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LETTER

# Vitamin D potentiates STING agonist-induced antitumor immunity in NSCLC

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Non-small cell lung cancer (NSCLC) has emerged as a major model for the clinical success of immune checkpoint inhibitors, which have significantly improved survival in a subset of patients[1,2]. However, durable clinical benefits remain limited, because many patients exhibit primary or acquired resistance to immunotherapy[3]. The mechanisms governing therapeutic response and resistance within the tumor microenvironment (TME) remain incompletely understood.

The cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway has emerged as a central regulator of innate immunity and an attractive target for cancer immunotherapy[4-6]. However, aberrant activation of cGAS-STING signaling within the TME, particularly when it is excessive or sustained, can paradoxically induce immunosuppressive feedback, systemic inflammation, and treatment-associated toxicity[7,8].

Accumulating evidence further suggests that alleviating T cell exhaustion in the TME through nutritional or metabolic interventions is a critical strategy for restoring effective antitumor immunity[9-13]. Collectively, these observations underscore the need for a more nuanced understanding of STING biology, and further support the rational integration of nutritional strategies to safely and effectively harness STING-driven antitumor immune responses.

## Vitamin D potentiates STING-mediated antitumor immunity through CD8$^+$ T cells

To explore the clinical relevance of this concept, we retrospectively enrolled patients with NSCLC diagnosed between January 2020 and January 2021. Clinicopathological characteristics, treatment regimens, and follow-up data were systematically collected (**Figure 1A–C** and **Table S1**). Notably, expression of the vitamin D receptor (VDR) and STING in peripheral blood mononuclear cells (PBMCs) was significantly higher in responders (with complete response, partial response, or stable disease) than in non-responders (with progressive disease) (**Figure 1D, E**). Consistently, among patients treated with anti-PD-1 antibodies in combination with chemotherapy, responders exhibited significantly higher serum levels of 25-hydroxyvitamin $D_3$ [25(OH)$D_3$] than non-responders (**Figure 1F**). Immunohistochemical analysis further confirmed that a substantial number of CD8$^+$ T cells infiltrated tumor tissues in patients with NSCLC (**Figure 1G**). Furthermore, the proportions of IFN-$\gamma^+$ and TNF-$\alpha^+$ cells within circulating CD8$^+$ T cells were also higher in responders than non-responders (**Figure 1H, I**).

Extensive evidence indicates that antibiotic administration compromises immunotherapy efficacy in patients with cancer and correlates with diminished abundance of beneficial gut microbiota[14,15]. Interestingly, patients with NSCLC who did not receive antibiotic treatment exhibited higher serum 25(OH)$D_3$ levels than those who did (**Figure S1A**). Subsequent analysis further demonstrated that, within the responder subgroup, antibiotic-naïve patients had significantly higher serum 25(OH)$D_3$ concentrations than their antibiotic-treated counterparts (**Figure S1B, C**). Therefore, antibiotic exposure might alter serum vitamin D levels by modulating the gut microbiota,

