Supplemental Materials

Molecular Biology of the Cell

Hoffman et al.

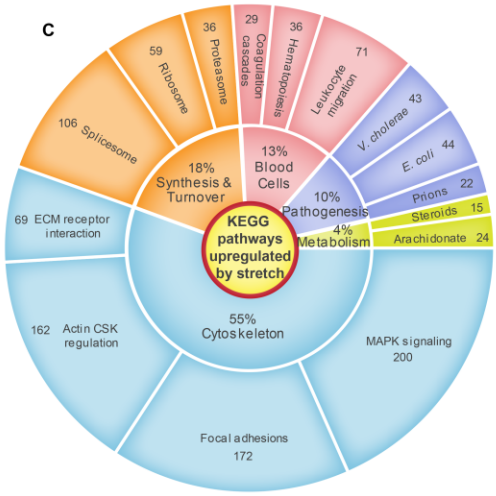
A

YAP/TAZ target genes	Descriptor	Log 2-fold change	p-value	x-fold up-regulation	Reference
AREG	Amphiregulin (EGFR ligand)	5.4	4.0e131	42	Zhang, Ji et al. 2009
CTGF	Connective tissue growth factor	2.6	1.3e9	6.1	Cordenonsi, Zanconato et al. 2011 Zanconato, Forcato et al. 2015
CYR61	Cysteine rich angiogenic inducer 61 or heparin binding protein	1.7	1.6e11	3.2	Cordenonsi, Zanconato et al. 2011 Zanconato, Forcato et al. 2015
DUSP1	Dual specificity phosphatase 1	2.4	3.2e17	5.3	Cordenonsi, Zanconato et al. 2011
FGF2	Fibroblast growth factor	3.0	1.7e73	8.0	Cordenonsi, Zanconato et al. 2011
FOSL1	Fos-like 1 (AP-1 transcription factor)	2.3	3.4e27	4.9	Zanconato, Forcato et al. 2015
GADD45B	Growth arrest and DNA damage inducible beta	2.6	1.4e35	6.1	Cordenonsi, Zanconato et al. 2011
SERPINE1	Serine protease inhibitor 1	3.3	2.4e32	10.6	Cordenonsi, Zanconato et al. 2011
TNNT2	Troponin T2 (Tropomyosin-binding)	2.7	9.9e20	6.5	Cordenonsi, Zanconato et al. 2011

