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Supporting information for article:

Structure of the type VI secretion phospholipase effector Tle1 provides insights into its hydrolysis and membrane targeting

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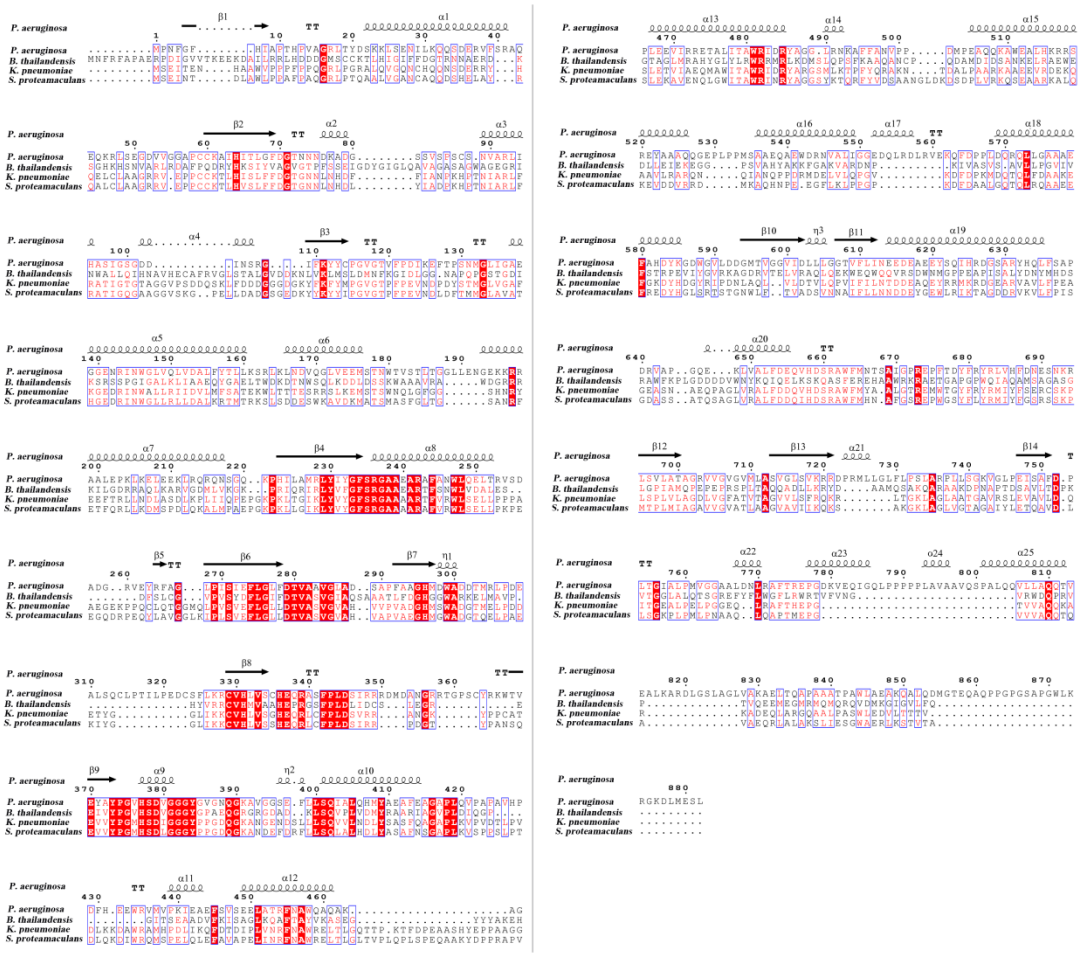

Figure S1 Structure-based sequence alignment for Tle1 family members. They are from *Pseudomonas aeruginosa* (*P. aeruginosa* PAO1, NP_251980), *Burkholderia thailandensis* (*B. thailandensis* E264, YP_443213), *Serratia proteamaculans* (*S. proteamaculans* 568, YP_001478026) and *Klebsiella pneumoniae* (*K. pneumoniae* subsp. pneumoniae DSM 30104, YP_005954437), and the alignment is performed using clustal X (version 1.81) and ESPript 3.0 (http://espript.ibcp.fr). The conserved residues are boxed in blue, identical conserved and low conserved residues are highlighted in red background and red letters, respectively.

