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Supplemental information

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Deciphering the molecular organization of GET pathway chaperones through native intact dissociation of multiprotein complexes

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SUPPLEMENAL INFORMATION

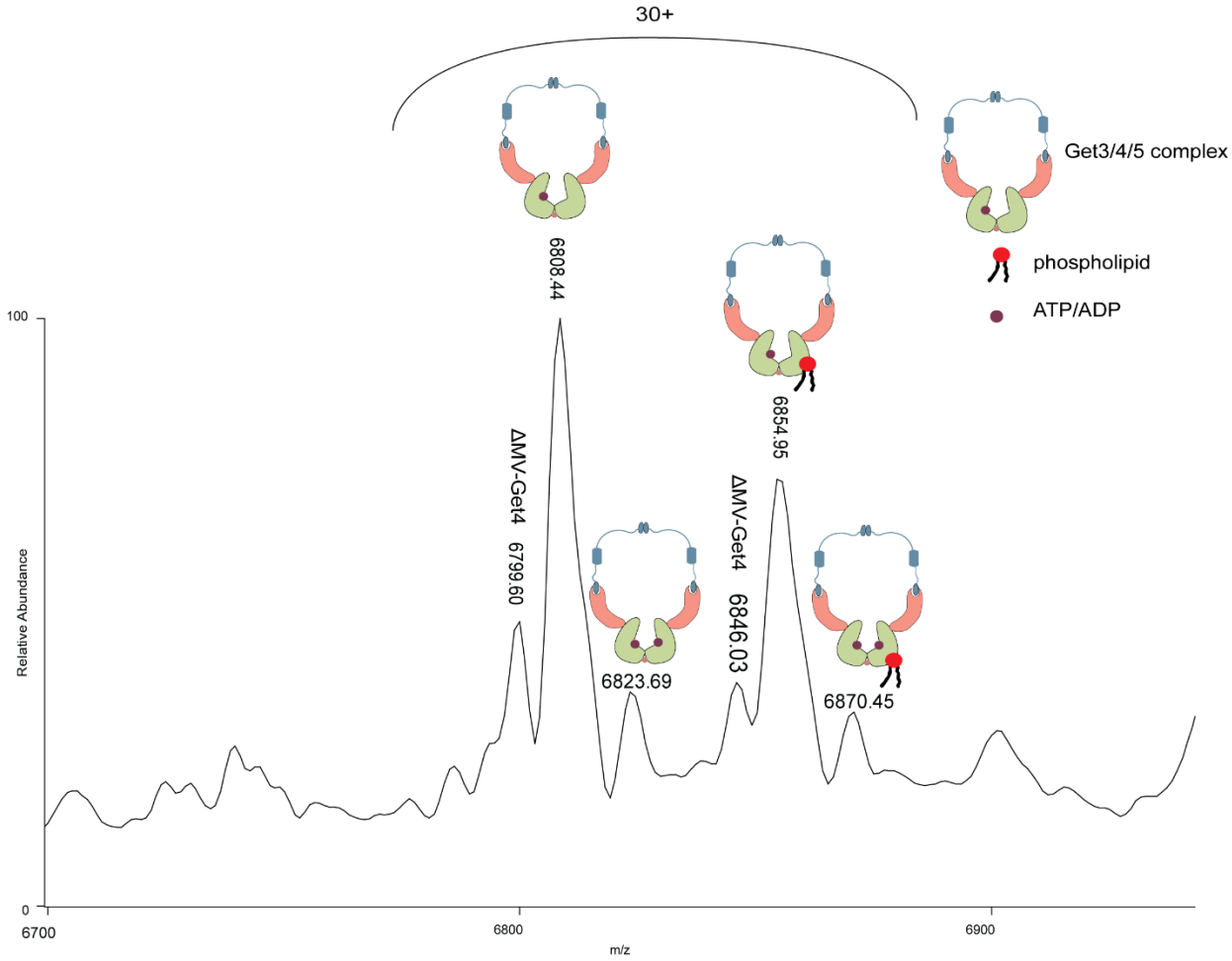

Figure S1. Zoom of the 30+ charge state of Get3/4/5 complex. Peak 6808.44 is the main peak of the complex with one ADP or ATP molecule bound. Peak 6854.95 is formed by Get3/4/5 bound to a phospholipid. Peaks 6799.60 and 6846.03 are formed by the Get3/4/5 complex with one truncated Get4 molecule. The difference in mass corresponds to the methionineand valine in the N-terminus of Get4. Peaks 6823.69 and 6870.45 are formed by the Get3/4/5 with two ADP or ATP molecules.

