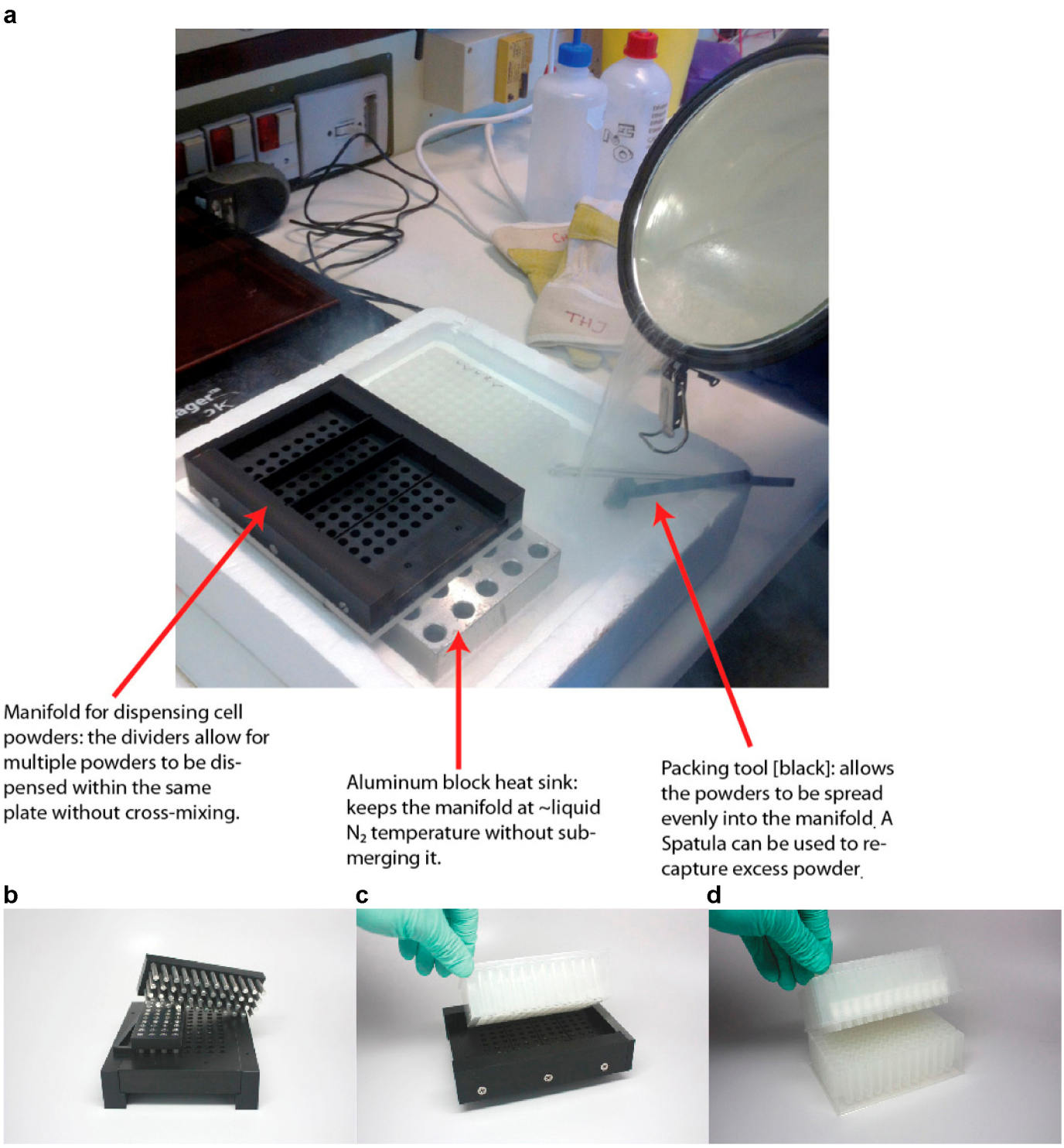
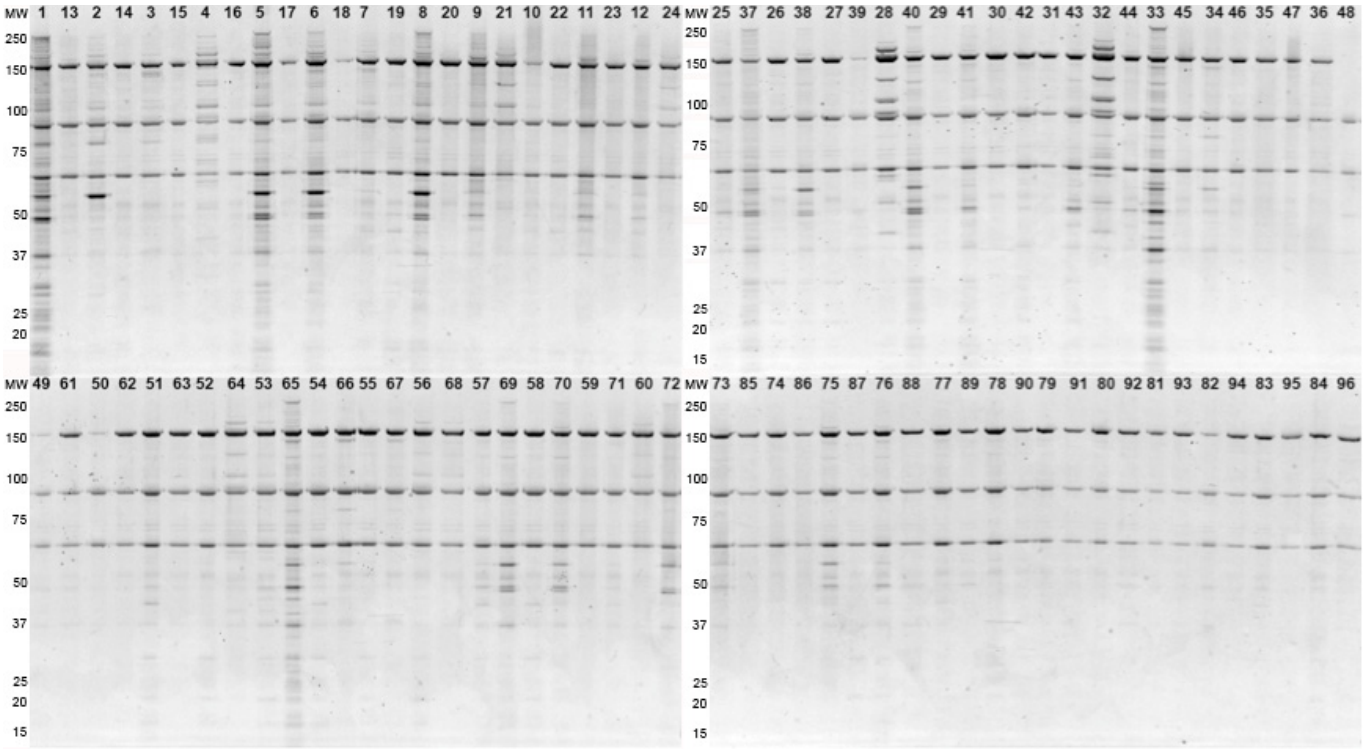


Supplementary Figure 1

Photographs of the powder-dispensing manifold and filter plate.

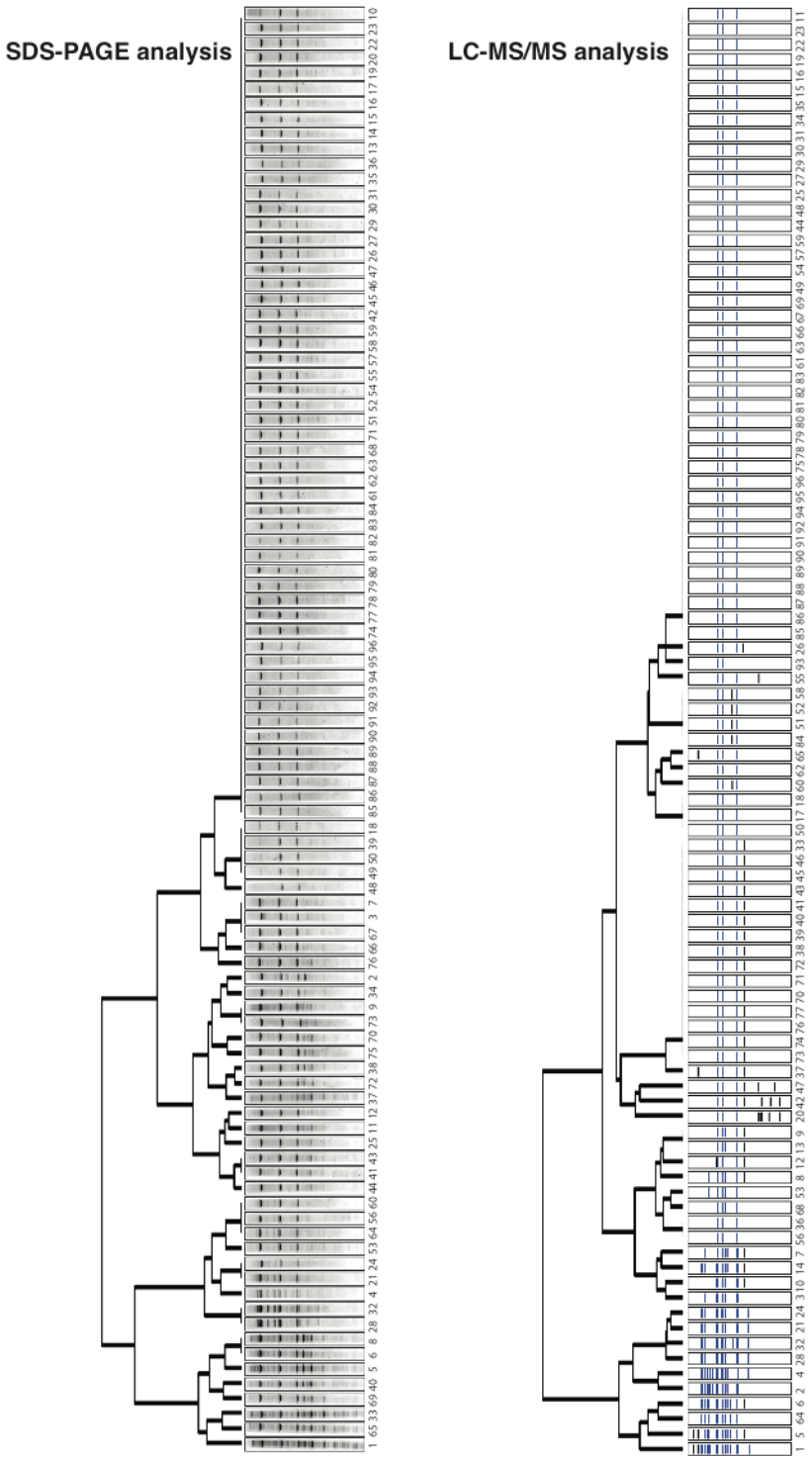
(a) Preparing to use the powder dispensing manifold; pre-cooling with liquid N_2; **(b)** adjustable volume dispensing manifold, shown bottom up; **(c)** dispensing manifold with 96-well deep-well plate atop, cell material transfer is achieved upon inversion of this assembly; **(d)** a 96-well filtration device atop a 96-well, deep well collection plate.



Supplementary Figure 2

Nup1p-SpA 96-well screen.

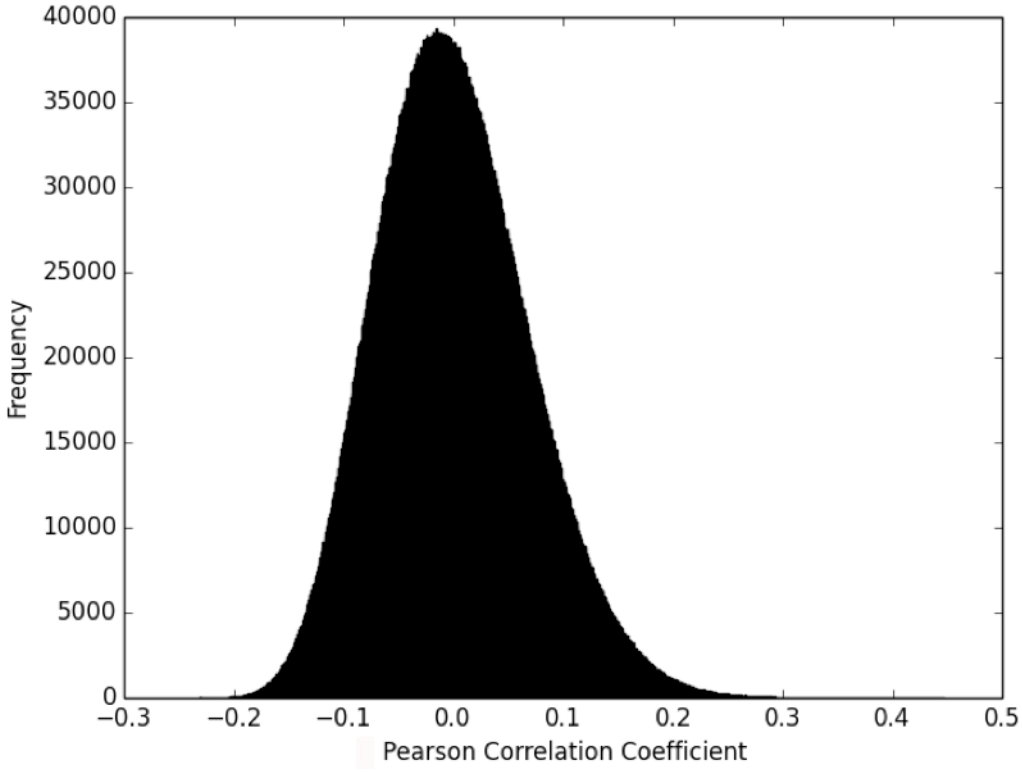
Coomassie stained SDS-polyacrylamide gels of Nup1p-SpA screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**.



Supplementary Figure 3

Comparison of SDS-PAGE and direct-to-MS analyses.

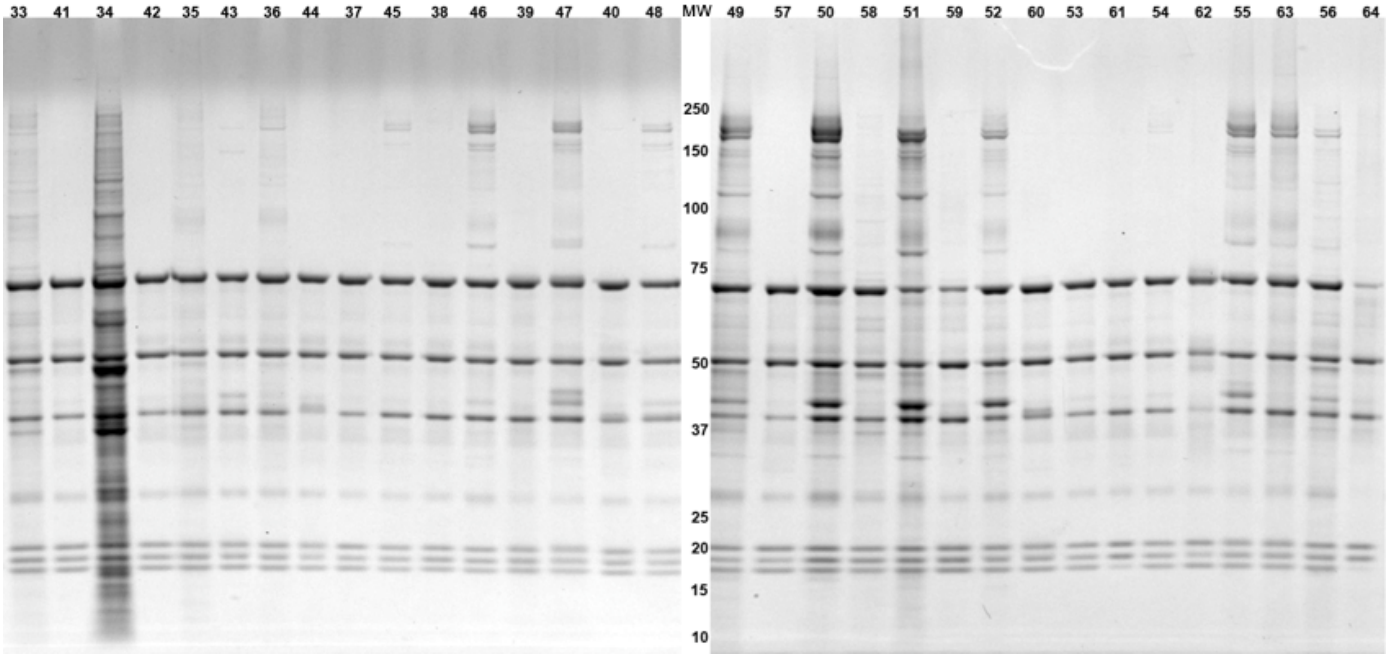
SDS-PAGE and LC-MS/MS clustering analysis of Nup1p-Spa 96-well purification. Numbers below each lane indicate the extractant formulation, presented in **Supplementary Table 1**.



Supplementary Figure 4

Correlation of SDS-PAGE and direct-to-MS analyses.

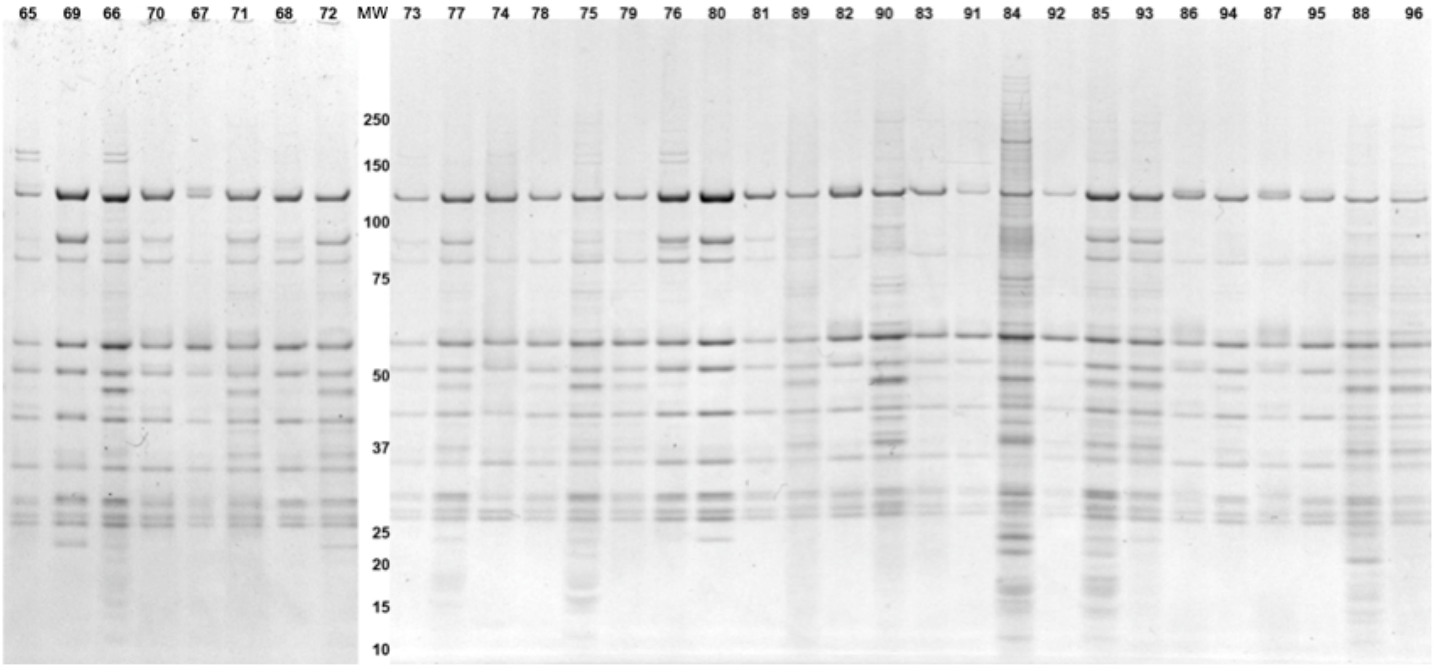
Frequency distribution of correlation coefficients between the gel dendrogram and 10 million permutations of the MS dendrogram.



Supplementary Figure 5

Arp2p-GFP 32-well screen.

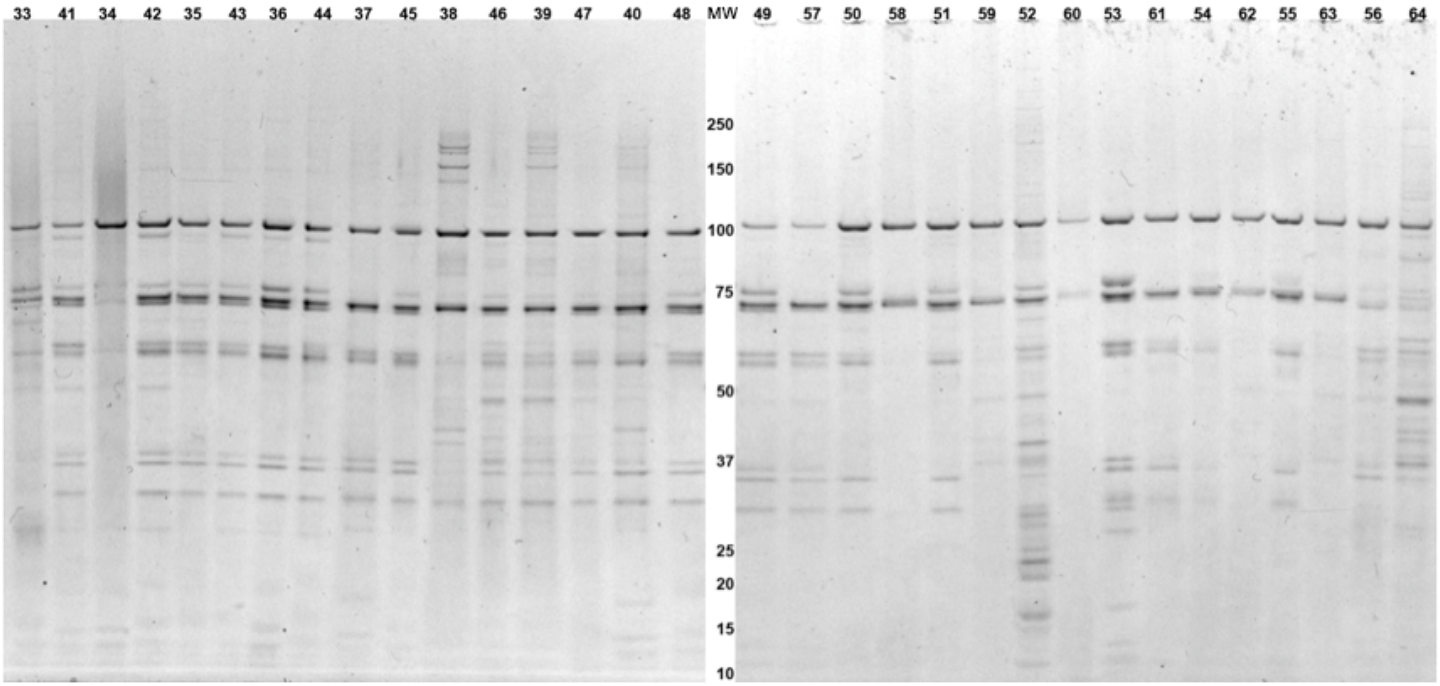
Coomassie stained SDS-polyacrylamide gels of Arp2p-GFP screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**. Reference condition # 64.



