Supporting Information

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SI Materials and Methods

Protein Expression and Purification. The SSL7 gene from S. *aureus* strain ATCC 12598 was amplified by PCR and cloned into the pET-32 Ek/LIC vector (Novagen) with primers containing a TEV protease site. Mutants of strain ATCC 12598 were generated by the use of the QuikChange Lightning Kit (Stratagene). Protein was expressed in BL21 (DE3) cells overnight in LB media. SSL7 was purified by Ni-affinity chromatography on a HisTrap HP column (GE Healthcare) followed by cleavage with TEV protease. Subsequently, the tag was removed by chromatography on a HisTrap HP column and SSL7 was dialyzed against 10 mM Hepes/100 mM NaCl (pH 7.4) and stored at −20 °C. The SSL7′ was generated from strain 4427 (a local clinical isolate obtained from Greenlane Hospital, Auckland) as described in ref. 1. The C-terminal domain SSL7′c (residues S129 –I231) was generated by PCR from the full-length gene, and SSL7 mutants were produced by the technique of overlap PCR as described in ref. 2. SSL7′ protein and its mutated proteins were produced from the vector pET-32a-3C in the *E. coli* K12 strain AD494(DE3)pLysS. Thioredoxin fusion proteins were purified from bacterial lysates by metal chelate chromatography, cleaved with 3C protease, and further purified by cation exchange chromatography as described in ref. 1. SSL7′ possesses and extra G-P-G at the N terminus due to the requirements of protease 3-C cleavage. Before crystallization, C5 and SSL7 or SSL7′c were mixed in a 1:2 molar ratio and purified on a Superdex 200 10/300 column (GE Healthcare) equilibrated in 20 mM Hepes/100 mM NaCl (pH 7.4). For purification of CVF, lyophilized venom (0.2 g) from *Naja naja siamensis* (Miami Serpentarium) was dissolved in 4 mL of running buffer (50 mM Tris·HC1/150 mM NaC1 pH 7.5). Subsequently, the venom solution was stirred for 12 h at room temperature, centrifuged for 10 min at 16,000 × *g*, and filtered using a 0.2-μm filter. The filtrate was applied to a Superdex 200 26/60 (GE Healthcare) gel-filtration column equilibrated with running buffer at 4 °C at a linear flow rate of 1.5 mL/min. Fractions (3 mL) containing purified CVF as assessed by 7.5% SDS/PAGE under nonreducing conditions were pooled and quantified on the basis of SDS/PAGE gels or using the BCA protein assay.

Structure Determination. Before data collection, crystals were transferred to a cryo-protection buffer containing 50 mM $MgAc_2$, 50 mM Mes (pH 6.2), 37.5% (wt/vol) PEG400, and 10 μM $CdCl_2$. The structure of C5-SSL7 was determined by molecular replacement using PHASER (3) with C5 (residue 22–1514) and SSL7 (RCSB entry 1V10) as independent search models. However, direct positioning of SSL7 by PHASER was unsuccessful, and SSL7 was manually docked into a $2mF_o$-DF_c electron density by hand using "O" (4). The structure of C5-SSL7′c was determined by molecular replacement using the structure of C5-SSL7 without the OB-domain (residue 40–128) of SSL7 as search model in PHASER. The electron density for one copy of the C345C domain located on the noncrystallographic twofold axis in the structure of the C5-SSL7 complex was weak, but comparable to that observed for the C5 structure (5). As the C5 and C5-SSL7 structures are almost perfectly isomorphous, the $2mF_o$-DF_c electron-density maps could be directly averaged, which somewhat improved the density for the C345C domain, and this resulted in significant rebuilding of the loop 1569-1575. In the structure of the C5-SSL7′c complex, the electron density for the C345C domain is too weak to justify modeling, and the domain is therefore not included in the deposited RCSB Protein Data Bank file.

C5b-SSL7 Pull-Down. C5b was generated by mixing C5:CVF:fB:fD in the molar ratio 1:0.05:0.1:0.001 for 14 h, resulting in C5 cleavage being at least 95% complete as measured by comparison of the intensities for the C5 α-chain and the C5b α′-chain. C5 or C5b were incubated 20 μL Ni^{2+}-NTA agarose (Qiagen) washed in 20 mM Tris·HCl/100 mM NaCl (pH 7.8) with either (*i*) 10 μg of C5 + 30 μg of SSL7-6His, (*ii*) 20 μg of C5b + 30 μg of SSL7-6His, (*iii*) 10 μg of C5, or (*iv*) 20 μg of C5b for 30 min at room temperature. Each sample of Ni^{2+}-beads was first washed with 1 mL of 20 mM Tris/100 mM NaCl (pH 7.8) and then eluted with 30 μL of 20 mM Tris/100 mM NaCl/300 mM imidazole (pH 7.8) by incubation for 30 min at room temperature. The eluted supernatants were analyzed by 4–20% SDS/PAGE. The SSL7-His_6 for these experiments was cloned from strain 12598, expressed in pET32 Ek/LIC, and therefore contains an N-terminal Trx-tag, a 6His-tag, and an S-tag.

Gel-Filtration Analysis of Reconstituted C5-SSL7 and C5-SSL7-IgA Fc Complexes. To generate the C5-SSL7 complex, C5 was mixed with a 4-fold molar excess of SSL7 in a total volume of 450 μL and incubated for 20 min at 22 °C. For analysis of the C5-SSL7-IgA complexes, C5 was incubated with a 2-fold molar excess of SSL7 and IgA-Fc for 20 min at 22 °C in a total volume of 450 μL. The complexes were separately analyzed by gel filtration on a 24-mL Superose 6 10/300 GL column equilibrated in 20 mM Hepes pH 7.4, 100 mM NaCl. The elution profile was examined by 12% SDS/PAGE.

