Supplementary data



Supplementary Fig. S1. Opening flower morphology of the parent plants 181A and H5 under relatively low temperature. A, the whole flowers of sterile parent 181A; B, the sterile flower with petals and sepals peeled off; C, the whole flowers of fertile parent H5; D, the fertile flower with petals and sepals peeled off. The petals of sterile parent are shrinked (A) and the stamens of sterile parent are shorter than pistil (B).

 $-1890 \quad \text{ATAACCAGGTTTGTCTTTTGTTCTGTGCGTGTATATTTTACTCTCTTTATTGAACTAGTCAATCATTTGTTAATATGCTTTCACAGATT$ -1800 -1620 TAGGGAGAACGGACTAGATGCCATAGAGGTAAAAAGCTTACACGTAATCCAAGAGGTCTCCACAGATGTTAACAACCAGAAGAAATAATA -1530ATAATGTTTTGCCGTACTGGTTCAGTGTTTTTTGTTTCTGTAATGACTATCAACAAAACTTTTGTTAGACAAGCAATGAAAACGCAACGC -1440 GAATATTTATCAAAAAAATTTGAAGTAGACTCATGTTTTGTTCCAGTAAGTTTTCTCTTAAACCAACTAAATATTTGTGTACAAAAATTTA -1350-1260 -1170 TGTGTGTATATCAGTGAAATTGTCTTTTTCTATCGATGATCTTTTATCTCTCCTTCAATAGCTTTAGGAGATAAAGATGCATGATCATAA -1080-990 -900 -810 AAAATTTAAGTGTATTTCTTTAAGTGAAGTTAAAAAGATACAAATCACTCATTTTGCAACTCTAAAAGTTATATCAAGAATCTCTAACCAA -720 AATTTCTAAAGCATACTTGAAACCAGGATATTCTTTTGTGTTTCACAATATGATTAACACCGTGAGTTGCATTGAATTTCTTTAGCAAAT -630 AGATATGTAGGGGTAATGAATGAATTTGAATTAGCGTTGTTTCTATGAAGCTTTAAGCAAAACCGGAAATGATGGGGGGTTAATAACCAAGAA -540 -450AACTGAAATTGTGTAAAAACAGAACTAAAATGAAATCGGACATGTTACTACGACATATCTCCTGTAATTATTAACATTGAAAATGAAAATGAAATAA -360 ATAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAACGGGACATGATATAACGCGGTCTGCTTTTATACAAACCAGATTCGTACCGCTTCTGAAAACCA -270 -180 $\tt CCGGGATCTACAAAGTTAACGATGGAATGTTTCTGCTGTAGATAAAATTCGTCTGGTGATGAAGATGTTTTTGTGGTGAAAAGGCGAAGAA$ -90 1 atgttgtgtcggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttgcaactcctttgctctcttttatcagatcc C R R L V L V S R I P L S P V G T L A T P L LSFTR 91 ERGYSG L G S D R N L S S Y K E R L R S G L 181 aagaaggatgctgtagctctgtttcagtccatgattaggtctcgtcctcttcctacggtcatggatttcaataaactgtttagtgcagtaD A V A L F O S M I R S R P L P T V M D F N K L F S A V к к 271 A R T K O Y D L V L D L C K Q M E L Q G I A H S I Y T L S 361 atgatcaattgcttctgccgcctccagaaactcggttttgctttttctgtgatgggaaagatgttgaagcttggggtatgagcccgacaca