

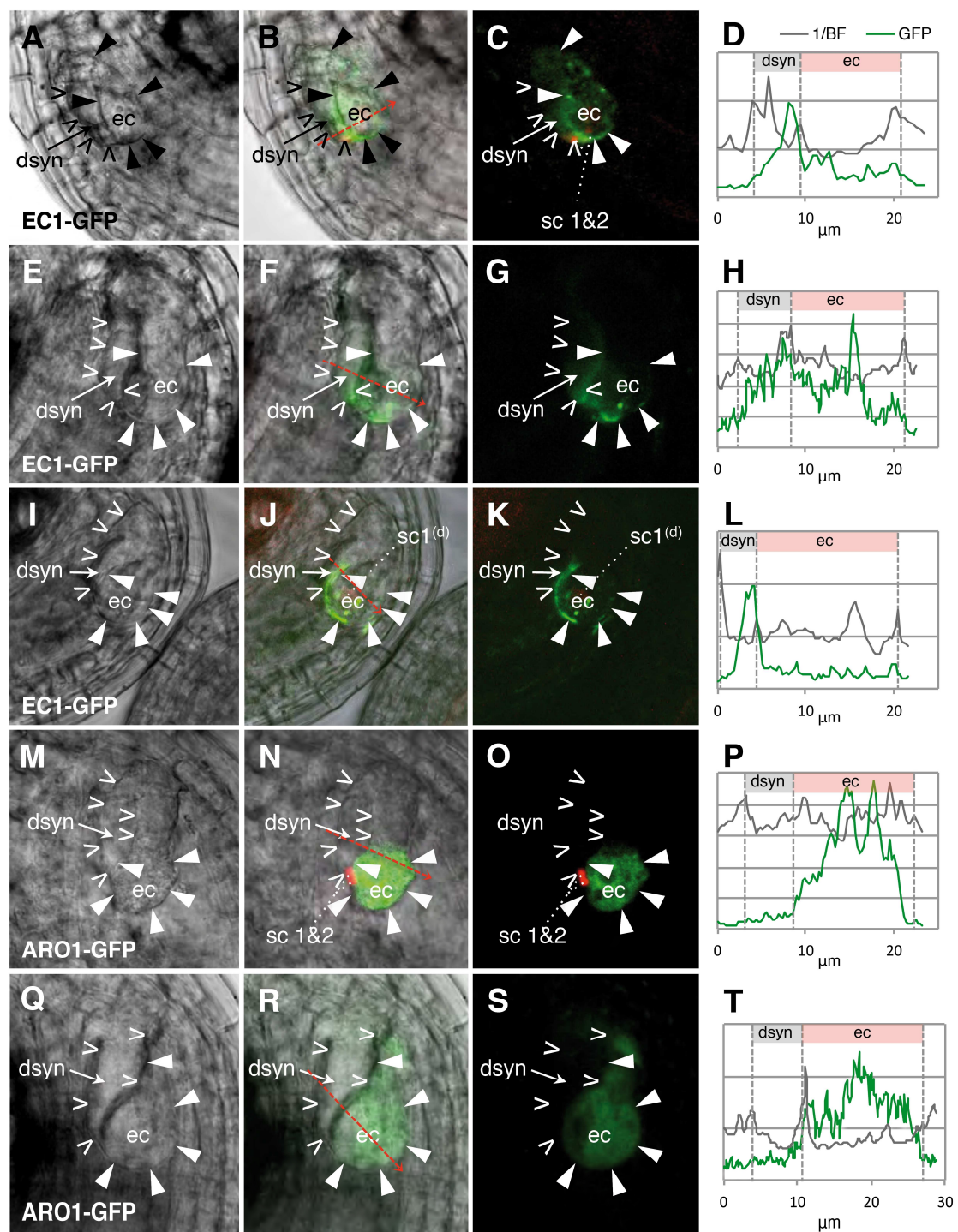


Figure S6.4 | Secretion of EC1-GFP is triggered during the short-lived event of double fertilization.

Emasculated flowers of plants expressing EC1-GFP (green fluorescence) were hand-pollinated with the sperm cell marker HTR10-mRFP (red fluorescence of sperm cell nuclei). Six to ten hours after pollination (hap), pistils were harvested and prepared for confocal microscopy. As controls, emasculated flowers of plants expressing the cytoplasmic

ARO1 (ARMADILLO REPEAT ONLY 1)-GFP fusion protein in the egg cell (Gebert et al., 2008) were hand-pollinated and analyzed in the same way at 6 to 10 hap. **(A)** to **(L)** Upon sperm cell arrival and during double fertilization, EC1-GFP signals are detected extracellularly, especially near the apical tip of the degenerating synergid cell (dsyn). Fluorescence intensities are gradually increasing toward the egg cell membrane **(D)**, **(H)**, and **(L)**, indicating regulated exocytosis of EC1-GFP by the egg cell (ec) during gamete interactions. **(I)** to **(L)** At karyogamy, shown by the decondensation of the paternal red fluorescent chromatin inside the egg nucleus, remnants of EC1-GFP are still present on the egg surface facing towards the central cell and the degenerating synergid cell. Similar observations to the data shown in in **Figure 6.2 F** to **H** and **Figure S6.4 A** to **L** were made in all pollen tube-targeted ovules that have been caught at the right moment during double fertilization and that showed a sufficient strong fluorescence of EC1-GFP in the egg cell (16 ovules; $n = 6$ independent experiments with 5 to 8 pistils each). **(M)** to **(T)** By contrast, cytoplasmic ARO1-GFP was never detected outside the egg cell upon sperm cell arrival and during double fertilization ($n = 3$ independent experiments with 5 to 8 pistils each). **(A)**, **(E)**, **(I)**, **(M)** and **(Q)** are bright field (BF) images, **(B)**, **(F)**, **(J)**, **(N)** and **(R)** show merged bright field and fluorescence images at a single z-plane. The visible cell borders of the egg cell (triangles) and the degenerating synergid cell (arrowheads) are indicated. **(C)**, **(G)**, **(K)**, **(O)** and **(S)** represent the respective fluorescence signals at a single z-plane. Sperm chromatin is decondensed and nuclei are partly out of focus in post-karyogamy stages shown in **(F)** and **(G)** and **(R)** and **(S)**. Plot profiles in **(D)**, **(H)**, **(L)**, **(P)** and **(T)** show the relative signal intensities along a line (red arrow) within the respective z-slice. The x-axis represents the distance (in μm) along the line. The y-axis displays the relative signal intensity of GFP (green line) and the reciprocal grayscale pixel intensity of the bright field (1/BF; gray line) signal, respectively. Cell borders of the egg cell and the degenerating synergid cell were identified in the pixel-sized grayscale image and are indicated by dashed lines in the plot profiles. Abbreviations: dsyn, degenerating synergid cell; ec, egg cell; sc, sperm cell nucleus; $sc^{(d)}$, decondensed sperm cell nucleus.