Gel-Filtration Analysis of IgA-SSL7-C5 Complexes Isolated from Serum. Ninety-six-well MaxiSorp immunoassay plates (Nunc) were precoated overnight at 4 °C with 1 μg/mL affinity purified mouse anti-SSL7 in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.01% NaN_3 and blocked with 1% BSA/PBS (pH 7.4) for 1 h at room temperature, then washed extensively with PBS-Tween. 50 μl aliquots of fresh human serum were incubated for 30 min at room temperature with 10 μg each of SSL7′, SSL7′-IgA$^-$, or SSL7′-C5$^-$ protein. Samples were individually resolved on a Superdex 200 5/150 GL analytical column (GE Healthcare). The 50-μL eluted fractions were diluted to 2 mL with PBS and 50 μL added to the anti-SSL7-coated plates and incubated for 1 h at room temperature before washing and blocking as above. Complement C5 was detected with a 1:5,000 dilution of a rabbit anti-human C5 antibody (Dako) followed by 1:2,000 dilution of HRP-conjugated goat anti-rabbit. Peroxidase activity was detected with 0.5 mg/mL *o*-phenylenediamine dihydrochloridein/50 mM citric acid/100 mM Na_2HPO_4/0.012% H_2O_2. Reactions were stopped with 10% HCl, and the absorbance was measured at OD at 490 nm.

Biophysical Measurements. All experiments were conducted with SSL7 from the 12598 strain or its mutants. Before ITC, the C5 and SSL7 preparations were dialyzed overnight against 10 mM Hepes/100 mM NaCl (pH 7.5) overnight at 4 °C and degassed before analysis. The concentration of C5 was 1.88–2.32 μM, determined using A_{280} = 1.08 for a 1 mg/mL solution and a molecular weight of 190 kDa. The concentrations of wild-type SSL7 and its variants were 46–111 μM, determined using A_{280} = 0.52 for 1 mg/mL and a molecular weight of 23 kDa. Isothermal titration calorimetry was performed in a MicroCal VP-ITC instrument operated according to the manufacturer's instructions at 30 °C. Titration data were analyzed using the Origin software package. Correction for heat of mixing was performed by determining the average value of the

deflections after saturation was reached and subtracting this value from the data points.

Serum Hemolytic Assay. RBCs from either human or sheep (Invitrogen; Alsevers sheep blood) were combined with human and rabbit serum, respectively. The optimal concentration of serum was first determined by dilution assay and for human serum. This was normally ~20% but varied slightly between donors. Five milliliters of RBCs was added to 45 mL of GHB [150 mM NaCl/5mM Hepes/0.11 (wt/vol) Gelatin from bovine skin type B] with 2 mM $MgCl_2$ and 0.2 mM EGTA and incubated at 37 °C for 15 min to lyse any unstable RBCs. Cells were centrifuged at 1,250 × *g* for 10 min at 4 °C, the supernatant was removed, and the cells were resuspended in 50 mL of ice-cold GHB/MgEGTA buffer. This was repeated until the supernatant was clear following centrifugation. The RBCs were standardized to 2×10^8 cells per mL. SSL7′ protein was added to a 96 well U-bottom tissue culture plates (Falcon) to give a 2-fold dilution series. One hundred microliters of serum diluted with GHB/MgEGTA was added. Fifty microliters of RBCs was added and the plate was incubated for 1 h at 37 °C with periodic shaking. The cells were pelleted by centrifugation at 1,250 × *g* for 5 min at 4 °C. One hundred microliters of the supernatant was added to 150 μL of ice-cold 150 mM NaCl in 96-well, flat-bottom tissue culture plates (Falcon), and the absorbance measured at 412 nm was measured using a uQuant plate reader (BioTek).

Serum Cell-Free Bactericidal Assay. Sera from normal volunteers were allowed to coagulate for 1 h at room temperature. Serum was centrifuged at 5,000 × *g* for 5 min before use to remove any particulate or cells. Cell-free serum bactericidal activity was assessed using a fresh overnight culture of *E. coli* K12 strain DH5α. SSL7′ protein was preincubated with 5% normal human serum diluted in Hanks's buffered saline solution (HBSS) (Sigma Aldrich) for 30 min at 37 °C in 5-mL borosilicate glass tubes and then incubated with ~1×10^7 stationary-phase (A_{600} ~ 0.15) DH5α for 90 min at 37 °C. Five percent normal human serum was typically 99.99% bactericidal. Tubes were placed in ice to stop reactions and then a dilution series of each was prepared in HBSS, plated in triplicate on LB agar, and incubated O/N at 37 °C. Colony forming units (CFU) were enumerated the following day on a BacCount (Bio-Tek) and expressed as mean CFU ± SD. Bacteria incubated with buffer alone were included as a no lysis control.

Detection of Serum C5a Production. Blood from healthy donors was clotted at room temperature for 1 h, centrifuged for 5 min at 1,250 × *g*, and diluted to 25% with 0.5% BSA, PBS (pH 7.3) (PBS-B). Duplicate 100-μL samples of 25% serum were mixed with 50 μL of serially diluted SSL7 or SSL7 mutant proteins before the addition of 100 μL of PBS-B containing 10^7 heat-killed *S. aureus* (Wood 46 strain). Plates were incubated at 37 °C for 30 min and then centrifuged for 5 min at 1,250 × *g* to pellet bacteria. The supernatants were further diluted to 1% with PBS-B, and 100 μL was added to the C5a sandwich ELISA. The anti-human C5a mAb (MAB2037; R&D Systems) was adsorbed at 2 μg/mL to 96-well MaxiSorp immunoassay plates (Nunc) overnight at 4 °C in 15 mM Na_2CO_3/35 mM $NaHCO_3$/0.01% NaN_3. Unbound antibody was removed, and the plates washed four times with PBS-T (0.5% vol/vol Tween-20/PBS, pH 7.4). Wells were blocked with 1% BSA/PBS (pH 7.4) for 1 h at room temperature and then washed extensively with PBS-T and stored at 4 °C until required. Diluted serum samples (100 μl) were incubated for 1 h at room temperature in the ELISA plates before extensive washing with PBS-T followed by 1:5,000 dilution of anti-C5a rabbit serum pAB (Calbiochem) in PBS-B for 1 h at room temperature and a 1:2,000 dilution of HRP-conjugated goat anti-rabbit. Peroxidase activity was detected with 0.5 mg/mL *o*-phenylenediamine dihydrochloride (OPD) in 50 mM citric acid/100 mM Na_2HPO_4/0.012% H_2O_2. Reactions were stopped with 10% HCl, and the absorbance was measured at OD of 490 nm.

