

SUPPLEMENTARY FIGURES

A

apopt.	G1	S	G2/M	excess							Cell count	% <2N DNA	% G1 phase	% S phase	% G2/M	% ≥5N DNA	Gene
				<2N	2N	4N	≥5N	~12N	400 - max								
0 - 16																	
17 - 32																	
33 - 49																	
50 - 66																	
67 - 82																	
83 - 99																	
100 - 116																	
117 - 132																	
133 - 149																	
150 - 166																	
167 - 182																	
183 - 199																	
200 - 216																	
217 - 232																	
233 - 149																	
250 - 266																	
267 - 282																	
283 - 299																	
300 - 316																	
317 - 332																	
333 - 349																	
350 - 366																	
367 - 382																	
383 - 399																	
400 - max																	
										5074	11	39	8	17	11	control	
										375	14	8	6	10	62	FBXO5	
										513	8	8	11	20	53	TPX2	
										419	12	17	9	16	47	INCENP	
										765	16	17	8	13	47	GMNN	
										962	14	17	12	12	46	BIRC5	
										1606	14	18	10	13	45	ESPL1	
										1800	11	16	13	17	43	TOP2A	
										569	13	20	11	14	42	CDCA5	
										959	14	20	10	14	42	CDCA8	
										1104	10	20	9	19	42	CUL1	
										228	12	14	11	22	40	PLK1	
										245	8	9	6	33	39	KIF11	
										869	10	22	11	19	38	ANLN	
										500	15	23	12	12	37	AURKB	
										1577	9	26	15	16	34	PRC1	
										721	5	25	14	22	34	LOC728689	
										773	10	24	13	21	33	CASC5	
										2876	6	30	14	18	33	PPP2R1A	
										1329	7	22	14	25	33	NEDD8	
										552	10	22	11	24	33	CHMP2A	
										2873	11	20	13	24	32	LIN54	
										3381	9	27	13	19	32	SGOL1	
										2177	8	31	13	16	32	BBC3	
										1300	13	19	15	22	32	FLJ40448	
										2398	6	17	14	33	31	LIN9	
										1154	16	22	13	18	31	KIF23	
										895	10	31	13	14	31	ARD1A	
										1136	9	19	19	23	31	METT11D1	
										1475	12	28	17	13	31	WDR78	
										993	10	29	13	16	31	KIAA1383	
										1825	7	29	12	22	31	LIN37	
										3590	7	21	15	28	30	CDC26	
										555	8	18	16	27	30	CDC27	
										772	11	29	12	19	30	CEP192	
										2702	12	25	15	19	30	BUB3	
										1898	7	21	19	23	30	RBX1	
										854	10	16	14	32	30	DTL	
										786	12	29	14	15	30	MSRB2	
										1305	8	34	12	17	30	CRKL	
										1420	13	29	5	21	29	CCNB1	
										1746	9	26	16	21	29	WDR51A	
										2179	9	26	15	21	29	BUB1B	

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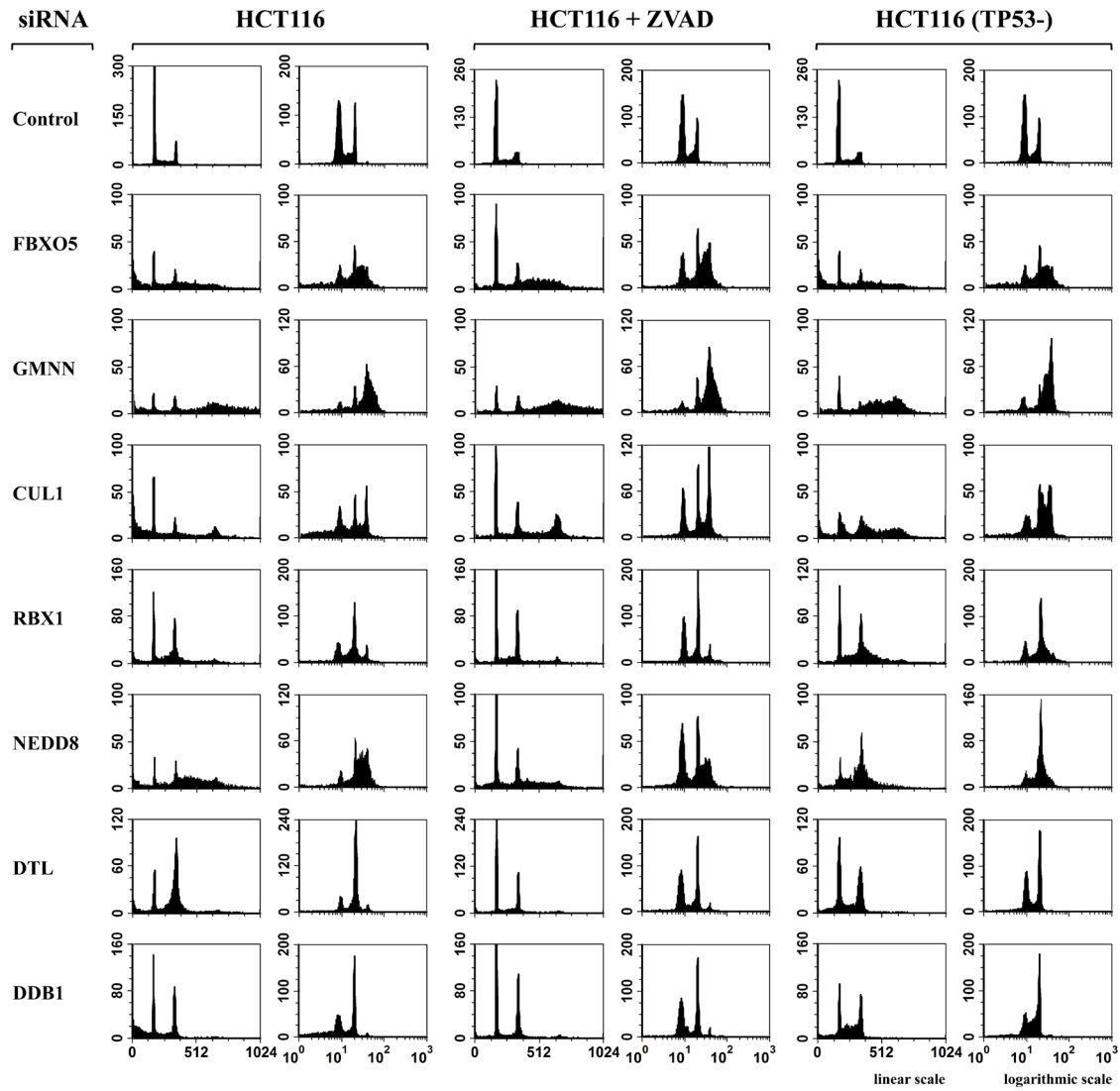
B

