STRUCTURAL DETERMINANTS OF THE INTEGRIN-BINDING SITE (IBS2) IN THE TALIN ROD

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SUPPLEMENTARY METHODS

Sequence conservation of talin 1974-2293. The sequence of talin residues 1974-2293 has been aligned across species. The alignment was performed using MultAlin (1) using the following sequences: *Mus musculus* talin-1; *Mus musculus* talin-2; *Homo sapiens*; *Gallus gallus*; *Danio rerio*; *Drosophila melanogaster*; and *Caneorhabditis elegans*. The map of surface exposed conserved residues is shown in Figure S2C - >90% conservation is indicated in magenta.

SUPPLEMENTARY FIGURE LEGENDS

FIGURE S1. Small angle X-ray scattering on talin 1974-2293 - Guinier plot. The linearity of the Guinier plot indicates that the protein solutions were homogeneous and no protein aggregation was detected**.**

FIGURE S2. Crystal structure of talin residues 1974-2293. (A) Cartoon representation of the talin 1974-2293 crystal structure. The lower panel is rotated by 180 degrees. The upper 5-helix bundle is called IBS2-A and the lower one IBS2-B. The helix numbers shown in brackets are for full-length talin. (B) Electrostatic potential surface representation in the same orientation as shown in A. The areas of contact between the different molecules in the crystal are indicated by a dashed line in the lower panel. (C) Map of surface exposed conserved residues - invariant residues are shown in magenta.

FIGURE S3. Comparison of the two talin IBS2 5-helix bundles structures. Optimal overlays of the talin IBS2-A 5-helix bundle (green) with (A) the IBS2-B 5-helix bundle (blue) - 140 residues aligned with an rmsd of 2.4Å, and (B) the talin 482-655 5-helix bundle (blue) (PDB entry 1SJ7) - 114 residues aligned with an rmsd of 2.5Å as determined with Coot (2). The topology of the 5-helix bundles is shown below. Solid and dashed lines represent connecting loops on opposite ends of the helices.

FIGURE S4. NMR characterisation of the individual IBS2-A and IBS2-B domains and the IBS2 double-domain. ^{15}N-HSQC spectra of talin polypeptides spanning residues (A) 1974-2293, (B) 1974-2140, and (C) 2137-2293.

SUPPLEMENTARY REFERENCES

1. Corpet, F. (1988) *Nucleic Acids Res* 16(22), 10881-10890
2. Emsley, P., and Cowtan, K. (2004) *Acta Crystallogr D Biol Crystallogr* 60(Pt 12 Pt 1), 2126-2132

