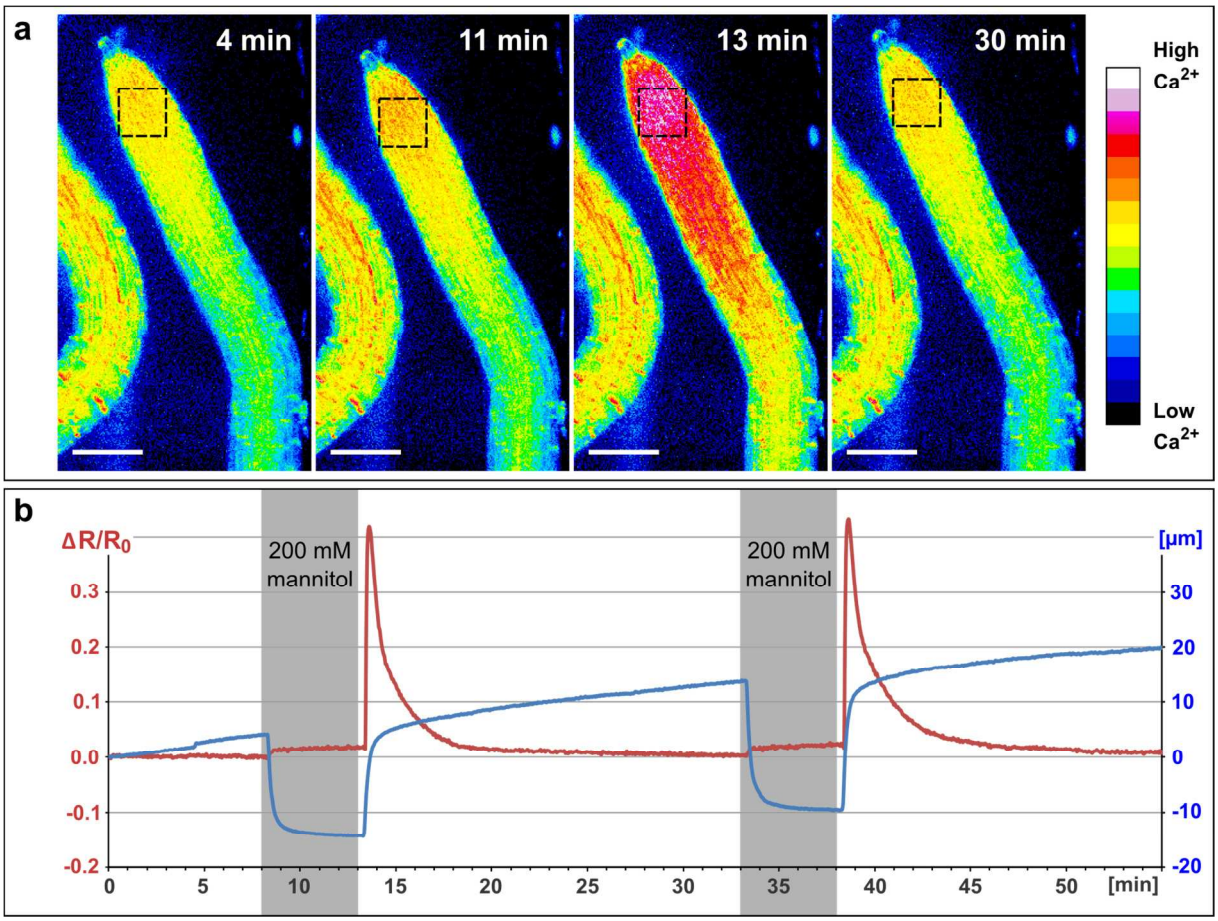
Supplementary Figures



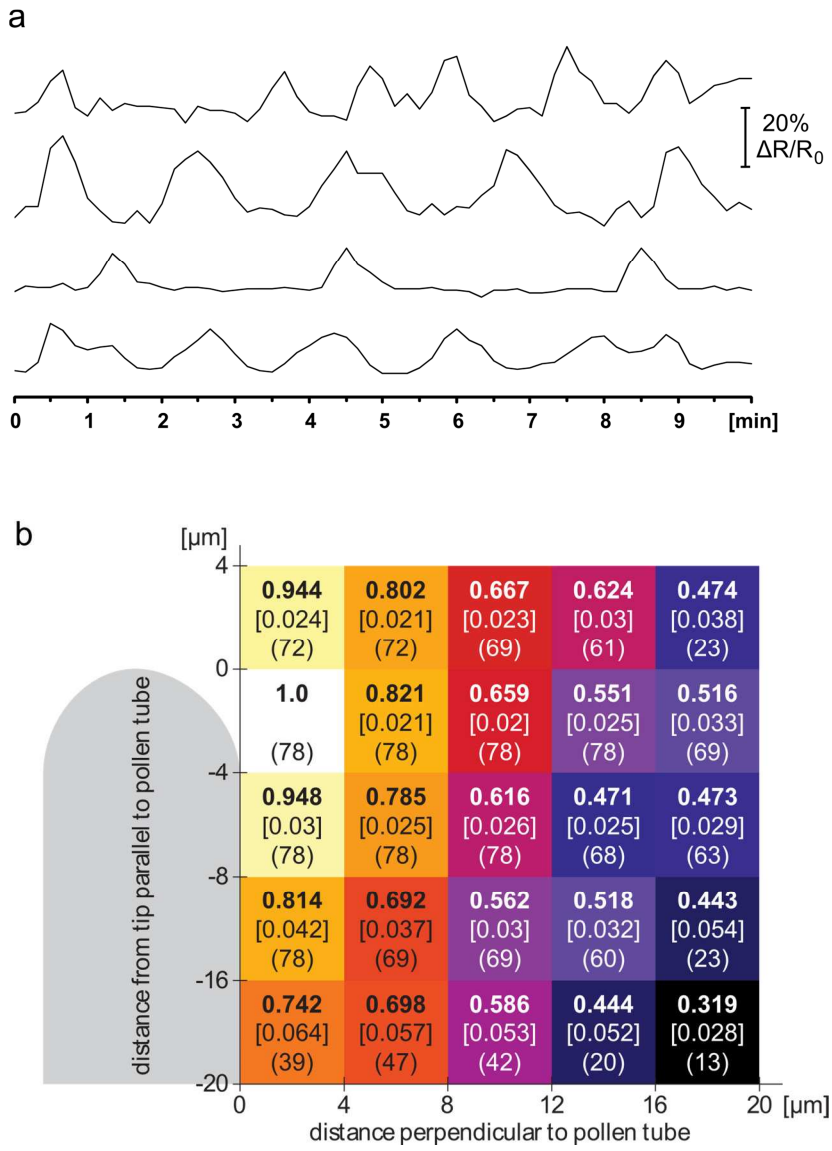
Supplementary Figure 1: Characterization of CerTN-L15 expressed in Arabidopsis roots.

a. Ratiometric images of CerTN-L15 in roots under osmotic stress

Ratiometric images show representative time points before (4 min), during (11 min), immediately after (13 min) and at 30 min after treatment with 200 mM mannitol of a root expressing p35S::CerTN-L15. The dotted square marks the region used for measurement shown in b. Scale bars: 100 µm.

b. Ratio changes of CerTN-L15 and growth behaviour in roots under osmotic stress

Normalized ratio change of CerTN-L15 in the root tip (red) and root length (blue) during two repeated osmotic stress treatments (experiment shown in a). Grey regions show the time of perfusion with 200 mM mannitol.



