Supplemental information

Non-protective immune imprint underlies failure of *S. aureus* IsdB vaccine

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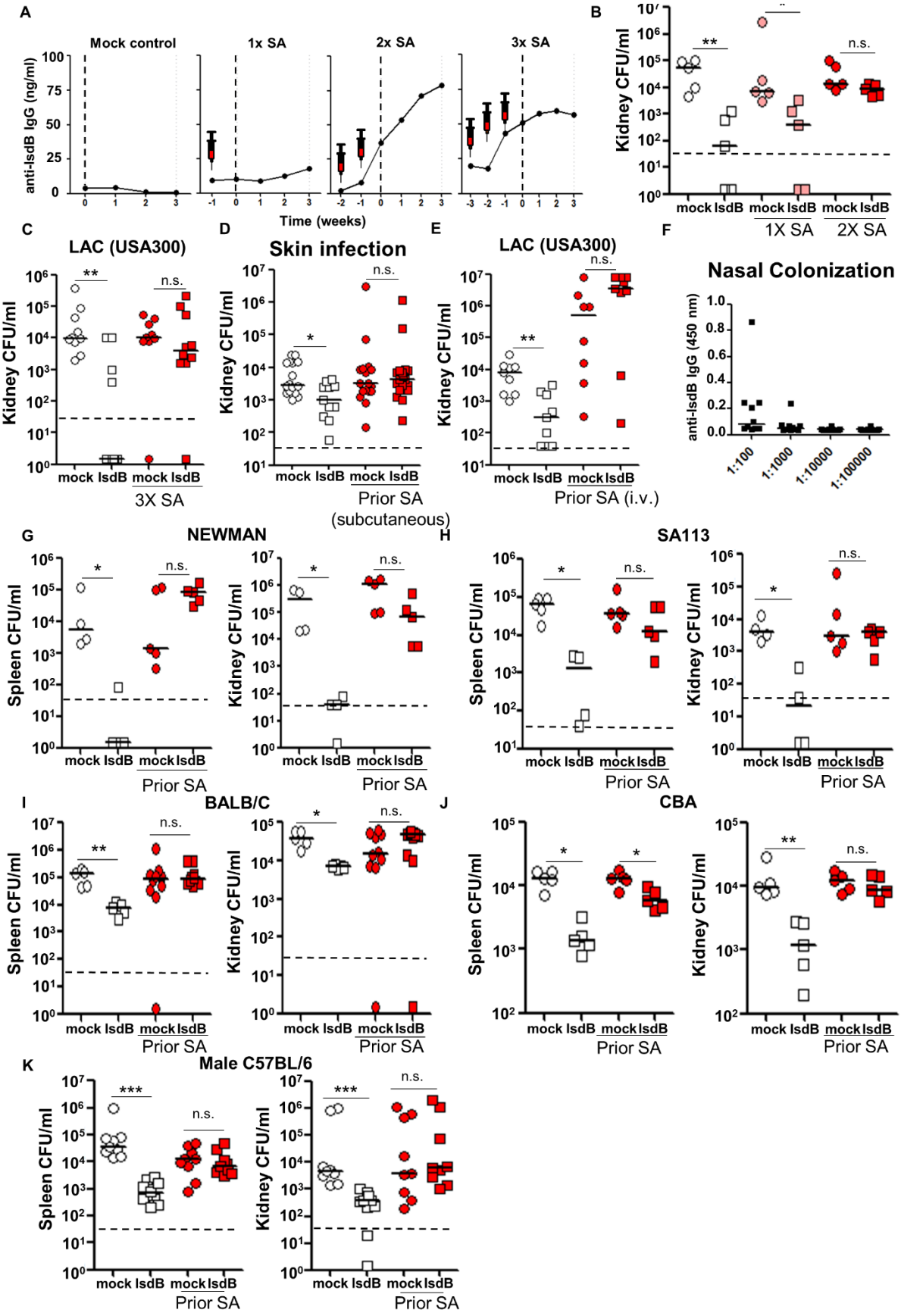

A
Mock control
1x SA
2x SA
3x SA
anti-IsdB IgG (ng/ml)
Time (weeks)
B
Kidney CFU/ml
mock IsdB mock IsdB mock IsdB
1X SA
2X SA
C
LAC (USA300)
Kidney CFU/ml
3X SA
D
Skin infection
Prior SA
(subcutaneous)
E
LAC (USA300)
Prior SA (i.v.)
F
Nasal Colonization
anti-IsdB IgG (450 nm)
1:100
1:1000
1:10000
1:100000
G
NEWMAN
Spleen CFU/ml
Kidney CFU/ml
Prior SA
H
SA113
I
BALB/C
J
CBA
K
Male C57BL/6
n.s.

