Supplemental Information

Supplemental Tables and Figures

Supplemental Table 1: Biochemical characterization of FTT0941c and FTT0941c variants.

Substrate	**3**			**11**			
	k_{cat} (10^{-3} s^{-1})[a]	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (10^{-3} s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	T_m (°C)[b]
Wild-Type	0.85 ± 0.06	2.2 ± 0.4	400 ± 50	8.7 ± 0.7	17 ± 2	530 ± 50	53.3 ± 1.0
D90A	12 ± 4	36 ± 3	320 ± 30	0.077 ± 0.04	4.4 ± 0.8	18 ± 4	51.8 ± 0.9
S151A	ND[c]	ND	ND	ND	ND	ND	50.6 ± 1.5
D248A	ND	ND	ND	ND	ND	ND	52.7 ± 1.0
D252A	ND	ND	ND	ND	ND	ND	52.0 ± 0.8
H278A	ND	ND	ND	ND	ND	ND	45.7 ± 1.8

[a]Kinetic constants for substrates **3** and **11** were determined by measuring the increase in fluorogenic enzyme substrate fluorescence over time. Data were fitted to a standard Michaelis-Menten equation to determine the values for k_{cat}, K_M, and k_{cat}/K_M. Kinetic measurements for each substrate were repeated three times and the values are given ± SE.

[b]Values for T_m were determined by following the change in Sypro Orange fluorescence with increasing temperature. Melting curves were repeated three times for each variant and the T_m values reported ± SE.

[c]Not determinable. The catalytic activity for each of these FTT0941c variants was below the detection limit for the fluorogenic enzyme assay.

Supplemental Table 2: Pairwise sequence alignment of FTT941c to homologous bacterial esterases. The regions surrounding the three catalytic amino acids and the less conserved aspartate (Asp90) used as a control are shown.

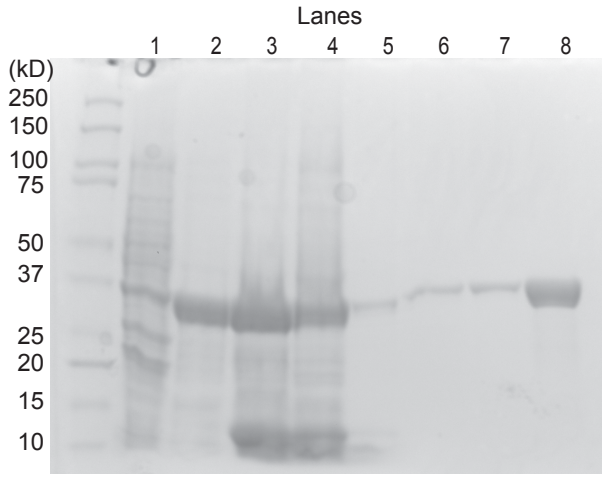
Bacterial species	Residues 77-90*	148-157	246-254	275-280	% Identity
Francisella tularensis	H G G G W C L G S I N T Y D	M G D S A G G N L V	T H D I L I D G I Y E	M F H G F	
Francisella novicida	H G G G W C L G S I N T Y D	M G D S A G G N L V	T H D I L I D G I Y E	M F H G F	98
Francisella philomiragia	H G G G W C L S S I D N Y D	M G D S A G G N L V	T H D I L I D G I Y E	M Y H G F	85
Clostridium botulinum	H G G F W I G G N V D T I D	V G D S A G G N L S	E I D P L R D E G E G	I T H G F	27
Listeria monocytogenes	H G G G F V L G G L Q T H D	A G D S V G G N L A	E F D P L R D Q G E K	V P H G F	21
Sulfolobus solfataricus	H G G G F V I G D V E S Y D	A G D S A G G N L A	E Y D P L R D Q G E N	V I H G F	29
Archaeoglobus fulgidus	H G G G F V I C S I E S H D	G G D S A G G N L A	E Y D P L R D E G E G	V L H G F	23
Pseudomonas aeruginosa	H G G G Y V V G S L D S H D	A G D S V G G S L C	E C D P L H D E G I G	M T H D F	20
Pseudomonas putida	H G G G Y V V G S L D S H D	V G D S V G G S L A	E C D P L H D E G I G	V T H D F	27
Oceanicola batsensis	H G G G W I Q G S I E T H D	G G D S A G G N L A	G H D P L W D E G L G	Q V H G F	28
Mycobacterium tuberculosis	H G G G W T L G D L D T H D	G G D S A G G N L S	G F D P L R D E G E S	L T H G F	26
Mycobacterium tuberculosis	H G G G W S L G G L D T H D	A G D S A G G N I S	E H D P L R D D G A T	M V H G Y	22
Deinococcus maricopensis	H G G G F V A Y D I D T H D	A G D S A G A N L A	E F D P L R D Q G R G	L I H G F	25
Bacillus megaterium	H G G G W V L G S L D T H D	G G D S A G G N L A	Q Y D P L R D V G K T	M I H G F	28
Alicyclobacillus acidocaldarius	H G G G W V V G D L E T H D	G G D S A G G N L A	Q Y D P L R D V G K D	L I H G F	28
Shewanella frigidimarina	H G G G G V I G S I N T H D	G G D S A G G Y L A	G Y D P L R D D G I D	C M H G F	24
Aspergillus oryzae	H G G G W V L G N I N T E N	G G S S A G G N L A	E L D V L R V E G E G	M P H P F	25
Penicillium chrysogenum	H G G G W V L G N I D T E N	G G S S A G G N L A	E L D V L R T E G E G	M P H P F	24

*Numbering refers to the amino acid position in FTT941c. All amino acid alignments performed using Clustal Omega.

