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Supporting information for article:

The mechanism of substrate-controlled allosteric regulation of SAMHD1 activated by GTP

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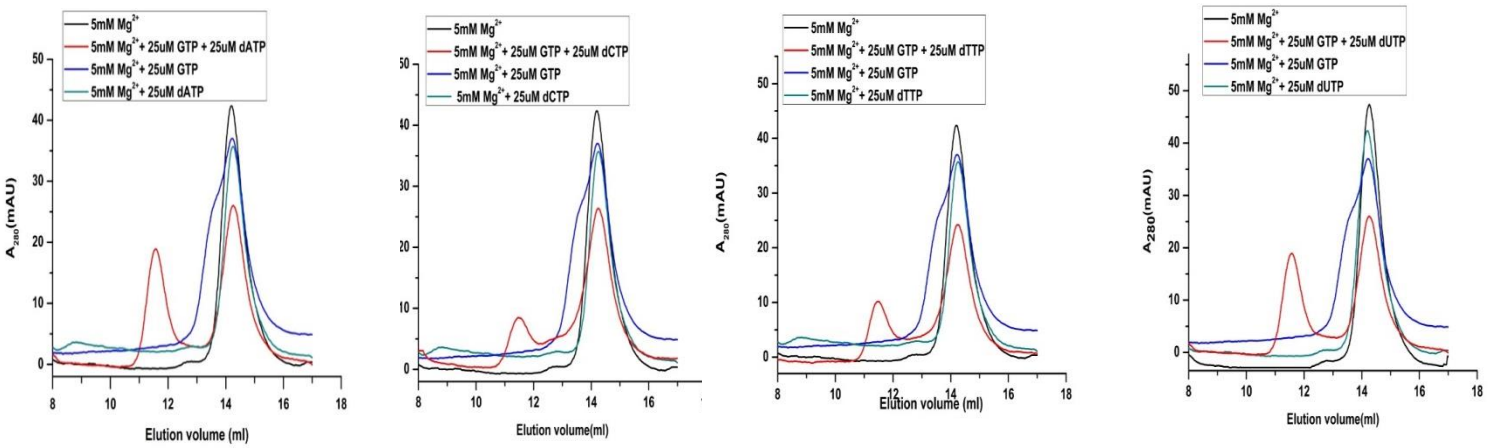
Supporting information:



(a) ***(b)*** ***(c)*** ***(d)***

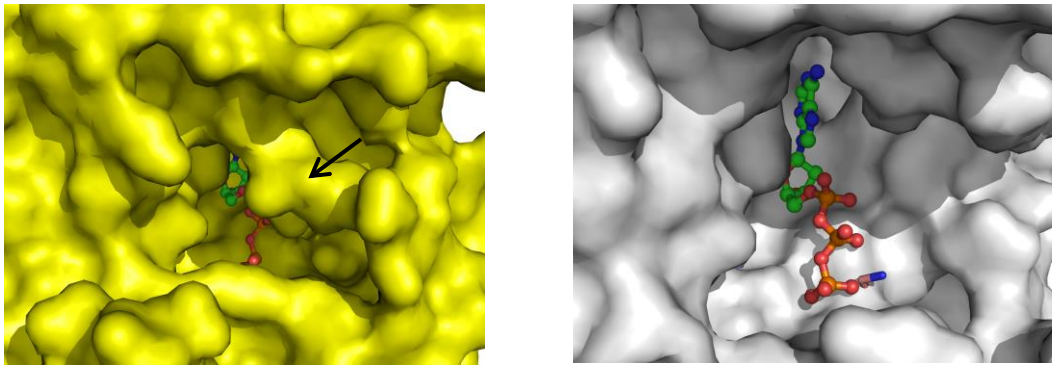
Fig. S1 Size-exclusion chromatography elution profiles of SAMHD1.

(*a*) Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone (blue line), 25 μM dATP alone (green line), or 25 μM GTP+25 μM dATP (red line). Protein samples were separated by size-exclusion chromatography. The elution profiles were monitored by ultraviolet absorbance at A280 nm and compared with known molecular size markers.

(b) Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone, 25 μM dCTP alone, or 25 μM GTP+25 μM dCTP (red line).

(c) Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone, 25 μM dTTP alone, or 25 μM GTP+25 μM dTTP (red line).

(d) Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone, 25 μM dUTP alone, or 25 μM GTP+25 μM dUTP (red line).



