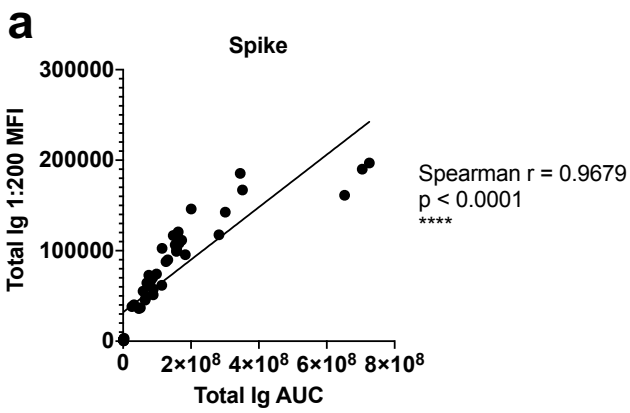
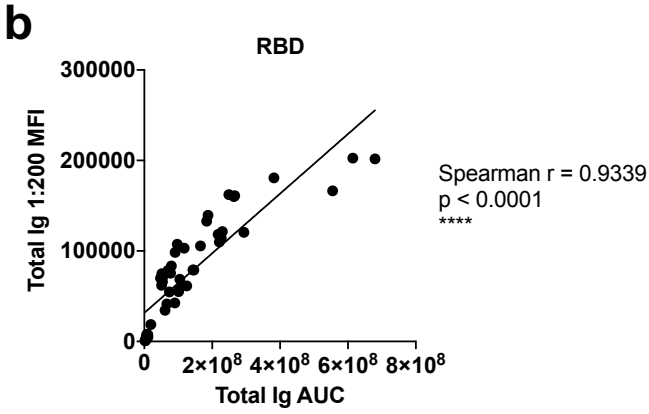
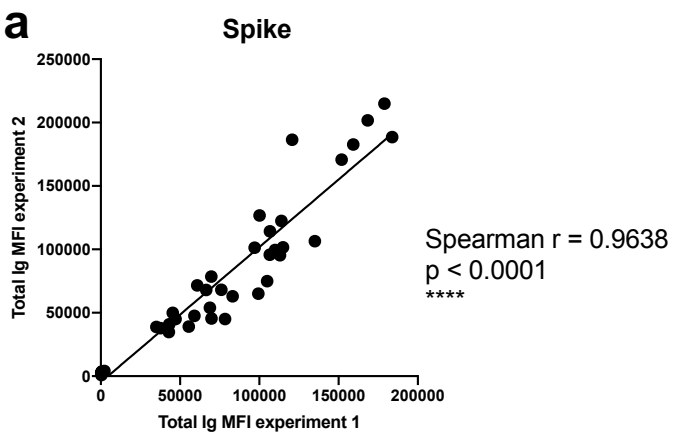
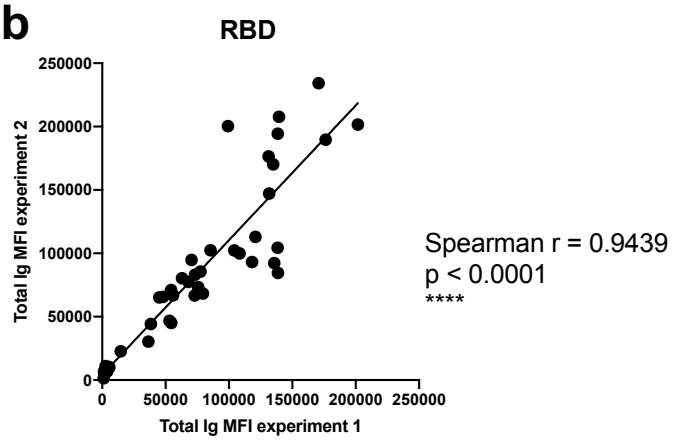

a
Spike
Total Ig 1:200 MFI
300000
200000
100000
0
0
2×10^8 4×10^8 6×10^8 8×10^8
Total Ig AUC
Spearman r = 0.9679
p < 0.0001
****

