Cell Reports Medicine, Volume *4*

Supplemental information

An mRNA-based platform for the delivery of pathogen-specific IgA into mucosal secretions

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Supplementary Material.

Table S1.

Abbreviations used:	Description
$_{m}$IgA	Monomeric IgA – referred to in results when monomeric portion has been isolated and used
$_{d}$IgA	Dimeric IgA – referred to in results when dimeric portion has been isolated and used
IgA_{mRNA}	Resultant IgA protein made from synthetic mRNA that, unless otherwise stated to be without JC, will consist of monomer, dimer and polymers.
IgG_{mRNA}	Resultant IgG protein made from synthetic mRNA
IgA_{R}	Resultant IgA protein made from traditional recombinant production and purification means involving transfection of DNA into EXPI293 cells and purifying protein. Unless otherwise indicated, these protein preparations will include monomer, dimer and polymer
$_{m}IgA_{R}$	Isolated monomeric portion of IgA protein made from traditional recombinant production and purification means.
$_{d}IgA_{R}$	Isolated dimeric portion of IgA protein made from traditional recombinant production and purification means
IgA2	Referring to IgA of the IgA2 isotype
IgA1	Referring to IgA of the IgA1 isotype

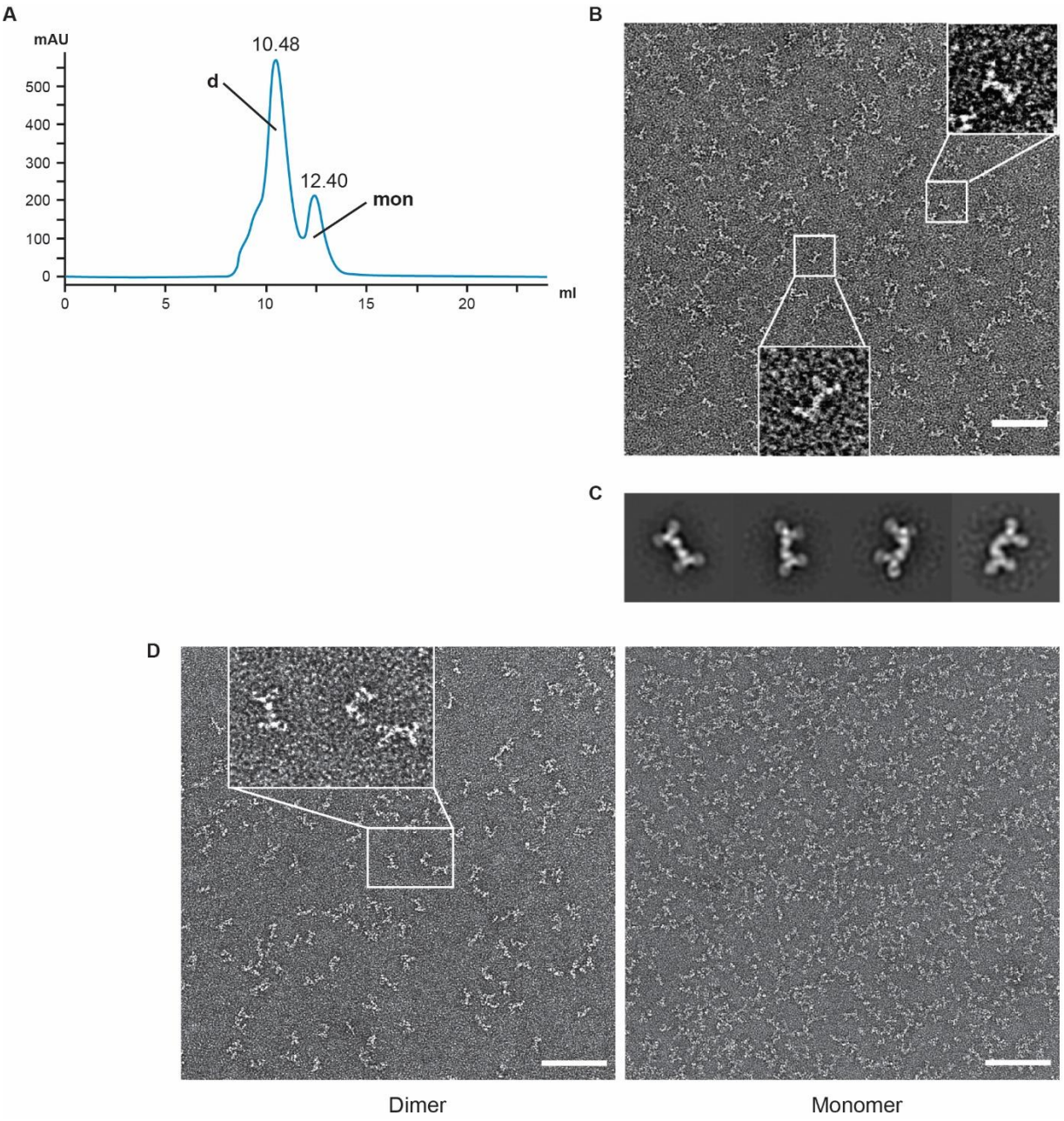

Figure S1. *In vitro* characterization and comparison of Sal4 IgA2$_R$ and IgA2$_{mRNA}$. Related to Fig 1. (A) Analytical size exclusion chromatogram of affinity-purified recombinant IgA2 (IgA2$_R$) from DNA plasmid transient transfection. Dimer (d) and monomer (mon) peaks are denoted. **(B)** Representative images of negative stain EM (nsEM) of the IgA2$_R$ dimeric peak. Scale bar: 50nm. **(C)** Reference free 2D class averages of IgA2$_R$ dimers. (**D**) Raw nsEM of IgA2$_{mRNA}$ dimer and monomer. Scale bar 50nm.

