Supporting Information

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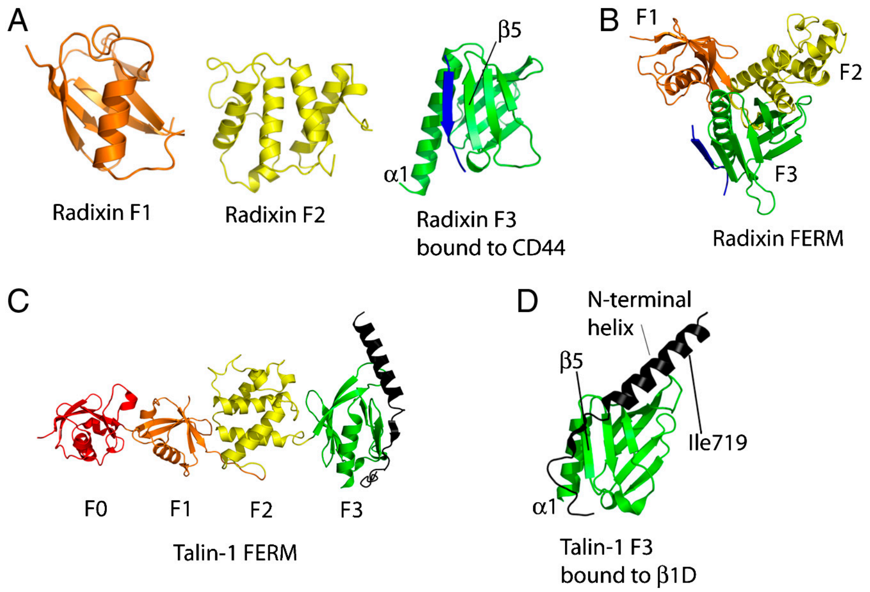

Fig. S1. Comparison of the talin-1 and radixin FERM domains. (*A*) The radixin FERM domain is composed of three subdomains: F1 (*Left*, orange), F2 (*Center*, yellow), and F3 (*Right*, green). The CD44 cytoplasmic tail (blue) binds to a cleft in F3 formed by helix α1 and strand β5. (*B*) The radixin subdomains adopt a canonical trefoil shape. (*C*) The talin-1 FERM domain contains F1 (orange), F2 (yellow), and F3 (green) domains, as well as an F0 subdomain (red) that precedes F1. Instead of a trefoil, the talin-1 FERM subdomains are arrayed linearly. (*D*) Model of talin-1 F3 (green) bound to the integrin β1D CT (black). Two regions of the β1D CT interact with talin-1 F3: Its C-terminal portion adopts an extended conformation and packs against a ridge formed by the interface of helix α1 and strand β5 in F3, while an N-terminal α-helix and a contiguous portion of the β1 transmembrane (TM) domain packs against the top of F3. Radixin structures were generated from Protein Data Bank (PDB) ID code 2ZPY. Talin-1 structures were modeled using PDB ID codes 3G9W and 3IVF.

