

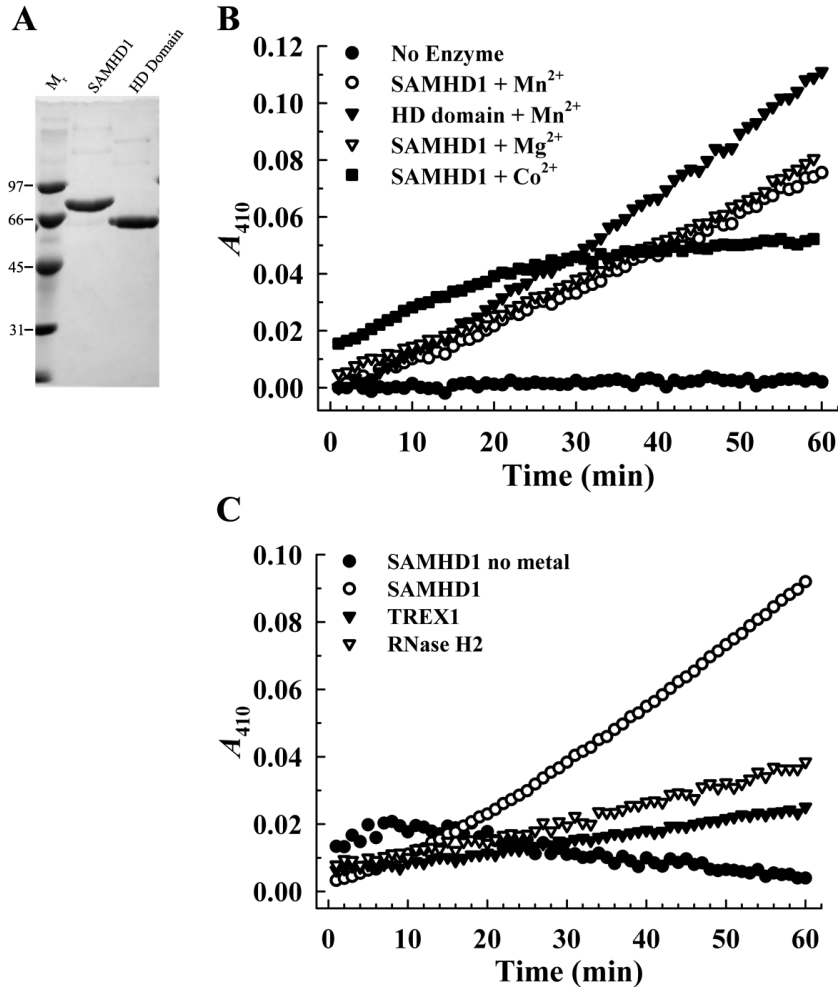
The Aicardi-Goutières syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase

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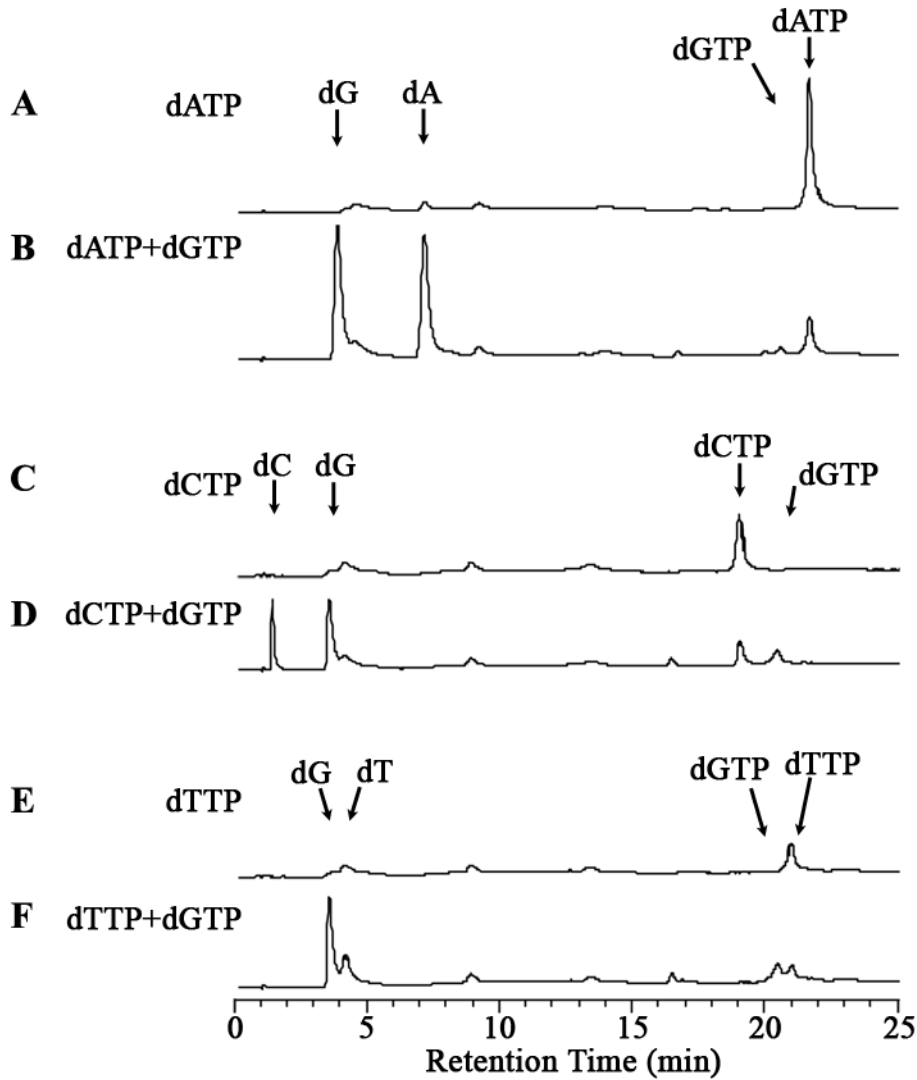
Supplemental Information

Fig. S1



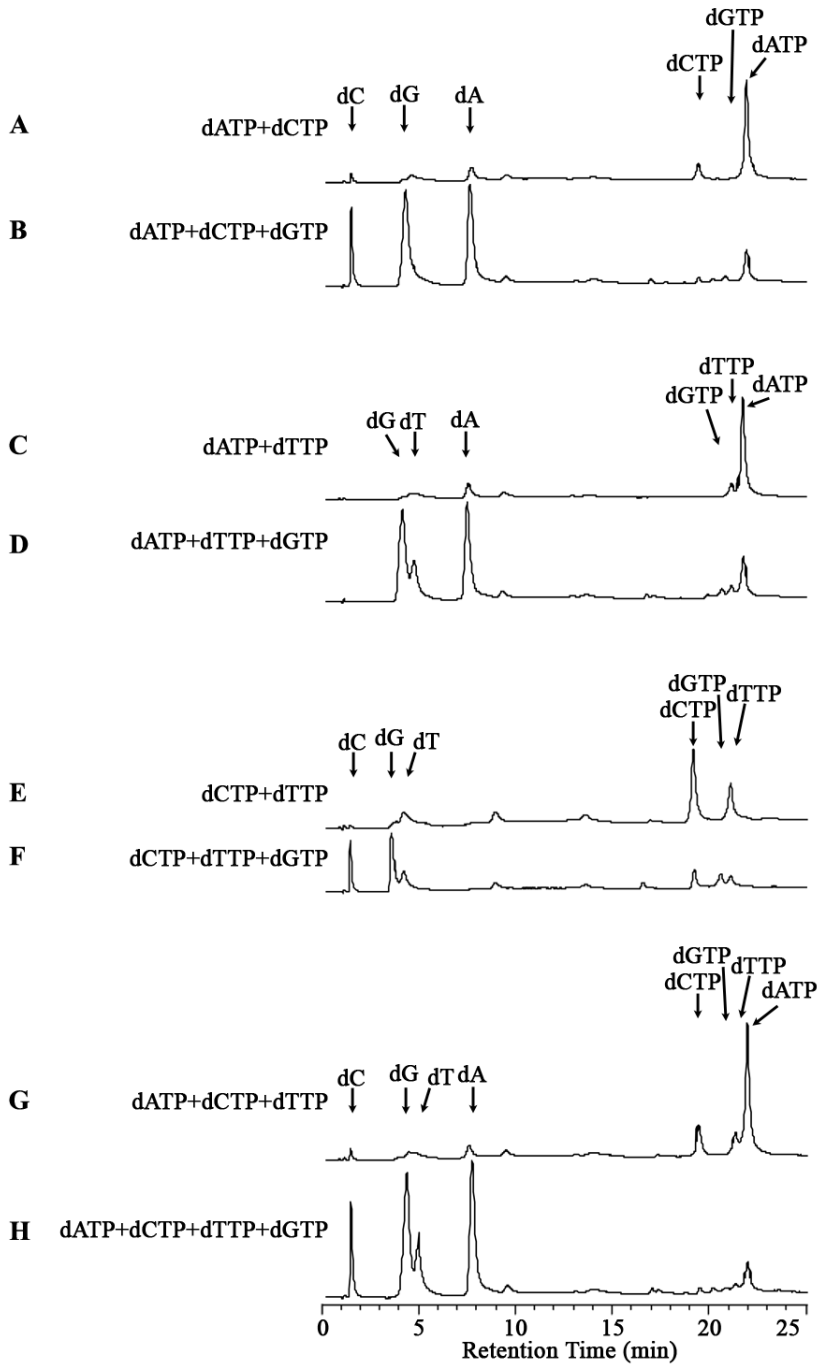
Supplemental Fig. S1. *SAMHD1 is a Phosphohydrolase* The SAMHD1 and SAMHD1 HD-domain were purified as described under “EXPERIMENTAL PROCEDURES.” *A*, approximately 5 μ g of the SAMHD1 (*lane 2*) and SAMHD1 HD-domain (*lane 3*) were subjected to 10% SDS-PAGE, and the gel was stained with Coomassie Brilliant Blue. The molecular weight standards (*lane 1*) are indicated. *B* and *C*, phosphodiesterase reactions (100 μ l) were performed in 96-well microplates containing 50 mM Tricine (pH 8.5), 4 mM *bis-p*-nitrophenyl phosphate, 5 mM of the indicated divalent metal ion, and 500 nM SAMHD1, TREX1, or RNase H2. Activity at 25°C was monitored every minute at A_{410} using a Tecan Safire2TM.

