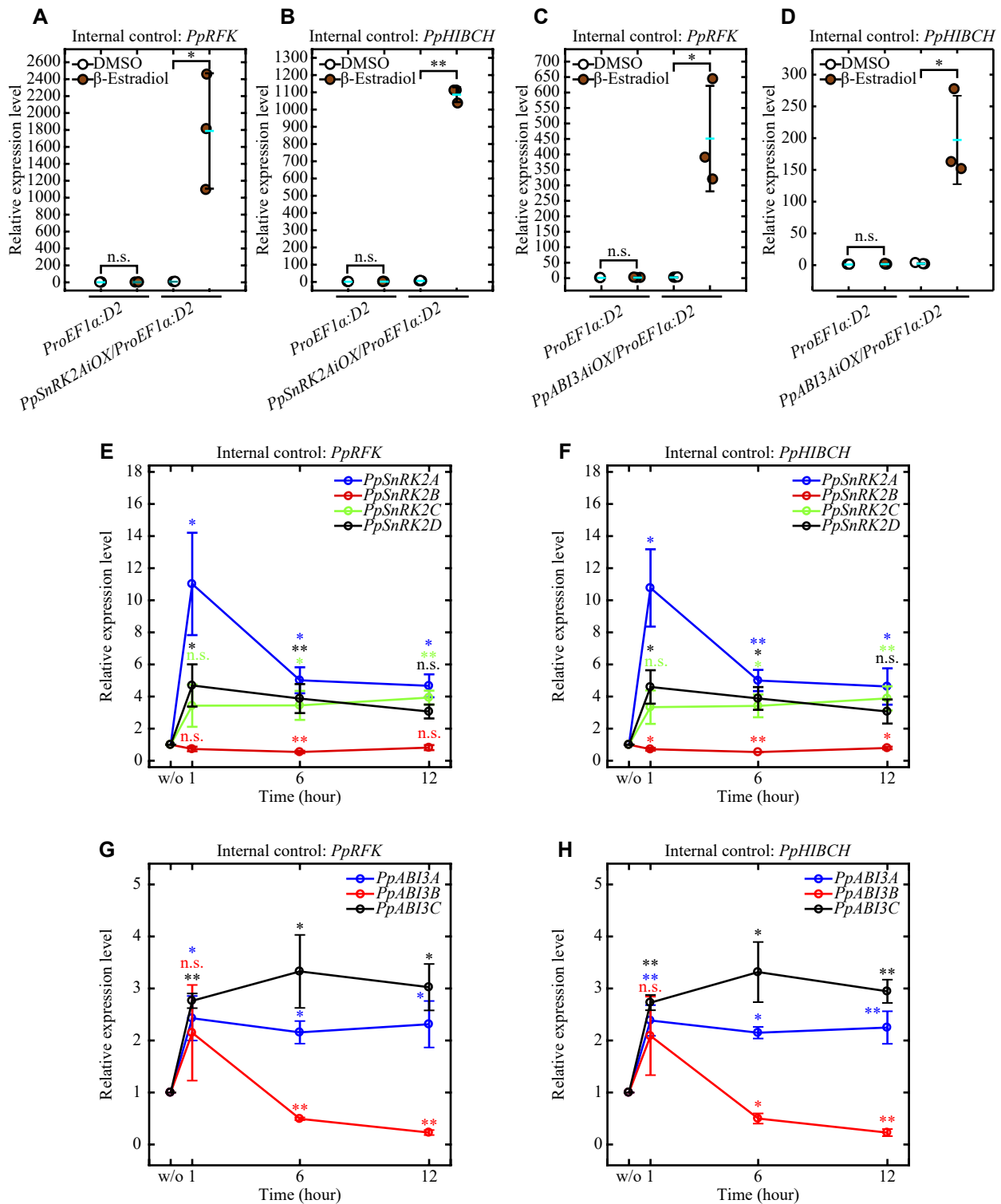




Title	Quantitative Imaging Reveals Distinct Contributions of SnRK2 and ABI3 in Plasmodesmatal Permeability in <i>Physcomitrella patens</i>
Author(s)	Tomoi, Takumi; Kawade, Kensuke; Kitagawa, Munenori; Sakata, Yoichi; Tsukaya, Hirokazu; Fujita, Tomomichi
Citation	Plant and Cell Physiology, 61(5), 942-956 https://doi.org/10.1093/pcp/pcaa021
Issue Date	2020-05
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Rights	This is a pre-copyedited, author-produced version of an article accepted for publication in Plant and cell physiology following peer review. The version of record Plant Cell Physiol (2020) 61 (5): 942–956 is available online at: https://doi.org/10.1093/pcp/pcaa021 .
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Supplementary data.pdf

