

Supplementary Material For:

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

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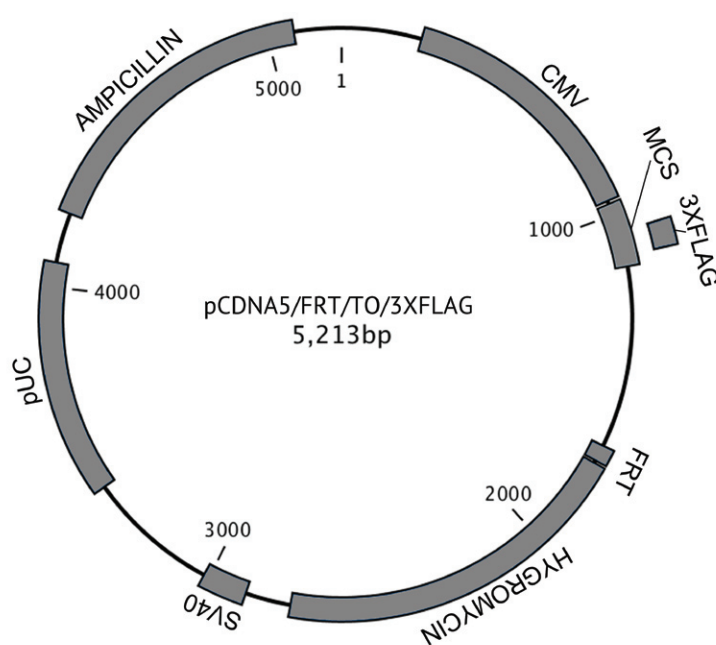


Figure S1: Map of a modified pcDNA5/FRT/TO vector for C-terminal 3xFLAG tagging.

3xFLAG coding sequence: GAC TAC AAG GAC CAC GAC GGT GAC TAC AAG GAC CAC GAC ATC GAC TAC AAG GAC GAC GAC AAG TGA. GenBank file of entire vector sequence provided as a supplement, pcDNA5_FRT_TO.gb

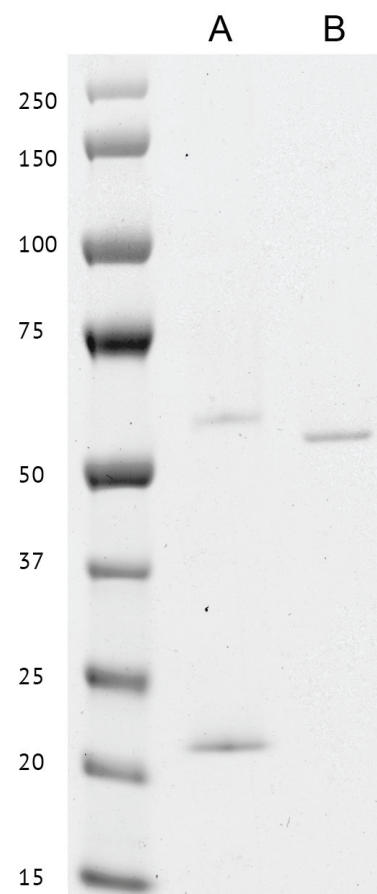


Figure S2: Migration of BAP-FLAG protein compared to anti-FLAG IgG chains. As IgG chains can sometimes leak from conjugated beads during elution with LDS, and as BAP-FLAG shares a similar mass to IgG heavy chain (HC), the following comparison was made: 5 μ l M2-coupled Dynabeads slurry were incubated 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.

