

1 **Supplementary Information**

2 **A point mutation in AgrC determines cytotoxic or colonizing properties associated**  
3 **with phenotypic variants of ST22 MRSA strains.**

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26 **Supplementary Methods**

27 **Gel Filtration Chromatography for AgrC<sub>Y223C</sub>**

28 Analytical size exclusion experiments were conducted using Superdex S200 column to  
29 determine the oligomeric status of AgrC<sub>Y223C</sub>. Also, AgrC<sub>Y223C</sub> was incubated with DTT to  
30 examine their effect on the Cysteine 223 and therefore on the oligomeric status of AgrC<sub>Y223C</sub>.

31 **Toxin ELISAs**

32 Levels of  $\alpha$ -toxin and PVL were determined by ELISA using toxin-specific antibodies  
33 provided by GSK Vaccines (Belgium) and bioMérieux R&D Immunodiagnostic (France),  
34 respectively<sup>1,2</sup>.

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36 **SupplementaryTable 1. Strains and plasmids used in this study**

Strain/plasmid	Clinical diagnosis	MRSA/MSSA	Response Profile*	$\alpha$ -toxin ( $\mu\text{g/ml}$ ) <sup>#</sup>	PVL ( $\mu\text{g/ml}$ ) <sup>#</sup>	Reference/Source
<b>(A) <i>S. aureus</i> strains</b>						
PUNE08 (Sa08)	SSTI	MRSA-IV	C	75	19.6	<sup>3</sup>
PUNE08 agrC-Y223C	-	MRSA-IV	P	N.D	N.D	This study
M37 (Sa37)	SSTI	MRSA-IV	P	9	1.5	<sup>3</sup>
M37 agrC-C223Y	-	MRSA-IV	C	N.D	N.D	This study
Sa6454	SSTI	MRSA-IV	C	18	1.6	This study
SaN08	Meningitis	MRSA-IV	C	32	8	<sup>3</sup>
Sa113	Brain abscess	MRSA-IV	C	20	3.2	<sup>3</sup>
Sa114	Cerebral abscess	MRSA-IV	C	23	4	<sup>3</sup>
Sa754	Invasive infection	MRSA-IV	C	20	1	<sup>3</sup>
Sa165	colonizing strain	MRSA-IV	C	19.3	2.3	<sup>3</sup>
Sa115	colonizing strain	MRSA-IV	P	0.2	0.08	<sup>3</sup>
<b>(B) EMRSA-15 reference <i>S. aureus</i> strains</b>						
HAR22	SSTI	MRSA-IV	C	N.D	N.D	Provided by Dr. H. Lencastre <sup>4</sup>
NCTC 13142	SSTI	MRSA-IV	C	N.D	N.D	Provided by A Kearns <sup>5</sup>
<b>(C) <i>E. coli</i> strains</b>						
BL21 (DE3)	-	-				NEB
IM08B	-	-				<sup>6</sup>
<b>(D) Plasmids</b>						
pIMAY-Z	-	-				<sup>6</sup>
pET22b						Novagen Inc.
pET28a						<sup>7</sup>

37 MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; P, proliferative; C, cytotoxic; SSTI, skin and soft tissue infection. N.D, Not  
38 determined; Names in parenthesis for strains are nomenclature based on previous publication

39 \* The response profile is determined by the pattern of proliferative responses elicited by different dilutions of bacterial supernatants against PBMC. A  
40 proliferative profile was denoted if all dilutions elicited a proliferative response. A cytotoxic pattern was denoted if a) proliferation was only noted at the highest  
41 dilution of the supernatants, and b) the supernatants (1:50 dilution) resulted in inhibition of PHA-induced responses in co-stimulation experiments.

42 #Amounts of toxins present in supernatants cultures grown to stationary phase (17hours)

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57 **SupplementaryTable 2. Toxin gene profile of the clinical ST22 *S. aureus* strains.**

Strains	ST/SPA type	<i>agr</i> type	<i>se/tsst-1/egc</i>	<i>pvl</i>	<i>lukD/E</i>	Staphylokinase	Hemolysins	Proteases	SSL genes	MSCRAMM/ Adhesin	Capsule/ Biofilm
PUNE08	ST22/t852	I	-/-/+	+	-	+	<i>hla,hlb,hld,</i>	<i>aur, sspA,B,P</i>	<i>ssl 5,7,9</i>	<i>clf A,B, cna, eno, fnbA, sdrC,</i>	5/ <i>ica A,C,D</i>
M37	ST22/t852	I	-/-/+	+	-	+	<i>hla,hlb,hld,</i>	<i>aur, sspA,B,P</i>	<i>ssl 5,7,9</i>	<i>clf A,B, cna, eno, fnbA, sdrC</i>	5/ <i>ica A,C,D</i>

