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Supplemental Data
A Structure-Based Mechanism for Vesicle Capture by the Multisubunit Tethering Complex Dsl1

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Supplemental Experimental Procedures

Purification of Tip20(L28C)–Dsl1(L48C)–Sec39 complexes for EM studies

To stabilize the Dsl1 complex for EM studies, a disulfide cross-link between Tip20 (L28C) and Dsl1 (L48C) was designed based on our previous Tip20-Dsl1 fusion protein structure (PDB 3ETV) (Tripathi et al., 2009). Tip20 (L28C), Dsl1 (L48C), and Sec39 were individually purified from cell lysates by Ni^{2+}-affinity and anion exchange chromatography. In order to form the disulfide bond, Tip20 (L28C) and Dsl1 (L48C) were incubated overnight in 10 mM Tris, pH 8.0, 150 mM NaCl, 1 mM 2-mercaptoethanol at 4 °C. Soon after adding Sec39, the protein mixture was purified over anion exchange chromatography, yielding a mixture of crosslinked and uncrosslinked Tip20(L28C)–Dsl1(L48C)–Sec39 complex. The protein was loaded onto a S200 16/60 column equilibrated in high ionic strength buffer (10 mM Tris, pH 8.0, 400 mM NaCl), under which conditions the Tip20–Dsl1 interaction was significantly weakened. Protein fractions containing the crosslinked complex were pooled and loaded onto a S200 10/30 column equilibrated in 10 mM Tris, pH 8.0, 150 mM NaCl. Fractions enriched for the crosslinked complex (~ 70% purity) were examined using negative stain EM.

Table S1. X-ray Data Collection and Refinement Statistics for Dsl1C$_{lactis}$–Sec39

	Dsl1C$_{lactis}$–Sec39 SeMet		
Data collection			
Space group	$P2_12_12_1$		
Cell dimensions			
a, *b*, *c* (Å)	72.02, 90.82, 213.88		
α, β, γ (°)	90.0, 90.0, 90.0		
	Peak	*Inflection*	*Remote*
Wavelength (Å)	0.9793	0.9795	0.9641
Resolution (Å)	100-2.60 (2.69-2.60)	100-2.60 (2.69-2.60)	100-2.70 (2.80-2.70)
R_{sym} (%)	6.0 (42.1)	6.8 (65.3)	6.4 (73.7)
$\langle I / \sigma_I \rangle$	15.6 (3.7)	14.0 (2.5)	12.3 (2.2)
Completeness (%)	99.2 (100.0)	99.8 (100.0)	99.9 (100.0)
Redundancy	3.8 (3.8)	3.8(3.8)	3.8 (3.8)
Refinement			
Resolution (Å)	35-2.6		
No. reflections	78583		
$R_{work/}$ R_{free} (%)	0.205/0.271		
No. atoms			
Protein	7373		
Water	3		
B-factors (Å^2)			
Main-chain	59.1		
Side-chain	63.4		
Water	56.2		
R.m.s deviations			
Bond lengths (Å)	0.008		
Bond angles (º)	1.170		
B-factors (m/c)	6.4		
B-factors (s/c)	7.1		
Ramachandran (%)			
Within favored	95.8		
Within allowed	99.4		
Outliers	0.6		

Values in parentheses are for the highest resolution shell.

Figure S1. Sequence Alignment and Mutagenesis Studies
(A) Sequence alignment of *S. cerevisiae* Dsl1 and *K. lactis* Dsl1. The disordered region, based on the structure of Dsl1C$_{lactis}$–Sec39, is indicated with gray shading. Tryptophan residues in this region, presumably involved in binding to COPI coat proteins, are indicated with blue shading. Residues involved in Dsl1–Sec39 binding are marked with stars.
(B) Mutations at the Dsl1–Sec39 binding interface abolish their interaction.

Figure S2. Negative Stain EM of the Dsl1 complex
(A) Class averages of the Dsl1/Sec39 complex. Side length of each class average is 36 nm.
(B) Class averages of the Tip20 subunit. Side length of each class average is 29 nm.
(C) Left: representative image of Tip20–Dsl1–Sec39 (scale bar = 50 nm). In this sample, the Tip20–Dsl1 interaction was stabilized by an engineered disulfide bond between Tip20 L28C and Dsl1 L48C. Right: selected particles of the Tip20–Dsl1–Sec39 complex (side length = 54 nm). Surface representation of the Dsl1 complex model was oriented to mimic particles in closed conformations. Manual adjustment at the Dsl1 subunit domain B-C hinge (see Figure 2C) allows fitting of the model into open conformations.