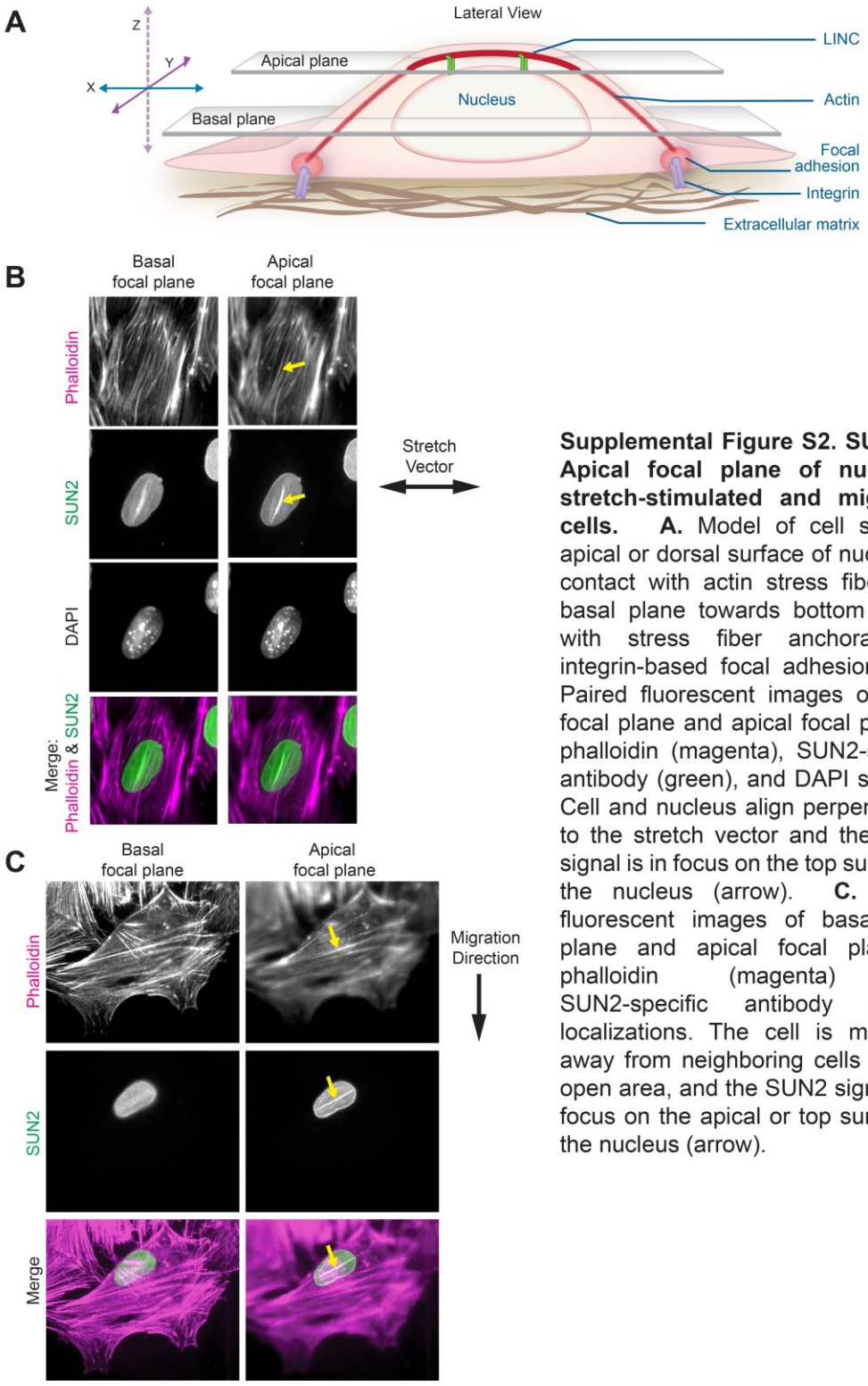
B

MRTF-A target genes	Descriptor	Log 2-fold change	p-value	x-fold up-regulation	Reference
ATF3	Activating transcription factor 3 (cAMP-dep)	6.3	1.3e51	74	Gegenfurtner 2018 Yu 2018
CSRNP1	Cys/Ser-rich nuclear prot1 (xtion activator)	4.7	2.3e171	26	Yu 2018
CTGF	Connective tissue growth factor (matricellular)	2.6	4.2e11	6.1	Yu 2018 Foster 2018
CYR61	Cys-rich angiogenic inducer 61 (matricellular)	1.7	4.6e13	3.2	Yu 2018 Foster 2018
DLX2	Distal-less homeobox prot2 (xtion activator)	1.6	3.8e17	3.0	Yu 2018
DUSP1	Dual specificity phosphatase 1	2.4	3.2e17	5.3	Yu 2018
DUSP5	Dual specificity phosphatase 5	3.9	4.3e50	15	Gegenfurtner 2018 Yu 2018
EGR3	Early growth response 3 (xtion factor)	1.9	3.5e15	3.7	Gegenfurtner 2018 Yu 2018
ERRFI1	ERBB receptor feedback inhibitor1 (neg reg ERBB)	2.1	5.7e19	4.3	Yu 2018
FOSB	Oncogene? (AP-1 xtion cofactor)	7.0	4.6e61	128	Gegenfurtner 2018
HBEGF	Heparin-binding EGF-like growth factor	4.2	5.3e57	18	Yu 2018
HIVEP3	Human immunodeficiency virus enhancer binding protein 3 (xtion factor)	2.0	5.8e13	4.0	Gegenfurtner 2018