58 Based on gene microarray data: *se*, staphylococcal enterotoxin; *pvl*, Panton-Valentine leucocidin; *hla*, alpha-hemolysin; *hnb*, beta-hemolysin; *hld*, delta-  
59 hemolysin; *spl*, serine protease like; *sspA*, serine protease; *sspB*, cysteine protease staphopain B; *sspP*, staphopain A precursor; *ssl*, staphylococcal  
60 superantigen-like; *clf*, clumping factor; *cna*, collagen adhesins; *eno*, enolase; *fib*, fibrinogen binding-protein; *fnbA*, fibronectin A; *sdrC*, serine-aspartate repeat-  
61 containing protein C

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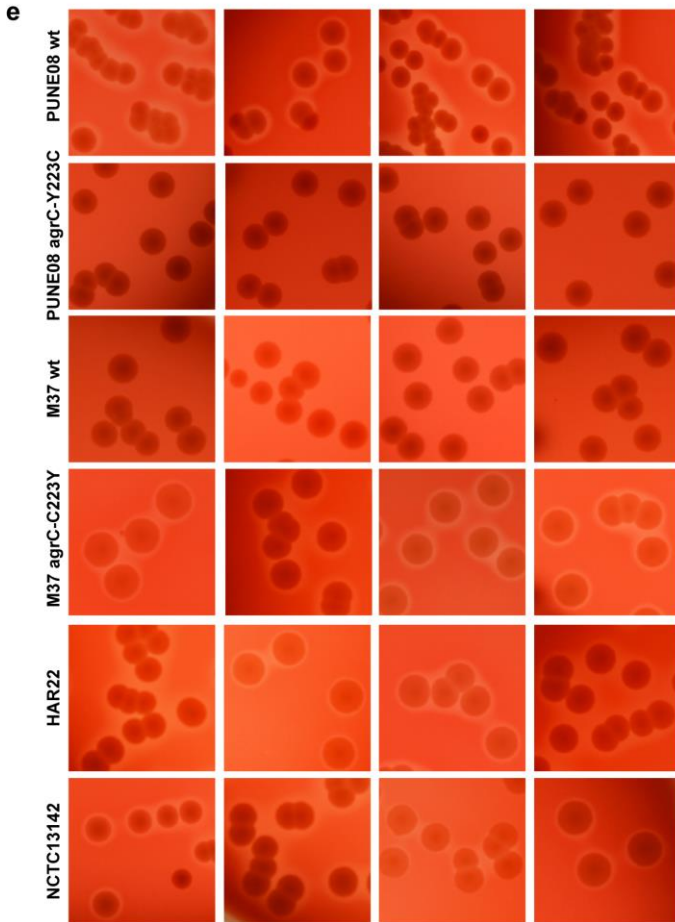
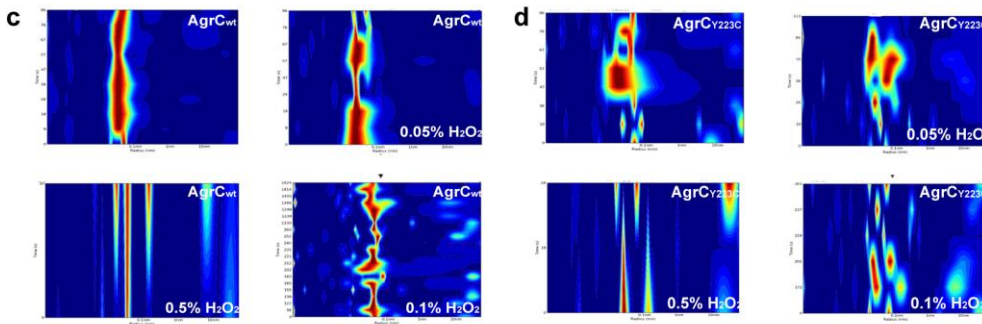
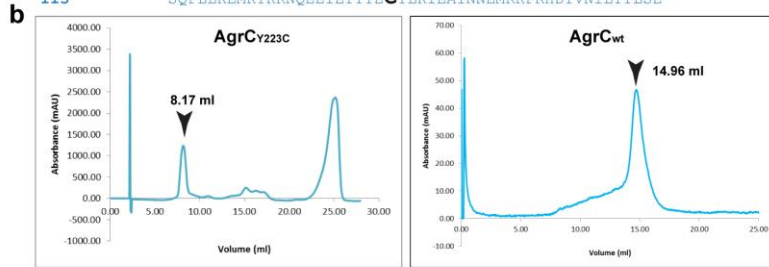
63 **Supplementary Table 3. qRT-PCR and *agr*-sequencing primers used in this study**

