

In situ measurement of PARP1 activity and trapping at single-strand DNA breaks



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Abstract

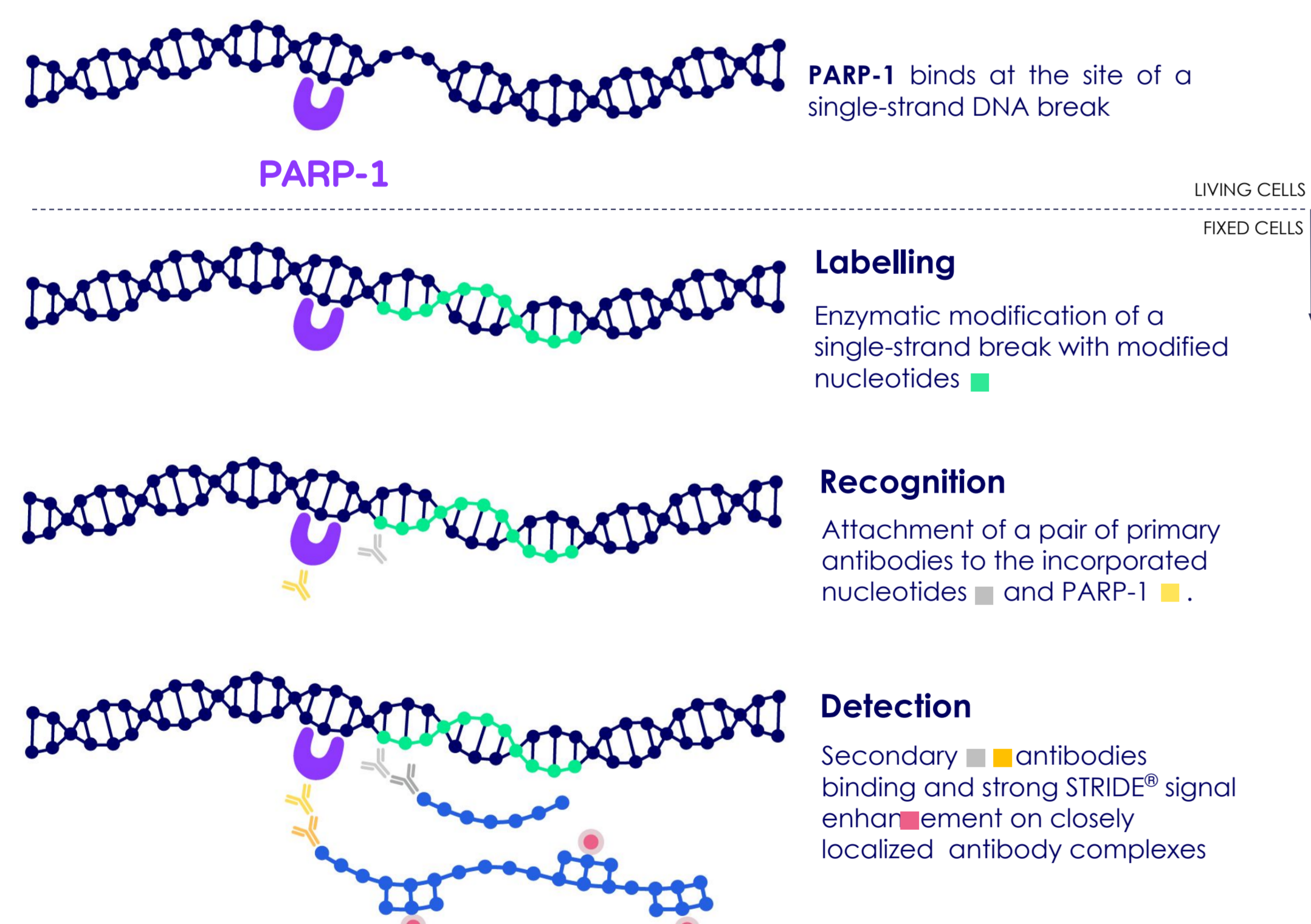
PARP1 trapping at single-strand DNA breaks (SSBs) is a fundamental mechanism of action for PARP inhibitors (PARPi) and a key determinant of therapeutic efficacy. Despite this clinical importance, direct methods to measure *in situ* PARP1 engagement are lacking, often relying on indirect surrogates like global PARylation. To address this we developed **sSTRIDE-PARP1**, an image-based assay utilizing the STRIDE platform to directly visualize and quantify PARP1 localized at SSBs at single-cell resolution. This approach enables precise measurement of PARPi trapping capacity and provides a translational platform for developing functional biomarkers to guide DNA damage response therapies.

