Figure S1 Data showing protocol for efficiently precipitating transgenic Cdk1(WT) proteins from wing disc extracts with rabbit antibodies against GFP. (S1A) Precipitate (P) and supernatant (S) fractions were probed with antibodies against Cyclin B which detect a single band of 63 kD, then stripped and re-probed with mouse anti-GFP antibodies, showing that Cdk1WT-VFP was efficiently precipitated (and depleted from the supernatant). The blot shows two experimental replicates. Panel S1B shows aliquots from one experiment, where Cdk1 fusion proteins were immunoprecipitated from larval wing discs with rabbit anti-GFP antibodies (yw as a negative control) and tested for kinase activity using histone H1 as a substrate. Anti-histone H1 and mouse anti-Cdk1 antibodies were used for labeling the blot as positive controls. Each sample represents immunoprecipitated protein from a protein extract representing 180 wing discs per genotype. The relative kinase activity for each Cdk1 variant was determined by normalizing kinase activity per unit of total immunoprecipitated protein. Panels S1C and S1D show western blots of protein extracts from cdc2ts mutants rescued with Cdk1(WT)-VFP or Cdk1(T14A) expressed using Tub-Gal4 and Actin-Gal4, respectively. Control extracts (cdk1ts/+), probed with anti-GFP, anti-Cdk(PSTAIR) or anti-Actin antibody showed no transgenic protein, whereas extracts from cdc2B47/cdc2E24;UAS-Cdk1WT-VFP/tubulin-Gal4, cdc2B47/cdc2E24;UAS-Cdk1A-VFP/tubulin-Gal4, cdc2B47/cdc2E24;UAS-Cdk1A-VFP/actin-Gal4 showed that the transgenic proteins were well expressed. Panel S1E shows a compilation of progeny data resulting from genetic crosses used to test whether the Cdk1-VFP transgenes ubiquitously expressed with either tubulin-Gal4 or actin-Gal4 could rescue pupal lethality in a cdk1 temperature-sensitive mutant background (w; cdc2B47/cdc2E24; Cdk1-VFP/tubulin-Gal4). Flies were cultured at 25 °C throughout development. The expected ratio of A:C:D classes of progeny that would represent full rescue was 1:2:4. Expression of Cdk1(WT)-VFP completely rescued cdk1 lethality as indicated by progeny in Class A. Expression of Cdk1(T14A)-VFP also completely rescued the temperature sensitive cdk1 lethality (see Class A). In contrast, neither expression of Cdk1(Y15F)-VFP nor Cdk1(T14A,Y15F)-VFP were capable of rescuing the lethality of cdk1 mutants (no progeny in Class A).