Supporting Information

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SI Methods

Microscopic Observation. Phloem exudation was observed by bright-field and fluorescence stereo microscopy (MZFLIII; Leica). To increase working distance below objectives, the microscope head was mounted on a metal stand, below which potted plants were placed. To allow for immediate observation, focus was preadjusted before the stem or petiole was cut transversely with a clean scalpel. There was no noticeable difference in exudation pattern from upper and lower faces of the cut stem. For continued observation of exudation, it was necessary to remove further petiole or stem slices of ~1–10 mm when the exudation became slow or stopped. This is the normal procedure used for such observations. Illumination was enhanced with fiber-optic light sources (KL1500; Leica), adjusted for maximum contrast to facilitate identification of phloem tissues.

The detailed phloem structure was observed with alkaline decolorized aniline blue (DAB) staining of callose using a fluorescence microscope (BX41; Olympus) under UV excitation with UV filter cube (U-M41012, migrated from Olympus AX70 microscopy system: excitation filter, 330–385 nm; barrier filter, 420 nm). DAB was prepared as 0.01–0.5% wt/vol solutions (pH 8.5) by dissolving its ammonium salt (Sigma) in 10 mM Na_2HPO_4 buffer. Free-hand sections were immersed in DAB solution for ~1 min and mounted in water for observation For cut petiole and stem surfaces, DAB-wetted tissue paper was used to gently touch the surface, followed by observation under UV with UV filter set.

For the phloem tracer experiments, carboxyfluorescein-5(6) [CF-5(6)] was prepared according to ref. 1 and used according to ref. 2. We used CF-5(6) in preference to the diacetate form because there was no noticeable difference in phloem exudate or phloem tissue labeling patterns between the two forms and because the former was more cost-effective in terms of the large amount necessary to replicate the experiments. CF-5(6) was applied to the leaf surface for 1–2 h before plants were transported to the microscopy laboratory for observation. CF-5(6) was applied to stem or petiole surfaces for an overnight incubation before microscopy analysis, which is when CF-5(6) signals in exudates were strong enough for photographing. A GFP filter set (excitation filter, 480 ± 20 nm; barrier filter, >510 nm) was used to visualize CF-5(6).

Metabolite Analysis. ***Sampling.*** For metabolite profiling and measurement, phloem exudates were collected from fresh tissue, and dissected phloem tissues from freeze-dried, fully developed stem vascular bundles.

Phloem exudates were collected according to ref. 3. Immediately after de-topping of plants, the earliest initial exudate was removed by clean tissue paper to avoid possible contamination from either broken cells or unknown sources. The subsequent exudate was collected by pipette and immediately transferred to microcentrifuge tubes containing extraction solvent for phloem exudates as described below.

To sample phloem tissues, stem segments were cut from fully elongated, thick basal internodes and immediately frozen in liquid nitrogen, then freeze-dried (Christ Alpha Loc-1m) for 3 d and stored at –20 °C in plastic bags filled with nitrogen. The plastic bag was placed in a desiccator with silica gel desiccant until use. Before extraction, the entire desiccator was taken out of –20 °C, filled with nitrogen, and warmed to room temperature before opening of the plastic bag. Because the dissection procedure is lengthy, the stem segments were kept in other smaller desiccators in the microscopy lab until immediately before dissection.

Dissection. Double-layered facemasks were worn during the dissecting process to avoid introduction of breath moisture to the desiccated phloem tissues being dissected. Dried stems were cut to 1- to 2-cm lengths, then sliced longitudinally to leave one or two vascular bundles intact for further dissection. The microscope stage was covered with clean wax-paper backing from Parafilm, which facilitated the identification of any contaminating fibers and kept the workspace clean. The following dissection procedure required extreme care at all stages: (*i*) each cut was done with a new clean razor blade or new blade position; (*ii*) any area that contained visible fibers on the wax-paper backing was removed immediately (or vascular bundles being dissected were removed) and the wax-paper backing was replaced; and (*iii*) clean syringe needles and small forceps were used for handling vascular bundles and dissected phloem tissues.

