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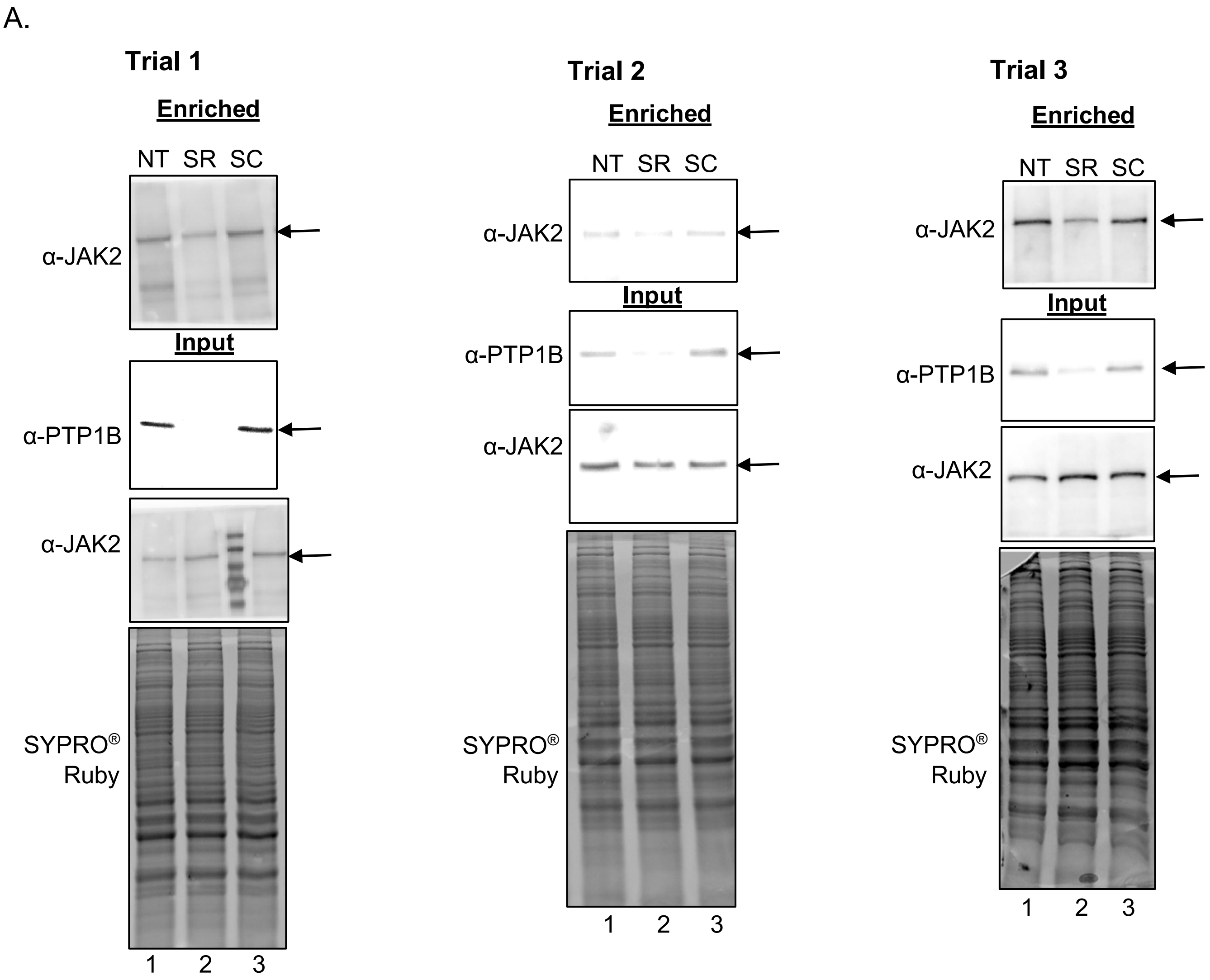


