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Supplemental information

The structural basis of *Salmonella* A_2B_5 toxin neutralization by antibodies targeting the glycan-receptor binding subunits

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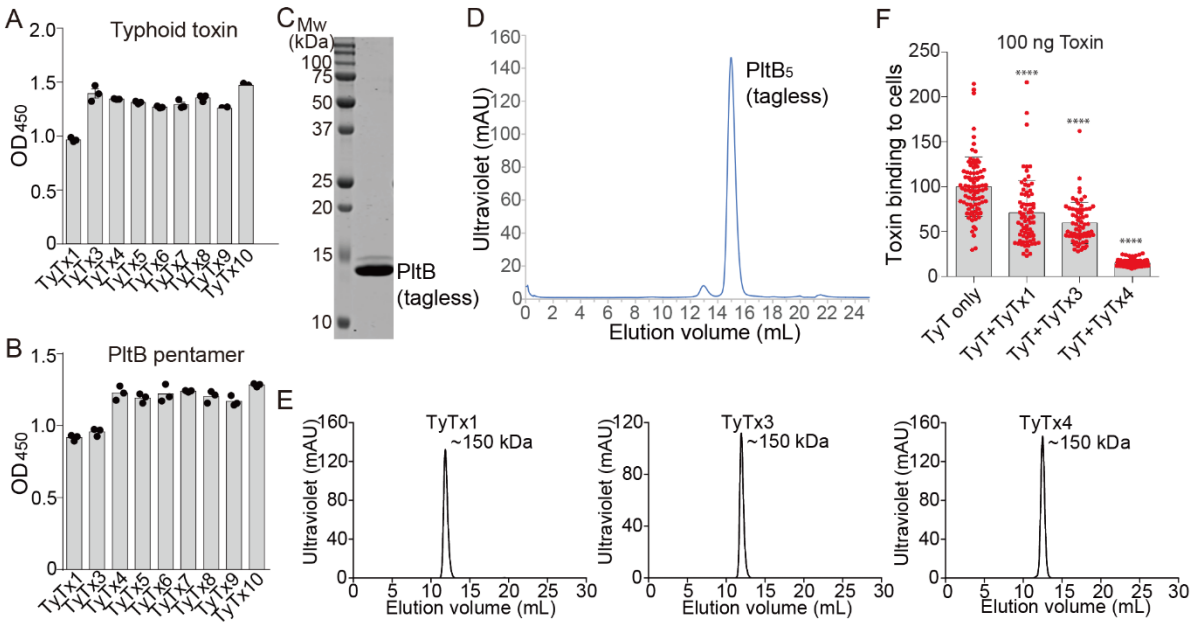

Figure S1. MAb ELISAs, Size-exclusion chromatograms, MAb-mediated inhibition of typhoid toxin binding to Henle-407 cells, and purified recombinant tagless PltB, related to Fig. 1A-C and 4. A-B, MAb ELISAs were performed to determine their specificities towards typhoid toxin (**A**) and tagless PltB pentamer (**B**). Bars represent the mean values of three replicates, which were obtained by measuring the absorbance at 450 nm. **C-D,** SDS-PAGE (**C**) and size-exclusion chromatograms (**D**) of purified recombinant PltB (tagless). See also the experimental procedure section for details. **E,** SD200 chromatograms of TyTx1 (left panel), TyTx3 (middle), and TyTx4 (right). Superdex 200 Increase prepacked columns (Cytiva) was used. **F.** Henle-407 cells grown on coverslips were precooled to 4°C, incubated with 100 ng of typhoid toxin-AF555 in the presence or absence of 2 µg MAb for 30 min, counterstained with DAPI, and analyzed by fluorescent microscopy. Bars represent the mean ± SEM obtained from at least three independent experiments. Each dot reflects the typhoid toxin signal intensity per image. n=60-88. ****, p<0.0001, compared to the TyT only group. Two-tailed unpaired t-tests were performed.

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TyTx7LCVariable       -MGPHKLRESLKMVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVSFSCRASQS
TyTx8LCVariable       ------------MVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVSFSCRASQS
TyTx10LCVariable      ------------MVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVSFSCRASQS
TyTx6LCVariable       ------------MVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVSFSCRASQS
TyTx9LCVariable       MGGPHKLRESLKMVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVIFSCRASQS
TyTx5LCVariable       MGGPHKLRESLKMVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERVSFSCRASQS
TyTx4LCVariable       MGGPHKLRESLKMVSTPQFLVFLLFWIPASRGDILLTQSPSILSVSPGERASFSCRASQS
TyTx3LCVariable       ------------MRPSIQFLGLLLFWLHGAQCDIQMTQSPSSLSASLGGKVTITCKASQD
TyTx1LCVariable       ------------MDSQAQVLILLLLWVSGTCGDIVMSQSPSSLAVSAGEKVTMSCKSSQS
                                   *    *.* :**: : .   ** ::**.* *:.* * :. ::*::*:

TyTx7LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx8LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx10LCVariable      IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx6LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx9LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx5LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx4LCVariable       IG------TSIHWYQQKPNGSPRLLIQYASQSISGIPSRFSGSGSGTDFTLTINSVESED
TyTx3LCVariable       IN------KYIAWYQHKPGKGPRLLIHYTSTLQPDIPSRFSGSGSGRDYSFSISNLEPED
TyTx1LCVariable       LFNSRTRKNHLAWYQQKPGQSPKLLIYWASTGESGVPDRFTGSGSGTDFTLTISSVQAED
                      :       . : ***:**. .*:***  ::   ..:*.**:***** *::::*..::.**

TyTx7LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK
TyTx8LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK
TyTx10LCVariable      IADYYCQHN-GWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK
TyTx6LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP-
TyTx9LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP-
TyTx5LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP-
TyTx4LCVariable       IADYYCQHTNGWPYTFGGGTTLEINRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK
TyTx3LCVariable       IATYYCLQYDTFLRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYP-
TyTx1LCVariable       LAVYFCKQSYNRALTFGSGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK
                      :* *:* :      ***.**.**::**********************************

TyTx7LCVariable       DINVMVYLLLKVKQNYSRGN-
TyTx8LCVariable       DINVMVYLLLKVKQNYSEGIS
TyTx10LCVariable      DINVMVYLLLKVKQNIREF--
TyTx6LCVariable       DINVMVYLLLKVNKIFRGN--
TyTx9LCVariable       DINVMVYLLLKVNKIFQRGIS
TyTx5LCVariable       DINVMVYLLLKVKQNIRGS--
TyTx4LCVariable       DINVMVYLLLKVNKIFRGEFR
TyTx3LCVariable       DINVMVYLLLKVKQNIQREFL
TyTx1LCVariable       DINVMVYLLLKVKQNYSGNS-
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Figure S2. Amino acid sequences of the light (kappa) chain variable region of TyTx1-10, related to Figs. 1-3. Highlighted in bold are the MAbs whose epitopes have been defined via cryo-EM in this study. The first amino acid residues indicated in the cryo-EM structures deposited are highlighted in red. Amino acid residues that bind to the toxin are highlighted in cyan.

Figure S3. Amino acid sequences of the heavy chain variable region of TyTx1-10, related to Figs. 1-3. Highlighted in bold are the antibodies whose epitopes have been defined via cryo-EM in this study. The first amino acid residues indicated in the cryo-EM structures deposited are highlighted in red. Amino acid residues that bind to the toxin are highlighted in purple.

