

Table 1 Suppl. Forward and reverse primer sequences used for site-directed mutagenesis of *Nbexo70D1*. \* - Underlined sequences represent the restriction enzyme sites for cloning (AAGCTT HindIII, GGATCC BamHI). \*\* - *Underlined codons* indicate mutant sites in the *NbExo70D1* domain D.

Primers	Forward Primer Sequence (5'-->3')	Reverse Primer Sequence (5'-->3')
NbExo70D1 full-length sequence	CACCATGGAACCACCGGAGAACG	CTGAGATCTTCTTCTTATGTGTTG
NbExo70D1-RE full-length sequence*	CCC <u>AAGCTT</u> CTATGGAACCACCGGAG	CGG <u>GATCC</u> CTCACTGAGATCTTCTTC
NbExo70D1 partial sequence	GAGTTGGAGCATCAAGAGAG	AGCCTGAACTCTAATTGACTCC
nbexo70d1 domain D-1**	GTGCATTGGCAGAGGCGTTCGCAACGTTCA	TGAACGTTGCGAACGCCTCTGCCAATGCAC
nbexo70d1 domain D-2**	TTCTCGGAGCGTTCGCGAGTGCTATTGAGA	TCTCAATAGCACTCGCGAACGCTCCGAGAA
NbExo70D1 D-domain	CAGAGATCAACCTGGGTGAG	CTGAGATCTTCTTCTTATGTGTTG
NbExo70D1 promoter	CACCCCTCCTCAATGTAGTGTGTGTTCA	ATTTTCATCGACGGCTTGCAGG
Endogenous NbExo70D1 D-domain	CAGAGATCAACCTGGGTGAG	CCACAAAACAATTCAAAGAATGTCC
NbEF1- $\alpha$	TGTCCCCATCTCTGGTTTCG	AATCTGGTCAAGAGCCTCAAGAA
NbATG8f	ATGGCAAAGAGTTCATTCAAGC	CACCAAGTTAAAGTCCCCAA'

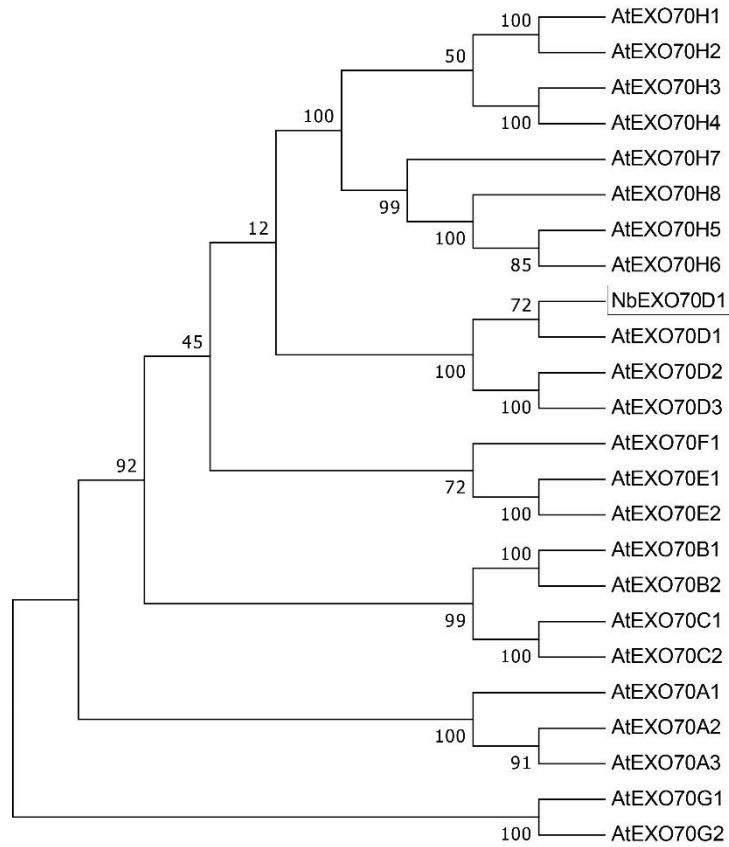


Fig. 1 Suppl. Phylogenetic relationships of Exo70 proteins from *Arabidopsis thaliana* NbExo70D1 and AtExo70. AtExo70 protein sequences were retrieved from the *TAIR* (The Arabidopsis Information Resource, <https://www.arabidopsis.org>) and aligned using the *ClustalW* program (<https://www.genome.jp/tools-bin/clustalw>). A phylogenetic tree was generated using the neighbor-joining method in the *MEGA 7.0* program. The numbers above or below the branches are bootstrap values from 1 000 replicates. NbExo70D1 is boxed.

AtExo70D1 1 -----MEPHDQTHGGDGGEGENCSSGSDSAEKVIIIRWDPAASEAR-----ERMIFN-  
AtExo70D2 1 -----MATPETRGVDSDETAEKIIIRWDSASEAR-----ENHIFC-  
AtExo70D3 1 -----MEPPENSLSDSGSEDAERIIIRWDSAADGAR-----GKMIFC-  
OsExo70D1 1 -----MMDGSTAAAEAEAEAEVVRWDSASASVGGGGGGGQMTFDG-  
OsExo70D2 1 -----MDGSAAEAEAEVVRWDSASAS-DEPMFDG-  
NbExo70D1 1 -----MEPPENDVAESAETIIIRWDSASEAR-----ERMIFAG-  
SacExo70 1 -----MPAEIDIDEADVVLVLSQELQKTSKLTFFINKSKRLAATSNQSSQLFPILARNNVLTTLQRNIES-  
MmExo70 1 MIPPEQASARRREIDKLLKQEEETLSFIRDSLEKSDQLTKNMVSISSSESRMLRMENSIIIPVHKQENLQRLQENVEK-  
DmExo70 1 -----MNNLDSSLQAHNKLEKATNLALLKDRVDKYLHDLSTQMSSIDAFIKRLGNDEQTLFVYQETEQGLKRCQNEAE-

