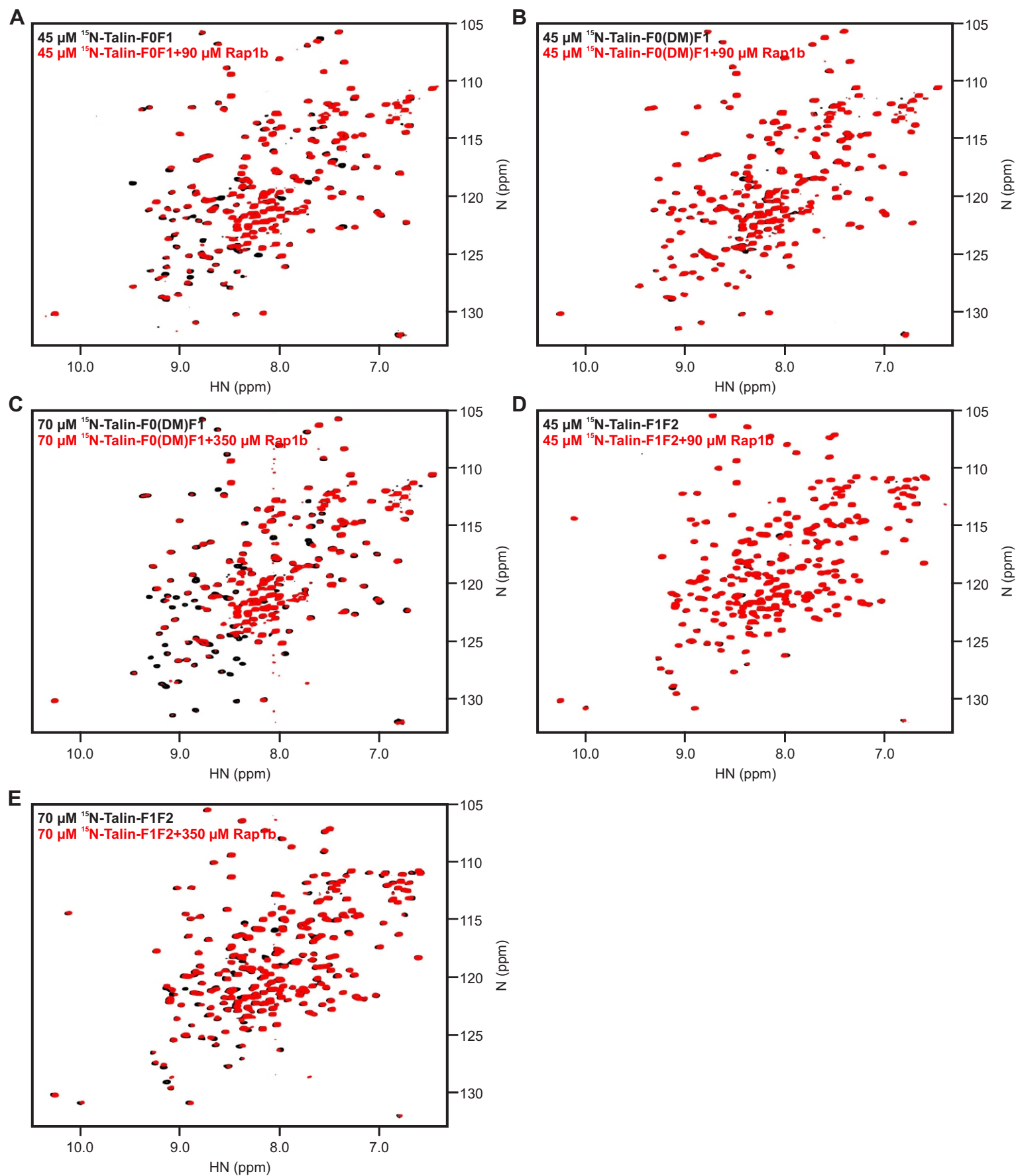# Figure S1

Figure S1: (A) The HSQC spectra of 45 µM ^{15}N-labeled talin-F0F1 in the absence (black) and presence of 90 µM GMP-PNP loaded Rap1b (red). (B) The HSQC spectra of 45 µM ^{15}N-labeled talin-F0(DM)F1 (K15A, R35A) in the absence (black) and presence of 90 µM GMP-PNP loaded Rap1b (red). (C) The HSQC spectra of 70 µM ^{15}N-labeled talin-F0(DM)F1 (K15A, R35A) in the absence (black) and presence of 350 µM GMP-PNP loaded Rap1b (red). (D) The HSQC spectra of 45 µM ^{15}N-labeled talin-F1F2 in the absence (black) and presence of 90 µM GMP-PNP loaded Rap1b (red). (E) The HSQC spectra of 70 µM ^{15}N-labeled talin-F1F2 in the absence (black) and presence of 350 µM GMP-PNP loaded Rap1b (red).
