

Supplemental Table 1. Phage proteins identified by Mascot in the tryptic digests of the phage gel bands that reacted with glycostain as shown in Figure 1.

Table S1. Phage proteins identified by Mascot in the tryptic digests of the phage gel bands that reacted with glycostain as shown in Figure 1.

Gel Band	Gene	MW (kDa)	Mascot Protein Score	Function ¹	C-terminal seq.
Che8	6	29.0	27602	Major Capsid protein	...NKTGS
Che8	11	29.9	1380	Major Tail Subunit protein	...YSDGS
Che8	17	29.1	969	Minor Tail Protein	...VLPLS
Che8	24	21.6	128	Minor Tail Protein	...YVVVP
Che8	4	27.7	52	Capsid Maturation Protease	...RSIQR
Che8	18	36.5	42	Minor Tail Protein	...GAVDD
Che8	27	10.4	27	Hypothetical protein	... TTEPW
Che8	51	7.9	23	Hypothetical protein	... MRETR
Corndog	49	29.9	6779	Major Tail Subunit protein	...IKGDS
Corndog	34	45.5	803	Portal protein	...SGGGV
Corndog	60	29.6	207	Minor Tail protein	...VQPIP
Corndog	43	22.4	79	Head-to-Tail Adapter protein	...CVEFL
Corndog	58	63.5	55	Minor Tail protein	...RRWPM
Corndog	115	7.5	31	Hypothetical protein	---LAVRR
Myrna	99	37.4	6279	Major Capsid protein	...ILRKA
Myrna	98	19.0	1189	Hypothetical protein	...LAGGS
Myrna	111	31.5	494	Putative tail fibre	...VVWKL
Myrna	132	105.6	237	Lysin A	...YSWMY
Myrna	122	53.1	207	Tail Sheath protein	...TDTAI
Myrna	121	26.7	216	Hypothetical protein	...RFDWQ
Myrna	239	119.1	182	Capsid Decoration protein	...VVVMG
Myrna	117	59.4	150	Hypothetical protein	...TYTGP
Myrna	241	27.8	121	Hypothetical protein	...VFPGF
Myrna	137	50.1	116	Hypothetical protein	...NFVRS
Myrna	78	54.6	58	Hypothetical protein	...GVVHP
Myrna	138	141.7	39	Hypothetical protein	...PHYLK
Myrna	3	32.8	27	Nucleotidyltransferase	... ANGPD
Myrna	98	19.0	2058	Minor capsid protein	...LAGGS
Myrna	99	37.4	781	Major Capsid protein	...ILRKA
Myrna	138	141.7	662	Hypothetical protein	...PHYLK
Myrna	131	22.5	514	Hypothetical protein	... PAVGR
Myrna	137	50.1	494	Hypothetical protein	...NFVRS
Myrna	121	26.7	424	Hypothetical protein	...RFDWQ
Myrna	117	59.4	371	Hypothetical protein	...TYTGP
Myrna	242	25.6	274	Hypothetical protein	... ARTAL
Myrna	129	26.9	271	Hypothetical protein	... PTPGR
Myrna	241	27.8	236	Hypothetical protein	...VFPGF
Myrna	239	119.1	190	Capsid Decoration protein	...VVVMG
Myrna	240	47.4	86	Hypothetical protein	... MAVPA
Myrna	118	36.9	80	Hypothetical protein	... TGDAR
Myrna	122	76	53.1	Tail Sheath protein	...TDTAI
Myrna	133	34.2	63	Hypothetical protein	... RAVLN
Myrna	97	129.8	38	Hypothetical protein	... SLMFG
Myrna	111	31.5	31	Putative Tail Fibre	...VVWKL
Myrna	255	29.9	23	Hypothetical protein	... PGDLN

¹Protein functions are based on homology with other phage proteins in the NCBI nr protein database.

Supplemental Table 2. Che8 proteins identified by mass spectrometry following chymotrypsin digestion of proteins in Fig. 2D.

Table S2. Che8 proteins identified by mass spectrometry following chymotrypsin digestion of proteins in Fig. 2D.

Gel Band # ¹	Gene #	Calc. M.W. (kDa)	Mascot Score ²	Function ³	C-terminal seq.
Che8 band 1	6	29.05	4754	Major capsid	...NKTGS
Che8 band 1	17	28.98	101	Minor Tail Protein	...VLPLS
Che8 band 1	11	29.78	64	Tail tube	...YSDGS
Che8 band 1	25	30.83	56	Hypothetical Protein	...VGITR
Che8 band 1	52	7.47	23	DNA Binding protein	...EPRSA
Che8 band 2	6	29.05	6957	Major capsid	...NKTGS
Che8 band 2	17	28.98	84	Minor Tail Protein	...VLPLS
Che8 band 2	11	29.78	63	Tail tube	...YSDGS
Che8 band 2	24	21.55	20	Minor Tail protein	...YVVVP
Che8 band 3	6	29.05	5098	Major capsid	...NKTGS
Che8 band 3	11	29.78	56	Tail tube	...YSDGS
Che8 band 4	6	29.05	7702	Major capsid	...NKTGS
Che8 band 4	11	29.78	182	Tail tube	...YSDGS
Che8 band 4	32	47.41	20	Lysin A	...VKGKS
Che8 band 4	21	36.70	18	Minor Tail protein	...PLNPV
Che8 band 5	6	29.05	7469	Major capsid	...NKTGS
Che8 band 5	11	29.78	306	Tail tube	...YSDGS
Che8 band 5	21	36.70	63	Minor Tail protein	...PLNPV
Che8 band 5	59	29.03	26	Hypothetical Protein	...QEVAE
Che8 band 5	16	63.84	23	Minor Tail protein	...LSPQG
Che8 band 5	15	63.40	17	Minor Tail protein	...RRYPM
Che8 band 6	6	29.05	4654	Major capsid	...NKTGS
Che8 band 6	11	29.78	130	Tail tube	...YSDGS
Che8 band 7	6	29.05	3772	Major capsid	...NKTGS
Che8 band 7	11	29.78	108	Tail tube	...YSDGS
Che8 band 7	2	60.99	17	Terminase Large Subunit	...PRRIY
Che8 Δ 110-1	6	29.05	6513	Major capsid	...NKTGS
Che8 Δ 110-1	11	29.78	805	Tail tube	...YSDGS
Che8 Δ 110-1	24	21.44	35	Minor Tail Protein	...YVVVP
Che8 Δ 110-1	59	29.03	26	Hypothetical Protein	...QEVAE

¹Bands were excised as labeled in Figure 2D. The band from the Che8 Δ 110-1 is the major band from Figure 2C.

²The score designated by Mascot, reflecting both the number of peptides identified and quality of the match of the MS/MS fragment ions to the amino acid sequences, is shown.

³The putative functions of the proteins identified are shown.

Supplemental Table 3. Details of groups of mice used for the studies shown in Figure 4 and 5.

Table S3. Details of groups of mice used for the studies shown in Figure 4 and 5.

C57L/6J mouse study (Figs. 4, 5, S2, S3, S4)

Group	# of mice	Sex of mice	Animal designators*	Age of mice	Inoculum
1	3	female	1-1, 1-2, 1-3	11 weeks	100 μ L PBS++ (mock)
2	3	male	2-1, 2-2, 2-3	10 weeks	1 μ g (100 μ L) wild type Che8
	3	female	2-4, 2-5, 2-6	10 weeks	1 μ g (100 μ L) wild type Che8
3	3	male	3-1, 3-2, 3-3	10 weeks	1 μ g (100 μ L) Che8 Δ 110-1
	2	female	3-4, 3-5	10 weeks	1 μ g (100 μ L) Che8 Δ 110-1

IFN β -EYFP reporter mouse (C57BL/6J background) study (Figs. S4, S5, S6, S7)

Group	# of mice	Sex of mice	Animal designators*	Age of mice	Inoculum
1	1	female	1-1	6 weeks	1 μ g (100 μ L) wild type Che8
	4	male	1-2, 1-3, 1-4, 1-5		
2	1	female	2-6	6 weeks	1 μ g (100 μ L) Che8 Δ 110-1
	4	male	2-7, 2-8, 2-9, 2-10		

*Each animal was individually tracked through the studies. Some figures use the animal designators in the tables above to show time course responses for individual mice.