apopt.	G1	S	G2/M	excess		Gene
				<2N	≥5N	
0-16	2N		4N	≥5N	~12N	
17-32						
33-49						
50-66						
67-82						
83-99						
100-116						
117-132						
133-149						
150-166						
167-182						
183-199						
200-216						
217-232						
233-249						
250-266						
267-282						
283-299						
300-316						
317-332						
333-349						
350-366						
367-382						
383-399						
400-max						
Cell count	% <2N DNA	% G1 phase	% S phase	% G2/M	% ≥5N DNA	
5074	11	39	8	17	11	control
1627	10	30	11	20	29	SMC2
2561	8	29	15	20	29	RACGAP1
814	6	36	13	16	29	FAU
1463	6	37	13	15	29	SMAD2
1341	11	29	15	17	29	NCAPD3
848	9	27	15	20	29	CDC40
1250	7	36	13	16	29	ISG15
1606	7	31	16	18	29	PHF17
2124	10	31	15	16	29	SC65
1860	8	30	15	18	29	SFRS2IP
1969	8	32	16	15	29	C2orf63
2826	6	30	15	21	28	MASTL
335	13	25	13	22	28	AURKA
1175	14	25	15	19	28	ECT2
3520	9	29	16	19	28	C1orf158
1366	10	28	14	20	28	REXO4
1323	10	31	14	17	28	LOX
2627	6	31	15	20	28	RANBP2
2724	6	32	13	22	28	RBM47
2579	11	27	15	18	28	MIPOL1
573	8	30	16	19	28	MUM1
1689	6	25	13	27	28	SAMD3
1553	11	27	14	20	28	UBQLNL
881	12	28	14	19	27	MAD2L1
1793	13	26	12	22	27	SMC4
2006	10	27	15	20	27	TTK
1797	10	31	13	18	27	CHMP4B
3755	7	34	14	17	27	FBXO22
1141	10	36	13	15	27	TELO2
3853	6	33	17	17	27	MGC16169
2134	7	36	15	16	27	FERMT2
584	14	28	13	18	27	BCAR1
1573	11	32	14	15	27	TACC1
1645	8	29	18	19	27	ZNF334
2175	7	28	16	22	26	CDC16
927	16	28	6	18	26	NUF2
1318	4	21	16	34	26	DDB1
3294	5	26	8	28	26	COP2
1337	15	28	6	18	26	FANCD2
2349	5	36	17	16	26	CSE1L
1091	11	30	15	18	26	RAD21
2661	7	25	16	26	26	FBXL13
1857	8	39	15	12	26	FBXL17

Supplementary Figure S1: DNA content analysis results for 85 genes selected for validation. Each histogram of the DNA content per cell in each well was extrapolated to represent 1000 cells. The fluorescence intensities were distributed into 25 intervals that covered the entire range of detected fluorescence. The results for each siRNA were then plotted as heat-maps in which the intensity of the color was proportional to the number of cells in the interval. In some cases, the position of nuclei with 2N DNA content (G1 phase cells) differed from the controls in the same plate. Differences in the amount of fluorescence per DNA unit that causes shifts in the DNA peaks positions of cell populations are routinely corrected during FACS by adjusting the laser PMT voltage of the instrument until the positions of the 2N or the 4N peak are the same for each sample (1). These data allowed calculation of fraction of cells with nuclear DNA content equivalent to G1 phase (2N), S phase (>2N<4N), G2/M phase (4N), apoptosis (<2N), or EDR (>5N). Gene names are from the Human Gene Nomenclature Committee (HUGO).

