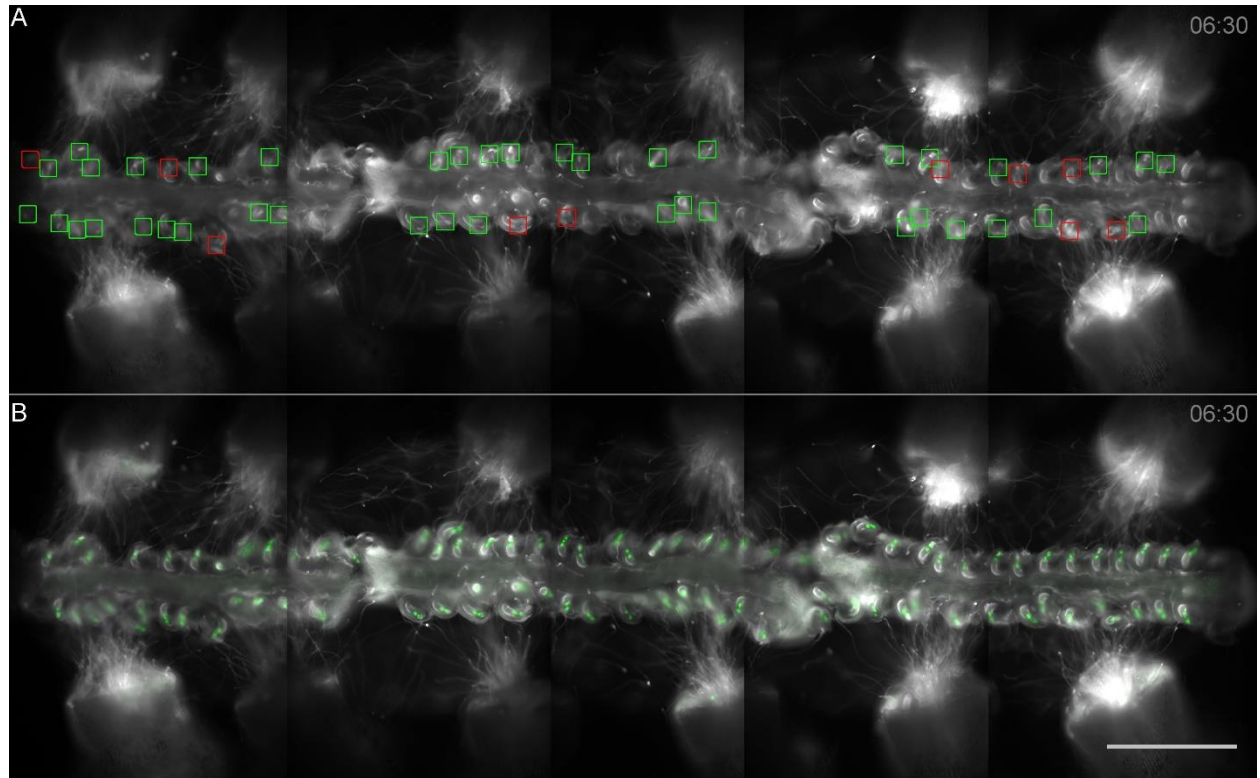
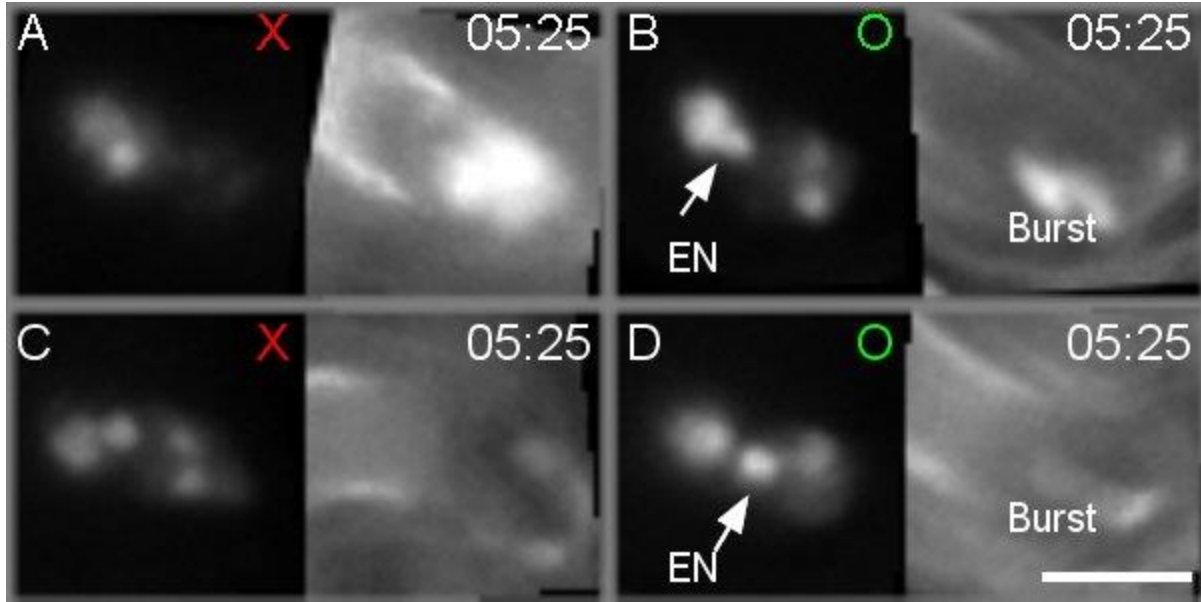


Supplementary Figure 1: Results of the SIV excised ovules method. Merged images from eight timelapses (see **Video 1**) of SIV using excised ovules showing the growth of pollen tubes (harboring *pLAT52:R-GECO*) and pollen tube reception within the ovules. The 20 green boxes indicate ovules with instances of explosive pollen tube burst in the synergids, and the 51 red boxes indicate ovules with failed pollen tube burst and overgrowth. Scale bars = 100 μm .



Supplementary Figure 2: Results of the SIV *cum septum* method. (A) Merged images at time 6:30 (h:min) from timelapse of five overview areas (see **Video 1** and **Figure 3**) of pollen tube growth and reception within the ovules with just the RFP channel (Channel 1). The pollen is harboring *pLAT52:R-GECO* and the ovules *pFG:roGFP2-ORP1-NLS*; *pMYB98:roGFP2-ORP1*. The 41 green boxes indicate ovules with explosive pollen tube burst, and the 10 red boxes indicates ovules with failed pollen tube burst and pollen tube overgrowth. (B) Merged images of the same timelapse with both the RFP and GFP channels (Channel 1 and Channel 2). The numbers on the top right indicate the time (h:min). Scale bar = 500 μm .



Supplementary Figure 3: Examples of positive and negative outcomes from *SIV cum septum*. (A,C) Still images of example ovules taken from **Video 2** showing two instances of failed fertilization. In **A**, the pollen tubes fail to arrest growth and continue coiling in the embryo sac. In **C**, a second category of pollen tube overgrowth is seen, where the first pollen tube undergoes fast growth as it grows into the synergid but arrests growth without rupturing. (B,D) Still images of example ovules taken from **Video 2** showing two instances of successful pollen tube reception. The wild-type-like pollen tube burst shows explosive rupture of the pollen tube in the synergids, and the migration of the egg cell nucleus toward the micropyle indicates karyogamy. The numbers on the top right indicate the time (h:min). Arrows indicate the migration of the egg cell nucleus (EN) after pollen tube burst (Burst). Scale bar = 30 μm .