

# Utilization of naïve VHH phage display library & integrated antibody engineering platform to generate antibody leads for CAR-T therapy

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## ABSTRACT

Due to its small size and stable, single-domain nature VHHs or single-domain antibody (sdAb) has become an ideal building block in cell therapy and achieved encouraging success clinically, such as in the case of CAR-T therapy based on anti-BCMA VHH (CARVYKTI® by Legend/J&J). However, VHH discovery based on the conventional immunized phage library is sometimes hampered by the poor sequence/epitope diversity and low immune response due the high AA sequence homology of the target protein between human and the host species. To address these limitations, here in this report, we present an alternative approach utilizing an alpaca naïve VHH library combined with antibody engineering by affinity maturation.

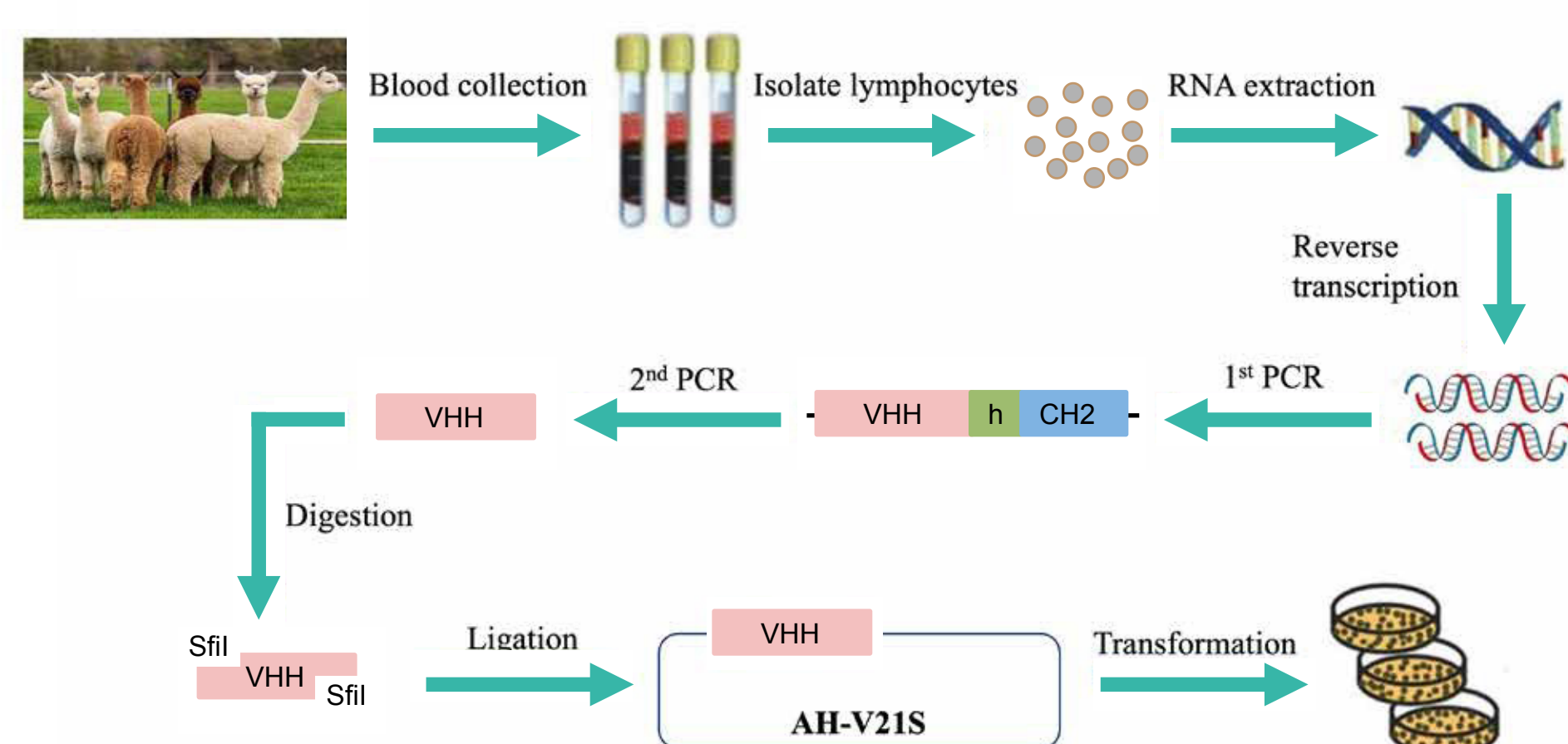
## INTRODUCTION

In this case study, an alpaca naïve VHH library was panned and screened by ROR1 proteins of 3 different ECDs and CHO-K1/ROR1 cell line to identify ROR1 VHH candidates. These VHH candidates