Supporting Information

A Robust Workflow for Native Mass Spectrometric Analysis of Affinity-isolated Endogenous Protein Assemblies

Paul Dominic B. Olinares,[†] Amelia D. Dunn,[†] Júlio C. Padovan,[†] Javier Fernandez-Martinez,[§] Michael P. Rout,[§] Brian T. Chait[*,†]

[†]Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, NY 10065 United States

[§]Laboratory of Cellular and Structural Biology, The Rockefeller University, New York, NY 10065 United States

***E-mail: chait@rockefeller.edu**

Supporting Information: Experimental Section

Cell culture, cryolysis and affinity isolation

Budding yeast strains containing constructs with the affinity tag protein A from *Staphylococcus aureus* (SpA) were cultured using standard procedures, harvested, flash-frozen in liquid nitrogen and cryomilled using a planetary ball mill (Retsch) as previously described.[1] The resulting cryomilled cell powder was stored indefinitely at –80 °C or lower until sample processing. Typically, we obtain 2–2.5 g of cell powder per 1 liter of yeast culture grown to midlog phase. The strains used included: Psf2-SpA,[2,3] Nup84-SpA with a cleavage site for HRV 3C protease,[4] and Csl4-TAP (*Sc* BY4741 cell line).[5]

Affinity isolations were performed using antibody-conjugated magnetic beads as previously detailed[1,6] with some modifications. Polyclonal rabbit IgG (MP Cappel) was conjugated to Epoxy M270 Dynabeads (Life Technologies) using the protocol outlined previously.[7] Briefly, conjugation (10 μg IgG per milligram of magnetic beads) in 1.5 M ammonium sulfate, 0.1 M phosphate buffer, pH 7.4 was performed overnight at 30 °C on a rotating wheel. The beads were washed of unconjugated material and the unreacted epoxy groups were quenched. After several additional washing cycles, the beads were then resuspended in PBS with 50% glycerol at 0.1 mg/μL concentration and stored at -20 °C.

For affinity isolation, the appropriate amount of frozen grindate (typically 0.5–2 g) was quickly resuspended in the appropriate extraction buffer with protease inhibitor cocktail (Roche), and centrifuged at 13,200 rpm at 4 °C for 10 min. The respective extraction buffers used included Psf2-SpA: 20 mM HEPES pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 0.1% Tween-20; Nup84-SpA: 20 mM HEPES pH 7.4, 500 mM NaCl, 2mM $MgCl_2$, 0.1% (w/v) CHAPS, and Csl4-TAP: 20 mM HEPES pH 7.4, 150 mM NaCl, 1.5 mM $MgCl_2$, 0.15% Nonidet P40. The supernatant was collected and IgG-conjugated magnetic beads were added at a ratio of 8 mg beads to 1 gram of frozen grindate. Incubation of the lysate was performed for 30–60 min at 4 °C with gentle rotation. Afterwards, the beads were separated from the lysate using a magnet and washed several times with extraction buffer and with the buffer used for native elution. The protein complexes bound to the magnetic beads were then eluted either by addition of peptide (PEGylOx) or by protease cleavage.

Nondenaturing peptide elution

PEGylOx preparation and elution was performed as described previously.[8] The PEGylOx peptide (DCAWHLGELVWCT, Cys-2 bridged to Cys-12, and four PEG units at the N-terminus) was synthesized (The Rockefeller University Proteomics Resource Center, New York, NY or 21st Century Biochemicals) and was resuspended in PEGylOx buffer (20 mM Tris pH 8.0, 100 mM NaCl, 2 mM EDTA, 0.01% Tween-20, 5% EtOH). Tween-20 was added from a 10% stock (Surfact Amps 20, Pierce). The mixture was then centrifuged at 14,000 rpm for 5 min at room temperature. The supernatant was collected, aliquoted and stored at -20 °C. For the Psf2-SpA capture (1 g cryogrindate starting cell powder), 15 μL of 2 mM PEGylOx was added to the beads containing the bound protein complexes and elution was achieved by gentle rotation for 15 min at room temperature.

For elution by protease release, the beads containing bound protein complexes were incubated with 0.5–2 μg of the protease (i.e., 1 μg protease/g of frozen cell powder) in 10–30 μL of protease digestion buffer (HRV 3C protease: 20 mM HEPES pH 7.4, 150 mM NaCl, 0.05% Tween-20, 1 mM DTT; or TEV protease: 50 mM Tris pH 8, 0.5 mM EDTA, 1 mM DTT, 100 mM NaCl, 0.05% Tween-20). Incubation was performed for one hour at 4 °C with gentle rotation. Depending on

the available engineered cleavage site, either the His-tagged HRV 3C protease (1 μg/μL stock; EMD Biosciences) or the His-tagged AcTEV protease (1 μg/μL stock; Life Technologies) was used.

Removal of elution reagent and buffer exchange

Depletion of the PEGylOx elution reagent and buffer exchange were performed using a Zeba desalting micro spin column, 40-kDa molecular weight cutoff (MWCO) (Thermo Fisher Scientific).[8] First, the column was equilibrated with 50 µL of the desired native MS buffer four times by centrifugation at 1,500×g for 1 min each time at room temperature. The PEGylOx-eluted sample (volume 10–13 µL) was then loaded on the column, spun for 2 min, and collected. Note that complete removal of the 1.7-kDa PEGylOx has only been successful with spin columns with molecular weight cutoff of 40-kDa or higher. The use of 10-kDa MWCO or lower only depletes PEGylOx by 10-20%.[8]

The protease-eluted sample was collected and the beads were washed with 10–30 µL filtration buffer (FB: 20 mM HEPES pH 7.4, 0.01% Tween-20). The wash was pooled with the sample and the volume adjusted to 150 µL with FB. The mixture was then loaded onto a 0.5 mL filtration membrane Microcon, 100-kDa MWCO (Ultracel YM-100 from Millipore), pre-washed twice with FB. The Microcon was centrifuged for 5 min at 12,000 rpm at 4 °C. Afterwards, 150–200 µL FB was added and another round of centrifugation was performed until the final volume was less than 20 µL. Buffer exchange into the respective native MS buffer was performed similar to that outlined for PEGylOx removal, except that it was performed at 4 °C instead of at room temperature.

We also tested size-exclusion chromatography (SEC) spin columns such as the Ultramicrospin (The Nest Group) and self-packed spin columns with Sephadex G-75 (GE Healthcare) or Biogel P-100 (BioRad) resins. These gel-filtration spin columns with MWCO resins above 75-kDa MWCO were not successful for the removal of the 22-kDa protease (data not shown). We surmise that SEC fractionation with spin columns to deplete the protease was significantly inefficient because of the short running time and short bed volume. We did not attempt to prepare longer columns with the packing SEC resins since their large bed volumes would lead to increased sample dilution and inevitably larger sample losses.

Protein Quantification

For the gel-based quantification performed in Figure 2A, 10 µL of a 1 µM IgG sample was buffer-exchanged into 150 mM ammonium acetate with increasing concentrations of Tween-20 using a Zeba desalting micro spin column with a 40-kDa MWCO (Thermo Fisher Scientific). The equivalent of 100 ng of bovine serum albumin (BSA) was then added as a loading control to the buffer-exchanged IgG samples. Prior to SDS-PAGE, the samples were diluted in NuPAGE sample loading buffer (Life Technologies), incubated with 20 mM dithiothreitol (DTT) for two hours at 55 °C and loaded on a 4-12% acrylamide Bis-Tris NuPAGE gel (Invitrogen) for separation. The gels were then Coomassie-stained with GelCode Blue (Pierce) and visualized on an LAS-3000 camera system (Fujifilm) with a linear detection range. The band intensities for BSA (66 kDa) and the heavy chain of IgG (55 kDa) were quantified using the ImageJ software (National Institutes of Health). The coefficient of variation for the measured BSA band intensities as loading control across the gel lanes analyzed ranged from 5% to 7%.

Native MS analysis of monoclonal antibody samples for Tween-20 study

To test the effect of increasing amounts of Tween-20 on native MS spectra (Figures S-1B and S-1C), 15 µM of a monoclonal antibody sample (Waters mAb intact protein standard) was buffer exchanged into 150 mM ammonium acetate, 0.001% Tween-20 using a Zeba micro spin desalting column with a 40-kDa MWCO (Thermo Fisher Scientific). The resulting

sample was then diluted to 500 nM final concentration with 150 mM ammonium acetate containing 0.001%, 0.01%, 0.05%, and 0.1% Tween-20. Native MS analysis was then performed with the following parameters: in-source dissociation, 200 V; HCD CE, 10 V; S-Lens, 200; UHV pressure, $5x10^{-10}$ mbar; capillary temperature, 200 °C; AGC target value: $5x10^5$; injection time, 200 ms; AGC mode, prescan; averaging, 1; source DC, 25 V; transfer multipole offset, 0V; C-trap lens, 0V; number of microscans, 5; total number of scans, 100; resolution setting, 35,000 at 200 *m/z* corresponding to 128 ms analyzer transient time. The inject flatapole voltage was set to 8 V. For IgG (Figure S-1B), the interflatapole and bent flatapole voltages were 7 V and 6 V, respectively. For larger assemblies (Figure S-1C), the interflatapole and bent flatapole voltages were both set to 4 V.

