

Defining the Contribution of POLQ to Replicative Repair

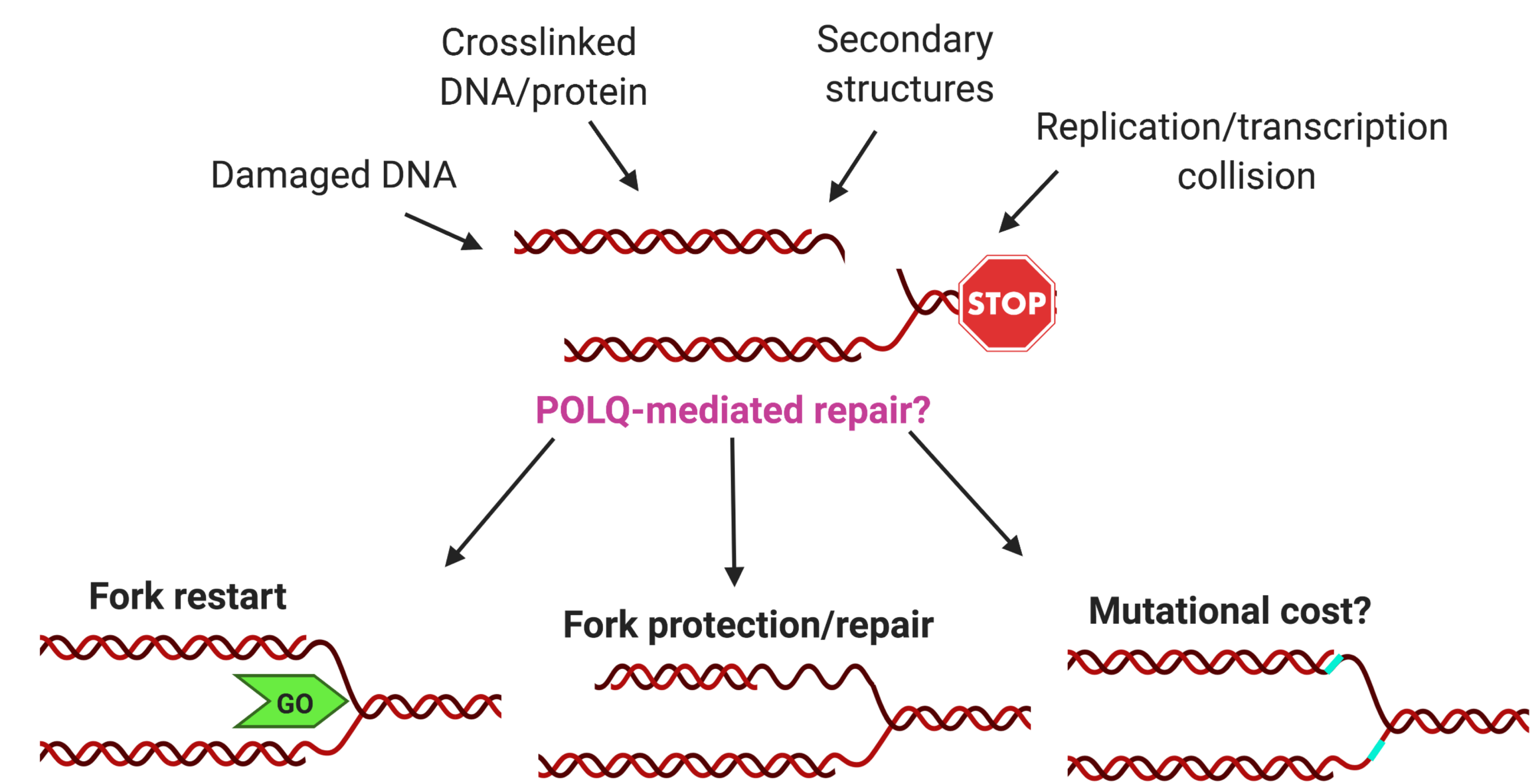
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POLQ's role in replicative outcomes is unknown



