

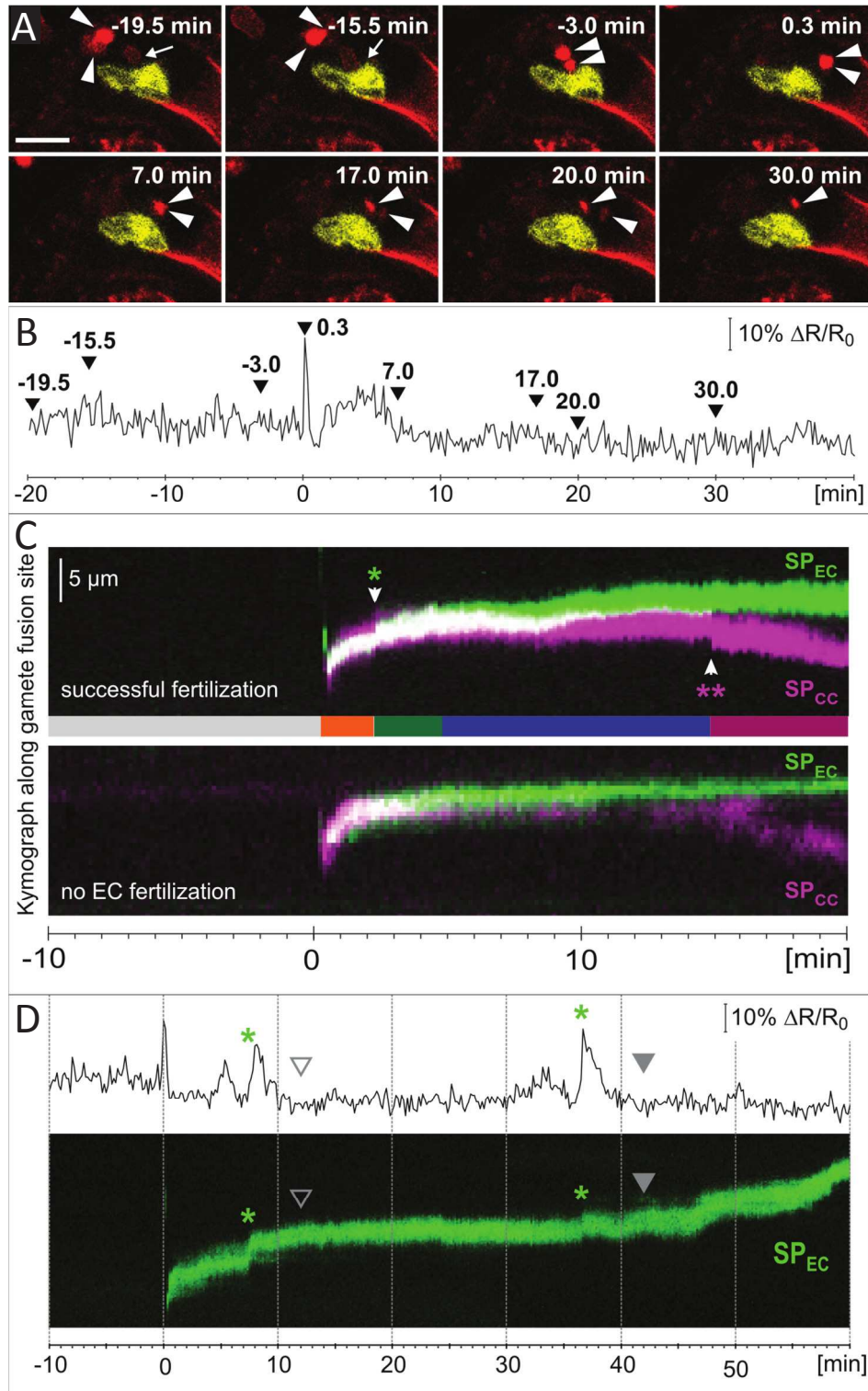


Figure S5.4 | Sperm cell movement in comparison to plasmogamy success and $\text{Ca}^{2+}_{\text{cyto}}$ elevations in egg cells.

(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 5.5**.

(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 **Figure 5.4 A, B**. White colour in the overlay indicates mostly parallel movement of SPEC (green) and SPCC (magenta). Shifts of SPs are indicated by asterisk (SPEC) and double asterisk (SPCC). **(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 SPEC (no signal from SPCC was detected). Asterisks show fast sideward movements of SPEC and arrowheads indicate movement towards the EC attempting gamete fusion. After SP release, an elevation of $\text{Ca}^{2+}_{\text{cyto}}$ can be observed, followed by an arrest and slow movement at the fusion site. After 7.3 min a fast sideward movement of SPEC was observed (green asterisk) with a subsequent $\text{Ca}^{2+}_{\text{cyto}}$ elevation in the EC, followed by a movement of SPEC 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 SPEC (36.3 min, 2nd green asterisk), an additional $\text{Ca}^{2+}_{\text{cyto}}$ elevation could be observed in the EC, followed by a movement of SPEC into the EC about six minutes later (grey arrowhead).