Supporting Information

Comparative activity and off-target effects in cells of the CHK1 inhibitors MK-8776, SRA737 and LY2606368

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Table S1A. Average values for Figure 1										
					GI50					
	MK-8776 (μM)			SRA737 (μM)			LY2606368 (nM)			
cell line	24 h	48 h	continuous	24 h	48 h	continuous	24 h	48 h	continuous	
HCT116	50	30	5	22	14	12	300	100	80	
U251	30	9	2.5	30	30	9	75	22	12	
UACC257	25	15	5	50	25	15	20	10	9	
SW620	25	10	5	15	11	7	18	15	15	
HCT15	25	5	1.5	30	4	3	12.5	12.5	10	
SKMEL28	15	9	2.5	25	20	2	30	12	5	
PC3	15	7	4	30	15	12	35	10	9	
H322M	12	12	0.5	12	2	1	6	5	5	
UACC62	12	8	4	25	20	12	1000	800	100	
MDA-MB-231	12	4	1	14	4	2	13	8	8	
OVCAR5	12	0.8	0.6	12	1.5	1.2	8	5	5	
MDA-MB-435	0.6	0.3	0.2	1.5	1	1	2.5	2	2	
OVCAR3	0.5	0.3	0.3	0.8	0.6	0.4	3	2	1	
AsPC-1	0.5	0.3	0.2	0.3	0.2	0.2	5	3.5	3.5	
CAKI-1	0.2	0.15	0.15	0.4	0.3	0.1	1.75	1.75	1.75	
U2OS	0.1	0.1	0.1	0.2	0.15	0.15	2.5	2.5	2.5	

Table S1B. Individual values for Figure 1									
					GI50				
	MK-8776 (μM)			SRA737 (μM)			LY2606368 (nM)		
cell line	24 h	48 h	continuous	24 h	48 h	continuous	24 h	48 h	continuous
HCT116	>10, 75, 25	>10, 75, 10	2, 10, 5	30, 15	12, 15	8, 15	>100, >100, 300	>100, >100, 70	>100, 60, 30
U251	30	9	2.5	30	30	9	100, 50	30, 15	15, 10
UACC257	>10, 25	>10, 15	>10, 5	50	25	15	16,25	8,12	6,12
SW620	25, 25	10, 10	4, 5	15, 15	10, 12	5, 10	12, 20, 25	10, 15, 20	10, 15, 20
HCT15	25, 25	1, 8	1.5, 1.5	30	4	3	12.5	12.5	10
SKMEL28	15	9	2.5	25	20	2	30	12	5
PC3	15, 12	8, 6	5, 3	30, 35	20, 12	15, 12	100, 50, 10, 35	25, 12, 7, 8	25, 10, 7, 8
H322M	12, 20, 8	15, 18, 0.8	0.5, 2, 0.4	18, 12, 5	2, 2, 1.5	1, 1, 0.8	6, 6	5, 4	5, 4
UACC62	12	8	4	25	20	12	>100, >1000	>100, 800	>100, 100
MDA-MB-231	12, 10, 15, 20	5, 2.5 ,6, 3	0.8, 1.8, 0.8, 1	17, 10, 25	7.5, 1.5	3, 1	15, 12.5, 12.5, 15	10, 8, 7, 10	10, 8, 7, 6
OVCAR5	12	0.8	0.6	12	1.5	1.2	8	5	5
MDA-MB-435	0.6	0.3	0.2	1.5	1	1	2.5, 2	2, 1.5	2, 1.5
OVCAR3	0.5	0.3	0.3	0.8	0.6	0.4	3	2	1
AsPC-1	0.45, 0.5	0.35. 0.2	0.2, 0.2	0.3	0.2	0.2	5.5, 4	4, 3	4, 3
CAKI-1	<0.4, 0.2, <0.4	0.15, <0.4	0.15, <0.4	0.4, <0.4	0.3, <0.4	0.1, <0.4	2, 1.5	2, 1.5	2, 1.5
U2OS	<0.4, 0.1	0.1	0.1	0.2	0.15	0.15	2.5	2.5	2.5

Table S2A. Values for Figure 7A (GI50)

		GI50								
		MK-8776 (μM)			SRA737 (μM)			LY2606368 (nM)		
parent cell	**derivative**	**24 h**	**48 h**	**continuous**	**24 h**	**48 h**	**continuous**	**24 h**	**48 h**	**continuous**
AsPC-1	wt	0.45	0.35	0.2	0.3	0.2	0.2	5.5	3.5	3.5
AsPC-1	776R	20	8.5	2.5	40	30	15	50	40	40
AsPC-1	SRA-R	10	10	2	40	40	20	40	30	30
AsPC-1	LY-R	10	6	3	30	25	20	2000	1000	1000
MDA-MB-231	wt	25	8	1.4	25	8	1.4	12.5	8	7
MDA-MB-231	SRA-R	35	20	15	25	6	3	600	200	40
MDA-MB-231	LY-R	40	40	20	20	10	4	1800	1500	1500
H322M	wt	12	12	0.5	12	2	1	6	5	5
H322M	SRA-R	20	10	2	35	30	15	30	10	8
H322M	LY-R	15	10	5	25	20	15	900	300	80

