Supporting Information Appendix

GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense

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Summary of SI Appendix

Text

Movement of macromolecules from the hemocoel into salivary glands and ingestion of saliva

Because aphids do not have Malpighian tubules, the excretory system found in most insects, it has been speculated that the salivary glands function as a combined secretory and excretory organ (1, 2). The aphid salivary glands are composed of a pair of principle and accessory glands, each with several cells (2). Both gland types have been implicated in excretory function. Dyes injected into aphid hemolymph are taken up by specialized cells in the principle salivary gland (2). The accessory gland, on the other hand, has extensive microvilli externally directed towards the hemocoel, suggesting transport of material from the hemocoel into the gland cells (2). Therefore, we speculate that GroEL and other *Buchnera* proteins, as well as the aphid proteins not predicted for secretion, moved from the hemocoel into the saliva through the salivary glands.

Recently, using proteome analysis, GroEL and other *Buchnera* proteins were identified in the aphid honeydew collected from aphids while feeding on the host plant (3). The likely source of the *Buchnera* GroEL in the honeydew is saliva. Saliva is secreted in the stylet path and into vascular and non-vascular cells. It is also well documented that aphid ingest saliva before saliva exits the stylet tip. The aphid stylets are composed of two canals, the salivary canal and the food canal (4). The outlet of the salivary canal is at some distance from the stylet tip providing a chamber where both saliva and ingested plant sap meet. While sap is ingested, the sap flow pushes the saliva into the food canal and hence into the gut (4). Consequently, saliva is ingested before and after being released into the plant milieu. Therefore, the presence of *Buchnera* proteins including GroEL in both saliva and honeydew indicates that these endosymbiont proteins move through the salivary glands into the saliva.

Salivary protein identification

Searching mass spectrometry (MS) spectra against the predicted potato aphid (*Macrosiphum euphorbiae*) protein database, derived from transcriptome sequences (accession number SRX339176), identified 131 potato aphid contigs. Since the potato

aphid transcriptome sequences are not full-length, we used these contigs in BLAST analysis against the pea aphid (*Acyrthosiphon pisum*) genome and identified their orthologs. The 131 potato aphid contigs corresponded to 90 pea aphid genes, indicating that a subset of the potato aphid contigs correspond to non-overlapping regions of a single pea aphid gene (Dataset S1). In addition, we identified four potato aphid contigs with no pea aphid homologs (TBLASTX, e-value ≤ 1e-3) indicating they are specific to potato aphids (Dataset S1).

Materials and Methods

Plant material and growth conditions. Tomato cultivar Moneymaker plants were maintained as described previousely (5). *Arabidopsis thaliana bak1-5* mutant, in Col-0 genetic background (6) and wild-type Arabidopsis Col-0 plants were grown under 12 h light photoperiod. Unless mentioned otherwise, five-week-old tomato and Arabidopsis plants were used for assays.

Aphid colonies and growth conditions. Colonies of the parthenogenetic potato aphid (*Macrosiphum euphorbiae*) and green peach aphid (*Myzus persicae*) were reared on tomato cv. UC82B and mustard India plants, respectively, and maintained as described in (5, 7). Age-synchronized one-day-old adult aphids were produced as described in (8).

Saliva collection from potato aphids

To collect saliva, potato aphids were fed on ultra pure sterile water in parafilm pouches as described previously (9). About 100 aphids were exposed to a single pouch, containing 150 µl of water, for 16 h under yellow light at 23°C. The liquid content of the pouches was collected with a fine pipette tip. To collect gelling saliva, the parafilm pouches were opened, rinsed well in sterile water and the internal surface was scraped using the dull side of a sterile surgical blade. Saliva was collected from an estimated 100,000 aphids and stored at -80°C until ready for mass spectroscopy (MS).

Saliva preparation. Saliva was heated at 94°C for 5 min to denature the proteins. A final concentration of 50 mM Hepes was added to the saliva to adjust the pH to 7.2. Cysteines were reduced and alkylated using 0.5 mM Tris(2-carboxyethyl) phosphine (Fisher, AC36383) at 94°C for 5 minutes then 1.25 mM iodoacetamide (Fisher, AC12227) at 37°C in dark for 15 min. Proteins were digested with 20 µg trypsin (Roche, 03 708 969 001) at 37°C overnight. Digested peptides were desalted and concentrated by Sep-Pak tC18 solid phase extraction cartridge (Waters, WAT054925). Eluted peptides were dried in a vacuum concentrator at 4°C. Peptides were re-suspended in 500 µl water and ready for MS analysis.

Liquid chromatography (LC)-MS analysis. Automated 2D nanoflow LC-MS analysis was performed using LTQ tandem MS (LC-MS/MS) (Thermo Electron Corporation, San Jose, CA) employing automated data-dependent acquisition as described in (10). Raw data were extracted and searched using Spectrum Mill (Agilent, version B.04.00). MS/MS spectra with a sequence tag length of 1 or less were considered as poor spectra and discarded. The enzyme parameter was limited to full tryptic peptides with a maximum mis-cleavage of 1. All other search parameters were set to default settings of Spectrum Mill [carbamidomethylation of cysteines, iTRAQ modification, +/- 2.5 Da for precursor ions, +/- 0.7 Da for fragment ions, and a minimum matched peak intensity (SPI%) of 50%]. Ox-Met and n-term pyro-Gln were defined as variable modifications for total proteome data. A maximum of 1 modification per peptide was used. The filtered MS/MS spectra were searched against two databases: 1) a NCBI non-redundant protein database limited to *Buchnera* taxonomy; and 2) a home-made potato aphid transcriptome database. The potato aphid transcriptome database was constructed by performing 6-frame translation of the potato aphid transcripts. ORFs with protein length less than 6 amino acids were discarded. For each of the two databases a 1:1 concatenated forward-reverse database was constructed to calculate the false discovery rate (FDR). The tryptic peptides in the reverse database were compared to the forward database, and were shuffled if they matched to any tryptic peptides from the forward database. The details of the two databases are summarised in Table S2A. Cutoff scores (Tables S2B-S2E) were dynamically assigned to each dataset to maintain the FDR at 0% (Tables S82F and S2G), which means there were no proteins from

the decoy database passing the filtering criteria. For *Buchnera*, proteins that share common peptides were grouped to address the protein database redundancy issue. The proteins within the same group shared the same set or subset of unique peptides.

