Supplementary Table 1. Genome-wide two-hybrid screens using wild type and mutant *DAM1* as baits

Categories of the identified proteins	**Bait**			**Function**
	Wild type DAM1	dam1 (S to A)	dam1 (S to D)	
Meiotic SPB-associated	Ady3			Spore wall biosynthesis
	Spo21	Spo21	weak	Component of the meiotic plaque, spore wall assembly
Cell wall structure	Ecm11	Ecm11	Ecm11	Cell wall organization and biosynthesis
Nuclear pore		Nup82	weak	Nuclear pore protein
RNA	Lcp5	Lcp5	Lcp5	rRNA processing
			Srb7	RNA polymerase II transcription mediator
Signalling	Ste50	Ste50	Ste50	MAP kinase regulator, MAPKKK cascade
	Mkk2	Mkk2		MAP kinase kinase
			Bmh1	RAS signal transduction, pseudohyphal growth
Polarity	Kel2		Kel2	Mating and morphology

	Bud6	Bud6	Bud6	Rho signal transduction, bud site selection
Transcription	Not5	Not5	Not5	Negatively regulates transcription of TATA-less promoters
	Yap7	weak	Yap7	Transcriptional activator
Secretory pathway	Sec34	Sec34	Sec34	ER-Golgi transport
Autophagy	Apg17 [1]	Apg17	Apg17	Autophagy
Unknown	She1	She1		Unknown, null mutant is benomyl sensitive.
	Ygl079w [1]	Ygl079w	Ygl079w	Unknown

- This table shows Dam1p interacting proteins that are not identified as kinetochore or spindle components.
- Table notes: “weak” indicates that this protein shows weak interaction with wild type or mutant Dam1p. Blank cell means no interaction.

[1] Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., and Sakaki, Y. (2001). A comprehensive two-hybrid analysis to explore the yeast protein interactome. [Comment In: Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4277-8 UI: 21192614]. Proceedings of the National Academy of Sciences of the United States of America *98*, 4569-4574.

Supplementary Table 2. Protein interactions of the Dam1 complex identified by two-hybrid and/or *in vitro* binding assays

Query Proteins	Interacting Proteins Identified			
	by *in vitro* binding		by two-hybrid	
			genome-wide	direct assay
Dam1	Dam1	Cse4	Dam1	
	Duo1 [5]	Bim1	Duo1 [1,2,4,5]	
	Spc34	Stu2	Spc34 [1,4]	
	Dad4 (vw)	Ipl1	Spc19	
	Ndc80	Sli15	Dad1 [1,4]	
	Ctf19	Bir1	Dad2	
	Okp1	Mps1	Ndc80 [1,4]	
	Mcm22	Mad1	Bim1	
	Mcm17	Mad3	Sli15 [1, 6]	
	Ndc10	Bub1	Mcm16 [4]	
	Ctf13	Bub2		
	Cep3			
Duo1	Dam1 [5]		Dam1 [1,2,4,5]	Duo1 [1]
	Duo1		Spc34 [3,4]	
	Spc34		Dad1 [2,3,4,5]	
	Dad1		Bim1 [2,4]	
	Ask1 (vw)		Sli15 [6]	
Spc34	Dam1		Dam1 [1,4]	
	Duo1		Duo1 [3,4]	
	Spc19		Dad1 [3,4]	
			Spc19 [2,3,4]	
			Mcm22 [8]	
Spc19	Spc34		Dam1	Spc19 [7]
			Dad2 [3,4]	Nuf2 [7]
			Spc34 [2,3,4]	
			Ndc80 [2]	
Ask1	Duo1 (vw)		Dad2 [2,3,4]	
Dad1	Duo1		Duo1 [2,3,4]	
	Dad3		Dam1 [1,4]	
			Spc34 [3,4]	
Dad2			Dam1	
			Spc19 [3,4]	
			Ask1 [2,3,4]	
Dad3	Dad1			
Dad4	Dam1 (vw)			

Intact Dam1 Complex	Ndc80	Ndc10
	Ctf19	Ctf13
	Okp1	Cse4

For the two-hybrid assays, only those interacting proteins that are also kinetochore proteins or that have related functions are included. Genome-wide two-hybrid indicates that the interacting proteins were identified in genome-wide screen against certain baits or in high-throughput screens. Direct assays indicates that the interactions were identified when two specific proteins were tested directly for binding by fusing one protein to a DNA-binding domain and the other protein to an activation domain.

Abbreviations: vw, very weak binding (only ~10% of IVT protein was pulled down by GST-Dam1p); MT, microtubule; SPB, spindle pole body.

Sources of the identified interactions: [1], this study; [2], (Uetz *et al*., 2000); [3], (Ito *et al*., 2000); [4], (Ito *et al*., 2001); [5], (Hofmann *et al*., 1998); [6], (Kang *et al*., 2001); [7], (Newman *et al*., 2000)); [8], (Measday *et al*., 2002).