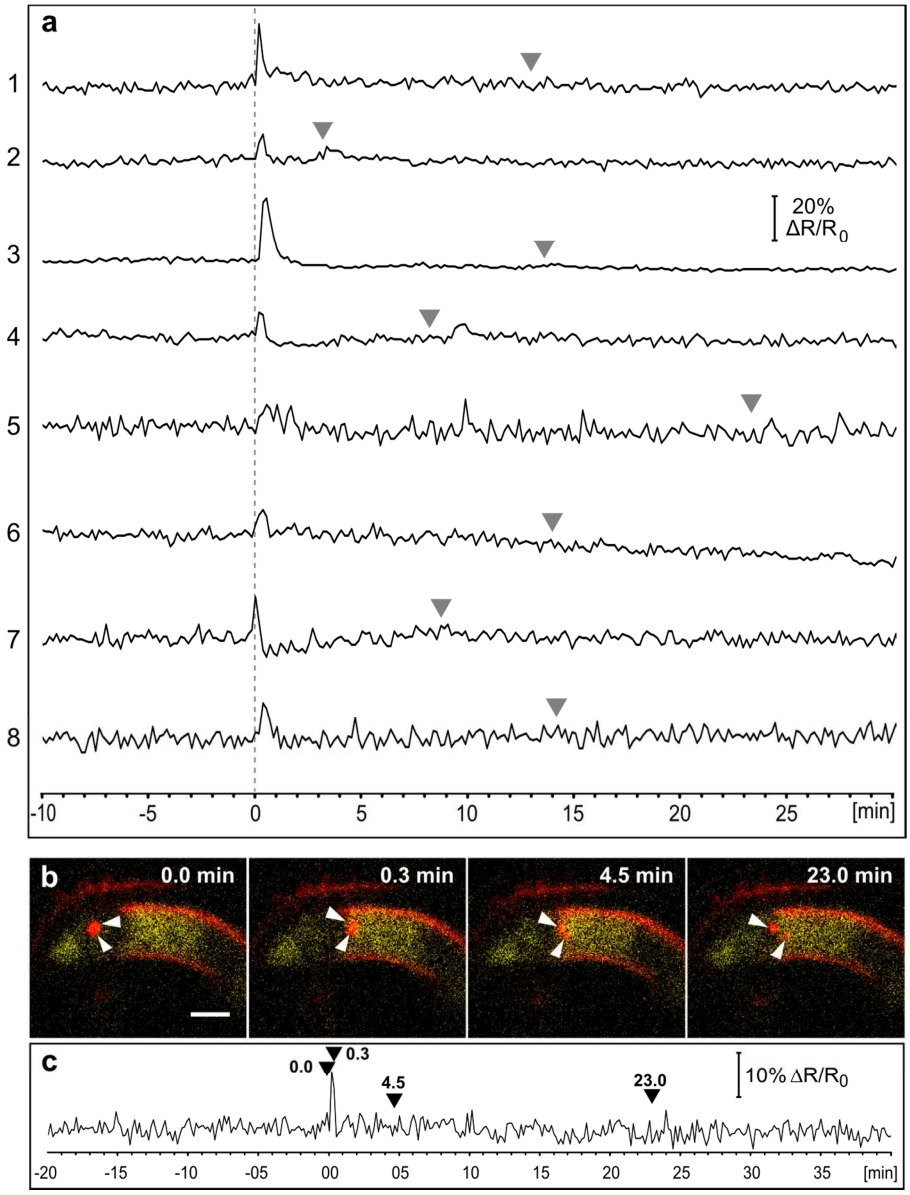
Supplementary Figure 2: Ca^{2+}_{cyto} signatures detected in synergid cells.

a. Structure of the Ca^{2+}_{cyto} oscillations in synergid cells shown in Fig. 1d.

During the interaction phase with a pollen tube similar amplitudes of the Ca^{2+}_{cyto} oscillations were detected in individual cells. The oscillations showed high variability in period among individual cells.

b. Mapping of Ca^{2+}_{cyto} amplitudes in distance to the PT tip.