Technology and Methods

STRIDE® methodology & analysis

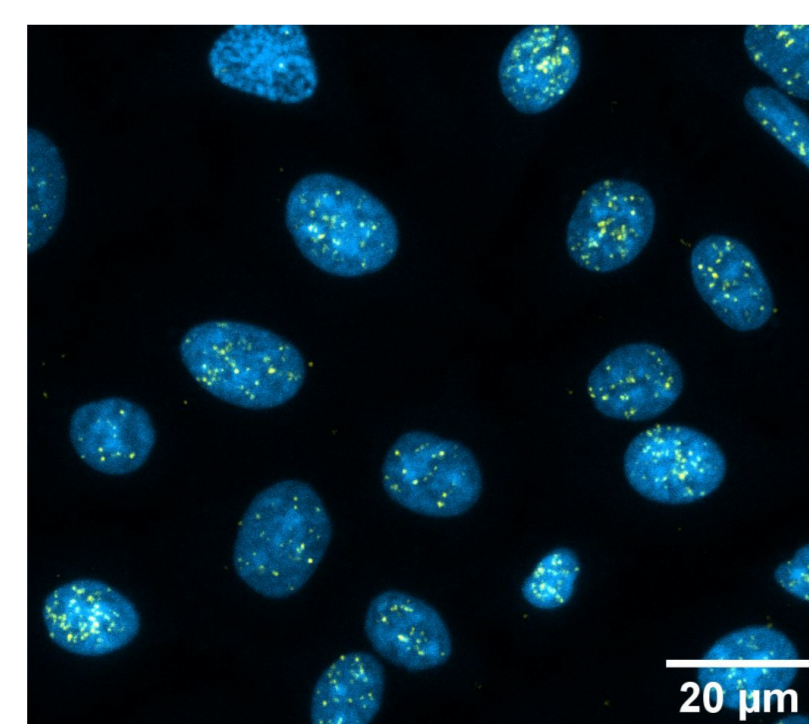
- **Direct detection:** High-sensitivity *in situ* labeling for the detection and visualization of DNA strand breaks
- **Single-cell resolution:** Quantitative functional profiling at the individual cell level
- **Sample versatility:** Validated across coverslips, 96-well plates, and clinical tissue (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

