Corrections

BIOCHEMISTRY

Correction for "Streamlined analysis schema for high-throughput identification of endogenous protein complexes," by Anna Malovannaya, Yehua Li, Yaroslava Bulynko, Sung Yun Jung, Yi Wang, Rainer B. Lanz, Bert W. O'Malley, and Jun Qin, which appeared in issue 6, February 9, 2010, of *Proc Natl Acad Sci USA* (107:2431–2436; first published January 22, 2010; 10.1073/pnas.0912599106).

The authors note that the following acknowledgments were omitted from the article:

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www.pnas.org/cgi/doi/10.1073/pnas.1001159107

EVOLUTION

Correction for "Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear," by Charlotte Lindqvist, Stephan C. Schuster, Yazhou Sun, Sandra L. Talbot, Ji Qi, Aakrosh Ratan, Lynn P. Tomsho, Lindsay Kasson, Eve Zeyl, Jon Aars, Webb Miller, Ólafur Ingólfsson, Lutz Bachmann, and Øystein Wiig, which appeared in issue 11, March 16, 2010, of *Proc Natl Acad Sci USA* (107:5053–5057; first published March 1, 2010; 10.1073/pnas.0914266107).

The authors note that, due to a printer's error, on page 5054, right column, second paragraph, eighth line, "Within this clade, we estimated the mean age of the split between the ABC bears and the polar bears to be 152 ky, and the mean age for all polar bears as 134 ky, near the **end** of the Eemian interglacial period and completely in line with the stratigraphically determined age of the Poolepynten subfossil (11)," should instead appear as "Within this clade, we estimated the mean age of the split between the ABC bears and the polar bears to be 152 ky, and the mean age for all polar bears as 134 ky, near the **beginning** of the Eemian interglacial period and completely in line with the stratigraphically determined age of the Poolepynten subfossil (11)." This error does not affect the conclusions of the article. This error has been corrected online and in print.

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EVOLUTION

Correction for "Paleobiology and the origins of avian flight," by John Ruben, which appeared in issue 7, February 16, 2009, of *Proc Natl Acad Sci USA* (107:2733–2734; first published February 9, 2010; 10.1073/pnas.0915099107).

Due to a printer's error, the first sentence of this Commentary appeared incorrectly and should read: "When interpreting the paleobiology of long extinct taxa, new fossils, and reinterpretations of well-known fossils, sharply at odds with conventional wisdom never seem to cease popping up." The online version has been corrected.

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IMMUNOLOGY

Correction for "Activation state and intracellular trafficking contribute to the repertoire of endogenous glycosphinogolipids presented by CD1d," by Karen Muindi, Manuela Cernadas, Gerald F. M. Watts, Louise Royle, David C. A. Neville, Raymond A. Dwek, Gurdyal S. Besra, Pauline M. Rudd, Terry D. Butters, and Michael B. Brenner, which appeared in issue 7, February 16, 2010, of *Proc Natl Acad Sci USA* (107:3052–3057; first published January 28, 2010; 10.1073/pnas.0915056107).

The authors note that the title of their manuscript appeared incorrectly. The title should instead appear as "Activation state and intracellular trafficking contribute to the repertoire of endogenous glycosphingolipids presented by CD1d." The title has been corrected online. Additionally, on page 3055, left column, second paragraph, line 14, "However, the GM2 peak that was a prominent GSL glycan eluted from soluble mCD1d was not observed in the mCD1d-TEV eluates (Figs. 2*A* and 4; Table 2)" should instead appear as "However, the GM2 peak that was a prominent GSL glycan eluted from soluble mCD1d was not observed in the mCD1d-TEV eluates (Figs. 2*B* and 4; Table 2)." This error does not affect the conclusions of the article.

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Streamlined analysis schema for high-throughput identification of endogenous protein complexes

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Contributed by Bert W. O'Malley, November 4, 2009 (sent for review July 28, 2009)

Immunoprecipitation followed by mass spectrometry (IP/MS) has recently emerged as a preferred method in the analysis of protein complex components and cellular protein networks. Targeting endogenous protein complexes of higher eukaryotes, particularly in large-scale efforts, has been challenging due to cellular heterogeneity, high proteome complexity, and, compared to lower organisms, lack of efficient in-locus epitope-tagging techniques. It is further complicated by variability in nonspecific identifications and cross-reactivity of primary antibodies. Still, the study of endogenous human protein networks is highly desired despite its challenges. Here we describe a streamlined IP/MS protocol for the purification and identification of extended endogenous protein complexes. We investigate the sources of nonspecific protein binding and develop semiquantitative specificity filters that are based on peptide spectral count measurements. We also outline logical constraints for the derivation of accurate complex composition from IP/MS data and demonstrate the effectiveness of this approach by presenting our analyses of different transcriptional coregulator complexes. We show consistent purification of novel components for the Integrator complex, analyze the composition of the Mediator complex solely from our data to demonstrate the wide usability of spectral counts, and deconvolute heterogeneous HDAC1/2 networks into core complex modules and several novel subcomplex interactions.

antibody cross-reactivity | complex heterogeneity | protein complex | protein–protein interactions | transcriptional coregulators

It is now accepted that most transcriptional regulators assemble into multisubunit complexes, and these may be their minimal biologically active units (1–3). Transcription in the cell can be viewed as a network of ordered interactions between different protein complexes. Some protein complexes have a stable core module where the components of the core appear together and with a constant stoicheometry in biochemical purifications. Other proteins interact transiently or weakly and often regulate and fine tune the function of the core complex module(s). Transcriptional regulator complexes respond to cellular signals through a variety of posttranslational modifications on multiple subunits to deduce an integrated response to a particular change of cell state (4, 5). Thus, with the goal of an unbiased study of transcriptional protein complex networks, it is desirable to obtain biochemical information not only about the core complexes, but also about their transient interactors and regulators, as well as their intercore complex interactions.

To date, the most extensive studies on endogenous protein interaction networks were done in yeast, where in-locus epitope-tagging of the complete ORFeome is feasible through homologous recombination (6–8). This is advantageous because the proteins are under the regulation of endogenous promoters, a single kind of high-affinity epitope antibody can be used to isolate the complexes, and, subsequently, cross-reacting proteins are easily distinguished from true associated proteins. Such approaches define a protein complex as all proteins that reproducibly copurify with the tagged "bait" antigen. The data derived from these efforts have been used to construct protein interaction networks.

In comparison to yeast, large-scale genetic manipulations in mammalian cells are limited. A few large-scale IP/MS datasets were obtained from human cell lines with overexpressed epitope-tagged proteins in recent years (9–11). Such experiments are limited to moderate size nontoxic proteins and may produce false-positive associations due to the overexpression of bait antigens or the epitope tag itself (12). More recent methodological studies have attempted to address these issues by improving the efficiency of tagging procedures, regulating levels of expression, devising quantitative measures for differentiation of nonspecific interactions, and by increasing experimental reproducibility (13–18). Still, these attempts cannot resolve a need for studying endogenous protein complexes and for performing large-scale comparative analyses between different cell types. Global IP/MS studies of endogenous protein complexes generally have not been attempted because of major concerns associated with variable cross-reactivity of primary antibodies, limited availability of antibodies that are suitable for affinity purification, and the complexity of nonspecific protein associations.

Here we report a comprehensive workflow for the identification of affinity-purified endogenous human protein complexes. We optimized several experimental parameters, standardized the IP/MS protocol, and evaluated different strategies to address the aforementioned concerns. With this workflow, we carried out >1,000 endogenous human IP/MS studies and found that it is now feasible to isolate complete endogenous human protein complex interaction networks in a standardized high-throughput manner. We analyze three coregulators of pol-II-driven transcription, to demonstrate consistent preservation and recovery of complete protein complex modules with previously uncharacterized subunits. Furthermore, we describe a tailored set of logical constraints for analysis of IP/MS data, which allows filtering of nonspecific proteins, derivation of core protein complex modules for the Integrator, Mediator, HDAC1/2, CHD4, SIN3A, KDM1, and PBRM1/BRD7, and the deconvolution of intercomplex interaction in the heterogenous HDAC1/2 network.

Results

Optimization of IP/MS Potocol for Deep Proteome Coverage and Preservation of Weak Protein–Protein Interactions. To establish a standardized procedure for isolation and identification of endogenous steady-state protein complexes that ultimately aims at high-throughput analyses, we chose to first target regulatory proteins in the nuclear extract (NE) of HeLa S3 cells. These cells are easily grown in suspension and can be cost-effectively

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The authors declare no conflict of interest.

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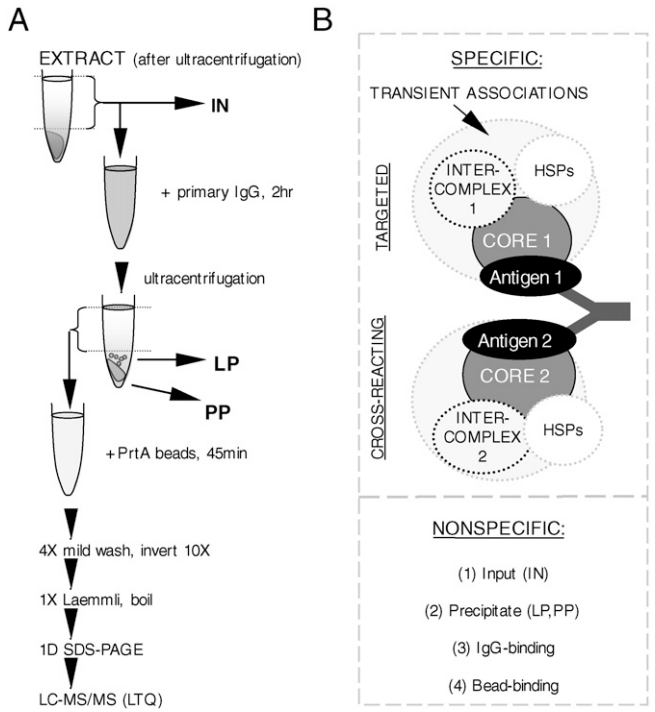

Fig. 1. IP/MS optimization for deep interactome coverage. (*A*) Immunoprecipitation procedure for purification of extended endogenous complexes. (*B*) Proteins in IP/MS result can be separated into the specific and nonspecific categories. Specific proteins constitute antibody affinities, including targeted (intended) and nontargeted (secondary, cross-reacting) complexes.

expanded for large-scale NE production within an individual laboratory (19, 20).

We first streamlined the IP protocol for preservation of weak interactions during protein complex isolation (Fig. 1*A*). We use a two-step IP protocol with 2-h primary antibody incubation and subsequent 45-min incubation with ProteinA Sepharose beads, where preservation of weaker affinities depends greatly on the quality of the extract and the length and stringency of bead washes. We use a reduced-detergent (0.5% NP-40) wash buffer and greatly limit the washing time by briefly inverting tubes 10 times and eliminating incubation in the wash buffer. Because some nonspecific proteins are retained by this procedure, additional filtering of nonspecific components is addressed *in silico* through data mining.

To maximize the number of protein identifications per IP, we resolve the immunocomplexes on SDS/PAGE and split each gel lane into six regions for subsequent sequencing in separate mass spectrometry runs. This size separation of proteins reduces the complexity of the protein mixture significantly and sufficiently to match the resolving power of the 35-min LC runs and enriches the identification of the minor components in the immunocomplex, such as auxiliary transcription factors and regulatory enzymes. It takes 6 h of machine time to analyze one IP experiment and about three additional hours to search and manually verify the identifications. With this streamlined procedure, we are now able to isolate and analyze on average three immunocomplexes per day and routinely identify 100–300 proteins (both specific and nonspecific) per IP/MS experiment.

Origins of Nonspecific Proteins in IP/MS and Definition of Corresponding Specificity Filters. We found that nonspecific proteins can originate from three major sources: (*i*) overly abundant proteins in the NE [input (IN)], (*ii*) proteins that aggregate and precipitate out of solution during primary antibody incubation [loose (LP) and packed (PP) precipitates], and (*iii*) proteins that preferentially bind to immunoglobulins (IgG) and ProteinA Sepharose (Fig. 1*A* and *B*).

Precipitates that accumulate during antibody incubation are the primary source of sporadic nonspecific contaminating proteins. This precipitate can be largely cleared by ultracentrifugation at $100,000 \times g$ prior to bead incubation. Substantial amounts of LP aggregates are suspended immediately above the PP after ultracentrifugation, and we normally avoid the whole bottom 0.1 mL at the cost of about 10% immunocomplex (Fig. 1*A*). To obtain a semiquantitative composition filter for LP we repacked and measured LP proteins from four different IPs and identified 712 unique proteins in one or more of these experiments (Table S1). For each protein, we summed their spectral counts (SPCs; peptide number parameters assigned by SeQuest Software) across repeats to obtain a semiquantitative composition filter for LP (see *SI Text*). We also measured the packed precipitate and IN material (1 μL NE) to determine the most abundant proteins in these fractions, which resulted in the identification of 413 unique "PP proteins" and 1,228 unique "input proteins" (Table S1). In contrast to LP and PP proteins that stick to beads, soluble input proteins are more readily washed away during the process of protein complex isolation.

After examining multiple IP/MS we noticed that likely nonspecific proteins appear as frequently identified proteins with distinguishably different distributions of their total SPCs. We performed statistical quartile analyses of SPC frequency distributions for all proteins identified in our IPs (*SI Text* and Table S2) and identified the upper-hand extreme outlier value as a suitable E_{cutoff} filter threshold, eliminating proteins with lower SPC values as nonspecific while preserving highly enriched proteins as specific interactors.

By combining the described filters, we were able to reduce ≈170,000 protein identifications in over 1,000 IPs to ≈60,000 likely specific interactions. This filtered dataset represents significantly enriched specific proteins from 6,548 unique human genes, which constitutes ≈25% of the human genome.

Extended Core Complexes Can be Derived by Reciprocal Co-occurrence. We first use the pol-II-regulatory Integrator complex to illustrate the high reproducibility of our approach. It is customary to use reciprocal IPs for verification of interactions in immunoprecipitation. Representative IP/MS data for the Integrator subunits INTS1, −3, −5, and −6 are shown in Fig. 2 and Table S3. A simple co-occurrence test for proteins identified in these IPs

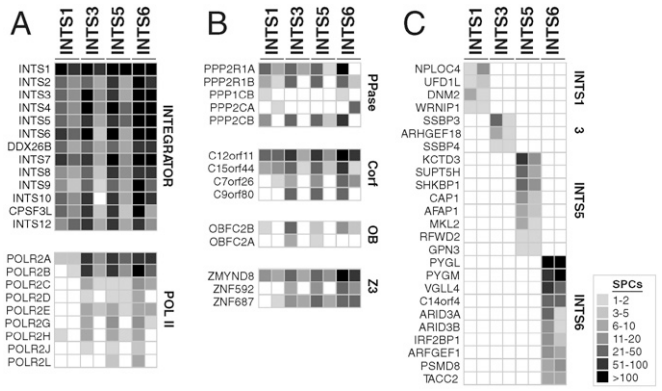

Fig. 2. Extended Integrator interactome. (*A*) Reciprocal IPs against Integrator subunits retrieve previously known core module and interacting polymerase subunits. (*B*) Multiple new interactors are discovered consistently with the Integrator: a phosphatase module, OBFC2A/B, four uncharacterized predicted proteins, and a unique Z3 complex consisting of ZMYND8, ZNF687, and ZNF592. (*C*) Reproducible antibody-specific identifications contain potential antibody cross-reactivity.

revealed all 12 known subunits and DDX26B in these purifications, which were previously found by epitope tag affinity purification (21). In addition, preservation of weak interactions by our IP protocol resulted in consistent purification of an extended pol II module (up to nine subunits), a complete phosphatase module (PPP1CB, PPP2CA/B, and PPP2R1A/B), four uncharacterized proteins C12orf11, C15orf44, C7orf26, C9orf80, the OB-fold nuclear acid binding proteins OBFC2A and OBFC2B, and a set of zinc-finger proteins (ZMYND8, ZNF687, and ZNF592).

C12orf11, C7orf26, C15orf44, C9orf80, and OBFC2A/B proteins are most likely to be specific new components of the Integrator complex, because they appear to have great positive correlation and specificity toward Integrator purifications. In contrast, although ZMYND8, ZNF687, and ZNF592 correlate well with Integrator subunits, we also found them in several Integrator-independent associations and thus consider the proteins to form a hitherto unidentified core complex module, which we termed Z3.

Furthermore, sorting proteins by co-occurrence across IP/MS experiments also allowed us to distinguish potential antibody-specific cross-reacting proteins. In Fig. 2*C* we show four subsets of proteins that are specific to each and only one antibody for INTS subunits. Because core subunits generally repeat across different antibodies targeted at the components of the same complex, antibody-specific identifications, which contain antibody cross-reactivity, can be easily avoided *in silico* during core complex assignment by comparing reciprocal IPs and omitting proteins with antibody-specific occurrences.

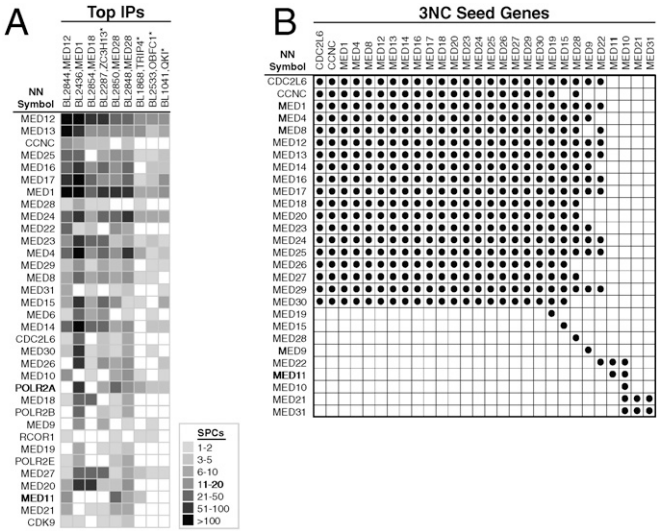

Fig. 3. Core complex subunits of Mediator are defined by 3N analysis. (*A*) Top IPs where MED12 is present at highest levels (>5 peptides) were clustered with 3N constraints (see text). BL#, antibody IDs; ∗ identifies primary antibodies where Mediator is a secondary interacting or cross-reacting complex. (*B*) 3N analysis was performed for all Mediator subunits with sufficient number of identification in our dataset. Protein neighbors that are copresent in multiple reciprocal 3Ns (•) define potential core complex clusters for Mediator. Mediator-interacting polymerase is effectively stratified from the Mediator core by this analysis.