Additional information to the schematic of a pollen tube tip in contact with a synergid cell given in Fig. 2d. Squares represent 4x4 µm regions used for analysis. Numbers in each square display the normalized average amplitude height, the standard error of mean in brackets and the number of measured Ca^{2+}_{cyto} elevations (n) in parentheses of five independent experiments.

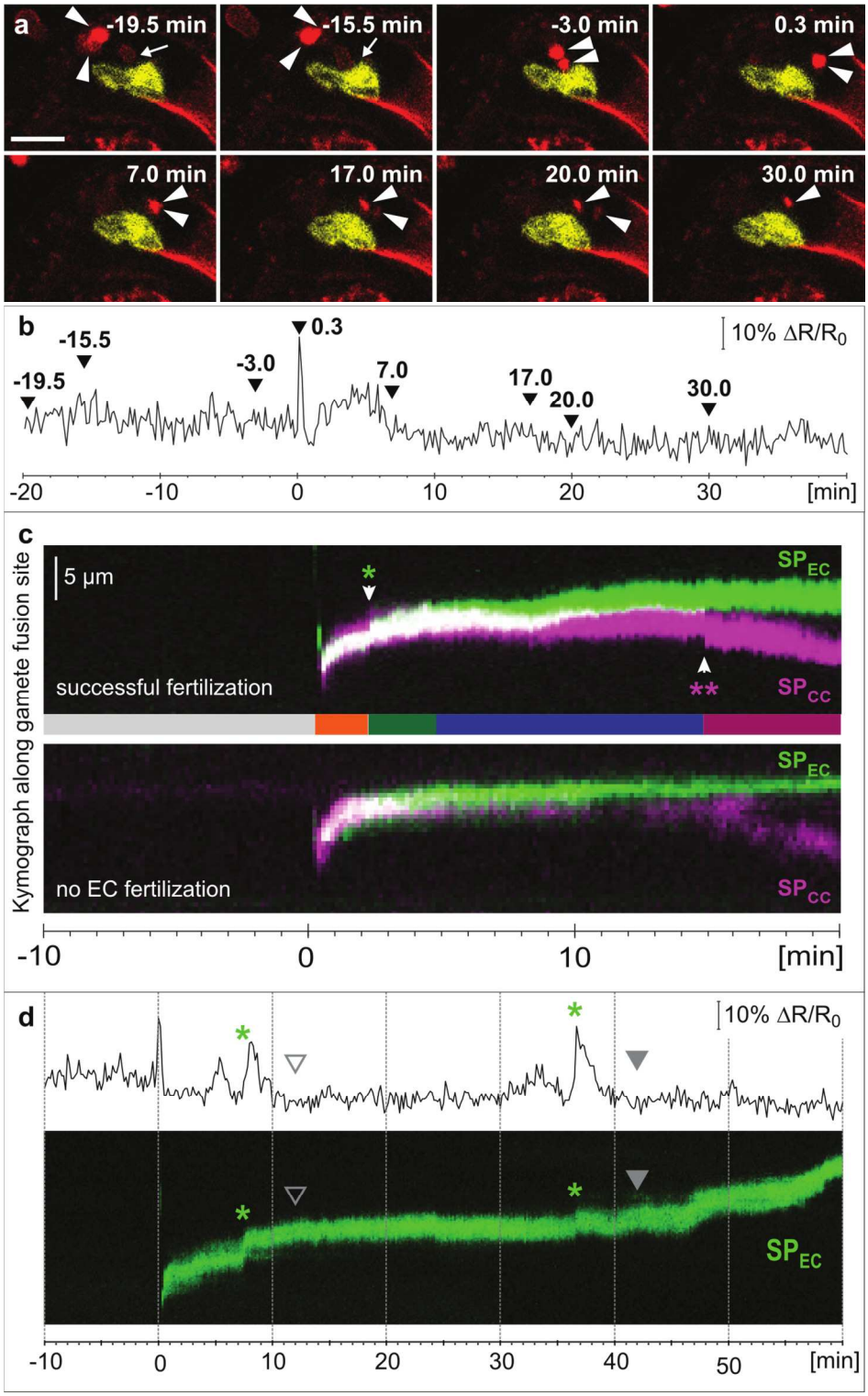


Supplementary Figure 3: Ca^{2+}_{cyto} elevations in central cells.