Figure S1 IsdB immunization is not protective in mice previously infected with *S. aureus*, Related to Figure 1

(A) Serum IsdB-specific IgG levels in mice infected 1-3 times with SA (LAC) and bled at the indicated times.

(B) IsdB vaccination after 1 or 2 SA infections: Kidney SA burden from experiment Figure 1C (*n*=5 per mouse group).

(C) IsdB vaccination after 3 SA infections: Kidney SA burden from experiment Figure 1D (*n*=10 per mouse group).

(D) Kidney bacterial burden in mice infected subcutaneously (twice 2 weeks apart) with SA (LAC), IsdB immunized two weeks later and then LAC challenged i.p. per Figure 1A (*n*=11-19 per mouse group).

(E) Kidney bacterial burden in mice infected once i.v. with SA, treated for 5 days with vancomycin and then immunized and LAC challenged i.p. per Figure 1A (*n*=9 per mouse group).

(F) Serum anti-IsdB IgG titer after 3 weekly SA nasal applications (*n*=9 per mouse group).

(G and H) Tissue bacterial burden in (C57BL/6) mice infected with SA Newman or SA113, then immunized and challenged with the same SA strain as per Figure 1A (*n*=4-5 per mouse group).

(I and J) Tissue bacterial burden in BALB/c and CBA mice pre-infected with LAC, then immunized and LAC challenged as per Figure 1A (*n*=5 per mouse group).

(K) Tissue bacterial burden in male mice infected with LAC, then immunized and LAC challenged as per Figure 1A (*n*=9-10 per mouse group).

Each data point represents an individual mouse; bars denote median and dashed lines indicate the limit of detection. n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; one way ANOVA (B to E and G to K).

