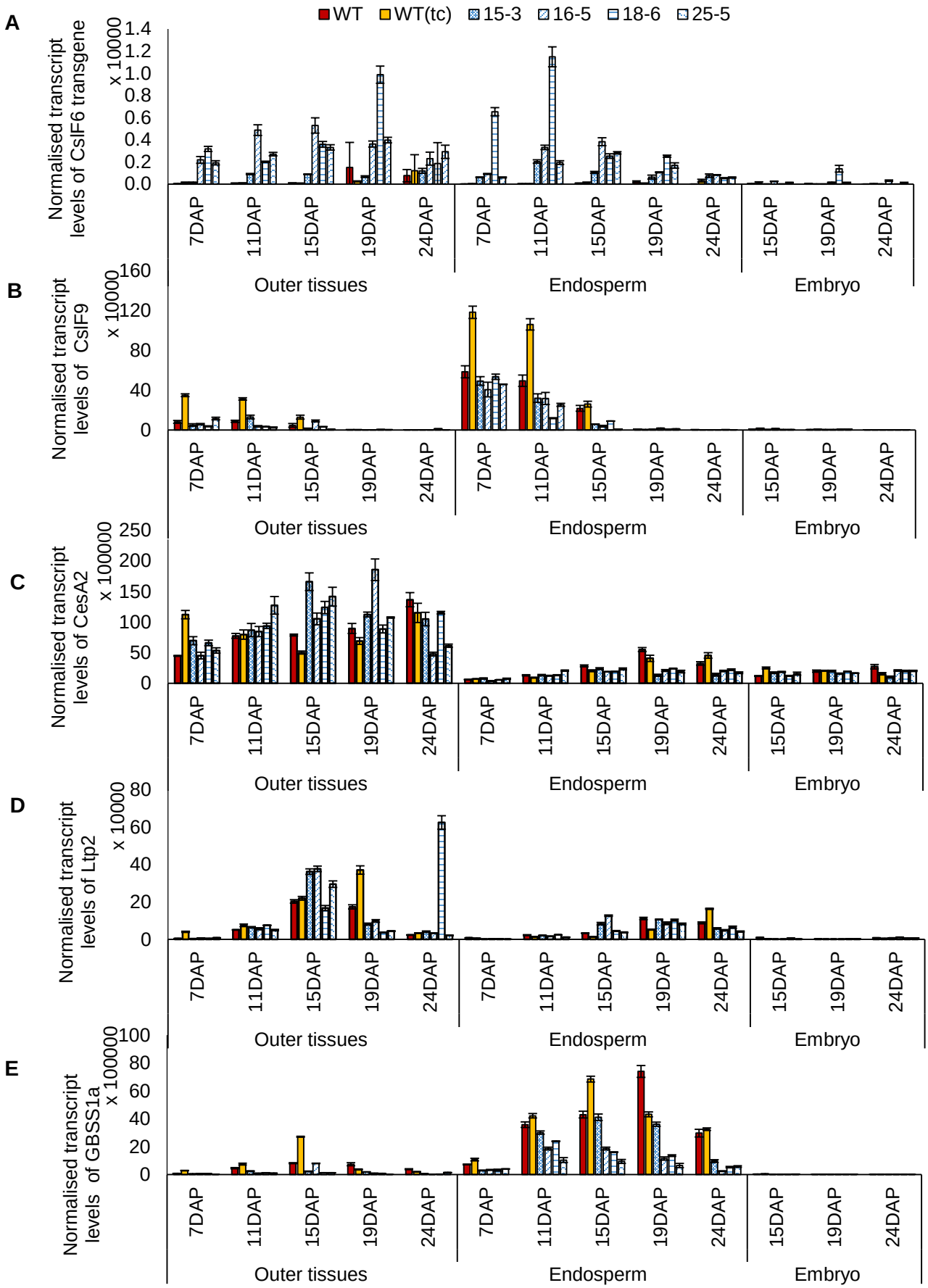

Fig. S1. Normalized transcript levels of (A) *HvCslF6* transgene, (B) *HvCslF9,* (C) *HvCesA2,* (D) *HvLtp2,* and (E) *HvGBSS1a* in developing grain tissues. The error bars show the standard deviation of the mean. Y-axis in arbitrary units.

