STRIDE™: Overcoming the limitations of γH2AX as a DNA damage detection method

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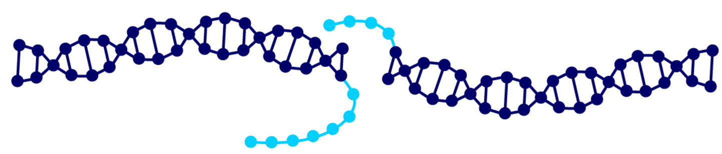
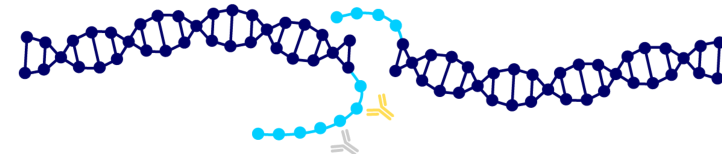
Introduction STRIDE™ directly labels the sites of DNA breaks, making it the most proximal marker available to date. Our technology addresses challenges commonly encountered with immunofluorescence-based techniques in DNA damage analysis. Quantifying immunostained DNA damage-associated proteins can be difficult due to poor staining quality and low signal-to-noise ratio. In contrast, distinct STRIDE™ foci can be accurately quantified using our in-house developed algorithms. Moreover, STRIDE™ is highly specific and functions independently of the complex DNA damage response (DDR) mechanisms.

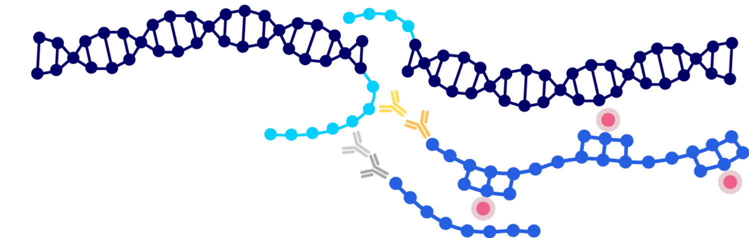
Schematic representation of the classic dSTRIDE™ assay. Proprietary STRIDE™ technology is a fluorescence-based method that directly labels DNA damage sites and detects individual DNA lesions. Its strong signal amplification enables robust and reliable quantification of DNA damage.



LABELING. Direct enzymatic incorporation of nucleotide analogues ■ to DNA ends within double-strand breaks.