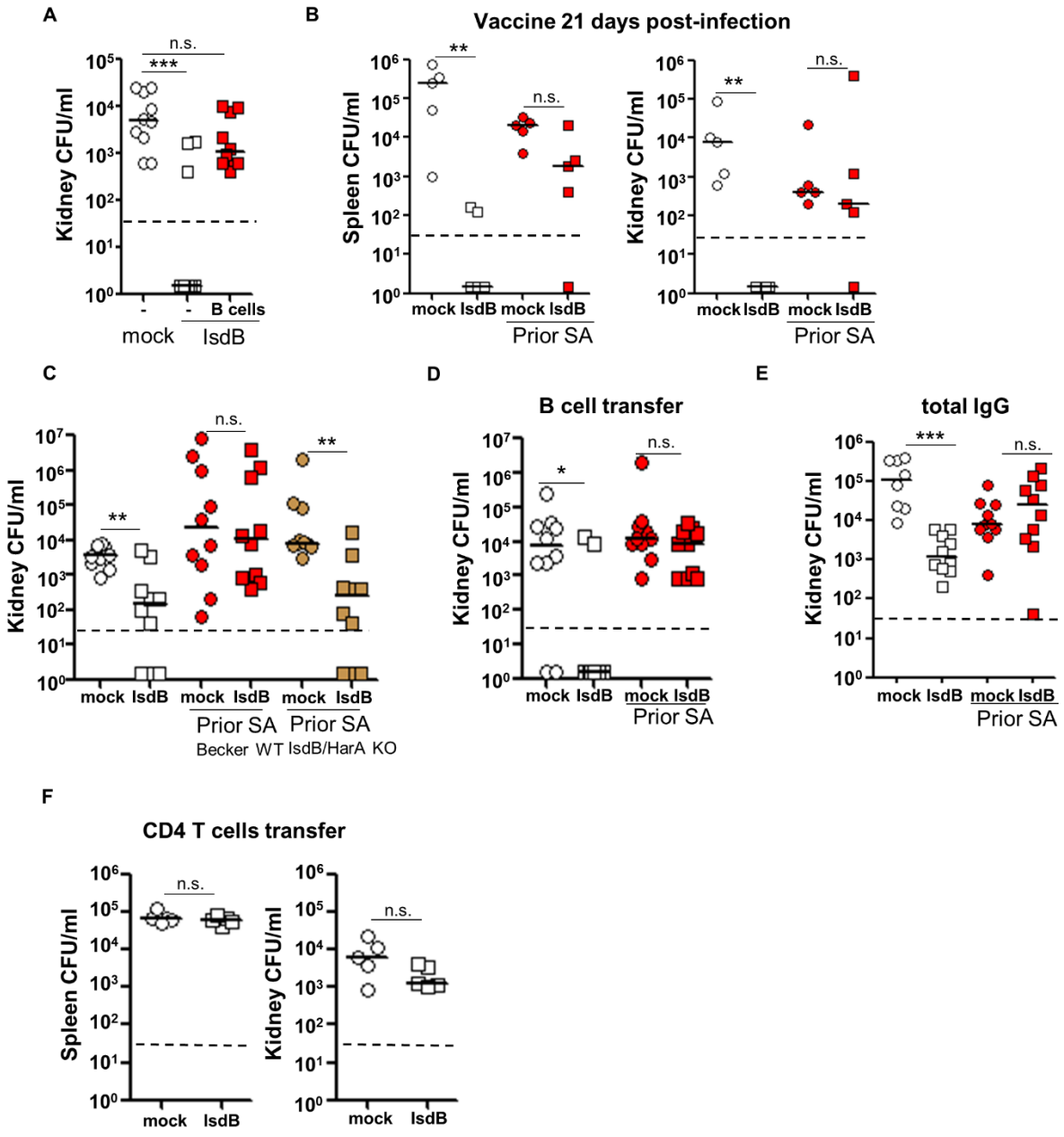


Figure S2 Parameters of IsdB vaccination and vaccine interference, Related to Figure 2

(A) B cells adoptively transferred from SA (LAC) infected mice blunt IsdB vaccine efficacy in the recipient mice. Recipient mice were immunized with IsdB as per Figure 1A, 24h after B cell transfer (*n*=10 per mouse group).

(B) Effect of extending the time interval between SA infection and IsdB vaccination to 21 days. Infection and immunization were otherwise performed as in Figure 1A (*n*=5 per mouse group).

(C) Specificity of vaccine suppression. Kidney SA burden from experiment Figure 2B (*n*=10 per mouse group).

(D) Adoptively transferred B cells: Kidney SA burden from experiment Figure 2C (*n*=10 per mouse group).

(E) Adoptively transferred total IgG: Kidney SA burden from experiment Figure 2D (*n*=8-10 per mouse group).

(F) $CD4^+$ T cell transfer from IsdB vaccinated SA-naïve mice is not protective (*n*=5 per mouse group).

Each data point represents an individual mouse; bars denote median and dashed lines indicate the limit of detection (A to F). n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; one way ANOVA (A to F).

