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Supplemental Information

The Starch Utilization System Assembles around Stationary Starch-Binding Proteins

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Supporting Information for:

The Starch Utilization System Assembles Around Stationary Starch-Binding Proteins

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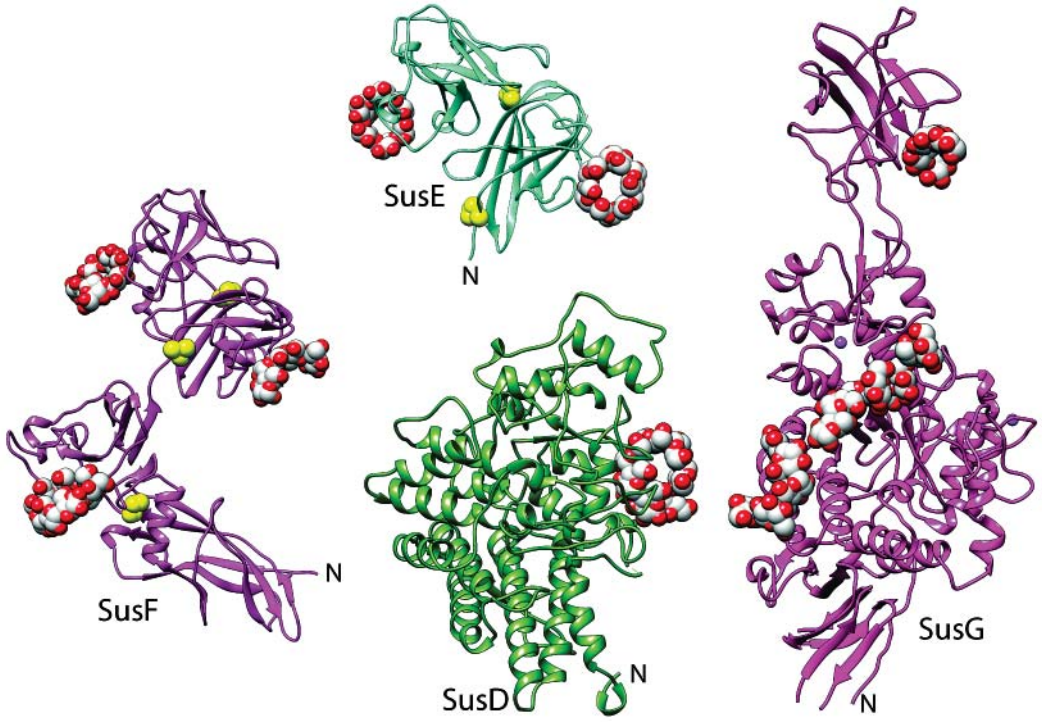

Figure S1. The crystal structures of SusD (PDB 3CK9), SusE (PDB 4FEM), SusF (PDB 4FE9), and SusG (PDB 3K8L). Co-crystallized maltooligosaccharides are shown with grey and red spheres, prolines in SusE and SusF are shown with yellow spheres. The 15 – 20 residue N-terminal linkers that connect each protein to the lipidated cysteine for tethering to the membrane were not resolved in the crystal structures. The N-terminal domain of SusE (residues 38-167) was not resolved in the crystal structure.

