Supporting Information

Structural Insights into a Novel Esterase from the East Pacific Rise and its Improved Thermostability by Semi-rational Design

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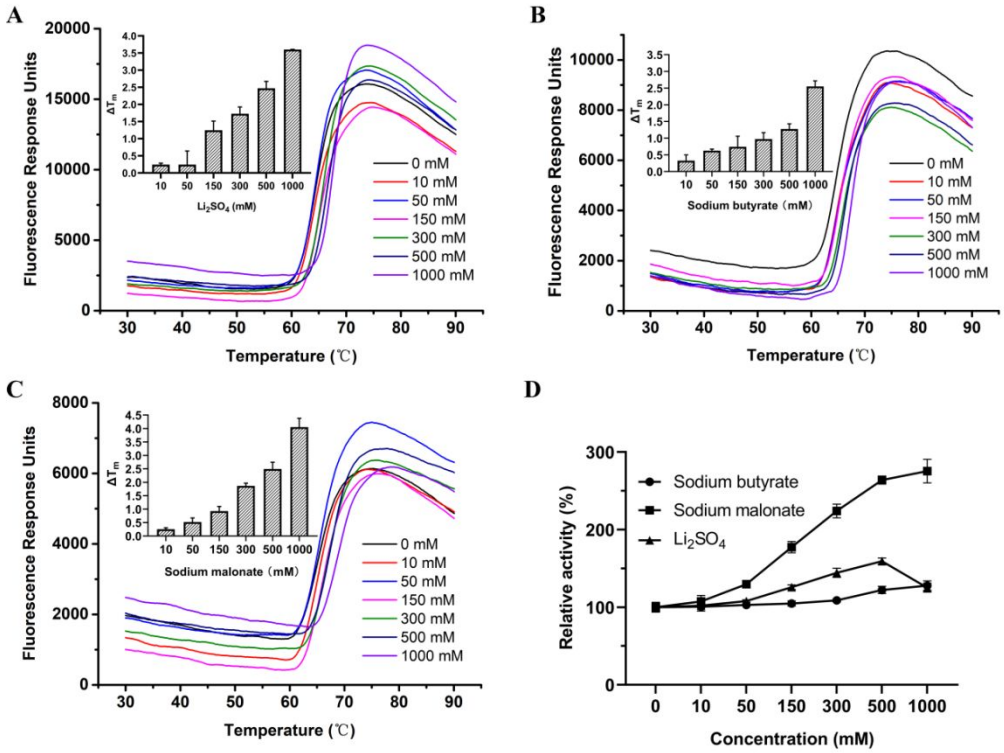

Figure S1. Influence of small molecule ligands on thermostability and activity of EprEst. The differential scanning fluorescence (DSF) was performed to screen the potential small molecules interacted with EprEst. (A) Li_2SO_4, (B) Sodium butyrate, (C) Sodium malonate. (D) The relative activity of EprEst with added different concentrations of Li_2SO_4, sodium butyrate or sodium malonate. The relative activity of EprEst is identified as 100%. The samples were determined in triplicate, and data are mean ± s.d. (n = 3).

