







SUPPLEMENTAL FIGURES

LEGENDS

Supplemental Figure 1: A) Amino acid sequence alignment of NtWLIM2 and NtWLIM1. The two proteins show 52% identity and 66 % similarity. Similar amino acids are highlighted by black shading. The position of the two LIM domains is indicated by the black bar above the aligned sequences. B) Schematic illustration of NtWLIM2 indicating the position of the two LIM domains (black boxes), the N-terminal part, the interLIM region and the C-terminus (white boxes). Sources of data: NCBI accession AF184886 (NtWLIM2) and AF184109 (NtWLIM1).

Supplemental Figure 2: NtWLIM2 interacts with the actin cytoskeleton in BY-2 cells. Co-labeling experiments of GFP-NtWLIM2 (A) with rhodamine-phalloidin (B). Co-localization in merged image (C) is indicated by yellow color (arrows). Bar: 20 μ m.

Supplemental Figure 3: Immunoblot detection of recombinant NtWLIM2 and GFP-NtWLIM2. Either 10 ng of recombinant protein or 20 μ g of total protein extract from 3-day-old BY-2 cells were separated by SDS-PAGE and transferred onto a PVDF membrane. Recombinant NtWLIM2 (lane 1, lower arrow) was detected at the expected size of \approx 22 kD using a home made primary polyclonal anti-NtWLIM2 antibody from mouse and a secondary HRP-coupled anti-rabbit antibody from goat (Jackson ImmunoResearch Laboratories, USA). No signal was obtained for recombinant NtWLIM1 (lane 2) and *Arabidopsis* WLIM1 protein (lane 3). The same antibody combination detected endogenous NtWLIM2 protein in the extracts of wildtype (lane 4), as well as transgenic GFP-NtWLIM1 (lane 5) and GFP-NtWLIM2 BY-2 cells (lane 6). GFP-NtWLIM2 fusion protein was detected at the expected size of \approx 47 kD (lane 6, upper arrow) in the GFP-NtWLIM2 BY-2 cell extract, whereas both wild type and GFP-NtWLIM1 extracts yielded no signal.

Supplemental Figure 4: Characterization of *Arabidopsis* WLIM1 and WLIM2a proteins: Test of transactivation ability in protoplasts and analysis of *in vitro* DNA binding activity by EMSA. A) Reporter and effector constructs used in the protoplast study. In the *H4* reporter construct,

LUC expression was controlled by a 604-bp long fragment of the *Arabidopsis* histone *H4A748* gene promoter. Effector constructs allowed cDNA expression of either full-length *AtWLIM1* (*At1g10200*) or *AtWLIM2a* (*At2g39900*) under control of the CaMV 35S promoter (35S). The *AtWLIM1* amino acid sequence shows 78% identity to *NtWLIM1*, whereas the comparison between *AtWLIM2a* and *NtWIM2* amino acid sequences revealed an identity of 75%. B) Analysis of *pH4A748*-controlled *LUC* expression in protoplasts. Effector and reporter constructs were co-transfected into *Arabidopsis* protoplasts. *LUC* activity in protoplast extracts was measured 20 hours after transfection and normalized against GUS activity. The signal of reporter activity in the presence of each effector is given relative to the signal measured with same concentrations of an empty vector, which is set to 100% (*AtWLIM1*: white bars, *AtWLIM2a*: black bars). Data represent mean values of three independent transfections ($n = 3$) and error bars indicate standard deviations. C) EMSA to test the DNA binding activity of *AtWLIM2a*. One ng of DIG-labeled *Arabidopsis H4A748* Probe 1 was incubated with either DNA binding buffer (lane 1) or purified recombinant *AtWLIM2a* (3 μ g, lanes 2 and 3), without or supplemented with a 10-fold molar excess of unlabeled Probe 1 (lane 2 and 3, respectively). The arrow marks the migrated free probe.