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Chimeric Interleukin 15 Bovine Ultralong Antibody Fusion For Potential Cancer Therapy

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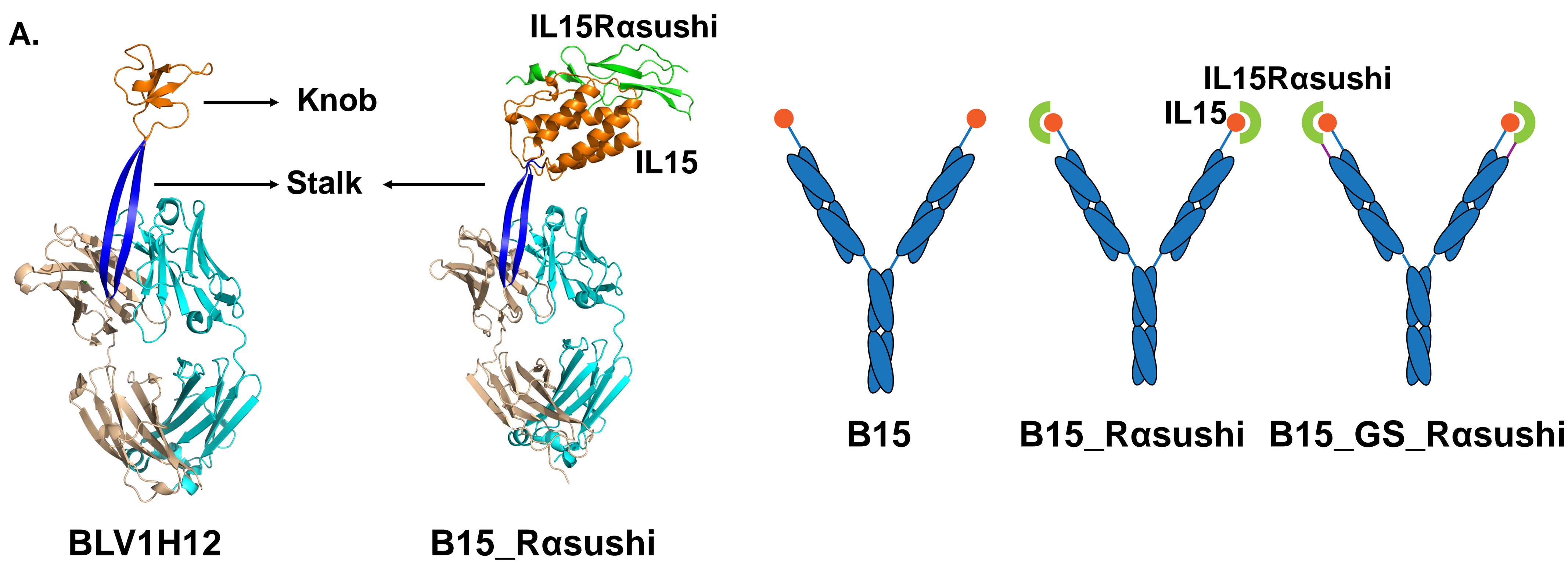
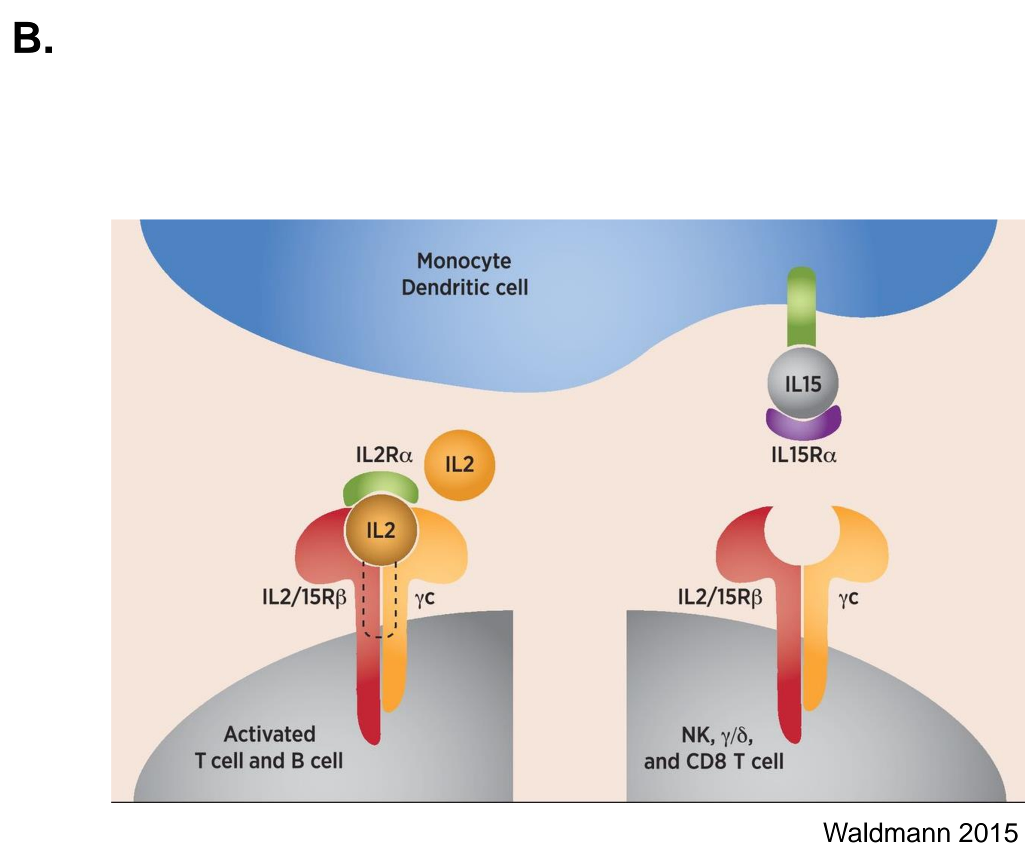
Introduction

