The DEAD-box Protein DDX43 (HAGE) Is a Dual RNA-DNA Helicase and Has a K-homology Domain Required for Full Nucleic Acid Unwinding Activity

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Running Title: Characterization of DDX43 helicase

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Included as Supplementary Information are:

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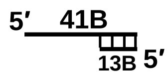
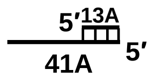
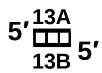
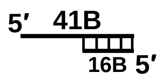
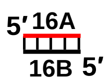
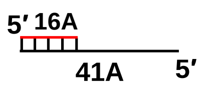
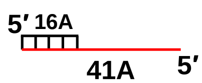
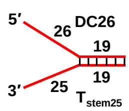
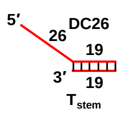
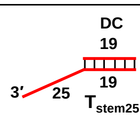
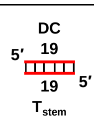
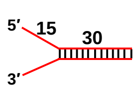
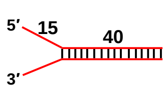
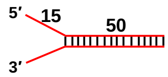
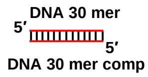
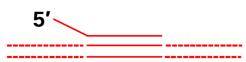
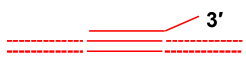
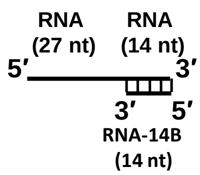
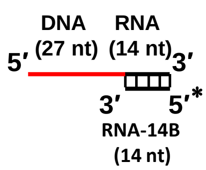
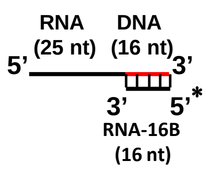
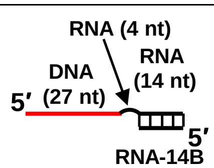
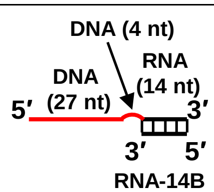
Table S1. DNA and RNA substrates and oligonucleotides used in this study

Substrate name	Structure or description[a]	Nucleotide sequence (5′→3′)
5′ tailed dsRNA (13 bp)		RNA-13B: GCGUCUUUACGGU RNA-41B: AAAACAAAACAAAACAAAACAAAAUAGCACC GUAAAGACGC
3′ tailed dsRNA (13 bp)		RNA-13A: ACCGUAAAGACGC RNA-41A: GCGUCUUUACGGUGCUUAAAACAAAACAAAA CAAAACAAAA
Blunt-end dsRNA (13 bp)		RNA-13B: GCGUCUUUACGGU RNA-13A: ACCGUAAAGACGC
5′ tailed dsRNA (16 bp)		RNA-16B: GCGUCUUUACGGUGCU RNA-41B: AAAACAAAACAAAACAAAACAAAAUAGCACC GUAAAGACGC
DNA:RNA hybrid (16 bp)_ blunt end		DNA-16A: AGCACCGTAAAGACGC RNA-16B: GCGUCUUUACGGUGCU
DNA:RNA hybrid (16 bp)_ RNA bottom strand		DNA-16A: AGCACCGTAAAGACGC RNA-41A: GCGUCUUUACGGUGCUUAAAACAAAACAAAA CAAAACAAAA
DNA:RNA hybrid (16 bp)_ DNA bottom strand		RNA-16A: AGCACCGUAAAGACGC DNA-41A: GCGTCTTTACGGTGCTTAAAACAAAACAAAACAAAACAAAA
Fork dsDNA (19 bp)		DC26: TTTTTTTTTTTTTTTTTTTTTTTTTCCCAGTAAAACGA CGGCCAGTGC T_{stem25}: GCGGTCCCAAAAGGGTCAGTGCTGGCATTTTGCT GCCGGTCACG
5′ tailed dsDNA (19 bp)		DC26: TTTTTTTTTTTTTTTTTTTTTTTTTCCCAGTAAAACGA CGGCCAGTGC T_{stem}: GCACTGGCCGTCGTTTTAC
3′ tailed dsDNA (19 bp)		DC: GTAAAACGACGGCCAGTGC T_{stem25}: GCGGTCCCAAAAGGGTCAGTGCTGGCATTTTGCT GCCGGTCACG
Blunt-end dsDNA (19 bp)		DC: GTAAAACGACGGCCAGTGC T_{stem}: GCACTGGCCGTCGTTTTAC
Fork dsDNA (30 bp)		Fork 30/15-T: TTTTTTTTTTTTTTTGGTGATGGTGTATTGAGT GGGATGCATGCA Fork 30/15-B: TGCATGCATCCCACTCAATACACCATCACCTT TTTTTTTTTTTTT
Fork dsDNA (40 bp)		Fork 40/15-T: TTTTTTTTTTTTTTTACGAGCTAAATTAGAGC GACTGCACAACTGTAAGGTCCGT Fork 40/15-B: ACGGACCTTACAGTTGTGCAGTCGCTCT AATTTAGCTCGTTTTTTTTTTTTTTT

Fork dsDNA (50 bp)		Fork 50/15-T: TTTTTTTTTTTTTTTTACGAGCTAAATTAGAGCGA CTGCACAACTGTAAGGTCCGTTGGTCAGCCT Fork 50/15-B: AGGCTGACCAACGGACCTTACAGTTGTGCAGTC GCTCTAATTTAGCTCGTTTTTTTTTTTTTTTT
Blunt-end dsDNA (30 bp)		DNA 30 mer: GAGCTACCAGCTACCCCGTATGTCAGAGAG DNA 30 mer comp: CTCTCTGACATACGGGGTAGCTGGTAGCTC
X12-1-28-BioT37[b]	DNA substrate with biotin	TTTTTTTTTTTTTTTTGACGCTGCCGAA-BiodT-TCTGGCTTGCTAGGACATCTTTGCCCACGTTGACCCG
dT_{30}	Used as ssDNA	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
rU_{30}	Used as ssRNA	UUUUUUUUUUUUUUUUUUUUUUUUUUUUUU
5′ Tail plasmid triplex		Plasmid pSupF5 is linearized by digestion with *Nde* I, and the third strand is annealed to triplex target site in duplex. 5′ tail TC30 (TGACGCTCCGTACGATCTTTTTCTTTCTTTTCTT CTTTTTTCTTT) is used as third strand.
3′ Tail plasmid triplex		Plasmid pSupF5 is linearized by digestion with *Nde* I, and the third strand is annealed to triplex target site in duplex. 3′ tail TC30 (TCTTTTTCTTTCTTTTCTTCTTTTTTCTTTTCACG CTCCGTACGA) is used as third strand.
dsRNA (14 bp)_ 5′ tail RNA		RNA-41B (top strand): AAAACAAAACAAAACAAAACAAAAUAGCACCGUAAAGACG C RNA-14B: GCGUCUUUACGGUG
dsRNA (14 bp)_ 5′ tail DNA		DNA27/RNA14-41B: AAAACAAAACAAAACAAAACAAAATAGCACCGUAAAGACG C RNA-14B: GCGUCUUUACGGUG
DNA:RNA hybrid (16 bp)_ 5′ tail RNA		RNA 25/DNA16: AAAACAAAACAAAACAAAACAAAAUAGCACCGTAAAGACG C RNA-16B: GCGUCUUUACGGUGCU
Chimeric dsRNA(14 bp)_RNA adjacent to duplex		DNA27/RNA18 (top strand) AAAACAAAACAAAACAAAACAAAATAGCACAGACCGUAAA GACGC RNA-14B: GCGUCUUUACGGUG
Chimeric dsRNA (14 bp)_DNA adjacent to duplex		DNA31/RNA14 (top strand): AAAACAAAACAAAACAAAACAAAATAGCACAGACCGUAAA GACGC RNA-14B: GCGUCUUUACGGUG

[a] Line and sequence in black are RNA, line and sequence in red are DNA; [b] BiodT, an internal biotin-conjugated dT.