were used to construct CAR-T cells and further evaluated by vitro functional assays, based on which final leads were selected for optimization by affinity maturation and ready for the development of CAR-T therapies.

## MATERIALS AND METHODS

### 1.Generation of naïve phage display library

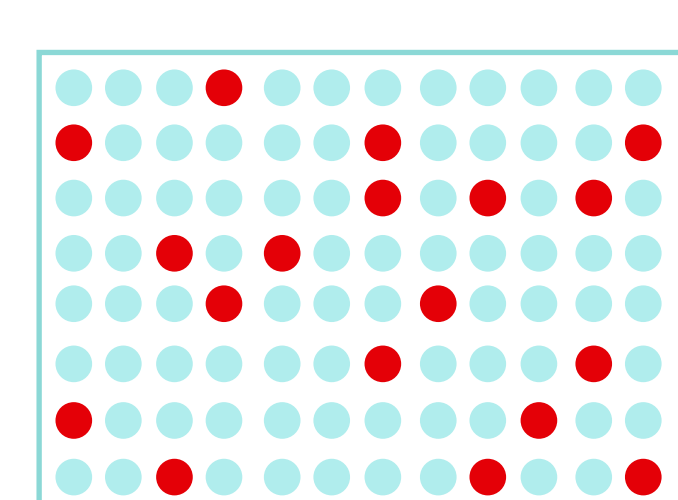
- PBMCs from hundreds of naïve alpaca donors were collected and used to build a naïve VHH phage library with the size of  $2 \times 10^{11}$ .



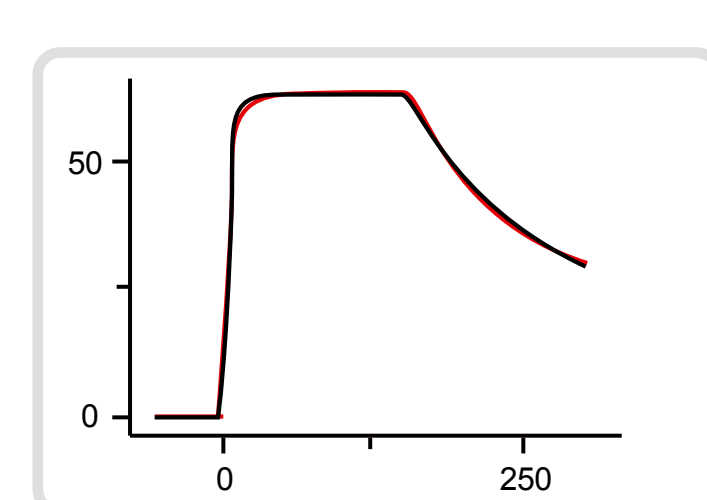
- Comparing with immunized library, whose success is often limited by poor epitope & sequence diversity and low immune response when the AA sequence homology of the target is high between hu Ag and alpaca, such as in the case of ROR1, the naïve library may offer better epitope/sequence diversity and faster turnaround time.

### 2.Phage library panning & screening

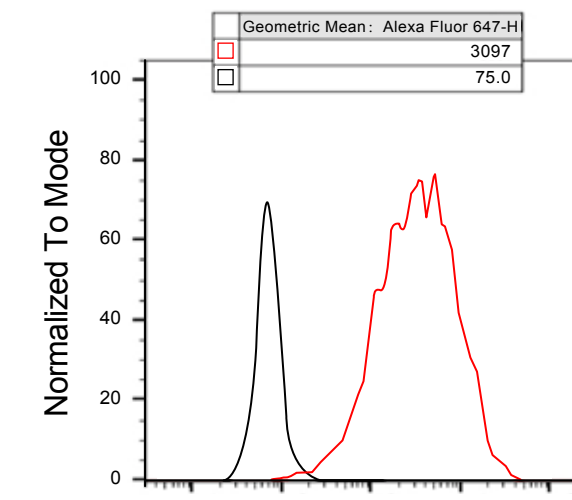
- To obtain VHH candidates recognizing different ECDs, the naïve phage library was panned and screened by three different ECD proteins and ROR1 overexpressing cell line, followed by phage monoclonal screening via ELISA, SPR and FACS.



