SUPPLEMENTAL MATERIAL

Substrate recognition and modification by a pathogen-associated aminoglycoside-resistance 16S rRNA methyltransferase

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SUPPLEMENTAL FIGURES S1-S5

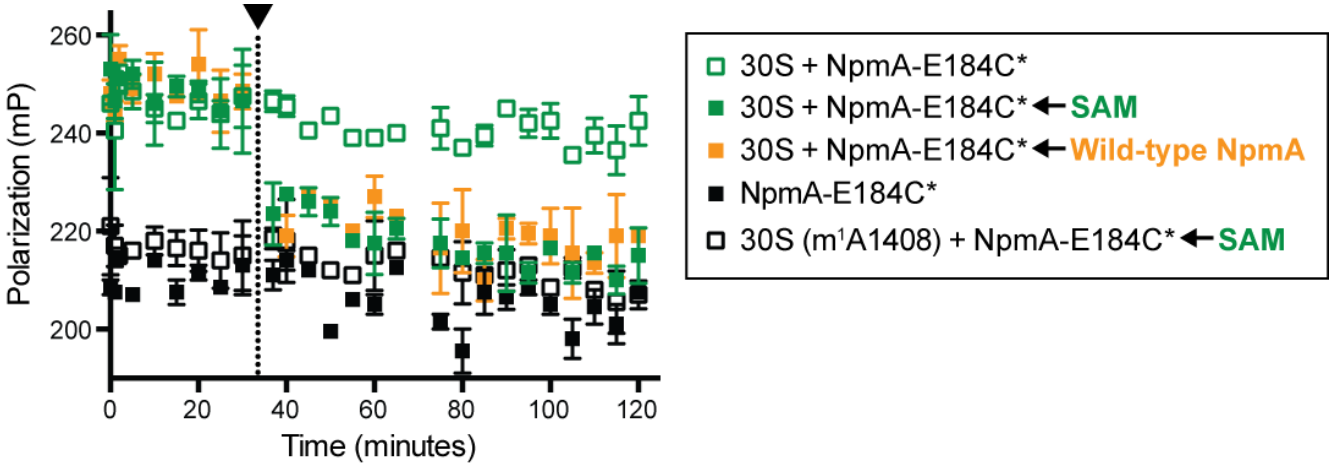

FIG S1 NpmA-E184C* binds only 30S subunits with unmodified A1408 and is displaced by addition of SAM and/or wild-type NpmA. NpmA-E184C* alone (black) and pre-methylated 30S with NpmA-E184C* (open black squares) controls and binding reactions containing 30S and NpmA-E184C* (green open squares) subject to addition of SAM (green closed squares), addition of wild-type NpmA (orange closed squares) or no treatment (green open squares) at the point indicated by the arrowhead and dotted vertical line.

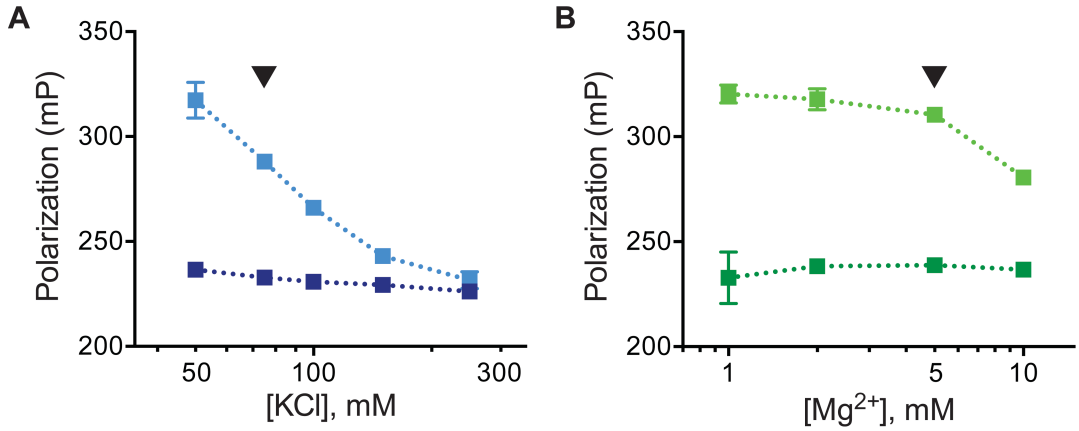

FIG S2 Solution optimization and effect of ionic strength on 30S-NpmA interaction. Fluorescence polarization comparison of free NpmA-E184C* (dark blue/ green) with 30S-bound (light blue/ green) NpmA-E184C* with increasing concentrations of (A) KCl and (B) Mg^{2+}. In each panel, error bars indicate standard deviation of averaged measurements and the arrow indicates the concentration of each component used in the final competition binding assays with wild-type and variant NpmA proteins.

