Supplemental Material to:

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Species-specific interaction of EA1 with the maize pollen tube apex

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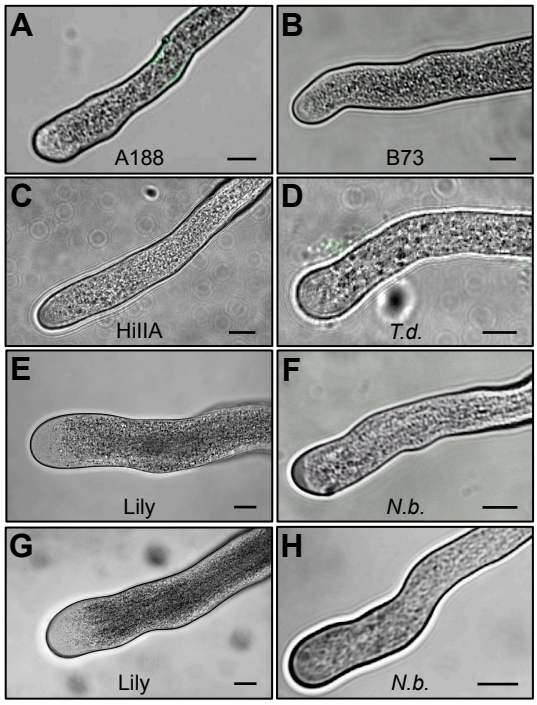

Figure S1. EA1-related EAL2 peptide does not interact with maize and *Tripsacum dactyloides* pollen tube tips. Moreover, lily (*Lilium* "Stargazer") and tobacco (*N. benthamiana*) pollen tubes are neither bound by EA1 nor EAL2, respectively. *In vitro* pollen tube binding assays of the maize inbred lines A188, B73 and HiIIA, *Tripsacum dactyloides* (*T.d.*), lily and *Nicotiana benthamiana* (*N.b*.) were performed as controls at the same concentration and condition like in Figure 1 either with synthetic predicted mature EAL2 (**A**, **B**, **C**, **D**, **E** and **F**) or EA1 (**G** and **H**) peptides both labeled with the green fluorophore DyLight 488 NHS Ester. Each panel shows merged micrographs of bright field and fluorescence Z-projections of confocal image stacks of 10 to 20 µm thick sections, respectively. Scale bars represent 10 µm.

SUPPLEMENTARY MATERIALS

Chemical labeling of synthetic peptides

Predicted mature EA1 peptide of 49 amino acids[1] was chemically synthesized with 80%-90% HPLC-purity by Centic Biotec. Successful synthesis of the linear peptide was shown by LC-MS. Chemically labeling of the synthetic EA1 peptide was performed with fluorophore DyLight 488 NHS Ester (Thermo Scientific) according to the manufacturer's protocol with a number of modifications. 0.5 mg peptide was first dissolved in 100% DMSO and then diluted stepwise in 0,1 M sodium phosphate, 0,15 NaCl (pH 7,4) to a final concentration of 1% DMSO. The protein solution was mixed with an aliquot of the fluorophore DyLight 488 NHS Ester. After 1.5 h incubation at room temperature the mixture was dialyzed against 0,1 M sodium phosphate, 0,15 NaCl (pH 7,4) with a final concentration of DMSO not exceeding 1% using a Slide-A-Lyzer® MINI Dialysis Unit (Thermo Scientific). Precipitates were removed by centrifugation at 10.000 rcf. Predicted mature EAL2 consisting of 52 amino acids[2] was chemically synthesized with 80%-90% HPLC-purity by Centic Biotec. The chemical labeling of the peptide was performed using the same method described above.

Pollen tube binding assay

The pollen tube binding assay was conducted as follows: in order to obtain pollen tubes, pollen grains of maize inbred lines or *Tripsacum dactyloides* were shaken shortly after anthesis directly onto 10 µl droplets of 1xPGM[3] containing a final concentration of 1% DMSO placed in a 35 mm plastic Petri dish. Pollen of *Nicotiana benthamiana* was transferred directly from a freshly opened anther onto drops of 1xPGM with 1% DMSO using forceps. Pollen of lily was directly taken one day after anthesis, dried at room temperature for at least 1 day and transferred directly into 1,5 ml of 1xPGM containing 1% DMSO in a 25 mm plastic Petri dish using forceps. Germination of maize and *T. dactyloides* pollen was performed for 30 to 45 min, while *N. benthamiana* pollen was germinated for 60 to 90 minutes and lily pollen over night for 14-15 hours. All incubations were performed at room temperature. Further on, 1 µl of chemically labeled EA1 or EAL2 were added to each droplet containing germinated pollen of maize, *T. dactyloides* or *N. benthamiana*. Germinated lily pollen were transferred step by step onto an object slide using a cut pipette tip and the media was removed to concentrate pollen into a final volume of 100 µl. Subsequently 10 µl of chemically labeled peptide were

added. After incubation of germinated pollen of maize, *T. dactyloides* and *N. benthamiana* the content of each droplet was transferred carefully using a cut pipette tip onto an object slide, to which 1xPGM containing 1% DMSO was added to a final volume of 200 µl. Samples were then washed on the object slide very carefully 3 times using each time 200 µl 1xPGM with 1% DMSO and resuspended in 500 µl 1xPGM with 1% DMSO. Microscopic analysis was initiated immediately after washing using a Zeiss Axiovert 200M LSM 510 META confocal laser scanning microscope.

References

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