Supplemental Table 4. Non-Actinobacteriophages coding for two or more Glycosyltransferases supporting data shown in Fig. 6.

Table S4. Non-Actinobacteriophages coding for two or more Glycosyltransferases supporting data shown in Fig. 6.

Phage	Host	Genome (bp)	Accession	Coordinates ¹	Subtype ²
ACG-2014f	Synechococcus	228,143	NC_026927.1	17316..18407 164077..164871 165637..166368 170033..170911 220090..220707	Group 1 Family 11 Family 6 Family 25 Family 2
BCepSauron	Burkholderia	262,653	NC_049851.1	c 21943..22635 203351..203692 c 237158..237586	Family 32 Family 36 Family A
BCP8-2	Bacillus	159,071	NC_027355.1	c 97556..97753 c 135238..135444	
Bellamy	Synechococcus	204,930	NC_047838.1	159088..159894 159891..160607 175169..176122 181292..182128	
Fnu1	Fusobacterium	130,914	NC_055035.1	c 56733..56978 119268..119579	
P-SSM2	Prochlorococcus	252,407	NC_006883.2	196236..196967 196964..197866 198463..199605 200233..201027 202494..203189 204709..205500	Family 2 Family 2 Family 1 Family 11 Family 6 Family 25
P-TIM68	Prochlorococcus	197,361	NC_028955.1	159647..160543 162196..162996 172254..173051	
S-CAM7	Synechococcus	216,121	NC_031927.1	17930..18910 141185..141934 165157..165951 168723..169460	Group 1 Family 11 Family 25 Family 6
S-CAM9	Synechococcus	174,830	NC_031922.1	147673..148491 148488..149204 154597..155550	
S-PRM1	Synechococcus	144,311	NC_055761.1	c 65221..65421 65791..65994 68778..69596 69593..70309 75553..76506 76496..77260	
S-SM2	Synechococcus	190,789	NC_015279.1	159988..160836 161639..162454 162426..163289	Family 11 Family 11 Family 25
S-SSM7	Synechococcus	232,878	NC_015287.1	206946..207755 207755..208681 209505..210362 210843..211652	Family 11 Family 11 Family 11 Family 25
Sfl	Enterobacteria	38,389	NC_027339.1	c18227..19747 c19750..20670	Family 2
vB_FspM_immuto_2-6A	Flavobacterium	160,410	NC_055915.1	49172..50614 50617..51927 52406..53029 58798..59988	RfaG Family 2 WcaA

¹Gene coordinates of predicted glycosyltransferases are shown. C denotes coding on the complementary strand

²Glycosyltransferase subtypes are shown where provided in genome annotations.

Figure S1. NanoLC-MS/MS analysis of glycostain-reactive gel bands, related to Figures 1 and 2. Glycopeptide mass spectra from the in-gel tryptic digests of the glycostain-reactive bands from **(A)** Corndog, **(B)** Che8 and **(C)** Myrna phages. MS/MS fragment spectra acquired on selected glycopeptide ions from each phage are presented in Figure 1. **D – J.** Glycopeptide mass spectra from the in-gel chymotryptic digest of seven glycostain-reactive bands in the high-resolution SDS-PAGE gel of Che8 proteins. These glycopeptide ions are all derived from the Major Capsid protein (gp6) and show a stepwise increase in mass corresponding to a HexNAc-Hex pair from one band to the next. The additional complexity associated with each ion group is due to partial methylation as well as non-covalent adduction by ammonia. The latter may be due to the presence of ammonium bicarbonate in the digest buffer. **K – M.** NanoLC-MS/MS analysis of the in-gel chymotryptic digests of Che8 glycoproteins. HCD-MS/MS spectra of the triply charged ions **(K)** at m/z 1284.9 corresponding to the chymotryptic glycopeptide from the Major Capsid protein (gene 6) and **(L and M)** at m/z 1687.3 and m/z 1741.7, respectively, corresponding to glycopeptides from the Major Tail protein (gene 11). The amino acid sequence of the two glycopeptides from the Major Tail protein differ by an N-terminal tyrosine.

