SUPPLEMENTARY INFORMATION

Structure and assembly of the S-layer in C. difficile

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Supplementary Fig. 1: H/L complex topology

Topology of the mature SIpA_{CD630} H/L complex, retrieved from structure analysis with PBDsum and Coot. SLP_L represented in gold and SLP_H coloured in blue. Colour shading represents different domains/motifs. Numbering of secondary structure components based on PDB ID 7ACY, subscripts indicate the relevant subunit. D1 – SLP_L domain 1, D2 – SLP_L domain 2, LID – SLP_L interacting domain, HID – SLP_H interacting domain, CWB2₁ – cell wall binding domain 1, CWB2₂ – cell wall binding domain 2, CWB2₃ – cell wall binding domain 3.



Supplementary Fig. 2: Charge distribution across CWB2 motifs in CWP6 and CWP8, two minor components of the *C. difficile* S-layer

Comparison of the Poisson-Boltzmann electrostatic potential calculated for CWB2 motifs from Cwp6 (a) and Cwp8 (b). The triangular CWB2 motifs of each CWP were superimposed onto the SlpA_{CD630} CWB2s to determine orientation of Cwp6 and Cwp8. Views are shown from the extracellular and cell wall surfaces, followed by side views of the lateral faces defined by two interacting CWB2s, as per SlpA_{CD630} H/L complex orientation at the cell surface. Electronegativity gradient: positive in blue to negative in red.



Supplementary Fig. 3: Residues required for H/L complex

a, Key interactions identified at the interface of the LID/HID complex from strain R7404 (SLCT-7b) (left; identified with PDBePISA in PDB ID: 7ACW) informed site directed mutagenesis for functional assessment.

b, Effects of point substitution mutations in SLP_L (left) or SLP_H (right) on complex formation were tested by ELISA. Graphs represent mean ± SD of n = 3 experiments, with least squares curve fit. Source data provided in Source Data file.