- Bovine ultralong CDR3 antibodies have a unique "stalk and knob" structure in which two antiparallel β-strands support a disulfide bonded knob protruding out of the antibody surface and forms a mini antigen binding domain (Figure 1A).
- Interleukin (IL) 15 stimulates the proliferation and cytotoxicity of cytotoxic T lymphocytes and nature killer (NK) cells, similar to the action of IL2, and thus is an immunotherapeutic candidate for cancer treatment.
- IL15 may be a better candidate drug than IL2 because it doesn't cause vascular leak syndrome or stimulate regulatory T cells.
- However, IL15 is difficult to express as a stable soluble protein and has a short half-life in vitro and in vivo.
- To solve the problem, we designed a chimeric IL15 ultralong bovine antibody fusion by replacing the knob of the bovine antibody BLV1H12 with IL15 (Figure 1A).
- BLV1H12_IL15 (**B15**) functions the same as IL15 in in vitro signaling assays but can be easily produced in mammalian cells and with increased stability.
- IL15 needs its high affinity receptor α (IL15Rα) for increased *trans* signaling to the receptor β and γ subunits (IL2/15Rβ and γc) (Figure 1B), two more variant candidates were produced by either co-expressing IL15Rα sushi domain with the chimeric IgG (**B15_Rαsushi**) or fusing the IL15Rα sushi domain to the light chain through a linker (**B15_GS_Rαsushi**). Both variants exhibit higher activities in in vitro signaling and NK92 cell expansion than B15 alone.

A.



B.