Table S2B. Values for Figure 7B (fold resistance)

		fold resistance								
		MK-8776			SRA737			LY2606368		
parent cell	**derivative**	**24 h**	**48 h**	**continuous**	**24 h**	**48 h**	**continuous**	**24 h**	**48 h**	**continuous**
AsPC-1	wt	1	1	1	1	1	1	1	1	1
AsPC-1	776R	44	24	12.5	133	150	75	9	11.4	11.4
AsPC-1	SRA-R	22	28	10	133	200	100	7.2	8.6	8.6
AsPC-1	LY-R	22	17	15	100	125	125	262	285	285
MDA-MB-231	wt	1	1	1	1	1	1	1	1	1
MDA-MB-231	SRA-R	1	0.75	2.1	1.4	0.8	1.2	48	25	5.7
MDA-MB-231	LY-R	0.8	1.25	2.9	1.6	1.6	1.6	144	188	214
H322M	wt	1	1	1	1	1	1	1	1	1
H322M	SRA-R	1.7	0.8	4	2.9	15	15	5	2	1.6
H322M	LY-R	1.25	0.8	10	2.1	10	15	150	60	16

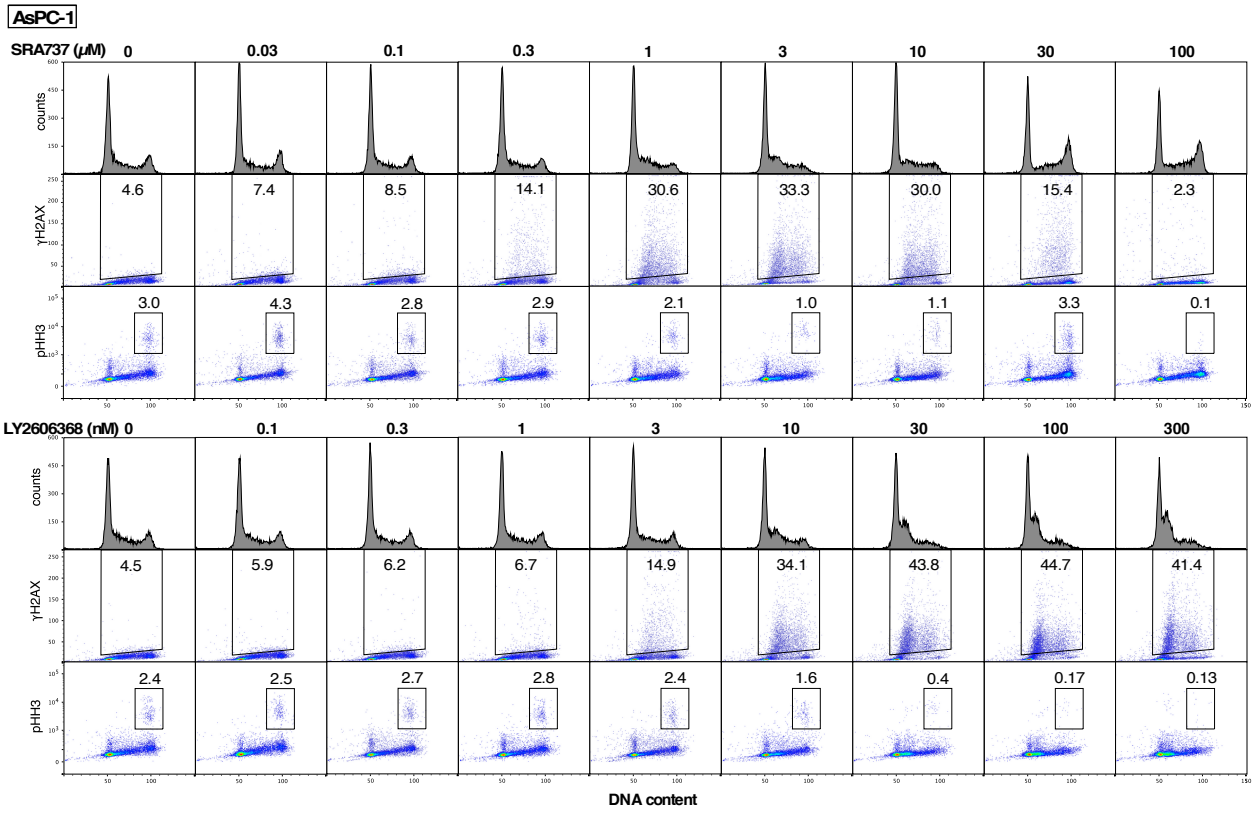

Figure S1A

Figure S1: Induction of γH2AX by SRA737 and LY2606368. A. ASPC-1 cells were incubated with the indicated concentrations of SRA737 (upper panel) or LY2606368 (lower panel) for 6 h, then fixed and analyzed by flow cytometry for DNA content, γH2AX and pHH3. **B-D.** MDA-MB-231, SW620 and H322M cells were incubated with the indicated concentrations of SRA737 (upper panel) or LY2606368 (lower panel) for 6 and 24 h and analyzed as in A. The percentages for γH2AX are graphed in Figure 4.

