



1737

Abstract

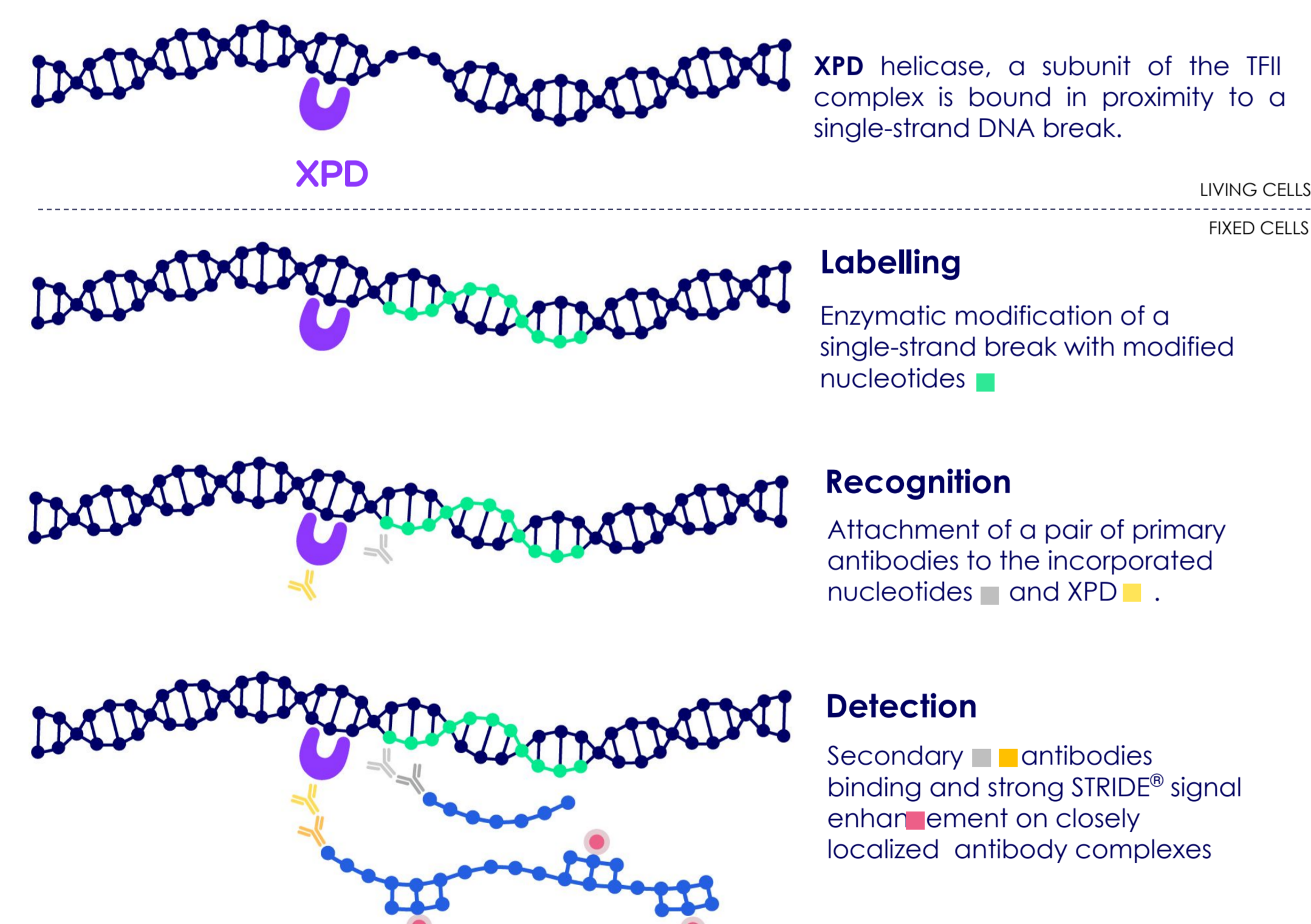
Nucleotide Excision Repair (NER) removes bulky DNA lesions generated by UV or chemotherapeutics such as cisplatin. NER activity can influence tumor response but despite its clinical importance, current methods often rely on bulk or indirect measurements that fail to capture single-cell repair dynamics. To address this, we developed **sSTRIDE-NER**, an image-based assay utilizing the STRIDE platform. By targeting the **XPD helicase** at active repair sites, the assay enables the direct visualization and quantification of transient single-strand breaks (SSBs) generated during NER. This approach provides the first single-cell readout of functional NER activity.