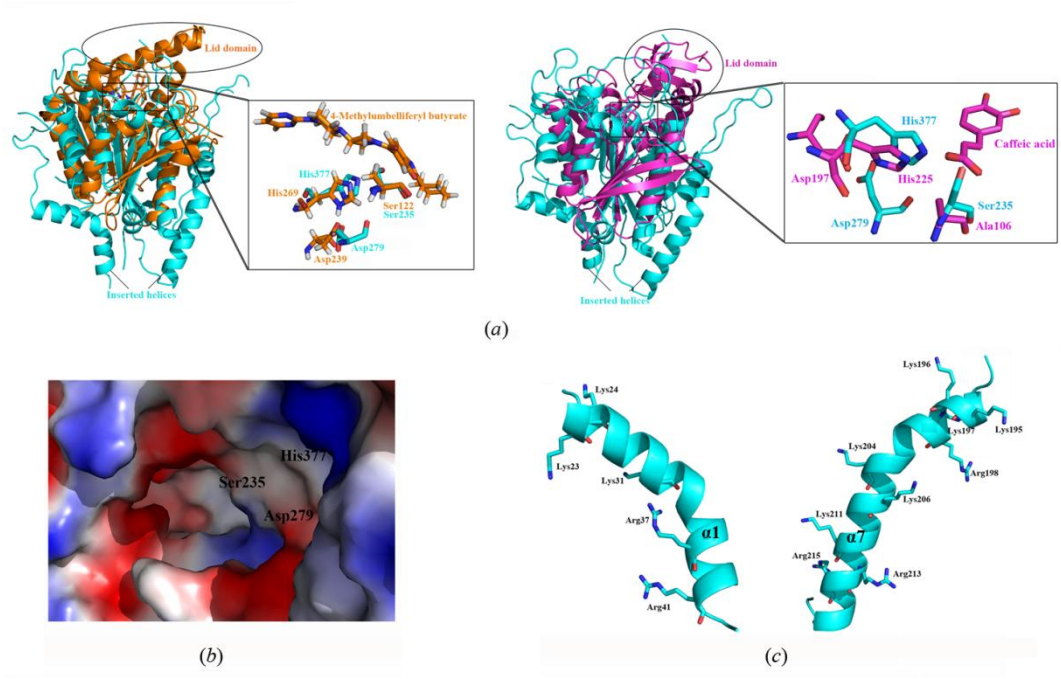

Figure S2 Characteristics of the catalytic module in Tle1. (**a**) Structural superposition of the phospholipase catalytic module (cyan) with hMGL in complex with its inhibitor methyl arachidonyl fluorophosphonate (orange, PDB entry 3PE6), and with LJ0536 in complex with caffeic acid (magenta, PDB entry 3S2Z), related to Fig. 2a. (**b**) The molecular surface representation of the catalytic pocket (blue, +7.1 KT; red, -7.1KT) colored by their local electrostatic potential. The relative locations of the catalytic triad Ser235-Asp279-His377 are labeled. (**c**) Distribution of the basic residues (cyan sticks) in the inserted helices α1 and α7 in the catalytic module of Tle1.

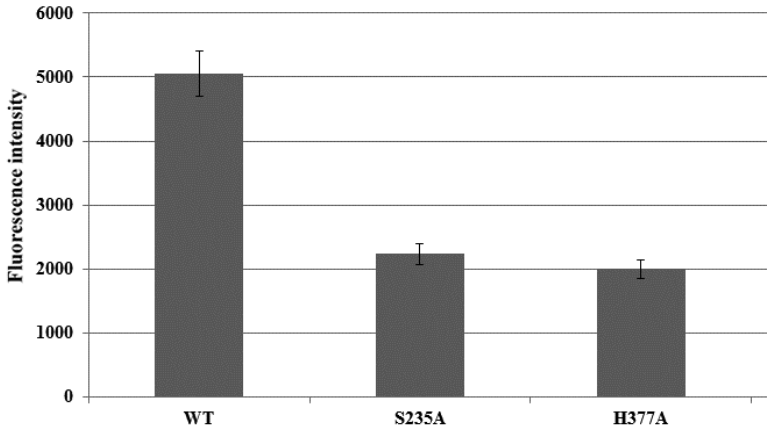

Figure S3 Enzymatic activity assay of purified S235A, H377A and wild-type of Tle1 (5 mg/mL) under the same conditions with those in Fig. 1*c*. The fluorogenic intensities were determined after 30 minutes. Error bars represent standard deviation from three independent experiments.

