FIGURE 1

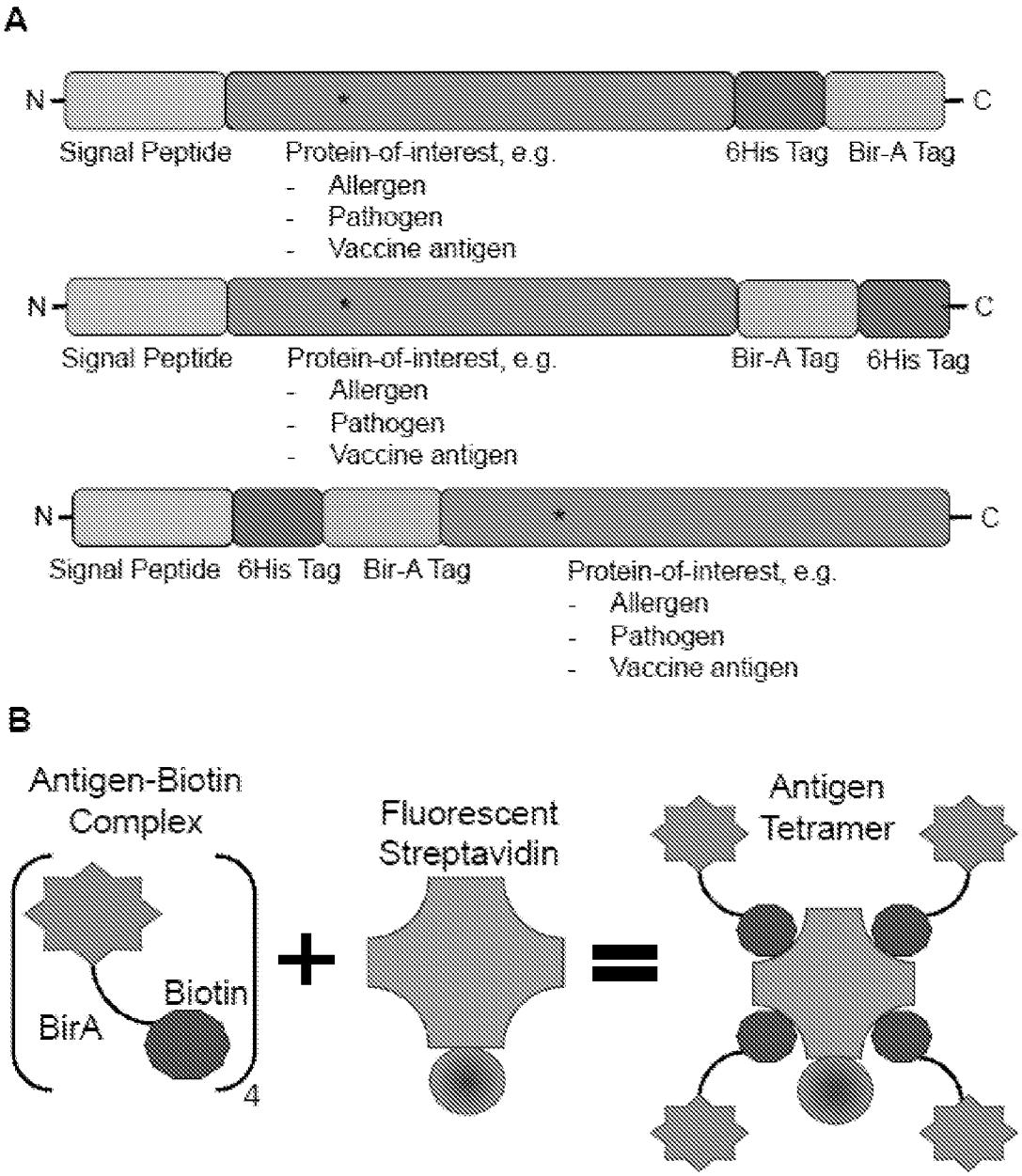

A
N
C
Signal Peptide
Protein-of-interest, e.g.
- Allergen
- Pathogen
- Vaccine antigen
6His Tag
Bir-A Tag
N
C
Signal Peptide
Protein-of-interest, e.g.
- Allergen
- Pathogen
- Vaccine antigen
Bir-A Tag
6His Tag
N
C
Signal Peptide
6His Tag
Bir-A Tag
Protein-of-interest, e.g.
- Allergen
- Pathogen
- Vaccine antigen
B
Antigen-Biotin Complex
BirA
Biotin
4
Fluorescent Streptavidin
Antigen Tetramer

FIGURE 2

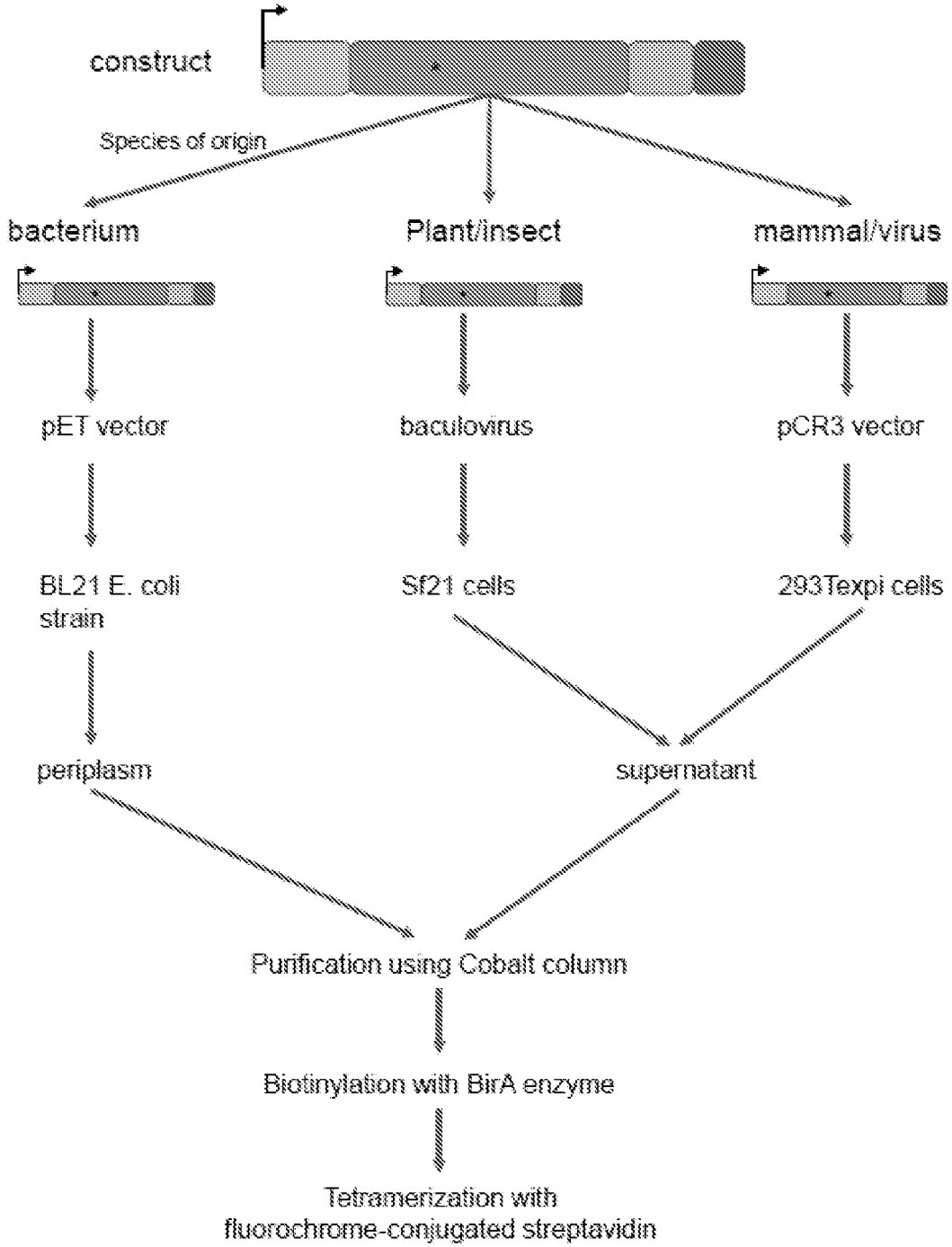

construct
Species of origin
bacterium
Plant/insect
mammal/virus
pET vector
baculovirus
pCR3 vector
BL21 E. coli strain
Sf21 cells
293Texpi cells
periplasm
supernatant
Purification using Cobalt column
Biotinylation with BirA enzyme
Tetramerization with fluorochrome-conjugated streptavidin

