Supporting Information

Cingöz and Goff 10.1073/pnas.1720431115

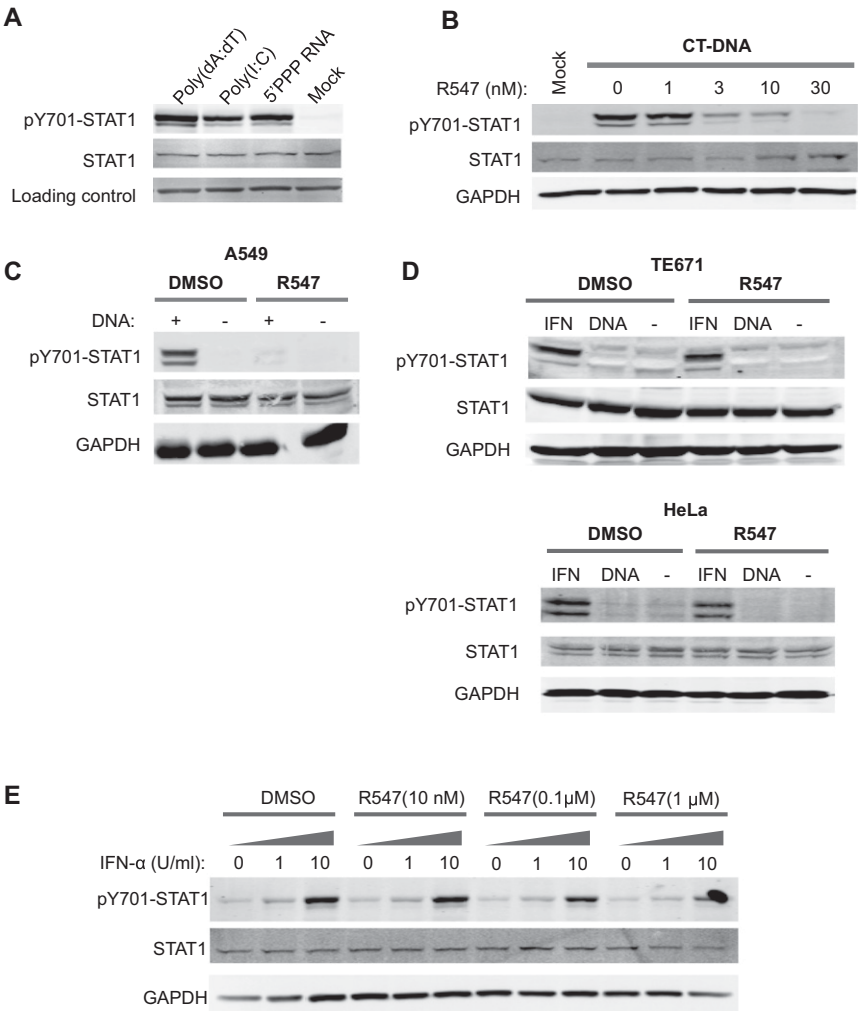

Fig. S1. The effect of CDK inhibition on STAT activation. (*A*) THP-1 cells were transfected with 4 μg/mL of poly(dA:dT), poly(I:C), 5′ triphosphate RNA (5′PPP RNA), or mock. Total cell lysates were analyzed for STAT1 activation by Western blot 2 h posttransfection. (*B*) THP-1 cells were treated with the indicated amounts of the CDK inhibitor R547 (0–30 nM) and challenged with 4 μg/mL DNA transfection. Lysates were analyzed as in *A*. (*C*) A549 cells were transfected with poly(I:C) (4 μg/mL) or mock, in the presence of R547 (3 nM) or DMSO. Lysates were analyzed as in *A*. (*D*) TE671 and HeLa cells were treated with IFN-α (10 U/mL) or transfected with DNA (4 μg/mL) in the presence of R547 (10 nM) or DMSO. Lysates were analyzed as in *A*. (*E*) THP-1 cells were challenged with different concentrations of IFN-α (0, 1, 10 U/mL) in the presence of different concentrations of R547 (0, 10 nM, 100 nM, 1 μM). Lysates were collected 30 min later and analyzed by Western blot. Results are representative of at least two independent experiments.