Near-Neighbor Network Analysis for Antigen/Antibody-Independent Protein Complex Assignment. Having carried out multiple coregulator IPs under similar assay conditions, we sought to develop a robust strategy for data-driven core complex assignments. Here we outline a semiquantitative approach we call near-neighbor network (3N) analysis that is sufficient and effective for this task (summarized in Fig. S1). To illustrate this method, we use an example of another pol II coregulator, the Mediator complex, which is well suited for this proof-of-principle study, as it has been exhaustively described in the literature (22–24).

To define a core complex de novo from IP/MS data, we introduced four major constraints to the co-occurrence analysis: (*i*) protein-centered top IP subset selection, (*ii*) positive co-occurrence requirement, (*iii*) limited number of antibody repeats, and (*iv*) statistical distance-based interaction proximity cutoff.

For each protein of interest ("seed" protein), we first selected multiple IPs containing this protein at highest SPCs (top IPs) regardless of the original targeted antigens. Then, proteins that passed all specificity filters in top IPs were sorted by their co-occurrence with the seed. True interactors are required to copurify three or more times with the seed protein. Furthermore, in our top IP selection, we allow a maximum of two repeat IPs for each antibody. Because, as illustrated for Integrator in Fig. 2*C*, cross-reacting proteins can be reproduced within antibody repeats, but not in reciprocal experiments, cross-reacting proteins are automatically omitted during analysis through combination of the imposed constraints.

MED12 is present in 25 IP/MS experiments that we performed. Clustering of the top nine IPs that contain the highest spectral counts of MED12 yields ≈40 proteins that cooccur in at least four experiments. Thus, these 40 proteins are likely to be associated with the Mediator complex, and, in fact, most are known Mediator subunits (Fig. 3*A*). This is a greatly reduced list from the original 503 unique proteins that pass all specificity filters in these top nine IPs.

We then sought a method to further constrain true core complex components. We reasoned that proteins forming core modules should not only copurify and be detected most times, but the ratio between the components of the core complex in different experiments should also be similar, although the amount of the protein complex can be different. A simple way to describe this relationship mathematically is the cosine similarity—each protein occurrence across selected IPs can be represented as a vector, where the coordinates are protein SPCs, and the angle between each pair of SPC vectors (U and V) is calculated according to standard definition $[\arccos(U \cdot V/\|U\| \times \|V\|)]$ (25). We observed that when 5–15 top IPs are used for calculations, true complex components are likely to fall within 65° from the seed protein. We then used 65° as a cutoff for near-neighbor interactors of each seed protein.

A major advantage of the 3N analysis is that it does not require antigen information or rigorous characterizations of cross-reactivity. Of the nine top MED12 experiments, only five were carried out using antibodies against known Mediator subunits; the other four experiments recovered the Mediator complex via intercomplex interactions or as a cross-reacting complex with no relation to the intended antigen.

To distinguish minimal core complex components from frequent interactors that have functions independent of, or in addition to, the core complex, we further compiled sets of related "reciprocal" 3Ns (Table S4). Near neighbors that are copresent in multiple 3N networks define core modules. Indeed, iterative comparison of the reciprocal 3Ns using different seed proteins (summarized in Fig. S1*B*) can reveal different complex associations and distinguish minimal core complex components from frequently interacting proteins. Such analysis further stratified the Mediator core complex and suggested that, in HeLa S3 cells, MED22, MED10, MED11, MED21, and MED31 are likely to form a distinct Mediator submodule (Fig. 3*B*).

Deconvolution of the Heterogeneous HDAC1/2 Networks with 3N Analysis. Next, we investigated whether 3N analysis can stratify heterogeneous complexes. Because HDAC1/2 is known to work in context of several different corepressor complexes (26–29), we applied 3N analysis to deconvolute the HDAC1/2 interactome.

Using HDAC1 as a seed, we found known HDAC1/2 interactors CHD4, KDM1, and SIN3A as its near neighbors (Table S5). Analogous to the 3N analysis of Mediator, we then used these near neighbors as seeds, found all the reciprocal 3N networks for CHD4, SIN3A, and KDM1, and organized these complexes into core modules based on co-occurrence of complex subunits in multiple neighbor networks (Fig. 4*A*, Fig. S2, and Table S5). Whereas Mediator and Integrator complexes mingle within a relatively uniform pool of subunits, it is apparent that HDAC-containing complexes are quite heterogeneous and separate from each other. Thus, 3N analysis is able to segregate different HDAC complexes from a limited number of related experiments where many of these complexes are copresent, albeit at different relative levels.

CHD4/NURD module. Using CHD4 as a seed, we recovered a multisubunit NURD-like complex (30) with CHD3, MTA1/2/3, MBD2/3, GATAD2A/B, RBBP7, CDK2AP1, and CDK2AP2 (Fig. 4*A* and Fig. S2). CDK2AP1, but not CDK2AP2, was previously identified in an MBD3-containing complex, and it has a repressive function on OCT4 expression (31, 32); CDK2AP proteins were separately shown to interact with each other (33).

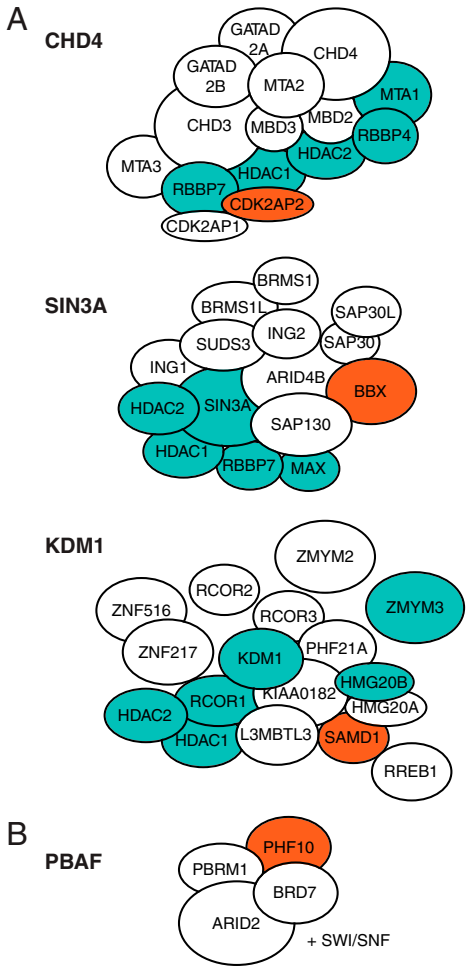

Fig. 4. De novo IP/MS deconvolution of human HDAC1/2 corepressor complex network. (*A*) HDAC1-containing CHD4, SIN3A, and RCOR1 complexes were defined by comparison of reciprocal 3Ns. Heterogeneity of HDAC1/2 complexes is revealed as these modules break apart from each other in 3N analysis. Proteins that were directly targeted as antigens are shaded in blue; unique core complex associations are highlighted in orange. (*B*) Subunit assignment for the HDAC1/2 network intercomplex interactor PBRM1/BRD7 complex.

SIN3A module. 3N of top SIN3A-containing IPs returns multiple known SIN3A-associated proteins including HDAC1/2, MAX, and the H2A/B module (Tables S5). Among them, MAX is a known SIN3A interacting transcription factor (34, 35), whereas bobby sox homolog, BBX, is a previously unknown interactor of SIN3A. When reciprocal 3Ns for all proteins in SIN3A 3N are compared, a cluster of 15 proteins persists, defining high-confidence subunits of the core SIN3A complex (Fig. 4*A* and Fig. S2). BBX remains in this complex, suggesting that it is a new core SIN3A complex subunit.

KDM1 complexes. HDAC1 and HDAC2 IPs recovered a large network of proteins associated with KDM1 (36). Based on reciprocal 3N analysis, KDM1-containing complexes can be stratified into several cores that share 15 proteins, including a previously unidentified subunit SAMD1. Several components—RCOR2, ZMYM2/3, RREB1, ZNF217, and ZNF516—are copresent with several, but not all, KDM1 interactors under the same 3N constraints (Fig. 4*A*). Thus, it is likely that KDM1 also resides in heterogeneous protein complexes, alike to HDAC1/2.

BRD7-containing SWI/SNF-interacting complex is observed reproducibly in the HDAC1/2 network. We also noticed the persistence of PBRM1 in the HDAC1/2 3N network. It exhibits good angle-based proximity with HDACs and CHD4, but the SPCs for PBRM1 are low in all HDAC-containing experiments, suggesting that PBRM1 is not a core component of the HDAC complex, but rather, it exists in its own HDAC-interacting complex. Indeed, PBRM1 3N analysis identified BRD7, ARID2, and PHF10, as well as the SWI/SNF complex as the closest interactors of PBRM1 (Fig. 4*B* and Table S6). Consistent with these data, BRD7 and ARID2 were recently shown to be a part of PBAF complex (37, 38). The composition of the PBRM1 complex and SWI/SNF complexes is defined by other experiments in our dataset which contain higher levels of these respective complexes than the HDAC1/2 experiments. Our data suggest that BRD7, ARID2, PBRM1, and PHF10 form a distinct four-subunit module; and SWI/SNF proteins form a strong multisubunit core aside from PBRM1, although PBRM1-containing IPs almost always contain SWI/SNF.

We would like to note here that none of BRD7 complex subunits were actually targeted as antigens in our IP/MS effort. This complex core is defined solely based on intercomplex interaction data and 3N analysis. These results, together with the assignments of CHD4, SIN3A, and KDM1 complexes, illustrate the ability of our data analysis schema to extract core complex information with high accuracy and to identify previously unidentified interactors in an unbiased way.

Discussion

In this study, we report a previously unidentified workflow for identification of endogenous human protein complexes. This workflow addresses and resolves major issues associated with large-scale antibody affinity-based complex purifications, namely, (*i*) reliable stratification of specific and nonspecific interactions, (*ii*) variable cross-reactivity of primary antibodies, and (*iii*) requirement for multiple repeat IPs.

We have approached these issues by (*i*) optimizing IP/MS protocols for better protein complex coverage and (*ii*) developing computational data analysis tools that provide flexible interrogation of IP/MS data for dissection of protein complexes. Our optimized IP/MS workflow allows preservation of weak interactions and thus maximizes deep proteome coverage resulting in identification of less abundant peripheral and regulatory protein complex components. For this purpose, we identified and fulfilled

three major requirements: (*i*) high-quality subcellular fractionation to enrich protein complexes, (*ii*) a uniform IP/MS protocol that preserves weak affinities, and (*iii*) matching resolving power of SDS/PAGE with LC-MS/MS. For data analysis, we used a protein-centered, antigen-independent core complex assignment algorithm that maximizes information output from IP/MS datasets with inherently uneven coverage and enables building endogenous protein complex networks, while eliminating the impact of two major sources of false interaction assignments: nonspecific and cross-reacting proteins.

We found that the major contributor to sporadic nonspecific identifications is the precipitate that forms during primary antibody incubation. Adding an ultracentrifugation step and sacrificing a substantial fraction of extract proximal to the pellet allows successful avoidance these protein aggregates. We measured the approximate composition of the input and precipitates. Proteins that are exceptionally enriched in the IP, as compared to the precipitate composition, are deemed true interactors.

Furthermore, we have instituted an E_{cutoff} filter that examines SPC distribution for each protein across all IPs and allows us to calculate SPC enrichment threshold for each protein. This filter preserves frequent proteins that appear at low levels across multiple IPs but may be greatly enriched in other experiments. This filter provides an improvement to cutoffs where judgment of protein specificity is based solely on frequency of protein occurrence in a dataset. To our knowledge, this is a previously undescribed in-depth study of origins of nonspecificity in IP/MS experiments; this work also establishes a basis for more just stratification of specific and nonspecific components.

Using the aforementioned specificity filters, we were able to use our IP/MS data for unbiased interrogation of core complexes and intercomplex interactions. In deriving core protein complex modules, the underlining premise is that true complex components should cooccur in the IPs, especially in cases when at least one of the subunits is abundant. Based on this assumption and empirical observations, we found it necessary to impose three types of constraints in our data analysis: (*i*) choosing a subgroup of IPs (5–15) where complex components are present at highest levels, (*ii*) emphasizing reciprocity by requiring co-occurrence in multiple IPs against different antigens rather than simple repeat experiments, and (*iii*) limiting true interactors by their proximity to the protein of interest, based on angles between corresponding SPC distribution vectors across selected IPs.

Although SPCs are semiquantitative at best as a measure for protein abundance, when compared across multiple IPs, they can be used effectively for correlation analysis, returning multiple known interactions with high accuracy. Although exact angle values alone cannot be used to imply accurate order between subunits of the complex, it is generally true that smaller angles suggest potential direct binders, and that smaller angle neighbors have a better chance to reside in the same complex.

Importantly, because our data selection process does not use information about intended antigens, potential false identifications resulting from antibody cross-reactivity are eliminated during analysis. Thus, we are able to take advantage of any antibody that has an affinity to a protein, targeted as well as nontargeted. As shown for MED12 analysis, TOP3A and QKI antibodies cross-react to different components of the Mediator, and these results aided our assignment of Mediator core. Additional benefit from this workflow is antibody cross-reactivity characterization, which has tremendous value for the scientific community.

To define protein complexes, it is not necessary to target complex subunits directly. Here, the SIN3A module is refined through comparison of >30 experiments, whereas only three of them were actually targeting the SIN3A complex. In a more drastic example, the PBRM1/BRD7 complex was never targeted, yet it was easily assigned based on intercomplex data alone. It is clear that, in the pursuit of an endogenous complexome, exhaustive targeting of all other subunits of SIN3A or PBRM1/BRD7 complexes will not be as beneficial as targeting some other proteins where coverage density lacks. Consequently, the presumed inability of obtaining antibodies to some proteins of interest ceases to be an issue, because they may be recovered indirectly, as shown in multiple examples here.

Together, the experimental improvements, data filtering, and analysis constraints described in this work comprise a major methodological breakthrough in antibody affinity purification of endogenous protein complexes. After filtering of nonspecific and cross-reacting contaminants, a typical immunocomplex identified in our purifications can be viewed as an extended interaction network with a central stable core (often seen in biochemical purifications in the past) and a multitude of peripheral components that interact with the core subunits transiently and/or weakly. Our custom data analysis schema allows dissection of these two classes of protein network components. At the next level of complexity, by comparing co-occurrence between core modules, we were able to initiate depiction of intercomplex relationships. Ultimately, incorporation of more diverse IP/MS experiments in such analyses can lead to a complete coverage of the endogenous human proteome with defined core complexes for all proteins and interaction networks among them. Because we demonstrate the feasibility of this approach using transcriptional regulatory proteins, which are in moderate abundance, we believe that our approach is applicable for most of the regulatory proteins in the cell. Therefore, the workflow protocol and data described here set the stage for an unbiased high-throughput endogenous complexome characterization, thereby benefiting the biological research community as a whole.

Materials and Methods

Cell Culture and Nuclear Extraction. HeLa S3 were cultured in suspension in RPMI-1640 media with 5% FBS. Cultures were grown to a final density of 0.5×10^6 cells/mL; a 20 L culture was raised for each nuclear extraction preparation. Nuclear extraction was carried out as previously described (20).

Immunoprecipitation. Immunoprecipitation protocol is discussed in detail in *Results*. Antibodies that are relevant to data in this publication are listed in Table S7.

SDS/PAGE and Mass Spectrometry. IPs were resolved on 4–20% precast Novex Tris-Glycine gels to half-length. Gels were minimally stained with Coomassie brilliant blue to differentiate IgG bands. Each lane was then cut into 10 molecular weight regions and a heavy chain band. These bands were digested with 100 ng of trypsin overnight, extracted twice with 100% acetonitrile, and dried in a Savant Speed-Vac. Peptides were then resuspended in 5% methanol and loaded onto a BioBasic C18 column. Thermo-Finnigan LC/LC-ESI-LTQ was run in a data-dependent mode, where each sample was eluted in a 35-min 0–80% acetonitrile gradient, and each full mass scan was followed by 15 MS/MS scans of most abundant ions. Spectral data were then searched against human protein RefSeq database with SeQuest software.

Multiconcensus result files of protein accession (GI) identifiers were compiled for each IP with the following filters: xCorr/*z* of 1.5 ($z = 1$), 1.8 ($z = 2$), and 2.5 ($z = 3$), peptide probability of 0.01, protein probability of 0.001, and minimum protein xCorr score of 10.0. All protein identifications were thereafter manually verified.

IP/MS Database and Software Design. IP/MS results were imported into a custom-built FileMaker-based database where protein GIs were converted to the GeneID identifiers according to the National Center for Biotechnology Information "gene2accession" table. Data filtering and clustering was performed as described in *Results* and *SI Text*.

