

The nuclear factor-κ B inhibitor SN50 enhances the efficacy of B-cell maturation antigen-targeted chimeric antigen receptor T-cell therapy

Authors

Jinlan Long,^{1*} Shufeng Xie,^{2*} Wenjie Zhang,^{1*} Weiyu Dou,³ Yixin Wang,² Ruiheng Wang,² Han Liu^{2#} and Zhenshu Xu^{1#}

¹Fujian Institute of Hematology, Fujian Provincial Key Laboratory of Hematology, Fujian Medical University Union Hospital, Fuzhou;
²Shanghai Institute of Hematology, State Key Laboratory of Medical Genomics, National Research Center for Translational Medicine at Shanghai, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine and School of Life Sciences and Biotechnology, Shanghai and ³Emergency Department, Chinese PLA Southern Theater Command General Hospital, Guangzhou, China

**JL, SX, and WZ contributed equally as first authors.*

#ZX and HL contributed equally as senior authors.

Correspondence:
Z. XU - xuzs@fjmu.edu.cn
H. LIU - liuhan68@sjtu.edu.cn
J. LONG - longjinlan2023@163.com
<https://doi.org/10.3324/haematol.2024.286689>

Received: September 21, 2024.
Accepted: January 14, 2025.
Early view: January 23, 2025.


©2025 Ferrata Storti Foundation
Published under a CC BY-NC license 

Figure S1. Sorting patterns of B-cell maturation antigen-targeted chimeric antigen receptor T-cells and MM cell lines co-culture and nuclear factor- κ B pathway activation after treatment with co-culture supernatants. (A) Multiple myeloma (MM) cells were labeled with CellTrace Violet dye before co-culturing and co-cultured with T-cells or B-cell maturation antigen (BCMA)-targeted chimeric antigen receptor (CAR) T-cells (353T, 917T, 353/917T) at an effector-to-target ratio of 1:50 for 16 hours. MM cells were sorted using a flow cytometer based on the BV421 channel, and the purity of the sorted MM cells was verified by flow cytometry analysis. (B) MM cell lines were co-cultured with T-cells or BCMA CAR T-cells (353T, 917T, 353/917T) at an effector-to-target ratio of 1:50 for 16 hours. Supernatants were collected from these co-cultures and used to treat fresh MM cell lines for 16 hours. After treatment, proteins were extracted from MM cells and subjected to Western Blot analysis to assess the activation of the nuclear factor-kappa B pathway.

Figure S2. Effects of SN50 on apoptosis, memory phenotype, exhaustion, activation and cytokine secretion in T-cells and B-cell maturation antigen-targeted chimeric antigen receptor T-cells. (A) Apoptosis in T-cells and B-cell maturation antigen (BCMA)-targeted chimeric antigen receptor (CAR) T-cells (353T, 917T, 353/917T) treated with DPBS or SN50 at concentrations of 0, 2.5, 5.0, and 10 μ M for 16 hours. (B) Memory phenotype changes in T-cells and BCMA CAR T-cells (353T, 917T, 353/917T) after 72 hours of co-culture with MM.1S cells at an effector-to-target (E:T) ratio of 1:1 in the presence of 2.0 μ M SN50 or DPBS, including naïve T-cells, central memory T-cells, effector memory T-cells, and terminally differentiated effector memory T-cells. (C) LAG-3 expression on T-cells and BCMA CAR T-cells (353T, 917T, 353/917T) after 48 hours of co-culture with MM.1S cells at an E:T ratio of 1:1 in the presence of 2.0 μ M SN50 or DPBS. (D) CD69 expression on T-cells and BCMA CAR T-cells (353T, 917T, 353/917T) after 16 hours of co-culture with MM.1S cells at an E:T ratio of 1:1 in the presence of 2 μ M SN50 or DPBS. (E-H) Cytokine secretion by BCMA CAR T-cells (353T, 917T,

353/917T) after 16 hours of co-culture with MM.1S cells at an E:T ratio of 1:10 in the presence of 2.0 μ M SN50 or DPBS. The supernatants were collected for detection of cytokines by LEGENDplexTM Human CD8/NK Panel. Data are presented as mean \pm SD from three independent experiments. Statistical significance was determined by t-test, with p-values indicated as follows: **p < 0.01, ****p < 0.0001, ns represent no significant.

