

1 **SUPPLEMENTAL MATERIAL**

2 **Supplemental Table 1.** Average gene copy per NCBI Staphylococcal Genomes

3 analyzed for P_i transporters.

Species	No. of Genomes	<i>pstS</i>	<i>pstC</i>	<i>pstA</i>	<i>pstB</i>	<i>pit</i>	<i>nptA</i>	<i>phoU</i>	<i>phoB1</i>	<i>phoR</i>
<i>Staphylococcus lutrae</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus pseudintermedius</i>	11	1	1	1	1	1	1	2	1	1
<i>Staphylococcus delphini</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus intermedius</i>	2	1	1	1	1	1	1	2	1	1
<i>Staphylococcus schleiferi</i>	5	1	1	1	1	1	1	2	1	1
<i>Staphylococcus agnetis</i>	4	1	1	1	1	1	1	2	1	1
<i>Staphylococcus hyicus</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus chromogenes</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus microti</i>	1	0	0	0	0	1	1	1	1	1
<i>Staphylococcus massiliensis</i>	2	1	1	1	1	1	1	2	1	1
<i>Staphylococcus capitis</i>	37	1	1	1	1	1	0	2	1	1
<i>Staphylococcus caprae</i>	2	1	1	1	1	1	0	2	1	1
<i>Staphylococcus epidermidis</i>	372	1	0.99	1	0.99	0.99	0	1.99	1	0.99
<i>Staphylococcus lugdunensis</i>	12	1	1	1	1	1	0	2	0.92	1
<i>Staphylococcus</i>	42	1	1	0.98	1	1	0	2	1	1
<i>Staphylococcus haemolyticus</i>	159	1	1	1	0.99	1	0	2	1	1
<i>Staphylococcus warneri</i>	19	1	1	1	1	1	0	2	1	1
<i>Staphylococcus pasteurii</i>	3	1	1	1	1	1	0	2	1	1
<i>Staphylococcus simiae</i>	1	1	1	1	1	1	0	2	1	1
<i>Staphylococcus schweitzeri</i>	3	1	1	1	1	1	1	2	1	1
<i>Staphylococcus argenteus</i>	106	1	1	1	1	1	1	2	1	0.99
<i>Staphylococcus aureus</i>	7792	0.99	1	1	1	1	0.99	2	1	1
<i>Staphylococcus pettenkoferi</i>	4	1	1	1	1	1	0	2	1	1
<i>Staphylococcus auricularis</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus equorum</i>	21	1	1	1	1	1	1	2	1	1
<i>Staphylococcus cohnii</i>	14	1	1	1	1	1	1	2	1	1
<i>Staphylococcus saprophyticus</i>	40	1	1	1	1	1	0.88	2	1	1
<i>Staphylococcus xylosus</i>	14	1	1	1	1	1	1	2	1	1
<i>Staphylococcus succinus</i>	4	1	1	1	1	1	1	2	1	1
<i>Staphylococcus gallinarum</i>	1	1	1	1	1	1	0	2	1	1
<i>Staphylococcus arlettae</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus simulans</i>	5	1	1	1	1	1	0	2	1	1
<i>Staphylococcus condimentii</i>	3	1	1	1	1	1	0	2	1	1
<i>Staphylococcus carnosus</i>	4	1	1	1	1	1	0	2	1	1
<i>Staphylococcus lentus</i>	4	1	1	1	1	1	1	2	1	1
<i>Staphylococcus sciuri</i>	15	1	1	1	1	1	1	2	1	1
<i>Staphylococcus vitulinus</i>	1	1	1	1	1	1	1	2	1	1
<i>Staphylococcus fleurettii</i>	1	1	1	1	1	1	1	2	1	1

Fractional numbers are mostly artifacts of incomplete genomes sequences, except for *S. saprophyticus*, of which 5 strains have partial gene deletions of *nptA*

5 **Supplemental Table 2.** Inorganic phosphate transporters, phosphate regulatory, and universally conserved gene
6 queries.

