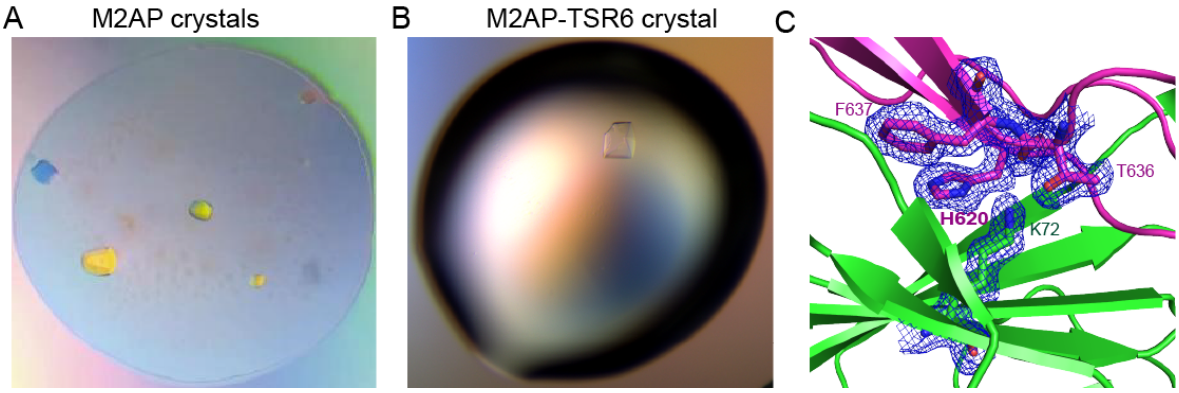

Figure S1. The crystals and electron density map.

A-B. M2AP and M2AP-TSR6 crystals. C. The 2mFo-DFc electron density map of the key residues in the complex. The residues His-620, Thr-636 and Phe-637 in TSR6 and K72 in M2AP are shown as sticks. 2mFo-DFc contoured at 1σ (blue).

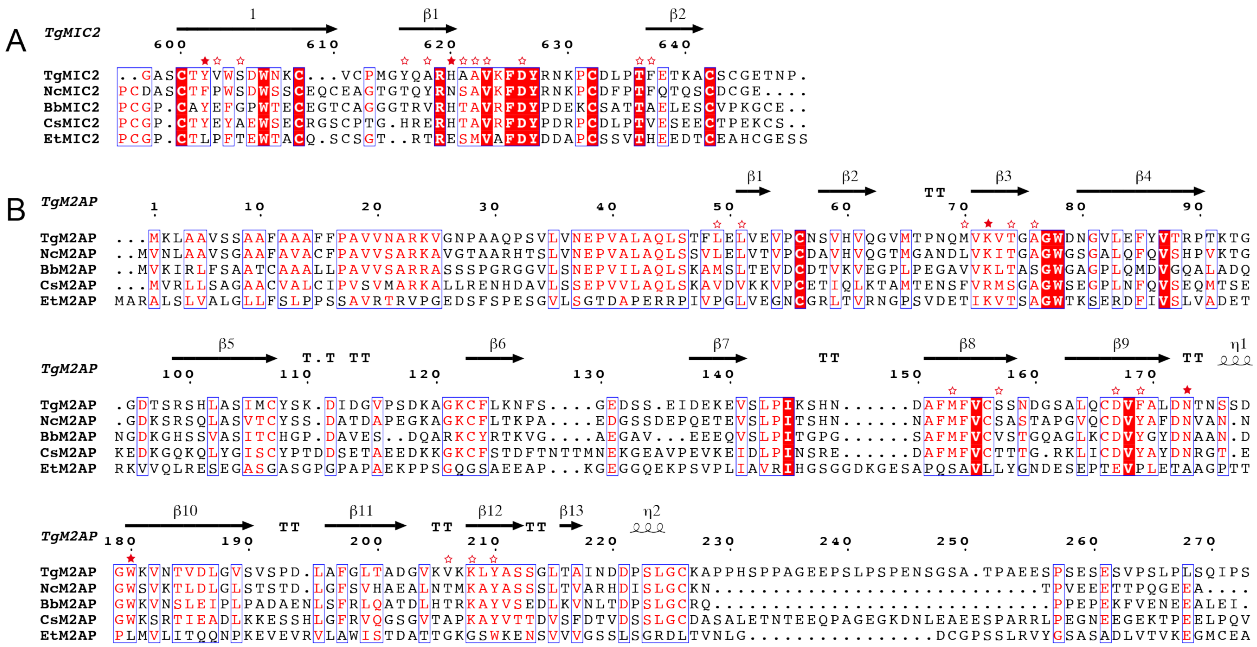

Figure S2. Sequence alignment of TSR6 (A) and M2AP (B).

