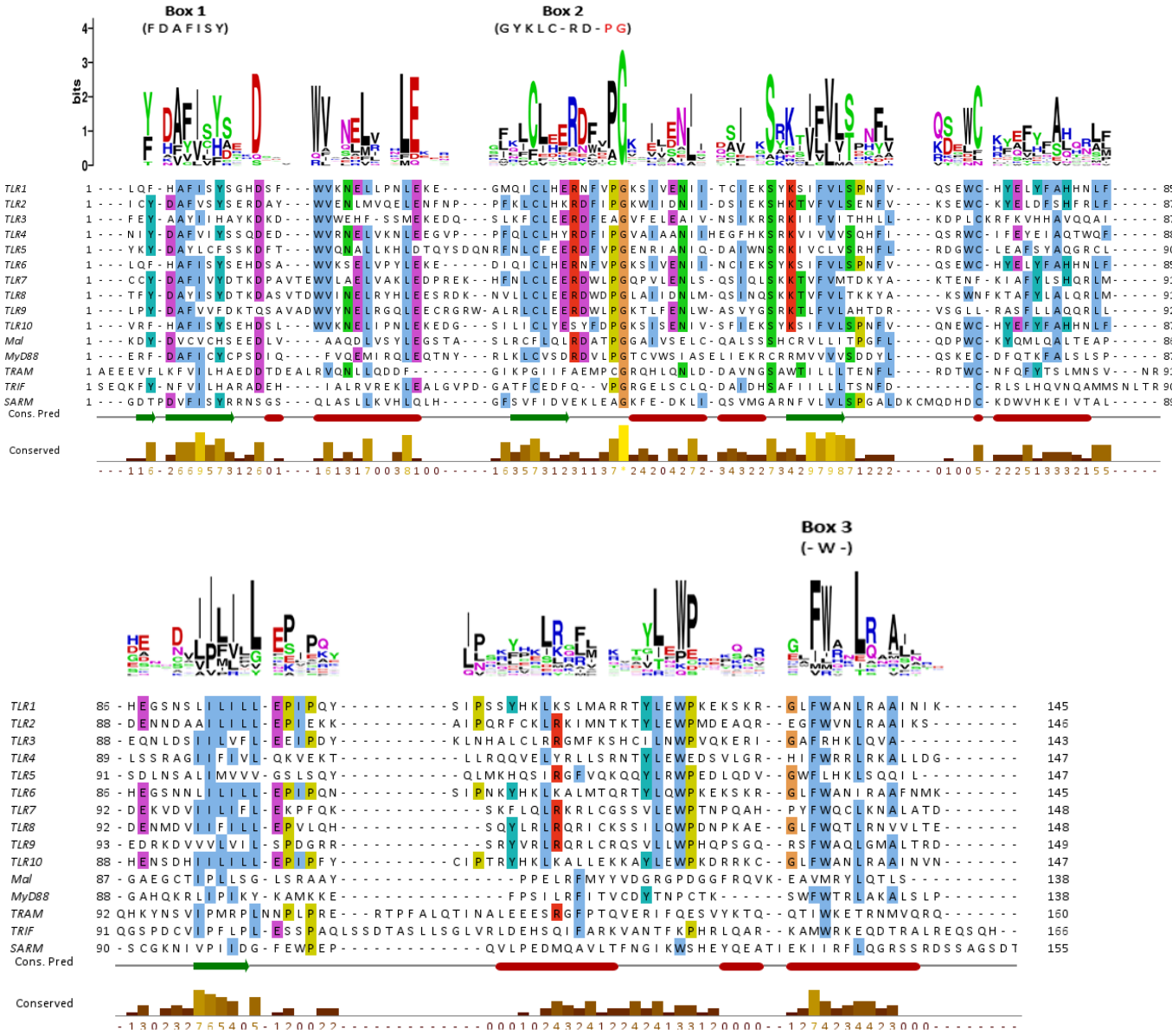
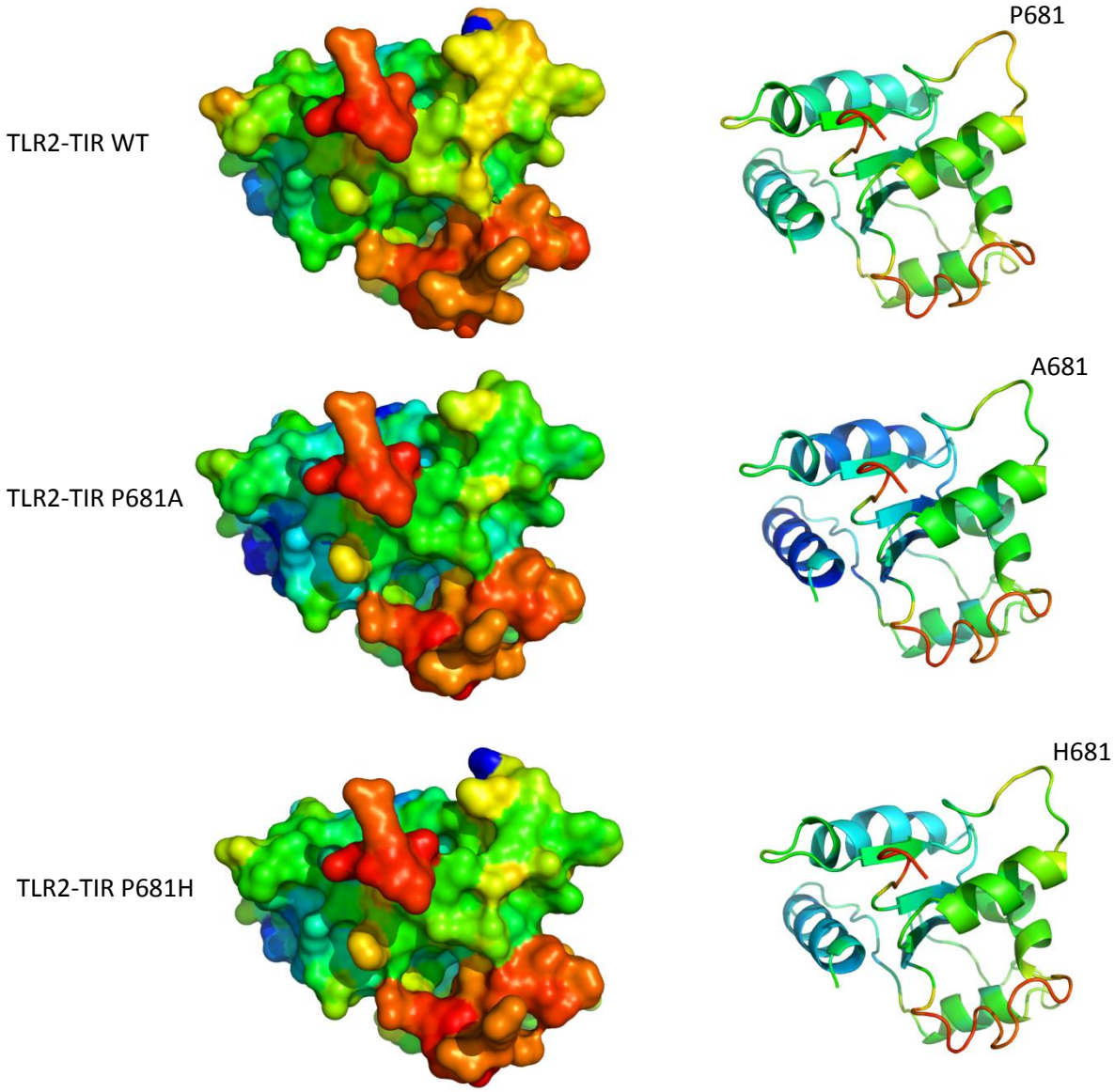


