed

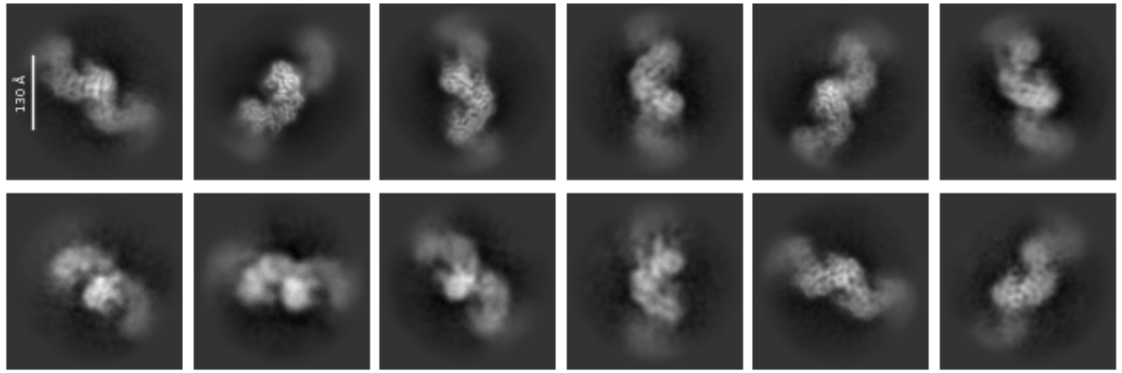


WT

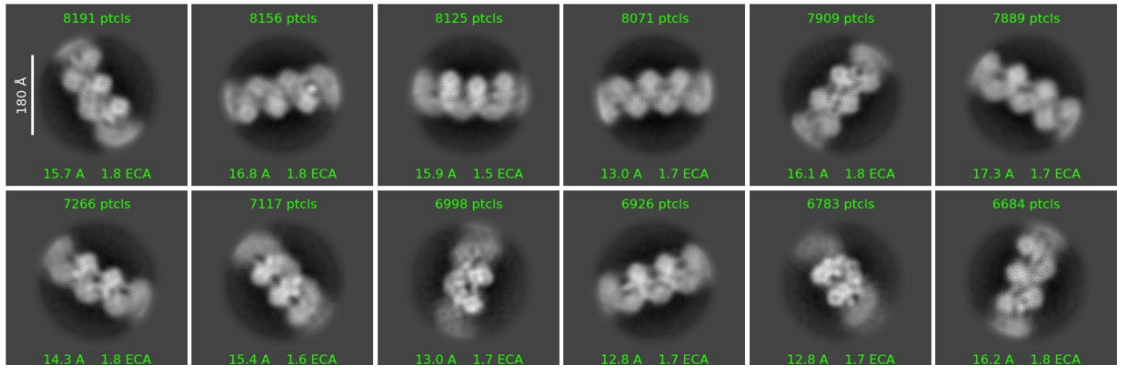


S296L

WT

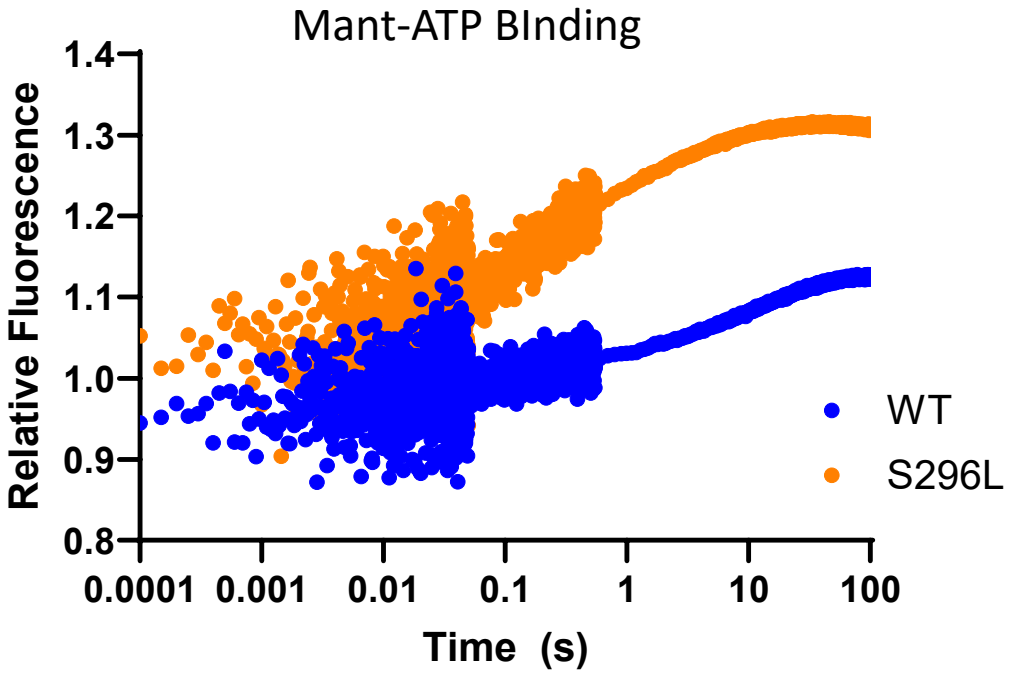


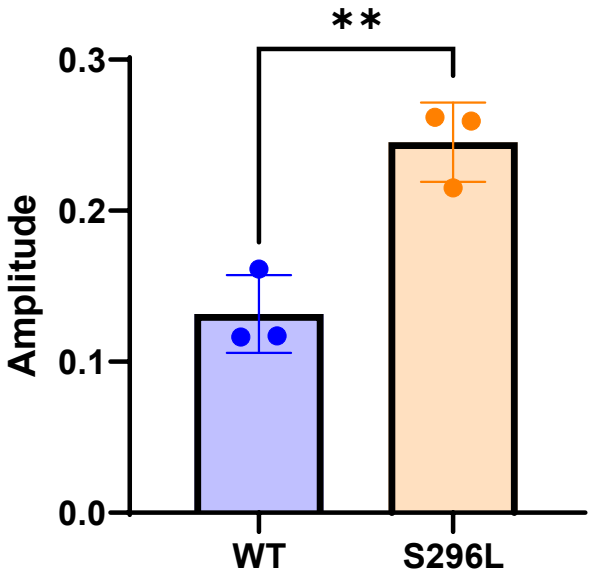
S296L



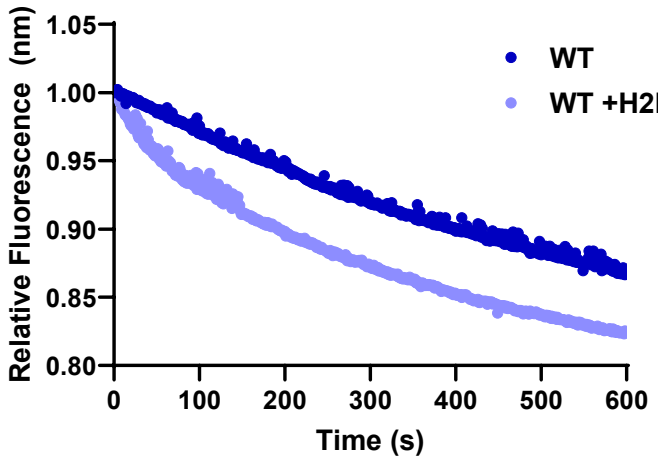
We have been unable to resolve the S296L structure any further

