oNKo-innate
DEFINING INNATE IMMUNOTHERAPY

Systematic target discovery in human NK cells using functional genomics

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Introduction

Natural killer (NK) cells are a major focus of novel anti-cancer therapies. Target discovery in NK cells has been so far hampered by their resistance to genetic manipulation. Here we present an integrated CRISPR-based framework for large-scale screening and drug target discovery in human and murine NK cells.

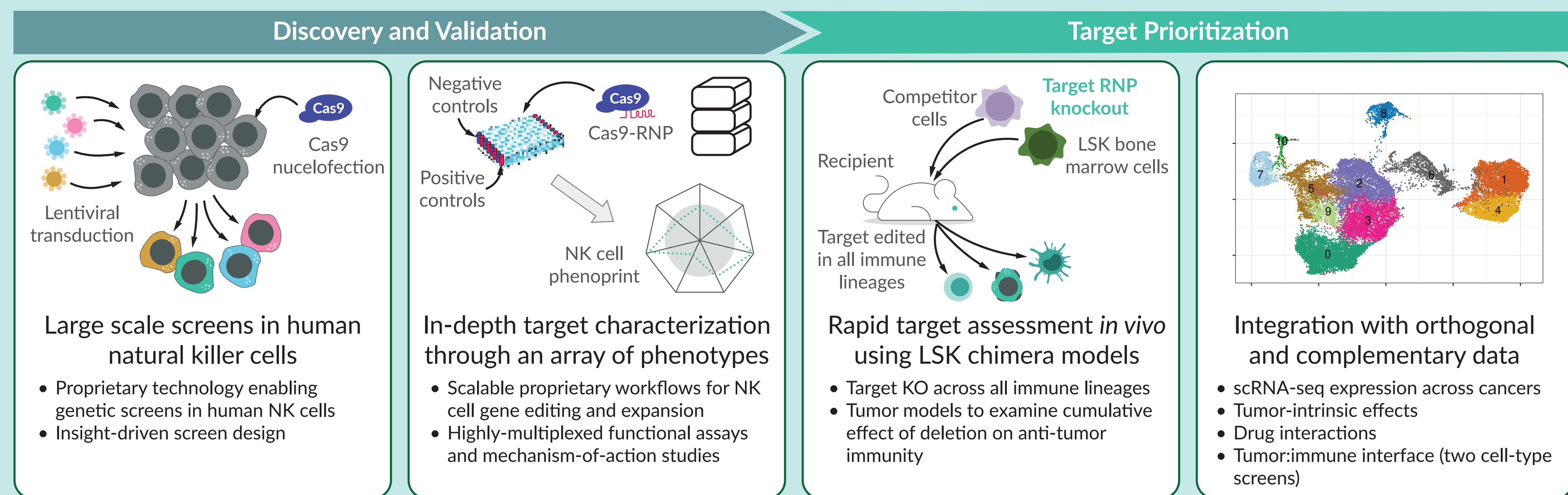

Figure 1. Schematic outline of oNKo-Innate NK cell screening platform.

Key takeaways

- Systematic functional screening in human NK cells reveals novel regulators of IL-15 axis, NK cell cytotoxicity and IFNγ release
- Target deletion boosts NK cell proliferation and chronic killing capacity as well as NK cell anti-metastatic function *in vivo*.

Biological question → CRISPR screen

1. NK cell survival/persistence → IL-15 axis regulators
2. NK cell cytotoxicity → NKG2D regulators
3. Cytokine production → IFNγ regulators

