# The cGAS-STING screening cascade facilitates new drug discovery

Kang Sun, Peng Liu, Yueqiang Xu, Dongmei Zhao, Tao Li, Qiang Xia, Tiejun Bing
ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, China 100176
Email: bingtj@ice-biosci.com

ICE Innovative CRO+Explorer ICE Bioscience

## Introduction

As one of the important abnormal cytoplasmic DNA monitoring mechanisms, cGAS-STING signaling pathway plays a unique and critical role in host defense against pathogens, tumor immunity, autoimmune diseases, and aging-related inflammation. Sustained activation of the cGAS- STING signaling pathway drives diseases such as autoimmune diseases, aging-associated inflammation, and neurodegenerative pathologies. Therefore, designing drugs that activate/inhibit cGAS or STING signaling may be helpful in anti-cancer, anti-pathogen, anti-inflammatory and other fields. On this basis, we established a series of in vitro screening platforms related to cGAS-STING signaling pathways to facilitate the rapid identification of cGAS/STING agonists/inhibitors.

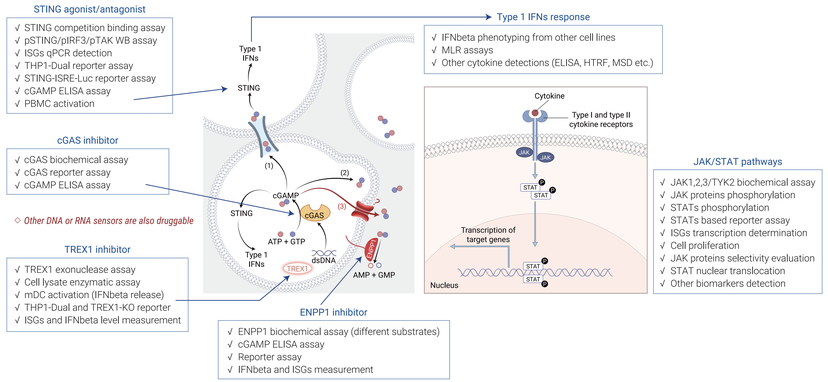


Figure 1. Innate Immunology platform of ICE

## Biochemistry Assay

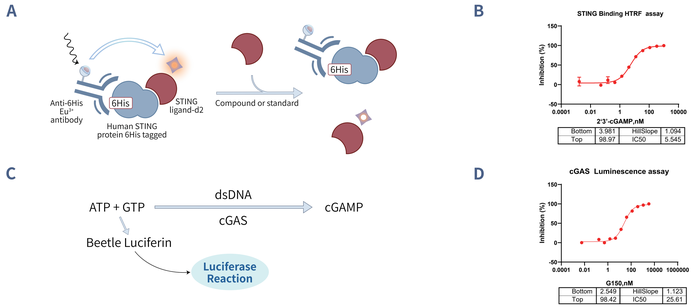


Figure 2. We established in vitro biochemical experiments of cGAS and STING, Here are the experimental principle descriptions and positive reference data of (A, B)STING and (C, D)cGAS.

## cGAS-STING activation/inhibition in reporter cell

### cGAS Atagonist/Agonist Reporter Assay

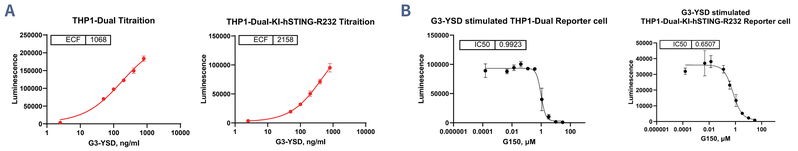


Figure 3. A. The cGAS agonist G3-YSD elicits significant activation effects in THP1-Dual and THp1-dual-ki-Hsting-R232 reporter cells. B. The inhibitory effect of cGAS antagonist G150 on THP1-Dual and THp1-dual-ki-HSTING-R232 cells activated by G3-YSD was observed.