Table S2 PCR primers used in this study

Primer	Sequence (5'-3')	Used in this study
DDX43-F-NdeI	ACGTCATATGTCCCACCACGGAGGAGCTCCC	Froward primer to PCR amplify full-length DDX43 gene or its N terminus (NT) for cloning in *Nde* I site of pET28a vector
DDX43-R-XhoI	GCATCTCGAGATGAAACTTCTTGGGCCTTCCTTG	Reverse primer to PCR amplify full-length DDX43 gene or its C-terminal helicase domain (HD) for cloning in *Xho* I site of pET28a vector
DDX43-NT-R-XhoI	GCATCTCGAGAACCTCAGGATAACATTGAAAG	Reverse primer to PCR amplify DDX43 gene N terminus (NT) for cloning in *Xho* I site of pET28a vector
DDX43-HD-F-NdeI	ACGTCATATGGAAAACATTAAAAAGGCAGG	Forward primer to PCR amplify DDX43 C-terminal helicase domain (HD) for cloning in *Nde* I site of pET28a vector
DDX43-KH-F-NdeI	ACGTCATATGCCGCTGTGTTTTGCTTTGAAG	Forward primer to PCR amplify DDX43 KH domain for cloning in *Nde* I site of pET28a vector
DDX43-KH-R-XhoI	GCATCTCGAGTTCTGAATTGTAATTTTCTTC	Reverse primer to PCR amplify DDX43 gene KH domain for cloning in *Xho* I site of pET28a vector
DDX43-G46D-F	GAGGGATATAGTGTCGACAGAGGTGGTCGCTGG	Forward primer for site-directed mutagenesis of DDX43-G46D
DDX43-G46D-R	CCAGCGACCACCTCTGTCGACACTATATCCCTC	Reverse primer for site-directed mutagenesis of DDX43-G46D
DDX43-G84D-F	GTTGGCGCGGTAATCGATCGTGGTGGGTCAAAAAT	Forward primer for site-directed mutagenesis of DDX43-G84D
DDX43-G84D-R	ATTTTTGACCCACCACGATCGATTACCGCGCCAAC	Reverse primer for site-directed mutagenesis of DDX43-G84D
DDX43-G154D-F	CATTCCAACCTTCTGTTGATAAAGATGGAAGCACAGATAAC	Forward primer for site-directed mutagenesis of DDX43-G154D
DDX43-G154D-R	GTTATCTGTGCTTCCATCTTTATCAACAGAAGGTTGGAATG	Reverse primer for site-directed mutagenesis of DDX43-G154D
DDX43-K292A-F	CAGACTGGAACAGGAGCGACATTGTGTTATTTA	Forward primer for site-directed mutagenesis of DDX43-K292A
DDX43-K292A-R	TAAATAACACAATGTCGCTCCTGTTCCAGTCTG	Reverse primer for site-directed mutagenesis of DDX43-K292A
DDX43-D396A-F	CCTACTTGGTTTTAGCTGAAGCAGACAAGATG	Forward primer for site-directed mutagenesis of DDX43-D396A
DDX43-D396A-R	CATCTTGTCTGCTTCAGCTAAAACCAAGTAGG	Reverse primer for site-directed mutagenesis of DDX43-D396A

Restriction digestion sites are underlined.

Supplemental Data Figure Legends

Figure S1. Structure and sequence of DDX43 protein.
(**A**) Cartoon depicting DDX43 protein with the conserved helicase motifs (in yellow), one potential KH domain (pink), and two GXXG motifs (olive green). (**B**) Sequence of DDX43 protein. Engineered mutants G46D, G84D, G154D, K292A, and D396A are indicated.

Figure S2. Chromatographic profiles of standard proteins.
Chromatographic profiles of standard proteins [blue dextran (2000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), beta amylase (200 kDa), aldolase (158 kDa) and albumin (67 kDa) eluting from a Sephacryl S-300 column and its calibration curve (inset).

Figure S3. Purification and characterization of mutants K292A and D396A proteins.
(**A**) SDS-PAGE analysis of DDX43 K292A and D396A proteins eluting from a Sephacryl S-300 HR column. (**B, C**) Representative images of helicase reactions performed by incubating 0.5 nM 5′ tail 13 bp duplex RNA substrate with increasing protein concentration of K292A (**B**) or D396A (**C**) at 37°C for 15 min. (**D, E**) Representative images of helicase reactions performed by incubating 0.5 nM 19 bp forked dsDNA substrate with increasing protein concentration of K292A (**D**) or D396A (**E**) at 37°C for 15 min. NE, no enzyme; WT, wild-type; filled triangle, heat denatured substrate control.

Figure S4. Unwinding activity of DDX43 protein on DNA:RNA hybrid substrates.
Representative images of helicase reactions by incubating increasing protein concentration (0-3 µM) at 37°C for 15 min with 0.5 nM of various 16 bp DNA:RNA hybrid substrares, blunt-end (**A**), bottom strand with RNA (**B**), bottom strand with DNA (**C**). (**D**) Quantitative analyses of DNA unwinding of DDX43 in panel A-C.

Figure S5. Annealing activity of DDX43 protein.
Representative image of annealing reactions with or without ATP for RNA substrate (**A**) and DNA substrate (**B**). NE: no enzyme; filled triangle: heat denatured RNA or DNA substrate control.

Figure S6. Effects of single-stranded tail length on DDX43 unwinding activity.
(A, B) Representative images of helicase reactions performed by incubating 0.5 nM of 8 nt 5′ tail (**A**) or 18 nt 5′ tail (**B**) 13-bp duplex RNA substrate with increasing protein concentration (0-3 µM) at 37°C for 15 min. (**C**) Quantitative analyses of RNA unwinding of DDX43 in panel A-B and Figure 2D. **(D-E**) Representative images of helicase reactions performed by incubating 0.5 nM of 15 nt 3′ tail (**D**) or 25 nt 3′ tail (**E**) 19-bp duplex DNA substrate with increasing protein concentration (0-3 µM) at 37°C for 15 min. (**F**) Quantitative analyses of DNA unwinding of DDX43 in panel D-E.

Figure S7. Optimization of cations and ATP:Mg^{2+} ratio for DDX43 unwinding.
(**A, B**) Representative images of helicase reactions performed by incubating 0.5 nM 5′ tailed 13 bp duplex RNA (**A**) or 19 bp forked duplex DNA substrate (**B**) with indicated cations (2 mM) at the protein concentration of 3 µM at 37°C for 15 min. (**C, D**) Representative images of helicase reactions performed by incubating 0.5 nM 5′ tailed 13 bp duplex RNA (**C**) or 19 bp forked duplex DNA substrate (**D**) with indicated ATP:Mg^{2+} ratio at the protein concentration of 3 µM at 37°C for 15 min.

Figure S8. Alignment of human DDX43 KH domain with others.
(**A**) Alignment of human DDX43 KH domain with other known KH domains. (**B**) Alignment of the KH domains from human DDX43 and its orthologues across species. The consensus were indicated below, and conserved amino acids are colored.

Figure S9. Sequence specificity binding of DDX43 KH domain.

Representative EMSA images for DDX43 KH domain proteins (0-3 µM) incubated with 0.5 nM of dA_{30} (left), dC_{30} (middle), and 30-mer random DNA (right).

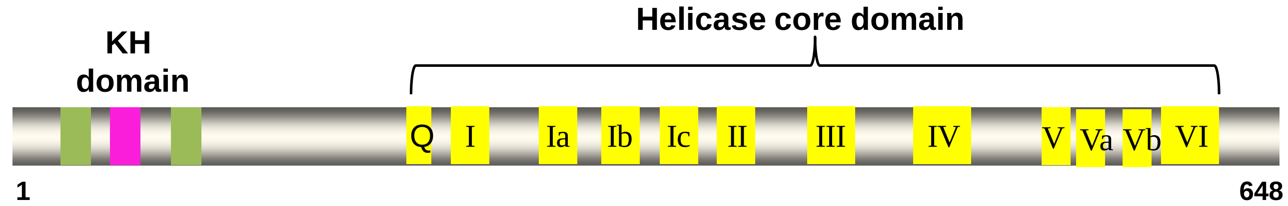
Figure S10. Purification and characterization of DDX43 N-terminal domain.
(**A**) Schematic representation of full-length DDX43 and its N-terminal domain (NT, top), and purified NT proteins (wild type and mutants, 1 µg each) shown on Coomassie stained SDS-PAGE gel (bottom). (**B-E**) Representative EMSA images for the N-terminal domain proteins binding with 0.5 nM of 19 bp forked duplex DNA (**B**), ssDNA dT_{30} (**C**), dsRNA (**D**), and blunt-end dsDNA (**E**). Arrow indicates the loading wells.