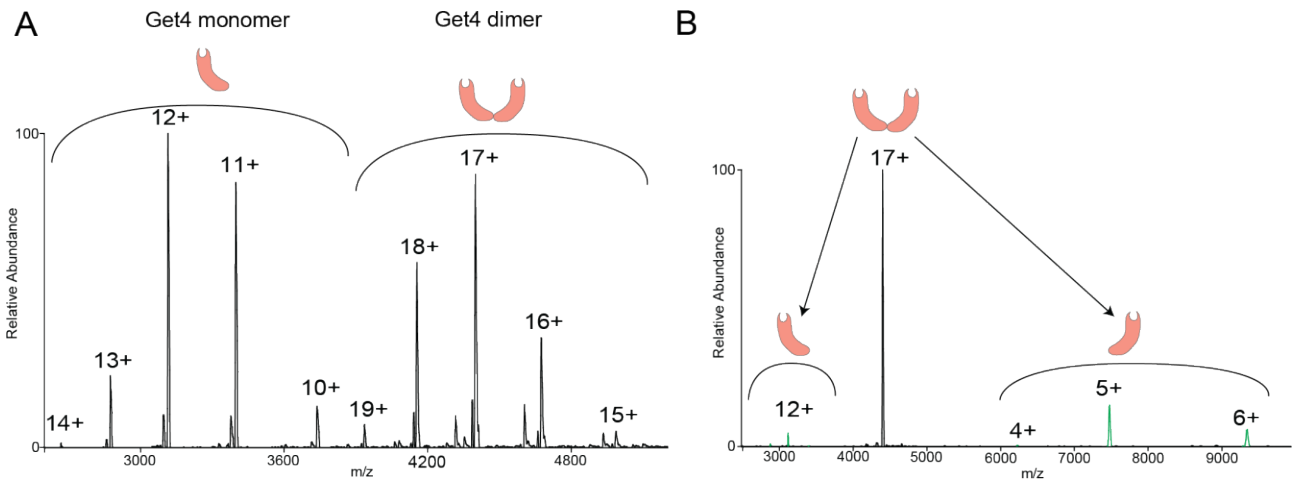

Figure S2. Get4 dimerizes. (A) Get4 purified from *E. coli* was analyzed with native mass spectrometry. The obtained spectrum shows that Get4 exists as a monomer and dimer. (B) MS/MS spectrum of Get4 dimer. CID caused dissociation of Get4 dimer into monomers. Peaks formed by the Get4 monomers are shown in green.