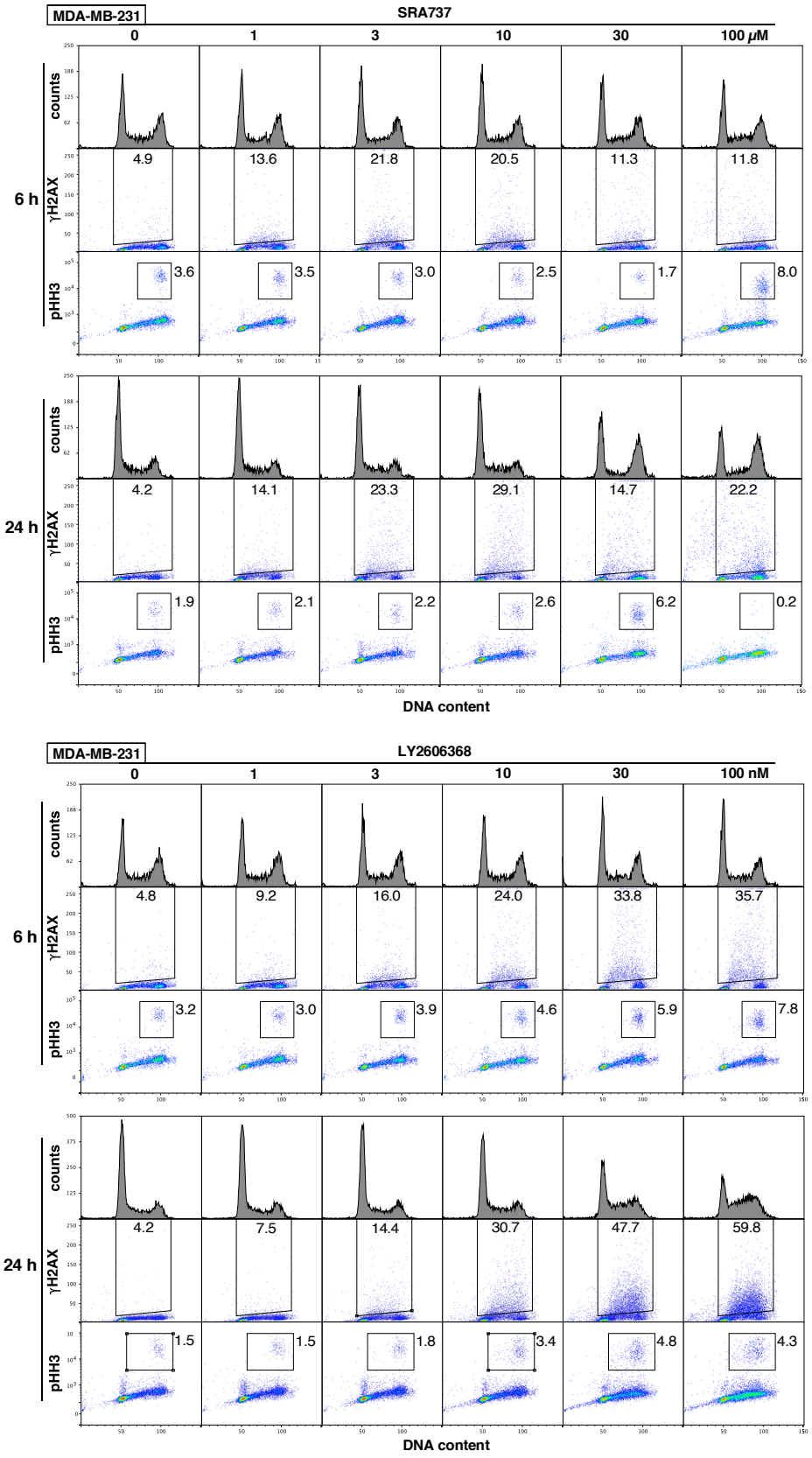
MDA-MB-231
SRA737
0
1
3
10
30
100 μM
6 h
24 h
counts
γH2AX
pHH3
4.9
13.6
21.8
20.5
11.3
11.8
3.6
3.5
3.0
2.5
1.7
8.0
4.2
14.1
23.3
29.1
14.7
22.2
1.9
2.1
2.2
2.6
6.2
0.2
DNA content
MDA-MB-231
LY2606368
0
1
3
10
30
100 nM
6 h
24 h
counts
γH2AX
pHH3
4.8
9.2
16.0
24.0
33.8
35.7
3.2
3.0
3.9
4.6
5.9
7.8
4.2
7.5
14.4
30.7
47.7
59.8
1.5
1.5
1.8
3.4
4.8
4.3
DNA content