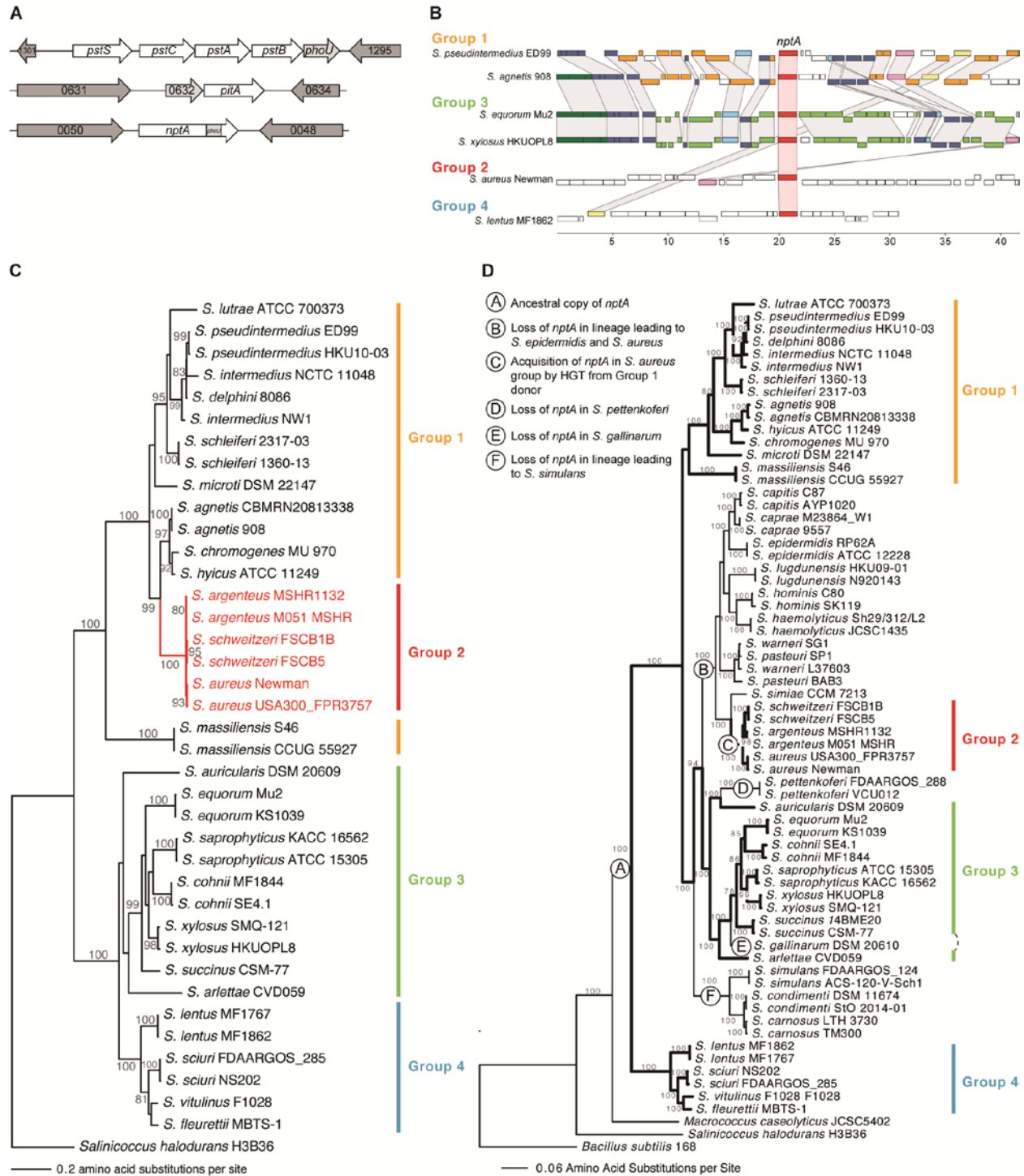
Gene name(s)	HMM(s)	KEGG Orthology	Gene description	<i>E. coli</i> ^a	<i>V. cholerae</i> ^b	<i>B. subtilis</i> ^c	<i>S. aureus</i> ^d
<i>pstB</i>	TIGR00972, PF00005	K02036	phosphate transport system ATP-binding protein	b3725	VC0726, VCA0073	BSU24950, BSU24960	NWMN_1297
<i>pstA</i>	TIGR00974, PF00528	K02038	phosphate transport system permease protein	b3726	VC0725, VCA0072	BSU24970	NWMN_1298
<i>pstC</i>	TIGR02138, PF00528	K02037	phosphate transport system permease protein	b3727	VC0724, VCA0071	BSU24980	NWMN_1299
<i>pstS</i>	TIGR02136, TIGR00975, TIGR04505, PF12849	K02040	phosphate transport system substrate-binding protein	b3728	VC0721, VCA0070	BSU24990	NWMN_1300
<i>pitA, pitB</i>	PF01384	K16322	low-affinity inorganic phosphate transporter	b2987, b3493	–	–	–
<i>pit</i>	PF01384	K03306	inorganic phosphate transporter, PiT family	–	VC2442	BSU12840	NWMN_0633
<i>yjbB</i>	TIGR01013, TIGR00704, PF02690, PF01895	K03324	phosphate:Na ⁺ symporter	b4020	–	BSU25420	NWMN_0049
<i>nptA</i>	PF02690	K14683	solute carrier family 34 (sodium-dependent phosphate cotransporter)	–	VC0676	–	–
<i>phoU</i>	TIGR02135, PF01895	K02039	phosphate transport system regulatory protein	b3724	VC0727	–	NWMN_1296
	TIGR00153, PF01865	K07220	uncharacterized protein with PhoU-like domain	–	VC2441	BSU12850	NWMN_0632
<i>phoB</i>	TIGR02154, PF00072, PF00486	K07657	two-component system, OmpR family, phosphate regulon response regulator PhoB	b0399	VC0719	–	–
<i>phoP, phoB1</i>	phoB1, PF00072, PF00486	K07658	two-component system, OmpR family, alkaline phosphatase synthesis response regulator PhoP	–	–	BSU29110	NWMN_1586