Aphid proteins with at least two peptides in either saliva were reported (Dataset S1). All peptides matching to proteins from the endosymbiont *Buchnera* were manually validated (Table S3) and reported (Dataset S1).

Annotation, gene ontology classification and signal peptide prediction. Potato aphid transcripts matching to the sequenced peptides were annotated by performing reciprocal TBLASTX analyses with pea aphid predicted sequences (aphidbase_2.1_mRNA) and against NCBI nucleotide (nt/nr) database. The annotated sequences were assigned to different gene ontology (GO) categories based on available database containing GO assignments of all the publicly available pea aphid expressed sequence tags (EST).

Amino-acid sequences of putative full-length pea aphid orthologs of the potato aphid secreted proteins were subjected to *de novo* signal peptide prediction analysis using SignalP 4.0 (11) and TargetP 1.1 (12) programs. Hidden Markov model scores higher than 0.45 were considered for SignalP prediction while for TargetP predictions were determined by predefined set of cutoffs that yielded specificity >0.95 on the TargetP test sets.

DAPI staining. Aphid saliva was collected in water as described above. Saliva from five feeding chambers (fed on by about 1,000 aphids) was pooled and vacuum concentrated. Ovaries, dissected from adults aphids, and saliva were fixed in 1% paraformaldehyde in PBS and nuclei were stained in the dark for 20 min with 1 µg/ml of 4′,6′-diamino-2-phenylindole (DAPI, Sigma). Samples were observed under a fluorescence microscope (Nikon Eclipse Ti).

Cloning in pVSP *Ps*SPdes vector and aphid bioassays. *groEL* (accession number KF366417) and *β-glucuronidase* (*GUS*) were PCR amplified from *M. euphorbiae* gDNA and pENTR-GUS (Invitrogen), respectively, using Phusion High fidelity DNA Polymerase (New England BioLabs). A stop codon was introduced at end of the end of

both fragments. Sequences were cloned into pVSP *Ps*SPdes vector (13) as described previously (7). A *Pseudomonas fluorescens* (*Pfo)* strain (EtHAn), engineered to deliver effectors into plant cells by T3SS (type three secretion system) (14), and wild type *Pfo* were transformed with the recombinant pVSP *Ps*SPdes vectors and grown as described previously (7).

Every rosette leaf of five-week-old Arabidopsis plants was infiltrated with a suspension of *Pfo* at a density of 1 x 10^4 colony forming units (cfu) ml^{-1}, in 10 mM $MgCl_2$, using a needleless syringe. Twenty-four hours after infiltration each plant was infested with a single age-synchronized one-day-old adult green peach aphids and the plant was caged inside a clear plastic tubing, pushed inside the soil and covered at the top with a white gauze secured with a rubber band. Aphid fecundity was assessed by counting and removing the nymphs daily for a period of five days. Fifteen plants were used per treatment and the experiment was repeated twice to generate data from three independent replicated experiments.

Tomato assays were performed as described previously (7). Briefly, five-week-old tomato plants were vacuum infiltrated at 1 x 10^4 cfu ml^{-1} in 10 mM $MgCl_2$ and 0.02% Silwet L-77. Twenty-four hours after infiltration, each tomato plant was infested with nine age-synchronized one-day-old adult potato aphids. Aphid fecundity was assessed by counting and removing the nymphs daily for a period of five days. Six plants were used per treatment and the experiment was repeated twice to generate data from three independent replicated experiments.

Construction of transgenic plants expressing GroEL. Arabidopsis Col-0 plants were used to generate GroEL transgenic lines. The full-length *groEL* in pENTR221 vector was used to perform an LR reaction with the GATEWAY® compatible binary vector pMDC32 or pMDC7 having a β-estradiol-inducible G1090::XVE promoter (15). The resulting clone was transformed into *Agrobacterium tumefaciens* strains GV3101 and stable transgenic Arabidopsis lines were generated using *A. tumefaciens*-mediated floral-dip transformation (16). Independent transformed plant pools were kept separate for selection of independent transgenic lines. Transgenic plants selected on hygromycin (25 mg L^{-1}) and by PCR for the presence of *groEL* transgene, were screened in the T2

generation for single locus insertions of *groEL* transgene and were propagated to successive generations to obtain homozygosity for the transgene.

Aphid bioassays on transgenic Arabidopsis. Five-week-old transgenic plants grown in soil were sprayed with 20 μM β-estradiol solution containing 0.02% silwet L-77 to induce the *groEL* transgene expression. Twenty-four hours after induction, each plant was infested with a single age-synchronized one-day-old adult green peach aphid as described earlier and allowed to feed on the induced plants for 24 h. The adult aphids were moved to a fresh transgenic plant sprayed with β-estradiol for two more times. At each move and on the last day (days 2, 3, 4 and 5), the number of nymphs was counted and nymphs were removed. Ten plants were used per treatment and the experiment was repeated twice to generate data from three independent replicated experiments.

Five-week-old Arabidopsis transgenic lines expressing *groEL* constitutively were infested with a single age-synchronized one-day-old adult green peach aphid per plant as described earlier. Aphid fecundity was assessed by counting and removing the nymphs daily for a period of five days. Ten plants were used per treatment and the experiment was repeated twice to generate data from three independent replicated experiments.