	CWB2 ₁					CWB2 ₂					CV	VB2 ₃
	α5	β6	α6	β7			β8	α8	β9	α9	β10	aĭ0
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SLCT11_strain_0x247	VVTELESMGL	KVERFSGDDRY KVTRLSGFDRY	ETSLKIADEIGL	D. NDKAYVVGG	GLADAMSIASVAS	TKLDGNGVVDRTNGHA	TPIVVVDGKAD	K I S D D L D S F L G S	S.ADVDIIGGF	ASVSEKMEEALSDAT	GKGVTRVKGDDRQ	DINSEVIKT. YYAN
SLCI/D_Strain_R/404		KVIRLSGEDRY KVTRISGDDRY	ATSIETADETGL		GLADAMSIAPVAS		TRIVVVDGKAK		AOVDIIGGKI	NSVSKEIEESIDSAI	GKTPDRISGDDRU	
SICT6 strain 19123		KVTRI SGDDRV	ATSIETADETGI		GLADAMSTAPVAS		TPTVVVDGKAK	FISSAAFDEIDI	SOVDITECK	SVSKDMEDATDDAT	GKSPNRVSGDDRO	
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SLCT2_strain_0x858	VENELKDMGL	KVTRLSGDDRY	ETSLAIADEVGLI	D.NDKAFVVGGT	GLADAMSIAPVAS	QL KKSNGDLDVVDG DA	TPIVVVDGKAK	TINNETEDELNI	N. AQVDIIGGE	NSVSKDVEKSIDDAT	GKEPNRTSGDDRQ	ATNAEVMKETDYFEK
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SLCT12_strain_CD062	VQKAIEDMGV	KVERLSGDDRY	ATSLKIADKVELI	NDKDKAFVVGGT	GLADAMSIAPVAS	Q LV	TPIVVVDGKAD	KLSSDASDELDS	SAKEVDIIGGE	NSVSNKVKDSIKDAI	GRSVDRISGDDRO	ATNAÈVIKEYYEN
SLCT9_strain_TL178	VÄNELKDMGL	KVERLSGDDRY	ATSLEIADEIGLI	N . H N K V F V V G G T	GLADAMSIASVAS	ŇKE	M P I V V V D G K G K	DLSTDAKDFIGS	S.AY VDIIGG KS	SVSEDMEDAIDDAT	GKSPERVSGDDRQ	DTNAEVIKT YFEK
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SLC77_strain_CD630 SLC77_strain_CD630 SLC71_strain_Ox247 SLC75_strain_Ox1437a SLC75_strain_Ox1437a SLC76_strain_01437a SLC76_Strain_01437a SLC71_strain_01910 SLC71_strain_0x58 SLC74_strain_R20291 SLC78_strain_0x1396 SLC710_strain_liv22 SLC73_strain_0x121 SLC712_strain_C0662 SLC79_strain_L178 SLC713_strain_0142	DTEIAKAAVL	CLASS GASSSDA CLASS GASSSDA GG GG GG GG GG GG GG GG GG GG GG GG GG	β11 290 300 EVVNYFVAKDGS GVENFYVAKDGS GVENFYVAKDGS AV. AV. NYFVAKDGS AV. NYFVAKDGS AV. NYFVAKDGS SVINFVAKDGS SV SVINFVAKDGS SV SVINFFVAKDGS SV SVINFFVAKDGS SV SVINFFLAKDGS SV SVNFFLAKDGS SV SVNFFLAKDGS SV SVNFFLAKDGS SV GVKNFFVAKDGS GVKNFFVAKDGS GVKNFFVAKDGS GVKNFFVAKDGS	η4 0 310 ΤΚΕΡΟ Ι ΥΔΑΙΑΥ ΤΚΕΡΟ Ι ΥΔΑΥΑΥ	CWB23 APIA GRF APIA GRF APIA GRF APIA GRF APVAA HFE APVAA HFG TYN I AAIA GNFG YTVDN APVAANFG STYDO APVAANFG STYDO APVAANFG STYDO APVAANFG STYDO APVAANFG STYDO AAVA GNFG SKHO AAVA GNLG LSAG AAVA GNLG LSAG AAVA GNLG LSAG	K D K D E G K P T V A D E G K P T V A D D G K P V D K D G K V L T G S D D G D Q D E D	320 	β12 330 VULATDSLSSD VULATDSLSSD IILATDSLSSD IILATDSLSSD IILATDSLSSD IILATDSLSSD IVLATDSLSSD IVLATDSLSSD IILATDNLSSE IVLATDNLSSE IVLATDNLSSE IVLATDSLSSD	AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2	β13 350 266TNLVQVGKGT 266TNLVQVGKGT 358.60NLVQVGGGT 368.60NLVQVGGGT 368.60NLVQVGGGT 368.60NLVQVGGGT 369KNLVQVGGGT 369KKLVQVGGGT 369KKLVQVGGGT 369KKLTQVGKT 369KKLTQVGKT 369KKLTQVGKT 369KKLTQVGKT	413 379 ASSVINKMKDLLD ANSVINKMKDLLD ASSVINKMKDLLD ASSVINKMKDLLD ASSVIKKKLLD ASSVIKKLKDLLD ASSVIKKLKDLLS ASSVIKKLKDLLS ATSVYKLKDLLS ADSVVKLKDLLG ADSVVKLKDLLG ADSVIKLKDLLG ADSVIKKLKDLLG ADSVIKKLKDLLG ADSVIKKLKDLLG ADSVIKKLKDLLG	Key – salt bridge ⑨ helix-helix ● hydrogen bond ♦ SLCT-specific
SLC77_strain_CD630 SLC77_strain_CD630 SLC71_strain_R7404 SLC75_strain_R7404 SLC75_strain_D123 SLC76_fH2_strain_D123 SLC76_fH2_strain_D108 SLC71_strain_D108 SLC74_strain_0x358 SLC74_strain_0x1306 SLC710_strain_liv22 SLC73_strain_Ox121 SLC712_strain_CD625 SLC712_strain_CD625 SLC713_strain_19142	DTĖIAKAAVL NL DN KD	DKDSGASSSDA G DKDSGASSSDG G G NNDK G S NNDK S S S S S S S S S S S S S S S S S S S	β11 190 300 EVVNYFVAXDGS GVFNFYVAXDGS GVFNFYVAXDGS AV.NYFVAXDGS AV.NYFVAXDGS AV.NYFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFVAXDGS SVINFFLAXDGS GVKNFFVAXDGS GVKNFVAXDGS GVKNFVAXDGS GAE DFFVAXDGS	η4 00 310 TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA TKEDQLVDALA	CWB23 COUCCO CAPYAGYKL APYAGYKL APYAAFFE APYAAFFGTTVNI APYAAFGTTVNI APYAAFGSTTVG APYAAFGSTTVG AAYAGNFGSTTVG AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE AAYAGNFGSTKNE	K D	320 	AL2 330 VVLATDSLSSD VVLATDSLSSD IILATDTLSSD IILATDSLSSD IILATDSLSSD IVLATDSL	AL2 AL2 AL2 AL2 AL2 AL2 AL2 AL2	β13 350 560	a13 370 ASSVINKICLLD ANSVINKICLLD ANSVINKICLLD ANSVINKICLLD ANSVINKICLLD ASSVINKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLD ASSVIKICLLLD ASSVIKICLLLD ASSVIKICLLLD ASSVIKICLLLD ASSVIKICLLLD	 Key salt bridge Ø helix-helix hydrogen bond ♦ SLCT-specific