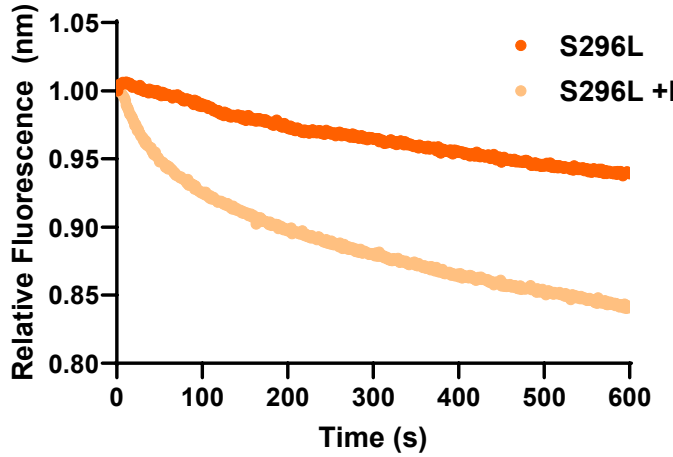
ATP Binding Pocket is Altered

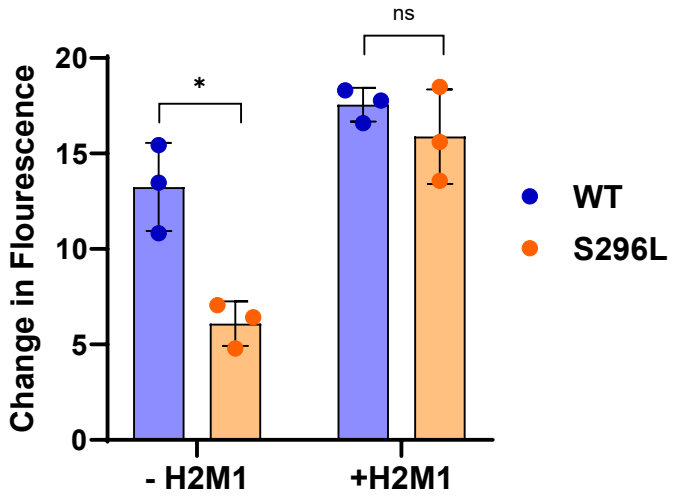




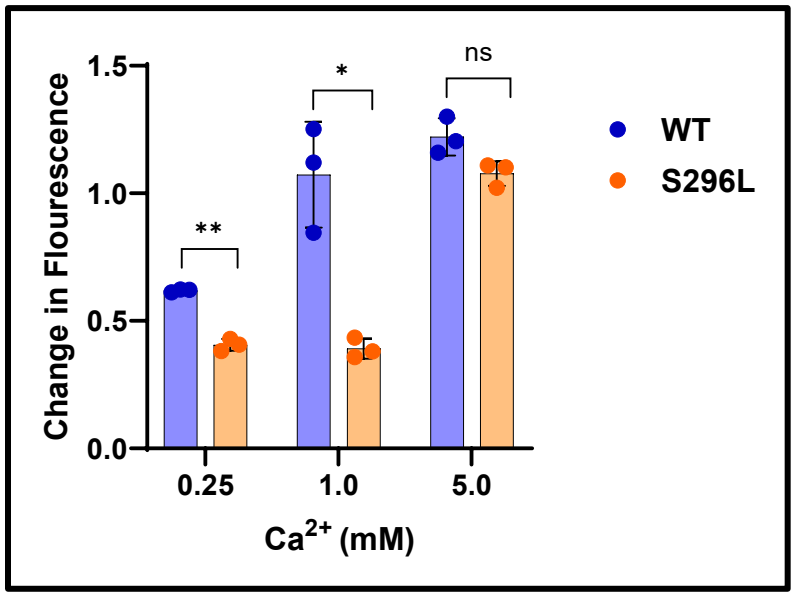
ssDNA/dsDNA Interaction is Defective