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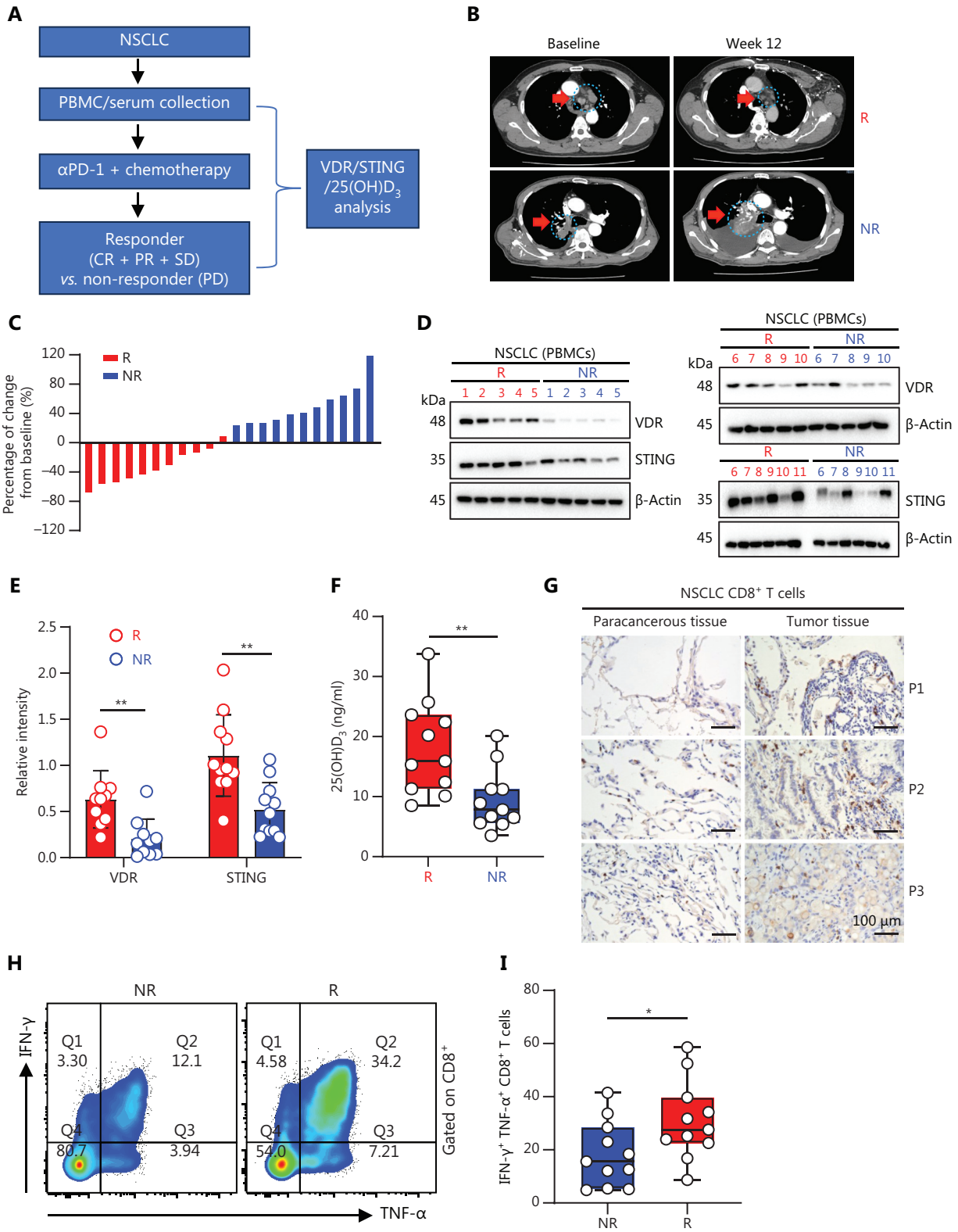


