

## Supplementary Materials and Methods

### Plant materials

The mutants and transgenic plants used in the present study were as follows: E361 (from Alex Webb's laboratory); *tmm-1*, *pTMM::TMM-GFP* and *flp-1* (from Fred D. Sack's laboratory); *sdd1-1* (from Thomas Altmann's laboratory); *mute*, *erl105*, *erl1-2 erl2-1*, *erl105 erl2-1*, *erl105 erl1-2 erl2-1*, and *scrm2-1* (SAIL\_808\_B10) (from Keiko U. Torii's laboratory); *fama-1* and *spch-1* (from Dominique C. Bergmann's laboratory); *epf2-1* (SALK\_102777), *ice1-2* (SALK\_003155), *myb88* (SALK\_068691) and *nrpb3-2* (SALK\_008220) (from ABRC); *nrpb2-3* (from Xuemei Chen's lab); *TMM<sub>pro</sub>::nucGFP*, *MUTE<sub>pro</sub>::GFP*, and *FAMA<sub>pro</sub>::nucGFP* (contrasted by our lab).

### Plasmid Construction

Gateway technology was employed for most manipulations. For complementation test and expression pattern analysis, 3.5 kb of genomic sequence including full *NRPB3* genomic sequence and its 1.6 kb upstream sequence was amplified from Col-0 template DNA with primers listed in Table S1 and cloned into the *pBIB-BASTA-GWR-GFP* and *pBIB-BASTA-GWR-GUS* vector by *in vitro* DNA recombination, respectively. For overexpression construct, the CDS sequence of *NRPB3* was amplified with primers listed in supplementary material Table S1 and introduced into the destination vector pB35GWF with the help of Gateway technology. For transient expression experiment, the cDNA of *NRPB3* was amplified with primers listed in supplementary material Table S1 and cloned into vector PA7-YFP. To make Dex-inducible *GVG-NRPB3RNAi*, the region corresponding to 388 to 622 bp of *NRPB3* was used as the inverted repeats and inserted into the XhoI/SpeI sites of pTA7002 vector (Aoyama and Chua, 1997). To create *amiR-NRPB3* plants, two amiRNA was created using the primers listed in supplementary material Table S1 and then introduced into the destination vector pB35GWF with the help of Gateway technology. To create *FAMA<sub>pro</sub>::amiR-NRPB3-2* plants, *amiR-NRPB3-2* was cloned into vector pCAMBIA-1305 at the SalI and HindIII sites, and *FAMA* promoter was

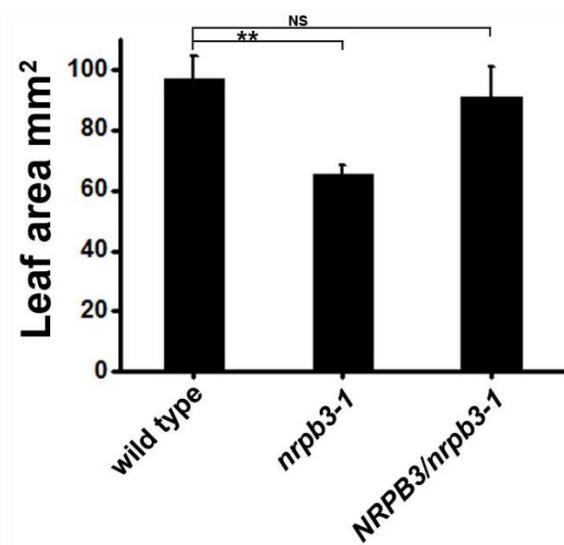
cloned into the vector at KpnI and SalI sites. See Table S1 for primer DNA sequence. All the cloned sequences were confirmed by sequencing analysis.

### **Yeast two-hybrid assay and two-hybrid screen with $\Delta N$ -NRPB3**

Yeast two-hybrid assay was done using the MATCHMAKER two-hybrid system 3 (Clontech, Shiga, Japan). Full-length or the N-terminally deleted NRPB3 ( $\Delta N$ -NRPB3, 68 to 319 amino acids) fused to the DNA-binding domain of GAL4 was used as the bait protein and *SPCH*, *MUTE*, *FAMA*, *ICE1*, *SCRM2*, *FLP* or *MYB88* fused to the transcriptional activation domain of GAL4 was used as the prey protein. Bait and prey constructs were transformed into yeast strain Y190, and  $\beta$ -gal activity was assayed according to the manufacturer's protocol (Clontech, Shiga, Japan). Each interaction was tested at least three times. See Table S1 in supplementary material for primer DNA sequence. For yeast two-hybrid screen, yeast strain Y190 transformed with bait pGBK- $\Delta N$ -NRPB3 was retransformed with a prey library made from 3-d-old seedlings in pACT (ABRC stock CD4-22), and  $\beta$ -gal activity was assayed according to the manufacturer's protocol (Clontech, Shiga, Japan). In the alternative Y2H system, full-length NRPB3, nrpb3 or RBR was cloned into the pGBKT7 vector and *SPCH*, *MUTE*, *FAMA*, *ICE1*, *SCRM2*, *FLP*, *MYB88*, or RBR was cloned into the pGADGH vector. BD and AD plasmids were transformed into yeast strain AH109. Protein–protein interaction was measured by growth of yeast. For serial dilution assay, yeast cells at exponential stage were adjusted to OD<sub>600</sub> = 0.5 and diluted to 1/10, 1/100, and 1/1000 with sterilized double-distilled water. Each interaction was tested at least three times. See Table S1 in supplementary material for primer DNA sequence.

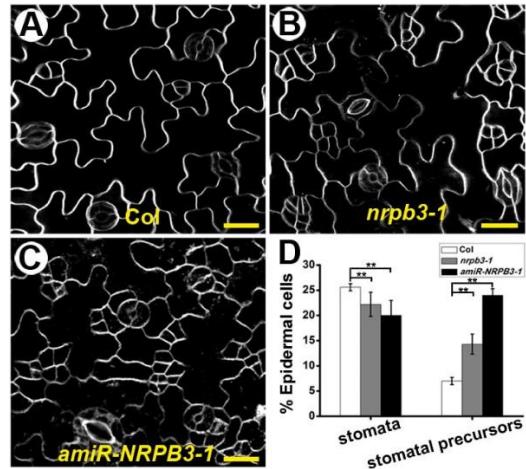
### **BiFC**

pXY106 and pXY104 described by Liu and Howell previously (Liu and Howell, 2010) were used in our BiFC analysis. NRPB3 or nrpb3 was cloned into pXY106 at XbaI and SalI sites to produce nYFP-NRPB3 or nYFP-nrpb3 fusion protein. ICE1 or FAMA was cloned into pXY104 at BamHI and SalI sites to make the ICE1-cYFP or FAMA-cYFP fusion protein. See Table S1 in supplementary material for primer DNA sequence.

