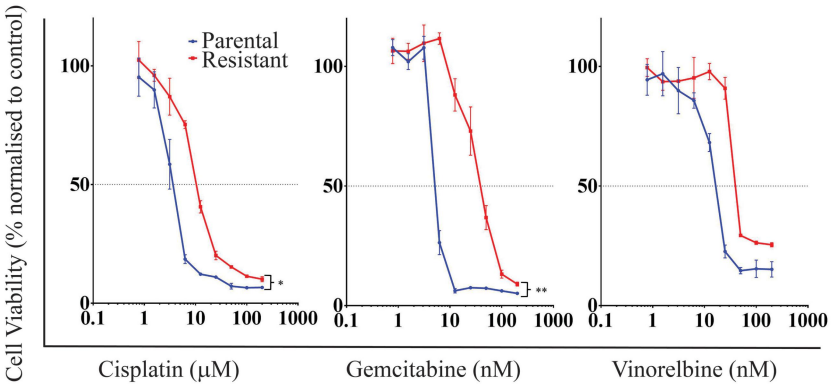
Cancer Drug Resistance

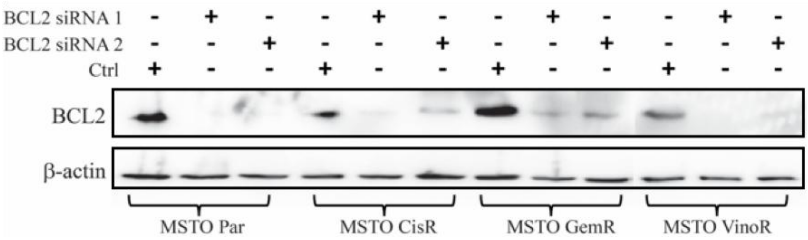
Supplementary Table 1. Assays, primers and miRNA mimic/siRNA sequences

TaqMan assays		
MicroRNA	TaqMan Assay ID	
miR-15a-5p	000389	
miR-16-5p	000391	
miR-34a-5p	000426	
miR-34b-3p	000427	
RNU6B	001973	
Primers for mRNA expression		
Target	Forward primer (5'-3')	Reverse primer (5'-3')
BCL2	ACAGAGGATCATGCTGTAC	TTATTTCATGAGGCACGTT
18S	GCCGCTAGAGGTGAAATTC	
Primers for reporter assay		
Insert region	Forward primer (5'-3')	Reverse primer (5'-3')
BCL2 3'UTR	ATCGCTCGAGGTATGAAGC	ATCGGCGGCCGCCAACAA
MicroRNA mimics		
MicroRNA	Antisense strand (5'-3')	Sense strand (5'-3')
miR-15a-5p	UAGCAGCACAUAAUGGUU	CAAACCAUUAUGUGCUGC
miR-16-5p	UAGCAGCACGUAAAUAUU	CCAAUAUUUACGUGCUGC
miR-34a-5p	UGGCAGUGUCULIAGCUG	AACCAGCUAAGACACUGC
siRNAs		
Target	Antisense strand (5'-3')	Sense strand (5'-3')
Control	AAGCAACUUGGUAAGACU	CCACACGAGUCUUACCAA
BCL2 siRNA-1	GAGAUAGUGAUGAAGUA	AAUGGAUGUACUUCAUCA
BCL2 siRNA-2	GCCCCAGCAUGCGGCCUC	AAUCAAACAGAGGCCGCA

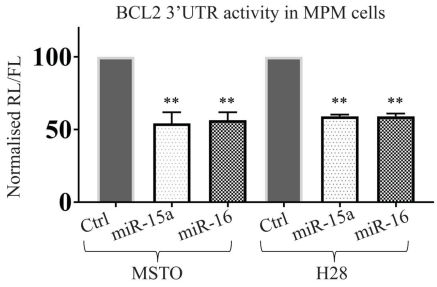
MiRNA TaqMan IDs are listed together with primers for mRNA expression, reporter assay cloning and miRNA mimic/siRNA sequences



Supplementary Figure 1. Basal drug sensitivities in MSTO parental and drug resistant cell lines. MSTO parental and drug resistant cells (MSTO-CisR, MSTO-GemR, MSTO-VinoR) were treated with 2-fold serial dilutions with the indicated start concentrations of cisplatin, gemcitabine and vinorelbine respectively. Cells were assayed for proliferation 96 h following drug treatment. Drug treated cells were normalised to the values of untreated cells (untreated = 100%). Data are mean ± SD of duplicate measurements and are representative of > 3 experiments producing similar results. *$P \leq 0.05$



Supplementary Figure 2. Confirmation of BCL2 reduction with BCL2 siRNA transfection. MSTO-Par, MSTO-CisR, MSTO-GemR and MSTO-VinoR were transfected with 1nM of BCL2 siRNAs and after 48 h BCL2 protein expression was measured using western blotting. β-actin was included as a control for equal loading



Supplementary Figure 3. Direct regulation of the BCL2 mRNA by miR-15a/16-1. miR-15a and miR-16 reduce luciferase activity of a reporter containing the 3'UTR region of BCL2 mRNA in MSTO-Par and an additional MPM cell line H28. Data is the average of 3 replicate experiments ± SD. *$P \leq 0.05$, **$P \leq 0.01$