

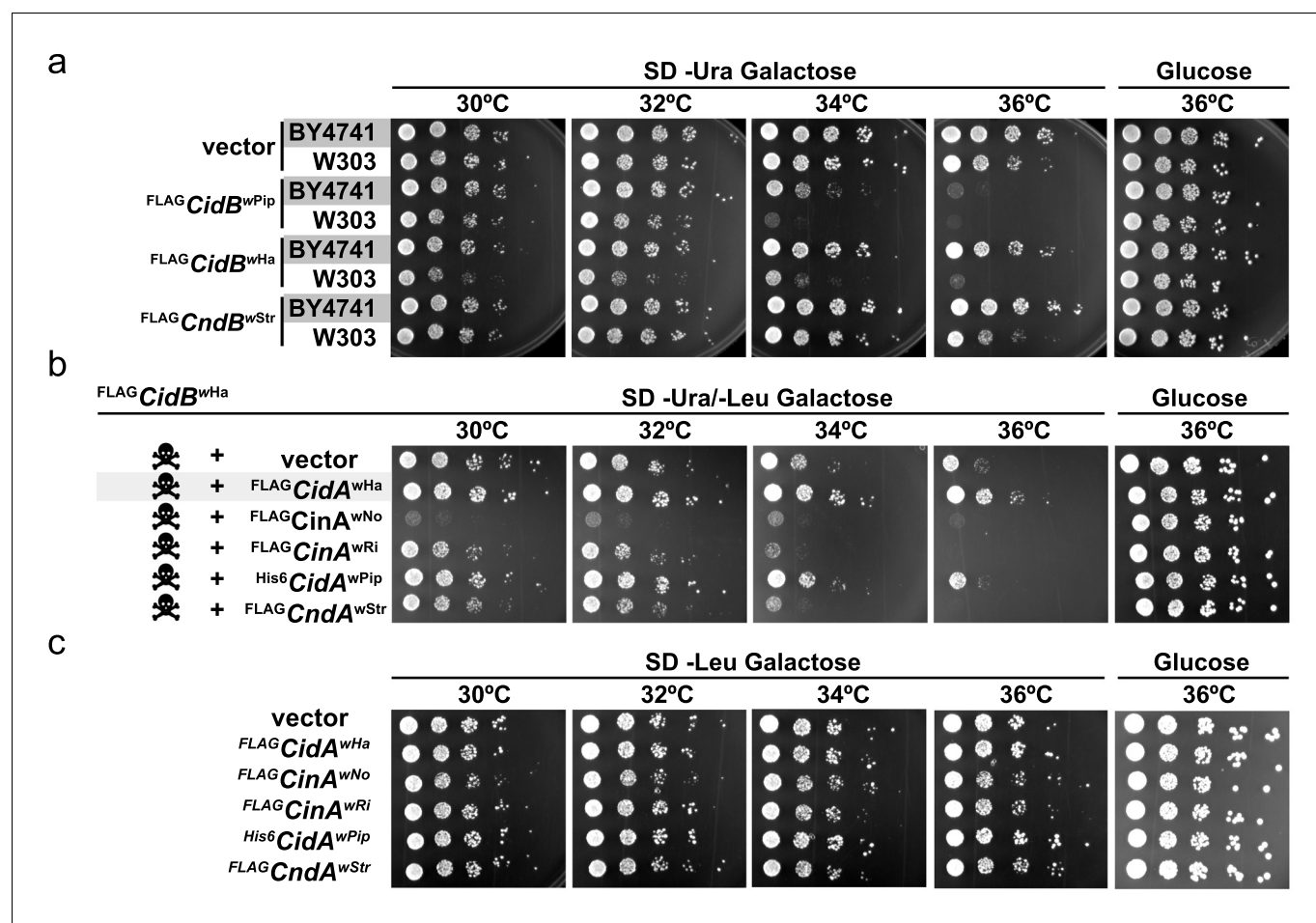


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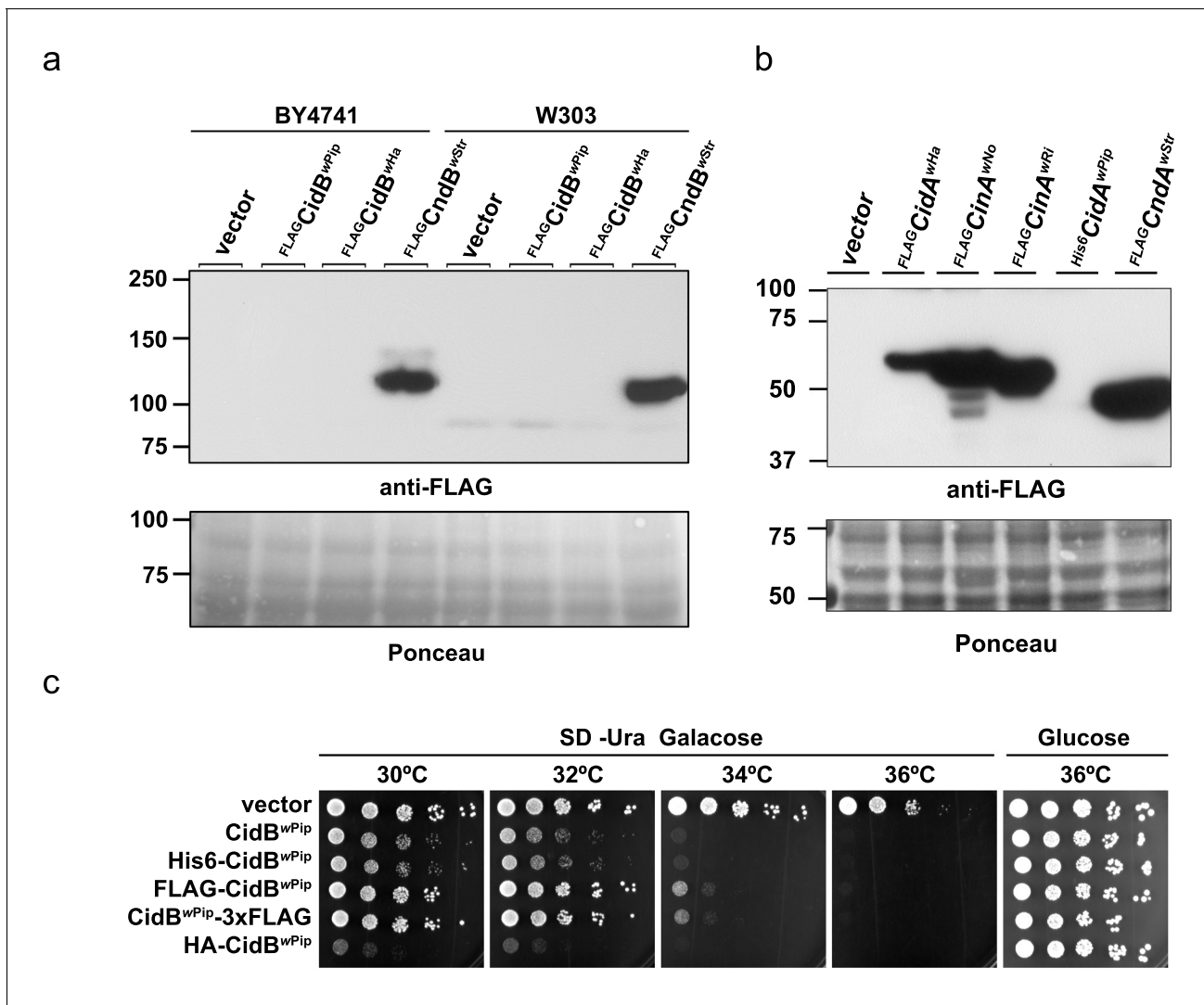
## Figures and figure supplements