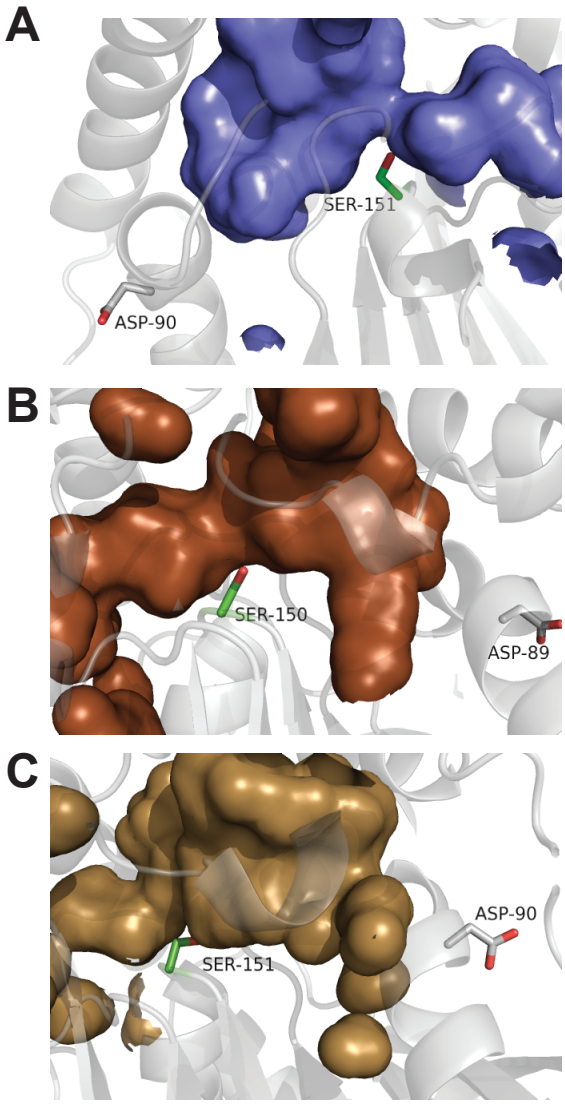
Supplemental Table 3: PCR primers used for site-directed mutagenesis.[a]

FTT0941c Variant	Primer nucleotide sequence
D90A	5'- GGT TCG ATA AAT ACT TAT GCT CAT GTA TGT AGG TAC C -3'
S151A	5'- GCT GAA AAT ATC TTT GTA ATG GGT GAT GCC GCT GGA GGT AAT CTA GTG AC -3'
D248A	5'- GTA GCA GCT ACT CAT GCT ATC CTA ATA GAT GG -3'
D252A	5'- GCT ACT CAT GAT ATC CTA ATA GCT GGT ATT TAT GCT TAT G -3'
H278A	5'- CGA TGA TGA AAT GTT TGC TGG TTT CAT GGG CAT ACT TGG -3'

[a]Primers for mutagenesis are one of the two complementary primers used in the mutagenesis reaction. Mutagenic PCR reactions were subjected to the following thermal cycle using a BIO-RAD MyCycler™: 1) initial denaturation at 95°C for 30 s, 2) denaturation at 95°C for 30 s, 3) annealing at temperatures between 52 – 60°C for 60 s, 4) extension at 68°C for 14 min. Steps 2-4 were repeated 18 times.



Supplemental Figure 1: Purification of FTT0941c. An SDS–PAGE gel (4-20%) showing the purification and final purity of FTT0941c. Lanes shown are (1) before induction with IPTG, (2) after induction with IPTG (0.5 mM), (3) supernatant after centrifugation, (4) Ni-NTA column flowthrough, (5) imidazole wash (10 mM), (6) imidazole wash (25 mM), (7) imidazole wash (50 mM), and (8) protein elution (250 mM imidazole). The expected molecular weight of FTT0941c is 35.5 kDa. The molecular weight was confirmed by comparison to the Kaleidoscope prestained protein standard (Bio-rad laboratories).



Supplemental Figure 2: Active site of FTT041c and relative positioning of Asp90. Zoomed in images of the binding pocket of FTT0941c from the homology models from Figure 3. In each model, the surface accessible binding pocket is colored and the nucleophilic serine and Asp90 are shown in sticks and labeled. A) Homology model of FTT0941c from Swiss-Model. B) Homology model of FTT0941c from RaptorX. C) Phyre2 heuristic model of FTT0941c.