

Menin inhibitor ziftomenib (KO-539) synergizes with drugs targeting chromatin regulation or apoptosis and sensitizes acute myeloid leukemia with *MLL* rearrangement or *NPM1* mutation to venetoclax

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Supplemental-Figures

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Author Contributions

M.W.M.K. designed the research, analyzed and interpreted the data, wrote and revised the manuscript, and supervised the study; J.R. performed the experiments, analyzed and interpreted the data, wrote the original draft of the manuscript and edited the manuscript. M.M.D. and N.D. performed experiments, analyzed and interpreted the data; S.R.B provided experimental support for BH3 profiling and revised the manuscript; K.K., C.L., and H.L.S. performed experiments and analyzed the data; D.S. analyzed and interpreted the RNA-seq data; F.B. und L.K. provided the menin-MLL inhibitor ziftomenib and revised the manuscript; H.E. provided NSG mice and revised the manuscript; M.T. and T.K. provided administrative support and revised the manuscript, K.D. provided genetic characterization of AML samples.

Competing Interests statement: M.W.M.K. receives honoraria and is consultant for Pfizer, Kura Oncology, Jazz Pharmaceuticals, Bristol-Myers Squibb / Celgene and Abbvie, is in the speakers bureau of Gilead and receives travel support from Daiichi Sankyo. J.R. has received travel support from Abbvie. L.K. and F. B. are employed by Kura Oncology and are current equity holders. D.S. receives honoraria from Bristol-Myers-Squibb, Astra Zeneca and Abbvie. K.D. receives honoraria and is on the advisory board of Novartis, Janssen, BMS/Celgene, Daiichi Sankyo, Jazz and Roche, is member of advisory board of Abbvie and receives research funding of Novartis, BMS/Celgene, Agios and Astellas. The remaining authors declare no competing financial interests. This work was supported by a grant from the German Research Foundation (DFG) (KU-2688/2-1 und DFG 318346496, SFB1292/2 TP12).

Supplemental Figures

Suppl.-Fig.1 Characterization of ziftomenib

A Relative mRNA expression of *MEF2C*, *MEIS1*, *PBX3* and *FLT3* after 5 days of treatment with 150nM ziftomenib in MOLM13 and OCI-AML3 / 4 days in MV411; mean of 3 experiments performed in technical triplicates \pm SD. **B** Cytospins of MV411, MOLM13 and OCI-AML3 after 5 days of treatment with ziftomenib in Giemsa staining. Pictures were taken in 60X magnification. **C** Surface expression of CD14 and CD11b after 5 days of treatment with ziftomenib assessed by flow cytometry; mean of at least 3 independent experiments performed in technical triplicates \pm SD. **D** Geneset enrichment analysis was performed with GSEA Software (Version 4.3.0) from Broad Institute. Genesets were chosen from the MSigDB Database (FDR<0.1). **E** VENN Diagram displays genes that were >1.5-fold downregulated in RNA sequencing data after treatment with ziftomenib (150nM), MI-503 (2,5 μ M) for 4 days (MOLM13, OCI-AML3) / 3 days (MV411) or VTP-50469 (3 days with 50nM in MV411 and MOLM13, 4 days with 100nM in OCI-AML3), adjusted P<0.05. Data was previously published for VTP-50469 treatment in MV411 and MOLM13 (Aubrey et al.¹⁰) and MI-503 in all three cell lines (Dzama et al.⁹). Data of ziftomenib treatment in MV411, MOLM13 and OCI-AML3 and VTP-50469 in OCI-AML3 can be accessed at GSE228307.

