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Supplementary Materials for

Inhibition of protective immunity against *Staphylococcus aureus* infection by MHC-restricted immunodominance is overcome by vaccination

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SUPPORTING INFORMATION

Table S1. Genetic background and MHC haplotypes of the mice used in this study

Strain	Background	Igh-1 Allele	MHC Haplotype	MHC Genes				
				K	I-A	I-E	D	L
C57BL/6	C57BL/6	b	H-2^b	b	b	-	b	-
B6.H2^d	C57BL/6	b	H-2^d	d	d	d	d	d
BALB/c	BALB/c	a	H-2^d	d	d	d	d	d
C.B10-H2^b	BALB/c	a	H-2^b	b	b	-	b	-
CB.17	BALB/c	b	H-2^d	d	d	d	d	d

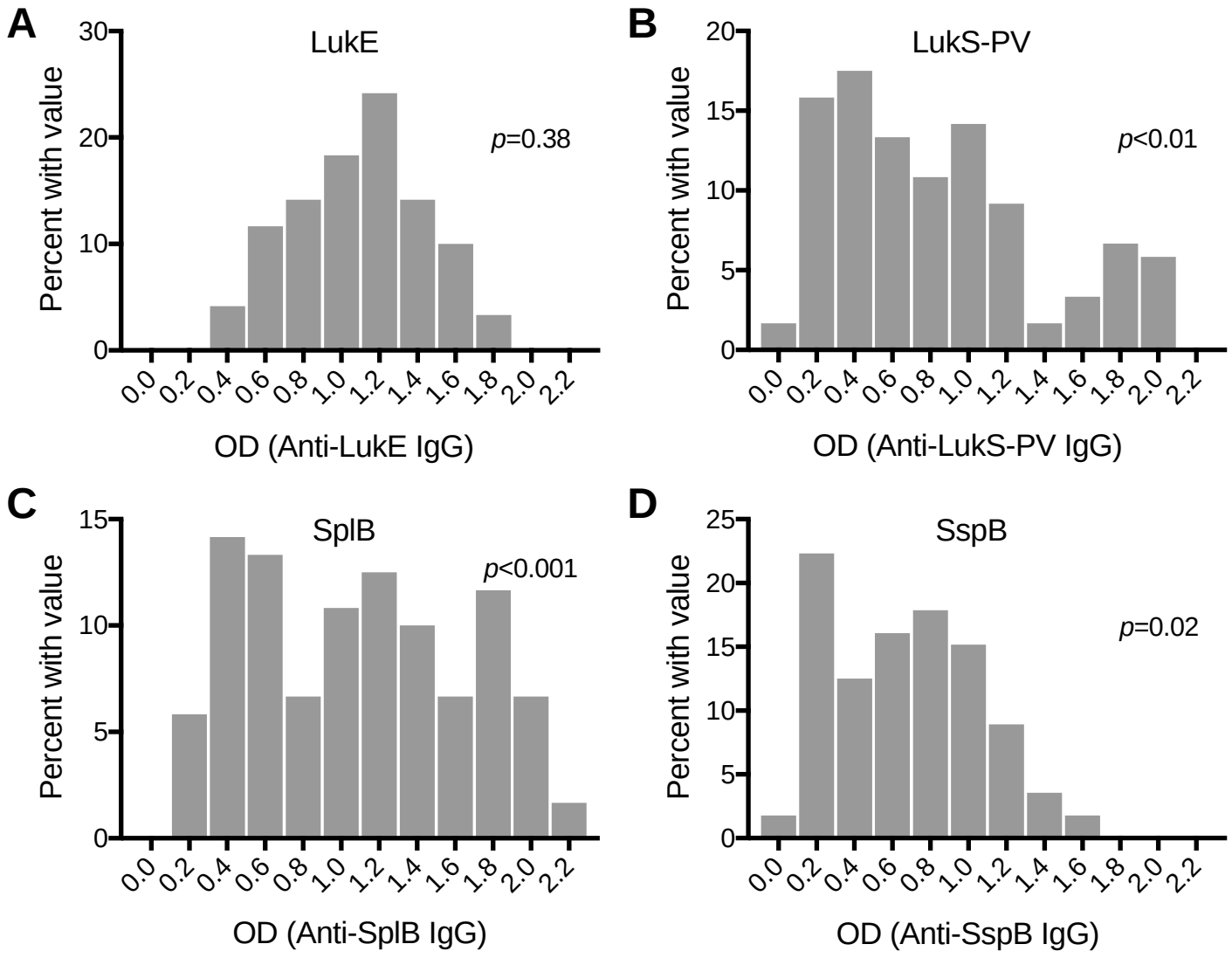

Figure S1. Antibody levels against *sae*-regulated antigens in 120 healthy adults. (A) Anti-LukE IgG levels were normally distributed (p=0.38, D'Agostino & Pearson normality test). In contrast, antibody levels against LukS-PV (B), SplB (C), and SspB (D) were not normally distributed.

