Vinblastine resets tumor-associated macrophages toward M1 phenotype and promotes antitumor immune response

Yi-Na Wang[1], Yuan-Yuan Wang[1], Jin Wang[1], Wen-Juan Bai[1], Nai-Jun Miao[1,2], Jing Wang[1,2*]

Affiliations:

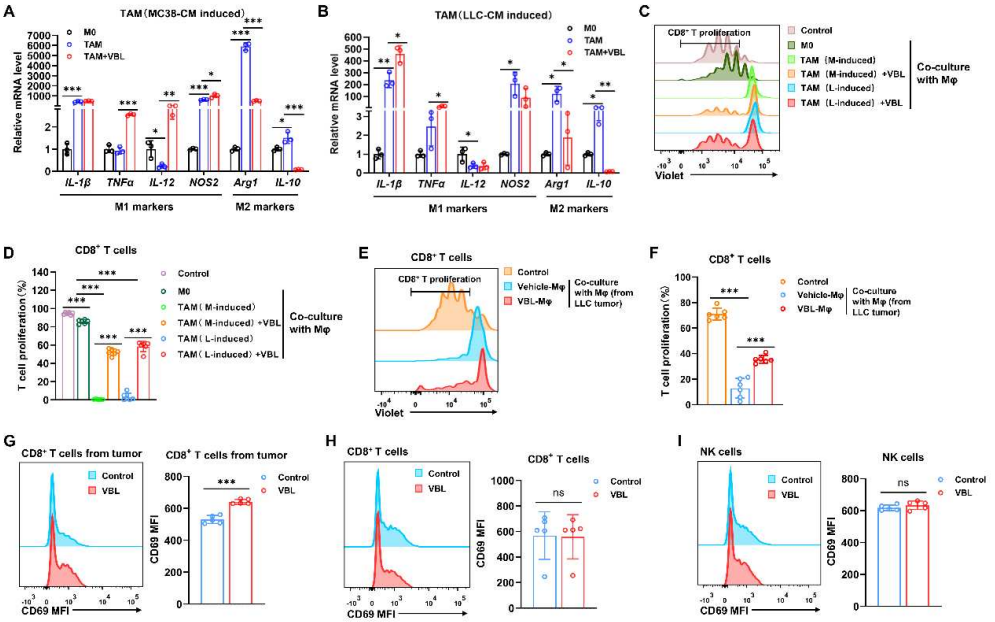
[1]Shanghai Institute of Immunology, Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

[2]Center for Immune-related Diseases at Shanghai Institute of Immunology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

*Correspondence to: Jing Wang. E-mail: jingwang@shsmu.edu.cn; Address: #5-603, Chongqingnan Rd. 280, Huangpu, Shanghai, China, 300025.

Wang Y-N, *et al. J Immunother Cancer* 2023; 11:e007253. doi: 10.1136/jitc-2023-007253

Supplementary Figures



Supplementary Figure 1: VBL reprograms TAMs to the antitumor M1-like phenotype and activates T cells

A-B. Gene expression of IL-1β, TNFα, IL-12, NOS2, Arg1, and IL-10 in MC38-CM-induced (A) and LLC-CM-induced (B) TAMs with or without VBL treatment for 24h (n=3 per group).

C. Representative flow cytometry results of T cell proliferation after co-culture with MC38-CM-induced or LLC-CM-induced TAMs with or without VBL treatment for 72h.