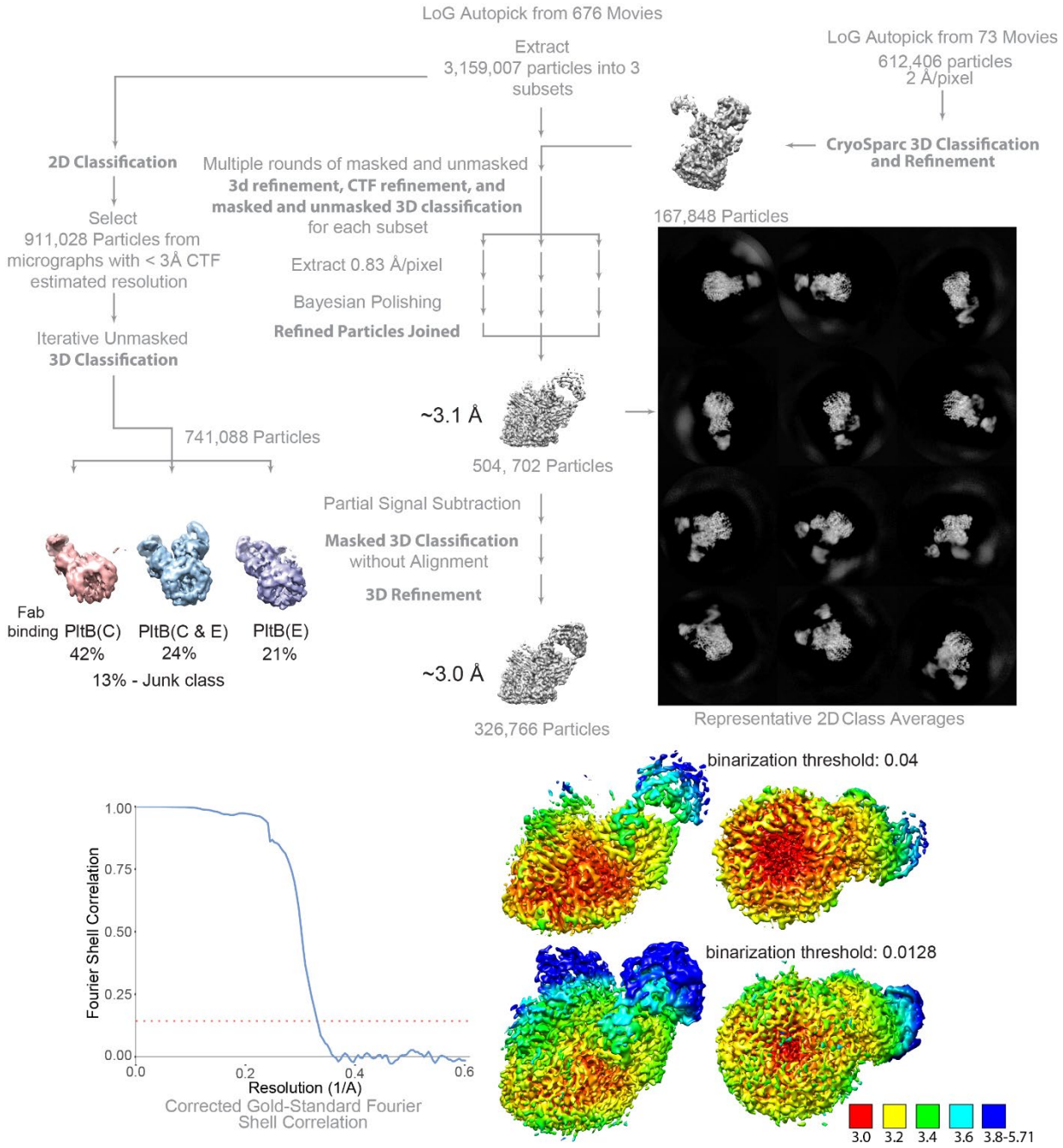

Figure S4. Flow chart depicting the cryo-EM workflow of the TyTx1 Fab-toxin complexes, related to Fig. 2. Details are described in the experimental procedure section under cryo-EM, data collection, and structure determination. Color-coded local resolution map is shown on the bottom right. The resolution map generated using 'binarization threshold=0.04' (top panel) is shown in the flow chart, while the map generated using 'binarization threshold=0.0128' is shown in the bottom panel that matches with Fig. 2B. Note that the FSC curve remains the same because the FSC curve was generated as part of post-processing of cryo-EM image refinement before any visualization steps for preparing figure panels. The appearance of the resolution maps can be different depending on the 'binarization threshold' value used during visualization. The local resolution maps were generated by Relion and visualized by Chimera.

