COMPREHENSIVE EGG-COAT PROTEOME OF AN ASCIDIAN *CIONA INTESTINALIS* REVEALS GAMATE RECOGNITION MOLECULES INVOLVED IN SELF-STERILITY

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SUPPLEMENTAL DATA

Fig. S1. Immunocytochemistry and Far Western Analysis of Ci-ApoBL
(A) An immunoblot of the FT and VC samples detected with an anti-Ci-ApoBL antiserum. (B-E) Localization of Ci-ApoBL protein on the VC and egg cytoplasm. Left column (B, D), immunostained images; right column (C, E), bright field images of the same sections. (B-C) An image of a cross section specimen stained with the Ci-ApoBL antiserum, (D-E) a control specimen stained with a preimmune serum. Scale bar, 100 μm. (F) Sperm homogenate was blotted onto a nitrocellulose membrane and subjected to the Far western analysis in the presence (left lane, FWB) or absence (right lane, WB) of incubation with Ci-ApoBL.

Fig. S2. Apparent Molecular Masses of CiVC Proteins in 1D SDS-PAGE
Total score of detected peptides derived from the protein for each gel slice is shown as a histogram. Mobility of molecular size markers is indicated on the left. Theoretical molecular mass of each protein is shown in parentheses at the top. (A) CiVC15 and CiVC66. Note that observed molecular masses of CiVC15 and CiVC66 were much higher than the predicted ones, suggesting that they are highly N-glycosylated because they have multiple putative N-glycosylation sites (VC15, four; VC66, three; see Fig. 3). (B) CiVC57. Domain architecture of the full-length product of CiVC57 is shown at the top. Regions from which detected peptides are derived are color-coded. Left histogram, peptide score. Right histogram, proportional peptide score normalized to 100% in each gel slice. Processing patterns of CiVC57, which are speculated from the profile of detected peptides from each gel slice, are shown on the right.

Fig. S3. Detection of v-Themis Alleles by LC/MS/MS
Schematic architectures of v-Themis alleles are shown at the top with potential N-glycosylation sites. Theoretical molecular mass of each allele is shown in parentheses at the top. N.D., not determined. Observed molecular masses of v-Themis alleles were always higher than the predicted ones and showed considerable variations among alleles.

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Table S1. **Full List of Identified VC Proteins**

Table S2. **Categories Used for Functional Classification of the VC Proteins**

Table S3. **Proteins Identified in the FT Fraction**

Table S4. **VC-Enriched Proteins**

Table S5. Proteins Identified in the AE Sample

Note: This analysis revealed that most components of the VC in *C. intestinalis* were scarcely extracted by the acid treatment except for Ci-ApoBL. Therefore, most proteins that appear in this list are considered to be contaminants from non-VC derived materials.

