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

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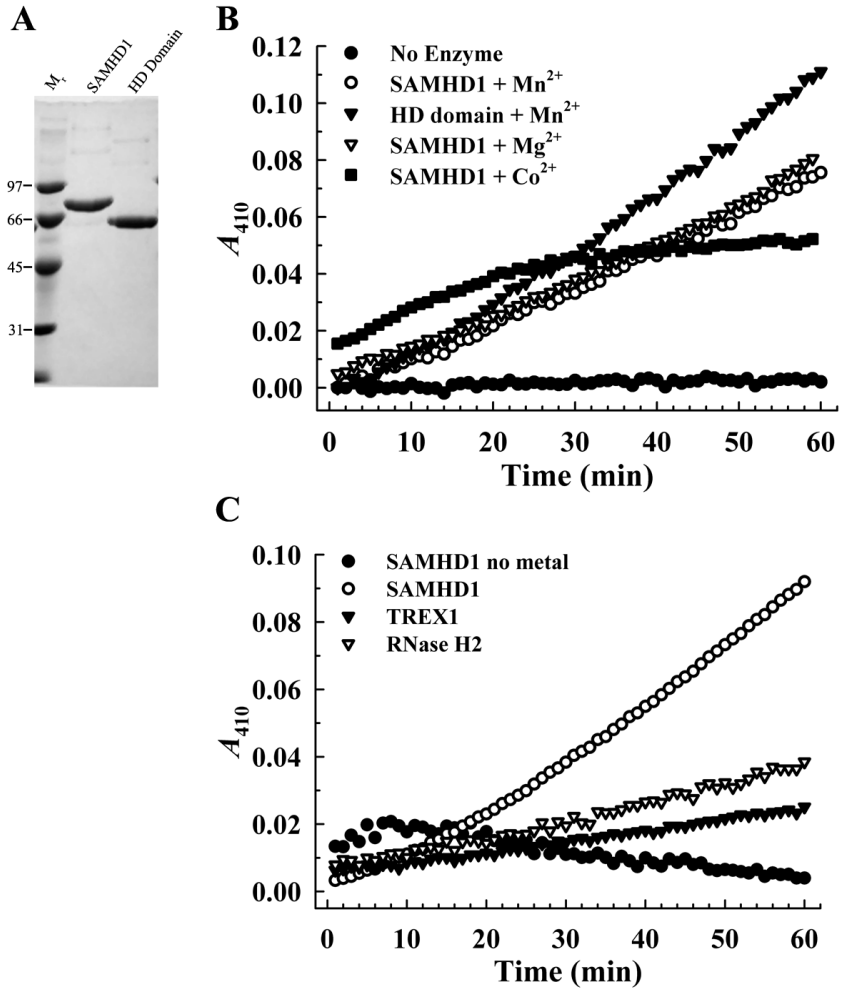
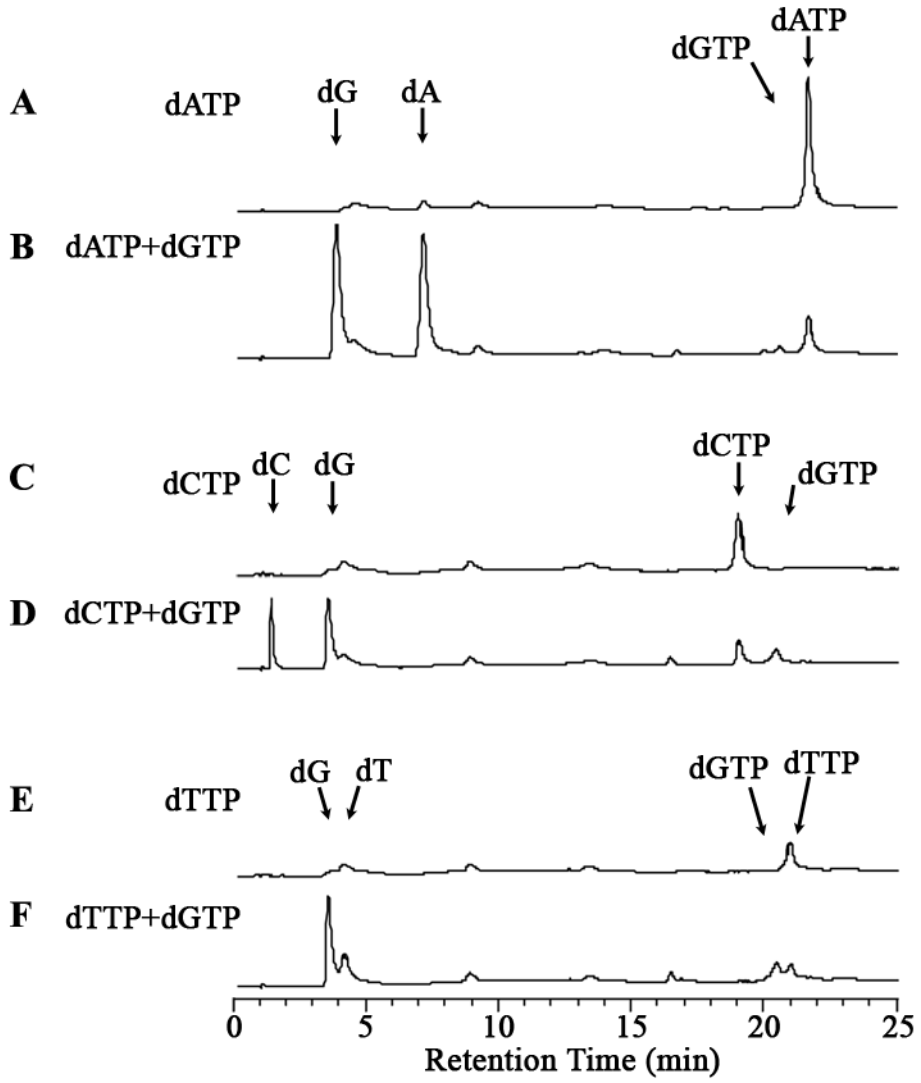
Supplemental Information