1. Langley R, et al. (2005) The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. *J Immunol* 174:2926–2933.
2. Hudson KR, Robinson H, Fraser JD (1993) Two adjacent residues in staphylococcal enterotoxins A and E determine T cell receptor V beta specificity. *J Exp Med* 177:175–184.
3. McCoy AJ (2007) Solving structures of protein complexes by molecular replacement with Phaser. *Acta Crystallogr D Biol Crystallogr* 63:32–41.
4. Jones TA, Zou JY, Cowan SW, Kjeldgaard M (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr A* 47:110–119.
5. Fredslund F, et al. (2008) Structure of and influence of a tick complement inhibitor on human complement component 5. *Nat Immunol* 9:753–760.

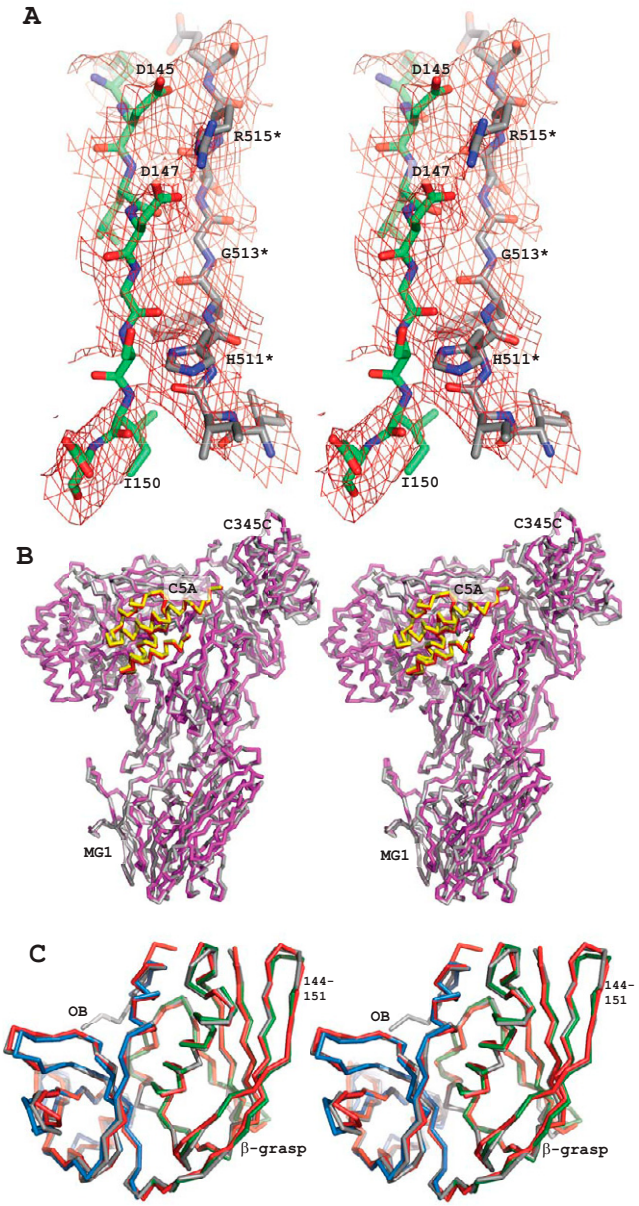

Fig. S1. Electron density of the complex and comparison with other structures. (*A*) Stereoview of a $2mF_o - DF_c$ electron density around the interaction between the β-sheets in SSL7 (green carbon atoms) and C5 (gray carbon atoms, residues marked with *). The unit cell parameters of crystals of both the C5-SSL7 and the C5-SSL7′c complexes are almost identical to those found for crystals of C5 [Fredslund F, et al. (2008) *Nat Immunol* 9:753–760], and they also contain the same pseudo-crystallographic twofold axis with one copy of the C345C domain located directly on this axis. All atoms shown were omitted for map calculation, and the resulting map was then averaged over the twofold noncrystallographic axis. The map is contoured at 1 σ. (*B*) C5 from the SSL7 complex (gray) superimposed on free C5 (magenta, RCSB entry 3CU7) with the C5a domain colored red/yellow (SSL7 bound/free C5). Least square superposition yields a root mean square deviation (rmsd) of 1.22 Å over residues 22–1510 between the structure of C5 and the structure of C5 bound to SSL7. In particular, the rmsd for residues 673–760, which includes C5a and N terminus of the C5 α′-chain is 0.44 Å, suggesting that C5a is essentially unaffected by the presence of SSL7. (*C*) Comparison of SSL7 from the C5 complex (OB domain blue, β-grasp domain green), isolated SSL7 (gray, RCSB entry 1V1O), and SSL7 in complex with IgA Fc (red, RCSB entry 2QEJ). The rmsd between 190 Cα atoms in SSL7 bound to either C5 or IgA Fc is 1.41 Å, and the rmsd between 190 Cα atoms in free SSL7 and C5-bound SSL7 is 1.29 Å.

