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Supplemental information

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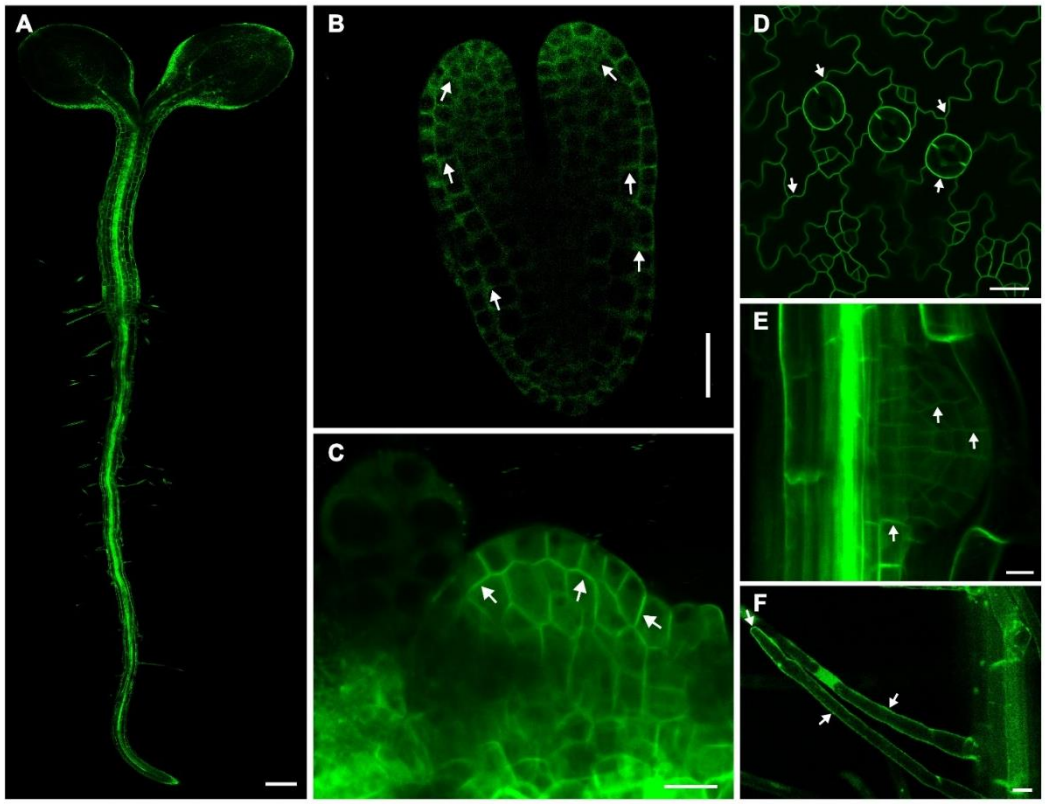
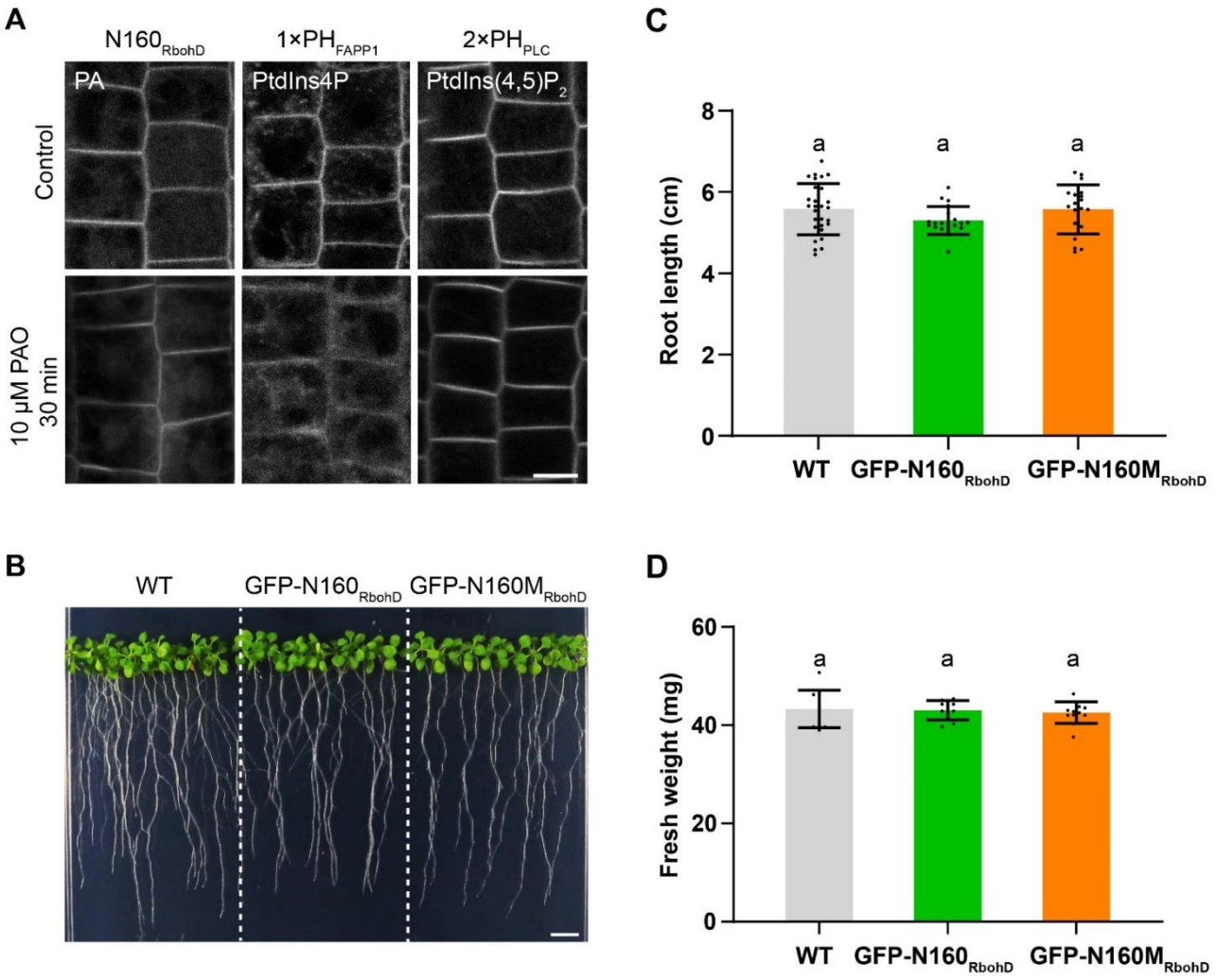
Supplemental Figures 1~8;
Supplemental Tables 1~2



