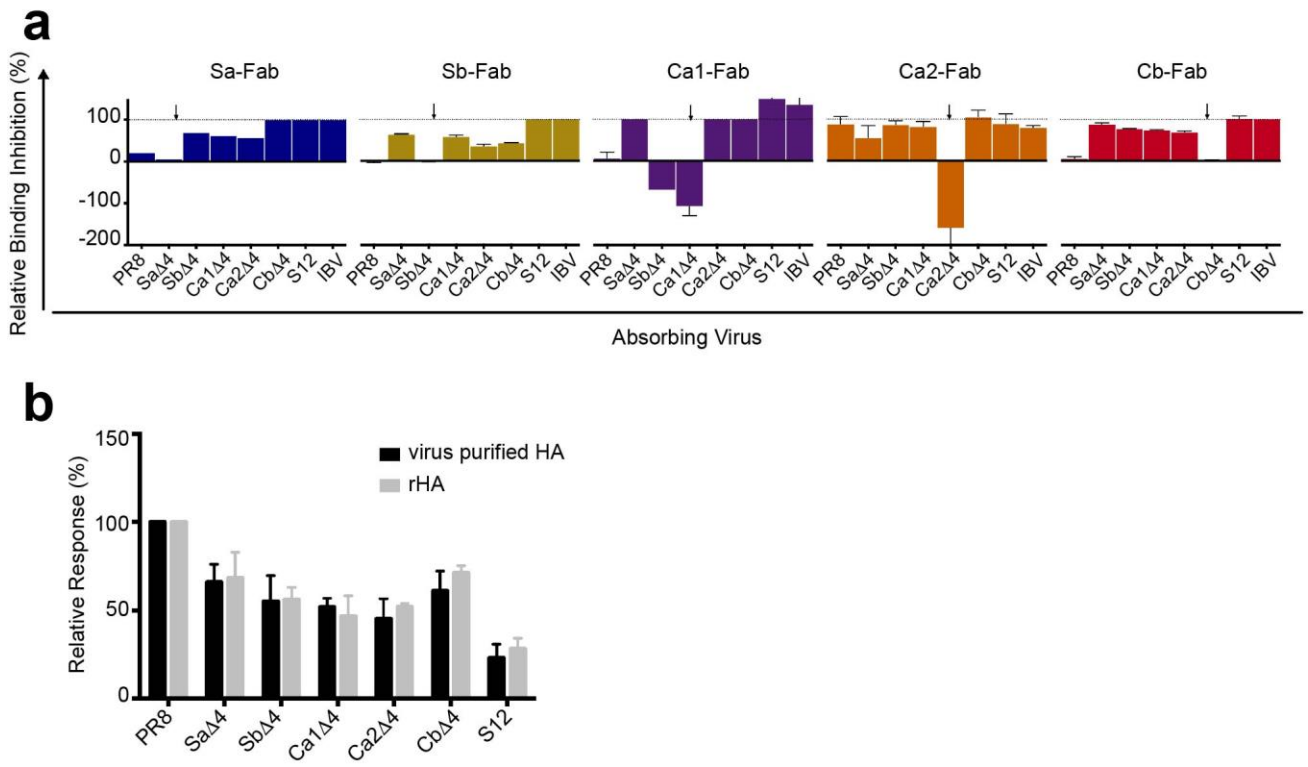
