Supplemental Data for

Combined Inhibition of BCL-2 and MCL-1 Overcomes BAX Deficiency-Mediated Resistance of *TP53*-mutant Acute Myeloid Leukemia to Individual BH3 Mimetics

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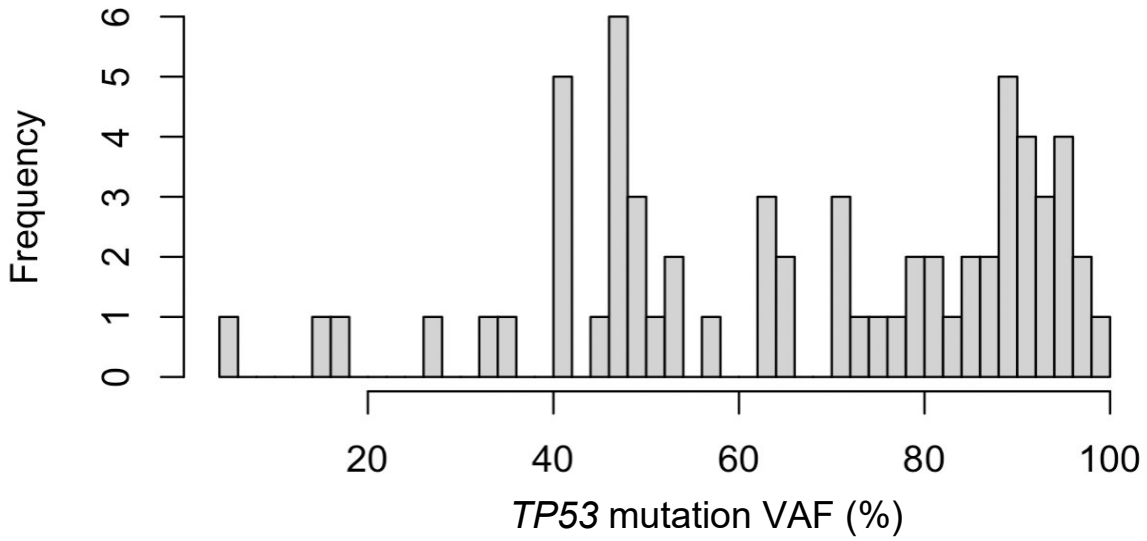
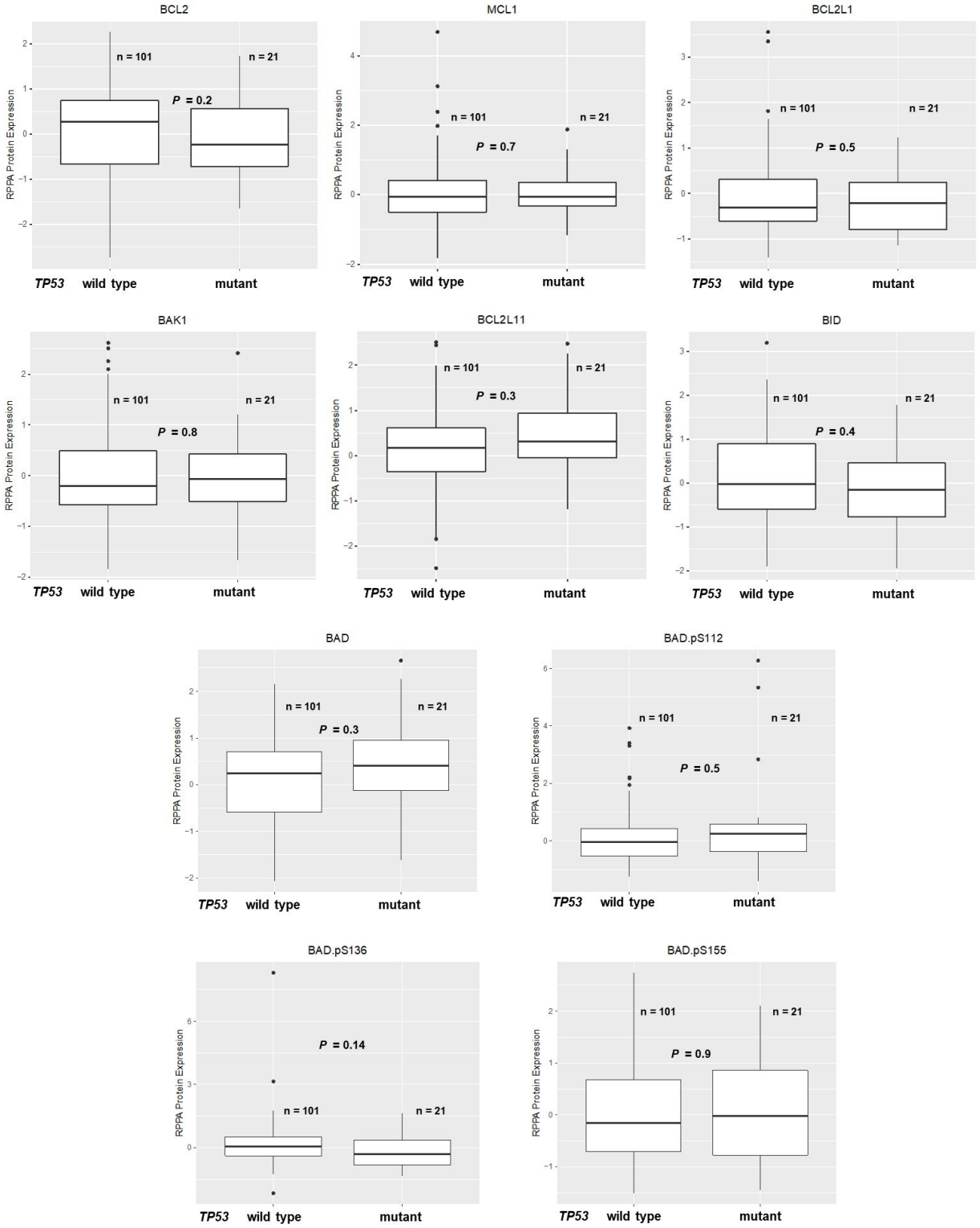
Supplemental Table 1. Combination index (CI) values of patient samples treated with venetoclax and AMG176 for 48 hours