#### Domain A

AtExo70D1 49 -----EECEVNLVNAVDEQCVYSSG-----G-----EIEENRANSAQAMARIEDEFNRNLT-  
AtExo70D2 49 GGDPEVNRILNAVDEQCVSSISIS-----DEVKATQAMARIEDEFNRNLT-  
AtExo70D3 42 -----DPEVNRILNAVDEQCVSSVSFSSSSSSAATSAAATVVD-----EHEVRNQAQAMARIEDEFNRNLT-  
OsExo70D1 44 GGDPEVNRILNAVDEQCVLRAFFSPATVGSFPRRTSSAS-----GGGGAASAVQAMARIEDEFNRNLT-  
OsExo70D2 32 -----DPEVNRILNAVDEQCVLRAFFSPATVGSFPRRLSSGSSSVSA-----GGGGAASAVQAMARIEDEFNRNLT-  
NbExo70D1 38 -----DPEVNRILNAVDEQCVSFTFLS-----DDLNRANSAQAMARIEDEFNRNLT-  
SacExo70 68 LNSVASIKDLANEASKYVEIKQKIN-----QVGLKQYTOVHKRDDMLEICSGQAN-  
MmExo70 81 LSCLDHVISIKVASITETIKREPTG-RLEEYLGSMARIQKAVEYFQDNSPDSPELNKVKLLFEPEKESLESTTSAT-  
DmExo70 77 LNCLESMLSHYDVSCETCCOHQCPVEGNISVFLDALAKLRDANDYFRHNSQSVELENVTSLFTCEGSCQHSMLK-

AtExo70D1 98 SHESFENSQMLSSSSSHLEVDEDDSSSSNNNGNEEDQOEEETDILLKRSGSSASTGSATRIPTGCRSRSSTSSIRE-  
AtExo70D2 90 SCSTTFPSLLDSSLSSSFASSSRTELEDTCDDGNEEEEQQQVLDLVPDGSSDSGSRRLRFRSRSSTSSIRE-  
AtExo70D3 108 SHHTTFPSFLFEE-----FVSPSLTVEVGEDTTVTPEELN-----SPGSGSSRLRFRSRSSTSSIRE-  
OsExo70D1 108 SRALDTEISAADITSLSMCSORTNSADVAEEAAADDDSSSSVGRSRSYRSLRSIRE-  
OsExo70D2 99 SRALDTEISAADIGSLSLISSDRSNSASSAELOQVVDSSSSVGRSRSYRSLRSIRE-  
NbExo70D1 89 AHSFTEASGTDTSNEDYHDYNSVDSPILTKELEHQSNSS-----GYRSSTSSIRE-  
SacExo70 121 REENSEFHGITHTEQLIKKSAQLRVYFISILNSIKPFPQIN-----IKKMPFIYEDDQCGA-  
MmExo70 160 RHFVKVSPVLDDISADDELEVQEDVLEHLEPSVLRVVRISRWLVEYG---RNDQFNWYVYQIRSLRSIKGLLE-  
DmExo70 157 KHSAPKPVLDLIYIEDD-SSDEYTSFRLSQTTRELYTISHWLEQNL---R-EYTNVAMERGEVVRSLQLAK-

#### Domain B

AtExo70D1 178 IELTPIESWIHSMIARMVSGYIRECTOVYSSVRKSAVSSFRRLGTEKISIGDVORINWEALECKIRRWIRAAKICV-  
AtExo70D2 170 IDLVTFEAVSOLRSIACRMICAGYSRECOVYVNRKSAEMIFNCLGVKISIGDVORLEWEVEGKIRRWIRAAKICV-  
AtExo70D3 173 MDLISFEAVSOLRSIVCRMVAGYSRECOVYVNRKSAEMIFNCLGVKISIGDVORLEWEVEGKIRRWIRAAKICV-  
OsExo70D1 168 IDLLPADALSOLHAIASRMVAGYSRECOVYVNRKSAVSSALRRLGTEKISIGDVORLEWEVEGKIRRWIRAAKICV-  
OsExo70D2 159 IDLLPADAVSOLRAIASRMVAGYSRECOVYVNRKSAVSSALRRLGTEKISIGDVORLEWEVEGKIRRWIRAAKICV-  
NbExo70D1 142 IDLLPADAVSOLRAIASRMVAGYSRECOVYVNRKSAVSSFRRLGTEKISIGDVORLEWEVEGKIRRWIRAAKICV-  
SacExo70 182 ISWILDYFHNSESGSIIDILVGRSKLILKCMAPLEPFAREISTAKNAPYKSGSGMNSYTBALGLIANEKSLVDDLY-  
MmExo70 237 HFFKSSSSSGVPYS-----PAIPNKKDKDTPTKKPIKRPETIRKAQNLKQYSQHLGEGKGSNIPLEGEDMDL-DWET-  
DmExo70 231 HQRSNSWHEALRPRHSGRQTEPKKTSARQQIFERANKLYLATQTIEGSGFSIKASSHSHTLSTEDMDDQET-

AtExo70D1 258 RVVFASEKRLCEHVESVGA-----NIHACCEMEVVKSAFOLENFAEALS-----ISRRSEPEKFLKILDHDA-  
AtExo70D2 250 RVVFASEKRLCECFEG-----TMEETCEMEVVKSAFOLENFAEALS-----ISRRSEPEKFLKILDHDA-  
AtExo70D3 253 RVVFASEKRLCECFDGH-CT-----AMDETCEMEVVKSAFOLENFAEALS-----ISRRSEPEKFLKILDHDA-  
OsExo70D1 248 RVVFASEKRLCFILFHDPLSSSTTTAT---HDAFSEAVKGAALOLEGFAEALS-----HGRSEPEKFLKILDHDA-  
OsExo70D2 239 RVVFASEKRLCFILFHDPLSNTTAAAPATHDTFSEAVKGAALOLEGFAEALS-----HGRSEPEKFLKILDHDA-  
NbExo70D1 222 RVVFASEKRLCECFEGGTA-----TDAFSEAVKSAFOLENFAEALS-----ISRRSEPEKFLKILDHDA-  
SacExo70 262 SQYTESKPHVLSCLSPFISAYAKIFG---ANLKIVRNENFEGFSSBELVESIN-----DVKKSLRGLRQNYNL-  
MmExo70 311 DAYIHCVSAFVKLAQSEYRLMEIPE---HHQKKTGDSLQDALDGLALEENHVAARKAIHHDFTSLVFFLHR-  
DmExo70 311 DKYLVMLLGQRLNWERAIMIDIPO---SKHNEVSATLAYNAIDLVKFAEATQORILRCSREWTSAICFSALR-