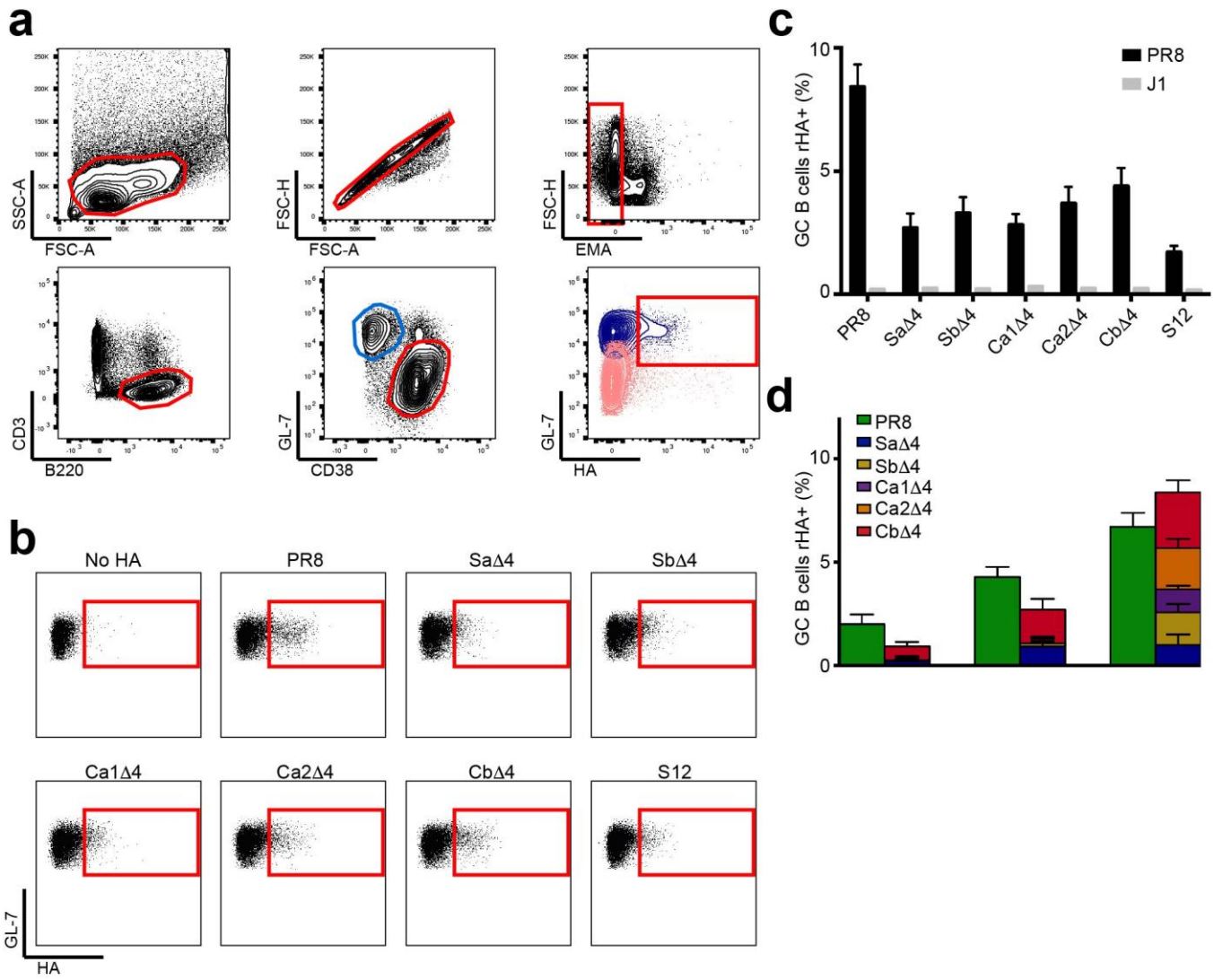


