

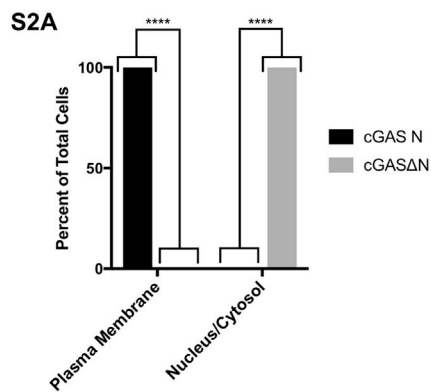
**Figure S1. Related to Figure 1**

S1A) Validation of two cGAS antibodies by western blot analysis with cGAS<sup>+/+</sup> and cGAS<sup>-/-</sup> THP1 lysates. Actin was used as a loading control. The cGAS-CST antibody was used for all other western blots. S1B) Validation of cGAS<sup>+/+</sup> and cGAS<sup>-/-</sup> THP1 cells through measurement of *IFNβ1* expression via qRT-PCR 3 hours after CT-DNA transfection (1 μg/mL). S1C) Confocal micrograph of cGAS<sup>+/+</sup> and cGAS<sup>-/-</sup> THP1 cells using the cGAS-CST antibody. S1D) Confocal micrograph of cGAS<sup>+/+</sup> and cGAS<sup>-/-</sup> THP1 cells using the cGAS-Sigma antibody. S1E) Quantification of endogenous cGAS localization. Ventral membrane indicates the plasma membrane-coverslip contact site. S1F) Western blot analysis to compare total cGAS expression in WT THP1 or WT BMDM as compared to cGAS-HA expressing THP1 or BMDM, respectively. S1G) Confocal micrographs of CST and Sigma cGAS antibodies detecting C-terminally tagged

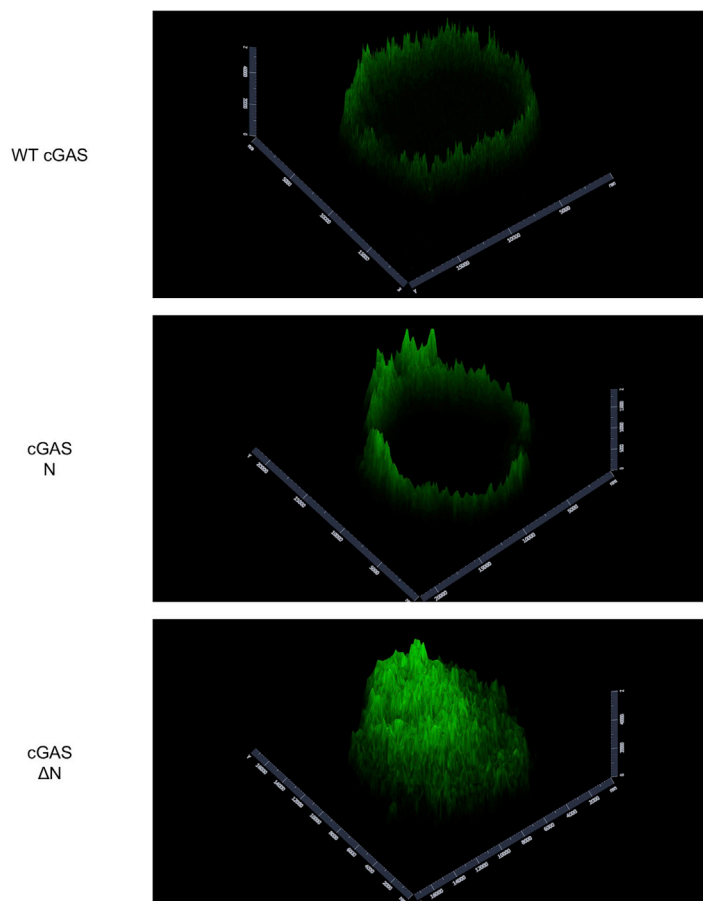
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cGAS-HA in THP1 cells. S1H) Quantification of cGAS-HA localization in various cell lines. S1I) Wide field views of cGAS-HA in various cell lines. S1J) cGAS-HA localization in iBMDMs before or 30 minutes after transfection with 1  $\mu\text{g}/\text{mL}$  CT-DNA. Experiments shown are representative of or averages of  $n = 3$  biological replicates, and statistical analysis was performed using a Student's  $t$  test. Data with error bars represent the mean with SEM. Asterisk coding is as follows: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ; \*\*\*\* $p \leq 0.0001$ .

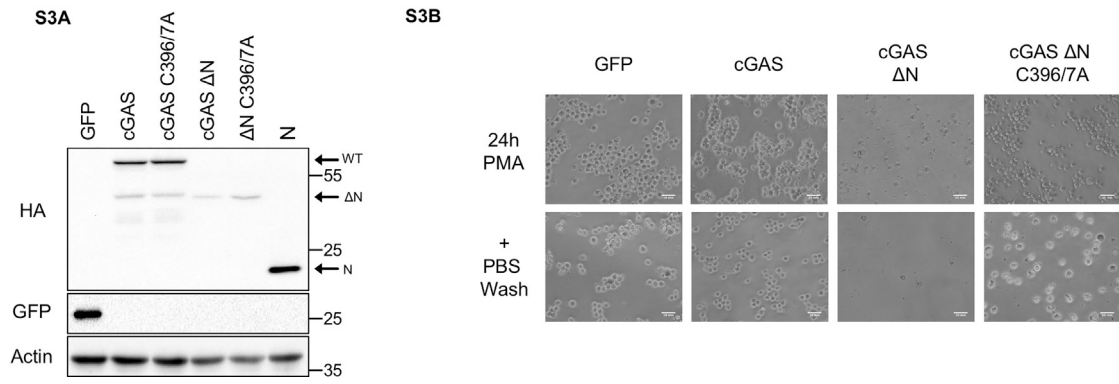


**S2B**



**Figure S2. Related to Figure 2**

S2A) Quantification of cGAS-N-HA and cGAS $\Delta$ N-HA localization stably expressed in iBMDMs. S2B) Pseudo-3D renderings of cGAS, cGAS N, and cGAS $\Delta$ N expressed in iBMDMs. A 2D confocal micrograph of each construct is on the x and y axes, while the pixel intensity is plotted on the z axis. Images were created using Zen software. Experiments shown are representative of or averages of  $n = 3$  biological replicates, and statistical analysis was performed using a Student's t test. Data with error bars represent the mean with SEM. Asterisk coding is as follows: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ; \*\*\*\* $p \leq 0.0001$ .



**Figure S3. Related to Figure 4**

S3A) western blot analysis of expression of cGAS mutant panel stable expression in THP1 cells. S3B) Phase contrast microscopy of THP1 cell lines treated overnight with 50ng/mL PMA before and after a gentle PBS wash. Experiments shown are representative of  $n = 3$  biological replicates.