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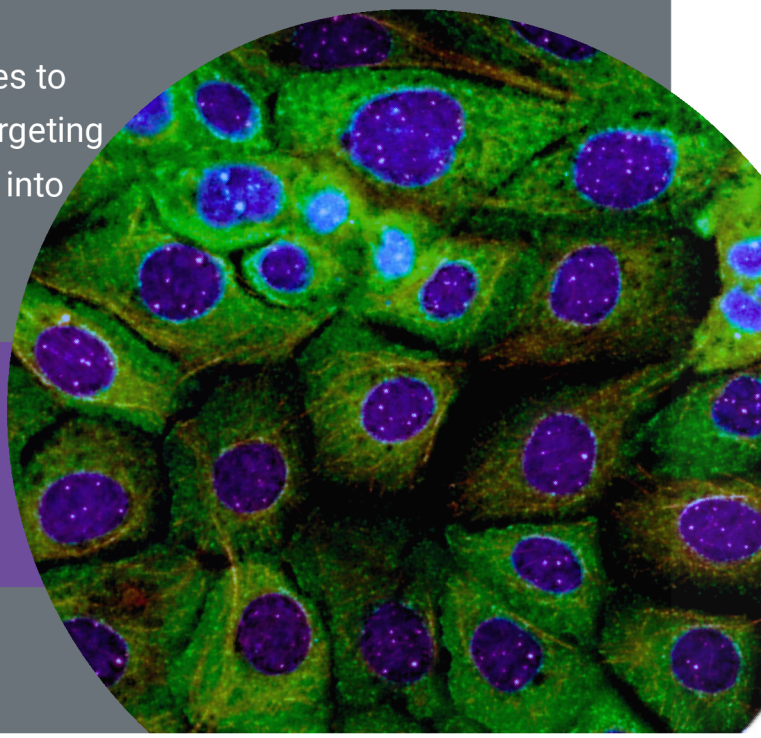
Mechanism of Action: DNA Damage

DNA damage is one of the most ubiquitous mechanisms of action exploited in cancer therapy. Rapid proliferation rates and the presence of damaging mutations in DNA repair pathway genes make cancer cells highly susceptible to insult to their genomic material, and classic radiation and chemotherapy treatments exploit this weakness. More targeted approaches to induce damage or prevent repair seek to overcome resistance by exploiting new targets. Conversely, DNA damage quantification is crucial to determine potential genotoxicity of new compounds, which is a key concern in assessing their safety profile.

Our team can deploy state-of-the-art cellular techniques to define the mechanism of action of candidate drugs targeting DNA damage pathways, delivering actionable insights into cellular responses to treatment.



- DNA damage and replication stress
- Mitotic perturbations
- Cell cycle and cell division outcomes

