Supplementary data

Methods

BH3 profiling. The BH3 peptides – PUMA, BIM, BAD, MS1, and HRK were purchased from New England Peptide, Inc. and stock solutions were prepared using DMSO (#D8418; Sigma-Aldrich). The peptides were diluted in staining solution that was prepared using oligomycin (#O4876; Sigma), digitonin (#D5628; Sigma), JC-1 (#89166-014; VWR), 2-mercaptoethanol (#M3148; Sigma-Aldrich), and MEB buffer.

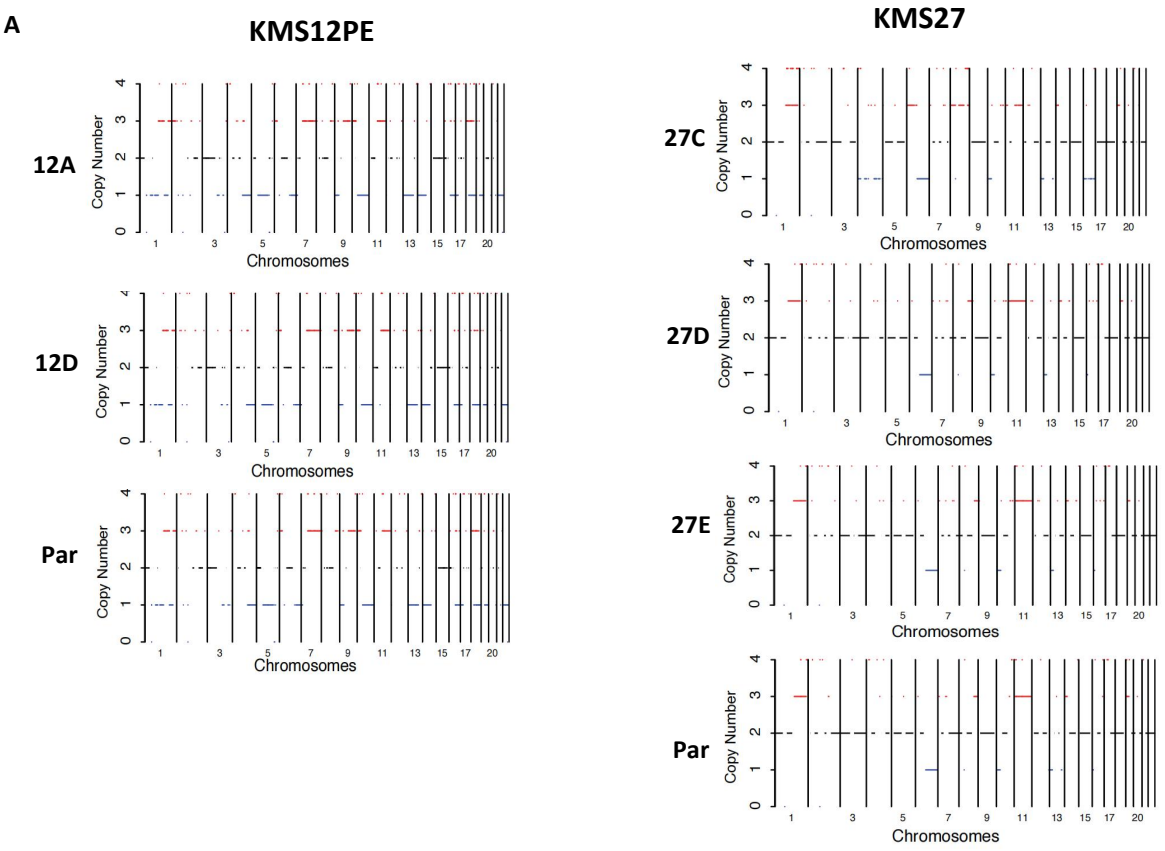
Cell viability assay for drug screening. Cells (5 x10^3/100 µL) were cultured in 96-well plates and treated with Venetoclax (#S8048; Selleckchem), Lenalidomide (#S1029; Selleckchem), Pomalidomide (#S1567; Selleckchem), Melphalan (#S8266; Selleckchem), Bendamustine (#S1212; Selleckchem), Dexamethasone (#S1322; Selleckchem), Bortezomib (#S1013; Selleckchem), Carfilzomib (#S2853; Selleckchem), S63845 (#S8383; Selleckchem), AZD5991 (#S8643; Selleckchem), or A1155463 (#E2926; Selleckchem) at different concentrations and times. Cell viability was assessed using CellTiter-Glo Luminescent Cell Viability Assay (CTG, #G7572; Promega).