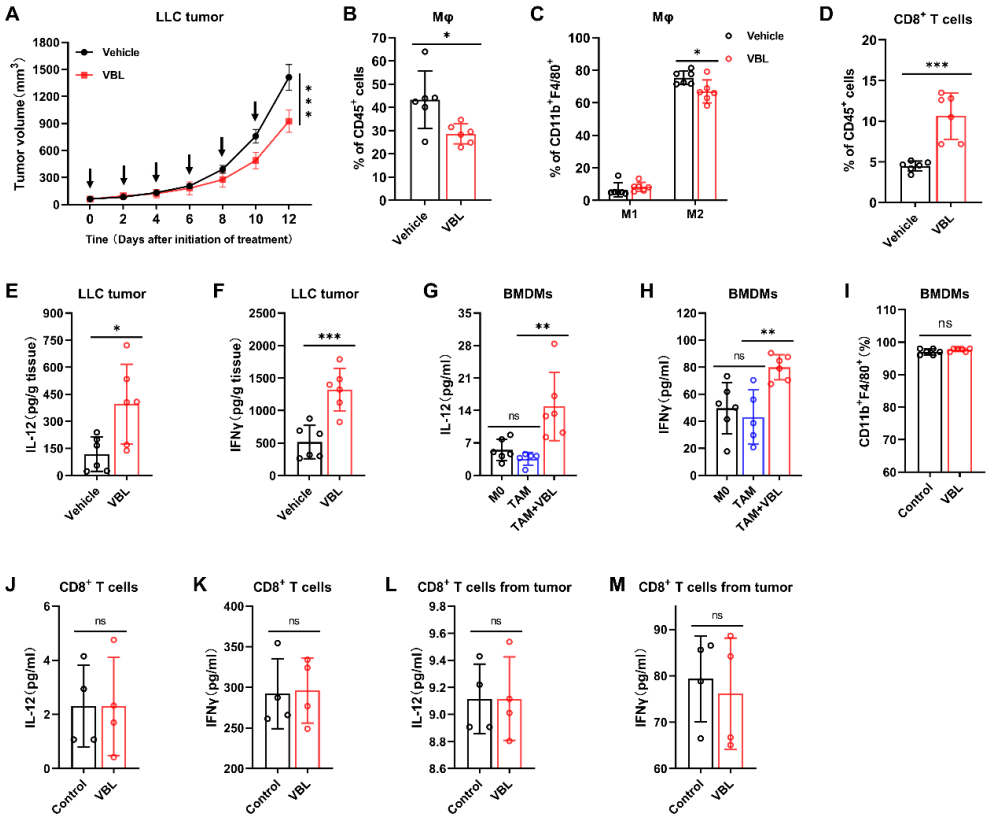
D. Statistical results of T cell proliferation in panel (C) (n=6 per group).

E. Representative flow cytometry results of T cell proliferation after co-culture with macrophages sorted from LLC tumors for 72h.

F. Statistical results of T cell proliferation in panel (E) (n=6 per group).

G-I. Flow cytometry analysis for CD69 MFI in $CD8^+$ T cells sorted from tumors (G), $CD8^+$ T cells cultured in vitro (H) and NK cells cultured in vitro (I).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 2: VBL monotherapy suppresses tumor growth and re-polarizes macrophages to the M1-like phenotype

A. Tumor growth curve of LLC tumor during VBL treatment (1.25mg/kg weight, every other day) (n=6 per group); arrows indicate the treatment time.

B-D. Flow cytometry analysis for total macrophages (B), M1 or M2 cells (C), and T cells (D) in LLC tumors after VBL (1.25mg/kg weight, every other day for 2w) treatment (n=6 per group).

E-F. ELISA analysis of IL-12 (E) and IFNγ (F) levels in LLC tumor homogenate (n=6 per group).