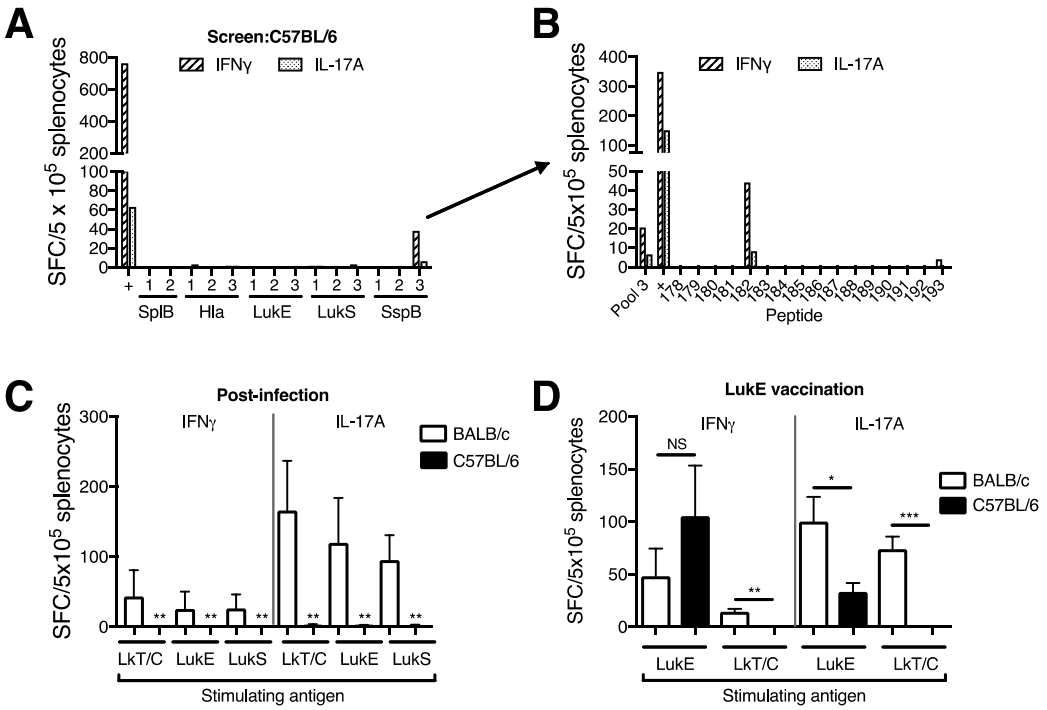

Figure S2. Immunogenicity of infection and vaccination. (A) Screening for immunogenic epitopes following infection in C57BL/6 mice identified one pool of peptides from SspB (pool 3) that stimulated moderate IFNγ responses by ELISpot. (B) Deconvolution of this peptide pool identified one moderately immunogenic peptide (#182, $SspB_{297\text{-}312}$; GRDIHYQEGVPSYEQV) that binds I-A^b and I-A^d with moderate affinity (IC_{50} – 616 nM and 606 nM, respectively) (3 mice/group from one representative experiment). (C) Following infection of BALB/c and C57BL/6 mice, LukE-specific IFNγ and IL-17A responses were driven by LkT/C in BALB/c mice, but there were no LukE or LkT/C-specific responses in C57BL/6 mice (6 mice/group pooled from two separate experiments). Data were analyzed by comparing responses for BALB/c and C57BL/6 mice for each condition. (D) Following vaccination with LukE, there were strong LukE-specific IL-17A and IFNγ responses in BALB/c and C57BL/6 mice, but only BALB/c mice had LkT/C-specific responses (4 mice/group from one representative experiment). "+" indicates concanavalin A, a positive control; SFC indicates spot forming colonies. Data were compared using student's T test. Data presented as mean ± SEM. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$.

