

## SUPPLEMENTARY MATERIAL

### Supplementary Materials and Methods

#### Yeast strains, plasmids and media

Full-length *AtXRN2* cDNA (p1486, {Kastenmayer, 2000 #59}) and a fragment encoding first 546 amino acids of *AtXRN2* had been inserted into a p424TEF vector {Mumberg, 1995 #195} between sites *SpeI*-*EcoRI* and introduced into the yeast *xrn1Δ* strain (yRP884) as described {Gietz, 1992 #197}. Strains were grown at 30°C in synthetic complete (SC, 0.67% yeast nitrogen base, 2% galactose, supplemented with required amount of amino acids and nucleotide bases) or in SC media without tryptophan.

#### Southern blot analysis

The number of T-DNA insertions in the genome was determined by Southern blot analysis using genomic DNA isolated from 2-week-old plants with Plant DNAzol Reagent (Invitrogen). Digested DNA (8 μg) was separated on a 0.8% agarose gels, depurinated in 0.25 M HCl, and blotted onto Hybond-N<sup>+</sup> membranes (GE Healthcare) by capillary transfer. DNA probes were labelled with α-<sup>32</sup>P by random hexamer priming (GE Healthcare) using gel-purified PCR products as templates.

#### Exoribonuclease activity in yeast

Analysis of reporter poly(G)-containing *PGK1* and *MFA2* mRNAs expressed in wild-type, *xrn1Δ*, *xrn1Δ* + [p1486/*AtXRN2*] and *xrn1Δ* + [p424TEF/5'*AtXRN2*] was performed as described {Kastenmayer, 2000 #59}.

#### Microarray analysis and half-life measurements

Genome-wide expression and mRNA stability profiles in wild-type and *xrn2-3* plants were performed using Affymetrix ATH1 Gene Chips on total RNA from seedlings treated with cordycepin. Time-course experiments were carried out as described {Souret, 2004 #109}. 2-week-old seedlings were transferred to flasks containing a buffer (1mM Pipes pH 6.25, 1mM sodium citrate, 1mM KCl, 15mM sucrose) and incubated for 30 min. Cordycepin was added (150mg/l) and incubation was continued for 120 min. Total RNA samples corresponding to 0 and 120 minute time points after transcriptional inhibition were extracted using RNeasy Plant Mini Kit (Qiagen). Biotin-labeled target RNA was prepared from 15 μg of total RNA, fragmented, and hybridized according to manufacturer's instructions (Affymetrix). Samples were stained with streptavidin-phycoerythrin conjugate using a GeneChip Fluidics Station 400 and probe arrays were scanned with Agilent GeneArray Scanner. Obtained data were analyzed using Affymetrix GeneChip Operating Software.

#### Supplementary Table S1: Oligonucleotides used in this study.

Oligo	Region	Sequence
p1	5'ETS-1	5'-CCTAGGCGGATCCATGCTTTCCAAC
p2	5'ETS-2	5'-CATCGATCACGGCAATCCCCGC
p3	5'ITS1	5'-GGTCGTTCTGTTTTGGACAGGTATC
p4	3'ITS1 (27SA)	5'-CGTTTTAGACTTCAGTTCGCAG
p5	5'ITS2	5'-GCAAAGGATGGTGAGGGACGACG
p6	3'ETS	5'-CGTTAAGGAGCTGTTGCTTTGTTAGTGTAG
p7	5.8S	5'-GATTCTGCAATTCACACCAAGTATC
p8	25S-5'	5'-CTCCGCTTATTGATATGCTTAAAC
p9	25S-3'	5'-GATGACCAATTGTGCGAATCAACGG
p10	18S-5'	5'-CATATGACTACTGGCAGGATCAACC
p11	18S-3'	5'-GATCCTTCCGCAGGTTACCTACG
p12	5S	5'-GCACGCTTAACTGCGGAGTTCTG
p13	7SL	5'-ACTGGGCAGCCAGAAACATGC
p15	XRN2-5'F	5'-ATGGGAGTCCGTCGTTCTACAG

