Supplemental Data

SLC19A1 is an importer of the immunotransmitter cGAMP

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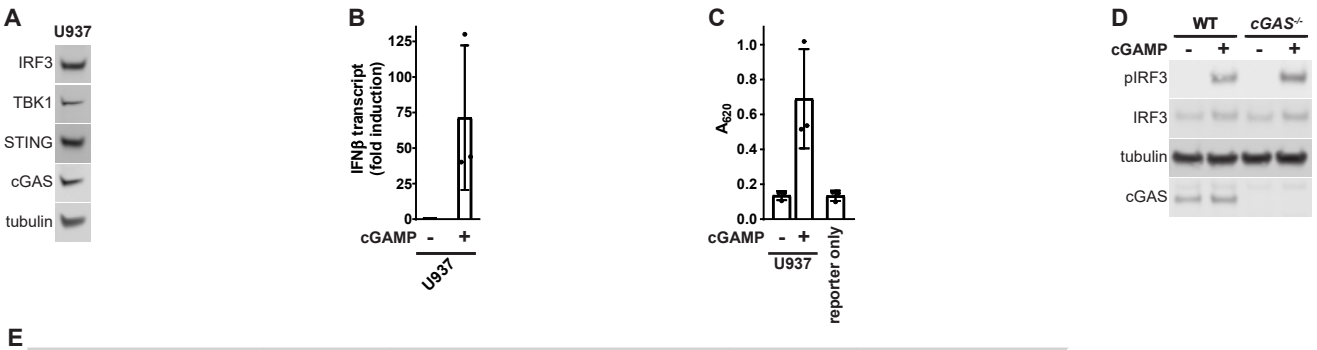

E

	LD_{50} Screen			LD_{30} Screen		
	casTLE Effect	casTLE Score	casTLE p-value (Maximum Estimate)	casTLE Effect	casTLE Score	casTLE p-value (Maximum Estimate)
TMEM173	8.6	537	9.08×10^{-6}	4.8	459	1.00×10^{-5}
IRF3	8	507	9.08×10^{-6}	4.7	372	1.00×10^{-5}
TBK1	7.6	465	9.08×10^{-6}	4.3	308	1.00×10^{-5}
SLC19A1	4.8	187	9.08×10^{-6}	3.5	226	1.00×10^{-5}

Figure S1. A Genetic Screen Identifies Putative Components of the Extracellular cGAMP-STING Pathway, Related to Figure 1.

(A) STING pathway protein levels in U937 cells. Cells were probed for TBK1, IRF3, STING, and cGAS levels using Western blots.

(B) Production of IFN-β mRNA in response to cGAMP treatment. U937 cells were treated with 100 μM cGAMP for 6 h. Total RNA was isolated and fold induction of IFN-β over untreated cells was quantified by RT-qPCR (n = 3 biological replicates).

(C) Production of IFN-β protein in response to cGAMP treatment. U937 cells were treated with 100 μM cGAMP for 6 h. Supernatant containing secreted IFN-β was then collected and added to HEK-Blue IFN-α/β reporter cells to quantify IFN-β protein levels (n = 3 biological replicates).

(D) Effect of cGAS on extracellular cGAMP signaling. U937 WT and *cGAS*$^{-/-}$ cells were treated with 100 μM cGAMP for 90 min and probed for pIRF3.

(E) Table showing the casTLE effects, scores, and p-values for the STING pathway genes *TMEM173*, *IRF3*, and *TBK1*, as well as the candidate cGAMP importer *SLC19A1*. All values were calculated using the methods described in Morgens *et al.,* 2016.

For (B) and (C) data are shown as mean ± SD.