IL-15 axis regulators

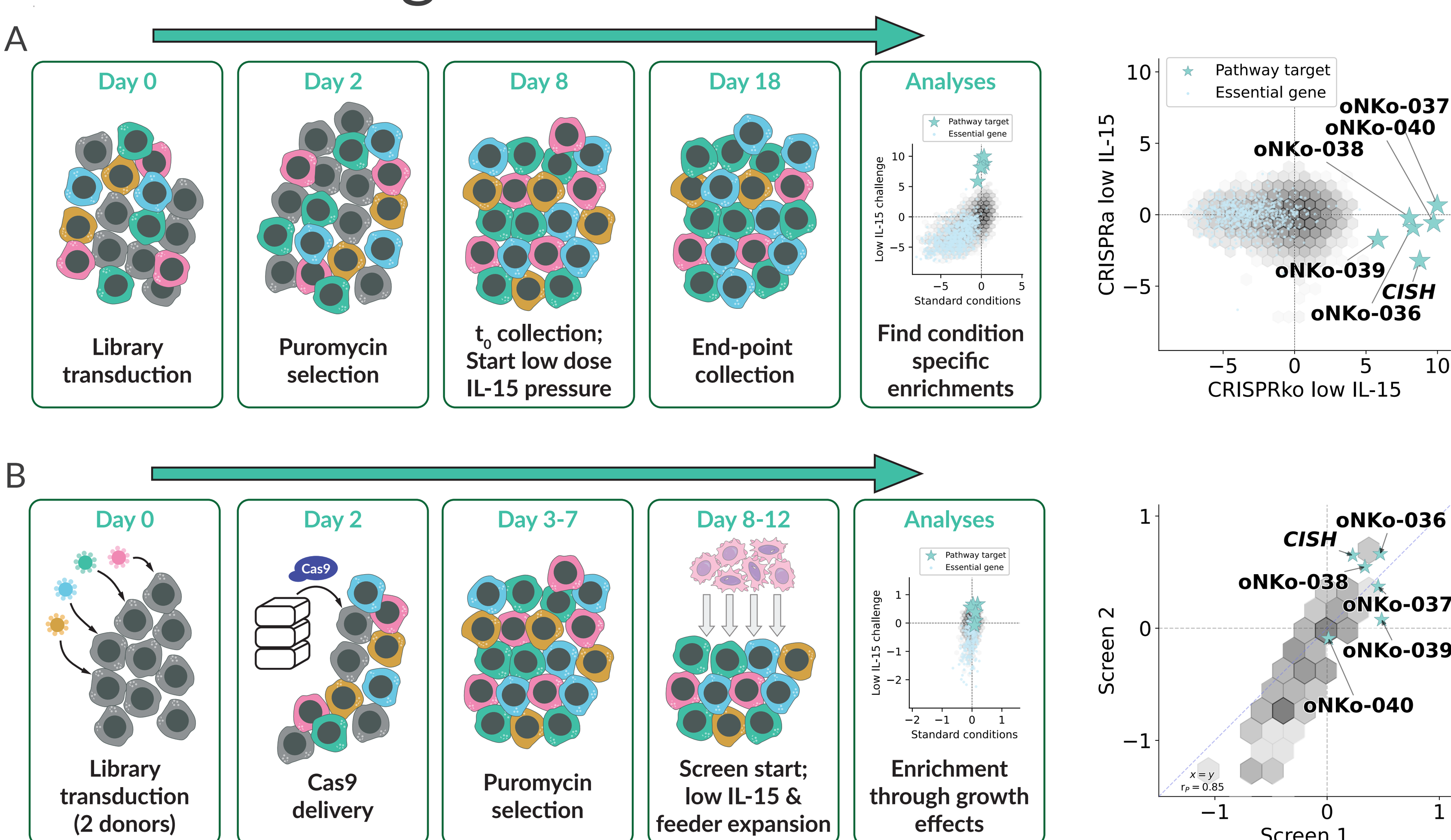

Figure 2. Enrichment screens in human NK cells. (**A**) Cas9-NK92 (KO) or dCas9-VP64-NK92 (activation) were transduced with a GW guide library and cultured under low or optimal IL-15 conditions. Remaining clones were isolated and sequenced. (**B**) 2 NK cell donors were transduced with a custom druggable library, edited and expanded on feeders in low IL-15. Surviving clones were isolated and sequenced.

