Paper 01

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Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions

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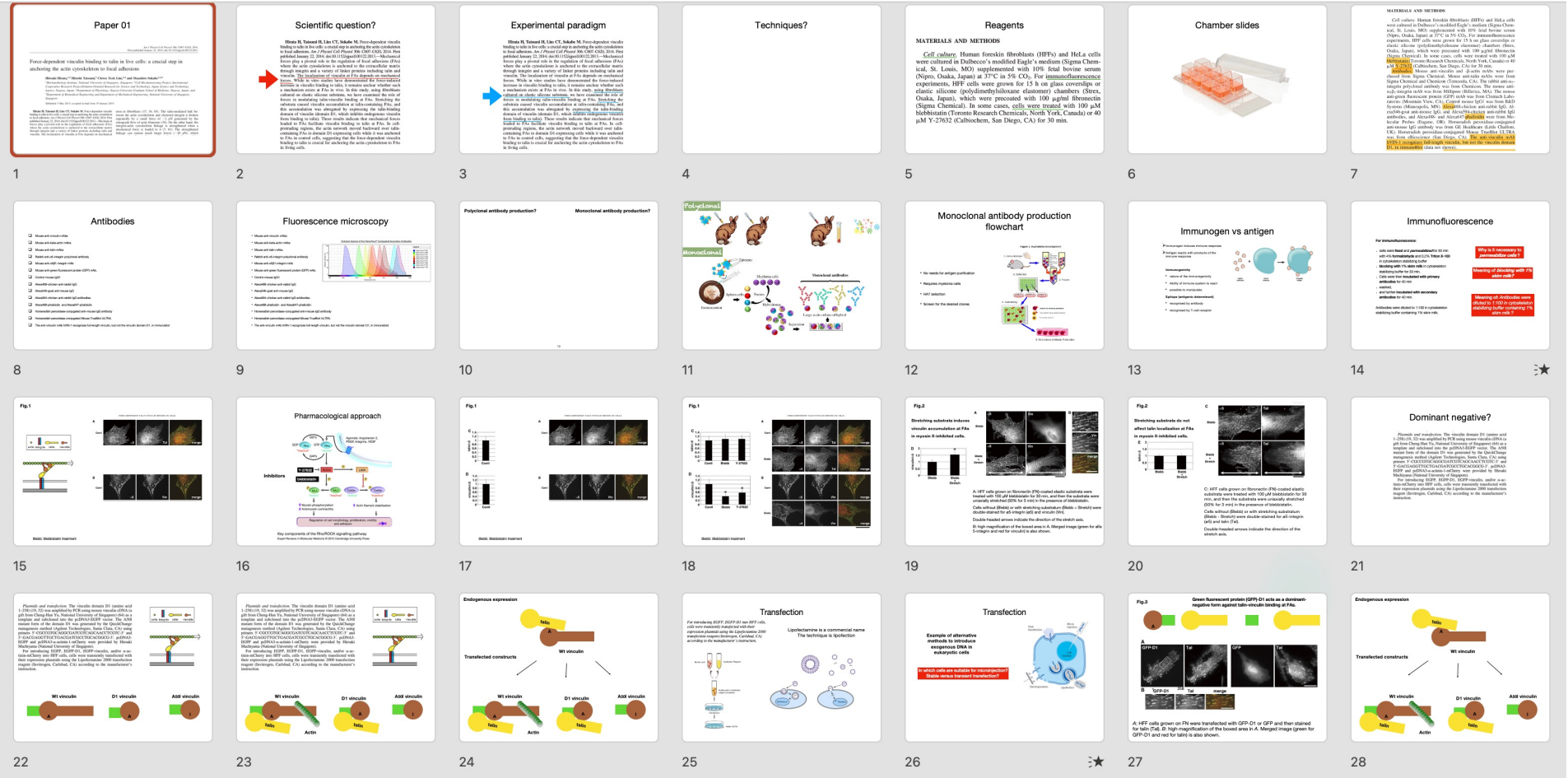
Hirata H, Tatsumi H, Lim CT, Sokabe M. Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions. *Am J Physiol Cell Physiol* 306: C607–C620, 2014. First published January 22, 2014; doi:10.1152/ajpcell.00122.2013.—Mechanical forces play a pivotal role in the regulation of focal adhesions (FAs) where the actin cytoskeleton is anchored to the extracellular matrix through integrin and a variety of linker proteins including talin and vinculin. The localization of vinculin at FAs depends on mechanical

eton in fibroblasts (17, 34, 65). The talin-mediated link between the actin cytoskeleton and clustered integrin is broken repeatedly by a small force of ~2 pN generated by the retrograde flow of actin filaments (34). On the other hand, the integrin-actin cytoskeleton linkage is strengthened when a mechanical force is loaded to it (7, 61). The strengthened linkage can sustain much larger forces (~20 pN), which

Where are we...



1 2 3 4 5 6 7

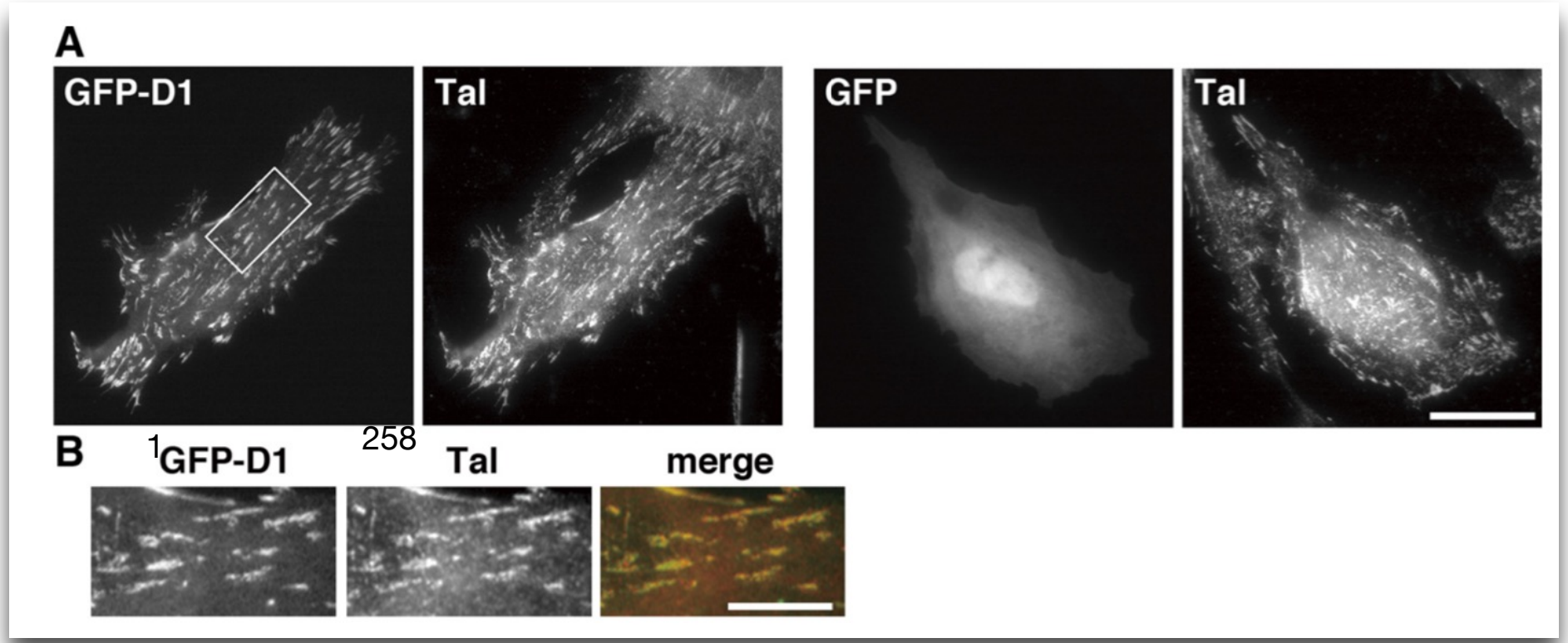
8 9 10 11 12 13 14 ★

15 16 17 18 19 20 21

22 23 24 25 26 ★ 27 28

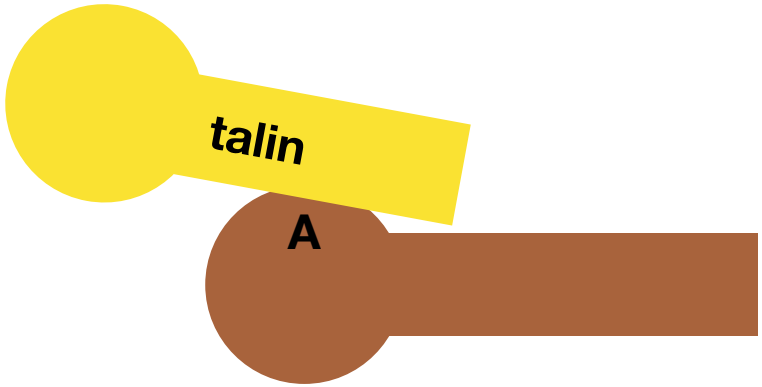
Fig.3

Green fluorescent protein (GFP)-D1 acts as a dominant-negative form against talin-vinculin binding at FAs.



