



Figure 1, Figure Supplement 1. Characterization of clonal cGAS KO HeLa cells and microscopy conditions for cGAS visualization.

(A) HeLa cells were transduced with LentiCRISPR encoding H1 non-targeting control gRNA or cGAS-targeted gRNA, cloned by limiting dilution, and tested for production of cGAMP in cell lysates four hours after transfection of calf thymus DNA (CT-DNA). (B) Lysates from H1 control and cGAS KO clonal HeLa cells were separated into cytosol (C) and nuclear pellet (NP), and then blotted for endogenous cGAS. (C) H1 control and cGAS KO HeLa cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained for endogenous cGAS. (D) cGAS KO HeLa cells were transduced with pSLIK lentivirus encoding doxycycline-inducible GFP-mouse cGAS (mcGAS), and then treated with doxycycline to induce GFP-mcGAS expression. Cells were then stained with DRAQ5 and visualized with an imaging flow cytometer (Amnis ImageStream). Representative images shown in D. (E) Analysis of ImageStream data for thousands of individual cells showing percent nuclear and cytosolic localization of GFP-mcGAS.