Supplementary Figure 6

Csl4p-TAP 32-well screen.

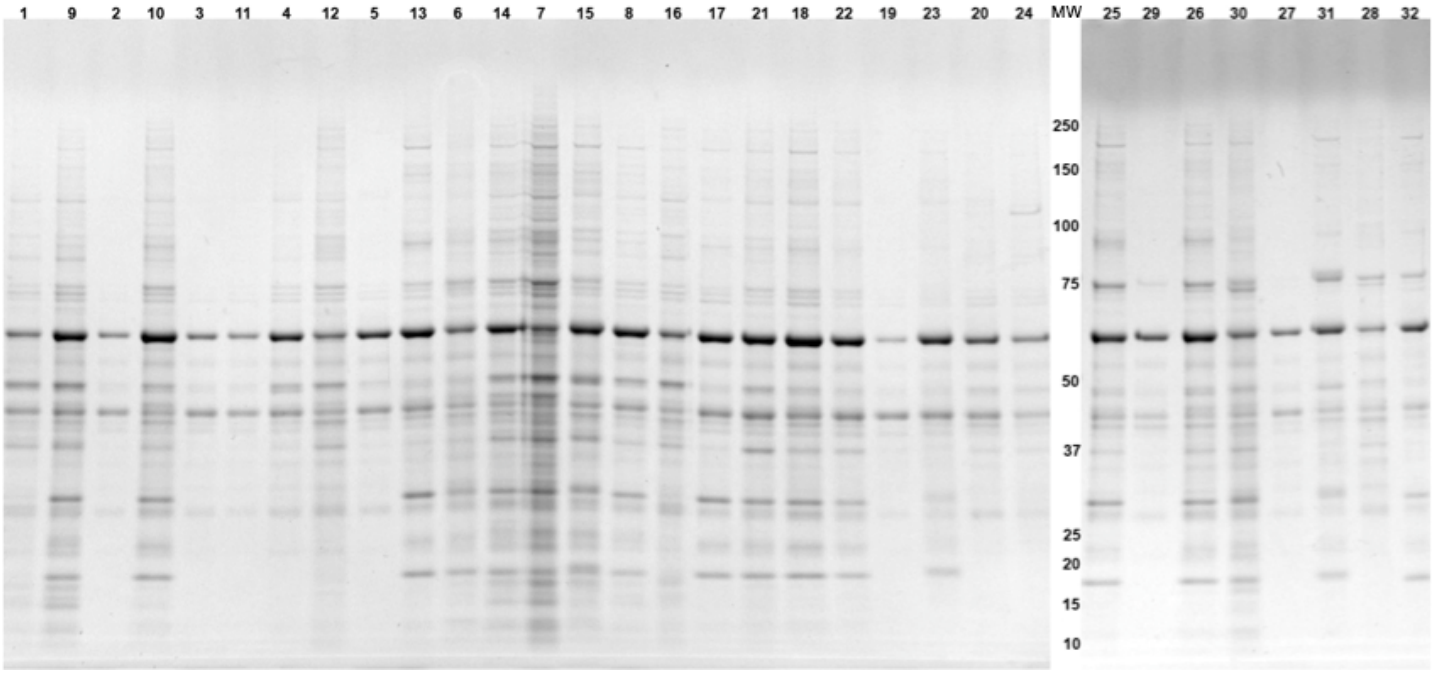
Coomassie stained SDS-polyacrylamide gels of Csl4p-TAP screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**. Reference condition # 65.



Supplementary Figure 7

Snu71p-TAP 32-well screen.

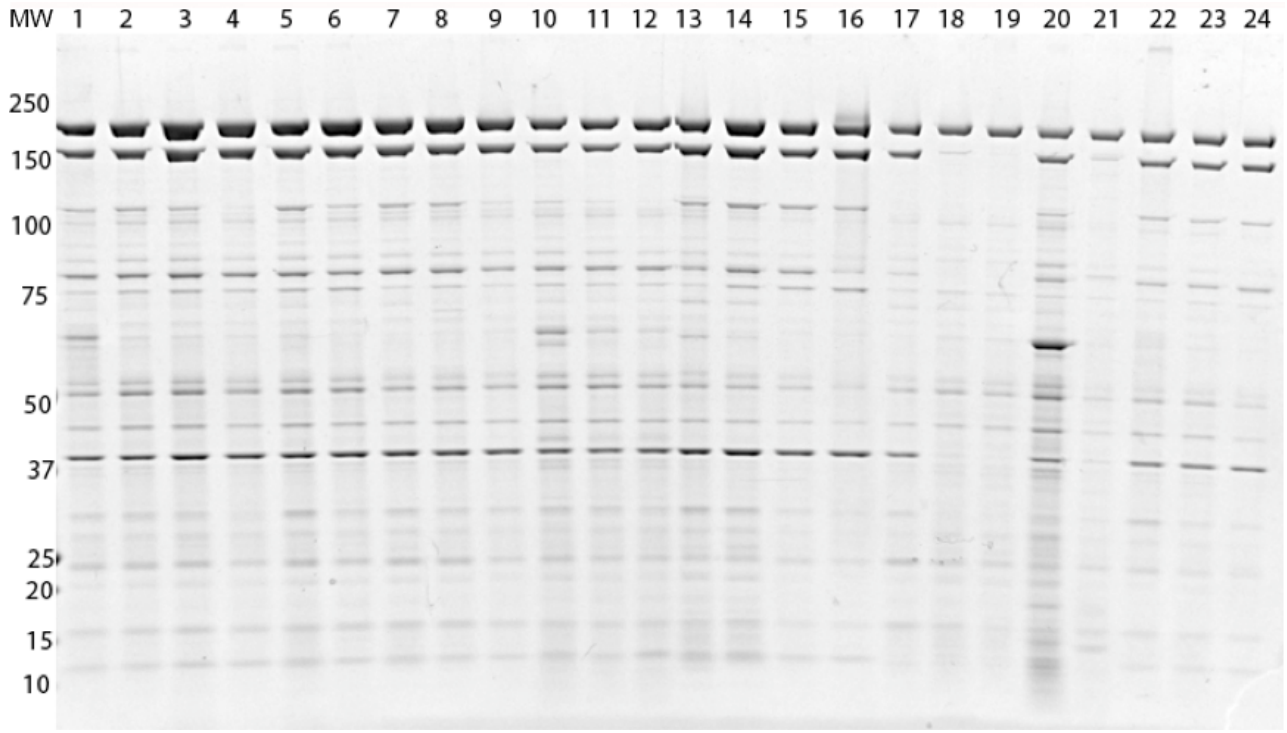
Coomassie stained SDS-polyacrylamide gels of Snu71p-TAP screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**. Reference condition # 33.



Supplementary Figure 8

Rtn1p-GFP 32-well screen.

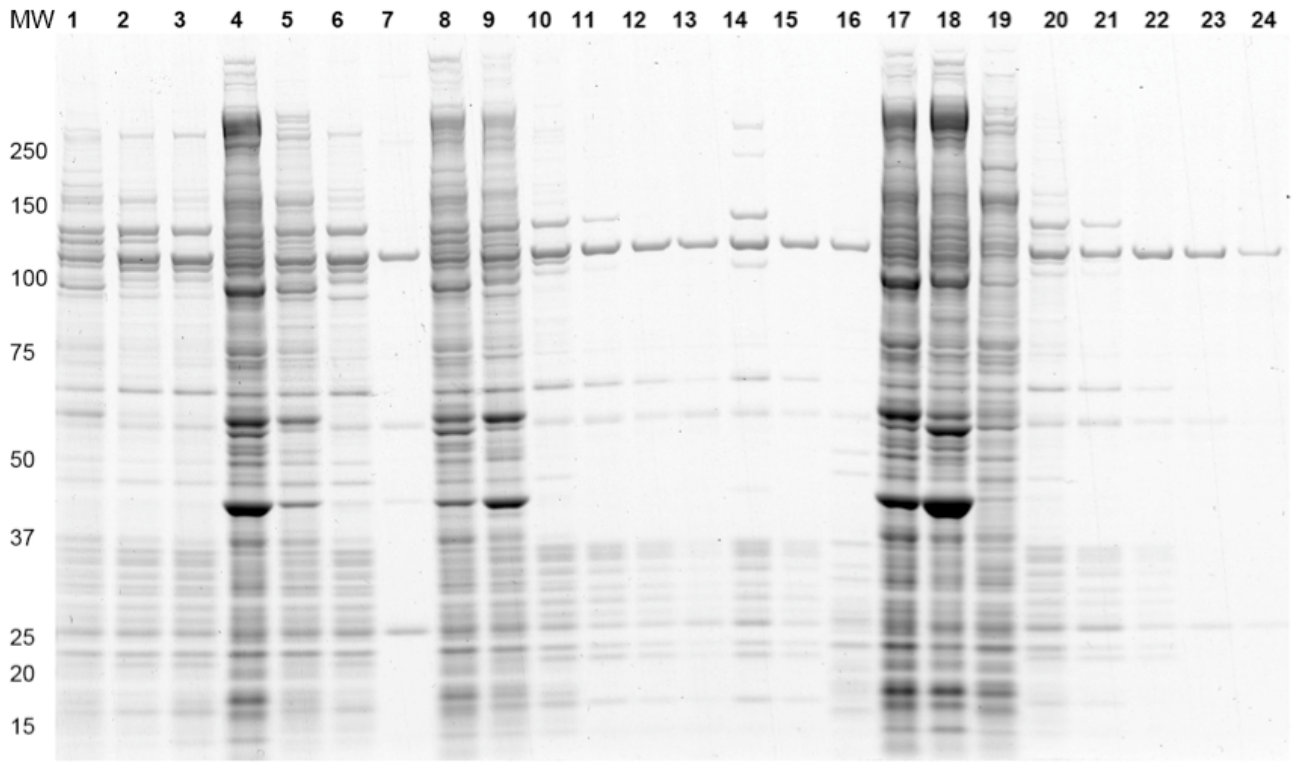
Coomassie stained SDS-polyacrylamide gels of Rtn1p-GFP screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**.



Supplementary Figure 9

RpoC-SpA 24-well screen.

Coomassie stained SDS-polyacrylamide gel of RpoC-SpA screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**.

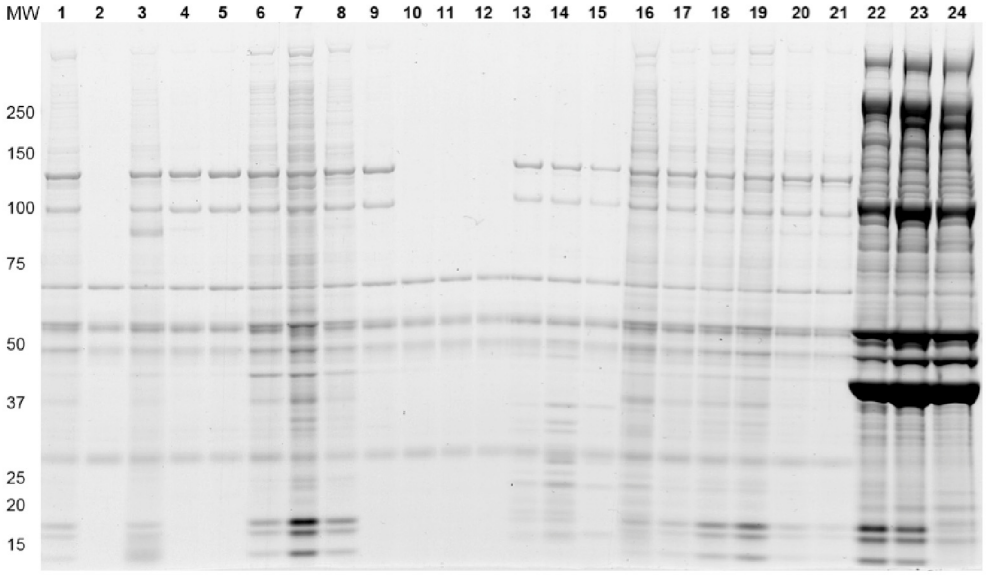


Supplementary Figure 10

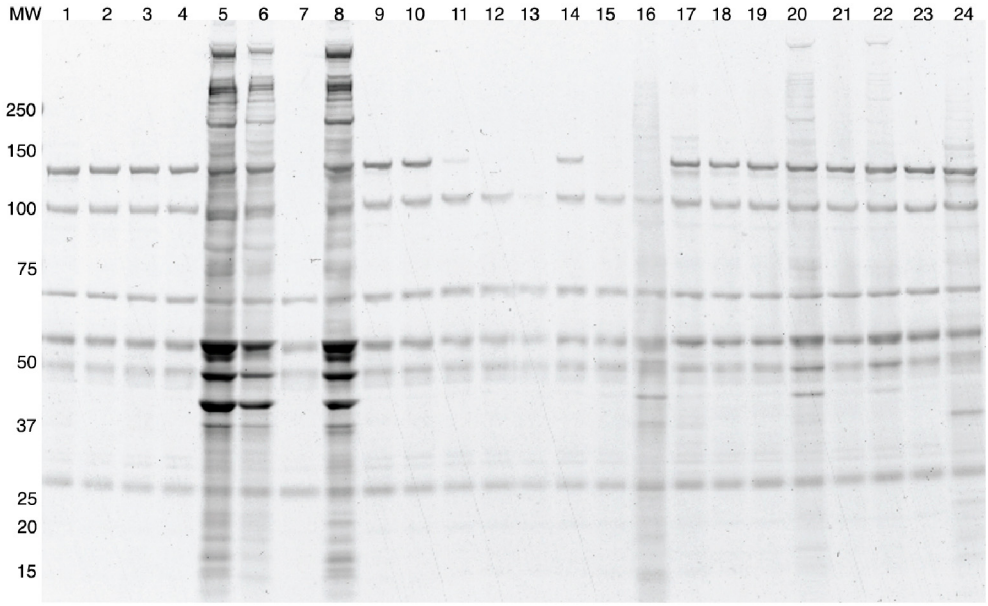
RRP6-3×Flag 24-well screen.

Coomassie stained SDS-polyacrylamide gel of RRP6-3xFLAG screen. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**.

Screen A



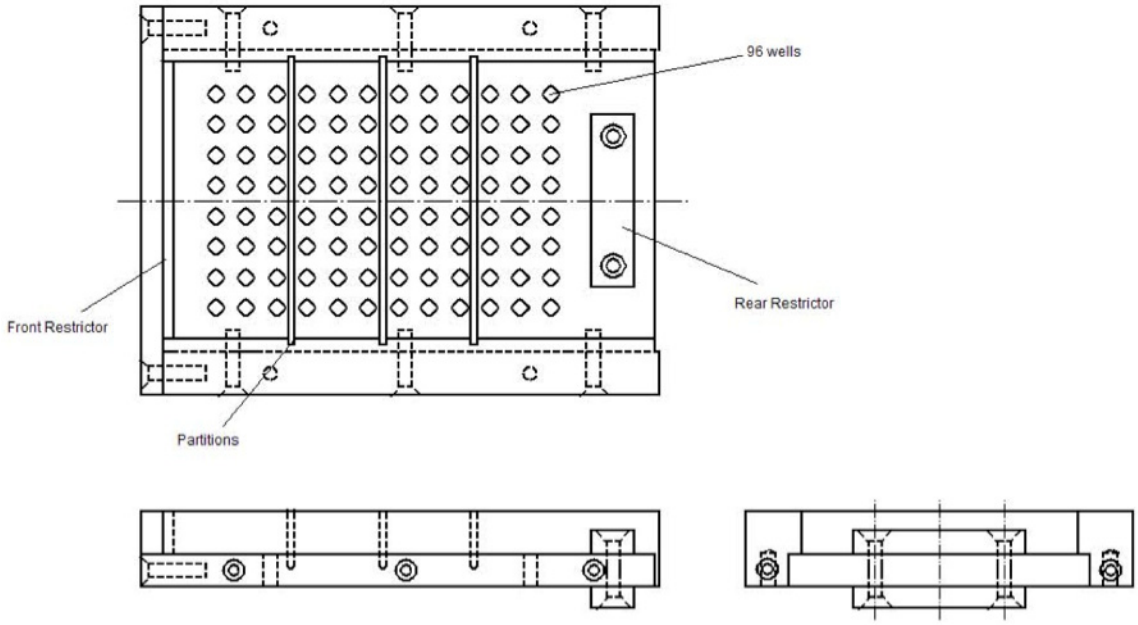
Screen B



Supplementary Figure 11

RBM7-LAP two 24-well screens.

Coomassie stained SDS-polyacrylamide gels of RBM7-LAP screens. Numbers above each lane indicate the extractant formulation, presented in **Supplementary Table 1**.



Supplementary Figure 12

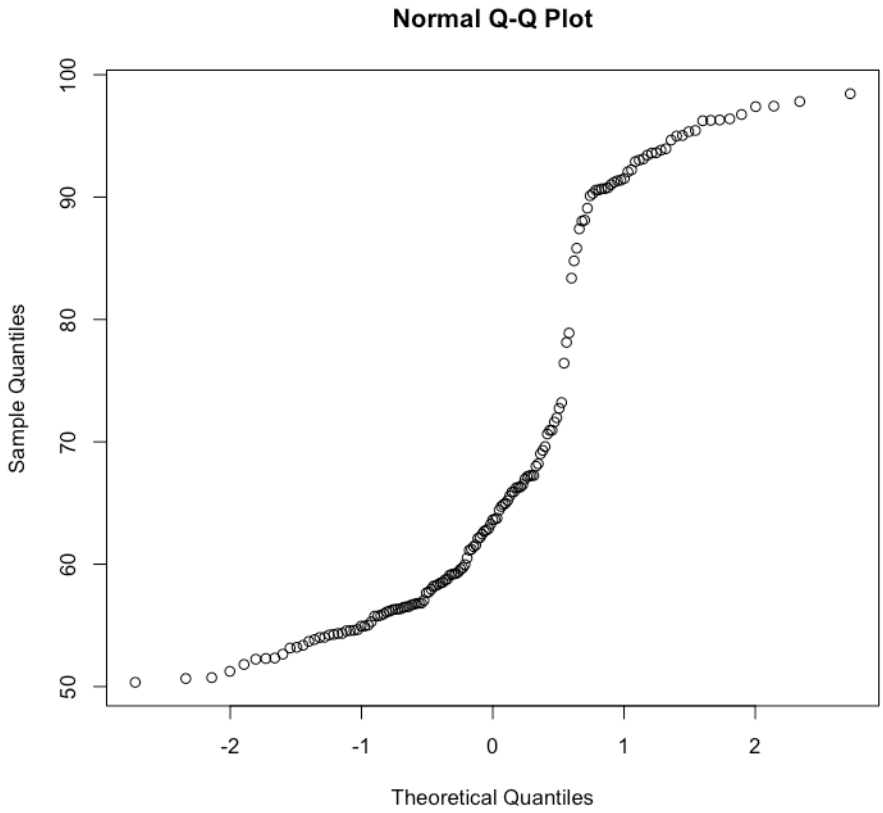
Dispensing manifold, basic engineering diagram.

The manifold is constructed of Black Delrin (acetal) that tolerates liquid nitrogen temperatures; additional engineering diagrams with more detailed specifications are available upon request.

Supplementary Figure 13

Bead-dispensing manifold.

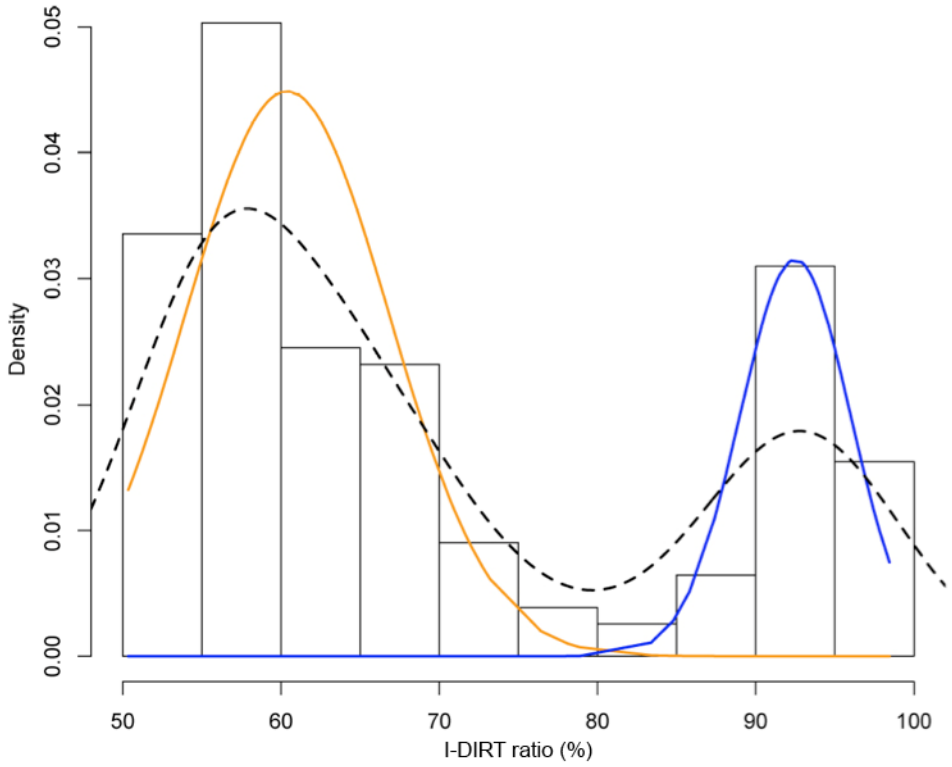
For 96-well screens with yeast, extract homogenization is assisted by vortexing in the presence of 2 mm Ø steel balls. This manifold provides for parallel dispensing of precisely 2 balls to each well. When placed atop a 96-well plate, removal of the sliding bottom allows the balls to be deposited into the wells of the plate.



