Gaggioli et al., http://www.jcb.org/cgi/content/full/jcb.201310083/DC1

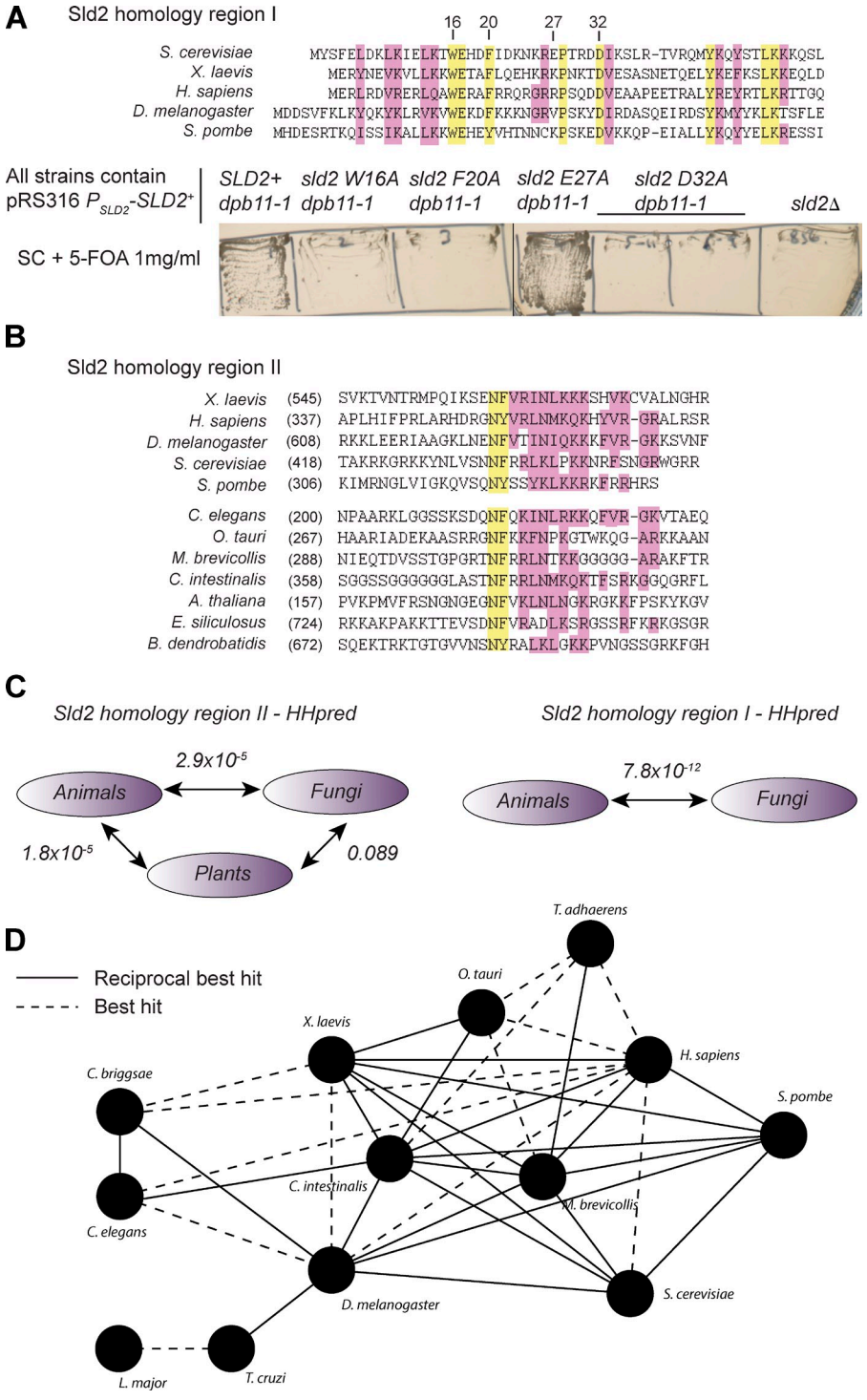

Figure S1. **Significance of the Sld2 alignment in Fig. 1 B and the subsequent bioinformatic screen for Sld2 orthologues.** (A, top) Alignment of Sld2 homology region I from five characterized Sld2/RecQ4 proteins as in Fig. 1 B. The top numbers indicate the *S. cerevisiae* Sld2 residues that were mutated to alanine to test the significance of this alignment. (bottom) Growth analysis of budding yeast strains with the indicated Sld2 mutant together with the temperature-sensitive *dpb11-1* allele. All strains express a wild-type copy of *SLD2* on the episomal plasmid pRS316. The indicated growth medium contains 5-fluoroorotic acid (5-FOA), which selects against the pRS316 plasmid. As a result, only strains that can survive without this plasmid will grow on this medium. Growth was at 24°C. SC, synthetic complete. (B) Alignment of homology region II from characterized Sld2/RecQ4 proteins (top) and newly discovered homologues (bottom). Identical and near-identical residues are highlighted in yellow, and regions of similarity are highlighted in pink. (C) HHpred profile-to-profile comparison between HMMs for homology region I (right) and homology region II (left). Numbers shown are p-values. Arrows indicate the profile search direction. (D) Reciprocal best-hit analysis between Sld2/RecQ4 proteins. Full-length Sld2 proteins or the N termini of RecQ4 proteins were individually BLASTed against the entire proteome of each organism represented in this diagram. The N termini of the RecQ4 proteins used in this best-hit analysis were as follows: *D. melanogaster*, 1–673 aa; *H. sapiens*, 1–394 aa; *C. intestinalis*, 1–428 aa; *X. laevis*, 1–605 aa; *O. tauri*, 1–313 aa; and *M. brevicollis*, 1–315 aa. For all other Sld2 proteins, the full-length protein was used. Reciprocal best hit means that both proteins were the most significant hit from both genomes. Best hit indicates that a protein was the most significant hit against the corresponding genome but not vice versa. Best hit rather than reciprocal best hit can be explained because the extent of similarity between some Sld2 homologues is too low for BLAST. *L. major*, *Leishmania major*; *C. briggsae*, *Caenorhabditis briggsae*.

