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Off-the-Shelf CAR-NK Cells Engineered to Express Calibrated Release IL-15 Exhibit Enhanced Persistence and Anti-Tumor Activity

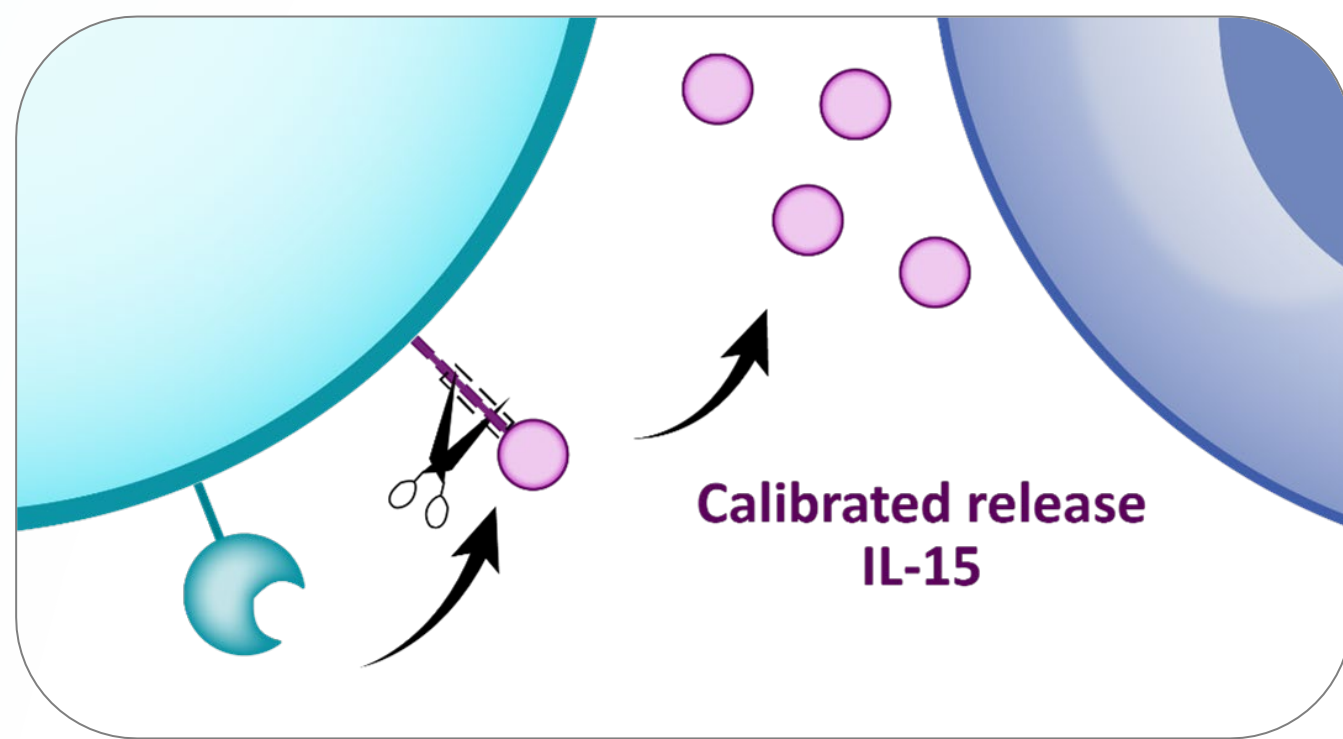
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AACR Annual Meeting
2023, Orlando, FL
Abstract Presentation #2902