A: HFF cells grown on FN were transfected with GFP-D1 or GFP and then stained for talin (Tal). *B*: high magnification of the boxed area in *A*. Merged image (green for GFP-D1 and red for talin) is also shown.

Endogenous expression



Wt vinculin

Transfected constructs

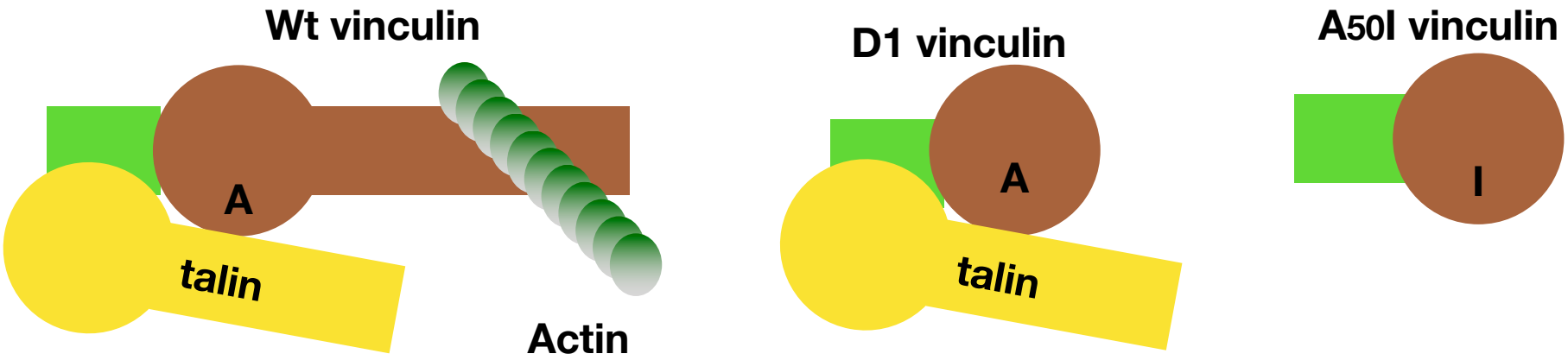
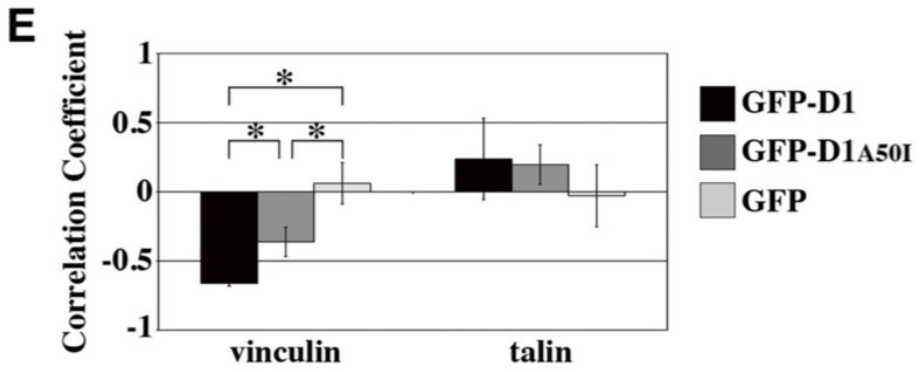
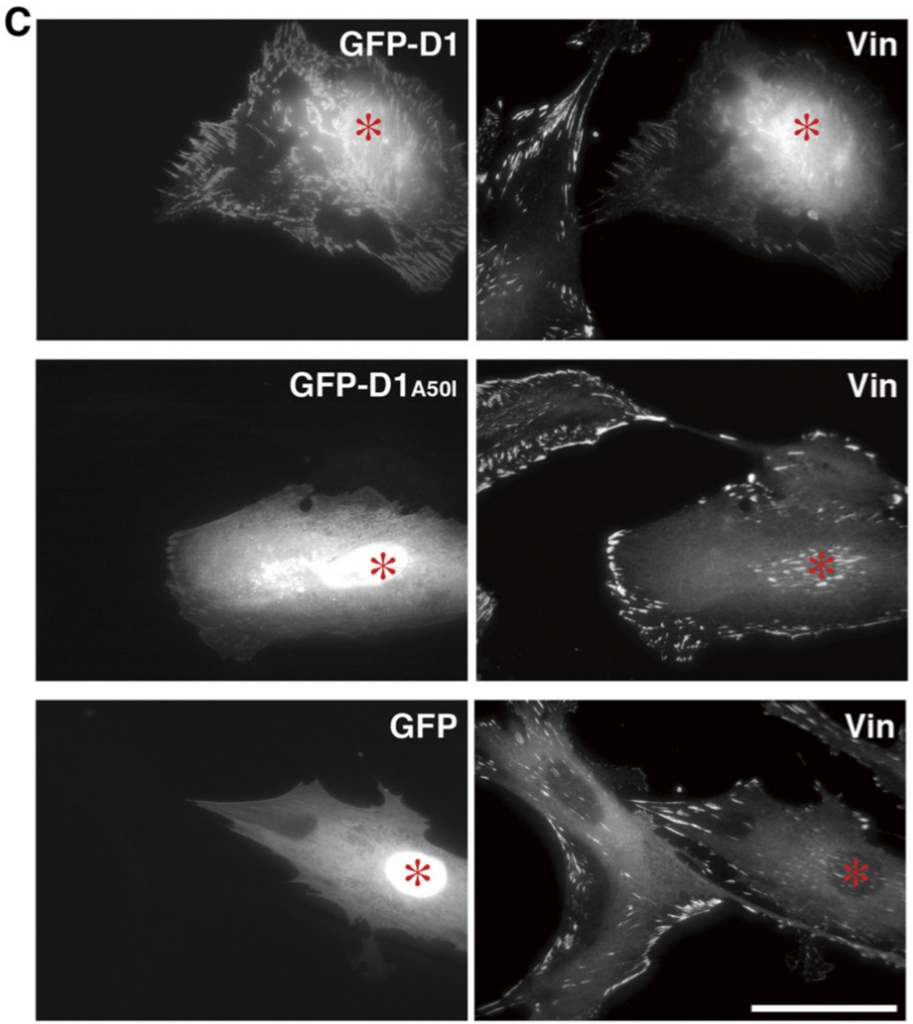