Supplementary Fig. 4: Sequence alignment of representatives of each S-layer cassette type (SLCT)

Secondary structural elements identified in the H/L CD630 complex (PDB ID: 7ACY) are indicated by looped lines – α -helices and arrows – β -

sheets. SLP_L D1, D2 and LID domains and SLP_H HID domain and CWB2 motifs are highlighted, coloured as in Fig. 1. Residues involved in

interactions are marked as per key (see Supplementary Fig. 5 for details), with interacting residues marked with the same colour. Strictly

conserved residues across all SLCTs are highlighted in black background, partially conserved groups are delimited by a box, with residues

conserved within each group highlighted in bold, as per default in ESPript3 (http://espript.ibcp.fr).

SLCT7 - strain CD630*; SLCT1 - strain 1912; SLCT2 - strain Ox858; SLCT3 - Ox1121; SLCT4 - strain R20291*; SLCT5 - Ox1437a; SLCT6 - strain 19123; SLCT6/H2 - strain M120; SLCT7b - R7404*; SLCT8 - Ox1396; SLCT9 - strain TL178; SLCT10 - strain Liv22; SLCT11 - strain Ox247; SLCT12 strain CD062; SLCT13 - strain 19142.

*indicates strains with structural models included in this work.



Supplementary Fig. 5: Interactions between neighbouring molecules in S-layer packing

a, Details of the interaction network between 7 SlpA_{CD630} H/L complexes within the 2D array, with each molecule represented as cartoon, interacting residues as sticks (coloured as in schematic for molecule 1, white for neighbouring residues, with molecule number identifier

in parenthesis) and interactions as dashed lines. The interface depicted in each panel is marked by a corresponding box within the array representation

b, Clustermap of predicted conservation across known SLCTs for sidechain-sidechain interactions found in SlpA_{CD630} H/L. Representatives of each SLCT were aligned (Supplementary Fig. 4), SWISS-MODEL structural homology models were generated and superimposed. The residues corresponding to interactions identified in SlpA_{CD630} H/L were analyzed and interaction conservation compared to SLCT-7 was depicted based on residue conservation and prediction of similar or different type of possible interaction. Key: 0 – no residue conservation, unstructured region; 1 – one conserved residue, unstructured region;
2 – no residue conservation, no interaction; 3 – one conserved residue, no predicted interaction; 4 – no residue conservation, different interaction type; 5 – one conserved residue, different interaction type; 6 – no residue conservation, same interaction type; 7 – one conserved residue, same interaction type; 8 – residues and interaction conserved.









Fitted D2 - SlpA_{R2D291}, SlpA_{RAD2}



Fitted D2 SlpA_{CD630}



Fitted D2 - SIpA_{R20291}



Fitted D2 - SlpA_{R20291}, SlpA_{RAD2}



Supplementary Fig. 6: Rotation of D2 domain allows optimised fit of H/L X-ray structure

into the *in situ* packing of the native S-layer

Fitted D2 SLPL

a, H/L planar crystallographic array (left, PDB ID 7ACY, cartoon representation, SLP_L - gold,

SLP_H - slate blue, views as defined in Fig. 1b) fitting into the 3D reconstruction of negatively

stained S-layer ghost indicating the overall envelope in the native lattice (grey map, middle). Right panels show the native S-layer reconstruction as red chicken wire, overlayed on grey map for the 3D reconstruction from $SlpA_{R\Delta D2}$ ghosts after rigid body fitting of the X-ray models. Reconstructions and arrays are shown from the environment (top panels) and side views in the 2D plane (bottom panels).

