SUPPLEMENTARY DATA

EXPERIMENTAL PROCEDURES

Non-covalent MS analysis

Samples of gp19.1 and gp19.1-gp21$_{1\text{-}404}$ were dialysed against 200 mM ammonium acetate solution (pH 6.8) using dialysis buttons (Hampton Research, Aliso Viejo, CA, USA). Mass spectra were acquired using a Synapt HDMS quadrupole-travelling wave orthogonal acceleration TOF mass spectrometer (Micromass UK Ltd., Waters Corpn., Manchester, United Kingdom). Samples were analysed by nano-electrospray ionization from platinum/gold-plated borosilicate capillaries fabricated in-house using a P-97 micropipette puller (Sutter Instrument Company, Novato, CA, USA) and a sputter coater (Polaron SC7620; Quorum Technologies Ltd, Kent, United Kingdom). Instrument conditions were optimized for the transmission and preservation of large non-covalent complexes in the gas-phase. ESI capillary voltages of 1.7-1.9 kV, and sample cone voltages of 80-100 V were used. The instrument was operated at a source pressure of 5 mbar. For the gp19.1-gp21$_{1\text{-}404}$ samples, a trap voltage of 150 V was required in order to reduce salt adduction and therefore gain sufficient peak resolution for confident mass measurement. All spectra were calibrated using cluster ions generated from a separate introduction of aqueous caesium iodide solution. Mass accuracy for the protein complexes was found to be within ≤0.03 % error.

Negative-staining electron microscopy, image processing and 3D reconstruction.

Three microliters of purified gp19.1-gp21$_{1\text{-}552}$ complex at 0.05 mg/mL were deposited onto a collodion/carbon grid and incubated for 2 minutes at room temperature. The sample was blotted and rinsed twice with 4 μL of uranyl acetate 1%. Grids were transferred into a 200 kV FEG electron microscope (JEOL JEM 2200FS) using an EM-21010 single tilt holder. Images were recorded with an UltraScan4000 CCD (Gatan inc.) camera at 50,000 × magnification in low-dose condition and with 1.0 to 3 μm defocus range. Magnification was calibrated using the 23 Å layer-line of tobacco mosaic virus. Particles were semi-automatically extracted using boxer from EMAN package. The defocus and astigmatism values of each micrograph were determined with the program CTFFIND3 and images were processed with IMAGIC-5.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Native ESI-MS spectrum of SPP1 Dit and complex. A) spectrum of Dit alone. The monomer (blue) has an observed mass of 28492.77 Da, which agrees within 0.01% . The dodecamer is detected (red) with a mass of 342,121 ± 23 Da, as well as multimers of twice (light green) and three times (violet) its mass (molecular weights are 661578 and 1045437 Da, respectively). B) Spectrum of the high mass signal from the Dit-Tal1-404 complex at low (B) and high (C) collision voltage. The mass of the complex is determined to be 618,937 Da. This agrees with theoretical mass of 618,796 Da. The ejected species is dit (28491 Da).

Figure S2: Electron microscopy of SPP1 gp19.1: $gp21_{1-552}$ complex negatively stained. **A.** Typical negative stain image of complexes. The vast majority of particles correspond to the side view of complexes. Insets display class averages corresponding to side and top views. Scale bar, 50 nm. **B.** The 3D reconstruction of gp19.1-$gp21_{1-552}$ after negative staining showed two back-to-back rings of hexameric Dit surrounded by six extra-densities. Scale bar, 5 nm. **C.** Fit of the gp19.1 atomic structure in the negative stain EM map. Two hexamers of gp19.1 have been fitted independently in the EM reconstruction. The purple volume corresponds to the volume of gp19.1 computed at 30Å resolution.

Figure S3: Calcium effect on the SPP1 gp19.1: $gp21_{1-552}$ complex structure. The cryo-EM map computed at 30Å resolution of the gp19.1: $gp21_{1-552}$ complex show that 100 mM Ca^{2+} induces structural modifications, notably with the $gp21_{1-552}$ trimer opening of the closed state. Scale bar, 5 nm.