G-H. ELISA analysis of IL-12 (G) and IFNγ (H) levels in BMDMs conditioned

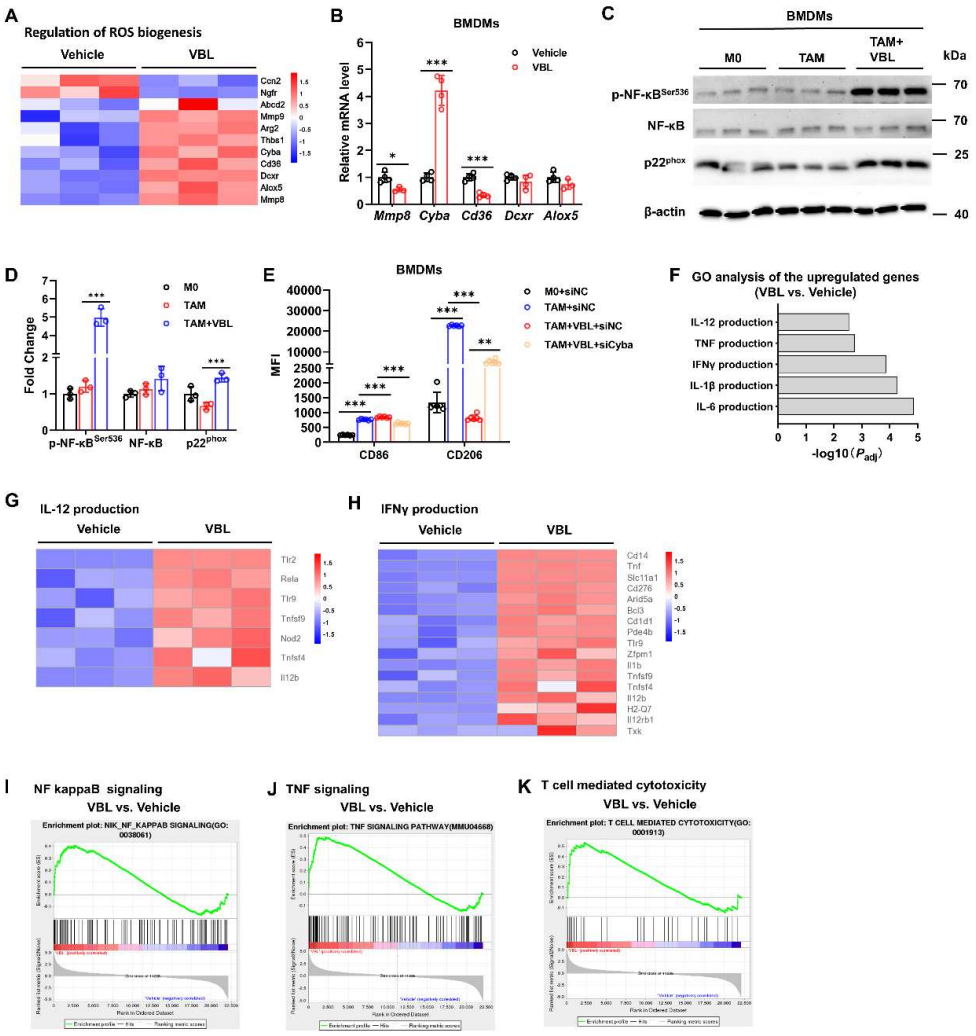
medium after VBL treatment for 24h (n=6 per group).

I. Flow cytometry analysis for percentage of $CD11b^+F4/80^+$ macrophages differentiated from BMDM with or without VBL treatment for 24h (n=6 per group).

J-K. ELISA of IL-12 (J) and IFNγ (K) levels in CM of $CD8^+$ T cells cultured in vitro (n=4 per group).

L-M. ELISA of IL-12 (L) and IFNγ (M) levels in CM of $CD8^+$ T cells sorted from tumors (n=4 per group).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 3: VBL targets NF-κB-Cyba to activate CD8$^+$ T cells

A. RNA-sequencing data was collected from macrophages (Mϕ in vivo) sorted and analyzed from Vehicle/VBL treated tumors, and the heat map was created based on normalized FPKM of genes in the regulation of the ROS production pathway.

B. Gene expression of Mmp8, Cyba, Cd36, Dcxr, and Alox5 in BMDMs with or without VBL treatment for 24h (n=4 per group).

C. Representative western blotting against p-NF-κB, NF-κB, p22phox, and β-actin in BMDMs with or without VBL treatment for 24h.