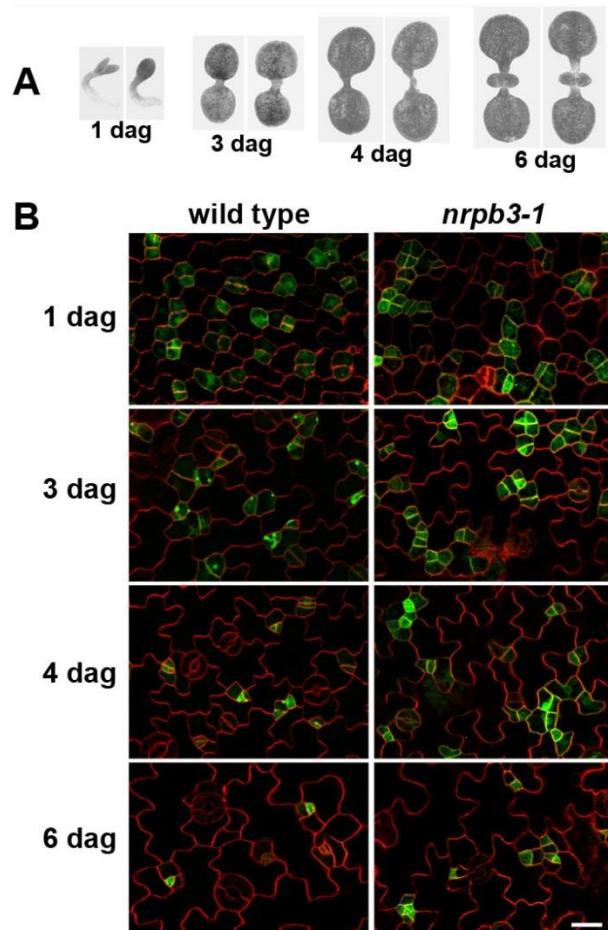


**Fig. S1.** The leaf area of the seventh fully expanded leaves in wild type, *nrpb3-1*, and *NRPB3/nrpb3-1*. Error bars

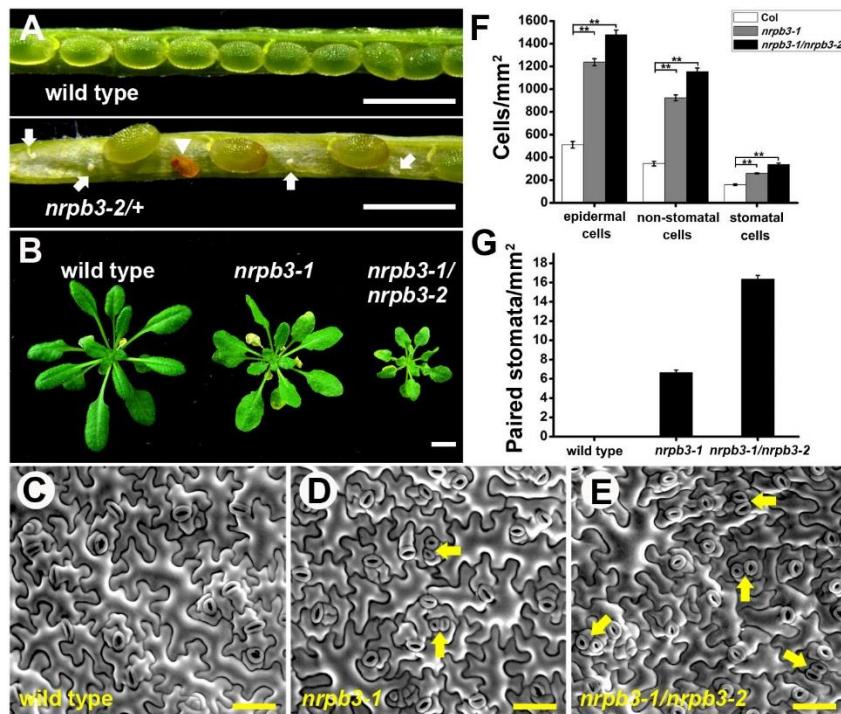
indicate s.e.m.; NS indicate no significance; \*\*, P<0.01 by Student's test. n=20 per genotype.



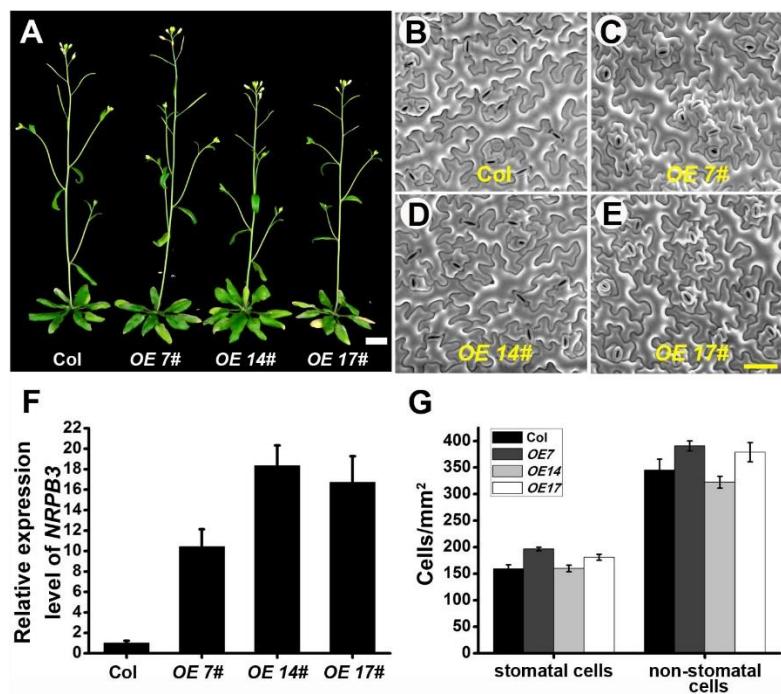
**Fig. S2. The proportion of stomata and stomatal precursors in the cotyledons of *nrpb3* mutants. (A–C)** Abaxial epidermis of cotyledons of wild type (A), *nrpb3-1* (B), and *amiR-NRPB3-1* (C) at 6 days after germination (dag). Bars = 20  $\mu$ m. **(D)** The proportion of stomata and stomatal precursors on the abaxial epidermis of cotyledons at 6 dag. Error bars indicate s.e.m.; \*\*, P<0.01 by Student's test. n=15 per genotype.



