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Supplementary Information for

A single mutation weakens symbiont-induced reproductive manipulation through reductions in deubiquitylation efficiency

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This PDF file includes:

Figures S1 to S6

Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

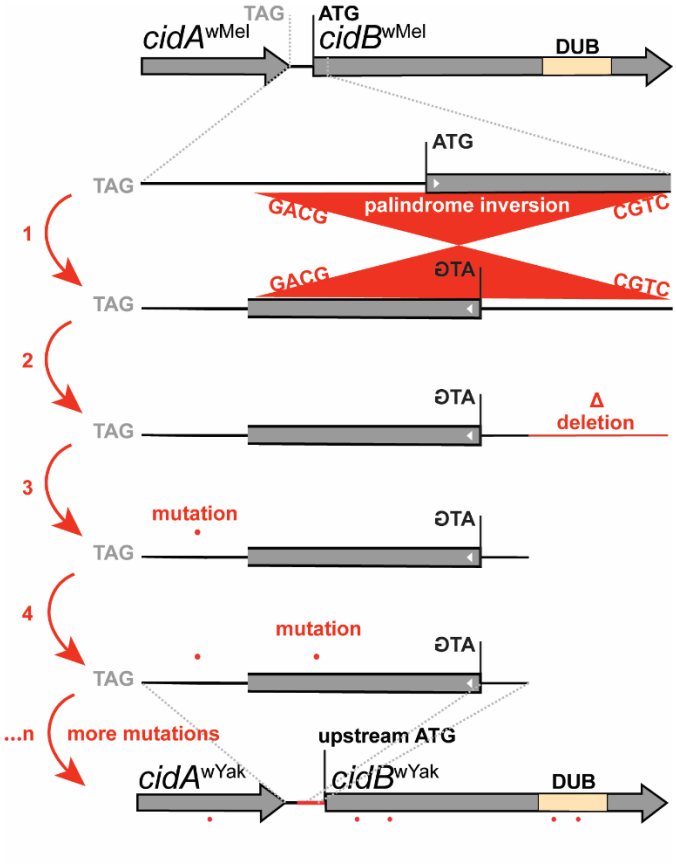

Fig. S1. Enumeration of putative genomic events diverging a common ancestor of *cid*^{wMel} and *cid*^{wYak} into the extant *w*Yak variants. Beginning with the *w*Mel backbone: 1) an inversion strikes the operon at a GACG palindrome. 2) a deletion removes a portion of the inversion. 3 and 4) are non-coding snp mutations found within the inversion. N) Nonsynonymous mutations accumulate in undiscernible order. Six total nonsynonymous coding mutations permutate the operon. From this analysis we cannot infer a definitive order of events, except that the palindrome inversion must occur prior to the deletion because the deletion iteratively removed the CGTC palindrome end.

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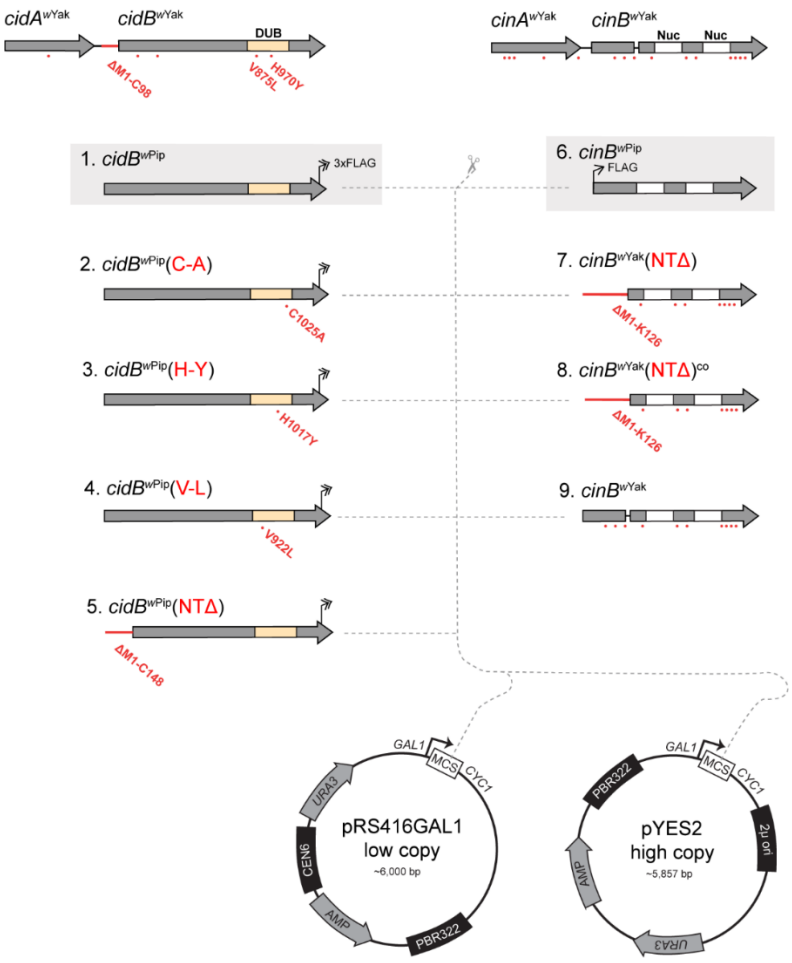