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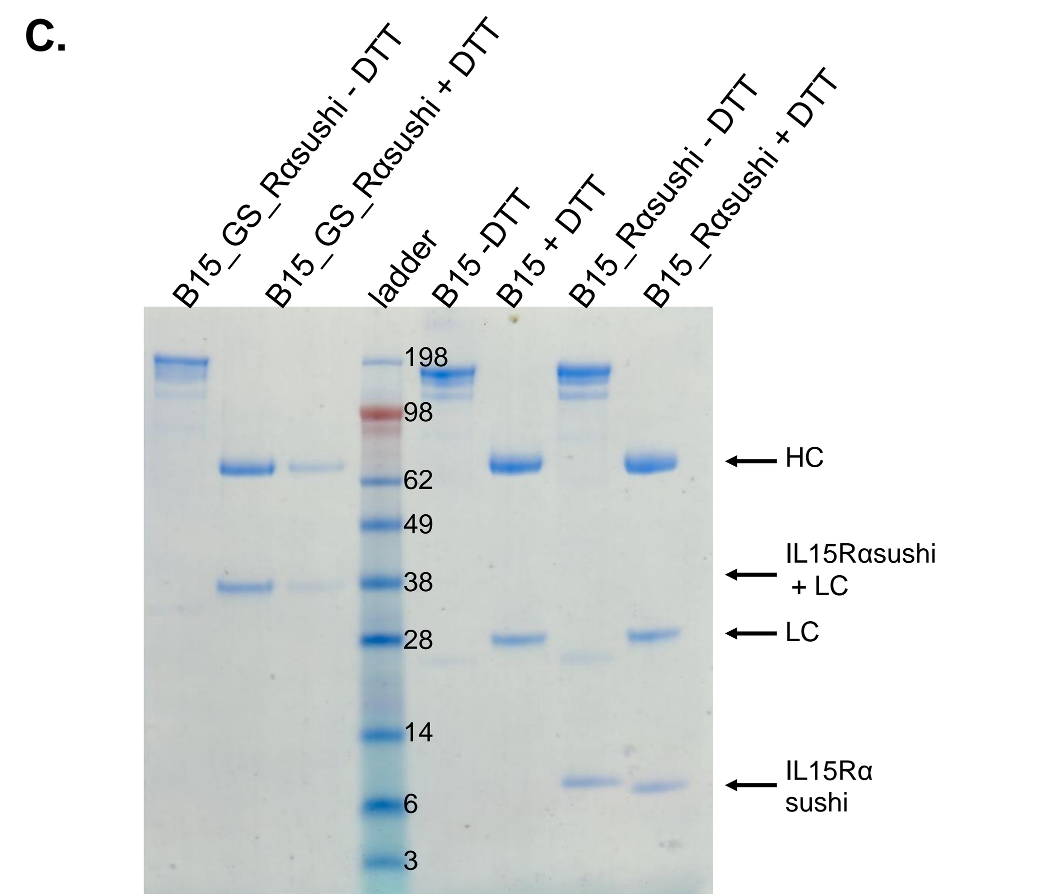

Figure 1. B15 and its variants can be easily expressed in mammalian cells and purified using standard antibody purification method. A. Structures of BLV1H12 and B15_Rαsushi and schematic illustration of B15 variants. B. IL15 is *trans* presented from antigen-presenting cells to NK or CD8⁺ T cells. C. SDS-PAGE gel showing purified B15 variants.

Methods

- B15 variants were expressed in freestyle HEK293 cells (ThermoScientific) and were subsequently purified using CaptureSelect CH1-XL affinity resin (ThermoScientific).
- In vitro STAT5 signaling assays were done using HEK-Blue IL2 reporter cells (InvivoGen).
- NK92 cell expansion assays were done using MTT assay kit (Promega).