<i>phoR</i>	phoR, TIGR02966, PF02518, PF00512	K07636	two-component system, OmpR family, phosphate regulon sensor histidine kinase PhoR	b0400	VC0720	BSU29100	NWMN_1585
<i>aspS</i>	TIGR00459	K01876	aspartyl-tRNA synthetase	b1866	VC1166	BSU27550	NWMN_1532
			Signal Recognition Particle (SRP) component with 4.5S RNA (<i>ffs</i>)				
<i>ffh</i>	TIGR00959	K03106	4.5S RNA (<i>ffs</i>)	b2610	VC0560	BSU15980	NWMN_1147
<i>fusA</i>	TIGR00484	K02355	elongation factor G	b3340	VC0361	BSU01120	NWMN_0509
<i>gltX</i>	TIGR00464	K01885	glutamyl-tRNA synthetase	b2400	VC2214	BSU00920	NWMN_0490
			fused protein chain initiation factor 2, IF2: membrane protein/conserved protein				
<i>infB</i>	TIGR00487	K02519	fused protein chain initiation factor 2, IF2: membrane protein/conserved protein	b3168	VC0643	BSU16630	NWMN_1178
<i>leuS</i>	TIGR00396	K01869	leucyl-tRNA synthetase	b0642	VC0956	BSU30320	NWMN_1651
<i>rplB</i>	TIGR01171	K02886	50S ribosomal subunit protein L2	b3317	VC2593	BSU01190	NWMN_2149
<i>rpoB</i>	TIGR02013	K03043	RNA polymerase, beta subunit	b3987	VC0328	BSU01070	NWMN_0504
<i>rpsE</i>	TIGR01021	K02988	30S ribosomal subunit protein S5	b3303	VC2579	BSU01330	NWMN_2135
<i>rpsH</i>	PF00410	K02994	30S ribosomal subunit protein S8	b3306	VC2582	BSU01300	NWMN_2138
<i>rpsK</i>	PF00411	K02948	30S ribosomal subunit protein S11	b3297	VC2573	BSU01420	NWMN_2127
<i>topA</i>	TIGR01051	K03168	DNA topoisomerase I	b1274	VC1730	BSU16120	NWMN_1160
<i>tufA</i>	TIGR00485	K02358	elongation factor Tu	b3339	VC0362	BSU01130	NWMN_0510

