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SUPPLEMENTARY INFORMATION

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The establishment of resident memory B cells in the lung requires local antigen encounter

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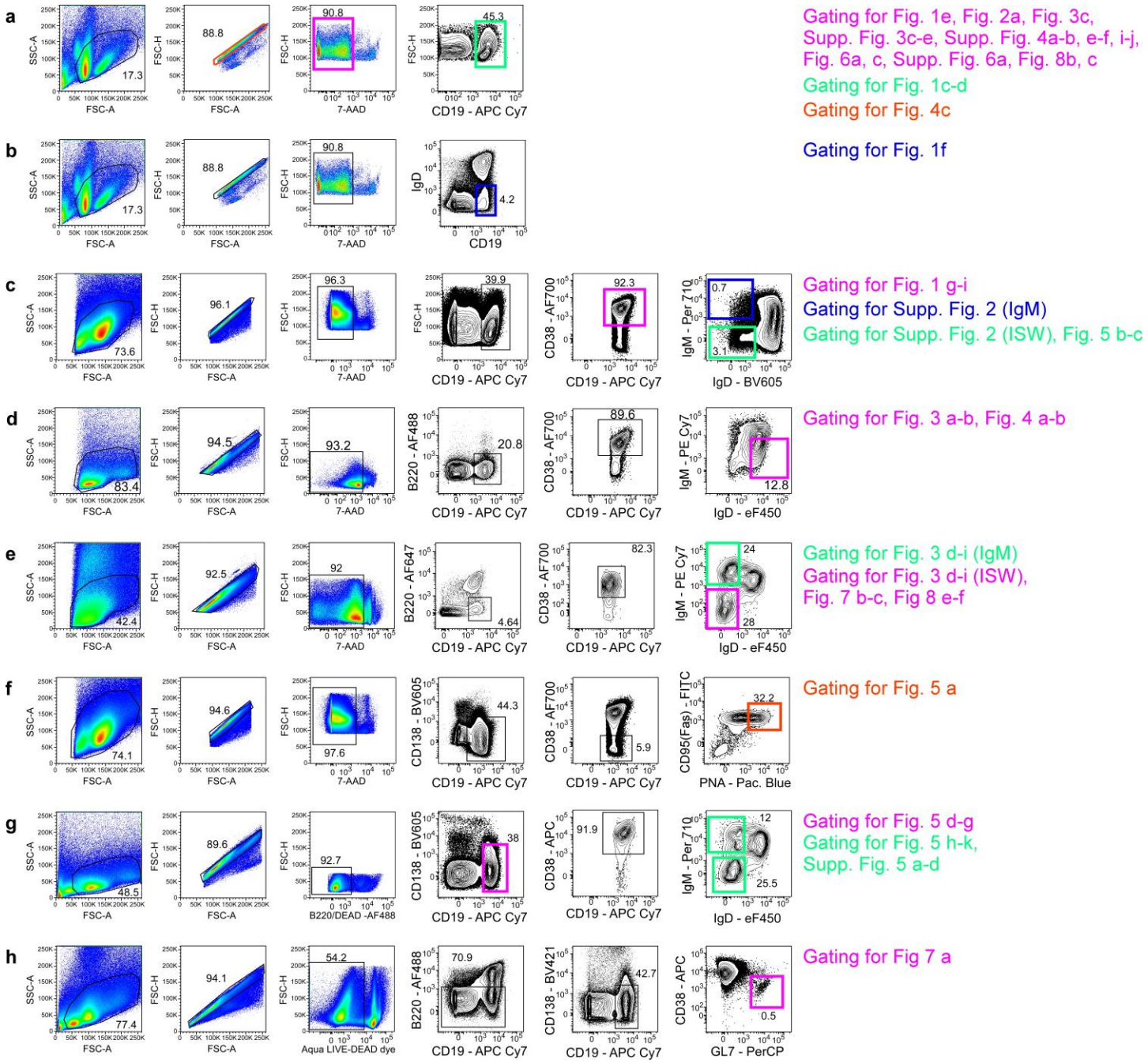


Gating for Fig. 1e, Fig. 2a, Fig. 3c, Supp. Fig. 3c-e, Supp. Fig. 4a-b, e-f, i-j, Fig. 6a, c, Supp. Fig. 6a, Fig. 8b, c