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Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
1	GAPDH	2597	**ILP**	113	181	**294**	9	6	8	10	**33**	**12**
2	ENO1	2023	**ILP**	89	205	**294**	1	1	3	4	**9**	**10**
3	HSP90AA1	3320	**ILP**	143	106	**249**	6	13	10	11	**40**	**5**
4	PKM2	5315	**ILP**	58	132	**190**				2	**2**	**5**
5	EEF1A2	1917	**ILP**	154	31	**185**	2	4		9	**15**	**7**
6	PRDX1	5052	**ILP**	154	31	**185**	4	1	2	3	**10**	**1**
7	LDHA	3939	**ILP**	116	62	**178**				1	**1**	**2**
8	HSP90AB1	3326	**ILP**	82	88	**170**	4	3	6	10	**23**	**2**
9	PRKDC	5591	**ILP**	87	64	**151**	41	9	16	44	**110**	**11**
10	HSPA8	3312	**ILP**	78	58	**136**	17	8	11	18	**54**	**15**
11	PRDX2	7001	**ILP**	97	28	**125**	2	1	2	6	**11**	**4**
12	HSPA5	3309	**ILP**	56	67	**123**	20	25	18	34	**97**	**14**
13	TKT	7086	**ILP**	46	77	**123**				3	**3**	**4**
14	TPI1	7167	**ILP**	43	77	**120**			2	3	**5**	**3**
15	HSPA1B	3304	**ILP**	63	46	**109**	6	7	7	9	**29**	**6**
16	PGK1	5230	**ILP**	44	65	**109**			2		**2**	**7**
17	DYNC1H1	1778	**ILP**	92	16	**108**				22	**22**	**2**
18	GPI	2821	**ILP**	18	82	**100**			1	5	**6**	**4**
19	FASN	2194	**ILP**	38	62	**100**				5	**5**	**3**
20	HSP90B1	7184	**ILP**	37	59	**96**	3	4	6	4	**17**	**2**
21	ALDOA	226	**ILP**	42	50	**92**				5	**5**	**2**
22	ACTC1	70	**ILP**	34	52	**86**	24	17	14	40	**95**	**9**
23	NUMA1	4926	**ILP**	21	59	**80**	24	6	5	85	**120**	**20**
24	TUBA1B	10376	**ILP**	31	49	**80**	4	6	3	90	**103**	**6**
25	ACTB	60	**ILP**	29	51	**80**	11	12	10	54	**87**	**14**
26	ANXA2	302	**ILP**	19	58	**77**	18	8	9	32	**67**	**2**
27	TUBA3E	112714	**ILP**	22	52	**74**	16	16	16	125	**173**	**50**
28	RPS27A	6233	**ILP**	22	50	**72**	10	12	9	11	**42**	**5**
29	PARP1	142	**ILP**	27	42	**69**		2	4	7	**13**	**5**
30	TCOF1	6949	**ILP**	36	28	**64**	4		1	15	**20**	**4**
31	TUBB	203068	**ILP**	13	50	**63**	29	16	16	141	**202**	**36**
32	NCL	4691	**ILP**	28	33	**61**	5			14	**19**	**4**
33	TRAP1	10131	**ILP**	38	22	**60**		1	1	2	**4**	**2**
34	TUBB4	10382	**ILP**	17	41	**58**	11	8	8	45	**72**	**10**
35	HNRNPK	3190	**ILP**	26	31	**57**	13	9	9	15	**46**	**20**
36	NPM1	4869	**ILP**	8	48	**56**	17	6	3	90	**116**	**20**
37	GTF2I	2969	**ILP**	21	33	**54**				2	**2**	**1**
38	ASCC3L1	23020	**ILP**	18	35	**53**	21	7	8	37	**73**	**22**
39	LDHB	3945	**ILP**	31	22	**53**				2	**2**	**1**
40	GCN1L1	10985	**ILP**	16	36	**52**				10	**10**	**1**
41	LMNA	4000	**ILP**	24	27	**51**			2	1	**3**	**4**
42	SF3B3	23450	**ILP**	22	28	**50**	7	4	2	26	**39**	**18**
43	CLTC	1213	**ILP**	16	34	**50**	12	1	1	8	**22**	**5**
44	HSPB1	3315	**ILP**	23	22	**45**	6	3	4	22	**35**	**16**
45	PPIB	5479	**ILP**	32	13	**45**	5		3	4	**12**	**5**
46	PPIA	5478	**ILP**		45	**45**	5		2	4	**11**	**7**
47	PRPF8	10594	**ILP**	12	30	**42**	16	5	1	44	**66**	**22**
48	HNRNPA2B1	3181	**ILP**	16	25	**41**	37	16	16	95	**164**	**49**
49	SFPQ	6421	**ILP**	22	19	**41**	3	7	2	18	**30**	**2**
50	ALDOC	230	**ILP**	17	20	**37**				2	**2**	**2**
51	HNRNPA1	3178	**ILP**	2	34	**36**	41	17	11	85	**154**	**40**
52	PTBP1	5725	**ILP**	11	22	**33**	7	6		11	**24**	**7**
53	EEF1A1	1915	**ILP**	11	22	**33**	5	2	2	1	**10**	**2**
54	SF3B1	23451	**ILP**	15	17	**32**	7	6	4	25	**42**	**4**
55	PDIA6	10130	**ILP**	20	12	**32**	3		3	3	**9**	**2**
56	P4HB	5034	**ILP**	6	26	**32**	4	2		2	**8**	**2**
57	EIF4A2	1974	**ILP**	22	10	**32**	2	2	1		**5**	**1**
58	TOP2A	7153	**ILP**	5	26	**31**	21	12	9	39	**81**	**18**
59	RPS9	6203	**ILP**	29	1	**30**	2			10	**12**	**9**
60	DSP	1832	**ILP**	11	18	**29**	24	24	11	25	**84**	**6**
61	DDX46	9879	**ILP**	11	18	**29**				7	**7**	**9**
62	EEF1G	1937	**ILP**	21	8	**29**				3	**3**	**1**
63	SNRPE	6635	**ILP**	18	10	**28**	11	4	4	14	**33**	**8**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
64	KPNB1	3837	**ILP**	10	18	**28**	1	1		7	**9**	**1**
65	AHNAK	79026	**ILP**	18	9	**27**	15	5	6	75	**101**	**2**
66	RP11-631M21.2	347688	**ILP**	11	16	**27**	13	8	6	41	**68**	**28**
67	HNRNPF	3185	**ILP**	7	20	**27**	5		3	55	**63**	**11**
68	PHGDH	26227	**ILP**	16	11	**27**	1				**1**	**2**
69	VIM	7431	**ILP**	12	13	**25**	33	30	19	37	**119**	**15**
70	DDX39	10212	**ILP**	12	13	**25**				2	**2**	**2**
71	XRCC6	2547	**ILP**	3	21	**24**	10	5		5	**20**	**3**
72	SF3A1	10291	**ILP**	11	13	**24**	2	2	1	9	**14**	**8**
73	NONO	4841	**ILP**	6	16	**22**	6	6	2	10	**24**	**4**
74	EFTUD2	9343	**ILP**	4	17	**21**	3	1	1	17	**22**	**11**
75	SND1	27044	**ILP**	9	12	**21**	1		1	5	**7**	**1**
76	DDX17	10521	**ILP**	6	14	**20**	14	14	11	7	**46**	**6**
77	MATR3	9782	**ILP**	5	14	**19**	12	11	10	44	**77**	**18**
78	DHX15	1665	**ILP**	13	6	**19**	7	5		14	**26**	**10**
79	HNRNPU	3192	**ILP**	4	14	**18**	15	13	9	43	**80**	**42**
80	SF3B2	10992	**ILP**	6	12	**18**				8	**8**	**7**
81	HCFC1	3054	**ILP**	4	14	**18**				2	**2**	**1**
82	SNRPD1	6632	**ILP**	9	8	**17**	2	2	2	5	**11**	**1**
83	FTH1	2495	**ILP**	10	7	**17**	3			1	**4**	**1**
84	EEF1D	1936	**ILP**	7	10	**17**	2				**2**	**2**
85	NACA	4666	**ILP**	10	6	**16**	2	4	1	3	**10**	**6**
86	SNRPN	6638	**ILP**	10	6	**16**	5	2	2		**9**	**2**
87	COPA	1314	**ILP**	6	10	**16**				1	**1**	**1**
88	PCBP2	5094	**ILP**	7	8	**15**	2		1	6	**9**	**2**
89	PABPC1	26986	**ILP**	8	7	**15**	3	2		3	**8**	**5**
90	PDCD6	10016	**ILP**	2	13	**15**	2				**2**	**5**
91	CCT5	22948	**ILP**	3	12	**15**		1			**1**	**1**
92	HNRNPM	4670	**ILP**	5	9	**14**	23	12	12	20	**67**	**25**
93	RUVBL2	10856	**ILP**	4	10	**14**	3	4	4	10	**21**	**4**
94	EIF4A1	1973	**ILP**	8	6	**14**				3	**3**	**3**
95	EIF4A3	9775	**ILP**	7	6	**13**	7	4	3	11	**25**	**14**
96	RPS16	6217	**ILP**	11	2	**13**	4	3	4	8	**19**	**9**
97	CCT7	10574	**ILP**	1	12	**13**				1	**1**	**1**
98	DHX9	1660	**ILP**	5	7	**12**	9	6	2	41	**58**	**33**
99	SNRPD2	6633	**ILP**	6	6	**12**	3	2	1	11	**17**	**9**
100	PA2G4	5036	**ILP**	5	7	**12**	5	4	3	2	**14**	**3**
101	SNRPA	6626	**ILP**	5	7	**12**	4	2	3	3	**12**	**2**
102	RUVBL1	8607	**ILP**	6	6	**12**	2	3		4	**9**	**4**
103	HNRNPR	10236	**ILP**	3	8	**11**	21	11	4	30	**66**	**32**
104	RPS3	6188	**ILP**	5	6	**11**	10	6	5	16	**37**	**10**
105	HNRNPD	3184	**ILP**	6	5	**11**	4		3	9	**16**	**5**
106	DEK	7913	**ILP**	4	7	**11**	3	1		3	**7**	**2**
107	M6PRBP1	10226	**ILP**	3	8	**11**	2	2	2		**6**	**4**
108	TPD52L2	7165	**ILP**	4	7	**11**	2	3	1		**6**	**2**
109	SYNCRIP	10492	**ILP**	2	8	**10**	13	5	3	11	**32**	**10**
110	TOP2B	7155	**ILP**		10	**10**	4	4	4	13	**25**	**1**
111	TUBB2C	10383	**ILP**	3	7	**10**	2	2	2	16	**22**	**7**
112	RPS19	6223	**ILP**	8	2	**10**	4	3	2	8	**17**	**9**
113	SNRPA1	6627	**ILP**	4	6	**10**	4	3		4	**11**	**2**
114	CLTCL1	8218	**ILP**		10	**10**	3	2		4	**9**	**5**
115	YWHAG	7532	**ILP**	8	2	**10**	1			3	**4**	**2**
116	EIF3B	8662	**ILP**	2	8	**10**				1	**1**	**3**
117	HNRNPH2	3188	**ILP**		9	**9**	6	3	3	25	**37**	**24**
118	NUDT21	11051	**ILP**	5	4	**9**	3	3	3	16	**25**	**7**
119	RPS13	6207	**ILP**	6	3	**9**	3	2		11	**16**	**9**
120	HIST4H4	121504	**ILP**	5	4	**9**	11			5	**16**	**8**
121	MYH9	4627	**ILP**		8	**8**	13	6	5	18	**42**	**1**
122	RP11-556K13.1	730029	**ILP**	5	3	**8**	7	5	6	16	**34**	**15**
123	DDX5	1655	**ILP**	5	3	**8**	9	7	4	7	**27**	**8**
124	HIST1H2BI	8346	**ILP**	5	3	**8**	4	2	3	18	**27**	**3**
125	RPS14	6208	**ILP**	5	3	**8**	7	4	3	7	**21**	**9**
126	RPS7	6201	**ILP**	6	2	**8**	2	3	1	12	**18**	**3**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
127	SERBP1	26135	**ILP**	2	6	**8**	4	6	2	5	**17**	**4**
128	CPSF6	11052	**ILP**	4	4	**8**	3	3	2	8	**16**	**4**
129	RPS8	6202	**ILP**	7	1	**8**	2	3	3	7	**15**	**9**
130	SNRPD3	6634	**ILP**	5	3	**8**	2		2	6	**10**	**8**
131	SF1	7536	**ILP**		8	**8**	2			3	**5**	**2**
132	HIST1H1A	3024	**ILP**	5	3	**8**				4	**4**	**2**
133	RFC4	5984	**ILP**	6	2	**8**				4	**4**	**2**
134	USP7	7874	**ILP**		8	**8**				2	**2**	**1**
135	API5	8539	**ILP**	2	6	**8**	1				**1**	**1**
136	HNRNPL	3191	**ILP**		7	**7**	13	7	6	14	**40**	**7**
137	GNB2L1	10399	**ILP**	4	3	**7**	9	5		3	**17**	**6**
138	MDN1	23195	**ILP**	7		**7**				17	**17**	**4**
139	THOC4	10189	**ILP**	2	5	**7**	3	2	2	7	**14**	**8**
140	RPL11	6135	**ILP**	7		**7**	2	1	2	5	**10**	**5**
141	ARS2	51593	**ILP**		7	**7**	3	2	1	2	**8**	**1**
142	USP39	10713	**ILP**	1	6	**7**	3			1	**4**	**3**
143	ABCF1	23	**ILP**	2	5	**7**				2	**2**	**2**
144	ILF3	3609	**ILP**		6	**6**	5	6	6	14	**31**	**7**
145	HNRNPAB	3182	**ILP**	2	4	**6**	8	7	3	9	**27**	**11**
146	RPL18	6141	**ILP**	4	2	**6**	1		2	22	**25**	**10**
147	RPLP0	6175	**ILP**	4	2	**6**	3	6	5	11	**25**	**8**
148	RBM25	58517	**ILP**	3	3	**6**	3	3	2	7	**15**	**8**
149	RPS25	6230	**ILP**	6		**6**				7	**7**	**3**
150	HIST1H1D	3007	**ILP**	5	1	**6**	2			4	**6**	**4**
151	EIF6	3692	**ILP**		6	**6**	2	2		1	**5**	**1**
152	NAP1L1	4673	**ILP**	2	4	**6**				1	**1**	**1**
153	SRRM2	23524	**ILP**	5		**5**	11	1	5	36	**53**	**41**
154	ELAVL1	1994	**ILP**	2	3	**5**	14	4	5	19	**42**	**21**
155	TARDBP	23435	**ILP**	1	4	**5**	8	11	7	3	**29**	**6**
156	DDX21	9188	**ILP**	3	2	**5**	1		1	22	**24**	**14**
157	RPL22	6146	**ILP**	3	2	**5**	4	5	2	9	**20**	**8**
158	PUF60	22827	**ILP**		5	**5**	4	4	4	5	**17**	**7**
159	TUBB6	84617	**ILP**		5	**5**		2		15	**17**	**6**
160	RPL12	6136	**ILP**	2	3	**5**	3		1	12	**16**	**8**
161	RPL35	11224	**ILP**	4	1	**5**				6	**6**	**8**
162	RPL38	6169	**ILP**	3	2	**5**			1	4	**5**	**5**
163	LSM2	57819	**ILP**	3	2	**5**				2	**2**	**1**
164	RPLP1	6176	**ILP**	3	1	**4**	4	3	3	14	**24**	**1**
165	RAB7A	7879	**ILP**	1	3	**4**	5	2	4	5	**16**	**2**
166	SRP14	6727	**ILP**	1	3	**4**	3	2	2	5	**12**	**3**
167	CDC5L	988	**ILP**		4	**4**	3	1		7	**11**	**5**
168	U2AF2	11338	**ILP**		4	**4**	2		1	4	**7**	**3**
169	RPS4X	6191	**ILP**	4		**4**	1			6	**7**	**2**
170	RCN1	5954	**ILP**		4	**4**	2			5	**7**	**2**
171	CALM3	808	**ILP**		4	**4**	1			6	**7**	**1**
172	KHDRBS1	10657	**ILP**	2	2	**4**				4	**4**	**3**
173	FUBP3	8939	**ILP**	4		**4**	2			1	**3**	**2**
174	DDX3Y	8653	**ILP**		4	**4**				3	**3**	**1**
175	RPS11	6205	**ILP**	4		**4**				2	**2**	**2**
176	DDX3X	1654	**ILP**	4		**4**	1			1	**2**	**1**
177	ACIN1	22985	**ILP**		3	**3**	12		1	21	**34**	**24**
178	HNRNPA3	220988	**ILP**		3	**3**	12	6	5	11	**34**	**10**
179	HIST1H2AE	3012	**ILP**	2	1	**3**	7	4	1	11	**23**	**5**
180	RPS18	6222	**ILP**		3	**3**		1	1	12	**14**	**7**
181	RPS10	6204	**ILP**		3	**3**	3	3	1	6	**13**	**4**
182	RBM10	8241	**ILP**		3	**3**	2			4	**6**	**3**
183	MAGOHB	55110	**ILP**	2	1	**3**	1			3	**4**	**1**
184	SART1	9092	**ILP**	3		**3**				3	**3**	**1**
185	SEC31A	22872	**ILP**		3	**3**	1				**1**	**2**
186	LUC7L	55692	**ILP**	1	2	**3**				1	**1**	**1**
187	HNRNPC	3183	**ILP**		2	**2**	27	16	5	53	**101**	**74**
188	RPLP2	6181	**ILP**		2	**2**	9	11	5	23	**48**	**9**
189	HNRNPH3	3189	**ILP**		2	**2**	14	7	4	22	**47**	**8**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
190	ILF2	3608	**ILP**		2	**2**	9	4	8	12	**33**	**18**
191	SFRS7	6432	**ILP**		2	**2**	11	4	1	9	**25**	**6**
192	RPL31	6160	**ILP**	2		**2**	4	2	2	14	**22**	**6**
193	SEC22B	9554	**ILP**		2	**2**	7	3	4	7	**21**	**3**
194	RPL10	6134	**ILP**		2	**2**	8	2		6	**16**	**2**
195	TOP1	7150	**ILP**		2	**2**	4	1		8	**13**	**6**
196	RPL7A	6130	**ILP**		2	**2**	3			8	**11**	**9**
197	HNRNPH1	3187	**ILP**		2	**2**	4		2	5	**11**	**2**
198	PRPF19	27339	**ILP**		2	**2**	3	2		5	**10**	**4**
199	HNRNPA0	10949	**ILP**		2	**2**	1			9	**10**	**3**
200	RPL24	6152	**ILP**	2		**2**	2	2		5	**9**	**2**
201	RPL23	9349	**ILP**	1	1	**2**	1	2	1	3	**7**	**3**
202	SFRS2	6427	**ILP**		2	**2**	2			5	**7**	**3**
203	HSPA6	3310	**ILP**		2	**2**	2	2	1	2	**7**	**1**
204	DDX23	9416	**ILP**		2	**2**				7	**7**	**1**
205	WDR57	9410	**ILP**		2	**2**	3			3	**6**	**3**
206	RPL27A	6157	**ILP**	2		**2**			1	5	**6**	**2**
207	RPS2	6187	**ILP**		2	**2**	3			2	**5**	**5**
208	RPL19	6143	**ILP**	2		**2**				5	**5**	**4**
209	EIF3CL	728689	**ILP**		2	**2**			1	4	**5**	**1**
210	U2AF1	7307	**ILP**		2	**2**				4	**4**	**2**
211	EIF3F	8665	**ILP**		2	**2**	2			2	**4**	**2**
212	NOLA3	55505	**ILP**		2	**2**	2	1			**3**	**2**
213	CPSF1	29894	**ILP**		2	**2**	1			2	**3**	**1**
214	RPL29	6159	**ILP**	2		**2**				2	**2**	**2**
215	RAP1B	5908	**ILP**		2	**2**				2	**2**	**1**
216	SF3A3	10946	**ILP**		2	**2**				1	**1**	**2**
217	DKC1	1736	**ILP**		2	**2**				1	**1**	**2**
218	FNBP4	23360	**ILP**		2	**2**				1	**1**	**2**
219	CBX3	11335	**ILP**		2	**2**				1	**1**	**1**
220	HRNR	388697	**ILP**		1	**1**	24	27	8	2	**61**	**6**
221	DSG1	1828	**ILP**		1	**1**	14	19	22		**55**	**7**
222	ADAR	103	**ILP**		1	**1**	4	5	4	7	**20**	**10**
223	RPL21	6144	**ILP**		1	**1**	5	1	1	9	**16**	**7**
224	PRPF40A	55660	**ILP**		1	**1**	4		1	10	**15**	**2**
225	SFRS3	6428	**ILP**	1		**1**		2		12	**14**	**8**
226	HNRPDL	9987	**ILP**		1	**1**	5	2	2	3	**12**	**3**
227	RPL10A	4736	**ILP**		1	**1**		1	2	6	**9**	**5**
228	SAFB	6294	**ILP**		1	**1**	4			5	**9**	**2**
229	RPS23	6228	**ILP**		1	**1**	1	1	2	4	**8**	**3**
230	RPL14	9045	**ILP**	1		**1**	2			6	**8**	**3**
231	RPS28	6234	**ILP**		1	**1**	2	3	1	2	**8**	**2**
232	RPL4	6124	**ILP**	1		**1**				7	**7**	**6**
233	RPL9	6133	**ILP**		1	**1**	1		1	5	**7**	**4**
234	HNRNPUL2	221092	**ILP**		1	**1**	2		2	3	**7**	**3**
235	RPL13	6137	**ILP**	1		**1**				6	**6**	**7**
236	RFC1	5981	**ILP**		1	**1**				6	**6**	**1**
237	RPL8	6132	**ILP**	1		**1**	2			3	**5**	**5**
238	SFRS2IP	9169	**ILP**		1	**1**	2			3	**5**	**3**
239	AQR	9716	**ILP**		1	**1**	2			3	**5**	**2**
240	RPS17	6218	**ILP**		1	**1**		1		3	**4**	**2**
241	ATL3	25923	**ILP**		1	**1**	2			2	**4**	**2**
242	RFC2	5982	**ILP**	1		**1**			1	3	**4**	**1**
243	C1orf57	84284	**ILP**	1		**1**				4	**4**	**1**
244	RBM39	9584	**ILP**		1	**1**				3	**3**	**5**
245	NOLA2	55651	**ILP**		1	**1**				2	**2**	**3**
246	FLJ12529	79869	**ILP**		1	**1**		1		1	**2**	**3**
247	RPL28	6158	**ILP**		1	**1**				1	**1**	**4**
248	SFRS11	9295	**ILP**		1	**1**				1	**1**	**3**
249	PWP2	5822	**ILP**		1	**1**				1	**1**	**1**
250	ENO3	2027	**IL**	46	70	**116**				4	**4**	
251	GAPDHL6	729403	**IL**	21	82	**103**	1			5	**6**	
252	ANXA1	301	**IL**	37	56	**93**	1	4	1		**6**	