R L Q K L G F A F S V M G K M L K L G M I N C F C Y E P D 451 ITFSTLINGLCLV G R V S K A V E L V D H M V D M 541 ${\tt gttattccaaatctcatcattacttaacactattgtcaatgggctttgtctccaagatagactctctgaagcaatgtctttgatacatcga$ I P N L I I L N T I V N G L C L O D R L S E A M S L I H R atgttggctaatgggtgccaacccgacgcagttacatatggtccggttttgaccagaatgtgtaagtcaggggaacactgcctcggccttg M L A N G C Q P D A V T Y G P V L N R M C K S G N T A S A L 631 721 gateteeteggaaagatggaaettagaaagateaageeteaagtagteaeatateateateattgaeagtetttgeaaggatgggageD L L G K M E L R K I K P Q V V T Y N I I I D S L C K D G 811 ${\tt ctcgaagatgcactctgccttttcaatgaaatggaaaccaaaggaatcaaagcaaatgtcattacctacacctctctcataggaggcttt$ L E D A L C L F N E M E T K G I K A N V I T Y T S L I G G F 901 tqtaqtqccqqaaqatqqqqatqatqqtqcacaqttqctqaqqqqatatqattacaaqqqqqaqtcacccctaacqttqtcactttcaatqct D G A Q L L R D M I T R G V T P N V V G R W D 991 $\tt ttgattgatagttttgtgaaagaaggacagcttaccgaggctaaaaaattgtacaatgagatgatcacaagaggcacagatccaaatatc$ I D S F V K E G Q L T E A K K L Y N E M I T R G T D P N I 1081 attacatataactctttgatatatgggatgtgcatggacaactgcctagatgaggccaaccagatgctggatatgatggttagccagggaТ YNSLI YGMC M D N C L D E A N Q M L D M M V S K 1171 ${\tt tctatcctgatattgtgacgtttaatatccttatcaacggatactgtaaggctaaacaggtcgatgaaggtacgagacttttccgcaat$ FNILINGYC КАКО VDEGTR DIV Т L F 1261 atgtgtttaagaggagtggttgctgatacagtcacttataacactctcatccaagggttttgtcaatcgggaaaacttaatgttgccaaaM C L R G V V A D T V T Y N T L I Q G F C Q S G K L N V A K gaactettecaggagatggtetttgttggtgtgecacceagtgttgtgaettatagtattttgetggatgggttgtggaeaatggggaa1351 ETFOEMVFVGVPPSVVTYSTT, DGTCDNG 1441 LEKĂLEILDQMLKŠKMELDIĞIYSIIIH 1531 C N A S K I D D A W D L F C S L P L K G V K P E V R T Y N 1621 atgattggaggattatgtaagaaaggctcgctgcctgaagcggtcttgttgtttagaaagatgggagaggctggggttgcgccaagcagtM I G G L C K K G S L P E A V L L F R K M G E A G V A P S S 1711 ggtacatacaacacactaatacgaggtcatctcagaggtggtgacttaacaaaatcagctgaacttatcgaagaaatgaagaggtgtggggYNTLIR AHLR G DLTKSAELIEEM 1801 F S A D A S T I K I V M D M L L D G R M K K S F L D M L S *

