Spatial Control as a Third Axis of Immune Regulation: Signal Placement Acts Independently of Receptor Identity and Signal Strength

Noelle Lim, Javi Bravo-Alegria, Andres Campos, Ayomide Oloyede, Jinchun Wang, Siddharth Subham, Andries Zijlstra, Colleen M O'Connor

StimulusBio, Houston, TX, United States

Main Conclusion

Immune cells respond not only to what signals they receive and how much they receive, but also to how those signals are spatially organized.

Background

- The immune set point has been understood as a contest between opposing signals, such as CD28 and CTLA-4, since the discovery of the co-stimulatory checkpoint. Under this model, cell communication or response depends on receptor identity and signal strength.
- A third variable, the spatial control of bioactive units on the presenting surface, has been difficult to separate from composition and dose, and so has remained largely untested.
- Signal spatial organization is intrinsic to every synthetic immune interface and shapes cell activation, yet remains uncontrolled in current platforms.
- sbPCI™ understands immune synapse architecture and presents molecules at defined density and organization.

Rationale

Receptor identity and signal strength are established determinants of immune outcome, but the role of signal arrangement remains poorly defined. sbPCI enables independent control of signal quantity, ratio, and position to evaluate the impact of arrangement on immune cell function.

Methods

- **Configurations**

sbPCI Formulation	Figure
IL-21	2A, 3A-B, 5A
IL-2	2A, 3A-B
CD3/CD28	2A-B,4C-F
CD28/CD3	4C-F
CD3/28/137	2A
CD137/IL-15	5B-D
CD137/IL-21	5B-D

- **Presenting cells:** < 1 × 10^6 RBCs modified with sbPCI.
- **Design variables:** Quantity, ratio, and axial position fixed at assembly; arrangement varied.
- **Co-localization:** Anti-CD3 and anti-CD28 on separate interfaces or co-localized on one; anti-CD28:anti-CD137 ratio then varied.
- **Density titration:** 1 × 10^6 RBC with anti-CD3 (1.18 pmol) and anti-CD28 (0.25, 0.75,1.5 pmol).
- **Flow Readouts:** pSTAT3 over time; NKG2D, NKp46, CD56, CD8, CD4, CD25, CD69, CD3 and Granzyme B.
- **Cell sources:** RBCs, T and NK cells from Gulf Coast Blood.

Controlling Presentation, Distance/Position, Stoichiometry, Density

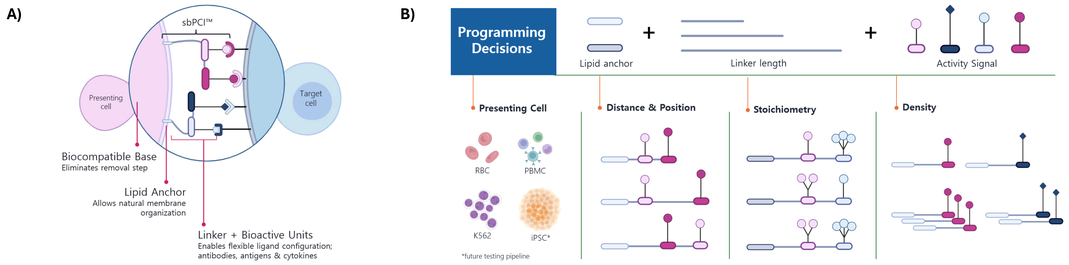


Figure 1: Assembly and characterization of programmable cellular interfaces (sbPCI). (A) Schematic of the sbPCI architecture, consisting of a biocompatible cell surface, lipid anchor, and modular bioactive ligands that enable controlled presentation of molecules to direct immune synapse signaling. **B)** Technology enables the quantitative control and spatial organization for controlled cell-to-cell communication.

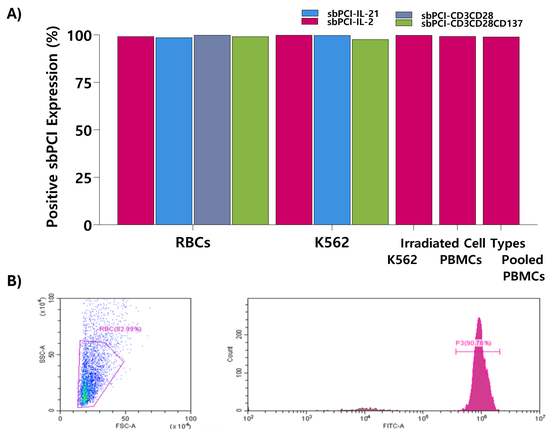


Figure 2: Presenting Cell. (A) Flow cytometric analysis of several different sbPCI presentation on RBCs, K562, irradiated K562, PBMCs, and pooled PBMCs shows consistent and efficient presentation across cell types. **(B)** sbPCI-CD3/CD28 expressing RBCs flow data.