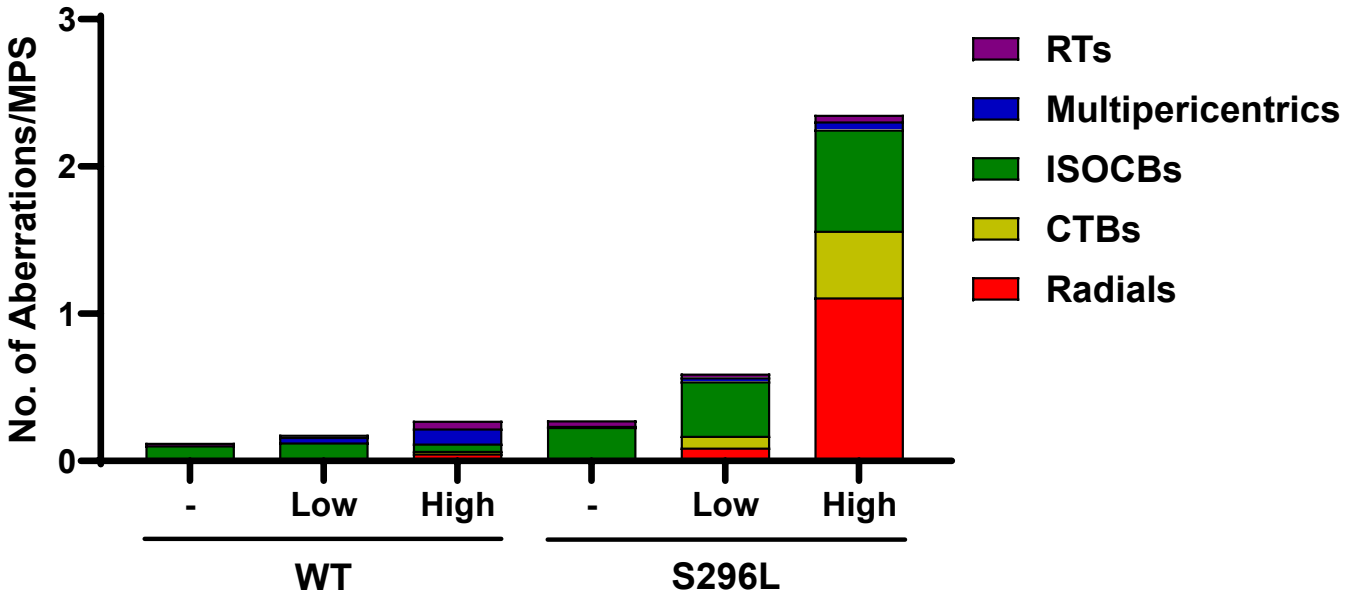


1mM Ca^{2+}



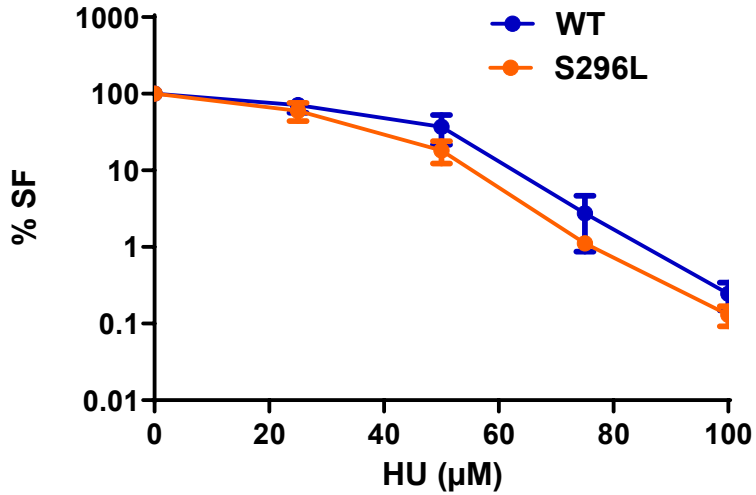
Stopped Flow Data

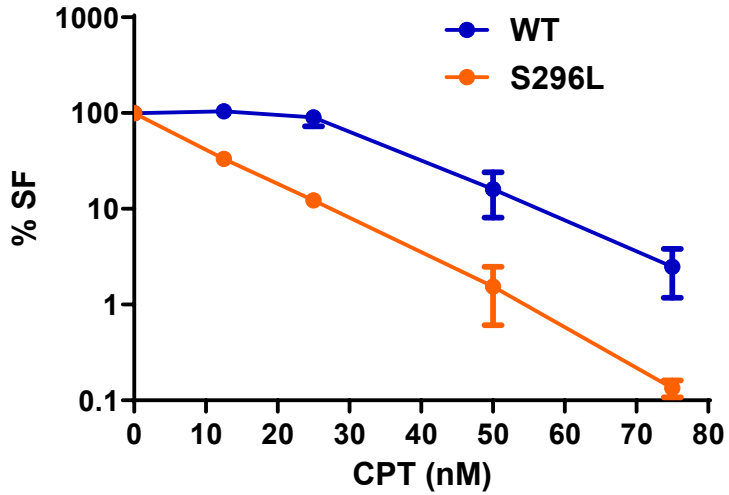
Mutation leads to Chromosomal Instability