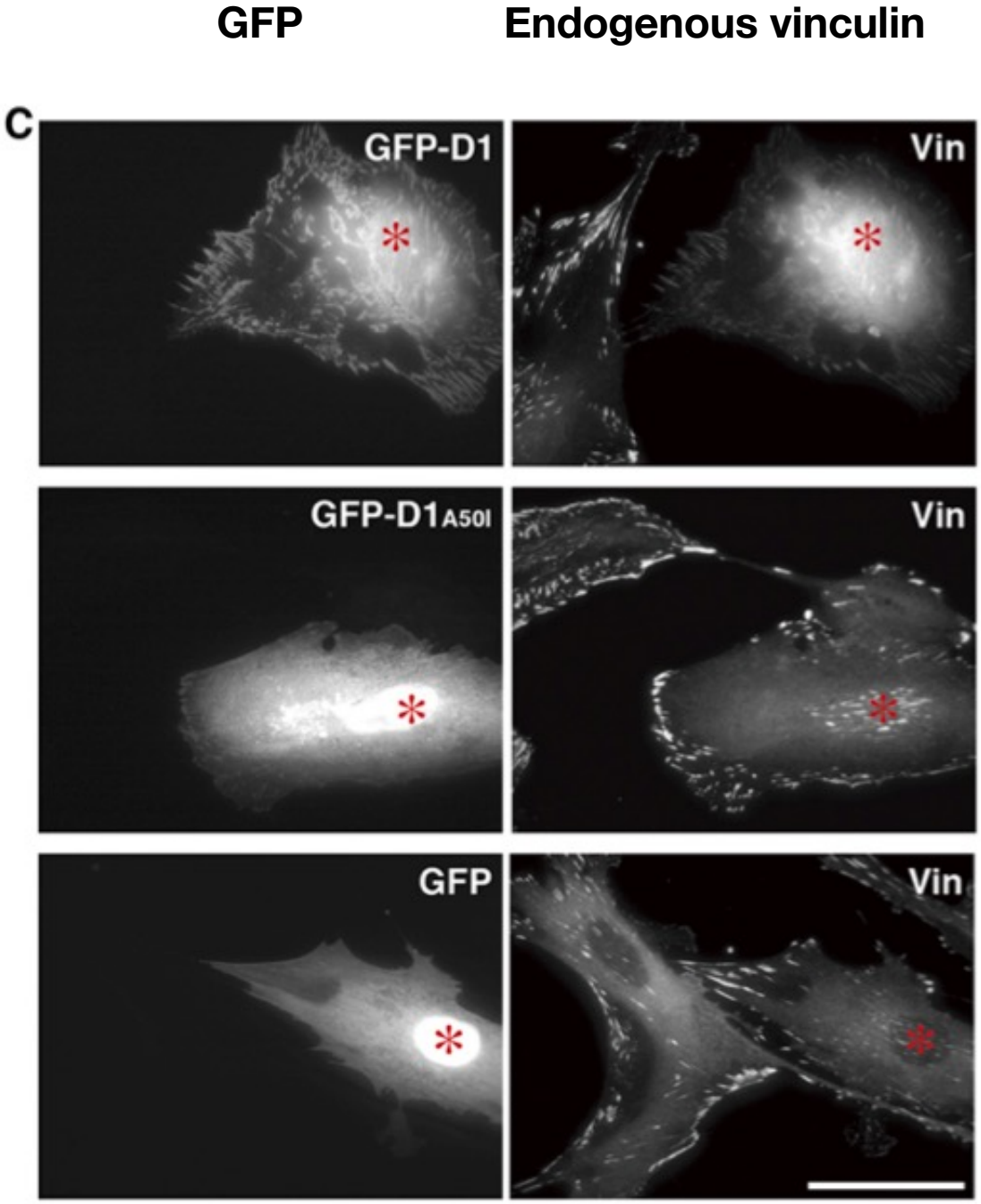
Fig.3

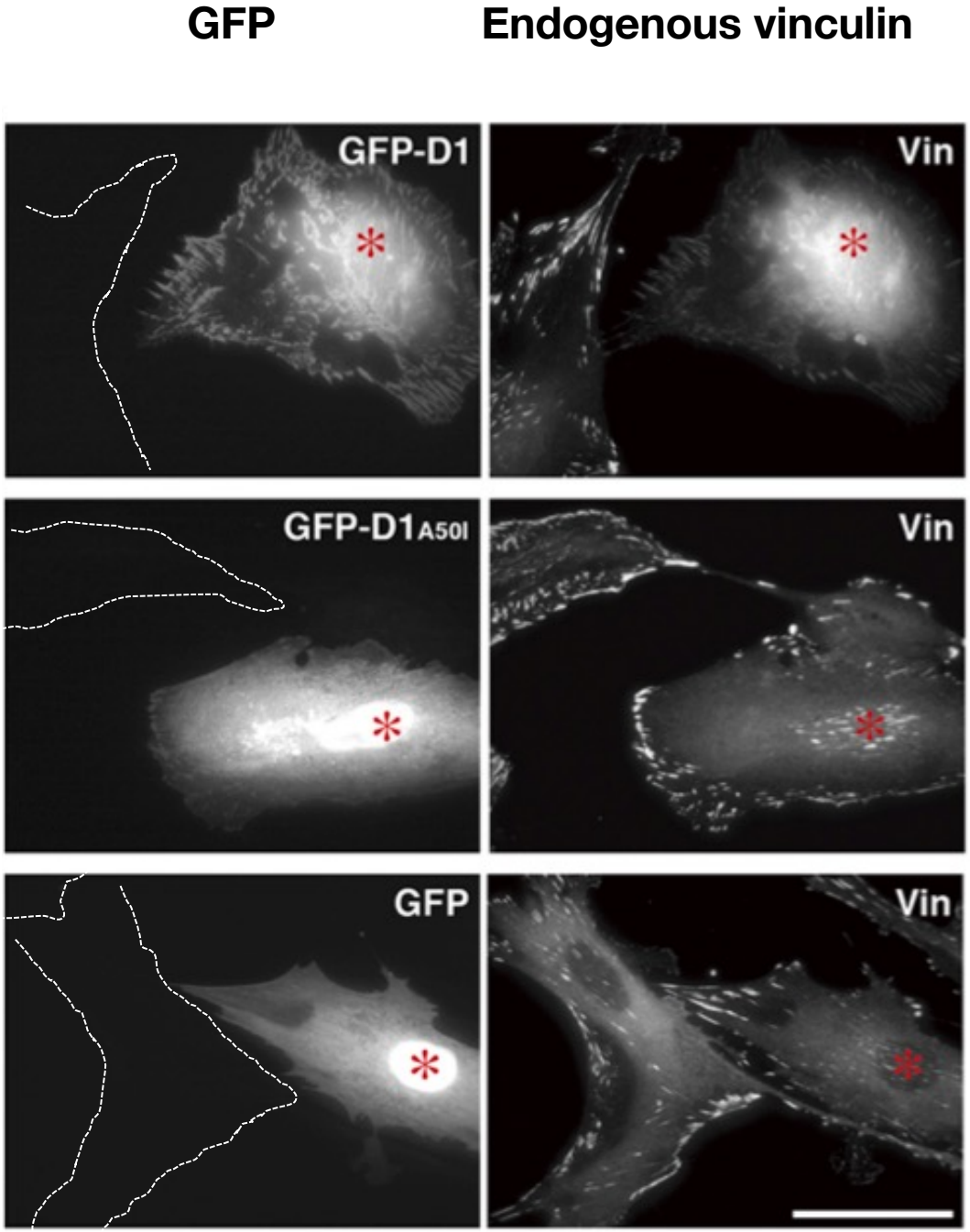
HFF cells were transfected with GFP-D1, GFP-D1$_{A50I}$ or GFP, and then stained for endogenous vinculin (Vin).





* Cells expressing the exogenous molecules.