**Figure 1** Continued

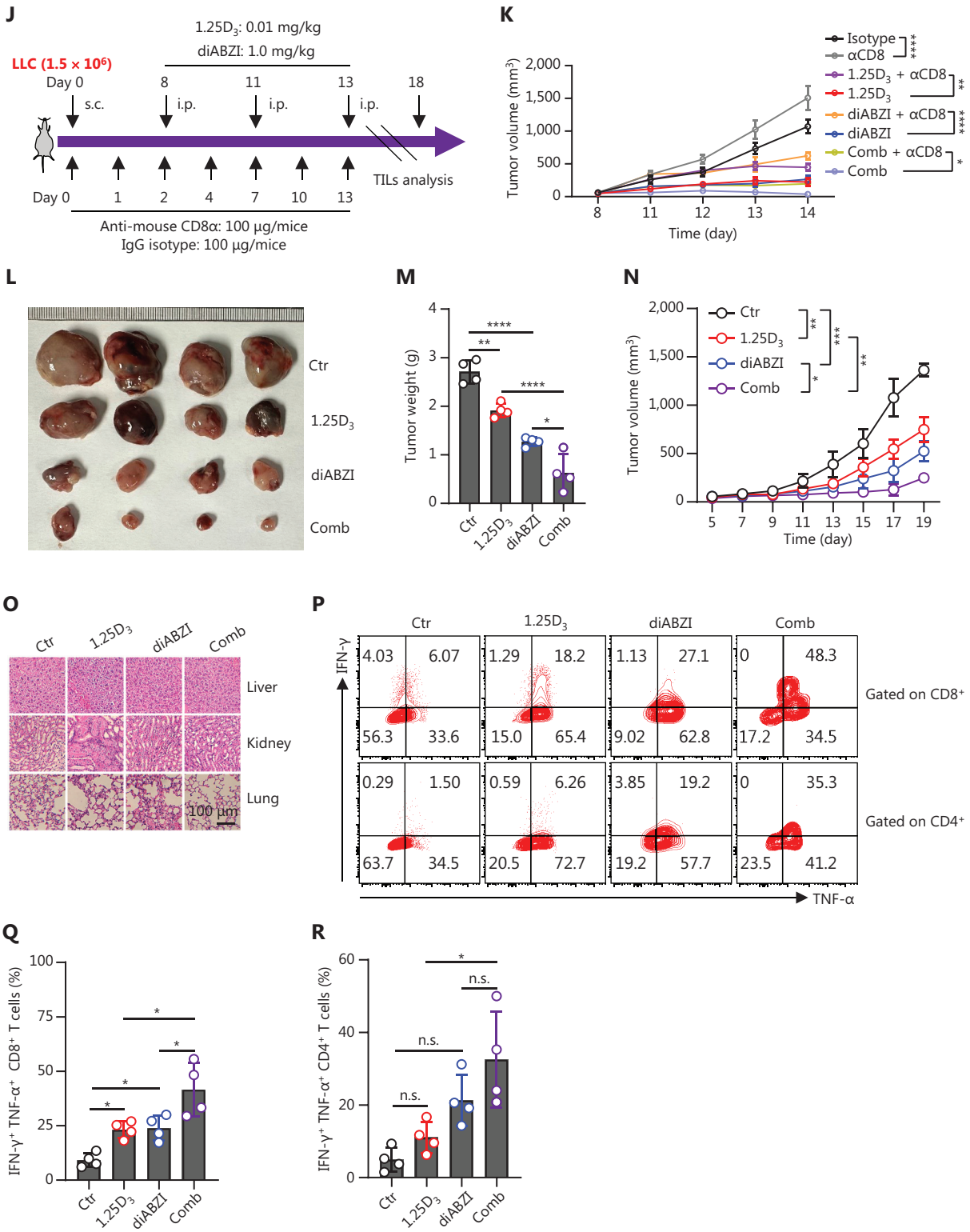


**Figure 1** Vitamin D, in synergy with STING activation, potentiates CD8⁺ T cell-mediated antitumor immunity. (A) Overview of experimental design. (B) CT scans showing changes in tumor sizes in patients with NSCLC. Responder (R) *vs*. non-responder (NR). (C) Waterfall plot depicting changes in tumor size in patients with NSCLC who received the specified treatment ($n$ = 11 per group). (D) VDR ($n$ = 10) and STING ($n$ = 11) expression levels in PBMCs from patients with NSCLC. (E) Relative levels of VDR and STING in R *vs.* NR groups, determined by western blotting.