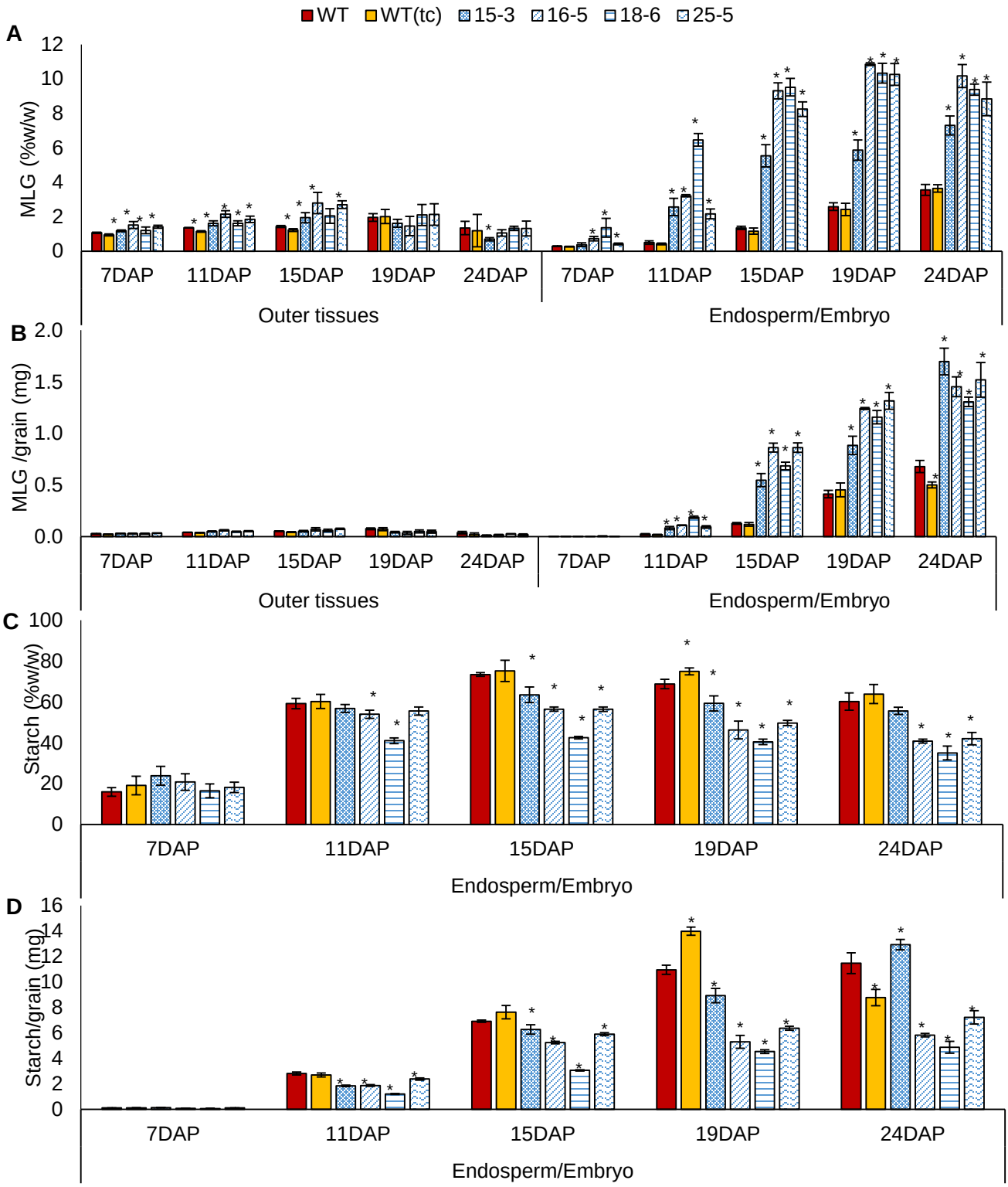

Fig. S2. (A) and (B) MLG and (C) and (D) starch content in developing barley grain as percentage on a weight per weight basis (w/w) and on a per grain basis (milligram, mg). For MLG and starch measurements from developing grain 'outer tissues' and endosperm were analysed together. The embryo, containing low amounts of storage carbohydrates, was removed and used for soluble sugar analysis. Error bars show the standard deviation of the mean. * indicates a significant difference from the WT by t-test with P-values of < 0.05.

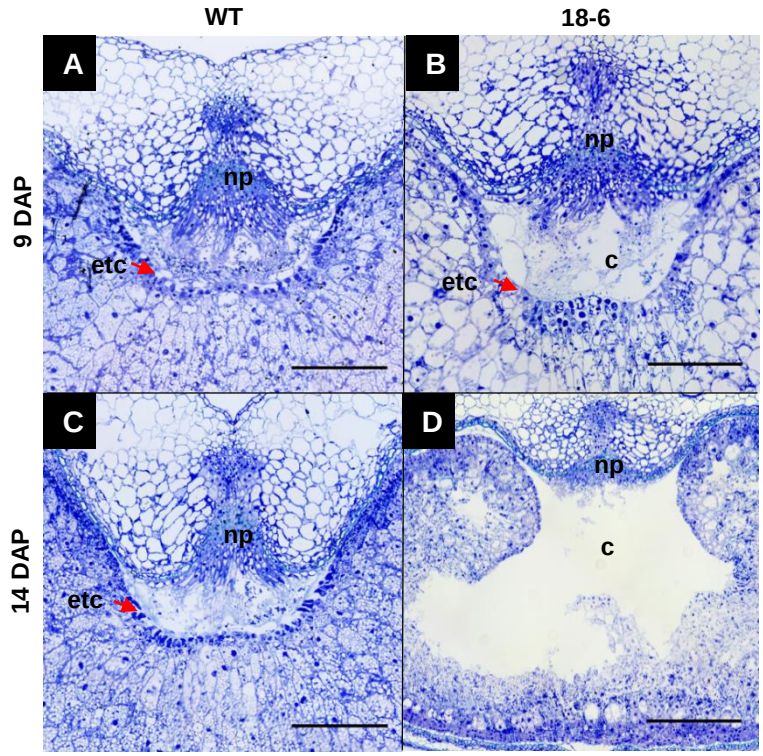

Fig. S3. Morphology of endosperm cavity in WT and 18-6 transgenic grain at (A) and (B) 9 DAP and (C) and (D) 14 DAP. Grain sections are stained with toluidine blue. All images are under 4x magnification with scale bars equivalent to 100 µm. Sections were prepared in the equatorial part of the grain for two biological replicates with two technical replicates each. Abbreviations: nucellar projection (np), cavity (c), endosperm transfer cells (etc), endosperm (e).

