Identification of Novel Inhibitor(s) Targeting NLRP3 Inflammasome Activation

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BACKGROUND

- Cytidine/uridine monophosphate kinase 2 (CMPK2) is a critical node in the NLRP3 inflammasome pathway (**Figure 1**)
  - CMPK2 is involved in mitochondrial DNA (mtDNA) synthesis[1,2]
  - Oxidization of new mtDNA activates NLRP3 inflammasome[1,2]

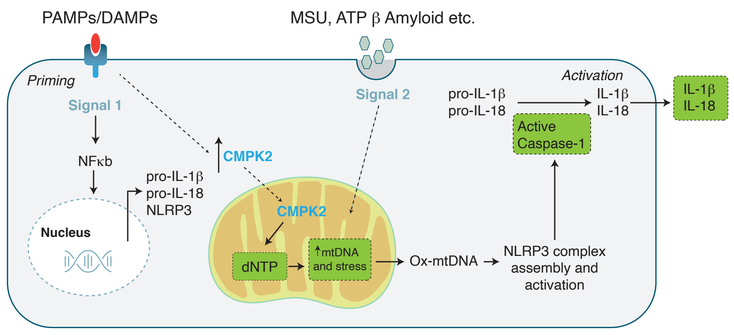
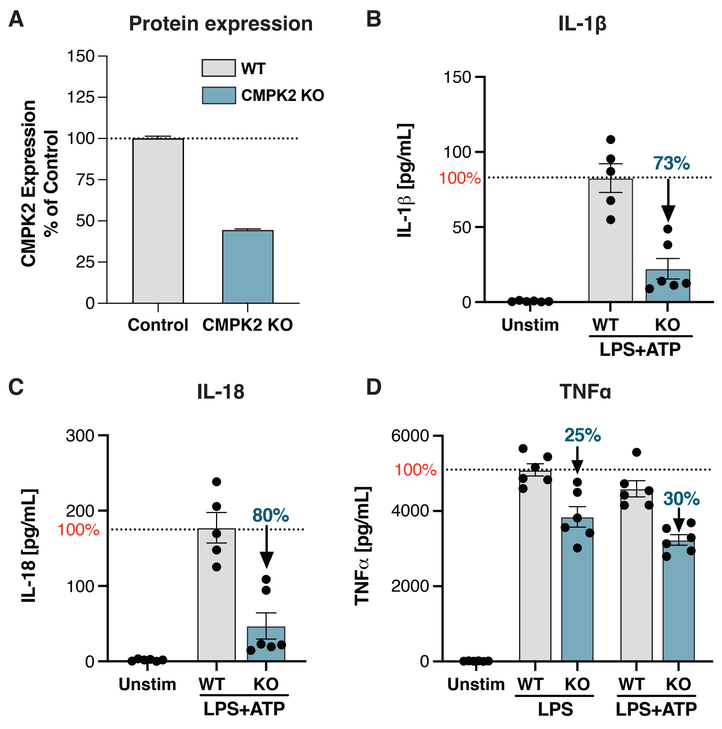


Figure 1. Role of CMPK2 in NLRP3 inflammasome activation

VALIDATION OF CMPK2 ROLE IN INFLAMMASOME PATHWAY

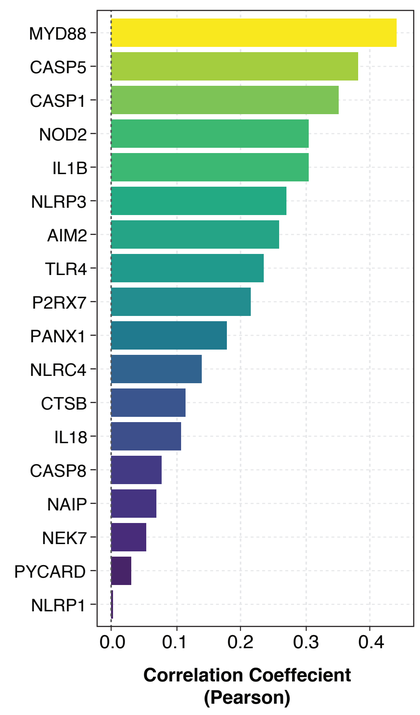
- CMPK2 expression is induced in human primary myeloid cells following treatment with inflammatory stimuli (**Figure 2A**)
- CMPK2 deletion dramatically decreases inflammasome-mediated IL-1β and IL-18 cytokine secretion, and impacts TNFα production (**Figure 2B,C,D**)
- Bioinformatic analysis suggests correlation between CMPK2 and the inflammasome pathway (**Figure 3**)

Figure 2. Validation of CMPK2 KO effect in pro-inflammatory cytokine production



CRISPR knockout of CMPK2 under basal conditions (**A**) in primary human macrophages (polyclonal) leads to substantial decrease in IL-1β (**B**) and IL-18 (C) secretion, but has less of an impact on TNFα secretion (**D**). A similar trend is observed with THP-1 cells (data not shown). The results emphasize the importance of CMPK2 as a key node in the NLRP3 pathway. Data shown here are representative from three donors.