B. PTP1B knockdown quantification (%)

Trial	1	2	3	Mean	SE
NT	100	100	100	100	0
SR	0	10	15	8.2	4.3
SC	120	180	70	120	32

C. JAK2 enrichment quantification (%)

Trial	1	2	3	Mean	SE
NT	100	100	100	100	0
SR	50	36	49	45	5
SC	99	89	76	88	7

Figure S1: JAK2 validation by K-BIPS. A) HEK293 cells were untreated (NT) or transfected with PTP1B siRNA (SR) or scrambled control siRNA (SC). After lysis, proteins in lysates were incubated with ATP-biotin. Biotinylated proteins were enriched with streptavidin resin and separated by SDS-PAGE. Input lysates before enrichment were separated as load and expression controls. JAK2 and PTP1B levels were visualized by immunoblotting with specific antibodies. Total proteins were visualized using SYPRO® Ruby staining. The JAK Western blot image from Trial 1 contains a ladder lane between lanes 2 and 3. Truncated images of Western Blot gels from trial 3 are shown in Figure 2A. B) PTP1B bands from input samples were quantified using ImageJ and normalized as a percentage to NT samples (set to 100%), with mean and standard error (SE) shown. C) JAK2 bands from enriched samples were quantified using ImageJ and normalized as a percentage to NT samples (set to 100%), with mean and standard error (SE) shown. A histogram with the % JAK2-biotin enriched from the table is shown in Figure 2B.

