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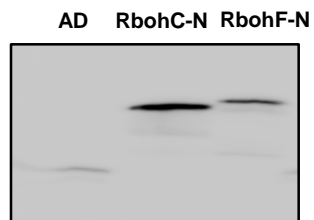
## Supplementary Material

***Arabidopsis thaliana* constitutively active ROP11 interacts with the NADPH oxidase respiratory burst oxidase homologue F to regulate reactive oxygen species production in root hairs**

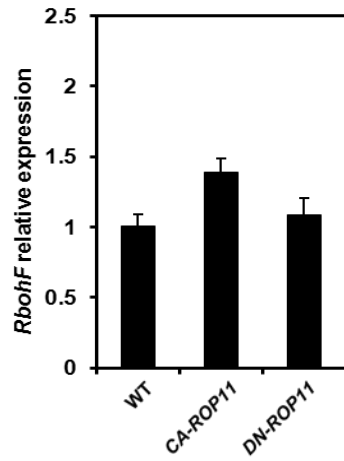
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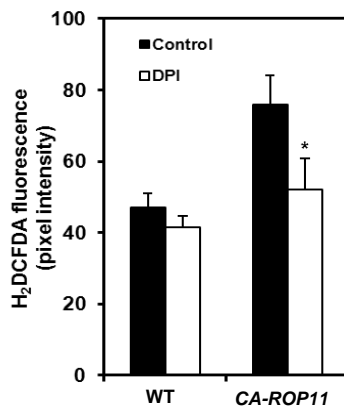
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**Fig. S1.** Immunoblotting of HA-tagged RbohC-N and RbohF-N proteins expressed in yeast.



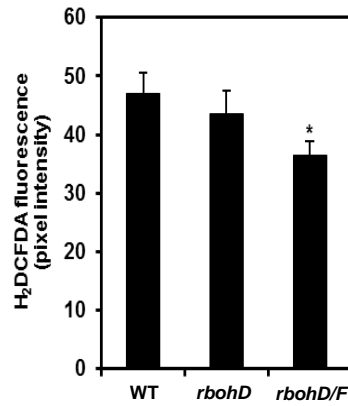
**Fig. S2.** qRT-PCR analysis of *RbohF* relative expression in wild-type, *CA-ROP11*, and *DN-ROP11* plants. *Actin2* was used as an internal control.



**Fig. S3.** The NADPH oxidase inhibitor DPI resulted in reduced ROS production by root hairs in *CA-ROP11* plants. The data are means  $\pm$  SE from three independent experiments;  $n = 50$  of each genotype per experiment. Significant difference from the control is indicated by one asterisk (Student's  $t$ -test; \*,  $P < 0.05$ ).

<b>RbohF (residues 325–344)</b>	L	Y	ELWQ	T	* *	KDTYL
<b>RbohC (residues 287–306)</b>	I	Y	MLES	T		AATQS
<b>RbohD (residues 314–333)</b>	I	F	MIEN	M		APNQS

**Fig. S4.** Sequence alignment of RbohF, RbohC and RbohD proteins. The two Leu residues of RbohF involved in CA-ROP11 binding are denoted by asterisks.



**Fig. S5.** *rbohD/F* double mutant showed lower ROS levels in root hairs than the wild-type plants. The data are means  $\pm$  SE from three independent experiments;  $n = 50$  of each genotype per experiment. Significant difference from the control is indicated by one asterisk based on Duncan's multiple range test (\*,  $P < 0.05$ ).

