



Supplementary materials

Table S1. Expression of *Rboh* genes in syncytia.

		Syncytium	Root
<i>At5g07390</i>	<i>RbohA</i>	3.9	6.6
<i>At1g09090</i>	<i>RbohB</i>	1.9	12.0
<i>At5g51060</i>	<i>RbohC</i>	5.7	8.8
<i>At5g47910</i>	<i>RbohD</i>	8.0	8.4
<i>At1g19230</i>	<i>RbohE</i>	2.9	4.6
<i>At1g64060</i>	<i>RbohF</i>	5.5	7.3
<i>At4g25090</i>	<i>RbohG</i>	3.3	4.4
<i>At5g60010</i>	<i>RbohH</i>	2.7	2.8
<i>At4g11230</i>	<i>RbohI</i>	2.3	3.1
<i>At3g45810</i>	<i>RbohJ</i>	2.7	3.0

Expression data from Szakasits et al. (The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots (2009) Plant Journal, **57**, 771–784) from a GeneChip analysis of *H. schachtii* syncytia in Arabidopsis roots showing normalized data on a log2 scale. Significant downregulation (as from the original data analysis) in syncytia compared to control roots is marked blue.

Table S2. Characteristics of Arabidopsis *Rboh* family genes and proteins.

Gene name	Accession Number	Chromosome #	Exons	Gene position on chromosome	Orientation	gDNA (bp)	CD (bp)	Protein (aa)	MW (Da)	pI	GRAVY	SPCS
AtRbohA	At5g07390	5	12	2335895-2339913	Reverse	4019	2709	902	102934.6	9.54	-0.229	No
AtRbohB	At1g09090	1	12	2932739-2936586	Forward	3848	2532	843	96389.3	9.59	-0.156	No
AtRbohC	At5g51060	5	11	2075728-2076243	Reverse	5148	2718	905	102517.4	9.91	-0.211	No
AtRbohD	At5g47910	5	8	1939744-1940206	Forward	4619	2766	921	103907.7	9.68	-0.241	No
AtRbohE	At1g19230	1	14	6643942-6649149	Forward	5208	2859	952	107701.6	8.9	-0.207	No
AtRbohF	At1g64060	1	14	2376977-2377698	Forward	7211	2835	944	108417.3	9.54	-0.287	No
AtRbohG	At4g25090	4	11	1287866-1288380	Reverse	5139	2550	849	96861.7	9.41	-0.196	No
AtRbohH	At5g60010	5	11	2416027-2416505	Forward	4783	2661	886	100626.7	9.5627	-0.196	No

AtRbohI	At4g11230	4	10	6840473-6845627	Reverse	5155	2826	941	106951	8.58	-0.230	No
AtRbohJ	At3g45810	3	13	16832726-16837792	Reverse	5067	2739	912	102936.2	9.9	-0.203	No

gDNA = genomic DNA, CD = coding sequence, MW = molecular weight, pI = isoelectric point, GRAVY = grand average of hydropathy, SPCS = signal peptide cleavage site.

Table S3. Description of the motifs identified in Arabidopsis Rboh proteins.

Motif	E-value	Sites	Width	Consensus sequence
1	3.5 ^{e-773}	10	136	KRAYFYWVTREQGSFDWFKGVMBEIAEYDKKGVIELHNYLTSVYEEGDARSALITMLQSLNHAKNGVDIVSGTRVTRTHFARNPWRKV FKKIAVKHPNARIGVFYCGAPTLVKELKKLAQDFSHKTSTRFEFHKENF
2	3.0 ^{e-470}	10	93	RAFRSSIKAVKILKVAVYPGNVLSLHMSKPTGFKYKSGQYMFVNCPAVSPFEWHPFSITSAPGDDYLSVHIRALGDWTEELRSVFSEVCK PPP
3	6.3 ^{e-382}	10	77	YFLLDNWKRIWVLALWIIIMAILFTWKFIZYKRKPAYEVMGYCVCVAKGAAETLKLNMALILLPVCRTITWLRSKT
4	3.2 ^{e-382}	9	90	ITKEZLKEFWEQITDKSFD SRLQIFFDMVDKBEDGRJTEEEVKEIIVLSASANKLSNJKKQADEYAALIMEELDPDNJGYIELEQLETL
5	6.5 ^{e-236}	10	39	DGPYGAPAQDYKKYDVLLLVLGIGATPFISILKDJLNN
6	3.2 ^{e-184}	20	28	PFDDNIWFHKTIWMYJAVPVLLYAGERL
7	9.3 ^{e-145}	10	33	LPKPLKKLTGFNAFWYSHHLFVIVYILLIVHGY
8	2.1 ^{e-115}	10	38	QPPTYLGLVKGPEGITGILMVILMLIAFTLATTWFRRN
9	6.2 ^{e-072}	10	38	TGGLPKRKFGGCGIMMDSKEFALELFDALARRRRRIKGE
10	1.1 ^{e-037}	10	26	ARLDRTKSSAERALKGLKFISKTDGG
11	8.4 ^{e-036}	10	20	CDFPRLJAATEDEYEPYAKY
12	4. ^{2e-015}	7	28	SGEKKKLSQMLSQKLIPTDRNPLKRWY
13	1.6 ^{e-007}	6	23	SRNRDEEYVEITLDIQDDSVSVH
14	8.4 ^{e-005}	2	28	PKPNDLIRMETRARGVNPHEESQVLFP
15	2.3 ^{e-004}	2	41	KNVSKNLGVGSIIRSLSVNKWRKSGNLGSPSTRKSGNLGPP

Table S4. Primers used in this work (restriction sites are underlined).

Name	Sequence	Amplification
At1g09090forNco	TAGT <u>CCATG</u> GGGAGGAAGAAATGG	Protein coding sequence of <i>At1g09090</i> from cDNA
At1g09090revBam	TGAT <u>GGATC</u> CATAGAATCGAAACTA	
At1g09090Mrev	ATAAGAACCAC <u>CAT</u> TGTAACACCGGT	Eliminate internal NcoI site
At1g09090Mfor	ACCGGTGTTACA <u>ATG</u> GTGGTTCTTAT	
pRBOHBforKpn	GTT <u>GGTAC</u> CTCTTCTCGATCTCG	Promoter fragment of <i>At1g09090</i>
pRBOHBrevNco	CCTCC <u>CCATG</u> GACTACAATTAC	
RbohRTfor	ACCGGTGTTACAATGGTGGTTCTTAT	RT-PCR of Rboh overexpression lines
RbohRTrev	TTGAGTTGCGATGTCCAGTC	