<b>Gene</b>	<b>Primer</b>	<b>Sequence (5'-3')</b>
<i>agrC</i>	AgrC_F	TTATGACTTCGCAGATTATTCTGTACT
	AgrC_R	TAGAAAAACTTTCTTGGAAACAATTCAT
<i>hla</i>	hla_F	CGGCACATTTGCACCAATAAGGC
	hla_R	GGTTTAGCCTGGCCTTCAGC
<i>RNAIII</i>	RNAIII_F	GTGATGGAAAATAGTTGATGAGTTGTTT
	RNAIII_R	GAATTTGTTCAGTGTGTCGATAATCC
<i>psma</i>	psma_F	TATCAAAAAGCTTAATCGAACAATTC
	psma_R	CCCCTTCAAATAAGATGTTTCATATC
<i>lukSPV</i>	lukSPV_F	GTGTTGTTCTTCTAGTAGCATGAGT
	lukSPV_R	ATCACATCATTAGGTAAAATGTCTG
<i>spa</i>	spa_F	CAACGGAGTACATGTCGTTA
	spa_R	GCTTGAGCTTTGTTAGCATC
<i>clfA</i>	clfA_F	CAAAGGTGATTTAGCTTTACGTTCAACTT
	clfA_R	TGCTACTTCGTTGTCCCATGAC
<i>clfB</i>	clfB_F	AATGTGTTACCACTTTGATTAGGGTCAA
	clfB_R	GCTGCTGATGCTAAAGGTACAAATG
<i>fnbA</i>	fnbA_F	AACCATTATCCCAAGTTAAGGTATATCCTCT
	fnbA_R	TGGTACTGATGAAGTTGATTTTAGAACACA
<i>IL-6</i>	hIL-6_F	AATTCGGTACATCCTCGACGG
	hIL-6_R	GGTTGTTTTCTGCCAGTGCCT
<i>TNF</i>	hTNF_F	TGGCCCAGGCAGTCAGA
	hTNF_R	GGTTTGCTACAACATGGGCTACA
<i>IL-1<math>\beta</math></i>	hIL-1 $\beta$ _F	GCCCTAAACAGATGAAGTGCTC
	hIL-1 $\beta$ _R	GAACCAGCATCTTCCTCAG
<i>CXCL8</i>	hIL-8_F	GAGCACTCCATAAGGCACAAA
	hIL-8_R	ATGGTTCCTTCCGGTGGT
<i>LL37</i>	hLL37-F	TCGGATGCTAACCTCTACCG
	hLL37-R	GTCTGGGTCCCCATCCAT
<i>beta-Actin</i>	hbetaAct_F	CTCTTCCAGCCTTCCTTCCCT
	hbetaAct_R	AGCACTGTGTTGGCGTACAG