POLQ is required for replicative break repair

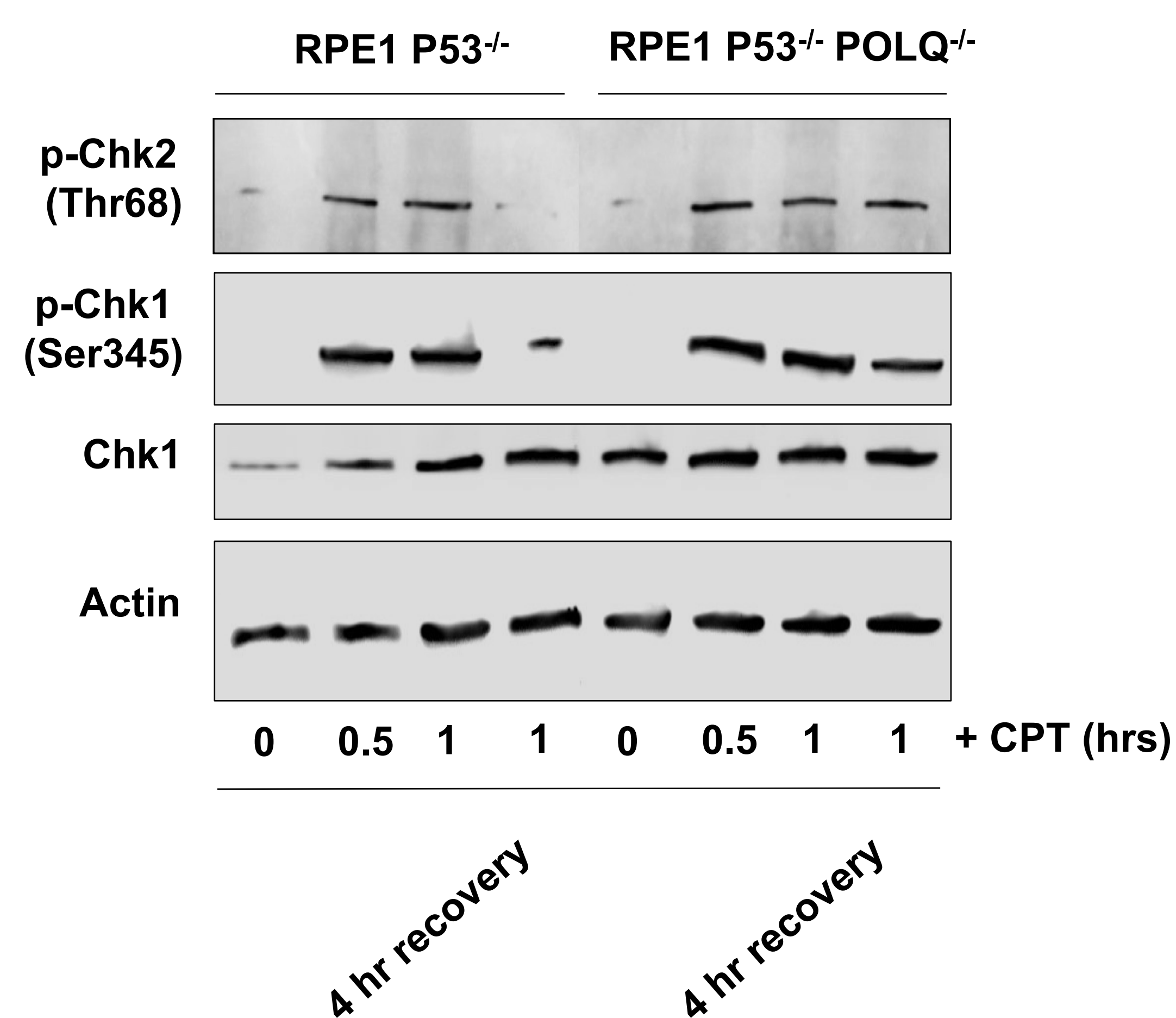
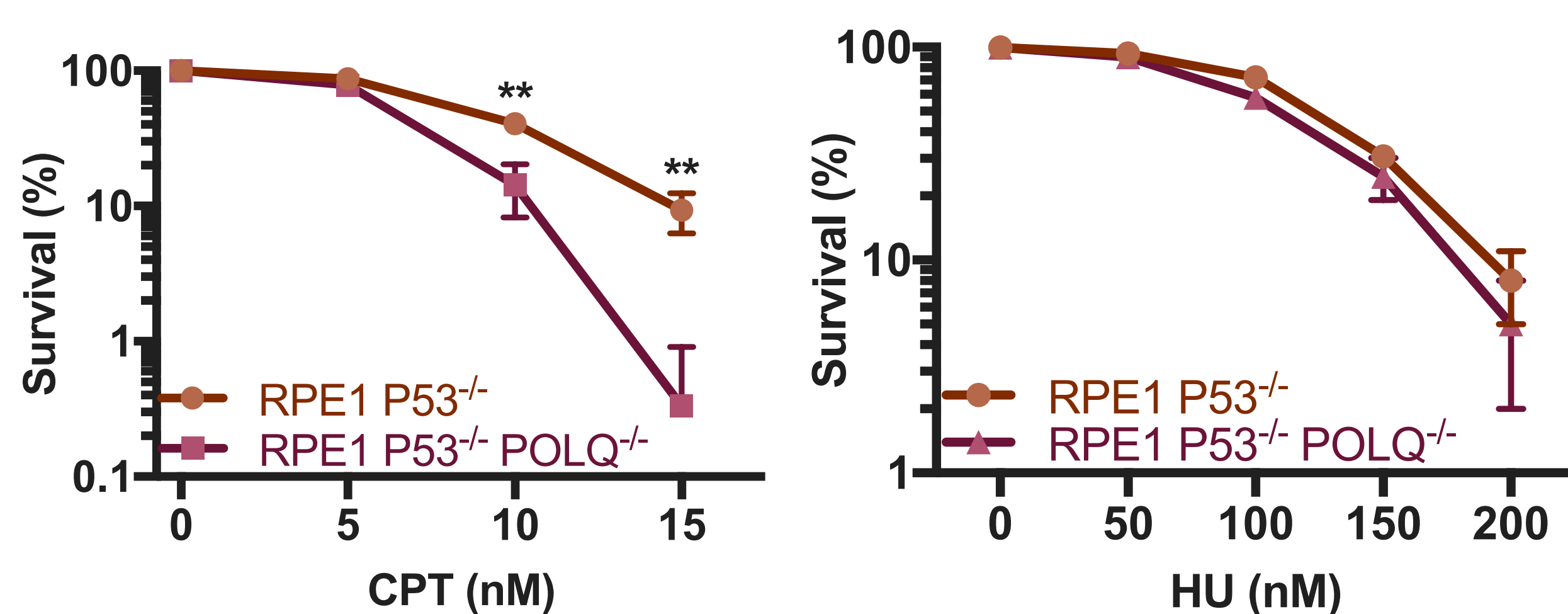