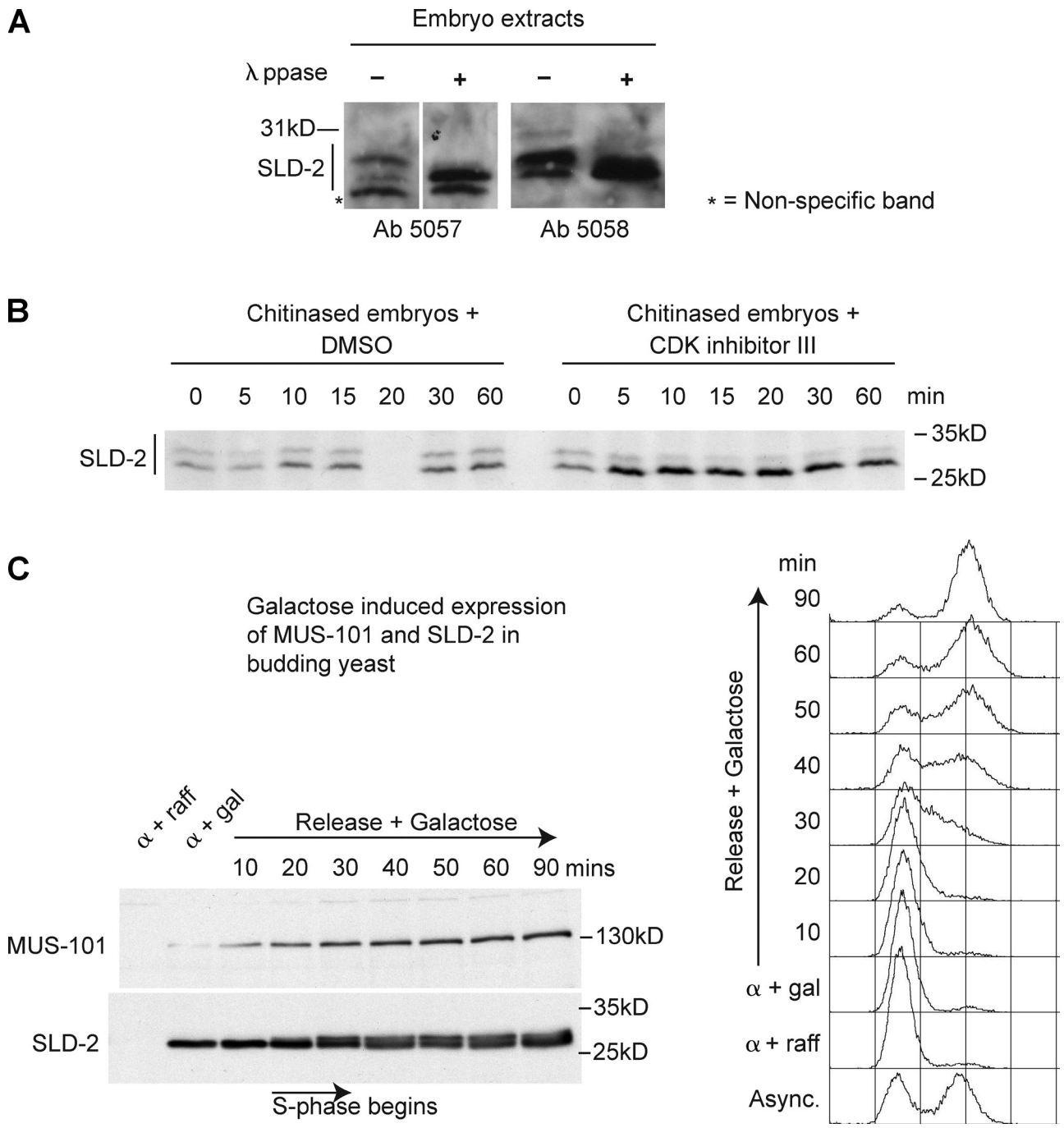

Figure S2. **Two separate rabbit polyclonal antibodies identify phosphorylated forms of SLD-2 in worm extracts, and this protein is modified in a cell cycle–dependent manner when expressed in budding yeast.** (A) Side by side, anti–SLD-2 Western blot with two different rabbit polyclonals, Ab 5057 and 5058. The right hand blot is also represented in Fig. 4 B. λ ppase, λ phosphatase. (B) Anti–SLD-2 Western blot of chitinase-treated worm embryos treated with or without CDK inhibitor III for the indicated times. (C) *C. elegans* MUS-101 and SLD-2 were expressed from the GAL1–10 galactose-inducible promoter in synchronized budding yeast cells. (right) Flow cytometry of α-factor arrest of yeast cells in G1 phase and release into S phase in galactose (gal)-containing medium. raff, raffinose. (left) Anti–MUS-101 and anti–SLD-2 Western blot from the synchronized yeast cells. Note that SLD-2 is modified at the same time as cells enter S phase by flow cytometry. This is consistent with SLD-2 being preferentially phosphorylated by the S-phase cyclin-CDK. Async., asynchronized.