Metaphase Plate Spread using MEFs

Low HU = 0.5mM, 1.5hrs
High HU = 4mM, 5hrs





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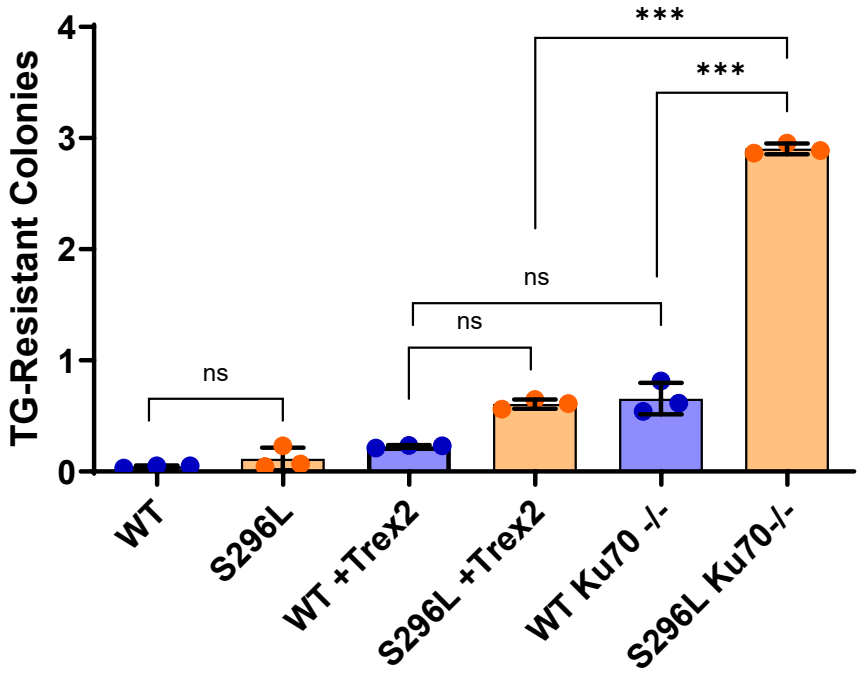
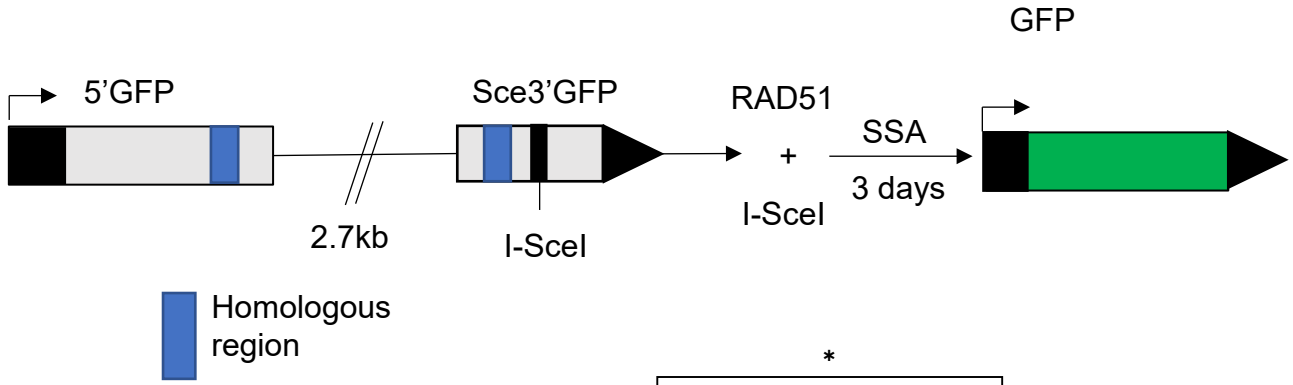
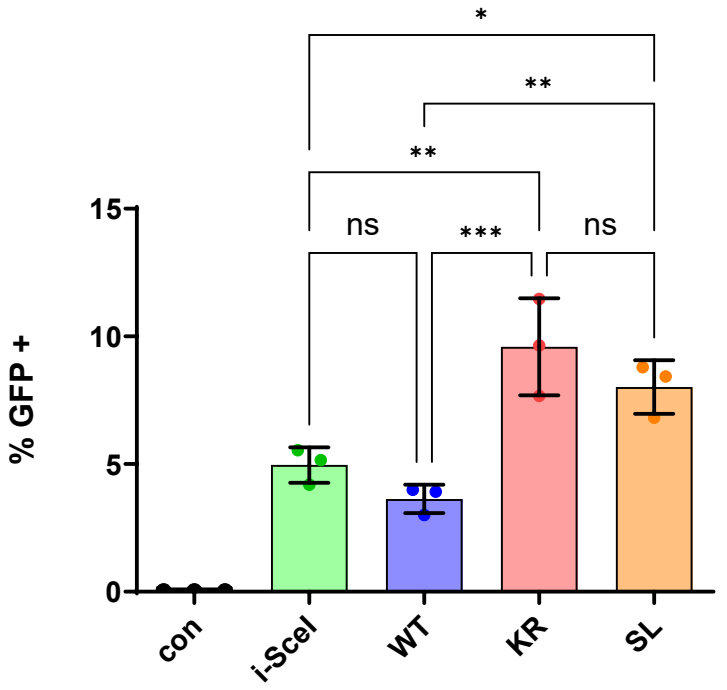
S296L Uses Alternative Repair Pathways