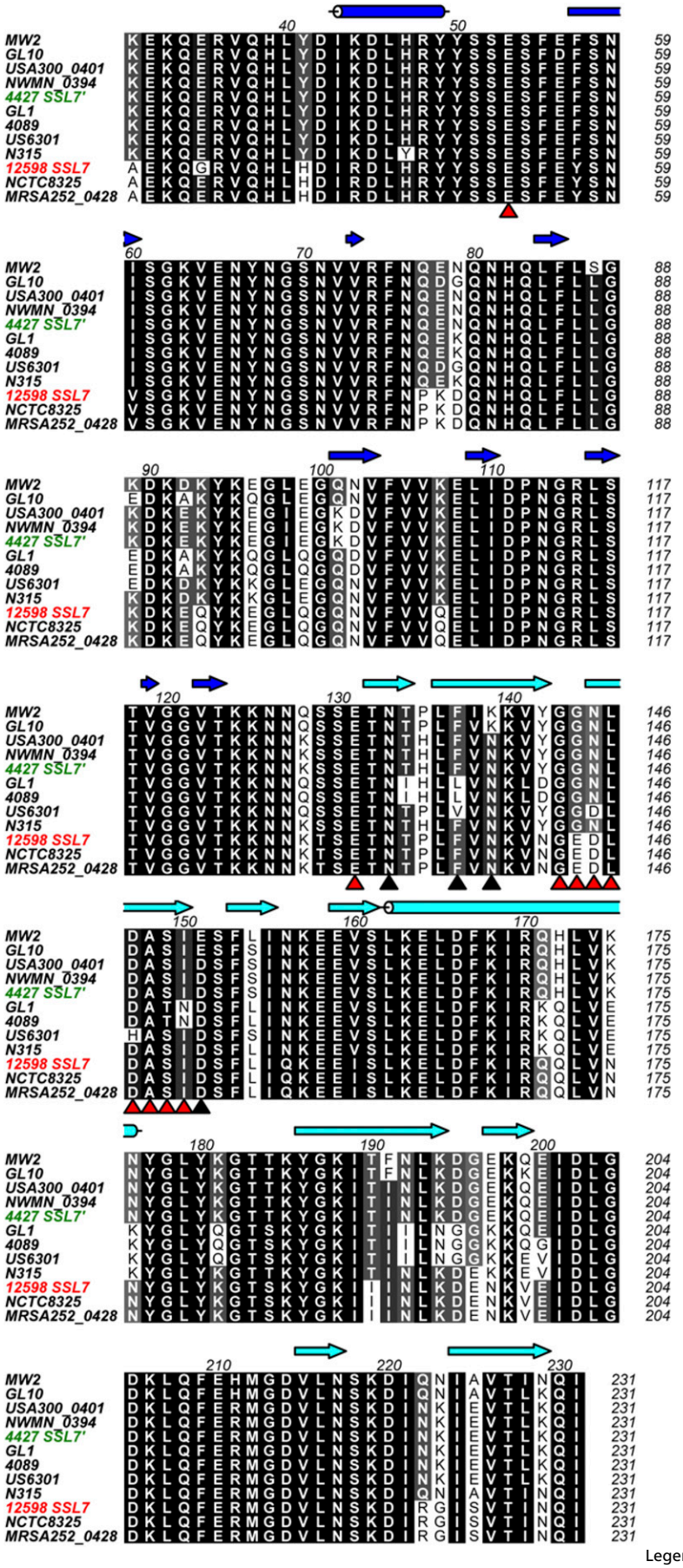

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Fig. S2. Sequence alignment of selected SSL7 sequences with secondary structure elements derived from the C5-SSL7 structure shown above. The label for the 12589 SSL7 and the 4427 SSL7′ sequences are colored red or green. Helices and β-strands are shown as rectangles and arrows, respectively. Secondary structure elements in the OB-domain are blue, and those in the β-grasp domain are colored green. Red triangles indicate residues forming hydrogen bonds or electrostatic interactions with C5; residues with black triangles only form van der Waal interactions.

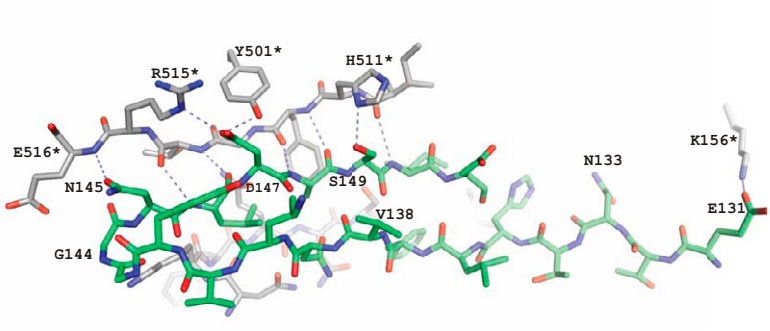
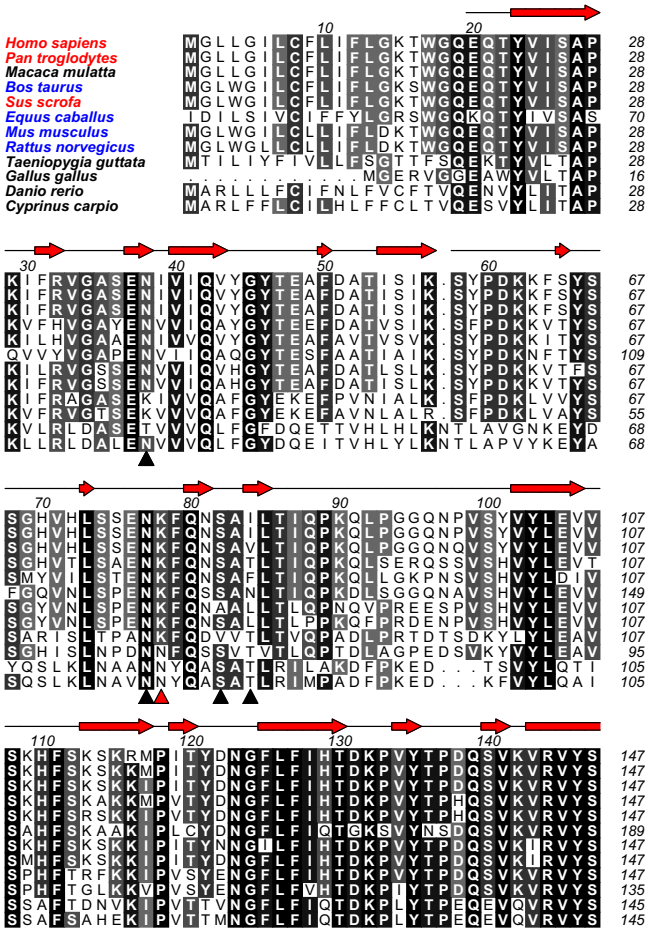


Fig. S3. Selected atomic interactions between C5 (gray carbon atoms, * after residue number) and SSL7′c (green carbon atoms). The view is identical to that in Fig. 2A showing the C5-SSL7 interface. Intermolecular hydrogen bonds and electrostatic interactions are shown with dotted lines.



