

Unique Carrier-Free Immunization Can Generate Precision Antibodies Targeting High-Homology Short Target Sequences in Mice



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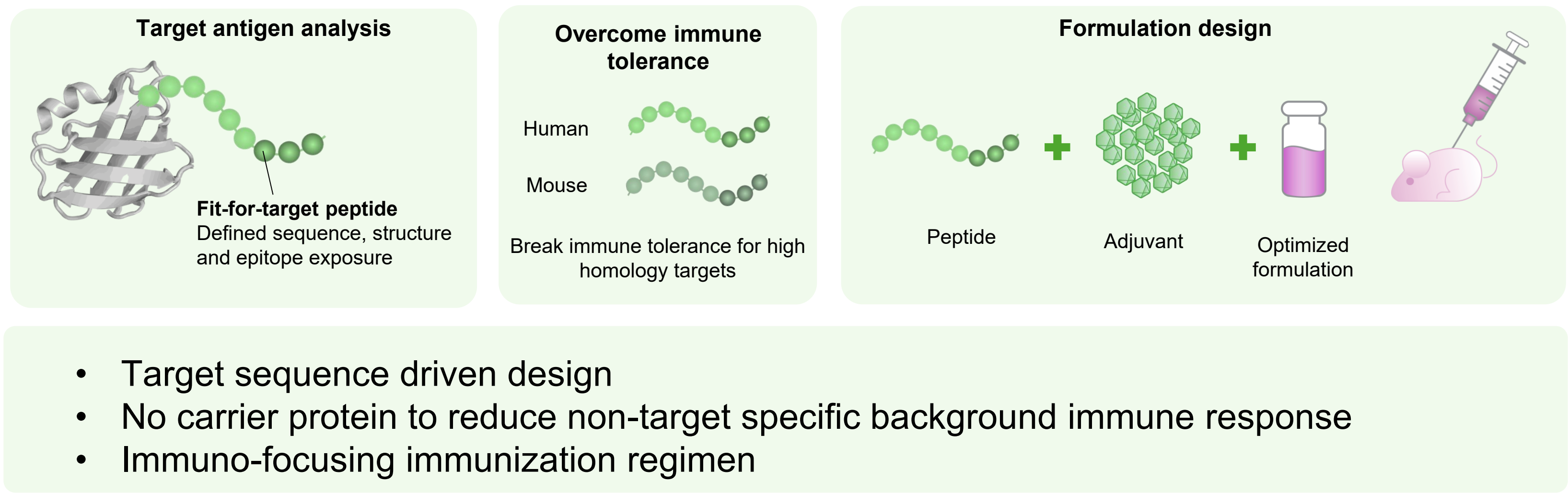
Abstract

Generating highly specific antibodies against short peptide epitopes remains challenging, particularly when target sequences are highly conserved across species. Conventional peptide immunization typically relies on carrier proteins to enhance immunogenicity; however, strong immune responses against the carrier can compete with or distract from responses directed toward the intended target epitope. Conigen has developed a unique carrier-free immunization technology designed to overcome these limitations and enable precision antibody generation against challenging peptide targets.

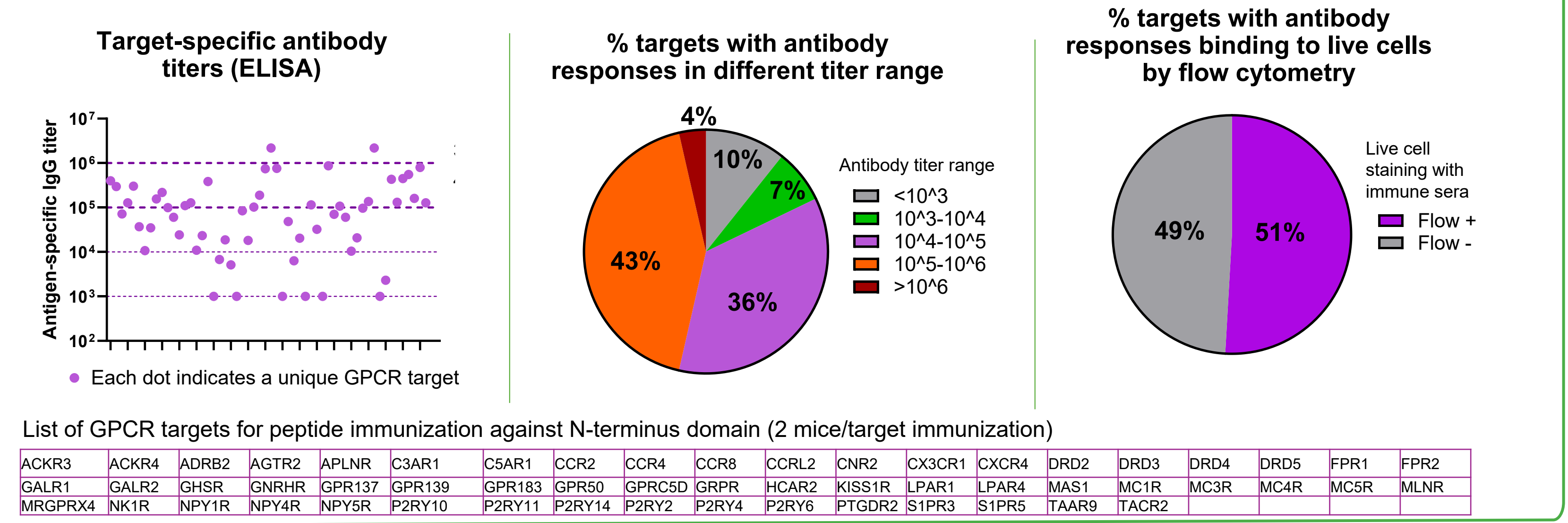
By eliminating conventional carrier proteins, this approach minimizes carrier-induced immune interference and focuses the immune response directly on the target peptide. The platform is particularly valuable for short, highly conserved sequences where immune tolerance and limited epitope availability can make antibody generation difficult. Using this strategy, Conigen has successfully generated highly specific and sensitive antibodies against numerous challenging targets, including conserved epitopes within G protein-coupled receptors (GPCRs) and isoform-specific antibody responses against FGFR4. The resulting antibodies can recognize defined target regions while providing the specificity required to distinguish closely related proteins or homologous sequences.