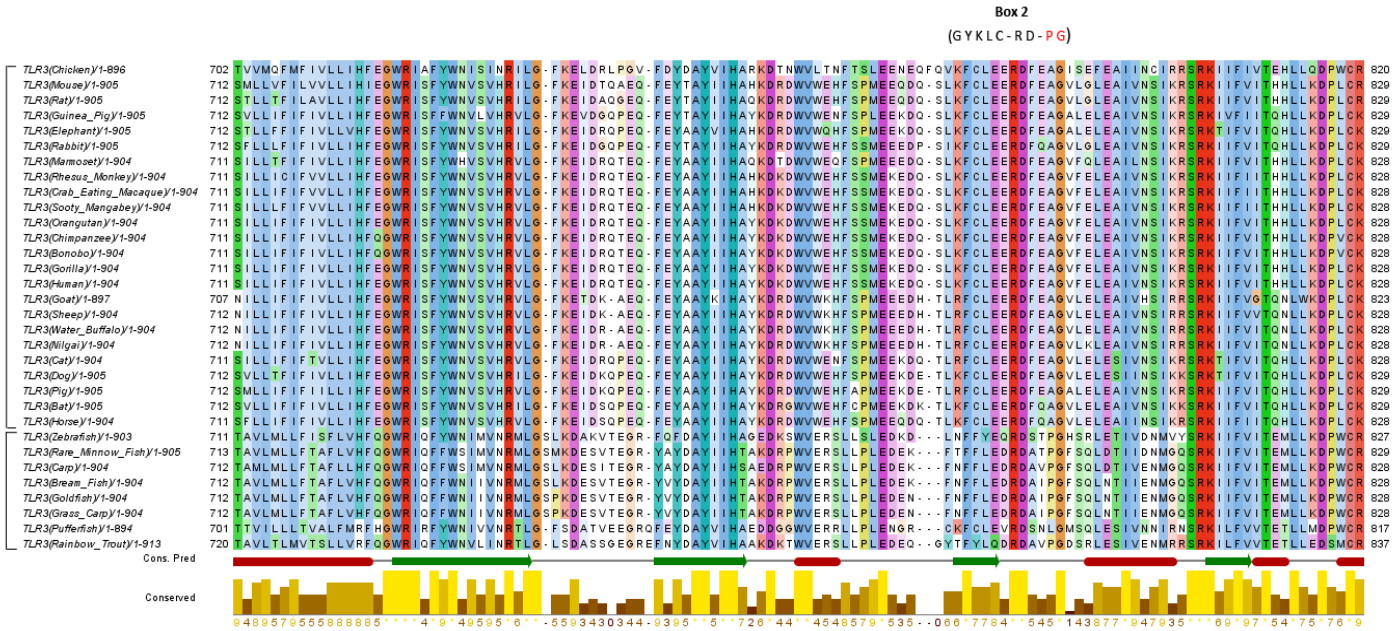
Supplementary Figure 1. Amino acid sequence alignment of TIR domains in human TLRs and their adaptor proteins

