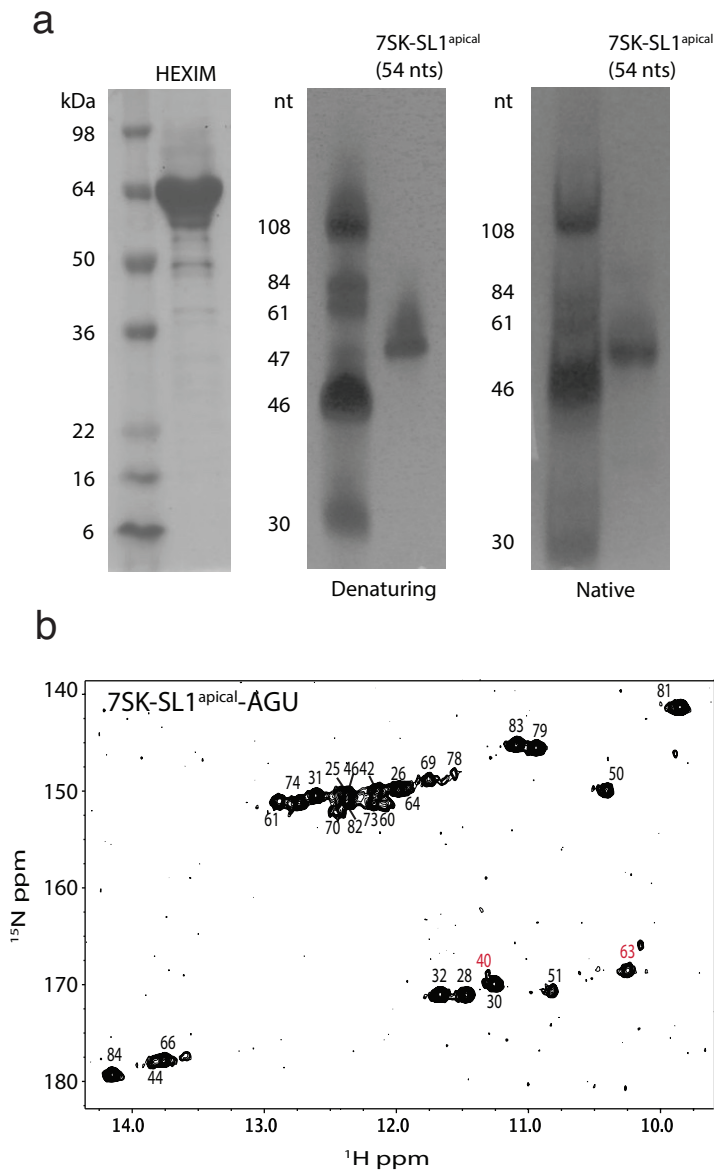