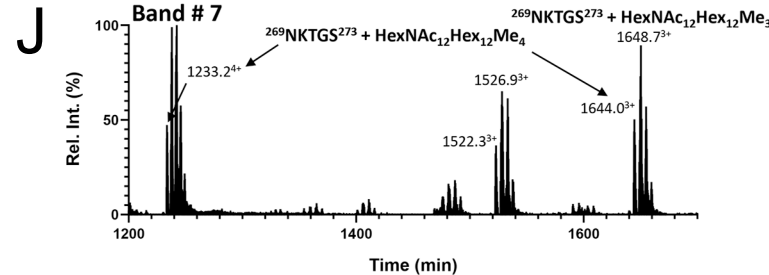
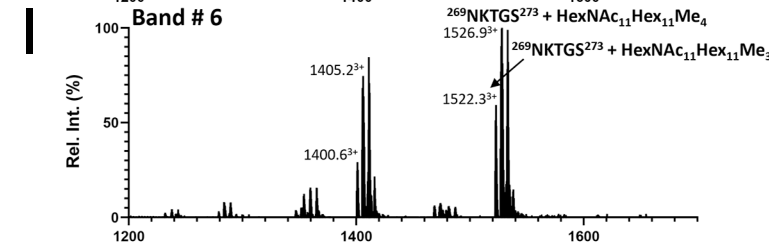
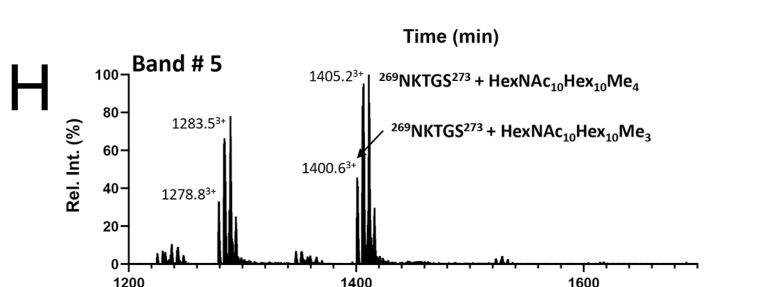
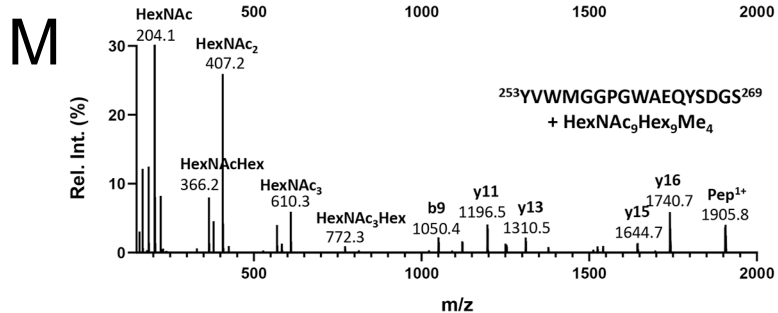
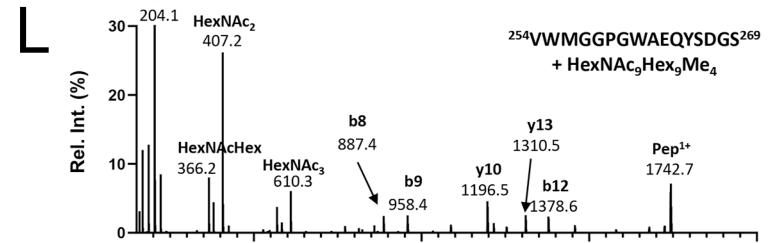
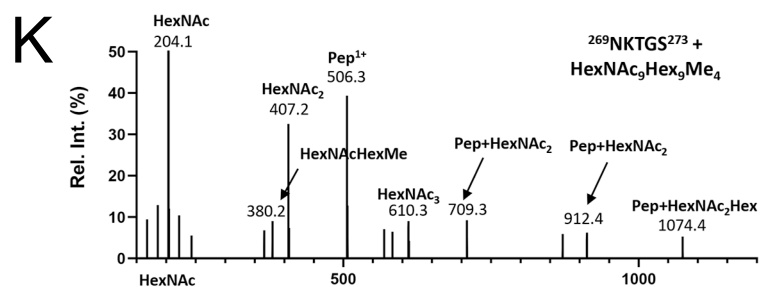
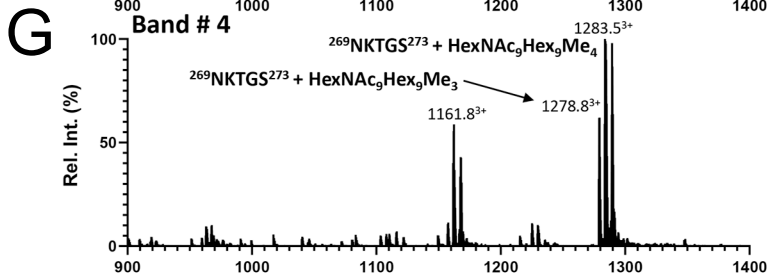
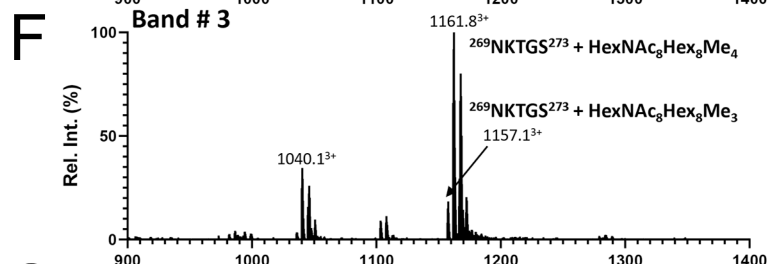
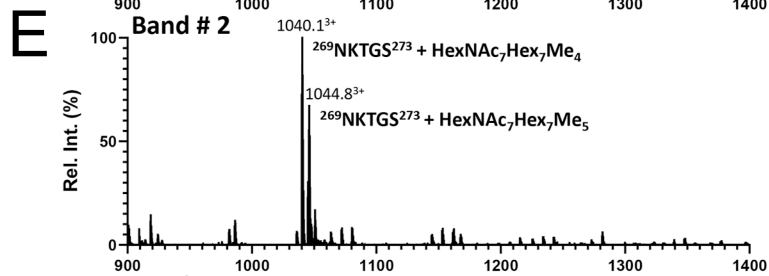
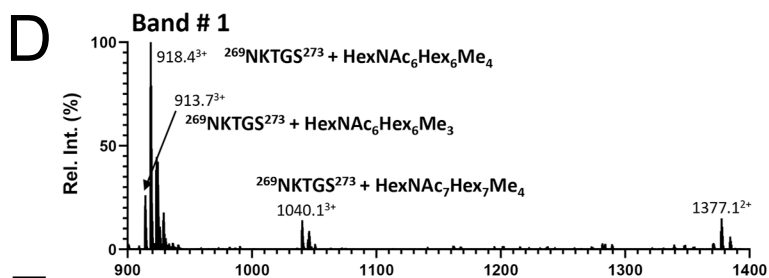
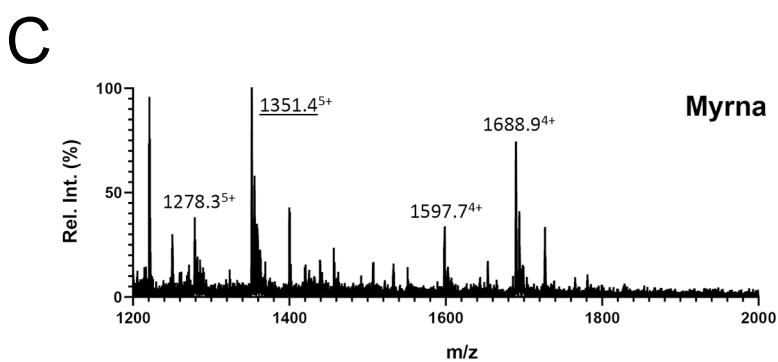
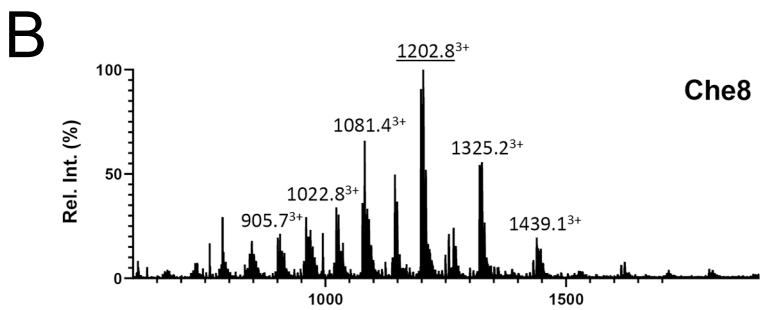
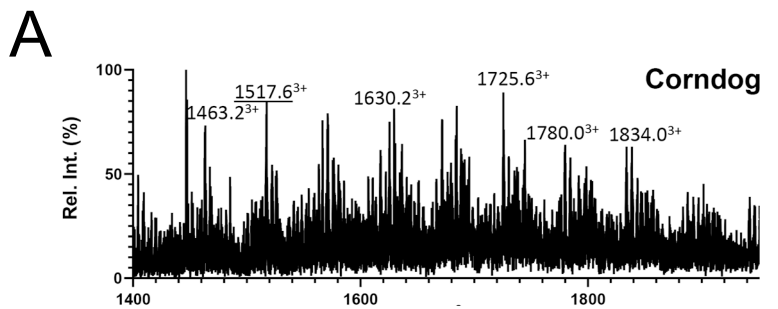


Figure S1

Figure S2. ELISA curves at each timepoint for individual animals in C57BL/6J mouse

study, related to Figures 4 and 5. A. For each individual mouse (Table S3), binding antibodies of IgM isotype are quantified by ELISA OD₄₅₀ versus serum dilutions. Binding to uncoated wells as a background measurement is quantified by the black and gray data/lines while binding to wild-type (wt) and mutant ($\Delta 110-1$) Che8 are shown in red and blue, respectively. Logistic fits of the data yield half-maximal serum dilutions. These values are used only for datasets with at least one datapoint > 4X the background level established by the uncoated wells; for any dataset not meeting this signal:noise threshold, the half-maximal value is set of 1 (half the limit of quantitation). **B.** Binding antibodies of IgG isotype, with data display, fitting, and analysis as in A.