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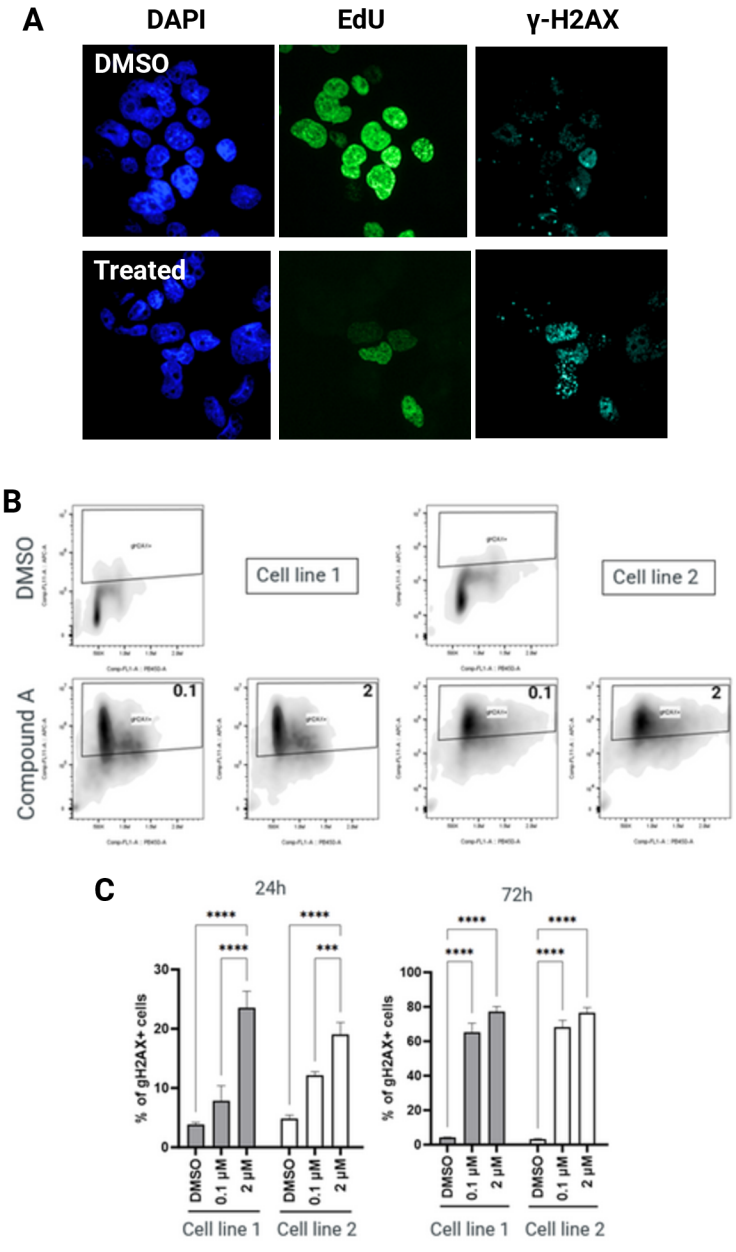
DNA Damage and Replication Stress

γ-H2AX analysis

Detection of γ-H2AX is a highly specific and sensitive method to monitor DNA double strand breaks (DSB) [1]. Quantitation of γ-H2AX foci is a powerful tool to evaluate the efficacy of compounds acting directly on DNA damage pathways, whereas global γ-H2AX levels are indicative of DSB arising both from DNA damage and DNA fragmentation during apoptosis.

Fig 1. Increase in γ-H2AX positive cells measured by microscopy and flow cytometry.

*Microscopy acquisition performed on Nikon Ti2 fluorescent microscope **(A)**. Flow cytometry performed on CytoFLEX S (Beckman Coulter) **(B)**. Quantification of flow B cytometry gamma-H2AX signal **(C)**.*