The *Wolbachia* cytoplasmic incompatibility enzyme CidB targets nuclear import and protamine-histone exchange factors

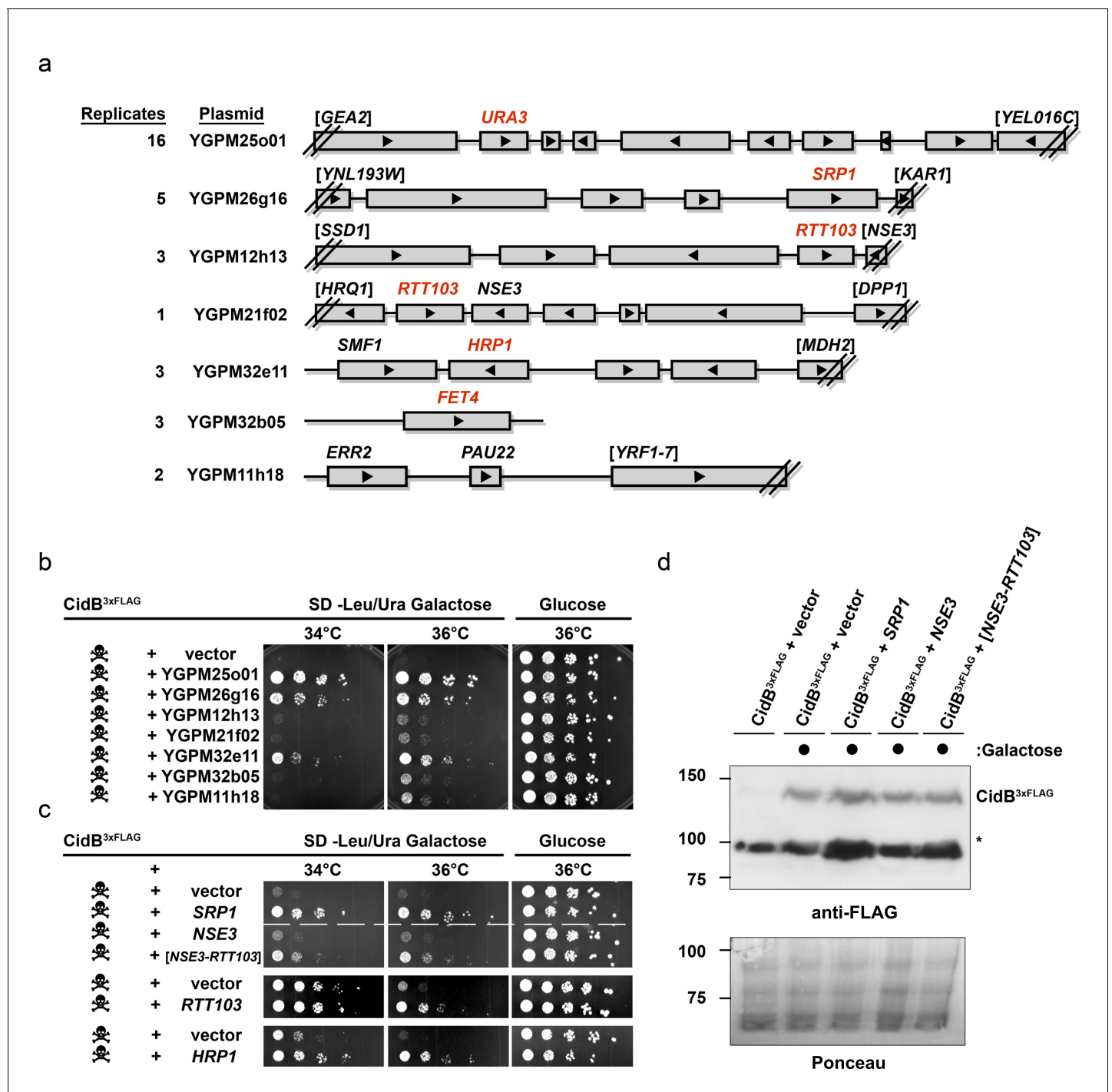
**John Frederick Beckmann et al**



**Figure 1.** Cif toxicity in *S. cerevisiae*. (a) Five-fold dilutions of yeasts BY4741 and W303-1A carrying galactose-inducible epitope-tagged *Wolbachia* genes on pRS416GAL1. Three Cif homologs from *Wolbachia* strains wPip, wHa, and wStr showed strong to mild toxicity. All three showed increased toxicity in W303-1A compared to BY4741 (three replicates). (b) Toxin-antidote behavior was exhibited by the *cidAB*<sup>wHa</sup> operon. FLAG *CidB*<sup>wHa</sup> exhibited toxicity at 36°C when expressed from pRS416GAL1. Co-expression of cognate partner FLAG *CidA*<sup>wHa</sup> from the 2-micron plasmid pRS425GAL1 rescues growth. Non-cognate partners did not rescue. Conversely, expression of FLAG *CinA*<sup>wNo</sup> from a bidirectionally incompatible *Wolbachia* strain wNo, enhanced toxicity of FLAG *CidB*<sup>wHa</sup> (four replicates). (c) CifA expression alone was nontoxic (three replicates).

