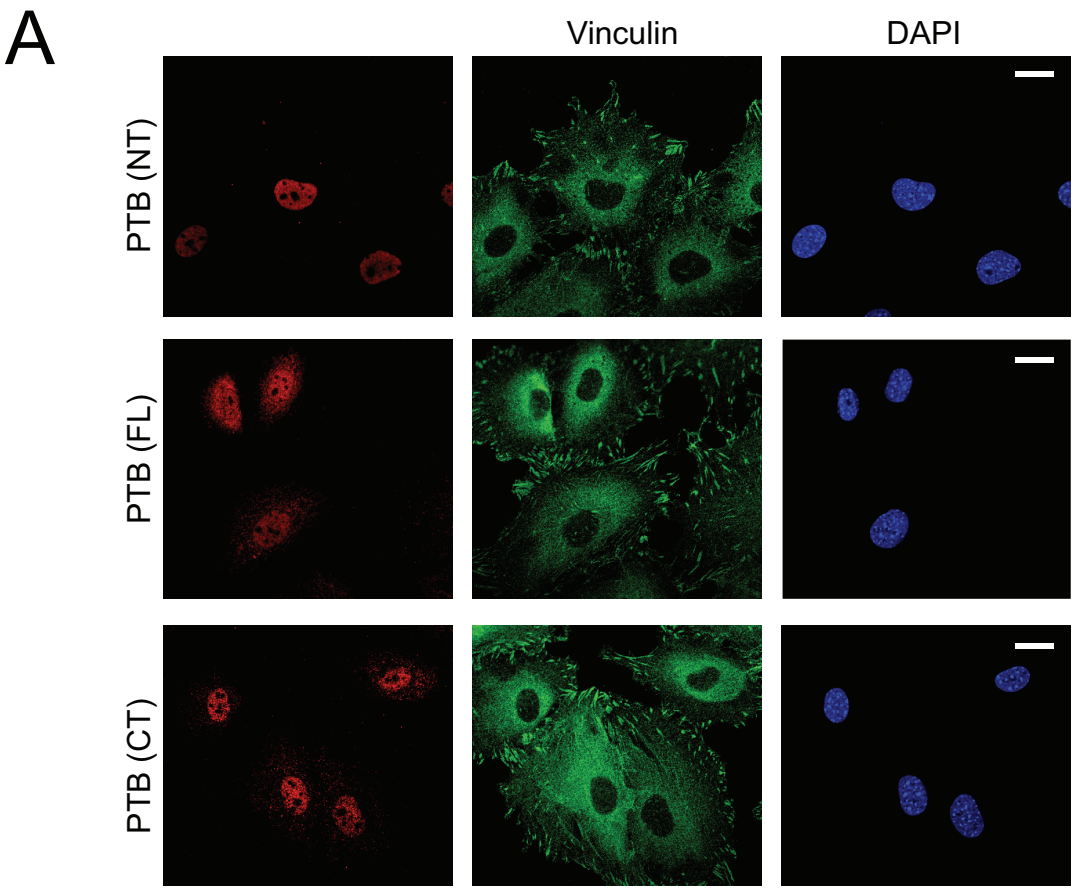
FIG. S1. All PTB antibodies detect PTB re-localization to the cytoplasm of MEFs adhering to fibronectin. (A) MEFs grown overnight were fixed and stained with the indicated antibodies. In the fully spread MEFs all PTB antibodies detect PTB mostly in the nucleus. (B) MEFs were lifted and maintained in suspension and replated on fibronectin. Cells adhered for 12 min and were fixed and stained with the indicated antibodies. Arrowheads indicate PTB vinculin co-localization at possible spreading initiation centers. Scale bars are 20 μm.

