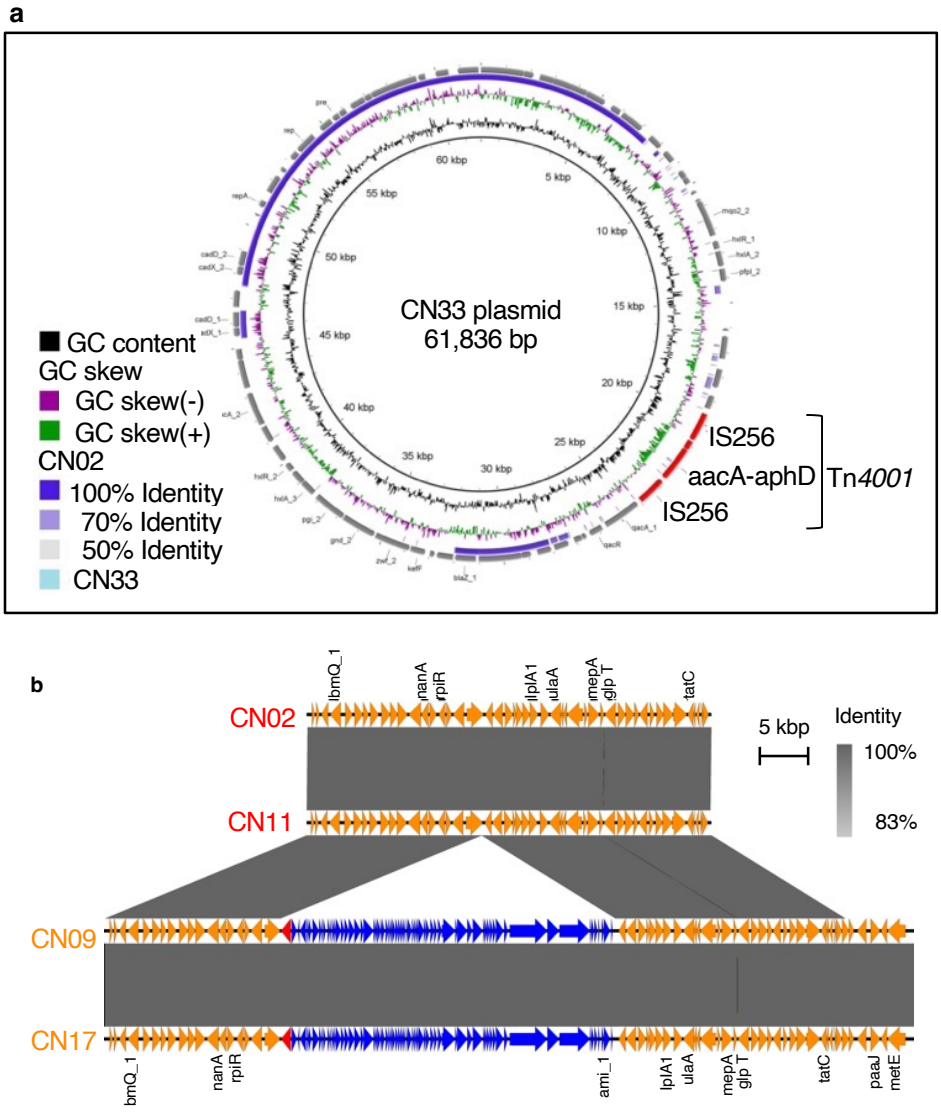