p16	XRN2-5'R	5'-TGTTCTCCATTCCTGACCTGAACC
p17	XRN2-3'F	5'-CATGCTCTCCCAGAGTGCTATAGG
p18	XRN2-3'R	5'-ATGAGATGCCTTCCCAAGCCTACC
p19	XRN3F	5'-AACGCAGCACATCGTCTTGTTTCC
p20	XRN3R	5'-CAATATCGATGTCTAGGATTCC
p21	eIF-4E-F	5'-TCATGAGAGCTTTGATGCCATGG
p22	eIF-4E-R	5'-GATGAGAACACGGGAGGAACCAG
p23	35S-53P	5'-GTTCCAACACTCTACCGAAGTAC
p24	ETSa-280	5'-GTGTAAACCAAACCTCAACAATTCC
p25	5'ETSas3	5'-GGAGAATCCATGTCAGCCCATG
p26	5'ETS-seq2	5'-CGAGTTTTGTTGATGTGTTTCCGAG
p27	18S-56	5'-GTCTGAATTCGTTCACTACTACAC
p28	RT-PCR ETS-seq	5'-CATGGGCTGACATGGATTCTCC
p29	RT-PCR 3-ETS	5'-GGAGTGATTTAGGGGAGGGTCG
p30	RT-PCR 25S-3'	5'-GATCCTTCGATGTCGGCTCTTCC
p31	cRT-PCR-ETS-P	5'-CCTAGGCGGATCCATGCTTTCCAAC
p32	cRT-PCR-5ETSa3	5'-GGAGAATCCATGTCAGCCCATG
p33	cRT-PCR-ETS-s2	5'-CGAGTTTTGTTGATGTGTTTCCGAG
p34	5'ETS	5'-CCGGACGGTCGGTCATTCCTCGTG
p35	ITS2	5'-CGTCGTCCTCACCATCCTTTGC
p36	25S	5'-CGCCCGATTGGGGCTGCATTCC
p37	U14	5'-CATTA ACTCTCAAGCCTGGCGAAAG
p38	U4	5'-TTGAAATAGTTTTCAACCAGC
p39	U3pe b	5'-TCAAGGAAACAGAGGTACGAGC
p40	snoR10	5'-GTCATTCTCATAACAGTAAACTG
p41	snoR30	5'-GATTCTGCCAGCAATTCTCTCAGC
p42	ITS1	5'-CCACGGATCCGGCGGGCAAGG
p43	ITS1	5'-ATGCCAGCCGTTTCGTTTGCATG
p44	3'ITS2	5'-GGACTTTGGGTCATCTACAGCTTC
X3-1	X3Hind1s	5'-ACTGATAAGCTTGATCTGCTAAAGATGCATCGG
X3-2	X3Xba_a1	5'-TTGACTTCTAGAGAATCCTCATCTCAAGCTCCAG
X3-3	X3Kpn_s1	5'-TTGACTGGTACCGATCTGCTAAAGATGCATCGG
X3-4	X3Xho_a1	5'-TTGACTCTCGAGGAATCCTCATCTCAAGCTCCAG
X3-5	X3Xho22a	5'-TTGACTCTCGAGGCCTTGTCACCAGGTGGAGCC
X3-6	X3Kpn22s	5'-TTGACTGGTACCCTCATCGTCTAGCTGAACTCGTC
X3-7	X3Hind2s	5'-ACTGATAAGCTTCTCATCGTCTAGCTGAACTCGTC
X3-8	X3Xba22a	5'-TTGACTTCTAGAGCCTTGTCACCAGGTGGAGCC
pROK_LB2		5'-TTGCTGCAACTCTCTCAGGGCCAGG
SALK_258		5'-GTAAGTCATCTCGTATCCGAGG
X2endoAS		5'-TGAGGATGACCAGAAACTGACC
SALK_657		5'-AGGAAACTACACAGTAGAAACC
X3endoAS		5'-GGTAAAACGACGGTACACCCATTC
AtXrnl		5'-GCGGGTACCATGGGAGTTCCGTCGTTCTAC
AtXrnR		5'-GCGCGGCCGCTCAAGCTGTTTTGGGAGGAG
ETS_a		5'-CATCGATCACGGCAATTCCTCCG
ETS-seq2		5'-CGAGTTTTGTTGATGTGTTTCCGAG
ETS_S		5'-GTA CTTCGGTAGAGTAGTTGGAAC
18S900		5'-CATAAATCCAAGAATTCACCTCTG
15s	eIF-4E	5'-TCATGAGAGCTTTGATGCCATGG
15as	eIF-4E	5'-GATGAGAACACGGGAGGAACCAG
NPTs	NPTII	5'-CTCCGGCCGCTTGGGTGGAGAGGC
NPTas	NPTII	5'-CAAGAAGGCGATAGAAGGCGATGCGC
AP2-s1	AP2	5'-GCTGCAGCATCATCAGGATTCTC
AP2-as	AP2	5'-CAAGAAGGTCTCATGAGAGG
ARF8-s1	ARF8	5'-CTTAGATCAGGCTGGCAGCTTG