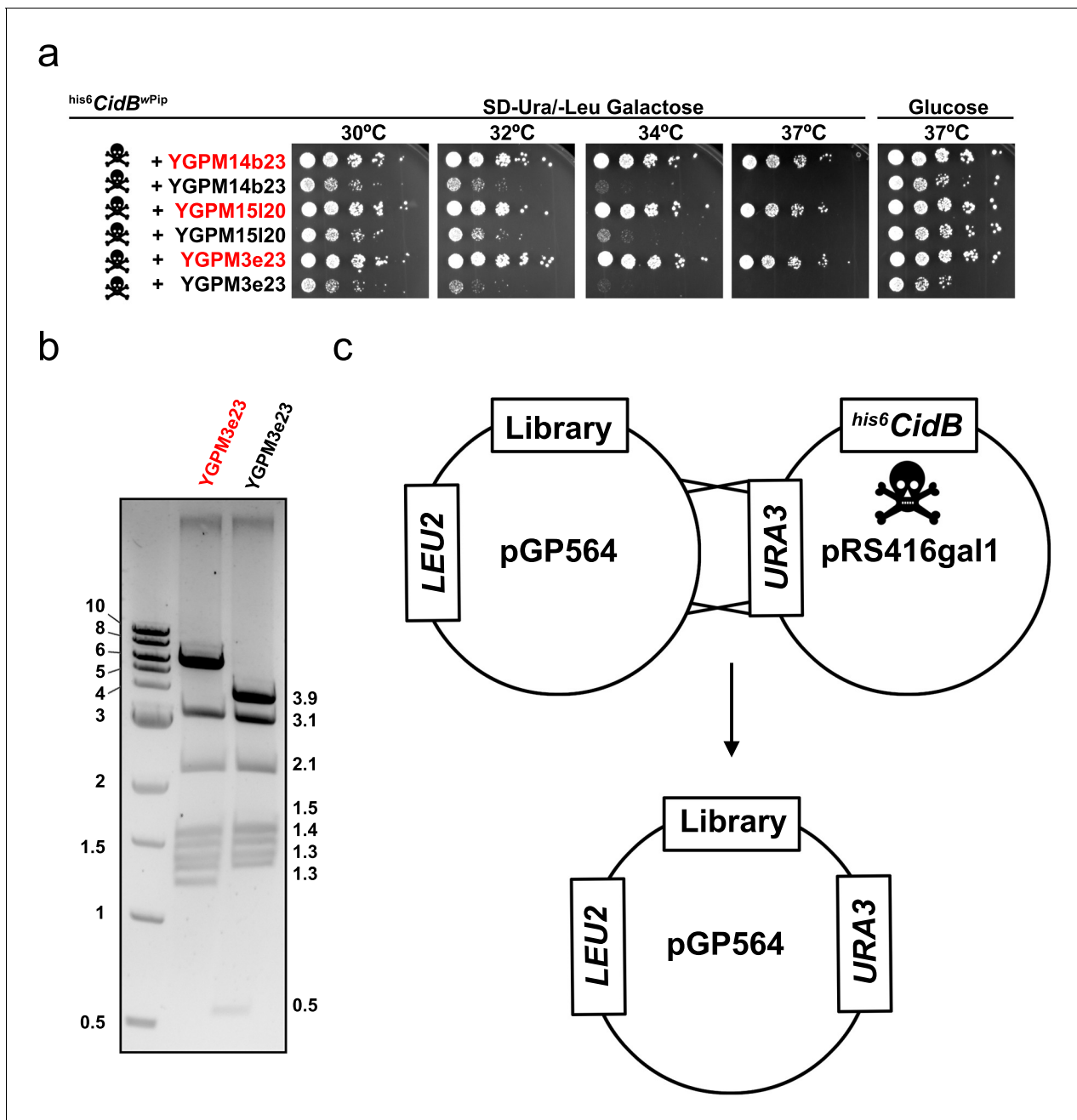


**Figure 1—figure supplement 1.** Expression Analysis of CI Factors in Yeast. (a) Western blots of total yeast extracts show that some CidB orthologs are not expressed well (three replicates). However, the weakly toxic truncated FLAG-CndB<sup>wStr</sup>(1–1085) is readily detected. Ponceau S staining indicates relative sample loading. (b) CifA orthologs express well and are detected by anti-FLAG Western blots (three replicates). His6-CidA<sup>wPip</sup> serves as negative control. (c) Serial dilutions comparing effects of recombinant protein tags on CidB<sup>wPip</sup>. FLAG and 3xFLAG tags weaken toxicity of CidB whereas an HA tag enhances toxicity. His6-CidB phenocopies wildtype (untagged). None of these proteins could be detected, except the 3xFLAG-tag which gave sporadic detection and also weakened the phenotypic penetrance (representative of 4 replicates; pictures taken on day 3). All orthologs were expressed from a Gal1 promoter on the CEN vector pRS416GAL1.

