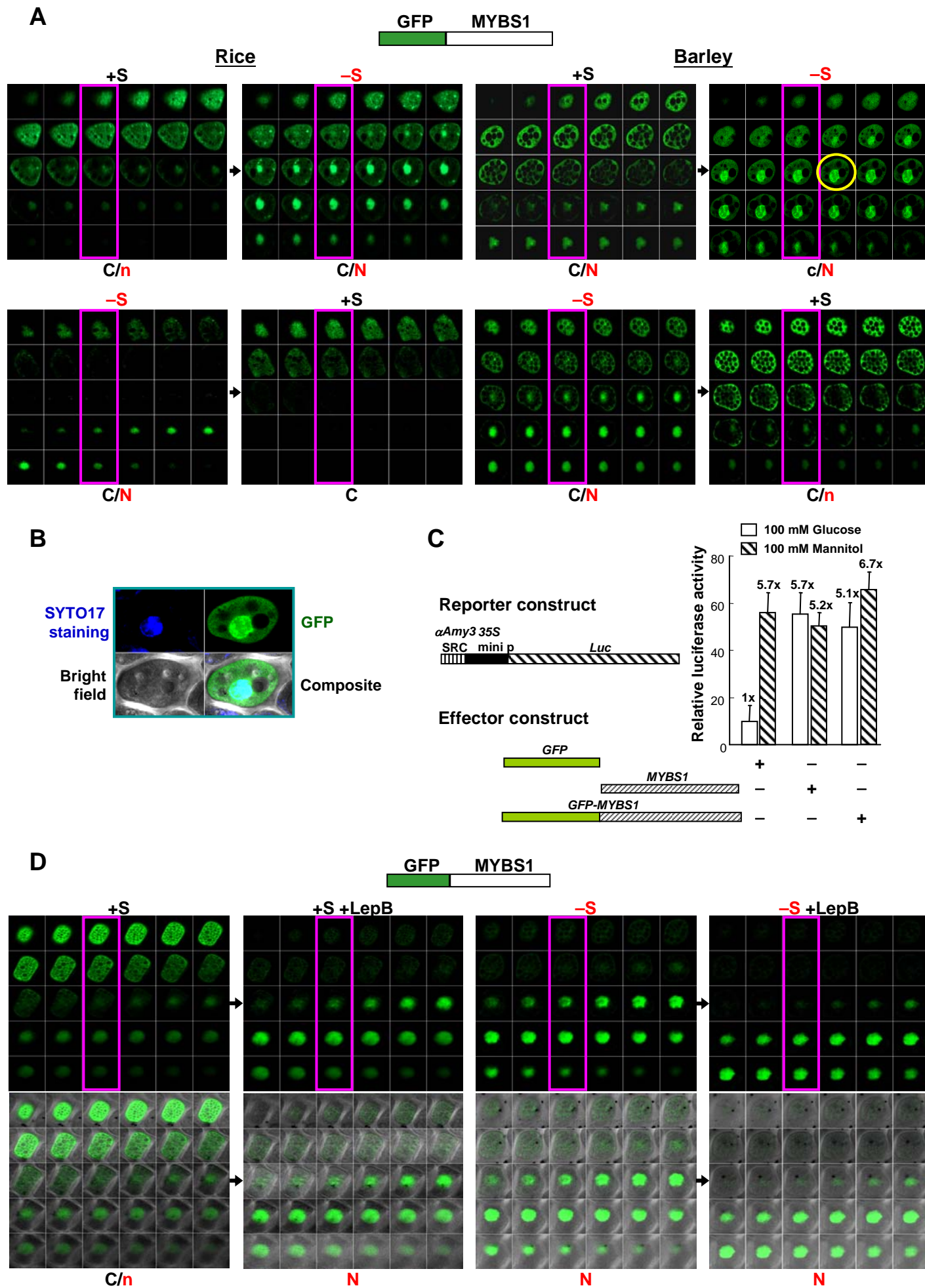


Figure S1 (Hong)



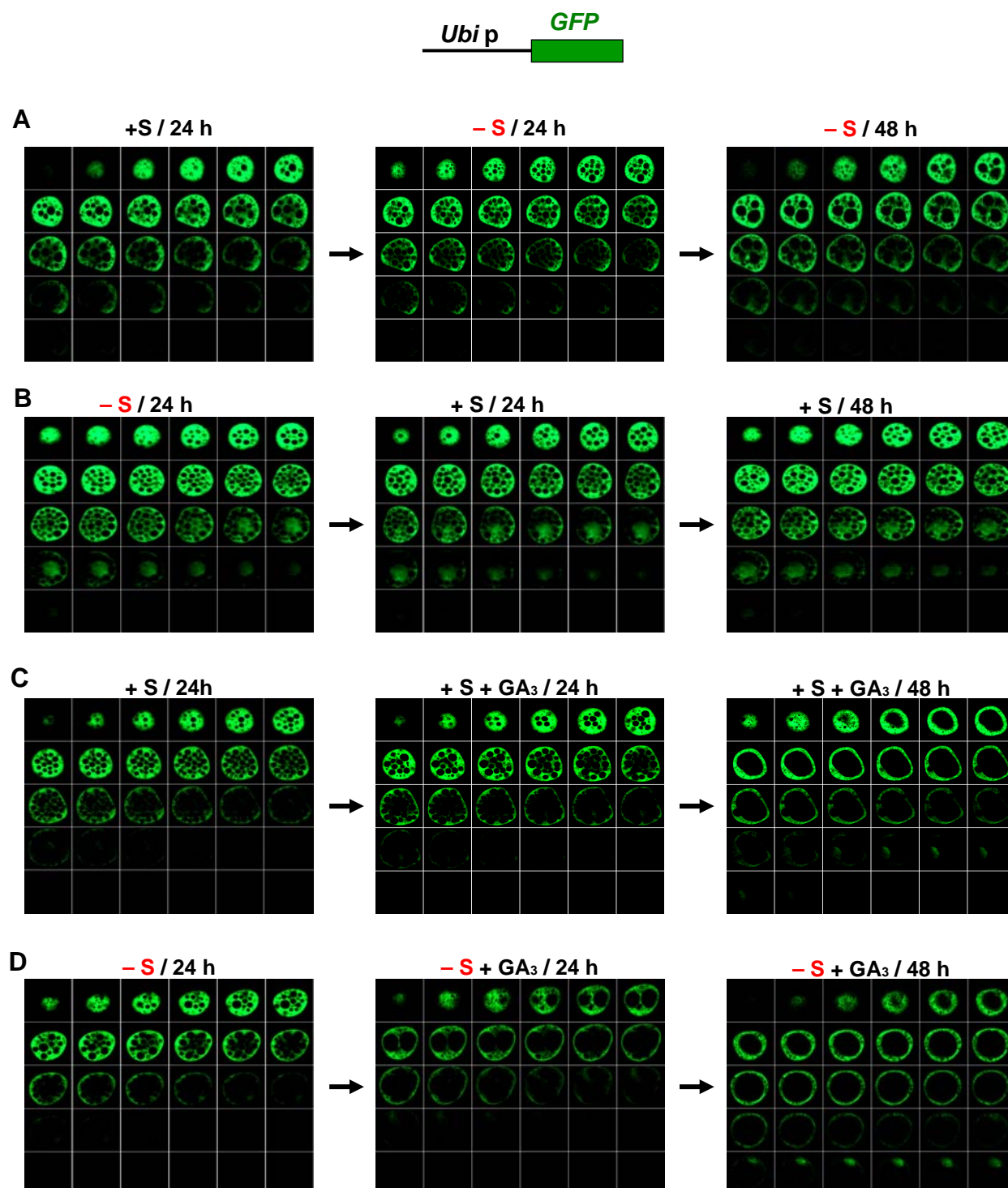
Supplemental Figure 1. Glucose Regulates the Nucleocytoplasmic Shuttling of MYBS1, Related to Figures 1A and 1C.

(A) Glucose inhibits the nuclear localization of MYBS1. Rice and barley aleurones were transfected with *Ubi:GFP-OsMYBS1*, incubated in +S or –S medium for 24 h, and then switched from +S to –S medium or from –S to +S medium and incubated for another 24 h. Boxes indicate images shown in Figure 1A. C and N indicate higher and c and n indicate lower GFP signals in the cytoplasm and nucleus, respectively.

