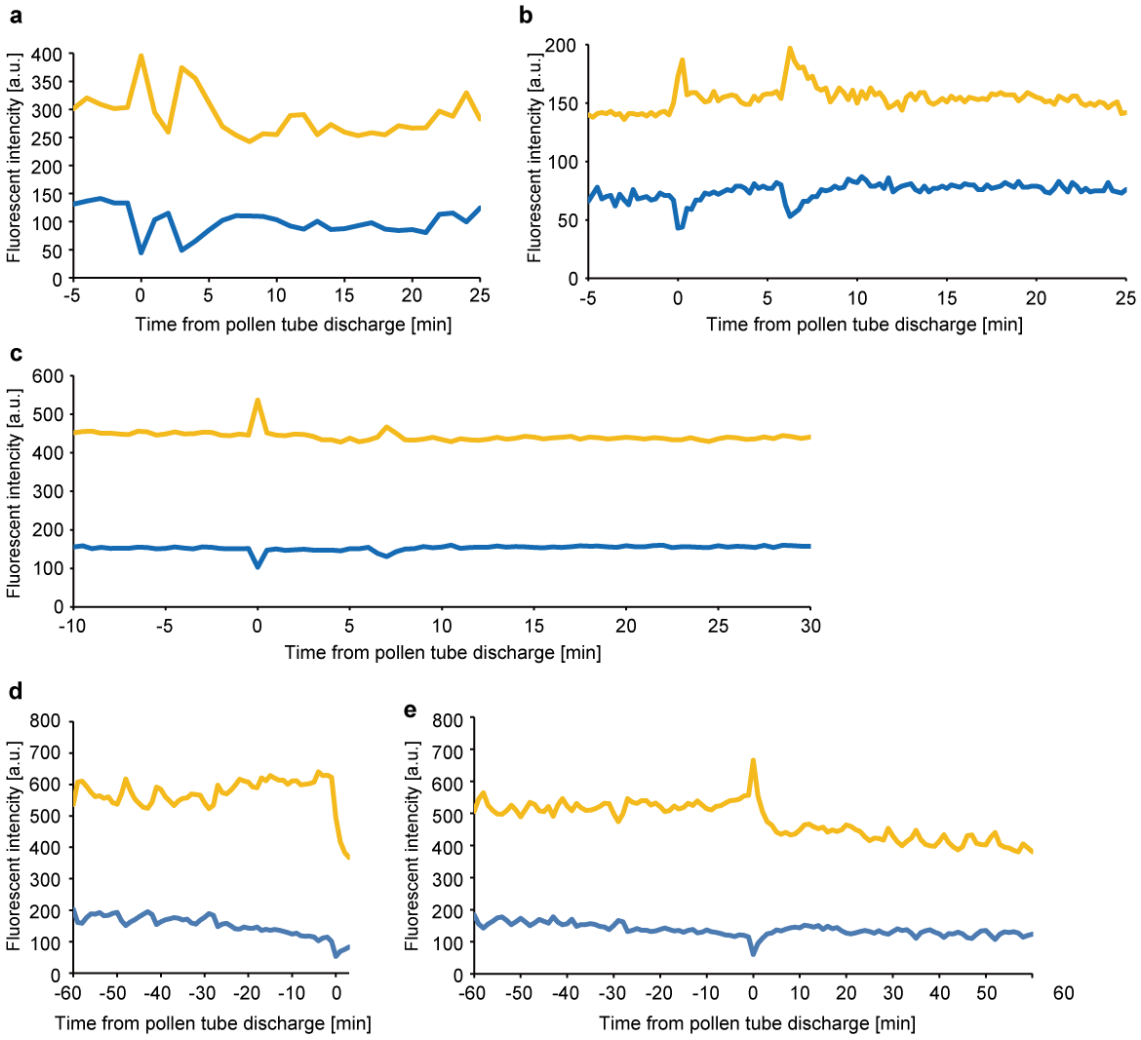
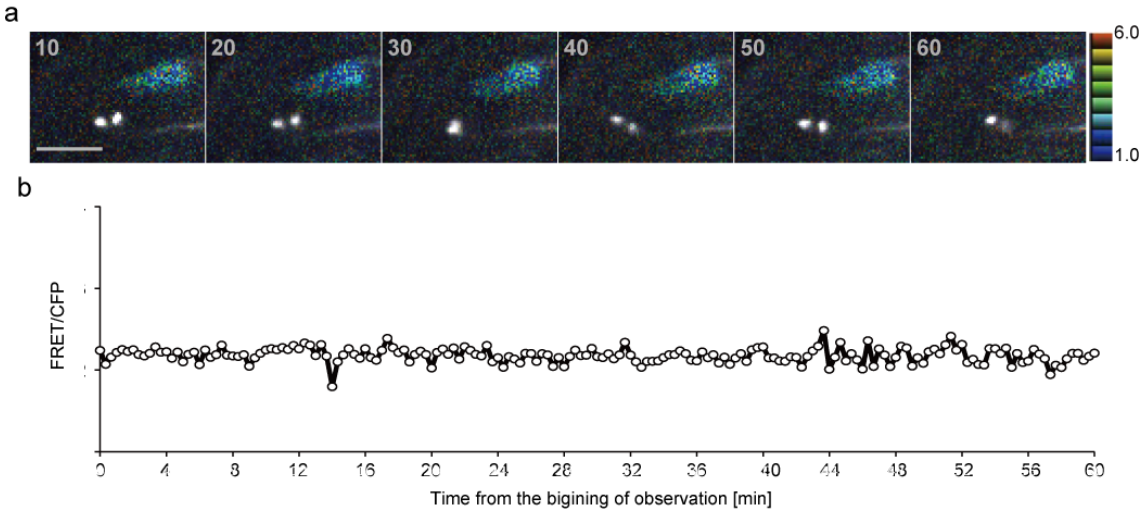
Supplementary informations



