Human senataxin is a bona fide R-loop resolving enzyme and transcription termination factor

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Supplementary material:

Figures S1-S7.

Table S1-S2.

Supplementary methods.

Supplementary references.

Supplementary figure 1 Hasanova *et* al.

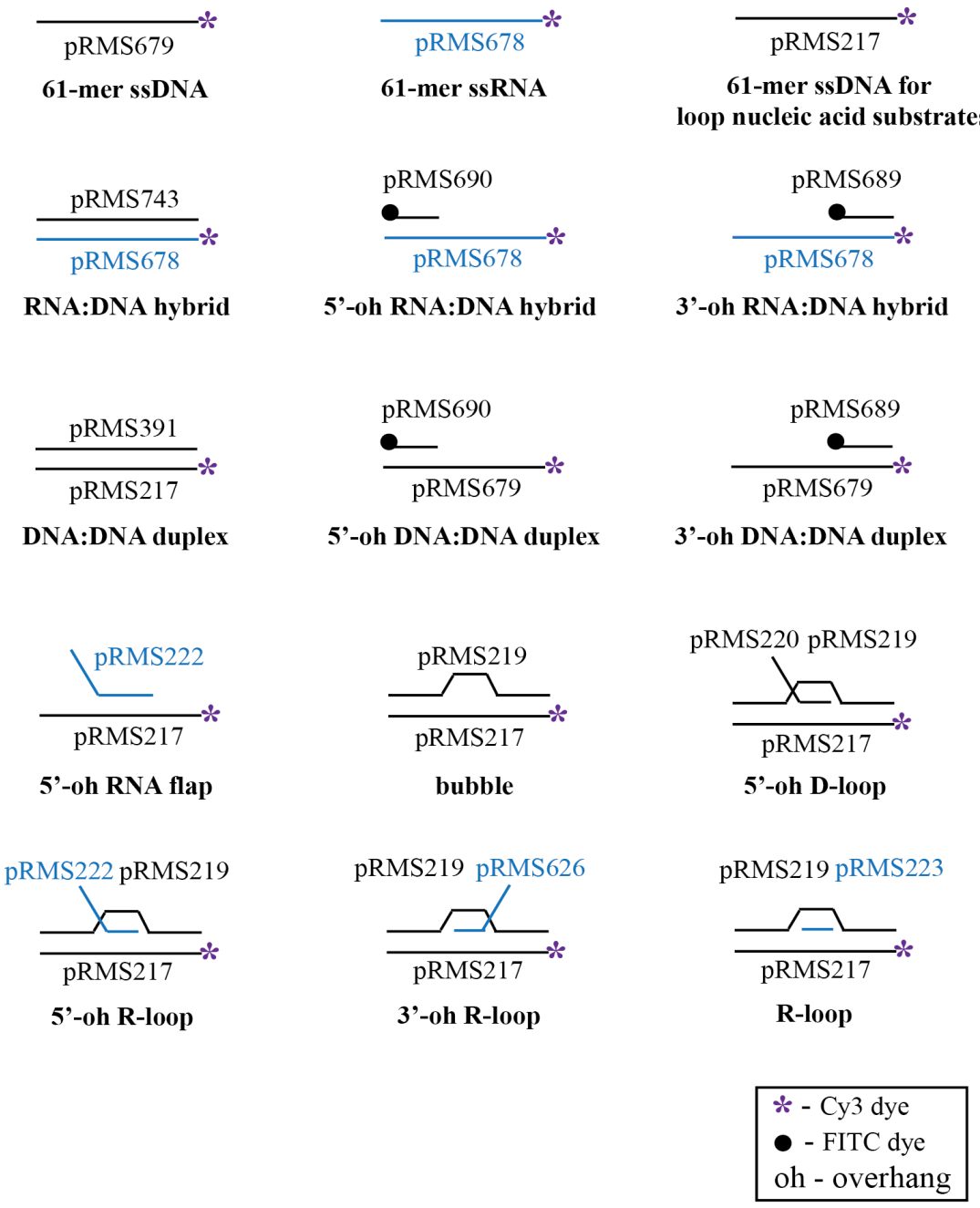

Figure S1: **Scheme of used nucleic acid substrates - supplementary figure for Material and Methods.** Depicted are nucleic acid substrates for electrophoretic mobility-shift assays and helicase assays. The names of the constituent oligonucleotides are indicated (see Supplementary table S1 for sequence details). DNA is depicted in black, RNA in blue, Cy3 fluorescent dye with a purple asterisk, and FITC fluorescent dye with a black circle. oh stands for overhang.

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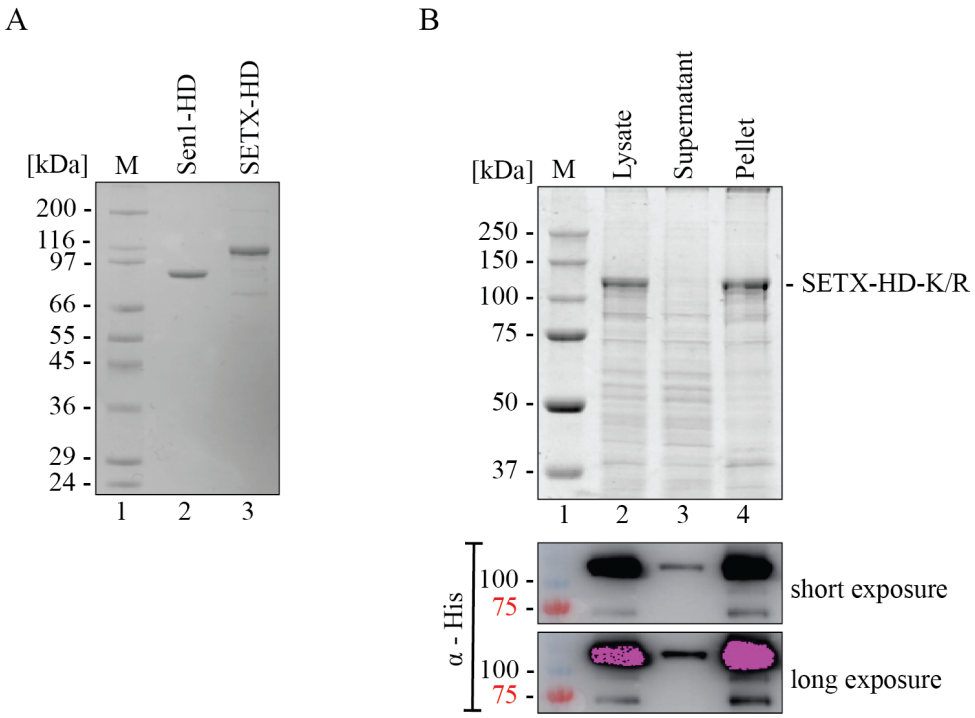

Figure S2: Supplementary figure related to main Figure 1: (A) SDS-PAGE gel, analysis of purified Sen1-HD and SETX-HD. M: molecular weight marker. **(B)** Solubility test for the mutant version of SETX-HD containing K1969R substitution in the Walker A motif. Top: Coomassie-stained SDS-PAGE gel showing the presence of the SETX-HD-K1969R (K/R) mutant in the cell lysate and in the supernatant and pellet fractions after centrifugation. Bottom: Immunodetection of $(His)_6$-tagged SETX-HD-K/R with an α-His antibody localised SETX-HD-K/R primarily in the pellet fraction (lane 4). M: molecular weight marker.