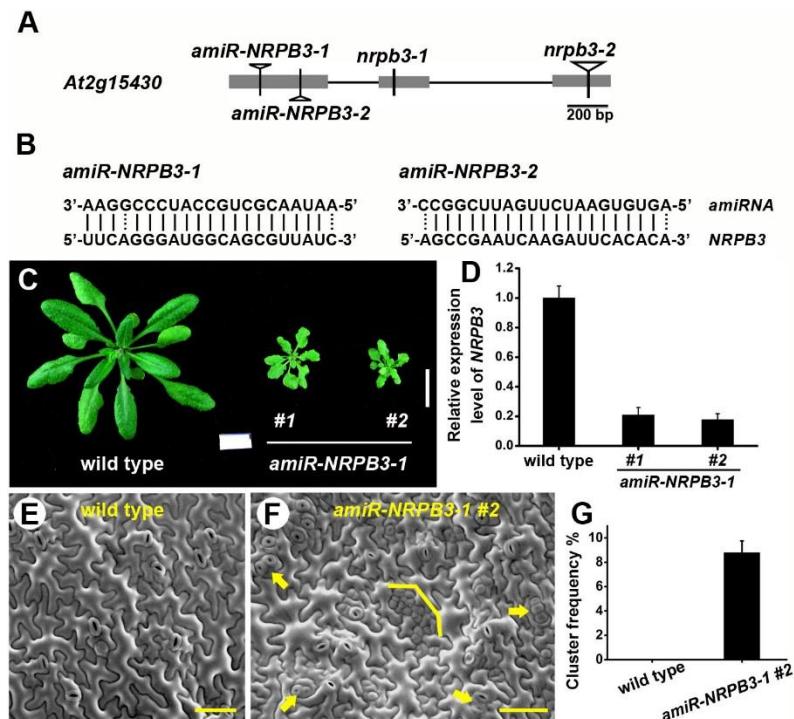
**Fig. S3. Time sequence of stomatal differentiation in *nrpb3-1* and wild type.** Images of the abaxial epidermis of wild-type and *nrpb3-1* cotyledons were taken from different cotyledons over time. *TMM<sub>pro</sub>::TMM-GFP* was used to monitor stomatal lineage cells. (A) Seedlings of wild type and *nrpb3-1* at 1 dag, 3 dag, 4 dag, and 6 dag. Wild type is on the left, and *nrpb3-1* is on the right. (B) Expression of *TMM<sub>pro</sub>::TMM-GFP* in the abaxial epidermis of cotyledons at 1 dag, 3 dag, 4 dag, and 6 dag. Bar = 20  $\mu$ m.



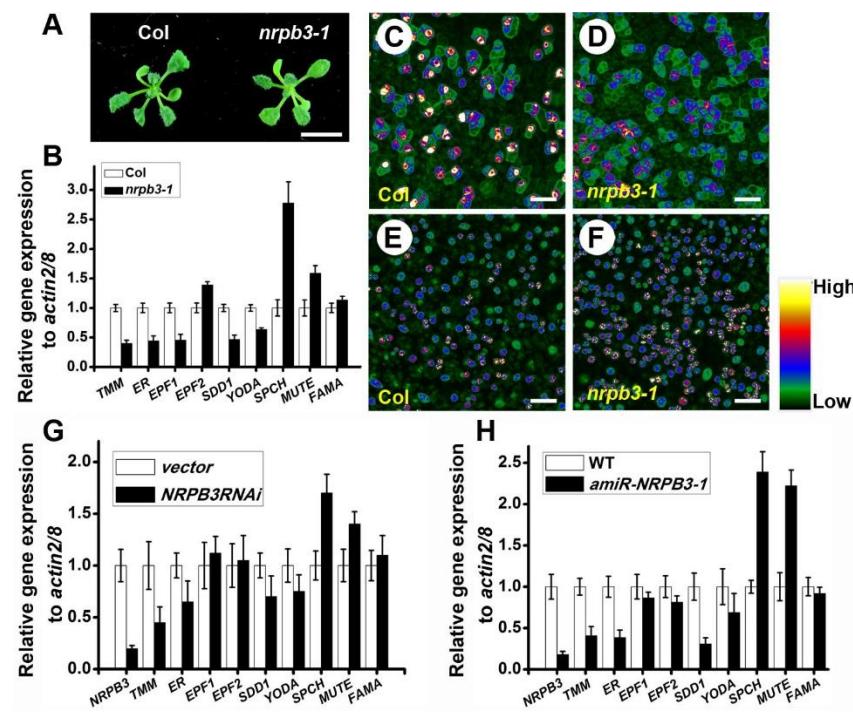
**Fig. S4. Phenotype analysis of *nrpb3-2* and *nrpb3-1/nrpb3-2*.** (A) Siliques of *nrpb3-2/+* plants contain normally developed seeds as well as arrested ovules (arrow) and aborted seeds (arrowheads). Bars = 1 mm. (B) Three-week-old seedlings of wild-type, *nrpb3-1*, and *nrpb3-1/nrpb3-2*. Bar = 1 cm. (C–E) The abaxial epidermis of the seventh fully expanded rosette leaf of wild type (C), *nrpb3-1* (D), and *nrpb3-1/nrpb3-2* (E). Arrow, paired stomata. Bars = 50 µm. (F,G) Densities of epidermal cells, non-stomatal cells, stomatal cells (F), and paired stomata (G) on the abaxial epidermis of the seventh mature leaves. Error bars indicate s.e.m.; \*\*, P<0.01 by Student's test. n=30 per genotype.



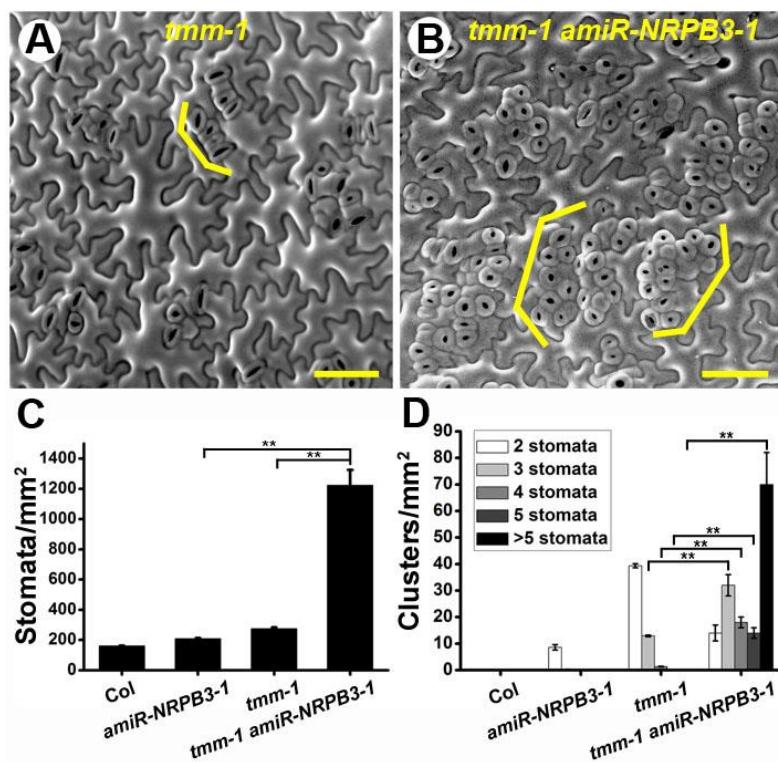
**Fig. S5. *NRPB3* overexpression phenotypes.** (A) Five-week-old plants of wild-type, *OE7#*, *OE14#*, and *OE17#*. Bar = 1 cm. (B–E) The abaxial epidermis of the seventh fully expanded rosette leaf of Col (B), *OE 7#*(C), *OE 14#*(D), and *OE 17#*(E). Bar = 50 µm. (F) Relative gene expression of *NRPB3* in Col, *OE 7#*, *OE 14#*, and *OE 17#*. (G) Densities of stomatal cells and non-stomatal cells on the abaxial epidermis of the seventh mature leaves. Error bars indicate s.e.m.; n=30 per genotype.