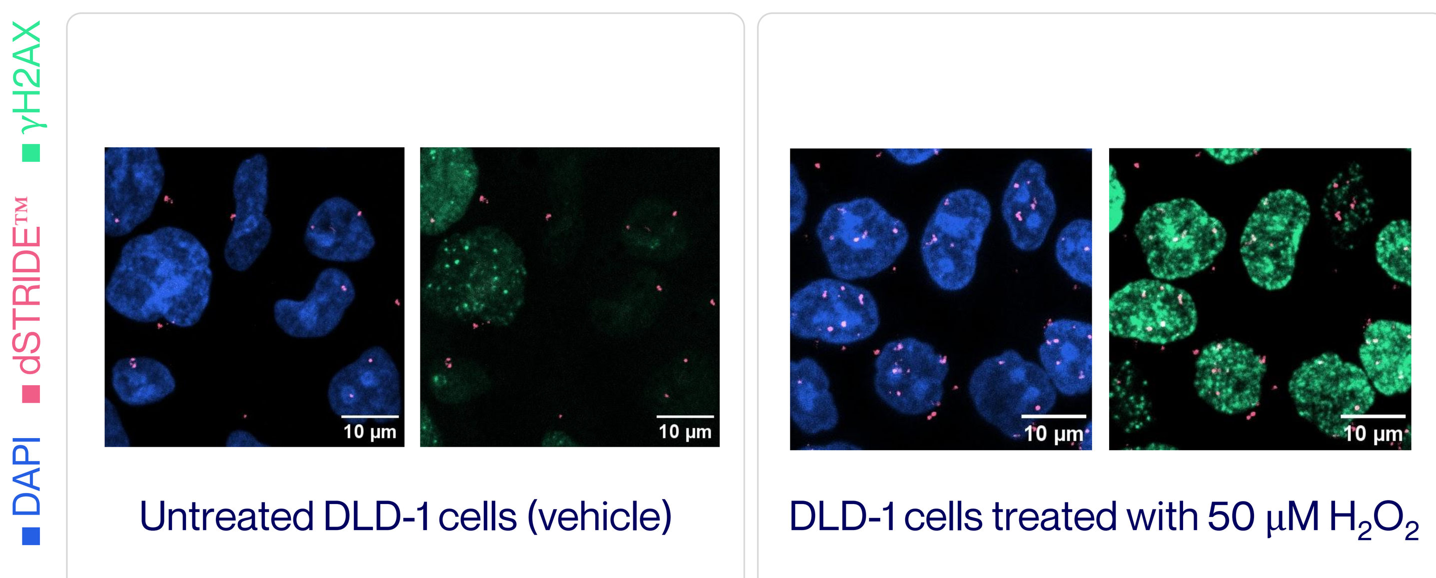


RECOGNITION. Attachment of a pair of primary antibodies ■ ■ to the labelled DNA ends.

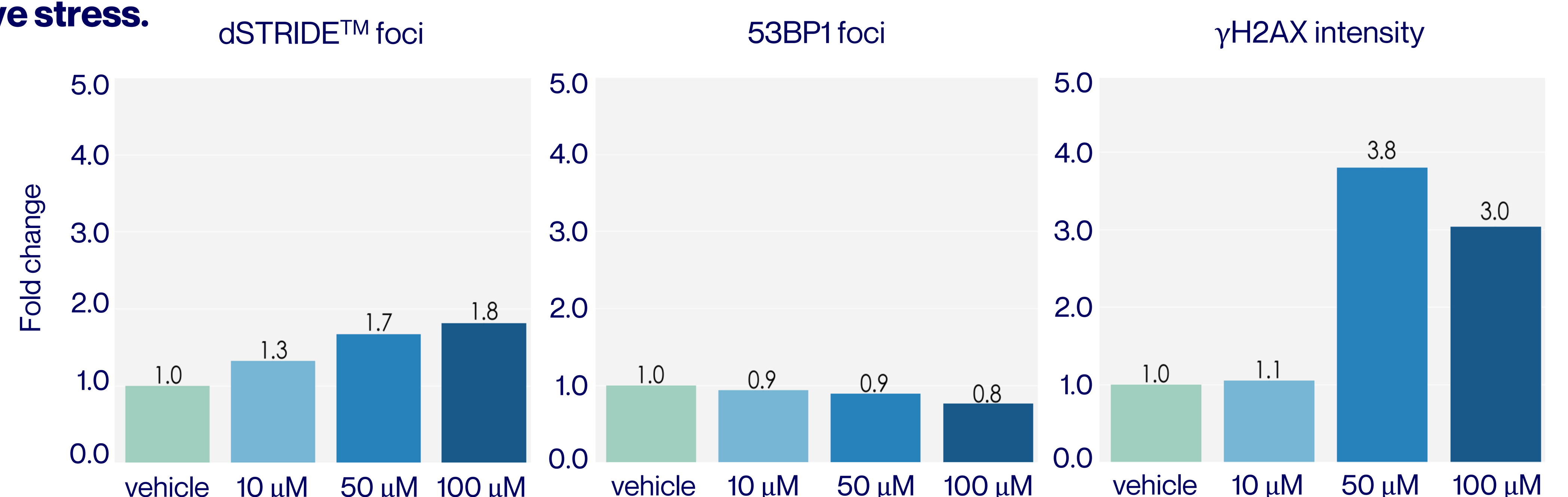


DETECTION. Secondary antibodies ■ ■ binding and strong STRIDE™ signal ■ enhancement on closely localized antibody complexes.

01 dSTRIDE™ foci form specifically and directly at the sites of DNA double-strand breaks (DSBs). In contrast, H2AX phosphorylation can occur independently of DSB formation, such as in response to oxidative stress.