Technology and Methods

STRIDE® methodology & analysis

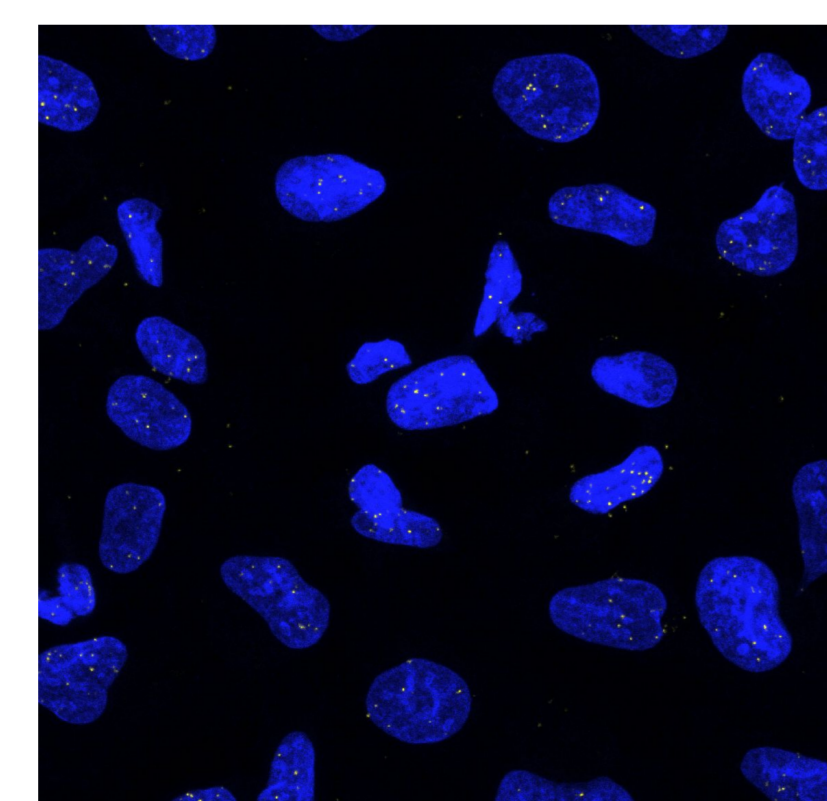
- **Direct detection:** High-sensitivity *in situ* labeling for the detection and visualization of DNA strand breaks
- **Single-cell resolution:** Quantitative functional profiling at the individual cell level
- **Sample versatility:** Validated across coverslips, 96-well plates, and clinical tissue (FFPE & fresh frozen).
- **High-dimensional multiplexing:** Seamless integration with cell cycle (Geminin, Ki67, PCNA) and DDR markers.
- **AI-driven quantification:** Automated analysis via CABLES, a custom AI solution for robust, high-throughput image scoring

Model of how sSTRIDE-based assays work



Experimental design

U2OS cells were cultured for 24 hours and then treated with cisplatin at 5 μM for 6 hours or 24 hours. Then, the cells were fixed and subjected to sSTRIDE protocol. Cell nuclei were co-stained with DAPI. 3D confocal stacks were acquired, and the images were analyzed by an in-house build software (CABLES). First, 3D nuclear masks were created based on the DAPI signal and then fluorescence foci were quantified in the 3D space the nuclear masks.