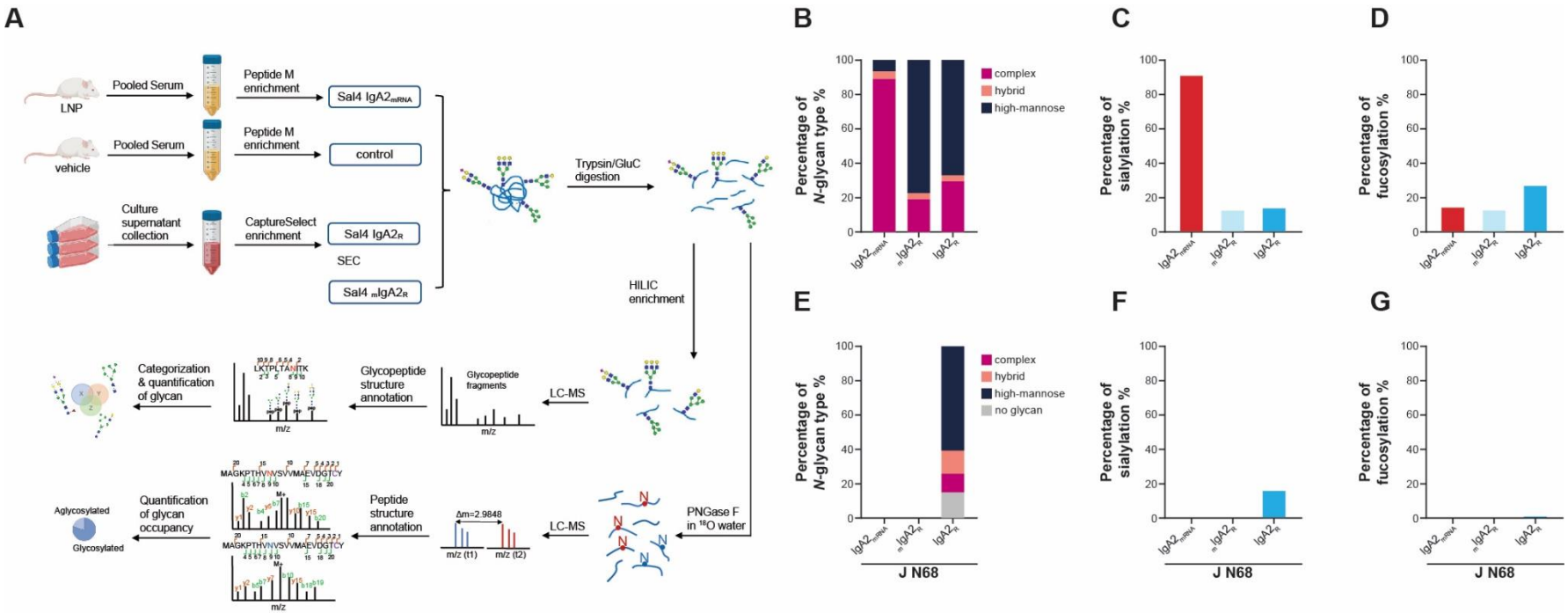

Figure S2. Site-specific N-glycosylation analysis of Sal4 IgA2 expressed in mice or EXPI293 cells. Related to Fig 2C-F. (**A**) Workflow schematic of site-specific N-glycosylation analysis of Sal4 IgA2 expressed in mice via mRNA administration ($IgA2_{mRNA}$) or in EXPI293 cells via cDNA transfection ($IgA2_R$). 50 Balb/c mice were treated with formulated mRNA encoding Sal4 IgA2, serum was collected after 24 h, and peptide M enrichment was conducted to purify Sal4 $IgA2_{mRNA}$. The control group was treated under the same conditions but without mRNA formulated in the vehicle. Sal4 $IgA2_R$ was synthesized by GeneArt (ThermoFisher) by transfecting EXPI293 cells with cDNA encoding Sal4 IgA2. Cell culture supernatant was collected and both monomeric and dimeric recombinant antibody was purified using CaptureSelect affinity chromatography column (ThermoFisher). To further isolate Sal4 $mIgA2_R$, SEC separation was performed on Sal4 $IgA2_R$. After isolation, both proteins were digested to obtain a peptide and glycopeptide mixture. The

18O label method was used to determine the glycan occupancy of each glycosylation site, and HILIC was applied to enrich glycopeptides. Following data acquisition in LC-MS, data analysis was performed to quantify the glycosylation of IgA2. (**B-D**) N-glycosylation analysis of Sal4 IgA2 heavy chain expressed in mice1081 via mRNA administration or in EXIP293 cells via cDNA transfection. Glycosylation was measured as the sum of glycosylation levels at four asparagine residues in the heavy chain (N190, N274, N348, and N470; panel **B** shows IgA2 N-glycan compositions; panel **C** shows sialylation of IgA2 asparagine residues, expressed as a percentage of complex and hybrid glycans that are sialylated; panel **D** shows fucosylation of IgA2 asparagine residues, expressed as a percentage of complex, hybrid, and high-mannose glycans that are fucosylated. (**E-G**) N-glycosylation analysis of Sal4 IgA2 J chain (N68) expressed in mice1092 via mRNA administration or in EXPI293 cells via cDNA transfection; panel **E** shows IgA2 J chain N-glycan compositions at N68; panel **F** shows sialylation of IgA2 J chain at N68, expressed as a percentage of complex and hybrid glycans that are sialylated; and panel **G** shows fucosylation of IgA2 J chain at N68, expressed as a percentage of complex, hybrid, and high-mannose glycans that are fucosylated.