a. Ca^{2+}_{cyto} responses of central cells during sperm cell delivery and successful gamete fusion.
Normalized ratio change of CerTN-L15 in central cells during pollen tube burst and the following gamete fusion events. Graphs are aligned to the frame of pollen tube burst (dotted line, time point 0 min). Arrowheads indicate the initiation of sperm nucleus movement into the central cell. See supplemental Movies 3, which corresponds to graph 2.

b. Fertilization event with unsuccessful sperm–central cell fusion.
Time series of sperm cell nuclei (red, arrowheads) arriving at the gamete fusion sites. Sperm nuclei separate, but one sperm cell stayed at the arrested position and failed to fuse with the central cell (yellow, YFP signal of pDD65::CerTN-L15). Scale bar : 20 µm.

c. Normalized ratio change of CerTN-L15 in the central cell shown in b.
Arrowheads mark time points shown in b. 0 min marks the time of pollen tube burst.



Supplementary Figure 4: Sperm cell movement in comparison to plasmogamy success and Ca^{2+}_{cyto} elevations in egg cells.

a. Unsuccessful fertilization attempt of the egg cell.

Time series of an LHR pollen tube (red sperm nuclei) in an pEC1:CerTN-L15 expressing ovule. The LHR pollen tube approaches (-19.5 min) and gets in direct contact (-15.5 min) with the egg cell (yellow). White arrows indicate the tip of the pollen tube. The LHR sperm cells (arrowheads) get

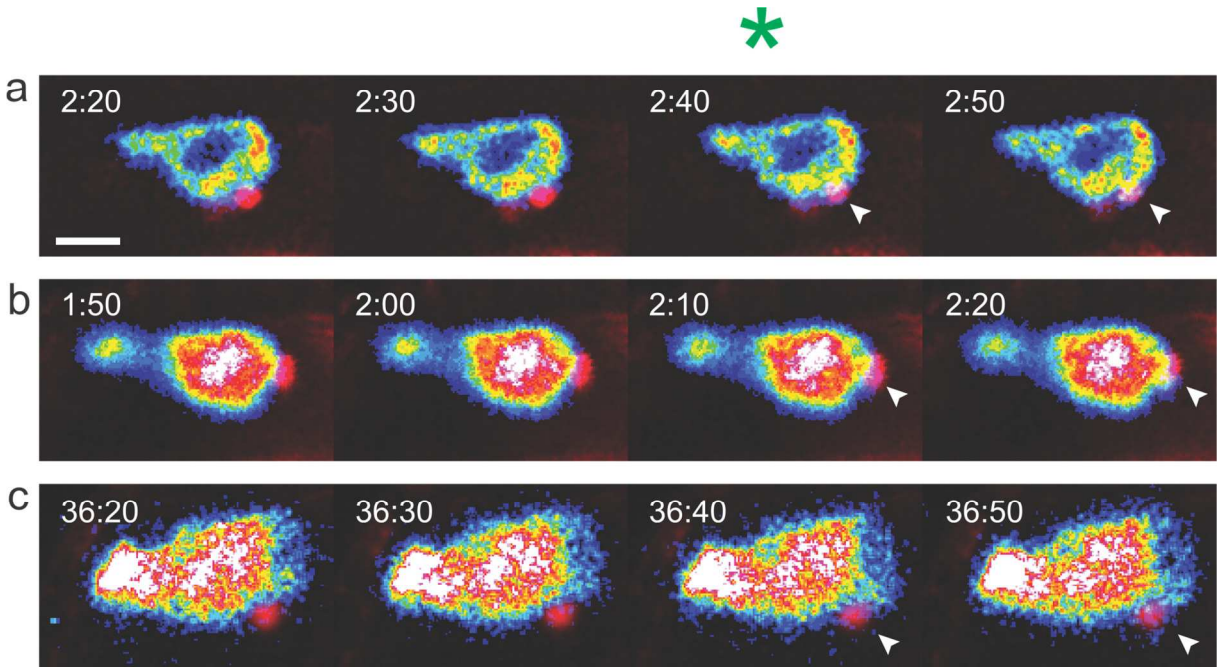
released from the pollen tube and rapidly reach the gamete fusion sites (0.3 min). The sperm cells stay at this position and do not appear to come into physical contact with the egg cell (7.0 min). 17.0 min after pollen tube burst, one sperm nucleus starts to move into the central cell (20.0 min), while the other sperm cell fails to fuse with the egg cell and remains at its position (30.0 min). See supplemental Movie S5

