Supplementary data

Ntann12 annexin expression is induced by auxin in tobacco roots

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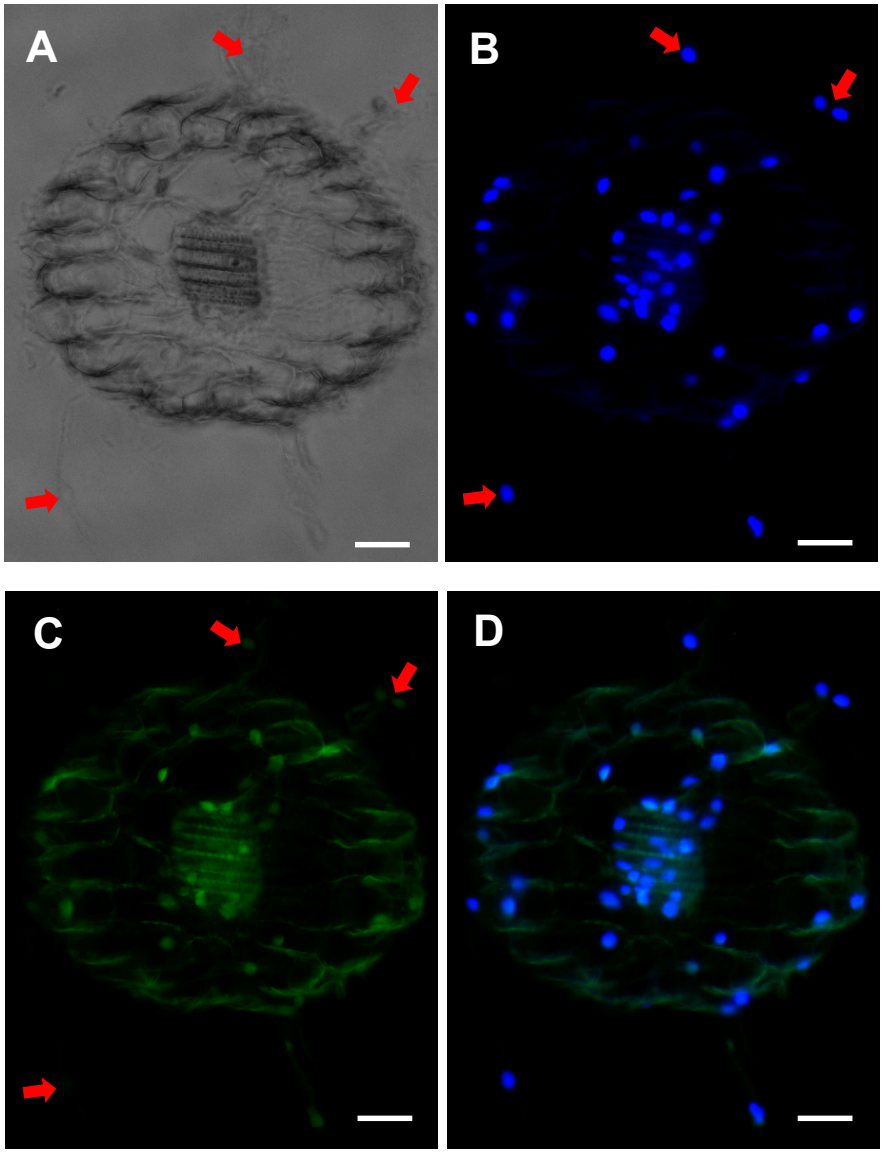

Fig. S1. Ntann12 immunolocalization in tobacco root 100 µm cross sections visualized by fluorescence microscopy. A: phase contrast. B: DAPI DNA staining. C: immunostaining with TOYOPEARL AF-Amino-650M-affinity-purified antiserum against Ntann12. D: superposition of B and C. In Panels C-D the green fluorescence is indicative of the presence of Ntann12, and shows that it is not due to DAPI fluorescence contamination since some DAPI-stained nuclei do not show green fluorescence (arrows), also, the pattern of fluorescence of the two fluorochromes is different. Bar = 50 µm (A-D).

