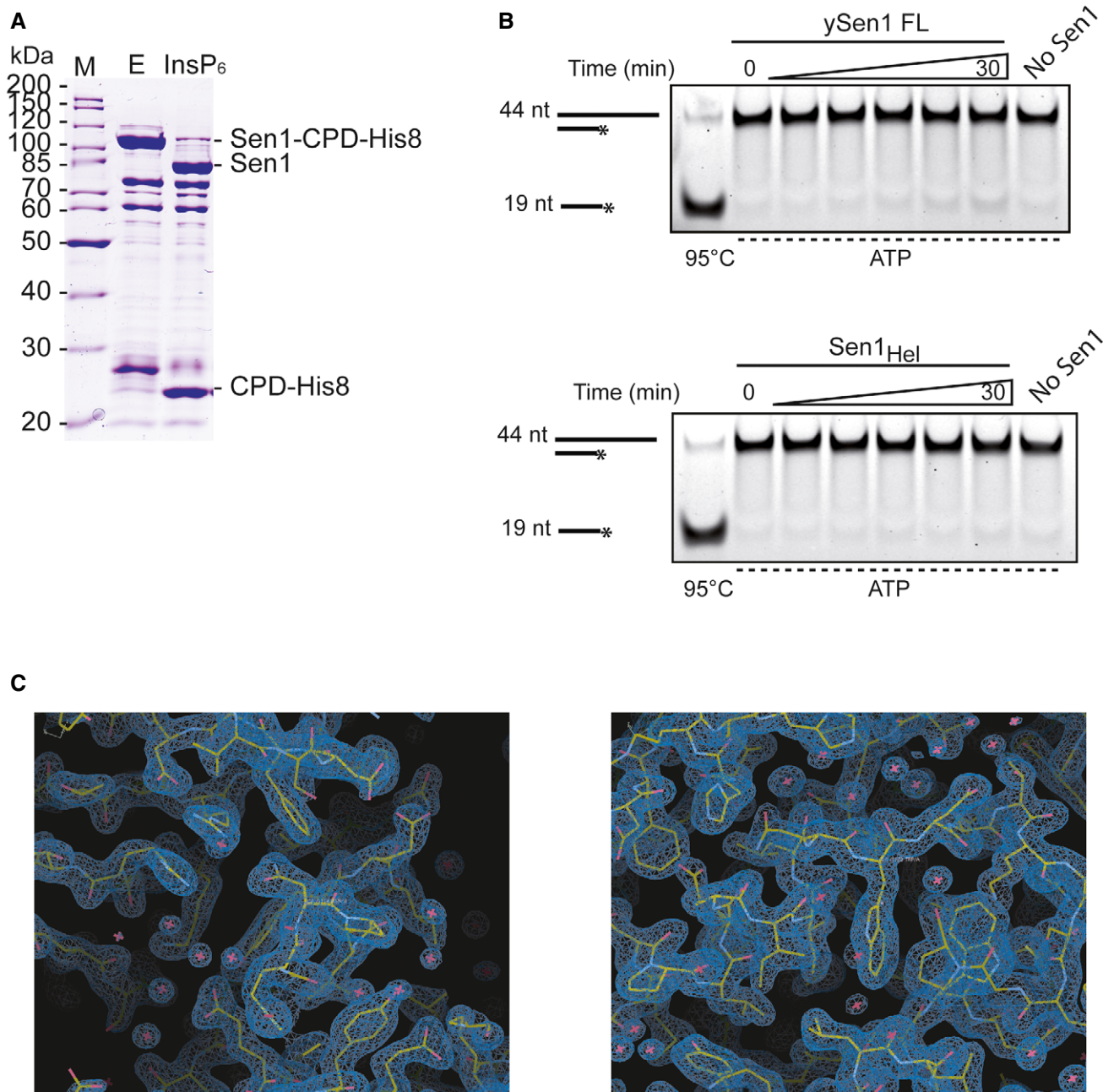# Expanded View Figures

Figure EV1. Identification and characterization of a recombinant helicase core of *Saccharomyces cerevisiae* Sen1 suitable for structural studies.

