# Supporting information

Table S1: Factors and levels tested in the *N. tabacum* BY-2 PCP extraction DoE approach.

| Factor | Levels | | | |
|---|---|---|---|---|
| A-Incubation time [dpi] | 4 | 5 | 6 | 7 |
| B-Time of incubation with EB [min] | 0 | 5 | 10 | |
| C-Triton X-100 concentration [g $L^{-1}$] | 0.000 | 0.005 | 0.050 | |
| D-pH of EB [-] | 6.0 | 8.0 | 9.0 | |
| E-Conductivity of EB [mS $cm^{-1}$] | 15 | 35 | 55 | |

EB: extraction buffer.

Table S2: Analysis of variance table of *N. tabacum* BY-2 PCP extraction DoE

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Block | 0.26 | 3 | 0.088 | | | |
| Model | 4.46 | 5 | 0.89 | 69.21 | < 0.0001 | significant |
| A-Incubation time [dpi] | *3.96* | *1* | *3.96* | *306.98* | *< 0.0001* | |
| D-pH of EB [-] | *3.055E-003* | *1* | *3.055E-003* | *0.24* | *0.6283* | |
| E-Conductivity of EB [mS $cm^{-1}$] | *9.696E-003* | *1* | *9.696E-003* | *0.75* | *0.3894* | |
| *DE* | *0.14* | *1* | *0.14* | *11.09* | *0.0015* | |
| $A^2$ | *0.23* | *1* | *0.23* | *18.20* | *< 0.0001* | |
| Residual | 0.79 | 61 | 0.013 | | | |
| *Lack of Fit* | *0.59* | *50* | *0.012* | *0.65* | *0.8500* | *not significant* |
| *Pure Error* | *0.20* | *11* | *0.018* | | | |
| Cor Total | 5.51 | 69 | | | | |

EB: extraction buffer.