Native MS analysis of affinity-isolated endogenous protein complexes

An aliquot (2–3 µL) of the sample was loaded into an in-house fabricated gold-coated quartz capillary and sprayed using a static nanospray source into the Exactive Plus EMR instrument (Thermo Fisher Scientific).[9] Calibration was performed using cesium iodide. Typical MS parameters included spray voltage, 0.9–1.2 kV; capillary temperature, 100 °C to 150 °C; S-lens RF level, 200; resolving power, 8,750 or 17,500 at *m/z* of 200 corresponding to 32 or 64 ms analyzer transient duration, respectively; AGC target, $5x10^5$; number of microscans, 5; maximum injection time, 200 ms; injection flatapole, 8 V; interflatapole, 4 V; bent flatapole, 4 V; ultrahigh vacuum pressure, $8–10\times10^{-10}$ mbar; total number of scans, 100. The in-source dissociation (ISD) and high energy collision dissociation (HCD) parameters were varied accordingly. RAW files were processed manually using Thermo Xcalibur Qual Browser (version 3.0.63). We estimated the charge state distributions of the protein complexes in their native or native-like states based on their molecular masses using the empirically derived expression $z = 1.638 \times MM^{0.5497}$, where z is the base-peak (most intense) charge state and MM is the molecular weight in kDa.[10]

Bottom-up LC MS analysis

Protein samples were diluted in NuPAGE gel-loading buffer (Life Technologies) and was reduced with 20 mM DTT by heating at 70 °C for 10 min. The reduced cysteines were then alkylated with 50 mM iodoacetamide in the dark for 30 min at room temperature. The resulting sample was loaded on an SDS-PAGE gel (4-12% acrylamide Bis-Tris) and run briefly to let the proteins migrate into the gel (5–10 mm) with minimal separation. The gel was quickly stained with GelCode Blue (Pierce). After destaining, the corresponding gel pieces containing the protein bands were excised, destained, and washed. Trypsin (Promega, Madison, WI) was added (200–400 ng per gel piece) in 25 mM ammonium bicarbonate, 10% acetonitrile and in-gel trypsin digested overnight at 37 °C. Digestion was terminated by adding 50% acetonitrile, 5% formic acid to the digest. The resulting peptides were extracted several times, dried by vacuum centrifugation, and stored at -20 °C. Prior to MS analysis, the peptide sample was desalted using a C_{18} Stagetip.[11,12]

The peptide sample was resuspended in 0.1% v/v formic acid and was loaded into an EASY-Spray C_{18} column using an EASY-nLC 1000 system (Thermo Fisher Scientific) for LC separation coupled with the Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) for analysis. The gradient started with 5 min of 0% B, then 60 min from 0% to 40% B, followed by 10 min from 40% B to 100% B and stayed at 100% B for another 10 min with solvent A = 0.1% aqueous formic acid and solvent B = 0.1% formic acid in acetonitrile at a flow rate of 300nL/min. The ten most abundant ions in each full scan were selected for fragmentation by high-collision dissociation (HCD) at normalized collision energy (NCE) setting of 30. Additional MS parameters include MS scan resolution setting: 70,000, AGC target: $1x10^6$, 1 microscan, and profile

mode. For the MS/MS analysis, the settings were scan resolution, 17,500; AGC target, 2 x 10^5; maximum injection time, 60 ms; isolation width, 2 Th, dynamic exclusion, 14 s; charge exclusion, 1+ and unassigned, and profile mode.

For the affinity-isolated GINS complex, the bound protein complexes were eluted from the beads using a solution of 1% (w/v) SDS and 20 mM Tris, pH 8. Disulfide bonds in the denatured, affinity-isolated proteins were reduced by treatment with 20 mM DTT at 60 °C for 1 h. Free cysteinyl residues were alkylated for 40 min at room temperature in the dark with freshly prepared 50 mM iodoacetamide. Proteins were separated from detergents and other small molecules by methanol-chloroform precipitation.[13] An aliquot of 20 µl of an 8 M urea solution in 100 mM Tris pH 8 was added to the protein pellet, and the solution was subjected to an ultrasonic bath for 5 min. The solution obtained was diluted with 140 µL of 25 mM ammonium bicarbonate. An aliquot containing 100 ng of trypsin was added to each sample, and the solution was incubated at 37 °C overnight. The peptide mixture was desalted using a C18 Stage tip.[14] Liquid chromatography was performed using an Ultimate HPLC system equipped with a FAMOS autosampler (Thermo Scientific). The system consisted of a trap column—5 mm (L) × 0.3 mm (ID), 10 µm particle size, PepMap C_{18}-resin (LC Packings)—and an in-house packed PicoFrit column—13 cm (L) × 75 µm (ID), 3 µm particle size, C_{18} Reprosil Pur C_{18}-AQ (Dr. Maisch GmbH). The system was operated with a measured flow rate of 200 nl/min and the column eluate was electrosprayed at 1.8 kV into the heated ion transfer capillary (275 °C) of the mass spectrometer (ESI/ETD-LTQ XL-Orbitrap, Thermo). Peptide mixtures were separated using a linear 1-hour acetonitrile gradient. Solvent A consisted of 5% acetonitrile and 0.1% formic acid in water, and solvent B of 5% water and 0.1% formic acid in acetonitrile. Eluted peptides were MS analyzed either using a method for fragmentation of the ten most abundant ions measured from a Top-10 MS scan or an MS-only method. Precursor masses were measured on the Orbitrap analyzer at a resolution of 60,000. MS/MS experiments were performed with CID activation, and fragment ions were analyzed in the linear ion trap analyzer.

For data processing, the acquired RAW files were converted to MGF format using the MM File Conversion tool (http://www.massmatrix.net/mm-cgi/downloads.py/) and searched against the *Saccharomyces cerevisiae* database with the Global Proteome Machine Cyclone X!Tandem[15] (GPM: http://h.thegpm.org/tandem/thegpm_tandem.html). The search settings included fragment and parent mass error of 10 ppm; Cys carbamidomethylation as fixed modification; Met and Trp oxidation, and Asn and Gln deamidation as variable modifications.

The resulting list of identified proteins was then used to determine the top proteins that co-isolated with the tagged protein. Additional filtering was performed including removal of proteins that had less than three unique peptides and also those that were identified in the Contaminant Repository for Affinity Purification as common contaminants from affinity isolations from budding yeast (www.crapome.org).[16] The overall bottom-up MS results for the GINS, Nup84 and exosome complexes are shown in Tables S-4, S-5, and S-6, respectively.

The masses of the protein subunits that were used for matching the measured masses from deconvolution of the native MS spectra were determined based on their primary sequence and from N-terminal processing or co-translational modifications, such as removal of N-terminal methionine and N-terminal acetylation verified by both bottom-up LC-MS analysis and from publicly available proteomic databases including SGD (www.yeastgenome.org), GPM (www.thegpm.org) and Uniprot (www.uniprot.org). Additional mass information for the exosome proteins from budding yeast was also obtained from the literature.[17,18]

