

Insights Into Nipocalimab-Neonatal Fragment Crystallizable Receptor Structure (FcRn), Binding Affinity, and Inhibition of Immunoglobulin G Recycling: Comparison With Efgartigimod

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Introduction

- Nipocalimab is a fully human, aglycosylated monoclonal antibody with high affinity and specificity for neonatal fragment crystallizable receptor (FcRn) with a dissociation constant (K_D) of ≤31.7 pM at pH 6.0, and K_D ≤57.8 pM at pH 7.4.¹
- Nipocalimab blocks the binding of immunoglobulin G (IgG) to FcRn, thereby inhibiting FcRn-mediated recycling of IgG and reducing circulating IgG levels, including pathogenic IgG.¹
- Nipocalimab demonstrated a dose-dependent, pharmacokinetic-pharmacodynamic-receptor occupancy relationship resulting in rapid and sustained reductions in IgG, aligning with clinical response observed in patients with gMG.¹
- Nipocalimab is being studied in several autoantibody and alloantibody diseases and has been approved by the U.S. Food and Drug Administration for use in anti-acetylcholine receptor antibodies (AChR) and anti-muscle-specific kinase (MuSK) antibody-positive patients (adults and adolescents aged ≥12 years) with generalized myasthenia gravis (gMG).²
- Efgartigimod is the Fc portion of an IgG1 antibody engineered for affinity to FcRn and is another FcRn inhibitor approved for gMG treatment.³

Objective

- To compare the FcRn blockers, nipocalimab vs efgartigimod, with respect to their structural interactions with FcRn, binding affinities to FcRn, and potency of inhibition of IgG recycling in human aortic endothelial cells (HAECs).

Methods

- Nipocalimab binding epitopes on FcRn were determined using X-ray crystallography (PDB ID: 9MI6).
- Efgartigimod-FcRn complex structure was downloaded from the Protein Data Bank (PDB ID: 7Q15).
- Binding affinities were determined using surface plasmon resonance.
- Imaging pulse-chase and IgG recycling assays were performed using HAECs which endogenously express FcRn.

