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Supporting information for article:

Structural and functional studies of SAV1707 from *Staphylococcus aureus* elucidate its distinct metal-dependent activity and a crucial residue for catalysis

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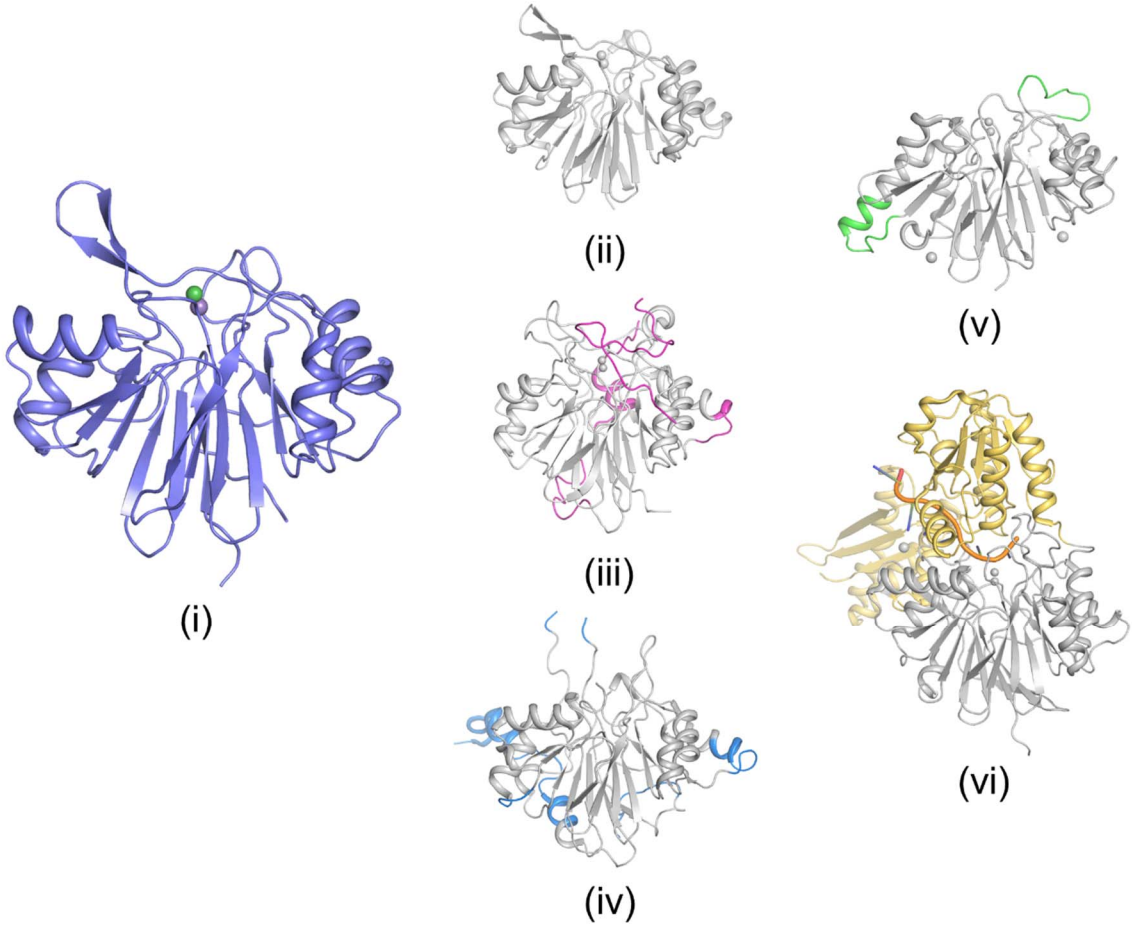

