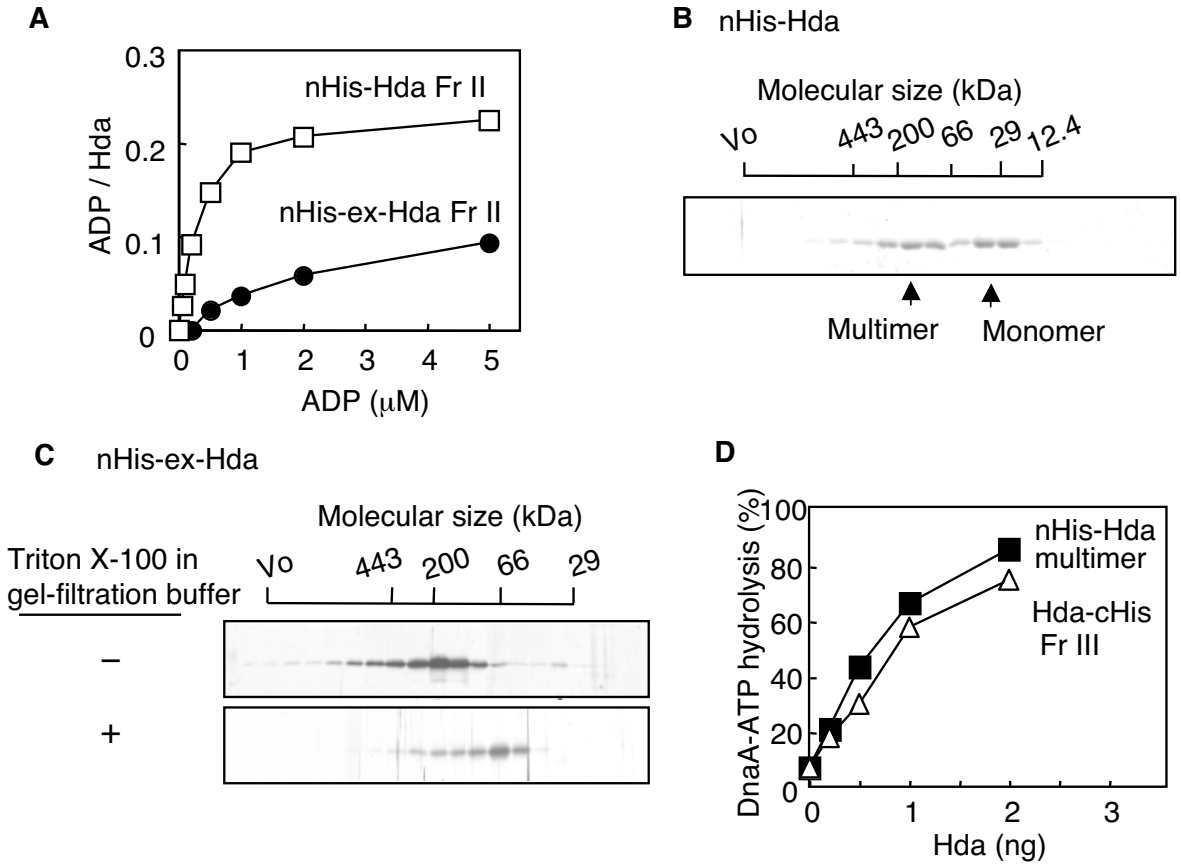
Supplemental table S1 **Nucleotide specificity of Hda-cHis Fr III**

Competitor	Bound ADP (% of control)	Competitor	Bound ADP (% of control)
None	[100]	AMP	97
ADP	1.0	dATP	95
ATP	37	dGTP	105
GTP	107	dCTP	97
CTP	93	dTTP	90
UTP	94	ppGpp	106
GDP	107	cAMP	105
CDP	93		
UDP	94		

Nucleotide competition with ADP was measured using Hda-cHis Fr III as described for Table 1. The error range with each experiment was 0.5-6.5%.