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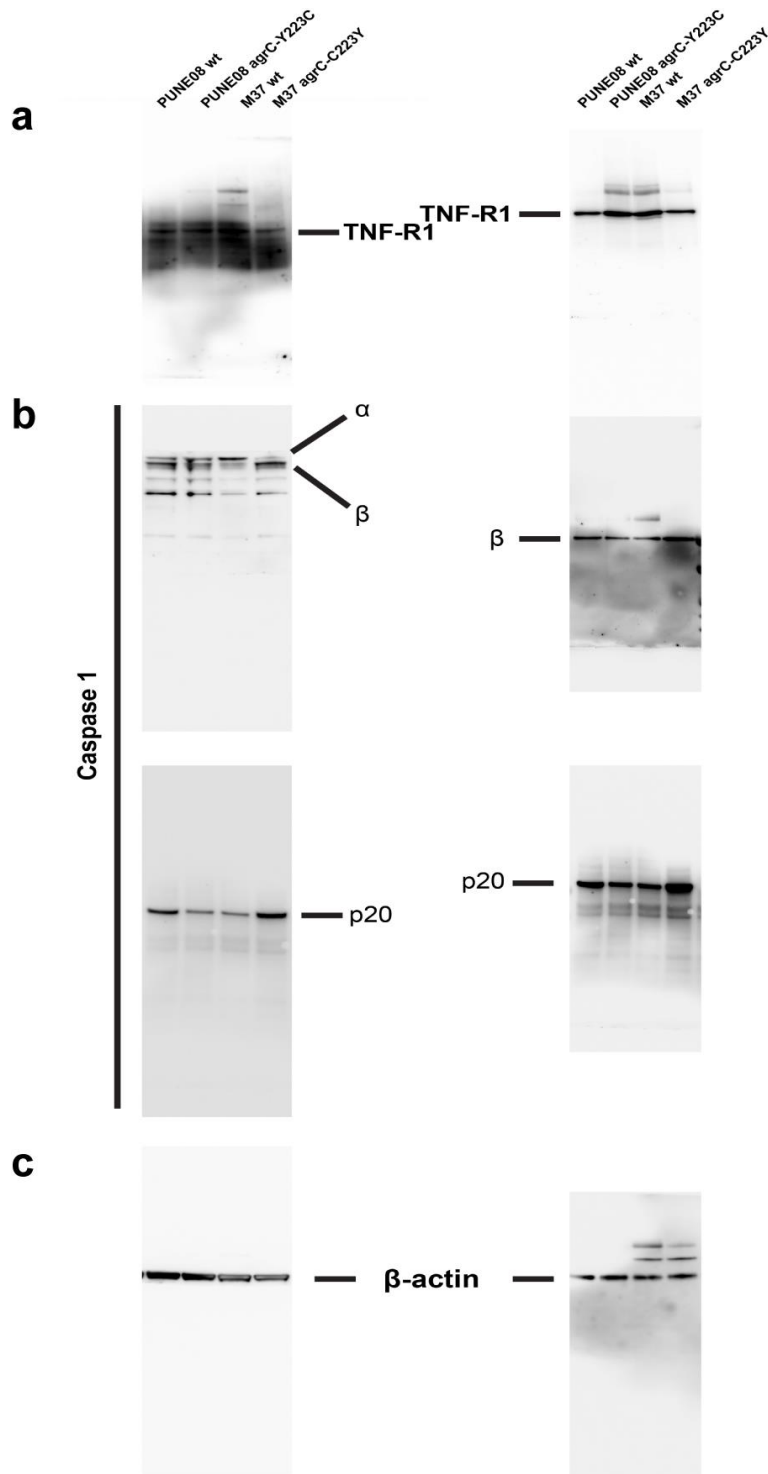
**a**

PUNE08	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
M37	SQFLKEMKYKRNQEEIETYYE <del>CT</del> LKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
HAR22	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
NCTC13142	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
NIM08	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
NP113	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
NP114	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
GH754	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
165	SQFLKEMKYKRNQEEIETYYEYTLKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE
115	SQFLKEMKYKRNQEEIETYYE <del>CT</del> LKIEAINNEMRKRFRHDYVNI <del>LT</del> TLSE



