

Interleukin (IL)-15 and IL-21 synergistically enhance NK and CD8+T cell responses

Phoi Tiet¹, Valeria Marrocco¹, Dalena Chu¹, Nisar Farhat¹, Stephen Connelly¹, Cherie Ng¹

¹ Equillum Inc., La Jolla, CA 92037

Introduction

- The common γ c chain (γ c, CD132) is a receptor subunit shared by a group of interleukin family members. These cytokines are crucial in regulating major immune responses. Among these cytokines, IL-15 and IL-21, have roles in activation, development, and survival of NK cells and CD8 T cells [1].
- IL-15 is an apex cytokine involved in tissue homeostasis and inflammation. Major inflammatory cytokine that activates tissue resident innate and adaptive immune cells.
- IL-21 is a broad pleiotropic cytokine targeting T, B and epithelial cells. Potent regulatory effects on immune cells including CD8, NK and Th17.
- Stimulation with both IL-15 and IL-21 were found to enhance CD8+ T and NK cytolytic activities and IFN γ production [2,3], suggesting that targeting these two pathways can amplify cell-based immunity.
- New cancer immunotherapy strategies have focused on cytokine pathways with the goal of enhancing anti-tumoral immune responses. Yet, to date, cytokine monotherapy has shown low efficacy and translatability to the clinic [4]. To improve these outcome, a more nuanced, modified approach is needed. One strategy is to target combinations of cytokines with cooperative effects to improve and refine the therapeutic potency.

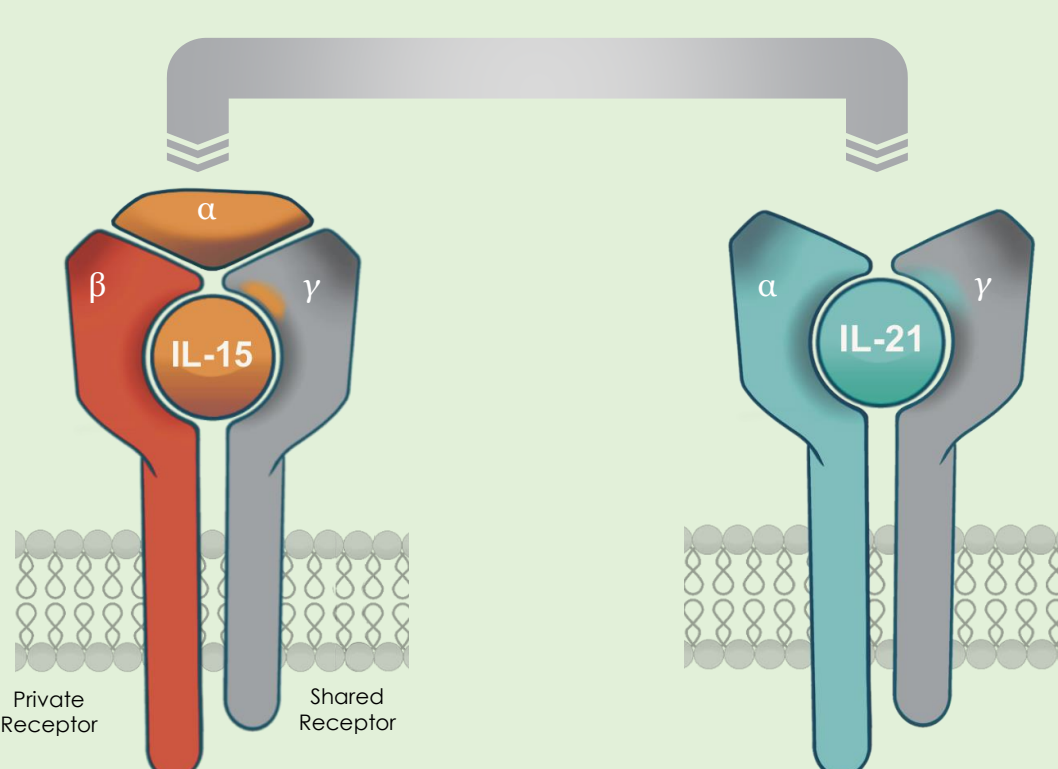
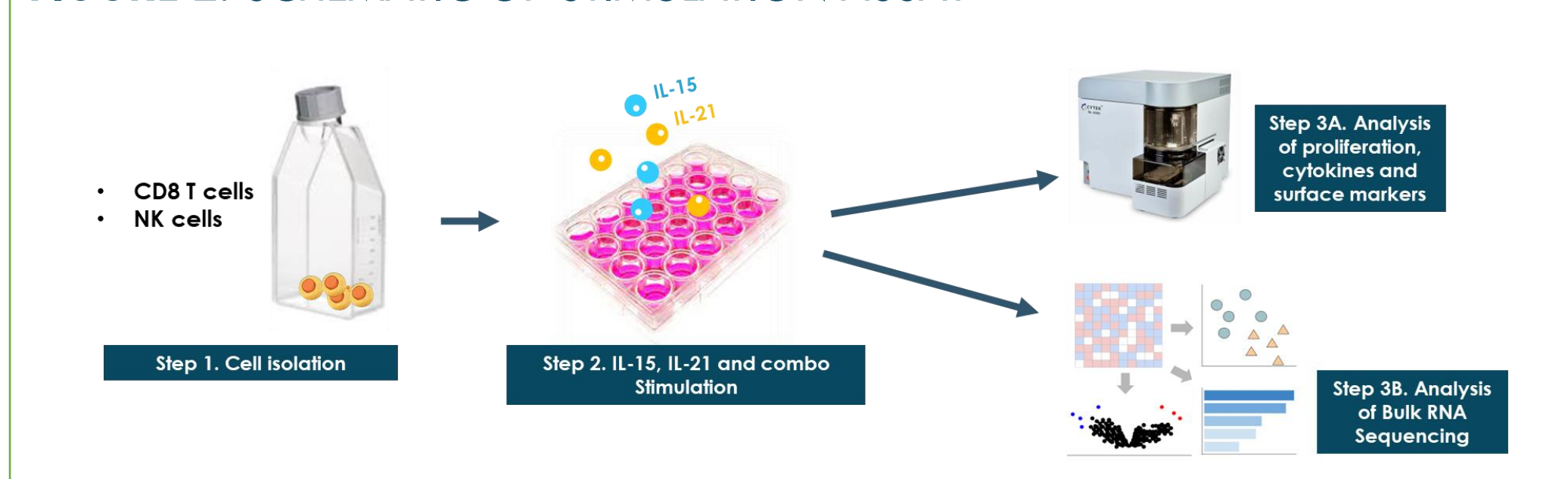