**Table S1. Primers used for PCR, site-directed mutagenesis, and transgenic plant construction**

<b>Primer name</b>	<b>Sequence (5'-3')</b>
ROP11 (Q66L) F	GGGACACTGCTGGGCTGGAAGACTATAACAGA
ROP11 (Q66L) R	TCTGTTATAGTCTTCCAGCCCAGCAGTGTCCC
ROP11 (D123A) F	GTTGGTACCAAATTAGCTCTTCGTGAAGATAAGC
ROP11 (D123A) R	GCTTATCTTCACGAAGAGCTAATTTGGTACCAAC
BD-(CA/DN)-ROP11 F	GGAATTCATGGCTTCAAGTGCCTCAAAGTTC
BD-(CA/DN)-ROP11 R	GCGTCGACTCAATGCCGAGTCACTATCCTCCCAGACAGAACATTTGATAA AGAGCC
AD-RbohC (1-306) F	CGGAATTCATGTCTAGAGTGAGTTTTGAAGTG
AD-RbohC (1-306) R	CGCTCGAGTTAAGACTGTGTTGCCGCTTGCAAAAG
AD-RbohD (1-333) F	GGAATTCATGAAAATGAGACGAGGCAATTCAAG
AD-RbohD (1-333) R	CGGATCCTTACGACTGGTTTGGTGCTTGTAATAG
AD-RbohF (1-344) F	CGGAATTCATGAAACCGTTCTCAAAGAACG
AD-RbohF (1-344) R	CGGGATCCTTAGAGGTATGTGTCTTTTTGTAGAAG
AD-RbohF (1-264) R	CGGGATCCGACTCTCGTCGTTGATTTGTGACCA
AD-RbohF (1-304) R	CGGGATCCGCTTGTATAGCTTATTTGCAGATGC
AD-RbohF (1-324) R	CGGGATCCTTATCTTTCAGGATCTAACTCTTCCAT
AD-RbohF (1-334) R	CGGGATCCTTACTCTAGTTGCCATAGCTCTATGTA
AD-RbohF (1-339) R	CGGGATCCTTATTGTAGAAGCAAAGTCTCTAGTTG
TL(335, 336)AA F	CTATGGCAACTAGAGGCTGCGCTTCTACAAAAGAC
TL(335, 336)AA R	GTCTTTTTGTAGAAGCGCAGCCTCTAGTTGCCATAG
LL(337, 338)AA F	CAACTAGAGACTTTGGCTGCACAAAAGACACATAC
LL(337, 338)AA R	GTATGTGTCTTTTTGTGCAGCCAAAGTCTCTAGTTG
QK(339, 340)AA F	GAGACTTTGCTTCTAGCAGCAGACACATACCTCTAA
QK(339, 340)AA R	TTAGAGGTATGTGTCTGCTGCTAGAAGCAAAGTCTC
DT(341, 342)AA F	TTGCTTCTACAAAAGCCGCATACCTCTAAGGATCC
DT(341, 342)AA R	GGATCCTTAGAGGTATGCGGCTTTTTGTAGAAGCAA
YL(343, 344)AA F	CTACAAAAGACACAGCCGCTAAGGATCCATCGAG
YL(343, 344)AA R	CTCGATGGATCCTTAGGCGGCTGTGTCTTTTTGTAG
LL(336, 337)AA F	CTATGGCAACTAGAGACTGCGGCTTCTACAAAAGAC
LL(336, 337)AA R	GTCTTTTTGTAGAGCCGAGTCTCTAGTTGCCATAG
T335A F	CTATGGCAACTAGAGGCTTTGCTTCTACAAAAG
T335A R	CTTTTTGTAGAAGCAAAGCCTCTAGTTGCCATAG
L336A F	TGGCAACTAGAGACTGCGCTTCTACAAAAGAC
L336A R	GTCTTTTTGTAGAAGCGCAGTCTCTAGTTGCCA
L337A F	CAACTAGAGACTTTGGCTTCTACAAAAGACAC
L337A R	GTGTCTTTTTGTAGAGCCAAAGTCTCTAGTTG
L338A F	CTAGAGACTTTGCTTGCACAAAAGACACATAC
L338A R	GTATGTGTCTTTTTGTGCAAGCAAAGTCTCTAG