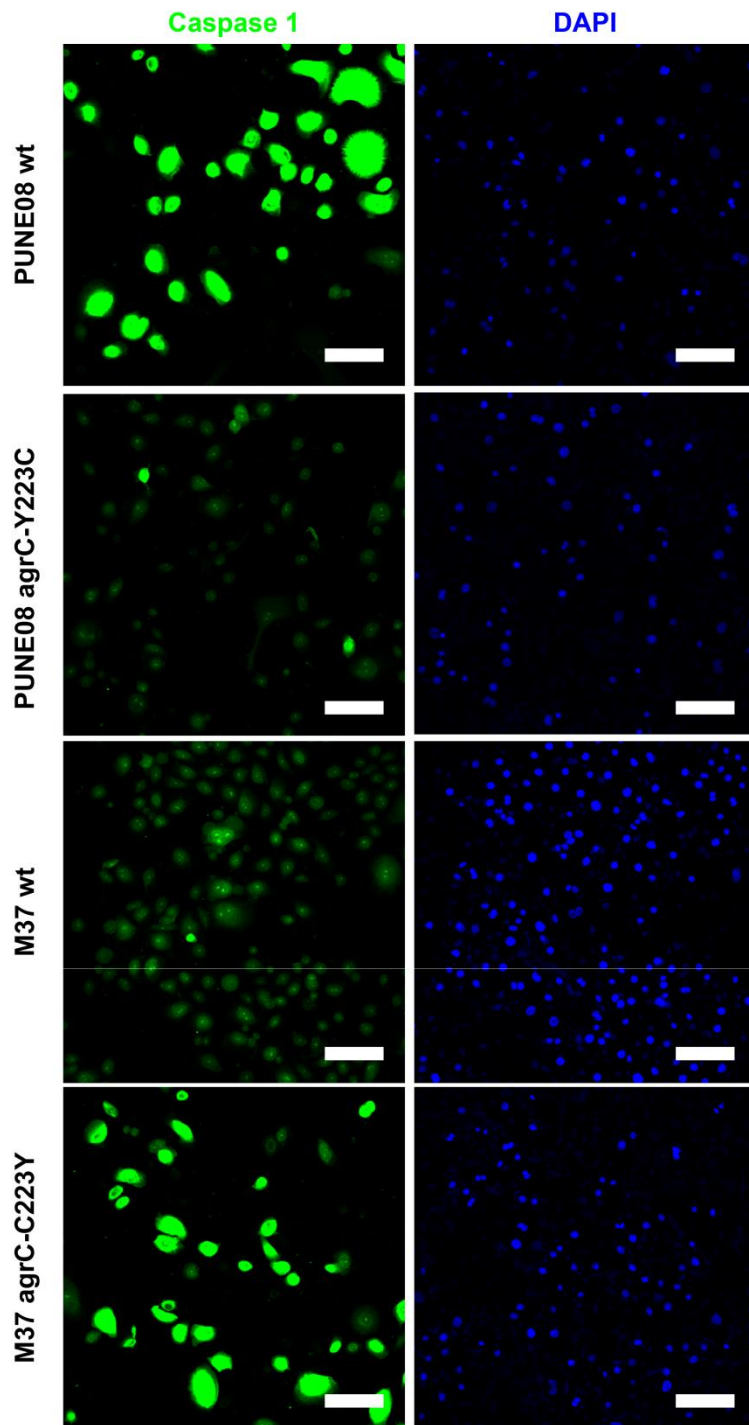
66 **Supplementary Figure 1.** Amino acid sequence variation in AgrC results in differential  
67 phenotypic response profiles of clinical ST22 *S. aureus* strains. **(a)** Amino acid sequence  
68 analysis of AgrC from indicated strains at specified positions. **(b)** Size exclusion profile of  
69 AgrC<sub>Y223C</sub> and wild-type AgrC. The chromatograms reveal that the AgrC<sub>Y223C</sub> protein is prone  
70 to aggregation and elutes in the void volume of the experiment. **(c)** DLS profile for wild-type  
71 AgrC<sub>t</sub> with increasing H<sub>2</sub>O<sub>2</sub>. **(d)** DLS profile for AgrC<sub>Y223C</sub> with increasing H<sub>2</sub>O<sub>2</sub> concentration.  
72 Addition of H<sub>2</sub>O<sub>2</sub> seemed to drastically increase the aggregation of AgrC<sub>Y223C</sub> protein. **(e)**  
73 Representative images of hemolysis on blood agar plates induced by indicated clinical and  
74 respective mutant *S. aureus* strains.  
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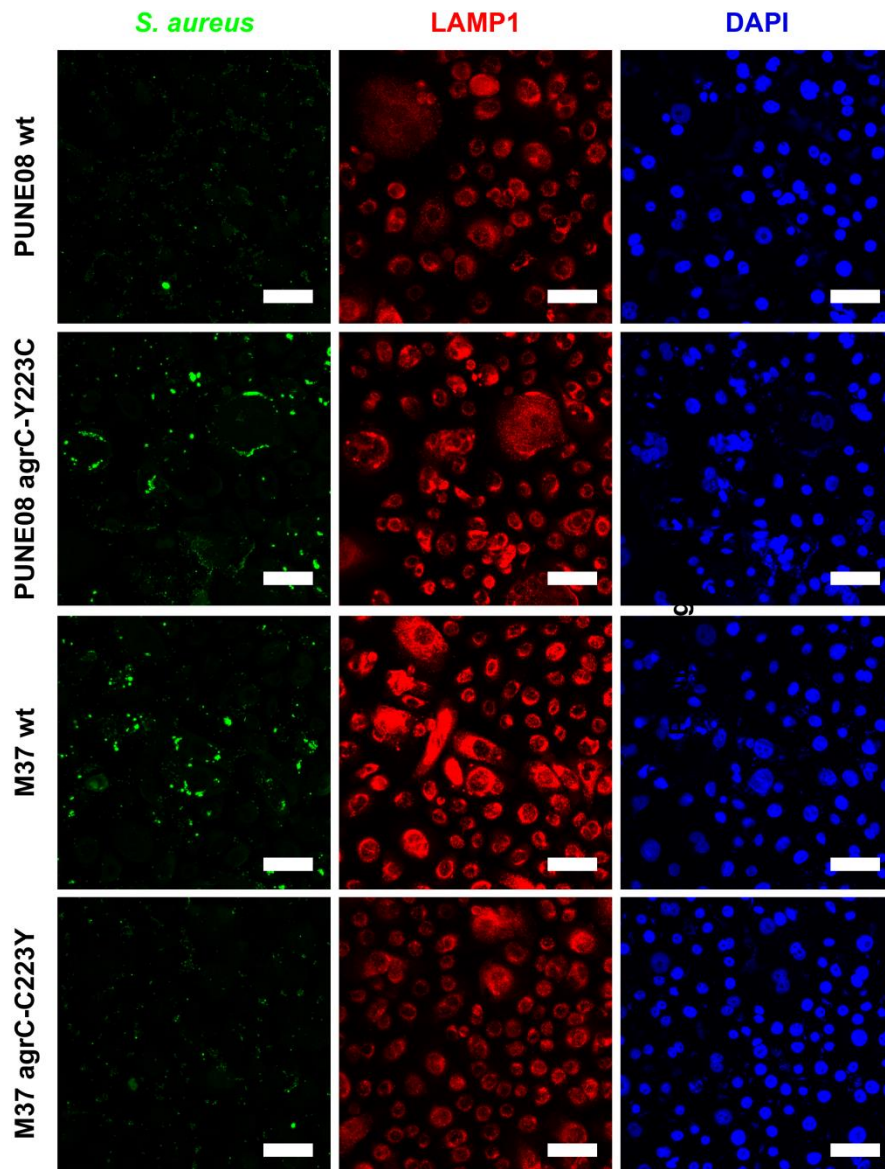
77 **Supplementary Figure 2.** Representative images of Western Blot analyses of (a) TNF-R1  
 78 and (b) Caspase 1 subunits expression after 2 h (left panel) and 4 h (right panel) of  
 79 keratinocytes infection. (c) β-Actin was used as a loading control (n=4). Uncropped full-size  
 80 blots are shown.