GFP
Endogenous vinculin
C
GFP-D1
Vin
GFP-D1A50I
Vin
GFP
Vin


GFP
Endogenous vinculin
GFP-D1
Vin
GFP-D1A50I
Vin
GFP
Vin

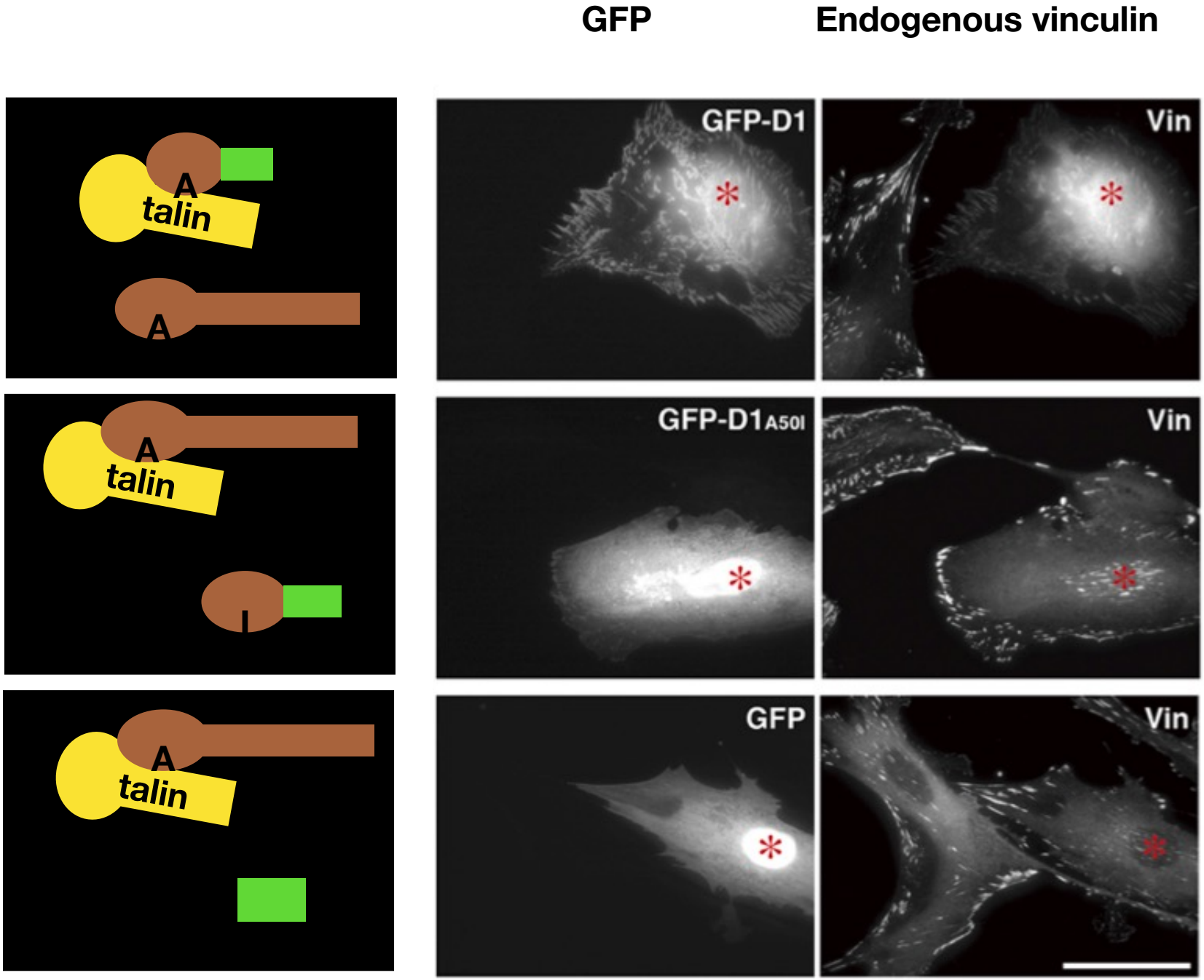
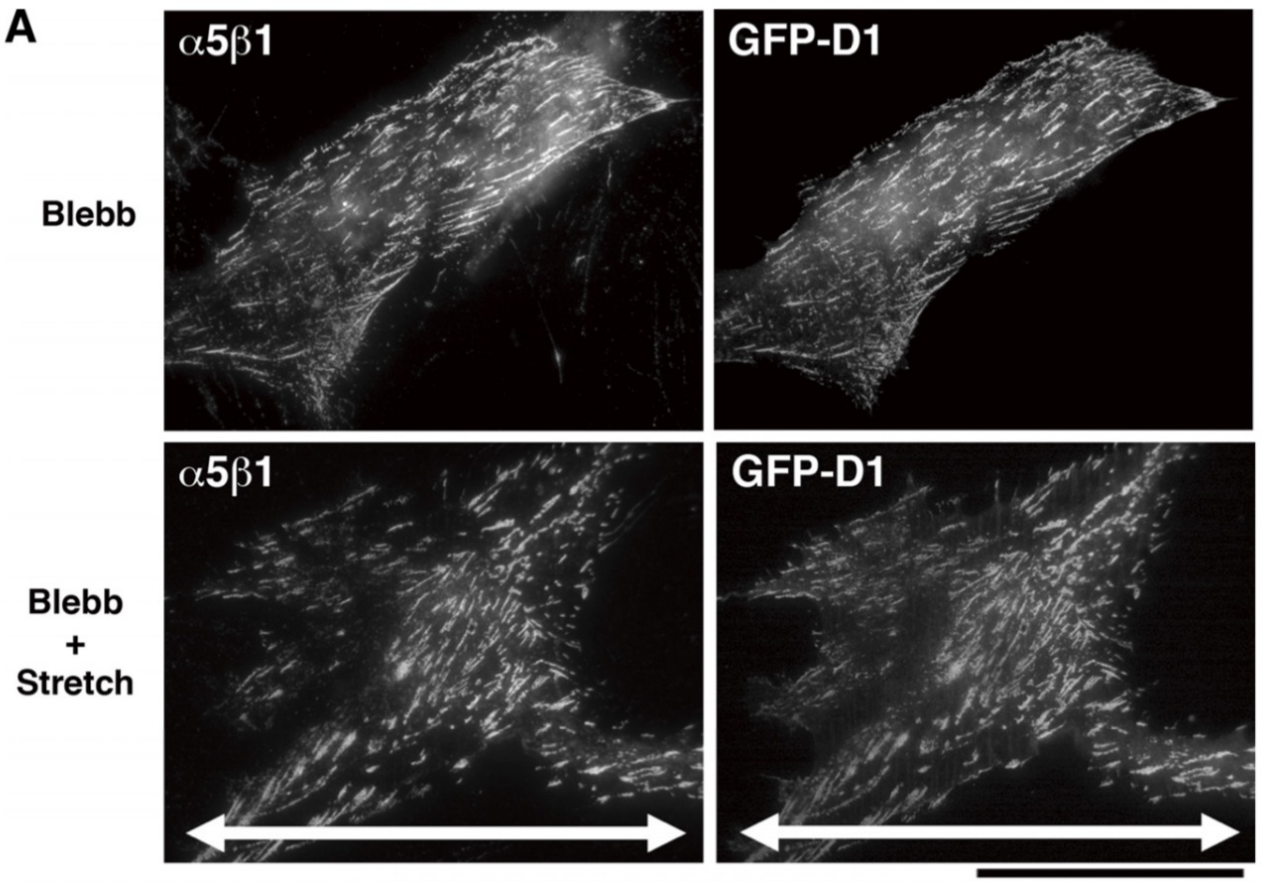

GFP
Endogenous vinculin
A
talin
A
A
talin
I
A
talin
GFP-D1
Vin
GFP-D1A50I
Vin
GFP
Vin

