

CRISPR knock-out cell lines for drug discovery:

reagent validation, MOA studies,
and assay development



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Using high-quality reagents increases efficiency of drug discovery and development by improving experimental reproducibility and reducing wasted resources.

Knock-out (KO) cell lines have become critically important not only to study biological processes but also as tools to validate other reagents. In this application note, we will describe our cell lines manufacturing process and provide examples of how our KOs have advanced R&D efforts for our customers. Abcam has developed a high-throughput cell editing platform to manufacture CRISPR KO cell lines at scale.

Our platform can also generate CRISPR knock-in cell lines overexpressing target proteins and markers. By using this efficient, automated platform, we can generate engineered cell lines with the consistency and quality required by biomedical and drug discovery researchers.

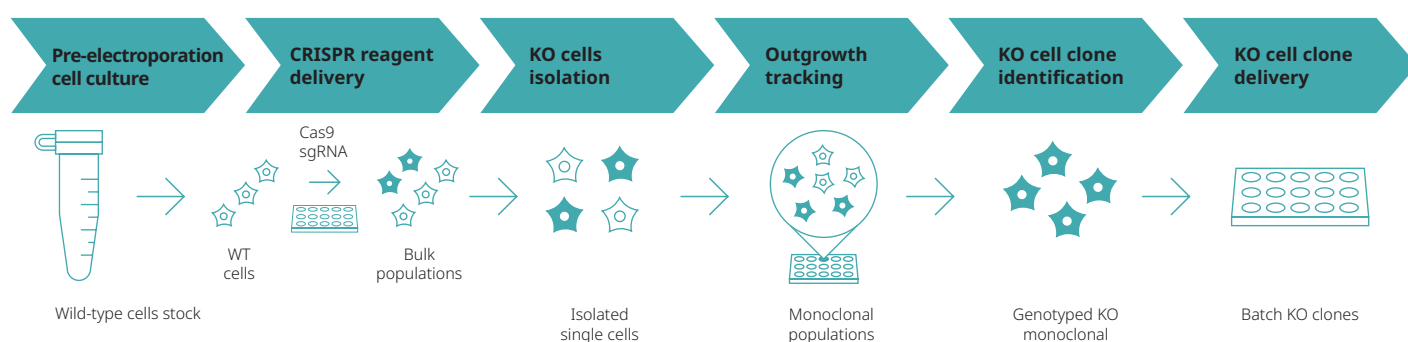


Figure 1. Schematic of abcam's high-throughput cell editing platform.

Our KO cell lines are individually cloned and sequenced. They are characterized, subjected to rigorous quality control measures, and tested for mycoplasma. All products are shipped with wild-type controls.