Supplementary Figure 14

Testing normality of I-DIRT ratio distribution.

Q-Q plot of the measured I-DIRT ratios (normalized to 100%) quantiles vs. theoretical quantiles.



Supplementary Figure 15

Fitted bimodal distribution of I-DIRT ratios of proteins copurifying with Rtn1p.

Orange and blue solid lines – fitted curves; dashed line – kernel density estimate of the total distribution; histogram – frequency distribution of I-DIRT ratios.

Supplementary Table 1

Extraction solvents used in affinity capture screens.

The extraction solvents used for each protein purification are presented in separate tabels, labeled with the name of the tagged protein. The first column in each tabel, "Condition #," corresponds to the number above each lane in corresponding gels of Supplementary Figures 2, 5-11 and lanes in Supplementary Data. The second column, "Components," contains the expected pH and final concentration of the reagents in the extraction solvents. Third column "Reference" provides the source of the extraction solvent composition, providing the appropriate reference if it was selected from the literature. The forth column "Main figure" contains Roman numerals next to a few select conditions which correspond to the conditions used in purifications of protien complexes presented in the main text Figure 4.

Nature Methods: doi:10.1038/nmeth.3395

Nup1p-SpA Extraction Solvents

Condition #	Components	Reference
1	125mM AmAc, pH 7, 15mM Triton X-100	This study
2	250mM AmAc, pH 7, 15mM Triton X-100	This study
3	500mM AmAc, pH 7, 15mM Triton X-100	This study
4	1500mM AmAc, pH 7, 15mM Triton X-100	This study
5	20mM HEPES-K, pH7.4, 50mM KAc, 15mM Triton X-100	This study
6	20mM HEPES-K, pH7.4, 125mM KAc, 15mM Triton X-100	This study
7	20mM HEPES-K, pH7.4, 250mM KAc, 15mM Triton X-100	This study
8	20mM HEPES-K, pH7.4, 50mM KAc, 20mM KCl, 15mM Triton X-100	This study
9	20mM HEPES-K, pH7.4, 125mM KAc, 20mM KCl, 15mM Triton X-100	This study
10	20mM HEPES-K, pH7.4, 250mM KAc, 20mM KCl, 15mM Triton X-100	This study
11	20mM HEPES-K, pH7.4,100mM KCl, 15mM Triton X-100	This study
12	20mM HEPES-K, pH7.4, 50mM KAc, 100mM KCl, 15mM Triton X-100	This study
13	20mM HEPES-K, pH7.4, 125mM KAc, 100mM KCl, 15mM Triton X-100	This study
14	20mM HEPES-K, pH7.4, 250mM KAc, 100mM KCl, 15mM Triton X-100	This study
15	20mM HEPES-K, pH7.4,500mM KCl, 15mM Triton X-100	This study
16	20mM HEPES-K, pH7.4, 50mM KAc, 500mM KCl, 15mM Triton X-100	This study
17	20mM HEPES-K, pH7.4, 125mM KAc, 500mM KCl, 15mM Triton X-100	This study
18	20mM HEPES-K, pH7.4, 250mM KAc, 500mM KCl, 15mM Triton X-100	This study
19	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 15mM Triton X-100	This study
20	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 15mM Triton X-100	This study
21	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 15mM Triton X-100	This study
22	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 20mM NaCl, 15mM Triton X-100	This study
23	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 20mM NaCl, 15mM Triton X-100	This study
24	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 20mM NaCl, 15mM Triton X-100	This study
25	40mM TRIS-Cl, pH 8, 100mM NaCl, 15mM Triton X-100	This study
26	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 100mM NaCl, 15mM Triton X-100	This study
27	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 100mM NaCl, 15mM Triton X-100	This study
28	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 100mM NaCl, 15mM Triton X-100	This study
29	40mM TRIS-Cl, pH 8, 500mM NaCl, 15mM Triton X-100	This study

30	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 500mM NaCl, 15mM Triton X-100	This study
31	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 500mM NaCl, 15mM Triton X-100	This study
32	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 500mM NaCl, 15mM Triton X-100	This study
33	125mM AmAc, pH 7, 10mM Tween 20	This study
34	250mM AmAc, pH 7, 10mM Tween 20	This study
35	500mM AmAc, pH 7, 10mM Tween 20	This study
36	1500mM AmAc, pH 7, 10mM Tween 20	This study
37	20mM HEPES-K, pH7.4, 50mM KAc, 10mM Tween 20	This study
38	20mM HEPES-K, pH7.4, 125mM KAc, 10mM Tween 20	This study
39	20mM HEPES-K, pH7.4, 250mM KAc, 10mM Tween 20	This study
40	20mM HEPES-K, pH7.4, 50mM KAc, 20mM KCl, 10mM Tween 20	This study
41	20mM HEPES-K, pH7.4, 125mM KAc, 20mM KCl, 10mM Tween 20	This study
42	20mM HEPES-K, pH7.4, 250mM KAc, 20mM KCl, 10mM Tween 20	This study
43	20mM HEPES-K, pH7.4,100mM KCl, 10mM Tween 20	This study
44	20mM HEPES-K, pH7.4, 50mM KAc, 100mM KCl, 10mM Tween 20	This study
45	20mM HEPES-K, pH7.4, 125mM KAc, 100mM KCl, 10mM Tween 20	This study
46	20mM HEPES-K, pH7.4, 250mM KAc, 100mM KCl, 10mM Tween 20	This study
47	20mM HEPES-K, pH7.4,500mM KCl, 10mM Tween 20	This study
48	20mM HEPES-K, pH7.4, 50mM KAc, 500mM KCl, 10mM Tween 20	This study
49	20mM HEPES-K, pH7.4, 125mM KAc, 500mM KCl, 10mM Tween 20	This study
50	20mM HEPES-K, pH7.4, 250mM KAc, 500mM KCl, 10mM Tween 20	This study
51	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 10mM Tween 20	This study
52	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 10mM Tween 20	This study
53	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 10mM Tween 20	This study
54	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 20mM NaCl, 10mM Tween 20	This study
55	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 20mM NaCl, 10mM Tween 20	This study
56	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 20mM NaCl, 10mM Tween 20	This study
57	40mM TRIS-Cl, pH 8, 100mM NaCl, 10mM Tween 20	This study
58	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 100mM NaCl, 10mM Tween 20	This study
59	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 100mM NaCl, 10mM Tween 20	This study
60	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 100mM NaCl, 10mM Tween 20	This study

61	40mM TRIS-Cl, pH 8, 500mM NaCl, 10mM Tween 20	This study
62	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 500mM NaCl, 10mM Tween 20	This study
63	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 500mM NaCl, 10mM Tween 20	This study
64	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 500mM NaCl, 10mM Tween 20	This study
65	125mM AmAc, pH 7, 5mM CHAPS	This study
66	250mM AmAc, pH 7, 5mM CHAPS	This study
67	500mM AmAc, pH 7, 5mM CHAPS	This study
68	1500mM AmAc, pH 7, 5mM CHAPS	This study
69	20mM HEPES-K, pH7.4, 50mM KAc, 5mM CHAPS	This study
70	20mM HEPES-K, pH7.4, 125mM KAc, 5mM CHAPS	This study
71	20mM HEPES-K, pH7.4, 250mM KAc, 5mM CHAPS	This study
72	20mM HEPES-K, pH7.4, 50mM KAc, 20mM KCl, 5mM CHAPS	This study
73	20mM HEPES-K, pH7.4, 125mM KAc, 20mM KCl, 5mM CHAPS	This study
74	20mM HEPES-K, pH7.4, 250mM KAc, 20mM KCl, 5mM CHAPS	This study
75	20mM HEPES-K, pH7.4,100mM KCl, 5mM CHAPS	This study
76	20mM HEPES-K, pH7.4, 50mM KAc, 100mM KCl, 5mM CHAPS	This study
77	20mM HEPES-K, pH7.4, 125mM KAc, 100mM KCl, 5mM CHAPS	This study
78	20mM HEPES-K, pH7.4, 250mM KAc, 100mM KCl, 5mM CHAPS	This study
79	20mM HEPES-K, pH7.4,500mM KCl, 5mM CHAPS	This study
80	20mM HEPES-K, pH7.4, 50mM KAc, 500mM KCl, 5mM CHAPS	This study
81	20mM HEPES-K, pH7.4, 125mM KAc, 500mM KCl, 5mM CHAPS	This study
82	20mM HEPES-K, pH7.4, 250mM KAc, 500mM KCl, 5mM CHAPS	This study
83	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 5mM CHAPS	This study
84	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 5mM CHAPS	This study
85	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 5mM CHAPS	This study
86	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 20mM NaCl, 5mM CHAPS	This study
87	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 20mM NaCl, 5mM CHAPS	This study
88	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 20mM NaCl, 5mM CHAPS	This study
89	40mM TRIS-Cl, pH 8, 100mM NaCl, 5mM CHAPS	This study
90	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 100mM NaCl, 5mM CHAPS	This study
91	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 100mM NaCl, 5mM CHAPS	This study

92	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 100mM NaCl, 5mM CHAPS	This study
93	40mM TRIS-Cl, pH 8, 500mM NaCl, 5mM CHAPS	This study
94	40mM TRIS-Cl, pH 8, 50mM Na3 Cit, 500mM NaCl, 5mM CHAPS	This study
95	40mM TRIS-Cl, pH 8, 125mM Na3 Cit, 500mM NaCl, 5mM CHAPS	This study
96	40mM TRIS-Cl, pH 8, 250mM Na3 Cit, 500mM NaCl, 5mM CHAPS	This study

Arp2p-GFP Extraction Solvents

Condition #	Components	Reference	Main Figure
33	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 5mM CHAPS	This study	
34	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 10mM d-BCHAP	This study	
35	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 15mM Triton X-100	This study	
36	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 0.5mM LMNG	This study	
37	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 5mM CHAPS	This study	
38	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 10mM d-BCHAP	This study	
39	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 10mM Tween 20	This study	
40	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 0.5mM LMNG	This study	
41	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 5mM CHAPS	This study	
42	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 10mM d-BCHAP	This study	ii
43	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 15mM Triton X-100	This study	
44	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 0.5mM LMNG	This study	
45	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 5mM CHAPS	This study	
46	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 10mM d-BCHAP	This study	
47	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 10mM Tween 20	This study	
48	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 0.5mM LMNG	This study	i
49	125mM NaPhos, pH 7, 5mM CHAPS	This study	
50	125mM NaPhos, pH 7, 10mM d-BCHAP	This study	
51	125mM NaPhos, pH 7, 15mM Triton X-100	This study	
52	125mM NaPhos, pH 7, 0.5mM LMNG	This study	
53	250mM NaPhos, pH 7, 5mM CHAPS	This study	
54	250mM NaPhos, pH 7, 10mM d-BCHAP	This study	

55	250mM NaPhos, pH 7, 10mM Tween 20	This study	
56	250mM NaPhos, pH 7, 0.5mM LMNG	This study	
57	1.5M NH4 Acet, pH 7, 5mM CHAPS	This study	
58	1.5M NH4 Acet, pH 7, 10mM d-BCHAP	This study	
59	1.5M NH4 Acet, pH 7, 15mM Triton X-100	This study	
60	1.5M NH4 Acet, pH 7, 0.5mM LMNG	This study	
61	1.5M NH4 Acet, pH 7, 5mM ASB14	This study	
62	1.5M NH4 Acet, pH 7, 20mM Saponin	This study	
63	1.5M NH4 Acet, pH 7, 10mM Tween 20	This study	
64	20mM HEPES-K, pH 7.4, 110mM K Acet, 2mM MgCl2, 8.5mM Triton X-100, 0.7mM Tween 20, 150mM NaCl	Cristea *et al*, 2005	

Csl4p-TAP Extraction Solvents

Condition #	Components	Reference	Main Figure
65	20mM HEPES-K, pH 7.4, 100mM NaCl, 1.5mM MgCl2, 1mM DTT, 3mM NP-40	Synowsky *et al*, 2006	
66	20mM HEPES-K, pH7.4, 150mM KCl, 8mM d-BCHAP, 2mM EDTA	This study	
67	40mM TRIS-Cl, pH 8, 150mM NaCl, 2mM CHAPS	This study	
68	40mM TRIS-Cl, pH 8, 150mM NaCl, 10mM Brij 58, 0.1mM MgCl2	This study	
69	1.5M NH4 Acet, pH 7, 10mM Tween 20, 2mM EDTA	This study	
70	1.5M NH4 Acet, pH 7, 8mM d-BCHAP, 2mM EDTA	This study	
71	1.5M NH4 Acet, pH 7, 2mM CHAPS, 0.1mM MgCl2	This study	
72	1.5M NH4 Acet, pH 7, 10mM Brij 58, 0.1mM MgCl2	This study	
73	125mM Na Phos, pH 7, 15mM Triton X-100	This study	
74	125mM Na Phos, pH 7, 8mM d-BCHAP	This study	
75	125mM Na Phos, pH 7, 2mM CHAPS	This study	
76	125mM Na Phos, pH 7, 10mM Brij 58	This study	i
77	250mM Na Phos, pH 7, 10mM Tween 20	This study	
78	250mM Na Phos, pH 7, 8mM d-BCHAP	This study	
79	250mM Na Phos, pH 7, 2mM CHAPS	This study	iii

80	250mM Na Phos, pH 7, 10mM Brij 58	This study	ii
81	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 15mM Triton X-100, 0.1mM MgCl2	This study	
82	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 8mM d-BCHAP, 0.1mM MgCl2	This study	iv
83	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 2mM CHAPS	This study	
84	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 5mM Taurodeo	This study	
85	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 10mM Tween 20, 2mM EDTA	This study	
86	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 8mM d-BCHAP, 2mM EDTA	This study	
87	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 2mM CHAPS	This study	
88	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 1mM Zwittergent 3-14	This study	
89	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 15mM Triton X-100, 0.1mM MgCl2	This study	
90	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 8mM d-BCHAP, 0.1mM MgCl2	This study	
91	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 2mM CHAPS	This study	
92	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 5mM Taurodeo	This study	
93	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 10mM Tween 20, 2mM EDTA	This study	
94	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 8mM d-BCHAP, 2mM EDTA	This study	
95	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 2mM CHAPS	This study	
96	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 1mM Zwittergent 3-14	This study	

Snu71p-TAP Extraction Solvents

Condition #	**Components**	**Reference**	**Main Figure**
33	20mM HEPES-K, pH 7.4, 125mM KCl, 2mM NP-40	Gornemann *et al*, 2011	
34	20mM HEPES-K, pH7.4, 150mM KCl, 8mM d-BCHAP, 2mM EDTA	This study	
35	40mM TRIS-Cl, pH 8, 150mM NaCl, 2mM CHAPS	This study	
36	40mM TRIS-Cl, pH 8, 150mM NaCl, 10mM Brij 58, 0.1mM MgCl2	This study	i
37	1.5M NH4 Acet, pH 7, 10mM Tween 20, 2mM EDTA	This study	
38	1.5M NH4 Acet, pH 7, 8mM d-BCHAP, 2mM EDTA	This study	
39	1.5M NH4 Acet, pH 7, 2mM CHAPS, 0.1mM MgCl2	This study	

40	1.5M NH4 Acet, pH 7, 10mM Brij 58, 0.1mM MgCl2	This study	
41	125mM Na Phos, pH 7, 15mM Triton X-100	This study	
42	125mM Na Phos, pH 7, 8mM d-BCHAP	This study	
43	125mM Na Phos, pH 7, 2mM CHAPS	This study	
44	125mM Na Phos, pH 7, 10mM Brij 58	This study	
45	250mM Na Phos, pH 7, 10mM Tween 20	This study	
46	250mM Na Phos, pH 7, 8mM d-BCHAP	This study	
47	250mM Na Phos, pH 7, 2mM CHAPS	This study	
48	250mM Na Phos, pH 7, 10mM Brij 58	This study	
49	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 15mM Triton X-100, 0.1mM MgCl2	This study	
50	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 8mM d-BCHAP, 0.1mM MgCl2	This study	
51	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 2mM CHAPS	This study	
52	20mM HEPES-K, pH7.4, 150mM KCl, 125mM K Acet, 5mM Taurode	This study	
53	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 10mM Tween 20, 2mM EDTA	This study	
54	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 8mM d-BCHAP, 2mM EDTA	This study	
55	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 2mM CHAPS	This study	
56	20mM HEPES-K, pH7.4, 150mM KCl, 250mM K Acet, 1mM Zwittergent 3-14	This study	
57	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 15mM Triton X-100, 0.1mM MgCl2	This study	
58	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 8mM d-BCHAP, 0.1mM MgCl2	This study	iii
59	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 2mM CHAPS	This study	
60	40mM TRIS-Cl, pH 8, 150mM NaCl, 125mM Na3 Cit, 5mM Taurode	This study	
61	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 10mM Tween 20, 2mM EDTA	This study	ii
62	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 8mM d-BCHAP, 2mM EDTA	This study	
63	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 2mM CHAPS	This study	
64	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 1mM Zwittergent 3-14	This study	

Rnt1p-GFP Extraction Solvents

Condition #	**Components**	**Reference**	**Main Figure**

1	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM Triton X-100, 5mM Taurode	This study	
2	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM Triton X-100, 5mM Zwit 3-10	This study	
3	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM Triton X-100, 0.5mM ASB-14	This study	
4	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM Triton X-100, 5mM d-BCHAP	This study	
5	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM DDM	This study	
6	20mM HEPES-K, pH7.4, 150mM NaCl, 20mM Saponin	This study	
7	20mM HEPES-K, pH7.4, 150mM NaCl, 15mM Mega-10	This study	
8	20mM HEPES-K, pH7.4, 150mM NaCl, 10mM Brij 58	This study	
9	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 5mM CHAPS	This study	
10	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 10mM d-BCHAP	This study	
11	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 15mM Triton X-100	This study	
12	20mM HEPES-K, pH7.4, 150mM NaCl, 50mM K Acet, 0.5mM LMNG	This study	
13	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 5mM CHAPS	This study	
14	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 10mM d-BCHAP	This study	
15	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 10mM Tween 20	This study	
16	20mM HEPES-K, pH7.4, 150mM NaCl, 250mM K Acet, 0.5mM LMNG	This study	
17	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 5mM CHAPS	This study	i
18	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 10mM d-BCHAP	This study	
19	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 15mM Triton X-100	This study	
20	40mM TRIS-Cl, pH 8, 150mM NaCl, 50mM Na3 Cit, 0.5mM LMNG	This study	
21	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit 5mM CHAPS	This study	
22	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit 10mM d-BCHAP	This study	
23	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 10mM Tween 20	This study	
24	40mM TRIS-Cl, pH 8, 150mM NaCl, 250mM Na3 Cit, 0.5mM LMNG	This study	
25	1.5M NH4 Acet, pH 7, 5mM CHAPS	This study	
26	1.5M NH4 Acet, pH 7, 10mM d-BCHAP	This study	
27	1.5M NH4 Acet, pH 7, 15mM Triton X-100	This study	
28	1.5M NH4 Acet, pH 7, 0.5mM LMNG	This study	
29	1.5M NH4 Acet, pH 7, 5mM ASB-14	This study	
30	1.5M NH4 Acet, pH 7, 20mM Saponin	This study	
31	1.5M NH4 Acet, pH 7, 10mM Tween 20	This study	

32	1.5M NH4 Acet, pH 7, 10mM Brij 58	This study	

RpoC-SpA Extraction Solvents

Condition #	Components	Reference	Main Figure
1	20mM HEPES pH 7.4, 2mM MgCl2, 150mM NaCl, 0.1% Tween 20	This study	
2	20mM HEPES pH 7.4, 2mM MgCl2, 300mM NaCl, 0.1% Tween 20	This study	
3	20mM HEPES pH 7.4, 2mM MgCl2, 500mM NaCl, 0.1% Tween 20	This study	
4	20mM HEPES pH 7.4, 2mM MgCl2, 1M NaCl, 0.1% Tween 20	This study	
5	20mM HEPES pH 7.4, 2mM EDTA, 150mM NaCl, 0.1% Tween 20	This study	
6	20mM HEPES pH 7.4, 2mM EDTA, 500mM NaCl, 0.1% Tween 20	This study	
7	20mM HEPES pH 7.4, 2mM MgCl2, 150mM NaCl, 1% Triton X-100	This study	
8	20mM HEPES pH 7.4, 2mM MgCl2, 300mM NaCl, 1% Triton X-100	This study	
9	20mM HEPES pH 7.4, 2mM MgCl2, 500mM NaCl, 1% Triton X-100	This study	
10	20mM HEPES pH 7.4, 2mM MgCl2, 150mM NaCl, 5mM CHAPS	This study	
11	20mM HEPES pH 7.4, 2mM MgCl2, 300mM NaCl, 5mM CHAPS	This study	
12	20mM HEPES pH 7.4, 2mM MgCl2, 500mM NaCl, 5mM CHAPS	This study	
13	150mM AmAc, 1% Triton X-100	This study	
14	300mM AmAc, 1% Triton X-100	This study	
15	600mM AmAc, 1% Triton X-100	This study	i
16	1.2M AmAc, 1% Triton X-100	This study	
17	40mM Tris pH 8.0, 1M urea, 1% Triton X-100	This study	
18	40mM Tris pH 8.0, 2M urea, 1% Triton X-100	This study	
19	40mM Tris pH 8.0, 3M urea, 1% Triton X-100	This study	ii
20	40mM Tris pH 8.0, 150mM NaCl, 0.5mM sarkosyl	This study	
21	40mM Tris pH 8.0, 150mM NaCl, 5mM sarkosyl	This study	
22	40mM Tris pH 8.0, 100mM Na3Cit, 1% Triton X-100	This study	
23	40mM Tris pH 8.0, 200mM Na3Cit, 1% Triton X-100	This study	
24	40mM Tris pH 8.0, 300mM Na3Cit, 1% Triton X-100	This study	

