## **Supporting Information**

## Orzalli et al. 10.1073/pnas.1424637112

DNAS



**Fig. S1.** Localization of cGAS in human cells. (*A*) Localization of endogenous cGAS in HFF and NOK cells. Cells were grown on coverslips and stained with cGAS and Lamin B2 antibodies, the latter to define nuclei. (*B*) Localization of cGAS in HEK293 cells stably expressing Flag-human cGAS. Cells were fixed and stained with Flag and Lamin-B1 antibodies. (*C*) Immunofluoresence of endogenous cGAS and IF116 in HFF following siRNA-mediated depletion. Cells were grown on coverslips and siRNA-depleted for 72 h and then stained with cGAS and IF116 antibodies. Images were captured with an Olympus Fluoview Confocal Microscope. (Magnification: 63×.)



**Fig. 52.** Intracellular cGAS and IFI16 relocalization following DNA transfection. (*A*) HFF cells were transfected with 200 ng of LabelIT Cy3 plasmid delivery control (Mirus) and fixed and stained for cGAS and IFI16 at indicated time points. (*B*) Quantification of cGAS and IFI16 foci/filaments following DNA transfection. One hundred cells were examined and foci or filaments were visually scored for the presence or absence of IFI16 or cGAS. Results are an average of three independent experiments and are presented as a percentage of total foci examined. Images were captured by Olympus Fluoview Confocal Microscope. (Magnification: 63×.)

## **Bipartite NLS**

## Monopartite NLS

CGAS	(Homo sapiens)	21	KASARNARGAPMDPTESPAAPEAALPKAGKF	51	295	DVIMKRKRGGS	300
CGAS	(Mus musculus)	12	RAKKPSAKRAPTQPSRT-RAHAESC	35	281	DVSVEKEKPGS	291

Fig. S3. Results of cGAS nuclear localization sequences (NLS) prediction. Putative bipartite and monopartite NLS within human and mouse cGAS were identified by NLSmapper (1). Mouse and Human cGAS sequences were aligned with uniprot alignment software. Lysine and arginine residues in human cGAS are highlighted in red.

1. Kosugi, et al. (2009).