(B) Accumulation of GFP-MYBS1 in nuclei was confirmed by staining with the red fluorescent dye SYTO17 that specifically stains nuclei. Rice aleurones were transfected with *Ubi:GFP-OsMYBS1* and incubated in -S medium containing SYTO17 for 24 h. The circled cell in (A) was examined for localization of the nucleus and GFP signal.

(C) The GFP-MYBS1 fusion protein was as active as MYBS1. *Ubi:GFP*, *Ubi:OsMYBS1*, or *Ubi:GFP-OsMYBS1* served as the effector and α .*Amy3 SRC-CaMV35S:Luc* served as a reporter. Rice embryos were co-transfected with effector and reporter, and incubated with glucose or mannitol for 24 h. Subsequently, luciferase activity was assayed. The luciferase activity in rice aleurones transfected with reporter constructs and *Ubi:GFP* was set as 1X, and other values were calculated relative to this value. Error bars indicate the SE for three replicate experiments

(D) MYBS1 accumulated exclusively in the nucleus in both +S and –S medium in the presence of LepB. Rice aleurones were transfected with *Ubi:GFP-OsMYBS1* and incubated in +S or –S medium for 24 h. After addition of LepB, aleurones were incubated for another 24 h. Boxes indicate images shown in Figure 1C. C and N indicate higher and c and n indicate lower GFP signals in the cytoplasm and nucleus, respectively.



Supplemental Figure 2. GFP without MYBS1 Was Distributed In Both the Cytoplasm and the Nucleus of Aleurone Cells Regardless of the Medium (+S or -S) or the Presence or Absence of GA₃, Related to Figures 1A and 3A.

As negative controls of Figures 1A/Supplemental Figure 1A and Figures 3A/Supplemental Figure 5A, rice aleurones were transfected with *Ubi:GFP* and incubated in:

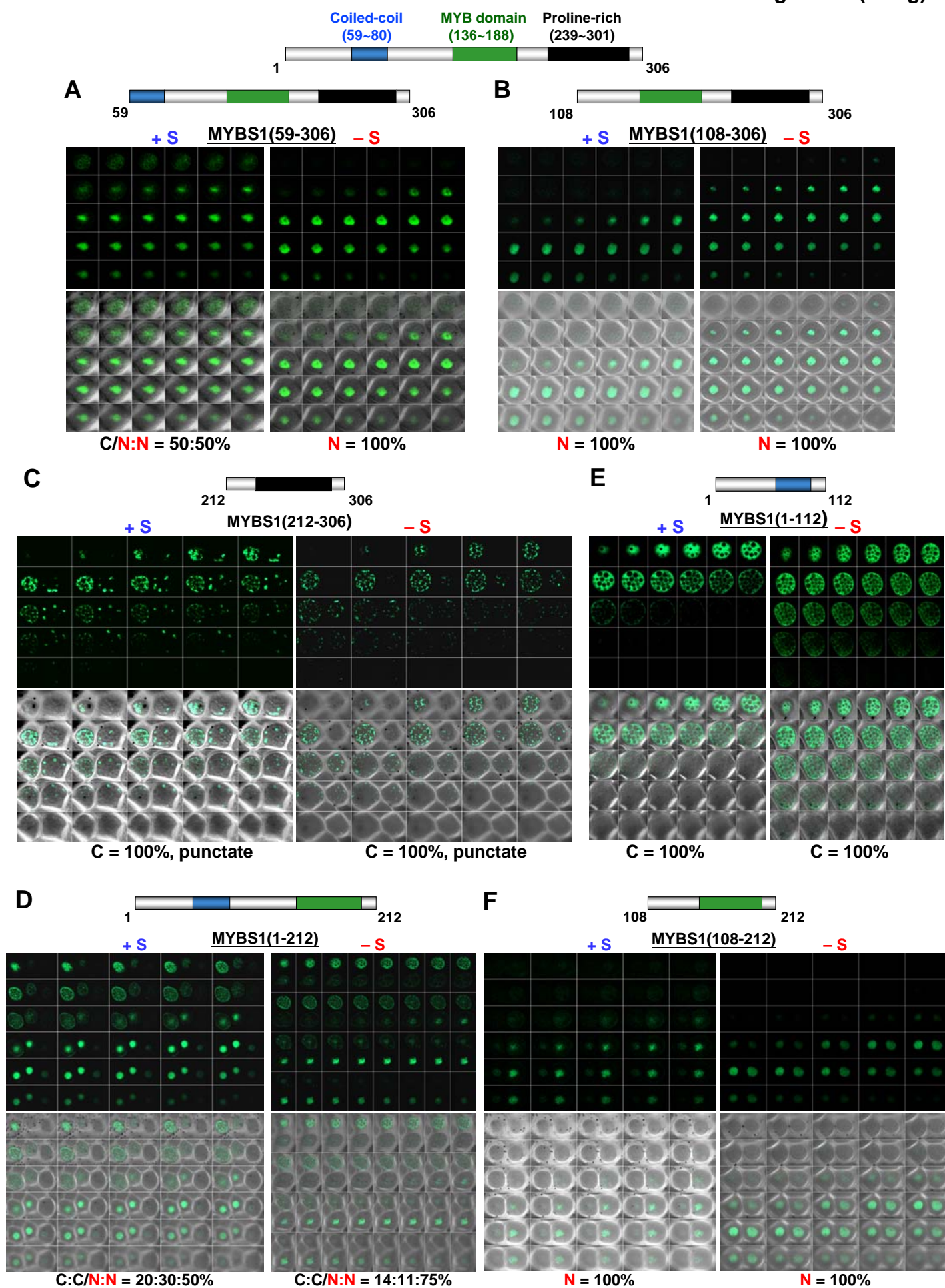
(A) +S medium for 24 h, and then switched to -S medium for another 24 and 48 h.

(B) -S medium for 24 h, and then switched to +S medium for another 24 and 48 h.

(C) +S medium for 24 h, followed by incubation for another 24 and 48 h after addition of GA₃.

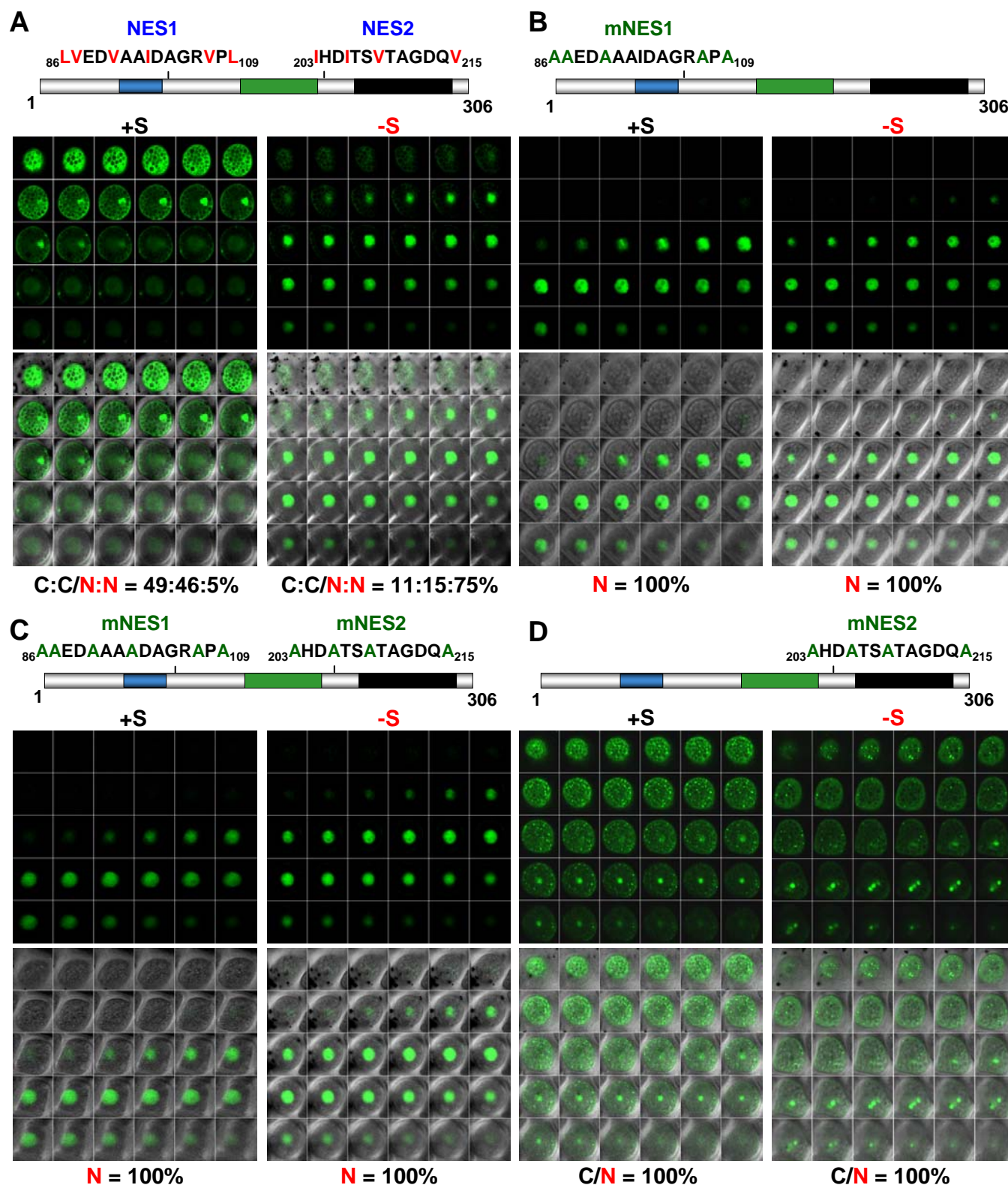
(D) -S medium for 24 h, followed by incubation for another 24 and 48 h after addition of GA₃.