Figure S1B

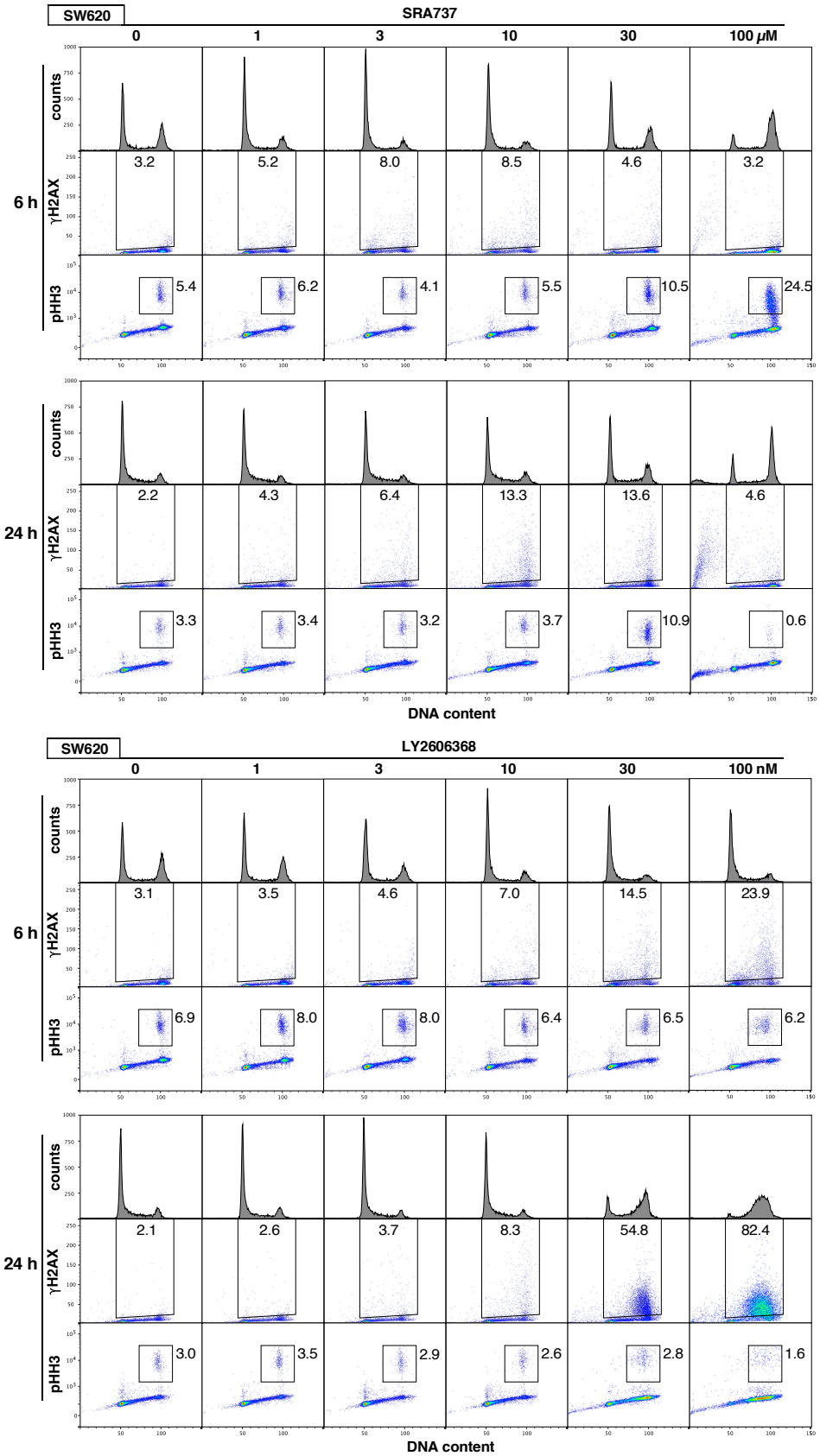
SW620
SRA737
0
1
3
10
30
100 μM
6 h
24 h
counts
γH2AX
pHH3
3.2
5.2
8.0
8.5
4.6
3.2
5.4
6.2
4.1
5.5
10.5
24.5
2.2
4.3
6.4
13.3
13.6
4.6
3.3
3.4
3.2
3.7
10.9
0.6
DNA content
SW620
LY2606368
0
1
3
10
30
100 nM
6 h
24 h
counts
γH2AX
pHH3
3.1
3.5
4.6
7.0
14.5
23.9
6.9
8.0
8.0
6.4
6.5
6.2
2.1
2.6
3.7
8.3
54.8
82.4
3.0
3.5
2.9
2.6
2.8
1.6
DNA content