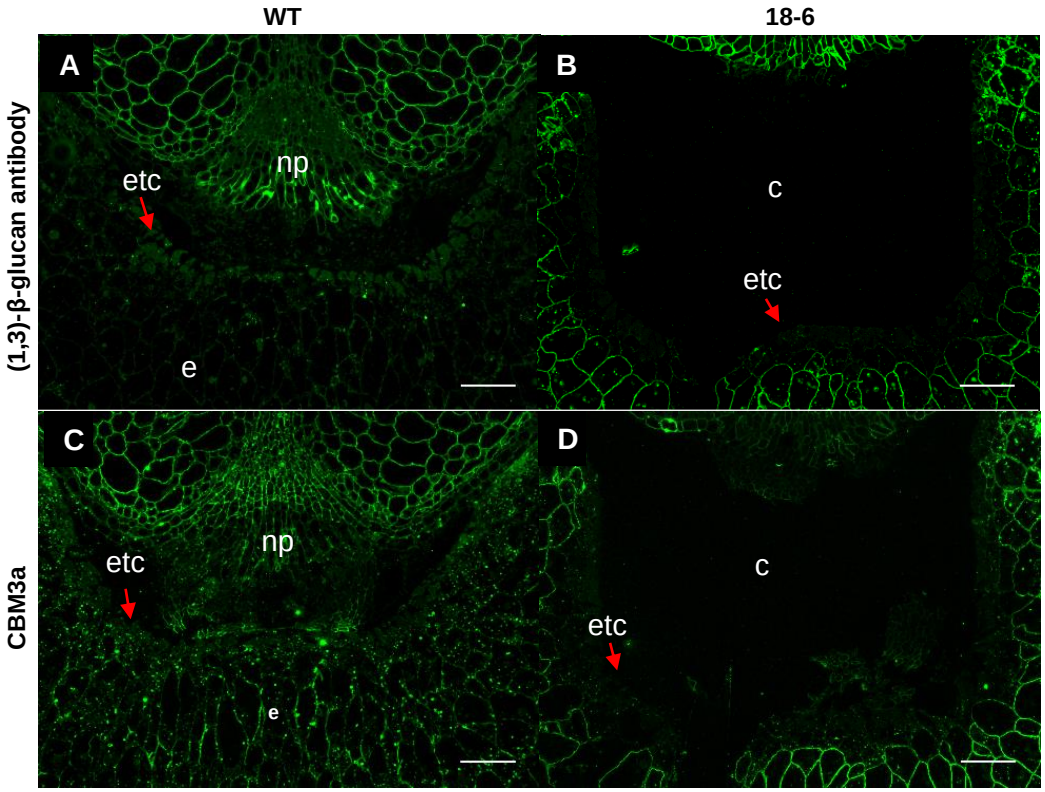

Fig. S4. Immuno-histochemical analysis of callose [(1,3)-β-glucan antibody] and crystalline cellulose (CBM3a) in ETC walls at 10 DAP. The fluorescence signals from (1,3)-β-glucan antibody and CBM3a are absent in the endosperm transfer cells in both WT and transgenic grain. All images are under 10x magnification with scale bars equivalent to 100µm. Sections were prepared in the equatorial part of the grain for two biological replicates with two technical replicates each. Abbreviations: nucellar projection (np), endosperm transfer cells (etc), cavity (c), endosperm (e), pericarp (p), aleurone (al).