Supplementary Fig. S2. The putative promoter region and coding sequence of *Rfn* and its deduced amino acids from the restorer line H5.

1	atgttgtgtcggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttgcaactcctttgctctcttttatcagatcctgttggtactcttgcaactcctttgctactcttttatcagatcctgttggtactcttggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttggagattgggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttgcaactcctttgcaactcctttgctactcttttatcagatcccctgagtcctgttggtactcttggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttggagattggtgcttgtgtctcgtatccccctgagtcctgttggtactcttggagattgggagtcctgtgtgctcctgtggagattgggagtcctgtggagattgggagattgggagtcctgtgtggtactcttgcaactcctttgcaactcctttgctactcttttatcagatcccqagatcctgttggtactcttggagattggagattggtgcttgtgtctctgtggagattgggagattgggagattgggagattgggagattgggagattgggagagagagagagagagagagagagagagagagagaga
	M L C R R L V L V S R I P L S P V G T L A T P L L S F I R S
91	tcatgcgaacgaggctactctggtctcggcagcgatagaaatctctcatcttacaaagagagactgagaagtggtctggtcgatatcaag
	S C E R G Y S G L G S D R N L S S Y K E R L R S G L V D I K
181	aagaaggatgctgtagctctgtttcagtccatgattaggtctcgtcctcttcctacggtcatggatttcaataaactgtttagtgcagta
	K K D A V A L F Q S M I R S R P L P T V M D F N K L F S A V
271	gccagaacgaaacagtatgatctcgtgttggatctctgcaagcaa
	A R T K Q Y D L V L D L C K Q M E L Q G I A H S I Y T L S I
361	atgatcaattgcttctgccg <mark>t</mark> ct <mark>gcggga</mark> actcggttttgctttttctgtggg <mark>t</mark> aagatgttg <mark>agg</mark> cttgggtatgagcc <mark>t</mark> gacaca
	M I N C F C R L <mark>R E</mark> L G F A F S V M G K M L R L G Y E P D T
451	atcacatteteaactttgatcaacggtttatgtetggtgggtagagtttee <mark>g</mark> aagetgtggggttagttggtggattagttggtggga <mark>a</mark> atggag
	I T F S T L I N G L C L V G R V S E A V E L V D R M V E M E
541	gttat <mark>a</mark> ccaaatctcatca <mark>cg</mark> ct <mark>c</mark> aacactattgtcaatgg <mark>a</mark> ctttgtctccaaggtgaagtgtctgaggcaatggctttgatcga
	V I P N L I T L N T I V N G L C L Q G E V S E A M A L I D R
631	atgatggataatggatgccaacccaatgaacgtacctatggtccggttttgaacagaatgtgcaagtcaggtaacactgccttggccttg
	M M D N G C Q P N E R T Y G P V L N R M C K S G N T A L A L
721	gatctgctcagaaagatggaacacagaaagatcaagctcgatgcagtcacatacaatttcatcattgacagtctttgcaaagatgggag
	D L L R K M E H R K I K L D A V T Y N F I I D S L C K D G S
811	ctcgaagatgcactcagcctttcaatgaaatggaaaccaaaggtatcaaaccaaatgtctttacctacaactctctattagaggcttc
	LEDALSLFNEMETKGIKPNVFTYNSLIRGF
901	tgtagtgctggaagatgggatggtggtggtggtggtgctgggggattagatcacaaggggaatcacccccccc
	C S A G R W D D G A P L L R D M I T R G I T P T V I T F N S
991	ttgattgatagttttgtgaaagtgggaaagcttactgaggctcaagattgtacaacgagatgatcacaagaggcacatatcctgatatc
1001	
1081	
1171	
11/1	
1261	
1201	M S V K G V V A N T V T V N T L T O G F C O S G K L N V A K
1351	
1001	E L F O E M V S O G V H P D I I T Y K I L L D G L C D N G E
1441	
1111	V E E A I, G I I, D O M H K S N M E I, D I G I, Y N I I I H G M
1531	
1001	C N A N K V D D A W S L F C S L R S K G V K P D V K T Y T T
1621	atgattggaggattgtgtaagaaaggatggctgtctgaaggggcatgttatgtaagaagatggaaggggatgggattgcgccaaatgat
1021	M I G G L C K K G S L S E A G M L C K K M E E D G I A P N D
1711	tgtacatacaacactcttatcagggcacatctccgagatggtgacttaacaaaatcagcaaaacttatcgaagaaatgaagaggtgtgggcacatctccgagagaggtgtgggcacatctccgagagaggtgtggggcacatctccgagagaggtgtgggggtgtgggggggg
	CTYNTLIRAHLRDGDLTKSAKLIEEMKRCG
1801	${\tt ttctctgcagatgcttcaaccataaagattgttatggatatgttatcggatggtagaatgaagaaaagctttctqqatatqctttcttaccataagatggtagaatgaagaaaagctttcttqqatatqctttcttaccataagatggtagaatgaagaaaagctttcttqqatatqctttcttaccataagatggtagaagaaagaatggtagaagaaagctttcttqqatatqctttcttaccataagatggtagaagaaaagctttctqqatatqctttcttaccataagatggtagaagaaagaatggtagaagaaagctttctqqatatqctttcttaccataagatggtagaagaaagaaagctttctqqatatqctttcttaccataagatggtagaagaaagctttctqqatatqctttcttaccatagaagaaagaaagctttctqqatatqctttcttaccatagaagaaagaaagctttctqqatatqctttcttaccatagaagaaagaaagctttctqqatatqctttcttaccatagaagaaagaaagaaagctttctqqatatqctttcttaccatagaagaaaagaa$
	FSADASTIKIVMDML <mark>S</mark> DGRMKKSFLDMLS*

Supplementary Fig. S3. Nucleotide sequence of recessive *rfn* allele from 181A and the deduced amino acids. The mutations are indicated by red.