FIGURE 3

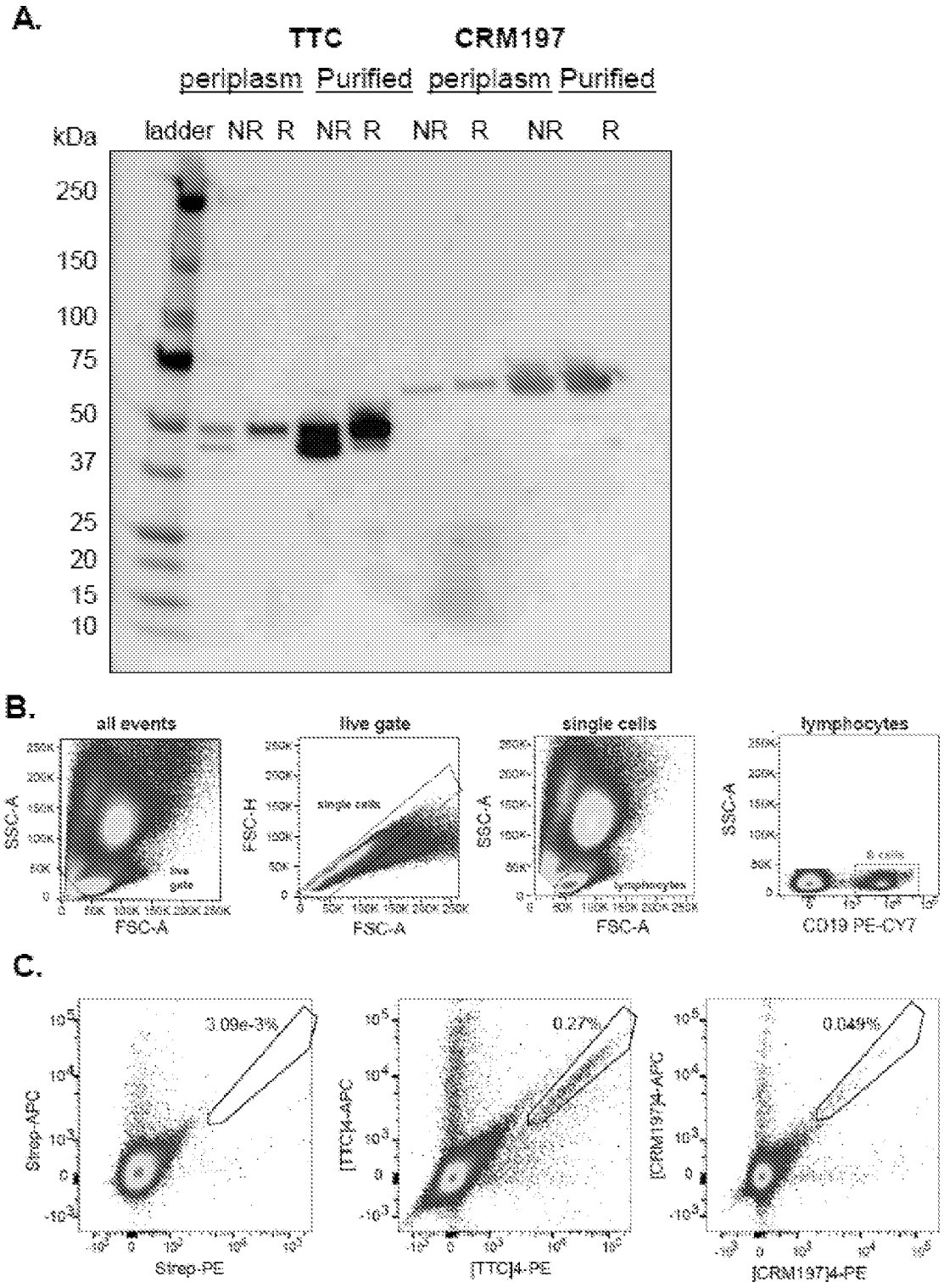

A.
TTC
CRM197
periplasm Purified periplasm Purified
kDa
ladder NR R NR R NR R NR R
250
150
100
75
50
37
25
20
15
10
B.
all events
live gate
single cells
lymphocytes
SSC-A
FSC-A
FSC-H
CD19 PE-CY7
C.
3.09e-3%
0.27%
0.049%
Strep-APC
Strep-PE
[TTC]4-APC
[TTC]4-PE
[CRM197]4-APC
[CRM197]4-PE

FIGURE 4

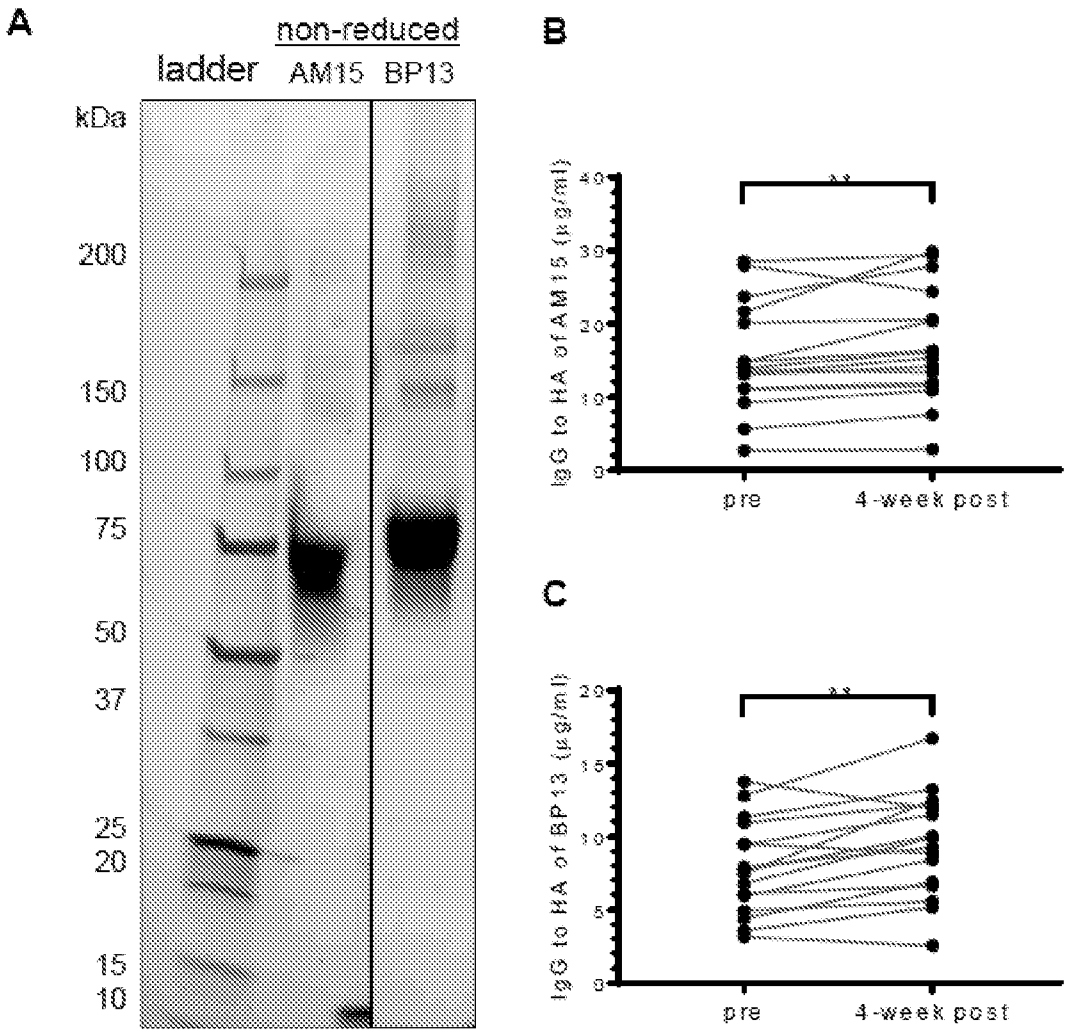

A
non-reduced
ladder
AM15
BP13
kDa
200
150
100
75
50
37
25
20
15
10
B
IgG to HA of AM15 (µg/ml)
pre
4-week post
C
IgG to HA of BP13 (µg/ml)
pre
4-week post