A   SDS–PAGE analysis of Sen1$_{976-1880}$ tagged with C-terminal CPD-His$_8$. Lane E shows the elution fraction after Ni^{2+}-affinity purification step, and lane InsP6 shows the tag cleavage after the protein was incubated with 400 μM inositol hexakisphosphate (InsP6) for 20 min at 4°C. The protein before and after tag cleavage is smaller than expected: Theoretical molecular weights of Sen1$_{976-1880}$-CPD-His$_8$ and Sen1$_{976-1880}$ are ~126 kDa and ~102 kDa, respectively. Left lane M shows a molecular weight marker.

B   Time course analysis of the ATP-dependent 3′-5′ duplex unwinding activity of Sen1 proteins. Reactions were performed in the presence of 5 nM of Sen1 and 2 nM of substrate. An RNA:DNA duplex composed of a 44-mer RNA annealed to a 19-mer DNA molecule to provide a 3′-end 25-nt single-strand overhang was used as the substrate (see Appendix Table S1 for sequence details). The asterisk (*) denotes the presence of a FAM at the 5′ end of the DNA.

C   Snapshots of the electron density maps at important regions of the structure described in the text. The 2Fo-Fc maps are contoured at 1.7σ.

Figure EV2. Biochemical and structural properties of Sen1, and comparison with Upf1.

A   Zoom-in view of the nucleotide binding site in Sen1 (left) and Upf1 (right) (PDB: 2XZO, Chakrabarti *et al*, 2011). The adenine ring is sandwiched between an apolar surface of RecA1 and an aromatic residue protruding from the short linker that connects RecA1 to RecA2 (Tyr1655, corresponding to $Tyr638_{Upf1}$ and $Tyr442_{IMGMBP2}$). In addition, the conserved side chain of Gln1339 (corresponding to $Gln413_{Upf1}$ and $Gln196_{IMGMBP2}$) forms a bidentate hydrogen-bond interaction with the N6 and N7 moieties of the adenine ring.

B   Comparison of the structures of yeast $Sen1_{Hel}$-ADP, human $UPF1_{Hel}$-AMPPNPP (PDB: 2GJK, Cheng *et al*, 2007), $UPF1_{Hel}$-ADP:AlF_4^--RNA (PDB: 2XZO, Chakrabarti *et al*, 2011), and yeast $Upf1_{Hel-CH}$-ADP:AlF_4^--RNA (PDB: 2XZL, Chakrabarti *et al*, 2011). The molecules in a side-view orientation (90° clockwise rotation around a vertical axis with respect to the front-view in Fig 4A).

C   Comparison of the RNA-binding sites of Sen1 (left) and Upf1 (right) (PDB: 2XZO, Chakrabarti *et al*, 2011).