b. Ratio change of CerTN-L15 in the egg cell shown in a.

Arrowheads in graph mark the time points shown in b. The graph is aligned to time point 0 min (pollen tube burst).

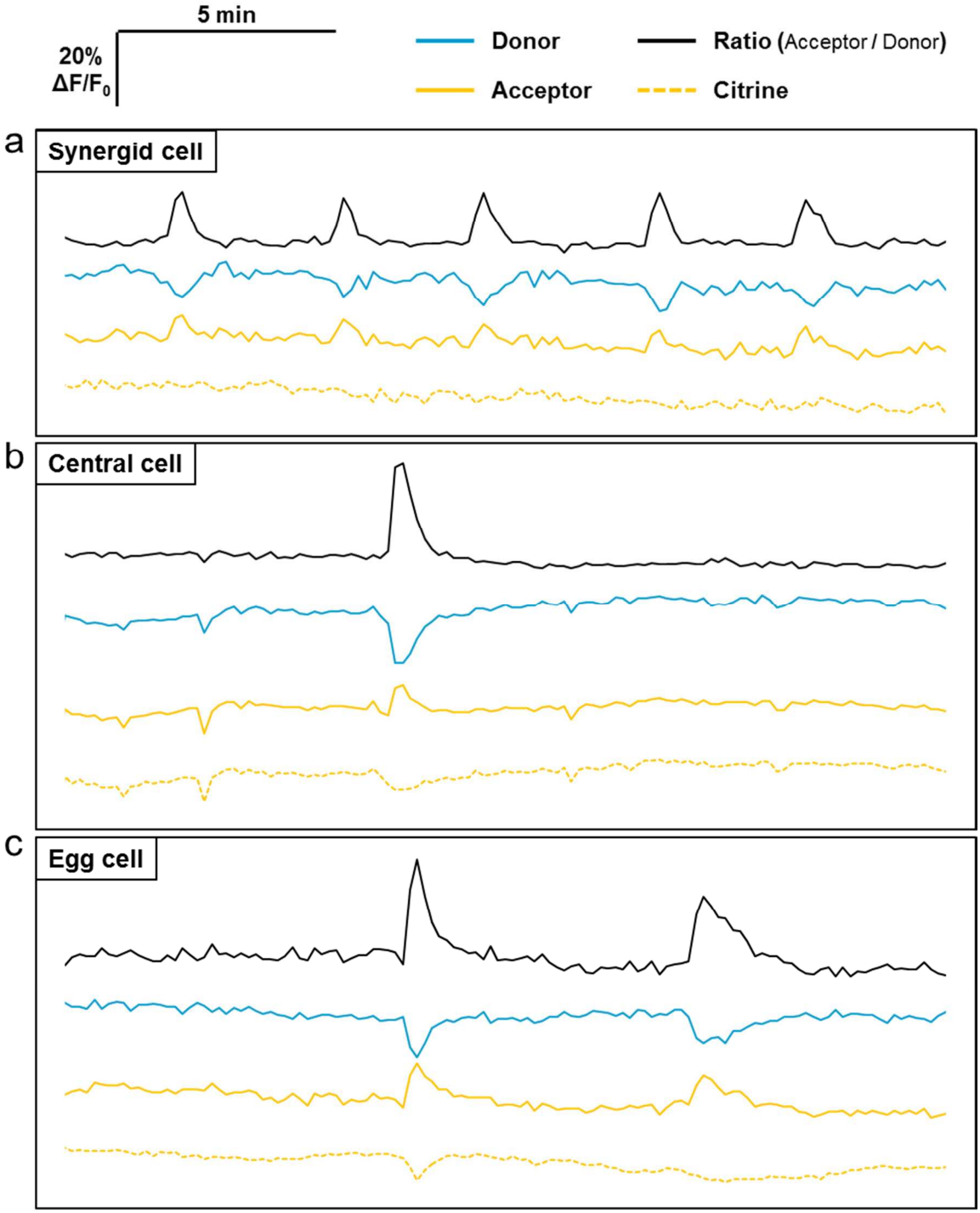
c. Characteristic sperm cell movement during fertilization. Kymograph visualizing SP movement over time during successful (upper) and unsuccessful (lower) fertilization. Each SP was analysed separately and kymographs were overlaid. Coloured bars correspond to phases shown in Fig 4a,b. White colour in the overlay indicates mostly parallel movement of SP_{EC} (green) and SP_{CC} (magenta). Shifts of SPs are indicated by asterisk (SP_{EC}) and double asterisk (SP_{CC}).