Figure S11. Comparison of nucleic acids binding ability between DDX43 N-terminal domain and helicase domain.
Representative EMSA images for DDX43 N-terminal domain (NT) and helicase core domain (HD) proteins (0-3 µM) incubated with 0.5 nM 19 bp fork duplex DNA (upper) or 13 bp 5′ tailed duplex RNA (bottom).

Figure S12. Unwinding activity of DDX43 protein on chimeric substrates.
(**A-F**) Representative images of helicase reactions by incubating increasing protein concentration (0-3 µM) at 37°C for 15 min with 0.5 nM substrates of 14 bp dsRNA with RNA tail (**A**), 14 bp dsRNA with DNA tail (**B**), 16 bp DNA:RNA hybrid with RNA tail (**C**), 16 bp DNA:RNA hybrid with DNA tail (**D**),14 bp dsRNA with RNA adjacent to duplex region (**E**), or 14 bp dsRNA with DNA adjacent to duplex region (**F**). (**G**) Quantitative analyses of the unwinding of DDX43 in panel A-F.

Fig. S1

A



B

MSHHGGAPKASTWVVASRRSSTVSRAPERRPAEELNRTGPEGYSVGRGGR-50
G46D

WRGTSRPPEDVAAGHEELPLCFALKSHFVGAVIGRGGSKIKNIQSTTNTT-100
G84D

IQIIQEQPESLVKIFGSKAMQTKAKAVIDNFVKKLEENYNSECGIDTAFQ-150

PSVGKDGSTDNNVVAGDRPLIDWDQIREEGLKWQKTKWADLPPIKKNFYK-200
G154D

ESTATSAMSKVEADSWRKENFNITWDDLKDGEKRPIPNPTCTFDDAFQCY-250

Q I

PEVMENIKKAGFQKPTPIQSQAWPIVLQGIDLIGVAQTGTGKTLCYLMPG-300
