SUPPLEMENTAL METHODS

Recombinant DNA. Plasmids to express recombinant proteins were generated by PCR with the following primer pairs and template DNAs, and gel-eluted PCR product and indicated plasmid were digested with the indicated restriction enzymes. The primer sequences are provided in Supplementary Table 1. All plasmid constructions were verified by restriction mapping and DNA sequencing. pGex2TN-term: primers KB1109, KB1110 and pcDNAFLRHA (C.G Lee, University of Dentistry and Medicine of New Jersey), EcoRI digestion and ligation to pGEx2T (GE). pGex2TDEIH: primers KB1733, KB 1734 and template pcDNAFLRHA, EcoRI and BamHI digestion and ligation to pGex2T. pT7.7HisC-term: primers KB1661, KB1662 and template pcDNAFLRHA, NdeI and HindIII digestion and ligation to pT7.7 (USB). pFLAG-N-term: primers KB1726, KB1727 and template pcDNAFLRHA, EcoRV and NotI digestion and ligation to pcDNA3.1. PCR-based site-directed mutagenesis (Stratagene) introduced indicated mutations by the indicated primers and template DNA: pGEX2TK54A/55A: KB1674, KB1675 and pGex2TN-term; pGEX2TN-termK236E: KB1440, KB1441 and pGex2TN-term; pGEX2T N-termK54A/55A/236E: KB1440, KB1441 and pGEX2TN-termK54A/55A. To generate pMH110, junD sequences were amplified by PCE with primers KB1377, KB1378 and template genomic DNA from HEK293 cells; gel-elution, EcoRI digestion and ligation to pCRII-TOPO (Invitrogen).

In vitro transcription. T7 RNA polymerase was used to synthesize EMSA RNA probes (Promega). Template DNA was generated by PCR with the following primer/template DNA combinations. SNV PCE: primers KB588, KB 664/pYW100 (15); mutant SNV PCE: KB1352, KB588/pAC'Aall (1); junD PCE: KB 1702, KB 1378/pMH110.

LEGENDS SUPPLEMENTAL FIGURE

Fig S1. **Evaluation of equilibrium binding conditions.** Representative electrophoretic mobility shift assays to establish equilibrium conditions upon varied incubation time and temperature. (A) Comparison of incubation time on binding of N-term to ^{32}P-SNV PCE; 30 minutes (lanes 1-7) versus 60 minutes (lanes 8-14) (B) Comparison of incubation temperature on binding of N-term to ^{32}P-SNV PCE; ice (lanes 1-7) versus room temperature (lanes 8-14).

Fig S2. **RHA C-term and DEIH exhibit weak and undetectable interaction with PCE RNA, respectively.** Representative results of 3 or more electrophoretic mobility shift assays with indicated recombinant protein and ^{32}P-labeled RNA. (A) DEIH and ^{32}P-junD PCE. (B) DEIH and ^{32}P-SNV PCE (C) C-term and ^{32}P-junD PCE (D) C-term and ^{32}P-SNV PCE (E) C-term and ^{32}P-mutant SNV PCE.

Fig S3. **DEIH domain lacks detectable binding to PCE RNA**. Representative FA assays with DEIH and indicated RNA. (A) PCE^{AB}. (B) PCE^{AC}. (C) mutAC.

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Supplemental Table 1: Sequence of DNA oligonucleotides used in indicated Experimental Procedure