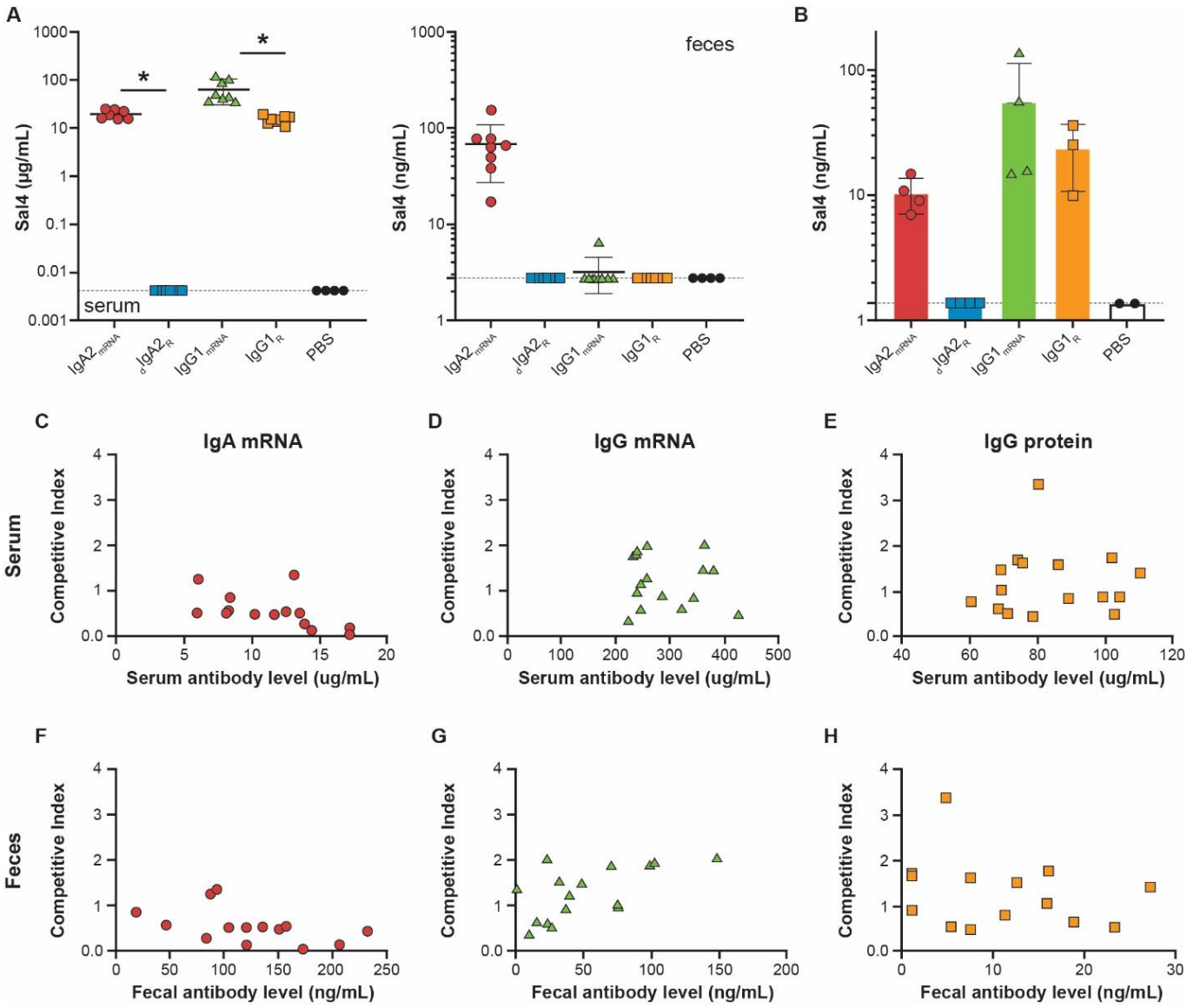

Figure S3. IgA2$_{mRNA}$ expresses in serum and traffics to mucosa and the correlations of circulating antibody levels to competitive index. Related to Fig 3.

(A-C) Balb/c mice were injected intravenously with 1 mg/kg of formulated mRNA encoded antibody, 2.5 mg/kg of IgG1$_R$ or 2.5 mg/kg of $_d$IgA2$_R$ (relates to Fig 3A-C). **(A)** Concentrations of antibody were in serum (left) and feces (right) at 24 hours post injection or **(B)** homogenized intestinal tissue at 168 hours post injection. Each symbol represents an individual mouse, and data is graphed mean ± SD. *P< 0.05 with one-way ANOVA Kruskal-Wallis test. **(C-H)** Correlations of circulating antibody levels to competitive index **(C-E)** serum and **(F-H)** feces for **(C, F)** IgA2$_{mRNA}$, **(D, G)** IgG$_{mRNA}$ and **(E, H)** IgG$_R$.