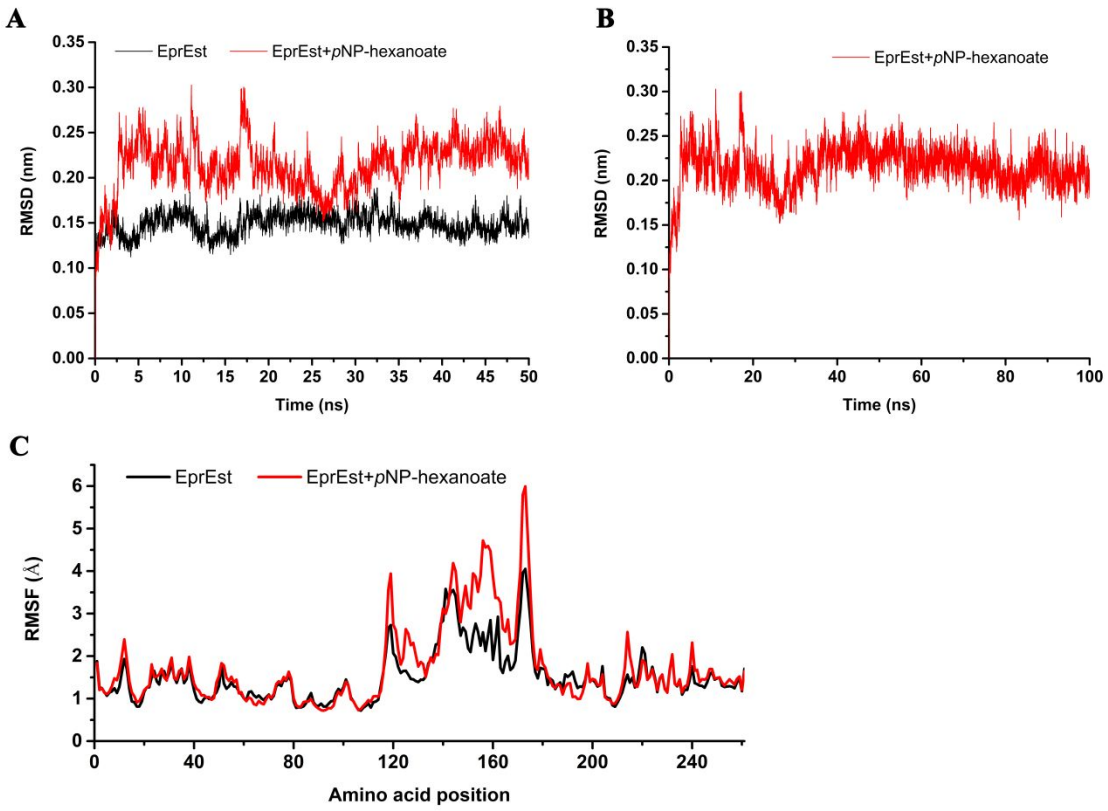

Figure S2. The RMSD and RMSF values of EprEst and its complex with *p*NP-hexanoate. (A) The MD simulations were performed for 50 ns at 298 K. (B) 100 ns. (C) The RMSF values.

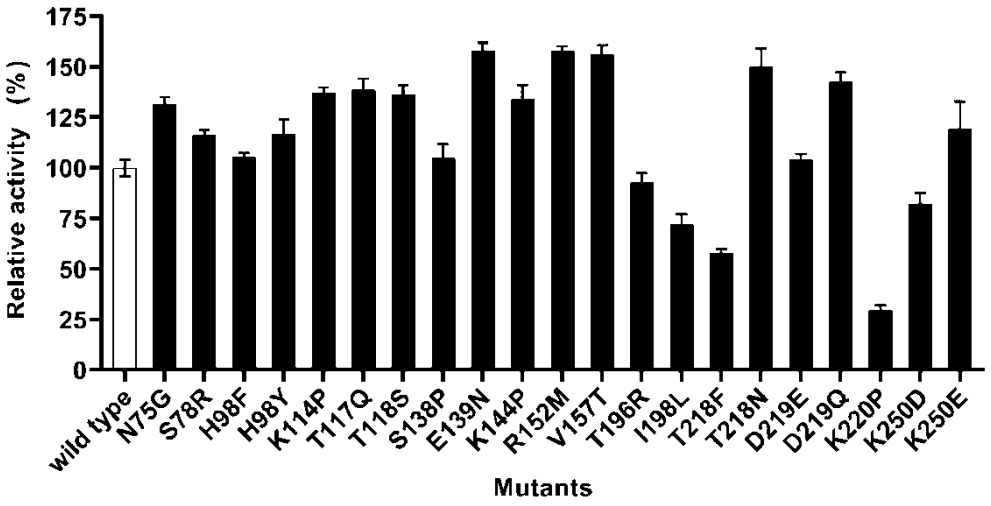

Figure S3. The relative activities of the mutants were determined through the first round of screening. All the mutants were incubated at the optimal temperature of 50 °C, the relative activity of wild type was identified as 100%.

Table S1. The ligands for molecular docking

Ligand	PubChem CID	Lowest Binding Energy (kcal mol^{-1})
*p*NP-butyrate ester	75834	-10.00
*p*NP-hexanoate ester	567162	-9.53
*p*NP-octanoate ester	97416	-7.23

