

Supporting information

## ***Staphylococcus aureus* small RNAs possess dephospho-CoA 5'-caps, but no CoAlation marks.**

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### **Supporting figures**

**Figure S1** Selective quantification of non-thioesterified CoA.

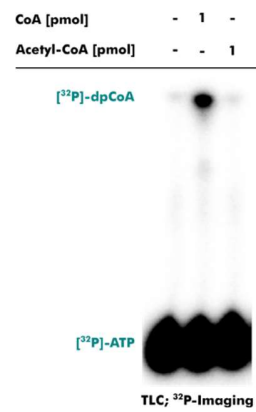
**Figure S2** Quantification of dpCoA-RNA and RNA CoAlation in *S. aureus*, grown in the presence of diamide.

**Figure S3** Synthesis of dpCoA-RNA disulfides.

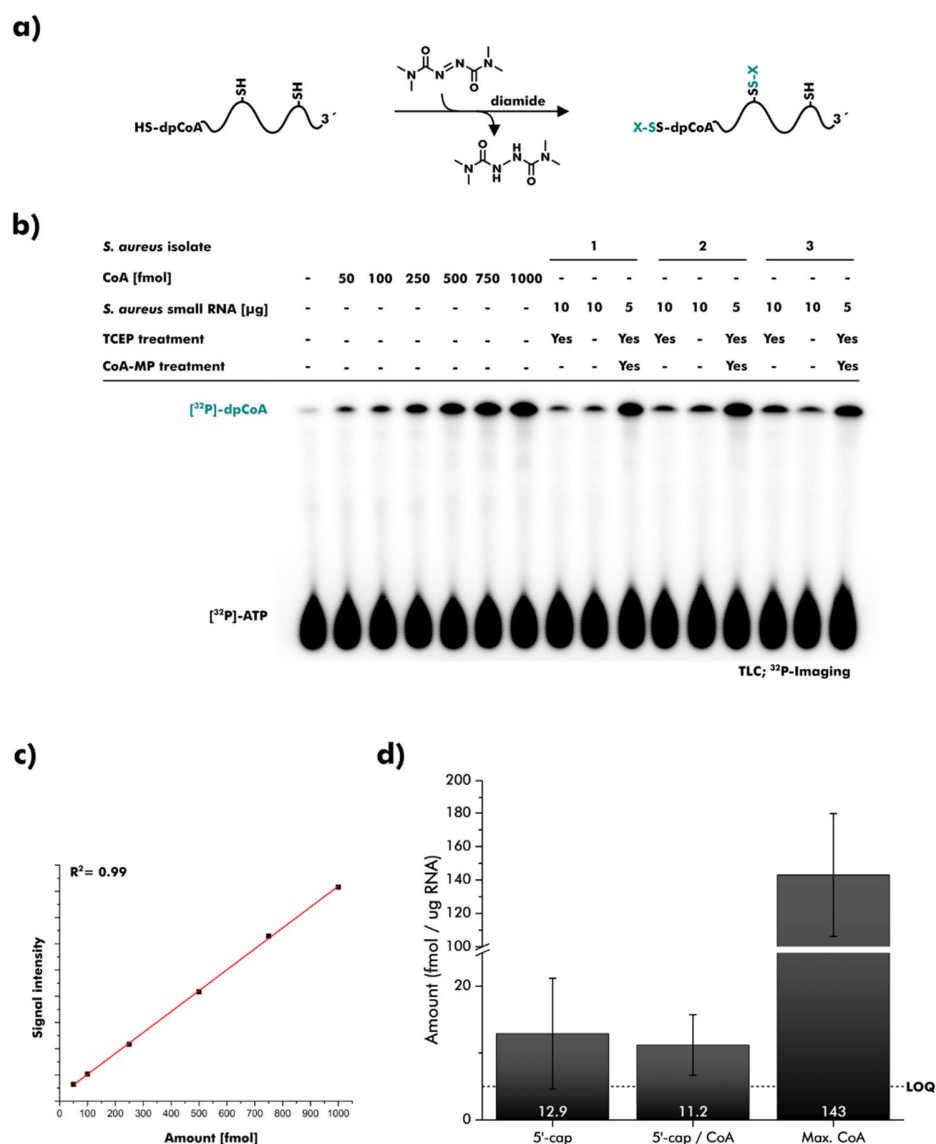
**Figure S4** Fluorescent labeling of dpCoA-RNA.