FIGURE 5

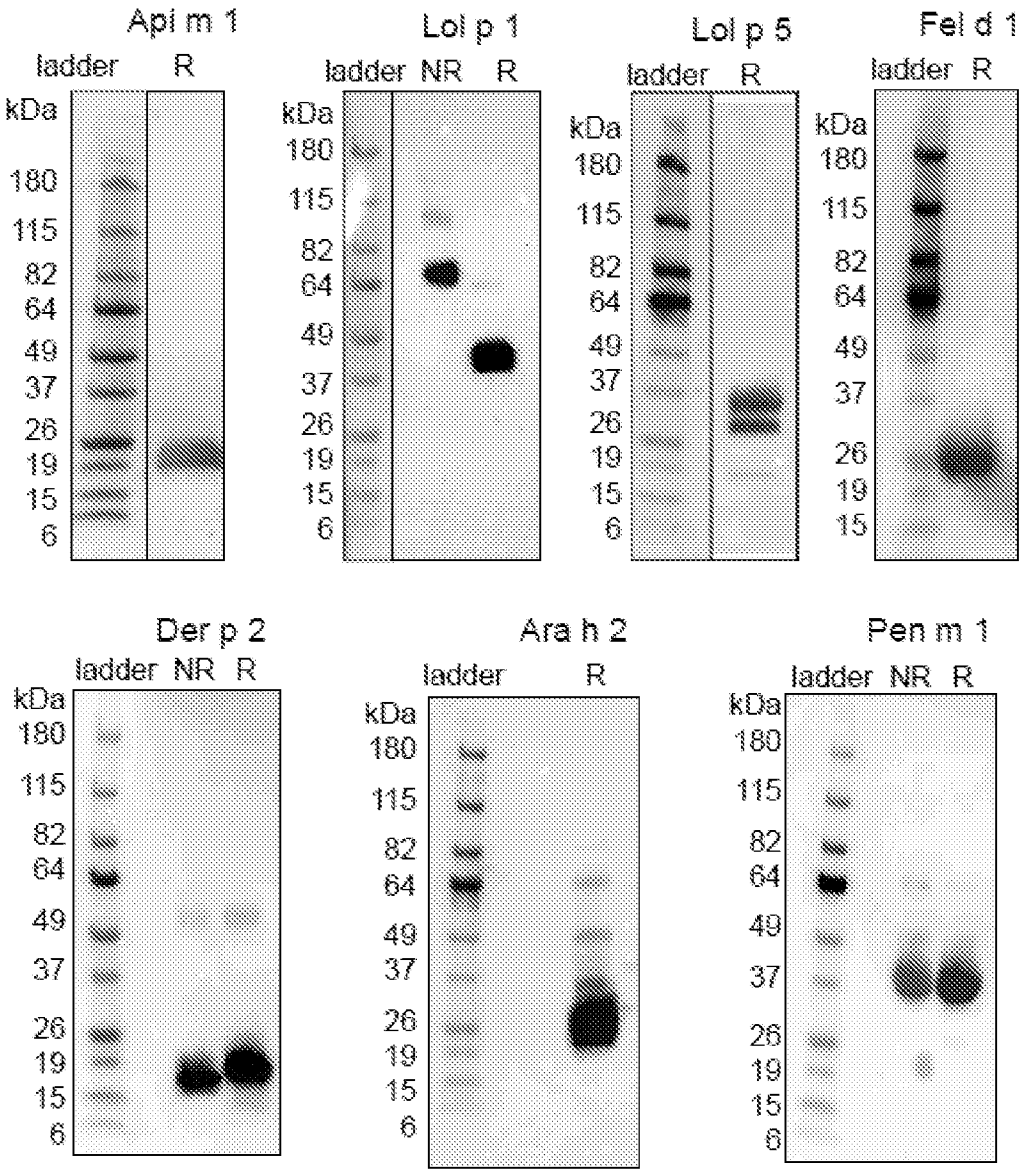

Api m 1
ladder R
kDa
180
115
82
64
49
37
26
19
15
6
Lol p 1
ladder NR R
Lol p 5
ladder R
Fel d 1
ladder R
Der p 2
ladder NR R
Ara h 2
ladder R
Pen m 1
ladder NR R

FIGURE 6

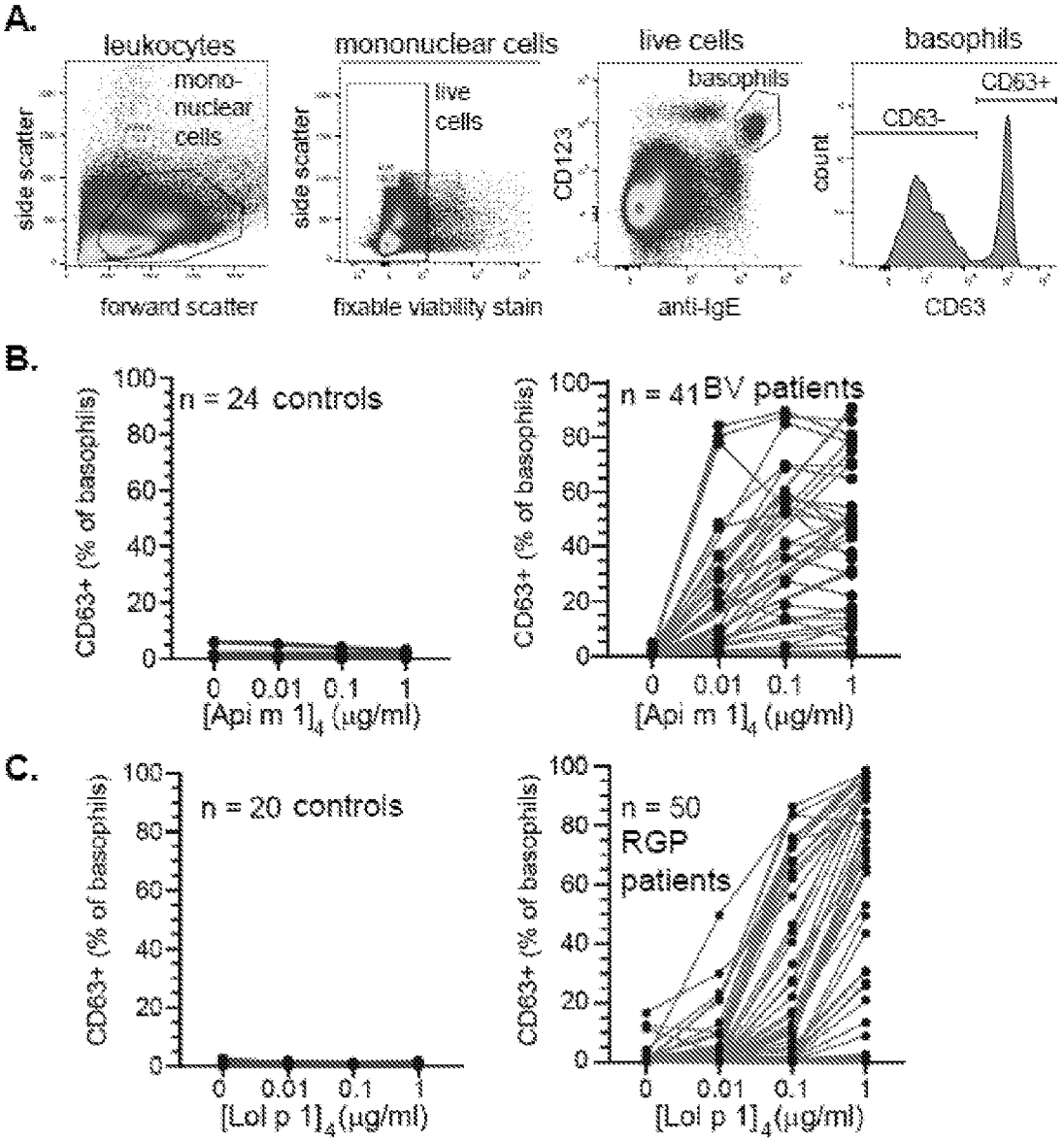

A.
leukocytes
mono-
nuclear
cells
side scatter
forward scatter
mononuclear cells
live
cells
side scatter
fixable viability stain
live cells
basophils
CD123
anti-IgE
basophils
CD63+
CD63-
count
CD63
B.
n = 24 controls
CD63+ (% of basophils)
[Api m 1]4 (µg/ml)
n = 41 BV patients
CD63+ (% of basophils)
[Api m 1]4 (µg/ml)
C.
n = 20 controls
CD63+ (% of basophils)
[Lol p 1]4 (µg/ml)
n = 50
RGP
patients
CD63+ (% of basophils)
[Lol p 1]4 (µg/ml)

