Supplemental Figures:

Figure S1: Characterization of 96 Well Plates. (A) Image of 96 well glass-bottom plate used for cell culture and high content imaging. (B) Plot of atomic force microscope-determined stiffness of 5% and 10% acrylamide hydrogels, i.e. myogenic- and osteogenic-inducing hydrogels, respectively. (C) Schematic illustrating the diffusion based technique used to determine hydrogel thickness. Red circles indicate Texas Red-conjugated beads. (D) Plot of hydrogel thickness for 5% and 10% acrylamide hydrogels.

Figure S2: CellProfiler Pipeline. (A) Nuclei are identified first from the DAPI channel. (B) Using the nuclei as seed regions, cell outlines are identified for each nucleus. (C) Representative images and their average nuclear and cytoplasmic fluorescence are shown to indicate marker expression and distribution information obtained through CellProlifer. Cells with high nuclear expression of the transcription factor are considered to have expressed and localized the factor correctly.

Figure S3: ScanSite Analysis of Candidate Mechanosensors. (A) Plots of surface accessibility reveal regions corresponding to predicted MAPK1 domains. Green highlighted regions denote surface inaccessible binding sites predicted to bind MAPK1 domains. (B) List of sites and surface accessibility values for given predicted MAPK1 binding domains. Note that a surface accessible site has a value above 1 and a completely inaccessible site has a value of 0 in this analysis.

Figure S4: Secondary Metrics from High Content Image Analysis. (A) Average cell area and (B) cell eccentricity, calculated as the ratio between the distance between the two foci of a fitted ellipse and the major axis length of the cell, are plotted as a function of siRNA treatment. For WT, Vinc, p130, Fil, SORBS3, Pax, and SORBS1 in (A) and (B), n=39, 31, 43, 24, 30, 35, 29, and 35, respectively. (C) Cell migration rose plots on tissue culture plastic for each knockdown condition. (D) Staining for Vinculin and Paxillin in the indicated siRNA conditions reveals no substantial differences in focal adhesion morphology as a result of siRNA induced knockdown.

Figure S5: Western Blotting of MAPK1 in hMSCs. Western blots of (A) MAPK1 and (B) GAPDH for the indicated hMSC culture conditions.

Supplemental Tables

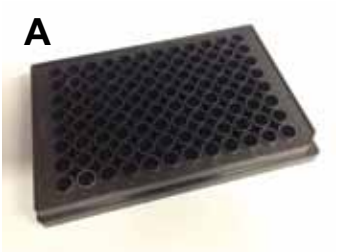
Supplemental Table 1: siRNA sequences used for transient knockdown.

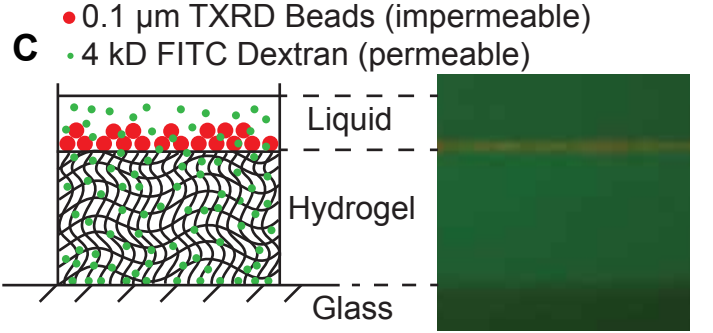
Gene	Accession Number	Sequences
Vinculin	P18206	CAGCAUUUAUUAAGGUUGA, GCCAAGCAGUGCACAGAUA, GAGCGAAUCCCAACCAUAA, UGAGAUAAUUCGUGUGUUA
p130Cas	P56945	GGUCGACAGUGGUGUGUAU, GGCCACAGGACAUCUAUGA, GCAAUGCUGCCCACACAUC, CCAGAUGGGCAGUACGAGA
Paxillin	P49023	GAGCUAACAUCCAUAUUUA, GUGCAACUGUCUUUAAUAU, CCAGUAACUUUCACAUGUA, GAGUUUAUCUGGAGUGUAG
Filamin	P21333	GCAGGAGGCUGGCGAGUAU, GCACCCAGACCGUCAAUUA, GCACAUGUUCCGUGUCCUA, GAAUGGCGUUUACCUGAUU
SORBS1	Q9BX66	CAAGAGCAUUUACGAAUAU, GAGAUGAGCUACAUUGAUG, UAUACCAGCUGAUUACUUG, GAAGAGCACUCAGGACUUA
SORBS3	O60504	GAGAGGCUGUGGCCCAGUA, CAUCUUCCCUGCUAAUUAU, CCAAGGAGCUGACUCUGCA, CCUAACACCUCUCAGAUAC