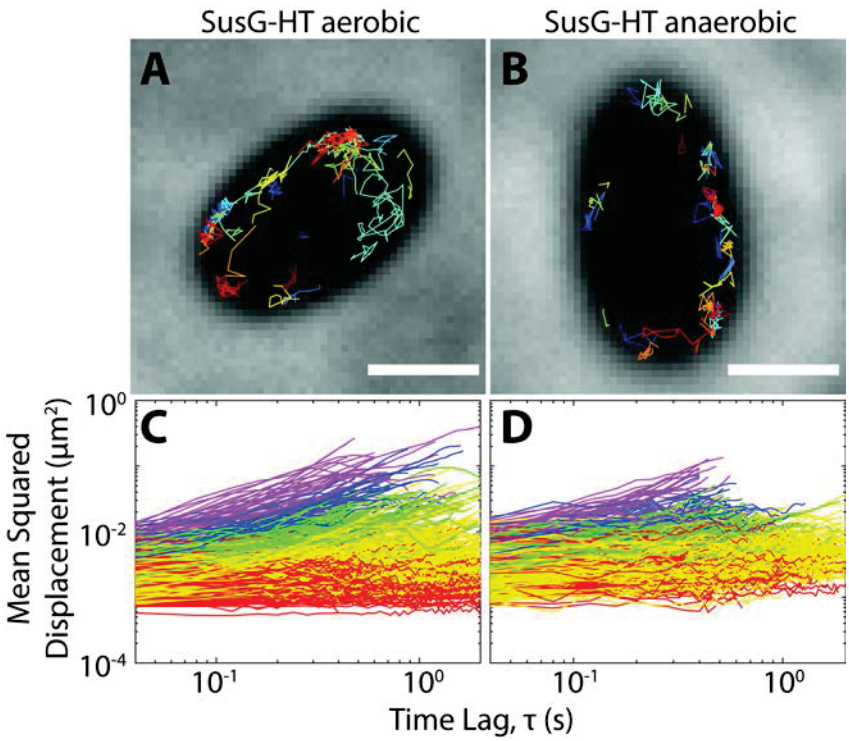

Figure S2. SusG-HT is mobile on the cell surface whether imaged under aerobic or anaerobic conditions. (A-B): each image shows a cell with 35 SusG-HT single-molecule tracks plotted in random colors. Scale bars = 1 μm. (C-D): the mean squared displacement of all tracks lasting longer than 20 frames is plotted for each protein fusion. Red: effective diffusion coefficient (D) ≤ 0.001 μm^2/s; yellow: 0.001 μm^2/s < D ≤ 0.02 μm^2/s; green: 0.02 μm^2/s < D ≤ 0.05 μm^2/s; blue: 0.05 μm^2/s < D ≤ 0.1 μm^2/s; purple: D > 0.1 μm^2/s.