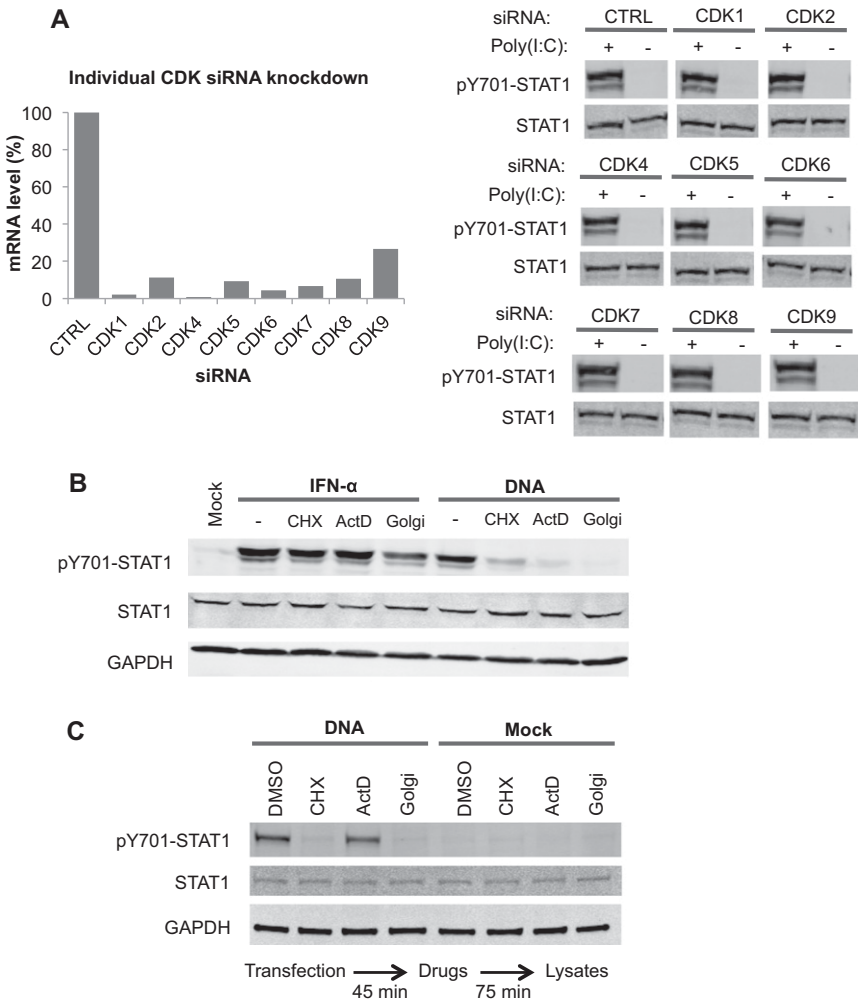

Fig. S2. Individual CDK knockdown has no effect on nucleic acid-induced STAT1 activation. (*A*) NHDF cells were transfected with siRNAs against CDKs 1, 2, 4, 5, 6, 7, 8, or 9 individually. Knockdown levels were determined at the RNA level by qRT-PCR and normalized to GAPDH (*Left*). Knockdown cells were transfected with 4 μg/mL poly(I:C) and analyzed by Western blot 3 h later (*Right*). (*B*) THP-1 cells pretreated with cycloheximide (CHX), actinomycin D (ActD), BD GolgiPlug (Golgi), or DMSO (-) were treated with IFN-α, or transfected with DNA (4 μg/mL). Lysates were collected 2 h later and analyzed by Western blot. (*C*) Experiment performed as in *B*, except THP-1 cells were first transfected with DNA or mock, and the inhibitors were added 45 min later. Lysates were collected 75 min later and analyzed by Western blot.

