

Table S1. **Mean ratio of fluorescence signal from GFP-tagged kinetochore proteins in G2/M and in anaphase/telophase cells to ScCse4p-GFP fluorescence signal**

<i>S. cerevisiae</i>	<i>S. pombe</i>	G2/M ratio		<i>S. pombe</i>		<i>S. cerevisiae</i>	Anaphase ratio	
		Mean	SD	Number per kinetochore	Number per attachment	Number per attachment	Mean	SD
Cse4p	cnp1	0.94	0.1	5.01		2	2.86	0.6
Mif2p	mif2	0.69	0.14	3.68	1–2	1–2	2.03	0.02
Mcm21p	mal2	2.01	0.04	9.07	3	2	2.45	0.0
-	sim4	2.25	0.29	12.75	4	-	2.72	0.01
-	fta1	0.69	-	3.7	1	-	2.39	-
-	fta2	1.8	-	9.6	3	-	2.52	-
-	fta3	2.29	0.06	12.21	4	-	2.56	0.07
Mtw1p	mis12	2.9	0.24	15.47	5	6–7	2.03	0.03
Spc105p	spc7	2.16	0.11	11.52	4	5	2.09	0.1
Ndc80p	ndc80	4.11	0.34	21.93	7–8	8	1.75	0.05
Dad1p	dad1	0.62	-	1.66	<1	16–20	0.62	-
Dam1p	dam1 ^a	-	-	7.56 ^a	2 ^a	16–20	0.71 ^a	-
Ask1p	ask1 ^a	-	-	7.36 ^a	2 ^a	16–20	0.69 ^a	-

A ratio was obtained from each experiment, with a minimum of 20 measurements of ScCse4p-GFP and G2/M as well as anaphase/telophase signal for the fission yeast protein of interest, as described in Materials and methods. Using Cnp1p as an example, the number of molecules per kinetochore was obtained as the following: Cnp1 per kinetochore = $0.94 \times 32/6 = 5.01$, with 32 as the number of ScCse4p in an anaphase cluster. This number was further divided by 3, the mean number of microtubule attachments per kinetochore, to estimate the number of molecules per attachment.

^aDad1p is the only protein from the Dam1–DASH complex that is localized to the kinetochores in G2 cells and was the only protein available for signal measurement. This strain contained the wild-type Dad1 gene along with an extra Dad1p-GFP gene. We therefore assume that the fluorescence measurement detects only half the number of molecules. The anaphase number shows that this may not be a valid assumption because the number of Dam1–DASH complexes increases from two in G2/M to seven to eight per kinetochore.