D–F   Functional analysis of the $Sen1_{Hel}$ T1289A, R1293A mutant harboring substitutions at conserved positions at the predicted RNA-binding surface. (D) Fluorescence anisotropy assays. Curves represent three independent measurements. (E) ATP hydrolysis assays. Values correspond to the average and SD of three independent experiments. (F) IVTT assays performed in the same conditions as in Fig 1C. The images correspond to different gels migrated and processed in parallel. The values of nascent RNA released correspond to one out of two independent experiments.
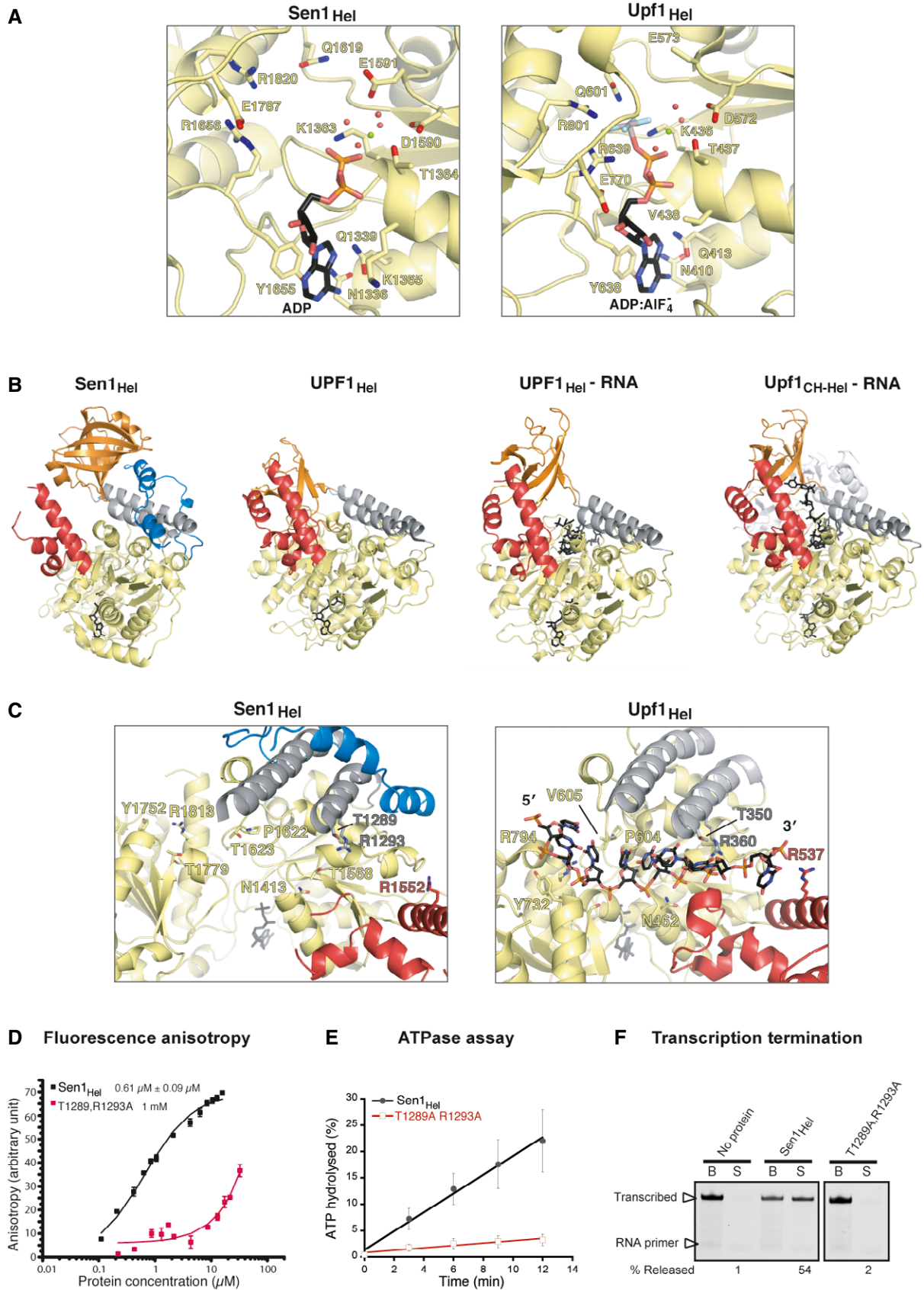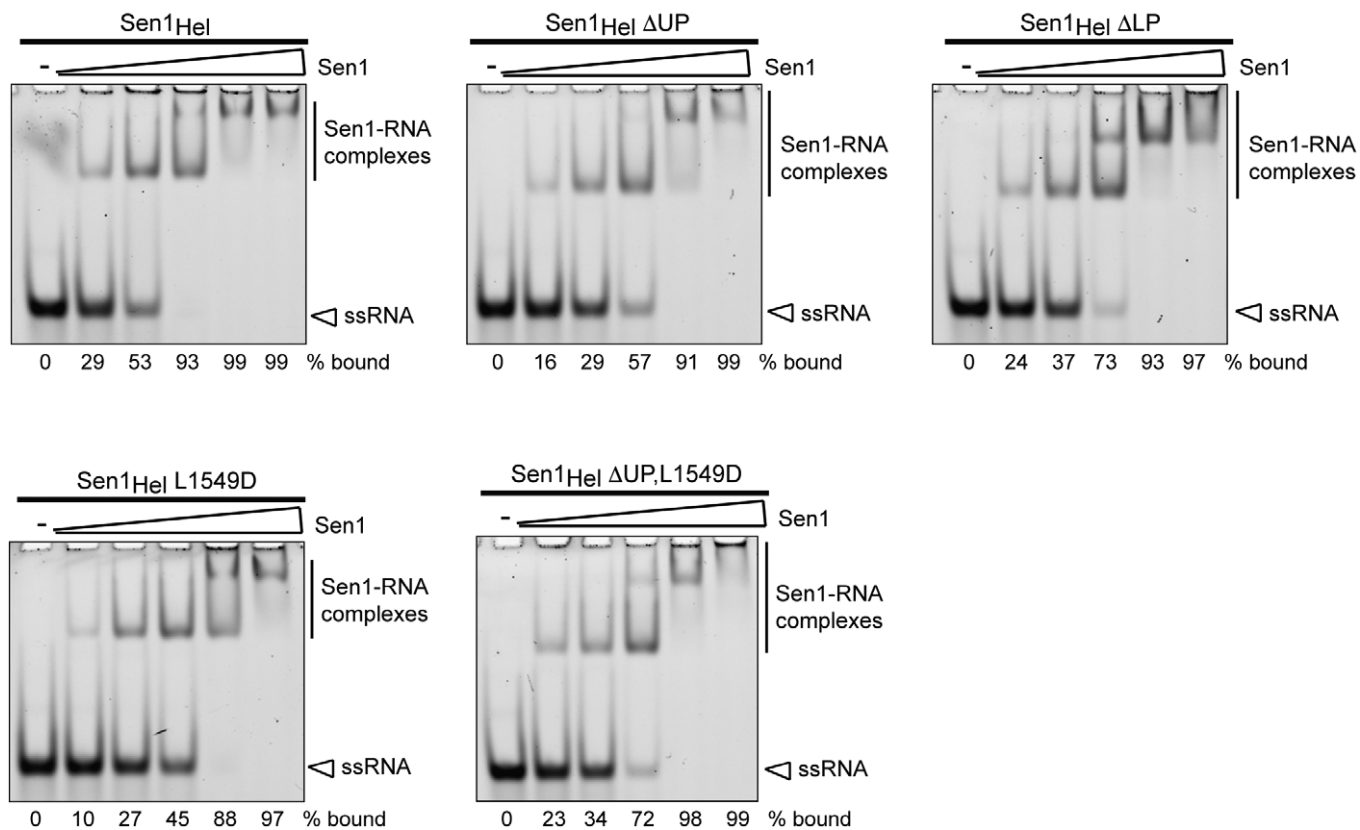
Figure EV2.