ARF8-as	ARF8	5'-CTAGAGATGGGTCGGGTTTTGC
2995ARFs	ARF10	5'-ATATCTAGAGCAGGAATACAGGGAGCCAG
2995ARFa	ARF10	5'-TCATCCAAGCTTAGCCTGATAATGATGATGC
SPL10-s1	SPL10	5'-CTACAGTGCTCTCTCTCTCTG
SPL10-as	SPL10	5'-CAGATGAAATGACTAGGGAAAG
ACT1	yeast <i>ACT1</i>	5'-TCTTGTCTACCGACGATAGATGGGAAGACAGCA
PGK1pG	<i>PGK1</i> +poly(G)	5'-AATTGATCTATCGAGGAATTCC
MFA2pG	<i>ACT1</i> +poly(G)	5'-ATATTGATTAGATCAGGAATTCC

## Supplementary Results

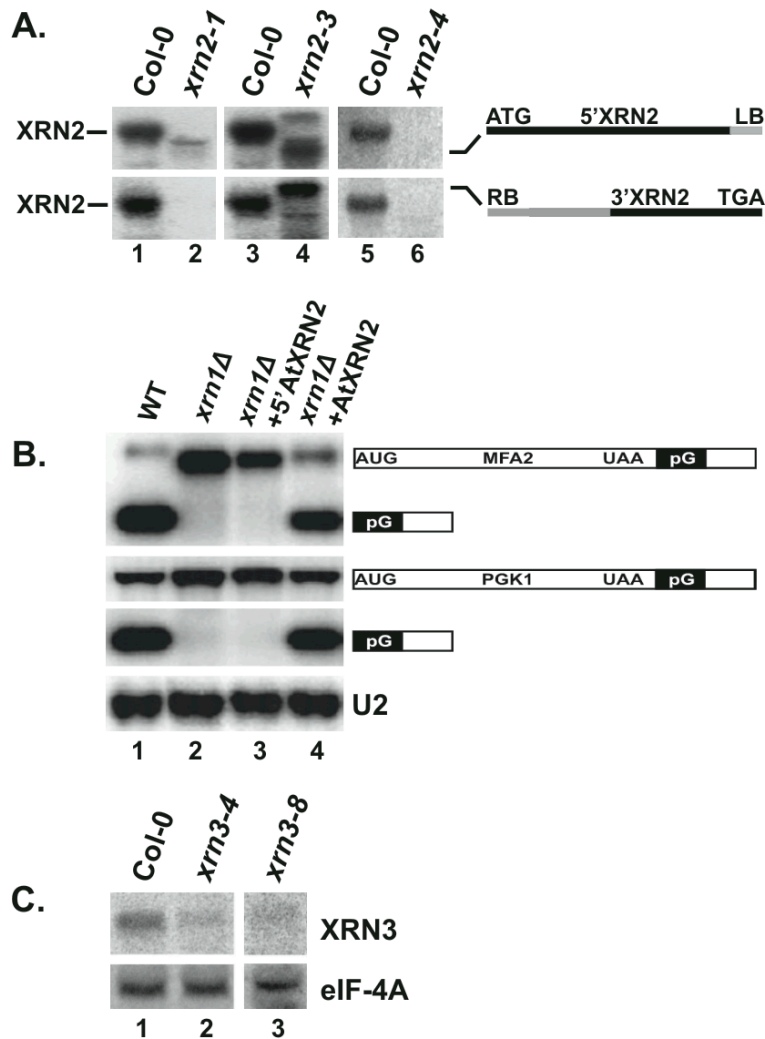
### *Isolation of mutant lines in AtXRN2 and AtXRN3 genes*

Three *xrn2* mutant lines were tested by PCR screening and Southern hybridization. Only one of them, *xrn2-3*, had a single T-DNA insert disrupting the 12<sup>th</sup> exon in *AtXRN2*; the other two, *xrn2-1* and *xrn2-4*, had further inserts at unknown locations in addition to inserts in the 12<sup>th</sup> exon and 11<sup>th</sup> intron, respectively (data not shown). Total RNA was isolated from *xrn2-1*, *xrn2-3* and *xrn2-4* seedlings to test for the expression of *AtXRN2* mRNA using probes directed against its different regions (Supplementary Figure S1A). The full-length mRNA was present only in wild-type plants (Col-0 ecotype), however, a considerable amount of shorter and longer RNAs containing *AtXRN2* sequences was detected in *xrn2-3* and some residual mRNA in *xrn2-1*, but none in *xrn2-4* mutants (Supplementary Figure S1A). The identity of the two transcripts in the *xrn2-3* line was confirmed by hybridization with a probe against *NPT2* from the T-DNA insert and by cloning and sequencing cDNAs corresponding to these RNAs. The truncated RNA contains the 5' end of the *AtXRN2* transcript that extends to the insert and is fused to the sequence originating from the T-DNA. The longer RNA initiates within the T-DNA and continues into the 3' end of *AtXRN2*. These RNAs could potentially encode proteins that retain some exonucleolytic activity. This is, however, unlikely for the 3'-*AtXRN2* transcript, since it contains hardly any of the sequences that encode conserved domains of the Xrn2/Rat1 family {Kastenmayer, 2000 #59}.