7 Strains ^a*Escherichia coli* K-12 MG1655, ^b*Vibrio cholerae* O1 biovar El Tor N16961, ^c*Bacillus subtilis* subsp. *subtilis* 168, ^d*Staphylococcus aureus* subsp. *aureus* Newman

8 **Supplemental Table 3.** PCR primers used in this study.

Name	Sequence
pst KO 5'F	GACGACAAGTTTGTACAAAAAAGCAGGCTGAATAGGCTGGGACATTAAGTTCTTAGGC
pst KO 5'R	TCAACCAAACCTTCCTGAAAATTGCCATTTTTTCATTGAAATATCC
pst KO 3'F	ATGAAAAAATGGCAATTTTCAGGAAGGTTTGGTTGATATATAATGGC
pst KO 3'R	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGTGTAGACATTACTACAGAACAGCC
nptA KO 5'F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGACCCACTTGCAGGCAAAACAGTTACAG
nptA KO 5'R	ATTTCTTCATCTGTAGGTACGACATTTCCATTATTTACCTCAAATAAGC
nptA KO 3'F	GGGGACCACTTTGTACAAGAAAGCTGGGTCGGTGTTCAGGTACATTAATTGTAGCG
nptA KO 3'R	GGTGAAATAATGAAAATGTCGTACCTACAGATGAAGAAAATTGCAACAAC
pst comp 5'F	CAGTGGTACCGGGAGGATATTTCAATGAAAAAATGGC
pst comp 5'R	GGACTTATAGCATTAAACTCTCCAAGACCTG
pst comp 3'F	GGTTTCTTTGCATTAACCTTTGTTACACCAG
pst comp 3'R	CAGTGAGCTCGCCATTATATATCAACCAAACCTTCC
pitA comp F	GCGTGAGCTCGGGGAGTATATATTTATGTCATATATAATCATCG
pitA comp R	GCGTGGTACCCAATTAGAAAAATAAGTTAAGTATATAGAATAGTAAACC
nptA comp F	GACGGAGCTCTTGAGGTGAAATAATGGAAATGTCGGTTACA
nptA comp R	GATGGAATTCCAGTAAAAATTAATTTTTCAGTTGTTGCAATTTT
pst prom F	GGGGCTGCAGTACATGTTAATACGTAGTATTAATGGCGAGAC
pst prom R	GGGGGGTACCTGAAATATCCTCCCTGTATGAACAACAA
pitA prom F	GGGGCTGCAGAATATACAGATGGCTTTCAGTAGAGTAGTGG
pitA prom R	GGGGGGTACCTTAAAAATCCTCCATTTAAGCGATTGTCACC
nptA prom F	GGGGCTGCAGCGAAAACCATTAATAGATTTTTATTTGGTGATTTCAAAT
nptA prom R	GGGGGGTACCTATTTTACCTCAAATAAGCTTTATATTTAGATTATCGC

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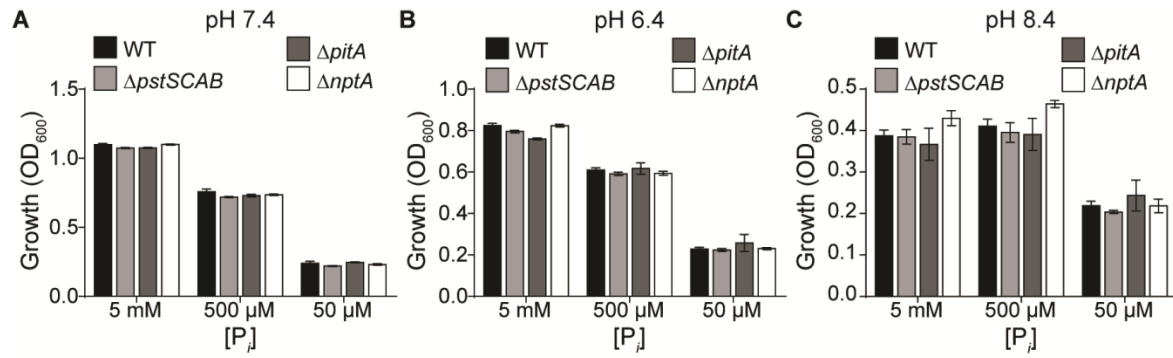


10 Supplemental Figure 1

11 **Supplemental Figure 1. *Staphylococcus aureus* reacquired *nptA* after ancestral**