Supplementary Figure 1

Quantification of the virus-purified HAs and their specificities.

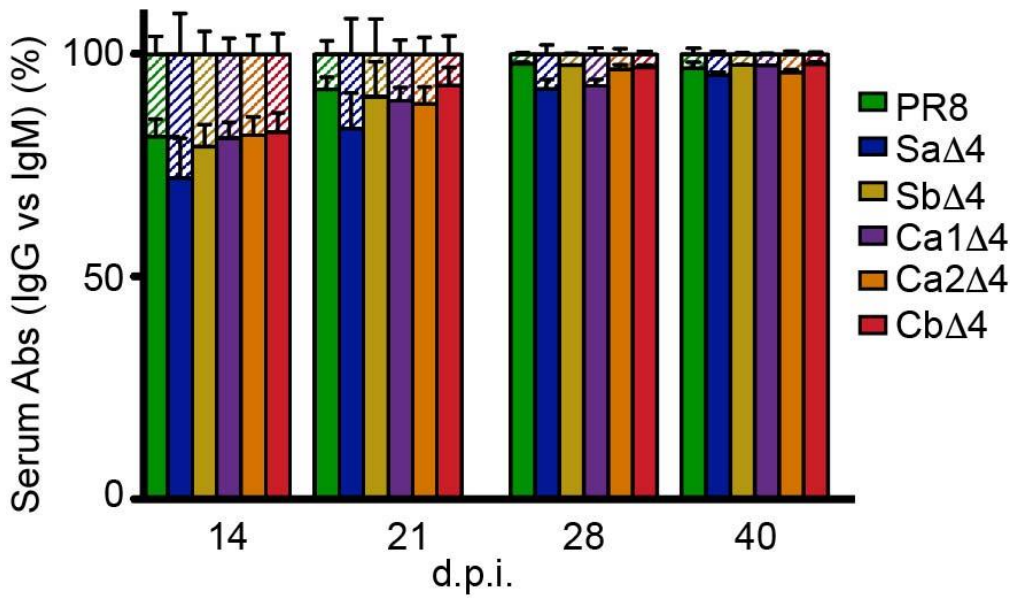
(**a**) Sera from two individual i.n. infected guinea pigs were absorbed on the viruses indicated on the X axis. The depleted sera was tested for its capacity to block binding of site-specific Fabs. The results are presented as relative binding inhibition, where binding of Fab to PR8 virus pre incubated with neat serum equals 100% and binding inhibition to virus with no competing serum is zero. Results are from three technical replicates of two individual guinea pigs. Columns represent means and SEM (bars). (**b**) ELISA comparison between the virus-purified HAs and rHA showing accurate quantification of the purified glycoproteins. Results are normalized to PR8 AUC set as 100%. Columns represent mean and SEM (bars) of 5 distinct sera. We also used total protein amount, Coomassie staining, western blotting, head-specific mAbs and anti-stem mAbs (not shown) to confirm that similar amounts of antigen were used in ELISA assays for all PR8 derived HAs.



Supplementary Figure 2

Gating strategy and specificity of rHA probes.

