

Supplemental Materials

Molecular Biology of the Cell

Zhou et al.

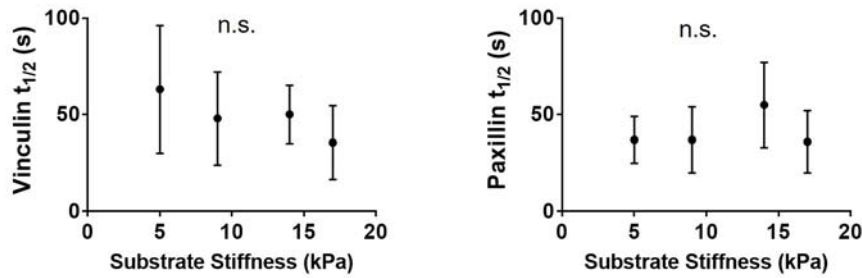


Figure S1: Relationship between substrate stiffness and vinculin $t_{1/2}$ and paxillin $t_{1/2}$ (average \pm SD, $n = 18-25$ FAs) at single FAs for MEFs cultured on different substrate stiffness. No relationship between substrate stiffness and vinculin or paxillin residence time at FAs was detected.

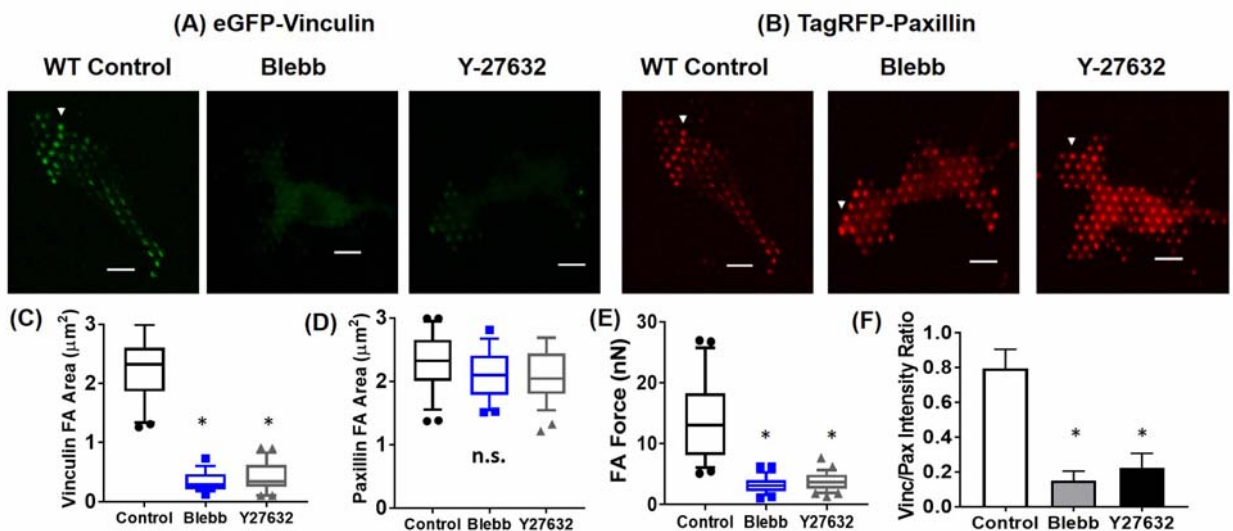


Figure S2: Comparison between blebbistatin and Y-27632 effects on FA assembly and traction force generation. (A) Vinculin (green) and (B) paxillin (red) on mPADs of 14 kPa in the presence of no inhibitor, 20 μ M blebbistatin for 1 h, or 50 μ M Y-27632 for 1 h. White arrowheads indicate FAs. Scale bar 10 μ m. Effects of inhibitors (C-F) on vinculin FA area (C), paxillin FA area (D), and traction force at single FAs (E), plotted as box-whisker plots (median, 10th, 25th, 75th, and 90th percentile, $n > 25$). (F) Effects of substrate stiffness and actomyosin contractility on vinculin/paxillin intensity ratio at single FAs ($n > 25$, mean \pm SD). * $P < 0.01$ vs. WT control.

Table S1: Summary of linear regression results between vinculin-paxillin area and force at single FAs. Data presented are results from figures 3 (WT vinculin and paxillin) and 4 (T12 vinculin and WT paxillin). A-B represent WT and T12 vinculin and force. C-D represent paxillin and force for WT and T12 vinculin expressing MEFs.

(A) WT Vinculin and Force			
Stiffness	Blebbistatin	R²	P-value vs. Zero Slope
3 kPa	Control	0.02	0.44
3 kPa	Treatment	0.01	0.88
5 kPa	Control	0.07	0.16
5 kPa	Treatment	0.01	0.54
14 kPa	Control	0.01	0.93
14 kPa	Treatment	0.05	0.26

(B) T12 Vinculin and Force			
Stiffness	Blebbistatin	R²	P-value vs. Zero Slope
3 kPa	Control	0.06	0.21
3 kPa	Treatment	0.06	0.17
5 kPa	Control	0.05	0.29
5 kPa	Treatment	0.01	0.87
14 kPa	Control	0.07	0.24
14 kPa	Treatment	0.11	0.07

(C) Paxillin and Force for WT Vinculin MEF			
Stiffness	Blebbistatin	R²	P-value vs. Zero Slope
3 kPa	Control	0.01	0.52
3 kPa	Treatment	0.07	0.13
5 kPa	Control	0.07	0.17
5 kPa	Treatment	0.01	0.61
14 kPa	Control	0.01	0.65
14 kPa	Treatment	0.01	0.78

(D) Paxillin and Force for T12 Vinculin MEF			
Stiffness	Blebbistatin	R²	P-value vs. Zero Slope
3 kPa	Control	0.08	0.23
3 kPa	Treatment	0.04	0.15
5 kPa	Control	0.07	0.32
5 kPa	Treatment	0.01	0.91
14 kPa	Control	0.09	0.31
14 kPa	Treatment	0.14	0.08