FIG. S2. MEFs transfected with shPTB(A) show decreased cell spreading and fewer protrusions. (A) MEFs were transfected with shPTB(A) and EGFP or empty vector and EGFP. After 3 days cells were lifted and re-adhered to fibronectin for 90 min. The cells were fixed and stained for PTB and vinculin. Arrowheads identify transfected cells. (B) Quantitation of cell surface area of transfected cells (n=50 for control and n=48 for shPTB(A)). The red bar in the plot shows the mean, and asterisks indicate statistical significance measured by paired *t*-test (***P<0.0001). (C) Cells were also scored for number of protrusions formed during spreading. The graph shows the percent of transfected cells having 5 or more protrusions or less than 5 protrusions. Scale bars are 20 μm.

FIG. S3. MEFs overexpressing PTB show increased cell spreading. (A) MEFs were transfected with myc tagged PTB or empty vector along with EGFP. After 2 days cells were lifted and re-adhered to fibronectin for 90 min. The cells were fixed and stained for myc and vinculin. Arrowheads identify transfected cells. (B) Quantitation of cell surface area for EGFP and myc-PTB or EGFP and empty vector transfected cells (n=51 for control and n=51 for PTB transfected cells). The red bar in the plot shows the mean, and asterisks indicate statistical significance measured by paired *t*-test (***P<0.0002). Scale bars are 20 μm.

FIG. S4. PTB co-localizes with vinculin mRNA in protrusions formed during early cell spreading. Cells were lifted and maintained in suspension for 1 hour and replated on fibronectin. After 20 min adhered cells were fixed and immunofluorescence performed with anti-PTB (CT) antibody. Cells were post-fixed with PFA and *in situ* hybridization was performed with Cy3 labeled vinculin ribo-probes-3 and -5 combined. Shown are 3 representative images. The arrowhead in the enlarged image identifies regions of co-localization in newly formed protrusions. Scale bars are 20 μm except in the enlarged images to the right which are 5 μm.


A
Vinculin
DAPI
PTB (NT)
PTB (FL)
PTB (CT)