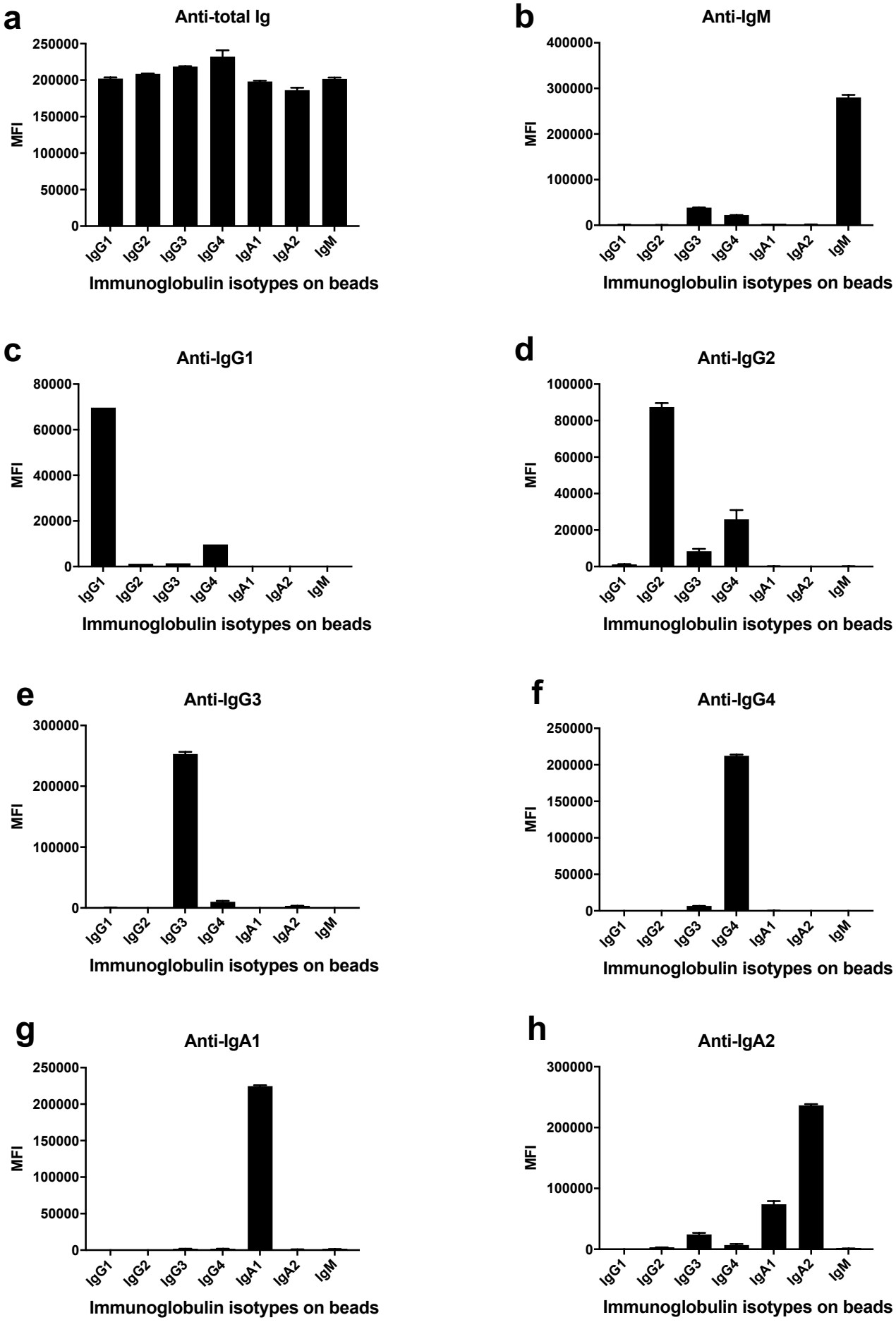

b
RBD
Total Ig 1:200 MFI
300000
200000
100000
0
0
2×10^8 4×10^8 6×10^8 8×10^8
Total Ig AUC
Spearman r = 0.9339
p < 0.0001
****

Supplementary Fig. 1. Spearman correlations of the area under the curves (AUCs) of (a) spike- or (b) RBD-specific total Ig versus total Ig MFI values at a 1:200 dilution.