Figure EV3. Analysis of the impact of the "prong" mutations on the affinity of Sen1$_{Hel}$ for the RNA.
Electrophoretic mobility shift assay (EMSA) using a 5′-end fluorescently labeled 44-mer RNA as the substrate (DL3316, see Appendix Table S1) at 2 nM and Sen1$_{Hel}$ variants at 10, 20, 40, 80, and 160 nM at the final concentrations. Gels were migrated and processed in parallel. The values correspond to the mean of two independent experiments. At high protein concentrations, Sen1$_{Hel}$ forms high-order complexes with the RNA that are retained in the wells of the gel.
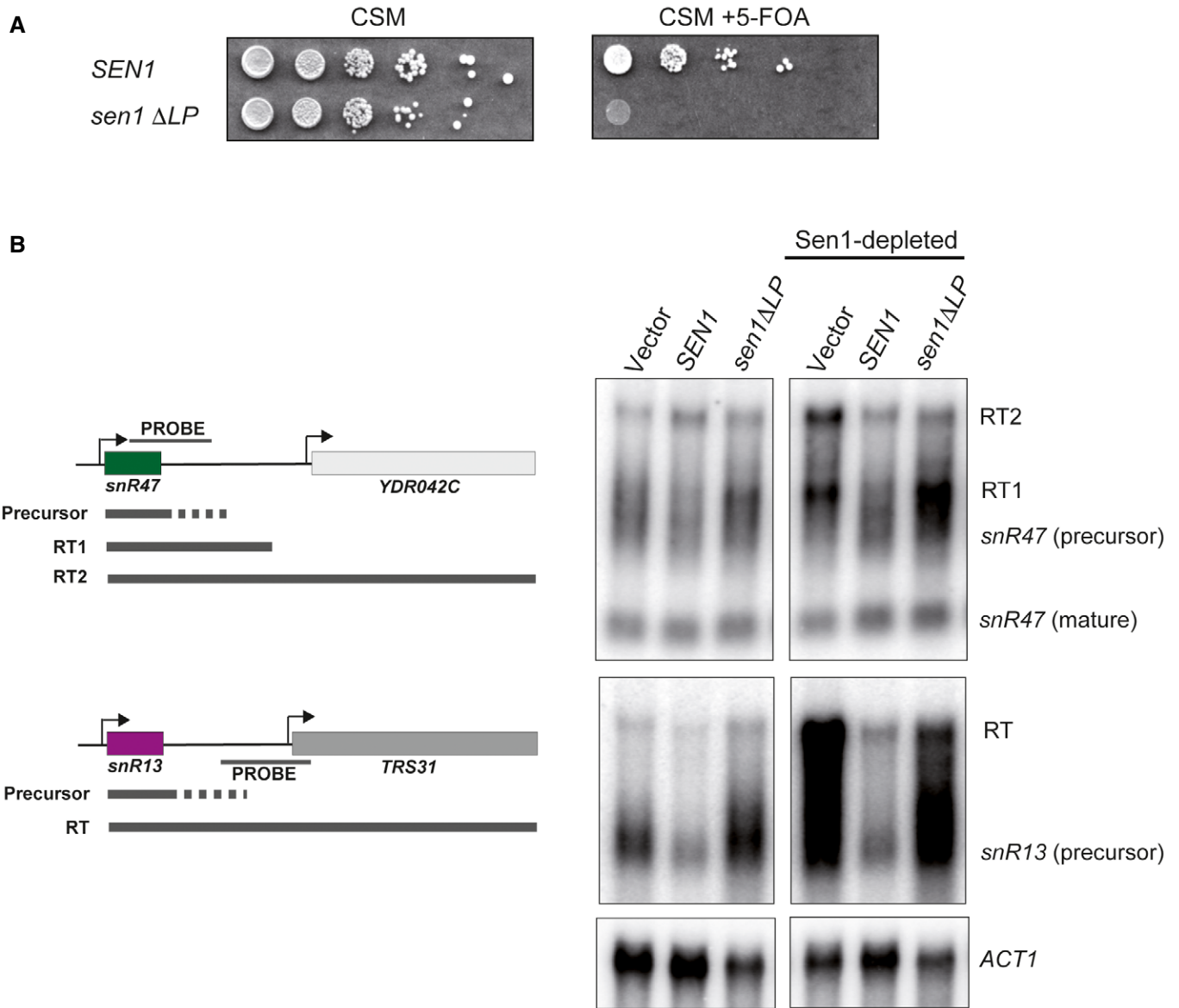
Figure EV4. Analysis of the phenotype of the ΔLP mutant *in vivo*.