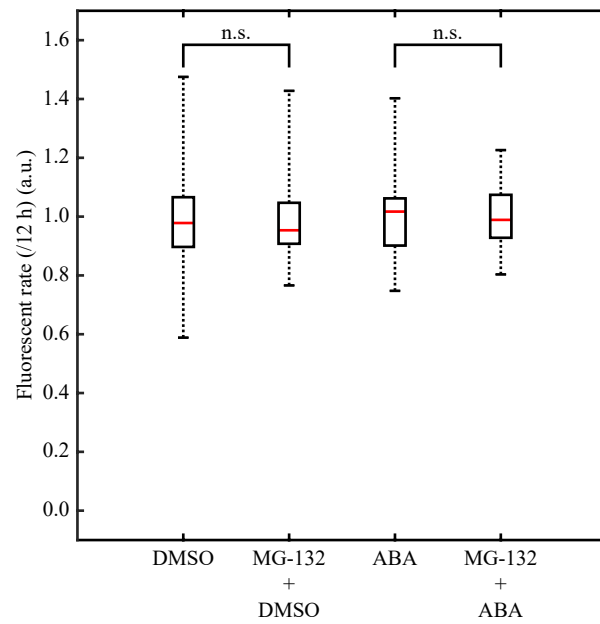


[Instructions for use](#)



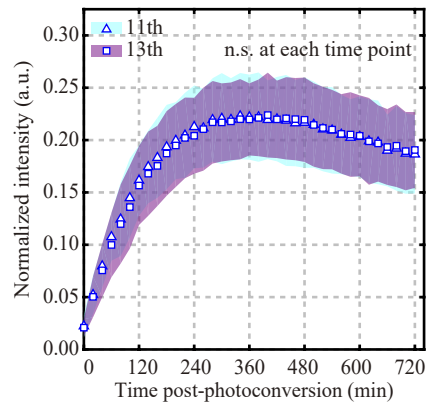
Supplemental Figure S1. Expression level of *PpSnRK2* and *PpABI3* in protonemal tissues.

A to D, Relative abundance of *PpSnRK2A* and *PpABI3A* transcripts with DMSO or β -estradiol treatment. E to H, Expression of *PpSnRK2A-D* and *PpABI3A-C* without and after 1-, 6- and 12-hour ABA treatment. The transcript levels were normalized against riboflavin kinase gene (*RFK*) (A, C, E and G) or 3-hydroxyisobutyryl-CoA hydrolase gene (*HIBCH*) gene (B, D, F and H). Data are mean \pm SD ($n = 3$ for biological replicates, with analytical triplicate in each sample). The P value was determined by the Welch's t -test, n.s., non-significance ($P \geq 0.05$), * $P < 0.05$, ** $P < 0.01$.



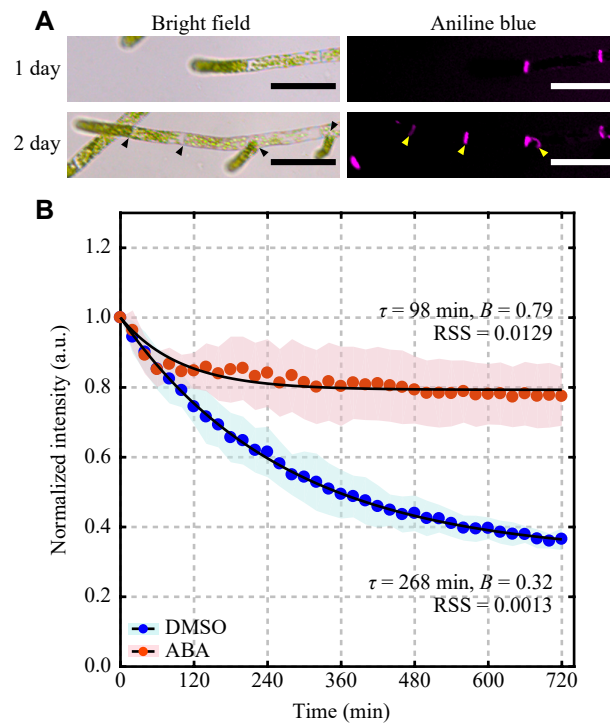
Supplemental Figure S2. Measurement of the Dendra2 degradation rate.