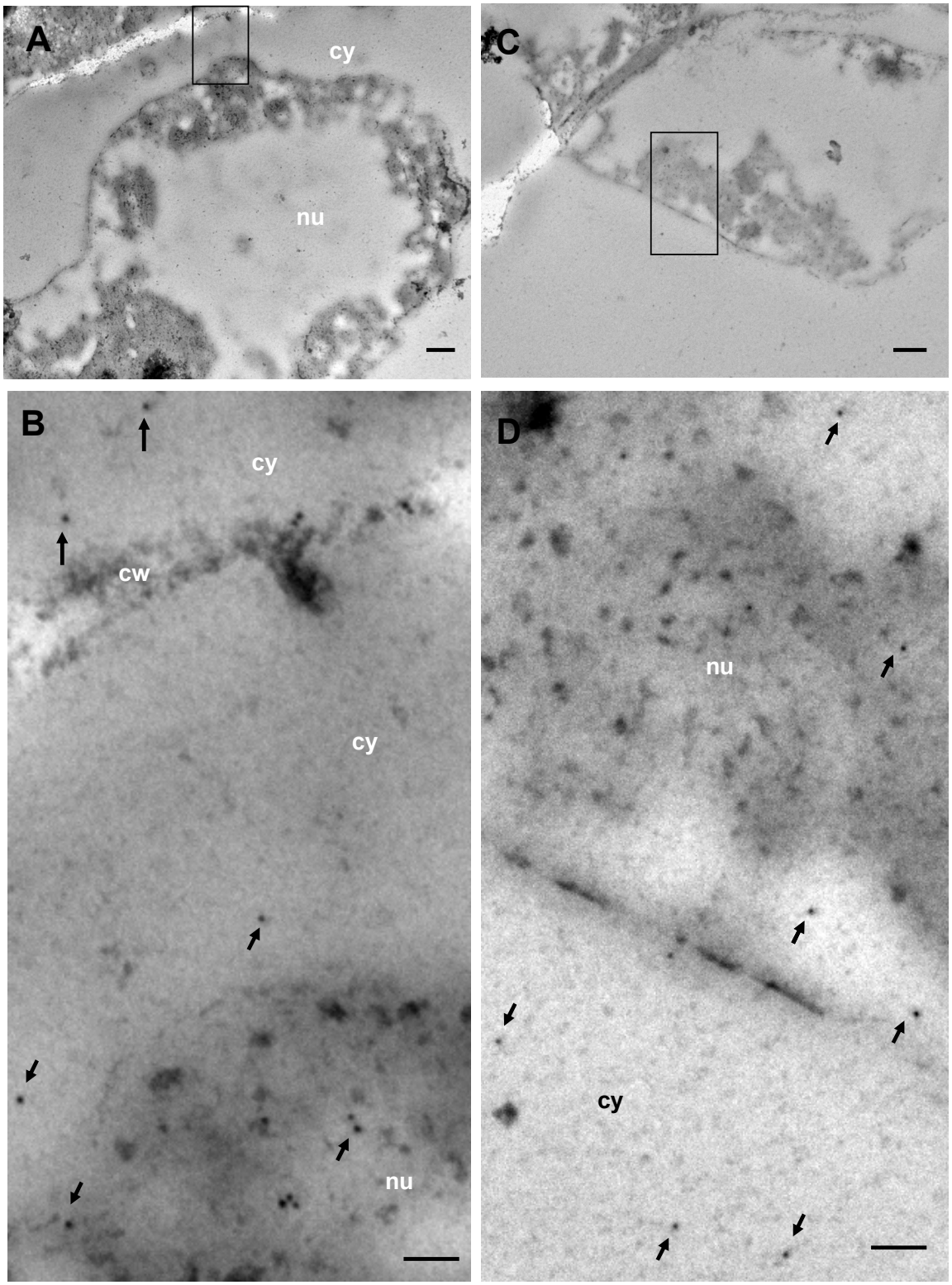

Fig. S2. Transmission electron micrographs of Ntann12 immunogold labelling in section of root cells. (A, C, E, G) Sections in root cells. (B, D, F, H) Details of (A, C,E,G) respectively showing labelling in the cytoplasm and in the nucleus (see arrows). Cw, cell wall; cy, cytoplasm; nu nucleus. Bars = 400 nm (A, C, E, G), 50 nm (B, D, F, H). The boxes indicate the details selection.