Supplemental Figure 1. Confocal microscopy of GFP-N160$_{RbohD}$ expression patterns in *Arabidopsis*.

(A) Confocal laser scanning microscopy images of transgenic *A. thaliana* fluorescence with the GFP-N160$_{RbohD}$ construct in 4-day-old seedlings. Scale bars = 1 mm.

(B–F) GFP-N160$_{RbohD}$ expression patterns in the developing embryo (B), apical meristem (C), cotyledon epidermal cells (D), lateral root primordium (E), and root hair (F). White arrowheads indicate PA at the plasma membrane (PM). Scale bars = 25 μm.

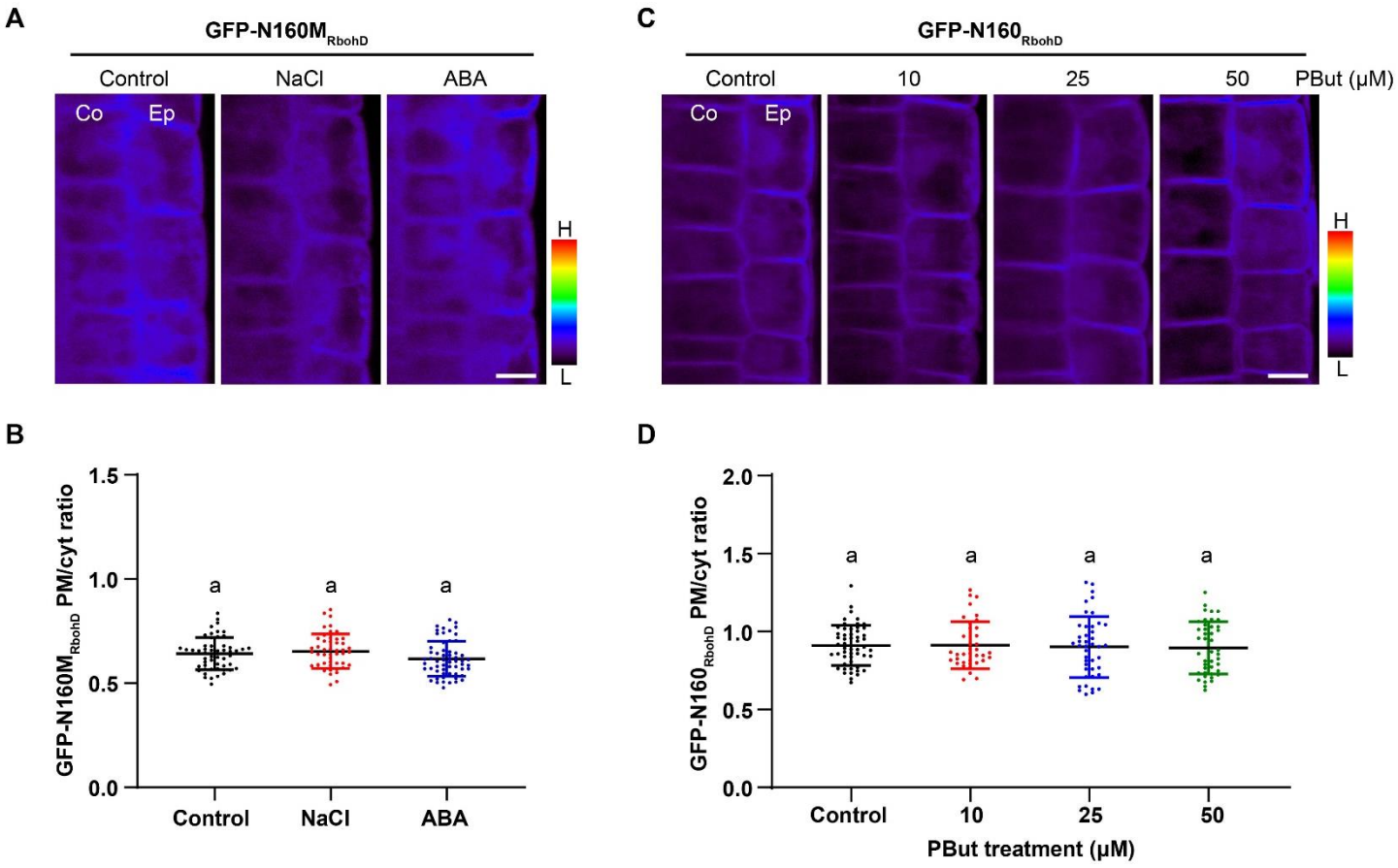


Supplemental Figure 2. PAO treatment and seedling phenotypes.

(A) Confocal observation of lipid biosensors in the presence of PAO. Four-day-old seedlings expressing GFP-N160$_{RbohD}$, mCIT-1×PH$_{FAPP1}$, or mCIT-2×PH$_{PLC}$ were treated with 10 μM PAO for 30 min prior to confocal observation. Bars = 25 μm.

(B) Phenotypes of WT, GFP-N160$_{RbohD}$, and GFP-N160M$_{RbohD}$ transgenic *A. thaliana*. Seedlings were grown on 1/2 MS medium for 12 days before photographs were acquired. Scale bars = 5 mm.

(C, D) Primary root length (C) and fresh weight (D) of WT, GFP-N160$_{RbohD}$, and GFP-N160M$_{RbohD}$ plants indicated in (B). Data are means ± SDs of three independent experiments. Similar letters indicate no statistically significant difference as determined by one-way ANOVA; $P < 0.05$.