A   A Sen1 variant harboring the ΔLP cannot support cell viability. A Δ*sen1* strain (YDL2767) covered by an *URA3*-containing plasmid (pFL38) expressing wild-type (wt) Sen1 was transformed with a *TRP1*-plasmid (pFL39) carrying either the wt or a ΔLP version of *SEN1*. After over-night growth in non-selective medium, cells initially harboring both plasmids were plated on minimal medium (CSM) containing 5-fluoroorotic acid (5-FOA) to select for cells that have lost the *URA3* plasmid (and can therefore survive thanks to the *TRP1* plasmid-borne *SEN1* copy). The absence of cells growing in 5-FOA and containing the *TRP1* plasmid expressing Sen1 ΔLP indicates that the ΔLP deletion is lethal.

B   The Sen1ΔLP mutant is strongly defective in transcription termination *in vivo*. Northern blot analyses of two well-characterized NNS-targets, snR47 and snR13, in a Sen1-AID (auxin-induced degron, Nishimura *et al*, 2009) strain carrying a plasmid (pFL39) expressing either the wt or a ΔLP version of *SEN1*. A strain harboring an empty vector was included as a positive control for termination defects. To detect the primary products of NNS-dependent termination that are processed/degraded by the exosome, the strain was also deleted in the exonuclease *RRP6*. Sen1-AID was depleted for 1 h by the addition of 100 μM indole-3-acetic acid (a natural auxin) to monitor the capacity of the plasmid-borne versions of *SEN1* to induce transcription termination. The strong accumulation of longer RNA species in the *sen1ΔLP* mutant compared to the wt is indicative of major termination defects. Under non-depletion conditions, the strain harboring the mutant protein exhibits a dominant-negative phenotype (partial termination defects), indicating that Sen1ΔLP has similar expression levels compared to the endogenous Sen1. The *ACT1* transcript is used as a loading control.