Figure S1C

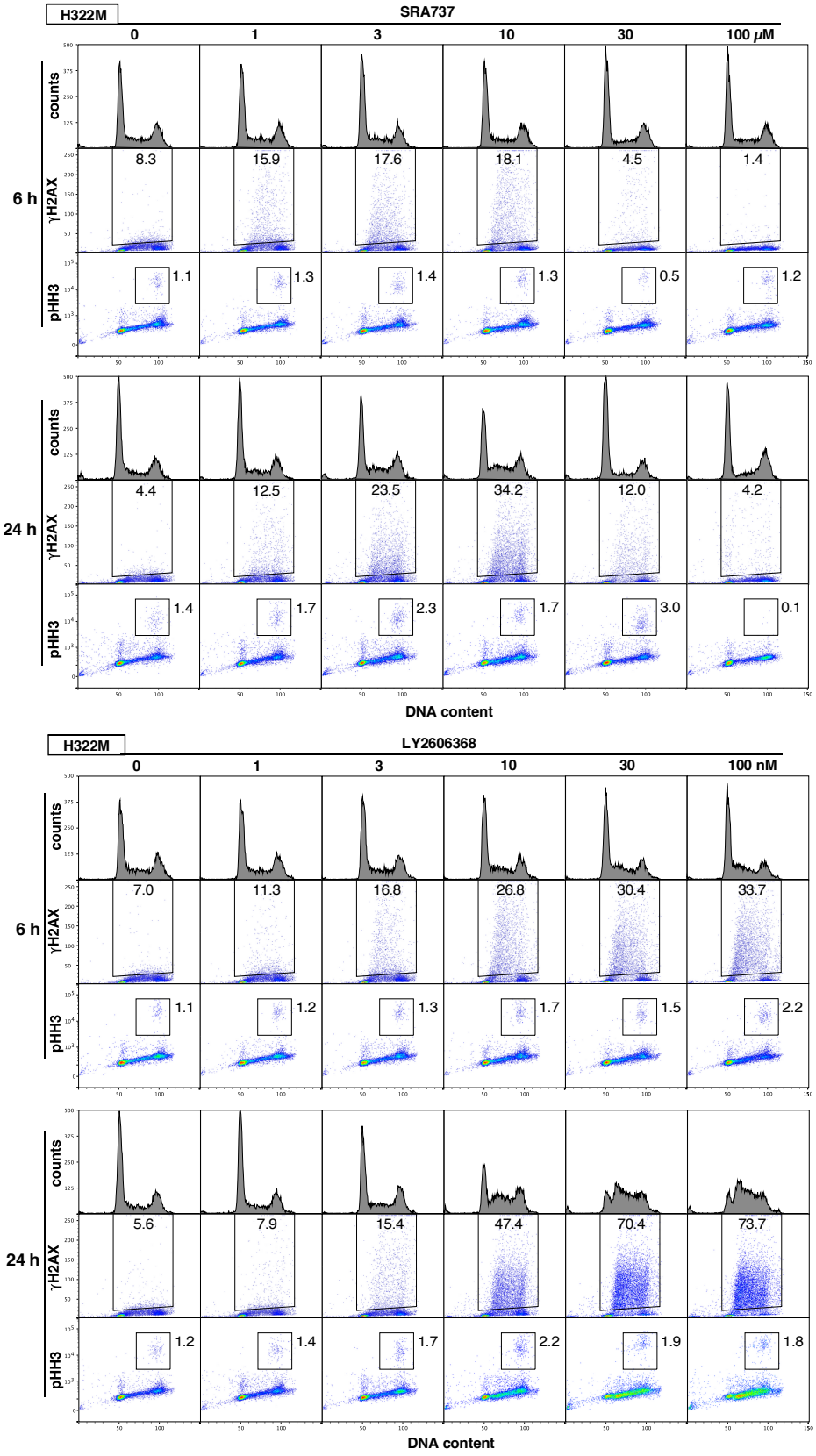
H322M
SRA737
0
1
3
10
30
100 μM
6 h
24 h
counts
γH2AX
pHH3
8.3
15.9
17.6
18.1
4.5
1.4
1.1
1.3
1.4
1.3
0.5
1.2
4.4
12.5
23.5
34.2
12.0
4.2
1.4
1.7
2.3
1.7
3.0
0.1
DNA content
H322M
LY2606368
0
1
3
10
30
100 nM
7.0
11.3
16.8
26.8
30.4
33.7
1.1
1.2
1.3
1.7
1.5
2.2
5.6
7.9
15.4
47.4
70.4
73.7
1.2
1.4
1.7
2.2
1.9
1.8
DNA content

