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 Supplementary Tables Showing Raw Data For Cell-Free Initiation Experiments

Late G1 nuclei preparation 1 (used in experiments 1-10) contained 17% S phase nuclei and 34% ‘replication competent’ nuclei. Preparation 2 (used in experiments 11 and 12) contained 12% S phase nuclei and 32% ‘replication competent’ nuclei. ‘% initiation’ is calculated from ‘% replication’ for each data point by subtracting ‘% S phase’ and dividing by ‘% replication competent’. For the first data point in the table below this is  $(31-17)/34 \times 100 = 41\%$ .

**T(191/2)A** (data for Fig. 3C)

Concentration of recombinant Ciz1 in replication assays (nm)	Labelled nuclei/total (nuclei prep.1)			% Replication *	% Initiation **
	Expt. 6	Expt. 7	Expt. 9		
0.00	39/132	57/172		31***	41
0.01			54/200	27	29
0.1		24/84		29	35
0.25		49/154		32	44
0.4			49/156	31	41
0.6	61/137			45	82
1.3	53/142	28/62	74/152	44	79
6.3	89/196	53/122		45	82
12.7		61/148	44/100	42	74
25.4	48/125	44/105		40	68
63.5			55/115	48	91

**T(293)A** (data for Fig. 3E)

Concentration of recombinant Ciz1 in replication assays (nm)	Labelled nuclei/total (nuclei prep.2)		% Replication *	% Initiation **
	Expt. 11	Expt. 12		
0.00	24/104	38/142	25	41
0.01		24/93	26	44
0.06		28/100	28	50
0.13	60/146	29/75	40	88
0.6		30/105	29	53
1.3	32/135		24	38
12.7	30/134		22	31

**Nterm442 (data for Fig. 3F)**

Concentration of recombinant Ciz1in replication assays (nm)	Labelled nuclei/total (nuclei prep.1)				% Replication *	% Initiation **
	Expt. 1	Expt. 2	Expt. 3	Expt. 4		
0.0	54/181	24/75	38/121	41/137	31***	41
0.2		30/94			32	44
0.7			19/57		33	47
1.0	76/175	102/242	62/152	51/93	44	79
1.3				44/97	45	82
2.1		45/117	20/70		35	53
10.3	53/183				29	35
103.3	37/107				35	53
1033.0	29/87				33	47

**Cterm274 (data for Fig. 3G)**

Concentration of recombinant Ciz1in replication assays (nm)	Labelled nuclei/total (nuclei prep.1)				% Replication *	% Initiation **
	Expt. 1	Expt. 2	Expt. 8	Expt. 5		
0.00	54/181	24/75	47/150		31***	41
0.01				53/163	33	47
0.05				54/168	32	44
0.1				39/138	28	32
0.3		30/100	21/69		30	38
1.6		46/162	33/99		30	38
5.0	36/108				33	47
6.5			24/75		32	44
10.0	30/98				31	41
13.0			20/68		29	35
26.0			44/136		32	44
50.0	40/122				33	47
165.0		36/112			32	44
1653		31/90			34	50
4958	30/96				31	41

### ECiz1 (data for Fig. 3B)

Concentration of recombinant Ciz1in replication assays (nm)	Labelled nuclei/total (nuclei prep.1)				Labelled nuclei/total (nuclei prep.2)				Mean % initiation
	Expt. 9	Expt 5	% replication *	% initiation **	Expt. 11	Expt. 12	% replication *	% initiation **	
0.00			31 ***	41	24/104	38/142	25.2	41.2	41
0.01	38/113		33.6	48.8		37/149	24.8	40.0	44
0.13	33/111		29.7	37.3					37
0.63	34/116	27/98	28.5	33.8					34
1.02		44/138	31.9	43.8	39/120	35/96	34.3	69.7	57
1.14						55/125	44.0	100	100
1.3	114/264	54/136	42.0	73.5	64/169	50/120	39.5	85.9	80
2.5					26/102		25.5	42.2	42
3.8		30/111	27.0	29.4	32/132		24.2	38.1	34
6.3	56/188		29.8	37.6					38
12.7	30/105		28.6	34.1					34
63.0	30/102		29.4	36.4					36

\*Proportion of nuclei that incorporated labelled nucleotides in vitro.

\*\*Proportion of ‘initiation competent’ nuclei that initiated in vitro.

\*\*\*Mean % replication, calculated from control samples from experiments 1-10.