FIGURE 1: IL-15 AND IL-21 SHARED CYTOKINE SIGNALING HUB

Methods

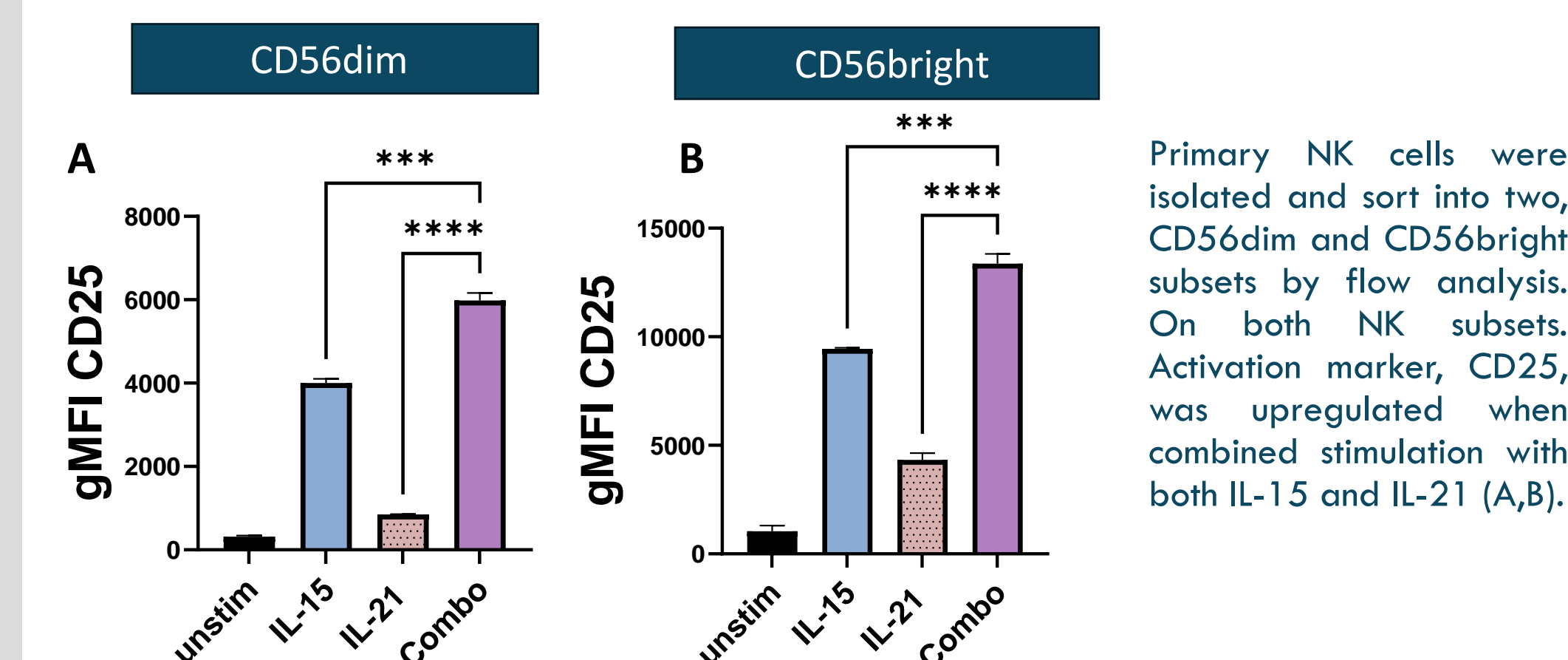
- NK and CD8+ T cell Isolation:** Primary NK and CD8+ T cells were isolated from fresh PBMCs (peripheral blood mononuclear cells) by negative selection using magnetic isolation kits (STEMCELL). Cells were reconstituted at desire density and left to rest on ice during experiment prep.
- Cytokine Stimulation:** NK cells were stimulated with IL-15 alone, IL-21 alone and IL-15 and IL-21 together for 24hr and 72hr at 37°C. While CD8 T cells were stimulated for a total of 7 days.
- Confirmation of Stimulation:** Cells were collected for flow cytometry staining, where additional intracellular staining was performed for CD8 T cells. Supernatant were collected for both cell types and accessed for cytokines by BioLegend's LEGENDplex ELISA. Additional CD8 T cell experiment were set-up for bulk RNA sequencing.
- T cells Exhaustion:** Total PBMCs were incubated with 1ug/mL CEF peptide pool for a total of 14 days. Media exchange with fresh peptide occurred on Day 7. Flow staining was performed on days 0, 7, and 14 to monitor progression of T cell exhaustion.
- On Day 14, cells were collected and re-stimulated with CEF peptide in the presence/absence of IL-15 alone or in combination with IL-21. Cells were incubated for 7 days.