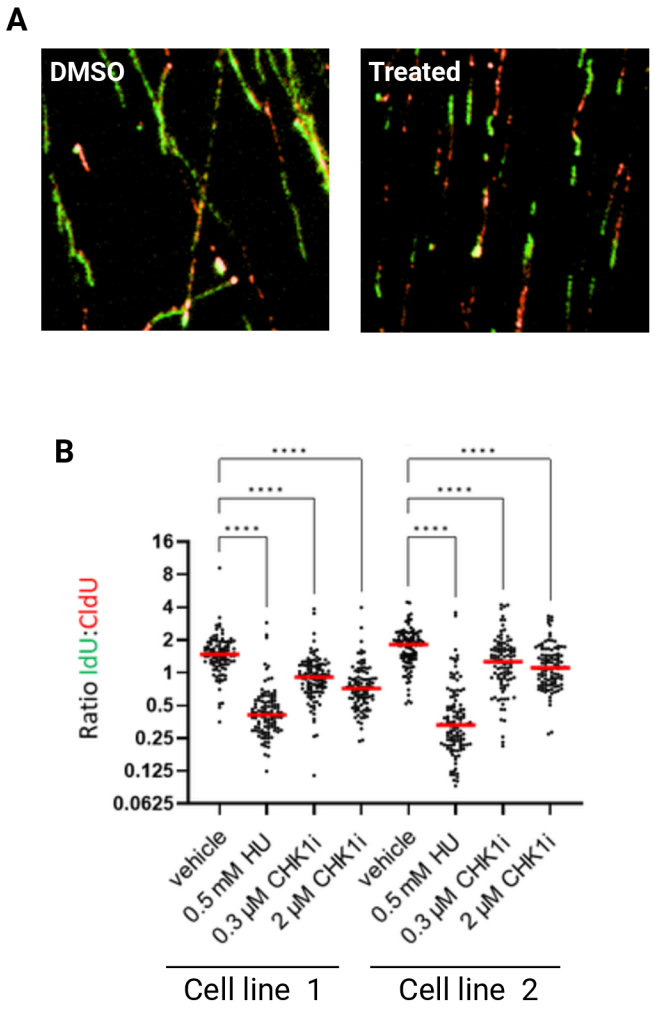


DNA fiber assay

The DNA fiber assay is a powerful tool for studying DNA replication fork dynamics at the single-molecule level. It enables visualization and quantification of the speed, frequency, and stability of DNA replication, providing insights into the mechanisms of action of compounds that induce replication stress. By analyzing fork progression, stalling, and restart, this assay helps us understand how cancer cells maintain genome stability or develop resistance to therapies targeting the replication machinery [2].

Fig 2. Treatment with hydroxiuridine or CHK1 inhibitor reduced fork progression after fork arrest and restart.

Top. Acquisition performed on Olympus BX41 microscope. Bottom. Quantification of IdU/CIdU ratio.



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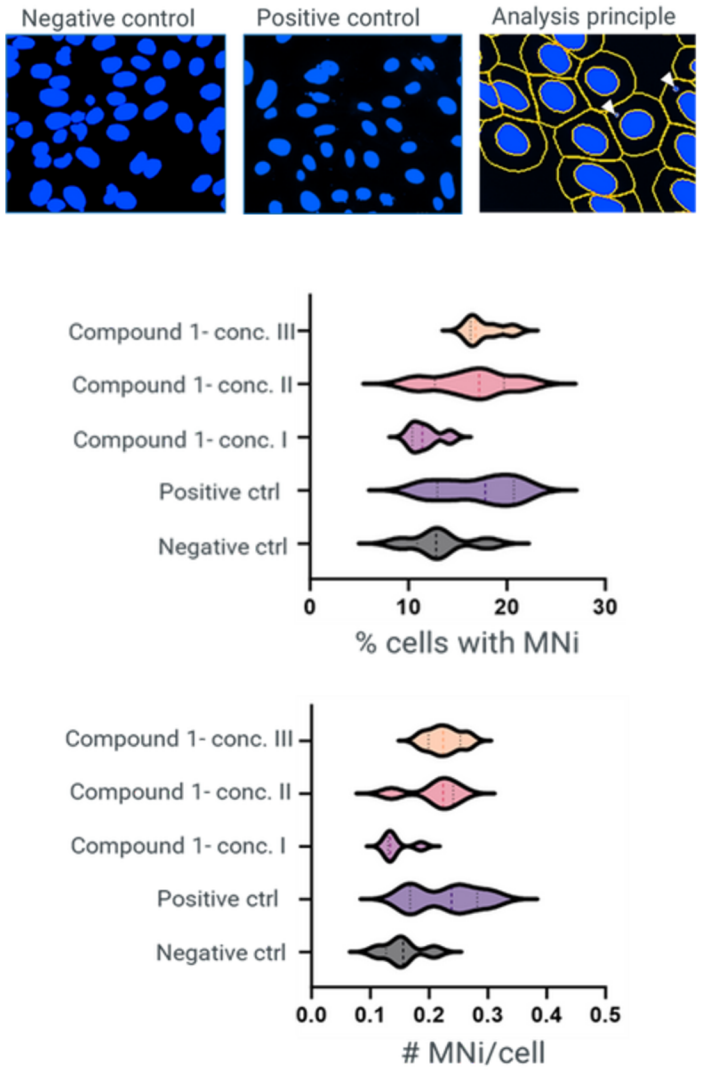
Mitotic perturbations

Micronuclei quantification

Micronuclei (MNi) analysis is a critical tool in genotoxicity research, as it provides a reliable measure of aneugenicity. Additionally, it serves as a marker of genomic instability and DNA damage, phenomena often targeted by cancer therapeutics. In oncology, MNi analysis can offer mechanistic clues e.g. on the regulation of centrosome biology or the spindle assembly checkpoint [3]. To enhance throughput, we have automated micronuclei quantification, enabling the screening of compound libraries for genotoxic or mitotic effects.

