

Table S1. NQO1, NQO2, P450R, and b5R activity in all transfected MDA468 clones.

Clones		Enzyme Activity			
Description	Name	NQO1 ^a	P450R ^b	b5R ^c	NQO2 ^d
Parental	MDA468	ND ^e	3.0 ± 0.9	270.8 ± 32.2	2.3 ± 0.3
NQO1 Knock-in	NQ16	1061.4 ± 50.5	3.0 ± 0.2	271.8 ± 30.8	2.0 ± 0.4
	R3	ND	8.3 ± 0.4	324.0 ± 19.0	1.5 ± 0.4
P450R Knock-in clones	R5	ND	89.3 ± 2.7	338.6 ± 31.0	2.5 ± 0.4
	R7	ND	16.8 ± 4.3	310.6 ± 21.2	2.9 ± 1.7
	R10	ND	77.9 ± 1.2	345.0 ± 9.3	2.7 ± 0.4
	R12	ND	17.8 ± 2.9	328.5 ± 42.0	2.2 ± 0.6
b5R Knock-in clones	C	ND	5.1 ± 0.1	1216.4 ± 49.1	2.7 ± 0.1
	D	ND	4.2 ± 0.6	1637.1 ± 143.4	4.0 ± 0.6
	H	ND	5.0 ± 0.4	889.9 ± 39.3	2.4 ± 0.4
NQO2 Knock-in clones	NQ2C2	ND	3.3 ± 1.1	281.9 ± 63.3	77.0 ± 7.0
	NQ2C3	ND	3.3 ± 1.4	268.5 ± 28.6	28.8 ± 2.6
	NQ2C4	ND	3.2 ± 0.1	268.3 ± 48.1	73.3 ± 6.8
	NQ2C5	ND	2.3 ± 1.3	286.3 ± 78.2	38.2 ± 1.1

^aNQO1 activity was expressed as nmole DCPIP reduced/min/mg protein; ^bP450R activity was expressed as nmole cytochrome *c* reduced/min/mg protein; ^cb5R activity was expressed as nmole potassium ferricyanide reduced/min/mg protein; ^dNQO2 activity was expressed as nmole MTT formazan/min/mg protein; ^eND, non-detectable, < 5 nmole DCPIP/min/mg protein.

Table S2. Percent of DNA in comet tail for all the transfected clones following RH1 treatment.

MDA468 Clones		Modified Comet Assay (Cross-linking)					Alkaline Comet Assay (Single Strand Breaks)				
Description	Name	Control	H ₂ O ₂ ^a				Control	Menadione ^b	RH1 (nM)		
			0	50	100	500			50	100	500
Parental	MDA468	9.1	71.4	72.7	68.3	54.2	7.2	60.6	8.1	8.4	7.6
NQO1-KI ^c	NQ16	6.4	71.1	36.5	22.1	6.9			ND ^d		
P450R-KI	R12	6.6	72.8	71.0	65.7	55.7	6.1	71.2	6.5	6.2	8.3
	R10	6.8	73.9	68.0	59.0	35.2	7.6	75.0	7.2	7.8	6.4
	R5	5.4	68.8	54.4	61.4	26.7	6.9	82.7	6.8	7.3	7.4
b5R-KI	D	8.2	72.1	64.5	59.1	46.3	8.1	72.2	8.6	9.6	8.3
NQO2-KI	NQ2C3	10.0	70.4	62.5	54.7	36.6			ND ^d		
	NQ2C4	9.7	72.3	55.7	43.0	28.2			ND ^d		

^aSamples were treated with 200 μ M of H₂O₂ for 20 min on ice post-RH1 treatment; ^b2 μ M menadione was used as positive control for DNA single strand breaks; ^cKI, knock in; ^dND, not determined. Percent of DNA in comet tail was recorded by the Komet software; values listed in the table represent the mean value of three independent determinations.