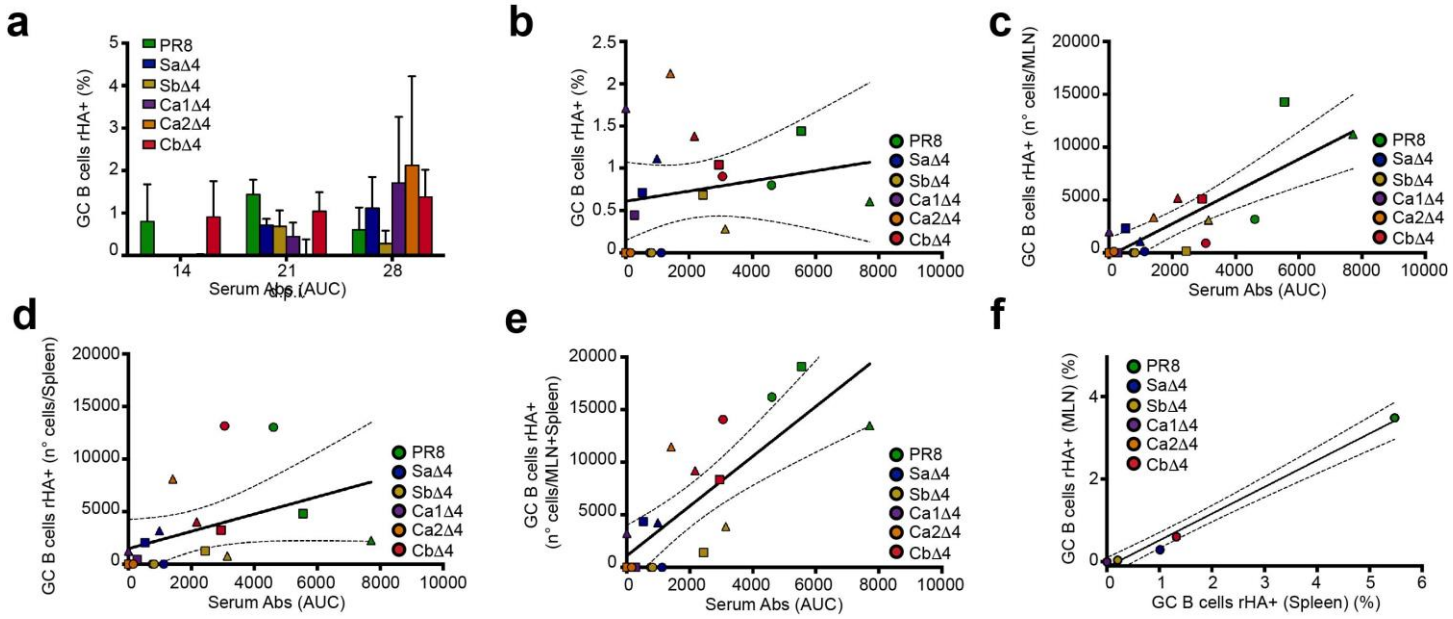
We infected mice i.n. with 50 $TCID_{50}$ PR8. (**a**) Flow plot depicting gating strategy to identify GC B cells based on live/dead EMA^- and $CD3\varepsilon^-$ $B220^+$ $CD38^-$ $GL7^+$ surface expression. Only GC B cells specifically bind rHA. (**b**) The dot plot depicts one representative experiment showing the % of GC B cells that are stained by the recombinant probes. Shown is one representative example of three independent experiments with 5 pooled mice each (for both **a** and **b**). (**c**) We infected mice were i.n. with 50 $TCID_{50}$ of either PR8 or J1, a reassortant virus with all the gene segments from PR8 except for segment 4 encoding H3 HA. At 28 d p.i, we pooled MLN from 5 infected mice and stained as above. Shown are results at 28 d.p.i. of three independent experiments with 5 mice pooled each (PR8) or one experiment with 5 mice pooled (J1).Columns represent means and SEM (bars). (**d**) Bar graph showing cumulative MLN GC B cell responses. After S12 AUC subtraction, the sum of the B cell frequency to the 5 antigenic sites is similar to the PR8 response. Columns represent means and SEM (bars).



Supplementary Figure 3

Heavy-chain composition of immune sera.