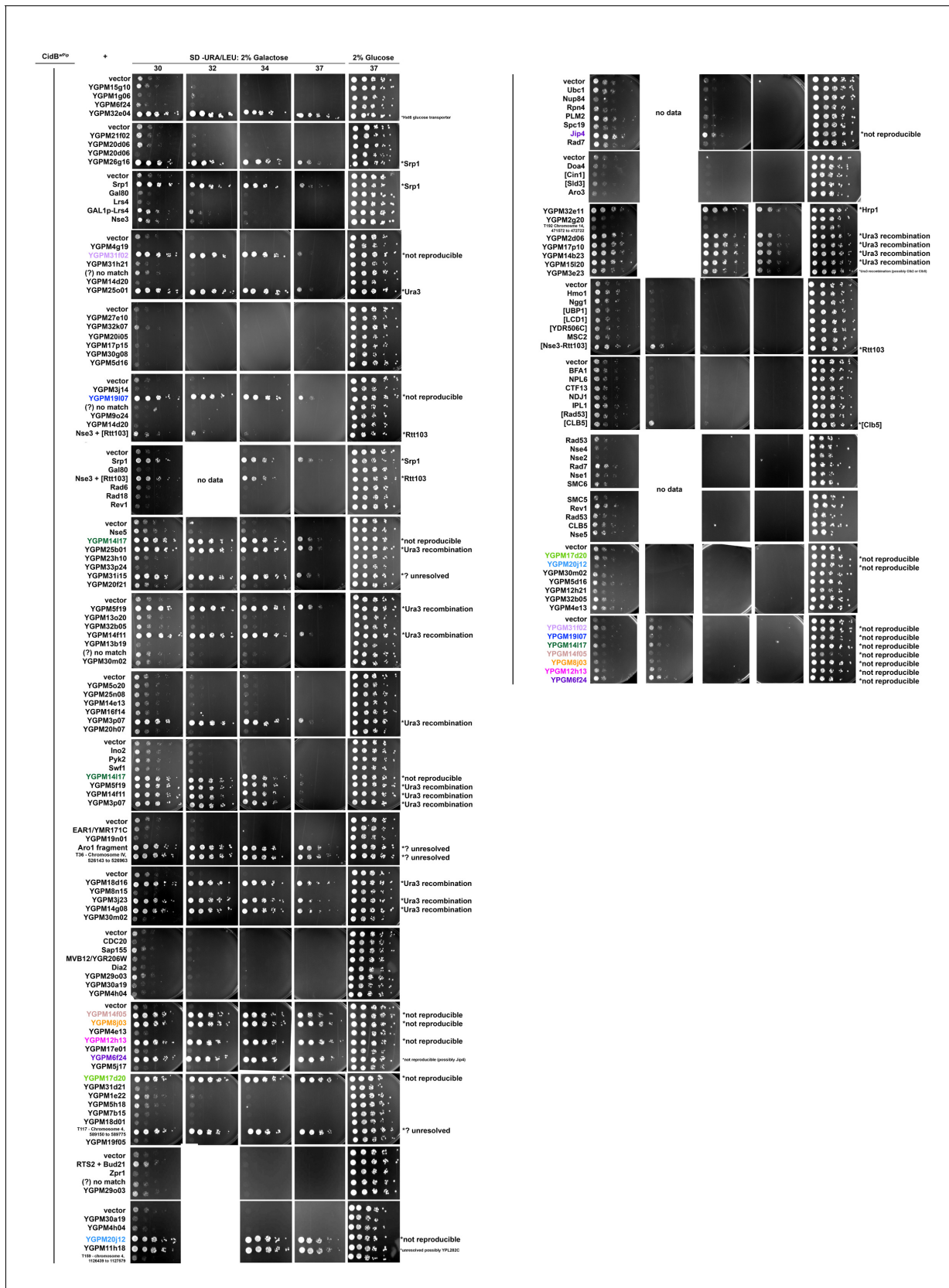


**Figure 2.** Yeast Suppressors of CidB. (a) Seven library plasmids were high-copy suppressors of CidB<sup>wPip</sup> toxicity. Red genes suppressed when individually sub-cloned. Library plasmid YGPM25o01 includes *URA3* and measures screen efficiency since it is an expected suppressor; Backslashes and brackets denote ORF truncations. (b) Five-fold serial dilutions of yeast (W303-1A) with recovered suppressing library plasmids co-transformed with pRS416GAL1-CidB<sup>3xFLAG-wPip</sup>. Library plasmid suppression varied. Suppression by YGPM25o01 (*URA3* control), YGPM26g16, and YGPM32e11 was strong and consistent (three replicates). Plasmids YGPM12h13, YGPM21f02, YGPM32b05, and YGPM11h18, showed weaker and less consistent suppression across four replicates. (c) Individual yeast genes *SRP1*, *RTT103*, and *HRP1* suppressed CidB<sup>wPip</sup> toxicity (three replicates). (d) Immunoblot analysis confirmed that suppressor plasmids do not reduce CidB expression. CidB and suppressors were controlled by *GAL1* and endogenous promoters, respectively. Asterisk, an unknown cross-reacting yeast protein. Ponceau S staining indicated relative sample loading.