AtExo70D1 323 LIELLPDIESVFDL-RSSSIRVQAEILSRLEAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
AtExo70D2 311 IDLLPDMEIFDS-SSSSSTVQATEIQSRLAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
AtExo70D3 317 IDLLPDIEAFIFDS-SSSIAIRQAEILSRLEAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
OsExo70D1 319 IDLLPDIEAFIFDS-SSSIAIRQAEILSRLEAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
OsExo70D2 313 IDLLPDIEAFIFDS-SSSIAIRQAEILSRLEAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
NbExo70D1 286 LSOLLDEIFVFS-RSSSIRVQAEILSRLEAAARGILSEFENAVLREPSI-----VFPVGGTIHPLTRVVMNYSL-  
SacExo70 330 LQCTCEVQVQTSFLRDAIRIKKANSTIPISNNVTEATVDTSLRKFSS-----EYKNGCGLGAMDNITRENWL-  
MmExo70 388 LQCTCEVQVQVLOG---AAASTNKLPGLISSETIKAKALEADNENEDR-----EYNMPKDTVHETLSNATLCOQ-  
DmExo70 388 WILLCEDIDRTYD-----PACREELKKYLLKQHTSKALEHLDVVKCESSTNIVGQSNVPKDATVHETLSNATLCOQ-

#### Domain C

AtExo70D1 396 ISHYRFTLIDLINSK---SNATSNTPDFDSLENN---KGPLAHMIWIVLLENLEKSKYVNAALSHLFTM-  
AtExo70D2 384 ISDYKFTLIDLIMTEKFCRLGCTNFRNDPMDISELG---ISPLAHMIWIVLLENLEKSKYVNAALSHLFTM-  
AtExo70D3 390 ISDYKFTLIDLINSNPS---TGSFNTPMDMFTELS---KSPIDHMIWIVLLENLEKSKYVNAALSHLFTM-  
OsExo70D1 393 ISDYKFTLIDLINSNPS---TGSFNTPMDMFTELS---KSPIDHMIWIVLLENLEKSKYVNAALSHLFTM-  
OsExo70D2 387 ISDYKFTLIDLINSNPS---TGSFNTPMDMFTELS---KSPIDHMIWIVLLENLEKSKYVNAALSHLFTM-  
NbExo70D1 359 ISDYKFTLIDLINSNPS---TGSFNTPMDMFTELS---KSPIDHMIWIVLLENLEKSKYVNAALSHLFTM-  
SacExo70 403 ISNYKEKEYTONEALNWDENVLLSCFISDCIDTAVN---LERKACHALPNOEPDVANPSSKNHKKORIGFTIL-  
MmExo70 462 ILDEQFAGAMIAQVGLDYNIPLDPRETSSSATSYSSEF-SKRLISTYCKVIGNLCLINLSKSVSEBPAISALFH-  
DmExo70 463 IYHFDVVGSIQAQDVLYSQDITILMKALP---VEER-NKALLATYVKKAAELLSMNNCECYNQATRLRLR-

AtExo70D1 469 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
AtExo70D2 459 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
AtExo70D3 461 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
OsExo70D1 473 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
OsExo70D2 467 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
NbExo70D1 434 NNHYIYVQRIRGS---PELREMGIDYLRKLTGKFRCAATYQRAAMVVKLYCLRDEGLHTK---GGFSSGVSKSAIRERFK-  
SacExo70 478 NNHTLWEQIVKES---ELNMLAGEGSRLELRKRYISMYSDWRDTANLMDSVFIDS---SGKSKDKREQERFK-  
MmExo70 541 NNHYIYKSPKSELICLVATQKTABSYREIEQIQIQYQ---SWIKVTDYDCKNLPWFQFGVKLRDKDPMKREKFK-  
DmExo70 537 NNHYIYKSPKORSNLIQLVTAEPECESYMEMIRKASLYQ---HSKRLVGYSLDELFKPVGKVKDKDSVIRERFS-

#### Domain D

AtExo70D1 545 SFNALFEEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVEDLETLVLLFEGYSA-  
AtExo70D2 535 AFNTMFEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVENLETLVLLFEGYTT-  
AtExo70D3 537 AFNTMFEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVEDLETLVLLFEGYTT-  
OsExo70D1 549 SFNALFEEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVEDLETLVLLFEGYTT-  
OsExo70D2 543 SFNALFEEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVEDLETLVLLFEGYTT-  
NbExo70D1 510 SFNALFEEVHRVQSCWVDPDOLREELNISILEKLPAYRSFLGRFRSHIESGRHPENYIRSVEDLETLVLLFEGYTT-  
SacExo70 551 FPNQGFEDVSKTKQKLSPELVTLSEFISLVNMYERYYSKQDSF---KNP---HIAFPELVTLSEFISLVNMY-  
MmExo70 620 CFNDGLFECKICKVAPDTECRKHKQACKIVKETYGNFHRG-SVPTKNPEYIRSVQCGMDLRLTSA-  
DmExo70 616 NFNRDLEACKICRGISLPELVTLSEFISLVNMYERYYSKQDSF---KNP---HIAFPELVTLSEFISLVNMY-

AtExo70D1 625 TCHHRRRSSE-  
AtExo70D2 615 AFHRRSQ--  
AtExo70D3 617 PFHRRR--  
OsExo70D1 629 SLHRRRRSHG  
OsExo70D2 623 SFHRRRRSHS  
NbExo70D1 590 SCHHRRRSQ-  
SacExo70  
MmExo70  
DmExo70