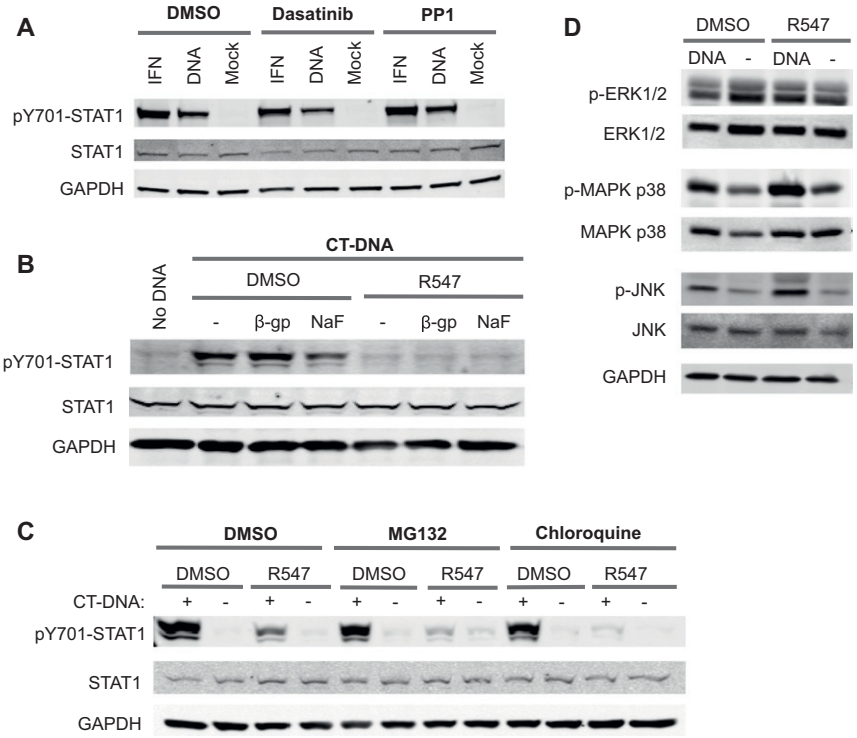

Fig. S3. CDK involvement in STAT activation is independent of phosphatases, proteasomes, lysosomes, or the MAPK pathway. (*A*) THP-1 cells were treated with dasatinib or PP1 (both at 100 nM), and challenged with IFN-α (25 U/mL) or DNA transfection (4 μg/mL). Total cell lysates were collected 2 h later and analyzed by Western blot. (*B*) THP-1 cells were treated with 1 mM of Ser/Thr phosphatase inhibitors β-glycerolphosphate (β-gp) or sodium fluoride (NaF), and then transfected with DNA (4 μg/mL) in the presence of R547 (10 nM) or DMSO. Lysates were analyzed by Western blot 2 h later. (*C*) THP-1 cells were treated with the proteasome and lysosome inhibitors MG132 or chloroquine, transfected with DNA (4 μg/mL) in the presence of R547 (10 nM) or DMSO. Lysates were analyzed by Western blot 2 h later. (*D*) THP-1 cells were treated with R547 (10 nM) or DMSO, and transfected with DNA (4 μg/mL) or mock. Lysates were collected 2 h posttransfection, analyzed by Western blot with antibodies against total and phosphorylated MAPK pathway components: ERK1/2, p38, and JNK. Results are representative of at least two independent experiments.