This carrier-free precision immunization platform expands access to previously difficult antibody targets, such as conserved, high-homology targets like GPCRs. Additionally, it provides a versatile approach for generating epitope-focused, isoform-specific antibodies like FGFR4. The technology can support antibody discovery across biomarker research, diagnostic assay development, therapeutic antibody programs, target validation, and basic biomedical research.

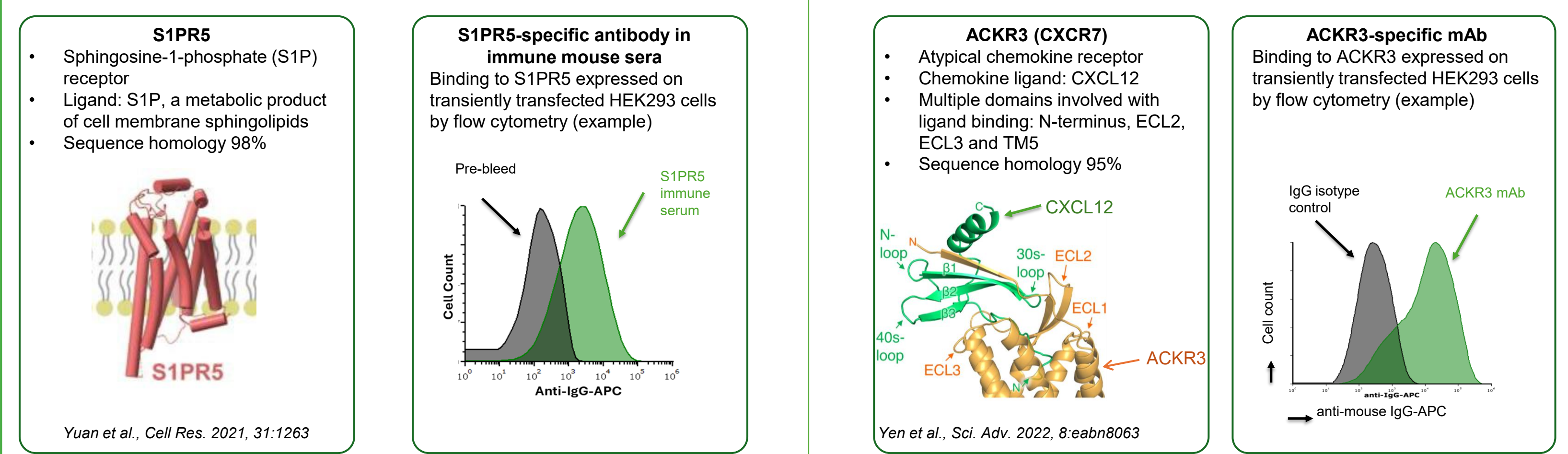
1. Carrier-Free Peptide Immunization for Short Target Sequence