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Sen1_S.cerevisiae     1095 A E L - A K Q E - - L E H M R K R L N V D M N P L Y E I I L Q W D Y T R N S E Y P D D E P I G N - - - - - Y S D V K D 1145
SETX_H.sapiens        1680 K Y F P S S S P - - V N I L L - S S Q S V S D T F V K E V L K W K Y E M F L N F G Q C G P P A S L C Q S I S R P V P V 1735
Upf1_S.cerevisiae      176 S W V - A E Q P T E E E K L K A R L - I T P S Q I S K L E A K W R S N K D A T I N D I D A P E E - - Q E A I P P L L L 230
IGHMBP2_H.sapiens          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Sen1_S.cerevisiae     1146 F F N S P A D Y Q K V M K P L L L L E S W Q G L - - - - C S S R D - - R E - D Y K P F S I I V G N R T A V S D F Y D V 1197
SETX_H.sapiens        1736 R F H N Y G D Y F N V F F P L M V L N T F E T V A Q E W L N S P N - - R E N F Y Q - - - L - - Q V R K F P A D Y I K Y 1787
Upf1_S.cerevisiae      231 R Y Q D A Y E Y Q R S Y G P L I K L E A D Y D K Q L - - K E S Q A - - L E H I S V S W S L A L N N R H L A S F T L S T 285
IGHMBP2_H.sapiens        1 - - - - - - - - - - - M A S A A V E S F V T K Q L D L L E L E R D A E V E E R R S W Q E N I S L K E L Q S R G - - - 44

Sen1_S.cerevisiae     1198 Y A S V A K - - - - - - - Q V I Q D C G I S E S D - L I V M A Y L P D F R P D K - - - - - - R L S S D D - - - - - F K 1237
SETX_H.sapiens        1788 W E F A - - - - - - - - - V Y L E E C E L A K Q L - Y P K E N D L V F L A P E R I N E E K K D T E R N D - - - - - I Q 1831
Upf1_S.cerevisiae      286 F E S N E L K V A I G D E M I L W Y S G M Q H P D - W E G R G Y I V R L P - - - - - - - - - - - N S - - - - - F Q 325
IGHMBP2_H.sapiens       45 - - - - - - - - - - - - - V C L L K L Q V S S Q R T G L Y G R L L V T F E P R R Y G S A A - A L P S N S F T S G D I V 89

Sen1_S.cerevisiae     1238 - - - K - - - - - - - - A Q H T C L A K V R T L K N T K G G N V D V T L R I H R N H S F S K F L T L R S E I Y C V K V M 1286
SETX_H.sapiens        1832 D L H E - - - - - - - Y H S G Y V H K F R R T S V M R N G K T E C Y L S I Q T Q E N F P A N L - - N E L V N C I V I S 1881
Upf1_S.cerevisiae      326 - - - D - - - - - - - - T - - - F T L E L K P S K T P - - - - - - - - - P P T H L T T G F T A E F I W K G T S Y D R M Q D 363
IGHMBP2_H.sapiens       90 G L Y D A A N E G S Q L A T G I L T R V T Q - - - - - - - - K S V T V A F D E S H D F Q L S L D R E N S Y R L L K L A 140

Sen1_S.cerevisiae     1287 Q M T T I E R E Y S T L E G L E Y Y D L V G Q I L Q - A K - - P - S P P V N V D A A - - - - - - - - - E I E T V K K - S 1332
SETX_H.sapiens        1882 S L V T T Q R K L K A M - - - - - - S L L G S R N Q L A R - - A V L N P N P M D F C T K D L L T T T S E R I I A Y - L 1931
Upf1_S.cerevisiae      364 N D V T Y R R L K K A L I A L K K Y H S G P A S S L I E V L F G R S A P S P A S - - - - - - - - - - E I H P L T F F N 406
IGHMBP2_H.sapiens      141 N D V T Y R R L K K A L I A L K K Y H S G P A S S L I E V L F G R S A P S P A S - - - - - - - - - - E I H P L T F F N 189

Sen1_S.cerevisiae     1333 Y K L N T S Q A E - - - - - - - A I V N S V S K E G F S L I Q G P P G T G K T K T I L G I I G Y F L S T K N A S S S N 1384
SETX_H.sapiens        1932 R D F N E D Q K K A I E T A Y A M V K H S P S V A K I C L I H G P P G T G K S K T I V G L L Y R L L T E N Q R K G - - 1988
Upf1_S.cerevisiae      407 A Q L N S S Q S - - - - - - - - N A V S H V L Q R P L S L I Q G P P G T G K T V T S A T I V Y - H L - - - - - - - - - 447
IGHMBP2_H.sapiens      190 T C L D T S Q K E - - - - - - - A V L F A L S Q K E L A I I H G P P G T G K T T T V V E I I L Q A V - - - - - - - - - 232

