SUPPLEMENTARY DATA

Deciphering the molecular basis for nucleotide selection by the West Nile virus RNA helicase

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Supplementary Table 1. Phosphohydrolysis of ATP analogs by the various WNV NS3 proteins harboring mutations in the vicinity of the active site.

Protein (Proximity to purine position)	Substrate (positions harboring modifications compared with ATP)	Phosphohydrolysis (pmol mg^{-1})	Normalized hydrolysis
WT	ATP	25.9	1.00
	P_5 (2'OH)	4.66	0.18
	P_7 (N1)	17.1	0.66
	P_8 (C2)	26.7	1.03
	P_9 (C2)	10.4	0.40
	P_{10} (N6)	11.2	0.43
	P_{11} (N6)	12.7	0.49
	P_{12} (N6)	31.6	1.22
	P_{15} (C8)	32.2	1.24
	P_{29} (C2, N6, N7)	3.6	0.14
	P_{31} (C2, N6, C8)	4.9	0.19
	P_{32} (C2, N6, C8)	14.0	0.54
R202A (N6, N7, C8)	ATP	0.6	1.00
	P_5 (2'OH)	0.2	0.36
	P_8 (C2)	0.7	1.13
	P_9 (C2)	0.2	0.28
	P_{10} (N6)	0.7	1.02
	P_{11} (N6)	0.7	1.09
	P_{15} (C8)	1.0	1.53
	P_{29} (C2, N6, N7)	0.3	0.43
	P_{31} (C2, N6, C8)	0.3	0.44
	P_{32} (C2, N6, C8)	0.5	0.80
Y395A (N6)	ATP	4.6	1.00
	P_{10} (N6)	12.2	2.67
	P_{11} (N6)	5.6	1.23
K399A (N6)	ATP	6.9	1.00
	P_5 (2'OH)	3.3	0.48
	P_8 (C2)	7.9	1.14
	P_9 (C2)	1.2	0.17
	P_{10} (N6)	9.7	1.41
	P_{11} (N6)	5.0	0.73
K419A (N1, C2)	ATP	8.5	1.00
	P_7 (N1)	5.5	0.65
	P_8 (C2)	8.8	1.04
	P_9 (C2)	4.5	0.53
	P_{12} (N6)	8.3	0.98

The phosphohydrolysis assays were performed a minimum of three times separately. Results are shown as the means of these independent experiments.

Supplementary Table 2. Phosphohydrolysis of GTP analogs by the various WNV NS3 proteins harboring mutations in the vicinity of the active site.

Protein (Purine position proximity)	Substrate (positions harboring modifications compared with GTP)	Phosphohydrolysis (pmol mg^{-1})	Normalized hydrolysis
WT	GTP	12.7	1.00
	P_{27} (N6)	10.4	0.82
	P_{29} (N7)	3.7	0.29
R202A (N6, N7, C8)	GTP	0.4	1.00
	P_{27} (N6)	0.3	0.90
	P_{29} (N7)	0.3	0.75
Y395A (N6)	GTP	4.9	1.00
	P_{27} (N6)	3.1	0.64
K399A (N6)	GTP	4.76	1.00
	P_{27} (N6)	2.71	0.57

The phosphohydrolysis assays were performed a minimum of three times separately. Results are shown as the means of these independent experiments.

Supplementary Table 3. RNA binding activity of catalytically inactive mutants of the WNV NS3 protein.