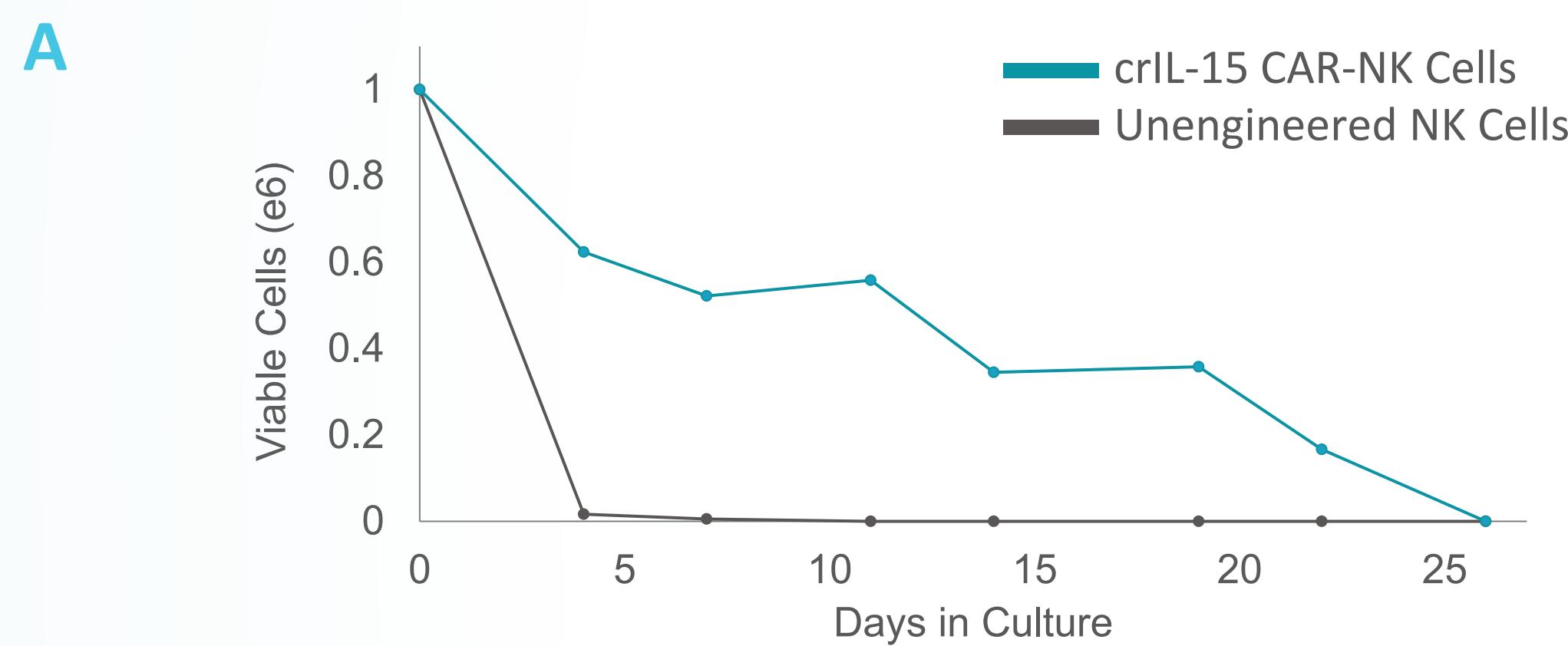
Arming CAR-NK Cells with Calibrated Release IL15 (crIL-15)

crIL-15: calibrated release of IL15 by local proteases -> enhanced killing activity