Figure 1. (A) Colony formation results demonstrating CPT sensitivity in POLQ^{-/-} cells. (B) Colony formation results after HU treatment. (C) Western blot demonstrating prolonged p-Chk1 and p-Chk2 response to CPT in POLQ^{-/-} cells.

POLQ repairs and restarts broken forks

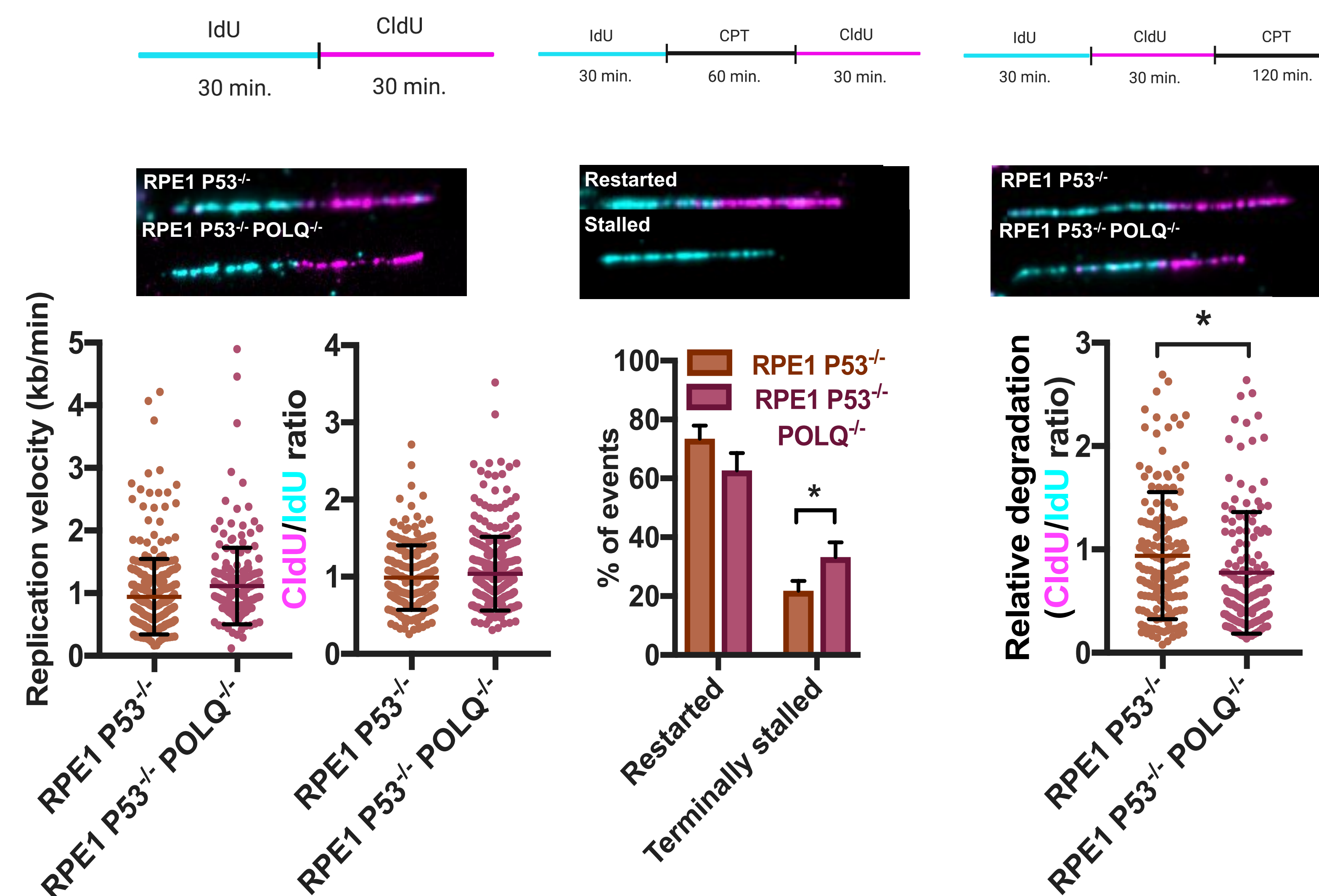


Figure 2. (A) DNA combing identifying comparable replication speed and dynamics in POLQ^{-/-} cells. (B) DNA combing with a 500 nM CPT stall documenting increased terminally stalled forks in the POLQ^{-/-} background. (C) DNA combing identifying increased fork degradation in POLQ^{-/-} cells after prolonged 500 nM CPT treatment.