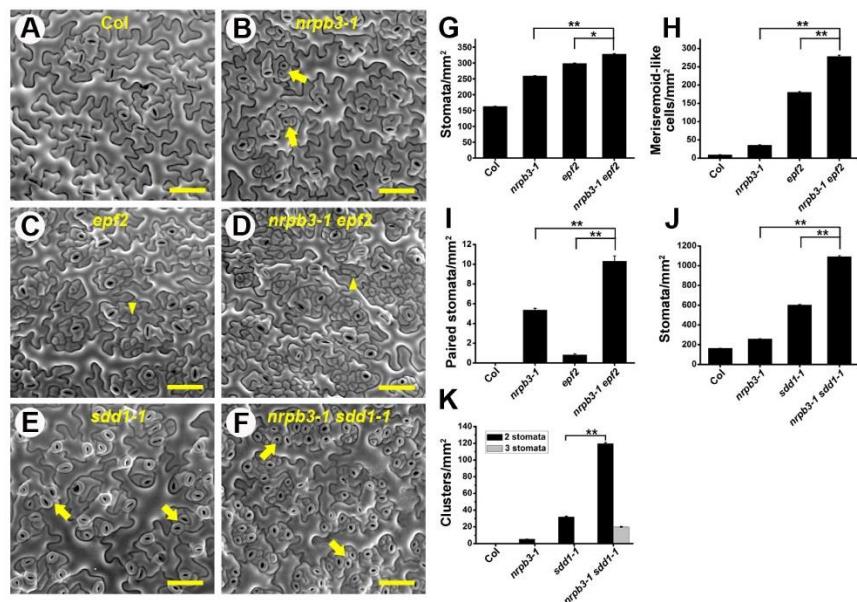
**Fig. S6. Creation and phenotype analysis of *amiR-NRPB3* plants.** (A) The *Arabidopsis* *NRPB3* locus. Boxes, exons; lines, introns. The specific targets of *amiR-NRPB3-1* and *amiR-NRPB3-2* are indicated by triangles. (B) The top *amiRNA* sequence was used to regulate the bottom target sequence of *NRPB3*. Solid lines, perfect matches; dashed lines, mismatches. (C) Four-week-old plants of wild type and *amiR-NRPB3-1*. Bar = 1 cm. (D) Relative gene expression of *NRPB3* in wild type and *amiR-NRPB3-1*. (E,F) The abaxial epidermis of the sixth immature rosette leaf of wild type (E) and *amiR-NRPB3-1* (F). Arrow, clustered stomata; Bracket, cluster of small highly divided meristemoid-like cells. Bars = 50  $\mu$ m. (G) *amiR-NRPB3-1* exhibited a much higher frequency of stomatal clusters. Error bars indicate s.e.m.; n=20 per genotype.



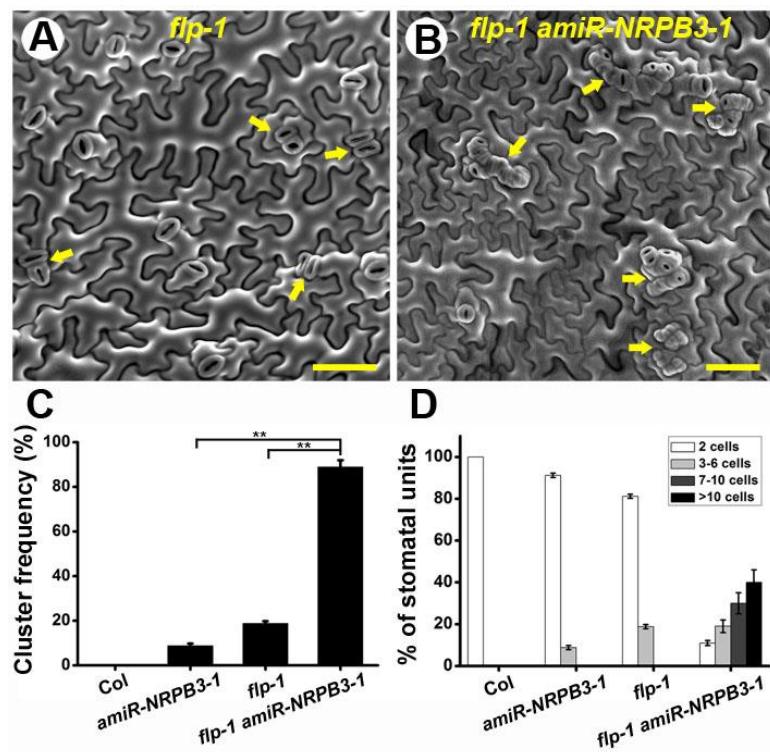
**Fig. S7. Proper expression of stomatal development genes was disturbed in the *nrpb3* mutants.** (A) Two-week-old Col and *nrpb3-1* seedlings used for isolation of total RNA. Bar = 5 mm. (B) Relative expression of stomatal development genes in Col and *nrpb3-1* seedlings. (C–F) The expression of *TMM<sub>pro</sub>::TMM-GFP* (C,D) and *SPCH<sub>pro</sub>::nucGFP* (E,F) in the abaxial epidermis of Col and *nrpb3-1*. Images were taken from the leaf base of the fifth rosette leaf that is 4 mm long under the same conditions. Bars = 20  $\mu$ m. (G) Relative expression of stomatal development genes in vector control and *GVG-NRPB3RNAi*/transgenic plants. Two weeks old plants were treated with Dex for 4 days continuously, and total RNA was isolated. (H) Relative expression of stomatal development genes in Col and *amiR-NRPB3-1* transgenic plants. Two weeks old plants were used for isolation of total RNA.



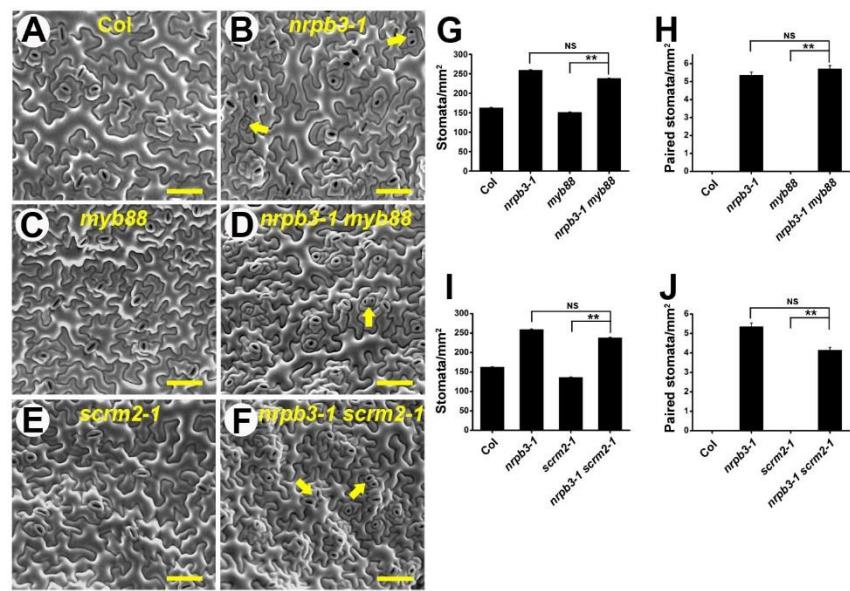
**Fig. S8. *amiR-NRPB3-1* exaggerated the stomatal phenotypes of *tmm-1*.** (A,B) The abaxial epidermis of the seventh fully expanded rosette leaf of *tmm-1* (A) and *tmm-1 amiR-NRPB3-1* (B). (C,D) Densities of stomata (C) and stomatal clusters (D) on the abaxial surface of the seventh fully expanded rosette leaves. Bracket, clustered stomata; Error bars indicate s.e.m.; \*\*, P<0.01 by Student's test. n=15 per genotype. Bars = 50  $\mu\text{m}$ .



