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## **Supplementary Information for**

## Pseudouridine modifications influence binding of aminoglycosides to helix 69 of bacterial ribosomes

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**Fig. S1** Footprinting analysis of H69 with different gentamicin concentrations is shown. Percent protection of position G1922 is given at the bottom of the gel.



**Fig. S2** Autoradiogram for DMS footprinting (no aniline) of h44 of the 30S subunit is shown. The same rRNA sample from the footprinting experiment in Figure 2a was used. In this case, position 1402 was used to normalize band intensities. Percent protection at position 1402 is indicated at the bottom of the gel.



**Fig. S3** Ribosome profiles after (A) 70S ribosome isolation in 6 mM Mg<sup>2+</sup> conditions and (B) 50S/30S subunit isolation in 1 mM Mg<sup>2+</sup> buffer, analyzed by sucrose density gradients. (C) The 50S subunit purity was analyzed by sucrose density gradients. (D) Ribosomal RNAs extracted from purified subunits or 70S ribosomes were analyzed by agarose gel electrophoresis.