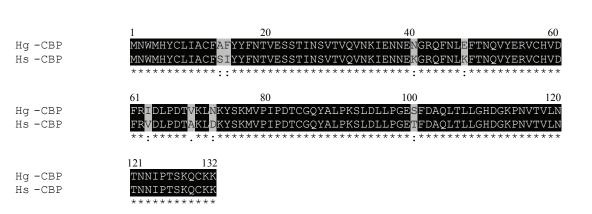
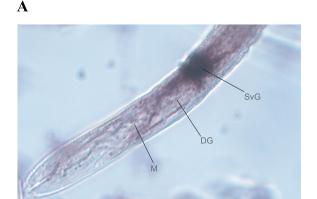
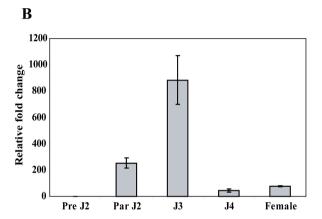
Supplemental Data. Hewezi et al. (2008) Cellulose Binding Protein from the parasitic nematode *Heterodera schachtii* interacts with Arabidopsis pectin methylesterase: cooperative cell wall modification during parasitism.



Supplemental Figure 1. Sequence alignment of Hg -CBP and Hs -CBP.

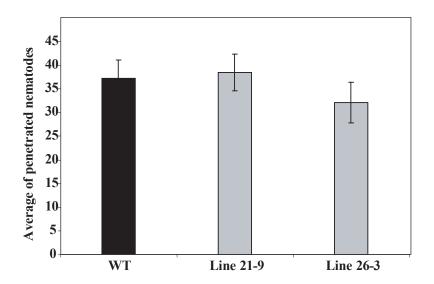
The full length cDNAs encode 132 amino acids with an N-terminal signal peptide terminated immediately upstream of a protease cleavage site between amino acids Ser22 and Ser23, and a cellulose binding domain of 107 amino acids (amino acids 23-129). Black shading indicates sequence identity and grey shading sequence similarity.



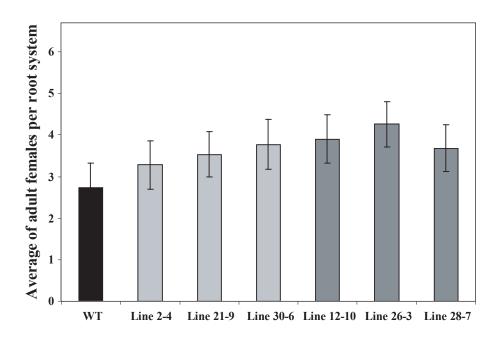


Supplemental Figure 2. *In situ* hybridization and developmental expression level of Hs -*CBP*.

- (A) Hybridization of digoxigenin-labeled antisense cDNA probe (dark staining) of CBP to mRNA expressed exclusively in the secretory subventral gland cells of H. schachtii. No hybridization was observed with the control sense cDNA probe. DG = dorsal gland cell, M = metacorpus, SvG = subventral gland cells.
- (B) Developmental expression level of *CBP*. The relative mRNA expression level of *CBP* was quantified using real time RT PCR in six different *H. schachtii* developmental stages and normalized using β-actin as an internal control. The fold-change values were calculated using the $2^{-\Delta\Delta CT}$ method and represent changes of mRNA abundance in pre-J2, par-J2, J3, J4 and females relative to that of eggs. Shown data are averages of three biologically independent experiments, each consisting of three technical replicates.

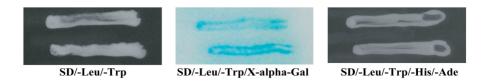


Supplemental Figure 3. Hs –*CBP* expression in *A. thaliana* does not alter nematode penetration into roots. The total number of penetrating J2 nematodes was determined in transgenic plants expressing *CBP* with (line 21-9) or without (line 26-3) the native signal peptide as well as in wild-type control (C24) as described in the Methods. No statically significant differences between the transgenic lines and the wild-type control as determined by modified t-test in SAS were detected.

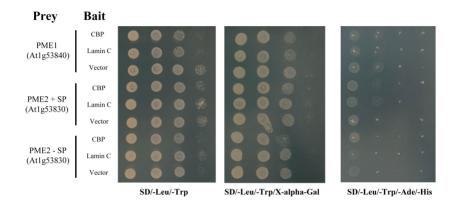


Supplemental Figure 4. Hs –CBP expression in A. thaliana does not alter susceptibility to the root-knot nematode. All tested lines showed no statistically significant differences (as determined by unadjusted paired t-tests (P < 0.05) compared to the wild-type control. Homozygous T3 lines expressing either CBP with (Lines 2-4, 21-9 and 30-6) or without (lines 12-10, 26-3 and 28-7) the native signal peptide were planted along with wild-type C24 were planted on modified Knop's medium, and two-week-old seedlings were inoculated with approximately 250 surface sterilized J2 $Meloidogyne\ incognita$ nematodes. Four weeks post inoculation, the number of adult egg-laying female nematodes was determined. Data are presented as the mean \pm the standard error. Identical results were obtained from two independent experiments.





B



Supplemental Figure 5. Hs -CBP specifically interacts with PME3.

(A) CBP/PME3 interaction: Yeast strain AH109 co transformed with bait and prey plasmids was plated and grown for 3 days on three synthetic dropout (SD) media (SD/-Leu/-Trp; SD/-Leu/-Trp/X-alpha-Gal and SD/-Leu/-Trp/-His/-Ade). Bait and prey protein–protein interaction resulted in the activation of the *MEL1* gene encoding α -galactosidase and the *Ade* and *His* nutritional selective genes. Shown are two independent yeast transformants.

(**B**) Specificity of the CBP/PME3 interaction.

PME1 (At1g53840) and *PME2* (At1g53830, with and without signal peptide) were cloned into the prey vector and transformed into yeast strain AH109 in combination with the *CBP* bait vector or pGBKT7-lam, expressing lamin C as a GAL-4 DNA-BD fusion or the empty pGBKT7 bait vector. Yeast cells containing both bait and prey plasmids were streaked onto SD/-Leu/-Trp/X-alpha-Gal and SD/-Leu/-Trp-/Ade/-His media to test the interaction. The co-transformed cells did not activate expression of the selectable marker to any detectable degree.

Supplemental Table 1. Quantification of Hs -*CBP* expression levels in transgenic Arabidopsis lines using quantitative real-time RT-PCR.

Hs-CBP expressing lines	Ct value (CBP)		Ct value (actin)		ΔCt	
	Mean	SD	Mean	SD	Mean	SD
Line 28-7	22.80	0.22	20.78	0.17	2.03	0.28
Line 26-3	24.65	0.21	21.53	0.15	3.13	0.26
Line 12-10	26.50	0.22	20.90	0.08	5.60	0.23
Line 21-9	28.40	0.39	24.80	0.42	3.60	0.58
Line 2-4	24.00	0.17	19.83	0.05	4.18	0.18
Line 30-6	23.70	0.10	17.93	0.13	5.78	0.16

The expression level of CBP was determined in transgenic lines by relative quantification using the comparative Ct (cycle threshold) method. Ct values represent the number of PCR cycles required for the fluorescent signal to cross the threshold. Δ CT represents the Ct values of Hs-CBP that was normalized to an endogenous reference (Arabidopsis actin). Low Δ Ct values indicate high expression level and vise versa. Data are averages of three biologically independent samples each consisting of three technical replicates.