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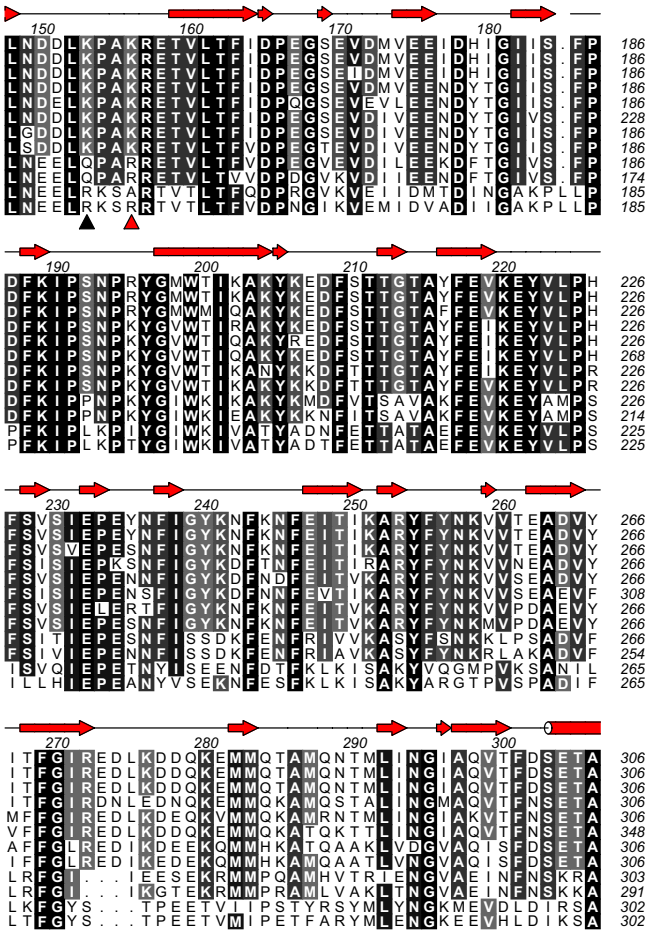

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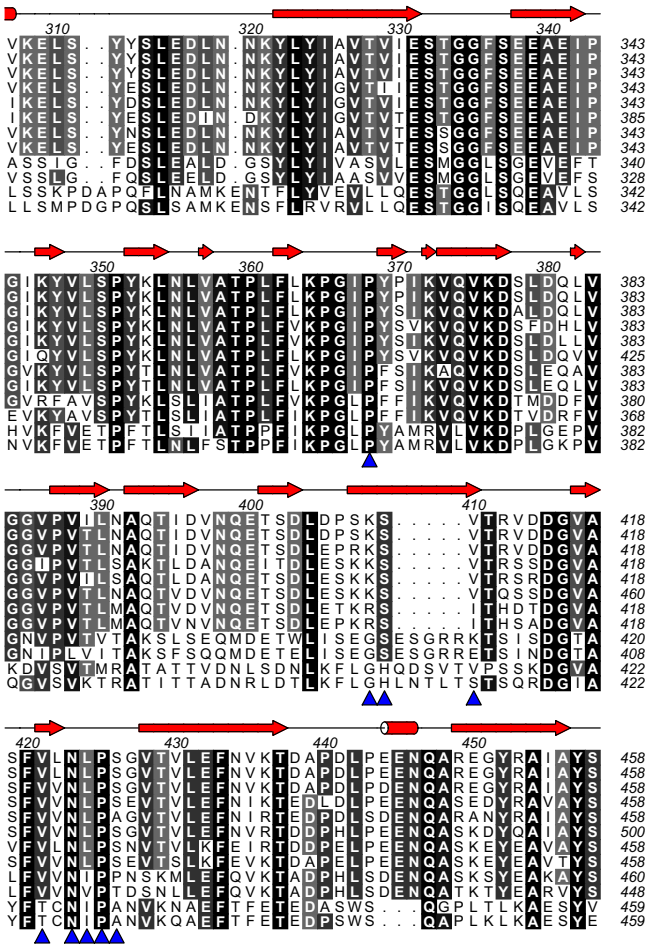

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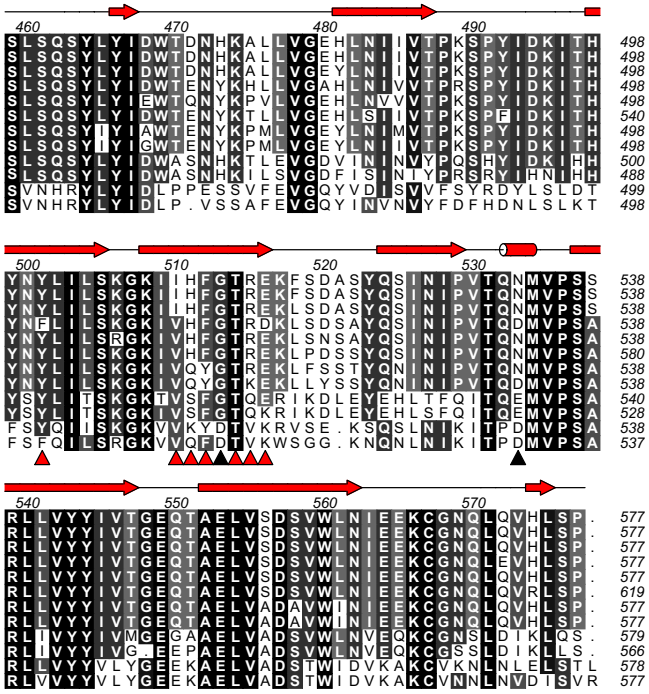
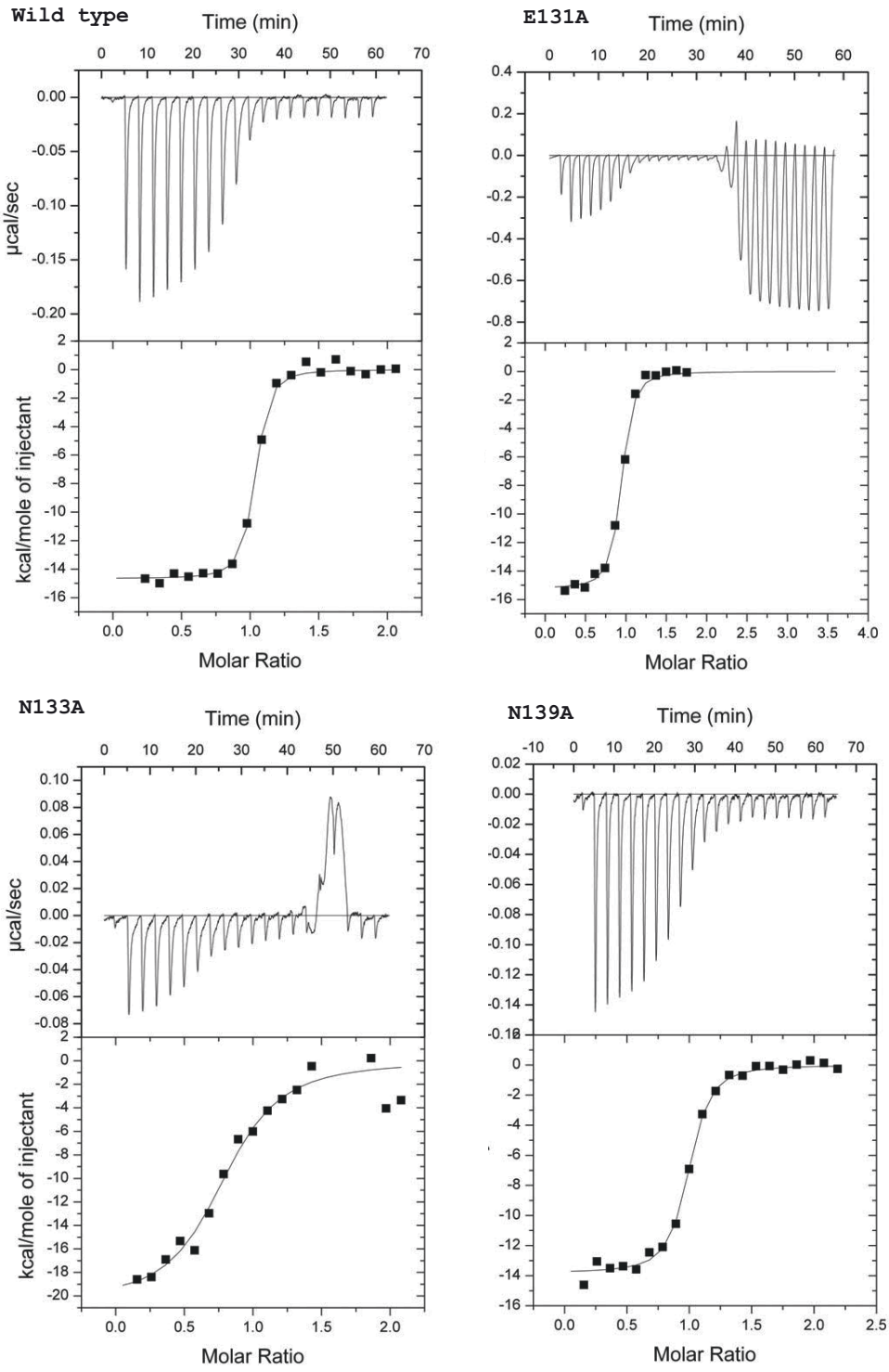