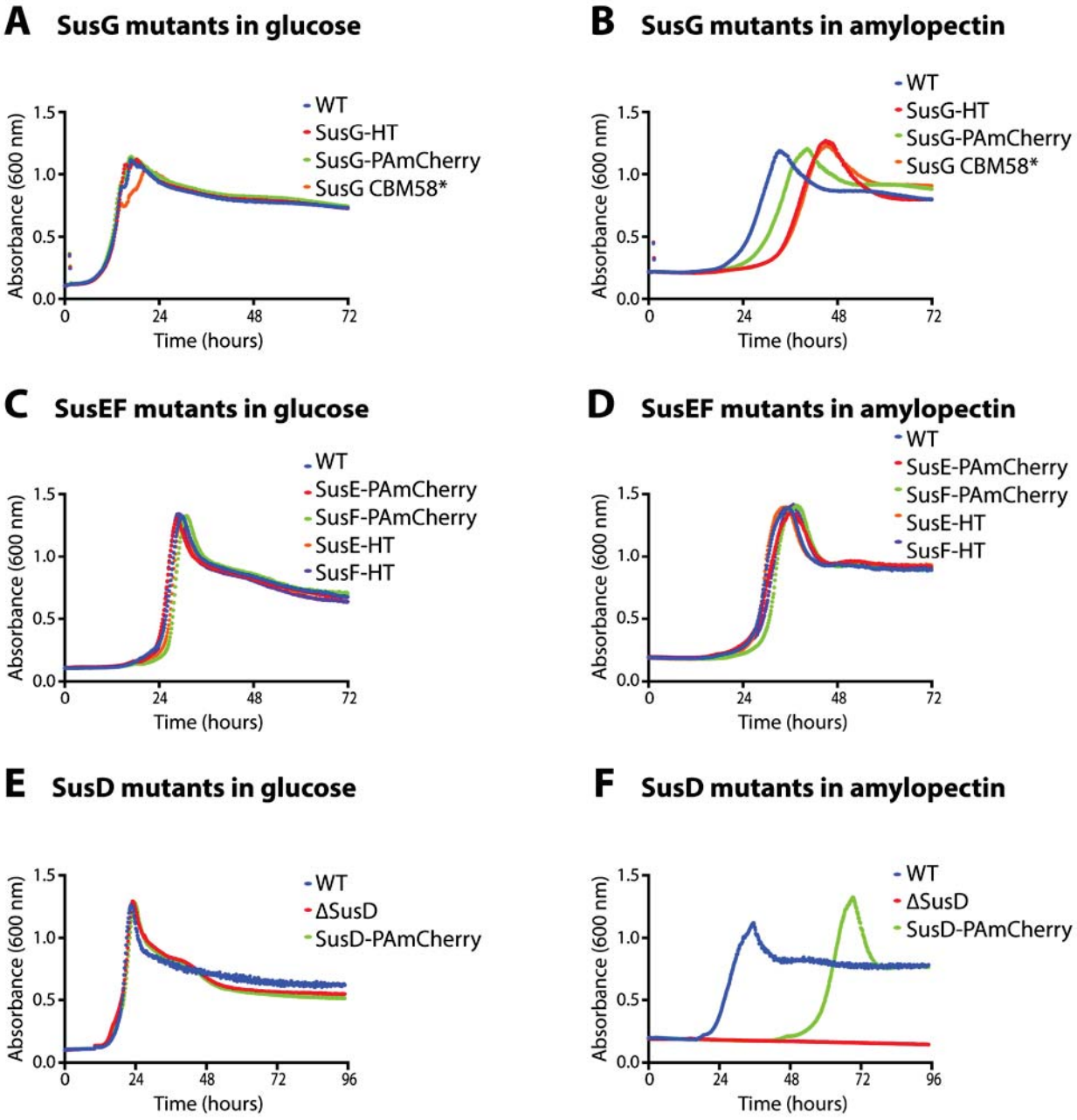

Figure S3. The fluorescently labeled SusG strains support growth on starch. Growth was measured in minimal medium containing 5 mg/mL glucose (*left*) or maize amylopectin (*right*) as the sole carbon source. (A, B): SusG-HT and SusG-PAmCherry were made by replacing the starch-binding CBM58 domain with HT or PAmCherry; CBM58 is not required for growth on starch as evidenced by the normal growth of SusG CBM58*, which contains a starch-bindingdeficient version of CBM58. (C, D): SusE-HT, SusE-PAmCherry, SusF-HT, and SusF-PAmCherry are C-terminal protein fusions with 3-alanine linkers. (E, F): SusD-PAmCherry is a C-terminal protein fusion with a 20-alanine linker. The ΔSusD strain was used as a negative control for growth on starch.