Figure S1

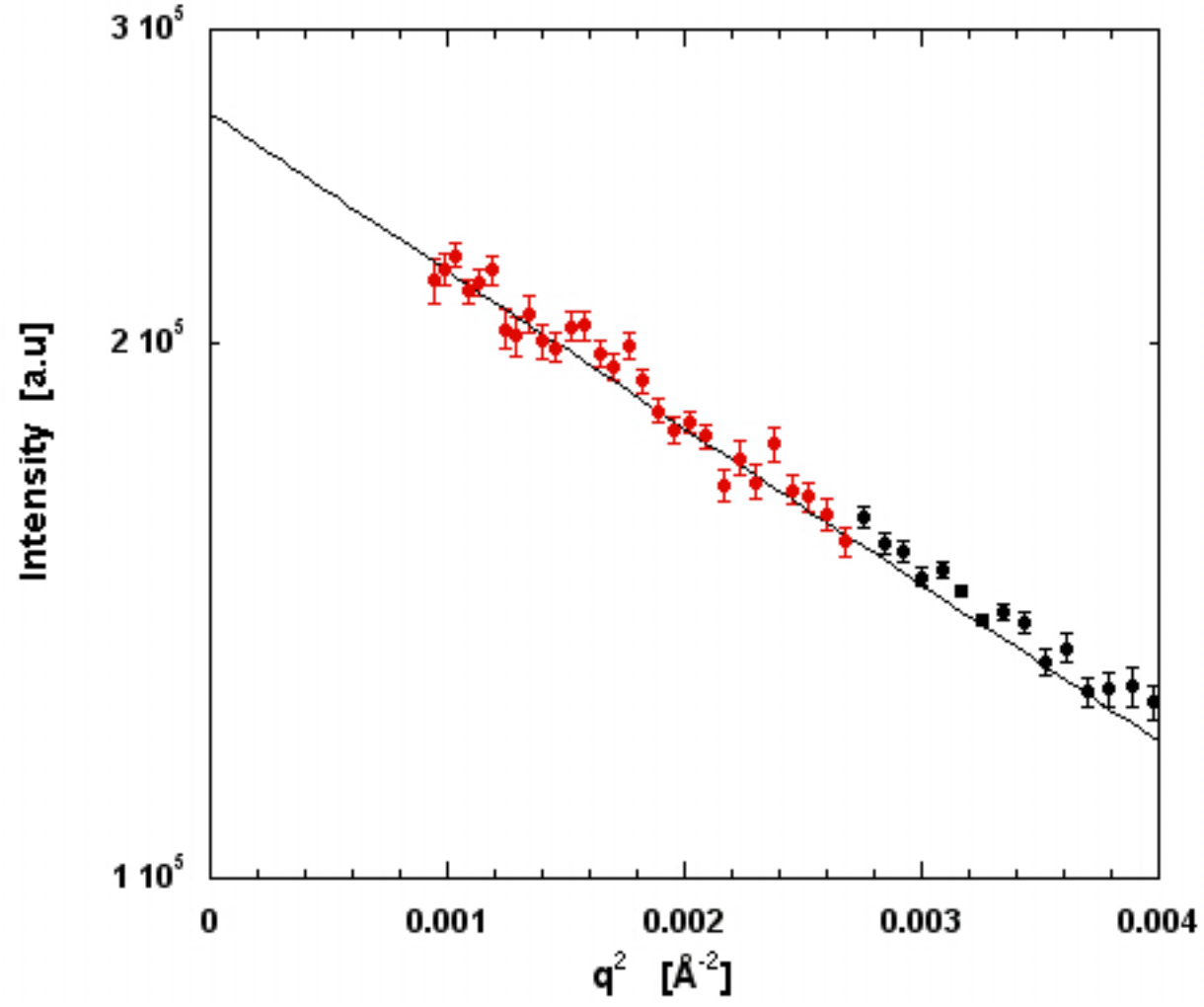


Figure S2

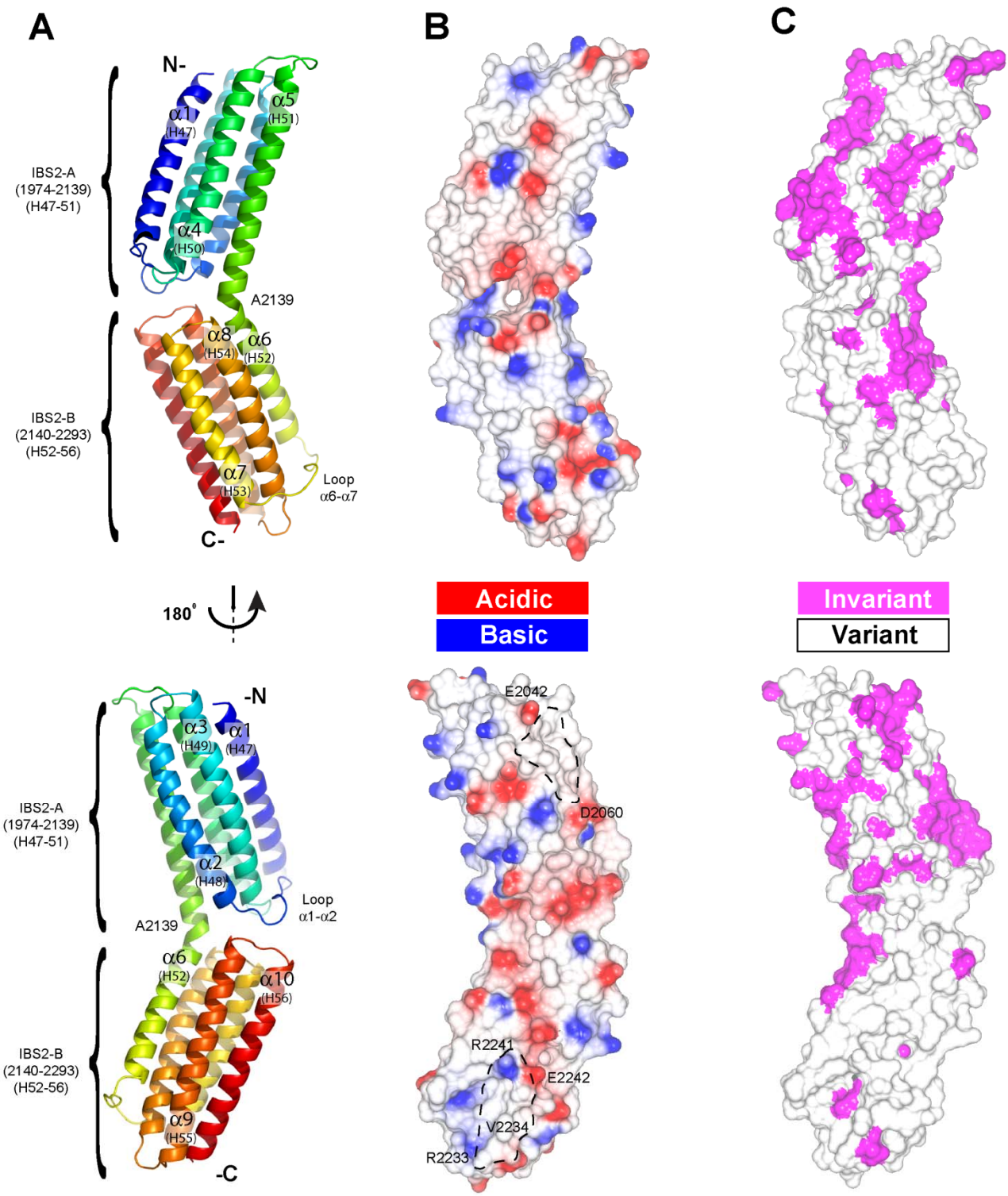

A
B
C
N-
α1
(H47)
α5
(H51)
IBS2-A
(1974-2139)
(H47-51)
α4
(H50)
A2139
α8
(H54)
α6
(H52)
IBS2-B
(2140-2293)
(H52-56)
α7
(H53)
Loop
α6-α7
C-
180°
Acidic
Basic
Invariant
Variant
-N
α3
(H49)
α1
(H47)
IBS2-A
(1974-2139)
(H47-51)
α2
(H48)
Loop
α1-α2
A2139
α6
(H52)
α10
(H56)
IBS2-B
(2140-2293)
(H52-56)
α9
(H55)
-C
E2042
D2060
R2241
E2242
V2234
R2233

