Supplementary Material

Structural Characterization of the RNase E S1 Domain and Identification of its Oligonucleotide-Binding and Dimerization Interfaces; Mario Schubert, Robert E. Edge, Paula Lario, Michael A. Cook, Natalie C.J. Strynadka, George A. Mackie, and Lawrence P. McIntosh

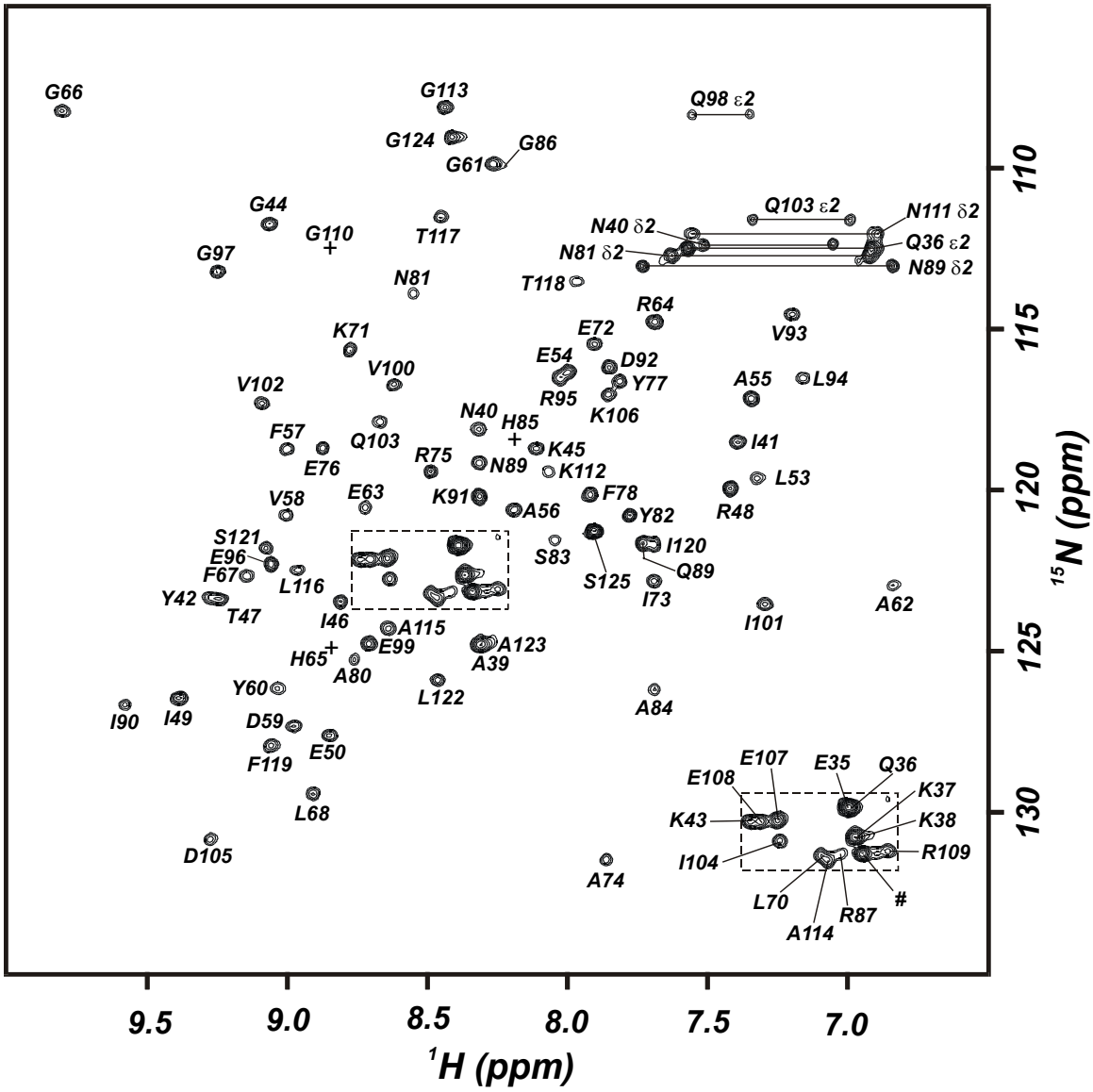

Figure S1: The assigned ^{1}H-^{15}N HSQC spectrum of RneS1$^{35-125}$ (pH 6.5, 30 ^{o}C, 0.4 mM protein). The peaks from the Asn and Gln side chain $^{15}NH_2$ groups are connected by a horizontal line. Assignments of the