Supplementary figure 3

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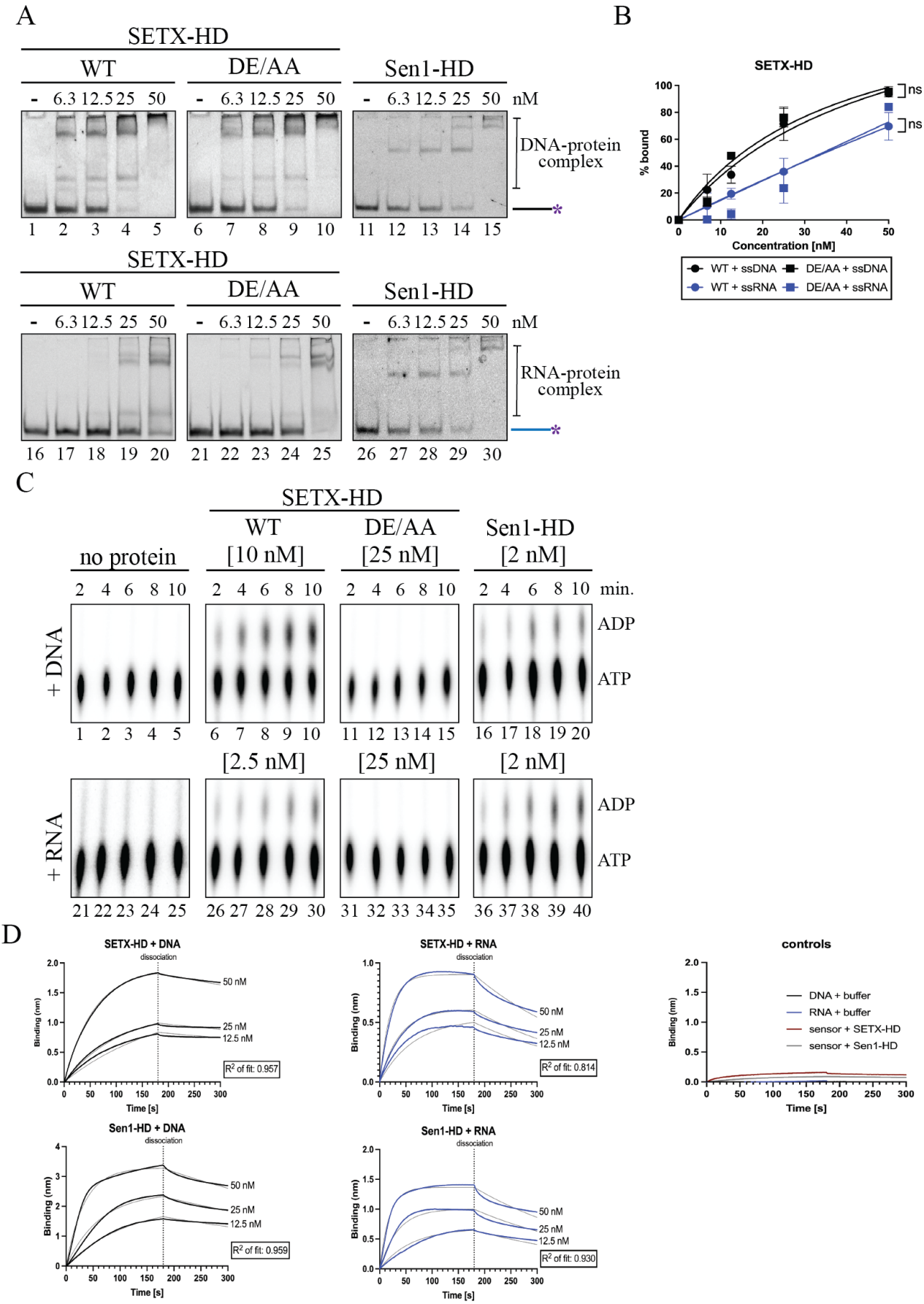

Figure S3: Supplementary figure related to main Figure 2: Biochemical characterisation of SETX-HD-WT and SETX-HD-D2181A, E2182A (DE/AA). (A) Electrophoretic mobility-shift assays (EMSA) were performed with the indicated concentrations of SETX-HD-WT, SETX-HD-DE/AA, and Sen1-HD, respectively, in the presence of 10 nM fluorescently labelled ssDNA (pRMS679, 61 nt, lanes 1-15) and ssRNA (pRMS678, 61 nt, lanes 16-30). **(B)** Quantification of nucleic acid binding affinity of SETX-HD-DE/AA compared to SETX-HD-WT from experiments shown in (A). The data points in the graphs represent the mean of 3 independent experiments (for WT + ssDNA: n = 4) and the error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$. **(C)** Determination of the ATPase activity of SETX-HD. ATPase assays containing SETX-HD-WT [10 nM or 2.5 nM, as indicated], SETX-HD-DE/AA [25 nM], and Sen1-HD [2 nM], respectively, were performed in the presence of single-stranded DNA (DL3701, 44 nt, lanes 1-20) and RNA (DL3316, 44 nt, lanes 21-40). The position of α[^{32}P]-ATP and hydrolysed α[^{32}P]-ADP is depicted. **(D)** Sensorgrams from bio-layer interferometry (BLI) assay. Twelve fmoles of biotinylated ssDNA (pRMS739, 41 nt) and ssRNA (pRMS740, 41 nt), respectively, were bound to a SAX biosensor. SETX-HD and Sen1-HD, respectively, were then submerged into the biosensor in three concentrations (12.5, 25, and 50 nM), respectively, to measure the protein association rate (180 s). The protein association rate to nucleic acids is represented as the change in light interferometry (in nm). The protein dissociation from nucleic acids was measured in BLI buffer for 120 s. Binding curves (black for DNA and blue for RNA) represent the mean from triplicate measurements for each concentration generated in Prism GraphPad 9. Grey lines indicate the non-linear regression fitting curve using the "Association then dissociation" model in global analysis mode. Quality of the data fit (R^2) is depicted in each graph. Control graph shows association and dissociation of BLI buffer with ssDNA and RNA, respectively, and a sensor without bound nucleic acids with SETX-HD and Sen1-HD, respectively.