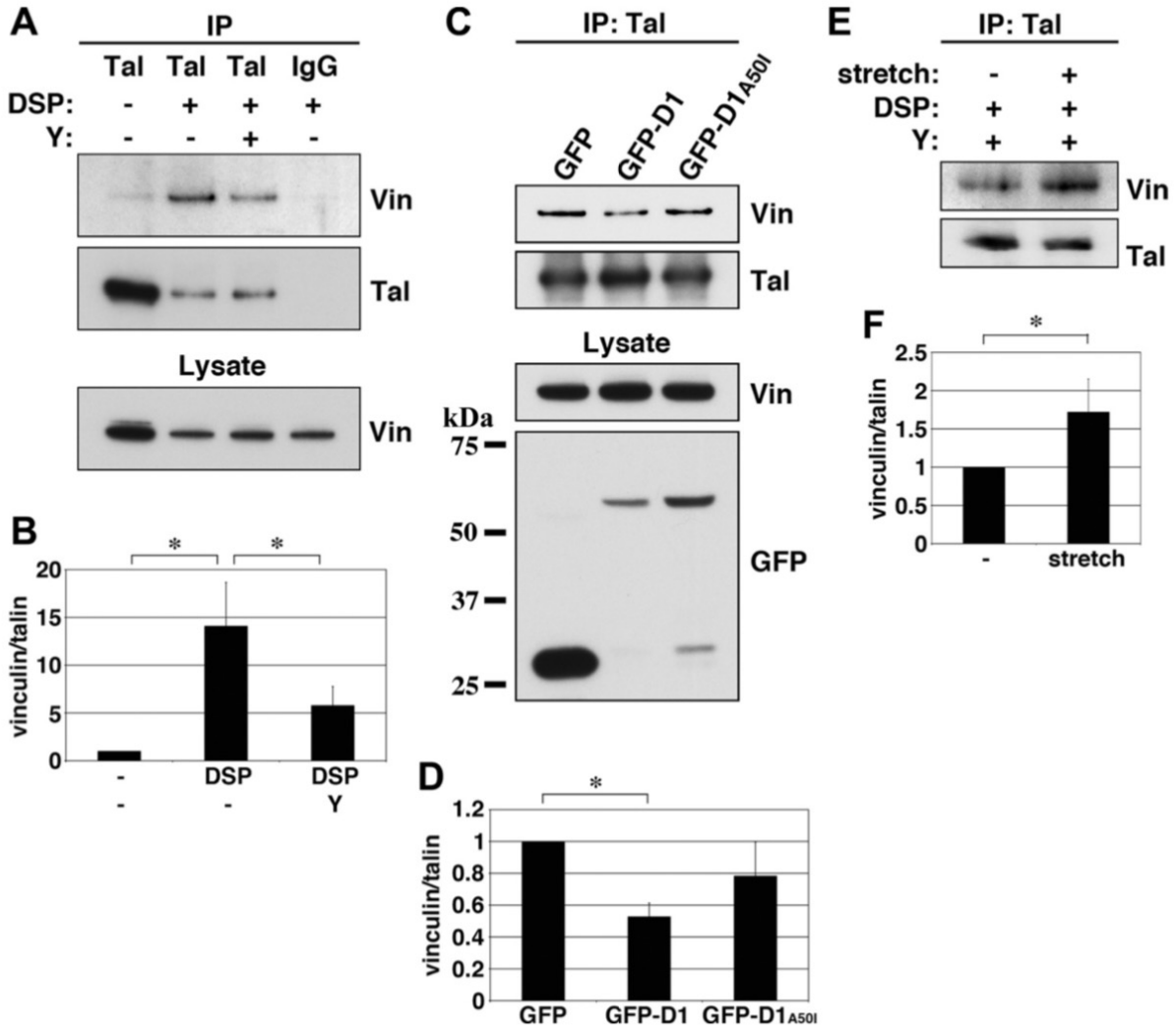
Fig.4

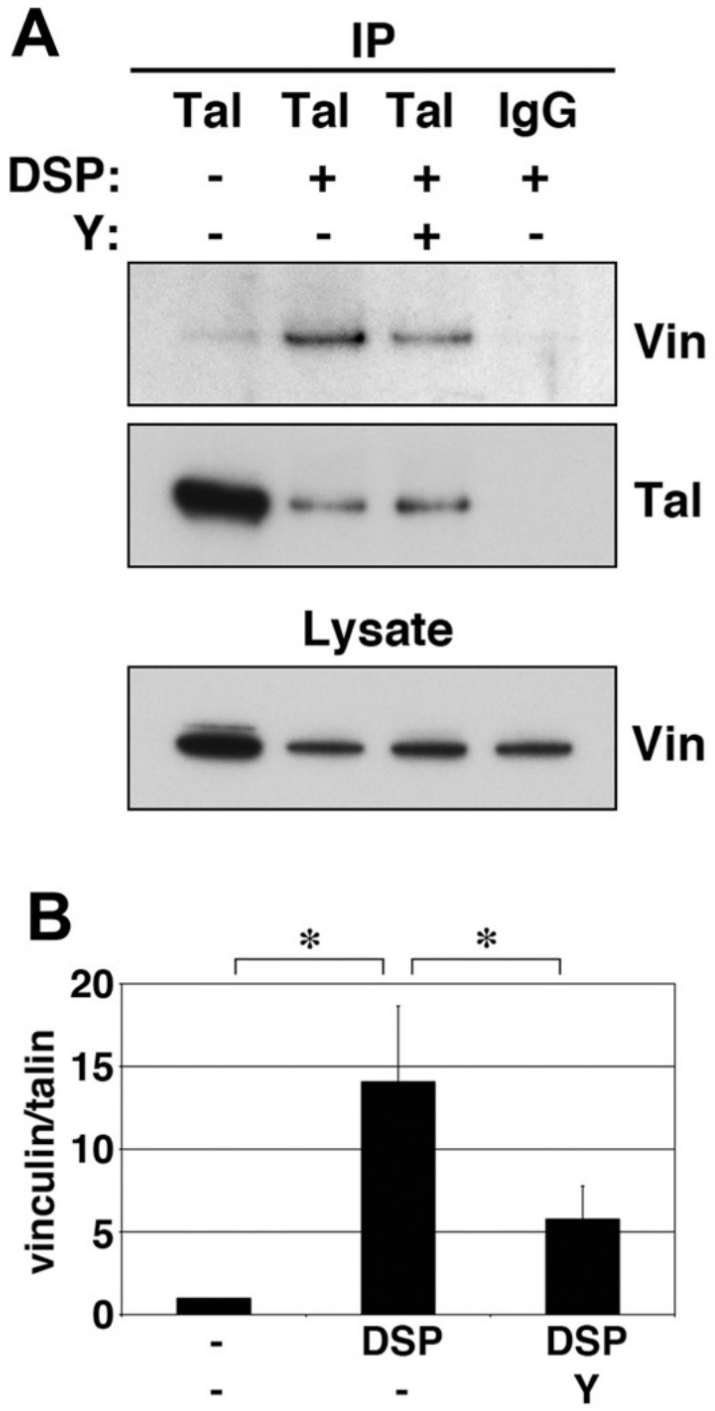
Inhibition of talin-vinculin binding abrogates the stretch-induced vinculin accumulation at FAs.



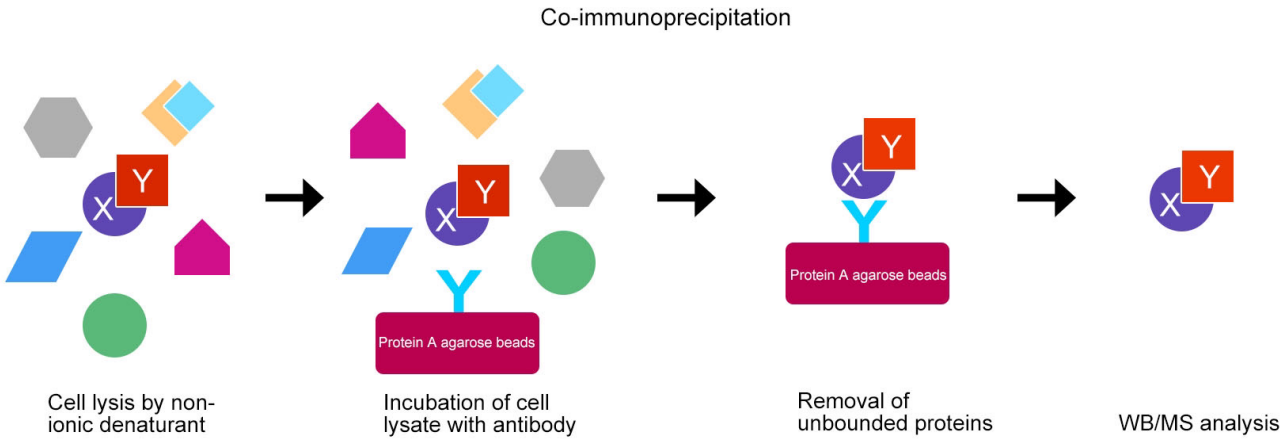
A: HFF cells grown on FN-coated elastic substrata and transfected with GFP-D1 were treated with 100 □M blebbistatin for 30 min, and then the substrata were uniaxially stretched (50% for 3 min) in the presence of blebbistatin. Cells without (Blebb) or with stretching substratum (Blebb □ Stretch) were stained for □α5β1□-integrin.