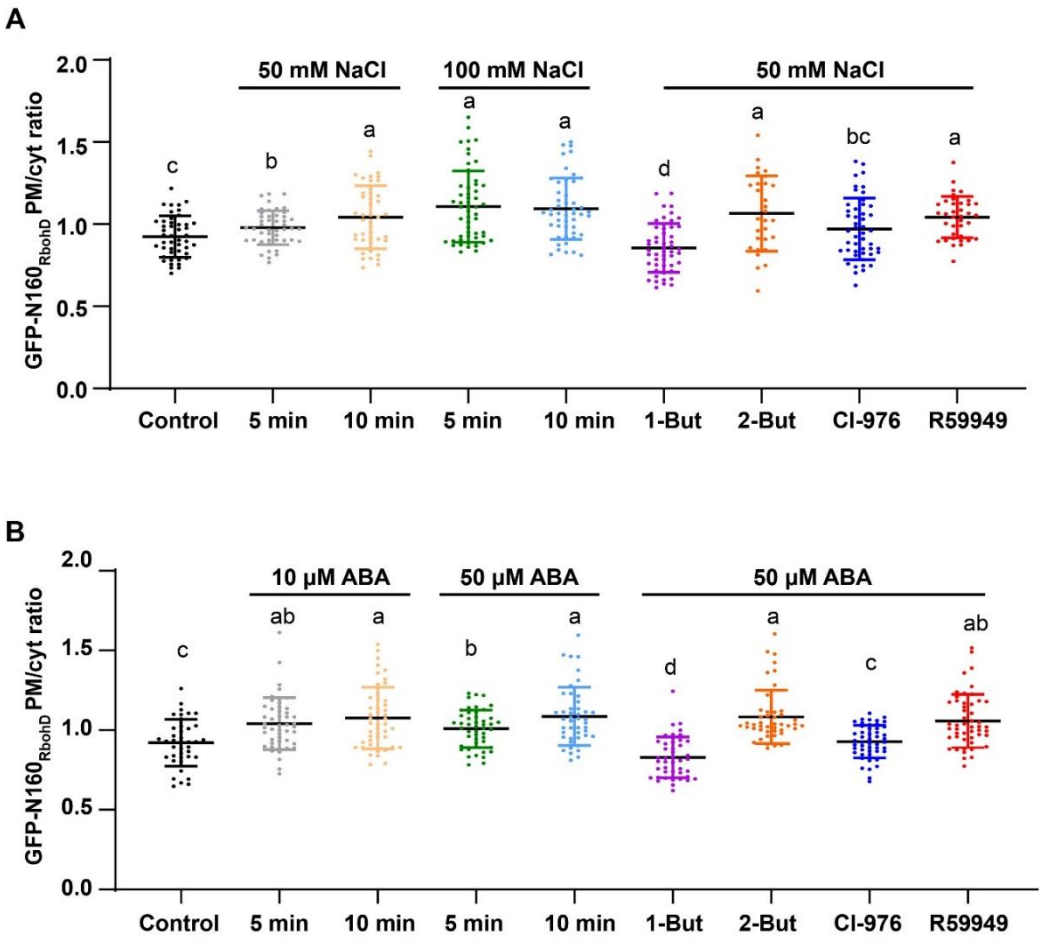
Supplemental Figure 3. Responses of PA sensors to stress or phosphatidylbutanol treatment.

(A) Confocal images of GFP-N160M$_{RbohD}$ fluorescence in roots upon NaCl or ABA treatment. Four-day-old plants were exposed to 50 mM NaCl or 50 µM ABA for 10 min prior to confocal observation. Co, cortex; Ep, epidermis. Scale bars = 25 µm.

