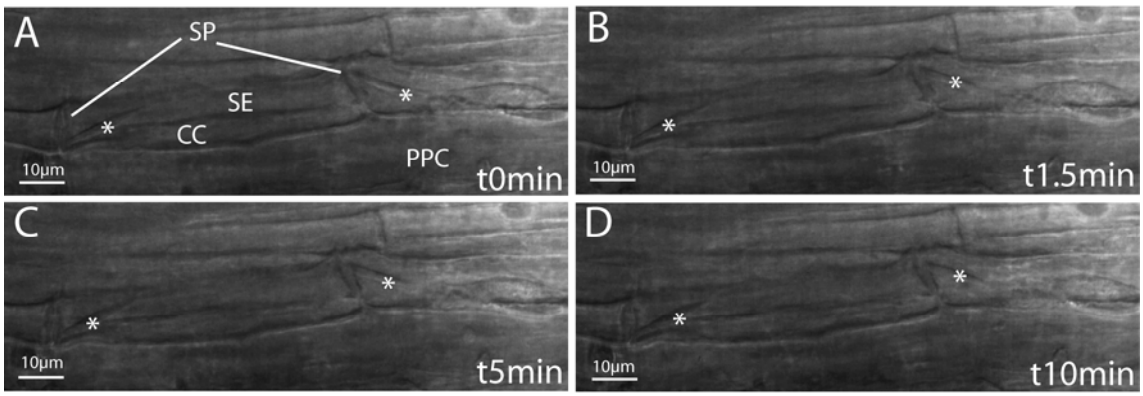
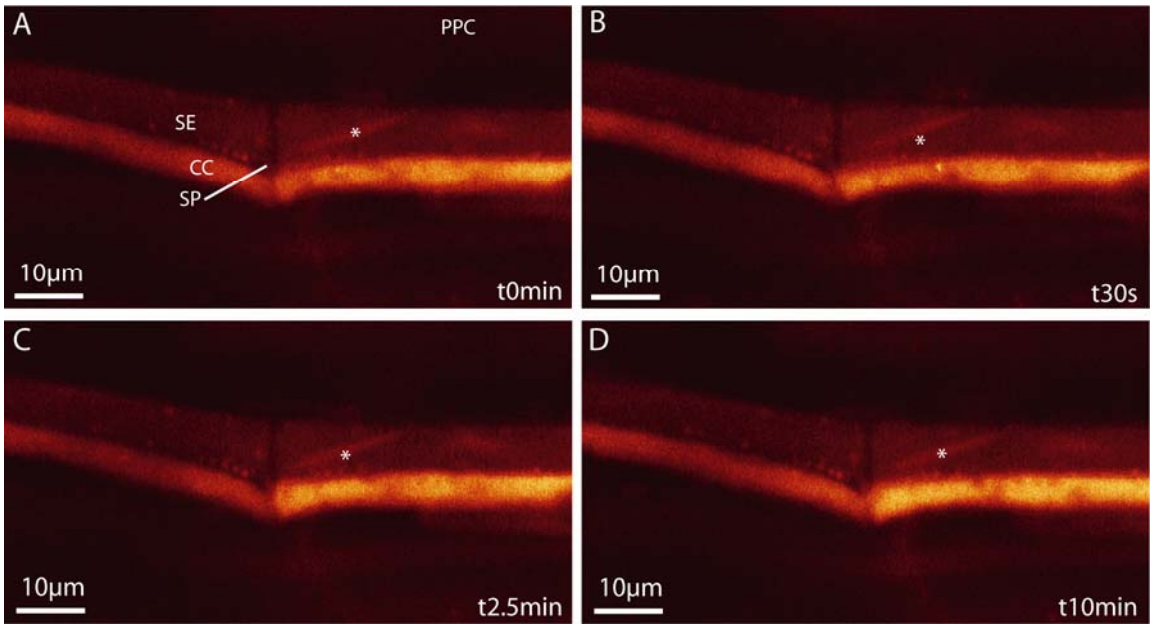
Supplemental Data. Furch et al. (2009). Sieve-element Ca^{2+} channels as relay stations between remote stimuli and sieve-tube occlusion in *Vicia faba*.