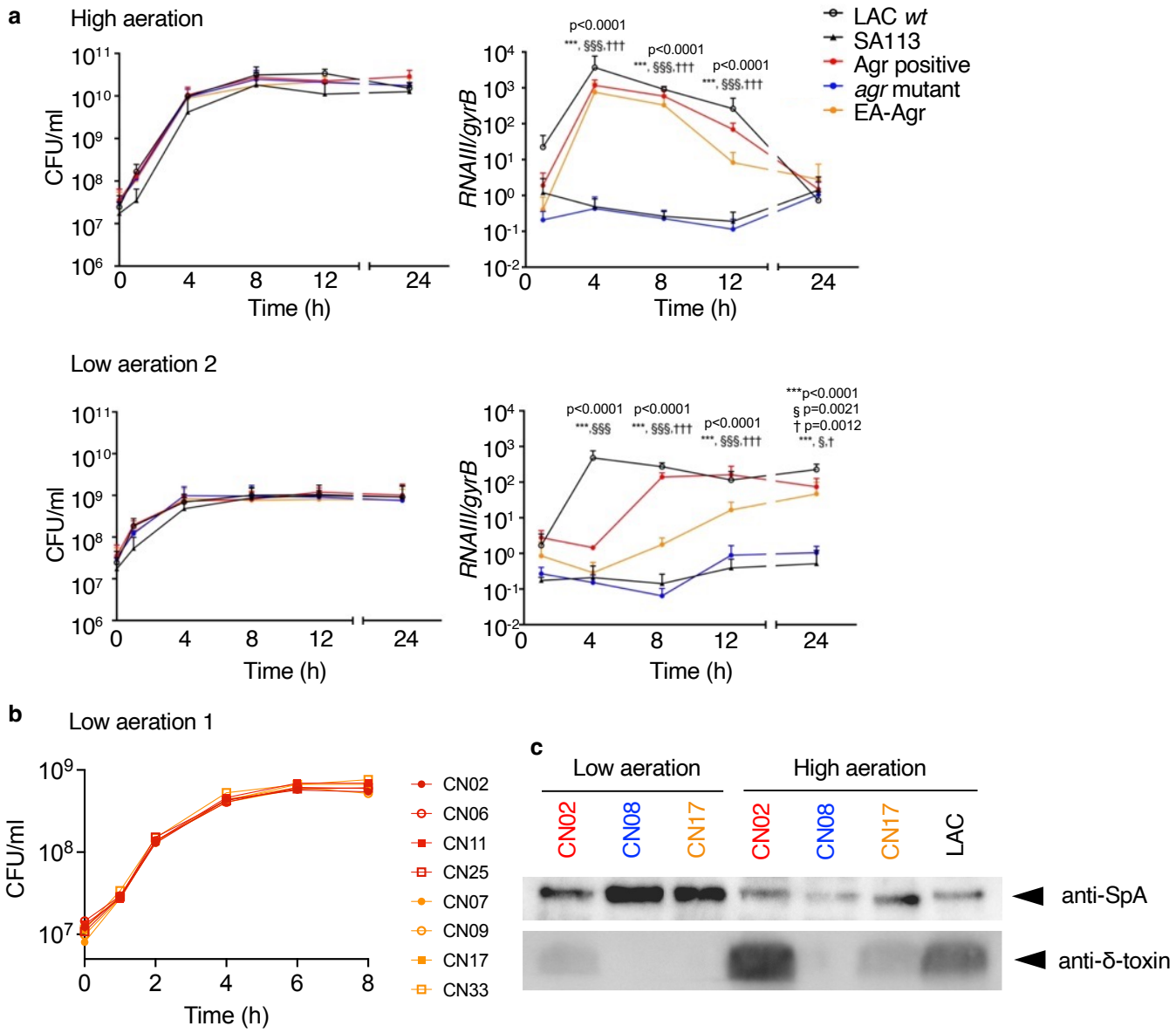