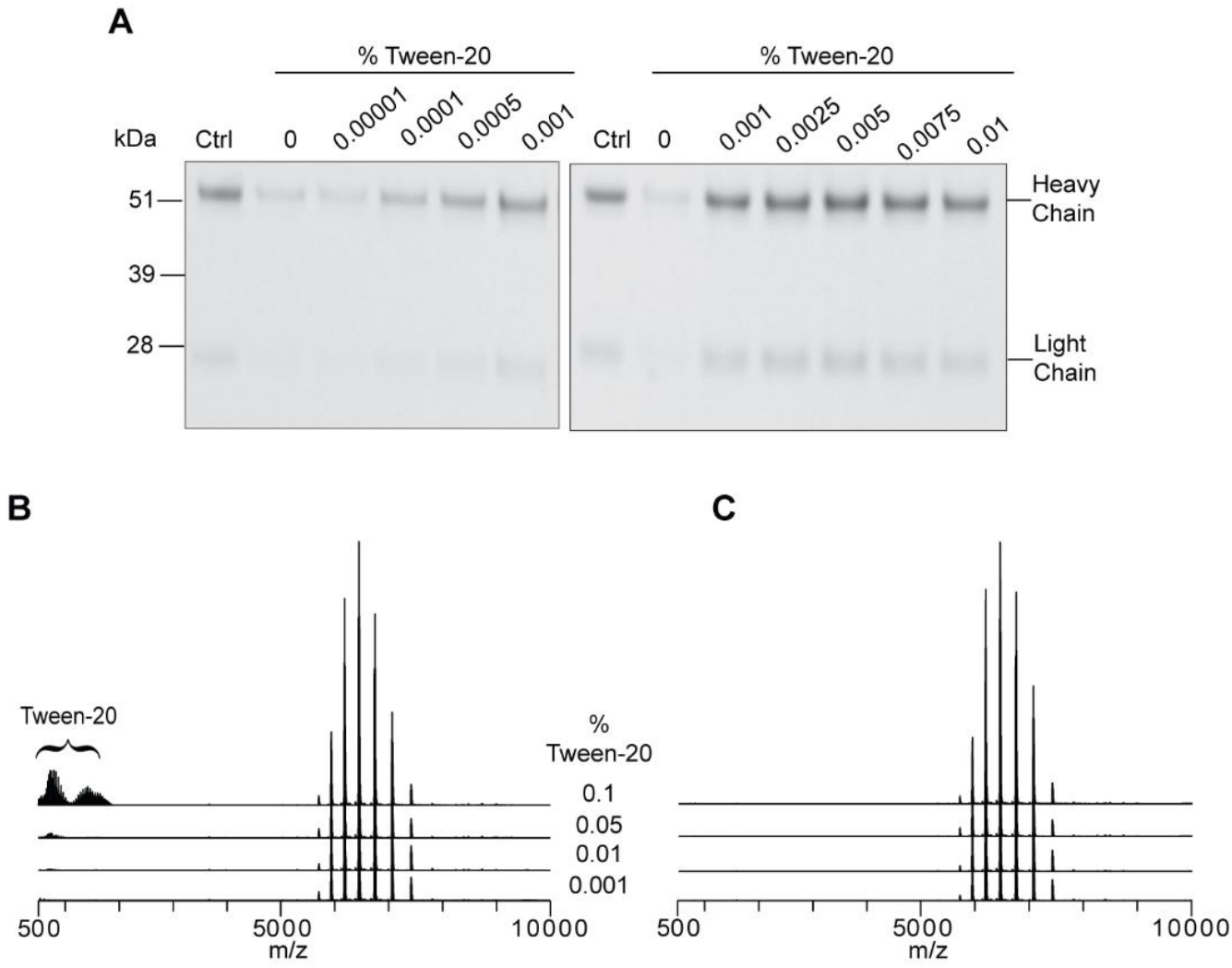

Figure S-1. Effect of increasing Tween-20 concentration on sample retention during buffer exchange and native MS analysis. (A) SDS-PAGE separation and Coomassie staining of 1 μM rabbit polyclonal IgG that was buffer-exchanged into 150 mM ammonium acetate with increasing concentrations of Tween-20 using desalting microspin columns. The control samples did not undergo buffer exchange. Quantification of IgG recovery was performed by measuring the protein band intensity of the IgG heavy chain (55 kDa band). As shown here, only about 20% of the IgG sample was recovered when no Tween-20 was present during buffer exchange. Recoveries increased with addition of Tween-20 and reached maximum at about 0.001%. (B) and (C) native MS spectra of 500 nM monoclonal antibody in 150 mM ammonium acetate, pH 7.5 with increasing Tween-20 concentrations at two different Exactive EMR tune settings. The tune settings for (B) are used for analyzing 150-kDa antibodies (Inject flatapole, 8 V; interflatapole, 7 V, and bent flatapole: 6 V). The tune settings for (C) are used for affinity-isolated endogenous protein assemblies which are typically larger assemblies with higher masses than IgG (Inject flatapole, 8 V; interflatapole, 4 V, and bent flatapole: 4 V). The resolution setting was 35,000 at 200 *m/z* corresponding to 128 ms analyzer transient time and each spectrum is an average of 100 scans (with 5 microscans each). Negligible signal interference was observed for both tune settings at concentrations at or below 0.01% Tween-20.

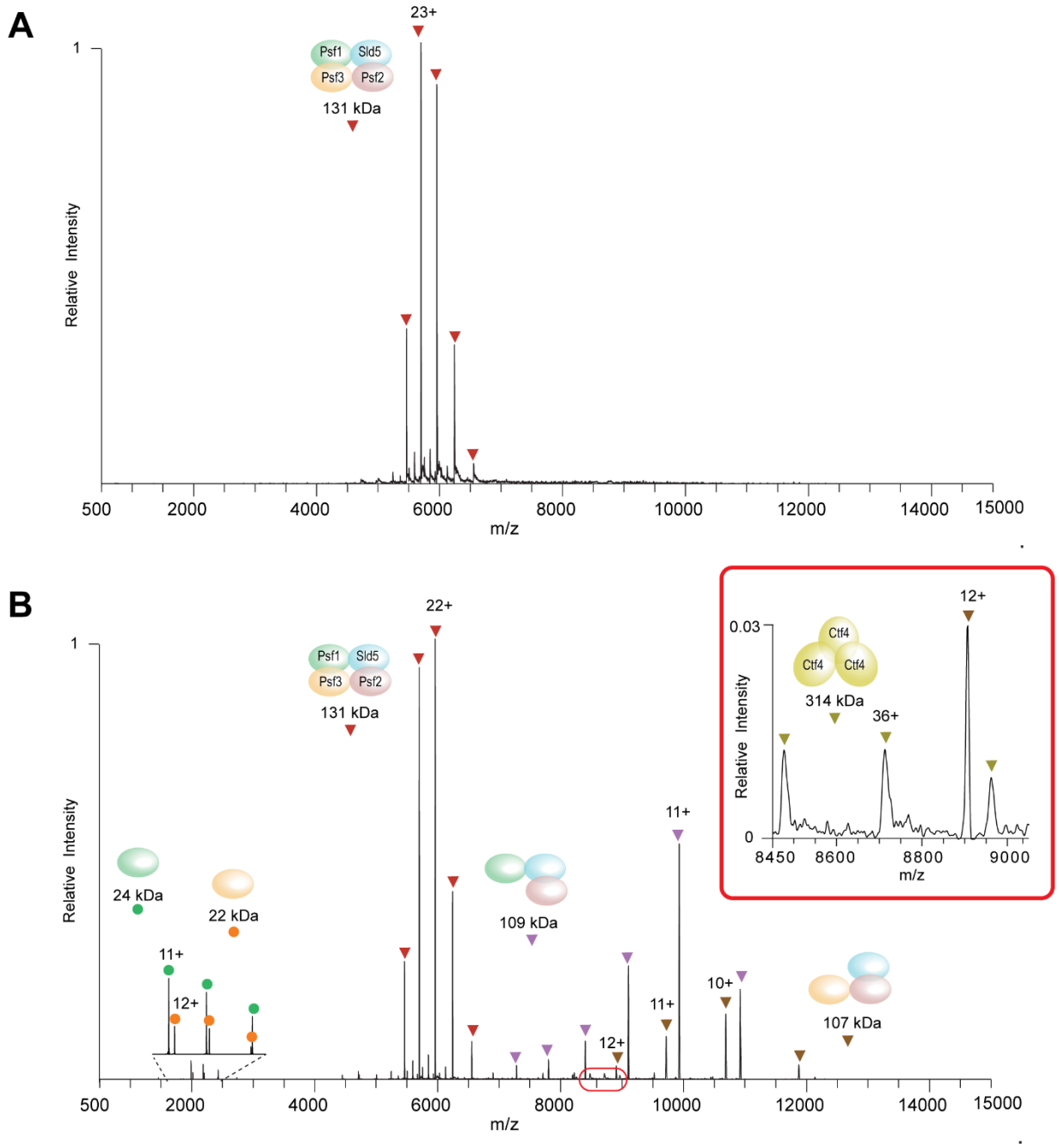

Figure S-2. Native MS analysis of the endogenous GINS complex from budding yeast. (A) Spectrum acquired with minimal activation (ISD of 50 eV and HCD voltage offset of 75 V) at nominal resolving power of 8,750 at *m/z* of 200. (B) Spectrum acquired with maximum HCD voltage setting (200 V) inducing gas phase dissociation of the the GINS complex. The ISD parameter and resolving power setting were the same as in (A). A low intensity peak series for the Ctf4 trimer was observed (see inset panel, red border).

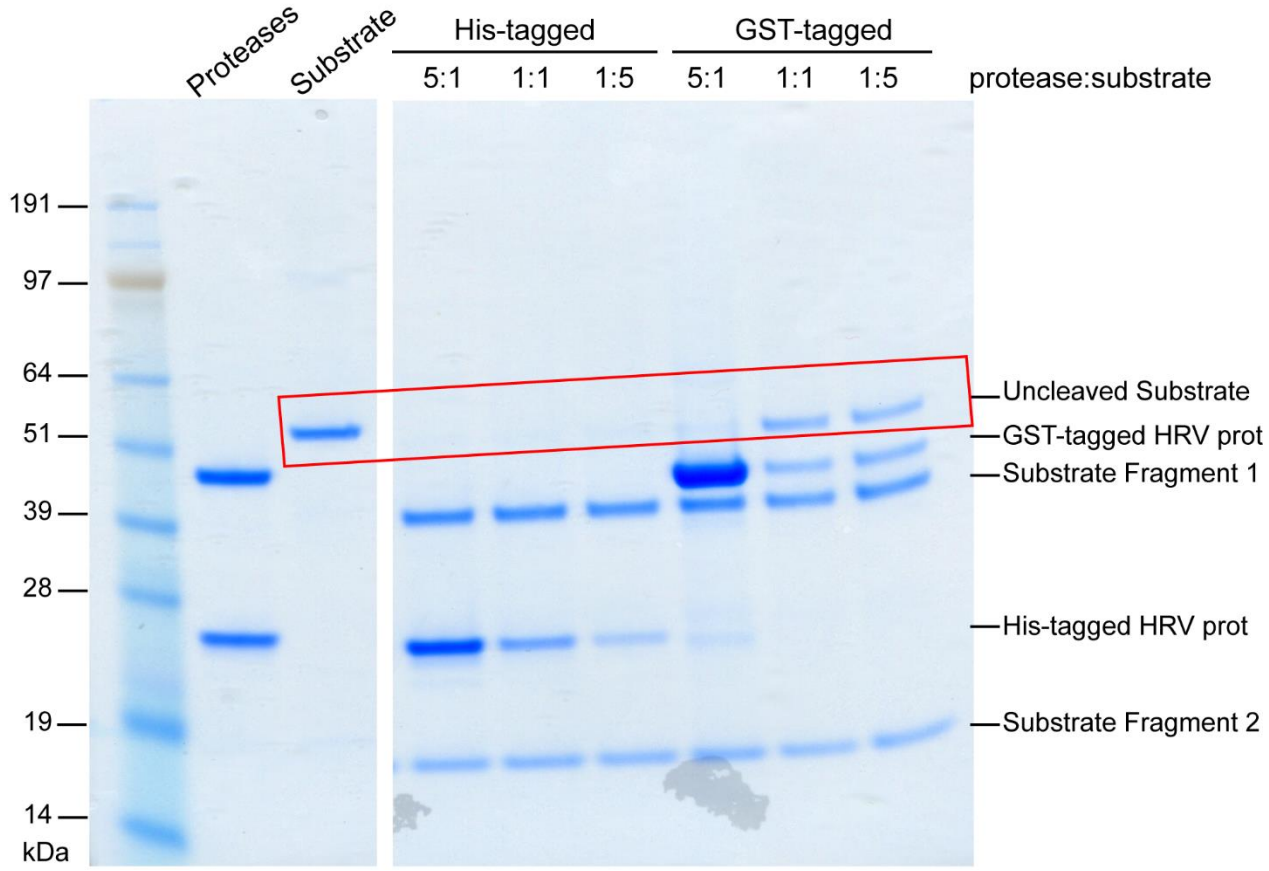

Figure S-3. Comparison of commercially available HRV 3C proteases. The equivalent of 500 ng of a 53-kDa substrate (Novagen HRV 3C protease Cleavage Control Protein, shown here in red) was incubated with increasing equivalent amounts of the protease (5:1, 1:1 and 1:5 protease-to-substrate molar ratio) in 20 mM HEPES pH 7.4, 500 mM NaCl, 2 mM $MgCl_2$, 0.01% Tween-20, 1 mM DTT at 4 °C for 1 hr. Samples were then analyzed by SDS-PAGE and stained with Coommassie Blue. The His-tagged HRV 3C protease (EMD Biosciences) was more efficient across the protease-to-substrate ratios tested than the GST-tagged version (GE, also called Prescission Protease or PPX).