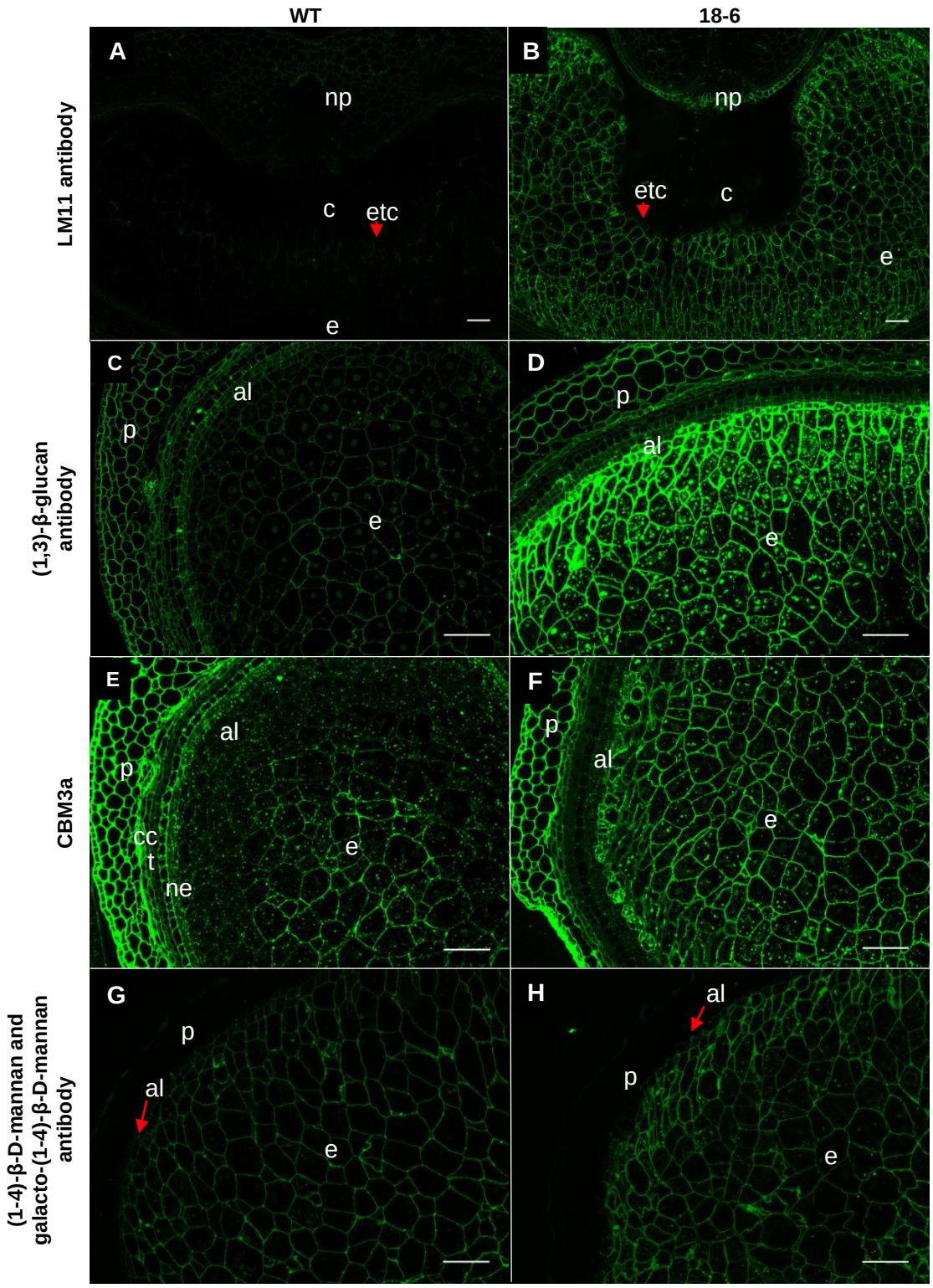

Fig. S5. Immuno-histochemical analysis of arabinoxylan (LM11antibody), callose [(1,3)-β-glucan antibody], crystalline cellulose (CBM3a) and mannan [(1-4)-β-D-mannan and galacto-(1-4)-β-D-mannan antibody] in endosperm cell walls at 10 DAP. The intensities of fluorescent signals from arabinoxylan and (1,3)-β-glucan are stronger in 18-6 transgenic grain relative to WT. Fluorescent signals from CBM3a and mannan are labelled uniformly in the transgenic endosperm relative to WT. The fluorescence signals from LM11 antibody are absent in the endosperm transfer cells in both WT and transgenic grain. (A) and (B) are under 5x magnification. (C) to (H) are under 10x magnification. All images have scale bars equivalent to 100µm. Sections were prepared in the equatorial part of the grain; two biological replicates with two technical replicates each. Abbreviations: nucellar projection (np), endosperm transfer cells (etc), cavity (c), endosperm (e), pericarp (p), aleurone (al), cross cells (cc), testa (t), nucellar epidermis (ne).

