

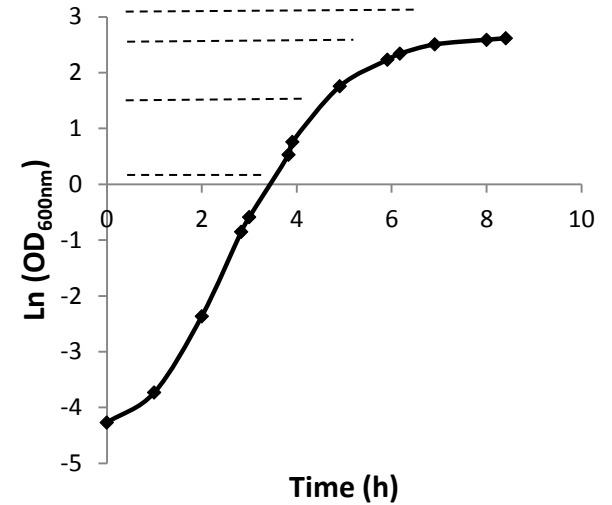
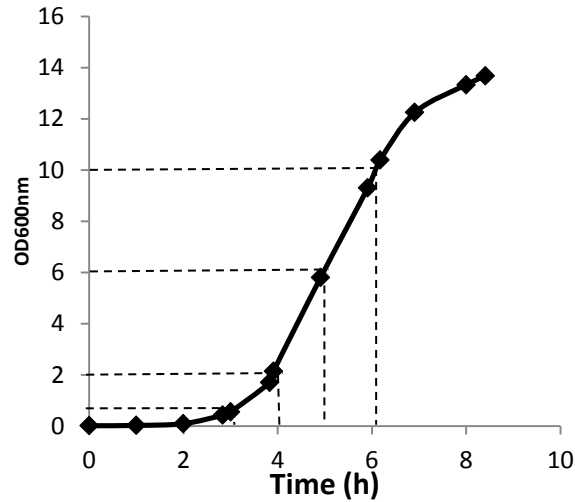
**sRNA and *cis*-antisense sRNA identification in *Staphylococcus aureus* highlights an unusual sRNA gene cluster with one encoding a secreted peptide**

Julie Bronsard, Gaetan Pascreau, Mohamed Sassi, Tony Mauro, Yoann Augagneur\*, and Brice Felden

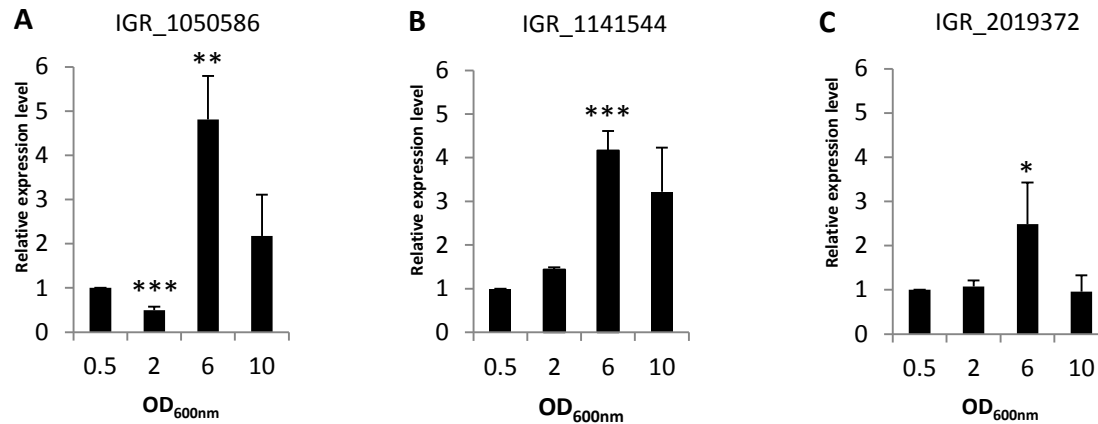
Inserm U1230 Biochimie Pharmaceutique, Université de Rennes 1, Rennes, France

\*Address correspondence to Yoann Augagneur, [yoann.augagneur@univ-rennes1.fr](mailto:yoann.augagneur@univ-rennes1.fr) (or alternatively to [brice.felden@univ-rennes1.fr](mailto:brice.felden@univ-rennes1.fr)).