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
253	IQGAP1	8826	**IL**	25	45	**70**	2			5	**7**	
254	RAN	5901	**IL**	39	25	**64**		1		1	**2**	
255	GANAB	23193	**IL**	20	39	**59**			2	2	**4**	
256	EPRS	2058	**IL**	17	32	**49**		1		1	**2**	
257	KHSRP	8570	**IL**	25	21	**46**				1	**1**	
258	SPTBN1	6711	**IL**	9	36	**45**				2	**2**	
259	TXN	7295	**IL**	18	21	**39**		2	1		**3**	
260	EIF5AL1	143244	**IL**	33	6	**39**				1	**1**	
261	MSH6	2956	**IL**	12	25	**37**				5	**5**	
262	YWHAZ	7534	**IL**	17	17	**34**				1	**1**	
263	ARF1	375	**IL**	19	14	**33**	1			1	**2**	
264	SPTAN1	6709	**IL**	13	19	**32**			1		**1**	
265	PDIA3	2923	**IL**	6	25	**31**	6	5	5	3	**19**	
266	SMC1A	8243	**IL**	14	16	**30**	2	1		1	**4**	
267	IARS	3376	**IL**	12	18	**30**				1	**1**	
268	VCP	7415	**IL**	8	15	**23**		2		3	**5**	
269	MSN	4478	**IL**	8	14	**22**				3	**3**	
270	YWHAQ	10971	**IL**	10	12	**22**		1			**1**	
271	LARS	51520	**IL**	4	16	**20**				1	**1**	
272	CSE1L	1434	**IL**	6	13	**19**				6	**6**	
273	SNHG3-RCC1	751867	**IL**	11	8	**19**		1			**1**	
274	XRCC5	7520	**IL**	6	11	**17**	3		3	7	**13**	
275	HSPA1L	3305	**IL**	13	4	**17**	1	1	1		**3**	
276	TRIM28	10155	**IL**	9	7	**16**				3	**3**	
277	CCT2	10576	**IL**	3	13	**16**	1			1	**2**	
278	PRKCSH	5589	**IL**	1	14	**15**		2	2		**4**	
279	XPO1	7514	**IL**		15	**15**				2	**2**	
280	YWHAB	7529	**IL**	8	7	**15**				1	**1**	
281	GMPS	8833	**IL**	4	11	**15**				1	**1**	
282	EIF3A	8661	**IL**		14	**14**				5	**5**	
283	SMARCA4	6597	**IL**	3	11	**14**				2	**2**	
284	EIF5B	9669	**IL**	3	11	**14**				1	**1**	
285	SNRPB2	6629	**IL**	8	5	**13**	6	1		1	**8**	
286	FTL	2512	**IL**	8	5	**13**		1	2	3	**6**	
287	UBE2N	7334	**IL**	9	4	**13**				1	**1**	
288	HSPA9	3313	**IL**	2	11	**13**				1	**1**	
289	EZR	7430	**IL**	7	5	**12**			2	6	**8**	
290	NOLC1	9221	**IL**	9	3	**12**				7	**7**	
291	RPA1	6117	**IL**	5	7	**12**				1	**1**	
292	LGALS3	3958	**IL**	6	5	**11**				3	**3**	
293	SMARCC2	6601	**IL**	2	9	**11**				3	**3**	
294	MYL6	4637	**IL**	6	4	**10**	3		2	5	**10**	
295	SFRS15	57466	**IL**	6	4	**10**				6	**6**	
296	PLEC1	5339	**IL**	5	4	**9**				8	**8**	
297	RANBP2	5903	**IL**	1	8	**9**				7	**7**	
298	XPO5	57510	**IL**		9	**9**				5	**5**	
299	CALR	811	**IL**		9	**9**		3	1		**4**	
300	APEX1	328	**IL**	3	6	**9**				1	**1**	
301	DNM2	1785	**IL**		9	**9**				1	**1**	
302	CRB1	23418	**IL**		8	**8**			24		**24**	
303	RIF1	55183	**IL**	5	3	**8**			1	6	**7**	
304	EIF2S1	1965	**IL**	3	5	**8**		1		1	**2**	
305	BUB3	9184	**IL**	2	6	**8**				2	**2**	
306	ACTL6A	86	**IL**	4	4	**8**				1	**1**	
307	VAT1	10493	**IL**	3	5	**8**				1	**1**	
308	ZCCHC11	23318	**IL**		7	**7**	37		34		**71**	
309	GTF3C1	2975	**IL**	3	4	**7**				2	**2**	
310	PLOD3	8985	**IL**	2	5	**7**			1		**1**	
311	IPO5	3843	**IL**		7	**7**				1	**1**	
312	STAT3	6774	**IL**	1	5	**6**			4		**4**	
313	CENPB	1059	**IL**		5	**5**			6	1	**7**	
314	FUS	2521	**IL**	1	4	**5**				2	**2**	
315	GAPVD1	26130	**IL**		5	**5**				2	**2**	

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
316	EPB41L2	2037	**IL**		5	**5**				1	**1**	
317	TNPO3	23534	**IL**		5	**5**				1	**1**	
318	FAF1	11124	**IL**		5	**5**				1	**1**	
319	RPS6	6194	**IL**	1	3	**4**	2	1		5	**8**	
320	PCBP1	5093	**IL**		4	**4**			1	7	**8**	
321	TUBA4A	7277	**IL**	2	2	**4**	1	1	1		**3**	
322	TSR1	55720	**IL**	1	3	**4**				2	**2**	
323	PLOD1	5351	**IL**		4	**4**				1	**1**	
324	EXOSC2	23404	**IL**	4		**4**	1				**1**	
325	DARS	1615	**IL**		4	**4**		1			**1**	
326	POLR2A	5430	**IL**		4	**4**				1	**1**	
327	NUP153	9972	**IL**		4	**4**				1	**1**	
328	RTN4	57142	**IL**		3	**3**	2	2	2	4	**10**	
329	RPS15A	6210	**IL**	1	2	**3**	1	2		5	**8**	
330	RPS24	6229	**IL**		3	**3**	1	1		4	**6**	
331	RAB11A	8766	**IL**	3		**3**	1		1	3	**5**	
332	SR140	23350	**IL**	2	1	**3**	1			2	**3**	
333	SYMPK	8189	**IL**	2	1	**3**				3	**3**	
334	EIF2S3	1968	**IL**	2	1	**3**				3	**3**	
335	COPB2	9276	**IL**	2	1	**3**				3	**3**	
336	PDAP1	11333	**IL**		3	**3**			1	1	**2**	
337	PPP1CC	5501	**IL**		3	**3**				2	**2**	
338	BAT2D1	23215	**IL**	3		**3**				1	**1**	
339	ARL1	400	**IL**	1	2	**3**				1	**1**	
340	HLTF	6596	**IL**		3	**3**				1	**1**	
341	RRBP1	6238	**IL**		3	**3**				1	**1**	
342	ALPI	248	**IL**	2		**2**	27	15	11	41	**94**	
343	TMPO	7112	**IL**		2	**2**				8	**8**	
344	RPL23A	6147	**IL**	1	1	**2**	3	2	1	1	**7**	
345	SNRPG	6637	**IL**		2	**2**	2	1	1		**4**	
346	RPS27	6232	**IL**		2	**2**	2	1	1		**4**	
347	SF3B4	10262	**IL**		2	**2**	1	2		1	**4**	
348	RAB8A	4218	**IL**		2	**2**				4	**4**	
349	RTN3	10313	**IL**		2	**2**				4	**4**	
350	FAM62A	23344	**IL**		2	**2**			1	2	**3**	
351	FIS1	51024	**IL**		2	**2**			1	2	**3**	
352	TBL3	10607	**IL**		2	**2**				3	**3**	
353	RGPD3	653489	**IL**		2	**2**				2	**2**	
354	TAF15	8148	**IL**		2	**2**		2			**2**	
355	EIF3EIP	51386	**IL**		2	**2**				2	**2**	
356	S100A10	6281	**IL**		2	**2**				2	**2**	
357	RFC5	5985	**IL**	2		**2**				2	**2**	
358	RFC3	5983	**IL**		2	**2**				2	**2**	
359	RAB14	51552	**IL**		2	**2**				2	**2**	
360	SETD1A	9739	**IL**		2	**2**				1	**1**	
361	SKP1	6500	**IL**		2	**2**	1				**1**	
362	DNAJA1	3301	**IL**		2	**2**				1	**1**	
363	MAPRE1	22919	**IL**	2		**2**				1	**1**	
364	EIF3E	3646	**IL**		2	**2**		1			**1**	
365	NCAPG	64151	**IL**		2	**2**				1	**1**	
366	CANX	821	**IL**		1	**1**	9	3	3	28	**43**	
367	RPL27	6155	**IL**		1	**1**	4	3		4	**11**	
368	VAMP2	6844	**IL**		1	**1**	3	2	1	2	**8**	
369	RAB1B	81876	**IL**		1	**1**	3			5	**8**	
370	RAB5C	5878	**IL**		1	**1**	3		1	1	**5**	
371	DIDO1	11083	**IL**		1	**1**	1			4	**5**	
372	RPS20	6224	**IL**	1		**1**	3	1	1		**5**	
373	C16orf80	29105	**IL**		1	**1**	3	2			**5**	
374	LYZ	4069	**IL**		1	**1**		4	1		**5**	
375	RAB1A	5861	**IL**		1	**1**	2	2			**4**	
376	RAVER1	125950	**IL**		1	**1**				4	**4**	
377	SF3B14	51639	**IL**		1	**1**				2	**2**	
378	LGALS1	3956	**IL**		1	**1**				2	**2**	