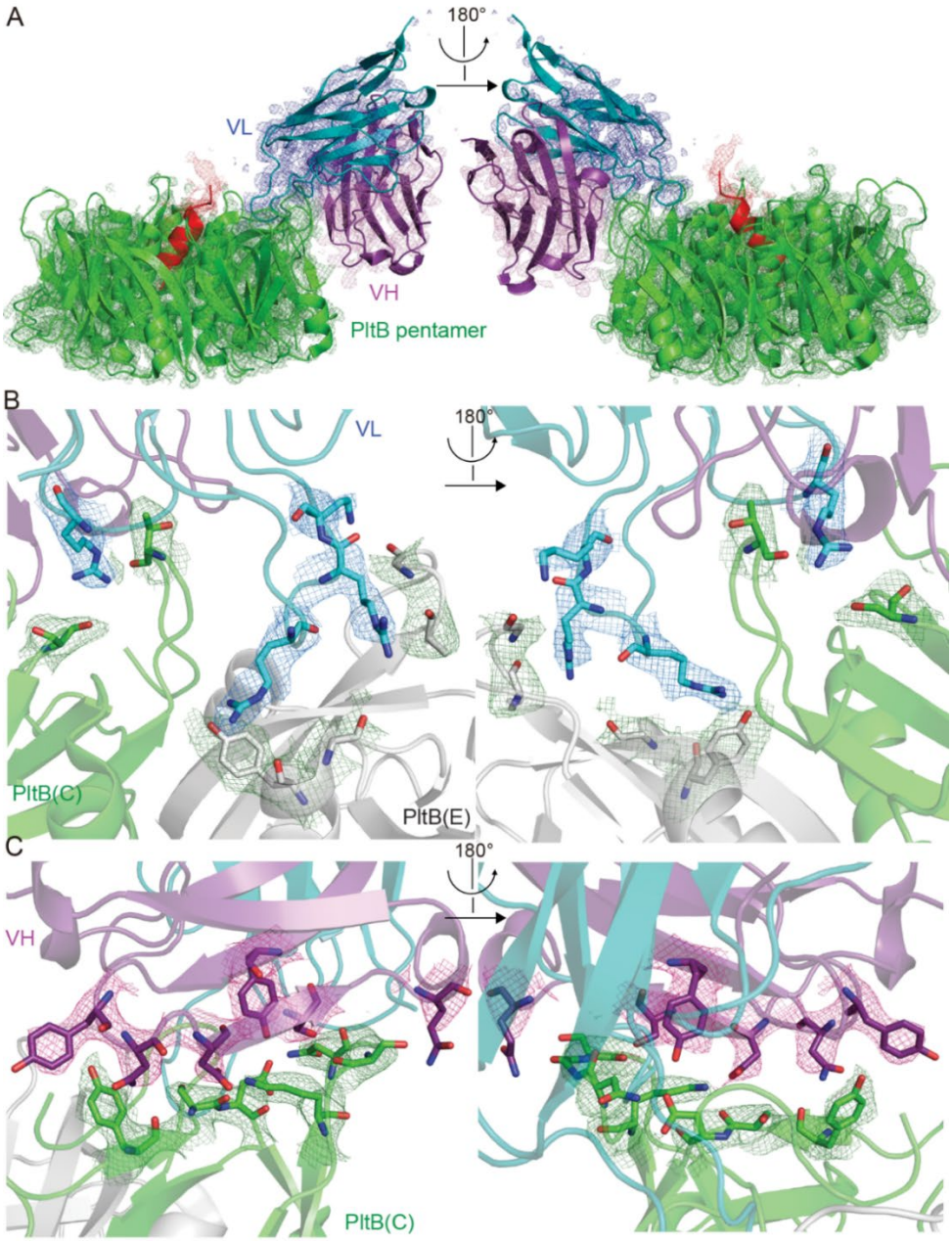

Figure S5. Cryo-EM density maps of typhoid toxin PltB pentamer bound to TyTx1 Fab variable regions, related to Fig. 2B-E. A, Overall cryo-EM density maps of PltB pentamer bound to TyTx1 variable regions of the light chain (VL, blue) and the heavy chain (VH, purple). The red helix is the tail part/C-terminal α-helix of PltA. **B-C**, Density maps of the interface between PltB subunits (C chain, green and E chain, grey) and TyTx1 VL (blue) (**B**) and between PltB subunits and TyTx1 VH (purple) (**C**). The density mesh was generated through Pymol based on the CryoEM density map around the built molecular structure. Color-coded local resolution map is shown in Fig S4, explaining the missing density in some parts.

