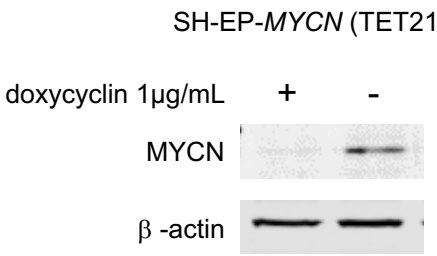
Figure S1

A



B

	YM155 IC_{50} (nM)
- doxycyclin (1µg/mL)	5.31 ± 3.21
+ doxycyclin (1µg/mL)	4.40 ± 1.69

Figure S1. Effects of MYCN expression on neuroblastoma cell sensitivity to YM155. A) MYCN levels in SH-EP-*MYCN* (TET21N) cells in the absence or presence of doxycycline; B) YM155 concentrations that reduce the viability of SH-EP-*MYCN* (TET21N) cells by 50% (IC_{50}) in the absence or presence of doxycycline as determined by MTT assay after a 5-day treatment period.

Figure S2

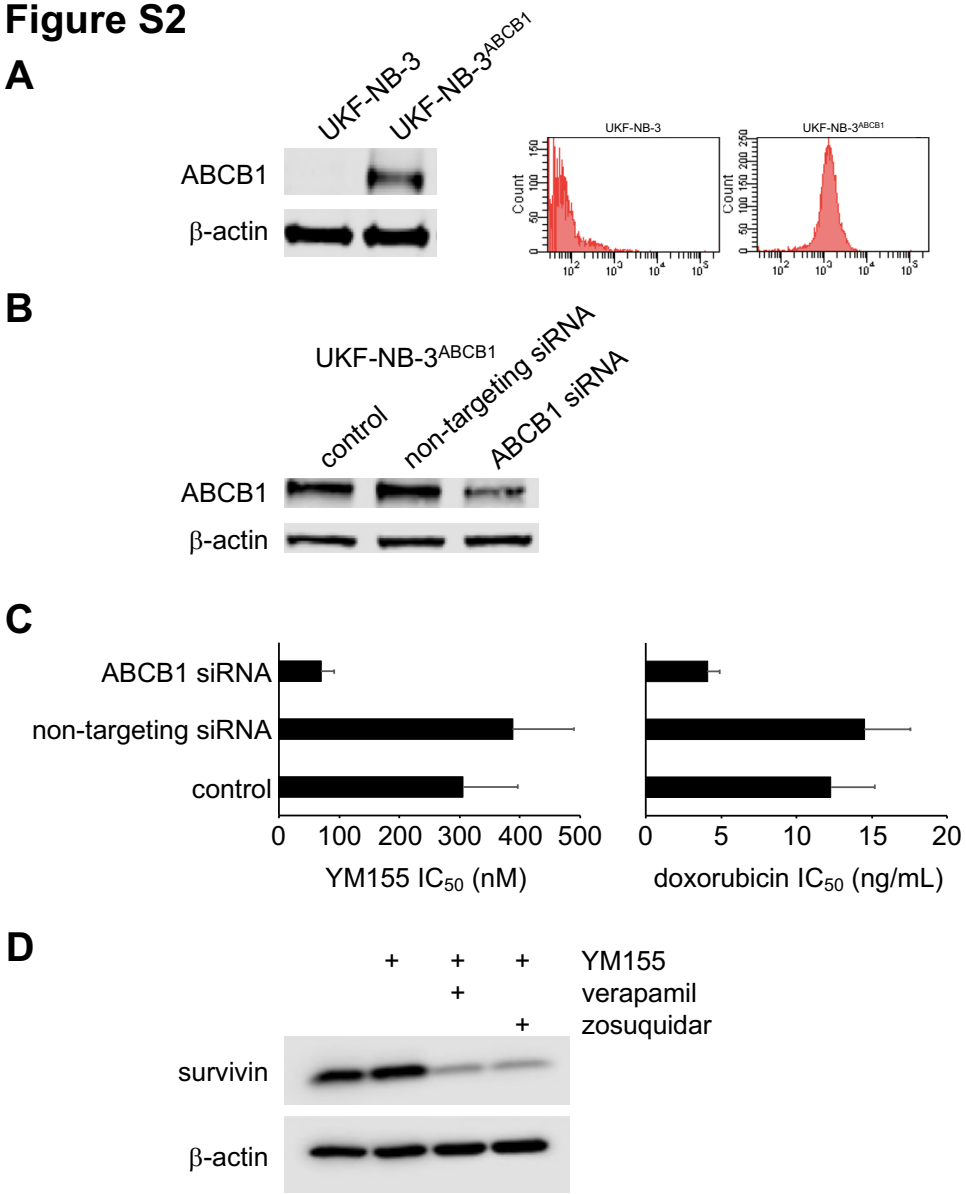


Figure S2. Effects of YM155 in ABCB1-transduced cells. A) Representative Western blots and flow cytometry histograms indicating ABCB1 levels in UKF-NB-3 cells and in UKF-NB-3 transduced with a lentiviral vector encoding ABCB1 (UKF-NB-3^{ABCB1}) . B) Effect of siRNA directed against ABCB1 on cellular ABCB1 levels in UKF-NB-3^{ABCB1} cells. C) Concentrations of YM155 and