

Discovery of diverse GPCR-targeted antibodies using integrated technologies

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BACKGROUND

GPCRs are difficult targets.

Despite being high-value therapeutic targets, G protein-coupled receptors (GPCRs) have been largely intractable to conventional antibody discovery technologies.¹ Due to the deep complexity in GPCR structure and function, there are challenges at each stage of discovery.

Generation of robust, target-specific immune responses is limited by high cross-species homology and a small number of available epitopes.¹ In addition, target-binding hits are extremely rare, reducing the chances of finding functional and developable leads.²

AIM

Find diverse GPCR-targeted antibodies.

Here, we present a case study in which we leveraged our discovery and development engine to find diverse antibodies against an established GPCR target, chemokine receptor 8 (CCR8).

CCR8 is highly and selectively expressed on tumor-associated regulatory T cells in multiple cancer types.³ Antibodies against CCR8 represent a promising therapy to treat solid tumors that have progressed on standard immune checkpoint inhibitors.^{4,5,6}

To be effective, antibodies against CCR8 should be human/humanized and show favorable developability properties, highly specific binding, and potent antibody-dependent cellular cytotoxicity (ADCC) reporter activity.

METHODS

Amplify diversity, enrich for potency, and fine-tune leads.

A combination of protein and DNA immunization campaigns was used to generate robust immune responses. We captured hundreds of diverse antibodies using deep single-cell screening. High-throughput function-based screening was then used to down-select antibodies based on ADCC reporter activity. Selected antibodies were optimized for developability using structure-guided protein engineering technologies paired with high-throughput antibody assessment.

RESULTS

A diverse panel of anti-CCR8 antibodies optimized for developability and function.

Selected molecules displayed desired properties, including:

- Diversity:** derived from two animal species, including two humanized mouse strains
- Potency:** strong ADCC reporter activity that is similar to a clinical benchmark
- Developability:** favorable biophysical properties that have been de-risked by pre-emptive remediation

272 diverse CCR8-binding hits

Immunization and deep single-cell screening technologies were used to find hundreds of diverse sequences.



Figure 1. 272 diverse CCR8-specific antibodies were identified from immunization and deep single-cell screening. (A) Two strains of humanized mice and two strains of wild-type rats were immunized using recombinant protein- or genetic-based formulations. Antibody titers were measured by flow cytometry using cells expressing human CCR8. (B) Single-cell screening assays were used to screen 2.6 million single cells and identify CCR8-specific antibodies. Representative output images of a CCR8-specific hit in a single chamber from our single-cell screen are shown. Antibodies that bind to CCR8-expressing cells were detected using fluorescence microscopy and images were analyzed using machine vision. (C) Antibody sequence diversity was visualized using Celium™, and 272 CCR8-binding antibodies were selected for high-throughput expression based on clonal diversity. Clusters represent clonal families based on V genes, J genes, and CDR3 lengths.

194 functional anti-CCR8 antibodies

High-throughput functional screening and liability assessment guided our down-selection and pre-emptive remediation strategy.

