Figure S1. Development of YK resistant cell lines. (A) A4573-R tolerated a maximum YK concentration of 3.475 μM with the most recent IC_{50} value of 14.90 μM. (B) TC71-R tolerated a maximum YK concentration of 0.876 μM with the most recent IC_{50} value of 1.857 μM. Drops in YK concentration in media represent the time points at which resistant cells were frozen and thawed. YK, YK-4-279; R, resistant.

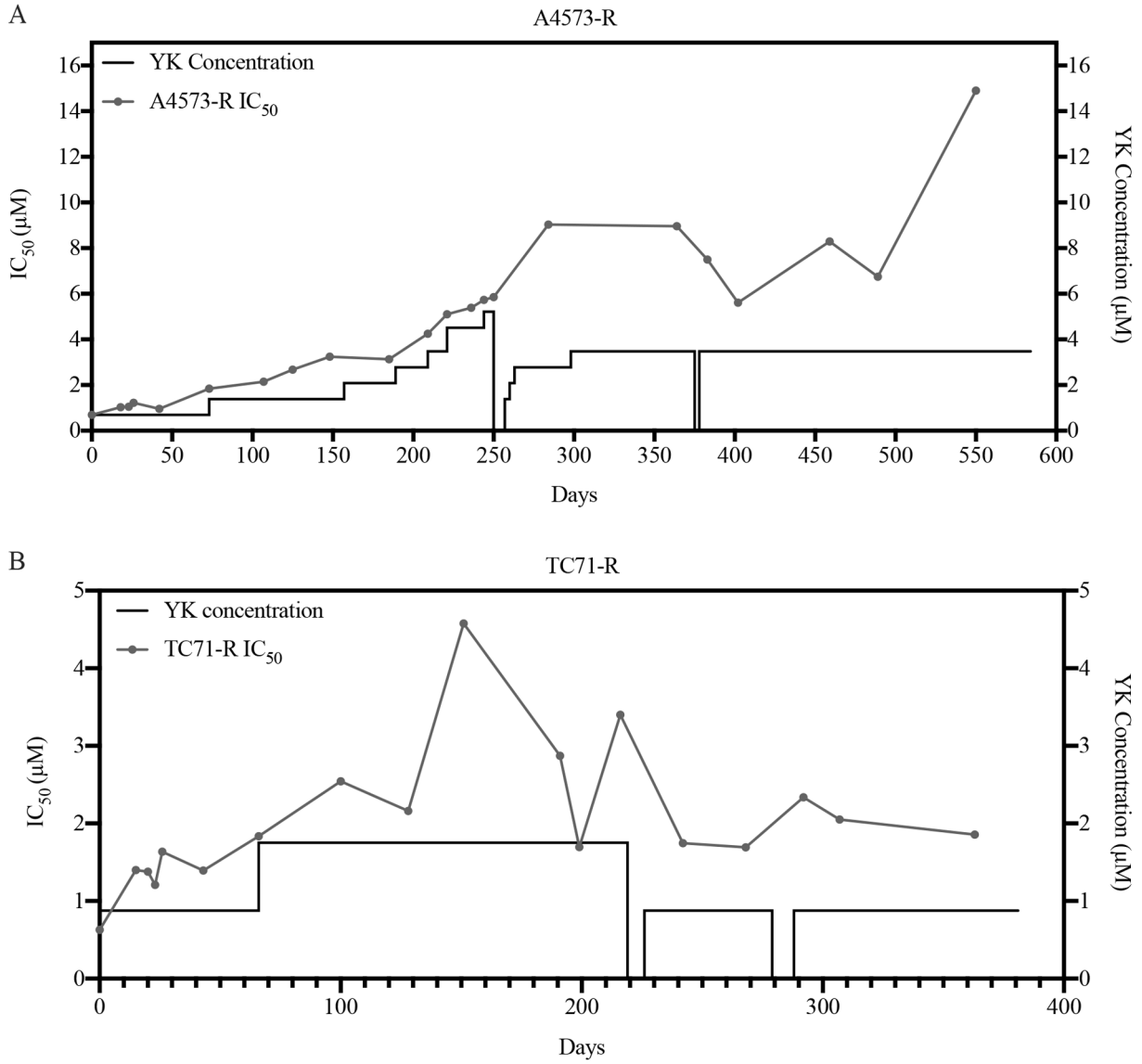

Figure S2. Viability curves comparing sensitive and resistant TC71 cell lines with (A) YK, (B) doxorubicin, (C) etoposide and (D) vincristine. IC_{50} values are presented. Resistant cells demonstrated significantly reduced sensitivity to YK (P<0.0001 vs. sensitive). YK resistant cells were significantly also resistant doxorubicin (P<0.0001 vs. sensitive), but did not demonstrate cross-resistance to etoposide or vincristine. YK, YK-4-279.