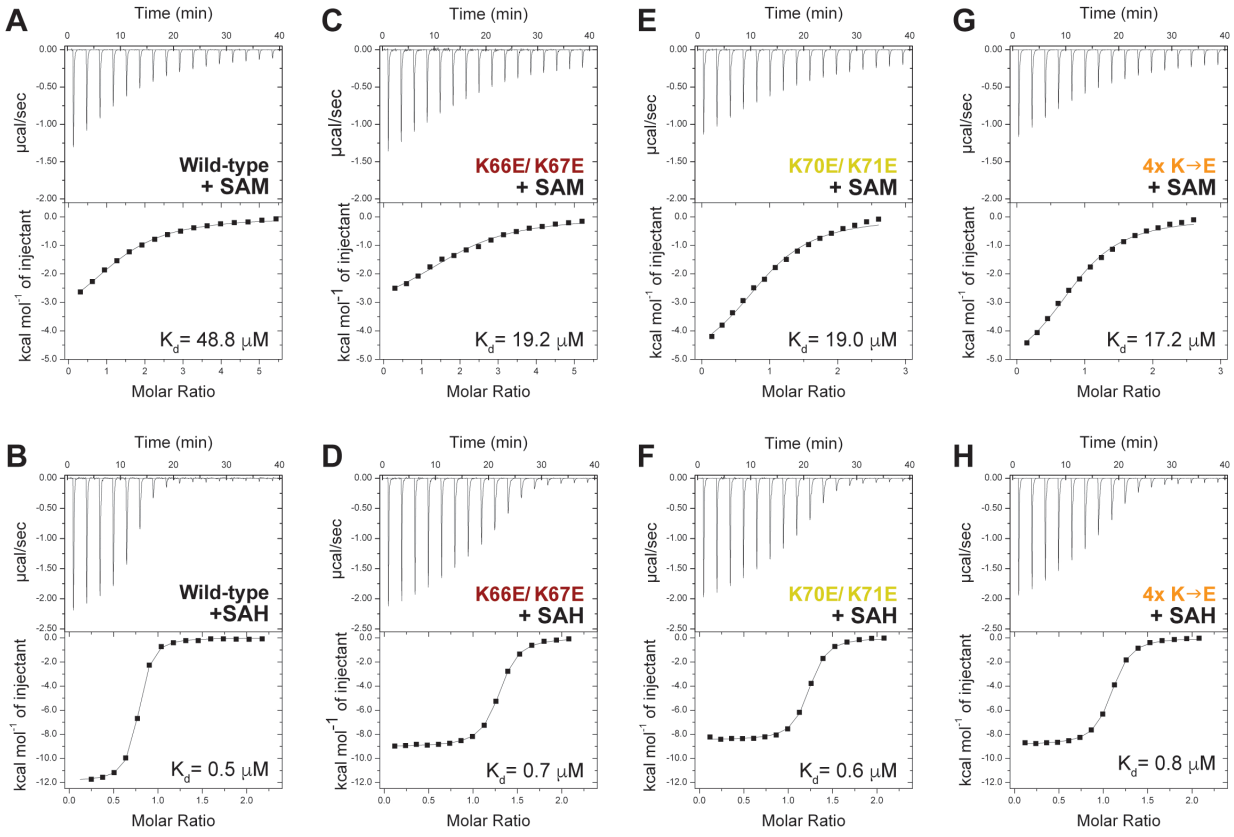

FIG S3 Measurement of NpmA β2/3 linker variant binding affinity for cosubstrate SAM and reaction by-product SAH. ITC titrations for each protein-ligand pair tested: (A),(B) wild-type NpmA; (C),(D) NpmA-K66E/K67E; (E),(F) NpmA-K70E/K71E; and, (G),(H) NpmA-K66E/K67E/K70E/K71E (4x K→E) with SAM and SAH, respectively. Values for binding affinity (K_d) are those derived from the individual titration shown.

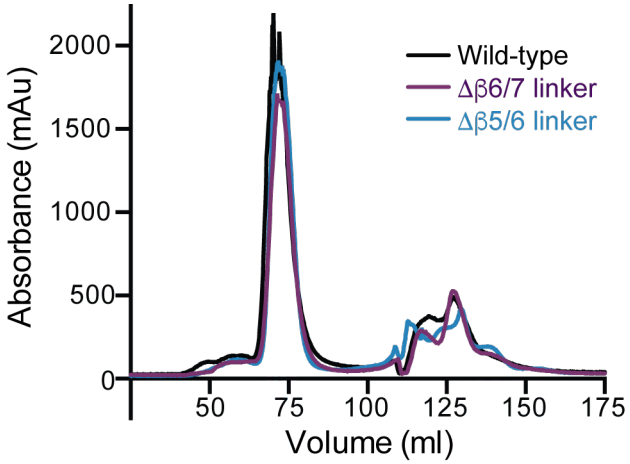

FIG S4 Linker deletion variants are folded and monomeric. Gel filtration chromatograms showing the essentially identical elution profile of wild-type NpmA and the NpmA-Δβ5/6 and NpmA-Δβ6/7 linker deletion variants during protein purification.