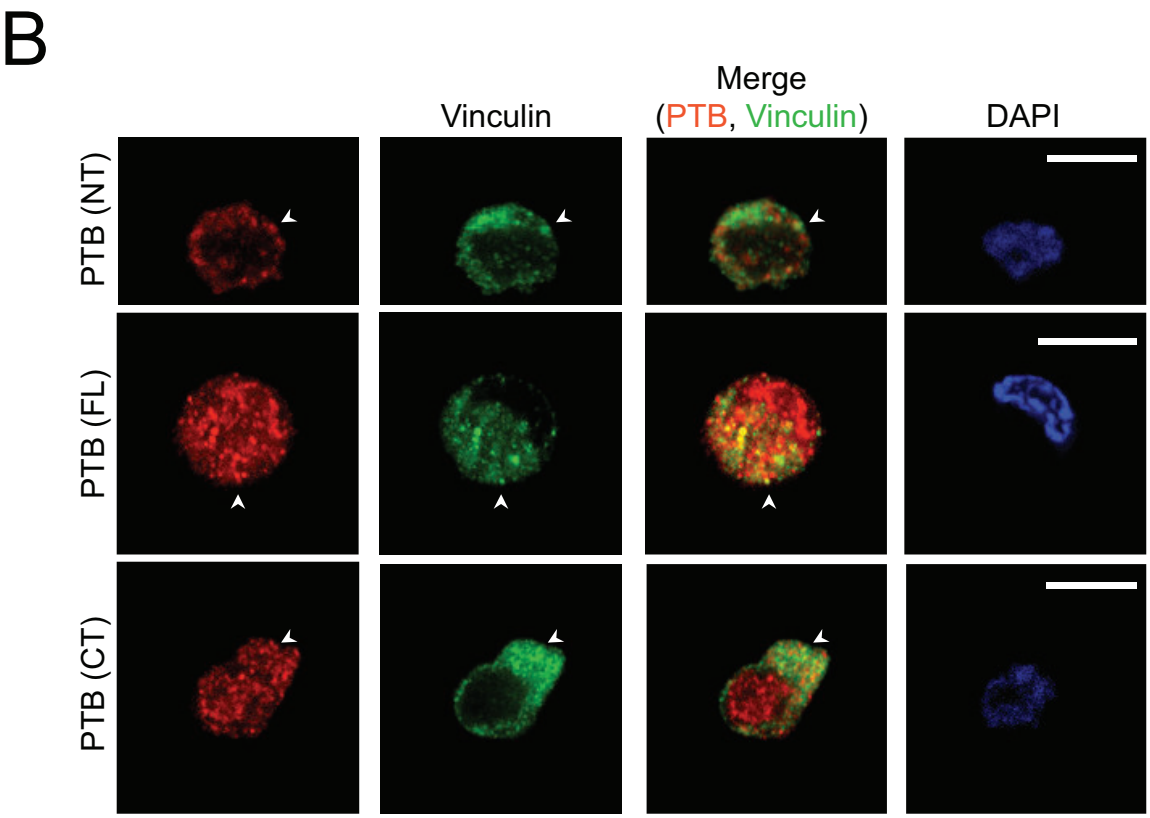

B
Merge
(PTB, Vinculin)
Vinculin
DAPI
PTB (NT)
PTB (FL)
PTB (CT)

