Supplementary information, Figure S1

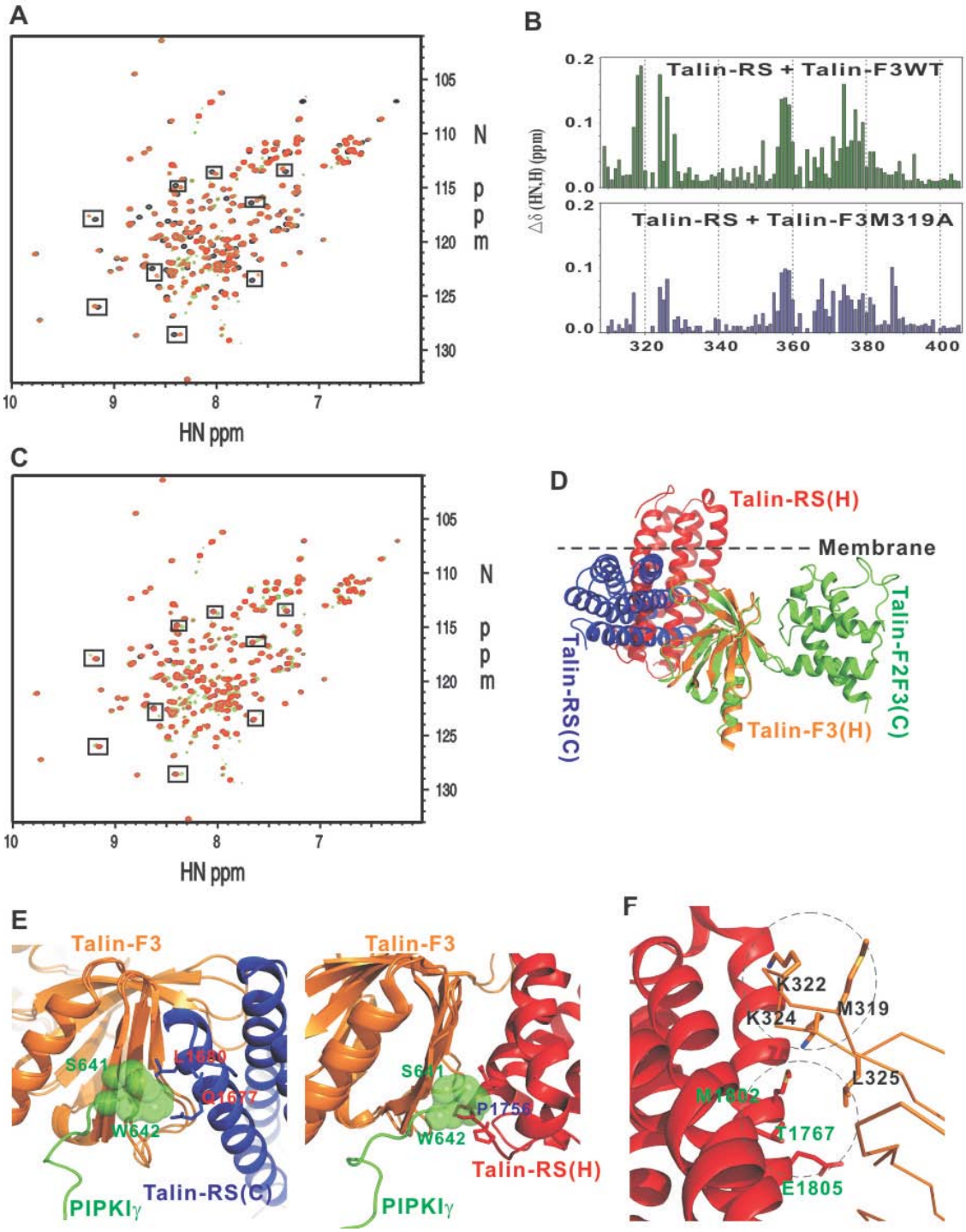

Figure S1 Structural analysis of talin-F2F3/talin-RS complex. **(A)** HSQC of ^{15}N-labeled talin-RS in the absence (black) and presence of equivalent talin-F3 (red) or talin-F2F3 (green), showing that the binding patterns are the same. **(B)** Binding of M^{319}A to talin-RS as

examined by NMR. In the presence of talin-RS (0.2 mM), chemical shift changes of $M^{319}A$ (0.1 mM) are significantly less than those of wild type talin-F3 (0.1 mM). This is consistent with reduced binding of $M^{319}A$ to talin-RS ($K_D \sim 42$ μM) than wild type talin-F3 ($K_D \sim 3.6$ μM) as determined by SPR. **(C)** HSQC of ^{15}N-labeled talin-RS in the absence (black) and presence of WT talin-F2F3 (green) or talin-F2F3 K322A/K324A (red) showing that the latter has diminished binding to talin-RS. **(D)** A nearly 90^o rotation of talin-RS in the crystal structure (blue) vs in the HADDOCK model [20] (red) when talin-F3 in both structures are superimposed. The orientation of the talin-RS in the HADDOCK model would make the talin-RS buried nearly 30% inside the membrane surface when talin-F3 approaches to membrane for binding to integrin β CT (see the dotted line). This would cause dramatic steric clash of talin-RS with membrane at high energetic cost. By contrast, talin-RS of inactive talin in the crystal structure faces to membrane via a negatively charged surface, resulting in charge-charge repulsion, thereby inhibiting talin access to integrin and maintaining inactive talin in the cytosol (see the text). Consistently, talin-RS has no interaction with membrane vesicles (data not shown). **(E)** Left, talin-F3 in the crystal structure is superimposed with that bound to PIPKIγ (de Pereda, J.M., Wegener, K.L., Santelli, E., *et al*. Structural basis for phosphatidylinositol phosphate kinase type Igamma binding to talin at focal adhesions. *J Biol Chem* 2005; **280**:8381-8386) that mimics the talin-bound β3 membrane-distal (MD) portion, showing clearly that talin-RS has little steric clash with PIPKIγ, which is fully consistent with previous competition data where PIPKIγ does not interfere with the talin-F3/talin-RS complex [19]. Right, by contrast, PIPKIγ, significantly clashes with S641-W642, would strongly perturb the talin-F3/talin-RS interface in the HADDOCK model [20]. **(F)** The HADDOCK model [20] showing that L325 in talin-F3 interacts extensively with T1767, Q1805, and M1802 and thus its mutation to R would significantly reduce the talin-F3/talin-RS interaction. However, L325R had little effect on the talin-F3/talin-RS interaction [19]. The L325R mutation data are consistent with the crystal structure where L325 has no interaction with talin-RS (see Figure 1C). Also notice that M319 in the HADDOCK model is not involved in any intermolecular or intramolecular interaction, which fails to explain the binding data here and previous data [19]. By contrast, the M319 conformation in the crystal structure (Figure 1C) can explain both the mutagenesis and binding data (see text).