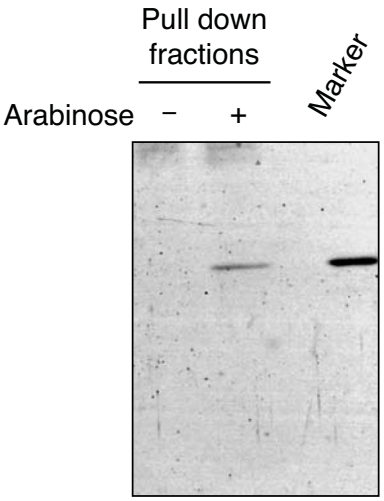
Supplemental Figure S1



SUPPLEMENTAL FIGURE S1 **Inhibitory effect of the N-terminal extra region of nHis-ex-Hda on ADP binding**

A, ADP binding activities of nHis-Hda and nHis-ex-Hda. nHis-Hda Fr II and nHis-ex-Hda Fr II (5 pmol) were incubated at 30 °C for 20 min in buffer (10 μl) containing the indicated concentration of [^{3}H]ADP, followed by filter-retention as described for Fig. 3*A*. Bound ADP molecules per Hda monomer are presented. See *Experimental procedures* for purification of nHis-Hda Fr II. *B*, nHis-Hda Fr II (28 μg) was subjected to Superose-12 PC 3.2/30 gel filtration at a flow rate of 40 μl/min. Eluted proteins were collected in 60 μl fractions and portions (5 μl) were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. The position of molecular size markers were determined under the same conditions and are indicated. Peak fractions of multimers (*Multimer*) and monomers (*Monomer*) are also indicated. *C*, nHis-ex-Hda Fr II (3.2 μg) was subjected to a Superdex-200 PC 3.2/30 gel filtration column equilibrated with buffer containing (+) or excluding (–) 0.1% Triton X-100 at a flow rate of 5 μl/min. Eluted proteins were collected in 30 μl fractions and portions (5 μl) were analyzed by SDS-PAGE and silver staining. *D*, DnaA-ATP hydrolysis activities of nHis-Hda multimers purified in *panel B* and Hda-cHis Fr III were assessed in a staged RIDA reconstituted system. The indicated amounts of Hda were incubated at 30 °C for 20 min in the presence of the DNA-loaded clamp (100 fmol as clamp), 0.5 pmol [^{32}P]ATP-DnaA and 30 μM ADP.

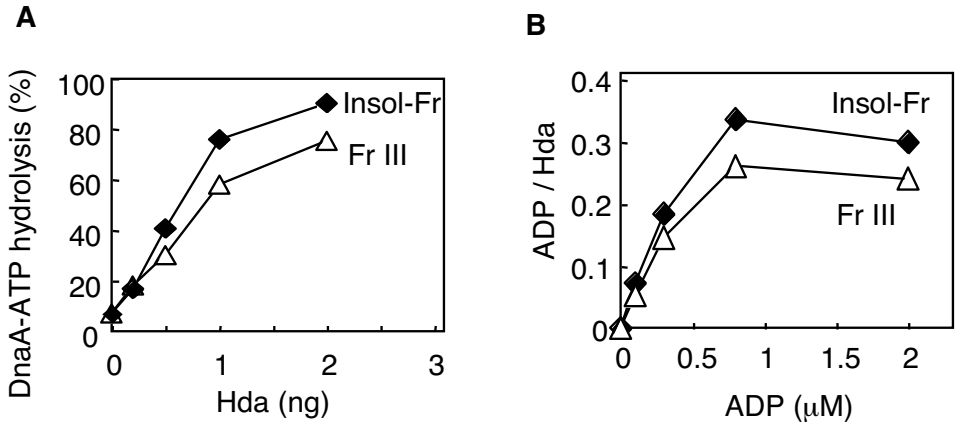
Supplemental Figure S2



SUPPLEMENTAL FIGURE S2 **Specific recovery of nHis-Hda from MC1061 harboring pBAD/nHis-Hda**

Pull down experiments were performed as described for Figure 5. Briefly, MC1061 cells harboring pBAD/nHis-Hda were grown at 37 °C in modified TG medium containing 100 μg/mL ampicillin.When A_{600} of the culture reached 0.1, 0.5 % arabinose was added and incubation continued for 10 min. Cells in culture (10 mL) were collected, washed, and lysed as described in *Experimental Procedures*. Pull down experiments were performed and recovered proteins were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. *Marker* indicates purified nHis-Hda protein.

Supplemental Figure S3



SUPPLEMENTAL FIGURE S3 **Activity of Hda-cHis purified from an insoluble fraction of cell lysate for ADP binding and DnaA-ATP hydrolysis**

Hda-cHis was purified from an insoluble fraction of cell lysate (*Insol-Fr*), as described in the *Experimental procedures*. *A*, DnaA-ATP hydrolysis activity. This experiment was simultaneously performed with that of supplemental Fig. S1*D* and; thus, for Hda-cHis Fr III (*Fr III*), the identical data is shown. *B*, ADP binding activity. Hda proteins (1 pmol each) were incubated at 30 °C for 20 min in buffer (12.5 μl) containing the indicated concentrations of [^{3}H]ADP, followed by the filter-retention as described for Fig. 3*A*. Bound ADP molecules per Hda monomer are presented.