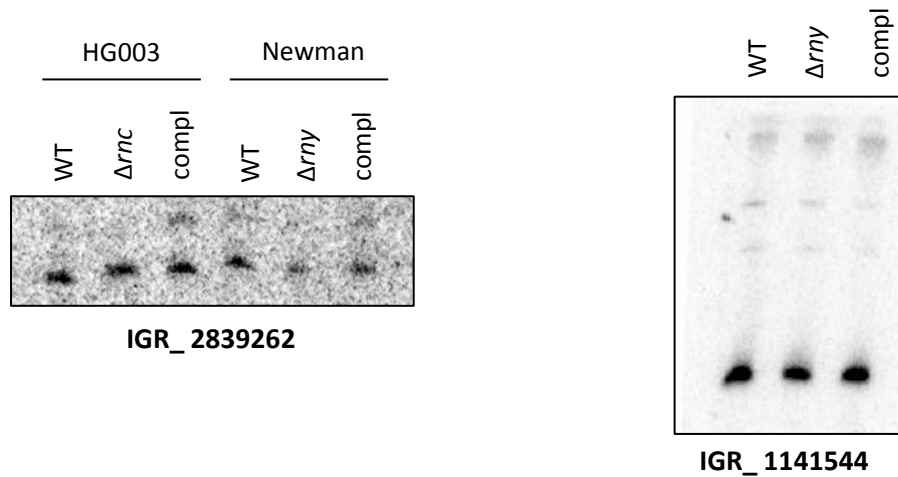
**Supplementary Information**



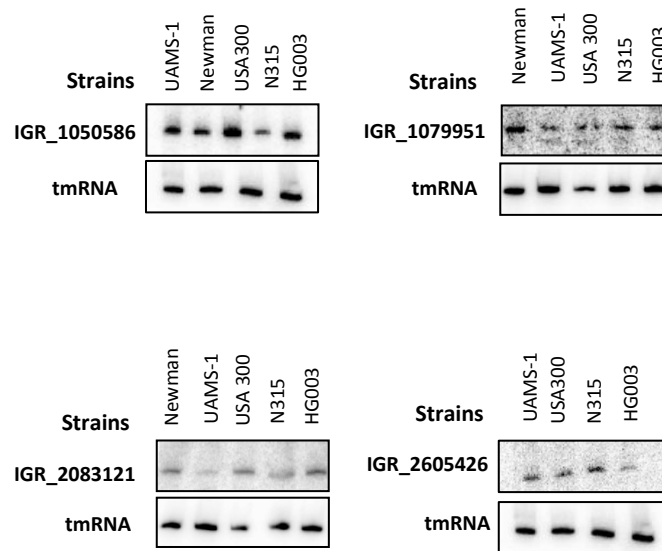
**Figure S1:** Growth curve of *Staphylococcus aureus* Newman in BHI broth. Cells were inoculated at an OD<sub>600nm</sub> of 0.02 and cultured at 37°C under agitation at 160 rpm. The dotted lines correspond to the time in hours (h) when cells were harvested prior RNA extraction.



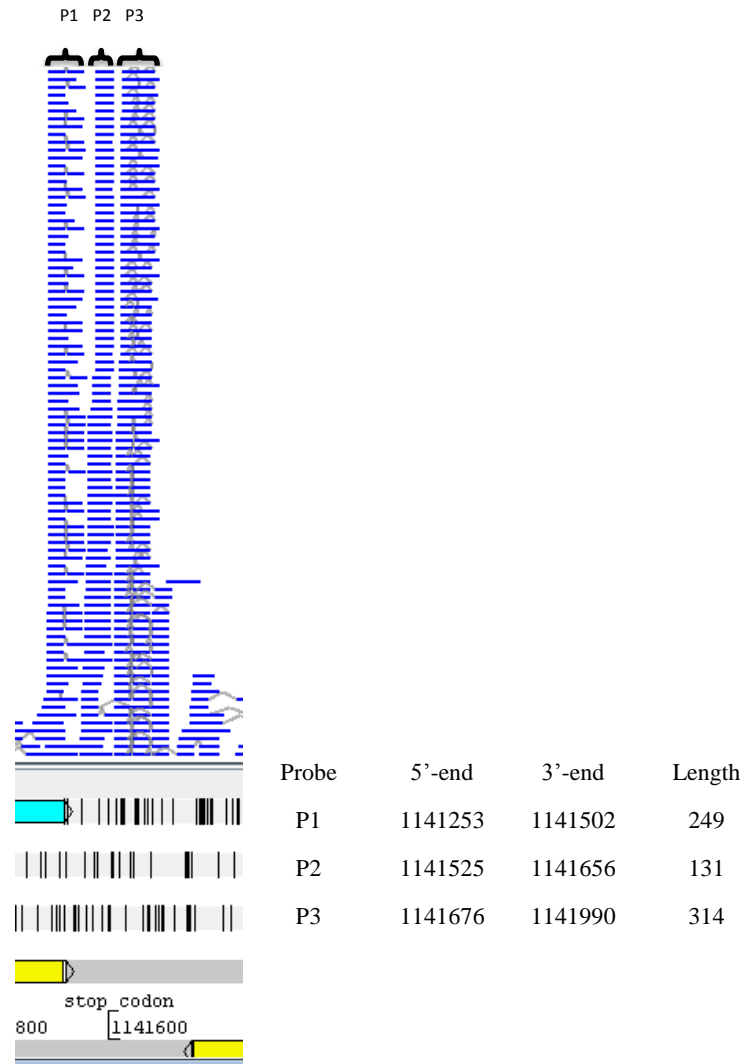
**Figure S2:** Relative RNA levels of (A) IGR\_1050586, (B) IGR\_1141544, and (C) IGR\_2019372 in *Staphylococcus aureus* Newman based on northern blots. Cells were grown in BHI medium and harvested at 0.5, 2, 6, and 10 OD<sub>600nm</sub>. tmRNA was used as an internal loading control, and the relative expression level determined at an OD<sub>600nm</sub> of 0.5 was set to one for each gene tested. Data presented are the means of three independent experiments. A student t-test was done to determine significant differences with the condition at OD<sub>600nm</sub> of 0.5.