Gating for Fig. 1c-d

Gating for Fig. 4c

Gating for Fig. 1f

Gating for Fig. 1 g-i

Gating for Supp. Fig. 2 (IgM)

Gating for Supp. Fig. 2 (ISW), Fig. 5 b-c

Gating for Fig. 3 a-b, Fig. 4 a-b

Gating for Fig. 3 d-i (IgM)

Gating for Fig. 3 d-i (ISW), Fig. 7 b-c, Fig 8 e-f

Gating for Fig. 5 a

Gating for Fig. 5 d-g

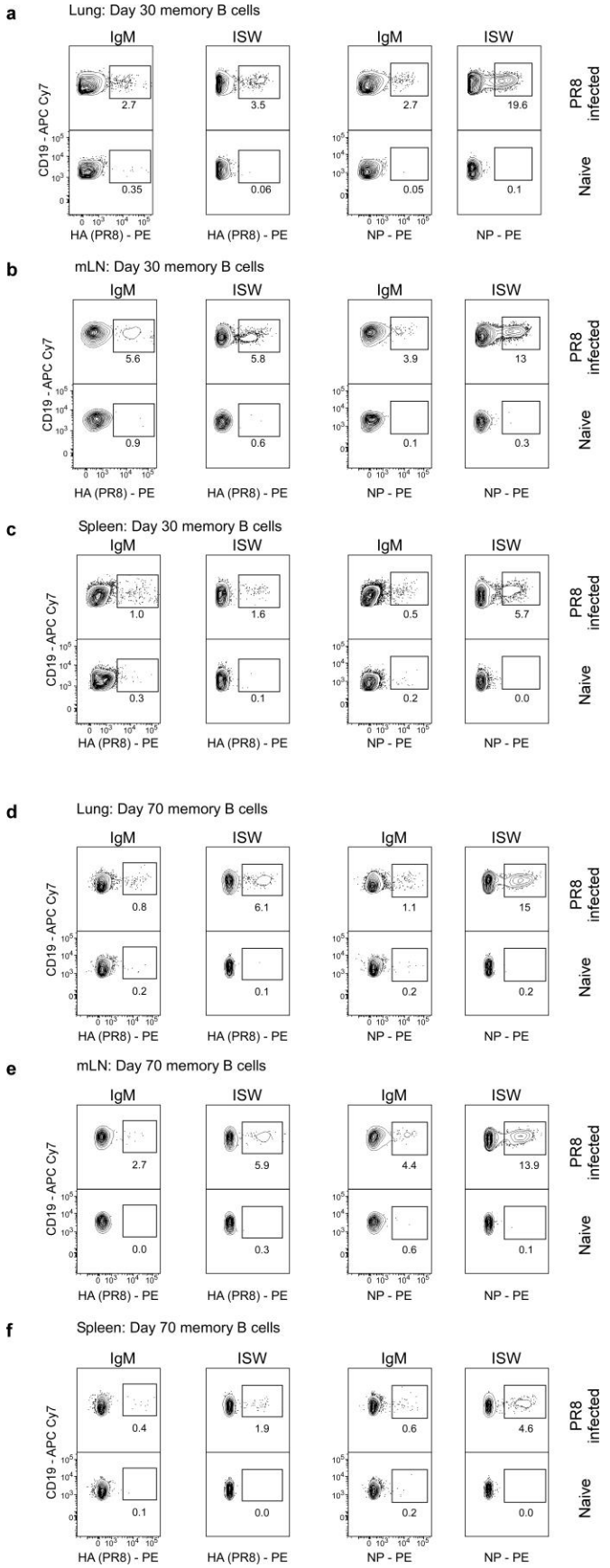
Gating for Fig. 5 h-k, Supp. Fig. 5 a-d

Gating for Fig 7 a

Supplementary Figure 1

Gating strategies.