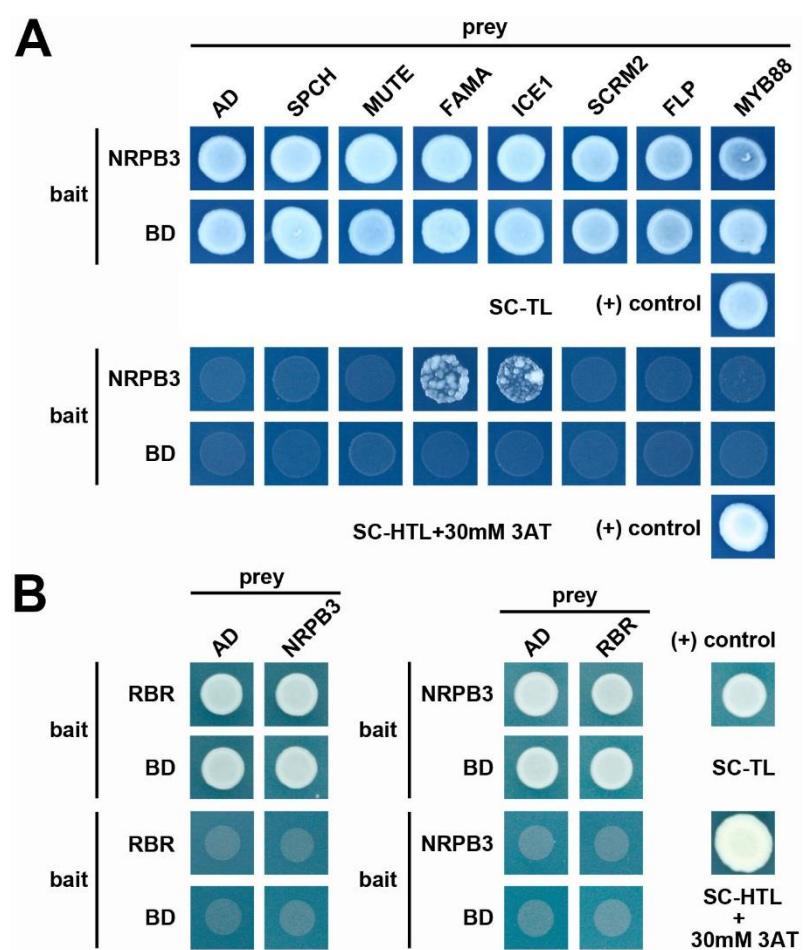
**Fig. S9. Genetic interactions of *nrpb3-1* with *epf2* and *sdd1-1*.** (A–F) The abaxial epidermis of the seventh fully expanded leaf of Col (A), *nrpb3-1* (B), *epf2* (C), *nrpb3-1 epf2* (D), *sdd1-1* (E), and *nrpb3-1 sdd1-1* (F). (G–K) Densities of stomata (G,J), meristemoid-like cells (H), paired stomata (I), and stomatal clusters (K) on the abaxial surface of the seventh fully expanded rosette leaves. Error bars indicate s.e.m.; \*, P<0.05; \*\*, P<0.01 by Student's test. n=30 per genotype. Bars = 50 μm.



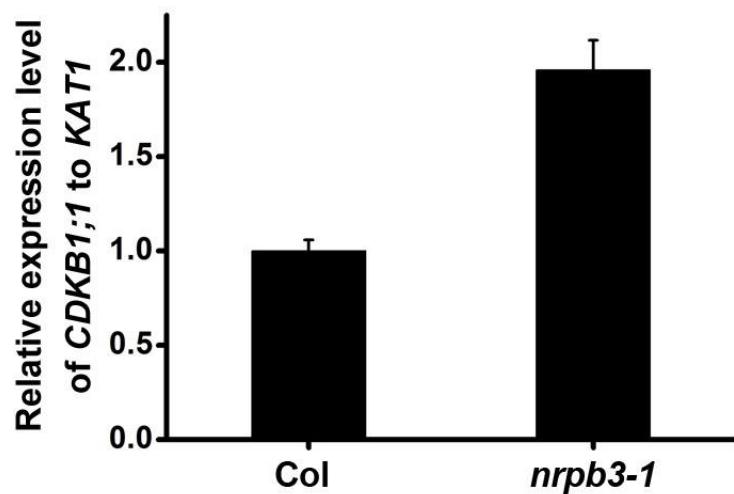
**Fig. S10. *amir-NRPB3-1* exaggerated the stomatal phenotypes of *fip-1*.** (A,B) The abaxial epidermis of the seventh fully expanded leaf of *fip-1* (A) and *fip-1 amir-NRPB3-1* (B). (C) Frequencies of clusters per area. (D) The relative means of cells per cluster and of normal stomata in each genotype. Arrow, stomatal cluster. Error bars indicate s.e.m.; \*\*, P<0.01 by Student's test. n=15 per genotype. Bars = 50 µm.



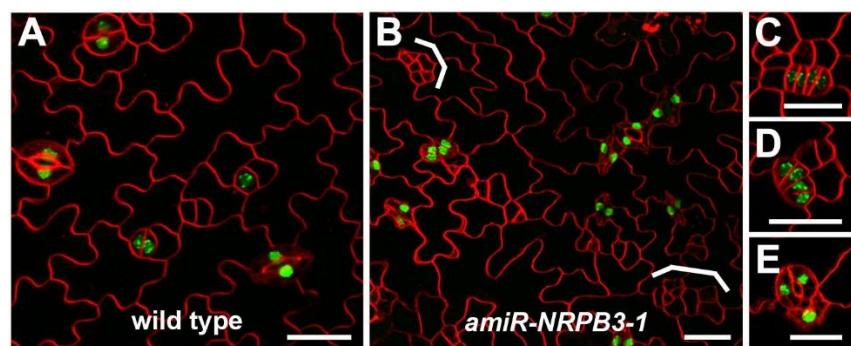
**Fig. S11. Genetic interactions of *nrpb3-1* with *myb88* and *scrm2*.** (A–F) The abaxial epidermis of the seventh fully expanded rosette leaf of Col (A), *nrpb3-1* (B), *myb88* (C), *nrpb3-1 myb88* (D), *scrm2-1* (E), and *nrpb3-1 scrm2-1* (F). (G–J) Densities of stomata (G,I) and paired stomata (H,J) on the abaxial surface of the seventh fully expanded rosette leaves. Arrow, paired stomata. Error bars indicate s.e.m.; NS indicate no significance; \*\*, P<0.01 by Student's test. n=30 per genotype. Bars = 50 µm.



