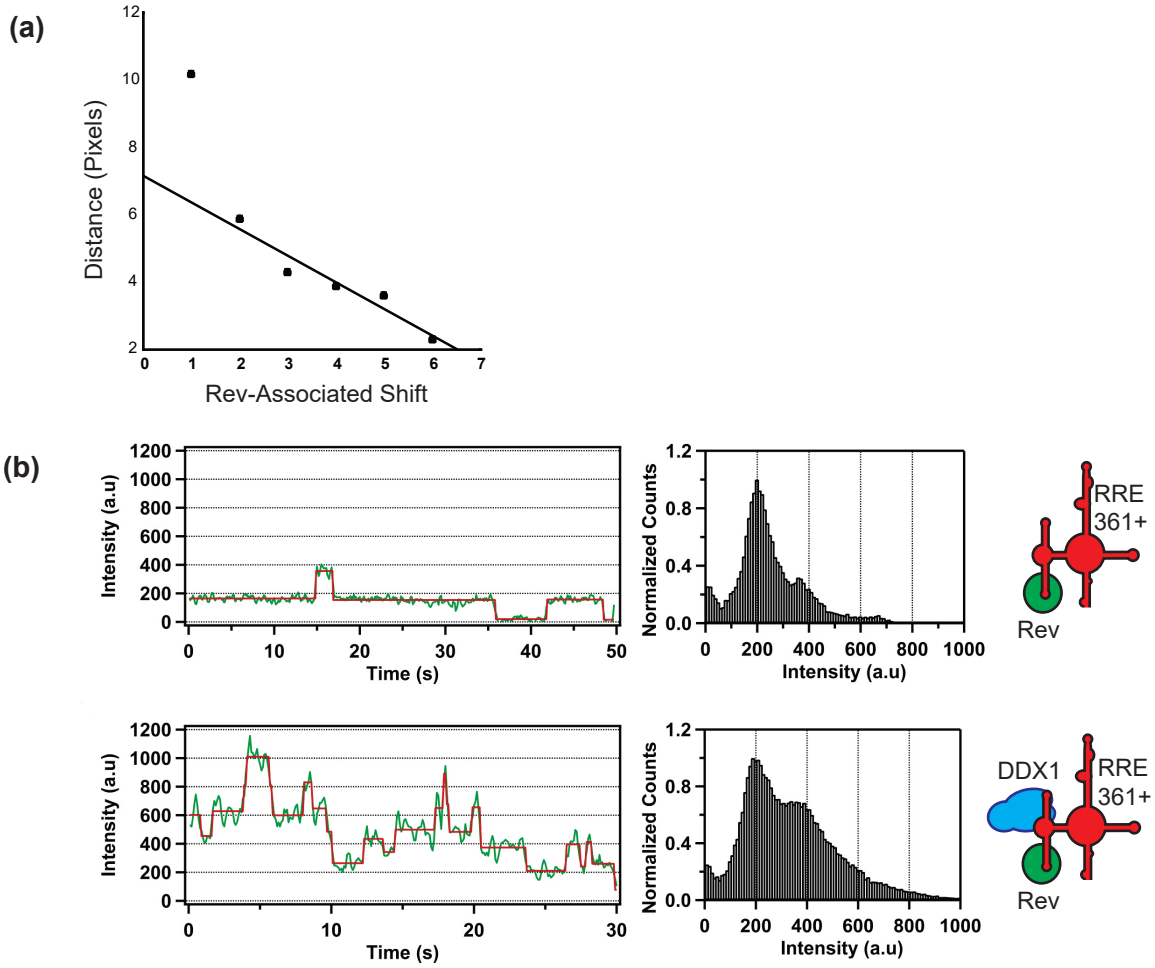
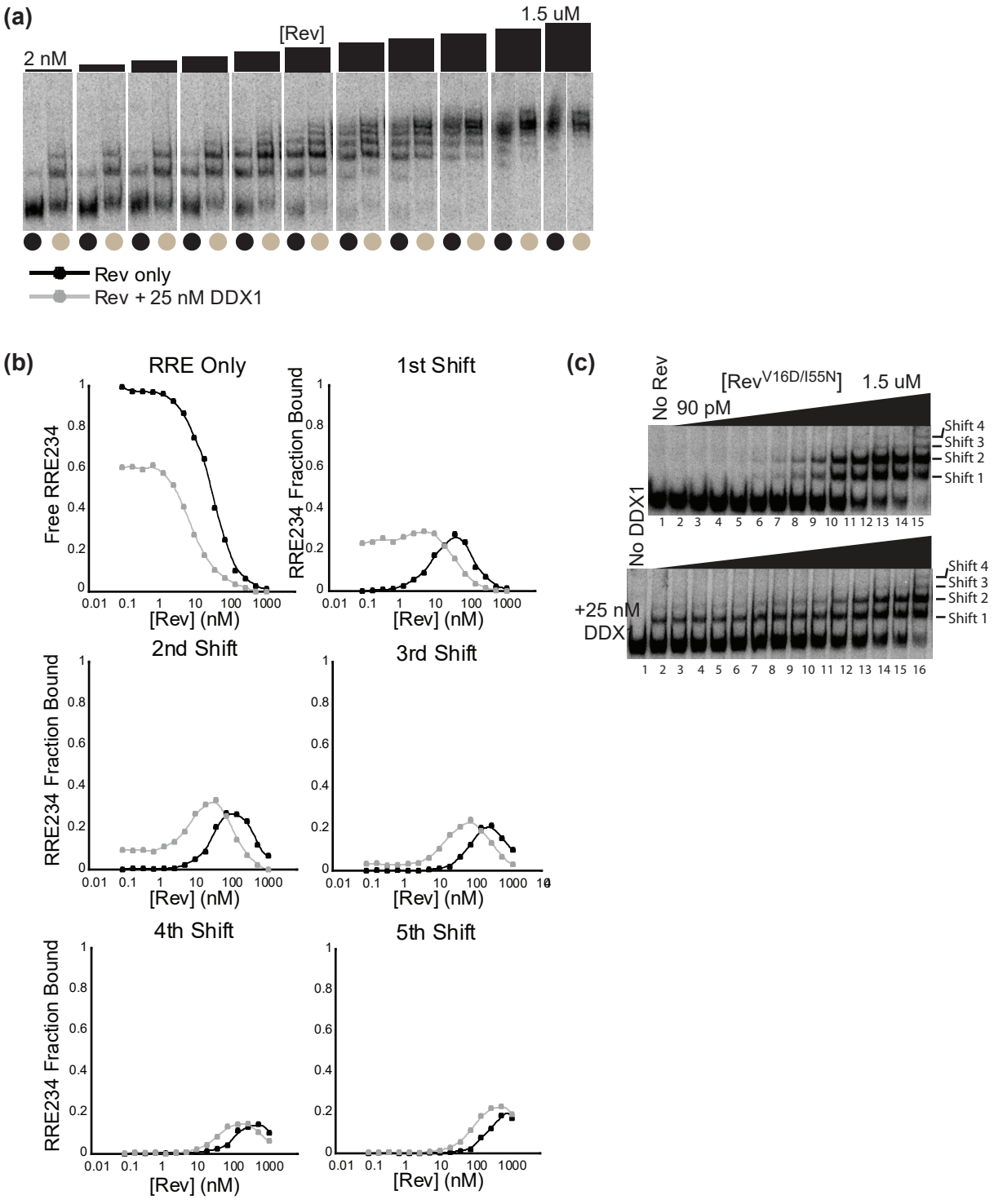
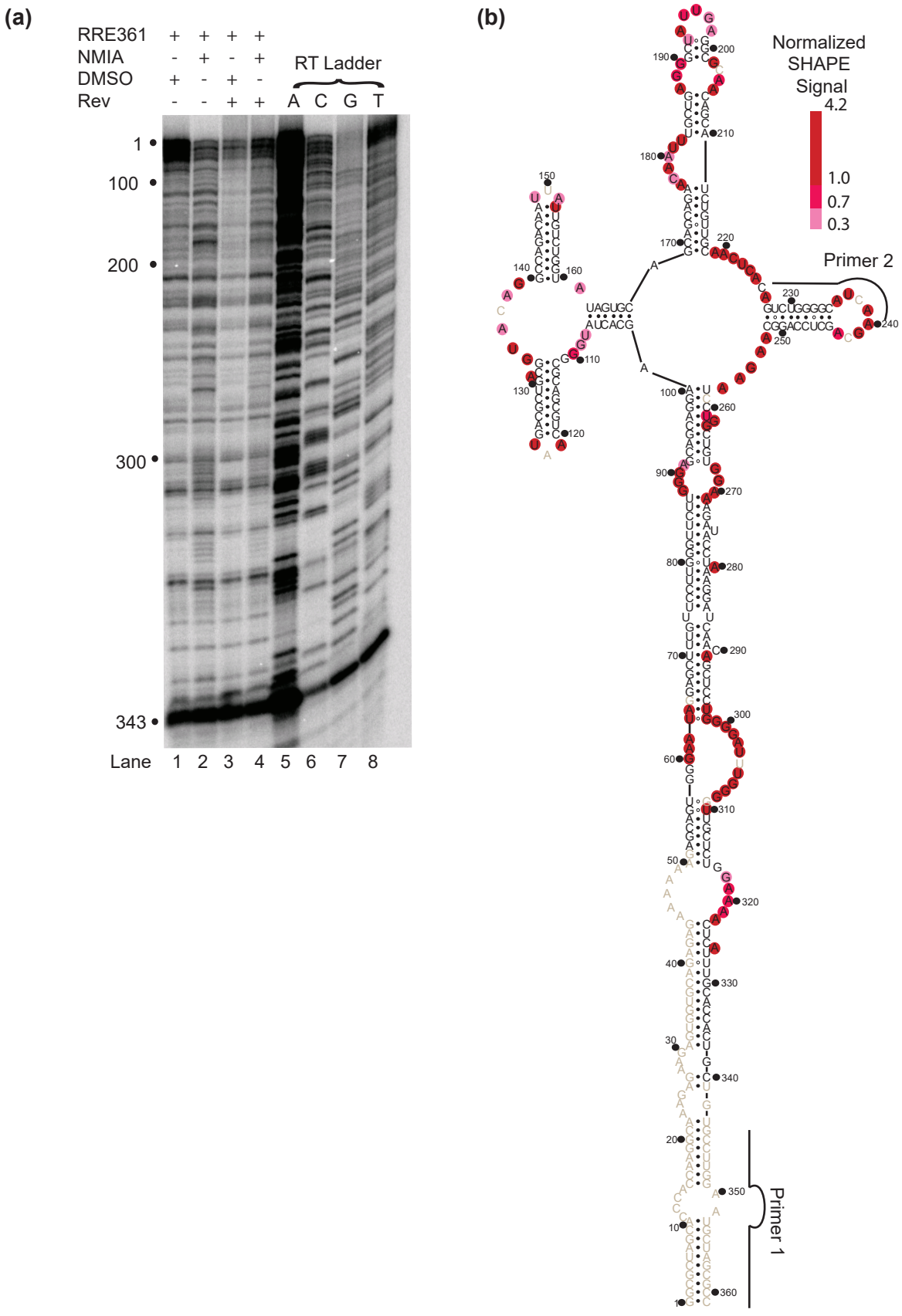
Supplemental Figure 1. (a) A distance migration plot of Rev/RRE bands of Figure 1d Points represent distance between oligomerization states of Rev/RRE interaction in Figure 1d Line is a simple y=mx+b fit of Rev oligomerization states 2-6. (b) Rev oligomerization assay using TIRF microscopy. Representative emission vs. time trace (green) for A555-labelled Rev molecules interacting with a single immobilized RRE molecule, and Hidden Markov Modeling fitted data (red) on left. Binned histograms of total Rev fluorescent signal in middle and schematic of factors added to each reaction to the right. Top histogram represent 1 nM Rev-Alexa555 alone, while the bottom includes 50 nM MBP-DDX1.