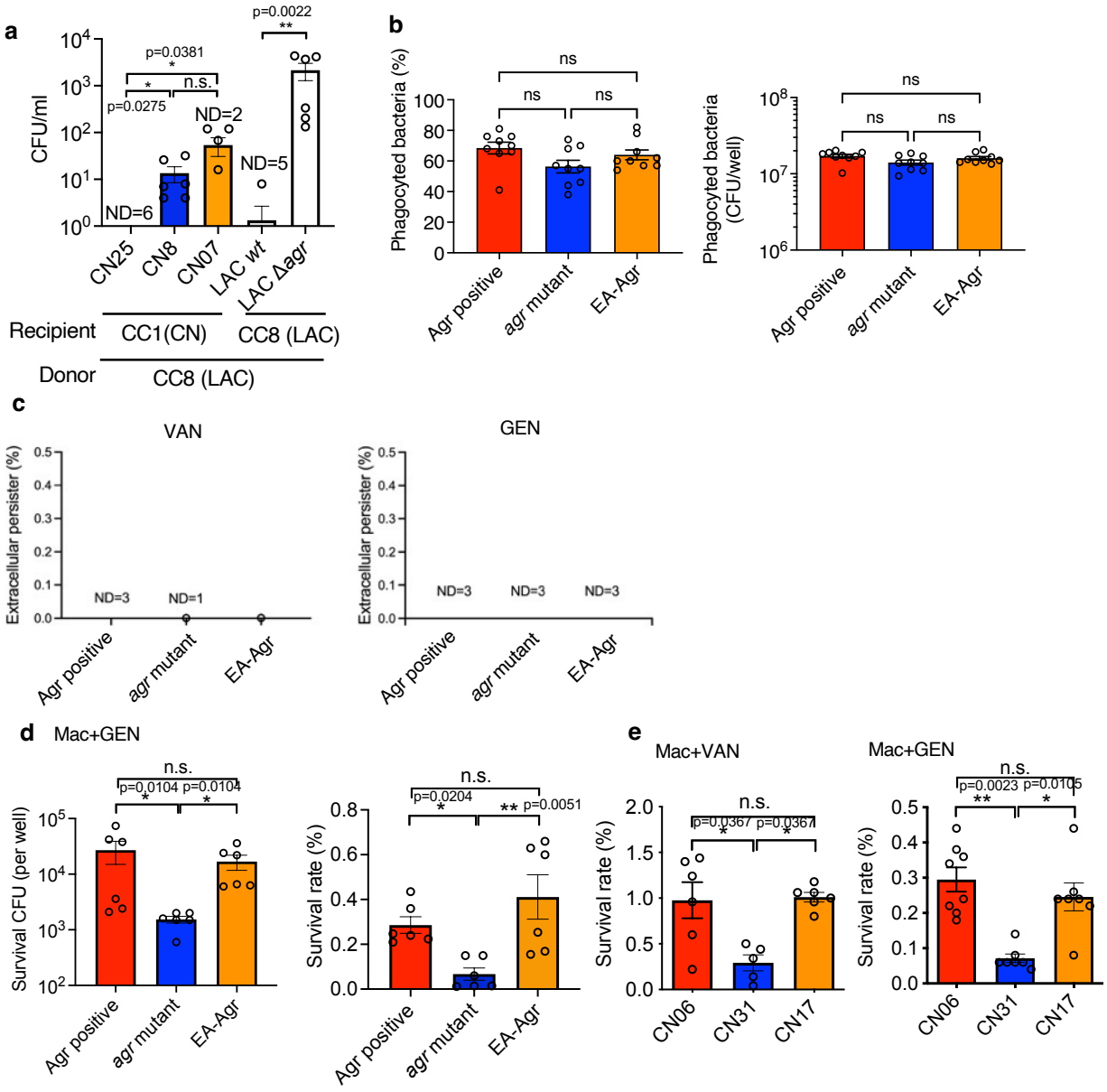
Supplementary figure 1. Maps of plasmids and mobile gene elements acquired by outbreak lineage in representative isolates

