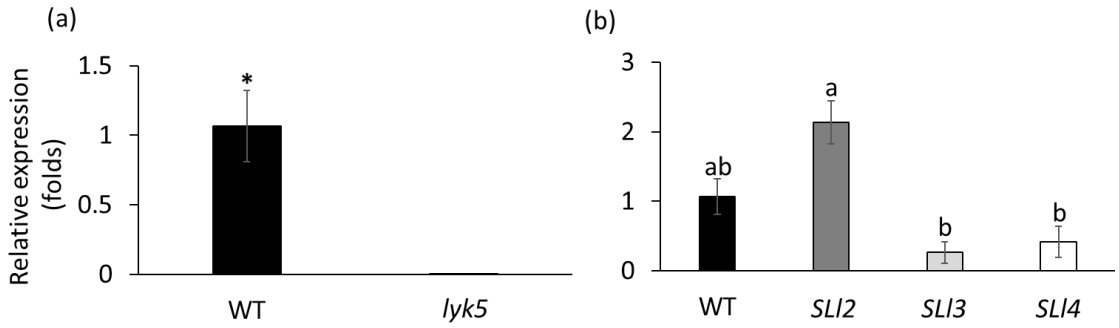

Figure S1. (a) qPCR was used to validate the loss of the gene from the *lyk5* mutant and (b) confirm the presence of the transgene in SCR::LYK5/*lyk5* plants. An asterisk and different letters above the columns represent significant differences between lines (*t*-test and Tukey's HSD test respectively, $P < 0.05$). Data are means (±SE) of at least three independent experiments.

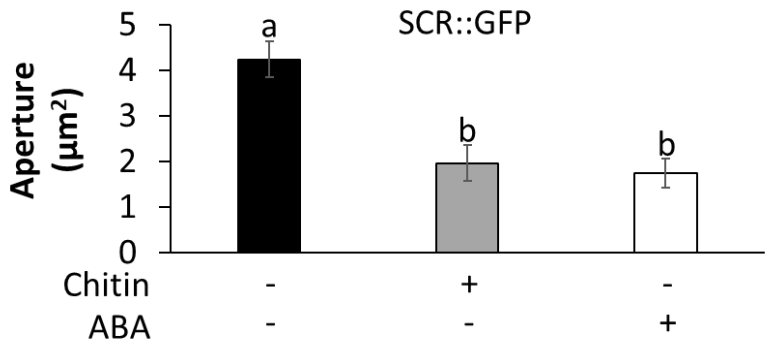

Figure S2. The size of the SCR::GFP stomata decreased in response to the application of chitin. To validate the chitin sensing of SCR::GFP plants, we evaluated stomatal closure in response to chitin application on epidermal peels. We used ABA as a positive control to ensure the physiological reliability of the experimental setup. Epidermal peels were incubated with stomata-opening solution, 100 µg/mL chitin and 1 µM ABA as a positive control for 1.5 h prior to the measurement. Different letters above the columns represent significant differences between treatments (Tukey's HSD test, $P < 0.05$). Data are means (±SE) from at least three independent experiments. (n >60)

