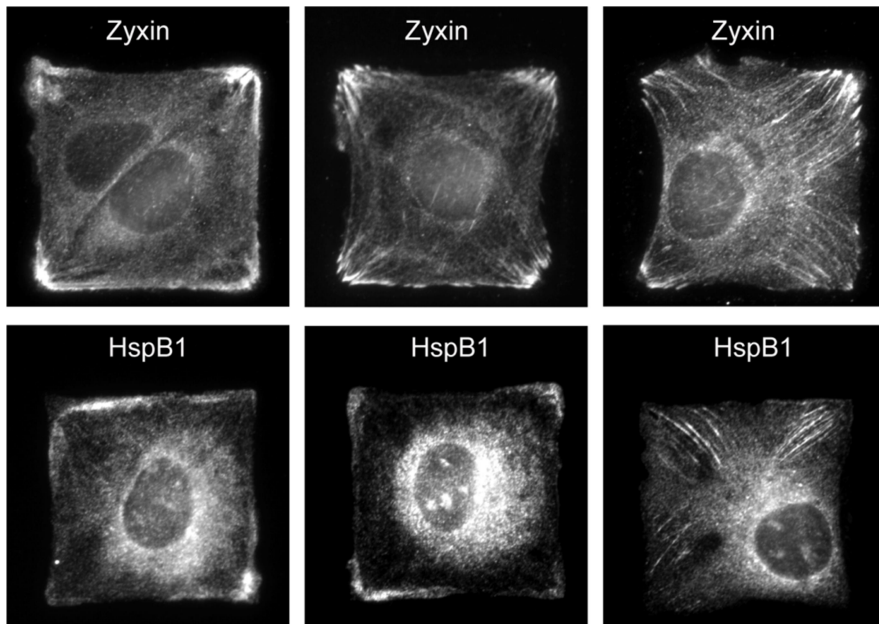


# Supplementary Materials

*Molecular Biology of the Cell*

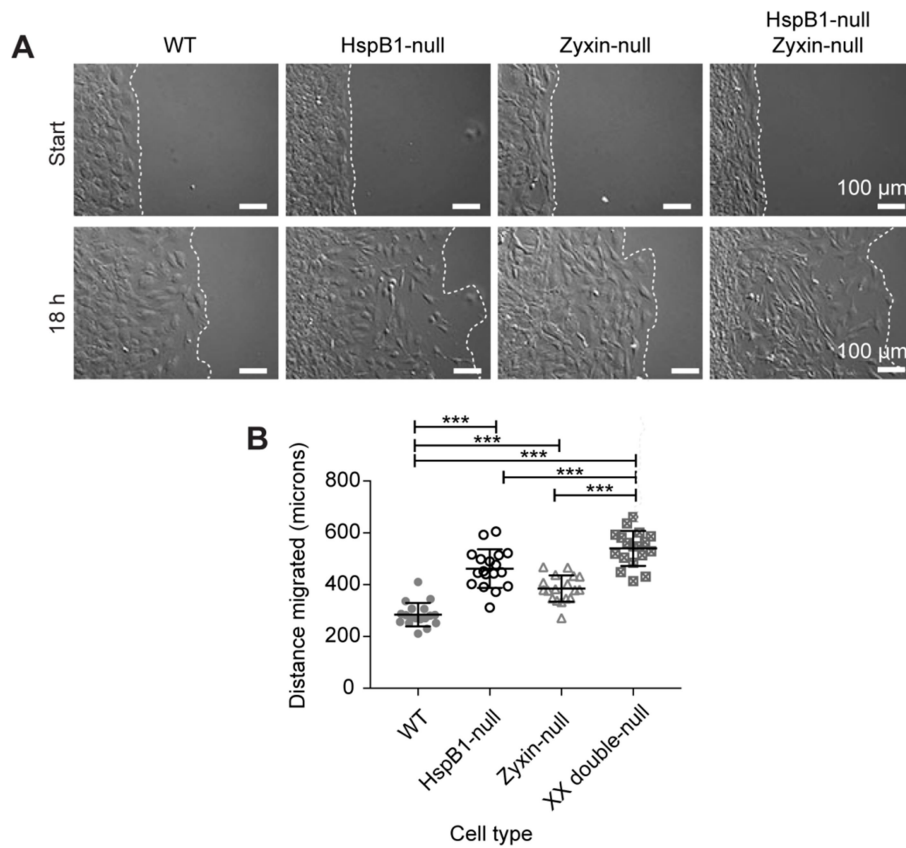
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**Supplemental Figure S1. Zyxin and HspB1 in geometrically constrained cells.**

Indirect immunofluorescence widefield microscopy of cells on fibronectin-coated islands of 47  $\mu\text{m}$  X 47  $\mu\text{m}$  dimension.