Sen1_S.cerevisiae     1385 V I K V P L E K N S S N T E Q L L K K Q K I L I C A P S N A A V D E I C L R L K S G V Y D K Q - - - - - - G H Q F K 1436
SETX_H.sapiens        1989 - - - - - - H S D E N S N A K I K Q N R V L V C A P S N A A V D E L M K K I I L E F K E K C K D K K N P L G N C G D 2040
Upf1_S.cerevisiae      448 - - - - - - - - - - - - - S K I H K D R I L V C A P S N V A V D H L A A K L R D L G L K - - - - - - - - - - - - - - 478
IGHMBP2_H.sapiens      233 - - - - - - - - - - - - - - - K Q G L K V L C C A P S N I A V D N L V E R L A L C K Q R - - - - - - - - - - - - - - 261

Sen1_S.cerevisiae     1437 P Q L V R V G R - - S D V V N V A I K D L T L E E L V D K R I G E R N Y E I - - - - R T D P - E L E R K F N N A V T K 1488
SETX_H.sapiens        2041 I N L V R L G P E - - K S I N S E V L K F S L D S Q V N H R M K K E L P S H V Q A M H K R K E F L D Y Q L D E L S R Q 2097
Upf1_S.cerevisiae      479 - - V V R L T A K S R E D V E S S V S N L A L H N L V G R G - - - - - - - - - - - - - - - - - - - - - - - - - 506
IGHMBP2_H.sapiens      262 - - I L R L G H P A R - - L L E S I Q Q H S L D A V L A R S D S A - - - Q I V A D I R K D I - - - - - - - D Q V F V K 306

Sen1_S.cerevisiae     1489 R R E L R G K L D S E S G N P E S P M S T E D I S K L Q L K I R E L S K I I N E L G R D R D E M R E K N S V N Y R N R 1547
SETX_H.sapiens        2098 R A L C R G G R E - - - - - - - - - - - - - - - - I - - - - - Q R Q E L D E N I S K V S K E R Q E - - - - L A S K I K E V 2133
Upf1_S.cerevisiae      507 - - - A K G E L - - - - - - - - - - - - - - - - - - - - - - - K N L L K L K D E V G E L - - - - - - S A S D T K R F 532
IGHMBP2_H.sapiens      307 N K K T Q D K - - - - - - - - - - - - - - - - - - - - - - - - R E K S N F R N E I K L L R K E L K E R - - - - - - - - 333

Sen1_S.cerevisiae     1548 D L D R R N A Q A H I L A V S D I I C S T L S G S A H D V L A - - - - - T M G I K F D T V I I D E A C Q C T E L S S I 1601
SETX_H.sapiens        2134 Q G R P Q K T Q S I I I I L E S H I I C C T L S T S G G L L L E S A F R G Q G G V P F S C V I V D E A G Q S C E I E T L 2192
Upf1_S.cerevisiae      533 V K L V R K T E A E I L N K A D V V C C T C V G A G D K R - - - - - - - L D T K F R T V L I D E S T Q A S E P E C L 583
IGHMBP2_H.sapiens      334 - - - E E A A M L E S L T S A N V V L A T N T G - - - A S A D G P L K L L P E S Y F D V V V I D E C A Q A L E A S C 386

Sen1_S.cerevisiae     1602 I P L R Y G G K R C I M V G D P N Q L P P T V L S G A A S N F K Y N Q S L F V R M E - K N - - - - - - - - - - - S S P 1648
SETX_H.sapiens        2193 T P L I H R C N K L I L V G D P K Q L P P T V I S M K A Q E Y G Y D Q S M M A R F C R L L E E N V E H N M I S R L P I 2251
Upf1_S.cerevisiae      584 I P I V K G A K Q V I L V G D H Q Q L G P V I L E R K A A D A G L K Q S L F E R L I S L G - - - - - - - - - - H V P 631
IGHMBP2_H.sapiens      387 I P L L K - A R K C I L A G D H K Q L P P T T V S H K A A L A G L S L S L M E R L A E E Y G A - - - - - - - - R V V 435