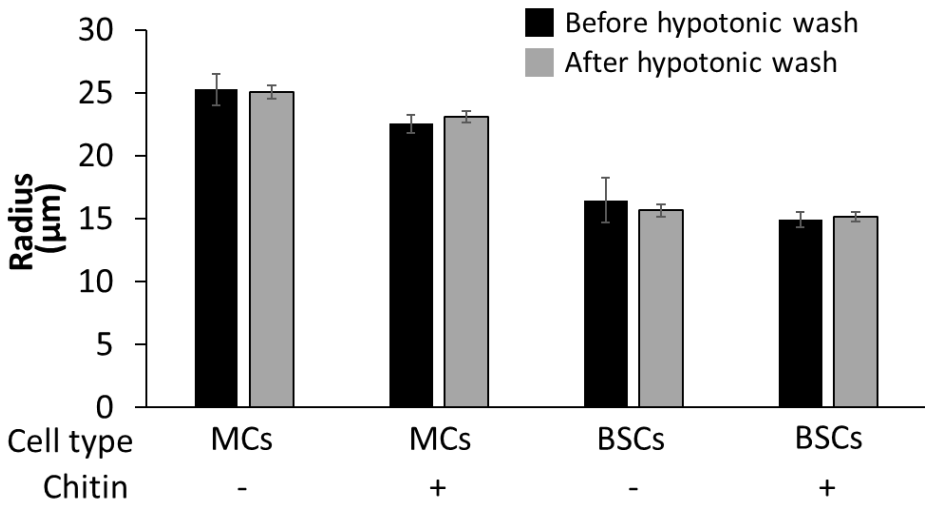

Figure S3. Chitin application had no impact on cells' ability to act as osmometers. Mesophyll cells (MCs) and bundle-sheath cells (BSCs) were incubated for 5 min in hypertonic solution after the wash, with or without chitin. No changes in cell radius were found, which indicates that the cells maintained their ability to act as perfect osmometers. Data are shown as means ± SE. (n >20)

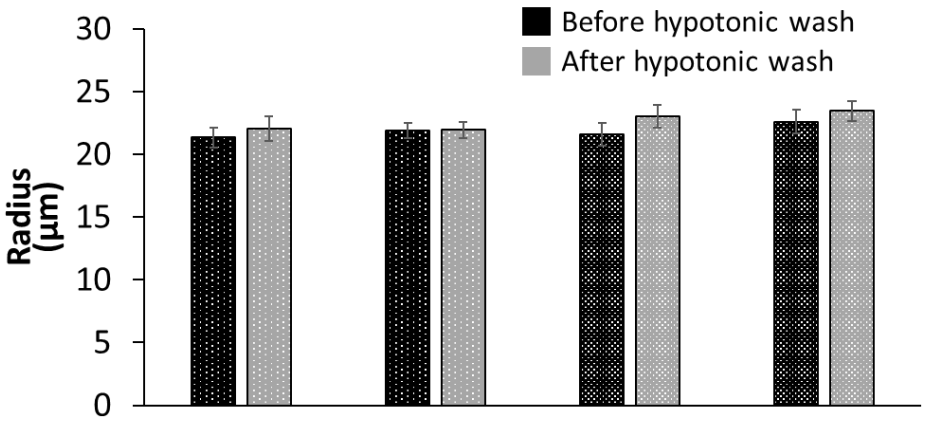

Figure S4. PEG transformation and chitin application had no impact on cell osmometer behavior. Cells were incubated for 5 min in hypertonic solution after the wash. No changes in cell radius were found, which indicates that the cells maintained their ability to act as perfect osmometers. Data are shown as means ± SE. (n >18)