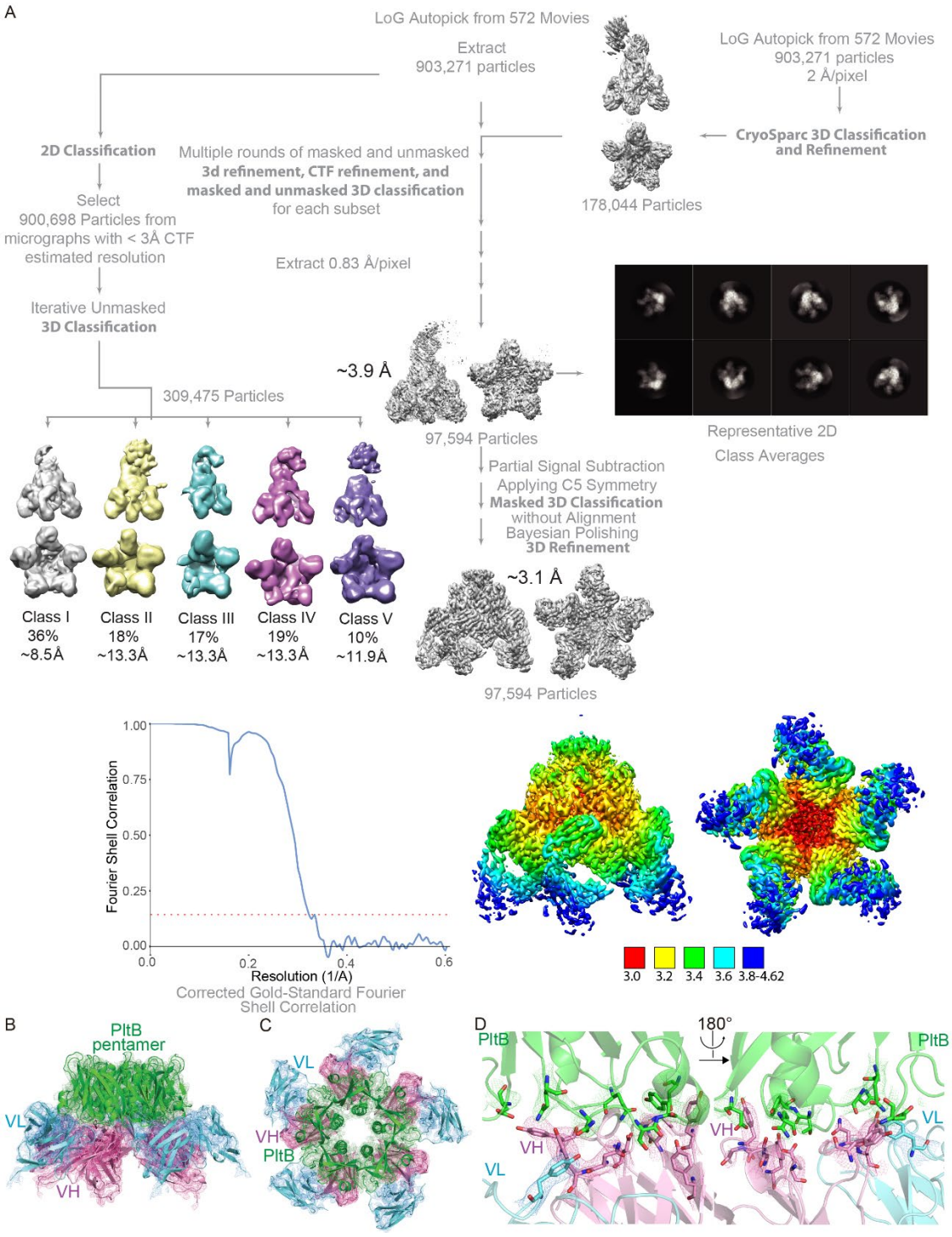

Figure S6. Cryo-EM workflow of the TyTx4 Fab-toxin complexes and cryo-EM density maps, related to Fig. 3A-D. A, Cryo-EM workflow. Details are described in the experimental procedure section under cryo-EM, data collection, and structure determination. Color-coded local resolution map is shown on the bottom right. **B-C,** Side (**B**) and top views (**C**) of overall cryo-EM density maps of PltB pentamer bound to TyTx4 variable regions of the light chain (VL, light blue) and the heavy chain (VH, pink). **D**, Density maps of the interface between PltB pentamer (green) and TyTx4 VL (light blue) and VH (pink). The density mesh was generated through Pymol based on the CryoEM density map around the built molecular structure.