RRP6-3xFLAG Extraction Solvents

Condition #	Components	Reference	Main Figure
1	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM CHAPS	This study	
2	20mM HEPES-Na, pH 7.4, 150mM NaCl, 5mM CHAPS	This study	i
3	20mM HEPES-Na, pH 7.4, 200mM NaCl, 5mM CHAPS	This study	
4	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.5% Triton X-100	This study	
5	20mM HEPES-Na, pH 7.4, 150mM NaCl, 0.5% Triton X-100	This study	
6	20mM HEPES-Na, pH 7.4, 200mM NaCl, 0.5% Triton X-100	This study	
7	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM Sarkosyl	This study	iv
8	20mM HEPES-Na, pH 7.4, 100mM NaCl, 1% Triton X-100	This study	
9	20mM HEPES-Na, pH 7.4, 200mM NaCl, 1% Triton X-100	This study	
10	20mM HEPES-Na, pH 7.4, 300mM NaCl, 1% Triton X-100	This study	ii
11	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1% Triton X-100	This study	
12	20mM HEPES-Na, pH 7.4, 1000mM NaCl, 1% Triton X-100	This study	iii
13	20mM HEPES-Na, pH 7.4, 2000mM NaCl, 1% Triton X-100	This study	
14	20mM HEPES-Na, pH 7.4, 100mM MgCl2, 1% Triton X-100	This study	
15	20mM HEPES-Na, pH 7.4, 250mM MgCl2, 1% Triton X-100	This study	
16	20mM HEPES-Na, pH 7.4, 500mM MgCl2, 1% Triton X-100	This study	
17	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.02mM MgCl2, 1% Triton X-100	This study	
18	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.5mM EDTA, 1% Triton X-100	This study	
19	250mM NH4 Acet, pH 7, 1% Triton X-100	This study	
20	500mM NH4 Acet, pH 7, 1% Triton X-100	This study	
21	1000mM NH4 Acet, pH 7, 1% Triton X-100	This study	
22	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1M UREA, 1% Triton X-100	This study	
23	20mM HEPES-Na, pH 7.4, 500mM NaCl, 2M UREA, 1% Triton X-100	This study	
24	20mM HEPES-Na, pH 7.4, 500mM NaCl, 4M UREA, 1% Triton X-100	This study	

RBM7-LAP Screen A

Condition #	Components	Reference	Main Figure
1	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM CHAPS	This study	

2	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM Sarkosyl	This study	iv
3	20mM HEPES-Na, pH 7.4, 100mM NaCl, 1% Triton X-100	This study	
4	20mM HEPES-Na, pH 7.4, 250mM NaCl, 1% Triton X-100	This study	
5	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1% Triton X-100	This study	ii
6	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.5mM EDTA 1% Triton X-100	This study	
7	20mM HEPES-Na, pH 7.4, 100mM NaCl, 20uM ZnCl2, 1% Triton X-100	This study	
8	20mM HEPES-Na, pH 7.4, 250mM NaCl, 20uM ZnCl2, 1% Triton X-100	This study	
9	20mM HEPES-Na, pH 7.4, 500mM NaCl, 20uM ZnCl2, 1% Triton X-100	This study	
10	20mM HEPES-Na, pH 7.4, 250mM NaCl, 1M UREA, 3mM Sarkosyl	This study	
11	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1M UREA, 3mM Sarkosyl	This study	
12	20mM HEPES-Na, pH 7.4, 1000mM NaCl, 1M UREA, 3mM Sarkosyl	This study	
13	20mM HEPES-Na, pH 7.4, 200mM MgCl2, 1% Triton X-100	This study	
14	20mM HEPES-Na, pH 7.4, 400mM MgCl2, 1% Triton X-100	This study	
15	20mM HEPES-Na, pH 7.4, 800mM MgCl2, 1% Triton X-100	This study	
16	100mM NH4 Acet, pH 7, 1% Triton X-100	This study	
17	200mM NH4 Acet, pH 7, 1% Triton X-100	This study	
18	400mM NH4 Acet, pH 7, 1% Triton X-100	This study	
19	100mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
20	200mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
21	400mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
22	20mM HEPES-Na, pH 7.4, 100mM KPO4, 1% Triton X-100	This study	
23	20mM HEPES-Na, pH 7.4, 200mM KPO4, 1% Triton X-100	This study	
24	20mM HEPES-Na, pH 7.4, 400mM KPO4, 1% Triton X-100	This study	

RBM7-LAP Screen B

Condition #	**Components**	**Reference**	**Main Figure**
1	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM CHAPS	This study	
2	20mM HEPES-Na, pH 7.4, 250mM NaCl, 5mM CHAPS	This study	
3	20mM HEPES-Na, pH 7.4, 100mM NaCl, 25mM CHAPS	This study	
4	20mM HEPES-Na, pH 7.4, 250mM NaCl, 25mM CHAPS	This study	

5	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.05mM Sarkosyl	This study	
6	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0.5mM Sarkosyl	This study	
7	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM Sarkosyl	This study	
8	20mM HEPES-Na, pH 7.4, 100mM NaCl, 1% Triton X-100	This study	
9	20mM HEPES-Na, pH 7.4, 250mM NaCl, 1% Triton X-100	This study	
10	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1% Triton X-100	This study	
11	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1M UREA, 1% Triton X-100	This study	
12	20mM HEPES-Na, pH 7.4, 500mM NaCl, 2M UREA, 1% Triton X-100	This study	
13	20mM HEPES-Na, pH 7.4, 500mM NaCl, 4M UREA, 1% Triton X-100	This study	
14	20mM HEPES-Na, pH 7.4, 1000mM NaCl, 1M UREA, 1% Triton X-100	This study	
15	20mM HEPES-Na, pH 7.4, 1000mM NaCl, 2M UREA, 1% Triton X-100	This study	iii
16	20mM HEPES-Na, pH 7.4, 1000mM NaCl, 4M UREA, 1% Triton X-100	This study	
17	100mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
18	200mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
19	400mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
20	800mM NH4 Acet, pH 7, 100mM NaCl, 1% Triton X-100	This study	
21	100mM NH4 Acet, pH 7, 250mM NaCl, 1% Triton X-100	This study	
22	200mM NH4 Acet, pH 7, 250mM NaCl, 1% Triton X-100	This study	
23	400mM NH4 Acet, pH 7, 250mM NaCl, 1% Triton X-100	This study	
24	20mM HEPES-Na, pH7.4, 5% Triton X-100	This study	i

Supplementary Table 3

Standard Name	Systematic Name	Codon Adaptation Index
Act1	YFL039C	0.71
Cdc19	YAL038W	0.89
Tdh3	YGR192C	0.92
Tdh2	YJR009C	0.91
Eno2	YHR174W	0.89
Eno1	YGR254W	0.87
Pdc1	YLR044C	0.9
Tef1	YPR080W	0.87
Adh1	YOL086C	0.81
Tdh1	YJL052W	0.86
TEF2	YBR118W	0.88
PGK1	YCR012W	0.81
SSA2	YLL024C	0.8
SSA1	YAL005C	0.71
FBA1	YKL060C	0.87
GPM1	YKL152C	0.81
ADH2	YMR303C	0.51
SSB2	YNL209W	0.77
SSB1	YDL229W	0.82
TPI1	YDR050C	0.82
EFT1	YOR133W	0.8
HSC82	YMR186W	0.58
HSP82	YPL240C	0.52
PGI1	YBR196C	0.68
RPS8B	YER102W	0.72
RPS6B	YBR181C	0.85
RPS11B	YBR048W	0.73
RPL4A	YBR031W	0.8
PMA1	YGL008C	0.73
RPL3	YOR063W	0.83

Supplementary Table 5

Tagged protein	Gene or Systematic name	Strain / Cell line	Reference
Arp2p-GFP	YDL029W	*Sc* BY4741	Huh *et al*, 2003
Dpm1p-GFP	YPR183W	*Sc* BY4741	Huh et al, 2003
Ent2p-GFP	YLR206W	*Sc* BY4741	Huh *et al*, 2003
End3p-GFP	YNL084C	*Sc* BY4741	Huh *et al*, 2003
Rtn1p-GFP	YDR233C	*Sc* BY4741	Huh *et al*, 2003
Tcb1p-GFP	YOR086C	*Sc* BY4741	Huh *et al*, 2003
Tcb2p-GFP	YNL087W	*Sc* BY4741	Huh *et al*, 2003
Tcb3p-GFP	YML072C	*Sc* BY4741	Huh *et al*, 2003
Csl4p-TAP	YNL232W	*Sc* BY4741	Ghaemmaghami *et al*, 2003
Rtn1p-TAP	YDR233C	*Sc* BY4741	Ghaemmaghami *et al*, 2003
Snu71p-TAP	YGR013W	*Sc* BY4741	Ghaemmaghami *et al*, 2003
Nup1p-SpA	YOR098C	*Sc* DF5	Rout *et al*, 2000
Nup53p-SpA	YMR153W	*Sc* DF5	Rout *et al*, 2000
Pom152p-SpA	YMR129W	*Sc* DF5	Rout *et al*, 2000
RpoC-SpA	*rpoC*	*Ec* MG1655	LaCava *et al*, 2013
RRP6-3xFLAG	EXOSC10	HEK 293	Domanski *et al*, 2012
RBM7-LAP	RBM7	HeLa Kyoto	Domanski *et al*, 2012

Supplementary Note 1

New Database and Minimum Information Standard for the Curation of SDS-Polyacrylamide Gel Images in Affinity Proteomics.

This note describes a new informational web portal and database for the curation of SDS-polyacrylamide gel images displaying the results of protein affinity capture experiments – www.copurification.org. In conjunction with this service, we propose an extension to the minimum information about proteomics experiment (MIAPE) standard[1] that is specifically designated for affinity capture experiments. The proposed extension, MIAPE-AC, is currently in development and will integrate with existing MIAPE standards, including those for gel electrophoresis[2-4] and gel informatics[5], but is designed for the curation of specific data and metadata essential for analyzing, understanding, replicating, and developing affinity capture experiments – a technique that comprises a cornerstone of interactomic analyses.

Although in-solution approaches to the characterization of protein samples by mass spectrometry grow increasingly common, gel-based approaches[6, 7] remain valuable and effective. SDS-PAGE coupled with dye-based protein staining serves as more than a dimension of pre-fractionation to reduce sample complexity and maximize depth of coverage for subsequent MS analyses. For example, a single sample requires several hours to analyze by LC-MS/MS. Being a serial technique, comparing multiple samples, including the needed replicates, is both time and labor intensive. In contrast, we commonly analyze 100 samples at a time by SDS-PAGE and protein staining within the space of a few hours, and we use this as a platform to assess sample quality. This permits rigorous selection criteria

to be enforced on samples prior to MS and/or other analytical and preparative procedures that represent a significant investment of both time and effort, ensuring that our downstream experiments are carried out at a high standard of quality with an efficient use of human effort and instrument time. Moreover, there are technical difficulties associated with assigning relative abundances of different proteins within samples by MS because the MS signal intensity is highly dependent on the physicochemical properties of the peptides and the solvents used. Stable isotope methods based on spiking the sample with heavy isotope labeled chimeric proteins that contain a few peptides for each protein of interest have been used to determine stoichiometry[8, 9] but this method is time consuming and labor intensive and it is therefore not suited for screening. In contrast, staining proteins separated upon a gel commonly facilitates accurate estimates of the constituent relative and absolute abundances within the sample[10] (and see e.g.[11, 12]). Hence, PAGE and protein staining are highly complementary to MS and as a result, they rightfully remain commonplace in the development of affinity capture-based interactomic approaches. Despite this, and despite previous efforts to capitalize on the value of these data through reporting and standardization[2-5, 13], these data remain without effective informatics tools for data curation and management – preventing comparisons of work carried out within and between different laboratories, encouraging the duplication of work by different researchers in search of effective affinity capture conditions, and stymieing progress in mapping protein copurification profiles.

Our newly launched web portal and database addresses this need. After registering an account on the site, users are able to upload gel images displaying

the results of affinity capture experiments. Along with the gel images, users will also provide descriptive metadata. In the current version (v0.9, public beta release), these metadata are contained within three separate files: i) an optional "Experimental Procedures File" that should contain organized, human readable, text-based information about the protein expression system, the preparation of the sample and the affinity capture procedure; ii) an optional "Gel Details File" describing the gel electrophoresis, protein staining and imaging – this should preferably be MIAPE-GE[2, 4] compliant; and iii) a required "Sample Descriptions File" comprising a tab delimited text file describing the basics of the affinity capture, including the conditions of the protein purification as well as the affinity reagents used to achieve the purification. These descriptive metadata within the Sample Descriptions File are provided for every sample on the gel to be curated (summarized in **Table 1**).

Upon the version 1.0 release, the Experimental Procedures File and the Sample Descriptions File will be merged into a single, required document utilizing an XML format implementing controlled vocabulary. This would comprise an initial first attempt at a comprehensive minimum information standard for affinity capture experiments. Integration with other MIAPE[1] / MIBBI[14] standards will be ongoing with continuous updates, starting with MIAPE-GE[2] and -GI[5]; eventually yielding a comprehensive web form system that allows users to produce MIAPE[1] / MIBBI[14] compliant XML documents from data entered into our system.

This beta release provides public access to the affinity capture data produced as part of the accompanying main text and allows users to curate their own data privately for their own in-house use. Our site will always consist of a

private interface for in-house use by any registered user, with relaxed requirements regarding information standards, and a public interface where all data are subject to minimum information standards prior to acceptance for final curation and open public access. The ability for users to make their data publicly searchable on our system (e.g. in conjunction with publication) will be activated along with the initial completed standard, the XML format and the accompanying version 1.0 release including web forms to ease user data submission.

Detailed instructions on how to use our site are provided within the HOWTOs located at www.copurification.org. In return for providing these data and metadata the user and the community are granted access to advanced tools for indexing and sorting experimental results, enabling comparisons of experiments based on broad or more selective criteria. As a result, researchers can inform their decisions regarding the conditions of handling of their protein complexes for a wide range of analytical and preparative applications from this archive of the prior art; an example output is displayed in Figure 1.

Gel file name	Name of the gel image file corresponding to these data
Lane # on gel	Gel lane where the sample has been loaded
Total # of lanes on gel	Total number of lanes
Buffer, Concentration, Units*	Three separate entries: the chemical used as a buffer, and the magnitude and units of concentration
Salt, Concentration, Units*	Three separate entries: the chemical used as a salt, and the magnitude and units of concentration
Detergent, Concentration, Units*	Three separate entries: the chemical used as a detergent, and the magnitude and units of concentration
Other, Concentration, Units*	Three separate entries: the chemical, and the magnitude and units of concentration
pH	The final pH of the solution
Mass Ladder	The masses in kDa of the species in the ladder
Quantity Std Amount	The name, molecular mass, quantity loaded and units
Over-expression	Is this over-expressed? – yes / no / unknown

Protein Systematic Name	For S. cerevisiae, SGD systematic name; for Human and E. coli, GenBank Accession, RefSeq or Gene ID
Tag Type	Name of the affinity tag used
Tag location	Is this tag C-terminal, N-terminal or internal?
Antibody	The antibody reagent used for capture
Other capture	The non-antibody reagent used, or second antibody in the case of a tandem capture
Notes	Any additional informative notes

Table 1 | Sample descriptions file. This table summarizes the contents of the Sample Descriptions File, required to be submitted along with a gel image displaying protein copurification patterns. *As many buffer, salts, detergents or other additives can be described as were used in the affinity capture procedure – instructions and an example file are provided at www.copurifcation.org.

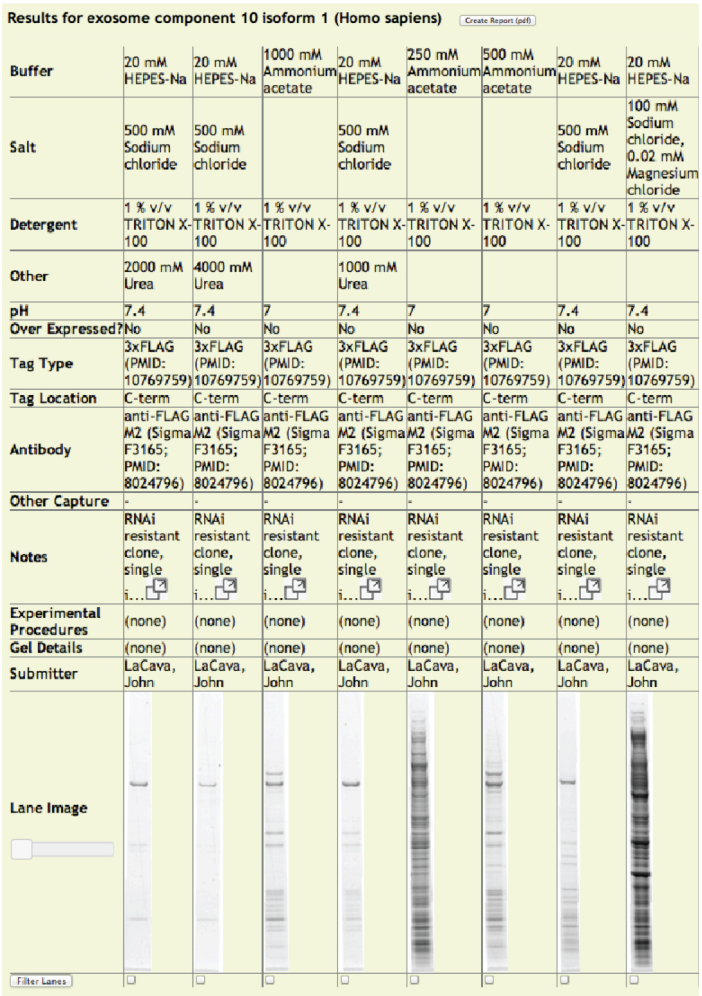


Figure 1 | Example database query return. A subset of result returned on an open search of purification profiles associated with human RRP6 (exosome component 10 isoform 1).

The software itself consists of three main functional parts: (1) a Relational Database where the user and project information, as well as gel descriptive information (contained within the Sample Descriptions File, Table 1) are stored, (2) a set of perl CGI scripts that encompass the main functionality of the web application, and (3) the lane clustering and image processing tools, i.e. lane detection, band detection, and band quantification.

Currently, the Relational Database implemented in MySQL stores all information pertaining to users that have registered an account with the system. Gel images and other descriptive information are grouped into Projects and Experiments, as specified by the user through our web interface, and these associations are also cataloged within the database. The descriptive information for each sample, submitted by the user in the tab-delimited Sample Descriptions text file, is stored in the database for each lane of each gel submitted, allowing subsequent queries to be performed based on these fields. Mass and quantity information is also stored for each lane, following lane sectioning and band detection. If a user has uploaded files describing the experimental procedures and or gel electrophoretic details, these will be cataloged as being associated with an Experiment in the Database. The contents of these files are not stored in the Database but are stored in a directory assigned to the user on the file system of the server.

The main functionality of the web application is implemented as a set of perl CGI scripts. Javascript is also used on the front-end to implement interactive forms and validation of input. In addition to producing the HTML pages to interact with the user, there are separate modules to perform the following functions: (1) verification of the data in the tab-delimited text file accompanying the gel image(s), (2) online verification of protein Systematic Names entered in the text file, and (3) an object-oriented interface to the MySQL database.

An important function of the main program is to provide the Gel Search feature for both public gels and a user's own collection of gels. When performing a search, multiple constraints may be chosen, such as Species, Protein, Submitter

(the person from whom the data originated), Reagents, and pH. The selections are translated into a series of SQL statements to be queried against the database and the results are returned in tabular format, with an option to convert to PDF. The shell utility, 'wkhtmltopdf' is utilized for the generation of the PDF file.

An additional feature provided to registered users is our lane-clustering tool. Once gels have been submitted by the user, clicking on the provided hyperlink will open a new page with a composite image of the gel lanes arranged in order of similarity of banding patterns. This grouping helps to highlight the degree of difference between profiles observed by simple visual inspection. In order to cluster the lanes, the algorithm calculates the dot product of band intensities among lanes and then groups these lanes based on their similarity score. A planned improvement to this tool will display a dendrogram to better illustrate the hierarchical nature of the relationship between lanes.

The algorithms for lane sectioning and band detection and quantification were developed in-house and are implemented in perl and make use of the image-processing tool ImageMagick (http://www.imagemagick.org). Since lanes are neither straight nor uniformly spaced, it is necessary to develop an algorithm to find the best division between lanes. Our algorithm searches for the line of minimum intensity between lanes in order to find this division. Once a lane has been sectioned, the middle 50% of the lane is used to locate the bands. The algorithm converts the image to a 1-dimensional array, where the x-values are the pixel positions along the length of the lane, and the y-values are the average pixel intensities. Smoothing and background subtraction are applied. First, the sum of pixel intensity at each point on the y-axis is calculated for the lane. Then, to obtain

background, these values are partitioned into bins and a minimum is found for each bin. The background subtracted from the sum at each point is the minimum of its current bin and a weighted average of the 2 surrounding bins. To detect the bands, the sum is calculated over every 3 points of the y-axis signal (intensity sum minus background), and sorted from largest to smallest. Each item in this list is considered as a peak indicating a band until 5% of the top sum is reached, at which point the band detection is halted. The width of the band is determined using a cutoff of 25% of the signal; the band must be at least 3 pixels wide to be detected. The sum of the intensity of pixels over the band width correspond to quantity. The absolute quantity data is calculated for each band based on the calibration lane(s) in the gel (if present). The ladder information given by the user is assigned to the most intense bands detected on the calibration lane. To calculate molecular weight (MW) for an experimental band, a line is fitted to the calibration masses (centroid pixel position vs. MW) and the MW is assigned based on where the band in question would fall on this line. Further implementations details can be found from the source code, which is available publicly on github at https://github.com/FenyoLab/copurification.

In summary, this software currently provides curation of affinity capture SDS-PAGE data to guide the understanding of protein copurification banding patterns and how they change with conditions, the selection of conditions for preparative and analytical procedures, and the development of new conditions of purification better suiting the needs of the experimentalist. However, future versions of this service will address a number of additional needs.

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Supplementary Note 2

Observations and biochemical validations

Nup53p: observations from automated affinity capture screening

Three distinct profiles were observed over the range of concentrations explored: at low trisodium citrate, a composite comprised of mutually exclusive dimers of Nup53p/Nup170p and Nup53p/Kap121p[31] (**Fig. 3c**, iv); as the concentration increased, the Kap121p interaction was lost (**Fig. 3c**, v); and as it increased further a larger subcomplex of the NPC was copurified (**Fig. 3c**, vi) consisting of, the inner ring scaffold, FG-Nups and transport factors Kap60p and Kap95p.

Arp2p: secondary affinity capture of Ent2p and End3p

In addition to the Arp2/3 complex components, Arp2p-GFP co-purified Pan1p and Chc1p as well as End3p (with established genetic links to Arp2p[9]) and Ent2p (with no previously established direct physical links to Arp2p). However, taken together, Ent2p, Pan1p, and End3p have biochemical and functional links to both clathrin (Chc1p) coated vesicles and the actin (Act1p, Arp2/3 complex) cytoskeleton[10-14]. We therefore performed a secondary affinity capture with GFP-tagged Ent2p and End3p. Both copurified Pan1p (a bona fide physical interactor of Arp2p[13]) and Chc1p (in common with Arp2p-GFP) and Sla1p (as expected[10,15]). Additionally, Ent2p copurified Myo2p (type V myosin) and End3p-GFP copurified Ent2p (in common with Arp2p-GFP). Although Ent2p

and End3p did not reciprocally co-purify Arp2p, there are a number of reasons why a reciprocal affinity capture (when a protein discovered as prey is subsequently used as bait) may not precisely recapitulate the initial result[16]. Importantly, however, Pan1p and Chc1p, were identified as common to all three affinity capture experiments, and End3p also purified Ent2p, demonstrating that Arp2p, Ent2p, and End3p, have overlapping interactomes.