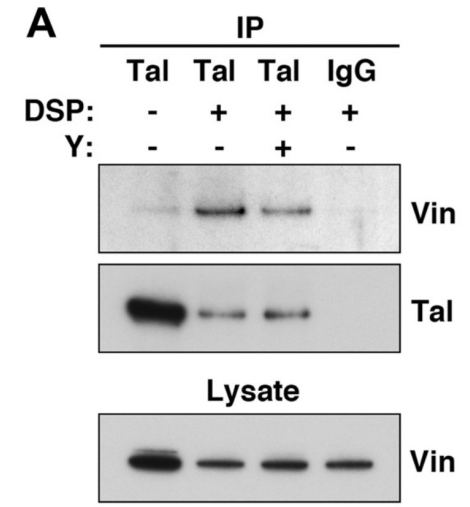
Explain the results of figure 6C

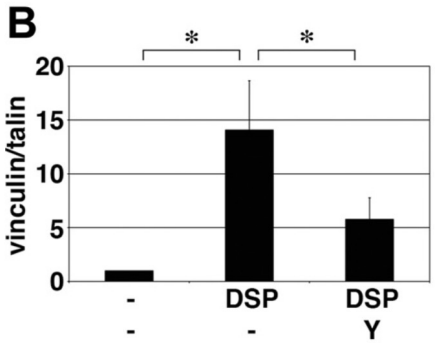


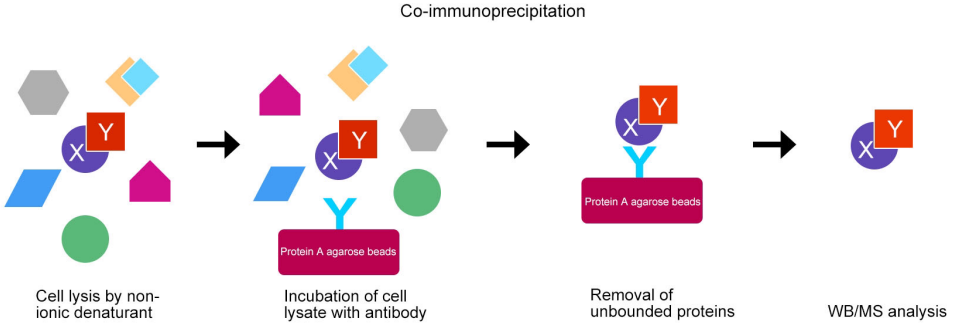

A
IP
Tal
Tal
Tal
IgG
DSP:
-
+
+
+
Y:
-
-
+
-
Vin
Tal
Lysate
Vin
B
*
*
20
15
10
5
0
vinculin/talin
-
-
DSP
-
DSP
Y

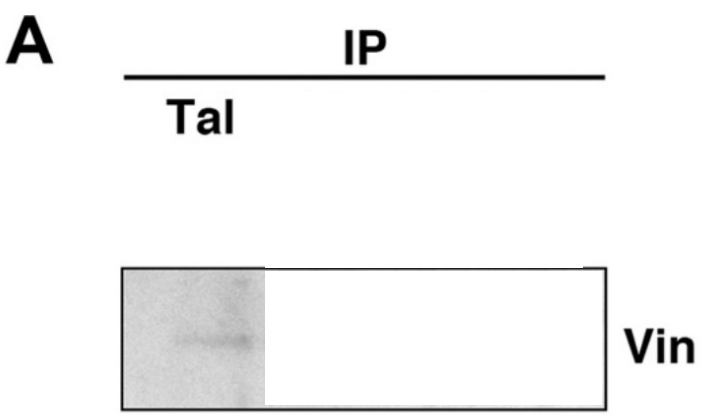
Immunoprecipitation

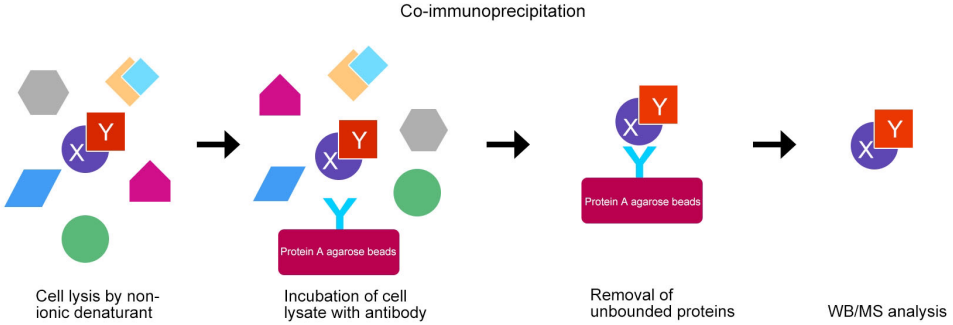


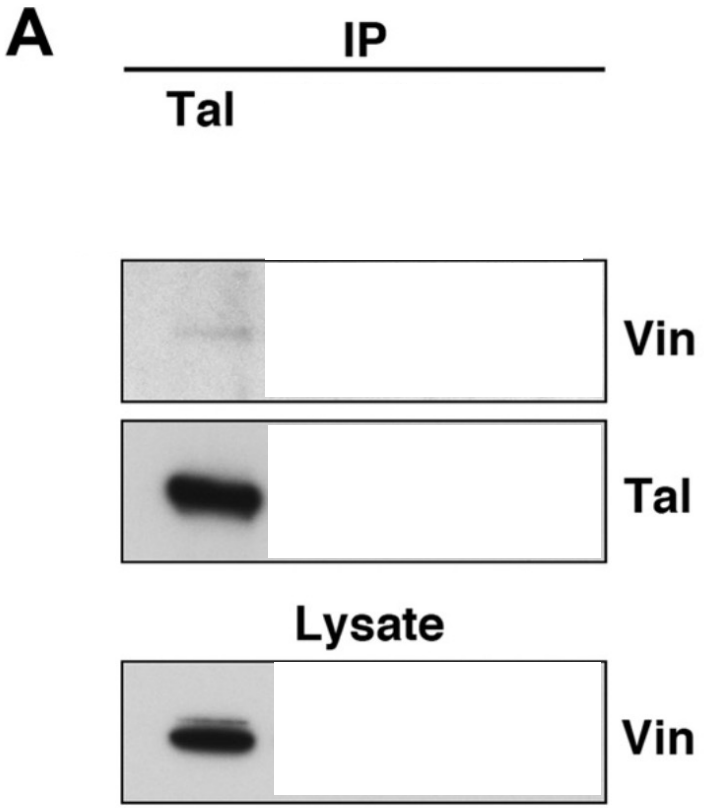




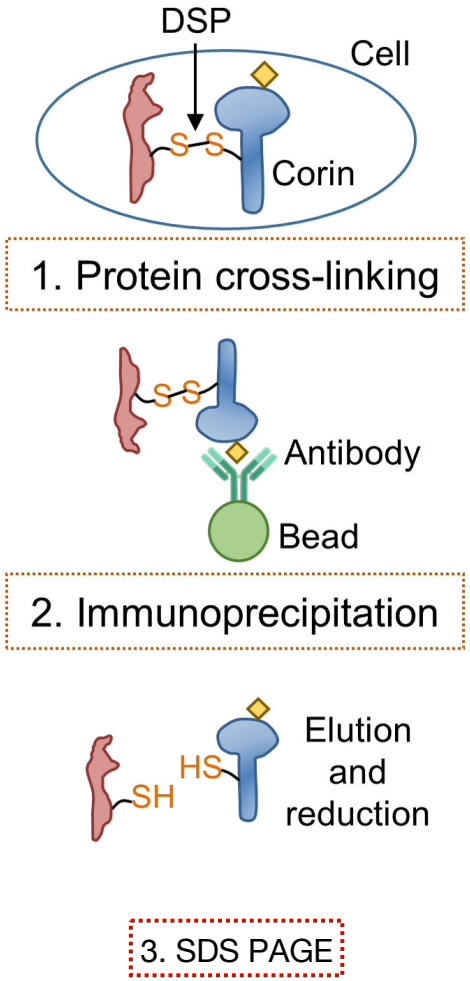

Co-immunoprecipitation
Y
X
Y
Protein A agarose beads
Y
X
Y
Protein A agarose beads
Y
X
Y
Cell lysis by non-ionic denaturant
Incubation of cell lysate with antibody
Removal of unbounded proteins
WB/MS analysis