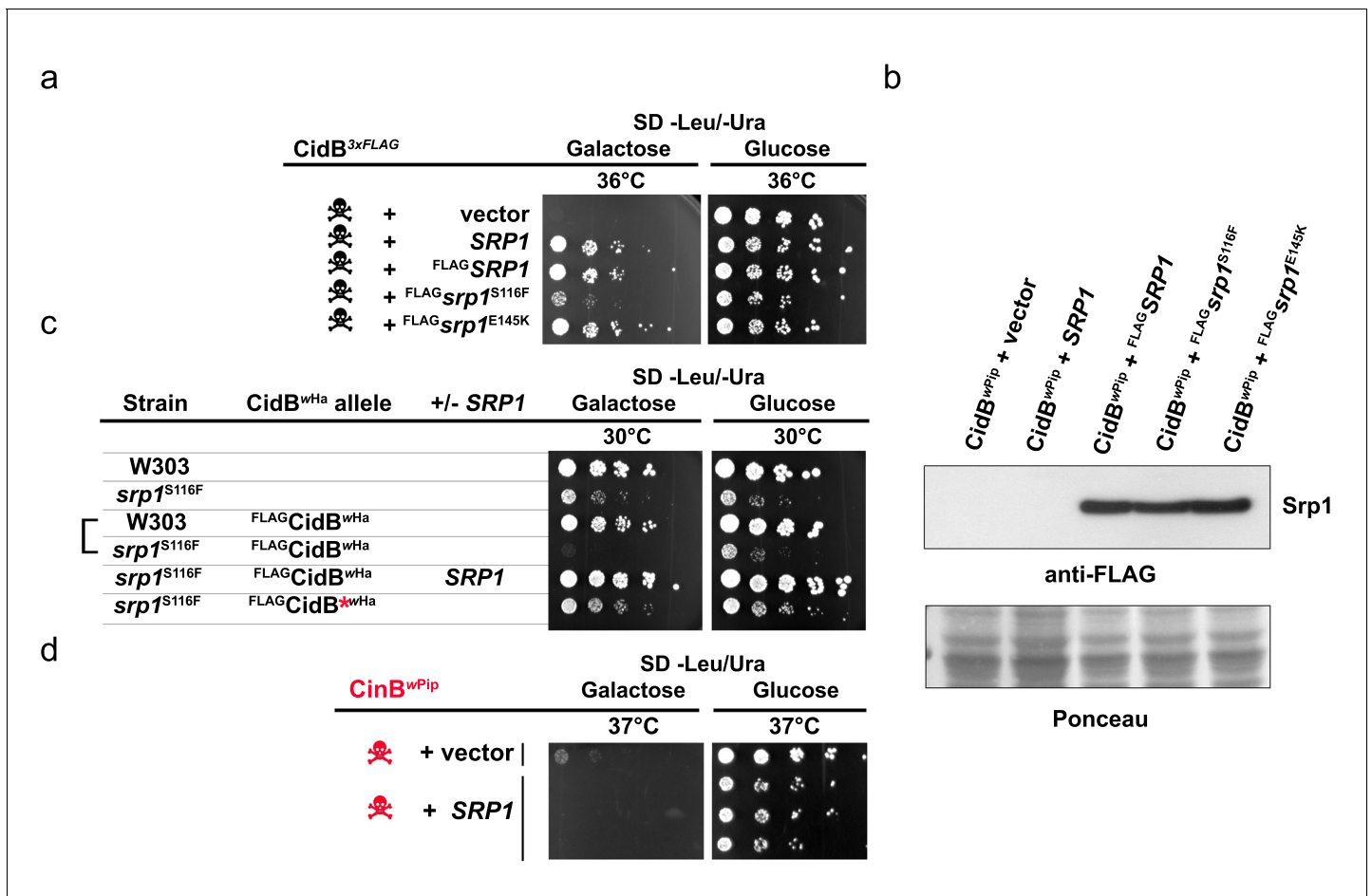




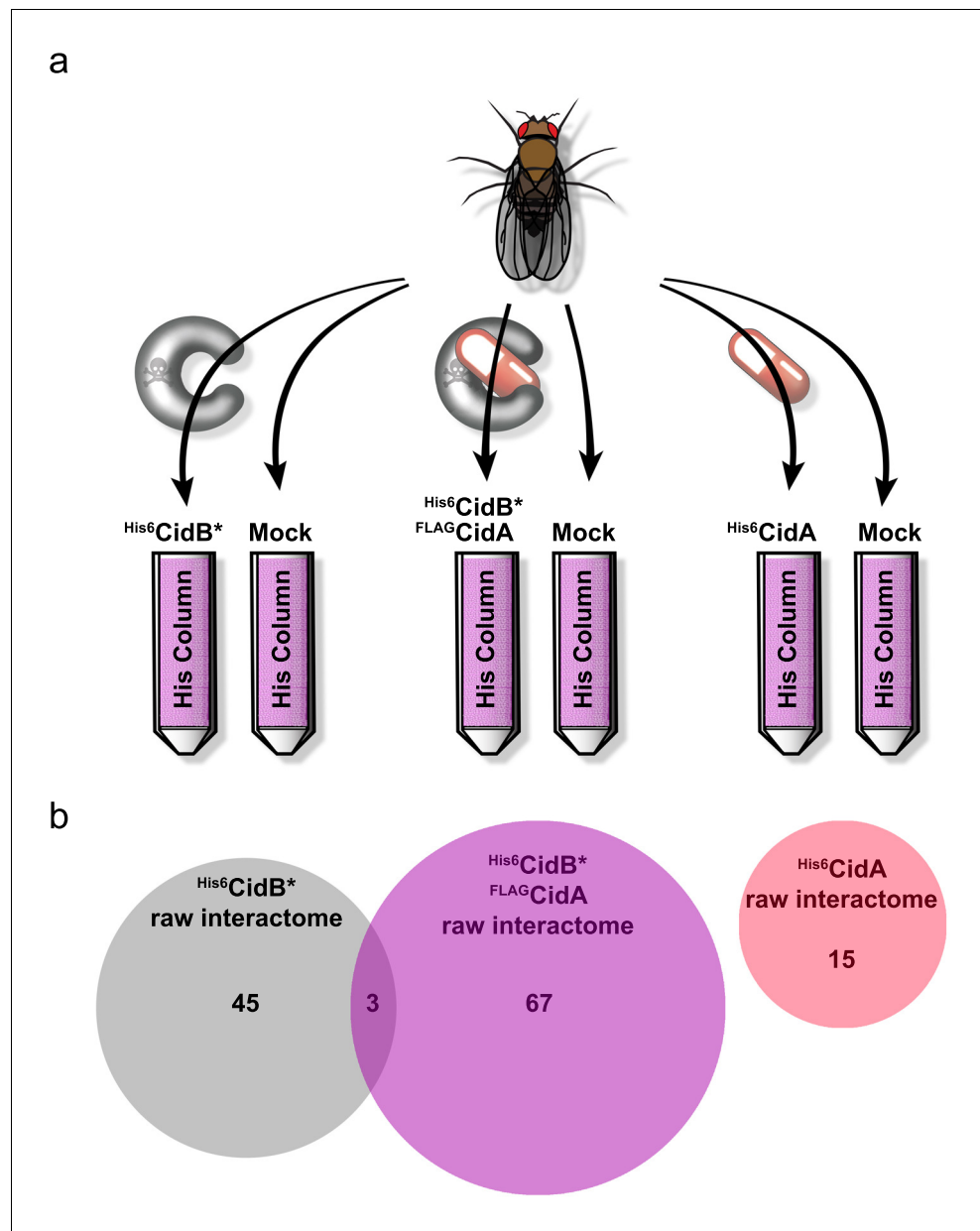
**Figure 2—figure supplement 1.** Eliminating false positives from the high-copy *His6 CidB<sup>wPip</sup>* suppression screen. Plasmids are named YGPMxxxx. (a) Five-fold serial dilution comparing three false positive suppressor plasmids (pulled directly from the screen; red) to the cognate plasmids (pulled directly from the source library; black) showed suppression only after passage through yeast during the screen. Lack of suppression by the original plasmids suggested the screening procedure could produce false positive artifacts (three replicates). Yeast background is BY4741. (b) Restriction digests comparing a false positive with the originating library plasmid. DNAs were simultaneously cleaved with XhoI, ApaI, and XcmI. Differences in banding patterns indicated the pGP564 plasmid backbone had increased the size of the 3.9 and 0.5 kb fragments (one experiment). (c) Sequencing the plasmid revealed that the *URA3* marker in pRS416GAL1-*His6 CidB<sup>wPip</sup>* had recombined into the *LEU2* library plasmid (pGP564). This unique recombination event occurred independently in 13 false-positive library plasmids. The recombination event produces a false positive by allowing yeast to lose the *CidB* toxin plasmid through equivalent growth support on media lacking uracil by the recombinant *URA3* marker in the library plasmid. We culled hits with this recombination from our final list.



