

Supporting Information for:

Roles of Active-Site Aromatic Residues in Cold Adaptation of *Sphingomonas glacialis* Esterase EstSP1

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Table S1. Mutagenic primers

Mutations	Primers	
Y191A	Forward	5'-ctgcccttcGCCccagcg-3'
	Reverse	5'-cgctggGGCgaagggcag-3'
Y191F	Forward	5'-ctgcccttcTTCccagcgac-3'
	Reverse	5'-gtcgctggGAAGAagggcag-3'
Y191H	Forward	5'-ctgcccttcCACccagcg-3'
	Reverse	5'-cgctggGTGgaagggcag-3'
Y191W	Forward	5'-ctgcccttcTGGccagcg-3'
	Reverse	5'-cgctggCCAgaagggcag-3'
I287A	Forward	5'-gcttcGCTacgctgcgcaag-3'
	Reverse	5'-gcagcgtAGCgaagccatgaatg-3'
I287P	Forward	5'-gcttcCCTacgctgcgcaag-3'
	Reverse	5'-gcagcgtAGGgaagccatgaatg-3'

The capital letters specify the codon for the substituted amino acid.

Table S2. Ramachandran plot statistics for WT and mutant EstSP1 enzymes.

	Percentage of residues in regions (%)			
	Most favoured	Additional allowed	Generously allowed	Disallowed
WT	87.3	10.7	0.4	1.6
Y191A	88.0	10.4	0.0	1.6
Y191F	87.7	10.3	0.4	1.6
Y191H	87.7	10.3	0.4	1.6
Y191W	88.1	9.9	0.4	1.6

Table S3. PMDB accession numbers and substrate-binding pocket volumes of for WT and mutant EstSP1 homology models.

	PMDB accession number	Substrate-binding pocket volume ($\text{\AA}^3$)	Δ Volume ($\text{\AA}^3$)
WT	PM0081182	1382	0
Y191A	PM0081183	1498	116
Y191F	PM0081184	1380	2
Y191H	PM0081185	1144	238
Y191W	PM0081186	1440	58