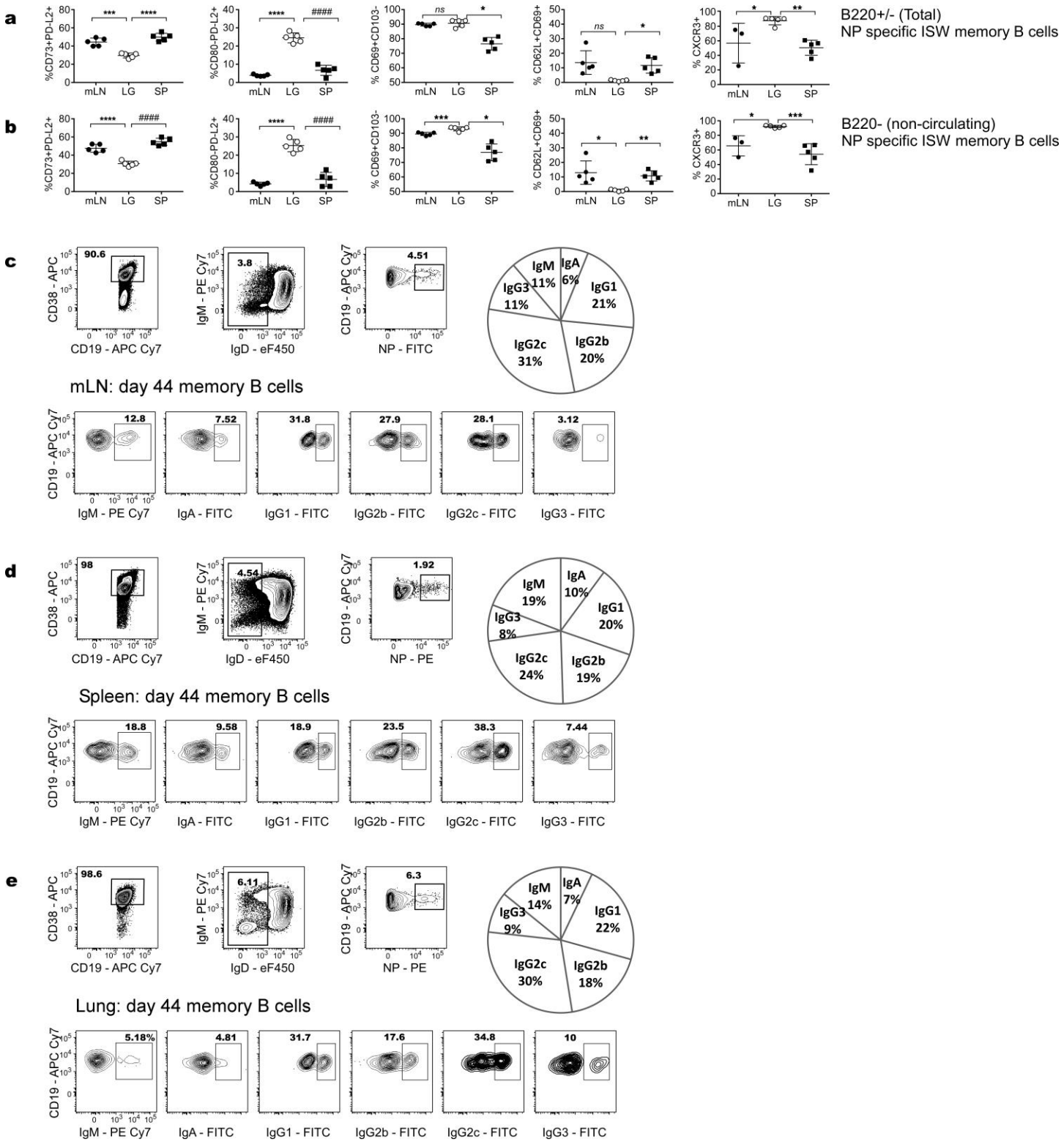
Gates are shown sequentially from left to right. Colored gates are used in the figures listed to the right in font of the same color.


a
Lung: Day 30 memory B cells
b
mLN: Day 30 memory B cells
c
Spleen: Day 30 memory B cells
d
Lung: Day 70 memory B cells
e
mLN: Day 70 memory B cells
f
Spleen: Day 70 memory B cells
IgM
ISW
CD19 - APC Cy7
HA (PR8) - PE
NP - PE
PR8 infected
Naive

Supplementary Figure 2

Identification of influenza-specific memory B cells.

a–**f**, Cells from the lungs (**a**,**d**), mLNs (**b**,**e**) and spleens (**c**,**f**) of naive mice or PR8-infected mice were gated on live, singlet lymphocytes and then on $CD19^{+}CD38^{hi}IgD^{-}IgM^{+}$ (IgM memory) or $CD19^{+}CD38^{hi}IgD^{-}IgM^{-}$ (ISW memory) (Supplementary Fig. 1c) and analyzed for NP-specific or HA-specific memory B cells on day 30 (**a**–**c**) or day 70 (**d**–**f**) after infection. Data are representative of four experiments with five mice/time point.