(F) Serum levels of $25(OH)D_3$ in R *vs*. NR groups ($n$ = 11 per group). (G) Immunohistochemistry detection of CD8 expression in tumor tissues from patients with NSCLC. Scale bar = 100 μm. (H, I) Expression of IFN-$\gamma^+$ and TNF-$\alpha^+$ in circulating $CD8^+$ T cells isolated from patients with NSCLC classified as responders or non-responders ($n$ = 11 per group). (J) Experimental design for LLC tumor models. (K) Tumor growth in mice in each group at the end of the experiment ($n$ = 6 per group). Comb: $1\alpha,25(OH)_2D_3$ + diABZI. (L–N) tumor weight (L, M) and tumor growth (N) ($n$ = 4 per group). (O) Tissue pathology of the specified organs, assessed through hematoxylin and eosin staining on day 18 post-tumor implantation. Scale bar = 100 μm. (P–R) Levels of IFN-γ and TNF-α in TILs-$CD4^+$/$CD8^+$ T cells after $1\alpha,25(OH)_2D_3$ ($1.25D_3$) or diABZI treatment, alone or in combination ($n$ = 4 per group). All mouse experiments were conducted as independent biological replicates. Two-tailed unpaired Student's $t$-test (E, F, and I). Two-way ANOVA with Tukey's multiple comparisons test (K and N). One-way ANOVA with Tukey's multiple comparisons test (M, Q, and R). Data are presented as mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, n.s., not significant.

which in turn is closely associated with the therapeutic efficacy of immunotherapy.

To determine whether the observed antitumor effects were mediated by $CD8^+$ T cells, we subcutaneously inoculated mice with LLC tumor cells. $CD8^+$ T cells were depleted with anti-mouse CD8α antibodies and subsequent treatment with vitamin D, the STING agonist diABZI, or both (**Figure 1J**). Depletion of $CD8^+$ T cells markedly attenuated the antitumor efficacy of the combined vitamin D and diABZI treatment (**Figure 1K**). In agreement with these findings, vitamin D in combination with diABZI significantly suppressed tumor growth in mouse lung cancer models (**Figure 1L–N**). Hematoxylin and eosin staining revealed no overt tissue damage in mice receiving vitamin D, diABZI, or combination therapy (**Figure 1O**). Flow cytometric analysis revealed that the frequencies of tumor-infiltrating lymphocytes (TILs), including $CD4^+$ and $CD8^+$ T cells, were not significantly altered by vitamin D, diABZI, or their combination (**Figure S2A–D**). In contrast, vitamin D supplementation significantly alleviated exhaustion in $CD8^+$ TILs (**Figure S2E–G**). Moreover, combined vitamin D and diABZI treatment, compared with either monotherapy, further enhanced the proportion of IFN-$\gamma^+$ and TNF-$\alpha^+$ $CD8^+$ TILs (**Figure 1P–R**).

## Vitamin D enhances T cell receptor signaling and alleviates exhaustion in $CD8^+$ T cells

To further elucidate the molecular mechanisms underlying these effects, we treated $CD8^+$ T cells from healthy donors with vitamin D, diABZI, or their combination, and conducted RNA sequencing analysis. Differentially expressed genes were identified and analyzed with gene set enrichment analysis (GSEA). Compared with single-agent treatments, the combination of vitamin D and diABZI significantly upregulated cytokine genes, including *IL2*, *IL15*, and *IFNG* (**Figure 2A**). GSEA further revealed enrichment in pathways associated with oxidative phosphorylation and cytosolic DNA sensing in response to the combined treatment (**Figure 2B**). Importantly, vitamin D combined with diABZI led to significantly greater IFN-γ production in human $CD8^+$ T cells than either agent alone, whereas expression of TNF-α, perforin, and granzyme B remained unchanged after αCD3/CD28 stimulation (**Figures 2C, D and S3A**). Specifically, vitamin D significantly decreased the expression of PD-1, Tim-3, TIGIT, and LAG3, and enhanced the level of CD28 on human $CD8^+$ T cells (**Figure S3B–D**). At the protein level, vitamin D markedly decreased PD-1 expression on human $CD8^+$ T cells, whereas diABZI alone did not significantly alter PD-1 or Tim-3 expression *in vitro* (**Figure S3E–G**). To directly assess the effects of vitamin D on T cell exhaustion, we established an *in vitro* $CD8^+$ T cell exhaustion model through prolonged stimulation with plate-bound anti-CD3 antibodies (**Figure 2E**). Vitamin D treatment significantly alleviated T cell exhaustion, as evidenced by decreased frequencies of PD-$1^+$Tim-$3^+$ and PD-$1^+$TIGIT$^+$ $CD8^+$ T cells (**Figures 2F–H and S3H**).