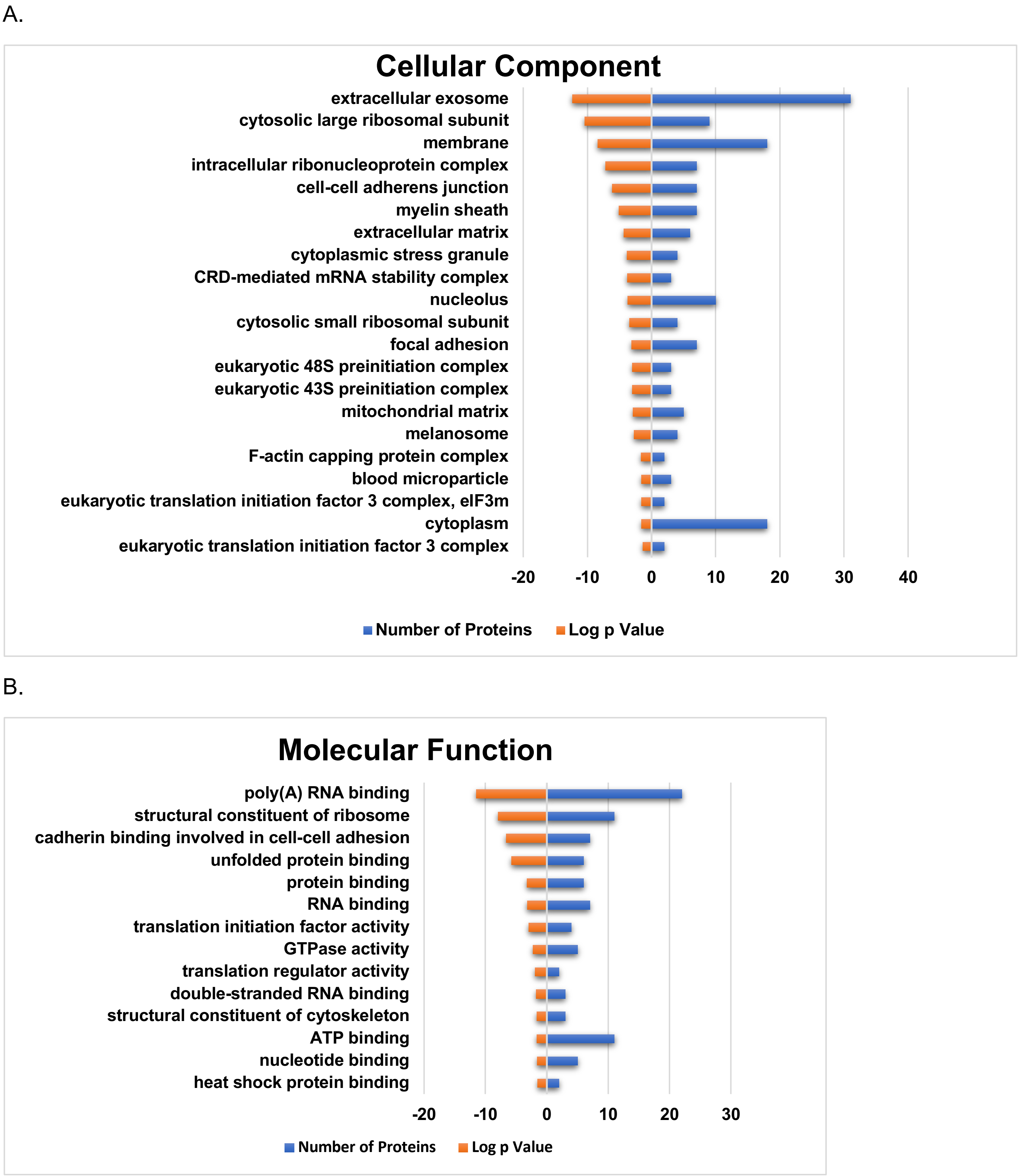


Figure S2: Cellular component and Molecular function classification K-BIPS hits using DAVID 6.8. The 53 K-BIPS hit proteins were classified according to cellular compartment (A) and molecular function (B) using DAVID 6.8. Only the cellular component categories showing both a significant p value (>0.5) and at least 5 proteins are shown Figure 3A, with the full data shown in here in part A.