The square shape of the external phloem facilitates cutting down of its four longitudinal sides along its borders to leave only the external fascicular phloem region. Note that it is difficult to obtain perfect cuts in a controlled manner and that imperfect cuts invading adjacent tissues resulted in samples that were not useful for further dissection and therefore discarded. Unsatisfactory, imprecise dissections were discerned by the different colors, shapes, and sizes of cells of the fascicular phloem. Mature (secondary) sieve elements in the fascicular phloem are large and readily identified among surrounding cells without staining. The white plug material on sieve plates and white crystal material in sieve elements are also good indicators. After successful dissection, both ends of the dissected fascicular phloem were recut to remove any potential contamination acquired during the original cutting of the stem segments. The dissected fascicular phloem tissues were photographed (along the length and from both ends) for subsequent volume estimation (see below). Samples were then transferred to individual microcentrifuge tubes and stored immediately in liquid nitrogen. Both removed ends were then stained with DAB solution for visualizing possible contamination from an unsuccessful cut and discarded after observation and photographing. If contamination was detected, the sample was discarded.

Xylem tissue and internal fascicular phloem dissection was also performed similarly. However, the latter is mostly curved shaped and more fragile due to its smaller size, which greatly reduced the success rate, such that data from only two internal fascicular phloem samples are reported here.

Estimation of dissected tissue volumes and phloem sieve element percentage. The volume of dissected tissues and the percentage by volume of sieve elements in dissected phloem tissues were estimated by analyzing microscopic images of dissected tissues with MetaMorph offline (version 4.6r9; Universal Imaging). The areas of both ends were estimated by MetaMorph software. Because dissected tissues are close to an elongated cuboid shape, the length of the dissected tissue was multiplied by the average area of both ends to obtain the estimated tissue volume.

To estimate the proportion of sieve elements in dissected fascicular phloem tissues, freeze-dried, undissected stem segments were stained with DAB and photographed by UV microscopy. Sieve element regions were selected and their area calculated by MetaMorph software. The ratio of sieve element area to the total area for counting sieve elements was used to estimate the volume ratio of sieve elements in the total fascicular phloem tissue. Approximately 45% of the total cross-sectional

area of the fascicular phloem comprised sieve elements and companion cells (SE/CCC).

Analytical procedure for metabolite analysis. Metabolite profiling and identification from both phloem exudates and dissected tissues were done according to refs. 3–5. For metabolite extraction from exudate, 1 μL of phloem exudate was extracted with water and chloroform (400:300 μL) and vortexed briefly. After centrifugation at 15,800 × *g* for 2 min, the polar (upper) phase was aliquoted into two parts, one for GC/MS and the other for LC/MS analysis. Metabolite extraction from dissected tissues was processed as described in ref. 6, except that homogenization steps were omitted. To avoid possible sample loss during processing, sample presence in the microcentrifuge tube was visibly confirmed both before and after adding extraction solvents. Aliquots for GC/MS were lyophilized and processed exactly as described in ref. 3. LC-MS analysis was performed as in ref. 4.

Protein Composition Analysis. ***Sampling.*** For protein analysis, phloem exudates were collected into empty polypropylene centrifuge tubes and stored at −80 °C. Ten millimolar DTT was added to inhibit gelling, which otherwise impairs resampling. One to 2 μL of phloem exudate was used for gel analysis. Approximately 50–100 μg of phloem exudate proteins was loaded onto the gels.

For dissecting phloem tissues, frozen stems stored at −80 °C were partially thawed at 4 °C. The vascular bundle was incised through the xylem into two parts. The xylem was then removed and the external phloem strands were stripped out by forceps. Phloem strands of up to a few centimeters from a single vascular bundle can be sampled this way. For internal phloem tissues, xylem was first removed and then visible pith, as judged by the naked eye, was removed by razor blade. Because these samples were for control purposes only, we did not further improve this technique for obtaining a larger amount of pure phloem tissue, as the needle-tip sampling approach (described below) proved more practical. Two to three dissected phloem vascular bundles (2–3 cm in length) were used for each protein extraction.

To sample relatively pure phloem proteins by needle-tip, frozen stems were partially thawed at 4 °C and cut transversely with a razor blade into segments a few millimeters in thickness. Segments were immediately immersed for 1–5 min in ice-cold 0.1% (wt/vol) amido black in 10 mM EDTA (pH ∼6.8) for visualizing the phloem proteins, washed briefly with 10 mM EDTA (pH 6.8), and stored on ice during dissection.

Under the stereomicroscope, the stained fascicular phloem proteins in large sieve elements were readily visible as blue strands of phloem protein aggregate in the longitudinal sections. Because the staining procedure does not use fixatives, the aggregates retained their elasticity and stickiness and could thus be readily picked up with syringe needle-tips for sampling. The needle-tip was then dipped into a water solution containing ice-cold 10 mM EDTA (pH 8.0) in a microcentrifuge tube. Fascicular phloem proteins from ∼20 stem sections were sampled in this way and pooled as one sample (of 50–90 μg).

