
Fig. S1. Structural comparison of the carbohydrate recognition domains (CRDs) of ERGIC-53 and its homologous lectins. (A) Sequence alignment of the CRDs of VIP36, VIPL, and ERGIC-53 from different mammalian species. The secondary structural elements are shown for human ERGIC-53-CRD. The amino acid residues involved in contact 1 are marked with asterisks. Residues conserved among the three CRDs are shown in red, whereas residues conserved only among ERGIC-53 sequences are shown in blue. (B) Superposition of the crystal structures of ERGIC-53-CRD (gray) in complex with MCFD2 (green) and two VIP36-CRD molecules occupying an asymmetric unit (1) (cyan and pink). Manα1-2Man in complex with one VIP36 (pink) molecule is colored in red. The bound Ca^{2+} ions are shown as spheres. A close-up view of the boxed area is shown in the bottom panel.
Fig. S2. Close-up view of the contact 2 interface between, showing intermolecular contacts. ERGIC-53-CRD is colored gray, whereas MCFD2 is pale blue. The bound Ca\textsuperscript{2+} ions are shown as orange spheres. Hydrogen bonds are represented by dotted lines.

Fig. S3. Sedimentation velocity (SV) and sedimentation equilibrium (SE) analyses of MCFD2 and ERGIC-53-CRD mixture under different protein concentration conditions. (A) \(C(s)\) distribution of 100-\(\mu\)M solution of MCFD2 and ERGIC-53-CRD mixture with a 1:1 molar ratio. \(C(s)\) of MCFD2 alone and ERGIC-53-CRD alone are also displayed. (B and C) Equilibrium concentration gradients for 10- (B) and 100-\(\mu\)M (C) solutions of MCFD2 and ERGIC-53-CRD mixture with a 1:1 molar ratio are shown. Result of UV absorbance optics (B) and Rayleigh interference optics (C) are shown. The best fit curve of nonlinear fitting employing a single species model and the residual of the fit are also indicated.
Fig. S4. $^1$H-$^{15}$N heteronuclear single-quantum coherence spectra of the D89A mutant of MCFD2. The spectra were measured in the absence (black) and presence (red) of 5-fold molar excess of the contact 1 peptide corresponding to the ERGIC-53-CRD segment Arg44–His56.

Fig. S5. Evaluation of the effects of the mutations on the complex formation between MCFD2 and ERGIC-53-CRD by analytical ultracentrifugation. Equimolar mixtures of wild-type or mutated MCFD2 and ERGIC-53 (5 μM each) were subjected to SV analyses. C(s) distributions obtained from the analysis of SV experiments are shown. (A) Wild-type; (B) D122V(MCFD2); (C) E116A(MCFD2); (D) D128A(MCFD2); (E) R45A(ERGIC-53-CRD); (F) Y199A(ERGIC-53-CRD); (G) K224A(ERGIC-53-CRD).
Fig. S6. Evaluation of the effects of the mutations on the stability of the complex between MCFD2 and ERGIC-53-CRD by nanoflow electrospray ionization mass spectrometric analysis. (A) Wild-type; (B) D122V(MCFD2); (C) E116A(MCFD2); (D) D128A(MCFD2); (E) R45A(ERGIC-53-CRD); (F) Y199A(ERGIC-53-CRD); (G) K224A(ERGIC-53-CRD). Peaks corresponding to MCFD2, ERGIC-53-CRD, and their 1∶1 complex are indicated by green, blue, and red dots, respectively. The stoichiometry and composition were confirmed the wild-type complex by tandem mass spectrometry as shown in the Inset, where the +12 charge state of the complex isolated in the quadrupole dissociates with symmetric and asymmetric charge partitioning into +6, +7, and +3 charge states of MCFD2 and +6, +5, and +9 charge states of ERGIC-53-CRD upon collision-induced dissociation.
Fig. S7. Isothermal titration calorimetry analysis of MCFD2 binding of ERGIC-53-CRD. The Upper panel displays the raw data, whereas the Lower panel shows the integrated heat values corrected for the heat of dilution and fit to a one-site binding model (Solid Line). ERGIC-53-CRD or its complex with the sugar ligand (300 μL) in buffer (20 mM Tris-HCl, 100 mM CaCl₂, pH 7.5) in a sample cell was titrated with MCFD2, by using a 40 μL stirrer syringe with injections of 2 μL (19 total). The concentrations of the samples injected were as follows: 0.045 mM ERGIC-53-CRD (plus 0.045 mM Manα1-2Man or 0.1 mM Man₈GlcNAc₂-PA) titrated with 0.45 mM MCFD2. The titrations were performed while samples were being stirred at 1,000 rpm at 20 °C. Injections were separated by 3-min intervals in order to stabilize the baseline. The data were analyzed by use of Microcal Origin software. The obtained $K_a$ for MCFD2 with no oligosaccharide, Manα1-2Man, and Man₈GlcNAc₂-PA are $3.03 \times 10^6$ (±0.64), $3.89 \times 10^6$ (±0.73), and $4.47 \times 10^6$ (±0.77) M⁻¹, respectively.
Table S1. Hydrodynamic properties of MCFD2, ERGIC-53-CRD, and their complex determined from SE experiments

<table>
<thead>
<tr>
<th>Protein</th>
<th>$S_{20,w}^*$</th>
<th>$S_n^†$</th>
<th>$f/f_0^‡$</th>
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<tr>
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<td>1.73</td>
<td>1.67§</td>
<td>1.34</td>
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<tr>
<td>ERGIC-53-CRD</td>
<td>2.70</td>
<td>2.93¶</td>
<td>1.21</td>
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<td>MCFD2/ERGIC-53-CRD</td>
<td>3.24</td>
<td>3.39∥, 3.43**</td>
<td>1.27</td>
</tr>
</tbody>
</table>

*Corrected to standard condition (water, 20 °C).
†Sedimentation coefficient determined for beads modeling using the SOMO module implemented in UltraScanII.
‡Frictional ratio obtained and molar mass calculated by using Sednterp.
§Calculated by using the NMR structure of MCFD2 (Protein Data Bank accession code 2VRG).
¶Calculated by using the crystal structure of ERGIC-53-CRD (Protein Data Bank accession code 1RIZ).
∥Calculated by using the structure of the 1:1 complex by contact 1 of the present study.
**Calculated by using the structure of the 1:1 complex by contact 2 of the present study.