crowded central region are given in the lower right corner (dashed box). The only signal detectable from the N-terminal GSHML sequence is that of the Leu (indicated by a '#'). The peaks from H65, H85, and G110 (+) are too weak to be seen at this contour level.

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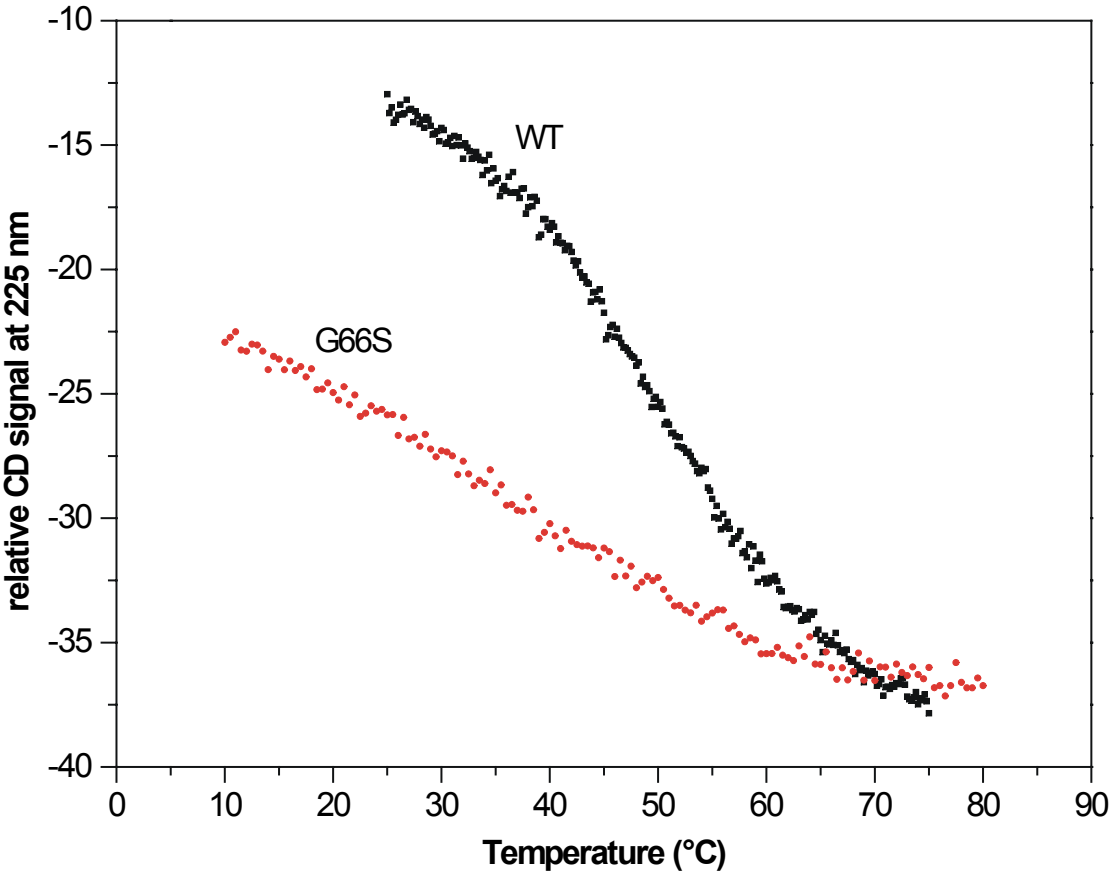

Figure S2: The thermal denaturation profiles of WT and G66S RneS1$^{35\text{-}125}$ in 20 mM phosphate buffer, pH 6.5, and 50 mM NaCl, monitored by CD spectropolarimetry (225 nm, 2 ^{0}C/min heating).