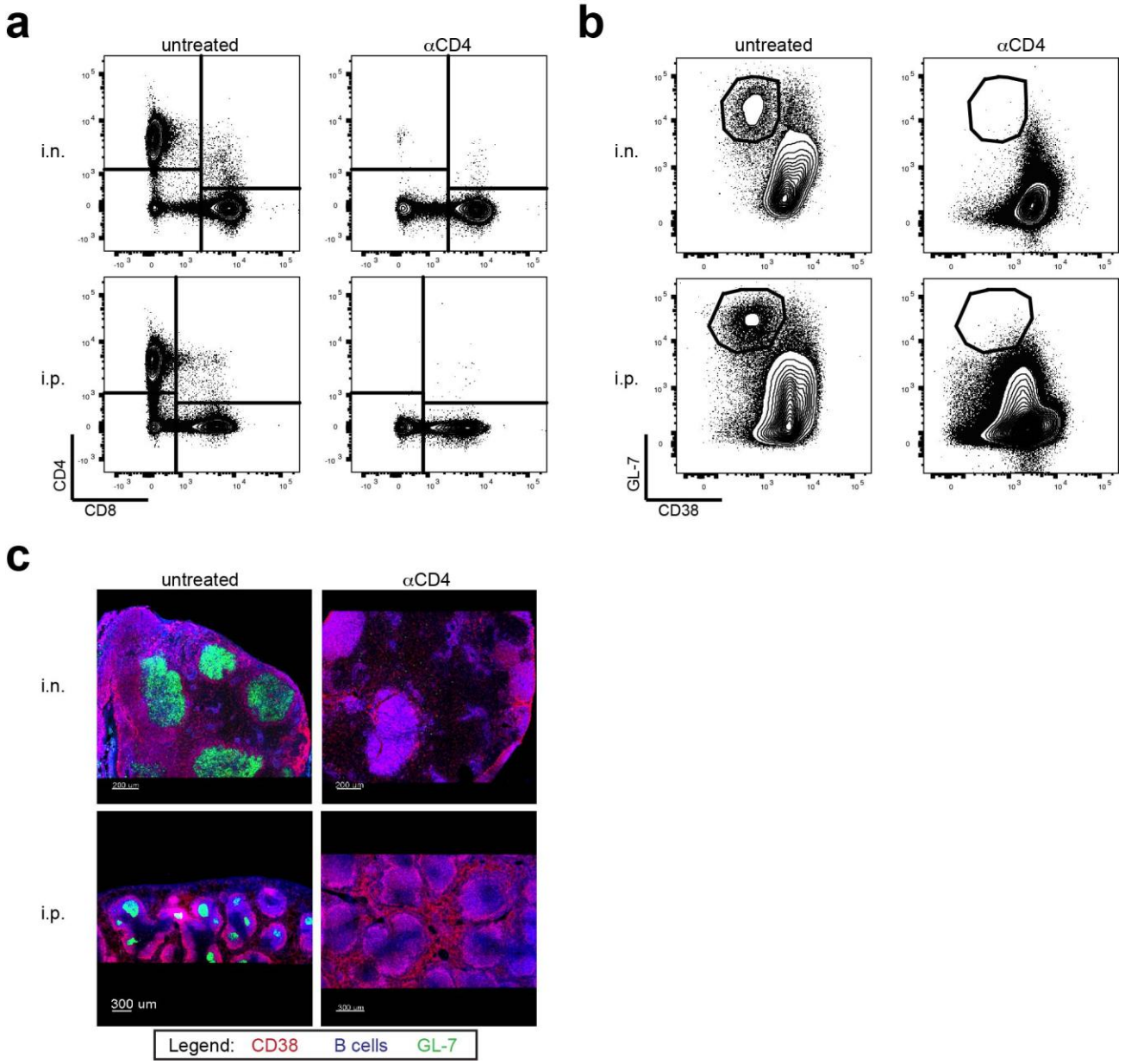
We infected mice infected i.n. with 50 $TCID_{50}$ PR8. The sera analyzed in Figure 2C were subjected to heavy chain ELISA to determine the proportion of antigenic site-specific IgG (filled bars) *vs.* IgM (striped bars). Columns represent means and SEM (bars).



Supplementary Figure 4

Splenic B cell kinetics and immunodominance in IAV-infected and immunized mice.

