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where DNA breaks, our insight begins

Replication stress detection using STRIDE technology platform

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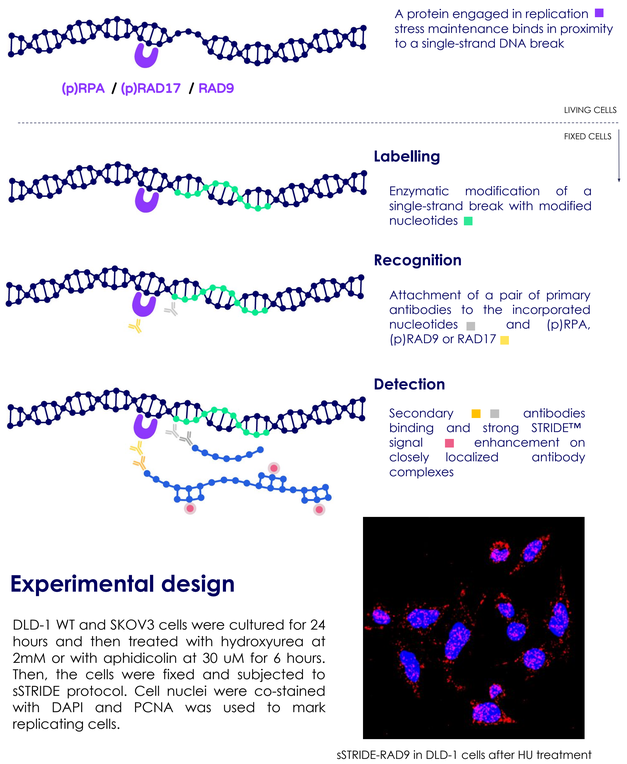
Replication stress is a common feature of cancer cells and a major contributor to genomic instability. Accurate detection of replication stress is essential for understanding tumor biology and for guiding the use of DNA damage response (DDR) inhibitors, such as ATR kinase inhibitors. Traditional methods lack the specificity and sensitivity needed to capture replication stress in diverse biological contexts. To address this, we developed novel assays using our STRIDE technology, combining direct detection of single-strand DNA breaks (SSBs) with replication stress-associated proteins. This approach enables a more precise assessment of replication stress levels, with potential applications in both research and clinical settings.

Model of how sSTRIDE based assays work



Experimental design

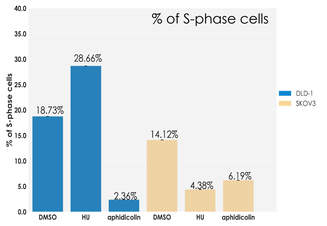
DLD-1 WT and SKOV3 cells were cultured for 24 hours and then treated with hydroxyurea at 2mM or with aphidicolin at 30 uM for 6 hours. Then, the cells were fixed and subjected to sSTRIDE protocol. Cell nuclei were co-stained with DAPI and PCNA was used to mark replicating cells.

sSTRIDE-RAD9 in DLD-1 cells after HU treatment

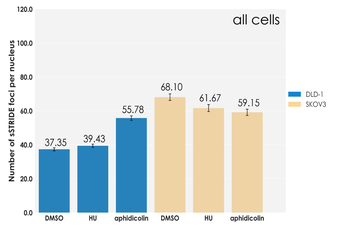
Aphidicolin and hydroxyurea influence on cell cycle and SSBs levels

Aphidicolin reduces the number of replicating DLD-1 and SKOV3 cells.

Hydroxyurea increases the number of replicating DLD-1 cells.

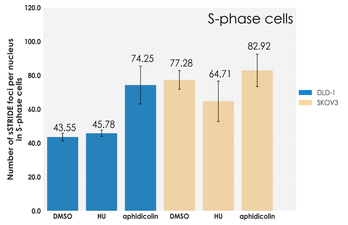


Only aphidicolin treatment leads to a significant global increase in the level of SSBs in DLD-1 cells.

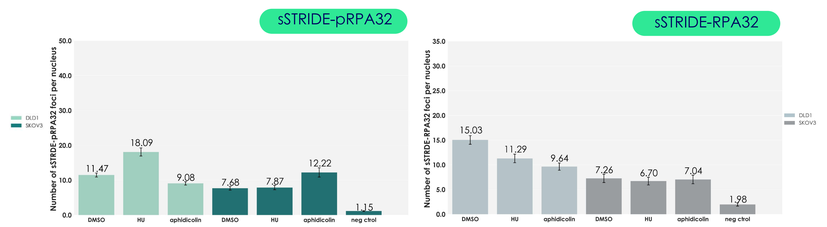


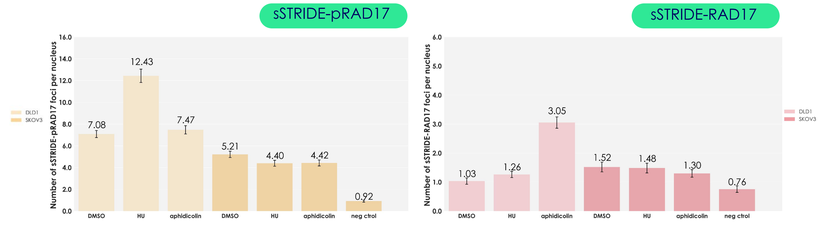
The level of SSBs is elevated in untreated DLD-1 and SKOV3 cells.

A higher response to aphidicolin is observed in replicating DLD-1 cells.



Recruitment of replication stress-associated proteins to SSBs

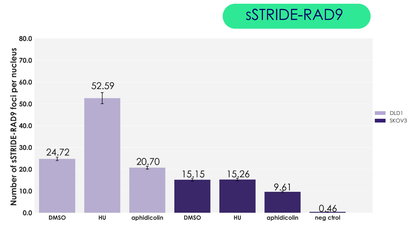




HU treatment leads to enhanced occurrence of RAD9, pRPA32 and pRAD17 at sites of SSBs in DLD-1 cells.

Only RAD17 is engaged in repair of aphidicolin induced SSBs in DLD-1 cells.

Phosphorylation of RPA occurs in response to aphidicolin treatment in SKOV3 cells.



We have shown here that STRIDE technology platform can be leveraged to develop assays measuring cellular events related to replication stress. Using a panel of new assays, we demonstrated for the first time that depending on the cell and treatment type, various proteins known to play a role in response to replication stress, can be engaged. While full validation is needed, the newly developed assays can become useful tools to inform about treatment outcomes and potentially, guide therapeutic strategy in the future.