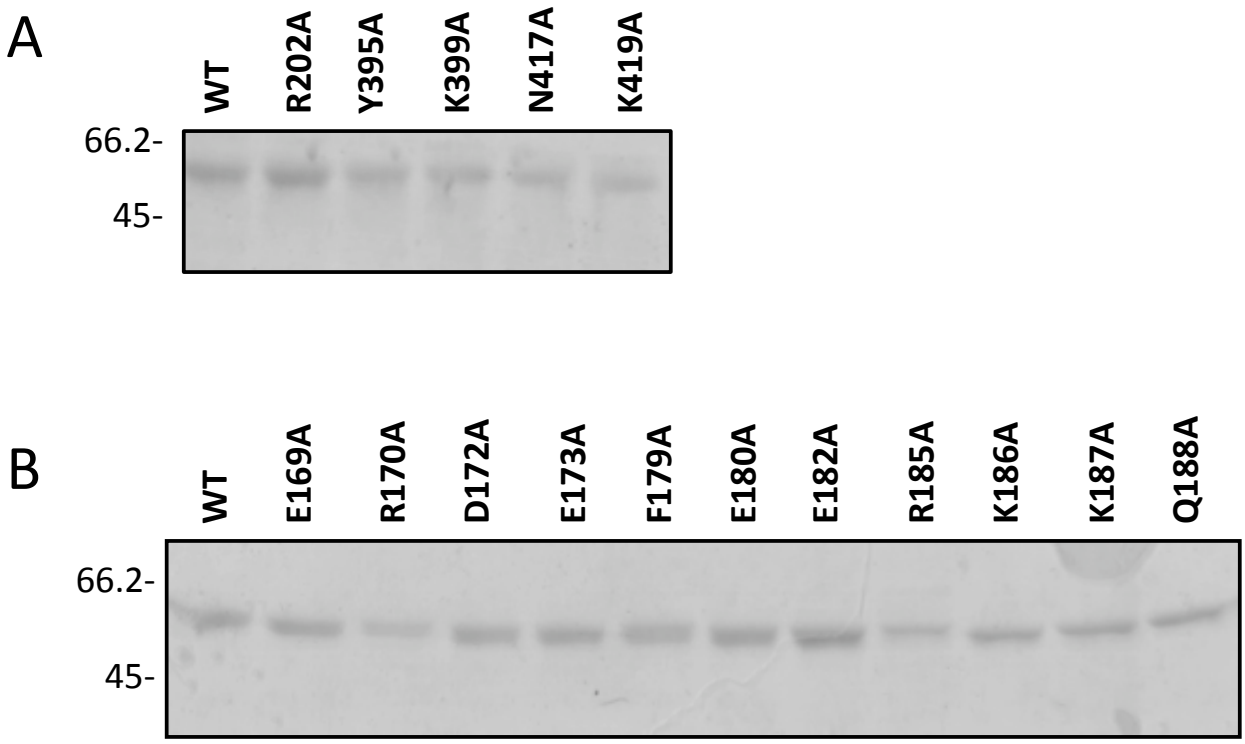
Protein	$\Delta F/F_{max}$ [a]	Apparent K_d (μM) [b]
WT	0.054	6.0 ± 4,2
R185A	0.036	9.1 ± 4,2
R202A	0.010	31.4 ± 9,9
N417A	0.093	3.5 ± 0,5

The assays were performed a minimum of three times separately. Results are shown as the means of these independent experiments.