Secondary structures of tgTSR6 and tgM2AP are highlighted on the top of the alignment, respectively. Residues involved in direct hydrogen bond interactions are shown as solid star, while other residues within the interface are shown as empty star.

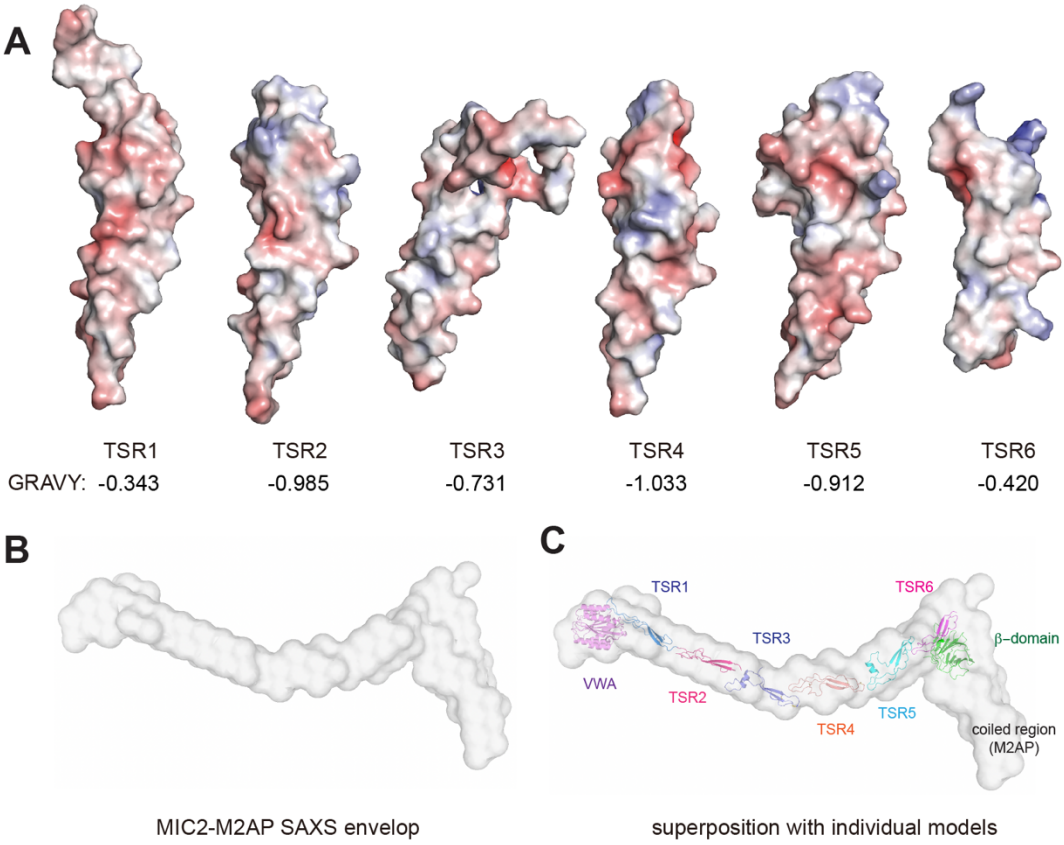

Figure S3. Electrostatics of MIC2 TSR domains and full-length architecture.

A. Electrostatics of TSR6 and other TSR domains within MIC2. The M2AP-binding face is shown for TSR6 and other TSR domains are shown in identical orientations. TSR1-5 structures are from the full-length of Alphafold2 MIC2 model. Electrostatic solvent accessible surfaces were calculated with scale of [−5 (red) to +5 (blue) kT/e]. B. The SAXS envelop of MIC2-M2AP from reference 9. C. Alignment of individual domains into the SAXS envelop of MIC2-M2AP complex. The coiled region of M2AP was predicted to be flexible by AF2 so not fitted.