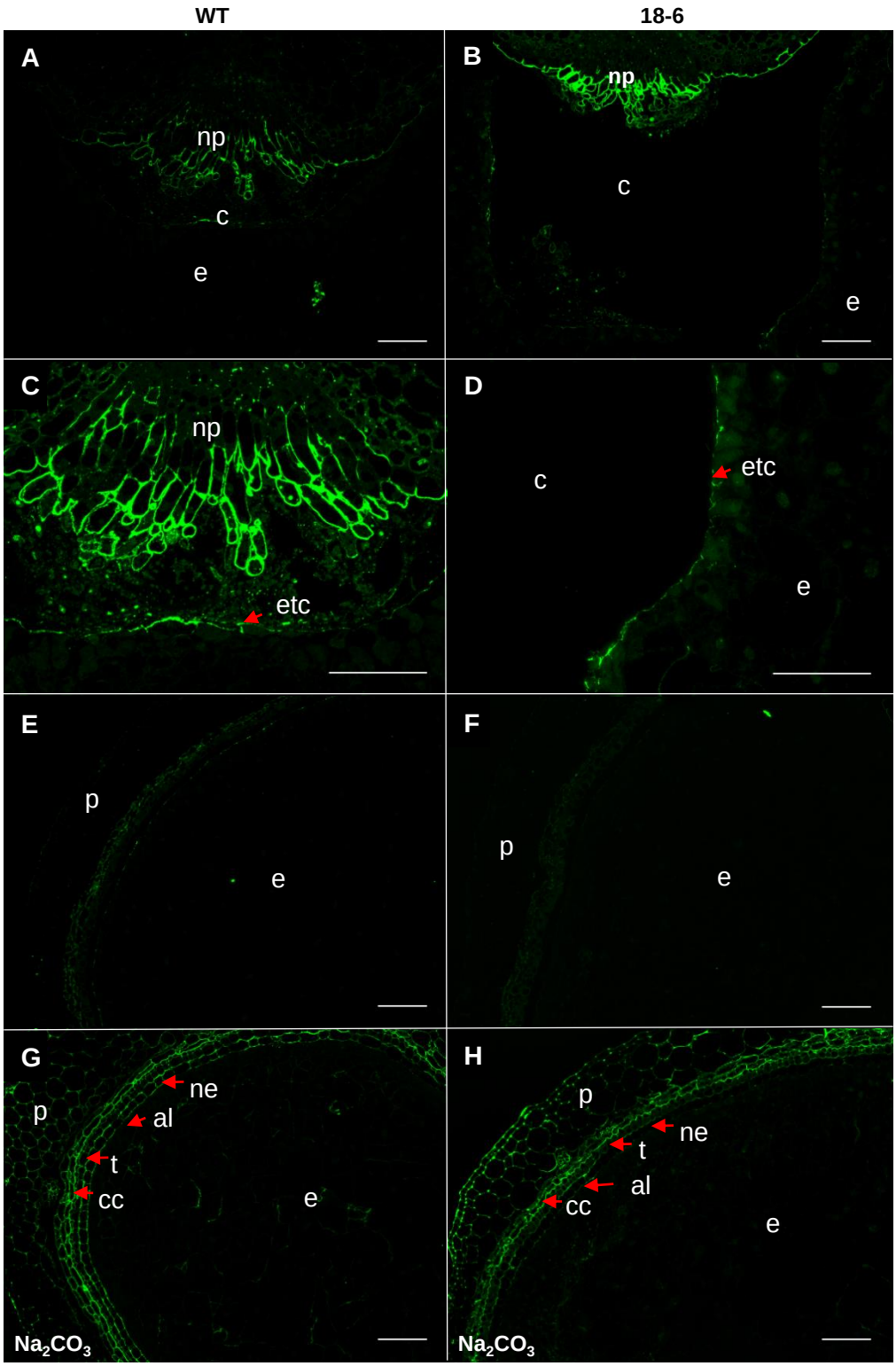

Fig. S6. Immuno-histochemical analyses of pectins with LM19 antibody on barley grain sections at 10 DAP. (A) to (D) The fluorescent signals from LM19 antibody labelled the cell walls of nucellar projection and the walls of endosperm transfer cells adjacent to the cavity in both transgenic and WT grain. In comparison to the untreated sections (E) and (F), grain sections pre-treated with 0.1 M sodium carbonate (Na_2CO_3) for 1 h prior to LM19 antibody labelling show stronger labelling intensities in the pericarp, cross cells, testa and nucellar epidermis (G) and (H) of both WT and transgenic grain. (A), (B), (E), (F), (G) and (H) are under 10x magnification. (C) and (D) are under 20x magnification. Scale bars are equivalent to 100µm. Sections were prepared in the equatorial part of the grain; two biological replicates with two technical replicates each. Abbreviations: nucellar projection (np), endosperm transfer cells (etc), cavity (c), endosperm (e), pericarp (p), nucellar epidermis (ne), testa (t),cross cells (cc) and aleurone (al).