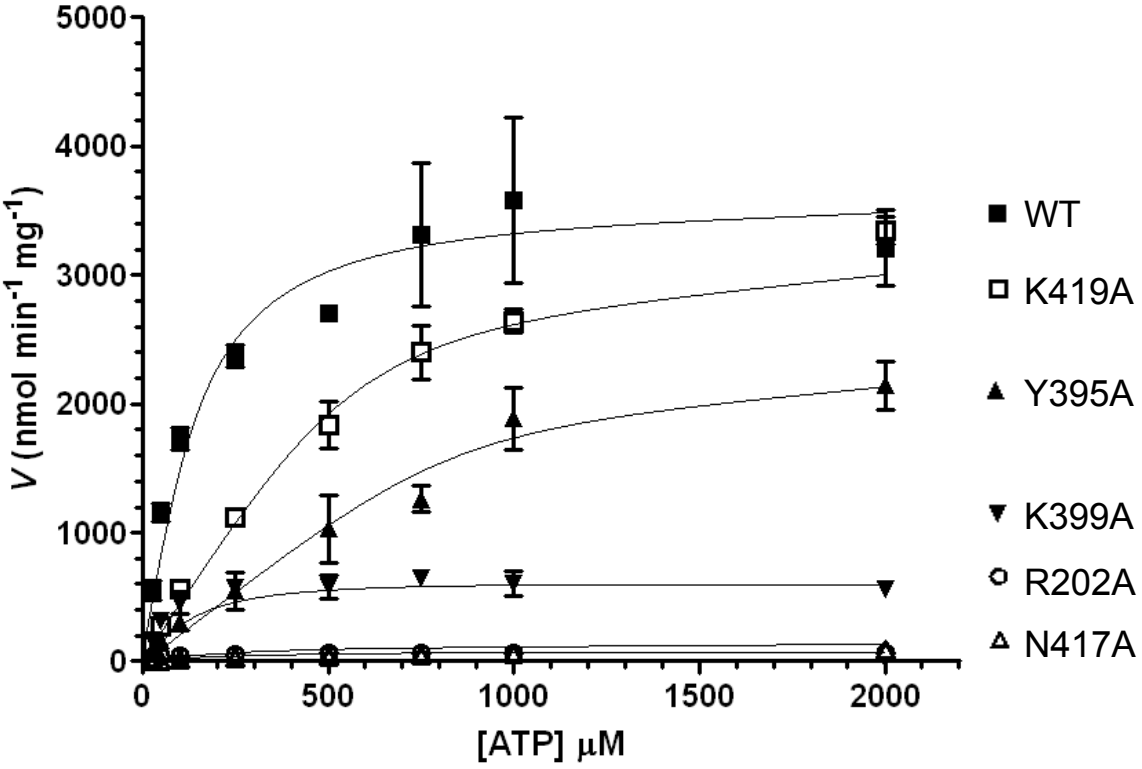
[a] Fluorescence was measured using an Hitachi F-2500 fluorescence spectrophotometer. Ligand binding was determined by monitoring the fluorescence emission of a fixed concentration of proteins and titrating with increasing concentrations of RNA. Excitation was performed at 290 nm and emisssion was monitored at 340 nm. $\Delta F/F_{max}$ is the difference at infinite [RNA].

[b] The K_d values were determined from a nonlinear least square regression analysis of titration data by using:
$\Delta F/F_{max} = [RNA]_{tot}/(K_d + [RNA]_{tot})$