Monoclonal ELISA



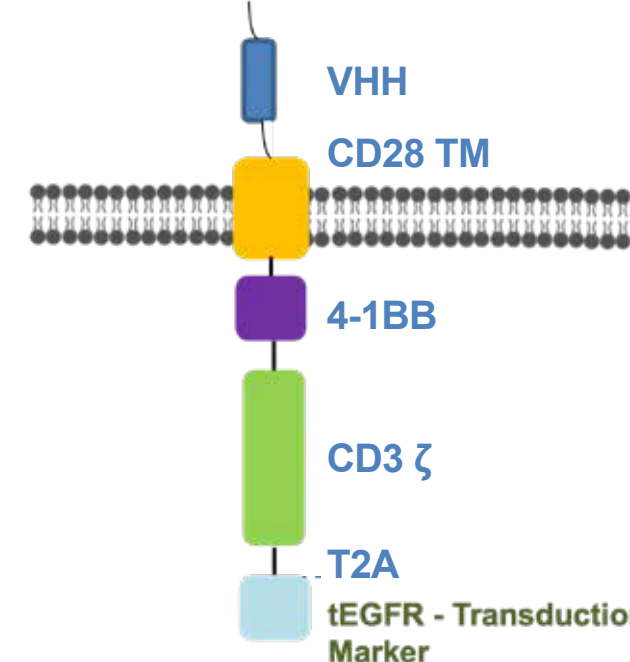
SPR Ranking



Monoclonal FACS

### 3.CAR-T cell construction and functional evaluation

- VHH candidates were used to build CAR domain construct via transduction of T-cells to make CAR-T cells, and further evaluated in cell killing and cytokine release assays in vitro.