The Dendra2 intensity in protoplasts from *ProEF1 α :D2* was quantified just after and 12 hours after photoconversion to evaluate proteolytic degradation. As the negative control, proteasome inhibitor MG-132 was pretreated 1.5 hours before photoconversion. Treatment of DMSO or ABA was performed just after photoconversion. Box plot shows the distribution of the data with median as a red horizontal line, interquartile range as a black box, and data range as whiskers. $n > 46$. Statistical significance difference was tested by the Mann-Whitney U -test. n.s., non-significance ($P \geq 0.05$).



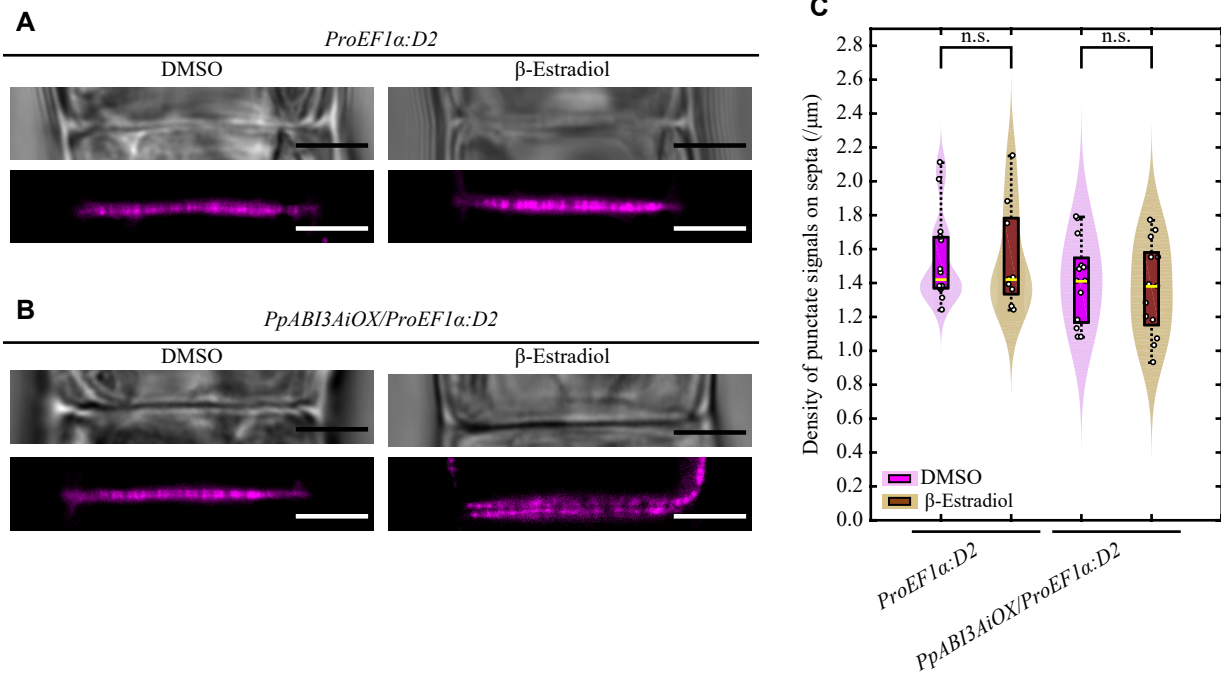
Supplemental Figure S3. Change in fluorescence intensity of Dendra2 in the neighboring cells of the photoconverted cells.

Mean fluorescence intensity of Dendra2 in the neighboring 11th and 13th cells of *ProEF1a:D2*. SD is indicated by the shaded areas. The 11th ($n = 15$) or 13th ($n = 14$) cell. The P value was determined by the Welch's t -test, n.s., non-significance ($P \geq 0.05$).



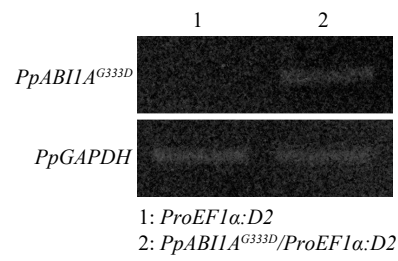
Supplemental Figure S4. Callose signal on newly formed cross walls and intercellular Dendra2 diffusivity under the condition of callose staining with aniline blue.