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
379	RAB35	11021	**IL**		1	**1**				2	**2**	
380	SNRPF	6636	**IL**		1	**1**	2				**2**	
381	DHX8	1659	**IL**		1	**1**				2	**2**	
382	POLR2E	5434	**IL**		1	**1**				1	**1**	
383	HDAC1	3065	**IL**		1	**1**				1	**1**	
384	WDR82	80335	**IL**		1	**1**				1	**1**	
385	RAC1	5879	**IL**		1	**1**			1		**1**	
386	SAR1B	51128	**IL**		1	**1**				1	**1**	
387	EXOSC10	5394	**IL**		1	**1**				1	**1**	
388	RAB5A	5868	**IL**	1		**1**				1	**1**	
389	EMG1	10436	**IL**	1		**1**				1	**1**	
390	PSMD6	9861	**IL**		1	**1**		1			**1**	
391	ZNF687	57592	**IL**		1	**1**				1	**1**	
392	MYO1F	4542	**IL**		1	**1**				1	**1**	
393	BAX	581	**IL**		1	**1**				1	**1**	
394	SMU1	55234	**IL**		1	**1**				1	**1**	
395	FERMT2	10979	**IL**		1	**1**				1	**1**	
396	NKRF	55922	**IL**		1	**1**				1	**1**	
397	CHRAC1	54108	**IL**		1	**1**				1	**1**	
398	UBA1	7317	**IP**	59	83	**142**						**3**
399	FLNA	2316	**IP**	58	68	**126**						**1**
400	PFN1	5216	**IP**	43	38	**81**						**3**
401	EEF2	1938	**IP**	35	30	**65**						**5**
402	PEBP1	5037	**IP**	35	20	**55**						**1**
403	CPS1	1373	**IP**	19	34	**53**						**1**
404	PRDX6	9588	**IP**	29	18	**47**						**1**
405	TAGLN2	8407	**IP**	29	17	**46**						**1**
406	SOD1	6647	**IP**	27	7	**34**						**1**
407	PCNA	5111	**IP**	18	11	**29**						**1**
408	CLIC1	1192	**IP**	13	9	**22**						**1**
409	CCT8	10694	**IP**	12	8	**20**						**1**
410	CIP29	84324	**IP**	9	7	**16**						**1**
411	PGD	5226	**IP**	5	11	**16**						**1**
412	CCT6A	908	**IP**	3	11	**14**						**1**
413	NSUN2	54888	**IP**	2	8	**10**						**2**
414	CSRP1	1465	**IP**	5	5	**10**						**1**
415	IMPDH2	3615	**IP**	3	5	**8**						**1**
416	MTPN	136319	**IP**	3	4	**7**						**1**
417	SEPT2	4735	**IP**		6	**6**						**1**
418	RBBP4	5928	**IP**	3	2	**5**						**1**
419	SUMO4	387082	**IP**	3	1	**4**						**1**
420	QKI	9444	**IP**	2	2	**4**						**1**
421	PPIAP19	390006	**IP**		3	**3**						**1**
422	SF3A2	8175	**IP**		3	**3**						**1**
423	PYGL	5836	**IP**		2	**2**						**5**
424	C14orf166	51637	**IP**		2	**2**						**2**
425	SUPT16H	11198	**IP**		2	**2**						**2**
426	HEATR1	55127	**IP**		2	**2**						**1**
427	PRPF3	9129	**IP**		1	**1**						**2**
428	FIP1L1	81608	**IP**	1		**1**						**2**
429	HP1BP3	50809	**IP**	1		**1**						**1**
430	G3BP1	10146	**IP**		1	**1**						**1**
431	RPS21	6227	**IP**	1		**1**						**1**
432	TIMM13	26517	**IP**		1	**1**						**1**
433	DCD	117159	**LP**				27	31	36	7	**101**	**20**
434	ALB	213	**LP**					43	38	4	**85**	**43**
435	SLC3A2	6520	**LP**				11	11	14	43	**79**	**1**
436	SFRS1	6426	**LP**				15	8	4	14	**41**	**14**
437	LDLR	3949	**LP**				14	7	9	8	**38**	**2**
438	TUBB3	10381	**LP**				4		3	24	**31**	**5**
439	TFRC	7037	**LP**				6	5	6	14	**31**	**1**
440	MKI67	4288	**LP**							28	**28**	**7**
441	CSTA	1475	**LP**					12	13		**25**	**5**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
442	RBM14	10432	**LP**				12	2	2	4	**20**	**6**
443	S100A9	6280	**LP**				5	6	8	1	**20**	**5**
444	CDSN	1041	**LP**					9	10		**19**	**3**
445	RALY	22913	**LP**				4		6	8	**18**	**3**
446	RPL7	6129	**LP**				3		1	13	**17**	**6**
447	RPL6	6128	**LP**							16	**16**	**5**
448	DDX50	79009	**LP**				2		2	10	**14**	**8**
449	SFRS10	6434	**LP**				5	2		7	**14**	**2**
450	CCBL2	56267	**LP**				5	2		6	**13**	**12**
451	THOC6	79228	**LP**				8	3		2	**13**	**4**
452	MYBBP1A	10514	**LP**				1			12	**13**	**1**
453	ZFR	51663	**LP**				3			9	**12**	**4**
454	RPN1	6184	**LP**				6	1	2	2	**11**	**1**
455	SRRM1	10250	**LP**							9	**9**	**6**
456	THOC1	9984	**LP**				3	2	2	2	**9**	**3**
457	RPL15	6138	**LP**				1			8	**9**	**3**
458	PGRMC2	10424	**LP**				2	1	2	3	**8**	**1**
459	RPS3A	6189	**LP**				2	1		4	**7**	**8**
460	SFRS9	8683	**LP**				4		1	2	**7**	**7**
461	THOC2	57187	**LP**				2			5	**7**	**7**
462	RBMX	27316	**LP**				2			5	**7**	**5**
463	RPL30	6156	**LP**				3	1		3	**7**	**4**
464	YBX1	4904	**LP**				2	1	2	2	**7**	**3**
465	SCAMP3	10067	**LP**				1			6	**7**	**2**
466	PGRMC1	10857	**LP**				1		2	4	**7**	**1**
467	SFRS6	6431	**LP**							6	**6**	**9**
468	POLDIP3	84271	**LP**				2			4	**6**	**6**
469	C1orf77	26097	**LP**				3			3	**6**	**5**
470	RPSA	3921	**LP**				1	2		3	**6**	**3**
471	HMGA1	3159	**LP**				2	2		2	**6**	**2**
472	SSR4	6748	**LP**				2	2		2	**6**	**1**
473	RPL3	6122	**LP**							5	**5**	**5**
474	NOL5A	10528	**LP**				2			3	**5**	**4**
475	HNRNPUL1	11100	**LP**				1		3	1	**5**	**3**
476	M6PR	4074	**LP**				2		1	2	**5**	**2**
477	PNN	5411	**LP**					1		4	**5**	**2**
478	TMEM109	79073	**LP**				2	2	1		**5**	**1**
479	BANF1	8815	**LP**				3			2	**5**	**1**
480	SEC16A	9919	**LP**							5	**5**	**1**
481	RPS15	6209	**LP**							5	**5**	**1**
482	RBM4	5936	**LP**				1			3	**4**	**4**
483	RPL18A	6142	**LP**				2			2	**4**	**3**
484	EBNA1BP2	10969	**LP**							4	**4**	**3**
485	RPL13A	23521	**LP**							4	**4**	**2**
486	ZC3H18	124245	**LP**				3			1	**4**	**1**
487	TRA2A	29896	**LP**				1	1		2	**4**	**1**
488	ATP2A1	487	**LP**				1		1	2	**4**	**1**
489	LPCAT1	79888	**LP**							4	**4**	**1**
490	ZNF638	27332	**LP**							3	**3**	**8**
491	RPS26	6231	**LP**							3	**3**	**3**
492	PRPF4B	8899	**LP**							3	**3**	**2**
493	LUC7L2	51631	**LP**							3	**3**	**2**
494	SAFB2	9667	**LP**							3	**3**	**2**
495	H1FX	8971	**LP**				2			1	**3**	**1**
496	BXDC2	55299	**LP**				1			2	**3**	**1**
497	PABPN1	8106	**LP**					1		2	**3**	**1**
498	PLA2G4A	5321	**LP**							3	**3**	**1**
499	NCOA5	57727	**LP**				2				**2**	**7**
500	RBM15	64783	**LP**							2	**2**	**4**
501	SON	6651	**LP**							2	**2**	**3**
502	RPL34	6164	**LP**							2	**2**	**2**
503	RBM3	5935	**LP**							2	**2**	**2**
504	WBP11	51729	**LP**							2	**2**	**2**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	**Symbol**	**GeneID**	**Category**	**1**	**2**	**IN**	**1**	**2**	**3**	**4**	**LP**	**PP**
505	POR	5447	**LP**					1	1		**2**	**1**
506	LAS1L	81887	**LP**							2	**2**	**1**
507	GTF3C2	2976	**LP**							2	**2**	**1**
508	RBM17	84991	**LP**				1	1			**2**	**1**
509	LYAR	55646	**LP**							2	**2**	**1**
510	XAB2	56949	**LP**							1	**1**	**4**
511	NOL1	4839	**LP**							1	**1**	**3**
512	RPL26L1	51121	**LP**							1	**1**	**3**
513	RBM27	54439	**LP**							1	**1**	**3**
514	WDR36	134430	**LP**						1		**1**	**2**
515	NAPA	8775	**LP**						1		**1**	**2**
516	SNW1	22938	**LP**							1	**1**	**2**
517	ATP2A2	488	**LP**							1	**1**	**2**
518	C7orf50	84310	**LP**							1	**1**	**1**
519	NHP2L1	4809	**LP**				1				**1**	**1**
520	SSRP1	6749	**LP**					1			**1**	**1**
521	BAG2	9532	**LP**							1	**1**	**1**
522	NAT10	55226	**LP**							1	**1**	**1**
523	RPS12	6206	**LP**							1	**1**	**1**
524	SSR3	6747	**LP**				1				**1**	**1**
525	FKBP8	23770	**LP**						1		**1**	**1**
526	SENP3	26168	**LP**							1	**1**	**1**
527	FAM120A	23196	**LP**							1	**1**	**1**
528	FLNB	2317	**I**	47	37	**84**						
529	TPR	7175	**I**	45	37	**82**						
530	LOC729708	729708	**I**	67		**67**						
531	LOC654188	654188	**I**	66		**66**						
532	CFL1	1072	**I**	44	17	**61**						
533	ACTN4	81	**I**	21	40	**61**						
534	ANXA5	308	**I**	35	17	**52**						
535	YWHAE	7531	**I**	28	23	**51**						
536	AARS	16	**I**	14	34	**48**						
537	GARS	2617	**I**	20	21	**41**						
538	HSPA4	3308	**I**	11	28	**39**						
539	ACLY	47	**I**	16	22	**38**						
540	TARS	6897	**I**	19	18	**37**						
541	PGAM1	5223	**I**	19	17	**36**						
542	CAND1	55832	**I**	15	21	**36**						
543	FUBP1	8880	**I**	18	17	**35**						
544	TLN1	7094	**I**	9	21	**30**						
545	PDIA4	9601	**I**	7	23	**30**						
546	SERPINB1	1992	**I**	11	18	**29**						
547	NME1	4830	**I**	18	10	**28**						
548	SERPINB6	5269	**I**	12	16	**28**						
549	S100A11	6282	**I**	6	22	**28**						
550	NDRG1	10397	**I**	5	22	**27**						
551	CBR1	873	**I**	13	13	**26**						
552	PSAT1	29968	**I**	16	10	**26**						
553	VARS	7407	**I**	13	11	**24**						
554	ASS1	445	**I**	8	16	**24**						
555	MAP4	4134	**I**		24	**24**						
556	HYOU1	10525	**I**	11	12	**23**						
557	PFKP	5214	**I**	10	13	**23**						
558	HDGF	3068	**I**	6	17	**23**						
559	VCL	7414	**I**	5	18	**23**						
560	ACP1	52	**I**	12	10	**22**						
561	MTHFD1	4522	**I**	12	10	**22**						
562	CCT4	10575	**I**	10	12	**22**						
563	EIF4G1	1981	**I**	9	13	**22**						
564	PSMB5	5693	**I**	21	1	**22**						
565	SMC3	9126	**I**	6	16	**22**						
566	CDC2	983	**I**	12	10	**22**						
567	EPPK1	83481	**I**	16	5	**21**						

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
568	SET	6418	I	2	19	**21**						
569	PPA1	5464	I	8	13	**21**						
570	PRDX5	25824	I	14	6	**20**						
571	DDB1	1642	I	9	11	**20**						
572	AHCY	191	I	14	6	**20**						
573	SUB1	10923	I	10	9	**19**						
574	IPO7	10527	I	5	14	**19**						
575	PSMB2	5690	I	13	6	**19**						
576	ANXA3	306	I	15	4	**19**						
577	MDH1	4190	I	11	8	**19**						
578	ANLN	54443	I	9	10	**19**						
579	ERP29	10961	I	9	9	**18**						
580	MSH2	4436	I	7	11	**18**						
581	RAD50	10111	I	10	8	**18**						
582	HSPD1	3329	I	6	12	**18**						
583	GART	2618	I	7	11	**18**						
584	KIAA0368	23392	I	3	15	**18**						
585	GSTO1	9446	I	13	4	**17**						
586	BLVRB	645	I	9	8	**17**						
587	GDI2	2665	I	4	13	**17**						
588	NME2	4831	I	7	9	**16**						
589	KIAA1967	57805	I	5	11	**16**						
590	NQO1	1728	I	8	8	**16**						
591	MARS	4141	I	4	12	**16**						
592	hCG_1983058	644820	I	8	7	**15**						
593	PRDX4	10549	I	6	9	**15**						
594	LDHAL3	442013	I	11	4	**15**						
595	HUWE1	10075	I	13	2	**15**						
596	CKAP5	9793	I	4	11	**15**						
597	TPM3	7170	I	2	12	**14**						
598	LASP1	3927	I	7	7	**14**						
599	STIP1	10963	I	3	11	**14**						
600	NUDT5	11164	I	10	4	**14**						
601	HSPH1	10808	I	6	8	**14**						
602	G6PD	2539	I	3	11	**14**						
603	CACYBP	27101	I	5	8	**13**						
604	VPS35	55737	I	5	8	**13**						
605	CHD4	1108	I	3	10	**13**						
606	CTTN	2017	I	9	4	**13**						
607	GLO1	2739	I	7	6	**13**						
608	MCM2	4171	I	5	8	**13**						
609	EFHD2	79180	I	8	5	**13**						
610	PAICS	10606	I	8	5	**13**						
611	RARS	5917	I	7	6	**13**						
612	HDLBP	3069	I	7	6	**13**						
613	TALDO1	6888	I	7	6	**13**						
614	NUDC	10726	I	4	9	**13**						
615	FAM129B	64855	I	3	10	**13**						
616	LOC645691	645691	I	13		**13**						
617	PSMB6	5694	I	13		**13**						
618	CCT3	7203	I	4	8	**12**						
619	SEPT9	10801	I	4	8	**12**						
620	AKR1B1	231	I	7	5	**12**						
621	PDCD6IP	10015	I	6	6	**12**						
622	KARS	3735	I	2	10	**12**						
623	PTGES3	10728	I	10	2	**12**						
624	NP	4860	I	8	4	**12**						
625	CAPG	822	I	4	8	**12**						
626	FKBP4	2288	I	3	9	**12**						
627	DNMT1	1786	I	2	10	**12**						
628	TXNRD1	7296	I		12	**12**						
629	FLYWCH2	114984	I	8	3	**11**						
630	PSMB1	5689	I	4	7	**11**						

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No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
631	PSMA5	5686	I	6	5	**11**						
632	FSCN1	6624	I	3	8	**11**						
633	ARPC4	10093	I	9	2	**11**						
634	PLS3	5358	I	2	9	**11**						
635	ACACA	31	I		11	**11**						
636	HMG1L1	10357	I	5	5	**10**						
637	ANP32B	10541	I	6	4	**10**						
638	PARK7	11315	I	6	4	**10**						
639	FEN1	2237	I	5	5	**10**						
640	STMN1	3925	I	4	6	**10**						
641	ANXA4	307	I	3	7	**10**						
642	S100A6	6277	I	7	3	**10**						
643	DUT	1854	I	6	4	**10**						
644	PSMD3	5709	I	2	8	**10**						
645	DIS3	22894	I	2	8	**10**						
646	LOC100133486	100133486	I	10		**10**						
647	TCP1	6950	I		10	**10**						
648	UBE2M	9040	I	10		**10**						
649	CAD	790	I		10	**10**						
650	PSMA6	5687	I	6	3	**9**						
651	SFN	2810	I	5	4	**9**						
652	MAT2A	4144	I	4	5	**9**						
653	CSTB	1476	I	4	5	**9**						
654	ENO2	2026	I	1	8	**9**						
655	GSPT1	2935	I	6	3	**9**						
656	PDS5A	23244	I	5	4	**9**						
657	APRT	353	I	4	5	**9**						
658	SMC2	10592	I	3	6	**9**						
659	XPOT	11260	I	2	7	**9**						
660	UGCGL1	56886	I	1	8	**9**						
661	ANP32A	8125	I		9	**9**						
662	TCERG1	10915	I		9	**9**						
663	TRRAP	8295	I	9		**9**						
664	SMC4	10051	I		9	**9**						
665	EEA1	8411	I		9	**9**						
666	CYFIP1	23191	I		9	**9**						
667	PCNP	57092	I	5	3	**8**						
668	C7orf24	79017	I	5	3	**8**						
669	TRIP13	9319	I	5	3	**8**						
670	TCEB2	6923	I	4	4	**8**						
671	NNMT	4837	I	3	5	**8**						
672	SERPINH1	871	I	1	7	**8**						
673	PSMA1	5682	I		8	**8**						
674	IDH1	3417	I	5	3	**8**						
675	SUPT5H	6829	I	2	6	**8**						
676	CYCS	54205	I	1	7	**8**						
677	LOC653658	653658	I	8		**8**						
678	LOC100133665	100133665	I	8		**8**						
679	NUP214	8021	I		8	**8**						
680	PSMA2	5683	I	1	6	**7**						
681	PSMB4	5692	I	4	3	**7**						
682	EIF4H	7458	I	2	5	**7**						
683	SEPT7	989	I	2	5	**7**						
684	ACTN3	89	I	1	6	**7**						
685	PPM1G	5496	I	4	3	**7**						
686	ANP32E	81611	I	4	3	**7**						
687	MAGED2	10916	I	4	3	**7**						
688	MAP2K1	5604	I	4	3	**7**						
689	HSPE1	3336	I	3	4	**7**						
690	HINT1	3094	I	2	5	**7**						
691	MDH2	4191	I	1	6	**7**						
692	PSMB3	5691	I		7	**7**						
693	OLA1	29789	I	6	1	**7**						

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
694	MRE11A	4361	I	4	3	**7**						
695	GNPNAT1	64841	I	4	3	**7**						
696	SLC9A3R1	9368	I	3	4	**7**						
697	NQO2	4835	I	3	4	**7**						
698	C19orf10	56005	I	3	4	**7**						
699	UPF1	5976	I	2	5	**7**						
700	MCM3	4172	I	2	5	**7**						
701	STRAP	11171	I	2	5	**7**						
702	PSME2	5721	I	2	5	**7**						
703	CDK2	1017	I	2	5	**7**						
704	LOC646817	646817	I	7		**7**						
705	LOC100133951	100133951	I	7		**7**						
706	ERO1L	30001	I		7	**7**						
707	NPEPPS	9520	I		7	**7**						
708	ACTR2	10097	I		7	**7**						
709	RECQL	5965	I		7	**7**						
710	POLD1	5424	I		7	**7**						
711	HMGB2	3148	I	2	4	**6**						
712	SPR	6697	I	4	2	**6**						
713	YWHAH	7533	I	3	3	**6**						
714	TP53BP1	7158	I	4	2	**6**						
715	ST13	6767	I	2	4	**6**						
716	EIF4B	1975	I	4	2	**6**						
717	PSMD7	5713	I	3	3	**6**						
718	PRPS1	5631	I	2	4	**6**						
719	ANXA6	309	I	2	4	**6**						
720	UBE2V1	7335	I	4	2	**6**						
721	ASNS	440	I	3	3	**6**						
722	PSME1	5720	I	3	3	**6**						
723	CUL1	8454	I	3	3	**6**						
724	ZYX	7791	I	2	4	**6**						
725	MAT2B	27430	I	2	4	**6**						
726	SORD	6652	I	2	4	**6**						
727	UBE2I	7329	I	2	4	**6**						
728	CCAR1	55749	I	1	5	**6**						
729	VPS26A	9559	I	1	5	**6**						
730	DCPS	28960	I	1	5	**6**						
731	ATP6V1A	523	I	1	5	**6**						
732	TOMM34	10953	I	1	5	**6**						
733	PGLS	25796	I	6		**6**						
734	P4HA1	5033	I		6	**6**						
735	FAM49B	51571	I		6	**6**						
736	IPO9	55705	I		6	**6**						
737	CNPY2	10330	I		6	**6**						
738	CMPK1	51727	I		6	**6**						
739	PSMA8	143471	I	4	1	**5**						
740	S100P	6286	I	2	3	**5**						
741	TPD52	7163	I	2	3	**5**						
742	NCOR1	9611	I	3	2	**5**						
743	AK2	204	I		5	**5**						
744	ZNF207	7756	I	3	2	**5**						
745	PSMD11	5717	I	3	2	**5**						
746	RDX	5962	I	2	3	**5**						
747	SOD2	6648	I	2	3	**5**						
748	PSMD13	5719	I	1	4	**5**						
749	DDX42	11325	I		5	**5**						
750	DDX1	1653	I		5	**5**						
751	NASP	4678	I	4	1	**5**						
752	CDC37	11140	I	3	2	**5**						
753	BAT3	7917	I	3	2	**5**						
754	PDCD10	11235	I	3	2	**5**						
755	CDK4	1019	I	3	2	**5**						
756	MTA2	9219	I	2	3	**5**						