Results

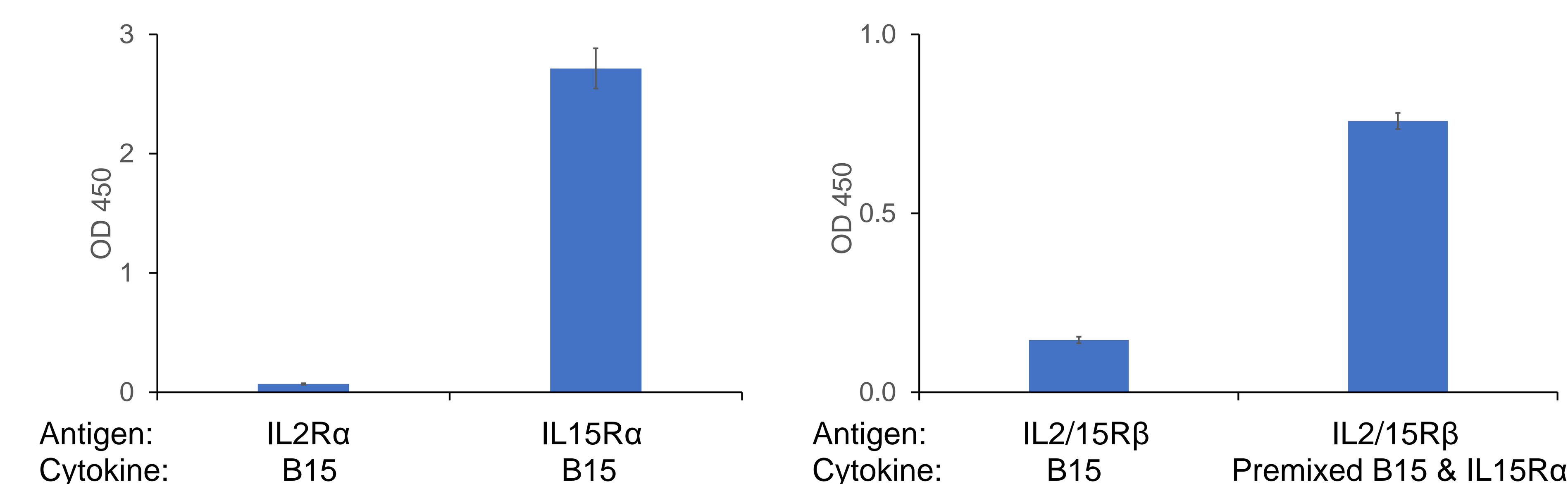

Figure 2. ELISA assays showed that B15 could bind to both IL15Rα and IL2/15Rβ subunits, and the IL15Rα subunit could improve B15 binding to the IL2/15 Rβ subunit.

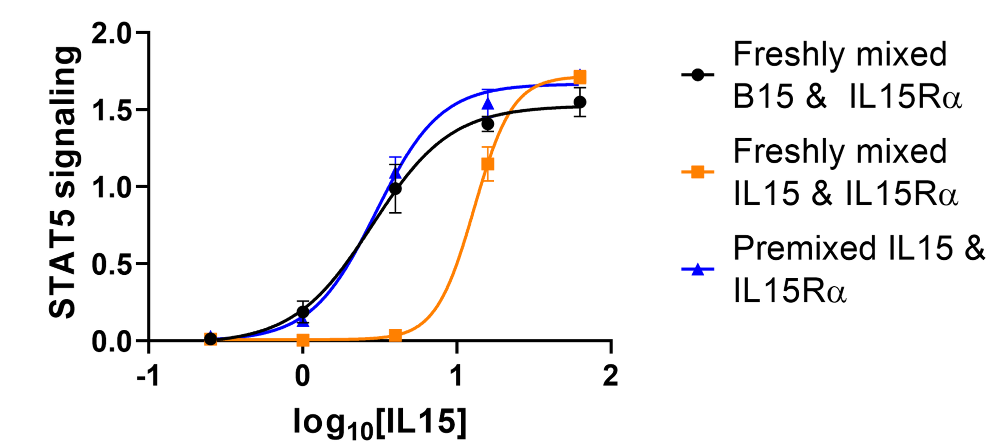

Figure 3. In vitro STAT5 signaling assays indicated that B15 could associate with 15Rα much faster than IL15 monomer. As there is no IL15Rα subunit expressed in the HEK-Blue IL2 reporter cells, IL15RαFc (R&D Systems) was mixed with IL15 (or B15) to increase its binding to the IL2/15Rβ and γc. IL15 was mixed with IL15Rα at 4°C overnight to achieve full signaling potency, while B15 mixed with IL15Rα right before the assay. As B15 is bivalent, only half molar concentration of B15 was used compared to IL15 monomer.