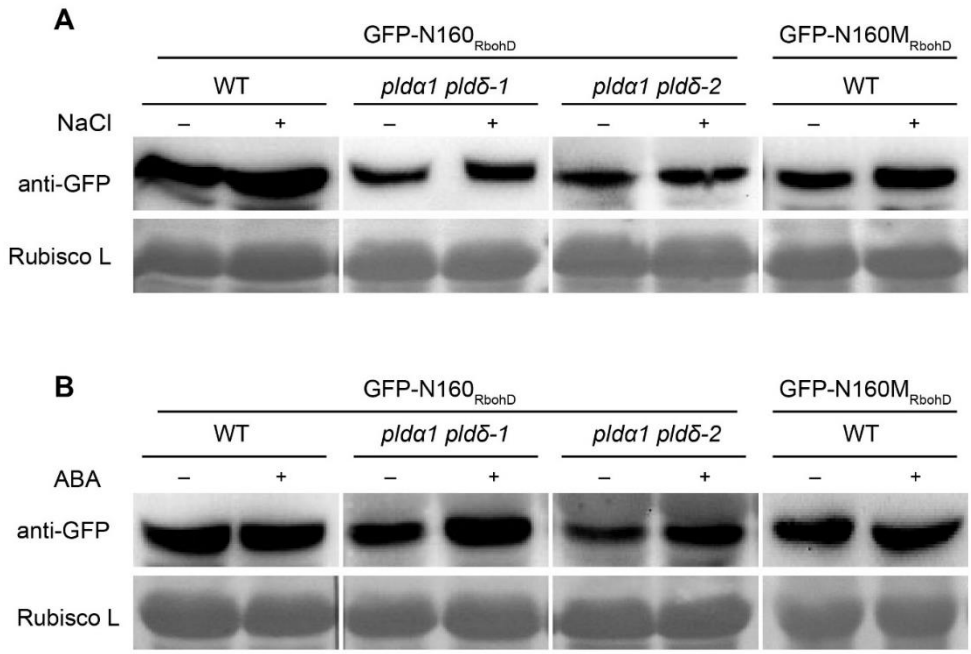
(B) Quantification of PM-associated versus cytoplasmic (PM/Cyt) intensity ratios of GFP-N160M$_{RbohD}$ after treatments indicated in (A). Data are means ± SDs of three independent experiments (n > 40 cells from at least 8 roots). Similar letters indicate no statistically significant difference as determined by one-way ANOVA; $P < 0.05$.

(C) Phosphatidylbutanol (PBut) did not affect GFP-N160$_{RbohD}$ fluorescence. Four-day-old plants were exposed to PBut for 30 min prior to confocal observation. Scale bars = 25 µm.

(D) Quantification of PM-associated vs. cytoplasmic (PM/Cyt) intensity ratios of GFP-N160$_{RbohD}$ after the treatments indicated in (C). Similar letters indicate no statistically significant difference, as determined by one-way ANOVA; $P < 0.05$.