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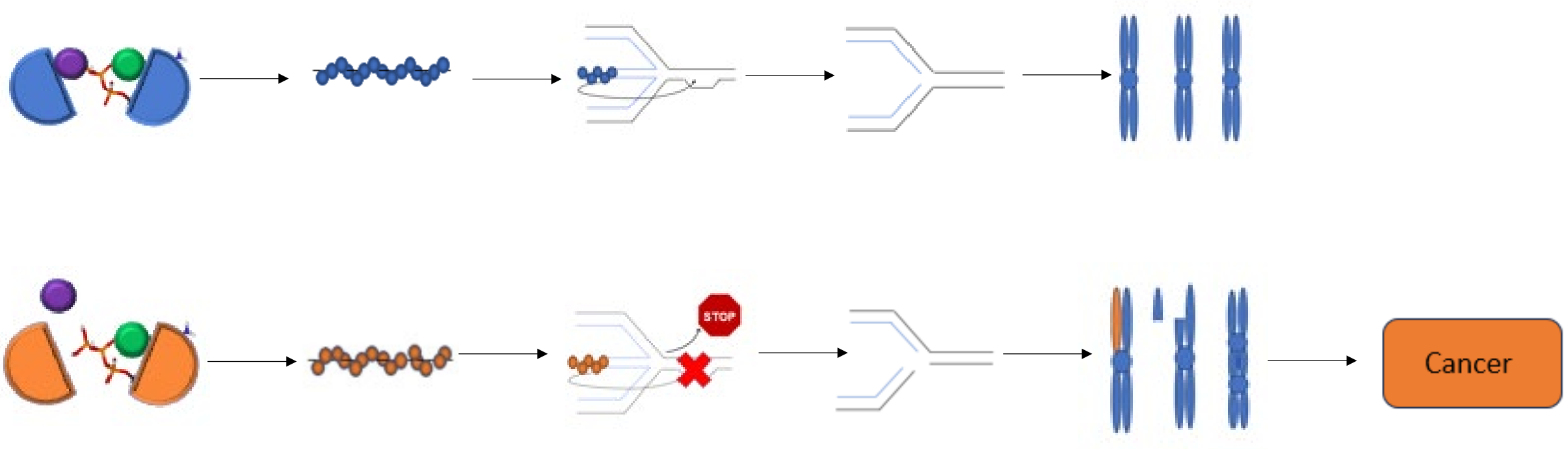
Model



RAD51 S296L has a diminished ability to coordinate the L2 Loop through Ca2+ ion which interacts with ATP

This defect leads to aberrant filament formation and structural changes

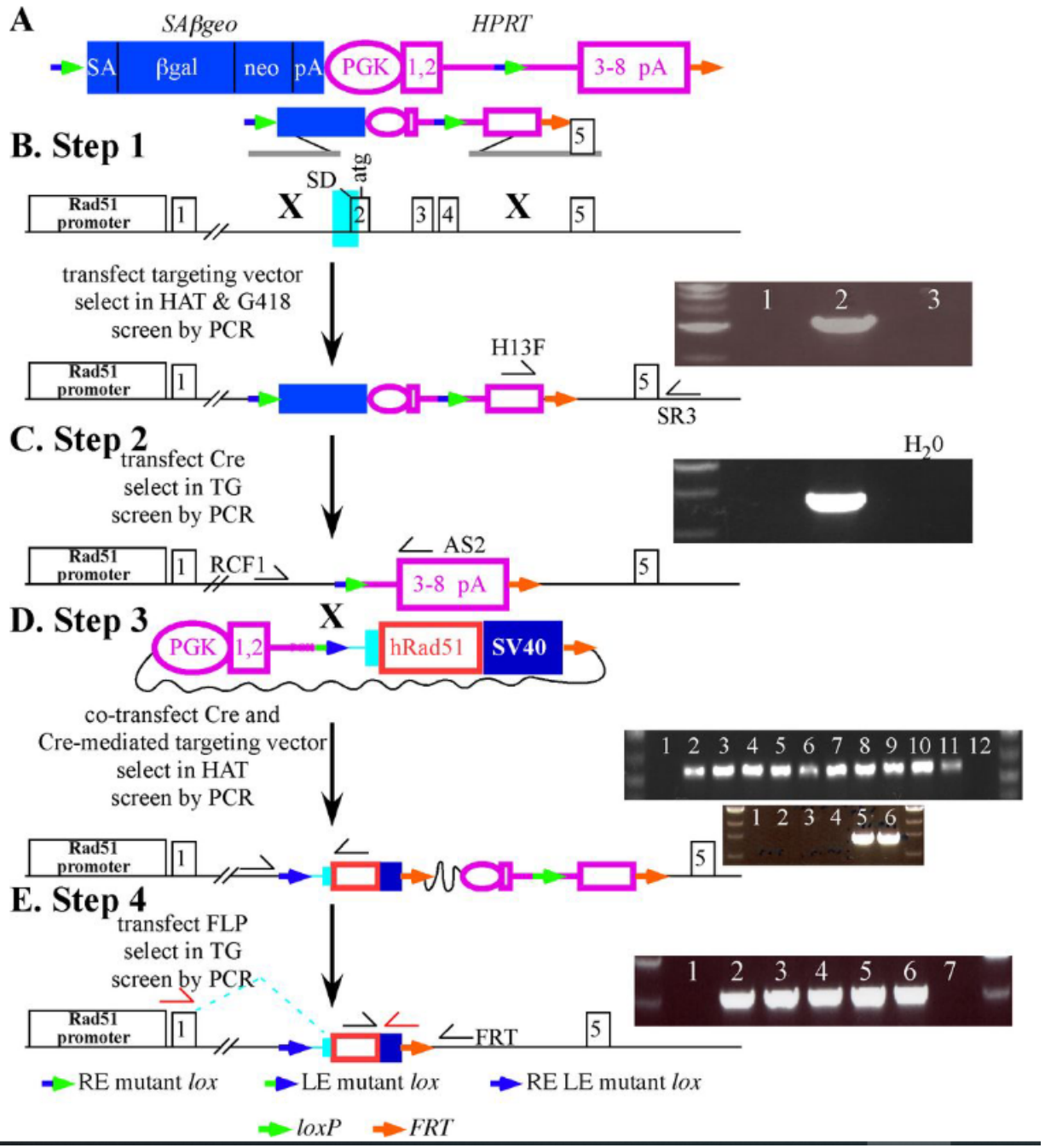
Filament defects affect D-Loop formation, resulting in stalled forks that can't be restarted