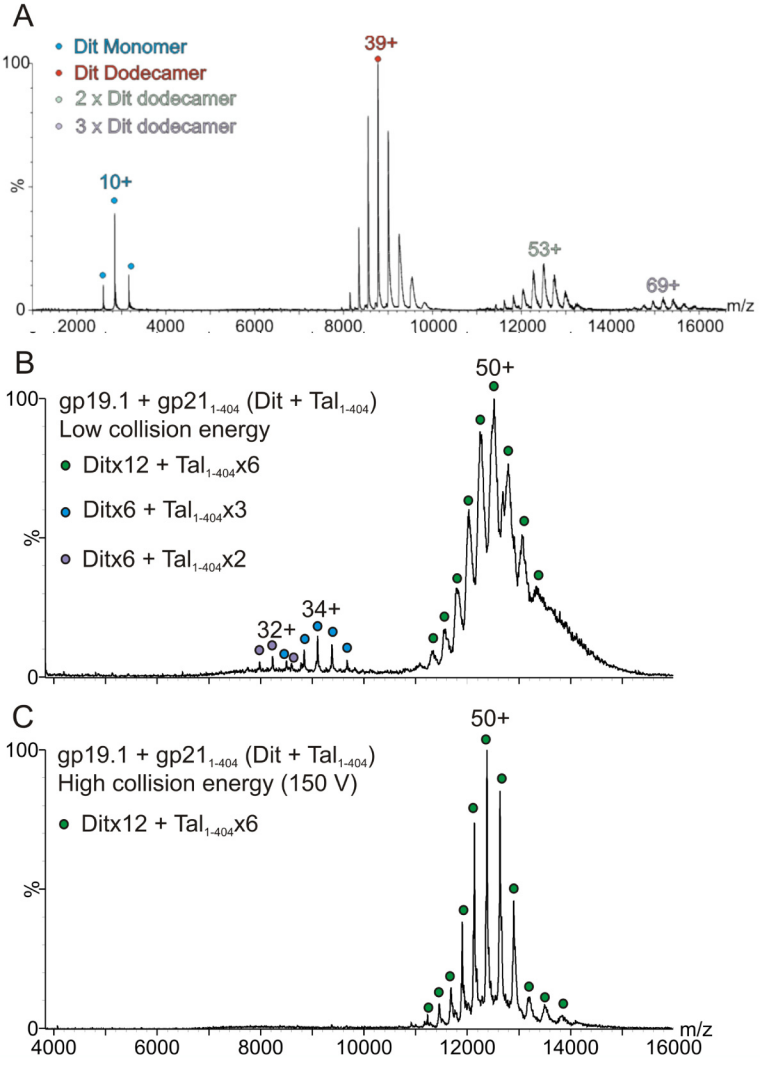
A
Dit Monomer
Dit Dodecamer
2 x Dit dodecamer
3 x Dit dodecamer
39+
10+
53+
69+
100
%
0
2000
4000
6000
8000
10000
12000
14000
16000
m/z
B
50+
gp19.1 + gp21$_{1-404}$ (Dit + Tal$_{1-404}$)
Low collision energy
Ditx12 + Tal$_{1-404}$x6
Ditx6 + Tal$_{1-404}$x3
Ditx6 + Tal$_{1-404}$x2
34+
32+
C
50+
gp19.1 + gp21$_{1-404}$ (Dit + Tal$_{1-404}$)
High collision energy (150 V)
Ditx12 + Tal$_{1-404}$x6