To assess the enzymatic activity conferred by the 5'-truncated *AtXRN2*, a complementation test was performed *in vivo* {Souret, 2004 #109}. This was based on the fact that active *AtXRN* proteins, when expressed in yeast cells, are able to replace endogenous Xrn1p and carry out its function in mRNA degradation. cDNA corresponding to the 5'-*AtXRN2* fragment present in *xrn2-3* was cloned into a high-copy number yeast vector and introduced into the *xrn1Δ* strain expressing reporter *PGK1* and *MFA2* transcripts with poly(G) tracts that block the progression of 5'→3' exonucleases, resulting in the appearance of specific products. In wild-type yeast and in cells expressing full-length *AtXRN2*, degradation intermediates derived from poly(G)-containing mRNAs were clearly detectable, indicating 5'→3' exonucleolytic activity (Supplementary Figure S1B, lanes 1 and 4). In contrast, in the absence of any XRN protein, as well as when only the 5'-truncated *AtXRN2* was produced, there was no similar accumulation, showing that the 5' portion of *AtXRN2* expressed in *xrn2-3* does not possess enzymatic activity (Supplementary Figure S1B, lanes 2 and 3).

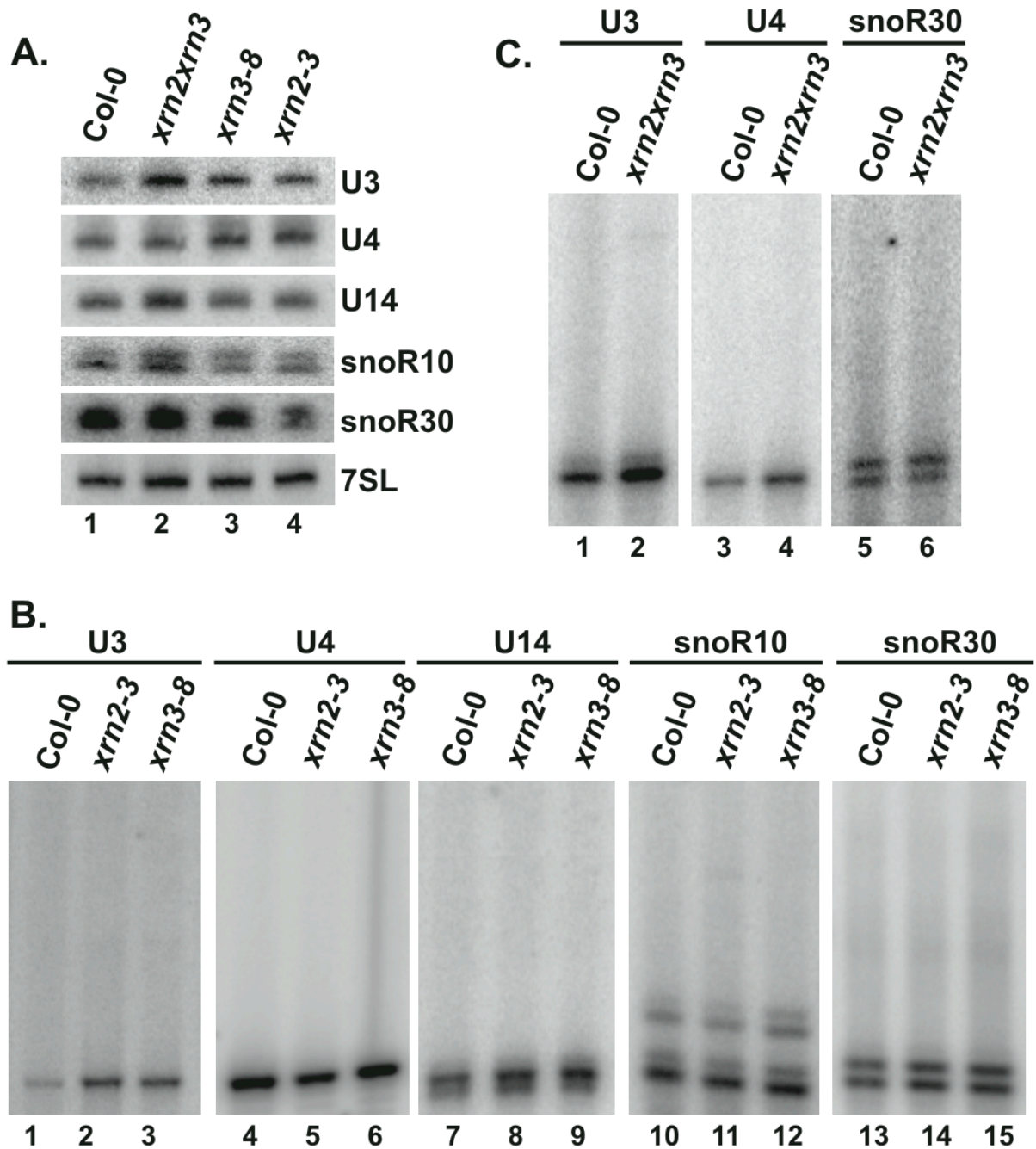
During selection of *xrn3* RNAi transformants, following preliminary screening five *xrn3* lines RNAi were tested for the reduction of *AtXRN3* mRNA by northern blot and RT-PCR and two transgenic lines exhibited 52% and 43% of mRNA depletion, respectively (Supplementary Figure S1C and data not shown). Silencing effects in *xrn3* lines were stably inherited and actually improved in the next generations.