However, how vitamin D and STING agonists synergistically exert antitumor activity remained unclear. To investigate this mechanism, we pretreated $CD8^+$ T cells with vitamin D or vehicle, then activated them with anti-CD3/CD28 for 120 s at 37°C. The cells were then subjected to phosphoproteomic analysis (**Figure 2I, J**). Differential phosphorylation sites associated with the T cell receptor signaling pathway, including LAT (S84), LCK (Y394), and CD3γ (S148), were upregulated in vitamin D-pretreated $CD8^+$ T cells (**Figure 2K, L**). On the basis of fold changes, we categorized differentially modified sites into four groups: Q1, Q2, Q3, and Q4. Notably, significantly upregulated phosphorylation sites were highly enriched in natural killer cell-mediated cytotoxicity signaling and T cell receptor signaling pathways (**Figures 2M, N and S4A–D**). Our model highlights vitamin D supplementation as a promising strategy to enhance both the efficacy and safety of STING-based immunotherapy (**Figure 2O**).

In summary, our findings uncovered a previously unrecognized crosstalk between vitamin D signaling and the STING pathway, and identified a vitamin D–STING axis as a critical regulator of antitumor immunity. Vitamin D supplementation might therefore offer a rational and safe strategy to optimize STING-based immunotherapies and enhance their efficacy while mitigating immune exhaustion and toxicity.