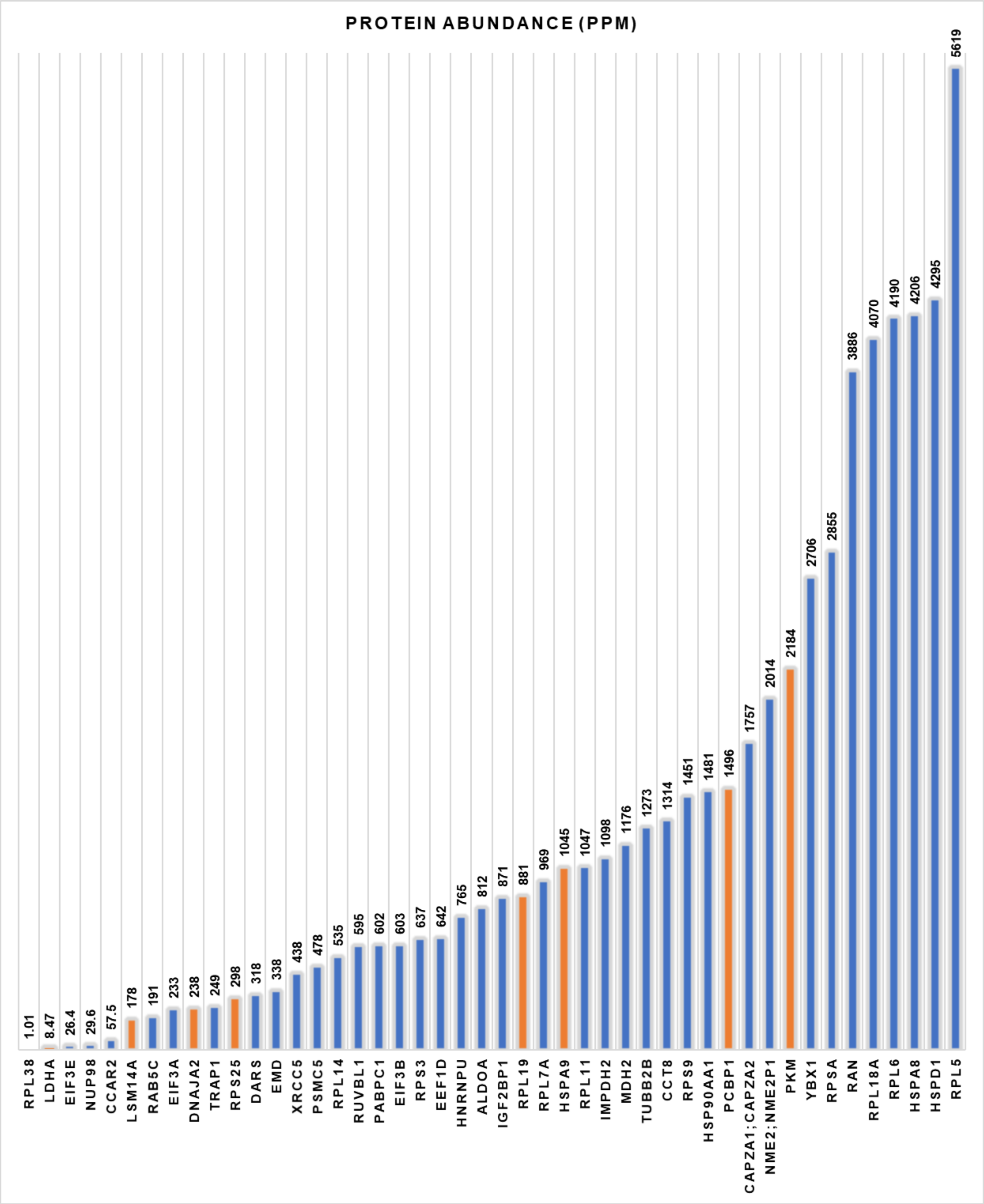
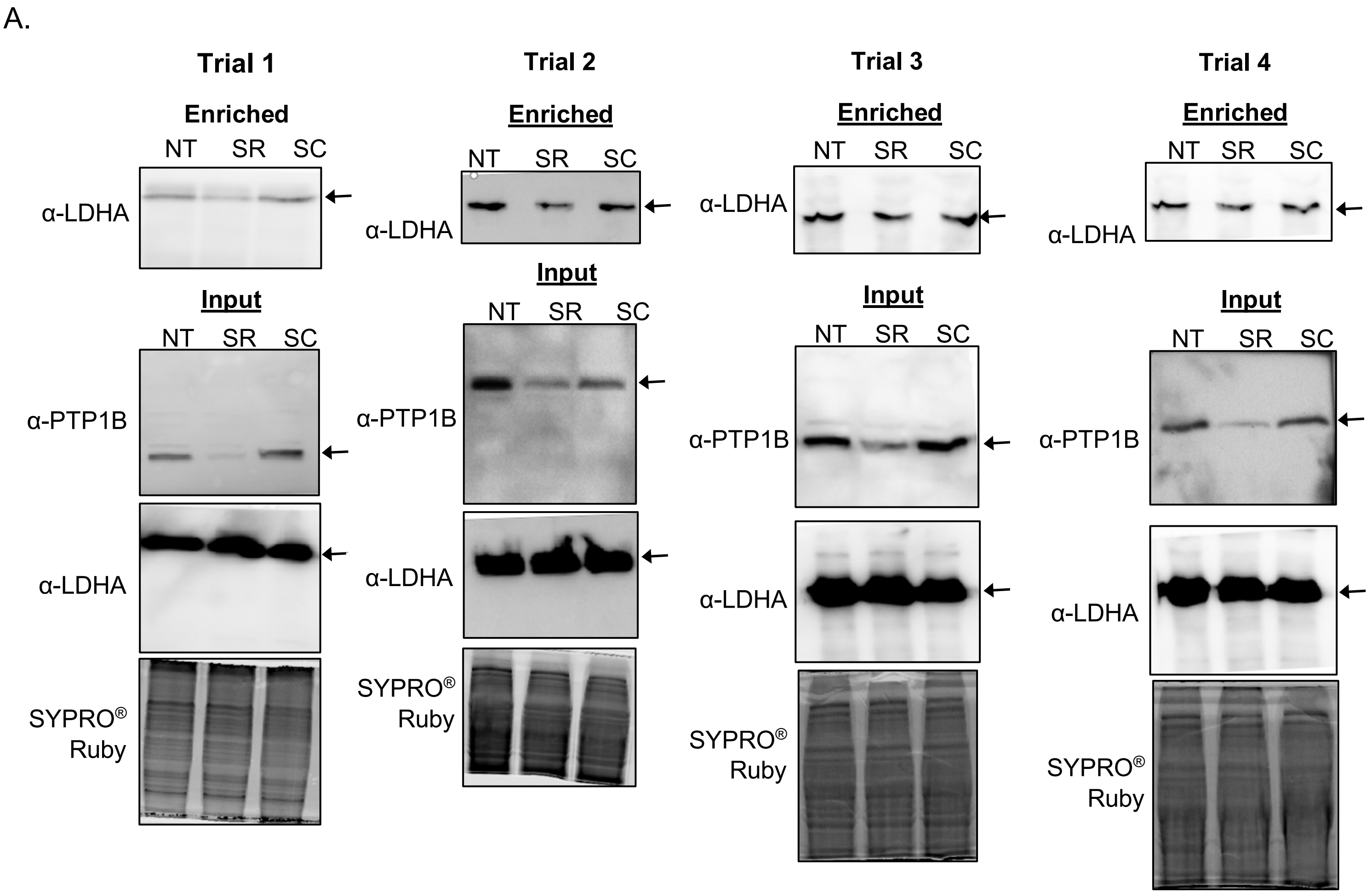