Figure S1 Homolog search of SAV1707 using the Dali server. The similar MβL domain is colored in gray (i) Monomeric structure of SAV1707 (PDB entry 7e3v) colored in light blue (ii) MβL from *Thermotoga maritima* (PDB entry 3x30; Z-scores of 36.9, r.m.s. deviations of 1.2 Å, and a sequence identity of 48% for 224 equivalent Cα pairs) in gray (iii) NAPE-hydrolyzing phospholipase D from *Homo sapiens* (PDB entry 4qn9; Z-scores of 24.8, r.m.s. deviations of 2.3 Å, and a sequence identity of 23% for 225 equivalent Cα pairs) in gray and pink (iv) L-ascorbate-6-P lactonase from *E. coli* (PDB entry 2wyl; Z-scores of 24.4, r.m.s. deviations of 1.9 Å, and a sequence identity of 21% for 214 equivalent Cα pairs) in gray and marine (v) MβL fold phosphodiesterase from *Bacillus subtilis subsp. spizizenii* (PDB entry 6kns; Z-scores of 20.9, r.m.s. deviations of 2.5 Å, and a sequence identity of 16% for 203 equivalent Cα pairs) in gray and light green (vi) RNase J from *Deinococcus radiodurans* (PDB entry 4xww; Z-scores of 20.8, r.m.s. deviations of 2.6 Å, and a sequence identity of 17% for 206 equivalent Cα pairs) in gray and yellow.

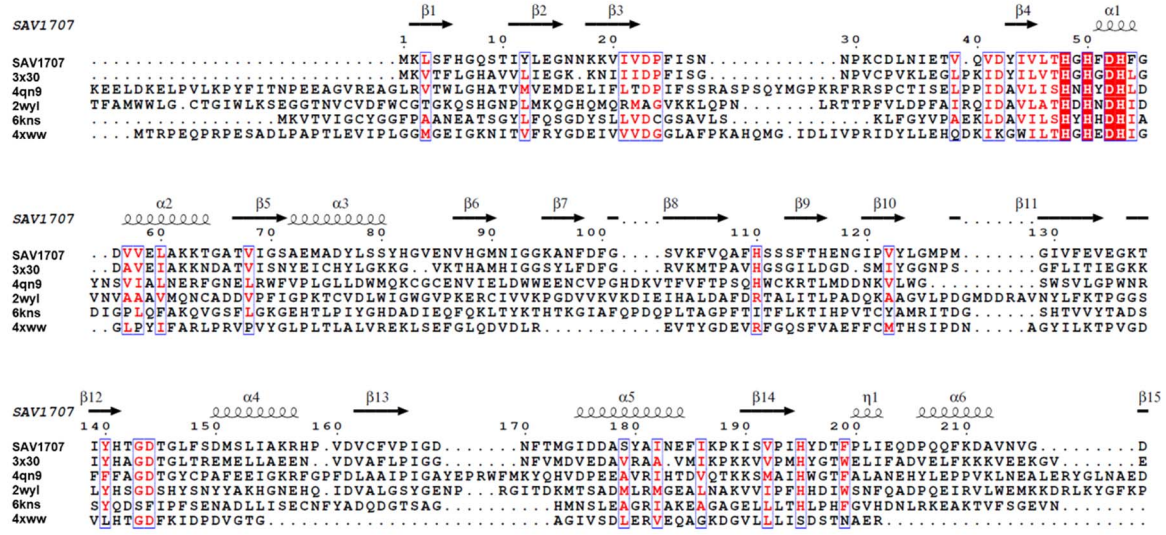

Figure S2 Multiple sequence alignment of homologs was performed using ClustalW and ESPript. The conserved residues and similar residues are presented in white font in the red-filled box and red font in the unfilled blue box. Although they have a high structural similarity with SAV1707, the alignment shows that only the H-X-H-X-D-H motif (located in His48, His50, Asp52 and His53 of SAV1707) is conserved in all homologs.

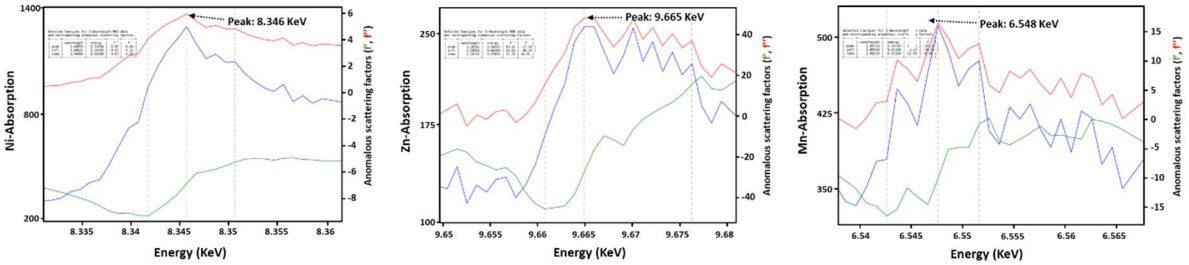

Figure S3 X-ray absorption spectroscopy of Ni^{2+}, Zn^{2+}, and Mn^{2+} ions. All experiments were performed using SAV1707 crystals. The Ni^{2+}, Zn^{2+}, and Mn^{2+} absorption edges were measured at beamline PAL-11C (MX) in Pohang Light Source. Red and green lines indicate anomalous scattering factor f'', f', respectively, and blue line indicates absorption intensity.