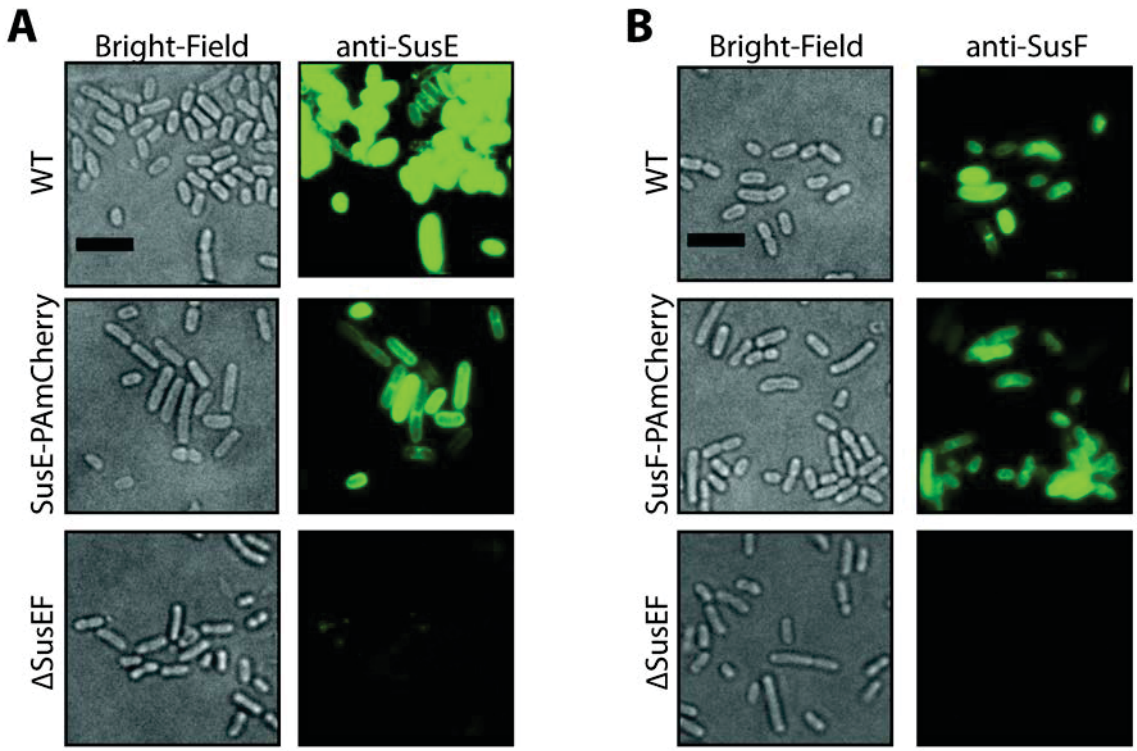

Figure S4. SusE-PAmCherry and SusF-PAmCherry visualized by immunofluorescence. Formalin-fixed, non-permeabilized *Bt* strains were grown in minimal media supplemented with maltose and labeled with custom rabbit polyclonal antibodies to SusE and SusF and then stained with a secondary antibody conjugated to Alexa Fluor 488 goat anti-rabbit IgG. The side-by-side panels display bright-field and fluorescence images for each strain labeled with (A) anti-SusE serum and (B) anti-SusF serum. Scale bars = 5 µm.

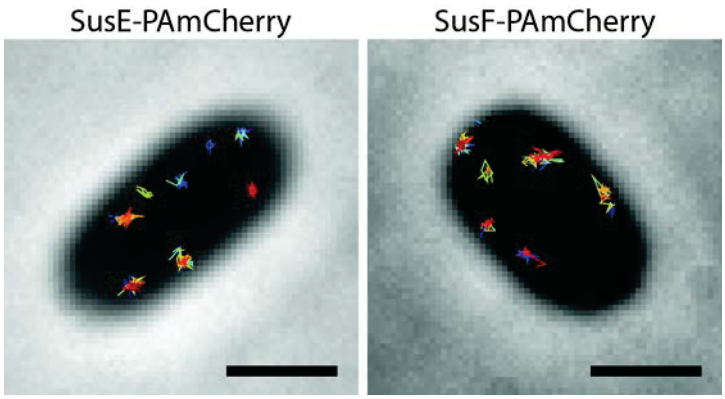

Figure S5. SusE-PAmCherry and SusF-PAmCherry remain highly confined when imaged for longer periods of time via time-lapse imaging. These figures show tracks of molecules that remain in place for 3 – 47 s, with one 40 ms frame acquired every 1 second. Each image shows a cell with 35 single-molecule tracks plotted in random colors. See also the corresponding Movies S1 and S2 of SusE-PAmCherry and SusF-PAmCherry, respectively. Scale bars = 1 µm.