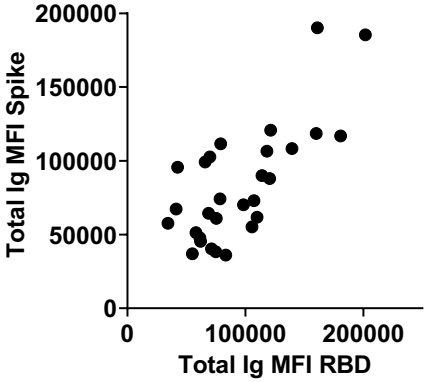

a
Spike
Total Ig MFI experiment 2
Total Ig MFI experiment 1
0
50000
100000
150000
200000
250000
Spearman r = 0.9638
p < 0.0001
****


b
RBD
Total Ig MFI experiment 2
Total Ig MFI experiment 1
0
50000
100000
150000
200000
250000
Spearman r = 0.9439
p < 0.0001
****

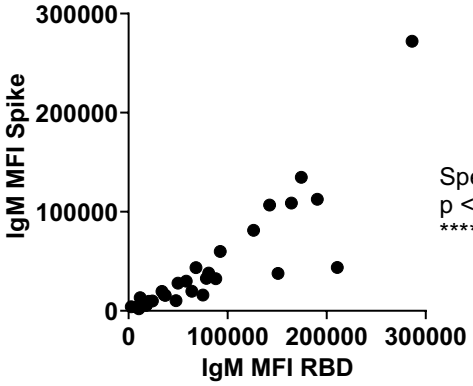
Supplementary Fig. 2. Spearman correlations between (a) spike-specific or (b) RBD-specific total Ig MFI values from two independent experiments to show the degree of assay reproducibility.


a
Anti-total Ig
MFI
250000
200000
150000
100000
50000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
b
Anti-IgM
MFI
400000
300000
200000
100000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
c
Anti-IgG1
MFI
80000
60000
40000
20000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
d
Anti-IgG2
MFI
100000
80000
60000
40000
20000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
e
Anti-IgG3
MFI
300000
200000
100000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
f
Anti-IgG4
MFI
250000
200000
150000
100000
50000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
g
Anti-IgA1
MFI
250000
200000
150000
100000
50000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads
h
Anti-IgA2
MFI
300000
200000
100000
0
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
IgM
Immunoglobulin isotypes on beads

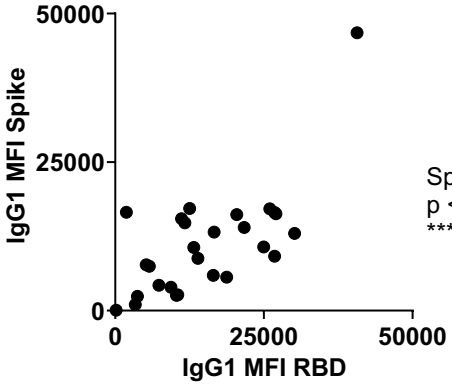
Supplementary Fig. 3. Isotyping validation was performed by coating Luminex beads with IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM myeloma proteins and detecting each specifically with eight different secondary Abs against (a) total Ig, (b) IgM, (c) IgG1, (d) IgG2, (e) IgG3, (f) IgG4, (g) IgA1 and (h) IgA2. The data are shown as mean MFI + SD of duplicate.