d. Trace of two plasmogamy attempts. The graph shows normalized ratio of CerTN-L15 in the EC of a time series with two plasmogamy attempts. The kymograph shows the movement of SP_{EC} (no signal from SP_{CC} was detected). Asterisks show fast sideward movements of SP_{EC} and arrowheads indicate movement towards the EC attempting gamete fusion. After SP release, an elevation of Ca^{2+}_{cyto} can be observed, followed by an arrest and slow movement at the fusion site. After 7.3 min a fast sideward movement of SP_{EC} was observed (green asterisk) with a subsequent Ca^{2+}_{cyto} elevation in the EC, followed by a movement of SP_{EC} towards the EC after additional 5 min (empty grey arrowhead). As plasmogamy failed in this attempt, the SPs further remained at the fusion site. After a second fast sideward movement of SP_{EC} (36.3 min, 2nd green asterisk), an additional Ca^{2+}_{cyto} elevation could be observed in the EC, followed by a movement of SP_{EC} into the EC about six minutes later (grey arrowhead).



Supplementary Figure 5. Diffusion of fluorophore molecules from egg cells into sperm cells during plasmogamy.

Additional examples of detected citrine signal in SP_{EC} as shown in Fig. 4f. Citrine intensity of egg cells is shown in false colours, sperm cells are shown in red. Image series (a) corresponds to the calcium recordings given in graph 7 in Fig. 4c, (b) corresponds to graph 8, (c) corresponds to Fig. 4e. The green asterisk represents SP_{EC} shift in all measurements. The arrow heads indicate the positions of sperm cells and the appearances of citrine signal outside the egg cell. Time format: mm:ss. Scale bar: 10 µm.



Supplementary Figure 6. Traces of individual fluorophores of CerTNL15 during Ca^{2+}_{cyto} measurements.

Representative examples of traces in synergid cell (a), central cell (b), and egg cell (c) are given as normalized donor (cerulean, blue line) and acceptor (citrine, yellow line) emission upon donor excitation. Calculated ratios of both fluorophores are given as black line. Acceptor (citrine) emission upon acceptor excitation is traced as yellow dotted line and served as control measurement in all experiments.