A

clone	allele	mutations in *SLC19A1* gene
SLC19A1$^{-/-}$ #1	1	Δ10548-10554
	2	Δ10548-10554
SLC19A1$^{-/-}$ #2	1	A10292G; Δ10548-10557
	2	G10226C; Δ10227-10556

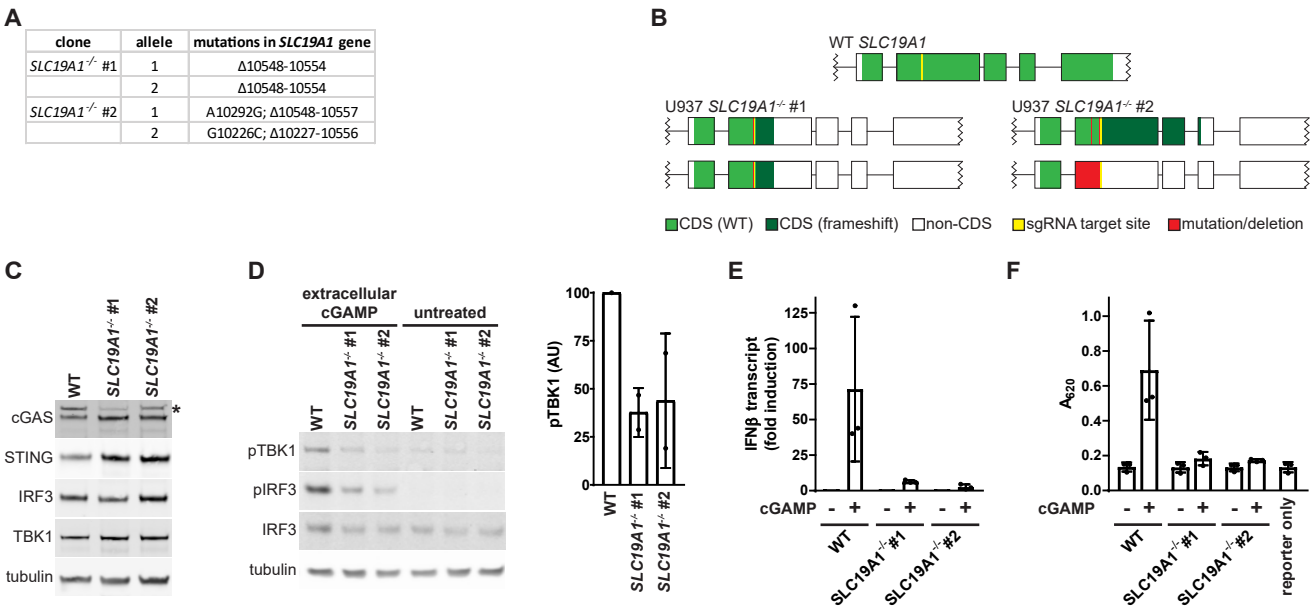

Figure S2. SLC19A1 Is Essential for Robust Extracellular cGAMP Signaling in U937 Cells, Related to Figure 2.

(A) Mutations at SLC19A1 loci in U937 *SLC19A1*$^{-/-}$ cell lines. Genomic DNA was isolated from U937 *SLC19A1*$^{-/-}$ cells and 800 bp of DNA flanking the *SLC19A1* sgRNA target site was sequenced. Note that both alleles in U937 *SLC19A1*$^{-/-}$ #1 contain the same mutation, whereas the alleles in U937 *SLC19A1*$^{-/-}$ #2 contain different mutations.

(B) Diagrams depicting the predicted coding DNA sequence of *SLC19A1* alleles in *SLC19A1*$^{-/-}$ cells. Boxes represent coding exons of *SLC19A1* and horizontal lines represent introns.

(C) STING pathway protein levels in U937 *SLC19A1*$^{-/-}$ cells. Cells were probed for TBK1, IRF3, STING, and cGAS levels by Western blot. * indicates nonspecific band.

(D) Effect of *SLC19A1* knockout on TBK1 phosphorylation in response to extracellular cGAMP. U937 WT and *SLC19A1*$^{-/-}$ cells were treated with 100 μM cGAMP for 2 h and then probed for pTBK1 (n = 2 biological replicates).

(E) Effect of SLC19A1 knockout on IFN-β mRNA production in response to extracellular cGAMP. U937 WT and *SLC19A1*$^{-/-}$ cells were treated with 100 μM cGAMP for 6 h. Total RNA was isolated and fold induction of IFN-β over untreated cells was quantified by RT-qPCR (n = 3 biological replicates).