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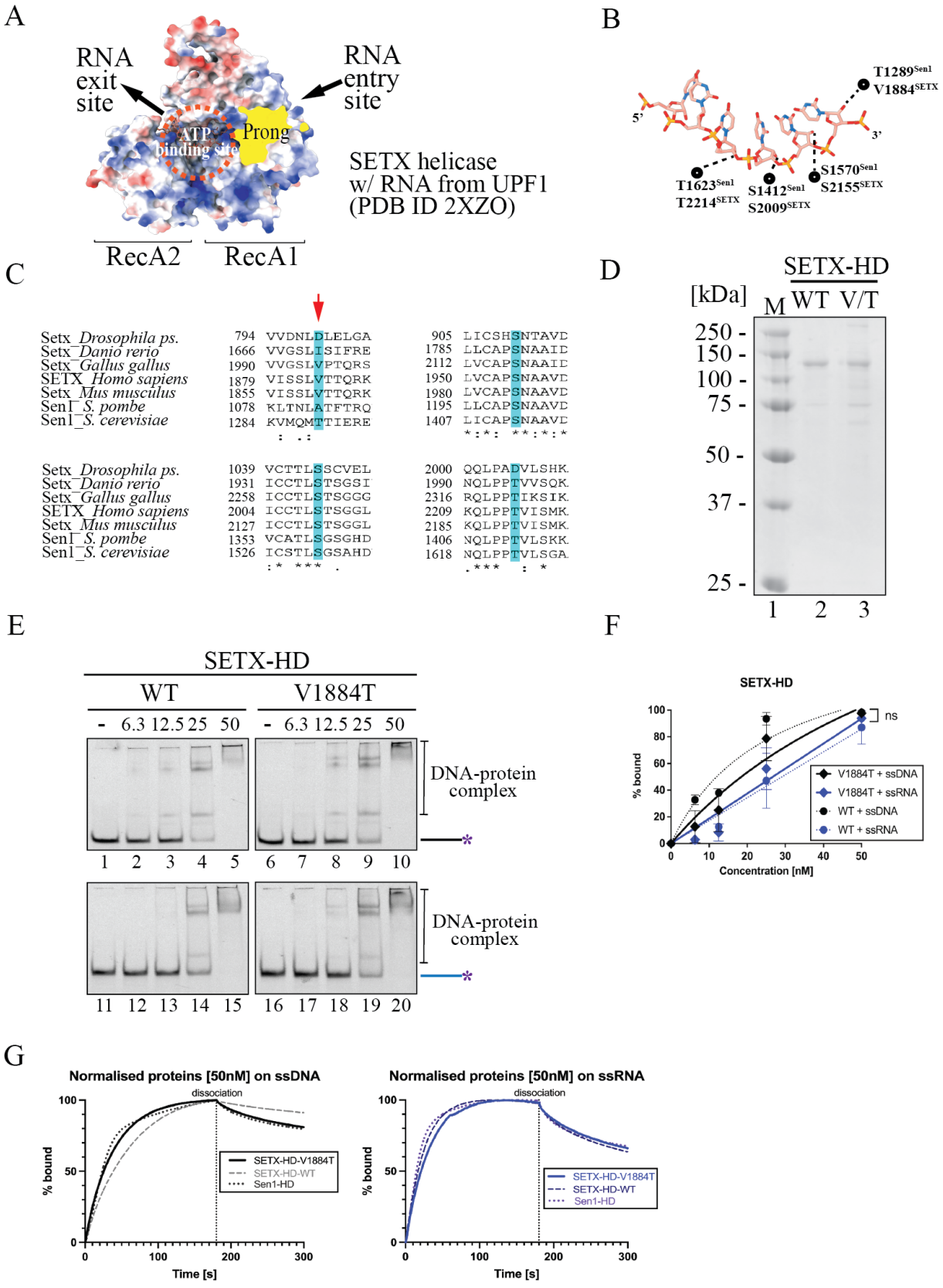

Figure S4: Supplementary figure related to main Figure 2: SETX-HD-V1884T mutant has similar binding kinetics to nucleic acids as Sen1-HD. (A) Model of SETX bound to RNA (shown as electrostatic surface) based on the structure of UPF1-RNA complex (2XZO, (1)). RNA was placed in the helicase channel based on the overlay of the SETX and Upf1 structures, which are highly conserved. **(B)** Recognition of the RNA sugar moieties (shown as sticks) by residues located in the helicase channel of SETX and Sen1. **(C)** Multiple sequence alignment of RNA sugar binding sites in Sen1/SETX in various organisms performed using the ClustalW online tool. The area (+/- 5 amino acids) around the RNA sugar recognition sites (highlighted in blue) of the helicase channel (presented in (B)) for *Drosophila pseudoobscura*, *Danio rerio*, *Gallus gallus*, *Homo sapiens*, *Mus musculus*, *Schizosaccharomyces pombe*, and *Saccharomyces cerevisiae*. The red arrow points to the position of the RNA recognition site (V1884 in humans) analysed here. **(D)** SDS-PAGE gel analysis of purified SETX-HD-V1884T variant and SETX-HD-WT. M: molecular weight marker. **(E)** EMSA were performed with the indicated concentrations of SETX-HD-WT and SETX-HD-V1884T, respectively, and 10 nM fluorescently labelled ssDNA (pRMS679, 61 nt, lanes 1-10) or ssRNA (pRMS678, 61 nt, lanes 11-20) as substrate. **(F)** Quantification of the nucleic acid binding affinity of SETX-HD-V1884T (solid line) and SETX-HD-WT (dotted line) from experiments shown in (E). The data points represent the mean of several independent experiments (n = 3 for WT + ssDNA; n = 4 for WT + ssRNA; n = 8 for V1884T + ssDNA; and n = 7 for V1884T + ssRNA) and the error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$. **(G)** SETX-HD-V1884T dissociates from DNA more rapidly than the WT. Sensorgrams from BLI assays representing the mean of 3 measurements for each condition. Twelve fmoles of biotinylated ssDNA (pRMS739, 41 nt) and ssRNA (pRMS740, 41 nt), respectively, were bound to a SAX biosensor. SETX-HD-V1884T (50 nM) was then submerged into the biosensor, to measure protein association for 180 s. Protein dissociation from nucleic acids was measured in BLI buffer for 120 s. Sensorgrams depict the rates of association and dissociation of SETX-HD-V1884T to ssDNA (left) and ssRNA (right). For comparison, SETX-HD-WT (dashed line) and Sen1-HD (dotted line) association/dissociation curves from Figure 2C were added to the graph. Binding curves were normalised in Prism GraphPad 9.