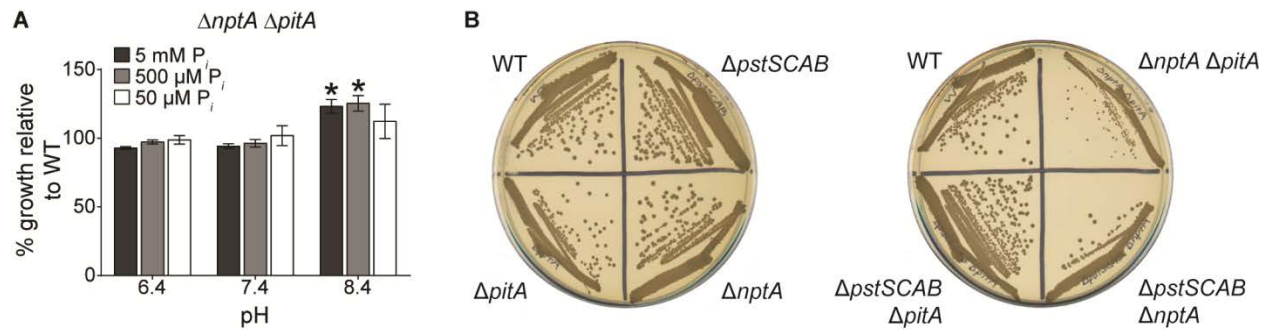
12 **loss. (A) BLAST analysis of the *S. aureus* Newman genome identified three putative P_i**

13 acquisition system loci: *pstSCAB* (top), *pitA* (middle), and *nptA* (bottom). Structural
14 genes of the transporters are in white. Each locus contains a *phoU* (light gray) gene
15 (*pstSCAB* and *pitA*) or domain (*nptA*). Flanking genes are in dark gray. Locus numbers
16 correspond to the *S. aureus* Newman genome annotation. Genes and intergenic
17 regions are not drawn to scale. **(B)** Schematic representation of *nptA* encoding regions
18 from six staphylococcal species aligned with MAUVE illustrates variable syntenic genes
19 flanking *nptA* (red). Species are assigned groups 1-4 based on the degree of synteny
20 observed. Colored rectangles are shared homologous genes with greater than 50% nt
21 identity, white are unique. **(C)** Maximum likelihood phylogeny generated from aligned
22 Staphylococcal *nptA* alleles suggests *nptA* was present in the common ancestor of the
23 genus *Staphylococcus* and that presence of *nptA* in *S. aureus* is due to HGT (red). **(D)**
24 Possible gains and losses of *nptA* in the genus *Staphylococcus* are overlaid on a
25 maximum likelihood phylogeny for 38 *Staphylococcus* spp. generated from 13
26 conserved core genes. Thick lines indicate *Staphylococcus* lineages with *nptA* and thin
27 lines indicate those without. Circled letters indicate predicted gain/loss events based on
28 parsimony. In **(C)** and **(D)**, branch lengths reflect the number of amino acid substitutions
29 per site, and bootstrap values ≥ 75 are indicated at the relevant nodes.



30 Supplemental Figure 2

31 **Supplemental Figure 2. The *S. aureus* P_i transporters compensate for one another**
 32 ***in vitro*. A-C.** Growth measured by OD₆₀₀ of wild type, ΔpstSCAB, ΔpitA, ΔnptA after 12
 33 hours in PFM9 supplemented with various P_i concentrations adjusted to pH 7.4 (A), 6.4
 34 (B), or 8.4 (C). n=3; error bars indicate SEM.