Figure S3: Protein abundance of the 53 K-BIPS hits. Protein abundance values were reported in PAXdb: Protein Abundance Database.[1] The y-axis shows the protein abundance in ppm, with the protein names are on the x-axis. According to the data, 1.01 - 5619 ppm range was observed among the 53 hit proteins. The abundances of all proteins in HEK293 cell is 0.01 to 10,000 ppm.[2] Seven proteins were not observed in the PAXdb database (CHN2, EIF2B4, KRT1, QKI, RBM43, RPL22, and TUBA1B; TUBA4A).



B. PTP1B knockdown quantification (%)

Trial	1	2	3	4	Mean	SE
NT	100	100	100	100	100	0
SR	12	14	18	10	14	2
SC	140	27	130	43	85	29

C. LDHA enrichment quantification (%)

Trial	1	2	3	4	Mean	SE
NT	100	100	100	100	100	0
SR	42	54	73	73	61	8
SC	120	80	93	95	96	7

Figure S4: Secondary confirmation of LDHA as a K-BIPS hit. HEK293 cells were untreated (NT) or transfected with PTP1B siRNA (SR) or scrambled control siRNA (SC). After lysis, proteins in lysates were incubated with ATP-biotin. Biotinylated proteins were enriched with streptavidin resin and separated by SDS-PAGE. Input lysates before enrichment were separated as load and expression control. LDHA and PTP1B levels were visualized by immunoblotting with specific antibodies. Total protein levels were visualized with SYPRO® Ruby staining. Truncated images of Western Blot gels from trial 1 are shown in Figure 4A. B) PTP1B bands from input samples were quantified using ImageJ and normalized as a percentage to NT samples (set to 100%), with mean and standard error (SE) shown. C) LDHA bands from enriched samples were quantified using ImageJ and normalized as a percentage to NT samples (set to 100%), with mean and standard error (SE) shown. A histogram with the % LDHA-biotin enrichment in the table is shown in Figure 4B.