Analytical procedure for protein analysis. For proteins from dissected fascicular phloem tissues, samples were frozen in liquid nitrogen, homogenized to a fine powder and then extracted by phenol (7). Protein samples collected from fascicular phloem tissue by needle-tip were processed either by direct acetone precipitation or phenol extraction (7). Phloem exudate protein samples were processed in the same way in parallel. Extracted proteins were analyzed by 1D SDS/PAGE according to ref. 8 or 2D gel electrophoresis according to standard procedures. For 1D gel analysis, 0.75- to 1-cm-thick 10% SDS/PAGE was used. Replicates of samples for 1D gel analysis were as follows: (*i*) needle-dissected fascicular phloem aggregates, four replicates; (*ii*) Total external phloem tissues: four replicates; (*iii*) phloem exudates: three replicates; (*iv*) total internal fascicular phloem tissues, two replicates.

For 2D gels, samples were suspended in 100 μL of sample buffer and all (50–90 μg) was loaded onto a pH 3–10 nonlinear strip for IEF. Eight percent to 16% gradient gels were used for the second dimensional separation by SDS/PAGE (refer to ref. 9 for detailed procedure on 2D gel analysis including IEF focusing, SDS/PAGE, gel staining and imaging, spot picking, and trypsin digestion). Protein bands or spots from the needle-dissected samples were excised from the gels, digested with trypsin according to standard procedures, then analyzed by nano-LC-ESI-MS/MS. Two replicates were performed for 2D gel analysis for sampled fascicular phloem aggregates and phloem exudates.

One replicate of needle-dissected fascicular phloem aggregates was used for direct trypsin digest without gel electrophoresis separation. The trypsin digest of needle-dissected fascicular phloem aggregates was loaded onto a nano-LC-ESI-MS/MS.

Nano-LC was performed with an Eksigent 2D nano-LC system equipped with a Dionex C18 PepMap100 column (75 μm i.d.) flowing at 180 nL/min. Peptides (5-μL injections) were resolved on a gradient that started at 95% solvent A (5% acetonitrile, 0.1% formic acid) and 5% solvent B (95% acetonitrile, 0.075% formic acid in Milli-Q water) for 3 min, then increasing from 5 to 25% B over 5 min, from 25 to 60% B over the next 32 min, and from 60 to 95% B over the final 5 min. Mass spectrometric analysis was performed on an ABI QSTAR XL (Applied Biosystems/MDS Sciex) hybrid QTOF MS/MS mass spectrometer equipped with a nanoelectrospray source (Protana XYZ manipulator). Positive-mode nanoelectrospray was generated from fused-silica PicoTip emitters with a 10-μm aperture (New Objective) at 2.5 kV. The *m*/*z* response of the instrument was calibrated daily with manufacturer standards. TOF mass and product ion spectra were acquired using information-dependent data acquisition (IDA) in Analyst QS v1.1 with the following parameters: mass ranges for TOF MS and MS/MS were *m*/*z* 300–2,000 and 70–2,000, respectively. Every second, a TOF MS precursor ion spectrum was accumulated, followed by three product ion spectra, each for 3 s. The switching from TOF MS to MS/MS is triggered by the mass range of peptides (*m*/*z* 300–2,000), precursor charge state (2–4), and ion intensity (>50 counts). The DP, DP2, and FP settings were 60, 10, and 230, respectively, and rolling collision energy was used.

Mascot database searching was according to standard procedures (9), and de novo sequencing was conducted with methods reported in ref. 8. Two databases were used for initial database searches: (*i*) nonredundant protein sequence database and (*ii*) EST and Unigene sequences from cucumber (version 1), melon (version 1), and watermelon (version 1) (www.icugi.org). Peptides were also TBLASTN searched against the cucumber genome (10) and BLASTP searched against the pumpkin sap proteome (11). Multiple sequence alignments were generated by ClustalW2 (12).

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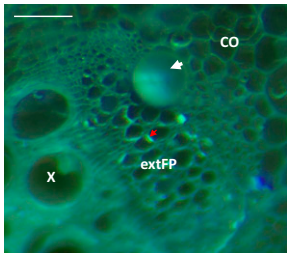

Fig. S1. Confirming location of fascicular phloem relative to sites of exudation with decolorized aniline blue staining UV fluorescence image shows part of the vascular structure after DAB staining during direct observation of phloem exudation. Callose deposits on the walls of sieve tubes in external central phloem can be clearly observed (red arrow). Arrowhead: one droplet of exudate was still formed from extrafascicular phloem, even after staining. (Scale bar: 200 μm.) CO, cortex; extFP, external fascicular phloem; X, xylem.