IER2	Immediate early response 2 (xtion factor)	2.0	6.2e22	4.0	Yu 2018
ITGA5	Integrin alpha5 (FN receptor)	2.1	3.4e32	4.3	Foster 2018
KDM6B	Lysine demethylase 6B (H3 K27 epigenet)	2.0	9.3e25	4.0	Gegenfurtner 2018 Yu 2018
LIF	Leukemia inhibitory factor (cytokine)	2.7	1.7e20	6.5	Yu 2018
MAFF	Musculo Aponeurotic Fibrosarcoma Oncogene Homolog F (xtion reg)	3.8	4.7e86	14	Yu 2018
NFKBIZ	NF-kappaB-zeta (xtion reg)	2.5	5.3e10	5.7	Yu 2018
NR4A1	Nuclear receptor subfamily4 groupA member 1 (xtion reg)	4.7	1.5e51	26	Gegenfurtner 2018 Yu 2018
NR4A2	Nuclear receptor subfamily4 groupA member 2 (xtion reg)	1.9	7.5e26	3.7	Yu 2018
NR4A3	Nuclear receptor subfamily4 groupA member 2 (xtion reg)	3.9	4.1e18	15	Yu 2018
PPP1R15A	Protein phosphatase 1 regulatory subunit 15A	4.8	5.8e130	28	Yu 2018
PTGER4	Prostaglandin E2 receptor 4 (g-prot regulated)	4.2	3.8e93	18	Yu 2018
TUFT1	Tuftelin (glycoprotein involved in dental enamelization?)	2.6	2.4e63	6.1	Yu 2018
ZFP36	Zinc finger protein 36 (RNA binding)	2.4	1.2e62	5.3	Yu 2018