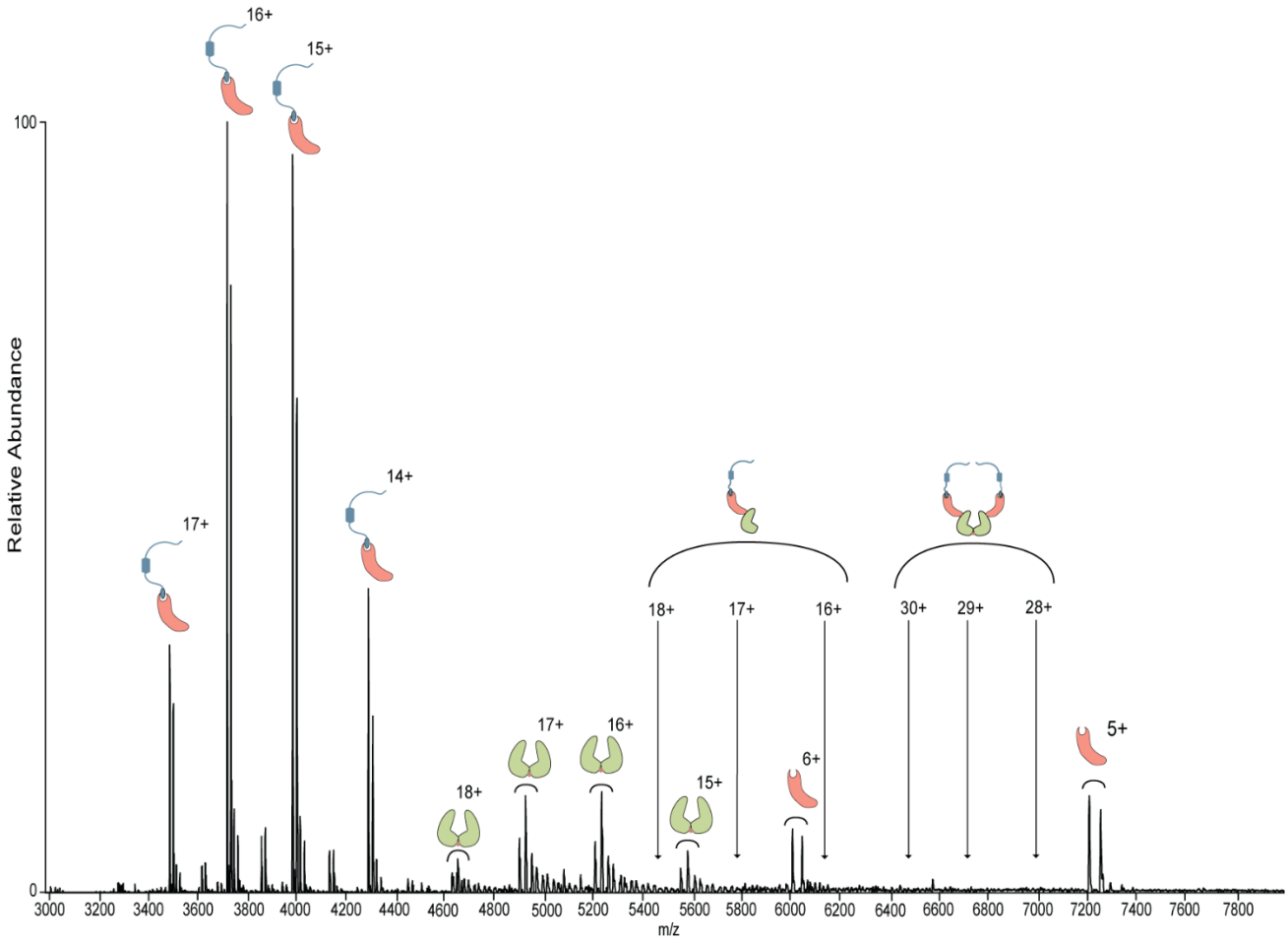

Figure S3. Get3/4/5* is not detected during mass spectrometry analysis. Thespectrum was obtained after mixing Get3 with Get4/Get5*.Arrows indicate m/z values for the predicted Ge3/Get4/Get5* heterotrimer and Ge3/Get4/Get5* heterotetramer.