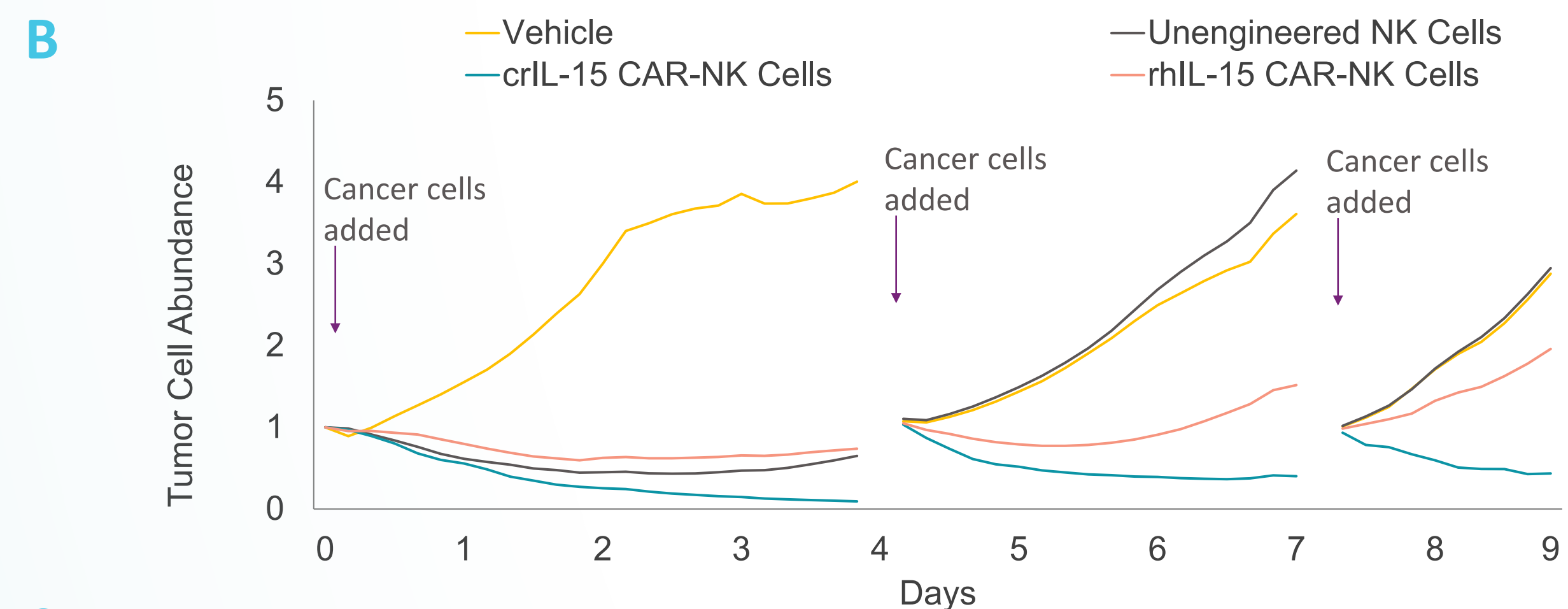


crIL-15 Increases Persistence and Activation of Both CAR-NK and