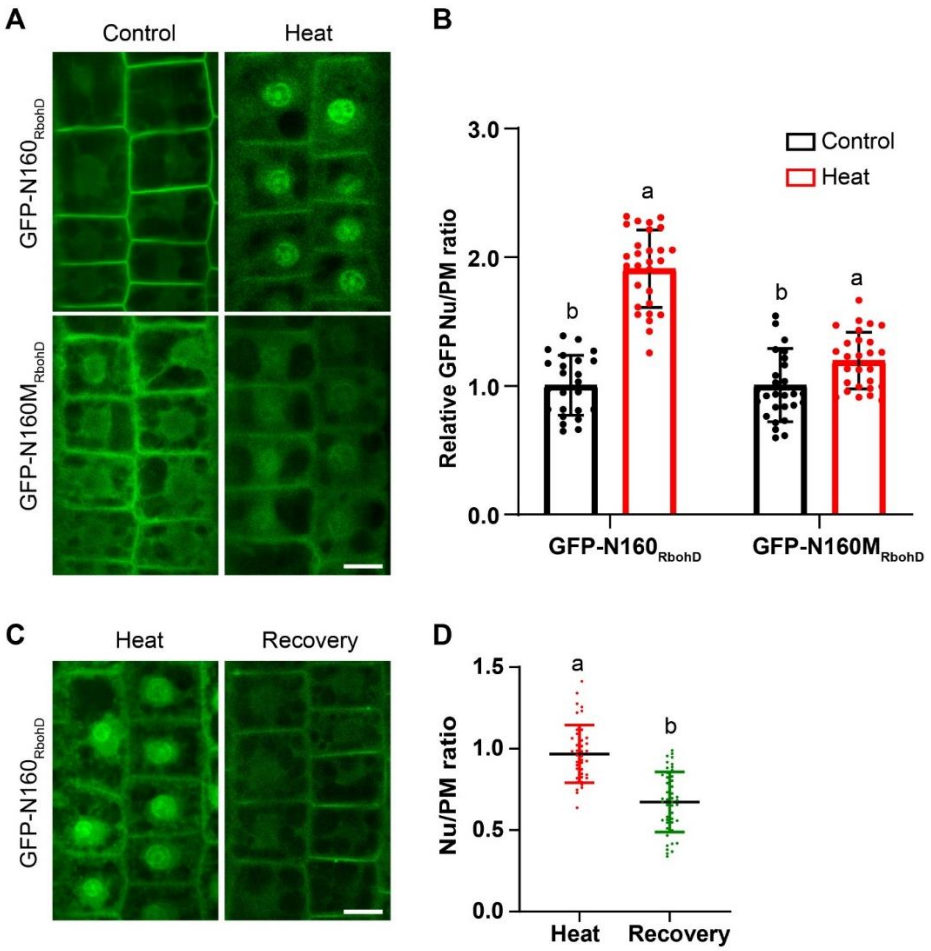


Supplemental Figure 4. Quantification of GFP-N160$_{RbohD}$ PM/Cyt intensity ratios after NaCl (A) and ABA (B) treatment. Data are means ± SDs of three independent experiments (n > 40 cells from at least 10 roots). Different letters indicate statistically significant differences, as determined by one-way ANOVA; $P < 0.05$.



Supplemental Figure 5. Immunoblot analysis of PA sensors with NaCl or ABA treatment.

Seven-day-old seedlings expressing sensors were exposed to 50 mM NaCl (A) or 50 μM ABA (B) for 10 min. PA sensor expression levels in GFP-N160$_{RbohD}$ and GFP-N160M$_{RbohD}$ seedlings were detected using an anti-GFP antibody. Rubisco L was used as the internal control.



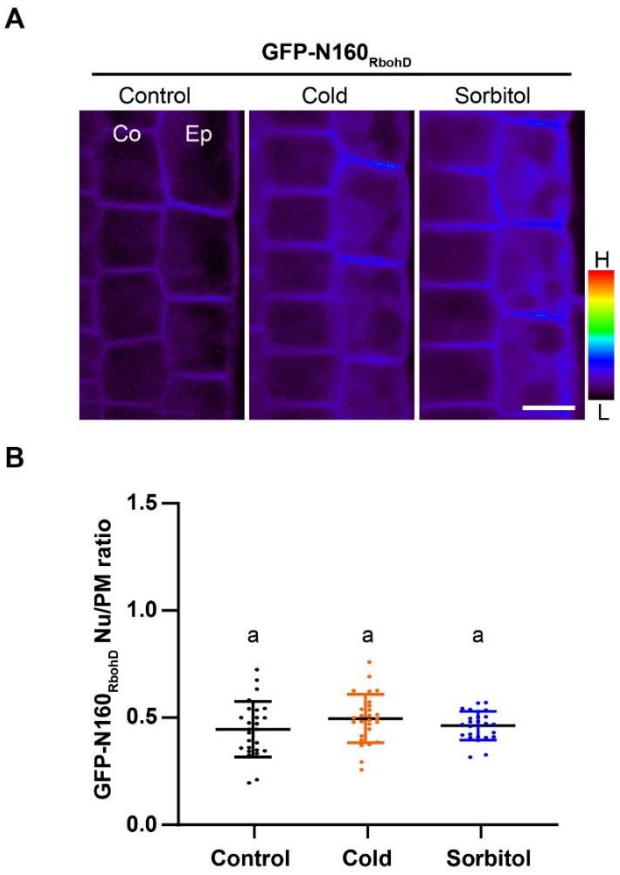
Supplemental Figure 6. GFP-N160M$_{RbohD}$ responses and PA translocation during recovery after heat stress.