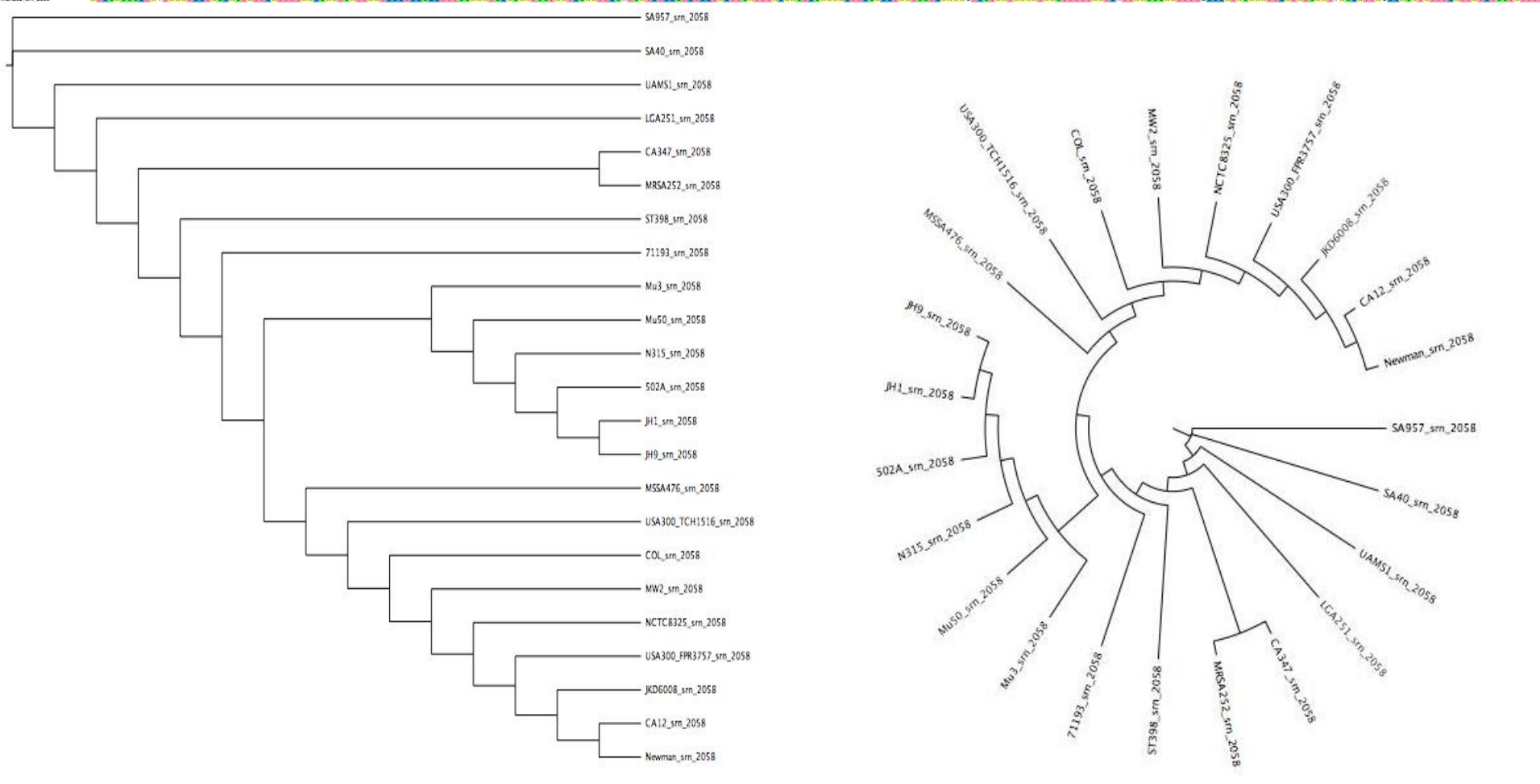
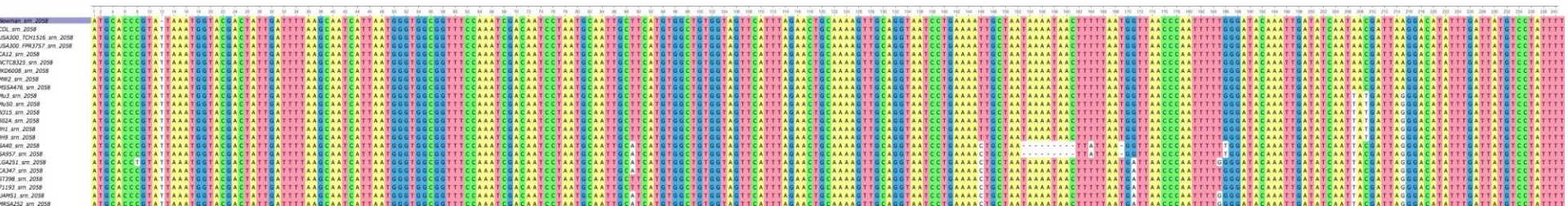
**Figure S3** : Expression profile of IGR\_2839262 and IGR\_1141544 RNase-deficient strains. Northern blot were performed using probes specific to the targets on RNAs extracted from HG003 ( $\Delta rnc$ , RNaseIII mutant) or Newman ( $\Delta rny$ : RNaseY mutant) cultured in BHI medium. The expression profile of IGR\_2839262 was monitored in HG003  $\Delta rnaselIII$  and in Newman  $\Delta rnaselY$  mutants of *S. aureus*, while IGR\_1141544 expression was only monitored in Newman  $\Delta rnaselY$  since the targeted region is absent in HG003. WT, wild-type; compl, complemented strain.



