

Table S1. Statistical analysis of qualitative live cell imaging data. Fisher’s exact p-values for qualitative analysis. Mitotic BS-C-1 cells expressing GFP-tagged constructs were visually scored for the presence or absence of chromosome association. P-values comparing the frequency of cells exhibiting chromosome association were determined using a Fisher’s exact test as described in the Methods. Abbreviations: N1(ON CHR) = number of cells expressing construct 1 with positive chromosome association; N1(OFF CHR + CC) = number of cells expressing construct 1 that had exclusion from mitotic chromosome or were “cannot call”. N2(ON CHR) = number of cells expressing construct 2 with positive chromosome association; N2(OFF CHR + CC) = number of cells expressing construct 2 that had exclusion from mitotic chromosome or were “cannot call”. The “OFF CHR” and “Cannot Call” were combined in order to have a 2-variable analysis that compared the frequency of cells that exhibited chromosome association.

Figure	Construct 1	N1 (ON CHR)	N1 (OFF CHR +CC)	Construct 2	N2 (ON CHR)	N2 (OFF CHR +CC)	Fisher’s Exact Test p-value
Figure S1B	WT ¹⁻⁴³³	308	193	GFP	0	84	2.15 x 10 ⁻³¹
				WT ¹⁻¹⁵	0	93	2.79 x 10 ⁻³⁴
				WT ¹⁻²⁰	154	54	1.35 x 10 ⁻³
				WT ¹⁻⁴¹	314	34	4.01 x 10 ⁻²²
				WT ¹⁻⁶³	99	5	1.32 x 10 ⁻¹³
				WT ¹⁻¹¹⁰	162	10	9.42 x 10 ⁻¹⁹
				WT ¹⁻¹⁶⁶	258	17	1.49 x 10 ⁻²⁵
Figure S1C	WT ¹⁻⁴³³	308	193	WT ²¹⁻⁴³³	0	24	2.82 x 10 ⁻¹⁰
				WT ⁴²⁻⁴³³	0	77	4.19 x 10 ⁻²⁹
	WT ¹⁻¹¹⁰	162	10	WT ²¹⁻¹¹⁰	56	18	9.33 x 10 ⁻⁵
				WT ⁴²⁻¹¹⁰	0	46	8.82 x 10 ⁻³⁸
	WT ¹⁻¹⁶⁶	258	17	WT ²¹⁻¹⁶⁶	3	30	3.13 x 10 ⁻²⁶
				WT ⁴²⁻¹⁶⁶	0	118	1.85 x 10 ⁻⁸²
Figure S4A	WT ¹⁻⁴³³	308	193	Δ3-8 ¹⁻⁴³³	0	157	4.31 x 10 ⁻⁵³
				R4A ¹⁻⁴³³	0	97	1.53 x 10 ⁻³⁵
				R4K ¹⁻⁴³³	4	66	2.50 x 10 ⁻²⁰
				T6A ¹⁻⁴³³	2	84	2.22 x 10 ⁻²⁸
				T6D ¹⁻⁴³³	0	33	1.12 x 10 ⁻¹³
				T6E ¹⁻⁴³³	0	50	7.30 x 10 ⁻²⁰

Table S1. Con't.

Figure	Construct 1	N1 (ON CHR)	N1 (OFF CHR +CC)	Construct 2	N2 (ON CHR)	N2 (OFF CHR +CC)	Fisher's Exact Test p-value
Figure S4A (con't)	WT ¹⁻⁴³³	308	193	R7A ¹⁻⁴³³	0	59	5.10 x 10 ⁻²³
				R7K ¹⁻⁴³³	0	53	6.35 x 10 ⁻²¹
				N8A ¹⁻⁴³³	21	18	0.4
				S9A ¹⁻⁴³³	40	18	0.32
				S9D ¹⁻⁴³³	0	64	9.72 x 10 ⁻²⁵
				S9E ¹⁻⁴³³	0	43	2.36 x 10 ⁻¹⁷
				E14A ¹⁻⁴³³	36	13	0.12
				N15A ¹⁻⁴³³	44	24	0.69
Figure S1B vs. Figure S4B	WT ¹⁻¹⁶⁶	258	17	Δ3-8 ¹⁻¹⁶⁶	0	222	4.90 x 10 ⁻¹²²
	WT ¹⁻¹¹⁰	162	10	Δ3-8 ¹⁻¹¹⁰	59	33	3.69 x 10 ⁻⁹
	WT ¹⁻⁶³	99	5	Δ3-8 ¹⁻⁶³	48	26	1.58 x 10 ⁻⁷
	WT ¹⁻⁴¹	314	34	Δ3-8 ¹⁻⁴¹	0	61	1.89 x 10 ⁻⁴⁸
Figure S6A	WT ¹⁻²⁰	154	54	R4A ¹⁻²⁰	0	125	6.19 x 10 ⁻⁴⁹
				R4K ¹⁻²⁰	0	68	3.70 x 10 ⁻³¹
				T6A ¹⁻²⁰	0	124	2.95 x 10 ⁻⁴⁸
				T6D ¹⁻²⁰	0	103	1.89 x 10 ⁻⁴²
				T6E ¹⁻²⁰	0	125	1.23 x 10 ⁻⁴⁸
				R7A ¹⁻²⁰	0	129	9.79 x 10 ⁻⁵⁰
				R7K ¹⁻²⁰	0	63	2.30 x 10 ⁻²⁹
				N8A ¹⁻²⁰	57	11	0.14
				S9A ¹⁻²⁰	36	20	0.18
				S9D ¹⁻²⁰	0	88	7.30 x 10 ⁻³⁸
S9E ¹⁻²⁰	0	106	3.39 x 10 ⁻⁴³				

Table S1. Con't.