Fig. S1



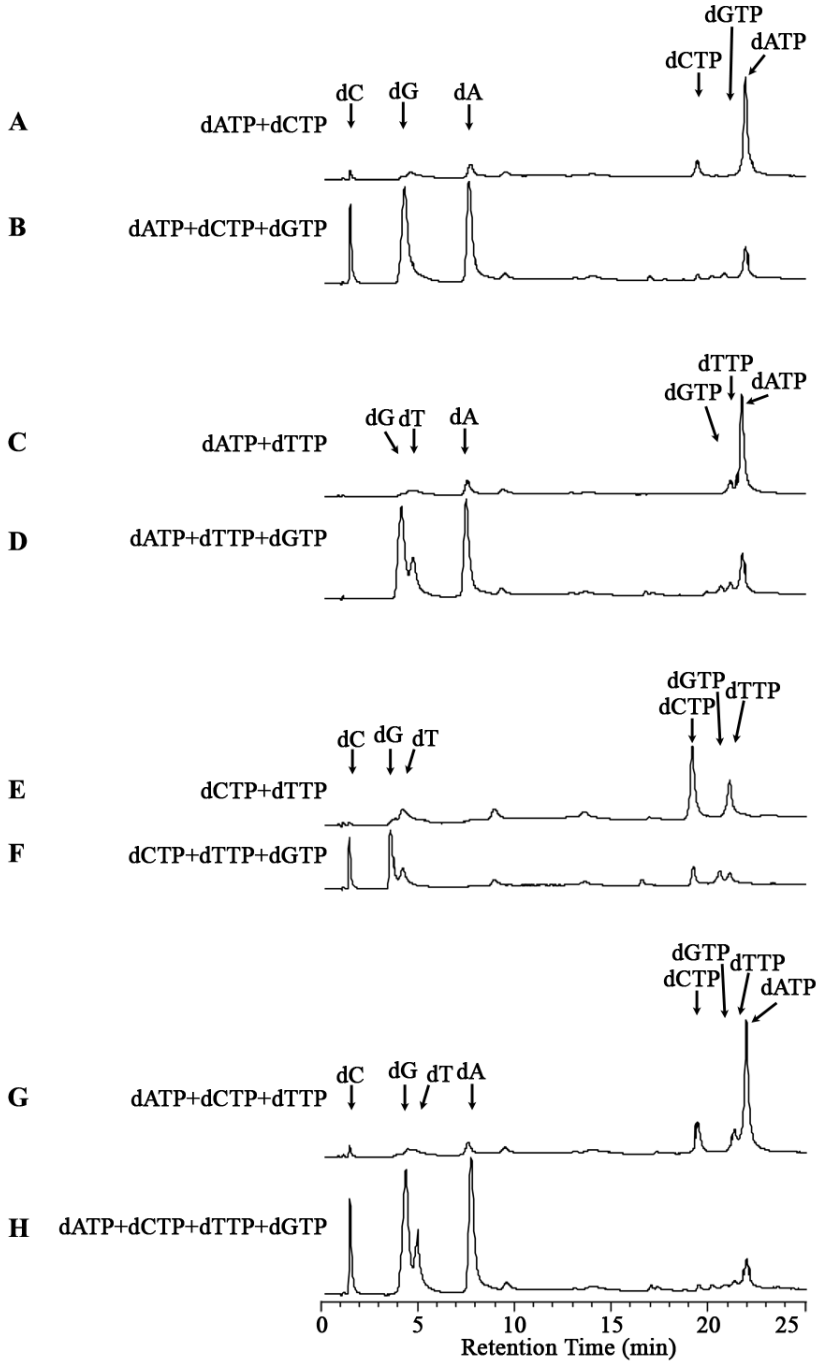
Supplemental Fig. S1. *SAMHD1 is a Phosphohydrolase* The SAMHD1 and SAMHD1 HD-domain were purified as described under "EXPERIMENTAL PROCEDURES." *A*, approximately 5 μg of the SAMHD1 (*lane 2*) and SAMHD1 HD-domain (*lane 3*) were subjected to 10% SDS-PAGE, and the gel was stained with Coomassie Brilliant Blue. The molecular weight standards (*lane 1*) are indicated. *B* and *C*, phosphodiesterase reactions (100 μl) 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 Safire2™.