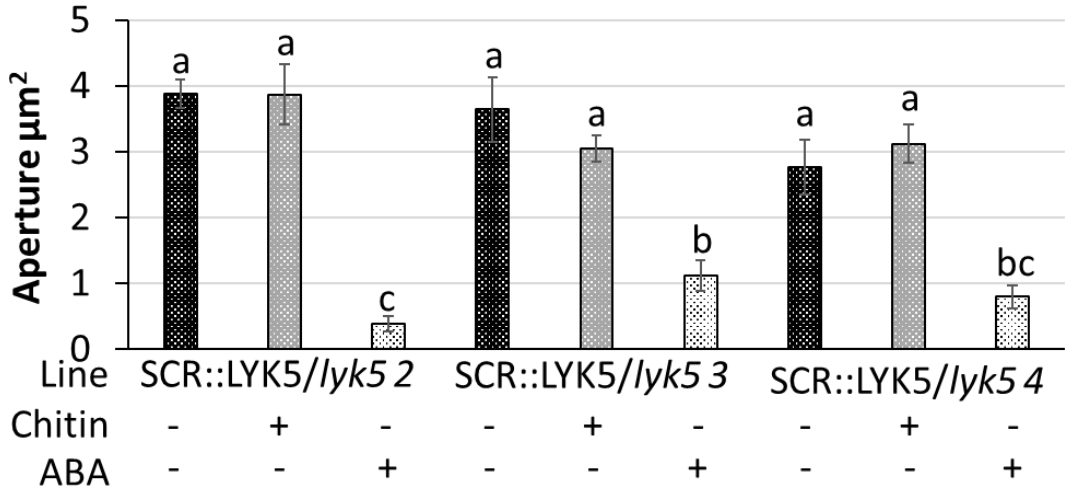

Figure S5. Chitin application did not affect stomatal aperture in epidermal peels of any of the independent lines of SCR::LYK5/*lyk5*. Images of abaxial epidermis were taken from leaves of 3 independent 8-week-old SCR::LYK5-complemented *lyk5* lines. ABA-treated peels were used as a control. Data are shown as means ± SE. Different letters above the columns indicate significant differences between treatments, according to Tukey's HSD test ($P < 0.05$). (n>60)