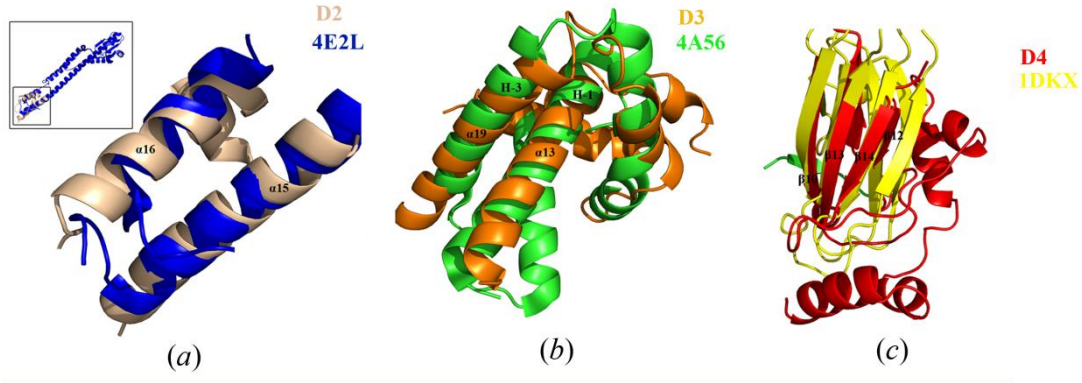

Figure S4 Structural superpositions of the domains D2-D4 with their analogues. (**a**) Superposition of D2 (wheat) with the periplasmic protein PCP (PDB entry 4E2L, chain G, blue). (**b**) Superposition of D3 (orange) with the lipoprotein OutS (PDB entry 4A56green) from bacterial T2SS. (**c**) Superposition of D4 (red) with the substrate-binding domain of DnaK (PDB entry 1DKX, yellow) binding a polypeptide segment (green).