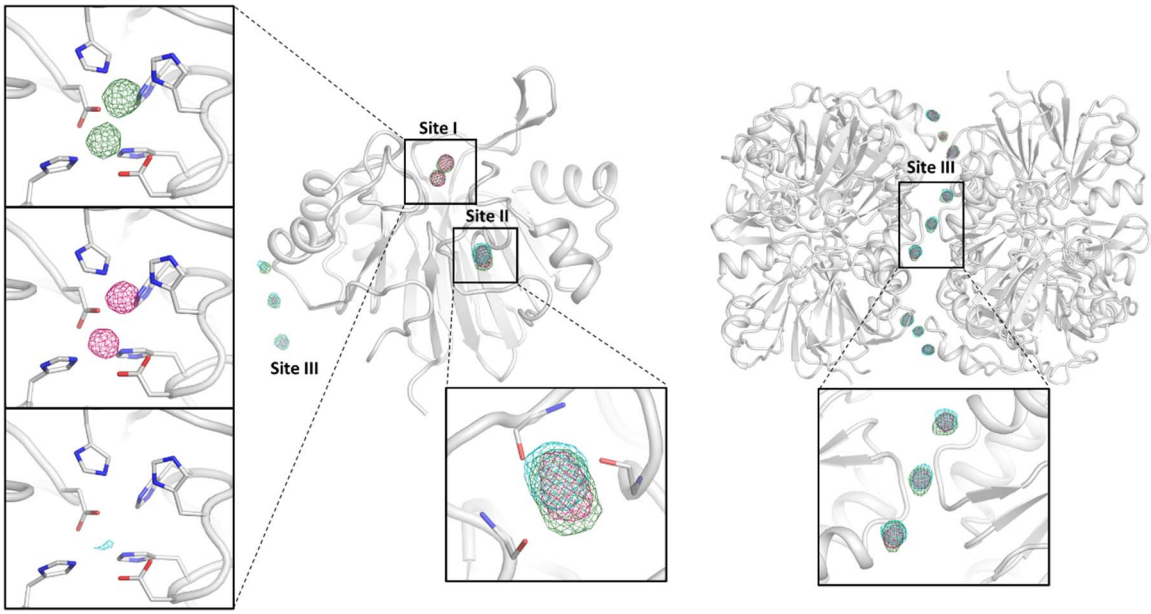

Figure S4 The anomalous difference maps in SAV1707 structure. Anomalous difference maps derived from anomalous datasets of Ni^{2+}, Zn^{2+}, and Mn^{2+} are shown as green, magenta, and cyan meshes, respectively. The maps were contoured at 7.0 σ in Site I and II (cyan map in Site I was contoured at 3.0 σ), and at 4.0 σ in Site III.