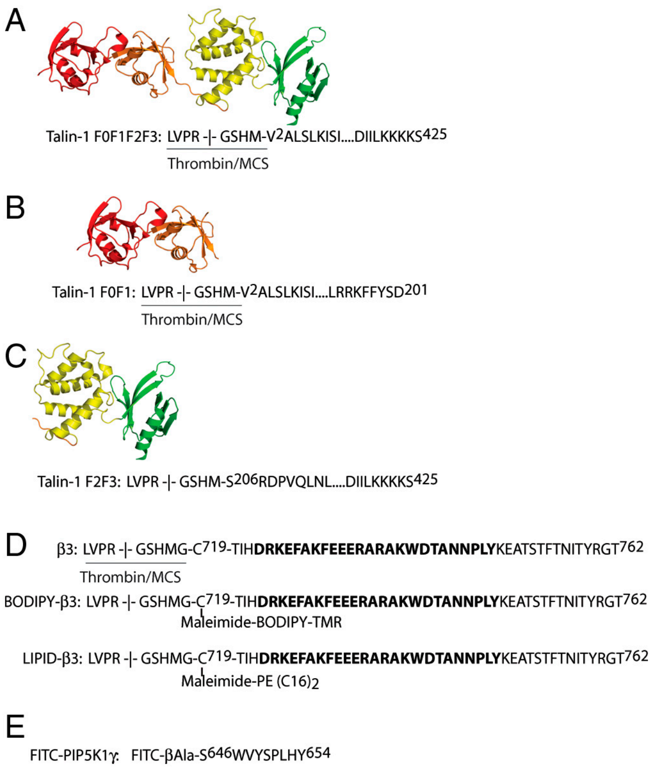

Fig. S2. Talin-1 constructs encompassing (*A*) the F0, F1, F2, and F3 subdomains, (*B*) the F0 and F1 subdomains, and (*C*) the F2 and F3 subdomains. Each construct contains an N-terminal Gly-Ser-His-Met (GSHM) motif enabling cleavage by thrombin. (*D*) β3 CT constructs include the entire CT including the talin-1 recognition motif (bold) and preceded by a Cys residue at position 719 as well as an N-terminal GSHMG sequence for cleavage by thrombin (*Top*); a fluorescent β3 CT construct in which a maleimide-BODIPY-TMR moiety is appended to C719 (*Middle*); and a β3 CT construct in Cys719 was covalently modified with maleimide-functionalized phosphatidylethanolamine (*Bottom*). (*E*) FITC-labeled peptide derived from PIP5KIγ.

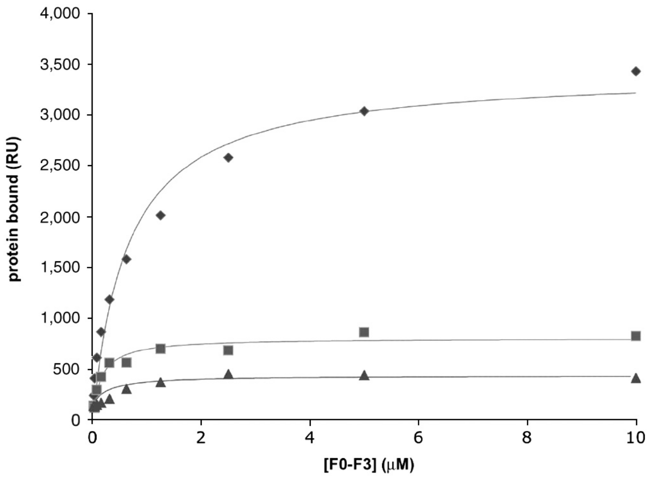

Fig. S3. Steady-state binding isotherms for F0-F3 binding to 1% (triangles), 3% (squares), and 10% (diamonds) PtdIns(4,5)P_2 immobilized on a Biacore L1 SPR chip.

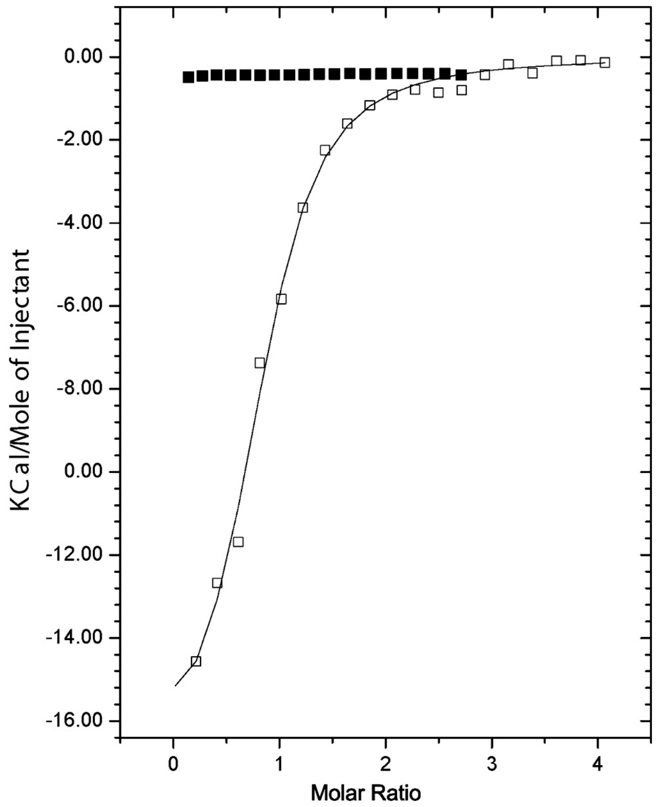

Fig. S4. ITC titrations of 2 mM Ins(1,4,5)P_3 into 150 μM F0-F3 (filled squares) and 120 μM Ins(1,4,5)P_3 into 6.0 μM PLC-δ1 (open squares). The titration into PLC-δ1 could be fit to a OneSite model.

