

## *Supplemental Movie Legends Chapter 2*

### **Supplemental Movie 2.1 | Time lapse imaging of pollen germinating in the multi-well micro-germination slide**

Imaging of ARO1-GFP expressing pollen in an exemplary 10 well micro-germination setup was performed over 6 hours. Note that position B1 was readjusted after 1 h PT growth. Time stamper shows hours and minutes. Scale bar = 50  $\mu\text{m}$ . The movie is accessible on [www.youtube.com/watch?v=M9H2XVKIE1s](http://www.youtube.com/watch?v=M9H2XVKIE1s).

### **Supplemental Movie 2.2 | Vesicle-associated ARO1-GFP is enriched in the inverted cone-shaped "clear zone" of the pollen tube tip and shows reverse fountain streaming pattern**

Time lapse Spinning Disc confocal imaging of ARO1-GFP at the PT tip. Royal LUT was used to highlight increasing signal intensities (low to high: blue to yellow to red to white). Time stamper shows minutes and seconds. Scale bar = 5  $\mu\text{m}$ .

### **Supplemental Movie 2.3 | Polar vesicle accumulation during pollen germination and PT tip growth, indicated by ARO1-GFP intensity changes**

Time lapse imaging of a representative pollen expressing ARO1-GFP before germination, during bulging and PT growth. PT outlines are drawn in cyan, ARO1-GFP low-intensity threshold in red (70 % highest intensities) and signal maxima in yellow (0.5 % highest intensities). White arrowheads point to ARO1-GFP maxima in the ungerminated pollen grain, green arrows indicate ARO1-GFP maxima after germination. Time stamper shows minutes pre/post germination. Scale bar = 10  $\mu\text{m}$ .

### **Supplemental Movie 2.4 | F-actin dynamics during pollen germination and PT tip growth**

Time lapse imaging of tagRFP-T-Lifeact expressing pollen before germination and during PT growth. Solid arrowheads highlight F-actin assembly at the periphery of the pollen cell, opposite to the later germination site. Empty arrowheads point at the massive accumulation of F-actin bundles at the PT base, during rapid PT growth. Time stamper shows minutes pre/post germination. Scale bar = 15  $\mu\text{m}$ .

### **Supplemental Movie 2.5 | Sperm cell translocation into the growing PT**

Time lapse imaging of germinating pollen with fluorescently labeled sperm cell nuclei and plasma membranes. Solid arrowheads highlight the sperm plasma membrane protrusion that connects one sperm cell to the vegetative cell nucleus. Time stamper shows minutes pre/post germination. Scale bar = 15  $\mu\text{m}$ .