Stalled forks eventually collapse, and repair is completed through error-prone pathways

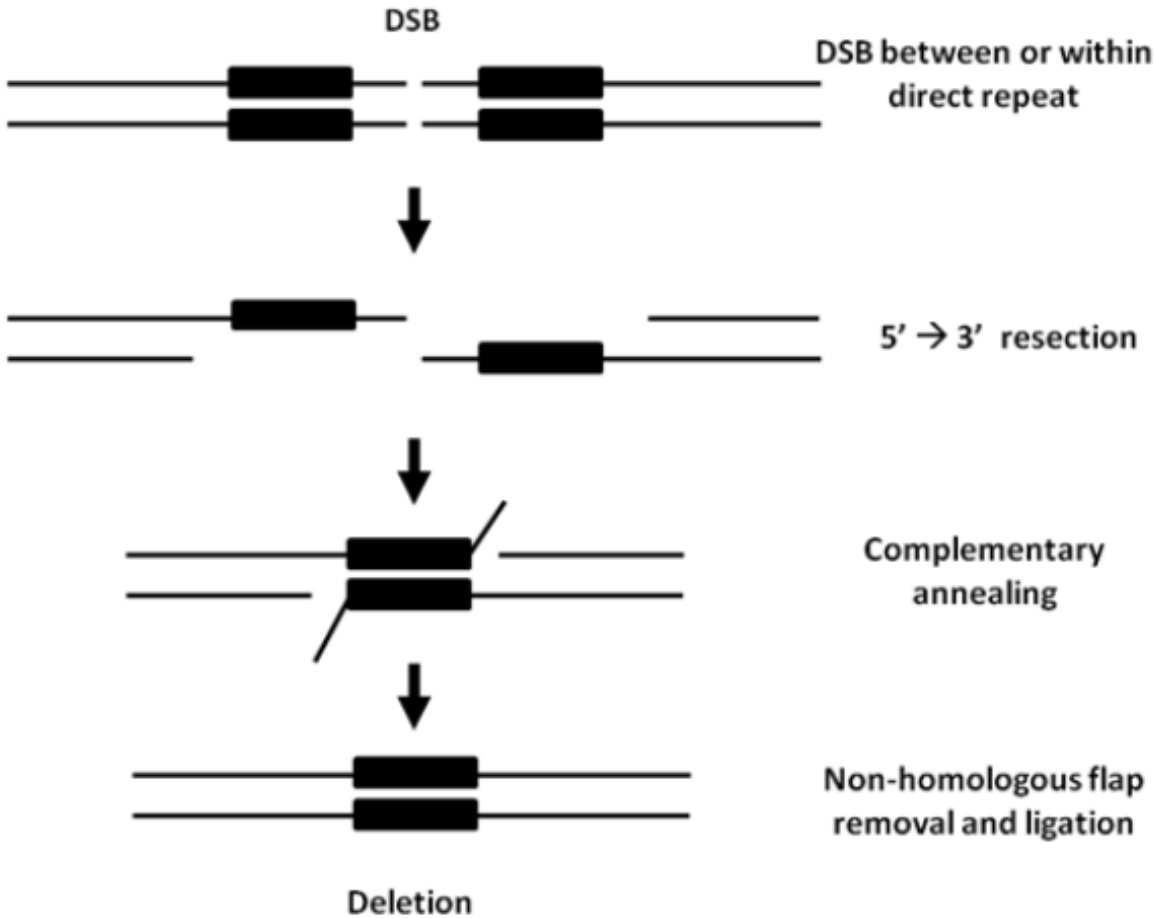
Error-prone pathways cause chromosomal aberrations

Chromosomal aberrations lead to cancer

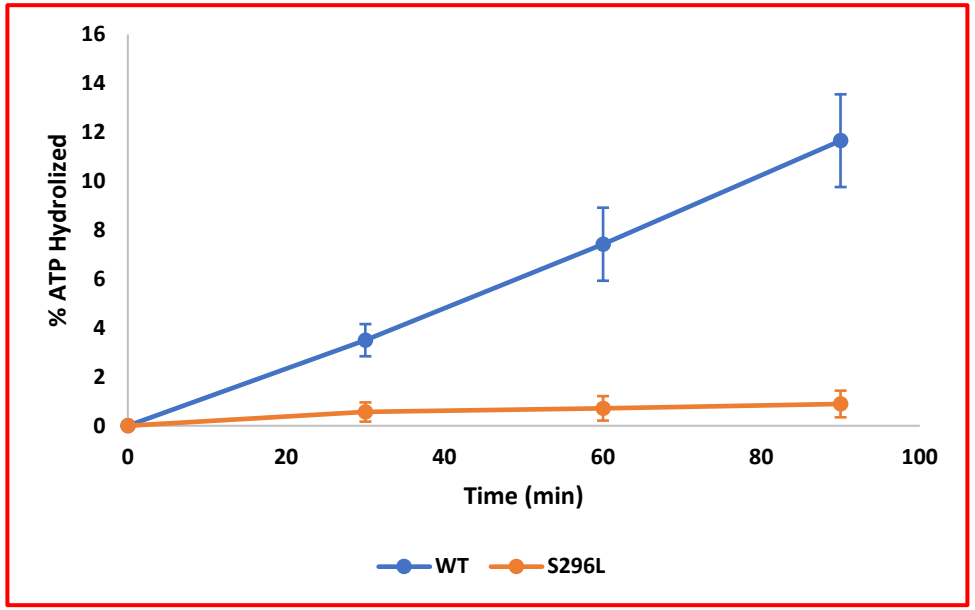
Thank You!