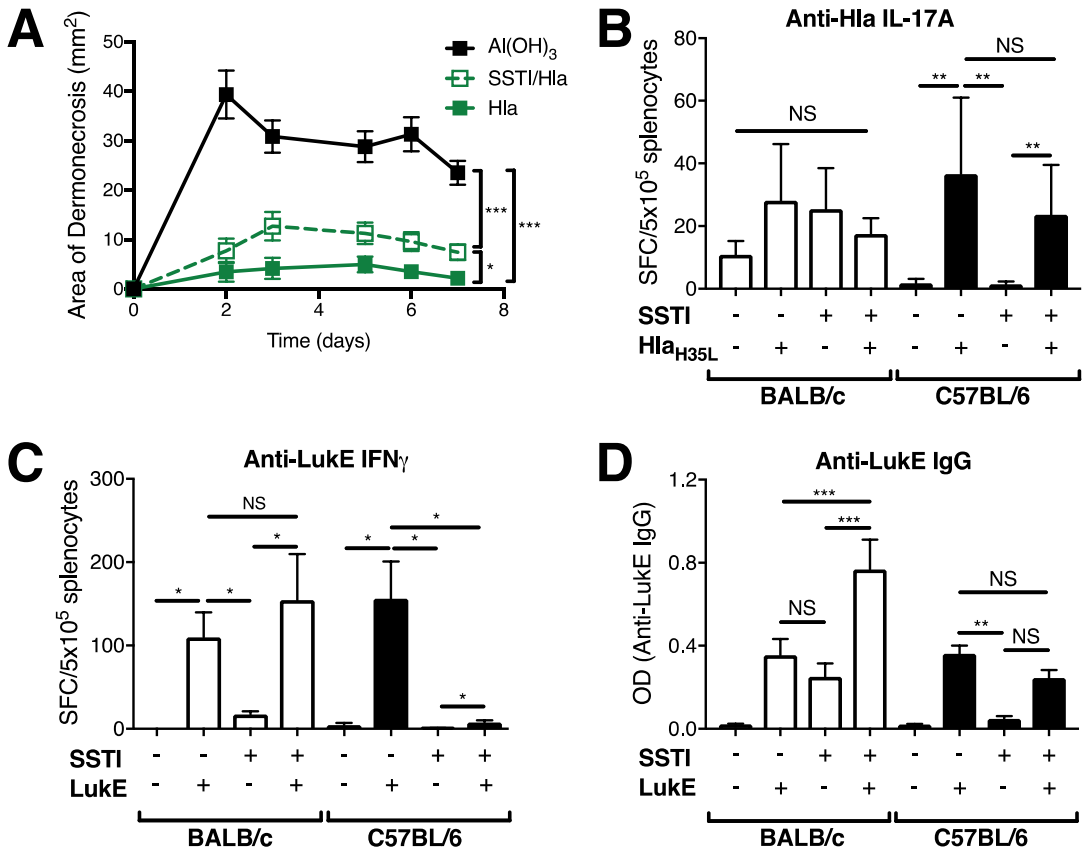

Figure S3. Protection and immune responses in concomitant infection/vaccination. (A) C57BL/6 mice were vaccinated with Hla_{H35L} alone, received concomitant SSTI and Hla_{H35L} vaccination (SSTI/Hla), or control. 4 weeks later, mice were infected with SSTI. Compared with Hla_{H35L} vaccination, concomitant SSTI/Hla resulted in inhibition of protection against SSTI, although protection was still observed, compared with controls (12-13 mice/group pooled from two experiments). (B) There was no impact of concomitant vaccination/infection on Hla-specific IL-17A responses in BALB/c or C57BL/6 mice, compared with vaccination alone (4-5 mice/group pooled from two experiments). (C) As we observed with LukE-specific IL-17A responses, concomitant SSTI/LukE vaccination of BALB/c mice resulted in a trend toward higher anti-LukE IFNγ responses, compared with vaccination alone, but the differences were not significant. In contrast, concomitant vaccination/infection resulted in strongly decreased anti-LukE responses in C57BL/6 mice, compared with vaccination alone (4 mice/group from one representative experiment). (D) Although anti-LukE IgG levels were higher in BALB/c mice following concomitant infection/vaccination, compared with

vaccination alone, there were no significant differences in C57BL/6 mice (7-10 mice/group pooled from two experiments). SFC indicates spot forming colonies. Data were compared using two-way ANOVA with repeated measures and Tukey's post test (A) or one-way ANOVA with Tukey's post test (B-D). All data are plotted as mean ± SEM. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, NS indicates not significant.