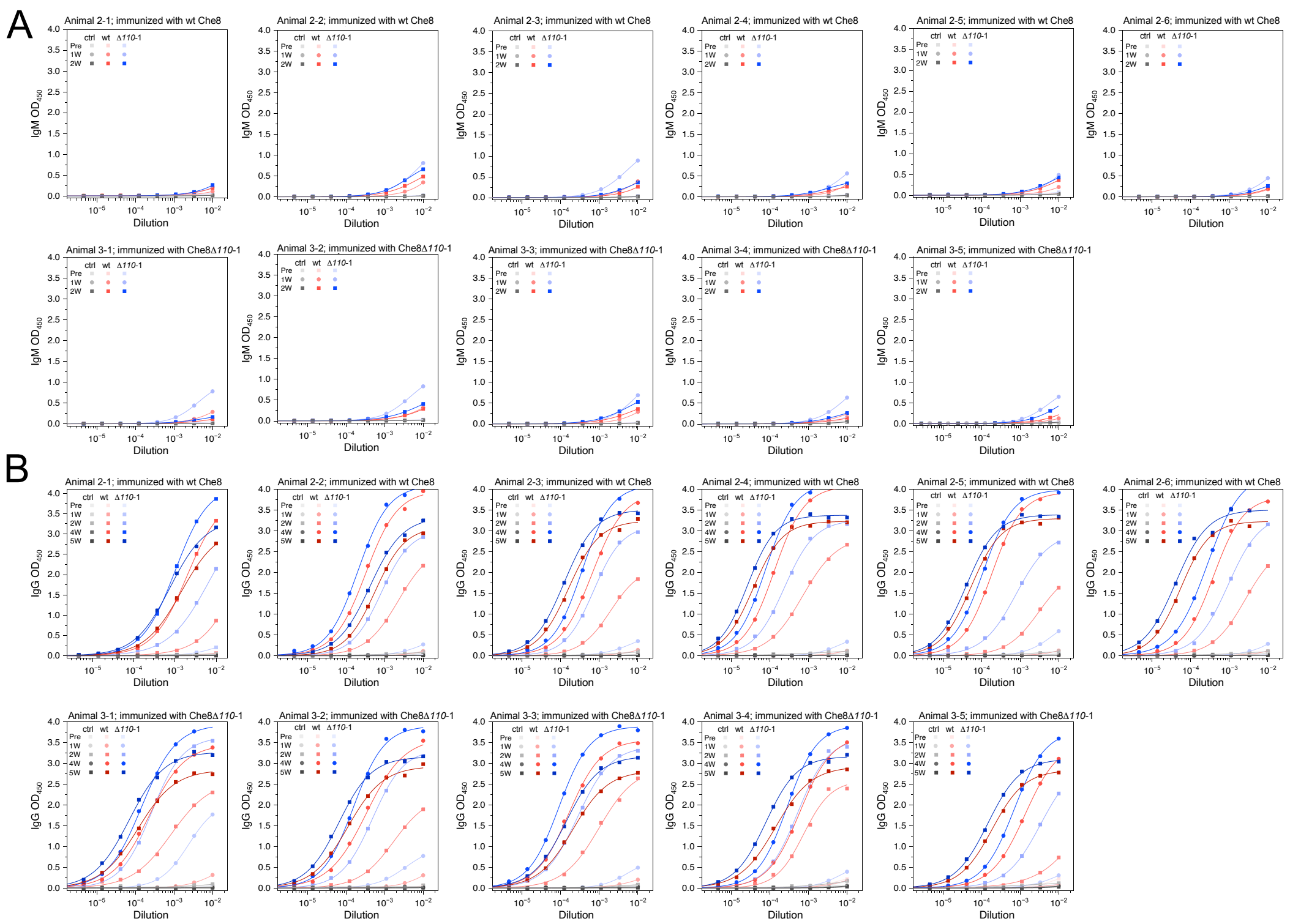


Figure S2

Figure S3. Neutralization assay spot titer plates for C57BL/6J mouse study, related to Figures 4 and 5. The neutralizing power of serum from each individual mouse inoculated with phage (Table S3) was assessed by incubating 10^9 pfu/mL of each phage with a 1:10 dilution of each serum sample. After 24 hours, the samples were serially diluted and 2uL of each dilution was spotted on bacterial lawns. Neutralization at each timepoint, from pre-phage (Pre) through Week 5 (5W), is shown. A no-serum control is also shown. The left side of the figure shows neutralization of both phages (as indicated with labels at the top) by serum from mice receiving glycosylated, wild-type Che8. The top right side of the figure shows neutralization by serum from mice receiving non-glycosylated Che8 $\Delta 110-1$. The bottom right corner shows controls of phages incubated with serum from mice inoculated with PBS.

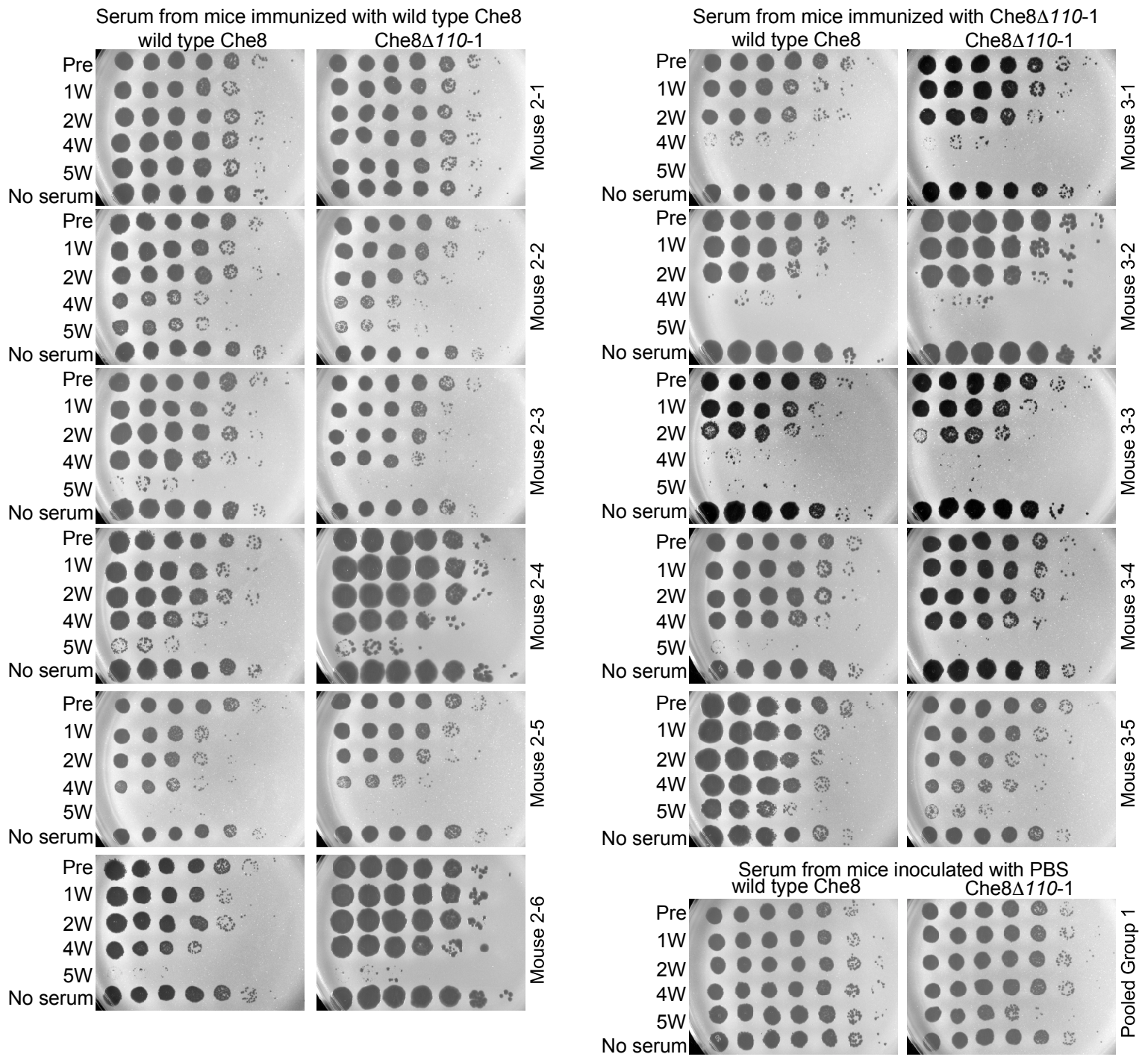


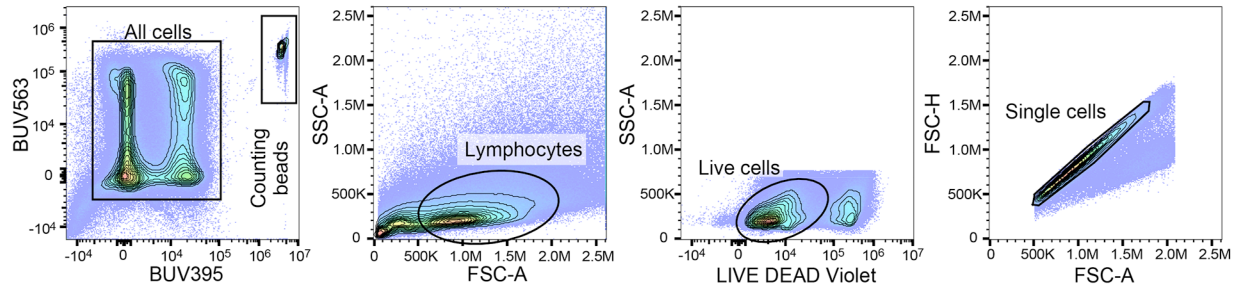
Figure S3

Figure S4. Flow cytometry gating strategy for representative animal in both mouse

