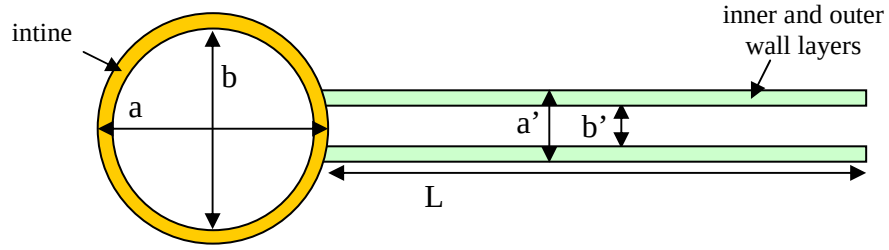
Estimation of the amount of the pollen grain wall *versus* pollen tube wall

From TEM pictures, we measured the diameter of the pollen grains and pollen tubes, the thickness of the intine wall and pollen tube cell wall (inner and outer wall) as shown in the drawing of Table SI.A. We considered (1) that the dehydration step used for sample preparation will have the same effect on both pollen grains and pollen tubes and (2) the thickness of the pollen tube wall is constant throughout the length of the tube. We calculated the volumes of the intine wall (using the formula $4/3\pi r^3$) and the pollen tube wall (using the formula $L\pi r^2$, with an average of L (averaged pollen tube length) = 1248 µm (1248 µm ± 374) (Table SI.B). We considered the pollen tube as a cylinder and did not take into account the tip which is negligible. We calculated the contribution of the pollen tube wall and pollen grain intine wall based on the average of 67% germination rate (67% ± 12) (Table SI. B).

Table SI. (A) Measurement of pollen grain and pollen tube diameters, (B) calculations of cell wall volumes and estimation of the contribution of the pollen tube wall *versus* pollen grain wall

A



B

	Pollen grain	Pollen tube
External diameter (µm), (a and a')	a = 18.1 µm ± 4.53	a' = 3.74 µm ± 0.17
Internal diameter (µm), (b and b')	b = 17.82 µm ± 4.55	b' = 3.52 µm ± 0.18
Thickness of wall (µm)	a-b = 0.28 µm ± 0.06	a'-b' = 0.22 µm ± 0.03
Volume formula	$V = 4/3\pi r^3$	$V = L\pi r^2$ with L = 1248 µm
Volume with wall (μm^3)	3104 μm^3	13710.32 μm^3
Volume without wall (μm^3)	2963 μm^3	12144.78 μm^3
Volume of wall (μm^3)	141 μm^3	1565.54 μm^3
Volume of wall for 67% germination rate		1048.91 μm^3
Ratio volume of wall from pollen tube / volume of wall from pollen grain		7.44 (for 67 % germination)