Engineering a replicative break reporter

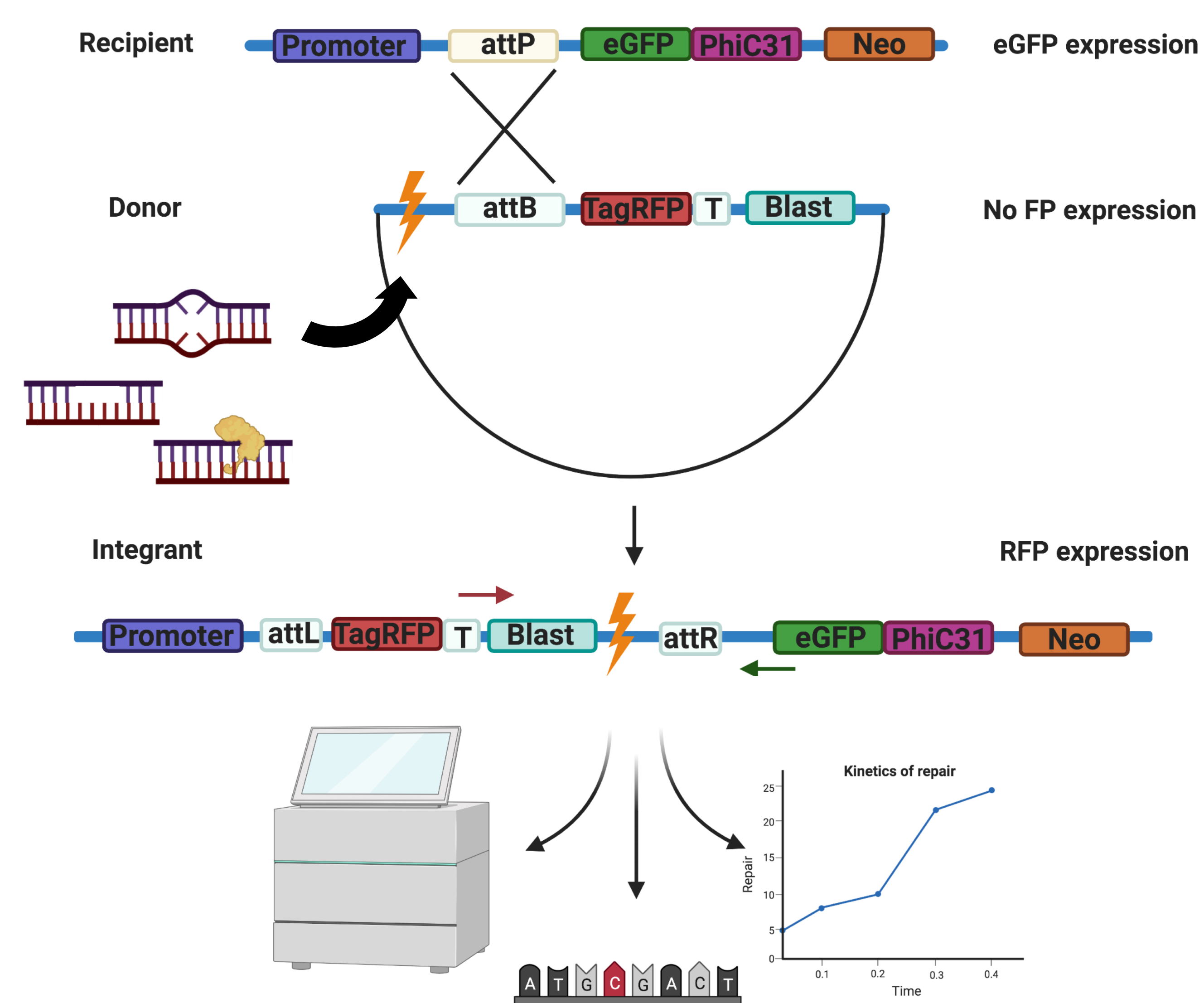


Figure 3. Diagram of the replicative break reporter system. The recipient is stably integrated into the genome and the donor is transiently introduced. PhiC31 will recombine the donor into the recipient to form integrants. The lightning bolt represents the site of the interchangeable DNA lesion.