FIGURE 7

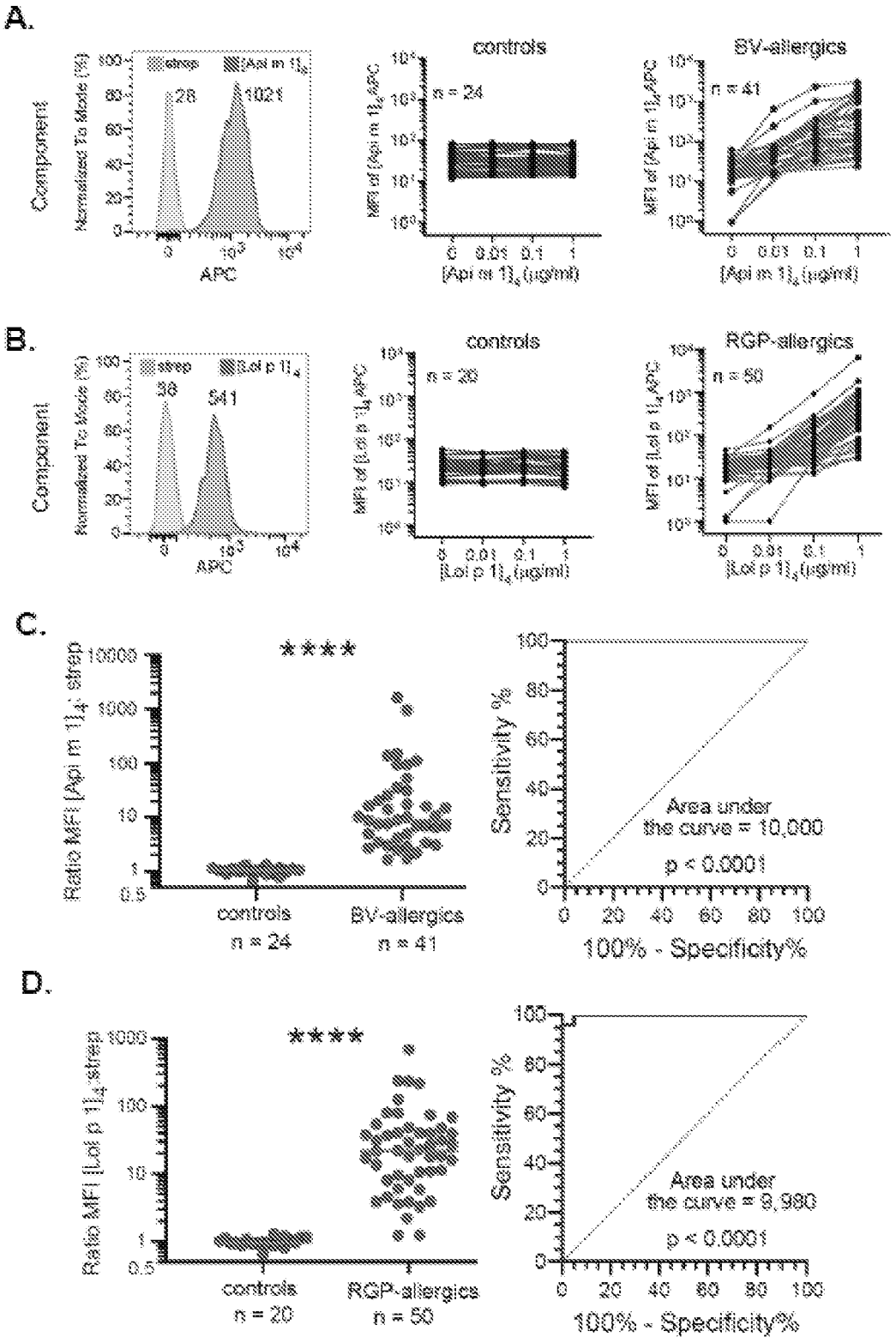

A.
Component
Normalised To Mode (%)
strep
[Api m 1]4
28
1021
APC
controls
n = 24
MFI of [Api m 1]4 APC
[Api m 1]4 (µg/ml)
SV-allergics
n = 41
B.
Component
Normalised To Mode (%)
strep
[Lol p 1]4
38
541
APC
controls
n = 20
MFI of [Lol p 1]4 APC
[Lol p 1]4 (µg/ml)
RGP-allergics
n = 50
C.
Ratio MFI [Api m 1]4: strep
****
controls
n = 24
SV-allergics
n = 41
Sensitivity %
Area under
the curve = 10,000
p < 0.0001
100% - Specificity%
D.
Ratio MFI [Lol p 1]4:strep
****
controls
n = 20
RGP-allergics
n = 50
Sensitivity %
Area under
the curve = 9,980
p < 0.0001
100% - Specificity%

FIGURE 8

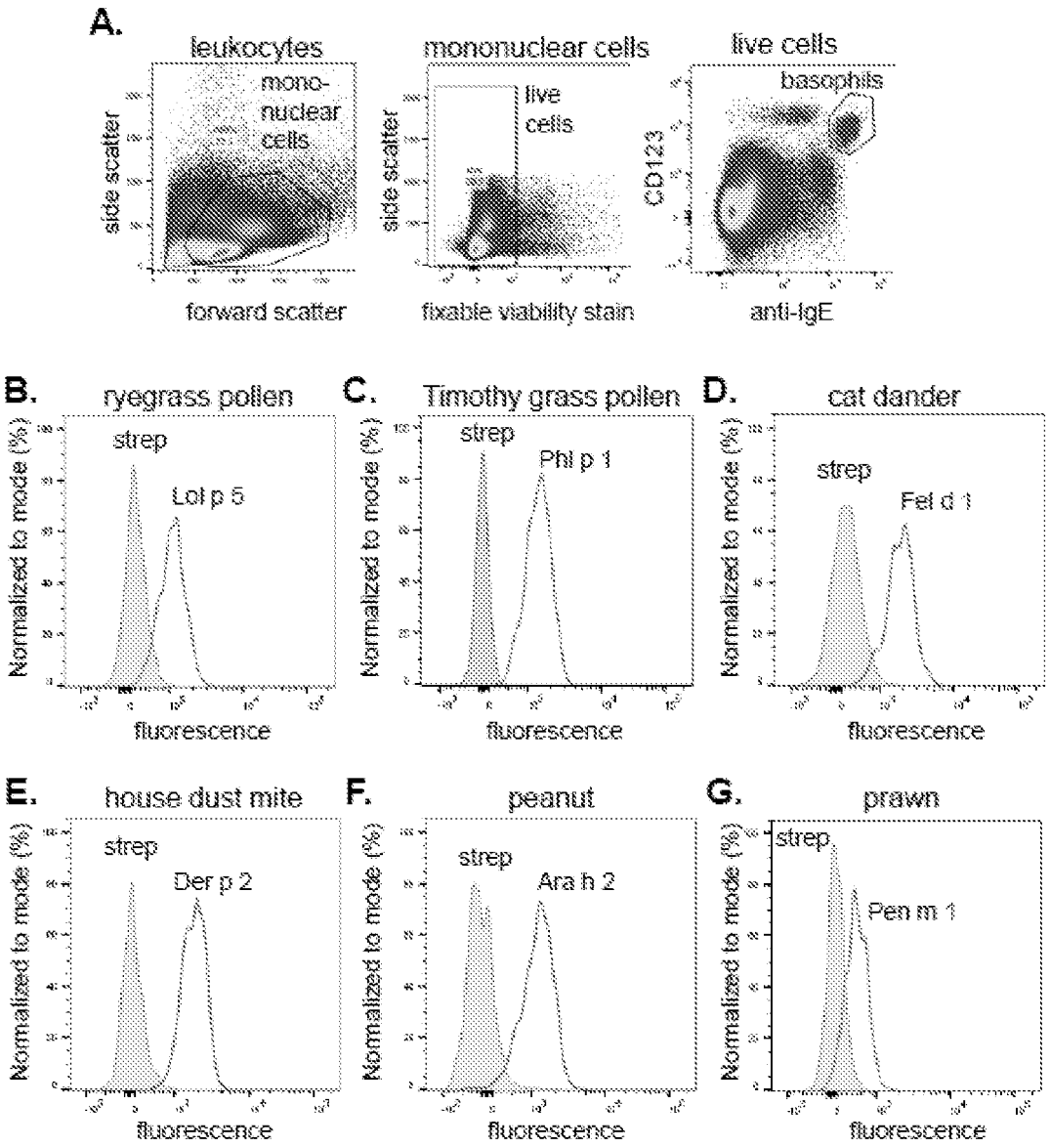

A.
leukocytes
mono-
nuclear
cells
side scatter
forward scatter
mononuclear cells
live
cells
side scatter
fixable viability stain
live cells
basophils
CD123
anti-IgE
B.
ryegrass pollen
strep
Lol p 5
Normalized to mode (%)
fluorescence
C.
Timothy grass pollen
strep
Phl p 1
Normalized to mode (%)
fluorescence
D.
cat dander
strep
Fel d 1
Normalized to mode (%)
fluorescence
E.
house dust mite
strep
Der p 2
Normalized to mode (%)
fluorescence
F.
peanut
strep
Ara h 2
Normalized to mode (%)
fluorescence
G.
prawn
strep
Pen m 1
Normalized to mode (%)
fluorescence

FIGURE 9

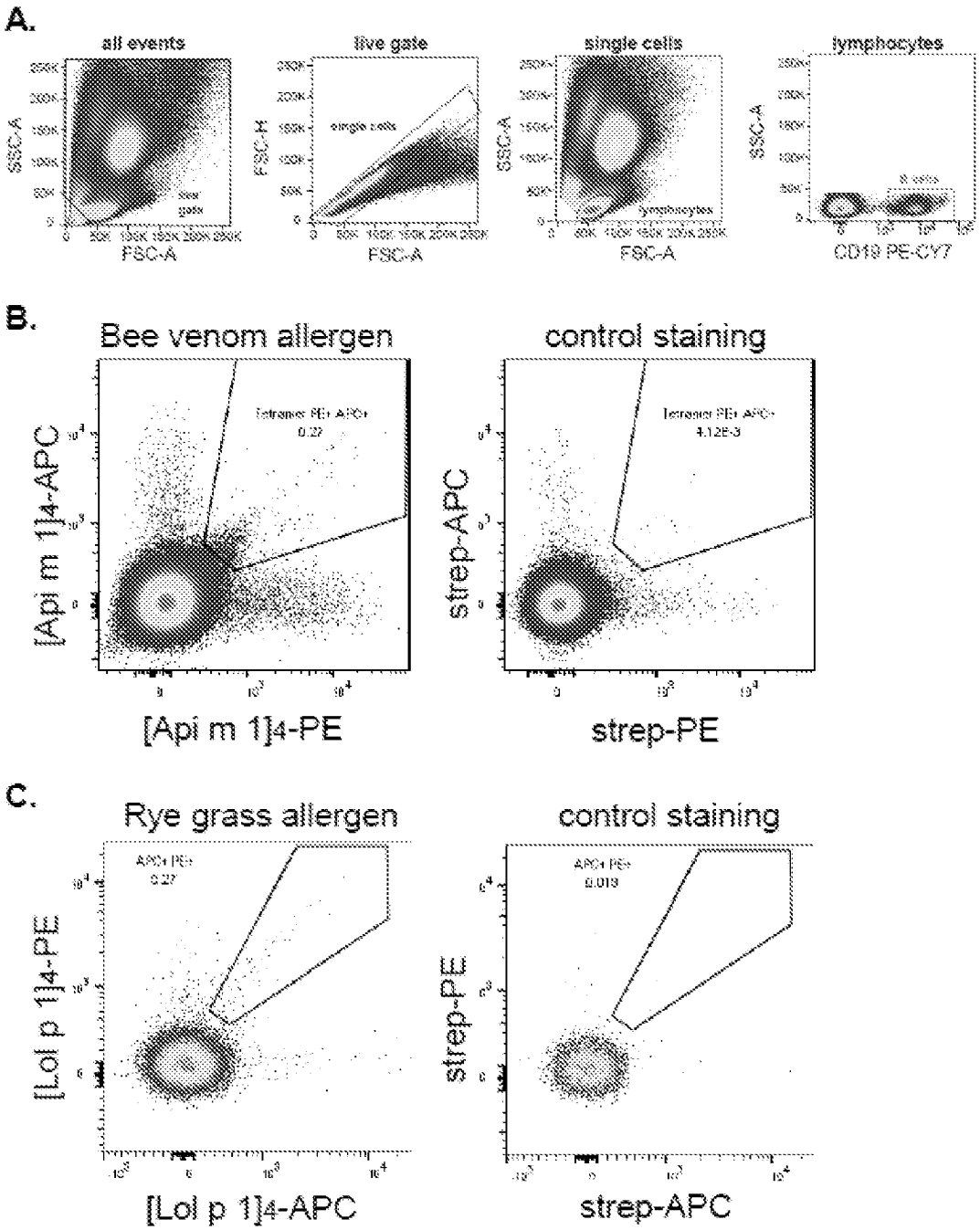

A.
all events
live gate
single cells
lymphocytes
SSC-A
FSC-A
FSC-H
CD19 PE-CY7
B.
Bee venom allergen
control staining
[Api m 1]4-APC
[Api m 1]4-PE
strep-APC
strep-PE
C.
Rye grass allergen
control staining
[Lol p 1]4-PE
[Lol p 1]4-APC
strep-PE
strep-APC