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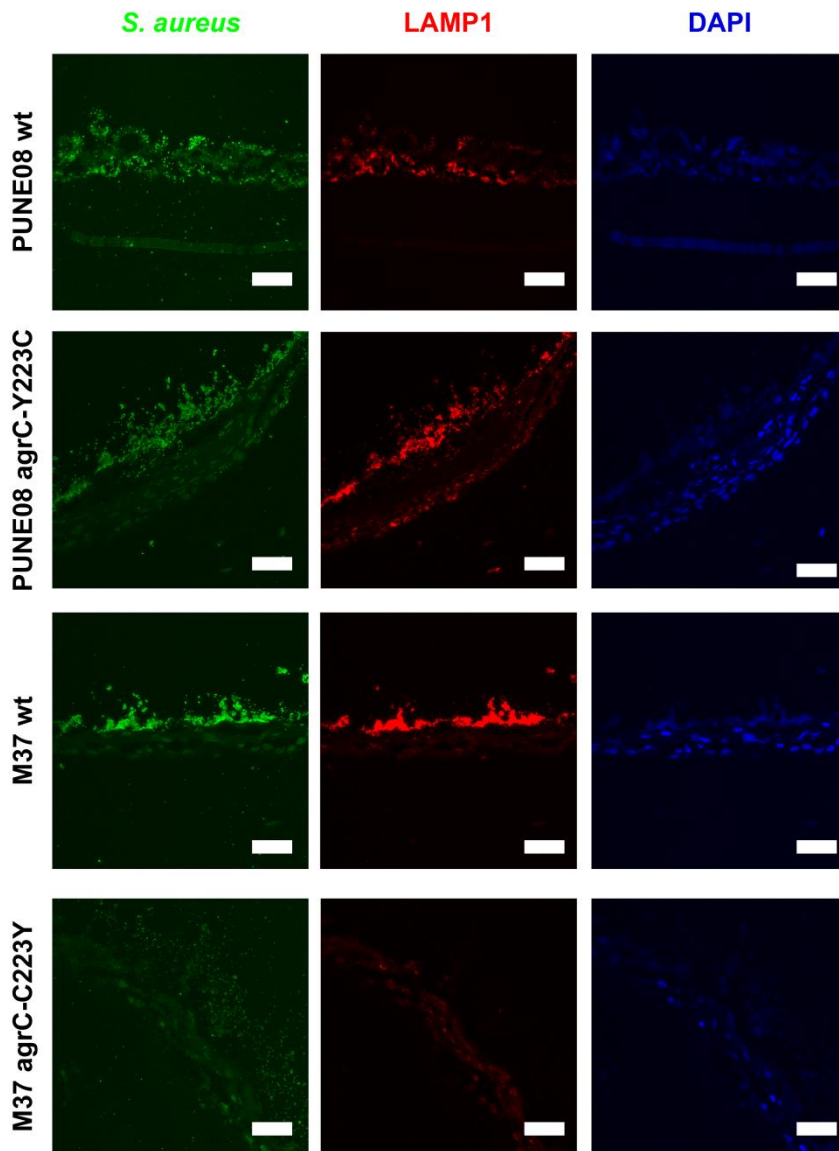
82 **Supplementary Figure 3.** Cytotoxic strains induce caspase 1 activation in infected  
 83 keratinocytes. Representative single channel images of Caspase 1 (green; left panel)  
 84 secretion and DAPI (blue; right panel) after 2 h of keratinocytes monolayer infection with  
 85 indicated strains. The scale bars in images equal 60  $\mu$ m.