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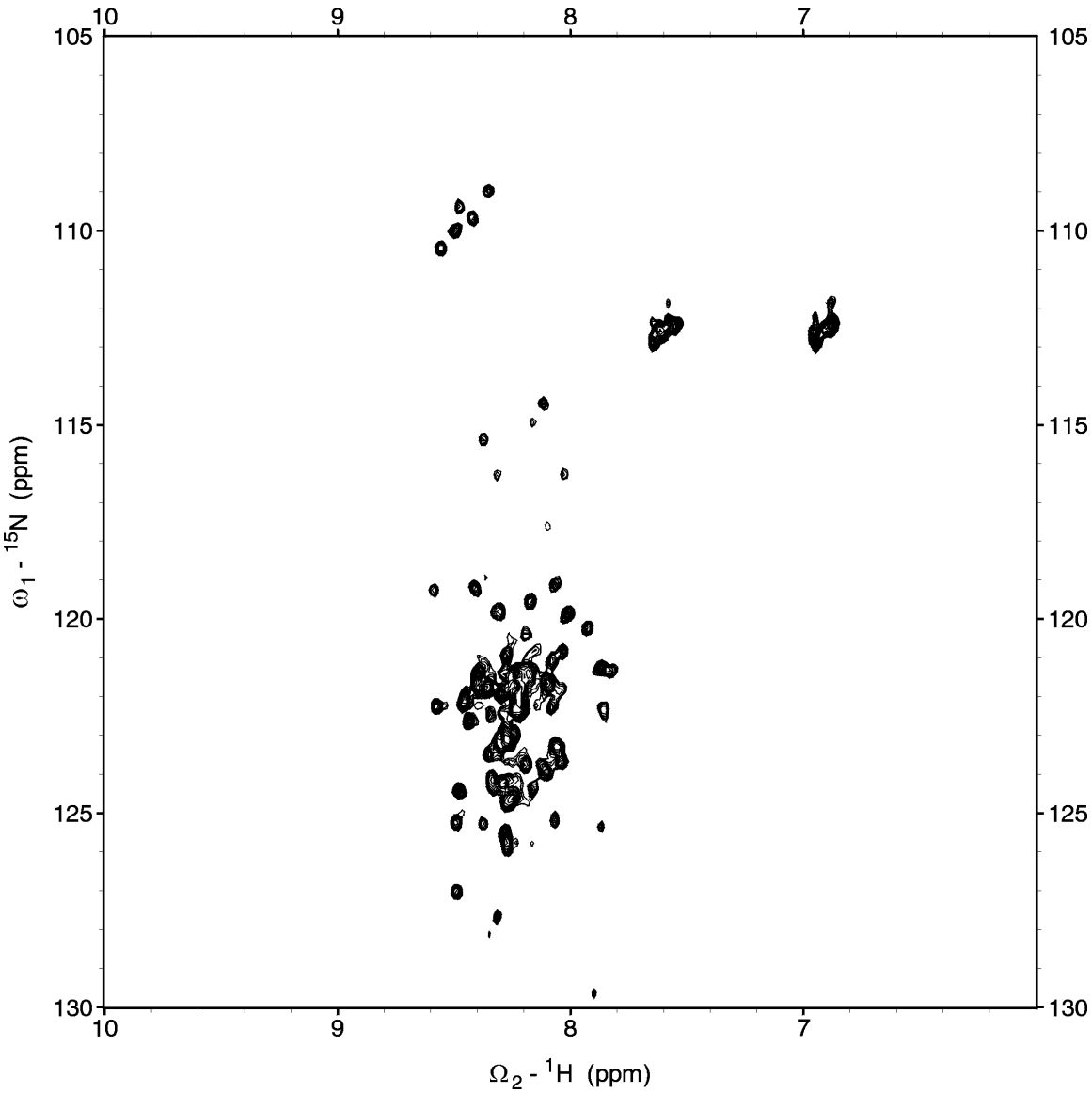

Figure S3: The ^{1}H-^{15}N HSQC spectrum of G66S RneS1$^{35-125}$ indicates that the single amino acid substitution leads to protein unfolding at pH 6.5 and 30 ^{o}C.