Suppl.-Fig.2 Synergy Drug Screen

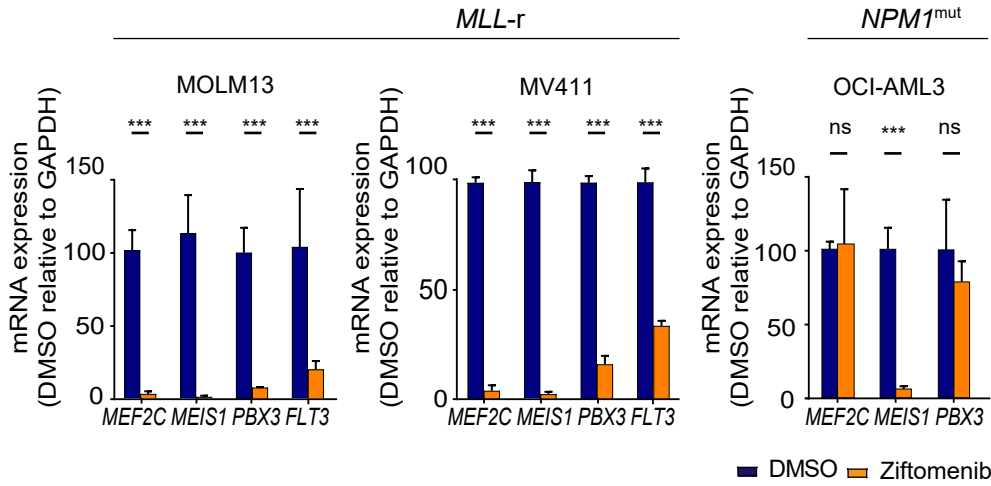
A List of targeted compounds that were assessed in combination with ziftomenib. **B** Cells were pretreated with different concentrations of ziftomenib for 2.5 days and then exposed to 4 concentrations of each small molecule inhibitor for 2 days combinational treatment. Viable cells compared to DMSO were assessed by flow cytometry on day 4.5. The screen was performed in at least 2 independent experiments without technical replicates. **C+D** Combination indices were calculated by the Chou Talalay method and weighted as described before. **E** Dose-response curve from a combinational cell viability assay in the *MLL*- and *NPM1* wildtype cell line U937 comparing 150nM ziftomenib (5 days), 100nM ATRA (5 days), and combinational treatment (5 days / 5 days). **F** Dose response curves for *MLL*-r *FLT3*^{TD} MOLM13 and MV411 after 4 day treatment with ziftomenib, 24 h with gilteritinib or combined treatment assessed by flow cytometry. One independent experiment in technical triplicates.

Suppl.-Fig.3 Synergy of ziftomenib and venetoclax

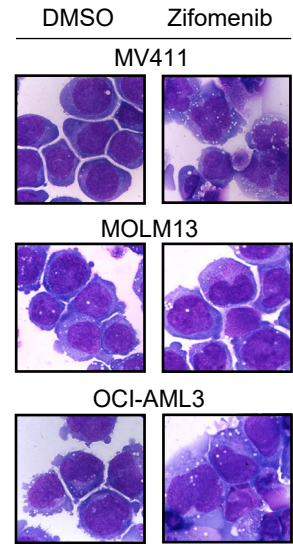
A Dose-response curve of AML cell lines after treatment for 48 hours (1000nM max., 1:2 dilutions). Mean of 3 experiments performed in technical triplicates \pm SD. **B** Proliferation assay of *MLL*- and *NPM1*- wildtype NB4 cells with ziftomenib, venetoclax and both drugs. Viability was assessed after 5 days / 24 h. Mean of 3 independent experiments. **C** Display of synergy (CI<1) of different IC concentrations for synergistic cell lines calculated by the Chou Talalay method. **D** Proliferation assay of *NPM1*^{mut} OCI-AML3 cells with ziftomenib (150nM), venetoclax (100nM) and both drugs. Viability was assessed after 5 days / 24 h. Mean of 3 independent experiments. **E** Proliferation assay of *NPM1*^{mut} OCI-AML3 cells with ziftomenib (150nM), venetoclax (10 000nM) and both drugs. Viability was assessed after 5 days / 24 h. Mean of 3 independent experiments. **F** Dose-response curves of MOLM13 cells treated with venetoclax for 24 h, comparing cells transduced with shRNAs against *MEN1* with control-transduced cells (LUC). mRNA expression levels of *MEN1*, *MEIS1*, and *BCL2* in MOLM13 were assessed 48 h after transduction to confirm knockdown. Data represent mean of 2 independent experiments in technical triplicates \pm SD. **G** Assessment of leukemia burden (defined as human CD45 positive bone marrow cells) in a *NPM1*^{mut} OCI-AMI3 xenotransplantation model (n=5 mice/group) upon treatment with drug vehicles, ziftomenib (50 mg/kg; PO; once daily, starting day 22), venetoclax (100 mg/kg; PO; once daily, starting day 16), or combination. Readout was on day 33. **H** Evaluation of differentiation defined as CD11b expression in hCD45+ positive cells of the experiment displayed in G.