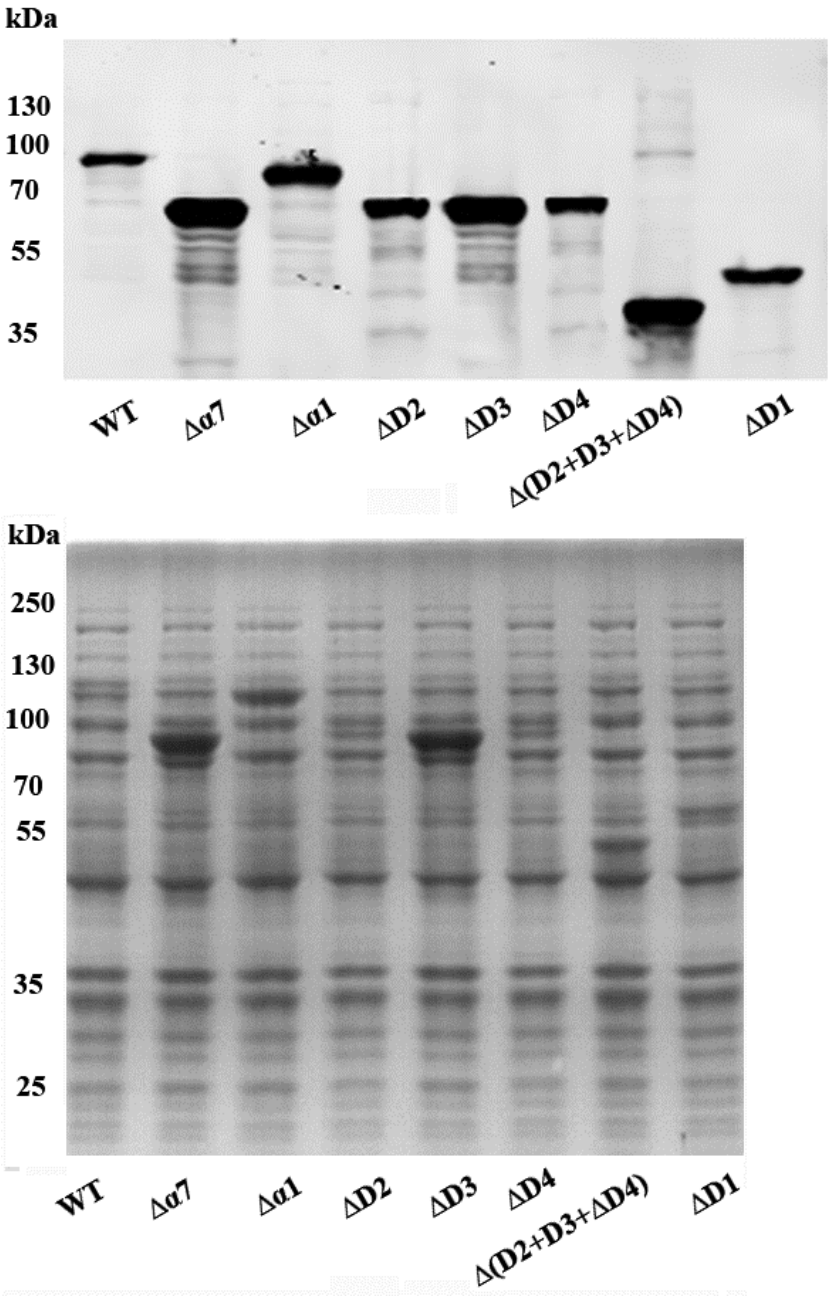

Figure S5 Determination of the expression levels of Tle1 truncations in the periplasm. Upper: Western blot analyses of *E. coli* expressing the full-length and truncations of His-tagged Tle1 directed to the periplasm, under induction identical to Fig. 5. Lower: Control: SDS-PAGE analysis of the expression of the full-length and truncations of Tle1 in the periplasm above visualized by Coomassie Blue staining.

Table S1 Structural similarity searches of the full-length and D1 of Tle1 performed by the DALI web server.

Full-length.

NO.	PDB entry	PDB description	Z score	rmsd	%id
1	3s2z-A	CINNAMOYL ESTERASE	10.9	3.1	11
2	2wtm-A	EST1E	10.8	3.4	11
3	4ke7-B	THERMOSTABLE MONOACYLGLYCEROL LIPASE	10.8	3.3	12
4	4fbl-D	LIPS LIPOLYTIC ENZYME	10.5	3.2	12
5	4ke7-B	THERMOSTABLE MONOACYLGLYCEROL LIPASE	10.5	3.3	12
6	3ksr-A	PUTATIVE SERINE HYDROLASE	10.5	4.3	8
7	3pe6-A	MONOGLYCERIDE LIPASE	10.4	3.3	9
8	3hju-B	MONOGLYCERIDE LIPASE	10.4	3.3	10
9	4ke6-D	THERMOSTABLE MONOACYLGLYCEROL LIPASE	10.4	3.4	10
10	4fbm-B	LIPS LIPOLYTIC ENZYME	10.4	3.4	13

D1.

NO.	PDB entry	PDB description	Z score	rmsd	%id
1	3ksr-A	CINNAMOYL ESTERASE	11.6	4.2	9
2	3f98-C	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE	11.5	3.9	11
3	2wtm-A	EST1E	11.4	3.3	10
4	3s2z-A	CINNAMOYL ESTERASE	11.4	3.2	11
5	3pf8-B	CINNAMOYL ESTERASE	11.4	3.3	12
6	1tqh-A	CARBOXYLESTERASE PRECURSOR	11.2	3.3	12
7	4fbl-B	MONOGLYCERIDE LIPASE	11.1	3.2	13
8	3f9c-A	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE	11.0	4.1	11
9	3aim-D	THERMOSTABLE MONOACYLGLYCEROL LIPASE	10.8	3.7	8
10	2wtm-D	EST1E	10.8	3.4	10