## Supplementary Figures

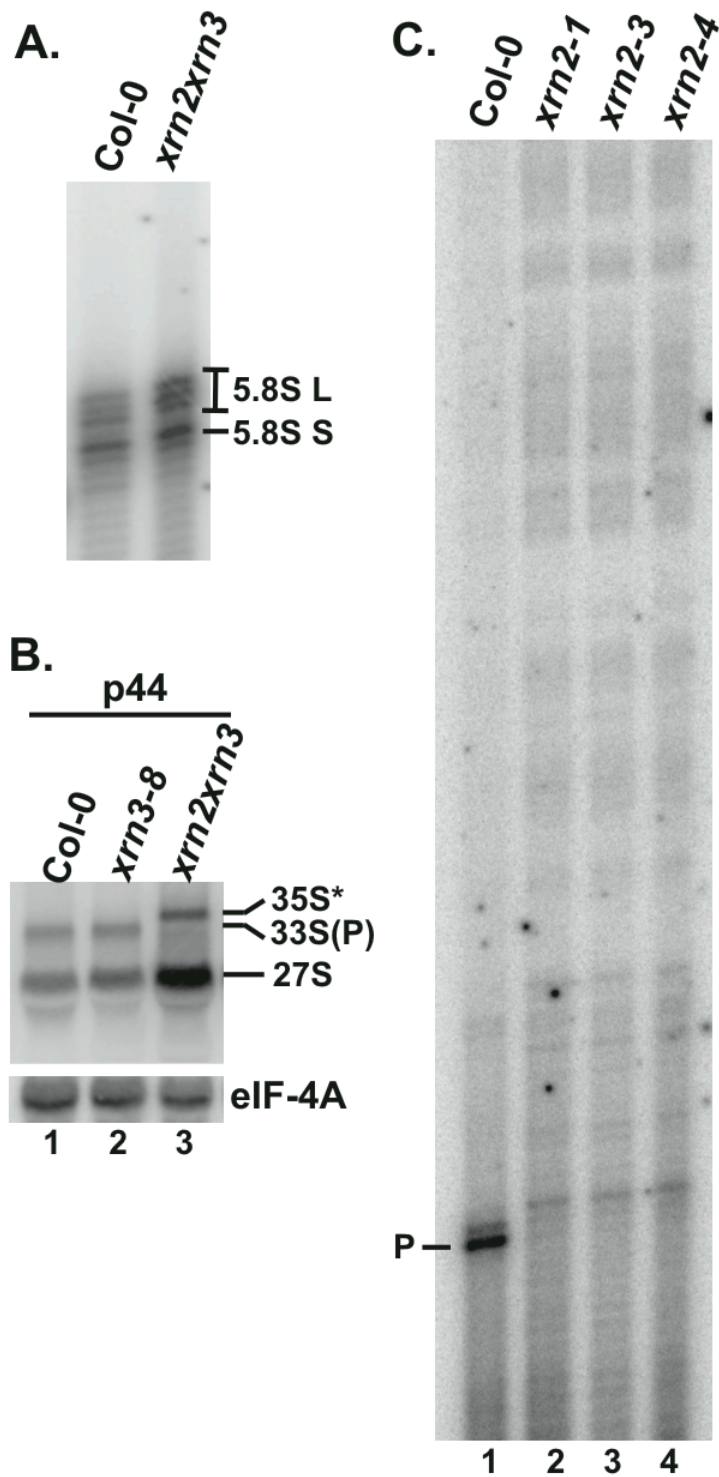


**Supplementary Figure S1.** (A) Northern analysis of *AtXRN2* mRNA in wild-type and mutant plants. PCR products covering the 5' (0.7 kb, top panel) or 3' (1.5 kb, bottom panel) portion of *AtXRN2* were used as probes. Schematic representation on the right shows *AtXRN2* transcripts detected in the *xrn2-3* mutant: the shorter RNA contains residues 1–1640 of *AtXRN2* (546 amino acids) and enters the T-DNA left border; the longer transcript initiates within the T-DNA and covers residues 1663–3039 of *AtXRN2* (458 amino acids). (B) 5'→3' exoribonucleolytic activity in yeast strains expressing *MFA2* (top panel) and *PGK1* (bottom panel) reporters containing poly(G) tracts analyzed by northern blot. Strains used are wild-type, *xrn1Δ* and *xrn1Δ* expressing full-length or truncated *AtXRN2*, respectively. Structures of detected poly(G)-containing RNAs are shown on the right. (C) Northern analysis of *AtXRN3* mRNA in *xrn3* RNAi-silenced mutants using probes specific for *AtXRN3* and a loading control, *eIF-4A*. The level of *AtXRN3* silencing is 52% and 43% in *xrn3-4* and *xrn3-8*, respectively.





**Supplementary Figure S2.** AtXRN2 and AtXRN3 do not participate in snoRNA 5' processing. (A) Northern analysis of total RNA extracted from 14-day old seedlings of wild-type and mutants: *xrn2-3*, *xrn3-8* and *xrn2-1 xrn3-3*. Oligonucleotide probes specific for snoRNAs and for 7SL RNA (loading control) were used for hybridizations. (B-C) Primer extension detecting snoRNA 5' ends performed on total RNA from wild-type (*Col-0*), *xrn2-3* and *xrn3-8* (B) and from *Col-0* and *xrn2-1 xrn3-3* (C) plants.