(a) *(b)*

Fig. S2. Comparison of the GTP-bound SAMHD1 dimer and the GTP-absent SAMHD1 dimer. (a) The GTP-bound SAMHD1 dimer with a hypothetical substrate. (b) The GTP-absent dimer (PDB: 3U1N) with a hypothetical substrate. The region aa502-510, which was one of regions not traced in 3U1N, is indicated by an arrow.

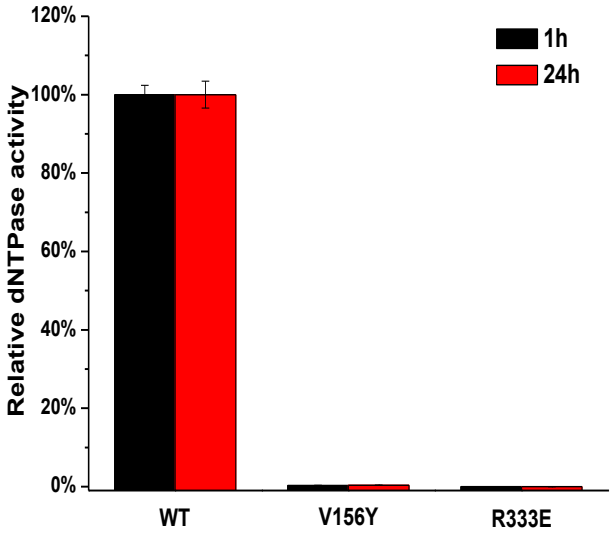
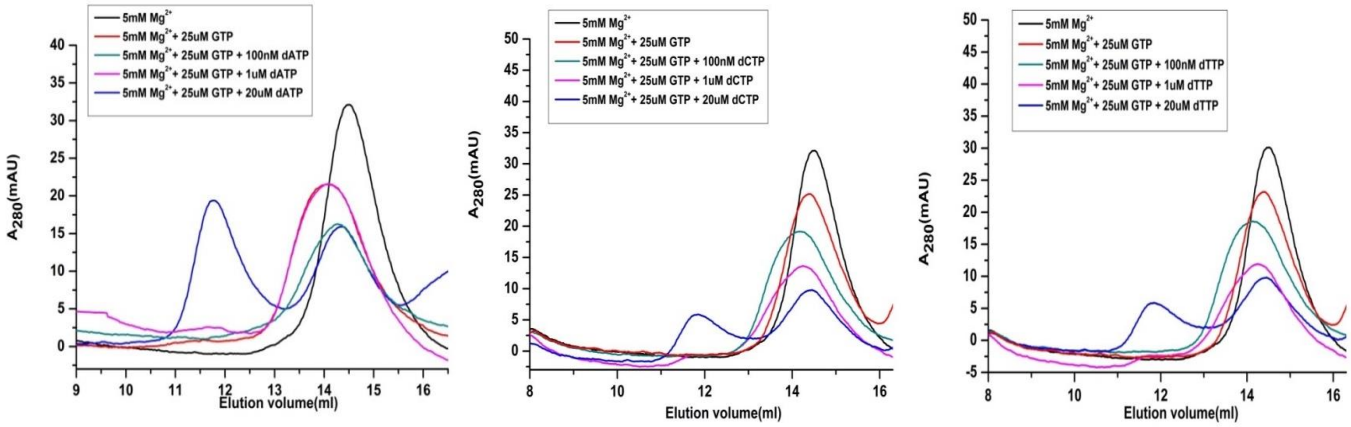


Fig. S3. Mutation of residues involved in dATP binding at the S site of SAMHD1 affects dNTPase activity. Purified recombinant SAMHD1 and SAMHD1 mutant proteins were analyzed for dNTPase activity and the activity of SAMHD1 was set to 100%. The standard error (s.e.) from triplicate samples (n=3) is shown.



(a) ***(b)*** ***(c)***

Fig. S4. **Formation of SAMHD1 tetramer dependents on the substrate concentration**

(a) Activation of the SAMHD1 tetramer depends on the concentration of dATP. Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone or with 25 μM GTP plus various concentrations of dATP. Protein samples were separated by size-exclusion chromatography. The elution profiles were monitored by ultraviolet absorbance at A280 nm and compared with known molecular size markers.

(b) Activation of the SAMHD1 tetramer depends on the concentration of dTTP. Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone or with 25 μM GTP plus various concentrations of dTTP and analyzed by size-exclusion chromatography.

(c) Activation of the SAMHD1 tetramer depends on the concentration of dCTP. Purified protein of human SAMHD1 catalytic core was incubated with 25 μM GTP alone or with 25 μM GTP plus various concentrations of dCTP and analyzed by size-exclusion chromatography.