Figure S1

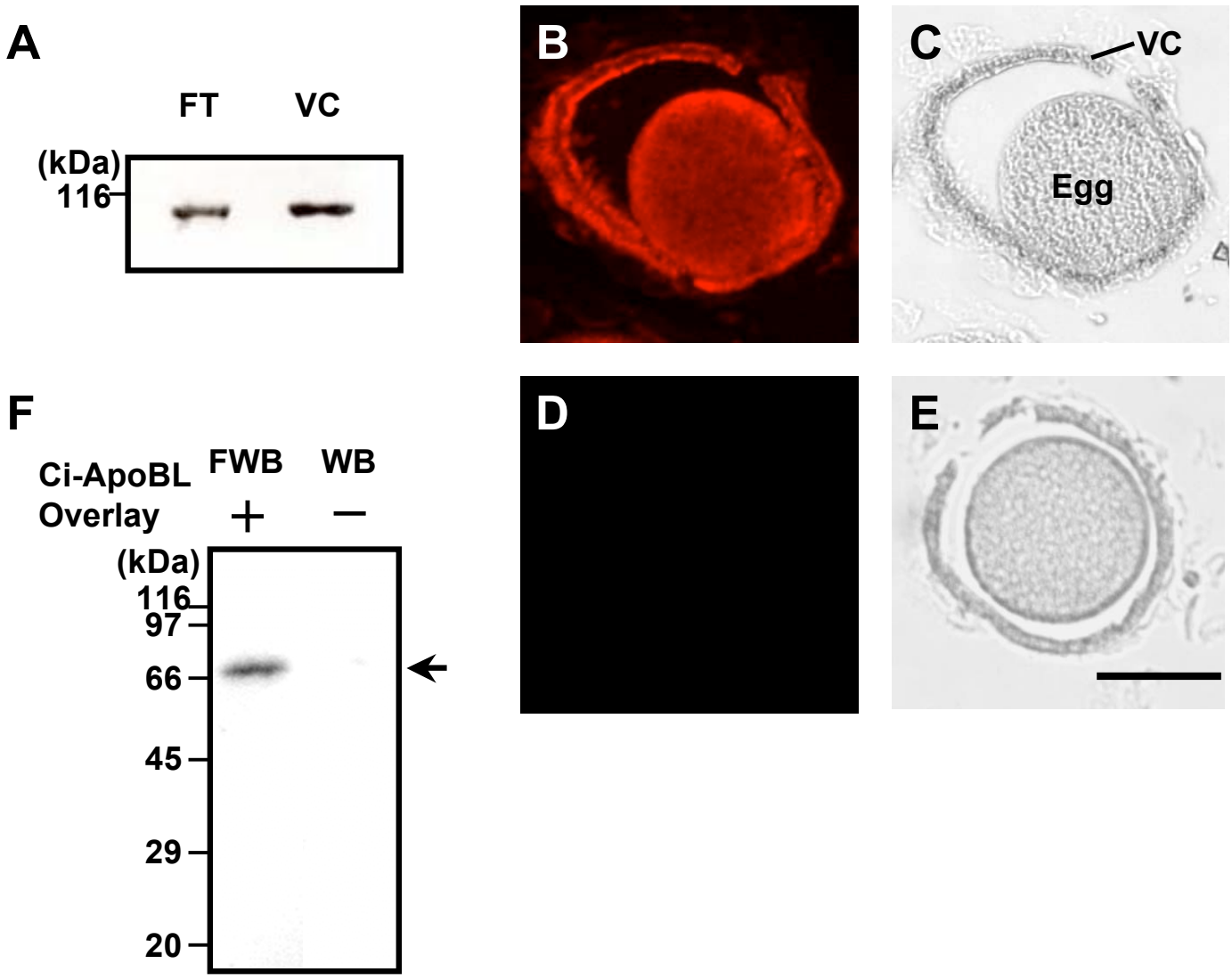

A
FT
VC
(kDa)
116
B
C
VC
Egg
F
Ci-ApoBL
Overlay
FWB
WB
+
−
(kDa)
116
97
66
45
29
20
D
E