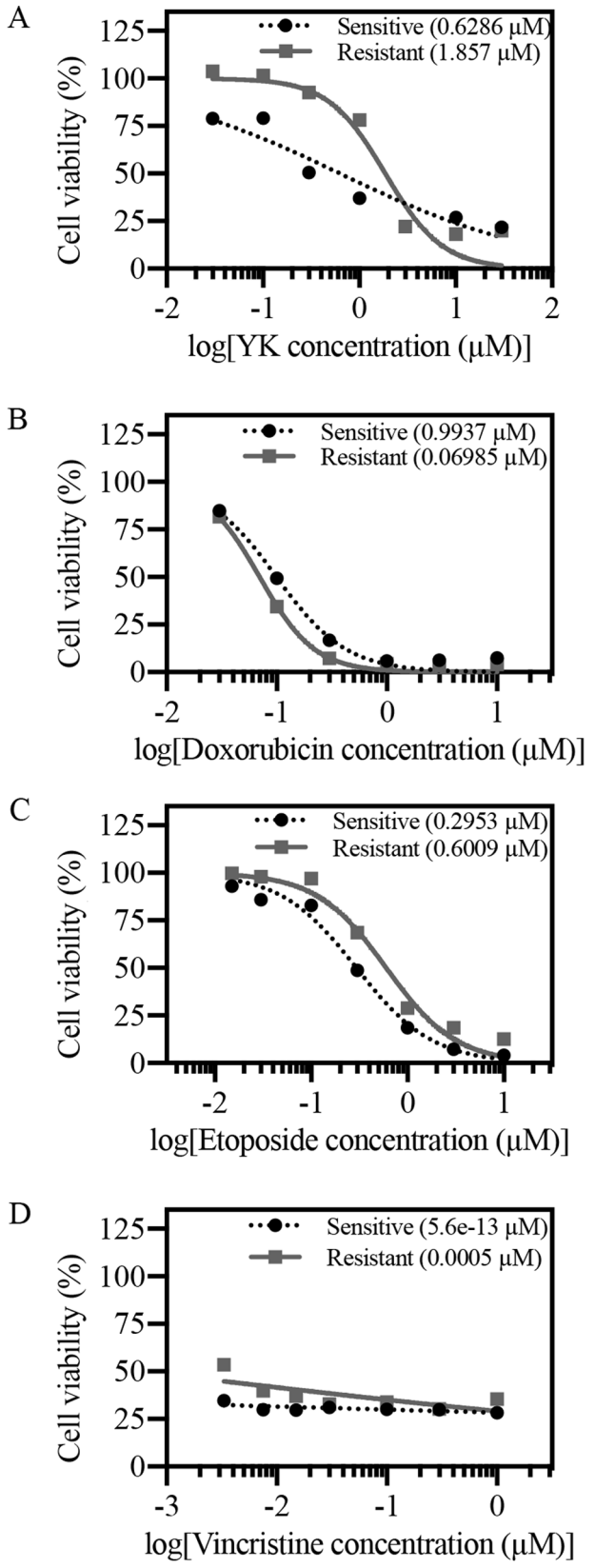

Figure S3. CD99 expression is increased in TC71-R cells. (A) Quantitative PCR analysis for EF, CD99 and 18S ribosomal RNA in TC71 sensitive cells and resistant cells at multiple time points. CD99 RNA expression is significantly elevated when compared with sensitive cells at all time points. ***P<0.001 and ****P<0.0001 vs. sensitive. (B) Western blot analysis of CD99 and EF in TC71 sensitive and resistant cells. Expression levels were calculated using densitometry analysis relative to sensitive cell lines. EF, EWS-FLI1.