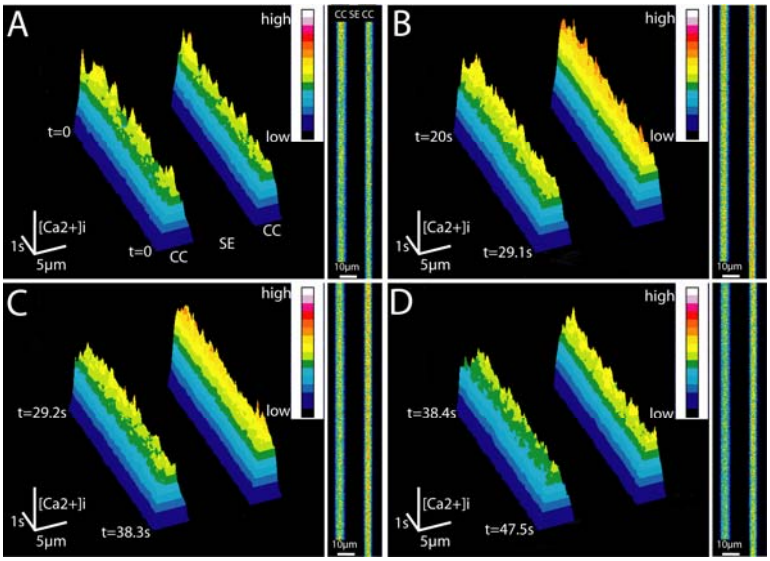
Supplemental Figure 1. The effect of the Ca^{2+}-channel blocker Gd^{3+} on the forisome reaction in phloem tissue.

Forisome reaction was observed microscopically during the passage of an electropotential wave in response to a remote burning stimulus, in the presence of 2 mM Gd^{3+}. Forisome conformation before (**A**) and after a burning stimulus (**B**-**D**). The time after stimulus application is indicated in each photomicrograph. The cell-impermeant Ca^{2+}-channel blocker abolished the forisome (marked with an asterisk) dispersion. CC, companion cells; PPC, phloem parenchyma cell; SE, sieve element; SP, sieve plate.



Supplemental Figure 2. The effect of the Ca^{2+}-channel blocker verapamil on the forisome reaction in phloem tissue.

CLSM micrographs of *Vicia faba* phloem tissue, stained with the fluorescence dye CMEDA/CMFDA, to highlight the forisome (marked with an asterisk). The cell-permeant Ca^{2+}-channel blocker verapamil (100 µM) was sufficient to inhibit forisome dispersion in response to remote burning stimuli in intact phloem tissue. Forisome conformation before (**A**) and after a burning stimulus (**B**-**D**). The time after stimulus application is indicated in each photomicrograph. CC, companion cells; PPC, phloem parenchyma cell; SE, sieve element; SP, sieve plate.



Supplemental Figure 3. Rapid line scan measurements of Ca^{2+} transients.
Line scan measurements (right rectangle of each figure) recorded from an CC/SE/CC region in intact phloem tissue stained with the Ca^{2+}-dependent fluorescence dye Oregon Green. For clearness, line scans were converted into surface plots (left side of each figure) using Image J software. Ca^{2+} concentration (relative quantity) is coded by the height and the color of the surface. (**A**-**D**) Visualization of release of free Ca^{2+} triggered by a 100 mM KCl stimulus. (A) The resting Ca^{2+} level (scanned during 9.1s) before applying the stimulus. (**B**-**D**) A continuous scan between 20 and 47.5s after application of KCl to the apical window.

Supplemental Methods

Rapid line scan measurements of Ca^{2+} transients - To reach a higher temporal resolution of Ca^{2+} dynamics in case of distant KCl treatments, the line-scan mode of CLSM was used, e.g. a single line was chosen (17.6 msec per line) and repeatedly scanned with a frequency of 57 Hz. The scanned lines were sequentially plotted next to one another to construct a (*x*,*t*) linescan image. Typically 512 successive line-

scans were executed. These images contain spatial information in one dimension at a high temporal resolution (Bischofberger and Schild, 1995).