## RESULTS

### 1.Library panning & screening funnel

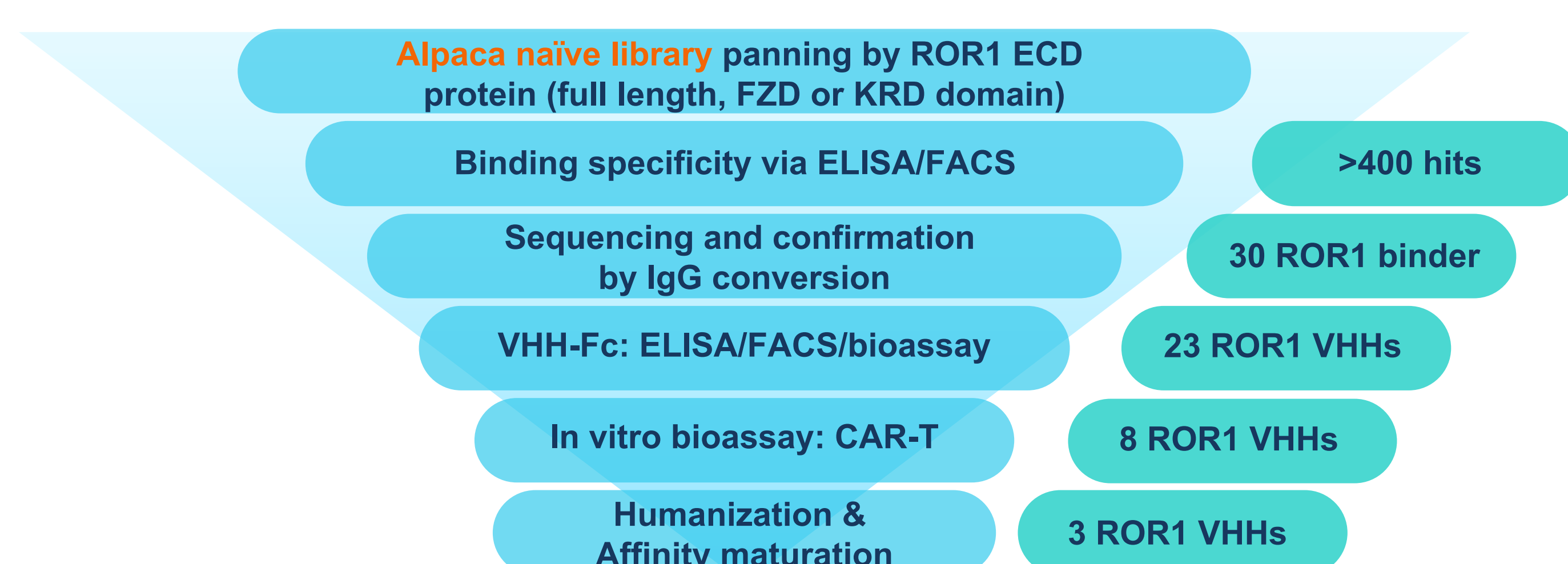


Figure 1. The alpaca naïve VHH library was panned & screened by three ROR1 ECD proteins and CHO-K1/ROR1 stable cell line, followed by functional evaluation in a CAR-T setting. The top three candidates were selected for antibody optimization by humanization and affinity maturation as final leads.