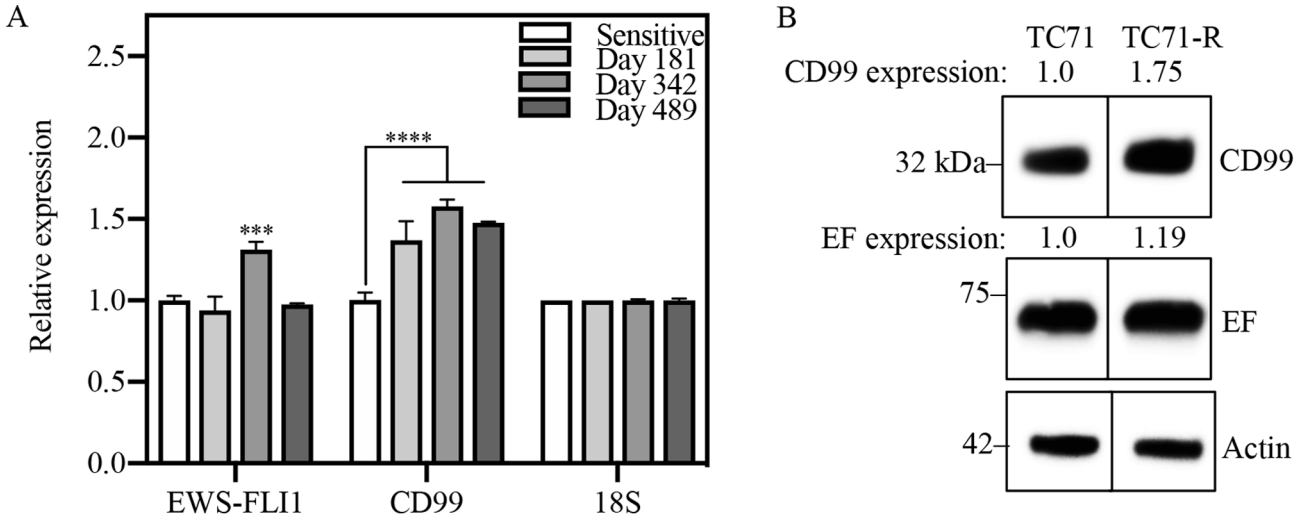

Figure S4. Quantitative PCR for upregulated genes identified through RNA sequencing in TC71 cells. Relative expression was evaluated at multiple time points in resistant cells compared with sensitive cell expression. (A) Upregulated genes included ANO1, BRSK2 and IGSF21. (B) Downregulated genes included COL24A1, PRSS23 and RAB38. *P<0.05 and **P<0.01. ANO1, anoctamin 1; BRSK2, BR serine/threonine kinase 2; IGSF21, immunoglobulin superfamily member 21; COL24A1, collagen type XXIV alpha 1 chain; PRSS23, serine protease 23; RAB38, Ras-related protein Rab-38.

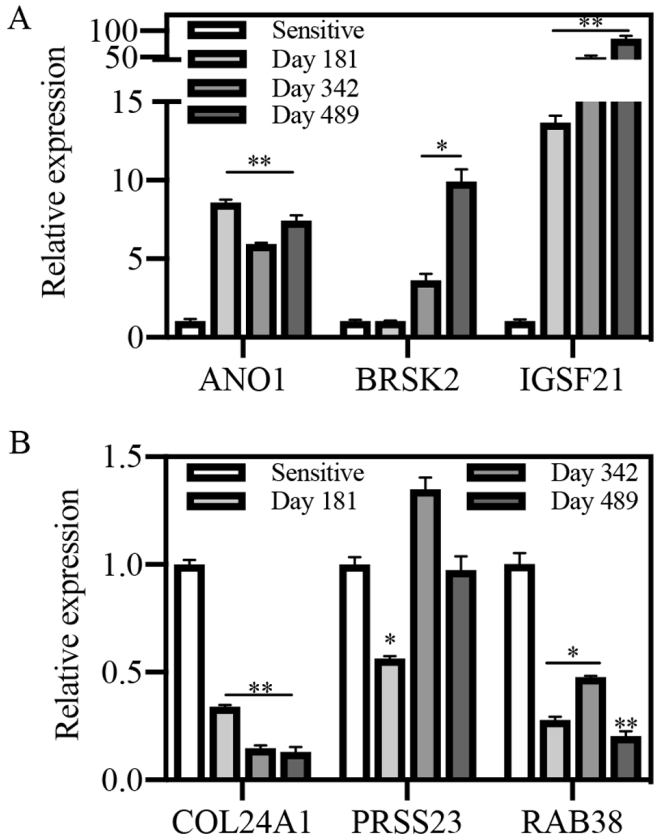

Table SI. List of primers used in PCR.