**Figure S4** : IGR 1050586, 1079951, 2083121, and 2605426 expression in five *Staphylococcus aureus* strains. Northern blots were performed using target-specific probes on RNA extracted from UAMS-1, Newman, USA300, N315, and HG003 cultured in BHI medium until  $OD_{600nm} = 6$ . tmRNA was used as an internal loading control.



**Figure S5:** Artemis visualization of paired-end reads mapped onto the IGR\_1141544 locus.

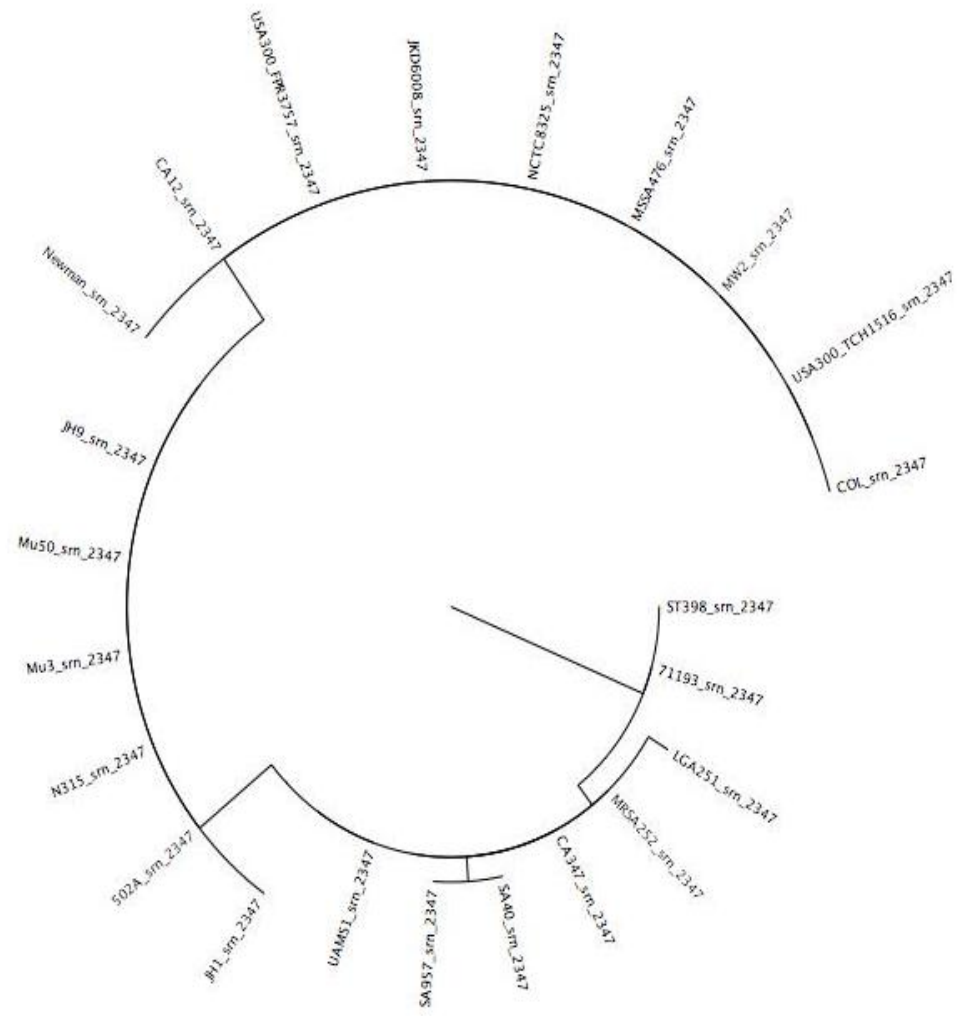
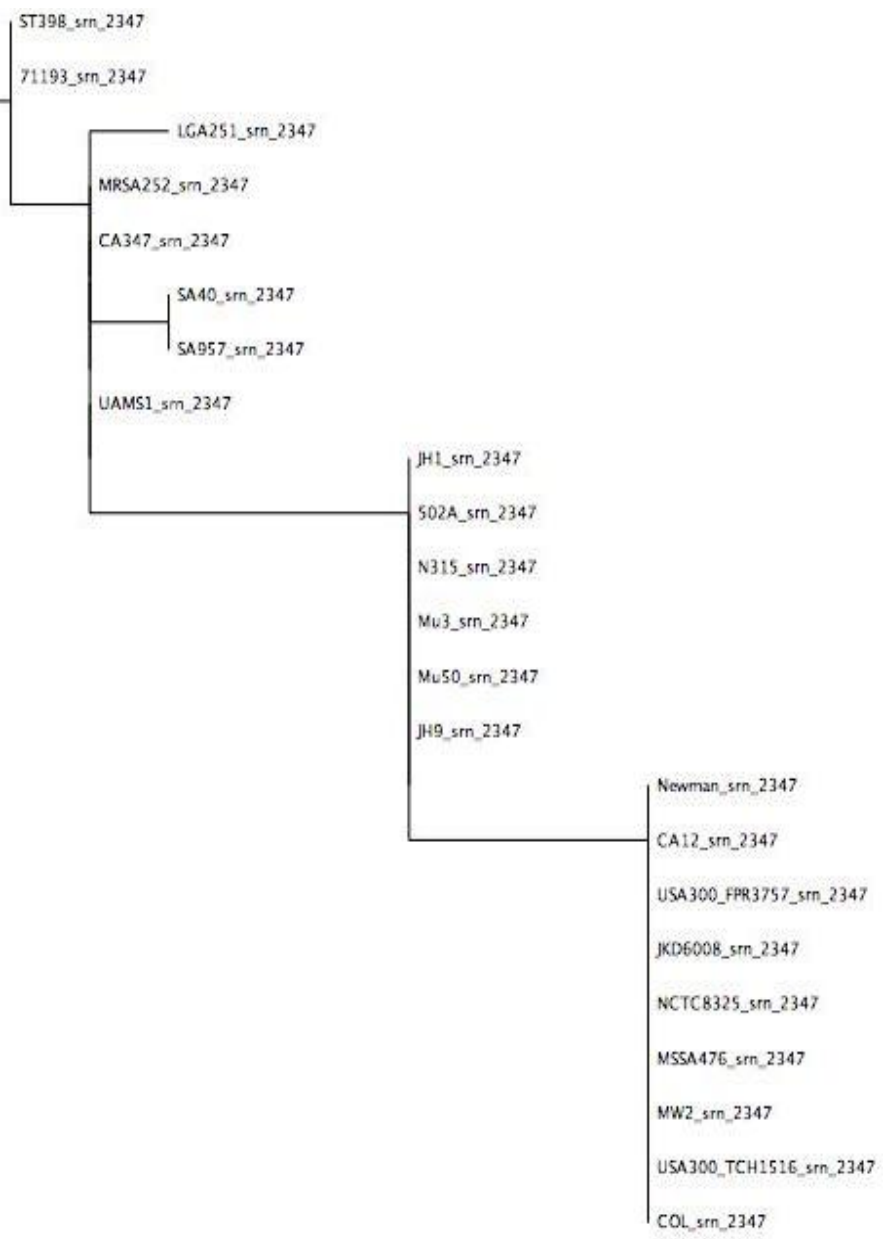
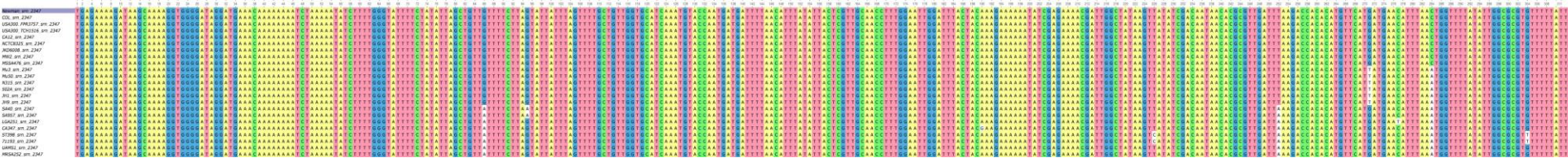