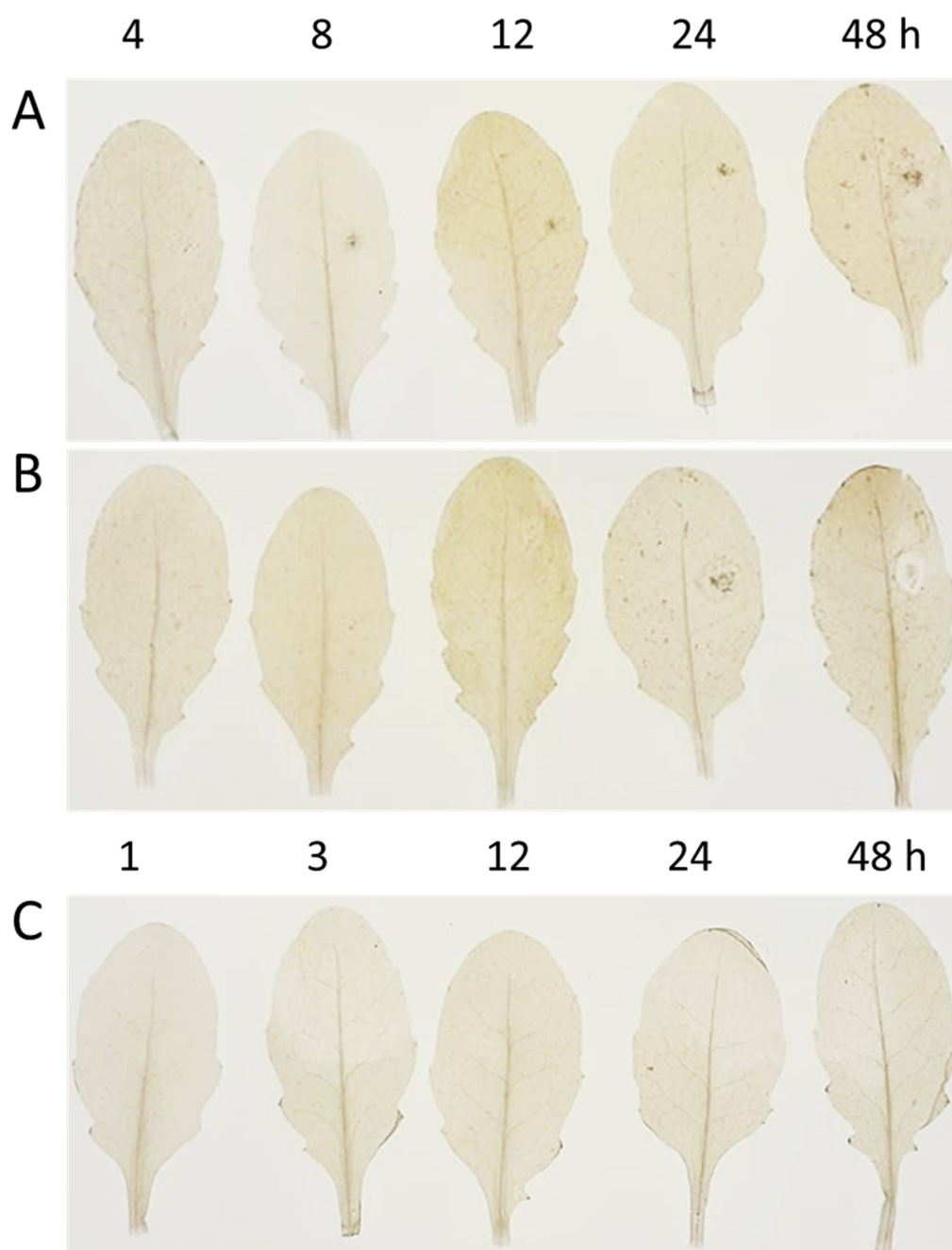
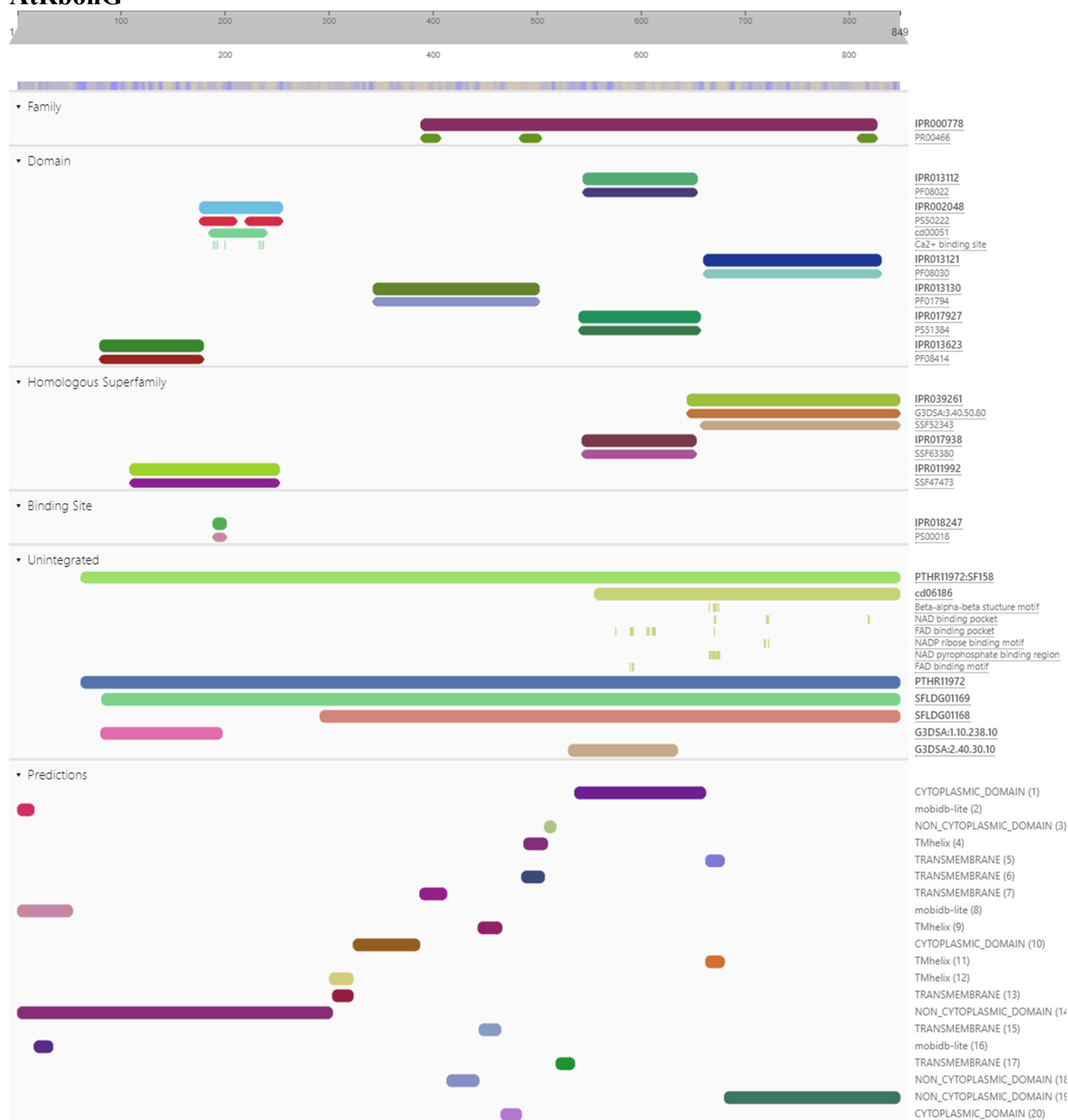


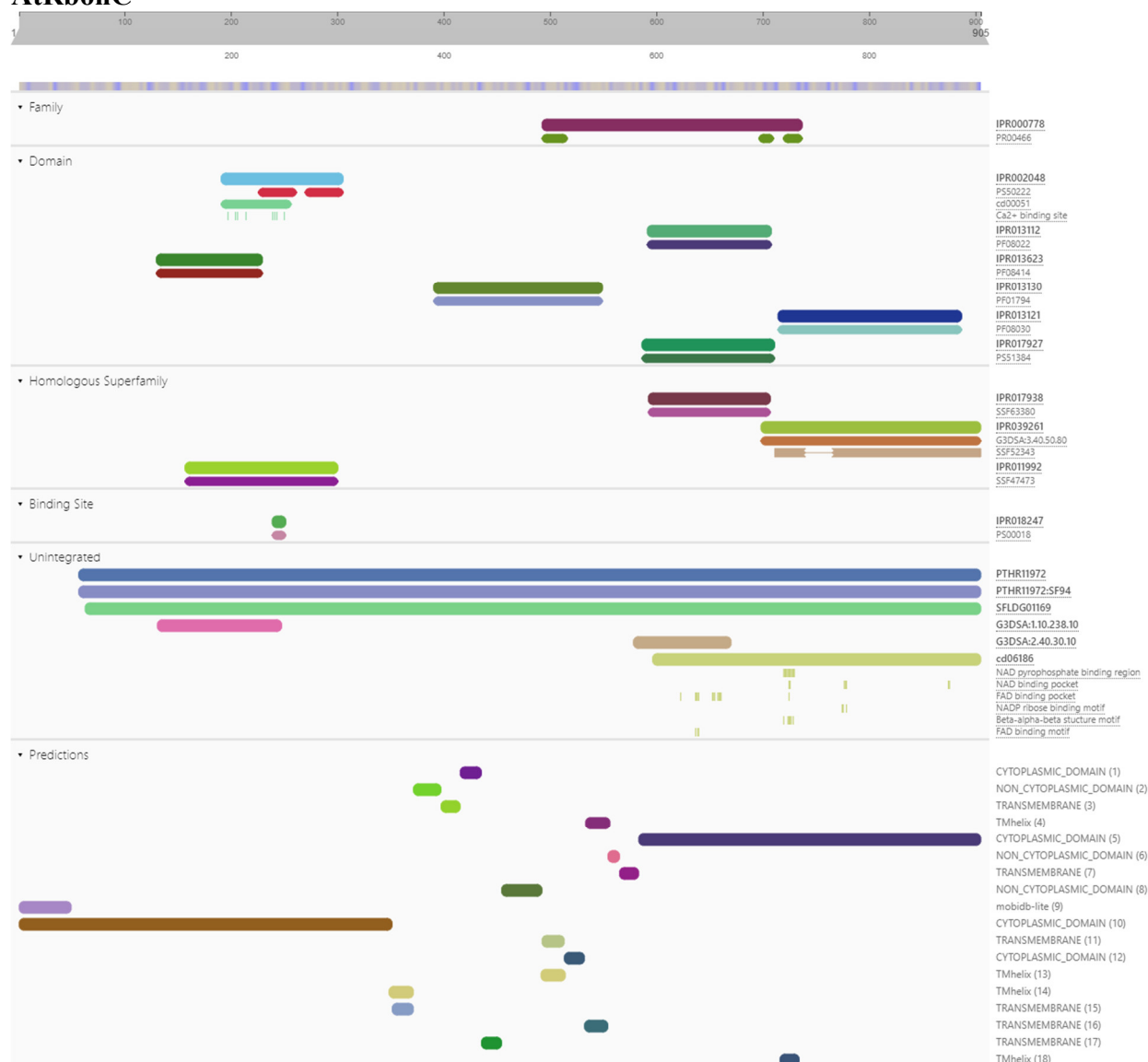
Figure S1. Leaves of the *RbohB* promoter::GUS line were stained for GUS at different time points after infection with A, *A. brassicicola*; B, *B. cinerea*; C, *P. syringae*.



