

Supplementary Information

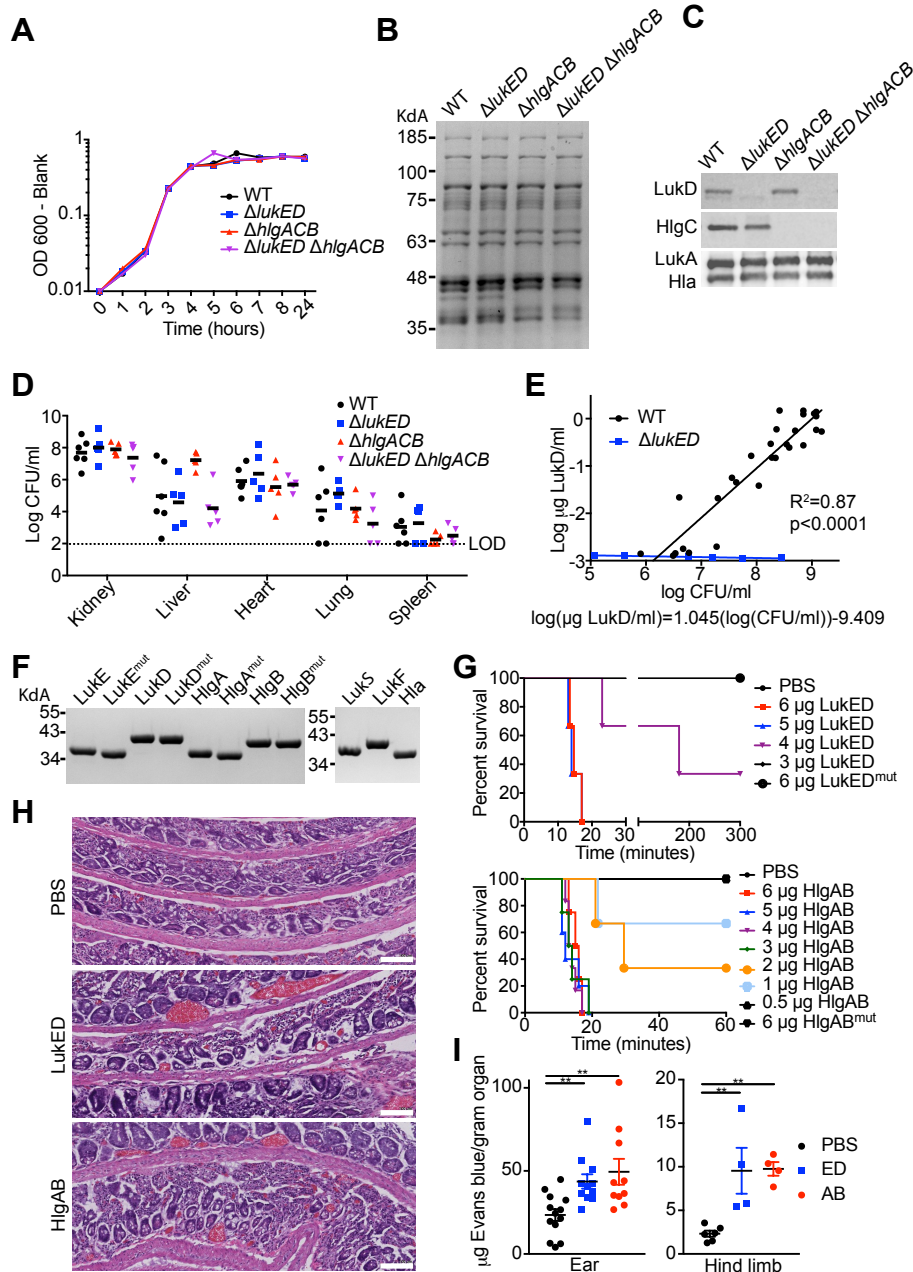


Figure S1 - Related to Figure 1: Infection model: (A) Growth curve in Tryptic Soy Broth of the strains of *S. aureus* Newman used for infection. (B) Coomassie Blue stained SDS-PAGE gel of TCA-precipitated exoproteins produced by the indicated strains during 5-hour subcultures in TSB. (C) Immunoblots of TCA-precipitated exoproteins for LukD, HlgC, LukA, and Hla, samples generated as in (B). (D) CFU from the indicated organs of Swiss-Webster mice infected intravenously with $2-3.5 \times 10^7$ CFU of the indicated strains