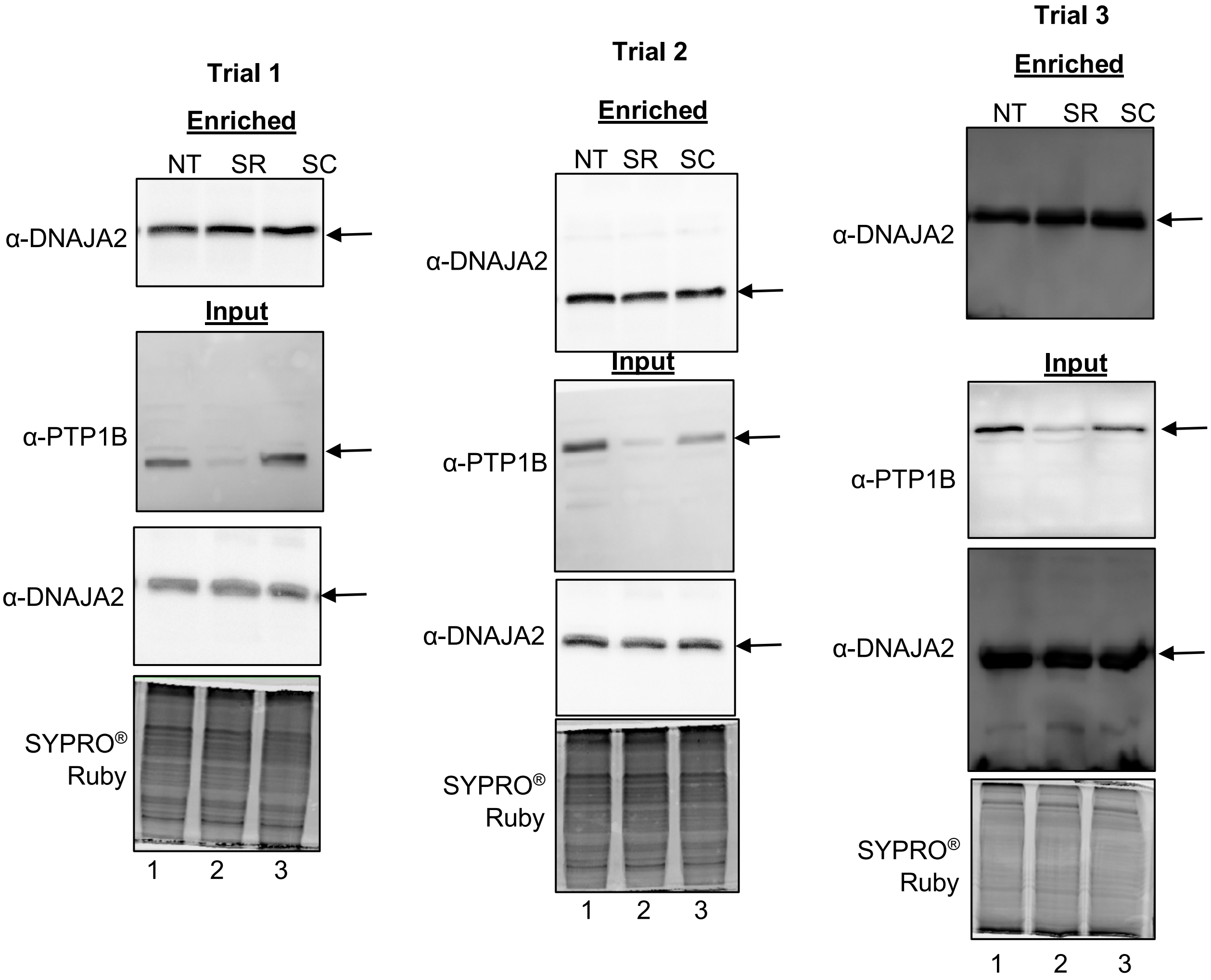
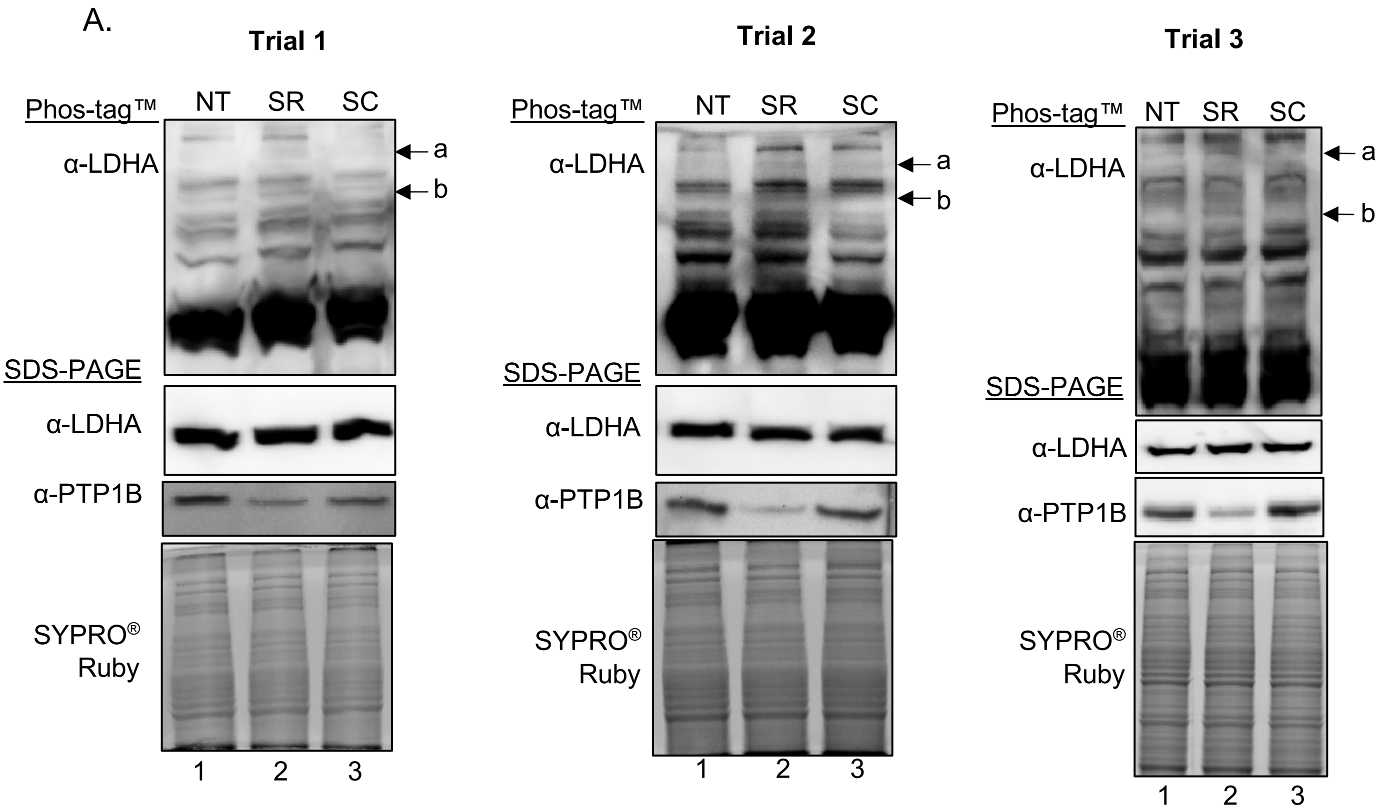