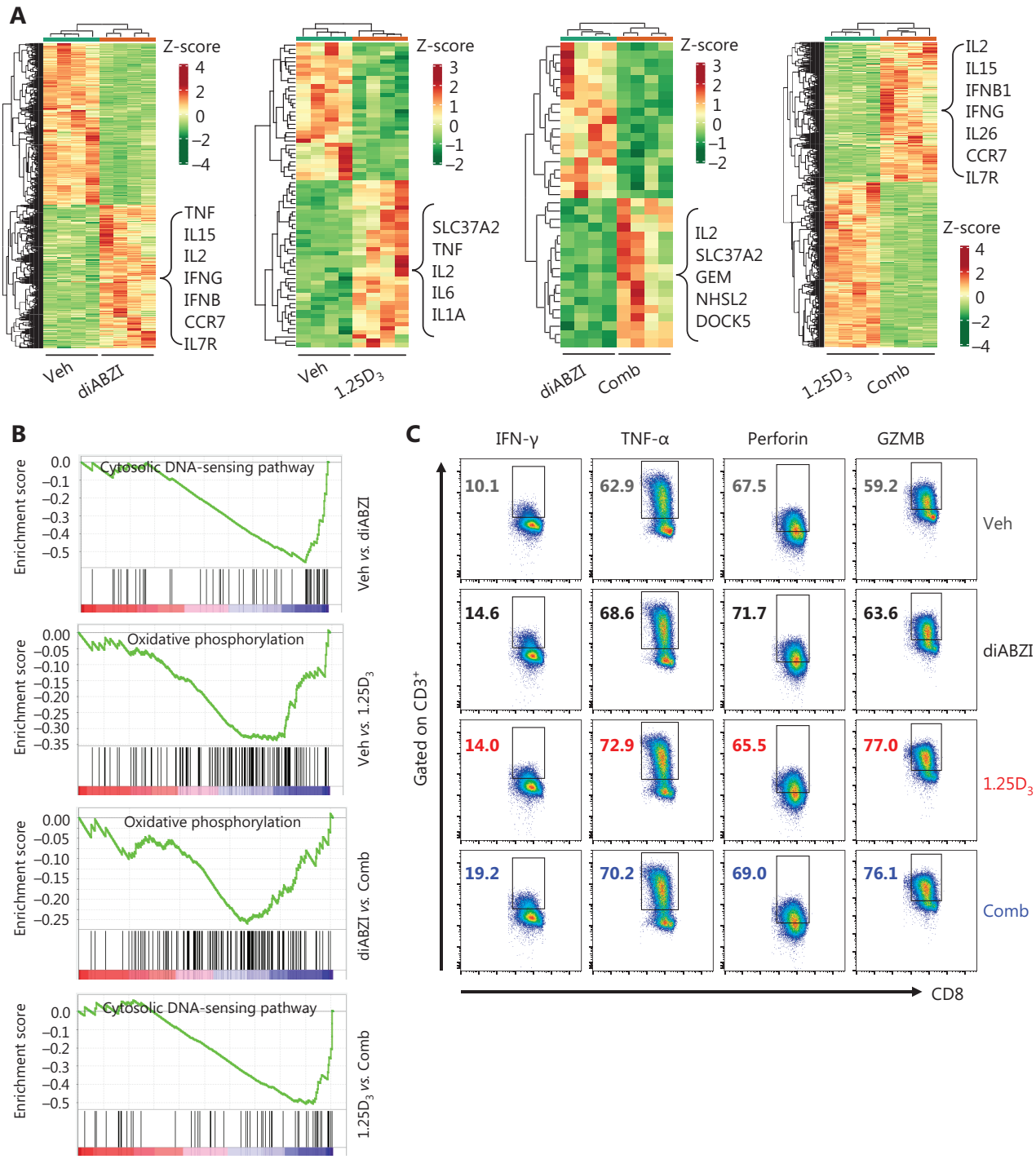


**Figure 2** Continued