(a) Plasmid map of CN33. The purple rings show the position of BLAST hits within the plasmid in CN02. The outermost rings show the coding sequences of each plasmid indicated by arrowheads. The red bars in outermost rings indicate resistant genes. The second outermost rings display GC skewing, and the black ring displays the GC content. Tn*4001*, a transposon containing *aacA-aphD* in plasmid CN33 is indicated. aacA-aphD: aminoglycoside modifying enzyme. **(b)** Genomic comparison of the representative isolates. The arrows show the orientation of genes. Red arrows: integrase. Indigo arrows: inserted bacteriophage genes.



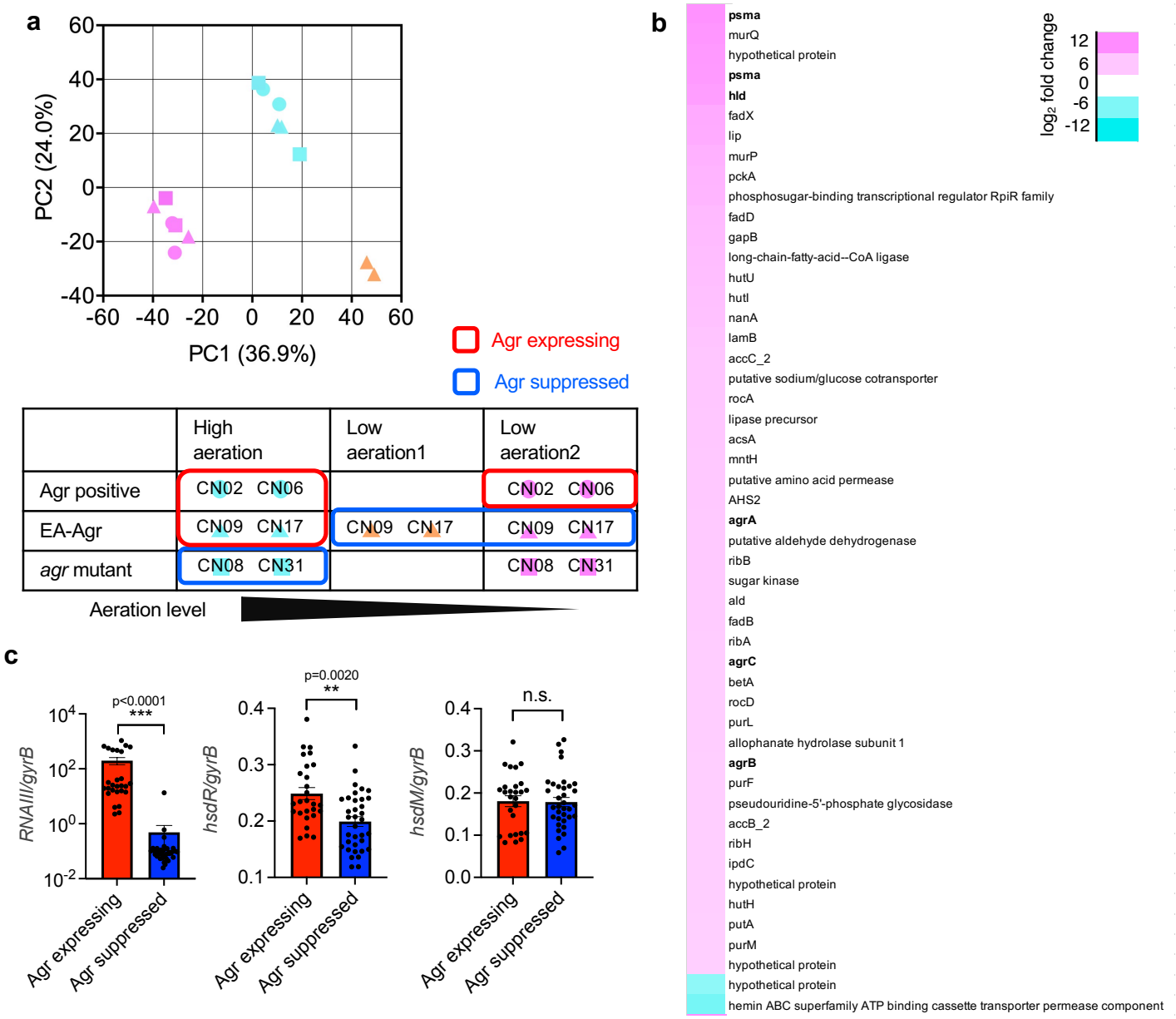
Supplementary figure 2. Time-course analysis of *RNAIII* expression.
(a) Growth curve and the time-course *RNAIII* expression under high aeration condition or low aeration condition 2. For each time point, a significant difference is represented as: § between Agr positive and *agr* mutant, † between EA-Agr and *agr* mutant, * between wildtype (*wt*) LAC and SA113 calculated by one-way ANOVA with two-tailed Dunn's post-hoc test. *p<0.05, **p< 0.01, ***p<0.001. **(b)** Growth curves for each isolate of Agr positive and EA-Agr under low-aeration condition 1. **(c)** δ-toxin and Staphylococcus protein A (SpA) production in 12 hours culture supernatants ($2x10^7$ CFU per well) detected by immunoblotting. Representative images from three independent experiments are shown.

Yamazaki_Supplementary figure 2



Supplementary figure 3. Electroporation assay and macrophage phagocytosis assay.
(a) Competency level of representative isolates measured as the CFU of the transformants after electroporation of pMK4 prepared from CC8 (LAC) strain. 6 experimental replicates are used for each group. ND; not detected. **(b)** Phagocyted bacterial rate (%) and number (CFU) inside macrophages after phagocytosis was determined as described in methods. **(c)** Extracellular persisters within supernatant after incubation with vancomycin (VAN) or gentamicin (GEN). ND; not detected. 3 experimental replicates are used for each group. **(d)** Bacterial survival rate (%) and number (CFU) inside macrophages (Mac) 24 hours after phagocytosis and treatment with gentamicin (GEN). ND; not detected. (**b-d**) Agr positive: CN02, *agr* mutant: CN08, EA-Agr: CN17. (**e**) Bacterial survival rate (%) of CN06, CN31 and CN07 inside macrophages (Mac) 24 hours after phagocytosis and treatment with vancomycin (VAN) or gentamicin (GEN). Data are the mean ± SEM. Each dot represents an experimental replicate. n.s.; not significant, *p<0.05, **p< 0.01, one-way ANOVA with two-tailed Dunn's post-hoc test. The dots represent the number (n) of biological measurements.