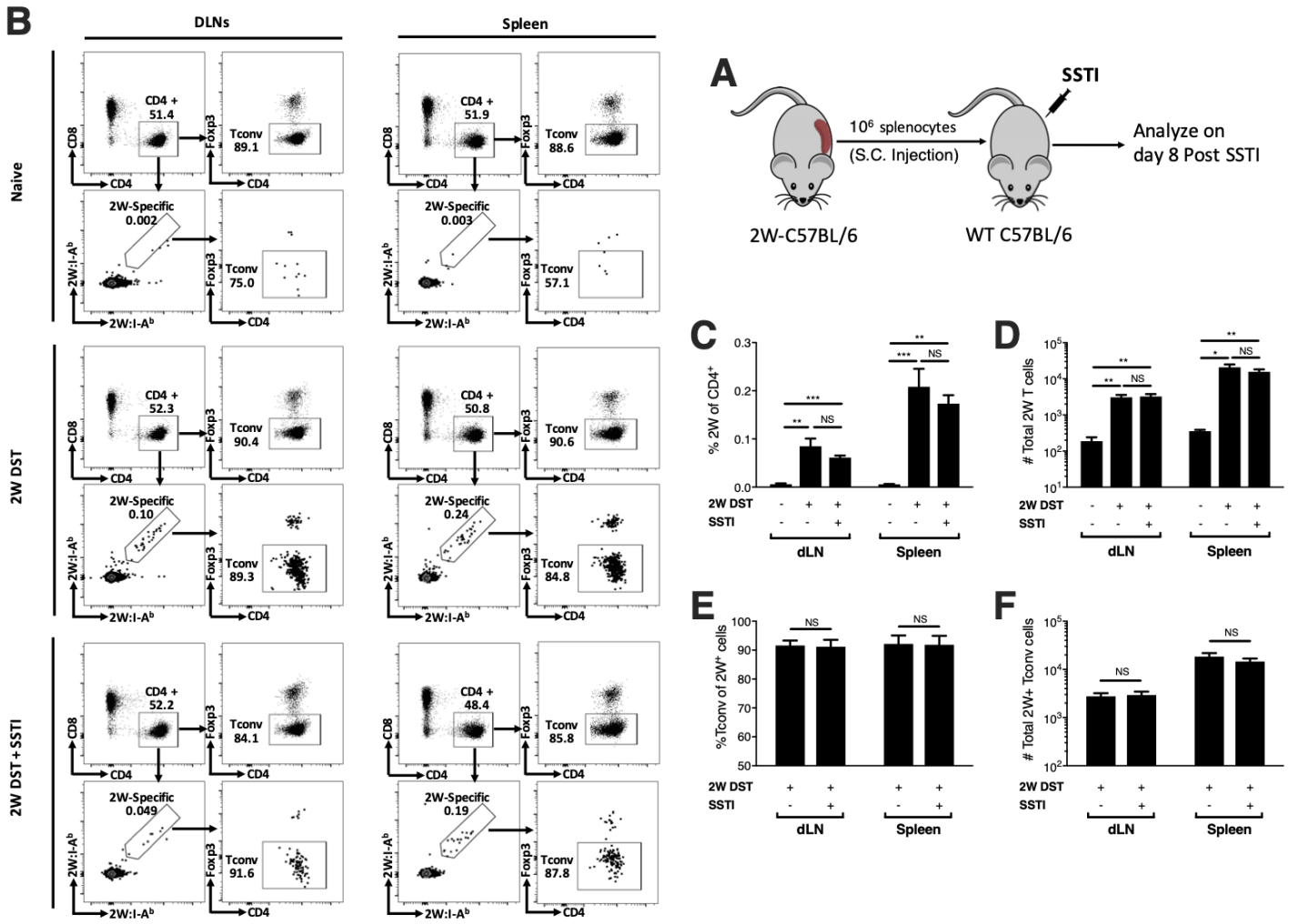

Figure S4. Immunodominance of *S. aureus* SSTI did not affect CD4+ T cell responses to the model antigen 2W-OVA. To test whether immunodominant antigens presented during *S. aureus* SSTI interfere with responses against an irrelevant antigen, 2W-OVA splenocyte immunization (s.c.) was performed using cells from 2W-OVA transgenic C57BL/6 mice (donor-specific transfusion, DST), with or without concomitant *S. aureus* SSTI. (A) Experimental model: 2W-specific splenocytes were harvested and transferred into naïve C57BL/6 mice, with concomitant *S. aureus* SSTI (or sham infection with PBS). 8 days later, T cells were harvested and flow cytometry was performed using 2W-specific I-A^b pMHC tetramers. (B) Representative flow cytometry plots displaying 2W-specific T cells and conventional T cells (Tconv) in draining lymph nodes (dLNs, left) and splenocytes (right). 2W-specific Tconvs were identified as $Foxp3^-$ cells. Regardless of whether mic received DST alone

or DST + SSTI, there were no significant differences in (C) the percentage of 2W-specific $CD4^+$ T cells, (D) the number of 2W-specific $CD4^+$ T cells, (E) the percentage of Tconv 2W-specific cells, or (F) the number of 2W-specific Tconvs. There were 6 mice/group pooled from 2 experiments. Data were compared using one-way ANOVA with Tukey's post test (C,D) or student's T test (E,F). All data are plotted as mean ± SEM. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, NS indicates not significant.