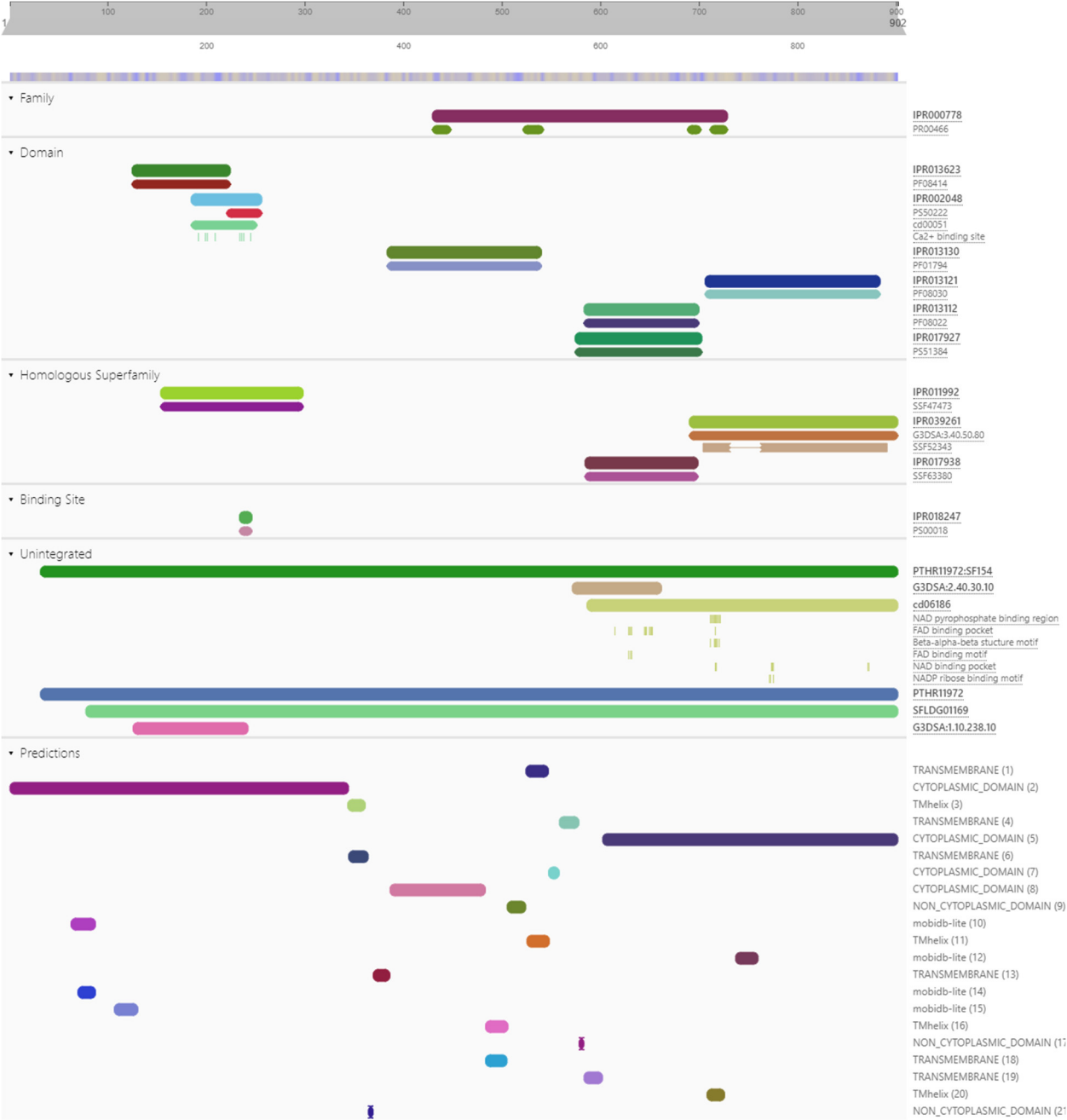
Figure S2. Sequence logos of the predicted 15 motifs in Rboh protein sequences. Colors of the motifs are the same as in Figure 11. Each letter shows one letter amino acid code. Amino acids with higher letters are more conserved.