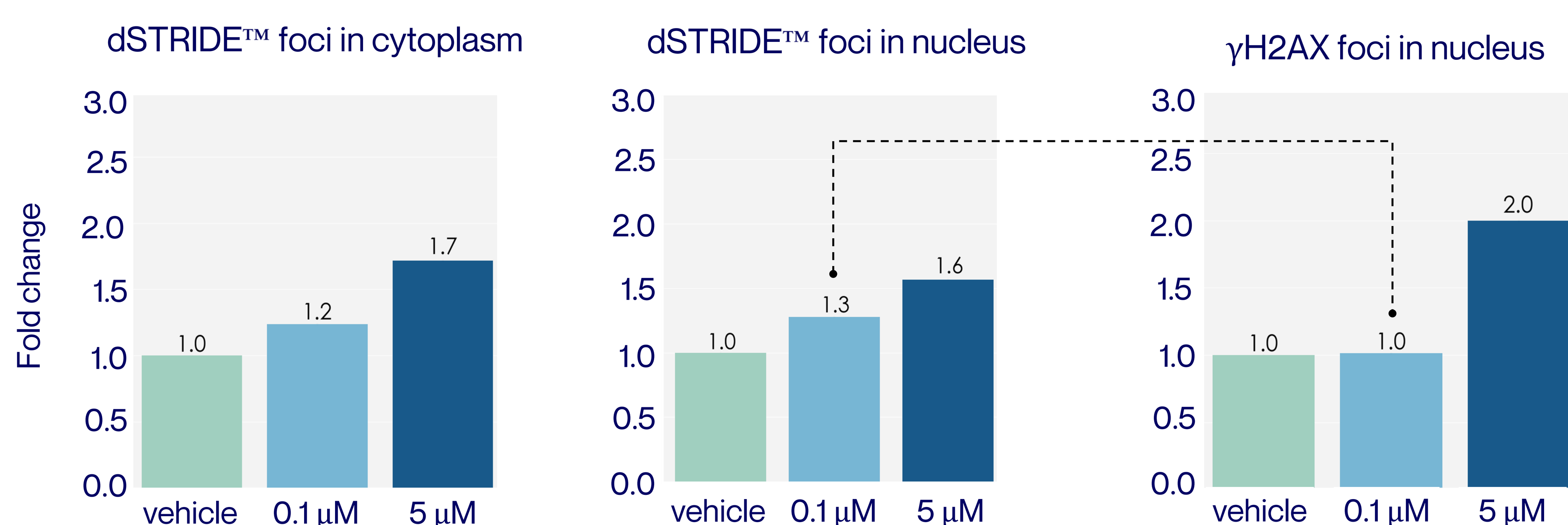


High levels of γH2AX do not correlate with dSTRIDE™. High signal-to-noise ratio of dSTRIDE™ allows for precise and unbiased quantification of DSBs. In contrast, the diffuse nuclear-wide pattern of γH2AX, without distinct foci, makes quantification challenging.



DLD-1 cells treated with H_2O_2. The exposure to H_2O_2 resulted in little/no response in dSTRIDE™ and 53BP1, while it markedly increased γH2AX. This suggests that H2AX phosphorylation may overestimate the degree of DNA damage under oxidative stress. Fold change normalized to the vehicle control, n=3.