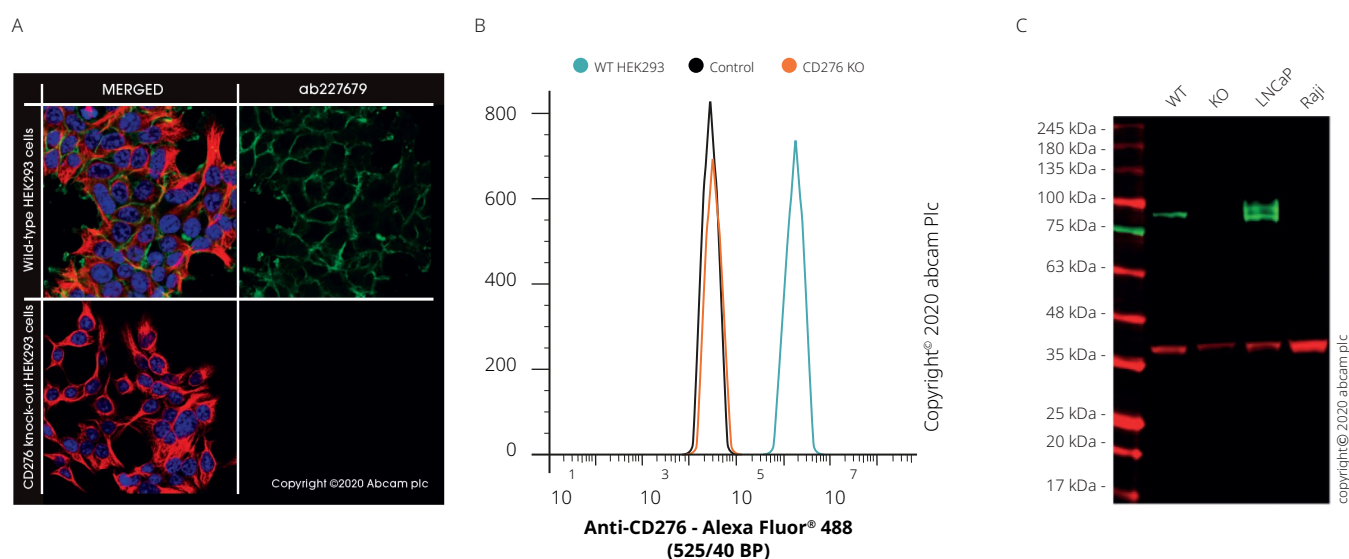
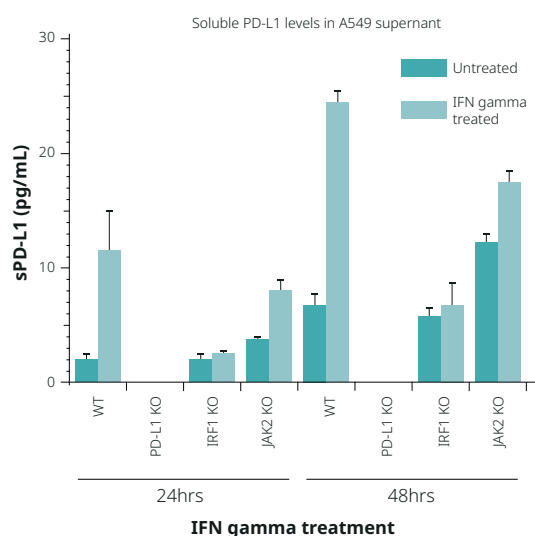


Figure 2. Human CD276 knock-out HEK293T (ab266658) characterization data. Panels: (A) ICC, (B) FC, and (C) WB.

Using KO cell lines for antibody validation

KO cell lines are the gold standard for antibody validation and are utilized to validate our extensive portfolio of antibodies and antibody pairs (used in our immunoassays).

Figure 3. Demonstration that soluble levels of PD-L1 were attenuated by knock-out of IRF1 (ab267042) and JAK2 (ab267113). PD-L1 SimpleStep® ELISA kit (ab277712) was validated with the PD-L1 KO (ab267054).



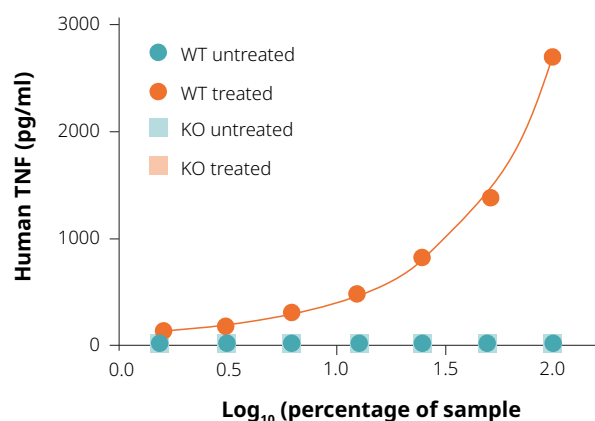
To advance our mission of supplying the life science research community with reliable reagents and tools, we have partnered with YCharOS, an open science company with the mission of characterizing commercially available antibodies.

CASE STUDY

Supporting biomarker assay development

KO cell lines are valuable tools for determining the best antibody pair combinations during immunoassay development. For any given immunoassay, a KO and its isogenic WT pair can be used to validate the specificity of the antibody to its target (Fig 5).

Figure 5. Human TNF-α ELISA kit (ab181421) characterization data of cell culture supernatant. WT THP-1 cells and TNF-α KO THP-1 cells (ab273761) were assessed in duplicate (n=2). Cells were treated with 100 ng/ml LPS for 16 h to induce expression of TNF-α.