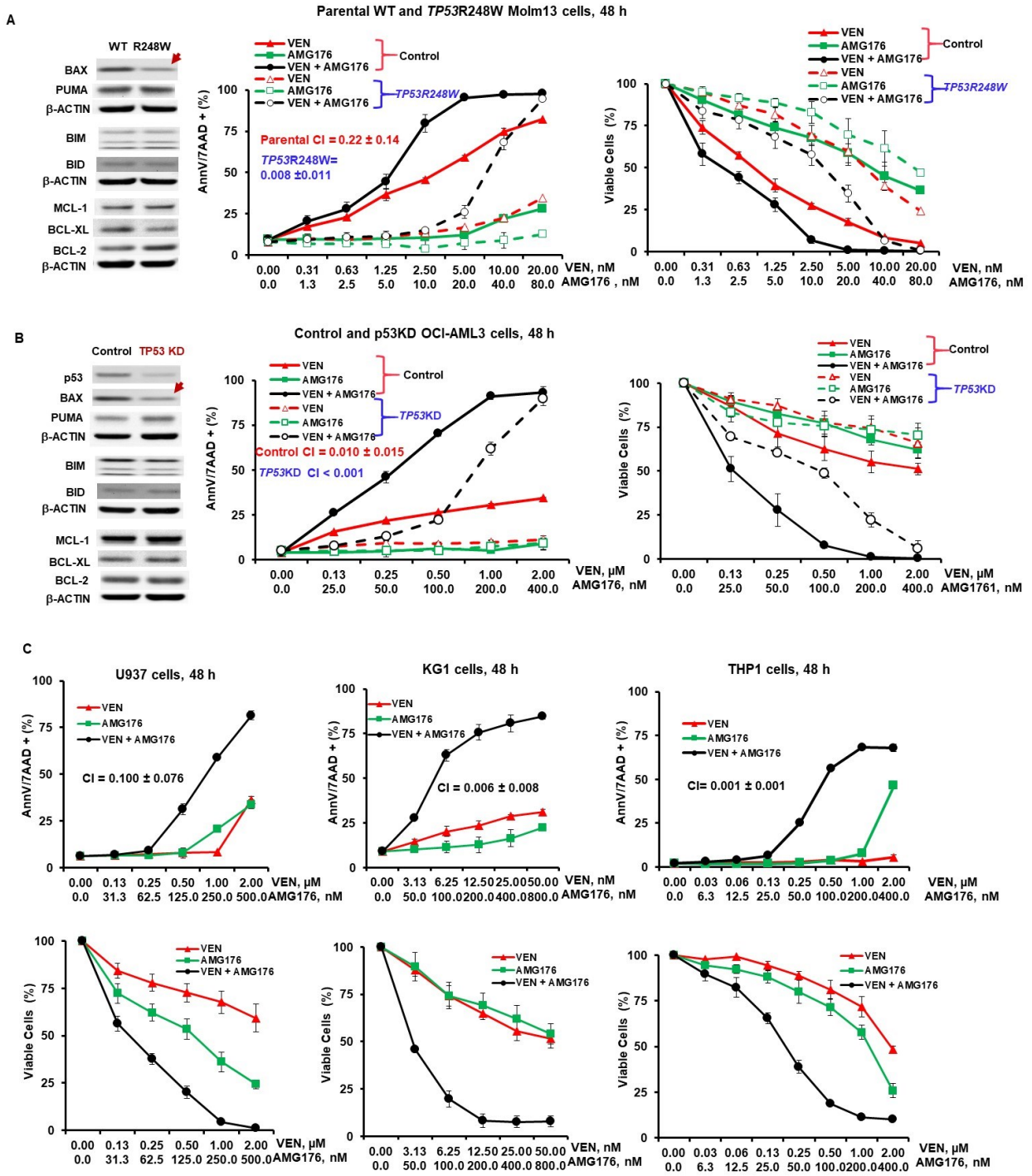
	CI values	
Patient no.	$CD45^+$ cells	$CD34^+$ cells
26	0.23 ± 0.03	0.28 ± 0.14
28	0.20 ± 0.05	0.17 ± 0.05
29	0.34 ± 0.10	0.22 ± 0.08
30	0.25 ± 0.19	0.22 ± 0.12
32	0.15 ± 0.07	0.07 ± 0.08
33	0.04 ± 0.04	0.24 ± 0.16
34	0.29 ± 0.07	0.21 ± 0.07
35	0.19 ± 0.15	0.15 ± 0.03