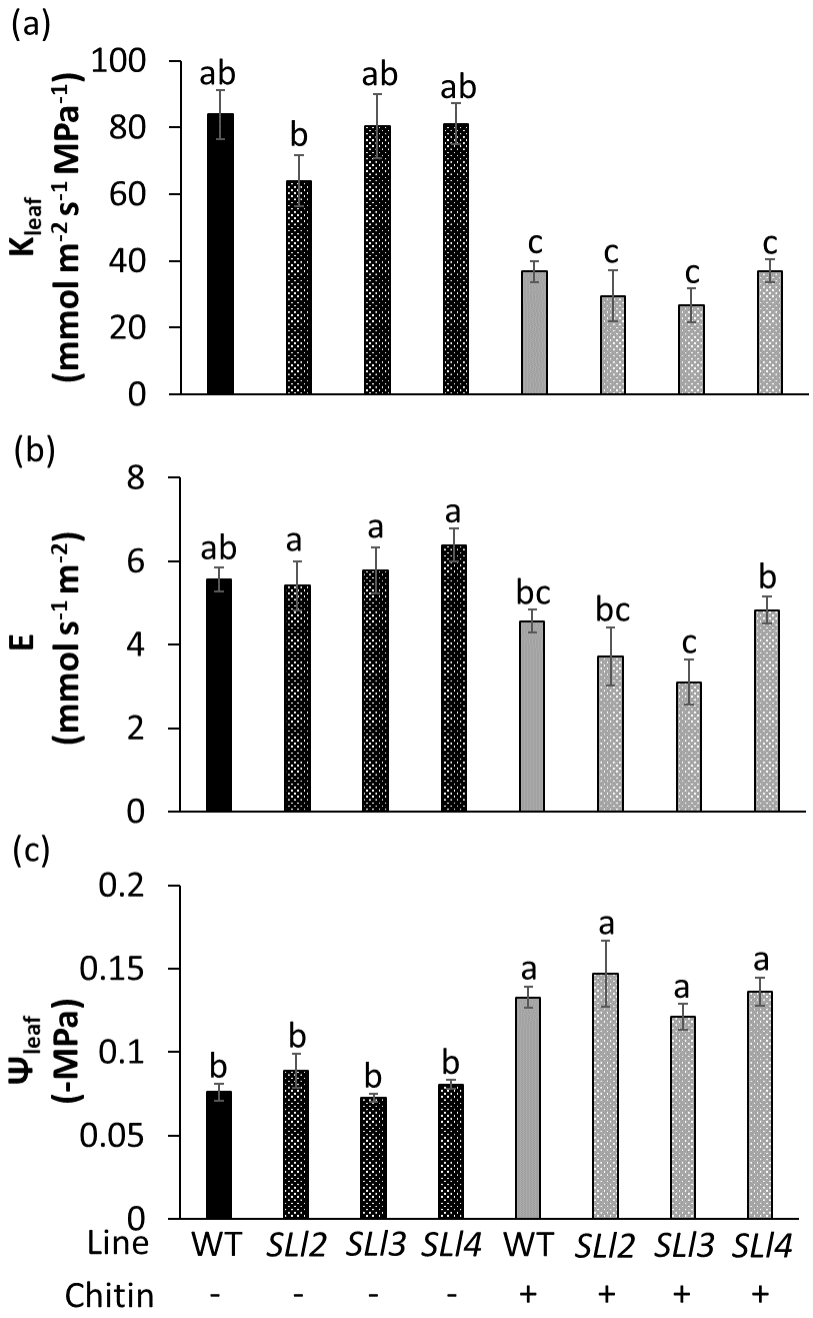

Figure S6. Complemented SCR::LYK5/*lyk5* *(SlI)* leaf hydraulic conductance (K_{leaf}) decreased in response to xylem-fed chitin in three independent lines. In order to validate the chitin sensitivity of the BS-specific complemented mutant, we analyzed the effects of chitin on K_{leaf}, *E* and Ψ_{leaf} in all three independent transgenic lines; we evaluated each line separately. All leaves were harvested before dawn and then fed AXS or 100 μg/mL chitin through the petiole.

After 2–4 h, (a) K_{leaf} was calculated for each individual leaf by dividing (b) the whole-leaf transpiration rate *E* by (c) the absolute value of the leaf water potential Ψ_{leaf}. Different letters above the columns represent significant differences between treatments (Tukey's HSD test, $P < 0.05$). Data are means (±SEs) from at least three independent experiments. ($n > 3$)

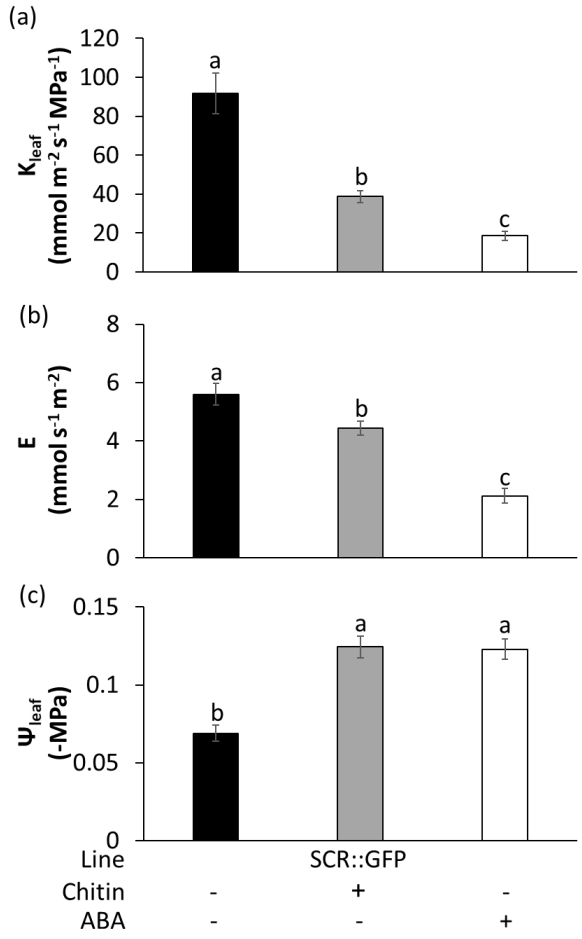

Figure S7. SCR::GFP exhibited reduced K_{leaf} following chitin application. To validate the chitin sensing of SCR::GFP plants, we evaluated the K_{leaf}, *E* and Ψ_{leaf} of leaves fed chitin through their petioles. We used ABA as a positive control to ensure the physiological reliability of the experimental setup. Leaves were harvested before dawn and then fed AXS, 100 μg/mL chitin or 1 μM ABA through the petiole. After 2–4 h, (a) K_{leaf} was calculated for each individual leaf by dividing (b) the whole-leaf transpiration rate *E* by (c) the absolute value of the leaf water potential Ψ_{leaf}. Different letters above the columns represent significant differences between treatments (Tukey's HSD test, $P < 0.05$). Data are means (±SEs) from at least three independent experiments. (n >20)