Figure S3. Yeast Bearing an N-terminal Truncation of *use1* are Temperature-Sensitive
S. cerevisiae bearing wild type *USE1* or *use1Δ2-35* (which disrupts the Use1–Sec39 interaction) on a *CEN-LEU* plasmid were spotted on YPD plates and incubated at the indicated temperatures for 2-3 days.

Figure S4. All Four SNARE Proteins are Required for Complex Formation
SNARE complexes did not assemble if any ER SNARE (Sec20 in (A), Ufe1 in (B), or Use1 in (C)) was omitted.

Figure S5. Fluorescence Polarization Studies of SNARE Complex Assembly
Experiments were carried out as in Figure 5A, with incubation at 23°C for 8 hr. Sec39 accelerated SNARE assembly, as judged by comparing the first two bars. The removal of the trigger factor tag from Use1, however, further accelerated the reaction, indicating that the tag itself affects SNARE assembly. Nonetheless, it is notable that the Dsl1 complex accelerated SNARE assembly compared with Sec39 alone, whether the Use1 TF tag was removed or not.

A

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Sc-Dsl1 : -------MESLFPNKGEIIRELLKDPLILKNDSKRSNGSELELDSSDLLQREAILANELNILDNLKTFLNLIKEVKTNLNILELENCYY :  82
Kl-Dsl1 : MATLEKLLNDLLSDTSALSQAIEADPQFKSSDSVHPKPNSKLLE--EVLREDSDLTKDLAKYNQYKILSDLLKEFNTNYELNELENCYY :  87

Sc-Dsl1 : SLQSLRKKMRNNAAYLKQSFNFQQSISTYVDTLHLELVSTLYKILTNGFWKITENSIQFTPTVEWGKDKVHIEYDTFMDFVAQQYFPKG : 171
Kl-Dsl1 : SLQNIHKKFKDV-NISGESFRFQQSVATYIDCLHLNVLER-FKEVSNMFYTISGNEMMFKKTIEL--DSVEFNFDEMVELFENNFFDHN : 172

Sc-Dsl1 : SLDNQAWFILDMTSADSQEQVRAKLNTIMKEYMNLSRIVSMIKNSIFISGKEISYENEKNILVFSKSSSHGQHCVSTVLTSFEAVCDFM : 260
Kl-Dsl1 : MIDQTHWFIASTNLETYKDKEIVLLQELLDDTVKLNPLIKFFKEFVEDEKCIMVRAEPG-KLTLATSEYNSL----DQIKSYQTLTTFI : 256

Sc-Dsl1 : LDGLAFRDRKTLSYELGPLFNTEFTKFVKNNASIILESLDSPLKNLVSVINNKLTRLVAKSEVTNWTHSGKEIQDLLMNKQLYYNLLLD : 349
Kl-Dsl1 : ENDLSKHAKESVMIHFGNVLLTELLKIIRANPE-LLSNDKEQTTEIISSIHDSLTKI---SEETEWAYDGFELNRILKDENIYTTLKFE : 341

Sc-Dsl1 : KVLESHISEIRSIFEDPKKSWQNLEVVELTTSNTNTMSEKIGKNDSDVQNEKELHNAVSKDDDWNWEVEDDDA--DAWGDEIDVNIDDE : 436
Kl-Dsl1 : SMMQQRVIQIRSI---PEEEYHELVSVQFKTDGGK--YEKGEKQDLEL-SEKKTENG-KDTESWGWNENQDSDEHDGWDEELDIDVDNV : 423
              *  *   *

Sc-Dsl1 : EEKTNQEKEKEPEEEENAWDEAWAIDENIDDASLENGKEHLKAHDVGSLDKDHIEVTQLPKLFLAISQNFKSS---FADSHVDEQYFAY : 522
Kl-Dsl1 : ------------------------------------------------------PIQVSVFVQSAAKVFTEFEQGCDTIGRSKVESIYL-Y : 459
                                                                                                  *