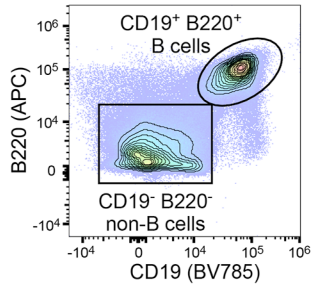
studies, related to Figure 4. A. For the C57BL/6J mouse study, splenocytes were first separated from the highly fluorescent counting beads, then sorted according to size to isolate the lymphocyte population. Live and dead lymphocytes were separated with Live/Dead stain and live cells were further plotted as cell height by cell area to gate the diagonal of single cells. From there, live, single lymphocytes were sorted by B220 and CD19 signals to gate B cells (B220⁺ CD19⁺) and non-B cells (B220⁻ CD19⁻). The non-B cells were further plotted by NK1.1 and CD3 signals, allowing gating of the natural killer cells (CD3⁻ NK1.1⁺) and T cells (CD3⁺). T cell subsets were identified by plotting the CD3⁺ population by CD4 and CD8 signals; helper T cell (CD4⁺) and cytotoxic T cell (CD8⁺) populations were gated as shown. Within the CD4⁺ population, plotting by FOXP3 signal allows gating of the regulatory T cells (FOXP3⁺). Finally, the subtypes of CD8⁺ populations were quantified by plotting by CD62L and CD44 signals and dividing the plot into quadrants for effector (CD62L^{high}CD44^{low}), central memory (CD62L^{high}CD44^{high}), naïve (CD62L^{low}CD44^{high}), and P4 (CD62L^{low}CD44^{low}) T cells. **B.** For the second mouse study the gating was performed as above except the non-B cells were first plotted by the CD3 signal, allowing gating of T cells (CD3⁺) and CD3⁻ cells, then CD3⁻ cells were further gated into a population of natural killer cells (CD3⁻ NK1.1⁺),

A

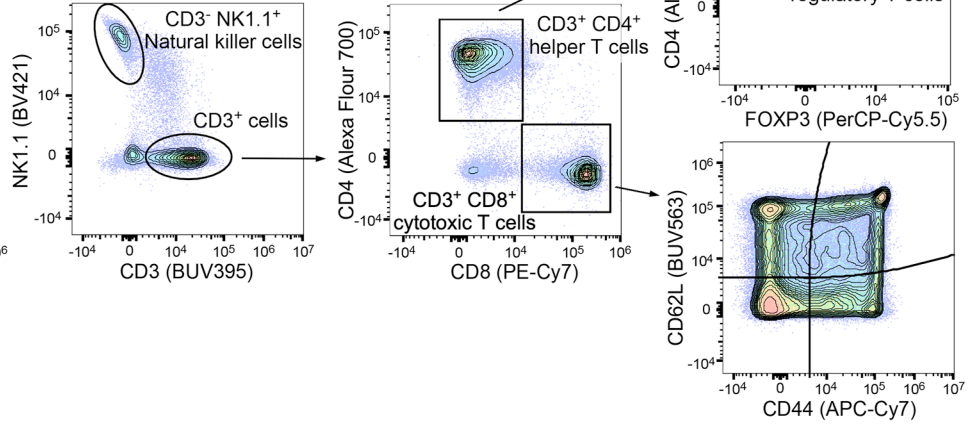
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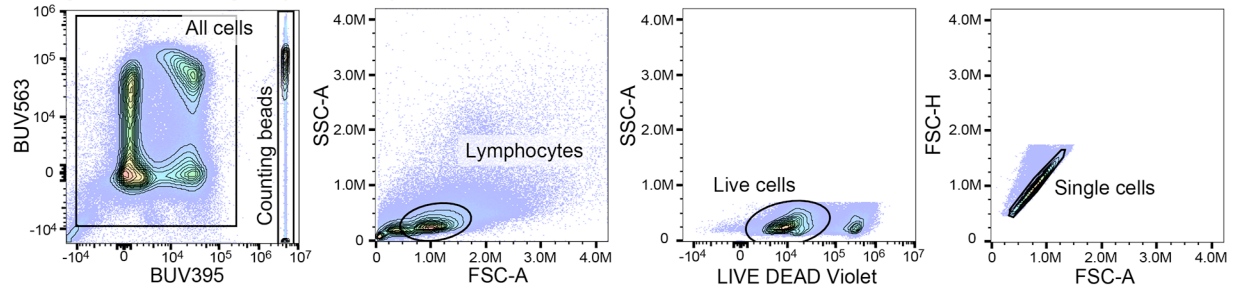
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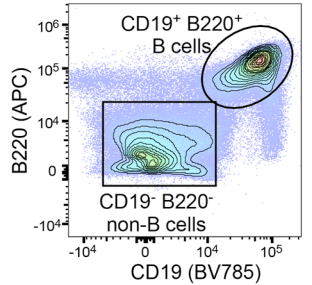
Gating non-B cells:

**B**

Gating all live, single lymphocytes:



Gating B cells:



Gating non-B cells:

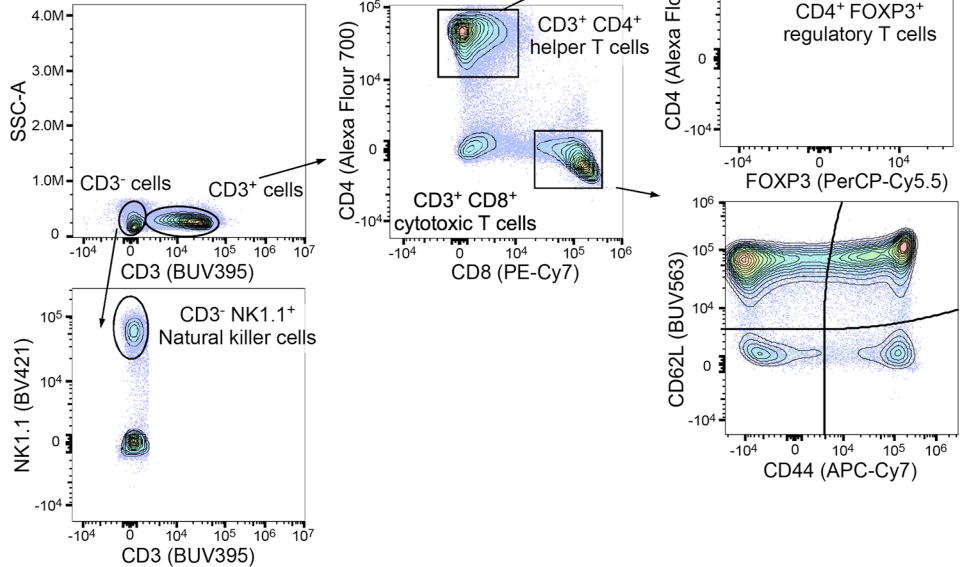


Figure S4

Figure S5. Mouse study with B6-like IFN β reporter mice, related to Figures 4 and 5. A. A graphical timeline of the mouse study. At day 0, groups of four male mice and one female mouse received 1 μ g of either wild-type (wt) Che8 or the non-glycosylated mutant, Che8 Δ 110-1 (Table S3). Serum was collected from each mouse via submandibular bleed before phage administration and one, two, three, four, and six weeks post-administration. A second dose of phage was given to each mouse at 7.5 weeks, after a cheek bleed. The mice were sacrificed at week 8.5 and terminal serum and spleens were harvested. **B and C.** IgM (B) and IgG (C) titers were tracked with ELISAs using serum from each mouse receiving phage at each timepoint, as labelled below each plot. For these and all later panels with box and data plots, measurements from individual mice are shown as datapoints and the mean values are shown with a line. The box extends the mean value \pm 1 standard error. Measurements below the limit of detection were set to half of the limit of detection ($1\log_{10}$ for ELISAs and $-7\log_{10}$ for neutralization assays) to allow statistical analysis. Two sample t-tests were performed between relevant pairs to evaluate significance, and any significant differences between related pairs of measurements are indicated with *P* values. The half-maximal titers (\log_{10}) of serum antibodies binding wild-type Che8 (red) and Che8 Δ 110-1 (blue) are shown. **D.** Neutralization assays with an input phage titer of 1×10^9 pfu/mL and a final serum dilution of 1:10. The efficiency of plaquing is plotted as a function of time for wt Che8 (left) and Che8 Δ 110-1 (right). Each panel presents the neutralization by sera from the three groups of mice, as indicated in the legend. **E.** Western blots to test reactivity to wild-type (wt, red) and mutant (Δ , blue) were performed with week 8.5 serum from each experimental mouse. Exposures are the same for all blots. **F.** Multicolor flow cytometry was performed with splenocytes after sacrifice at week 8.5 and the data were gated according to the strategy in Figure S4.