Expression and purification of proteins. *groEL* and *GUS* constructs in pDONR221 were recombined into the pDEST17 expression vector (Invitrogen) using GATEWAY® cloning technology to generate His-fusion proteins. The clones were transformed into *E. coli* BL21 cells and grown at 37°C to an OD_{600} of 0.7. Protein was expressed and purified using a Ni-NTA column (QIAGEN) as described previously (17). The column was washed ten times in Buffer A with 40 mM Imidazole. The bound protein was eluted in Buffer A with 250 mM Imidazole. Pooled elutes were concentrated and equilibrated to phosphate buffered saline (PBS) (pH 6.8) using an Amicon concentrator. Eluted GroEL protein was further fractionated on a 4.3 ml BioFox 17Q anion exchange column. The sample was loaded onto the resin and washed with 10 column volumes of 20 mM Tris-Cl pH 8.0 at 0.5 ml min^{-1}. Bound protein was eluted with a linear gradient of 0 to 1 M NaCl in 20 mM Tris-Cl pH 8.0. Protein concentration was measured using

Bradford assay. Anion exchange purification was performed by AthenaES (Athena Enzyme Systems Group, Baltimore, MD).

Aphid bioassays on Arabidopsis with purified GroEL protein. In preliminary experiments, defense gene induction after His-epitope tagged GroEL infiltration of Arabidopsis leaves indicated transient expression of *PR1* gene reaching maximum levels at 24 h and returning to pre-induction levels at 48 h. Based on this result, aphids were exposed to GroEL infiltrated leaves for only 48 h. A single leaf per plant was infiltrated with PBS or 1.5 μM of GroEL or GUS. Soon after infiltration, the infiltrated leaf was infested with a single age-synchronized one-day-old adult green peach aphid using a clip cage. After 48 h, the adult aphids were moved to a new plant with a freshly infiltrated leaf and were allowed to feed for an additional 48 h. Nymphs were counted and removed daily for a total of four days. Ten plants were used per treatment. Experiment was repeated twice to generate data from three independent replicated experiments.

Saliva collection from green peach aphid and Arabidopsis treatment. Green peach aphid saliva was collected in a diet containing sucrose and amino acids (18) as described for potato aphids. Diet fed on aphids and control (diet without aphids) were infiltrated into Arabidopsis leaves using a 1 ml needle less syringe. A single rosette leaf was infiltrated per plant and three plants were used per treatment. Leaves were harvested immediately after infiltration at 0 h and at 3 h and 6 h post treatment. Experiment was repeated once to generate data from two independent replicated experiments.

Quantitative real-time PCR analysis. RNA extraction, sample preparation for quantitative RT-PCR was performed as described earlier (5) using gene-specific primers (Table S1). Relative expression of genes was calculated using actin (*ACT-2*) as a standard gene for Arabidopsis and ubiquitin (*Ubi3*) for tomato (Table S1).

For gene expression in transgenic Arabidopsis lines, five-week-old plants were sprayed with 20 μM β-estradiol or three-week-old seedlings, germinated on MS media, were transferred to MS supplemented with 5 μM β-estradiol. Leaf samples were harvested at the indicated times post treatment. A leaf from three plants were used for

each independent transgenic line and experiment was repeated once to generate data from two independent replicated experiments.

For gene induction by purified GroEL, five-week-old Arabidopsis Col-0 and *bak1-5* plants were infiltrated with 1.5 µM of GroEL, GUS or PBS. A single rosette leaf was infiltrated per plant and three plants were used per treatment. Leaf samples were harvested at the indicated times post treatment. Experiment was repeated once to generate data from two independent replicated experiments.

For gene expression after *Pfo* infection in tomato, plants were infected at a density of 1 x 10^9 cfu ml^{-1} as described earlier (19). Leaf samples were harvested at 6 h post treatment. Three plants were vacuum infiltrated per treatment and one leaflet was harvested per plant. Experiment was repeated once to generate data from two independent replicated experiments.

Oxidative burst measurement. ROS burst was determined by a luminol-based assay as described previously (20) with modifications. Three to four-week-old Arabidopsis plants were excised into 2 mm slices and incubated overnight in a 96-well plate with 200 µl H_2O. H_2O was replaced with 200 µl of 20 µM of luminol and 5 µg ml^{-1} of horseradish peroxidase (Sigma) supplemented with 1.5 µM of GroEL, boiled and snap-chilled GroEL or GUS and measurement was conducted with a luminometer (Mithras LB 940 Multimode Reader luminometer, Berthold Technologies). Experiment was repeated at least twice to generate data from three independent replicated experiments.

Callose deposition. Callose deposition was performed as described previously (21). Callose deposits were visualized under a UV filter using a fluorescence microscope and counted using ImageJ 1.43U software (22), (http://rsb.info.nih.gov/ij/) as described previously (23). Two leaves was infiltrated per plant and four plants were used per treatment. Experiment was repeated once to generate data from two independent replicated experiments.

Table S1. List of primer sequences used in this study.