**Figure S6:** Alignment of the retained new sRNAs in a set of *S. aureus* strains.

**srn\_2058**

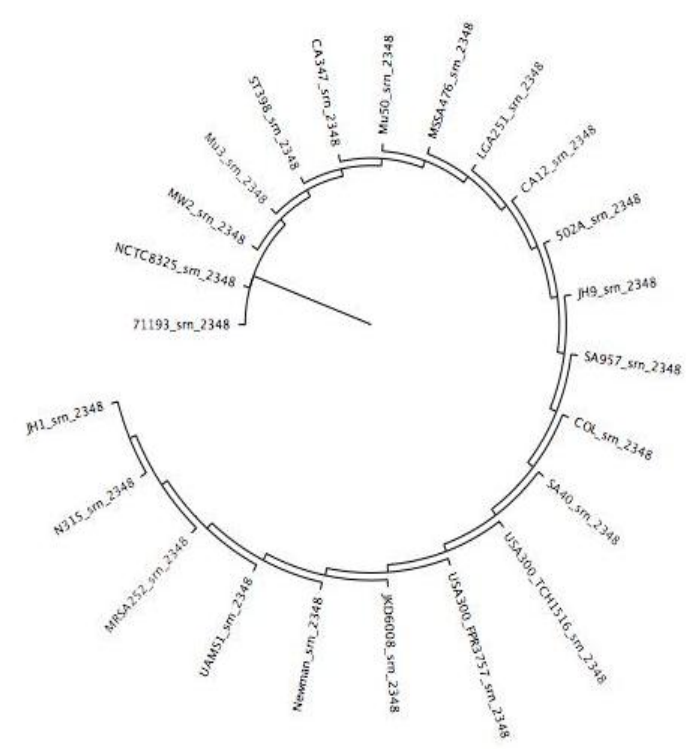
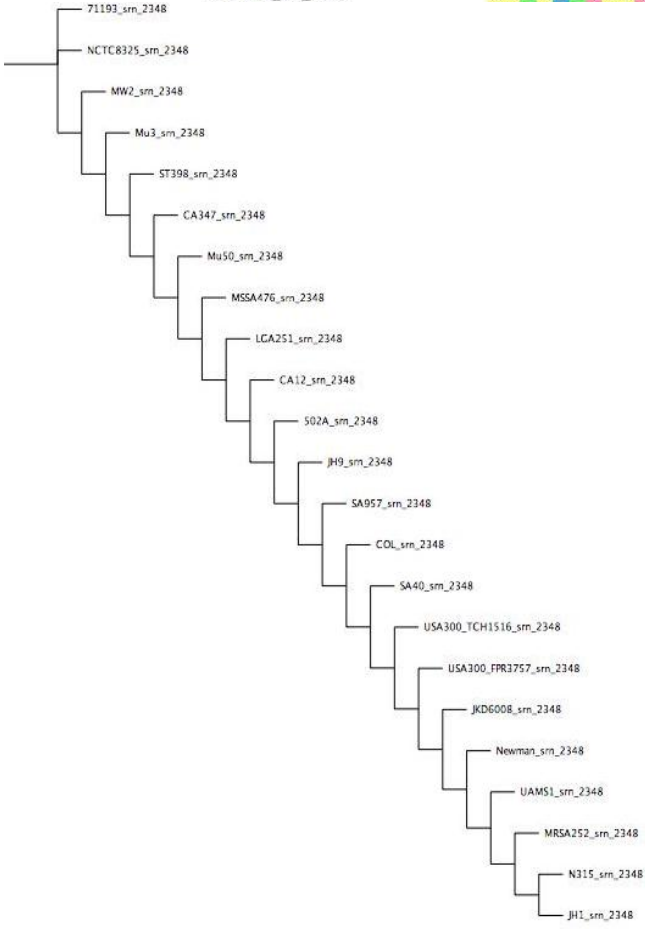
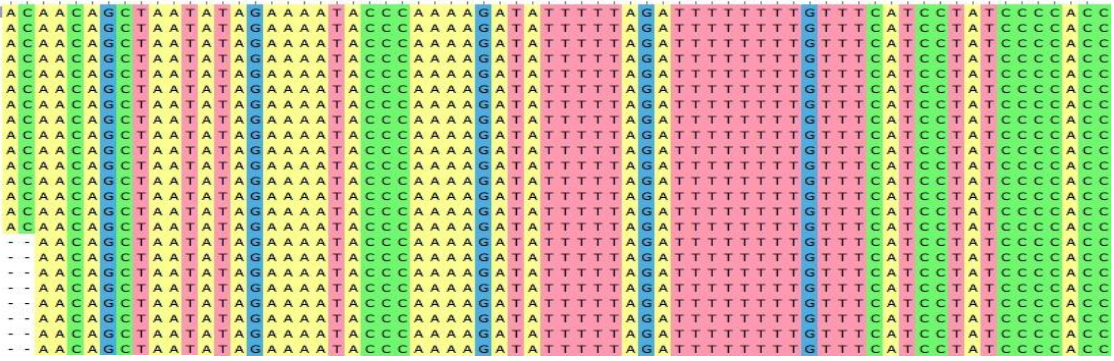