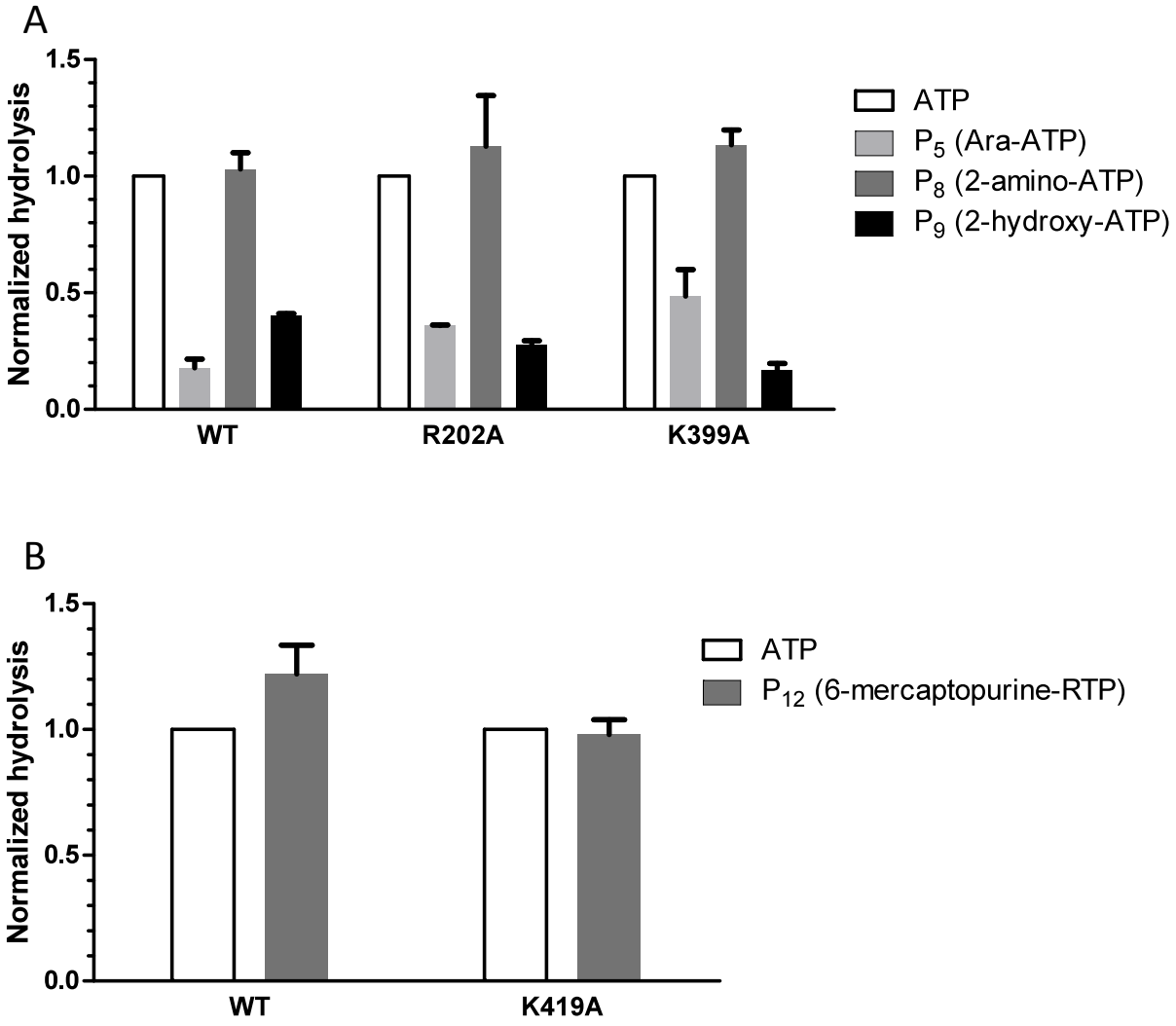
where K_d is the apparent dissociation constant, $\Delta F/F_{max}$ is the difference at infinite [RNA], and $[RNA]_{tot}$ is the total RNA concentration.



