

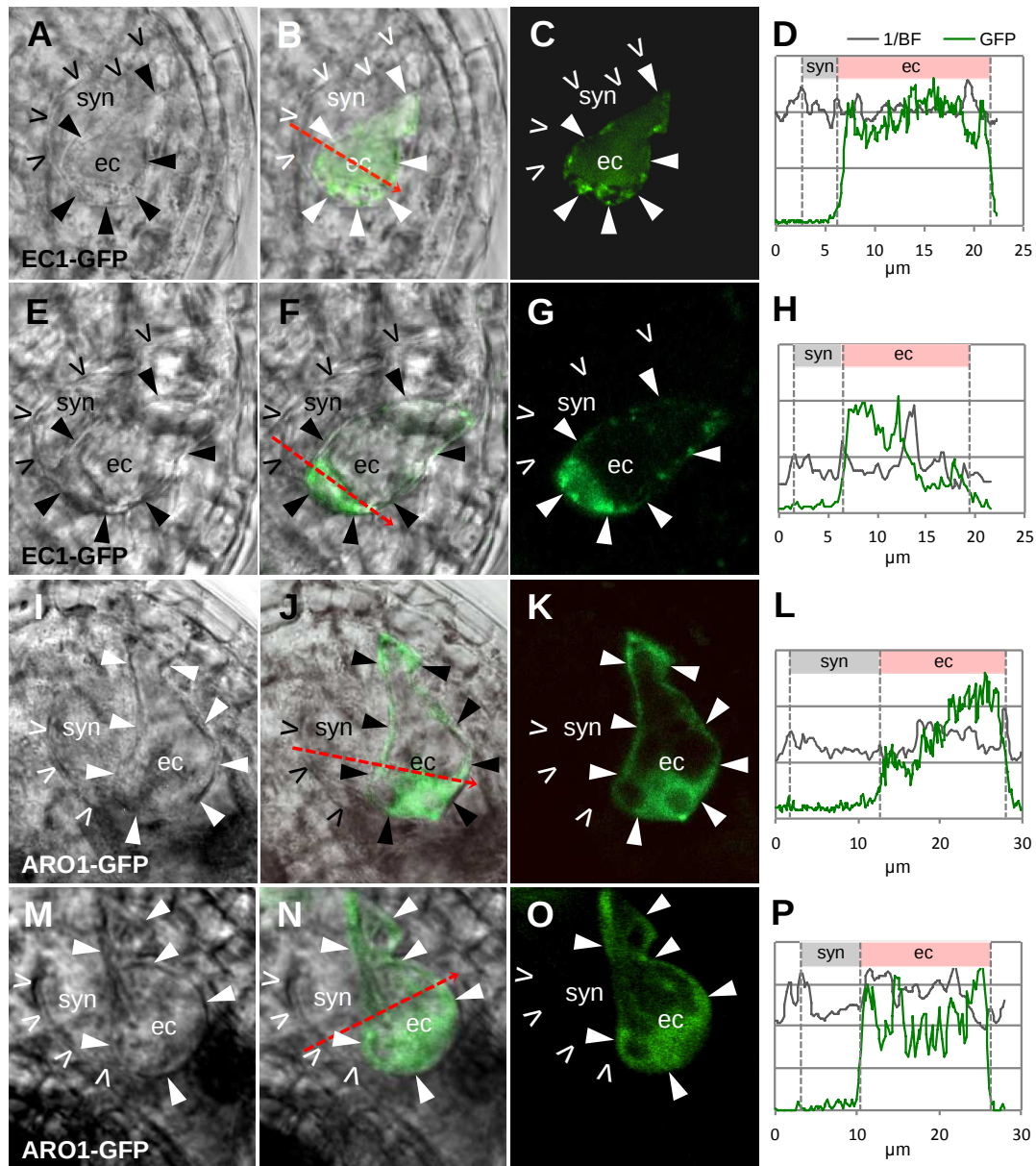


Figure S6.3 | Subcellular localization of EC1-GFP in mature but unfertilized *Arabidopsis* egg cells, analyzed by CLSM.

(A) to (H) EC1-GFP signals cannot be detected extracellularly, e.g. in the region of the synergid cells (syn) flanking the egg cell (ec) but only within the egg cell. (I) to (P) Unfertilized egg cells expressing a cytoplasmic ARO1 (ARMADILLO REPEAT ONLY 1)-GFP fusion protein which is partially also associated to the endomembrane system (Gebert et al., 2008), served as controls. (A), (E), (I), and (M) are bright field (BF) images, (B), (F), (J), and (N) show merged bright field and fluorescence images at a single z-plane. The visible cell borders of the egg cell (triangles) and of one of the two adjacent synergid cells (arrowheads) are indicated. (C), (G), (K), and (O) represent the corresponding fluorescence

images. (D), (H), (L), and (P) Plot profiles showing the relative signal intensities along a line (indicated by the red arrow) within the respective z-slices shown in (B), (F), (J), and (N). The x-axis represents the distance (in μm) along the line. The y-axis displays the relative fluorescence intensity of GFP (green line) and the reciprocal grayscale intensity of the bright field (1/BF; gray line) signal, respectively. Cell borders of the egg cell and the adjacent synergid cell were identified in the pixel-sized grayscale image and are indicated by dashed lines in the plot profiles. Extracellular signals were neither detected in EC1-GFP expressing egg cells, nor in ARO1-GFP control egg cells (EC1-GFP: $n = \geq 8$ independent experiments with 3 to 5 pistils each; ARO1-GFP: $n = 4$ independent experiments with 3 to 5 pistils each). Abbreviations: ec, egg cell; syn, synergid cell.