(A) Effect of heat stress on the nuclear translocation of GFP-N160M$_{RbohD}$. Four-day-old seedlings expressing GFP-N160M$_{RbohD}$ were exposed to 45°C for 10 min before confocal observation. Bars = 25 µm.

(B) Quantification of the Nu/PM intensity ratios of GFP-N160M$_{RbohD}$ indicated in (A). Different letters indicate statistically significant differences, as determined by one-way ANOVA; $P < 0.05$.

(C) Recovery of GFP-N160$_{RbohD}$ fluorescence in roots following heat stress. For recovery experiments, seedlings treated with 45°C for 10 min were transferred to normal growth conditions for 24 h before confocal observation. Bars = 25 µm.

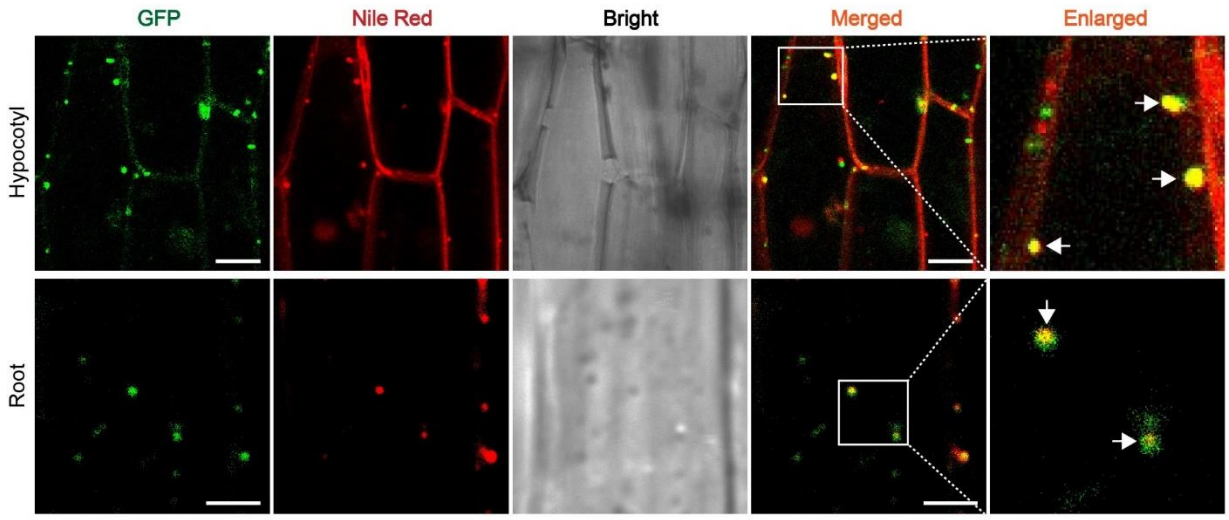
(D) Quantification of the Nu/PM intensity ratios of GFP-N160$_{RbohD}$ indicated in (C). Different letters indicate statistically significant differences, as determined by one-way ANOVA; $P < 0.05$.



Supplemental Figure 7. Cold and hyperosmotic stress do not affect the subcellular localization of GFP-N160$_{RbohD}$.

(A) Confocal images of GFP-N160$_{RbohD}$ fluorescence after low temperature or sorbitol stress. Four-day-old plants were exposed to 4°C or 300 mM sorbitol for 30 min prior to confocal observation. Co, cortex; Ep, epidermis. Scale bars = 25 µm.

(B) Quantification of nuclear versus PM-associated (Nu/PM) intensity ratios of GFP-N160$_{RbohD}$ after treatments indicated in (A). Data are means ± SDs of three independent experiments (n > 30 cells from at least 8 roots). Similar letters indicate no statistically significant difference as determined by one-way ANOVA; $P < 0.05$.



Supplemental Figure 8. PA is involved in lipid droplet formation in darkness.