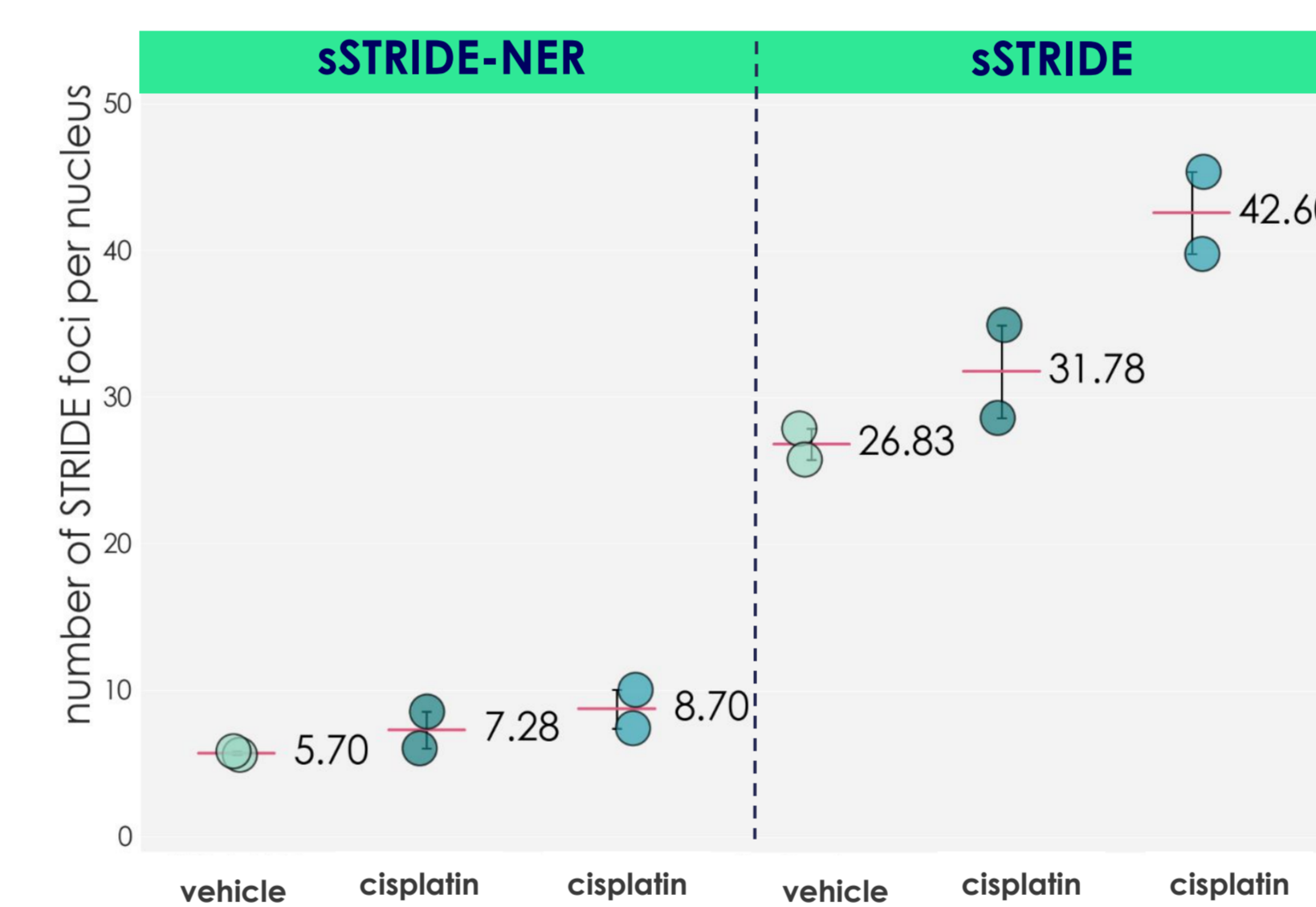
sSTRIDE-NER in U2OS cells after cisplatin treatment.