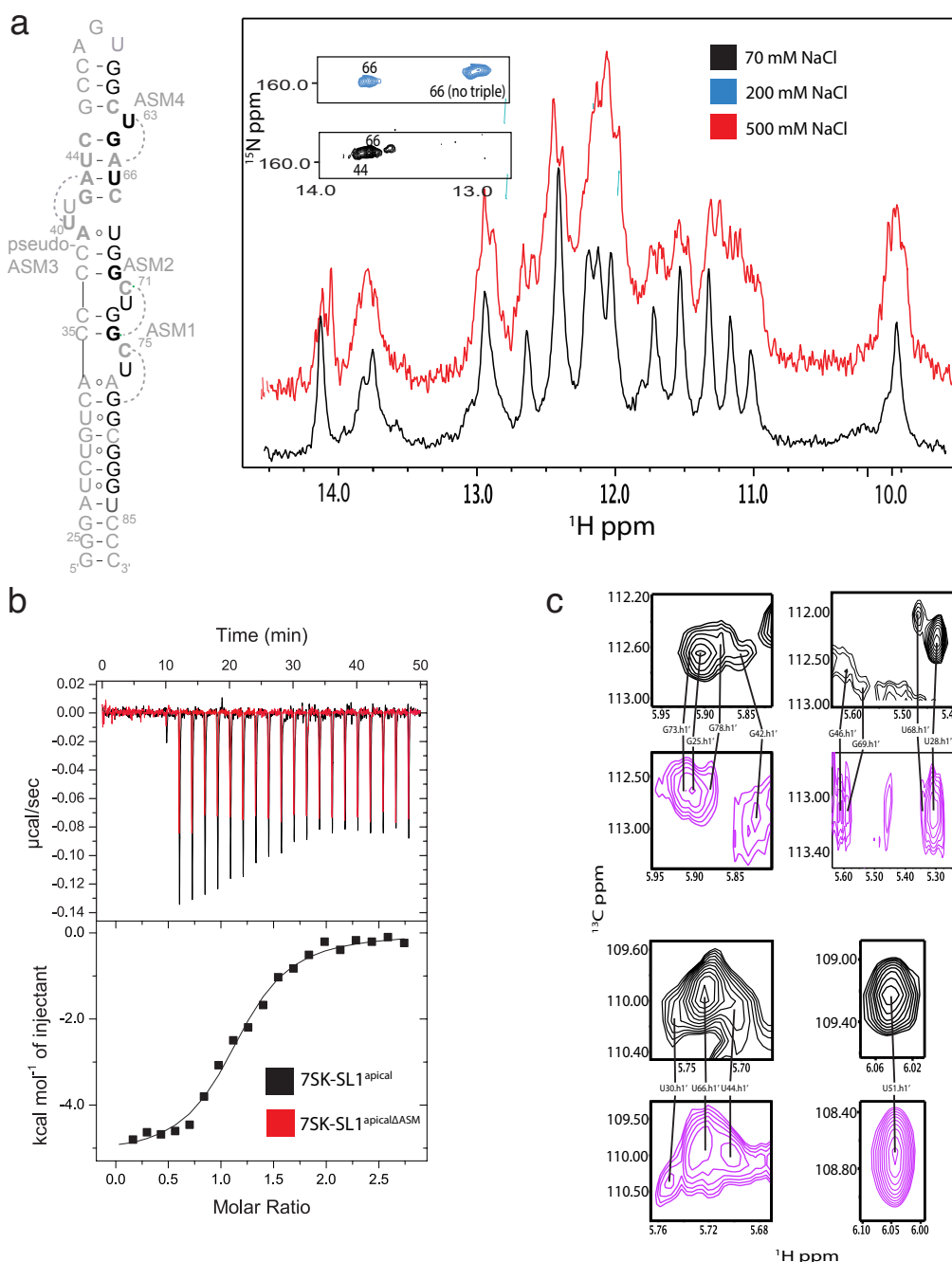