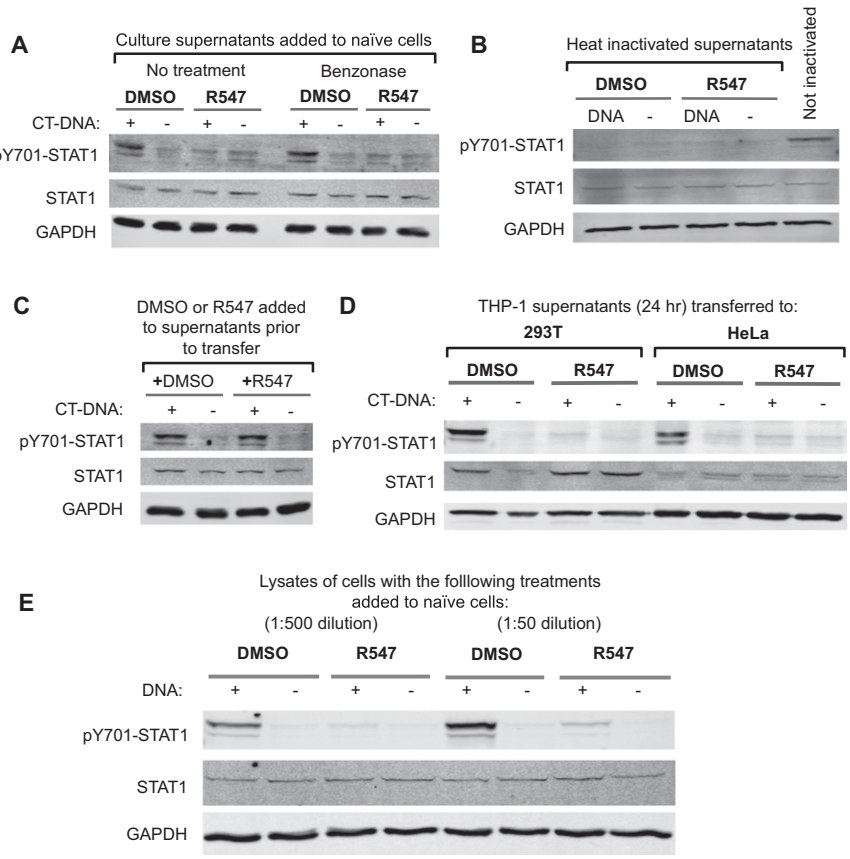

Fig. S4. CDK inhibition prevents the production of a cytokine in culture supernatants. (*A*) THP-1 cells were treated with R547 (10 nM) or DMSO, and transfected with DNA (4 μg/mL) or mock. Supernatants were collected 4 h later, treated with benzonase or left untreated, and applied to naïve THP-1 cells. Lysates were analyzed by Western blot 2 h after transfer. (*B*) Experiment was performed as in *A* except supernatants were heat inactivated at 95 °C for 10 min and allowed to cool to room temperature before their application to recipient cells. (*C*) Culture supernatants from DNA- or mock-transfected THP-1 cells (without drugs) were collected 4 h after transfection. R547 or DMSO were then added to these supernatants, and they were applied to recipient cells. Lysates were analyzed as in *A*. (*D*) Experiment performed as in *A*, where the recipient cells were HEK293T or HeLa. (*E*) THP-1 cells were treated with R547 (10 nM) or DMSO, and transfected with DNA (4 μg/mL) or mock. Cells were lysed, lysates diluted 1:50 or 1:500 in media, then added onto recipient cells. Lysates of the recipient cells were analyzed by Western blot 1 h later. Results are representative of at least two independent experiments.

