# **Expanded View Figures**

#### Figure EV1. The Sen1 NIM is conserved in close yeast species.

Saccharomyces cerevisiae Sen1 protein sequence was submitted to blastp excluding the genus Saccharomyces from the search. The ten most conserved protein sequences together with Sen1 orthologues from *Homo sapiens* (SETX) and *Schizosaccharomyces pombe* were aligned to *S. cerevisiae* Sen1 using clustal omega. Visualization of the alignment and calculation of the consensus sequences ware performed with Jalview (Waterhouse *et al*, 2009). Amino acids are coloured according to clustal colour scheme. The protein identifiers for the proteins used in the alignment are the following: Q7Z333 (*H. sapiens*), Q92355 (*S. pombe*), XP\_001644478.1 (*Vanderwaltozyma polyspora*), SMN21961.1 (*Kazachstania saulgeensis*), SCV99407.1 (*Lachancea fermentati*), CCK69072.1 (*Kazachstania naganishii*), XP\_004181671.1 (*Tetrapisispora blattae*), XP\_003955130.1 (*Kazachstania Africana*), XP\_003672383.1 (*Naumovozyma dairenensis*), Q00416 (*S. cerevisiae*), XP\_003680903.1 (*Torulaspora delbrueckii*), CDF91445.1 (*Zygosaccharomyces bailii*) and GAV52597.1 (*Zygosaccharomyces rouxii*). The NIM is indicated by a red box.

n. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae T. delbrueckii Z. bailii Z. rouxii	2495 L DSG FAKTSVAA - SLYHT PS - DSKEIT - LTVTSKDPERP VHD- 1675 - ANSE SKEIRQ - RS 1894 K - RKY 1895 - PAISESLSMQ PQKK KDI PI I EI EL AEGEEKRLKREEKQRKKEKKE RDK - K - EN - KEKKEK K 1877 - YVEKR - RGKRKH - PR - NONSQLKSEAST KDSN - NAKNLSTD - KAG RSD PE KRE 1862 L - QS - DAK PD - VVT - QKEKGPE - GEQ - SLE - QKSKKLKKSK K 1879 SDDMF PE PK KFAKSENNEK I DRRESKD SGVT KE - AVEKKEKKEK RDK - KRI KEKKDKKOKNKKEK 1870 - KREFQEDSSTFE PARKRAKDVQ - HD - SALKKVKSKKDKKDK - KD - KKDC KEKKNKSVK 1863 - PLK - RKDLD - E - PLSKNVKKAKKEK RDK - T - VSEDKQKKONKTER DR 1896 - QG - PD - E - VKSNKDT KRR V - VDEGEE - ADKAVKKK K K 1860 - VK	2534 1687 1913 1924 1897 1928 1924 1928 1924 1928 1865 1869 1870
Consensus	LD++F+EKRK+ASSSLS+EP++KRSKDI++KP+ELAEGEEKRLK++EK+VKSEKKEKRDKH+ESEDNK+K+VSEGKEKKDKKVKKKKK	
H. sapiens S. pombe	2535 Q LQ DPRLLKRM <mark>G</mark>   EVKGG   FLWDPQPSSPQHPGATPPTGEPGFPV VHQDLSH   QQPAAVVAAL	2597
V. polyspora K. saulgeensis	1938 SSIF <mark>G</mark> APN-LSEN <mark>G</mark> KPDDVSSSKKSKLASST <mark>KKK</mark> SSIFGGPSLAKEISKTM <mark>PY</mark> IKDTSNNTEVKNSLKNKD <mark>K</mark> HVRISEKIEIIP 1914 KERKDKKEKKDNNEK <mark>G</mark> FEKENKN <mark>G</mark> SSS <mark>GT<mark>KRKSS</mark>FFGPT<mark>KSPSP</mark>STAPNNNIYINDEKIKKQRKSSSLKDN<mark>RHL</mark>KFVDDIIAIP</mark>	2020 1997
L. fermentati K. naganishii T. blattae	1925 - K Y DNQNNESAN I KFNKGL VKGT KKKSS I FGAPSLA DO VSPVETVANQ RVL DDENTSKHVSFPDNL HGGV 1898 EKRESSKTAKKSDT LNTATGT KKKSS I FGAPSLEDS L PK - NLYINDEASKE KKKRERKAVKH I TFSETPTS I P 1929 EKARDOPSSTCANSSNTVASGT KKKSS I FGAPSL VTE - I LDTKTREDSNAT VPKKKK LDTHVSFSOTSKKY I P	1993 1969 2002
K. africana N. dairenensis	1925 - KSDDTT <mark>G</mark> DKENIEKQKKKVKITIN <mark>G</mark> EVS <mark>GTRKKSAIFGNTAFDT</mark> N TSTRGLYINDHNKKN NKQAKEKK <mark>R</mark> HISFSEHSTVIP 1908 KQKKDKKD - RKDKIDISKKKNDSLSVLSA <mark>GTKKKSSIFGGPSLST</mark> D - VPSTK <mark>GSVS</mark> ID EKSKKKRRKKKNCHVSFSDDITFIP	2005 1988
S. cerevisiae T. delbrueckii Z bailii	1929 EKKKEKK-SKADDKKKNNKKAESPSISSGIKKKSSIFGGISVPSA-VVPKIPDVDSNKKAAAVQKKKNNKHVCFSDDVSFIP 1866 HEETNASG-KALGRGNTPSAAGAVGVKKKKSSIFGGPSVEA-GKHANGPYIKEKGSSKQV-ETSTKATDNRHVLFSDNVQVIP 1870 HEDWDAQT-RGINNEKKRKETSSSALOGKKRKSSLFGGPTIANDIGNSGPYIKBKGSSKQV-BTNPKKSENTNPKTKHVGFSDNVLIP	2011 1946 1954
Z. rouxii	1871 NDNSDTTA-RKMTINEKKRKDEKTLAASOGKKKKSSIFSGPSIANEVTSSGPYIKEKGKSENK-KESLNPKKNRHVGFSDNLEIIP	1954
Consensus	+EKKD+KGDRK++++NEKK+KK+SS+G+SSGTKKKSSIFGGPSLAT++VIPT+GPYIKDKGKK++K+KKK+KKK+NRHVSFSDNV+IIP	
H. sapiens S. pombe	2598 SSHK <mark>PP</mark> VRTCQSKCD	2623
V. polyspora K. saulgeensis	2021 NINEQENLKIVDTNETNHENKIPD	2061 2084
L. termentati K. naganishii T. blattae	1994 NLSNTGSSGKAAM TPVIFESRRA	2035 2011 2045
K. africana N. dairenensis	2006 DS <mark>GE</mark> LKKLAPSS <mark>B I PVP</mark> - NKKASKKSYESS ATEEKK	2047 2025
S. cerevisiae T. delbrueckii Z bailii	2012 RNDEPEIKVTRSLSSVL-KEKQLGLKE-TR	2052 1972 1993
Z. rouxii	1955 R <mark>G</mark> NENEHADNTASPPTEQKENK-S- <mark>G</mark> RGSPPPQ	1992
Consensus	NSGE++SLPPS+S+PTP++SK+++KP+ER+DNKEDSNTKGSPPP++++++ISKSNTRETDTLNKEAKRSNVSASHANDLI+++D++SD	
H. sapiens S. pombe	2624 DPEEELCHRREARAESEGEQEKCGS.ETH.HTRNSRWDKRTLEQEDSS.SKKRK	2675
V. polyspora K. saulgeensis	2062 EDEDDYKLPTSVTPTEVDKNRVS	2129 2171
L. termentati K. naganishii T. blattae	2036   NSNDAFSSNIGH - L - SDGSTGAHNTT 2012   KDNDDYIPTIGPSSIER ANP - AI RDV KKSSG - TV GKTTKPTHESDNN	2062 2061 2095
K. africana N. dairenensis	2048 <mark>DSEDDYSPSLP</mark> STHVSERRV-TEEDQN <mark>PSG</mark> ALCDSKKAGEIVRANSALN <mark>PSG</mark> NVT- 2026 <mark>DANDDYIP</mark> SEVEKVVVTDAK- <mark>P</mark> TETERPKIKKQIELSKYNSKNAHS <mark>S</mark> TTEV <mark>P</mark> KERPTAPSAQ	2101 2086
S. cerevisiae T. delbrueckii Z bailii	2053 DDEDDYTPSISDSSLMKSEA-NGRNNRVASH-NQNFSASIYDDPQVS-QAKQTQ 1973 DDENDYVPRVEMKTDLRHQ-ESESVAD-EDDYHPADAP 1994 DDENDYTPRPDFFRIPP	2103 2009 2044
Z. rouxii	1993 EEENDYTPRPPEDTVLP GSK-KQRDA GQSFY-DD-YQP	2033