K292A

Ia

FIHLVLQPSLKGQRNRPGMLVLTPTRELALQVEGECCKYSYKGLRSVCVY-350

Ib Ic II

GGGNRDEQIEELKKGVDIIIATPGRLNDLQMSNFVNLKNITYLVLDEADK-400
D396A

III

MLDMGFEPQIMKILLDVRPDRQTVMTSATWPHSVHRLAQSYLKEPMIVYV-450

IV

GTLDLVAVSSVKQNIIVTTEEEKWSHMQTFLQSMSSTDKVIVFVSRKAVA-500

V Va

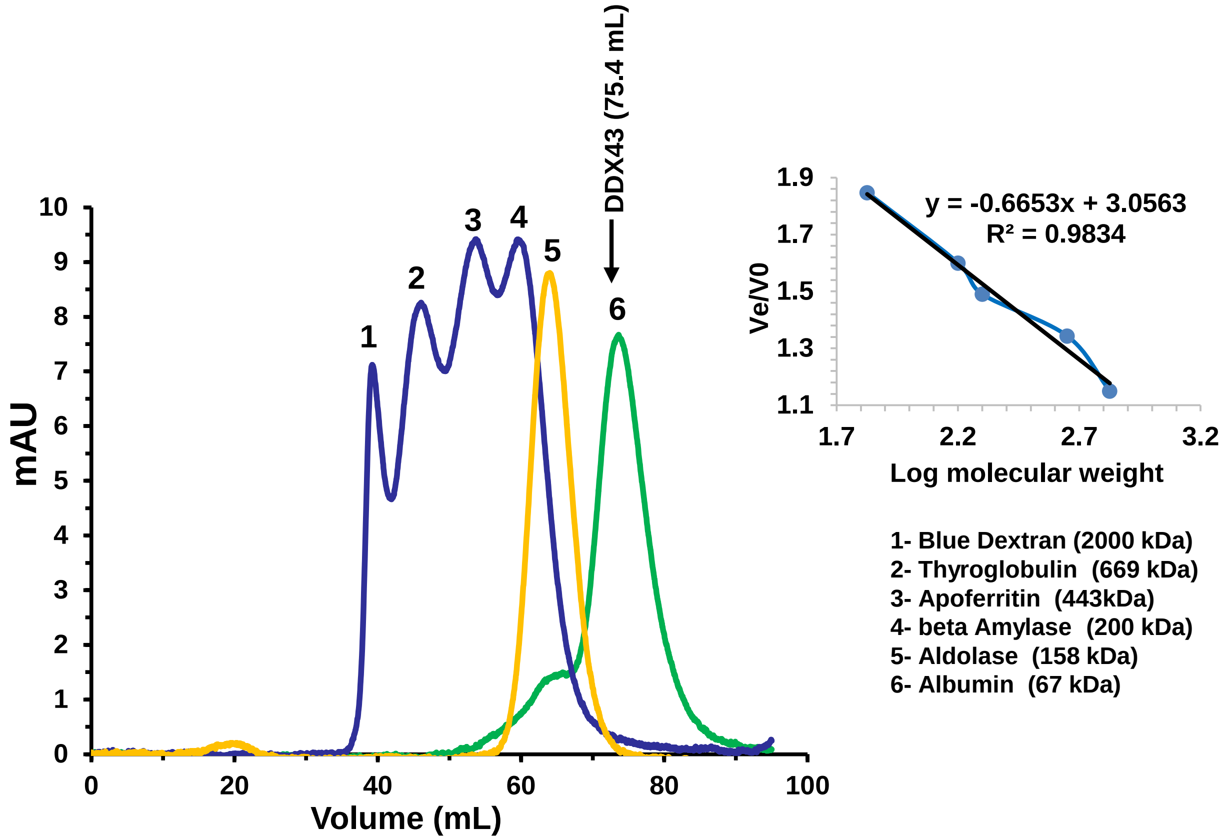
DHLSSDLILGNISVESLHGDREQRDREKALENFKTGKVRILIATDLASRG-550

Vb VI

LDVHDVTHVYNFDFPRNIEEYVHRIGRTGRAGRTGVSITTLTRNDWRVAS-600

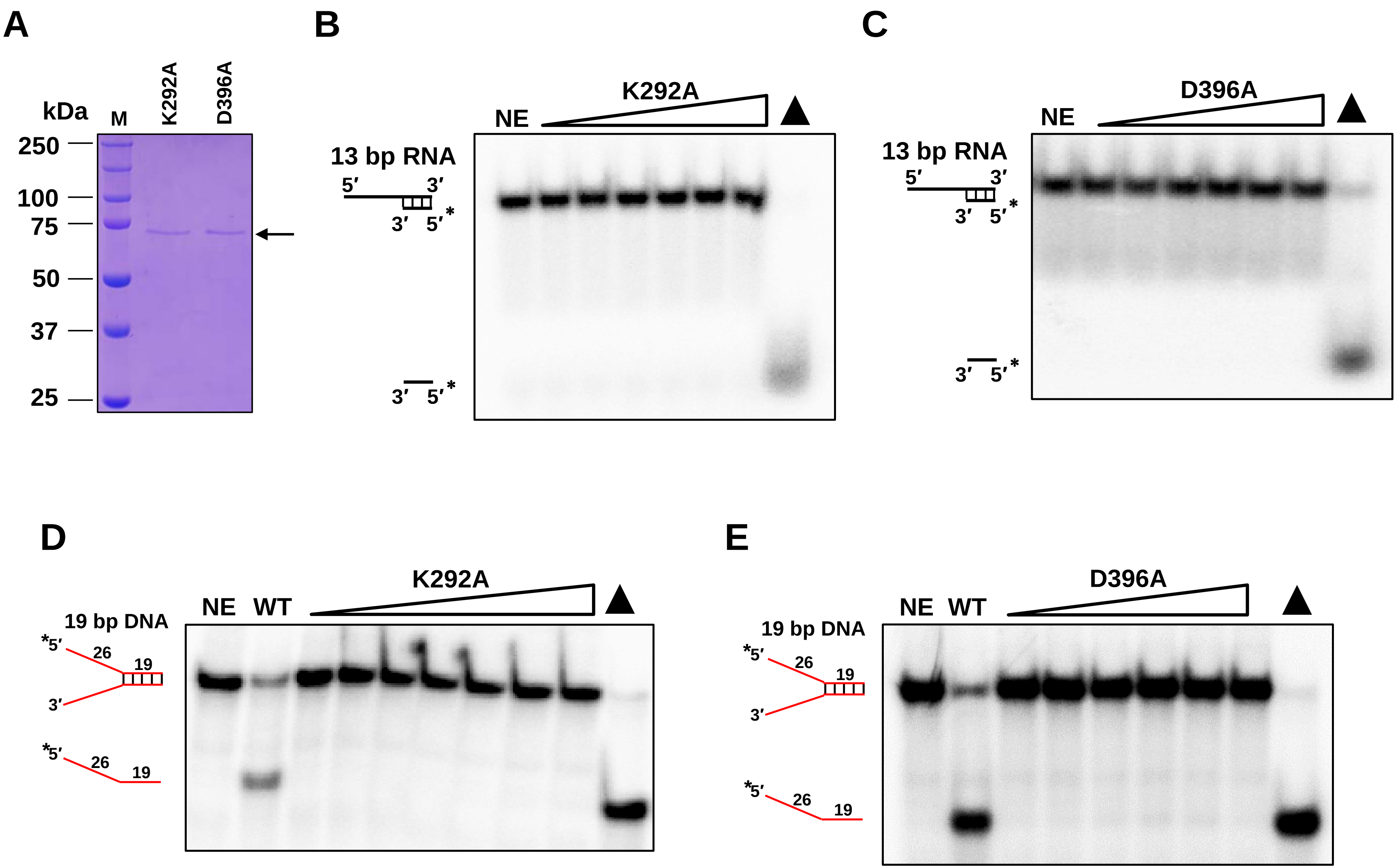
ELINILERANQSIPEELVSMAERFKAHQQKREMERKMERPQGRPKKFH---648

Fig. S2



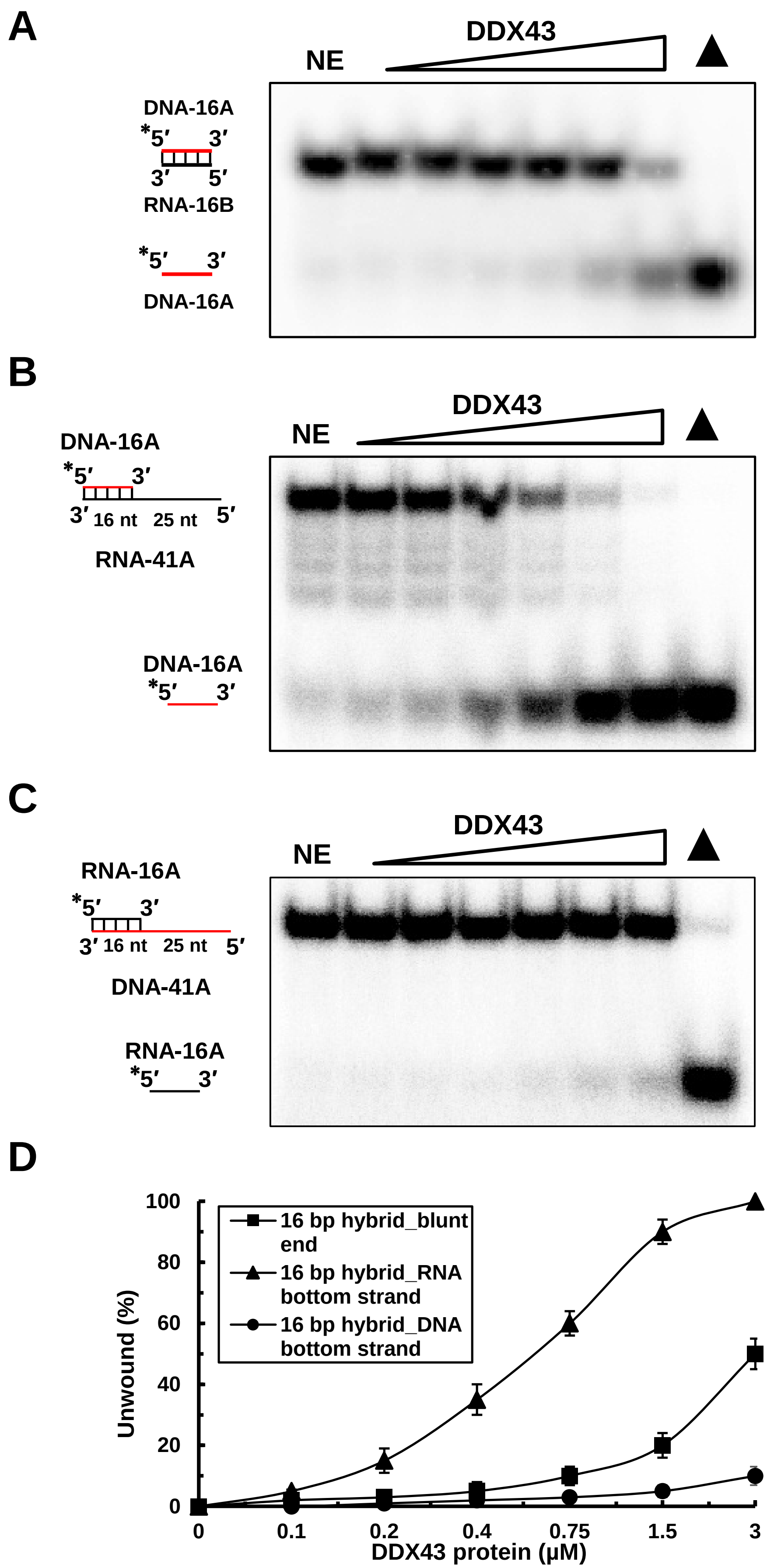
1- Blue Dextran (2000 kDa)
2- Thyroglobulin (669 kDa)
3- Apoferritin (443kDa)
4- beta Amylase (200 kDa)
5- Aldolase (158 kDa)
6- Albumin (67 kDa)

Fig. S3



A
kDa
M
K292A
D396A
250
100
75
50
37
25
B
13 bp RNA
5′
3′
3′
5′*
NE
K292A
3′
5′*
C
13 bp RNA
NE
D396A
D
19 bp DNA
NE
WT
K292A
*5′
26
19
3′
E
19 bp DNA
NE
WT
D396A

Fig. S4



A
DDX43
NE
DNA-16A
*5′ 3′
3′ 5′
RNA-16B
*5′ 3′
DNA-16A
B
DDX43
NE
DNA-16A
*5′ 3′
3′ 16 nt 25 nt 5′
RNA-41A
DNA-16A
*5′ 3′
C
DDX43
NE
RNA-16A
*5′ 3′
3′ 16 nt 25 nt 5′
DNA-41A
RNA-16A
*5′ 3′
D
16 bp hybrid_blunt end
16 bp hybrid_RNA bottom strand
16 bp hybrid_DNA bottom strand
Unwound (%)
100
80
60
40
20
0
0
0.1
0.2
0.4
0.75
1.5
3
DDX43 protein (μM)

