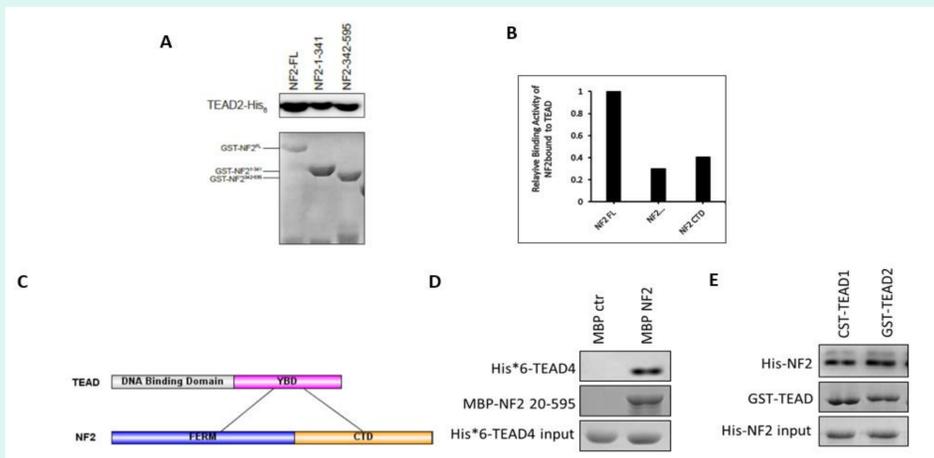


## NF2-dependent regulation of TEAD's stability and activity

Mengying wu, liqiao hu, wei tian

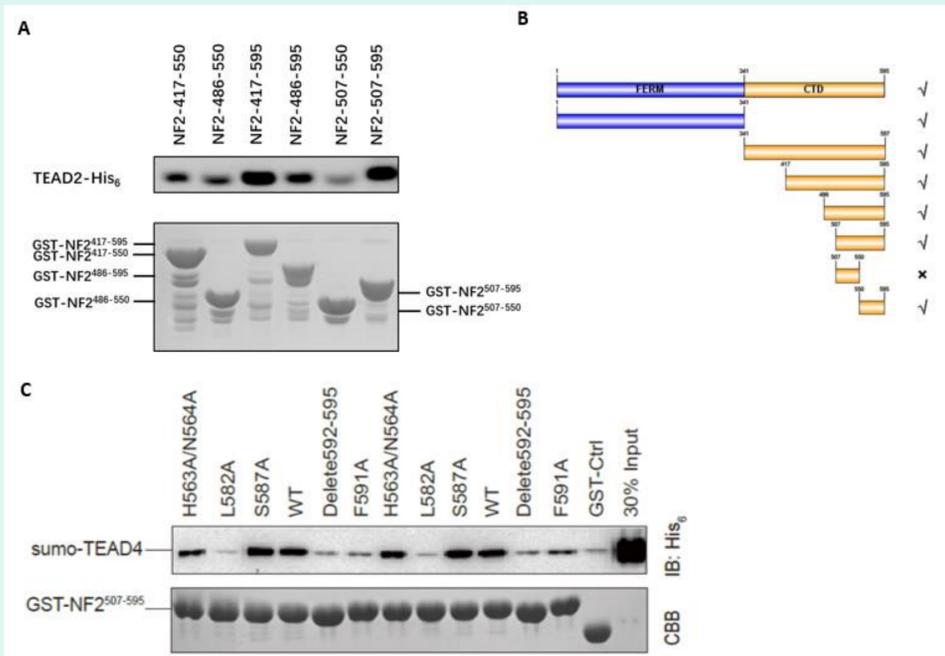
Hippo pathway plays a crucial role in cell proliferation, cell differentiation and organ size control by restricting activities of TEAD and its coactivator Yap. Dysregulation of TEAD/Yap contribute to tumorigenesis. NF2 as a tumor suppressor inhibits the activity of TEAD by promoting phosphorylation of YAP. In this study, we identify that NF2 competes with YAP for binding TEAD. NF2's FERM domain and CTD domain are both essential and sufficient for its binding TEAD. Importantly, NF2 A585W which can't inactivate Yap because of its closed conformation not only increases the interaction between NF2 and TEAD but also inhibits TEAD/Yap-induced overgrowth. It demonstrates that NF2 functions as a transcriptional repressor of TEAD. In addition, we confirm that NF2 accelerates depalmitoylation-induced degradation of TEAD. Our results reveal a novel regulation and function of NF2 in Hippo pathway.