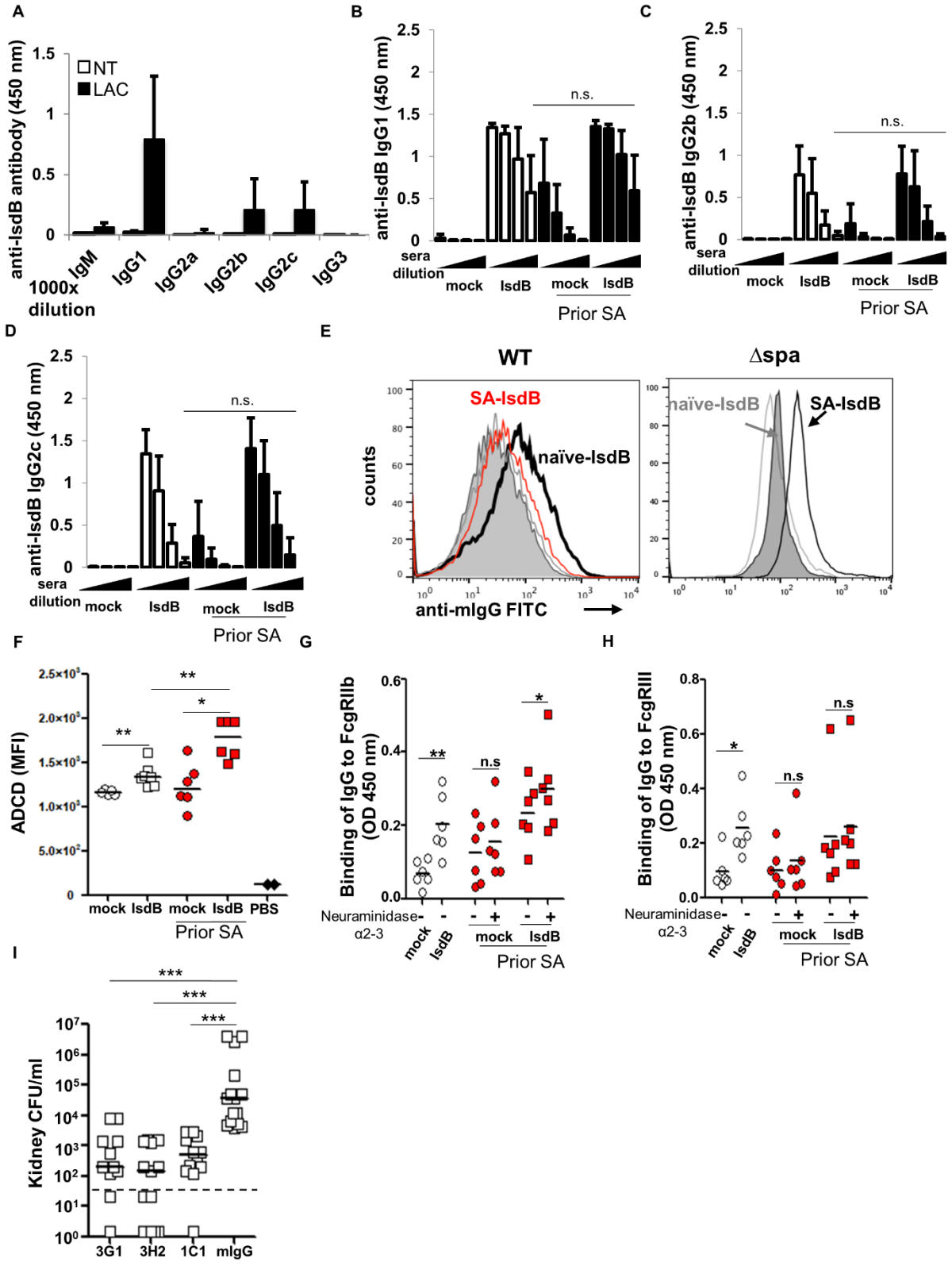

A
anti-IsdB antibody (450 nm)
NT
LAC
IgM
IgG1
IgG2a
IgG2b
IgG2c
IgG3
1000x dilution
B
anti-IsdB IgG1 (450 nm)
n.s.
sera dilution
mock
IsdB
mock
IsdB
Prior SA
C
anti-IsdB IgG2b (450 nm)
n.s.
sera dilution
mock
IsdB
mock
IsdB
Prior SA
D
anti-IsdB IgG2c (450 nm)
n.s.
sera dilution
mock
IsdB
mock
IsdB
Prior SA
E
WT
Δspa
SA-IsdB
naïve-IsdB
naïve-IsdB
SA-IsdB
counts
anti-mIgG FITC
F
ADCD (MFI)
mock
IsdB
mock
IsdB
PBS
Prior SA
G
Binding of IgG to FcgRIIb (OD 450 nm)
Neuraminidase α2-3
mock
IsdB
mock
IsdB
Prior SA
H
Binding of IgG to FcgRIII (OD 450 nm)
n.s
Neuraminidase α2-3
mock
IsdB
mock
IsdB
Prior SA
I
Kidney CFU/ml
3G1
3H2
1C1
mIgG

Figure S3 Characterization of IsdB-specific antibodies from naïve or *S. aureus*-infected mice that have been immunized with IsdB, Related to Figure 3

(A) Titers of IsdB-specific Ig subclasses (IgM, IgG1, IgG2a, IgG2b, IgG2c and IgG3) in the sera of naïve or SA-infected mice at 1:1000 dilution.

(B to D) Titers of IsdB-specific IgG1, IgG2b and IgG2c in the sera of mice treated as in Figure 1A.

(E) Binding of purified anti-IsdB antibodies to WT LAC or isogenic spa mutant analyzed by flow cytometry. Grey: stain with FITC-conjugated anti-mouse IgG only.

(F) Ab-dependent complement deposition assay.

(G) in vivo function of adoptively transferred IsdB-specific mAbs: Kidney SA burden from experiment Figure 3J.

(H and I) Binding of IgG to FcgRIIb (G) or FcgRIII (H) analyzed by ELISA.

Data are plotted as mean ± SD (A to D). n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; One way ANOVA (A to D, F to I).