NK cell cytotoxicity and IFNγ regulators

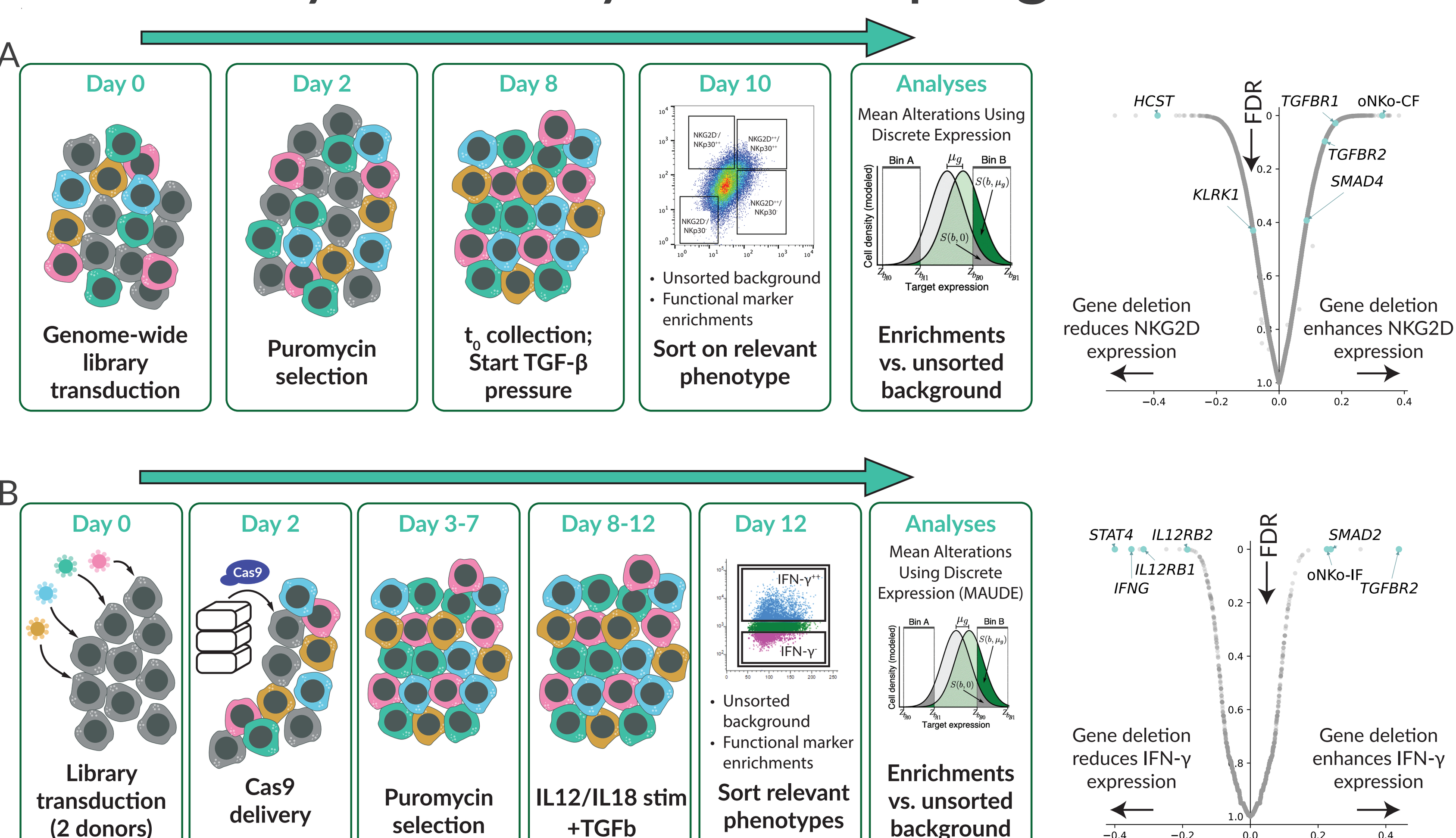

Figure 3. Phenotypic screens in human NK cells. (**A**) Cas9-NK92 were transduced with a GW guide library and puromycin selected. To identify regulators of NK cell cytotoxicity, NKG2D high and low subpopulations were sorted and sequenced. (**B**) 2 NK cells donors were transduced with a custom druggable library, edited, and IFNγ was induced using IL12/IL18 stim. IFNγ high and low subpopulations were sorted and sequenced. In both screens, TGF-β pressure was applied to increase the screen resolution and mimic the effects occurring in the tumor microenvironment.

Methods

Validation assays in NK cells donors

After isolation and expansion on K562 feeders, NK cells were RNP edited and expanded on feeders for another round before setting up the functional assays. In CAR-NK cell experiments, NK cells were first lentivirally transduced with an Abecma CAR construct and 4 days later RNP edited. To assess the edited NK cell function, proliferation was measured using an EdU uptake assay and cytokine release using LegendPlex FACS-based bead assay. For serial challenges, the NK cells were exposed to the target cell challenge every 24h for 3-4 rounds (R1-R4 in graphs) and the target cell killing was quantified either by FACS (Fig.4) or Incucyte (Fig. 5). Statistical tests were performed using one-way or two-way ANOVA, error=SD, *p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001

In vivo validation in target KO chimeric mice

To assess the gene target function in vivo, murine LSKs were RNP edited with the indicated sgRNA and transferred into lethally irradiated mouse recipients. 8 weeks post-reconstitution, splenic NK cells were phenotyped and the mice were challenged with the B16F10 melanoma cells. Metastatic burden was assessed by quantifying established lung metastatic nodules.