b, Rotation of SLP_L domain D2 by 11° towards the D1 domain allows for an improved fitting of H/L array into the native S-layer. The overall crystal packing is maintained (left, colours and views as in **a**) and a more compact SLP_L fits the native lattice (middle panel), matching most of the density of the ridges, as seen in the side views (bottom panels). Comparison of the SlpA_{RΔD2} (right, grey surface) and SlpA_{R20291} (right, red chicken wire) reconstructions with the fitted D2 model further demonstrates that the missing density in the mutant S-layer reconstruction corresponds to the deleted D2 domain.

c, Superimposition of the crystallographic H/L complex (top left, grey) and the fitted (top left, coloured as in **a** and **b**) models shows a similar overall fold of the complex, with negligible reorientation in SLP_H, D1 and LID/HID (RMSD between original and fitted subdomains is <1Å, see Methods for details) but a significant rotation of D2 towards D1 by 11°.

Zoomed views of the structural models with original (top) and reoriented D2 domains (bottom) fitted into $SlpA_{R20291}$ (middle panels) and $SlpA_{R\Delta D2}$ (left panels) S-layer ghosts, as presented in **a** and **b** illustrate how a more compact packing is possible.

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Supplementary Fig. 7: Absence of D2 does not affect overall structure and packing
a, 2D tiling representation of SlpA_{RΔD2} assembly in crystal packing (PDB ID: 7ACZ), with
identified interactions represented as symbols defined in the key (as in Fig. 3).
b, Cartoon representation of the SlpA_{RΔD2} array (SLP_L coloured in gold and SLP_H in slate blue)
in top and a side view (as defined in Fig. 3).



Supplementary Fig. 8: Cryo-electron microscopy of wild type and SlpA_{RΔD2} S-layer ghosts. a, Projection map of frozen hydrated native S-layer ghost from strain R20291 at 8.7 Å resolution. Contours represent density greater than mean density, contour interval 0.5 RMS density, as per gradient (0 – blue to pink – 250). **b**, Superimposition of the reconstruction of negatively stained native S-layer (grey surface) on the projection in **a**.

c, Projection map at 8.7 Å resolution of frozen hydrated *C. difficile* S-layer ghost containing SIpA_{RAD2}, depicted as in **a**.

d, Wild type minus SlpA_{RΔD2} difference projection map. Positive difference density corresponds to the projection of the ridge-like density in the 3D reconstruction shown in b.
e, Superposition of isolated domain D2 crystal structure (gold surface) on the difference projection map in d. Pseudo-symmetrically related structures are shown together in the central density.



Supplementary Fig. 9: Patches of S-layer present in cryo-electron tomographic slice of extracted S-layer ghosts

a, Distinct S-layer lattice patches can be seen in tomographic slices of S-layer ghosts, with 'fault lines' present where patches intersect (white arrows). Tomogram representative of 52 tomograms collected.

b, Annotated patches from **a** (dotted lines) have distinct orientations on the surface, with unit cell axes of the different lattices highlighted (black arrows). Scale bar: 25 nm.

