LETTER TO THE EDITOR

Pirtobrutinib and venetoclax combination overcomes resistance to targeted and chimeric antigen receptor T-cell therapy in aggressive mantle cell lymphoma

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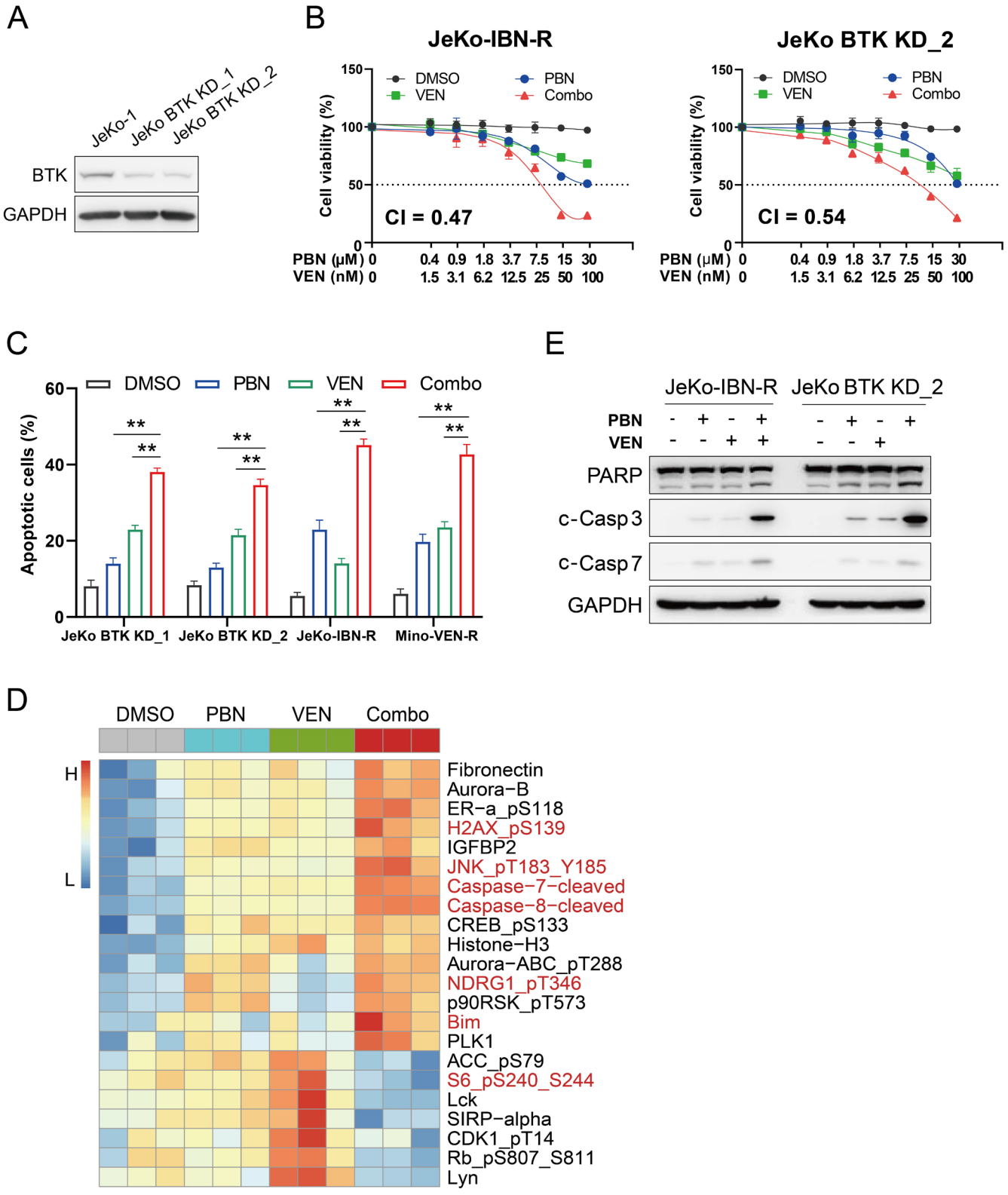
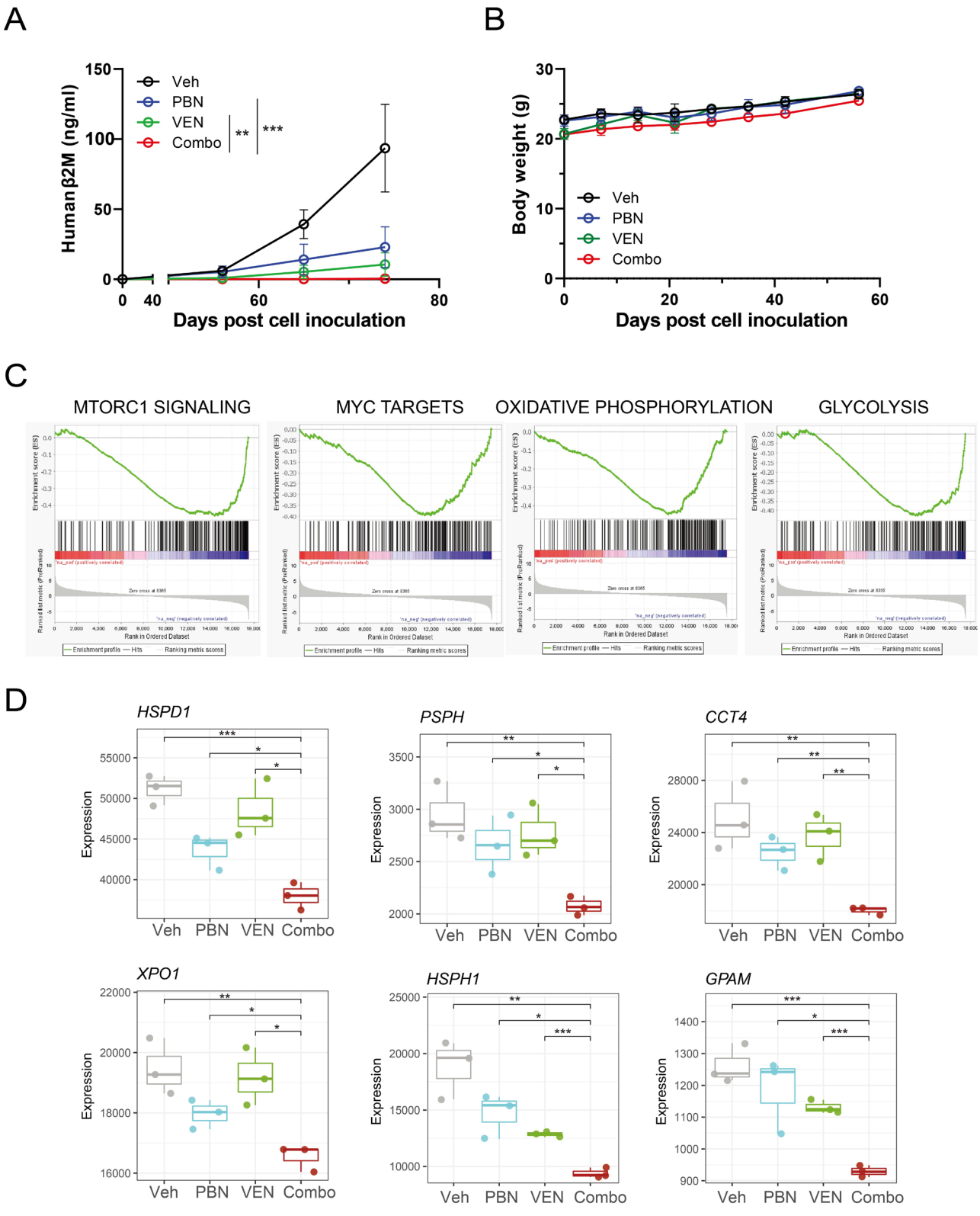
Supplementary Data