Fig. S1

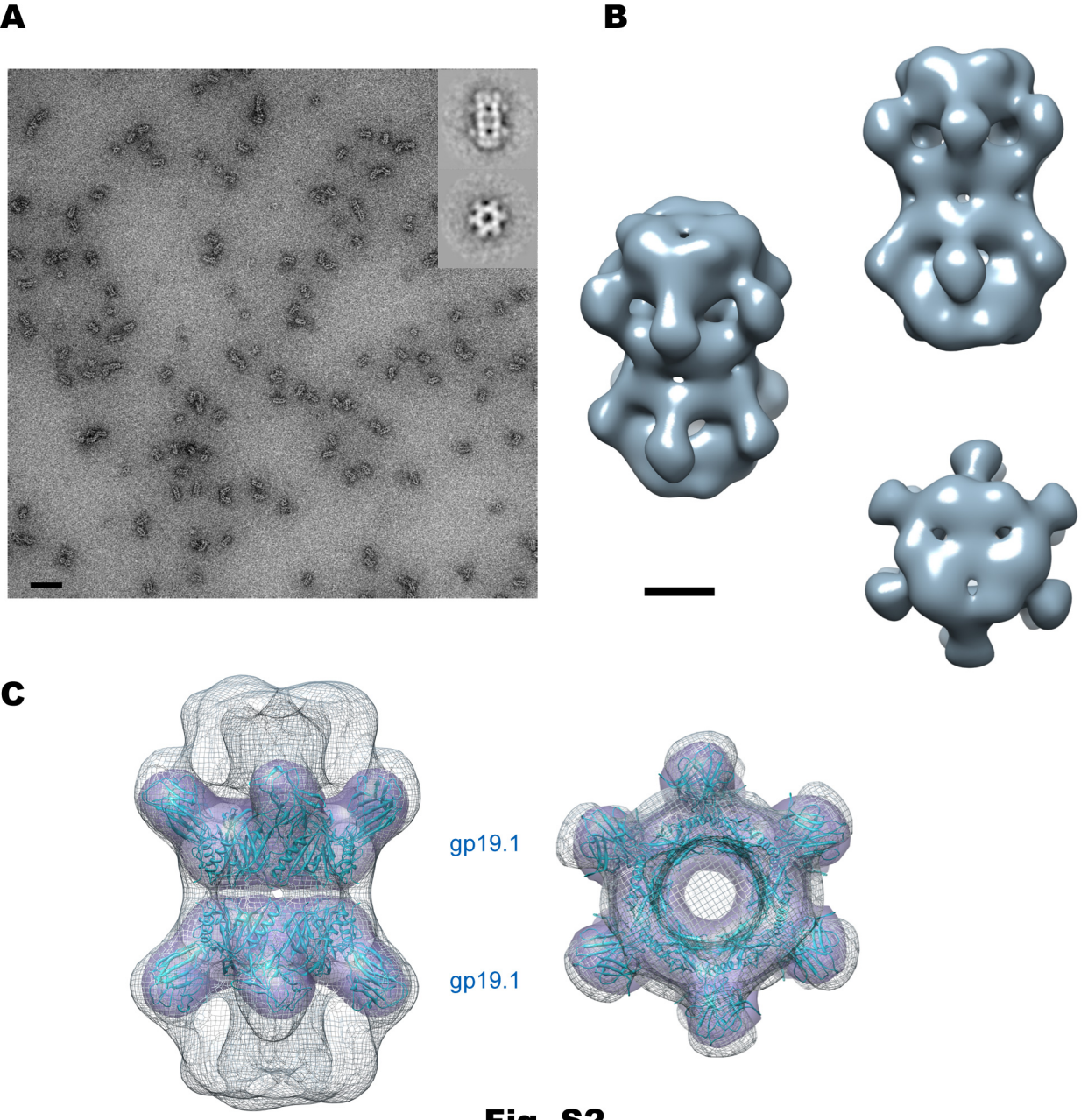

Fig. S2

PH 7

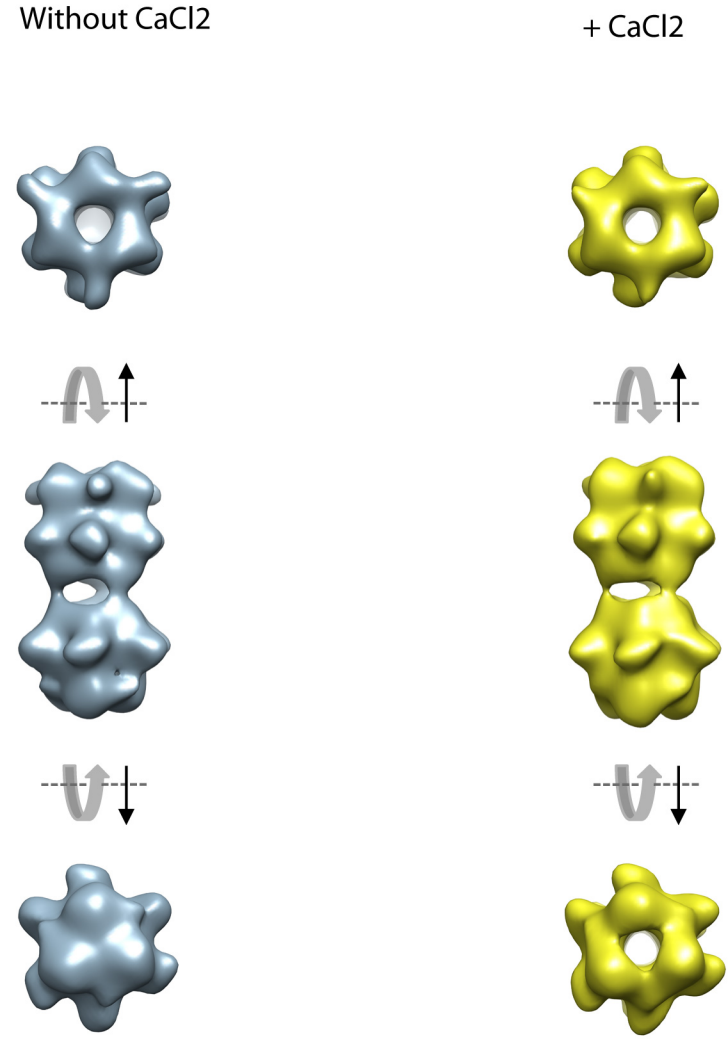

Fig S3.