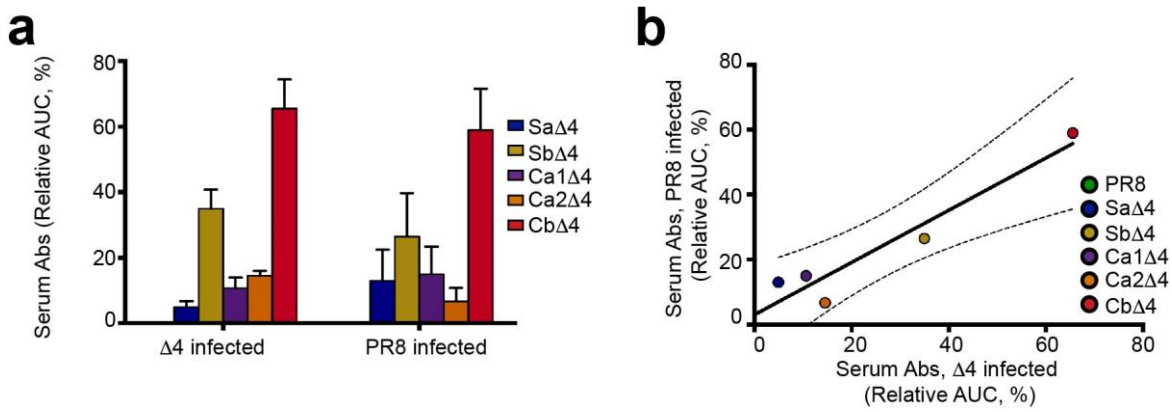
(**a**) Bar graph showing the frequency of antigenic site-specific germinal center B cells at different d.p.i. S12 frequencies were considered baseline and subtracted from PR8 and Δ4 values. Column represent mean and SEM (bars). Shown are three independent experiments with three pooled spleens each. (**b**) Scatter plot showing the correlation between ELISA recognition of the different HAs (**Fig. 2c**) and frequency of splenic GC B cells P=0.4319 r=0.1976. Dashed lines are 95% confidence intervals. Scatter plots showing the correlation between ELISA recognition of different HAs (**Fig. 2c**) and the different cell numbers. Data is the same presented in **Fig. 2b** and **Supplementary Fig. 3a** but expressed as total number of cells in the MLN P<0.0001 r=0.8095 (**c**), spleen P=0.0838 r=0.4186 (**d**) or total cell number (MLN+spleen) P=0.0001 r=0.7865 (**e**). Circles represent 14 d p.i., squares, 21 d p.i. and triangles, 28 d p.i.. Dashed lines represent 95% confidence intervals. (**f**) We immunized mice i.p. with 2500 HAU of UV-inactivated PR8. Scatter plot shows correlation between the frequency of MLN versus splenic GC B cells. P<0.0001 r=0.9941. Shown is the average from two independent experiments with five pooled mice each.



Supplementary Figure 5

$CD4^+$ T cell depletion.

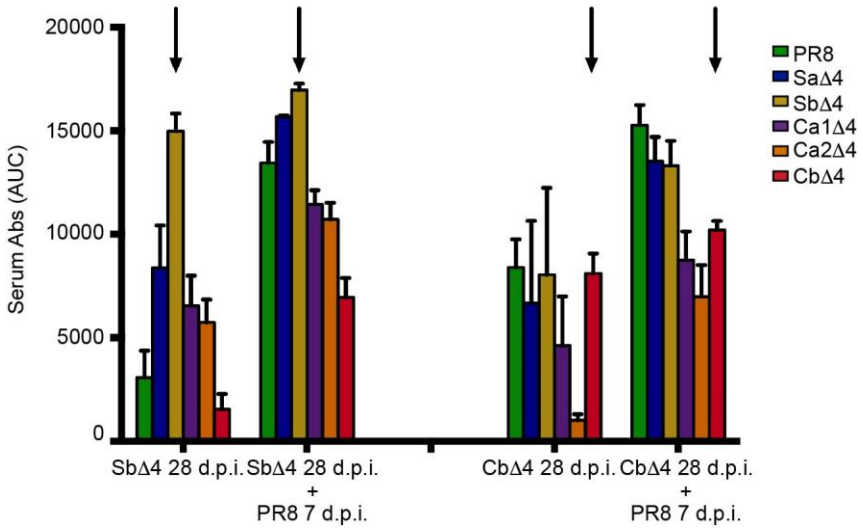
We depleted $CD4^+$ T cells as described in **Fig. 4**. Shown are representative flow cytometry plots showing CD4+ and CD8+ T cells (gated on live, $CD3^+$, $B220^-$) (**a**) and GC B cells (gated on live, $CD3^-$, $B220^+$) (**b**) in untreated versus CD4-depleted animal at 14 d.p.i.. (**c**) LN sectioning and immunofluorescent staining comparing GC formation in untreated versus CD4-depleted animals in LN following i.n. infection and spleen following i.p. immunization. Representative flow cytometry of one spleen from two independent experiments with five and three mice per group (**a**), one spleen from one experiment with three mice per group (**b**). Representative microscopy of one spleen from one experiment with three mice per group (**c**)