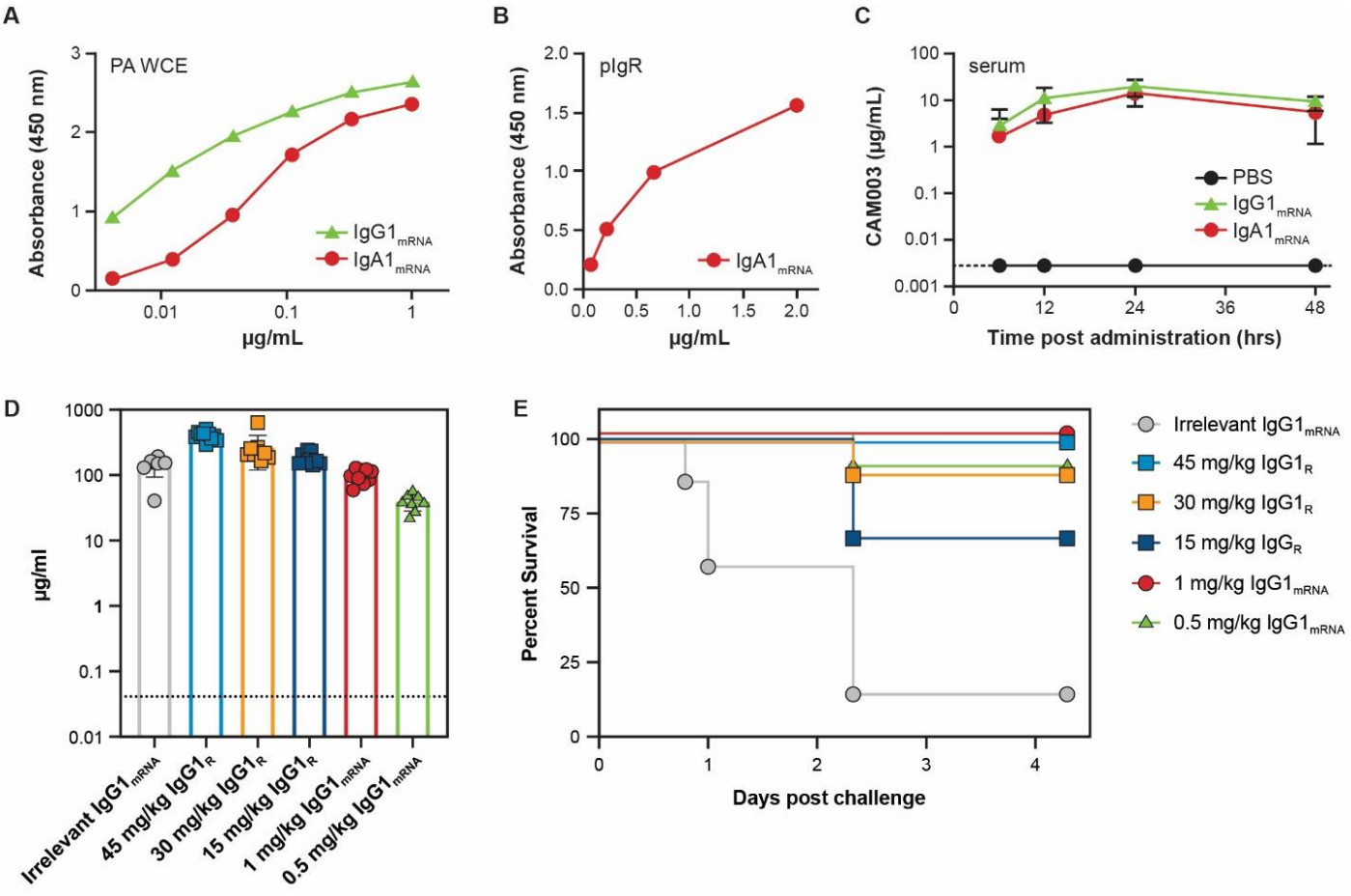

Figure S4. IgG1$_{mRNA}$ and IgA1$_{mRNA}$ from in vitro transfection binds to *P. aeruginosa* (PA), pIgR and expresses *in vivo*. Related to Fig 4. (A) Binding of mRNA transfection supernatant to PA of CAM003 as an IgG or IgA1 isotype. **(B)** Binding of mRNA transfection supernatant of CAM003 IgA1 to human pIgR. **(C)** C57Bl/6 mice were injected IV with 0.5 mg/kg of formulated IgG1$_{mRNA}$ or a IgA1$_{mRNA}$. Concentrations of antibody were measured in serum over time by isotype-specific ELISA. **(D)** Adult Balb/c mice were IV administered irrelevant IgG1$_{mRNA}$, CAM003 IgG1$_{R}$, or IgG1$_{mRNA}$ at the indicated doses 1 day before intranasal challenge with 6.88 $\log_{10}$ CFU of *P. aeruginosa* strain PA01. Circulating antibody concentration in serum was determined before challenge via isotype-specific ELISA (n=10/group). **(C and D)** Each symbol represents an individual mouse, and data is graphed mean ± SD. **(E)** Kaplan-Meier survival curves.