srn\_2347

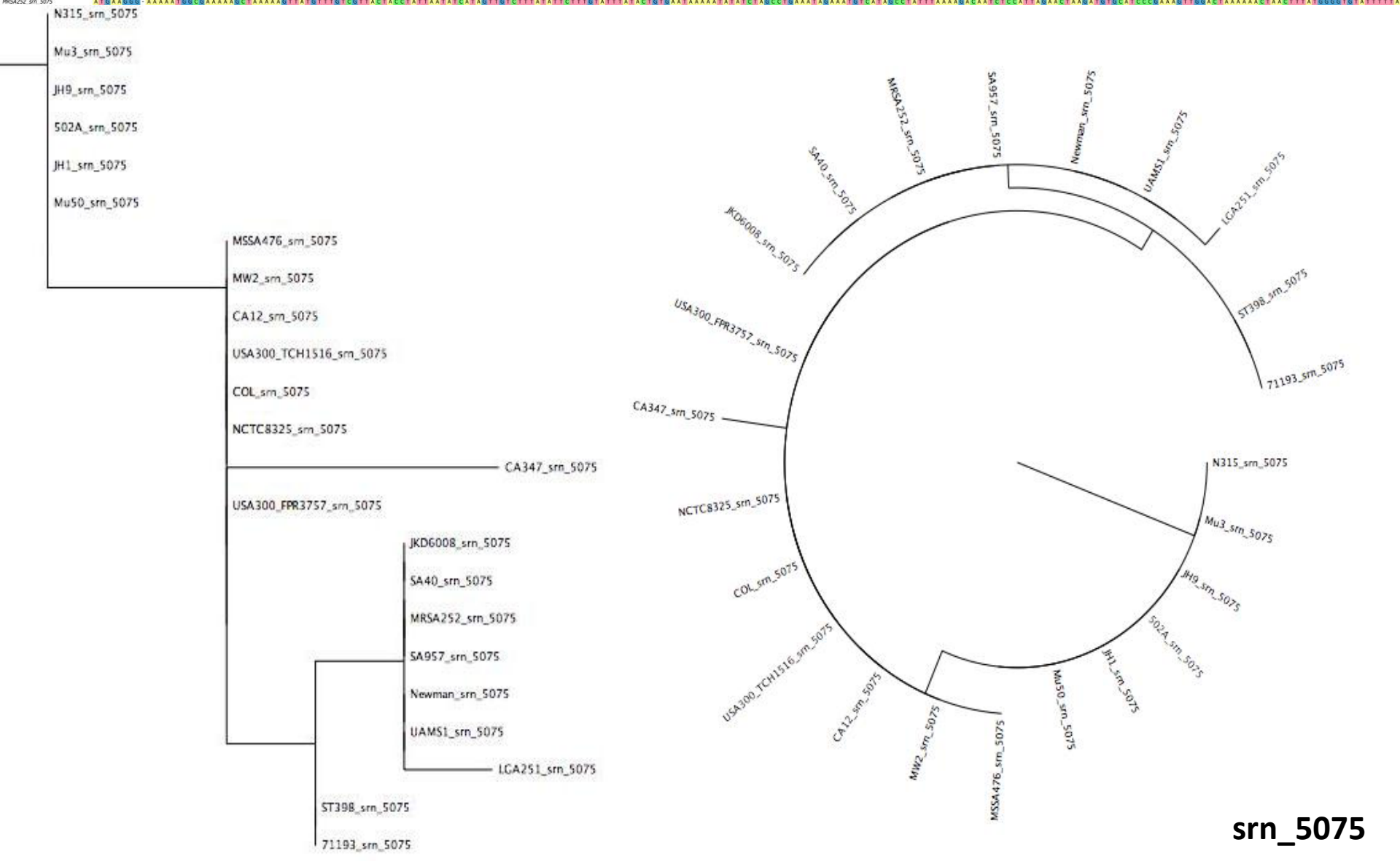
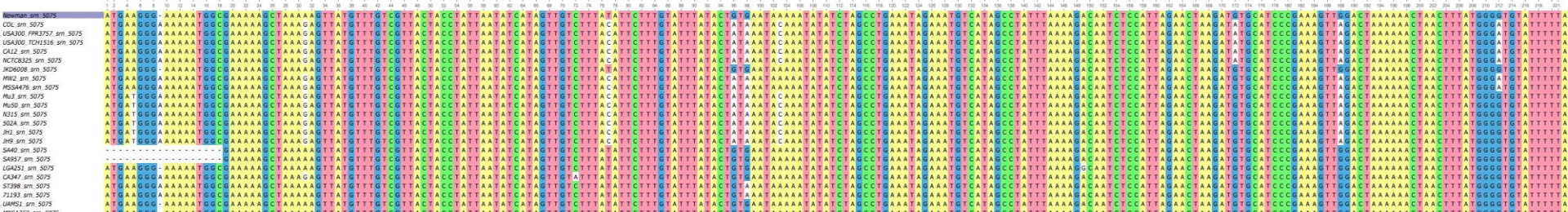
*Newman\_srn\_2348*  
*COL\_srn\_2348*  
*USA300\_FPR3757\_srn\_2348*  
*USA300\_TCH1516\_srn\_2348*  
*CA12\_srn\_2348*  
*NCTC8325\_srn\_2348*  
*JKD6008\_srn\_2348*  
*MW2\_srn\_2348*  
*MSSA476\_srn\_2348*  
*Mu3\_srn\_2348*  
*Mu50\_srn\_2348*  
*N315\_srn\_2348*  
*502A\_srn\_2348*  
*JH1\_srn\_2348*  
*JH9\_srn\_2348*  
*SA40\_srn\_2348*  
*SA957\_srn\_2348*  
*LGA251\_srn\_2348*  
*CA347\_srn\_2348*  
*ST398\_srn\_2348*  
*71193\_srn\_2348*  
*UAMS1\_srn\_2348*  
*MRSA252\_srn\_2348*