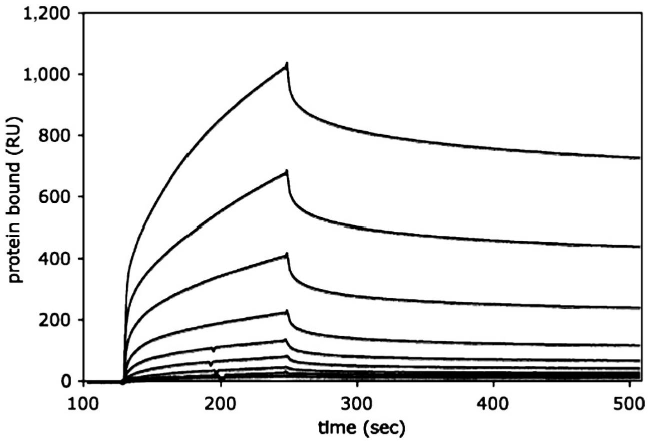

Fig. S5. Talin-1 FERM domain binding to the β3 CT immobilized on negatively charged surfaces or free in solution. The β3 CT (600 RU) was immobilized on a carboxy-methylated dextran SPR chip and F0-F3 to maximum concentration of 1 μM was flowed over the chip.

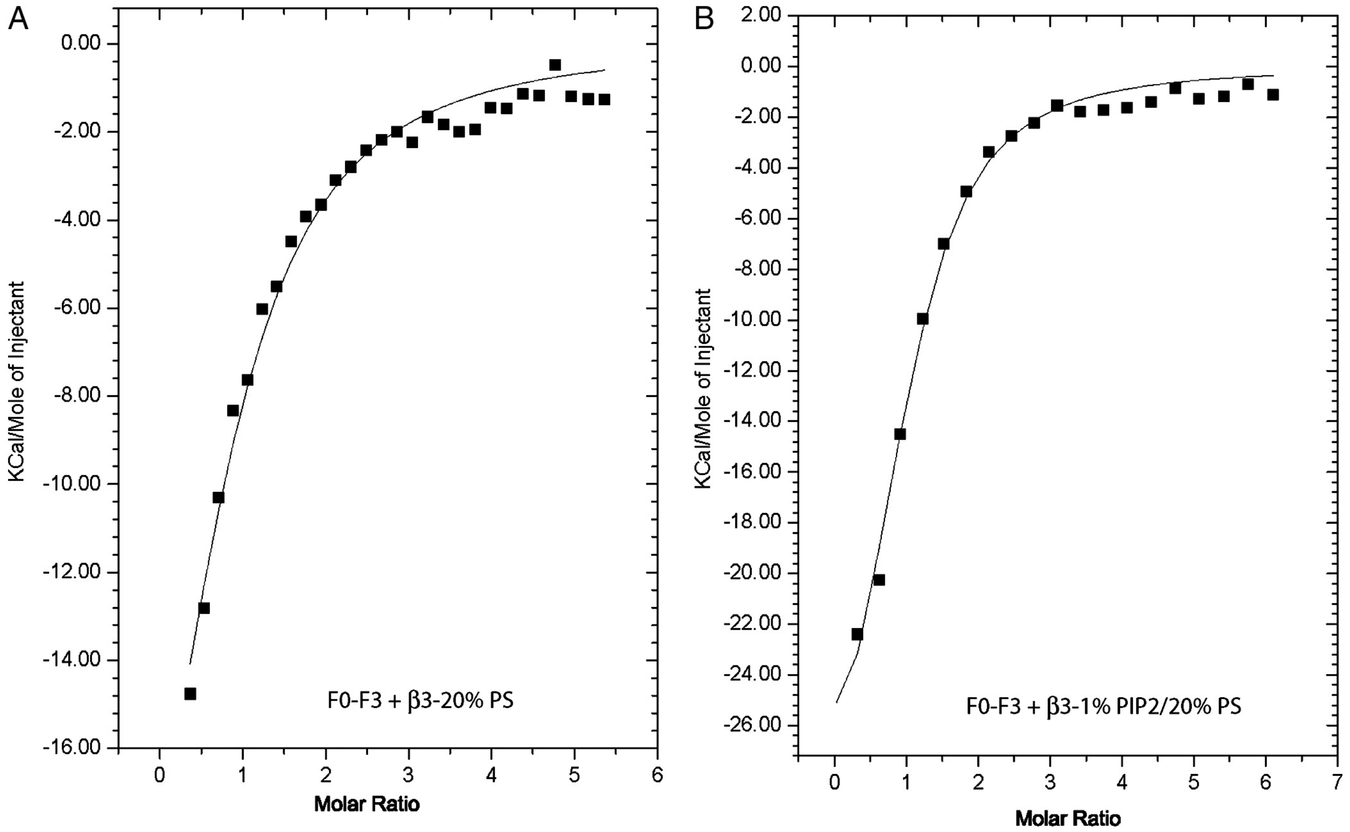

Fig. S6. FERM domain binding to the β3 CT conjugated to negatively charged phospholipid bilayers. The talin-1 FERM domain (F0-F3, 150 μM) was titrated at 20 °C into a buffer-matched suspension of LUVs composed of (*A*) 20% phosphatidylserine (PS), and (*B*) 20% PS + 1% PtdIns(4,5)P_2 and to which 5 μM β3 CT had been conjugated via a maleimide-functionalized lipid head group. The titrations were globally fit to a OneSite binding model using MicroCal/Origin.

Table S1. ITC measurements of talin-1 FERM domain and PLC-δ1 binding to PtdIns(4,5)P_2 and Ins(1,4,5)P_3

	K_a, ×10^6 M^{-1}	K_d, μM	ΔH, kcal/mol	ΔS, cal · mol^{-1} · K^{-1}	Stoichiometry
F0-F3 + 1% PtdIns(4,5)P_2	—	—	—	—	—
F0-F3 + 3% PtdIns(4,5)P_2	—	—	—	—	—
F0-F3 + 10% PtdIns(4,5)P_2	1.3 ± 0.6	0.781	−3.3 ± 0.4	−17.0	0.33 ± 0.03
F0-F3 + 2 mM Ins(1,4,5)P_3	—	—	—	—	—
PLC-δ1 + 1% PtdIns(4,5)P_2	0.36 ± 0.08	2.82	−11.7 ± 1.0	−14.0	1.13 ± 0.07
PLC-δ1 ± 3% PtdIns(4,5)P_2	0.47 ± 0.03	2.11	−10.2 ± 0.2	−8.37	0.97 ± 0.01