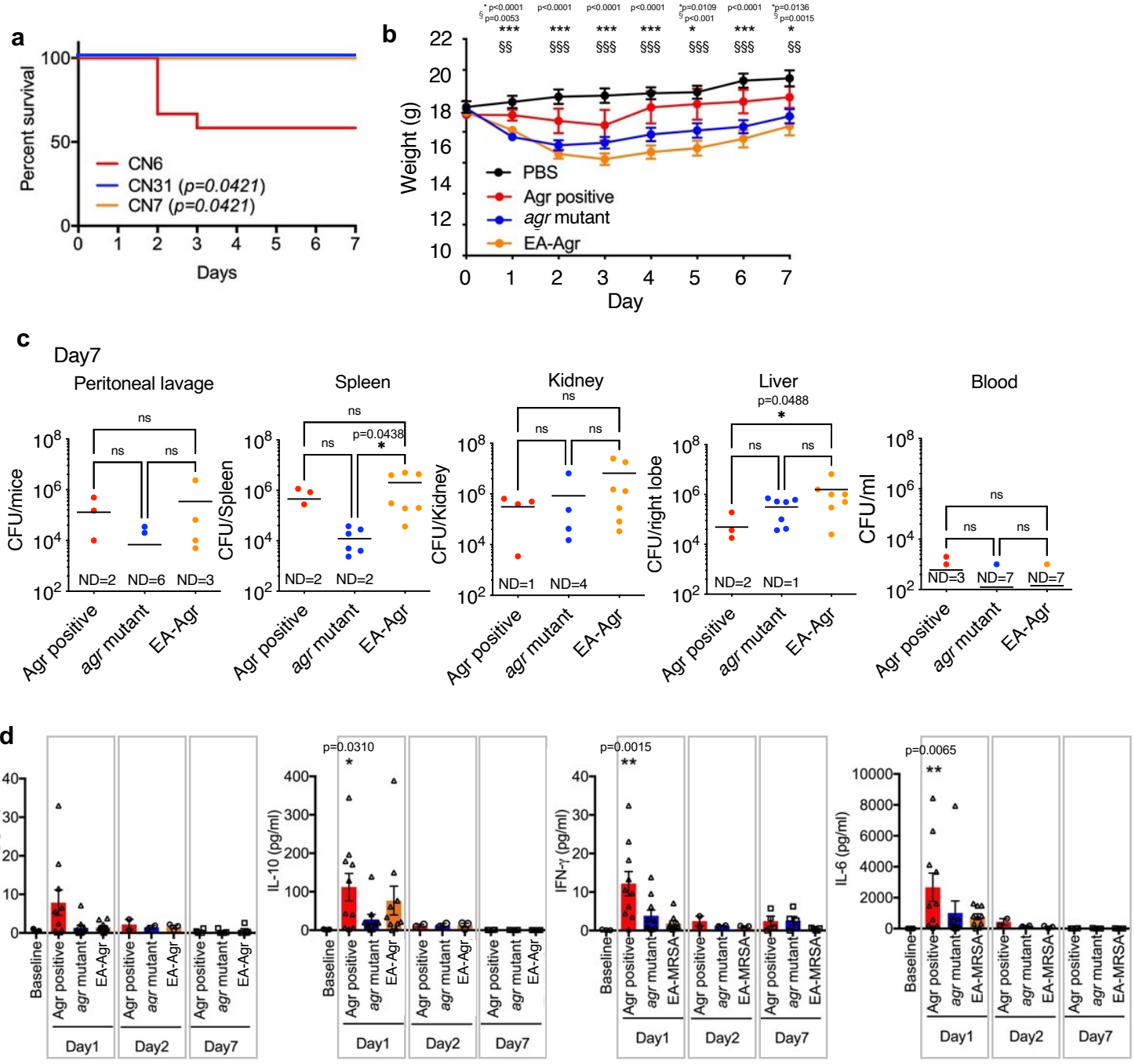
Yamazaki_Supplementary figure 3



Supplementary figure 4. Identification of gene expression changes dependent on Agr