Figure	Construct 1	N1 (ON CHR)	N1 (OFF CHR +CC)	Construct 2	N2 (ON CHR)	N2 (OFF CHR +CC)	Fisher's Exact Test p-value
Figure S6B	WT ¹⁻⁴¹	314	34	R4A ¹⁻⁴¹	0	82	5.07 x 10 ⁻⁶¹
				R4K ¹⁻⁴¹	69	3	0.17
				T6A ¹⁻⁴¹	93	44	1.25 x 10 ⁻⁸
				T6D ¹⁻⁴¹	0	90	1.77 x 10 ⁻⁶⁵
				T6E ¹⁻⁴¹	0	72	3.33 x 10 ⁻⁵⁵
				R7A ¹⁻⁴¹	0	65	5.94 x 10 ⁻⁵¹
				R7K ¹⁻⁴¹	31	25	2.12 x 10 ⁻⁹
				N8A ¹⁻⁴¹	64	5	0.65
				S9A ¹⁻⁴¹	30	2	0.75
				S9D ¹⁻⁴¹	0	64	2.48 x 10 ⁻⁵⁰
S9E ¹⁻⁴¹	0	65	5.94 x 10 ⁻⁵¹				
Figure S6C	WT ¹⁻¹⁶⁶	258	17	R4A ¹⁻¹⁶⁶	19	87	5.01 x 10 ⁻⁴⁹
				R4K ¹⁻¹⁶⁶	34	10	1.30 x 10 ⁻³
				T6A ¹⁻¹⁶⁶	87	29	5.84 x 10 ⁻⁷
				T6D ¹⁻¹⁶⁶	0	110	1.10 x 10 ⁻⁷⁸
				T6E ¹⁻¹⁶⁶	4	138	6.35 x 10 ⁻⁸⁵
				R7A ¹⁻¹⁶⁶	0	122	2.71 x 10 ⁻⁸⁴
				R7K ¹⁻¹⁶⁶	26	7	7.85 x 10 ⁻³
				N8A ¹⁻¹⁶⁶	89	5	1
				S9A ¹⁻¹⁶⁶	21	3	0.21
				S9D ¹⁻¹⁶⁶	12	95	1.19 x 10 ⁻⁵⁷
S9E ¹⁻¹⁶⁶	29	94	7.07 x 10 ⁻⁴⁷				

Table S1. Con't.

Figure	Construct 1	N1 (ON CHR)	N1 (OFF CHR +CC)	Construct 2	N2 (ON CHR)	N2 (OFF CHR +CC)	Fisher's Exact Test p-value
Figure S7	WT ¹⁻¹¹⁰	162	10	R4A ¹⁻¹¹⁰	50	10	0.02
				T6A ¹⁻¹¹⁰	47	14	4.26 x 10 ⁻⁴
				T6D ¹⁻¹¹⁰	21	9	3.60 x 10 ⁻⁴
				T6E ¹⁻¹¹⁰	16	10	2.13 x 10 ⁻⁵
				R7A ¹⁻¹¹⁰	43	18	6.21 x 10 ⁻⁶
				N8A ¹⁻¹¹⁰	27	1	1
				S9A ¹⁻¹¹⁰	43	1	0.47
				S9D ¹⁻¹¹⁰	45	7	0.08
				S9E ¹⁻¹¹⁰	43	4	0.51
Figure S8A	WT ¹⁻⁴³³	308	193	R40A ¹⁻⁴³³	1	64	3.45 x 10 ⁻²³
				R42A ¹⁻⁴³³	0	88	1.10 x 10 ⁻³²
				L45A ¹⁻⁴³³	33	10	0.02
				ΔDB ¹⁻⁴³³	62	53	0.14
				ΔDB/ K42A ¹⁻⁴³³	3	29	3.70 x 10 ⁻⁹
	R42A ¹⁻⁴³³	0	88	R40A ¹⁻⁴³³	1	64	0.42
				ΔDB ¹⁻⁴³³	62	53	2.08 x 10 ⁻²⁰
	ΔDB ¹⁻⁴³³	62	53	ΔDB/ K42A ¹⁻⁴³³	3	29	4.42 x 10 ⁻⁶
Figure S8B	WT ¹⁻⁶³	99	5	R42A ¹⁻⁶³	73	8	0.25
				Δ3-8/ R42A ¹⁻⁶³	0	107	7.59 x 10 ⁻⁵⁵
	WT ¹⁻¹¹⁰	162	10	R42A ¹⁻¹¹⁰	101	10	0.35
				Δ3-8/ R42A ¹⁻¹¹⁰	0	121	3.32 x 10 ⁻⁷¹
	WT ²¹⁻¹¹⁰	56	18	R42A ²¹⁻¹¹⁰	0	63	6.66 x 10 ⁻²³