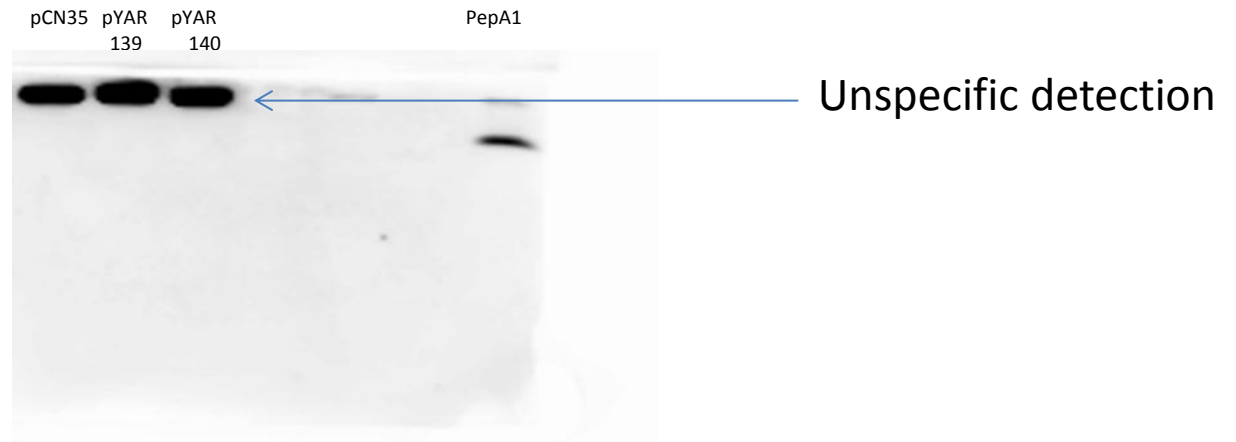
srn\_2348







## A- Cellular extract



## B- Extracellular proteins



**Figure S7:** Western blots performed on cell extracts or secreted proteins of Newman strain transformed with appropriate plasmids.