Fig. S2



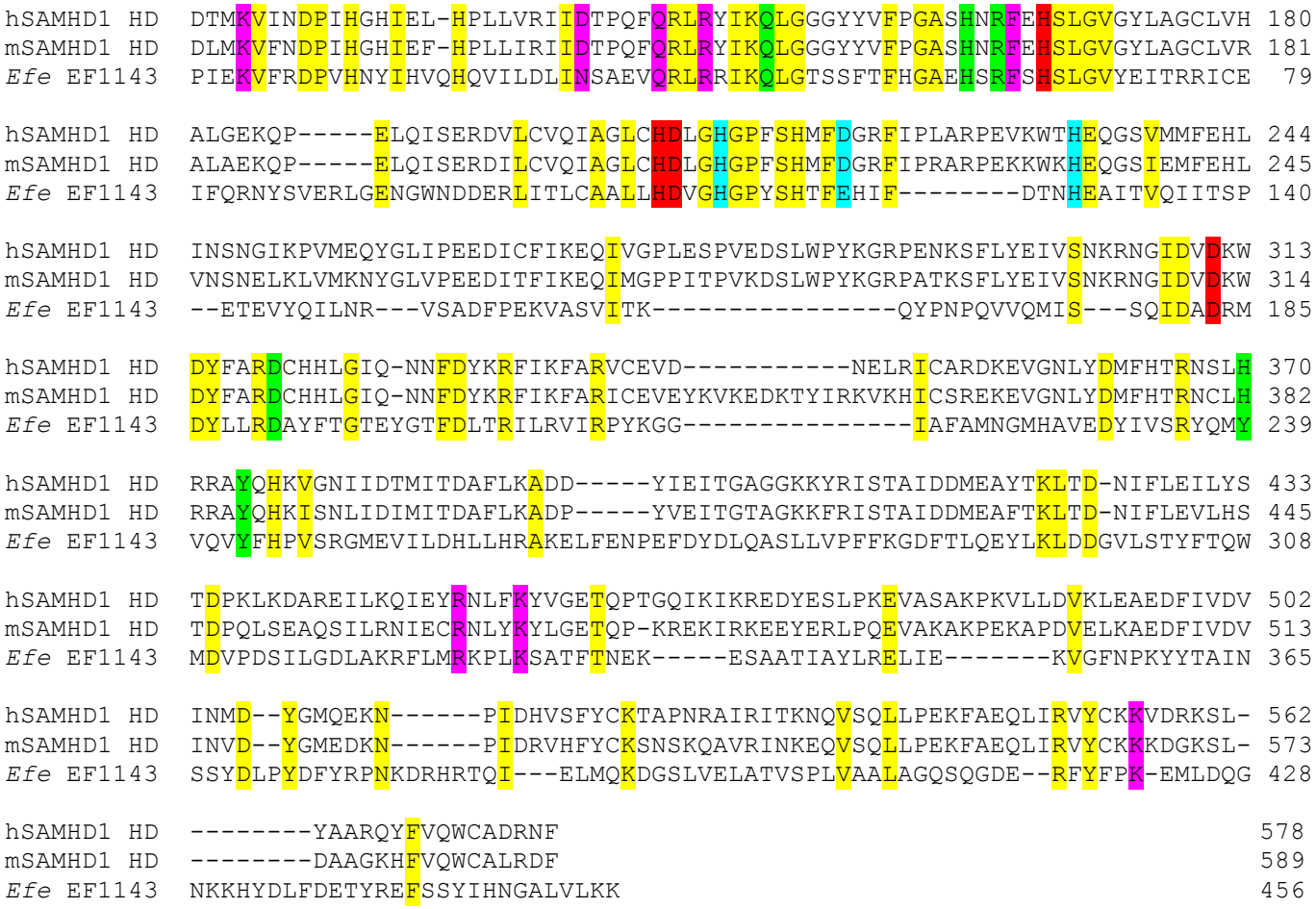
Supplemental Fig. S2. *SAMHD1 nucleotide triphosphohydrolase activity is dGTP dependent* - Reactions containing 400 nM SAMHD1, 200 μM dATP (*A*), dGTP and dATP (*B*), dCTP (*C*), dGTP and dCTP (*D*), dTTP (*E*) dGTP and dTTP (*F*) and 5 mM $MgCl_2$ were performed for 20 minutes at 25°C and products were fractioned 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 μM 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 $MgCl_2$ were performed for 20 minutes at 25°C and products were fractioned by HPLC as described under "EXPERIMENTAL PROCEDURES." The positions of migration of the substrates and products are indicated.

Fig. S4



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.