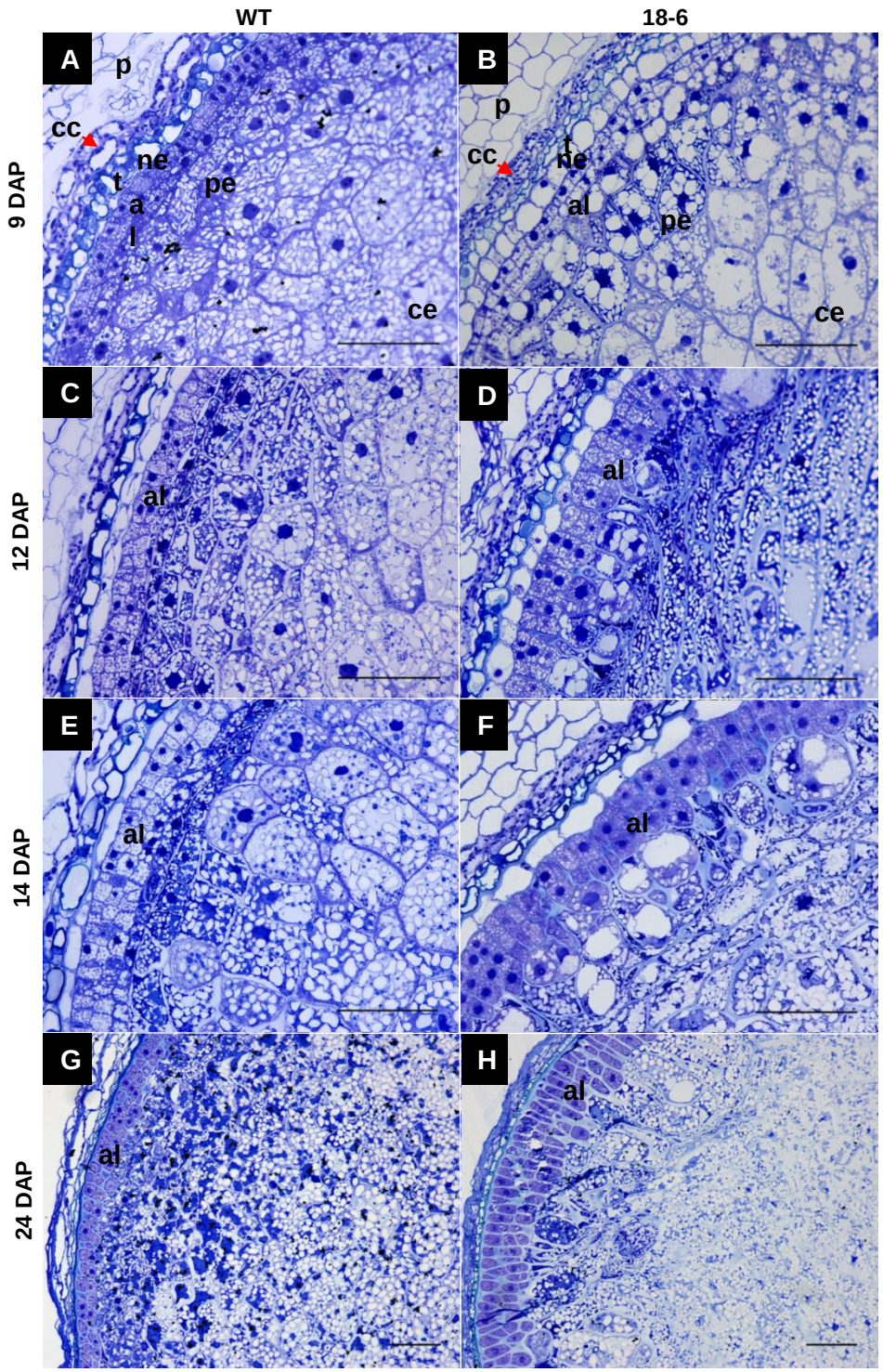

Fig. S7. Morphology of aleurone cells in WT and 18-6 transgenic grain during grain development. Grain sections are stained with toluidine blue. (A) to (F) are under 20x magnification. (G) and (H) are under 10x magnification. All images have scale bars equivalent to 100 µm. Sections were prepared in the equatorial part of the grain and images taken from the dorsal region for two biological replicates with two technical replicates each. Abbreviations: pericarp (p), cross cells (cc), testa (t), nucellar epidermis (ne), aleurone (al), peripheral endosperm (pe), central endosperm (ce).

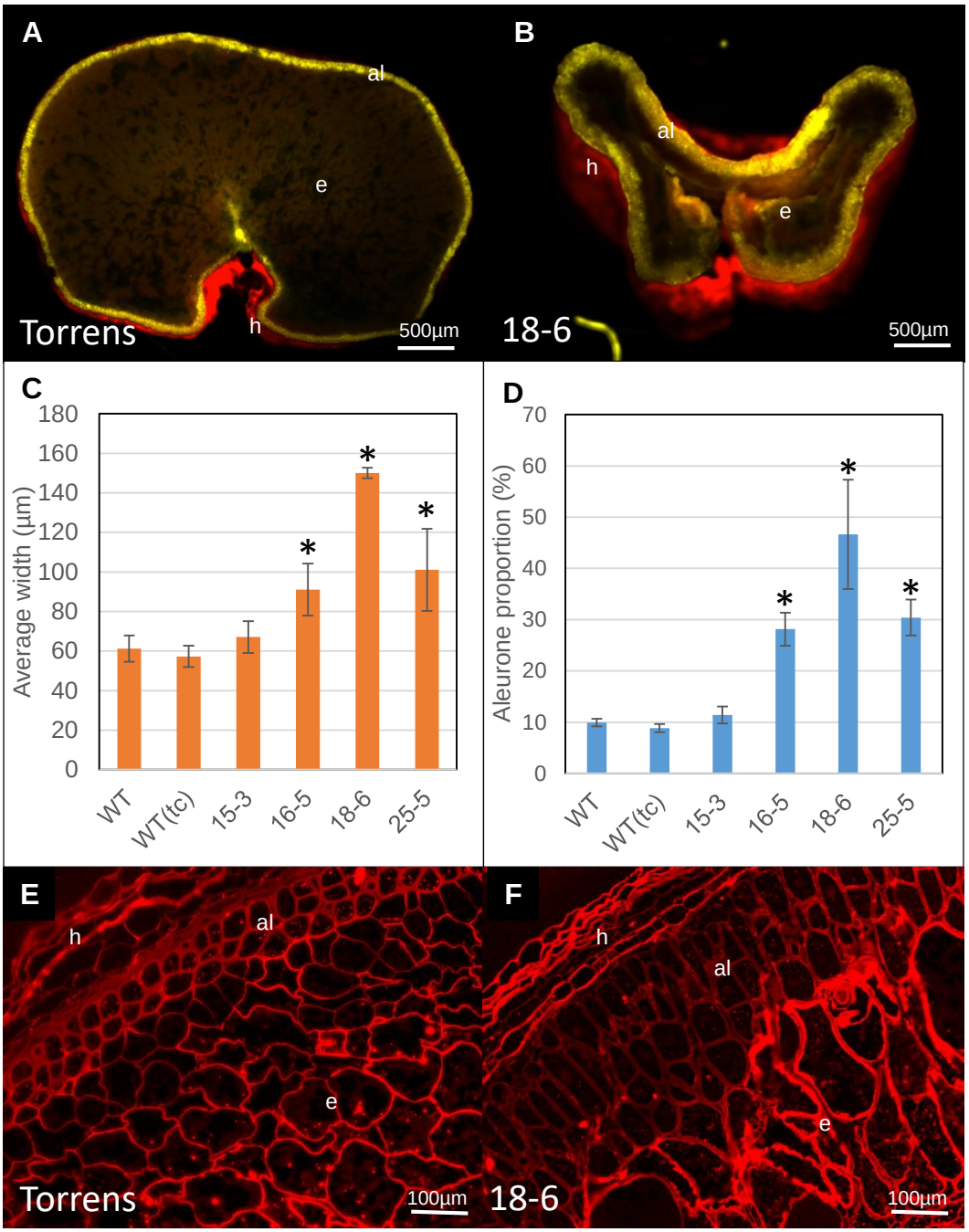

Fig. S8. Morphology of aleurone cells in WT and 18-6 transgenic grain at grain maturation and during grain development. (A and B) Mature, wholegrain transverse sections viewed at 1× magnification using Zeiss filter sets 46 (false-coloured red) and 49 (DAPI; false-coloured yellow). (C) Average width of the aleurone layer in mature grain in transverse sections. (D) Proportion of aleurone area of the total transverse endosperm area. * indicate significant differences to WT by t-test with $P < 0.05$. (E and F) Immunodetection of MLG in 24 DAP developing barley grain (false-coloured red). Abbreviations: aleurone (al), endosperm (e), husk (h).