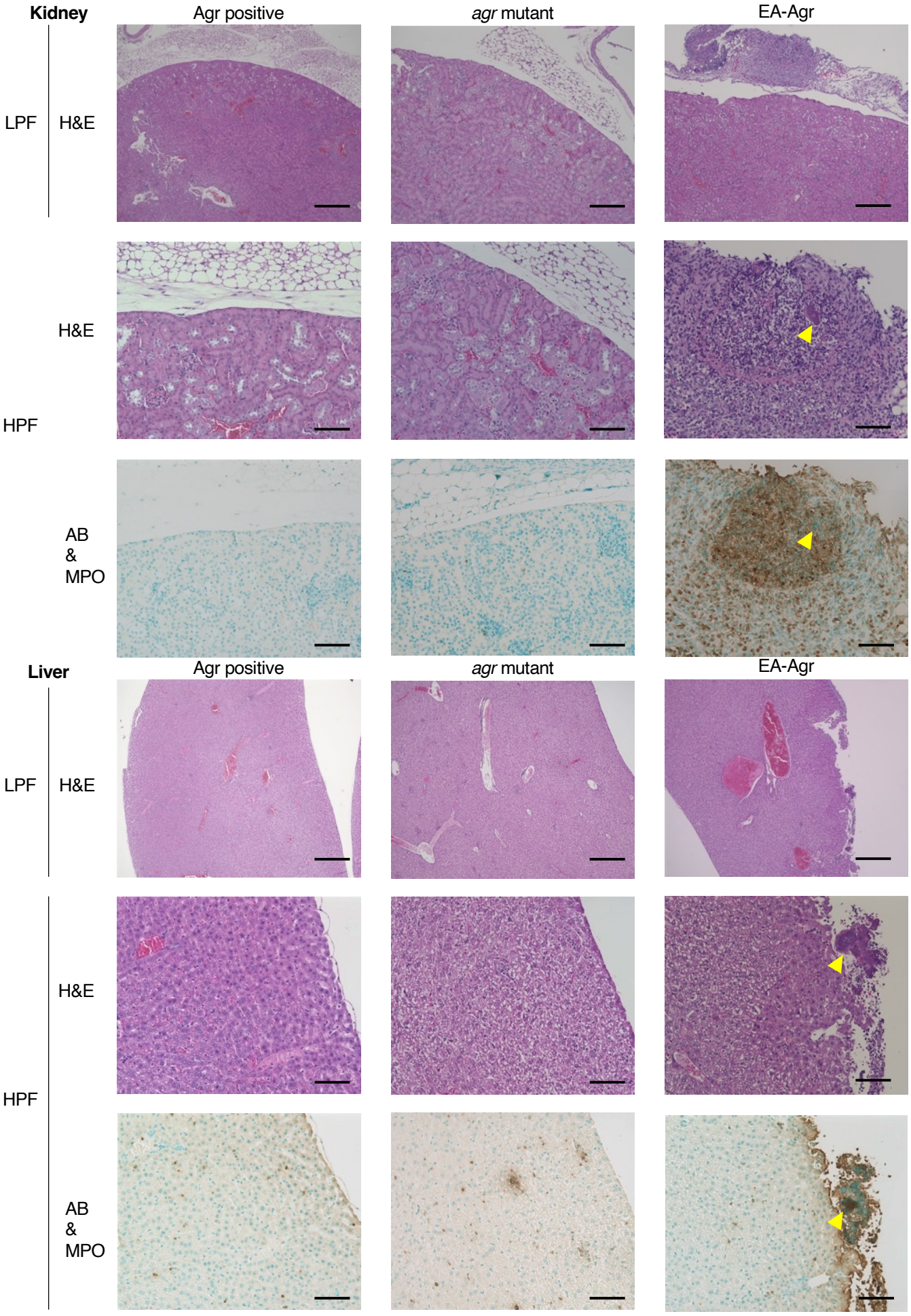
(a) Top: Principal component analysis (PCA) of the differential gene expression in Agr positive (circle), EA-Agr (triangle), and *agr* mutant (square), under either low aeration (pink and orange) or high aeration (cyan) conditions. Data points representing individual samples are colour-coded based on growth condition. The PCA showed that most gene expressions change in a culture condition-dependent manner. Bottom: The samples surrounded by the red and blue lines indicate Agr expressing and Agr suppressed groups used for DESeq2 analysis, respectively. **(b)** The top genes with an absolute log2 fold change greater than 5. *Agr* and *psma* genes shown in Figure 2f are highlighted in bold. **(c)** *RNAIII*, *hsdR* and *hsdM* expressions by qPCR in Agr expressing（CN02, CN06, CN07, CN17, 3-6 experimental replicates） and Agr suppressed (CN09, CN17, CN08, CN31, 3-12 experimental replicates) groups. Agr expressing and Agr suppressed groups were set to mix culture conditions, as shown in the bottom of (a). n.s.; not significant, **p<0.01, ***p< 0.001, two-tailed, Mann-Whitney test. The dots represent the number (n) of biological measurements.