FIGURE 2: SCHEMATIC OF STIMULATION ASSAY



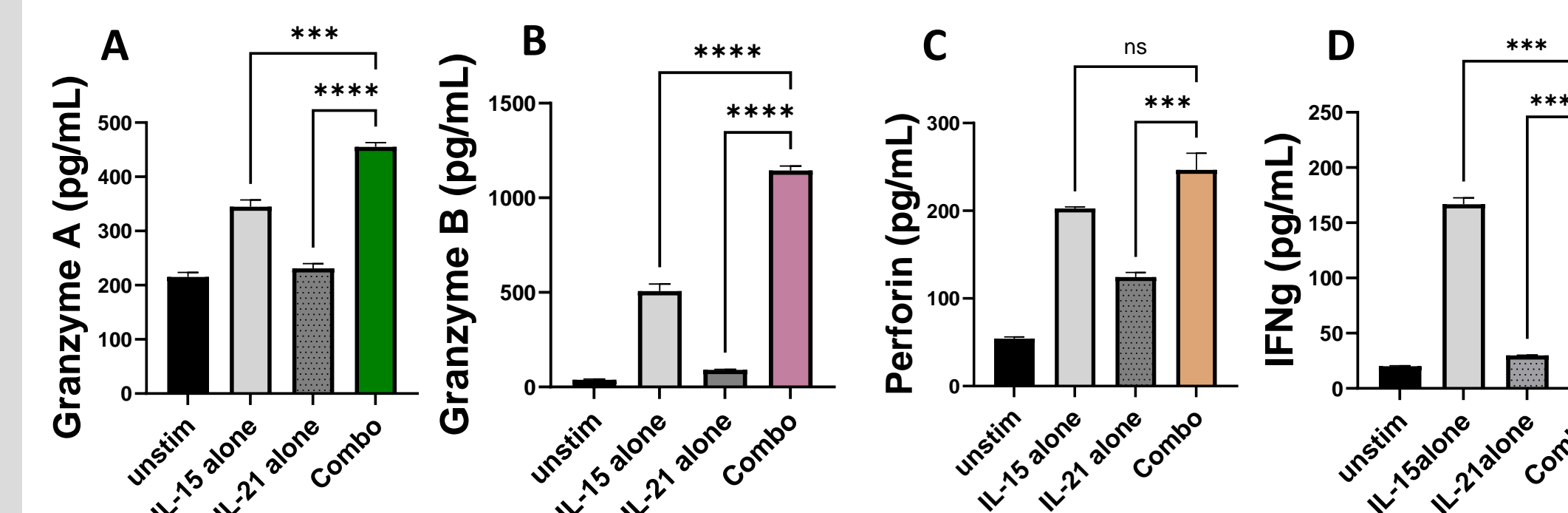
Results

FIGURE 3. IL-15 AND IL-21 COOPERATIVELY INDUCED ACTIVATION IN PRIMARY NK CELLS



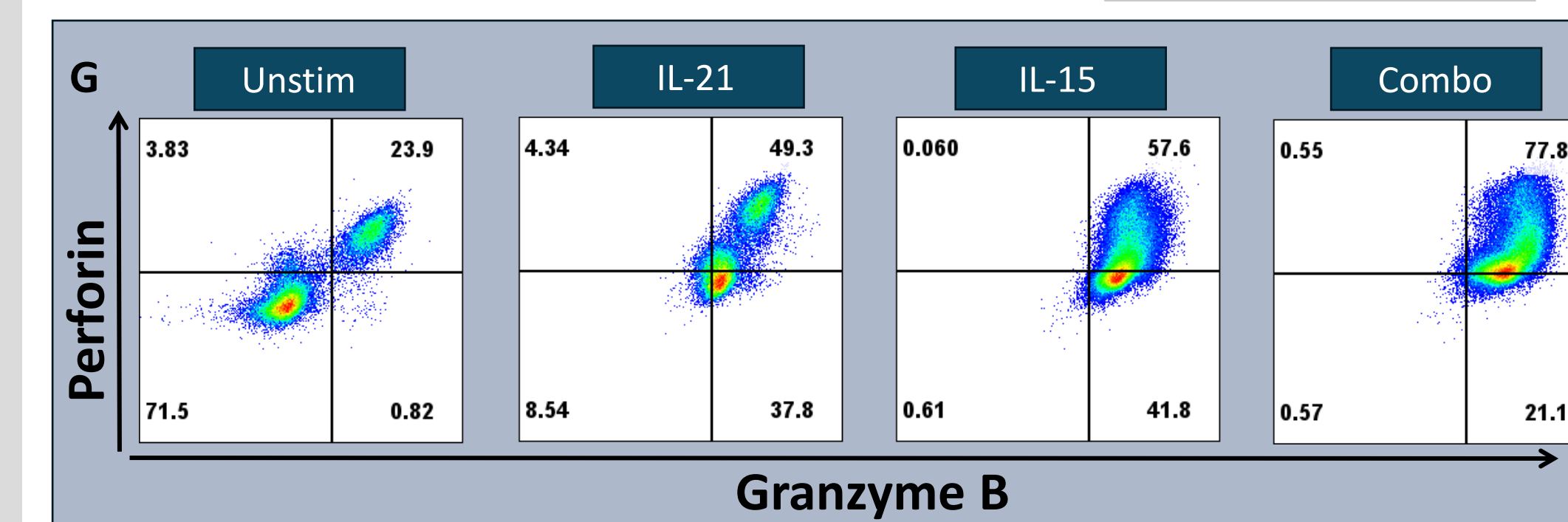
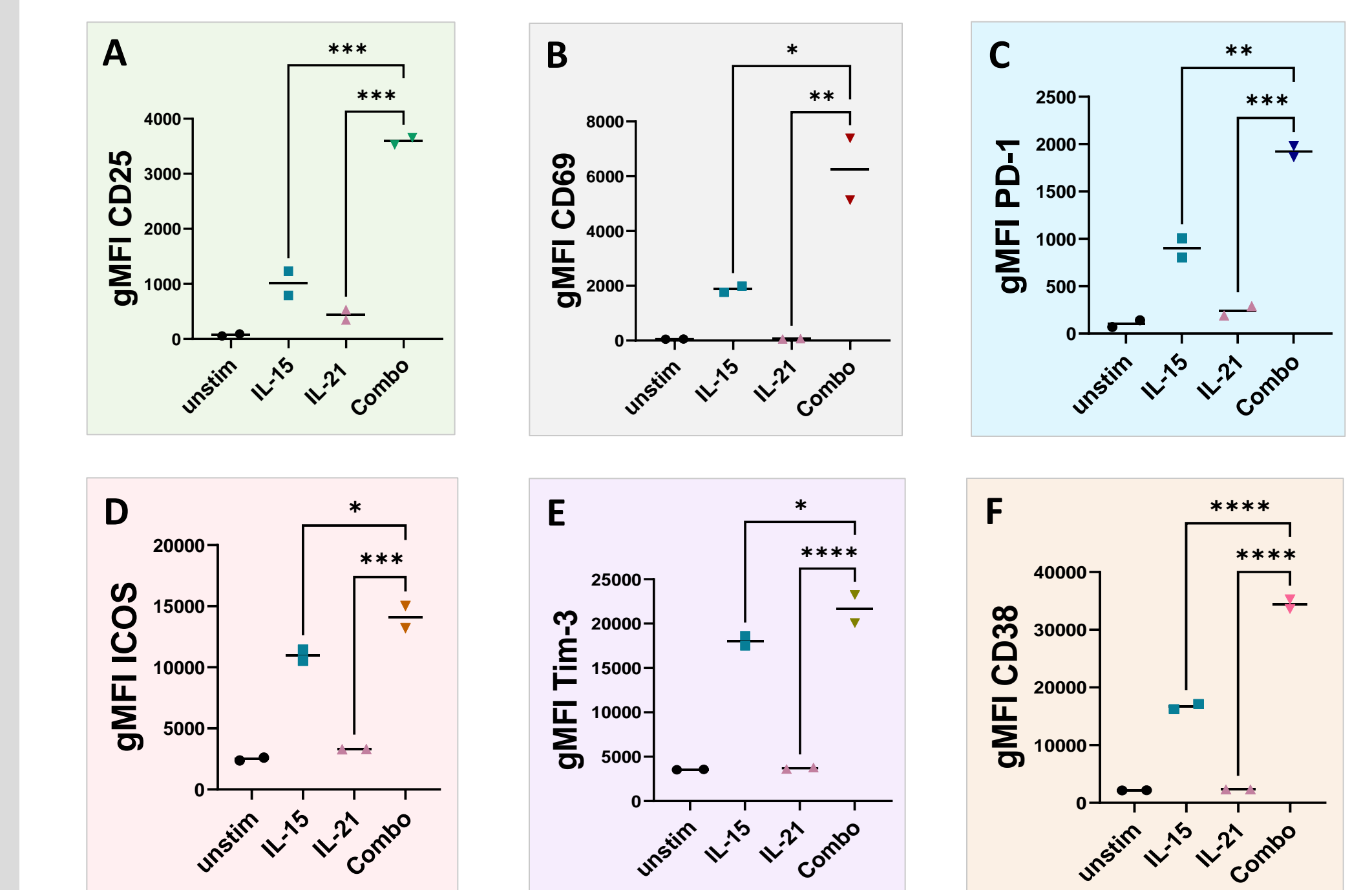
Primary NK cells were isolated and sort into two, CD56dim and CD56bright subsets by flow analysis. On both NK subsets, Activation marker, CD25, was upregulated when combined stimulation with both IL-15 and IL-21 (A,B).