RFL2	<u>MVKLMIRRLSSQVSKF</u> VQPRLLETGTLRIALINCPNELSFCCERGFSAFSD-R-NLS	55
RFN	<u>MLCRRLVLVSRIP</u> LSPVGTLATPLLSFIRSSCERGYSGLGSDRNL <mark>SS</mark>	47
RFP	<u>MVLRTQRWNRLTTLRLVHLRSTETGTLRNA</u> AFFQSPYEFFFCVQGFSGLTSDRKMSS	57
	* *** * * *	
RFL2	YRERLRSGLVDIKADDAIDLFRDMIHSRPLPTVIDFSRLFSAIAKTKQYDLVLALCKQME	115
RFN	YKERLRSGLVDIKKKDAVALFQSMIRSRPLPTVMDFNKLFSAVARTKQYDLVLDLCKQME	107
RFP	YKERLRSGLVDIKKDDAVALFQSMLRSRPLPTVIDFNRLFGLLARTKQYDLVLALCKQME	117
	* ********** ** ** ** * ****** ** ** **	
RFL2	LKGIAHNLYTLSIMINCFCRCRKLCLAFSAMGKIIKLGYEPNTITFSTLINGLCLEGRVS	175
RFN	LQGIAHSIYTLSIMINCFCRLQKLGFAFSVMGKMLKLGYEPDTITFSTLINGLCLVGRVS	167
RFP	LKGIAYDLYTLNIMINCFCRRRKLGFAFSAMGKIFKLGYEPNTVTFNTLLNGLCLEGRVF	177
	* *** *** ******** ** *** *** **** *****	
RFL2	EALELVDRMVEMGHKPDLITINTLVNGLCLSGKEAEAMLLIDKMVEYGCQPNAVTYGPVL	235
RFN	KAVELVDHMVDMKVIPNLIILNTIVNGLCLQDRLSEAMSLIHRMLANGCQPDAVTYGPVL	227
RFP	EAVELVDCMVLSQHVPDLITLNTIVNGLCLKDRVSEAVDLIARMMANGCQPNQFTYGPIL	237
	* **** ** * ** ** ****** ** ** * ****	
RFL2	NVMCKSGQTALAMELLRKMEERNIKLDAVKYSIIIDGLCKHGSLDNAFNLFNEMEMKGIT	295
RFN	NRMCKSGNTASALDLLGKMELRKIKPQVVTYNIIIDSLCKDGSLEDALCLFNEMETKGIK	287
RFP	NRMCKSGNTASALDLLRKMEHRKIKPHVVTYTIIIDNLCKDGRLDDALSFFSEMETKGIK	297
	* ***** ** * ** *** * ** * * * **** *** *	
RFL2	TNIITYNILIGGFCNAGRWDDGAKLLRDMIKRKINPNVVTFSVLIDSFVKEGKLREAEEL	355
RFN	ANVITYTSLIGGFCSAGRWDDGAQLLRDMITRGVTPNVVTFNALIDSFVKEGQLTEAKKL	347
RFP	ANVFTYNSLIGSFCSFGRWDDGAQLLRDMITRKITPNVVTFSALIDSLVKEGKLTEAKDL	357
	* ** *** ** ******* ****** * ******	
RFL2	HKEMIHRGIAPDTITYTSLIDGFCKENHLDKANQMVDLMVSKGCDPNIRTFNILINGYCK	415
RFN	YNEMITRGTDPNIITYNSLIYGMCMDNCLDEANQMLDMMVSKGFYPDIVTFNILINGYCK	407
RFP	YNEMITRGIEPNTITYNSLIYGLCNDKRLDEANQMMDLMVSKGCDPDIWTYNILINGFCK	417
	*** ** * *** *** * ** ** ***** * ***** *	
RFL2	ANRIDDGLELFRKMSLRGVVADTVTYNTLIQGFCELGKLNVAKELFQEMVSRKVPPNIVT	475
RFN	AKQVDEGTRLFRNMCLRGVVADTVTYNTLIQGFCQSGKLNVAKELFQEMVFVGVPPSVVT	467
RFP	AKQVDDGMRLFRKMSLRGMIADTVTYSTLIQGFCQSRKLIVAKKVFQEMVSQGVHPGIMT	477
	* * * *** * *** ****** ****** ** ** *** ***	
RFL2	YKILLDGLCDNGESEKALEIFEKIEKSKMELDIGIYNIIIHGMCNASKVDDAWDLFCSLP	535
RFN	YSILLDGLCDNGELEKALEILDQMLKSKMELDIGIYSIIIHGMCNASKIDDAWDLFCSLP	527
RFP	YAILLDGLCDNGELEEALGILDQMHKCKMELDIGIYNIIIHGMCNANKVDDAWSLFCSLS	537
	* ********* * ** * * * * * *******	
RFL2	LKGVKPGVKTYNIMIGGLCKKGPLSEAELLFRKMEEDGHAPDGWTYNILIRAHLGDGDAT	595
RFN	LKGVKPEVRTYNIMIGGLCKKGSLPEAVLLFRKMGEAGVAPSSGTYNTLIRAHLRGGDLT	587
RFP	LKGVKRDIQSYNIMLSGLCKRSSLSEADALFRKMKEDGYEPDGCTYNTLIRAHLRGNDIT	597
	***** **** **** * ** **** * * * ****** *	
RFL2	KSVKLIEELKRCGFSVDASTIKMVIDMLSDGRLKKSFLDMLS	637
RFN	KSAELIEEMKRCGFSADASTIKIVMDMLLDGRMKKSFLDMLS	629
RFP	TSVQLIEEMKRCGFSSDASTVKIVMDMLSSGELDKSFLNMLSGPFGDKSSSLD	650
	* **** ****** **** * * *** * * ****	

Supplementary Fig. S4. Alignment of the amino acids sequences of RFN, RFP and their homologous protein RFL2 in *Arabidopsis*. The PPR repeats are highlighted by red and blue. The predicted mitochondrial signals are underline.