Other Immune Cells

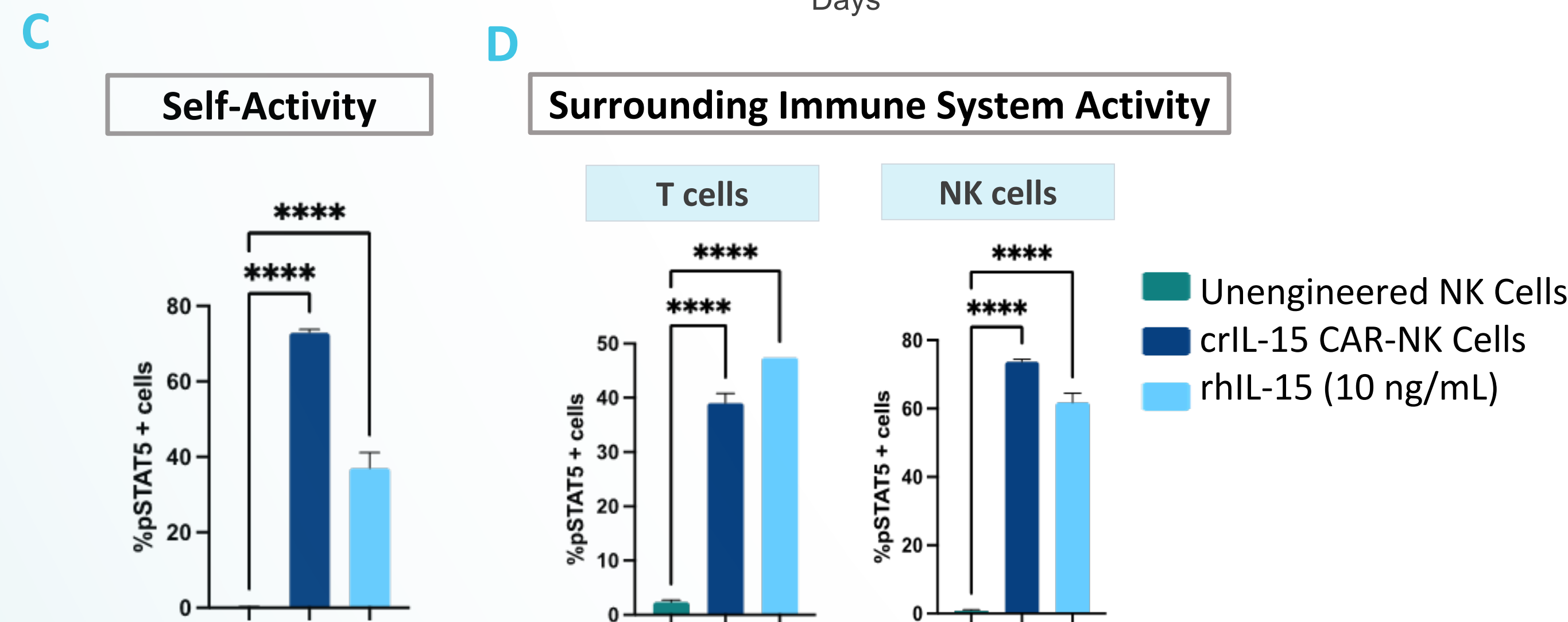
crIL-15 increases persistence of CAR-NK cells