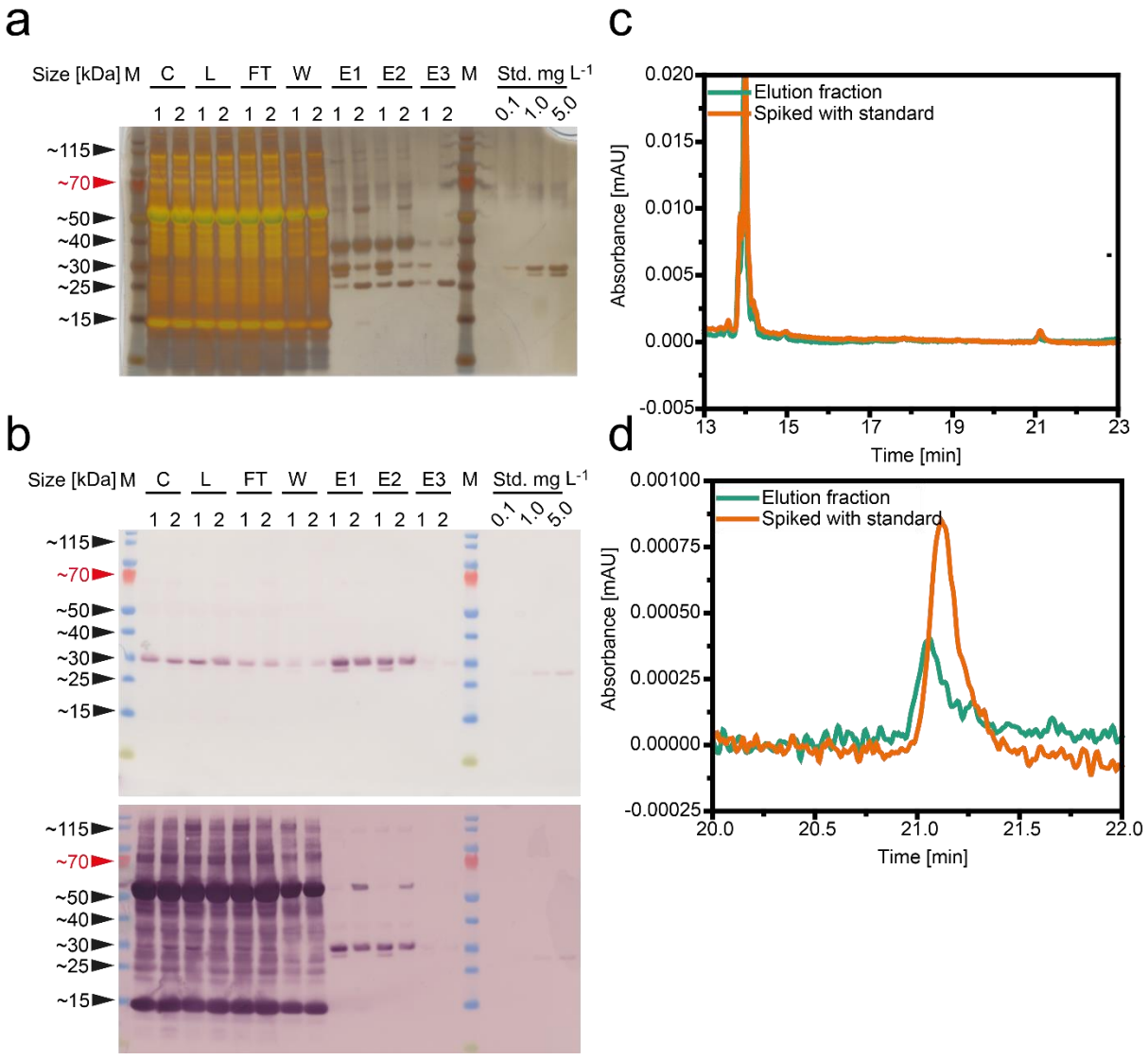


Figure S1: Protein composition analysis of purification intermediates by LDS-PAGE. a. Silver staining. C: centrate; L: affinity chromatography load; FT: flow-through; W: wash; E: elution fraction 1-3. M: Marker; 1 and 2: extract batches of two biological replicates. Std.: refolded viscumin (non-glycosylated) purified from *E. coli*. b. Western blot using primary mAb TA-5 (top) and an alkaline phosphatase-labeled goat anti-mouse IgG secondary antibody. The blot was then stained again with an anti-host cell protein polyclonal antibody as primary antibody (Arfi et al. 2016) and the same secondary antibody as before (bottom). c. Electropherogram of a sample of affinity purified, plant-derived viscumin (green) and the same sample spiked with 20 mg $L^{-1}$ bacterial viscumin standard (orange). The peak at 14 min retention time corresponds to the buffer components, whereas the peak at ~21 min

corresponds to viscumin. d. A zoomed-in section of the data shown in c focusing on the recombinant viscumin and avoiding the system peak at 14 mL. Colors are as in c.

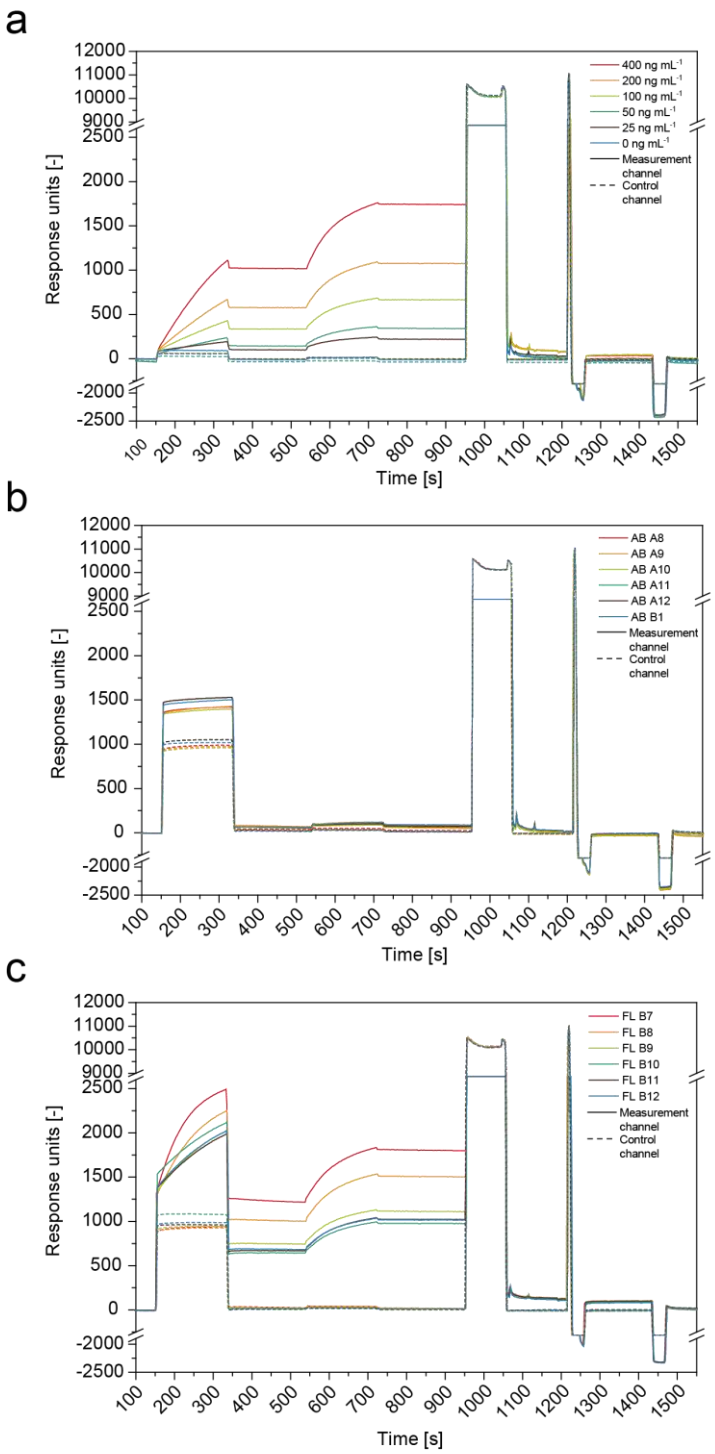


Figure S2: SPR sensograms of viscumin-containing *N. benthamiana* leaf extracts obtained 5 days post infiltration. Six biological replicates per infiltration set are shown. Each sample was injected at 150 s on chip with B chain-specific 36-2-0 mAb. After a wash, A chain-specific

mAb TA-5 was injected at 550 s and chip regeneration was started at 950 s. The measurement channel is shown as a solid line whereas the control channel is as dotted line. a. Measurement of viscumin standard ranging from 0 to 400 ng $mL^{-1}$ using refolded viscumin (non-glycosylated) purified from *E. coli*. b. visA and visB co-infiltration samples (loaded from well A8-B1 of the SPR device). c. visFL infiltration samples (loaded from well B7-B12 of the SPR device).