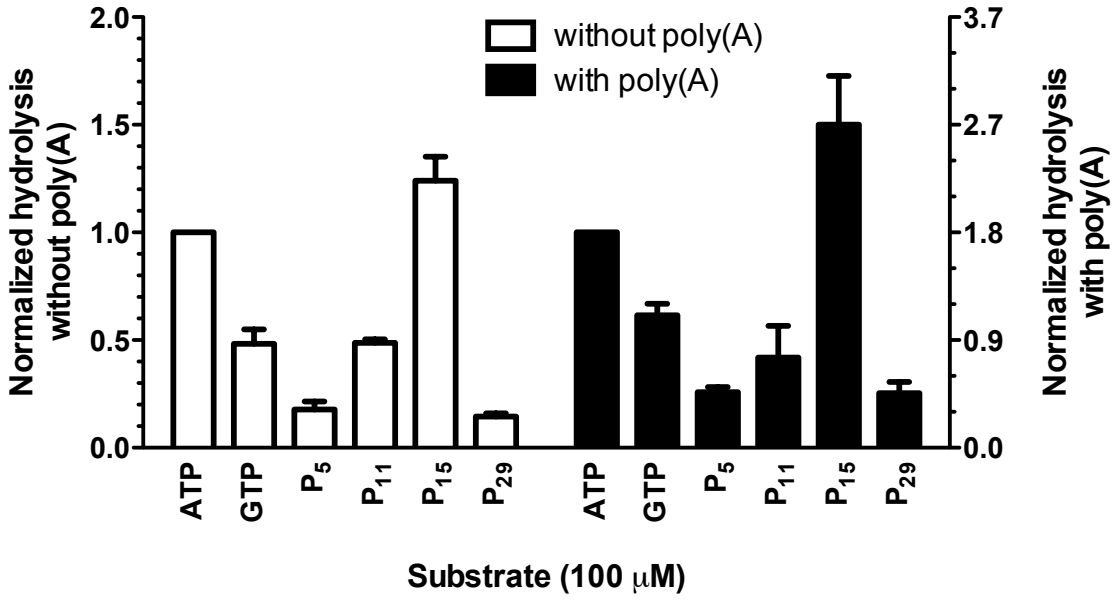
Supplementary figure 1. Expression and purification of the WNV RNA helicase mutants. The purified proteins were analysed by electrophoresis on 12.5% PAGE gels containing 0.1% SDS and visualized by staining with Coomassie Blue. Aliquots (3 µg) of the various purified proteins were analyzed. The positions and sizes (in kDa) of the molecular-mass markers are indicated on the left.



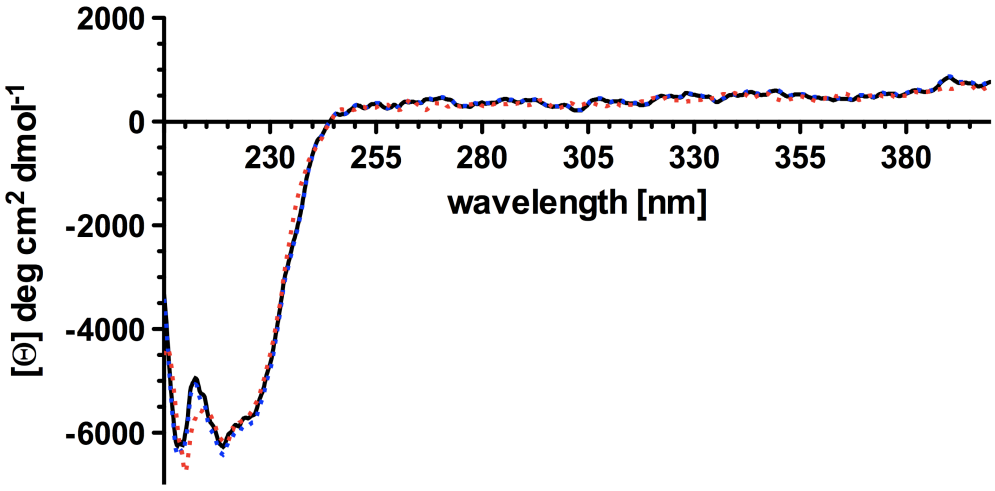
Supplementary figure 2. Phosphohydrolase activity of the active site mutants. The WNV NS3 proteins (R202A (○), Y395A (▲), K399A (▼), N417A (Δ), K419A (□)) harboring mutations to amino acids in the active site were incubated in the presence of increasing concentrations of ATP as described under 'Materials and Methods'. Each value represents the average of at least three independent experiments. Error bars represent the S.D. of the mean values.



