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RALY is a member of the heterogeneous nuclear ribonucleoproteins, a family of RNA-binding proteins generally involved in many processes of mRNA metabolism. No quantitative proteomic analysis of RALY-containing ribonucleoparticles (RNPs) has been performed so far, and the biological role of RALY remains elusive. Here, we present a workflow for the characterization of RALY's interaction partners, termed iBioPQ, that involves in vivo biotinylation of biotin acceptor peptide (BAP)-fused protein in the presence of the prokaryotic biotin holoenzyme synthetase of BirA so that it can be purified using streptavidin-coated magnetic beads, circumventing the need for specific antibodies and providing efficient pulldowns. Protein eluates were subjected to tryptic digestion and identified using data-independent acquisition on an ion-mobility enabled high-resolution nanoUPLC-QTOF system. Using label-free quantification, we identified 143 proteins displaying at least 2-fold difference in pulldown compared to controls. Gene Ontology overrepresentation analysis revealed an enrichment of proteins involved in mRNA metabolism and translational control. Among the most abundant interacting proteins, we confirmed RNA-dependent interactions of RALY with MATR3, PABP1 and ELAVL1. Comparative analysis of pulldowns after RNase treatment revealed a protein-protein interaction of RALY with eIF4AIII, FMRP, and hnRNP-C. Our data show that RALY-containing RNPs are much more heterogeneous than previously hypothesized.