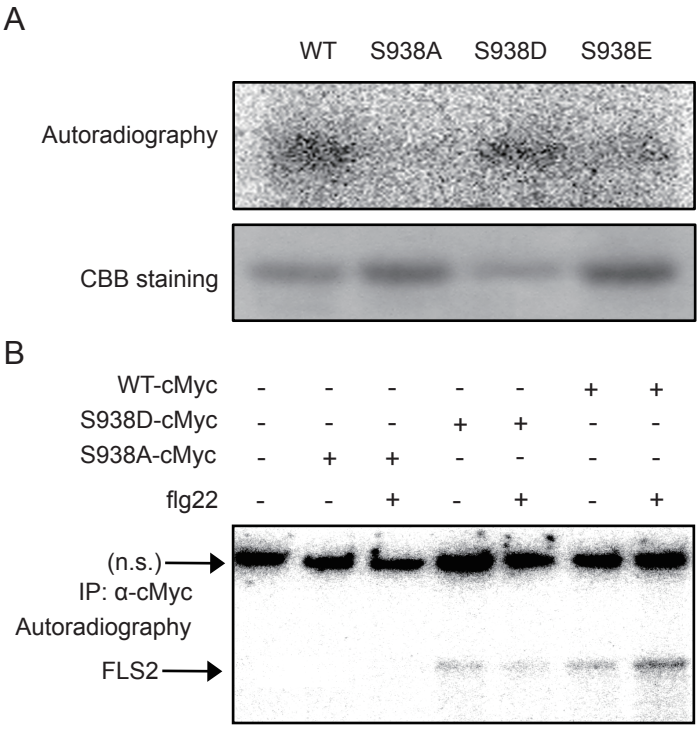

Supplemental Figure 8. Ser-938 may be important for phosphorylation of FLS2 in vitro and in vivo.

A. In vitro autophosphorylation activity of purified FLS2 kinase domains expressed from *E. coli* (GST tag removed during purification). Samples were separated by SDS-PAGE and then subjected to autoradiographic detection of ^{32}P. Lower panel: same samples, stained with Coomassie brilliant blue. Previous studies have also reported that FLS2 protein kinase activity is weak and/or difficult to detect in vitro [14,43,44]. In our experiments, FLS2^{WT} had very low autophosphorylation activity that was only detected in some experiments, using high sensitivity settings on the phosphorimager. However, in experiments where autophosphorylation activity of FLS2^{WT} was detected, FLS2^{S938A} lacked activity, suggesting that Ser-938 may be required for the autophosphorylation activity of FLS2. FLS2^{S938D} and FLS2^{S938E} apparently retained very weak autophosphorylation activity.

B. In vivo phosphorylation of FLS2^{WT}, FLS2^{S938A}, and FLS2^{S938D} before (-) or 15 min. after (+) exposure to 1 μM flg22. Col-0 *fls2-101* protoplasts transformed with the indicated full-length FLS2-myc alleles or PEG-only negative control were incubated for 10 hours in media carrying ^{32}P inorganic phosphate prior to collection, immunoprecipitation using anti-cMyc antibody, separation by SDS-PAGE and autoradiographic detection of ^{32}P. Note that gel run was very long (lower full-length FLS2 band migrates at ~170 kDa); upper band next to interface with stacking gel migrates at an extremely high apparent M.W. and is non-specific (n.s.; also present in control with no myc tag). The observed phosphorylation of FLS2^{S938D} indicates that Ser-938 is not the only phosphorylation site on FLS2. The absence of phosphorylation of FLS2^{S938A} is a more tentative observation because, although similar overall protein amounts were loaded in all lanes, the presence of ^{32}P prevented use of x-ray film to confirm presence of FLS2^{S938A} using anti-myc antibody. However, FLS2-S938D-myc and FLS2-WT-myc are visible in this experiment due to ^{32}P labeling, and in other experiments with FLS2^{S938A} its levels were similar to those of FLS2^{S938D} and FLS2^{WT} (e.g., Fig. 5, Fig. 6, Supplemental Fig. 2 (which includes Endo H tests for normal ER processing), and unpublished experiments).