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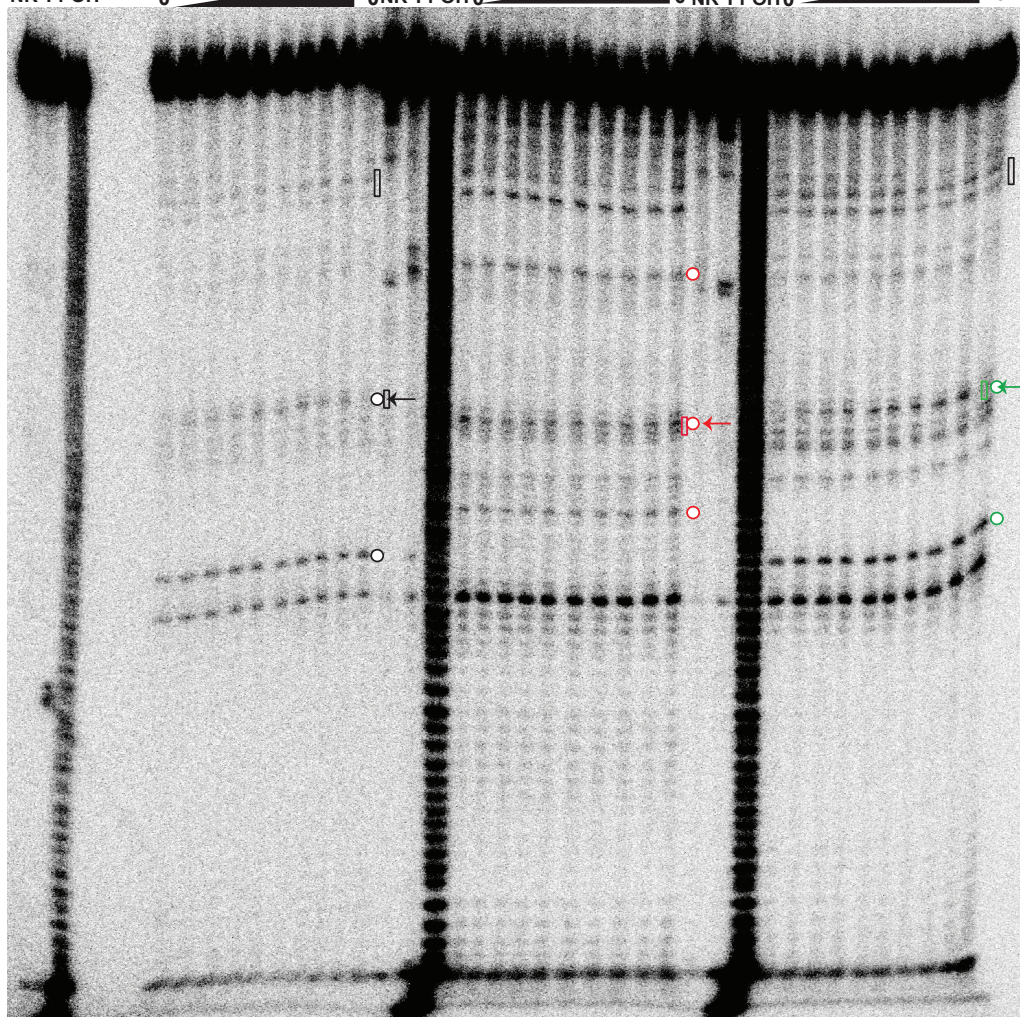
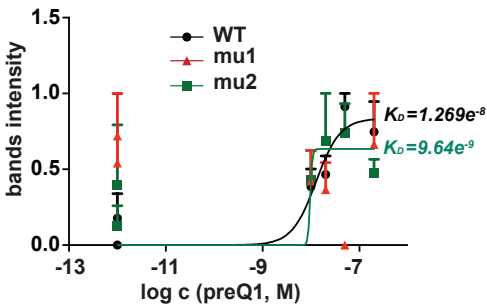


Figure S3 PreQ1-III riboswitch. (A) left: consensus secondary structure of the preQ1-III riboswitch, stars mark the chosen mutation position; right, single sequence from *Faecalibacterium prausnitzii* secondary structure. Red boxes show the disruptive mutation (mu1), green boxes show the compensatory mutation (mu2), Q1 illustrates the preQ1 binding sites, adapted from (Lieberman et al. 2015) . (B) One of the In-line probing results of three constructs: wild type, mu1 (disruptive mutant) and mu2 (compensatory mutant). Circles mark the differences in structure between the three constructs. Rectangles mark the structure change as the ligand concentration increases. Arrows indicate the points used to calculate the K_D values. (C) Plot of the fraction of RNAs whose cleavage sites (mark on B with arrow) modulate with different concentrations of preQ1 present during the in-line probing reaction. The black curve represents wild type binding interaction with a K_D of 13 ± 4.8 nM. The green curve represents the rescue mutation construction binding interaction with a K_D of 9 ± 0.96 nM. No K_D could be calculated for mu2, as expected if no binding occurs. Note: the start concentration is 0.

Lieberman JA, Suddala KC, Aytenfisu A, Chan D, Belashov IA, Salim M, Mathews DH, Spitale RC, Walter NG, Wedekind JE. 2015. Structural analysis of a class III preQ1 riboswitch reveals an aptamer distant from a ribosome-binding site regulated by fast dynamics. *Proc Natl Acad Sci U S A* 112: E3485-3494.