FIGURE 10

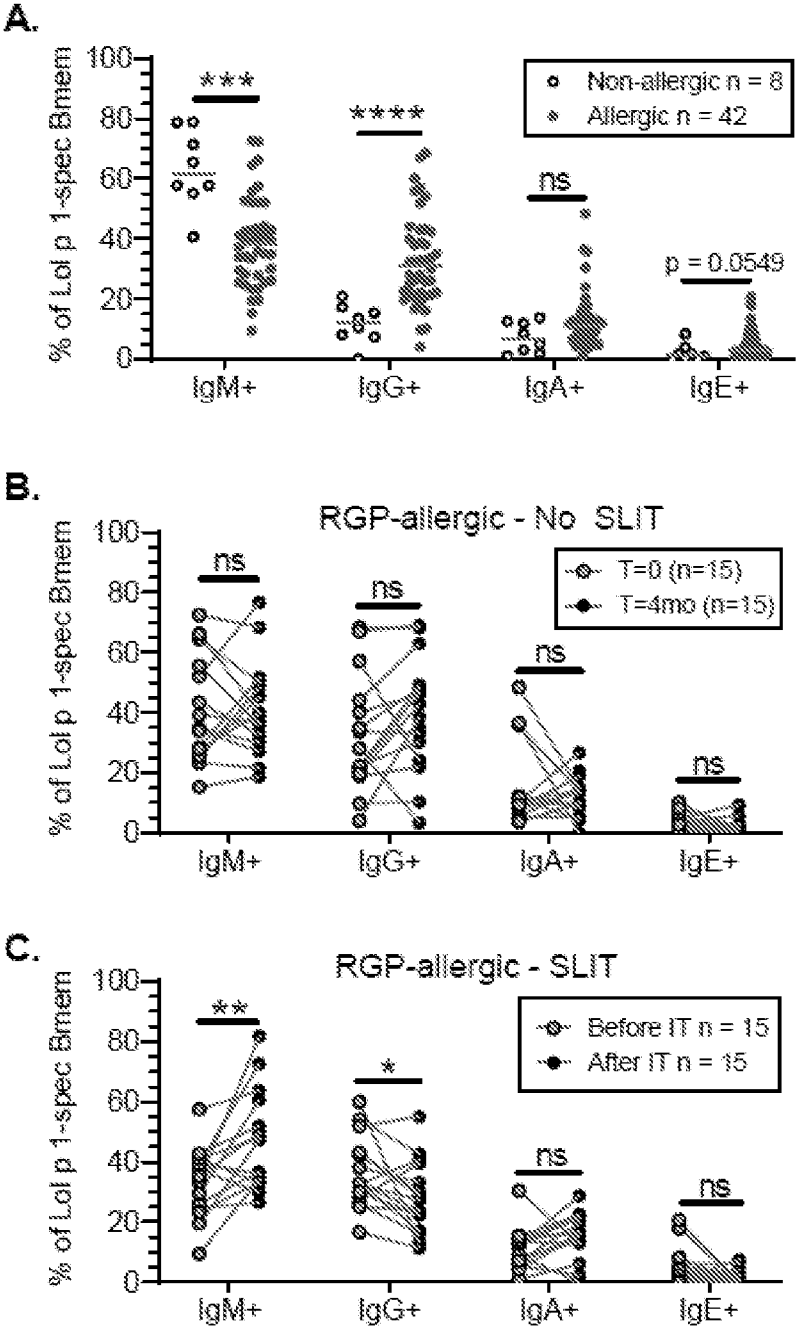

A.
% of Lol p 1-spec Bmem
100
80
60
40
20
0
***
****
ns
p = 0.0549
Non-allergic n = 8
Allergic n = 42
IgM+
IgG+
IgA+
IgE+
B.
RGP-allergic - No SLIT
T=0 (n=15)
T=4mo (n=15)
ns
C.
RGP-allergic - SLIT
Before IT n = 15
After IT n = 15
**
*