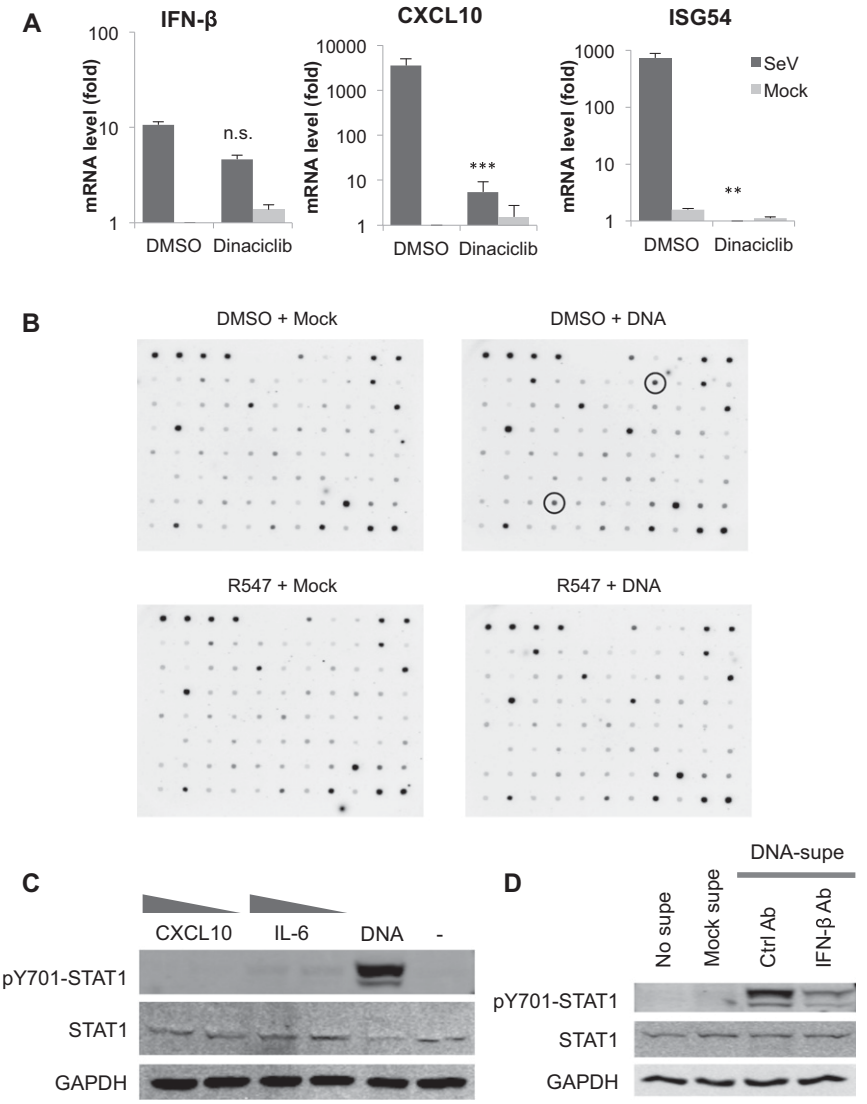

Fig. S5. The effect of CDK inhibition on the expression of IFN-β, ISGs and other cytokines. (*A*) THP-1 cells were treated with DMSO or dinaciclib (10 nM) and infected with SeV. Total RNA was harvested 4 h postinfection, and IFN-β, CXCL10, and ISG54 mRNA levels were determined by qRT-PCR (normalized to GAPDH). (*B*) THP-1 cells were transfected with DNA or mock, in the presence or absence of R547 (10 nM). Supernatants were collected 4 h later and incubated with a membrane containing a human cytokine antibody array. Circles indicate signals corresponding to IL-6 and CXCL10. (*C*) THP-1 cells were treated with recombinant CXCL10 (0.2–1 μg/mL) or IL-6 (0.1–0.5 μg/mL). Lysates were collected 2 h after treatment and analyzed by Western blot. (*D*) Supernatants from DNA-transfected THP-1 cells were preincubated with an antibody against IFN-β for 1 h and added to recipient cells. Lysates were collected 1 h later and analyzed by Western blot. Untreated supernatants from DNA or mock-transfected cells were included as controls. Statistical significance was determined by unpaired Student's *t* test, compared with vehicle treated samples: $^{**}P < 0.01$, $^{***}P < 0.001$, n.s. not significant.

Table S1. CDK inhibitors used in this study

Inhibitor name	Target	IC50 Value* (nM)	Reference
R547	CDK1	2 (K_i)	Depinto et al. (8)
	CDK2	3 (K_i)	
	CDK4	1 (K_i)	
Dinaciclib (SCH727965)	CDK1	3	Parry et al. (12)
	CDK2	1	
	CDK5	1	
	CDK9	4	
AZD5438	CDK1	16	Byth et al. (13)
	CDK2	6	
	CDK9	20	
SNS-032 (BMS-387032)	CDK2	38–48	Conroy et al. (14)
	CDK7	62	
	CDK9	4	
Palbociclib (PD-0332991)	CDK4	9–11	Fry et al. (15)
	CDK6	15	

*Concentrations for R547 are given as K_i values.