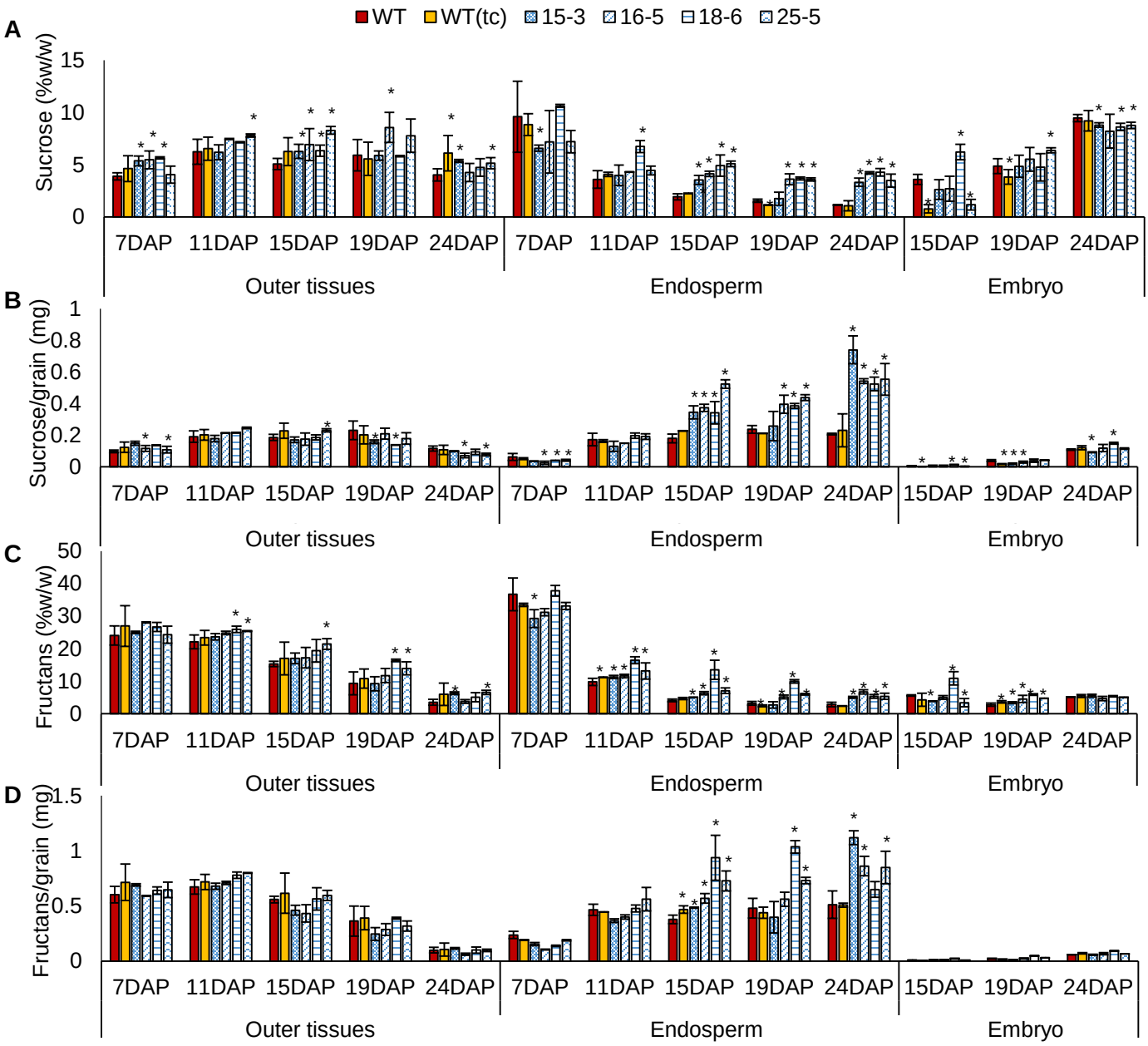

Fig. S9. (A) and (B) Sucrose and (C) and (D) total fructan content in developing barley grain as percentage on a weight per weight basis (w/w) and on a per grain basis (milligram, mg). For soluble sugar measurements, developing grains were separated in 'outer tissues', endosperm and embryo, and two biological replicates with two technical replicates each were analysed. The error bars show the standard deviation of the mean. * indicates a significant difference from the WT by t-test with P-values of < 0.05.