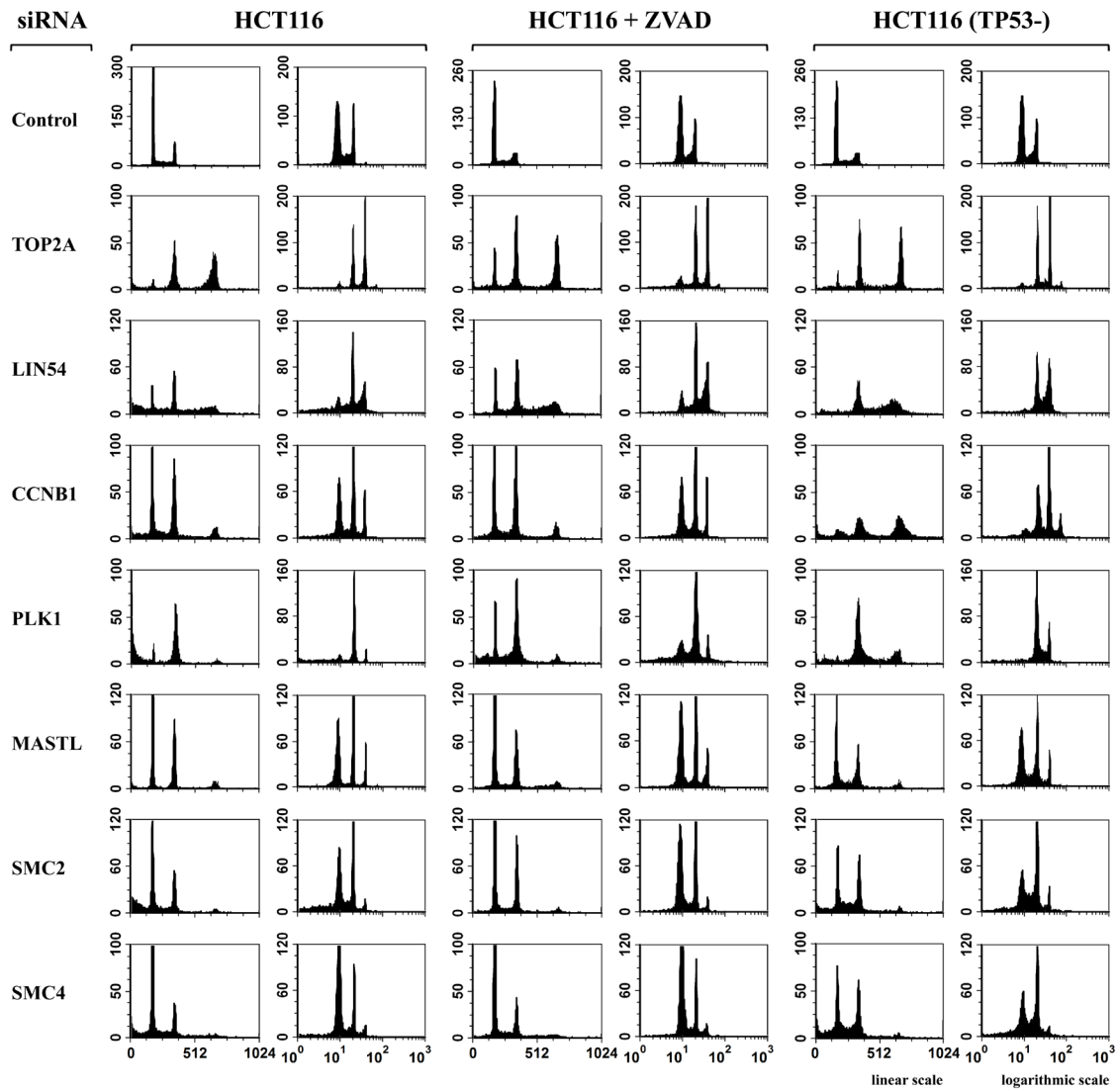
A

Origin Licensing Inhibition



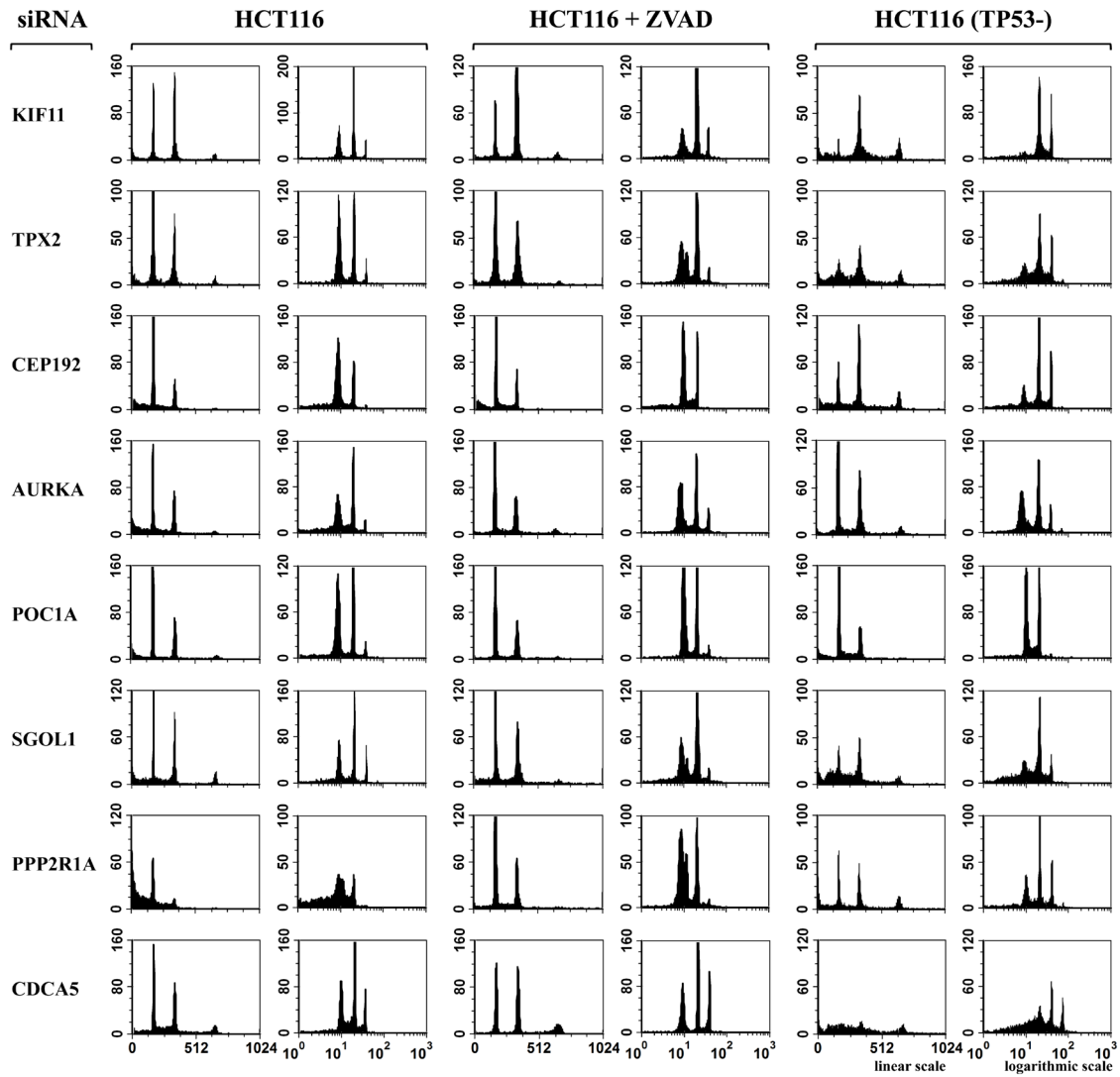
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B Chromatin Untangling, and