Yamazaki_Supplementary figure 4



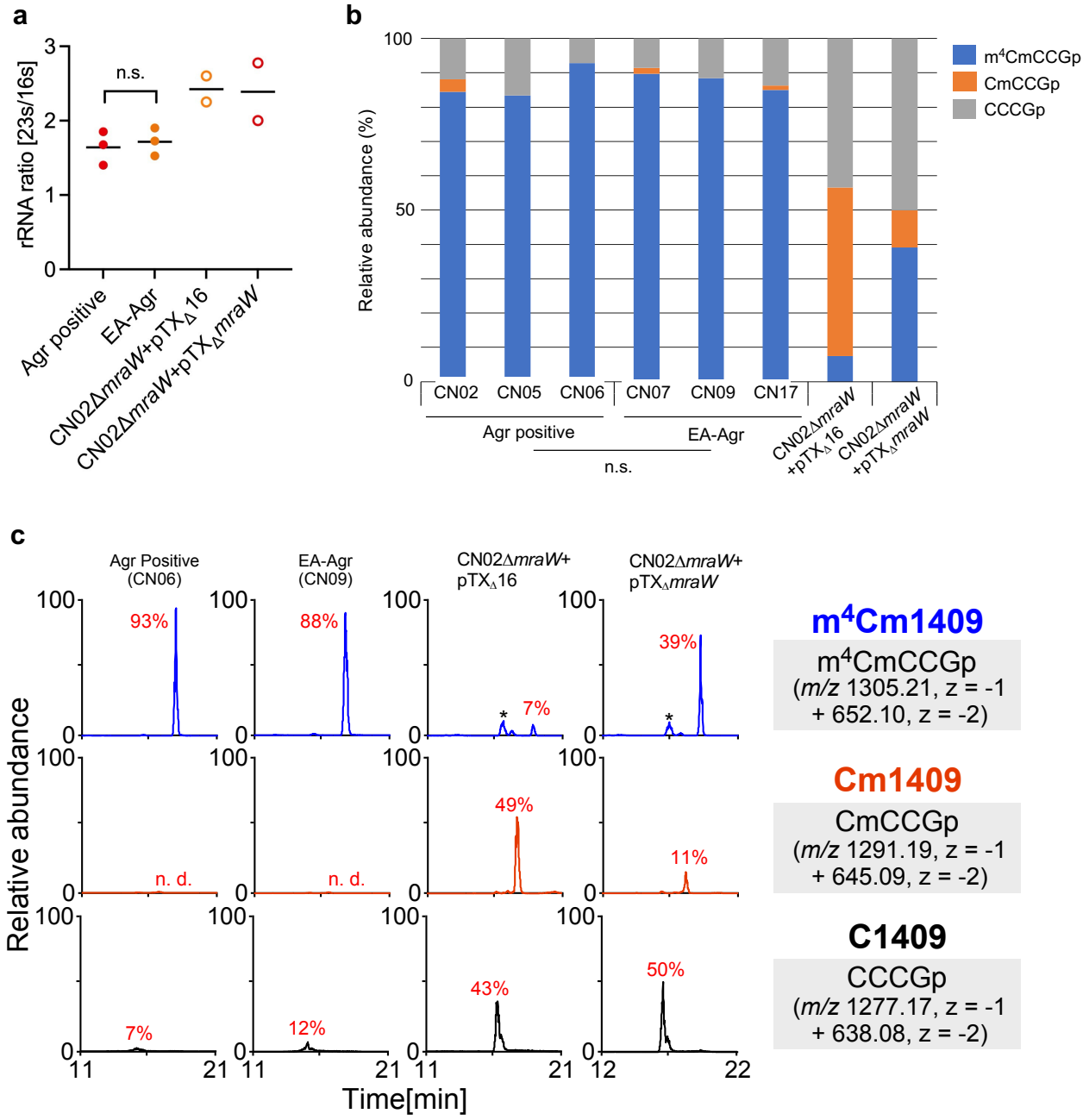
Supplementary figure 5. Mice systemic infection assay.
(a) Survival of C57BL/6 mice after intraperitoneal inoculation with Agr positive (CN06, n=10), agr mutant (CN31, n=8), or EA-Agr (CN07, n=8) subclones. **(b)** Body weight of C57BL/6 mice after intraperitoneal inoculation with Agr positive (CN2, n=17), agr mutant (CN8, n=20) or EA-Agr (CN17, n=20), or PBS (n=13). * between Agr positive and PBS, † between EA-Agr and PBS, and § between agr mutant and PBS. One-way ANOVA with Tukey's multiple comparisons test. **(c)** Bacterial loads in peritoneal lavage, spleen, kidney, liver and blood on day 7 after bacterial inoculation. Agr positive (n=5), agr mutant (n=8) and EA-Agr (n=7). **(d)** The levels of inflammatory cytokines in the peritoneal lavage fluid of mice intraperitoneally inoculated with Agr positive (CN02), *agr* mutant (CN08), and EA-Agr (CN17) (n=10 for infected groups, n=3 for baseline). ND; not detected. Data are the mean ±SEM. Each dot represents an individual mouse. ns; not significant, *p< 0.05, **p< 0.01, one-way ANOVA with two-tailed Dunn's post-hoc test.