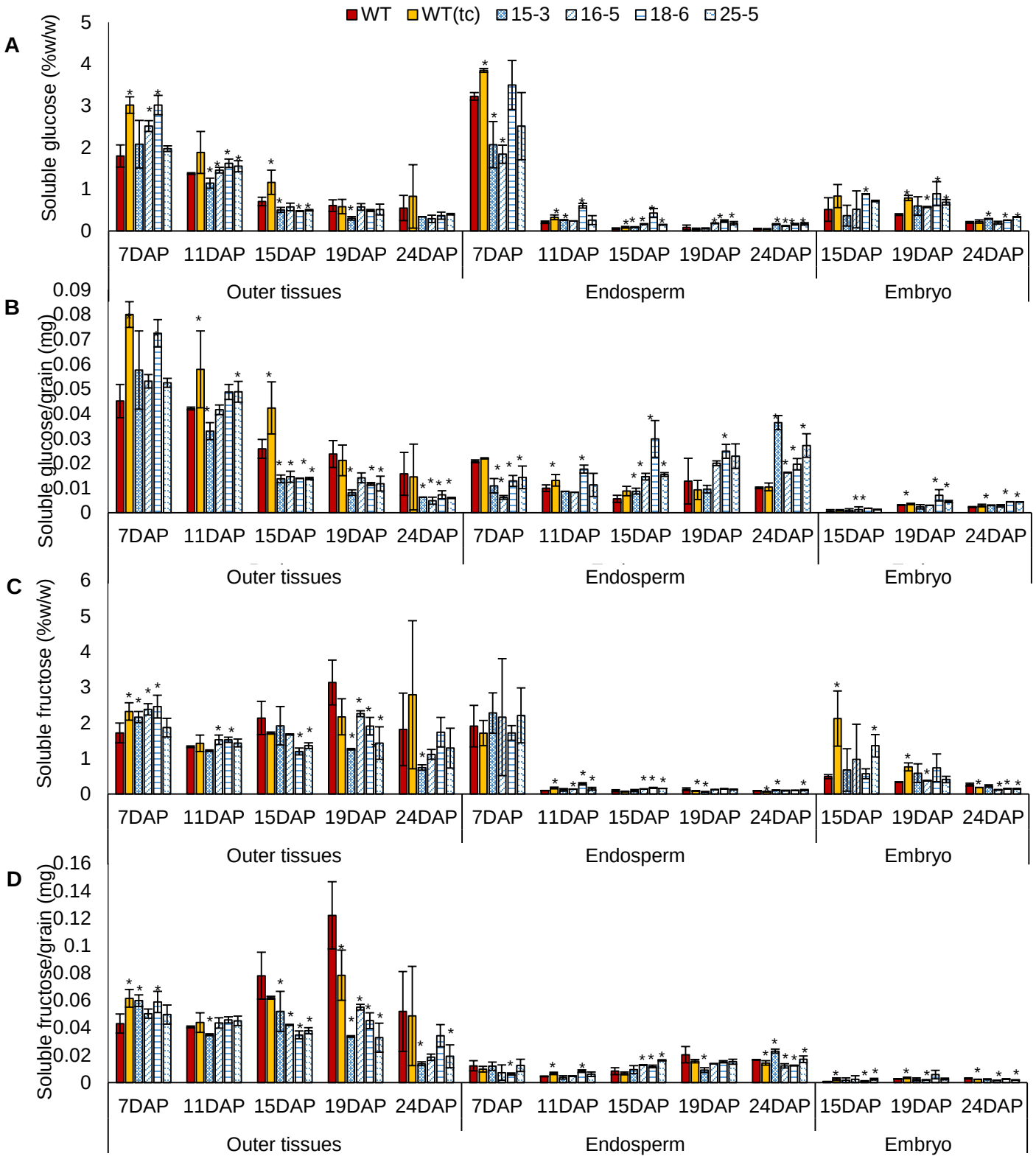

Fig. S10. (A) and (B) Glucose and (C) and (D) Fructose content in developing barley grain as percentage on a weight per weight basis (w/w) and on a per grain basis (milligram, mg). For soluble sugar measurements, developing grains were separated in 'outer tissues', endosperm and embryo, and two biological replicates with two technical replicates each were analysed. Error bars show the standard deviation of the mean. * indicates a significant difference from the WT by t-test with P-values of < 0.05.

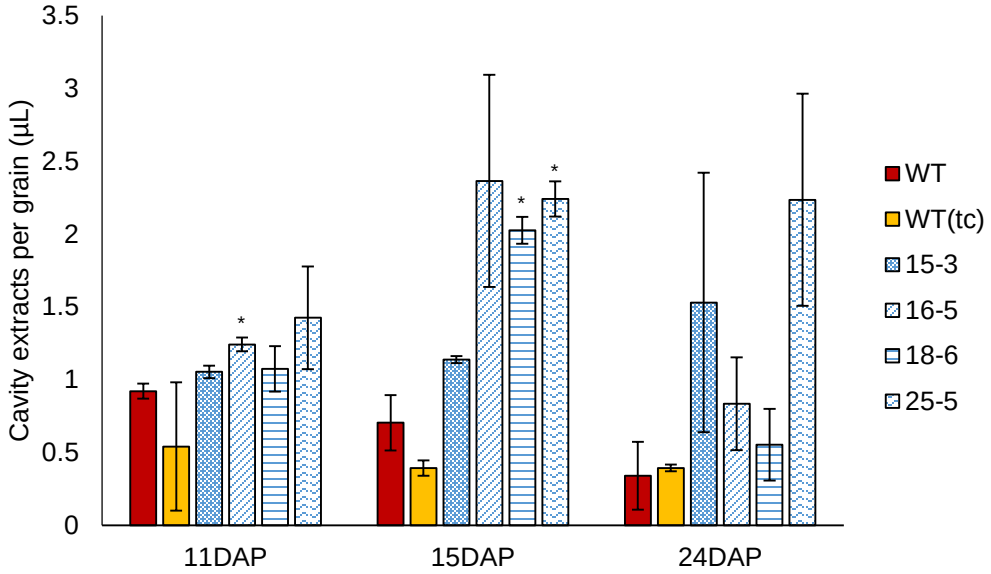

Fig. S11. Total volume of fluids in the endosperm cavity extracted from a single grain (microliter, µL). *pAsGlo1:HvCslF6* transgenic grain contain a greater amount of fluids in the endosperm cavity of a single grain at 15 DAP relative to WT. The error bars show the standard deviation of the mean of ten grains.*indicates a significant difference from the WT by t-test with P-values of < 0.05.