A, Representative images of protonemata 1 day and 2 days after treatment of the aniline blue solution in *ProEF1 α :D2*. Scale bars = 200 μ m. Arrows indicate newly formed cross walls after the cell division. B, Mean fluorescence intensity of Dendra2 at 20-min intervals after photoconversion in the protonemal cells in the presence of aniline blue solution. *ProEF1 α :D2* treated with DMSO ($n = 4$) or ABA ($n = 13$). SD is indicated by the shaded areas. The time constant (τ) and immobile fraction (B) were determined by fitting the exponential function to the kinetics of mean fluorescence intensity (black solid lines).



Supplemental Figure S5. Live-cell imaging of callose staining in *PpABI3AiOX/ProEF1α:D2*.

A and B, Representative images of DIC and fluorescence from callose with aniline blue in *ProEF1α:D2* (A) and *PpABI3AiOX/ProEF1α:D2* (B) for detection of punctate callose signals with DMSO or β -estradiol treatment. Scale bars = 5 μm . (C) The density of punctate signals on cross walls. Each violin plot shows the density distribution of the data by the box plot (median as a yellow horizontal line, interquartile range as a box, and data range as whiskers). *ProEF1α:D2* with DMSO ($n = 14$) and β -estradiol ($n = 19$), and *PpABI3AiOX/ProEF1α:D2* with DMSO ($n = 13$) and β -estradiol ($n = 13$). The P value was determined by the Mann-Whitney U -test, n.s., non-significance ($P \geq 0.05$).



Supplemental Figure S6. Expression of *PpABI1A^{G333D}* in protonemal tissues of *PpABI1A^{G333D}OX/ProEF1α:D2*.

Constitutive expression of *PpABI1A^{G333D}* in *PpABI1A^{G333D}OX/ProEF1α:D2* was confirmed by RT-PCR. The glutaraldehyde dehydrogenase gene (*GAPDH*) was used as an internal control. The number of PCR cycles was 28.

Supplemental Table S1. Primer sequences in qPCR

Gene Name (annotation in the v1.6; http://www.cosmos.org/)	Sequence
<i>PpABI3A</i> (Pp1s7_115V6.1)	(F) 5'-AAAGAGGCGGAGGTTCACTTAC-3'
	(R) 5'-TTCTCGAGCAAGTACATCCGAC-3'
<i>PpABI3B</i> (Pp1s173_143V6.1)	(F) 5'-AGAAGCCGAGGCGCACTTAC-3'
	(R) 5'-CGACTCTTGTGTTTCGGCCAG-3'
<i>PpABI3C</i> (Pp1s143_82V6.1)	(F) 5'-CTTCGCCCAAGTGATGTTGGC-3'
	(R) 5'-ACTAAGGAAGGGCATGTGCTG-3'
<i>PpSnRK2A</i> (Pp1s218_59V6)	(F) 5'-CACGTTGCTTGATGGAAGCC-3'
	(R) 5'-GGGTGCGAGTGCAACAAGG-3'
<i>PpSnRK2B</i> (Pp1s240_91V6)	(F) 5'-AGGATCCCGATGACCCAAGG-3'
	(R) 5'-GCCTGCACTCCACGGAAATG-3'
<i>PpSnRK2C</i> (Pp1s113_72V6)	(F) 5'-ACCTAAGTCGACCGTGGGAAC-3'
	(R) 5'-CAAAGTCACACCGCACGACC-3'
<i>PpSnRK2D</i> (Pp1s116_98V6)	(F) 5'-TGTTGCACTCGCAACCGAAG-3'
	(R) 5'-GTACAAGGTCACACCGCACG-3'
<i>PpRFK</i> (Pp1s56_240V6)	(F) 5'-AGTGCTGGGTTTCATTCGAC-3'
	(R) 5'-AGCCATGTTACATCCGAAAA-3'
<i>PpHIBCH</i> (Pp1s13_134V6)	(F) 5'-CATGATTGATCGCTTGTTGG-3'
	(R) 5'-ACCGCGATCTTTATCACCTG-3'

(F): Forward primer

(R): Reverse primer