FILE S1

Supporting Materials and Methods

Sequence alignments: We determined the reciprocal best-match BLAST hits between *Drosophila* and human cyclins (Table S1). BLAST searches were conducted with each of the *Drosophila* cyclins listed below to identify the top matching human cyclins. In cases where a gene had multiple protein isoforms, the longest isoform that had a cyclin domain was used. The top matching human proteins were then used in BLAST searches against the *Drosophila melanogaster* annotated proteins and the top matching protein was identified. Reciprocal best-match BLAST hits are listed in Table S1. An example of how reciprocal best-match hits are interpreted is as follows: Human CCND1, CCND2, and CCND3 are the human proteins most similar to *Drosophila* CycD, and *Drosophila* CycD is the *Drosophila* protein most similar to human CCND1, CCND2, or CCND3, according to BLAST. Multiple sequence alignment was performed using ClustalX version 2 (LARKIN *et al.* 2007; THOMPSON *et al.* 2002). Pair-wise percent identity was determined by dividing the number of identical sites in the alignment by the length of the alignment, including gaps and unaligned ends. The dendrogram shown in Figure S1A was constructed using ClustalX with the neighbor-joining algorithm. For Figure S1B, the reciprocal best-match BLAST hits between *Drosophila* CycY and proteins from several divergent species were aligned using ClustalW followed by manual corrections to improve identities. Only the top matching CycY-like protein from each species is shown. The proteins aligned were as follows, where Genbank accession numbers are in parentheses: *Aedes aegypti* hypothetical protein AaeL_AAEL010543 (XP_001660900.1); *Caenorhabditis elegans* hypothetical protein ZK353.1a (NP_498858.2); *Danio rerio* hypothetical protein LOC767752 (NP_001070188.1); *Drosophila melanogaster* CG14939-PA (NP_609519.1); *Gallus gallus* CCNYL1 cyclin Y-like 1 (XP_425973.2); *Homo sapiens* cyclin fold protein 1 variant b (AAL78999.1); *Mus musculus* cyclin fold protein 1 (NP_080760.2); *Xenopus laevis* hypothetical protein LOC431857 (NP_001084816.1); *Nematostella vectensis* predicted protein (XP_001641126); *Trichoplax adhaerens* hypothetical protein (XP_002116466); *Monosiga brevicollis* hypothetical protein (XP_001750168).

To identify proteins with similarity to CycY in more distant species, reciprocal best-match BLAST hits between *Drosophila* CycY and proteins in the species listed below were determined. The identified proteins were also determined to be reciprocal best-match BLAST hits with the human *CCNY* protein. For all of the identified proteins the sequence similarity with the human or *Drosophila CycY* proteins was restricted to the annotated cyclin domain (MARCHLER-BAUER *et al.* 2009) and immediate flanking regions, referred to as the "cyclin+" region in Figure S2. The cyclin+ regions were aligned using ClustalW and a consensus sequence was determined by identifying residues that were found in >50% of the sequences (Figure S2A). The dendrogram shown in Figure S2B was obtained by aligning the cyclin+ region of the proteins most similar to CycY, and the annotated cyclin domains of reciprocal best-match hits of *Drosophila* CycA and CycB for the species shown. Only the top matching CycY-like protein from each species is shown; gene or genome duplications in some lineages have resulted in several

parologous CycY-like proteins (not shown). The following proteins from non-metazoan species were reciprocal best-match hits of *Drosophila* CycY or the human *CCNY* protein, where Genbank accession numbers are in parentheses: *Arabidopsis thaliana* CYCP4;3 (NP_196362.1); *Coprinopsis cinerea* predicted protein (XP_001832875); *Cryptococcus neoformans* cyclin (XP_566770); *Dictyostelium discoideum* cyclin domain-containing protein (XP_642568); *Giardia intestinalis* Cyclin fold protein 1 (EET00183.1); *Laccaria bicolor* predicted protein (XP_001886042); *Medicago truncatula* unknown (ACJ84314); *Paramecium tetraurelia* hypothetical protein (XP_001460214); *Perkinsus marinus* hypothetical protein (EER16009); *Phaeodactylum tricornutum* CYCP1 (XP_002182703.1); *Phytophthora infestans* cyclin-Y-like (EEY67633.1); *Populus trichocarpa* predicted protein (XP_002302113); *Ricinus communis* cyclin (XP_002520742.1); *Saccharomyces cerevisiae* PCL1 (NP_014110.1); *Tetrahymena thermophila* Cyclin, N-terminal domain containing protein (EAS05969); *Toxoplasma gondii* cyclin, N-terminal domain-containing protein (EEE19730); *Trypanosoma cruzi* cyclin 6 (AAG44389.1); *Yarrowia lipolytica* hypothetical protein (XP_505742).

Yeast two-hybrid assays: Yeast two-hybrid assays (FIELDS and SONG 1989) were performed using the LexA system (GYURIS *et al.* 1993) and interaction mating assays (FINLEY and BRENT 1994). Yeast strains and vectors, the protocol for one-on-one mating assays, and the reporter scoring methods were previously described (ZHONG *et al.* 2003). All of the cyclins tested were expressed as activation domain (AD) fusions, whereas all of the Cdks were expressed as DNA-binding domain (BD) fusions. AD and BD strains were obtained from the arrays of LexA-based yeast two-hybrid clones previously described (STANYON *et al.* 2004).

Supporting References

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