Supplementary Figure 1: HEXIM1 and 7SK-SL1^{apical}-AGU purification

(a) Coomassie stained SDS-PAGE showing HEXIM after purification (left). HEXIM has a molecular weight of 40.4 kDa, but has been shown to run with an apparent molecular weight closer to 67 kDa³⁵. Denaturing (middle) and native (right) gels of the 56 nucleotide 7SK-SL1^{apical}-AGU purified RNA product. (b) Two-dimensional ¹H-¹⁵N HSQC spectra for 7SK-SL1^{apical} with an AGU triloop (top) and a native loop (bottom) showing that all major motifs in the apical stem are unaffected by the change in the loop. Expected chemical shifts match the HSQC spectra of 7SK-SL1^{apical}-WT is from previous publication⁴⁵.



11

12 **Supplementary Figure 2: Determination of experimental conditions for characterizing**

13 **the minimal 7SK-SL1^{apical}-AGU:HEXIM N-ARM interaction.**

14 (a) Two-dimensional ^1H - ^{15}N HSQC spectra of 7SK-SL1^{apical}-AGU where residues labeled in

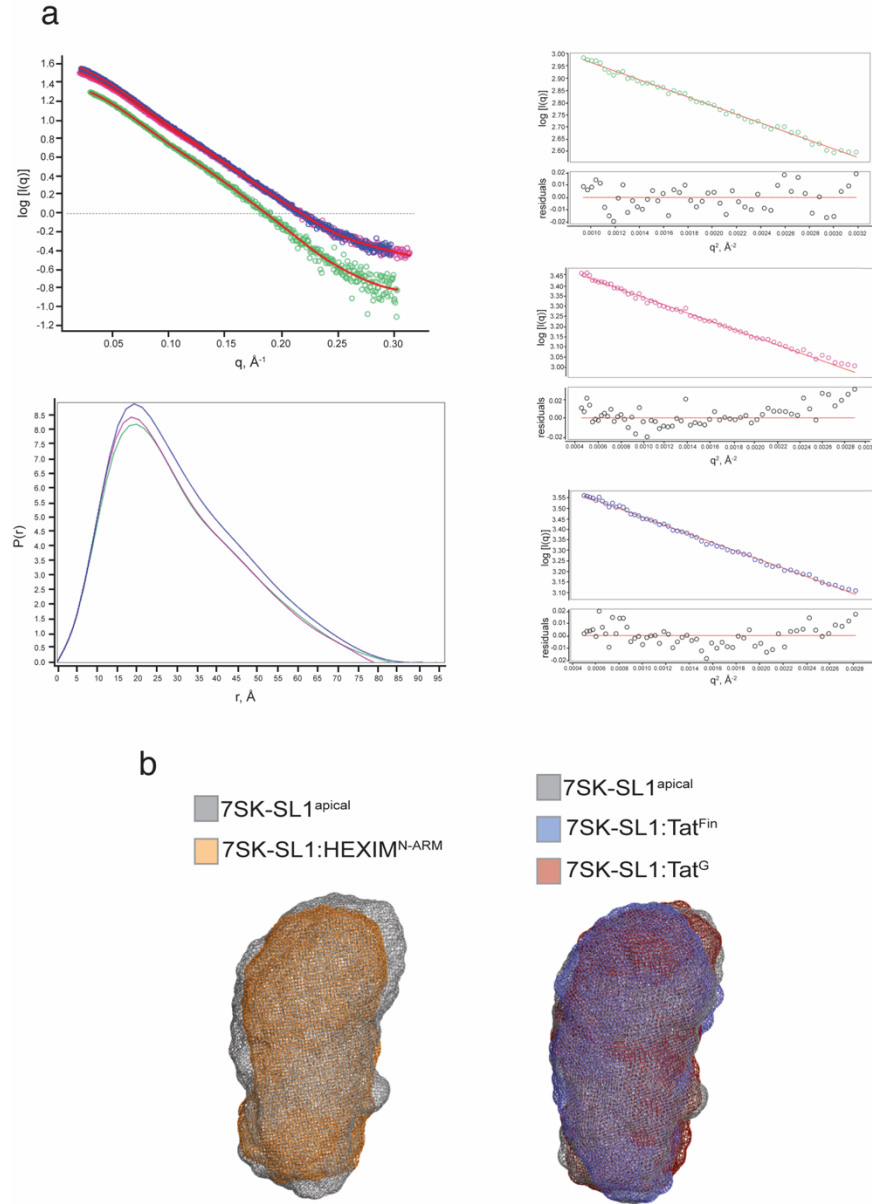
15 black were specifically labeled under high salt conditions. The emergence of a non-tripled

16 based U₆₆ imino at ~13.1ppm (inset), the disappearance of the U₆₃ imino, and the upfield

17 shift of the G₇₉ imino to ~10.2ppm shows the dissolution of the ASMs under high salt