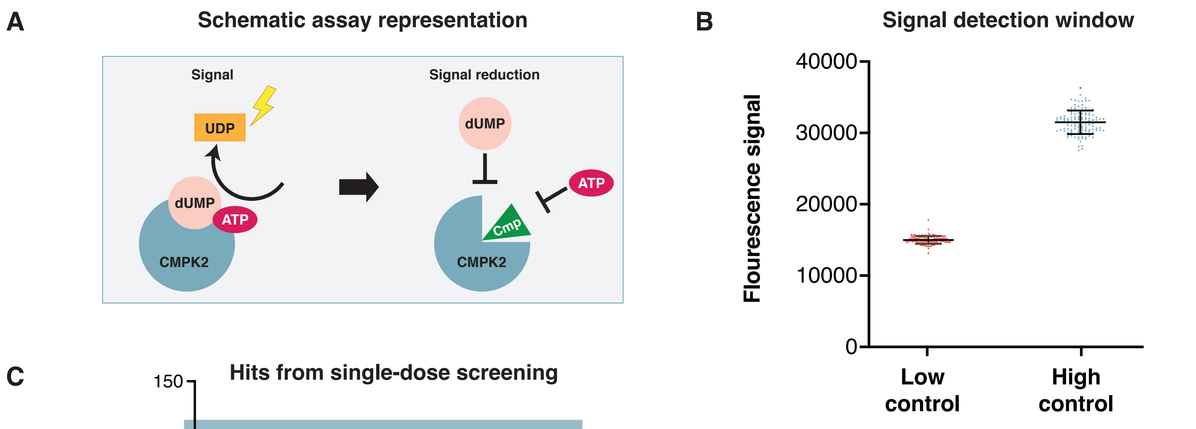
Figure 3. Bioinformatic analysis suggests correlation of CMPK2 with inflammasome pathway genes.



Among genes associated with the inflammasome pathway, caspase-1, IL-1β and NLRP3 correlate with CMPK2. In addition, NLRP3 is more highly correlated with CMPK2 than NLRC4 or NLRP1. Gene expression data is from OmicSoft HumanDisease DB.

SCREENING OF FOCUSED LIBRARIES YIELDED ATTRACTIVE STARTING POINTS

Figure 4. Identification of hits from CMPK2 biochemical enzyme assay.

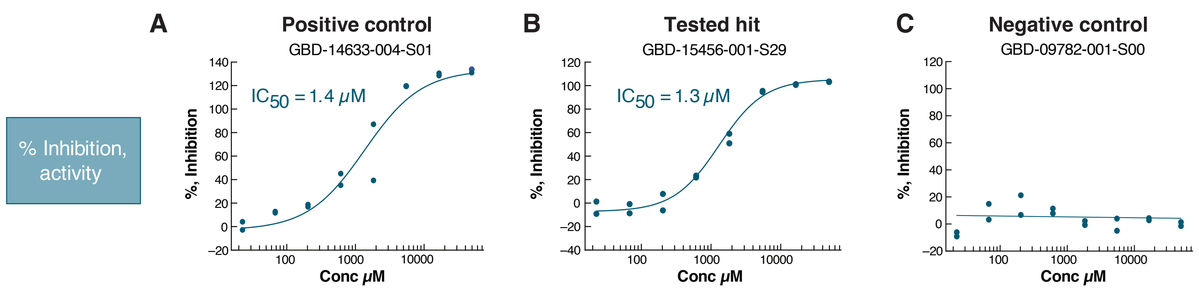


(**A**) The assay robustly detects changes in UDP (signal) production in presence of compound. Inhibitory effect of compounds leads to reduction in signal detected.

(**B**) Evaluation of the CMPK2 biochemical enzymatic assay window. High vs. low controls were included in the screening assay to assure a statistically meaningful assay window. Z' values were also calculated to confirm assay robustness and high-throughput suitability. Typical window and z' values for the assay are 2-fold and >0.7, respectively.

(**C**) Primary focused-library screening (FLS) of three small molecule libraries identified compounds that could inhibit CMPK2 activity by ≥ 50% (highlighted in blue).

Figure 5. Characterization of single-dose hit(s) and controls in dose-response screening.