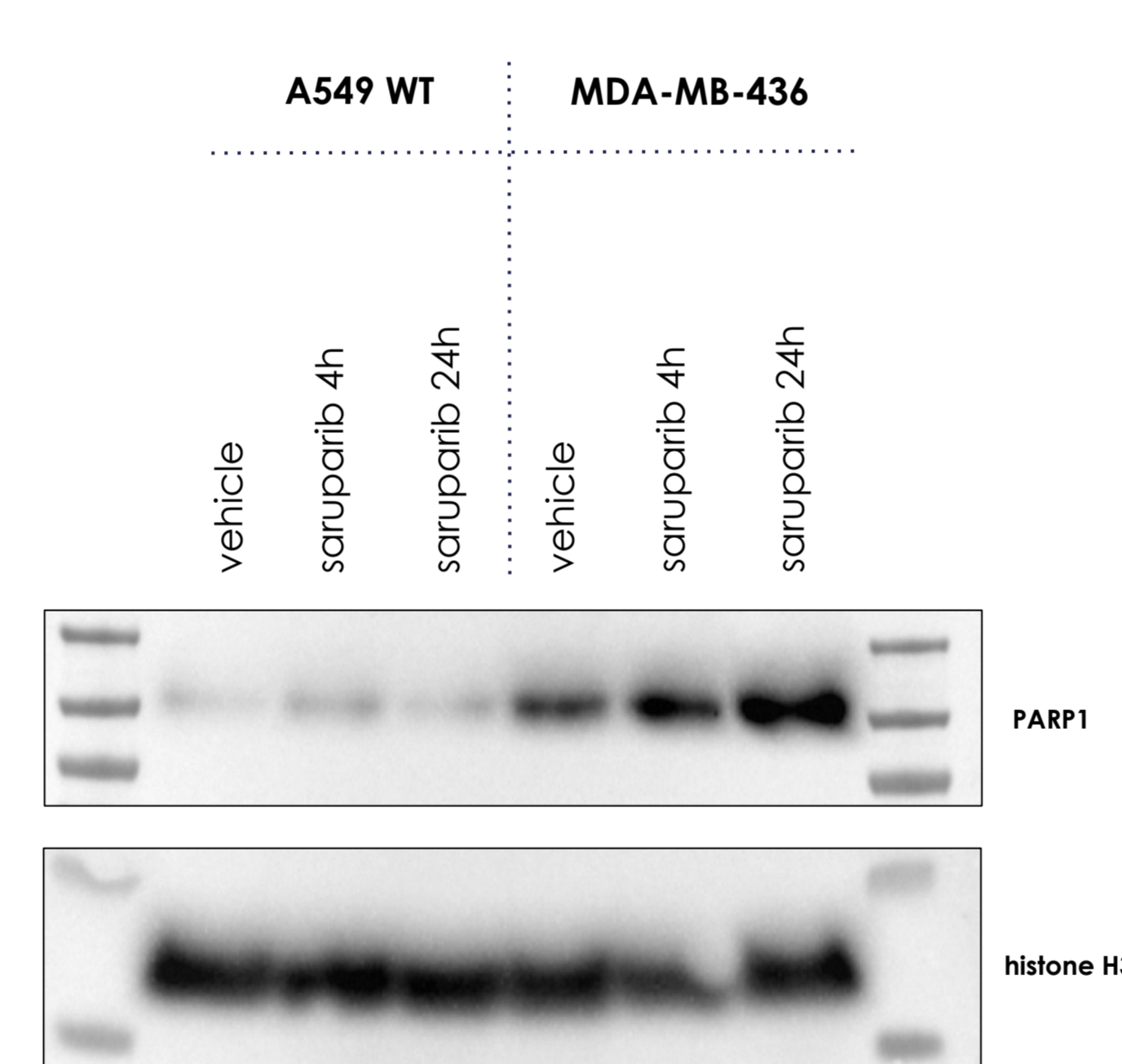
A549 WT, A549 PARP1-KO and MDA-MB-436 cells were cultured for 24 hours and then treated with saruparib at 350 nM (6h or 24 hrs) or 50 nM MMS. Cells were then processed for chromatin fractionation or subjected to sSTRIDE-PARP1 protocol. Cell nuclei were co-stained with DAPI. 3D confocal stacks were acquired 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-PARP1 in A549 cells after saruparib treatment.