**Figure S5** Reduction of different dpCoA-RNA disulfides by *S. aureus* CoADR.

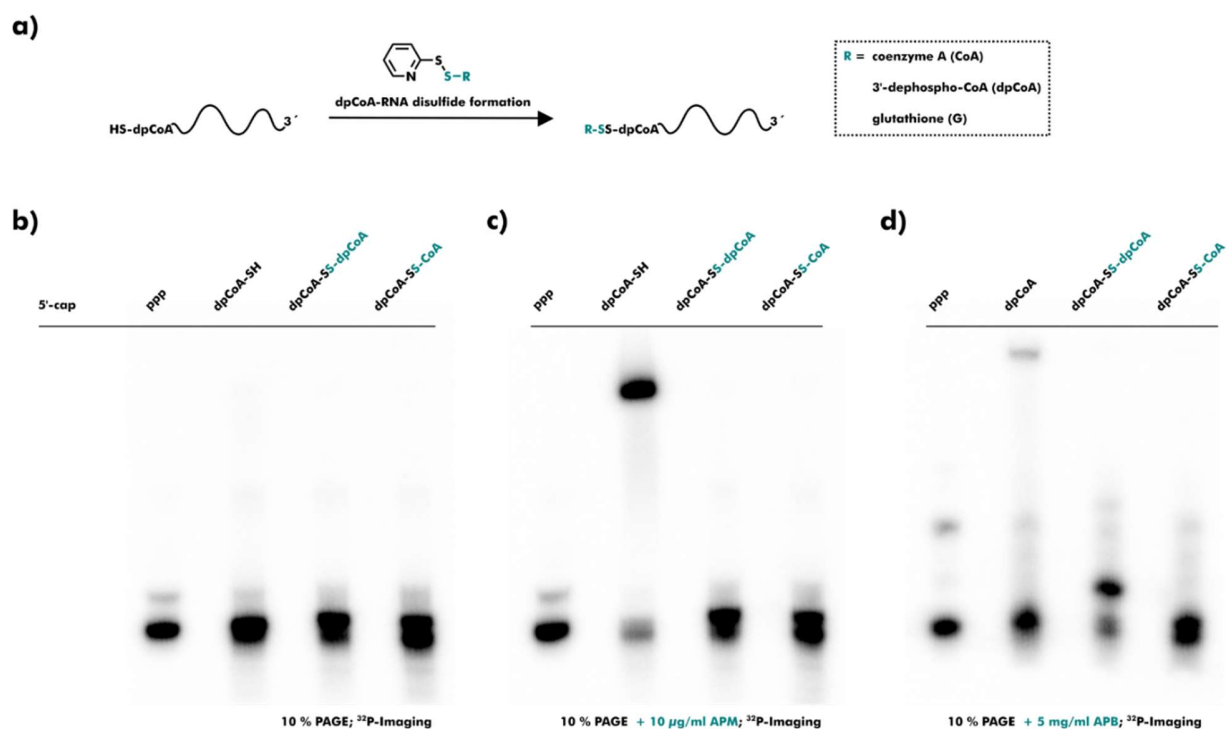
**Figure S6** Substrate preferences of *S. aureus* CoADR.