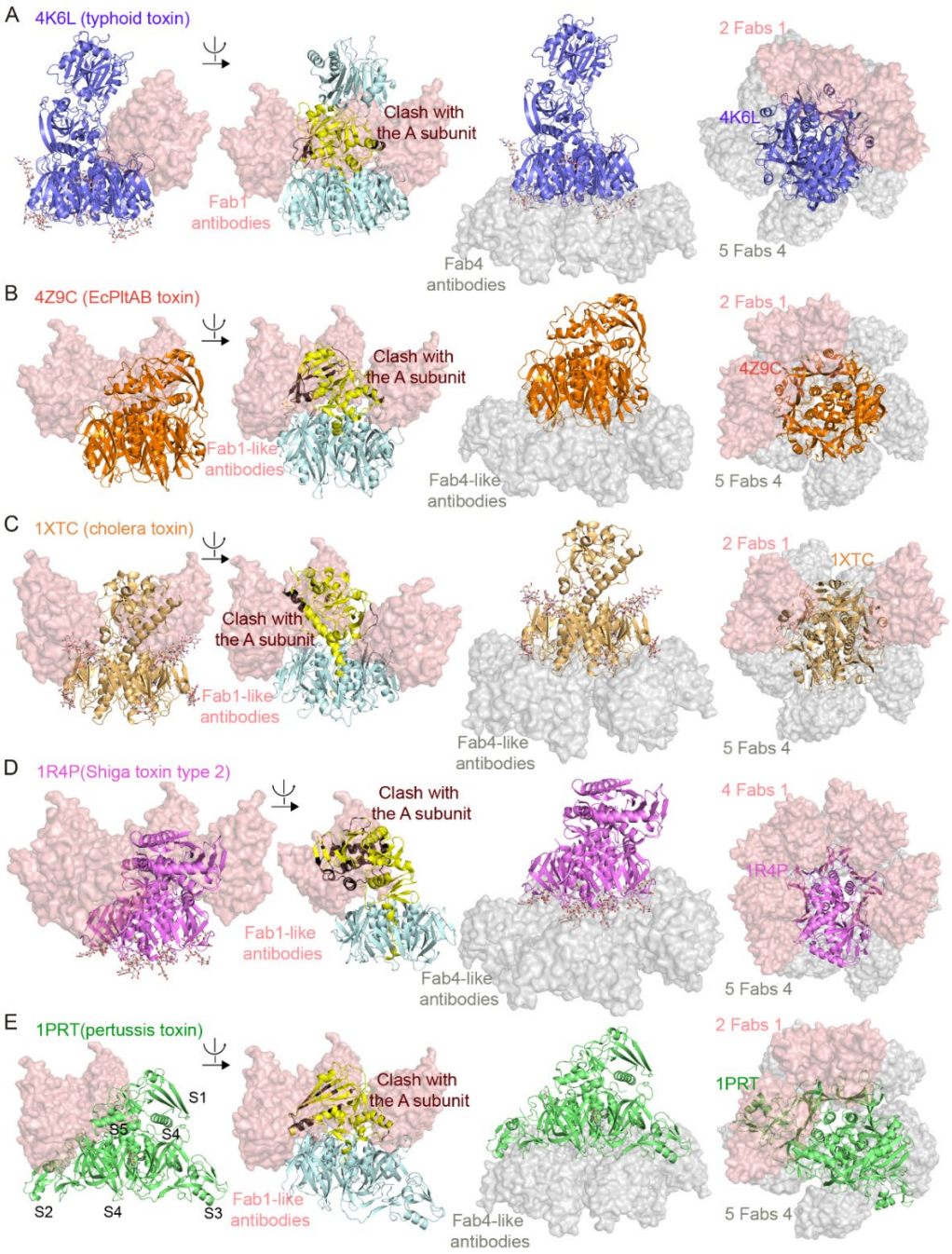

Figure S7. Secondary structure matching (SSM) analyses of bacterial AB_5 toxins, related to Fig. 7. A-E, Typhoid toxin (**A**), *E. coli* PltAB toxin (**B**), cholera toxin (**C**), Shiga toxin type 2 (**D**), and pertussis toxin (**E**) are complexed with TyTx1 Fabs (pink volume) and TyTx4 Fabs (grey volume). The region of toxin encroaching the TyTx1 Fab volume was manually identified and highlighted in brown (clashes with the A subunit in yellow). Total numbers of Fabs that can bind to the indicated toxins without clashes are indicated in the top view images that overlay toxin, Fabs of TyTx1, and Fabs of TyTx4 (the far right panel). Glycan receptor moieties are also indicated if known; See also Table S5. Note that unlike all other $A_{(2)}B_5$ toxins investigated in this manuscript (where up to 2 Fabs 1 or up to 5 Fabs 4 can fit in), our SSM analyses predict that 4 Fabs 1 or 5 Fabs 4 can fit in the region around the B subunit of Shiga toxin without clashes. We hypothesize that the A-subunit mediated asymmetry combined with a relatively smaller Shiga toxin contributes to this prediction result. This theoretical prediction appears to be highly conservative based on a recent observation by Bernedo-Navarro and colleagues (Bernedo-Navarro et al., 2018).

Table S1. Cryo-EM data collection, refinement, and validation statistics, related to Figs. 2-3.

	TyTx1-Toxin Complex (EMDB-22699) (PDB 7K7H)	TyTx4-Toxin Complex (EMDB-22700) (PDB 7K7I)
Data collection and processing		
Magnification	49,000	49,000
Voltage (kV)	200	200
Electron exposure (e–/Å^2)	53.0	51.5
Defocus range (μm)	-0.8 to -1.5	-0.8 to -1.5
Pixel size (Å)	1.66	1.66
Symmetry imposed	C1	C5
Initial particle images (no.)	3,159,007	903,271
Final particle images (no.)	326,766	97,594
Map resolution (Å)	3.00	3.13
FSC threshold	0.143	0.143
Map resolution range (Å)	2.6 to 3.4	3.0 to 3.7
Refinement		
Initial models used (PDB code)	4RHR, 1MHH, 4H20	4RHR, 2OZ4, 4M48,
Model resolution (Å)	3.00	3.13
FSC threshold	0.143	0.143
Model resolution range (Å)	2.63 to 3.38	2.97 to 3.69
Map sharpening *B* factor (Å^2)	83.57	101.11
Model composition		
Non-hydrogen atoms	6320	13195
Protein residues	816	1705
Ligands	0	0
B factors (Å^2)		
Protein	36.89	55.14
Ligand	0	0
R.m.s. deviations		
Bond lengths (Å)	0.003	0.003
Bond angles (°)	0.517	0.530
Validation		
MolProbity score	1.95	2.17
Clashscore	8.45	11.29
Poor rotamers (%)	0	0
Ramachandran plot		
Favored (%)	91.88	87.82
Allowed (%)	8.12	12.18
Disallowed (%)	0	0

Table S2. PltB amino acid residues known to interact with glycan-receptors, related to Figs. 2-3. Note that binding pocket BS1 is located on the lateral side of PltB pentamer, whereas binding pockets BS2 and BS3 are located on the bottom side of PltB pentamer.

Glycan receptors*	Methods used	Residues in BS1**	Residues in BS2**	Residues in BS3**	References
Neu5Ac	Molecular docking	Y33, **S35**, K59			(Song et al., 2013)
Neu5Acα2-6Galβ1-4GlcNAc	Co-crystal structure	Y33, Y34, S35, K59, R100			(Lee et al., 2020)
Neu5,9Ac$_2$α2-6Galβ1-4GlcNAc	Co-crystal structure	Y34, S35, K59, T65, R100			(Nguyen et al., 2020)
Neu5Acα2-3Galβ1-4GlcNAc	Co-crystal structure	Y33, Y34, **S35**, D36, **K59**, R100	Q75, I107, **W108**, **T109**, Y110, **F113**	E24, **D28**, **N29**, D48, Y110	(Lee et al., 2020)
Neu5,9Ac$_2$α2-3Galβ1-4GlcNAc	Co-crystal structure	Y33, Y34, S35, K59, N61, T65, R100	Q75, I107, W108, T109, Y110, F113		(Nguyen et al., 2020)
Neu4,5Ac$_2$α2-3Galβ1-4GlcNAc	Co-crystal structure	Y33, Y34, S35, D36, K59, T65	Q75, W108, T109, Y110		(Nguyen et al., 2020)
GD2 glycan: GalNAcβ1-4(Neu5Acα2-8Neu5Acα2-3)Galβ1-4Glc	Co-crystal structure	**Y33**, Y34, **S35**, **K59**, T65, R100			(Deng et al., 2014)

* Neu5Ac, N-acetylneuraminic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Neu5,9Ac$_2$, 9-*O*-acetyl Neu5Ac; Neu4,5Ac$_2$, 4-*O*-acetyl Neu5Ac; GalNAc, N-acetylgalactosamine; Glc, glucose.