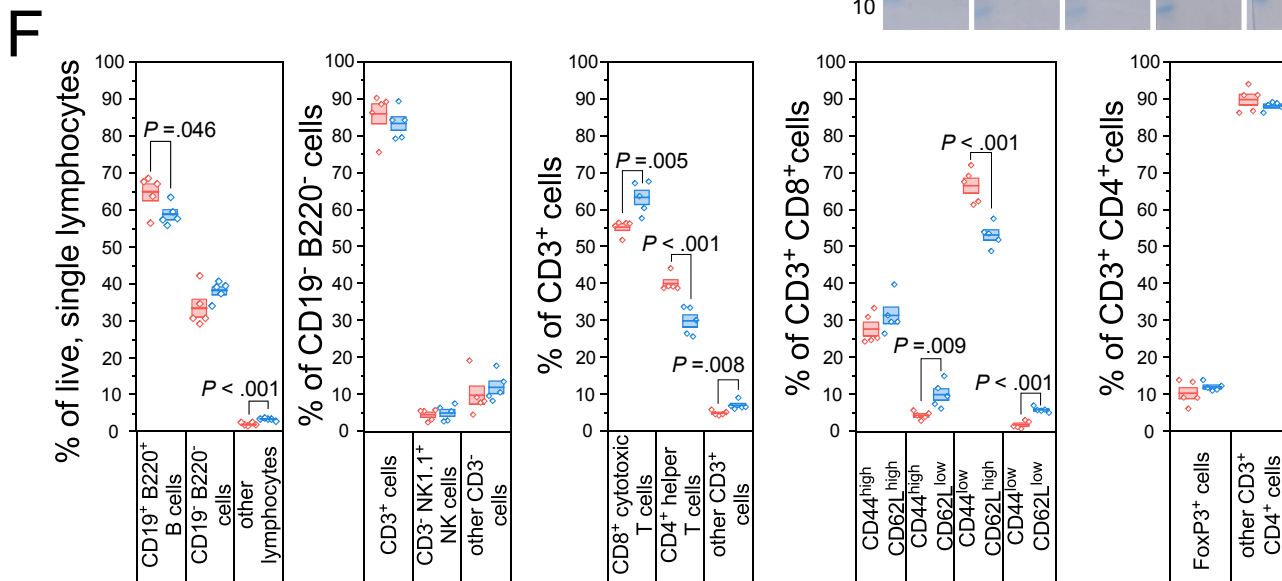
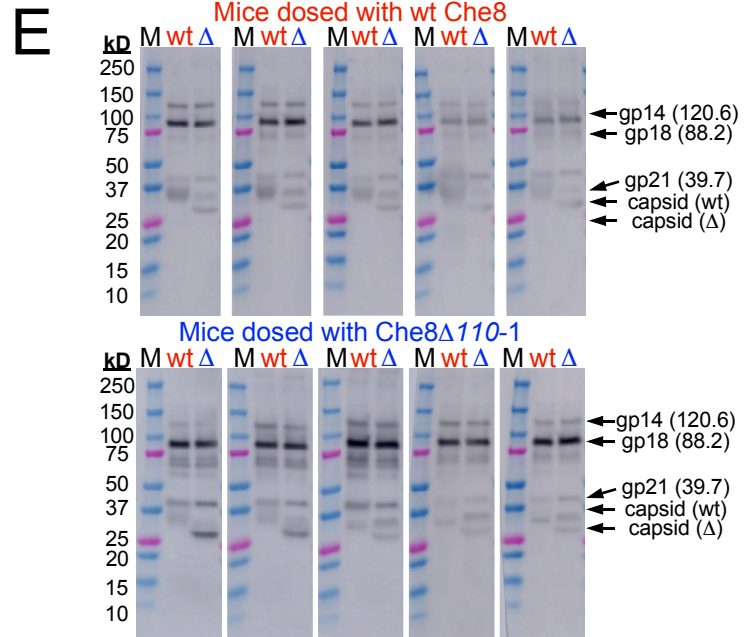
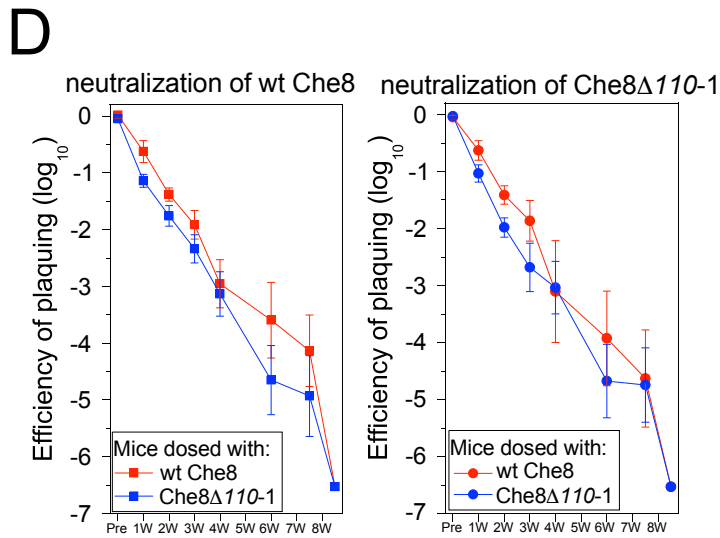
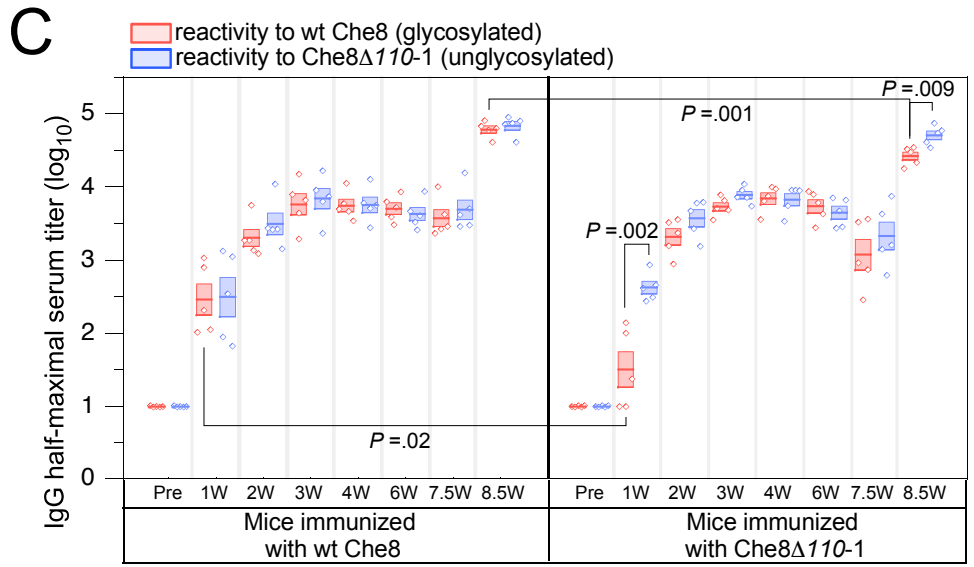
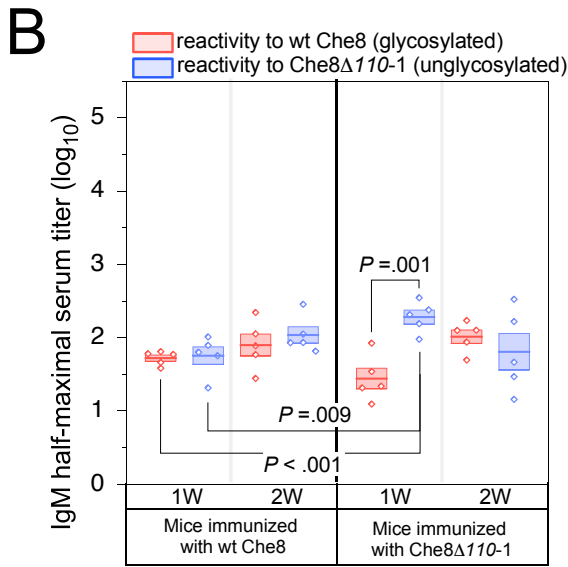
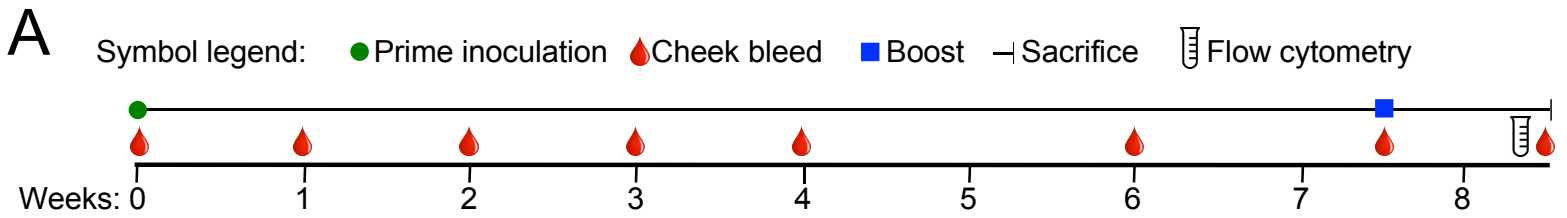