Figure S1

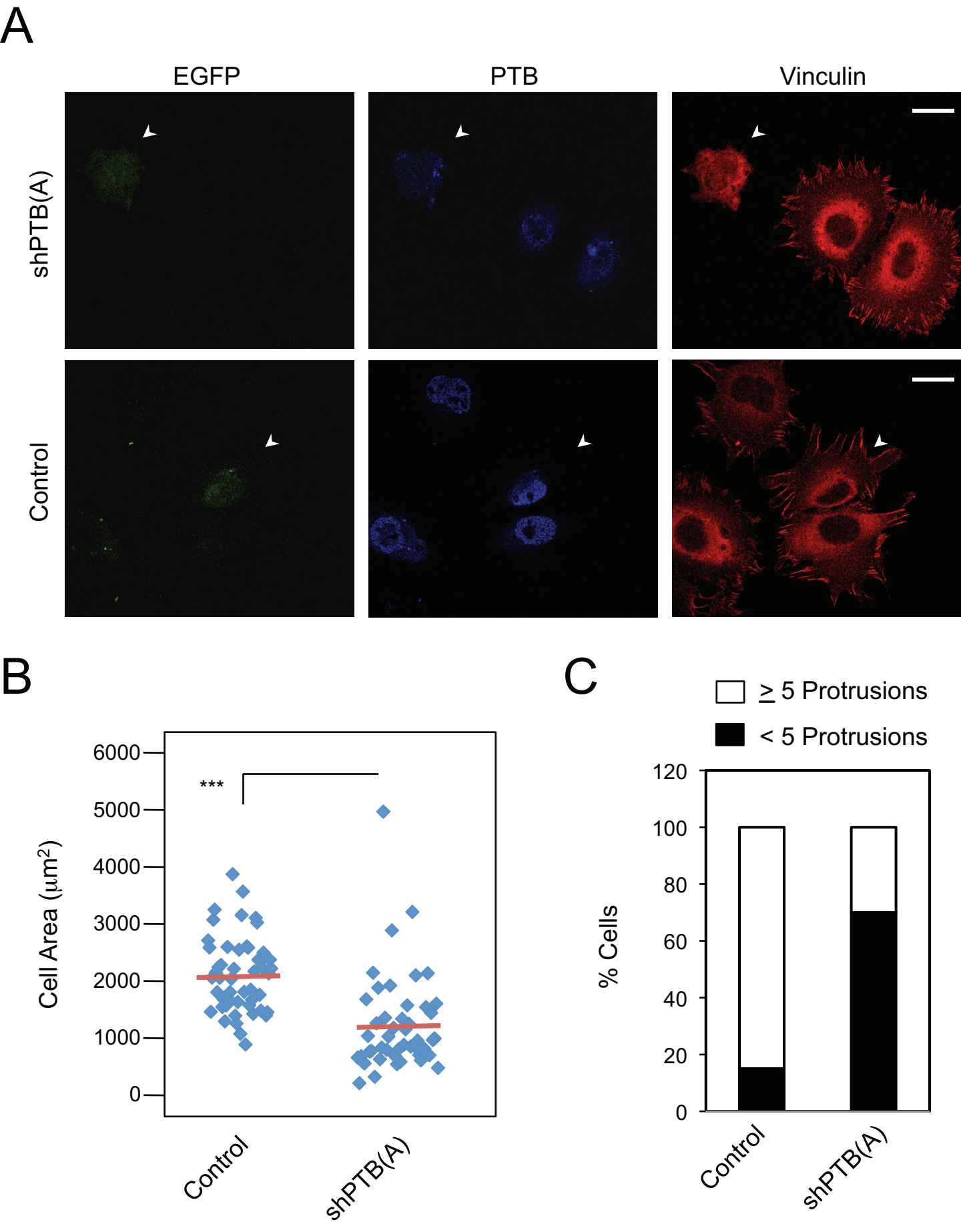