Fig. S2



Supplemental Fig. S2. *SAMHD1* nucleotide triphosphohydrolase activity is dGTP dependent - Reactions containing 400 nM SAMHD1, 200 μ M dATP (A), dGTP and dATP (B), dCTP (C), dGTP and dCTP (D), dTTP (E) dGTP and dTTP (F) and 5 mM MgCl₂ were performed for 20 minutes at 25°C and products were fractionated by HPLC as described under “EXPERIMENTAL PROCEDURES.” The positions of migration of the substrates and products are indicated.

Fig. S3



Supplemental Fig. S3. *SAMHD1* nucleotide triphosphohydrolase activity is dGTP dependent - Reactions containing 400 nM SAMHD1, 200 μ M dATP, dCTP (A), dGTP, dATP, dCTP (B), dATP, dTTP (C), dGTP, dATP, dTTP (D), dCTP, dTTP (E), dGTP, dCTP, dTTP (F), dATP, dCTP, dTTP (G), dGTP, dATP, dCTP, dTTP (H) and 5 mM MgCl₂ were performed for 20 minutes at 25°C and products were fractionated by HPLC as described under “EXPERIMENTAL PROCEDURES.” The positions of migration of the substrates and products are indicated.

Fig. S4

hSAMHD1 HD	DTMKVINDPIHGHIEL-HPLLVRITDTPQFORLRYIKQLGGGYVFPGASHNRFESLGVGYLAGCLVH	180
mSAMHD1 HD	DLMKVFNDPIHGHIIEF-HPLLIRIIDTPQFORLRYIKQLGGGYVFPGASHNRFESLGVGYLAGCLVR	181
<i>Efe</i> EF1143	PIEKVFRDPVHNYIHVQHQVILDILNSAEVQRLRRIKQLGTSSFTFHGAESHRSLSLGVYEITRRICE	79
hSAMHD1 HD	ALGEKQP-----ELQISERDVL CVQIAGLCHDLGHGPF SHMFDGRFIPLARPEVKWTHEQGSVMMFEHL	244
mSAMHD1 HD	ALAEKQP-----ELQISERDIL CVQIAGLCHDLGHGPF SHMFDGRFI PRARPEKKWKHEQGSLEMFEHL	245
<i>Efe</i> EF1143	IFQRNYSVERLGENGWNDERLITLCAALLHGVGHGYSHTFEHIF-----DTNHEAITVQIITSP	140
hSAMHD1 HD	INSGIKPVMEQYGLIPEEDICFIKEQIVGPLESPVEDSLWPYKGRPENKSFLYEIVSNKRNGIDVQKW	313
mSAMHD1 HD	VNSNELKLVMKNYGLVPEEDITFIKEQIMGPPITPVKDSLWPYKGRPATKSFLYEIVSNKRNGIDVQKW	314
<i>Efe</i> EF1143	--ETEYVQILNR---VSADFPEKVASVITK-----QYPNPQVVQMIS---SQIDADR	185
hSAMHD1 HD	DYFARDCHHLGIQ-NNFDYKRFIKFARVCEVD-----NELRICARDKEVGNLYDMFHTRNSLH	370
mSAMHD1 HD	DYFARDCHHLGIQ-NNFDYKRFIKFARICEVEYKVKEDKTYIRKVKHICSREKEVGNLYDMFHTRNCLH	382
<i>Efe</i> EF1143	DYLLRLAYFTGTTEYGTFDLTRLILRVIRPYKGG-----IAFAMNGMHAVEDYIVSRYQMY	239
hSAMHD1 HD	RRAYQHKVGNIIDTMITDAFLKADD-----YIEITGAGGKKYRISTAIDDMEAYTKLTD-NIFLEILYS	433
mSAMHD1 HD	RRAYQHKISNLIDIMITDAFLKADP-----YVEITGTAGKKFRISTAIDDMEAF TKLTD-NIFLEVLHS	445
<i>Efe</i> EF1143	VQVYFHPVSRGMEVILDHLLHRAKELFENPEFDYDLQASLLVPPFFKGDFTLQEYLLKDDGVLSTYFTQW	308
hSAMHD1 HD	TDPKLDAREILKQIEYRNLFKYVGETQPTGQIKIKREDYESLPKEVASAKPKVLLDVKLEAEDFIVDV	502
mSAMHD1 HD	TDPQLSEAQSILRNIECRNLYKYLGETQP-KREKIRKEEYERLPQEVAKAKPEKAPDVELKAEDFIVDV	513
<i>Efe</i> EF1143	MDVPDSILGDLAKRFLMRKPLK SATFTNEK-----ESAATIAYLRELIE-----KVGFNPKYYTAIN	365
hSAMHD1 HD	INMD--YGMQEK-----PIDHVSFYCKTAPNRAIRITKNQVSQLLPEKFAEQLIRVYCKKVDKSL-	562
mSAMHD1 HD	INVD--YGMEDK-----PIDRVHFYCKSNSKQAVRINKEQVSQLLPEKFAEQLIRVYCKKDKGKSL-	573
<i>Efe</i> EF1143	SSYDLPYDFYRPNKDRHRTQI---ELMQKDGSLVELATVSPIVAALAGQSQGDE--RFYFPK-EMLDQG	428
hSAMHD1 HD	-----YAARQYFVQWCADRNF	578
mSAMHD1 HD	-----DAAGKH FVQWCALRDF	589
<i>Efe</i> EF1143	NKKHYDLFDETYRE FSSYIHNGALVLKK	456

Supplemental Fig. S4. *SAMHD1* HD-domain structural model - The complete human SAMHD1 (hSAMHD1) and mouse SAMHD1 (mSAMHD1) amino acid sequences were used to query the program Phyre (see references 36, 37 in text). Structural models and alignments for human (amino acids 113-578) and mouse (amino acids 114-589) SAMHD1 HD-domains were generated. The residues conserved between hSAMHD1, mSAMHD1, and *Enterococcus faecalis* EF1143 protein (*Efe* EF1143) are indicated in yellow. The likely SAMHD1 active site metal-binding residues (red), the proposed nucleophile-generating residues (cyan), residues in direct contact with the dGTP ligand (pink), and residues likely responsible for conferring deoxyribose substrate specificity (green) are highlighted.