Zyxin (top row) and HspB1 (bottom row) display several patterns, including accumulation at edges and corners of the square cells, and along linear streaks emanating from the corners.



**Supplemental Figure S2. Enhanced motility in cells double-null for HspB1 and zyxin.**

A. Time-lapse microscopy (10 minute intervals for 18 hour total time) of Start and Finish (18 h) of edge migration assay for parental WT cells, HspB1-null cells, zyxin-null cells, and XX HspB1/zyxin-double null cells. B. Graph of distance migrated over 18 h period for each cell type (mean with standard deviations). Unpaired t-tests were used to determine p-values \*\*\*p < 0.0001.

**Supplemental Table I. Western Immunoblot Quantitation**

Western immunoblot films were scanned into digital images, then quantified using FIJI gel scan analysis tool.

Figure	Antibody	Experiment	Intensity (counts)	Change (X-fold)
1B	pS86-HspB1	Unstretched	44834	1x
1B	pS86-HspB1	Stretch 60 mins	85892	1.9x
1B	Total HspB1	Unstretched	76068	1x
1B	Total HspB1	Stretch 60 mins	74845	1x
1B	Vinculin	Unstretched	57375	1x
1B	Vinculin	Stretch 60 mins	65349	1.1x
1E	pS86-HspB1	Unstretched	19600	1x
1E	pS86-HspB1	Stretch 15 mins	53449	2.7x
1E	pS86-HspB1	Stretch 60 mins	24188	1.2x
1E	Vinculin	Unstretched	56054	1x
1E	Vinculin	Stretch 15 mins	49893	0.9x
1E	Vinculin	Stretch 60 mins	30372	0.5x
1E	pS86-HspB1	SB203580+Unstretched	31641	1x
1E	pS86-HspB1	SB203580+Stretch 15 mins	22268	0.7x
1E	pS86-HspB1	SB203580+Stretch 60 mins	23626	0.7x
1E	Vinculin	SB203580+Unstretched	36960	1x
1E	Vinculin	SB203580+Stretch 15 mins	42627	1.2x
1E	Vinculin	SB203580+Stretch 60 mins	16450	0.4x
3A	Total HspB1	WT cells	129556	1x
3A	Total HspB1	HspB1-null cells	0	0
3A	Total HspB1	WT HspB1	70838	0.5x
3A	Total HspB1	S15,86A HspB1	62016	0.5x
3A	Total HspB1	S15,86E HspB1	60733	0.5x
3A	Vinculin	WT cells	78700	1x
3A	Vinculin	HspB1-null cells	58910	0.8x
3A	Vinculin	WT HspB1	75050	1x
3A	Vinculin	S15,86A HspB1	59696	0.8x
3A	Vinculin	S15,86E HspB1	41513	0.5x
5A	Phospho-p38	WT Unstretched	32716	1x
5A	Phospho-p38	WT Stretch 15 mins	110646	3.4x
5A	Phospho-p38	WT Stretch 60 mins	111140	3.4x
5A	Phospho-p38	Null Unstretched	48211	1x
5A	Phospho-p38	Null Stretch 15 mins	82982	1.7x
5A	Phospho-p38	Null Stretch 60 mins	93861	1.9x
5A	Total p38	WT Unstretched	73180	1x
5A	Total p38	WT Stretch 15 mins	77129	1x
5A	Total p38	WT Stretch 60 mins	81840	1x
5A	Total p38	Null Unstretched	73171	1x
5A	Total p38	Null Stretch 15 mins	72454	1x
5A	Total p38	Null Stretch 60 mins	124318	1.7x
5A	pS86-HspB1	WT Unstretched	20129	1x
5A	pS86-HspB1	WT Stretch 15 mins	52635	2.6x
5A	pS86-HspB1	WT Stretch 60 mins	46928	2.3x
5A	Total HspB1	WT Unstretched	46440	1x
5A	Total HspB1	WT Stretch 15 mins	38253	0.8x
5A	Total HspB1	WT Stretch 60 mins	53040	1.1x

