Supplemental Data and Materials

Table S1: X-ray data collection and refinement statistics

Wavelength (Å)	1
Resolution range (Å)	49.4 - 3.92 (4.06 - 3.92)
Space group	P 4_1 2_1 2
Unit cell	a = 116.721, b = 116.721, c = 371.057; α, β, γ = 90
Total reflections	46,740 (4,579)
Unique reflections	23,566 (2,317)
Multiplicity	2.0 (2.0)
Completeness (%)	97.61 (94.06)
Mean I/sigma(I)	5.74 (0.82)
Wilson B-factor	147.58
R_{merge}	0.09953 (0.9323)
R_{meas}	0.1408 (1.319)
R_{pim}	0.09953 (0.9323)
CC1/2	0.994 (0.342)
CC*	0.999 (0.714)
Reflections used in refinement	23,430 (2201)
Reflections used for R_{free}	1,991 (188)
R_{work}	0.2257 (0.3685)
R_{free}	0.3220 (0.4313)
Number of non-hydrogen atoms	13,605
macromolecules	13,498
ligands	106
solvent	1
Protein residues	1,694
RMS (bonds)	0.004
RMS (angles)	0.78
Ramachandran favored (%)	74.76
Ramachandran allowed (%)	19.78
Ramachandran outliers (%)	5.46
Rotamer outliers (%)	0.13
Clashscore	17.61
Average B-factor	148.55
macromolecules	148.29
ligands	182.18
solvent	108.76

Table S2. Interfacial contacts between ET3i C1 domain and 2A9 light chain.

C1 Domain	Light Chain
P2067	P55
F2068	L45, Y33, F48, F49
Q2100	Y31
I2102	Y31, F49
T2122	N30
V2125	Y31
T2154	Y31
H2155	S51, F49
Y2156	L53

Table S3. Interfacial contacts between ET3i C1 domain and 2A9 heavy chain.

C1 Domain	Heavy Chain
S2040	Y32
E2066	Y109
W2070	Y102
I2102	V103
M2104	Y102, V103
K2110	E54
W2112	Y102
R2150	D31

Table S4. Interfacial contacts between ET3i A3 domain and 2A9 light chain.

A3 Domain	Light Chain
D1740	S28
S1742	T26
T1744	N2, S92
Q1745	T91
Y1748	T91
N1810-NAG	K65

Data sharing: Public deposit in the Protein Databank (PDBID: 7K66)

Figure S1. Binding sites on the C1 domain. Crystal structure of the C1 domain reported in this study (grey) with highlighted regions showing multiple binding sites that were previously reported using hydrogen/deuterium exchange. The 2A9 epitope (red) and vWF binding site (yellow) share a similar binding loop (2062-2069) that is distinct from the lipid binding region (cyan).

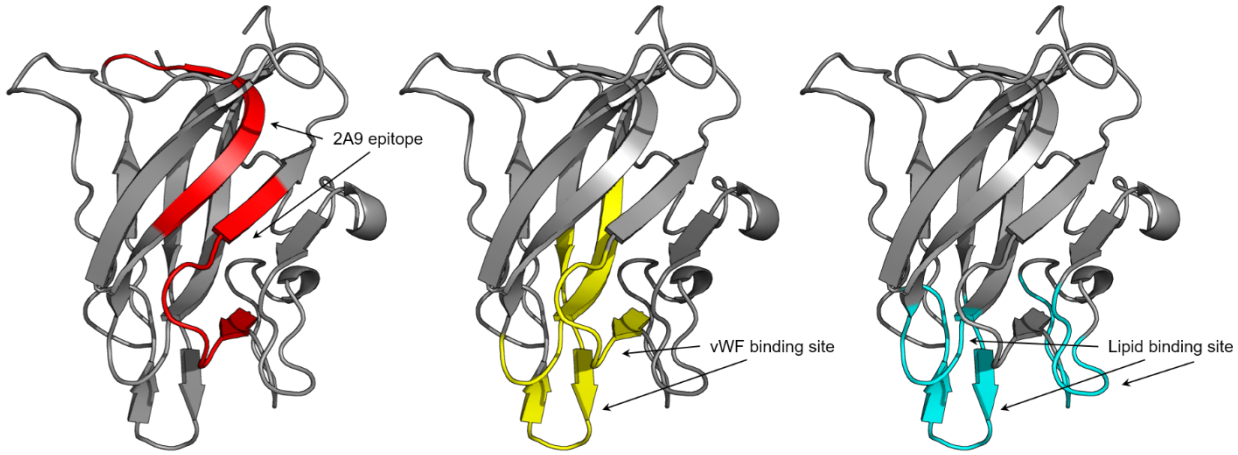

Figure S2. Conformational changes of the fVIII C2 domain. (A) Structural superposition of fVIII reported in this study (yellow, pdb#: 7K66) with the previously reported crystal structure of fVIII in the canonical conformation (pink, pdb#: 6MF0 model A). Structural superposition of each fVIII structure highlights the conserved domain architecture of the A1-A3 and C1 domains. (B) Structural superposition of models A (pink) and B (cyan) from the recently published 3.2 Å structure of ET3i (pdb#: 6MF0). By contrast to the C2 domain translocation in (A), the conformational change of the C2 domain illustrates a barrel-like rotation about an axis central to the C2 domain structure.