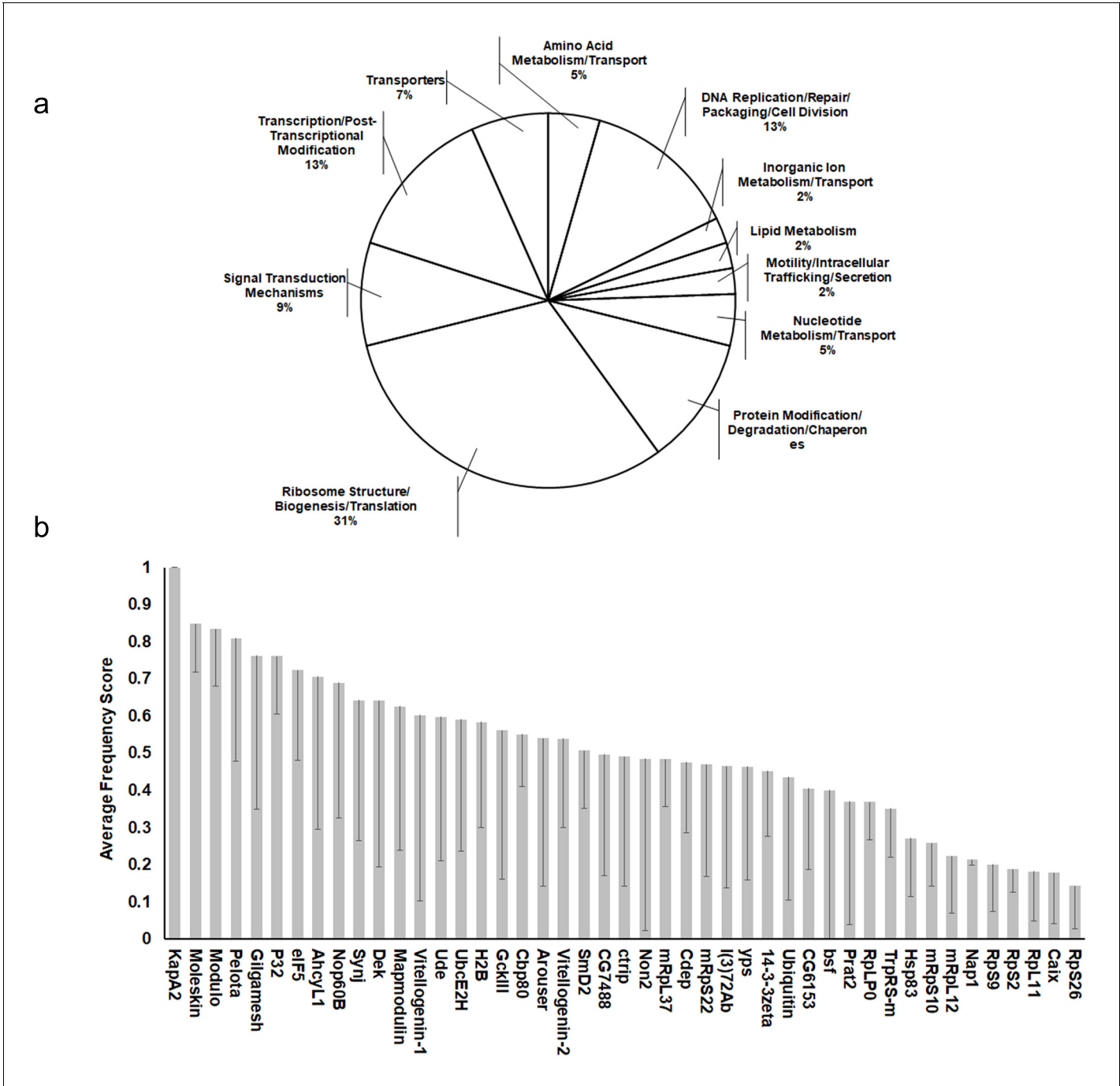
**Figure 2—figure supplement 2.** Five-fold serial dilutions of yeast (BY4741) with recovered suppressing library plasmids co-transformed with pRS416GAL1-<sup>HIS6</sup>CidB. These data include all the originally isolated plasmids that were then re-screened in our analysis. Colored plasmids show results that were not reproducible between experiments.



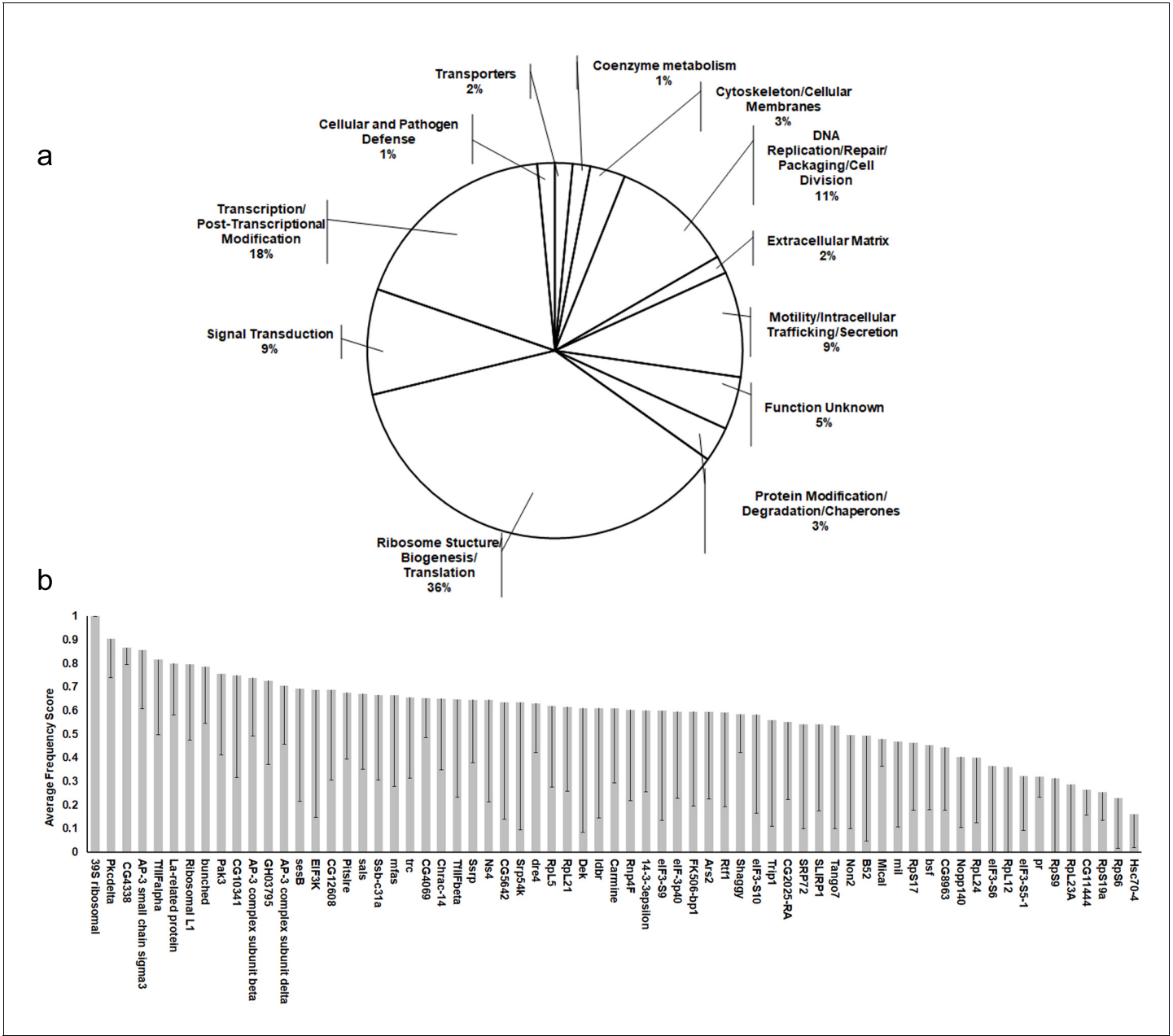
**Figure 3.** Analysis of high-copy *SRP1* suppression of CidB<sup>wPip</sup> toxicity in yeast. (a) Differential impact of mutations affecting distinct Srp1 functions. An *srp1* mutation impairing NLS binding (S116F) weakened suppression in W303-1A. E145K, which inhibits cotranslational protein degradation, did not impact suppression (three replicates). (b) Immunoblot analysis showed equivalent protein levels in *srp1* mutants. Ponceau S staining demonstrated similar loading (three replicates). (c) The *srp1*-S116F mutation sensitized W303-1A yeast to FLAG CidB<sup>wHa</sup>-induced toxicity in 6/7 replicates. Wild-type *SRP1* complemented the mutation (5<sup>th</sup> row). Red \* indicates an inactive DUB catalytic mutant control (6<sup>th</sup> row). Blank columns are empty vectors. (d) High-copy *SRP1* did not suppress CinB<sup>wPip</sup> toxicity in BY4741 yeast (three replicates).