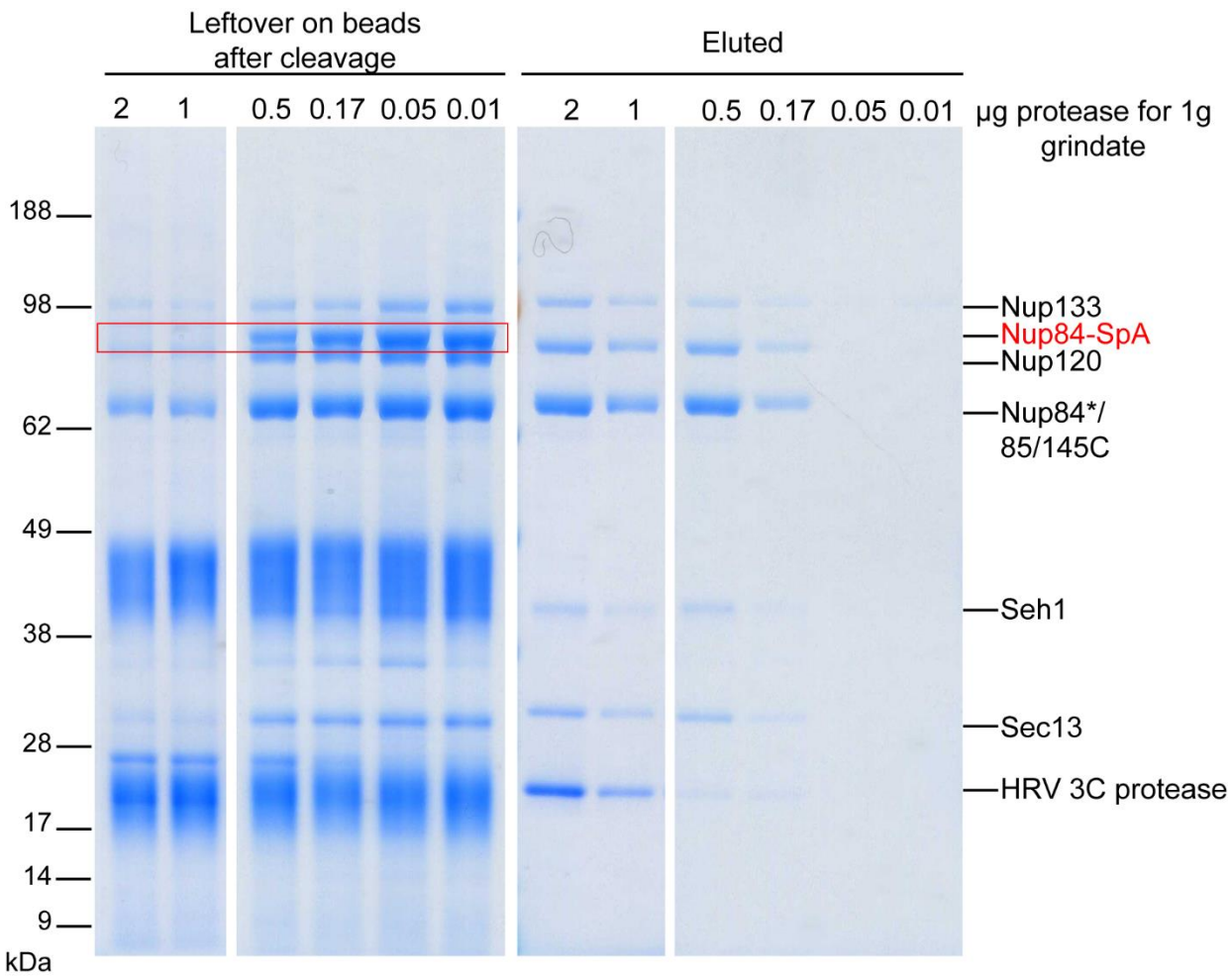

Figure S-4. Optimization of the conditions for on-bead nondenaturing cleavage elution using HRV 3C protease. Native elution of the affinity-isolated Nup84 complex from 1 g of frozen grindate with decreasing amounts of the HRV 3C protease (2 µg to 10 ng). The His-tagged version of the protease was used (EMD Biosciences). Efficient elution was achieved with 1 µg protease or higher as indicated by the disappearance of the Nup84-SpA band (highlighted in red) on the lanes showing the bead-bound fractions, and an increase in protein band intensities on the corresponding gel lanes for eluted samples. Protease cleavage was performed in 10 µL digestion buffer (20 mM HEPES pH 7.4, 2 mM $MgCl_2$, 500 mM NaCl, 1 mM DTT, 0.01% Tween-20) for 1 hr at 4 °C.

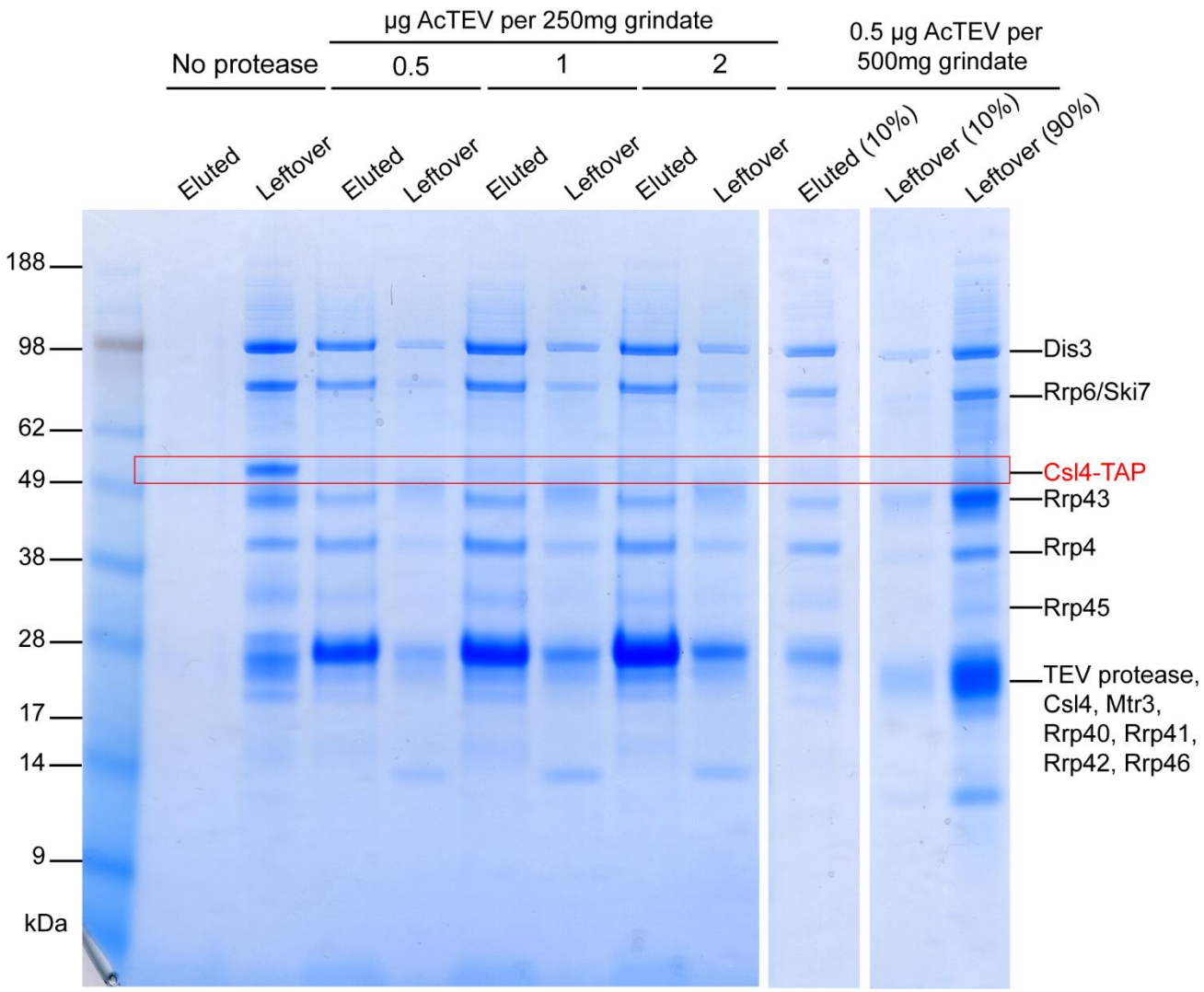

Figure S-5. Optimization of the conditiions for on-bead nondenaturing cleavage elution using TEV protease. Native elution of the affinity-isolated exosome complex from either 250 or 500 mg of Csl4-TAP frozen grindate with increasing amounts of the TEV protease (AcTEv, Invitrogen). Efficient elution was achieved with the equivalent of 1 µg protease per gram of frozen grindate, or higher, as indicated by the disappearance of the Csl4-TAP band (highlighted in red) on the Bead-bound fractions. Protease cleavage was performed in 10 µL TEV buffer (50 mM Tris pH 8, 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 0.05% Tween-20) for 1 hr at 4 °C.