Primer	Sequences (5'-3')*	Purpose	Reference
GroEL-F	GGGGACAAGTTTGTACAAAAAAGCAGG CTccATGGCCGCTAAAGATGTGAAAT	Gateway cloning of *groEL*	This study
GroEL-R	GGGCACCACTTTGTACAAGAAAGCTGGG TTTACATCATTCCGCCCATGCC	Gateway cloning of *groEL*	This study
GroEL-HA-R	GGGCACCACTTTGTACAAGAAAGCTGGG TCATCATTCCGCCCATGCC	Gateway cloning of *groEL*	This study
GUS-F	GGGGACAAGTTTGTACAAAAAAGCAGG CTccATGGTCCGTCCTGTAGAAAC	Gateway cloning of GUS	This study
GUS-R	GGGCACCACTTTGTACAAGAAAGCTGGG TTTATTGTTTGCCTCCCTGCT	Gateway cloning of GUS	This study
GroEL-qPCR-F	AATATCTAATTTACGTGGTCAAAATGA	qPCR	This study
GroEL-qPCR-R	CATCATTCCGCCCATGCC	qPCR	This study
pMDC32-F	TCCTTCGCAAGACCCTTCCTC	Genotyping	This study
pMDC7-F	GGTGGTAATGCCATGTAATATGC	Genotyping	This study
AtAct2-F	GATGAGGCAGGTCCAGGAATC	qPCR	(24)
AtAct2-R	GTTTGTCACACACAAGTGCATC	qPCR	(24)
AtFrk1-F	AAGAGAGGTTGAAGATATCA	qPCR	This study
AtFrk1-R	CATCTTCGCTTGGAGCTTCT	qPCR	This study
AtWrky29-F	GCGTAAATACGGGCAGAAAC	qPCR	(25)
AtWrky29-R	GGTTTGGGTTGGGAAGTTTT	qPCR	(25)
AtPR1-F	GTTGCAGCCTATGCTCGGAG	qPCR	(26)
AtPR1-R	CCGCTACCCCAGGCTAAGTT	qPCR	(26)
SlUbi3-F	GTGTGGGCTCACCTACGTTT	qPCR	(5)
SlUbi3-R	CCGTTCATTCGACAAAAAGAA	qPCR	(5)
SlWrky28-F	ACAGATGCAGCTACCTCATCCTCA	qPCR	(27)
SlWrky28-F	GTGCTCAAAGCCTCATGGTTCTTG	qPCR	(27)
SlLrr22-F	AAGATTGGAGGTTGCCATTGGAGC	qPCR	(28)
SlLrr22-R	ATCGCGATGAATGATCGGTGGAGT	qPCR	(28)
SlPti5-F	ATTCGCGATTCGGCTAGACATGGT	qPCR	(28)
SlPti5-R	AGTAGTGCCTTAGCACCTCGCATT	qPCR	(28)

Table S2. MS spectra analysis.

A. Summary of the two protein databases used in the database searches.

Database	Protein sequences #	Decoy sequences #	Common contaminants #	Total protein sequences #
Potato aphid	2,622,342	2,622,342	28	5,244,712
Buchnera	16,419	16,419	28	32,866

B. Filtering Criteria for autovalidation of soluble *Buchnera* database search results.

mode	Protein score	1+ peptide	2+ peptide	3+ peptide
Protein Details	>11.6	>11.6	>15.9	>16.4
Peptide	NA	>11.6	>15.9	>16.4

C. Filtering Criteria for autovalidation of gelling *Buchnera* database search results.

mode	Protein score	1+ peptide	2+ peptide	3+ peptide
Protein Details	>9.6	>9.6	>14.6	>15.4
Peptide	NA	>9.6	>14.6	>15.4

D. Filtering Criteria for autovalidation of soluble potato aphid transcript database search results.

mode	Protein score	1+ peptide	2+ peptide	3+ peptide
Protein Details	>14.7	>14.7	>15.4	>16.7
Peptide	NA	>14.7	>17.4	>19.0

E. Filtering Criteria for autovalidation of gelling potato aphid transcript database search results.

mode	Protein score	1+ peptide	2+ peptide	3+ peptide
Protein Details	>12.7	>12.7	>17.2	>17.1
Peptide	NA	>12.7	>15.2	>17.1

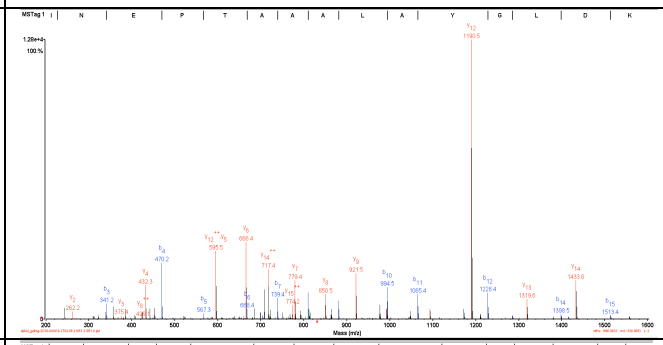
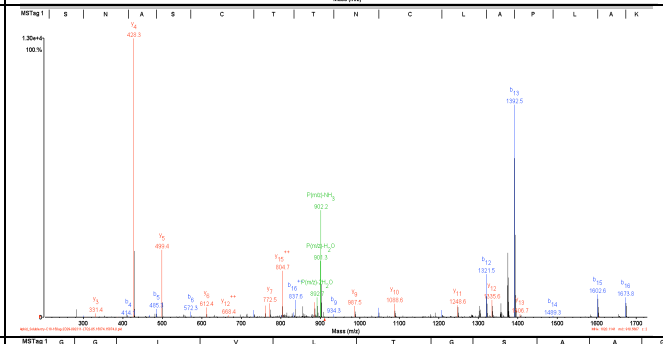
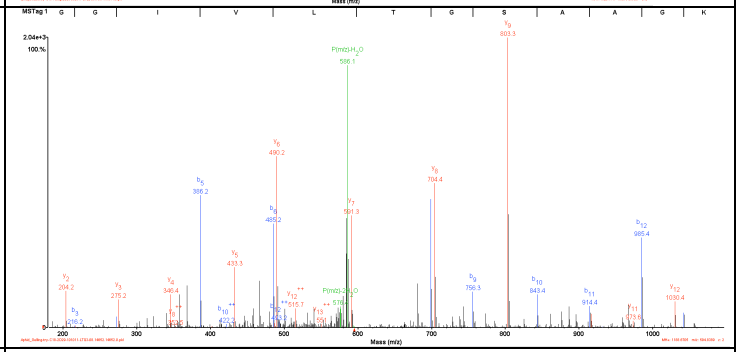
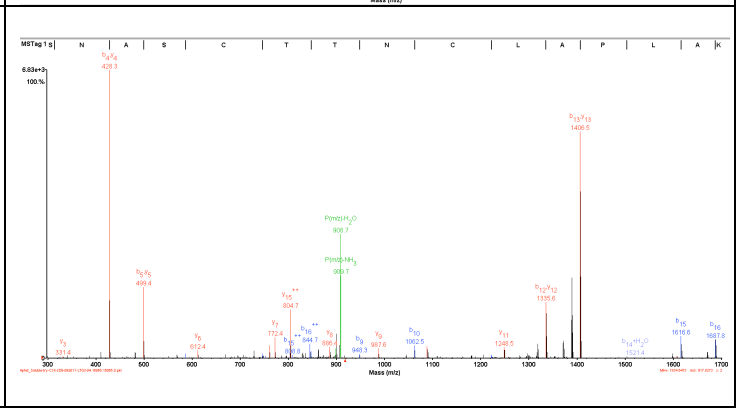
F. Summary of soluble saliva protein database search results.

