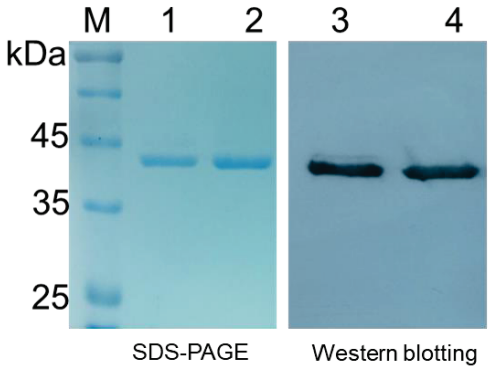
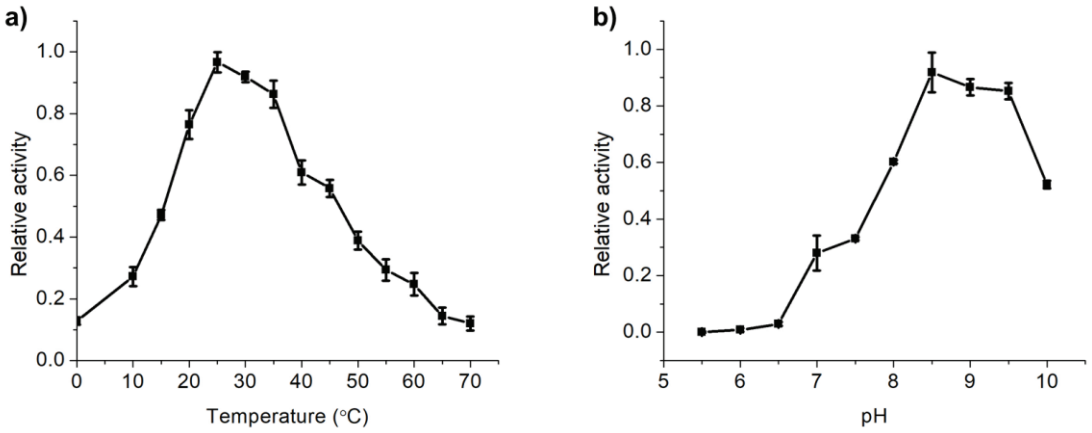
SUPPLEMENTARY METHOD

SDS-PAGE and western blotting of purified *Dm*AK WT and H284A

The purified samples were separated on 12% polyacrylamide gels with 5% stacking gels and stained with Sun-Gel Staining solution (LPS solution). For western blotting, we transferred samples separated by SDS-PAGE to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% skim milk in TBS-T (TBS plus Tween 20). An anti-His-tag monoclonal antibody (1:6,000 dilution) and a goat anti-mouse IgG F(ab′)2 polyclonal antibody (HRP conjugate) (1:12,000 dilution) were used as primary and second antibodies, respectively. The reactive bands were revealed by ECL solution (1 ml of A: 99.30 mM Tris, 196.6 μM p-coumaric acid, 1.241 mM luminol, and 3 μl of B: 3% H_2O_2).



Supplementary Fig. S1. The purified proteins were loaded into 12% SDS-PAGE and confirmed by western blotting. One microgram of purified proteins was loaded into well. Lane 1 and 3: *Dm*AK WT, Lane 2 and 4: H284A.



Supplementary Fig. S2. The optimal temperature and pH on *Dm*AK WT activities. a) The relative activity of *Dm*AK WT depend on temperature. b) The relative activity of *Dm*AK WT versus pH. We used three different buffer system; the sodium citrate/citrate buffer in the range of pH < 7.0; the Tris-HCl buffer in pH 7.0-9.0; the glycine-NaOH buffer in pH > 9.0.

Supplementary Table S1. Data collection and refinement statistics for *Dm*AKs

Structure	***Dm*AKWT$_{apo}$**[a] (PDB entry: 6KY2)	**H284A-Arg**[a] (PDB entry: 6KY3)
Data collection		
Space group	C2	C2
Cell dimensions (Å)		
a, b, c (Å)	78.00, 57.89, 74.45	77.94, 57.84, 74.86
α, β, γ (°)	90.0, 100.09, 90.0	90.00, 100.46, 90.00
Resolution (Å)	46.23-1.87 (1.97-1.87)[b]	46.17-1.34 (1.14-1.34)[b]
R_{merge}[c]	0.081 (0.474)	0.038 (0.370)
I/σ (I)	10.3 (3.1)	16.1 (3.3)
Completeness (%)	98.6 (99.1)	96.6 (93.1)
Redundancy	3.7 (3.7)	3.8 (3.7)
Refinement		
Resolution (Å)	46.23-1.87	46.17-1.34
Number of reflections	25,313	67,028
R_{work}/R_{free} (%)	18.73/23.01	17.35/21.83
Number of non-H atoms	3096	3366
Protein	2856	2844
L-Arg		12
PO_4^{2-}	5	10
K^+		2
Water	235	498
Average B-factor (Å^2)	26.91	20.05
Wilson B factor (Å^2)	25.10	15.40
RMS deviations:		
Bond lengths (Å)	0.010	0.007
Bond Angles (°)	1.363	1.224
Ramachandran plot (%)[d]:		
Most favored	96.9	97.1
Allowed	2.5	2.0
Disallowed	0.6	0.9

[a]One crystal was used.

[b]Values in parentheses are for the highest_resolution shell.

[c]$R_{merge} = \Sigma_{hkl} \Sigma j|I_{hkl}(j) - < I_{hkl} >|/\Sigma_{hkl} \Sigma j\ I_{hkl}(j)$, where $< I_{hkl} >$ is the mean intensity and $I_{hkl}(j)$ are individual intensity measurements of the reflection (*hkl*).

[d]As defined in the program using the PDB server (https://validate-rcsb-2.wwpdb.org/).