Sup. Table S1. *Paramecium PTIWI* genes. **(A)** The table shows the accession numbers of *PTIWI* genes in ParameciumDB (http://*Paramecium*.cgm.cnrs-gif.fr/) (Arnaiz et al. 2007 *Nucleic Acids Res* **35** (Database issue): D439-444), the sizes of the genes, the numbers of introns, the sizes and positions (in gene models) of the fragments used for dsRNA production (also used as probes in Fig. 2), the sizes and positions (in gene models) of the fragments used as probes in Sup. Fig. S3 and Fig. 3), the sizes of the deduced proteins, and the positions of the PAZ, MID and PIWI domains. For the *PTIWI04* pseudogene, the protein data (marked with *) are from the virtual protein that was created by correcting nonsense and frameshift mutations in the coding sequence, using the *PTIWI05* paralog. **(B)** The table describes Smith-Waterman (Smith, T.F. and Waterman, M.S. 1981 *J Mol Biol* **147**(1): 195-197) alignments of each dsRNA fragment (lines) to each of the entire gene models (columns). The upper number is the overall percentage of sequence identity, and the lower number is the maximum length of perfect identity.

A

gene	accession	gene				protein			
		size (bp) ATG-TGA	intron number	size (bp) and position of dsRNA fragment	size (bp) and position of probe fragment	protein size (aa)	PAZ domain position	MID domain position	PIWI domain position
PTIWI08	GSPATG00021288001	2430	3	645 (269-913)	146 (1451-1596)	784	204-312	320-531	540-784
PTIWI14	PTETG16300003001	2428	3	723 (285-1007)	145 (1454-1598)	784	204-312	320-531	540-784
PTIWI13	PTETG4800007001	2483	3	683 (105-787)	200 (1101-1300)	802	217-324	332-546	545-802
PTIWI04cor	PTETPS1700010001	2297	0	614 (484-1097)		765*	211-312*	320-527*	536-765*
PTIWI05	GSPATG00038263001	2325	0	532 (572-1103)		774	213-321	328-536	545-774
PTIWI02	PTETG3700002001	2295	0	586 (96-681)		764	206-311	318-526	535-764
PTIWI01	PTETG7100004001	2397	3	401 (41-441)	675 (1756-2430)	774	180-284	291-525	536-774
PTIWI09	GSPATG00020796001	2398	3	390 (50-439)	867 (1773-2639)	774	180-284	291-525	536-774
PTIWI03	GSPATG00001395001	2417	3	659 (179-837)		781	186-290	298-532	543-781
PTIWI12	GSPATG00001709001	2315	1	588 (307-894)	146 (1032-1177)	763	173-276	284-515	526-763
PTIWI15	GSPATG00005370001	2315	1	620 (307-926)	146 (1032-1177)	763	173-276	284-515	526-763
PTIWI10	GSPATG00009468001	2456	5	403 (22-424)		781	179-284	291-514	523-781
PTIWI11	GSPATG00019939001	2456	5	406 (23-438)		773	179-284	291-514	523-773
PTIWI06	GSPATG00002500001	2443	5	686 (160-845)		773	180-284	291-514	523-773
PTIWI07	PTETG2500007001	2807	7	726 (139-864)		875	285-407	412-639	648-875

B

gene dsRNA	01	02	03	04cor	05	06	07	08	09	10	11	12	13	14	15
01	**100** **401**	50.0 11	51.5 11	44.6 12	40.5 11	46.8 9	45.1 12	46.6 11	85.5 19	39.1 9	39.9 9	44.2 11	45.7 11	45.6 11	52.5 11
02	41.8 11	**100** **586**	44.7 10	51.9 12	54.3 11	41.1 10	40.5 12	38.3 13	43.0 11	44.1 10	38.1 10	37.7 11	43.6 11	39.0 12	39.7 11
03	57.8 14	44.0 10	**100** **659**	43.3 15	42.5 14	39.7 10	38.9 12	45.6 11	54.6 14	44.1 10	44.4 10	53.7 12	45.7 15	43.1 11	51.3 12
04	41.4 12	46.8 13	42.3 15	**100** **614**	63.0 18	45.1 10	41.0 10	39.8 12	37.4 11	48.2 11	46.9 11	38.3 11	52.4 10	41.7 15	37.7 11
05	41.2 11	46.2 11	41.5 12	61.9 16	**100** **532**	41.1 11	49.4 12	50.2 11	39.5 12	47.9 11	44.5 11	44.2 11	42.8 14	49.6 11	44.2 11
06	40.0 11	39.5 11	43.6 10	38.5 12	47.9 11	**100** **686**	43.3 10	41.8 11	40.7 12	72.6 17	72.0 15	36.4 11	40.5 11	44.3 10	37.3 12
07	42.8 12	41.2 10	44.0 12	40.7 10	46.4 11	34.6 13	**100** **726**	39.5 12	38.8 12	37.6 13	43.0 13	42.8 10	42.0 13	41.7 11	38.4 11
08	44.8 11	39.1 12	40.4 12	40.1 12	47.5 13	45.6 12	44.4 11	**100** **645**	43.9 11	40.9 13	40.5 13	41.4 11	50.4 12	77.4 19	35.8 15
09	85.1 19	42.9 10	47.5 11	44.0 11	46.5 9	46.7 10	43.0 10	43.5 10	**100** **390**	42.3 11	43.8 11	42.7 10	40.9 11	43.0 11	51.1 10
10	37.8 11	42.6 9	45.2 10	36.6 11	37.7 12	75.8 17	40.7 13	44.4 11	43.1 10	**100** **403**	85.1 20	40.4 10	39.4 10	42.3 12	35.0 9
11	37.7 10	38.2 9	36.2 13	43.4 10	38.3 10	72.6 14	37.9 10	41.9 10	38.5 10	85.0 20	**100** **406**	43.3 10	43.2 10	39.6 10	33.9 11
12	56.9 11	41.0 11	57.6 12	39.7 11	41.5 11	49.1 11	42.9 10	40.5 11	54.3 11	44.0 12	50.6 12	**100** **588**	41.2 11	47.3 17	81.5 18
13	42.0 10	43.3 11	41.4 14	47.1 12	39.3 11	38.4 12	38.2 11	51.2 10	36.0 12	36.4 11	42.2 11	42.5 11	**100** **683**	44.3 11	44.0 11
14	42.4 11	40.0 10	48.6 10	44.4 12	47.1 12	44.4 13	41.7 11	79.0 19	45.3 10	39.5 12	42.1 13	50.3 17	47.0 11	**100** **723**	49.3 11
15	56.0 11	40.4 10	54.6 14	40.1 12	44.0 16	51.1 12	41.6 12	46.7 15	52.9 10	50.4 15	45.4 12	81.6 18	41.5 11	41.1 11	**100** **620**