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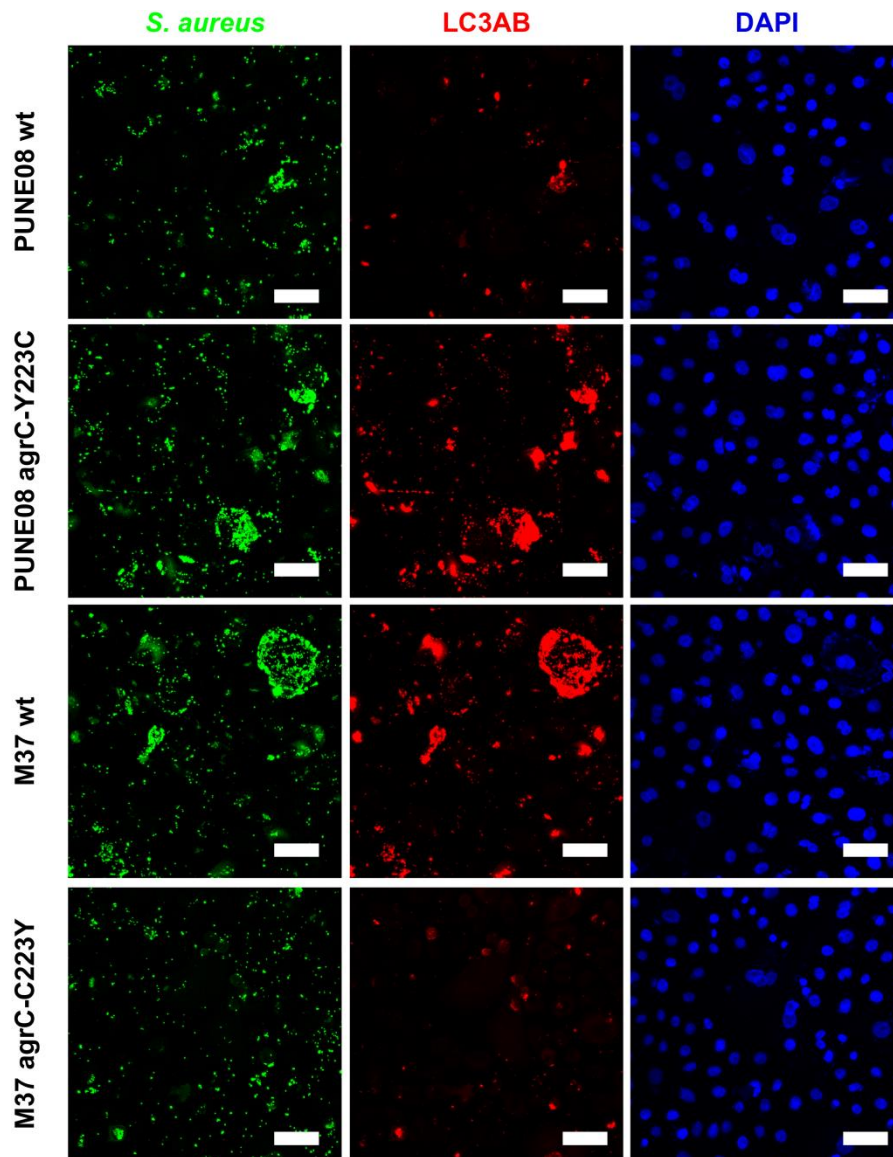
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88 **Supplementary Figure 4.** *S. aureus* strains exhibiting colonizing phenotype reside within  
 89 phagolysosome in infected Keratinocytes. Representative single channel images of LAMP1  
 90 (red; center panel), DAPI (blue; right panel) and *S. aureus* (green; left panel) co-localization  
 91 in infected keratinocytes monolayers after 2 h of infection. The scale bars in images equal  
 92 60  $\mu\text{m}$ .