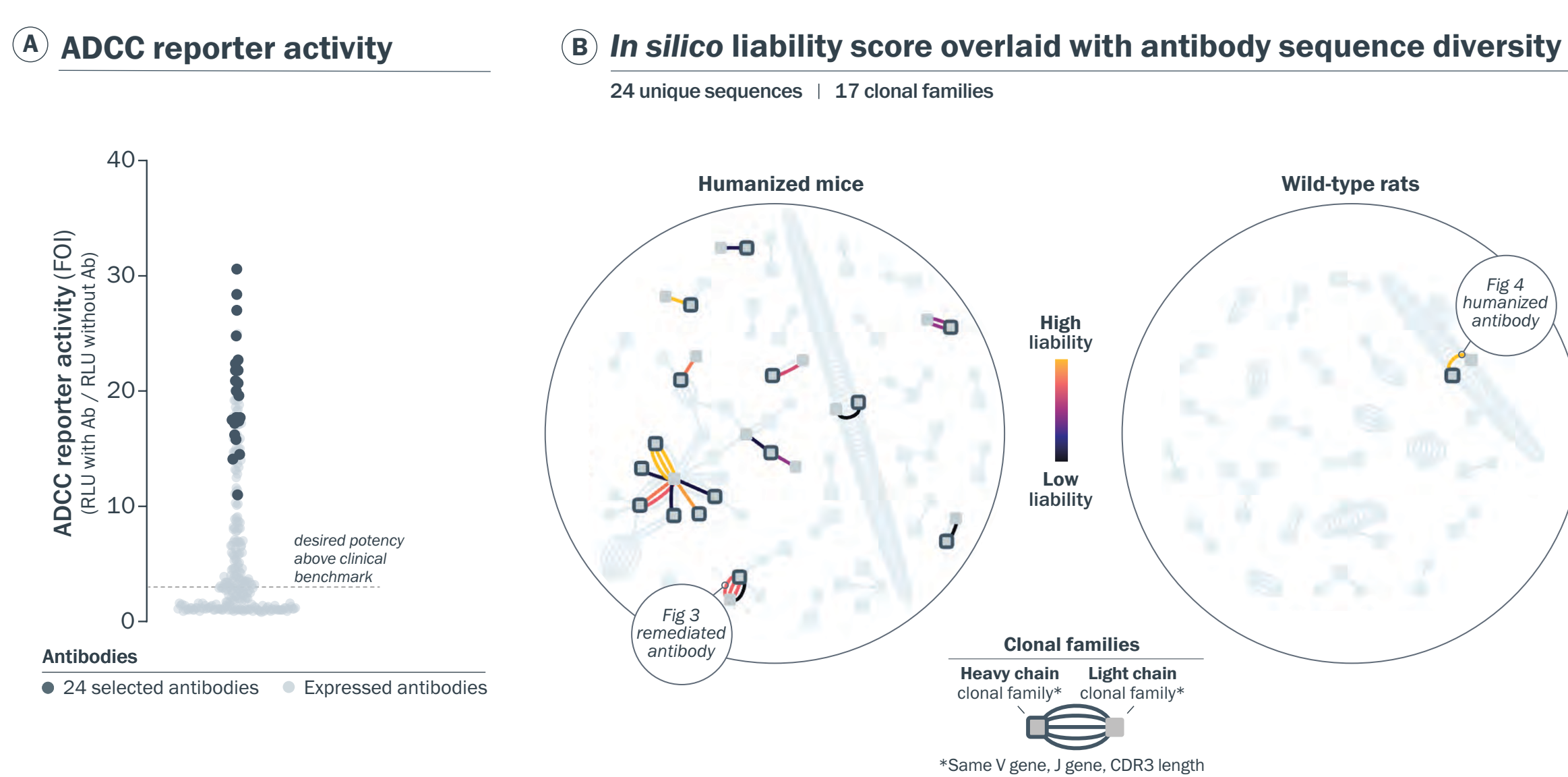


Figure 2. From 194 functional antibodies, 24 highly potent candidates were selected for high-throughput characterization and liabilities were mapped to guide pre-emptive remediation. (A) 194 of 272 expressed antibodies met the desired functional activity, measured in an ADCC reporter assay with activity quantified using luminescence. (B) Potential sequence liabilities of the 24 selected antibodies were identified *in silico* and mapped using proprietary bioinformatics pipelines. Antibody clonal diversity and liabilities were visualized using Celium™.

RLU: Relative luminescence units; FOI: Fold of induction.

24 highly potent antibodies optimized for developability

Remediation of sequence liabilities in a humanized mouse-derived antibody.