Fig. S2. Design of yeast constructs which mimic naturally occurring *cif* polymorphisms found between *w*Yak, *w*Mel, and *w*Pip. At top are the native *w*Yak operons. Grey boxes are positive controls. **1)** Positive toxicity inducing control, *cidB*^{wPip}. **2)** Catalytic inactive negative control, *cidB*^{wPip}(C-A).[7] **3)** *cidB*^{wPip}(H-Y) mutant. H970Y in *w*Yak aligns to H1017Y in *w*Pip. **4)** *cidB*^{wPip}(V-L) mutant. V875L in *w*Yak aligns with V922L in *w*Pip. **5)** *cidB*^{wPip}(NTD) mutant. M99 in *w*Yak aligns with M149 in *w*Pip and serves as start codon. **6)** Positive toxicity inducing control, *cinB*^{wPip}. **7)** *cinB*^{wYak}(NTD) line that mimics native *w*Yak translation from an upstream methionine M127. **8)** similar to **7)** but codon optimized, *cinB*^{wYak}(NTD)^{co}. **9)** Endogenous sequence of *cinB*^{wYak} containing both parts of *cinB*^{wYak} as is (includes the tandem duplication). At bottom are two destination plasmids designed for low copy (left, pRS416*GAL1*) and high copy (right, pYES2) expression of transgenes. Dotted lines indicate cloning reactions.

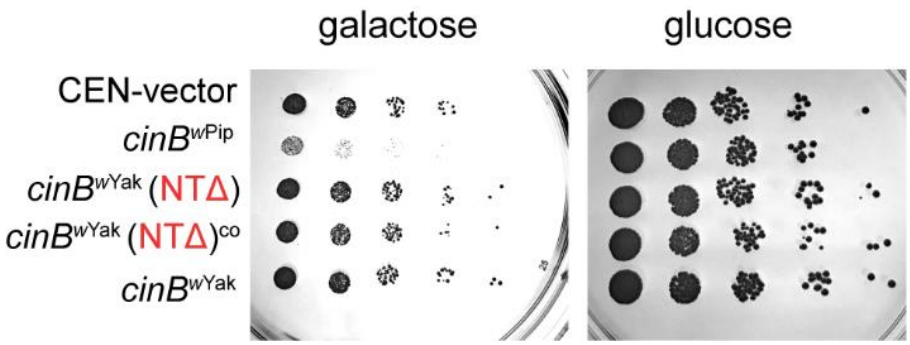

Fig. S3. Serial dilution of *cif* permutations from *w*Yak and *w*Pip expressed within low copy centromeric plasmids. *cinB*^{wYak} alleles in yeast didn't produce phenotypes for three variant constructs including *cinB*^{wYak} beginning after the tandem duplication - (NTΔ) and wild type sequence containing the tandem duplication. (NTΔ) is endogenous sequence and (NTΔ)^{co} is codon optimized. *cinB*^{wPip} is a positive control and CEN-vector is an empty pRS416gal1 negative control plasmid. Serial dilutions were performed at 34°C. Media is synthetic defined lacking uracil with galactose or glucose as inducer and repressor. Figures are representative of triplicates.

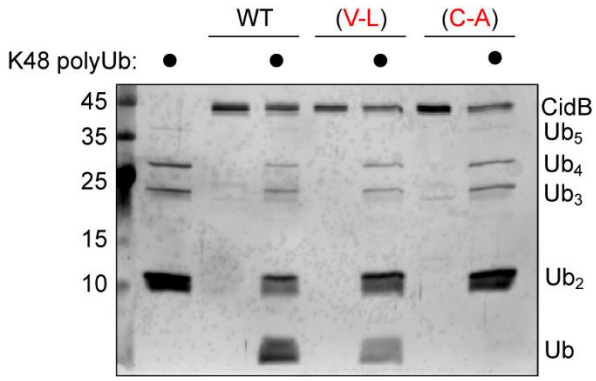

Fig. S4. Poly-Ubiquitin cleavage assays with CidB^{wMel} variants. All CidB^{wMel} proteins are identical to those described in **Fig. 3**. **A-B.** Silver stained SDS-PAGE analysis of one-hour digests with poly-K48 linked Ubiquitin (Ub_{2-5}). The (V-L) mutant shows reduced DUB activity in comparison to wildtype (WT).