Figure S1D

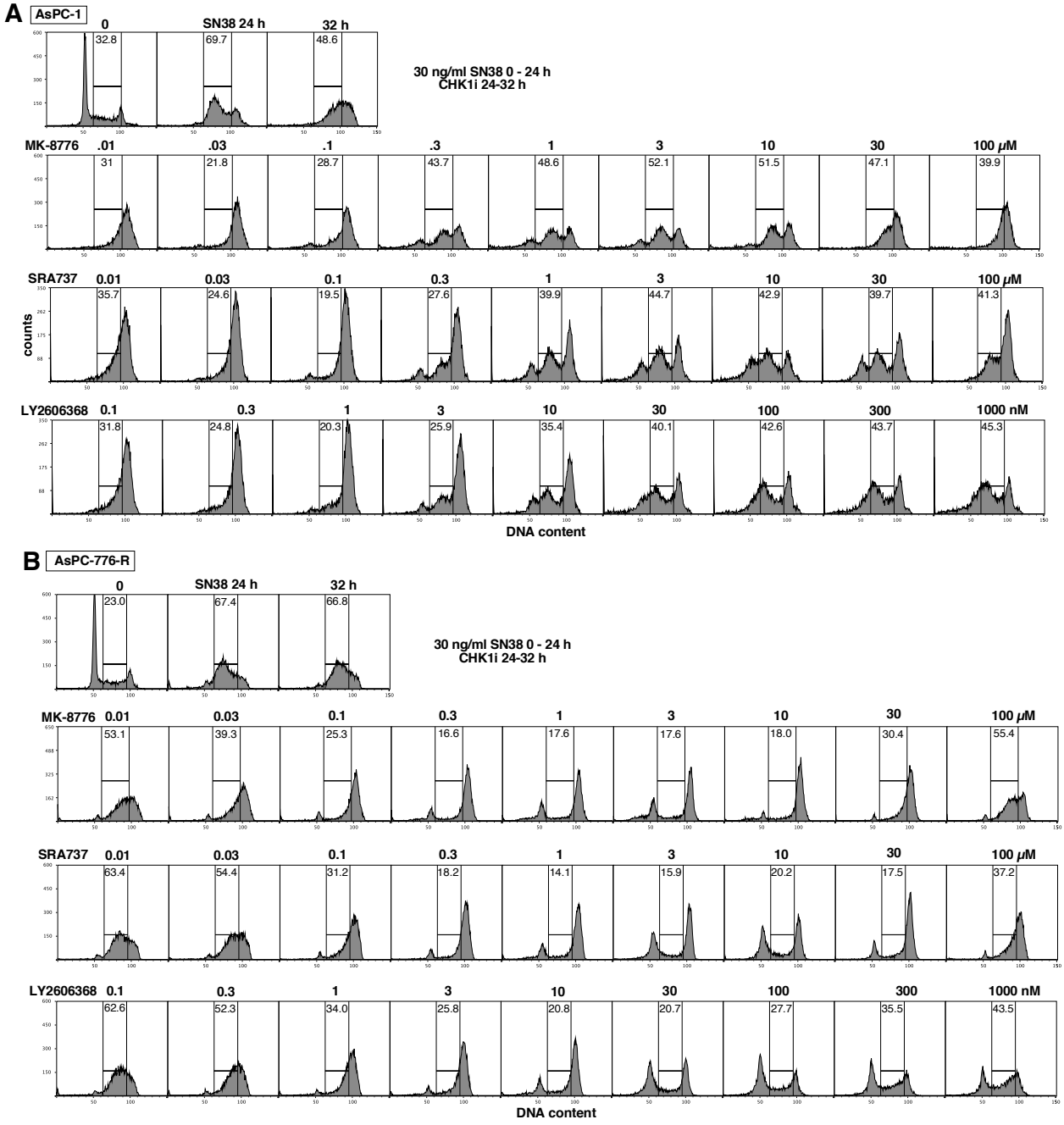

Figure S2: Abrogation of DNA damage-induced cell cycle arrest by CHK1i in sensitive and resistant cell lines. A. ASPC-1 cells were incubated with 30 ng/mL SN38 (75 nM) for 24 h. SN38 was then removed and cells were incubated with the indicated concentrations of MK8776 (second row), SRA737 (third row), or LY2606368 (bottom row) from 24-32 h. Cells were then fixed and analyzed by flow cytometry for DNA content. **B and C.** AsPC-1 cells with acquired resistance to MK-8776 or LY2606368 were similarly analyzed. **D.** MDA-MB-231 cells with acquired resistance to LY2606368 were incubated with 10 ng/ml SN38 for 24 h followed by each CHK1i from 24-30 h and analyzed as above. The % of cells in S phase are shown, and graphed in Figure 5.