(a)
Distance (Pixels)
Rev-Associated Shift
(b)
Intensity (a.u)
Time (s)
Normalized Counts
Intensity (a.u)
RRE
361+
Rev
DDX1

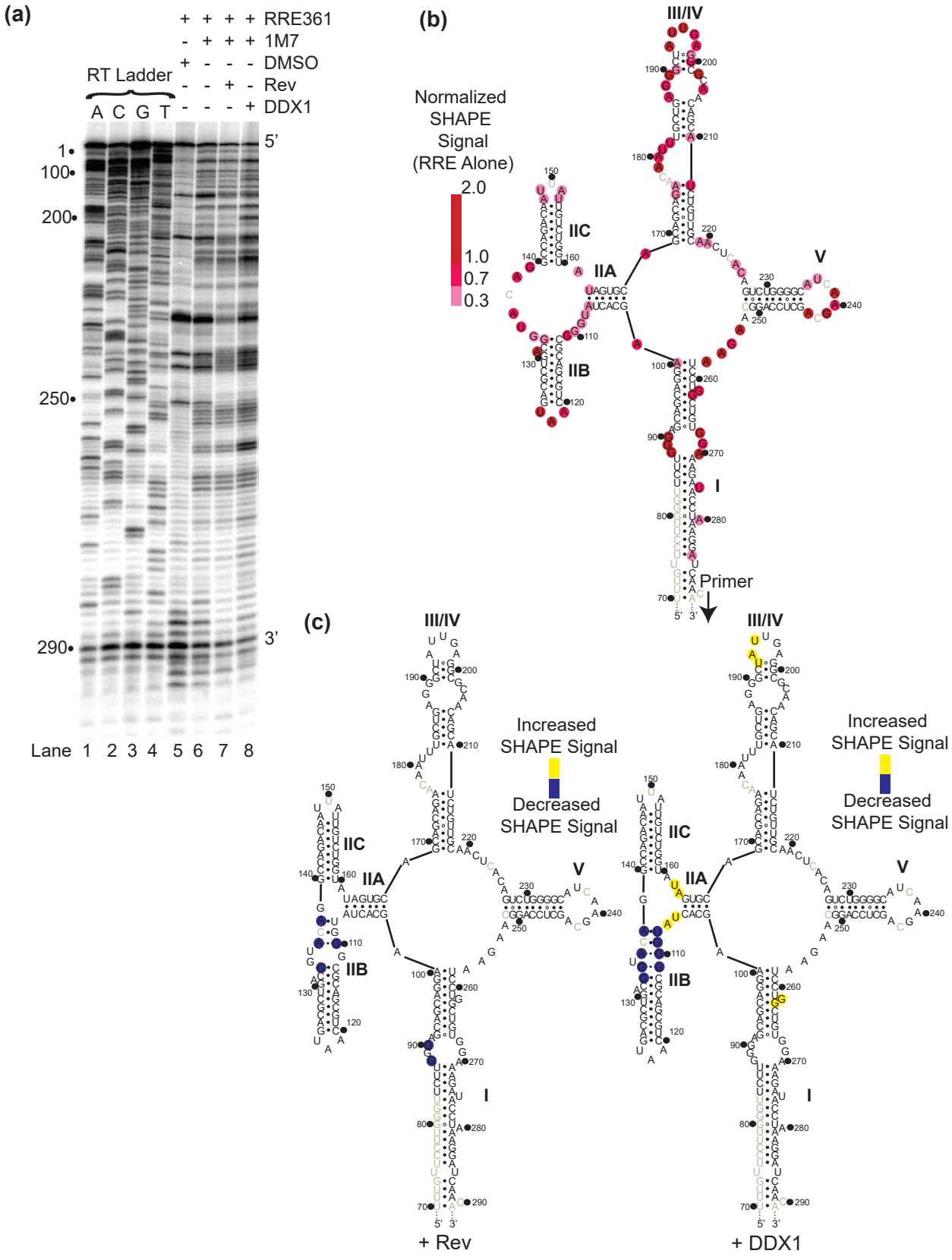
Supplemental Figure 2. (a) Electrophoretic gel mobility shift titration of Rev into radiolabeled RRE234 shown in Figure 2A. Lanes have been restructured to show similar Rev concentrations side by side. Black circles represent lanes found in Figure 2a top panel, and grey circles represent lanes found in Figure 2a lower panel. (b) Plots of fraction bound for each Rev/RRE species found in Figure 2a. Fits are simple smooth fits to show general trends. Black dots are data found in Figure 2a top panel, and grey in Figure 2a bottom panel. (c) Identical to Figure 2c. Contrast has been adjusted to more clearly display less prominent RRE species.