Rtn1p: observations from I-DIRT

In addition to supporting the *in vivo* association of previously uncharacterized interactors Dpm1p and Tcb1-3p with Rtn1p, I-DIRT also revealed a number of previously known physical interactors including Yop1p[17], Sey1p[18], Lnp1p[19], Rtn2p[20], Sac1p, and Scs2p[21]. Deeper interactomic analyses revealed that the proteins copurifying with Rtn1p-GFP segregated into two I-DIRT populations (**Fig. 5a**). Abundant housekeeping proteins dominated the first population and exhibited isotopic ratios consistent with interactions formed post-extraction (as expected)[22-24]. This population included some common contaminants seen as bands by SDS-PAGE, consistent with their high cellular abundance and frequency of occurrence in affinity capture experiments[5,23,25]. In contrast, the second population, which exhibited isotopic ratios consistent with enrichment for interactions formed *in vivo*, mainly contained ER and lipid biosynthesis proteins; corroborating the predominant ER localization of Rtn1p and the role of ER in lipid biosynthesis (reviewed in[26]). This population included the above-mentioned previously characterized and uncharacterized interactors.

Exosome complex: observations from affinity capture screening

Screening the exosome complex from both yeast and human cells provided us the opportunity to make some interactomic comparisons. Among other differences from the yeast complex, copurification of the key enzyme component, hDIS3, along with the core complex has proven particularly challenging in human cell model systems[27-29]. Notably, we were able todemonstrate affinity capture conditions where the hDIS3 protein was readily copurified with the hRRP6-containing exosome complex (**Fig. 4c**, RRP6-3xFLAG, compare profile I to II) even at salt concentrations previously thought incompatible with such cocapture[28]. Another difference between the yeast and human exosome is the tenacious retention of SKIV2L2 (hMTR4) within the purified human exosome (e.g. compare **Fig. 4c**, Csl4p-TAP to RRP6-3xFLAG, I and II). Despite its stability we were able to fractionate (SKIV2L2) away from RRP6-containing exosomes – yielding an endogenous exosome preparation lacking this putative helicase (**Fig. 4c**, RRP6-3xFLAG, III) – and we also isolated RRP6-3xFLAG, alone (**Fig. 4c**, RRP6-3xFLAG, IV).

NEXT complex: observations from affinity capture screening

Although we have previously described the set of interactors including NCBP1 (CBP80), SRRT (ARS2), and ZC3H18 (NHN1) (Fig. 4c, RBM7-LAP, I), we were only able to observe them by deep digging in quantitative tandem MS, utilizing approximately seven times more material per purification[30], whereas

during the present screening we observed these proteins above background by Coomassie blue staining.

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Supplementary Data

Mass spectrometry analysis of selected bands from affinity capture screens.

This supplement contains tables sumarizing the MS results generated during the course of this study. Each table corresponds to the results displayed in a main figure, indicated in the top left of the table. The corresponding gel from which the protein bands were excised for analysis is shown below the table. The tables are presented in the same order as the results appear in main text.

Images of the original gels resulting from affinity capture screening are presented in Supplementary Figures 2, 5-11. In some cases, analyzed lanes are presented that are in addition to those in main text, to convey the breadth of our examination. Analyzed bands were either cut directly from the gel produced in the screen, or from a subsequent gel run using a sample prepared under identical conditions as determined through the screen. Each analyzed protein band is indicated on the image of the actual gel lane from which it was obtained. The numbers above the lanes of the imaged gel correspond to the extraction solvent used to produce that affinity capture profile, and can be crossreferenced to Supplementary Table 1 and Methods. Where duplicate lanes are presented, the bands in both lanes were excised and pooled for Mass spectrometric analysis.

The search engine used is indicated at the top of each list of protein IDs. For ProFound search results from MALDI-TOF data we provide the E-value, the coverage (% of the protein sequence observed) and # of different peptides assigned to the protein ID. An ID by PMF was considered significant if it yielded an E-value of below 0.01 and at least 3 different peptide masses could be assigned to the protein. For X! Tandem search results from MS/MS sequencing data we provide the log E-value, the coverage (% residues / % corrected - which encompasses the % of residues observed and the % residues correct for those generally expected to be observed), and the number of unique peptides assigned to the protein. An ID by MS/MS was considered significant if it yielded a log E-value better than the first false positive retrieved from a decoy database of reversed protein sequence and had at least 4 unique peptides assigned to it. Protein IDs meeting these criteria are listed in the table. Keratins, BSA and trypsin were ignored in reporting search results.

All raw data and full search engine results are available on request.

Figure 3a Nup1p, Nup53p, Pom152p

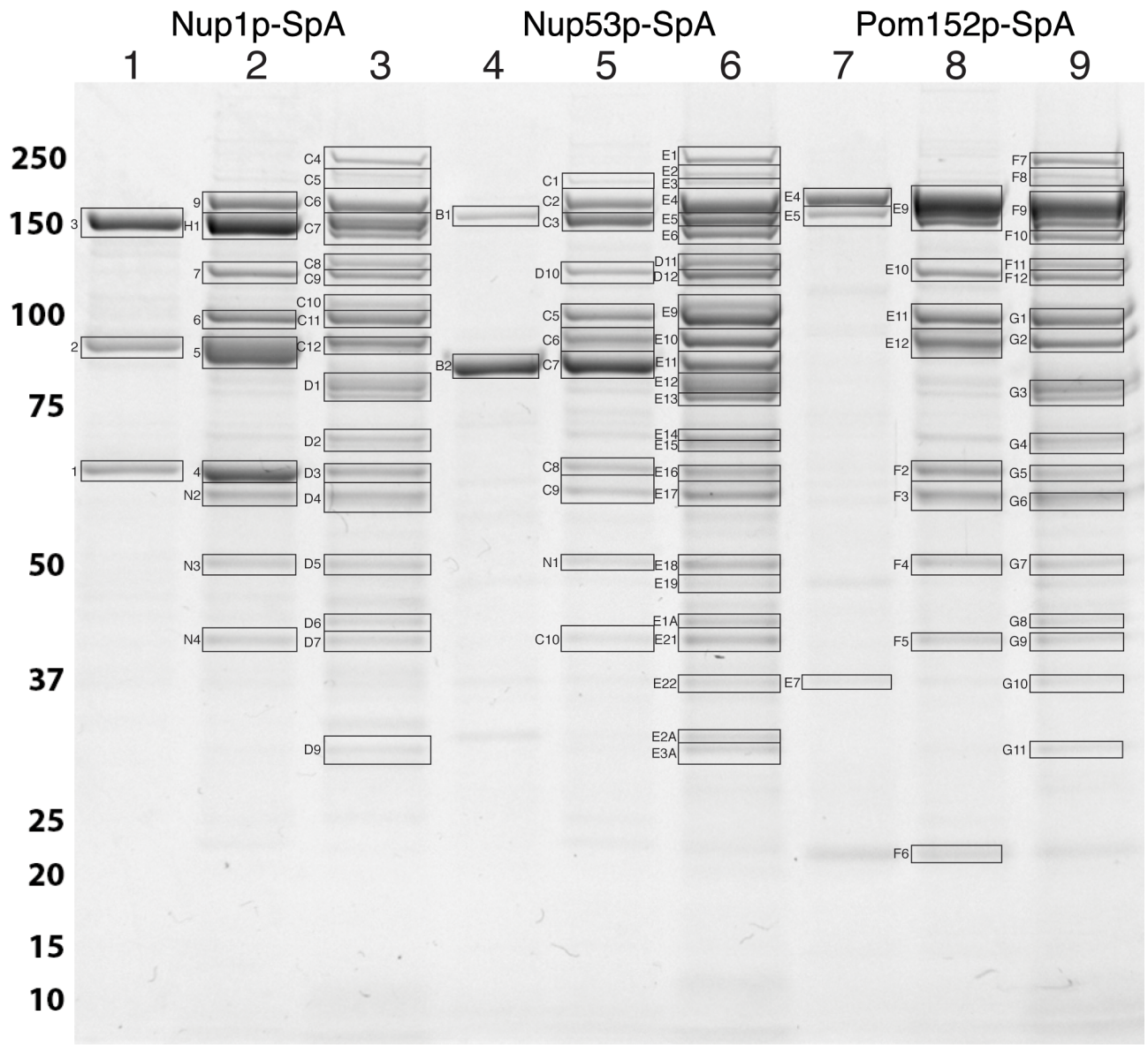
MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
1	3	Nup1p	139.6	8.9E-05	15	8	tagged protein
1	2	Kap95p	94.8	5.3E-07	24	10	transport factor
1	1	Kap60p (Srp1p)	60.4	7.0E-08	34	9	transport factor
2	9	Nup188, Nup192	188.6, 191.5	3.9E-08, 3.9E-05	15, 8	11, 8	
2	H1	Nup1, Nup170	139.6, 169.5	8.0E-06, 1.3E-03	29, 13	17, 13	tagged protein (Nup1p)
2	7	Nup116p	116.2	7.7E-18	8	4	
2	6	Nsp1p	86.5	6.8E-03	8	3	< 4 peptides
2	5	Kap95p, Nic96p	94.8, 96.2	1.8E-10, 2.4E-03	27, 8	10, 4	transport factor (Kap95p)
2	4	Kap60p (Srp1p)	60.4	5.0E-10	35	10	transport factor
2	N2	Nup57p	57.5	3.8E-05	17	8	
2	N3	Nup49p	49.1	9.8E-05	10	4	
2	N4	Gle2p	40.5	1.5E-03	12	4	transport factor
3	C4	Mlp1p	218.5	6.2E-08	12	13	
3	C5	Mlp2p	195.1	6.4E-04	8	7	
3	C6	Nup192p, Nup188p	191.5, 188.6	3.5E-12, 9.6E-12	22, 19	24, 16	
3	C7	Nup170p, Nup157p, Nup1p	169.5, 156.7, 139.6	6.8E-09, 3.0E-07, 8.9E-05	20, 14, 16	18, 12, 7	tagged protein (Nup1p)
3	C8	Nup133p	133.3	1.2E-15	35	20	
3	C9	Nup116p	116.2	9.7E-04	11	7	
3	C10	Kap120p	119.6	3.5E-08	21	13	transport factor
3	C11	Nup120p	120.4	2.4E-08	24	14	
3	C12	Nic96p, Kap95p	96.2, 94.8	2.5E-06, 1.0E-08	23, 26	14, 10	transport factor (Kap95p)
3	D1	Nup84p, Nup82p, Nup85p, Nup145C	83.6, 82.1, 84.9, 81.1	7.3E-06, 3.8E-04, 1.1E-03, 9.4E-04	32, 25, 18, 12	15, 13, 9, 10	
3	D2	Mex67p, Nup145N	67.4, 64.6	6.7E-05, 2.3E-04	36, 14	11, 11	transport factor (Mex67p)
3	D3	Nup59p, Kap60p (Srp1p), Gle1p	58.8, 60.4, 62.1	2.1E-06, 6.6E-10, 8.9E-03	31, 29, 15	11, 11, 5	transport factors (Kap60p, Gle1p)

3	D4	Nup53p, Nup57p	52.6, 57.5	1.5E-04, 1.8E-04	38, 22	10, 9	
3	D5	Nup49p	49.1	7.4E-06	30	8	
3	D6	Seh1p	39.1	1.3E-08	44	12	
3	D7	Gle2p	40.5	7.0E-07	48	11	transport factor
3	D9	Sec13p	33	5.6E-08	65	14	
4	B1	Nup170p	169.5	4.2E-21	32	28	
4	B2	Nup53p	78.6	7.6E-04	43	10	tagged protein
5	C1	Nup159p	158.9	2.8E-05	16	11	
5	C2	Nup192p, Nup188p	191.5, 188.6	5.3E-09, 1.4E-07	23, 18	22, 16	
5	C3	Nup170p	169.5	9.2E-13	36	37	
5	D10	Nup116p	116.2	1.9E-08	15	12	
5	C5	Nsp1p, Nup100p	86.5, 100	8.0E-14, 2.2E-03	37, 13	17, 5	
5	C6	Nic96p, Kap95p	96.2, 94.8	1.1E-10, 1.3E-08	27, 33	22, 14	transport factor (Kap95p)
5	C7	Nup53p	78.6	7.7E-04	31	7	tagged protein
5	C8	Nup59p, Kap60p (Srp1p)	58.8, 60.4	4.4E-07, 1.4E-04	38, 30	11, 8	transport factor (Kap60p)
5	C9	Nup57p	57.5	1.4E-04	21	11	
5	N1	Nup49p	49.1	2.4E-04	10	5	
5	C10	Gle2p	40.5	1.2E-07	47	11	transport factor
6	E1	Mlp1p	218.5	8.8E-06	15	19	
6	E2	Mlp2p	195.1	4.5E-04	10	11	
6	E3	Nup159p	158.9	2.9E-07	15	12	
6	E4	Nup188p, Nup192p	188.6, 191.5	1.8E-11, 1.4E-14	32, 28	29, 28	
6	E5	Nup170p	169.5	1.5E-17	42	45	
6	E6	Nup157p	156.7	4.2E-15	46	39	
6	D11	Nup133p, Nup116p	133.3, 116.2	4.8E-17, 2.8E-04	43, 10	34, 7	
6	D12	Nup116p, Nup133p	116.2, 133.3	7.3E-08, 7.4E-07	18, 12	14, 12	
6	E9	Nsp1p, Nup120p, Kap120p, Nup100p	86.5, 120.4, 119.6, 100	3.2E-10, 8.8E-06, 2.0E-03, 2.0E-04	53, 19, 15, 14	24, 13, 7, 6	transport factor (Kap120p)
6	E10	Nic96p	96.2	2.5E-07	35	23	

6	E11	Nup53p	78.6	2.6E-06	31	8	tagged protein
6	E12	Nup85p, Nup145C, Nup84p	84.9, 81.1, 83.6	1.6E-10, 1.9E-05, 9.0E-03	34, 11, 18	17, 10, 7	
6	E13	Nup82p, Nup84p, Nup145C	82.1, 83.6, 81.1	5.8E-09, 1.5E-09, 6.5E-05	41, 37, 10	22, 17, 8	
6	E14	Nup145N, Ssb1p/Ssb2p	64.6, 66.6/66.6	8.2E-10, 6.2E-03/ 6.2E-03	21, 15, 13	18, 5, 4	Ssb1/2p are abundant contaminants
6	E15	Mex67p	67.4	2.8E-10	32	13	transport factor
6	E16	Nup59p, Gle1p	58.8, 62.1	7.9E-10, 7.7E-03	21, 12	10, 5	transport factor (Gle1p)
6	E17	Nup57p	57.5	6.7E-09	29	14	
6	E18	Nup49p	49.1	1.5E-06	36	10	
6	E19	Eno2p	46.9	1.7E-07	62	22	abundant contaminant
6	E1A	Seh1p	39.1	2.0E-10	53	14	
6	E21	Gle2p	40.5	9.8E-09	48	11	
6	E22	Tdh3p	35.7	3.2E-06	48	11	abundant contaminant
6	E2A	Sec13p	33	3.3E-05	28	6	
6	E3A	Sec13p	33	4.0E-06	34	10	
7	E4	Pom152p	177.6	5.9E-11	30	29	tagged protein
7	E5	Pom152p	177.6	1.4E-12	32	30	tagged protein
7	E7	Tdh3p	35.7	1.7E-06	59	10	abundant contaminant
8	E9	Pom152p, Nup188p, Nup170p, Nup192p	177.6, 188.6, 169.5, 191.5	2.6E-07, 2.7E-03, 1.9E-03, 4.4E-03	27, 12, 12, 8	24, 10, 12, 10	tagged protein (Pom152p)
8	E10	Nup116p	116.2	1.2E-05	12	8	
8	E11	Nsp1p, Nup100p	86.5, 100	1.6E-14, 9.2E-03	46, 12	21, 5	
8	E12	Kap95p, Nic96p	94.8, 96.2	9.6E-07, 4.6E-11	32, 22	14, 15	transport factor (Kap95p)
8	F2	Kap60p (Srp1p)	60.4	2.3E-10	46	11	transport factor
8	F3	Nup57p, Nup53p	57.5, 52.6	9.5E-07, 4.9E-04	19, 28	10, 7	
8	F4	Nup49p	49.1	1.0E-03	21	10	
8	F5	Gle2p	40.5	3.5E-09	44	11	transport factor
8	F6	Lsm12p	21.3	1.2E-06	52	10	protein of unknown function

9	F7	Mlp1p	218.5	1.2E-10	20	23	
9	F8	Mlp2p	195.1	8.6E-05	15	18	
9	F9	Pom152p, Nup170p, Nup188p, Nup192p	177.6, 169.5, 188.6, 191.5	8.2E-10, 5.8E-09, 9.6E-05, 5.5E-03	17, 7, 6, 4	16, 9, 6, 6	tagged protein (Pom152p)
9	F10	Nup157p	156.7	1.8E-08	27	23	
9	F11	Nup133p	133.3	1.2E-11	26	27	
9	F12	Nup116p	116.2	1.8E-14	17	13	
9	G1	Nup120p, Kap120p	120.4, 119.6	1.7E-08, 6.0E-06	25, 16	16, 8	transport factor (Kap120p)
9	G2	Nic96p	96.2	1.3E-06	24	15	
9	G3	Nup84p, Nup82p	83.6, 82.1	2.6E-05, 7.0E-03	17, 10	7, 5	
9	G4	Mex67p	67.4	6.3E-03	16	6	transport factor
9	G5	Nup59p, Gle1p	58.8, 62.1	3.6E-07, 3.0E-06	36, 22	11, 8	transport factor (Gle1p)
9	G6	Nup57p	57.5	2.9E-05	18	8	
9	G7	Nup49p	49.1	8.3E-07	30	8	
9	G8	Seh1p	39.1	2.3E-06	33	7	
9	G9	Gle2p	40.5	3.6E-04	34	7	transport factor
9	G10	Tdh3p	35.7	9.6E-04	43	6	abundant contaminant
9	G11	Sec13p	33	8.7E-09	56	9	



Nature Methods: doi:10.1038/nmeth.3395

Figure 3c Nup53p-SpA

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
50 (iv)	1	Nup170p	170.2	1.3E-25	14%	15	
50 (iv)	2	Kap121p	121.9	2.4E-38	11	7	transport factor
50 (iv)	3	Nup53p	52.7	6.5E-03	17	5	tagged protein
250 (vi)	1	Nup192p, Nup170p, Nup188p	192.6, 170.2, 189.0	2.5E-24, 2.1E-19, 6.5E-15	5, 5, 5	6, 5, 4	
250 (vi)	2	Nup170p	170.2	3.5E-21	17	18	
250 (vi)	3	Nup116p	116.4	1.9E-20	8	6	
250 (vi)	4	Nup120p, Nsp1p	121.5, 84.5	1.8E-05, 1.0E-02	8, 6	7, 4	
250 (vi)	5	Nic96p	96.4	8.2E-44	15	10	
250 (vi)	6	Nup53p	52.66	7.5E-13	16	4	tagged protein

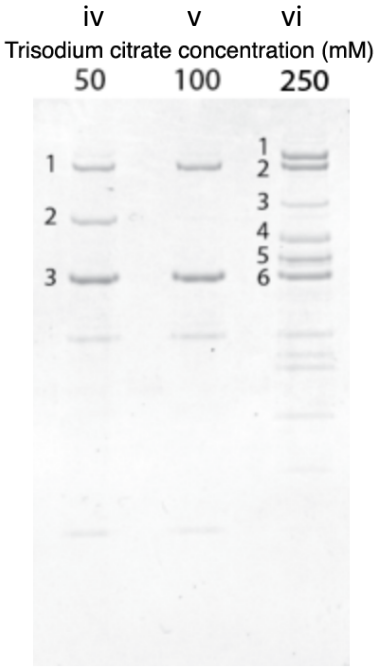


Figure 4a Ent2p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Myo2p	180.6	-1057.9	55/71	91	
1	1	Chc1p	187.1	-564.1	35/50	56	
1	1	Pan1p	160.2	-479.9	31/92	41	
1	2	Sla1p	135.8	-448.8	37/84	44	
1	3	Ent2p	71.8	-405.5	46/100+	36	tagged protein
1	4	Tdh3p	35.7	-335	47/55	31	

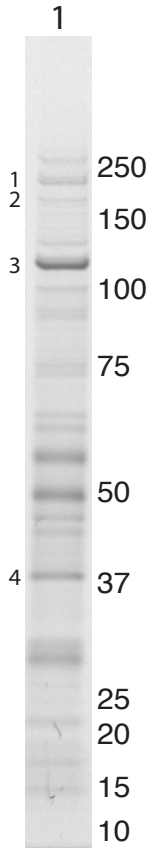
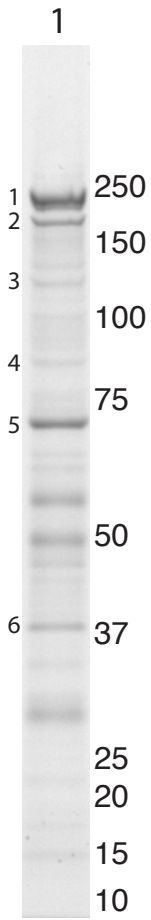


Figure 4a End3p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Pan1p	160.2	-907.9	49/100+	73	
1	1	Chc1p	187.1	-871.9	50/72	76.5	
1	2	Sla1p	135.8	-608.4	43/97	57	
1	2	Pan1p	160.2	-571.7	32/94	49	
1	3	Pan1p	160.2	-630.6	40/100+	52	
1	3	Sla2p	108.8	-484.5	46/70	42	
1	4	Pan1p	160.2	-454.5	29/86	40	
1	4	Ent2p	71.8	-315.5	41/93	26	
1	5	End3p	40.3	-483.3	64/88	40	tagged protein
1	6	Tdh3p	35.7	-328.1	60/70	30	