FIGURE 4. PRODUCTION OF CYTOLYTIC CYTOKINES SIGNIFICANTLY INCREASED WITH IL-15 AND IL-21 COMBO STIMULATION



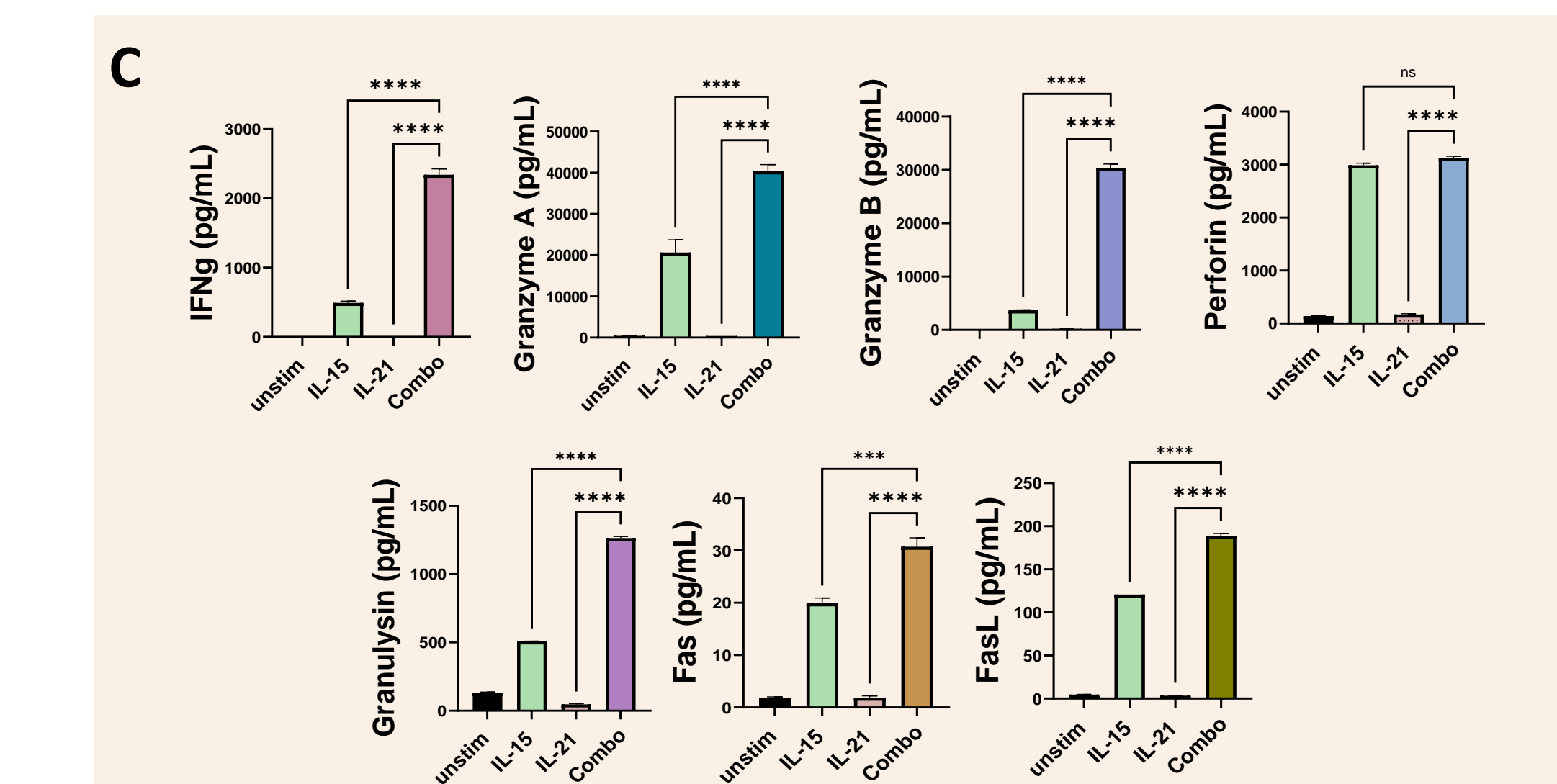
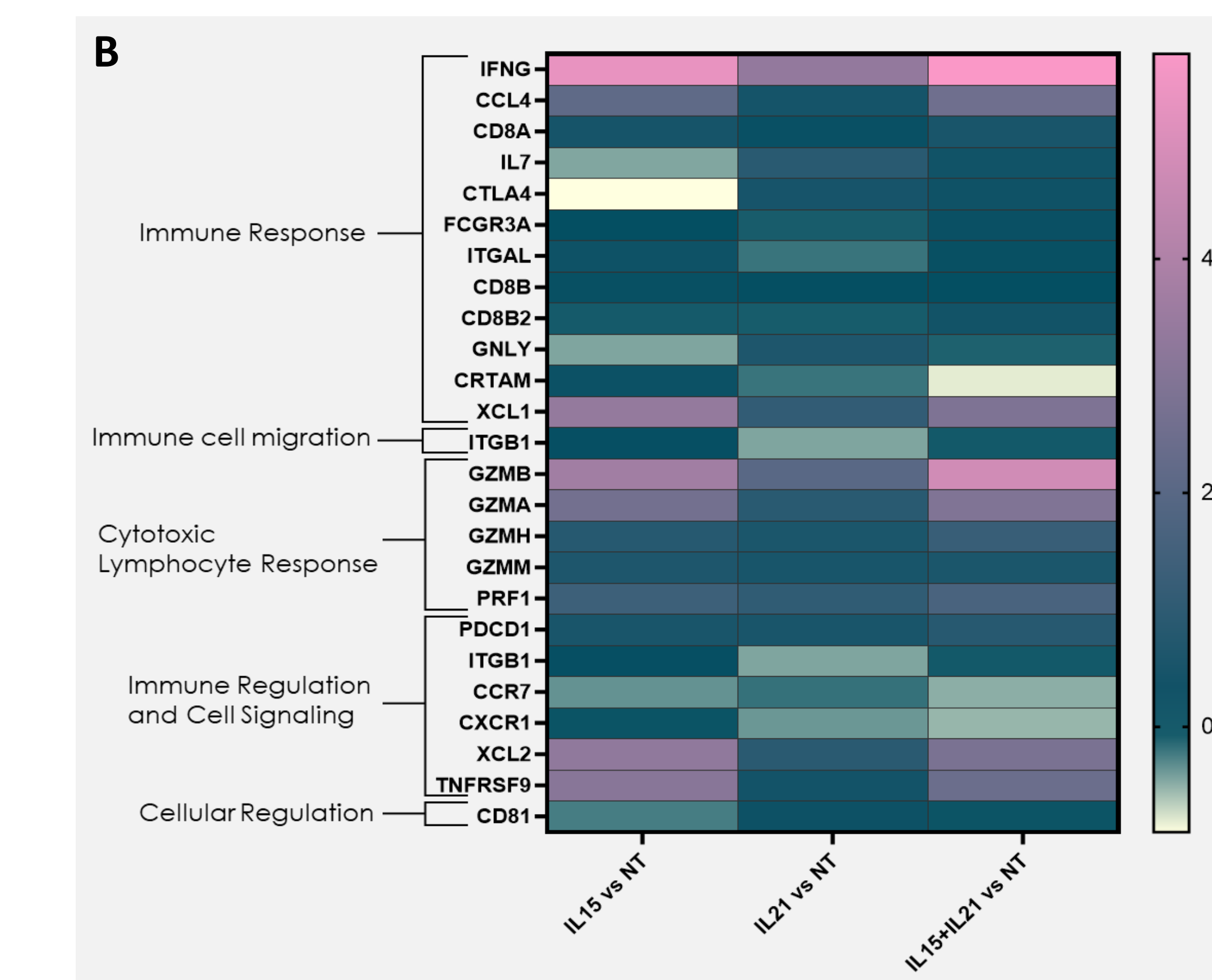
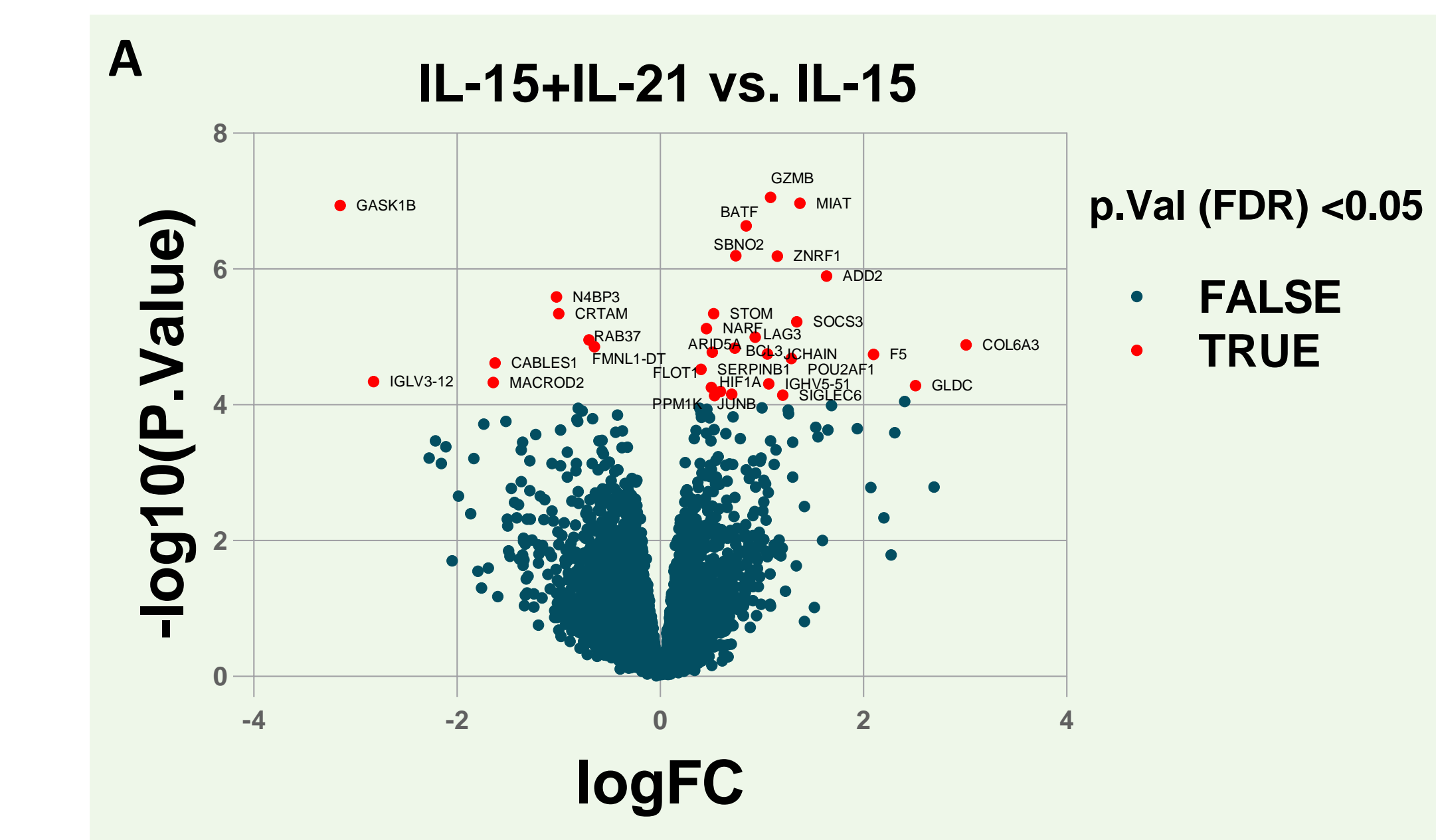
Primary NK cells were stimulated with IL-15 and/or IL-21, and then granzyme A, granzyme B and perforin were quantified from cell supernatant. Stimulation with IL-15 or IL-21 alone increased secretion cytolytic cytokines and granule, however, these levels were significantly heightened by the cooperative signaling of both IL-15 and IL-21 (A, B, C, D).