Fig. 2 Suppl. Sequence analyses of NbExo70D1. Exo70 amino acid sequences from *Saccharomyces cerevisiae*, *Mus musculus*, *Drosophila melanogaster*, *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Oryza sativa* were aligned (GenBank accessions: yeast, SacExo70, NP\_012450; *M. musculus*, MmExo70, NP\_058553; *D. melanogaster*, DmExo70, NP\_648222; *N. benthamiana*, KF280306; *A. thaliana*, AtExo70D1, NP\_177319; AtExo70D2, NP\_175811; AtExo70D3, NP\_566477; *O. sativa*, OsExo70D1, NP\_001063276; OsExo70D2, NP\_001061962). The *black boxes* indicate identical residues, and the *gray boxes* indicate conservative substitutions. *Hyphens* indicate gaps introduced to optimize alignments. The *colored lines* on the top indicate domains A, B, C, and D of the Exo70 protein sequence. The *numbers* on the left indicate amino acid residues in the NbExo70D1 protein sequence. The C-terminal conserved domain is highlighted with a *yellow frame*. The two conserved binding sites, an Arp2/3 complex and PtdIns(4,5)P2, are marked in *blue* and *red*, respectively. The alignment was done using the *ClustalW* program (<http://www.ch.embnet.org/software/ClustalW.html>) and *BoxShade* program ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)).

1396	AGGCAAGCAGCTATTA	ACTACCAGAGATCAACCTGGGTGAGGGTT
	R Q A A I N	Y Q R S T W V R V
1441	TTGTATTGTTT	GCGAGATGAGGGTTTACATGTTAAAGGCAACTTT
	L Y C L R D E G L H V K G N F	
1486	TCATCTGGGGTGTCTAAGAGTGCATTG	gcAGAGgcGTTcgcAACG
	S S G V S K S A L	A E A F A T
1531	TTCAATGCTATGTTTGAAGAGGTT	CATAGGACTCAGTCCACATGG
	F N A M F E E V H R T Q S T W	
1576	TTGATTCCGGATACTCAGCTCCGGGAGGAGCTGCGTATTTCTATA	
	L I P D T Q L R E E L R I S I	
1621	TCCGAGAAGTTGATCCCAGCTTATAGATCATTTCTCGGAAGGTTTC	
	S E K L I P A Y R S F L G R F	
1666	AGGAGTCATATTGAGAGCGGAGcGgcTCCGGA	AAACTACATCgcG
	R S H I E S G A A P E N Y I A	
1711	TATTCTGGTGAAGACATTGAGACTGCTGTCTTGGATTTCTTTGAG	
	Y S G E D I E T A V L D F F	E
1756	GGGTATCAGGTTT	CACAACACATAAGAAGAAGATCTCAGTGA 1797
	G Y Q V S Q H I R R R S Q	*

Fig. 3 Suppl. The position of site-directed mutants in the NbExo70D1 domain D. Nucleotide and amino acid sequences of NbExo70D1 domain D between residues 451 and 585 (amino acid residues inside the *gray box*). The C-terminus of NbExo70D1 is indicated by the *underlined* amino acid sequence. The *star* indicates the stop codon. Amino acid residue mutants are indicated by *colored bold letters*. Nucleotide mutants are represented in *bold lower case letters*. The amino acid residues with *bold red* and *bold green* letters indicate *nbexo70d1* domain D-1 and *nbexo70d1* domain D-2 mutants, respectively, which were created.



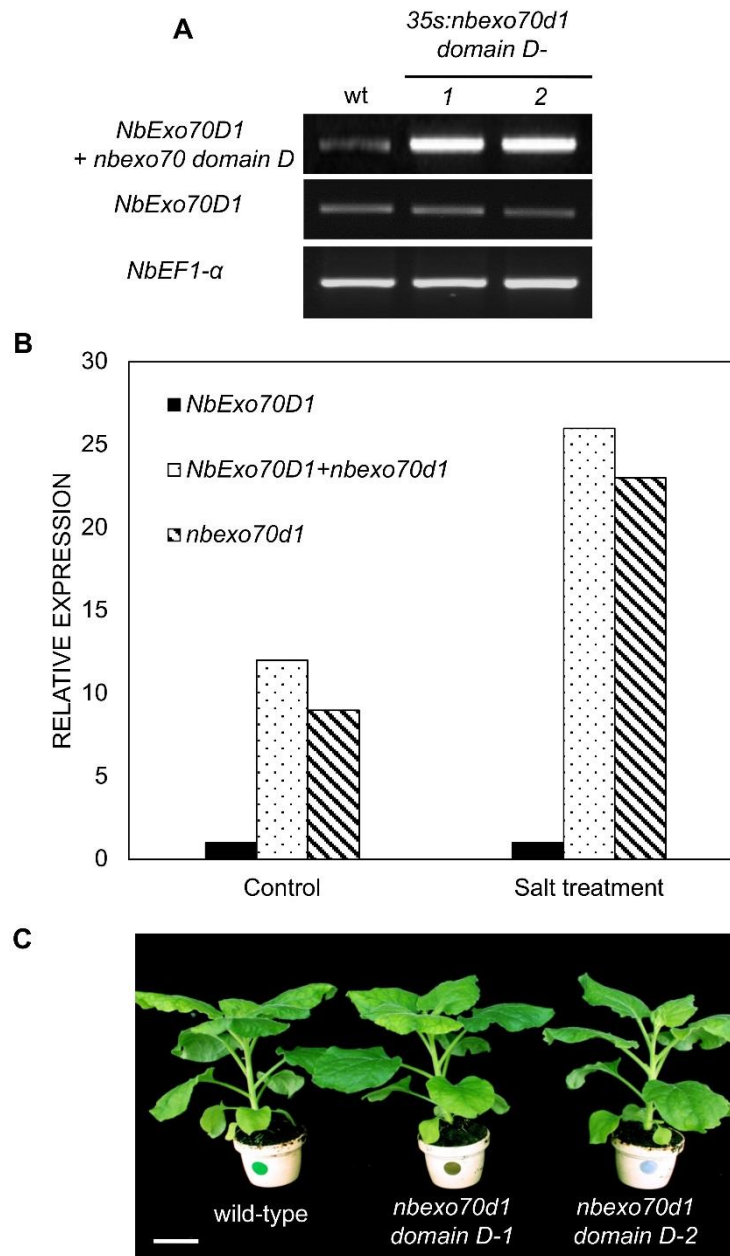


Fig. 4 Suppl. The phenotype and gene expression of the *nbexo70d1* domain D mutation in *Nicotiana benthamiana* plants. **A** - Expression of the *Nbexo70* mRNA, and expression of the *nbexo70d1* domain D mutant mRNA and the endogenous *NbExo70D1* mRNA in combination. Expression of the *NbEF1-α* gene was used as an internal loading control. **B** - Relative expression of *Nbexo70D1*, *nbexo70d1* domain D mutant and the combination. **C** - The phenotype of wild type and *nbexo70d1* domain D mutant plants (the white bar is 2 cm).