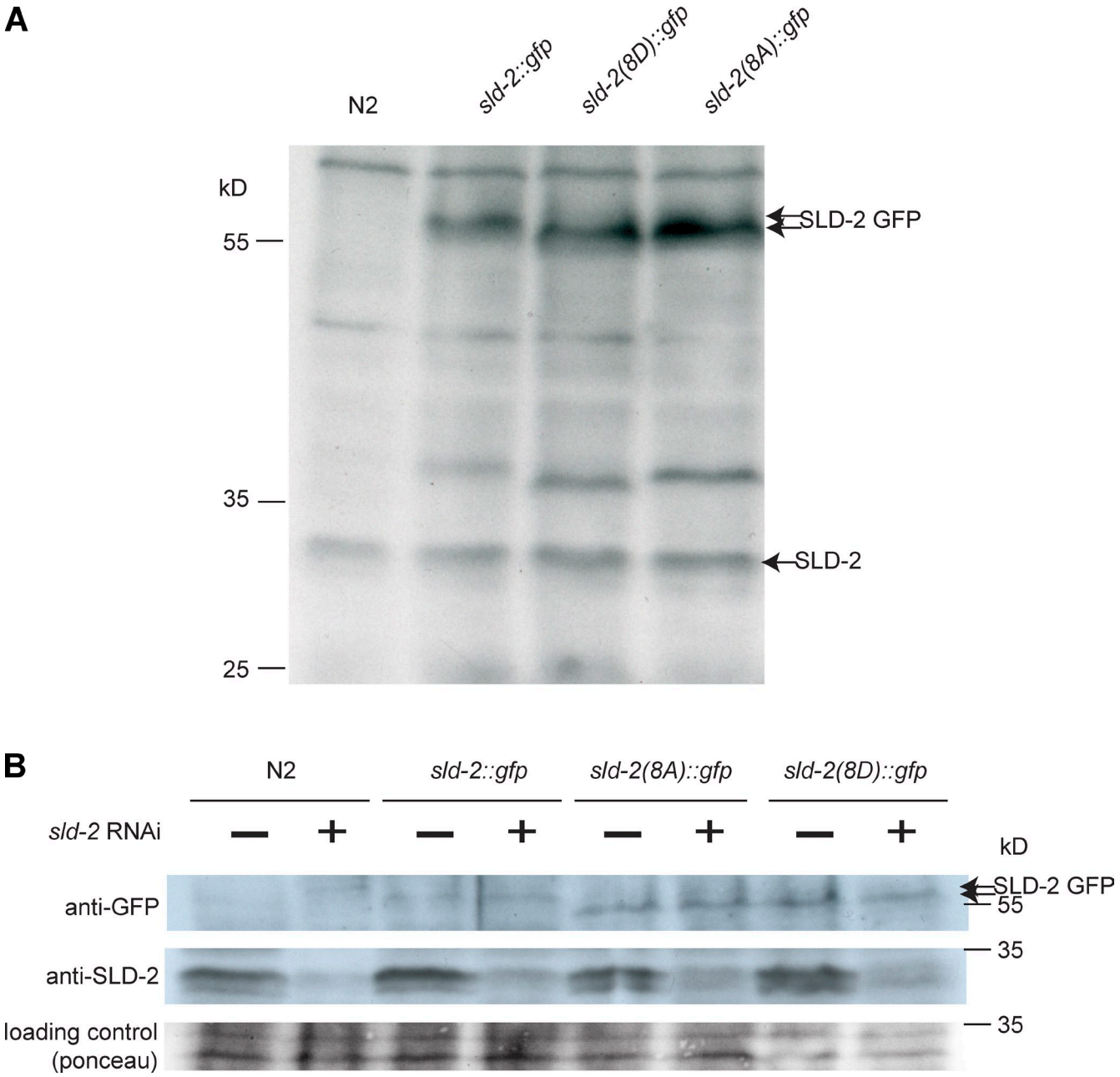

Figure S3. **The expression of codon-altered *sld-2::gfp* transgenes relative to endogenous SLD-2 and RNAi of endogenous *sld-2* does not affect the expression of these transgenes.** (A) Anti–SLD-2 Western blot comparing endogenous SLD-2 levels with the *mex-5* promoter-driven *sld-2::gfp* transgenes. (B) Western blot of endogenous SLD-2 and SLD-2 GFP after *sld-2* RNAi. The codon usage in these *sld-2* transgenes is altered to make them refractory to endogenous *sld-2* RNAi. Note that although endogenous SLD-2 levels are greatly reduced after RNAi, the GFP-fused constructs are not affected.