Consensus	DEDDYTPR++E++++E++++V+P++KAN+KTERDSNDKLSR+NGSKQ+E+SSY+S+++HES++N++SRL++RT+EV++++PSAPQTQ	
H. sapiens S. pombe	2676 LL	2677
V. polyspora K. saulgeensis	2130 NIISPOPMPFR-TNEIANSSHYNTN SVPSPNMSHQ QN MPYNNPGNNMNT 2172 ISYTNHEEPOSMSASFKDRITPISRYGTSSKESRFESTPSPGSTLPYKSR EYSNNFAQHNG QPSGFHGY-YNN 2022	2177 2243
K. naganishii T. blattae	2062 KPKTIPKTTDKDLASFSNENIITLPONDEYIPKPAVVVQTDKKTN	2123 2157
K. africana N. dairenensis	2102 I - YESR - NTRNTGHT	2159 2142
T. delbrueckii Z. bailii		2162
L. Duilli	2045	2152 2016 2082
Z. rouxii	2045	2152 2016 2082 2072
Z. rouxii Consensus	2045	2152 2016 2082 2072
Z. rouxii Consensus H. sapiens S. pombe	2045	2152 2016 2082 2072
Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati	2045	2152 2016 2082 2072 2215 2331 2125
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae	2045 - LYGYQV - AIPPPSTH - YRPG - GNGQQHQL - Q - Y - YPTN - GF YTE 2034 - ISSYSR - DGIRYPEN - FSTG - NDSQRPDI - NSEQ - RLPH - GS	22152 2016 2082 2072 2072 2215 2331 2125 2152 2201 2201
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae	2045 LY GYQV - AI PPPSTH	22152 2016 2082 2072 2072 2072 2331 2125 2152 2201 2200 2184 2187
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae T. delbrueckii Z. bailii Z. rouxii	2045	2152 2016 2082 2072 2072 2215 2331 2152 2201 2200 2184 2187 2031 2110 2088
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeonsis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae T. delbrueckii Z. bailii Z. rouxii	2045	22152 2016 2082 2072 2072 2331 2125 2152 2201 2200 2184 2187 2031 2110 2088
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae T. delbrueckii Z. rouxii Consensus	2045 LY GY QV - A I PPP STH	22152 20166 2082 2072 2072 2231 2152 2252 2252 2201 2200 2184 2187 2031 2187 2031 2187 2031 2088
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cervisiae T. delbrueckii Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora	2045 LY GY QV - A I PPPSTH YRPG GNGQQHQL - Q - Y YPPTN - GF - YTE 2034 ISSYSR - DG I REY EN	22152 2016 2082 2072 2072 2072 22072 22072 22072 22072 22072 2152 22072 2152 22072 2152 22072 2152 22072 2088 2088 2088 2267
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. dairenensis S. cerevisiae T. delbrueckii Z. bailii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentatii K. farenzietii	2045 LY GY QV . A I PPP STH	22152 2016 2082 2072 2072 2072 2331 2125 2257 2201 2200 2184 2187 2038 2187 2088 2267 2088
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae S. cerevisiae T. delbrueckii Z. touxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana	2045 LY GY QV - A I PPP STH YR PG	22152 20016 2082 2072 2072 2031 2152 2200 2184 22000 2184 22001 2088 22267 2384 2110 2088 22267 2384 2174 2174 2215
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana N. dairenensis S. cerevisiae Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana S. cerevisiae	2045 LY GY QY A I PEPSTH	22152 20016 2082 2072 20331 2152 2200 2184 2200 2184 2200 2184 2031 2031 2038 2038 22384 22384 22384 22384 22382 22382 22384 22382 22382 22384 22392 22384 22392 22384 22392 22384 22392 22384 22394 2
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati T. blattae K. africana N. dairenensis S. cerevisiae Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. africana S. cerevisiae T. delbrueckii Z. bailii T. blattae	2045 LYG YQY A I PPB STH YR G NB Q QHQL Q - Y Y PF TN	22152 20072 200 200
Z. rouxii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. dairenensis S. cerevisiae T. delbrueckii Z. rouxii Consensus H. sapiens S. pombe V. polyspora K. saulgeensis L. fermentati K. naganishii T. blattae K. faricana N. dairenensis S. cerevisiae T. delbrueckii Z. rouxii	2045	22152 2016 2082 2072 2072 2031 2152 2201 2152 2201 2187 2232 2152 2200 2184 2172 2088 22867 22384 2171 22384 2187 2235 22384 2187 2235 22382 22382 22382 22384 2171 2125 22384 2171 2172 2088 2215 2215 2215 2215 2215 2215 2215 22