Yamazaki_Supplementary figure 5



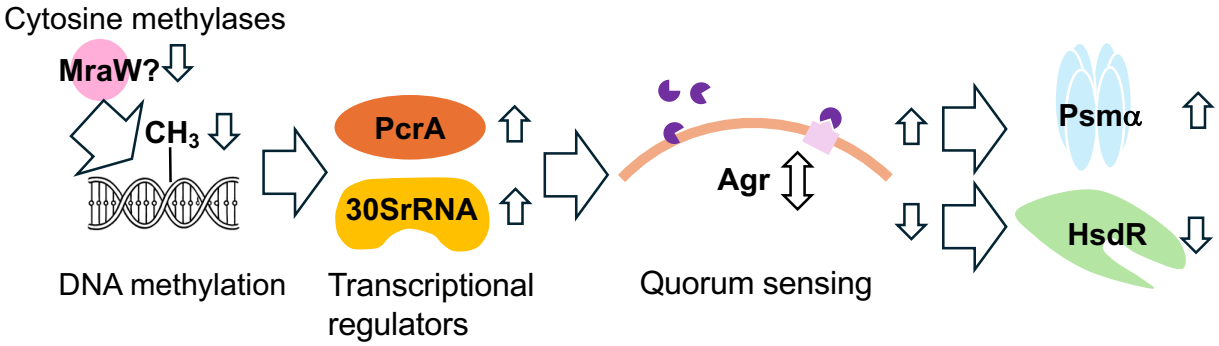
Supplementary figure 6. Histological evaluation of mice after intraperitoneal bacterial inoculation. Histological analysis of kidney and liver on day 7 after infection. LPF; low power field, HPF; high power field, H&E; hematoxylin and eosin, AB; alcian-blue, MPO; myeloperoxidase. Scale bar; 500 μm (LPF), 100 μm (HPF), yellow arrowheads indicate biofilm-like structures. Representative images from three independent experiments are shown.

Yamazaki_Supplementary figure 6



Supplementary figure 7. rRNA methylation analysis.
(a) 23s/16s rRNA ratio in Agr positive (CN02,CN05,CN06), EA-Agr (CN07,CN09,CN17), CN02Δ*mraW*+pTX$_{\Delta}$16 and CN02Δ*mraW*+pTX$_{\Delta}$*mraW* subclones. Each dot represents an individual subclones (Agr-positive and EA-Agr) and experimental replicates (CN02Δ*mraW*+pTX$_{\Delta}$16 and CN02Δ*mraW*+pTX$_{\Delta}$*mraW*). n.s.; not significant. two-tailed Mann-Whitney test. **(b)** LC/MS analyses of m4Cm1409 in 16S rRNA from Agr positive (CN02,CN05,CN06), EA-Agr (CN07,CN09,CN17), CN02ΔmraW+pTX$_{\Delta}$16 and CN02ΔmraW + pTX$_{\Delta}$mraW subclones. n.s.; not significant. two-tailed chi-square test. **(c)** Representative raw data of LC/MS analyses of (b). Extracted ion chromatograms (XICs) of the indicated negative ions of the RNase T1-digested fragments with m4Cm1409 (Upper), Cm1409 (Middle) or C1409 are shown. n.d.; not detected.

Yamazaki_Supplementary figure 7



Supplementary figure 8. A graphical representation of the main molecular mechanisms in EA-Agr *S. aureus*.

Yamazaki_Supplementary figure 8

Source Data

Supplementary figure 2c.