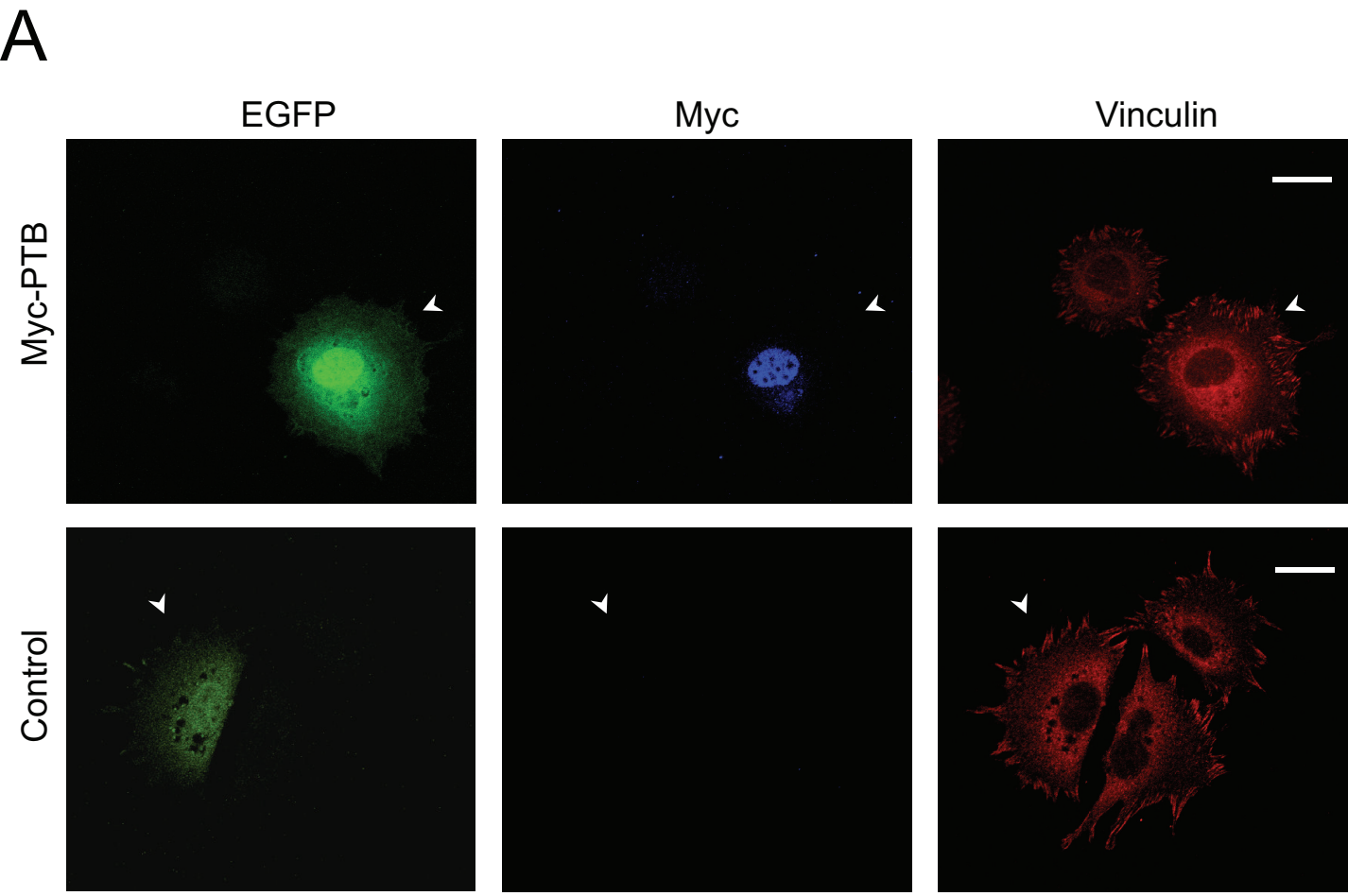