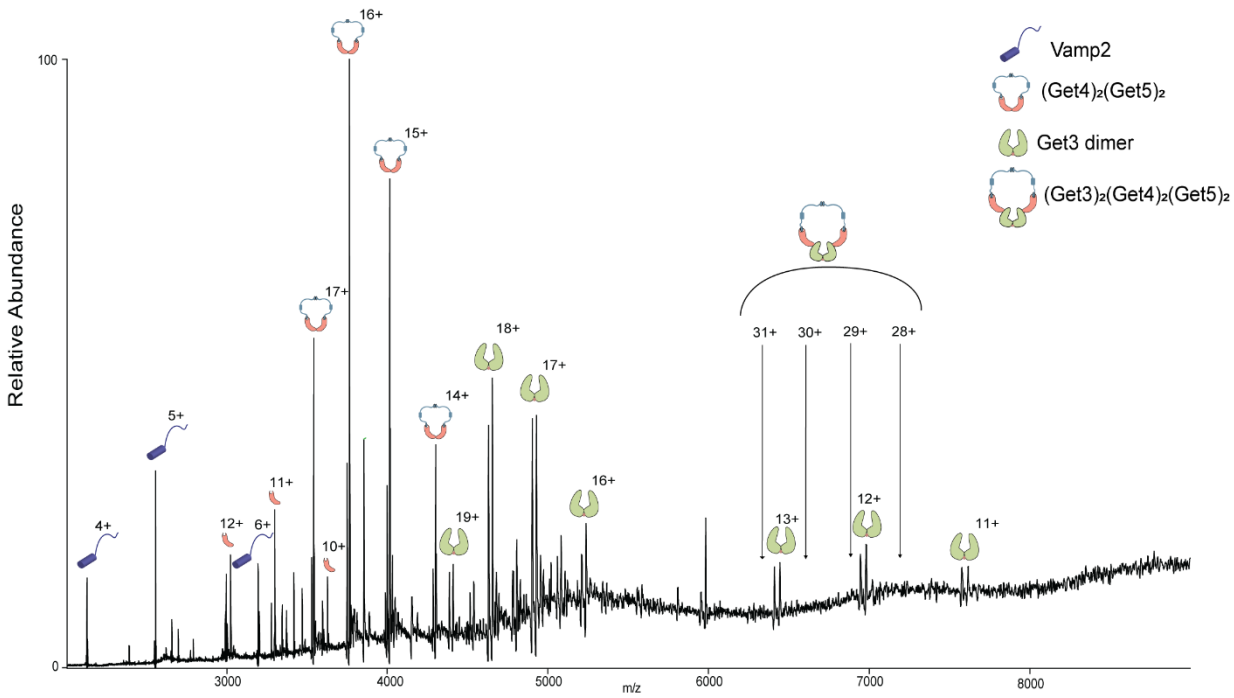

Figure S4. Substrate binding to Get3 dimer causes dissociation of the Get3/4/5 complex. Get3 was incubated with Get4/5 for one hour on ice. Next Vamp2 was addedto the protein mixture and the sample was incubated for another hour on ice. Then proteins weretransferred to buffer: 200 mM Ammonium Acetate, 2mM DTT, and mass spectrometry was performed. Get3/4/5 complex was not detected during the measurement. Arrows indicate the expected m/z values for particular charges of Get3/4/5 complexes detected from samples withoutVamp2.

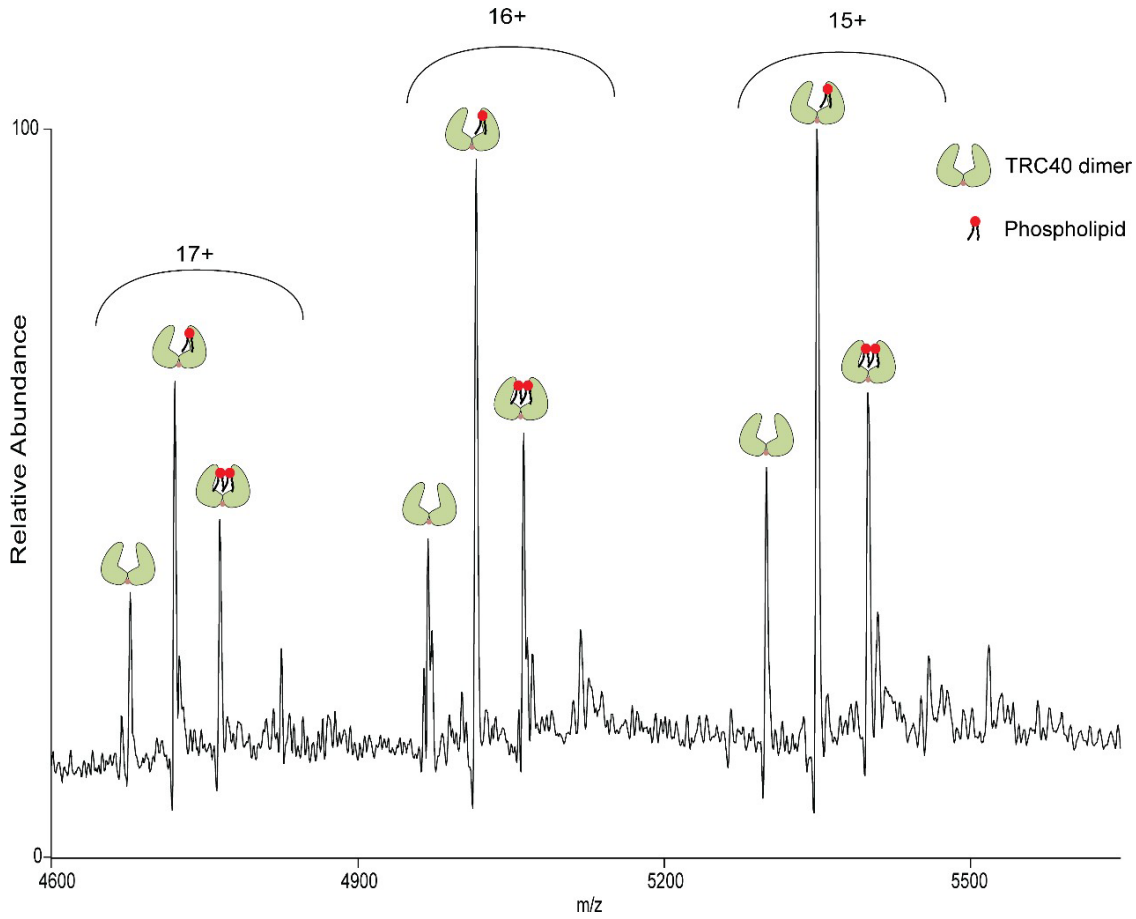

Figure S5. Human TRC40 co-purifies with phospholipids. Human TRC40 wasoverexpressed in HEK293T cells and purified with affinity chromatography. Obtained sample wasanalyzed with mass spectrometry. The spectrum shows that TRC40 is bound to one or two smallmolecules. The analysis of the spectra reveals that the mass of the molecules attached to TRC40 is between 743 Da and 751 Da. This mass is characteristic of glycerophospholipids.