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
757	C20orf77	58490	I	2	3	**5**						
758	SRI	6717	I	2	3	**5**						
759	YARS	8565	I	2	3	**5**						
760	NANS	54187	I	2	3	**5**						
761	COPG	22820	I	1	4	**5**						
762	KIF5B	3799	I	1	4	**5**						
763	PDCD5	9141	I	5		**5**						
764	NAMPT	10135	I		5	**5**						
765	HMGB3	3149	I		5	**5**						
766	CUTA	51596	I		5	**5**						
767	LTA4H	4048	I		5	**5**						
768	NCAPD2	9918	I	5		**5**						
769	SRM	6723	I	5		**5**						
770	LOC100130211	100130211	I	5		**5**						
771	PSME3	10197	I		5	**5**						
772	CLIP1	6249	I		5	**5**						
773	SMCHD1	23347	I		5	**5**						
774	GFPT1	2673	I		5	**5**						
775	UBE2K	3093	I		5	**5**						
776	AK1	203	I	1	3	**4**						
777	LOC652595	652595	I	4		**4**						
778	PSMA3	5684	I	4		**4**						
779	ARHGDIA	396	I		4	**4**						
780	CORO1C	23603	I		4	**4**						
781	MCM5	4174	I	2	2	**4**						
782	HPCAL1	3241	I		4	**4**						
783	SART3	9733	I		4	**4**						
784	MDC1	9656	I		4	**4**						
785	PSMA7	5688	I		4	**4**						
786	BAT1	7919	I		4	**4**						
787	QARS	5859	I	3	1	**4**						
788	ARF5	381	I	3	1	**4**						
789	PSPH	5723	I	3	1	**4**						
790	TAGLN3	29114	I	2	2	**4**						
791	TWF1	5756	I	2	2	**4**						
792	PSMC1	5700	I	2	2	**4**						
793	TSN	7247	I	2	2	**4**						
794	OXSR1	9943	I	2	2	**4**						
795	FAM50B	26240	I	2	2	**4**						
796	MOCOS	55034	I	2	2	**4**						
797	PPP2R4	5524	I	2	2	**4**						
798	SNX2	6643	I	1	3	**4**						
799	UGDH	7358	I	1	3	**4**						
800	BLVRA	644	I	1	3	**4**						
801	BZW1	9689	I	1	3	**4**						
802	AKR7A2	8574	I	1	3	**4**						
803	AKR1C3	8644	I	1	3	**4**						
804	EIF5A	1984	I	1	3	**4**						
805	LOC388339	388339	I	4		**4**						
806	LOC389901	389901	I	4		**4**						
807	LOC647000	647000	I	4		**4**						
808	PCMT1	5110	I		4	**4**						
809	AOF2	23028	I		4	**4**						
810	ATIC	471	I		4	**4**						
811	RNH1	6050	I		4	**4**						
812	NARS	4677	I		4	**4**						
813	POLD2	5425	I		4	**4**						
814	MCM4	4173	I		4	**4**						
815	EIF3G	8666	I		4	**4**						
816	DNAJC8	22826	I		4	**4**						
817	ATM	472	I	4		**4**						
818	MPG	4350	I	4		**4**						
819	ISOC1	51015	I	4		**4**						

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				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
820	VPS29	51699	I	4		**4**						
821	PSMB8	5696	I	4		**4**						
822	RANGAP1	5905	I		4	**4**						
823	COBRA1	25920	I		4	**4**						
824	CNOT1	23019	I		4	**4**						
825	INTS1	26173	I		4	**4**						
826	EHD1	10938	I		4	**4**						
827	GEMIN5	25929	I		4	**4**						
828	MCM6	4175	I		4	**4**						
829	NCKAP1	10787	I		4	**4**						
830	PDS5B	23047	I		4	**4**						
831	POLE	5426	I		4	**4**						
832	CAP1	10487	I		4	**4**						
833	WARS	7453	I		4	**4**						
834	ESD	2098	I		4	**4**						
835	FH	2271	I		4	**4**						
836	PIR	8544	I		4	**4**						
837	PDLIM1	9124	I		4	**4**						
838	LOC730032	730032	I		4	**4**						
839	C6orf108	10591	I		4	**4**						
840	RBM12	10137	I		4	**4**						
841	MESDC2	23184	I	1	2	**3**						
842	CRIP2	1397	I	2	1	**3**						
843	POLR2H	5437	I	2	1	**3**						
844	EWSR1	2130	I	1	2	**3**						
845	CMBL	134147	I	2	1	**3**						
846	GCLM	2730	I	2	1	**3**						
847	TBCA	6902	I	2	1	**3**						
848	MCM7	4176	I	1	2	**3**						
849	SSB	6741	I		3	**3**						
850	HK2	3099	I		3	**3**						
851	WNK1	65125	I	3		**3**						
852	ANAPC1	64682	I		3	**3**						
853	CSNK2A2	1459	I	2	1	**3**						
854	WAPAL	23063	I	2	1	**3**						
855	STAG2	10735	I	2	1	**3**						
856	BTF3	689	I	2	1	**3**						
857	ARF4	378	I	2	1	**3**						
858	SH3GL1	6455	I	2	1	**3**						
859	DUSP3	1845	I	2	1	**3**						
860	SAE1	10055	I	2	1	**3**						
861	GLOD4	51031	I	2	1	**3**						
862	UBE2L3	7332	I	2	1	**3**						
863	ETF1	2107	I	1	2	**3**						
864	PSMD12	5718	I	1	2	**3**						
865	COPS8	10920	I	1	2	**3**						
866	PREP	5550	I	1	2	**3**						
867	TCEB1	6921	I		3	**3**						
868	PSPC1	55269	I		3	**3**						
869	HK1	3098	I		3	**3**						
870	PPP2R1A	5518	I		3	**3**						
871	NPLOC4	55666	I		3	**3**						
872	GOT2	2806	I		3	**3**						
873	KIF2C	11004	I	3		**3**						
874	BZW2	28969	I	3		**3**						
875	CTPS	1503	I	3		**3**						
876	PFAS	5198	I	3		**3**						
877	ANXA7	310	I	3		**3**						
878	LOC730429	730429	I	3		**3**						
879	PLS1	5357	I	3		**3**						
880	XPNPEP3	63929	I	3		**3**						
881	PPCS	79717	I	3		**3**						
882	ZMYND8	23613	I		3	**3**						

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				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
883	DNAJC9	23234	I		3	**3**						
884	ARHGEF2	9181	I		3	**3**						
885	KIAA0460	23248	I		3	**3**						
886	SUPT6H	6830	I		3	**3**						
887	DFFA	1676	I		3	**3**						
888	TBC1D20	128637	I		3	**3**						
889	ATAD5	79915	I		3	**3**						
890	JARID1C	8242	I		3	**3**						
891	DCTN1	1639	I		3	**3**						
892	ACTN1	87	I		3	**3**						
893	NUP160	23279	I		3	**3**						
894	CUL4A	8451	I		3	**3**						
895	PQBP1	10084	I		3	**3**						
896	CYP24A1	1591	I		3	**3**						
897	HTATSF1	27336	I		3	**3**						
898	LRRFIP1	9208	I		3	**3**						
899	FLII	2314	I		3	**3**						
900	COPB1	1315	I		3	**3**						
901	USP14	9097	I		3	**3**						
902	GDA	9615	I		3	**3**						
903	PGM1	5236	I		3	**3**						
904	MTAP	4507	I		3	**3**						
905	PUS7	54517	I		3	**3**						
906	ARFGEF1	10565	I		3	**3**						
907	IQGAP2	10788	I		3	**3**						
908	ETFA	2108	I		3	**3**						
909	DHX16	8449	I		3	**3**						
910	GOT1	2805	I		3	**3**						
911	CAST	831	I		3	**3**						
912	PFDN5	5204	I		3	**3**						
913	ARFGAP1	55738	I		3	**3**						
914	CRK	1398	I		3	**3**						
915	GLRX3	10539	I		3	**3**						
916	KYNU	8942	I		3	**3**						
917	RCC2	55920	I		3	**3**						
918	THOP1	7064	I		3	**3**						
919	SERPINB5	5268	I		3	**3**						
920	SMS	6611	I		3	**3**						
921	USP8	9101	I		3	**3**						
922	GNPDA2	132789	I		3	**3**						
923	LTB4DH	22949	I		3	**3**						
924	C12orf10	60314	I		3	**3**						
925	LOC728564	728564	I		3	**3**						
926	STK25	10494	I		3	**3**						
927	GALE	2582	I		3	**3**						
928	EIF1AX	1964	I	1	1	**2**						
929	MAD1L1	8379	I	1	1	**2**						
930	PSMA4	5685	I	2		**2**						
931	HPRT1	3251	I		2	**2**						
932	TPT1	7178	I		2	**2**						
933	APOA1BP	128240	I		2	**2**						
934	BAT2	7916	I	1	1	**2**						
935	ARCN1	372	I	1	1	**2**						
936	RRM1	6240	I	1	1	**2**						
937	NSFL1C	55968	I	1	1	**2**						
938	ACO1	48	I	1	1	**2**						
939	ARPC5	10092	I	1	1	**2**						
940	FGFR3	2261	I	1	1	**2**						
941	GMDS	2762	I	1	1	**2**						
942	CARHSP1	23589	I	1	1	**2**						
943	AKR1A1	10327	I	1	1	**2**						
944	LOC643287	643287	I	2		**2**						
945	LOC100130553	100130553	I	2		**2**						

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				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
946	LOC388532	388532	I	2		**2**						
947	B2M	567	I	2		**2**						
948	LOC402057	402057	I	2		**2**						
949	LSM3	27258	I	2		**2**						
950	PFDN2	5202	I	2		**2**						
951	LOC100131863	100131863	I	2		**2**						
952	NIT2	56954	I	2		**2**						
953	GSTM3	2947	I		2	**2**						
954	HSPC152	51504	I		2	**2**						
955	PSIP1	11168	I		2	**2**						
956	DAZAP1	26528	I		2	**2**						
957	CARS	833	I		2	**2**						
958	GLT25D1	79709	I		2	**2**						
959	EIF3D	8664	I		2	**2**						
960	NUP155	9631	I		2	**2**						
961	HDGF2	84717	I		2	**2**						
962	FKBP2	2286	I		2	**2**						
963	GATAD2A	54815	I	2		**2**						
964	EIF2B4	8890	I	2		**2**						
965	PFN2	5217	I	2		**2**						
966	SRP72	6731	I	2		**2**						
967	PMM2	5373	I	2		**2**						
968	DHX29	54505	I	2		**2**						
969	DNAJC7	7266	I	2		**2**						
970	CAPN1	823	I	2		**2**						
971	ADSL	158	I	2		**2**						
972	SP100	6672	I	2		**2**						
973	ACAT2	39	I	2		**2**						
974	LOC100134349	100134349	I	2		**2**						
975	LOC100130561	100130561	I	2		**2**						
976	RANBP1	5902	I	2		**2**						
977	DDB2	1643	I	2		**2**						
978	HSPBP1	23640	I	2		**2**						
979	TXNDC17	84817	I	2		**2**						
980	UFC1	51506	I	2		**2**						
981	HRSP12	10247	I	2		**2**						
982	TAX1BP3	30851	I	2		**2**						
983	EXOSC1	51013	I	2		**2**						
984	POLR1A	25885	I		2	**2**						
985	CTBP2	1488	I		2	**2**						
986	RAD21	5885	I		2	**2**						
987	DYNC1LI1	51143	I		2	**2**						
988	MORC2	22880	I		2	**2**						
989	NBN	4683	I		2	**2**						
990	COPZ1	22818	I		2	**2**						
991	RRM2	6241	I		2	**2**						
992	UCHL5	51377	I		2	**2**						
993	ROCK2	9475	I		2	**2**						
994	SEPT6	23157	I		2	**2**						
995	LEO1	123169	I		2	**2**						
996	TARBP1	6894	I		2	**2**						
997	CRKRS	51755	I		2	**2**						
998	WHSC2	7469	I		2	**2**						
999	USP9X	8239	I		2	**2**						
1000	KIF5C	3800	I		2	**2**						
1001	PSMD14	10213	I		2	**2**						
1002	KIFC1	3833	I		2	**2**						
1003	NFIC	4782	I		2	**2**						
1004	NFKB2	4791	I		2	**2**						
1005	GPKOW	27238	I		2	**2**						
1006	EIF3J	8669	I		2	**2**						
1007	DIAPH1	1729	I		2	**2**						
1008	TRIM25	7706	I		2	**2**						

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No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
1009	SLK	9748	I		2	**2**						
1010	RNF20	56254	I		2	**2**						
1011	NCAPD3	23310	I		2	**2**						
1012	PDXDC1	23042	I		2	**2**						
1013	CRABP2	1382	I		2	**2**						
1014	C3	718	I		2	**2**						
1015	DACT1	51339	I		2	**2**						
1016	TERF2IP	54386	I		2	**2**						
1017	LOC128192	128192	I		2	**2**						
1018	PPP2R1B	5519	I		2	**2**						
1019	PTRF	284119	I		2	**2**						
1020	PSMC5	5705	I		2	**2**						
1021	AHSA1	10598	I		2	**2**						
1022	PSMC2	5701	I		2	**2**						
1023	EIF3H	8667	I		2	**2**						
1024	PPP1R2	5504	I		2	**2**						
1025	OTUB1	55611	I		2	**2**						
1026	LRPPRC	10128	I		2	**2**						
1027	HSPA4L	22824	I		2	**2**						
1028	RPA3	6119	I		2	**2**						
1029	GSR	2936	I		2	**2**						
1030	COPE	11316	I		2	**2**						
1031	COL6A6	131873	I		2	**2**						
1032	ZNF294	26046	I		2	**2**						
1033	CDV3	55573	I		2	**2**						
1034	GBE1	2632	I		2	**2**						
1035	PDXK	8566	I		2	**2**						
1036	LSM6	11157	I		2	**2**						
1037	DPP3	10072	I		2	**2**						
1038	LPP	4026	I		2	**2**						
1039	PFKM	5213	I		2	**2**						
1040	PLAA	9373	I		2	**2**						
1041	ACOT7	11332	I		2	**2**						
1042	TXNL1	9352	I		2	**2**						
1043	RNPEP	6051	I		2	**2**						
1044	ABHD14B	84836	I		2	**2**						
1045	CDK5	1020	I		2	**2**						
1046	LOC51035	51035	I		2	**2**						
1047	RAD23A	5886	I		2	**2**						
1048	COTL1	23406	I		2	**2**						
1049	UBA6	55236	I		2	**2**						
1050	PHPT1	29085	I		2	**2**						
1051	SARS	6301	I		2	**2**						
1052	DDX19A	55308	I		2	**2**						
1053	S100A4	6275	I		2	**2**						
1054	TCEA1	6917	I		2	**2**						
1055	HISPPD1	23262	I		2	**2**						
1056	C1orf41	51668	I		2	**2**						
1057	HEBP1	50865	I		2	**2**						
1058	PSMD10	5716	I		2	**2**						
1059	SLC4A1AP	22950	I		2	**2**						
1060	MCTS1	28985	I		2	**2**						
1061	LOC647020	647020	I		2	**2**						
1062	BOLA2B	654483	I		2	**2**						
1063	LOC391322	391322	I		2	**2**						
1064	ME1	4199	I		2	**2**						
1065	VBP1	7411	I		2	**2**						
1066	SBDS	51119	I		2	**2**						
1067	IPO11	51194	I		2	**2**						
1068	DOCK11	139818	I		2	**2**						
1069	CUL3	8452	I		2	**2**						
1070	TEGT	7009	I		2	**2**						
1071	LOC100128049	100128049	I		2	**2**						

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
1072	RHEB	6009	I		2	**2**						
1073	GCHFR	2644	I		2	**2**						
1074	RAB5B	5869	I	1		**1**						
1075	HMGB1	3146	I		1	**1**						
1076	LOC729595	729595	I	1		**1**						
1077	TPM4	7171	I		1	**1**						
1078	CSRP2	1466	I		1	**1**						
1079	DCI	1632	I		1	**1**						
1080	TPM2	7169	I		1	**1**						
1081	REXO2	25996	I		1	**1**						
1082	LOC100128936	100128936	I	1		**1**						
1083	LOC100133662	100133662	I	1		**1**						
1084	LOC388474	388474	I	1		**1**						
1085	LOC387867	387867	I	1		**1**						
1086	LOC644937	644937	I	1		**1**						
1087	LOC653889	653889	I	1		**1**						
1088	PSMD8	5714	I	1		**1**						
1089	CDKN2A	1029	I	1		**1**						
1090	LOC650788	650788	I	1		**1**						
1091	LOC119358	119358	I	1		**1**						
1092	CSNK2B	1460	I	1		**1**						
1093	HDAC2	3066	I		1	**1**						
1094	PSMD2	5708	I		1	**1**						
1095	C14orf38	729665	I		1	**1**						
1096	CSTF3	1479	I		1	**1**						
1097	LIG3	3980	I		1	**1**						
1098	RBBP7	5931	I		1	**1**						
1099	PBRM1	55193	I		1	**1**						
1100	PPP1R12A	4659	I		1	**1**						
1101	ENY2	56943	I		1	**1**						
1102	CLTA	1211	I		1	**1**						
1103	SCYE1	9255	I		1	**1**						
1104	TK1	7083	I		1	**1**						
1105	RHOC	389	I		1	**1**						
1106	VASP	7408	I	1		**1**						
1107	RBBP5	5929	I	1		**1**						
1108	MED22	6837	I	1		**1**						
1109	KIAA0664	23277	I	1		**1**						
1110	FAM44A	259282	I	1		**1**						
1111	TNRC6C	57690	I	1		**1**						
1112	ADNP	23394	I	1		**1**						
1113	LCP1	3936	I	1		**1**						
1114	FKBP5	2289	I	1		**1**						
1115	UBE2V2	7336	I	1		**1**						
1116	CROCC	9696	I	1		**1**						
1117	ADRM1	11047	I	1		**1**						
1118	FANCD2	2177	I	1		**1**						
1119	APOL2	23780	I	1		**1**						
1120	CTPS2	56474	I	1		**1**						
1121	PDLIM5	10611	I	1		**1**						
1122	PPAT	5471	I	1		**1**						
1123	LAP3	51056	I	1		**1**						
1124	GALK1	2584	I	1		**1**						
1125	LYPLA1	10434	I	1		**1**						
1126	PAFAH1B3	5050	I	1		**1**						
1127	ACYP1	97	I	1		**1**						
1128	S100A13	6284	I	1		**1**						
1129	CIAPIN1	57019	I	1		**1**						
1130	DKFZP686M0199	653238	I	1		**1**						
1131	TBCB	1155	I	1		**1**						
1132	LOC100129520	100129520	I	1		**1**						
1133	STAT5A	6776	I	1		**1**						
1134	XPO4	64328	I	1		**1**						