### 1. The FERM and CTD domain of NF2 bind to TEADs



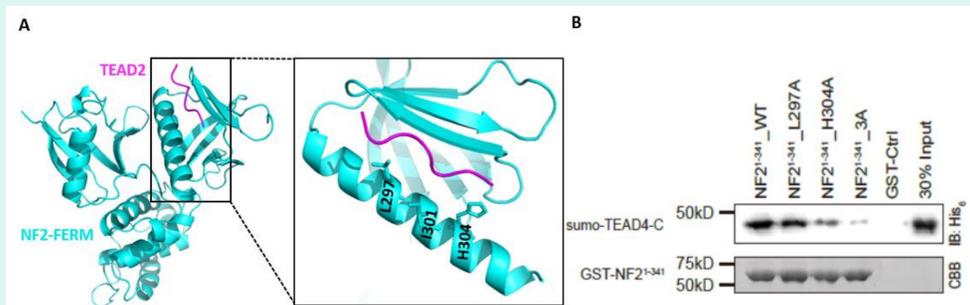
(A) Pull-down analysis between TEAD2 and various GST-NF2 constructs. (B) The relative binding activity of GST-NF2 (FL and constructs) bind sumo-TEAD2. (C) Schematic illustration of the domain organization for human NF2 and TEAD. (D) pull-down analysis between His-TEAD4 and MBP-NF2 FL. (E) pull-down analysis between His\*6-TEAD4 and His-NF2.

### 2. Conserved residues mediate CTD interaction with TEAD



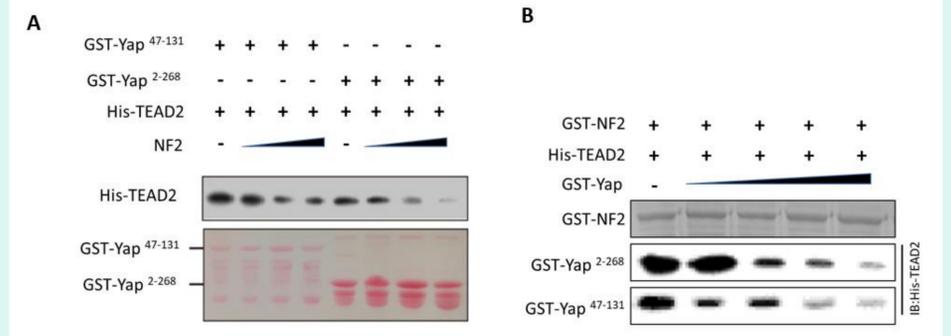
(A) Pull-down analysis between TEAD4 and various GST-NF2 CTD constructs. (B) Diagram of NF2 constructs used for binding TEAD. (C) Pull-down analysis between TEAD4 and various GST-NF2<sup>550-595</sup> mutations