Figure S5: DNAJA2 validation by K-BIPS. HEK293 cells were untreated (NT) or transfected with PTP1B siRNA (SR) or scrambled control siRNA (SC). After lysis, proteins in lysates were incubated with ATP-biotin. Biotinylated proteins were enriched with streptavidin resin and separated by SDS-PAGE. Input lysates before enrichment were separated as load and expression control. DNAJA2 and PTP1B levels were visualized by immunoblotting with specific antibodies. SYPRO® Ruby staining was used to visualized total protein.



B. PTP1B knockdown quantification (%)

Trial	1	2	3	Mean	SE
NT	100	100	100	100	0
SR	49	13	30	31	10
SC	81	43	130	86	26

C. LDHA enrichment quantification (%)

	Band	Trial 1	Trial 2	Trial 3	Mean	SE
NT	a (top)	60	72	32	54	12
	b (bottom)	80	58	70	69	6.0
SR	a (top)	100	100	100	100	0
	b (bottom)	100	100	100	100	0
SC	a (top)	47	43	15	35	10
	b (bottom)	57	37	31	42	7.8

Figure S6: LDHA validation by Phos-tag$^{\text{TM}}$ SDS-PAGE. A) After lysis, proteins in the lysates were separated using Phos-tag$^{\text{TM}}$ SDS-PAGE (top gel) or traditional SDS-PAGE (bottom gels). Separated proteins were transferred into the PVDF membrane, and LDHA and PTP1B levels were visualized with the appropriate antibodies. The arrowheads indicated new bands in the PTP1B knockdown lane. To confirm the equal level of protein, SYPRO® Ruby stained gel was shown. The third trial is shown in Figure 5A. B) PTP1B bands from input samples were quantified using ImageJ and normalized as a percentage to NT samples, with mean and standard error (SE) shown. C) The new LDHA bands in the Phos-tag™ gels were quantified using ImageJ and normalized as a percentage to SR samples (set to 100%), with mean and standard error (SE) shown. The histograms of % phosphorylated LDHA bands upper and lower bands) are shown in Figure 5B and C.

References

1. Wang, M.; Herrmann, C. J.; Simonovic, M.; Szklarczyk, D.; von Mering, C., Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **2015,** *15* (18), 3163-8.
2. Geiger, T.; Wehner, A.; Schaab, C.; Cox, J.; Mann, M., Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. *Molecular & cellular proteomics : MCP* **2012,** *11* (3), M111.014050-M111.014050.