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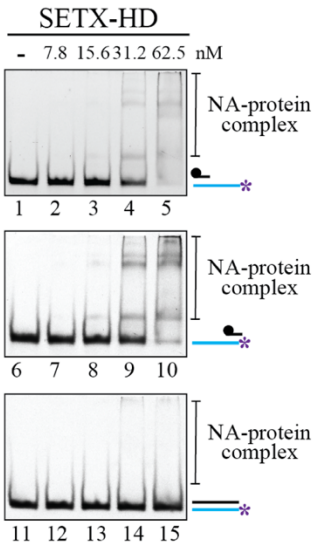
A



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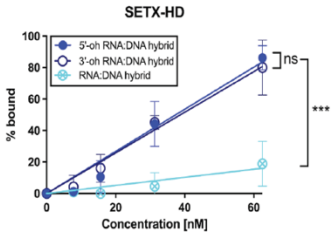

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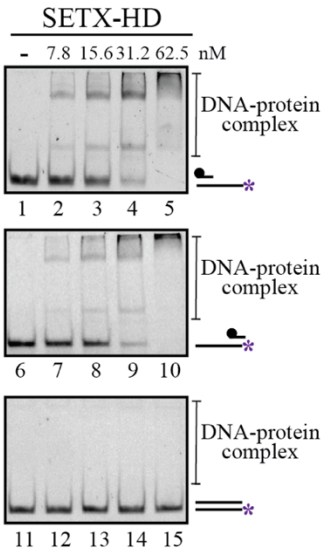

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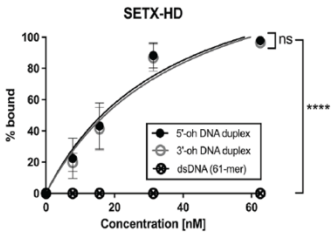

Figure S5: Supplementary figure related to main Figure 3: SETX-HD has similar binding affinity for RNA:DNA hybrids and DNA:DNA duplexes containing a single-stranded overhang. (A) Electrophoretic mobility-shift assays (EMSA) were performed with the indicated concentrations of SETX-HD and fluorescently labelled substrates: 5'-oh RNA:DNA hybrid (lanes 1-5), 3'-oh RNA:DNA hybrid (lanes 6-10), and RNA:DNA hybrid with blunt ends (lanes 11-15), all at 10 nM. **(B)** Quantification of the experiments shown in (A). The data points in the graph represent the mean of 3 independent experiments and error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$ and *** $P < 0.001$. **(C)** Electrophoretic mobility-shift assays (EMSA) were performed with the indicated concentrations of SETX-HD and fluorescently labelled substrates: 5'-oh DNA:DNA duplex (lanes 1-5), 3'-oh DNA:DNA duplex (lanes 6-10), and dsDNA (lanes 11-15), all at 10 nM. **(D)** Quantification of the experiments shown in (C). The data points in the graph represent the mean of 3 independent experiments (for SETX-HD + 5'-oh DNA:DNA duplex: $n = 4$) and error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$ and **** $P < 0.0001$.

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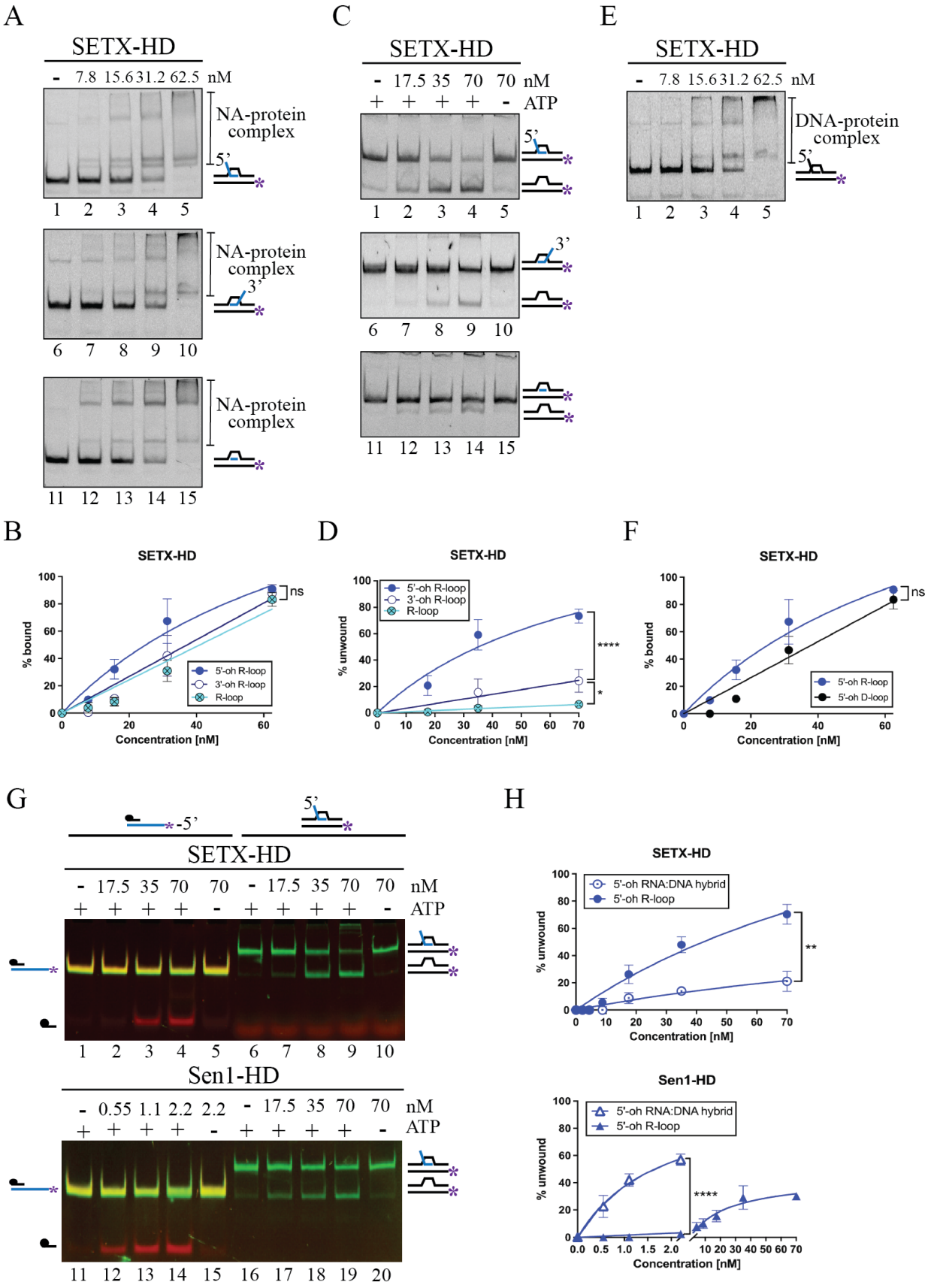