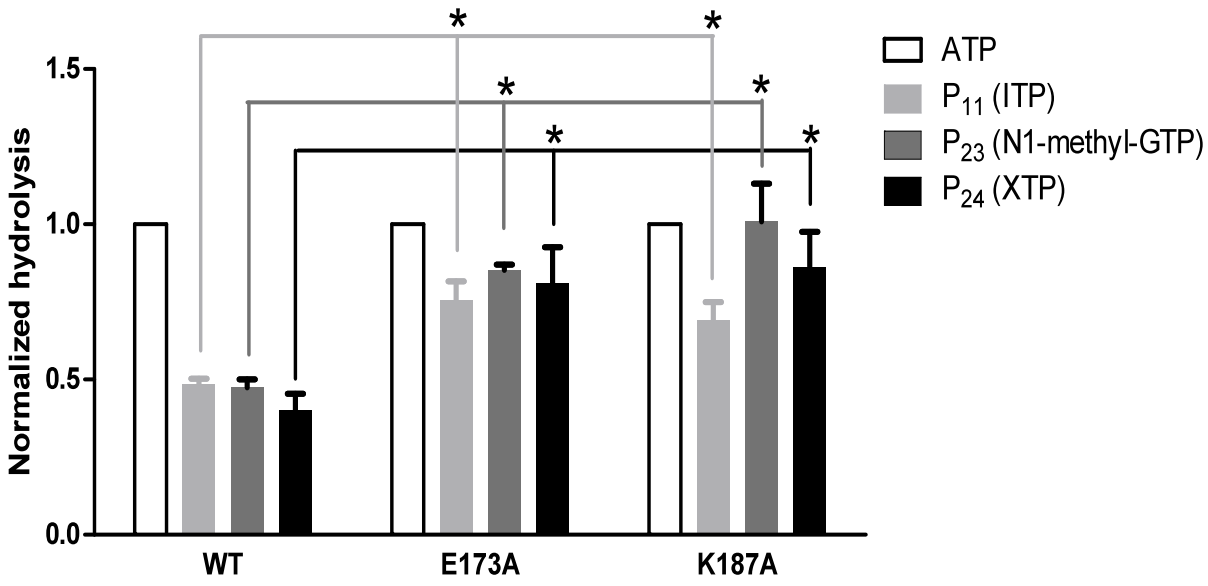
Supplementary figure 3. Relative phosphohydrolysis of nucleotide analogs by active site mutants. The wild-type WNV NS3 protein and three mutants (R202A K399A, and K419A) harboring mutations to amino acids in the active site were incubated in the presence of ATP and various nucleotide analogs as described under 'Materials and Methods'. Each value represents the average of at least three independent experiments. Error bars represent the S.D. of the mean values.



Supplementary figure 4. The molecular determinants involved in substrate recognition are identical in the presence of nucleic acids. The phosphohydrolase activity of the WNV NS3 protein was monitored using various substrates at a concentration of 100 μM. The assays were performed in the absence (white bars) or presence (black bars) of 150 ng/μl of poly(A). Each value represents the average of at least three independent experiments. Error bars represent the S.D. of the mean values.



Supplementary figure 5. The mutant polypeptides are correctly folded. Circular dichroism spectra were recorded for the wild-type WNV NS3 protein (black line), the F179A mutant (dotted blue line), and the Q188A mutant (dotted red line). In each case, the enzyme concentration was 2.5 μM, and the spectra were recorded from 200 to 400 nm. The averages of three wavelength scans are presented. The measurements were performed with a Jasco J-810 spectropolarimeter. The samples were analyzed in quartz cells with pathlengths of 1 mm. All of the dichroic spectra were corrected by subtraction of the background for the spectrum obtained with buffer alone The ellipticity results were expressed as mean residue ellipticity, [θ], in degrees·cm^2·$dmol^{-1}$. Similar spectra were also observed for all the WNV NS3 mutants generated in the current study.



Supplementary figure 6. Relative phosphohydrolysis of nucleotide analogs by mutants located in the nucleotide specificity region located upstream of motif 1. The wild-type WNV NS3 protein (WT) and two mutants (E173A and K187A) were incubated in the presence of ATP and various nucleotide analogs as described under 'Materials and Methods'. Each value represents the average of at least three independent experiments. Error bars represent the S.D. of the mean values.