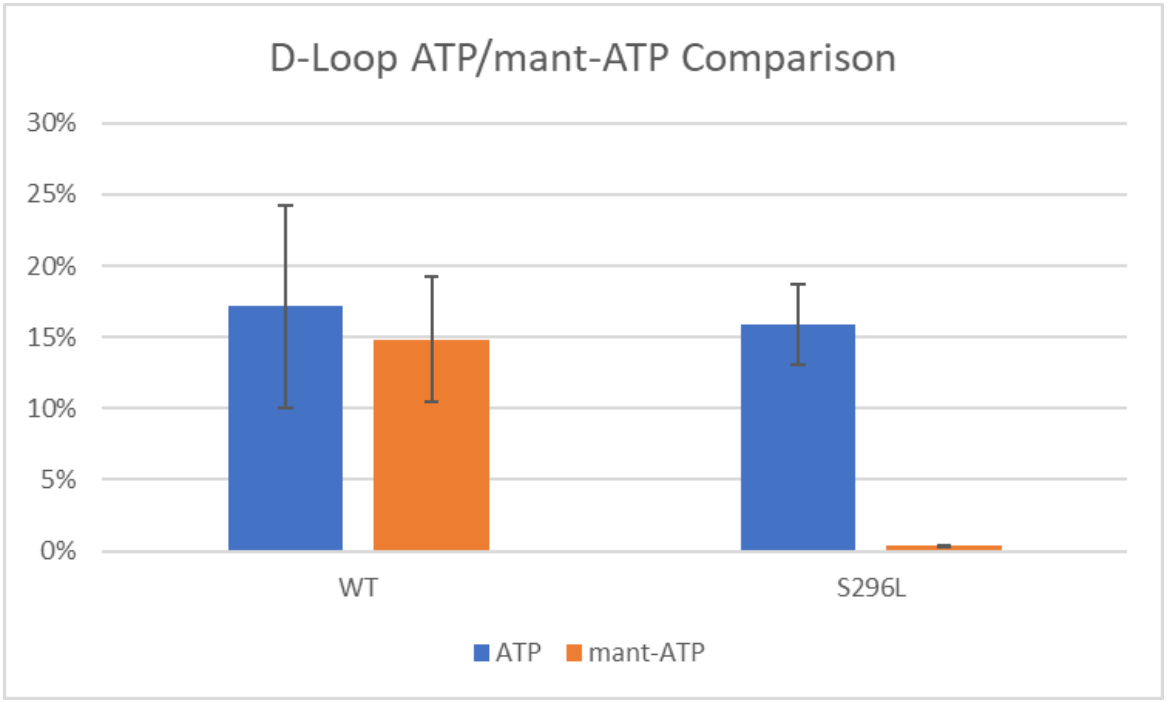


Kim et al, 2012

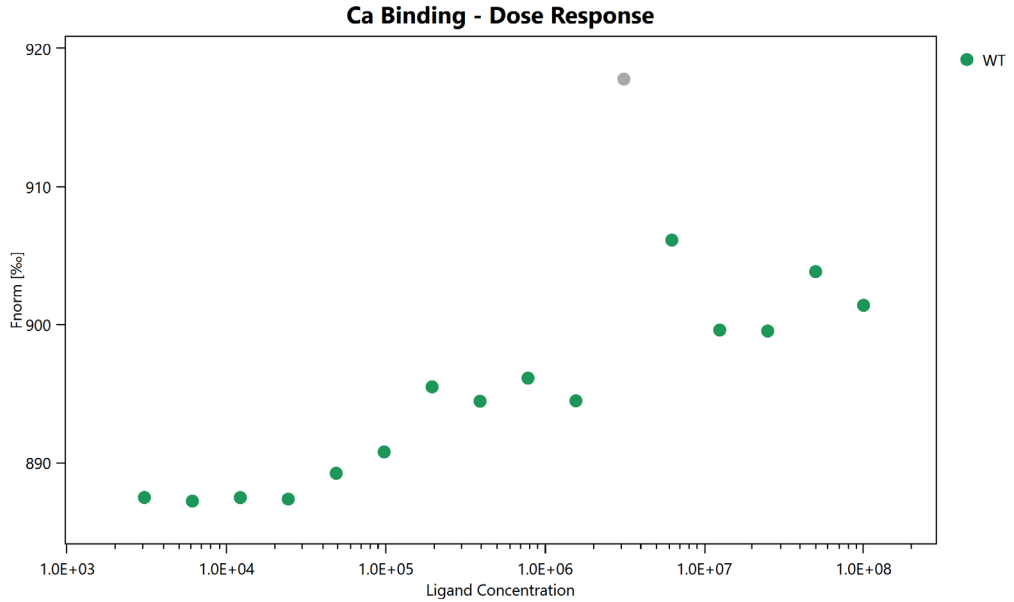

DSB
DSB between or within direct repeat
5' → 3' resection
Complementary annealing
Non-homologous flap removal and ligation
Deletion