Figure S3. Enhancement of B-cell maturation antigen-targeted chimeric antigen receptor T-cells cytotoxicity by the nuclear factor- κ B pathway inhibitor IKK γ NBD inhibitory peptide TFA. (A-C) Multiple myeloma cell lines MM.1S (A), ARP-1 (B), and ARD (C) were co-cultured with T-cells or B-cell maturation antigen-targeted chimeric antigen receptor T-cells (353T, 917T, and 353/917T) at an effector-to-target ratio of 1:10 for 16 hours in the presence of 50 nM IKK γ NBD inhibitory peptide TFA. The cytotoxic activity was assessed using flow cytometry by staining the cells with annexin V and 7-AAD, and the percentage of dead target cells was quantified. Data are presented as mean \pm SD from three independent experiments. Statistical significance was determined by t-test, with p-values indicated as follows: *p < 0.05, **p < 0.01, ns represent no significant.

Figure S1

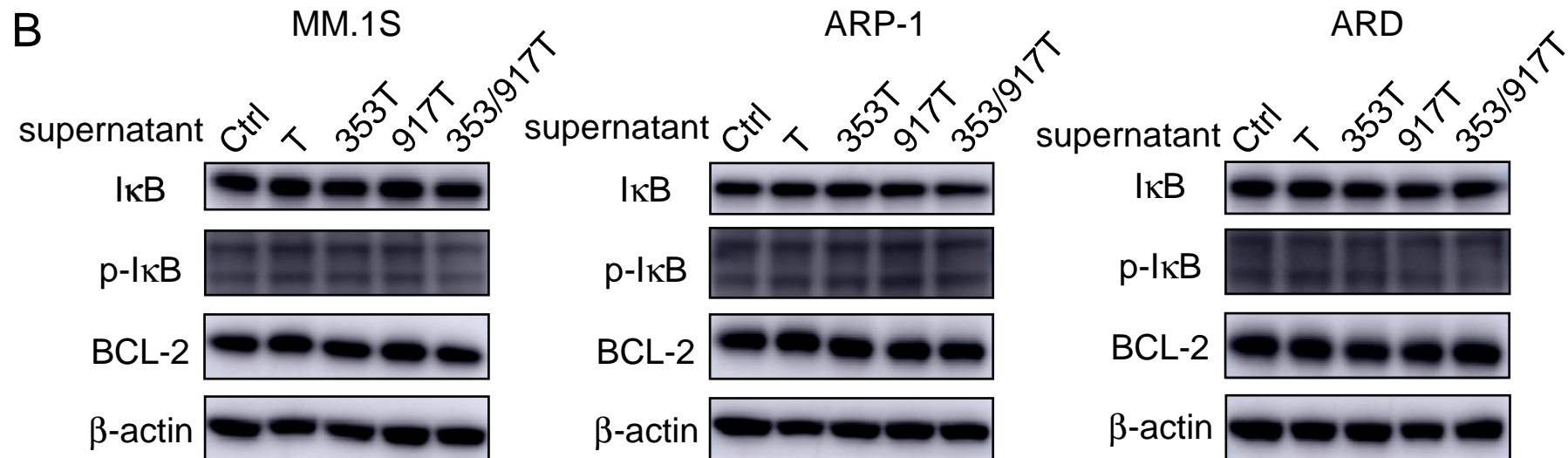
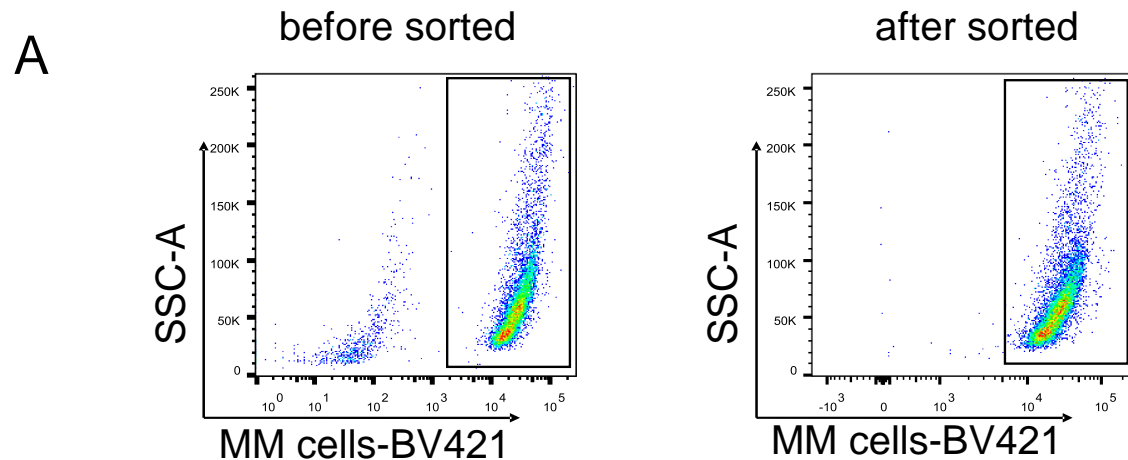