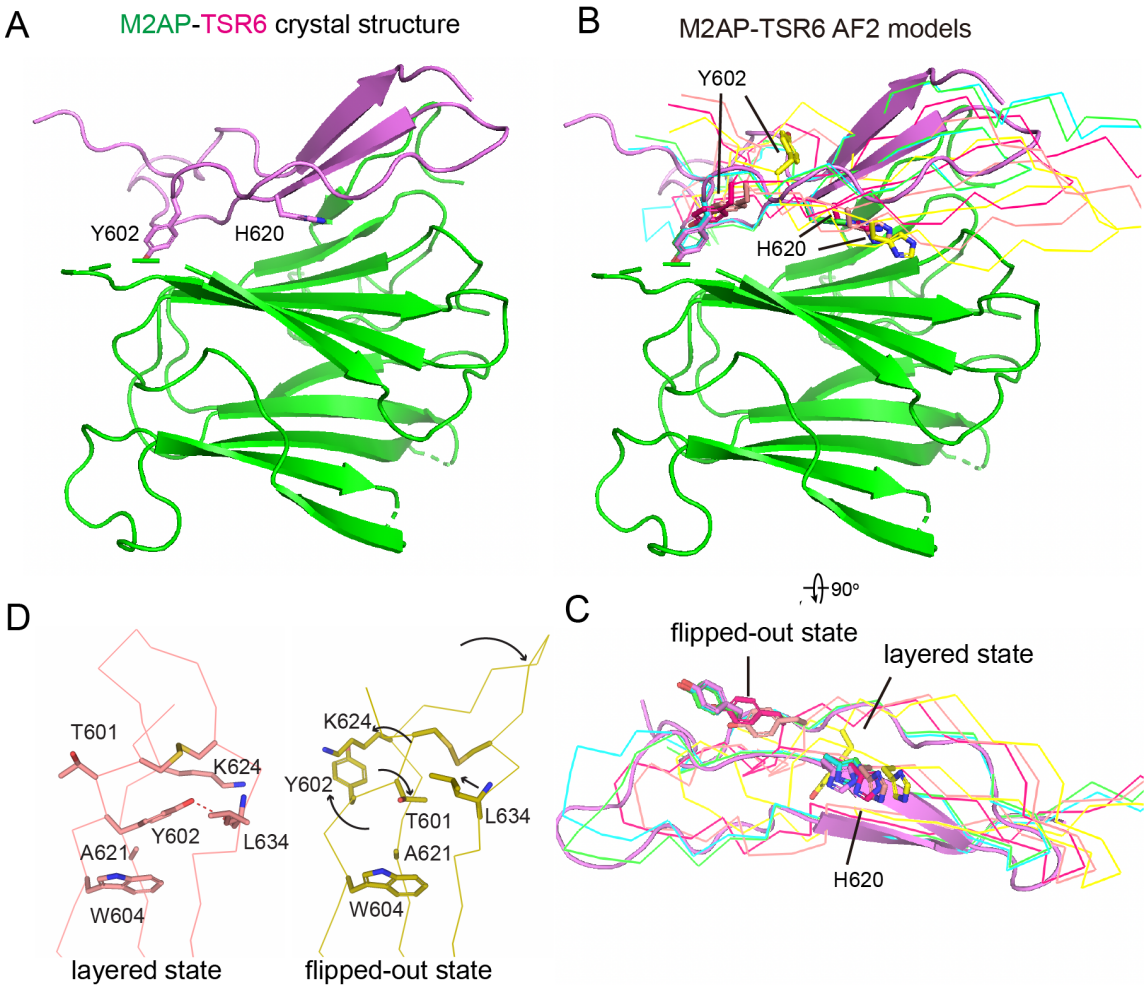

Figure S4. The predicted AF2 complex models and dynamics of Y602.

A. The determined crystal structure of MIC2-M2AP complex in current study. Two key residues are shown with sticks. B-C. The predicted complex models are superimposed with the crystal structure on M2AP. TSR6s in models are shown as ribbon and the M2APs are hidden for clarity. In panel C all M2APs are hidden to view the positions of Tyr-602 and His-620. D. Comformational changes around Tyr-602 of TSR6 between layered state and flipped-out state.