Apoptosis assay. Apoptosis was investigated by an Annexin V-FITC/PI flow cytometry assay using FACS CANTO II(BD Biosciences).

RNA-seq and WGS. DNA/RNA of parental/resistant clones were extracted using the Qiagen AllPrep DNA/RNA Mini Kit. WGS was performed at a depth of 60 to 100X for parental and clones respectively. Raw reads were aligned to hg38 reference genome following GATK best practices. ControlFREEC were used for tumor only copy number analysis and Mutect2 tumor only mode were used for SNV detections. All mutations detected in tumor only mode from parental cell lines were removed from respective resistant clones. All SNVs overlapping with SNPs were also removed. Remaining mutations in each clone with VAF > 5% were combined and respective locations in each BAM files were re-evaluated for alternative and reference alleles to reduce false positives. Only regions that had 0 alternative allele counts in the parental samples were kept for upset plots. RNA-seq libraries were produced using TruSeq Stranded RNA HT kit

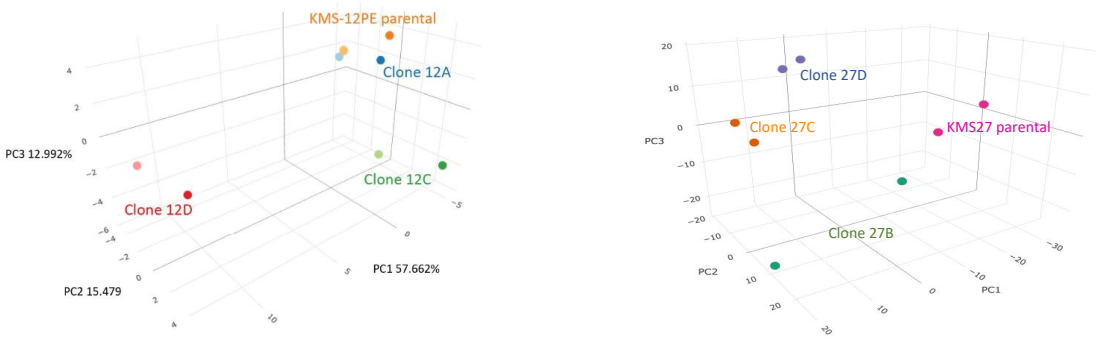
with 100 million targeted read depth in each sample. All sequencing was performed on Illumina insturments and all sequencing was paired end. After RNAseq is completed, samples were quantified using Salmon. tximport was used to summarize isoform level expression to gene level data and DESeq2 was used for differential expression analysis and PCA.