Figure S2

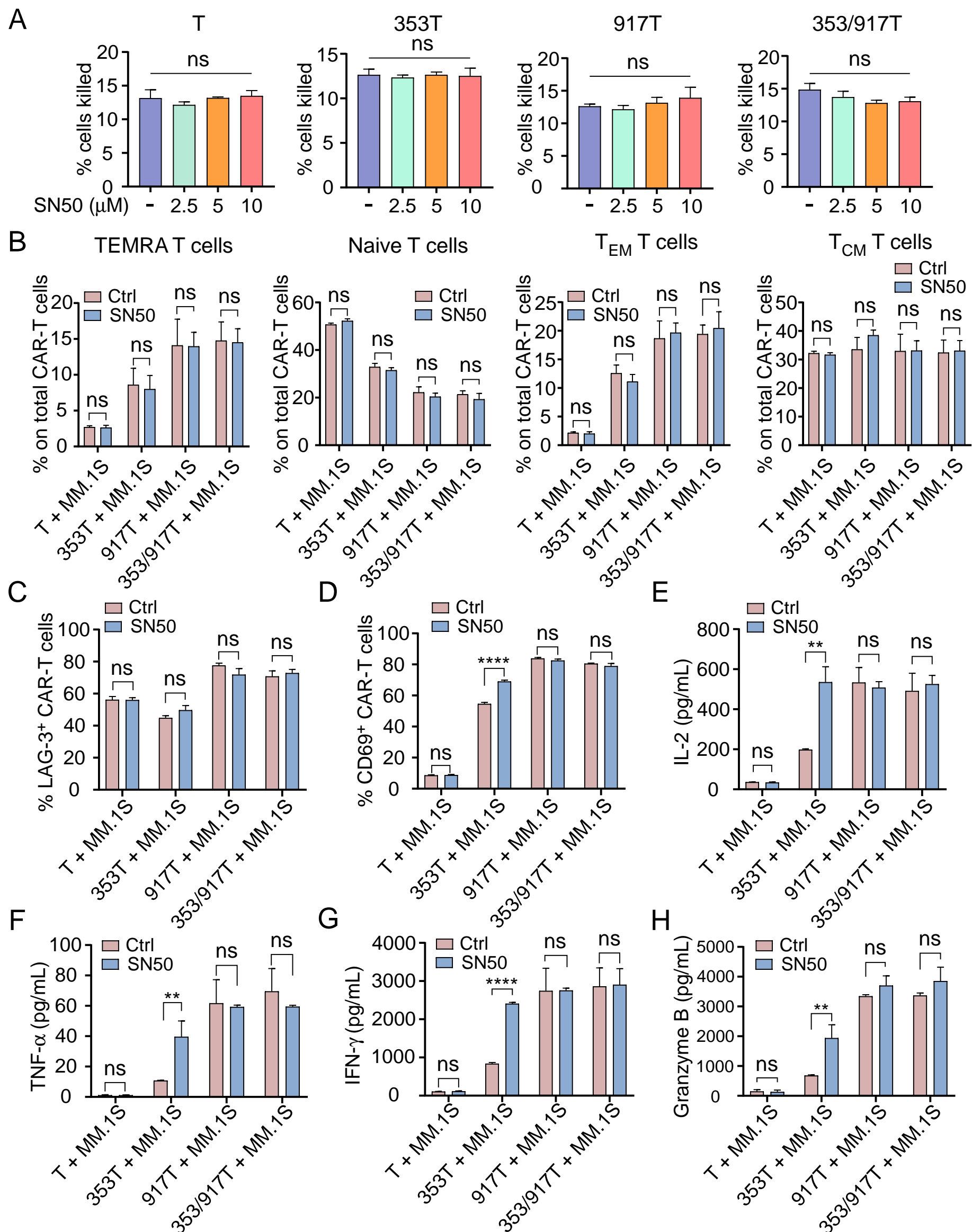
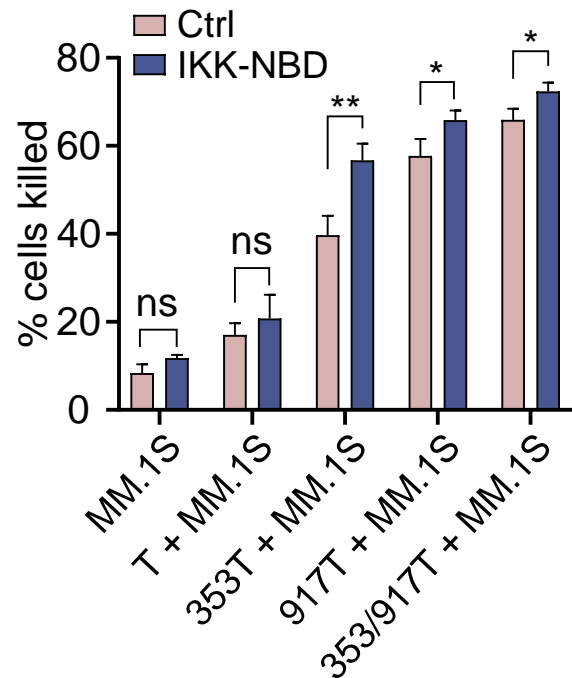