(F) Effect of SLC19A1 knockout on IFN-β protein production in response to extracellular cGAMP. U937 WT and *SLC19A1*$^{-/-}$ cells were treated with 100 μM cGAMP for 6 h. Supernatant containing secreted IFN-β was then collected and added to HEK-Blue IFN-α/β reporter cells to quantify IFN-β protein levels (n = 3 biological replicates).

For (D), (E), and (F) data are shown as mean ± SD.

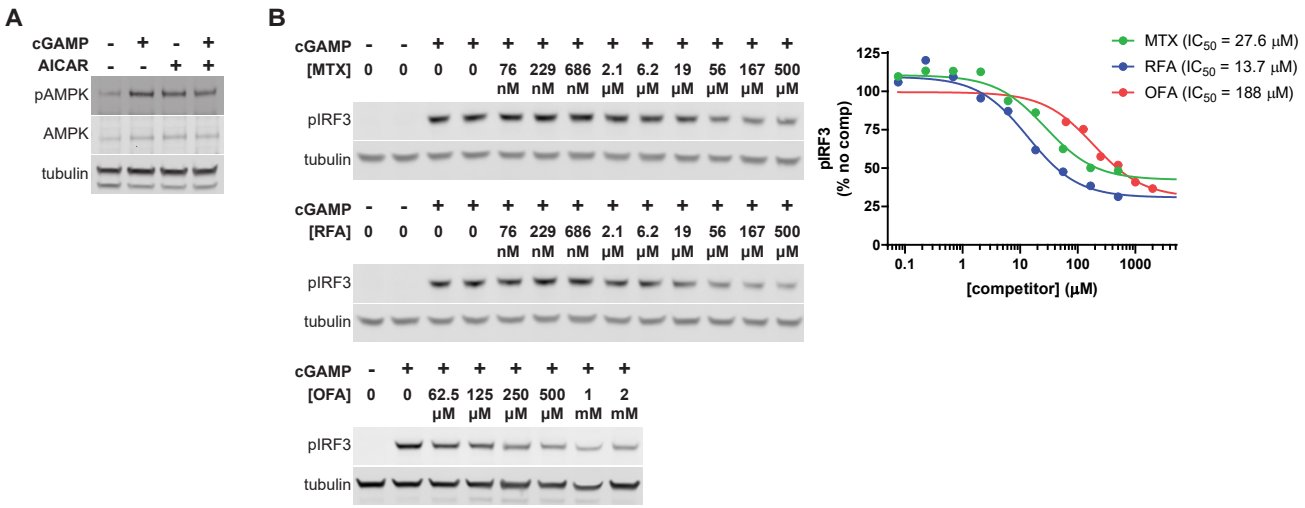

Figure S3. SLC19A1 Is a Direct cGAMP Importer, Related to Figure 4.

(A) Combining AICAR and cGAMP treatment does not cause increased pAMPK. U937 cells were pretreated with 1 mM AICAR for 15 min., and then treated with cGAMP for 90 min.

(B) Dose-dependent inhibition of SLC19A1 substrates on extracellular cGAMP signaling. U937 WT cells were treated with 100 µM cGAMP in the presence of the indicated concentrations of methotrexate (MTX), folinic acid (RFA), or folic acid (OFA) for 2 h, and then probed for pIRF3.

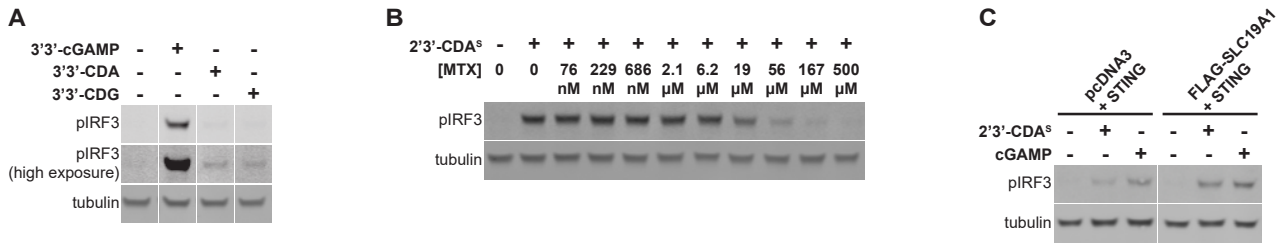

Figure S4. SLC19A1 Imports Bacterial and Synthetic Cyclic Dinucleotides, Including 2'3'-CDA^S, Related to Figure 5.