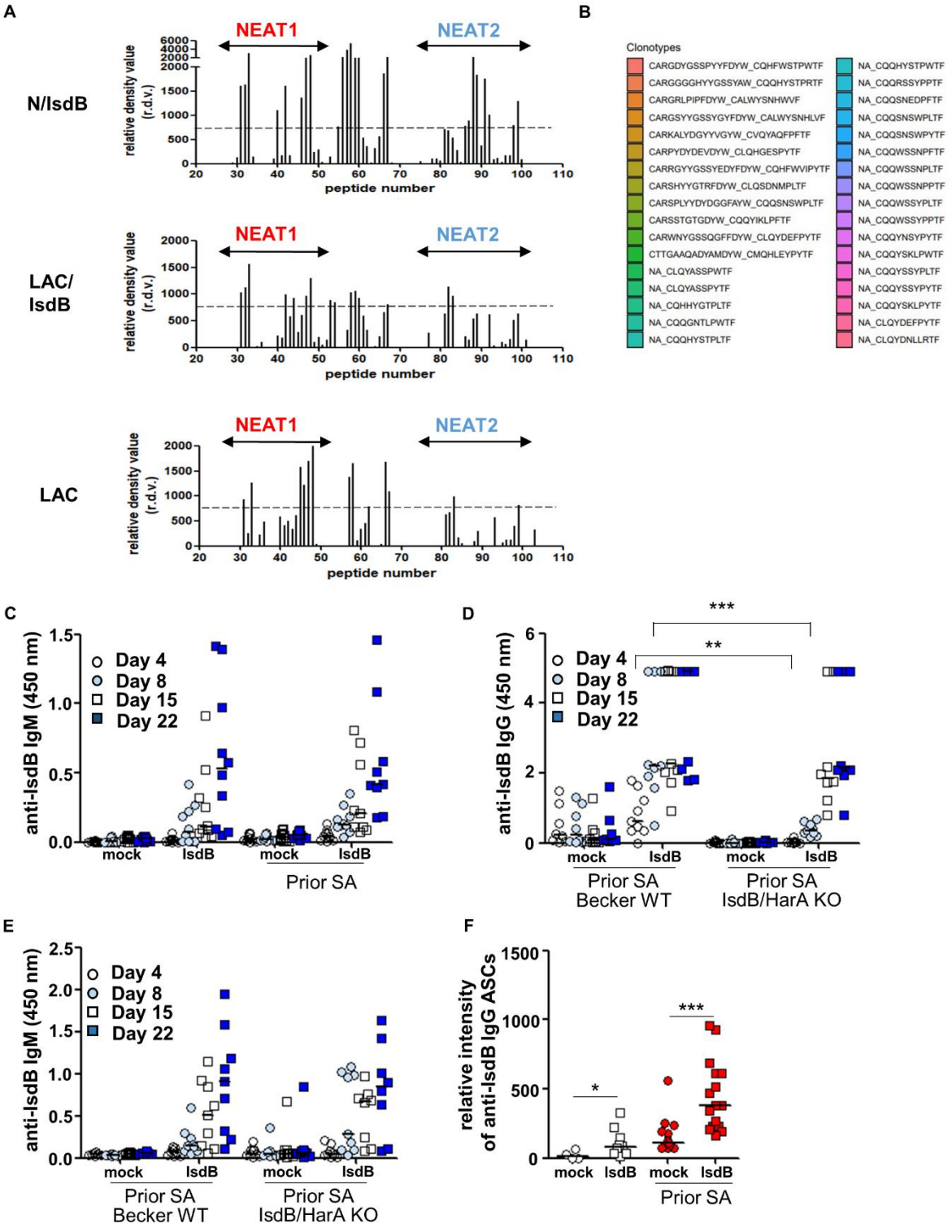

A
N/IsdB
LAC/
IsdB
LAC
NEAT1
NEAT2
relative density value
(r.d.v.)
peptide number
B
Clonotypes
CARGDYGSSPYYFDYW_CQHFWSTPWTF
CARGGGGHYYGSSYAW_CQQHYSTPRTF
CARGRLPIPFDYW_CALWYSNHWVF
CARGSYYGSSYGYFDYW_CALWYSNHLVF
CARKALYDGYYVGYW_CVQYAQFPFTF
CARPYDYDEVDYW_CLQHGESPYTF
CARRGYYGSSYEDYFDYW_CQHFWVIPYTF
CARSHYYGTRFDYW_CLQSDNMPLTF
CARSPLYYDYDGGFAYW_CQQSNSWPLTF
CARSSTGTGDYW_CQQYIKLPFTF
CARWNYGSSQGFFDYW_CLQYDEFPYTF
CTTGAAQADYAMDYW_CMQHLEYPYTF
NA_CLQYASSPWTF
NA_CLQYASSPYTF
NA_CQHHYGTPLTF
NA_CQQGNTLPWTF
NA_CQQHYSTPLTF
NA_CQQHYSTPWTF
NA_CQQRSSYPPTF
NA_CQQSNEDPFTF
NA_CQQSNSWPLTF
NA_CQQSNSWPYTF
NA_CQQWSSNPFTF
NA_CQQWSSNPLTF
NA_CQQWSSNPPTF
NA_CQQWSSYPLTF
NA_CQQWSSYPPTF
NA_CQQYNSYPYTF
NA_CQQYSKLPWTF
NA_CQQYSSYPLTF
NA_CQQYSSYPYTF
NA_CQQYSKLPYTF
NA_CLQYDEFPYTF
NA_CLQYDNLLRTF
C
anti-IsdB IgM (450 nm)
Day 4
Day 8
Day 15
Day 22
mock
IsdB
Prior SA
D
anti-IsdB IgG (450 nm)
***
**
Prior SA
Becker WT
Prior SA
IsdB/HarA KO
E
anti-IsdB IgM (450 nm)
F
relative intensity
of anti-IsdB IgG ASCs
*
***