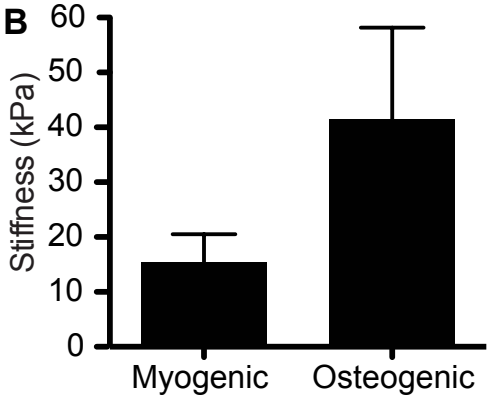
Supplemental Table 2: Forward and reverse primers for qPCR.

Primer set 1: targeting all isoforms of SORBS1	
Forward primer: GAAGGTAGTCAAGAGGTCGGC	T_m: 60.14 °C
Reverse primer: GGGGGTTCCCAGTCATTTCTT	T_m: 59.92 °C
Primer set 2: targeting SORBS1 isoforms containing L1033	
Forward primer: CACCTCGCCTTGTCACCAA	T_m: 60.23 °C
Reverse primer: GTGGGACGATCTGACCAACT	T_m: 59.39 °C

Figure S1


A


C
0.1 μm TXRD Beads (impermeable)
4 kD FITC Dextran (permeable)
Liquid
Hydrogel
Glass