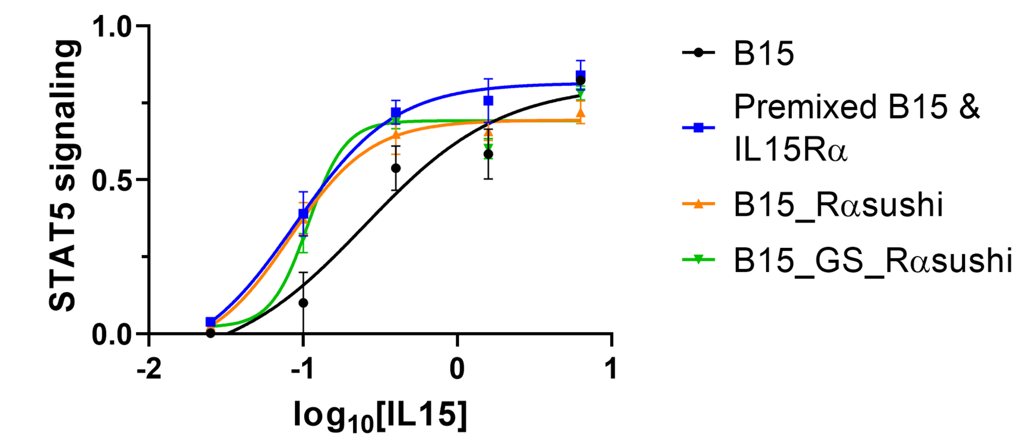

Figure 4. B15 variants expressed with IL15Rα sushi domain achieved the same signaling potency as premixed B15 and IL15RαFc, which all are much better than B15 without IL15Rα subunit.