Supplementary Figure 3

Phenotypic characteristics of non-circulating and total memory B cells.

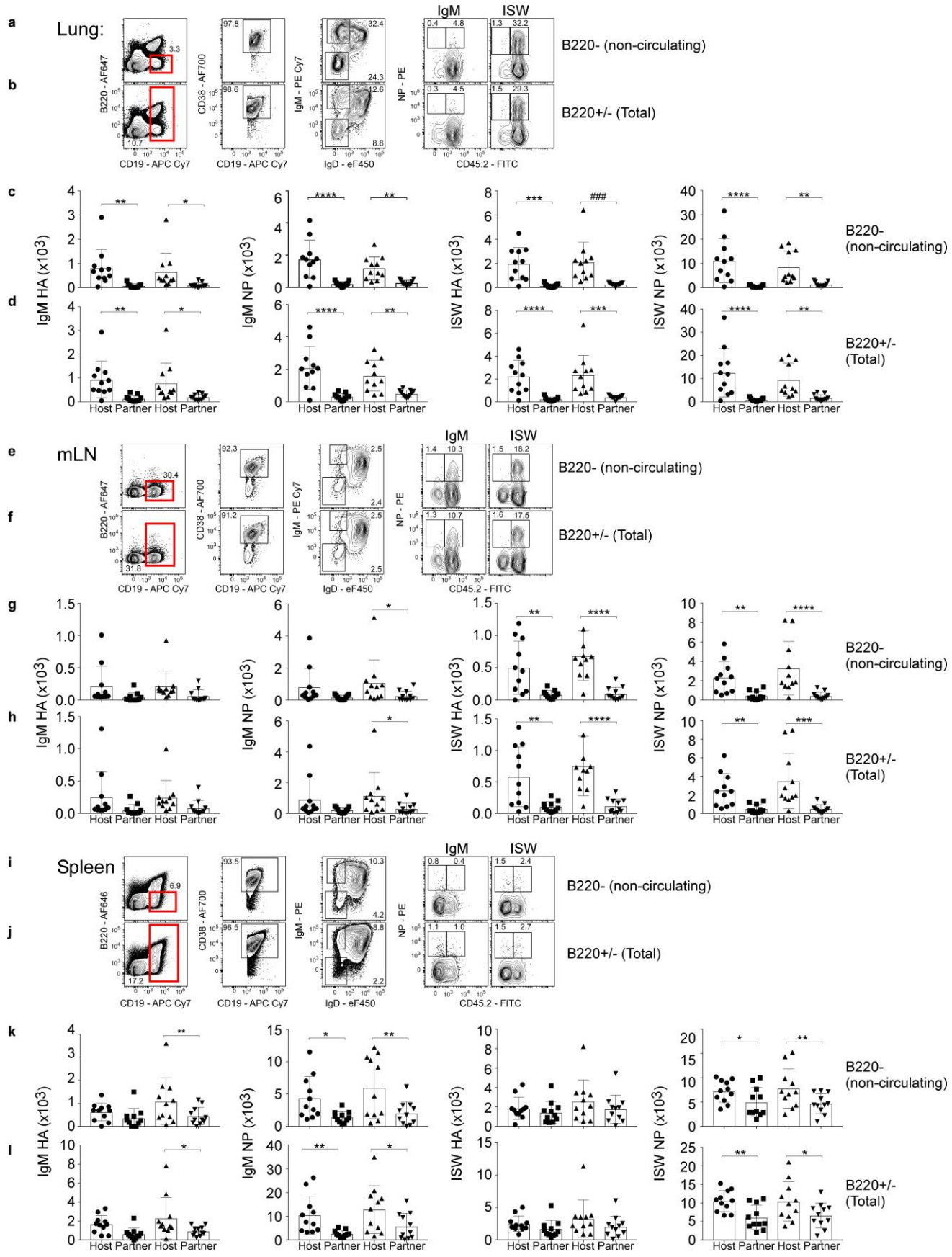
a,**b**, Mice were infected with PR8 influenza A virus and cells from the mLN, lung and spleen were gated on live, singlet lymphocytes and then on NP-specific $CD19^{+}CD38^{+}IgM^{-}IgD^{-}$ ISW memory B cells that were either $B220^{+/-}$ (combined circulating and non-circulating;

a) or B220^{-} (non-circulating; **b**) (gating strategy as in Fig. 2a–d). The phenotype within these populations was determined as in Fig. 2e–i. Data are representative of five experiments with five mice each. Graphs show individual data points (n = 5) as well as mean ± s.d. Data were analyzed by one-way ANOVA with Tukey's correction for multiple comparisons:

(**a**) CD73^{+}PD-L2^{+} ***P = 0.0002 ****P = 0.0001
(**a**) CD80^{-}PD-L2^{+} ****P = 0.0001 $^{\#\#\#\#}P$ = 0.0001
(**a**) CD69^{+}CD103^{-} ns = 0.4447 *P = 0.0129
(**a**) CD62L^{+}CD69^{+} ns = 0.0511 *P = 0.0237
(**a**) CXCR3^{+} *P = 0.0350 **P = 0.0054
(**b**) CD73^{+}PD-L2^{+} ****P = 0.0001 $^{\#\#\#\#}P$ = 0.0001
(**b**) CD80^{-}PD-L2^{+} ****P = 0.0001 $^{\#\#\#\#}P$ = 0.0001
(**b**) CD69^{+}CD103^{-} ***P = 0.0002 *P = 0.0105
(**b**) CD62L^{+}CD69^{+} *P = 0.0471 **P = 0.0042
(**b**) CXCR3^{+} *P = 0.0206 ***P = 0.0008

P < 0.05 is considered significant.

c–**e**, Cells from the mLN (**c**), spleen (**d**) and lung (**e**) of day 44 PR8-infected mice were gated on live, singlet lymphocytes (Supplementary Fig. 1a), and then on NP-specific CD19^{+}CD38^{+}IgD^{-} memory B cells and the frequency of IgM-, IgA-, IgG1-, IgG2b-, IgG2c- and IgG3-expressing cells was determined. Data are representative of three experiments with five mice.


a
Lung:
b
B220 - AF647
CD19 - APC Cy7
CD38 - AF700
IgM - PE Cy7
IgD - eF450
NP - PE
CD45.2 - FITC
IgM
ISW
B220- (non-circulating)
B220+/- (Total)
c
d
IgM HA (x10³)
IgM NP (x10³)
ISW HA (x10³)
ISW NP (x10³)
Host Partner Host Partner
e
mLN
f
g
h
i
Spleen
B220 - AF646
j
k
l