GA₃-induced severe vacuolation was observed in (C) and (D).



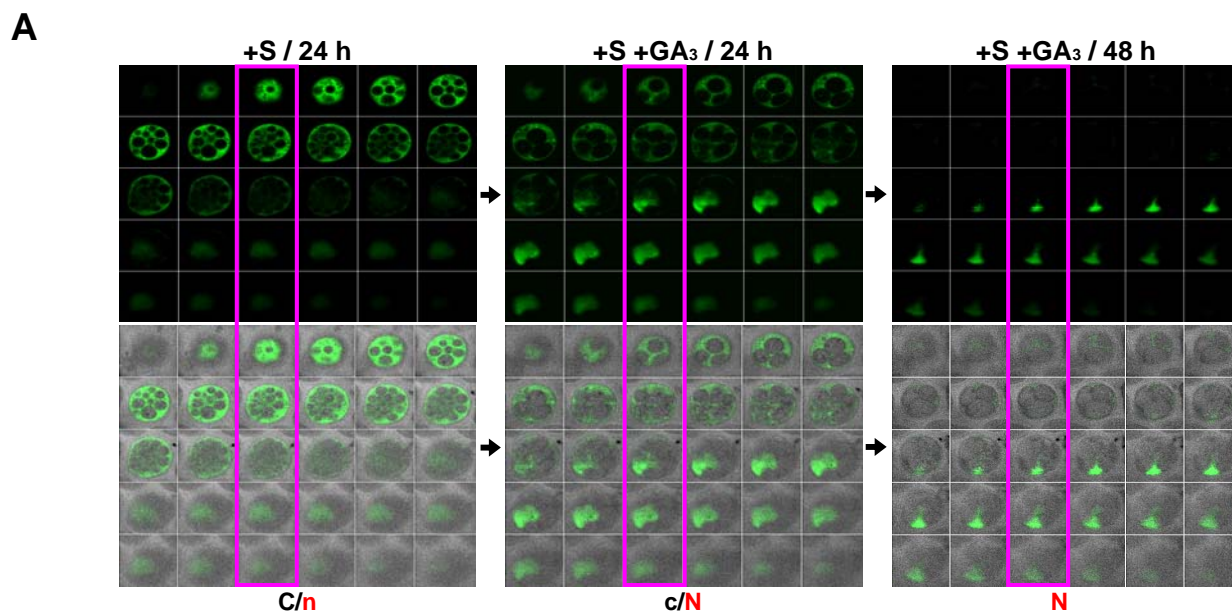
Supplemental Figure 3. OsMYBS1(108-212) Contains a Nuclear Localization Signal (NLS), and OsMYBS1(1-112) Contains a Cytoplasmic Retention Signals (CRS), Related to Figure 2A.

Barley aleurones were transfected with plasmids containing *GFP*-fused with **(A)** OsMYBS1(59-306), **(B)** OsMYBS1(108-306), **(C)** OsMYBS1(212-306), **(D)** OsMYBS1(1-212), **(E)** OsMYBS1(1-112), or **(F)** OsMYBS1(108-212), and incubated in +S or –S medium for 24 h. Percentage (%) = cells in the cytoplasm (C) or nucleus (N) per total cells expressing GFP examine

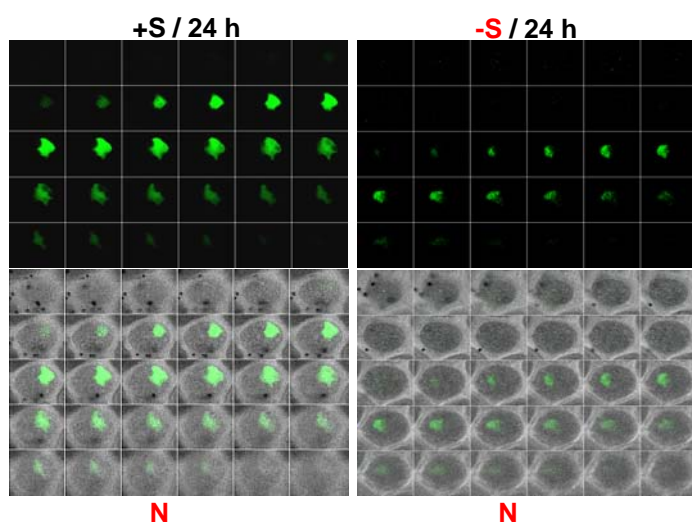


Supplemental Figure 4. NES1 Is a Major Nuclear Export Signal of OsMYBS1, Related to Figure 2C.

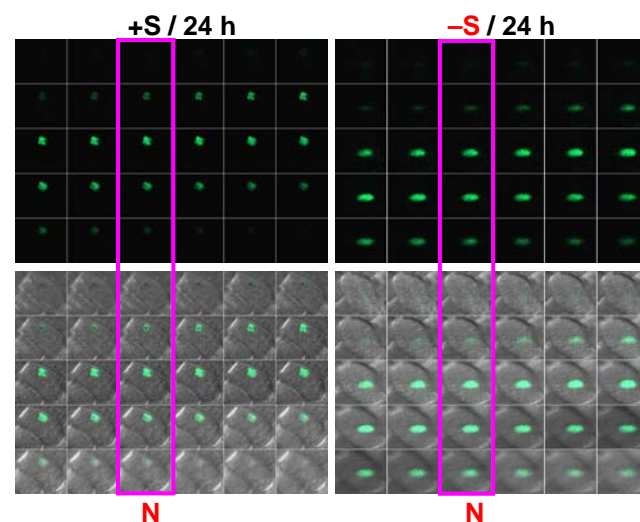
Barley aleurones were transfected with *Ubi:GFP-OsMYBS1* with (A) wild type NES, (B) mutations in NES1, (C) mutation in both NES1 and NES2, or (D) mutation in NES2, and incubated in +S or -S medium for 24 h. Percentage (%) = cells in the cytoplasm (C) or nucleus (N) per total cells expressing GFP examined. In (D), GFP-OsMYBS1 showed punctate pattern in nucleus and cytoplasm.