Figure S4 Binding profile of immune sera to overlapping IsdB peptide array and clonotype sequences and IsdB-specific antibody levels following vaccination of naïve and SA pre-infected mice, Related to Figure 4

(A) Binding profile of sera to overlapping IsdB peptide array. Mouse sera: NT/IsdB: Naïve mice vaccinated with IsdB, LAC/IsdB: LAC-infected mice vaccinated with IsdB; LAC: LAC-infected mice given adjuvant alone (LAC/alum). NEAT1 extends from peptide #24 to #55, and NEAT2 from peptide #76 to #103.

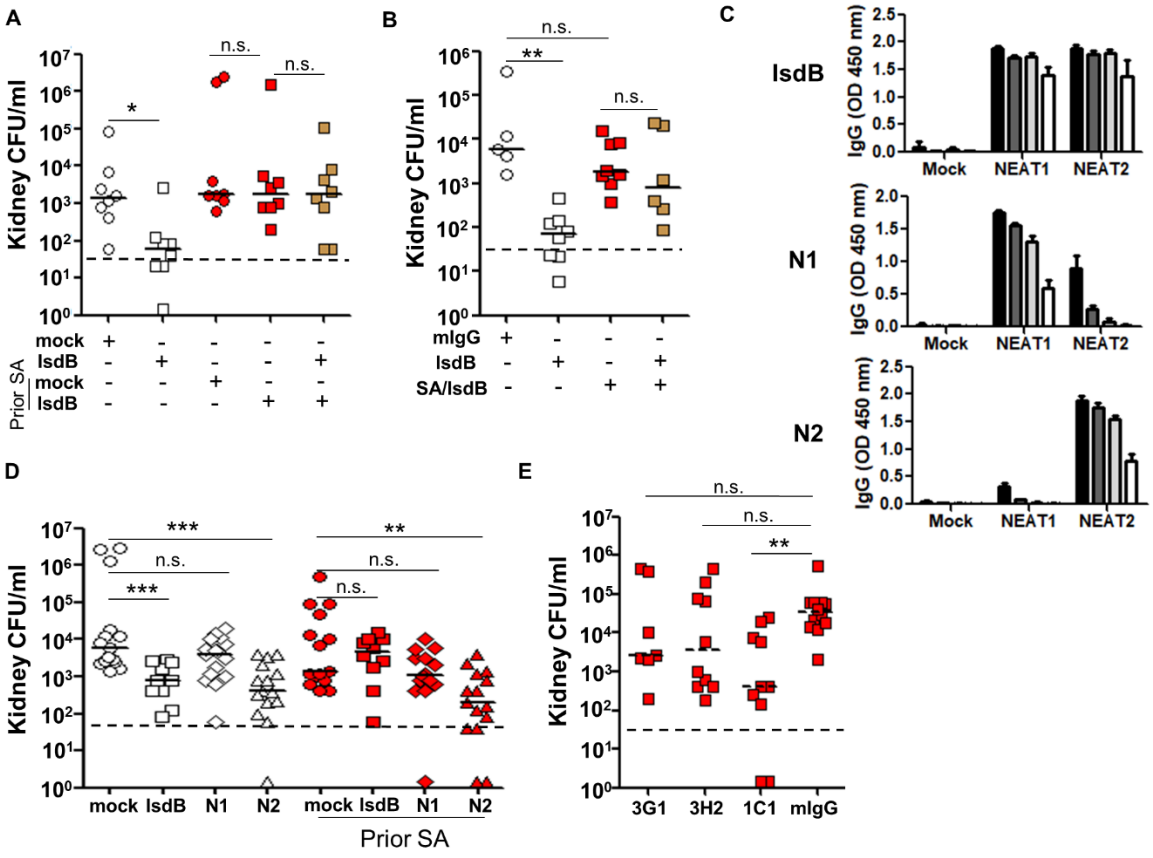
(B) Sequences of clonotypes from LAC, LAC/IsdB and N/IsdB, from experiment Figure 4A.