AtRbohG

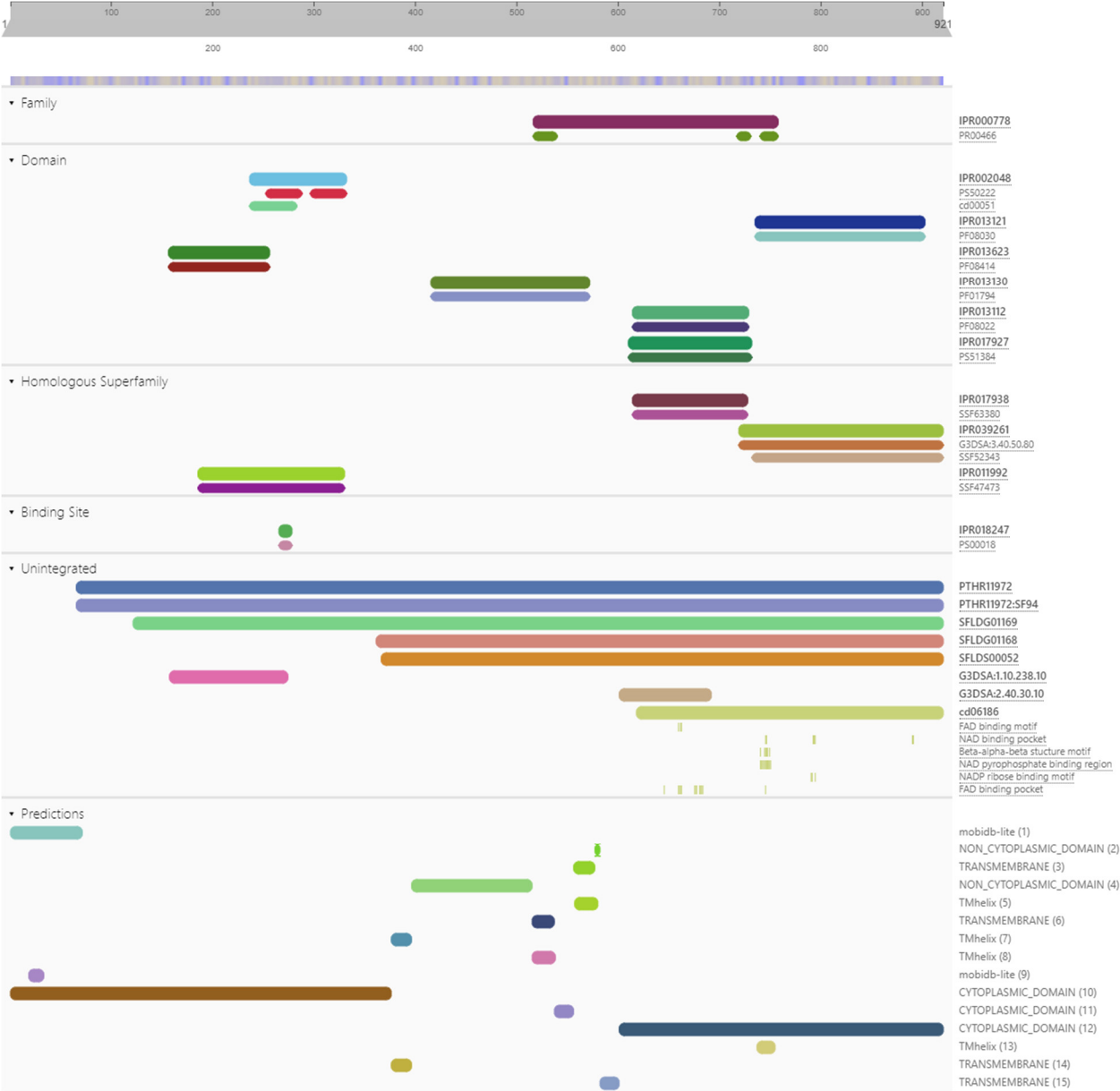
AtRbohC



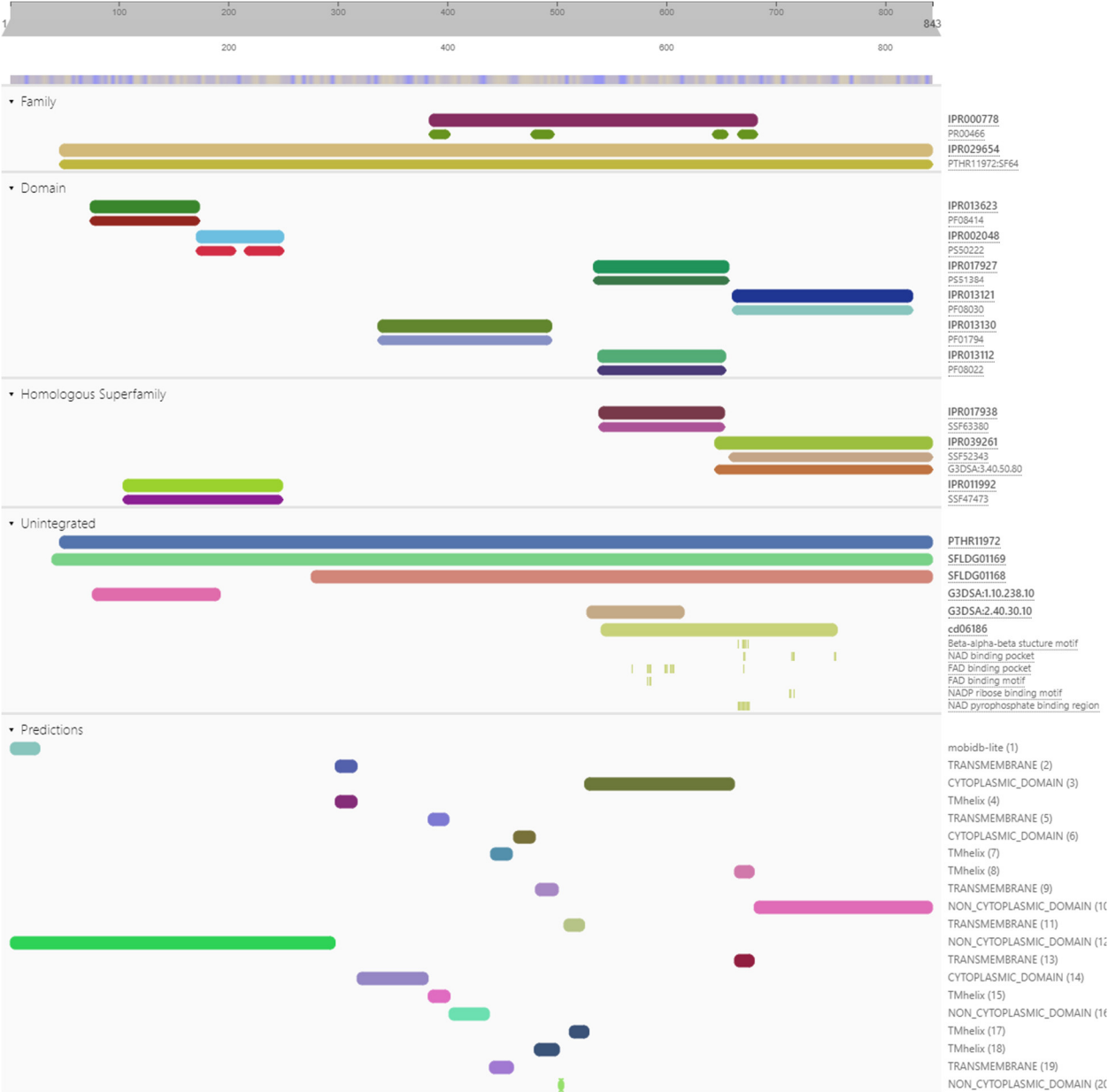
AtRbohA



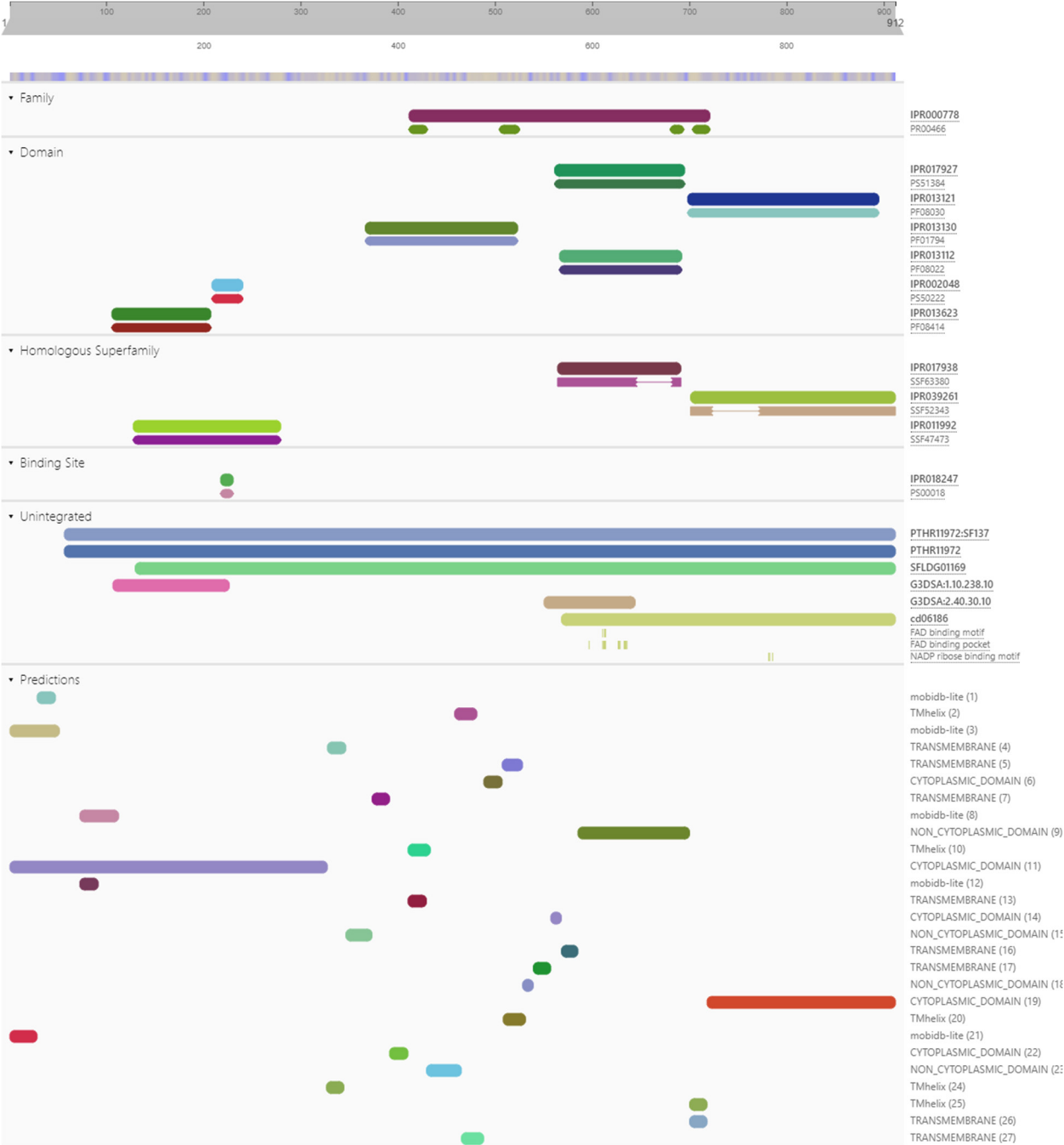
AtRbohD

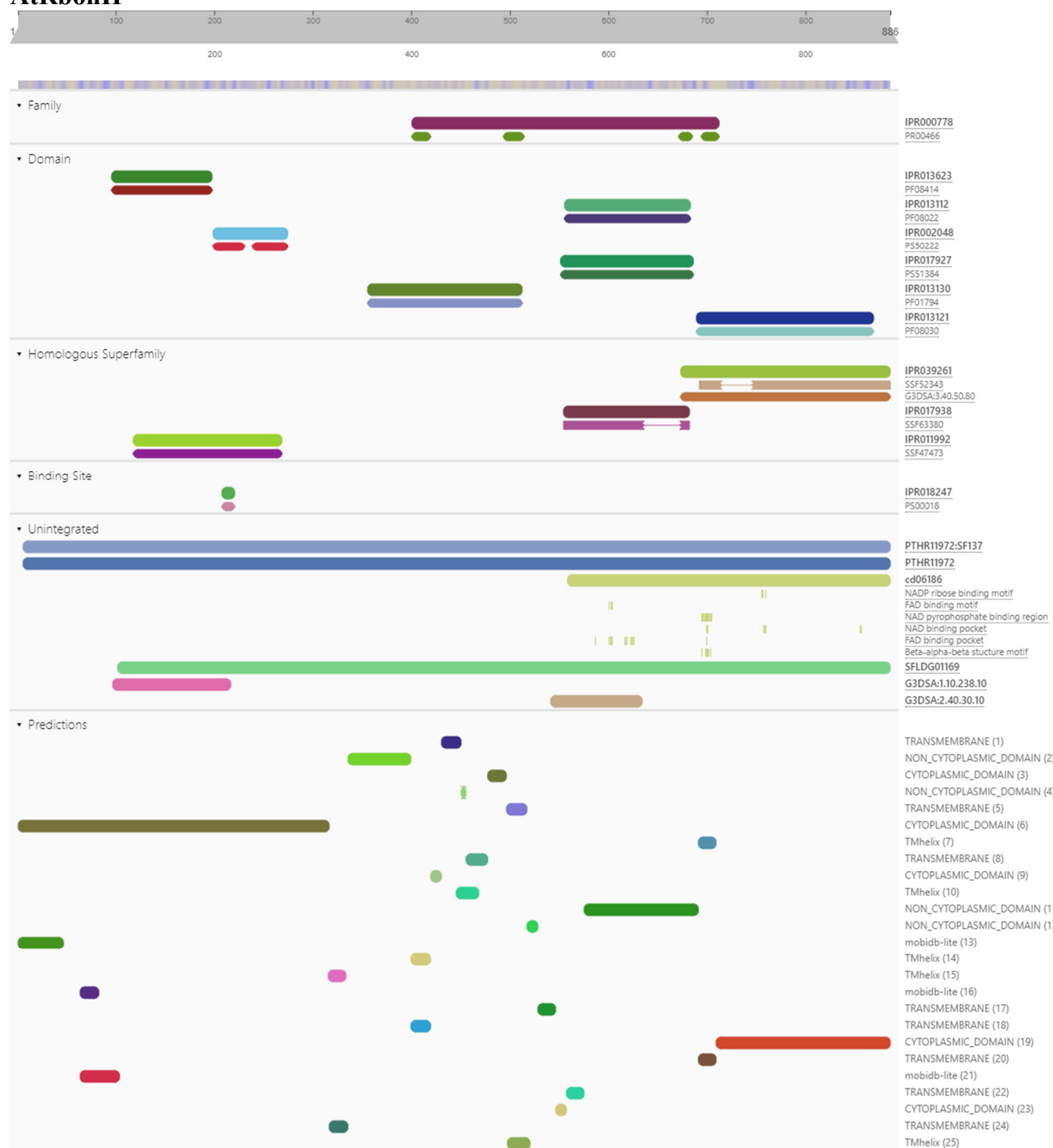


AtRbohB

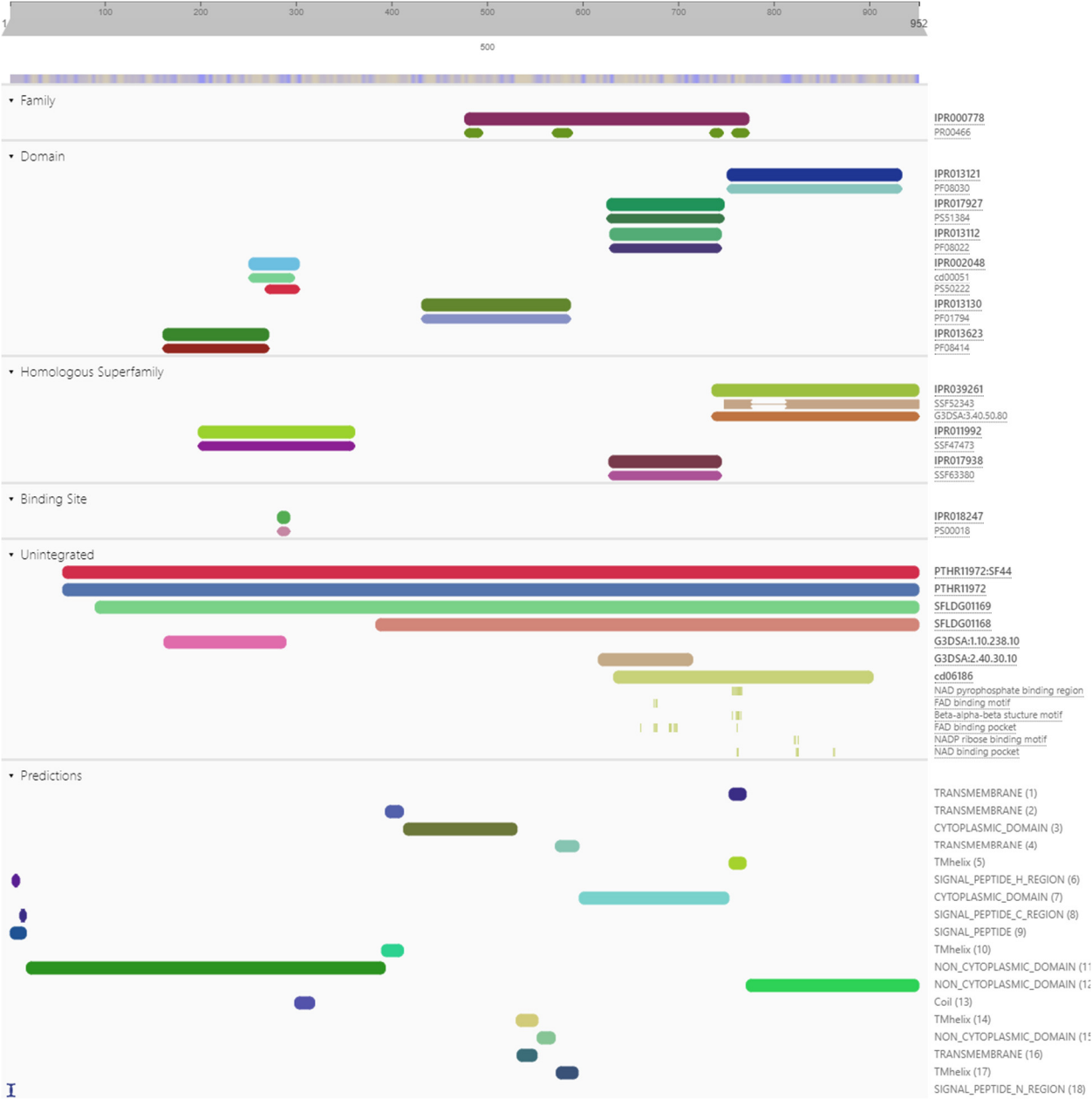


AtRbohJ

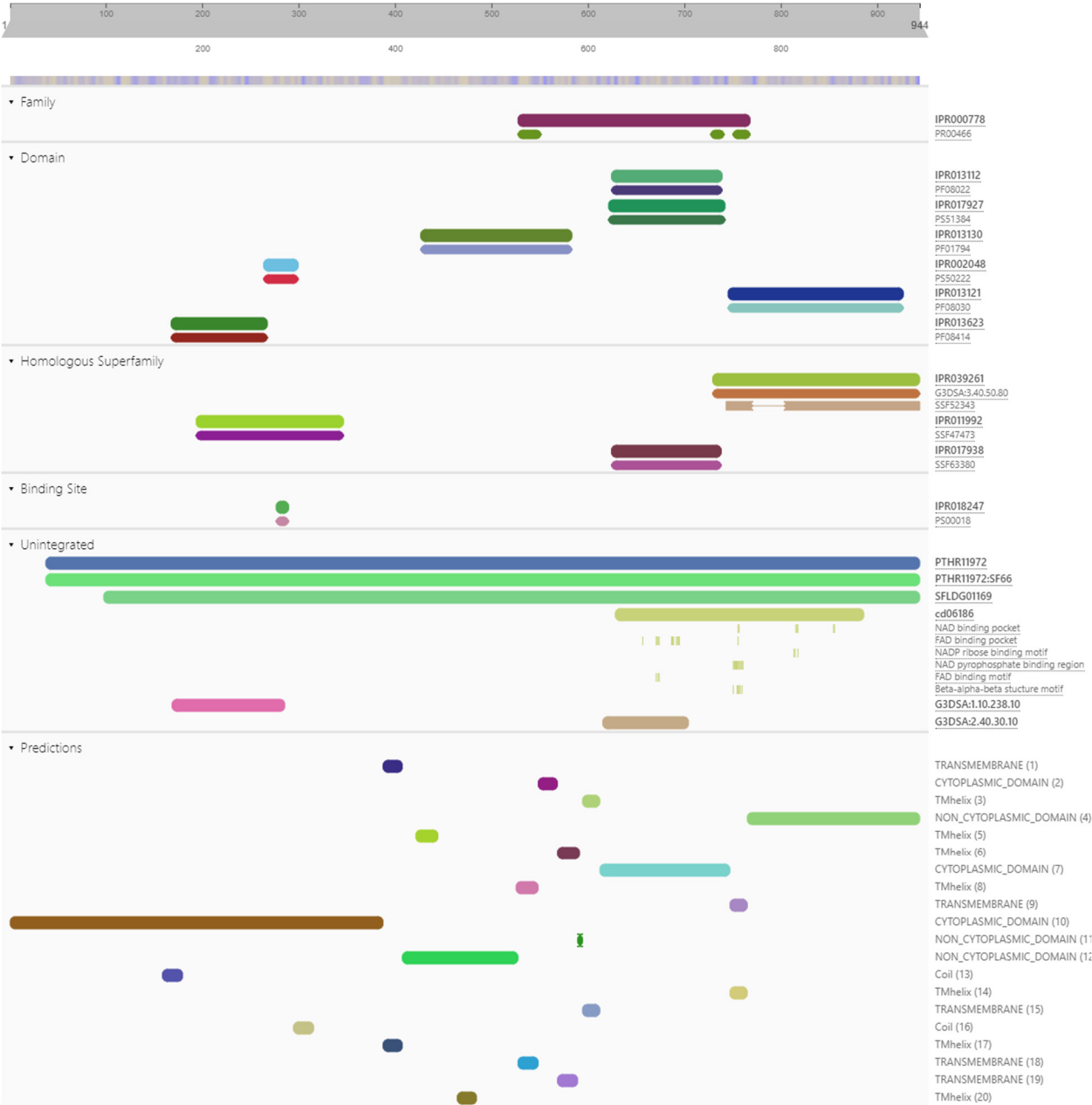