Table S2. Oligonucleotides used in the study

Primers, probe set or oligonucleotide		
Target	Sequence or assay ID	Source
SYBR green primers		
IFN-β (F)	AGGACAGGATGAACTTTGAC	Current paper
IFN-β (R)	TGATAGACATTAGCCAGGAG	Current paper
CXCL10 (F)	TGGCATTCAAGGAGTACCTC	Current paper
CXCL10 (R)	TTGTAGCAATGATCTCAACACG	Current paper
ISG54 (F)	TGTTCCATTCTTGCCAGCCTC	Current paper
ISG54 (R)	CAGTTGTTTCGCTACAGGAGTAAGC	Current paper
MX1 (F)	AGACAGGACCATCGGAATCTTG	Current paper
MX1 (R)	TTCTTCAGGTGGAACACGAGG	Current paper
IFIT1 (F)	CCTGGCTAAGCAAAACCCTG	Current paper
IFIT1 (R)	CATCGTCATCAATGGATAACTCCC	Current paper
ISG15 (F)	TCTGAACATCCTGGTGAGGAATAAC	Current paper
ISG15 (R)	AAGGTCAGCCAGAACAGGTCGTC	Current paper
GAPDH (F)	TCGGAGTCAACGGATTTG	Current paper
GAPDH (R)	GCATCGCCCCACTTGATT	Current paper
CCL5 (F)	CTCGCTGTCATCCTCATTGC	Current paper
CCL5 (R)	TACTCCTTGATGTGGGCACG	Current paper
CDK1 (F)	ACCTATGGAGTTGTGTATAAGG	Sigma
CDK1 (R)	GACTGACTATATTTGGATGACG	Sigma
CDK2 (F)	TGTTATCGCAAATGCTGC	Sigma
CDK2 (R)	TCAAGAAGGCTATCAGAGTC	Sigma
CDK4 (F)	AGAATCTACAGCTACCAGATG	Sigma
CDK4 (R)	AGAGTTTCCACAGAAGAGAG	Sigma
CDK5 (F)	CCTGAGATTGTAAAGTCATTCC	Sigma
CDK5 (R)	CCCCATTCCTGTTTATTAGC	Sigma
CDK6 (F)	GGATATGATGTTTCAGCTTCTC	Sigma
CDK6 (R)	TCTGGAAACTATAGATGCGG	Sigma
CDK7 (F)	CTAGGATGTATGGTGTAGGTG	Sigma
CDK7 (R)	AAGGAACCCTTAGAAGTAACTC	Sigma
CDK8 (F)	AGCAAGGCATTATACCAAAG	Sigma
CDK8 (R)	CTTTATCTGCAGGAAATCCC	Sigma
CDK9 (F)	TGAGATTTGTCGAACCAAAG	Sigma
CDK9 (R)	TTTCTGTGGATGTAGTAGAGG	Sigma
Taqman primer/probe sets		
IFN-β	Hs01077958_s1	Thermo Fisher
CXCL10	Hs00171042_m1	Thermo Fisher
ISG54	Hs00533665_m1	Thermo Fisher
Actin	Hs99999903_m1	Thermo Fisher
GAPDH	4310884E	Thermo Fisher
siRNAs		
CDK1	SASI_Hs01_00044053	Sigma
CDK2	SASI_Hs01_00060175	Sigma
CDK4	SASI_Hs01_00122489	Sigma
CDK5	SASI_Hs01_00159314	Sigma
CDK6	SASI_Hs01_00048790	Sigma
CDK7	SASI_Hs01_00214780	Sigma
CDK8	SASI_WI_00000018	Sigma
CDK9	SASI_Hs01_00112403	Sigma
Control	SIC001	Sigma
Immunostimulatory oligonucleotides		
526	GGGTACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACAGGG	Herzner et al. (1)
529	GGGTGTAGATCATGTACAGATCAGTCATAGATCACTAGTAGATCTGTAGGG	Herzner et al. (1)

1. Herzner AM, et al. (2015) Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. *Nat Immunol* 16:1025–1033.