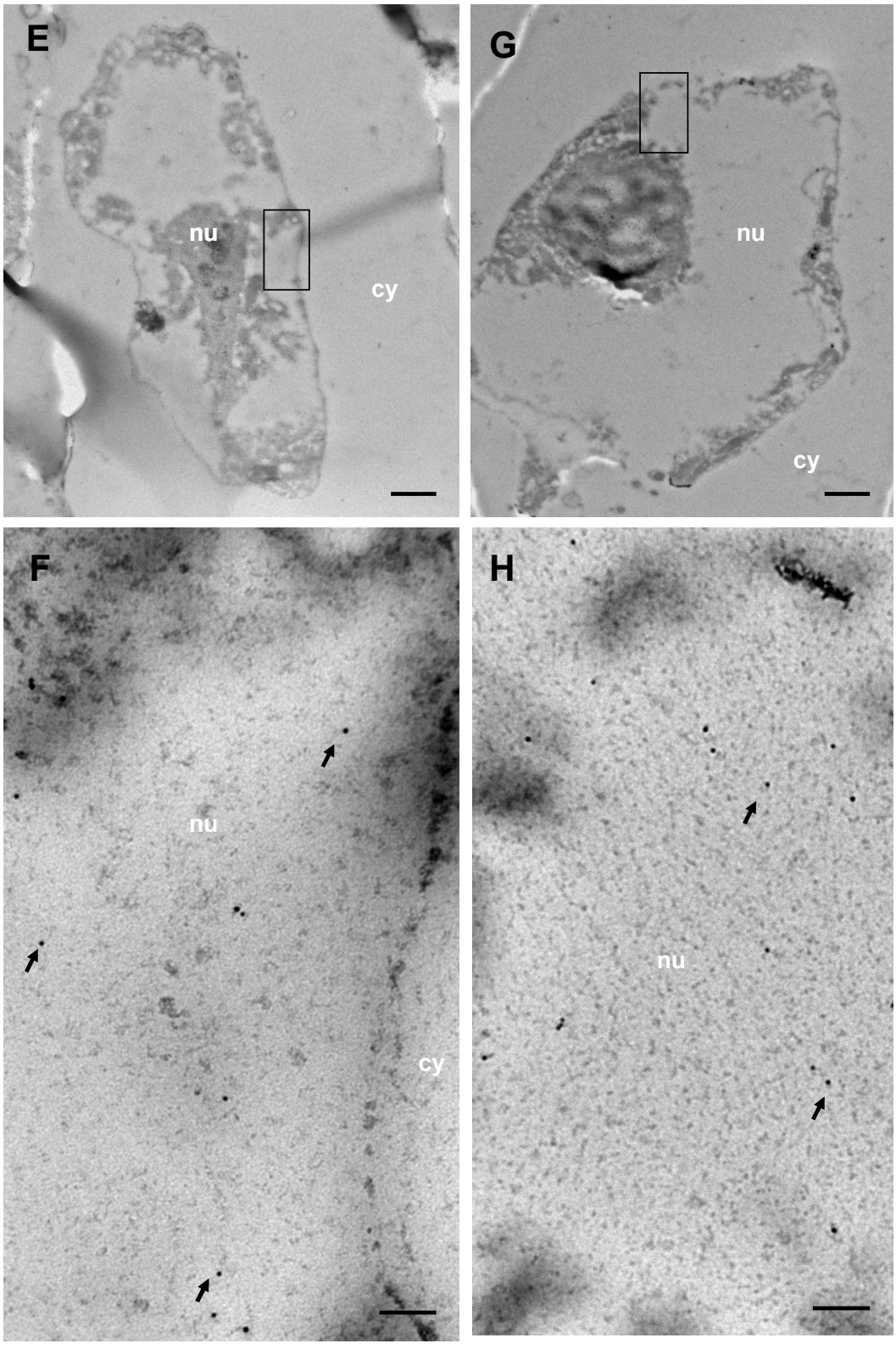

Fig S2 continued

Supplementary data S3.

Characterization of T2 transgenic tobacco progenies overexpressing and downregulating *Ntann12*

Material and methods

The entry clone BPNtann12 was recombined into the Gateway^{TM}-compatible binary T-DNA destination vectors pK7WG2 and pK7WIWG2, allowing respectively to increase (p*35S-Ntann12*) or to suppress expression (RNAi) (Karimi et *al.*, 2002). Plant transformation was performed as described in Vandeputte et al. (2007). Plants were either grown in solid MS medium or in soil. Transcriptional changes were calculated based on the comparative $\Delta\Delta C_T$ method as described by Livak and Schmittgen (2001) and are reported as ratios between expression in roots and in leaves of transgenic lines overexpressing (5.1, 6.1, 11.2, 16.2, 19.2) or downregulating (1.1, 4.2 and 11.4) *Ntann12* and wild type plants (see Materials and Methods of the the main manuscript). For the germination test, sterilized seeds were plated on MS medium. Various concentrations of NaCl were added (0, 75, 150 or 250 mM). Three days after sowing, germination (emergence of radicles) was scored daily for 3 days and 7 days after sowing, hypocotyls lengths were scored daily for 3 days. Plants were also cultured with 250 mM mannitol, 150 µM ABA, 50 µM IAA, TDZ/NAA (50 µM /5 µM), 2,4-D/kinetine (50 µM /5 µM) and darkness.