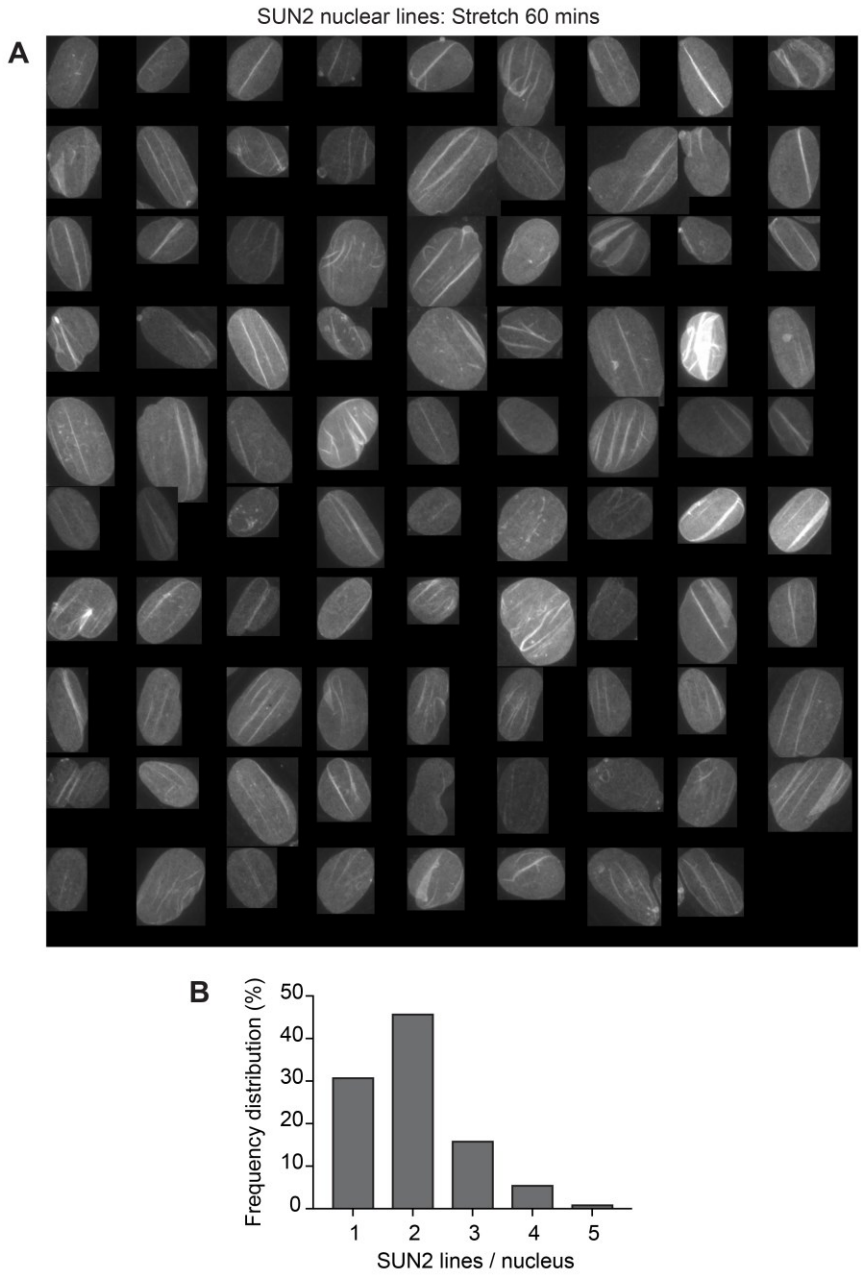
C



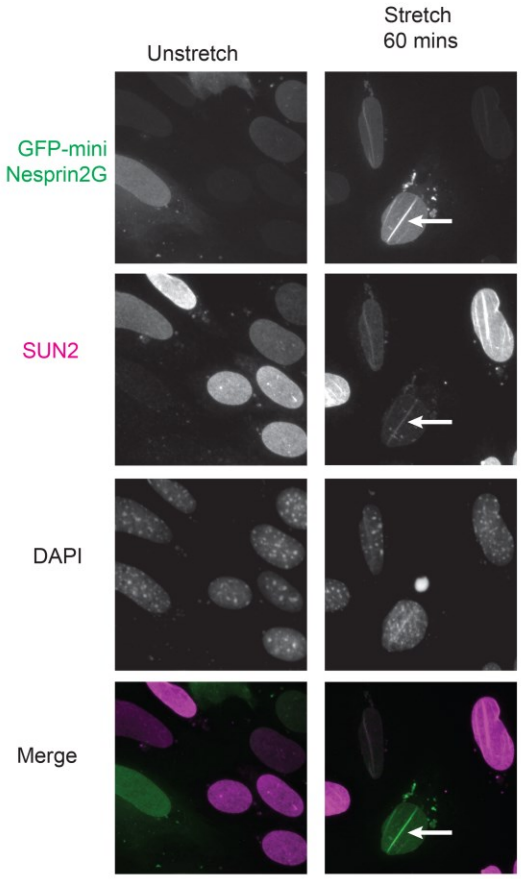
Supplemental Figure S1. Gene expression analysis of stretch-stimulated cells. mRNAs up-regulated by uniaxial cyclic stretch stimulation (15%, 0.5 Hz, 6hrs) of mouse fibroblasts were identified by RNA sequencing analysis of 4 pairs (unstretched and stretch-stimulated) cell samples. Searches for genes that were characterized as YAP/TAZ target genes and MRTF-A target genes in several published reports were identified. **A.** 9 YAP/TAZ target genes and **B.** 25 MRTF-A target genes upregulated by stretch stimulation. These genes are shown with log 2-fold change, p-values, references, and descriptors. **C.** KEGG pathway analysis of the RNAseq data including the top 15 pathways is summarized in a circular form. Pathways for MAPK signaling, Focal adhesions, Actin cytoskeleton regulation, and ECM receptor interaction are the most highly represented as stretch-stimulated pathways.