crIL-15 increases CAR-NK serial killing ability

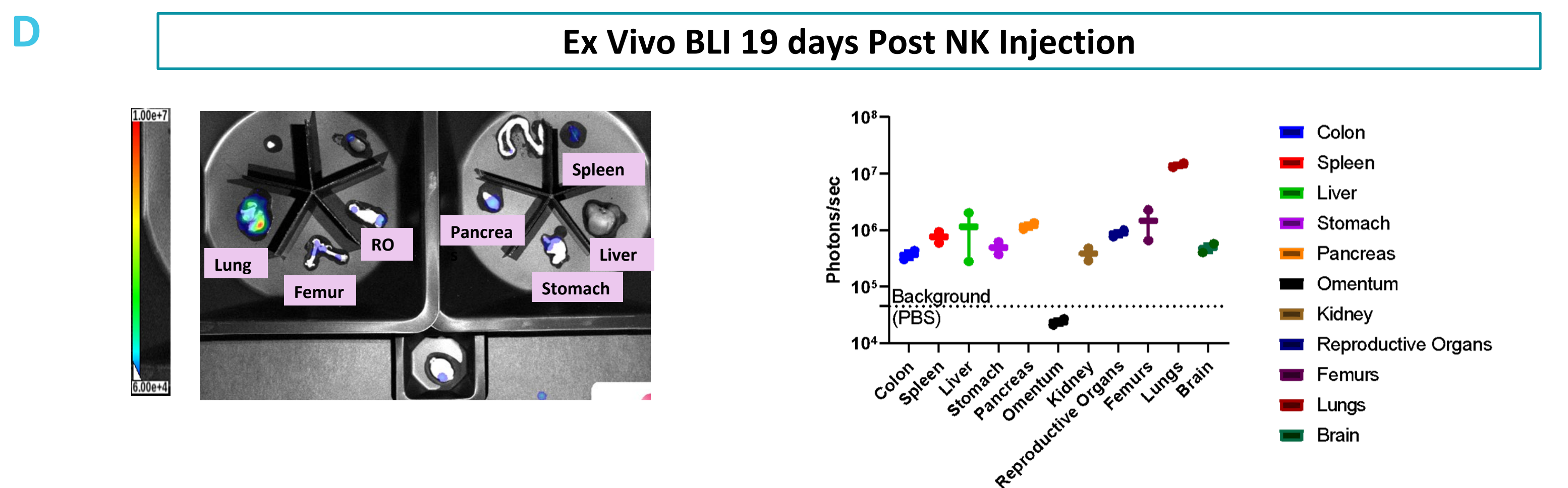
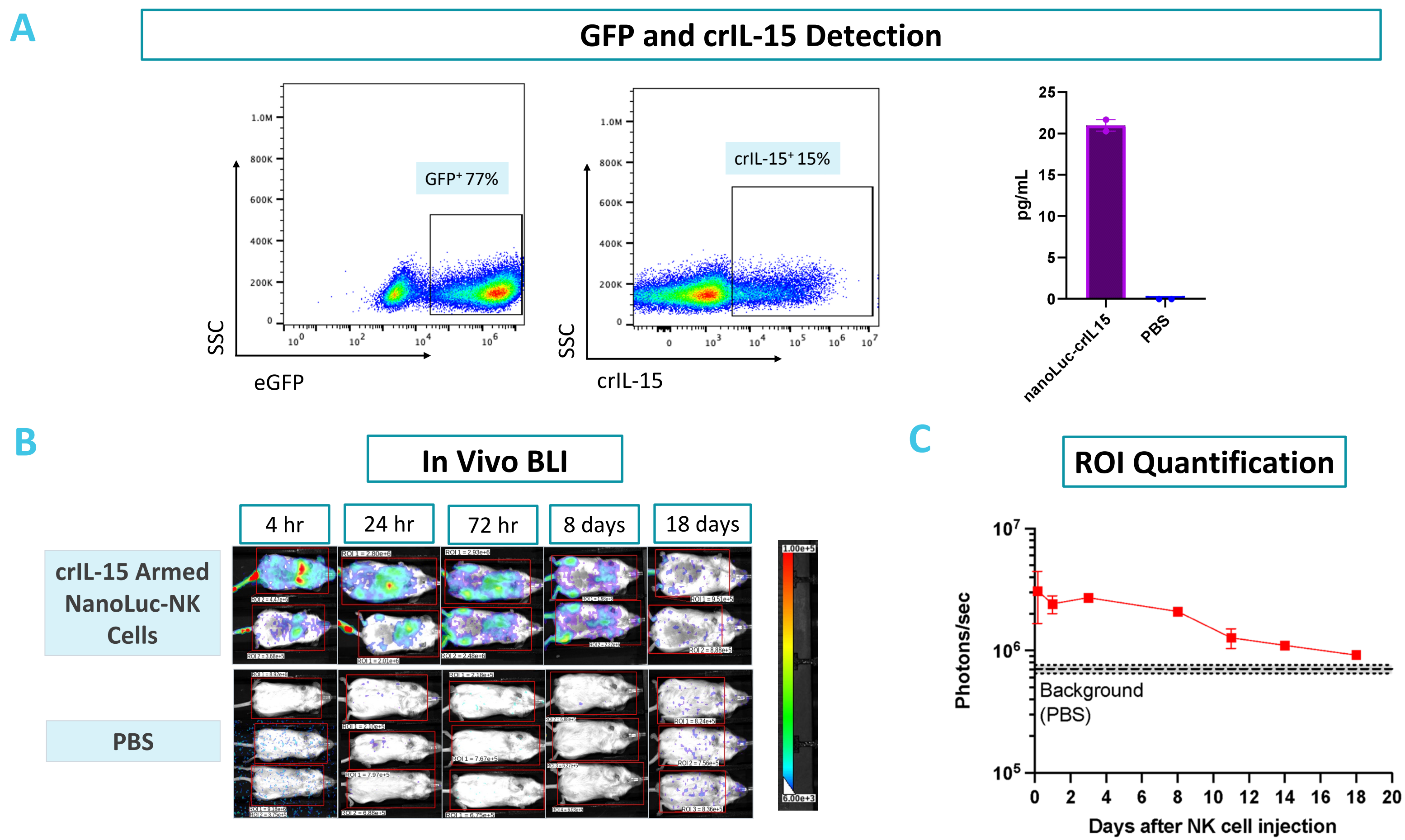