Validation of the replicative break reporter

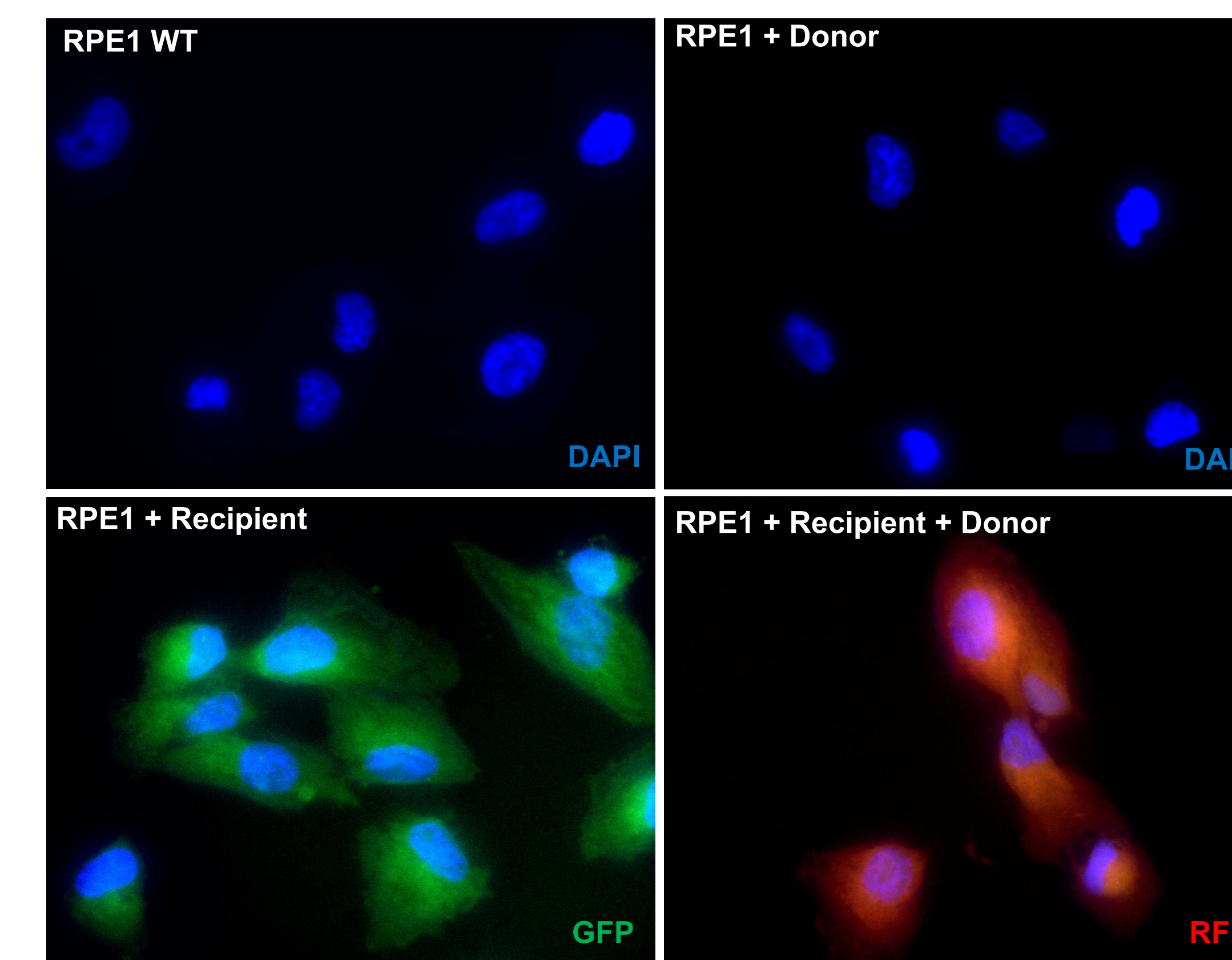


Figure 4. Microscopy confirming stable GFP expression of the recipient and integration and expression of the donor RFP.