Figure S3

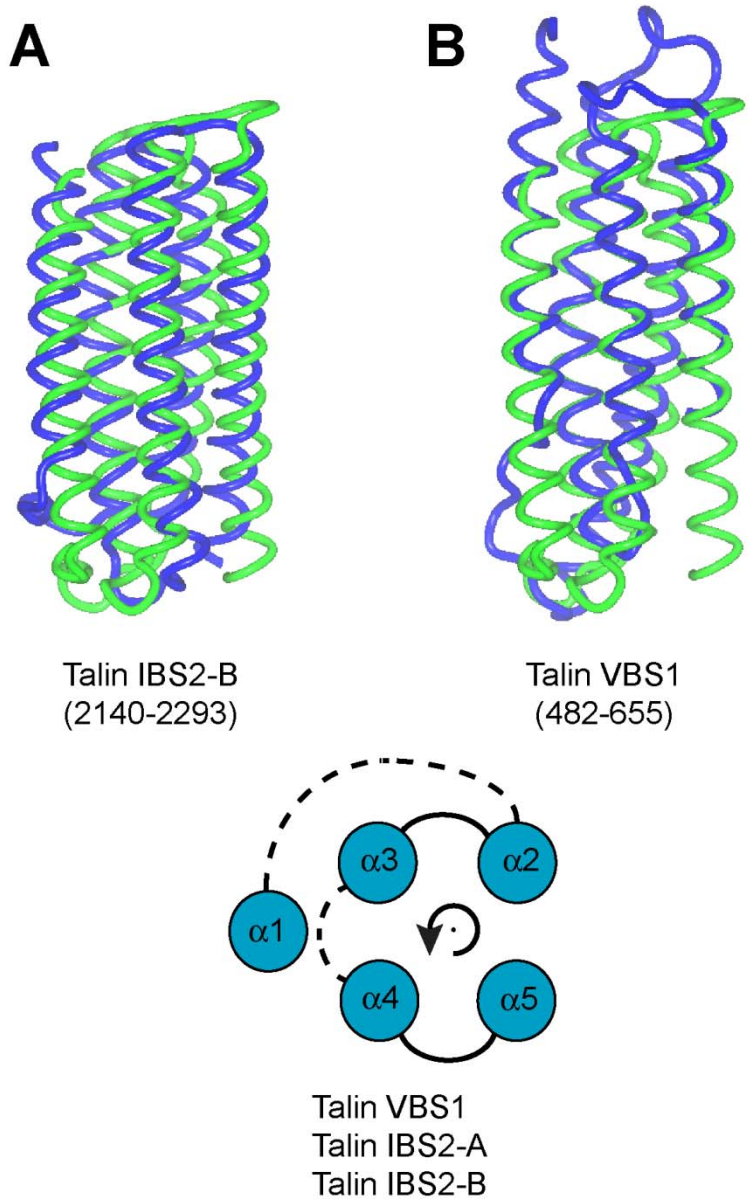
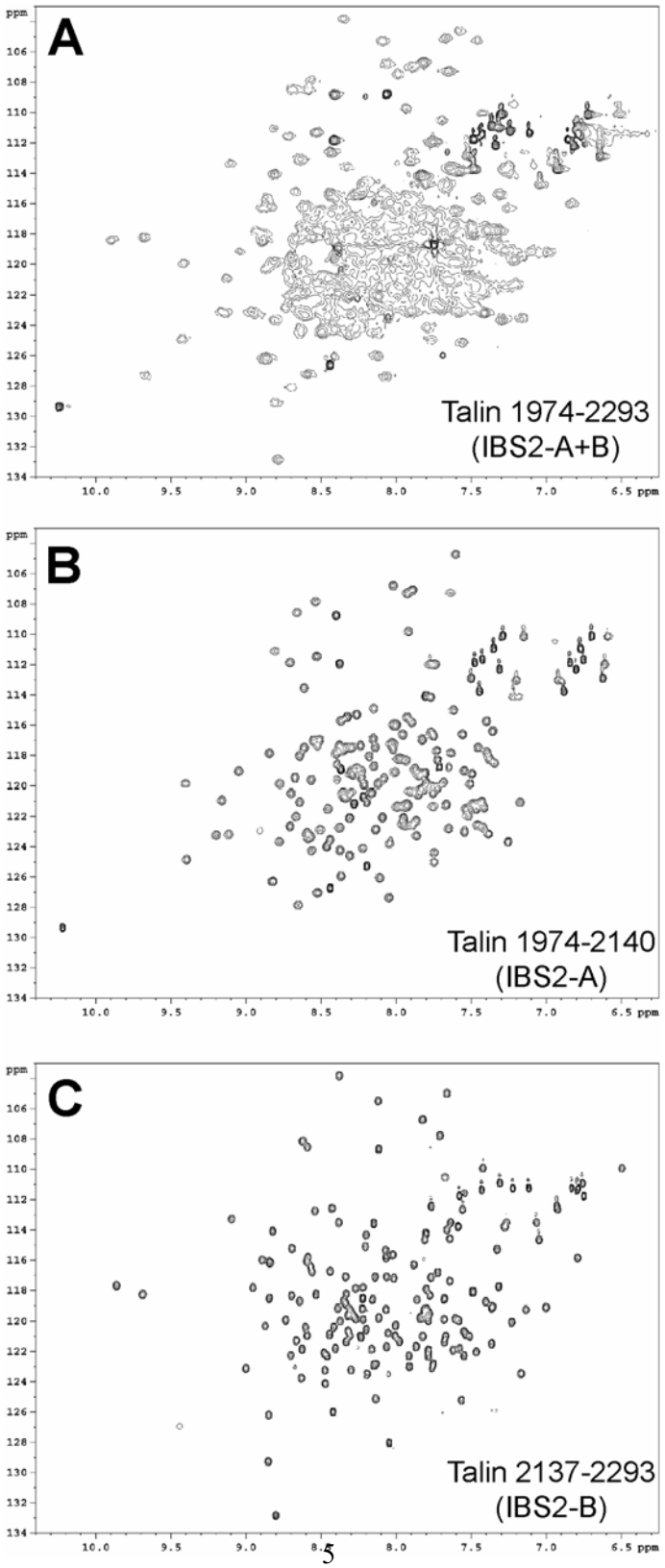

A
B
Talin IBS2-B
(2140-2293)
Talin VBS1
(482-655)
α3
α2
α1
α4
α5
Talin VBS1
Talin IBS2-A
Talin IBS2-B

Figure S4


A
Talin 1974-2293
(IBS2-A+B)
B
Talin 1974-2140
(IBS2-A)
C
Talin 2137-2293
(IBS2-B)
ppm
106
108
110
112
114
116
118
120
122
124
126
128
130
132
134
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5 ppm