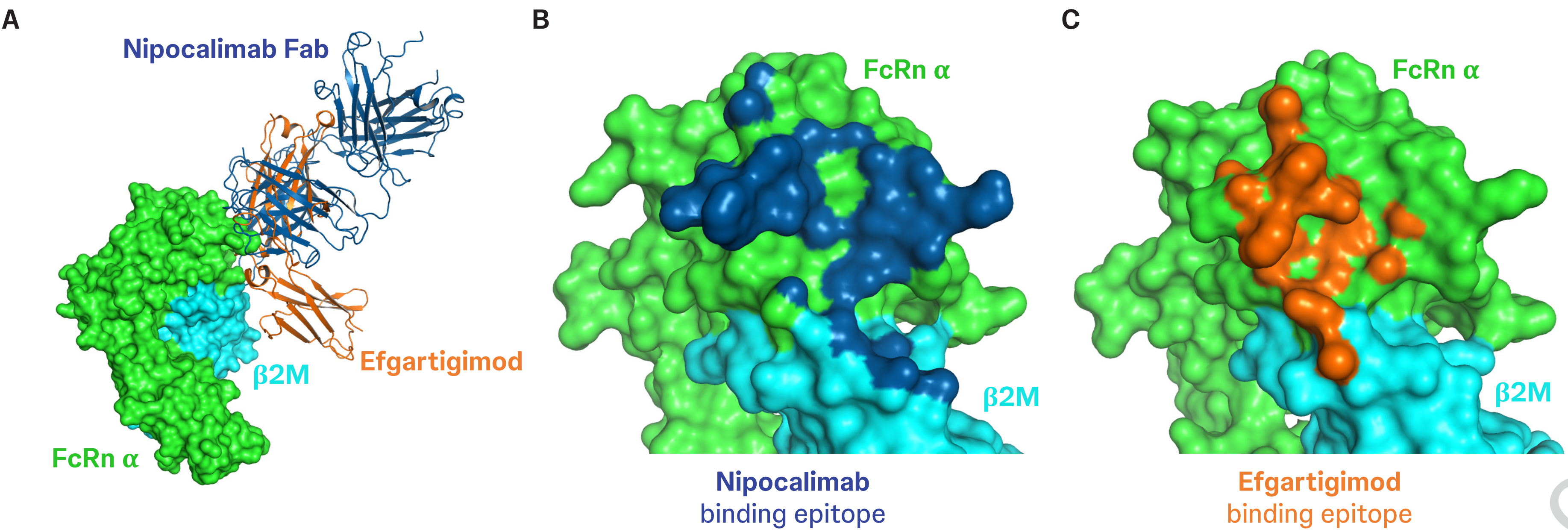
Key Takeaways

- There were differences between nipocalimab and efgartigimod with respect to their binding epitopes, binding affinities, and inhibition of IgG recycling.
- Nipocalimab demonstrated:
 - 30% larger binding interface with FcRn than efgartigimod.
 - Over 500-fold higher binding affinity at pH 6.0 and over 3,500-fold higher binding affinity at neutral pH versus efgartigimod
 - A longer intracellular residence time upto 18 hrs, consistent with its expected site of action (endosomal pathway) at lower doses than efgartigimod.
 - A ~300-fold greater in vitro inhibition of IgG recycling when compared to efgartigimod.

Results

Nipocalimab has a 30% larger binding interface with FcRn compared with efgartigimod

Figure 1: Nipocalimab has a larger binding interface with FcRn (1017.5 Å²) compared with efgartigimod (651.3 Å²)



Structural overlay of nipocalimab and efgartigimod bound FcRn suggests that both molecules target the Fc binding site of FcRn (Figure 1A). But Nipocalimab binding footprint on FcRn (Figure 1B) is larger than that of efgartigimod (Figure 1C) (1017.5 Å² vs 651.3 Å²).

Nipocalimab has over 500-fold greater binding affinity to FcRn at pH 6.0 and over 3,500-fold greater binding to FcRn at neutral pH compared with efgartigimod