D. Fold change of p-NF-κB, NF-κB, and p22phox protein levels in panel (C) (n=3 per

group).

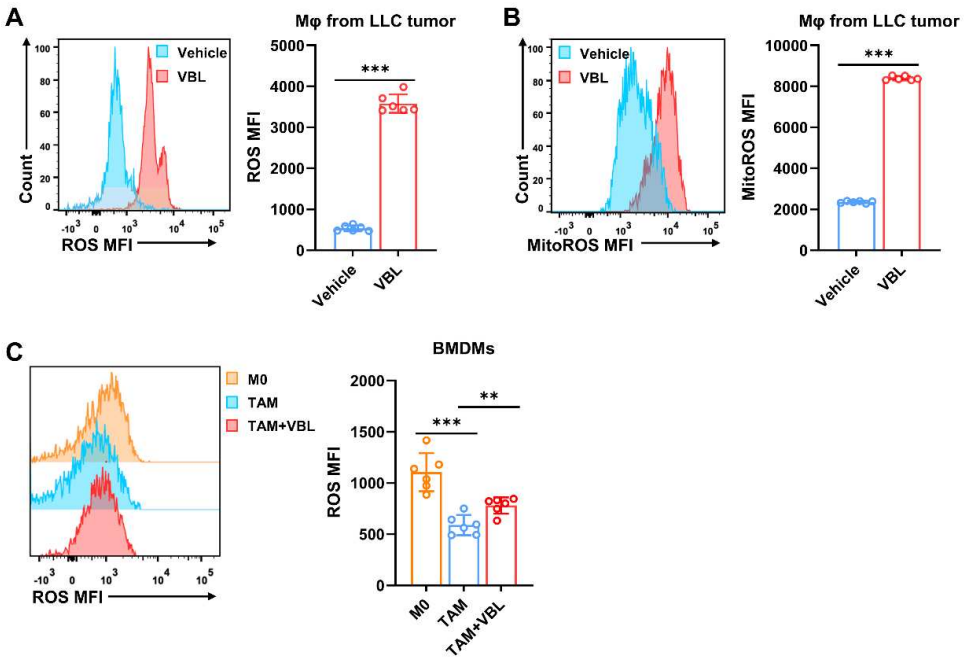
E. Flow cytometry analysis for CD86 and CD206 MFI of BMDMs after the corresponding treatment for 24h (n=6 per group).

F. Gene ontology analysis of RNA-sequencing data collected from BMDMs (Mϕ in vitro) with or without VBL treatment for 24h.

G-H. RNA-sequencing data was collected from BMDMs with or without VBL treatment for 24h, and the heat map was created based on normalized FPKM of genes in the IL-12 production pathway (G) and IFNγ production pathway I-K. (H).

I-K. RNA-sequencing data was collected from BMDMs with or without VBL treatment for 24h, and the GSEA maps were created based on normalized FPKM of genes in the NF-κB pathway (I), TNF signaling pathway (J) and T cell cytotoxicity pathway (K).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

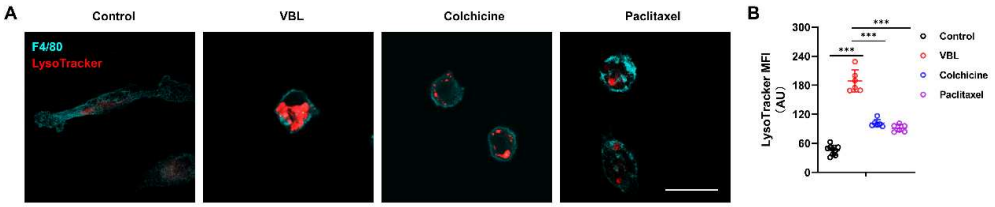


Supplementary Figure 4: VBL induces ROS generation through the Cyba pathway

A-B. Flow cytometry analysis of cytosolic ROS (A) and mitoROS (B) levels in macrophages sorted from Vehicle/VBL-treated LLC tumors (n=6 per group).

