

**Structure, Volume 24**

**Supplemental Information**

**Structural and Functional Analysis of a Talin  
Triple-Domain Module Suggests an Alternative  
Talin Autoinhibitory Configuration**

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## SUPPLEMENTAL INFORMATION

### Figure S1, related to Figure 2.

**A. Cross-linking of purified His-R7R8R9 protein and mutants.** Cross-linking reactions were terminated at 20 min or 40 min before subject to SDS-PAGE analysis. **B. Structural comparison of R7R8R9 and R7R8R9-3Y.** The R9 domain of R7R8R9-3Y is superimposed on that of the wild-type R7R8R9. The R9:R8' interfaces in the two structures are highly similar (*left*), despite a  $\sim 30^\circ$  rotation between the two R8' domains (*right*, orange for WT and lime for R7R8R9-3Y). **C. Electron density at the R9:R8' interface in a steric view.** *Upper:* electron density of R7R8R9. *Lower:* electron density of R7R8R9-3Y.

### Figure S2, related to Figures 3 and 4.

**A. Integrin activity analysis of talin-V1540Y.** *Left:* relative integrin activity of wild-type talin and talin-V1540Y in histogram. NS: not significant compared with talin WT. *Right:* expression levels of the transfected constructs were examined by anti-GFP Western. **B. Expression levels of talin in CHO-A5 cells for integrin activity analyses.** The corresponding figures are indicated.

### Figure S3, related to Figure 4.

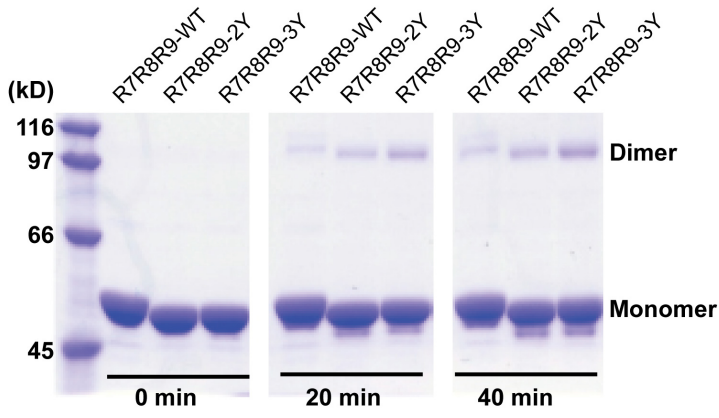
**Crystal contact prevents the alternative autoinhibitory configuration in the crystal structure of the F2F3:R9 complex.** The F2 domain of a symmetrically related F2F3 molecule (F2', in lime) makes contact with several residues in the  $\alpha 5$  helix of the R9 domain. This crystal contact of the F2 and R9 domains likely prevents the "mode B" autoinhibitory configuration.

### Figure S4, related to Figure 3.

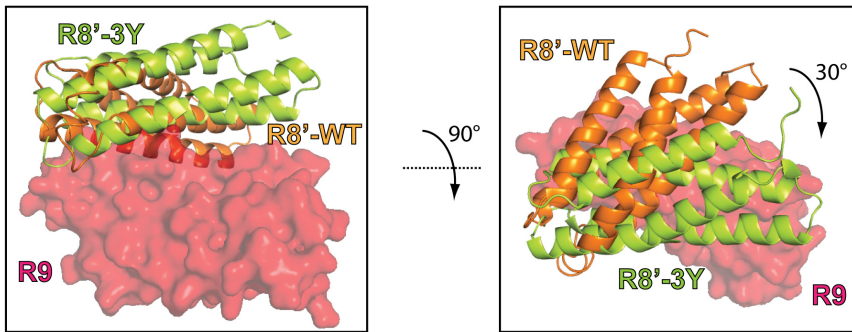
**Effect of PI(4,5)P<sub>2</sub> headgroup on the F2F3:R9 interaction.** **(A)** *In-vitro* pull-down of His-F2F3 by GST-R9 in the presence of IP<sub>3</sub> at various concentrations. His-F2F3 input and GST/GST-R9 pull-down were shown by Coomassie staining. Bound His-F2F3 was shown by anti-His Western. **(B)** *In-vitro* pull-down of His-F2F3-KKR by GST-R9 in the presence of IP<sub>3</sub> at various concentrations. Input proteins and bound His-F2F3 were shown by Coomassie staining.

