Supplemental material 1. Short-range movement of GFP silencing affects a nearly constant number of cells and is triggered from a small number of cells.

To determine the number of cells affected by short distance movement of GFP silencing in line 16c, we performed microscopic analyses of individual sectors encompassing silenced tissues that surrounded several independent patches infiltrated with the GFP-GUS strain of Agrobacterium (Figure S1 A). Observations were under a Nikon eclipse E800 microscope. Phase contrast was used to image individual epidermal cells (Figure S1 C). Images were retrieved digitally with a Sony colour video camera an analysed using the Biocom Visiolab 200 software. These analyses involved at least 4 independent patches of similar diameter, generated on either young developing (sink) or old, fully developed (source) leaves. 14 independent sectors were chosen randomly on each patch. Epidermal cells with characteristic jigsaw puzzle shapes served as references in these observations (Figure S1 C-D). These analyses revealed that the number of silenced epidermal cells located at the edge of each GUS-stained area was remarkably constant (Figure S1 E). The same regularity was also observed between individual patches, regardless of the age of infiltrated leaves (Figure S1 E). Overall, an average of 13 cells (±2 cells) appeared to be consistently affected.
Further experiments showed that this number remained the same if the diameter of the patches was significantly reduced (< 1cm) or enlarged (> 5cm), suggesting that the short-range movement of GFP silencing at the edge of the patches originated mainly from the outmost cell layer. This was confirmed in subsequent experiments in which a cork borer was used to remove most of the inner tissues of infiltrated patches immediately after pressure-injection of the *Agrobacterium* cell suspension. The layer of intact cells remaining after this treatment was usually less than 10-15 cells in width. As shown in Figure S1 F, there was no difference in the extent of the red border (13±2 cells) developing around those patches and around normal infiltrated areas. We conclude that movement of GFP silencing over 10-15 cells can be triggered at the single cell level.

**Legend:**

(A) Close-up view of an Agro-infiltrated patch, as depicted in Figure 1J. (B) Microscopic observation of the square inlay in (A) (X20). (C) Enlarged view of the square inlay in (B) (X40). The outline of epidermal cells is revealed by phase contrast. (D) Schematic representation of the view in (C). G: guard cells; V: veins. The diagonal bar encompasses 12 epidermal cells. Thus, in addition to the two cells in (B) (double-head arrow), a total of 14 cells are affected by movement of silencing. Bar: 100 µm. (E) Individual silenced patches were generated either on sink or source leaves of line 16c. For each patch, 14 randomly chosen sectors (X axis) located at the edge of the patch were analysed at 10 dpi, as described in (A-D). For each sector, the number of epidermal cells affected by short-range movement of silencing was recorded (indicated in the Y axis). The green bar represents the average value. S: silenced tissue; NS: nonsilenced tissue. (F) Short-distance spread of silencing originates from the outmost cells of infiltrated area. The images show leaf of line 16c that had been infiltrated with the GFP *Agrobacterium* strain 8 days previously. The inside of the patch was
removed with a cork borer immediately after injection. The border of red fluorescent tissue is
clearly visible at the edge of the remaining tissue (arrow) and is similar in width to the border
developing on intact infiltrated patches. The left panel was observed under normal light and
the right panel under UV light. Bar: 1.5 cm.
Supplemental material 2. A phloem-restricted VIGS vector based on PVX.

To create PVX-GFP-Δ25, an AvrII-BstBI restriction fragment from plasmid pTXS-GFP-ΔApa/Apa (Voinnet et al., 2000) was mobilised into AvrII-BstBI-digested PVX209, a PUC19-based version of PVX-GFP. This introduced an in-frame deletion in the 25kDa ORF. A SacI fragment of the resulting plasmid (PVX209-Δ25) was then mobilised into SacI-linearized pBin19 (Bevan, 1984), leading to PVX-GFP-Δ25. Digestion of PVX-GFP-Δ25 with BglII and religation created a 1729bp deletion in the replicase ORF, leading to PVX-GFP-Δrep-Δ25. PVX-GF:RbcS:P-Δ25 was created by inserting a 500bp PCR-amplified fragment of the *N.benthamiana* RbcS cDNA (Jones et al., 1999) into the GFP insert of PVX-GFP-Δ25 (PmlI blunt restriction site).

Phloem restricted viruses such as SMYEV are experimentally transmitted to host plants *via* Agrobacterium–mediated inoculation of infectious DNA clones (Lamprecht and Jelkmann, 1997). Phloem companion cells are directly transformed with the corresponding T-DNA, from which virus replication and systemic spread proceed. The Agrobacterium remains confined at or near the site of inoculation. PVX-GFP-Δ25 was thus mobilised into the T-DNA of the pBin19 binary vector (see above), under the control of the 35S promoter (Figure 4A) and inoculated on plants by leaf injection of an Agrobacterium culture.

References:


**Supplemental material 3.** Transversal sections of a mature leafs from a GFP142/sde3 plant transformed with the atSUC2-GF-FG construct. Observations were made under a confocal microscope.

**Legend:**

The split channels in the two first left panels show GFP and chlorophyll fluorescence, respectively. The last panel is an overlay between these split channels. (A) The central section of the leaf is uniformly silenced for GFP. (B-C) Green fluorescence is mainly detected at the extreme tips of the leaf, in agreement with the overall silencing phenotype, as depicted in Figure 6C-D.
**Supplemental material 4.** Effect of silencing suppressor proteins on the onset of long-distance, as opposed to short-range movement of GFP silencing

**Legend:**

Extensive silencing was monitored in systemic tissues of 16c plants that had received one of the treatments depicted in Figure 2A. The values are at 7dpi and correspond to three independent experiments involving 10 plants each.