Supplemental Figure 1. VAF frequency distribution of *TP53*-mutant AML patient samples used for RNA-seq analysis.

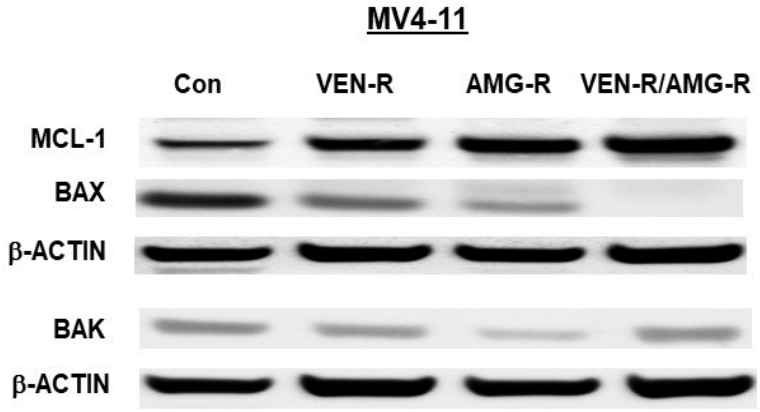


Supplemental Figure 2. RPPA analysis of BCL-2 family protein expression in patients with newly diagnosed *de novo TP53*-WT (n = 101) or *TP53*-mutant (n = 21) AML.