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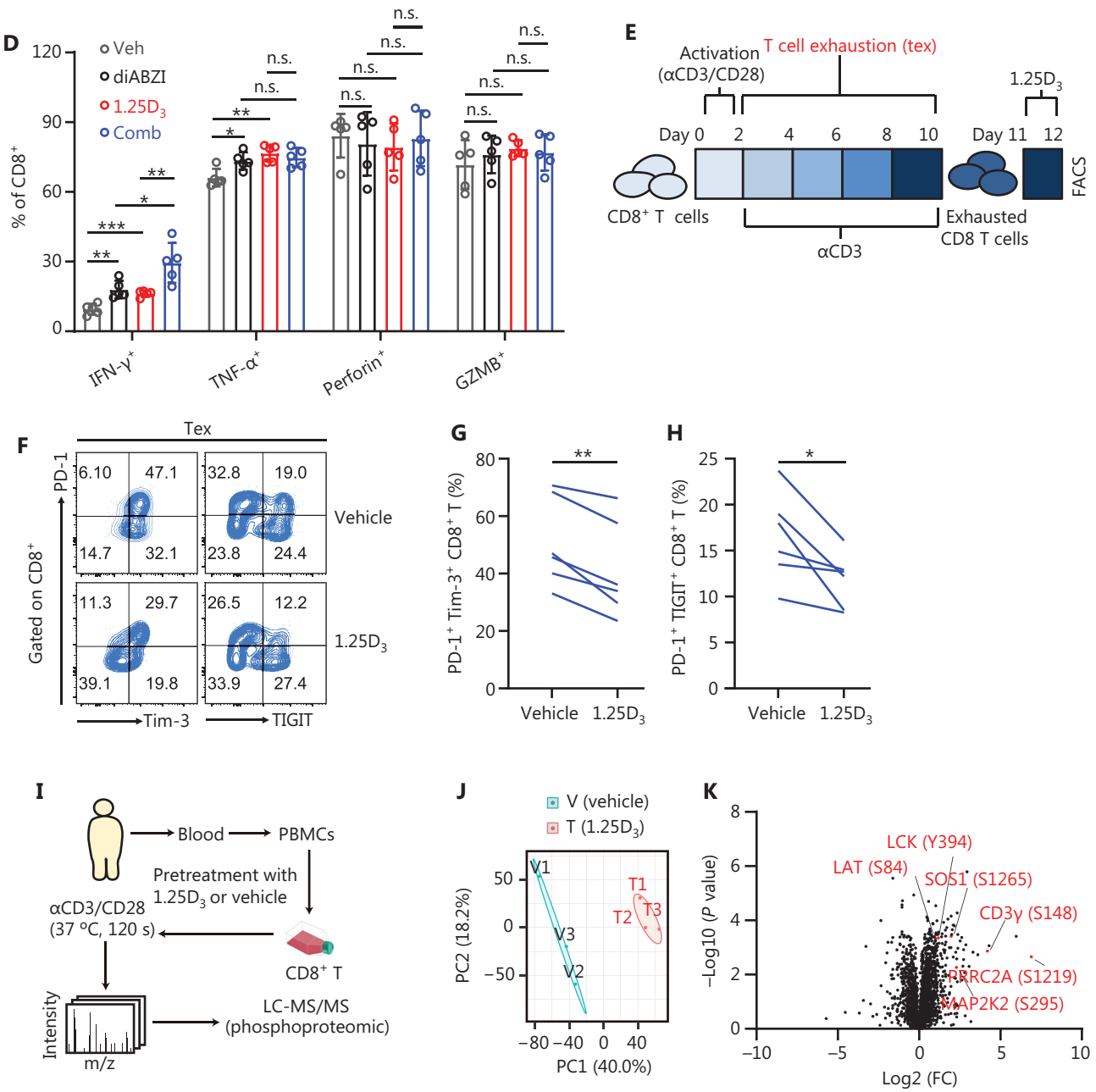