Representative compounds with sub-micromolar IC_{50} (**B**) were identified. Positive (**A**) and negative (**C**) controls are included in every run as quality assurance.

SCREENING STRATEGY FOR INHIBITORS OF CMPK2 ACTIVITY

- >7000 compounds were screened at a single concentration using a cell-free biochemical assay. Dose-dependent effects of hits were subsequently confirmed.
- A counter-assay was developed to detect signal interference (i.e. false positives)
- Hits identified by FLS were further validated for inflammasome inhibitory effects in primary human cells

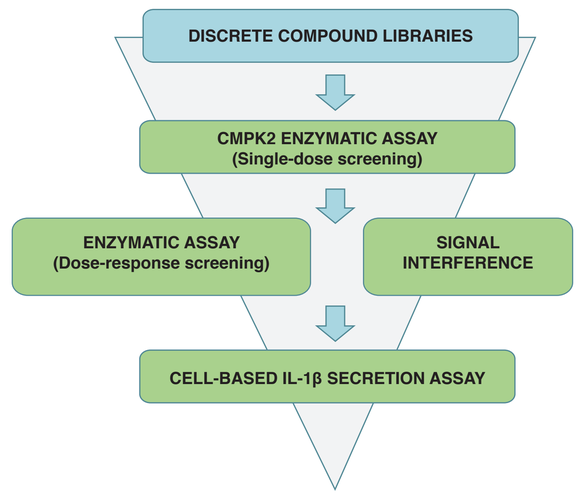


Table 1. Screening of focused libraries results in the identification of tractable chemical matter

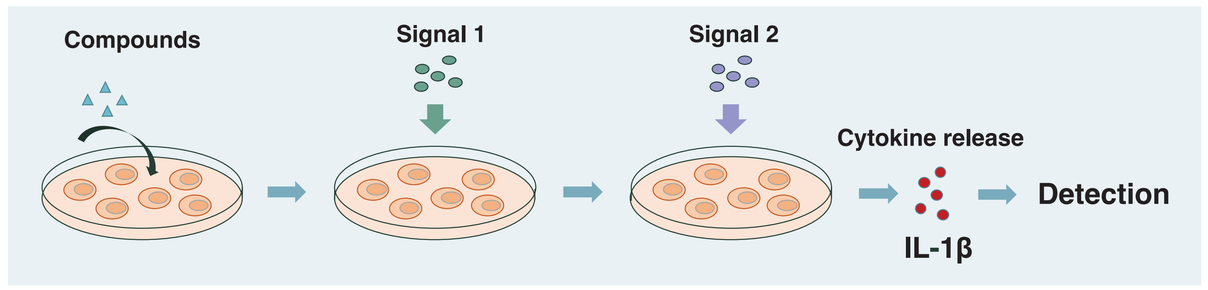
Overview of Primary Screening Results					
Number of compounds screened	7500				
Number of confirmed hits	1940				
Tractable Compound Series	Series 1	Series 2	Series 3	Series 4	Series 5
Current SAR follow-up?	Yes	Yes	Yes	No	No

Screening at a single concentration followed by dose-response re-confirmation resulted in identification of active compounds with diverse structures. Hits with IC_{50} <10 μM are being further characterized in structure activity relationship studies (SAR) and cellular assays.

FUNCTIONAL IMPACT OF HITS ON CYTOKINE PRODUCTION

- Tractable chemical series identified from the biochemical enzymatic assay were subject to further validation in primary human myeloid cells
- Cellular functional assays monitor the inhibitory effects of compounds on inflammatory cytokine production
- Several compounds representing diverse scaffolds demonstrated sub-micromolar potency with regard to inhibition of IL-1β

Figure 6. Hits from the biochemical FLS were active in a human functional assay.



(**A**) The assay robustly detects changes in IL-1β release in the presence of compounds. The inhibitory effect of compounds leads to a reduction in IL-1β detected. (**B**) Representative examples of biochemical selected compounds show inhibition of IL-1β production primary human monocytes. The potency of these inhibitors is in the sub-micromolar range.

SUMMARY

- CMPK2 is a key node in the NLRP3 inflammasome pathway and is inducible in primary human cells
- CMPK2 deletion leads to a dramatic decrease in pro-inflammatory cytokine production
- An initial screening campaign yielded multiple tractable chemical series from structurally diverse compound libraries
- Putative lead compounds reduce inflammasome-associated cytokine production
- Structure-activity relationship studies of compound series with drug-like properties are currently underway

REFERENCES

1. Shimada K, Crother TR, Karlin J, et al. *Immunity* 2012; 36(3): 401-14. 2. Zhong Z, Liang S, Sanchez-Lopez E, et al. *Nature* 2018; 560(7717): 198-203.

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