Improved methodology for the affinity isolation of human protein complexes expressed at near endogenous levels

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Supplementary Material For:

Figure S1: Map of a modified pcDNA5/FRT/TO vector for C-terminal 3xFLAG tagging.
3xFLAG coding sequence: GAC TAC AAG GAC CAC GAC GGT GAC TAC AAG GAC CAC GAC ATC GAC TAC AAG GAC GCC GAC CAC GAC ATC GAC TAC AAG GAC GCC GAC CAC GAC AAG TGA. GenBank file of entire vector sequence provided as a supplement, pcDNA5_FRT_TO.gbk

Figure S2: Migration of BAP-FLAG protein compared to anti-FLAG IgG chains. As IgG chains can sometimes leak from conjugated beads during elution with LDS, and as BAP-FLAG shares a similar mass to IgG heavy chain (HC), the following comparison was made: 5 μl M2-coupled Dynabeads slurry were incubated with 1x LDS, as described in methods, and loaded on the gel (A); 100ng BAP-FLAG protein was loaded on the gel (B) — same as fig. 2. This shows that IgG and BAP-FLAG are easily differentiated on the gel, and that BAP-FLAG — not IgG HC — were measured as indicators of DB performance.
Figure S3: Over-expression can lead to protein mislocalization. Cells induced for expression of SKIV2L2-FLAG (a.k.a. hMTR4-FLAG) using three different concentrations of tetracycline: (A) 30 ng/ml, (B) 100 ng/ml, and (C) 1000 ng/ml. At low expression in (A), SKIV2L2-FLAG is nuclear localized with accumulation in nucleoli. However, with increasing tetracycline concentration SKIV2L2-FLAG expression goes up and more signal is observed within the nucleus and the cytoplasm, whereas relatively less signal is accumulated within nucleoli – indicating protein mislocalization with increasing expression level. DAPI staining displays nuclei and SKIV2L2-FLAG localization was visualized by indirect immunofluorescence using anti-FLAG antibodies [16].

Figure S4: 3xFLAG exhibits superiority over 1xFLAG. Panel A shows the results of purification of RRP41 and RRP6 proteins tagged either with 1xFLAG (A, lane 1 and 2, respectively) or 3xFLAG (B, lane 3 and 4, respectively). Handle proteins marked with asterisk (in lane 1, RRP41-FLAG is not readily observed). All purification were performed at 300mM NaCl; the gel is Coomassie stained. Panel B shows the purification of SKIV2L2-FLAG at 100 and 300mM NaCl. The upper panel is a Commassie stained gel; the lower panel is a western blot using anti-FLAG antibodies. In either case, the SKIV2L2-FLAG signal is drastically diminished with increased salt.
Figure S5: Purification of RBM7-LAP using Dynabeads coupled to either llama polyclonal antibodies provided by Rout Lab (R) or nanobodies (N). Gel stained with Pierce Silver Stain Kit – cat. #24612 (Thermo Fisher Scientific, Rockford, IL).