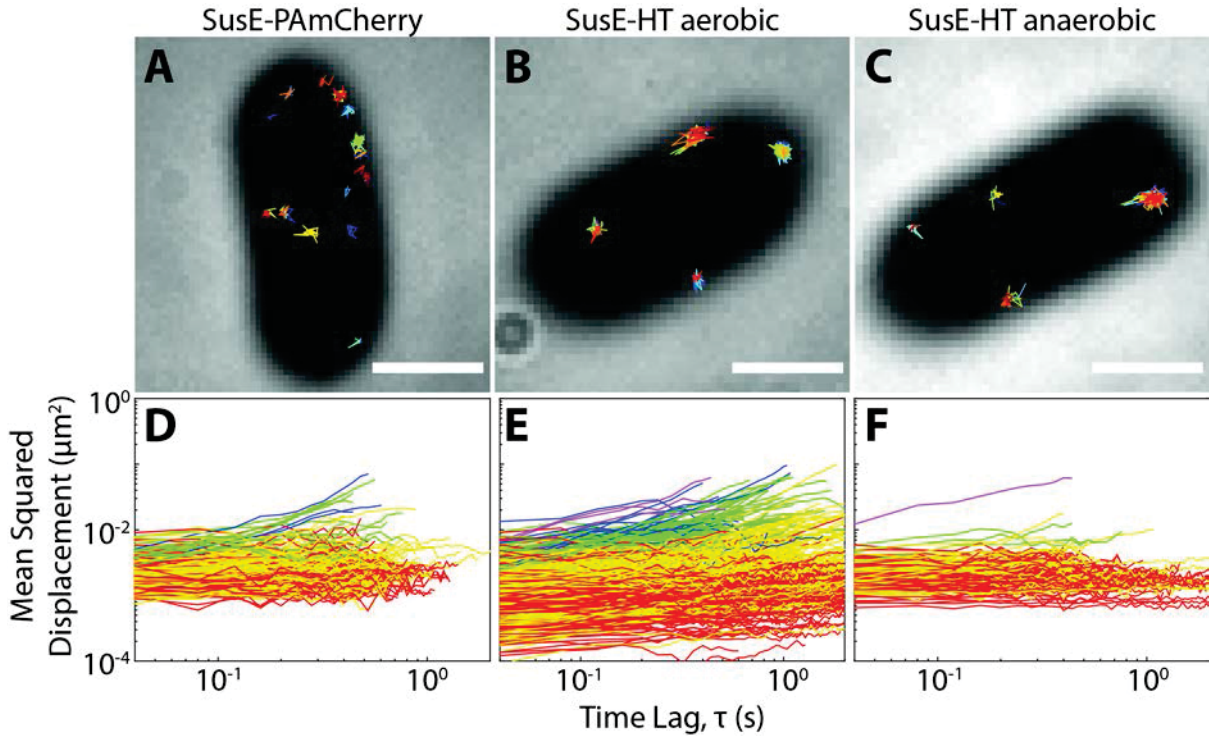

Figure S6. SusE-PAmCherry is highly confined when labeled with PAmCherry or HaloTag (HT) and whether imaged under aerobic or anaerobic conditions. (A-C): each image shows a cell with 35 single-molecule tracks plotted in random colors. Scale bars = 1 µm. (D-F): the mean squared displacement of all tracks lasting longer than 20 frames is plotted for each protein fusion. Red: effective diffusion coefficient (D) ≤ 0.001 µm^2/s; yellow: 0.001 µm^2/s < D ≤ 0.02 µm^2/s; green: 0.02 µm^2/s < D ≤ 0.05 µm^2/s; blue: 0.05 µm^2/s < D ≤ 0.1 µm^2/s; purple: D > 0.1 µm^2/s.