Mitotic Entry and Maintenance



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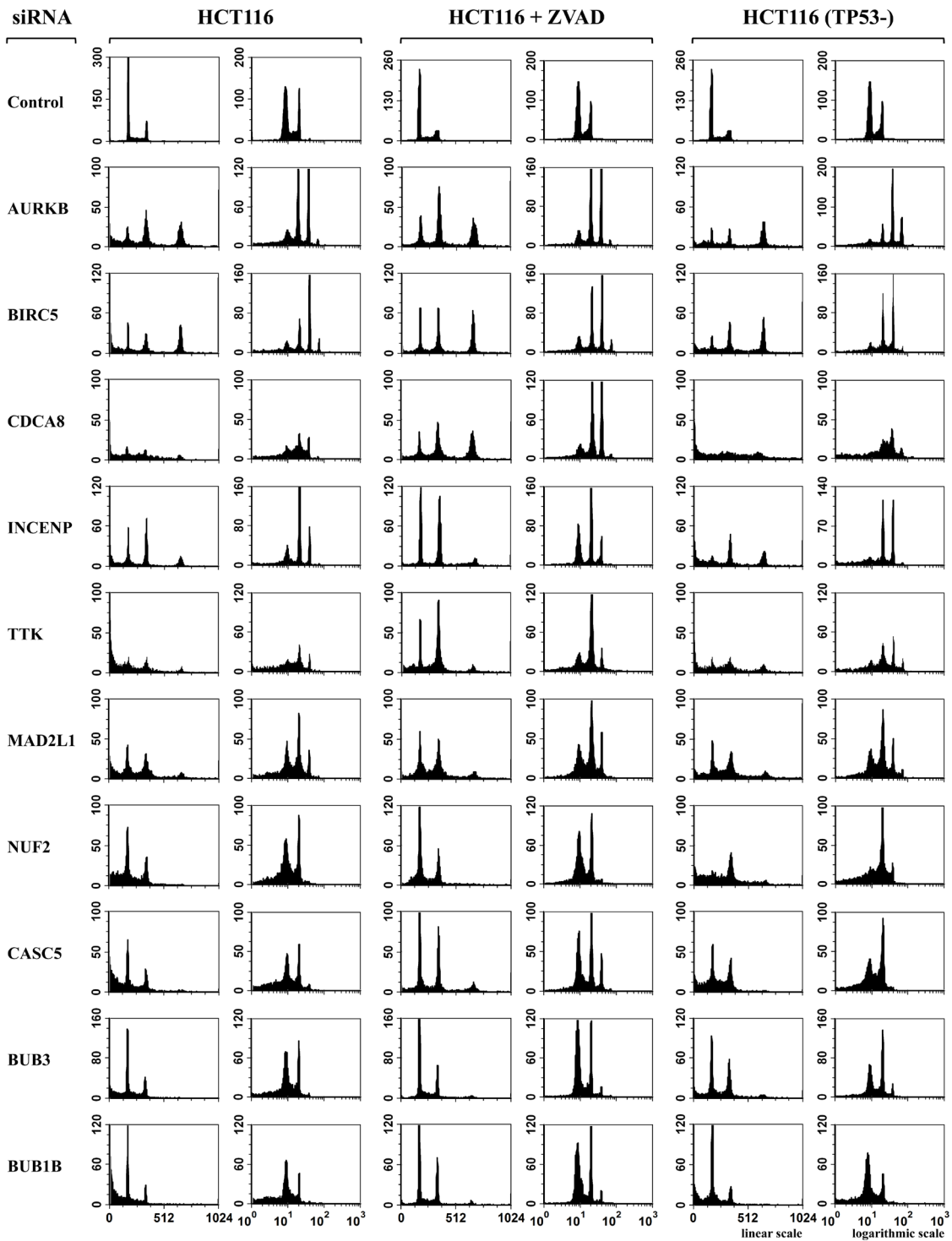
C Mitotic Spindle Assembly, Sister Chromatid Cohesion Maintenance