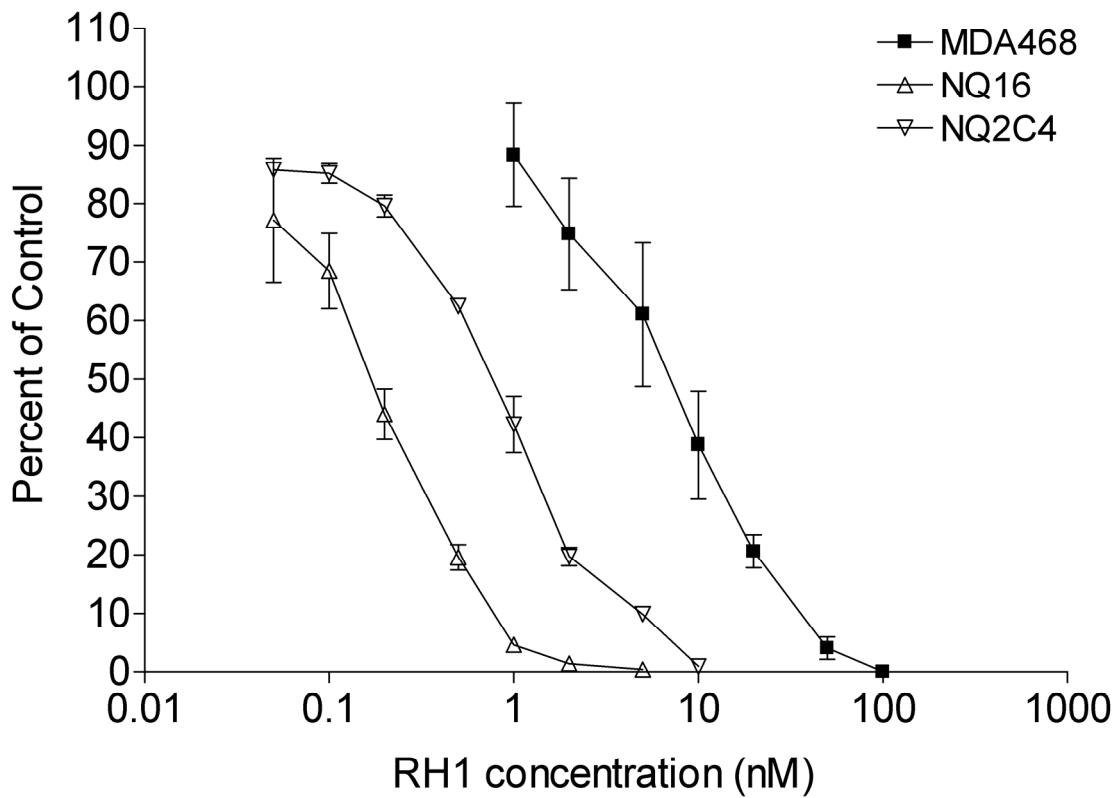


Figure S1. Effect of RH1 on colony formation in MDA468 and NQO1/NQO2 transfected clones. Colony forming ability of the parental MDA468, the NQO1 knock-in clone NQ16, and the NQO2 knock-in clone NQ2C4, was measured using the clonogenics assay following RH1 treatment. Data represents mean \pm standard deviation, n = 3.

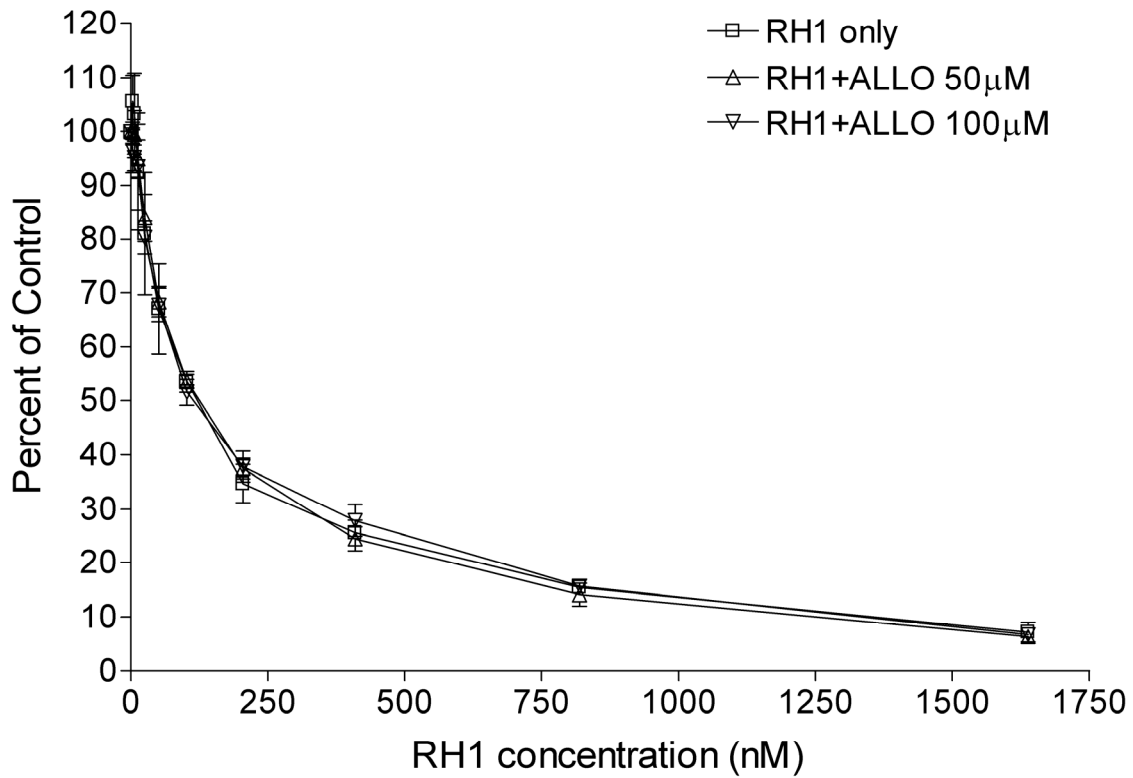


Figure S2. Effect of allopurinol pretreatment on RH1 cytotoxicity in MDA468 cells. Cells were seeded in 96-well plates and pretreated with 50 or 100 µM allopurinol for 30 min before 2 h of RH1 treatment. Growth inhibition was measured 3 d after incubation in complete medium using the MTT method. Data represents the mean \pm standard deviation of three independent determinations.