Fig 3. Quantification of MNi using high-content imaging.

Analysis method developed on Cytation5 (Agilent). Nuclear count is obtained based on nuclear mask. MNi count is obtained with spot detection analysis within a secondary mask, defined as a ring surrounding a nucleus.

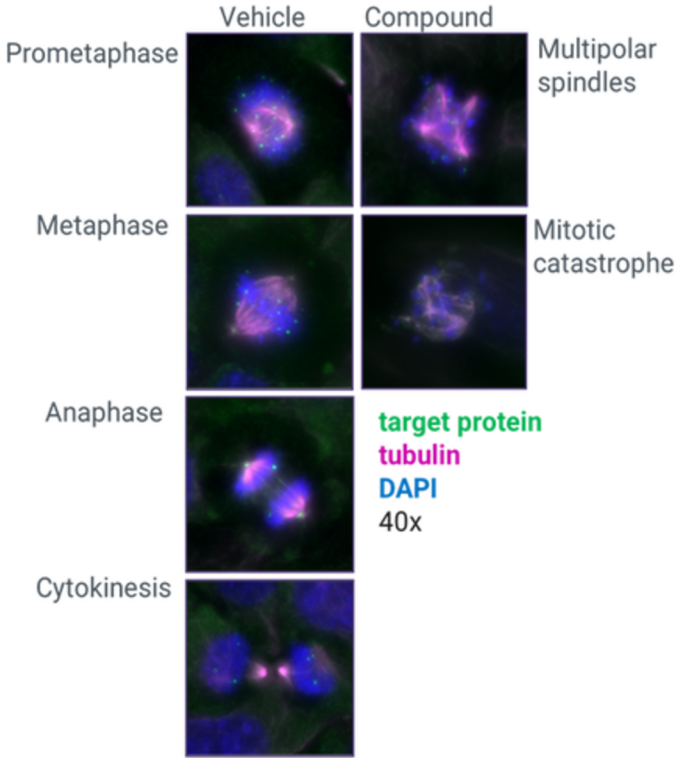


Mitotic aberrations

Analysis of mitotic aberrations can provide crucial insights into cellular processes controlling genome integrity. These errors during mitosis – including improper chromosome segregation, spindle formation, and cytokinesis – can result in aneuploidy and other chromosomal abnormalities [4]. Studying mitotic aberrations helps validate potential therapeutic targets by exploring their roles in pathways regulating mitosis, elucidating disease mechanisms, assessing genotoxicity, and more.

Fig 4. High-magnification fluorescence imaging of cell division process.

Acquisition performed on Nikon Eclipse Ti2-E microscope. Mitotic aberrations are scored after visual analysis of mitotic structures.



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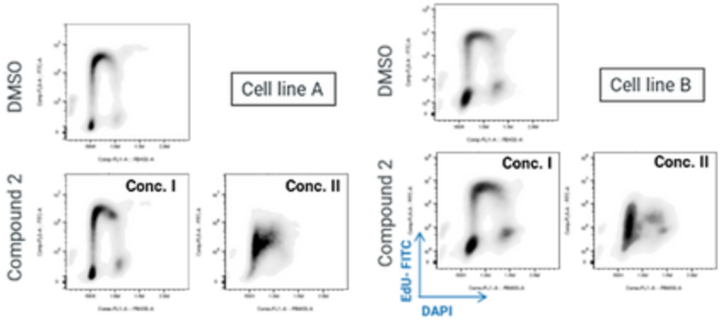
Cell cycle and cell division outcomes