FIGURE 5: IL-15 AND IL-21 INCREASED CYTOLYTIC ACTIVITY OF CD8+ T CELLS



CD8 T cells were assessed by flow surface and intracellular staining after 7 days of incubation. Compared to cells stimulated with IL-15 or IL-21 alone, those challenged with IL-15 and IL-21 in combination, had greater increase in CD8 T cell activation markers, CD25, CD69 (A, B) and immune checkpoints PD-1, ICOS, Tim-3 and CD38 (C, D, E, F). Analysis of intracellular staining show that upon stimulation with the cytokine combo, Perforin and Granzyme B were upregulated and cells morphology also shifted to become double positive expressors (G).

FIGURE 6. IL-15 AND IL-21 COMBO STIMULATION UPREGULATED A SURPLUS OF CYTOTOXIC CD8 GENES



Here we isolated CD8+ T cells from total PBMCs and introduced IL-15 and/or IL-21 for 48hr. After stimulation, samples were collected for RNA isolation and bulk RNA sequence analysis. The results displayed a collection of genes further amplified by the cytokines combo stimulation when compared to IL-15 stimulation alone (A). In addition, CD8+ specific cytotoxic genes were also upregulated (B). These genes play important roles in different biological processes such as regulation of cell cycle, cell communication, immune response, and protein metabolism etc. (A,B). Cells supernatant from the isolated CD8, 7day stimulation assay, were also collected to examine for cytokine production by ELISA. Analysis results revealed that stimulation with IL-15 alone enhanced the production of pro-inflammatory cytokines, however, co-stimulation with IL-21 further increased the production of these pro-inflammatory cytokines (C). This also validated the RNA sequence results showing upregulation of specific genes such as IFN γ , granzyme A and B, etc. (B).