Figure S6: Supplementary figure related to main Figure 4: SETX-HD preferentially unwinds R-loop structures with a 5'- overhang. (A) SETX-HD binds R-loop structures with similar affinity. Electrophoretic mobility-shift assays (EMSA), which were performed with the indicated amounts of SETX-HD and fluorescently labelled 5'-oh R- loop (lanes 1-5), 3'-oh R-loop (lanes 6-10), or R-loop (lanes 11-15), all at 10 nM. **(B)** Quantification of the experiments shown in (A). The data points in the graphs represent the mean of 3 independent experiments and the error bars represent the SD. The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$. **(C)** Helicase assays were performed in the presence of the indicated amounts of SETX-HD, and 10 nM fluorescently labelled 5'-oh R-loop (lanes 1-5), 3'-oh R-loop (lanes 6-10), or an R-loop with no overhang (lanes 11- 15). Where indicated, ATP was omitted. **(D)** Quantification of the experiments shown in (C). The data points in the graphs represent the mean of 3 independent experiments (for WT + 5'-oh R-loop: n = 5) and the error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by * $P < 0.05$ and **** $P < 0.0001$. **(E)** Electrophoretic mobility-shift assay (EMSA) was performed with the indicated amounts of SETX-HD and fluorescently labelled 5'-oh D-loop at 10 nM. **(F)** Quantification of the experiments shown in (E and A - 5'-oh R-loop - for comparison). The data points in the graphs represent the mean of 3 independent experiments and error bars represent the standard deviation (SD). The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ns (non-significant) $P > 0.05$. **(G)** SETX-HD, in contrast to Sen1-HD, preferentially unwinds triplex R-loop structures than duplex RNA:DNA hybrids. Helicase assays were performed in the presence of the indicated amounts of SETX-HD and Sen1-HD, respectively, and 10 nM fluorescently labelled 5'-oh RNA:DNA hybrid (lanes 1- 5 and 11-15) and 5'-oh R-loop (lanes 6-10 and 16-20). Where indicated, ATP was omitted. Scans are shown as an overlay of FITC (red) and Cy3 (green) signals generated in Multi Gauge software. **(H)** Quantification of the experiments shown in (G). Data points represent the mean of 3 independent experiments and error bars represent the SD. The values obtained for the highest concentration were compared using a two-tailed unpaired Student′s t-test. The resulting p-values (P) are represented by ** $P < 0.01$ and **** $P < 0.0001$.

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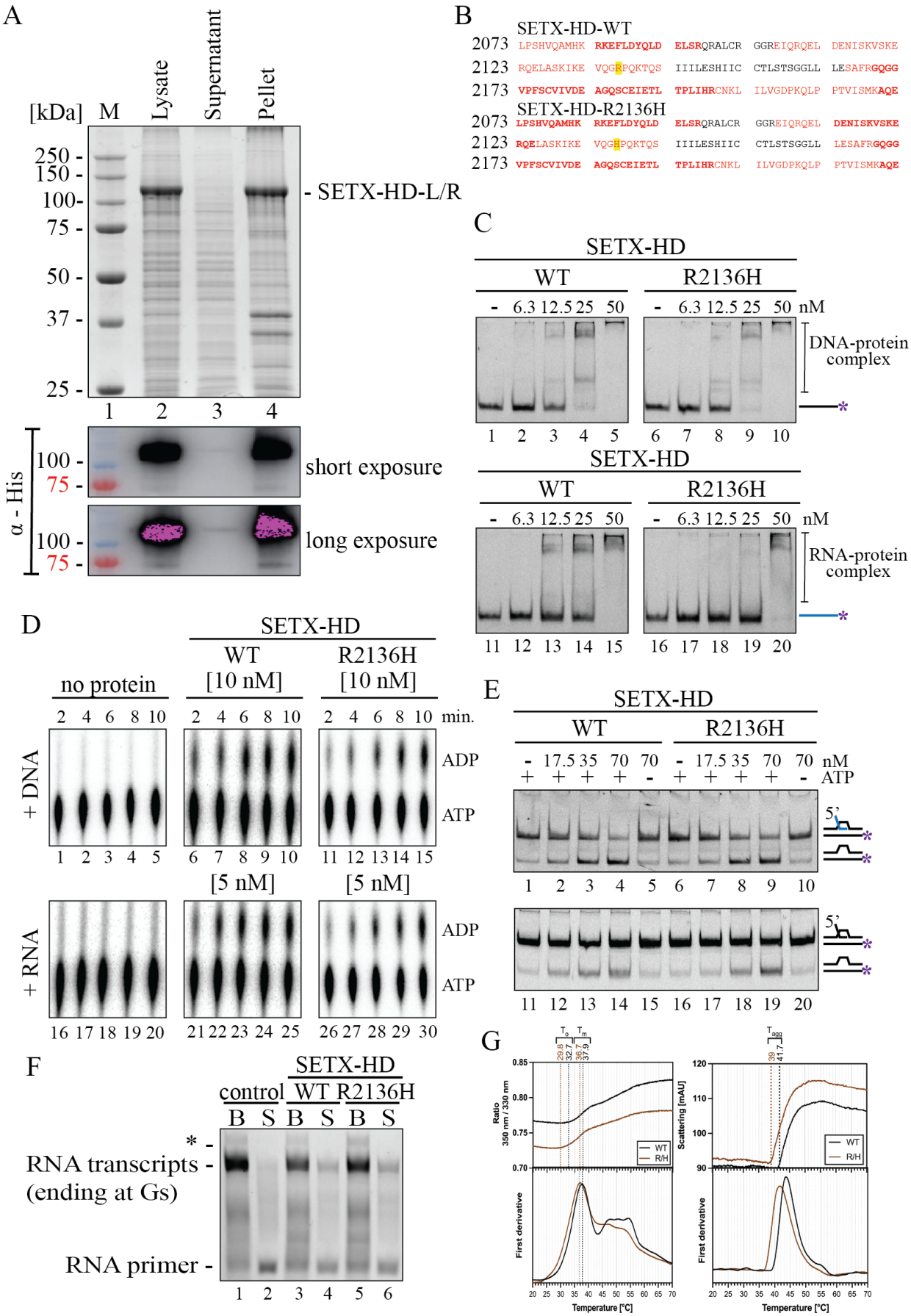