Results

Expression level of Ntann12 was evaluated in one-month-old T2 homozygous lines by immunoblot and qRT-PCR analysis in roots and leaves. As shown in Fig. S3, in

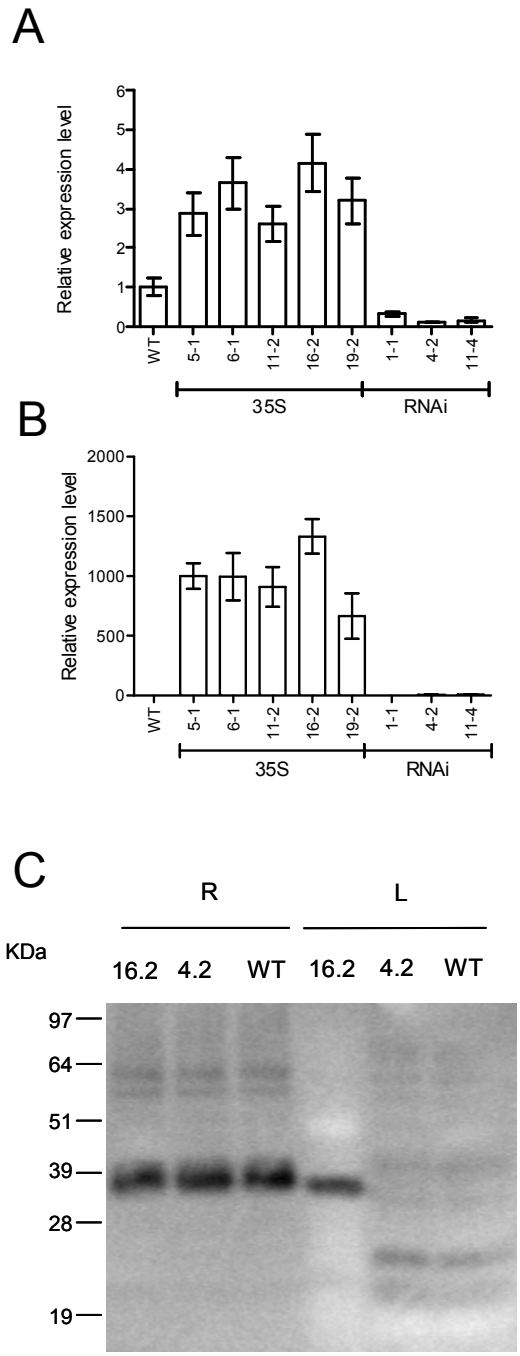

Figure S3: Characterization of T2 transgenic tobacco progenies overexpressing (35S) and downregulating (RNAi) *Ntann12*. (A) qRT-PCR analysis of *Ntann12* expression in roots. (B) qRT-PCR analysis of *Ntann12* expression in leaves. (C) Western blot analysis of total protein extracts (10 µg) from roots and leaves of transgenic lines overexpressing and downregulating *Ntann12* and wild type using the anti-Ntann12 antibodies. L, leaves; R, roots.

roots (Fig. S3A), *Ntann12* expression was estimated to be about 4 times more abundant in 35S plants than in non transgenic plants and about 4 times less abundant in RNAi plants than in non-transgenic plants. In leaves (Fig. S3B), *Ntann12* expression was estimated to be about 1000 times more abundant in 35S plants than in non-transgenic plants and no significant differences were measured for RNAi plants compared to in non-transgenic plants (Fig. S3B). One possible explanation of the lower ovexpression level of *Ntann12* in roots compared to leaves in the 35S lines is that, in WT plants, *Ntann12* expression level is very high in roots and almost not present in leaves (Fig. 2). At the protein level, Ntann12 was present in both roots and leaves of 35S lines whereas in RNAi and WT plants Ntann12 was not detected in the leaves but was present in the roots (Fig. S3C). The RNAi lines examined are therefore not sufficiently down-regulated for Ntann12. Finally, no phenotypic differences between 35S, RNAi and non-transgenic plants were observed during germination and plant growth after *R. fascians* infection, after organogenesis initiation and after treatments with diverse growth factors or abiotic stress.

References

Karimi, M., Inzé, D. and Depicker, A. (2002) GATEWAY^{TM} vectors for *Agrobacterium*-mediated plant transformation. *Trends in Plant Sciences* **7,** 193-195.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $-2^{\Delta\Delta C}{}_{T}$ method. *Methods* **25,** 402-408.

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