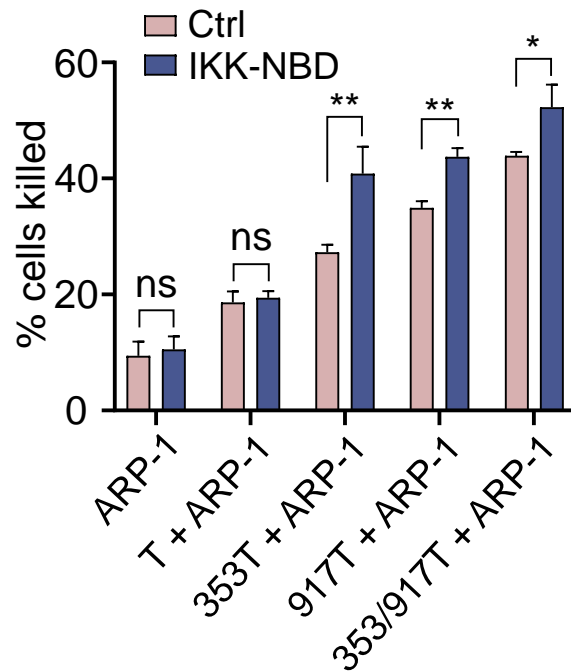


Figure S3

A



B



C