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5A	Vinculin	WT Unstretched	21375	1x
5A	Vinculin	WT Stretch 15 mins	18885	0.9x
5A	Vinculin	WT Stretch 60 mins	21584	1x
5A	Vinculin	Null Unstretched	20351	1x
5A	Vinculin	Null Stretch 15 mins	18206	0.9x
5A	Vinculin	Null Stretch 60 mins	22873	1x
5B	pS86-HspB1	WT HspB1 Unstretched	18445	1x
5B	pS86-HspB1	WT HspB1 Stretch 60m	77109	4.2x
5B	Total HspB1	WT HspB1 Unstretched	74916	1x
5B	Total HspB1	WT HspB1 Stretch 60m	101954	1.4x
5B	Total HspB1	S15,86A Unstretched	69119	1x
5B	Total HspB1	S15,86A Stretch 60m	58869	0.9x
5B	Total HspB1	S15,86E Unstretched	54054	1x
5B	Total HspB1	S15,86E Stretch 60m	66639	1.2x
5B	Vinculin	Null Unstretched	163297	1x
5B	Vinculin	Null Stretch 60m	95676	0.6x
5B	Vinculin	WT HspB1 Unstretched	104120	1x
5B	Vinculin	WT HspB1 Stretch 60m	134179	1.3x
5B	Vinculin	S15,86A Unstretched	73655	1x
5B	Vinculin	S15,86A Stretch 60m	75472	1x
5B	Vinculin	S15,86E Unstretched	76563	1x
5B	Vinculin	S15,86E Stretch 60m	49387	0.6x
6A	Total HspB1	WT HspB1 Unstretched	26249	1x
6A	Total HspB1	WT HspB1 Stretch 60m	34940	1.3x
6A	Total HspB1	S15A Unstretched	20559	1x
6A	Total HspB1	S15A Stretch 60m	20007	1x
6A	Total HspB1	S86A Unstretched	39292	1x
6A	Total HspB1	S86A Stretch 60m	39400	1x
6A	pS86-HspB1	WT HspB1 Unstretched	24500	1x
6A	pS86-HspB1	WT HspB1 Stretch 60m	42059	1.7x
6A	pS86-HspB1	S15A Unstretched	15371	1x
6A	pS86-HspB1	S15A Stretch 60m	33833	2.2x
6A	Total p38	WT HspB1 Unstretched	34983	1x
6A	Total p38	WT HspB1 Stretch 60m	31194	0.9x
6A	Total p38	S15A Unstretched	18286	1x
6A	Total p38	S15A Stretch 60m	17601	1x
6A	Total p38	S86A Unstretched	37738	1x
6A	Total p38	S86A Stretch 60m	35613	0.9x
6A	Phospho-p38	WT HspB1 Unstretched	10083	1x
6A	Phospho-p38	WT HspB1 Stretch 60m	39056	4x
6A	Phospho-p38	S15A Unstretched	12315	1x
6A	Phospho-p38	S15A Stretch 60m	29192	2.4x
6A	Phospho-p38	S86A Unstretched	7486	1x
6A	Phospho-p38	S86A Stretch 60m	26717	3.6x
6A	Phospho-zyxin	WT HspB1 Unstretched	8184	1x
6A	Phospho-zyxin	WT HspB1 Stretch 60m	39174	4.8x
6A	Phospho-zyxin	S15A Unstretched	21157	1x
6A	Phospho-zyxin	S15A Stretch 60m	30473	1.4x
6A	Phospho-zyxin	S86A Unstretched	18551	1x
6A	Phospho-zyxin	S86A Stretch 60m	31044	1.7x
6A	Vinculin	WT HspB1 Unstretched	30669	1x
6A	Vinculin	WT HspB1 Stretch 60m	37998	1.2x

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6A	Vinculin	S15A Unstretched	36171	1x
6A	Vinculin	S15A Stretch 60m	30599	0.8x
6A	Vinculin	S86A Unstretched	31256	1x
6A	Vinculin	S86A Stretch 60m	31899	1x
7C	Total HspB1	HspB1-null cells	0	
7C	Total HspB1	WT HspB1 rescue	26817	1x
7C	Total HspB1	S15,86A rescue	48079	1.8x
7C	Total HspB1	S15,86E rescue	35416	1.3x
7C	Vinculin	HspB1-null cells	37960	0.7x
7C	Vinculin	WT HspB1 rescue	58110	1x
7C	Vinculin	S15,86A rescue	51677	0.9x
7C	Vinculin	S15,86E rescue	55201	0.9x

**Supplemental Table II. HspB1 Reagents and Resources**

Resource Details for Materials and Methods used in this paper: Antibodies and detection reagents, cells and mutants, assays, microscopes, software and algorithms.