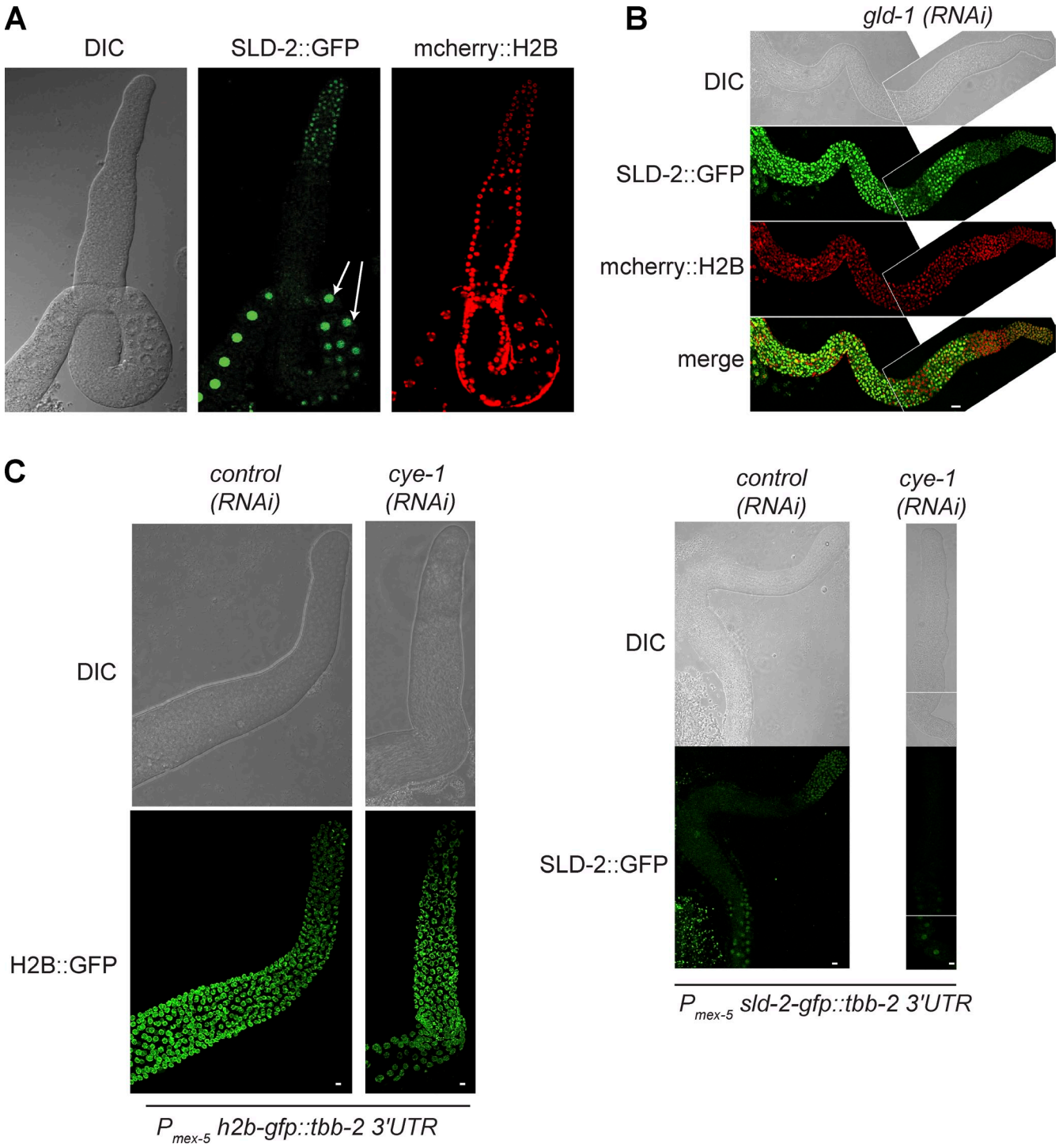

Figure S4. **The expression of SLD-2::GFP in the distal tip and loop region of the germline and the expression of SLD-2::GFP after *gld-1* and *cye-1* (RNAi), with a control showing that the effect of CYE-1 on SLD-2 localization is independent of the regulatory elements of the transgene.** (A) Image of *C. elegans* germline showing SLD-2 expression in the mitotic region of the gonad and in the loop region and oocyte nuclei. Notably, in wild-type worms, SLD-2 is largely absent from the transition zone and pachytene region of the germline but reappears in developing oocyte nuclei (arrows). This *sld-2::gfp* transgene is integrated as a single copy at a Mos transposon site and is expressed from the *mex-5* promoter with a *tbb-2* 3′UTR. (B) As in A, except after RNAi of *gld-1*. *gld-1*(RNAi) in L1 worms results in increased proliferation in the germline and increased expression of SLD-2::GFP. White lines indicate where separate images have been merged. (C) Extruded gonad of a worm expressing *Pmex-5::h2b-gfp::tbb-2 3′UTR* (left), which contains the same promoter, 3′ GFP ORF fusion, and 3′UTR as the *sld-2* transgenes (right), and is inserted at the same Mos transposon site. RNAi (control or *cye-1*) was performed by feeding in L3 worms. H2B::GFP is visible in all germ nuclei, and this expression pattern is not affected by *cye-1* (RNAi), whereas SLD-2::GFP is absent from distal nuclei after *cye-1* RNAi (right). DIC, differential interference contrast. Bars, 10 µM.