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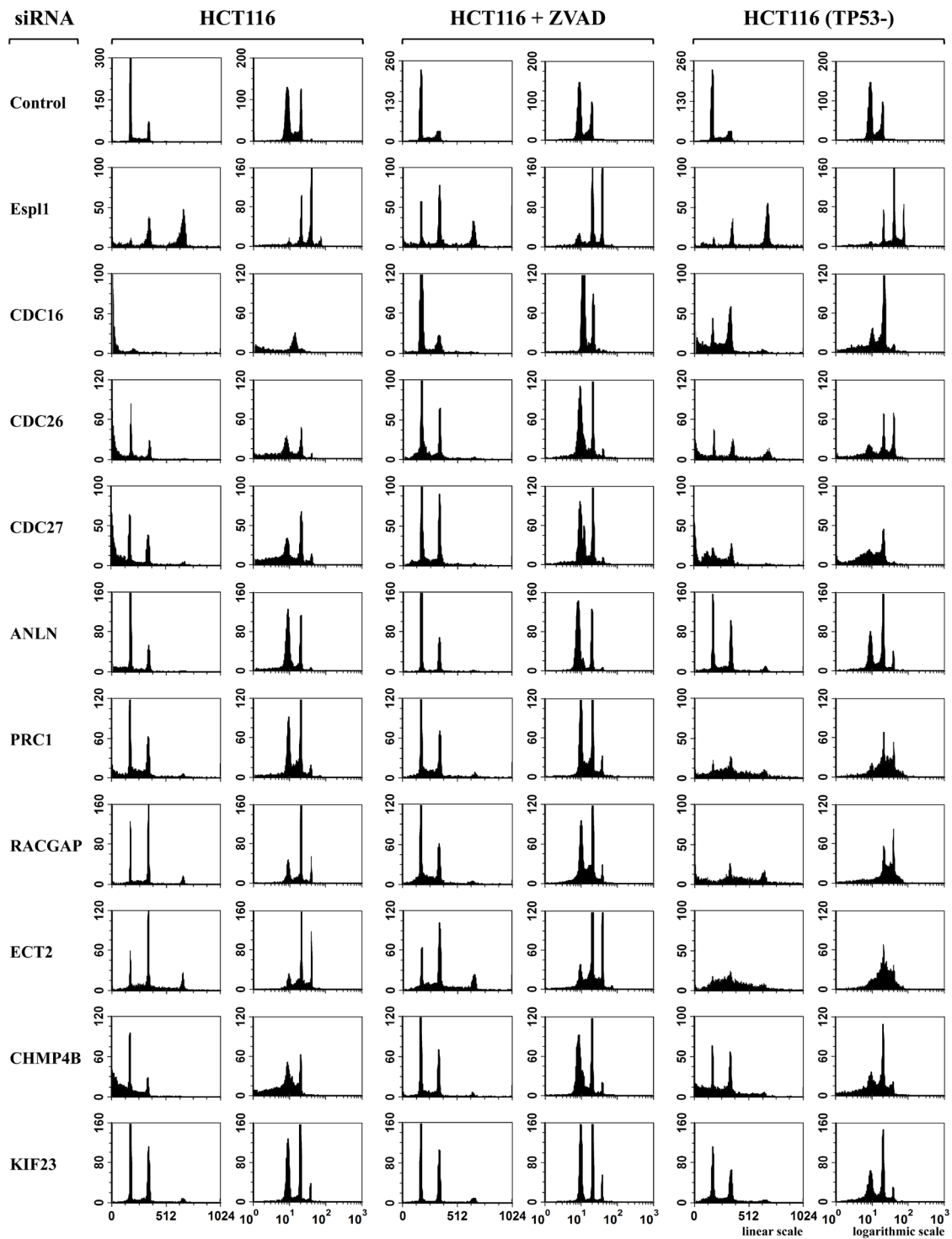
D