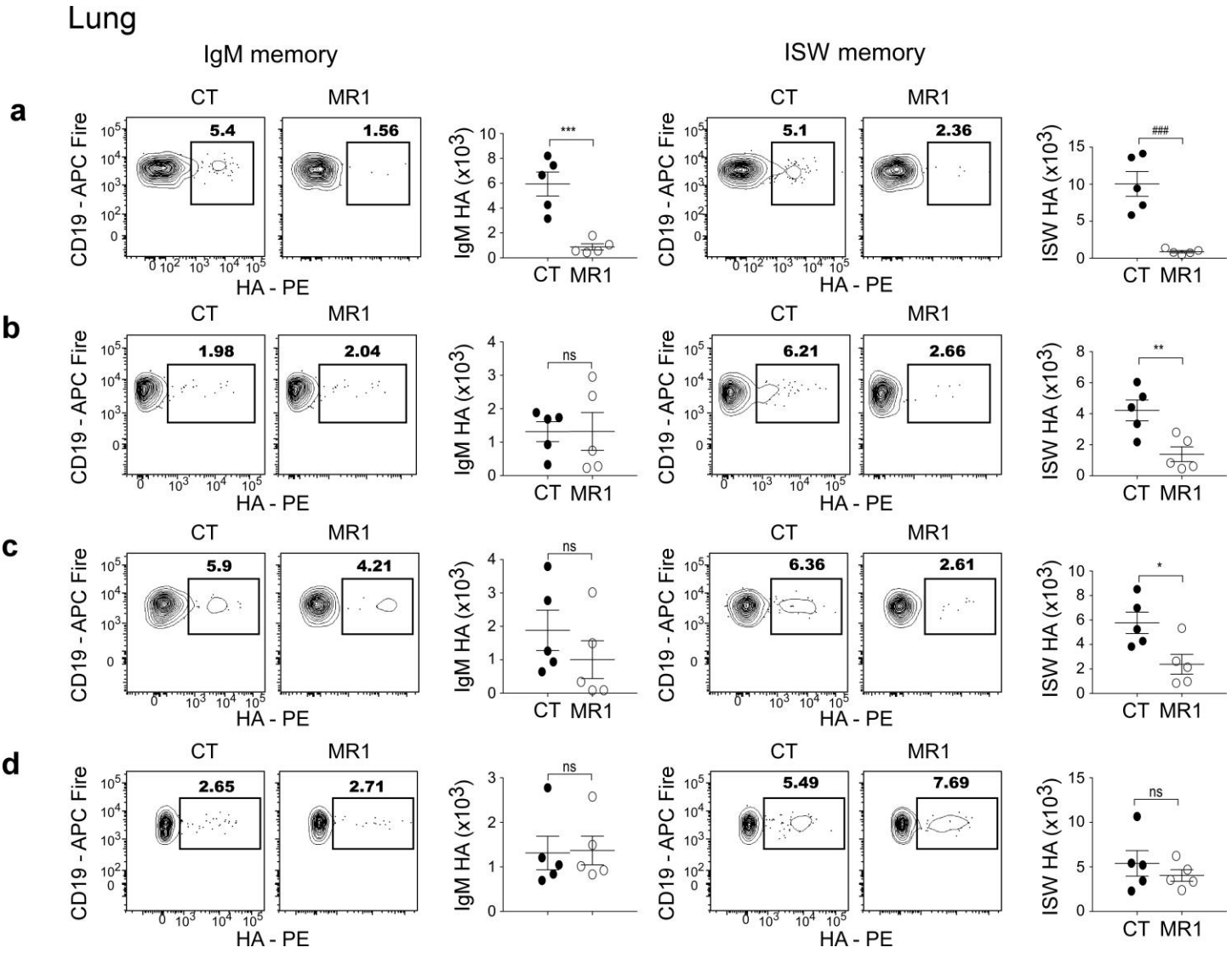
Supplementary Figure 4

Identification of influenza-specific, non-circulating and total memory B cells.

a–**l**, Mice were infected on day 0, surgically paired with partner mice on day 44 and analyzed on day 59. Cells from the lung (**a**–**d**), mLN (**e**–**h**) or spleen (**i**–**l**) were gated on live, singlet lymphocytes (Supplementary Fig. 1a) and subsequently gated on $CD19^{+}CD38^{+}IgD^{-}IgM^{+}$ (IgM) or $CD19^{+}CD38^{+}IgD^{-}IgM^{-}$ (ISW) NP-specific memory B cells in either the $B220^{-}$ (non-circulating; **a**,**c**,**e**,**g**,**i**,**k**) or $B220^{+/-}$ (combined circulating and non-circulating; **b**,**d**,**f**,**h**,**j**,**l**) fractions. The numbers of NP-specific memory B cells derived from host and partner mice in the lung (**c**,**d**), mLN (**g**,**h**) and spleen (**i**,**j**) are shown for both the non-circulating fraction and the total (combined circulating and non-circulating) fraction. Data are representative of three experiments combined, totaling 11 pairs of mice. Graphs show individual data as well as mean ± s.d. Significance was determined using one-way ANOVA followed by the Bonferroni–Sidak method for multiple comparisons:

(**c**) Lung, IgM HA $B220^{-}$	***P* = 0.0031	*p=0.0243
(**c**) Lung, IgM NP $B220^{-}$	*****P* = 0.0001	**p=0.0040
(**c**) Lung, ISW HA $B220^{-}$	****P* = 0.0003	###p=0.0002
(**c**) Lung, ISW NP $B220^{-}$	*****P* = 0.0001	**p=0.0022
(**d**) Lung, IgM HA $B220^{+/-}$	***P* = 0.0013	*p=0.0158
(**d**) Lung, IgM NP $B220^{+/-}$	*****P* = 0.0001	**p=0.0017
(**d**) Lung, ISW HA $B220^{+/-}$	*****P* = 0.0001	***p=0.0001
(**d**) Lung, ISW NP $B220^{+/-}$	*****P* = 0.0001	**p=0.0024
(**g**) mLN, IgM HA $B220^{-}$	*P* = 0.0618	p=0.1136
(**g**) mLN, IgM NP $B220^{-}$	*P* = 0.0783	*p=0.0183
(**g**) mLN, ISW HA $B220^{-}$	***P* = 0.0014	****p=0.0001
(**g**) mLN, ISW NP $B220^{-}$	***P* = 0.0079	****p=0.0001
(**h**) mLN, IgM HA $B220^{+/-}$	*P* = 0.0718	p=0.1631
(**h**) mLN, IgM NP $B220^{+/-}$	*P* = 0.0746	*p=0.0237
(**h**) mLN, ISW HA $B220^{+/-}$	***P* = 0.0022	****p=0.0001
(**h**) mLN, ISW NP $B220^{+/-}$	***P* = 0.0081	***p=0.0001
(**k**) Spleen, IgM HA $B220^{-}$	*P* = 0.2821	**p=0.0039
(**k**) Spleen, IgM NP $B220^{-}$	**P* = 0.0160	**p=0.0012
(**k**) Spleen, ISW HA $B220^{-}$	*P* = 0.6749	p=0.2475
(**k**) Spleen, ISW NP $B220^{-}$	**P* = 0.0337	**p=0.0028
(**l**) Spleen, IgM HA $B220^{+/-}$	*P* = 0.0662	*p=0.0116
(**l**) Spleen, IgM NP $B220^{+/-}$	***P* = 0.0078	*p=0.0156
(**l**) Spleen, ISW HA $B220^{+/-}$	*P* = 0.4171	p=0.1469
(**l**) Spleen, ISW NP $B220^{+/-}$	***P* = 0.0029	*p=0.0108

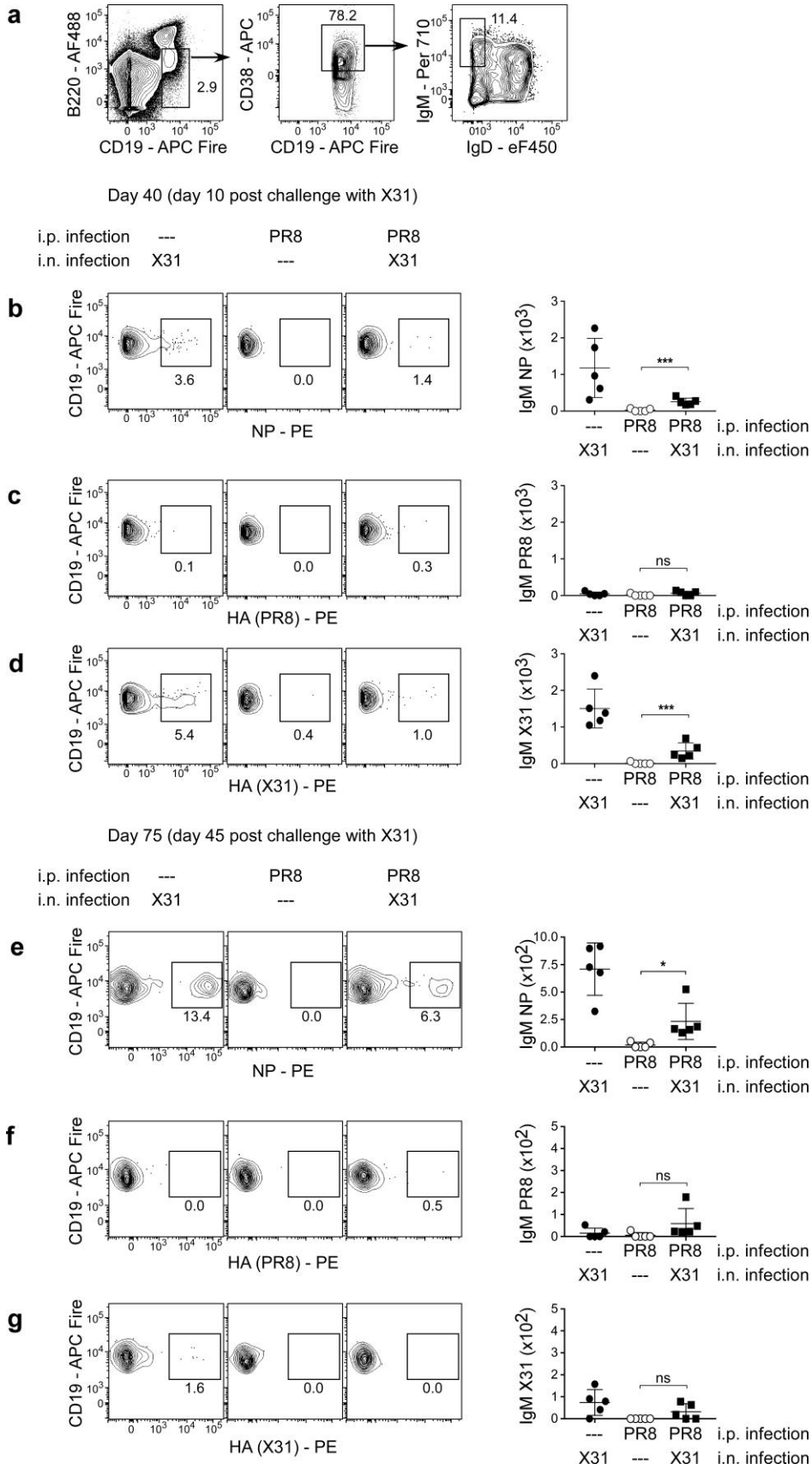
P < 0.05 is considered significant.