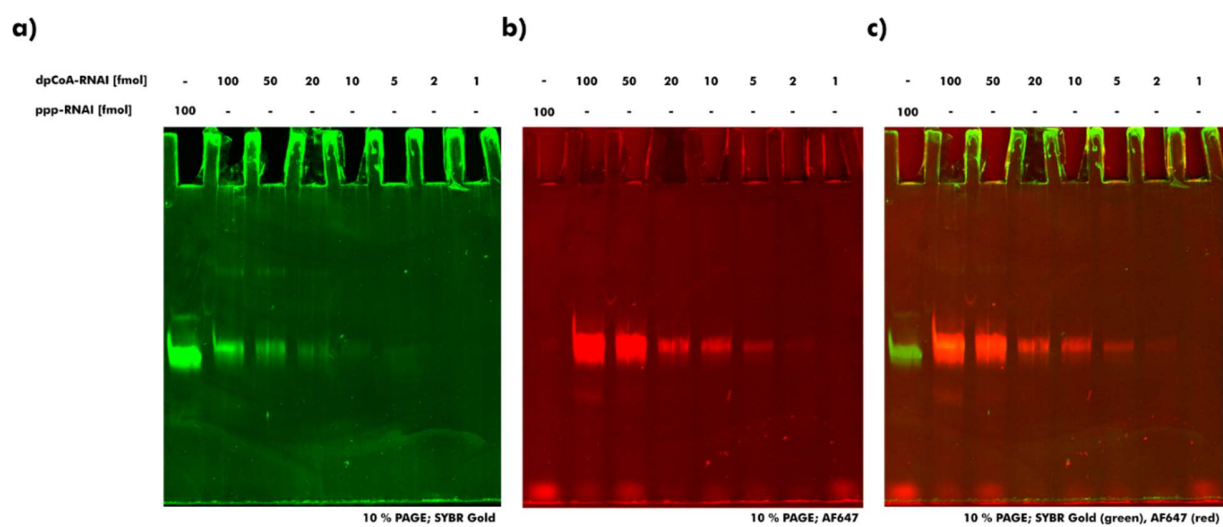
**Figure S1.** Selective quantification of non-thioesterified CoA. CoA and Acetyl-CoA were quantified with *E. coli* NudC, *E. coli* PPAT, and [ $\alpha$ -<sup>32</sup>P]-ATP. Arising [ $\alpha$ -<sup>32</sup>P]-dpCoA signals were separated by thin-layer chromatography (TLC) and visualized by <sup>32</sup>P-imaging.



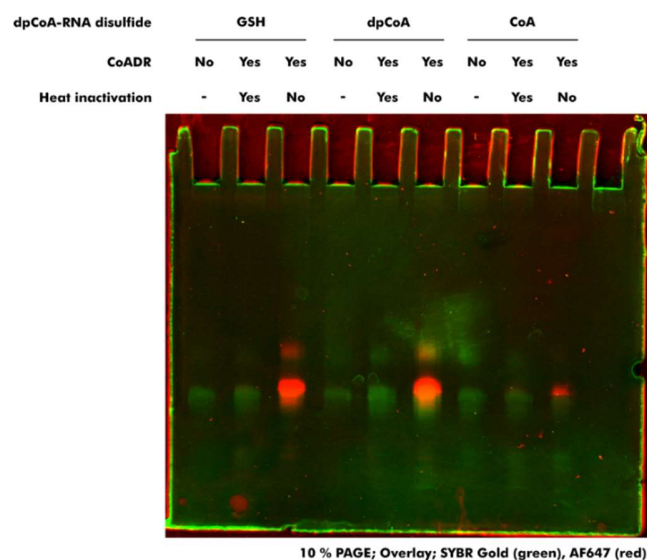
**Figure S2** Quantification of dpCoA-RNA and RNA CoAlation in *S. aureus*, grown in the presence of diamide. **a)** Principle of increased in vivo RNA disulfide formation by the oxidant diamide. **b)** dpCoA caps and CoAlation events were analyzed after separation by thin-layer chromatography (TLC). Signals were visualized by  $^{32}\text{P}$ -imaging.  $^{32}\text{P}$ -dpCoA signals were quantified. CoA-MP: 2-mercaptopyridine-activated CoA. **c)** CoA was used to create a calibration line for 50 – 1000 fmol CoA and dpCoA-RNA, respectively. **d)** The bar chart illustrates the amount of dpCoA-RNAs and CoA in one microgram small RNA of *S. aureus*. 5'-dpCoA caps (5'-cap) were quantified. A combination of 5'-dpCoA caps and *in vivo* CoAlation events (5'-cap/CoA) was quantified. Potential CoAlation sites (Max. CoA) in *S. aureus* small RNA were quantified. Error bars represent the standard deviation ( $n = 3$ ). The limit of quantification (LOQ) is 50 fmol.