S1

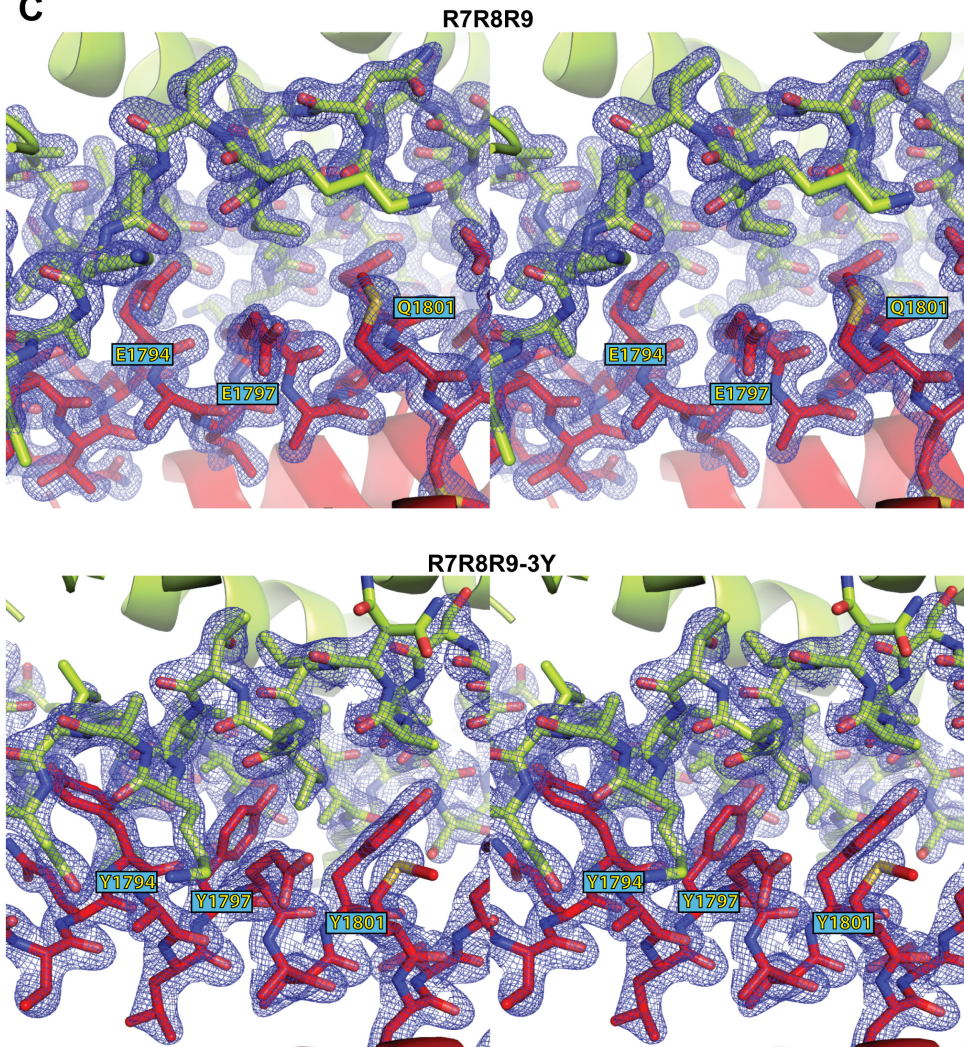
A



B

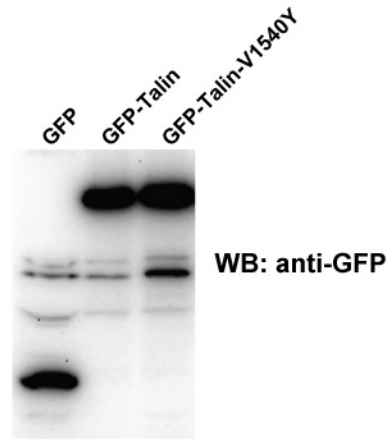
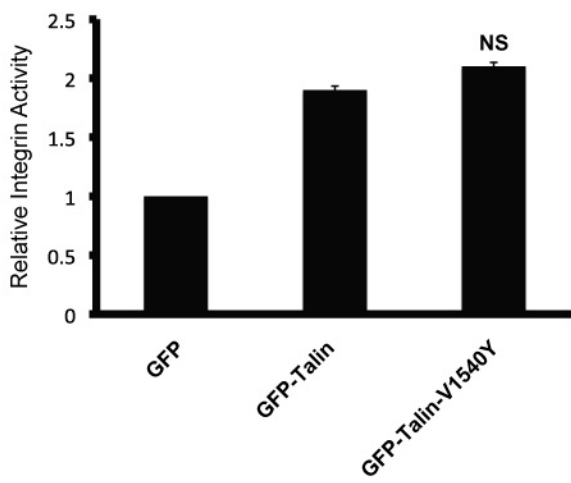