Sc-Dsl1 : KYNLLQTSYMAMCTANFSHNWCQLYVDMRYLIERDEKLYRIKELTRNLLETKLNMKYRIVCQLIRHQLTEFRENERNPSWDATIEKLLP : 611
Kl-Dsl1 : KFNLLQTAFFAMVSEKV-NDWTQLYKDVRYLYTENPKLLQLMELNSRRLDLNLNLIKKTIYKLVNDQLQELKDNERTPDWDITISSLLP : 547
          *  *  **  **  **                              *  **  *  **  *  *

Sc-Dsl1 : YILKEIVRPLQKIRGEEGSRYLLSFLNFLYNDCVTKEILKWQIISEVNSENLGELVSLLVNNTDIQLLAKEPSYKKMREKFATMGKFLP : 700
Kl-Dsl1 : YLKKTALPTLYKL---EDNTILVALIRYIVHDLVIDNILHWRVISEKSSENLSEFIMLLLSGLEIPRLNLIETYRHSREKLGILSKILT : 633
                                  *

Sc-Dsl1 : LHLKEIMEMFYNGDFYLFATDELIQWIELLFADTPLRRNAIDDIYEIRGTALDD : 754
Kl-Dsl1 : AHLKDILEMFYEGEEFLFETDEIVQWILLFADTPTRRDCIDEIRRVREEATD- : 686
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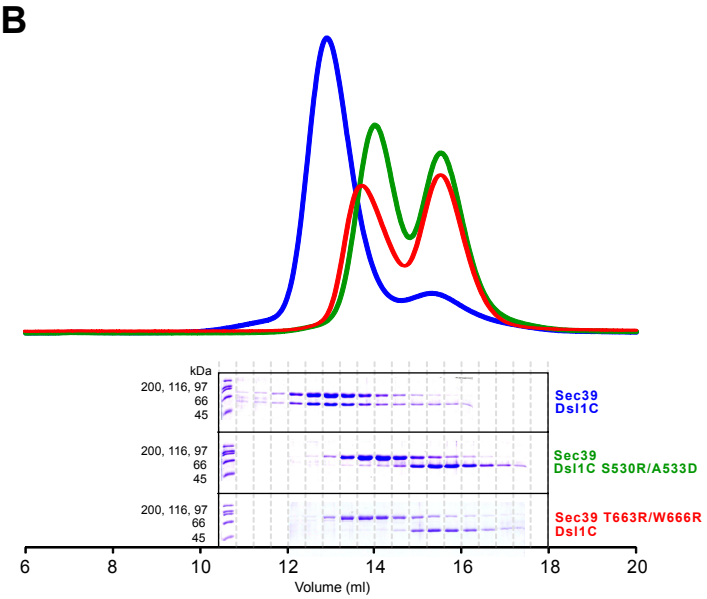
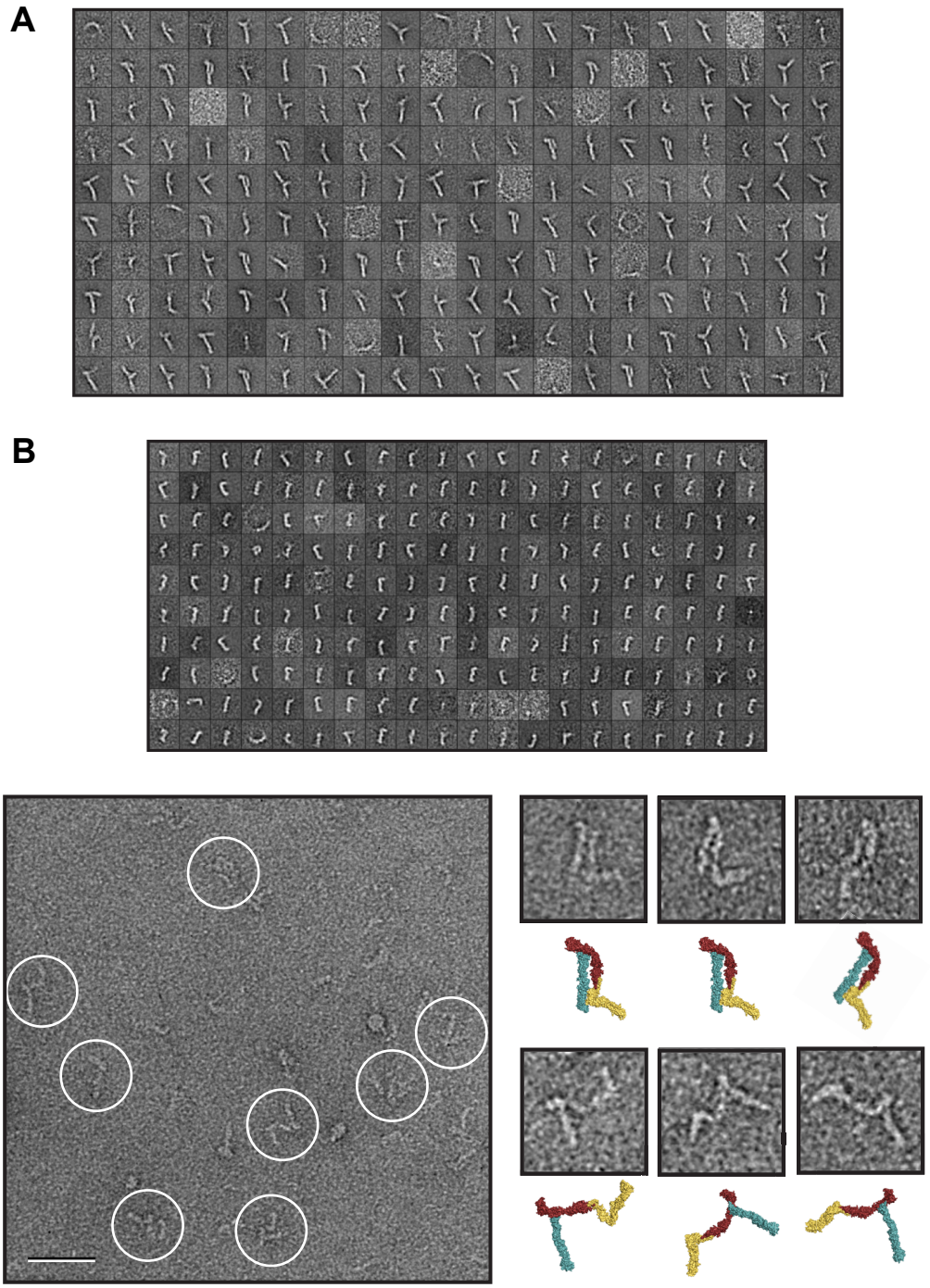
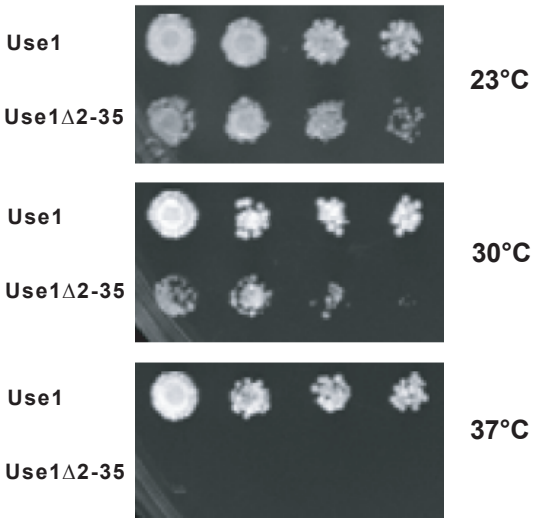
B



