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Subcellular localization and dynamics of the tobacco ROS-producing enzyme RBOHD upon cryptogein elicitation

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RBOHD1      MQNSENHHPHHQHHHSDTEIIGNDRASYSGPLSGPLNKRGGKKSARFNIPESTDIGTSVG 60
RBOHD2      MQNSENHHPHHHHHHSDTEIIGNDRASYSGPLSGPLNKRGGKKSARFNIPESTDIGTSVG 60
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RBOHD1      TGGKSNDDAYVEITLDVREDSVAVHSVKTAGGDDVEDPELALLAKGLEKKSTLGSSLVRN 120
RBOHD2      TGAKSNDDAYVEITLDVREDSVAVHSVKTAGGDDVEDPELALLAKGLEKKSTLGSSLVRN 120
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RBOHD1      ASSRIRQVSQELRRLASLNKRPIPTGRFDRNKSAAAHALKGLKFISKTDGGAGWAAVEKR 180
RBOHD2      ASSRIRQVSQELRRLASLNKRPIPTGRFDRNKSAAAHALKGLKFISKTDGGAGWAAVEKR 180
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RBOHD1      FDEITASTTGLLPRAKFGECIGMNKESKEFAVELYDALARRRNITTDSINKAQLKEFWDQ 240
RBOHD2      FDEITASTTGLLPRAKFGECIGMNKESKEFAVELYDALARRRNITTDSINKAQLKEFWDQ 240
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RBOHD1      VADQSFDSRLQTFFDMVDKDADGRITEEEVREIIGLSASANRLSTIQKQADEYAAMIMEE 300
RBOHD2      VADQSFDSRLQTFFDMVDKDADGRITEEEVREIIGLSASANRLSTIQKQADEYAAMIMEE 300
            ************************************************************

RBOHD1      LDPNNLGYIMIENLEMLLLQAPNQSVQRGGESRNLSQMLSQKLKHTQERNPIVRWYKSFM 360
RBOHD2      LDPNNLGYIMIENLEMLLLQAPNQSVQRGGESRNLSQMLSQKLKHTQERNPIVRWYKSFM 360
            ************************************************************

RBOHD1      YFLLDNWQRVWVLLLWIGIMAGLFTWKYIQYKEKAAYKVMGPCVCFAKGAAETLKLNMAI 420
RBOHD2      YFLLDNWQRVWVLLLWIGIMAGLFTWKYIQYKEKAAYKVMGPCVCFAKGAAETLKLNMAI 420
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RBOHD1      ILFPVCRNTITWLRNKTRLGAAVPFDDNLNFHKVIAVAIALGVGIHGLSHLTCDFPRLLN 480
RBOHD2      ILLPVCRNTITWLRSKTRLGAAVPFDDNLNFHKVIAVAIALGVGVHGLSHLTCDFPRLLN 480
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RBOHD1      ASEEEYEPMKYYFGDQPESYWWFIKGVEGVTGIIMVVLMAIAFTLATPWFRRNRVSLPKP 540
RBOHD2      ASEEEYEPMKYYFGDQPESYWWFIKGVEGVTGIIMVVLMAIAFTLATPWFRRNRVSLPKP 540
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RBOHD1      FHKLTGFNAFWYSHHLFVIVYTLFIVHGEKLYITKDWYKRTTWMYLTIPIILYASERLIR 600
RBOHD2      FHKLTGFNAFWYSHHLFVIVYTLFIVHGEKLYITKDWYKRTTWMYLTIPIILYASERLIR 600
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RBOHD1      AFRSSIKAVKILKVAVYPGNVLALHMSKPQGYKYKSGQYMFVNCAAVSPFEWHPFSITSA 660
RBOHD2      ALRSSIKAVKILKVAVYPGNVLALHMSKPQGYKYKSGQYMFVNCAAVSPFEWHPFSITSA 660
            *:**********************************************************

RBOHD1      PGDDYLSVHIRTLGDWTRQLKTVFSEVCQPPPNGKSGLLRADYLQGENNPNFPRVLIDGP 720
RBOHD2      PGDDYLSVHIRTLGDWTRQLKTVFSEVCQPPPNGKSGLLRADYLQGENNPNFPRVLIDGP 720
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RBOHD1      YGAPAQDYKKYEVVLLVGLGIGATPMISIVKDIVNNMKAMDEEENSLEDGHNNNMAPNSS 780
RBOHD2      YGAPAQDYKKYEVVLLVGLGIGATPMISIVKDIVNNMKAMDEEENSLENGDNNNMAQNSS 780
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RBOHD1      PNIAKNKGNKSGSASGGNNFNTRRAYFYWVTREQGSFDWFKGIMNEAAEMDHKGVIEMHN 840
RBOHD2      PNIAQKRGNKSDSASGRNNFNTRRAYFYWVTREQGSFDWFKGIMNEAAEMDHKGVIEMHN 840
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RBOHD1      YCTSVYEEGDARSALITMLQSLHHAKNGVDIVSGTRVKSHFAKPNWRNVYKRIALNHPEA 900
RBOHD2      YCTSVYEEGDARSALITMLQSLHHAKNGVDIVSGTRVKSHFAKPNWRNVYKRIALNHPDA 900
            ************************************************************:*