AtRbohH

AtRbohE



AtRbohF



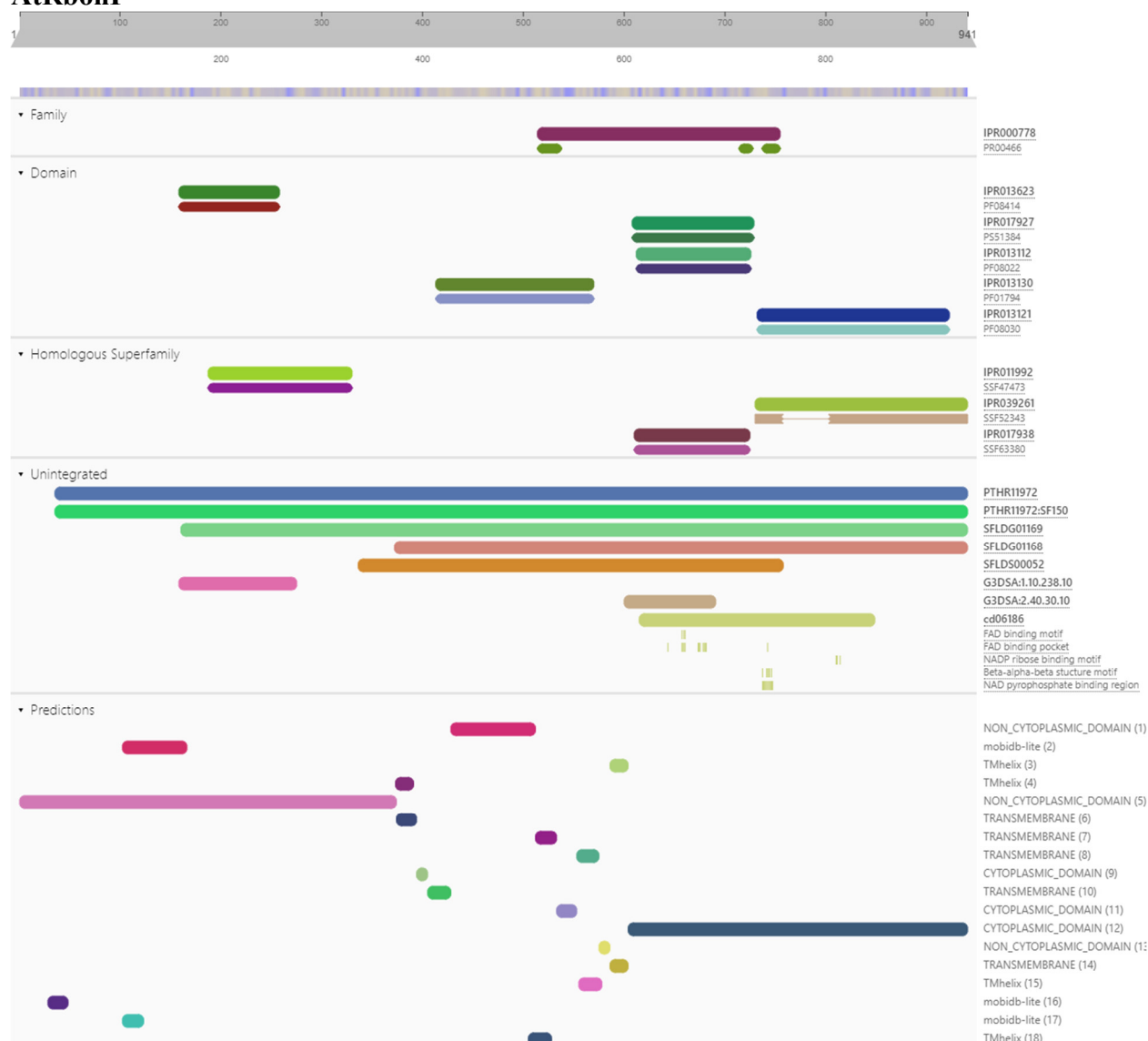
AtRbohI

Figure S3. Description of predicted domains in Arabidopsis Rboh proteins.

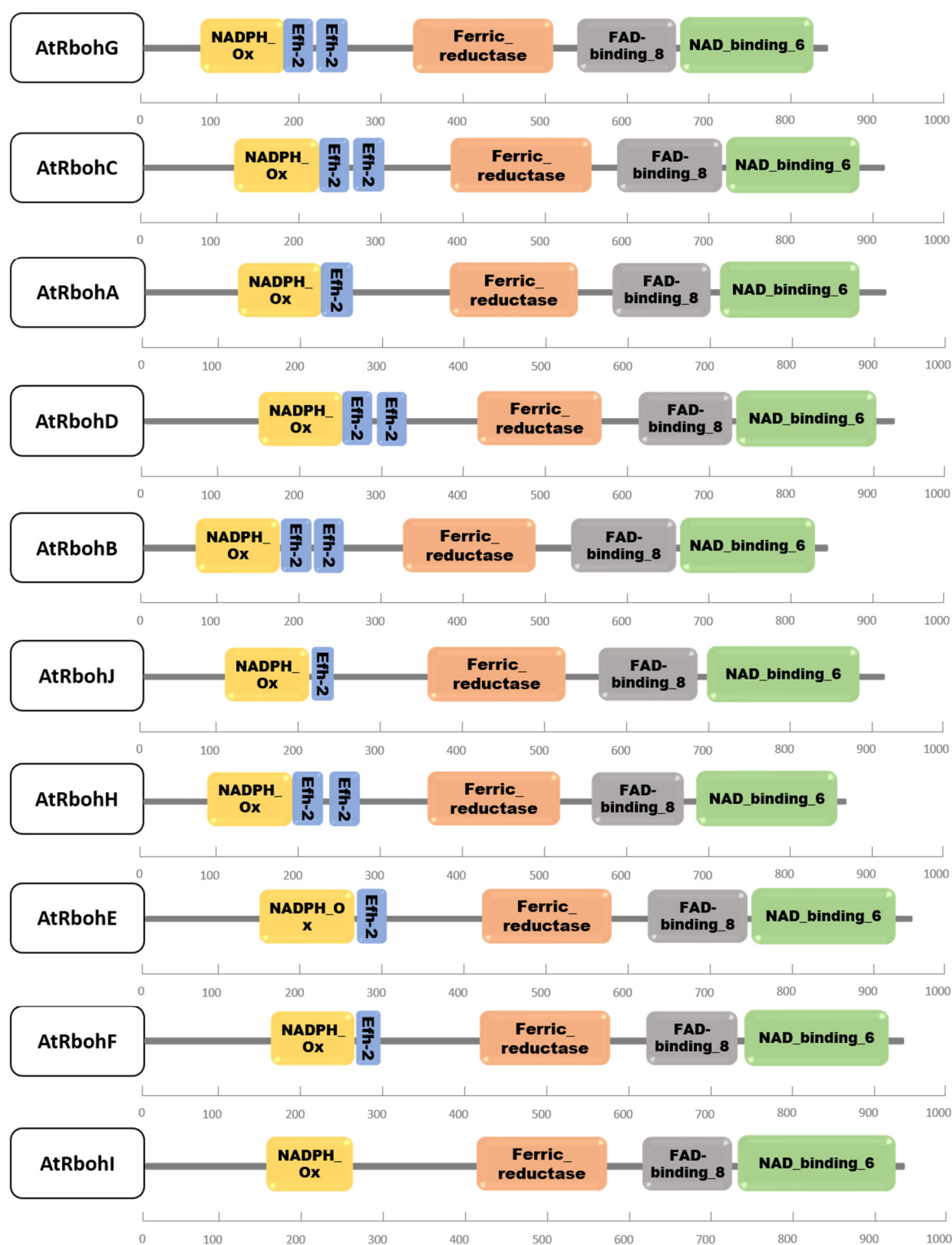


Figure S4. Conserved domains predicted in the Arabidopsis Rboh proteins. Scale below each sequence shows polypeptide length in amino acids. Blocks of different colors represent different domains as well as the EF_{hand}-2 motif. Width of each block directly correlates with the length of predicted domain in amino acids. X-axis shows the number of amino acids.