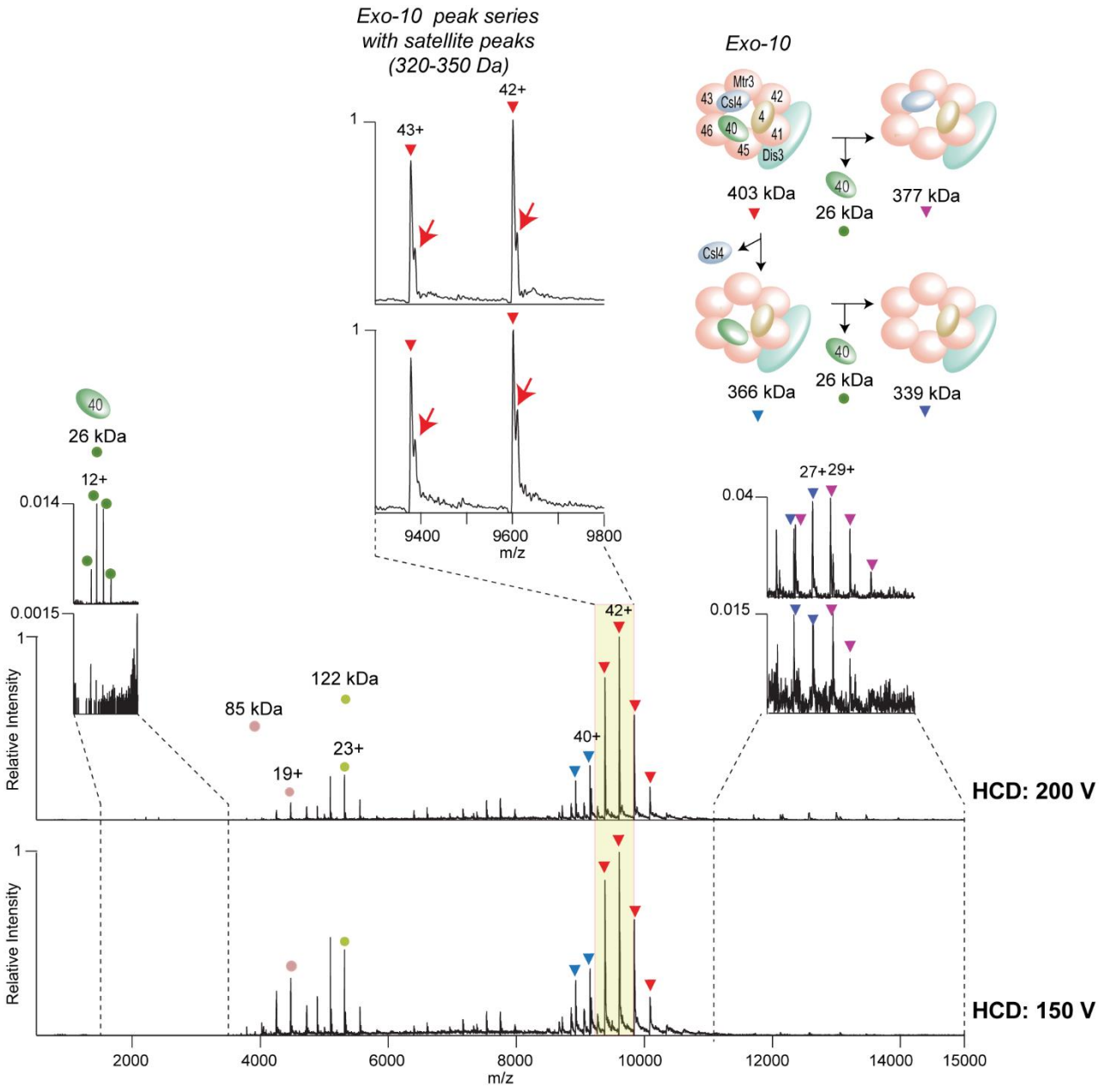

Figure S-6. Native MS analysis of the endogenous exosome assembly from budding yeast. Native MS spectra of the endogenous exosome assembly acquired at maximum HCD voltage (200 V, top spectrum) and at 150 V (bottom), with in-source dissociation voltage kept at 50 V in both experiments. The slightly lower HCD voltage yielded minimal gas-phase dissociation indicated by low signal intensities for the ejected subunit, Rrp 40 (zoom-in at left) and the charge-stripped subcomplexes (zoom-in at right). Tween-20 adduction was not observed in both spectra. In addition, we observed satellite peaks (see red arrows in the central inset panel) at lower relative intensity corresponding to mass shifts of 320-350 Da on all the observed intact assembly and subassembly peaks from the exosome sample (Table S-3). Despite repeated experiments using more stringent washes and buffer exchange steps, these satellite peaks remained unchanged. Thus, we speculate that they might result from adduction or presence of unknown post-translational modification(s).

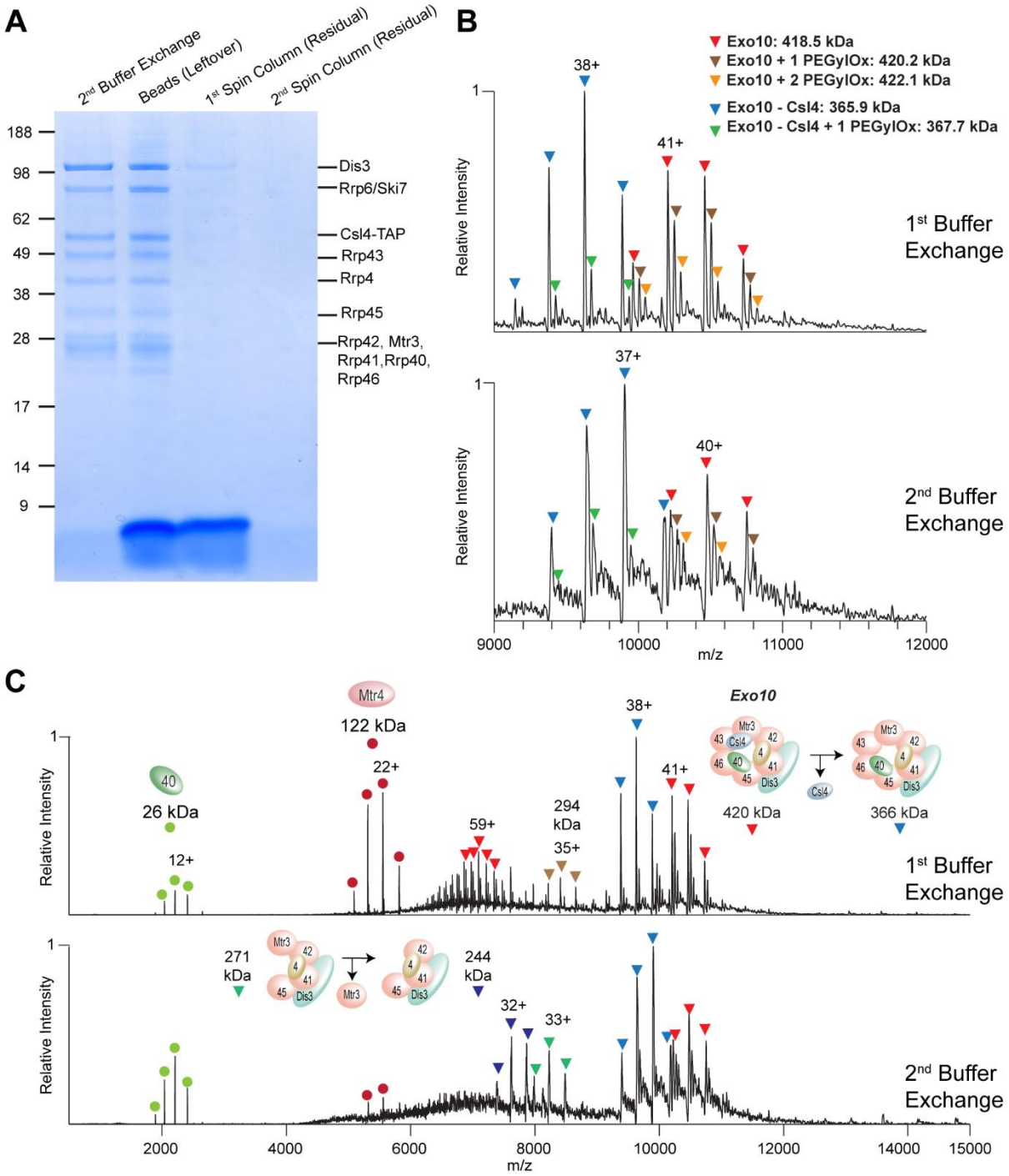

Figure S-7. Affinity capture, elution with PEGylOx, and subsequent native MS analysis of the yeast exosome assembly. (A) SDS-PAGE analysis of the second buffer exchange step, leftover bead-bound complex, and residual material from the gel filtration mini spin columns used for buffer exchange. The starting material was 0.5 g yeast cryogrindate of Csl4-TAP strain and elution was performed with 2 mM PEGylOx, which was later removed by buffer exchange into 400 mM ammonium acetate, 0.01 % Tween-20. Most PEGylOx were removed during the first buffer exchange step (see gel lane for 1st spin column residual). (B) Zoomed-in and (C) fully expanded native MS spectra of the exosome complex. The spectra show PEGylOx adduction. This issue was not resolved by a second round of buffer exchange indicating that there is considerable nonspecific interaction between PEGylOx and the exosome complex.

Table S-1. List of native MS studies on endogenous protein complexes from budding yeast.