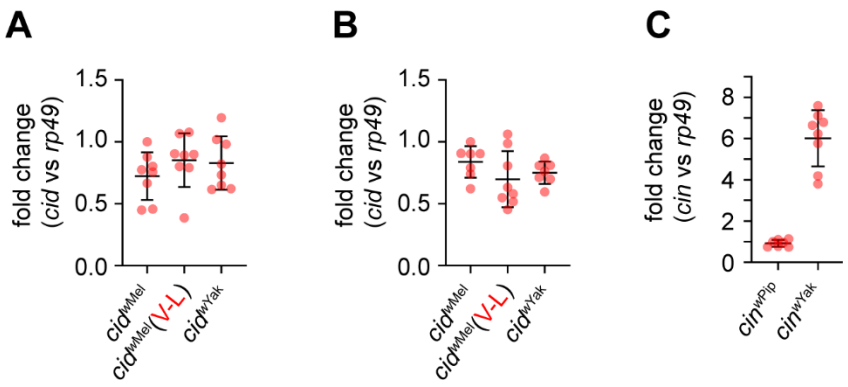

Fig. S5. Gene expression fold change of *cif* transgenes relative to the *Drosophila* housekeeping gene *rp49* was determined on a subset of abdomens from males using the equation $2^{-\Delta\Delta Ct}$. A. Relative expression of Cid^{wMel}, Cid^{wMel}(V-L), and Cid^{wYak} transgenes using the NGT driver. Although these three *cid* transgenes cause varying levels of CI, their expression does not vary (P = 0.35). **B.** Relative expression of the same transgenes as in (A) except, using the MTD driver. Again, relative expression does not vary across transgenes (P = 0.23). **C**. Relative expression of Cin^{wPip} and Cin^{wYak} transgenes using the MTD driver. Although Cin^{wPip} is expressed significantly less than Cin^{wYak} (P < 0.001) it causes CI, indicating relative transgene expression levels are unlikely to explain hatch rate phenotypes. A single asterisk denotes P < 0.001. P-values for (A) and (B) are calculated from a Kruskal-Wallis analysis, while P-values for (C) are calculated from a Wilcoxon test. Error bars represent standard deviations around mean.

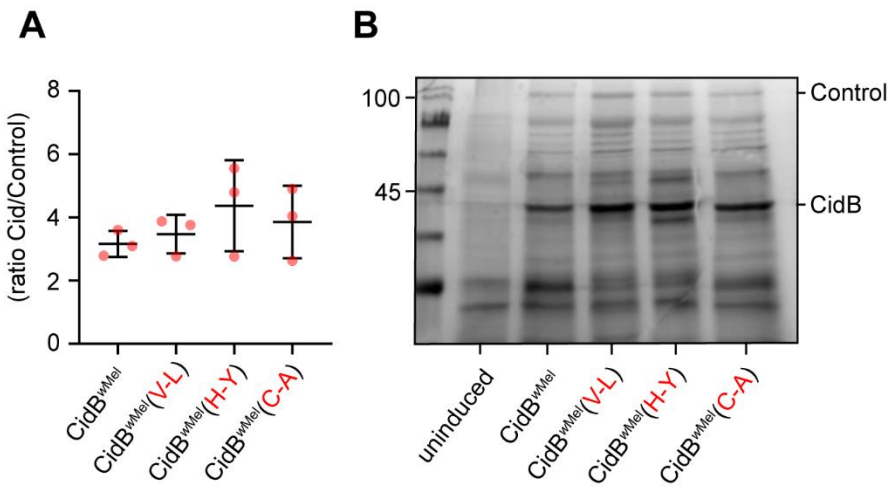

Fig. S6. Variant CidB DUB domain protein levels after recombinant protein expression in *E.coli.* A. Quantified ratios (CidB / 120 kDa Control band) of silver-stained SDS-PAGE gel bands. **B.** Example silver-stained SDS-PAGE gel. Integrated densities are measured for corresponding gel bands in ImageJ and plotted in Graphpad Prism. The gel is representative of triplicates. All mutants tested are not expressed less than native CidB^{wMel} DUB sequence. Error bars represent standard deviations around mean.