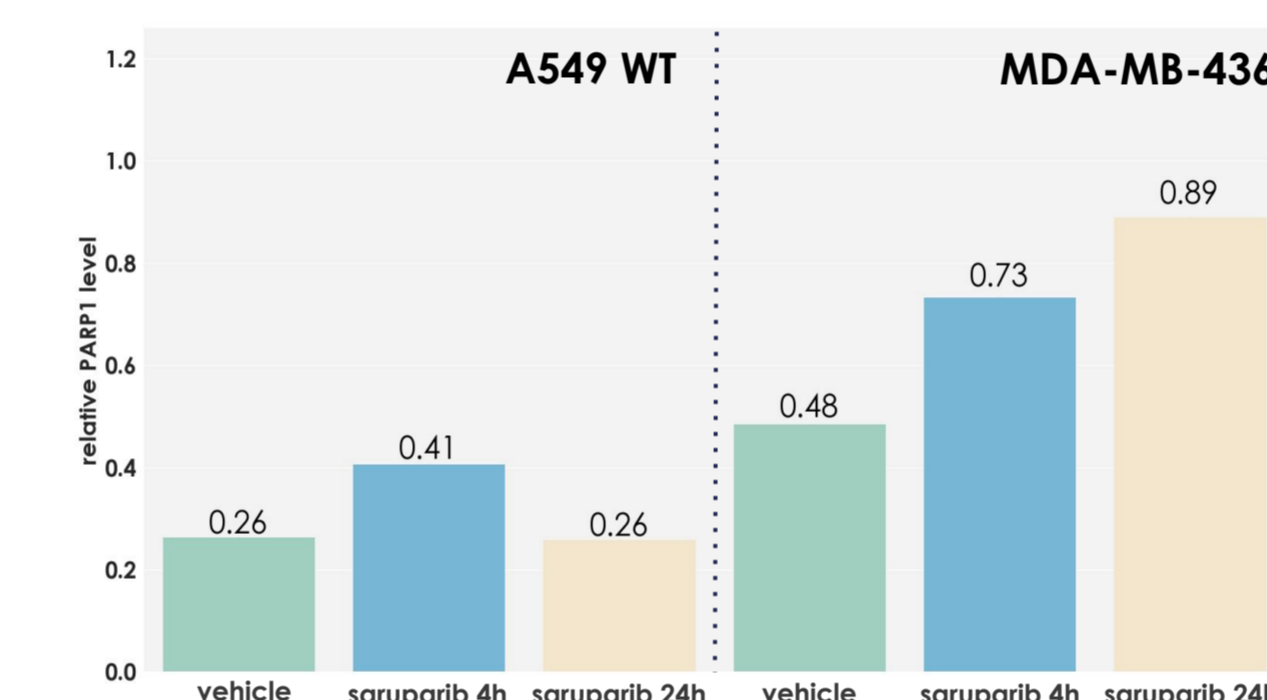
Results and Assay Performance

sSTRIDE-PARP1 detects PARPi trapping at SSBs



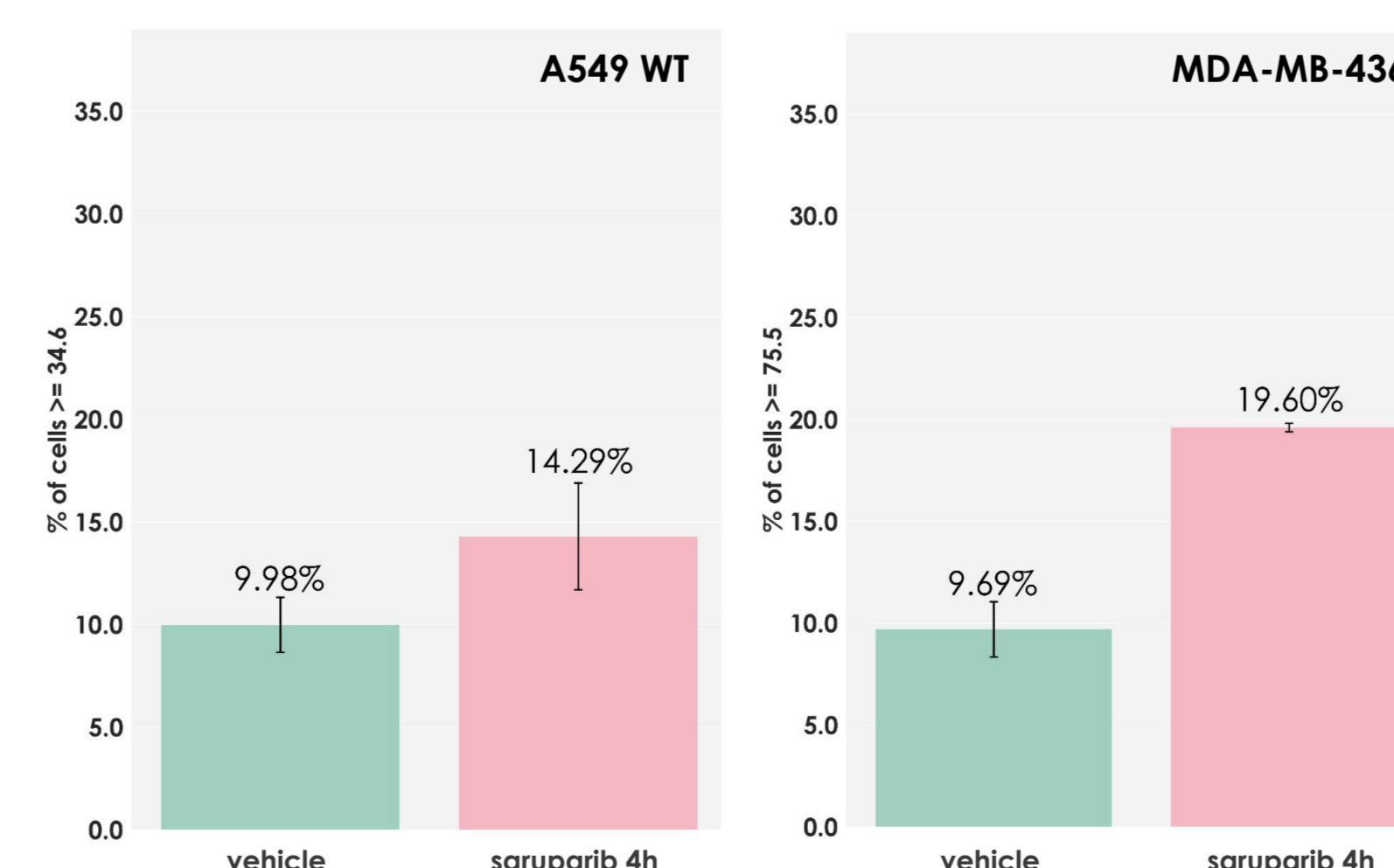
sSTRIDE-PARP1 was applied to evaluate the assay's sensitivity to PARP1-DNA complexes, cells were treated with the PARP1-selective inhibitor saruparib (350 nM, 4 h).

- The results demonstrate a clear increase in PARP1 engagement at single-strand breaks.
- Data analysis used a threshold based on the 90th percentile of the vehicle control; MDA-MB-436 cells showed a greater than 2-fold increase in the population of cells with high PARP1-SSB signal compared to A549 WT cells.