### 2.VHH candidates against three different ECD domains were obtained by differential panning/screening strategy

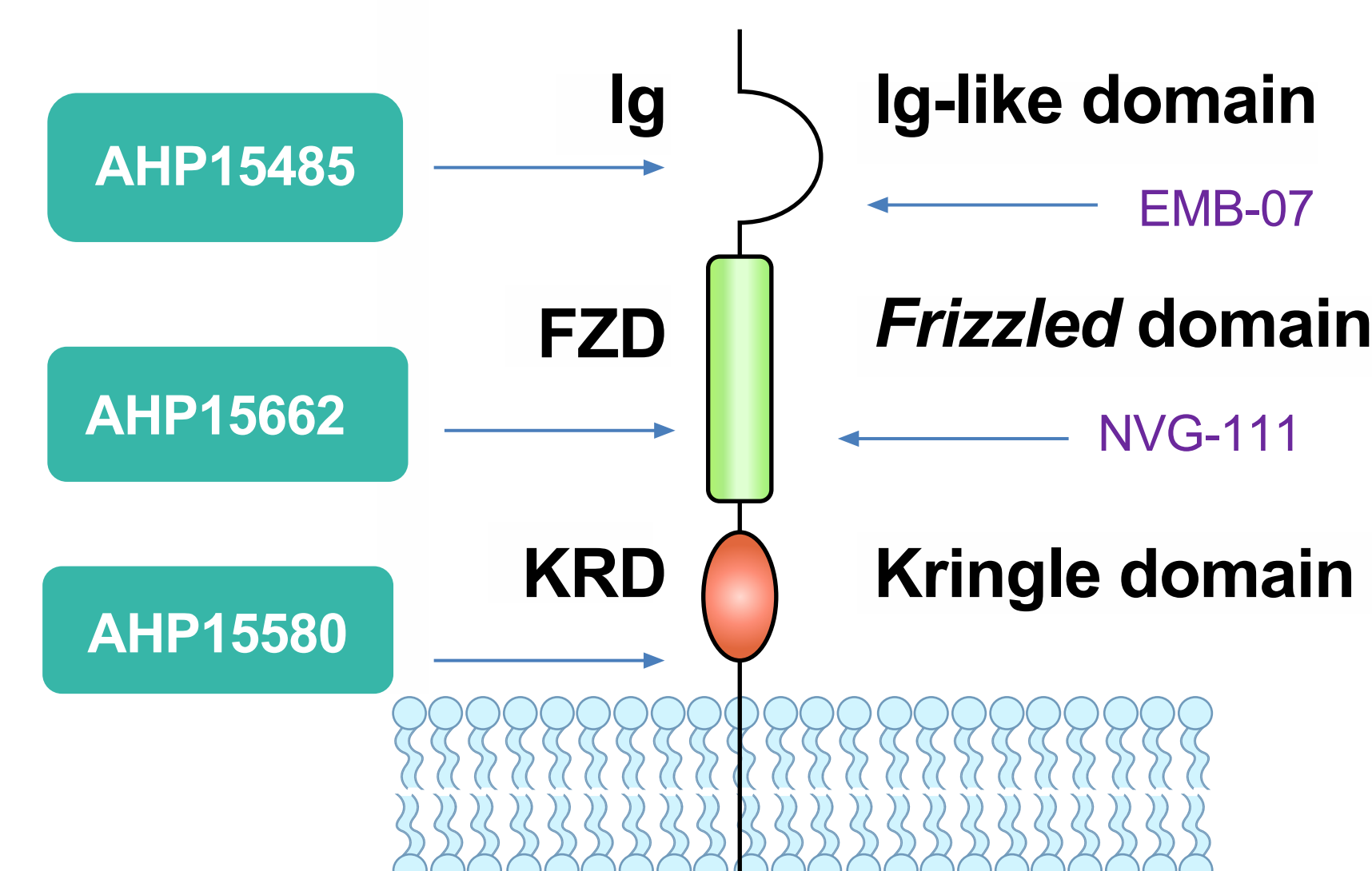


Figure 2. The naïve library gives a panel of diversified candidates, binding to three different domains of ROR1 extra-cellular region.