Table 1: SPR Binding Affinity (K_D) at (endosomal) pH 6.0

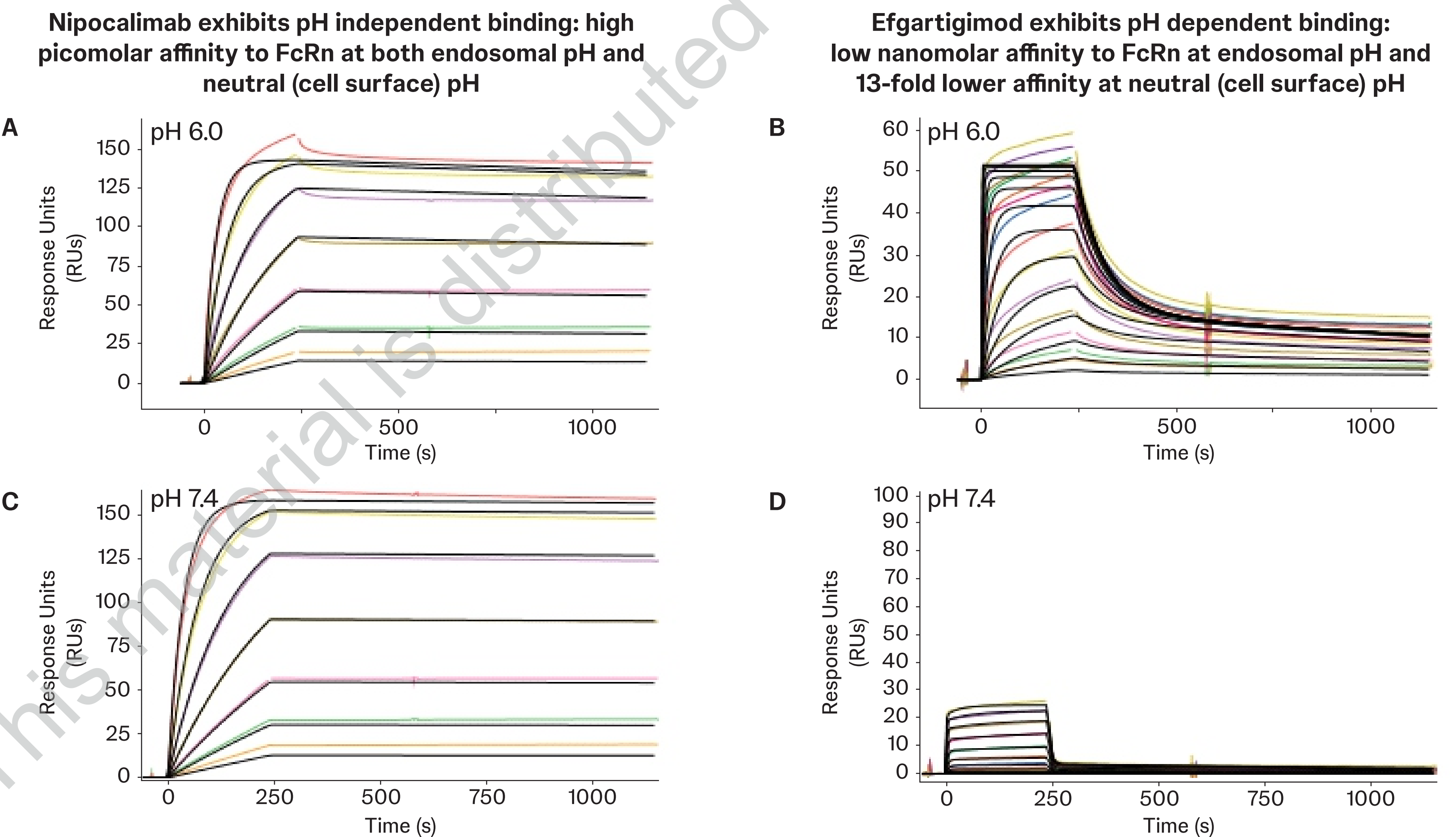
	n	k _a (M ⁻¹ s ⁻¹)	k _d (s ⁻¹)	K _D (pM)
Nipocalimab	20	2.05E+06	5.25E-05	≤32
Efgartigimod	4	8.52E+05	1.33E-02	16,100 (10,820–21,380)

- A large number of nipocalimab K_D values exceeded the limit of quantitation in this surface plasmon resonance (SPR) method, therefore the affinity reported is an upper limit.
- Efgartigimod binding data was modeled using a heterogenous-ligand binding model, to account for observed negative cooperativity in FcRn binding. Reported here is the dominant affinity interaction for efgartigimod (Figure 2).

Table 2: SPR Binding Affinity (K_D) at (cell surface) pH 7.4

	n	k _a (M ⁻¹ s ⁻¹)	k _d (s ⁻¹)	K _D (pM)
Nipocalimab	22	1.28E+06	7.42E-05	≤58
Efgartigimod	4	8.26E+05	1.72E-01	208,000 (188,600–227,400)