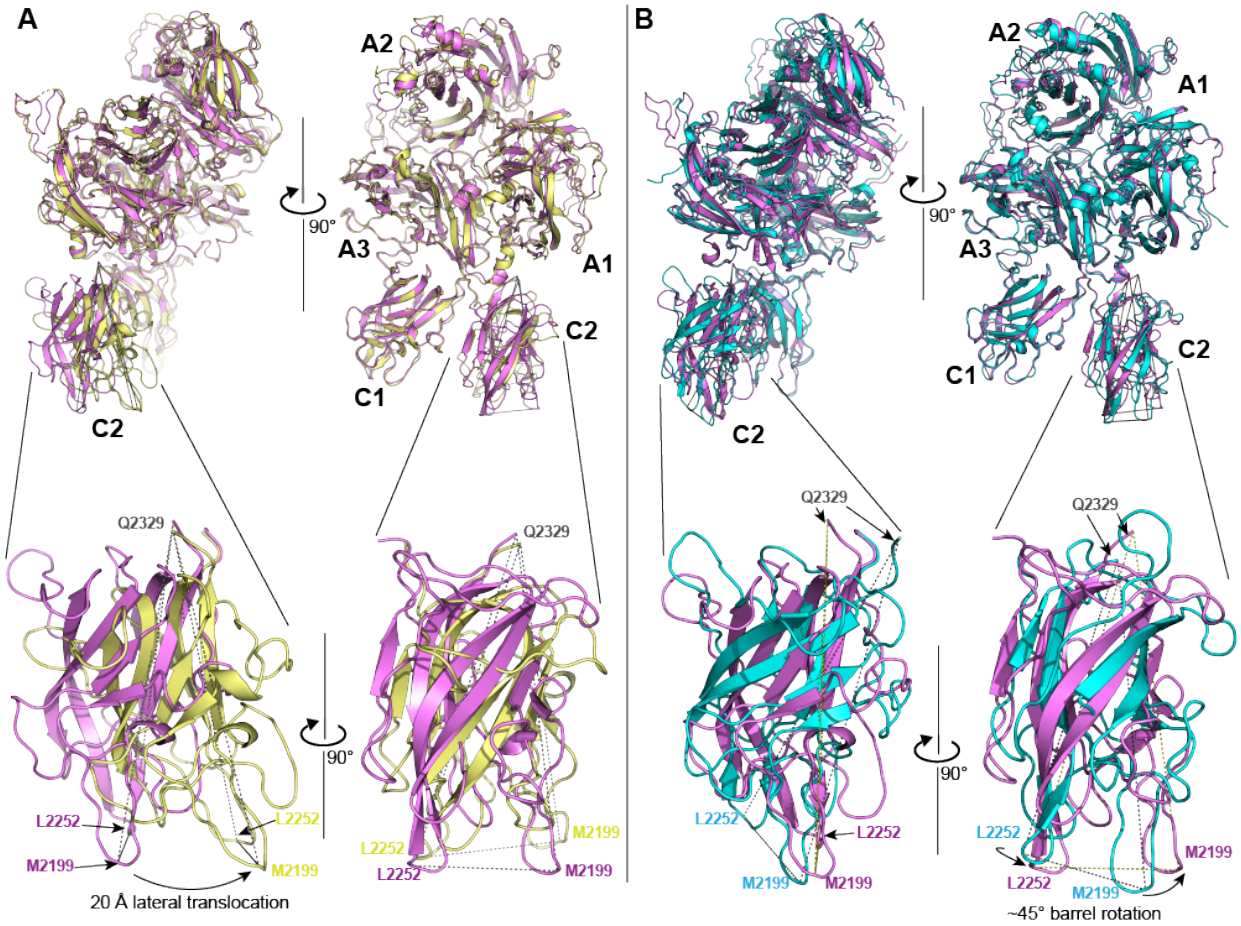

Figure S3. Interdomain contacts between the C2 Domain and the A1/C1 Domains. (A,B) Interdomain contacts within 5 Å between the C2 and A1 domains. (A) The A1/C2 domain interface in the ET3i/2A9 complex structure in this study (teal: A1, blue: C2; pdb#: 7K66). (B) the A1/C2 domain interface in the previously determined ET3i structure (light gray: A1, dark gray: C2; Smith, *et al*. (2020), pdb#: 6MF0). (C,D) Interdomain contacts within 5 Å between the C2 and C1 domains. (A) The C1/C2 domain interface in the ET3i/2A9 complex structure in this study (yellow: C1, blue: C2; pdb#: 7K66). (B) the C1/C2 domain interface in the previously determined ET3i structure (light gray: A1, dark gray: C2, Smith, *et al*. (2020), pdb#: 6MF0).