Identified hits regulate NK cell function

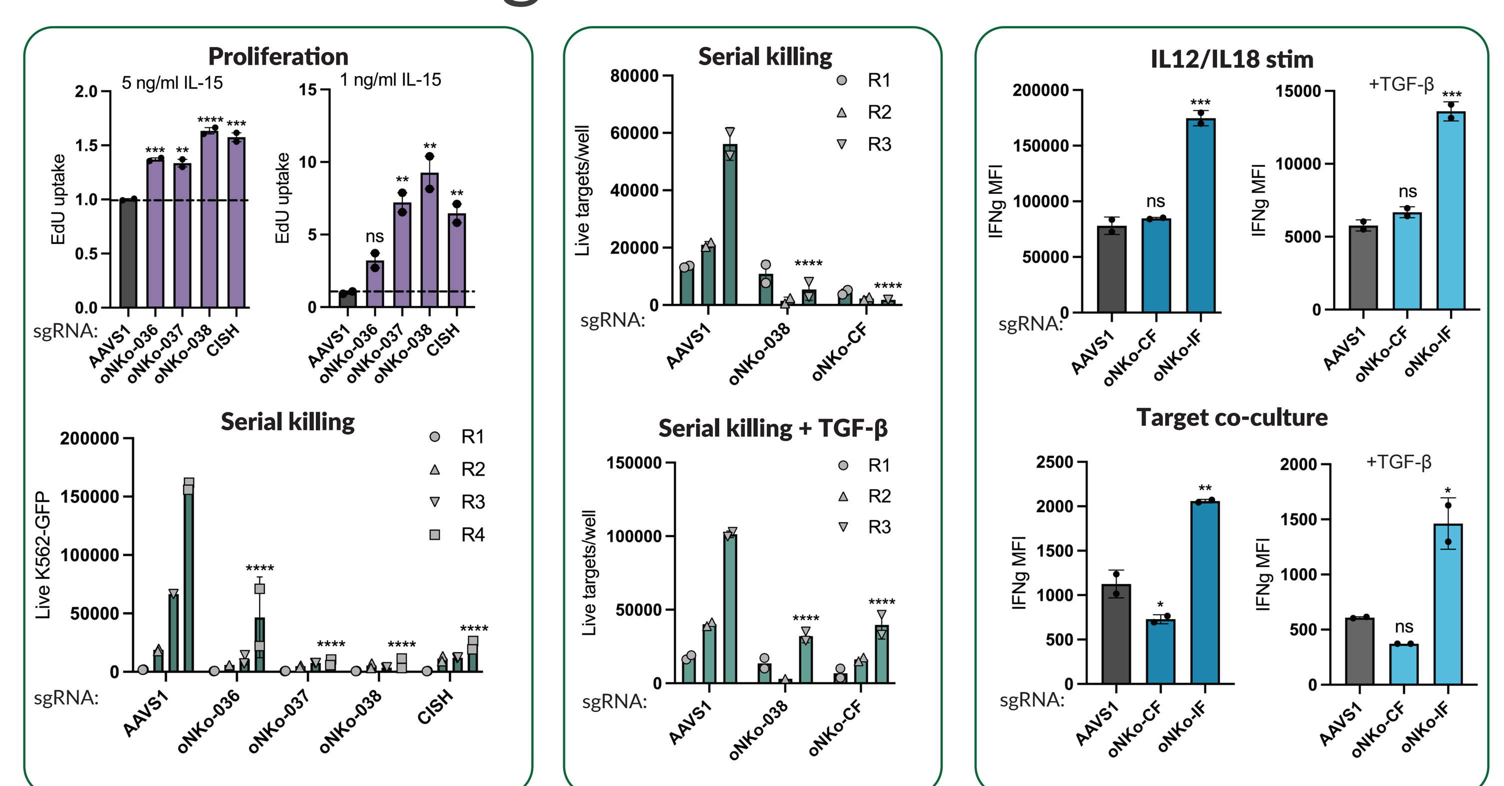

Figure 4. Identified hits modulate different aspects of the NK cell function. Target deletion in NK cell donors enhances their proliferation, serial killing w/o TGF-β (oNKo-036-38 and oNKo-CF), and IFNγ release induced by either IL-12/IL-18 or K562 co-culture (oNKo-IF).