of *S. aureus* Newman, means are shown (n= 5-6 mice per group). (E) Relationship between LukD levels and CFU burden in kidneys and hearts of C57BL/6J mice 96 hours post infection with 2.5×10^7 CFU of the indicated strains of *S. aureus* Newman by ELISA. LOD = limit of detection. **Toxin challenge model:** (F) Coomassie gel of 2 μ g of each of the purified leukotoxins. (G) Survival curve of Swiss-Webster mice challenged intravenously with indicated doses of purified toxin (n = 3-6 mice per group). (H) H&E staining of intestine from toxin challenged Swiss-Webster mice. Scale bars indicate 100 μ M. (I) Evans blue content in the indicated organs of toxin challenged Swiss Webster mice post perfusion with PBS (n = 10-13 mice per group for the ear and 4-6 mice per group for the hind limb).

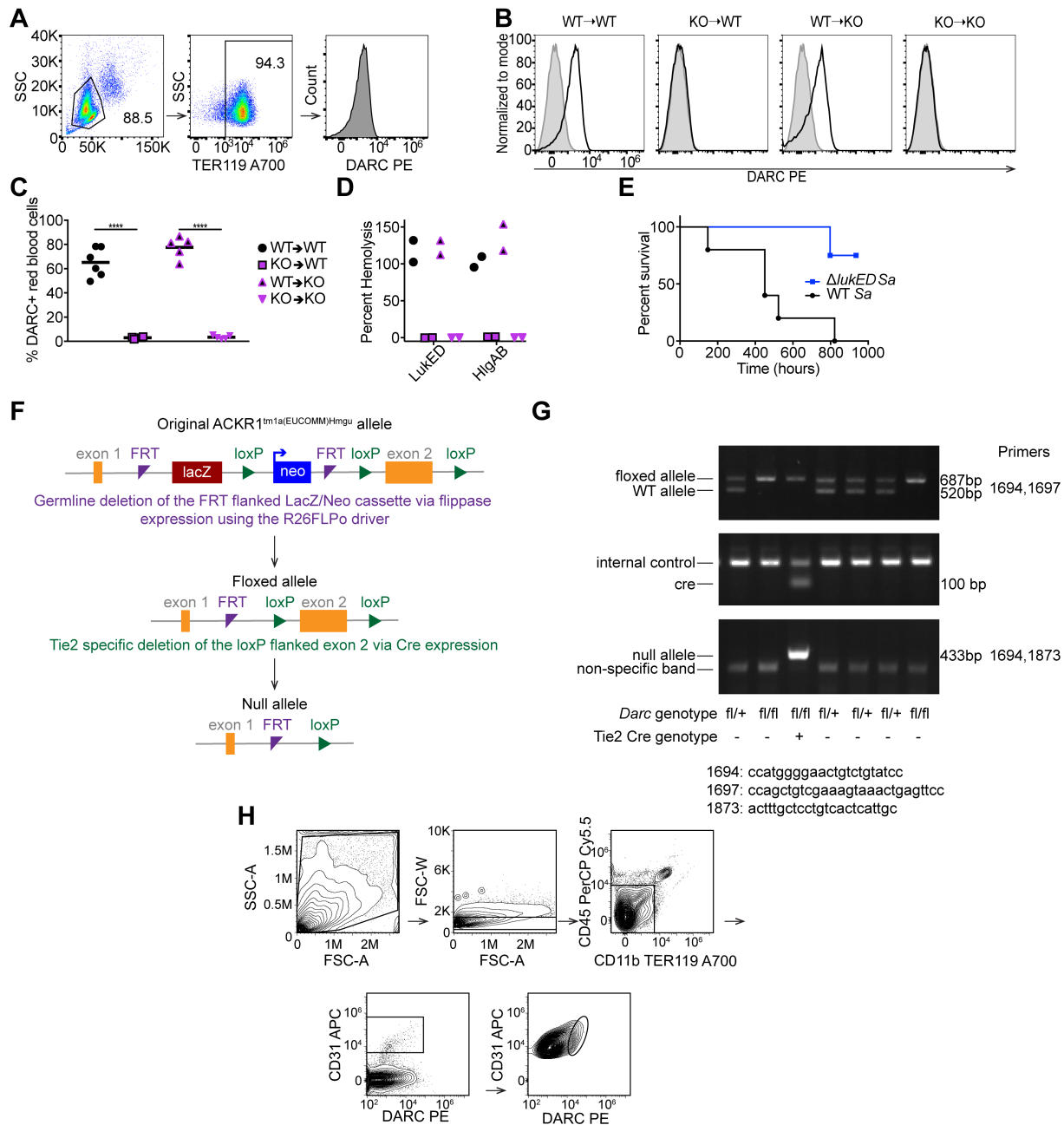


Figure S2 - Related to Figure 2: Bone marrow chimera model: (A) Gating strategy for erythrocytes in mouse blood. (B) Representative DARC staining (C) percent DARC positive cells and (D) hemolysis of erythrocytes in blood from bone marrow chimeric mice 11 weeks post bone marrow transfer. In (B) the isotype control is shown in gray. (E) Survival curve of female WT→WT bone marrow chimeric mice infected with $1-1.5 \times 10^7$ CFU WT and $\Delta lukED$ *S. aureus* Newman (n = 4-5 mice per group). **** p < 0.0001 C,

ANOVA with Tukey's correction. **Endothelial specific DARC KO model:** (F) Schematic of the generation of *DARC^{fl/fl}* and *DARC^{fl/fl} Tie2 Cre* mice. (G) Example of genotyping gels from PCRs using the indicated primers. The middle gel shows a PCR for the Tie2 Cre allele using the generic Cre PCR on the Jackson Laboratory website. (H) Gating strategy for measuring the DARC levels on endothelial cells from mouse skin.