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
1135	LOC401847	401847	I	1		1						
1136	PRPS2	5634	I	1		1						
1137	UBE2C	11065	I	1		1						
1138	LOC344382	344382	I	1		1						
1139	NUP37	79023	I	1		1						
1140	GAPDHS	26330	I	1		1						
1141	PMVK	10654	I	1		1						
1142	GATAD2B	57459	I		1	1						
1143	ARID2	196528	I		1	1						
1144	PPP1R10	5514	I		1	1						
1145	ZHX1	11244	I		1	1						
1146	SP1	6667	I		1	1						
1147	RP11-78J21.1	144983	I		1	1						
1148	WIZ	58525	I		1	1						
1149	TAF6	6878	I		1	1						
1150	WDHD1	11169	I		1	1						
1151	CARM1	10498	I		1	1						
1152	SMARCA2	6595	I		1	1						
1153	SMARCE1	6605	I		1	1						
1154	WDR5	11091	I		1	1						
1155	RBM16	22828	I		1	1						
1156	TCEB3	6924	I		1	1						
1157	CTR9	9646	I		1	1						
1158	WDR61	80349	I		1	1						
1159	ANK3	288	I		1	1						
1160	UBQLN4	56893	I		1	1						
1161	GARNL3	84253	I		1	1						
1162	UBQLN1	29979	I		1	1						
1163	IRF2BP2	359948	I		1	1						
1164	CCNB1	891	I		1	1						
1165	CAPZA1	829	I		1	1						
1166	IFI16	3428	I		1	1						
1167	GOLGA4	2803	I		1	1						
1168	ATXN2L	11273	I		1	1						
1169	ZMYM6	9204	I		1	1						
1170	SOS1	6654	I		1	1						
1171	XPO7	23039	I		1	1						
1172	CSDE1	7812	I		1	1						
1173	UBQLN2	29978	I		1	1						
1174	AP3B1	8546	I		1	1						
1175	PSMC6	5706	I		1	1						
1176	PRCC	5546	I		1	1						
1177	PFKL	5211	I		1	1						
1178	HEXIM1	10614	I		1	1						
1179	CAPZB	832	I		1	1						
1180	POU2F1	5451	I		1	1						
1181	AP1B1	162	I		1	1						
1182	PSMD4	5710	I		1	1						
1183	CTNND1	1500	I		1	1						
1184	FANCI	55215	I		1	1						
1185	HK3	3101	I		1	1						
1186	PLOD2	5352	I		1	1						
1187	WDR1	9948	I		1	1						
1188	XIRP2	129446	I		1	1						
1189	CALD1	800	I		1	1						
1190	A26C1A	445582	I		1	1						
1191	DCTN2	10540	I		1	1						
1192	EIF3K	27335	I		1	1						
1193	GPN1	11321	I		1	1						
1194	INTS9	55756	I		1	1						
1195	CALU	813	I		1	1						
1196	EHBP1L1	254102	I		1	1						
1197	DRG1	4733	I		1	1						

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No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
1198	IQGAP3	128239	I		1	**1**						
1199	EIF3M	10480	I		1	**1**						
1200	TUFM	7284	I		1	**1**						
1201	SPATA5	166378	I		1	**1**						
1202	ROCK1	6093	I		1	**1**						
1203	ARHGEF1	9138	I		1	**1**						
1204	NUP205	23165	I		1	**1**						
1205	SEC24C	9632	I		1	**1**						
1206	CNN2	1265	I		1	**1**						
1207	CKB	1152	I		1	**1**						
1208	EIF4G3	8672	I		1	**1**						
1209	VTA1	51534	I		1	**1**						
1210	SSBP1	6742	I		1	**1**						
1211	IPO4	79711	I		1	**1**						
1212	RAD23B	5887	I		1	**1**						
1213	PSMD5	5711	I		1	**1**						
1214	USP5	8078	I		1	**1**						
1215	PRDX3	10935	I		1	**1**						
1216	NUP43	348995	I		1	**1**						
1217	PPP2R2B	5521	I		1	**1**						
1218	UNC45A	55898	I		1	**1**						
1219	IPO8	10526	I		1	**1**						
1220	NES	10763	I		1	**1**						
1221	FTO	79068	I		1	**1**						
1222	ARFGEF2	10564	I		1	**1**						
1223	SH3GLB1	51100	I		1	**1**						
1224	POU2F3	25833	I		1	**1**						
1225	FAM21C	253725	I		1	**1**						
1226	ACSL4	2182	I		1	**1**						
1227	FARSB	10056	I		1	**1**						
1228	SAPS3	55291	I		1	**1**						
1229	SEC24B	10427	I		1	**1**						
1230	DDX6	1656	I		1	**1**						
1231	MGEA5	10724	I		1	**1**						
1232	DNAJB1	3337	I		1	**1**						
1233	CAPN2	824	I		1	**1**						
1234	CBFB	865	I		1	**1**						
1235	MAD2L1	4085	I		1	**1**						
1236	HSPA14	51182	I		1	**1**						
1237	GPD1L	23171	I		1	**1**						
1238	RNASEH2A	10535	I		1	**1**						
1239	ENOPH1	58478	I		1	**1**						
1240	UBE3C	9690	I		1	**1**						
1241	PPP1R8	5511	I		1	**1**						
1242	NOMO1	23420	I		1	**1**						
1243	SEPHS1	22929	I		1	**1**						
1244	YKT6	10652	I		1	**1**						
1245	HIBADH	11112	I		1	**1**						
1246	CPNE1	8904	I		1	**1**						
1247	JMJD1B	51780	I		1	**1**						
1248	RAP1GDS1	5910	I		1	**1**						
1249	METTL1	4234	I		1	**1**						
1250	TES	26136	I		1	**1**						
1251	PPA2	27068	I		1	**1**						
1252	HMBS	3145	I		1	**1**						
1253	NUDCD2	134492	I		1	**1**						
1254	IKBKAP	8518	I		1	**1**						
1255	ADSS	159	I		1	**1**						
1256	PROSC	11212	I		1	**1**						
1257	JTV1	7965	I		1	**1**						
1258	EIF5	1983	I		1	**1**						
1259	MLKL	197259	I		1	**1**						
1260	SAR1A	56681	I		1	**1**						

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No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
1261	PPP2CA	5515	I		1	1						
1262	NEDD8	4738	I		1	1						
1263	ADK	132	I		1	1						
1264	ADH5	128	I		1	1						
1265	SNAPIN	23557	I		1	1						
1266	THEM2	55856	I		1	1						
1267	DEPDC1	55635	I		1	1						
1268	PAFAH1B2	5049	I		1	1						
1269	MPST	4357	I		1	1						
1270	PIN4	5303	I		1	1						
1271	CFDP1	10428	I		1	1						
1272	SFRS12	140890	I		1	1						
1273	DDT	1652	I		1	1						
1274	ARPC3	10094	I		1	1						
1275	YPEL5	51646	I		1	1						
1276	GTF2E2	2961	I		1	1						
1277	ACTR3	10096	I		1	1						
1278	GALM	130589	I		1	1						
1279	LOC100129387	100129387	I		1	1						
1280	KIAA1797	54914	I		1	1						
1281	NT5DC1	221294	I		1	1						
1282	DNAH3	55567	I		1	1						
1283	RNMT	8731	I		1	1						
1284	C9orf78	51759	I		1	1						
1285	UBE2H	7328	I		1	1						
1286	FDPS	2224	I		1	1						
1287	TPP2	7174	I		1	1						
1288	STAT6	6778	I		1	1						
1289	GSS	2937	I		1	1						
1290	DRG2	1819	I		1	1						
1291	SYCP2	10388	I		1	1						
1292	ARL3	403	I		1	1						
1293	MEMO1	51072	I		1	1						
1294	SH3BGRL3	83442	I		1	1						
1295	NIF3L1	60491	I		1	1						
1296	FKBP1A	2280	I		1	1						
1297	NAE1	8883	I		1	1						
1298	RCADH5	642443	I		1	1						
1299	CCDC58	131076	I		1	1						
1300	STAT5B	6777	I		1	1						
1301	BZW1L1	151579	I		1	1						
1302	FAM105B	90268	I		1	1						
1303	XPNPEP1	7511	I		1	1						
1304	TTRAP	51567	I		1	1						
1305	UAP1	6675	I		1	1						
1306	FAM50A	9130	I		1	1						
1307	PRPF38B	55119	I		1	1						
1308	NIT1	4817	I		1	1						
1309	C4orf18	51313	I		1	1						
1310	KIAA2018	205717	I		1	1						
1311	GDF10	2662	I		1	1						
1312	PSMG2	56984	I		1	1						
1313	C6orf130	221443	I		1	1						
1314	MIF	4282	I		1	1						
1315	BRDT	676	I		1	1						
1316	GCLC	2729	I		1	1						
1317	PCNXL3	399909	I		1	1						
1318	CD247	919	I		1	1						
1319	ITGB5	3693	I		1	1						
1320	C11orf67	28971	I		1	1						
1321	LOC100131032	100131032	I		1	1						
1322	SYCE1	93426	I		1	1						
1323	ZNF614	80110	I		1	1						

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				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
1324	HBA2	3040	L				12	20	14		**46**	
1325	HBB	3043	L					21	15		**36**	
1326	FER1L3	26509	L				6	3	4	21	**34**	
1327	BASP1	10409	L				8	6	3	4	**21**	
1328	RAB2A	5862	L				4	3	4	4	**15**	
1329	HBD	3045	L				4	11			**15**	
1330	TMED10	10972	L				3	2	3	4	**12**	
1331	ATP1A1	476	L				1	1	3	7	**12**	
1332	JUP	3728	L					6	6		**12**	
1333	SLC7A5	8140	L				2	1	2	5	**10**	
1334	ATP1A2	477	L						1	9	**10**	
1335	LMAN1	3998	L				3	2	2	2	**9**	
1336	BSG	682	L				4	2		3	**9**	
1337	C19orf29	58509	L					8	1		**9**	
1338	RAB6A	5870	L				7			2	**9**	
1339	hCG_2004593	645296	L				3			5	**8**	
1340	CA9	768	L				2	2	2	2	**8**	
1341	RAB33B	83452	L				3	2		2	**7**	
1342	IFITM3	10410	L				1	2	2	2	**7**	
1343	GNAI2	2771	L					2	2	3	**7**	
1344	GNAI3	2773	L				1	1	2	3	**7**	
1345	STOM	2040	L				2			5	**7**	
1346	VDAC2	7417	L				3	2		2	**7**	
1347	MYO1C	4641	L							7	**7**	
1348	PKP1	5317	L				2		5		**7**	
1349	PTPRF	5792	L							7	**7**	
1350	CKAP4	10970	L				4	2			**6**	
1351	RPS4Y1	6192	L				4	1		1	**6**	
1352	CASP14	23581	L				2	3			**5**	
1353	LACRT	90070	L					4	1		**5**	
1354	LOC100131892	100131892	L					5			**5**	
1355	tcag7.23	392979	L							4	**4**	
1356	RALA	5898	L				1			3	**4**	
1357	PELP1	27043	L				2			2	**4**	
1358	MCAM	4162	L							4	**4**	
1359	CAT	847	L					2	2		**4**	
1360	DSC1	1823	L				4				**4**	
1361	HLA-C	3107	L				4				**4**	
1362	LOC342346	342346	L						4		**4**	
1363	AHCTF1	25909	L							4	**4**	
1364	CSDA	8531	L				1		2		**3**	
1365	RRP12	23223	L							3	**3**	
1366	ERLIN1	10613	L				1			2	**3**	
1367	CD44	960	L							3	**3**	
1368	VDAC1	7416	L							3	**3**	
1369	GNB1	2782	L							3	**3**	
1370	ATP1B3	483	L							3	**3**	
1371	CYB5R3	1727	L							3	**3**	
1372	FAU	2197	L							3	**3**	
1373	ITGA5	3678	L							3	**3**	
1374	RNPS1	10921	L							3	**3**	
1375	SERPINB13	5275	L					2	1		**3**	
1376	RAB9B	51209	L						2	1	**3**	
1377	THOC5	8563	L				3				**3**	
1378	CDGAP	57514	L						3		**3**	
1379	ISY1	57461	L							3	**3**	
1380	LOC442175	442175	L							3	**3**	
1381	SLC1A5	6510	L							3	**3**	
1382	NEFM	4741	L							3	**3**	
1383	EGFR	1956	L							3	**3**	
1384	RPL37A	6168	L							3	**3**	
1385	GNAI1	2770	L							3	**3**	
1386	GALNT2	2590	L							3	**3**	

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				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
1387	EXOSC6	118460	**L**				1	1			**2**	
1388	BCAP31	10134	**L**							2	**2**	
1389	SNRPC	6631	**L**				2				**2**	
1390	VAPA	9218	**L**				1			1	**2**	
1391	TMED9	54732	**L**				1			1	**2**	
1392	GNG12	55970	**L**						1	1	**2**	
1393	FYTTD1	84248	**L**				2				**2**	
1394	LTF	4057	**L**					2			**2**	
1395	RPL32	6161	**L**							2	**2**	
1396	CYB5B	80777	**L**				2				**2**	
1397	FUSIP1	10772	**L**				2				**2**	
1398	NCBP1	4686	**L**				2				**2**	
1399	ITGB1	3688	**L**							2	**2**	
1400	LAMP1	3916	**L**							2	**2**	
1401	HLA-F	3134	**L**							2	**2**	
1402	CD3EAP	10849	**L**							2	**2**	
1403	ATP5A1	498	**L**							2	**2**	
1404	TUBAL3	79861	**L**				1	1			**2**	
1405	PLP2	5355	**L**				1	1			**2**	
1406	SKIV2L2	23517	**L**				1		1		**2**	
1407	ALDH3A2	224	**L**				1		1		**2**	
1408	PKP3	11187	**L**				1			1	**2**	
1409	POP7	10248	**L**				1			1	**2**	
1410	CUGBP1	10658	**L**				1			1	**2**	
1411	ATP1A3	478	**L**						1	1	**2**	
1412	LARP1	23367	**L**				2				**2**	
1413	RANBP10	57610	**L**				2				**2**	
1414	LOC652614	652614	**L**				2				**2**	
1415	DHTKD1	55526	**L**				2				**2**	
1416	ITGA2	3673	**L**				2				**2**	
1417	LOC100132931	100132931	**L**				2				**2**	
1418	CST4	1472	**L**					2			**2**	
1419	SCGB2A1	4246	**L**					2			**2**	
1420	LOC100129540	100129540	**L**					2			**2**	
1421	SOX10	6663	**L**						2		**2**	
1422	MAGEE1	57692	**L**						2		**2**	
1423	TMEM14C	51522	**L**						2		**2**	
1424	POLR1C	9533	**L**							2	**2**	
1425	TEX10	54881	**L**							2	**2**	
1426	C14orf21	161424	**L**							2	**2**	
1427	PRPF31	26121	**L**							2	**2**	
1428	MLL	4297	**L**							2	**2**	
1429	AFAP1	60312	**L**							2	**2**	
1430	DNAJA2	10294	**L**							2	**2**	
1431	CHCHD9	645345	**L**							2	**2**	
1432	MAN2A1	4124	**L**							2	**2**	
1433	FAM62B	57488	**L**							2	**2**	
1434	LSM4	25804	**L**							2	**2**	
1435	STRBP	55342	**L**							2	**2**	
1436	RPL35A	6165	**L**							2	**2**	
1437	STX7	8417	**L**							2	**2**	
1438	EXOSC8	11340	**L**							2	**2**	
1439	ADARB1	104	**L**							2	**2**	
1440	PLXND1	23129	**L**							2	**2**	
1441	PON2	5445	**L**							2	**2**	
1442	RHOB	388	**L**							2	**2**	
1443	KRAS	3845	**L**							2	**2**	
1444	PDCD11	22984	**L**							1	**1**	
1445	ZC3HAV1	56829	**L**							1	**1**	
1446	IMP3	55272	**L**							1	**1**	
1447	FBL	2091	**L**							1	**1**	
1448	POLR1E	64425	**L**							1	**1**	
1449	RPL7L1	285855	**L**							1	**1**	

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
1450	LOC100133382	100133382	L				1				1	
1451	IGF2BP3	10643	L						1		1	
1452	TM4SF1	4071	L							1	1	
1453	LRRC59	55379	L							1	1	
1454	ACSL3	2181	L							1	1	
1455	ERH	2079	L							1	1	
1456	LOC728774	728774	L							1	1	
1457	HLA-G	3135	L							1	1	
1458	CISD2	493856	L							1	1	
1459	SAP30BP	29115	L				1				1	
1460	MRCL3	10627	L				1				1	
1461	NTHL1	4913	L				1				1	
1462	ATP2B2	491	L				1				1	
1463	TMED2	10959	L				1				1	
1464	RDH11	51109	L				1				1	
1465	UBOX5	22888	L				1				1	
1466	CALML5	51806	L					1			1	
1467	OCRL	4952	L					1			1	
1468	CHERP	10523	L					1			1	
1469	WDR18	57418	L					1			1	
1470	NXF1	10482	L					1			1	
1471	KIAA0152	9761	L					1			1	
1472	DMBT1	1755	L					1			1	
1473	PRIC285	85441	L					1			1	
1474	VDAC3	7419	L					1			1	
1475	WDR93	56964	L					1			1	
1476	FAM20C	56975	L					1			1	
1477	PRR4	11272	L						1		1	
1478	WTAP	9589	L						1		1	
1479	AIM1	202	L						1		1	
1480	MGST1	4257	L						1		1	
1481	PI4KA	5297	L						1		1	
1482	FAM83E	54854	L						1		1	
1483	LMAN2	10960	L						1		1	
1484	VAMP3	9341	L						1		1	
1485	RTN4R	65078	L						1		1	
1486	SCN8A	6334	L						1		1	
1487	HTRA2	27429	L						1		1	
1488	NFRKB	4798	L							1	1	
1489	TBL1XR1	79718	L							1	1	
1490	AP2A1	160	L							1	1	
1491	MVP	9961	L							1	1	
1492	DDX47	51202	L							1	1	
1493	ANAPC2	29882	L							1	1	
1494	MED21	9412	L							1	1	
1495	PHF3	23469	L							1	1	
1496	UBR4	23352	L							1	1	
1497	PRMT1	3276	L							1	1	
1498	CCDC131	196441	L							1	1	
1499	WDR33	55339	L							1	1	
1500	CDC2L1	984	L							1	1	
1501	UIMC1	51720	L							1	1	
1502	C20orf74	57186	L							1	1	
1503	MAP7	9053	L							1	1	
1504	C22orf28	51493	L							1	1	
1505	GOLGB1	2804	L							1	1	
1506	ASPH	444	L							1	1	
1507	C1QBP	708	L							1	1	
1508	DNAH10	196385	L							1	1	
1509	SLC2A1	6513	L							1	1	
1510	ABCF2	10061	L							1	1	
1511	GNAS	2778	L							1	1	
1512	SCRIB	23513	L							1	1	