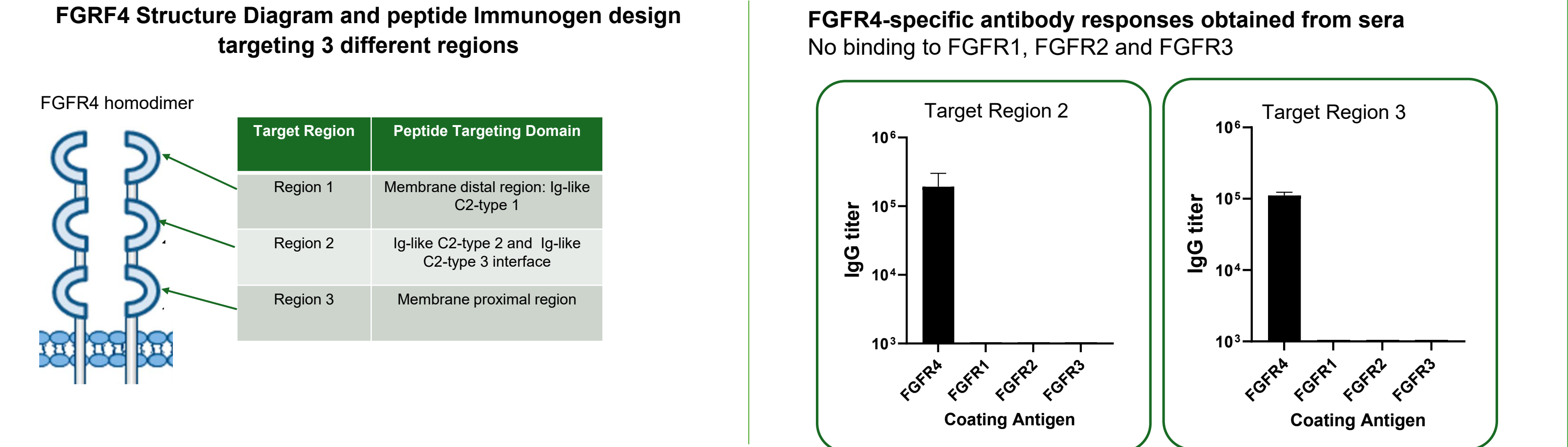
2. GPCR-Specific Antibody Responses by Peptide Immunization



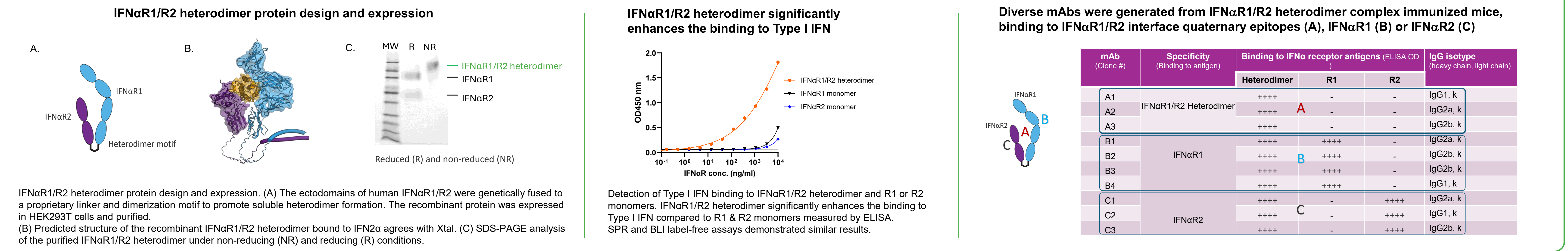
3. Peptide-Induced Antibodies Against High Homology GPCR Targets (Examples)



4. FGFR4 Isoform Specific Antibodies, Not Binding to FGFR1, FGFR2 and FGFR3



5. IFNαR1/R2 Heterodimer Complex Significantly Enhances Type I IFN Binding and Elicited Diverse Antibodies Targeting IFNαR1/R2 Interface, IFNαR1 and IFNαR2



IFNαR1/R2 heterodimer significantly enhances the binding to Type I IFN

OD450 nm

IFNαR conc. (ng/ml)

IFNαR1/R2 heterodimer
IFNαR1 monomer
IFNαR2 monomer

Detection of Type I IFN binding to IFNαR1/R2 heterodimer and R1 or R2 monomers. IFNαR1/R2 heterodimer significantly enhances the binding to Type I IFN compared to R1 & R2 monomers measured by ELISA. SPR and BLI label-free assays demonstrated similar results.

Diverse mAbs were generated from IFNαR1/R2 heterodimer complex immunized mice, binding to IFNαR1/R2 interface quaternary epitopes (A), IFNαR1 (B) or IFNαR2 (C)

IFNαR1
IFNαR2

mAb (Clone #)	Specificity (Binding to antigen)	Binding to IFNα receptor antigens (ELISA OD)			IgG isotype (heavy chain, light chain)
		Heterodimer	R1	R2	
A1	IFNαR1/R2 Heterodimer	++++	-	-	IgG1, k
A2		++++	A	-	IgG2a, k
A3		++++	-	-	IgG2b, k
B1	IFNαR1	++++	++++	-	IgG2a, k
B2		++++	B	-	IgG2b, k
B3		++++	++++	-	IgG2b, k
B4		++++	++++	-	IgG1, k
C1	IFNαR2	++++	-	++++	IgG2a, k
C2		++++	-	++++	IgG1, k
C3		++++	-	++++	IgG2b, k

Conclusions

- Conigen's carrier-free peptide immunization platform is very effective in:
 - Generating specific antibodies against short target sequences.
 - Breaking immune tolerance for conserved high homology targets like GPCRs.
 - Eliciting isoform-specific antibodies like antibodies only binding to FGFR4 not FGFR1, FGFR2 and FGFR3.
- The soluble IFNαR1/R2 heterodimer complex protein significantly increased the binding potency to Type I interferon cytokine compared to the respective monomeric receptor proteins.
- IFNαR1/R2 heterodimer as immunogen elicited diverse specific antibodies against IFNαR1, IFNαR2 and more importantly the IFNαR1/R2 interface quaternary epitopes.