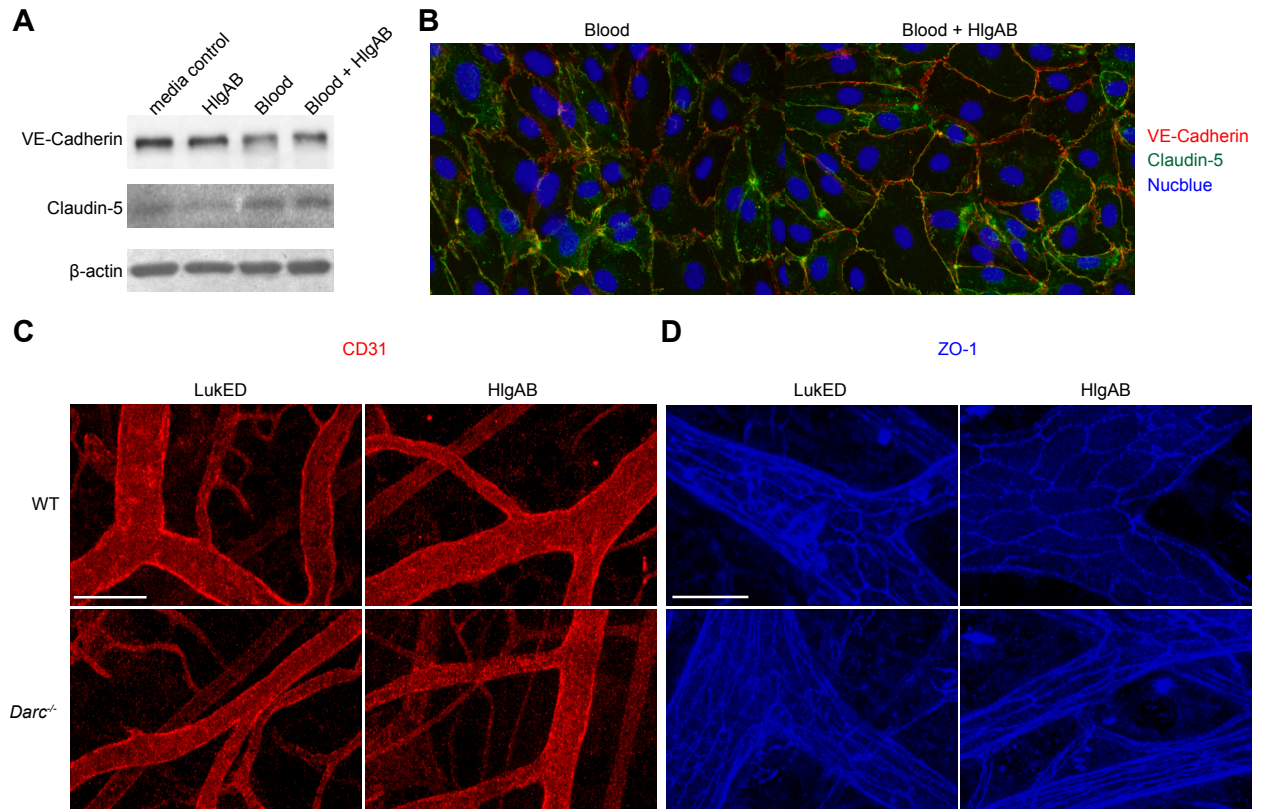


Figure S3 - Related to Figure 3: (A) Immunoblot of the indicated junctional proteins upon blood and toxin exposure. (B) Images of junctional proteins on endothelial cells post blood and toxin exposure. Blots and images are from 1 hour post toxin challenge; similar results were obtained at 15 minutes and 5 hours post toxin challenge. (C, D) Images of vessels in ears of toxin challenged mice. Scale bars indicate 80 μ M in (C) and 50 μ M in (D).