References from Main Text	Protein Complex(es)	Affinity Tag	Cell Lysis	Affinity Capture and Sample Preparation for Native MS
[14] Hanson *et. al.* , 2004	Ribosome stalk complex (77 kDa)	Untagged	Glass beads	Cell lysate was separated using a 20-40% sucrose gradient with centrifugation (20 hrs). Ribosomes were recovered from the pellet and resuspended in buffer. Sample was buffer exchanged prior to native MS.
[15] Hernandez *et. al.* , 2006	Scavenger decapping complex (92 kDa), Cap-binding complex (189 kDa),Exosome complex (403 kDa)	TAP	Glass beads	Incubate lysate with IgG Sepharose beads, elute bead-bound protein complexes with TEV protease, bind eluted protein complexes to calmodulin beads (second-step purification), and then elute using EGTA-containing buffer. Gel filtration was performed in some experiments prior to buffer exchange and native MS.
[16] Synowsky *et. al.* , 2006	Exosome complex (403 kDa)	TAP	Glass beads	Incubate lysate with IgG Sepharose beads (2.5 hrs), elute bead-bound protein complexes with TEV protease (1.5 hrs), bind eluted protein complexes to calmodulin beads (1 hr), and then elute using EGTA-containing buffer. A sample concentration step and buffer exchange were performed prior to native MS.
[17] Sharon *et. al.* , 2006	19S proteasome lid (376 kDa)	His-tagged	Glass beads	Ni-NTA purification followed by size exclusion chromatography. Sample concentration and buffer exchange were performed prior to native MS.
[18] Lorenzen *et. al.* , 2007	RNA polymerase II (517 kDa) and III (699 kDa)	Untagged	Glass beads	From cell lysate, perform multiple chromatography steps (heparin-Sepharose, separation on a monoclonal antibody column, and anion exchange). Concentration steps and with precipitation using ammonium sulfate were performed between these chromatography steps.
[19] Synowsky and Heck, 2008	Ski complex (404 kDa)	TAP	Glass beads	Incubate lysate with IgG Sepharose beads (2.5 hrs), elute bead-bound protein complexes with TEV protease (1.5 hrs), bind eluted protein complexes to calmodulin beads (1 hr), and then elute using EGTA-containing buffer. A sample concentration step and buffer exchange were performed prior to native MS.
[20] Zhou *et. al.* , 2008	eIF3 (411 kDa)	TAP	Flash-freezing then glass beads	Incubate lysate with IgG Sepharose beads (1 hr), elute bead-bound protein complexes with TEV protease (2 hrs), concentrate sample, and perform size exclusion chromatography. A sample concentration step and buffer exchange were performed prior to native MS.
[21] Synoswky *et. al.* , 2009	Nuclear- and cytoplasmic-specific exosome (729 kDa)	TAP	Glass beads	Incubate lysate with IgG Sepharose beads (2.5 hrs), elute bead-bound protein complexes with TEV protease (1.5 hrs), bind eluted protein complexes to calmodulin beads (1 hr), and then elute using EGTA-containing buffer. A sample concentration step and buffer exchange were performed prior to native MS.

Table S-1. (Cont.)

References from Main Text	Protein Complex(es)	Affinity Tag	Cell Lysis	Affinity Capture and Sample Preparation for Native MS
[22] Geiger *et. al.* , 2010	RNA polymerase I (590 kDa)	HA- and His- tagged	Glass beads	Ammonium sulfate precipitation, ultracentrifigation (1.5 hrs), dialysis (overnight), centrifugation (1 hr), Nickel-NTA purification, followed by successive anion exchange, cation exchange, and size exclusion chromatography steps.
[23] Lane *et. al.* , 2011	RNA polymerase I (600 kDa), RNA polymerase III (700 kDa)	TAP	Glass beads	Ammonium sulfate precipitation, binding to IgG Sepharose beads, elution with TEV protease, and gel filtration. A sample concentration step and buffer exchange were performed prior to native MS.
[24] Sakata *et. al.* , 2011	26S proteasome (2.6 MDa) and assembly intermediates	3X FLAG	Glass beads	Incubate lysate with anti-FLAG antibody (2 hr) and elute with 3X FLAG. Sample was treated with high salt and anion exchange chromatography was performed to obtain subcomplexes. Sample concentration and buffer exchange were performed prior to native MS.
[7] Politis *et. al.* , 2014	Proteasome lid (376 kDa) and subcomplexes as well as proteasome-interacting proteins (PIPs) (139 kDa)	3X FLAG, TAP	Glass beads	For FLAG-tagged: Incubate lysate with anti-FLAG antibody and elute with 3X FLAG. Sample was treated with high salt and anion exchange chromatography was performed to obtain subcomplexes. Sample concentration and buffer exchange were performed prior to native MS. For TAP-tagged: Incubate lysate with IgG-conjugated magnetic beads but elution conditions and subsequent sample work-up not fully mentioned.
[25] Politis *et. al.* , 2015	eIF3:eIF5 complex (410 kDa)	TAP	Glass beads	Incubate lysate with IgG Sepharose beads, elute with TEV protease (2 hrs), bind eluted protein complexes to calmodulin beads (overnight), and elute with EGTA-containing buffer. Gel filtration was also performed prior to buffer exchange.

Table S-2. Intact masses of the protein components from the affinity-isolated endogenous protein complexes.

Protein	Sequence Mass (Da)[a]	Expected Mass (Da)[b]	Modifications[c]
GINS complex			
Psf2-PrtA	51,012.1	50,922.9	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Psf1	24,203.6	24,203.6	
Psf3	21,952.2	21,821.0	(–) *N*-terminal Met
Sld5	33,947.7	33,989.7	(+) *N*-terminal acetylation
Ctf4	104,424.5	104,424.5	
Nup84 complex			
Nup84*	110,632.7	84,464.1	(+) *N*-acetyl, HRV 3C protease cleavage
Sec13	33,043.0	32,911.8	(–) *N*-terminal Met
Seh1	39,122.8	39,122.8	
Nup145C	81,068.0	81,068.0	
Nup85	84,897.8	84,766.6	(–) *N*-terminal Met
Nup120	120,447.9	120,316.7	(–) *N*-terminal Met
Nup133	133,320.4	133,189.2	(–) *N*-terminal Met
Exosome complex			
Csl4-TAP	52,631.6	37,460.0	(–) *N*-terminal Met, (+) *N*-terminal acetylation, TEV cleavage
Dis3	113,706.6	113,617.5	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp4	39,427.2	39,338.1	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp43	44,011.0	43,921.9	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp45	33,961.9	33,872.7	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp42	29,055.2	28,966.0	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Mtr3	27,577.0	27,619.0	(+) *N*-terminal acetylation
Rrp41	27,560.7	27,471.5	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp46	24,407.2	24,318.1	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp40	26,556.4	26,467.2	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Rrp6	84,038.5	83,949.3	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Ski7	84,778.8	84,689.7	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Lrp1	21,087.2	21,087.2	
Ski2	146,058.4	145,969.2	(–) *N*-terminal Met, (+) *N*-terminal acetylation
Ski8	44,231.6	44,231.6	
Ski3	163,725.8	163,725.8	
Mtr4	122,054.9	122,096.9	(+) *N*-terminal acetylation

[a] Mass from the primary sequence of the unprocessed protein.

[b] Mass of the mature protein obtained after accounting for *N*-terminal processing, and other modifications.

[c] This includes protease cleavage during native elution and also *N*-terminal processing or co-translational modifications, such as N-terminal methionine removal and acetylation obtained from bottom-up LC-MS analysis, nMS analysis, and publicly available proteomic databases: SGD (http://www.yeastgenome. org), GPM (http://www.thegpm.org) and Uniprot (http://www.uniprot.org). Information for the exosome proteins was also obtained from Synowsky et al. Mol. Cell. Prot. **2006**, *5*, 1581-1592 and Hernandez et. al. EMBO Rep. **2006**, *7*, 605-610.

Table S-3. Mass assignment and stoichiometry determination for complexes and subcomplexes analyzed by native MS.

Measured Mass ± SD (Da)[a]	Complex/Subcomplex	Expected Mass/Da[b]	ΔMass/ Da[c]	% Mass Error	Comments
GINS Complex					
Minimal activation (ISD: 50 V, HCD: 75 V, Nominal Resolving Power at 200 m/z: 8,750)					
131,094 ± 5	Psf1/2/3/Sld5	130,937	157	0.12	
128,661 ± 8	Psf1/Ctf4	128,628	33	0.03	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*
126,617 ± 7	Psf3/Ctf4	126,272	345	0.3	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*
High activation (ISD: 50 V, HCD: 200 V, Nominal Resolving Power at 200 m/z: 8,750)					
313,629 ± 9	Ctf4 trimer	313,273	355	0.11	
131,069 ± 6	Psf1/2/3/Sld5	130,937	132	0.10	
109,211 ± 3	Psf1/2/Sld5	109,116	95	0.09	
106,861 ± 3	Psf2/3/Sld5	106,734	127	0.12	
80,049.0 ± 1.0	Psf1/3/Sld5	80,014	35	0.04	
85,027 ± 24	Psf2/Sld5	84,912	114	0.13	
21,847.3 ± 0.1	Psf3	21,821	26.0	0.12	
24,202.9 ± 0.4	Psf1	24,204	–0.6	–0.003	
130,423 ± 6	Unmatched				could not match with any subunit mass combination
128,638 ± 5	Psf1/Ctf4	128,628	10	0.008	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*
126,589 ± 9	Psf3/Ctf4	126,272	–317	–0.3	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*
High activation (ISD: 0 V, HCD: 200 V, Nominal Resolving Power at 200 m/z: 17,500)					
131,060 ± 4	Psf1/2/3/Sld5	130,937	124	0.09	
109,205 ± 3	Psf1/2/Sld5	109,116	89	0.08	
106,853 ± 5	Psf2/3/Sld5	106,734	119	0.11	
80,042.7 ± 0.4	Psf1/3/Sld5	80,014	28.5	0.04	
85004 ± 4	Psf2/Sld5	84,913	91	0.11	
21,847.2 ± 0.7	Psf3	21,821	26.3	0.12	
24,202.8 ± 0.1	Psf1	24,204	–0.8	–0.003	
130,420 ± 6	Unmatched				could not match with any subunit mass combination
128,630 ± 6	Psf1/Ctf4	128,628	–2	–0.002	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*
126,576 ± 1	Psf3/Ctf4	126,272	–304	–0.24	unlikely since Ctf4 mainly exists as trimer and Ctf4 interacts with Sld5*

Table S-3. (Cont.)