Sen1_S.cerevisiae     1649 Y L L D V Q Y R M H P S I S K F P S S E F Y Q G R L K D G P G M D I L - - - - N K R P W H Q L E P L A P Y K F F D I - 1702
SETX_H.sapiens        2252 L Q L T V Q Y R M H P D I C L F P S N Y V Y N R N L K T N R Q T E A I R - - - C S S D W - - - - P F Q P Y L V F D V - 2302
Upf1_S.cerevisiae      632 I R L E V Q Y R M N P Y L S E F P S N M F Y E G S L Q N G V T I E Q R T V P N S K F P W P I R G - - I P M M F W - A - 686
IGHMBP2_H.sapiens      436 R T L T V Q Y R M H Q A I M R W A S D T M Y L G Q L T A H S S V A R H L L R D L P G V A A T E E T G V P L L L V D T A 494

Sen1_S.cerevisiae     1703 I S G R Q E Q - N A K T M S Y T N M E E I R V A I E L V D Y L F R K F D N K I D F T G K I G I I S P Y R E Q M Q K M R 1760
SETX_H.sapiens        2303 G D G S E R - - - R D N D S Y I N V Q E I K L V M E I I K L I K D K - - R K D V S F R N I G I I T H Y K A Q K T M I Q 2356
Upf1_S.cerevisiae      687 N Y G R E E I - S A N G T S F L N R I E A M N C E R I T K L F R D - - - - G V K P E Q I G V I T P Y E G Q R A Y I L 740
IGHMBP2_H.sapiens      495 G C G L F E L E E E D E Q S K G N P G E V R L V S L H I Q A L V - - - - D A G V P A R D I A V V S P Y N L Q V D L L R 549

Sen1_S.cerevisiae     1761 K E F - - A R Y F G G M I N K S I D F N T I D G F Q G G E K E I I L I S C V R A D D T K S S V G F L K D F R R M N V A 1817
SETX_H.sapiens        2357 K D L D - - K E F D - - R K G P A E V D T V D A F Q G R Q K D C V I V T C V R A N S I Q G S I G F L A S L Q R L N V T 2411
Upf1_S.cerevisiae      741 Q Y M Q M N G S L D K D L Y I K V E V A S V D A F Q G R E K D Y I I L S C V R A N E Q - Q A I G F L R D P R R L N V G 798
IGHMBP2_H.sapiens      550 Q S L V - - - - - - - H R H P E L E I K S V D G F Q G R E K E A V I L S F V R S N R K - G E V G F L A E D R R I N V A 600

Sen1_S.cerevisiae     1818 L T R A K T S I W W L G H Q R S L A K S K L W R D L I E D A K D R S C L A Y A C S - - - - - - - G F L D - - - - - - - 1862
SETX_H.sapiens        2412 I T R A K Y S L F I L G H L R T L M E N Q H W N Q L I Q D A Q K R G A I I K T C D - - - - - - - K N Y R H D A V K I L 2463
Upf1_S.cerevisiae      799 L T R A K Y G L V I L G N P R S L A R N T L W N H L L I H F R E K G C L V E G T L - - - - - - - D N L Q L C T V Q L V 850
IGHMBP2_H.sapiens      601 V T R A R R H V A V I C D S R T V N N H A F L K T L V E Y F T Q H G E V R T A F E Y L D D I V P E N Y S H E N S Q G S 659

Sen1_S.cerevisiae     1863 - P R N N R A - Q S I L R K F N V P V P S E Q E D D Y K L P M E Y - - - - - I T Q G - - - - - P D E V K S N        1904
SETX_H.sapiens        2464 K L K P V L Q - - - - - R S L T H P P T I A P E G S R - - P - - - - - - - - - - Q G - - - - - G L - - - -          2490
Upf1_S.cerevisiae      851 R P Q P R K T E R P M N A Q F N V E S E - - M - G D F P K F Q D F - - - - - D A Q S - - - - - M V S F S G Q          891
IGHMBP2_H.sapiens      660 S H A A - - - - - - - - T K P Q G P A T S T R T G S Q R Q E G G Q E A A A P A R Q G R K K P A G K S L A S E        705
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Figure EV5. Multiple sequence alignment of the helicase domain of Upf1-like helicases.
The multiple alignment was done using Clustal Omega, and the conservation was calculated using BLOSUM62 and is shown in purple.