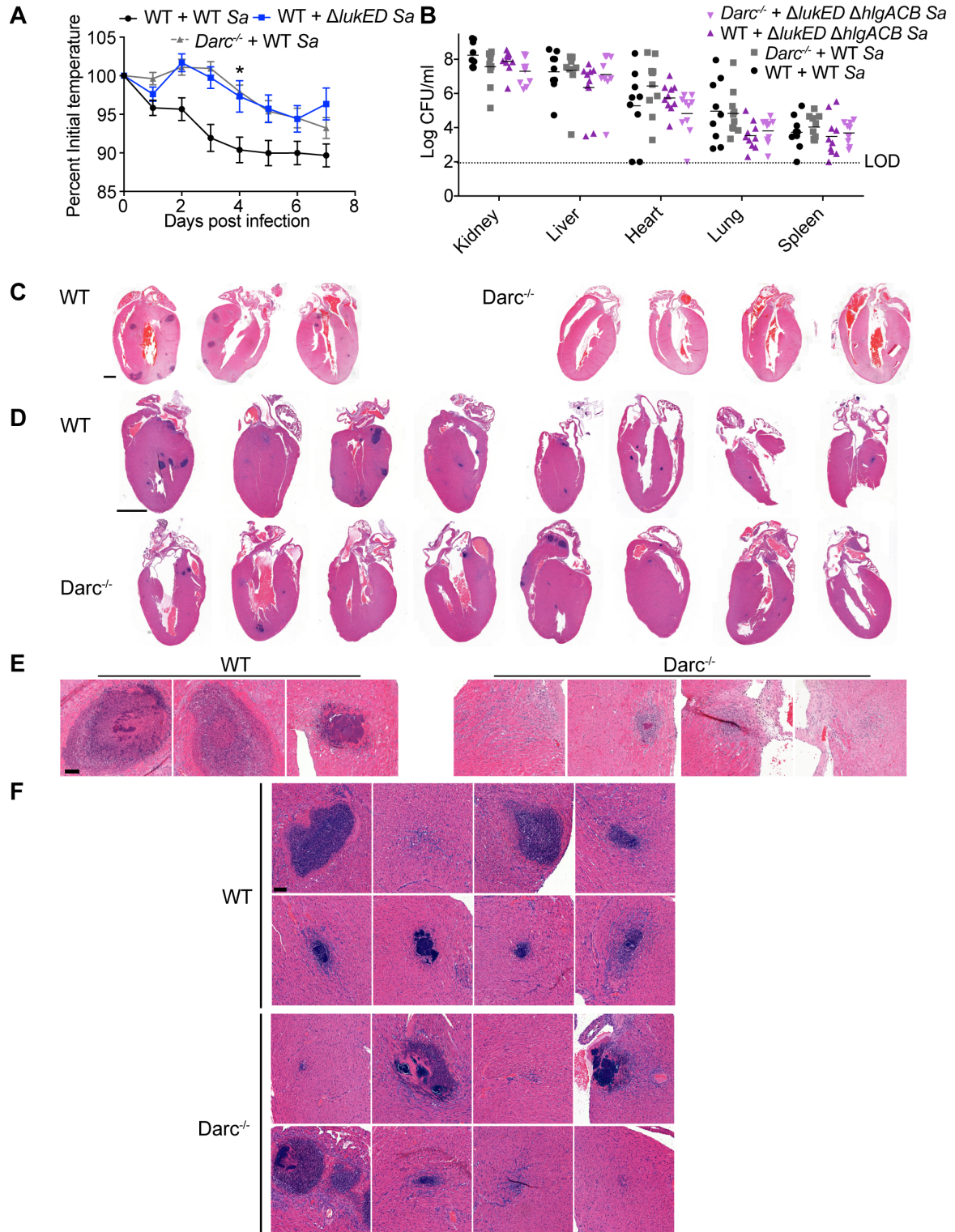


Figure S4 - Related to Figure 4: (A) Percent of initial temperature of WT and $Darc^{-/-}$ mice infected with $1-2.7 \times 10^7$ CFU of the indicated strains of MRSA USA500 strain BK2395 (n = 15-19 mice per group). * indicates significant differences between the WT and $Darc^{-/-}$

mice infected with WT *S. aureus*. (B) CFU from indicated organs at 72 hours post infection of WT and *Darc*^{-/-} mice infected with 2-3.5 x 10⁷ CFU of the indicated strains of *S. aureus* Newman (n = 9-10 mice per group). Data shown are pooled from two (B) or three (A) independent experiments. LOD = limit of detection. Where relevant, means ± SEM are shown. * p<0.05. (A, ANOVA with Tukey's correction). (C-F) H&E staining of hearts at 72- (females) or 96- (males) hours post infection with 2.5 x 10⁷ CFU of *S. aureus* Newman. (C, D) whole hearts, (E, F) 10x images that were chosen to represent the breadth of lesions in each group. Images in (E) are magnified from the images shown in (C), images in (F) are magnified from the images shown in (D). In total one unmagnified and one magnified image are shown from each mouse. Scale bars indicate 1mm (C, D), 100 μM (E, F).

Mouse/Condition	Cell type depleted	Result
WT (SW/B6)		Susceptible
Anti-Ly6G	Neutrophils	Susceptible
Anti-Ly6C	Monocytes	Susceptible
Anti-Gr1	Neutrophils + Monocytes	Susceptible
Clodronate Liposomes	Macrophages	Susceptible
<i>MyD88</i> ^{-/-}		Susceptible
<i>Caspase1/11</i> ^{-/-}		Susceptible
<i>Ccr5</i> ^{-/-}		Susceptible
<i>Ccr2</i> ^{-/-}		Susceptible
<i>Cxcr1</i> ^{-/-}		Susceptible
<i>Cxcr2</i> ^{-/-}		Susceptible
<i>Darc</i>^{-/-}		Resistant

Supplemental Table 1 - Related to Figure 2: DARC is the only leukocidin receptor required for lethality. Outcome of intravenous injection of toxin in cell depletion and genetic deletion mouse models.