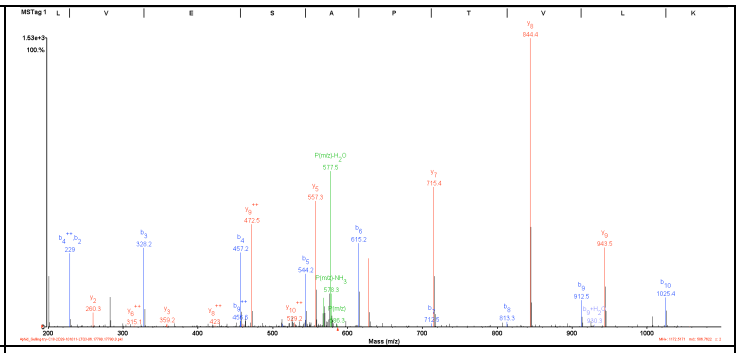
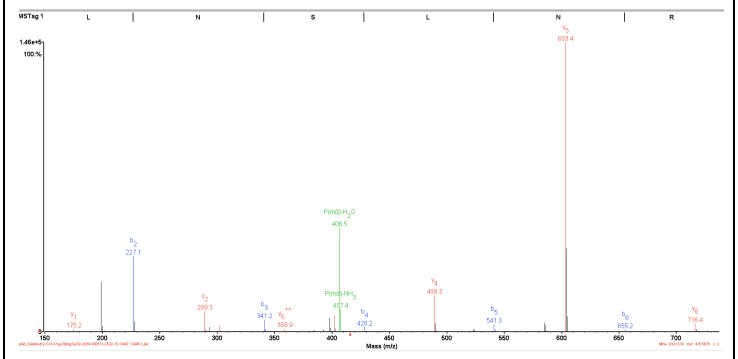
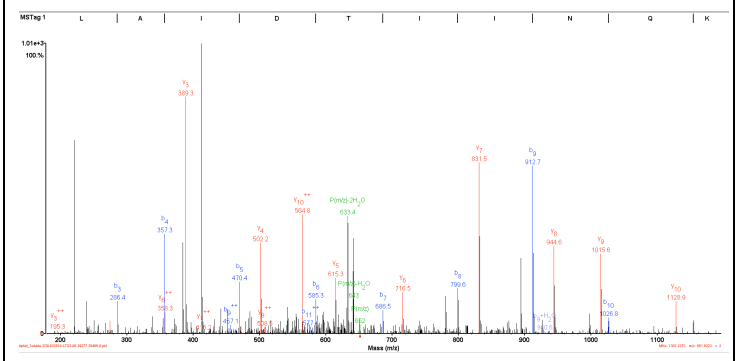
Database	Identified spectral #	Identified peptide #	Identified protein #	Identified protein group #	FDR
Potato Aphid	48,438	1,463	264	181	0%
Buchnera	3,902	26	56	13	0%

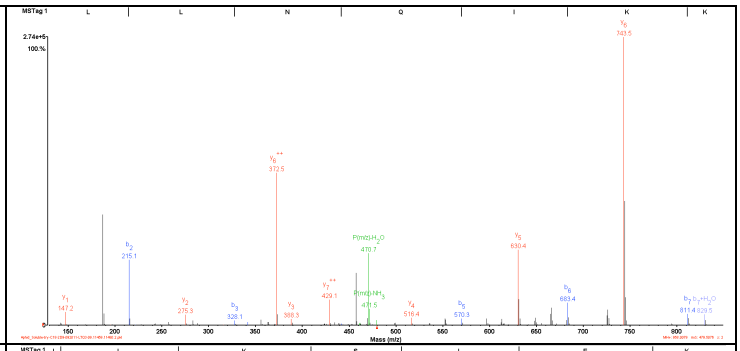
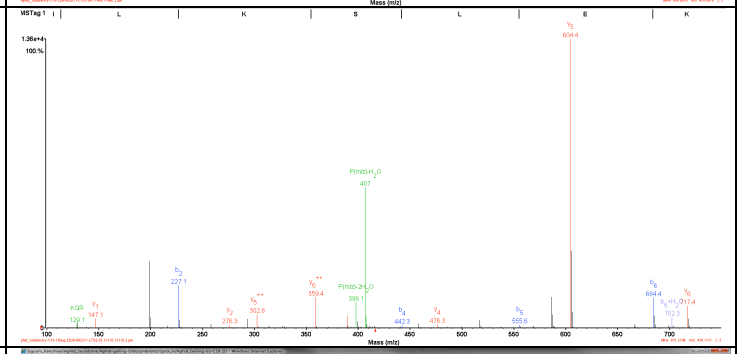
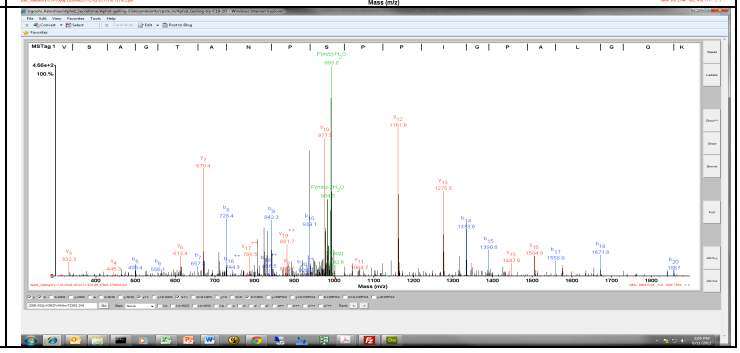
G. Summary of gelling saliva protein database search results.

