Figure S5

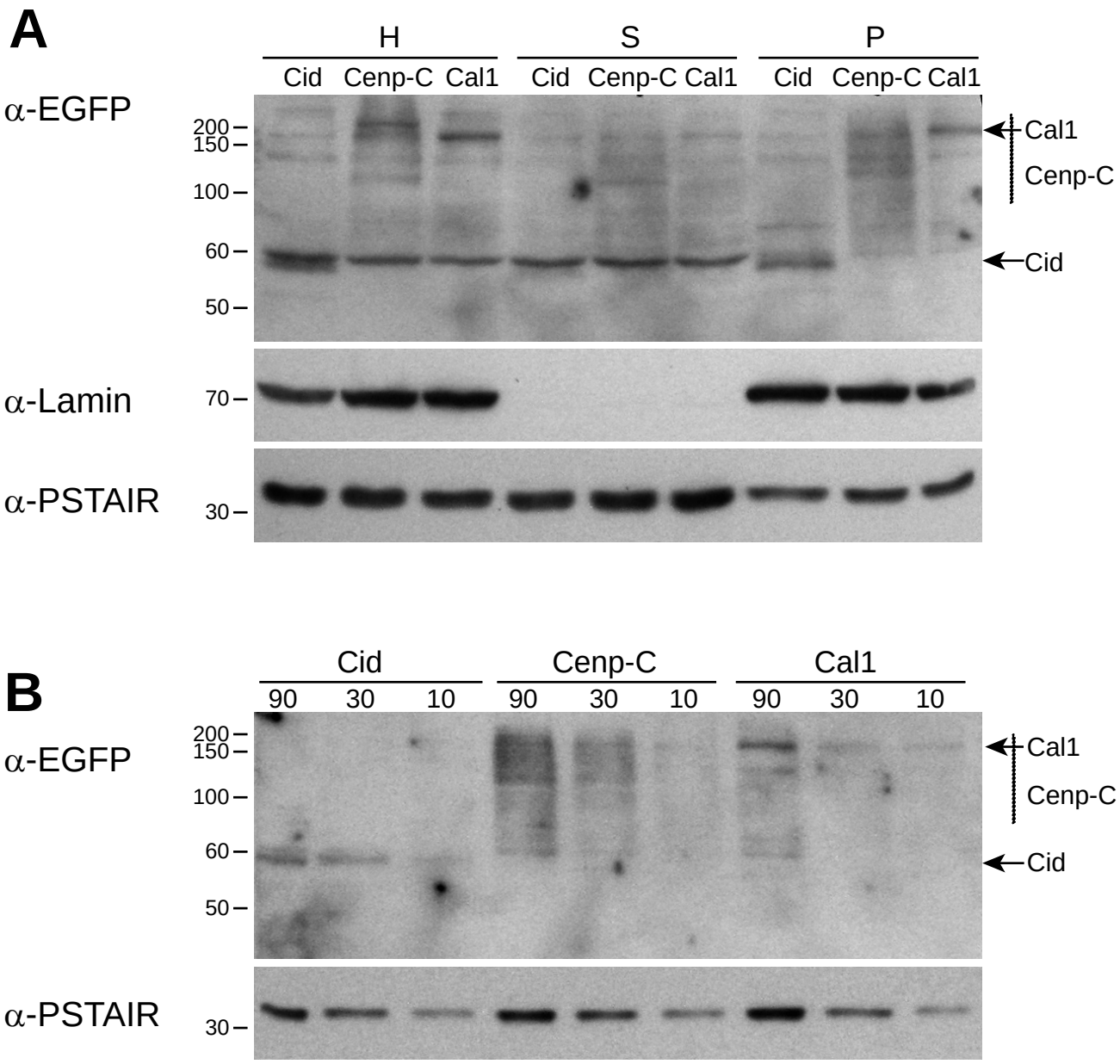

Figure S5. Expression levels EGFP fusion proteins of Cenp-A/Cid, Cenp-C and Cal1.
(A) Embryos were collected from strains with transgenes driving expression of EGFP fused to either Cenp-A/Cid, Cenp-C or Cal1 under control of the corresponding cis-regulatory regions in the corresponding null mutant backgrounds. 5-8 hour embryos were homogenized (H) followed by separation of a crude nuclear fraction (P) from the soluble material (S) by centrifugation. Immunoblotting with anti-EGFP (α-EGFP) was used to detect the different EGFP fusion proteins. Re-probing with anti-Lamin (α-Lamin) and anti-PSTAIR (α-PSTAIR) which reacts with Cdk1 was used to control the fractionation.
(B) For a comparison of expression levels, serial dilutions of crude nuclear fractions obtained from 90, 30, or 10 embryos, respectively, were immunoblotted with anti-EGFP (α-EGFP) and anti-PSTAIR (α-PSTAIR) as a loading control. Densitometric quantification indicated that the expression levels of Cenp-A/Cid-EGFP and Cal1-EGFP were 5.2 and 3.7 fold lower than that of Cenp-C-EGFP. Taking into account that only 3.3% of Cal1-EGFP is centromeric (Fig. S4H), this yields a stoichiometric ratio of centromeric Cenp-A/Cid, Cenp-C and Cal1 of about 20 : 100 : 0.9 compared to 60 : 100 : 1.9 obtained by purely microscopic EGFP signal detection and quantification (Table 1, Fig. 4C, Fig. S4A-C).