35 Supplemental Figure 3

36 **Supplemental Figure 3. PstSCAB is insufficient for growth of *S. aureus* on rich**

37 **media agar plates. (A)** Growth of *ΔnptA ΔpitA* in PFM9 adjusted to pH 6.4, 7.4, or 8.4

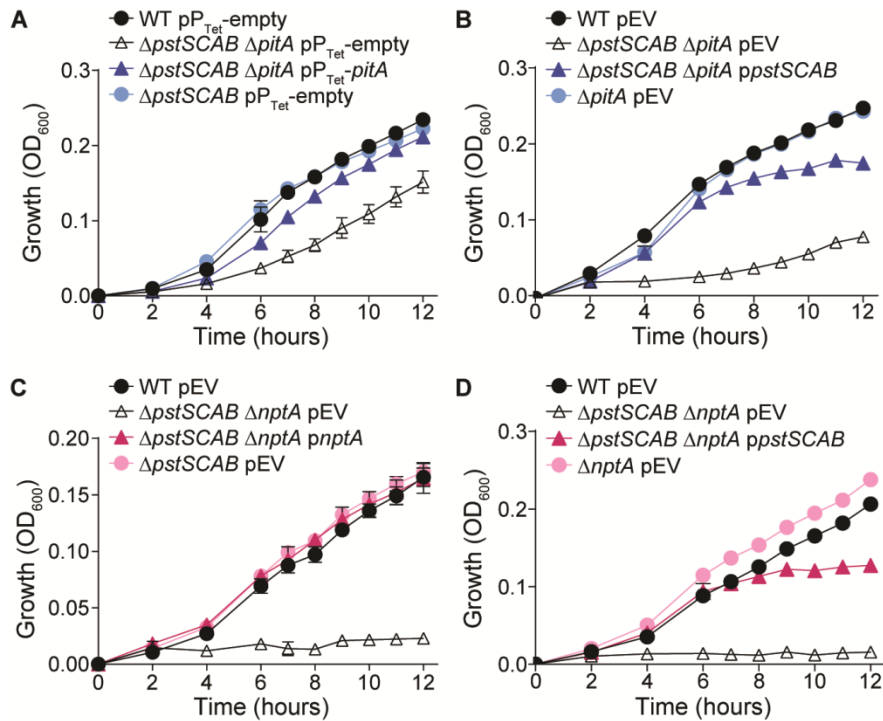
38 with different supplemental P_i concentrations was measured by OD_{600} and normalized

39 to wild type. $n=3$; error bars indicate SEM. * = $p < 0.05$ compared to wild type via two-

40 way ANOVA with Dunnett's posttest. **(B)** Wild type and the single transporter mutants

41 (*ΔpstSCAB*, *ΔpitA*, and *ΔnptA*) or the double transporter mutants (*ΔnptA ΔpitA*,

42 *ΔpstSCAB ΔpitA*, and *ΔpstSCAB ΔnptA*) were grown on tryptic soy agar plates.

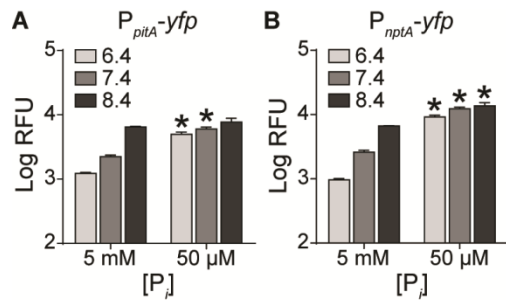


43 Supplemental Figure 4

44 **Supplemental Figure 4. Growth defects of $\Delta pstSCAB \Delta pitA$ and $\Delta pstSCAB \Delta nptA$**
 45 **can be complemented by ectopic expression of either deleted transporter.**