Database	Identified spectral #	Identified peptide #	Identified protein #	Identified protein group #	FDR
Potato Aphid	22,038	327	256	121	0%
Buchnera	13,724	44	229	16	0%

Table S3. List of *Buchnera* proteins identified with one peptide and manually validated.

Protein	Organism	Score	Peptide Sequence	MS/MS spectrum
gb-AEH39691.1- chaperone Hsp70	*Buchnera aphidicola (Cinara tujafilina)*	20.89	IINEPTAAALAYGLDK	
gb-AAO27001.1- glyceraldehyde 3-phosphate dehydrogenase	*Buchnera aphidicola str. Bp (Baizongia pistaciae)*	20.10	IVSNASCTTNCLAPLAK	
emb-CAC10483.1- GroES [Buchnera aphidicola (Thelaxes suberi)]	*Buchnera aphidicola (Thelaxes suberi)*	19.26	SAGGIVLTGSAAGK	
gb-ABJ90653.1- glyceraldehyde 3-phosphate dehydrogenase [Buchnera aphidicola str. Cc (Cinara cedri)]	*Buchnera aphidicola str. Cc (Cinara cedri)*	18.31	IISNASCTTNCLAPLAK	

ref-NP_660396.2- 50S ribosomal protein L7/L12 [Buchnera aphidicola str. Sg (Schizaphis graminum)]	*Buchnera aphidicola str. Sg (Schizaphis graminum)*	18.04	DLVESAPTVLK	
gb-AAM67773.1- UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [Buchnera aphidicola str. Sg (Schizaphis graminum)]	*Buchnera aphidicola str. Sg (Schizaphis graminum)*	16.98	ILNSLNR	
gb-AEO07841.1- F0F1 ATP synthase subunit alpha [Buchnera aphidicola str. Ua (Uroleucon ambrosiae)]	*Buchnera aphidicola str. Ua (Uroleucon ambrosiae)*	16.96	TALAIDTIINQK	

gb-AAG33952.1-AF197892_1 DNA polymerase III beta subunit [Buchnera aphidicola]	*Buchnera aphidicola*	16.58	TLLNQIKK	
gb-AEO08518.1- NADH dehydrogenase I chain F [Buchnera aphidicola str. Ak (Acyrthosiphon kondoi)]	*Buchnera aphidicola str. Ak (Acyrthosiphon kondoi)*	16.40	ILKSLEK	
emb-CBF84492.1- unnamed protein product [Buchnera aphidicola]	*Buchnera aphidicola*	16.26	LQVSAGTANPSPPIGPALGQK	

References

1. Miles PW (1972) The saliva of Hemiptera. *Adv Insect Physiol* **9**:183-255.
2. Ponsen MB (1972) The site of potato leafroll virus multiplication in its vector, *Myzus persicae* : an anatomical study. Meded Landbou Wagen **16**:1-147.
3. Sabri A, et al. (2013) Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins. *PloS one* **8**(9):e74656.
4. Tjallingii WF (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *J Exp Bot* **57**(4):739-745.
5. Bhattarai KK, Atamian HS, Kaloshian I, Eulgem T (2010) WRKY72-type transcription factors contribute to basal immunity in tomato and Arabidopsis as well as gene-for-gene resistance mediated by the tomato *R* gene *Mi-1*. *Plant J* **63**(2):229-240.
6. Roux M, et al. (2011) The *Arabidopsis* leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell* **23**(6):2440-2455.
7. Atamian HS, et al. (2013) In planta expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity. *Mol Plant-Microbe Interact* **26**(1):67-74.
8. Bhattarai KK, et al. (2007) *Coil*-dependent signaling pathway is not required for *Mi-1*-mediated potato aphid resistance. *Mol Plant-Microbe Interact* **20**(3):276-282.
9. Miles PW (1965) Studies on the salivary physiology of plant bugs: the salivary secretions of aphids. *J Insect Physiol* **11**(9):1261-1268.
10. Bellafiore S, et al. (2008) Direct identification of the *Meloidogyne incognita* secretome reveals proteins with host cell reprogramming potential. *PLoS Pathog* **4**(10):e1000192.
11. Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* **8**(10):785-786.
12. Emanuelsson O, Nielsen H, Brunak S, von Heijne G (2000) Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J Mol Biol* **300**(4):1005-1016.
13. Rentel MC, et al. (2008) Recognition of the *Hyaloperonospora parasitica* effector ATR13 triggers resistance against oomycete, bacterial, and viral pathogens. *Proc Natl Acad Sci USA* **105**(3):1091-1096.
14. Thomas WJ, Thireault CA, Kimbrel JA, Chang JH (2009) Recombineering and stable integration of the *Pseudomonas syringae* pv. *syringae* 61 *hrp/hrc* cluster into the genome of the soil bacterium *Pseudomonas fluorescens* Pf0-1. *Plant J* **60**(5):919-928.
15. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* **133**(2):462-469.
16. Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* **16**(6):735-743.
17. Kale SD, et al. (2010) External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell* **142**(2):284-295.