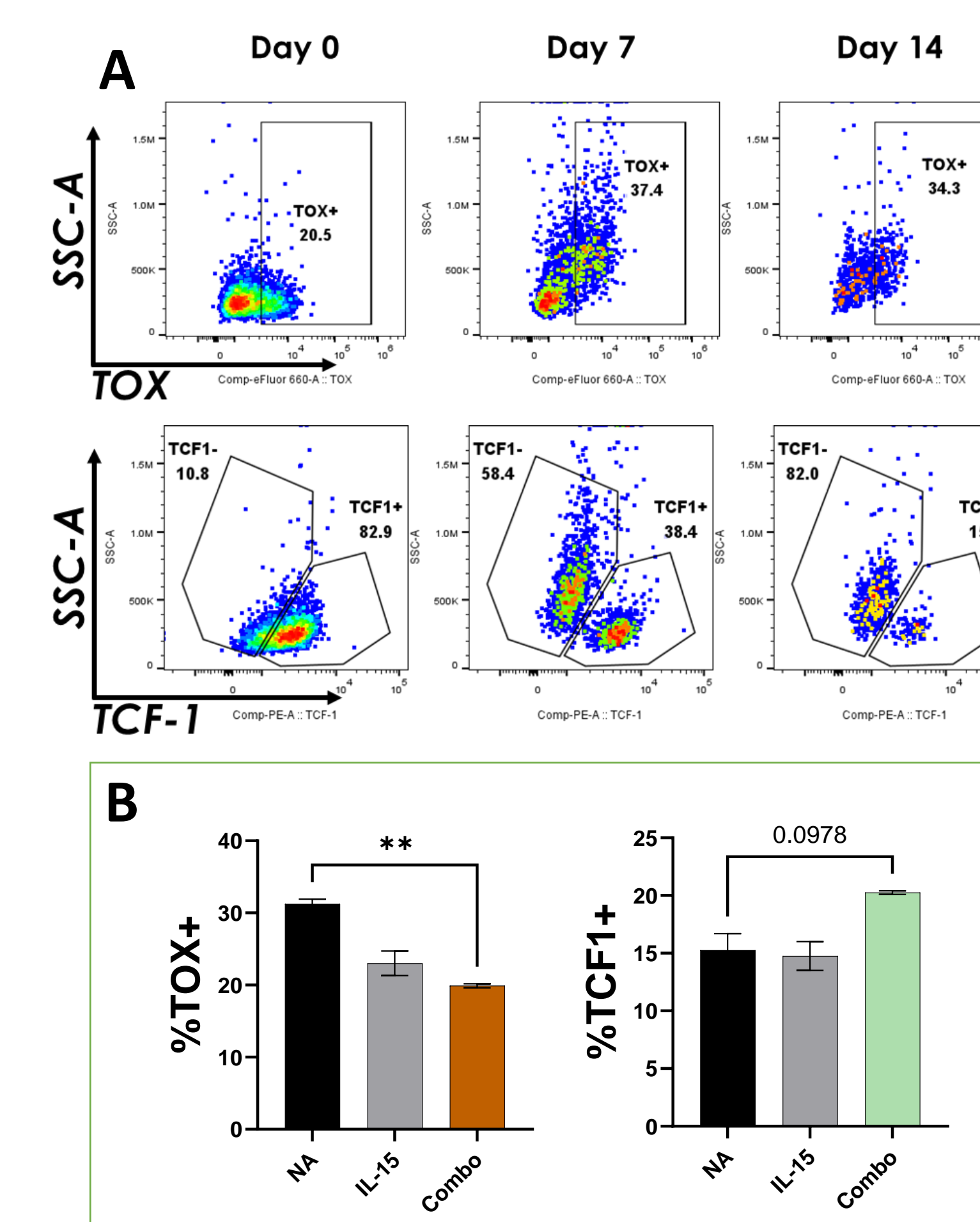


FIGURE 7: CEF-REACTIVE CD8+ T CELLS EXPRESS HIGHER LEVELS OF INHIBITORY RECEPTORS AND LOWER LEVEL OF TCF-1

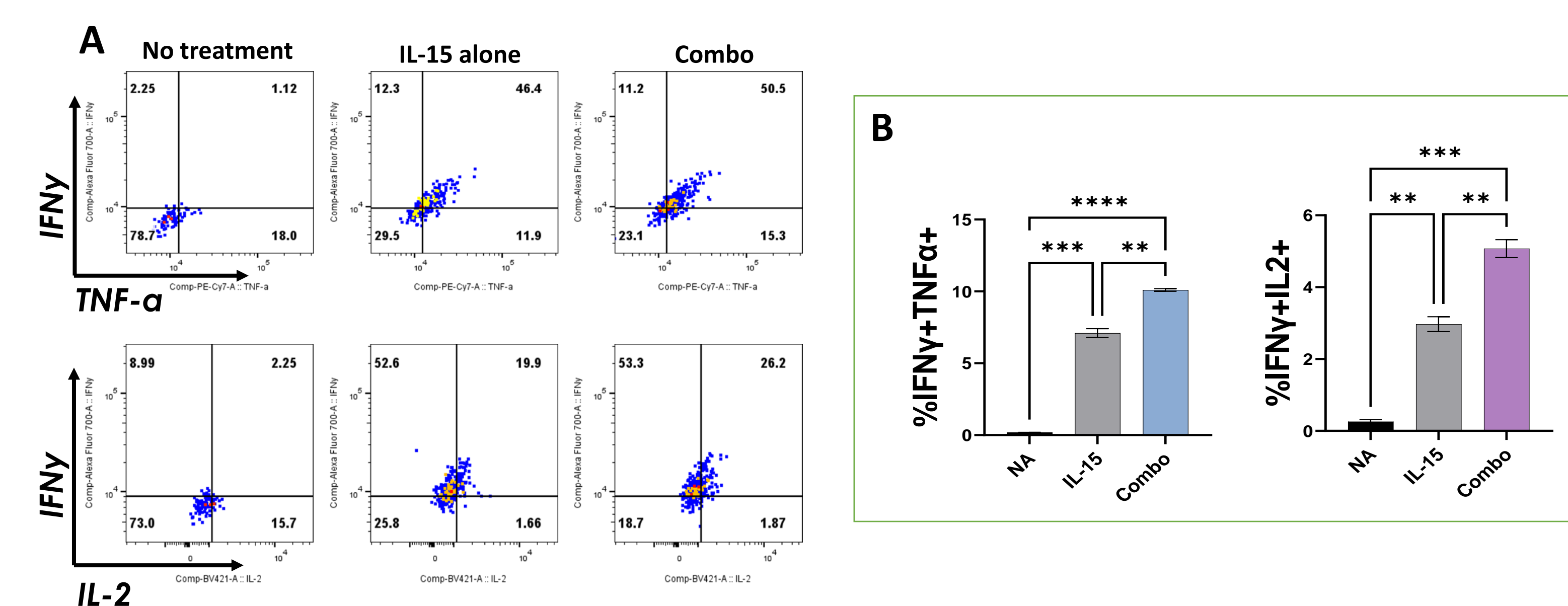
High TOX expression is a phenotypic characteristic of progenitor and terminally exhausted T cells [7]. Also, terminally exhausted T cells have been described to lose expression of the transcription factor TCF-1 [5]. After CEF peptide pool incubation, cells were assessed by flow analysis on days 0, 7, and 14 to monitor progression of T cell exhaustion.

Analysis results showed an increase of 84.5% in TOX expression on Day 7 and 69% on Day 14 compared to baseline (day 0) (A). Furthermore, 82.9% of CD8+ T cells express TCF-1 prior to exposure to antigen and the percentage of TCF-1+ cells declines as the cells progress through the progenitor exhausted T cell state (day 7) and enter the terminally exhausted T cell state (day 14) (A).

Exhausted T cells also upregulate and express multiple inhibitory receptors [6]. In this analysis, we also saw an increase of cells that are double positive for LAG-3 and TIM-3.

After 14 day of CEF peptide incubation, cells were treated with IL-15 and/or IL-21 for 7 days, cells were then collected for flow analysis. Flow results showed a reduction of frequency of CD8+ T cells that are positive for TOX with the combo cytokine treatment (B). We also observed IL-15 and IL-21 treatment modestly increased (~5%) frequency of CD8+T cells that are positive for TCF-1 expression compared to no cytokine treatment (B). Although this change is modest, it does support the potential for restoring cells to a less exhausted state.

FIGURE 8: IL-15+IL-21 STIMULATION INDUCES CO-EXPRESSION OF IFN γ /TNF α /IL-2



