

1 **Title:** Ribonuclease J is required for chloroplast and embryo development in *Arabidopsis*

2 **Running Title:** *AtRNJ* is required for embryogenesis

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12 Supplemental Materials

13 **Table S1.** Primers (5' to 3') used in this study.

1.1 Primers for mutant verification			14
	FP	RP	15
<i>rnj-1</i>	aaaggaaagctggcaacaaaact	gcgccaaggaagagtaatc	16
<i>rnj-2</i>	cctatacttcaccttcgcc	tgtattagggtccaatgctgg	17
<i>rnj-3</i>	tttgatgcgtgactactccc	agattgatgaagcaccattgg	18
CSLB	cccattggacgtgaatgtagacac		19
1.2 Primers for complementation			20
	FP	RP	21
RNJ-gDNA	cgcGGTACCctgggaccaagt gataccggaa	cgcGAGCTCcttgggattg attctgcgtt	22 23
1.3 Primers for GUS/GFP fusion constructs			24
	FP	RP	25
RNJ-Pro-GUS	gcgGTCGACctgggaccaag tgataccggaa	gcgAGATCTatcatcactgtat aacaaaacca	26 26
RNJ-Pro-GFP	gcgCTGCAGctgggaccaag tgataccggaa	gcgTCTAGAatcatcactgtat aacaaaacca	27 28
1.4 Primers for qRT-PCR			29
	FP	RP	30
GAPDH	gagtctactggtgtcttcactg	caaggctggacttgattcgtg	31
AtRNJ-qRT	ttggtggagaggtgtttcgtg	acctctccaattctccacg	32

33 SFig. 1 RNase J is a metallo-beta-lactamase and conserved in plants. (A) Phylogenetic tree analysis
34 of RNJ with TRZ, CPSF73-I and CPSF73-II from *Arabidopsis* and other organisms. The tree was
35 constructed by the Neighbor-Joining method with MEGA program 4.0. Branch numbers represent
36 the percentage of bootstrap values in 1000 sampling replicates. Abbreviations: At, *Arabidopsis*
37 *thaliana*; Hs, *Homo sapiens*; Os, *Oryza sativa*; Gm, *Glycine max*; Sb, *Sorghum bicolor*; Sc,
38 *Saccharomyces cerevisiae*; Vv, *Vitis vinifera*; Zm, *Zea mays*. (B) Protein comparison of AtTRZs,
39 AtCPSF73-I, AtCPSF73-II and AtRNJ showing the conserved motifs.

40
41 SFig. 2 The functional domains of RNJ with respect to the T-DNA insertions. The conserved
42 domains of RNJ as a metallo-beta-lactamase (I-IV; A-C) are indicated along with the diagnostic
43 amino acid residues. The Arabic number represents the specific amino acid position in the protein.

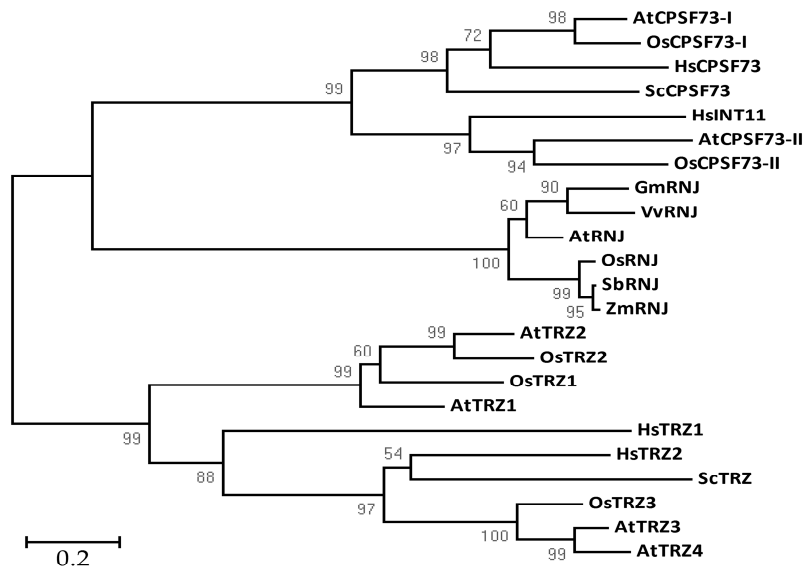
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45 SFig. 3 The embryo characteristics in wild-type, *rnj-1/+*, and *rnj-3/+* plants. (A-E) Embryos from
46 the globular stage to the bent cotyledon stage in wild-type ovules. (A) embryo in globular stage; (B)
47 embryo in transition stage; (C) embryo in heart stage; (D) embryo in torpedo stage; (E) embryo in
48 bent cotyledon stage. (F-J) The irregular globular *rnj-1* embryos from different development stages
49 as similar as wild-type embryos showed in (A-E). (K-O) The *rnj-1* embryos with abnormal
50 cotyledons from different development stages as similar as wild-type embryos showed in (A-E).
51 (P-T) The irregular globular *rnj-3* embryos from different development stages as similar as
52 wild-type embryos showed in (A-E). (U-Y) The *rnj-3* embryos with abnormal cotyledons from
53 different development stages as similar as wild-type embryos showed in (A-E). Scale bars = 20 μ m.

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55 SFig. 4 The down-regulation of *RNJ* expression level after 24h dark treatment. (A) Real-time
56 quantitative PCR analysis of *RNJ* expression in 7DAG seedlings. CK, control check; Dark, 24h
57 dark condition; DAG, day after germination. (B-C) GUS staining signals in 7DAG seedlings from
58 *pRNJ::GUS* transgenic plants. (B) 7DAG seedling in normal light cycles; (C) 7DAG seedling with
59 6 days normal light cycles and 24h dark condition.