Figure S2

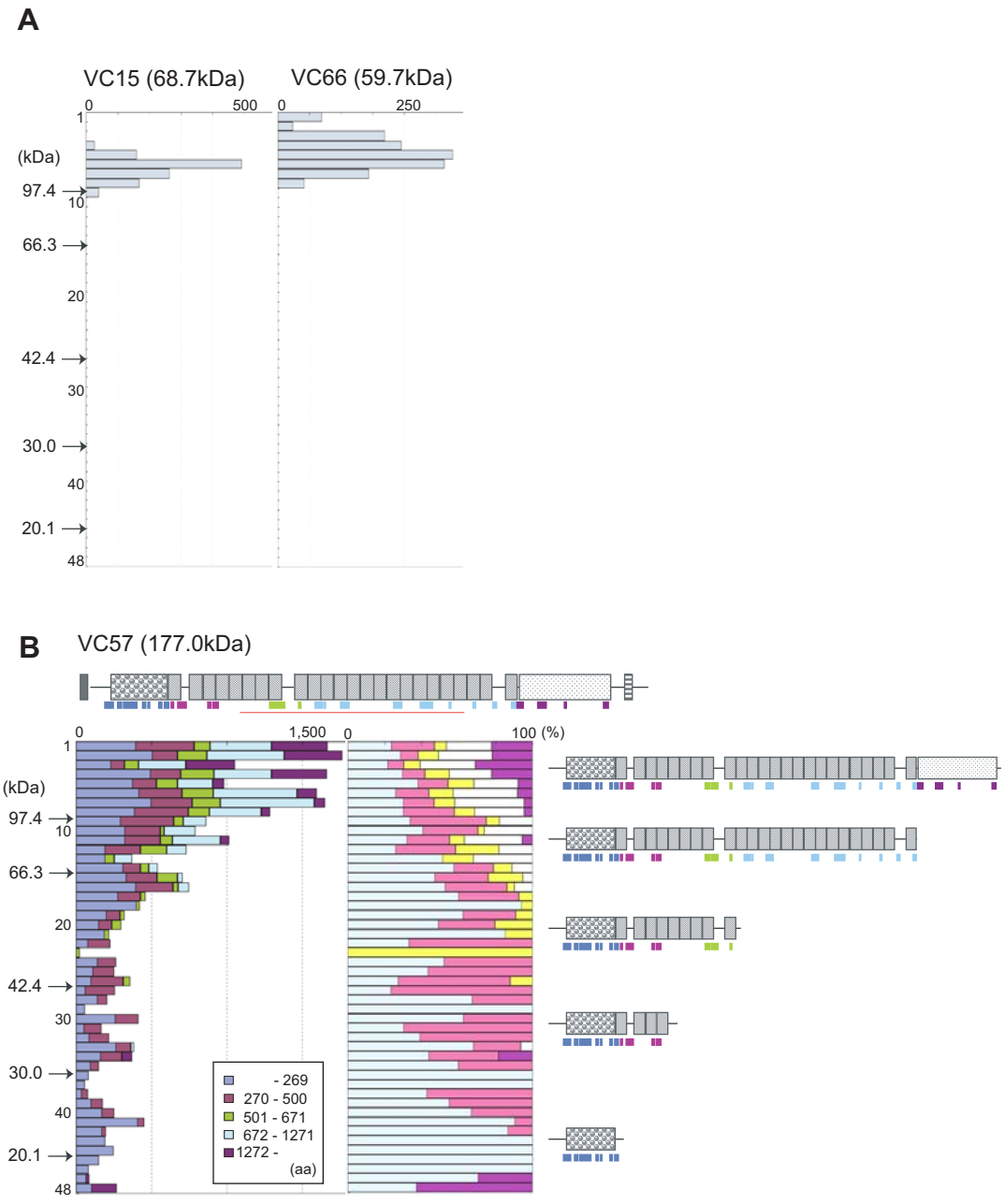
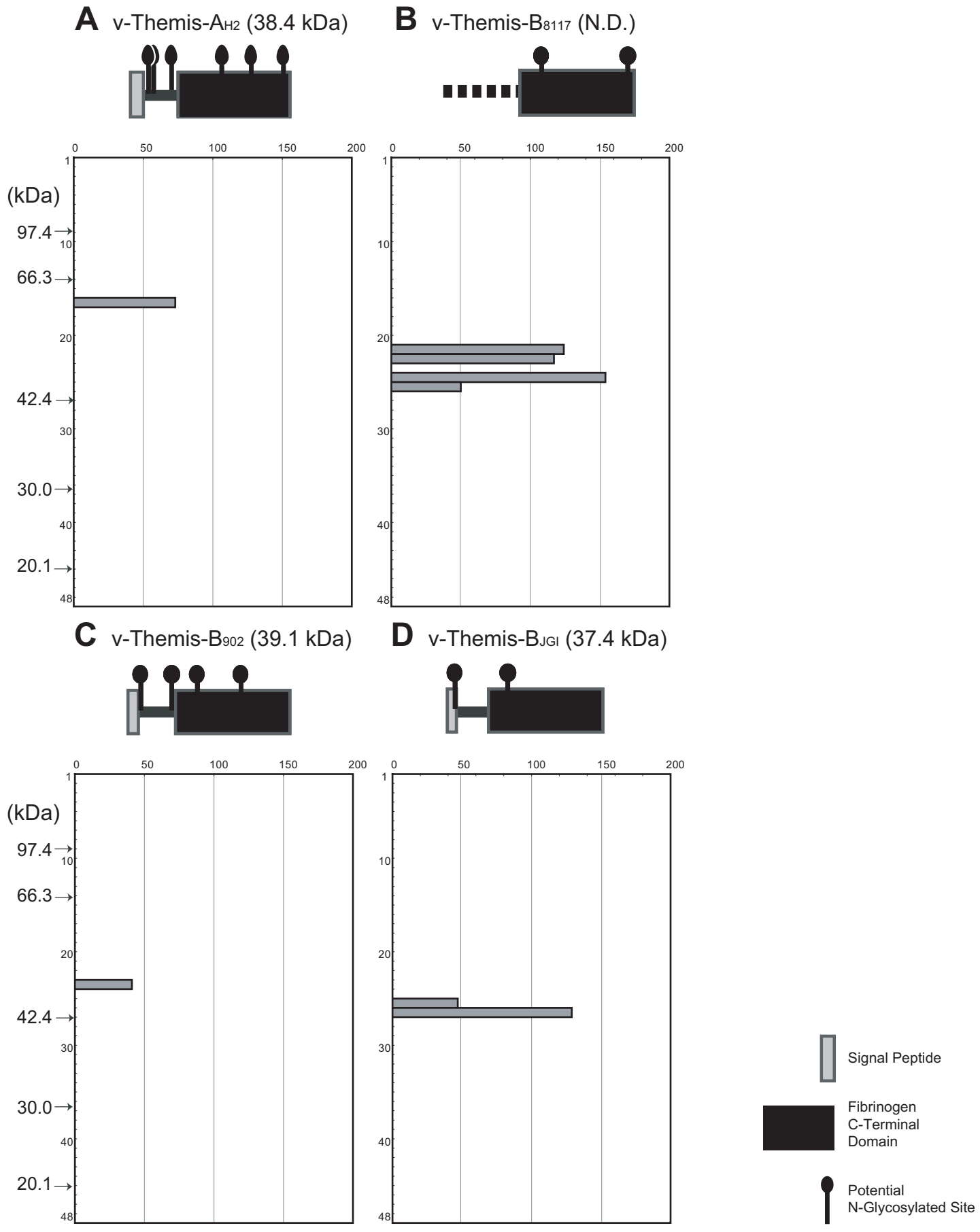

A
VC15 (68.7kDa)
VC66 (59.7kDa)
0
500
0
250
1
(kDa)
97.4
10
66.3
20
42.4
30
30.0
40
20.1
48
B
VC57 (177.0kDa)
0
1,500
0
100 (%)
1
(kDa)
97.4
10
66.3
20
42.4
30
30.0
40
20.1
48
- 269
270 - 500
501 - 671
672 - 1271
1272 -
(aa)

Figure S3


A v-Themis-AH2 (38.4 kDa)
B v-Themis-B8117 (N.D.)
C v-Themis-B902 (39.1 kDa)
D v-Themis-BJGI (37.4 kDa)
(kDa)
97.4
66.3
42.4
30.0
20.1
0
50
100
150
200
1
10
20
30
40
48
Signal Peptide
Fibrinogen C-Terminal Domain
Potential N-Glycosylated Site