Additionally, KO cell lines can be especially vital when developing assays to measure low-abundance protein biomarkers. Such was the case when Roche used abcam's KO cell lines to develop a highly sensitive assay that can reliably differentiate between true signal and background noise at the lower limits of detection. The Roche team was then able to utilize their assay in patient sample testing for potential diagnostics use.



Abcam's knock-out cell line was critical to the successful development and validation of our biomarker ELISA assay.



Elucidating the mechanism of action of a DNA-PK drug candidate¹

DNA-dependent protein kinase (DNA-PK) is an emerging therapeutic target in cancer that plays a critical role in the survival and proliferation of tumor cells². Along with related kinases, ATM and ATR, DNA-PK controls the DNA damage response in vertebrate cells³. In ZL-2201, Zai Lab had a promising small molecule inhibitor against DNA-PK but needed to further elucidate its mechanism of action.

The Zai Lab team characterized the *in vitro* pharmacology of ZL-2201 against PIKK family members and found that it had by far the greatest inhibitory activity against DNA-PK (Fig 4A). They showed that ATM-deficient NCI-H1703 cells demonstrated greater sensitivity to ZL-2201 when compared to ATM-proficient A549 cells (Fig 4B). To confirm this ATM dependency, they used abcam's A549 ATM KO cell line (ab276095) and observed that it was three times more sensitive to ZL-2201 versus the isogenic WT (Fig 4C).

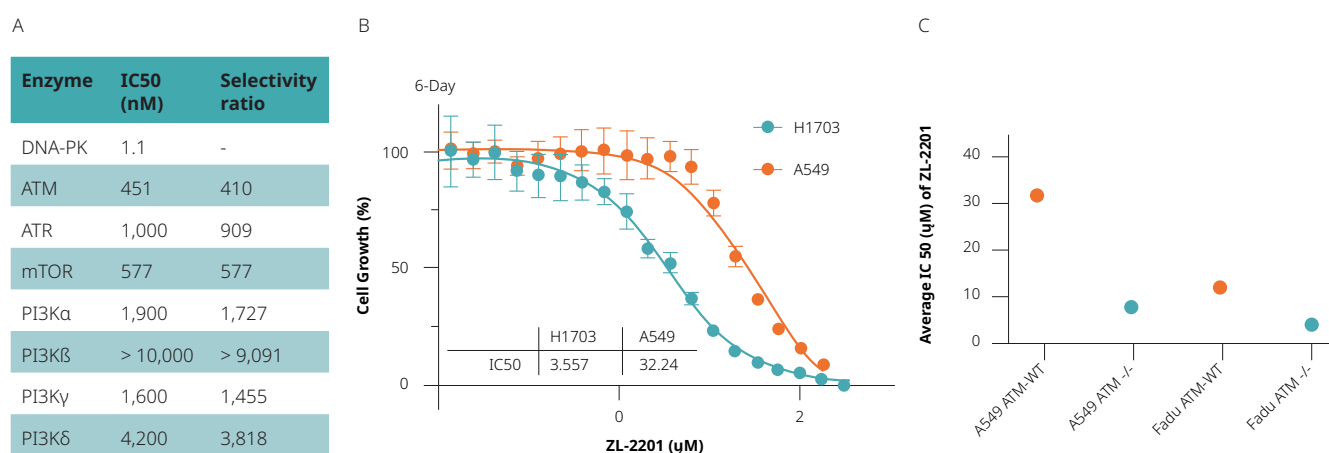


Figure 4. (A) *In vitro* pharmacology of ZL-2201 against PIKK family members. (B) Cellular response to ZL-2201 in ATM-proficient (A549) and ATM-deficient (NCI-H1703) human lung cancer cells. (C) Genetic ablation of ATM enhances the antiproliferative effect of ZL-2201.

1. Lal, et al. Discovery and characterization of ZL-2201, a potent, highly-selective, and orally bioavailable small-molecule DNA-PK inhibitor, AACR (2022)
2. Mohiuddin and Kang, Frontiers in Oncology (2019); 3. Blackford and Jackson, Molecular Cell (2017)

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