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

				INPUT			LOOSE PRECIPITATE					PACKED
No	Symbol	GeneID	Category	1	2	IN	1	2	3	4	LP	PP
1513	ARHGEF17	9828	**L**							1	**1**	
1514	GPATCH8	23131	**L**							1	**1**	
1515	HLA-A	3105	**L**							1	**1**	
1516	PRR12	57479	**L**							1	**1**	
1517	GNAO1	2775	**L**							1	**1**	
1518	LOC100129902	100129902	**L**							1	**1**	
1519	IGF2R	3482	**L**							1	**1**	
1520	AP1G1	164	**L**							1	**1**	
1521	DNAJC13	23317	**L**							1	**1**	
1522	HMOX2	3163	**L**							1	**1**	
1523	BRI3BP	140707	**L**							1	**1**	
1524	GLG1	2734	**L**							1	**1**	
1525	ECE1	1889	**L**							1	**1**	
1526	LOC729397	729397	**L**							1	**1**	
1527	RAB21	23011	**L**							1	**1**	
1528	KIAA0090	23065	**L**							1	**1**	
1529	GAL3ST3	89792	**L**							1	**1**	
1530	NIP7	51388	**L**							1	**1**	
1531	RAB10	10890	**L**							1	**1**	
1532	LOC100134229	100134229	**L**							1	**1**	
1533	MAP2K1IP1	8649	**L**							1	**1**	
1534	ATP11A	23250	**L**							1	**1**	
1535	GOLT1B	51026	**L**							1	**1**	
1536	PDZD2	23037	**L**							1	**1**	
1537	PIGG	54872	**L**							1	**1**	
1538	GNA12	2768	**L**							1	**1**	
1539	FYN	2534	**L**							1	**1**	
1540	IER3IP1	51124	**L**							1	**1**	
1541	SEC31B	25956	**L**							1	**1**	
1542	GDPD1	284161	**L**							1	**1**	
1543	APP	351	**L**							1	**1**	
1544	YLPM1	56252	**P**									**15**
1545	PRPF4	9128	**P**									**3**
1546	AGL	178	**P**									**3**
1547	CLINT1	9685	**P**									**3**
1548	GYS1	2997	**P**									**3**
1549	CROP	51747	**P**									**2**
1550	THRAP3	9967	**P**									**2**
1551	SPEN	23013	**P**									**2**
1552	CSTF1	1477	**P**									**2**
1553	SRPR	6734	**P**									**2**
1554	CWC15	51503	**P**									**2**
1555	TMEM33	55161	**P**									**2**
1556	RSL1D1	26156	**P**									**1**
1557	XRN2	22803	**P**									**1**
1558	DDX27	55661	**P**									**1**
1559	RPS5	6193	**P**									**1**
1560	BAZ2A	11176	**P**									**1**
1561	KPNA2	3838	**P**									**1**
1562	TFIP11	24144	**P**									**1**
1563	RBM9	23543	**P**									**1**
1564	RBBP6	5930	**P**									**1**
1565	NUP93	9688	**P**									**1**
1566	RPN2	6185	**P**									**1**
1567	DHX36	170506	**P**									**1**
1568	LOC727997	727997	**P**									**1**
1569	C17orf85	55421	**P**									**1**
1570	PYGB	5834	**P**									**1**
1571	ADAD2	161931	**P**									**1**
1572	LARP2	55132	**P**									**1**
1573	BLMH	642	**P**									**1**
1574	CEBPZ	10153	**P**									**1**
1575	RBM12B	389677	**P**									**1**

Supplementary Material 1. Identification of Abundant Proteins in IN, LP, and PP. Proteins are sorted and labeled (ILP categories) by presence across these fractions.

No	Symbol	GeneID	Category	INPUT			LOOSE PRECIPITATE					PACKED
				1	2	IN	1	2	3	4	LP	PP
1576	NMNAT1	64802	P									1
1577	CCDC68	80323	P									1

Supplementary Material S2. E-cutoff Values for Frequently Occuring (>25% of IPs) Proteins.

			SPCs		
No	**Symbol**	**GeneID**	**Q1**	**Q3**	**E-cutoff**
1	**HSPA5**	3309	7	34	**115**
2	**HSPA8**	3312	12	32	**92**
3	**HSPA1B**	3304	7	27	**87**
4	**NUMA1**	4926	0	21	**84**
5	**TUBB**	203068	2	18	**66**
6	**TOP2A**	7153	0	16	**64**
7	**MATR3**	9782	0	13	**52**
8	**HSP90AA1**	3320	0	13	**52**
9	**ACTC1**	70	0	12	**48**
10	**VIM**	7431	0	12	**48**
11	**RP11-631M21.2**	347688	0	11	**44**
12	**HNRNPU**	3192	2	12	**42**
13	**LMNA**	4000	0	10	**40**
14	**TUBB4**	10382	0	10	**40**
15	**TUBA1B**	10376	0	9	**36**
16	**DDX5**	1655	0	9	**36**
17	**HSP90AB1**	3326	0	9	**36**
18	**ACTB**	60	0	8	**32**
19	**HSPA9**	3313	0	7	**28**
20	**RIF1**	55183	0	7	**28**
21	**MKI67**	4288	0	6	**24**
22	**HNRNPK**	3190	0	6	**24**
23	**HSPB1**	3315	0	6	**24**
24	**NPM1**	4869	0	6	**24**
25	**DDX17**	10521	0	6	**24**
26	**TOP2B**	7155	0	6	**24**
27	**FLNA**	2316	0	5	**20**
28	**TCOF1**	6949	0	5	**20**
29	**PCBP2**	5094	0	5	**20**
30	**NCL**	4691	0	5	**20**
31	**RPLP0**	6175	0	5	**20**
32	**EEF1A2**	1917	0	5	**20**
33	**RPS27A**	6233	0	5	**20**
34	**HSPD1**	3329	0	4	**16**
35	**EEF1A1**	1915	0	4	**16**
36	**PCBP1**	5093	0	4	**16**
37	**TUBB2C**	10383	0	4	**16**
38	**TPR**	7175	0	4	**16**
39	**TOP1**	7150	0	4	**16**
40	**DDX21**	9188	0	4	**16**
41	**MYH9**	4627	0	4	**16**
42	**GTF2I**	2969	0	4	**16**
43	**GAPDH**	2597	0	4	**16**
44	**DHX9**	1660	0	4	**16**
45	**DDB1**	1642	0	4	**16**
46	**NOLC1**	9221	0	3	**12**
47	**RUVBL2**	10856	0	3	**12**

Supplementary Material S2. E-cutoff Values for Frequently Occuring (>25% of IPs) Proteins.

			SPCs		
No	**Symbol**	**GeneID**	**Q1**	**Q3**	**E-cutoff**
48	**PARP1**	142	0	3	**12**
49	**HNRNPR**	10236	0	3	**12**
50	**RACGAP1**	29127	0	3	**12**
51	**HSPA1L**	3305	0	3	**12**
52	**ALB**	213	0	3	**12**
53	**SFPQ**	6421	0	3	**12**
54	**CLTC**	1213	0	3	**12**
55	**RPL7**	6129	0	3	**12**
56	**EPRS**	2058	0	3	**12**
57	**HSP90B1**	7184	0	3	**12**
58	**HIST1H2AE**	3012	0	3	**12**
59	**HDAC1**	3065	0	2	**8**
60	**SPTAN1**	6709	0	2	**8**
61	**HCFC1**	3054	0	2	**8**
62	**XRCC6**	2547	0	2	**8**
63	**TRIM28**	10155	0	2	**8**
64	**SMARCC2**	6601	0	2	**8**
65	**ACTL6A**	86	0	2	**8**
66	**RUVBL1**	8607	0	2	**8**
67	**KIF23**	9493	0	2	**8**
68	**TUBB3**	10381	0	2	**8**
69	**HSPA6**	3310	0	2	**8**
70	**RFC3**	5983	0	2	**8**
71	**RFC4**	5984	0	2	**8**
72	**PTBP1**	5725	0	2	**8**
73	**RFC2**	5982	0	2	**8**
74	**RBM39**	9584	0	2	**8**
75	**THOC4**	10189	0	2	**8**
76	**RPLP2**	6181	0	2	**8**
77	**KPNB1**	3837	0	2	**8**
78	**SF3B3**	23450	0	2	**8**
79	**IQGAP1**	8826	0	2	**8**
80	**KIAA1967**	57805	0	2	**8**
81	**SNRNP200**	23020	0	2	**8**
82	**EEF1D**	1936	0	2	**8**
83	**FASN**	2194	0	2	**8**
84	**RPL19**	6143	0	2	**8**
85	**HIST1H2BI**	8346	0	2	**8**
86	**MTA2**	9219	0	1	**4**
87	**PRKDC**	5591	0	1	**4**
88	**DYNC1H1**	1778	0	1	**4**
89	**S100A9**	6280	0	1	**4**
90	**EFTUD2**	9343	0	1	**4**
91	**SMARCA4**	6597	0	1	**4**
92	**DIDO1**	11083	0	1	**4**
93	**DDX52**	11056	0	1	**4**
94	**SPTBN1**	6711	0	1	**4**

Supplementary Material S2. E-cutoff Values for Frequently Occuring (>25% of IPs) Proteins.

			SPCs		
No	**Symbol**	**GeneID**	**Q1**	**Q3**	**E-cutoff**
95	**CCT3**	7203	0	1	**4**
96	**NONO**	4841	0	1	**4**
97	**HNRNPD**	3184	0	1	**4**
98	**SF3B1**	23451	0	1	**4**
99	**CSTA**	1475	0	1	**4**
100	**HNRNPF**	3185	0	1	**4**
101	**CDC5L**	988	0	1	**4**
102	**FIP1L1**	81608	0	1	**4**
103	**EEF1G**	1937	0	1	**4**
104	**MDC1**	9656	0	1	**4**
105	**SEPT9**	10801	0	1	**4**
106	**HRNR**	388697	0	1	**4**
107	**DDX3X**	1654	0	1	**4**
108	**U2AF2**	11338	0	1	**4**
109	**RFC1**	5981	0	1	**4**
110	**CCT2**	10576	0	1	**4**
111	**ANXA2**	302	0	1	**4**
112	**EIF2S1**	1965	0	1	**4**
113	**EIF4A2**	1974	0	1	**4**
114	**CCT6A**	908	0	1	**4**
115	**SEPT2**	4735	0	1	**4**
116	**DHX15**	1665	0	1	**4**
117	**PRDX1**	5052	0	1	**4**
118	**ILF3**	3609	0	1	**4**
119	**ALDOA**	226	0	1	**4**
120	**NDRG1**	10397	0	1	**4**
121	**CCT8**	10694	0	1	**4**
122	**MAGED2**	10916	0	1	**4**
123	**SRP14**	6727	0	1	**4**
124	**RBMXL1**	494115	0	1	**4**

Supporting Information

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SI Text

We have defined four categories of specificity filters for the immunoprecipitation followed by mass spectrometry (IP/MS) data as follows:

(*i*) Three filters [input (IN), loose (LP), and packed (PP) precipitates] compare protein composition of an IP with that of extract and protein precipitates that form during the IP procedure. For these semiquantitative composition enrichment filters, approximate fractional contribution of each protein to either of the fractions or an IP experiment was defined as $FC_i = \mathrm{SPA}_i / \sum_{i=1}^{n} \mathrm{SPA}_i$, where $\mathrm{SPA\,(spectral\ abundance)}_i = \mathrm{SPC\,(spectral\ counts)}_i / MW$ (molecular weight), and n = total number of nonredundant protein identifications in a given experiment. The enriched identifications were defined as proteins with $FC_{i(\mathrm{IP})} \geq k \times FC_{i(\mathrm{IN,LP,\ or\ PP})}$, where k is a threshold multiplier. We examined several different thresholds, ranging from $k = 3$ to $k = 100$ for each of these filters, by manually evaluating the protein identifications which were "flagged" as nonspecific. For our dataset, $FC_{i(\mathrm{IP})} \geq 5 \times FC_{i(\mathrm{IN,LP,\ or\ PP})}$ ($k = 5$) is effective at pinpointing nonspecificity, particularly when used in combination with the E_{cutoff} filter described below.

(*ii*) The purpose of the SPC distribution E_{cutoff} filter is to differentiate background, or "noise," identifications from enriched proteins by examining levels at which each protein appears across all IPs in our dataset. For the E_{cutoff} filter, a standard statistical outlier test was applied as follows: for each protein, the interquartile region (IQR) was calculated as a difference between the 25th and 75th percentile SPCs in SPC distribution across IPs, and the extreme upper-hand outlier threshold was defined as a cutoff for specific identification level $E_{\mathrm{cutoff}(i)} = (75\mathrm{th\ percentile\ SPC}_i) + (3 \times \mathrm{IQR}_i)$. By this definition, proteins that are present in <25% of our IPs are always specific; more frequent proteins are omitted from IPs only when present at background levels. We find these constraints sufficient to this date.

(*iii*) Keratins, trypsin, and immunoglobulins are introduced during the IP/MS procedure. These identifications are deleted from the results.

(*iv*) For 3*N* analysis, a group of ribosomal, heat-shock, and cytoskeletal protein variants were omitted in addition to the dynamic composition and distribution filters that are described above for two reasons: (*i*) results from the automated search, such as SeQuest, often contain ambiguous gene identifications for these categories of proteins due to high homology between multitude of isoforms, which decreases accuracy of gene product-based dynamic filters for this particular list; (*ii*) often, the same set of heat-shock chaperone proteins specifically associates with different protein complexes that otherwise do not share biological functions; inclusion of these proteins in 3*N* analysis causes merging of functionally unrelated protein complexes, which is not desirable. Information about specific occurrences of heat-shock binding can be retrieved from the original experimental data, separately from 3*N* analysis.

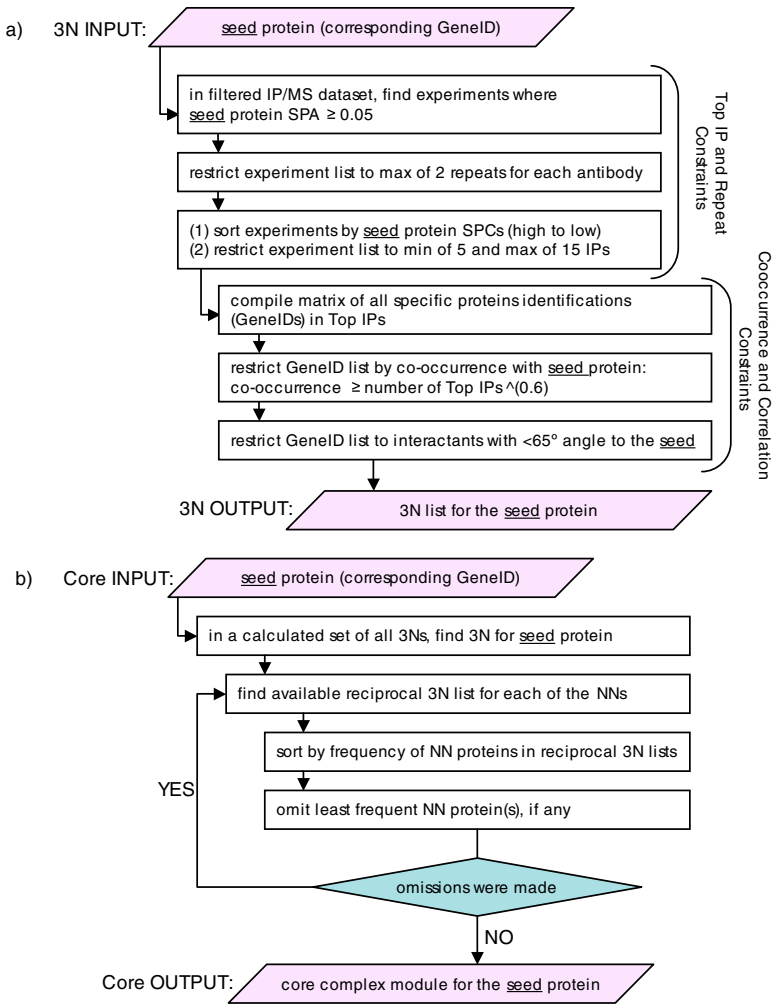

Fig. S1. Schematic of (*A*) 3*N* and (*B*) core complex cluster logic. NN = near neighbor.

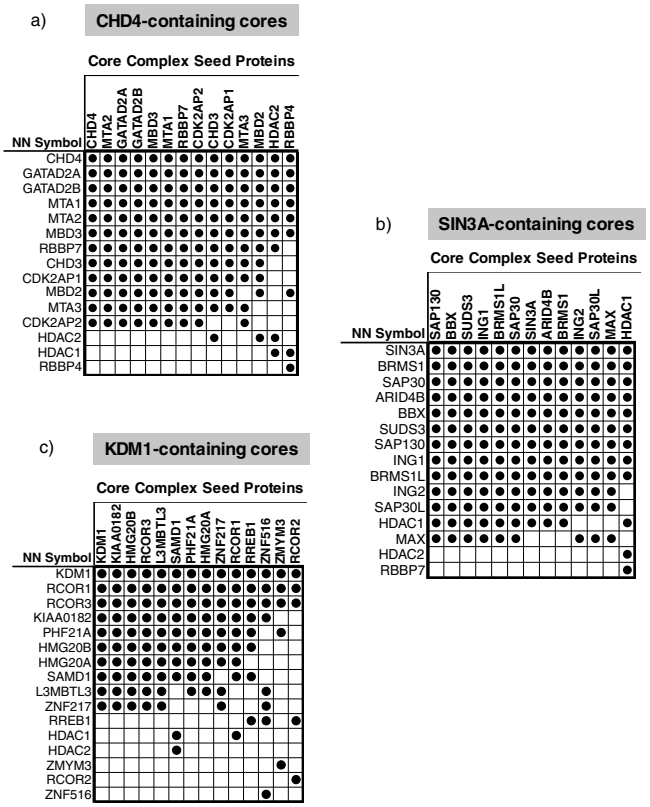

Fig. S2. 3*N* core complex components were assigned for each of the denoted seed proteins for the three HDAC-containing modules (CHD4, SIN3A, KDM1), and core module subunits were sorted by their copresence across multiple 3*N* clusters, analogous to the Mediator complex analysis shown in Fig. 3*B*. NN = near neighbor.

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (XLS)
Table S4 (XLS)
Table S5 (XLS)
Table S6 (XLS)
Table S7 (XLS)