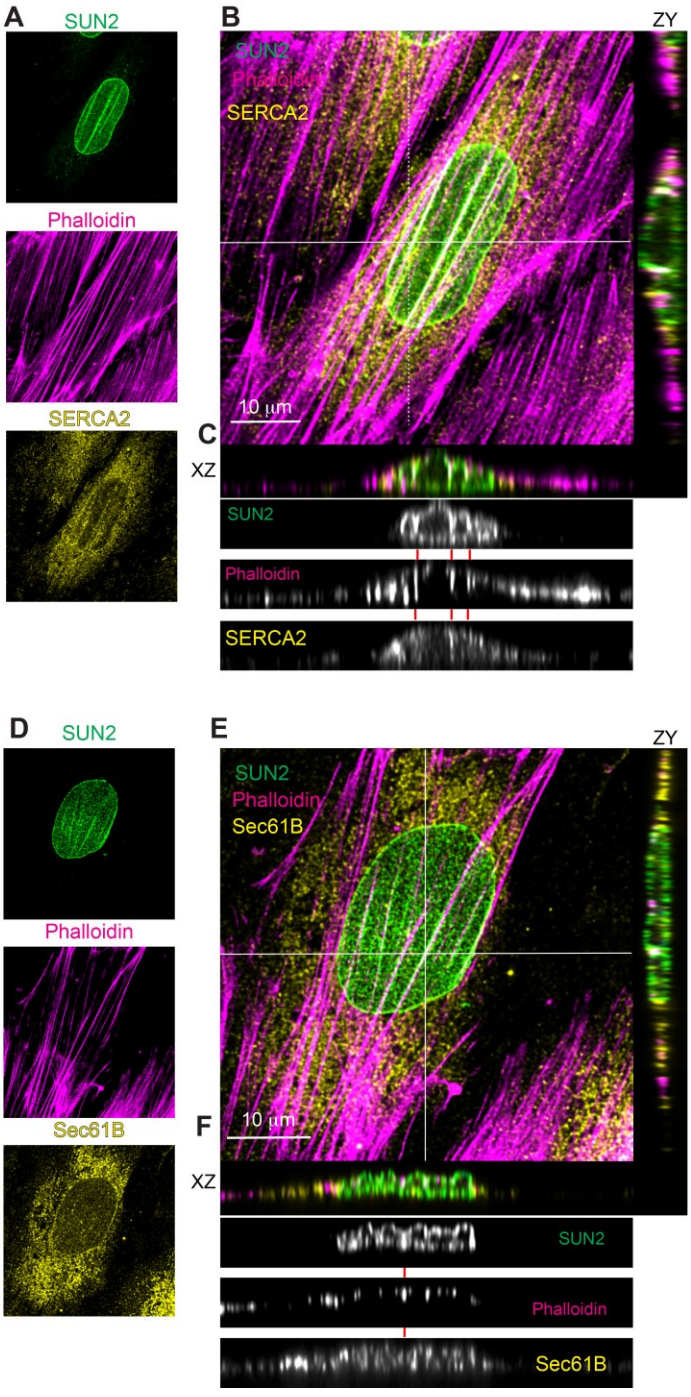
Supplemental Figure S2. SUN2 on Apical focal plane of nuclei in stretch-stimulated and migrating cells. **A.** Model of cell showing apical or dorsal surface of nucleus in contact with actin stress fiber, and basal plane towards bottom of cell with stress fiber anchorage at integrin-based focal adhesions. **B.** Paired fluorescent images of basal focal plane and apical focal plane of phalloidin (magenta), SUN2-specific antibody (green), and DAPI staining. Cell and nucleus align perpendicular to the stretch vector and the SUN2 signal is in focus on the top surface of the nucleus (arrow). **C.** Paired fluorescent images of basal focal plane and apical focal plane of phalloidin (magenta) and SUN2-specific antibody (green) localizations. The cell is migrating away from neighboring cells into an open area, and the SUN2 signal is in focus on the apical or top surface of the nucleus (arrow).