doxorubicin (alternative ABCB1 substrate used as control) that reduce the viability of UKF-NB-3^{ABCB1} cells by 50% (IC_{50}) as determined by MTT assay after 120h of incubation. D) Effects of YM155 (100nM) on survivin levels in UKF-NB-3^{ABCB1} cells after 24h of incubation in the presence or absence of verapamil (5μM) or zosuquidar (1.25μM).

Figure S3

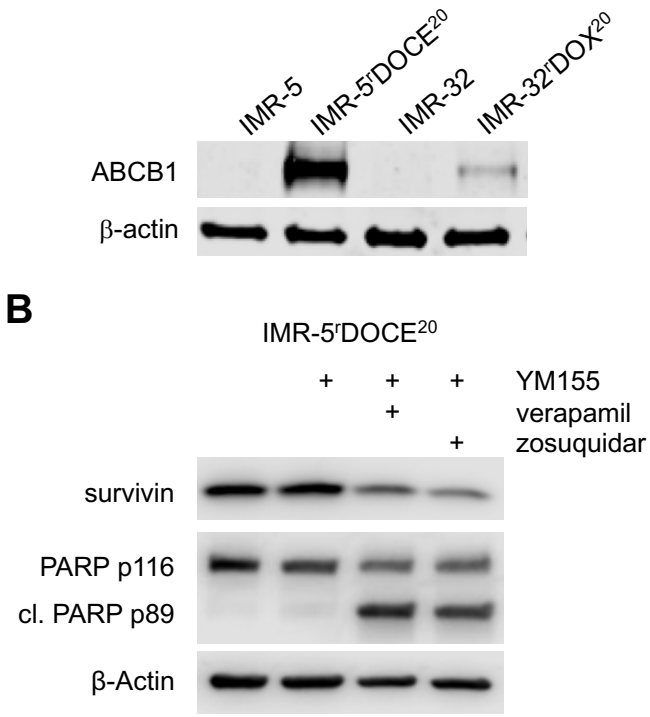


Figure S3. ABCB1 expression and YM155 activity in drug-adapted neuroblastoma cells. A) Representative Western blots indicating ABCB1 levels in IMR-5, IMR-5^{r}DOCE^{20}, IMR-32, and IMR-32^{r}DOX^{20}. B) Effects of YM155 (500nM) on survivin levels and PARP cleavage in IMR-5^{r}DOCE^{20} cells in the presence or absence of verapamil (5μM) or zosuquidar (1.25μM) after 24h of incubation.

Table S1. YM155 concentrations that reduce the viability of neuroblastoma cell lines by 50% (IC_{50}) as indicated by MTT assay after 120h of incubation.

