Supplemental Methods

Starch staining

For starch staining of pollen grains, inflorescences were first fixed in FAA solution (50% ethanol: acetic acid: formaldehyde, 18:1:1, v/v/v). Flower buds with pollen grains at different developmental stages were then isolated. Pollen grains from the same anther were stained with either Lugol's iodine solution (1% I₂-KI) for starch detection or DAPI for determining the pollen development stage. Lugol's iodine staining was performed as previously described (Su et al., 2017).

Yeast one-hybrid assay

Wild-type GPT1 promoter fragment containing all four W boxes was introduced in front of HIS2 reporter gene using inverted PCR amplification of pHIS2.1 vector with $\text{P}_{\text{GPT1}}$-$\text{pHIS2-FF}$ (5'-CAAAACTGACTCAATTCTAATTTTAGATTCCAGGTCAAACGTGATCCAgggggagctcacgc-3') and $\text{P}_{\text{GPT1}}$-$\text{pHIS2-BP}$ (5'-ATTGATATCAATTGACTATGAAATCATTTGGATCACAGTTTGAACTGGAATCTAAAagggagctcacgc-3') primer pair. Lower case letters indicate sequence that matches the pHIS2.1 vector. GPT1 promoter with all four W-boxes mutated was generated similarly using $m\text{P}_{\text{GPT1}}$-$\text{pHIS2-FF}$ (5'-CAAAACTGACTCAATTCTAATTTTAGATTCCAGGTCAAACGTGATCCAgggggagctcacgc-3') and $m\text{P}_{\text{GPT1}}$-$\text{pHIS2-BP}$ (5'-ATTGATATCAATTGACTATGAAATCATTTGGATCACAGTTTGAACTGGAATCTAAAagggagctcacgc-3') primer pair. PCR products were then end-phosphorylated and ligated before being transformation into E. coli. All constructs were confirmed by sequencing. WRKY34 coding region was PCR-amplified using WRKY34 CDS-FF (5'-CCGGAAATTCATGGCTGGTATTGATAATAAAGCTGC-3') and WRKY34 CDS-BP (5'-TCCCCCGGGTCATATCTGTCGTAATCTACTCAACATC-3'), and cloned into pGAD7-rec2 vector (CLONTECH), creating a translational fusion of GAL4 activation domain and WRKY34 transcription factor. Yeast one-hybrid assays were performed as described before (Yan et al., 2016). Briefly, yeast competent cells (Y187 strain) were prepared and transformed according to the Clontech Yeast Protocols Handbook. Transformations were plated on SD media-Leu-Trp and incubated at 28 °C for 4 days to select co-transformants. Transformed yeast cells were subsequently grown in SD-Trp-Leu liquid media overnight and adjusted to OD₆₀₀ of 0.1. The suspensions were then spotted on SD-Trp-Leu and SD-Trp-Leu-His media plates supplemented with 90 mM 3-amino-1, 2, 4-triazole (3-AT) (Sigma-Aldrich). The plates were incubated for 6 days at 28 °C.

Co-localization experiment

For co-localization study, we transformed plastid marker construct, pt-rk CD3-999 (Nelson et al., 2007), into $\text{P}_{\text{GPT1}}$:GPT1-eYFP transgenic background. Homozygous T3 plants were used for co-localization experiments. Because this plastid marker is driven
by 35S dual enhancer promoter, it is not expressed in pollen grains, which makes co-localization experiment in pollen grains impossible. As a result, we used epidermal cells to study the co-localization of GPT1-eYFP and mCherry plastid marker.

Supplemental References

