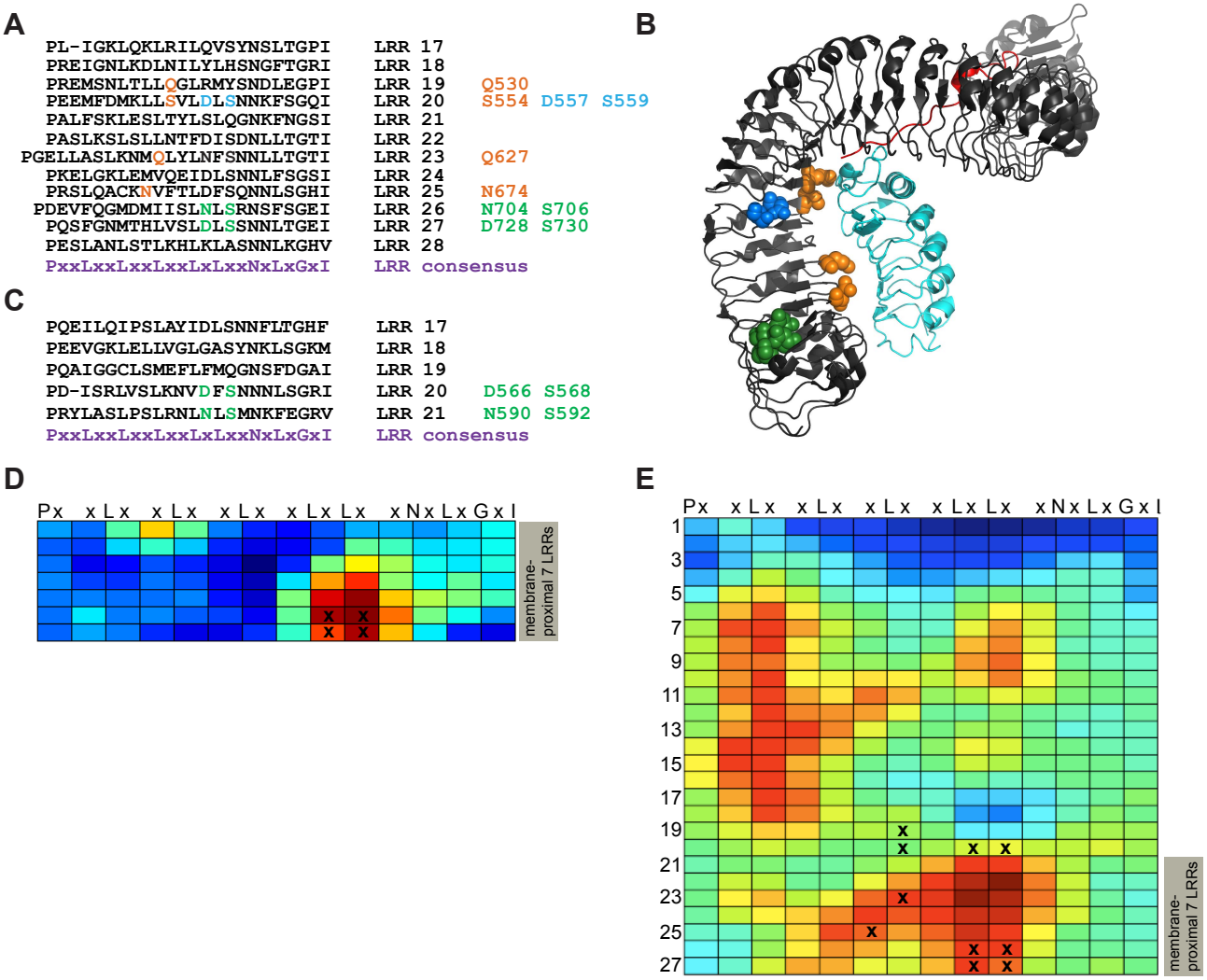
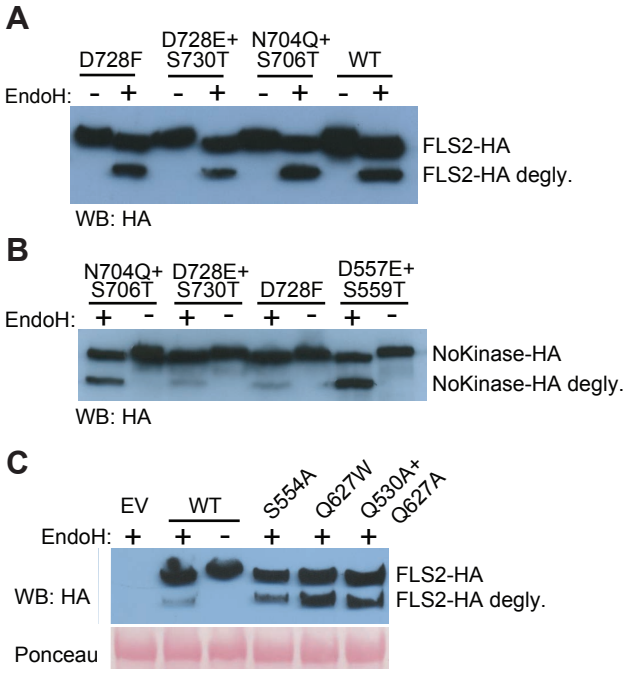
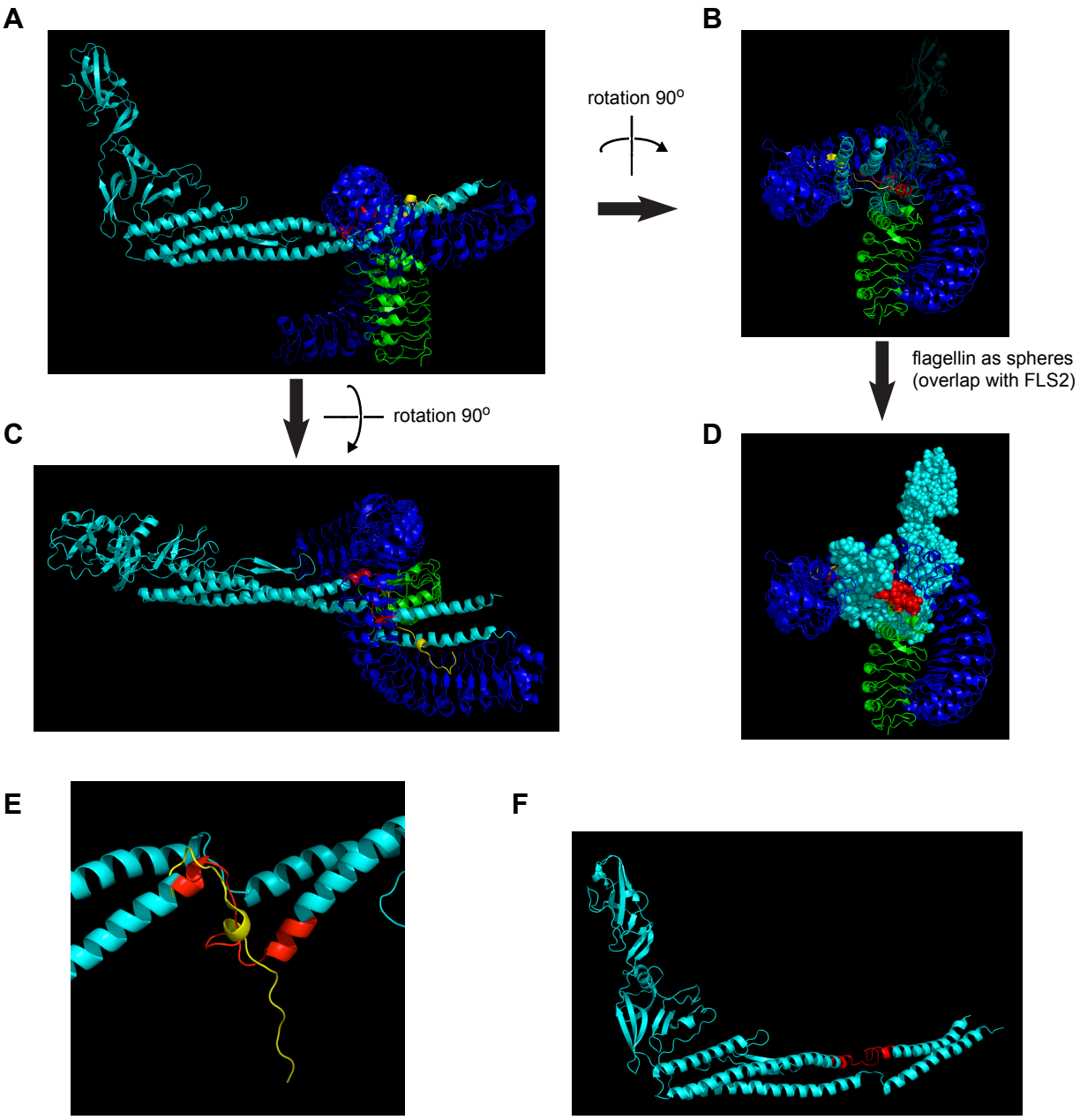


Supplemental Figure 1: Mutation sites in FLS2 and EFR ECDs. (A) Sites subjected to site-directed mutagenesis in the FLS2 LRR domain. Only repeats 17-28 are shown (FLS2: total 28 repeats). Green: mutation sites in the conserved LRR domain C-terminus (see also (B, D, E)). Blue: "control" mutations; sites similar to N704/D728 and S706/S730 but outside of the conserved region; blue "control" sites also adjacent to but outside of FLS2 BAK1-interaction site. Orange: mutation sites based on FLS2 BAK1-interaction sites in the FLS2-flg22-BAK1 ECD co-crystal structure. **(B)** Mutation sites as described in (A), using same color scheme as in (A). Structure is PDB ID: 4MN8 with FLS2 backbone as black ribbon, BAK1 backbone as light blue ribbon, and flg22 backbone as red ribbon. Space-filling spheres show side-chains only for mutagenized sites. **(C)** Mutation sites in the EFR LRR domain. Only repeats 17-21 are shown (EFR: total 21 repeats). Green: mutation sites in the conserved LRR domain C-terminus (see also (D)). **(D, E)** Regional LRR surface conservation maps from Arabidopsis FLS2, EFR, PEPR1 and BRI1 (D) or eleven non-Brassicaceae FLS2s (E), as shown and described in Figure 2, with x's at the FLS2 and EFR LRR domain amino acid positions described above that were subjected to site-directed mutagenesis in the present study.