(A) Extracellular 3'3'-CDN signaling in U937 cells. U937 WT cells were treated with either 200 µM 3'3'-cGAMP, 400 µM 3'3'-CDA, or 400 µM 3'3'-CDG for 3 h.

(B) Dose-dependent inhibition of MTX on extracellular 2'3'-CDA^S signaling. U937 cells were treated with 15 µM 2'3'-CDA^S in the presence of various concentrations of MTX for 2 h.

(C) Effect of SLC19A1 overexpression on extracellular 2'3'-CDA^S signaling. HEK 293T cells were transfected with pcDNA3-STING-HA and either an empty pcDNA3-FLAG-HA vector or pcDNA3-FLAG-HA-SLC19A1, and then were incubated for 24 h to allow for protein production. Transfected cells were then treated with 15 µM 2'3'-CDA^S for 2 h.

Lanes not relevant to the experiment are removed from (A) and (C) for clarity. Uncropped Western blots are available at http://dx.doi.org/10.17632/5bssvpns6h.1

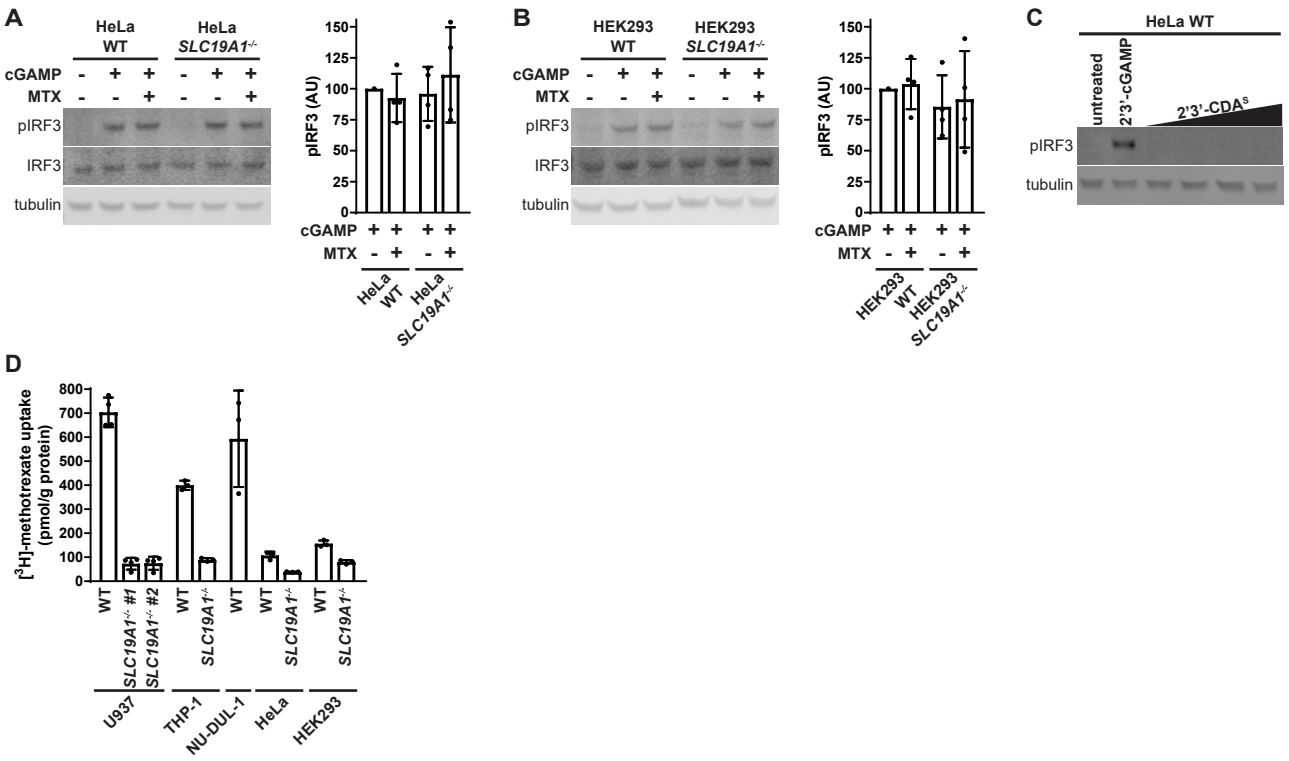

Figure S5. 2'3'-cGAMP Import Through SLC19A1 Varies Across Cell Lines and Primary Cells, Related to Figure 6.

(A-B) Role of SLC19A1 on extracellular cGAMP signaling in epithelial cell lines. HeLa (A) or HEK293 (B) WT and SLC19A1$^{-/-}$ cells were treated with 100 μM cGAMP for 2 h in the presence or absence of 500 μM MTX.

(C) Response of HeLa cells to extracellular 2'3'-CDA^{S}. HeLa cells were treated with either 100 μM cGAMP or 15, 20, 25, or 30 μM 2'3'-CDA^{S} for 2 h.