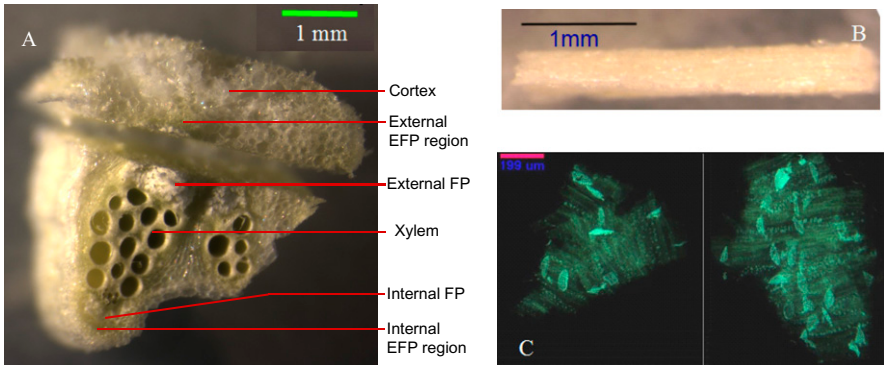

Fig. S2. Dissected pumpkin fascicular phloem tissues used for metabolite analysis. An example of the procedure used to dissect lyophilized central phloem tissue from vascular bundles. (*A*) Lyophilized stem segments were dissected under stereo microscope with sharp razor blades. (*B*) Dissected fascicular phloem tissues. (*C*) Purity was confirmed with decolorized aniline blue staining of both ends of dissected tissues. Stained callose deposits indicate sieve plates and pore-plasmodesmal units. EFP, extrafascicular phloem; FP, fascicular phloem.

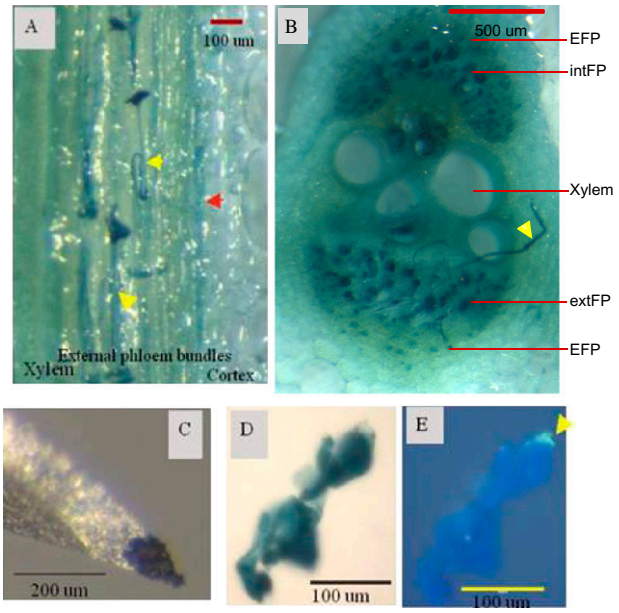

Fig. S3. Sampling fascicular phloem proteins by needle-tips. (*A*) Longitudinal free-hand sections of living stem vascular bundles, stained with amido black 10R for proteins. Red arrow, stained proteins in extrafascicular phloem; yellow arrows, protein strands from fascicular phloem, which were collected on needle-tips. (*B*) Stem cross-sections (for abbreviations see Fig. S2). Yellow arrow, FP proteins stretched by needle-tip. (*C*) Needle-tip with attached amido-black-stained fascicular phloem proteins (dark blue). (*D*) Sampled stained proteins under light microscope. (*E*) Sampled proteins under epifluorescent microscope. Yellow arrow, sieve plates with callose staining. intFP, internal fascicular phloem; extFP, external fascicular phloem.