Supplemental Figure 2: EndoH assay reveals no glycosylation defects in mutated FLS2 and FLS2-NoKinase. (A) Protein extracts from Arabidopsis *fls2*$^{-}$ leaves carrying P_{FLS2}*-FLS2-HA* with mutations as indicated or WT (no mutations), not digested (-) or digested (+) with endoglycosidaseH (EndoH). An EndoH-resistant protein pool (characteristic of mature glycosylated proteins) is visible in all EndoH-treated samples. **(B)** Protein extracts from *Nicotiana benthamiana* carrying *35S-FLS2-NoKinase-HA* with mutations as indicated, not digested (-) or digested (+) with endoglycosidaseH (EndoH). An EndoH-resistant protein pool is visible in all EndoH-treated samples. Mutations D557E+S559T were included as control mutations located in sites of a single LRR repeat analogous to D728E+S730T, but outside of the conserved LRR C-terminus. **(C)** Protein extracts from Arabidopsis *fls2*$^{-}$ seedlings carrying P_{FLS2}*-FLS2-HA* with mutations as indicated or WT (no mutations), digested with EndoH. An EndoH-resistant protein pool is visible in all EndoH-treated samples except for the empty vector (EV) control. Ponceau stained blot shows similar loading of total protein in all lanes including EV negative control. Degly.: FLS2 pools deglycosylated by EndoH.



