Supplemental Data for "*KAONASHI4* encodes a β -(1,3)-galactosyltransferase involved in the biosynthesis of type II arabinogalactan that mediates pollen exine development in *Arabidopsis thaliana*" by Suzuki and Narciso et al.



Supplemental Figure S1. The average exine thickness measured from CLSM and TEM images.

A, Exine thickness as measured from CLSM optical sections of 10 individual microspores of both WT and the *kns4-2* mutant at various developmental stages. Three measurements were made on each image. OF, open flower.

B, Exine and intine thickness as measured from TEM sections of WT (n=118) and *kns4-2* (n=134) microspores at stage **12M**.

Bar and error bar represent the average +/- SD.



Supplemental Figure S2. Enzyme activity of KNS4 using different UDP- sugar donors. RP-HPLC profiles of enzyme assay products after reaction with β -Gal-NBD (acceptor) and indicated UDP-sugars in the presence of KNS4-expressing MMs. Numbers above the peaks indicate the number of Gal residues.



Supplemental Figure S3. Transmission electron micrographs of stage 12M WT and *kns4-2* microspores labelled with either anti-AGP (JIM8, JIM13) or anti-callose gold-labelled antibodies.

A and D, JIM8 labelling in WT (A) and *kns4-2* (D) microspore walls.

B and **E**, JIM13 labelling in WT (**B**) and *kns4-2* (**E**) microspore walls. In the *kns4* mutant (**E**), labelling of the sporopollenin granules (white arrowheads) was also seen.

C and F, Callose labelling in WT (C) and kns4-2 (F) microspore walls.

G, Percentage of immuno-gold counts of JIM13-labelled sections of WT (102 images, 4788 gold particles) and *kns4-2* (89 images, 3180 gold particles) microspore walls.

Immuno-gold particles are indicated by arrows. Exine, ex; intine, in; nexine I, ne-I; nexine II, ne-II. Scale bars = $0.2 \ \mu m$.