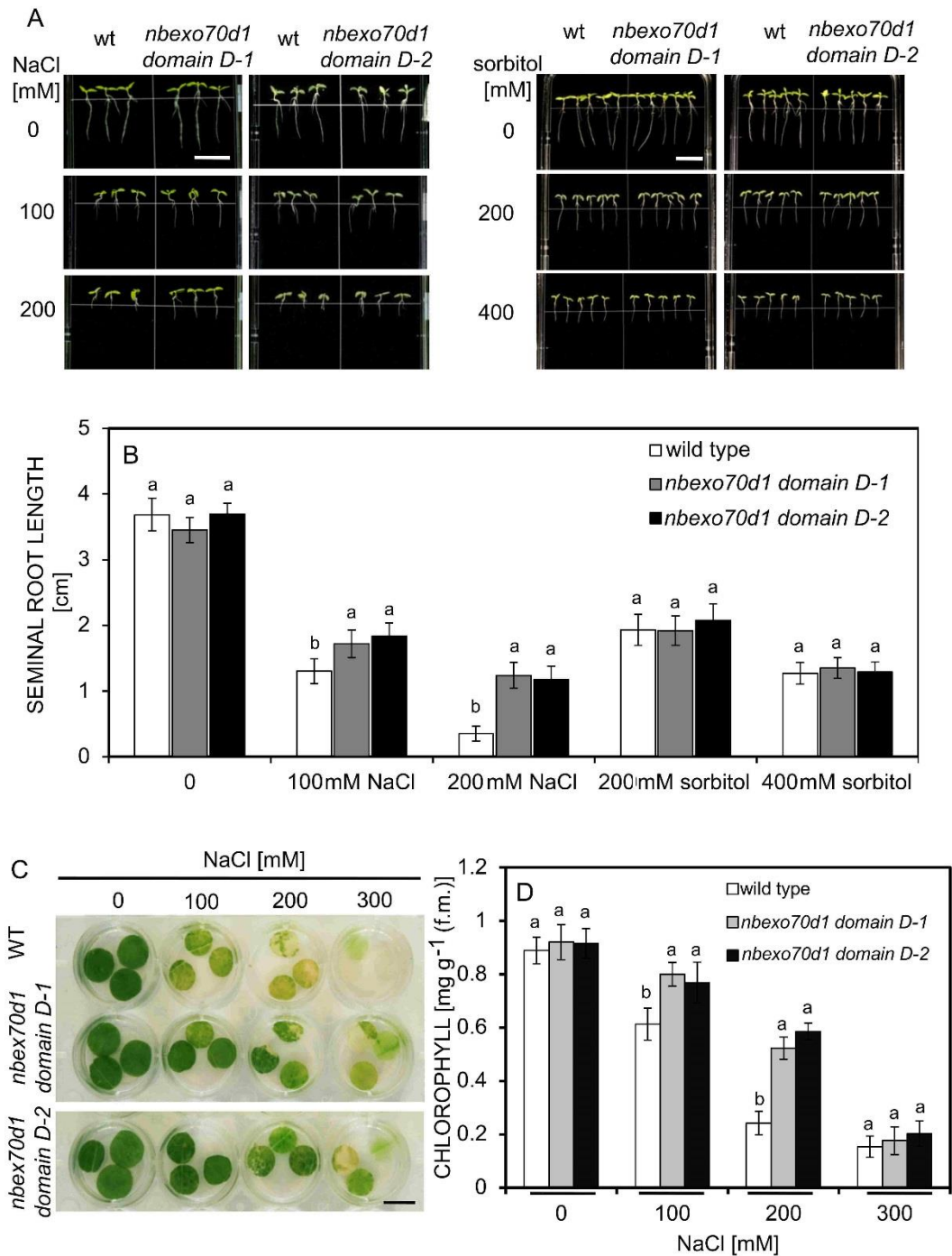


Fig. 5 Suppl. Increased salt tolerance in transgenic plants overexpressing a dominant negative *nbexo70d1* domain D mutation. **A** - Comparison of primary root length of wild type and *nbexo70d1* domain D mutant seedlings under salt stress and osmotic stress (the white bar is 3 cm). **B** - Comparison of root length measurements. Means  $\pm$  SDs,  $n = 9$ . **C** - Leaf discs assay under salt stress (the black bar is 1 cm). **D** - Chlorophyll content in leaf discs. Means  $\pm$  SDs,  $n = 3$ , different letters indicate significant differences (ANOVA with the Tukey post-hoc test;  $P < 0.05$ ).

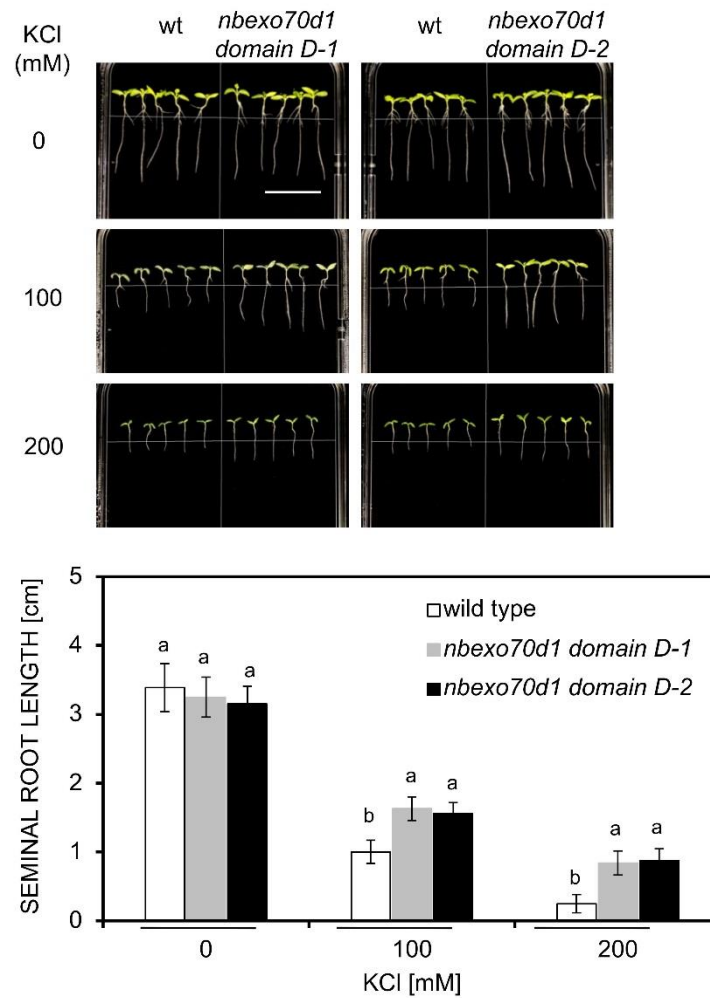


Fig. 6 Suppl. Effects of dominant negative *nbexo70d1 domain D* mutants on tolerance of transgenic tobacco seedlings to KCl stress. Means  $\pm$  SDs,  $n = 30$ , different letters indicate significant differences (ANOVA with the Tukey post-hoc test;  $P < 0.05$ ).

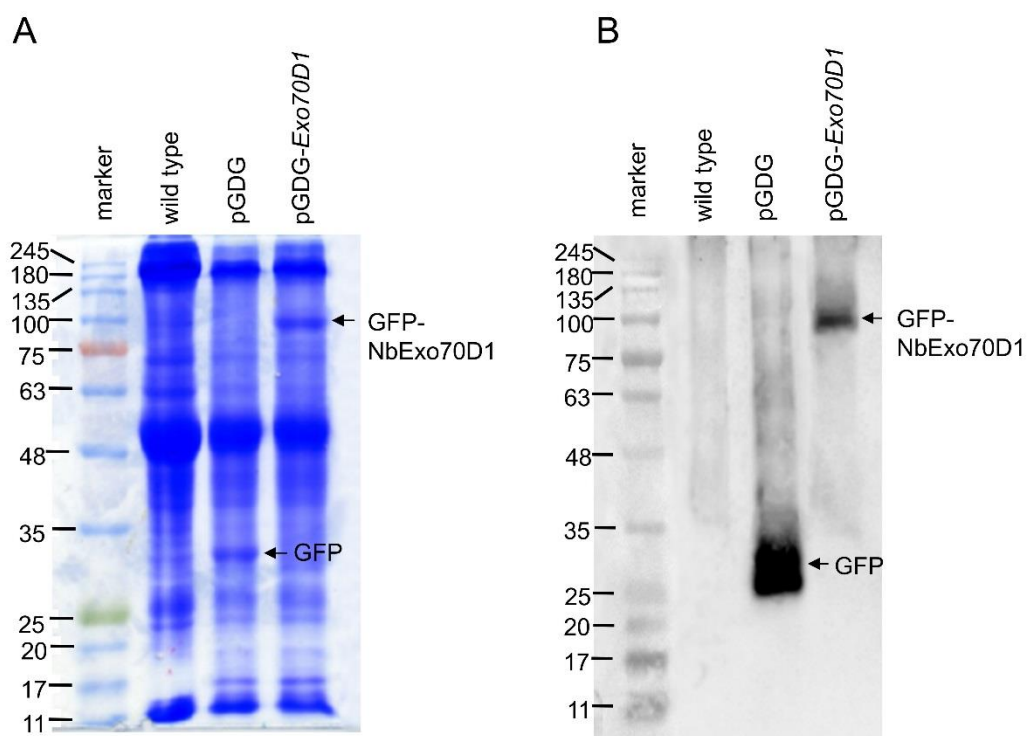


Fig. 7 Suppl. Immunoblot detection of green fluorescent protein (GFP) fusions with NbExo70D1 proteins expressed from pGDG vectors in agroinfiltrated *Nicotiana benthamiana* leaves. *A* - SDS-PAGE with Coomassie Brilliant Blue staining. *B* - Detection of GFP and GFP fusions with a GFP monoclonal antibody. M - marker, 1 - wild type *N. benthamiana* plants, 2 - pGDG control *N. benthamiana* plants, 3 - pGDG-NbExo70D1 *N. benthamiana* plants.



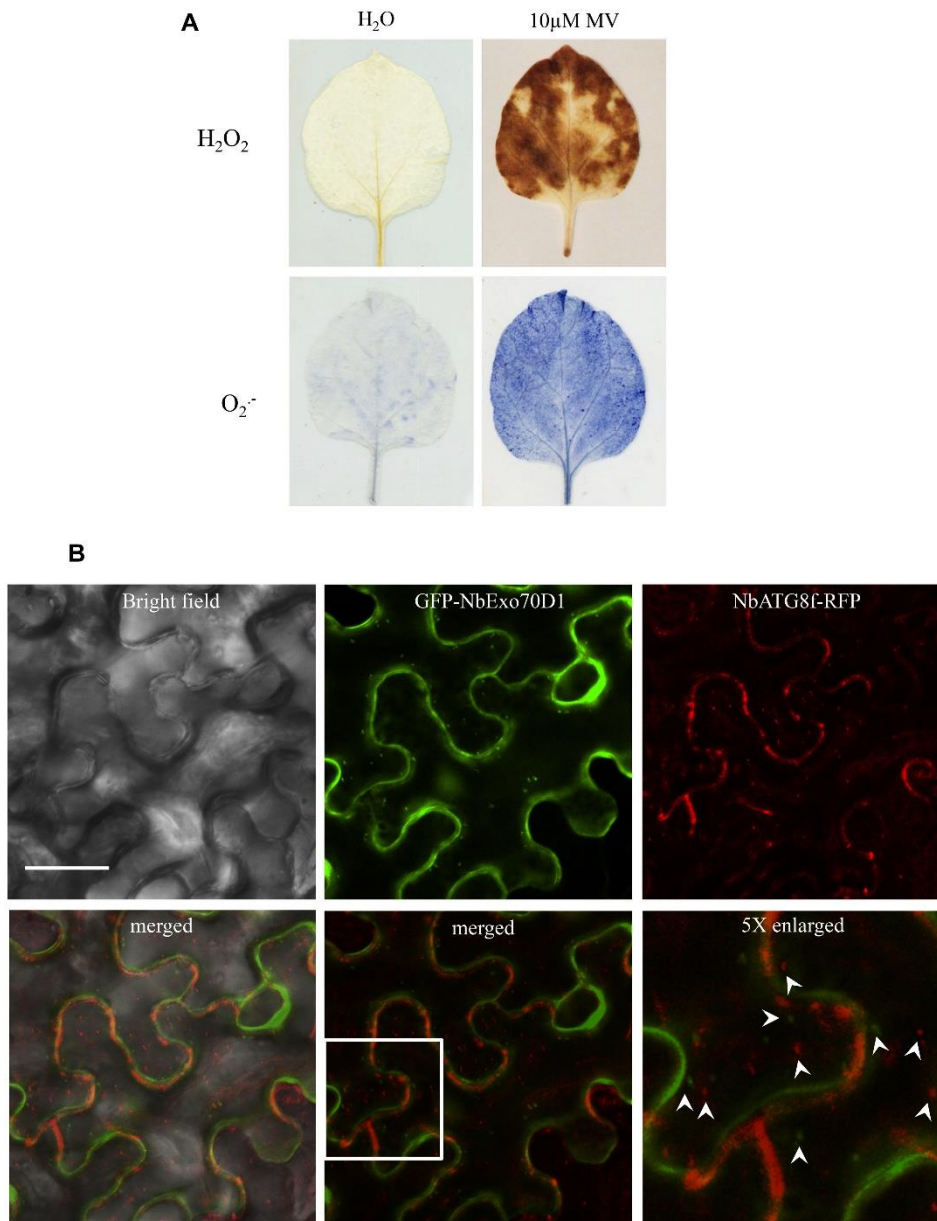


Fig. 8 Suppl. Treatment with methylviologen (MV, an inducer of reactive oxygen species) and subcellular localization of NbExo70D1 and NbATG8f in *Nicotiana benthamiana* leaf epidermal cells. *A* - H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> accumulations in tobacco leaves were detected by diaminobenzidine and nitroblue tetrazolium staining, respectively. *B* - Images of tobacco leaf epidermal cells from confocal laser-scanning microscopy. The cells expressed intracellular localization of the proteins NbExo70D1 and NbATG8f (the white bars are 5  $\mu$ m).

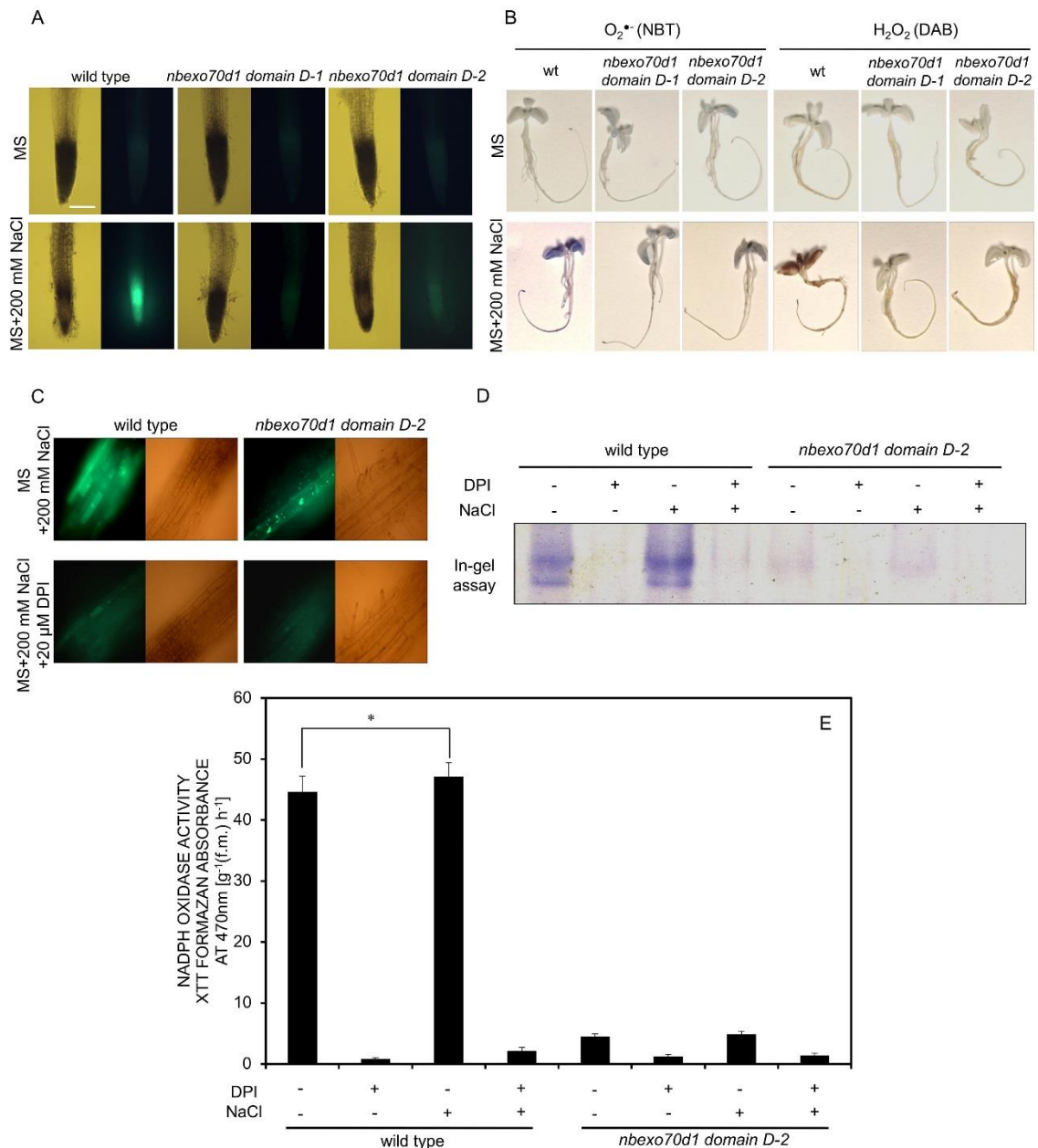


Fig. 9 Suppl. Histochemical assay of reactive oxygen species (ROS) generation and activity of NADPH oxidase in salt stressed *Nicotiana benthamiana* seedlings. Wild type and *nbexo70d1 domain D* mutant plants germinated on agar plates containing a one-half strength Murashige and Skoog medium. **A** - Production of ROS in tobacco roots under salt stress was detected using a *Leica MPS60* fluorescent microscope with the addition of 10  $\mu$ M *CM-H<sub>2</sub>DCFDA*. Pictures were taken 30 min after the addition of the dye (the white bar is 300  $\mu$ m). **B** - Diaminobenzidine and Nitroblue tetrazolium were used for staining to detect ROS accumulation in shoots and roots. **C** - Intracellular production of ROS in tobacco seedlings under 200 mM NaCl for 10 min. The magnified view of the root cells of the wild type and *nbexo70d1 domain D-2* mutant line (stained with *CM-H<sub>2</sub>DCFDA*) for 30 min. Results of one representative experiment (from three individual experiments with similar results). **D** - Effect of NaCl and diphenylene iodonium (DPI) on the activity of the NADPH oxidase in wild type and *nbexo70d1 domain D-2* mutant tobacco seedlings demonstrated by in-gel assay. **E** - sodium,3'-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT) assays. Means  $\pm$  SDs,  $n = 4$ , \* indicate a significant difference from the control (the Student *t*-test,  $P < 0.05$ ).

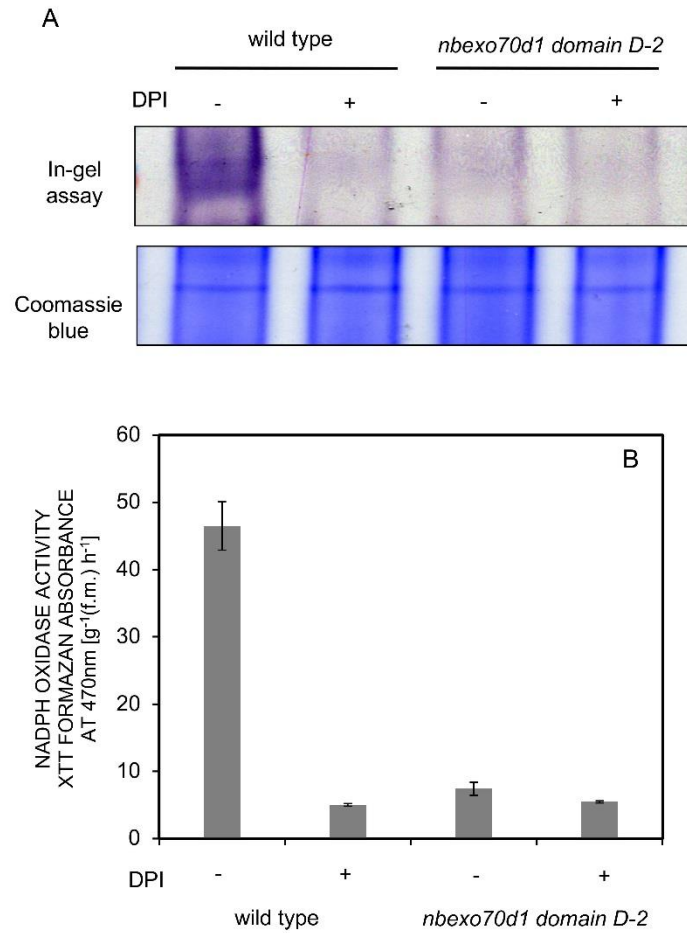


Fig. 10 Suppl. Activity of NADPH oxidase in *nbexo70d1* domain D mutant and wild type plants. Activity of NADPH oxidase in protein extracts from tobacco seedlings were analyzed by in-gel assay (A) and XTT assay (B). Data for NADPH oxidase activity are means  $\pm$  SDs,  $n = 4$ . Coomassie Brilliant Blue staining was used to check the amount and quantity of the total proteins.

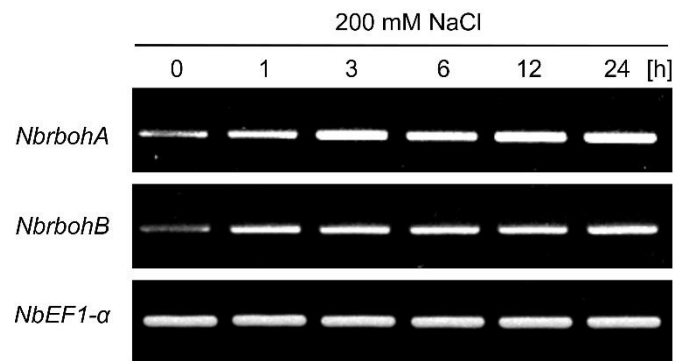


Fig. 11 Suppl. The expression profile of *NbrbohA* and *NbrbohB* genes in tobacco seedlings in response to 200 mM NaCl stress treatment for 0 to 24 h. *NbEF1-α* was used as a loading control.