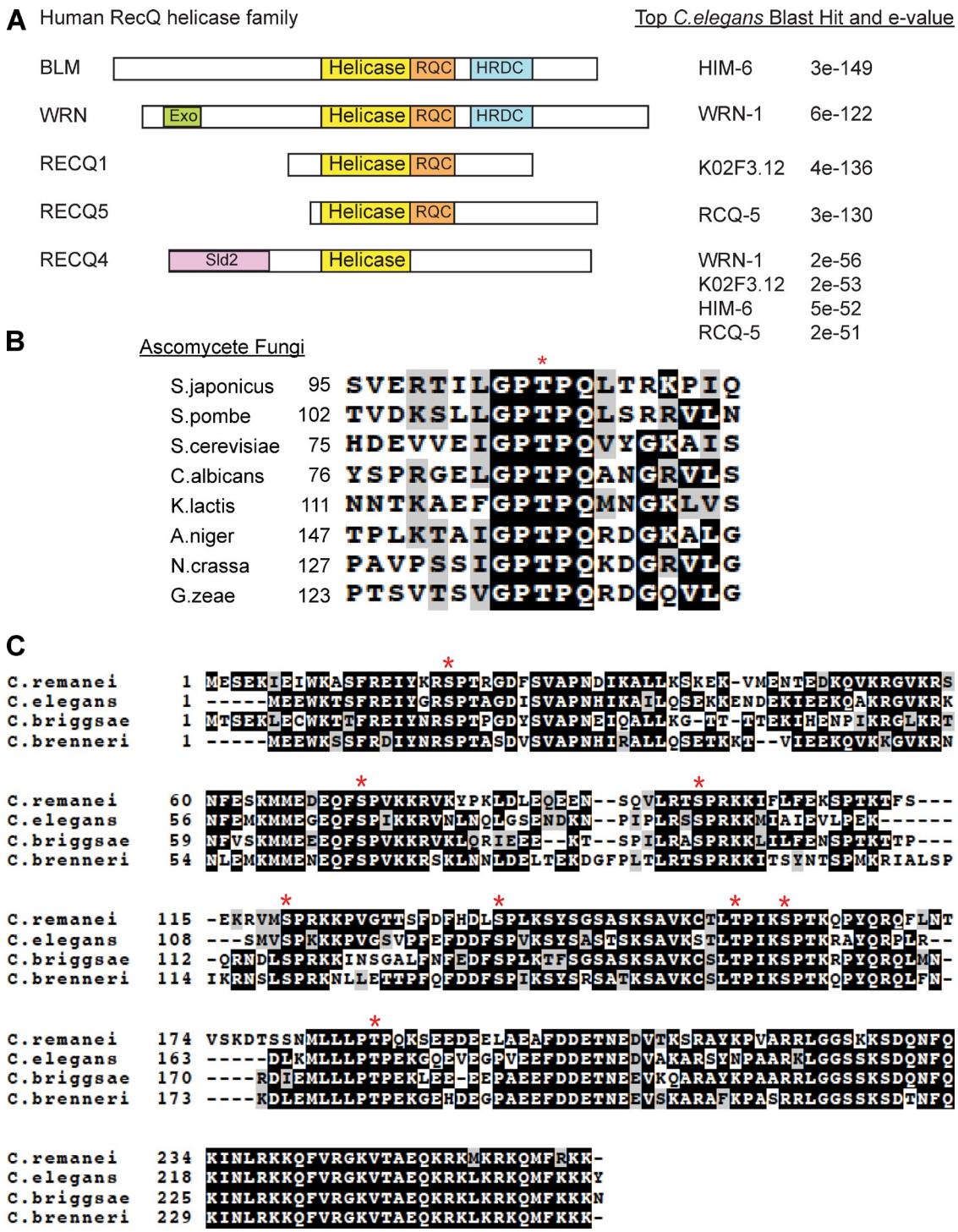

Figure S5. **The conservation of RecQ helicase family members in *C. elegans* and an alignment of the CDK sites in yeast and worm Sld2 proteins.** (A) Scale diagram of the five known RecQ helicase proteins in humans. Each human helicase was BLASTed against the *C. elegans* genome and the top hit, and the e-value for that hit is shown on the right. Notably, RecQ4 BLAST did not isolate any RecQ4 helicase proteins in *C. elegans*. Instead, the top hits for human RecQ4 in worm are the other RecQ helicase family members. Note that T12F5.1 (SLD-2) is not the top hit from this BLAST search because the DEAD box helicase domains are much more similar to each other than the Sld2 homology region at the N terminus of human RecQ4 is to SLD-2. This is why the reciprocal best-hit analysis in Fig. S1 D was performed with only the N termini of the RecQ helicase proteins. RQC, RecQ C-terminal domain; HRDC, helicase and RNase D C-terminal domain. (B) Alignment of fungal Sld2 proteins showing the conservation of the essential CDK site in budding yeast and fission yeast (asterisks). (C) Alignment of nematode SLD-2 proteins showing the sites of the eight *C. elegans* CDK consensus sites (asterisks). Black, identical residues. Gray, similar residues. *S. japonicus*, *Schizosaccharomyces japonicus*; *C. albicans*, *Candida albicans*; *K. lactis*, *Kluyveromyces lactis*; *A. niger*, *Aspergillus niger*; *N. crassa*, *Neurospora crassa*; *G. zeae*, *Gibberella zeae*; *C. remanei*, *Caenorhabditis remanei*; *C. brenneri*, *Caenorhabditis brenneri*.

Table S1 shows species names and gene identification numbers of new and characterized Sld2 and RecQ4 proteins and is provided online as an Excel file.