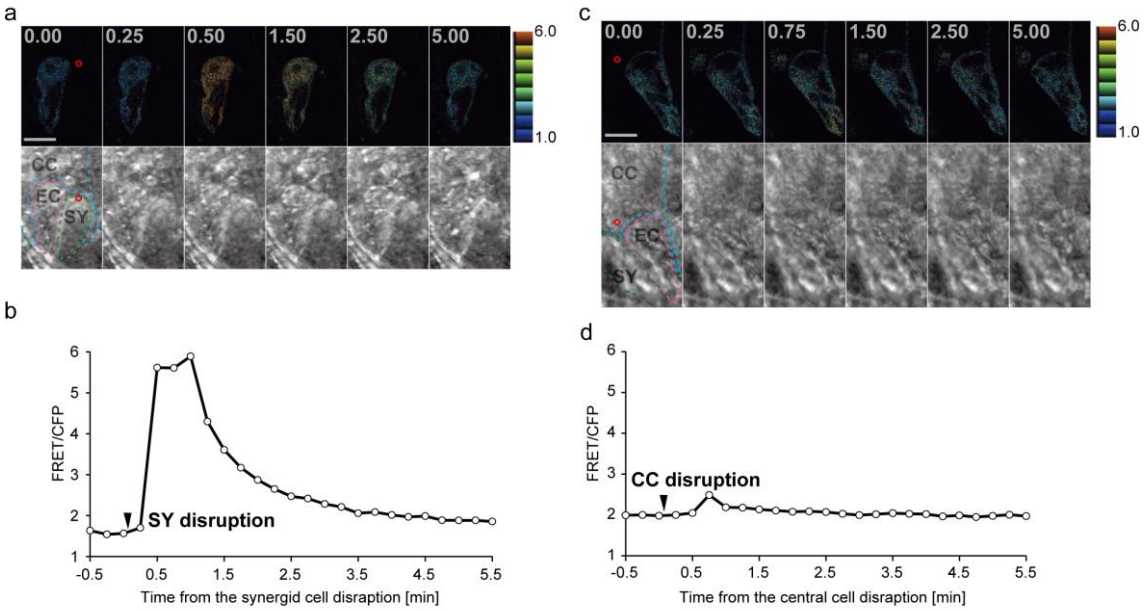
Supplementary Figure 1. Donor and acceptor signals in each cell type during double fertilization.

CFP and YFP fluorescence intensities in egg cell (a, shown in Figure 1b, 1c; b, shown in Figure 1d, 1e), and a central cell (c, shown in Figure 3a, 3b), and synergid cells (d, SY1; e, SY2 shown in Figure 3c-3e), respectively.