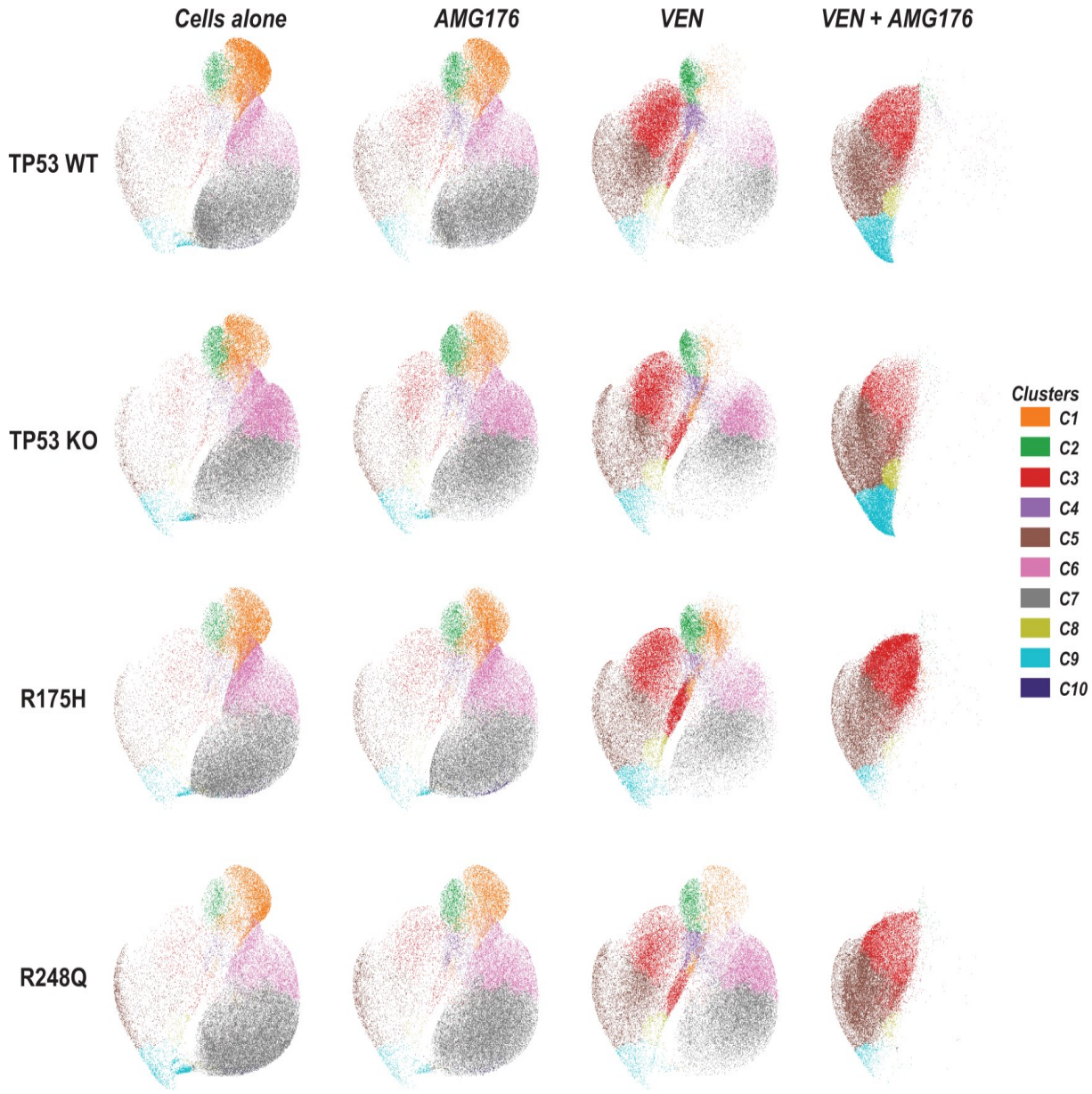


Supplemental Figure 3. The combined inhibition of BCL-2 and MCL-1 is highly synergistic against *TP53*-mutant and -KD leukemia cells. A–C. Control and *TP53*-R248W Molm13 cells (A), control and *TP53*-KD OCI-AML3 cells (B), and other *TP53*-mutant leukemia cells (C) were treated with venetoclax (VEN), AML176, or both. Cell death and viability were determined by flow cytometry. Experiments were performed in triplicates, and data are expressed as mean ± SEM.

Protein levels were determined by Western blot analysis and the arrows mark the decreased expression. AnnV, annexin V; 7AAD, 7-aminoactinomycin D.



Supplemental Figure 4. Expression of BCL-2 family proteins in MV4-11 cells with acquired resistance to venetoclax (VEN-R), AMG176 (AMG-R), or both (VEN-R/AMG-R), as assessed by Western blotting. Con, control.



Supplemental Figure 5. The combination of venetoclax plus AMG176 induces similar cell death and stress patterns in *TP53*-WT, -deficient, and -mutant AML cells. *TP53*-WT, -KO, and -mutant (R175H and R248Q) Molm13 cells were treated with DMSO, VEN, AMG176, or the combination and stained with an array of flow cytometry antibodies. The cells were then subjected to UMAP dimension reduction and projected on 2-dimensional plots. The FlowSOM algorithm was utilized for automated cell population identification; the colors indicate different FlowSOM clusters.