Cell line	YM155 IC_{50} (nM)	Cell line	YM155 IC_{50} (nM)
Be(2)-C	24.25 ± 2.59	NLF^{r}GEMCI^{20}	1.84 ± 0.47 (0.1)
CHP-134	2.64 ± 0.50	NLF^{r}IRINO^{1000}	6.93 ± 0.71 (0.3)
GIMEN	33.74 ± 2.26	NLF^{r}MEL^{3000}	15.36 ± 3.20 (0.6)
IMR-5	7.18 ± 1.04	NLF^{r}OXALI^{4000}	33.67 ± 2.67 (1.3)
IMR-5^{r}CARBO$^{5000\ (1)}$	8.55 ± 2.01 (1.2)^{2}	NLF^{r}VCR^{10}	334.5 ± 21.6 (12.5)
IMR-5^{r}CDDP^{1000}	19.71 ± 5.70 (2.7)	NLF^{r}VINB^{10}	38.10 ± 12.02 (1.4)
IMR-5^{r}DOCE^{20}	21549 ± 638 (3001)	NMB	6.41 ± 1.17
IMR-5^{r}DOX^{20}	116.3 ± 21.6 (16.2)	SHEP	10.15 ± 0.84
IMR-5^{r}ETO^{100}	8.29 ± 3.95 (1.2)	SHEP^{r}CDDP^{1000}	30.83 ± 2.24 (13.7)
IMR-5^{r}GEMCI^{20}	7.08 ± 1.20 (1.0)	SHEP^{r}ETO^{100}	20.24 ± 10.16 (2.0)
IMR-5^{r}MEL^{1000}	11.10 ± 1.57 (1.5)	SHEP^{r}VCR^{10}	20.95 ± 1.45 (2.1)
IMR-5^{r}OXALI^{4000}	10.18 ± 2.69 (1.4)	SH-SY5Y	31.8 ± 6.50
IMR-5^{r}TOPO^{20}	4.88 ± 1.72 (0.7)	SK-N-AS	3.55 ± 0.21
IMR-5^{r}VCR^{10}	472.9 ± 97.4 (65.9)	SK-N-SH	74.94 ± 19.52
IMR-5^{r}VINB^{20}	1608 ± 212 (224)	UKF-NB-2	4.18 ± 0.27
IMR-5^{r}VINOR^{20}	4978 ± 147 (693)	UKF-NB-2^{r}CARBO^{2000}	318.2 ± 42.7 (76.1)
IMR-32	1.40 ± 0.35	UKF-NB-2^{r}CDDP^{1000}	1.15 ± 1.21 (0.3)
IMR-32^{r}CARBO^{1000}	9.35 ± 0.97	UKF-NB-2^{r}DOCE^{10}	1108 ± 179 (265)
IMR-32^{r}DOX^{20}	35.63 ± 2.23 (5.0)	UKF-NB-2^{r}DOX^{20}	347.0 ± 55.2 (83.0)
IMR-32^{r}ETO^{100}	1.53 ± 0.13 (0.2)	UKF-NB-2^{r}OXALI^{600}	3.25 ± 0.64 (0.8)
IMR-32^{r}GEMCI^{20}	2.16 ± 0.22 (0.3)	UKF-NB-2^{r}VCR^{10}	5940 ± 247 (1421)
IMR-32^{r}OXALI^{800}	0.60 ± 0.02 (0.1)	UKF-NB-3	0.49 ± 0.10
IMR-32^{r}TOPO$^{7.5}$	0.45 ± 0.06 (0.1)	UKF-NB-3^{r}CARBO^{2000}	155.4 ± 24.6 (317)
IMR-32^{r}VINOR^{5}	16.43 ± 1.08 (2.3)	UKF-NB-3^{r}CDDP^{1000}	5.32 ± 1.21 (10.9)
LAN-6	248.1 ± 32.9	UKF-NB-3^{r}DOCE^{10}	469.6 ± 113.1 (958)
NB-S-124	76.66 ± 6.51	UKF-NB-3^{r}DOX^{20}	15,700 ± 1,019 (32041)
NGP	12.48 ± 3.01	UKF-NB-3^{r}ETO^{200}	7.97 ± 0.13 (16.3)
NGP^{r}CARBO^{5000}	112.3 ± 5.0 (9.0)	UKF-NB-3^{r}GEMCI^{10}	0.40 ± 0.01 (0.8)
NGP^{r}CDDP^{1000}	13.00 ± 0.42 (1.0)	UKF-NB-3^{r}Nutlin$^{10\mu M}$	1.18 ± 0.07 (2.4)
NGP^{r}DACARB^{18}	20.59 ± 1.84 (1.6)	UKF-NB-3^{r}OXALI^{4000}	1.80 ± 0.78 (3.7)
NGP^{r}DOCE^{20}	159.0 ± 19.5 (12.7)	UKF-NB-3^{r}TOPO^{20}	7.40 ± 0.71 (15.1)
NGP^{r}DOX^{20}	306.9 ± 78.5 (24.6)	UKF-NB-3^{r}VCR^{10}	26.59 ± 6.37 (54.3)
NGP^{r}ETO^{400}	59.20 ± 11.40 (4.7)	UKF-NB-6	0.65 ± 0.09
NGP^{r}GEMCI^{20}	41.55 ± 6.13 (3.3)	UKF-NB-6^{r}CARBO^{2000}	16.83 ± 1.62 (25.9)
NGP^{r}MEL^{3000}	26.10 ± 3.86 (2.1)	UKF-NB-6^{r}CDDP^{2000}	79.93 ± 7.14 (123)
NGP^{r}OXALI^{4000}	6.93 ± 0.28 (0.6)	UKF-NB-6^{r}DOCE^{10}	14.33 ± 4.08 (22.0)
NGP^{r}VCR^{20}	6986 ± 715 (560)	UKF-NB-6^{r}DOX^{20}	11.80 ± 1.56 (18.2)
NLF	26.78 ± 4.04	UKF-NB-6^{r}ETO^{200}	3.60 ± 0.01 (5.5)
NLF^{r}CARBO^{5000}	340.5 ± 34.5 (12.7)	UKF-NB-6^{r}GEMCI^{10}	2.10 ± 0.84 (3.2)
NLF^{r}CDDP^{500}	12.58 ± 5.39 (0.5)	UKF-NB-6^{r}OXALI^{4000}	5.34 ± 0.71 (8.2)
NLF^{r}DOCE^{20}	21.6 ± 5.98 (0.8)	UKF-NB-6^{r}TOPO^{20}	3.47 ± 0.81 (5.3)
NLF^{r}DOX^{40}	34.88 ± 4.33 (1.3)	UKF-NB-6^{r}VCR^{10}	49.30 ± 2.24 (75.8)
NLF^{r}ETO^{100}	7.40 ± 0.54 (0.3)	UKF-NB-6^{r}VINOR^{40}	228.5 ± 41.5 (352)