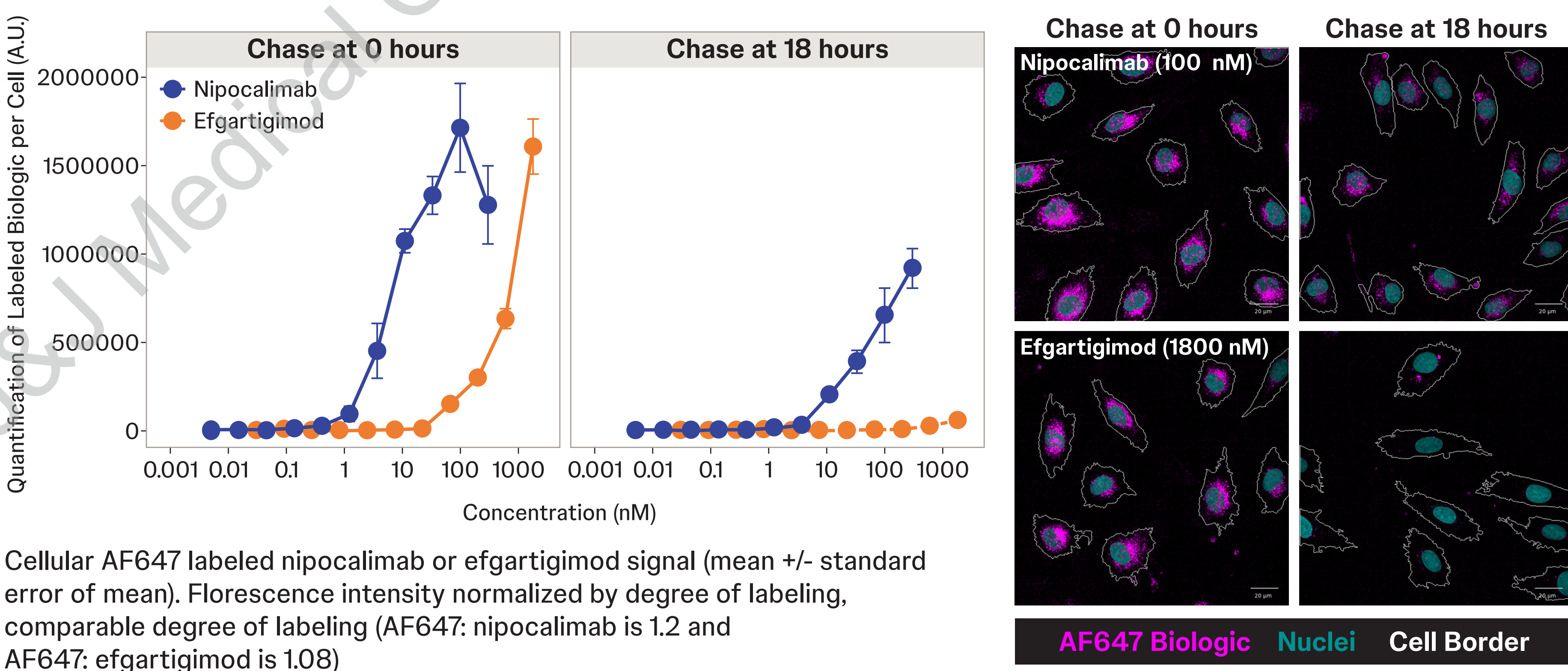
Figure 2: SPR sensograms showing binding of niopcalimab or efgartigimod to FcRn at pH 6.0 and pH 7.4



k_a=association rate constant, k_d=dissociation rate constant, K_D=Equilibrium dissociation constant; affinity of ligand/analyte binding, n=number of replicates, M=mole, s=second, pM=picomolar, SPR=surface plasmon resonance.

Nipocalimab exhibits longer intracellular residence time at lower doses versus efgartigimod

Figure 3: Pulse-Chase in HAECs



Cellular AF647 labeled nipocalimab or efgartigimod signal (mean +/- standard error of mean). Florescence intensity normalized by degree of labeling, comparable degree of labeling (AF647: nipocalimab is 1.2 and AF647: efgartigimod is 1.08)

HAECs expressing endogenous levels of FcRn were pulsed with different concentrations of Alexa Fluor 647-labeled nipocalimab or efgartigimod, for 1 hour and subsequently washed and chased for 0 or 18 hours. Cells were imaged using an Opera Phenix spinning disk confocal and analyzed in SlmA.

100 nM of AF647 labeled nipocalimab and 1800 nM of AF647 efgartigimod were used for the pulse, cells were washed and imaged at 0 and 18 hours.