Fig. S4. C5 sequence alignment of the MG1-MG6 domains. Names of species where C5 binds SSL7 are colored red; species where C5 does not bind SSL7 are shown in blue. Red triangles indicate residues forming hydrogen bonds or electrostatic interactions with SSL7; residues with black triangles only form van der Waal interactions. Residues marked by blue triangles are within 5 Å of C3b in a modeled C5-C5 convertase complex. Residue numbers for human C5 is shown below the secondary structure elements, where arrows and cylinders indicate β-strands and α-helices, respectively.



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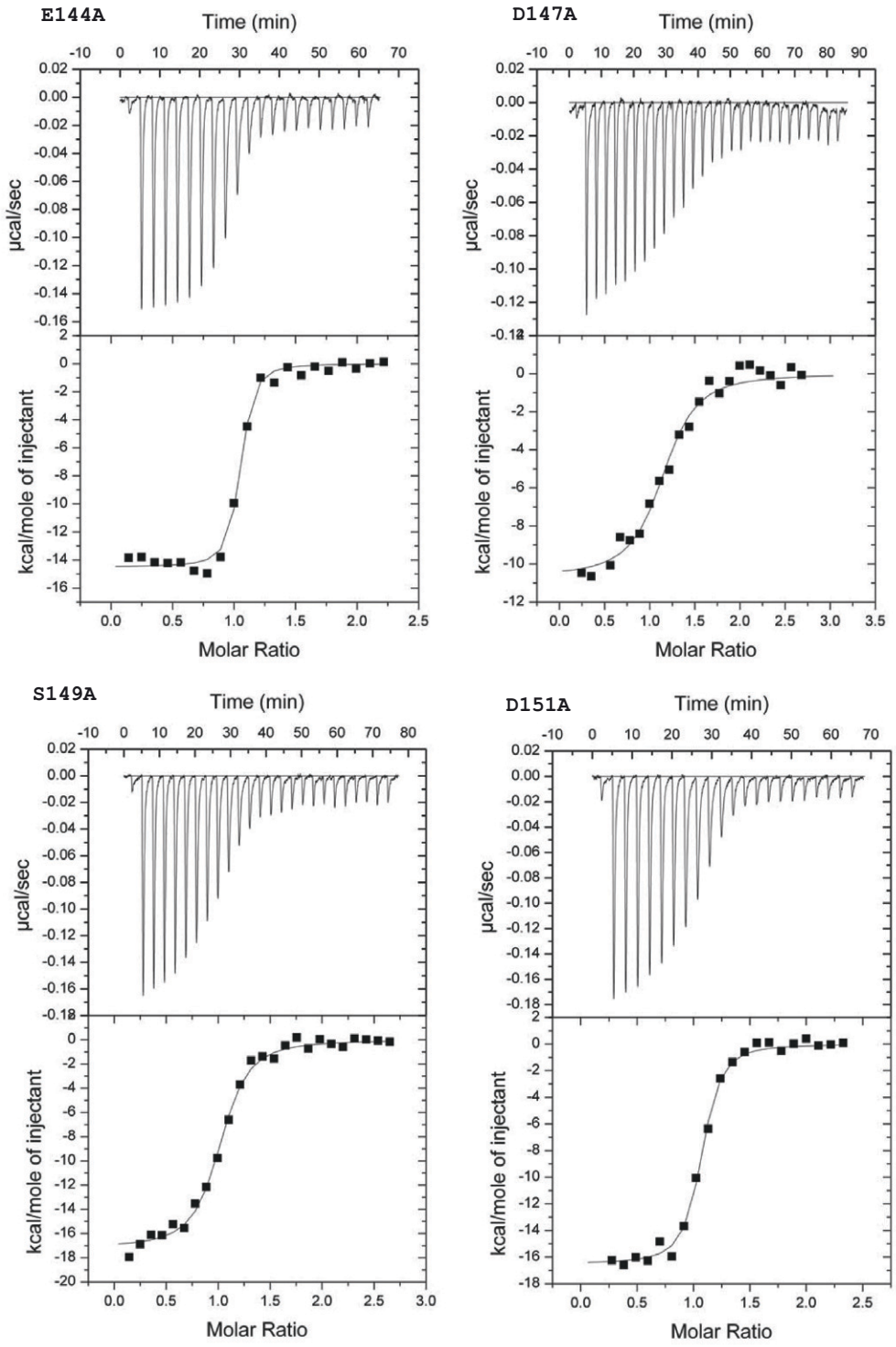

Fig. S5. ITC thermograms obtained upon titration of C5 with wild-type or mutant SSL7. The graphs were produced using the "ITC final" option of the Origin program. In two cases (E131A and N133A) the differential power used for maintaining temperature equilibration between the cells attained a value outside the range recommended by the manufacturer, and the instrument became unstable as revealed by oscillating power recordings. The corresponding data points were deleted.