Identified hits regulate CAR-NK cell function

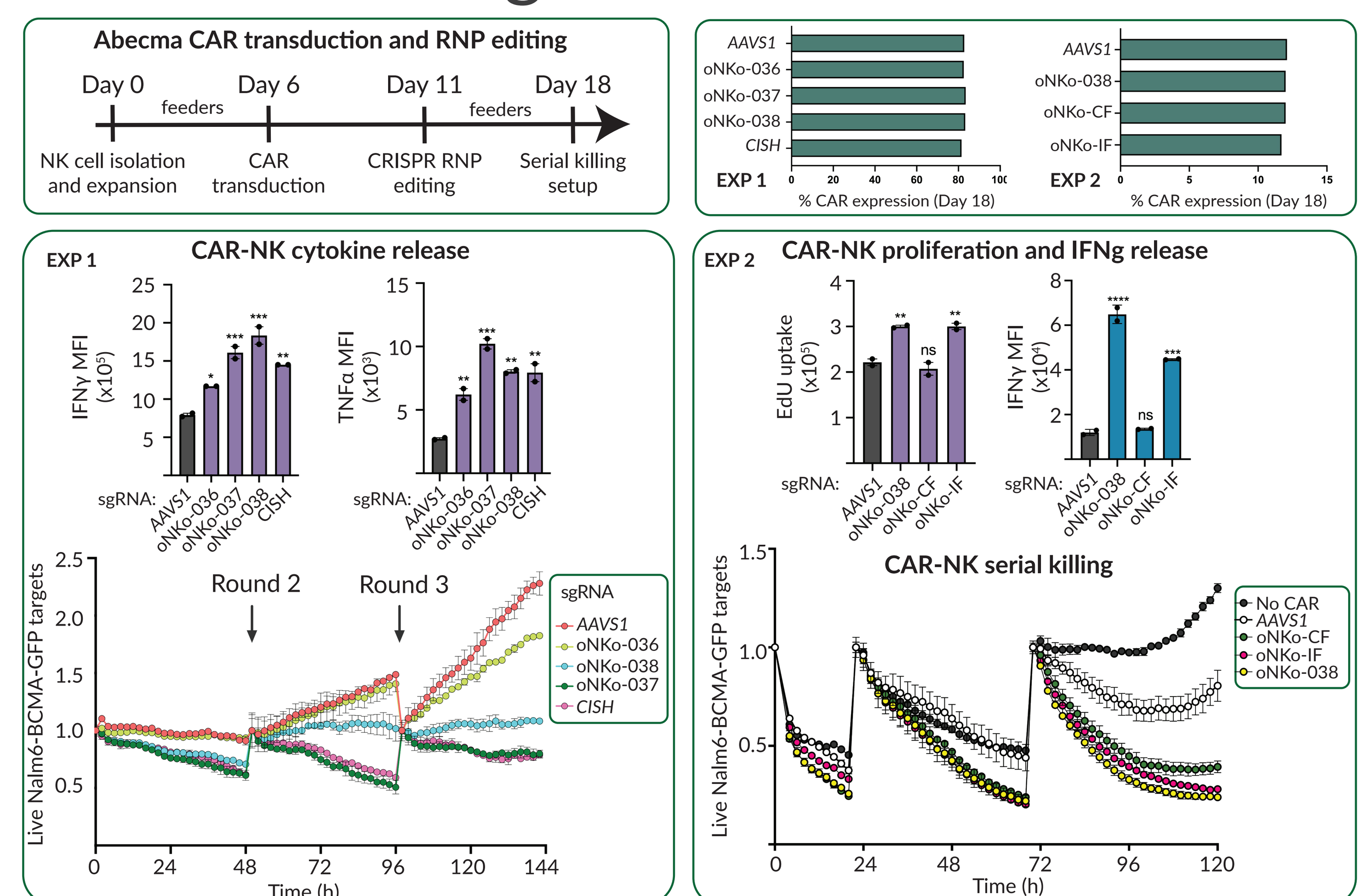

Figure 5. Identified hits modulate Abecma CAR-NK cell function. Target deletion in CAR-NK cell donors boosts their serial killing capacity and cytokine production.