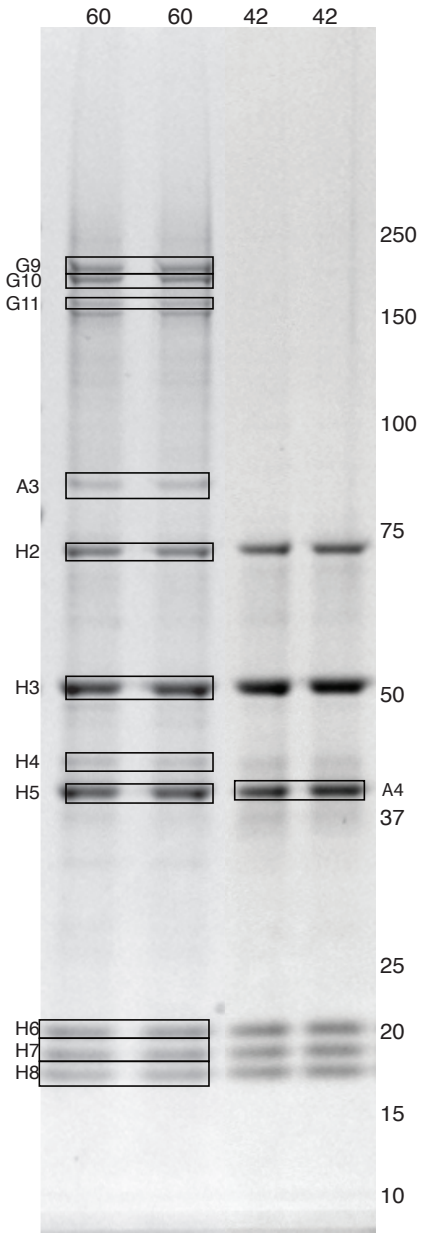


Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Arp2p-GFP

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
60	G9	Pan1p, Arc40p	160.3, 42.5	8.9E-09, 5.8E-02	26, 35	19, 5	
60	G10	Chc1p, Arc40p	187.2, 42.5	2.2E-10, 3.6E-03	34, 45	28, 7	
60	G11	Pan1p, Arc40p	160.3, 42.5	6.1E-06	14, 41	12, 6	
60	A3	Ent2p	71.8	6.7E-09	22	11	
60	H2	Arp2p, Arc40p, Arp3p	71.9, 42.5, 49.5	4.2E-04, 4.0E-03, 6.7E-03	37, 38, 39	11, 7, 7	tagged protein
60	H3	Arp3p	49.5	1.8E-09	57	16	
60	H4	Act1p, Act35p, Arp3p	41.7, 39.6, 49.5	4.3E-04, 9.2E-07, 4.5E-03	55, 39, 27	10, 9, 6	
60	H5	Arc35p, End3p	39.6, 40.3	1.4E-07, 3.3E-03	62, 30	16, 8	
60	H6	Arc19p	19.9	1.3E-07	73	10	
60	H7	Arc18p	20.6	1.1E-04	52	6	
60	H8	Arc15p	17.1	5.0E-06	69	9	
42	A4	Arp3p	49.5	3.0E-08	38	14	



Nature Methods: doi:10.1038/nmeth.3395

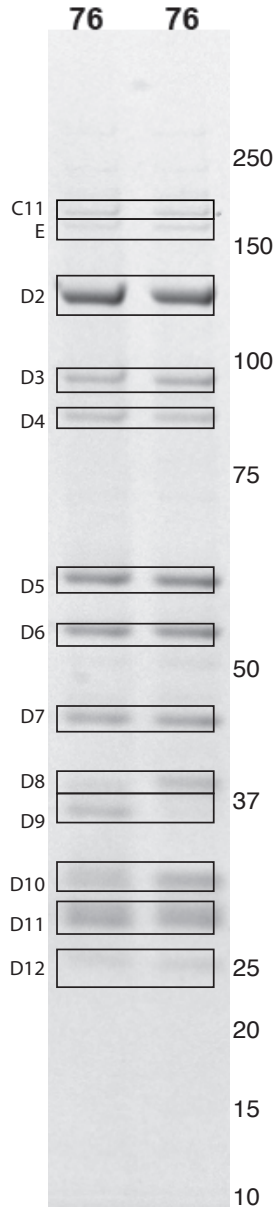
Figure 4a Csl4p-TAP

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
76	C11	Ski3p	163.7	2.0E-03	12	11	
76	D2	Dis3p	113.7	8.1E-12	43	30	
76	D3	Rrp6p	84	1.2E-10	34	17	
76	D4	Ski7p	84.8	8.8E-12	40	15	
76	D5	Csl4p	57.6	3.2E-04	32	8	tagged protein
76	D6	Rrp43p	44	9.6E-08	50	14	
76	D7	Rrp4p	39.4	5.7E-09	62	15	
76	D8	Rrp45p, Tdh3p	34, 35.7	9.2E-05, 6.7E-03	40, 35	10, 6	
76	D9	Rrp45p	34	5.2E-04	31	9	
76	D10	Rrp42p, Ski6p	29.1, 27.6	3.3E-05, 1.3E-03	60, 24	10, 8	
76	D11	Rrp40p, Rrp46p, Mtr3p	26.6, 24.4, 27.6	6.2E-05, 5.1E-03, 5.6E-03	54, 43, 48	11, 7, 7	
76	D12	Lrp1p	21	3.5E-06	56	11	

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
76	E	Ski2p	146.1	-417.2	42/60	51	



Nature Methods: doi:10.1038/nmeth.3395

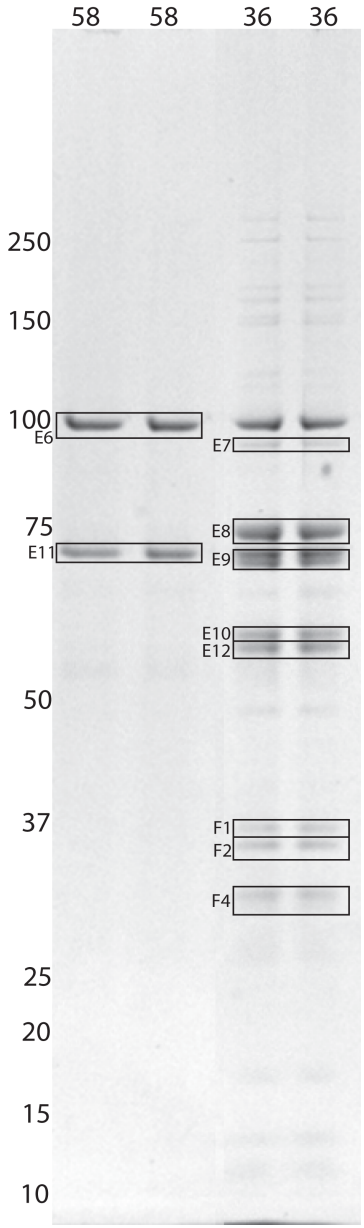
Figure 4a Snu71p-TAP

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
58	E6	Snu71p	92.4	4.9E-11	41	22	tagged protein
58	E11	Prp40p	69.1	4.0E-08	37	16	
36	E7	Sto1p	100	6.4E-10	40	19	
36	E9	Prp39p, Prp40p, Pab1p	74.7, 69.1, 64.3	3.7E-10, 2.2E-06, 8.6E-03	41, 26, 27	22, 14, 6	
36	E10	Prp42p, Nam8p	65.1, 57.0	3.4E-10, 2.1E-03	40, 28	19, 7	
36	E12	Nam8p, Snu56p	57.0, 56.5	2.2E-05, 8.1E-04	30, 28	8, 7	
36	F1	Snp1p	34.4	2.1E-05	37	10	
36	F4	Luc7p	30.2	3.7E-04	32	8	

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
36	E8	Gag_ScVLA	76	-201.1	25/51	17	S. cerevisiae virus L-A major capsid protein
36	F2	Mud1p	34.4	-290.2	65/88	30	

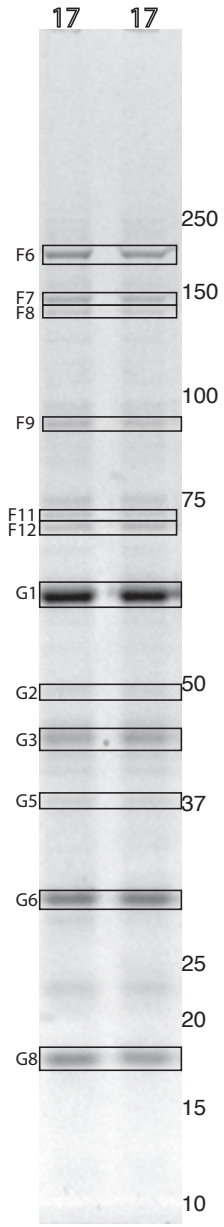


Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Rtn1p-GFP

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
17	F6	Tcb3p	171.1	5.30E-09	30	24	
17	F7	Tcb1p	133.6	3.50E-07	26	15	
17	F8	Tcb2p	132.5	6.50E-07	22	13	
17	F9	Pma1p	99.6	1.20E-04	23	12	
17	F11	SSa1p/ Ssa2p	69.7/69.5	1.60E-10	47/36	18/16	98% identical paralogs
17	F12	Ssb1p/Ssb2p	66.6/66.6	9.80E-10	51/48	19/18	paralogous proteins
17	G1	Rtn1p	60.7	6.60E-04	41	9	tagged protein
17	G2	Tef2p/Ted1p	50/ 50	3.40E-07	36	10	paralogous proteins
17	G3	Pep4p	44.5	1.30E-12	55	12	
17	G5	Tdh3p	35.7	1.20E-03	37	7	
17	G6	Dpm1	30.4	1.20E-04	57	14	
17	G8	Yop1p	20.3	9.30E-11	18	3	restricted search to 25KDa, < 4 peptides

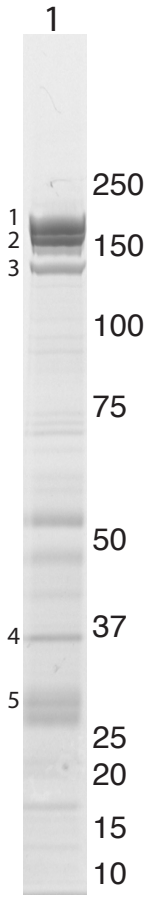


Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Tcb1p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Tcb3p	171	-1237.1	62/100+	100.5	
1	1	Tcb1p	133.5	-408.2	39/56	38	tagged protein
1	2	Tcb1p	133.5	-749.7	54/78	61	tagged protein
1	2	Tcb3p	171	-712.2	48/84	58.5	
1	3	Tcb2p	132.4	-723	46/65	70.2	
1	4	Rtn1p	32.9	-254.2	68/81	26	
1	4	Tdh3p	35.7	-195.5	52/61	20	
1	5	Dpm1p	30.3	-119.5	49/60	14	

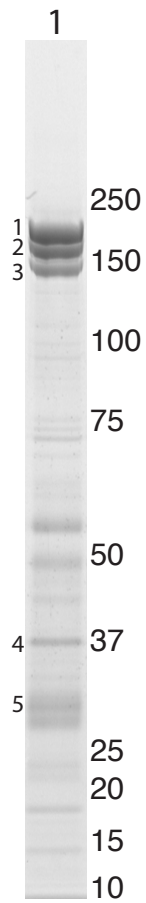


Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Tcb2p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Tcb3p	171	-1234.1	62/100+	100.5	
1	2	Tcb3p	171	-781.6	48/84	63	
1	2	Tcb2p	132.4	-750.4	54/77	69.5	tagged protein
1	3	Tcb1p	133.5	-743.2	52/74	61	
1	3	Tcb3p	171	-527.5	35/60	44.5	
1	3	Tcb2p	132.4	-498.2	41/59	47	tagged protein
1	4	Rtn1p	32.9	-252	68/80	26	
1	4	Tdh3p	35.7	-176.3	44/52	18	
1	5	Dpm1p	30.3	-119.9	49/60	14	

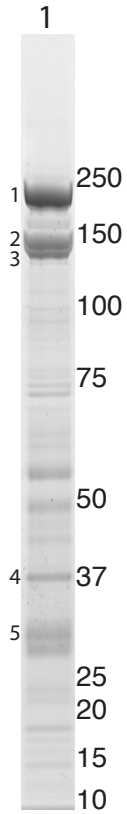


Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Tcb3p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Tcb3p	171	-1159.1	60/100+	93.5	tagged protein
1	2	Tcb1p	133.5	-835.5	53/76	67.5	
1	2	Tcb2p	132.4	-378	34/48	36	
1	3	Tcb2p	132.4	-815.8	52/75	74	
1	3	Tcb1p	133.5	-501.8	43/62	44.5	
1	4	Rtn1p	32.9	-255.5	68/81	26	
1	4	Tdh3p	35.7	-194.4	45/54	20	
1	5	Dpm1p	30.3	-115.4	49/60	13	
1	5	Rps3p	26.5	-114.4	49/62	15	



Nature Methods: doi:10.1038/nmeth.3395

Figure 4a Dpm1p-GFP

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
1	1	Dpm1p	30.3	-180.9	60/73	18	tagged protein
1	2	Tdh3p	35.7	-235.6	52/62	23	
1	2	Rtn1p	32.9	-207.6	57/67	23	

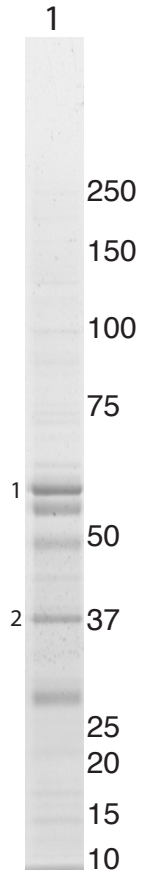


Figure 4b RpoC-SpA

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
16	1	RpoC	155.1	-518.2	40 / 50	61.5	tagged protein
16	2	RpoB	150.5	-727.8	56 / 69	82.5	
16	2	RpoC	155.1	-21.9	2.5 / 3	4	
16	3	RapA	109.7	-118.4	17 / 20	17	
16	4	RpoD	70.2	-241	35 / 72	31	Sigma 70
16	5	RpoA	36.5	-176.5	53 / 67	22	
16	5	OmpC	40.3	-50.1	23 / 24	8	outter membrane protein
16	5	usg	36.3	-23.5	9.8 / 16	4	predicted semialdehyde dehydrogenase
16	6	SpA	55.5	-167.1	29 / 35	20	SpA-tag breakdown product
16	7	RpoZ	10.2	-50.3	82 / 96	8	
21	1	SpA	55.5	-105	25 / 30	13	SpA-tag breakdown product
21	2	SpA	55.5	-100.9	23 / 28	14	SpA-tag breakdown product

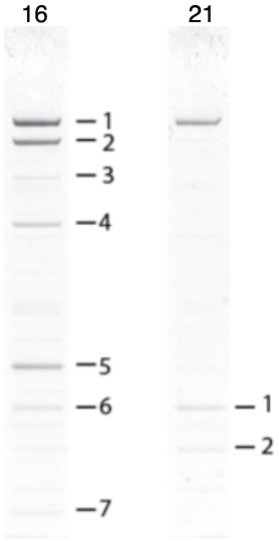


Figure 4c RRP6-3xFLAG

MALDI-TOF Searched in ProFound

lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
1	1	DIS3	110.12	2.7E-16	32	19	
1	2	SND1	102.66	6.7E-04	12	7	
1	3	SFPQ	76.25	1.6E-07	24	12	
2	4	NVL	85.99	2.4E-07	27	15	
2	4	PWP2H	103.4	2.0E-04	14	9	
2	4	EXOSC10	101.61	4.4E-05	17	8	RRP6; tagged protein
2	5	no significant hits					
2	6	SFPQ	68.73	4.3E-05	19	7	
2	7	TBL3	90.39	4.8E-14	32	16	
3	8	SKIV2L2	119.27	7.7E-17	28	22	
7	9	EXOSC10	101.61	1.0E-11	28	16	RRP6; tagged protein
10	10	NVL	96.06	7.3E-10	22	12	
10	10	EXOSC10	98.87	7.3E-05	15	7	RRP6
10	11	ZCCHC8	79.22	2.7E-06	26	6	NEXT complex xomponent
10	12	EXOSC9	51.41	3.4E-08	43	8	RRP45
10	13	Ribosomal protein L3	40.31	9.4E-03	23	5	
10	14	EXOSC7	32.31	6.9E-04	32	6	RRP42
10	15	EXOSC2	33	1.9E-06	57	10	RRP4
10	16	EXOSC3	30.01	2.0E-03	64	9	RRP40
10	17	EXOSC6	28.5	1.7E-02	50	9	MTR3
10	18	EXOSC4	26.65	1.4E-03	29	6	RRP41
10	18	EXOSC5	25.94	4.9E-04	46	5	RRP46
10	19	EXOSC5	25.94	9.5E-09	65	8	RRP46
10	20	EXOSC1	21.78	5.5E-05	39	6	CSL4
10	21	MPHOSPH6	19.06	4.8E-03	44	6	MPP6, RRP6 interactor
10	22	no significant hits					
10	23	no significant hits					
14	24	Myosin 10	229.95	7.8E-03	5	7	

14	25	Clathrin Heavy Chain 1	193.37	1.1E-11	14	15	

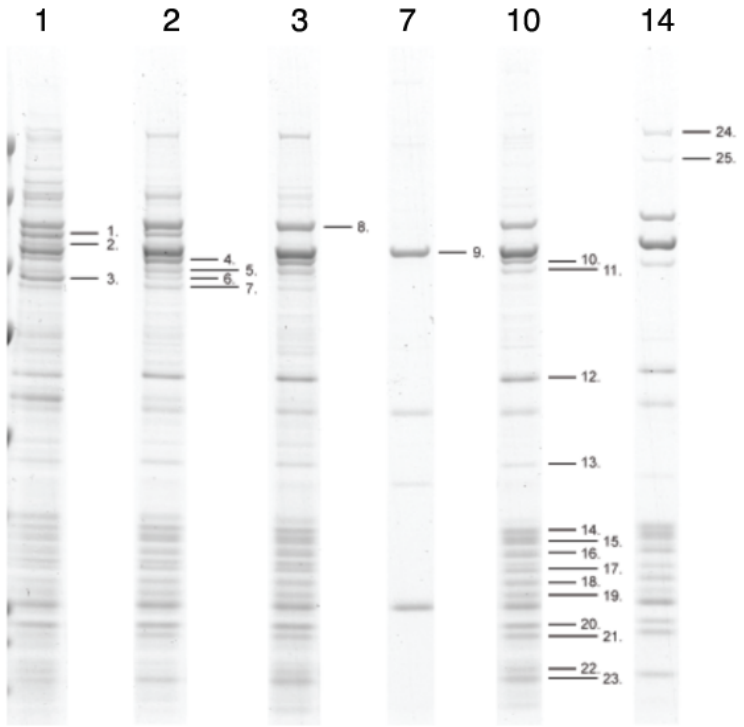


Figure 4c RBM7-LAP

MALDI-TOF Searched in ProFound

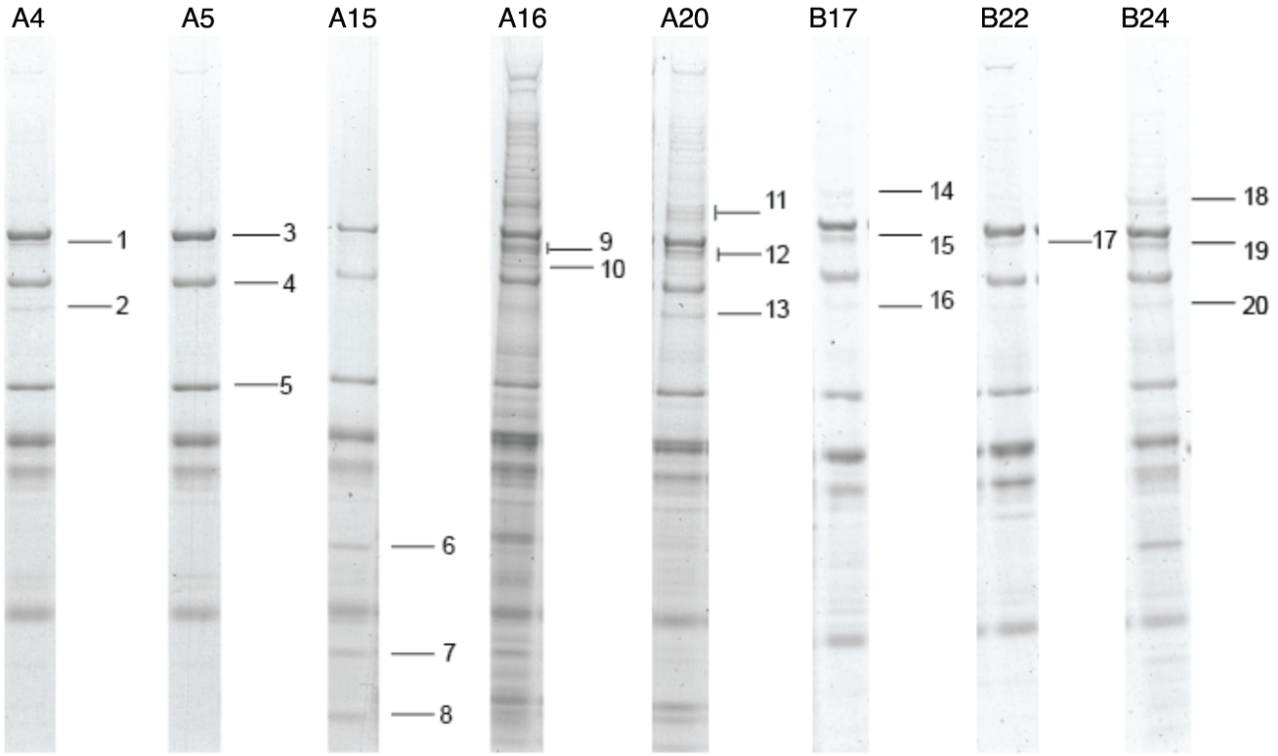
lane	band #	proteins	MW (kDa)	E-value	coverage	# peptides	Note
A4	1	SKIV2L2	118.94	8.3E-06	14	9	SRRT - not significant hit, but expected in this band
A4	2	NCBP1	84.8	6.1E-09	18	10	
A5	3	SKIV2L2	118.94	5.1E-16	35	25	
A5	4	ZCCHC8	79.08	1.0E-20	47	23	
A5	5	RBM7	30.49	6.2E-08	67	12	tagged protein
A15	6	Ribosomal Protein L5	28.25	7.0E-04	43	6	
A15	7	no significant hit					
A15	8	no significant hit					
A16	9	HNRNP U	87.58	1.0E-03	15	6	
A16	10	coatomer protein complex, subunit beta 2	99.88	1.1E-04	11	7	
A20	11	ZC3H18	106.59	2.6E-06	19	7	
A20	11	DHX9	143.48	2.9E-05	8	7	
A20	12	no significant hit					
A20	13	NCBP1	84.8	3.0E-13	22	13	
B17	15	SKIV2L2	118.94	2.8E-07	16	10	SRRT expected
B24	19	SKIV2L2	118.94	9.1E-04	8	6	SRRT expected

VMALDI Searched in X! Tandem

lane	band #	proteins	MW (kDa)	peptides searched	peptides matched	log E-value
A4	1	SRRT	100.2	VALSEPQPER, ISHGEVLEWQK	VALSEPQPER, ISHGEVLEWQK	-7.6
A20	12	SRRT	100.2	VALSEPQPER, ISHGEVLEWQK	ISHGEVLEWQK	-2.8

LC-MS/MS Searched in X! Tandem

lane	band #	proteins	MW (kDa)	log E-value	% residues / % corrected	# unique peptides	Note
B17	14	DHX9	140.9	-51.9	4.3 / 6	7.0	
B17	14	ZC3H18	106.3	-27.4	3.6 / 6	4.0	
B17	16	NCBP1	91.8	-83.6	11 / 19	8.0	
B17	16	ZCCHC8	78.5	-33.2	9.5 / 14	4.0	
B22	17	SKIV2L2	117.7	-321.8	27 / 37	27.0	
B22	17	SRRT	100.2	-88.1	11 / 19	9.0	
B24	18	DXH9	140.9	-184.2	12 / 18	17.0	
B24	18	LRPPRC	157.8	-118.0	8 / 10	13.5	
B24	18	ZC3H18	106.3	-39.5	3.9 / 7	5.0	
B24	20	HSP90AB1	83.2	-156.3	19 / 28	13.0	
B24	20	NCBP1	91.8	-72.7	11 / 20	8.0	
B24	20	KHSRP	72.9	-61.9	15 / 26	7.0	
B24	20	DDX1	82.4	-33.8	4.7 / 7	4.0	
B24	20	TARS	83.4	-28.8	5.1 / 7	4.0	



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Supplementary Protocol 1

BACKGROUND

This protocol utilizes cell powder, produced by cryogenic milling, and antibody conjugated magnetic beads. Full descriptions of procedures for preparing cell powder and antibody conjugated magnetic beads are located at http://www.ncdir.org/public-resources/protocols/.