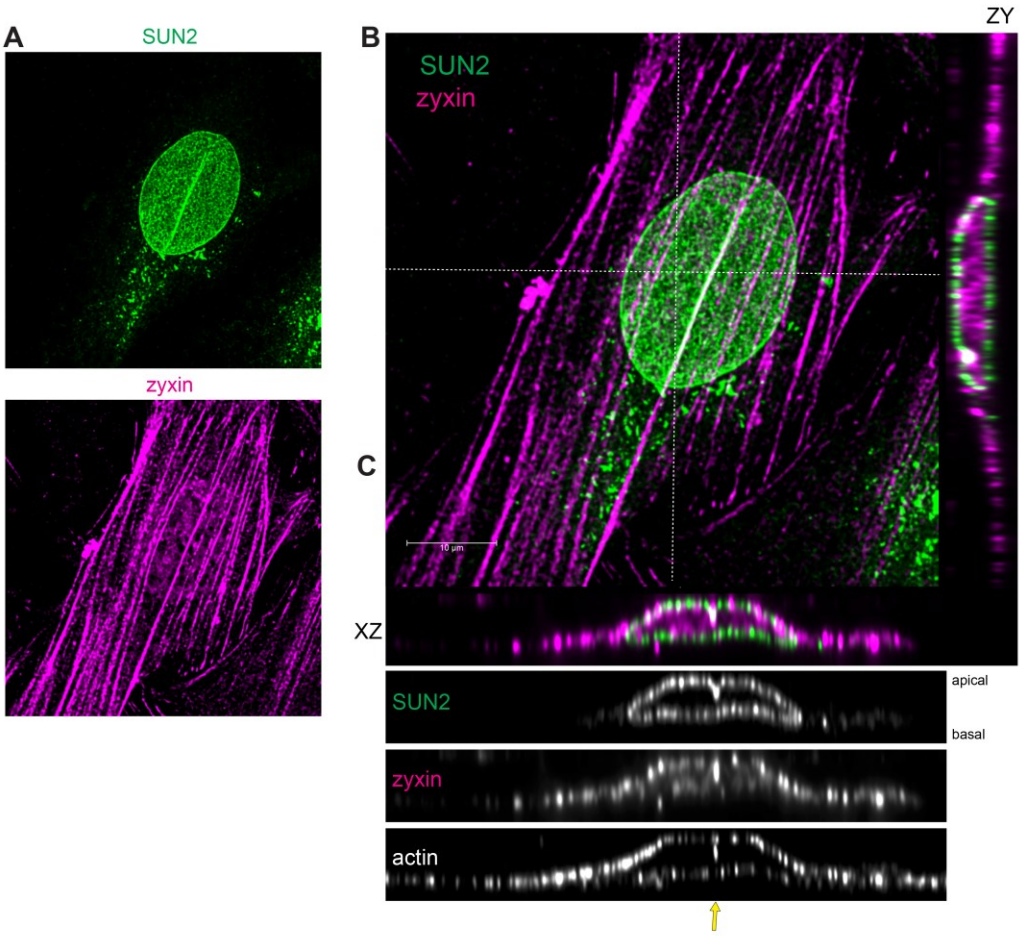
Supplemental Figure S3. SUN2 nuclear line quality and quantity. **A.** Stretch-stimulated (uniaxial cyclic stretch, 15% 0.5 Hz 60 mins) nuclei with immunolocalized SUN2 nuclear envelope proteins are presented as a montage to show the variety of SUN2 distributions observed. **B.** Nuclei were scored for number of SUN2 lines detectable in identically-captured unprocessed images. In a total of 87 nuclei scored from this typical experiment, 31% had single lines/nuclei and 46% had 2 lines/nuclei. There were small numbers of nuclei with 3 (16%), 4 (6%), and 5 (1%) lines/nuclei.