### 3.Functional evaluation of ROR1 VHH leads in a CAR-T setting

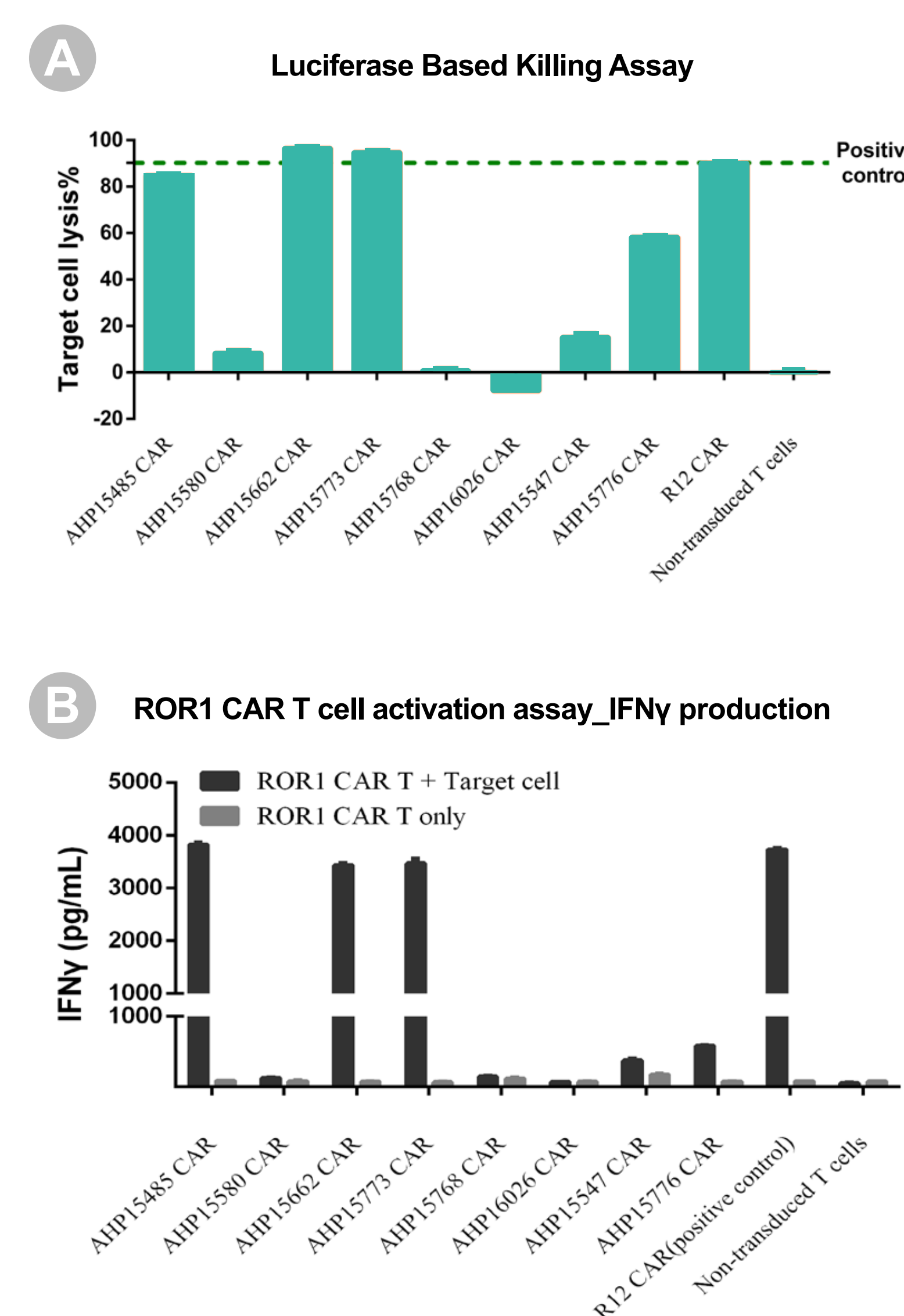


Figure 3. **Functional evaluation of ROR1 VHHs in a CAR-T setting in vitro.**

CAR-T cells was constructed by transduction of primary T cells with CAR constructs containing relevant VHH candidates, and evaluated by in vitro function assays.

(A) Cytotoxicity assay was performed after co-incubation of CAR-T cells (effectors) and CHO-K1/ROR1/Luc for 24 hours. The percentage of target cell lysis was calculated per luminescence signal.

(B) The release of representative cytokines (IFN- $\gamma$ ) by ROR1 CAR-T cells in response to co-incubation with target cells was measured by ELISA assays.

### 4.VHH lead optimization by humanization & affinity maturation

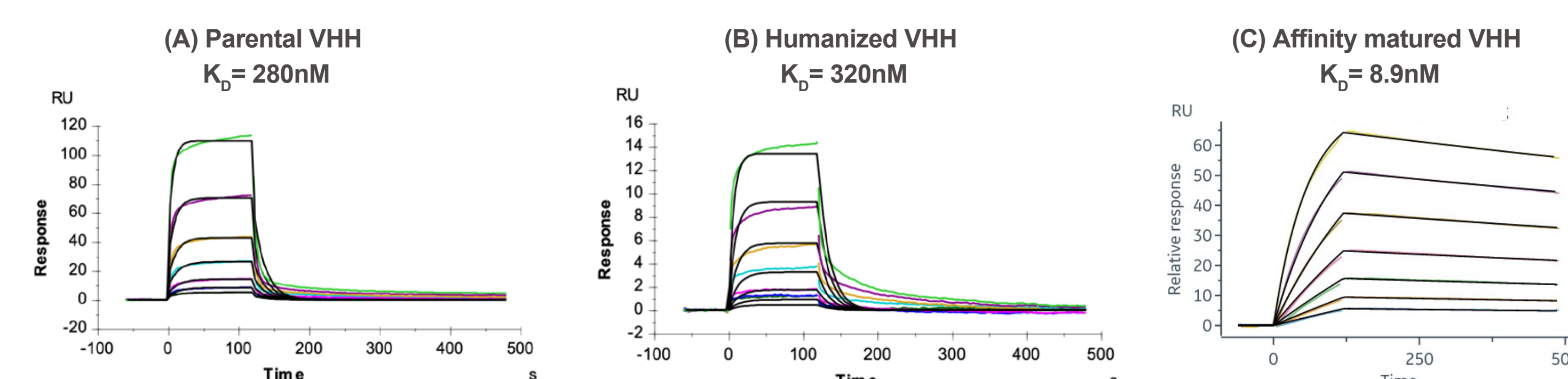


Figure 4. Full kinetic SPR analysis revealed that the affinity of a top candidate (AHP15485) was maintained after humanization, and greatly improved after affinity maturation (> 30 fold).

## CONCLUSION

In this report, to address some of the limitations of the conventional VHH lead discovery based on the immunized library, an alternative approach was presented, combining the advantages of alpaca naïve VHH library and Ab engineering by affinity maturation. We showed this approach successfully delivered multiple VHH leads targeting diverse ECD domains of ROR1, a challenging target for an immunized library approach due to its high AA sequence homology between human and alpaca. These VHH candidates showed promising characteristics in vitro and are ready for the development of CAR-T therapies.