[1] CARBO, carboplatin; CDDP, cisplatin; DACARB, dacarbazine; DOX, doxorubicin; ETO, etoposide; GEMCI, gemcitabine; IRINO, irinotecan; MEL, melphalan; Nutlin, nutlin-3; OXALI, oxaliplatin; TOPO, topotecan; VCR, vincristine; VINB, vinblastine; VINOR, vinorelbine

Table S2. YM155 concentrations that reduce the viability of MYCN-amplified and non-MYCN-amplified neuroblastoma cell lines by 50% (IC50) in the absence or presence of the ABCB1 inhibitors verapamil (5 µM) or zosuquidar (1.25 µM) as indicated by MTT assay after 120h of incubation.

Cell line	YM155 IC_{50} (nM)	+ verapamil (5 µM)[1] YM155 IC_{50} (nM)	+ zosuquidar (1.25 µM) YM155 IC_{50} (nM)
MYCN amplification			
CHP-134 (wt)[2]	2.64 ± 0.50	1.64 ± 0.27	1.85 ± 0.34
IMR-5 (wt)	7.18 ± 1.04	9.70 ± 1.97	10.64 ± 2.80
IMR-32 (wt)	1.40 ± 0.35	1.70 ± 0.41	1.80 ± 0.23
NB-S-124 (wt)	76.66 ± 6.51	12.52 ± 1.16	3.20 ± 0.40
NGP (wt)	12.48 ± 3.01	17.35 ± 4.97	24.95 ± 0.21
NLF (V203M)	26.78 ± 4.04	19.55 ± 1.20	45.30 ± 1.34
UKF-NB-2 (wt)	4.18 ± 0.27	4.55 ± 0.32	2.85 ± 0.14
UKF-NB-3 (wt)	0.49 ± 0.10	0.61 ± 0.13	0.74 ± 0.10
UKF-NB-6 (wt)	0.65 ± 0.09	0.58 ± 0.07	0.57 ± 0.07
no MYCN amplification			
GIMEN (wt)	33.74 ± 2.26	52.90 ± 8.62	50.87 ± 5.91
LAN-6 (wt)	248.1 ± 32.9	46.75 ± 2.33	24.35 ± 1.06
SHEP (wt)	10.15 ± 0.84	3.92 ± 0.11	3.20 ± 0.14
SK-N-AS (null)	3.55 ± 0.21	1.01 ± 0.26	1.31 ± 0.11
SK-N-SH (wt)	74.94 ± 19.52	6.80 ± 0.83	1.72 ± 0.15