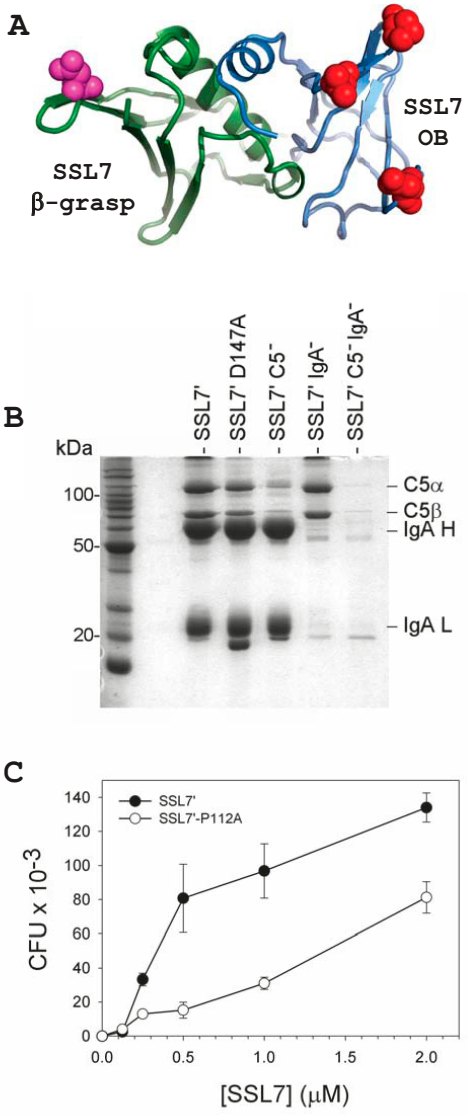

Fig. S6. (*A*) Position of mutations in SSL7′. Mutations D147K (magenta sphere) in the β-grasp domain (green) (SSL7′ $C5^{-}$) and N68T.L109A.P112A (red spheres) in the OB domain (blue). (*B*) Purified mutants immobilized to Sepharose fail to bind either IgA, C5, or both from human serum. (*C*) Inhibition of SSL7′ and SSL7′-P112A on the killing of *E. coli* cells in the presence of 5% cell free human serum in triplicate. In the absence of SSL7, 99.99% killing was achieved within 30 min of incubation at 37 °C. Addition of SSL7′ to 1 μM resulted in ∼20% survival. Note that the single mutation in the IgA binding site significantly reduces the inhibition of bacterial killing. Results are representative of three separate experiments with separate donors.

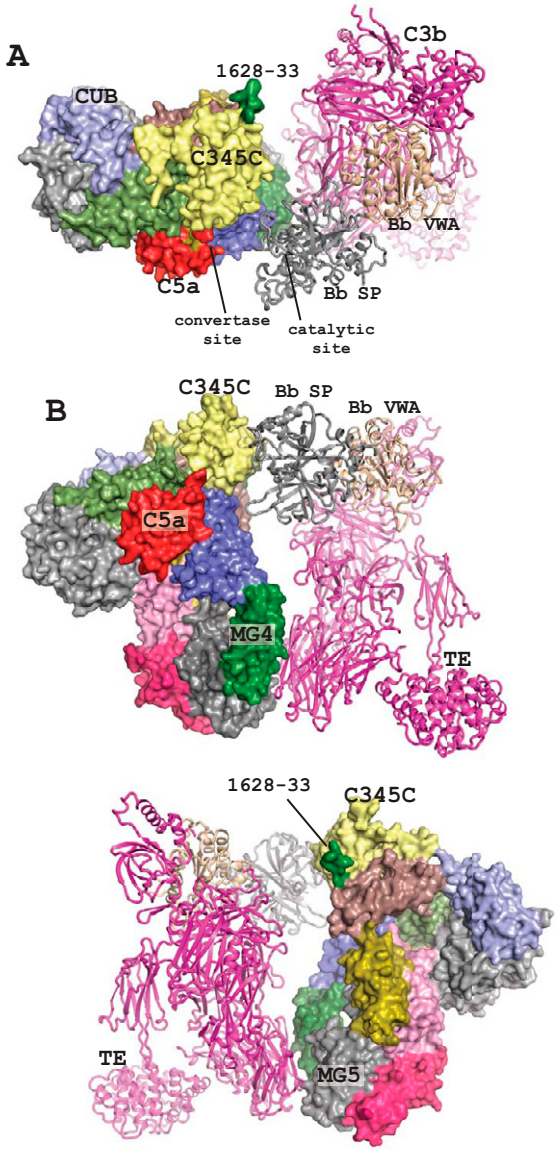

Fig. S7. C5 interactions with the convertase. (*A*) Model of complex between C5 (in surface representation, domains colored individually) and C3b-Bb (cartoon representation, C3b colored magenta, Bb von Willebrand domain wheat colored, and the Bb serine proteinase colored gray) taken from RCSB entry 2WIN [Rooijakkers SH, et al. (2009) *Nat Immunol* 10:721–727]. The model was created by superimposing the MG4 domain of C5 with the MG4 domain of C3b in the $(C3b\text{-}SCIN\text{-}Bb)_2$ complex. Notice that the position of the C345C domain may be a result of crystal packing in the structure of free C5 [Fredslund F, et al. (2008) *Nat Immunol* 9:753–760]. The C5 region 1628–33 (colored green), is likely to come in direct contact with the convertase [Sandoval A, et al. (2000) *J Immunol* 165:1066–1073]. (*B*) Two overall views of the modeled complex. (Upper) Related to A by rotation around a horizontal axis. (*Lower*) Related to *B Upper* by a rotation around a vertical axis.