18. Kim JH, Jander G (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *Plant J* **49**(6):1008-1019.
19. Wei HL, Chakravarthy S, Worley JN, Collmer A (2013) Consequences of flagellin export through the type III secretion system of *Pseudomonas syringae* reveal a major difference in the innate immune systems of mammals and the model plant *Nicotiana benthamiana*. *Cell Microbiol* **15**(4):601-618.
20. Keppler LD, Baker CJ (1989) O2--initiated lipid peroxidation in a bacteria-induced hypersensitive reaction in tobacco cell suspensions. *Phytopathol* **79**(5):555-562.
21. Adam L, Somerville SC (1996) Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant J* **9**(3):341-356.
22. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int* **11**(7):36-42.
23. Shang Y, et al. (2006) RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector AvrB. *Proc Natl Acad Sci USA* **103**(50):19200-19205.
24. Kettles GJ, et al. (2013) Resistance of *Arabidopsis thaliana* to the green peach aphid, *Myzus persicae*, involves camalexin and is regulated by microRNAs. *New Phytol* **198**(4):1178-1190.
25. Trujillo M, Ichimura K, Casais C, Shirasu K (2008) Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. *Curr Biol* **18**(18):1396-1401.
26. Chang S, Pikaard CS (2005) Transcript profiling in Arabidopsis reveals complex responses to global inhibition of DNA methylation and histone deacetylation. *J Biol Chem* **280**(1):796-804.
27. Kim JG, et al. (2009) *Xanthomonas* T3S effector XopN suppress PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1. *Plant Cell* **21**(4):1305-1323.
28. Nguyen HP, et al. (2010) Methods to study PAMP-triggered immunity using tomato and *Nicotiana benthamiana*. *Mol Plant-Microbe Interact* **23**(8):991-999.

Supporting Figures

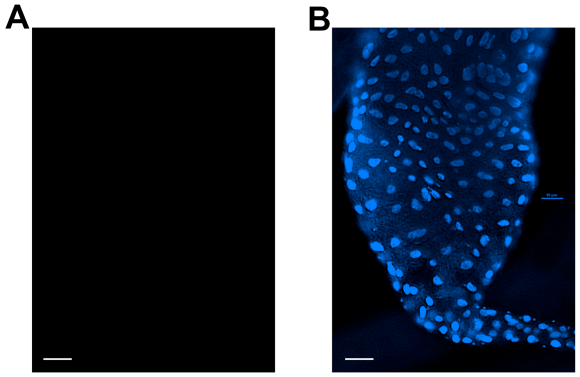


Fig. S1. No nuclei were detected in aphid saliva. DAPI staining of aphid saliva **(A)** and ovaries **(B;** control**)**. Saliva was collected from ~1000 aphids feeding for 24 hour on parafilm pouches containing sterile water. Samples were observed under an inverted microscope (Nikon Eclipse Ti). Scale bar = 10 μM.

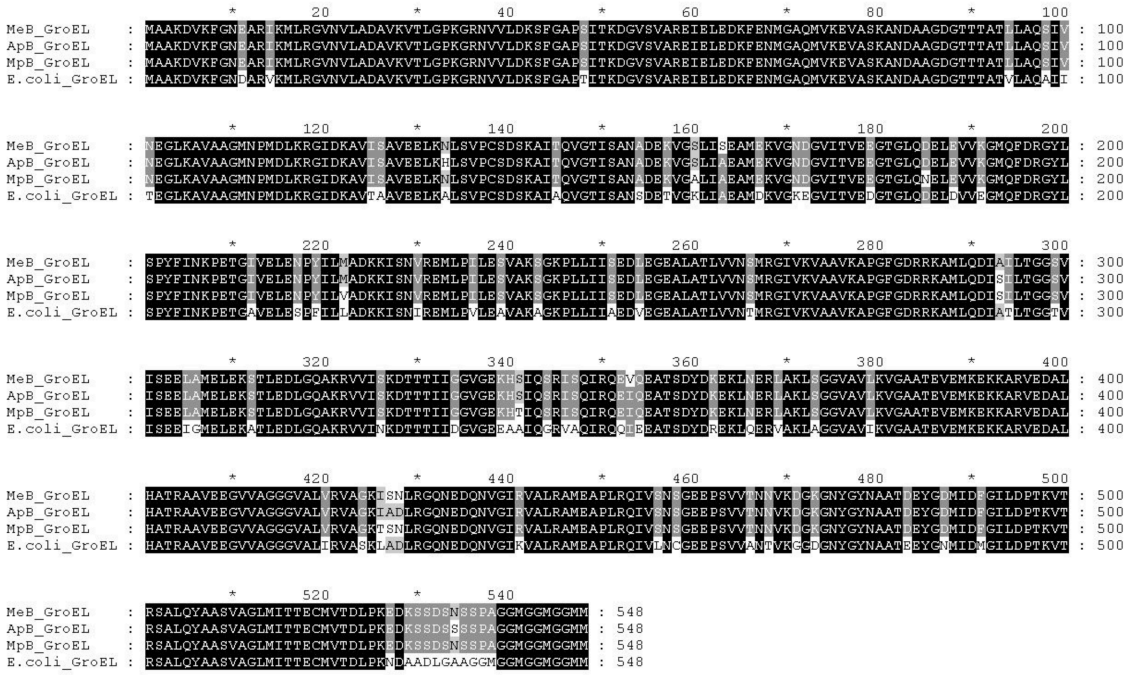


Fig. S2. Sequence alignment of GroEL proteins**.** The deduced amino acid sequences of *Buchnera aphidicola* (B) GroEL amplified from aphids (Me, *Macrosiphum euphorbiae* [KF366417]*;* Ap, *Acyrthosiphon pisum* [NP_239860] *;* Mp, *Myzus persicae* [AF003957]) and GroEL from *Escherichia coli* (*E. coli;* AAL55999). Black and grey shades indicate identical and highly conserved amino acids, respectively. *Buchnera* GroEL sequences from these aphids have 98-99% amino acid sequence identity. Sequences were aligned using GeneDoc 2.7.0.

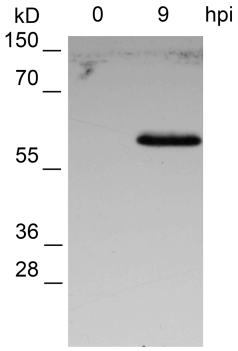


Fig. S3. GroEL is expressed by *Pseudomonas fluorescens (Pfo)*. Five-week-old tomato plants were infiltrated with 1 x 10^9 cfu ml^{-1} of *Pfo*+T3SS expressing GroEL::3xHA. Leaf samples were harvested at 0 and 9 hour post infiltration (hpi) for immunoblot analysis using anti-HA antibody. Samples were fractionated on 10% SDS-PAGE. Expected size of GroEL::3xHA is 60.6 kD.