	LID/HID	SLP _L /HID	H/L R7404 S-SAD	H/L R7404 ¹	H/L CD630	H/L R∆D2
Data collection						
Space group Cell dimensions	C2	C2	P21	P21	P1	P1
a, b, c (Å)	73.3, 56.7, 61.8	172.9, 29.5, 144.3	76.7, 134.7, 83.9	78.1, 137.9, 84.7	72.7, 78.3, 81.6	52.8, 80.4, 81.9
α, β, γ (°)	90.0, 122.7, 90.0	90.0, 94.2, 90.0	90.0, 100.8, 90.0	90.0, 100.7, 90.0	81.9, 67.0, 65.3	97.0, 90.2, 90.2
Wavelength (Å)	0.975	0.969	2.755	0.928	0.969	0.969
Resolution (Å)	41.74- 1.50 (1.55-1.50)	53.35-2.40 (2.49-2.40)	47.70-3.00 (3.16-3.00)	83.26-2.65 (2.75-2.65)	52.31-2.55 (2.64-2.55)	44.34-3.50 (3.63-3.50)
Ι/σΙ	8.1 (2.3)	5.8 (2.1)	16.5 (1.2)	12.1 (1.4)	7.0 (1.5)	10.0 (2.5)
CC1/2	0.998 (0.975)	0.992 (0.731)	0.998 (0.707)	0.712 (0.360)	0.992 (0.732)	0.627 (0.438)
Completeness (%) Redundancy	99 (97) 3.4 (2.6)	100 (100) 1.9 (1.9)	91 (80) 6.4 (6.1)	100 (100) 48.0 (43.0)	96 (97) 3.3 (3.4)	99 (97) 3.4 (3.5)
Anomalous completeness			91.2 (79.7)			
Anomalous multiplicity			3.3 (3.3)			
Refinement						
Resolution (Å)	41.74- 1.50	53.35-2.40		83.26-2.65	52.31-2.55	44.34-3.50
No. reflections	33987	29329		51027	47200	16606
Rwork / Rfree	18.1/21.0	25.3/29.9		22.6/27.8	24.3/26.5	25.5/27.6
No. atoms						
Protein	1709	3477		9945	10306	7890
Ligand/ion	-	-		40	15	-
Water	107	166		111	97	-
B-factors (Å ²)						
Protein	33.1	37.4		71.9	60.2	37.0
Ligand/ion	-	-		71.8	123.3	-
Water	37.6	33.1		59.4	46.0	-
Ramachandran %						
favoured	100.0	98.4		97.2	98.5	96.1
allowed	0.0	1.6		2.6	1.5	3.8
outliers	0.0	0.00		0.2	0.00	0.1
R.m.s. deviations						
Bond lengths (Å)	0.009	0.003		0.007	0.006	0.003
Bond angles (°)	1.14	0.58		1.19	1.01	0.60
PDB ID	7ACW	7ACV		7ACX	7ACY	7ACZ

Supplementary Table 1 - Data collection and refinement statistics

*Values in parentheses are for highest-resolution shell.

¹Two crystals were used to determine the structure of H/L complex R7404.

	SIpA _{R20291}	SIpA _{R∆D2}
3D merging statistics (EM reconstructions; negative staining)		
Resolution limit (Å)	20	20
No. structure factors	1085	667
Overall R-factor	0.33	0.33
Overall phase residual (°)	22.3	13.9
Phase residuals in CryoEM projections (p2-averaged Fourier terms)		
No. independent phases Resolution shell (Å) ∞ – 15 15 - 11 11 - 8.7 8.7 – 7.5	43 42 47 44	43 42 45
Mean value phase error [*] Resolution shell (Å) ∞ – 15 15 - 11 11 - 8.7 8.7 – 7.5	15.3 22.9 32.2 36.8	21.5 28.7 33.8
Standard error (°) Resolution shell (Å) ∞ – 15 15 - 11 11 - 8.7 8.7 – 7.5	2.4 2.8 3.6 3.9	3.5 4.1 3.6

Supplementary Table 2 - EM data and reconstruction statistics

*Mean value phase error against symmetry-imposed phase of 0° or 180° (45° is expected for random phases¹).

Plane Group ^{\dagger}	Phase residual (°) (random = 90°)	Target residual [‡] (°)
p1	27.0	-
p2*	37.5	39.7
p12b	77.6	29.8
p12a	55.1	29.8
p121b	57.2	29.8
p121a	51.0	29.8
c12b	77.6	29.8
c12a	55.1	29.8
p222	67.7	33.6
p2221b	52.7	33.6
p2221a	47.5	33.6
p2221a	62.2	33.6
c222	67.7	33.6
p4	46.8	33.6
p422	61.9	30.1
p4212	63.5	30.1
р3	52.5	27.0
p312	60.4	27.7
p321	63.0	28.5
p6	50.4	31.6
p622	58.1	29.3

Supplementary Table 3 – Symmetry table for cryo-EM reconstruction of SIpA_{R20291}

*Represents most likely plane group

⁺a and b represent the respective symmetry axis for the plane group

^{*}Target residual indicates the expected phase residual of each symmetry group based on the signalto-noise ratio of the respective reflections². Supplementary Table 4. Bacterial strains and plasmids used in x-ray crystallography and