### Supplementary Figure S3

(A) Primer extension for 5' ends of mature 5.8S rRNA species, 5.8S<sub>S</sub> and 5.8S<sub>L</sub>, in wild-type (Col-0) and *xrn2-1 xrn3-3* lines using primer *p7*.

(B) Northern analysis for wild-type (Col-0), *xrn3-8* and *xrn2-1 xrn3-3* lines using probe *p44* located upstream of the mature 25S rRNA 5' end. *eIF-4A* mRNA was used as a loading control.

(C) Primer extension for cleavage at site P and upstream of site P in wild-type (Col-0) and *xrn2* plants using primer *p23*.



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Rat1      1  MGVPSEFRWLSRKYFKILSPVLEEQFQIVDGG--VILFLDYASANPNG-FLDNLVLDMNGIVHPCSHPENKFPFETEDEMEL
Atxrn2   2  MGVPSEFYRWLIQRYPILTQEVIEEEPLEVNGGGVTFPIDSSKPNPNGYEYDNLVLDMNGIHPCHFHPEDKPSPTTFTEVF
Atxrn3   3  MGVPSEFYRWLAEKYPLLVDVIEEEVVEIEG--IKIPVDTSKPNPNLEEDNLVLDMNGIHPCHFHPEDRPSPTFEVF
Atxrn4   4  MGVEAFYRWLADRYPKSISDVVEEETDGGRC-DLIPVDITRPNPNGFEDNLVLDMNGIHPCHFHPGKPAFAIYDDVF

Rat1     78  LAVFEYTNRVLNARPRKVLVMAVDGVAPRAKMNQQRARRFRASARDAQIENEAREEELMRQREVEVEIIDDVA RNKKTWDS
Atxrn2  82  QCMFDYIDRLFVMVRPRKLLFMAIDGVAPRAKMNQQRARRFRAAKDAEAAAEAEELQREEFEREGKLPKPV-DSQVFDS
Atxrn3  81  QCMFDYIDRLFVMVRPRKLLYMAIDGVAPRAKMNQQRARRFRASAKDASDAEAEERLREEFEREGRLLPKPV-DSQVFDS
Atxrn4  83  KSMFEYIDHLEFLVLRPRKILYLAIDGVAPRAKMNQQRARRFRAAKDAEAEAEERLRKDFEMEGQTLISAKE-KAETCDS