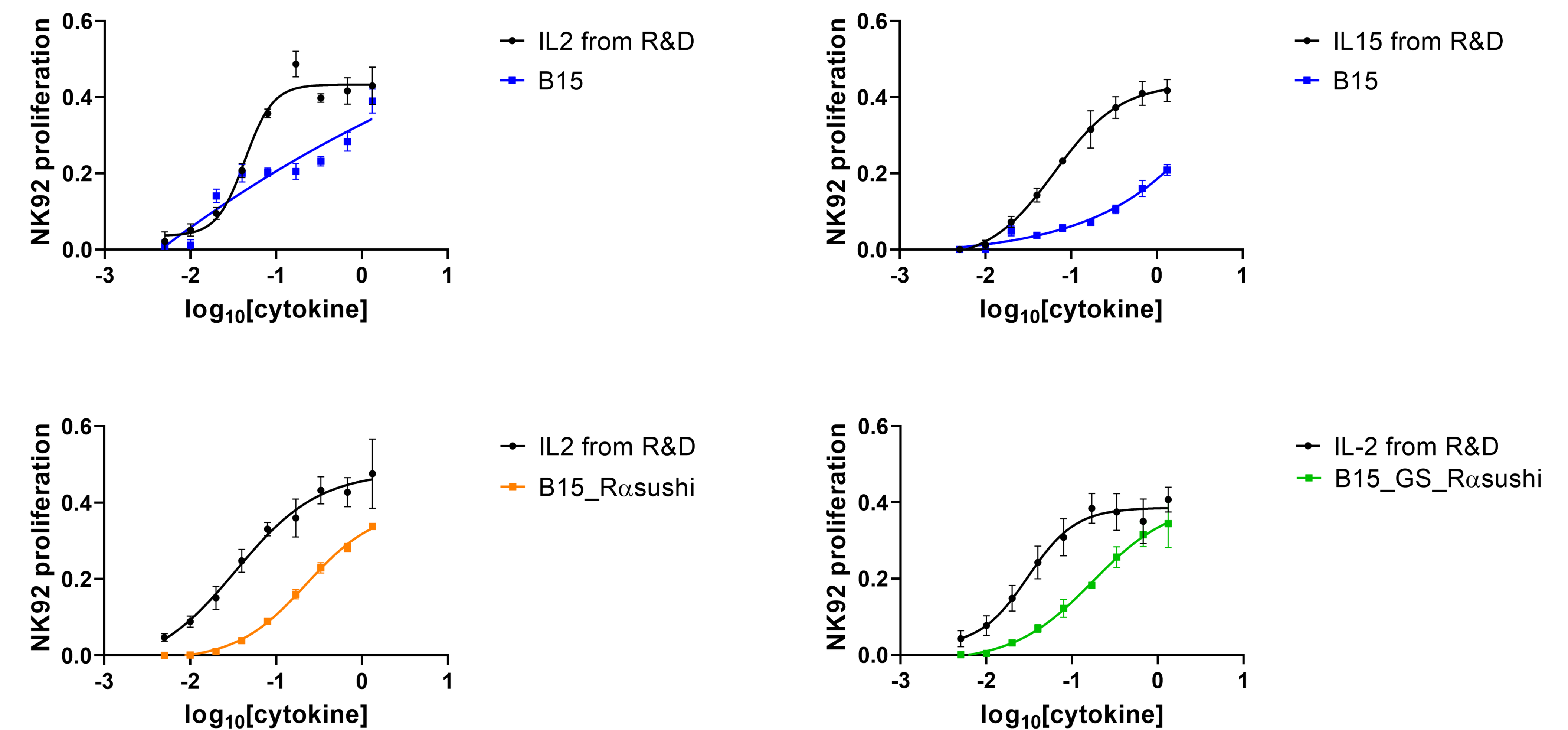

Figure 5. All B15 variants could expand NK92 cells, although to a lesser extent than either the IL2 or IL15 monomer. Half molar concentration of B15 was used compared to the IL2 or IL15 monomer.

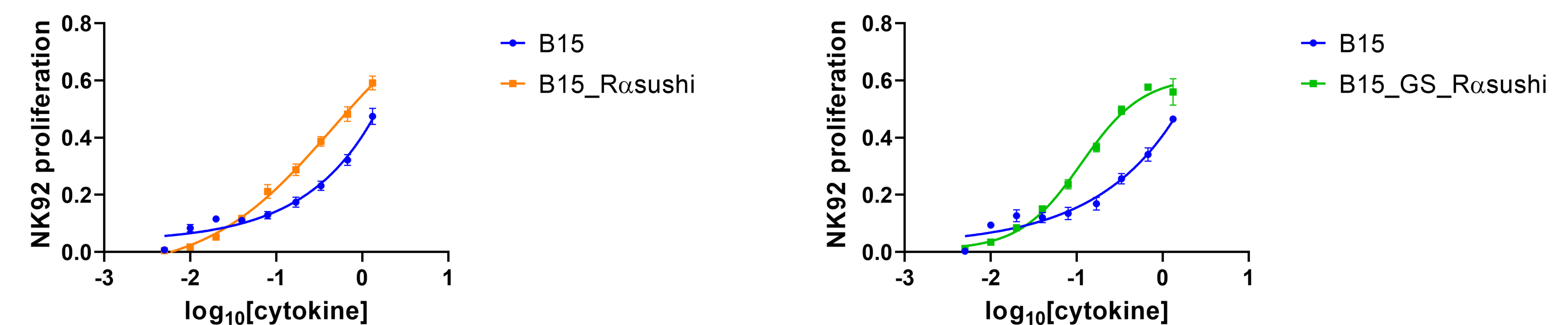

Figure 6. IL15Rα sushi domain improved B15 ability to expand NK92 cells.

Discussion and Future Directions

- B15 variants can be expressed and purified the same as typical human antibodies with high yield.
- IL15Rα or its sushi domain is required for efficient binding to IL2/15Rβ and γc subunits.
- It is unknown why B15 variants performed worse than the IL2 or IL15 monomer expressed in *E.coli* in NK92 cell expansion assay. However, our results are similar to other IL15-Fc fusion constructs.
- B15 variants will be further evaluated in:
  - — In vitro cell proliferation assay to test their cell targets in human PBMCs using flow cytometry.
  - — In vivo cell proliferation assay to test their cell targets in human PBMC-engrafted NSG mice.
  - — In vivo pharmacokinetic assay to test their half life.
  - — In vivo assay to test their antitumor responses in the tumor microenvironment in mice.