Fig. S5

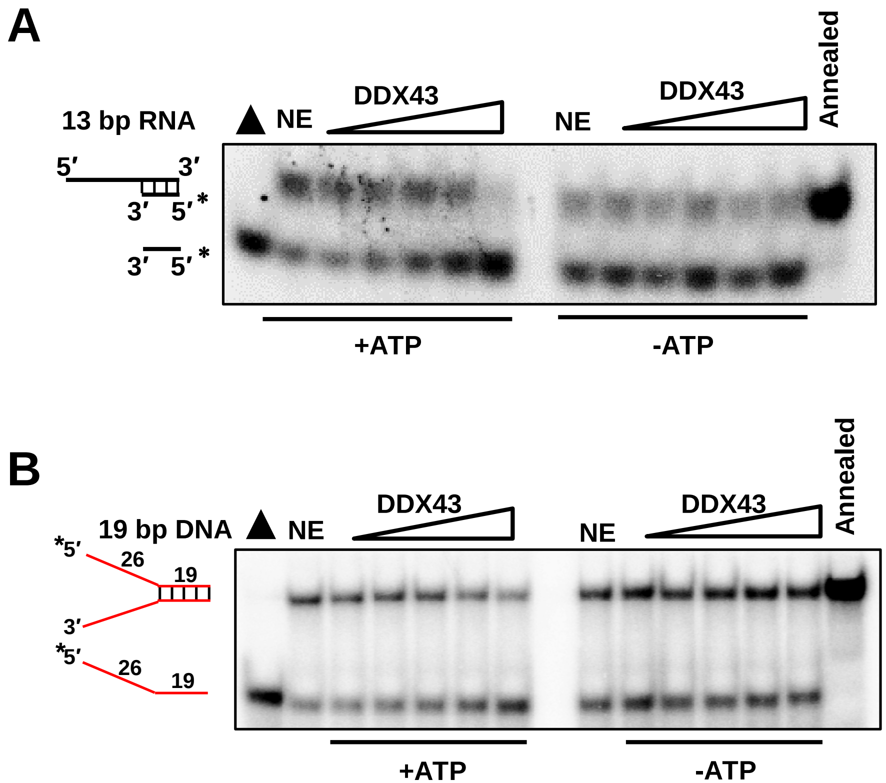
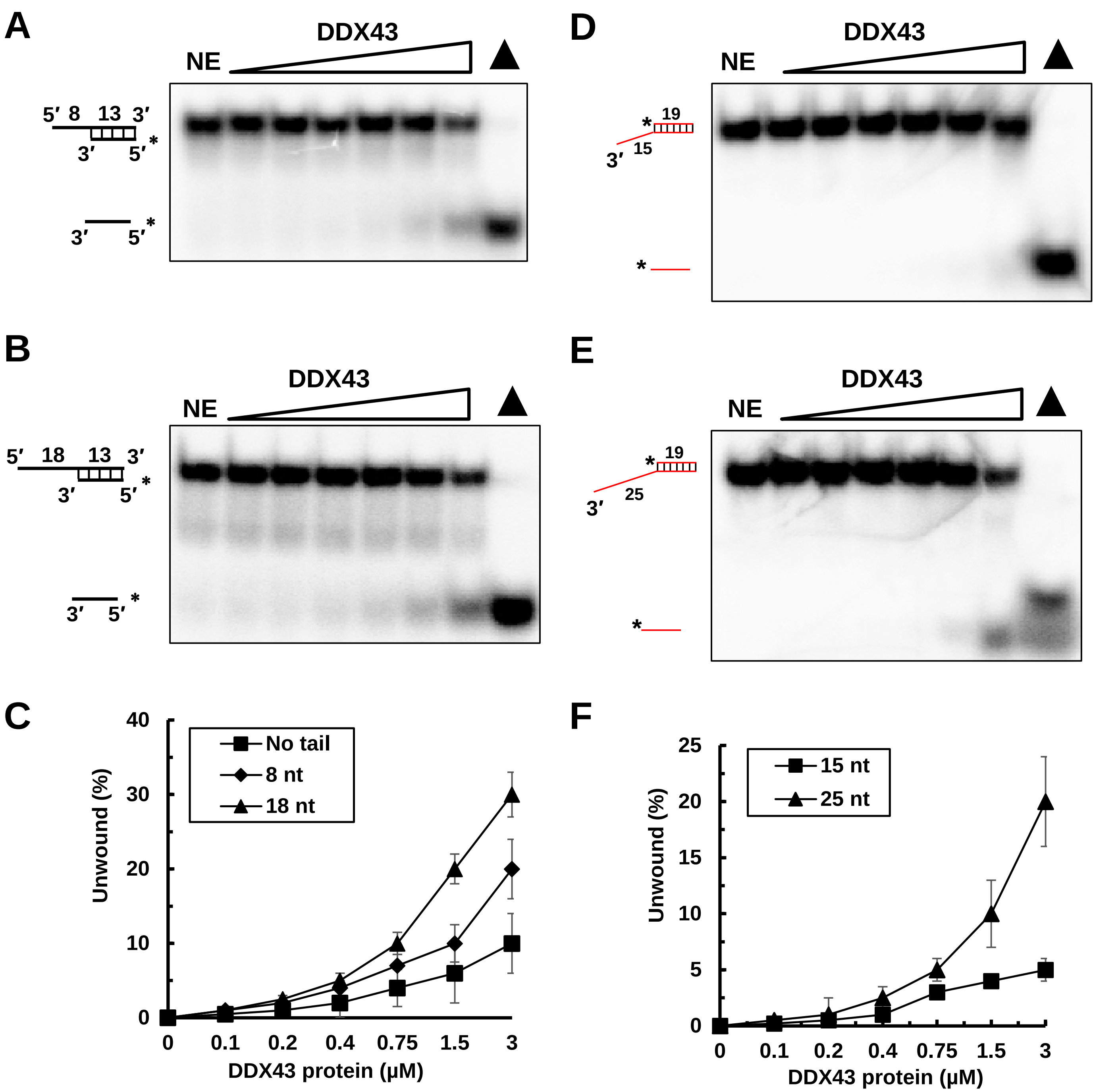

A
13 bp RNA
NE
DDX43
NE
DDX43
Annealed
5′
3′
3′
5′*
3′
5′*
+ATP
-ATP
B
19 bp DNA
NE
DDX43
NE
DDX43
Annealed
*5′
26
19
3′
*5′
26
19
+ATP
-ATP

Fig. S6



A
DDX43
NE
5′ 8 13 3′
3′ 5′ *
3′ 5′ *
B
DDX43
NE
5′ 18 13 3′
3′ 5′ *
3′ 5′ *
C
Unwound (%)
No tail
8 nt
18 nt
0
10
20
30
40
0 0.1 0.2 0.4 0.75 1.5 3
DDX43 protein (μM)
D
DDX43
NE
* 19
3′ 15
*
E
DDX43
NE
* 19
3′ 25
*
F
Unwound (%)
15 nt
25 nt
0
5
10
15
20
25
0 0.1 0.2 0.4 0.75 1.5 3
DDX43 protein (μM)