Nipocalimab demonstrated ~300-fold greater potency in inhibiting IgG recycling in a cell-based assay compared with efgartigimod

Figure 4: IgG recycling assays in HAECs

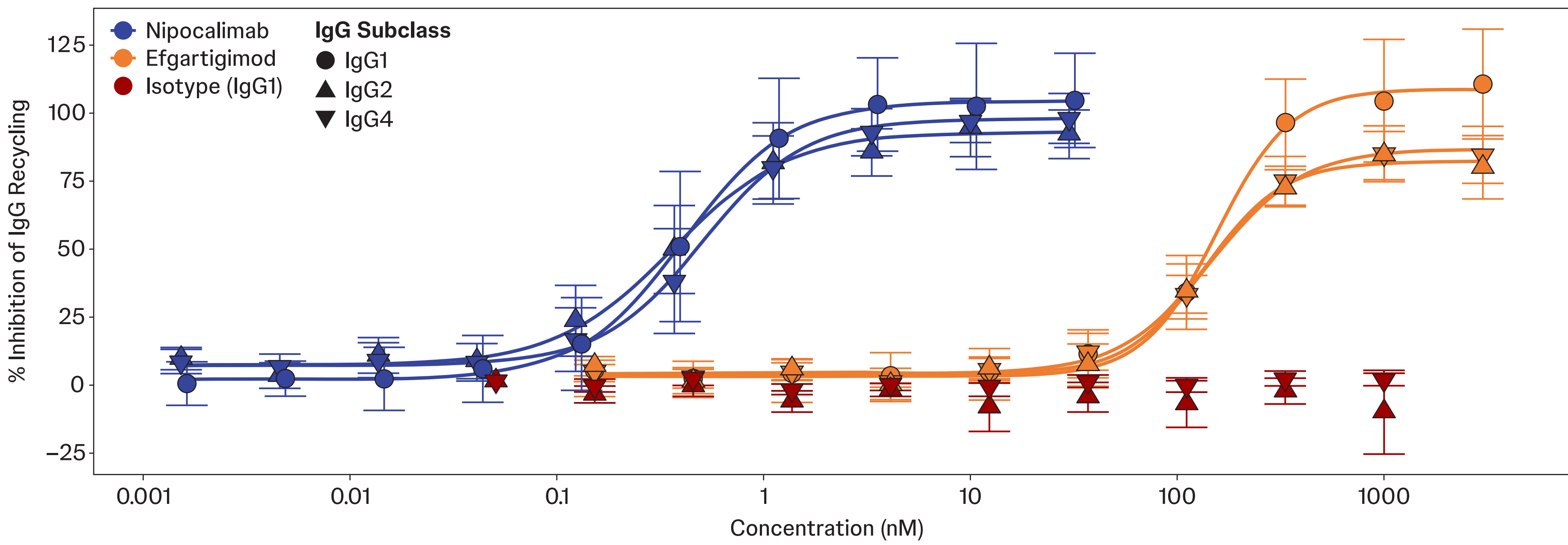


Table 3: IC₅₀s of nipocalimab and efgartigimod for inhibition of IgG subtypes

IgG	IgG1 (IC ₅₀)	IgG2 (IC ₅₀)	IgG4 (IC ₅₀)
Nipocalimab	0.41 nM	0.36 nM	0.51 nM
Efgartigimod	155 nM	136 nM	146 nM

- Concentration-response curves showing nipocalimab and efgartigimod potency against IgG subclasses IgG1, IgG2, and IgG4. Error bars represent standard deviation.
- IgG3 data was not generated due to lower binding affinity to FcRn, and poor cellular assay signals.

FcRn=neonatal fragment crystallizable receptor, IC=Inhibitory concentration, IgG=Immunoglobulin G, nM=nanomolar.

- Nipocalimab demonstrated 378-fold greater potency in inhibiting IgG1 and IgG2 recycling compared with efgartigimod.
- Nipocalimab demonstrated 286-fold greater potency in inhibiting IgG4 recycling compared with efgartigimod.