(a)
2 nM
[Rev]
1.5 uM
Rev only
Rev + 25 nM DDX1
(b)
RRE Only
1st Shift
2nd Shift
3rd Shift
4th Shift
5th Shift
Free RRE234
RRE234 Fraction Bound
[Rev] (nM)
(c)
No Rev
[Rev^V16D/I55N]
90 pM
1.5 uM
Shift 4
Shift 3
Shift 2
Shift 1
No DDX1
+25 nM DDX1

Supplementary Figure 3. (a) A representative sequencing polyacrylamide gel of a SHAPE experiment using NMIA. Lanes 1–8 contain RRE361 in RT reaction; Lanes 5–8 are sequencing lanes A, C, G and U sequentially. Lanes 1 and 3 are DMSO control lanes without NMIA reaction, and lanes 2 and 4 are NMIA-reacted RNA lanes. In lanes 3 and 4, reactions were performed in the presence of Rev. Approximate nucleotide positions are annotated on left. (b) Secondary structure model of RRE361 with normalized SHAPE signals annotated by colored circles. Grey nucleotides indicate areas not imaged because of primer placement, or because of very strong background (–) NMIA signal. Primers used for RT analysis are annotated by Primer 1 and Primer 2.


(a)
RRE361 + + + +
NMIA - + - +
DMSO + - + -
Rev - - + +
RT Ladder
A C G T
1
100
200
300
343
Lane 1 2 3 4 5 6 7 8
(b)
Normalized SHAPE Signal
4.2
1.0
0.7
0.3
Primer 2
UAGUGC
AUCACG
GUCUGGGGC
CGGACCUCG
Primer 1

Supplemental Figure 4. (a) A representative sequencing polyacrylamide gel of a SHAPE experiment using 1M7. Lanes 1–8, RT reactions using a RRE361 substrate; lanes 1–4, sequencing lanes A, C, G and U; lane4,DMSO control lane without 1M7; lanes 6-8, 1M7-reacted RNA lanes with RRE361 alone (lane 6), RRE361+Rev (lane 7) or RRE361+MBP-DDX1 (lane 8). Approximate nucleotide positions are designated on the left. (b) Secondary structure model of RRE361 using 1M7 SHAPE probing data. Red circles denote normalized 1M7 SHAPE signal. (c), (d) Secondary structure model of RRE361 using 1M7 SHAPE data in the presence of Rev (c) or DDX1 (). In each case, large increases in SHAPE signal above RRE361 alone are designated by yellow circles, and decreases by blue. Grey nucleotides indicate areas not imaged due to primer placement, or due to strong background RT signal in lane 5. Dotted lines indicate a predicted purine-purine interaction based on previously published NMR and X-ray crystallography data[5, 29].


(a)
RRE361
1M7
DMSO
Rev
DDX1
RT Ladder
A C G T
1
100
200
250
290
5'
3'
Lane 1 2 3 4 5 6 7 8
(b)
Normalized SHAPE Signal (RRE Alone)
2.0
1.0
0.7
0.3
III/IV
IIC
IIA
IIB
V
I
Primer
(c)
Increased SHAPE Signal
Decreased SHAPE Signal
+ Rev
+ DDX1