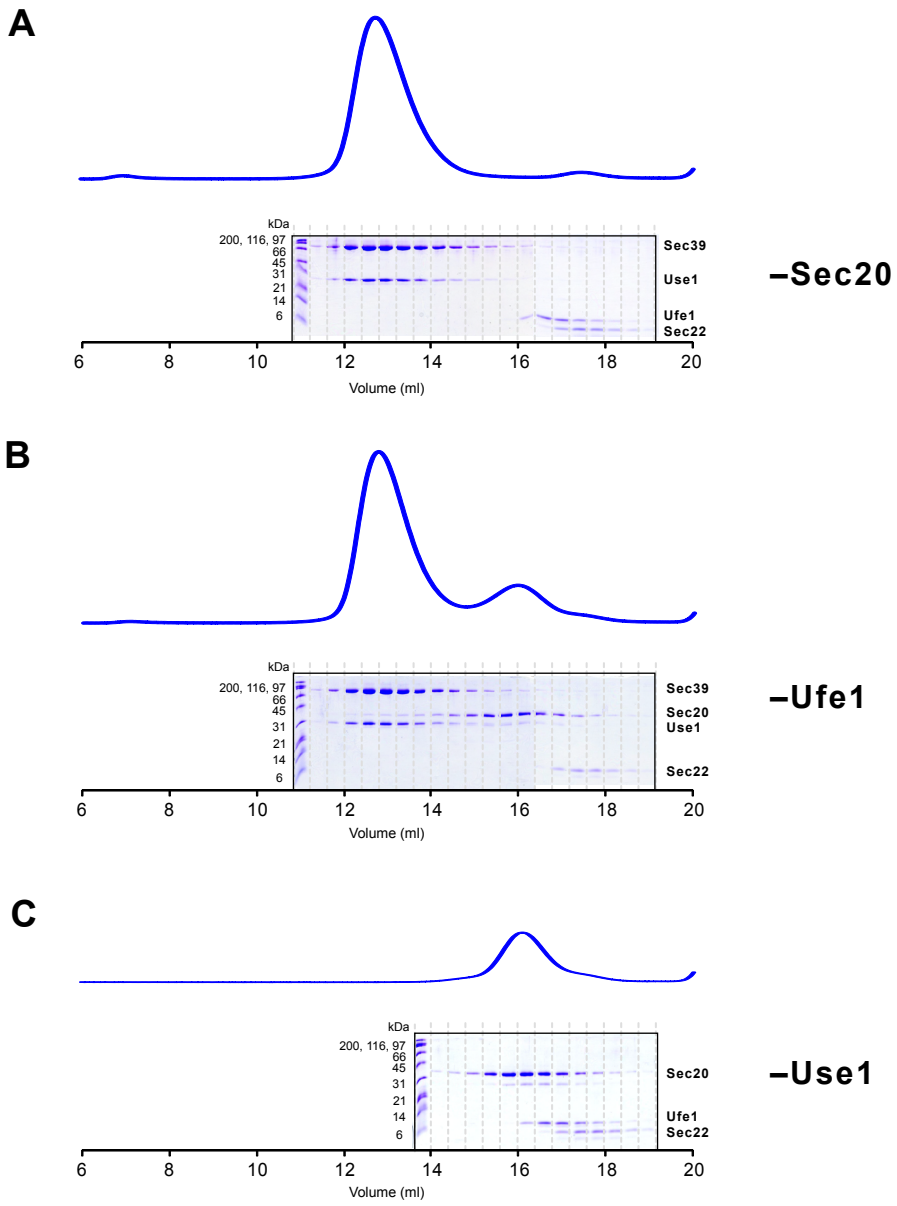
Supplementary Figure 1



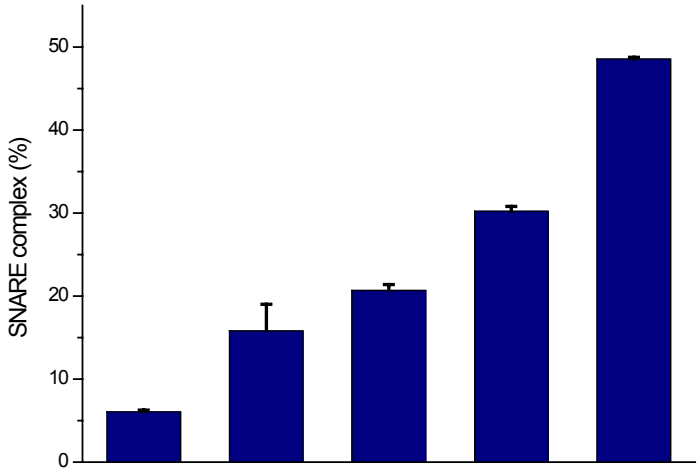
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



TF-Use1	+	+	+		
Use1				+	+
Sec20	+	+	+	+	+
Ufe1	+	+	+	+	+
Sec22	+	+	+	+	+
Tip20			+		+
Dsl1			+		+
Sec39		+	+	+	+

Supplementary Figure 5