



Figure S1. CENP-A^{Cnp1} overexpression in *S. pombe* cells.

(A) CENP-A^{Cnp1} can be detected when overexpressed from the *nmt1* promoter. Adapted from Castillo et al., 2007 (Figure S3). Western analyses of extracts from wildtype cells overexpressing CENP-A^{Cnp1} from *nmt81* (81xC), *nmt41* (41xC) or *nmt1* (3xC) promoter to give low, medium and high expression levels compared with cells containing endogenous levels (3x). **(B)** Western analysis of GFP-CENP-A^{Cnp1} levels in wt and *spt16-18* cells. From Choi et al., 2012 (Figure 1D). Western analysis of GFP-CENP-A^{Cnp1} levels in wt and *spt16-18* cells expressing GFP-CENP-A^{Cnp1} under endogenous, *nmt81* or *nmt41* promoter (upper panel). The intensities of GFP-CENP-A^{Cnp1} and TAT-1 (alpha-tubulin) signals were measured using LICOR Odyssey Infrared Imaging System software (Li-COR Bioscience) and the relative intensities of GFP-CENP-A^{Cnp1}/TAT-1 were quantified (bottom panel). GFP-CENP-A^{Cnp1} was expressed for 24 h at 25°C before harvest. **(C)** Comparison of CENP-A^{Cnp1} localisation in cells expressing CENP-A^{Cnp1} from different strengths of *nmt* promoter. Immunolocalization of CENP-A^{Cnp1} in cells expressing endogenous levels of CENP-A^{Cnp1} or extra CENP-A^{Cnp1} expressed from the *nmt81*, *nmt41* or *nmt1* promoters. Cells were grown in the absence of thiamine for 24 h at 36°C and stained with anti-CENP-A^{Cnp1} antibody (green) and DAPI (DNA: red). Images are displayed to indicate the relative signal intensity in cells expressing CENP-A^{Cnp1} from the four different promoters. Images have not been autoscaled. The images for endogenous (Endog.) and *nmt81* promoters therefore appear very faint compared to *nmt1*. Scale bar, 5 mm.

Figure S1

C