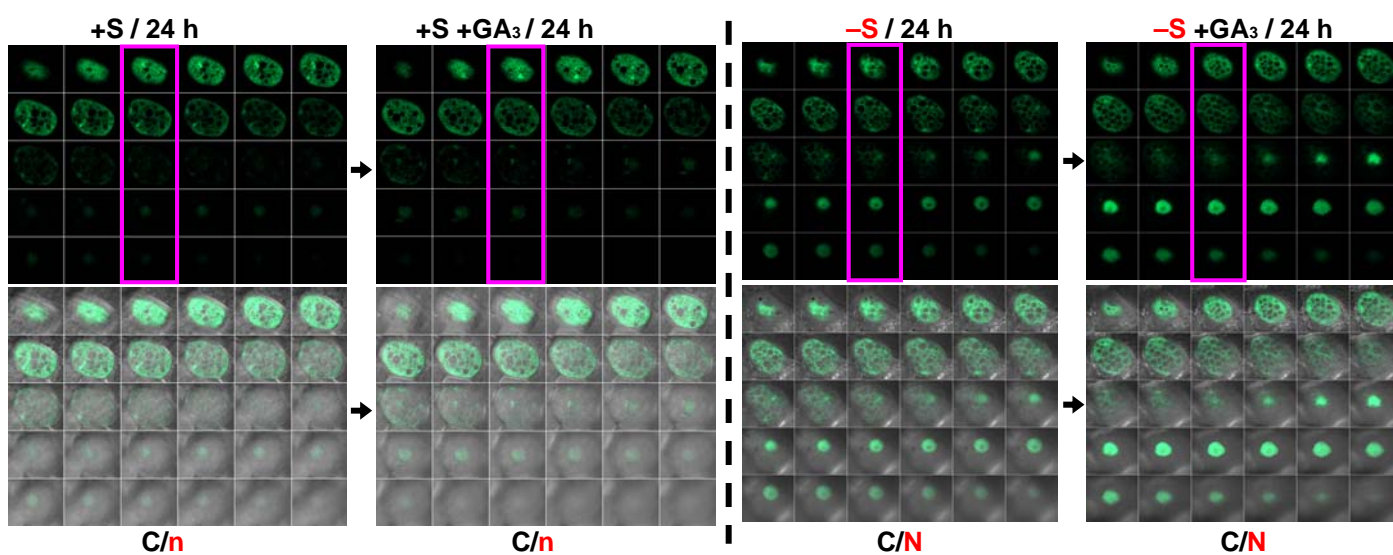
B MYBGA transient overexpression
in barley aleurones



C MYBGA stable overexpression
in transgenic rice aleurones



D MYBGA deficient mutant (*gamyb-2*)



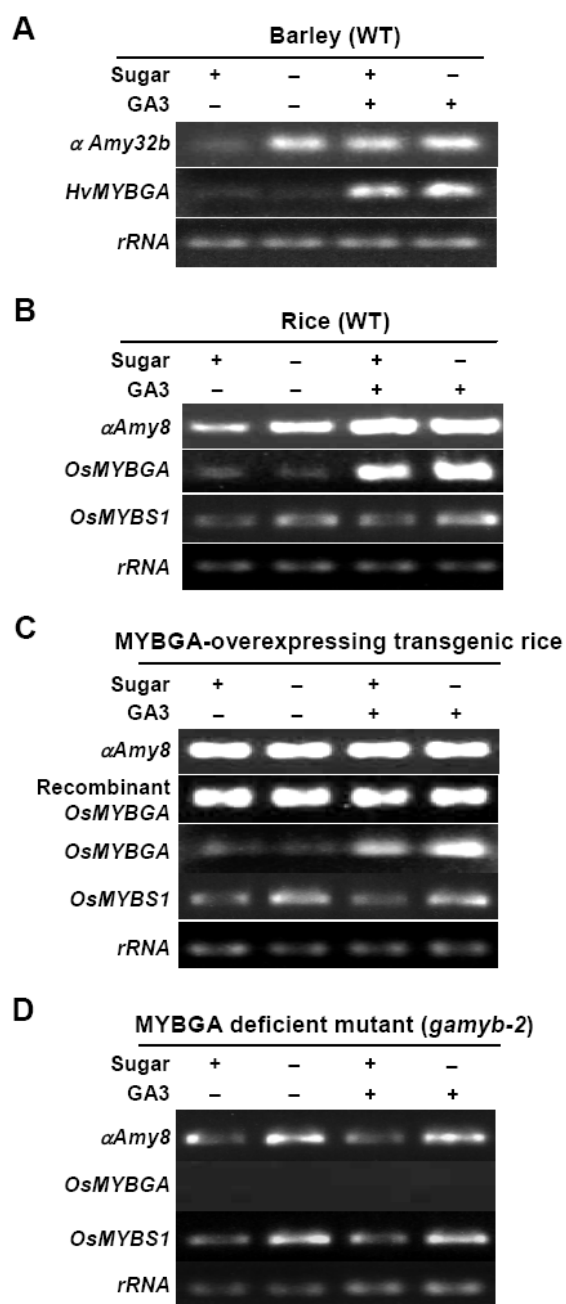
Supplemental Figure 5. GA₃ Promotes Nuclear Localization of MYBS1, and MYBGA Is Sufficient and Necessary for Promoting Nuclear Localization of MYBS1, Related to Figures 3A-C.

(A) GA₃ promotes nuclear localization of MYBS1 in the presence of glucose. Barley aleurones were transfected with *Ubi:GFP-OsMYBS1* and incubated in +S medium for 24 h. After addition of GA₃, they were incubated for another 24 and 48 h. Vacuolation in GA₃-treated aleurone cells was observed in bright field images. Boxes indicate images shown in Figure 3A.

(B) Transient overexpression of MYBGA promotes nuclear localization of MYBS1 in the presence of glucose. Barley aleurones were transfected with *Ubi:GFP-OsMYBS1* and *Ubi:OsMYBGA* and incubated in +S or –S medium for 24 h. Vacuolation in aleurone cells overexpressing MYBGA was observed in bright field images.

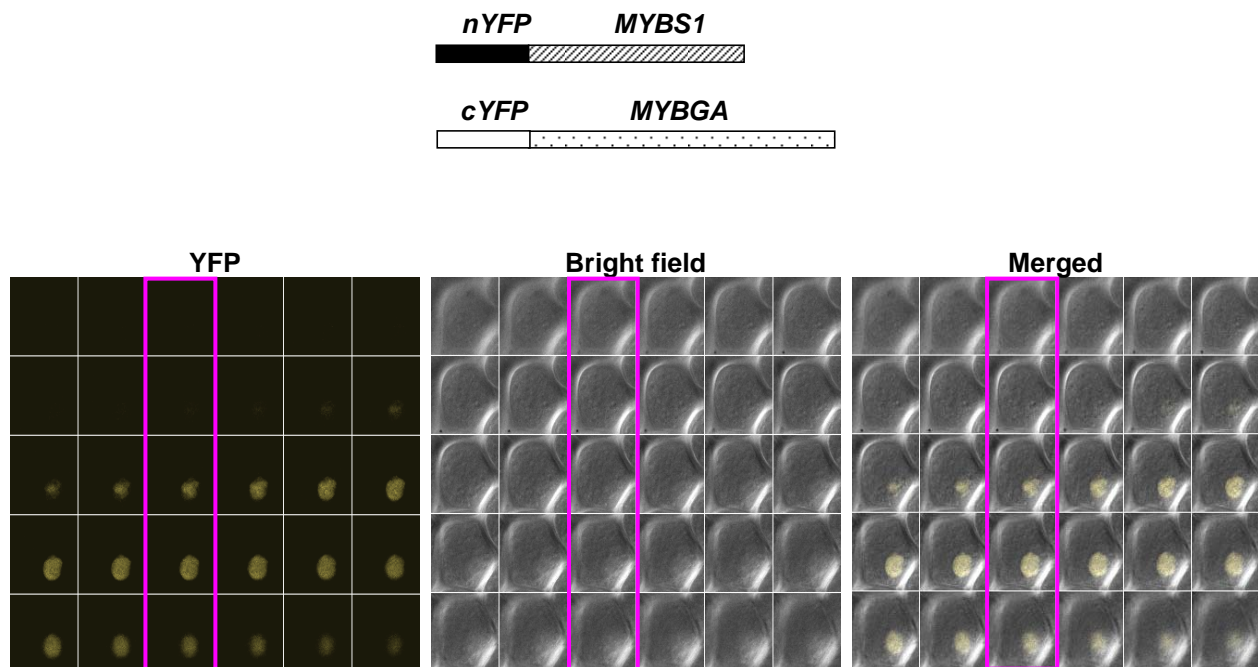
(C) Stable overexpression of MYBGA promotes nuclear localization of MYBS1 in the presence of glucose. Transgenic rice aleurones overexpressing OsMYBGA were transfected with *Ubi:GFP-OsMYBS1* and incubated in +S or –S medium for 24 h. Boxes indicate images shown in Figure 3B.