Figure EV1.



## Comparison of CSP in Nrd1 upon addition of Sen1 NIM and Trf4 NIM

**Figure EV2. Quantification of chemical shift perturbations (CSP) of Nrd1 CID upon binding to the Trf4 NIM (in red) and Sen1 NIM peptides (in blue).** The combined chemical shift perturbations are plotted versus the amino acid residue number. The black boxes indicate α-helices of Nrd1 CID.

#### Figure EV3. The interaction of Sen1 with Nrd1 and Nab3 is not essential for non-coding transcription termination (related to Fig 3).

- A Growth tests of the *sen1* $\Delta$ *NIM* mutant in either a wt or a  $\Delta$ *rrp*6 background.
- B–D Metagene analyses of RNAseq experiments performed in a  $\Delta rrp6$  background in the presence of the wt or the  $\Delta NIM$  version of Sen1. The profile corresponds to the median coverage (reads per 10<sup>7</sup> reads mapping at each genomic position) from 0.5 kb upstream to 0.5 kb downstream of the annotated transcription termination site (TTS) of protein-coding genes (B) and CUTs (D) or the 3' end of the mature snoRNAs (C). Experiments were performed in biological duplicates.
- E Deletion of Sen1 C-terminal domain completely abolishes the interaction of Sen1 with Nrd1. Top: scheme of proteins analysed in these experiments. Bottom: CoIP assays using Nrd1-TAP as the bait. Representative gel of one out of two independent experiments. Antibodies used for protein detection are detailed in Appendix Table S3.
- F Deletion of Sen1 Cter provokes minor transcription termination defects at typical NNS-dependent non-coding genes. Northern blot assays performed in a Δrrp6 background. Results correspond to one out of two independent biological replicates. The CAR2 and PHO5 RNAs are detected as a loading controls. Probes used for RNA detection are described in Appendix Table S6.





### Figure EV4. Quantification of transcription termination defects detected in the different Sen1 mutants at individual ncRNAs (related to Fig 4).

- A Plot representing the RT index of the indicated mutants relative to the wt version of Sen1 for each CUT. RT indexes were calculated as in Fig 4. CUTs are ranked in the decreasing order according to corresponding value in *sen1*Δ*Nter*. Data from *sen1*Δ*NIM* and *sen1*Δ*Nter*Δ*NIM* are shown by vertical bars while data from *sen1*Δ*Nter* are represented by a line.
- B-D Heatmaps representing the log2 of the fold change (FC) of the RNAPII signal in the indicated mutants relative to the wt at CUTs.
- E Graph representing the RT index, calculated as in Fig 4, of the indicated mutants relative to the wt for each snoRNA analysed.
- F-H Heatmaps showing the log2 FC of the signal in the indicated mutants relative to the wt at snoRNAs.



Figure EV4.



#### Figure EV5. Distribution of Sen1 signal at different types of RNAs (related to Fig 5).

- A, B Screenshots of particular genomic regions to illustrate the differences in the signals obtained with the untagged control, relative to a strain expressing the HTPtagged version of Sen1 allowing its purification (see Methods for details). The signal of the control is multiplied by 10 to allow a better comparison with that of Sen1-HTP. Values correspond to the data before normalization to total number of deletions. The data from one of the biological replicates of Sen1 wt is shown as Sen1-HTP.
- C Heatmap visualization of the distribution of Sen1 wt at mRNAs.
- D Metagene analysis of the distribution of the different Sen1 variants at CUTs as in Fig 6J but using the TTS as reference point.