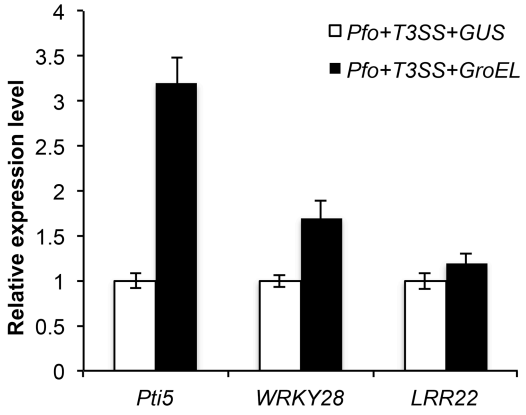


Fig. S4. *Pfo* expressing GroEL induces early-induced defense markers gene in tomato. Tomato plants were vacuum infiltrated with 1 x 10^9 cfu ml^{-1} of *Pfo*+T3SS+GUS or *Pfo*+T3SS+GroEL. Leaf samples were harvested 6 h post infiltration. Relative expression levels of defense marker genes were evaluated by qRT-PCR. Expression levels in samples infiltrated with *Pfo*+T3SS+GUS were designated as 1. Error bars represent $\pm$ SEM of six biological replicates and two technical replicates each.

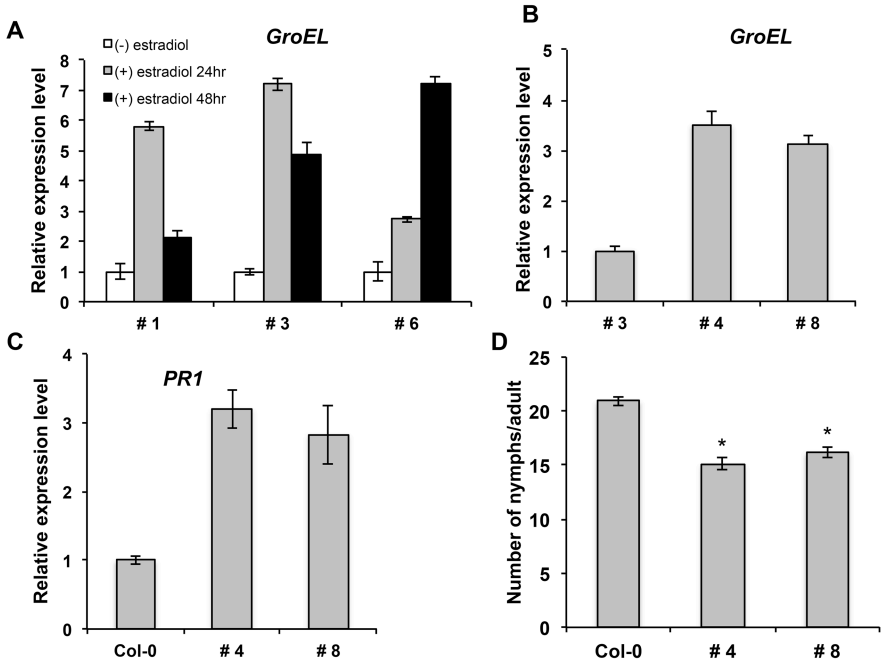


Fig. S5. Arabidopsis Col-0 transgenic lines expressing GroEL. *groEL* transcript levels were evaluated by qRT-PCR in (**A**) Arabidopsis transgenic lines (# 1, # 3 and # 6) expressing GroEL under the control of an estradiol-inducible *G1090::XVE* promoter and (**B**) in lines (# 3, # 4 and # 8) expressing GroEL constitutively. For inducible expression, transgenic plants were sprayed with 20 μM estradiol containing 0.02% silwet L-77 and harvested at the indicated time for expression analysis. (**C**) *PR-1* expression in Arabidopsis transgenic lines expressing GroEL constitutively. For expression analysis, error bars represent ± SEM of six biological replicates and two technical replicates. (**D**) Fecundity of green peach aphids on Arabidopsis transgenic lines expressing GroEL constitutively. Error bars represent ± SEM (n=30). * indicates significant differences (Student's *t*-test; $P < 0.05$).

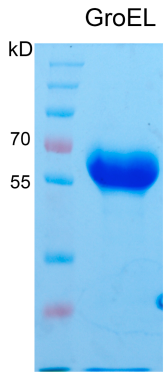


Fig. S6. SDS-PAGE of anion exchange purified recombinant His-GroEL protein expressed in *E. coli*. Twenty five µg of purified full-length 6xHis-GroEL protein was loaded onto 10% acrylamide gel and the gel was stained with Coomassie blue. Expected size of 6xHis-GroEL is 60.5 kD.

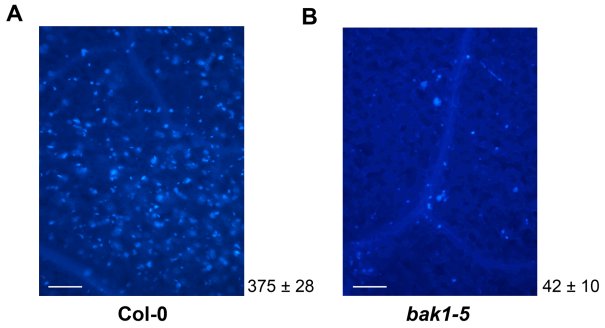


Fig. S7. GroEL induced callose deposition is *BAK1*-dependent. Callose deposition in the leaves of (**A**) Col-0 and (**B**) *bak1-5* plants. Arabidopsis Col-0 (**A**) and *bak1-5* (**B**) leaves were infiltrated with 1.5 µM GroEL and 24 h after infiltration leaves were stained with aniline blue and callose deposits were observed using an Olympus BX51 microscope, and images evaluated using ImageJ. The average numbers of callose deposits per field of view (0.1 mm^2) are displayed on right side of the image ± SEM (n=16). Scale bar = 100 µm.