Figure S7: Supplementary figure related to main Figure 6: Purification and characterisation of SETX-HD variants associated with AOA2 and ALS4. (A) Solubility test for the mutant version of SETX-HD containing the L1976R substitution associated with AOA2. Top: Coomassie-stained SDS-PAGE gel showing the presence of the SETX-HD-L1976R (L/R) mutant in the cell lysate and in the supernatant and pellet fractions after centrifugation. Bottom: Immunodetection of $(His)_6$-tagged SETX-HD-L/R with an α-His antibody localised SETX-HD-L/R primarily in the pellet fraction (lane 4). M: molecular weight marker. **(B)** Mass spectrometry analysis of SETX-HD-WT and SETX-HD-R/H, respectively. Combined amino acid coverage from MALDI-MS/MS and LC-MS/MS. In both proteins the amino acid position of interest (p. 2136) was covered (highlighted in yellow). Covered amino acids are shown in red (bold red depicts significant MASCOT score). **(C)** Electrophoretic mobility-shift assays (EMSA) were performed with the indicated amounts of SETX-HD-WT and SETX-HD-R/H, respectively, in the presence of 10 nM fluorescently labelled ssDNA (pRMS679, 61 nt, lanes 1-10) and ssRNA (pRMS678, 61 nt, lanes 11-20). **(D)** Determination of the ATPase activity of SETX-HD-R/H. ATPase assays containing indicated amounts of SETX-HD-WT and SETX-HD-R/H, respectively, were performed in the presence of single-stranded DNA (DL3701, 44 nt, lanes 1-15) and RNA (DL3316, 44 nt, lanes 16-30). The position of α[^{32}P]-ATP and α[^{32}P]-ADP is depicted. **(E)** SETX-HD-R/H unwinds 5'-oh R-loop and 5'-oh D-loop structures, respectively, to a similar extent as SETX-HD-WT. Helicase assays were performed in the presence of the indicated concentrations of SETX-HD-WT and SETX-HD-R/H (17.5, 35, and 70 nM), respectively, and 10 nM of fluorescently labelled 5'-oh R-loop (upper panel) or 5'-oh D-loop (lower panel) in the presence of ATP. Lanes 5, 10, 15, and 20 represent control experiments with no ATP. **(F)** SETX- HD-R/H induces transcription termination *in vitro* to a similar extent as the wild-type. *In vitro* transcription termination assays performed with mammalian RNAPII as in Figure 5. Asterisk denotes transcripts associated with RNAPIIs that over-read the G-stretch due to small GTP contaminations. **(G)** Representative nanoDSF thermogram for SETX-HD-WT (in black) and SETX-HD-R/H (in brown). Upper left panel shows fluorescence intensity as a 350/330 nm ratio indicating protein unfolding and the upper right panel shows light scattering (back reflected light intensity passing through the sample) indicating the onset of protein aggregation. Corresponding first derivatives are plotted in the bottom panel. Temperatures of onset of protein unfolding (T_o), melting temperature (T_m), and onset of protein aggregation (T_a) are shown as vertical lines.

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A

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Sen1_prong   1457LEELVDKRIGERNYEIRTDPELERKFNNAVTKRRE-LRGKLDSESGNPESPMSTEDISKL
SETX_prong   2061LDSQVNHRMKK---------ELPSHVQ-AMHKRKEFLDYQLDELS-RQRAL-CRGGRE-I
                 *:. *:.*:.:         **  :.: *: **:* *  :**. *   : .:  .. . :

Sen1_prong       QLKIRELSKIINELGRDRDEMREKNSVNYRNRDLDRRNAQAHILAV 1561
SETX_prong       Q-R-QELDENISKVSKERQELASKIK-EVQGR---PQKTQSIIILE 2147
                 * : :**.: *.::.::*:*: .* . : :.*    :::*: *::
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B

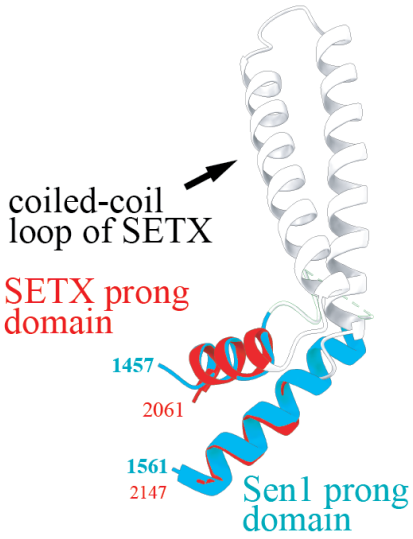

Figure S8: Comparison of the prong domain of Sen1 and SETX. (A) Structure-guided amino acid alignment of the prong domain of Sen1 and SETX. **(B)** Overlay of the prong domain from the crystal structure of Sen1 (PDB ID 5MZN, in blue) (1) and from the AlphaFold2 model of SETX (in red).

Supplementary table S1: List of oligonucleotides used in this work.