Supplemental-Figure 1

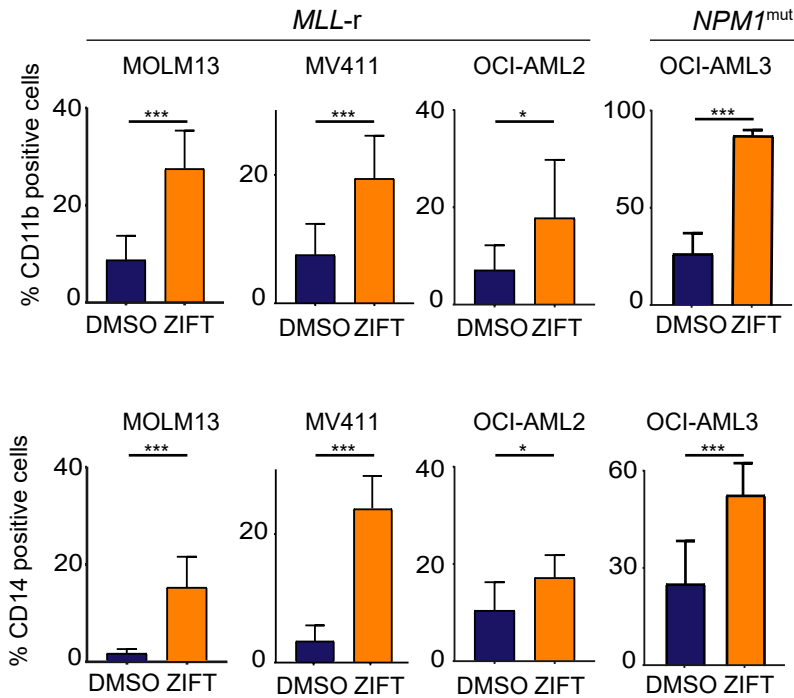
A Gene expression: day 5



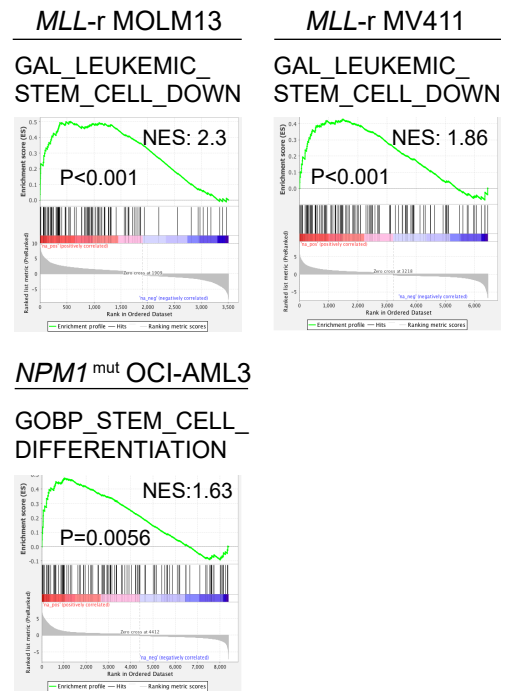
B Morphological changes



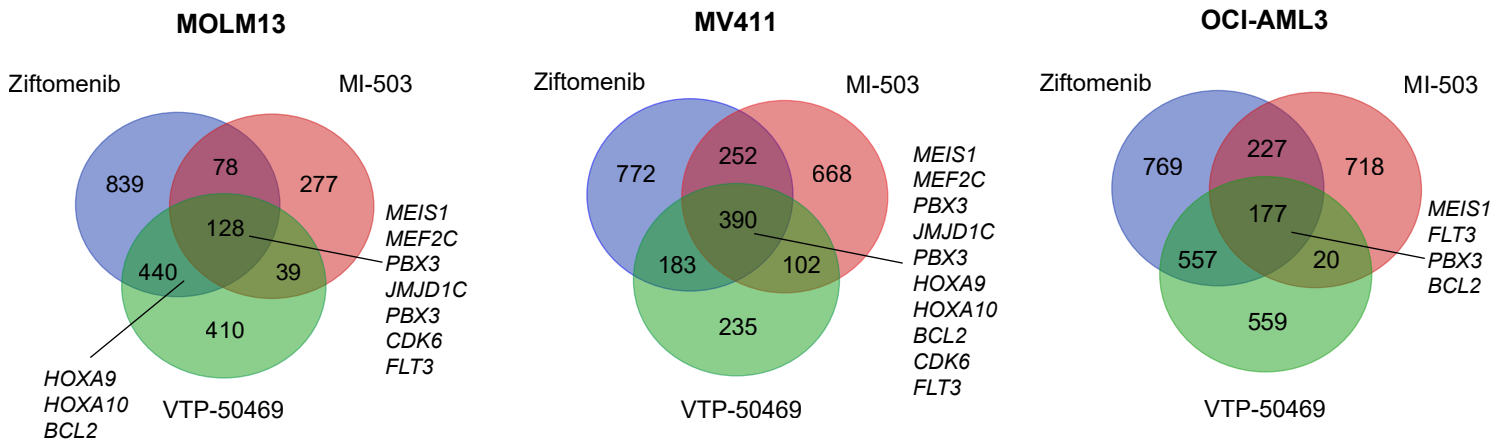
C Cell differentiation: day 5



D Geneset enrichment analysis



E VENN Diagramm of downregulated genes (>1.5-fold) upon menin inhibition



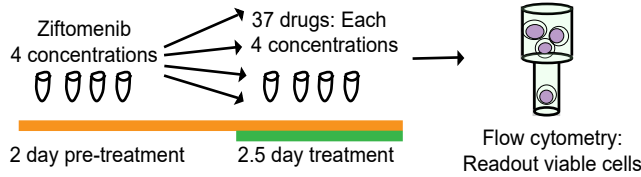
Supplemental-Figure 2

A Drug classes of the synergy screen

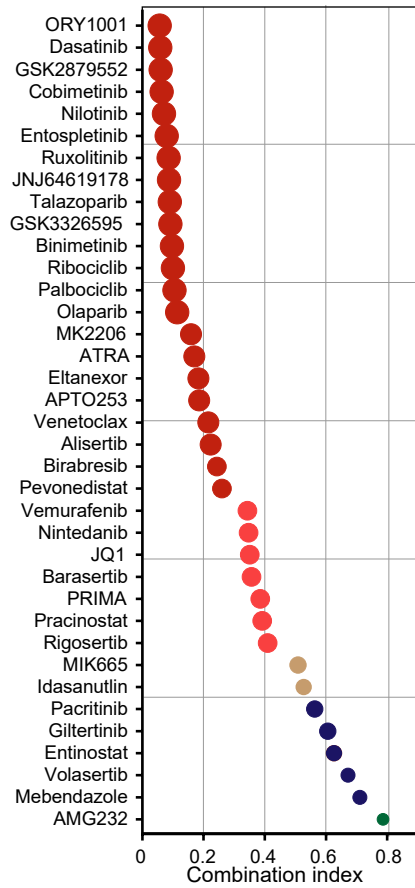
Apoptosis & cell cycle		Tyrosine kinase inhibitors		Epigenetics & DNA damage		Various targeted drugs		Intracellular pathway inhibitors	
Venetoclax	BCL2-i*	Ruxolitinib	JAK2-i	GSK2879552	LSD1-i	ATRA	RAR Agonist	Cobimetinib	MEK-i
MIK2206	AKT1-i	Pacritinib	JAK2-i	ORY1001	LSD1-i	Eltanexor	Exportin 1-i	Binimetinib	MEK-i
MIK665	MCL1-i	Nilotinib	c-KIT-i	Pracinostat	HDAC-i	Pevonedistat	NEDD8-i	Vemurafenib	BRAF-i
Idasanutlin	MDM2-i	Dasatinib	c-KIT-i	Entinostat	HDAC-i	Mebendazole	HSP70-i	Alisertib	Aurora Kinase A-i
AMG232	MDM2-i	Gilteritinib	FLT3-i	Birabresib	BRD2-i	APTO 253	MYC-i	Barasertib	Aurora Kinase B-i
Palbociclib	CDK4/6-i	Nintedanib	VEGFR-i	GSK3326595	PRMT5-i	PRIMA	mutant p53 activator		
Ribociclib	CDK4/6-i	Entospletinib	SYK-i	JNJ64619178	PRMT5-i				
Volasertib	PLK1-i			JQ1	BET-i				
Rigosertib	PLK1-i			Olaparib	PARP-i				
				Talazoparib	PARP-i				