Supplementary Figure 1



Supplementary Figure 1

C



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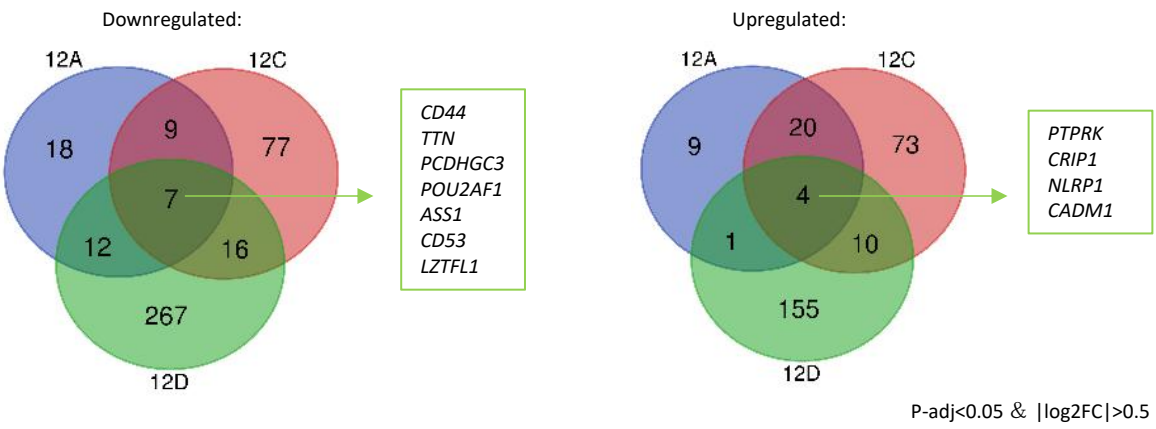

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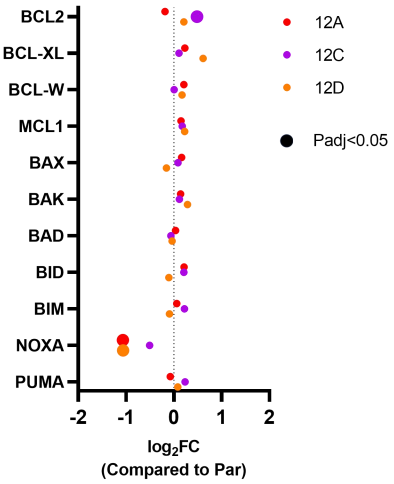

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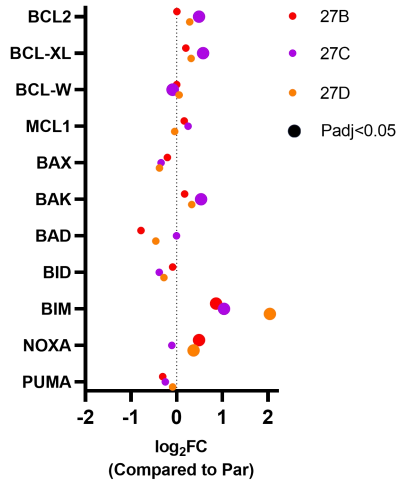


Figure Legends

Figure S1. Venetoclax resistant cells exhibit heterogeneous transcriptomic profiles and absence of genomic alterations. A. Copy number alterations estimated from WGS are shown as integer copy numbers (y-axis) in each model. Left panels illustrate two resistant clones derived from KMS12PE alongside parental cells. Right panel represents the KMS27 model. Red lines are copy number gains/amplifications and blue lines are copy number deletions. Diploid regions are shown with black colors. Chromosomes from chromosome 1 to X are shown on X axis. **B.** Novel SNVs detected in resistant clones but not in parental. All mutations and SNPs detected in parental cell line were removed. Bars on y-axis shows the total number of SNVs remaining after filtering. Bars in upper panels shows the intersections between clones and unique to each clone as represented by circles and connections between circles in the bottom section. Left panels illustrate two resistant clones derived from KMS12PE. Right panel represents 3 resistant clones derived from KMS27. **C.** Principal component analysis (PCA) of parental and Venetoclax resistant clones in KMS12PE model (Parental and clone 12A, 12C, and 12D) and KMS27 model (Parental and clone 27B, 27C, and 27D). **D**. Venn diagram of differentially expressed genes between parental and each resistant clone in KMS12PE model (Padj<0.05 & log2FC>0.5)**. E-F.** Differential expression of BCL2 family members in KMS12PE (**E**) and KMS27 model (**F**).