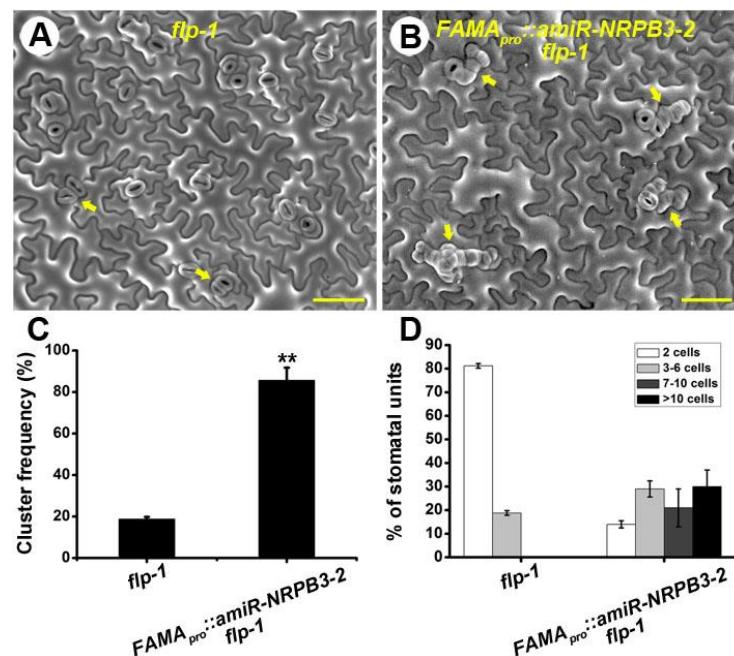
**Fig. S12. Yeast two-hybrid analysis.** **(A)** NRPB3 exhibited interactions with FAMA and ICE1, but no interactions with FLP, MYB88, SCRM2, MUTE, or SPCH. **(B)** NRPB3 exhibited no interactions with RBR. Full-length NRPB3 was used. BD, binding domain; AD, activation domain; SC-TL, synthetic complete medium lacking tryptophan and leucine; SC-HTL, synthetic complete medium lacking histidine,tryptophan and leucine; 3AT, 3-amino-1,2,4-triazole.



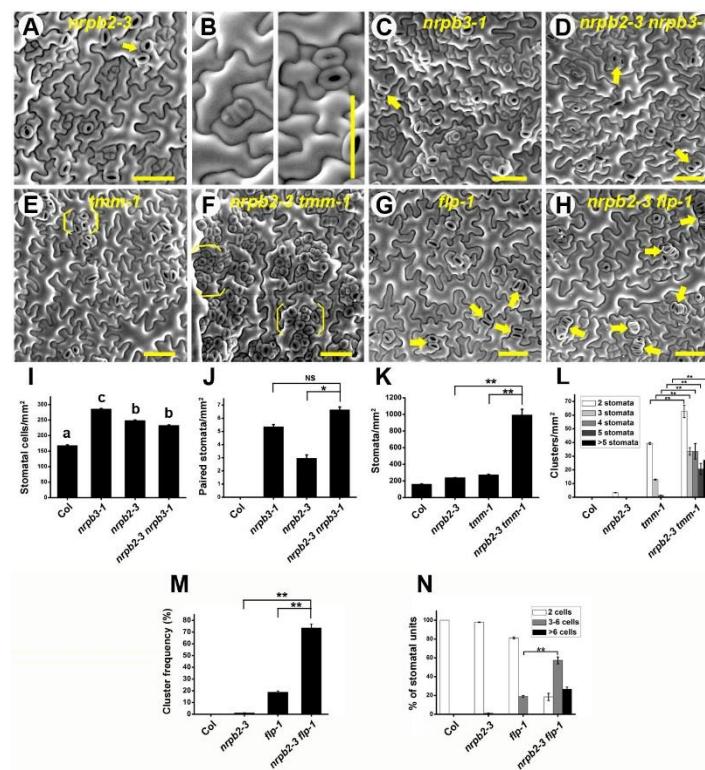
**Fig. S13. Relative expression of *CDKB1;1* in wild type and *nrpb3-1* seedlings.** Two weeks old plants were used for isolation of total RNA. To correct for the increased number of stomata in *nrpb3-1* mutants, mRNA level was normalized to *KAT1* which is a marker for guard cells (Nakamura et al., 1995; Xie et al., 2010).



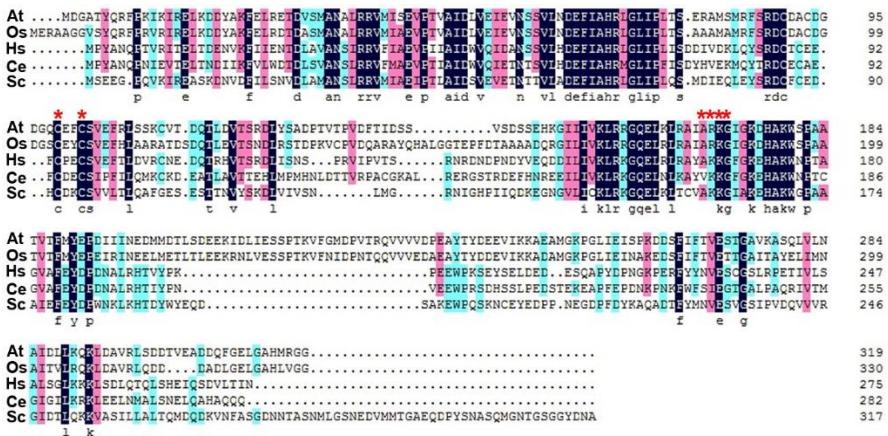
**Fig. S14. Expression of *FAMA<sub>pro</sub>::nucGFP* in *amiR-NRPB3-1* transgenic plants.** (A–E) Expression of *FAMA<sub>pro</sub>::nucGFP* in the abaxial epidermis of the sixth immature rosette leaf of wild type (A) and *amiR-NRPB3-1* transgenic plants (B–E). (C–E) Close-up of caterpillar-like structures (C,D) and aberrant GMC or GC (E) expressing *FAMA<sub>pro</sub>::nucGFP* in *amiR-NRPB3-1* transgenic plants. Bracket, clusters of small, highly divided meristemoid-like cells. Bars = 20  $\mu$ m.



**Fig. S15.** *FAMA<sub>pro</sub>::amiR-NRPB3-2* dramatically exaggerated the stomatal phenotypes of *flp-1*. **(A,B)** The abaxial epidermis of the seventh fully expanded leaf of *flp-1* (A) and *FAMA<sub>pro</sub>::amiR-NRPB3-2 flp-1* (B). **(C)** Frequencies of clusters per area. **(D)** The relative means of cells per cluster and of normal stomata in each genotype. Arrow, stomatal cluster. Error bars indicate s.e.m.; \*\*, P<0.01 by Student's test. n=15 per genotype. Bars = 50 µm.