Sup. Table S2. Comparison of the expression levels of *PTIWI* genes, as determined by a NimbleGen microarray analysis using GEO series GSE18002, between the vegetative samples of the wild-type (*Klebsiella*-fed) and *ND7* dsRNA-fed cultures (GSM450430 and GSM450436). Data preprocessing was as described in Mat. & Meth. The significance of differences in expression levels was assessed by a Welch Two Sample t-test comparing the mean signal (across the six probes) of each *PTIWI* gene in the *ND7* dsRNA *vs.* wild-type conditions. Significant differences are highlighted in bold.

gene	wild-type	*ND7* dsRNA	P values
PTIWI01	1222	1258	0,91
PTIWI02	3550	3078	0,62
PTIWI03	1039	971	0,80
PTIWI04	482	582	0,59
PTIWI05	**936**	**4028**	**1,4E-3**
PTIWI06	348	344	0,98
PTIWI07	260	331	0,44
PTIWI08	1089	1113	0,89
PTIWI09	1395	2051	0,23
PTIWI10	2342	2702	0,68
PTIWI11	1626	1885	0,33
PTIWI12	3009	2517	0,48
PTIWI13	**5611**	**10747**	**1,1E-3**
PTIWI14	4226	5999	0,31
PTIWI15	1930	1726	0,61

Sup. Table S3. Cumulative number of divisions, over 3 days at 27°C, of 12 individual post-autogamous clones isolated from populations of autogamous cells silenced for the indicated *PTIWI* genes by dsRNA feeding. ++, too many cells to estimate division numbers. D, all cells were dead. The significance of differences in the proportions of dead clones at day 3 (compared to the 'no silencing' control) was assessed by a Chi square test. Significant P values are shown in bold.

knockdowns		*PTIWI01*			*PTIWI03*			*PTIWI09*			*PTIWI01-03*			*PTIWI01-09*			*PTIWI03-09*			*PTIWI01-03-09*			no silencing		
days		day1	day2	day3	day1	day2	day3	day1	day2	day3	day1	day2	day3	day1	day2	day3	day1	day2	day3	day1	day2	day3	day1	day2	day3
post-autogamous clones	1	3-4	≥6	++	2-3	≥6	++	3-4	≥6	++	2-3	≥6	++	2-3	3-4	D	3	≥6	++	2	4-5	++	2-3	≥6	++
	2	3-4	≥6	++	3	≥6	++	3	≥6	++	2-3	≥6	++	2	4	D	3	≥6	++	1	4	D	3-4	≥6	++
	3	3	≥6	++	3	≥6	++	3-4	≥6	++	2-3	≥6	++	2	3-4	D	2-3	≥6	++	2	4-5	D	2-3	≥6	++
	4	3	≥6	++	2-3	≥6	++	2-3	≥6	++	2-3	≥6	++	2-3	4-5	++	3-4	≥6	++	2	4-5	D	3	≥6	++
	5	3	≥6	++	2-3	≥6	++	3	≥6	++	2-3	≥6	++	2-3	3-4	D	3	≥6	++	2	4-5	++	3	≥6	++
	6	2-3	≥6	++	3	≥6	++	2-3	≥6	++	3	≥6	++	1	3-4	D	3	≥6	++	1-2	4-5	D	2-3	≥6	++
	7	3	≥6	++	2-3	≥6	++	2-3	≥6	++	3	≥6	++	2-3	3-4	D	3	≥6	++	2	3-4	D	2-3	≥6	++
	8	3	≥6	++	2-3	≥6	++	2-3	≥6	++	3-4	≥6	++	1-2	3-4	+	2-3	≥6	++	2	4-5	++	3	≥6	++
	9	3-4	≥6	++	3-4	≥6	++	D	D	D	2-3	≥6	++	2	3-4	D	2-3	≥6	++	2	3-4	D	2-3	≥6	++
	10	3	≥6	++	2-3	≥6	++	2-3	≥6	++	2-3	≥6	++	2	4	+	2-3	≥6	++	2	3-4	D	3-4	≥6	++
	11	3	≥6	++	3	≥6	++	2-3	≥6	++	2-3	≥6	++	2	4-5	++	3	≥6	++	2	3-4	D	3	≥6	++
	12	3	2-3	3-4	3	≥6	++	2	3-4	+	2-3	≥6	++	1	2-3	D	2-3	≥6	++	2	3-4	D	2-3	≥6	++
clones dead at day 3		0/12			0/12			1/12			0/12			8/12			0/12			9/12			0/12		
P value		NA			NA			>0.99			NA			**2x10^{-3}**			NA			**7x10^{-4}**			NA		