oNKo-36/37 regulate NK cell metabolism and anti-metastatic activity

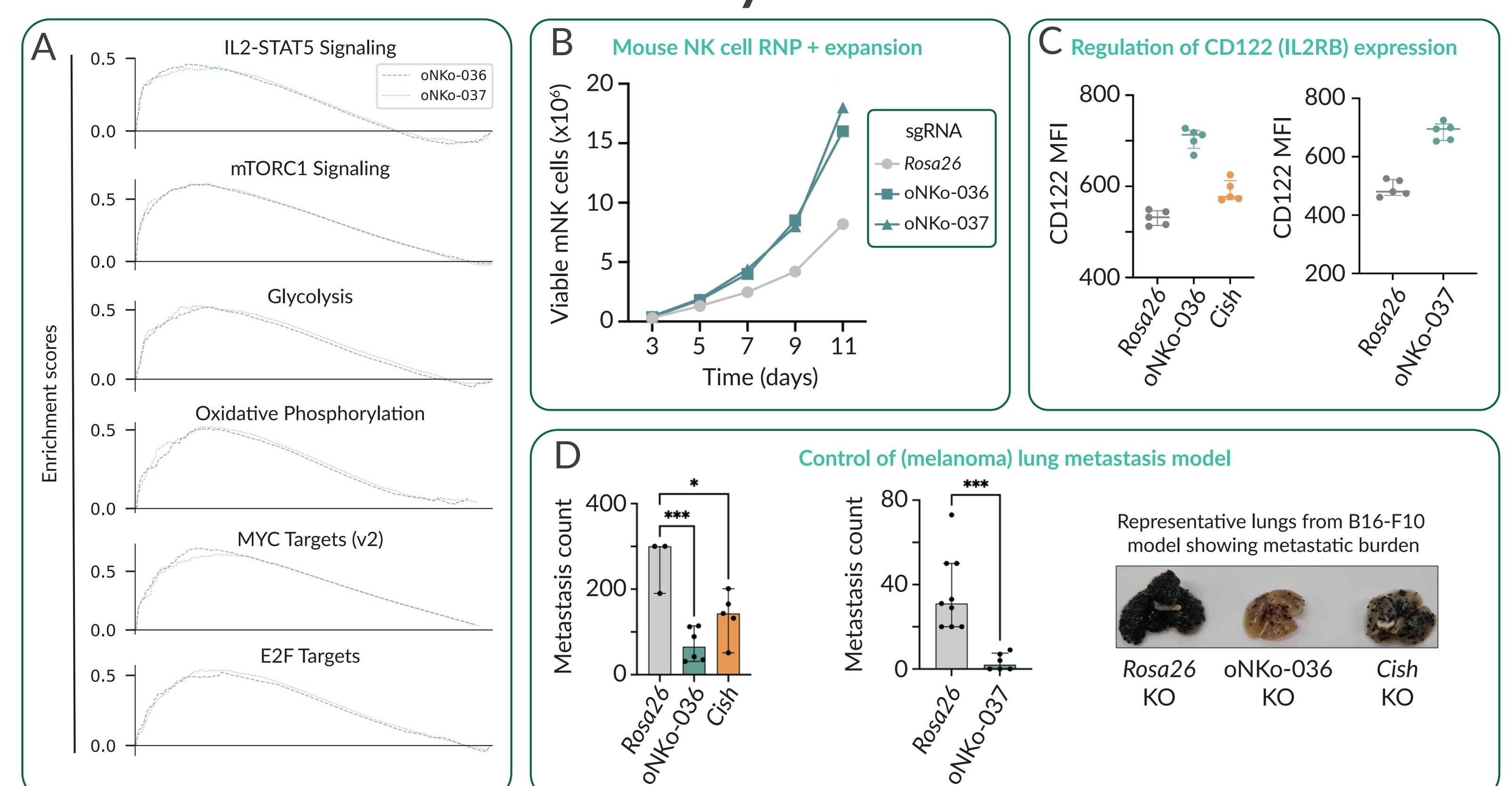

Figure 6. oNKo-36/37 KO enhances IL-15 signaling and supports murine NK cell anti-metastatic function. (**A**) Bulk RNA-seq data from target deleted NK cell donors indicates enhanced metabolic activity. (**B**) Target deletion in murine NK cells enhances their proliferation. (**C-D**) Target KO chimeric mice show splenic NK cells with increased CD122 (IL2Rb) expression (**C**) and enhanced control of B16F10 melanoma metastatic burden (**D**).