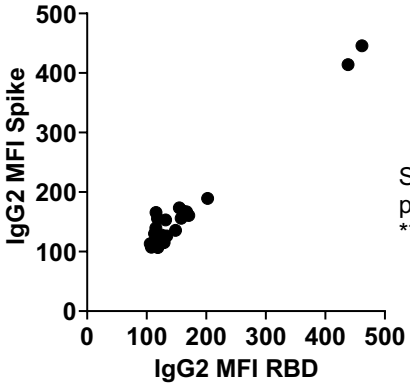
Spearman r = 0.6419
p = 0.0002
***



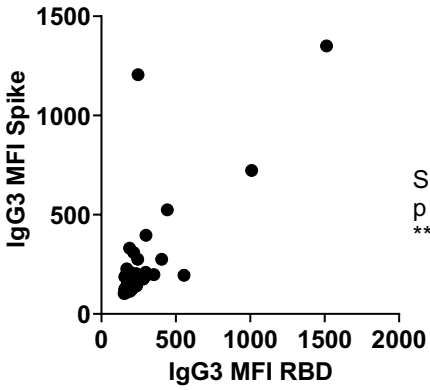
Spearman r = 0.9429
p < 0.0001
****



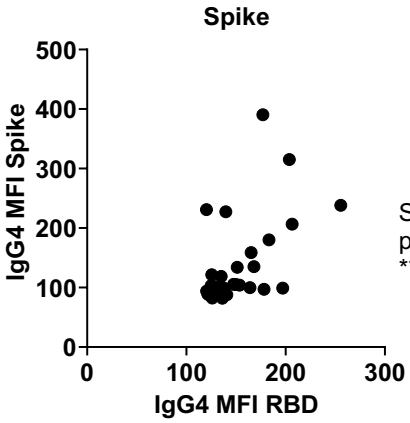
Spearman r = 0.6754
p < 0.0001
****



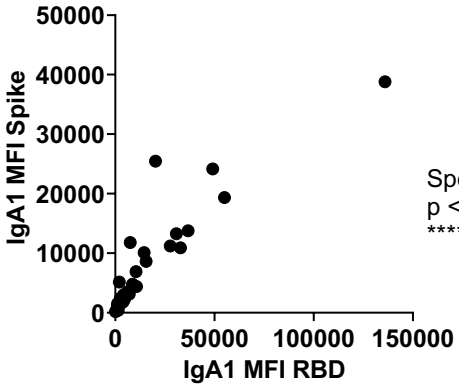
Spearman r = 0.6899
p < 0.0001
****



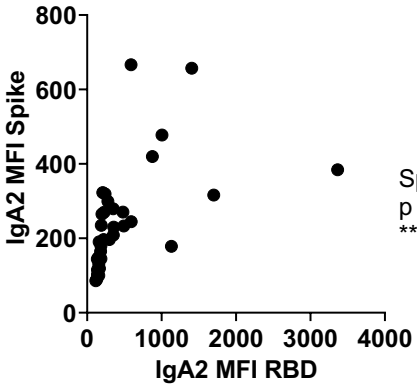
Spearman r = 0.6453
p < 0.0001
****



Spearman r = 0.5546
p = 0.0004
***

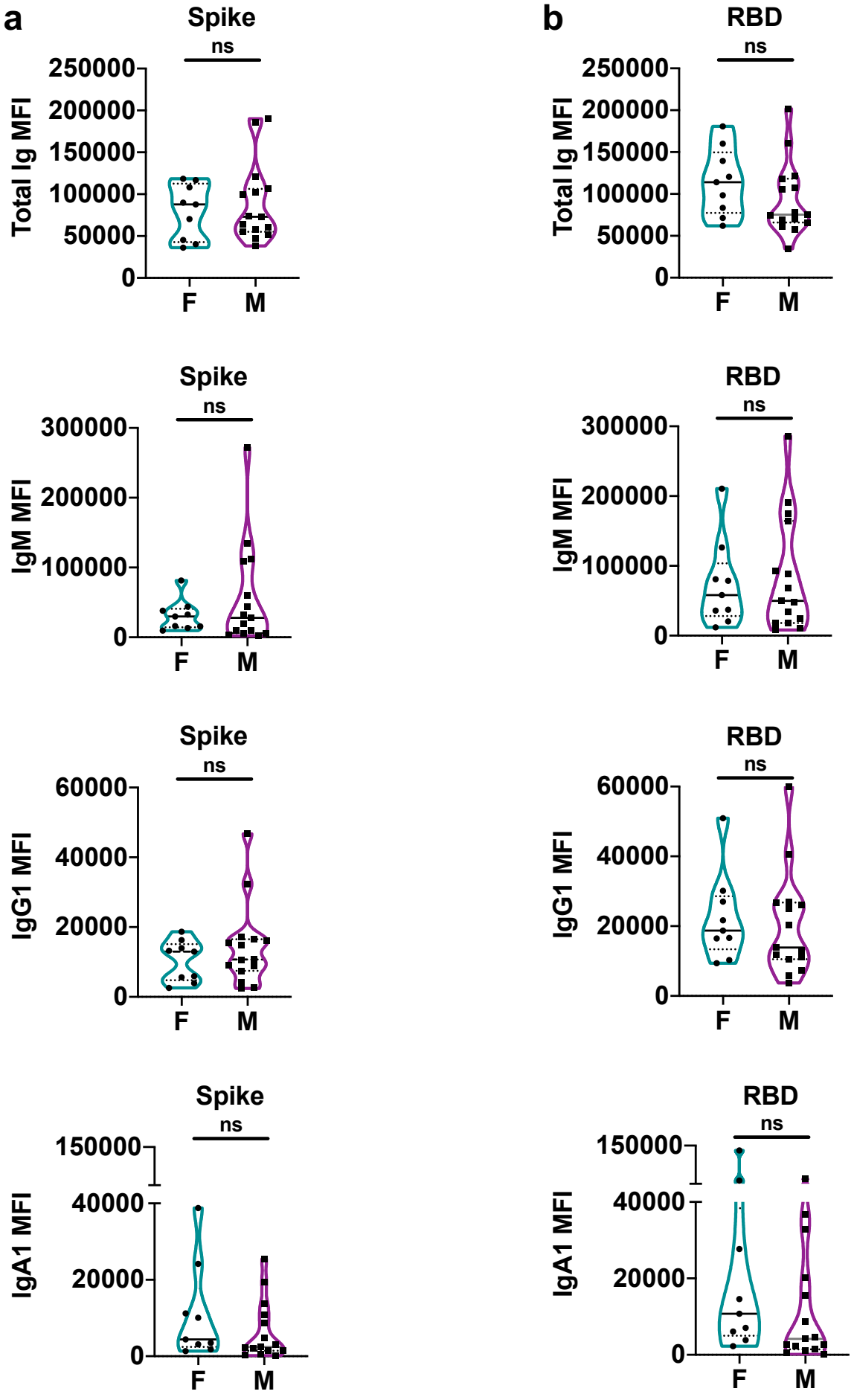


Spearman r = 0.9251
p < 0.0001
****

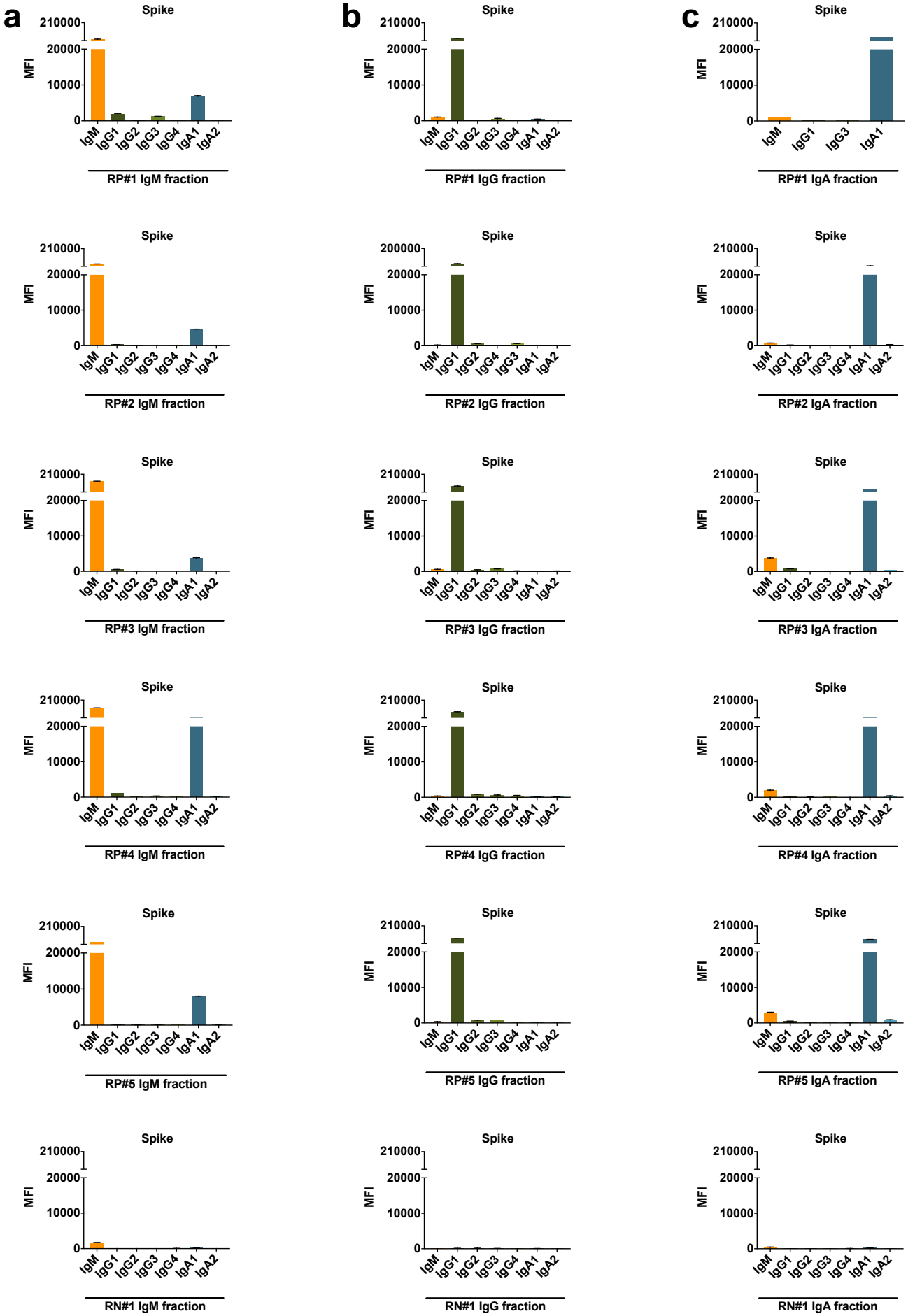


Spearman r = 0.8219
p < 0.0001
****

Supplementary Fig. 4. Spearman correlations between spike-specific versus RBD-specific total Ig, IgM, IgG1, IgG2, IgG3, IgG4, IgA1, or IgA2 MFI values.