Fluorescent probes - The voltage-sensitive styryl dye RH-414 (-N-(3-triethylammoniumpropyl)-4-(4-(4-(diethylamino)phenyl)butadienyl)pyridiniumdibromide) was used to label the PM. A 5 mg ml^{-1} stock solution was prepared in dimethyl sulfoxide (DMSO). Working solutions were prepared as a 1:2000 dilution to give a final concentration of 4.3 µM RH-414 and applied as 100-500 µl droplets.

A mixture of CellTracker Yellow-Green CMEDA (5-chloromethyleosin-diacetate) and CellTracker Green CMFDA (5-chloromethylfluorescein-diacetate) was used to highlight forisomes in SEs. Stock solutions of 7.8 mg ml^{-1} CMEDA and 4.65 mg ml^{-1} CMFDA were made up in DMSO. Immediately prior to use, 1 µl of each stock solution was mixed and diluted into 1 ml bathing medium.

To label Ca^{2+} channels with DM BODIPY dihydropyridine (fl-DHP), 25 µg of BODIPY DHP was dissolved in 5 µl DMSO and 495 µl bathing medium to give a stock solution. Immediately before use, 1 µl of the stock solution was diluted in 2 ml bathing medium to give a final concentration 0.036 µM (for Ca^{2+}-channel distribution). For fl-DHP binding studies, fl-DHP stock solution was diluted to various concentrations between 10 and 2000 nM using bathing medium. Binding affinity was obtained by measuring the averaged pixel density from the recorded fl-DHP images following background correction using Image J-software. Titration curve fitting was carried out using Microcal Origin 3.5 (Northampton, MA, USA).

To label the ER in SEs, ER-Tracker Green (glibenclamide BODIPY FL) and ER-Tracker Red (glibenclamide BODIPY TR) were used at a final concentration of 0.5 µM. Stock solutions were prepared by dissolving 100 µg of ER-Tracker Green in 128 µl DMSO and 100 µg of ER-Tracker Red in 110 µl DMSO. The stock solutions were diluted 1:2000 with bathing medium before incubating the tissue for at least 1h. In double-staining studies, intact phloem tissue was pre-incubated with 1 mM Pluronic F-127 (Molecular Probes, Eugene OR) to facilitate loading of ER-Tracker Red into the SE. To test unspecific binding of the fluorophore to phloem cells, tissue was saturated with 1 µM glibenclamide (dissolved in DMSO and diluted with bathing medium) 1h before application of the ER-Tracker to the exposed phloem tissue for 1h. Subsequent scanning by CLSM revealed no significant staining with ER-Tracker, indicating the respective receptor in the ER was saturated with unlabelled

glibenclamide. To co-localize Ca^{2+} channels with the ER, tissue was incubated for at least 1h with ER-Tracker Red. Successful staining of the phloem tissue by ER-Tracker Red was verified by CLSM, and the phloem tissue was then stained with DM-BODIPY dihydropyridine (fl-DHP) for 2h.
All fluorescent probes were purchased from Molecular Probes (Eugene, OR).

Isolation of SEPs - In brief, an upper internode from a 3-4 week old *Vicia faba* plant was excised and split by a tangential cut. For crude isolation of stem phloem strands comprising intact SE/CC complexes, tangential tissue slices with a thickness of ~300 µm were made with a razor blade from the cut face of the internode. The tissue slices were transferred into a wall digestion medium (Hafke et al., 2007) and incubated overnight at room temperature.

Electron microscopical preparation of phloem tissue - To obtain phloem tissue windows for slow fixation, cortical tissue was locally removed by manual paradermal slicing with a fresh razor blade, while avoiding damage to the phloem in intact plants. Large segments of about 5 cm length and 2 cm width containing the phloem window were excised under recovery medium (10 mM KCl, 10 mM $CaCl_2$, 5 mM NaCl) and remained submerged for 2h. At the start of fixation (during 9h at room temperature), the recovery medium was replaced by a 5% aldehyde solution. Following primary fixation, tissues were postfixed in 1% osmium tetroxide overnight, washed with H_2O, stained with 0.5% uranyl acetate, and then dehydrated in a graded ethanol series. Sections were cut with a diamond knife on a Reichert Om U2 ultramicrotome (Leica, Bensheim, Germany) and post-stained with 2% uranyl acetate and lead citrate (Reynolds, 1963).