FIGURE 11

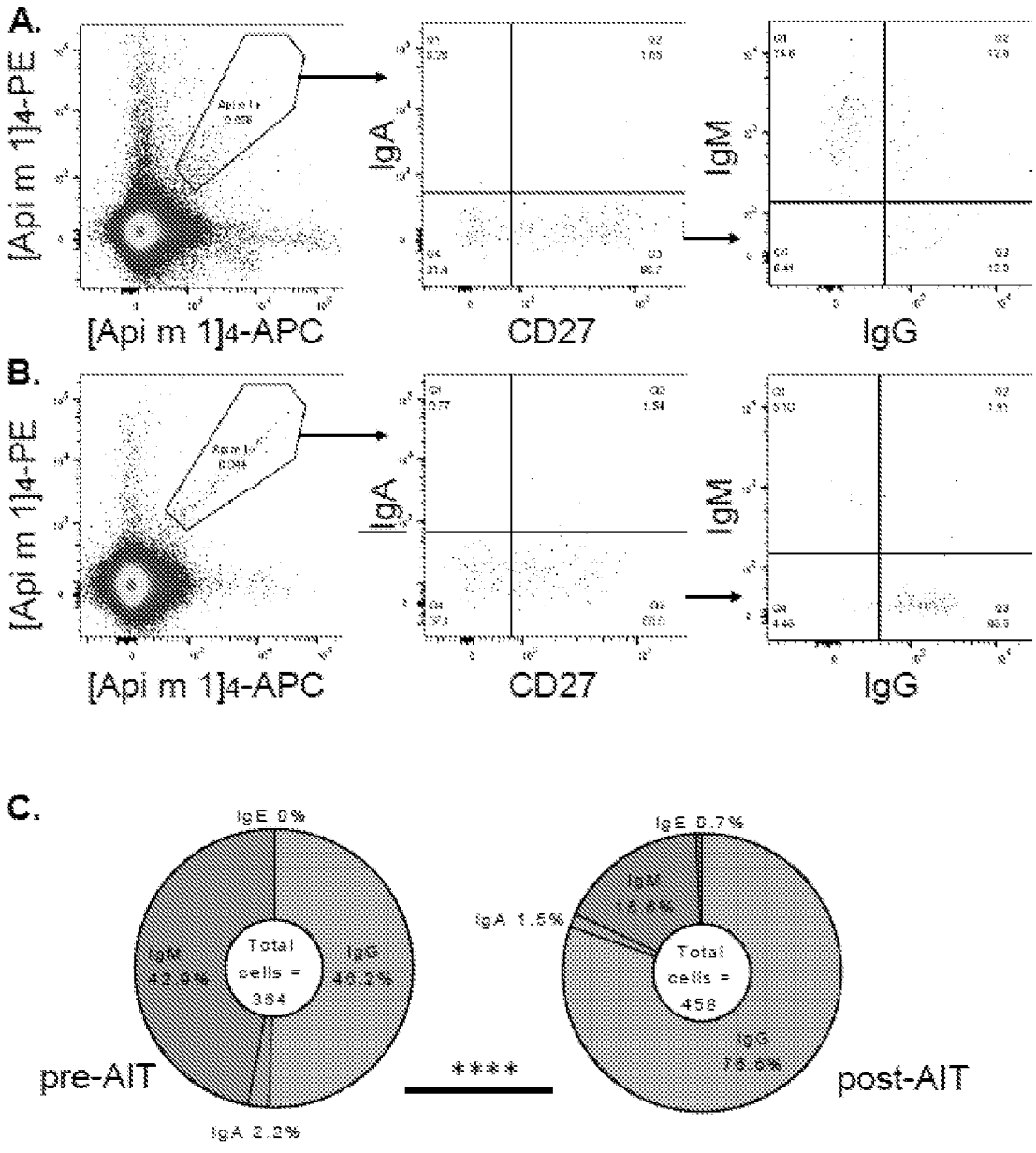
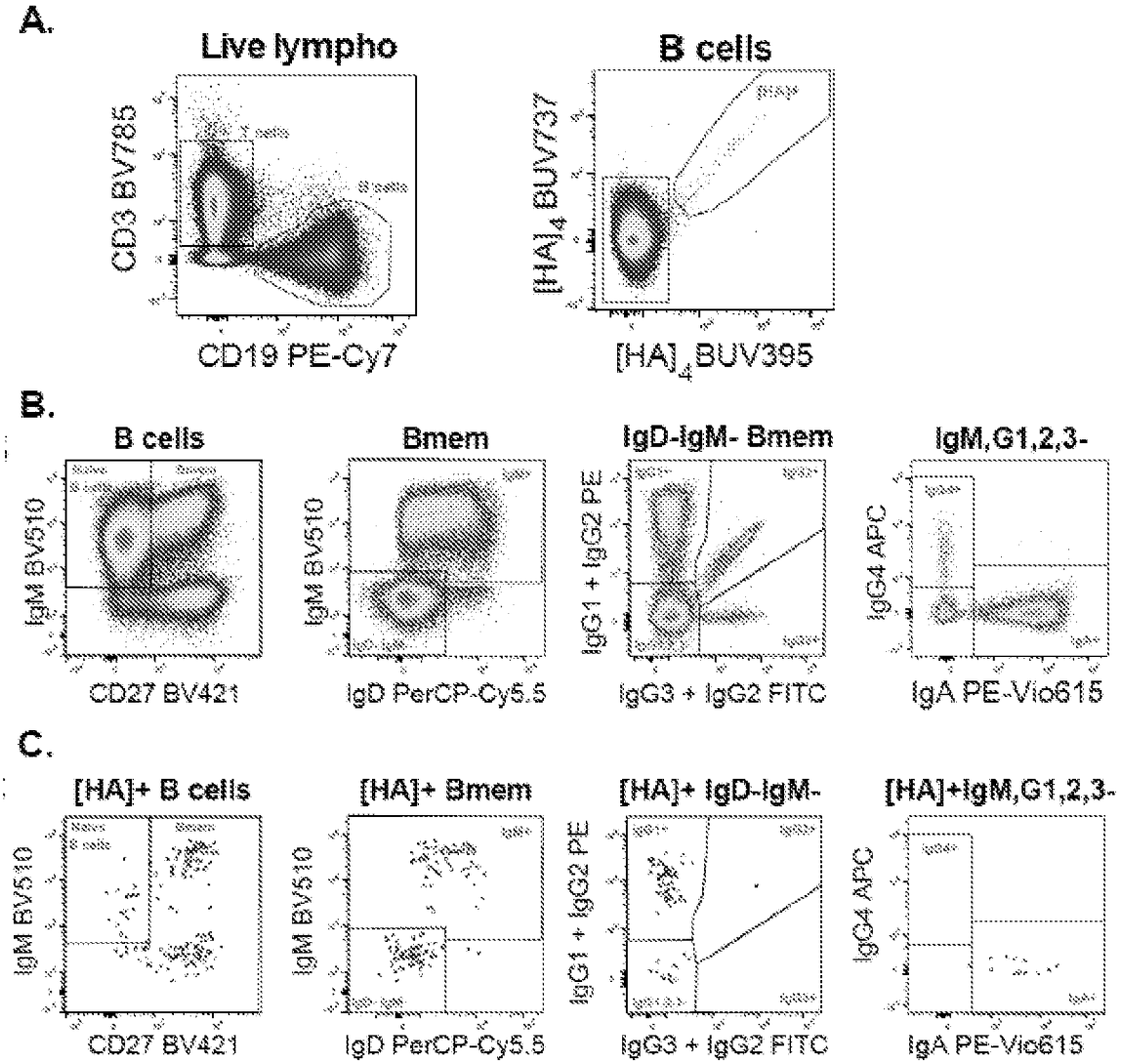
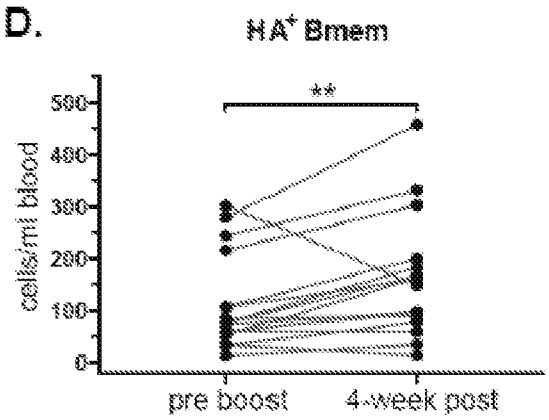

A.
[Api m 1]4-PE
[Api m 1]4-APC
IgA
CD27
IgM
IgG
B.
[Api m 1]4-PE
[Api m 1]4-APC
IgA
CD27
IgM
IgG
C.
IgE 0%
IgM 43.4%
Total cells = 364
IgG 46.2%
IgA 2.2%
pre-AIT
****
IgE 5.7%
IgA 1.5%
IgM 14.4%
Total cells = 456
IgG 78.8%
post-AIT

FIGURE 12


A.
Live lympho
CD3 BV785
CD19 PE-Cy7
B cells
[HA]4 BUV737
[HA]4 BUV395
B.
B cells
IgM BV510
CD27 BV421
Bmem
IgM BV510
IgD PerCP-Cy5.5
IgD-IgM- Bmem
IgG1 + IgG2 PE
IgG3 + IgG2 FITC
IgM,G1,2,3-
IgG4 APC
IgA PE-Vio615
C.
[HA]+ B cells
IgM BV510
CD27 BV421
[HA]+ Bmem
IgM BV510
IgD PerCP-Cy5.5
[HA]+ IgD-IgM-
IgG1 + IgG2 PE
IgG3 + IgG2 FITC
[HA]+IgM,G1,2,3-
IgG4 APC
IgA PE-Vio615


D.
HA+ Bmem
**
cells/ml blood
500
400
300
200
100
0
pre boost
4-week post

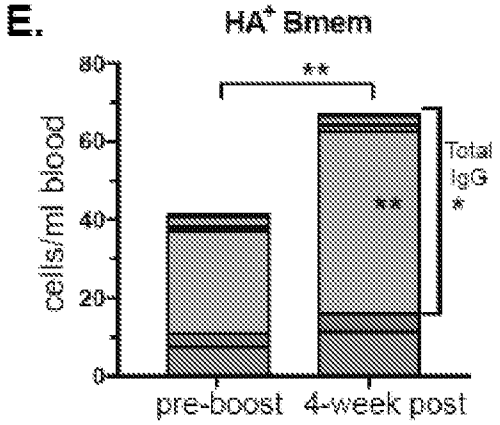

E.
HA+ Bmem
**
cells/ml blood
80
60
40
20
0
**
Total IgG
*
pre-boost
4-week post

FIGURE 13

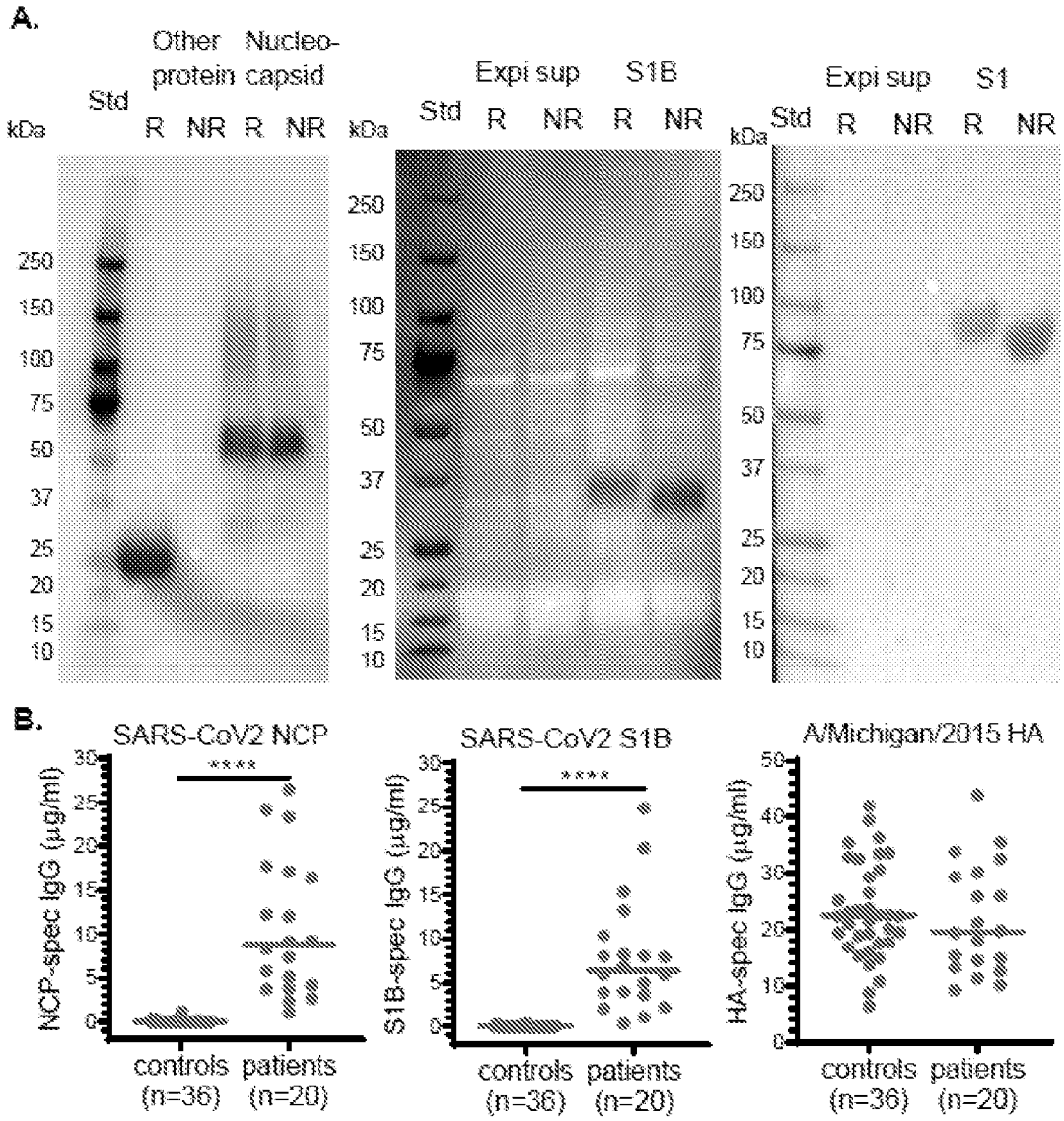

A.
Other protein
Nucleo-capsid
Expi sup
S1B
Expi sup
S1
Std
R
NR
kDa
250
150
100
75
50
37
25
20
15
10
B.
SARS-CoV2 NCP
SARS-CoV2 S1B
A/Michigan/2015 HA
NCP-spec IgG (µg/ml)
S1B-spec IgG (µg/ml)
HA-spec IgG (µg/ml)
****
controls (n=36)
patients (n=20)