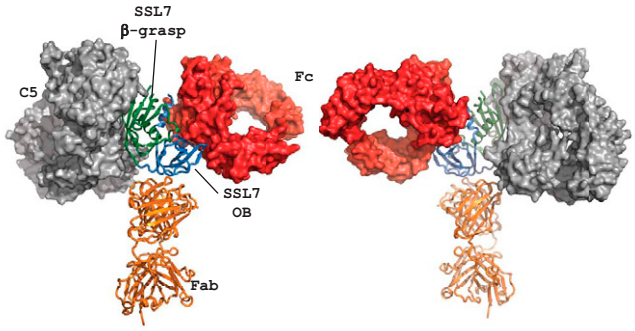

Fig. S8. Shielding of SSL7 epitopes. A model of an SSL7 molecule (green and blue ribbon) bound between a C5 molecule (gray surface, only MG1-MG6 domains and linker region is shown) and Fc (red surface, carbohydrate not included) is compared with a Fab fragment (orange cartoon). Large areas of SSL7 not in direct contact with C5 or IgA would not be accessible to a host antibody due to steric hindrance.

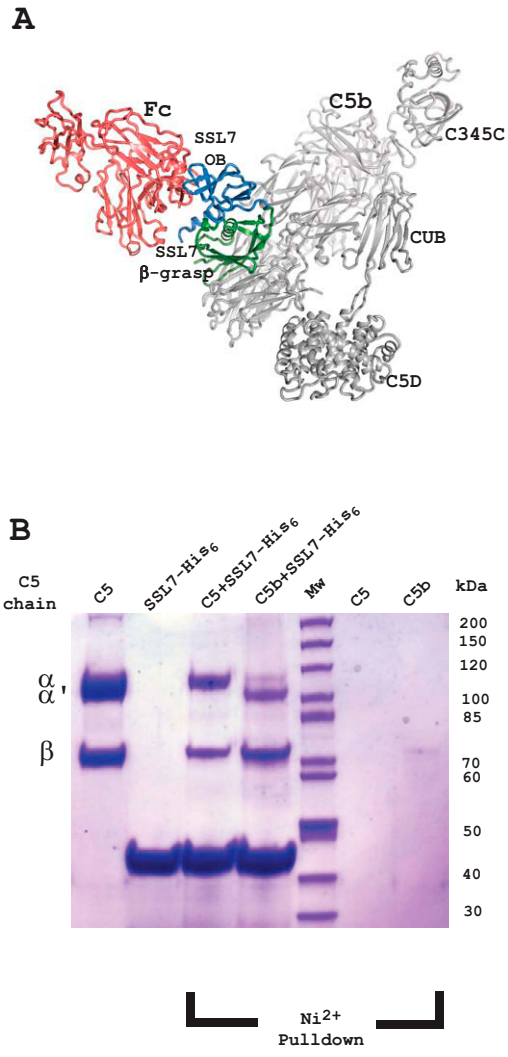

Fig. S9. Interaction of SSL7 with C5b. (*A*) A model of C5b (gray cartoon) based on the structure of C3b [Janssen BJ, et al. (2006) *Nature* 444:213–216] suggests that C5b can interact with SSL7 (green and blue cartoon) in the presence of IgA Fc (red cartoon). (*B*) Experimental verification of the C5b-SSL7 interaction with histidine-tagged SSL7 bound to Ni^{2+}-charged beads.

Table S1. Statistics for data collection and refinement

Data collection	C5-SSL7	C5-SSL7′c
Beamline	MAX-lab 911-2	SLS PXI
λ, Å	1.0379	0.9000
Space group	$P3_1$	$P3_1$
Unit cell parameters, Å	a = 143.88	a = 144.79
	c = 241.24	c = 245.28
Unique reflections	64,277	41,914
Resolution, Å	29–3.6 (3.8–3.6)	50–4.2 (4.4–4.2)
Completeness, %	99.4 (99.5)	99.8 (100)
Redundancy	2.9 (2.9)	5.3 (5.5)
Mean $I/\sigma(I)$	11.0 (2.0)	14.4 (2.1)
Reflections with $I/\sigma(I) > 3$, %	64 (23)	61 (21)
R_{sym},[b] %	8.5 (68.0)	14.1 (89.4)
Refinement		
Reflections: work set/test set	61,125/3,205	39,763/2,029
No. of atoms	27,684	24,804
rms deviation		
Bond lengths, Å	0.012	0.011
Bond angles, °	1.651	1.549
R-factor[a]/R_{free}-factor[3], %	19.6/26.2	22.7/29.6
Ramachandran plot: most favored/allowed/disallowed, %	71.0/28.2/0.8	67.0/32.4/0.6

[a]$R_{sym} = (\Sigma_h\Sigma_i|I(h)_i - \langle I(h)\rangle|/\Sigma_h\Sigma_i I(h)_i)$ for the intensity of reflection h measured N times. Values in parentheses are for outer resolution shell.
[b]R-factor = $(\Sigma_h||F_o| - k|F_c||/\Sigma_h|F_o|)$, where F_o and F_c are the observed and calculated structure factor, respectively, and k is a scaling factor.
[c]R_{free}-factor is identical to the R-factor on a subset of test reflections not used in refinement.

Table S2. Thermodynamic parameters for the dissociation equilibrium C5·SSL7 = C5 + SSL7 derived by isothermal titration calorimetry of human C5 with SSL7

SSL7	N, mol/mol	K_D, M	ΔH, kcal/mol
wt	0.99	5.46×10^{-9}	−14.7
E144A	1.00	5.13×10^{-9}	−14.5
N133A	0.91	9.43×10^{-9}	−11.1
D151A	1.03	1.36×10^{-8}	−16.5
N139A	0.95	1.46×10^{-8}	−13.8
E131A	0.89	1.79×10^{-8}	−15.2
S149A	0.99	3.65×10^{-8}	−17.2
D147A	1.13	6.21×10^{-8}	−10.7

Table S3. IC_{50} comparison of SSL7 inhibition across three C5-mediated functions

	% serum used	IC_{50}, μM	Serum IC_{50}, μM
Hemolysis	20	0.05	0.25
Bacteriolysis	5	0.4	8
C5a production	10	0.02	0.2