Supplementary Figure 2

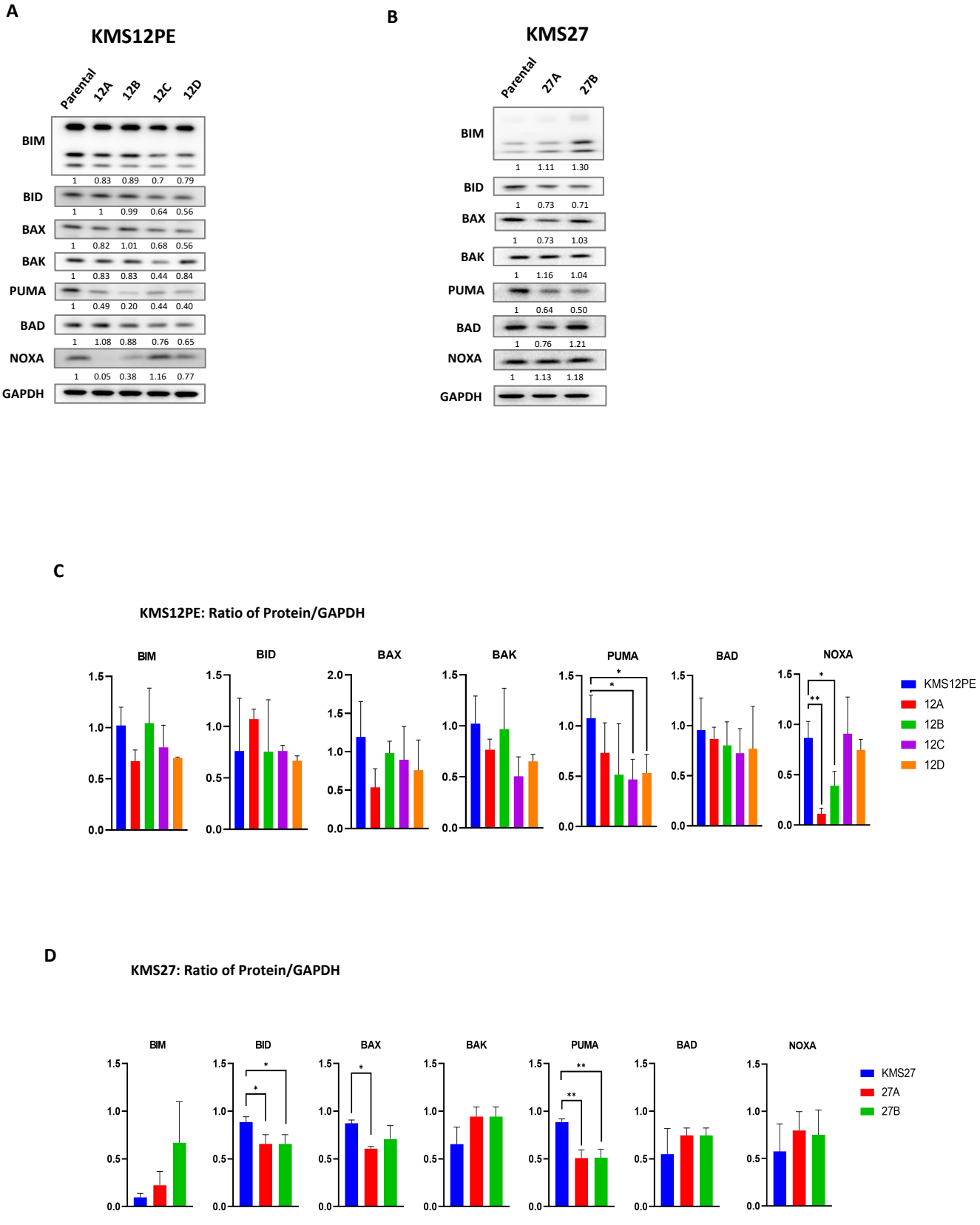