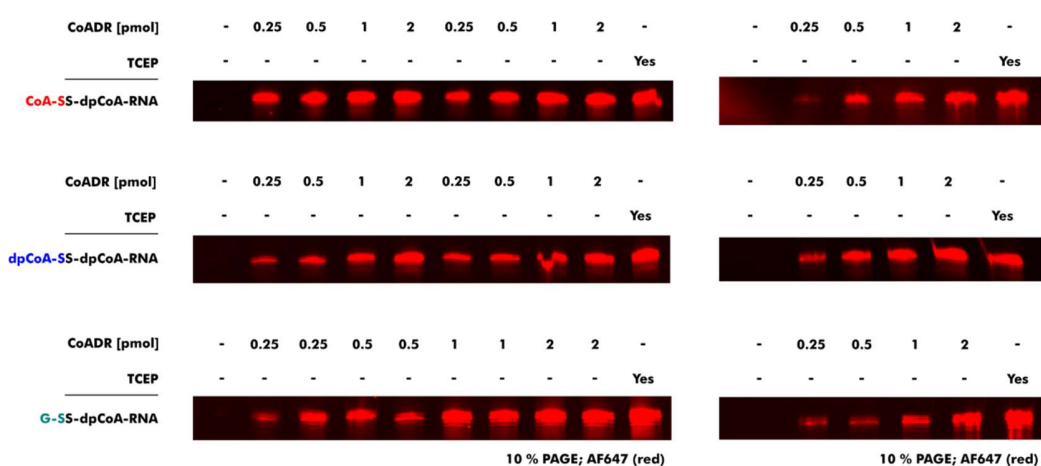
**Figure S3** Synthesis of dpCoA-RNA disulfides. **(a)** dpCoA-RNA disulfides were produced by the reaction of <sup>32</sup>P-bodylabeled dpCoA-RNA with 2-mercaptopyridine-activated CoA and dpCoA. **(b - d)** The products CoA-SS-dpCoA-RNA and dpCoA-SS-dpCoA-RNA were analyzed by 10 % PAGE **(b)**, 10 % APM-PAGE **(c)**, and 10 % APB-PAGE **(d)**. 5'-triphosphate-RNA (ppp-RNA) and the substrate dpCoA-RNA were analyzed simultaneously. Products were visualized by <sup>32</sup>P-imaging (excitation of phosphor storage screen at 635 nm).



**Figure S4** Fluorescent labeling of dpCoA-RNA. ppp-RNA and varying concentrations of dpCoA-RNA were incubated with Alexa Fluor 647-maleimide (AF647-maleimide). Products were and run on 10 % PAGE and stained with SYBR Gold. **a)** SYBR Gold visualization (excitation at 473 nm). **b)** AF647 visualization (excitation at 635 nm). **c)** SYBR Gold signals (green) and AF647 signals (red) were superimposed.



**Figure S5** Reduction of different dpCoA-RNA disulfides by *S. aureus* CoADR. RNA were left untreated or incubated with either heat-inactivated or active *S. aureus* CoADR. Samples were incubated with AF647-maleimide and separated by 10 % PAGE. RNA were stained with SYBR Gold. AF647 signals (red; excitation at 635 nm) and SYBR Gold signals (green; excitation at 473 nm) were superimposed.



**Figure S6** Substrate preferences of *S. aureus* CoADR. dpCoA-RNA disulfides with CoA (CoA-SS-dpCoA-RNA), dpCoA (dpCoA-SS-dpCoA-RNA) and glutathione (G-SS-dpCoA-RNA) were incubated with defined concentrations of *S. aureus* CoADR or TCEP for 1 h, then treated with AF647-maleimide. Gel segments with quantified AF647 signals (excitation at 635 nm) are depicted.