(D) MYBGA is necessary for promoting nuclear localization of MYBS1 in the presence of glucose. Aleurones of *gamyb-2* mutant rice seeds were transfected with *Ubi:GFP-OsMYBS1* and incubated in +S or –S medium for 24 h. After addition of GA₃, they were incubated for another 24 h. Boxes indicate images shown in Figure 3C. The image of upper panel is a fluorescent field and the lower panel is a composite of fluorescent and bright fields. C and N indicate higher and c and n indicate lower GFP signals in the cytoplasm and nucleus, respectively.



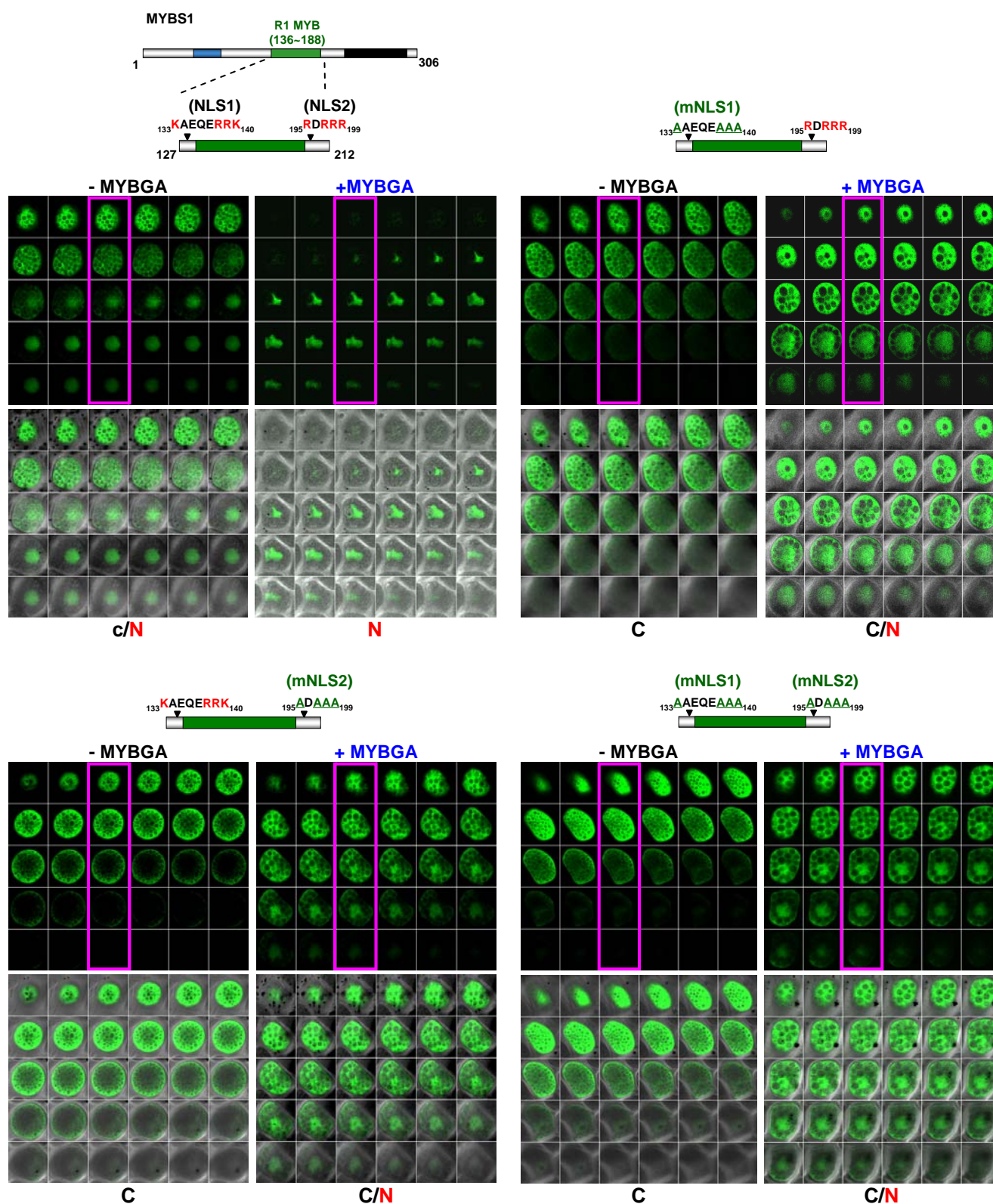
Supplemental Figure 6. GA and MYBGA Antagonize Sugar Repression of α -Amylase Expression. Related to Figures 3D-F.

(A) Accumulation of α Amy32b and HvMYBGA mRNA in barley aleurones. **(B)-(D)** Accumulation of α Amy8, OsMYBGA, OsMYBS1 and mRNA in aleurones of wild-type rice, MYBGA overexpressing transgenic rice, and *gamyb-2* mutant rice, respectively. Barley or rice aleurone layers were incubated in +S or -S medium with or without GA3 for 24 h. Total RNA was purified and subjected to RT-PCR analysis.



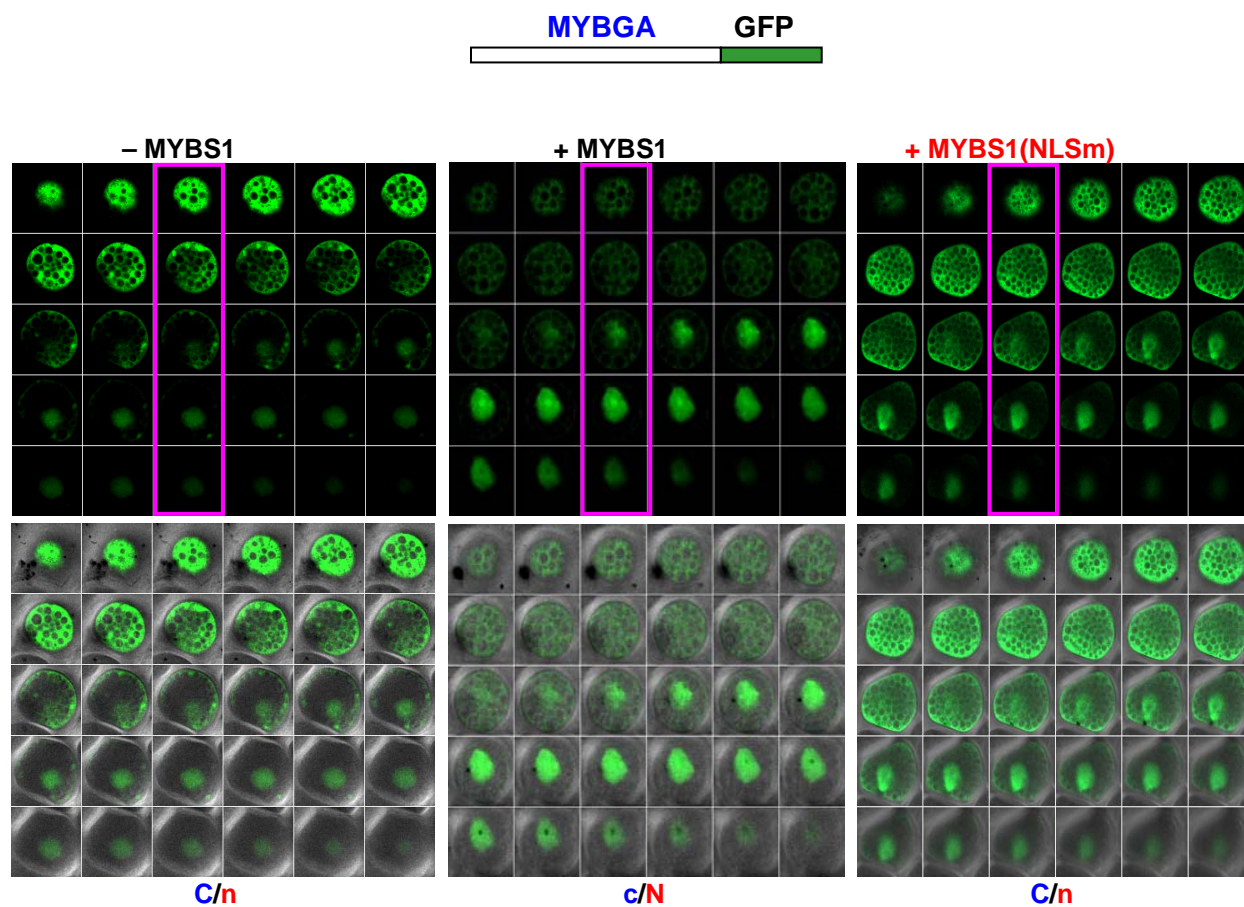
Supplemental Figure 7. BiFC Complementation Test Indicates MYBS1 and MYBGA Interact Physically, Related to Figure 4A.