(C) Serum IsdB-specific IgM level after IsdB vaccination, from experiment Figure 4C (*n*=10 per mouse group).

(D and E) Serum IsdB-specific IgG and IgM levels in IsdB vaccinated WT and IsdB/HarA mutant-infected mice (*n*=10 per mouse group).

(F) Relative intensity of anti-IsdB IgG ASCs 7 Days after the first IsdB vaccination, from experiment Figure 4D.

Each data point represents an individual mouse; bars denote median (C to E). n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; one way ANOVA (C to F).



F

Amino acid sequences of CDR3 of monoclonal antibodies

Amino acid sequencing data of IsdB-specific monoclonal antibodies

mAb	mouse VH family	CDR3	target
3G1	IGHV1	ARGPITTVVAYSFDC	N2
3H2	IGHV1	ARFGYDQDHFDY	N2
1C1	IGHV1	ARHPPYGNDWYFDV	N2

G

NEAT2
76-103

3G1			
74	82	90	98
75	83	91	99
76	84	92	100
77	85	93	101
78	86	94	102
79	87	95	103
80	88	96	104
81	89	97	105

3H2			
74	82	90	98
75	83	91	99
76	84	92	100
77	85	93	101
78	86	94	102
79	87	95	103
80	88	96	104
81	89	97	105

1C1			
74	82	90	98
75	83	91	99
76	84	92	100
77	85	93	101
78	86	94	102
79	87	95	103
80	88	96	104
81	89	97	105

Figure S5 Competition between non-protective and protective IsdB-specific antibodies determines outcome of staphylococcal infection, Related Figure 5

(A) Anti-SA immunity conferred by protective sera (NT-IsdB) in the presence or absence of non-protective sera (LAC-IsdB): Kidney SA burden from experiment Figure 5A (*n*=8 per mouse group).

(B) Anti-SA immunity conferred by IsdB-specific protective Antibodies (NT-IsdB) in the presence or absence of non-protective specific antibodies (LAC-IsdB): Kidney SA burden from experiment Figure 5B (*n*=5-8 per mouse group).

(C) Serum IsdB-, NEAT1- and NEAT2- specific IgG levels following IsdB, N2 or N2 immunization of naïve mice respectively.

(D) N2 vaccination confers anti-SA protection in naïve and SA pre-infected mice: Kidney SA burden from experiment Figure 5E (*n*=10-15 per mouse group).

(E) Anti-SA immunity conferred by anti-N2 mAbs in SA-pre-infected mice: Kidney SA burden from experiment Figure 5G (*n*=7-10 per mouse group).

(F) Table of amino acid sequence of CDR3 of neutralizing IsdB mAbs from Figure 3I and 3J.

(G) Mapping of IsdB-specific mAb interaction with IsdB NEAT2 domain using peptide array. Peptide index of NEAT2: #76-#103.

Data are plotted as mean ± SD (C). Each data point represents an individual mouse; bars denote median (A, B, D and E). n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; one way ANOVA (A, B, D and E).