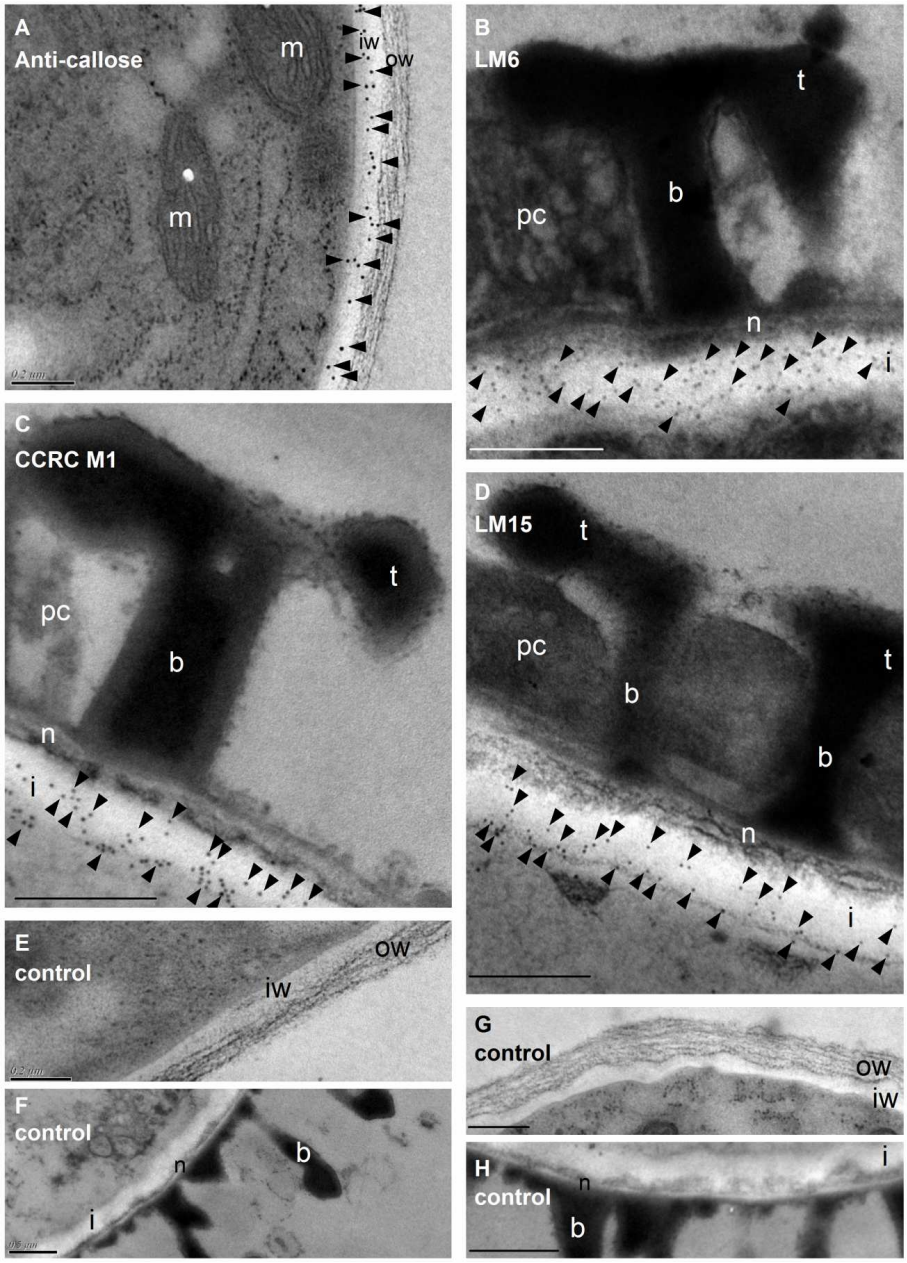


Figure S1. Immunogold localization of cell wall polymers in 6h-old Arabidopsis pollen tube (A), pollen grain (B-D) and controls (E-H). **A**, Localization of callose in pollen tube. Gold particles (arrowheads) are localized in the weakly electron-dense inner wall layer. **B**, Localization of (1→5)-α-L-arabinan epitopes with LM6 in pollen grain. Gold particles (arrowheads) are uniformly dispatched over the entire intine wall. The outer wall (bacula, nexine and tectum) forming the exine of the pollen grain and pollen coat (pc) are visible. **C**, Labeling of fucosylated XyG in the pollen grain with CCRC-M1. Gold particles (arrowheads) appeared located in the inner part of the intine wall. **D**, Localization of non galactosylated XyG in the pollen grain with LM15. The MAb also labeled the inner part of the intine wall, close to the plasma membrane. **E-F**, Controls with the anti-rat-gold conjugate in the pollen tube and pollen grain, respectively. No significant non-specific labeling was observed. **G-H**, Controls with the anti-mouse-gold conjugate in the pollen tube and pollen grain, respectively. No significant labeling was observed. b, bacula; i, intine; iw, inner wall; m, mitochondria; n, nexine; ow, outer wall; pc, pollen coat; t, tectum. Scale bars = 0.5 µm (B-E, G, I) and 0.2 µm (A, F, H).