(D) SLC19A1 mediated uptake of [^{3}H]-methotrexate in U937, THP-1, NU-DUL-1, HeLa, and HEK293 cells. U937 (n = 4 biological replicates), THP-1 (n = 3 biological replicates), NU-DUL-1 (n = 3 biological replicates), HeLa (n = 3 biological replicates), or HEK293 (n = 3 biological replicates) WT and *SLC19A1*$^{-/-}$ lines were treated with 17.5 nM [^{3}H]-methotrexate for 5 min. For (A), (B), and (D) data are shown as mean ± SD.

Name	Sequence (5'->3')
cDNA Cloning Primers	
SLC19A1 FWD	taagcatctagaATGGTGCCCTCCAGCCCAGC
SLC19A1 REV	gcgtgaggatccTCACTGGTTCACATTCTGAACACCG
sscGAS FWD	ctggaagttctgttccaggggccccatatgGGCGCCTGGAAGCTCCAGAC
sscGAS REV	gatctcagtggtggtggtggtggtgctcgagCCAAAAAACTGGAAATCCATTGT
CRISPR sgRNA Oligos	
cGAS sgRNA TOP	caccgGGCTTCCGCACGGAATGCCA
cGAS sgRNA BOT	aaacTGGCATTCCGTGCGGAAGCCc
SLC19A1 sgRNA TOP	caccgGCACGAGAGAGAAGATGT
SLC19A1 sgRNA BOT	aaacACATCTTCTCTCTCGTGCc
NGS Primers	
sgRNA FWD	aggcttggatttctataacttcgtatagcatacattatac
sgRNA REV	acatgcatggcggtaatacggttatc
Library prep FWD	caagcagaagacggcatacgagatgcacaaaaggaaactcaccct
Library prep ID 1 REV	aatgatacggcgaccaccgagatctacacGATCGGAAGAGCACACGTCTGAACTCCAGTC ACCTTGTACGACTCGGTGCCACTTTTTC
Library prep ID 2 REV	aatgatacggcgaccaccgagatctacacGATCGGAAGAGCACACGTCTGAACTCCAGTC ACGCCAATCGACTCGGTGCCACTTTTTC
Library prep ID 3 REV	aatgatacggcgaccaccgagatctacacGATCGGAAGAGCACACGTCTGAACTCCAGTC ACAGTTCCCGACTCGGTGCCACTTTTTC
Library prep ID 4 REV	aatgatacggcgaccaccgagatctacacGATCGGAAGAGCACACGTCTGAACTCCAGTC ACTAGCTTCGACTCGGTGCCACTTTTTC
qPCR Primers	
ACTB FWD	GGCATCCTCACCCTGAAGTA
ACTB REV	AGAGGCGTACAGGGATAGCA
IFNB FWD	AAACTCATGAGCAGTCTGCA
IFNB REV	AGGAGATCTTCAGTTTCGGAGG

Table S1. Oligonucleotides Used in This Study, Related to STAR Methods