Summary and future directions

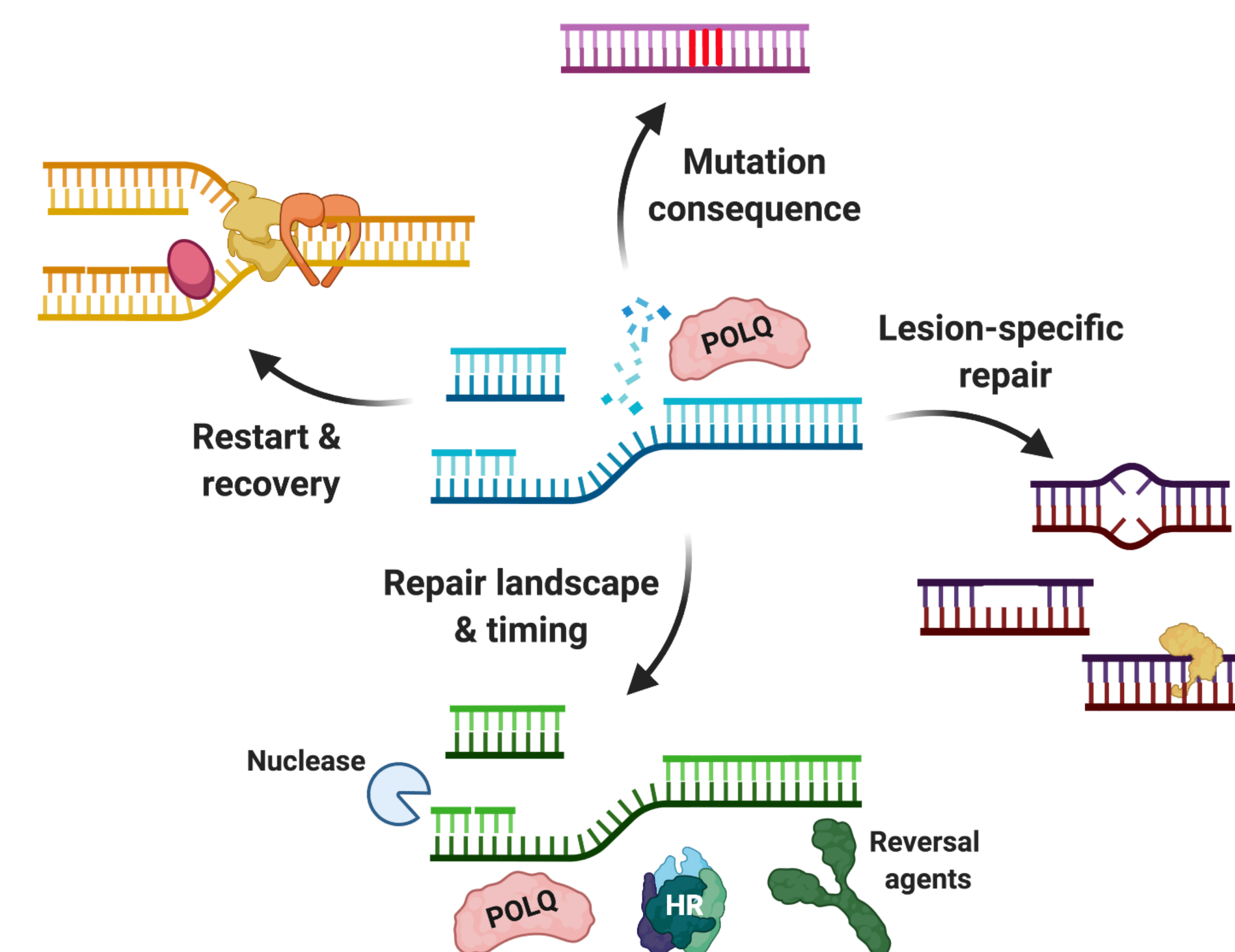


Figure 5. Graphical representation of project goals.

Future directions –

- Perform super resolution microscopy to monitor POLQ's requirements for recruitment to forks
- Identify mutational signatures at different replicative blocks (crosslinks, protein-DNA adducts, abasic sites, etc.)
- Determine how POLQ cooperates with other fork recovery pathways (Homologous Recombination, fork regression, etc.)