	Primer name	Primer Sequence
IN VITRO TRANSCRIPTION	KB588	GTACTACGGATTCAGTCCGG
	KB 664	AAATCTAGACGATAGAATTCTAATACGACTCACTATAGGGGGTCGCCGTCCTACACATTGTTGTT
	KB 1352	AGACGATAGAATTCTAATACGACTCACTATAGGGGGTGCGTGCAACACAAACAATGTGAC
	KB 1702	AAGGTAATACGACTCACTATAGGGAGGAGCCGCCGCCAGTGG
RECOMBINANT DNA	KB 1109	TTTTGCGAATTCCCATGGGTGACGTTAAAAATTTTCTG
	KB 1110	AAAACGGAATTCGGGGGCAAAATCTCAAGATTTAGC
	KB 1674	GGGAAATTCCACCAATGCAGCAGATGCACAAAGC
	KB 1675	GCTTTGTGCATCTGCTGCATTGGTGGAATTTCCC
	KB 1440	GGATCAAATAAGGAATTGGCAGCACAGTCC
	KB 1441	GGACTGTGCTGCCAATCCTTATTTGATCC
	KB 1733	AAAGGATCCGGGGCTACTGGATGTGGGC
	KB 1734	AAAGAATTCTCATCACCGGCCAGCTCGCCCTTTCC
	KB 1661	CTTAGGATCTAAGCTTGGAATTCCTTATTAATAGCCGCCACCTCCTCT
	KB 1662	CGTATCCAGCCATATGCATCATCATCATCATCATCCTCCCAAGATGGCCCGA
	KB 1377	CCGAGGCTATAAGAGGGCGC
	KB 1378	CCTCCGCCTCCCCCGCCGCG
RNA IP PCR	KB 1303	GTCGCCCCATCGACATGGACACG
	KB 1304	GCCGCTGTTGACGTGGCTGAGG
	KB 750	CTTTGGTATCGTGGAAGGACTC
	KB 752	GTTGCTGTAGCCAAATTCGTTG
REAL TIME pcr	KB1252	TCACCCACACTGTGCCCATCTACGA
	KB1253	CAGCGGAACCGCTCATTGCCAATGG
	KB1614	GTAAGAAAAAGGCACAGCAAGCAG
	KB1615	CATTTGCCCCTGGAGGTTCTG

A

Incubated on ice 30 mins

N-term (μM)

0 0.2 0.4 0.8 1.0 1.5 2.0

Incubated on ice 60 mins

N-term (μM)

0 0.2 0.4 0.8 1.0 1.5 2.0

α-^{32}P SNV PCE

1 2 3 4 5 6 7 8 9 10 11 12 13 14

B

Incubated on ice 30 mins

N-term (μM)

0 0.2 0.4 0.8 1.0 1.5 2.0

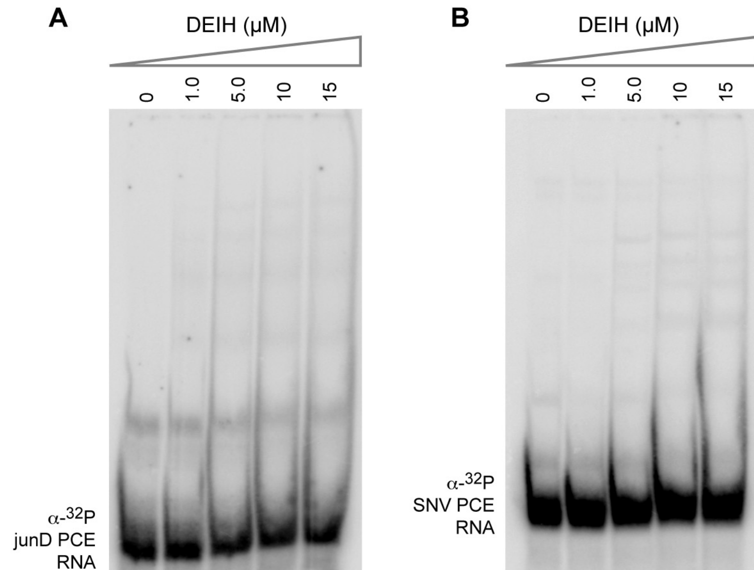
Incubated at room temperature 30 mins

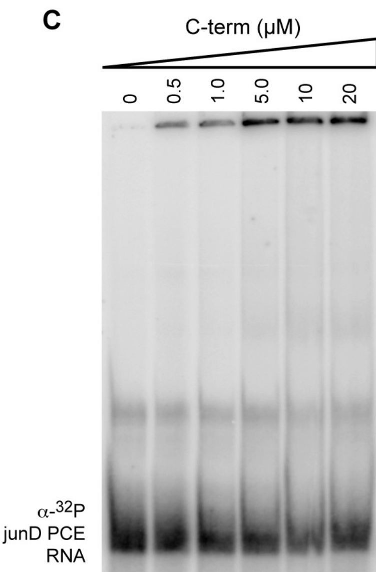
N-term (μM)