### 3. Structure of FERM bind to TEAD



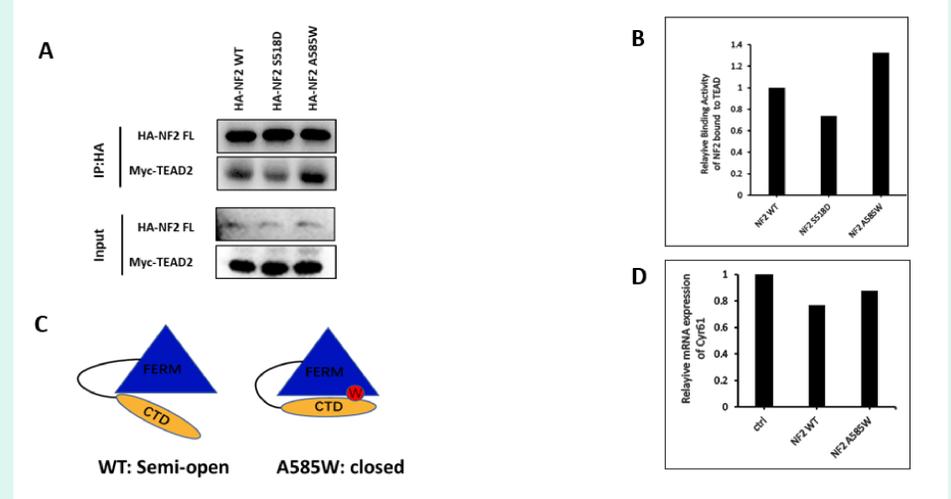
(A) Zoomed-in view of the binding interface between FERM and TEAD. (B) Pull-down analysis between TEAD4 and various NF2<sup>21-341</sup> mutations. 3A means L297/I301/H304A.

### 4. NF2 compete with YAP for binding TEAD



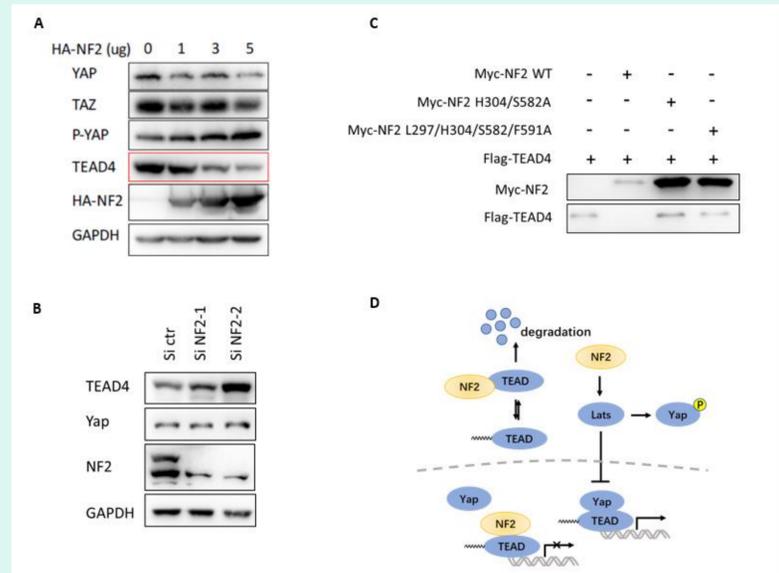
(A) Competitive pull-down assay to detect the effect of NF2 on GST-YAP<sup>47-131</sup> and GST-YAP<sup>2-263</sup> binding to His-TEAD2. (B) Competitive pull-down assay to detect the effect of GST-YAP<sup>47-131</sup> and GST-YAP<sup>2-263</sup> on NF2 binding to His-TEAD2

### 5. NF2 in closed conformation regulates TEAD activity



(A) Co-IP between Myc-TEAD and various HA-NF2 mutations in HEK293. (B) The relative binding activity of GST-NF2 (FL and mutations) bind sumo-TEAD2. (C) Schematic illustration the conformation exchange between NF2 WT and A585W mutation. (D) Transcriptional levels of TEAD target genes in cells after NF2 overexpression.

### 6. NF2 facilitates TEAD to degrade



(A) Proteins level are determined by Western blotting after overexpressing HA NF2. (B) Proteins level are determined by Western blotting after overexpressing Myc-NF2 WT and mutations. (C) Proteins level are determined by Western blotting after overexpressing Myc-NF2 WT and mutations. (D) NF2 as an upstream regulator of Hippo pathway negatively regulates TEAD. (1) NF2 facilitates Lats-dependent phosphorylation of YAP. (2) NF2 functions as a transcriptional repressor of Yap for binding TEAD. (3) NF2 binds to TEAD to accelerate TEAD degradation.