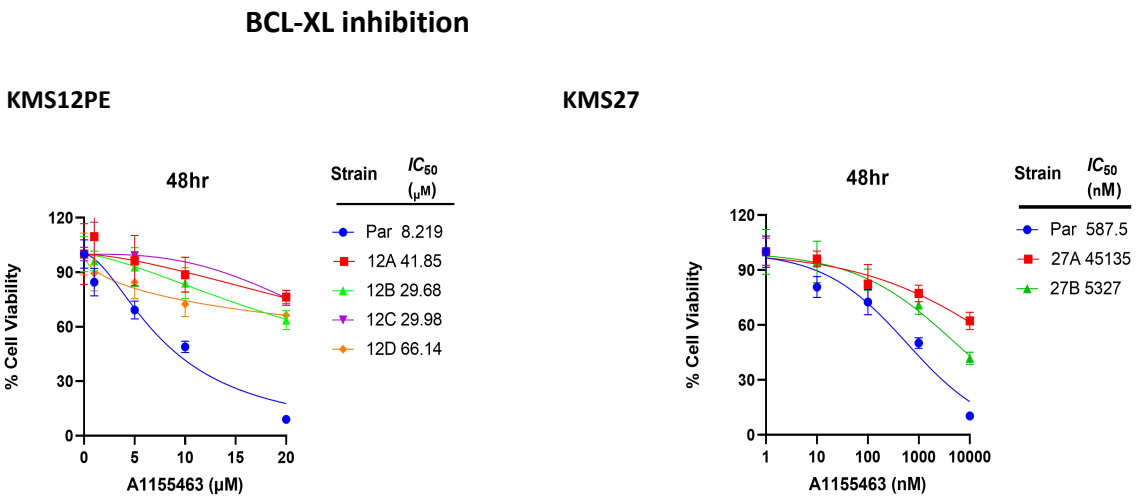


