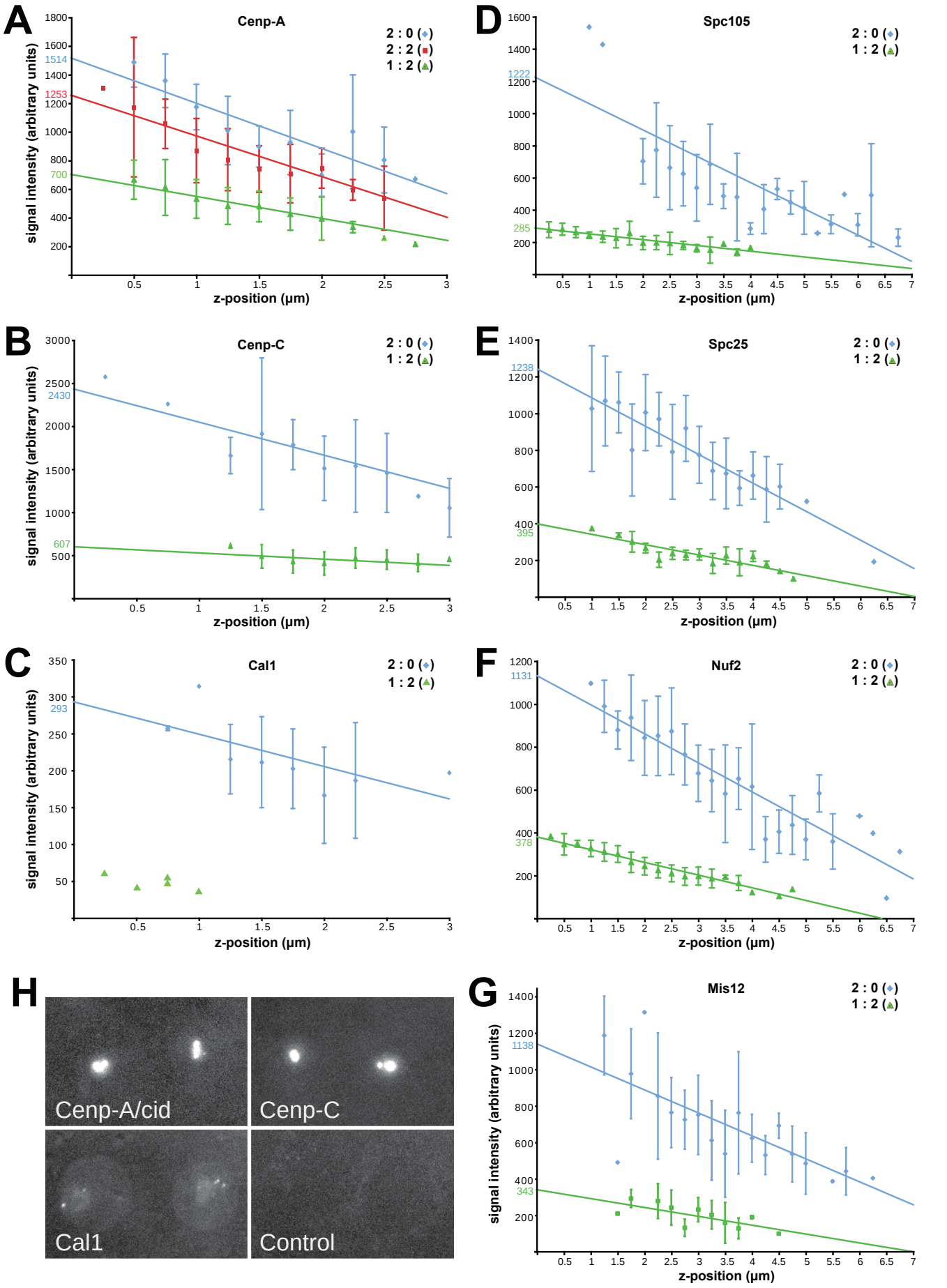
Figure S4


A
Cenp-A
2 : 0 (♦)
2 : 2 (■)
1 : 2 (▲)
signal intensity (arbitrary units)
z-position (µm)
1514
1253
700
B
Cenp-C
2 : 0 (♦)
1 : 2 (▲)
2430
607
C
Cal1
2 : 0 (♦)
1 : 2 (▲)
293
D
Spc105
2 : 0 (♦)
1 : 2 (▲)
1222
285
E
Spc25
2 : 0 (♦)
1 : 2 (▲)
1238
395
F
Nuf2
2 : 0 (♦)
1 : 2 (▲)
1131
378
G
Mis12
2 : 0 (♦)
1 : 2 (▲)
1138
343
H
Cenp-A/cid
Cenp-C
Cal1
Control