### STING Atagonist/Agonist Reporter Assay

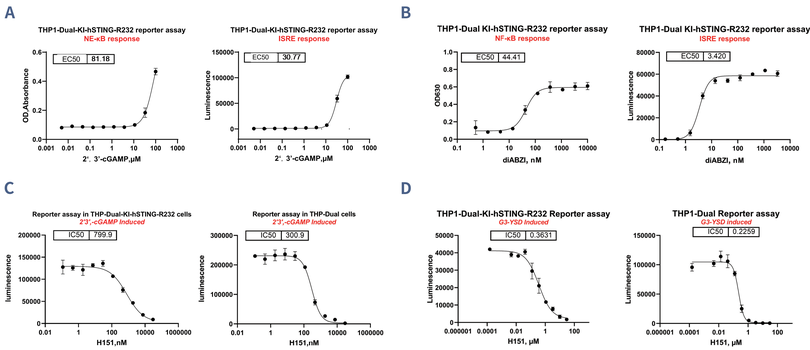


Figure 4. Activation of STING by (A) 2′,3′-cGAMP and (B) diABZI in THP1-Dual-KI-hSTING-R232 reporter cells. Inhibition of STING stimulated by (C) 2′,3′-cGAMP and (D) G3-YSD by H151 in THP1-Dual-KI-hSTING-R232 reporter cells.

## Cytokine release detection in THP-1 cells/human PBMC

### cGAS Atagonist/Agonist Cytokine release Assay

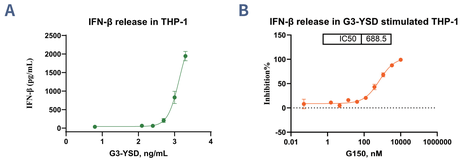


Figure 5. Data on the activity of different compounds, G3-YSD (A) and G150 (B) in activating/inhibiting the release of IFN-β in THP-1 cells.

### STING Agonist Cytokine release Assay

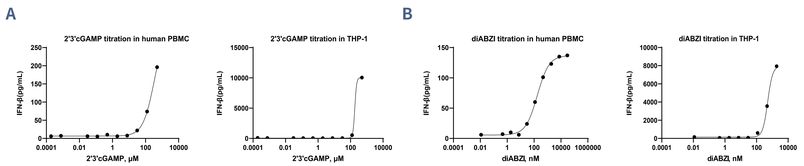


Figure 6. Data on the activity of different agonist, 2′,3′-cGAMP (A) and diABZI (B) in activating the release of IFN-β in human PBMC and THP-1 cells.

## Western blot assay of STING

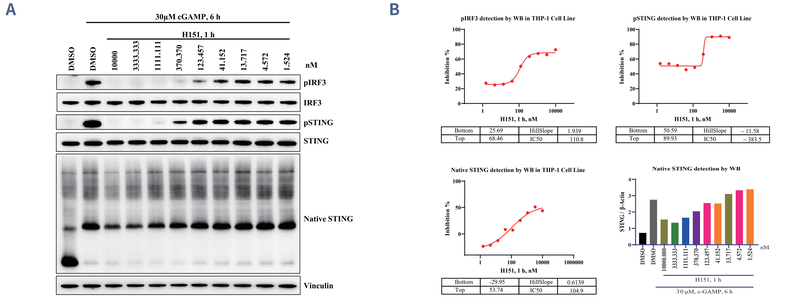


Figure 7. WB analysis of Native STING, pSTING and pIRF3 in H151 treated THP-1 cells.

## Summary

The cGAS-STING pathway has become increasingly important in the field of immunology and cell biology, and in-depth understanding of the molecular mechanism and regulation of this pathway will help to develop new immunotherapies, antiviral drugs and tumor treatment strategies. Our cGAS-STING screening cascade can provide comprehensive compound evaluation, thus serve as an efficient screening platform for new drug discovery.

## References

[1] Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. Nat Rev Immunol. 2021 Sep;21(9):548-569. doi: 10.1038/s41577-021-00524-z. Epub 2021 Apr 8. PMID: 33833439; PMCID: PMC8029610.

[2] Haag SM, Gulen MF, Reymond L, Gibelin A, Abrami L, Decout A, Heymann M, van der Goot FG, Turcatti G, Behrendt R, Ablasser A. Targeting STING with covalent small-molecule inhibitors. Nature. 2018 Jul;559(7713):269-273. doi: 10.1038/s41586-018-0287-8. Epub 2018 Jul 4. PMID: 29973723.