Rice aleurones were co-transfected with *Ubi:nYFP-OsMYBS1* and *Ubi:cYFP-OsMYBGA*, and incubated in +S medium for 24 h. YFP was detected by a confocal microscopy. Boxes indicate images shown in Figure 4A



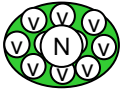
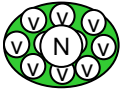
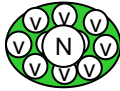
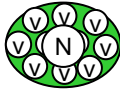
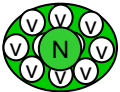








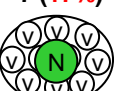

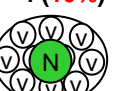
Supplemental Figure 8. MYBS1 and MYBGA Interact in the Cytoplasm Prior to Co-transport Into the Nucleus, Related to Figure 5A.

Barley aleurones were transfected with *Ubi:GFP-OsMYBS1* (wild-type or mutated in NLS1 and/or NLS2) and with or without *Ubi:OsMYBGA*, and incubated in -S medium for 48 h. Vacuolation was observed in the presence of MYBGA. Boxes indicate images shown in Figure 5A. C and N indicate higher and c and n indicate lower GFP signals in the cytoplasm and nucleus, respectively.



Supplemental Figure 9. Overexpression of MYBS1 Promotes Nuclear Localization of MYBGA, Related to Figure 5B

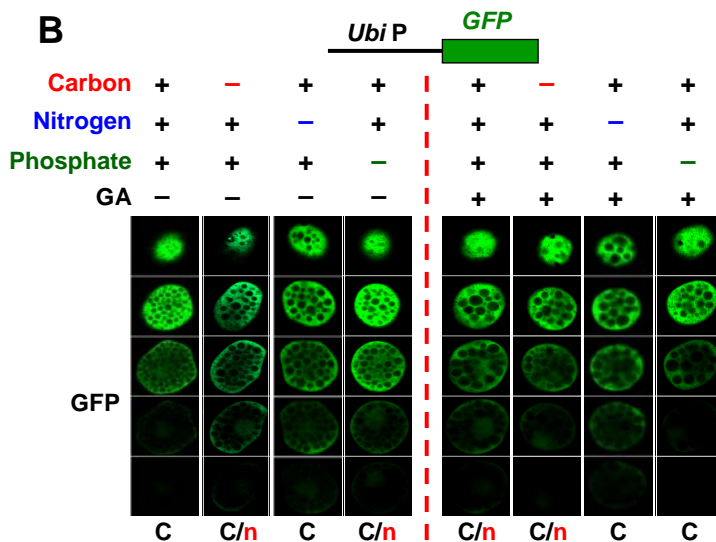
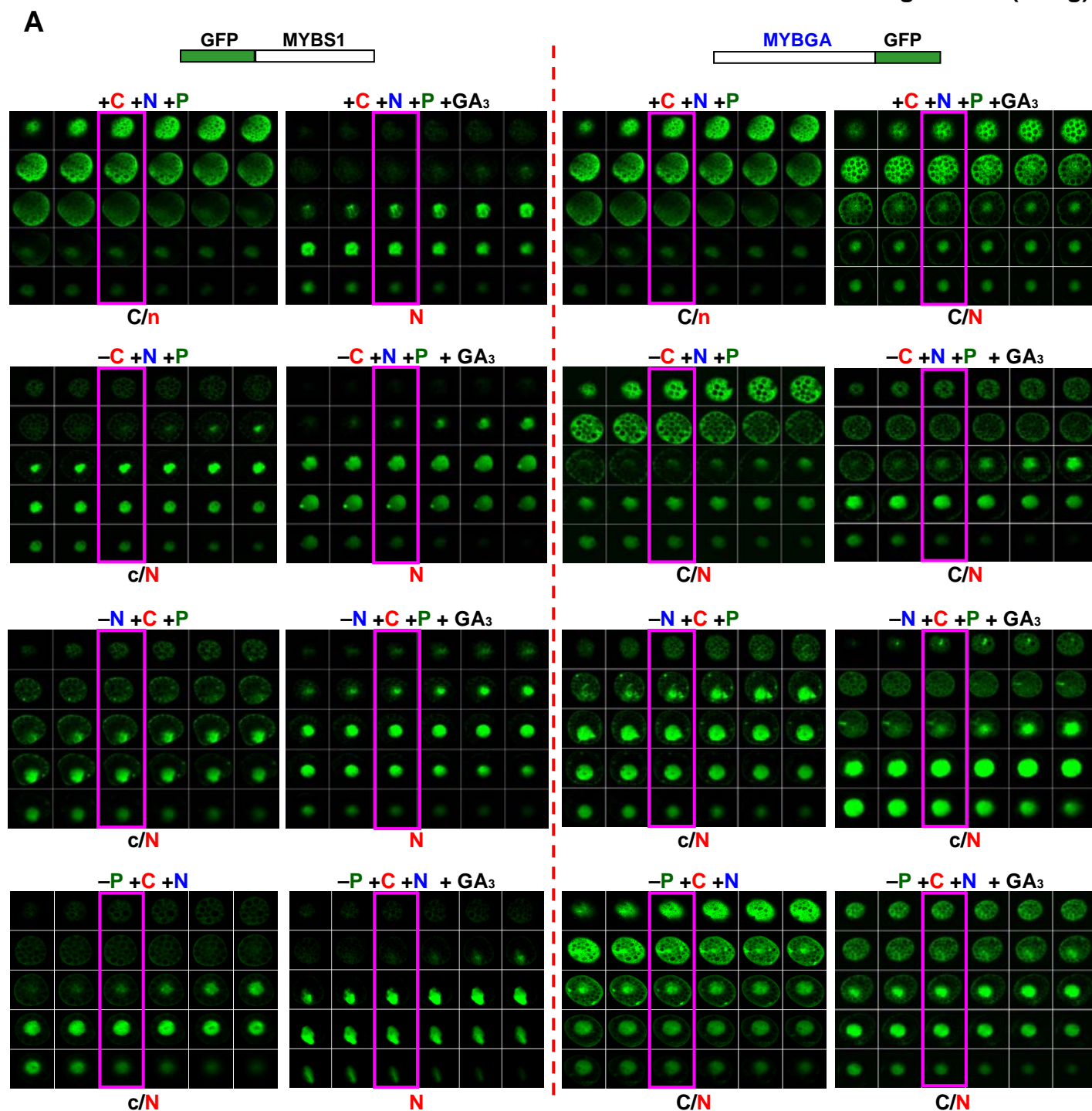
Barley aleurones were transfected with *Ubi:OsMYBS1* and *Ubi:OsMYBGA-GFP*, and incubated in +G medium for 24 h. The image of upper panel is a fluorescent field and the lower panel is a composite of fluorescent and bright fields. Boxes indicate images shown in Figure 5B.

