

**Table S1 EM data and refinement, related to Figure 3**

	Overall	Composite Model*	Core	DIS3-PIN	DIS3-RNBCS	MTR4	MTR4-dsDNA/RNA
<b>Data collection</b>							
Particles	122284		122284	122284	91483	122284	33967
Pixel size	1.07 Å/px						
Defocus range (µM)	-1.0 to -3.0						
Voltage (kV)	300						
Electron dose (Å)	85.23 e <sup>-</sup> /Å <sup>2</sup>						
<b>Reconstruction (RELION)</b>							
Accuracy of rotations (°)	1.35		2.07	1.65	1.40	1.47	1.82
Accuracy of translations (pixel)	0.81		0.88	0.78	1.04	0.98	1.34
Resolution (Å)	3.45		3.31	3.40	3.80	3.54	3.91
Map sharpening B-factor (Å <sup>2</sup> )	73.6		47.4	64.3	90.6	82.1	84.0
<b>Model composition</b>							
Non-hydrogen atoms	31633	31633	17826	1817	5469	6623	6623
Protein residues	3889	3889	2310	212	647	726	726
Nucleic acid residues	59	59	5	8	14	37	37
<b>Refinement</b>							
Map CC (around atoms)	0.738		0.754	0.688	0.744	0.729	0.741
FSC (map,model)=0.143 (Å)	3.39		3.25	3.33	3.66	3.46	3.79
FSC (map,model)=0.5 (Å)	3.62		3.40	4.04	3.95	3.77	4.06
<b>RMS deviations</b>							
Bond lengths (Å)	0.006	0.001					
Bond angles (°)	0.748	0.41					
<b>Validation</b>							
Molprobity score/Percentile	2.06/73 <sup>rd</sup>	2.03/74 <sup>th</sup>					
Clashscore/Percentile	9.78/73 <sup>rd</sup>	9.1/77 <sup>th</sup>					
Rotamer Outliers (%)	0.06	0.03					
C-beta deviations	0	0					
<b>Ramachandran plot</b>							
% favored	90.1	90.0					
% allowed	9.9	10.0					
% outliers	0.0	0.0					

\* Composite model generated by combining respective coordinates from each refinement in focused maps.

**Table S2 Oligonucleotides used in this study, related to Key Resources Table**

Type	Sequence 5' to 3'	Source
RNA	Fluorescein-AGC ACC GUA AAG ACG C	IDT
RNA	GCG UCU UUA CGG UGC UAA AAA AAA AAA AAA AAA AAA	IDT
RNA	AGC ACC GUA AAG ACG C	Dharmacon
RNA	Fluorescein-GCG UCU UUA CGG UGC UCA CCA CAC CAC ACC ACA CCA CAC CAC ACC ACA CCA CAC AAA AAA AA	Dharmacon
RNA-DNA chimera (DNA underlined)	<u>GCG UCU UUA CGG UGC UAA AAA AAA AAA AAA AAA AAA</u>	Dharmacon
RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC T CA CCA CAC CAC ACC</u> ACA CCA CAC CAC ACC ACA CCA CAC AAA AAA AA	Dharmacon
RNA-DNA chimera (DNA underlined)	<u>GCG TCT TTA CGG TGC T CA CCA CAC CAC ACC</u> ACA CCA CAC CAC ACC ACA CCA CAC AAA AAA AA	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC TAA AAA AAA AAA AAA</u> AAA A(4SU)A	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC TAA AAA AAA AA</u> (4SU) AAA AAA AAA	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC TAA AAA</u> (4SU)AA AAA AAA AAA AAA	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC T</u> (4SU)A AAA AAA AAA AAA AAA AAA	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC T CAC ACC ACA CCA</u> CAC CAC ACA AAA AA(4SU) A	Dharmacon
4SU-labeled RNA-DNA chimera (DNA underlined)	Fluorescein-GCG <u>TCT TTA CGG TGC T CAC CAC ACC ACA</u> CCA CAC CAC ACC ACA CCA CAC CAC ACA AAA AA(4-S-U) A	Dharmacon
DNA	<u>GTG TGG TGT GGT GTG GTG TGG TGT GGT GTG GTG TGG T</u>	IDT
DNA	Fluorescein- <u>GTG TGG TGT GGT GTG GTG TGG TGT GGT GTG GTG TGG T</u>	Dharmacon
DNA	<u>TTT TTT TTT TT</u>	IDT
DNA	<u>TTA TTT TTT TT</u>	IDT
DNA	<u>TTT TTT ATT TT</u>	IDT
DNA	<u>GTG TGG TGT GGT GTG GT GT</u>	IDT
DNA	<u>GCGTCTTACGGTGCT</u>	IDT
DNA	<u>ACCACACCACACCACAC</u>	IDT