We present two procedures for multi-well purifications. **Protocol A** provides for twenty-four simultaneous affinity capture optimization experiments, carried out in a 96-well, deep-well microplate. We have optimized this procedure for E. coli and for human tissue culture – but it is likely adaptable to essentially any material that can be successfully pulverized and extracted. A primary feature of this procedure is the use of microtip probe sonication. For both E. coli and human cell lines, protein extraction may result in viscous solutions. Moreover, human cell powders often initially yield a somewhat inhomogeneous extract containing aggregates that require a more vigorous effort to homogenize than simple vortex mixing. Both of these attributes are detrimental to affinity capture quality. However, brief microtip probe sonication can cut viscosity and disperse aggregates resulting in cell extracts amenable to high quality affinity capture. Although we have implemented ice-water bath sonication using a microplate horn to successfully extract and homogenize S. cerevisiae cell powders (described subsequently in **Protocol B**), the problems associated with E. coli and human

cell extracts were not readily resolved on a short time-scale by the same approach during our testing – necessitating a microtip probe. A second feature of this procedure is the use of high-speed centrifugation to clarify cell extracts. As such, established centrifugal clarification regiments may be applied while leveraging the throughput and convenience parallelization in the 96-well format. In principle, the only limiting factor in this procedure is the tedium of transferring more than ~twenty four reactions from the microplate to microfuge tubes for centrifugation and back for parallelized affinity capture; high-speed centrifugal clarification (e.g. 10 min at ~20k RCF, a typical regiment for thorough extract clarification prior to affinity capture) is not possible in microplates and standard bench top microcentrifuges accept only up to ~30 samples at a time. Using an 8-channel pipette with adjustable channel width simplifies and expedites the transfer from the microplate to 1.5 ml microcentrifuge tubes.

Protocol B provides for up to ninety-six simultaneous affinity capture purifications in this same format. The primary feature that allows this throughput to be achieved is the parallel clarification of extracts by filtration in a 96-well format, as opposed to centrifugation. Filtration of cell extracts is non-trivial. Insoluble material and aggregates, a natural byproduct of cell breakage and extraction, rapidly foul most conventional filters of appropriate specification (e.g. pore size) to provide a clarified extract comparable in quality to that achieved by high-speed centrifugation. Hence, in this procedure we have implemented a multi-staged filtration approach (described in the main text) that addresses this

common limitation. The filtration system presented was designed and optimized for S. cerevisiae cell extracts, but should be similarly effective on any extract generated from pulverized cell material in a similar fashion (i.e. extracted at comparable total mass and volume). Because yeast extracts do not typically exhibit issues related to viscosity, we chose sonication using a microplate horn in a chilled water bath to disperse and resuspend cell powders in the extraction solvents. Multi-microtip probe sonication can be equally applied here, as in **Protocol A**, but during our tests we found that a brief water bath sonication with intermittent mechanical mixing provided rapid, simultaneous resuspension of all wells.

Both procedures are elaborated below, including a multitude of alternative strategies we have tested at different steps to help facilitate implementation within most laboratories – where indicated, additional details may be found in the **NOTES** section at bottom.

SUPPLIERS

Here we indicate the suppliers of reagents and equipment we have used in the execution of these procedures. The product numbers are indicated where appropriate. However, in many cases an alternative supplier's (or self-made) product of comparable make and quality will equally suffice for these procedures. Naturally, initial testing should be carried out to ensure comparable performance.

Agilent Technologies (Santa Clara, CA); Beckman Coulter (Indianapolis IN); Corning (Corning, NY); Eppendorf (Hauppauge, NY); Life Technologies (Grand Island, NY); Rainin (Oakland, CA); Orochem Technologies (Naperville, IL); PHENIX Research Products (Asheville, NC); Promega (Madison, WI); QSonica (Newtown, CT); Roche Applied Science (Indianapolis, IN); Thermo Fisher Scientific (Waltham, MA); VWR (Radnor, PA);

REAGENTS

- Extraction solvents (see **Note 1**)
- Protease inhibitors cocktail (Roche #11 873 580 001, see **Note 2**)
- Liquid nitrogen (LN_2)
- 4x lithium dodecyl sulfate sample loading buffer (Life Technologies #NP0007)
- 500mM dithiothreitol (DTT)
- 4-12% Bis-Tris SDS midi-gels (Life Technologies #WG1403BX10) and appropriate running buffer

EQUIPMENT

Common to both protocols

- Dispensing manifold (**or** volumetric spoons, see **Note 3**)
- 2.5 ml 96-well deep-well microplates (square-well) with lids (VWR #37001-520)
- 96-well microplate cap mats (Thermo Scientific #4412-MTX)
- Sample rotator for microplates (Thermo Fisher #05-450-200, see **Note 4**)
- 96-well deep-well magnet (Promega #V3031)
- 96-well PCR plate magnet (Life Technologies #12331D).
- 250 µl 96-well PCR plate (Eppendorf #0030133390)
- PCR tube strip caps (Agilent Technologies #410096) or plate seals (Corning #3096)
- Vortex mixer w/ microplate adaptor (VWR # 58816-121 w/ #12620-878)
- Midi-gel system (Life Technologies #WR0100)

24-well screens including centrifugation – **Protocol A**

- 8-channel pipette with adjustable channel width (Rainin #LA8-1200XLS)
- 96-well deep-well microplates (polypropylene, round or conical bottom) – 0.8 ml 96-well deep-well microplates (Thermo Fisher Scientific #AB-0765)
- 96-well microplate cap mats, pierced (see **Note 5**)

- Sonicator with 8-microtip probe (QSonica S4000 or Q700) **or** traditional microtip probe sonicator
- 1.5 ml microfuge tubes
- Bench-top refrigerated micro-centrifuge (Eppendorf #5417R)
- Bench top centrifuge with microplate carrier (Beckman Coulter Allegra 6R with GH 3.8 rotor and SX4750*μ* microplate carrier or Beckman Coulter Avanti J-26 XP with JS-5.3 rotor)

96-well screens including filtration – **Protocol B**

- Standard 12-channel pipettes (**or** 96-channel pipette, see **Note 6**)
- 96-well deep-well microplates (polypropylene, round or conical bottom) – 1.2 ml 96-well deep-well microplates (PHENIX Research Products #M-0564)
- 96-well cell extract filtration unit (Orochem Technologies #OC21202DEPY)
- High-speed centrifuge with microplate carrier (Beckman Coulter Avanti J-26 XP with JS-5.3 rotor, see **Note 7**)
- (Optional) 2 mm Ø, stainless steel balls, Retsch part #22.455.0010 (see **Note 8**)
- Sonicator with Microplate horn sonicator (QSonica S4000 or Q700)
- (Optional) 300 µl 96-well 0.45 µm hydrophilic PVDF filter plate (Orochem Technologies #OC03PPT45) (see **Note 9**).

LEGEND

¡ATTENTION! – be careful here.

*HINT – added details that may make this easier.

✋REST – it is safe to stop here for some period.

These procedures use several multi-well plates indicated as they appear within the protocol:

SOLVENT PLATE – contains a matrix of extraction solvents.

PI PLATE – contains concentrated protease inhibitors.

BINDING PLATE – contains antibody-coupled magnetic media.

ELUTION PLATE – proteins are eluted from the affinity media using 1x LDS in this plate.

LOADING PLATE – Elutions are combined with 500 mM DTT (to ~50 mM final concentration) in this plate and subsequently loaded onto SDS-polyacrylamide gels.

Protocol A

Throughout this protocol a series of 24-wells within a 96-well plate (i.e. 3 consecutive columns) are used to hold extraction solvents, cell powders, cell extracts, magnetic beads, etc. in different microplates. The optimized procedure

implements 8-microtip probe sonication to homogenize extract volumes of ~500 µl in a 0.8 ml 96-well deep-well plate and an 8-channel adjustable-channel-width pipette for liquid manipulations. It has been successfully executed on E. coli and human cells (HEK 293 and HeLa Kyoto). It can also be carried out using a standard microtip sonication probe and a combination of a standard 8-channel and single-channel pipette. It is best to decide the orientation of the wells in advance – placing all e.g. solvents, cell powders, and magnetic beads in concordant wells of different plates will ensure that the orientation of the experiment is maintained throughout and the results will not be accidentally compromised. The 5 µl of magnetic beads slurry implemented in each affinity capture assumes a 15% w:v (300 mg : 2ml) slurry of antibody coupled Dynabeads M-270 or equivalent. In several steps, reagents must be dispensed to 24 wells from stock solutions, see **Note 10** for recommended practices.

1. Prepare an appropriate set of extraction solvents (see **Note 1**) to 2.2 ml each in a 2.5 ml 96-well deep-well microplate (SOLVENT PLATE) and set aside. This plate will be used in **Steps 4, 8, & 14-16**. After executing the following procedure ~400 µl will remain.

✋REST – We typically use 1x extraction solvents within ~24 hr of preparation. They may be held at RT if freshly made before use, or stored overnight, covered at 4°C. Prior to initiating the experiment, buffers should be equilibrated to RT for

the protein extraction, and then placed again at 4°C (or held on ice) for subsequent washing steps.

*HINT – Longer-term storage of solvents is possible, but remain vigilant of microbial contamination in non-sterile solutions and evaporative losses in unsealed storage plates.

2. Prepare a plate containing 2 µl of 500 mM DTT in each of 24 wells of a 250 µl 96-well PCR plate (LOADING PLATE). This plate will be included in the procedure by accepting fractions eluted in 1xLDS in **Steps 17 & 18** (see **Note 10**).

3. Prepare a 0.8 ml 96-well deep-well microplate with 5 µl of magnetic affinity medium slurry in each of 24 wells of a 0.8 ml 96-well deep-well microplate (BINDING PLATE). This will be used in **Steps 4 & 11-16**.

¡ATTENTION! – As beads may settle during dispensation, mix the stock periodically to ensure equivalent slurry is supplied to each well (see **Note 10**).

4. (OPTIONAL, see **Note 11**) Wash the beads in each well of the BINDING PLATE with 100 µl from the appropriate well of the SOLVENT PLATE and mix by pipetting up and down several times. Place the plate on the deep-well magnet, remove the washing solutions and hold covered at 4°C until ready for use (**Step 11**).

*HINT – Washed beads remain moist from the residual washing solutions and are stable for several hours. However, excessive drying of the beads should be avoided, as can occur due to evaporation on extended storage. If the BINDING PLATE is to be prepared more than several hours before the experiment, leave the beads within the storage or washing solutions, completing the preparation prior to executing the experiment.

5. Prepare protease inhibitors by dispensing 5 µl of 100x stock into each of 24 wells of a 0.8 ml 96-well deep-well microplate (PI PLATE, see **Note 12**). These inhibitors will be included in the procedure by combining with extraction solvent and transferring the mix to frozen cell powders in **Step 8**.

*HINT – Depending on the stability of the constituents of your protease inhibitors cocktail it may be feasible to prepare this plate and store frozen until use.

REST – In this way, it may be possible to execute steps 1-5 the day prior to the experiment. We typically carry out **Steps 2-5** the morning of the experiment and **Step 1** the night before or morning of.

6. Ensure that the SOLVENT PLATE and PI PLATE are equilibrated to RT.

7. Starting with cryomilled cell powder, dispense an appropriate amount to each well, using a dispensing manifold (described in the main text, **Fig. 1**, and **Supplementary Fig. 1a,b,c, and 12**) or a volumetric spoon (see **Note 3**).

Cell powders are dumped onto the manifold surface and packed into all the wells using a packing tool (**Supplementary Fig. 1a**). Excess powder is recovered using a spatula. A 0.8 ml 96-well deep-well microplate is placed on top of the manifold such that the openings of the wells of each device are aligned and face each other; and then the sandwich is inverted and given a firm tap from above (the underside of the manifold) – transferring the powders out of the wells of the manifold and into the wells of the 96-well plate (EXTRACTION PLATE). In case of any slight error or spillage during dispensing, a lint-free laboratory tissue paper or fine paintbrush can be used to brush away any powder that has accumulated between the wells of the deep-well microplate.

*HINT – We have found that ~25 mg of *E. coli* and ~50 mg of human cell powder can provide for robust colloidal Coomasie blue staining of all major components of a purified mixture. The procedures described here are optimized for the above given masses of cell powder. Very low molecular weight proteins (≥15 kDa) or proteins of very low abundance within the cell, or within the purified mixture, may fall below the limit of detection, however. In this case, utilizing a more sensitive stain may be appropriate. While using more cell powder is also possible, keep in mind this may require re-optimization of certain steps (e.g. **Step 9**).

¡ATTENTION! – Powders should always be held on LN_2 or dry ice when not in -80°C storage; dispensing occurs under LN_2 cooling and all utensils should be pre-cooled before initiating the procedure. It is useful to dispense your powders

within three consecutive columns, excluding A1-H1 and A12-H12 (i.e. those columns without a neighboring column on one side), of the deep-well microplate – we have observed that these edge columns can respond differently to sonication than inner rows.

✋REST – The microplate may be held on LN_2 until ready to proceed. Covering the plate with a cap mat may help reduce the accumulation of frozen condensate within the wells from moisture in the air during an extended pause – the mat will become rigid at subzero temperatures and does not need to be snuggly fitted.

8. Remove the EXTRACTION PLATE from LN_2 and allow it to stand ~2-3 minutes at RT. The powders will remain thoroughly frozen during this time. Add 475 µl (for 25 mg *E. coli* powder) or 450 µl (for 50 mg human cell powder) of each RT solvent from the SOLVENT PLATE to the PI PLATE – producing solvents including ~1x protease inhibitors. Transfer this entire mixture to the concordant wells of the EXTRACTION PLATE. Optionally, add one column (8-wells) of de-ionized water to the plate – this will allow the 8-microtip probe to be washed in between sonicating different samples (**Step 9**). Cover the plate with a cap mat that has had the caps from the appropriate columns pierced to allow the use of a multi-microtip probe sonication (see **Note 5**) and transfer the plate to 4°C.

¡ATTENTION! – Letting the plate stand briefly at RT and adding RT solvents helps ensure a more rapid and homogenous dispersal and extraction during the

sonication, and results in a cold extract which should be subsequently maintained at 4°C. Using solvents at 4°C for resuspension frequently results in the formation of ice at the powder / solvent interface and may significantly hinder subsequent resuspension. **Once RT buffers are added, all the remaining work prior to elution should be carried out within a controlled temperature room at 4°C.**

9. For ~50 mg of human cells, sonication is carried out for ~30 sec. using a QSonica S4000 with an 8-microtip probe using an amplitude setting of 15. For ~25 mg of *E. coli* use the same settings for 1 min. Apply sonication to one row, then proceed to the second row, and finally the third. By adding water to a column within the plate (**Step 8**), the 8-tip probe may be quickly and conveniently washed between sample columns to eliminate sample carryover (see **Note 13**).

¡ATTENTION! – This step may require empirical optimization for your particular sonication and microplate set-up. Please use these settings as a starting guide. Reasonable settings for human cell powder on most setups can probably be found between 15 sec – 1 min sonication at between 10 – 20 amplitude; similarly for *E. coli* at 1 – 3 min. We suggest keeping power low (below amplitude 20 on the QSonica S4000) and extending time as needed to avoid sample warming and loss due to excessive spraying or foaming. Complete resuspension can typically be assessed visually by holding the plate over head and looking at the bottom of the wells from underneath (to ensure materials is not trapped under ice). A

thorough resuspension should appear translucent and homogenous when viewed through the plate from underneath. Viscosity can be assessed by test pipetting selected wells – viscous wells will not pipette smoothly or completely and will appear snotty in consistency. Thus, in our procedure the minimum program applied to all samples should be that needed to resuspend and cut viscosity of the most demanding well – which varies according to extraction solvent – and thus some solvent matrices may be more or less demanding in sonication duration.

*HINT – This same procedure can be executed using a traditional sonicator and sequential sonication of each well – settings should be determined empirically. We have had success using a Misonix XL2020 with microtip probe on an amplitude setting of 2-3.

10. Using an 8-channel pipette with adjustable channel width, transfer crude extracts from the EXTRACTION PLATE to 24 x 1.5 ml tubes and centrifuge for 10 min, ~14k RPM at 4°C in a bench top microcentrifuge.

¡ATTENTION! – Number your tubes in advance, and array them in a tube holder in the same orientation as the multi-well plate (e.g. such that H1 is on far left, A1 is on far right when the plate is facing you oriented as 8-well rows).

11. Transfer the supernatants (clarified extracts) to the BINDING PLATE and cap the plate with a cap mat or other liquid-tight plate seal. Resuspend the beads

fully within each well by manually inverting the plate several times. Place the plate on a rotating wheel at moderate speed (just enough to prevent the beads from settling) for 30 min – 1 hr (see **Note 4** & **Note 14**).

*HINT – Transferring the clarified extracts back to a 96-well plate can be done using the same adjustable width 8-channel pipette, but one must be careful not to recapture pellet from within the 1.5 ml tubes, which may be a different proportion depending on the extraction solvent. We prefer to rapidly and precisely transfer supernatants back to a microplate from the 1.5 ml tubes, one-at-a-time, using a standard pipette. This maximizes extract quality by minimizing pellet carryover.

12. Remove the BINDING PLATE from the rotating wheel and briefly spin down to collect all liquid within the bottom of the well (Beckman Coulter Allegra 6R with GH 3.8 rotor and SX4750 microplate carrier or Beckman Coulter Avanti J-26 XP with JS5.3 rotor – 1 min at 2k RPM and 4°C).

13. Place the BINDING PLATE on a 96-well deep-well magnet to retain the beads at the well sides. Then remove the depleted extracts and remove the plate from the magnet.

14. Add 500 µl of concordant extraction solvent to each well of the BINDING PLATE (wash #1). Place the plate back on the magnet and alter its position several times to wash and mix. Park the plate back in the original position on

the magnet to collect and hold the beads at the well side and remove the wash. Then remove the plate from the magnet.

*HINT – By altering the position of the plate on the magnet, one can cause the beads to slide across the well interior to an adjacent position as well as diagonally across the well. We use 9 moves to thoroughly mix beads within each well. This avoid problems with resuspension by pipetting including foaming, incomplete resuspension, and spillage due to the displacement of volume in the well by the pipette tip.

¡ATTENTION! – With a standard magnet (including the deep-well magnet recommended in this procedure) one row of the plate may not be under the influence of the magnetism when placed at certain positions. Ensure that the appropriate movements are made that all the wells receive comparable mixing.

15. Repeat **Step 14** (wash #2), then proceed to **Step 16**

16. Add 200 µl of concordant extraction solvent to each well of the BINDING PLATE (wash #3). Pipette the solution in each well up and down several times to fully resuspend the beads and transfer them to a 250 µl 96-well PCR plate (ELUTION PLATE) placed on a 96-well PCR plate magnet. Removing the wash solution once the beads have adhered to the well sides. Remove the plate from the magnet.

¡ATTENTION! – The beads in the BINDING PLATE reside mostly adhered to the sides of the wells after wash #3 is removed, their position alternating to opposite sides of the well with respect to neighboring columns. By pipetting up and down against the appropriate side of the wells for each column, the beads will be easily resuspended in 200 µl volume within the fewest repetitions.

17. Remove the wash solution and add 18 µl of 1.1x LDS loading buffer to the sample. Cover with a strip tube caps or a plate seal and incubate the plate 5-10 min at between RT and 70°C with vigorous shaking. Re-equilibrate the plate to RT if heated. Briefly spin the plate to recapture all beads and loading buffer at the bottom of the wells as in **Step 12** (operation at RT is fine). Place the ELUTION PLATE on the 96-well PCR plate magnet and transfer the elutions to a plate containing 2 µl 500 mM DTT (LOADING PLATE).

*HINT – You may need to determine the best elution regime empirically. RT incubation minimizes the amount of IgG that co-elutes from the beads (typically below detectable levels by colloidal Coomassie blue staining) – elevating the temperature enhances the amount of IgG polypeptide chains can be observed in the eluate. Hence, RT elution is preferred. However, some tag/antibody interactions are of sufficiently high affinity that they require elevated temperature to be effectively eluted by ~1x LDS. Our observation is that affinity capture using either a Protein A based tag in combination with Rabbit IgG or the 3xFLAG-tag in combination with the M2 anti-FLAG antibody, elution can effectively be achieved

upon incubation at RT with ~1x LDS and vigorous shaking; whereas a GFP-tag in combination with our in-house generated llama polyclonal antibody requires incubation at 70°C to effectively elute.

18. Cover the sample-containing wells of the plate with strip caps or thermal seal and reduce the samples by heating to 70°C for 10 min. Cool to RT. Briefly spin the plate to recapture all the solutions at the bottom of the wells (as in **Step 17**).