Spindle Assembly Checkpoint



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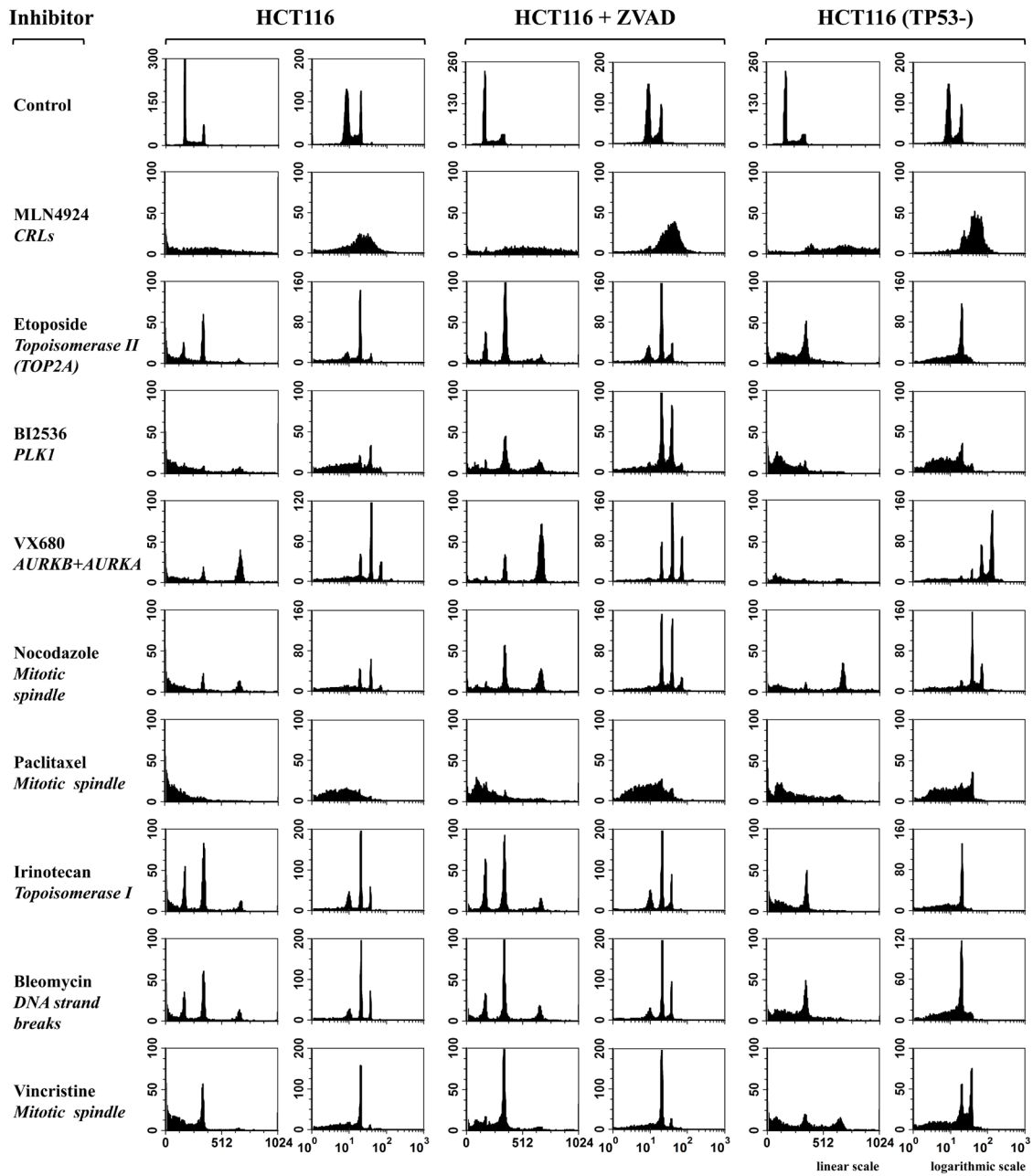
E Chromosome Segregation, Cytokinesis