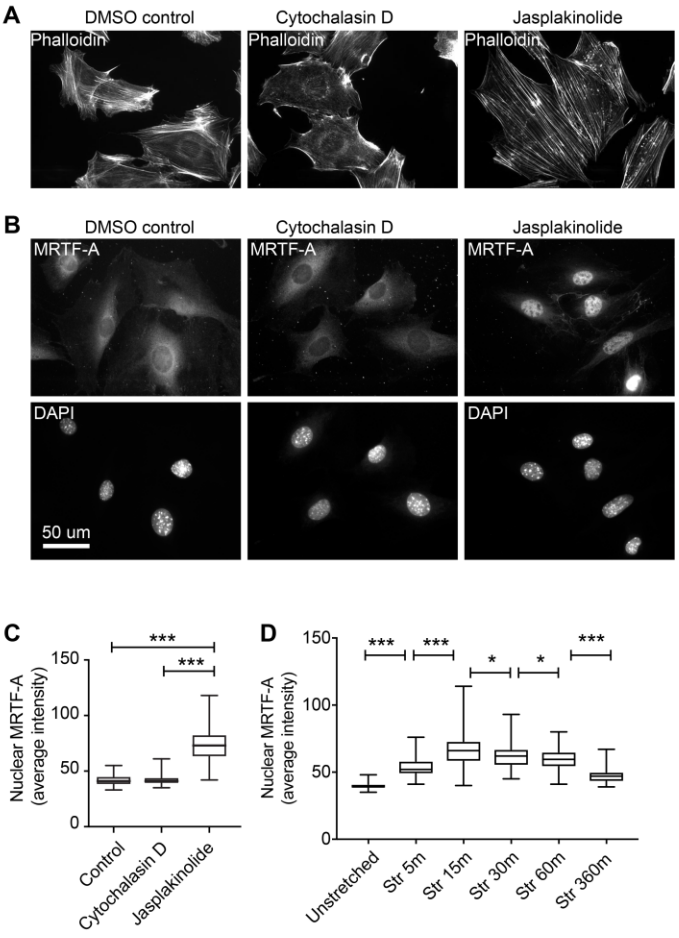
Supplemental Figure S4. Stretch-induced co-distribution of SUN2 and nesprin proteins. GFP-mini-Nesprin2G DNA was transiently transfected into fibroblasts, expressed for 48 hrs, then cells were seeded onto silicone membranes and stretch-stimulated (uniaxial cyclic, 15%, 0.5 Hz, 60 mins) and formaldehyde-fixed. SUN2 immunolocalization showed nuclear staining in cells with and without mNsp2G. In stretch-stimulated cells the mNsp2G and SUN2 co-distributed (arrow) along the same linear arrays.



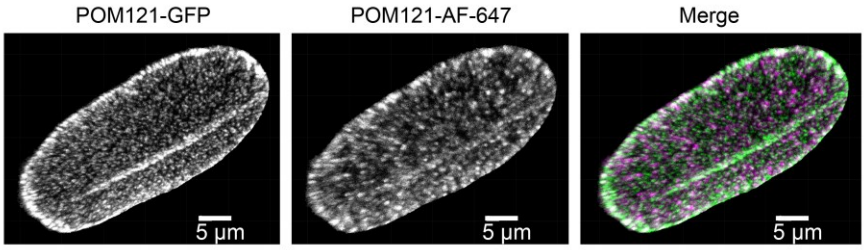
Supplemental Figure S5. Membrane proteins SERCA2 and Sec61B protein distributions.
Confocal microscopy of SUN2 (green, Burke mab#3.1E), F-actin (phalloidin,magenta) and endoplasmic reticulum-nuclear membrane proteins SERCA2 and Sec61B (yellow) in stretch-stimulated fibroblasts. A. Maximum intensity projections of SUN2, phalloidin, and SERCA2 confocal sections (0.35 micron steps). B. Orthogonal z-sections across the x (horizontal line, bottom of merge) and y (vertical line, right of merge) planes presented as stack of z sections with end-on view. C. Orthogonal view shows nuclear envelope distribution of SUN2, coincident with F-actin across the top of nucleus (red guidelines). SERCA2 is distributed in the ER around the nucleus and within the nuclear membrane. D. Maximum intensity projections of SUN2, phalloidin, and Sec61B confocal sections (0.35 micron steps). E. Orthogonal z-sections across the x (horizontal line, bottom of merge) and y (vertical line, right of merge) planes are shown in merged image. F. Orthogonal or end-on view shows nuclear envelope distribution of SUN2, coincident with F-actin across the top of nucleus (red guidelines). Sec61B is distributed in the ER around the nucleus and within the nuclear membrane. Scale bar is 10 microns. Associated Videos 4-11.



