



Yellow fluorescent protein was excited with 488 nm laser at the power of 24 %. Red fluorescent protein was excited with 561nm laser at the power of 1.5 %. (A) Raw image displayed with uncompressed intensity levels (B) Numerical compression of the intensity levels facilitates the visualization of the graft interface. Scale bars: 50 μ m



Supplemental Figure S2. Emission spectra of chlorophyll, mRFP and YFP in HM20 section of a graft interface.

When illuminated with 488 and 561 nm wavelengths, chlorophyll emission spectra were shifted toward the mRPF emission spectrum (*A*) Fluorescence emission spectra of mRFP (magenta), YFP (green) and chlorophyll (blue) illuminated with 488 and 561 nm lines (*B*) Confocal, (*C*) Correlated, and (*D*) TEM imaging allows the identification of chloroplasts. Dotted lines label the interface. White arrows show plastids. sc: scion, rt: rootstock. Scale bars: $5 \mu m$.



Supplemental Figure S3. Correlative Light Electron Microscopy as a tool to investigate the ultrastructure of the graft interface

The scion and the rootstock are respectively at the top and bottom positions (A, B). (A) Confocal acquisition of sieve elements (SE) and companion cells (CC) aligned at the interface, its corresponding stitched images with of several transmission electron micrographs and correlated light electron microscopy view. Stitching was performed with the photomerge function of Adobe Photoshop. (B) Confocal acquisition of xylem tracheary element (TE) properly aligned at the graft interface, its corresponding transmission electron microscopy view. White arrowheads denote "natural landmarks" i.e. plastids and nuclei, that were used to correlate confocal and transmission electron microscopy images. Images were rotated with respect to the graft orientation and peripheral empty regions were filled by default with black pixels. Scale bars: (A) 10 μ m, (B) 5 μ m



Supplemental Figure S4. Box plots of the thickness of the cell wall near to the plasmodesmata found at the graft interface of *Arabidopsis thaliana* by class of plasmodesmata.

Box plots showing the median 10^{th} , 25^{th} , 75^{th} and 90^{th} percentiles and outliers. Letters indicate the results of ANOVA on ranks and Dunn's tests (p-value < 0.001).



Supplemental Figure S5. Correlative light electron microscopy (CLEM) reveals cell wall gap through which organelle is moving from cell-to-cell close to the graft interface at three days after grafting

The scion and the rootstock are at the top and bottom positions respectively. CLEM reveals the presence of aligned bicolored cells (white asterisks) at the graft interface. (*A*-*C*) Overview of the graft interface. (*D*-*E*) higher magnifications of a white square in (A-C). (*E*) Magnification of a plastid crossing a cell wall opening (black arrowheads). Electron micrographs were the results of stitched images with the photomerge function of Adobe Photoshop. Images were rotated with respect to the graft orientation and peripheral empty regions were filled by default with black pixels. Scale bars: (A, B, C,) 20 μ m, (D, E) 5 μ m, (F) 2 μ m



Supplemental Figure S6. Bicolored cells at the graft interface of Arabidopsis thaliana 35S::HDEL_YFP (scion) on 35S::HDEL_mRFP (rootstock) three days after grafting

The scion and the rootstock are at the top and bottom positions respectively. (A) Overview of a 150 nm-thick section of a graft interface. (B) Magnification of the bicolored cell indicated by a white square in image (A). (C) The split YFP channel of (B). (D) The split RFP channel of (B). (E) Stitched tiles of transmission electron micrographs of the same cell. (F) CLEM on the same cell. Electron micrographs were the results of stitched images with the photomerge function of Adobe Photoshop. (G) Magnification of the correlated bicolored region. (H) The same cell is observed by TEM on following section. (I-J) CLEM on this cell shows the presence of a RFP-labelled nucleus. (K) Electron tomogram of the YFP- labelled nucleus observed in E-G. *(L)* Electron **RFP-labelled** tomogram of the nucleus observed reveals the nuclear envelope in H-I (black arrows). Images were rotated with respect to the graft orientation. For TEM images, peripheral empty regions were filled by default with black pixels. Scale bars: (A) 20 μ m, (B, C, D, E, F, H, I) 5 μm, (G, J) 2 μm, (K, L) 1 μm