**Figure 4.** *Drosophila* Interactome Analysis. **(a)** Experimental pipeline for defining CidA and CidB interactomes. Soluble lysates from *Drosophila* adults were passed over columns bound to the indicated recombinant proteins and washed. Remaining proteins were eluted and subjected to in-solution LC-MS/MS analysis. **(b)** Venn diagram of protein identifications from raw biological triplicate measurements. The <sup>His6</sup>CidB\* interactome was dramatically changed when it was bound to <sup>FLAG</sup>CidA. The interactome of <sup>His6</sup>CidA itself was modest and showed no overlap with the *Drosophila* proteins bound to either CidB\* or the CidA-CidB\* complex.

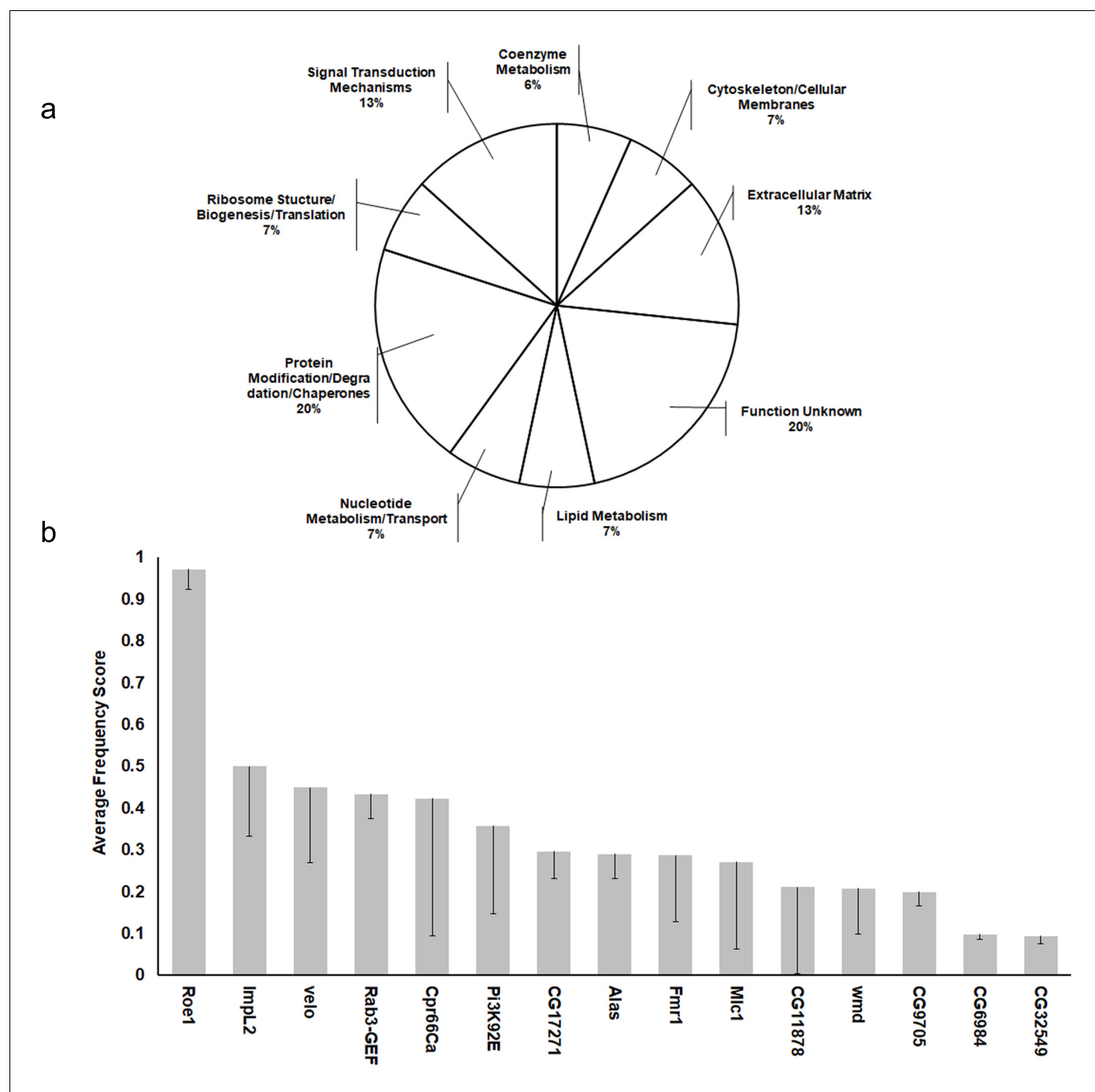


**Figure 4—figure supplement 1.** Triplicate Enrichment Interactome for  $\text{His6CidB}^{*wPip}$ . (a) Pie chart showing functional categories of protein hits. (b) 45 triplicate-enrichment hits ranked according to peptide frequency  $F$ .