Supplementary Figure 6

Δ4 virus infection generates Abs specific to the intact antigenic site.

(**a**) ELISA results showing the reactivity of Δ4 infected animals (from **Fig. 6**) on PR8 HA (Δ infected) and the reactivity of PR8 infected animals on Δ4 and S12 (PR8 infected). Column represent mean and bars SEM (n=4 for PR8, Sa, Ca1, Ca2; n=5 for Cb and n=8 for Sb). (**b**) Correlation between the AUC values presented in A. *P*=0.0105 *r*=0.9573. Dashed lines are 95% confidence intervals.



Supplementary Figure 7

Pre-existing Abs influence recall responses.

We infected mice i.n. with 50 $TCID_{50}$ Δ4Sb or Δ4Cb virus and challenged at 28 d.p.i. i.p. with 2000 HAU of PR8. We collected sera 7 d post challenge and tested by ELISA for binding to PR8, Δ4 and S12 HAs. The data are the same as **Fig. 7c**, but shown is the serum response to all antigenic sites 28 d after infection and 7 d after i.p. challenge. Arrows indicate the site corresponding to the primary virus. Data on graph represent mean and SEM (bars) of two independent experiments with 3 mice each.

Suppl Table 1. mAbs used for sequential selection of the Δ4 viruses

	Name	Selected with Abs	Cb	Ca1	Ca2	Sa	Sb
SaΔ4	dCb	H18-S48, H20-A15, H17-L7, H2-4C2	L75P, V77M, R78K, E124G				
	E11	H2-5B6, H18-L9, Y8-2D1	L75P, V77M, R78K, E124G		S145N		
	C6	H17-L2, H17-L10, H33-46, H33-23	L75P, V77M, R78K, E124G	G173E	S145N		
	SaΔ4	H2-6C4, H28-D14	L75P, V77M, R78K, E124G	G173E	S145N		E156K
SbΔ4	dCb	H16-S48, H20-A15, H17-L7, H2-4C2	L75P, V77M, R78K, E124G				
	E11	H2-5B6, H18-L9, Y8-2D1	L75P, V77M, R78K, E124G		S145N		
	C6	H17-L2, H17-L10, H33-46, H33-23	L75P, V77M, R78K, E124G	G173E	S145N		
	F1	Y8-1A6, H16-S53	L75P, V77M, R78K, E124G	G173E	S145N	S160L	
	3/F8	H9-B20, Y8-3B3	L75P, V77M, R78K, E124G	G173E	S145N	S160L, S167Y	
	1G11	H2-6A1	L75P, V77M, R78K, E124G	G173E	S145N	N129K, S160L, S167Y(lost)	
	SbΔ4	H36-101	L75P, V77M, R78K, E124G	G173E	S145N	N129K, S160L, K165E	
Ca1Δ4	dCb	H16-S48, H20-A15, H17-L7, H2-4C2	L75P, V77M, R78K, E124G				
	E11	H2-5B6, H18-L9, Y8-2D1	L75P, V77M, R78K, E124G		S145N		
	A1	H2-6C4, H28-D14, Y8-1C1, H35-C3	L75P, V77M, R78K, E124G		S145N		E156K
	F10	Y8-1A6, H16-S53	L75P, V77M, R78K, E124G		S145N	S160L	E156K
	4/G12	H9-B20, Y8-3B3	L75P, V77M, R78K, E124G		S145N	S160L, S188N	E156K
	Ca1Δ4	H36-104	L75P, V77M, R78K, E124G		S145N	S160L, K165E, S188N	E156K
Ca2Δ4	dCb	H16-S48, H20-A15, H17-L7, H2-4C2	L75P, V77M, R78K, E124G				
	H1	H17-L2, H17-L10, H33-46, H33-23	L75P, V77M, R78K, E124G	G173E			
	F9	H2-6C4, H28-D14	L75P, V77M, R78K, E124G	G173E		N248D	E156K
	Ca2Δ4	Y8-1A6, H16-S53	L75P, V77M, R78K, E124G	G173E		N248D, N131T (glyc), Y201H	E156K
CbΔ4	dSa	Y8-1A6, H9-B20, H16-S53, Y8-3B3, H9-A22, PEG-1				N129D, E158K, S167P	
	B2	H2-6C4, H28-D14, H35-C3, Y8-1C1				N129D, E158K, S167P	Q196R
	E3	H17-L2, H17-L10, H33-46, H33-23		G173E		N129D, E158K, S167P	Q196R
	CbΔ4	H2-5B6, Y8-2D1		G173E	S145N	N129D, E158K, S167P	Q196R