a
Spike
ns
Total Ig MFI
250000
200000
150000
100000
50000
0
F
M
b
RBD
ns
Total Ig MFI
250000
200000
150000
100000
50000
0
F
M
Spike
ns
IgM MFI
300000
200000
100000
0
F
M
RBD
ns
IgM MFI
300000
200000
100000
0
F
M
Spike
ns
IgG1 MFI
60000
40000
20000
0
F
M
RBD
ns
IgG1 MFI
60000
40000
20000
0
F
M
Spike
ns
IgA1 MFI
150000
40000
20000
0
F
M
RBD
ns
IgA1 MFI
150000
40000
20000
0
F
M

Supplementary Fig. 5. Violin plots of (a) spike-specific or (b) RBD-specific total Ig, IgM, IgG1, and IgA1 levels from nine COVID-19 convalescent female (F) and 15 male (M) subjects. The statistical significance was determined by a two-tailed Mann-Whitney test (ns: non-significant: $p > 0.05$).


a
b
c
Spike
MFI
210000
20000
10000
0
IgM
IgG1
IgG2
IgG3
IgG4
IgA1
IgA2
RP#1 IgM fraction
RP#1 IgG fraction
RP#1 IgA fraction
RP#2 IgM fraction
RP#2 IgG fraction
RP#2 IgA fraction
RP#3 IgM fraction
RP#3 IgG fraction
RP#3 IgA fraction
RP#4 IgM fraction
RP#4 IgG fraction
RP#4 IgA fraction
RP#5 IgM fraction
RP#5 IgG fraction
RP#5 IgA fraction
RN#1 IgM fraction
RN#1 IgG fraction
RN#1 IgA fraction

Supplementary Fig. 6. Enrichment of spike-specific IgM (a), IgG (b), and IgA in purified fractions from RP#1-5 and RN#1. Each fraction was measured for the presence of IgM, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 Abs using the isotyping method validated in Supplementary Fig. 3.