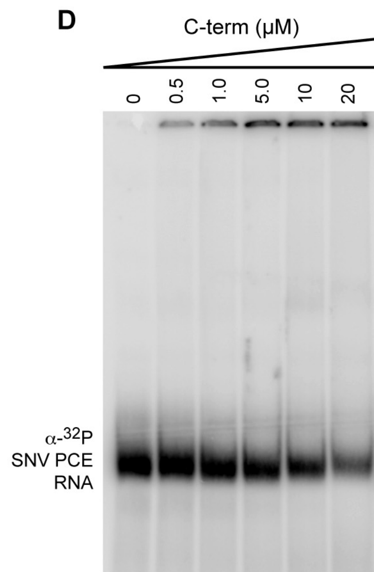
0 0.2 0.4 0.8 1.0 1.5 2.0

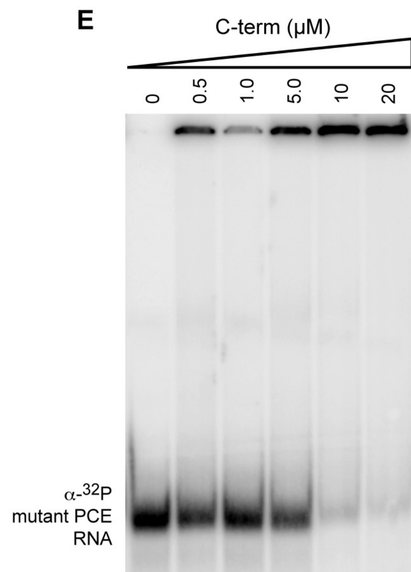
α-^{32}P SNV PCE

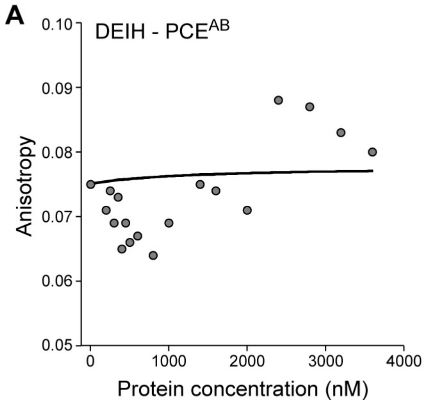
1 2 3 4 5 6 7 8 9 10 11 12 13 14

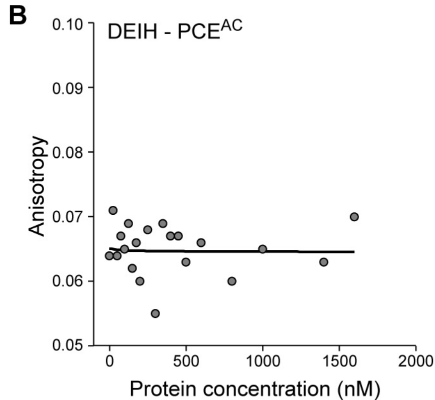

A
DEIH (μM)
0
1.0
5.0
10
15
α-32P
junD PCE
RNA
B
DEIH (μM)
0
1.0
5.0
10
15
α-32P
SNV PCE
RNA

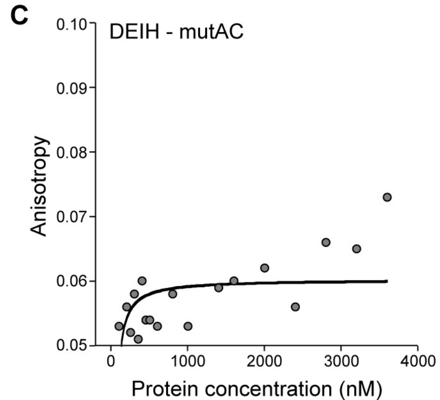

C
C-term (μM)
0
0.5
1.0
5.0
10
20
α-32P
junD PCE
RNA


D
C-term (μM)
0
0.5
1.0
5.0
10
20
α-32P
SNV PCE
RNA


E
C-term (μM)
0
0.5
1.0
5.0
10
20
α-32P
mutant PCE
RNA


A
DEIH - PCE^AB
0.10
0.09
0.08
0.07
0.06
0.05
Anisotropy
0
1000
2000
3000
4000
Protein concentration (nM)


B
DEIH - PCE^AC
0.10
0.09
0.08
0.07
0.06
0.05
Anisotropy
0
500
1000
1500
2000
Protein concentration (nM)


C
DEIH - mutAC
0.10
0.09
0.08
0.07
0.06
0.05
Anisotropy
0
1000
2000
3000
4000
Protein concentration (nM)