PLC-δ1 +10% PtdIns(4,5)P_2	0.24 ± .02	4.20	−12.8 ± 0.7	−18.3	0.75 ± 0.03
PLC-δ1 + 2 mM Ins(1,4,5)P_3	1.4 ± 0.2	0.72	−17.5 ± 0.7	−30.5	0.80 ± 0.02

Measurements were performed at 20 °C in 25 mM MOPS pH 6.8 with 100 mM NaCl. Background heats of dilution were subtracted. Titrations were fit to one-site binding isotherms, and errors are reported as fitting errors. There was no net enthalpy for titration of F0-F3 into 1% and 3% PtdIns(4,5)P_2 and 2 mM Ins(1,4,5)P_3. Data shown are the mean and standard deviation of three experiments.

Table S2. ITC measurements of talin-1 FERM domain binding to the β3 CT attached to large unilamellar phospholipid vesicles (LUVs)

	K_a, ×10^6 M^{-1}	K_d, μM	ΔH, kcal/mol	ΔS, cal · mol^{-1} · K^{-1}	Stoichiometry
β3-1% PtdIns(4,5)P_2	1.19 ± 0.21	0.86	−25.0 ± 1.5	−55.8	0.80 ± 0.04
β3-20% PS	0.29 ± 0.02	3.4	−32.3 ± 0.9	−83.3	0.8
β3-20%PS + 1% PtdIns(4,5)P_2	0.61 ± 0.11	1.6	−34.0 ± 2.8	−87.5	0.95 ± .06

The talin-1 FERM domain was titrated into LUV suspensions containing 5 μM immobilized β3 CT. All experiments were performed at 20 °C in 25 mM MOPS pH 6.8 with 100 mM NaClk, and background heats of dilution were subtracted. Titrations were fit to one-site binding isotherms. The data are the mean and standard deviations of three to six experiments.