RBOHD1      KVGVFYCGAPALTKELRQHALDFSHKTSTKFDFHKENF 938
RBOHD2      KVGVFYCGAPALTKELRQHALDFSHKTSTKFDFHKENF 938
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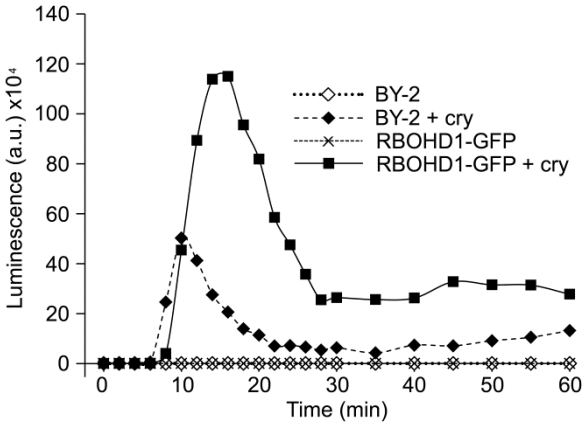
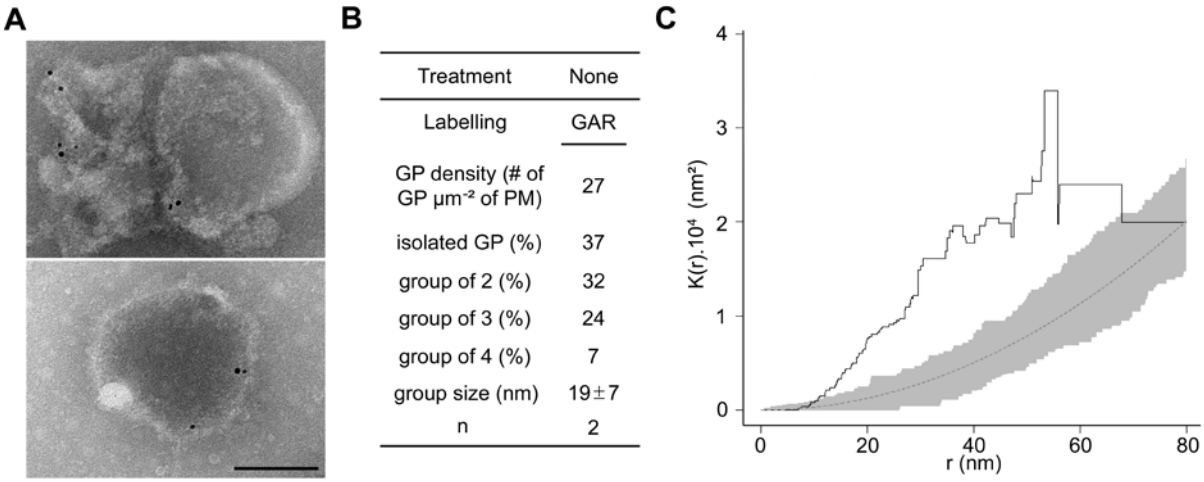
Supplementary Fig. S1. Alignment of RBOHD1 and RBOHD2 amino acid sequences. Alignment was realized using ClustalW (Larkin et al., 2007). Fourteen amino acid differences are found in the two tobacco genes.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*. **23,** 2947-2948.



Supplementary Fig. S2. Kinetics of ROS production upon elicitation by cryptogein. ROS burst was measured in wild-type and NtrbohD1-expressing BY-2 cells by chemiluminescence (a.u.: arbitrary units). Kinetics is representative of 3 independent experiments.



Supplementary Fig. S3. RBOHD-GFP protein forms clusters in the plasma membrane. (A) Representative transmission electron micrographs of PM vesicles from RBOHD-GFP-expressing cells and labelled with anti-GFP antibody and secondary IgG coupled to GPs of 5 nm (GAR). (Scale bar = 100 nm). (B) Labelling characteristics of PM vesicles. n = number of experiments. (C) Ripley's K-function analysis of RBOHD-GFP distribution on PM vesicles. Black line: sample K(r), dotted line: theoretical Poisson K(r), grey area: 99% Poisson simulation interval.

Supplementary Table S1. Primers used in this study

Cloning primers	
Primer name	**Sequence**
attB1-NoxD5	5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTAATGCAAAATTCGGAAAAT
attB2-NoxD1-2	5’-GGGGACCACTTTGTACAAGAAAGCTGGGTCAACCTGTGTCCTTAGCTG
attB2-NoxD2-1	5’-GGGGACCACTTTGTACAAGAAAGCTGGGTATAGGGAGAGGTGGTAGATTC
attB1-NtrbohD4	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTTTATTTAGGGCAAGTT
attB2-NtrbohD1	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTAGAAATTTTCTTTATGGAAATCAAAC
qRT-PCR primers	
Gene name	**Sequence**
EF-1a	Forward 5'-TGAGATGCACCACGAAGCTC
	Reverse 5'-CCAACATTGTCACCAGGAAGTG
L25	Forward 5'-CCCCTCACCACAGAGTCTGC
	Reverse 5'-AAGGGTGTTGTTGTCCTCAATCTT
PP2A	Forward 5'-GTGAAGCTGTAGGGCCTGAGC
	Reverse 5'-CATAGGCAGGCACCAAATCC
RBOHD1	Forward 5'-CATCAAAACAGCTAAGGACACAG
	Reverse 5'-GTACACAATAGGGAGAGTTGGTAGAC
RBOHD2	Forward 5'-AGATACCAAGGGAATTAAGAATGTG
	Reverse 5'-GGCACCCATCAAAAGAGG