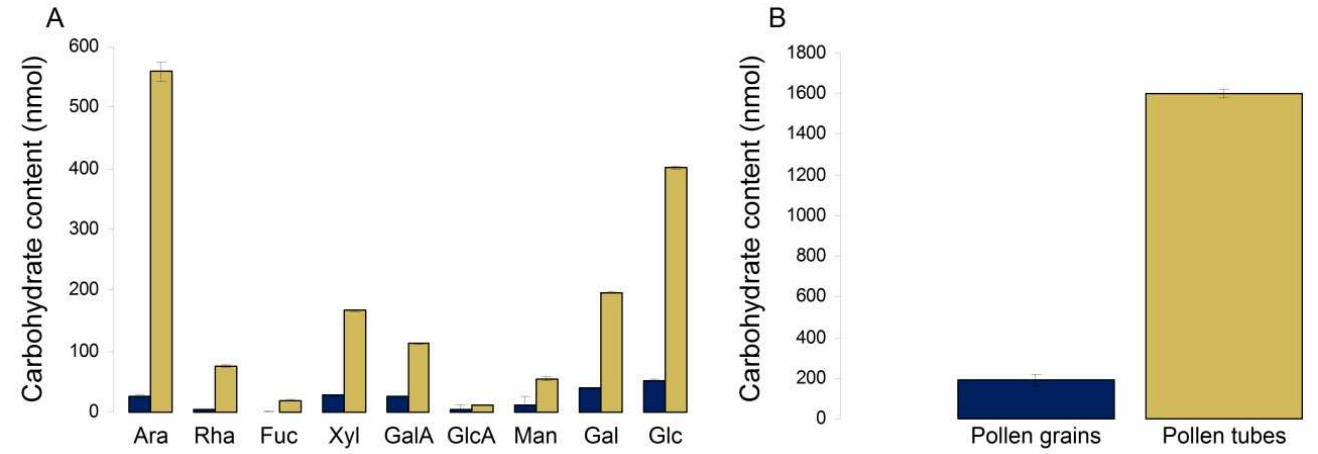


Figure S2. Monosaccharide composition (**A**) and carbohydrate content (**B**) of the cell wall of non-germinated pollen grains (blue) and 16h-*in vitro* grown pollen tubes (yellow) collected from 520 flowers.

Ara, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; GalA, galacturonic acid; GlcA, glucuronic acid; Man, mannose; Rha, rhamnose; Xyl, xylose.

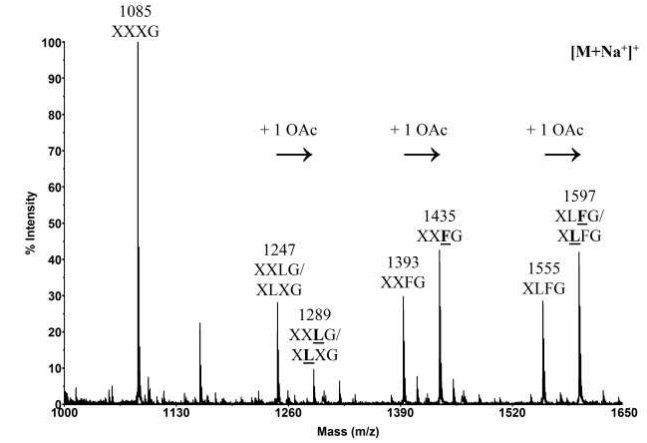


Figure S3. MALDI-TOF mass spectrum of *endo*-glucanase-generated XyG fragments from mature leaf cell wall. The structures of the XyG fragments are shown according to the nomenclature proposed by Fry et al., (1993). Underlined and bold structures represent *O*-acetylated side chains (+ 1 OAc).