AtRbohF	-----MKPF-----SKNDRRRWSFDSVSAGTA-----V	24
AtRbohB	-----MREEE-----ME-----S-----SSEG-----E	13
AtRbohD	MKMRRGNSSNDHELGLRGANSOTNSDTEIASDORGAFS---GPLGRPKRASKKNARFAD	57
AtRbohF	GSASTSPGTEYSINGDQEFVEVTIDLQDDITVLRSEPATAINVIGDISDONTGIMTPV	84
AtRbohB	TNKISRCKATGSDNPDEDYVEITLVRDETINTMKAKAT-----	52
AtRbohD	DLPKRSNSVAGGRGDDDEYVEITLDIRDDSVAVHSVQQAAGG---GGHLEDPELALLTKK	114
AtRbohF	SISRPTMKRTSSNRFRQFSQELKAEAVAKQLSQELKRFWSRSFSGNLTTTSTAANQ	144
AtRbohB	-LRS-VLS-----GRLLTM-----	64
AtRbohD	TLES-SLNN-----TTLSFFRSTSSRIKNASREL-	144
AtRbohF	SGGAGGGLVNSALEARALRKQRAQLDRT-RSSAQRALRGLRFISNKQKQNVGDVQSNF	203
AtRbohB	DKLAV--EGKLPSKFGHCIGMVE-SSEFVNELFEALVRRRTTSSSITKTELFEFWEQI	110
AtRbohD	-----RVFSRRSPAVRRFDRT-SSAAIHALKGLKFIATKT--AAMPVADQRF	189
AtRbohF	EKFEK--NGYIYRSDFAQCIGMKD-SKEFALELFDALSRRLKVEKINHDELYEWSQI	260
AtRbohB	DKLAV--EGKLPSKFGHCIGMVE-SSEFVNELFEALVRRRTTSSSITKTELFEFWEQI	167
AtRbohD	DKLSADSNGLLLSAKFWELGMNKESKDFADQLFRALARNNVSGDAITKEQLRIFWEQI	249
AtRbohF	NDESFDRLQIFFDIVDKNEDGRITEEEVKIIMLSASANKLSRLKEQAEEYAALIMEEL	320
AtRbohB	TGNSFDDRLQIFFDMVDKNDGRITGDEVKEIILASASANKLSKIKENVDEYAALIMEEL	227
AtRbohD	SDESFDRLQIFFDMVDKDEDGRVTEEEVAEIIISLSASANKLSNIQKQAKYAALIMEEL	309
AtRbohF	DPERLGYIELWQLETLQLLQKDTYLNYSQA---LSYTSQALSQNLQGLRGKSRHRMSSDF	377
AtRbohB	DRDNLGYIELHNLLETLLQVPSQSNSSPSSANKRALNKLMLQKLTPTKDRNPVKRFAMNI	287
AtRbohD	DPDNAGFIMIEIENLEMLLQAPNQSVR--MGDSRIISQMLSKQLRPAKESNPLVRWSEKI	366
AtRbohF	VYIMQENWKRIWVLSLWIMIMIGLFLWKFFQYKQDAFHVMGYCLLTAKGAAETLKFNMA	437
AtRbohB	SYFFLENWKRIWVLTWISICITLFTWKFQYKRTVFVVMGYCVTVAKGSAETLKFNMA	347
AtRbohD	KYFILDNNQRLWIMMLWLGICGGLFTYKFIQYKKAAYGVMGYCVCAKGAETLKFNMA	426
AtRbohF	LILFPVCRNITWLRSTR--LSYFVPFDDNINFHKIAGAIIVAVILHIGDHLACDFPRI	495
AtRbohB	LILLPVCRNITWLRTRSKLIGSVVPFDDNINFHKVAVFAGIAGVIGLHAISHLACDFPRL	407
AtRbohD	LILLPVCRNITWLRNKTG-LGTVPFDDNINFHKVAVFAGIAGVIGLHAISHLACDFPRL	485
AtRbohF	VRATEYDYNRYLFHYFQTKQPTYFDLVKGPEGITGLMVLMIISFTLATRWFRNRLVKL	555
AtRbohB	LHAKNVFEPFMKFFGDERPENYGMFMKGTGWTGVTMVVLMVLVAVVLAQSVFRNRANL	467
AtRbohD	IAADEDTYEPMEKYFGDQ-PTSYWVFMKVGEGWTGIMVVLMAIAFTLATPWFRNRLNKL	544
AtRbohF	PKPFDRLTGFAFWYSHHFLVIVYILLHIGIFLYFAKPWYVRTTMYLAVPVLLYGGER	615
AtRbohB	PKSLKRLTGFAFWYSHHFLVIVYLLIVHGYFVYLSKEWYKTTMYLAVPVLLYAFER	527
AtRbohD	PNFLKRLTGFAFWYTHHFLIIVYALLIVHGIKLYLTKIWYKTTMYLAVPILLYASER	604
AtRbohF	TLRYFRSGSYSVRLKVAIYPGNVLTLMKSKPTQFRYKSGQYMFVQCFAVSPFEWHPFISI	675
AtRbohB	LIRAFRPGAKAVKVLKAVVYPGNVLSLYMSKPKGFKYTSQGYIYINCSQVPLQWHPFISI	587
AtRbohD	LLRAFRRSSIKPVKMIKAVVYPGNVLSLHMTKPGQFKYKSGQFMLVNCRAVSPFEWHPFISI	664
AtRbohF	TSAPEDDYISIHRLQGLDWTQELKRVFSEVCEPPVGGKSGLLRADETTK--KSLPKLLI	732
AtRbohB	TSASGDDYLSVHIRTLDGWTSQLKSLYSKVCPLPSTQSGLFIADIGQANNITRFPRLI	647
AtRbohD	TSAPGDDYLSVHIRTLDGWTQELKRVFSEVCKPPTAGKSGLLRADGGDN--LPFPKLLI	722
AtRbohF	DGPYGAPAQDYRYKYDVLVLLVGLGIGATPFIISILKDLLNNIVKMEEHADSIQFSRSSEYS	792
AtRbohB	DGPYGAPAQDYRYKYDVLVLLVGLGIGATPFIISILKDLLNNIVKMEEHADSIQFSRSSEYS	693
AtRbohD	DGPYGAPAQDYRYKYDVLVLLVGLGIGATPFIISILKDIINNMGQPDQSDI-----	771
AtRbohF	TGSNGDTPRRKRIKTTNAYFYWVTRQGSFDFWFKGVMNEVAELDQRGVIEHMYLTSVY	852
AtRbohB	--ERGTNQHIKNYVATKRAYFYWVTRQGSLEWFEVMNEVAEYDSEGMIELHNYCTSVY	751
AtRbohD	--ENNNSNNNSKGFTRKAYFYWVTRQGSFEWFKGIMDEISELDEEGIEELHNYCTSVY	829
AtRbohF	EEGDARSALITMVQALNHAKNGDIVSGTRVTRTHFARNWKKVLTKLSSKHCNARIGVFI	912
AtRbohB	EEGDARSALITMLQSLHAKSGDIVSGTRVTRTHFARNWRSVFKHVAVNHVNRQVGFY	811
AtRbohD	EEGDARSALITMLQSLHAKNGDIVSGTRVKSHPAKPNWQVYKIAVQHPKRIQVFI	889
AtRbohF	CGVPVLGKELSKLCNTFNQKGSTKFEFHKEHF	944
AtRbohB	CGNTCIIGELKRLAQDFSRKTTTKFEFHKEHF	843
AtRbohD	CGMPGMKELKNLALDFSRKTTTKFEFHKEHF	921

Figure S5. Alignment of RbohB, RbohD and RbohF protein sequences.

Dataset: 48 anatomical parts from data selection: AT_AFFY_ATH1-5
 Showing 10 measure(s) of 10 gene(s) on selection: AT-0

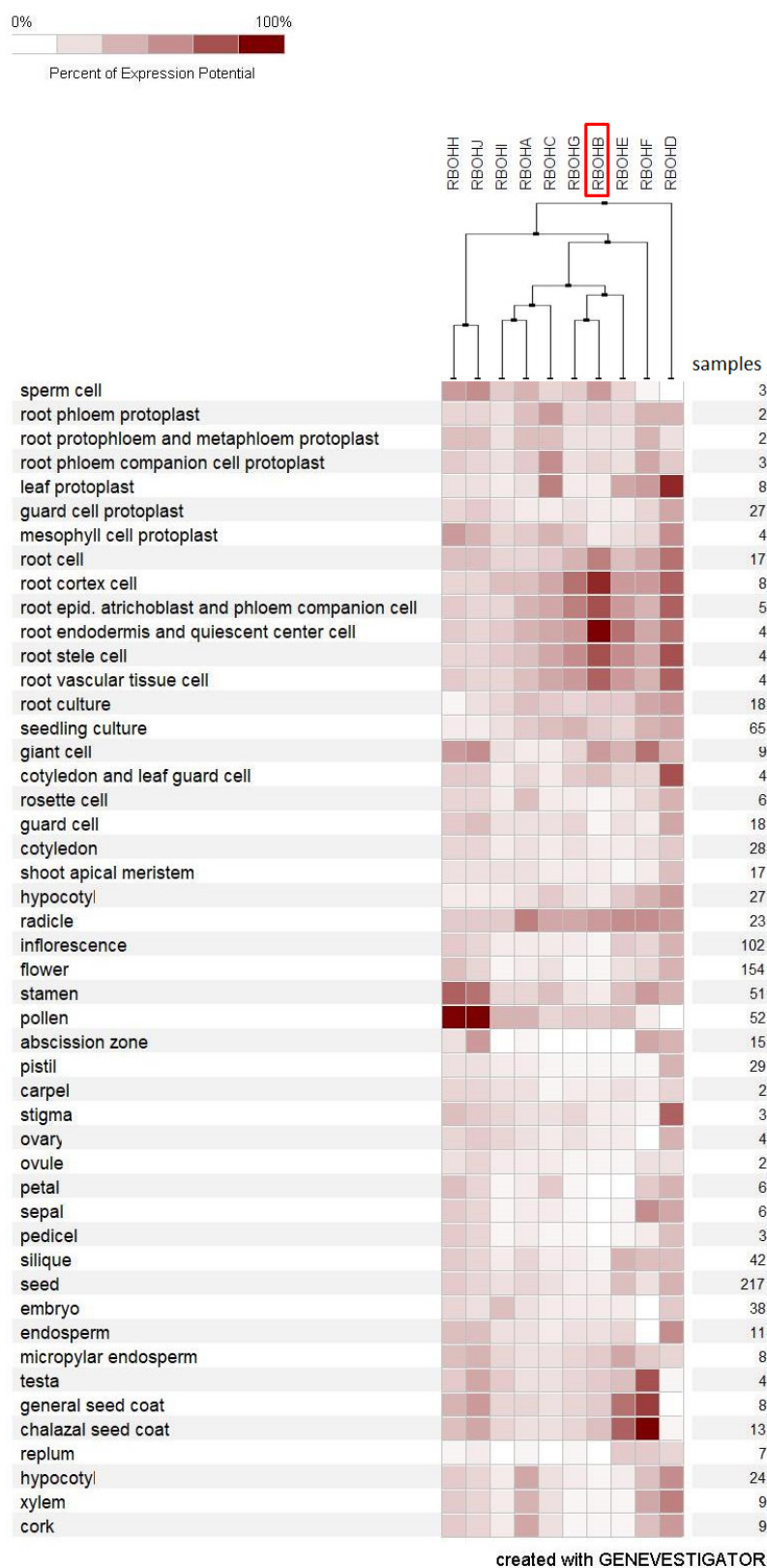


Figure S6. Expression of Arabidopsis Rboh genes in anatomical parts according to Genevestigator.