Supplemental Figure S6. SUN2 and zyxin co-distribution along nuclear lines. WT mouse fibroblasts were stretch stimulated (uniaxial cyclic stretch 15% 0.5 Hertz 1 hr) then fixed and immuno stained for SUN2 (Burke mab#3.1E@1:10) and zyxin (B71 @1:600). Cells on silicone membrane in PBS were imaged face down in a glass bottom dish by confocal microscopy (Leica SP8) with 0.5 micron steps. A. Maximum projections of a stack of confocal images for SUN2 (green) and zyxin (magenta). B. In Merged image the SUN2 nuclear signal is coincident with zyxin at the nuclear membrane, however zyxin extends along the entire actin filament. C. Orthogonal z sections across the x (horizontal line, bottom of merge) and y (vertical line, right of merge) planes are positioned as apical surface of cell on top and basal surface of cell on bottom for SUN2, zyxin, and F-actin. Scale bar 10 microns. Associated Videos 12-15.



Supplemental Figure S7. Nuclear translocation of MRTF-A induced by Jasplakinolide-stabilized F-actin. A. Cells were treated for 2 hours with DMSO control, Cytochalasin D (250 nM), and Jasplakinolide (100 nM) then fixed and stained with phalloidin to evaluate F-actin. Cytochalasin D blocks F-actin formation and Jasplakinolide induces robust F-actin stress fibers. B. In parallel experiments, immunolocalization of MRTF-A with DAPI stained nuclei show the cytoplasmic distribution of MRTF-A in control and Cytochalasin D-treated cells. MRTF-A translocates to the nucleus in Jasplakinolide-treated cells. C. DAPI images were used to threshold Regions of Interest which were then transferred onto the MRTF-A images. Quantitation of MRTF-A nuclear signal in control (47 nuclei), Cytochalasin D-treated (41 nuclei), and Jasplakinolide-treated (45 nuclei) cells shows the increased nuclear MRTF-A. D. Graph of nuclear MRTF-A signal following time course of uniaxial cyclic stretch (15% 0.5Hz) for 0, 5, 15, 30, 60 minutes and 6 hours with n= 93, 90, 111, 107, 106, 101 nuclei. Nuclei from the same time course were scored for SUN2 nuclear lines revealing 5, 13, 28, 28, 60, 55% of the nuclei with SUN2 nuclear lines. The peak nuclear translocation of MRTF-A (5-15 minutes) precedes the peak SUN2 nuclear lines (60-360 minutes). Box and whisker plots showing median with minimum to maximum range and p-values determined using unpaired student's t-test. *p<0.05 ***p<0.0001.Scale bar 50 microns.



Supplemental Figure S8. Nuclear pore protein POM121 localization by GFP and by antibody detection. Maximum intensity projection of a confocal stack of images shows the nuclear rim and nuclear line distribution of transiently transfected POM121-GFP (green) and the immunolocalization with a POM121 antibody (magenta). Scale bar is 5 microns.

Supplemental Videos

Video 1. Confocal stack of SUN2 images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure 4E).
Video 2. Confocal stack of Phalloidin/F-actin images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure 4E).
Video 3. Merged images of SUN2 (green) and phalloidin (magenta) from a stretch-stimulated cell (Figure 4E) with 3D rotation for spatial resolution.

Video 4. Confocal stack of SUN2 images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5A).
Video 5. Confocal stack of phalloidin images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5A).
Video 6. Confocal stack of SERCA2 images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5A).
Video 7. Confocal stack of merged images (green-SUN2, magenta-actin, yellow-SERCA2) from a stretch-stimulated cell (Figure S5A) with 3D rotation for spatial resolution.

Video 8. Confocal stack of SUN2 images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5D).
Video 9. Confocal stack of phalloidin images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5D).
Video 10. Confocal stack of Sec61B images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S5D).
Video 11. Confocal stack of merged images (green-SUN2, magenta-actin, yellow-Sec61B) from a stretch-stimulated cell (Figure S5D) with 3D rotation for spatial resolution.

Video 12. Confocal stack of SUN2 images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S6).
Video 13. Confocal stack of zyxin images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S6).
Video 14. Confocal stack of F-actin images from a stretch-stimulated cell going from top of the cell to the bottom at 0.35 micron steps (Figure S6).
Video 15. Confocal stack of merged images (green-SUN2, magenta-zyxin) from a stretch-stimulated cell (Figure S6) with 3D rotation for spatial resolution.