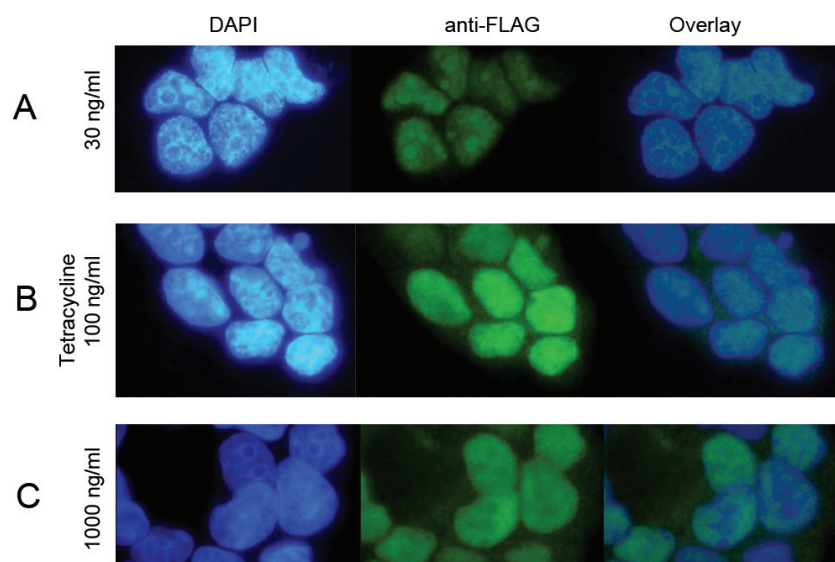


Figure S3: Over-expression can lead to protein mislocalization. Cells induced for expression of SKIV2L2-FLAG (a.k.a. hMTR4-FLAG) using three different concentrations of tetracycline: (A) 30 ng/ml, (B) 100 ng/ml, and (C) 1000 ng/ml. At low expression in (A), SKIV2L2-FLAG is nuclear localized with accumulation in nucleoli. However, with increasing tetracycline concentration SKIV2L2-FLAG expression goes up and more signal is observed within the nucleus and in 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 antibodies¹⁶.

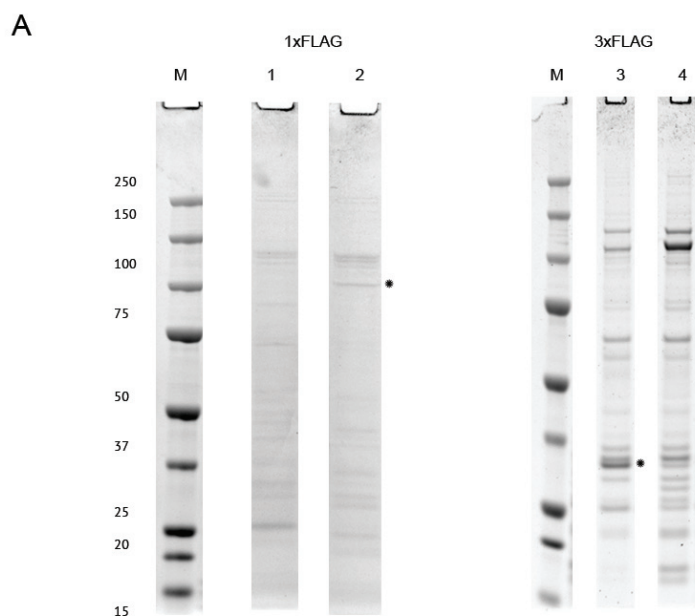
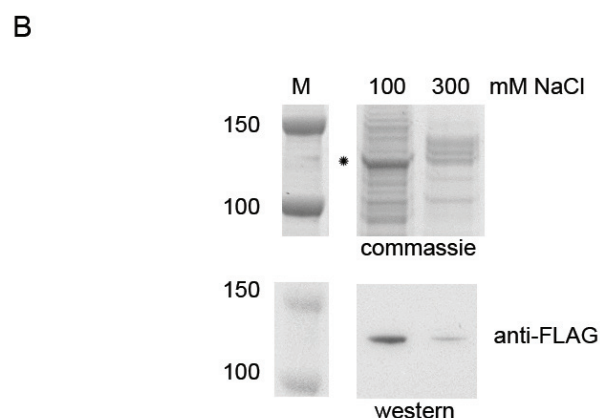


Figure S4: 3xFLAG exhibits superiority over 1xFLAG. Panel A shows the results of purification of RRP41 and RRP6 proteins tagged either with 1xFLAG (A, lane 1 and 2, respectively) or 3xFLAG (B, lane 3 and 4, respectively). Handle proteins marked with asterisk (in lane 1, RRP41-FLAG is not readily observed). 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 Coomassie 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.