**Fig. S16.** *nrpb2-3* exhibited paired stomata and exaggerated phenotypes of both *tmm-1* and *fip-1*. (A–H) The abaxial epidermis of the seventh fully expanded leaf of *nrpb2-3* (A,B), *nrpb3-1* (C), *nrpb2-3 nrpb3-1* (D), *tmm-1* (E), *nrpb2-3 tmm-1* (F), *fip-1* (G), and *nrpb2-3 fip-1* (H). (I–L) Densities of stomatal cells (I), paired stomata (J), stomata (K), and stomatal clusters (L) on abaxial surface of the seventh fully expanded rosette leaves. (M) Frequencies of clusters per area. (N) The relative means of cells per cluster and of normal stomata in each genotype. Arrows indicate paired stomata in (A,C,D,G) and stomatal clusters in (H); Bracket, stomatal clusters in (E,F); Error bars indicate s.e.m.; NS indicate no significance; \*, P<0.05; \*\*, P<0.01 by Student's test. n=20 per genotype. Bars = 50 µm.



**Fig. S17. Alignment of amino acids of NRPB3 in *Arabidopsis thaliana* (At) and related proteins in *Oryza sativa* (Os),**

***Homo sapiens* L (Hs), *Caenorhabditis elegans* (Ce), and *Saccharomyces cerevisiae* (Sc).** Asterisks indicate two special regions of RPB3, residues 92–95 and 159–162, which are required for activator-dependent transcription in yeast.

## Supplementary References

- Aoyama, T., and Chua, N.-H.** (1997). A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J.* **11**, 605–612.
- Liu, J. X. and Howell, S. H.** (2010). bZIP28 and NF-Y transcription factors are activated by ER stress and assemble into a transcriptional complex to regulate stress response genes in *Arabidopsis*. *Plant cell* **22**, 782–796.
- Nakamura, R.L., McKendree, W.L., Jr., Hirsch, R.E., Sedbrook, J.C., Gaber, R.F., and Sussman, M.R.** (1995). Expression of an *Arabidopsis* potassium channel gene in guard cells. *Plant Physiol.* **109**, 371–374.
- Xie, Z., Lee, E., Lucas, J.R., Morohashi, K., Li, D., Murray, J.A., Sack, F.D., and Grotewold, E.** (2010). Regulation of cell proliferation in the stomatal lineage by the *Arabidopsis* MYB FOUR LIPS via direct targeting of core cell cycle genes. *Plant Cell* **22**, 2306–2321.

**Table S1. Primers Used in This Study.**

Primer name	Sequence
<b>Complementation test and expression pattern analysis</b>	
<i>NRPB3-PB1</i>	aaaaaaaggcaggcttcCGACATCTGGCAAATATAGC
<i>NRPB3-PB2</i>	caagaaagctgggtTCCTCCACGCATATGGGCAC
<b>Genotyping</b>	
<i>nrpb3-2-F</i>	TGATGTGGTTAGTGCATTGC
<i>SALK_LBa1</i>	TGGTTCACGTAGTGGGCCATCG
<i>nrpb3-1-F</i>	TGTCATTATGAATCGATTGG
<i>nrpb3-1-R</i>	GGTACTACAAGTACTATATC
<i>tmm-1-F</i>	ATGGTGGCGATATTCAATCC
<i>tmm-1-R</i>	TCCTCGAGTTATCCCGTGAA
<i>fip-1-F</i>	AAGGTAACGGAGCGTCGAA
<i>fip-1-R</i>	GGTATGAGAGAGATGGTTGG
<i>mute-F</i>	AGTATCATGTCTCACATCGC
<i>mute-R</i>	ATCTTGAATCAACCTCTACG
<i>nrpb2-3-F</i>	GCAGTTGTCTGGTTTGCC
<i>nrpb2-3-R</i>	TTGACAATGGCAAAGAAGTC
<i>sdd1-F</i>	CAGTAGTTTCATTCCCTGG
<i>sdd1-R</i>	CCTTAGACTCCAATCCCAG
<i>erl1-2</i>	GTCACGTCTCAGCTATTGTAAGCTTGT
<i>JL202</i>	CATTTTATAATAACGCTGCGGACATCTAC
<i>erl2-1-R</i>	GTGACAATGAATTAACTGGG
<i>SAIL_LB3</i>	TAGCATCTGAATTCTATAACCAATCTCGATAACAC
<i>epf2-1-R</i>	AGCTCTAGATGGCACGTGATAG
<i>SALK_LBa1</i>	TGGTTCACGTAGTGGGCCATCG
<i>fama</i>	ATGTGTACCATTACACACCC
<i>SALK_LBa1</i>	TGGTTCACGTAGTGGGCCATCG

<i>spch</i>	AACCTGAAGAACATCTCAAGAGCC
<i>SAIL_LB3</i>	TAGCATCTGAATTCTATAACCAATCTCGATACAC
<i>ice1-2</i>	TGAGGAAGAGGGCTCGTGATAG
<i>SALK_LBa1</i>	TGGTTCACGTAGTGGGCCATCG
<i>scrm2-1</i>	TAACCTCCGGAGATTACCG
<i>SAIL_LB1</i>	GCCTTTCAGAAATGGATAAATAGCCTGCTCC
<i>myb88</i>	GATATGGCTGCAAACCTATGGAG
<i>T-DNA-R</i>	GGCAATCAGCTGTTGCCCGTCTCACTGGTG
<b>Overexpression</b>	
<i>NRPB3-PB1</i>	aaaaagcaggcttcATGGACGGTGCCACATACC
<i>NRPB3-PB2</i>	caagaaagctgggtTCCTCCACGCATATGGGCAC
<b>Transient expression</b>	
<i>NRPB3-Xba1-F</i>	tctagaATGGACGGTGCCACATACC
<i>NRPB3-Kpn1-R</i>	ggtaccTCCTCCACGCATATGGGC
<b>GVG-NRPB3RNAi</b>	
<i>NRPB3RNAi</i> -antisense-F	gaac gaattcCCTACTGTTACTCCTGTGG
<i>NRPB3RNAi</i> -antisense-R	gaac ctcgagCCTCGTCTGACAAAGTATCC
<i>NRPB3RNAi</i> -sense-F	taac ggatcc CCTACTGTTACTCCTGTGG
<i>NRPB3RNAi</i> -sense-R	gcag actagtCCTCGTCTGACAAAGTATCC
<b>amiR-NRPB3</b>	
<i>amiR-NRPB3-1</i> - I	gaTTATTGCGACGGTAGGGCCTTtctcttttgtattcc
<i>amiR-NRPB3-1</i> - II	gaAAGGCCCTACCGTCGCAATAAtcaaagagaatcaatga
<i>amiR-NRPB3-1</i> - III	gaAAAGCCCTACCGTGGCAATATtcacaggcgtgatatg
<i>amiR-NRPB3-1</i> - IV	gaATATTGCCACGGTAGGGCTTTtctacatatatattcct
<i>amiR-NRPB3-2</i> - I	gaTCACACTTAGAACTAACGCCGGtctcttttgtattcc
<i>amiR-NRPB3-2</i> - II	gaCCGGCTTAGTTCTAAGTGTGAtcaaagagaatcaatga
<i>amiR-NRPB3-2</i> - III	gaCCAGCTTAGTTCTAGTGTGTTcacaggcgtgatatg
<i>amiR-NRPB3-2</i> - IV	gaACACACTAAGAACTAACGCTGGtctacatatatattcct