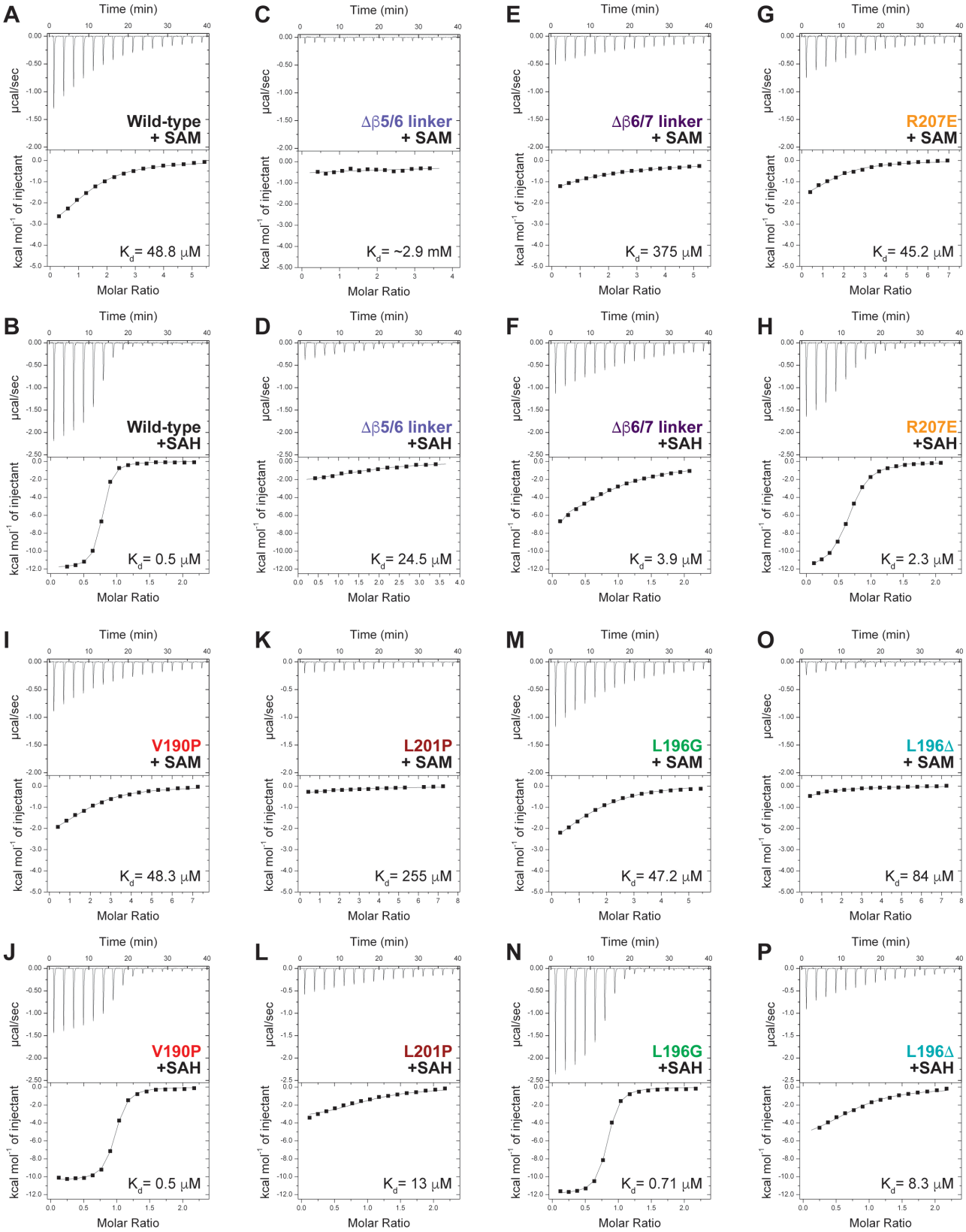

FIG S5 ITC analysis of SAM/SAH interaction with β5/6 and β6/7 linker deletion and single residue substitution NpmA variants. ITC titrations for each protein-ligand pair tested: (A),(B) wild-type NpmA (same data as shown in FIG S3); (C),(D) Δβ5/6 linker; (E),(F) Δβ6/7 linker; (G),(H) NpmA-R207E; (I),(J) NpmA-V190P; (K),(L) NpmA-L201P; (M),(N) NpmA-L196G; and, (O),(P) NpmA-L196Δ with SAM and SAH, respectively. Values for binding affinity (K_d) are those derived from the individual titration shown.