** Residues in bold, their importance was experimentally confirmed through mutagenesis studies in references indicated.

Table S3. Primers used in this study, related to Fig. 1 and STAR Methods.

Application	Primer name	Forward or reverse	Primer sequence (5′-3′)
Reverse transcription	Template-switch oligo	Universal forward primer	AGGCAGTGGTATCAACGCAGAGTACATGrGrGr
	mIGK RT	Reverse primer for kappa chain	TTGTCGTTCACTGCCATCAATC
	mIGL RT	Reverse primer for lambda chain	GGGGTACCATCTACCTTCCAG
	mIGHG RT	Reverse primer for heavy chain	AGCTGGGAAGGTGTGCACAC
PCR	ISPCR	Universal forward primer	AAGCAGTGGTATCAACGCAGAG
	mIGK PCR	Reverse primer for kappa chain	ACATTGATGTCTTTGGGGTAGAAG
	mIGL PCR	Reverse primer for lambda chain	ATCGTACACACCAGTGTGGC
	mIGHG PCR	Reverse primer for heavy chain	GGGATCCAGAGTTCCAGGTC

GrGrGr: 3 riboguanines. See Meyer et al, 2019 for details (Meyer et al., 2019).

Table S4. Kinetics of TyTx MAbs binding to typhoid toxin by surface plasmon resonance (SPR) assays, related to Fig. 5.

MAb	Isotype	Target	k_a ($M^{-1}s^{-1}$)[a]	k_d (s^{-1})[a]	K_D (M)[a]
TyTx1	IgG1	PltB	9.39×10^{4}	6.64×10^{-3}	7.11×10^{-8}
TyTx3	IgG1	PltB	1.73×10^{5}	1.68×10^{-2}	1.45×10^{-7}
TyTx4	IgG2a	PltB	1.03×10^{6}	6.80×10^{-6}	6.70×10^{-12}

[a] Mean values of three technical replicates.

Table S5. Secondary structure matching analyses of bacterial AB_5 toxins, related to Fig. 7.

Toxin name	Holotoxin PDB	Glycan complex PDB (glycan name)[1]	TyTx1-like IgGs per toxin (shoulder-located epitopes).	TyTx4-like IgGs per toxin (bottom-located epitopes)
Typhoid toxin (A_2B_5)	4K6L	6P4M (Neu5Acα2-3Galβ1-4GlcNAc)	1 MAb (2 Fabs)	3 MAbs (5 Fabs)
E. coli PltAB toxin (AB_5)	4Z9C	Unknown[2]	1 MAb (2 Fabs)	3 MAbs (5 Fabs)
Cholera toxin (AB_5)[3]	1XTC	5ELB (Lewis Y glycans); 2CHB (GM1 glycans)	1 MAb (2 Fabs)	3 MAbs (5 Fabs)
Shiga toxin type 2 (AB_5)[4]	1R4P	1BOS (α-D-galactopyranose-(1-4)-β-D-galactopyranose-(1-4)-β-D-glucopyranose).	2 MAbs (4 Fabs)	3 MAbs (5 Fabs)
Pertussis toxin (AB_5)[5]	1PRT	1PTO (Neu5Acα2-6-β-D-galactopyranose)	1 MAb (2 Fabs)	3 MAbs (5 Fabs)

[1]Additional glycan binding pockets may exist.
[2]Glycan bound structure is unavailable.
[3]Similar to typhoid toxin, glycan-binding pockets are located on both the shoulder and the bottom.
[4]Glycan binding pockets are located on the bottom side of the B pentamer.
[5]Glycan binding pockets on the shoulder regions in S2 and S3. Heteropentameric B subunits consisting of S2, S3, 2S4, and S5 can also be considered as an intrinsic immune subversion mechanism because their epitopes in the B heteropentamer are different.