Glucose		+	+	-	-
OsMYBGA-GFP		+	+	+	+
MYBS1		-	+	-	+
Number of cells with different locations of MYBGA-GFP	C > N (C/n)	44 (76%) 	3 (7%) 	31 (67%) 	0 
	C = N (C/N)	14 (24%) 	9 (22%) 	15 (33%) 	8 (20%) 
	C < N (c/N)	0 	21 (54%) 	0 	28 (70%) 
	N	0 	7 (17%) 	0 	4 (10%) 
Total cell no.		58	41	46	32

Supplemental Figure 10. The nuclear localization of MYBGA is enhanced by MYBS1

Barley aleurones were transfected with *Ubi:OsMYBGA-GFP* only or with *Ubi:OsMYBGA-GFP* plus *Ubi:OsMYBS1*, incubated in +sugar or -sugar medium for 24 h, and GFP signal was detected after 24 h. **N**: nucleus; C: cytoplasm; V: vacuole. C>N (C/n): signal higher in cytoplasm; C=N (C/N): signal similar between cytoplasm and nucleus; C<N (c/N): signal lower in cytoplasm. %: Percentage of number of cells with GFP distribution in the indicated category divided by total number of cells examined/

Figure S12 (Hong)



Supplemental Figure 12. C, N and P Starvation Promote the Nuclear Import of MYBS1 and MYBGA, Related to Figure 7B.

(A) Barley aleurones were transfected with *Ubi:GFP-OsMYBS1* (left panel) or *Ubi:OsMYBGA-GFP* (right panel). Boxes indicate images shown in Figure 7B.

(B) Barley aleurones were transfected with *Ubi:GFP*. Transfected aleurones were incubated in medium with or without glucose (C), nitrogen (N), or phosphate (P) in the absence or presence of GA₃ for 24 h. C and N indicate higher and c and n indicate lower GFP signals in the cytoplasm and nucleus, respectively.

Supplemental Table 1. Primer list.

Primer	Nucleotide sequence (5'→3')
S1-1(F)	CACCATGACCTCCCAGGCGGCGACG
S159(F)	CACCATGAGGTCTGGCGGAGGAGGTGCGGA
S1-108(F)	CACCATGTCCAAGGACGGCGGACAC
S1-127(F)	CACCATGGGATATCGCAAGAGCTGCTCCAAG
S1-212(F)	CACCATGGCCGGCGATCAGGTCGCCGCG
S1-112(R)	ACAGGATCCGCCGTCTTGGAC
S1-212(R)	TTACTAGCCGGCGGTGACGCTGGTGATGT
S1-306(R)	CTATTGGTGATCTTGGCCGGTGGC
S1-127(F)-133m	CACCATGGCGGCGAGAGCTGCTCCGAGGCGGAGCAGGAGGAGGAGGCCATCCCA TGGACGGAGGAA
S1-212R-196m	GGTGACGCTGGTGATGTCGTGGATGCTGGATTCTTCTTCGTCTTCGTTTCATGGAGGTGAG
S1-141(F)	GGGATCCCATGGACGGAGGAAGAGCACAGG
S1-145(R)-NLSm	ATGGATCCCATTTTCTTCTCCTGCTCCGCATTGGAGCAGCTCTTGCCGCCGTCGTA
S1-191(F)-NLSm	CTGAATTCCATGAACGAAGACGAAGAAGAATCCAGCATCCACGACATCA
S1-193(R)	CATGGAATTCAGGCGGATGAAGTACTTCTGCGCGT
S1-NES1m-(F)	GGCTGCAGACGCGGGCCGCGCTCCGGCTCCGCGCTACGCCGGGAGGAGTCCGCG
S1-NES1m-(R)	GTCTGCAGCCGCGAGGTCCTCAGCAGCCGCCTCGTAGTGCCTCCGCACCTCCTCCGC
S1-NES2m-(F)	GGCGATCAGGCGGCCGCGCAGCAGGGCGCCCGATCACCGG
S1-NESm-(R)	CTGCTGCGCGGCCGCTGATCGCCGGCGGTTGCGCTGGTTGCGTCGTGTGCGCTGGA GCGGCGGCGGTGCGGTTTCAT
S1-1(F)- <i>KpnI</i>	AAAGGTACCATGACCTCCCAGGCGGCGACG
GA-1(F)	CACCATGTATCGGGTGAAGAGCGAGA
GA-553(R)	AGATCATTTGAATTCTGACATTTT
GA-1(F)- <i>KpnI</i>	AAAGGTACCATGTATCGGGTGAAGAGCGAGA
YFP-342(R)- <i>KpnI</i>	AGAGGTACCGATAGATCTCTTGTACAGCTCGTCCAT
YFP155(R)- <i>KpnI</i>	CCAGGTACCGCCATGATATAGACGTTGTG
YFP-156(F)	CACCATGGACAAGCAGAAGAACGGCATC
YFP-1(F)	CACCATGGTGAGCAAGGGCGAGGAGCT
GFP-239(R)	CCCCTTGTACAGCTCGTCCATGCC
GFP-1(F)	TTAGCATGCATGGTGAGCAAGGGCGAGGAGCTGTT
GBD(R)	GATATCTCACTTAGGAAATTCGCCCGGAATTA
GBD(F)	CACCATGAAGCTACTGTCTTCTA
GAD(F)	CACCATGGATAAAGCGGAATTAATTCC
GAD(R)	GATATCTCACTTAGGAAATTCGCCCGGAATTA
AK059511-Q5	CAGCGACAAGATTTGGAACA
AK059511-Q3	CAGGTAGCTCCTGCAATCGT

AK061988-Q5	CTTTGGGGGCGGAAGTAA
AK061988-Q3	GGAGACTAGTTTAATACGACATGACAA
AK061237-Q5	GCCATCAAGGCCTGCTAC
AK061237-Q3	CGATGAAGAAGCGGTTGG
AK064300-Q5	TCTTCCAGGGGTTAACTGG
AK064300-Q3	ATCTCCTCCACCTTCTCGTG
AK069132-Q5	CATCAATCTTGGCTTTACTGAGG
AK069132-Q3	AGAATTGCAGGCACAGCAG
AK069691-Q5	CGGCTCGATCATTCTCTTC
AK069691-Q3	ACGGATCATTGACTGTCCTGTA
AK069697-Q5	GCGATCGTCATGGAGTACG
AK069697-Q3	GCTGCTGGAAGAAGAAACGA
AK070261-Q5	CCAATTACGCTTCTGGAGGA
AK070261-Q3	GCGAAGTACACGATTTGCTTT
AK072228-Q5	CGTCTACGACGTCAAGAGCA
AK072228-Q3	CAAGTATCCATGCGGACCAT
AF099203-Q5	CCGCAAATCCCAGCTATAAAT
AF099203-Q3	TTGGAATTGGAGGGTGAGG
AK100323-Q5	CTTCTGGAGAGCCTTGCAGT
AK100323-Q3	CCAGCATTAGCAAGTTGGTTG
AK106694-Q5	GGTGCTGCAGGTGGAAAT
AK106694-Q3	CGAGAAGAGGCCGTAGTCC
AK110902-Q5	ACCACAGCCGAGTGATCC
AK110902-Q3	ATGCCGCTAGCACTTGATCT
Lip2p-F	CACCATCTTGATTATTCATATCCTA
Lip2p-R	AGGTAGCTCGTGTGGGTTGATGCG
LIP1p-F	CACCCAGGGGGTTCAACTCCGGGCT
LIP1p-R	CCACGAAAGAACAAAGCGTAACGCA
PHO1-F	CACCGCTCCATTGTCTCCAACCTGC
PHO1-R	ATGCCATTTCGTAGAACTGTTTGTG
PHOSp-F	CACCTCGATACACACATGGCACATG
PHOSp-R	GAGGAGAATGGGAGGCCGCGGG

Supplemental Table 2. PCR pairs used for plasmid construction.