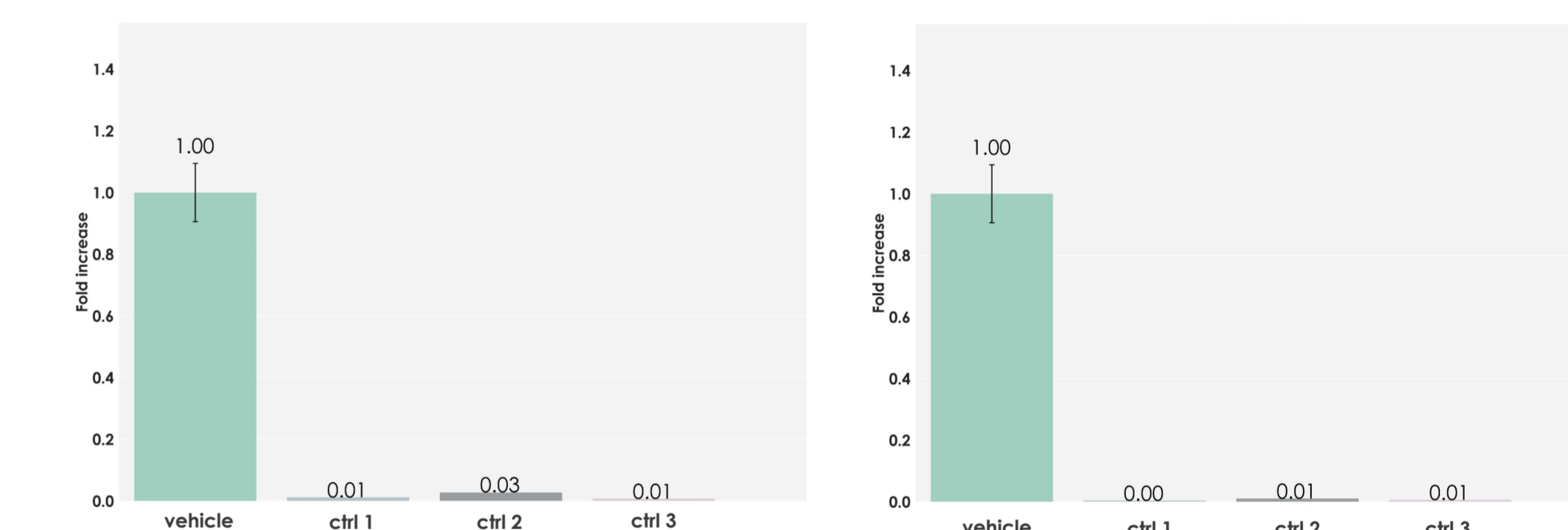


Chromatin fractionation was performed to evaluate the levels of chromatin-associated PARP1 across different cell lines.

- Basal levels: MDA-MB-436 cells exhibited higher constitutive levels of chromatin-bound PARP1 compared to A549 cells under untreated conditions.
- Response to treatment: Exposure to 350 nM saruparib induced a significant enrichment of PARP1 in the chromatin fraction in both cell lines. The magnitude and kinetics of this trapping effect varied, with MDA-MB-436 showing a more robust and sustained accumulation of PARP1 over 24 hours.