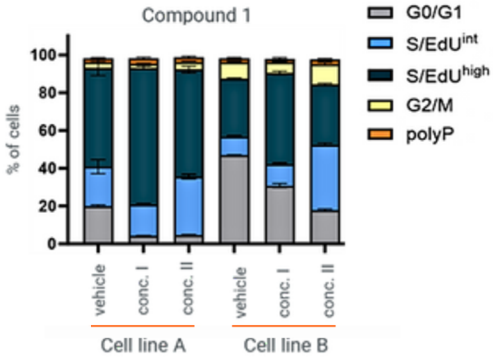
EdU/DAPI cell cycle by flow cytometry

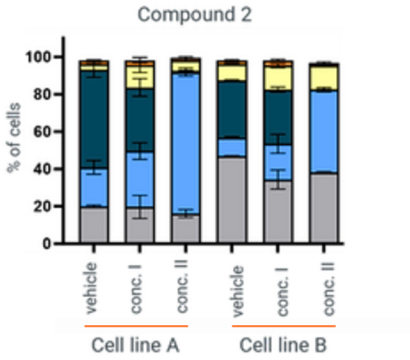
The combination of EdU incorporation and DAPI staining to analyze the cell cycle by flow cytometry provides a snapshot of both DNA synthesis and cell cycle distribution. This approach is particularly useful for mechanistic studies of drugs causing perturbation of cell cycle checkpoints and DNA damage and repair, as well as uncovering related resistance mechanisms. It can be multiplexed with intracellular staining of key markers. This method's detailed resolution makes it an ideal technique for studying drugs targeting cell cycle progression.

Fig 5. EdU/DAPI cell cycle analysis.

Analysis performed on CytoFLEX Flow Cytometer (Beckman Coulter) shows accumulation of cells in early S/full S phase as an effect of treatment with Compound 1 and accumulation of cells in early S phase and decrease in EdU MFI as an effect of Compound 2.





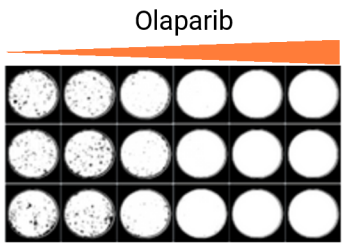


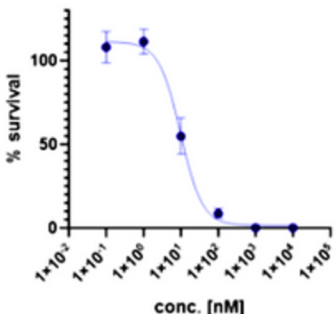
Automated colony formation assay

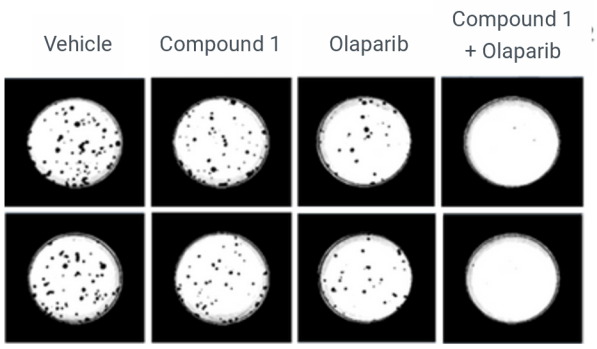
Colony formation or clonogenic assay measures the ability of a single cell to grow into a colony, reflecting long-term survival and reproductive viability. It is considered a gold standard for evaluating effects of DNA-damaging agents, as it is more sensitive in detecting sub-lethal damage affecting the ability of cells to proliferate over time, consequently revealing delayed effects of treatments that might not be apparent in short-term viability assays [5]. In the context of DNA damage repair, the clonogenic assay is an excellent tool for identifying synthetic lethal interactions. All the above make this technique particularly valuable for evaluating the efficacy of anticancer therapies and their combinations.

Fig 6. Olaparib dose-response and synergistic effect with test compound demonstrated in colony formation assay.

Automated acquisition of colonies stained with Janus Green B performed on BioTek Cytation5 (Agilent).







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