*inhibitor = -i

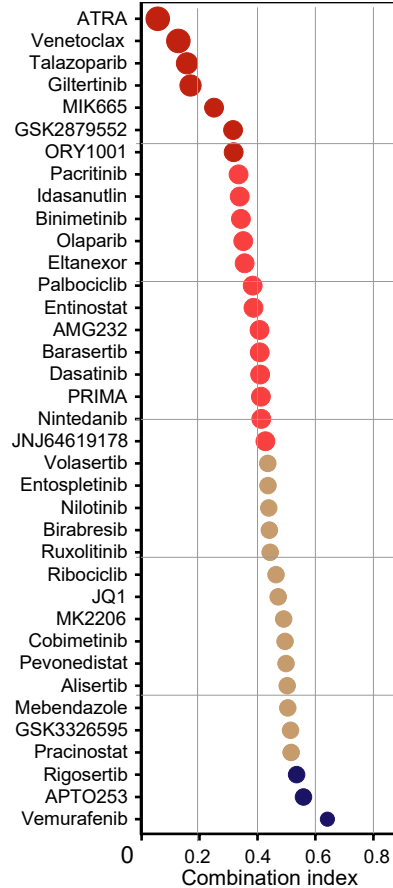
B Design of the synergy screen



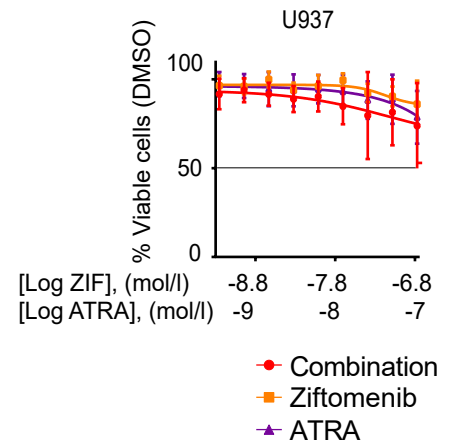
C Synergy screen of MLL-r MOLM13



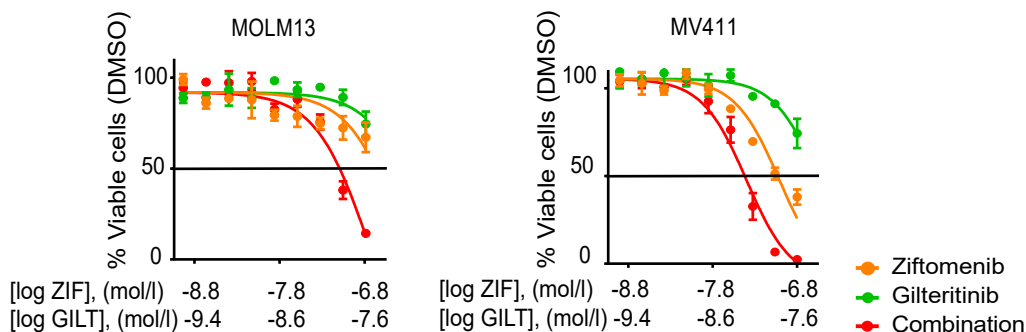
D Synergy screen of MLL-r MV411



E Combined menin inhibition and ATRA treatment

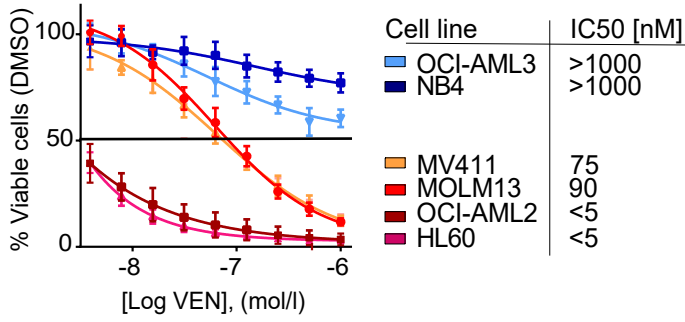