sSTRIDE-PARP1: technical and biological validation

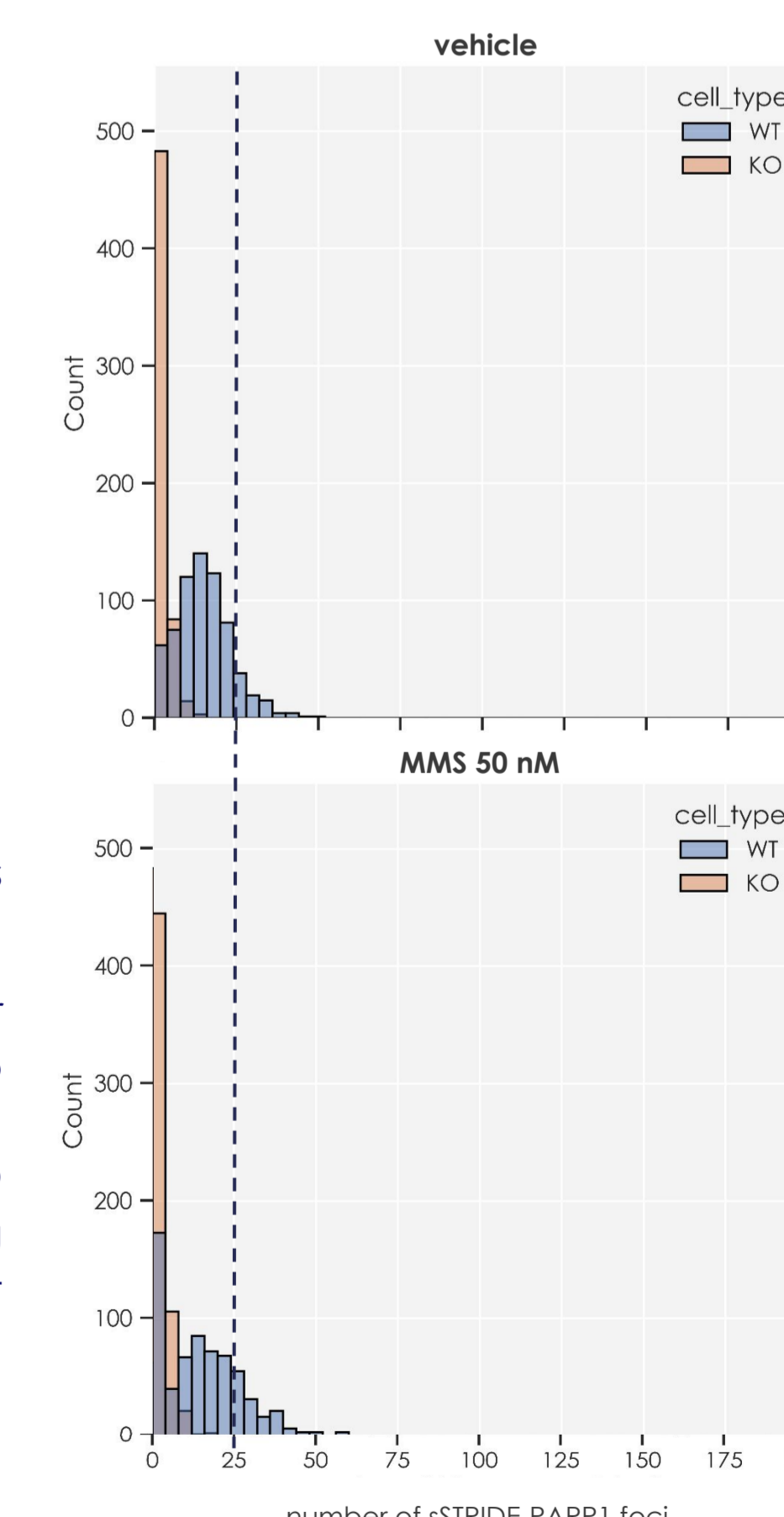


A panel of **technical negative controls** was implemented to ensure the high specificity of the sSTRIDE-PARP1 platform:

- Negative controls: systematic omission of primary/secondary antibodies or the use of isotype-matched controls resulted in negligible background signal.
- This confirms that the visualized signal is derived specifically from the targeted recognition of DNA ends and protein-specific antibodies.

The biological requirement for PARP1 was confirmed using **A549 PARP-1 KO cells**:

- sSTRIDE-PARP1 signal was significantly lower in PARP1 knockout (KO) cells compared to wild-type (WT) controls.
- No increase in signal was detected in KO cells following MMS treatment, confirming that the assay readout is strictly dependent on the presence of PARP1.



Conclusions

REFERENCES

1. Kordon 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, Szarek A, Beniak K, Szelest O, Solarczyk K, Tworzydło M, Wachsmann-Hoglu S, Vaahkolari 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, 118716 (2023).

sSTRIDE-PARP1 provides a definitive, single-cell readout of PARP1-DNA engagement. Validated through knockout models, technical controls and PARP1 inhibitors, the assay serves as a high-fidelity alternative to indirect markers. Having demonstrated robust performance in cell pellets, the assay's foundation on the STRIDE platform ensures seamless compatibility with clinical tumor FFPE samples. This may enable a clear distinction of trapping potencies across various inhibitors, providing deeper **mechanistic insight into their unique interactions with DNA damage sites.**