Supporting Information Legends

Figure S1. Screening for potential *T. urticae* salivary-gland specific genes. Expression values of 18 *T. urticae* genes in intact mites (grey bars) and in anterior body dissected mites (black bars). For each gene the bars were scaled to the lowest average i.e. the lowest bar in each plot is 1. Candidates showing an at least 10-fold statistically-significant lower expression in dissected mites were considered as potential salivary-gland specific genes (Tu19, Tu28, Tu84, Tu90, and Tu128). Statistical differences were analyzed using Student's *t*-test (* = $p < 0.05$).

Figure S2. Amino acid sequence alignment of Te28 and Tu28. Alignment of mature amino acid sequences (minus predicted signal peptide) of Te28 and Tu28. Clustal Omega was used to align both sequences. Black and grey shading indicate identical and similar residues, respectively. Lines above the alignment indicate the ARMADILLO domain (IPR016024) in Te28 (upper) and Tu28 (lower). The conserved 80-amino acid repeats in Te28 and Tu28 are indicated with a line below the alignment.

Figure S3. Detection of Te28 and Te84 gene expression in salivary glands of *T. evansi* using whole-mount *in situ* hybridization. DIG-labeled antisense riboprobes (left panel) were used for hybridization, while corresponding sense probes (right panel) served as negative controls. Signals were detected with alkaline phosphatase linked to an anti-DIG antibody. In situ hybridization experiments for Te28 and Te84 were repeated using both nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate (NBT/BCIP) (top row) or Fast Red (bottom row) as substrate. The pictures show mites representative for the samples. Specific colorization (arrows) in salivary glands was only seen in antisense probe samples and never in sense probe samples. Some background staining in e.g. stylet and legs was common in all samples. Scale bars indicate 0.5 mm. The black dark spots are spider mite midgut cells containing excretory material (e.g. chlorophyll) (first described by Wiesmann 1968, Blauvelt 1945).

Figure S4. Candidate effector Te28 causes tissue necrosis after agroinfiltration. Agroinfiltrated leaves expressing Te28 or with the EV. The arrow indicates a necrotic zone in the Te28-agroinfiltrated area. Necrosis after expression of Te28 occasionally occurred.

Figure S5. Agroinfiltration of candidate effectors from families 19, 90, or 128 does not induce chlorosis in *N. benthamiana*. Agroinfiltrated leaves after expression of candidates from families 19, 90, or 128. Infiltrated areas were delineated with a black marker. Leaves with the EV or expressing the candidate Te28 are shown as negative and positive control for tissue chlorosis, respectively. Pictures were taken at 5 DPI.

Figure S6. Relative gene expression of the SA-marker gene *PR1* after agroinfiltration of 4 candidate effectors or 35s:GFP as control. Relative expression of the SA-related marker *PR1* at 5 DPI after agroinfiltration of Te28, Tu28, Te84, Tu84, and 35s:GFP or infiltration of the mock control. Error bars denote SE. Statistical differences were analyzed using a General Linear Model, and are denoted as different letters ($p<0.05$).

Figure S7. Relative gene expression of the JA-marker gene *TPI* after agroinfiltration of 4 candidate effectors. Relative expression of the JA-related marker *TPI* after agroinfiltration of Te28, Tu28, Te84, Tu84, and EV or infiltration of the mock control, at 2 and 5 DPI. Error bars denote SE. Statistical differences were analyzed using a General Linear Model, and are denoted as different letters ($p<0.05$). The data are representative for 2 experiments.

Figure S8. Leaf-discs expressing candidate Te28 show intense chlorotic symptoms. The picture shows leaf-discs from agroinfiltrated *N.benthamiana* leaves expressing the candidate effectors or the control EV at the end of a *T. urticae* performance assay (6 DPI, 4 days of spider mite infestation).

Figure S9. Amino acid sequence alignment of Te84 and Tu84. Alignment of predicted amino acid sequences (minus predicted signal peptide) of Te84 and Tu84. Clustal Omega was used to align both

sequences. Black and grey shading indicate identical and similar residues, respectively. No known motifs were identified in Te84 or Tu84.

Figure S10. Agroinfiltrated *N. benthamiana* leaves after leaf-surface washing. (A) Picture of an intact agroinfiltrated *N. benthamiana* leaf showing high density of glandular trichomes (arrows) on the adaxial side. (B) Glandular trichomes were removed with a tissue paper soaked in water.

Figure S11. Exponential growth of two hypothetical populations growing on the SA-impaired *nah*G tomato plants or Moneymaker (MM) tomato plants. Despite the moderate differences in oviposition rates observed on both genotypes (Fig. 6), this has a big impact on population level. The intrinsic rate of increase (rm) was estimated using the regression model proposed in Jansen and Sabelis (1992) for *T. urticae:* $r_m = 0.131 + 0.011\ x$; where x is the oviposition rate (i.e. number of eggs per female, per day). The intrinsic rates of increase for mites reared on Moneymaker (MM) and *nah*G plants were: $r_{m\ MM} = 0.212$; and $r_{m\ nahG} = 0.220$. The values were used to parametrize the model: $n = 10^{(t*rm)}$; where n is the number of individuals; t is time in days; and r_m is the intrinsic rate of increase.

Table S1. Primers sequences used for gateway cloning and *in situ* hybridization

Table S2. *Nicotiana benthamiana* primer sequences used for qPCR in this study

Table S3. *Tetranychus urticae* and *Tetranychus evansi* primer sequences used for RT-PCR and/or qPCR in this study

Data S1. *Tetranychus evansi* and *T. urticae* clustered secretomes

Methods S1. High-throughput sequencing and *de novo* assembly

Methods S2. RNA isolation and RT-qPCR

Methods S3. Phytohormone extraction and LC-MS

Methods S4. *In situ* hybridization and confocal laser-scanning microscopy