A
EGFP
PTB
Vinculin
shPTB(A)
Control
B
Cell Area (μm²)
6000
5000
4000
3000
2000
1000
0
***
Control
shPTB(A)
C
≥ 5 Protrusions
< 5 Protrusions
% Cells
120
100
80
60
40
20
0
Control
shPTB(A)

Figure S2


A
EGFP
Myc
Vinculin
Myc-PTB
Control

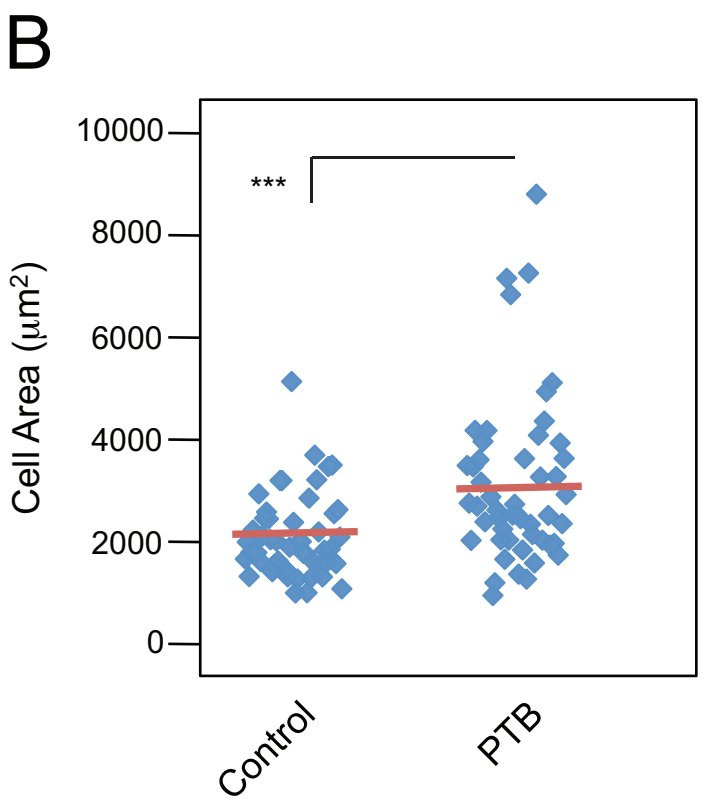

B
10000
8000
6000
4000
2000
0
Cell Area (μm²)
***
Control
PTB

Figure S3

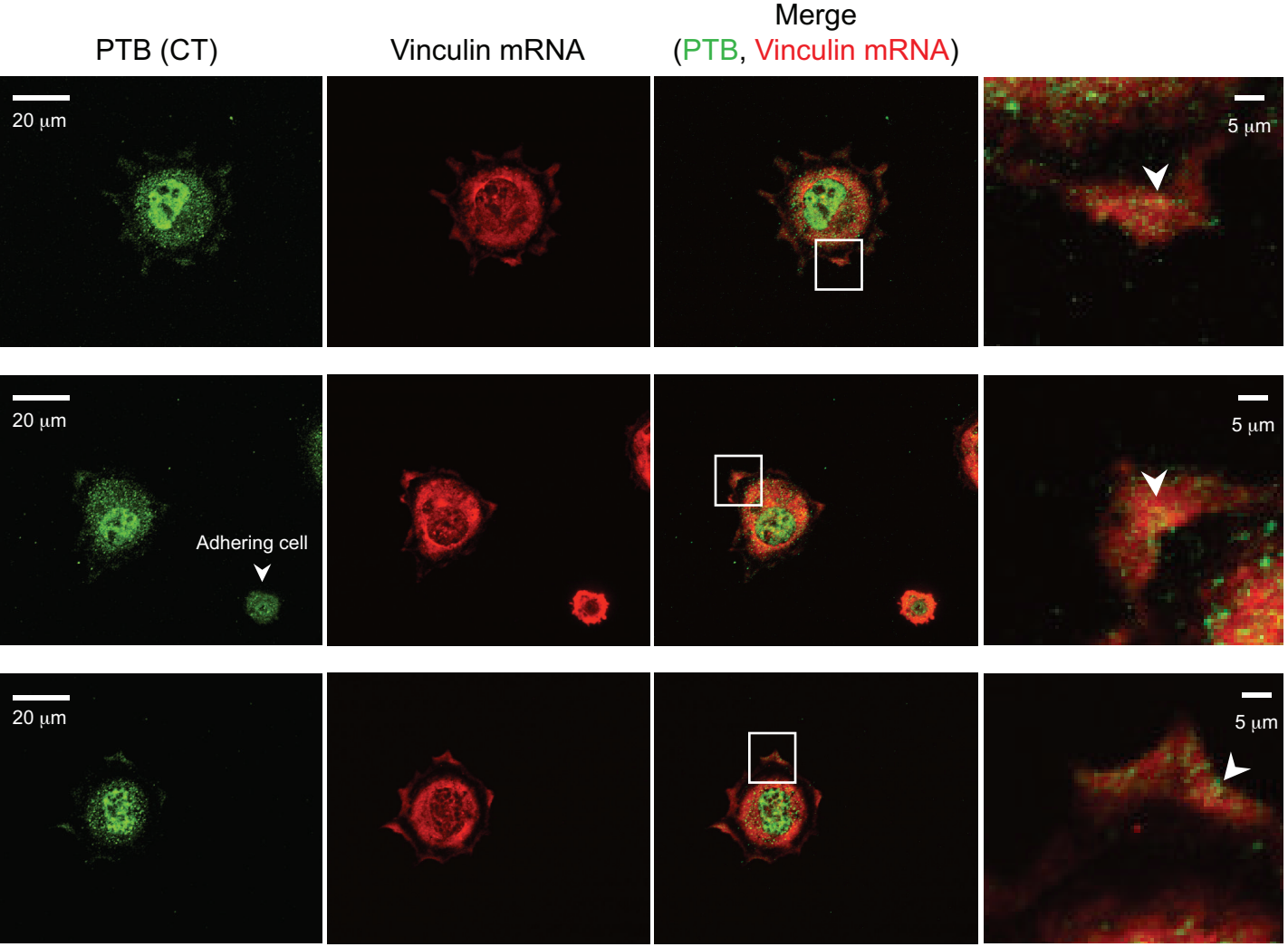

PTB (CT)
Vinculin mRNA
Merge
(PTB, Vinculin mRNA)
20 μm
5 μm
20 μm
5 μm
Adhering cell
20 μm
5 μm

Figure S4