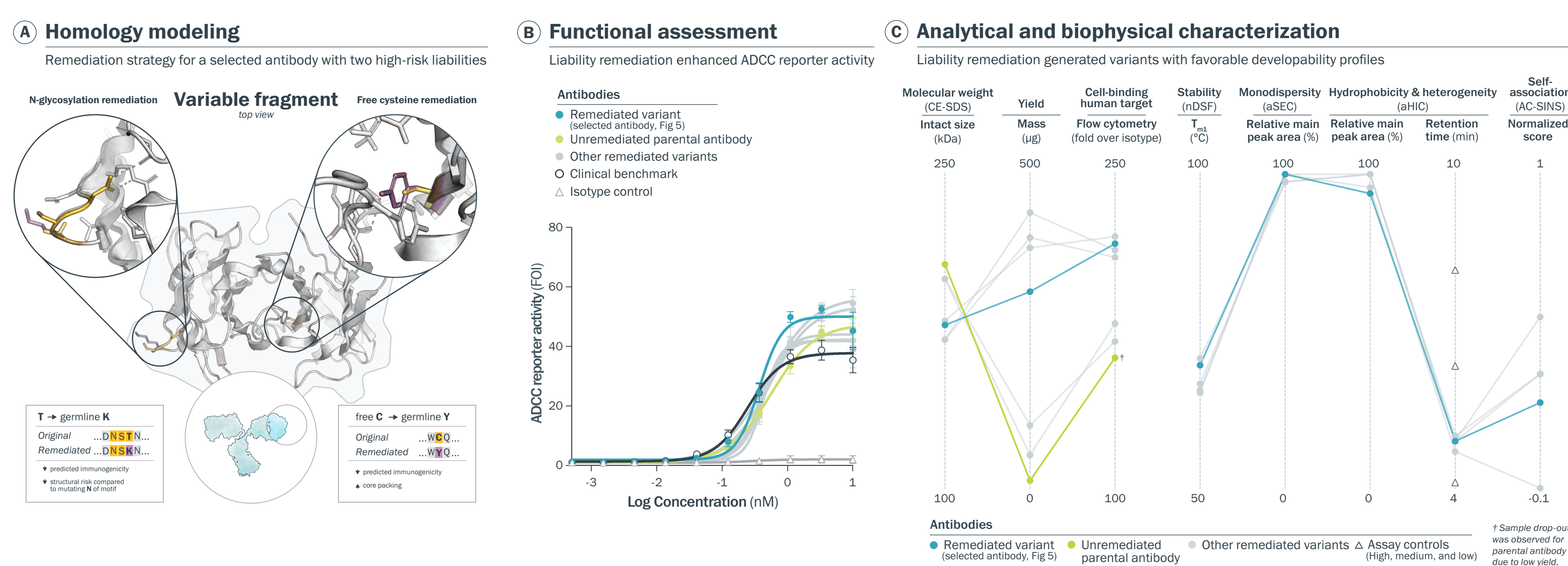


Figure 3. Remediated variants show enhanced function, affinity, and yield compared to the parental antibody. (A) Homology modeling of a humanized mouse-derived antibody guided the remediation strategy.

(B) Functional activity was measured in an ADCC reporter assay in three-fold serial dilutions starting at 10 nM and data were normalized to an isotype control; activity was quantified using luminescence.

(C) Variants with remediated N-glycosylation site (indicated by reduced apparent molecular weight in CE-SDS) displayed improved yield and cell-binding compared to the parental antibody. Extended developability profiles of remediated variants were favorable, including desired stability, high monodispersity, and low self-association.

Structure-guided humanization of a highly potent rat-derived antibody.