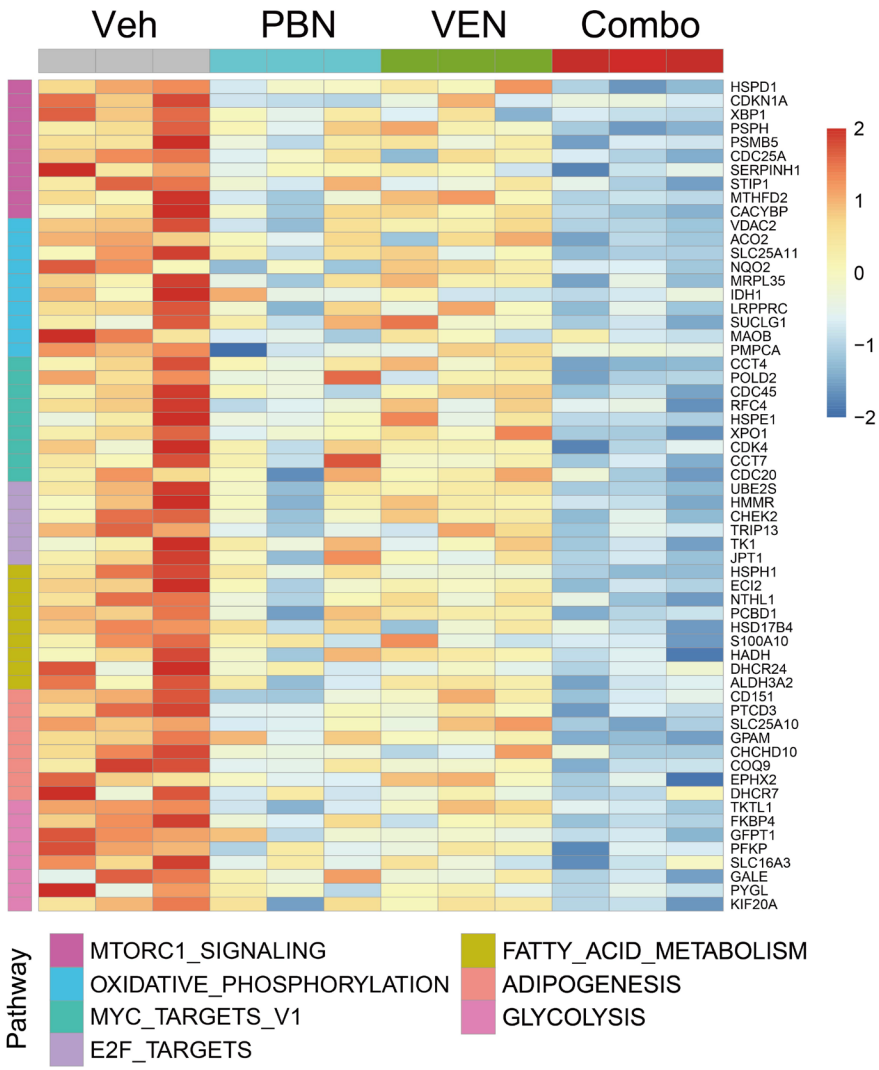


Supplementary Figure S1. *In vitro* anti-MCL activity of the pirtobrutinib-venetoclax combination in resistant MCL cell lines. (**A**) BTK expression levels for parental JeKo-1 cells and two BTK knockdown JeKo-1 clones. (**B**) Dose-response cell viability assay was performed for JeKo BTK KD_2 and JeKo-ibrutinib-R cells after a 72 h treatment using the CellTiter-Glo luminescence assay (Promega). The combination Index (CI) (where synergistic, additive, and antagonistic effects are defined by CI < 1, CI = 1, and CI > 1, respectively) is indicated in the individual panels. (**C**) Annexin V/PI apoptosis assay was performed for the indicated resistant MCL cell lines after 24 h treatment with DMSO, pirtobrutinib, venetoclax or the pirtobrutinib-venetoclax combination. **p < 0.01. (**D**) Heatmap showed RPPA-based protein expression profiles of JeKo-IBN-R cells in response to 12 h treatment of pirtobrutinib, venetoclax, and their combination. Names of proteins of interest are written in red. (**E**) JeKo-BTK KD_2 and JeKo-IBN-R cells were treated with pirtobrutinib or/and venetoclax for 24 h. Apoptosis induction is indicated by cleaved caspase 3/7 and PARP. GAPDH was used as a loading control.



Supplementary Figure S2. The pirtobrutinib-venetoclax combination showed enhanced anti-MCL efficacy in Mino-venetoclax-R xenograft model with a favorable safety profile. (**A**) Mouse tail vein blood was collected

periodically and β2M levels in the serum were measured with a β2M ELISA kit to assess the tumor burden. (**B**) Mouse body weight was monitored throughout the treatment period as indicated. (**C**) GSEA enrichment plots show downregulation of mTORC1, MYC targets, OXPHOS, and glycolysis pathways in the combination group compared to vehicle. (**D**) Box plots display the expression of representative differentially expressed genes in the combination group versus both single treatments and vehicle group. n = 3 per group. *p < 0.05. **p < 0.01. ***p < 0.001.



Supplementary Figure S3. Heatmap shows the expression of the most downregulated gene targets in oncogenic and metabolism pathways by combination treatment in Mino-venetoclax-R xenograft model. Veh = vehicle; PBN = pirtobrutinib; VEN = venetoclax; Combo = combination.