Supplementary Figure 2. No notable Ca^{2+} spike is observed in case of pollen tube discharge failure

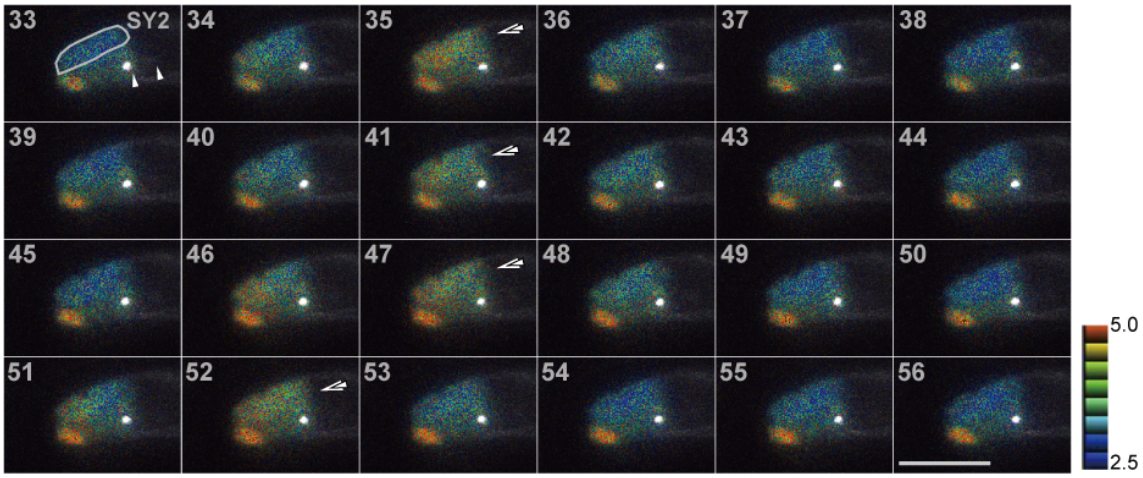
(**a**) Time lapse series of confocal images (maximum projections of three optical sections) of the egg cell expressing YC3.60 during pollen tube approach. Sperm cells nuclei are labeled by pHTR10::HTR10-mRFP (white in color). Representative FRET/CFP ratio images are shown as in Figure 1. Numbers indicate time (min) after starting observation. Scale bar, 20 μm. (**b**) Time course of the FRET/CFP ratio change in the egg cell expressing YC3.60 shown in (**a**).



Supplementary Figure 3. Synergid cell disruption results in a Ca^{2+} spike in the egg cell, but central cell disruption does not trigger a Ca^{2+} spike.

(**a, c**) Time lapse series of two-photon optical sections of the egg cell expressing YC3.60 after disruption of a synergid cell (**a**) or the central cell (**c**) by using femto-second pulse laser. Upper panel shows representative FRET/CFP ratio shown as in Figure 1. Lower panels show corresponding bright field images. The red circled region represents the point irradiated by the femto-second pulse laser. Scale bar, 10 μm. After the disruption of a synergid cell, the edge of the disrupted cell becomes fuzzy (0.25 min in the lower panel) whereas the edge of the other synergid cell remains sharp (2.50, 5.00 min in the lower panel) **(a**). Numbers indicate time (min) after the disruption. The femto-second pulse laser for cell disruption was irradiated just after the scanning of time

0.00 image. Scale bar, 20 μm. (**b, d**) Time course of the FRET/CFP ratio change in the egg cell expressing YC3.60 shown in (**a, c**) respectively.



Supplementary Figure 4. Persistent synergid cell shows Ca^{2+} oscillation after double fertilization.

Time lapse series of confocal images (maximum projections of four optical sections) of synergid cells expressing pMYB98:YC3.60 shows Ca^{2+} oscillation in the persistent synergid cell after double fertilization. Sperm cell nuclei labeled with pRPS5A::H2B-tdTomato are indicated by arrowheads in first panel (white in color). Representative FRET/CFP ratio images are shown as before. Double arrowheads show Ca^{2+} elevation in the persistent synergid cell similar to SY2 in Figure 3. Numbers indicate time (min) as in Figure 1. Scale bar, 20 μm.

Supplementary Table 1. The timing of the second Ca^{2+} peak in the central cell

Egg cell fertilization related	Central cell fertilization related	Non-related to fertilization	No second Ca^{2+} peak	N.D.*	Total
5 (2)	4 (2)	2	9	1	19

( ) shows the cases which egg cell and central cell fertilized simultaneously.

*timing of the two fertilization events could not be established in this samples..