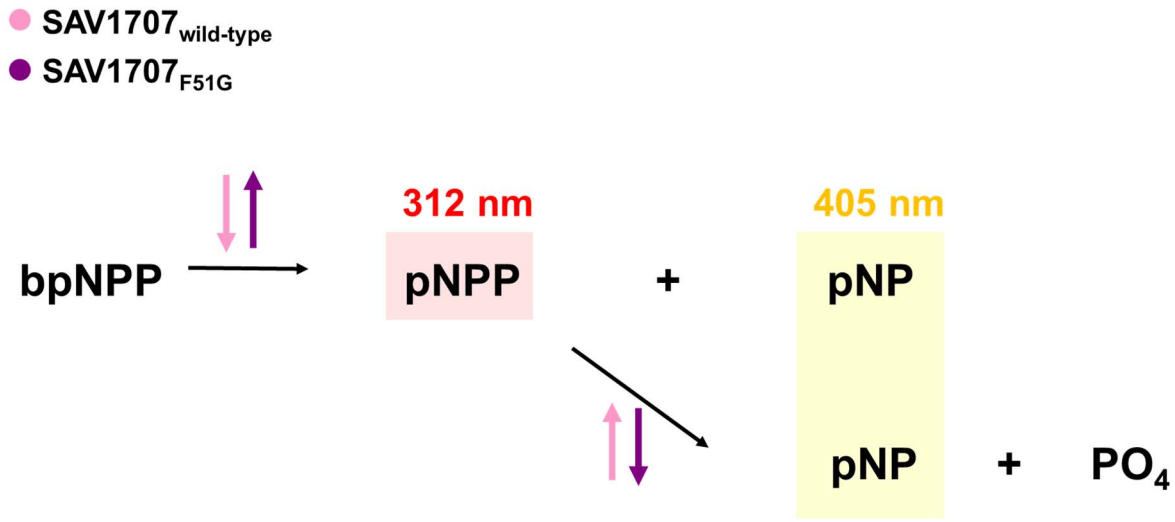

Figure S5 Relative catalytic activity of $SAV1707_{wild\text{-}type}$ and $SAV1707_{F51G}$. The arrows indicate the relative activity.

Table S1 Metal-binding interactions in the active site.

Residues	Metal	Distance (Å)
His48 (NE2)	M1	2.2
His50 (ND1)	M1	2.3
His110 (NE2)	M1	2.1
Asp144 (OD2)	M1	2.4
W1 (O)	M1	2.1
W2 (O)	M1	2.3
Asp52 (OD2)	M2	2.2
His53 (NE2)	M2	2.1
Asp144 (OD2)	M2	2.2
His195 (NE2)	M2	2.2
W1 (O)	M2	2.1
W3 (O)	M2	2.3

Table S2 ICP-MS analysis. ND indicates “not detected”, NA indicates “not analysed”.

Metal	Buffer for purification	SAV1707 protein solution	Crystals dissolved in the water
Manganese (II)	ND	5017	2229
Nickel (II)	ND	15025	5332
Zinc (II)	ND	8241	9547
Calcium (II)	ND	ND	NA
Magnesium (II)	ND	ND	NA

Table S3 Anomalous data collection statistics.

Data Sets	**Mn peak**	**Ni peak**	**Zn Peak**
X-ray wavelength (Å)	1.8935	1.4856	1.2828
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell length (a, b, c, Å)	70.45 121.12 187.38	70.21 121.75 187.08	70.21 121.74 187.17
Unit cell angle (α, β, γ, °)	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00
Resolution range (Å)	30.00–2.35 (2.39–2.35)[a]	30.00–2.25 (2.29–2.25)[a]	30.00–2.10 (2.14–2.10)[a]
Total / unique reflections	1,157,737 / 67,525	1,339,867 / 76,088	1,669,695 / 93,529
Completeness (%)	99.9 (99.9)[a]	99.9 (98.9)[a]	99.9 (100.0)[a]
$CC_{1/2}$[b]	0.975 (0.882)[a]	0.995 (0.929)[a]	0.986 (0.946)[a]
Redundancy	17.1 (16.2)[a]	17.6 (14.9)[a]	17.9 (17.8)[a]
I / σ_I	23.9 (4.5)[a]	27.8 (3.1)[a]	26.5 (4.5)[a]
R_{merge}[c]	0.226 (0.660)[a]	0.158 (0.682)[a]	0.150 (0.613)[a]

Table S4 Enzymatic assays for functional screening of SAV1707.

Enzyme type	Substrate	Reaction mixture	Absorbance	Activity
β-lactamase	Nitrocefin	50 mM Tris, pH 7.5 200 mM NaCl 1 mM nitrocefin	485 nm	X
Esterase	*p*-Nitrophenyl palmitate (pNP-palmitate)	50 mM Tris, pH 8.0 0.4% Triton X-100 1 mM pNP-palmitate	405 nm	X
Lactonase	Dihydrocoumarin	50 mM Tris, pH 8.0 1 mM 3,4-dihydrocoumarin	270 nm	X
Phosphodiesterase	Bis-*p*-nitrophenyl phosphate (bpNPP)	50 mM Tris, pH 7.5 1 mM bpNPP	405 nm	O
DNase	*SAV1707* genomic DNA	20 mM Tris, pH 7.0 150 mM NaCl 200 ng/µl genomic DNA	-	O
RNase	RNase Alert Kit (IDT)	-	Fluorescence Ex/Em=490/520	O

Enzyme assays using different substrates were performed to identify enzymatic activities of SAV1707. The assays were carried out based on the functions of structurally similar enzymes to SAV1707 protein. All products for assays were purchased from Sigma-Aldrich (Germany).