C. Flow cytometry analysis of cytosolic ROS levels in BMDMs after VBL treatment for 24h (n=6 per group).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

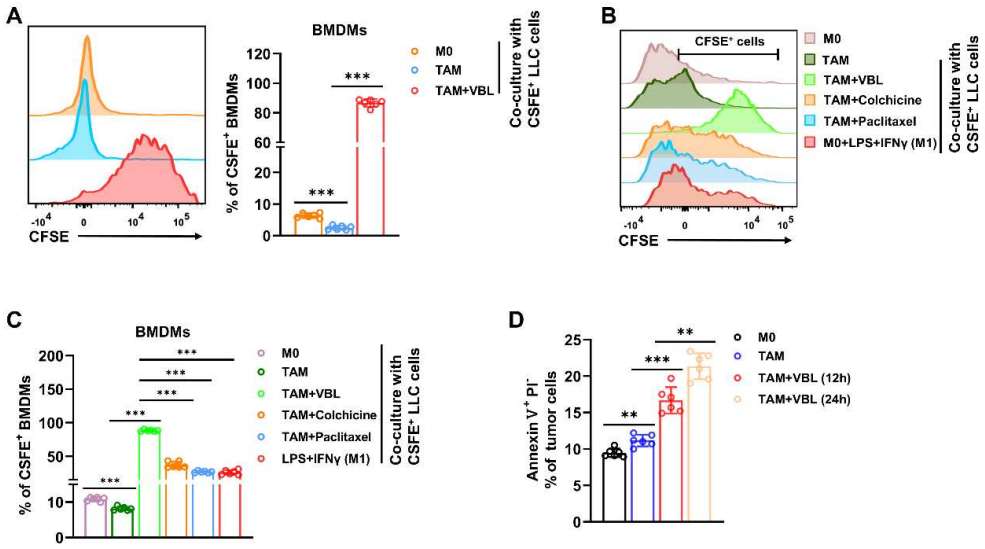


Supplementary Figure 5: VBL promotes lysosome activation and biogenesis

A. Immunostaining with F4/80 and LysoTracker showed the changes in lysosome levels in BMDMs after different treatments for 24h.

B. Statistical results of LysoTracker MFI in panel (A) (n=6 per group).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 6: VBL reprograms macrophages to be tumoricidal by increasing the phagocytic capacity

A. Flow cytometry analysis of CFSE+ BMDMs co-cultured with CFSE+ LLC tumor cells (n=6 per group).

B. Representative flow cytometry results of BMDMs after different treatments and co-culture with CFSE+ LLC tumor cells.

C. Statistical results of CFSE+ BMDMs in panel (B) (n=6 per group).

D. Flow cytometry analysis of apoptotic tumor cells (AnnexinV+PI-) after BMDM conditioned medium treatment for 12 or 24 hours (n=6 per group).

Data are presented as mean ± SD. P values were determined by a two-tailed Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Table 1. List of primers sequences used in this study.

Gene Name	Forward Primer Sequence	Reverse Primer Sequence
18S rRNA	CGCCGCTAGAGGTGAAATTCT	CATTCTTGGCAAATGCTTTCG
TNFα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
NOS2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Arg1	AGGAGCTGTCATTAGGGACATC	CTCCAAGCCAAAGTCCTTAGAG
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Arg2	TCCTCCACGGGCAAATTCC	TCCTCCACGGGCAAATTCC
IL-12	ATGCGTTACAAGCTCAAG	ATGGCTTCAGCTGCAAGTTC
Cyba	TGCCAGTGTGATCTATCTGCT	TCGGCTTCTTTCGGACCTCT
Mmp8	TGCCACGATGGTTGCAGAG	AGGCATTTCCATAATCCCCATTG