**Figure 2** Continued

## Conflict of interest statement

No potential conflicts of interest are disclosed.

## Author contributions

Conceived and designed the analysis: Peng Li, Ke Li, Guichao Liu.
Collected the data: Peng Li, Ke Li, Xinhai Zhu, Guichao Liu.
Contributed data or analysis tools: Wenhui Yuan, Xinhai Zhu.
Performed the analysis: Peng Li, Ke Li, Guichao Liu.
Wrote the paper: Peng Li, Ke Li.

## Data availability statement

The raw sequencing data reported herein have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics, 2021) at the National Genomics Data Center

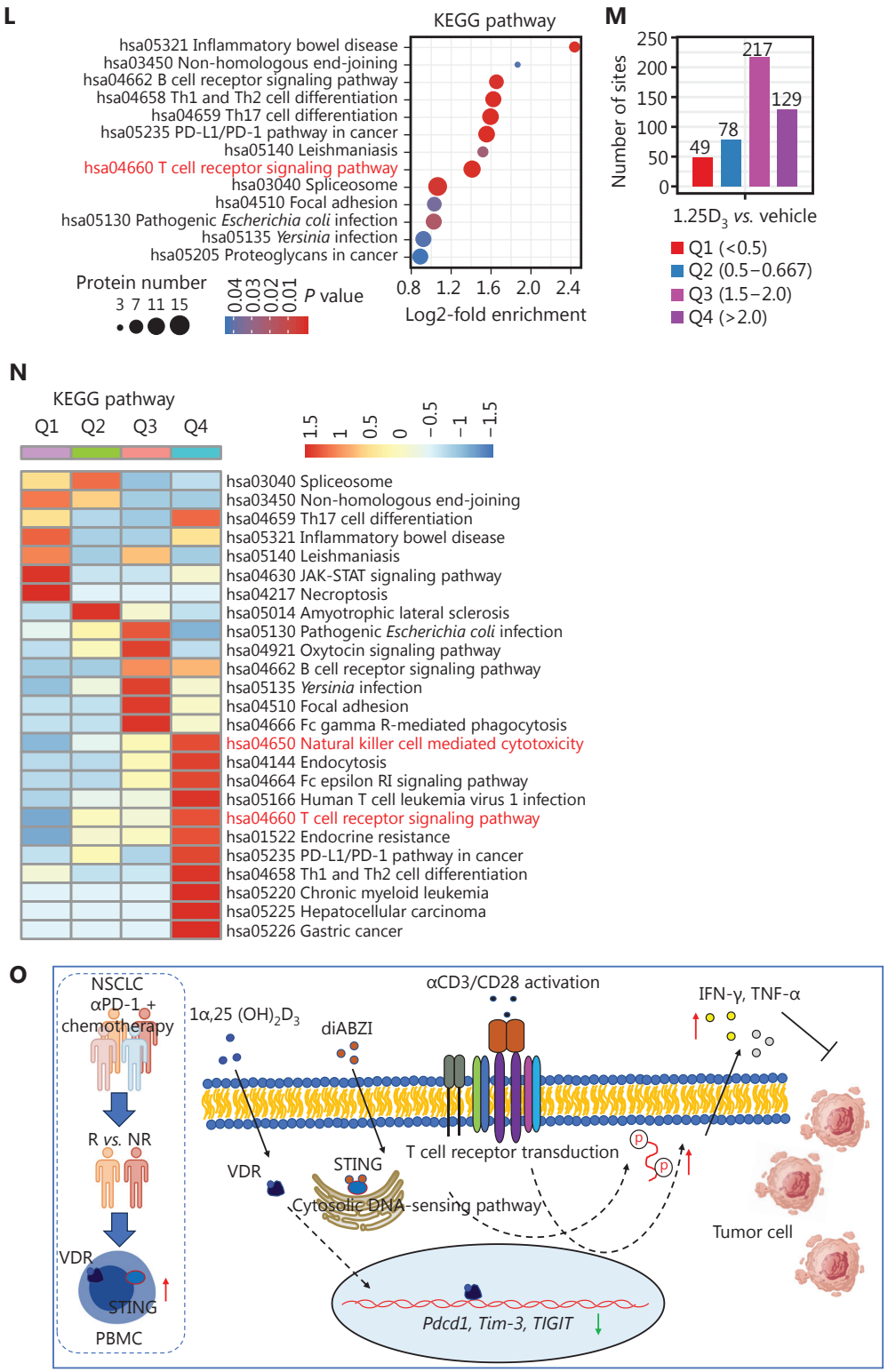


**Figure 2** Vitamin D alleviates immune exhaustion, enhances TCR signaling, and acts in synergy with STING agonists in potentiating antitumor immunity. (A) Heatmaps showing transcriptome analysis of human CD8$^+$ T cells after pretreatment with vehicle or 1.25D$_3$, and subsequent TCR stimulation under diABZI treatment for 4 h ($n$ = 4 per group). (B) GSEA of the cytosolic DNA-sensing pathway and oxidative phosphorylation signaling pathway. (C, D) Flow cytometric analysis of antitumor-related cytokine levels in human CD8$^+$ T cells ($n$ = 5). (E–H) Diagram of the

*in vitro* exhaustion assay. Surface phenotype of human $CD8^+$ T cells on day 13 of the T cell exhaustion assay ($n$ = 6). (I) Workflow for human $CD8^+$ T cell pretreatment and phosphoproteomic analysis. (J) Principal component analysis (PCA) of vehicle and $1.25D_3$ treatments ($n$ = 3). (K) Volcano plot and statistical chart showing differential phosphorylation modification sites. (L) KEGG pathway analysis. (M, N) Data divided into four sections (Q1–Q4) according to fold changes in differential expression (M), followed by KEGG pathway enrichment in each group (N). (O) Expression of the vitamin D receptor and STING is significantly elevated in patients responding to chemotherapy combined with anti-PD-1 therapy, and is associated with improved clinical outcomes in non-small cell lung cancer. Mechanistically, vitamin D mitigates $CD8^+$ T cell exhaustion and enhances T cell receptor signaling, thereby acting in synergy with STING agonists in eliciting durable and robust antitumor immunity. One-way ANOVA with Tukey's multiple comparisons test (D). Paired Student's $t$-test (G and H). Data are presented as mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, n.s., not significant.

(Nucleic Acids Research, 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA008264). The data are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. All data supporting the findings of this study are included herein. Further inquiries can be directed to the corresponding author.

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