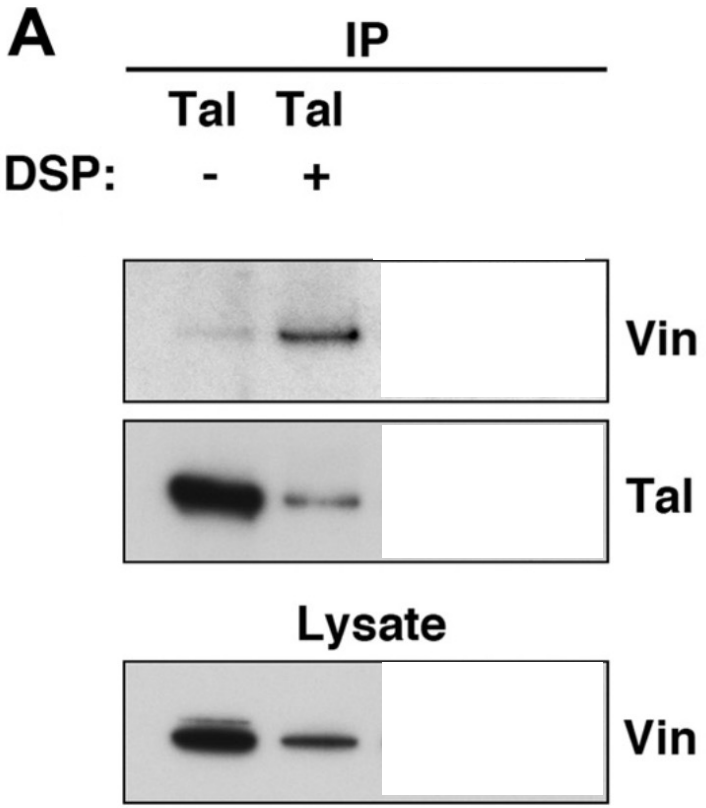

A
IP
Tal
Vin


Co-immunoprecipitation
X
Y
Protein A agarose beads
Cell lysis by non-ionic denaturant
Incubation of cell lysate with antibody
Removal of unbounded proteins
WB/MS analysis