¡ATTENTION! – If you would like to carry out proteomic analysis by in-gel digestion of excised bands after SDS-PAGE / protein staining, block cysteines by alkylation with iodoacetamide prior to loading on the gel (see **Note 15**).

19. Load all samples on a 26-well 4-12% Bis-Tris midi-gel and stain with colloidal Coomassie or by your preferred method.

*HINT – using a 26-well gel leave 2 free lanes, one for a molecular weight marker and one for a protein quantity standard. We typically load 10-50 ng of BSA as a quantity standard to help assess our protein recoveries within the screen.

¡ATTENTION! – If you would like to carry out proteomic analysis by in-gel digestion of excised bands after SDS-PAGE / protein staining, use an MS-compatible protein stain and handle the gel in a keratin and contamination conscious way (i.e. use clean solutions and containers and observe best practices for MS) (see **Note 15**).

Protocol B

This procedure facilitates the utilization of each well of a 96-well plate. The optimized procedure implements a combination of water bath sonications and vortex mixing to homogenize extract volumes of ~750 µl in a 1.2 ml 96-well deep-well plate and a 12- or 96-channel pipette for liquid manipulations. It has been successfully implemented on cell powder derived from baker's yeast. The water bath sonication / vortex mixing implemented permits that all reactions are resuspended simultaneously; hence one can process an entire multi-well plate without significant time-staggering of sample extraction, facilitating a single tagged strain to be screened for optimized affinity capture with 96 different extraction solvents or e.g. two strains with 48 solvents, three strains with 32 solvents, and four strains with 24 solvents. Generally, if there is little or no information about the purification of the protein of interest with an interacting partner available, one should adopt a 48- or 96-condition screen for the broadest coverage of the purification space. However, if desired, several factors may be screened in parallel against a smaller matrix of conditions when some prior knowledge exists. The 5 µl of magnetic beads slurry implemented in each affinity capture assumes a 15% w:v (300 mg : 2ml) slurry of antibody coupled Dynabeads M-270 or equivalent. In several steps, reagents must be dispensed to 96 wells from stock solutions, see **Note 10** for recommended practices.

1. Prepare an appropriate set of extraction solvents (see **Note 1**) to 2.2 ml each in a 2.5 ml 96-well deep-well microplate (SOLVENT PLATE) and set aside (see **Note 16**). This plate will be used in **Steps 4, 8, & 15-17**. After executing the following procedure ~400 µl will remain.

✋REST – We typically use 1x extraction solvents within ~24 hr of preparation. They may be held at RT if freshly made before use, or stored overnight, covered at 4°C. Prior to initiating the experiment, buffers should be equilibrated to RT for the protein extraction, and then placed again at 4°C (or held on ice) for subsequent washing steps.

*HINT – Longer-term storage of solvents is possible, but remain vigilant of microbial contamination in non-sterile solutions and evaporative losses in unsealed storage plates.

2. Prepare a plate containing 2 µl of 500 mM DTT in each of 96 wells (LOADING PLATE). This plate will be included in the procedure by accepting fractions eluted in 1xLDS in **Steps 18 & 19** (see **Note 10**).

3. Prepare a 1.2 ml 96-well deep-well microplate with 5 µl of magnetic affinity medium slurry per well (BINDING PLATE). This will be used in **Steps 4 & 11-17**.

¡ATTENTION! – As beads may settle during dispensation, mix the stock periodically to ensure equivalent slurry is supplied to each well (see **Note 10**).

4. (OPTIONAL, see **Note 11**) Wash the beads in each well of the BINDING PLATE with 100 µl from the appropriate well of the SOLVENT PLATE and mix by pipetting up and down several times. Place the plate on the deep-well magnet, remove the washing solutions and hold covered at 4°C until ready for use (**Step 11**).

*HINT – Handled in this way, the beads remain moist from the residual washing solutions and are stable for several hours. However, excessive drying of the beads should be avoided, as can occur due to evaporation on extended storage. If the BINDING PLATE is to be prepared more than several hours before the experiment, leave the beads within the storage or washing solutions, completing the preparation prior to executing the experiment.

5. Prepare protease inhibitors by dispensing 5 µl of 100x stock into each of 96 wells of a 1.2 ml 96-well deep-well microplate (PI PLATE, see **Note 12**). These inhibitors will be included in the procedure by combining with extraction solvent and transferring the mix to frozen cell powders in **Step 8**.

*HINT – Depending on the stability of the constituents of your protease inhibitors cocktail it may be feasible to prepare this plate and store frozen until use.

✋REST –In this way, it may be possible to execute steps 1-5 the day prior to the experiment. We typically carry out **Steps 2-5** the morning of the experiment and **Step 1** the night before or morning of.

6. Ensure that the SOLVENT PLATE and PI PLATE are equilibrated to RT.

7. Starting with cryomilled cell powder, dispense an appropriate amount to each well, using a dispensing manifold (described in the main article and **Supplementary Fig. 1 and 12**) or a volumetric spoon (see **Note 3**). Cell powders are dumped onto the manifold surface and packed into all the wells using a packing tool. If dispensing more than one tagged strain powder, dividers are inserted into grooves on the manifold to avoid cross-contamination (See **Note 17**). Excess powder is recovered using a spatula. A 1.2 ml 96-well deep-well microplate is placed on top of the manifold such that the openings of the wells of each device are aligned and face each other; and then the sandwich is inverted and given a firm tap from above (the underside of the manifold) – transferring the powders out of the wells of the manifold and into the wells of the 96-well plate (EXTRACTION PLATE). In case of any slight error or spillage during dispensing, a lint-free laboratory tissue paper or fine paintbrush can be used to brush away any powder that has accumulated between the wells of the deep-well microplate.

*HINT – We have found that 150 mg of yeast cell powder can provide for robust colloidal Coomasie blue staining of all major components of a purified mixture. Very low molecular weight proteins (≥15 kDa) or proteins of very low abundance

may approach or fall below the limit of detection, however. In this case using more material or a more sensitive stain may be appropriate.

¡ATTENTION! – Powders should always be held on LN_2 or dry ice when not in -80°C storage; dispensing occurs under LN_2 cooling and all utensils should be pre-cooled before initiating the procedure.

✋REST – The microplate may be held on LN_2 until ready to proceed. Covering the plate with a cap mat may help reduce the accumulation of frozen condensate within the wells from moisture in the air during an extended pause – the mat will become rigid at subzero temperatures and does not need to be fit snuggly.

8. Remove the EXTRACTION PLATE from LN_2 and allow it to stand ~2-3 minutes at RT. The powders will remain thoroughly frozen during this time. Add 600 µl of RT solvents from the SOLVENT PLATE to the PI PLATE – producing solvents including ~1x protease inhibitors. Transfer this entire mixture to the concordant wells of the EXTRACTION PLATE. See **Note 18**.

¡ATTENTION! – Letting the plate stand briefly at RT and adding RT solvents helps ensure a more rapid and homogenous dispersal and extraction during the sonication, and results in a cold extract which should be subsequently maintained at 4°C. Using solvents at 4°C for resuspension frequently results in the formation of ice at the powder / solvent interface and may significantly hinder subsequent resuspension. **Once RT buffers are added, all the remaining work prior to**

elution should be carried out within a controlled temperature room at 4°C if working with a 12-channel pipette.

9. (OPTIONAL, see **Note 8**) – add 2 x 2 mm Ø steel balls to each well (**Supplementary Fig. 13**).

*HINT – For protein extraction within each well, we utilize a sequence of water bath sonication and vortex mixing, described below; we include 2 x 2 mm Ø stainless steel balls (e.g. Retsch part #22.455.0010) in each well to expedite resuspension of the powder and homogenization of the resulting extract during this process.

10. Cover the plate with a clean cap mat and make sure all the caps fit snuggly inside the wells. Perform a sequence of 1 min ice bath sonication (QSonica S4000, microplate horn, amplitude: 95) and 1 min vortexing (we use an adapter for microplates). Usually 3 cycles are enough to achieve complete and homogenous resuspension, which can be monitored visually by looking at the bottom of the plate (the steel balls sink to the bottom of the well when the mixture has been homogenized).

¡ATTENTION! – This step may require empirical optimization for your particular sonication and microplate set-up. Please use these settings as a starting guide. When resuspending without steel balls more sonication and vortexing cycles may be necessary. Resuspension is complete when the crude extract appears

translucent and homogenous when the plate is viewed from underneath with a light source above.

11. Using a 12-channel pipette transfer the crude lysate onto the filter plate sitting on top of the BINDING PLATE. Spin 5 min at 3.5k RPM in Beckman Coulter Avanti J-26 XP with JS5.3 rotor. See **Note 7**.

¡ATTENTION! – With this step we have experienced problems using the VIAFLO 96; some channels do not retain the extract solution well. Use a 12-channel pipette if you observe inconsistent pipetting at this step.

12. Seal the plate with a cap mat or other liquid-tight plate seal. Resuspend the beads fully within each well by manually inverting the plate several times. Place the plate on a rotating wheel at moderate speed (just enough to prevent the beads from settling, but not too fast as to precipitate the beads) for 30 min – 1 h (see **Note 4** & **Note 14**).

13. Remove the BINDING PLATE from the rotating wheel and briefly spin down to collect all liquid within the bottom of the well (Beckman Coulter Allegra 6R with GH 3.8 rotor and SX4750 microplate carrier or Beckman Coulter Avanti J-26 XP with JS5.3 rotor – 1 min at 2k RPM and 4°C).

14. Place the BINDING PLATE on a 96-well deep-well magnet to retain the beads at the well sides. Then remove the depleted extracts and remove the plate from the magnet.

*HINT – when using a 96-well pipette, the procedure is fast enough to be performed at RT. Otherwise use a 12-channel pipette and work in the cold room.

15. Add 500 µl of concordant extraction solvent to each well of the BINDING PLATE (wash #1). Place the plate back on the magnet and alter its position several times to wash and mix. Park the plate back in the original position on the magnet to collect and hold the beads at the well side and remove the wash. Then remove the plate from the magnet.

*HINT – By altering the position of the plate on the magnet, one can cause the beads to slide across the well interior to an adjacent position as well as diagonally across the well. We use 9 moves to thoroughly mix beads within each well. This avoid problems with resuspension by pipetting including foaming, incomplete resuspension, and spillage due to the displacement of volume in the well by the pipette tip.

¡ATTENTION! – With a standard magnet (including the deep-well magnet recommended in this procedure) one row of the plate may not be under the influence of the magnetism when placed at certain positions. Ensure that the appropriate movements are made that all the wells receive comparable mixing.

*HINT – using a 96-well pipette, the beads can also be pipetted up and down to wash. However, for the second wash (**Step 16**), after resuspending the medium in the wash, place the BINDING PLATE on the magnet and pipette up and down to wash the tips of residual affinity medium before removing the wash from the well.

16. Repeat **Step 15** (wash #2), then proceed to **Step 17**

17. (a) Without filter plate - add 200 µl of concordant extraction solvent to each well of the BINDING PLATE (wash #3). Pipette the solution in each well up and down several times to fully resuspend the beads and transfer them to a 250 µl 96-well PCR plate (ELUTION PLATE) placed on a 96-well PCR plate magnet. Removing the wash solution once the beads have adhered to the well sides. Remove the plate from the magnet.

(b) (OPTIONAL) With filter plate - alternatively add 200 µl of concordant extraction solvent to each well of the BINDING PLATE (wash #3). Pipette the solution in each well up and down several times to fully resuspend the beads and transfer them to a 0.45 µ filter plate (e.g. Orochem Technologies, OC03PPT45). Put the filter plate on top of a microplate with > 300 µl well size for flow-through collection and spin down for 5 min at 3k RPM and 4°C

(Beckman Coulter Allegra 6R with GH 3.8 rotor and SX4750 microplate carrier or Beckman Coulter Avanti J-26 XP with JS5.3 rotor).

¡ATTENTION! – The beads in the BINDING PLATE reside mostly adhered to the sides of the wells after wash #3 is removed, their position alternating to opposite sides of the well with respect to neighboring columns. By pipetting up and down against the appropriate side of the wells for each column, the beads will be easily resuspended in 200 µl volume within the fewest repetitions.

*HINT – in our experience collecting the beads on the filter plate is the quickest and most efficient way. When choosing a filter of a different make one has to be aware of the protein binding capacity of the filter (i.e. use low binding materials).

18. (a) Without filter plate - add 18 µl of 1.1 x LDS loading buffer to the beads. Cover with a strip tube caps or a plate seal and incubate the plate 5-10 min at between RT and 70°C with vigorous shaking. Re-equilibrate the plate to RT if heated. Briefly spin the plate to recapture all beads and loading buffer at the bottom of the wells as in **Step 13** (operation at RT is fine). Place the ELUTION PLATE on the 96-well PCR plate magnet and transfer the elutions to a plate containing 2 µl 500 mM DTT (LOADING PLATE).

(b) With filter plate – Using a multi-channel pipette add 10 µL of 1.1x LDS to the beads on the surface of the filter in each well. Cover with a cap mat or a plate seal and incubate the plate 5-10 min at between RT and 70°C with

vigorous shaking. Re-equilibrate the plate to RT if heated. Place the filter on top of the LOADING PLATE and collect the eluate by briefly spinning the plate (Beckman Coulter Allegra 6R with GH 3.8 rotor and SX4750 microplate carrier or Beckman Coulter Avanti J-26 XP with JS5.3 rotor – 1 min at 2.5k RPM, RT). Repeat the incubation with 10 µl fresh 1.1x LDS and repeat the centrifugation to capture a second eluate fraction also in the LOADING PLATE, giving ~20 µl final combined elution.

*HINT – You may need to determine the best elution regime empirically. RT incubation minimizes the amount of IgG that co-elutes from the beads (typically below detectable levels by colloidal Coomassie blue staining) – elevating the temperature enhances the amount of IgG polypeptides can be observed in the eluate. Hence, RT elution is preferred. However, some tag/antibody interactions are of sufficiently high affinity that they require elevated temperature to be effectively eluted by ~1x LDS. Our observation is that affinity capture using either a Protein A based tag in combination with Rabbit IgG or the 3xFLAG-tag in combination with the M2 anti-FLAG antibody, elution can effectively be achieved upon incubation at RT with ~1x LDS and vigorous shaking; whereas a GFP-tag in combination with our in-house generated llama polyclonal antibody requires incubation at 70°C to effectively elute.

*HINT – when using the optional filter plate for elution, eluting twice with 10 µl gives a more consistent and effective elution than a single 20 µl volume.

19. Cover the LOADING PLATE with thermal seal and reduce the samples by heating to 70°C for 10 min. Cool to RT. Briefly spin the plate to recapture all the solutions at the bottom of the wells (as in **Step 13**; operation at RT is fine).

¡ATTENTION! – If you would like to carry out proteomic analysis by in-gel digestion of excised bands after SDS-PAGE / protein staining, block cysteines by alkylation with iodoacetamide prior to loading on the gel (see **Note 15**).

20. Load all samples on a 26-well 4-12% Bis-Tris midi-gel and stain with colloidal Coomassie blue or by your preferred method.

*HINT – using a 26-well gel leave 2 free lanes, one for a molecular weight marker and one for a protein quantity standard. We typically load 50-100 ng BSA to help assess our protein recoveries within the screen.

¡ATTENTION! – If you would like to carry out proteomic analysis by in-gel digestion of excised bands after SDS-PAGE / protein staining, use an MS-compatible protein stain and handle the gel in a keratin and contamination conscious way (i.e. use clean solutions and containers and observe best practices for MS) (see **Note 15**).

NOTES

1. Extraction solvents can be prepared by hand or by using robotic automation. We have successfully automated this procedure using The Formulator liquid handler, from Formulatrix (Waltham, MA), a device typically used for preparing protein crystallization screening matrices, and also using a Hamilton (Reno, NV) STAR liquid handling workstation (an example solvent mixing program is included within the Hamilton Methods Supplement accompanying this publication). Extraction solvent formulations used in the present study are provided in the Supplementary Table 1 and figure legends.

2. Use an EDTA-free protease inhibitor cocktail if you plan to explore the effect of this reagent or of divalent cations on affinity capture optimization. Although chelation will restrict the activity of metalloproteases, divalent cations are also important to many protein classes and chelation may affect interaction stability and co-purification profiles. Also, keep in mind that other reagents may chelate or sequester metal ions (e.g. phosphate or citrate).

3. In the absence of a dispensing manifold, volumetric spoons may be used to add powder to each well of a liquid N_2 cooled multi-well plate, one well at a time. Our favorite: Norpro 3080 Mini Measuring Spoons, 5 Piece Set available though www.amazon.com. Using this set we have found that, e.g. one heaping scoop using ‘smidgen’ gives ~50 mg and one level scoop with ‘dash’

gives ~250 mg. The user must achieve an initial feel for the 'size' of the scoop, but dispensing powders within +/- 20% by mass can generally be achieved with practice.

4. The product indicated is a wheel intended for mixing solutions within test tubes. However, a microplate fits securely on this wheel between the metallic test tube holders and the central knob. A setting of 40 RPM provides excellent mixing for batch binding.

5. The sonication of ~0.5 ml volumes in a 0.8 ml deep-well microplate can result in some sample spraying from the well depending on the power transmitted and the probe depth within the sample. An excellent implementation will minimize these effects, and best results are observed when the probe can be submerged to a depth at the mid-point or below within the sample. Using the 8-microtip probe described in **Protocol A**, the probe depth with respect to sample is suboptimal (a limitation in the current design of the 8-microtip probe) and it is therefore useful to have a splashguard to protect against inadvertent cross mixing of samples during sonication. A splashguard can be constructed with a scalpel by manually cutting an "X" into each cap on the mat corresponding to a well that you would like to sonicate. In a first test, fill the corresponding wells of a 96-well plate with water and then cover with the pierced cap mat. Gently lower the multi-microtip probe into the wells that have

been pierced and run at the maximum intended power and duration of use. This will result in the “X’s” cut into the caps becoming small holes of roughly the diameter of the sonication probe microtips. Each well of a subsequent experiment can now be sealed at the perimeter by using this mat, providing a reduced diameter orifice that allows probe insertion while limiting liquid escape.

6. We have successfully executed **Protocol B** using a VIAFLO 96, 96-channel pipette (Integra Biosciences AG, Zizers, Switzerland) – with the exception of **Step 11**. The use of a 96-channel pipette greatly expedites the processing of samples and is fast enough to be performed at RT without apparent deterioration of protein complexes.

7. When the 96-well cell extract filtration unit (Orochem Technologies #OC21202DEPY) is stacked upon the 1.2 ml 96-well deep-well microplates (PHENIX Research Products #M-0564) the resulting assembly is too tall to fit in carriage of the Beckman SX4750µ microplate carrier. Although SX4750µ microplate carrier can be operated without the carriages, we recommend using the Beckman Coulter JS-5.3 rotor for ease of handling and surety of operation.

8. These expedite the resuspension process initiated in **Step** 10. The balls may be added en masse at precisely 2 per well using a precision machined at manifold (similar to the one for dispensing powders – see **Supplementary Fig. 13**).

9. We have found that SpA-tagged proteins can be efficiently eluted from IgG coupled magnetic beads in a small volume of ~1x LDS at RT from within the well of a filter plate. Working from within this filter plate simplifies the elution stage of this procedure as described in **Step 18** of **Protocol B**.

10. When dispensing a single stock solution to multiple wells, it is helpful to first dispense this stock to a reservoir or strip tubes and then use a multi-channel pipette to dispense to the many wells of a 96-well plate. A reservoir requires excess reagent be added whereas the use of strip tubes necessitates pre-allocation of the reagent to the individual tubes on the strip, but creates less waste and greater storage flexibility for the remaining reagent. Our tendency is to dispense inexpensive reagents, e.g. 1x LDS (**Step 17, Protocol A; Step 18, Protocol B**), from a reservoir (Thermo Fisher #8075) and to dispense expensive reagents, e.g. magnetic affinity media (**Step 3**) from PCR strip tubes (Agilent Technologies #410092). The latter approach ensures that no beads are sacrificed and that the remainders can be returned to storage; the former is simply less effort – and remainders can often still be recaptured from

the reservoir as desired. In the case of dispensing magnetic beads, PCR strip tubes are especially recommended because the tubes enable the slurry to be maintained as a suspension by pipetting up and down periodically, whereas the beads tend to settle within a trough.

11. The 5 µl beads slurry is only ≲1% the total cell extract volume, and therefore the residual storage buffer is not likely to exert any discernable impact on the resulting purification profile. However, pre-equilibrating the beads is a best practice.

12. E.g. dissolve one Roche complete EDTA-free protease inhibitor tablet (product #11 873 580 001) in 500 µl of Milli-Q water (18 MΩ cm deionized water), giving a 100x solution.

13. It may be important to eliminate carryover between neighboring columns during probe sonication. Adding one column of water to the plate allows the multi-tip probe to be washed between samples by brief sonication (~5 sec) within the water, eliminating potential extract carryover. Also adding a row of cotton wool can provide for drying of the washed tips by brief touching to the tips, wicking away the water – although we do not implement this latter measure for our work.

14. We routinely use 1 hr binding for preliminary tests, but typically restrict binding to the shortest time giving ≳ 70% recovery of the tagged protein. Within limits, as the binding time increases the yield of the handle and co-purifying proteins also typically increase, however the non-specific background may increase disproportionately during long incubations. The optimal binding time has to be determined empirically.

15. While MS data can often be obtained directly from gel produced as a result of multi-well screening, promising results generated in multi-well format should be repeated in single tube format to verify the reproducibility of the result. If the reaction scale needs to be increased to promote detection by MS, we recommend pooling multiple identical affinity capture experiments rather than increasing scale for a single reaction.

16. Adding 1:1000 Antifoam B per well (right before use, as it is not sterile) will help reduce the foaming during sonication steps and we have not observed it to negatively affect the affinity capture.

17. Keep in mind that when processing different strains within the same plate, care must be taken not to cross-contaminate the samples, this is achieved by pre-weighing the powder to just in excess of that required to fill the cylinders within the manifold. For example, to fill 24 cylinders at 150 mg per purification

(for processing 4 strains in parallel across 24 extraction solvents), 3.8 g may be applied to each section of the manifold (consisting of 200 mg surplus powder to ensure each well is equally and completely filled). Carefully recover the small amount of remaining excess powder in each section, brush to the sides any minute remainders, before removing the dividers (see **Supplementary Fig. 1a**) and transferring the powder to the 96-well plate.

18. We use 1.2 ml deep-well plates to combine 150 mg of cell powder per well with 600 µl of buffer. The 1:4 ratio of cell material to extraction solvent is typically enough to stabilize the pH of the extract and has frequently exhibited improved yield when compared to more dilute proportions (such as our prior standard of 1:9 for bench-scale preparative work). The final lysate volume is ~750 µL.