Name	Sequence 5'-3'	Use
pRMS101	ATCCACGGACCACCTGGAACCGGAAGGTCT AAGACCATCGTCGGACTG	Forward primer for site-directed mutagenesis to introduce K1969R mutation.
pRMS102	CAGTCCGACGATGGTCTTAGACCTTCCGGTT CCAGGTGGTCCGTGGAT	Reverse primer for site-directed mutagenesis to introduce K1969R mutation.
pRMS103	CCCTTCTCTTGCGTGATCGTGGCCGCAGCCG GACAGTCTTGCGAAATCGAAACTC	Forward primer for site-directed mutagenesis to introduce DE2181, 2182AA mutation.
pRMS104	GAGTTTCGATTTCGCAAGACTGTCCGGCTGC GGCCACGATCACGCAAGAGAAGGG	Reverse primer for site-directed mutagenesis to introduce DE2181, 2182AA mutation.
pRMS114	ACCTCCTGCCTCTCTGTGC	Sequencing of SETX
pRMS115	GGACTTCAACGAGGACCAAA	Sequencing of SETX
pRMS116	CTGCTGCACCTTGTCTACCA	Sequencing of SETX
pRMS117	AGTCGACACCGTTGATGCTT	Sequencing of SETX
pRMS217	GACGCTGCCGAATTCTACCAGTGCCTTGCTA GGACATCTTTGCCCACCTGCAGGTTCACCC	61-mer DNA primer fluorescently labelled on 5' end with Cy3 used to create R-loop/D-loop/bubble substrates for EMSAs and helicase assays.
pRMS219	GGGTGAACCTGCAGGTGGGCGGCTGCTCAT CGTAGGTTAGTTGGTAGAATTCGGCAGCGTC	61-mer DNA primer used to create R-loop/D-loop/bubble substrates for EMSAs and helicase assays.
pRMS220	TAAGAGCAAGATGTTCTATAAAAGATGTCCT AGCAAGGCAC	41-mer DNA primer used to create a 5'-overhang D-loop substrate for EMSAs and helicase assays.
pRMS222	UAAGAGCAAGAUGUUCUAUAAAAGAUGUC CUAGCAAGGCAC	41-mer RNA primer used to create a 5'-overhang R-loop and RNA:DNA hybrid substrate for EMSAs and helicase assays.
pRMS223	AAAGAUGUCCUAGCAAGGCAC	21-mer RNA primer used to create an R-loop substrate for EMSAs and helicase assays.
pRMS323	TTATCCACTTCCAATGTTATTATTAGTGATG GTGATGGTGATG	Reverse primer for generating a 1674-2677 amino acid fragment of SETX for cloning into 438A plasmid.
pRMS391	GGGTGAACCTGCAGGTGGGCAAAGATGTCC TAGCAAGGCACTGGTAGAATTCGGCAGCGT C	61-mer DNA primer used to create a double stranded DNA substrate for EMSA and helicase assays.
pRMS590	TTTAAGAAGGAGATATAGTTCATGGTCCTGA ACACCTTCG	Forward primer for generating a 1674-2677 amino acid fragment of SETX for cloning into 2BcT plasmid.
pRMS591	GGATTGGAAGTAGAGGTTCTCGAGCAACTT ACGCTTCTTTG	Reverse primer for generating a 1674-2677 amino acid fragment of SETX for cloning into 2BcT plasmid.
pRMS592	TACTTCCAATCCAATCGATGGTCCTGAACAC CTTCG	Forward primer for generating a 1674-2677 amino acid fragment of SETX for cloning into 438A plasmid.
pRMS607	GGAAAGTCTAAGACCATCGTCGGACGGCTG TACAGGCTGCTCACC	Forward primer for site-directed mutagenesis to introduce a L1976R mutation.
pRMS608	GGTGAGCAGCCTGTACAGCCGTCCGACGAT GGTCTTAGACTTTCC	Reverse primer for site-directed mutagenesis to introduce a L1976R mutation.

Name	Sequence 5'-3'	Use
pRMS609	TCCAAGATCAAAGAGGTCCAGGGACACCCT CAGAAAACCCAGTCT	Forward primer for site-directed mutagenesis to introduce a R2136H mutation.
pRMS610	AGACTGGGTTTTCTGAGGGTGTCCCTGGACC TCTTTGATCTTGGA	Reverse primer for site-directed mutagenesis to introduce a R2136H mutation.
pRMS626	AAAGAUGUCCUAGCAAGGCACUAAGUCCU AGAUGUUCUAUA	41-mer RNA primer used to create a 3'-overhang R-loop substrate for EMSAs and helicase assays.
pRMS678	UUGUCUUCUCAUAAAUUUAAUCCCCGUAC GCUUAUACUCCUUUAACUACCCAGUCUUCC CG	61-mer RNA primer fluorescently labelled on 5' end with Cy3 used as a single-stranded RNA for EMSAs and to create RNA:DNA hybrid substrates for EMSAs and helicase assays.
pRMS679	TTGTCTTCTCATAAATTTAATCCCCGTACGCT TATACTCCTTTAACTACCCAGTCTTCCCG	61-mer DNA primer fluorescently labelled on 5' end with Cy3 used as a single-stranded DNA for EMSAs.
pRMS680	TTGTCTTCTCATAAATTTAATCCCCGTACGCT TATACTCCTTTAACTACCCAGTCTTCCCG	61-mer DNA primer used as a competitor in helicase assays with a RNA:DNA hybrid.
pRMS681	TTAAATTTATGAGAAGACAA	20-mer DNA primer used as a competitor in helicase assays with a 3'-overhang RNA:DNA hybrid.
pRMS682	CGGGAAGACTGGGTAGTTAA	20-mer DNA primer used as a competitor in helicase assays with a 5'-overhang RNA:DNA hybrid.
pRMS689	TTAAATTTATGAGAAGACAA	20-mer DNA primer fluorescently labelled on 5' end with FITC used to create a 3'-overhang RNA:DNA hybrid substrate for EMSAs and helicase assays.
pRMS690	CGGGAAGACTGGGTAGTTAA	20-mer DNA primer fluorescently labelled on 5' end with FITC used to create a 5'-overhang RNA:DNA hybrid substrate for EMSAs and helicase assays.
pRMS739	TTGTCTTCTCATAAATTTAATCCCCGTACGCT TATACTCCT	41-mer 5' biotin tagged DNA primer for BLI assay
pRMS740	UUGUCUUCUCAUAAAUUUAAUCCCCGUAC GCUUAUACUCCU	41-mer 5' biotin tagged RNA primer for BLI assay
pRMS741	CTGCATCGTCATCTCTTCCCTGACCACCACT CAGCGCAAGCTGAA	Forward primer for site-directed mutagenesis to introduce a V1884T mutation.
pRMS742	TTCAGCTTGCGCTGAGTGGTGGTCAGGGAA GAGATGACGATGCAG	Reverse primer for site-directed mutagenesis to introduce a V1884T mutation.
pRMS743	CGGGAAGACTGGGTAGTTAAAGGAGTATAA GCGTACGGGGATTAAATTTATGAGAAGACA A	61-mer DNA primer used to create a blunt ended RNA:DNA hybrid for EMSAs and helicase assays.
DL3701	TTCATTTCAGACCAGCACCCACTCACTACAA CTCACGACCAGGC	Primer used to stimulate nucleic-acid dependent SETX/Sen1 ATPase activity.
DL3316	UUCAUUUCAGACCAGCACCCACUCACUACA ACUCACGACCAGGC	Primer used to stimulate nucleic-acid dependent SETX/Sen1 ATPase activity.

Name	Sequence 5'-3'	Use
DL2492	UGCAUUUCGACCAGGC	5' FAM labelled RNA primer for performing IVTT assays on immobilized templates.
DL3352	CTAGAGGAAACAAACTATAGGAAACGACCAGGCCCTCAACATCTCTCACCCATCTCCACACGGGGGTTACCCGGCCTGCA	Non-template strand for IVTT assays.
DL3353	GGCCGGGTAACCCCCGTGTGGAGATGGGTGAGAGATGTTGAGGGCCTGGTCGTTTCCTATAGTTTGTTTCCT	Template strand for IVTT assays.