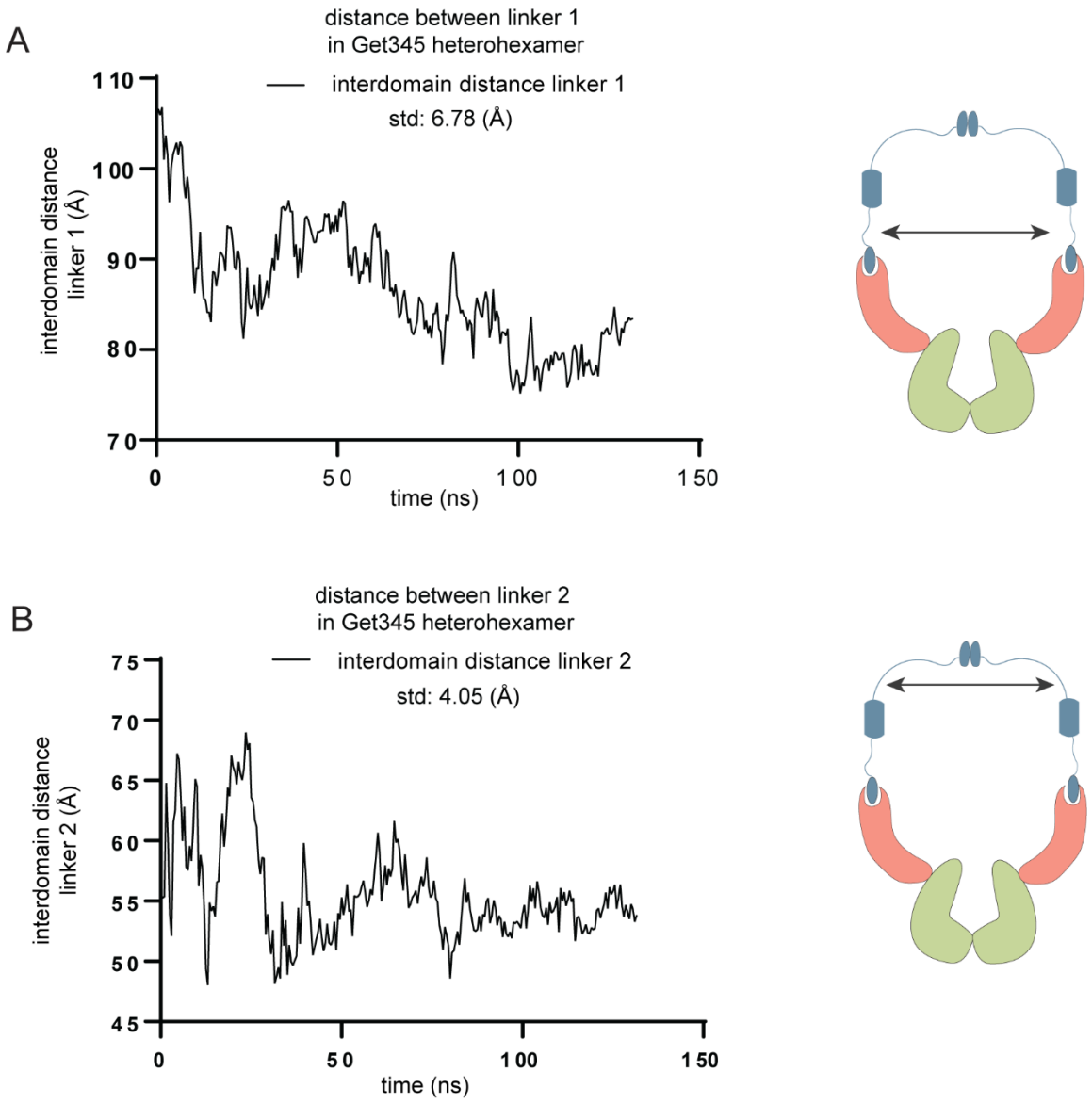

Figure S6. Analysis of the Get3/4/5 MD simulation trajectories. (A) Plot of the distance between the center-of-mass of the flexible linker 1 of Get5 (residues 31-64, Uniprot ID: Q12285) during the course of MD simulation. (B) Plot of the distance between the center-of-mass of the flexible linker 2 of Get5 (residues 152-156, Uniprot ID: Q12285) during the course of MD simulation. Standard deviation (std) of the distances are shown above each graph.

Legend for supplemental Movie S1. Video of a 120 ns molecular dynamics simulation of the modelled Get3/4/5 complex showing the dimeric interfaces between Get3 andGet5 remain intact during the course of the simulations.