Supplemental information

Supplementary Table S1: Paxillin peptides used in the study.

LD1	MDDLDALLADLES
LD2	SNLSELDRLLLELNAVQHN
LD3	SVESLLDELES
LD4	SATRELDELMASLSD
LD5	SQLDSMLGSLQSD
LD2/4	SNLSELDRLLLELNAVQHN*GSGSGGSGGSGGSGGSGGSGGSGGSGG*SATRELDELMASLSD

For anisotropy experiments peptides were labelled N-terminally with FITC. The non-native linker region connecting LD2 and LD4 sequences in peptide LD2/4 is in italic.

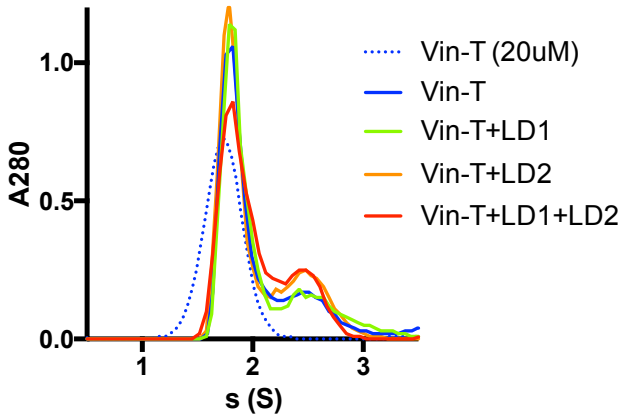
Supplementary Table S2. Crystallographic data collection and refinement statistics

	Vinculin-T+LD1+LD2
Data collection	
X-ray source	ALBA, BL13-XALOC
Wavelength (Å)	0.97926
Space group	C2
Cell dimensions	
a, b, c (Å)	177.38, 70.56, 117.37
α, β, γ (°)	90, 131.25, 90
Resolution (Å)	44.83-2.55 (2.69-2.55)*
Total reflections	109790 (16149)
Multiplicity	3.1 (3.1)
Unique reflections	35621 (5168)
Completeness (%)	99.6 (99.9)
R_{merge} (%)	10.1 (85.6)
R_{meas} (%)	12.4 (103.5)
R_{pim} (%)	6.9 (57.5)
CC(1/2)	99.5 (61.1)
$I / \sigma I$	7.3 (1.4)
Refinement	
Resolution (Å)	44.87-2.54
Reflections (total/test set)	34219/1677
R_{work} / R_{free} (%)	22.5/25.9
No. atoms	5926
Protein	5745
Solvent	101
Other	80
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.996
mean *B* value (Å^2)	70.1

*Values in parentheses are for highest-resolution shell.

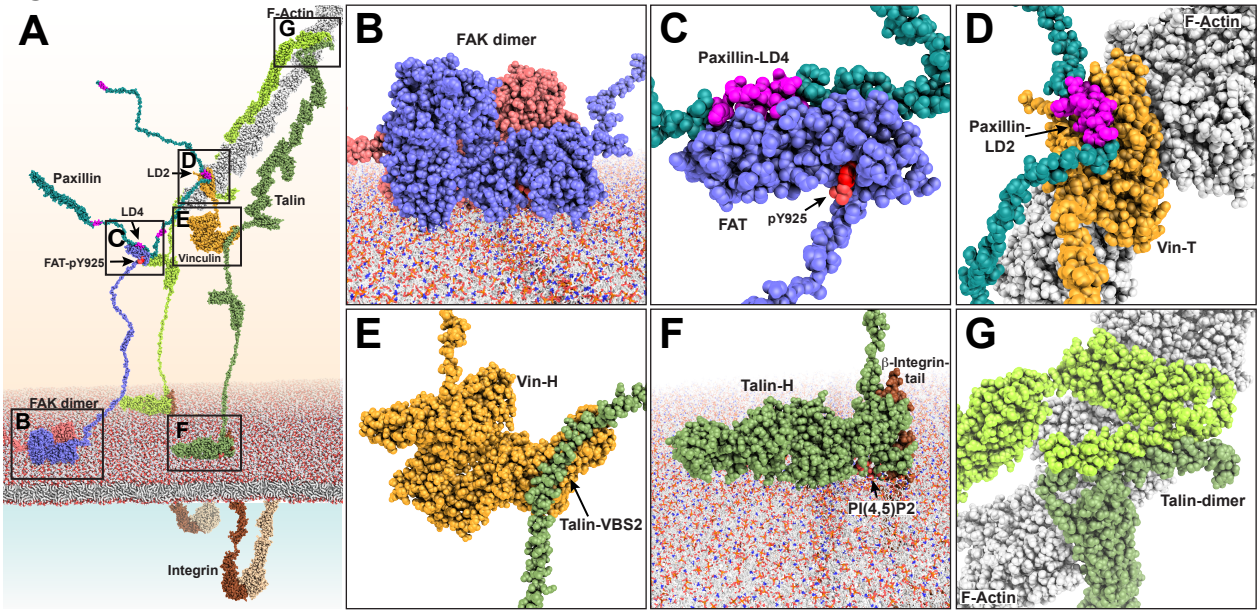
Supplementary Figures:

Figure S1:



Sedimentation velocity analytical ultracentrifugation (svAUC) analysis of vinculin tali (Vin-T) alone or bound to LD1 and/or LD2. Measurements are performed at 100 uM Vin-T and LD1/LD2 unless indicated. At 20 uM Vin-T appears as a homogenous monomer, while at 100 uM approximately 20% of Vin-T forms a dimer. Formation of Vin-T dimers or higher oligomers are not significantly affected by the presence of LD1 and/or LD2.

Figure S2:



Atomic model of FAK force activation. **(A)** Atomic model as in Fig.6A. Boxed are regions based on high-resolution structures, which are shown enlarged in panels B-G. Coloring as in Fig.6A. **(B)** Symmetric FAK dimer bound via FERM and kinase domains to the membrane with the kinase active site facing the membrane as in PDB 6TY4 (Acebrón *et al*, 2020). **(C)** Paxillin LD4 bound to the H23 site in FAT, as in 1OW7 (Hoellerer *et al*, 2003). Y925 (red) is modelled in a phosphorylated state resulting in an unbound H14 site. **(D)** Paxillin LD2 bound to Vin-T as reported in this study (Fig.5C right panel) and Vin-T bound to actin as in 3JBI (Kim *et al*, 2016). **(E)** Vin-H bound to talin VBS2 as in 1U6H (Fillingham *et al*, 2005). **(F)** The talin head (H) domain is bound to the membrane lipid PI(4,5)P2 as in 6MFS (Chinthalapudi *et al*, 2018) and to the β3-integrin tail as in 1MIZ (Garcia-Alvarez *et al*, 2003) and 2H7E (Wegener *et al*, 2007). **(G)** Talin C-terminal helices are dimerized as in 2QDQ (Gingras *et al*, 2008) and the talin rod domain R13 modelled bound to actin, loosely based on the low resolution structure in (Gingras *et al.*, 2008).

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