Forward primer	Reverse primer	Product (Plasmid)
MYBS1		
S1-1(F)	S1-112(R)	MYBS1(1-112) [pUbi-GFP-MYBS1(1-112)-Nos]
S1-1(F)	S1-212(R)	MYBS1(1-212)[pUbi-GFP-MYBS1(1-212)-Nos]
S1-108(F)	S1-212(R)	MYBS1(108-212)[pUbi-GFP-MYBS1(108-212)-Nos]
S1-108(F)	S1-306(R)	MYBS1(108-306)[pUbi-GFP-MYBS1(108-306)-Nos]
S1-212(F)	S1-306(R)	MYBS1(212-306)[pUbi-GFP-MYBS1(212-306)-Nos]
S1-127(F)	S1-212(R)	MYBS1(127-212)[pUbi-GFP-MYBS1(127-212)-Nos]
S1-127(F)-133m	S1-212(R)	MYBS1(127-212)(mNLS1)[pUbi-GFP-MYBS1(127-212)(mNLS1)-Nos]
S1-127(F)	S1-212(R)-196m	MYBS1(127-212)(mNLS2)[pUbi-GFP- MYBS1(127-212)(mNLS2)-Nos]
S1-127(F)-133m	S1-212R-196m	MYBS1(127-212)(mNLS1,2)[pUbi-GFP-(127-212)(mNLS1,2)-Nos]
S1-1 (F)	S1-145(R)-NLSm	MYBS1 (mNLS1)[pUbi-GFP-MYBS1 (mNLS1)-Nos]
S1-141(F)	S1-306(R)	
S1-1 (F)	S1-193(R)	MYBS1 (mNLS2)[pUbi-GFP-MYBS1 (mNLS2)-No2]
S1-191F-NLS2m	S1-306(R)	
S1-1 (F)	S1-145(R)-NLSm	MYBS1 (m NLS1,2)[pUbi-GFP-MYBS1 (mNLS1,2)-Nos]
S1-141F	S1-306(R)	Using pUbi-GFP-MYBS1 (mNLS2)-Nos as tamplate
S1-1 (F)	S1-145(R)-NLSm	MYBS1 (m NLS1)[pUbi- MYBS1 (m NLS1)-Nos]
S1-141(F)	S1-306(R)	
S1-1 (F)	S1-193(R)	MYBS1 (m NLS2)[pUbi- MYBS1 (m NLS2)-Nos]
S1-191F-NLS2m	S1-306(R)	
S1-1 (F)	S1-145(R)-NLSm	MYBS1 (mNLS1,2)[pUbi-MYBS1 (mNLS1,2)-Nos]
S1-141F	S1-306(R)	Using pUbi-MYBS1 (m NLS2)-Nos as tamplate
S1-59(F)	S1-306(R)	MYBS1(59-306)[pUbi-GFP-MYBS1(59-306)-Nos]
S1-1 (F)	S1-NES1m(R)	MYBS1 (m NES1)[pUbi-GFP-MYBS1 (m NES1)-Nos]
S1-NES1m(F)	S1-306(R)	
S1-1 (F)	S1-NES2m(R)	MYBS1 (m NES2)[pUbi-GFP-MYBS1 (m NES2)-Nos]
S1-NES2m(F)	S1-306(R)	
S1-1 (F)	S1-NES2m(R)	MYBS1 (m NES1, 2)[pUbi-GFP-MYBS1 (m NES1, 2)-Nos]
S1-NES2m(F)	S1-306(R)	
MYBGA		
GA-1(F)	OsGA553(R)+S	MYBGA (pUbi-GAD-MYBGA-Nos)
GA-1(F)	OsGA553(R)+S	MYBGA (pUbi-GFP-MYBGA-Nos)

BiFC

YFP-1(F)	YFP155(R)-xS- <i>KpnI</i>	nYFP-MYBS1 (pUbi-nGFP-MYBS1-Nos)
S1-1(F)- <i>KpnI</i>	S1-306 (R)	
YFP-156 F)	YFP342 (R)-xS- <i>KpnI</i>	cYFP-MYBS1 (pUbi-cGFP-MYBGA-Nos)
GA-1 (F)- <i>KpnI</i>	OsGA553(R)+S	

Promoter assay

Lip2p-GF	Lip2p-R	pLIP2 (pLip2-Luc-Nos)
PHOSp-GF	PHOSp-GF	pPhospho1 (pPhospho1-Luc-Nos)
LIP1p-GF	LIP1p-R	pLIP1 (pLip1-Luc-Nos)
PHO1-GF	PHO1-R	pPHO1 (pPHO1-Luc-Nos)

Quantitative RT-PCR

AK059511-Q5	AK059511-Q3	Lip2: GDSL-motif lipase
AK061237-Q5	AK061237-Q3	Phospho1: Phosphatase-like
AK061988-Q5	AK061988-Q3	UBQ
AK064300-Q5	AK064300-Q3	α Amy8/Ramy3E
AK069132-Q5	AK69132-Q3	ST: Sugar transporter family protein
AK069691-Q5	AK069691-Q3	NAP2: Nepenthesin-1 aspartic protease precursor 2
AK069697-Q5	AK069697-Q3	SAPK9: Serine/threonine protein kinase
AK070261-Q5	AK070261-Q3	Lip1: GDSL-motif lipase
AK072228-Q5	AK072228-Q3	NAP1: Nepenthesin-1 aspartic protease precursor 1
AF099203-Q5	AF099203-Q3	EP3A: Cysteine protease
AK100323-Q5	AK100323-Q3	PHO1: phosphate transporter
AK106694-Q5	AK106694-Q3	PT1: <i>Zea mays</i> phosphate transport protein
AK110902-Q5	AK110902-Q3	GLN: Putative β -1,3-glucanase

Supplemental Table 3. Quantitative analysis of α Amy8, MYBGA, and OsMYBS1 mRNA levels in aleurones of wild-type rice, MYBGA overexpressing transgenic rice, and MYBGA deficient mutant rice.

Sugar	+	–	+	–
GA ₃	–	–	+	+
WT				
<i>αAmy8</i>	8.13*10 ⁻³ ±1.71*10 ⁻³	1.06±7.87*10 ⁻²	27.30±1.20	26.78±1.91
<i>OsMYBGA</i>	4.25*10 ⁻² ±2.24*10 ⁻³	1.03*10 ⁻¹ ±2.15*10 ⁻²	1.79*10 ⁻¹ ±2.08*10 ⁻²	1.84*10 ⁻¹ ±1.15*10 ⁻⁴
<i>OsMYBS1</i>	2.48*10 ⁻² ±2.30*10 ⁻³	3.57*10 ⁻² ±1.11*10 ⁻³	2.67*10 ⁻² ±5.83*10 ⁻³	3.71*10 ⁻² ±3.79*10 ⁻³
MYBGA-overexpressing transgenic rice				
<i>αAmy8</i>	89.26±2.82	86.79±2.85	91.02±7.70	99.11±7.94
<i>OsMYBGA</i>	4.05*10 ⁻¹ ±7.58*10 ⁻³	4.38*10 ⁻¹ ±3.15*10 ⁻²	3.94*10 ⁻¹ ±3.18*10 ⁻²	3.78*10 ⁻¹ ±3.89*10 ⁻²
<i>OsMYBS1</i>	2.93*10 ⁻² ±2.40*10 ⁻³	4.25*10 ⁻² ±1.95*10 ⁻³	2.78*10 ⁻² ±3.54*10 ⁻³	4.37*10 ⁻² ±1.62*10 ⁻³
MYBGA deficient mutant (<i>gamyb-2</i>)				
<i>αAmy8</i>	4.13*10 ⁻⁴ ±4.32*10 ⁻⁵	2.78*10 ⁻² ±3.07*10 ⁻⁵	3.74*10 ⁻⁴ ±2.96*10 ⁻⁵	2.05*10 ⁻² ±3.38*10 ⁻³
<i>OsMYBGA</i>	ND	ND	ND	ND
<i>OsMYBS1</i>	5.52*10 ⁻³ ±8.17*10 ⁻⁴	1.19*10 ⁻² ±2.20*10 ⁻³	4.48*10 ⁻³ ±1.02*10 ⁻³	1.02*10 ⁻² ±1.98*10 ⁻³