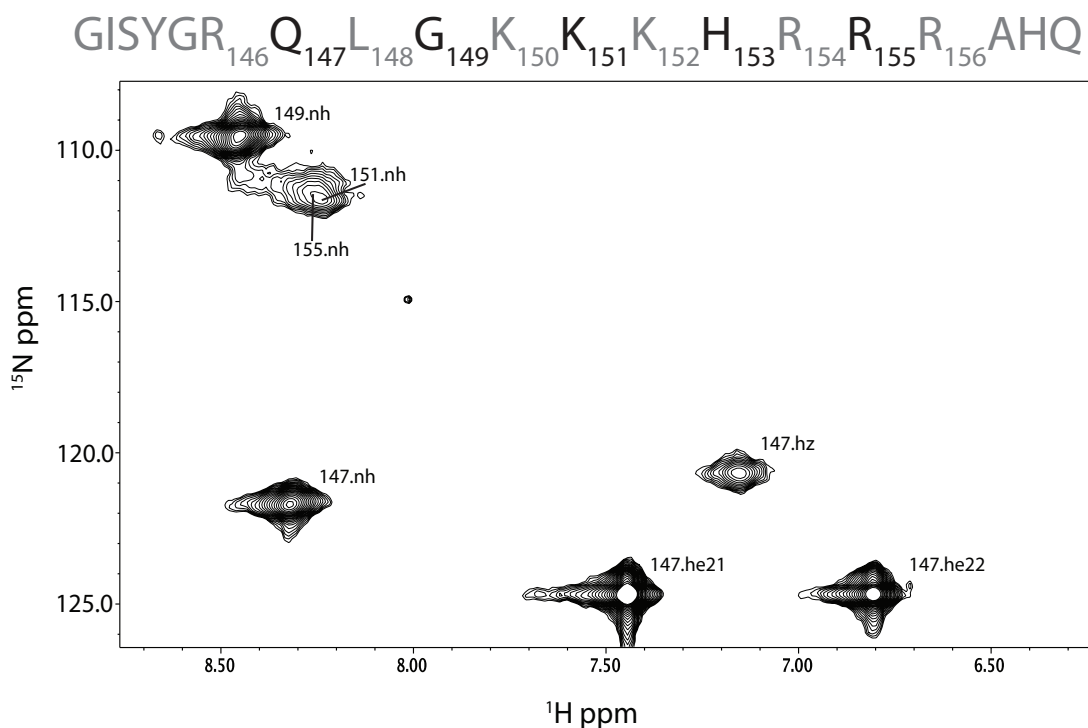
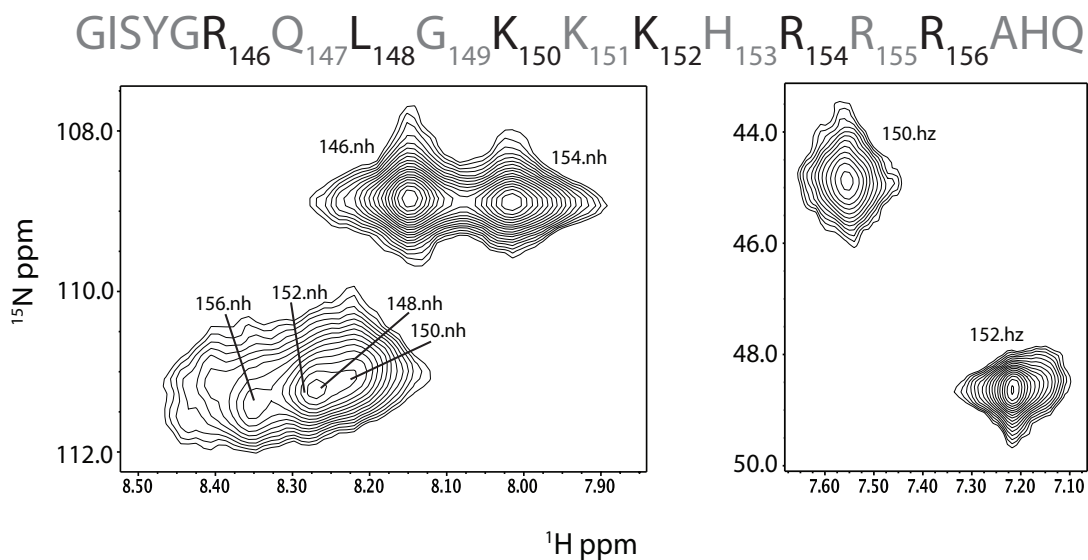
18 conditions. Comparison of 1D ^1H experiments of 7SK-SL1^{apical}-GAGA in 500mM NaCl (blue)

used in a previous study⁵² and 70mM NaCl (red). High salt leads to the significant broadening of peaks and the emergence of new imino resonances not seen near physiological conditions. **(b)** Representative ITC baseline subtraction for generation of ITC curves. Heat shown in red represent an ITC experiment of the HEXIM^{N-ARM} peptide titration into just a 7SK-SL1^{apical-AGU} construct lacking bulge pyrimidine residues with the AGU triloop. This baseline subtraction protocol was applied to all peptide ITC experiments. **(c)** Two-dimensional ¹H-¹³C HMQC spectra of GU-labeled 7SK-SL1^{apical-AGU} binding to 0.3 equivalents of full-length HEXIM1 (black) and 0.9 equivalents of HEXIM N-ARM (magenta). While there are slight differences in both carbon and proton chemical shifts due to the salt concentration of the buffers, the HEXIM N-ARM makes the same interactions with 7SK-SL1^{apical-AGU} as the full-length HEXIM1.