A functional characteristic of exhausted T cells is the reduction in cytokine production and ability to produce multiple cytotoxic cytokines [8]. Therefore, we also evaluate the co-expression of IFN γ /TNF α /IL-2 with no treatment, IL-15 and/or IL-21 (A). Following IL-15 treatment, we observed an increased in percentage of CD8+ T cells that are double positive for IFN γ /TNF α and IFN γ /IL-2, this was further upregulated from samples treated with combination of IL-15 and IL-21 cytokines (A,B).

Conclusions

- Cooperative effect of IL-15 and IL-21 enhanced markers of activity including CD25, CD38 and CD69, in addition to expression of granzyme A, granzyme B and perforin. IL-15 and IL-21 together significantly enhanced proliferation and cytolytic function of Primary NK and CD8+T cells.
- Transcriptomic analysis indicates that while the IL-15 signature is dominant, multiple inflammatory gene sets responded to a greater extent when in the presence of both IL-15 and IL-21 vs. either cytokine alone.
- Preliminary evaluation of IL-15 and IL-21 in an antigen-based T cell exhaustion model described here suggests that the combination of the two cytokines has a modest effect on reversing the phenotype and function of terminally exhausted CD8+ T cells as shown by the increase in the percentage of TCF-1 positive cells, decrease in the percentage of TOX positive cells, and increase the percentage of polyfunctional cells.
- These results indicate that the combination of IL-15 and IL-21 robustly augments NK and CD8 T cell activity. The ability to not only boost cytolytic function of naive and effector cells but to partially rescue the exhausted phenotype of cytotoxic CD8+ T cells is a promising therapeutic approach and addresses the challenges of immuno-oncology.

Acknowledgment

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References

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Disclosures

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