Mouse	Sa strain	AST	ALT	ALP	GGT	TBILI	BUN	CREAT	NA	K	CL	CA
	Units	U/l	U/l	U/l	U/l	mg/dl	mg/dl	mg/dl	mmol/l	mmol/l	mmol/l	mg/dl
B6	WT Newman	257	53	1205	0	0.01	120	2.3	148	7.5	93	13
B6	WT Newman	410	83	2255	10	1.22	105	2.8	152	11.5	93	12
B6	WT Newman	448	100	HI	0	0	197	2.3	147	9.7	95	11
B6	WT Newman	553	74	46	0	0	66	0.6	158	9.4	107	11
B6	WT Newman	433	83	1447	0	0	132	1.3	152	11.2	109	11
B6	PBS	348	56	125	0	0.39	27	0.5	159	8.1	114	11
DARC KO	WT Newman	147	34	22	0	0.02	61	0.7	153	7.1	101	12
DARC KO	WT Newman	270	44	23	29	1.41	65	0.9	152	6.9	97	13
DARC KO	WT Newman	143	42	18	0	0	14	0.3	153	7.5	109	10
DARC KO	WT Newman	253	37	28	0	0	40	0.4	162	8.3	110	11
DARC KO	WT Newman	357	74	805	0	0	108	0.8	158	7.8	107	11
DARC KO	WT Newman	269	50	614	0	0	142	0.8	152	8.9	103	12
DARC KO	PBS	693	159	80	0	0.15	16	0.5	153	7.7	110	10
DARC KO	PBS	439	69	122	0	0	20	0.6	150	10.3	111	9.4
DARC KO	PBS	194	49	104	0	0	21	0.5	150	8.9	109	9.5

Mouse	strain	PHOS	TP	ALB	GLU	CHOL	TRIG	GLOB	A/G	DBILI	IBILI
	Units	mg/dl	g/dl	g/dl	mg/d	mg/dl	mg/dl	g/dl	Ratio	mg/dl	mg/dl
B6	WT Newman	19.8	8.3	4.3	42	259	170	4	1.1	0	0.01
B6	WT Newman	18.9	7.1	3.7	20	231	408	3.4	1.1	1.03	0.19
B6	WT Newman	19.6	7.6	3.9	29	268	348	3.7	1.1	0	0
B6	WT Newman	9.6	7.8	4.1	56	172	39	3.7	1.1	0.01	-0.01
B6	WT Newman	17.6	7.3	3.9	59	189	77	3.4	1.1	0.03	-0.03
B6	PBS	9.3	6.3	3.8	172	97	48	2.5	1.5	0.26	0.13
DARC KO	WT Newman	10.6	8.1	4.2	48	209	92	3.9	1.1	0.01	0.01
DARC KO	WT Newman	11.9	7.6	4.1	60	209	85	3.5	1.2	1.08	0.33
DARC KO	WT Newman	8.90	5.6	3.10	82	158	58	2.50	1.2	0.01	-0.01
DARC KO	WT Newman	9.10	7.5	4.00	51	186	40	3.50	1.1	0.01	-0.01
DARC KO	WT Newman	14.80	7.7	4.10	42	194	72	3.60	1.1	0.01	-0.01
DARC KO	WT Newman	14.90	8.3	4.30	52	217	60	4.00	1.1	0.00	0
DARC KO	PBS	10	5.7	3.4	251	152	133	2.3	1.5	0.13	0.02
DARC KO	PBS	10.3	5.9	3.6	193	145	213	2.3	1.6	0.09	-0.09
DARC KO	PBS	8.3	5.5	3.3	219	145	236	2.2	1.5	0.05	-0.05

Supplemental Table 2 - Related to Figure 4: Full clinical chemistry panel on infected WT and DARC KO mice. Serum levels of the indicated analytes in mice at 96 hours post infection with 2.5×10^7 CFU of *S. aureus* Newman. AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase, TBILI = total bilirubin, BUN = blood urea nitrogen, CREAT = Creatinine, PHOS = phosphate, TP = total protein, ALB = albumin, GLU = glucose, CHOL = cholesterol, TRIG = triglycerides, GLOB = globulins, A/G = albumin / globulin ratio, DBILI = direct bilirubin, IBILI = indirect bilirubin. All samples were moderately to grossly hemolyzed.