F Combined menin-MLL and FLT3 inhibition

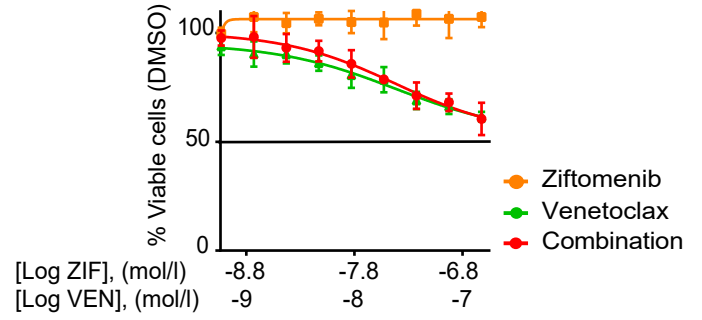


Supplemental-Figure 3

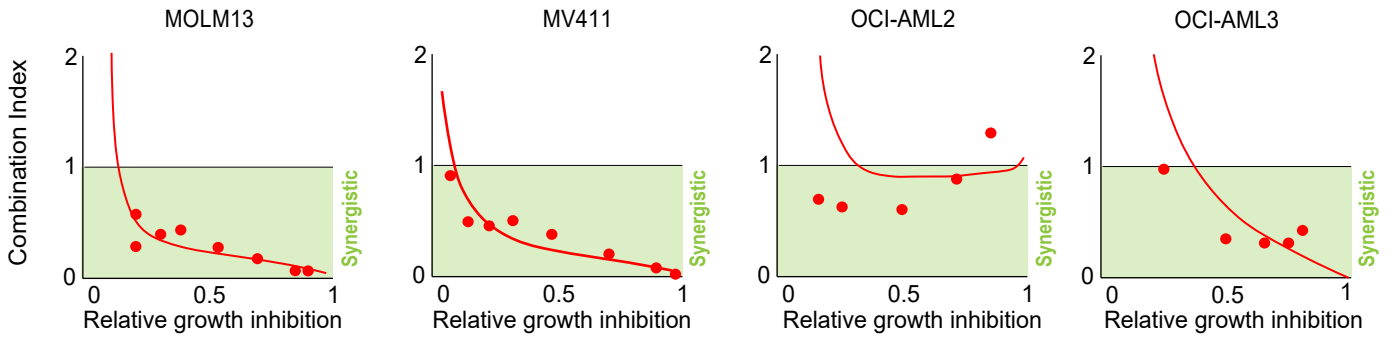
A Venetoclax treatment: 48 h



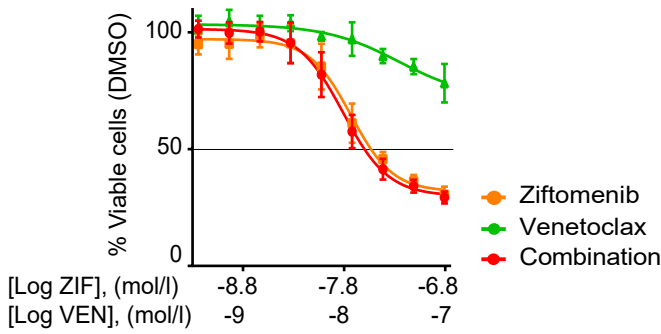
B NB4 (MLLwt, NPM1wt): 5d / 1d



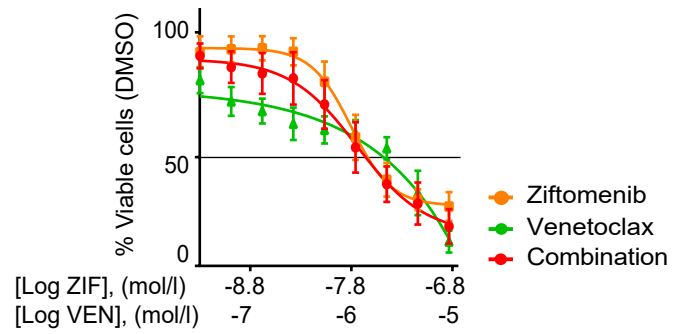
C Synergy of Ziftomenib and Venetoclax: combination index values