Table S6. Comparison of *S.* Typhi *pltB* sequences deposited in NCBI, related to Fig. 6. Shown are the first 100 hits from the BLAST search using *S.* Typhi CT18 *pltB*. Note that all *S.* Typhi strains encode *pltB* on their chromosomes and their DNA sequences are identical. (Adapted from (Lee et al., 2020))

Strain Name	Sequence ID	PltB gene location	Sequence Homology (%)
CT18	Sty1891	1788687-1788274	**100**
R19_2839	CP046429.1	2684230-2683817	**100**
2018K-0756	CP044007.1	2313144-2312731	**100**
WGS1146	CP040575.1	2684661-2684248	**100**
Ty2 4316STDY6559669	LR590081.1	2568088-2567675	**100**
311189_217186	CP029646.1	1787549-1787136	**100**
311189_201186	CP029958.1	1787568-1787155	**100**
311189_218186	CP029925.1	1788480-1788067	**100**
343078_273110	CP029846.1	1787719-1787306	**100**
343078_256191	CP029959.1	1726503-1726090	**100**
343078_251131	CP029960.1	1788099-1787686	**100**
343078_228140	CP029962.1	1787808-1787395	**100**
343078_223175	CP029964.1	1787907-1787494	**100**
343078_211126	CP029848.1	1787796-1787383	**100**
343078_203125	CP029850.1	1787691-1787278	**100**
343078_201101	CP029852.1	1788012-1787599	**100**
343077_292148	CP029855.1	1787884-1787471	**100**
343077_286126	CP029856.1	1787067-1786654	**100**
343077_285138	CP029858.1	1787839-1787426	**100**
343077_281186	CP029853.1	1726037-1725624	**100**
343077_278127	CP029863.1	1787499-1787086	**100**
343077_267164	CP029906.1	1790717-1790304	**100**
343077_260153	CP029861.1	1787799-1787386	**100**
343077_255118	CP029907.1	1790401-1789988	**100**
343077_228157	CP029864.1	1787663-1787250	**100**
343077_228140	CP029866.1	1827555-1827142	**100**
343077_215174	CP029868.1	1790707-1790294	**100**
343077_214162	CP029862.1	1788094-1787681	**100**
343077_214135	CP029872.1	1787901-1787488	**100**
343077_213147	CP029897.1	1882683-1882270	**100**
343077_212159	CP029870.1	1726862-1726449	**100**
343077_212138	CP029919.1	1790394-1789981	**100**
343077_211171	CP029873.1	1787670-1787257	**100**
343076_294172	CP029888.1	1788337-1787924	**100**
343076_269157	CP029881.1	1827552-1827139	**100**
343076_253155	CP029890.1	1827830-1827417	**100**
343076_252143	CP029892.1	1787952-1787539	**100**
343076_249107	CP029913.1	1787807-1787394	**100**
343076_248190	CP029882.1	1789926-1789513	**100**
343076_241106	CP029899.1	1790429-1790016	**100**

343076_232188	CP029900.1	1787868-1787455	**100**
343076_227128	CP029875.1	1787865-1787452	**100**
311189_282186	CP029920.1	1787761-1787348	**100**
343076_217103	CP029914.1	1790493-1790080	**100**
343076_202113	CP029915.1	1787839-1787426	**100**
311189_291186	CP029894.1	1787546-1787133	**100**
311189_269186	CP029922.1	1787670-1787257	**100**
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311189_252186	CP029896.1	1787765-1787352	**100**
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311189_223186	CP029880.1	1788850-1788437	**100**
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311189_221186	CP029923.1	1787191-1786778	**100**
311189_220186	CP029886.1	1787992-1787579	**100**
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311189_208103	CP029932.1	1788052-1787639	**100**
311189_207186	CP029938.1	1726111-1725698	**100**
311189_206186	CP029940.1	1789029-1788616	**100**
311189_205186	CP029950.1	1787384-1786971	**100**
311189_204186	CP029954.1	1787710-1787297	**100**
311189_203186	CP029942.1	1787052-1786639	**100**
311189_202186	CP029956.1	1827716-1827303	**100**
Ty21a	CP023975.1	2176424-2176837	**100**
LXYSH	CP030936.1	4243329-4243742	**100**
OVG_041	LT906560.1	516042-515629	**100**
SGB90	LT904870.2	2806254-2805841	**100**
ISP_03_07467_SGB110-sc-1979083	LT905060.2	2662091-2661678	**100**
1036491	LT906495.1	2657594-2657181	**100**

ERL024120	LT906494.1	509382-509795	**100**
1554-sc-2165329	LT906493.1	2657605-2657192	**100**
E98_3139-sc-1927833	LT905143.1	2366618-2367031	**100**
H12ESR00755-001A	LT905142.1	369292-368879	**100**
OVG_041	LT905141.1	1896510-1896923	**100**
ERL082356	LT905140.1	2441262-2441675	**100**
2010-007898	LT905139.1	2657610-2657197	**100**
ty3-243	LT905090.1	4566056-4566469	**100**
ERL024120	LT905088.1	1520236-1520649	**100**
1554-sc-2165329	LT905064.1	2657555-2657142	**100**
lupe_GEN0059-sc-1979081	LT905063.1	2072707-2073120	**100**
403Ty-sc-1979084	LT905062.1	4644368-4644781	**100**
ERL12960	LT904894.1	2657509-2657096	**100**
1016889	LT904893.1	2657582-2657169	**100**
80-2002	LT904891.1	4585640-4586053	**100**
H12ESR00394-001A	LT904890.1	2060046-2060459	**100**
129-0238-M	LT904888.1	4564955-4565368	**100**