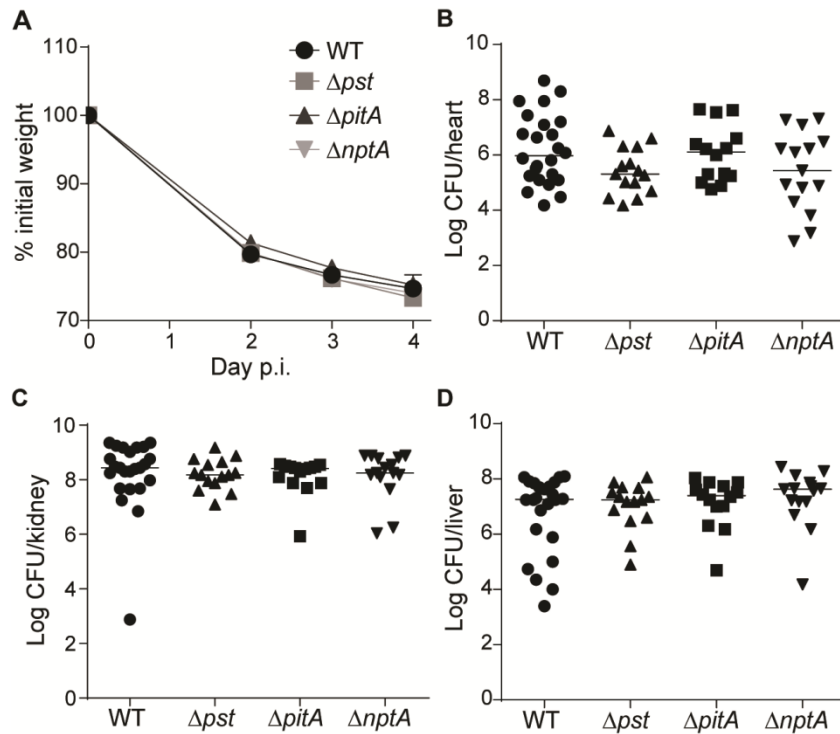
46 **A.** Growth of wild type *S. aureus*, $\Delta pstSCAB$, and $\Delta pstSCAB \Delta pitA$ carrying empty
 47 vector (pP_{Tet}-empty) and $\Delta pstSCAB \Delta pitA$ carrying pP_{Tet}-*pitA* in PFM9, pH 6.4, with 50
 48 $\mu M P_i$ as measured by OD₆₀₀. n=3; error bars indicate SEM and are frequently smaller
 49 than the symbols. **B.** Growth of wild type *S. aureus*, $\Delta pstSCAB$, and $\Delta pstSCAB \Delta pitA$
 50 carrying empty vector (pEV) and $\Delta pstSCAB \Delta pitA$ carrying *ppstSCAB* in PFM9, pH 6.4,
 51 with 50 $\mu M P_i$ as measured by OD₆₀₀. n=3; error bars indicate SEM and are frequently
 52 smaller than the symbols. **C.** Growth of wild type *S. aureus*, $\Delta pstSCAB$, and $\Delta pstSCAB$
 53 $\Delta nptA$ carrying empty vector (pEV) and $\Delta pstSCAB \Delta nptA$ carrying *pnptA* in PFM9, pH
 54 8.4, with 50 $\mu M P_i$ as measured by OD₆₀₀. n=3; error bars indicate SEM and are
 55 frequently smaller than the symbols. **D.** Growth of wild type *S. aureus*, $\Delta nptA$, and

56 $\Delta pstSCAB \Delta nptA$ carrying empty vector (pEV) and $\Delta pstSCAB \Delta nptA$ carrying
57 $ppstSCAB$ in PFM9, pH 8.4, with 50 μM P_i as measured by OD_{600} . n=3; error bars
58 indicate SEM and are frequently smaller than the symbols.



59 Supplemental Figure 5

60 **Supplemental Figure 5. *pitA* and *nptA* are expressed by *S. aureus* independent of**
 61 **pH. A-B.** Expression of *pitA* (A) and *nptA* (B) after 9 hrs of growth in PFM9
 62 supplemented with 5 mM (excess) or 50 μM (limiting) P_i adjusted to pH 6.4, 7.4, or 8.4.
 63 Expression was assessed using wild type *S. aureus* carrying the reporter plasmids P_{*pitA*}-
 64 *yfp* and P_{*nptA*}-*yfp*. * = $p < 0.05$ compared to 5 mM P_i at the same pH via two-way
 65 ANOVA with Dunnett's posttest. n=3; error bars indicate SEM.



66 Supplemental Figure 6

67 **Supplemental Figure 6. *S. aureus* P_i transporters compensate for one another in a**
 68 **systemic mouse model of infection.** Wild type C57BL/6J mice were infected with *S.*
 69 *aureus* wild type, $\Delta pstSCAB$, $\Delta pitA$, and $\Delta nptA$ ($\Delta pst = \Delta pstSCAB$). Weight loss was
 70 monitored (**A**) and bacterial burdens in the heart (**B**), kidney (**C**), and liver (**D**) were
 71 enumerated four days post-infection. Error bars indicate SEM. The lines indicate
 72 medians. The data are results from three independent experiments. $n \geq 14$ for each
 73 group.

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