Rat1    158  NAITPGTTFMDKIAAALRYWTAFLKLATDPGWKNLQVIISDATTVPGEGEHKIMNFIRSDRADPEYNPNTTHCIYGLDADLI
Atxrn2  161  NVITPGTEFMATLSFALRYIYHVRLLSDPGWKNIKVILSDANVPGEGEHKIMSYIRCNKNHFGYNPNTTHCLYGLDADLI
Atxrn3  160  NVITPGTEFMGVLSIALQYYVHLRLNHDVGVKNIKVILSDANVPGEGEHKIMSYIRLQRLNPGFDPNTRHCLYGLDADLI
Atxrn4  162  NVITPGTTFMAILSVALQYYIQSRLNHNHGWRYVKVILSDSNVPGEGEHKIMSYIRLQRLNPGFDPNTRHCLYGLDADLI

Rat1    238  FVGLATHEPHFKILREDVFAQDNRKRNNIKDTINM-----TFEEKQFLQKQNSEQFFLWVHINVLREYLSAE
Atxrn2  241  MSLATHEIHFHILREVVFFPGEEGKFLCGQEGHRRADCEGKIIRKRTGEMLDNTEADVVKKPYEFVNIWI LREYLEHD
Atxrn3  240  MGLATHEVHFSILREVYTPGQQRCEFLCGQMGHFASNCEGKPKKRAEESDEKGDGDFVKKPYFLHIWV LREYLELE
Atxrn4  242  MSLATHEVHFSILREVITYPGQQKCFVCGQTHGFASDCPGK----SGSNNAADIPHKKKYQFLNIWV LREYLQYE

Rat1    305  LWVFGLEPFTFDLERAIDWVEMCFEGNDLFLPHLFCIDVRENSDILLDIWKVVLVPLKLTVMTCDEVLNIPSVETLLQHL
Atxrn2  321  MQIPGA--KKNLDRLLIDDFIFICFFVGNDFLPHMPTLEIREGATELLMSVYKKNFRSAKYLTDSSKLNLRNVERFIKAV
Atxrn3  320  MRLENPFEITDLERIVDDFIFICFFVGNDFLPHMPTLEIREGAINLLMAVYKKEFRSFDGYLTDGCKPNLKRVEQFIQAV
Atxrn4  317  LAIEDPPFMNFERIIDDFVFLCFFVGNDFLPHMPTLEIREGAINLLMHVYRKEFTAMGGYLTDSGEVLLDRVHFHQAV

Rat1    385  GSREGDIFKTHIQEARKKEAFERRKAKQNMMSKGOHRHPTVA-----TEQIQMYDTQGNLAKGSWNLTTSDMVR
Atxrn2  399  GMYENQIFQKRAQVQRQSERFERRDKARDKARDNARDNAQASRQF-----SGKLVQLDSLDEVSDSLHSSSRKYLRL
Atxrn3  400  GSFEEDKIFQKRAMQHQRQAERVKRDKAGKATKRMDDEAPTVPDLVPPVAFSGSRLASAPTPSPFQNDGRSAHQKVRRL
Atxrn4  397  AVNEDKIFQKTRIKQSMDNNEEMKQSRRRDPSEVPPEPI-----

Rat1    454  IKKELMLANEGNFEAIAKVKQSSKNNELMKDISKEEIDDVAVSKANKNFNLAEVMKQKIINKKRRLEKDNEEEI AKDS
Atxrn2  471  LSLDDNIGVNVETENSIAEELDNEEDLKFKLLKLLRDKGDFERSNG-----
Atxrn3  480  LSPGSSVGAIVDVENSLSDRENKEELKTKLKEILREKSDAFNSDIT-----
Atxrn4  437  -----

Rat1    534  KVKTEKAESECDLDAEIKDEIVADVNDRENSETTEVSRDSVHSTVNVSEGPKNVDFDTDEFVKIFEPGYHERYYTAKF
Atxrn2  520  -----EQDKVKLNKVGWRERYEKEF
Atxrn3  529  -----EEDKVKLQPGWRERYEKEF
Atxrn4  437  -----DDKIKLGEFPGYKERYAEKF

Rat1    614  HV-TPQDIEQLRKDMVKCIEGVAWVLMYYYQGCASWNNWFYPYHYAPLAFTHFGFSSHLEIKFEESTPFLPYEQIMSVLPA
Atxrn2  541  AAKSVEMEQLRRDVLKYTEGLCWMHYYYHGVCSSWNNWFYPYHYAPFASDLKLEKLDIKFELGSPFKPFNLAVLPS
Atxrn3  550  SVVTPPEEMERVKDVLKYTEGLCWMHYYMEGVCSSWQWFYPYHYAPFASDLKLDLGMIDIKFELGTPFKPFNLVGFPA
Atxrn4  457  STTNPEETEQLKQDMVLKYVEGLCWVCRYYYQGCSSWQWFYPYHYAPFASDLKLNLPDLEITFFIIGEPFKPFDLIMGLTLP