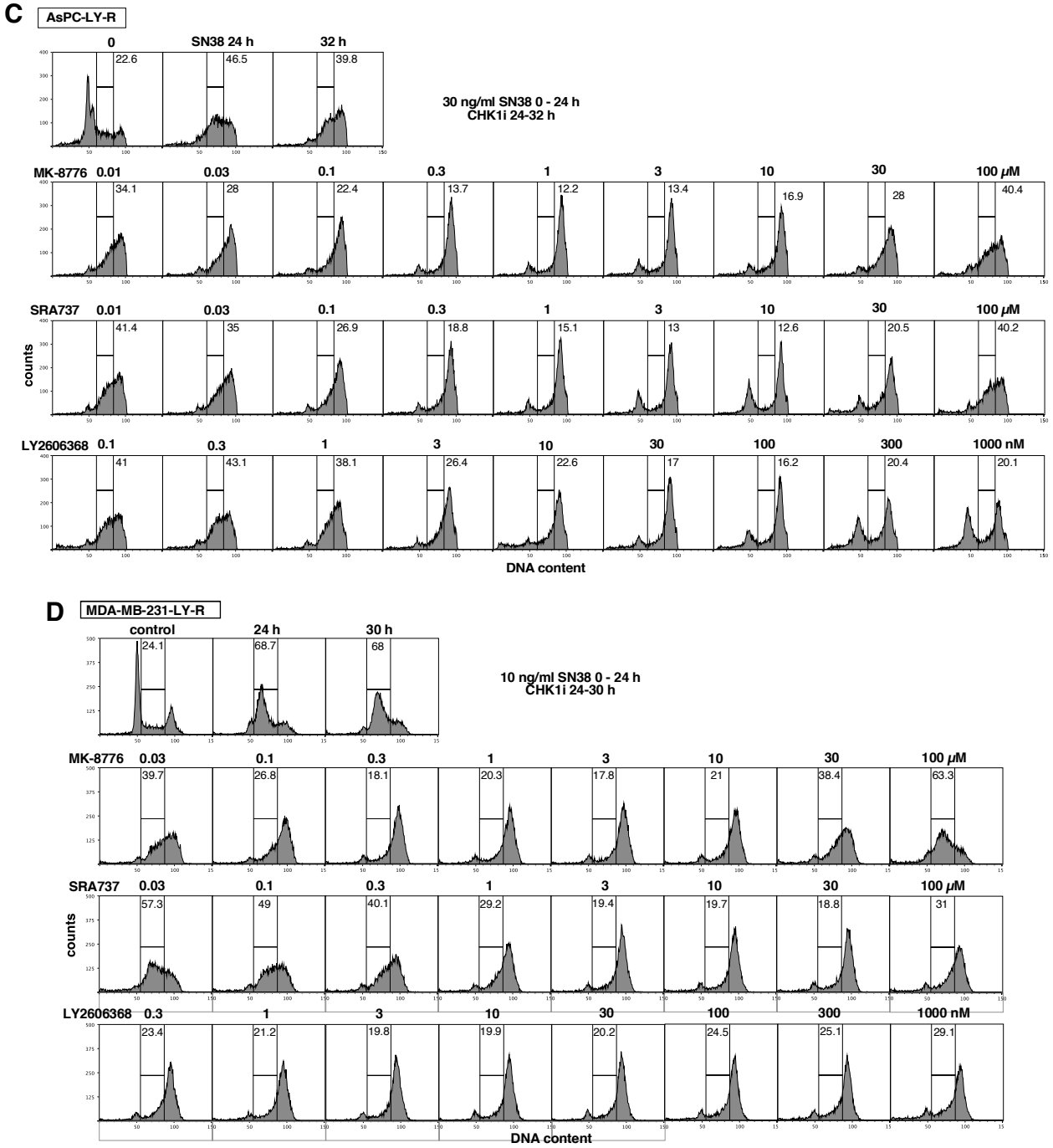
C
AsPC-LY-R
0
SN38 24 h
32 h
22.6
46.5
39.8
30 ng/ml SN38 0 - 24 h
CHK1i 24-32 h
MK-8776 0.01 0.03 0.1 0.3 1 3 10 30 100 μM
34.1 28 22.4 13.7 12.2 13.4 16.9 28 40.4
SRA737 0.01 0.03 0.1 0.3 1 3 10 30 100 μM
41.4 35 26.9 18.8 15.1 13 12.6 20.5 40.2
counts
LY2606368 0.1 0.3 1 3 10 30 100 300 1000 nM
41 43.1 38.1 26.4 22.6 17 16.2 20.4 20.1
DNA content
D
MDA-MB-231-LY-R
control
24 h
30 h
24.1
68.7
68
10 ng/ml SN38 0 - 24 h
CHK1i 24-30 h
MK-8776 0.03 0.1 0.3 1 3 10 30 100 μM
39.7 26.8 18.1 20.3 17.8 21 38.4 63.3
SRA737 0.03 0.1 0.3 1 3 10 30 100 μM
57.3 49 40.1 29.2 19.4 19.7 18.8 31
counts
LY2606368 0.3 1 3 10 30 100 300 1000 nM
23.4 21.2 19.8 19.9 20.2 24.5 25.1 29.1
DNA content