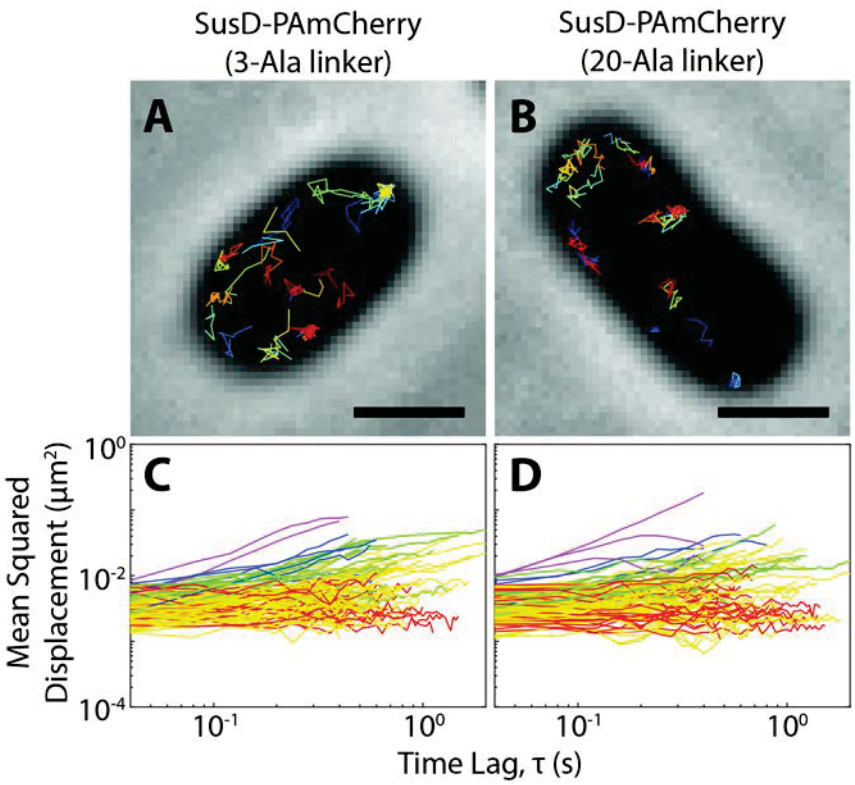

Figure S7. SusD-PAmCherry is mobile when the fluorescent label is attached via two different C-terminal linker lengths. (A, B): each image shows a cell with 35 single-molecule tracks plotted in random colors. Scale bars = 1 μm. (C, D): the mean squared displacement of all tracks lasting longer than 20 frames is plotted for each protein fusion. Red: effective diffusion coefficient (D) ≤ 0.001 μm^2/s; yellow: 0.001 μm^2/s < D ≤ 0.02 μm^2/s; green: 0.02 μm^2/s < D ≤ 0.05 μm^2/s; blue: 0.05 μm^2/s < D ≤ 0.1 μm^2/s; purple: D > 0.1 μm^2/s.

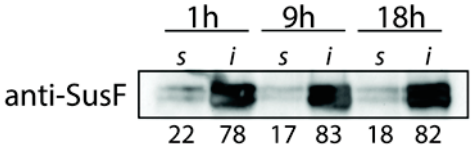

Figure S8. SusF remains insoluble during prolonged incubation with dodecyl maltoside. SusF is solubilized from the membrane as described in Methods, but incubated for 1, 9 or 19 h prior to centrifugation. The membrane-solubilized supernatant (*s*) and insoluble material (*i*) were run on a Western blot and SusF was detected with custom anti-SusF rabbit antibodies.

SI Movies

Movie S1. SusE-PAmCherry molecules remain immobile in a *Bt* cell on the timescale of seconds. Movies are acquired via time-lapse imaging, with one 40 ms frame acquired every 1 second. Scale bar = 1 µm.

Movie S2. SusF-PAmCherry molecules remain immobile in a *Bt* cell on the timescale of seconds. Movies are acquired via time-lapse imaging, with one 40 ms frame acquired every 1 second. Scale bar = 1 µm.