Supplementary Table 2. Proportion of Abs directed to the conserved stem of HA

Experimental protocol	Days post-exposure	Source of Data (Figure[1])	Head Abs[2] (AUC ± SEM)	Stem Abs[3] (AUC ± SEM)
Intranasal infection	14	2C	4608 ± 673	541 ± 155
	21		5548 ± 948	170 ± 50
	28		7712 ± 1011	385 ± 106
	40		10702 ± 1975	251 ± 9
Intramuscular immunization	14	3A	1388 ± 1388	541 ± 199
	28		7233 ± 2010	794 ± 217
Intraperitoneal immunization	14	3B	8693 ± 679	194 ± 194
	28		8808 ± 454	1267 ± 422
Intranasal infection + α-CD4 treatment	14	4A	1393 ± 469	493 ± 188
Intraperitoneal immunization + α-CD4 treatment	14	4A	1398 ± 437	124 ± 76
	28		855 ± 540	246 ± 53
Intraperitoneal immunization + Fab (Sb site specific)	14	7A	1733 ± 717	114 ± 51
	28		2061 ± 1112	135 ± 92
Intranasal infection (SbΔ4) + intraperitoneal immunization (PR8)	28	Sup. 7	14980 ± 857	368 ± 317
	28+7		16975 ± 302	746 ± 669
Intranasal infection (CbΔ4) + intraperitoneal immunization (PR8)	28	Sup.7	8107 ± 964	324 ± 94
	28+7		10205 ± 437	805 ± 346

[1]The sera used in the figures indicated were tested for stem Ab titers by ELISA

[2] Head Abs indicatesAbs to the five Ag sites and is obtained by subtracting S12-AUC to PR8-AUC (or SbΔ4- and CbΔ4-AUC for the challenge experiment). Average AUC ± SEM.

[3]Stem Abs are measured using a chimeric virus with H5 head and H1(PR8) stem. Average AUC ± SEM.

Suppl Table 3. Summary of site specific functional activity of mAb (unpublished data from Gerhard)

Antigenic site	n	Neutralization units/mg (log10)	HI units/mg (log10)
Sa	11	5.09 ± 0.85	4.67 ± 1.31
Sb	44	4.86 ± 0.68	5.22 ± 0.68
Ca1	7	5.61 ± 0.98	5.53 ± 0.28
Ca2	6	3.94 ± 0.06	4.64 ± 0.52
Cb	35	3.20 ± 0.81	3.71 ± 0.74