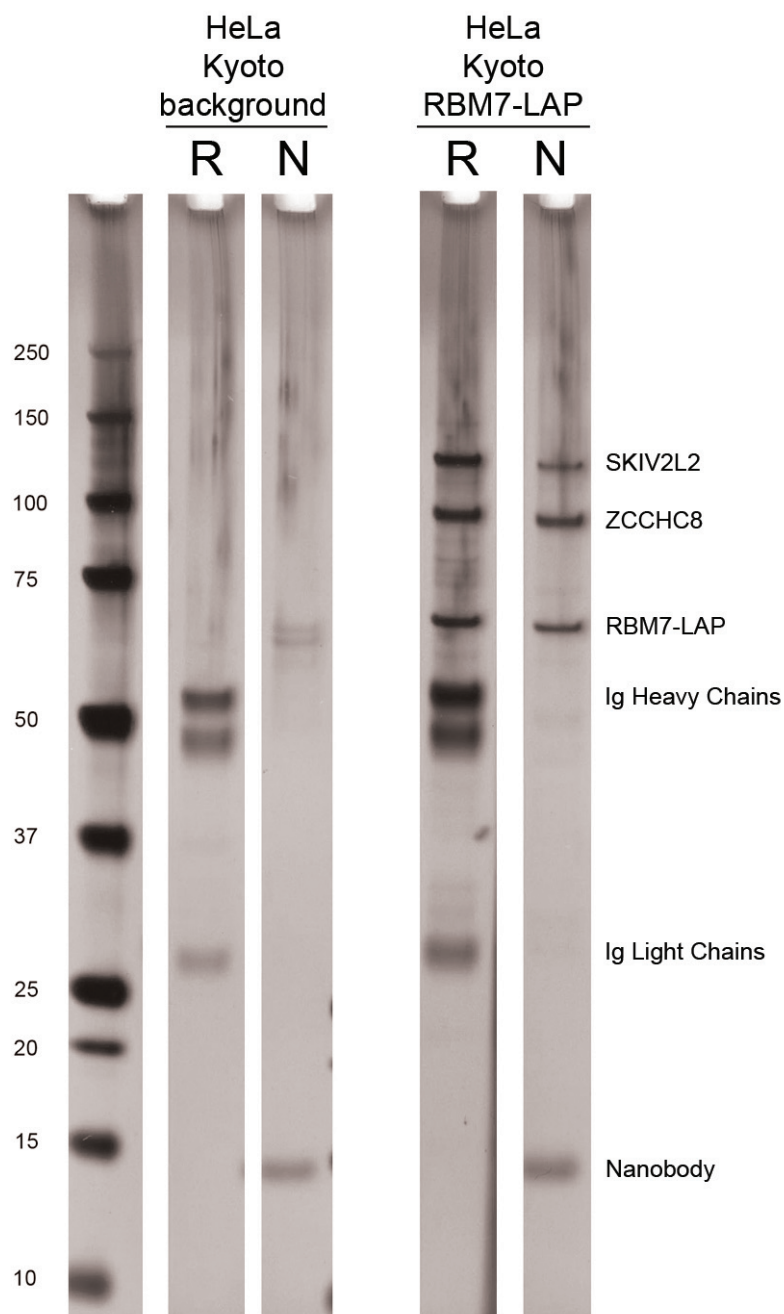


Figure S5: Purification of RBM7-LAP using Dynabeads coupled to either llama polyclonal antibodies provided by Rout Lab (R) or nanobodies (N). Gel stained with Pierce Silver Stain Kit – cat. #24612 (Thermo Fisher Scientific, Rockford, IL).