Supplementary Table 3. Kinetochore protein interactions identified by two-hybrid and/or *in vitro* binding assays

Functional category	**Query Proteins**	**Interacting Proteins Identified**		
		by *in vitro* binding	by two-hybrid	
			genome-wide	direct assay
Ndc80 complex	**Ndc80**	Dam1	Dam1[1,4] Nuf2[4] Spc24[9] Spc19[2]	Nuf2[7,10] Ndc80[7]
	Nuf2		Ndc80[4]	Nnf1[7] Spc19[7] Ndc80[7]
	Spc24		Spc25[9] Ndc80[9]	Spc24[7] Mcm21[10]
	Spc25		Spc24[9]	Mad1[7]
Ctf19 compex	**Ctf19**	Dam1 Dam1 complex		Ndc10[11] Cep3[11] Cse4[11] Mcm21[11] Okp1[11]
	Okp1	Dam1 Dam1 complex	Ame1[4]	Ctf19[11] Mcm21[11]
	Mcm19			Mcm17[12]
	Mcm22	Dam1	Mcm16[2,4] Spc34[8]	
	Mcm17	Dam1	Mif2[24]	Mmc19[12]
	Mcm21			Okp1[11] Ctf19[11] Mcm21[11] Mif2[11] Cbf1[11] Skp1[11] Spc24[10]
	Mcm16		Dam1[4] Mcm16[4] Mcm22[2,4]	
	Mtw1			
	Ame1	(nd)	Okp1[4]	
	Ctf3	(nd)	Mcm22[8]	
	Nkp1	(nd)	Nkp2[2]	
	Nkp2	(nd)	Nkp1[2]	

CBF3 complex	**Ndc10**	Dam1 Dam1 complex Cbf1 [15]		Bir1 [13] Ctf19 [11]
	Ctf13	Dam1 Dam1 complex Cbf1 [15]	Skp1 [2]	Bir1 [13]
	Cep3	Dam1 (w) Cbf1 [15]		Bir1 [13] Cep3 Ctf19 [11]
	Skp1		Ctf13 [2]	Bir1 [13] Mcm21[11]
DNA Binding	**Cse4**	Dam1 Dam1 complex		Ctf19 [11,16]
	Cbf1		Cbf1 [14] Mcm21 [11]	Ndc10 [15] Cep3 [15] Ctf13 [15] Mif2 [15]
	Mif2	(nd) Cbf1 [15]		Mcm21[11]
Kineto-chore spindle-associated proteins	**Bim1**	Dam1 MT [27]	Dam1 [1] Duo1 [2,4] Bik1 [4]	Tub1 [17] Tub3 [17]
	Bik1		Bim1 [4] Stu2 [4]	Tub1 [17] Kip2 [7]
	Stu2	Dam1 MT [19]	Bik1 [4]	Stu2 [18]
	Cin8	(nd)		Cin8 [7] Nuf2 [7]
Ipl1 Complex	**Ipl1**	Dam1 MT [6]	Sli15 [6]	
	Sli15	Dam1 MT [6]	Ipl1 [6] Duo1 [6]	Dam1[6]
	Bir1	Dam1		Ndc10 [13] Cep3 [13] Ctf13 [13] Skp1 [13]
Check-point Proteins (kineto-chore associated)	**Mps1**	Dam1		Stu1 [20]
	Mad1	Dam1		Mad1 Spc25 [7] Cdc20 [21]
	Mad2			Cdc20 [21]

	Mad3	Dam1	Bub3 [2] Cln3 [2]	Cdc20 [21]
	Bub1	Dam1	Bub3 [4]	Bub3 [23]
	Bub3		Bub1 [4] Mad3 [2]	Mad3 [22]
(SPB-associated)	**Bub2**	Dam1		
	Slk19			Slk19 [7]
	Nnf1	(nd)		Ndc80 [7] Nuf2 [7]
	Dsn1	(nd)		
	Nsl1	(nd)		

41 kinetochore proteins and their interacting partners are listed. Interacting proteins are identified either by *in vitro* binding or by two-hybrid assays, or both. In the binding assays, the kinetochore proteins were tested for their interactions with Dam1p, except in those cases in which they have not been determined (nd). For the two-hybrid assays, only those interacting proteins that are also kinetochore proteins or that have related functions are included. Genome-wide two-hybrid indicates that the interacting proteins were identified in genome-wide screen against certain baits or in high-throughput screens. Direct assay indicates that the interactions are found when two specific proteins were tested directly for binding by fusing one protein to a DNA-binding domain and the other protein to an activation domain.

- Abbreviations: w, weak binding (only ~20% of IVT protein was pulled down by GST-Dam1p); vw, very weak binding (only ~10% of IVT protein was pulled down by GST-Dam1p); MT, microtubule; SPB, spindle pole body.
- Sources of the identified interactions are referenced: [1], This study; [2], (Uetz *et al.*, 2000); [3], (Ito *et al.*, 2000); [4], (Ito *et al.*, 2001); [5], (Hofmann *et al.*, 1998); [6], (Kang *et al.*, 2001); [7], (Newman *et al.*, 2000); [8], (Measday *et al.*, 2002); [9], (Cho *et al.*, 1998); [10], (Janke *et al.*, 2001); [11], (Ortiz *et al.*, 1999); [12], (Ghosh *et al.*, 2001); [13], (Yoon and Carbon, 1999); [14], (Dowell *et al.*, 1992); [15], (Hemmerich *et al.*, 2000); [16], (Chen *et al.*, 2000); [17], (Schwartz *et al.*, 1997); [18], (Chen *et al.*, 1998); [19], (Wang and Huffaker, 1997); [20], (Jones *et al.*, 1999); [21], (Hwang *et al.*, 1998); [22], (Hardwick *et al.*, 2000); [23], (Roberts *et al.*, 1994); [24], (Pot *et al.*, 2003)