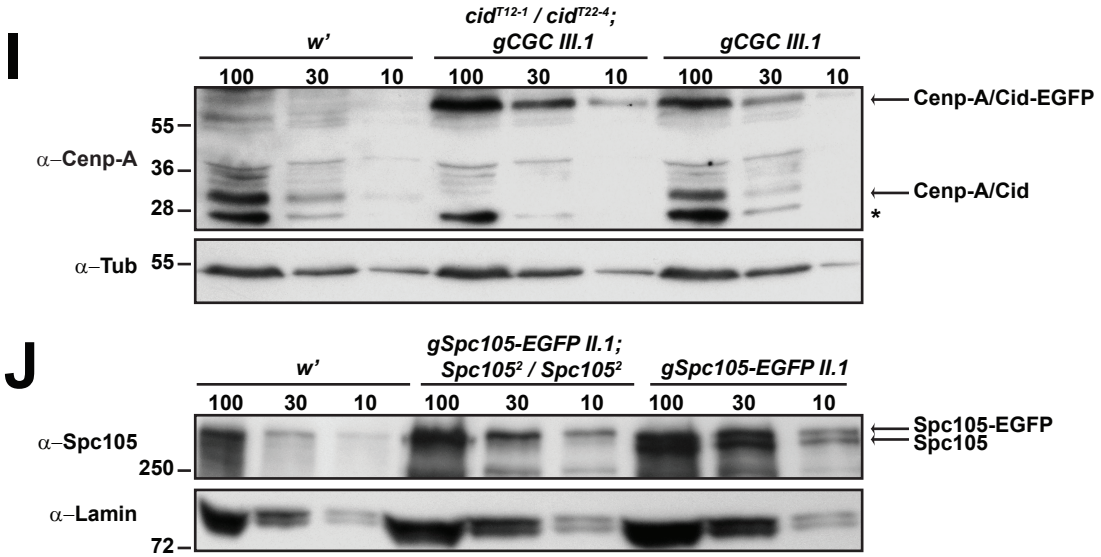


Figure S4. Stoichiometry of Drosophila centromere and kinetochore proteins.
(A-G) EGFP signal intensities observed in wing imaginal disc cells expressing EGFP fused to either the centromere proteins Cenp-A/Cid (A), Cenp-C (B) and Cal1 (C) or the kinetochore proteins Spc105 (D), Spc25 (E), Nuf2 (F) and Mis12 (G) were quantified and grouped according to their average focal depth. The average signal intensity (with s.d.) for each bin was plotted as a function of their z-position. Y intercepts of the linear regressions were used for comparisons of relative protein levels. To evaluate the accuracy of our quantifications, EGFP fusion proteins were expressed not only in a corresponding null mutant background but also in a background with functional endogenous genes. Untagged protein expressed from the endogenous genes is expected to compete with the EGFP-tagged protein and hence predicted to lower EGFP signal intensities at centromeres/kinetochores. Blue color represents data that was obtained with cells expressing two EGFP transgene copies and no functional endogenous copies (2:0), red color with cells expressing two EGFP transgene copies and two functional endogenous copies (2:2), and green color with cells expressing one EGFP transgene copy and two functional endogenous copies (1:2). In case of Cal1-EGFP, signals in the wild-type background were close to background and therefore difficult to detect, resulting in fewer data points which are shown individually as green triangles (C). Taking into account the observed relative expression levels of EGFP-tagged and untagged proteins (see also I and J) and assuming equal efficiency of incorporation into the centromere/kinetochore, the measured effects of competition deviate by less than 30% from the predicted competition effects.
(H) EGFP signals in live peripodial membrane cells of wing imaginal discs expressing either no EGFP (control) or EGFP fused to Cenp-A/Cid, Cenp-C or Cal1 in a corresponding null mutant background after identical acquisition and image processing (maximum projection). While Cal1-EGFP is detected not only at the centromere, but also in the nucleolus and weakly throughout the nucleus, strongly overexposed but exclusively centromeric signals are apparent in the case of Cenp-A/Cid-EGFP and Cenp-C-EGFP. Quantification of the Cal1-EGFP signals indicated that about 3.3% is centromeric, 21% nucleolar and 76% distributed throughout the nucleus (n = 5).
(I) Total extracts of 5-8 h old embryos (the exact genotypes are depicted above the lanes) were probed by immunoblotting with anti-Cenp-A/Cid (α-Cenp-A) and anti-α-Tubulin (α-Tub) to control for loading. The expression level of Cenp-A/Cid-EGFP was found to be approximately 3-fold higher than that of the endogenous Cenp-A/Cid, which explains the deviation between the expected and the observed centromeric incorporation of Cenp-A/Cid-EGFP in a null mutant compared to wild-type background (see also A). The numbers above the lanes indicate embryo equivalents loaded and the asterisk marks a prominent, unspecific band. The migration of the molecular weight marker (kDa) is indicated on the left side.
(J) Total extracts of 5-8 h old embryos (the exact genotypes are depicted above the lanes) were probed by immunoblotting with anti-Spc105 (α-Spc105) and anti-Lamin (α-Lamin), which served as a loading control. The expression levels of Spc105-EGFP and endogenous Spc105 were found to be similar, which is consistent with the observed decrease of centromeric incorporation of Spc105-EGFP in wild-type compared to null mutant background (see also D). The numbers indicate either embryo equivalents loaded (above the lanes) or the migration of the molecular weight marker (kDa; left side).