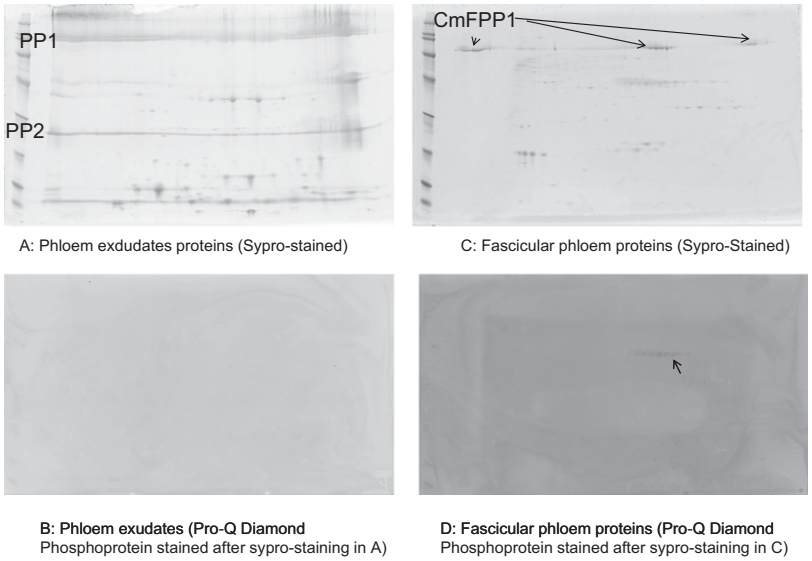

Fig. S4. 2D PAGE of phloem exudates and phospho-stained fascicular phloem proteins on 2D gel from *Cucurbita maxima*. (*A*) Under standard 2D PAGE electrophoresis conditions, the abundant PP1 and PP2 proteins appear as horizontal smear lines along IEF dimension (as indicated). However, smearing does not affect the identification of these proteins. Analysis conditions for proteins from phloem exudates were identical to those used for dissected fascicular phloem proteins shown in Fig. 4. (*B*) Subsequent phospho-protein staining of the same gel in *A* by Pro-Q Diamond to visualize possible phosphorylated proteins in exudate protein extracts. No phosphorylated proteins from phloem exudates were detected. (*C*) 2D PAGE of FP proteins (fascicular phloem proteins) visualized by Sypro-staining. CmFPP1 appears as horizontal smear lines along IEF dimension (as indicated). (*D*) Subsequent phospho-protein staining of the same gel in *C* by Pro-Q Diamond revealed one group of phosphorylated proteins from fascicular phloem (as indicated). IEF, pI 3–10 nonlinear; SDS/PAGE, 8–16%; MW markers, 200, 116, 97, 66, 45, 31, 21, 14, and 6 kDa.

Table S1. Summary of design, experimental evidence, and conclusions for all exudation and phloem tracer experiments, examining the origins of phloem exudation in cucurbits

Experiment	Figure	CF(5)6 feeding site	Cutting site	Cartoon [yellow = fed CF(5)6; — = cut site]	Labeling clearly visible in			Conclusion
					FP	EFP	Exudate	
1	Fig. 1 (and Movie S1)	—	Stem		n/a	n/a	n/a	Exudate is from EFP
2	Fig. 2 *A* and *B*	Leaf blade	Petiole		✓	✓	✓	FP and EFP can both transport CF
3	Fig. 2*C*	Leaf blade	Stem		✓	X	X	Exudate is from EFP
4	Fig. 2 *D–F*	Petiole	Petiole		X	✓	✓	Exudate is not from FP

In addition to video record (experiment 1), three different types of experiment were conducted using CF5(6) tracer (experiments 2–4).

Table S2. CLUSTAL alignment of partial sequences from cucumber genome and pumpkin proteome with peptides identified from CmFPP1