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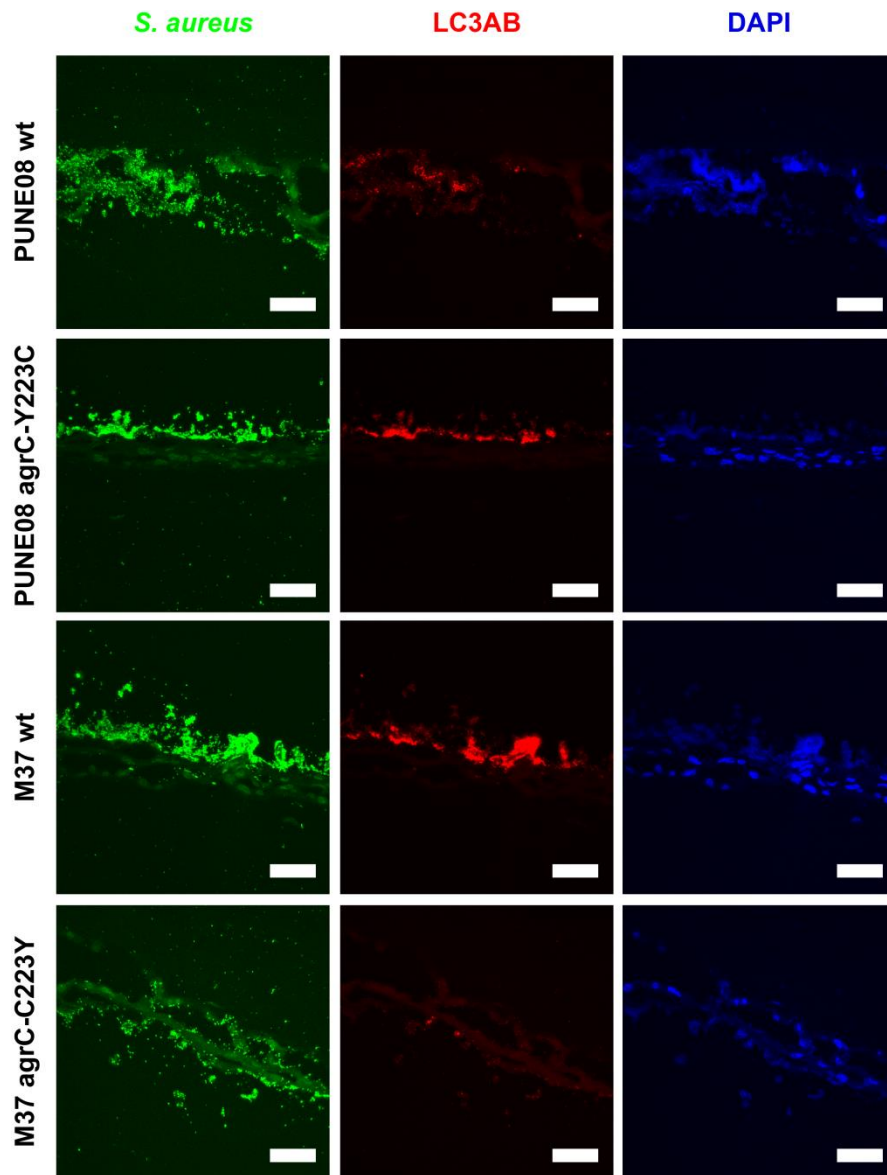
94 **Supplementary Figure 5.** *S. aureus* strains exhibiting colonizing phenotype reside within  
 95 phagolysosome in infected skin tissue models. Representative single channel images of  
 96 LAMP1 (red; center panel), DAPI (blue; right panel) and *S. aureus* (green; left panel) co-  
 97 localization in infected skin tissue models after 24 h of infection. The scale bars in images  
 98 equal 60  $\mu$ m.



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100 **Supplementary Figure 6.** *S. aureus* strains exhibiting colonizing phenotype induce  
 101 autophagy in infected Keratinocytes. Representative single channel images of LC3AB (red;  
 102 center panel), DAPI (blue; right panel) and *S. aureus* (green; left panel) co-localization in  
 103 infected keratinocytes monolayers after 2 h of infection. The scale bars in images equal  
 104 60  $\mu$ m.





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106 **Supplementary Figure 7.** *S. aureus* strains exhibiting colonizing phenotype induce  
 107 autophagy in infected skin tissue models. Representative single channel images of LC3AB  
 108 (red; center panel), DAPI (blue; right panel) and *S. aureus* (green; left panel) co-localization  
 109 in infected skin tissue models after 24 h of infection. The scale bars in images equal 60  $\mu$ m.

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