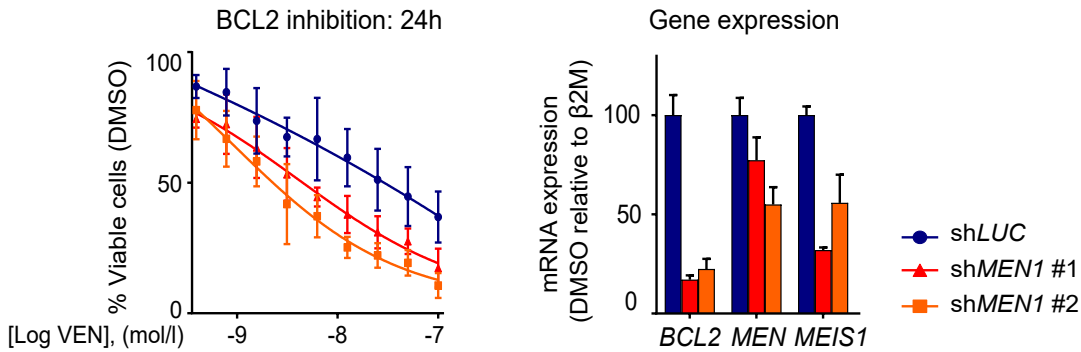
D NPM1mut OCI-AML3: 5d / 1d



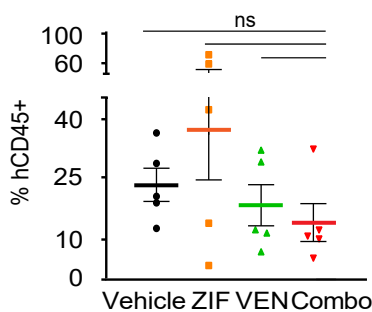
E NPM1mut OCI-AML3: high dose Venetoclax 5d / 1d



F ShRNA based menin knockdown in MOLM13



G Leukemia Burden: OCI-AML3 xenograft model



H In vivo CD11b expression: OCI-AML3 xenograft model