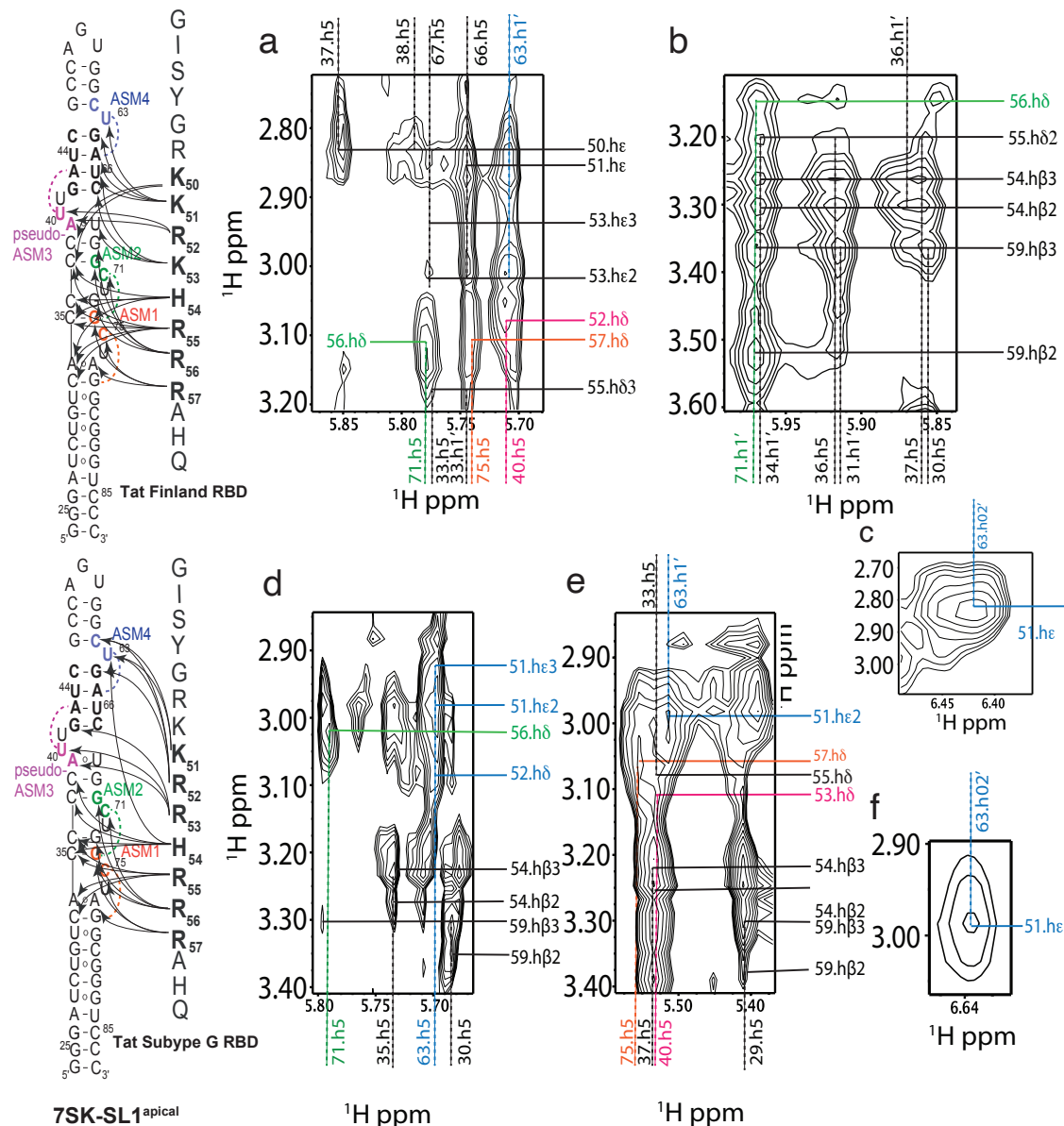
Supplementary Figure 3: SAXS analysis of 7SK-SL1^{apical}-AGU free and in complex with HEXIM and Tat ARMs.