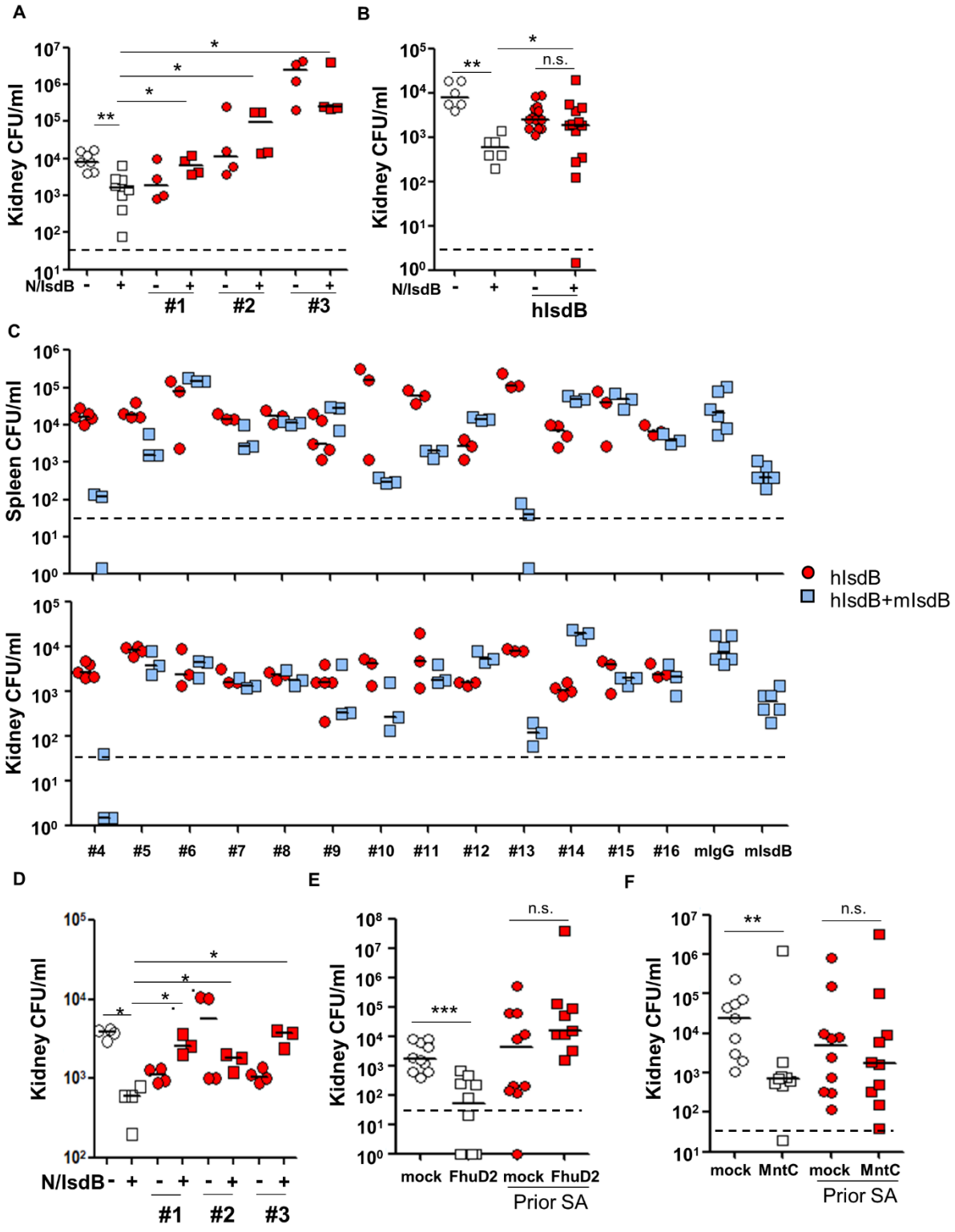

A
B
C
D
E
F
Kidney CFU/ml
Spleen CFU/ml
N/IsdB
#1
#2
#3
hIsdB
n.s.
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
mIgG
mIsdB
hIsdB
hIsdB+mIsdB
mock
FhuD2
Prior SA
MntC

Figure S6 Human serum anti-*S. aureus* antibodies blunting of specific protection conferred by anti-IsdB, and interference with FhuD2 and MntC vaccines, Related to Figure 6

(A) Human serum interference with protective anti-IsdB Ab function: Kidney SA burden from experiment Figure 6C (*n*=4-8 per mouse group, 3 human serum samples).

(B) Purified human anti-IsdB Ab interference with protective anti-IsdB Ab function: Kidney SA burden from experiment Figure 6D. Ratio of protective to non-protective Ab injected IV: 25 μg to 35 μg. (human serum, *n*=13).

(C) Data in (B) plotted to show individual human antibody competition with the protective Ab.

(D) Purified human anti-IsdB Ab interference with protective anti-IsdB Ab function: Kidney SA burden from experiment Figure 6E. Ratio of protective to non-protective Ab injected IV: 25 μg to 2.5 μg (human serum, *n* =3).

(E) FhuD2 vaccination in naïve or SA infected mice: Kidney SA burden from experiment Figure 6F (*n*=10 per mouse group).

(F) MntC vaccination in naïve or SA infected mice: Kidney SA burden from experiment Figure 6G. (*n*=10 per mouse group).

Each data point represents an individual mouse; bars denote median and dashed lines indicate the limit of detection (A to C). n.s., not significant, *P<0.05, **P<0.01, and ***P< 0.001; one way ANOVA (A, B and D to F)