Confocal images of GFP-N160$_{RbohD}$ fluorescence in hypocotyl and root upon exposure to darkness. Four-day-old seedlings were grown on 1/2 MS medium lacking sucrose. For dark treatment, the plants were covered with aluminum foil for 24 h prior to confocal observation. Nile Red was used to stain lipid droplets.

Supplemental Table 1. Peptide sequences used in this article.

Peptides	Sequences
K-Ras4B	AAGATGAGCAAAGATGGTAAAAAGAAGAAAAAGAAGTCAAAGACAAAGTGTGTAATTATGTAA
WPP	ATGGATCATTCAGCGAAAACCACACAGAACCGTGTTTTGTCAGTGAAGATGTGGCCACC
	GAGTAAGAGTACCCGTCTCATGCTTGTTGAGCGGATGACCAAGAACATTACCACCCCTT
	CCATCTTCTCCAGGAAGTACGGTCTTTTGTCTGTTGAAGAGGCTGAGCAAGACGCCAAG
	CGCATTGAAGATTTGGCCTTTGCTACTGCCAACAAACACTTCCAGAACGAGCCTGATGG
	TGATGGCACTTCTGCTGTTCACGTCTATGCTAAAGAATCCAGCAAGCTCATGCTTGATG
	TCATCAAACGTGGTCCACAGGAAGAATCCGAGGTTGAGGTGAGCAAGGATGGTGATGT
	CTTTTTTGATATATCTGGTGGGAGCAGAGCTTTTATTGAAGAAGAGGAGGCACGCGACC
	TTTTGAGGCCTCTGGCTGACCCGCGTAACTCCTATACC
HEDL	CACGATGAGCTTTGA
SKL	TCAAAGCTT
P2C3	TGGTATGCCAAAGGAGCAGGCTTGATGGTTGTGGGTGTGCTTTTAGGGTTCATTATCGG
	GTTTTTTACCCGGGGAAAGAAATCTTCGTCT

Supplemental Table 2. PCR primers used in this article.

Primers	sequences (5'-3')
$N160_{RbohD}$-His	Forward: TACCCTCGAGGGATCCGAATTCATGAAAATGAGACGAGGCAATTCAA
	Reverse: CAGGTCGACAAGCTTGAATTCGCGGTCAAACCGCCGCACGGCC
$N160M_{RbohD}$-His	Forward: TACCCTCGAGGGATCCGAATTCATGAAAATGAGACGAGGCAATTCAA
	Reverse: CAGGTCGACAAGCTTGAATTCGCGGTCAAACGCCGCCACGGCC
$N160_{RbohD}$-1301	Forward: GACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTAGCGGTCAAACCGCCGCACGGCC
$N160M_{RbohD}$-1301	Forward: GACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTAGCGGTCAAACGCCGCCACGGCC
GFP-$N160_{RbohD}$-1301	Forward: GAGCTCGGTACCCGGGGATCCATGGTGAGCAAGGGCGAGGAGCTGT
	Reverse: CAGGTCGACTCTAGAGGATCCCTTGTACAGCTCGTCCATGCCGAGA
UBQ-GFP-$N160_{RbohD}$-1301	Forward: TACGAATTCGAGCTCGGTACCGTCGACGAGTCAGTAATAAACGGCG
	Reverse: TCTAGAGGATCCCCGGGTACCCTGTTAATCAGAAAAACTCAGATTA
PM-1301	Forward: GACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTATTCTTCTTTTTACCATCTTTGCTCA
WPP-1301	Forward: CTGATTAACAGGGTACCCGGGATGGATCATTCAGCGAAAACC
	Reverse: TGCTCACCATGGATCCCCGGGGGGTATAGGAGTTACGCGGGTC
HEDL-1301	Forward: ATCCTCT GAGTCGACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTATCAAAGCTCATCGTGGCGGTCAAACCGCCGCACG
SKL-1301	Forward: GACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTAAAGCTTTGAGCGGTCAAACCGCCGCAC
P2C3-1301	Forward: GACCTGCAGGCATGCAAGCTTATGAAAATGAGACGAGGCAATTCAA
	Reverse: GGGGAAATTCGAGCTGGTGACCTTAAGACGAAGATTTCTTT