Fig. S7

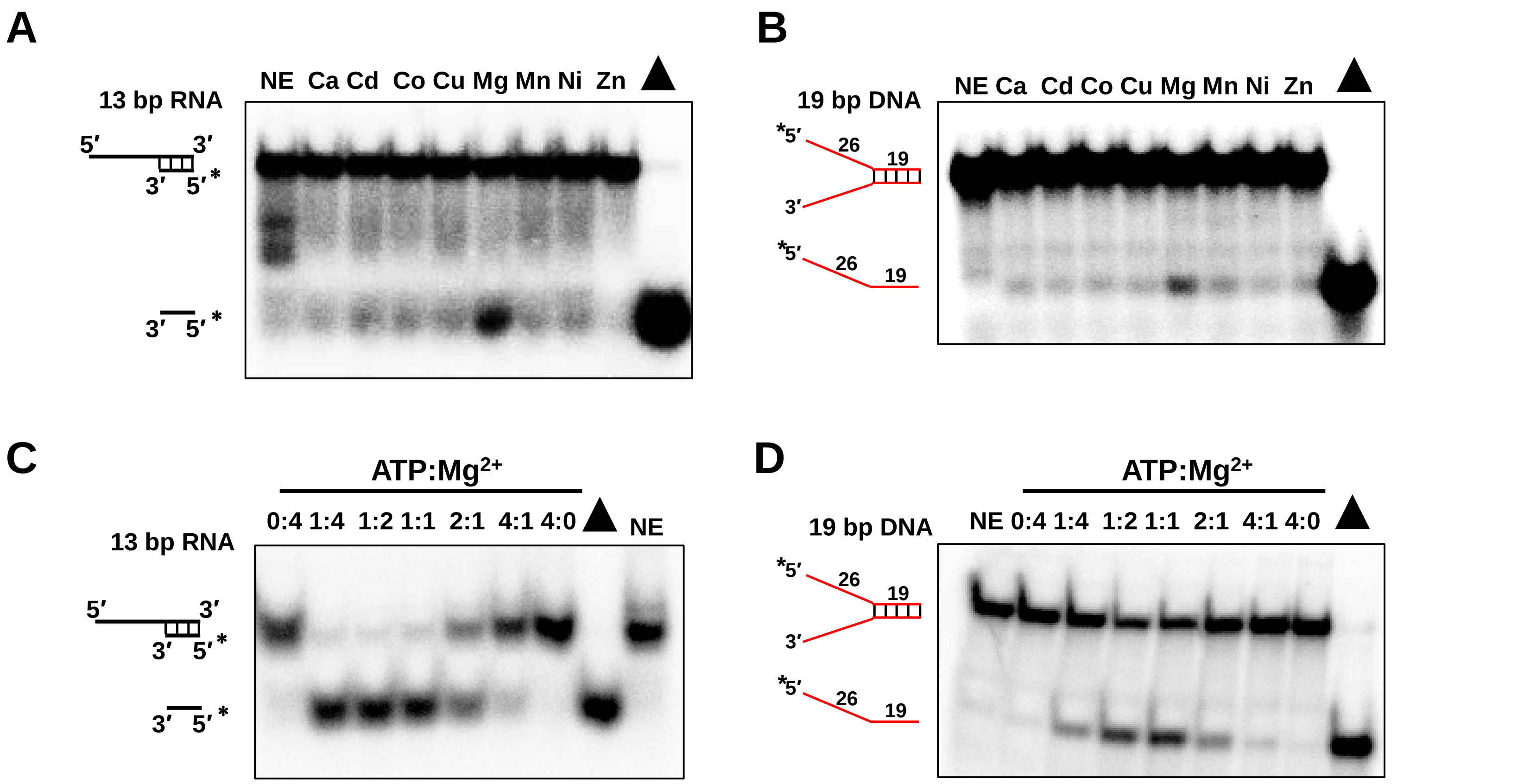

A
13 bp RNA
NE Ca Cd Co Cu Mg Mn Ni Zn
5′ 3′
3′ 5′ *
3′ 5′ *
B
19 bp DNA
NE Ca Cd Co Cu Mg Mn Ni Zn
*5′ 26 19
3′
*5′ 26 19
C
ATP:Mg2+
0:4 1:4 1:2 1:1 2:1 4:1 4:0 NE
13 bp RNA
5′ 3′
3′ 5′ *
3′ 5′ *
D
ATP:Mg2+
NE 0:4 1:4 1:2 1:1 2:1 4:1 4:0
19 bp DNA
*5′ 26 19
3′
*5′ 26 19

Fig. S8

A

```
                                               GXXG
hnRNP K (1) 41... EMVELRILLQSKNAGAVIGKGGKNIKALRTDYNASVSVPDSSGPERI------LSISADIETIGEI
hnRNP K (2) 143.. FDCELRLLIHQSLAGGIIGVKGAKIKELRENTQTTIKLFQECCPHSTDR---VVLIGGKPDRV
hnRNP K (3) 386.. PIITTQVTIPKDLAGSIIGKGGQRIKQIRHESGASIKIDEPLEGSED----RIITITGTQDQIQ
FMR-1   (1) 40... SRFHEQFIVREDLMGLAIGTHGANIQQARKVPGVTAIDLDEDTCT--------FHIYGEDQDAVKKAR
FMR-1   (2) 217.. EFAEDVIQVPRNLVGKVIGKNGKLIQEIVDKSGVVRVRIEAENEKNVPQEEEIMPPNSLP
NOVA    (1) 280.. GEYFLKVLIPSYAAGSIIGKGGQTIVQLQKETGATIKLSKSKDFYPGT-TERVCLVQGTAE
NOVA    (2) 31... RAKQAKLIVPNSTAGLIIGKGGATVKAVMEQSGAWVQLSQKPEGINLQE--RVVTVSGEPEQ
NOVA    (3) 405.. AKELVEIAVPENLVGAILGKGGKTLVEYQELTGARIQISKKGEFLPGTRN-RRVTITGSPA
SF1         265.. VMIPQDEYPEINFVGLLIGPRGNTLKNIEKECNAKIMIRGKGSVKEGK-----VGRKDGQMLPGE
DDX43       66... EELPLCFALKSHFVGAVIGRGGSKIKNIQSTTNTTIQIIQEQPESL-------VKIFGSKAMQTKAK
Consensus                 I    IG IIG  G I  I        I           I I G
                          L    V  L       L  L        V           V L
                          V    A  V       V  V                       V
```

B

```
                                                  GXXG
Homo sapiens            67..PLCFALKSHFVGAVIGRGGSKIKNIQSTTNTTIQIIQEQPESLVKIFGSKAMQTKAKAVIDNFVKKEENYNSE
Macaca mulatta          67..PLCFALKNHFVGAVIGRGGSKIKNIQSTTNTTIQIIQEQPESLVKIFGSKAMQTKAKAAIDNFVKKEENYNSE
Pan troglodytes         67..PLCFALKSHFVGAVIGRGGSKIKNIQSTTNTTIQIIQEQPESLVKIFGSKAMQTKAKAVIDNFVKKEENYNSE
Rattus norvegicus       66..PLCFGVKNDVVGAVIGRGGSKIREIQNTTNTRIQVIKGNPEAEIKIFGNKAMQTKAKTVIDNVVKKQQNYIPG
Mus musculus            66..PLCFGVKNDVVGAVIGRGGSKIREIQNTTNTRIQVIKGNPEAEIKIFGNKAMQTKAKTVIDNVVKKQQNYIPG
Felis catus             67..PLCFGLKNMWVGAVIGRGGSKIKDIQSTTNTKIQIIKGYPDAQVRIFGSKGMKAKAKTAIDNVVKQQESYSLK
Bos Taurus              67..PLSFKLKNDCVGAVIGRGGSNIKEIQSSTYTKIQIIRGYPEAEVRIFGTKAMQSKAKTVIDDLVKKQEEYKTE
Sus scrofa              67..PLSFRLKNDWVGVVIGRGGSKIKEIQNTTYTKIQIIKGYPEAEVRIFGTEEMQSKARIVIDNIVKKQEEYKSE
Ovis aries              67..LLSFKLKNDCVGAVIGRGGSNIKEIQSSTYTKIQIRRGCPEAEVRIFGTKAMQCKAKTVIDDLVKKQEEYKTE
Gallus gallus           77..PLCFLISSALAGVLIGRGGAKIKELEESSGSRIQVIKGTSEAEVKIFGSAAVQNKAKVLIDETVVSCGQSNPR
Anolis carolinensis     63..PLCFHLDNALVGALIGRGGSRIKELEDSSGCKIKVMRGDYEAEVKVFGDNDKKRKAKMLIDDLIDRAKQSSAG
Danio rerio            100..SVTLNVENSLVGRIIGRGGAKIRELQESSGASIKAI--ITRGEVLICGSCDAQHKAKDMIEDLVHEMGGSGYR
Consensus                        L    VGAVIGRGGSKI I      I         V I G      KAK
                                 V    A RL     AN L                I V
                                 I       I
```