Supplementary Fig. S5. GUS assay from different tissues of Pro_{Rfn} : GUS introduced *Arabidopsis* plants. A, seedling after two weeks screened by hygromycin; B-F, staining of the stem and leaf (B), inflorescence (C), flower (D) and silique (E) from reproductive stage. F-J, staining of the corresponding tissues from wild-type plants. Scale bars: A-C, E-H, J, 2 mm; D, I, 500 µm.

Prime name	Primer sequence (5'-3')	Purpose
BnSR02-F	CTCTTCTACGGTCAGTGGCA	Fine mapping
BnSR02-R	GAGATTGAGGATTGGGGGTTG	
BnSR28-F	TTTGGAAAGAGCGGTTATGA	Fine mapping
BnSR28-R	ACATGCTCGATCGTGCTTAT	
BnSR33-F	GCTTGGTATCGTTCTCCGTC	Fine mapping
BnSR33-R	TCTGGCTTGTTACTCAAGCG	
BrSC64-F	CTGCTGCGAGTTACTTGAAGC	Fine mapping and BAC
BrSC64-R	AGGATTCTGCTTTTGTGGTTTC	screening
BrSC65-F	ATAGCAGAAGCAGCATTGAAAG	Fine mapping and BAC
BrSC65-R	GCCTGGAGTGAVAAGAAGACG	screening
BrIP77-F	TGACGTGAGGGCGATTCTTG	Fine mapping
BrIP77-R	TAATGGACCAGGCGGTGATG	
BrIP81-F	TCGCTCCACCAACAATAAAC	Fine mapping
BrIP81-R	TCAGTCCATGAGATCAAGACG	
ORF2-F	CGgaattcGTCCAACCACAATGGCTTTAAC	Binary construct of Rfn
ORF2-R	CGggatccATTGCTAAGGCGAATCCGGTTG	
Rfn-F	GTTCTTGCTGTAAAGCGTTGT	Transgenic plants
Rfn-R	GTTGCGTTTTCATTGCTTGTC	detection
RfnRT-F	CTTGCAGAGATCCAACACGAGAT	qRT-PCR of Rfn or rfn
RfnRT-R	CAAAACCGAGTTTCTGGAGGC	
Pro-F	CC <u>ttaattaa</u> GTCCAACCACAATGGCTTTAAC	GUS-fusion
Pro-R	AggcgcgccCTGTTTGTTTTTTTTTTTCTTCAAATCTAC	
Rfn-Xba-F	GCtctagaATGTTGTGTCGGAGATTGGT	GFP-fusion
Rfn-Xba-R	GCtctagaATTTCTATCGCTGCCGAGAC	
insitu-F	ATGTAAGAAAGGCTCGCTGC	RNA in situ
insitu-R	TAAGTCACCACCTCTGAGATGAG	hybridization

Supplementary Table S1. The primers used in this study. Underlines indicate the restriction sites.

orf222-F	TGGTGGAAAAGATCGTACAAGT	RNA gel blot
orf222-R	AAGACCCGGAAGTGGTGATC	
orf139-F	CGATCCACAAACTGACGCAT	RNA gel blot
orf139-R	TTGTGCCGAGTCAAATCTGC	
BnActin-F	CCCTGGAATTGCTGACCGTA	qRT-PCR (control)
BnActin-R	TGGAAAGTGCTGAGGGATGC	

Supplementary Table S2. Fertility of T_1 progeny derived from five independent T_0 transgenic plants by selfing and test-cross.

No.	Source	Total	Fertile	Sterile	Expected	χ^2 value
		plants	plants	plants	ratio	а
T ₁ -04	selfing	61	47	14	3:1	0.05
T ₁ -20	selfing	96	70	26	3:1	0.13
T ₁ -21	selfing	132	125	7	15:1	0.07
T ₁ -29	selfing	122	111	11	15:1	1.16
T ₁ -46	selfing	99	91	8	15:1	0.30
T ₁ -04	test-cross	65	32	33	1:1	0
T ₁ -20	test-cross	88	51	37	1:1	1.92
T ₁ -21	test-cross	82	55	27	3:1	2.34
T ₁ -29	test-cross	78	60	18	3:1	0.07
T ₁ -46	test-cross	84	62	22	3:1	0.02

 $^{a}\chi^{2}(0.05, 1) = 3.84$