***FAMA<sub>pro</sub>::amiR-NRPB3***


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<i>FAMA</i> promoter-KpnI-F	ggtacc GGAAATTGATTTGGGATCACC
<i>FAMA</i> promoter-SalI-R	gtcgacTGCTATTCGTGGTAGTTGATTATAAACTGC

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**RT-PCR**

<i>NRPB3</i> -F	GATTGTGATGCTTGTGATGG
<i>NRPB3</i> -R	CCCTTGTGCTCGCTTGAATC
<i>TMM</i> -F	GATTGTGTGATGCAGAACGTC
<i>TMM</i> -R	TCTTCTTCTCTAGATAGGTGCTGGA
<i>ER</i> -F	TGACTTCAGAGGAGGGAGCA
<i>ER</i> -R	GCAACAAACATTGAAGGTGAC
<i>EPF1</i> -F	CCTCCCATCCAAGTCATCA
<i>EPF1</i> -R	GCTAACCATCACAAAGACGG
<i>EPF2</i> -F	ACGTAGTACTTGCTCCTATCATTCT
<i>EPF2</i> -R	GCGTACAAACTTCGTCATGTTA
<i>SDD1</i> -F	TGCTCTTATCCGGTCTGCAT
<i>SDD1</i> -R	ATCAATGCGGATTGATTGC
<i>YODA</i> -F	CAAGACGACGACGTGATGAG
<i>YODA</i> -R	ACGGACAATAGGACGAGGAA
<i>SPCH</i> -F	TTCTGCACTTAGTTGGCACTCAAT
<i>SPCH</i> -R	GCTGCTCTGAAGATTGGCTCT
<i>MUTE</i> -F	CGTTGTTAAGATAGGATTGGAGTG
<i>MUTE</i> -R	CAAAGCTTTCTGAACTTCAAGAGT
<i>FAMA</i> -F	GGAGCAATAGAGTTGTGAGAG
<i>FAMA</i> -R	TGGTCGCTACCGTAGTTATG
<i>Actin2/8</i> -F	GGTAACATTGTGCTCAGTGGTGG
<i>Actin2/8</i> -R	AACGACCTTAATCTTCATGCTGC

**Yeast two hybridization**

<i>NRPB3</i> -F	aaaaaaagcaggcttcATGGACGGTGCCACATACCAAAG
<i>NRPB3</i> -R	caagaaagctgggtTCCTCCACGCATATGGGCACCG
<i>SPCH</i> -F	aaaaaaagcaggcttcATGCAGGAGATAATACCGGATTTTC
<i>SPCH</i> -R	caagaaagctgggtGCAGAATGTTGCTGAATTGTTG
<i>MUTE</i> -F	aaaaaaagcaggcttcATGTCTCACATCGCTGTTGAAAG
<i>MUTE</i> -R	caagaaagctgggtATTGGTAGAGACGATCACTTCATC
<i>FAMA</i> -F	aaaaaaagcaggcttcATGGATAAAGATTACTCGGCACC
<i>FAMA</i> -R	caagaaagctgggtAGTAAACACAATATTCCCAGG
<i>ICE1</i> -F	aaaaaaagcaggcttcATGGGTCTTGACGGAAACAATGG
<i>ICE1</i> -R	caagaaagctgggtTCAGATCATACCAGCATAACCTG
<i>SCRM2</i> -F	aaaaaaagcaggcttcATGAACAGCGACGGTGGC
<i>SCRM2</i> -R	caagaaagctgggtAACCAAACCAGCGTAACCTGCTG
<i>FLP</i> -F	aaaaaaagcaggcttcATGGAAGATAACGAAGAAG
<i>FLP</i> -R	caagaaagctgggtCAAGCTATGGAGAAGGACTC
<i>MYB88</i> -F	aaaaaaagcaggcttcATGGAAGAGACAACTAAGC
<i>MYB88</i> -R	caagaaagctgggtCAAGCTATCGAGAAGGACTC
RBR-F	aaaaaaagcaggcttcATGGAAGAAGTCAGCCTCCAGTG
RBR-R	caagaaagctgggtTGAATCTGTTGGCTCGG

**Yeast two hybridization**

<i>NRPB3</i> -EcoR-F	gaattcATGGACGGTGCCACATACCAAAG
$\Delta N$ - <i>NRPB3</i> -EcoR-F	gaattcAACGACGAGTTCATTGCT
$\Delta N$ - <i>NRPB3</i> -Sal-R	gtcgacTCCTCCACGCATATGGGCACCG
<i>SPCH</i> -Nde-F	catatgATGCAGGAGATAATACCGGATTTTC
<i>SPCH</i> -EcoR-R	gaattcGCAGAATGTTGCTGAATTGTTG
<i>MUTE</i> -Nde-F	catatgATGTCTCACATCGCTGTTGAAAG
<i>MUTE</i> -EcoR-R	gaattcATTGGTAGAGACGATCACTTCATC
<i>FAMA</i> -Nde-F	catatgATGGATAAAGATTACTCGGCACC
<i>FAMA</i> -EcoR-R	gaattcAGTAAACACAATATTCCCAGG
<i>ICE1</i> -EcoR-F	gaattcATGGGTCTTGACGGAAACAATGG

<i>ICE1</i> -BamH1-R	ggatccTCAGATCATACCAGCATAACCTG
<i>SCRM2</i> -Nde-F	catatgATGAACAGCGACGGTGTGTTGGC
<i>SCRM2</i> -EcoR-R	gaattcAACCAAACCAGCGTAACCTGCTG
<i>FLP</i> -Nde-F	catatgATGGAAGATAACGAAGAAG
<i>FLP</i> -EcoR-R	gaattcCAAGCTATGGAGAAGGACTC
<i>MYB88</i> -Nde-F	catatgATGGAAGAGACAACTAAGC
<i>MYB88</i> -EcoR-R	gaattcCAAGCTATCGAGAAGGACTC

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### BiFC

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<i>NRPB3</i> -Xba1-F	tctagaATGGACGGTGCCACATACCAAAG
<i>NRPB3</i> -Sal1-R	gtcgacTCATCCTCCACGCATATGGGCACCG
<i>FAMA</i> -BamH1-F	ggatccATGGATAAAAGATTACTCGGCACC
<i>FAMA</i> -Sal1-R	gtcgacAGTAAACACAATATTCCCAGG
<i>ICE1</i> -BamH1-F	ggatccATGGGTCTTGACGGAAACAATGG
<i>ICE1</i> -Sal1-R	gtcgacGATCATACCAGCATAACCTG

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