Results and Assay Performance

sSTRIDE-NER: technical and biological validation

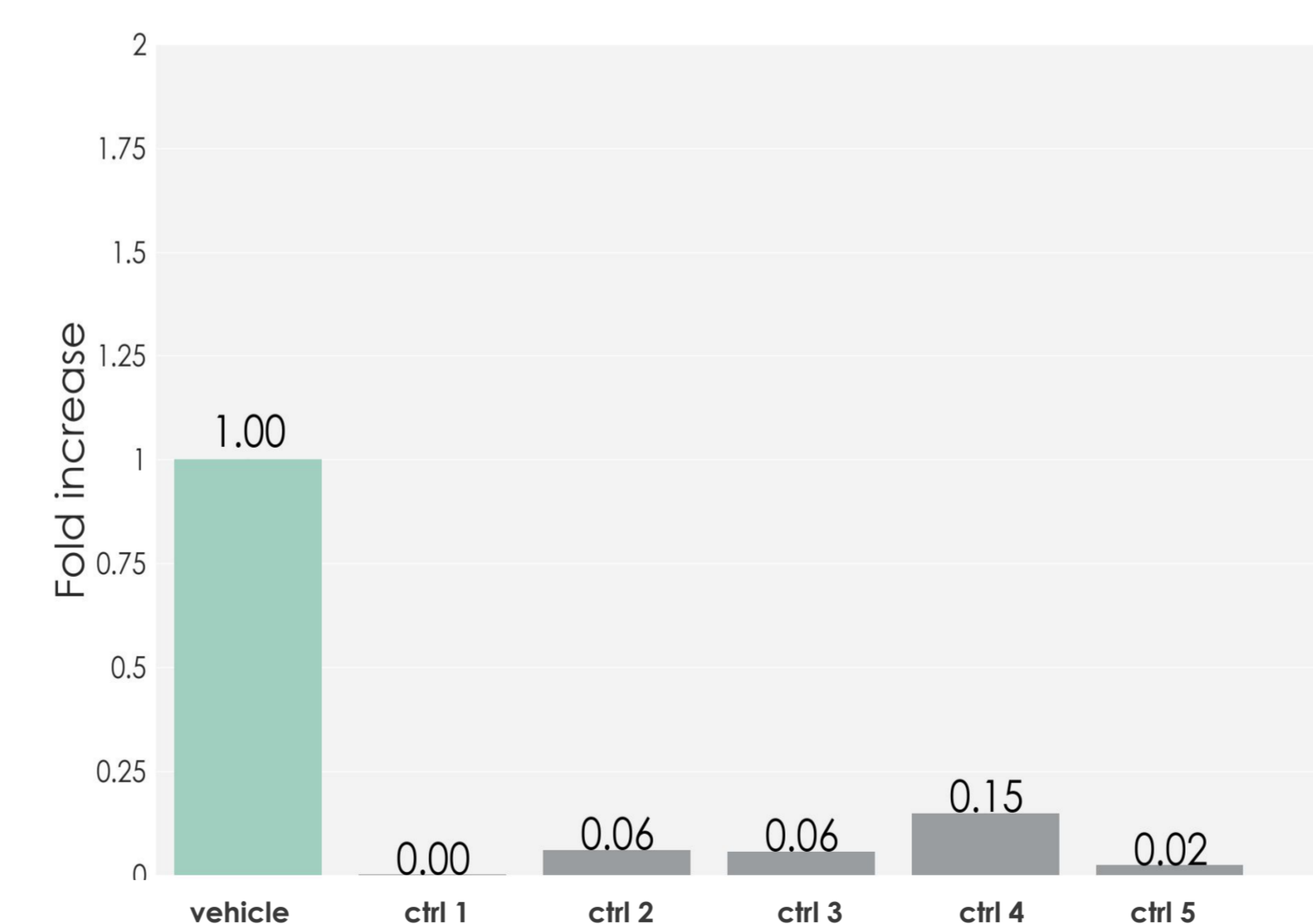
Treatment with 5 μM cisplatin induces a robust, time-dependent accumulation of both total single-strand breaks (sSTRIDE) and XPD-localized repair intermediates (sSTRIDE-NER).