(a) Intensity (top left), pairwise distance distribution function (bottom left), and Gunier plots (right) for 7SK-SL1^{apical}-AGU in complex with HEXIM^{N-ARM} (magenta), Tat^{Fin} (blue), and Tat^G (green) ARMs. I is scattering intensity and q is proportional to the scattering angle ($q = 4\sin\theta/\lambda$ where 2θ is the angle between the incident x-ray beam and the detector, and λ is the x-ray wavelength in $\text{\AA}$). (b) Overlay of reconstructed ab initio SAXS envelopes of free 7SK-SL1^{apical}-AGU (gray) and in complex with HEXIM^{N-ARM} (orange), Tat^{Fin} (blue), and Tat^G (red) ARMs, demonstrating the lack of global rearrangement of the RNA. Chi-squared values from FoXS analysis are 1.04 (7SK-SL1^{apical}-AGU), 1.68 (7SK-SL1^{apical}-AGU:HEXIM^{N-ARM}), 3.52 (7SK-SL1^{apical}-AGU:Tat^{Fin}), and 1.50 (7SK-SL1^{apical}-AGU:Tat^G).

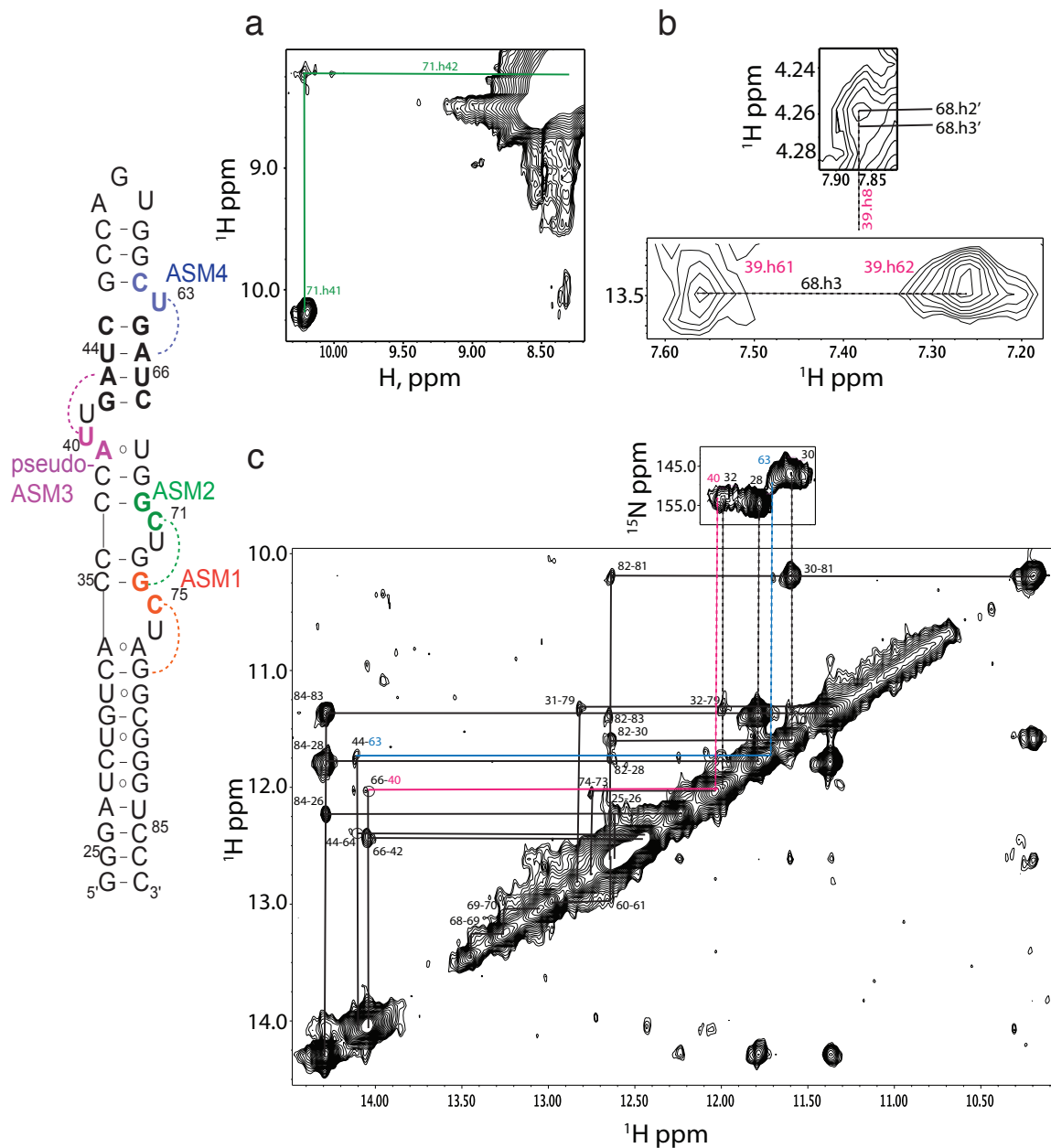


Supplementary Figure 5. Assignments of amino acid backbone and sidechain protons of the HEXIM N-ARM:7SK-SL1^{apical}-AGU interaction.

HSQC spectra of the two selectively ¹³C/¹⁵N- labeled HEXIM N-ARMs in complex with 7SK-SL1^{apical}-AGU. Bolded residues in the sequences are the amino acids that have been selectively labeled. All backbone amide protons and the side chain amino protons were unambiguously assigned with the help of this selective labeling strategy.



Supplementary Figure 7. Interactions between 7SK-SL1^{apical}-AGU and Tat^{Fin} and Tat^G RBDs: Secondary structure of the 7SK-SL1^{apical}-AGU showing connectivities to the RBDs. NOEs between: **(a)** R57, R56, and R52 Hδ of Tat^{Fin} protons and the C₇₅, C₇₁⁺, and U₄₀ caps position then into ASM₁, ASM₂, and remodeled ASM₃; K53 Hε and C₆₇ and U₆₆, and R55 Hδ and C₃₃ position these