Figure S2. Expression of pro-apoptotic proteins upon acquisition of venetoclax resistance. **A-B.** WB analysis of pro-apoptotic proteins in parental and venetoclax resistant clones of KMS12PE (**A**) and KMS27 model (**B**). GAPDH was used as the loading control. Protein expression densitometry values were calculated using ImageJ. **C-D.** Ratios of protein/GAPDH in WB analysis of KMS12PE (**C**) and KMS27 model (**D**) were illustrated.

Supplementary Figure 3

A



B

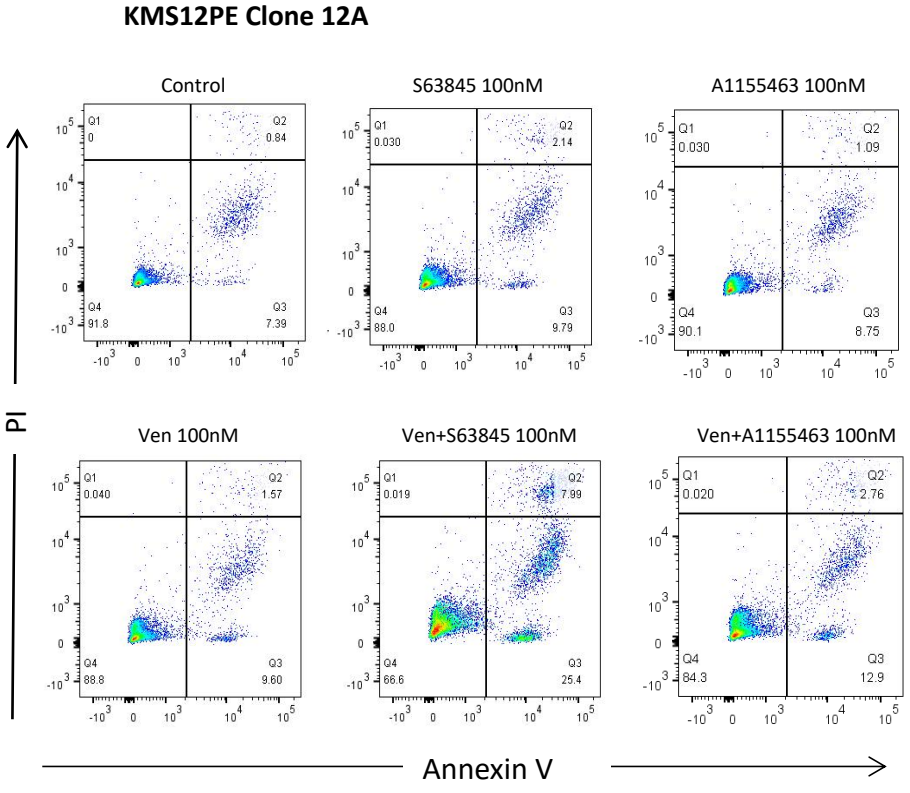


Figure S3. Dual Targeting of BCL2 anti-apoptotic members has anti-MM activity despite acquired venetoclax resistance. A. Parental cells and venetoclax-resistant clones were treated for 48h with different doses of BCL-XL inhibitor, A-1155463. Cell viability was assessed by CTG analysis. **B.** Venetoclax-resistant clones (KMS12PE model) were treated with a combination of different BH3 mimetic drugs for 24h and apoptosis was measured by flow cytometry following Annexin V-FITC and PI staining. One representative experiment in resistant clones is shown.

Supplementary Figure 4

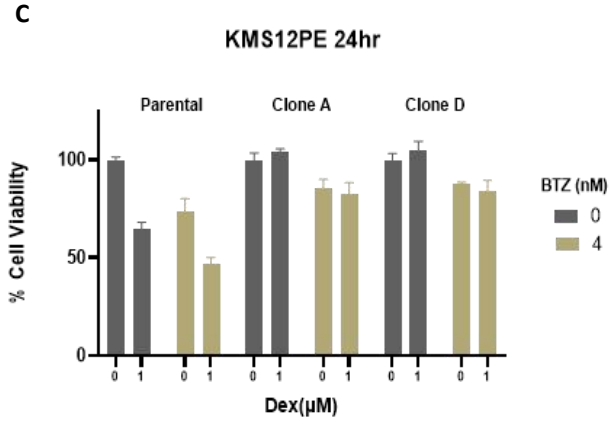
A

IC50	KMS12PE Parental	12A	12B	12C	12D
Bendamustine (μM)	65.13	79.58	89.84	88.79	78.37
Melphalan (μM)	5.571	19.45	12.88	7.78	15.8
Bortezomib (nM)	5.5708	9.970	8.958	8.385	9.647
Carfilzomib (nM)	4.55	12.62	7.754	8.234	5.257
Lenalidomide (μM)	65.53	75.2	104.3	96.05	105.1
Pomalidomide (μM)	72.52	100.3	146.7	124.6	328.1
Dexamethasone (μM)	3.15	20.08	7.67	39.25	8.37

B

IC50	KMS27 Parental	27B	27D
Bendamustine (μM)	30.92	119	97.15
Melphalan (μM)	2.866	11.44	42.97
Bortezomib (nM)	6.061	11.11	8.376
Carfilzomib (nM)	1.956	3.979	2.985
Lenalidomide (μM)	87.2	97.89	Not reached
Pomalidomide (μM)	0.5016	0.5	91.96
Dexamethasone (μM)	77.48	99.02	Not reached