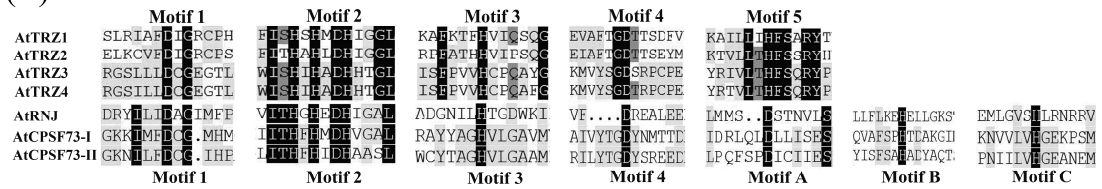
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Figure S1

(A)



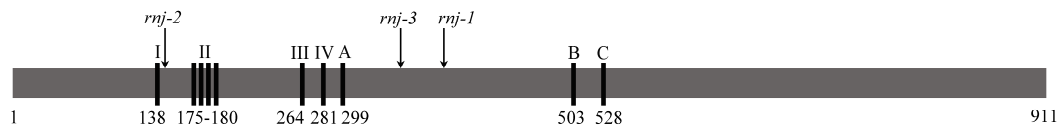
(B)



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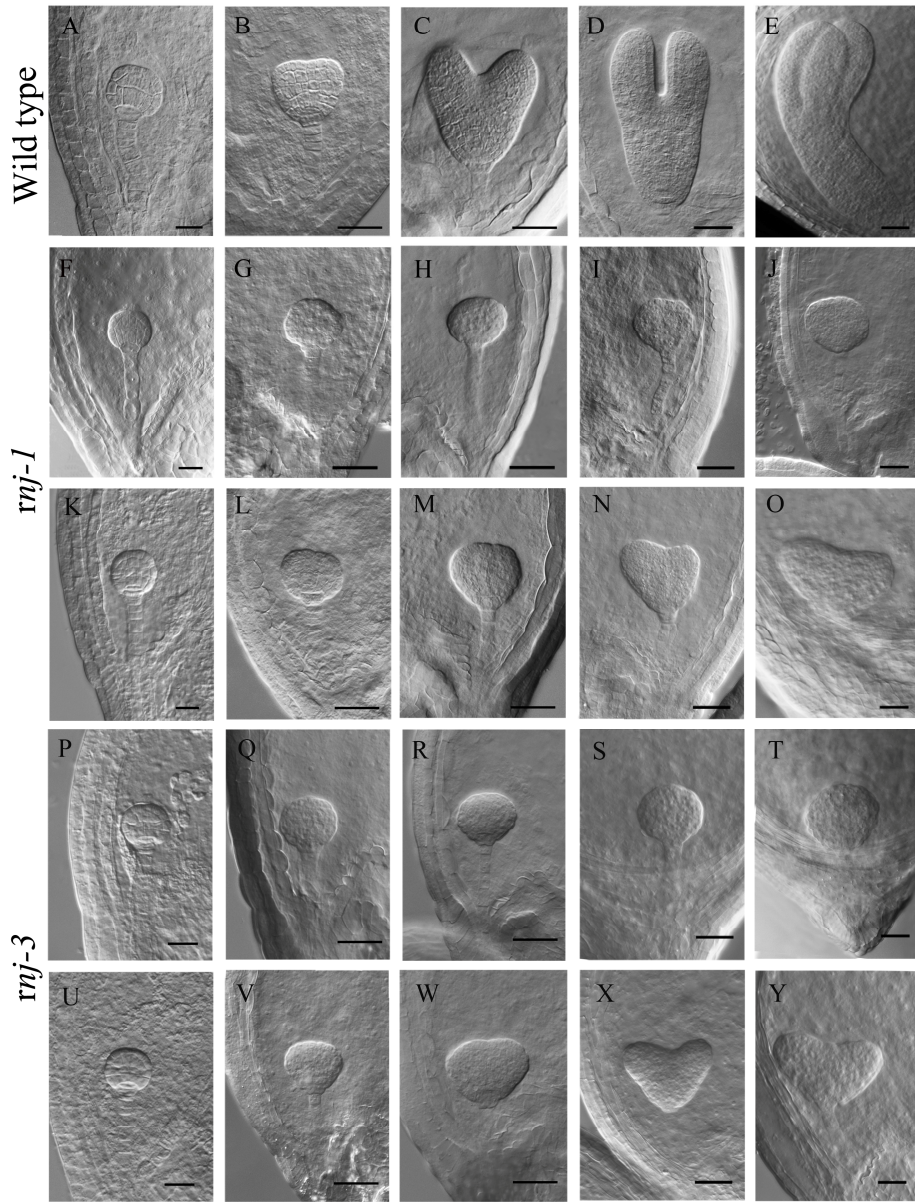
Figure S2



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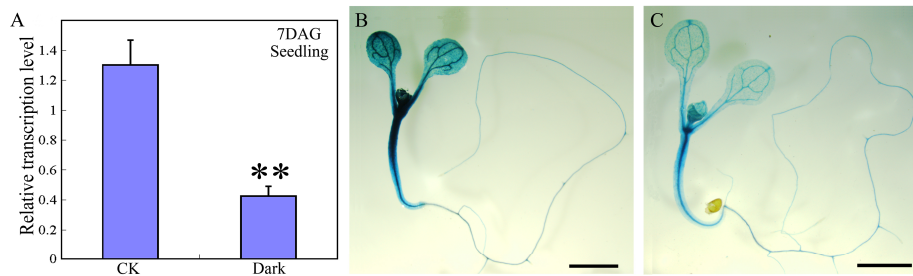
Figure S3



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Figure S4



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