SI Tables

Table S1. Bacterial strains used in this study

Strain Name	Organism	Mutations	Notes
Bt Δ*tdk*	*Bt*	Δ*tdk*	Ref. (13)
Bt SusG-HT	*Bt*	SusG-HT	Ref. (21)
MF001	*Bt*	SusG-PamCherry, Δ*tdk*	
MF002	*Bt*	SusE-HT, Δ*tdk*	
MF003	*Bt*	SusE-PamCherry, Δ*tdk*	
MF004	*Bt*	SusE-PAmCherry Δ*susC*, Δ*tdk*	
MF005	*Bt*	SusE-PAmCherry Δ*susD*, Δ*tdk*	
MF006	*Bt*	SusE-PAmCherry Δcps	
MF007	*Bt*	SusF-HT, Δ*tdk*	
MF008	*Bt*	SusF-PAmCherry Δ*susC*, Δ*tdk*	
MF009	*Bt*	SusF-PAmCherry Δ*susD*, Δ*tdk*	
MF010	*Bt*	SusF-PAmCherry Δ*cps*, Δ*tdk*	

Table S2. Oligonucleotides used in this study

Primer Name	Sequence (5′-3′)	Used for Construction of
SusD pAMCherry Up nest	GTGCAGACAAAGCCGCCAAC	SusD C-terminal PAmCherry fusion
SusD pAMCherry UpF Sal1	GCATGTCGACCTGGTTTGTGGTACTCGTGTAG	SusD C-terminal PAmCherry fusion
SusD pAMCherry UpR	GCGCATGAACTCCTTAATGATGGCTGCTGCTGCTTTATAGCCTTCATTTTGTG	SusD C-terminal PAmCherry fusion
pAMCherry SusD F	CACAAAATGAAGGCTATAAAGCAGCAGCAGCCATCATTAAGGAGTTCATGCGC	SusD C-terminal PAmCherry fusion
pAMCherry SusD R	CTTTTATATAAGGATGAACTCTTGGTTACTTGTACAGCTCGTCCATG	SusD C-terminal PAmCherry fusion
SusD pAMCherry DownF	CATGGACGAGCTGTACAAGTAACCAAGAGTTCATCCTTATATAAAAG	SusD C-terminal PAmCherry fusion
SusD pAMCherry DownR Xba1	GCATTCTAGATAACCGTCACGCGGTTGTCG	SusD C-terminal PAmCherry fusion
SusD pAMCherry Down nest	CCGCTTGCAGTCATGGCAGG	SusD C-terminal PAmCherry fusion
SusE pAMCherry Up nest	TGCCCAAACTGTAGAACTCA	SusE C-terminal PAmCherry fusion
SusE pAMCherry UpF Sal1	GCATGTCGACGTAGCTGTATATATCCGCCTG	SusE C-terminal PAmCherry fusion
SusE pAMCherry UpR	GCGCATGAACTCCTTAATGATGGCTGCTGCTGCCTTCTTTTCTCCTGTACC	SusE C-terminal PAmCherry fusion
pAMCherry SusE F	GGTACAGGAGAAAAGAAGGCAGCAGCAGCCATCATTAAGGAGTTCATGCGC	SusE C-terminal PAmCherry fusion
pAMCherry SusE R	GAATCGTTCTTTTTAAAGTTAATTACTTGTACAGCTCGTCCATG	SusE C-terminal PAmCherry fusion
SusE pAMCherry DownF	CATGGACGAGCTGTACAAGTAATTAACTTTAAAAAGAACGATTC	SusE C-terminal PAmCherry fusion
SusE pAMCherry DownR Xba1	GCATTCTAGACAGCTAACAAAATTATCACCGG	SusE C-terminal PAmCherry fusion
SusE pAMCherry DownR nest	CGGTGCAAATTTCACCTG	SusE C-terminal PAmCherry fusion
SusF pAMCherry Up nest	GCATCACCTTGCAGCCTGCC	SusF C-terminal PAmCherry fusion
SusF pAMCherry Up Sal1	GCATGTCGACCGGTGATAATTTTGTTAGCTGG	SusF C-terminal PAmCherry fusion
SusF pAMCherry UpR	GCGCATGAACTCCTTAATGATGGCTGCTGCTGCTTCGATACGGCCTGTTCCGTTGC	SusF C-terminal PAmCherry fusion
pAMCherry SusF F	GCAACGGAACAGGCCGTATCGAAGCAGCAGCAGCCATCATTAAGGAGTTCATGCGC	SusF C-terminal PAmCherry fusion
pAMCherry SusF R	CCTTGATTTCTTGTAGTATTACTTGTACAGCTCGTCCATG	SusF C-terminal PAmCherry fusion
SusF pAMCherry DownF	CATGGACGAGCTGTACAAGTAATACTACAAGAAATCAAGG	SusF C-terminal PAmCherry fusion
SusF pAMCherry Down R Xba1	GCATTCTAGACCTTTCACGGCAGCGGTC	SusF C-terminal PAmCherry fusion
SusF pAMCherry Down nest	GGAAGAGGCGCCGTATTTTG	SusF C-terminal PAmCherry fusion
HaloTag F	AGACCTGGGTTATTTCTTCGAC	HaloTag C terminal fusion