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FG227020_2_56kD     YCTDELK-REDNYKIVWIPMINSQGFDQE–SR 106
Cs_contig13022      EELK-REDNYKIVWIPVMNSEAFNEE–SH 27
Cs_contig12984      NEVK-REDKYKIVWIPVITVETEDEEEEAR 29
Cs_contig12982      DELG-REDKYKIVWIPIINPNEPYEE–NR 27
Cs_contig13025      HVYNELK-RDNKYEIVWIPIIPERYLEED—R 29
FG227702_3_81kD     NVYDELKNRGTNYEIVWIPIIPEPYHEDD—H 357
Cs_contig13028      EELK-RDNRYEIVWIPIIPEPYQEED—R 26
                    *: * * *****:::
FG227020_2_56kD     KRYELVRSTMKWYAVQYTTKIAGLRFLEEIWQVREDALMVVLDSKSKVKFSNAIHLLRVW 166
Cs_contig13022      KRYENLRSTMKWYAVQYGTKIAGLRFLEEIWQLRDDALMVVLDSKSKLKFSNAIHLLRVW 87
Cs_contig12984      KKYEYASSLMKWYIVPYTRKIAGWRYLEENWQLRQDPLIVVMNSKSRVEFNNAIHLIRVW 89
Cs_contig12982      RRYEYVISKMPWYIVQFTTKIAGWRFLEENWQLRDDPLVVVLDSTSKVEFTNAIHLIRVW 87
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Cs_contig13025      RRYEYLRSTMKWYSIQFTTRIAGMRYIEEKWQFREDPLVVVLNPQSKVEFTNAIHLIRVW 89
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Cs_contig13028      KRYEYLRSTMKWYSVEFTTKISGMRYIEEKWQLREDPLVVVLNPQSKVEFMNAIHLIRVW 86
                    ::** * * **:::::* *::** **.*:*.*:**:.. *.: * *****:***
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Cs_contig13022      GNNAIPFTLERANALLRKNWPESTIVKFTNQPRLQSWIID————————————————————Q 128
Cs_contig12984      GIDAIPFTNGRTNALLAKNWPESTLFKFIDQPRLMNWVSHV————————————————NQ 132
Cs_contig12982      GSEVVPFSNRKIDILLEKTWPESTILKFTDHPRVHNWVSK————————————————NQ 129
FG227362_3_45kD     GTEAIPFTHNRTEILLRKHWPESTLVKFTHQPRLLSW————————————————FNQ 97
Cs_contig13025      GTEAIPFTHNRTDYLLRKHWPESTLVKFTHQPRLLSWVRILFNMTRAPLFDNHFVLGFNQ 149
FG227702_3_81kD     GTEAIDFKEDRAKFLLRKNWPDSTLVKFTHQPRLQSWIK————————————————————Q 457
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Cs_contig12984      ERNIIFYGGKEPKWIQQFEDRIVEIKNDPYLKEKGNTFEIIRVGQNIK—GDSNDFTLT 189
Cs_contig12982      EKNIIFYGGKDPNWIQQFEEKVIDIKTDPWIRSKGITFEIVRIIRDD————DPKLM 181
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FG227702_3_81kD     EKSILFYGGKEPMWIQQFEERVEILKSDPLIRD-GGSFEIVRIGKNAK——GEDDPALM 512
Cs_contig13028      DKNILFYGGKDPLWIQQFEERAEILRSDPLIMD-GGSFEIVRIGKDAI——GQDDPALM 186
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Cs_contig12984      GVLQDFNKWKRNMNIKAFPDAFRDYFNELNLNFHICERMTLPGFSGWIPMIVNCPECPRF 309
Cs_contig12982      AVLQDFNKWKQILNIKSFPDSFRDYFNELALKTHQCDRVVLPGFSGWIPMVVNCPECPRF 301
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Cs_contig13025      RLLEDFPKWKQTLRLKGFPDAFREYFNELAAKNHQCDRVILPGFSGWIPMIVNCPECPRF 324
FG227702_3_81kD     KLLEEFPKWKQSLRLKAFPDAFREYFNELALKSHQCDRVILPGFSGWIPMIVNCPECPRF 632
Cs_contig13028      KLLEDFPKWKQNLRIKAFPDVFREYFNELAFSSHQCDRVILPGFSGWIPMIVNCPECPRF 306
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FG227020_2_56kD     METGINFNCCHGR 394
Cs_contig13022      METGINFNCCHGR 321
Cs_contig12984      METGISFKCNHGR 322
Cs_contig12982      MDTGISFKCCHGR 314
FG227362_3_45kD     METGISFKCCHGR 285
Cs_contig13025      METGISFKCCHG- 336
FG227702_3_81kD     METGISFKCCHGG 645
Cs_contig13028      METGISFKCCHG- 318
                    *:***.*:* **
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Cs_contigxxxxx cucumber sequences are from NCBI Genome Project ID 33619 by the Cucumber Genome Initiative [Huang S, et al. (2009) *Nat Genet* 41:1275–1281]. FGxxxxx_x sequences are from pumpkin sap proteome accessions with xxkD suffix indicating predicted molecular weight [Lin MK, et al. (2009) *Mol Cell Proteomics* 8:343–356]. Sequences in underlined red are peptides identified from CmFPP1 matched onto closest homologous sequence, FG227702_3_81kD. Shorter homologous sequences (not shown on alignment) were also found from watermelon and melon (accession nos. WMN55A06 and MU9468, respectively, at www.icugi.org).

Movie S1. Record of phloem exudation process in pumpkin. This movie is also available online at www.danforthcenter.org/cmaxima. Selected still images from the movie are shown in Fig. 1.

Movie S1