

Supplementary material

Anti-cancer flavonoids are mouse selective STING agonists

Sujeong Kim^{†1}, Lingyin Li^{†1}, Zoltan Maliga¹, Qian Yin², Hao Wu²

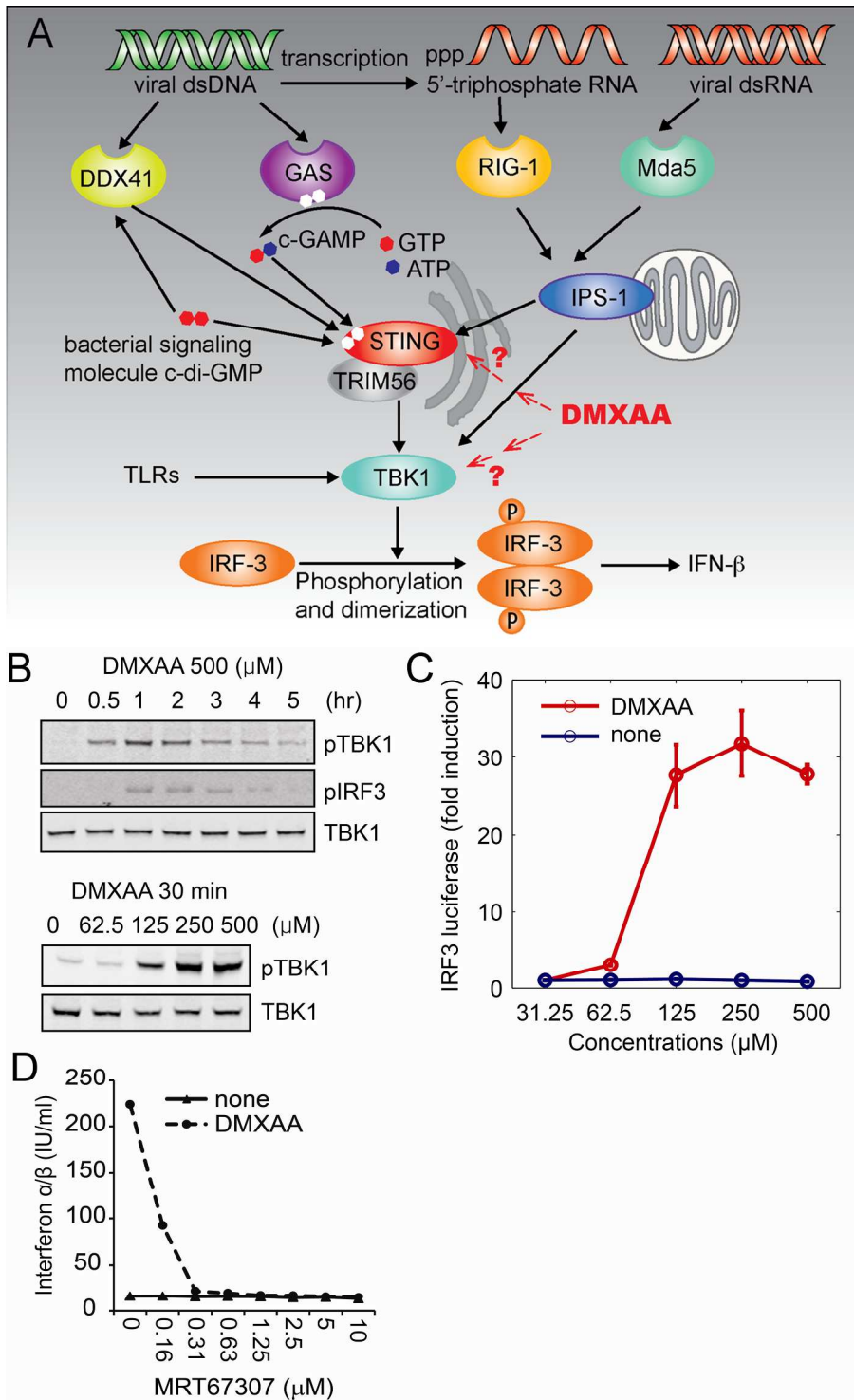
& Timothy J. Mitchison^{1*}

¹Department of Systems Biology, Harvard Medical School, Boston, USA.