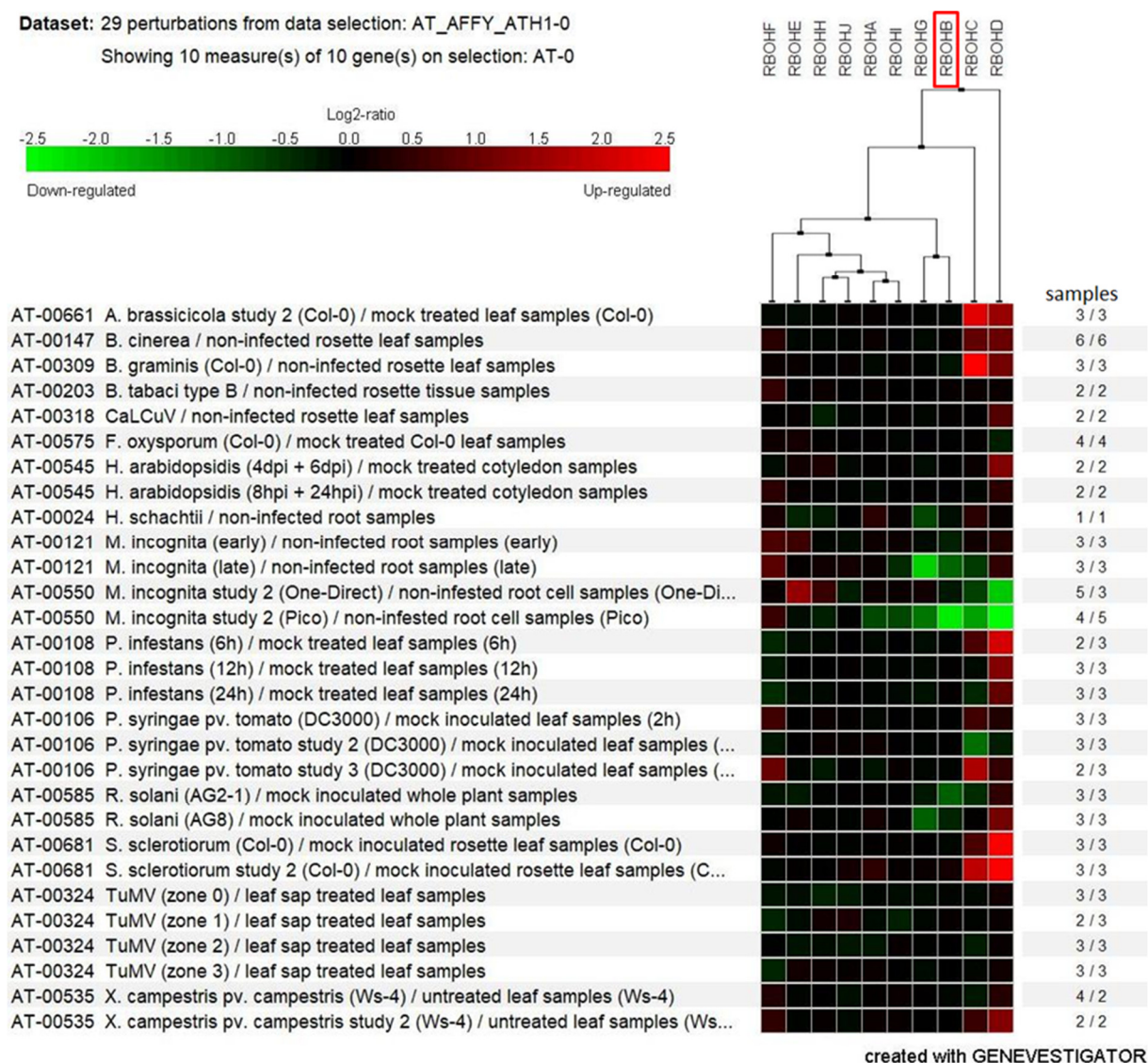


Figure S7. Expression of Arabidopsis Rboh genes after infection with pathogens according to Genevestigator.

Dataset: 12 perturbations from data selection: AT_AFFY_ATH1-1

Showing 10 measure(s) of 10 gene(s) on selection: AT-0

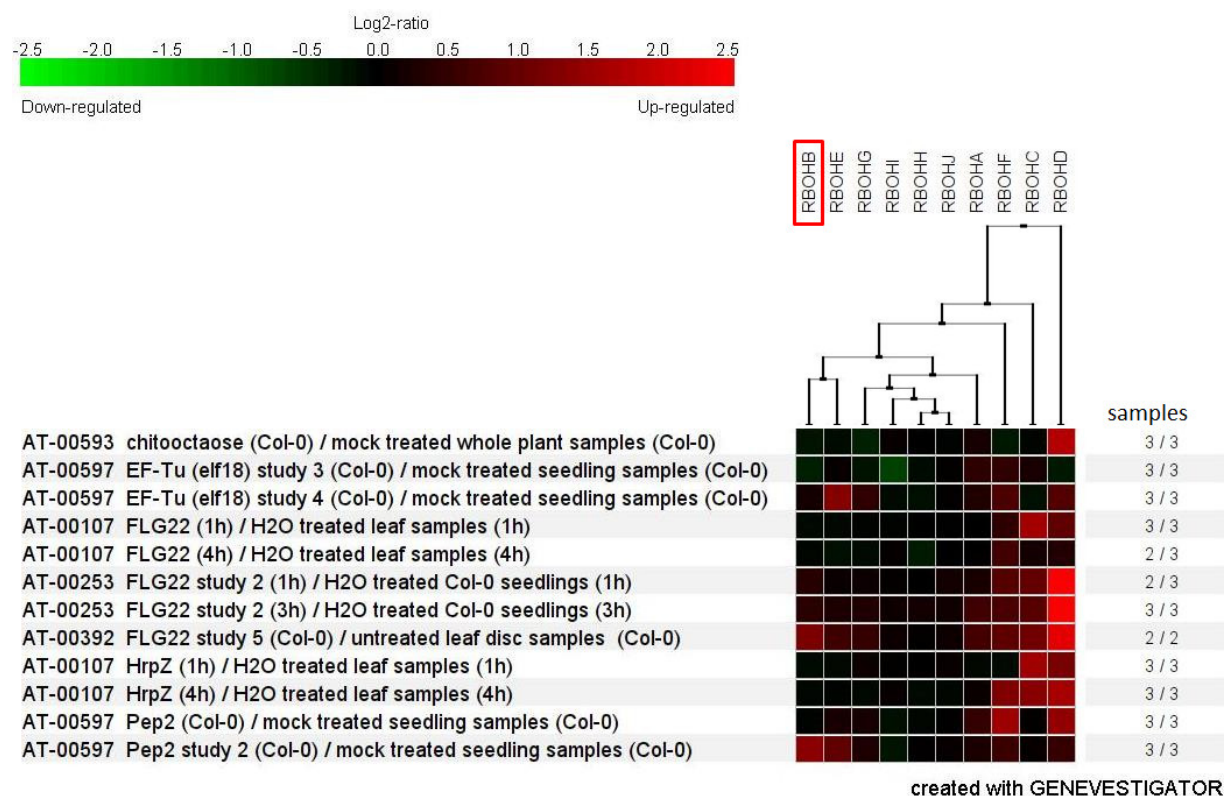


Figure S8. Expression of Arabidopsis Rboh genes after treatment with elicitors according to Genevestigator.

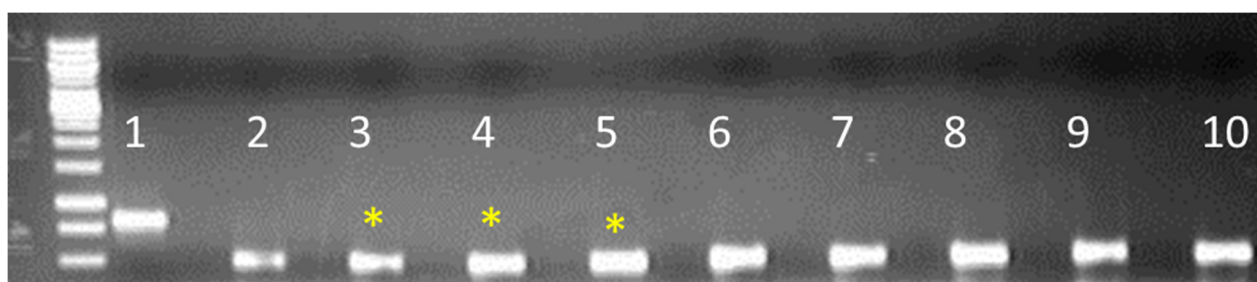


Figure S9. RT-PCR of overexpression lines of RbohB, where 1= gDNA WT, 2=cDNA of WT, 3=cDNA from overexpression line (OE) 1, 4 = cDNA from OE2, 5= cDNA from OE3, 6= cDNA from OE4, 7= cDNA from OE5, 8= cDNA from OE6, 9= cDNA from OE7, 10= cDNA from OE8. cDNA concentration for all the lines was equalized through the measurement of DNA concentrations using Nanodrop. Asterisks show the lines used for characterization. .



© 2020 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).