Rat1    693  ASGHALPKIFRSLMSEPDSEIIDFYPEEFPIDMNGKMSWQGIALLPFFIDQDRLLTAVRAQYPLSDAERARNIRGEPVL
Atxrn2  621  ASHALPERCYRSLMTPNSPIADFYPADFEIDMNGKRYSWQGISKLPFVEEKRLLEAAQVEKSLTNEEIRRNALFDML
Atxrn3  630  ASSHALPERYRSLMTPNSPIIDFYPTDFEVDMMNGKRFVWQGIKLPFIDERRLLEAVEVEFTLTDDEKRRNSRMCMDL
Atxrn4  537  ASSNALPEGEYRSLMTPNSPIIKFYPADFELDMNGKRFVWQGIKLPFIEKRLLEAVEVEFTLTDDEKRRNSVMDLL

Rat1    773  LISNKNANYRFSKLYSKENNNNNVVVKFQ--HFKSGLSGIVSKDVEGFELNGKICPIQGGSLPNISITLLIKMSYRI
Atxrn2  701  FVVASHPLGELRSLNSRTNLSNEERATIEKIDFGLSDGNGYIASGSDSQFSCFCSTVEGMEVLTNQVICATYKL
Atxrn3  710  FIATSHRLAELVFLDNHCRQLSARERVDFKVKIKKLSDGNGYITPSETHPPEVRSPEMGMEDILTNOVICIYRL
Atxrn4  617  YVHPAHFLGQRLQYYHFYQHMPPHCLPVM--IDFNSSQGMNGFLWFSERNGFQTRVDSVNGLPCIEQNRALNVTYLC

Rat1    851  IPL-PSRNKSIILNCFIPSEPVLTAYDLDSIMYKNNQNYSRWVNFN-----
Atxrn2  781  FEDIRGSEITHQIFRLALPKKTSISLDLSSGGLLWHDGDKRAPPKV-----IKIKFYNFESSISGRLGKASHR
Atxrn3  790  PDA--HEHITRPPPGVIFPKKTVIDGLKPGPALWHEDNGRPEMHNHGMHNNHGMHNNQGRQNPFGSVSGRHGLGNAHR
Atxrn4  695  PAK--HSHISEPFGAIIIDKILTSVDIKPFPLWHEDNSNRROARD-----RPQVVGATAGPSLGEAAHR

Rat1    898  -----DLKQNI VVPGPKGI-----TQYKPRTEGYRAFFYFAELSRNN
Atxrn2  852  LVLQINIAQPIYMNINSEPALCPNTVFQNERVPKKIHTFKDNGIQWISPPFSQ----ITFKKMSRQKAWKKEETPQS
Atxrn3  868  LVSNSLQMGTRYQTPDVP-----AGYGYNPPQYVPIFYQHGGYMAFPGAQCYAPAPYQNRGGYQP
Atxrn4  760  LTKNLTLMKSSSTGAASGLD-----PNGYRNVPNGNSYGGVNRPRAPGSEPYRKAVIDDSSSY

Rat1    935  VQPAHYGRNSYNSQFGFNNSRYDGGNNYRQNSYRNNNSYENRNSGQYSGNSYSRNNQSRYDNRANRF-----
Atxrn2  928  REKSKKLLKSSLVNPLKMKKTKSPQREFTREKKENITPQRKLTKAQRQVKHIRMMEEAKMIKQRKKEKYLKAKKYAQG
Atxrn3  933  RCPSEGRFSEPYQSRSREGQHASRGGYSGNHQNHQHQQQWHQGGSEQNNPRGYNGQHHHQGGHDDRGRGRGSHHHH
Atxrn4  819  YGKYNSTQGTFNNGERYYPSPNGSQDYRNNYNSKIVAEQHNRRGLGAGMGLSIEDNRSKQLYSYTEAANANLNPLP

Rat1    1008  APEKTA-----
Atxrn2  1013  DQGGNPRHRY-----
Atxrn3  1013  -----
Atxrn4  899  SPTQWIGTQPGGNFVGGYYRDGVGYSETNGKSVKVIYQAKTQPSHRGANL

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**Supplementary Figure S4.** Sequence alignment generated by CLUSTAL-W of Rat1, AtXRN2, AtXRN3 and AtXRN4 proteins. Identical and similar amino acids are marked blue and green, respectively; red arrows indicate nuclear localization signals (NLS) and a pink bracket shows the region of 8 amino acid deletion in AtXRN2 relative to AtXRN3.