Table S2. Mutagenic primers used in this work

Mutants	Primer	Sequence (5' to 3')
L21A	L21A -F	ATCATCCTGCACGGC**GCG**TACGGTAGC
	L21A -R	GCGCCGTGCAGGATGATCAGCGGTTCG
Y115A	Y115A -F	ATTGCGCCGGGCAAA**GCC**GATACCACC
	Y115A -R	GCTTTGCCCGGCGCAATATCCGCCACG
N121A	N121A -F	GATACCACCTCTCCG**GCC**GTTAAAACC
	N121A -R	GCCGGAGAGGTGGTATCGTATTTGCCC
H125A	H125A -F	CCGAACGTTAAAACC**GCC**AAAAAGATC
	H125A -R	GCGGTTTTAACGTTCGGAGAGGTGGTA
M192A	M192A -F	AACATCATCAACATC**GCA**AGCGGCATCA
	M192A -R	TGCGATGTTGATGATGTTTTTCTCAATG
W243A	M192A -F	CCGGGTGCGGGCCAC**GCG**CTGCACGCG
	M192A -R	GCGTGGCCCGCACCCGGAATGGTAACC
K114P	K114P -F	GATATTGCGCCGGGC**CCA**TACGATACC
	K114P -R	GGGCCCGGCGCAATATCCGCCACGATC
Q74N	Q74N-F	GAATTCATCAAAAGC**AAC**AACCTGGAAA
	Q74N-R	GTTGCTTTTGATGAATTCGTGCAGATC
N75G	N75G-F	TTCATCAAAAGCCAG**GGC**CTGGAAAGC
	N75G-R	CCCTGGCTTTTGATGAATTCGTGCAG
S78R	S78R-F	AGCCAGAACCTGGAA**CGC**GTGAACA
	S78R-R	GTTCCAGGTTCTGGCTTTTGATGAA
H98F	H98F-F	TGGTTCACCGCAGCG**TTC**CCGGATAA
	H98F-R	AACGCTGCGGTGAACCACATCGCGGT
H98Y	H98Y-F	TGGTTCACCGCAGCG**TTC**CCGGATA
	H98Y-R	ACGCTGCGGTGAACCACATCGCGGT
K114M	K114M-F	ATATTGCGCCGGGC**ATG**TACGATACC
	K114M-R	CATGCCCGGCGCAATATCCGCCACGA
T117L	T117L-F	CCGGGCAAATACGAT**CTG**ACCTCTCCGA
	T117L-R	CAGATCGTATTTGCCCGGCGCAATATC
T117Q	T117Q-F	CCGGGCAAATACGAT**CAG**ACCTCTCCGA
	T117Q-R	CTGATCGTATTTGCCCGGCGCAATATC
T118S	T118S-F	GCAAATACGATACC**AGC**TCTCCGAAC
	T118S-R	CTGGTATCGTATTTGCCCGGCGCAA

Mutants	Primer	Sequence (5’ to 3’)
S138P	S138P-F	AAAAGCATCGATCCG**CCG**GAAGCGAAAA
	S138P-R	CGGCGGATCGATGCTTTTCAGCGCCGC
E139N	E139N-F	AGCATCGATCCGTCT**AAC**GCGAAAACCC
	E139N-R	GTTAGACGGATCGATGCTTTTCAGCGC
K144P	K144P-F	GAAGCGAAAACCCGT**CCA**GAAATCGAA
	K144P-R	GGACGGGTTTTCGCTTCAGACGGATC
R152L	R152L-F	AAACCCAGCTGTCT**CTG**TACATCGATA
	R152L-R	CAGAGACAGCTGGGTTTCGATTTCTT
R152M	R152M-F	GAAACCCAGCTGTCT**ATG**TACATCGATA
	R152M-R	CATAGACAGCTGGGTTTCGATTTCTTT
V157T	V157T-F	CGTTACATCGATAAC**ACT**CAGCTGCGC
	V157T-R	GTGTTATCGATGTAACGAGACAGCTG
T196R	T196R-F	ATCATGAGCGGCATC**CGC**GGTATCGAC
	T196R-R	CGGATGCCGCTCATGATGTTGATGAT
I198L	I198L-F	AGCGGCATCACCGGT**CTG**ATCGACATCC
	I198L-R	CAGACCGGTGATGCCGCTCATGATGTT
T218N	T218N-F	TGAGCGATTACATC**AAC**GATAAAGAC
	T218N-R	TTGATGTAATCGCTCAGTTCACCTT
T218D	T218D-F	CTGAGCGATTACATC**GAT**GATAAAGACA
	T218D-R	ATCGATGTAATCGCTCAGTTCACCTTT
T218F	T218F-F	CTGAGCGATTACATC**TTC**GATAAAGAC
	T218F-R	AAGATGTAATCGCTCAGTTCACCTTT
D219E	D219E-F	CGATTACATCACC**GAA**AAAGACATCC
	D219E-R	TTCGGTGATGTAATCGCTCAGTTCA
D219Q	D219Q-F	AGCGATTACATCACC**CAA**AAAGACATCC
	D219Q-R	TTGGGTGATGTAATCGCTCAGTTCACC
K220P	K220P-F	GATTACATCACCGAT**CCG**GACATCCCGC
	K220P-R	CGGATCGGTGATGTAATCGCTCAGTTC
K250D	K250D-F	CACGCGCAGCAGCCG**GAT**ATCTTCATCC
	K250D-R	ATCCGGCTGCTGCGCGTGCAGCCAGTG
K250E	K250E-F	ACGCGCAGCAGCCG**GAA**ATCTTCATCC
	K250E-R	TTCCGGCTGCTGCGCGTGCAGCCAGT

Note: The underline represents the mutagenic position.