GST-CA-ROP11 F	GGAATTCATGGCTTCAAGTGCTTCAAAG
GST-CA-ROP11 R	CCGCTCGAGTCAATGCCGAGTCACTATCCTCCCAC
His-RbohF (1–344) F	CGGAATTCATGAAACCGTTCTCAAAGAACG
His-RbohF (1–344) R	GTGCTCGAGGAGGTATGTGTCTTTTTGTAGAAG
(CA/DN)-ROP11-YFP <sup>C</sup> F	GCTCTAGAATGGCTTCAAGTGCTTCAAAG
(CA/DN)-ROP11-YFP <sup>C</sup> R	CCGCTCGAGATGCCGAGTCACTATCCTCCC
YFP <sup>N</sup> -RbohF (WT/mutant) F	GCGGATCCATGAAACCGTTCTCAAAGAAC
YFP <sup>N</sup> -RbohF (WT/mutant) R	GGGGTACCGAAATGCTCCTTGTGAAATTC
GFP-(CA/DN)-ROP11-1300 F	GCGTGCACATGGCTTCAAGTGCTTCAAAG
GFP-(CA/DN)-ROP11-1300 R	GGACTAGTATGCCGAGTCACTATCC
Flag-RbohF (WT/mutant)-1300 F	GCTCTAGAATGAAACCGTTCTCAAAGAAC
Flag-RbohF (WT/mutant)-1300 R	GGGGTACCGAAATGCTCCTTGTGAAATTC
ROP11-RT F	ATGGCTTCAAGTGCTTCAAAGTTCA
ROP11-RT R	TCAATGCCGAGTCACTATCCTCCCA
RbohF-RT F	ATGAAACCGTTCTCAAAGAACGATCGGC
RbohF-RT R	GGCGAAACCGCAGGACATTGGACAAAC
Actin2 F	GACCTTGCTGGACGTGACCTTAC
Actin2 R	GTAGTCAACAGCAACAAAGGAGAGC
ROP11-GUS F	ACGCGTGCACAGCTGTTTAGCATATGTGGAA
ROP11-GUS R	CGCGGATCCTTTGATCTCAAACAAACATACAA
RbohF-qRT F	TTGCAGAACTTGACCAACGG
RbohF-qRT R	TGTTCTGACCCTAGTGCCAG

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**Table S2. The constructs used in this study**

<b>Construct name</b>	<b>Vector (5'-3')</b>	<b>Promoter</b>	<b>Tag</b>
BD-(CA/DN)-ROP11	BD, pGBKT7	T7	N-Myc
AD-RbohC (1-306)	AD, pGADT7	T7	N-HA
AD-RbohD (1-333)	AD, pGADT7	T7	N-HA
AD-RbohF (1-344)	AD, pGADT7	T7	N-HA
AD-RbohF (1-264)	AD, pGADT7	T7	N-HA
AD-RbohF (1-304)	AD, pGADT7	T7	N-HA
AD-RbohF (1-324)	AD, pGADT7	T7	N-HA
AD-RbohF (1-334)	AD, pGADT7	T7	N-HA
AD-RbohF (1-339)	AD, pGADT7	T7	N-HA
TL(335, 336)AA	AD, pGADT7	T7	N-HA
LL(337, 338)AA	AD, pGADT7	T7	N-HA
QK(339, 340)AA	AD, pGADT7	T7	N-HA
DT(341, 342)AA	AD, pGADT7	T7	N-HA
YL(343, 344)AA	AD, pGADT7	T7	N-HA
T335A	AD, pGADT7	T7	N-HA
L336A	AD, pGADT7	T7	N-HA
L337A	AD, pGADT7	T7	N-HA
L338A	AD, pGADT7	T7	N-HA
GST-CA-ROP11	pGEX4T-1	T7	N-GST
His-RbohF (1-344)	pET-28a (+)	T7	N-His
(CA/DN)-ROP11-YFP <sup>C</sup>	pSPYCE	35S	C-HA
YFP <sup>N</sup> -RbohF (WT/mutant)	pSPYNE	35S	N-Myc
GFP-(CA/DN)-ROP11-1300	GFP-pSuper1300	Super	N-GFP
Flag-RbohF (WT/mutant)-1300	Flag-pSuper1300	Super	N-Flag
ROP11-GUS	pBI101	<i>ROP11</i> promoter	GUS
(CA/DN)-ROP11-1300	pSuper1300	Super	-