- A significant increase in foci was observed at 6 and 24 hours, reflecting the progression of DNA damage and the subsequent recruitment of the NER machinery.
- Data representation: Individual data points represent the mean number of foci per nucleus across biological replicates, demonstrating high experimental reproducibility and the assay's ability to distinguish global damage from functional repair activity.



A panel of **technical controls** was utilized to validate the specificity of the sSTRIDE-NER platform.

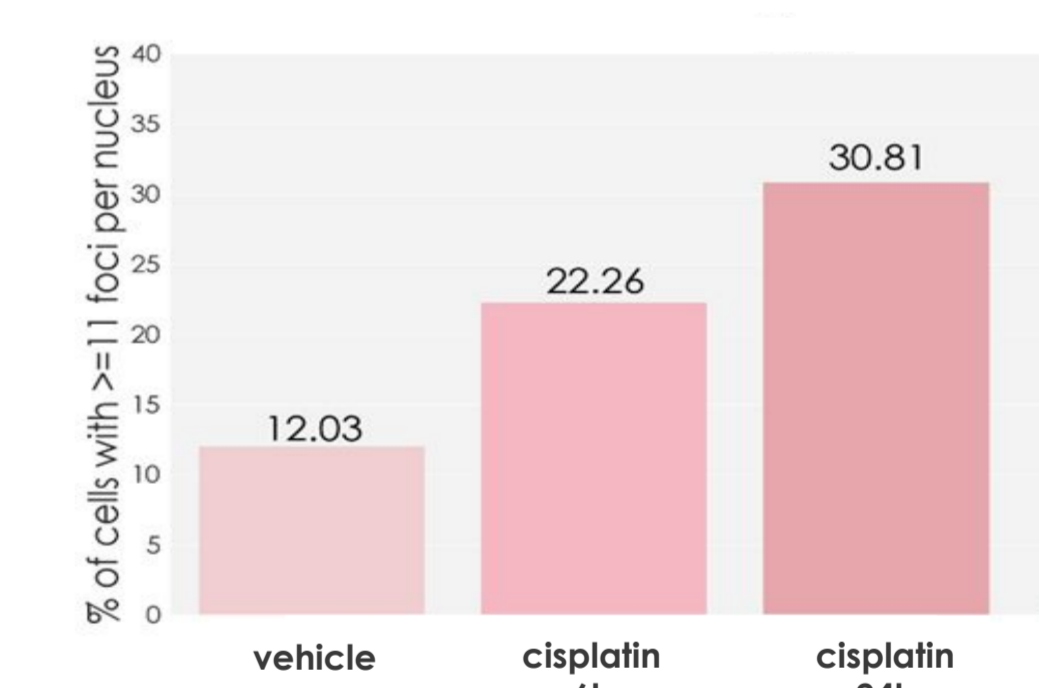
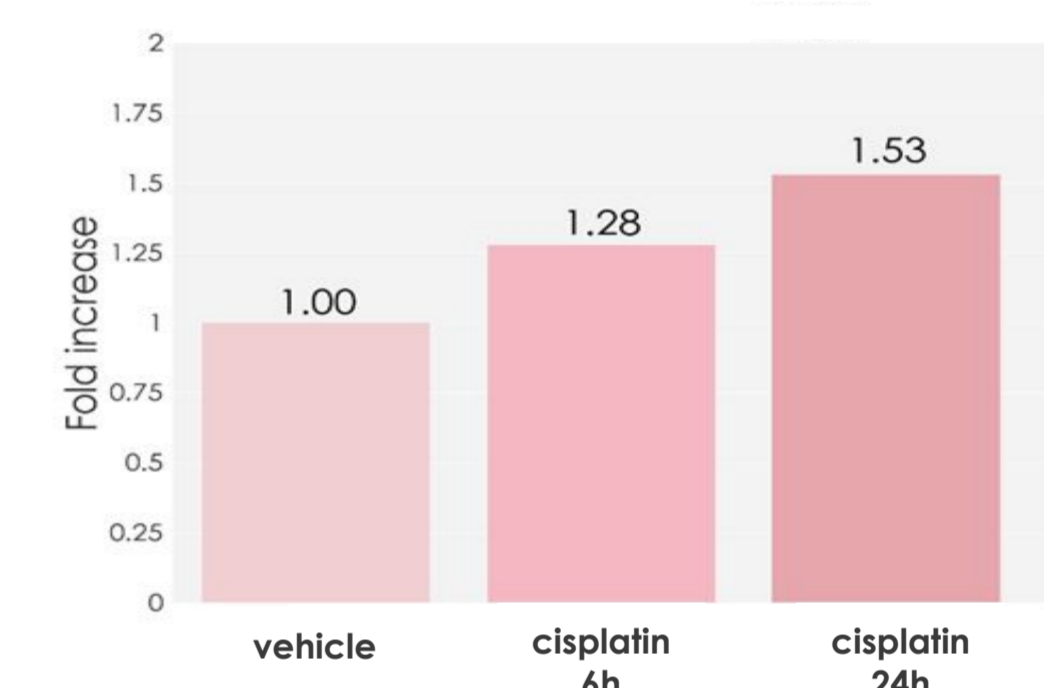
- Methodology: validation involved the systematic omission of primary or secondary antibodies and the introduction of isotype-matched controls.
- Results: Data are expressed as a fold-change relative to the vehicle control (complete protocol). The low background signal across all negative controls confirms that the visualized foci are derived from the specific recognition of XPD-DNA end complexes, ensuring a high signal-to-noise ratio for quantitative analysis.