Measured Mass ± SD (Da)[a]	Complex/Subcomplex	Expected Mass/Da[b]	ΔMass/ Da[c]	% Mass Error	Comments
Nup84 Complex					
Minimal activation (ISD: 50 V, HCD: 200 V, Nominal Resolving Power at 200 m/z: 17,500)					
442,892 ± 45	Nup84/120/85/145C/Sec13/ Seh1	442,650	242	0.05	
358,340 ± 19	Nup85/120/145C/Seh1/Sec13	358,186	154	0.04	
244,212 ± 11	Nup85/Seh1/Nup120	244,206	6	0.003	consistent with structure
	Nup84/Seh1/Nup120	243,904	308	0.13	this mass error is higher
133,188 ± 8	Nup133	133,189	–1	–0.0008	
133,373 ± 10	Nup133+Δ_{Unk}		185		
123,850 ± 10	Nup85/Seh1	123,889	–39	–0.03	consistent with structure
	Nup84/Seh1	123,587	263	0.2	this mass error is higher
84,464 ± 2	Nup84	84,464	–1	–0.0012	
Nup84 Complex (Activated)					
High activation (ISD: 200 V, HCD: 200 V, Nominal Resolving Power at 200 m/z: 17,500)					
442,820 ± 36	Nup84/120/85/145C/Sec13/ Seh1	442,650	170	0.04	
358,324 ± 37	Nup145C/120/85/Seh1/Sec13	358,186	138	0.04	
277,113 ± 8	Nup120/85/Seh1/Sec13	277,118	–5	–0.002	consistent with structure
	Nup120/84/Seh1/Sec13	276,815	297	0.11	mass error is inconsistent
244,191 ± 13	Nup120/85/Seh1	244,206	–15	–0.006	consistent with structure
	Nup120/84/Seh1	243904	–287	–0.12	this mass error is higher
204,810 ± 12	Nup145C/85/Seh1	204,957	–147	–0.07	consistent with structure
	Nup145C/84/Seh1	204,655	155	0.08	this mass error is higher
156,747 ± 21	Nup85/Seh1/Sec13	156,801	–54	–0.03	consistent with structure
	Nup84/Seh1/Sec13	156,490	248	0.16	this mass error is higher
133,182 ± 3	Nup133	133,189	–7	–0.005	
133,363 ± 8	Nup133+Δ_{Unk}		181		
123,827 ± 15	Nup85/Seh1	123,889	–63	–0.05	consistent with structure
	Nup84/Seh1	123,587	263	0.2	this mass error is higher
120,321 ± 15	Nup120	120,317	5	0.004	
120,358 ± 3	Nup120+Δ_{Unk}		37		
84,461 ± 3	Nup84	84,464	–3	–0.003	

Table S-3. (Cont.)

Measured Mass ± SD (Da)[a]	Complex/Subcomplex	Expected Mass/Da[b]	ΔMass/ Da[c]	% Mass Error	Comments
Exosome Complex (ISD: 50 V, HCD: 200 V, Nominal Resolving Power at 200 m/z: 17,500)					
403,235 ± 15	Exo10	403,052	183	0.05	
403,582 ± 39	Exo10+Δ_{Unk}		347		
365,762 ± 7	Exo10-Csl4	365,592	170	0.05	
366,115 ± 10	Exo10-Csl4+Δ_{Unk}		353		
376,793 ± 4	Exo10-Rrp40	376,587	208	0.06	
377,118 ± 37	Exo10-Rrp40+Δ_{Unk}		325		
339,310 ± 12	Exo10-Csl4-Rrp40	339,125	185	0.05	
339,625 ± 28	Exo10-Csl4-Rrp40+Δ_{Unk}		315		
398,229 ± 9	Ski2/Ski3/2Ski8	398,158	71	0.02	
398,582 ± 14	Ski2/Ski3/2Ski8+Δ_{Unk}		353		
398,929 ± 26	Ski2/Ski3/2Ski8+Δ_{Unk}		700		
271,032 ± 13	Dis3/Mtr3/Rrp4/41/42/45	270,885	147	0.05	
271,356 ± 7	Dis3/Mtr3/Rrp4/41/42/45+Δ_{Unk}		324		
271,685 ± 26	Dis3/Mtr3/Rrp4/41/42/45+Δ_{Unk}		653		
243,412 ± 3	Dis3/Rrp4/41/42/45	243,266	146	0.06	
243,742 ± 15	Dis3/Rrp4/41/42/45+Δ_{Unk}		330		
204,593 ± 3	Csl4/Mtr3/Rrp40/42/6	204,462	131	0.06	lowest SD but subunit connectivity inconsistent with known structure
	Lrp1/Rrp6/40/42/43	204,350	243	0.12	
	Csl4/Rrp6/40/41/42	204,314	279	0.14	
204,917 ± 20	205kDa complex+Δ_{Unk}		324		
122,089 ± 2	Mtr4	122,096	–7	–0.006	
122,173 ± 11	Mtr4+Δ_{Unk}		84		
122,435 ± 7	Mtr4+Δ_{Unk}		346		
122,522 ± 33	Mtr4+Δ_{Unk}		433		
122,783 ± 32	Mtr4+Δ_{Unk}		694		
84,737 ± 15	Lrp1/Rrp46/Rrp4	84,743	–6	–0.007	unlikely as Lrp1 only binds to Rrp6
	Ski7	84,779	–42	–0.05	most likely
	Rrp40/45/46	84,658	79	0.09	
84,812 ± 5	Ski7+Δ_{Unk} or Rrp40/45/46+Δ_{Unk}		75		
84,878 ± 3	Ski7+Δ_{Unk} or Rrp40/45/46+Δ_{Unk}		141		
84,944 ± 2	Ski7+Δ_{Unk} or Rrp40/45/46+Δ_{Unk}		207		
85,008 ± 9	Ski7+Δ_{Unk} or Rrp40/45/46+Δ_{Unk}		271		
85,061 ± 31	Ski7+Δ_{Unk} or Rrp40/45/46+Δ_{Unk}		324		
77,178 ± 3	Unmatched				
66,505 ± 2	Unmatched				
64,252 ± 1	Unmatched				
26,466.1 ± 0.4	Rrp40	26,467.2	–1.1	–0.004	

[a] Calculated from the S.D. from all the measured mass values present in the ion envelope ($n \geq 3$).

[b] Co-translational modifications, such as *N*-terminal methionine removal and acetylation, were included (see Table S-1 for details).

[c] For Species + Δ_{Unk}, the Δ Mass corresponds to (Measured Mass + Δ_{Unk}) – Measured Mass. The unknown extra mass, Δ_{Unk}, may correspond to post-translational modification(s), cofactor binding and/or adduction (see Figure S-6).

* Refer to Simon et al. Nature **2014**, *510*, 293-297.

Table S-4. Database search results for the affinity isolation of the yeast GINS complex.[a]

Accession Number	Protein	$-log_{10}E$	% Seq. coverage (measured)	% Seq. coverage (corrected)[b]	Unique Peptides	Total Peptides	Mass/ 10^3 Da	Non-specific Components[c]
YPR135W	CTF4:p	136.7	21	25	14	22	104.4	
YDR489W	SLD5:p	133.8	69	>100	14	18	33.9	
YHR174W	ENO2:p	95.1	31	41	10	11	46.9	most likely
YGR192C	TDH3:p	80.2	42	49	10	12	35.7	most likely
YJL072C	PSF2:p	74.3	48	96	7	9	25.0	
YBR118W	TEF2:p	61.6	19	31	9	9	50.0	most likely
YDR013W	PSF1:p	59.5	34	49	7	7	24.2	
YOL146W	PSF3:p	53.2	42	46	6	9	21.9	
YAL038W	CDC19:p	51.2	19	24	6	8	54.5	most likely
YLR044C	PDC1:p	50.5	20	31	7	7	61.5	most likely
YCR012W	PGK1:p	45.6	18	20	6	8	44.7	most likely

[a] Affinity isolation was performed using Psf2-SpA strain. LC-MS/MS spectra was searched using X! Tandem from the Global Proteome Machine (GPM). Only protein hits with more than three unique matched peptides are shown. Proteins highlighted in blue were used for native MS data analysis.

[b] Sequence coverage based on the primary protein sequence corrected for peptides that are not likely to be observed under normal peptide nano-LC/MS methods (e.g., long and short peptides and hydrophobic peptides). A corrected sequence coverage of >100% means that one or more of these atypical peptides were observed in addition to the rest of the matched peptides.

[c] Identification based on at least 10 out of 17 large-scale protein interactome studies in yeast with relatively high spectral counts in the CRAPOME database (http://www.crapome.org), which makes it most likely a non-specific interacting protein.

Table S-5. Database search results for the affinity isolated yeast Nup84 complex.[a]