REAGENT	RESOURCE	IDENTIFIER	NOTES
<b>Antibodies</b>			
Hsp25 rab (total HspB1)	Enzo	ADI-SPA-801	IF 1:200 WB 1:1,000
PhosphoSer82-HspB1 rab (P-HspB1) Human HspB1p-Ser82 & mouse HspB1p-Ser86	CST	9709	IF 1:150 WB 1:1,000
PhosphoSer15-HspB1 rab	CST	2404	NA
P38 rab	CST	4511	WB 1:1000
Phospho-p38 rab	CST	9212	WB 1:1000
Vinculin mab	Sigma	V-9131	IF 1:600 WB 1:10,000
Zyxin B72 rab	Beckerle Lab Millipore	(Hoffman, Nix et al. 2003) #1387	IF 1:600 WB 1:10,000
Phospho-zyxin huS142/143 rab	CST	#8467 #4863	WB 1:2000
AlexaFluor488- antibodies	Molecular Probes/ Invitrogen	488-anti-Mouse A11029 488-anti-Rabbit A11008	IF 1:100
AlexaFluor568- antibodies	Molecular Probes/ Invitrogen	568-anti-Mouse 11031 568-anti-Rabbit A11036	IF 1:100
AlexaFluor647-antibody	Molecular Probes/ Invitrogen	647-anti-Rabbit A21245 647-anti-Mouse A21236	IF 1:100
AlexaFluor-Phalloidin to detect F-actin	Molecular Probes/ Invitrogen	488-phalloidin A12379 568-phalloidin A1238 647-phalloidin A22287	IF 1:150-1:100
Far Red-SiR-actin Binds F-actin	Cytoskeleton	CY-SC001	1:3000
DAPI (Nucleus)	Molecular Probes/ Invitrogen	D-1306	0.5 uM
Hoechst (Nucleus)	Molecular Probes/ Invitrogen	H-1399	0.25 ug/ml
Horseradish- Peroxidase conjugated antibodies	GE Healthcare	NA934V (mouse) NA931V (rabbit)	
Blebbistatin	Millipore Sigma	CAS 856925-71-8 Calbiochem	50 uM
Fibronectin	Sigma	F1141	2.5-10 ug/ml
Collagen I	Sigma	354236	
Silicone membrane	AAA-Acme Rubber Co.	CASS-.020x36-64909	

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<b>Cells</b>			
WT mouse (parent) fibroblasts	Beckerle Lab	(Hoffman, Jensen et al. 2006) M28	
HspB1-null CRISPR fibroblasts	Beckerle Lab	(Hoffman, Jensen et al. 2017)	
HspB1-WTrescue fibroblasts	Beckerle Lab PAM-mutant WT HspB1		
HspB1-Ser15,86Ala phospho-mutant fibroblasts	Beckerle Lab PAM-mutant S2A HspB1 Double mut		
HspB1-Ser15,86Glu phospho-mutant fibroblasts	Beckerle Lab PAM-mutant S2E HspB1 Double mut		
HspB1-Ser15Ala single phospho-mutant fibroblasts	Beckerle Lab Single S15A		
HspB1-Ser86Ala single phospho-mutant fibroblasts	Beckerle Lab Single S86A		
HspB1/zyxin-double null cells	Beckerle Lab	(Hoffman, Jensen et al. 2017)	
Zyxin-null cells	Beckerle Lab	(Hoffman, Jensen et al. 2006)	
<b>Software and Algorithms</b>			
Adobe Photoshop version CC	Adobe Systems Inc.		
Adobe Illustrator version CC	Adobe Systems Inc.		
Stress Fiber Thickness Index	Masaaki Yoshigi	(Yoshigi, Hoffman et al. 2005)	SFTI
ImageJ FIJI	NIH		Line profiles
Axiovision v4.8.1	Zeiss		Image acquisition
Elements v.4.6	Nikon		Image acquisition & analysis
LASX v.3.5.7 Imaging & Lightning Deconvolution	Leica Microsystems		Image acquisition & analysis
Prism V7	GraphPad		Graphs and statistics
Endnote v.20.1	Clarivate		References
<b>Microscopes</b>			
Zeiss Axioskop2 mot plus Widefield Fluorescent Microscope	Zeiss Microscopy	Zeiss Axioskop2	For stretch
Nikon Widefield Fluorescent Microscope	Nikon Microscopy	Nikon Ti Eclipse	For areas, stretch, migration
Leica SP8 Spinning Disk Confocal Microscope	Leica Microsystems	Leica SP8 DMI8	For P-hsp & Squares & Live Retrograde



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