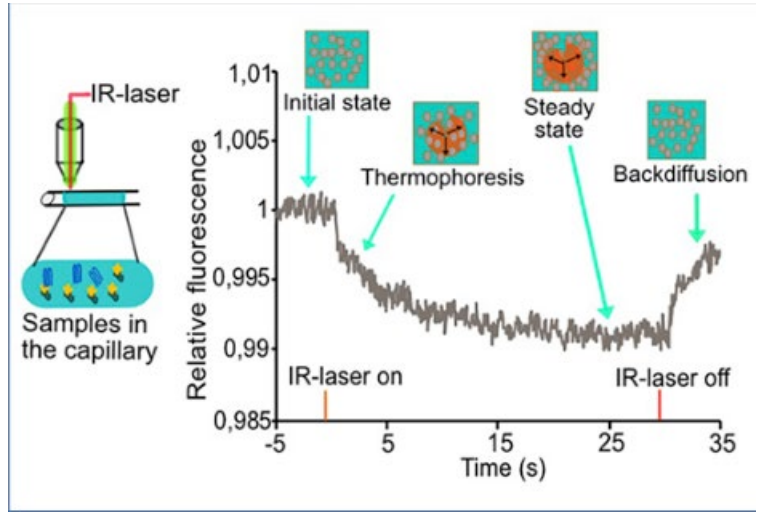
A. Sena, 2008


16
14
12
10
8
6
4
2
0
% ATP Hydrolized
0
20
40
60
80
100
Time (min)
WT
S296L

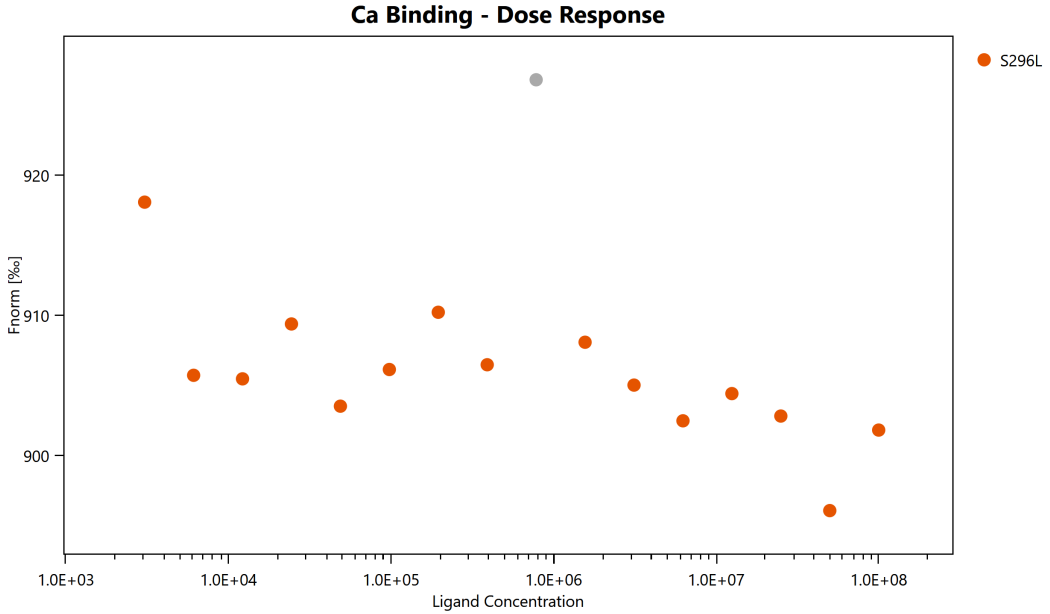

D-Loop ATP/mant-ATP Comparison
30%
25%
20%
15%
10%
5%
0%
WT
S296L
ATP
mant-ATP

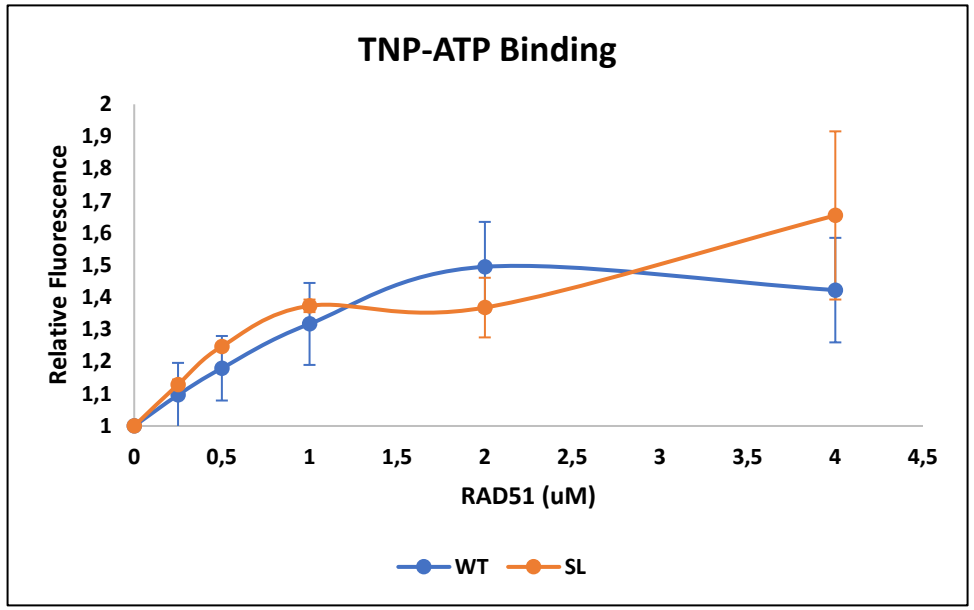
Defect in Calcium Binding, but not ATP Binding





Tamim Al-Jubair, 2022





ATP Binding Pocket is Altered