Accession Number	Protein	$-log_{10}E$	% Seq. coverage (measured)	% Seq. coverage (corrected)[b]	Unique Peptides	Total Peptides	Mass/ 10^3 Da	Non-specific Components[c]
YGL092W	NUP145:p	1052.8	34	53	85	309	145.6	
YKR082W	NUP133:p	1001.8	45	65	79	287	133.2	
YDL116W	NUP84:p	953.0	46	71	80	315	83.6	
YKL057C	NUP120:p	919.5	45	73	77	318	120.4	
YJR042W	NUP85:p	716.0	46	61	57	207	84.8	
YLR208W	SEC13:p	623.6	84	>100	47	235	33.0	
YGL100W	SEH1:p	486.9	57	79	39	180	39.1	
YLL024C	SSA2:p	386.7	41	58	28	93	69.4	most likely
YMR129W	POM152:p	243.6	20	27	25	28	151.6	
YDL229W	SSB1:p	124.2	20	28	11	14	66.6	most likely
YAL038W	CDC19:p	103.2	24	30	11	15	54.5	most likely
YML031W	NDC1:p	87.2	15	28	10	11	74.1	
YGR192C	TDH3:p	85.7	27	32	8	12	35.7	most likely
YPR080W	TEF1:p	75.4	19	31	9	12	50.0	most likely
YNL055C	POR1:p	74.2	33	38	8	9	30.4	
YPL036W	PMA2:p	73.6	7.8	15	8	8	102.1	most likely
YLR044C	PDC1:p	72.9	15	23	7	7	61.5	most likely
YNL178W	RPS3:p	72.3	29	37	7	7	26.5	most likely
YML063W	RPS1B:p	70.9	36	60	9	11	28.8	most likely
YDR385W	EFT2:p	68.1	9.3	12	8	8	93.2	most likely
YBR127C	VMA2:p	61.3	16	24	7	7	57.7	
YIL115C	NUP159:p	60.6	5.2	9	8	8	158.8	
YHR174W	ENO2:p	57.7	16	21	6	6	46.9	most likely
YLR249W	YEF3:p	57.3	7	10	7	9	115.9	most likely
YBR084C-A	RPL19A:p	56.7	23	58	5	6	21.7	most likely
YJR123W	RPS5:p	56.0	19	31	5	5	25.0	most likely
YML026C	RPS18B:p	50.4	32	42	5	6	17.0	most likely
YOR063W	RPL3:p	47.8	15	21	6	6	43.7	most likely
YBR048W	RPS11B:p	44.4	35	53	5	7	17.7	most likely
YJL041W	NSP1:p	43.8	7.2	14	5	5	86.5	
YJL061W	NUP82:p	43.5	8.3	17	6	6	82.0	
YLL039C	UBI4:p	43.2	8.7	11	5	11	42.8	
YJR121W	ATP2:p	41.1	9.2	12	4	4	54.8	
YFR031C-A	RPL2A:p	40.6	19	26	5	6	27.4	most likely
YBL099W	ATP1:p	38.5	10	14	5	5	58.6	
YBR106W	PHO88:p	37.6	31	39	4	5	21.1	
YDR098C-B	YDR098C-B:p	37.0	4.4	9	5	6	198.5	most likely
YBR191W	RPL21A:p	36.8	24	53	5	8	18.2	most likely
YBL079W	NUP170:p	33.9	3.5	5	4	4	169.4	

Table S-5. (Cont.)

Accession Number	Protein	$-log_{10}E$	% Seq. coverage (measured)	% Seq. coverage (corrected)[b]	Unique Peptides	Total Peptides	Mass/ 10^3 Da	Non-specific Components[c]
YLR075W	RPL10:p	31.9	17	22	4	4	25.3	most likely
YOL086C	ADH1:p	31.8	11	20	4	4	36.8	most likely
YDR012W	RPL4B:p	31.5	16	26	4	4	39.0	most likely
YJR145C	RPS4A:p	28.3	13	18	4	5	29.4	most likely
YPL081W	RPS9A:p	27.1	21	29	4	5	22.4	most likely
YBL030C	PET9:p	24.2	11	14	4	4	34.4	most likely

[a] Affinity isolation was performed using the Nup84-SpA strain. LC-MS/MS spectra was searched using X! Tandem from the Global Proteome Machine (GPM). Only protein hits with more than three unique matched peptides are shown. Proteins highlighted in blue were used for native MS data analysis.

[b] Sequence coverage based on the primary protein sequence corrected for peptides that are not likely to be observed under normal peptide nano-LC/MS methods (e.g., long and short peptides and hydrophobic peptides). A corrected sequence coverage of >100% means that one or more of these atypical peptides were observed in addition to the rest of the matched peptides.

[c] Identification based on at least 10 out of 17 large-scale protein interactome studies in yeast with relatively high spectral counts in the CRAPOME database (http://www.crapome.org), which makes it most likely a non-specific interacting protein.

Table S-6. Database search results for the affinity isolated yeast exosome assembly and associated protein factors.[a]

Accession Number	Protein	$-log_{10}E$	% Seq. coverage (measured)	% Seq. coverage (corrected)[b]	Unique Peptides	Total Peptides	Mass/ 10^3 Da	Non-specific Components[c]
YOL021C	DIS3:p	539.8	33	45	45	318	113.6	
YOR001W	RRP6:p	251.1	23	34	18	135	84.0	
YNL232W	CSL4:p	212.6	68	>100	19	97	31.6	
YCR035C	RRP43:p	182.3	29	42	16	122	44.0	
YHR069C	RRP4:p	166.2	31	49	14	91	39.4	
YGR095C	RRP46:p	138.3	33	47	13	64	24.4	
YDR280W	RRP45:p	116.6	29	43	11	75	33.9	
YMR229C	RRP5	116.4	7.5	12	14	16	193.0	most likely
YOL142W	RRP40:p	107.1	37	46	10	48	26.5	
YLR227W-B	YLR227W-B:p	99.9	5.7	10	9	17	198.3	most likely
YLR398C	SKI2:p	89.4	5.8	8	10	16	146.0	
YOR076C	SKI7:p	87.8	9	14	9	21	84.7	
YGL008C	PMA1:p	84.3	8	13	9	12	99.6	most likely
YPR189W	SKI3:p	77.0	5.5	7	8	11	163.6	
YLR197W	SIK1:p	75.7	10	16	7	13	56.8	most likely
YJL050W	MTR4:p	72.0	7.5	11	8	13	122.0	
YDL014W	NOP1:p	71.2	22	34	8	15	34.4	most likely
YNL209W	SSB2:p	70.6	12	17	8	11	66.6	most likely
YER165W	PAB1:p	70.5	13	19	7	14	64.3	most likely
YOR063W	RPL3:p	65.3	13	19	7	13	43.7	most likely
YHR081W	LRP1:p	64.3	31	47	7	31	21.0	
YPL198W	RPL7B:p	64.0	24	32	7	13	27.7	most likely
YLR441C	RPS1A:p	62.8	21	31	7	17	28.7	most likely
YJL109C	UTP10:p	60.6	3.4	5	6	6	200.0	most likely
YML056C	IMD4:p	60.4	9.9	15	6	16	56.4	most likely
YOR341W	RPA190	59.1	3.8	5	7	7	186.3	most likely
YJL076W	NET1:p	58.5	7.3	11	7	10	128.5	
YGR195W	SKI6:p	58.5	18	43	7	39	27.5	
YDR381W	YRA1:p	58.5	24	41	6	15	24.9	most likely
YML063W	RPS1B:p	56.9	4.7	8	1	1	28.8	most likely
YOR310C	NOP58:p	52.1	8.6	12	5	11	56.9	most likely
YBR181C	RPS6B:p	52.1	22	49	5	14	27.0	most likely
YAL038W	CDC19:p	51.8	12	15	6	12	54.5	most likely
YLR448W	RPL6B:p	50.9	24	31	6	8	20.0	most likely
YOL120C	RPL18A:p	50.0	24	37	6	10	20.6	most likely
YNR024W	YNR024W:p	48.8	26	40	4	16	21.1	
YGR192C	TDH3:p	47.4	14	16	4	6	35.7	most likely
YIL133C	RPL16A:p	47.1	24	41	5	8	22.2	most likely

Table S-6. (Cont.)

Accession Number	Protein	$-log_{10}E$	% Seq. coverage (measured)	% Seq. coverage (corrected)[b]	Unique Peptides	Total Peptides	Mass/ 10^3 Da	Non-specific Components[c]
YBR048W	RPS11B:p	46.8	26	39	6	9	17.7	most likely
YBR191W	RPL21A:p	41.0	24	53	5	11	18.2	most likely
YMR290C	HAS1	39.6	9.9	14	5	5	56.7	most likely
YBR084C-A	RPL19A:p	39.1	22	55	5	11	21.7	most likely
YCR031C	RPS14A:p	38.7	28	61	5	7	14.5	most likely
YGR158C	MTR3:p	38.5	14	28	5	6	27.6	
YOL127W	RPL25:p	38.4	30	61	4	8	15.7	most likely
YDL082W	RPL13A:p	38.3	18	30	4	11	22.5	most likely
YDR012W	RPL4B:p	36.6	12	20	4	5	39.0	most likely
YHL033C	RPL8A:p	34.4	14	19	4	8	28.1	most likely
YDL111C	RRP42:p	34.2	13	26	4	30	29.0	
YDR385W	EFT2:p	32.2	4.5	6	4	4	93.2	most likely
YNL132W	KRE33:p	30.9	3.5	5	4	4	119.3	most likely
YLR340W	RPP0:p	29.9	11	28	4	7	33.7	most likely
YDR064W	RPS13:p	29.5	21	27	4	7	17.0	most likely
YDR324C	UTP4	28.8	4.5	7	4	5	87.7	most likely
YPR080W	TEF1:p	28.6	6.3	10	4	7	50.0	most likely
YJR145C	RPS4A:p	28.5	12	17	4	6	29.4	most likely
YGL173C	KEM1:p	28.3	2	3	4	4	175.3	most likely
YGR178C	PBP1:p	27.8	5.5	10	4	4	78.7	most likely
YGL123W	RPS2:p	27.1	11	16	4	6	27.4	most likely
YMR143W	RPS16A:p	26.9	20	34	4	5	15.8	most likely
YMR242C	RPL20A:p	26.7	15	23	4	7	20.4	most likely
YGR034W	RPL26B:p	26.5	23	35	4	4	14.2	most likely
YBR189W	RPS9B:p	26.4	18	26	4	4	22.3	most likely
YGL213C	SKI8:p	20.2	4.5	11	4	5	44.2	

[a] Affinity isolation was performed through the Csl4-TAP strain. LC-MS/MS spectra was searched using X! Tandem from the Global Proteome Machine (GPM). Only protein hits with more than three unique matched peptides (except for Ski8 which has been shown to form a complex with Ski 2 and Ski3) are shown. Proteins highlighted in blue were used for native MS data analysis.

[b] Sequence coverage based on the primary protein sequence corrected for peptides that are not likely to be observed under normal peptide nano-LC/MS methods (e.g., long and short peptides and hydrophobic peptides). A corrected sequence coverage of >100% means that one or more of these atypical peptides were observed in addition to the rest of the matched peptides.

[c] Identification based on at least 10 out of 17 large-scale protein interactome studies in yeast with relatively high spectral counts in the CRAPOME database (http://www.crapome.org), which makes it most likely a non-specific interacting protein.

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