Time-dependent response to cisplatin

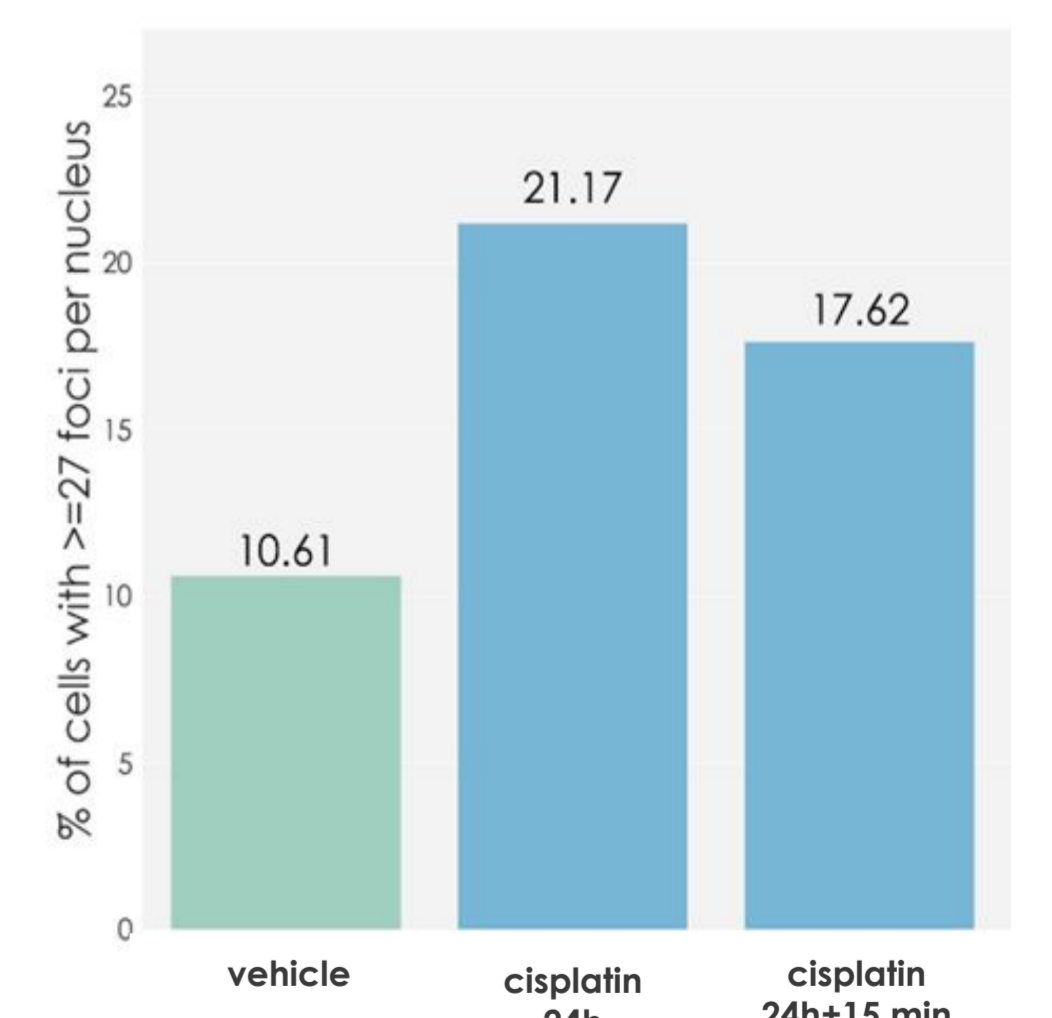
Cisplatin treatment triggers a significant increase in XPD-bound SSBs, characterized by both average induction and population-wide shifts:

- **Fold-change:** The ratio of mean nuclear foci reveals a robust, time-dependent accumulation of active NER sites relative to the vehicle control.
- **Population dynamics:** Analysis of "high-responder" cells - defined as those exceeding the 90th percentile threshold of the vehicle - shows a greater than 2-fold increase in the subpopulation actively undergoing repair.



To confirm that sSTRIDE-NER captures active repair events rather than static DNA damage, a washout experiment was conducted:

- **Experimental design:** Following 24 hours of cisplatin exposure, the drug was removed, and cells were incubated in fresh medium for a 15-minute recovery period.
- A measurable reduction in the number of sSTRIDE-NER foci was observed immediately following the washout.
- This rapid decrease highlights the transient nature of NER intermediates.



Conclusions

REFERENCES

1. Kordon, M. M., Zarebski, M., Solarczyk, K., Ma, H., Pederson, T. & Dobrucki, J. W. STRIDE—a fluorescence method for direct, specific *in situ* detection of individual single- or double-strand DNA breaks in fixed cells. *Nucleic Acids Res.* **48**, e14 (2020).
2. Kordon MM, Szczurek A, Berniak K, Szelest O, Solarczyk K, Tworzydło M, Wachsmann-Hoglu S, Vaahokari A, Cremer C, Pederson T, Dobrucki JW. PML-like subnuclear bodies, containing XRCC1, juxtaposed to DNA replication-based single-strand breaks. *Nucleic Acids Res.* **49**, 11930–11945 (2021).
3. Solarczyk, K. & Kordon-Kiszala, M. Let's not take DNA breaks for granted: the importance of direct detection of DNA breaks for the successful development of DDR inhibitors. *Front. Cell Dev. Biol.* **11**, 1118716 (2023).

sSTRIDE-NER expands the STRIDE platform's capabilities offering a robust and validated method for quantifying NER activity with high technical precision. By demonstrating a clear response to cisplatin treatment and maintaining high performance across technical replicates, this assay proves its **reliability for functional DNA repair studies**. Crucially, its seamless compatibility with routine FFPE processing positions sSTRIDE-NER as a translationally ready tool for **profiling patient sensitivity to platinum-based therapies**, offering a path toward personalized treatment strategies and the discovery of functional clinical biomarkers.