C



# S2

## A

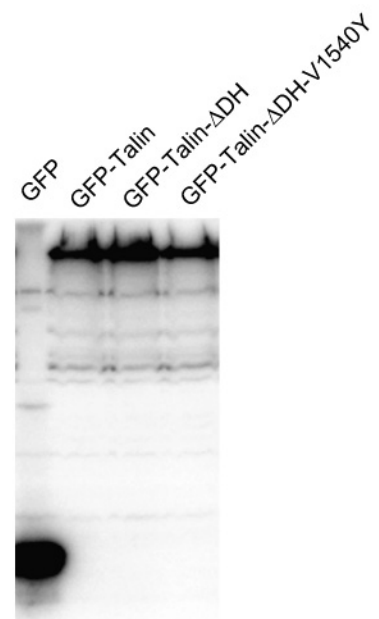
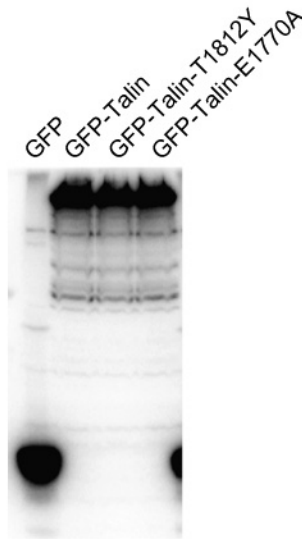
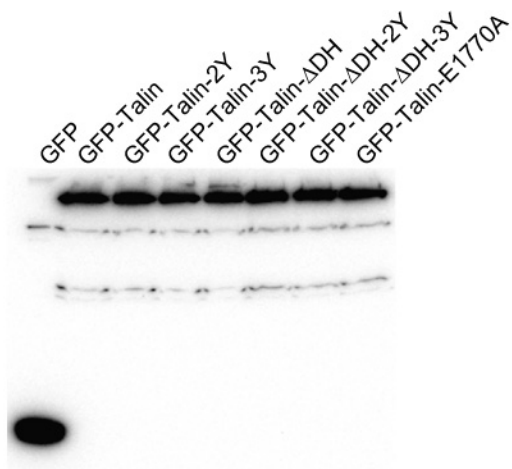


## B

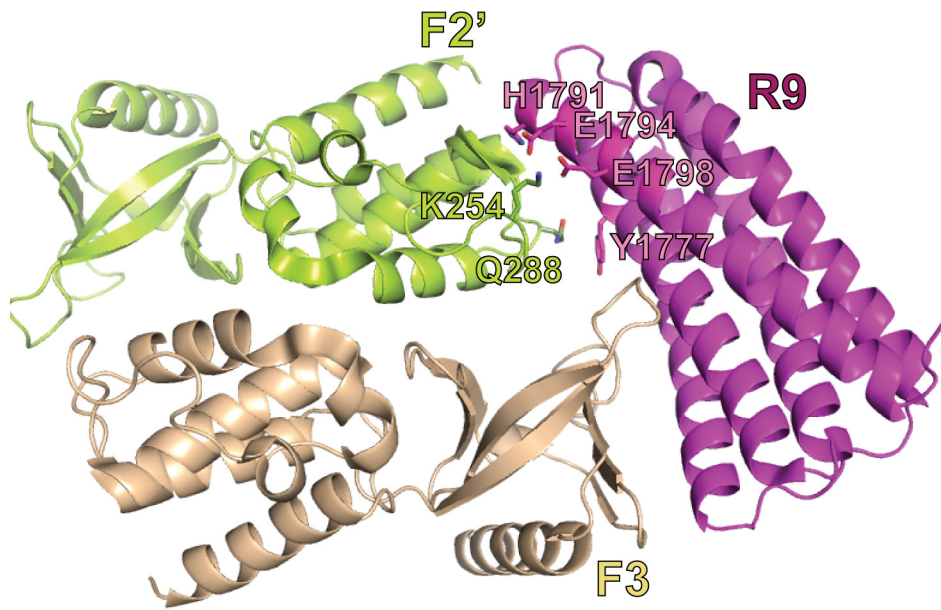
Talin levels in Figures 3E and 4B

Talin levels in Figures 3G

Talin levels in Figures 4C

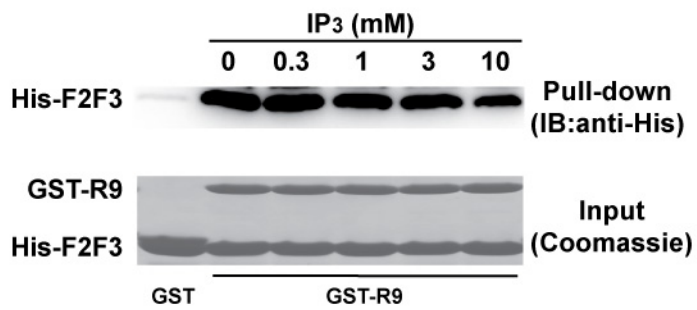


S3



S4

A



B