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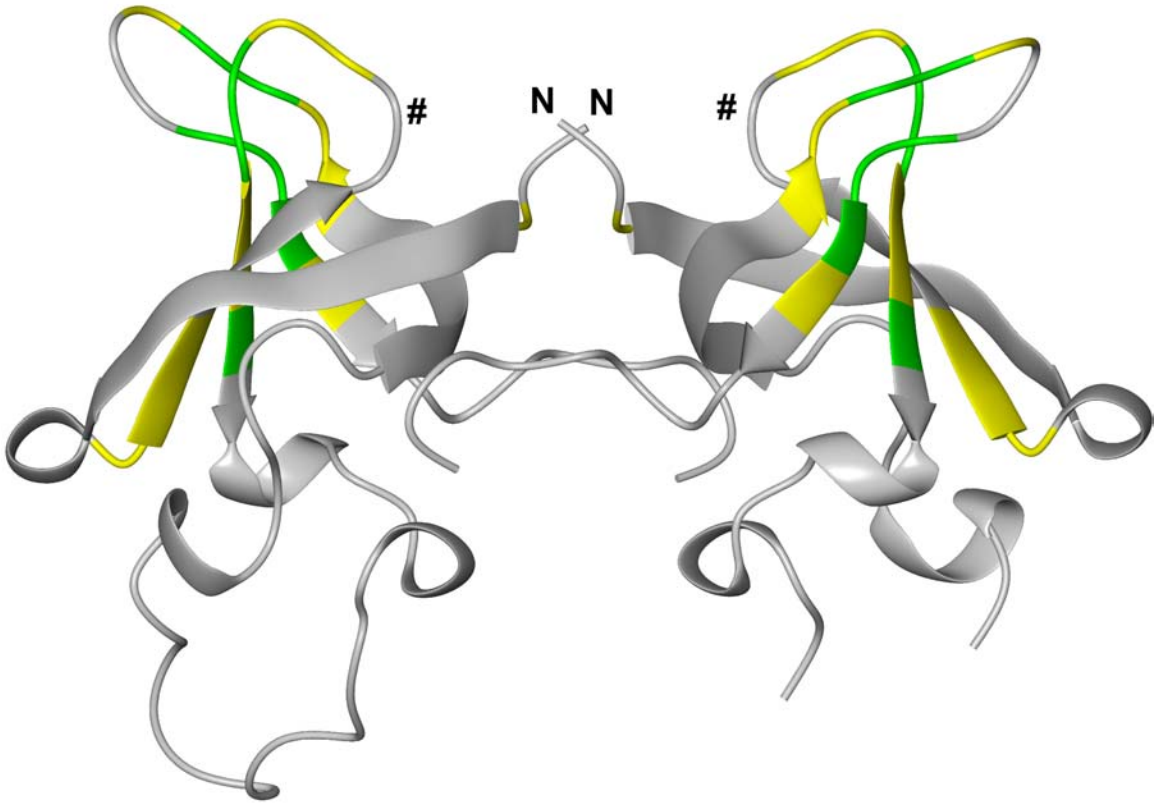

Figure S4: The position of the oligonucleotide-binding site, color coded by chemical shift perturbations as in Figure 7A is shown in context of the quaternary structure of crystalline RneS1$^{35\text{-}125}$. Two S1 domain mutants identified by Diwa et al. that affect RNase E autoregulation, but not catalytic activity, include Y60 (indicated by an #), and K37 which lies in the disordered N-terminal region of this construct (indicated by an N).