Strain or	Description	Reference/ Source
plasmid		Application
C. difficile strain	IS	
CD630	Ribotype 012, SLCT-7	3
R20291	Ribotype 027, SLCT-4	4
FM2.5	R20291 slpA 282_283insA	5
R∆D2	FM2.5 <i>slpA</i> ∆D2	This study
R7404	Ribotype 017, SLCT-7b	6
E. coli strains	·	
NEB5a	fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17	New England Biolabs
BL21 (DE3)	E. coli str. B F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacl lacUV5- T7p07 ind1 sam7 nin5]) [malB+]K- 12(λS)	Novagen
Rosetta (DE3)	F- ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (CamR)	Novagen
Plasmids		
pOB001	pMTL960-Ptet- <i>slpA</i> ∆D2 (R20291)	This study. RF102/ RF103
nRPE170	nMTI 960-Ptet-s/n4 (CD630)	This study.
pm 1 1 7 0		NF1414/ NF1415
pRPF233	pMTL960-Ptet- <i>slpA</i> (R20291)	5
Plasmids for rec	combinant expression of mature protein	
		This study.
pABS17	pET28a-SLP∟-6xHis-tag (R20291)	SLP∟ of R20291;
		oABS46/ oABS47
nABS18	nFT28a-SI Pu-6xHis-tag (R20291)	This study. SLP _H of R20291;
	heisog-strilloxuis-rgg (ksnsat)	oABS44/ oABS45
pABS19	pET28a-SLP _L -6xHis-tag (R7404)	This study. SLPL of R7404; oABS1/ oABS2

protein-protein interaction studies.

pABS20	pET28a-SLP _H -6xHis-tag (R7404)	This study. SLP _H of R7404; <i>Ncol/Xho</i> I subcloning from pJAK148 into pET28a
pABS21	pET28a-SLP∟ΔLID-6xHis-tag (R20291)	This study. SLP∟ of R20291 lacking LID; oABS31/oABS48
pABS22	pET28a-SLP _H ∆HID-6xHis-tag (R20291)	This study. SLP _H of R20291 lacking HID; oABS15/oABS16
pABS23	pET28a-SLP∟∆LID-6xHis-tag (R7404)	This study. SLP∟ of R7404 lacking LID; oABS31/oABS32
pABS24	pET28a-SLP _H ∆HID-6xHis-tag (R7404)	This study. SLP _H of R7404 lacking HID; oABS39/oABS16
pSLPH630	pET28a-SLP _H -6xHis-tag (CD630)	SLP _H of CD630
pSLPH∆1-40	pET28a-SLP _H ∆HID-6xHis-tag (CD630)	⁷ SLP _H of CD630 lacking the N- terminal HID
pSLPL630	pET28a-SLP _L -6xHis-tag (CD630)	⁷ SLP _L of CD630
pSLPLΔ260- 321	pET28a-SLPLΔLID-6xHis-tag (CD630)	⁷ SLP _L of CD630 lacking the C- terminal LID
pJAK149	pETDuet-1-HID-6xHis-tag –SLPL (R7404)	This study. Recombinant co- expression of mature SLP _L /HID of R7404; RF1396/ RF1397, RF1398/ RF1400, RF1394/ RF1395
pJAK147	pETDuet-1-HID-6xHis-tag – LID (R7404)	This study. Recombinant co- expression of mature LID/HID of R7404; RF1396/ RF1397, RF1398/ RF1400, RF1395/ RF1396
Plasmids for exp domain, as spec	pression of the mature protein with a po rified in subscript	int mutation in the interaction
pABS1	pET28a-SLP _{L F274A} -6xHis-tag (CD630)	This study. oABS33/ oABS34
pABS2	pET28a-SLP _{H Y27A} -6xHis-tag (R20291)	This study. oABS37/ oABS38
pABS3	pET28a-SLP _{L F273A} -6xHis-tag (R20291)	This study. oABS35/ oABS36
pABS4	рЕТ28а-SLP _{H Y26A} -6xHis-tag (R7404)	This study. oABS5/ oABS6

pABS5	pET28a-SLP _{L F270A} -6xHis-tag (R7404)	This study. oABS21/ oABS22
pABS6	рЕТ28а-SLP _{H N18A} -6xHis-tag (R7404)	This study. oABS3/ oABS4
pABS7	pET28a-SLP _{H D28A} -6xHis-tag (R7404)	This study. oABS7/ oABS8
pABS9	рЕТ28а-SLP _{H N34A} -6xHis-tag (R7404)	This study. oABS11/ oABS12
pABS11	pET28a-SLP _{L D254A} -6xHis-tag (R7404)	This study. oABS17/ oABS18
pABS12	pET28a-SLP _{L 1259A} -6xHis-tag (R7404)	This study. oABS19/ oABS20
pABS13	pET28a-SLP _{L Y279A} -6xHis-tag (R7404)	This study. oABS23/ oABS24
pABS14	pET28a-SLP _{L G300A} -6xHis-tag (R7404)	This study. oABS25/ oABS26
pABS16	pET28a-SLP _{L R312A} -6xHis-tag (R7404)	This study. oABS29/ oABS30
pSLPH_Y27A	pET28a-SLP _{H Y27A} -6xHis-tag (CD630)	This study. NF1386/ NF1387
pJAK186	рМТL960 Ptet- <i>slpA</i> SLP _{H Y27A} (CD630)	This study. NF1386/ NF1387
pRPF209	pMTL960-Ptet- <i>slpA</i> SLP _{L F274A} (CD630)	This study. NF1189/ NF1190