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F

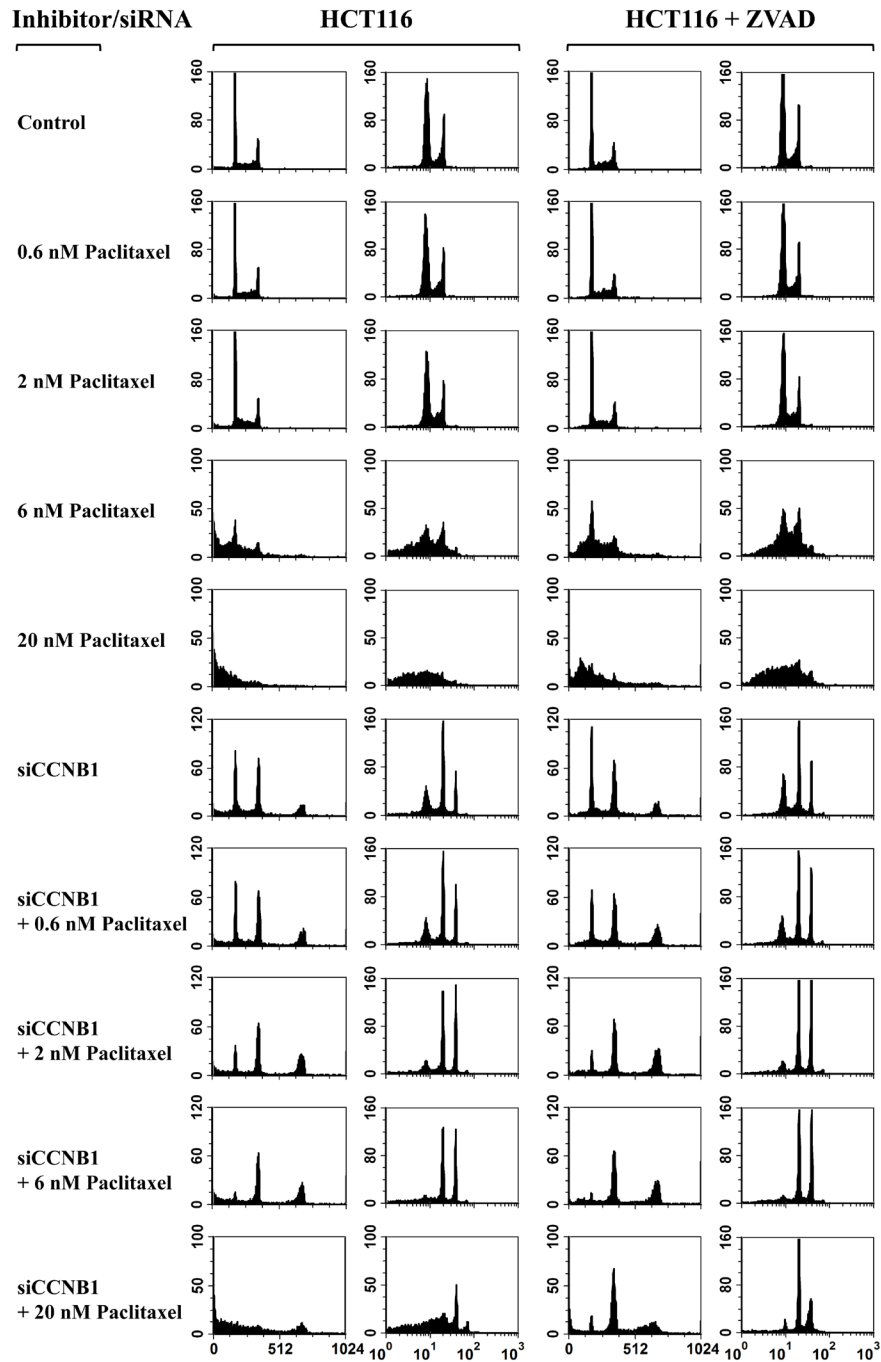
Chemical Inhibitors



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G

Synergistic effects between chemotherapeutic drugs and siRNA



Supplementary Figure S2: Validation FACS profiles of all genes confirmed as essential for prevention of EDR. The results of the three validation assays (TP53+ cells, TP53+ cells +ZVAD, and TP53- cells) carried out on each validation siRNA are included. The genes are arranged according to their known function during the cell cycle.