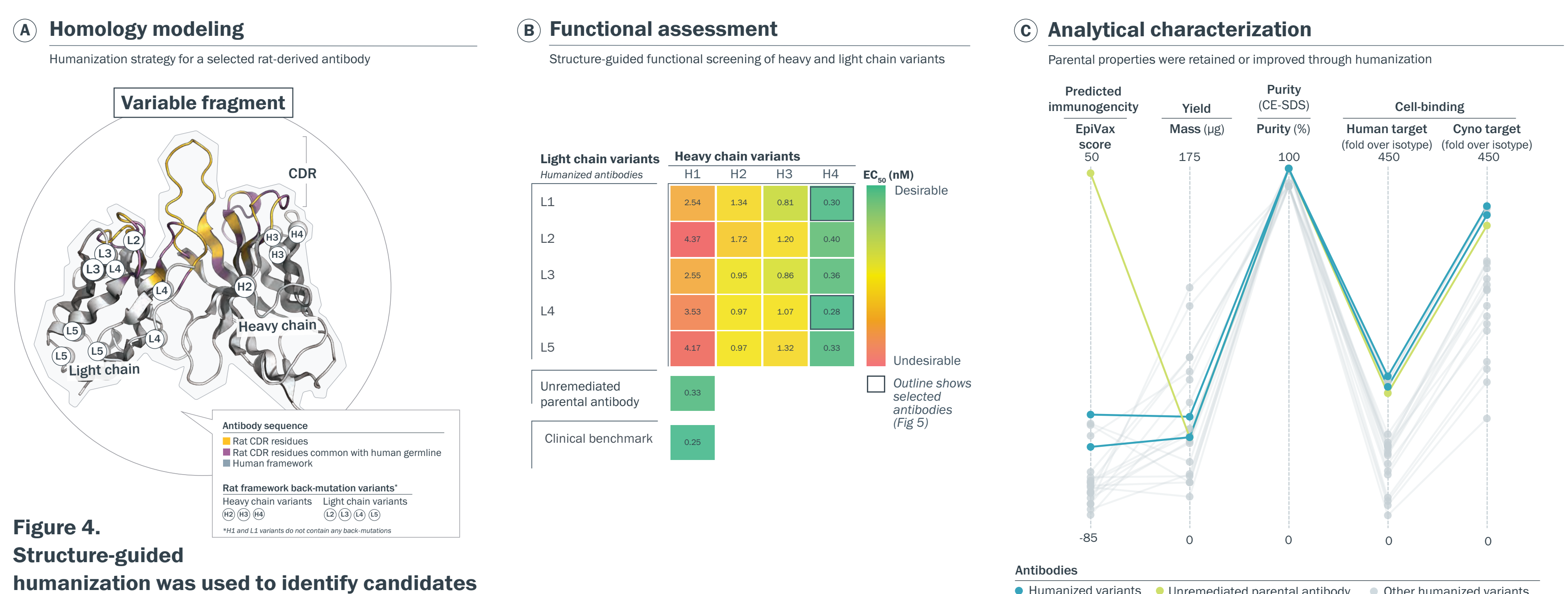


Figure 4. Structure-guided humanization was used to identify candidates with optimal profiles for immunogenicity and function. (A) Homology modeling guided the assessment of residues targeted for humanized back-mutations on the light (L1-5) and heavy chains (H1-4).

(B) Impact of heavy/light chain back-mutations on function was assessed in a rational, combinatorial approach and measured in an ADCC reporter assay as described in Figure 3B.

(C) Humanization generated variants with reduced predicted immunogenicity and maintained or improved target-binding, yield, and purity compared to the parental antibody.

Anti-CCR8 antibodies with profiles similar to a clinical benchmark

A panel of wild-type and remediated antibodies was selected based on:

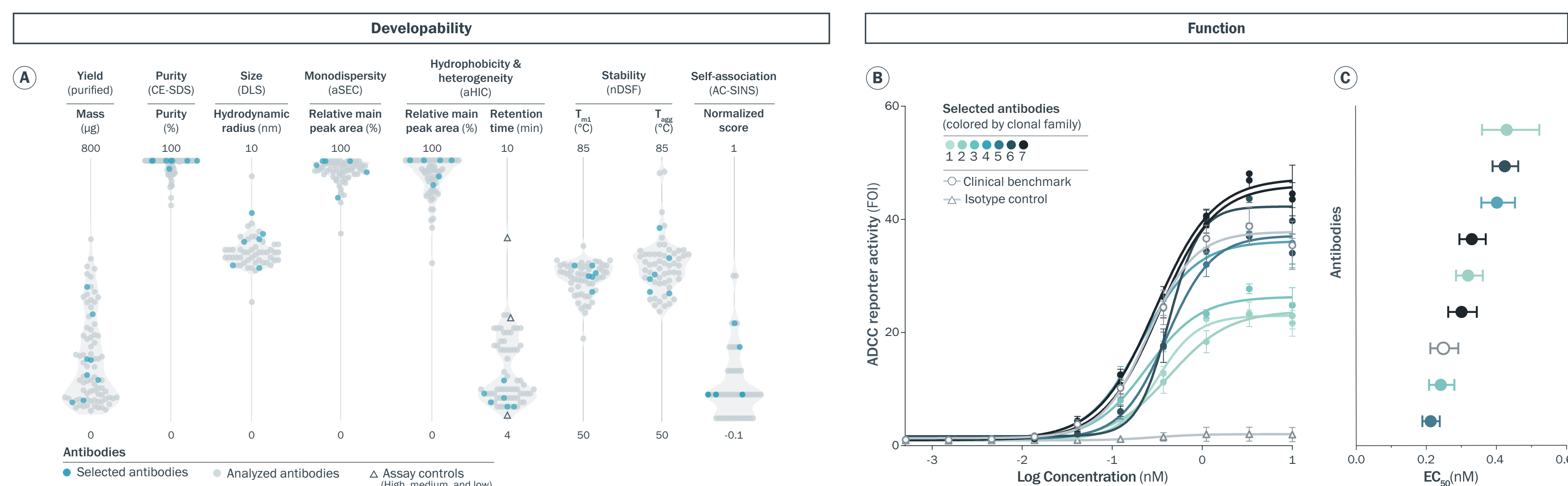


Figure 5. Strategic selection of diverse, developable antibodies with a range of potencies. (A) Selected antibodies have favorable developability profiles, including desired purity, monodispersity, relative surface hydrophobicity and heterogeneity, stability, hydrodynamics, and self-association with scores normalized to high and low controls.

(B) Selected antibodies were comparable to a clinical benchmark molecule in preliminary ADCC assessment. Functional activity was measured with an ADCC reporter assay as described in Figure 3B.

(C) ADCC assay EC_{50} values and 95% confidence intervals are shown.

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