Primer Name	SEQUENCE (5′-3′)	Used for Construction of
HaloTag R	GTCGAAGAAATAACCCAGGTCT	HaloTag C terminal fusion
SusG-PAmCherry UpF SalI	GCATGTCGACCAAGGAAACAGGGAATGGCCGTCGC	CBM58 swap with PAmCherry
SusG-PAmCherry UpR	GCTGCTGCATTGCCTGAGCCTGTCACGGCAGCGGTCTCGTCAG	CBM58 swap with PAmCherry
SusG-PAmCherry DownF	CAGCAGCAGGCAGCAACGGCGCGAACGGCCAGATCACCTATTTCCATTCTC	CBM58 swap with PAmCherry
SusG-PAmCherry DownR XbaI	GCATTCTAGAGTGAATGGGTATCGGCTTGTTGG	CBM58 swap with PAmCherry
PAmCherry SusG F	CAGGCTCAGGCAATGCAGCAGCAGCCATCATTAAGG	CBM58 swap with PAmCherry
PAmCherry SusG R	GTTGCTGCCTGCTGCTGCCTTGTACAGCTCGTCCATG	CBM58 swap with PAmCherry
PAmCherry-SusG F	CGCTGCCGTGACAGGCTCAGGCAATGCAGCAGCAGCCATCATTAAG	CBM58 swap with PAmCherry
PAmCherry-SusG R	CTGGCCGTTCGCGCCGTTGCTGCCTGCTGCTGCCTTGTACAGCTCGTCC	CBM58 swap with PAmCherry
SusG-PAmCherry-pXR	ATTGCCTGAGCCTGTCACGGCAGCGG	CBM58 swap with PAmCherry
SusG-PAmCherry-pXF	GGCAGCAACGGCGCGAACGG	CBM58 swap with PAmCherry
SusE-PAmCherry D*dFG DownF	GACGAGCTGTACAAGTAATTAACTTTAAAAAGAACGATTCATC	SusD* E-PAmCherry ΔFG
SusE-PAmCherry D*dFG DownR	GGCGGCCGCTCTAGAGAATGCGGAGTGATTATTC	SusD* E-PAmCherry ΔFG
SusE-PAmCherry-pXR	TTACTTGTACAGCTCGTCC	SusD* E-PAmCherry ΔFG
SusE-PAmCherry-pX F	TCTAGAGCGGCCGCCAC	SusD* E-PAmCherry ΔFG
SusG-PAmCherry pX NT swap F	TGGACCGCACTTACCGCC	SusE-Nterm-SusG-PAmCherry
SusG-PAmCherry pX NT swap R	AATGATGATGTATTAAAGAC	SusE-Nterm-SusG-PAmCherry
SusE NT F	TGTCTTTAATACATCATCATTATGAAAAAAATATCCAACATATTAC	SusE-Nterm-SusG-PAmCherry
SusE NT R	CGGTAAGTGCGGTCCAGTTCAGGATCGGATTGCTG	SusE-Nterm-SusG-PAmCherry

Table S3. Whole membrane proteomics. (Attached separately)

Table S4. Co-IP proteomics. (Attached separately)