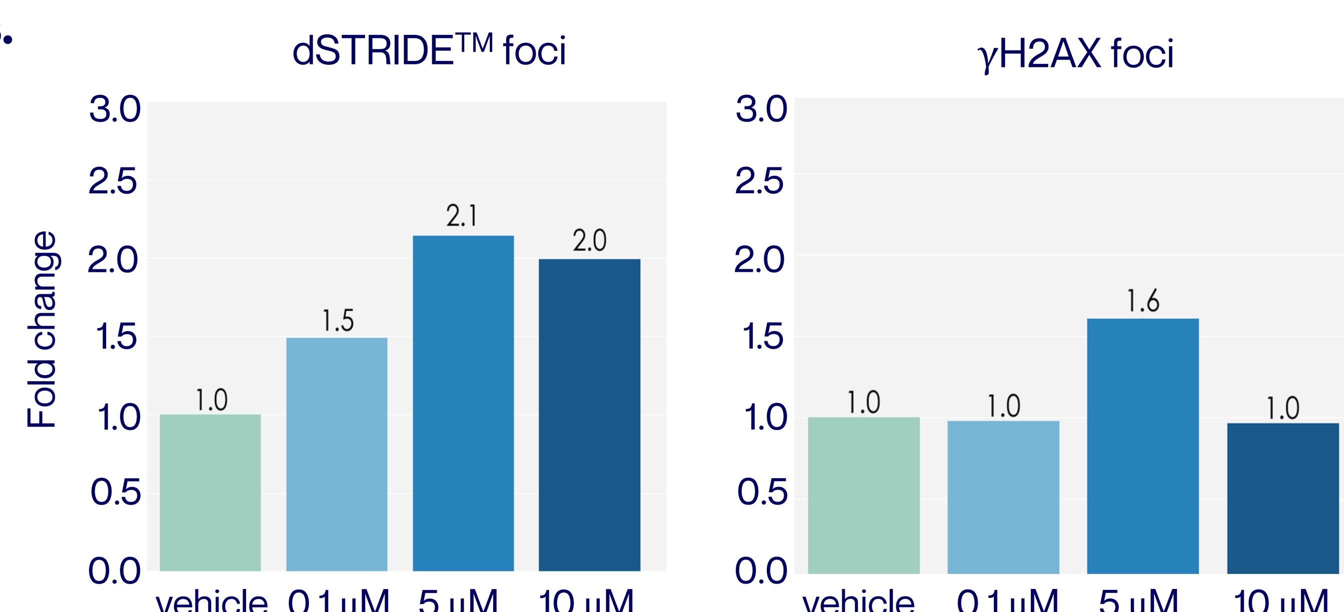
02 STRIDE™ superior sensitivity enables the detection of subtle changes in DNA damage caused by a DDR inhibitor.



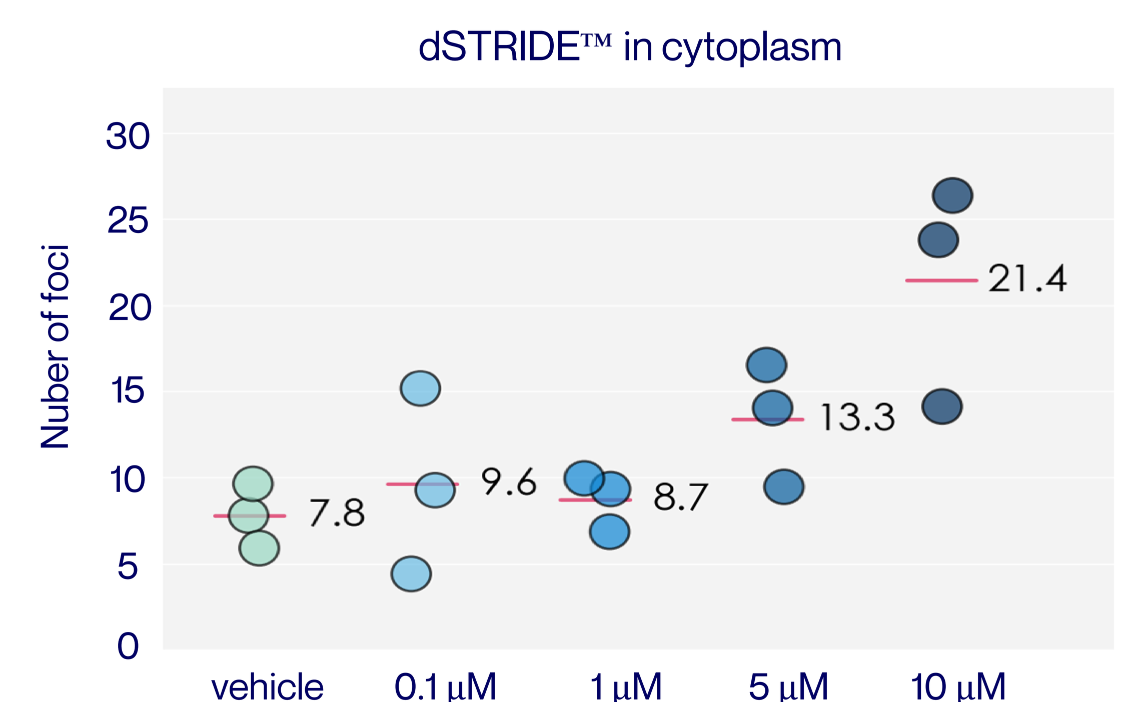
HCT116 cells treated with Olaparib. dSTRIDE™ detects DNA breaks even at a low concentration of Olaparib, where γH2AX may be less responsive due to its lower sensitivity. Additionally, the delayed engagement of the DNA repair machinery could contribute to the absence of γH2AX at the lower drug concentration. Fold change normalized to the vehicle control, n=3.