B
Stiffness (kPa)
60
50
40
30
20
10
0
Myogenic
Osteogenic

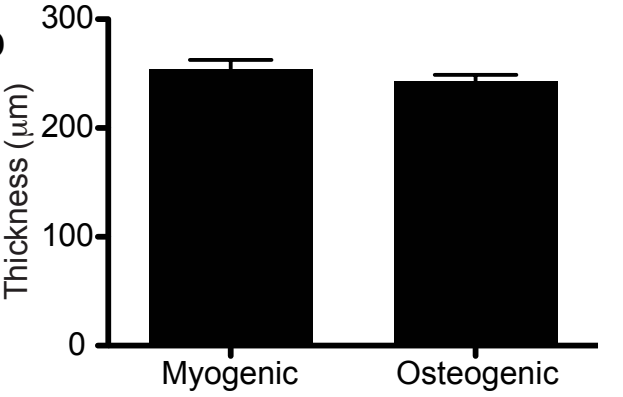

D
Thickness (μm)
300
200
100
0
Myogenic
Osteogenic

Figure S2

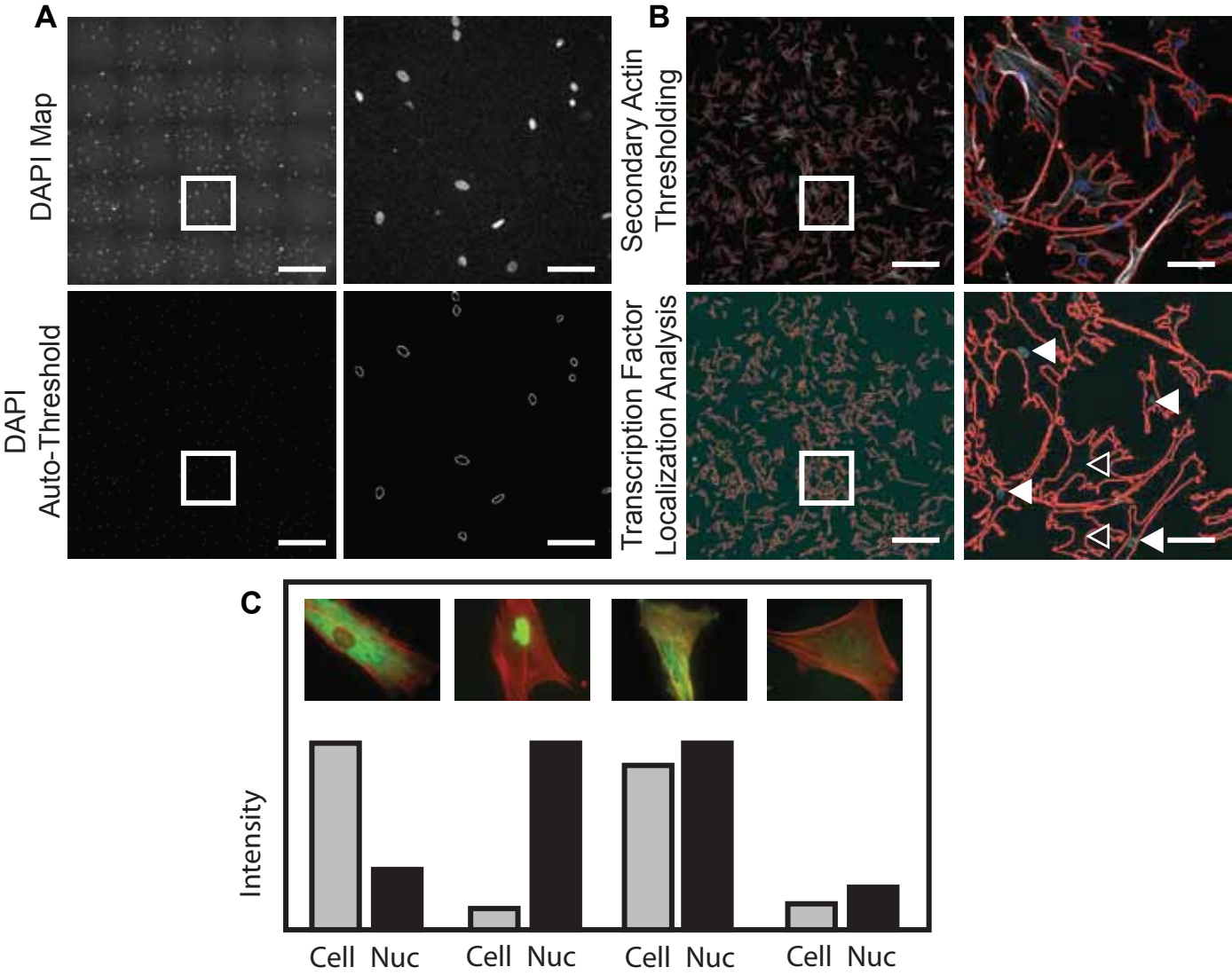
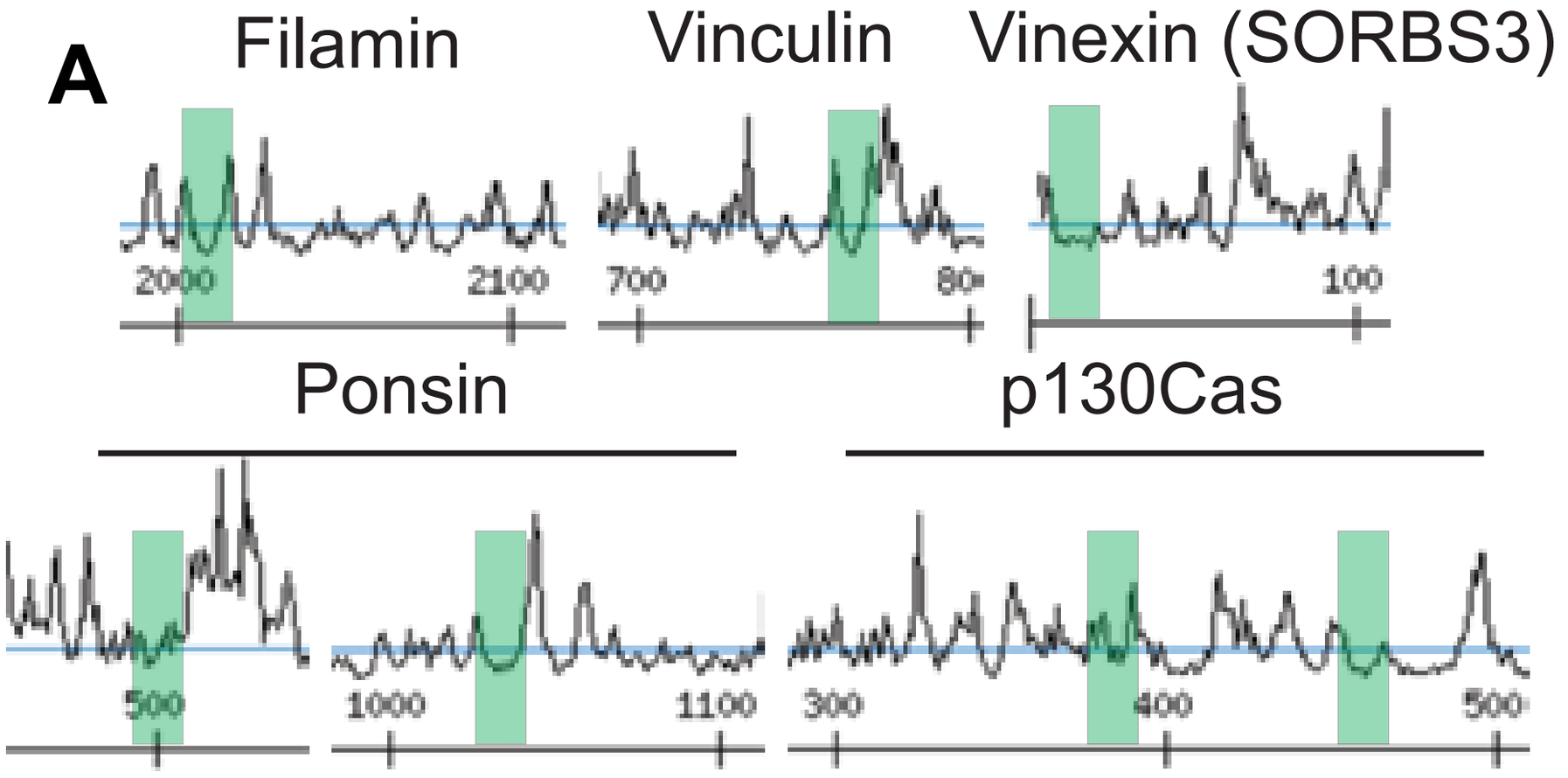