Gene symbol	Forward (5'-3')	Reverse (5'-3')
CD99	AACCCACCCAAACCGATGC	TGAAAAGCTACCGGAGGAACTA
EWS-FLI1	CAGCCTCCCACTAGTTACCC	GTTGAGGCCAGAATTCATG
ANO1	CTGATGCCGAGTGCAAGTATG	AGGGCCTCTTGTGATGGTACA
IGSF21	CCAACTACTCACACATGGAGAAC	CTCATAGGGACCATTGTCTGAGA
BRSK2	CGACGATGACAACTTGCGAC	ATGCCCCGTAGCAGACTCT
PRSS23	TGTGCTGTTGGGCAAGTGAG	AGTTCCCTTATGACACTGGGG
COL24A1	TGCTGTGGATCTCACAAACCA	TTTGCCTCACTGATGCGATGA
RAB38	TGACGAGGGTCTATTACCGAG	CAAACCATCCTACGAAACCGT
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Table SII. Differentially expressed genes.

Gene	$\log_2$ (Fold change)	
	A4573	TC71
AC116565.2	3.1036335	4.89793251
AC138904.1	1.62435102	1.02823268
AC144652.1	1.36666103	1.23987823
AC145098.2	-1.0226612	-1.0504629
ADGRL2	-1.5582041	-1.42754
ADM	-3.7193692	-4.1181448
AK7	1.63391455	1.19462477
AL645608.8	1.15636329	1.02415434
ANGPT1	-2.9624119	-3.2912802
ANO1	**7.68735211**	**2.20590622**
AP000648.3	-1.170692	-1.4163983
APOBEC3C	-2.1257189	-1.923566
ARHGAP23	-1.3526887	-1.6210497
ASAH2B	-1.3235138	-1.3373677
ATP1B2	1.32958409	1.28476596
ATP2B3	1.19534686	2.04972062
AVPR1A	3.00759594	2.09281006
BHLHE40	-1.4439505	-1.421994
BRSK2	**3.19879758**	**2.15356482**
CACNA2D2	1.26290219	1.00398238
CAMK2N1	1.70891158	2.12756208
CAV2	-1.1825717	-1.0459855
CCDC102B	-1.0120044	-1.6970542
CCNG2	1.5977705	1.52231313
CD24	1.38396639	1.80781702
CLCF1	1.22612694	1.37004018
CNTFR	1.02349661	2.82475675
COL16A1	-1.7284549	-3.2974658
COL24A1	**-2.4677996**	**-2.272875**
CORO2A	-1.013942	-1.0989503
CRLF1	2.36502281	1.23712437
CTSC	-1.0010546	-1.4257356
DNAJC22	-2.1999681	-1.1835929
DNM3OS	-1.1127452	-1.2627119
DOK3	-1.2680641	-1.0544036
DPF3	-1.1390319	-1.5217879
E2F2	1.04917706	1.34417793
EEF1A2	1.96410222	1.9980859
EMILIN1	-1.4064189	-1.0911143
EOGT	-1.0785892	-1.2623885
ETS2	1.80503006	2.42459332
FAM131B	1.90848712	1.91756709
FBLN5	-1.9724967	-2.0241841
FBXO32	1.02476866	2.27963074
FOXA1	-1.1901958	-1.1649783
FOXC1	-2.4333814	-2.7993554
FREM2	-1.3772687	-1.9362209
GABRA2	-1.2018386	-2.5355678
GABRD	4.40325141	3.06544439
GPR137C	1.2490235	1.15420452
HCN2	1.42122552	1.34802204
HGNC:18790	2.76421653	3.37158904
HS6ST2	-1.7596001	-1.3625964
IFI16	1.89455528	2.66496982
IFIH1	1.82571633	2.11706034
IGSF21	**3.28302631**	**6.17167095**
IMPA2	1.06135914	1.22565753

Table SII. Continued.