Figure S5

Figure S6. ELISA curves at each timepoint for individual animals in B6-like IFN β reporter mouse study, related to Figures 4 and 5. A. For each individual mouse (Table S3), binding antibodies of IgM isotype are quantified by ELISA OD₄₅₀ versus serum dilutions. Binding to uncoated wells as a background measurement is quantified by the black and gray data/lines while binding to wild-type (wt) and mutant ($\Delta 110-1$) Che8 are shown in red and blue, respectively. Logistic fits of the data yield half-maximal serum dilutions. These values are used only for datasets with at least one datapoint > 4X the background level established by the uncoated wells; for any dataset not meeting this signal:noise threshold, the half-maximal value is set of 1 (half the limit of quantitation). **B.** Binding antibodies of IgG isotype, with data display, fitting, and analysis as in A.

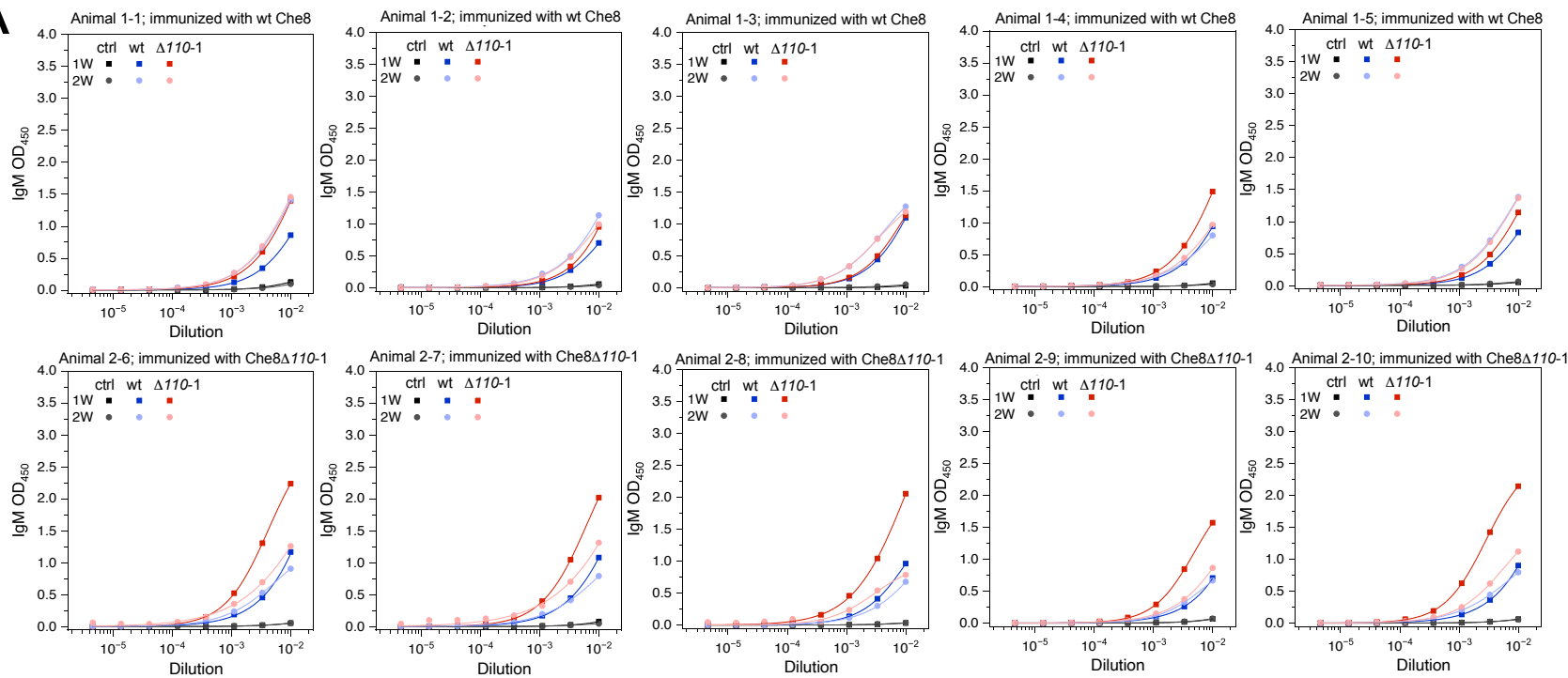
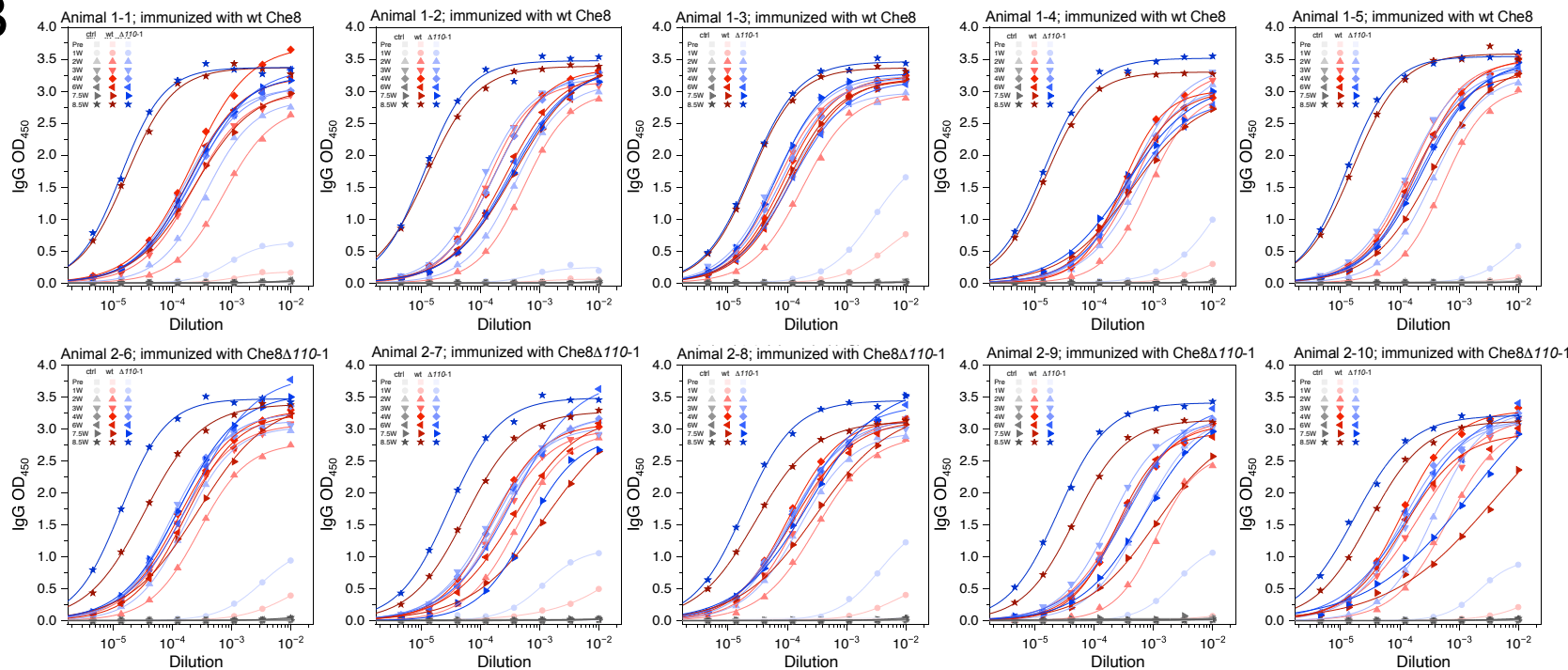
A**B**