Gene	HTS		HCT116(TP53+)						HCT116 (TP53-)			Total cells (% of control)			2N-4N cells (% of control)			effective siRNAs	ZVAD effect		Δ TP53 effect	
	siRNAs	% >5N DNA	(-)ZVAD			(+)ZVAD			≥5N DNA	<2N DNA	abnormal	TP53+	+ZVAD	TP53-	TP53+	+ZVAD	TP53-		Δ EDR	Δ Apoptosis	Δ EDR	Δ Apoptosis
			≥5N DNA	<2N DNA	abnormal	≥5N DNA	<2N DNA	abnormal														
control		11	1.3	3.4	4.7	1.5	5.3	6.8	1.9	3.8	5.7	100	100	100	95	93	94	0	2	1	0	
Origin Licensing Inhibition																						
FBXO5	7/7(5)	62	38	50	88	46	21	67	44	36	80	13	18	10	2	6	2	8	8	-29	6	-14
GMNN	7/7(5)	47	59	26	85	73	11	84	62	20	81	21	23	23	3	4	4	8	15	-16	3	-7
CUL1	3/7(5)	42	23	52	75	46	13	59	46	23	68	39	35	12	10	14	4	4	23	-40	23	-30
NEDD8	4/7(5)	33	56	17	73	57	10	67	29	11	40	17	19	32	5	6	19	5	1	-8	-27	-7
RBX1	5/7(5)	30	26	6	32	19	10	28	35	7	42	40	19	33	27	14	19	6	-8	4	9	1
DTL	2/3(3)	29	17	14	32	10	9	19	6	14	20	44	42	50	30	34	40	3	-8	-5	-11	0
DDB1	2/3(3)	26	8	22	30	11	10	21	7	20	26	35	31	63	25	24	47	3	3	-12	-1	-3
Chromatin Untangling																						
TOP2A	7/7(5)	43	47	22	68	46	14	60	57	10	66	16	31	25	5	12	8	8	-1	-8	10	-12
Mitotic Entry and Maintenance																						
LIN54	2/3(3)	32	29	35	63	45	6	51	56	11	68	35	40	27	13	20	9	3	16	-29	27	-24
CCNB1	4/7(5)	29	17	22	38	17	11	28	57	21	78	35	40	18	22	29	4	5	1	-11	40	-1
MASTL	2/3(3)	28	12	9	21	15	6	22	15	9	24	54	50	54	43	39	41	3	3	-3	3	0
PLK1	7/7(5)	40	11	56	67	18	13	31	56	11	68	28	32	30	9	22	10	8	7	-43	45	-44
SMC2	3/7(5)	29	6	34	40	9	6	15	13	18	31	40	45	55	24	38	38	4	2	-28	6	-16
SMC4	3/7(3)	27	8	22	30	9	7	15	12	24	36	49	47	64	34	40	41	4	1	-15	4	2
Mitotic Spindle Assembly																						
TPX2	5/7(5)	53	7	20	27	14	12	26	26	26	53	23	20	11	17	15	5	6	7	-8	19	6
KIF11	7/7(5)	39	10	32	42	15	15	30	31	27	58	10	13	6	6	9	3	8	5	-17	22	-6
CEP192	2/3(3)	30	2	35	37	3	9	12	26	26	52	37	65	44	23	57	21	3	1	-26	24	-9
AURKA	5/7(5)	29	11	27	38	14	8	22	15	12	27	39	38	40	24	30	29	6	2	-18	4	-15
POCIA	3/7(3)	29	6	27	33	7	11	17	4	19	23	18	25	28	12	21	22	4	0	-16	-2	-8
Spindle Assembly Checkpoint																						
INCENP	7/7(5)	47	14	39	53	17	11	28	22	49	71	62	66	56	29	48	16	8	3	-28	9	10
BIRC5	4/7(5)	46	31	23	54	43	12	55	42	23	65	30	44	22	14	20	8	5	12	-11	11	-1
CDCA8	5/7(5)	42	12	67	79	40	22	62	33	48	81	32	29	14	7	11	3	6	28	-45	22	-20
AURKB	7/7(5)	37	33	31	64	39	10	49	55	21	76	18	25	20	6	13	5	8	6	-21	22	-10
CASC5	4/7(5)	33	6	53	60	18	15	32	6	33	39	31	41	57	13	28	35	5	12	-39	0	-21
BUB3	2/3(3)	30	3	38	41	8	7	14	11	24	36	55	43	59	32	37	38	3	4	-31	8	-13
BUB1B	2/3(3)	29	2	65	67	7	8	15	4	32	36	57	54	56	19	46	36	3	5	-57	2	-33
MAD2L1	2/3(3)	27	17	39	55	25	16	41	27	21	48	34	25	49	15	15	26	3	9	-23	11	-18
TTK	4/7(5)	27	12	61	73	18	13	31	28	43	71	21	23	16	6	16	5	5	6	-49	16	-18
NUF2	2/3(3)	26	6	39	45	9	16	24	20	33	53	43	41	20	24	31	9	3	3	-23	14	-6
Sister Chromatid Cohesion Maintenance																						
CDCA5	5/7(5)	42	15	47	62	21	9	31	40	28	68	10	12	12	4	8	4	6	7	-38	25	-19
PPP2R1A	2/7(5)	33	3	18	20	8	12	20	17	47	64	28	30	23	22	24	8	3	5	-5	14	30
SGOL1	3/7(5)	32	11	28	39	11	18	29	17	30	47	14	33	22	8	23	12	4	0	-10	6	2
Chromosome Segregation																						
ESPL1	7/7(5)	45	51	19	69	46	21	67	68	12	80	22	20	20	7	7	4	8	-5	2	17	-7
CDC16	2/3(3)	26	2	94	95	7	11	17	8	33	40	87	107	82	4	88	49	3	5	-83	6	-61
CDC26	2/3(3)	30	3	62	65	7	14	21	28	39	67	48	69	32	17	55	11	3	4	-48	25	-23
CDC27	2/3(3)	30	5	59	64	9	11	20	5	62	67	73	80	40	26	64	13	3	4	-49	0	3
Cytokinesis																						
ANLN	2/7(5)	38	3	23	26	3	3	6	13	9	22	57	55	53	42	52	41	3	1	-21	10	-14
PRC1	3/7(5)	34	9	24	33	13	6	19	41	19	60	38	34	16	25	27	6	4	4	-18	32	-5
RACGAP1	2/7(5)	29	12	9	21	12	13	25	46	32	77	15	19	15	12	14	3	3	0	4	34	23
ECT2	4/7(5)	28	27	9	36	30	6	36	42	12	54	15	16	18	10	10	8	5	2	-3	15	3
CHMP4B	2/3(3)	27	2	57	59	8	12	20	16	36	53	54	45	20	22	36	10	3	6	-45	14	-21
KIF23	2/7(5)	26	8	11	19	10	5	15	11	14	25	17	12	18	14	10	14	3	2	-6	3	3

Supplementary Figure S3: Genes essential to prevent EDR in HCT116 cells. HTS siRNAs are the number of positive siRNAs/ number of siRNAs tested in HTS (\geq MAD value) from 'statistical analysis'. HTS \geq 5N DNA is the fraction (%) of cells with \geq 5N nuclear DNA content based on the median of the three siRNAs from DNA content analysis. Total cells are the number of cells counted relative to the number of cells in control (%) at end of experiment (3 days). 'Δ EDR' and 'Δ Apoptosis' are the differences \pm ZVAD. They were calculated by subtracting the corresponding percentage for the TP53+ assay from the ZVAD assay. 'Δ EDR' and 'Δ Apoptosis' for the TP53 effect were calculated by subtracting the corresponding percentage for the TP53+ assay from the TP53- assay. Red indicates a reduction in EDR or Apoptosis, whereas blue indicates an increase. Gene names are from the Human Gene Nomenclature Committee (HUGO).