Fig. S9

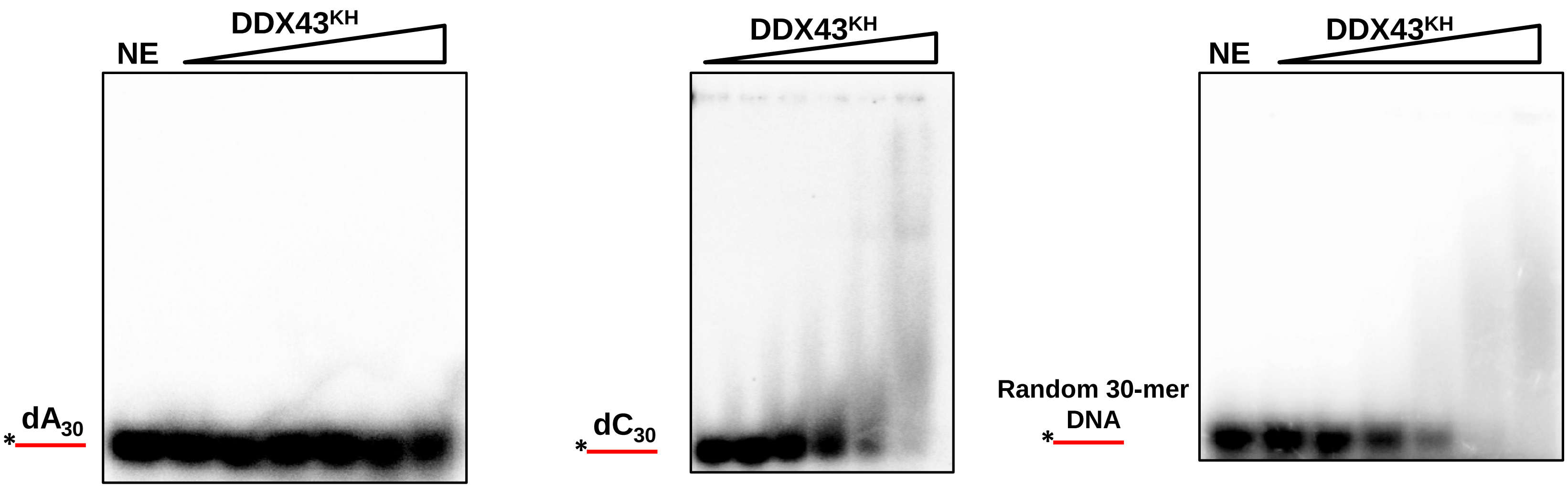
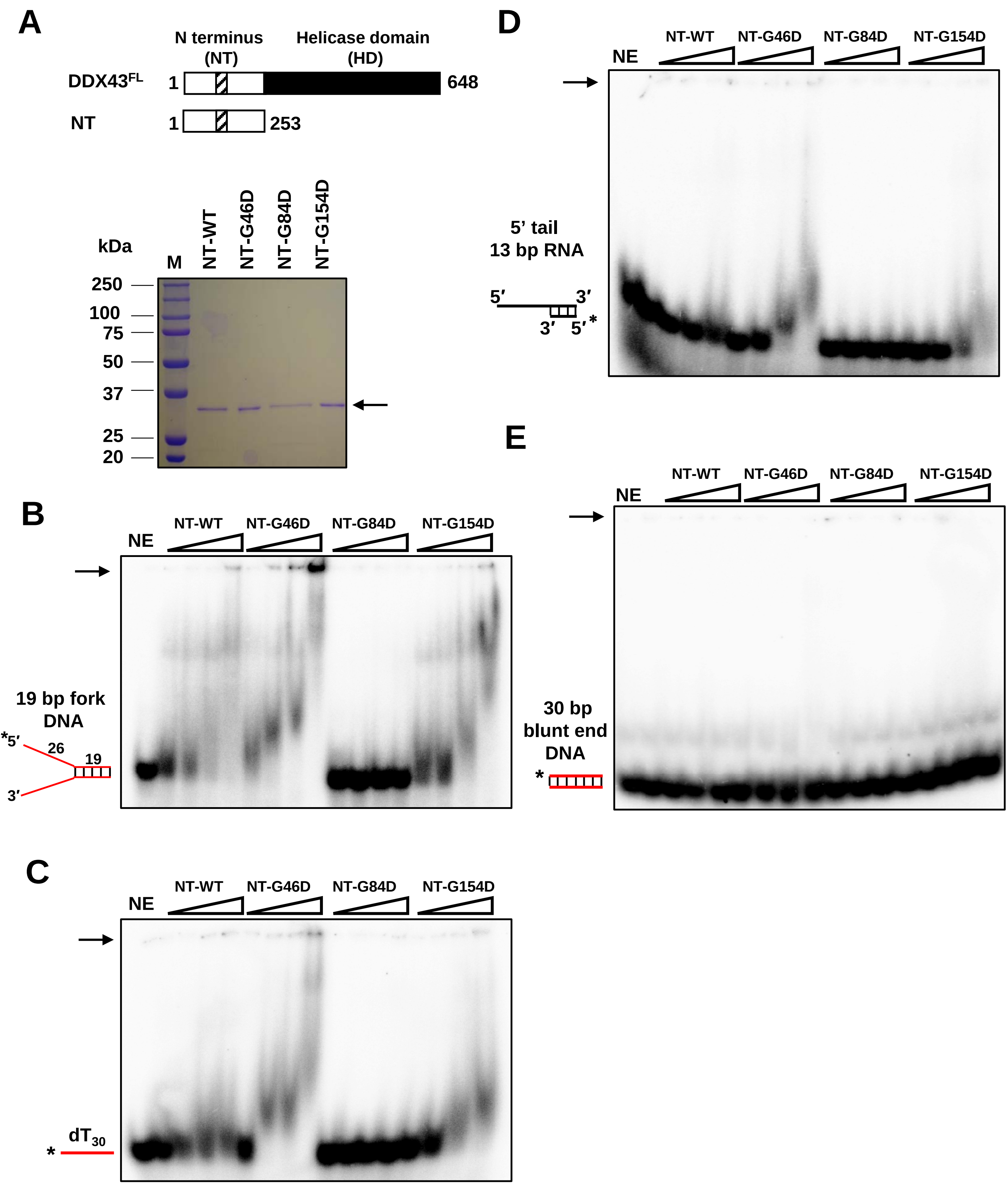
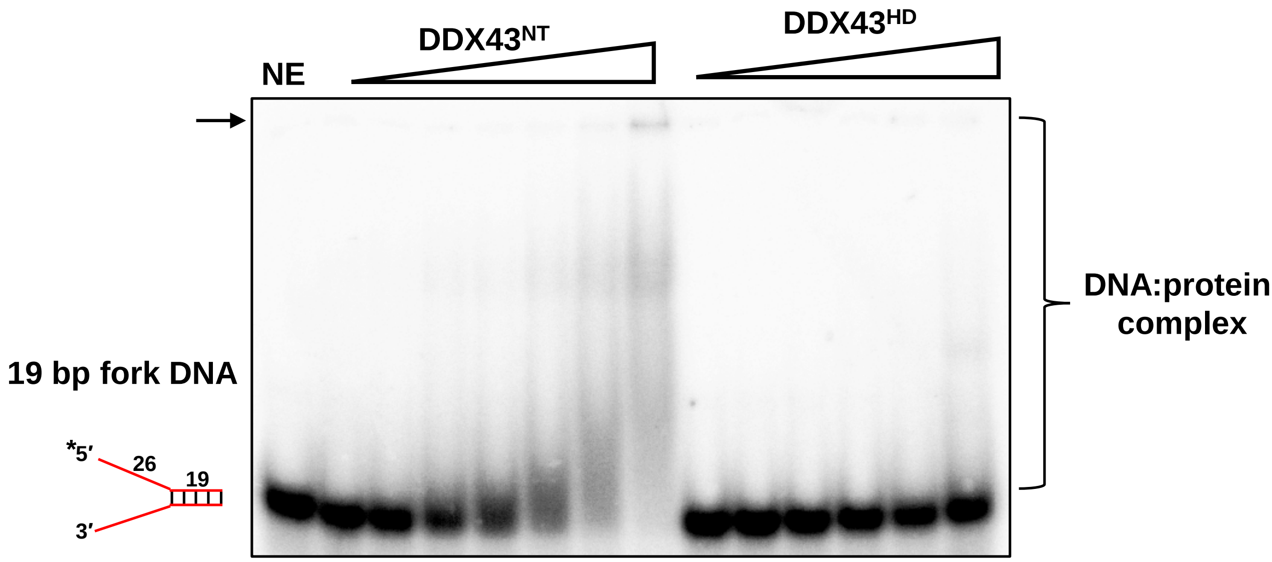


Fig. S10



A
N terminus (NT)
Helicase domain (HD)
DDX43FL
1
648
NT
1
253
kDa
M
NT-WT
NT-G46D
NT-G84D
NT-G154D
250
100
75
50
37
25
20
B
NE
NT-WT
NT-G46D
NT-G84D
NT-G154D
19 bp fork DNA
*5′
26
19
3′
C
NE
NT-WT
NT-G46D
NT-G84D
NT-G154D
dT30
*
D
NE
NT-WT
NT-G46D
NT-G84D
NT-G154D
5’ tail 13 bp RNA
5′
3′
3′
5′*
E
NE
NT-WT
NT-G46D
NT-G84D
NT-G154D
30 bp blunt end DNA
*