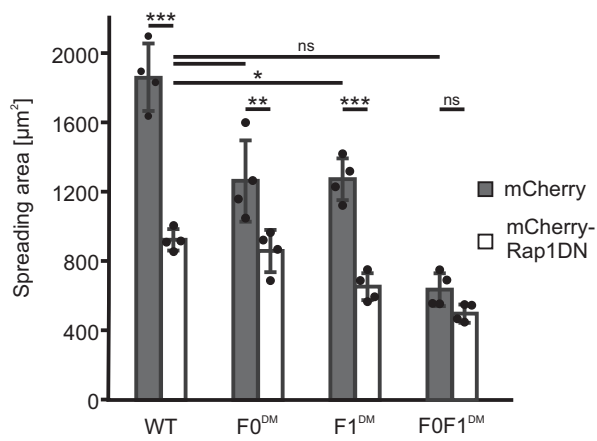
Figure S2

Figure S2: Talin-mediated cell spreading is highly dependent on Rap1. Spreading area of talin[1/2dKO] fibroblasts expressing ypet-talin WT, ypet-talin F0[DM], F1[DM] or F0F1[DM] combined with either mCherry alone or a mCherry-tagged dominant negative S17N Rap1a mutant (n=4). Spreading area was assessed 1 h after seeding the cells on fibronectin. Data are represented as mean±c.i.; statistical significance was tested by two-way ANOVA followed by Tukey's multiple comparison test.
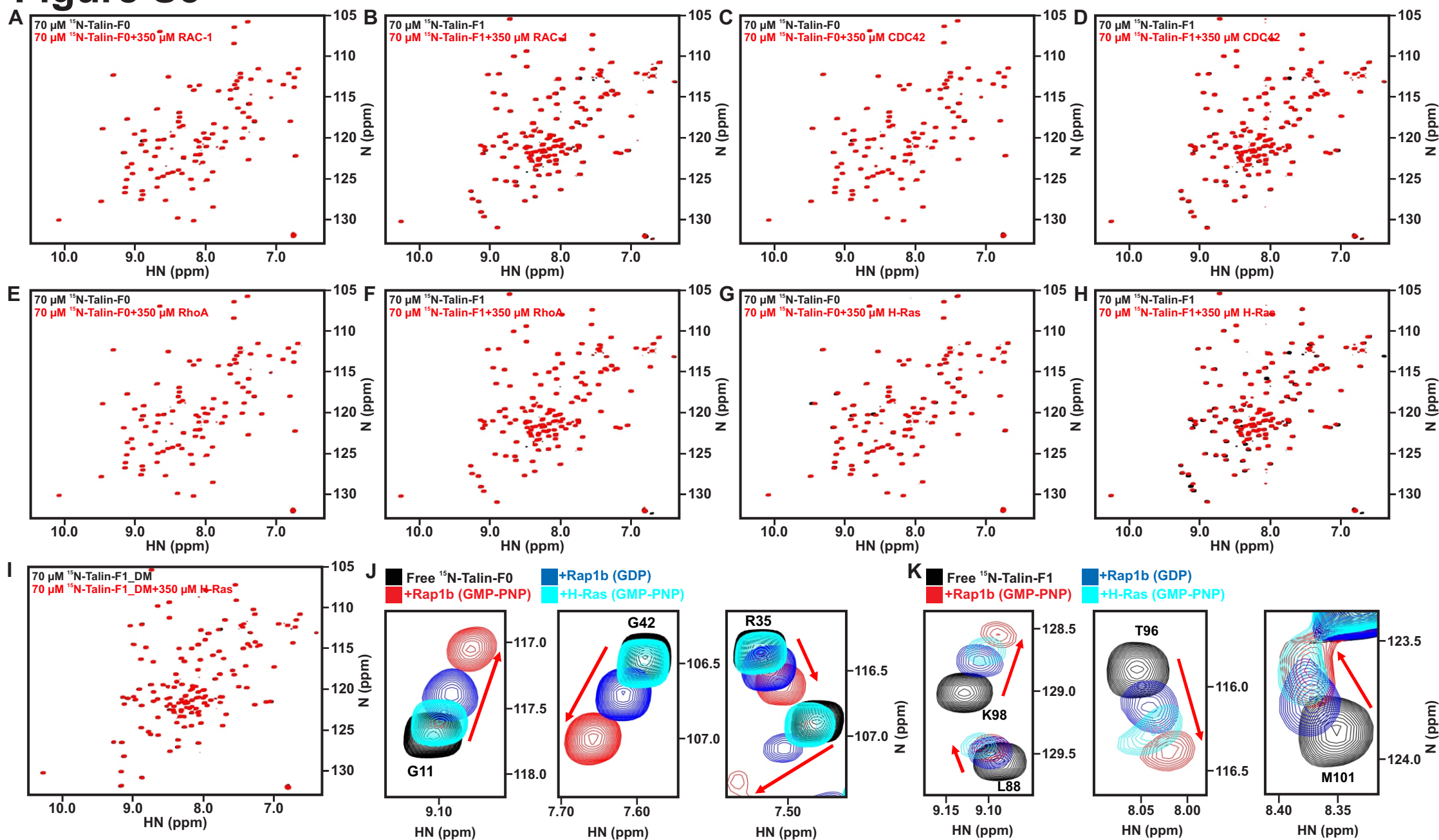
Figure S3

Figure S3: Interactions betweenTalin F0 or F1 domains with Rac-1, Cdc42, RhoA or H-Ras tested by NMR HSQC. The HSQC spectra of 70 μM ^{15}N-labeled talin-F0 (A/C/E/G) and 70 μM ^{15}N-labeled talin-F1 (B/D/F/H) in the absence (black) and presence (red) of 350 μM GMP-PNP loaded Rac-1 (A/B), Cdc42 (C/D), RhoA (E/F) or H-Ras (G/H). (I) The HSQC spectra of 70 μM ^{15}N-labeled talin-F1_DM (K98A, R118A) in the absence (black) and presence of 350 μM GMP-PNP loaded H-Ras. (J/K) Zoomed HSQC spectra (representative residues are shown) of 70 μM ^{15}N-labeled talin-F0 (I) and 70 μM ^{15}N-labeled talin-F1 (J) in the absence (black) and presence of 350 μM GDP loaded Rap1b (dark blue), GMP-PNP loaded Rap1b (red) or GMP-PNP loaded H-Ras (cyan). For binding talin-F0, Rap1b GMPPNP > Rap1b GDP >> H-Ras GMPPNP. For binding talin-F1, Rap1b GMPPNP > (slightly) HRas GMPPNP > Rap1b GDP. These suggest that talin F0 is more selective than F1 in binding GTPase.