Gene	$\log_2$ (Fold change)	
	A4573	TC71
KANK1	-1.2136154	-1.0364599
KCNE3	-1.6768461	-1.5840939
KCNG1	1.74300987	-1.3461115
KITLG	-1.4708565	-2.54629
KLHL14	-1.6793754	-2.5683667
KLHL35	1.1734682	1.14743572
LGALS8	-1.0883504	-1.9555023
LRRK1	-1.2018838	-1.7248554
LY96	1.84992727	1.86489839
LZTS1	1.72409707	1.21112362
MANSC1	-1.1090466	-2.24799
MAST1	1.27745185	1.13180925
MATN3	-1.713971	-1.3296733
MBD3L2	2.42749467	2.15770714
MBD3L3	6.30357261	1.44746376
MECOM	-1.9019499	-1.3483682
MID2	-1.3484544	-1.2915732
MOXD1	2.49641966	1.02997811
MSX1	1.08108528	1.78963468
MTHFS	-1.1194347	-1.2619389
MXD3	1.17944108	2.24519399
NEFL	-1.0185224	-1.6464463
NEURL1B	1.8549476	1.40782945
NGEF	3.12939716	1.99632604
NPAS1	1.20733315	1.02775584
OLFML3	-1.2685989	-1.4081912
OPTN	2.01361497	1.00344065
P2RY11	-1.0388228	-1.2813828
PCSK9	3.14196273	1.61992896
PGBD5	1.80142912	3.77609251
PGF	-2.1245325	-2.3958275
PHKA1	-1.0352164	-2.3503253
PI15	-2.8195604	-1.1202116
PPIC	-1.6083729	-3.394824
PPP1R16B	1.43669275	2.31498223
PRDM11	-1.0413972	-1.318713
PRKCZ	1.68301301	1.30397869
PRKD1	-1.8910929	-1.2155768
PRSS23	**-2.379889**	**-2.7531212**
PRSS35	-1.2897459	-2.2225325
RAB38	**-2.1333105**	**-6.2150882**
RAI14	-1.4690794	-1.130213
RDM1	1.12370277	1.56872004
REEP1	1.24917735	1.83143982
RELL1	1.05117568	1.14156291
RFX2	1.32580207	1.06981196
RGS5	-1.920895	-1.5820687
RTKN2	-1.0141592	-1.282864
RTN1	-1.0951412	-1.610215
SCN9A	1.15501877	1.54890379
SEMA3D	-1.8647638	-4.0137111
SEMA5B	1.05418495	2.49235963
SERPINF1	-1.0205335	-1.7633755
SHISA6	-1.7293635	-2.0117086
SHISA7	3.155656	1.77158215
SKAP2	1.48939581	1.02352229
SLC16A7	-1.2746379	-1.536979

Table SII. Continued.

Gene	log2 (Fold change)	
	A4573	TC71
SLC25A25-AS1	-1.4094252	-1.9561794
SLC30A3	1.70229876	1.38900682
SOWAHA	1.25328389	1.65614123
SPOCK1	-1.4035824	-1.4993066
SPX	-1.1513991	-4.8255943
ST6GALNAC3	-1.0643253	-1.0213197
STAMBPL1	-1.8370029	-2.3524324
STUM	1.21171591	1.47679938
SUSD5	-1.5410248	-1.8928099
SYNM	-1.2223885	-2.0369778
SYTL5	-1.5601473	-4.4123734
TEN1-CDK3	-1.1790016	-1.8390402
TFAP2A	1.1002679	1.10379992
TJP1	-1.0595533	-3.1099237
TLE2	2.18626529	1.09250312
TMEM140	-1.0063495	-1.0973178
TMEM151A	2.40102519	1.20934637
TMEM255A	1.12612793	3.05255688
TMSB15A	1.03820175	2.24462428
TNFAIP3	1.83498566	1.13853581
TNFAIP8L1	1.40604597	1.43489181
TPM1	-1.4307727	-1.2673826
TRAF3IP2	-1.3249538	-3.3268324
TRIM36	1.06708973	1.62697917
TRIM9	1.12469108	-1.7906791
UNC5A	2.31466769	2.15033145
UNC5B	-1.7854599	-1.462174
VGLL3	-4.1457506	-1.9667002
WHAMM	-1.0604845	-1.206954
WTIP	-1.7345728	-1.6468515
YJEFN3	-1.2549304	-1.3650542
YPEL1	2.55681204	3.08047572
ZMAT4	1.56137638	1.23900949
ZNF365	-1.2751959	-2.5708664
ZNF385D	-1.8839994	-4.309575
ZNF804A	-3.5328956	-1.0019524