C



D

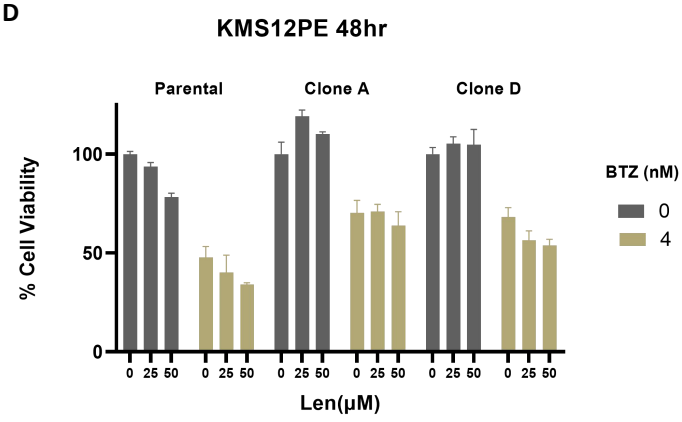
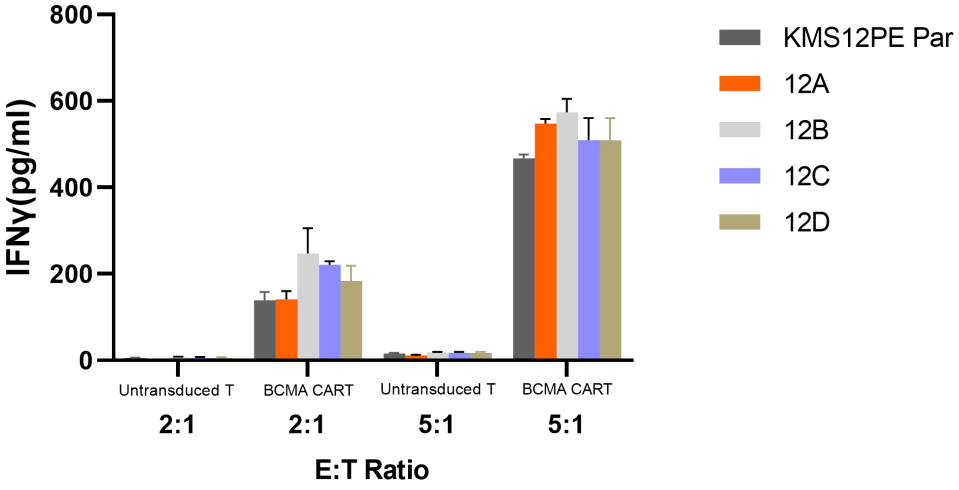


Figure S4. Decreased sensitivity to anti-MM agents is observed in Venetoclax resistant cells. **A-B.** IC50s in parental and resistant cells for indicated drugs of KMS12PE (**A**) and KMS27 model (**B**). **C-D.** Combinations of anti-myeloma agents were evaluated in KMS12PE parental and resistant clones (12A and 12D) (bortezomib plus dexamethasone in **Fig C**, bortezomib plus lenalidomide in **Fig D**). Cell viability was evaluated via CTG assay.

Supplementary Figure 5

A



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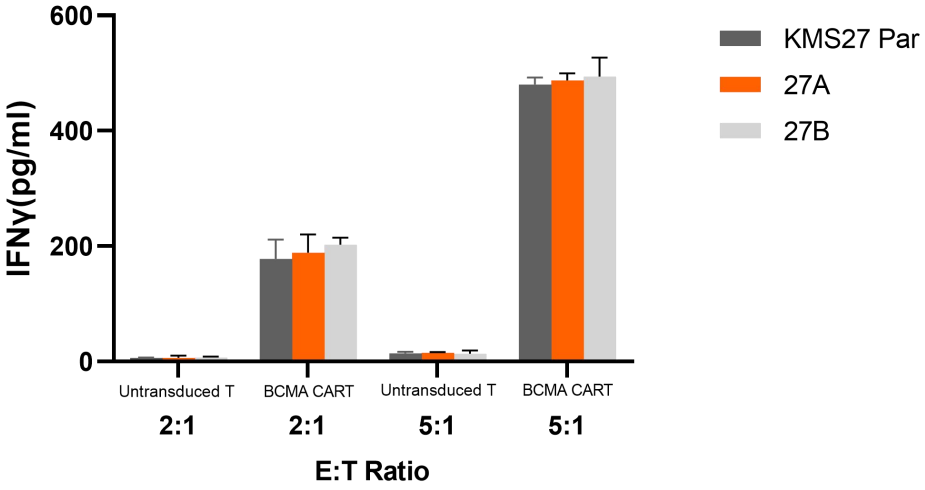

Figure S5. BCMA-CAR T cells efficiently induce IFNγ secretion in venetoclax resistant clones. **A-B.** Parental and venetoclax resistant clones were incubated with either untransduced T cells or BCMA-CAR T cells for 4 hours at the Effector:Target ratio of 2:1 or 5:1. IFNγ secretion in the culture supernatant after 4h co-culture was assessed by ELISA. KMS12PE model is illustrated in **Fig A** and KMS27 model is illustrated in **Fig B**.