Supplementary Figure 5

HA-specific BRM cells in the lung are generated from early CD40-dependent precursors.

a–**d**, Mice were infected with PR8 and administered anti-CD40L (MR1) or isotype control (CT) antibody every other day for 10 d starting on day 5 (**a**), day 10 (**b**), day 20 (**c**) or day 30 (**d**). Cells from the lung were gated on live, singlet lymphocyte, $B220^-CD19^+CD38^+IgM^+IgD^-$ IgM BRM cells or $B220^-CD19^+CD38^+IgM^-IgD^-$ ISW BRM cells (Supplementary Fig. 1g). Data are representative of three experiments with five mice/group per time point. Graphs show mean ± s.d. as well as individual data points. Significance was determined using an unpaired, two-tailed *t* test, ***P = 0.0009 $^{###}P$ = 0.0006 (**a**), **P = 0.0090 (**b**), *P = 0.0219 (**c**). $P < 0.05$ is considered significant.


a
B220 - AF488
CD19 - APC Fire
2.9
CD38 - APC
78.2
IgM - Per 710
11.4
IgD - eF450
Day 40 (day 10 post challenge with X31)
i.p. infection --- PR8 PR8
i.n. infection X31 --- X31
b
CD19 - APC Fire
NP - PE
3.6
0.0
1.4
IgM NP (x10³)
***
c
HA (PR8) - PE
0.1
0.0
0.3
IgM PR8 (x10³)
ns
d
HA (X31) - PE
5.4
0.4
1.0
IgM X31 (x10³)
***
Day 75 (day 45 post challenge with X31)
e
13.4
0.0
6.3
IgM NP (x10²)
*
f
0.0
0.0
0.5
IgM PR8 (x10²)
ns
g
1.6
0.0
0.0
IgM X31 (x10²)
ns

Supplementary Figure 6

Poor recruitment of antigen-non-specific IgM memory cells to inflamed lungs.

a–**g**, Mice were peritoneally infected with PR8 on day 0, intranasally challenged with X31 on day 30 and analyzed on day 40 (**a**–**d**) and day 75 (**e**–**g**). Cells from the lung were gated on live, singlet lymphocytes (Supplementary Fig. 1a) and subsequently gated on $CD19^{+}B220^{-}CD38^{+}IgD^{-}IgM^{+}$ IgM memory B cells (**a**). NP-specific (**b**,**e**), HA(PR8)-specific (**c**,**f**) and HA(X31)-specific (**d**,**g**) IgM BRM cells were enumerated on day 40 (**b**–**d**) and day 75 (**e**–**g**). Graphs show individual data points as well as mean ± s.d. These data are representative of two independent experiments with five mice/time point. Data were analyzed with a one-sided unpaired *t* test, ***P = 0.001 (**b**), ***P = 0.0098 (**d**, *P = 0.0205 (**e**). $P < 0.05$ is considered significant.