Supplementary Table 5. Oligonucleotides used in this study

Name	Sequence	Application
oABS1		Amplification of SLP _L R7404
	GATCCCATGGCAGATAGTAC	with Ncol site forward primer
- 4.0.02	CATCOTCCACACATTACTTC	Amplification of SLP _L R7404
UADSZ	GATCETEGAGAGATTTAGTTTE	with Xhol site reverse primer
04052		Introduction of N18A point
UADSS	GCTAAATTAAAAGATTAAAAGATTATGTAG	mutation in R7404 SLP _H
		Introduction of N18A point
UAD34	AGCITITATAGITATITTAGCIGG	mutation in R7404 SLP _H
OVDE	COTGETAGATGATTEAAAAAACATAC	Introduction of Y26A point
UADSS	GEIGTAGAIGAITTAAAAACATAC	mutation in R7404 SLP _H
04856		Introduction of Y26A point
UADJU	ATCHTTAAATCHTTAATTATTATTAGC	mutation in R7404 SLP $_{\rm H}$
0AP\$7	CCTCATTTAAAAACATACAATAATAC	Introduction of D28A point
UAD37	GEIGATTIAAAACATACAATAATAC	mutation in R7404 SLP _H
	ТАСАТААТСТТТТАААТСТТТТААТТТАТТАG	Introduction of D28A point
UADSO		mutation in R7404 SLP _H
0AP\$11	GCTAATACTTACTCAAATGTTGTAAC	Introduction of N34A point
UADSII		mutation in R7404 SLP _H
0AP\$12	GTATGTTTTAAATCATCTACATAATC	Introduction of N34A point
UADSIZ	GIAIGITITIAAATCATCIACATAATC	mutation in R7404 SLP _H
oABS15	GCGCGCACAGTAGCAGGAGAAGATAGAATAG	Deletion of HID in R7404 SLP _H
0AP\$16		Deletion of HID in SLP_H
UADSIU		(R7404, R20291)
0AP\$17	COTTON A CITICATA TA TA CICO	Introduction of D254A point
UADS17	Generaditeatatatadige	mutation in R7404 SLP_L
0/BS18		Introduction of D254A point
UAD310	CACATCAATAGATTCTTCTTTGC	mutation in R7404 SLPL
0AB\$10	CTACTCCAAAATTTACC	Introduction of I259A point
UAD313	GCTAGTGCTGAAAATTTAGC	mutation in R7404 SLP_L
0AB\$20	ΑΤΑΤΘΑΑCTΤΘΑΑΤΟΟΛΟΑΤΟ	Introduction of I259A point
070320	ATATGAACTIGAATCCACATC	mutation in R7404 SLP_L
0AB\$21	GCTAATCCTAAAGAGGTTTCTG	Introduction of F270A point
UADJZI		mutation in R7404 SLP _L
οΔBS22		Introduction of F270A point
		mutation in R7404 SLP _L
οΔ <u></u> Β\$23	GCTAATGCAATAGTTGCATTAC	Introduction of Y279A point
oABS23	GUTAATGUAATAGTTGUATTAC	mutation in R7404 SLP _L