Supplemental Figure 3: Hypothetical docking of full-length flagellin structure (PDB ID: 3A5X) to FLS2-flg22-BAK1 structure (PDB ID: 4MN8) illustrates minimal space for flagellin inside FLS2 LRR, and constraints to flg22 contact with FLS2 LRRs #3-15 if flg22 region is held within full-length flagellin. (A), (B), (C) Flagellin, hypothetically positioned so that flg22 residues within full-length flagellin are near the flg22 binding sites of FLS2 and BAK1. PDB structures 3A5X and 4MN8 superimposed at same scale; (B) and (C) are 90° rotated views of (A). Light blue: flagellin; red: flg22 residues within flagellin (3A5X). Dark blue: FLS2 LRR; green: BAK1 LRR; yellow: flg22 co-crystallized with FLS2 and BAK1 LRRs (4MN8). **(D)** Same view as (C), with space-filling representation of flagellin to more clearly illustrate impossible overlap of flagellin and FLS2 residues in same spatial locations in this arrangement (and other arrangements) of 3A5X and 4MN8. FLS2 and BAK1 side-chains omitted for clarity. **(E)** PyMol alignment of flg22 (yellow, in structure 4MN8) and flg22 region within flagellin (red, in structure 3A5X). Lower portions of flg22 in (E) (the yellow residues that are not proximal to red residues) are the N-terminal 7 residues of flg22 that associate with FLS2 LRRs #3-7 (FLS2 and BAK1 not shown, for clarity). **(F)** Full length flagellin (PDB structure 3A5X) colored as in (A) but shown by itself, showing that flg22 region forms a less-ordered hinge region between flanking pairs of alpha-helical bundles.