Figure S2C & D

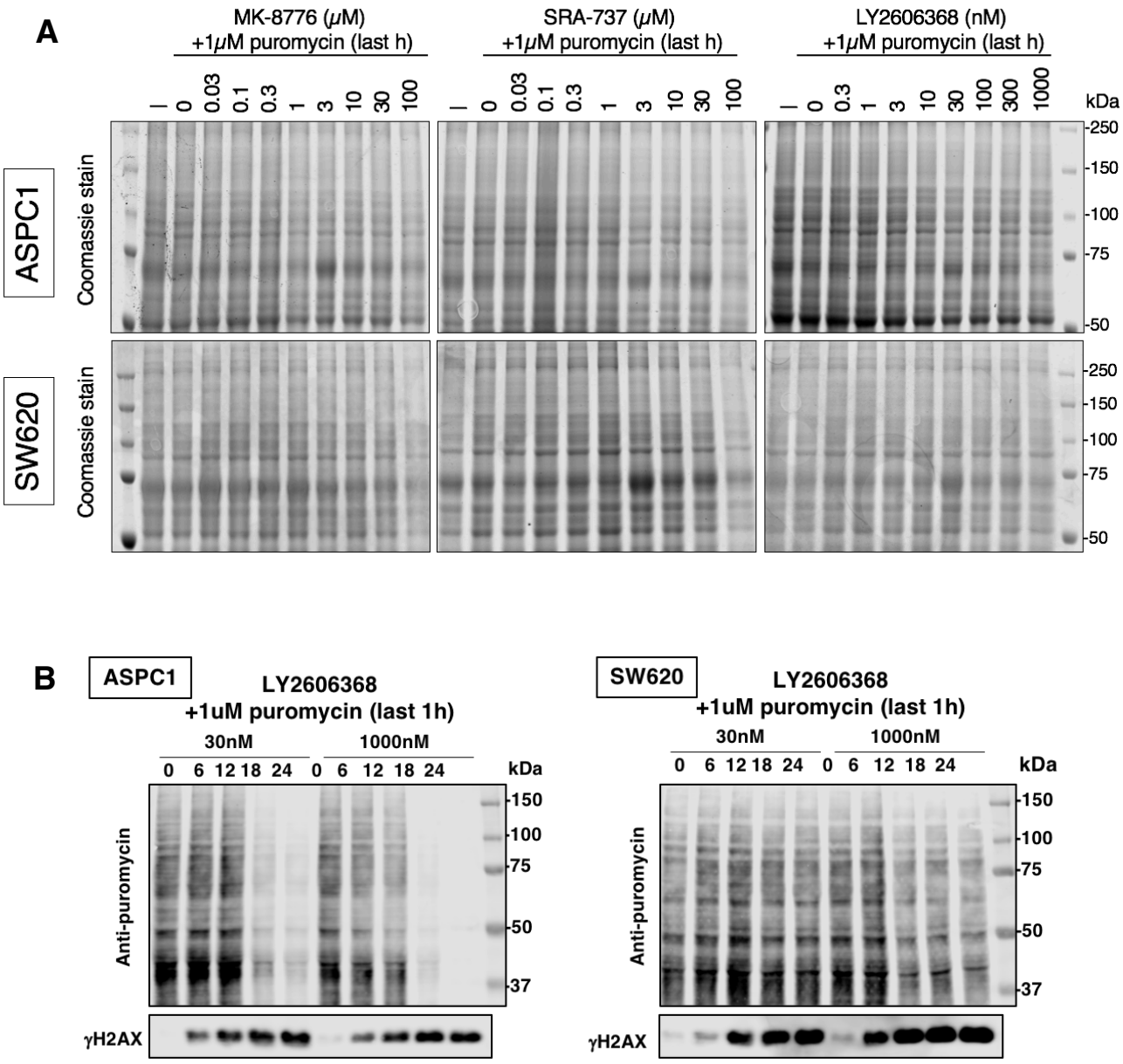

Figure S3: Inhibition of protein synthesis by CHK1i. **A.** Lysates from cells analyzed in Figure 7, were electrophoresed on a SDS-PAGE gel and stained with Coomassie blue to confirm that the inhibition of translation was not associated with an overall change in protein. **B.** AsPC-1 and SW620 cells were incubated with either 30 or 1000 nM LY2606368 for 0 – 24 h, and puromycin was added for the final hour. Lysates were analyzed as in Figure 8.