spacer residues in-between the lower and apical ASMs; **(b)** K51 Hε and U₆₃ and U₆₆, and K50 Hε and C₃₈ and C₃₇ make up the final interactions as the peptide exits the groove; H54 Hβ and the C₃₇ and C₃₆ H5 provide information for the final spacer residue of the Tat^{Fin}; **(c)** U₆₃ 2' hydroxyl and K51 Hε indicate a hydrogen bonding interaction; **(d)** R56, R52 Hδ of Tat^G and the C₇₁⁺ and U₆₃ caps show interaction with ASM₂ and ASM₁; **(e)** R57, R52 Hδ and the C₇₅ and U₄₀ caps show interaction with ASM₁ and the formation of ASM₃ by R52; **(f)** U₆₃ 2' hydroxyl proton and K51 Hε indicate K51 hydrogen bond with the U₆₃ ribose ring.



Supplementary Figure 8. Characterization of the A₃₉-U₆₈ base pair:

Portions of the ^1H - ^1H 2D NOESY spectra. (a) The C₇₁⁺ amino protons are downshifted due to their participation in a triple base interaction. (b) NOEs between the U₆₈ imino proton with the A₃₉ amino protons and the U₆₈ H2' and H3' protons with the A₃₉ H8 proton confirm that the A₃₉-U₆₈ base pair is in a cis Hoogsteen/sugar interaction. (c). Portion of the ^1H - ^{15}N 2D HSQC spectrum for ^{15}N , ^{13}C -labeled 7SK-SL1^{apical} supports the assignments of the U₄₀ and U₆₃ cap imino protons.

Complex	N-value	K _d
HEXIM1:7SK-SL1 ^{Full}	2.1 ± 0.2	209 ± 30 nM ^{*+}
HEXIM1:7SK-SL1 ^{Full-AGU}	2 ± 0.07	200.0 ± 20 nM [*]
HEXIM1:7SK-SL1 ^{apical-AGU}	1.8 ± 0.03	206 ± 60 nM ^{+∞}
HEXIM-N-ARM:7SK-SL1 ^{apical}	1 ± 0.1	229 ± 20 nM ^{∞£¢}
Tat-Finland-RBD:7SK-SL1 ^{apical}	1 ± 0.02	172 ± 10 nM ^{£π}
Tat-Subtype-G-RBD:7SK-SL1 ^{apical}	1.1 ± 0.1	81 ± 10 nM ^{£π}

*p=0.9990

⁺p=0.9999

[∞]p=0.9354

[£]p=0.2797

[¢]p=0.0008

^πp=0.0324

One-way ANOVA, Tukey's multiple comparisons test

Supplementary Table 1. ITC derived binding parameter: