

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*	CCCA <u>AAGCTT</u> CTATGGAACCACCGGAG	CGGG <u>ATCCT</u> CACTGAGATCTTCTTC
NbExo70D1 partial sequence	GAGTTGGAGCATCAAGAGAG	AGCCTGAACTCTAATTGACTCC
nbexo70d1 domain D-1**	GTGCATT <u>GGCAGAGGCGTTCGCAACGTTCA</u>	TGAACGTTGCGAACGCCCTGCCAATGCAC
nbexo70d1 domain D-2**	TTCTCGGAG <u>CGTTCGCGAGTGCTATTGAGA</u>	TCTCAATAGCACTCGCGAACGCTCCGAGAA
NbExo70D1 D-domain	CAGAGATCAACCTGGGTGAG	CTGAGATCTTCTTCTTATGTGTTG
NbExo70D1 promoter	CACCCCTCCTCAATGTAGTGGTGTCA	ATTTCATCGACGGCTTGCAGG
Endogenous NbExo70D1 D-domain	CAGAGATCAACCTGGGTGAG	CCACAAAACAATTCAAAGAATGTCC
NbEF1- α	TGTCCCCATCTCTGGTTCG	AATCTGGTCAAGAGCCTAAGAA
NbATG8f	ATGGCAAAGAGTTCATTCAAGC	CACCAAGTTAAAGTCCCCAA'

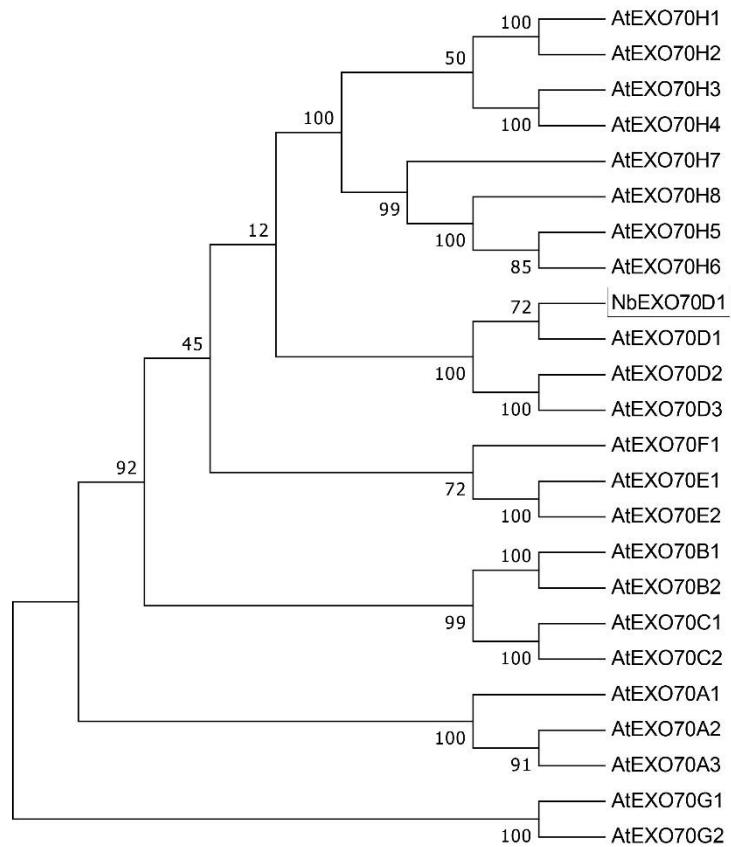


Fig. 1 Suppl. Phylogenetic relationships of Exo70 proteins from *Arabidopsis thaliana* NbExo70D1 and AtExo70. AtExo70 protein sequences were retrieved from the TAIR (The Arabidopsis Information Resource, <https://www.arabidopsis.org>) and aligned using the ClustalW program (<https://www.genome.jp/tools-bin/clustalw>). 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*.

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).

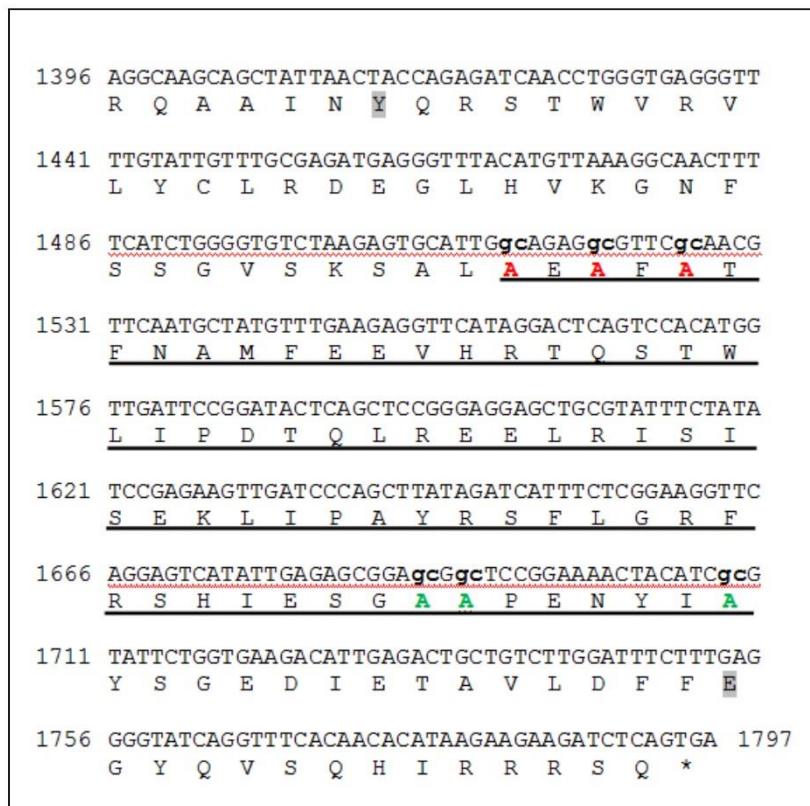


Fig. 3 Suppl. The position of site-directed mutants in the NbExo70D1 domain D. Nucleotide and amino acid sequences of NbExo70D1 domain D between residues 451 and 585 (amino acid residues inside the *gray box*). The C-terminus of NbExo70D1 is indicated by the *underlined* amino acid sequence. The *star* 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.

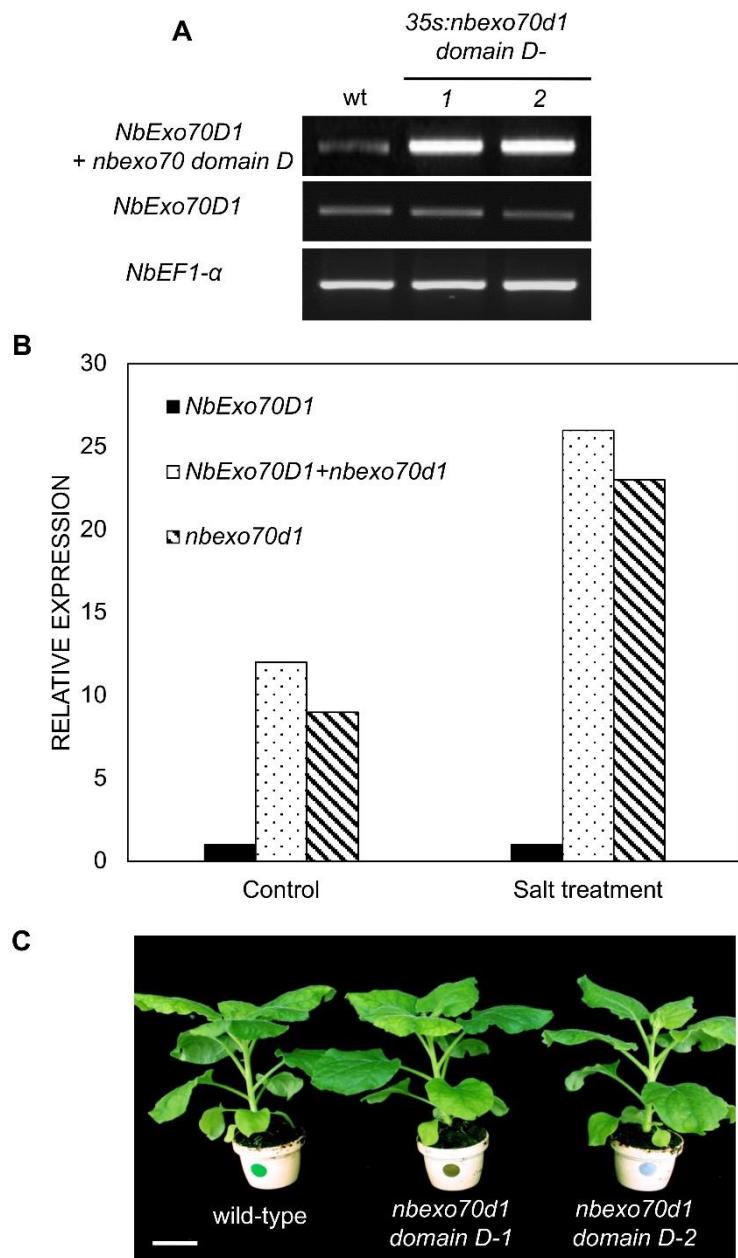


Fig. 4 Suppl. The phenotype and gene expression of the *nbexo70d1* domain D mutation in *Nicotiana benthamiana* plants. A - Expression of the *Nbexo70* mRNA, and expression of the *nbexo70d1* domain D mutant mRNA and the endogenous *NbExo70D1* mRNA in combination. Expression of the *NbEF1- α* gene was 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).

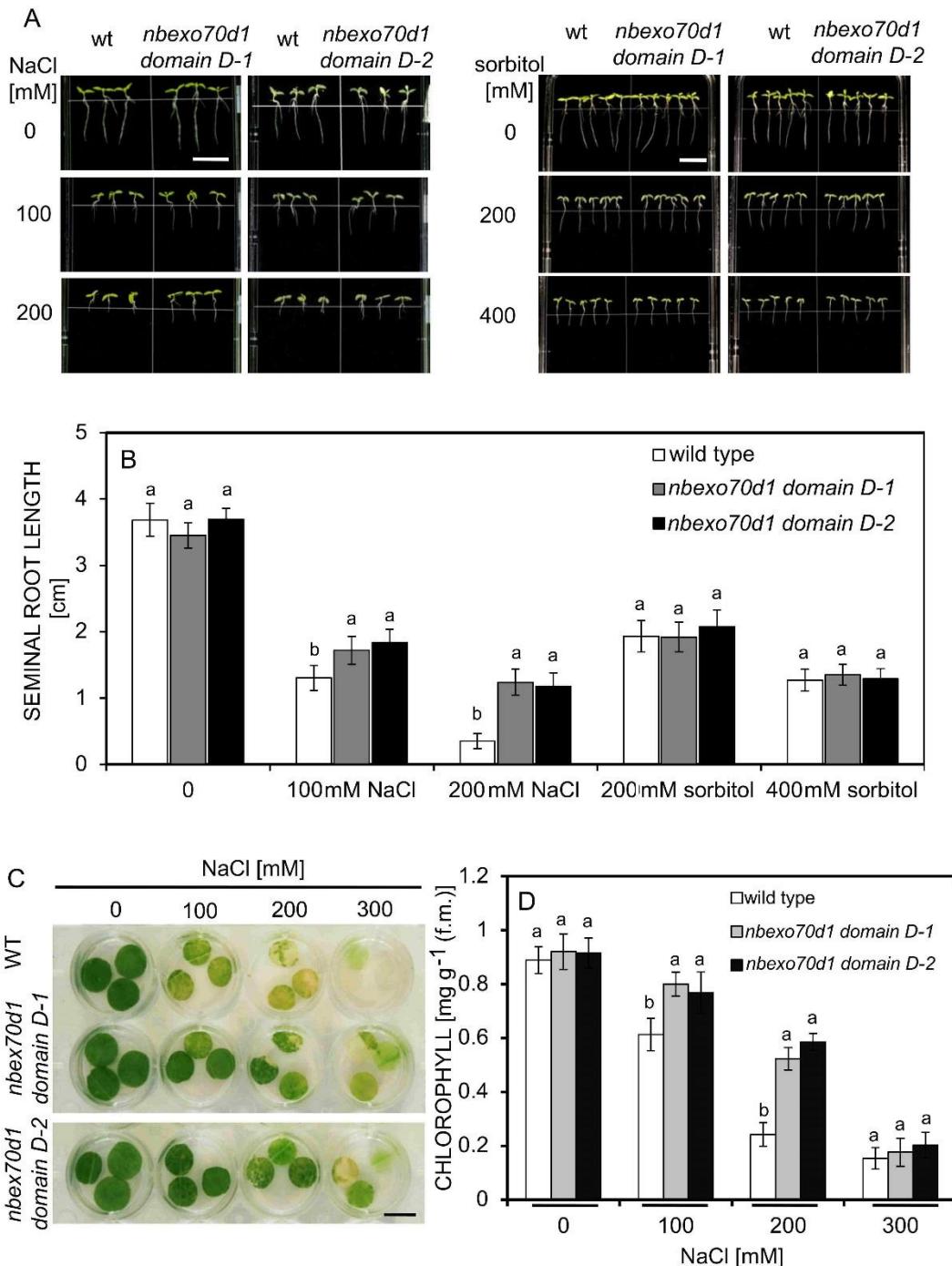


Fig. 5 Suppl. Increased salt tolerance in transgenic plants overexpressing a dominant negative *nbexo70d1* domain D mutation.
A - Comparison of primary root length of wild type and *nbexo70d1* domain D mutant seedlings under salt stress and osmotic stress (the white bar is 3 cm). **B** - Comparison of 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; $P < 0.05$).

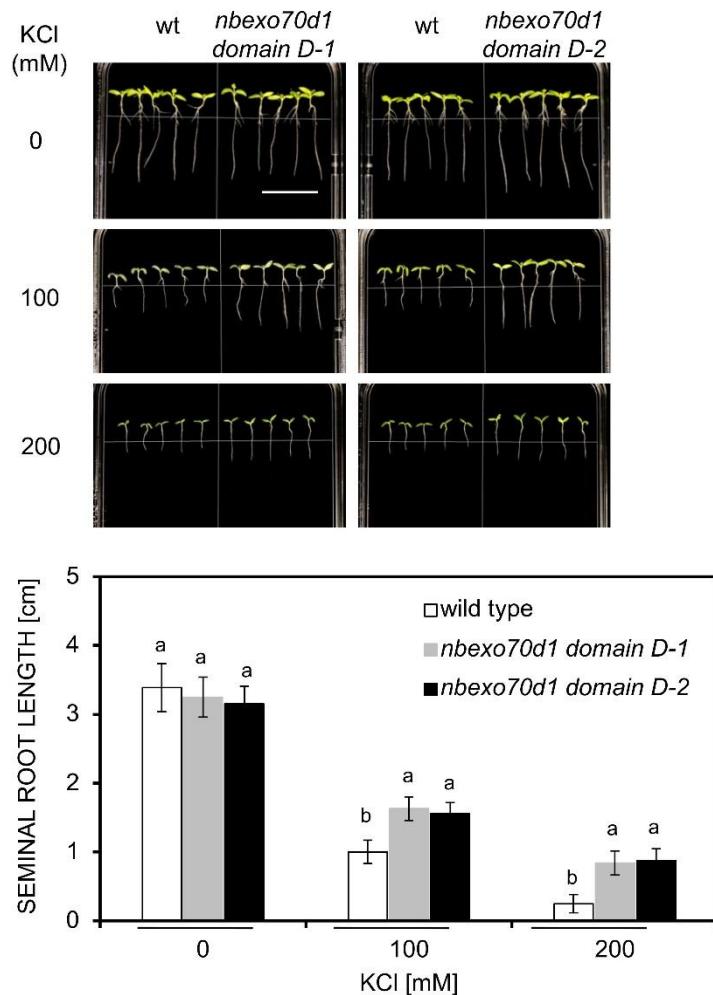


Fig. 6 Suppl. Effects of dominant negative *nbexo70d1* domain D mutants on tolerance of transgenic tobacco seedlings to KCl stress. Means \pm SDs, $n = 30$, different letters indicate significant differences (ANOVA with the Tukey post-hoc test; $P < 0.05$).

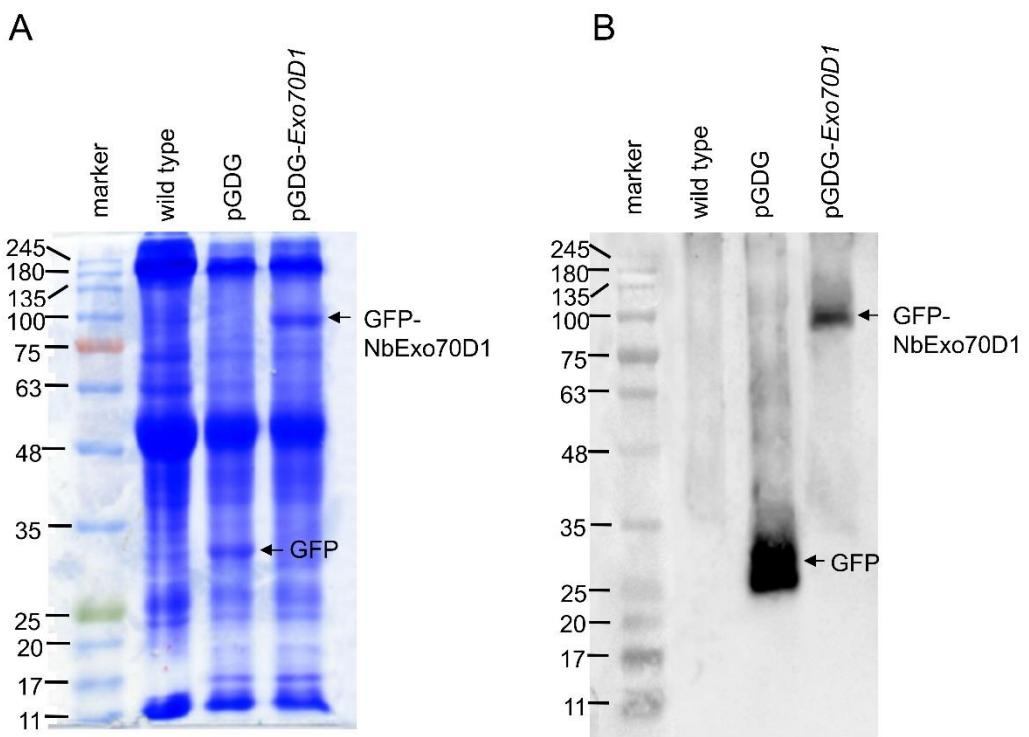


Fig. 7 Suppl. Immunoblot detection of green fluorescent protein (GFP) fusions with NbExo70D1 proteins expressed from pGDG vectors in agroinfiltrated *Nicotiana benthamiana* leaves. A - SDS-PAGE with Coomassie Brilliant Blue staining. B - Detection of GFP and GFP fusions with a GFP monoclonal antibody.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

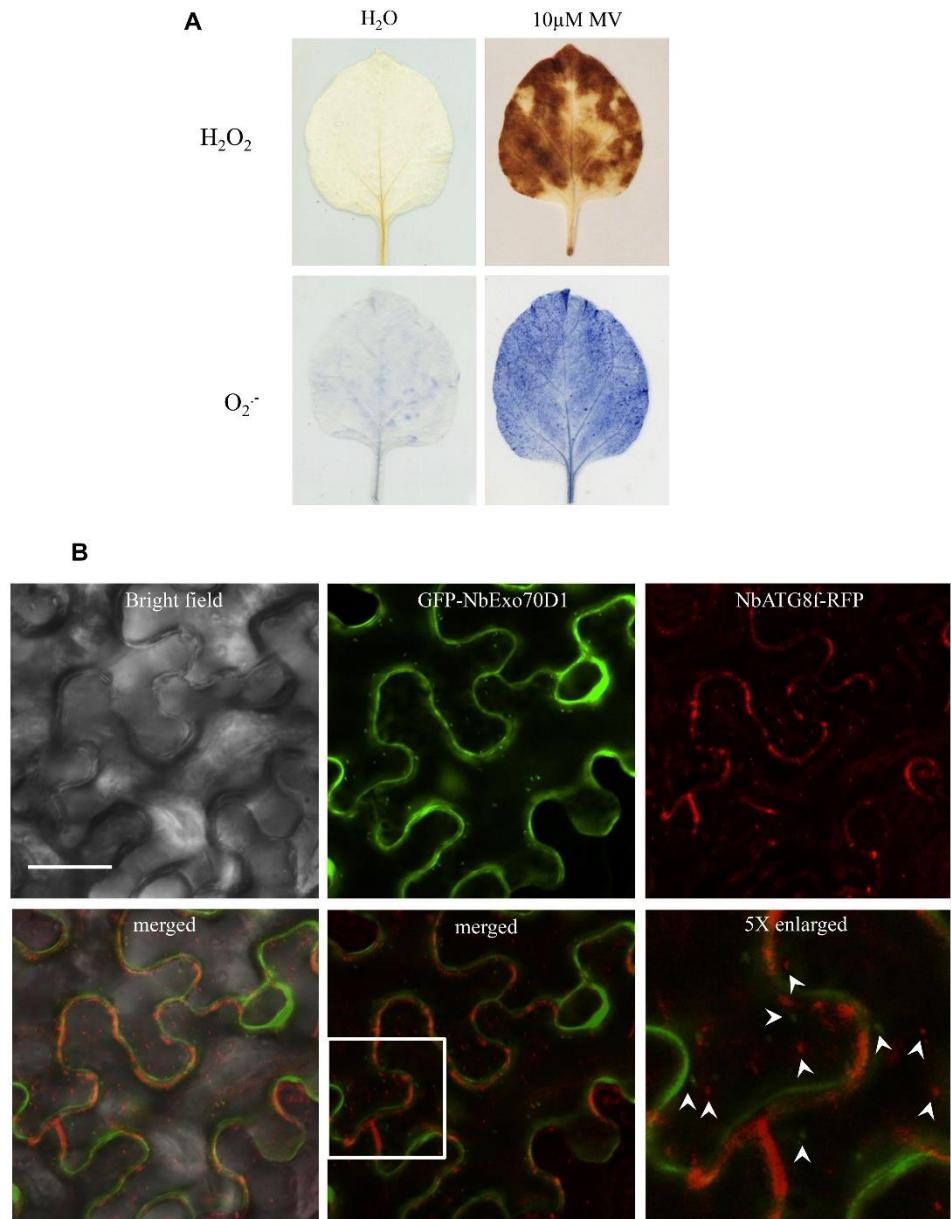


Fig. 8 Suppl. Treatment with methylviologen (MV, an inducer of reactive oxygen species) and subcellular localization of NbExo70D1 and NbATG8f in *Nicotiana benthamiana* leaf epidermal cells. A - H_2O_2 and O_2^- accumulations in tobacco leaves were detected by diaminobenzidine and nitroblue tetrazolium 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 μm).

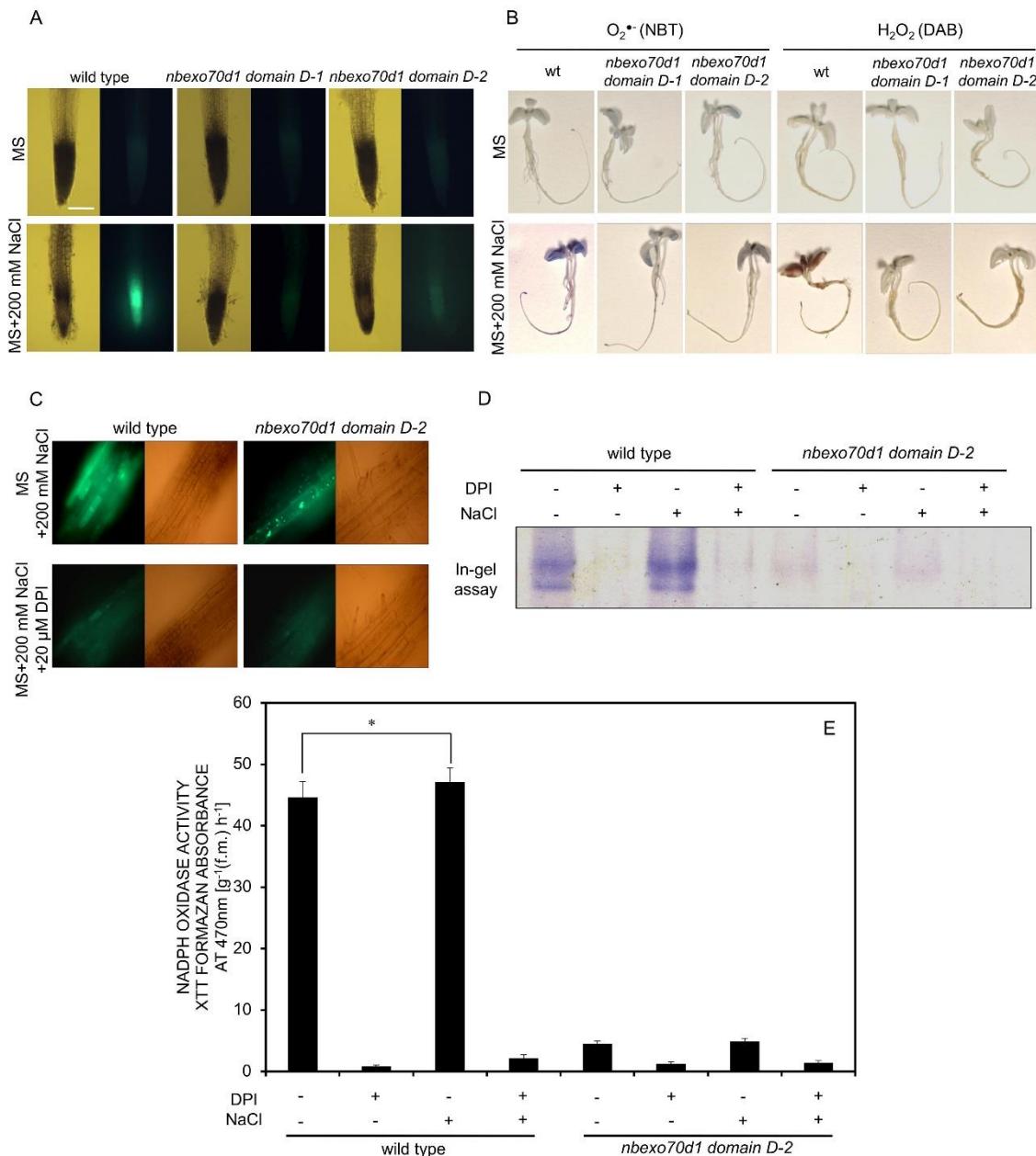


Fig. 9 Suppl. Histochemical assay of reactive oxygen species (ROS) generation and activity of NADPH oxidase in salt stressed *Nicotiana benthamiana* seedlings. Wild type and *nbexo70d1* mutant plants germinated on agar plates containing a one-half strength Murashige and Skoog medium. **A** - Production of ROS in tobacco roots under salt stress was detected using a Leica MPS60 fluorescent microscope with the addition of 10 μM CM-H₂DCFDA. Pictures were taken 30 min after the addition of the dye (the white bar is 300 μm). **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* mutant line (stained with CM-H₂DCFDA) 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* mutant tobacco seedlings demonstrated by in-gel assay. **E** - sodium,3'-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4- methoxy-6-nitro)benzenesulfonicacid hydrate (XTT) assays. Means \pm SDs, $n = 4$, * indicate a significant difference from the control (the Student *t*-test, $P < 0.05$).

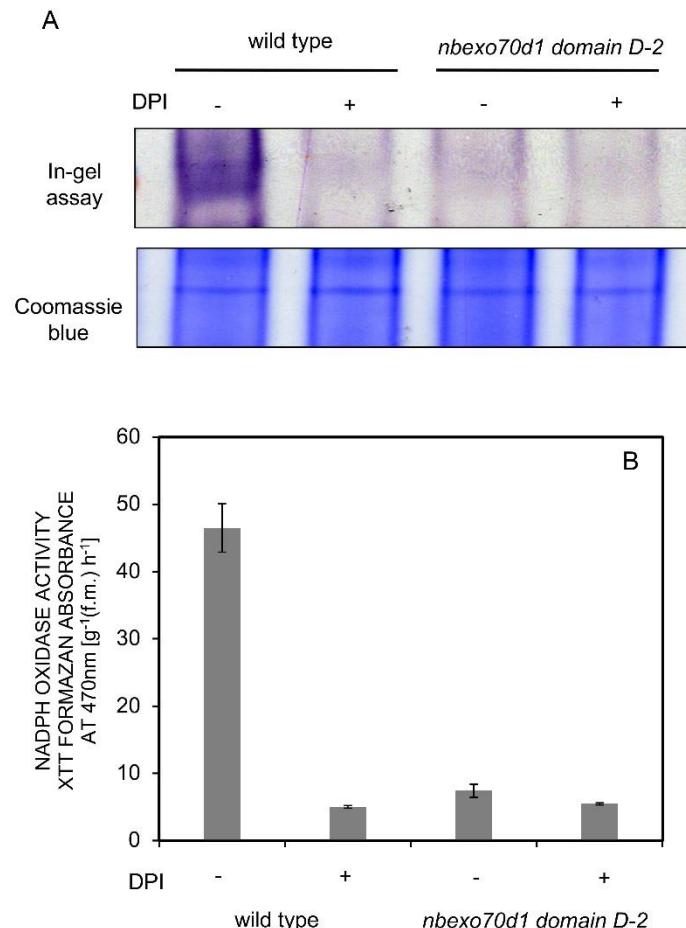


Fig. 10 Suppl. Activity of NADPH oxidase in *nbexo70d1 domain D* mutant and wild type plants. Activity of NADPH oxidase in protein extracts from tobacco seedlings were analyzed by in-gel assay (A) and XTT assay (B). Data for NADPH oxidase activity are means \pm SDs, $n = 4$. Coomassie Brilliant Blue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

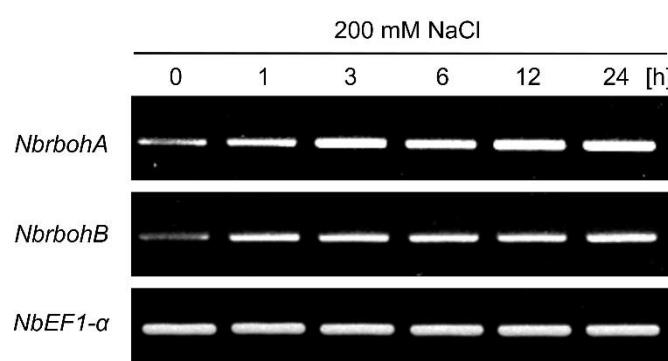


Fig. 11 Suppl. The expression profile of *NbrbohA* and *NbrbohB* genes in tobacco seedlings in response to 200 mM NaCl stress treatment for 0 to 24 h. *NbEF1- α* was used as a loading control.