Figure S3

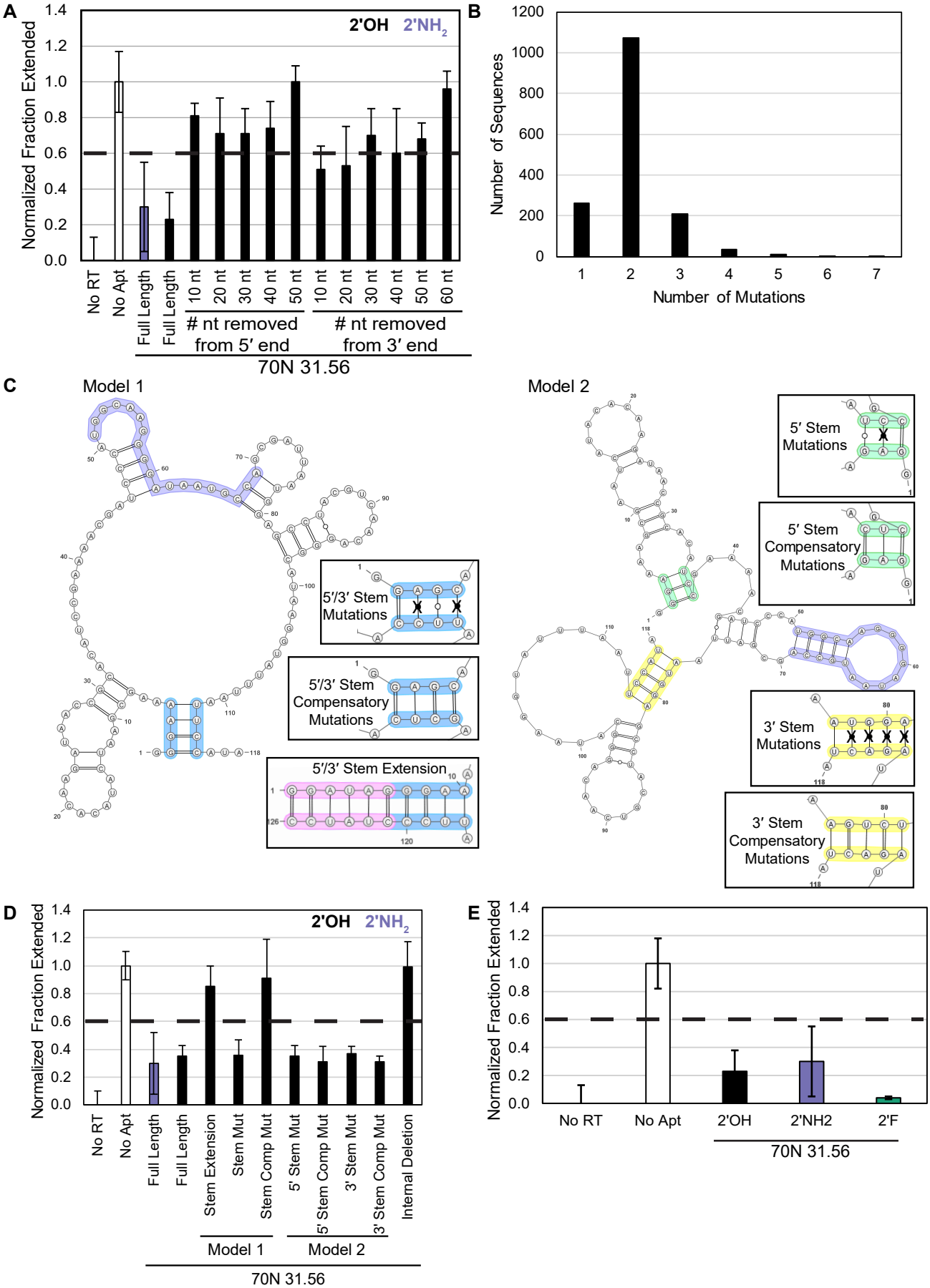
A
2'OH
2'NH2
Normalized Fraction Extended
No RT
No Apt
Full Length
Full Length
10 nt
20 nt
30 nt
40 nt
50 nt
10 nt
20 nt
30 nt
40 nt
50 nt
60 nt
nt removed from 5′ end
nt removed from 3′ end
70N 31.56
B
Number of Sequences
Number of Mutations
C
Model 1
5′/3′ Stem Mutations
5′/3′ Stem Compensatory Mutations
5′/3′ Stem Extension
Model 2
5′ Stem Mutations
5′ Stem Compensatory Mutations
3′ Stem Mutations
3′ Stem Compensatory Mutations
D
2'OH
2'NH2
Normalized Fraction Extended
No RT
No Apt
Full Length
Full Length
Stem Extension
Stem Mut
Stem Comp Mut
5' Stem Mut
5' Stem Comp Mut
3' Stem Mut
3' Stem Comp Mut
Internal Deletion
Model 1
Model 2
70N 31.56
E
Normalized Fraction Extended
No RT
No Apt
2'OH
2'NH2
2'F
70N 31.56

Figure S3. Investigating the Secondary Structure of Cluster 31 Aptamers. (A) Aptamer 70N 31.56 was systematically truncated by removing a block of 10 nucleotides from either the 5′ or 3′ end, and these truncations were used in primer extension assays. 2′-OH pyrimidines were used in these assays because the unmodified and 2′-NH_2Y versions of this RNA showed similar inhibition profiles. (B) Distribution of cluster 31 sequences showing number of sequences with the indicated number of mutations relative the seed sequence. The 300 most abundant sequences with at least two mutations were used to the generate the covariation models in (C). (C) A covariation model for the secondary structure of cluster 31 aptamers ("Model 1") determined using RNAalifold with parameters of weight of covariance of 0.6 and penalty for noncompatible sequences of 6. The 5′/3′ stem is highlighted in blue. To test this model, three variants were transcribed that mutate or extend the 5′/3′ stem and used in primer extension assays shown in (D). The changed regions of the variants are shown in the boxes. A second covariation model for the secondary structure of cluster 31 aptamers ("Model 2") determined using RNAalifold with parameters of weight of covariance of 1 and penalty for noncompatible sequences of 1. The 5′ and 3′ stems are highlighted in green and yellow, respectfully. To test this model, variants were transcribed that mutate the 5′ or 3′ stem and used in primer extension assays as shown in (D). The changed regions of the variants are shown in the boxes. The 19-nucleotide region highlighted in purple in both models was removed for the internal deletion variant tested in (D). The sequences used to generate the covariation models were first aligned using MAFFT(Katoh et al. 2002), and the output from MAFFT was used as the input for RNAalifold. (D) The variants of the two cluster 31 covariation models were used in primer extension assays to test the base-pairing hypotheses of the two models. (E) To test whether cluster 31 aptamers from the 2′-NH2-pyrimidine selection inhibit HIV-1 RT when transcribed with 2′-F pyrimidines, 70N 31.56 was transcribed using 2′-F pyrimidines (green) and used in primer extension assays (n = 4). Full-length 70N 31.56 transcribed with either 2′-OH (black) or 2′-NH_2 (purple) pyrimidines was included for comparison in (A), (D), and (E).