[1] Effects of verapamil or zosuquidar alone on cell viability are presented in Table S5.
[2] *TP53* status: wt, wild-type; otherwise type of mutation is provided

Table S3. YM155 concentrations that reduce the viability of neuroblastoma cell lines with varying p53 status by 50% (IC_{50}) as indicated by MTT assay after 120h of incubation.

Cell line	p53 status	YM155 IC_{50} (nM)
UKF-NB-3	wild-type	0.49 ± 0.10
UKF-NB-3^{r}Nutlin$^{10\mu M}$	G245C (homo)[1]	1.18 ± 0.07 (2.4)[2]
UKF-NB-3clone1	wild-type	0.35 ± 0.07
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$I	stop codon in exon 4	0.40 ± 0.12 (1.1)
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$III	R248W (het)	0.60 ± 0.08 (1.7)
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$IV	V173L (het)	0.45 ± 0.06 (1.3)
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$VI	R196Q (het)	0.55 ± 0.17 (1.6)
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$VIII	Y236C (het)	0.50 ± 0.14 (1.4)
UKF-NB-3clone1^{r}Nutlin$^{10\mu M}$X	P151R (het)	0.73 ± 0.08 (2.1)
UKF-NB-3clone3	wild-type	0.45 ± 0.06
UKF-NB-3clone3^{r}Nutlin$^{10\mu M}$I	P152L (het)	1.50 ± 0.06 (3.3)
UKF-NB-3clone3^{r}Nutlin$^{10\mu M}$VIII	N239S (het)	0.50 ± 0.08 (1.1)
UKF-NB-3clone3^{r}Nutlin$^{10\mu M}$IX	R280S (het)	1.03 ± 0.03 (2.3)
UKF-NB-3clone3^{r}Nutlin$^{10\mu M}$X	I251F (het)	0.58 ± 0.09 (1.3)
UKF-NB-6	wild-type	0.65 ± 0.09
UKF-NB-6^{r}Nutlin$^{10\mu M}$	K132N (het); P223L (hom)	0.64 ± 0.04 (1.0)
UKF-NB-6^{r}Nutlin$^{10\mu M}$I	S241F (hom)	0.57 ± 0.01 (0.9)
UKF-NB-6^{r}Nutlin$^{10\mu M}$IV	C135F (het); D281Y (het)	0.43 ± 0.04 (0.7)

[1] homo = homozygous, het = heterozygous

[2] fold change YM155 IC_{50} nutlin-3-resistant sub-line/ YM155 IC_{50} respective parental cell line

Table S4. Mean YM155 concentrations that reduce the viability of neuroblastoma cell lines with resistance to certain drug classes by 50% (IC_{50}) as indicated by MTT assay after 120h of incubation. Values are presented as mean ± S.D. Individual values are presented in Table 1.

Drug class	**YM155 IC_{50} (nM)**
topoisomerase I inhibitors	4.63 ± 2.52
parental	7.91 ± 8.27
nucleoside analogue (gemcitabine)	9.18 ± 14.62
alkylating agents	18.29 ± 5.63
platinum drugs	58.43 ± 98.80
topoisomerase II inhibitors	73.67 ± 112.42
including UKF-NB-3^{r}DOX^{20}	1190 ± 4026
taxane (docetaxel)	354 ± 411
including IMR-5^{r}DOCE^{20}	3889 ± 7908
vinca alkaloids	1725 ± 2519

Table S5. YM155 concentrations that reduce the viability of neuroblastoma cell lines by 50% (IC50) in the absence or presence of the ABCB1 inhibitors verapamil (5 μM) or zosuquidar (1.25 μM) as indicated by MTT assay after 120h of incubation.

		+ verapamil (5 μM)		+ zosuquidar (1.25 μM)	
Cell line	**YM155 IC_{50} (nM)**	**verapamil alone**	**YM155 IC_{50} (nM)**	**zosuquidar alone**	**YM155 IC_{50} (nM)**
CHP-134	2.64 ± 0.50	94 ± 13[1]	1.64 ± 0.27 (1.6)[2]	105 ± 6[1]	1.85 ± 0.34 (2.4)
GIMEN	33.74 ± 2.26	105 ± 3	52.90 ± 8.62 (0.6)	92 ± 7	50.87 ± 5.91 (0.7)
IMR-5	7.18 ± 1.04	109 ± 8	9.70 ± 1.97 (0.7)	104 ± 11	10.64 ± 2.80 (0.7)
IMR-5^{r}CARBO^{5000}	8.55 ± 2.01	91 ± 16	7.80 ± 0.28 (1.1)	105 ± 8	27.01 ± 3.04 (0.3)
IMR-5^{r}CDDP^{1000}	19.71 ± 5.70	88 ± 11	15.23 ± 4.21 (1.3)	100 ± 6	33.47 ± 6.84 (0.6)
IMR-5^{r}DOCE^{20}	21549 ± 638	90 ± 7	149.01 ± 1.99 (145)	112 ± 2	13.63 ± 5.54 (1581)
IMR-5^{r}DOX^{20}	116.3 ± 21.6	97 ± 9	17.60 ± 0.57 (6.6)	99 ± 8	13.45 ± 2.45 (8.6)
IMR-5^{r}ETO^{100}	8.29 ± 3.95	95 ± 10	6.99 ± 2.79 (1.2)	97 ± 4	18.26 ± 3.19 (0.5)
IMR-5^{r}GEMCI^{20}	7.08 ± 1.20	108 ± 6	7.90 ± 2.09 (0.9)	105 ± 8	12.73 ± 3.34 (0.6)
IMR-5^{r}MEL^{1000}	11.10 ± 1.57	92 ± 8	6.63 ± 1.30 (1.7)	107 ± 6	12.80 ± 1.22 (0.9)
IMR-5^{r}OXALI^{4000}	10.18 ± 2.69	96 ± 8	15.80 ± 1.77 (0.6)	110 ± 6	16.81 ± 2.71 (0.6)
IMR-5^{r}TOPO^{20}	4.88 ± 1.72	100 ± 13	5.94 ± 1.31 (0.8)	101 ± 7	11.77 ± 3.95 (0.4)
IMR-5^{r}VCR^{10}	472.9 ± 97.4	93 ± 8	13.05 ± 2.90 (36)	94 ± 5	19.35 ± 0.07 (24)
IMR-5^{r}VINB^{20}	1608 ± 212	93 ± 7	9.34 ± 0.94 (172)	93 ± 6	10.05 ± 1.06 (160)
IMR-32	1.40 ± 0.35	102 ± 7	1.70 ± 0.41 (0.8)	101 ± 3	1.80 ± 0.23 (0.8)
IMR-32^{r}DOX^{20}	35.63 ± 2.23	92 ± 15	1.75 ± 0.77 (20)	89 ± 13	0.94 ± 0.08 (38)
IMR-32^{r}ETO^{100}	1.53 ± 0.13	90 ± 5	1.60 ± 0.27 (1.0)	104 ± 6	3.55 ± 0.21 (2.2)
IMR-32^{r}GEMCI^{20}	2.16 ± 0.22	105 ± 8	1.15 ± 0.05 (1.9)	107 ± 11	4.20 ± 0.45 (0.5)
IMR-32^{r}OXALI^{800}	0.60 ± 0.02	99 ± 8	0.71 ± 0.08 (0.8)	105 ± 12	1.18 ± 0.07 (1.7)
IMR-32^{r}TOPO$^{7.5}$	0.45 ± 0.06	97 ± 10	0.61 ± 0.07 (0.7)	101 ± 2	0.97 ± 0.04 (0.5)
LAN-6	248.1 ± 32.9	99 ± 8	46.75 ± 2.33 (5.3)	103 ± 5	24.35 ± 1.06 (10.2)
NB-S-124	76.66 ± 6.51	103 ± 6	12.52 ± 1.16 (6.1)	110 ± 8	3.20 ± 0.40 (24.0)
NGP	12.48 ± 3.01	91 ± 8	17.35 ± 4.97 (0.7)	109 ± 2	24.95 ± 0.21 (0.5)
NGP^{r}CARBO^{5000}	112.3 ± 5.0	112 ± 9	76.10 ± 3.17 (1.5)	107 ± 5	158.24 ± 9.34 (0.7)
NGP^{r}CDDP^{1000}	13.00 ± 0.42	104 ± 5	19.61 ± 1.35 (0.7)	101 ± 18	17.80 ± 0.97 (0.7)
NGP^{r}DACARB^{18}	20.59 ± 1.84	107 ± 2	26.26 ± 4.77 (0.8)	103 ± 7	41.90 ± 5.27 (0.5)
NGP^{r}DOX^{20}	306.9 ± 78.5	92 ± 6	5.52 ± 0.35 (56)	90 ± 2	0.70 ± 0.04 (438)
NGP^{r}ETO^{400}	59.20 ± 11.40	98 ± 16	50.14 ± 16.45 (1.2)	98 ± 3	39.12 ± 7.87 (1.5)
NGP^{r}GEMCI^{20}	41.55 ± 6.13	94 ± 13	73.43 ± 16.41 (0.6)	105 ± 12	10.50 ± 1.34 (4.0)
NGP^{r}MEL^{3000}	26.10 ± 3.86	99 ± 10	24.34 ± 1.76 (1.1)	108 ± 9	18.75 ± 4.64 (1.4)
NGP^{r}OXALI^{4000}	6.93 ± 0.28	102 ± 8	12.25 ± 2.78 (0.6)	101 ± 12	8.21 ± 1.04 (0.8)
NGP^{r}VCR^{20}	6986 ± 715	100 ± 10	157.60 ± 11.79 (44)	106 ± 15	16.20 ± 1.74 (431)
NLF	4.18 ± 0.27	93 ± 8	4.55 ± 0.32 (0.9)	99 ± 5	2.85 ± 0.14 (1.5)

Table S6. YM155 concentrations that reduce the viability of neuroblastoma cell lines by 50% (IC_{50}) in the absence or presence of zosuquidar (1.25μM) as indicated by MTT assay or CellTiterGlo after 120h of incubation.

Cell line	YM155 IC_{50} (nM)	+ Zosuquidar[1]
IMR-5 (MTT)	7.18 ± 1.04	10.64 ± 2.80
IMR-5 (CellTiterGlo)	6.27 ± 1.56	19.99 ± 5.97
IMR-5^{r}DOCE^{20} (MTT)	21,549 ± 638	13.63 ± 5.54
IMR-5^{r}DOCE^{20} (CellTiterGlo)	32,946 ± 4360	14.82 ± 4.66
IMR-32 (MTT)	1.40 ± 0.35	1.80 ± 0.23
IMR-32 (CellTiterGlo)	1.22 ± 0.21	1.91 ± 1.16
IMR-32^{r}DOX^{20} (MTT)	35.53 ± 2.23	0.94 ± 0.08
IMR-32^{r}DOX^{20} (CellTiterGlo)	29.20 ± 3.56	0.86 ± 0.06

[1] The effects of zosuquidar alone are provided in Table S5.

Table S7. YM155 concentrations that reduce the viability of neuroblastoma cell lines by 50% (IC_{50}) in the absence or presence of the ABCC1 inhibitor MK571 (10μM) as indicated by MTT assay after 120h of incubation.

		+ MK571	
Cell line	**YM155 IC_{50} (nM)**	**MK571 alone[1]**	**YM155 IC_{50} (nM)**
NLF	26.3 ± 5.9	98 ± 15	25.6 ± 9.1
NLF^{r}VCR^{10}	324 ± 79	102 ± 17	141 ± 38

[1] Effect of MK571 (10μM) on cell viability in percentage relative to untreated control.