FIGURE 14

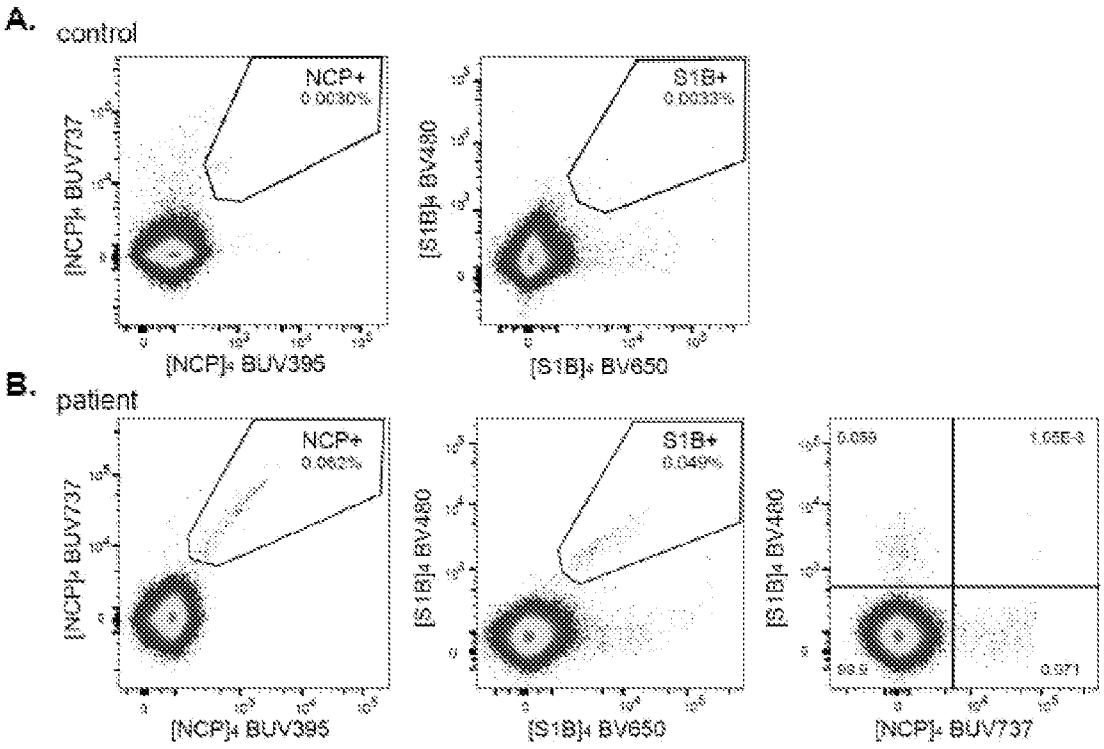
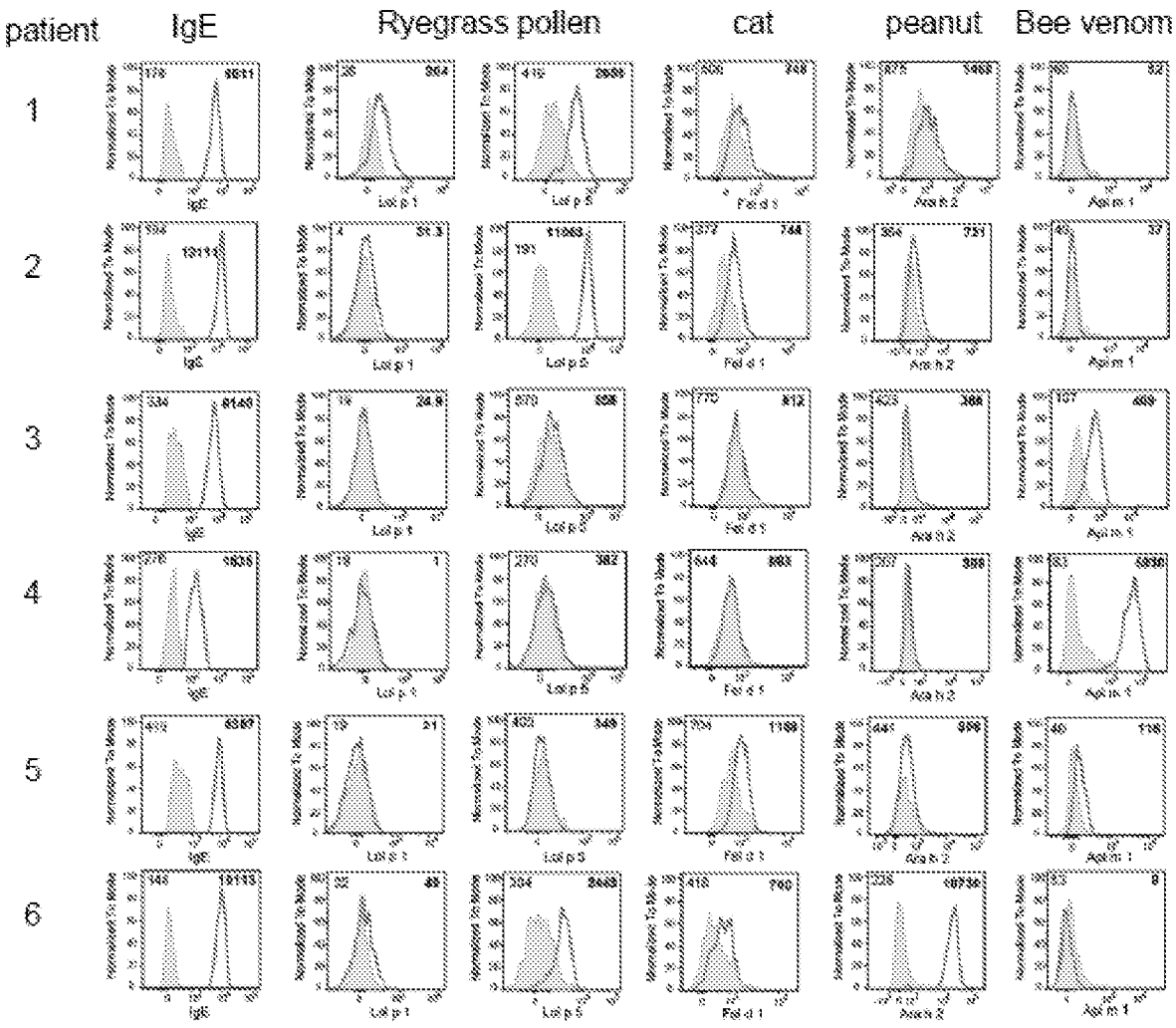

A.
control
NCP+
0.0030%
S1B+
0.0033%
[NCP]4 BUV737
[NCP]4 BUV395
[S1B]4 BV480
[S1B]4 BV650
B.
patient
NCP+
0.062%
S1B+
0.049%
0.059
1.05E-3
99.9
0.071
[NCP]4 BUV737
[NCP]4 BUV395
[S1B]4 BV480
[S1B]4 BV650

FIGURE 15

A

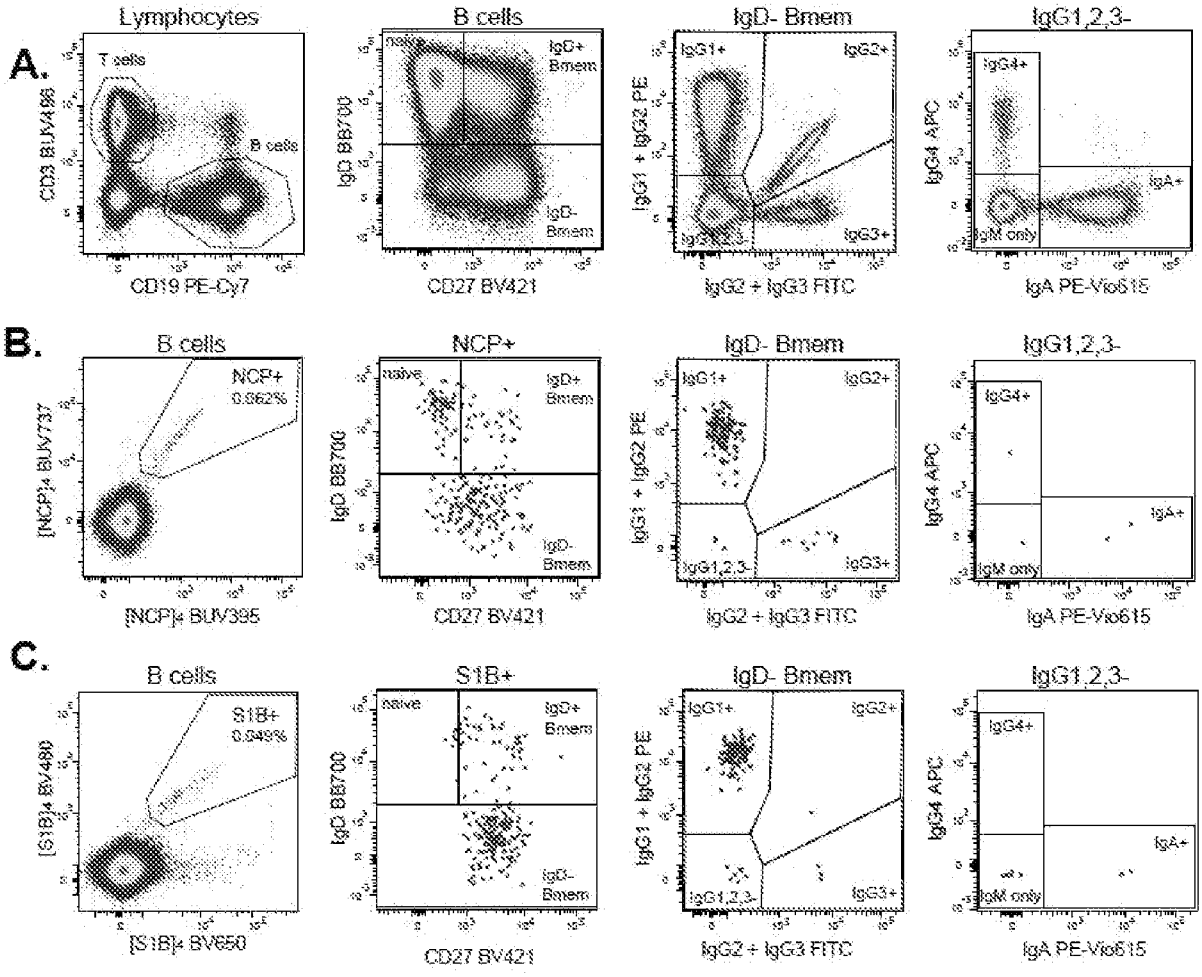
fluorochrome	BV421	BV480	BV605	BV711	FITC	PE	APC
mAb/antigen	Ara h 2	Fel d 1	CD123	Lol p 5	IgE	Api m 1	Lol p 1

B



SUBSTITUTE SHEET
(Rule 26) RO/AU

FIGURE 16


A.
Lymphocytes
T cells
B cells
CD3 BUV496
CD19 PE-Cy7
B cells
naive
IgD+ Bmem
IgD- Bmem
IgD BB700
CD27 BV421
IgD- Bmem
IgG1+
IgG2+
IgG3+
IgG1,2,3-
IgG1 + IgG2 PE
IgG2 + IgG3 FITC
IgG1,2,3-
IgG4+
IgA+
IgM only
IgG4 APC
IgA PE-Vio615
B.
B cells
NCP+
0.862%
[NCP]+ BUV737
[NCP]+ BUV395
NCP+
CD27 BV421
C.
B cells
S1B+
0.049%
[S1B]+ BV480
[S1B]+ BV650
S1B+
CD27 BV421

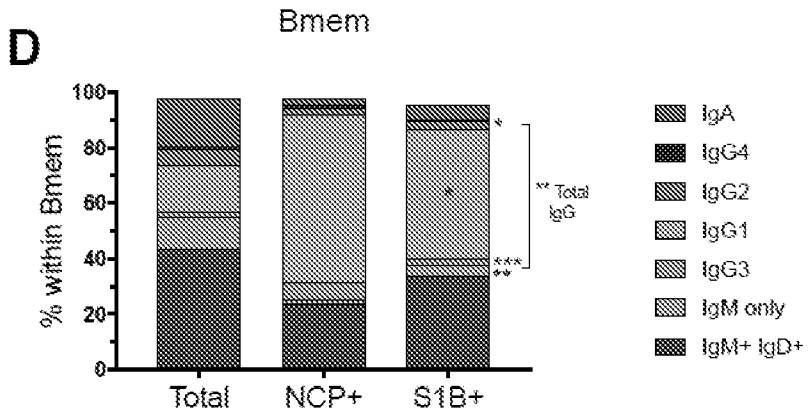
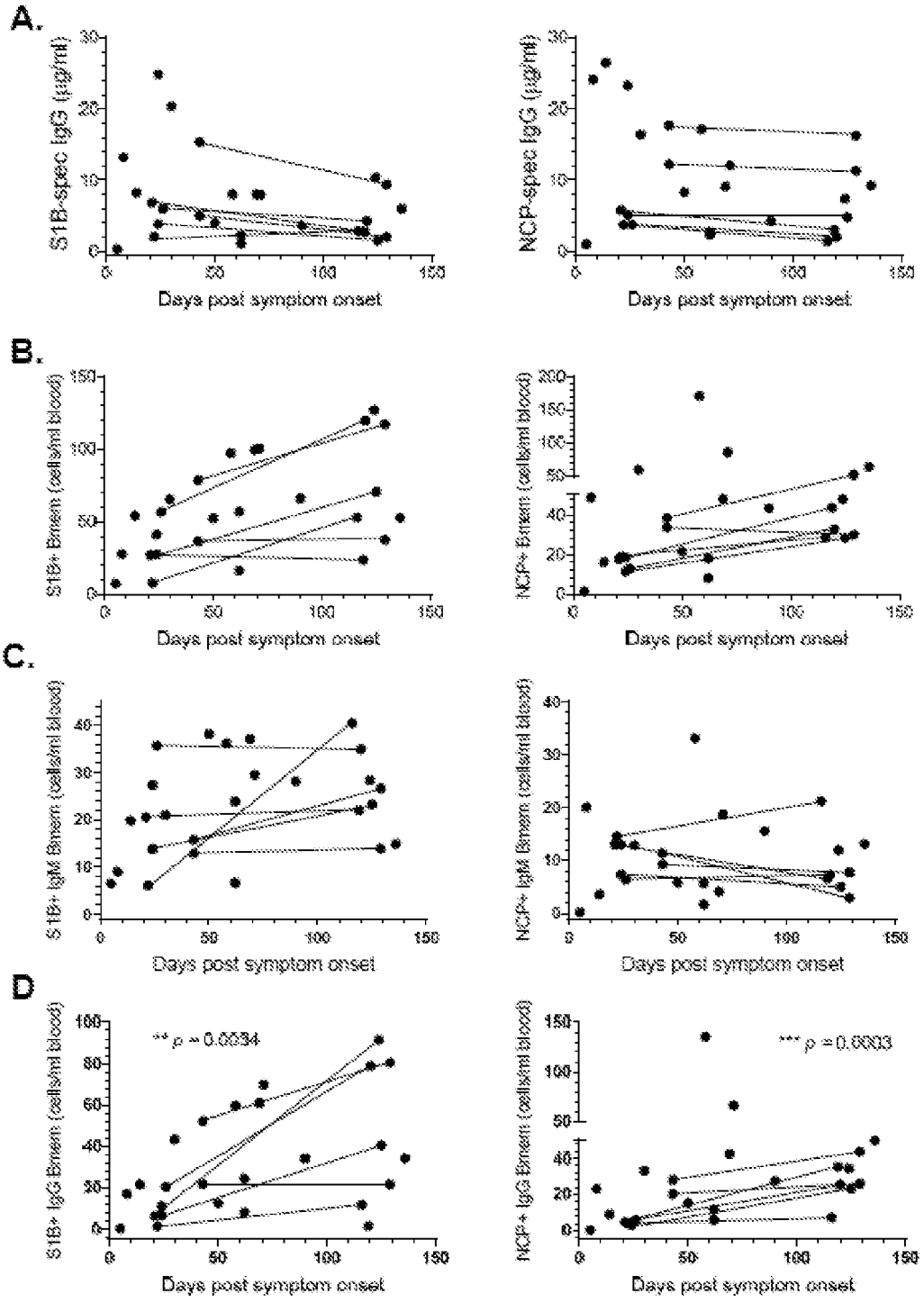

D
Bmem
% within Bmem
100
80
60
40
20
0
Total
NCP+
S1B+
** Total IgG
IgA
IgG4
IgG2
IgG1
IgG3
IgM only
IgM+ IgD+

FIGURE 17


A.
S1B-spec IgG (µg/ml)
NCP-spec IgG (µg/ml)
Days post symptom onset
B.
S1B+ Bmem (cells/ml blood)
NCP+ Bmem (cells/ml blood)
Days post symptom onset
C.
S1B+ IgM Bmem (cells/ml blood)
NCP+ IgM Bmem (cells/ml blood)
Days post symptom onset
D.
S1B+ IgG Bmem (cells/ml blood)
** p = 0.0034
NCP+ IgG Bmem (cells/ml blood)
*** p = 0.0003
Days post symptom onset