A
DAPI Map
DAPI
Auto-Threshold
B
Secondary Actin
Thresholding
Transcription Factor
Localization Analysis
C
Intensity
Cell Nuc
Cell Nuc
Cell Nuc
Cell Nuc

Figure S3

A



B

Protein	*Site*	*Sequence*	*Accessibility*
Vinculin	L765	RRANRILLVAKREVE	0.164
p130Cas	L386	RRPGPGTLYDVPRER	0.524
	V458	REPLELEVAVEALAR	0.315
Filamin	V2007	KRLRNGHVGISFVPK	0.213
Paxillin	N/A	N/A	N/A
Ponsin	L500	KRSATLPLPARSSSL	0.737
	L1033	RASPSLSLSLPHLSW	0.357
Vinexin	L12	PRSLRAGLSLDDFIP	0.349

Figure S4

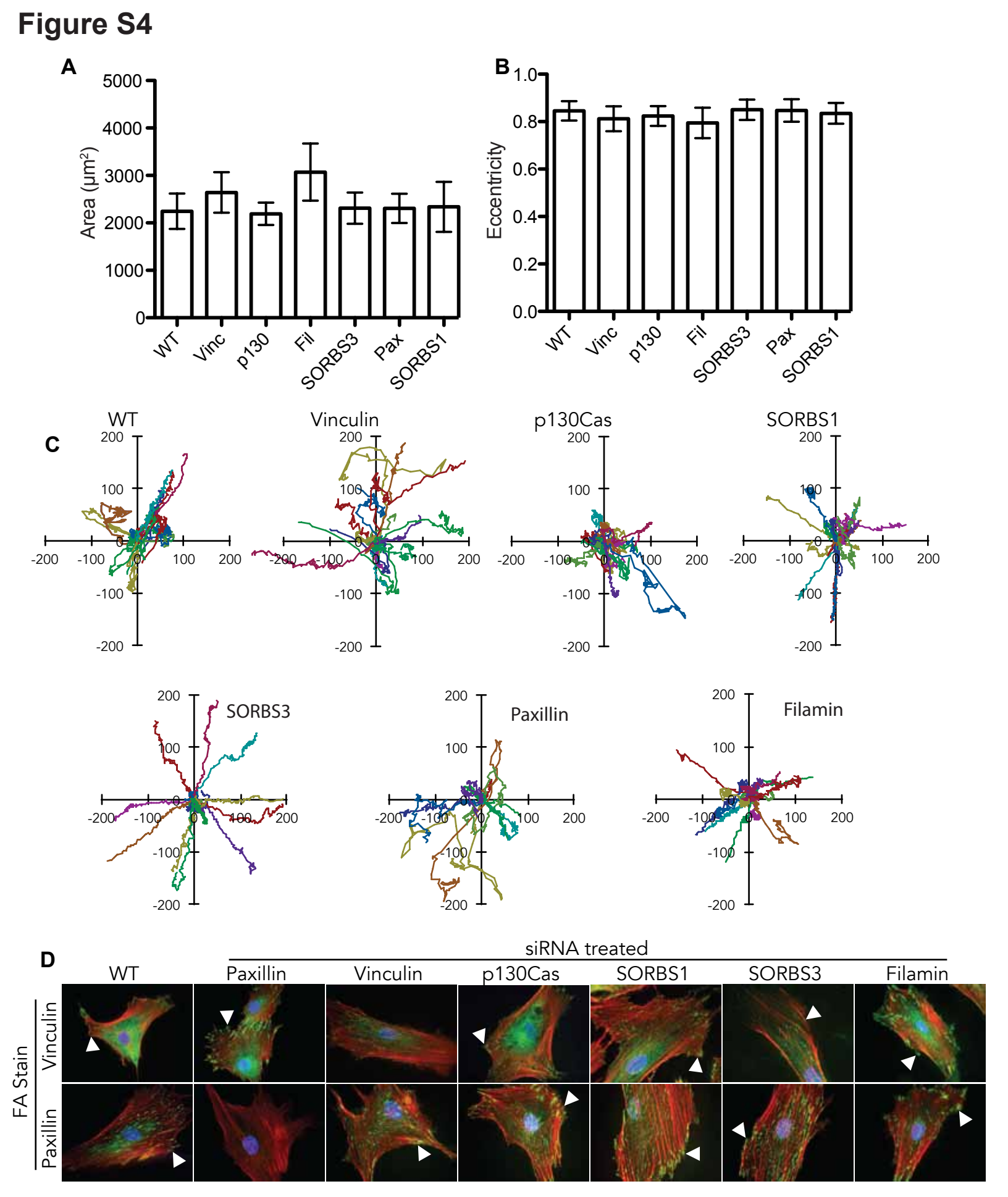

A
Area (μm²)
5000
4000
3000
2000
1000
0
WT
Vinc
p130
Fil
SORBS3
Pax
SORBS1
B
Eccentricity
1.0
0.8
0.6
0.4
0.2
0.0
C
WT
Vinculin
p130Cas
SORBS1
SORBS3
Paxillin
Filamin
D
siRNA treated
WT
Paxillin
Vinculin
p130Cas
SORBS1
SORBS3
Filamin
FA Stain
Vinculin
Paxillin

Figure S5