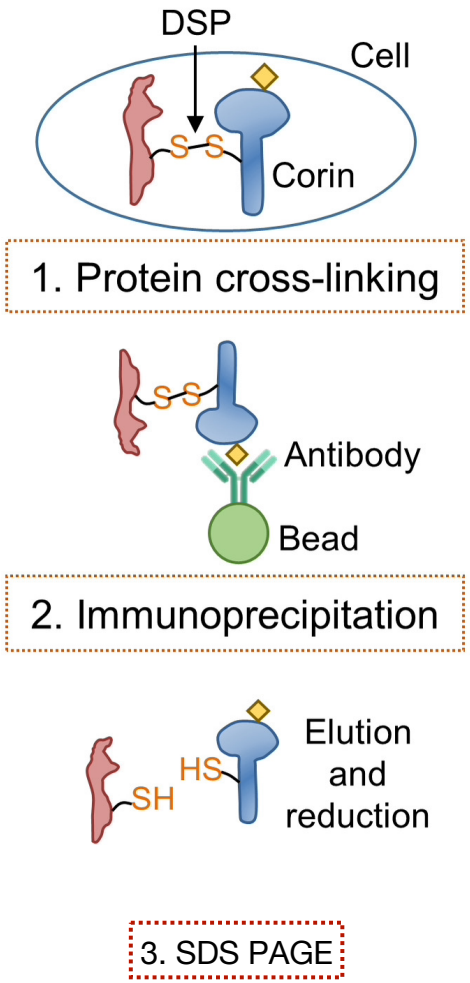

A
IP
Tal
Vin
Tal
Lysate
Vin

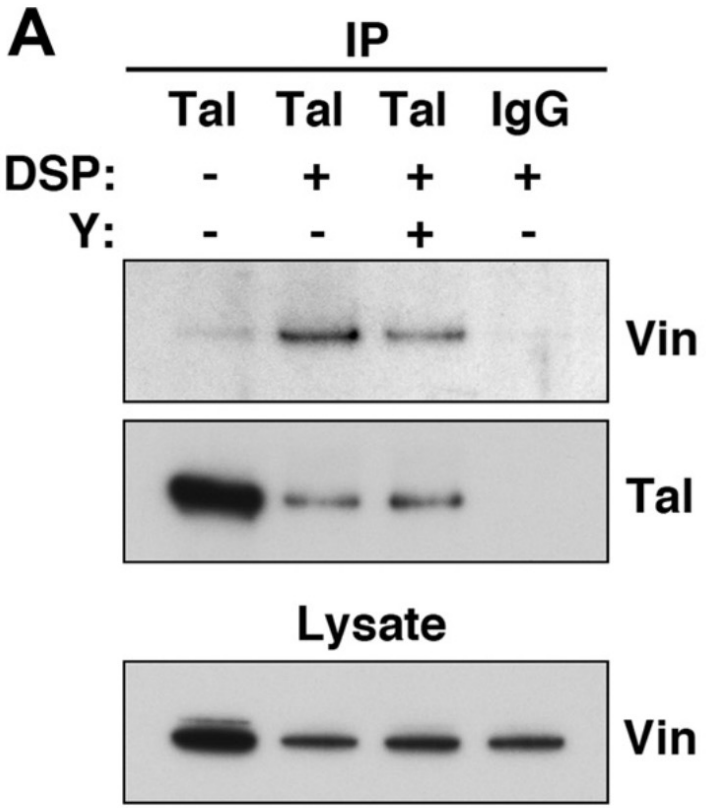
Crosslinking



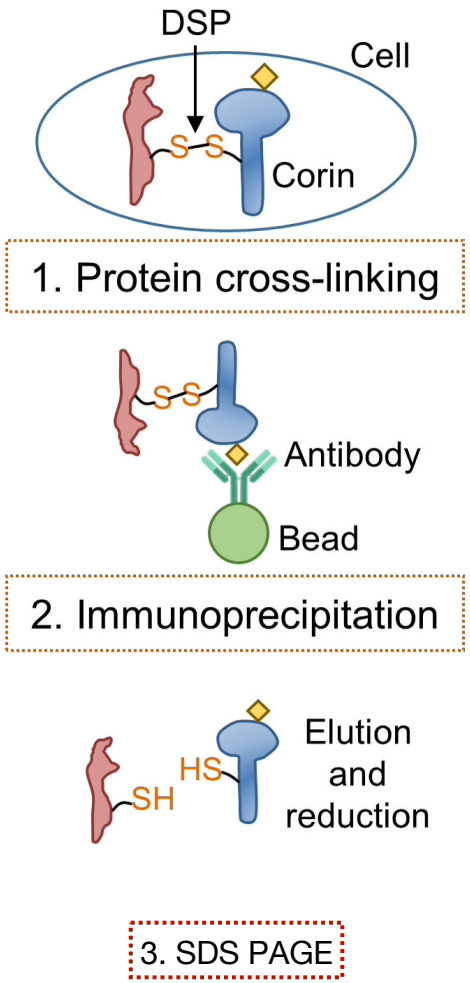


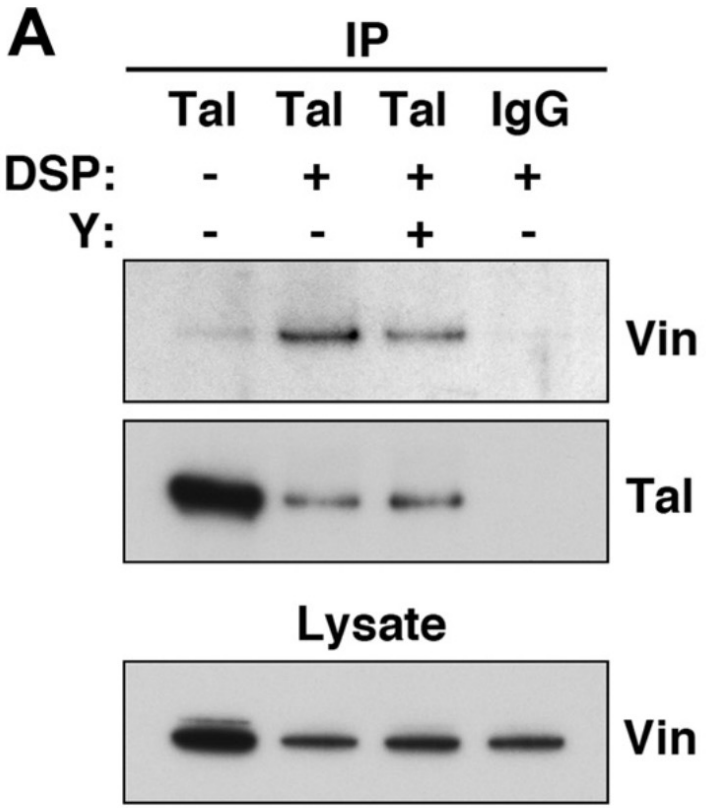
Crosslinking

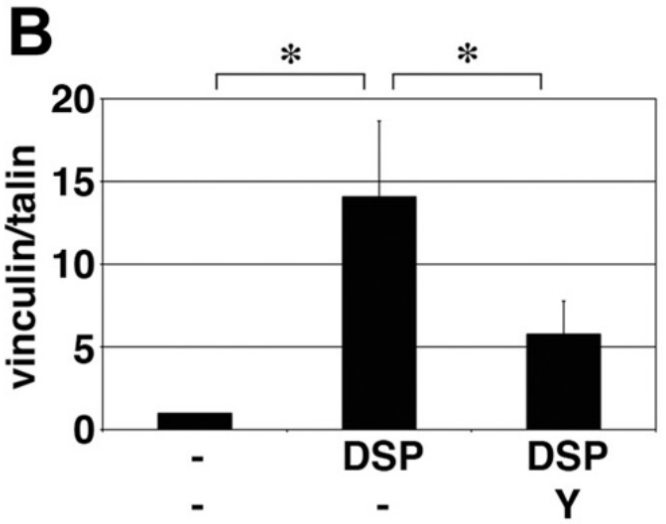




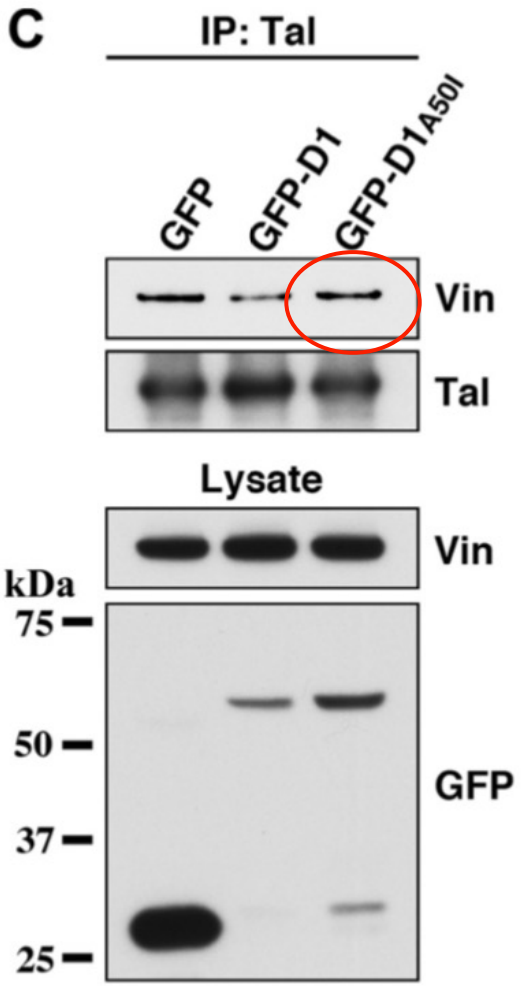
Crosslinking







Your turn:


C
IP: Tal
GFP
GFP-D1
GFP-D1A50I
Vin
Tal
Lysate
Vin
kDa
75
50
37
25
GFP

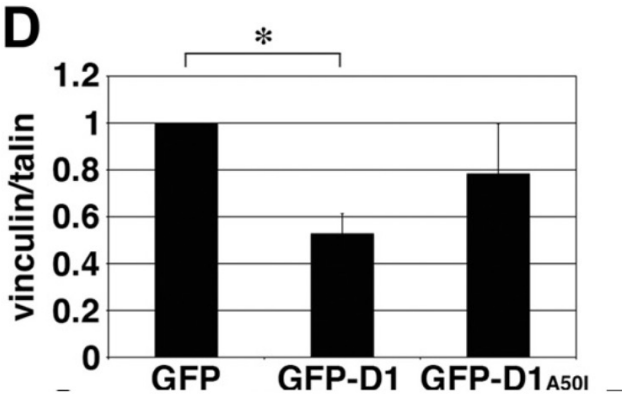
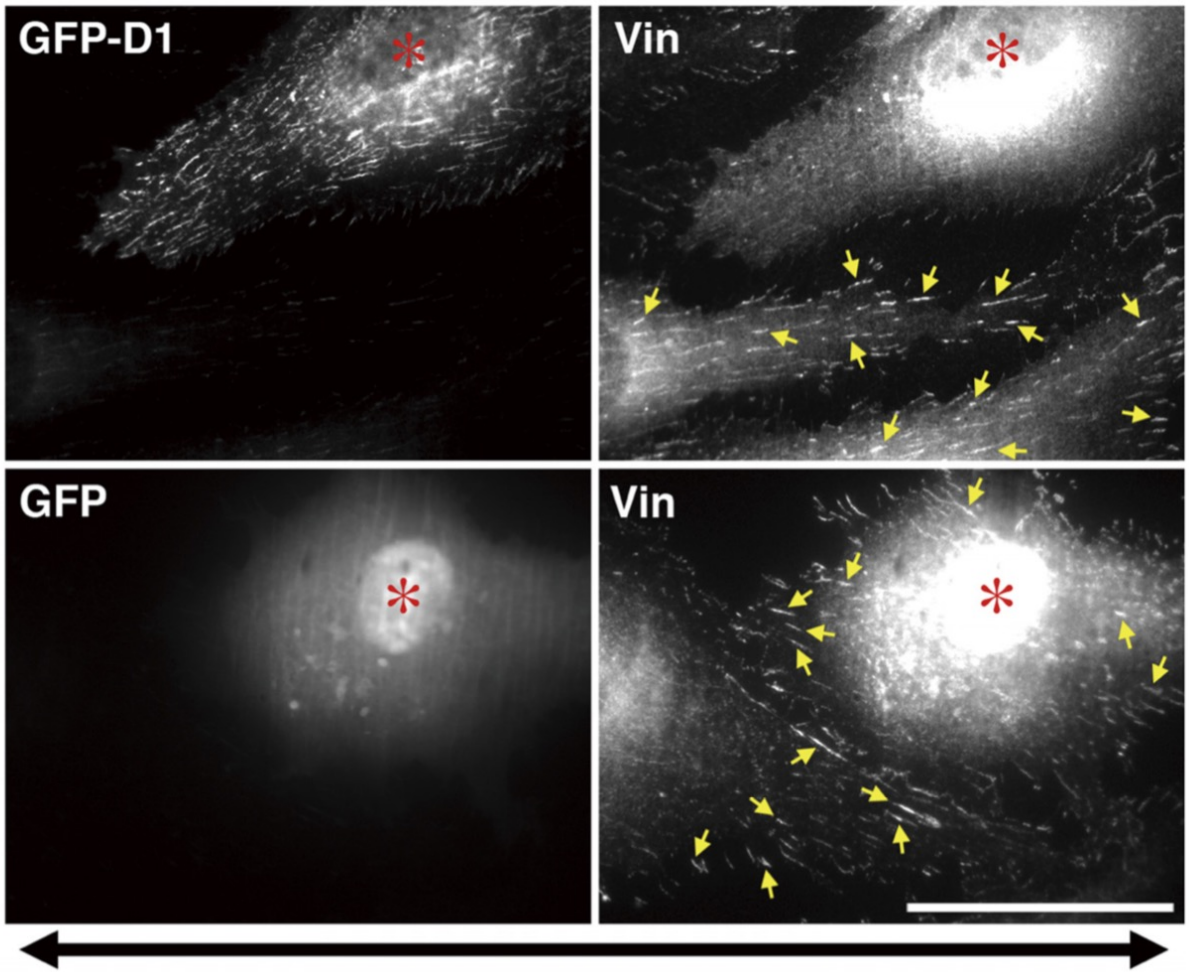

D
*
vinculin/talin
1.2
1
0.8
0.6
0.4
0.2
0
GFP
GFP-D1
GFP-D1A50I

Fig.4

Inhibition of talin-vinculin binding abrogates the stretch-induced vinculin accumulation at FAs.

* Cells expressing GFP-D1 or GFP.



HFF cells grown on FN-coated elastic substrata were transfected with GFP-D1 or GFP and treated with 100 μM blebbistatin for 30 min. The substrata were uniaxially stretched (50% for 3 min) in the presence of blebbistatin, and cells were stained for endogenous vinculin (Vin). Double-headed arrows indicate the direction of the stretch axis. Bars = 50 μm.