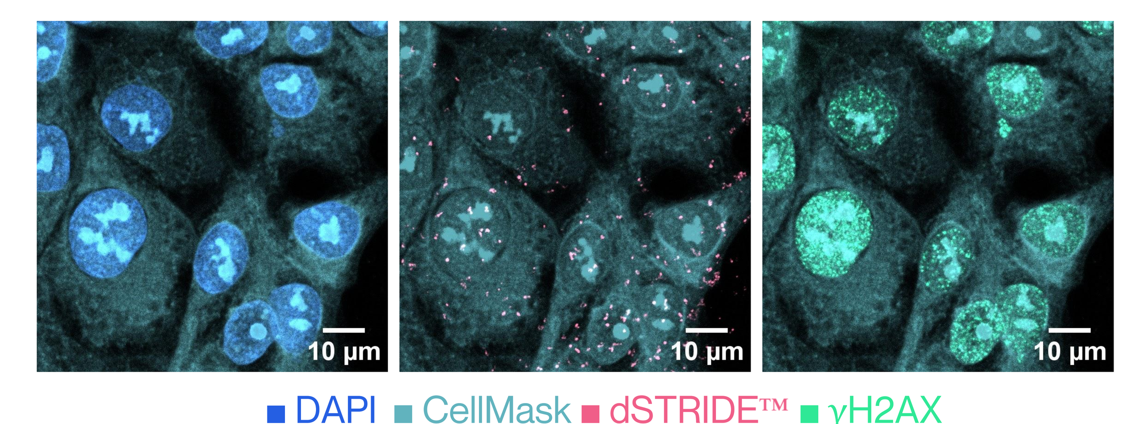
03 STRIDE™ is a direct marker that detects DNA lesions independently of the cell's DNA repair machinery, which is crucial when assessing the mechanism of action and efficacy of therapeutics that compromise DDR, such as ATR inhibitors.

DLD-1 cells treated with ATR inhibitor (VE-821). The increase in dSTRIDE™ foci reflects ATRi-induced DSBs. Mild or no response indicated by γH2AX could misleadingly suggest a reduction in DNA damage, when in reality, this may reflect disrupted repair machinery. Fold change normalized to the vehicle control, n=3.



04 STRIDE™ is a versatile technology that provides additional insights into DNA integrity across various cell compartments, including DNA fragments in the cytoplasm.





■ DAPI ■ CellMask ■ dSTRIDE™ ■ γH2AX

HCT116 cells treated with Olaparib. The treatment leads to a dose-dependent increase in the number of dSTRIDE™ foci in the cytoplasm. An increase in cytosolic DNA fragments is an important indicator of the therapeutic impact on cellular integrity, stress response, and potential efficacy in inducing cell death. The red line represents the mean of replicates, n=3.

Conclusions STRIDE™ technology provides a highly sensitive method for detecting even subtle changes in the levels of DNA breaks, enhancing our understanding of DNA damage response (DDR). Its versatility allows for the analysis of both single- and double-strand DNA breaks across various cellular compartments, making it an invaluable tool for studying the mechanism of action of DNA damaging agents and DDR inhibitors. Importantly, STRIDE™ offers specificity by directly measuring DNA breaks without interference from other cellular events, such as oxidative stress, and operates independently of DDR pathways. This combination of sensitivity, versatility, and specificity positions STRIDE™ as a crucial asset for advancing our knowledge of DNA damage mechanisms and therapeutic responses.