Figure S6

Figure S7. Neutralization assay spot titer plates for B6-like IFN β reporter mouse study, related to Figures 4 and 5. The neutralizing power of serum from each individual mouse (Table S3) inoculated with phage was assessed by incubating 10^9 pfu/mL of each phage with a 1:10 dilution of each serum sample. After 24 hours, the samples were serially diluted and 2 μ L of each dilution was spotted on bacterial lawns. Neutralization at each timepoint, from pre-phage (Pre) through Week 8.5 (8.5W), is shown. A no-serum control is also shown. The left side of the figure shows neutralization of both phages (as indicated with labels at the top) by serum from mice receiving glycosylated, wild-type Che8. The top right side of the figure shows neutralization by serum from mice receiving non-glycosylated Che8 $\Delta 110-1$.

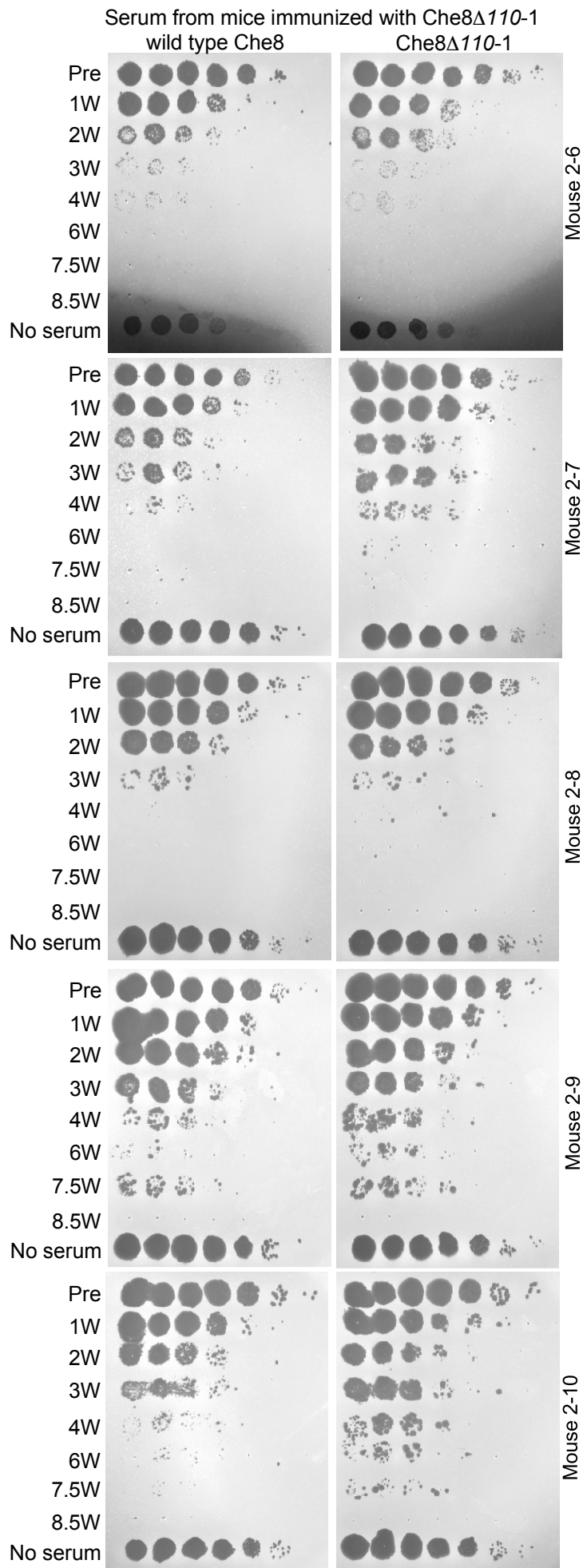
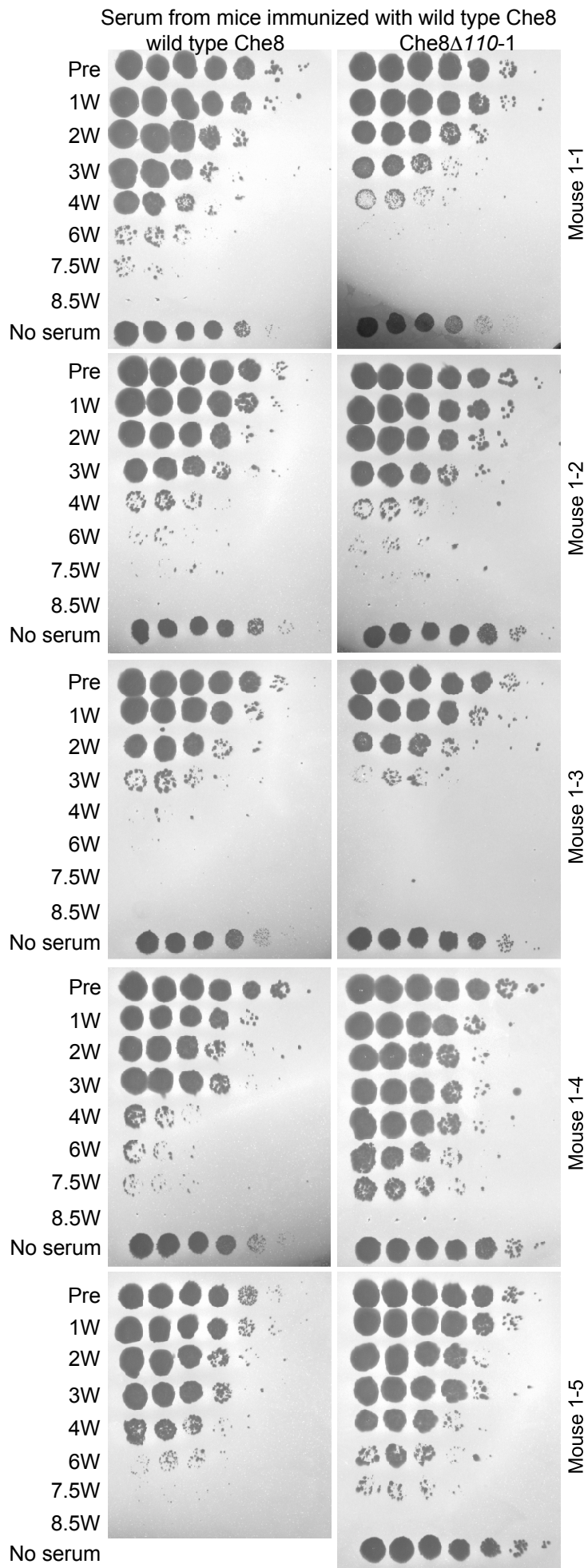


Figure S7