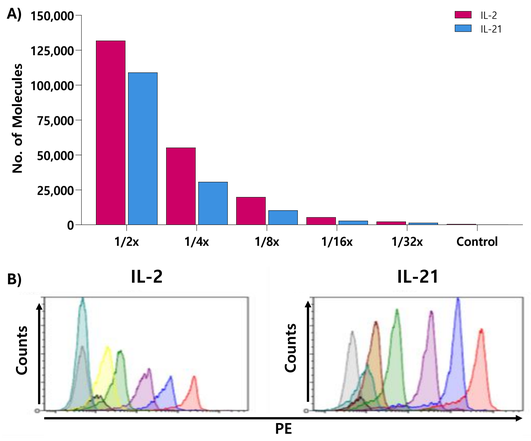


Figure 3 Density. A) IL-2 and IL-21 molecule number on the RBC surface is quantitatively controlled for defined cytokine presentation. **B)** Flow cytometry analysis demonstrated titration of sbPCI-presented IL-2 (left) and IL-21 (right).

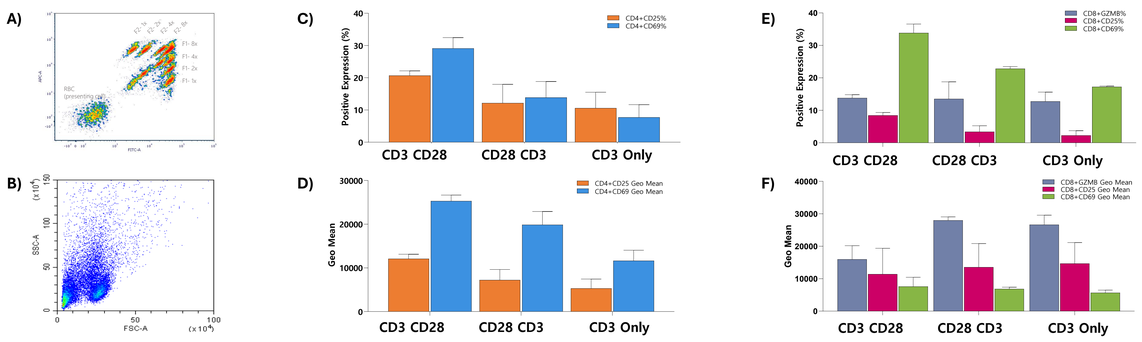


Figure 4: Stoichiometry, Distance and Position. A) Dual fluorescent labeling demonstrates precise stoichiometric control of sbPCI components. **B)** Forward and side scatter plot of T cells cocultured with sbPCI-RBC on Day 2, showing the initial gating used to separate T cell and sbPCI populations. **C-F)** CD28 density and CD3/CD28 positioning modulated early T cell activation. Effects were most pronounced at lower CD28 densities and altered positioning differentially modulated $CD8^+$ and $CD4^+$ activation responses.

Signal Placement Impacts Cell Response

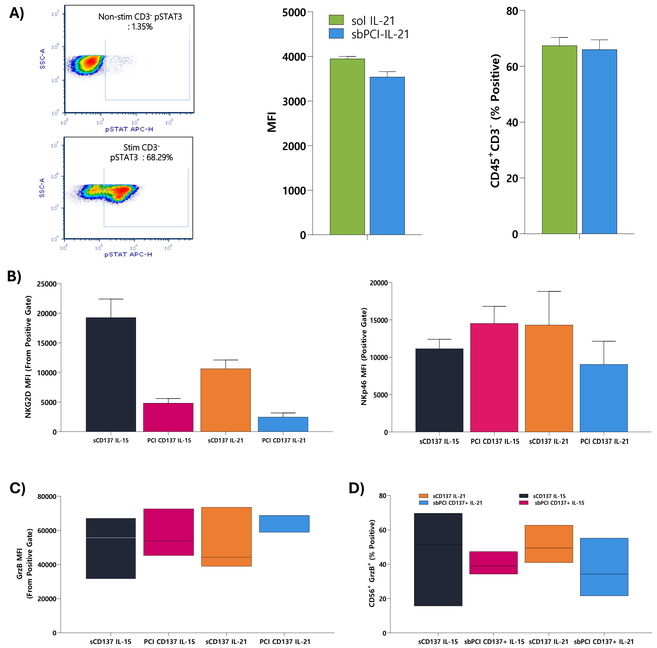


Figure 5. sbPCI configured for NK preferentially expands cells and modulates activating receptor expression in G-Rex. A) sbPCI-IL-21 induces STAT3 phosphorylation comparable to soluble IL-21. **B)** NKG2D expression is reduced in sbPCI groups, while NKp46 expression is maintained, indicating selective modulation of NK signaling pathways. **C, D)** The frequency of $GrzB^+$ NK cells was comparable across conditions, while GrzB expression trended higher in sbPCI groups, particularly PCI CD137/IL-21, suggesting enhanced per-cell cytotoxic arming. Together, these data indicate that signal presentation mode influences NK phenotype and function.

Conclusions

The same signaling molecules produce distinct immune outcomes when spatial organization is altered.

- Signal arrangement influences immune cell phenotype and function independent of receptor identity and signal strength.
- Signaling molecules produce distinct T cell and NK cell responses when presented in different spatial configurations.
- Signal geometry represents a programmable and previously underexplored determinant of immune cell outcome.
- In addition to receptor identity and signal strength, spatial organization when designing synthetic immune interfaces.

Future Directions:

- Identify quantitative design rules linking signal geometry to immune cell outcome. Apply spatial programming to define engineered immune interfaces for research and therapeutic applications.