Sequence alignment and secondary structure prediction of TIR domains was generated using MUSCLE and Jnet and viewed using Jalview. The conserved residues are coloured based on a clustalx colour scheme. The consensus prediction marks helices as red tubes, and sheets as dark green arrows. Sequence conservation is primarily confined to three short motifs denoted as boxes 1, 2, and 3. The highlighted proline-glycine region of box 2 indicates the glycine residue being conserved amongst all the TIR domains whilst the proline is found to be absent in TLR3 and adaptor proteins TRAM and SARM, all of which are all involved in TRIF-dependent signalling.



Supplementary Figure 2. Crystal structure of the TLR2 TIR domain and BB-loop mutations

Surface and cartoon representation of the crystal structure of TLR2 TIR domain (1FYX) and BB-loop mutations P681A and P681H (1FYW). P681A mutation was obtained using the mutate model script within Modeller. Coloured based on b-factor, where the rainbow-like colouring represents the thermal fluctuations of each atom in the solved crystal structure. High b-factors (coloured red) indicate the area has some flexibility 'floppy' regions, even in the context of the crystal, while low b-factor (blue) areas are very rigid.



Supplementary Figure 3. Amino acid sequence alignment of the box 2 domain from various TLR3 species

Sequence alignment and secondary structure prediction of the box 2 domain from various TLR3 species was generated using MUSCLE and Jnet and viewed using Jalview. The conserved residues are coloured based on a clustalx colour scheme. The highlighted box pinpoints the conserved alanine-glycine residues across all species of mammals and birds with the exception of fish. In fish this region is a conserved proline-glycine (except puffer fish), which is the same conserved residues as seen in all human TLRs.