οΔBS24		Introduction of Y279A point
070524		mutation in R7404 SLP _L
oABS25	GCAAAATATCAAGTTATTTCTATCC	Introduction of G300A point
		mutation in R7404 SLP _L
oABS25	ΑΤΤΑΑCΤΑΑΤΤΩΤΑCΤΑΑΑΤCAGATTC	Introduction of G300A point
040525		mutation in R7404 SLP _L
0ABS29	GCATTAGAAACTAAATCTCTCG	Introduction of R312A point
040525		mutation in R7404 SLP _L
0AB\$30	ΤΤΤΓΟ	Introduction of R312A point
0/10000		mutation in R7404 SLP _L
0AB\$31	GEGEGETEGAGEACEACEACE	Deletion of LID in SLP _L (R7404,
070551		R20291)
oABS32	TTTTATAGTACCTGTTGCAGCCATATC	Deletion of LID in R7404 SLP_L
04BS33	GCTGATCCAGATGAAATTTCTG	Introduction of F274A point
040333		mutation in CD630 SLP _L
OABS3/		Introduction of F274A point
040334	TACATATCITITAGCTAAATTTTCAGC	mutation in CD630 SLPL
04BS/1/	GATCCCATGGCTGCAAAGGCTTCAATTGCTG	Amplification of SLP _H R20291
070344		with Ncol site forward primer
04BS/15	GATCCTCGAGCATACTTAATAAATCTTTT	Amplification of SLP _H R20291
070343	AATTTATTATAACTG	with Xhol site reverse primer
0AD546	GATCCATGGCAGAAGATATGTCGAAAGTTG	Amplification of SLP _L R20291
070340		with 5' <i>Nco</i> l site
οΔ <u></u> ΒS47	GATCCTCGAGACTCTTAGTTGTAACTCTTTTCC	Amplification of SLP _L R20291
070347		with 3' <i>Xho</i> l site
04BS48	AGTTATTACTGGGCTTCCAGATTGTG	Introduction of deletion of LID
070340		in R20291 SLPL
0AB\$35	GCTAATAAAACAGATTTAAATACTCTTTAC	Introduction of F273A point
040555	GCTAATAAAACAGATTTAAATACTCTTTAC	mutation in R20291 SLP _L
0AB\$36		Introduction of F273A point
UAD330		mutation in R20291 SLP _L
0AB\$37		Introduction of Y27A point
UAD337		mutation in R20291 SLP _H
0VB238		Introduction of Y27A point
UAD330		mutation in R20291 SLP $_{\rm H}$
0VB230	CCCCCCCAACTACCACCACAACATAC	Deletion of HID in R20291
	GUGUGUGAAGTAGUAGGAGAAGATAG	SLP _H
NF1189		Introduction of F274A point
	GCAGATCCAGATGAAATTTCTGAAGC	mutation in CD630 SLP _L

NE1100		Introduction of F274A point
NF1190		mutation in CD630 SLP∟
NE1296	GCAGTAGATGATTTAAAAACATATAATAATACT	Introduction of Y27A point
NF1300	ТАТТС	mutation in CD630 SLP _H
NE1207	ΑΤCTTTTAAATCTTTTAATTTATTAGCTTTTATAA	Introduction of Y27A point
NF1207	С	mutation in CD630 SLP _H
	GATCGAGCTCTATAATGTTGGGAGGAATTTAAG	Amplification of <i>slpA</i> from
NF1414	AAATG	CD630 with 5' <i>Sac</i> I site
NE1/15	GATCGGATCCTTACATATCTAATAAATCTTTCAT	Amplification of <i>slpA</i> from
NI 1415	тттб	CD630 with 3' BamHI site
		Replacement of coding
RF102	GGTTCTGGAAGCCCAGTAATAACTAAAC	sequence of SLP _L domain 2
		with GGA GGT
		Replacement of coding
RF103	TCCAGAGCTTATTAAGAAATCTACATAATCC	sequence of SLP _L domain 2
		with GGA GGT
DE1202	CATCCATATECCACATACTACTACCCACC	Amplification of SLP _L for
KL1222	GATCCATATGGCAGATAGTACTACGCCAGG	insertion into pACYC-Duet1
		Amplification of LID and SLP_L
RF1394		for insertion into pACYC-
		Duet1
DE120E	GATCCATATGGTTAGAGTTACAAGTGCAAAAG	Amplification of LID for
KL1292	AAG	insertion into pACYC-Duet1
DE1206		Linearization of pACYC-Duet1
NI 1390		for insertion of HID
DE1207		Linearization of pACYC-Duet1
KL1221		for insertion of HID
DE1209	TGTTTAACTTTAATAAGGAGATATACCATGGCA	Amplification of HID for
11220	GATATAATAGCTGATGCAG	insertion into pACYC-Duet1
DE1400	ATCTCAGTGGTGGTGGTGGTGGTGGTGGTGGTG	Amplification of HID for
RF1400	CAACATTTGAGTAAGTATTATTGTATG	insertion into pACYC-Duet1

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