Supplementary table S2: List of proteins identified by MS analysis in a typical SETX-HD purification.

SETX-HD wild-type

NCBIprot entry	Protein	Score	Organism	Sequence coverage [%]
BAG10365.1	senataxin (fragment)	10135	*Homo sapiens*	82
XP_026726793.1	ras GTPase-activating protein-binding protein 2	1141	*Trichoplusia ni*	45
XP_026736716.1	cleavage and polyadenylation specificity factor subunit CG7185	543	*Trichoplusia ni*	19
XP_026740726.1	protein lingerer-like	511	*Trichoplusia ni*	23
XP_026743046.1	AMP deaminase 2-like isoform X6	503	*Trichoplusia ni*	34
XP_026748099.1	cytoplasmic FMR1-interacting protein	376	*Trichoplusia ni*	18
XP_026739518.1	transcription elongation factor SPT6	365	*Trichoplusia ni*	19
XP_026733954.1	protein LSM14 homolog A	323	*Trichoplusia ni*	22
XP_026747555.1	la-related protein 1-like	292	*Trichoplusia ni*	28
XP_026747903.1	cleavage and polyadenylation specificity factor subunit 5	265	*Trichoplusia ni*	35
XP_026730705.1	ataxin-2-like protein, partial	194	*Trichoplusia ni*	21

SETX-HD R2136H mutant

NCBIprot entry	Protein	Score	Organism	Sequence coverage [%]
BAG10365.1	senataxin (fragment)	11213	*Homo sapiens*	82
XP_026743046.1	AMP deaminase 2-like isoform X6	962	*Trichoplusia ni*	48
XP_026736716.1	cleavage and polyadenylation specificity factor subunit CG7185	672	*Trichoplusia ni*	18
XP_026726793.1	ras GTPase-activating protein-binding protein 2	613	*Trichoplusia ni*	38
XP_026739518.1	transcription elongation factor SPT6	474	*Trichoplusia ni*	25
XP_026747555.1	la-related protein 1-like	449	*Trichoplusia ni*	32
XP_026732337.1	T-complex protein 1 subunit delta	417	*Trichoplusia ni*	45
XP_026740726.1	protein lingerer-like	319	*Trichoplusia ni*	13

AAB06239.1	HSC70	259	*Trichoplusia ni*	39
XP_026747903.1	cleavage and polyadenylation specificity factor subunit 5	225	*Trichoplusia ni*	34
XP_026730705.1	ataxin-2-like protein, partial	194	*Trichoplusia ni*	21

SETX-HD V1884T mutant

NCBIprot entry	**Protein**	**Score**	**Organism**	**Sequence coverage [%]**
AAH32622.2	senataxin (fragment)	2079	*Homo sapiens*	50
XP_026739518.1	transcription elongation factor SPT6	1309	*Trichoplusia ni*	38
XP_026743039.1	AMP deaminase 2-like isoform X1	1164	*Trichoplusia ni*	56
XP_026747555.1	la-related protein 1-like	818	*Trichoplusia ni*	38
XP_026748099.1	cytoplasmic FMR1-interacting protein	693	*Trichoplusia ni*	35
XP_021197558.1	ras GTPase-activating protein-binding protein 2	544	*Trichoplusia ni*	33
ABH09732.1	HSP 70	476	*Trichoplusia ni*	69
XP_026730705.1	ataxin-2-like protein, partial	387	*Trichoplusia ni*	25
XP_026736716.1	cleavage and polyadenylation specificity factor subunit CG7185	356	*Trichoplusia ni*	23
XP_026728848.1	endoplasmic reticulum chaperone BiP	307	*Trichoplusia ni*	39
XP_026737278.1	membrane-associated protein Hem	279	*Trichoplusia ni*	25
XP_026740726.1	protein lingerer-like	270	*Trichoplusia ni*	20
XP_026725864.1	formin-binding protein 1-like isoform X1	233	*Trichoplusia ni*	28
XP_026729973.1	titin isoform X1	184	*Trichoplusia ni*	6
XP_026725810.1	LOW QUALITY PROTEIN: wiskott-Aldrich syndrome protein family member 3	167	*Trichoplusia ni*	37
XP_026730764.1	beta-parvin	158	*Trichoplusia ni*	11
XP_026732337.1	T-complex protein 1 subunit delta	156	*Trichoplusia ni*	22
XP_026738533.1	dedicator of cytokinesis protein 7 isoform X1	155	*Trichoplusia ni*	11
XP_026726855.1	rho-associated protein kinase 2	148	*Trichoplusia ni*	16
XP_026739466.1	protein flightless-1	137	*Trichoplusia ni*	14

Supplementary methods

Protein identification from 1D gel bands by LC-MS/MS

The proteins were subjected to incubation with trypsin or pepsin for 1 hour at 37 °C. The resulting peptides were analyzed by LC-MS/MS using RSLCnano system (Thermo Fisher Scientific) connected to Qq-Time-Of-Flight mass spectrometer Impact II (Bruker Daltonics). MS/MS data were searched against a custom database of relevant protein sequences in combination with cRAP contaminant database, and against NCBIprot database

(downloaded on 6th February 2021) without taxonomy restrictions, using an in-house Mascot search engine (Matrixscience; version 2.6).

Solubility test and immunodetection of SETX-HD variants by western blotting

Pelletted Hi5 insect cells expressing SETX-HD variants were lysed in lysis buffer (50 mM Tris-HCl, pH 8; 0.5 M NaCl; 10 % (v/v) glycerol; 1 mM DTT; 0.4 % (v/v) Triton-X; 10 mM imidazole), containing protease inhibitors (0.66 µg/ml pepstatin, 5 µg/ml benzamidine, 4.75 µg/ml leupeptin, 2 µg/ml aprotinin) and 25 U benzonase per ml of lysate. The lysate was cleared by centrifugation to obtain soluble proteins. The individual fractions – lysate, supernatant, and pellet after centrifugation were separated on SDS-PAGE and transferred on a PVDF membrane (AppliChem). Monoclonal mouse anti-His antibodies (SAB1305538) and goat anti-mouse IgG conjugated with HRP (A0168) were purchased from Sigma-Merck.

Supplementary references

1. Chakrabarti,S., Jayachandran,U., Bonneau,F., Fiorini,F., Basquin,C., Domcke,S., le Hir,H. and Conti,E. (2011) Molecular Mechanisms for the RNA-Dependent ATPase Activity of Upf1 and Its Regulation by Upf2. *Mol Cell*, **41**, 693–703.