Fig. S11


DDX43NT
DDX43HD
NE
DNA:protein
complex
19 bp fork DNA
*5′
26
19
3′

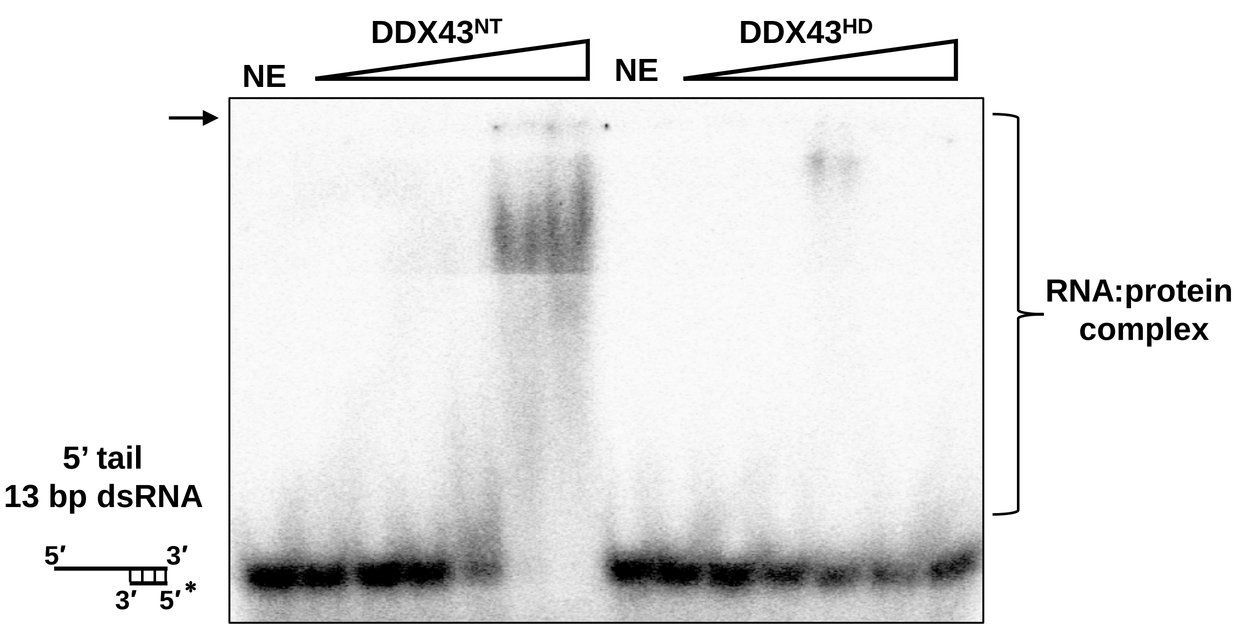
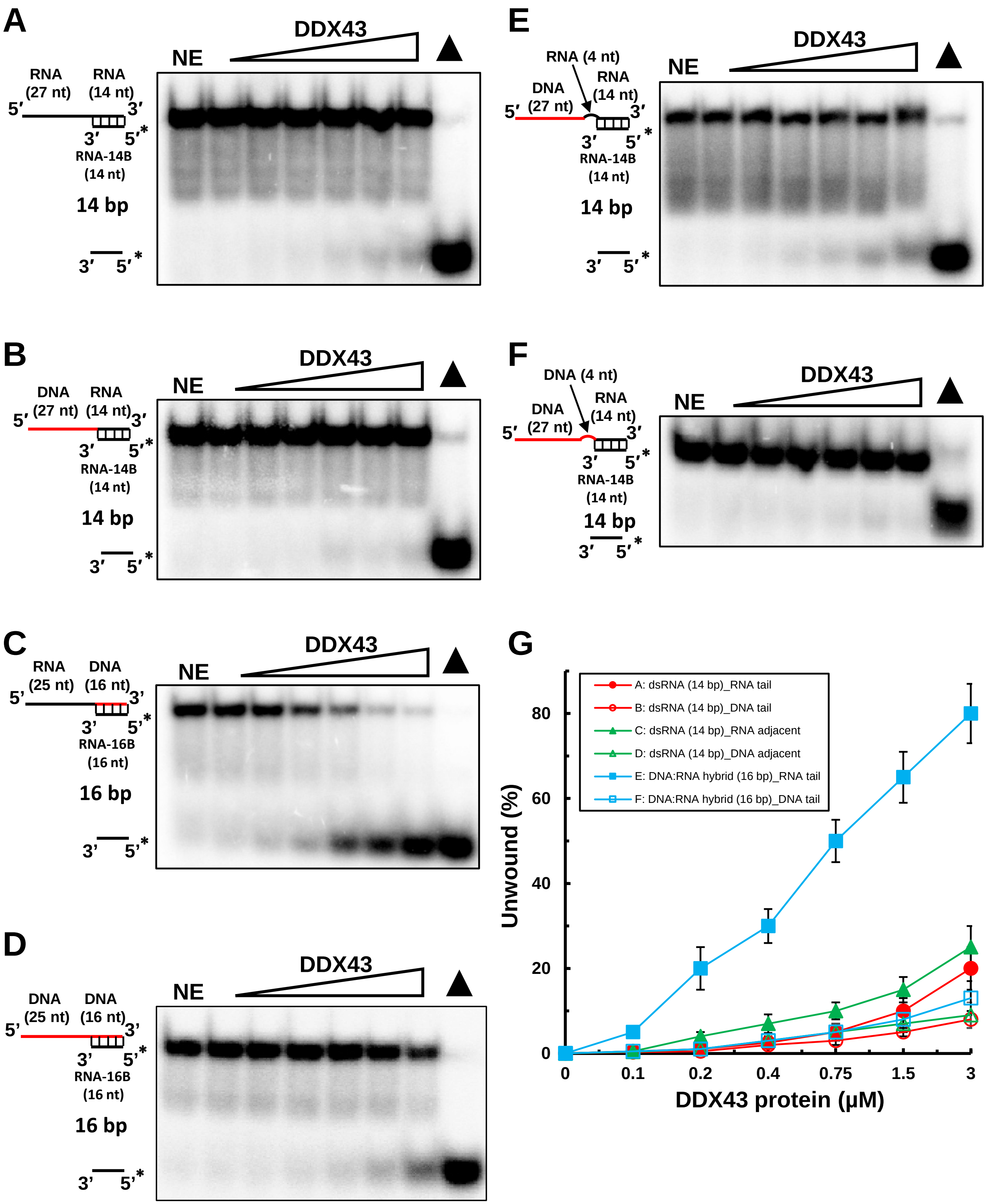

DDX43NT
DDX43HD
NE
NE
RNA:protein
complex
5’ tail
13 bp dsRNA
5′
3′
3′
5′*

Fig.S12


A
DDX43
NE
RNA
(27 nt)
RNA
(14 nt)
5′
3′
3′
5′*
RNA-14B
(14 nt)
14 bp
3′ 5′*
B
DDX43
NE
DNA
(27 nt)
RNA
(14 nt)
5′
3′
3′
5′*
RNA-14B
(14 nt)
14 bp
3′ 5′*
C
DDX43
NE
RNA
(25 nt)
DNA
(16 nt)
5′
3′
3′
5′*
RNA-16B
(16 nt)
16 bp
3′ 5′*
D
DDX43
NE
DNA
(25 nt)
DNA
(16 nt)
5′
3′
3′
5′*
RNA-16B
(16 nt)
16 bp
3′ 5′*
E
DDX43
NE
RNA (4 nt)
DNA
(27 nt)
RNA
(14 nt)
5′
3′
3′
5′*
RNA-14B
(14 nt)
14 bp
3′ 5′*
F
DDX43
NE
DNA (4 nt)
DNA
(27 nt)
RNA
(14 nt)
5′
3′
3′
5′*
RNA-14B
(14 nt)
14 bp
3′ 5′*
G
A: dsRNA (14 bp)_RNA tail
B: dsRNA (14 bp)_DNA tail
C: dsRNA (14 bp)_RNA adjacent
D: dsRNA (14 bp)_DNA adjacent
E: DNA:RNA hybrid (16 bp)_RNA tail
F: DNA:RNA hybrid (16 bp)_DNA tail
Unwound (%)
0
20
40
60
80
0
0.1
0.2
0.4
0.75
1.5
3
DDX43 protein (μM)