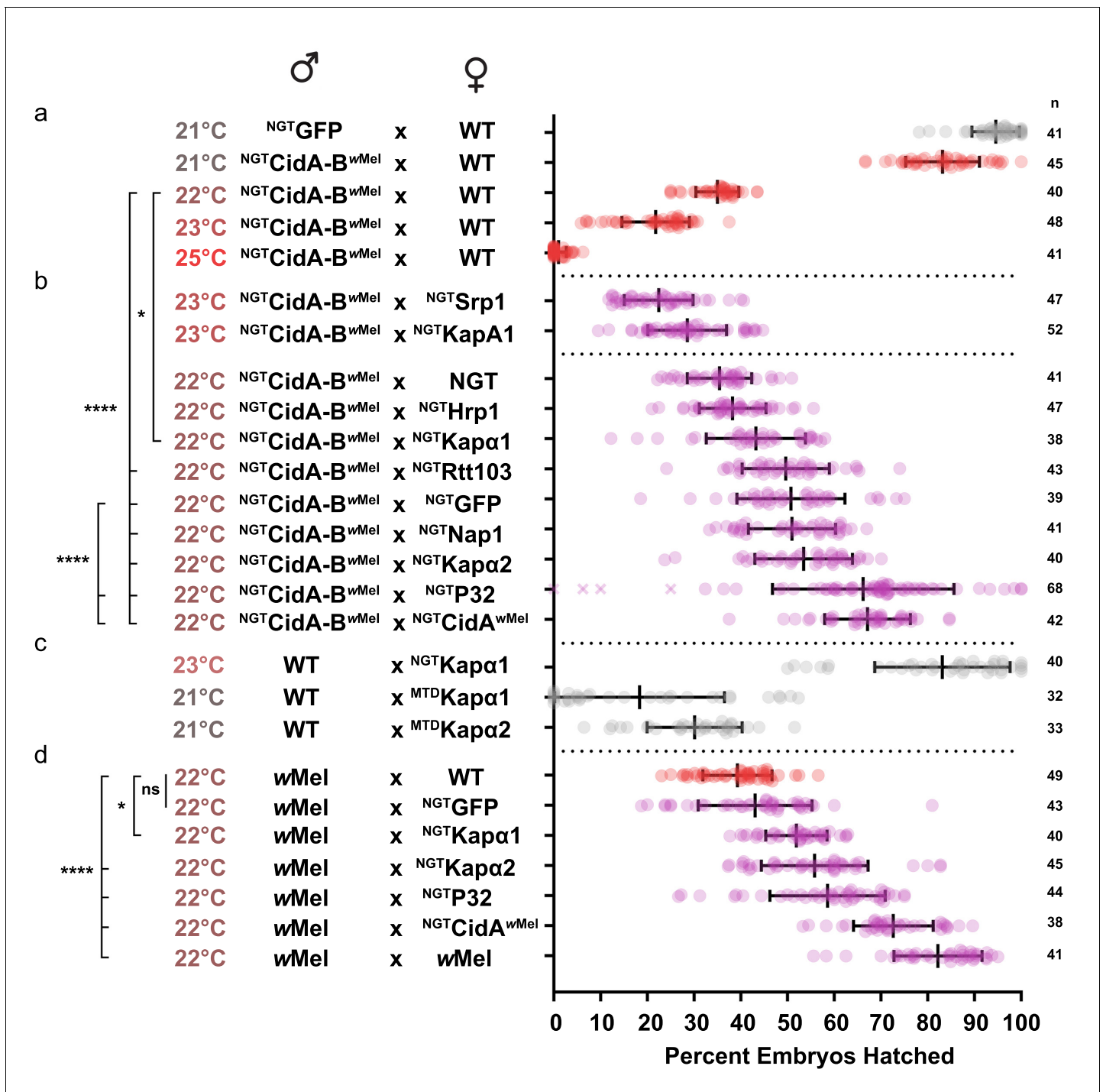


**Figure 4—figure supplement 2.** Triplicate Enrichment Interactome for the <sup>FLAG</sup>CidA<sup>wPip/His6</sup>CidB<sup>wPip</sup> complex. (a) Pie chart showing functional categories of protein hits. (b) 67 triplicate-enrichment hits ranked according to peptide frequency F.

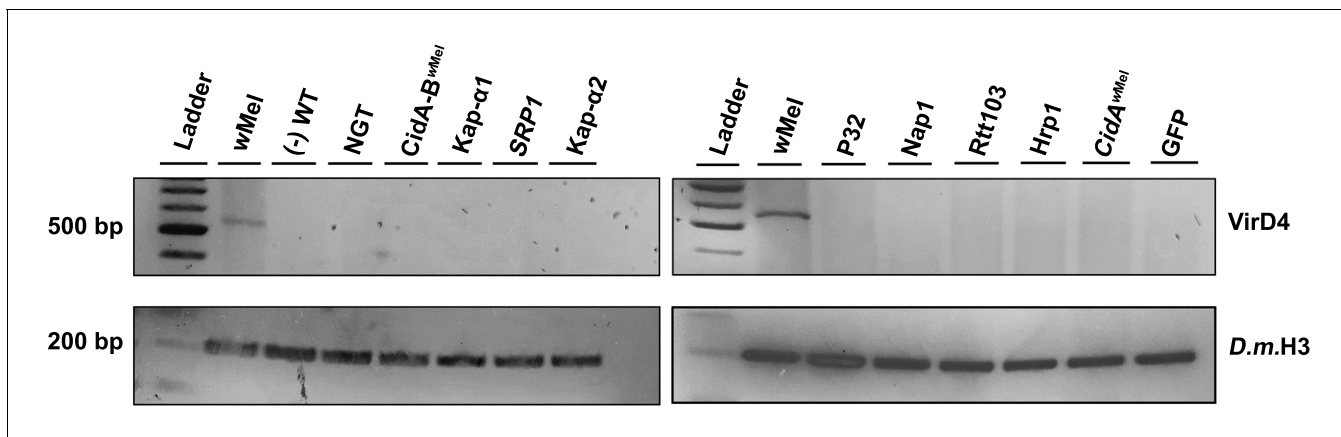




**Figure 4—figure supplement 3.** Triplicate Enrichment Interactome for  $\text{His}^6\text{CidA}^{\text{WPip}}$ . (a) Pie chart showing functional categories of protein hits. (b) 15 triplicate-enrichment hits ranked according to peptide frequency  $F$ .



**Figure 5.** Suppression of CI in *Drosophila*. (a) Transgenic CI was temperature sensitive. (b) Yeast *SRP1* and *HRP1* did not suppress CI in *Drosophila* and serve as negative controls. At 22°C, overexpression of *D.m.Kap-α1*, *S.c.Rtt103*, GFP, *D.m.Nap1*, *D.m.Kap-α2*, *D.m.P32* and *CidA<sup>wMel</sup>* suppressed transgenic CI relative to the control. Both *D.m.P32* and *CidA<sup>wMel</sup>* suppression were still highly significant when compared to the GFP control. (c) CI suppressive effects of karyopherin overexpression were countered by its maternal toxicity. (d) *D.m.* Karyopherins and *D.m.P32* significantly suppressed bacterial (*wMel*) CI; GFP did not. Error bars represent means  $\pm$  s.d. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  by ANOVA with multiple comparison between all groups and Tukey's post-hoc analysis; four outliers (x) removed by ROUT analysis.



**Figure 5—figure supplement 1.** PCR analysis demonstrates that transgenic flies used in this study are not infected with *Wolbachia*. VirD4 is a conserved *Wolbachia* gene. Amplification of the *Drosophila melanogaster* Histone three gene served as a positive control. NGT is the Nanos-Gal4-Tubulin driver (three replicates).