crIL-15 activates NK and resting immune cells



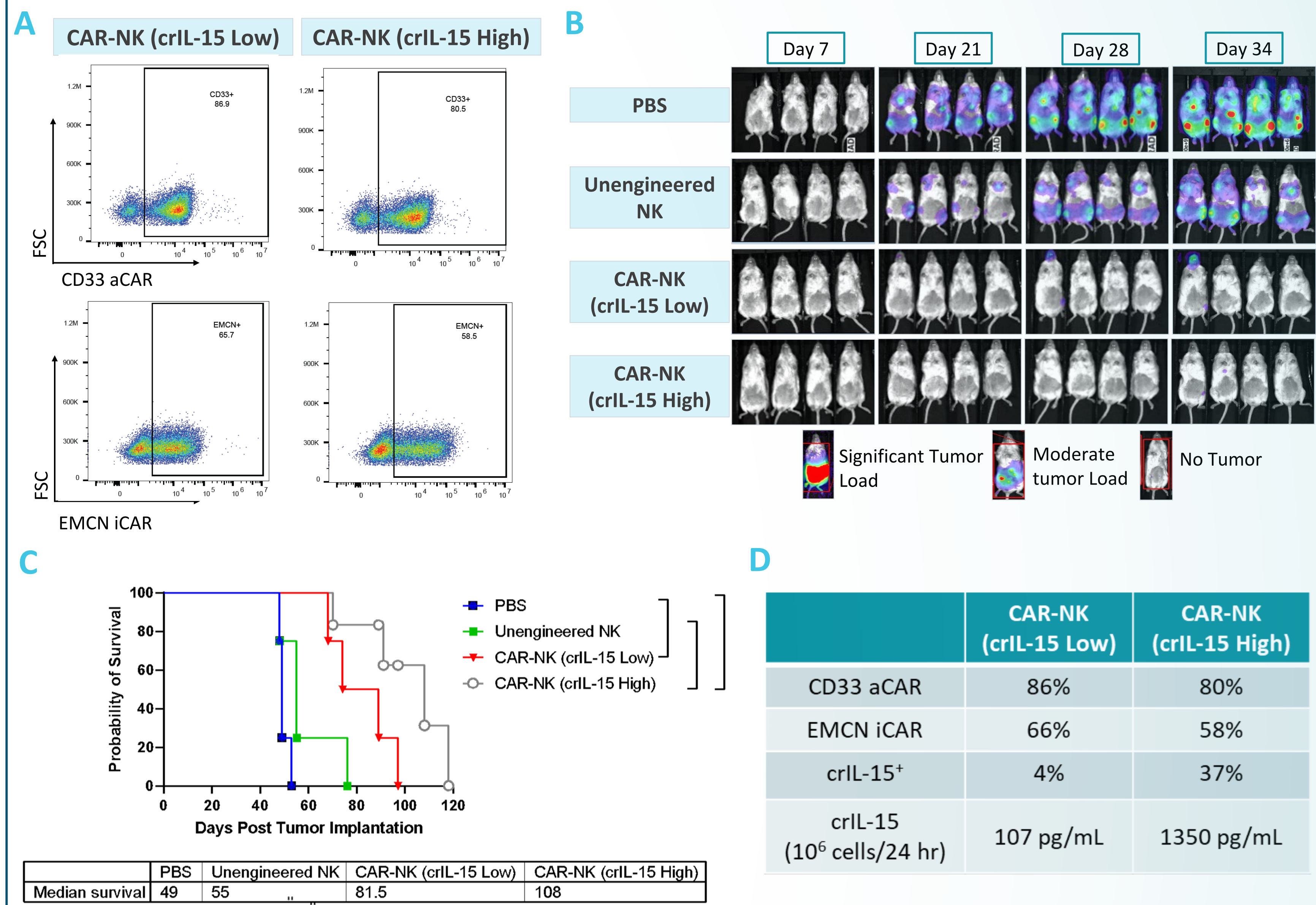
A. crIL-15 CAR-NK cells exhibited increased survival and expansion without exogenous cytokine support compared to unengineered NK cells. **B.** crIL-15 CAR-NK cells outperformed recombinant human IL-15 (rhIL-15) CAR-NK cells using an incu-cyte-based serial killing assay. crIL-15 CAR-NK cells demonstrated enhanced target cell killing and persistence in the secondary and tertiary re-challenges with additional cancer cells. **C-D.** crIL-15 pathway activation of NK and resting immune cells by phospho STAT5 (pSTAT5) detection.

Biodistribution and Persistence of crIL15 NK Cells In Vivo



A. NK cells were engineered to express GFP, NanoLuc and crIL-15. Expression was detected by flow cytometry and Luminex. **B-F.** crIL-15 armed NanoLuc NK cells (20×10^6) were intravenously injected into NSG mice. NK cells biodistribution and persistence was examined by bioluminescence imaging (**B-D**), human/mouse duplex PCR (**E**) and WPRE qPCR (**F**). crIL-15 NK cells were detected in the lung, femur, spleen, liver and stomach 1 day post injection and lasted up to 20 days in the lung.

Anti-Tumor Activity of crIL15 CAR-NK Cells In Vivo



A. NK cells were engineered to express a FLT3 and/or CD33 activating CAR, an EMCN inhibitory CAR and crIL-15. **B-C.** MV4-11-Fluc AML tumor cells were co-injected intravenously with CAR-NK cells expressing low or high crIL-15 or dose matched unengineered NK cells into NSG-Tg (Hu-IL15) mice. Tumor growth was monitored by bioluminescence imaging. CAR-NK cells significantly reduced MV4-11 tumor burden and prolonged mouse survival compared to unengineered NK cells. **D.** The improved anti-tumor functions correlated with IL-15 levels produced by the CAR-NK cells.

Summary

Senti has designed the calibrated release technology to simultaneously stimulate surrounding immune cells and promote CAR-NK cell expansion, persistence, and tumor killing.

- Effects of crIL-15 on CAR-NK cell functions in vitro:
 - crIL-15 increased in vitro persistence and target tumor cell killing ability of CAR-NK cells.
 - crIL-15 activated both NK and other immune cells.
- Effects of crIL-15 on CAR-NK cell functions in vivo:
 - crIL-15 CAR-NK cells significantly reduced MV4-11 tumor burden and prolonged mouse survival compared to unengineered NK cells.
 - The improved anti-tumor function correlated with IL-15 levels produced by the CAR-NK cells.

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