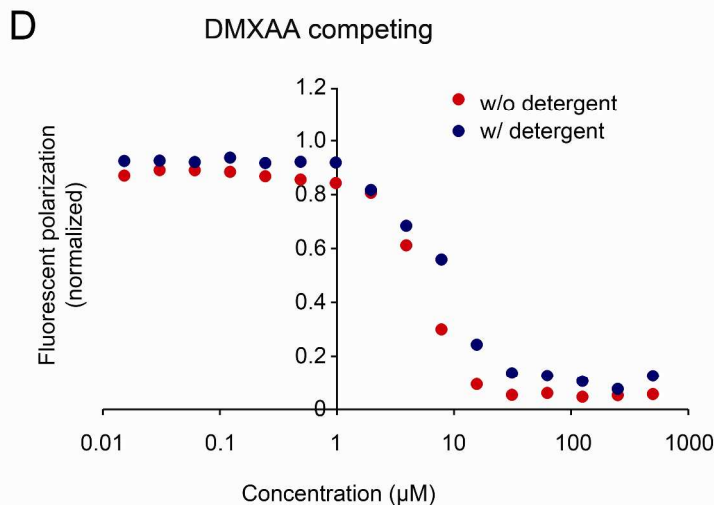
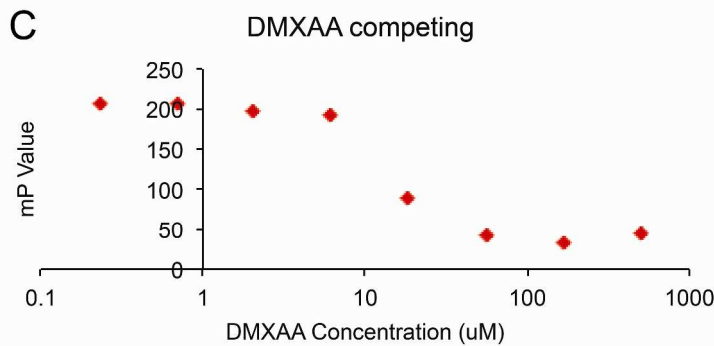
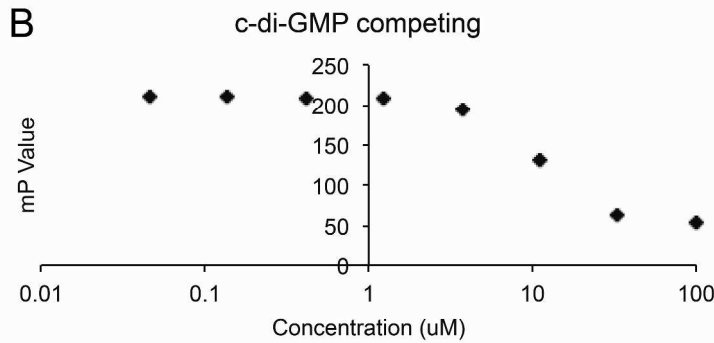
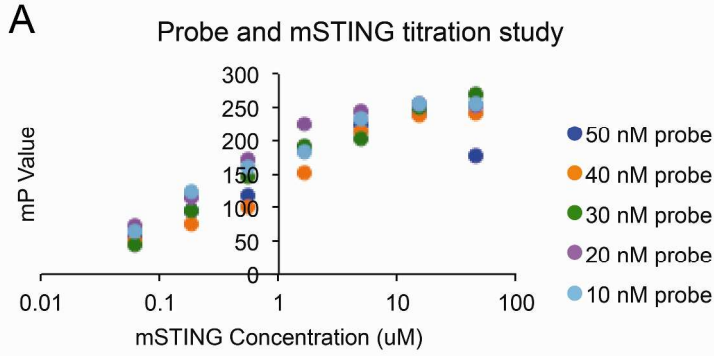
²Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, USA.

† these authors contributed equally to this work

* e-mail: timothy_mitchison@hms.harvard.edu

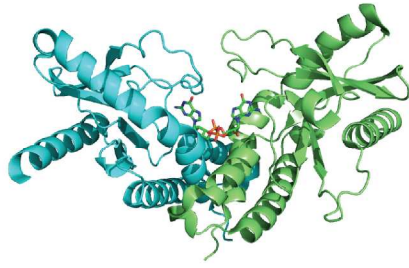


Supplementary figure 1 | Studying the role of DMXAA in the STING mediated innate immune response. (A) STING signaling network and potential targets for DMXAA. (B) Time course and dose studies of DMXAA activated TBK1 phosphorylation in Raw264.7 cells. (C) Dose studies of IRF3 activation in Raw264.7 cells. (D) Dose studies of TBK1 inhibitor, MRT67307 in DMXAA (500 μM) treated Raw264.7 cells.

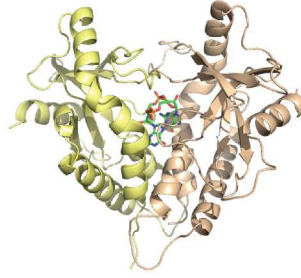


Supplementary figure 2| Competition studies using fluorescence polarization assays. (A) Probe and mSTING titration studies. (B) C-di-GMP competing with the fluorescein-labeled probe. (C) DMXAA competing with the probe. (D) DMXAA competing with the probe with or without detergent (0.01% (v/v) TritonX-100).

A

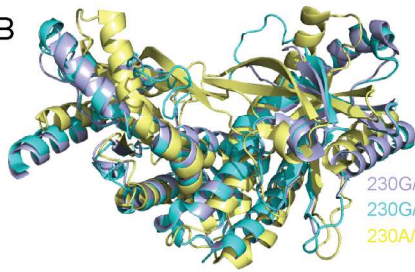


human STING WT CTD:c-di-GMP
PDB: 4F5Y



mouse STING CTD:c-di-GMP
PDB: 4G4D

B



230G/232H PDB:4EF4
230G/232R pDB: 4F5Y
230A/232R PDB:4F5D

Supplementary figure 3 | Investigating the binding ability of DMXAA to hSTING variants. (A) Structural comparison of hSTING-CTD and mSTING-CTD. (B) Structural comparison of natural occurring hSTING variants.