p<0.05, n=3) and LIV induced βcatenin-Nsk association was inhibited in siSUN treated MSCs (p<0.05, n=3). **e**) Similarly, siSUN decreased both basal Nsk-bound βcatenin to 70% (p<0.05, n=3) and inhibited the strain induced βcatenin-Nsk association (p<0.05, n=3). **f)** Nesprin-2 was immunoprecipitated immediately following LIV and probed against βcatenin. LIV decreased Nesprin-2- βcatenin association 50% (p<0.001, n=4). Please see Fig.S4 for a more detailed blot. Group comparisons were made using unpaired T-test (Figure 4f) and One-way ANOVA followed by a Newman-Keuls post-hoc test (Fig.4a-e). * p<0.05, ** p<0.01, *** p<0.001, against control and each other.

APPENDIX: Supplementary Figure Legends & Tables and Methods

Supplementary Figure Legends

Figure S1. Co-depletion of LINC elements Sun-1 and Sun-2 displaces both Nesprin-2 and βcatenin from nuclear envelope a) Immunoprecipitation of Nesprin-2 showed associations between βcatenin and Emerin. **b)** Representative images of siCtrl and siSUN treated cells showing Nesprin-2 (red) and βcatenin (green) distribution. In siCtrl treated cells, colocalization between Nesprin-2 and βcatenin (represented as yellow line) remains intranuclear while in siSUN treated cells colocalization was shifted into perinuclear area and remains outside of the nuclear boundary (DAPI, blue line). Representative images of **c)** siCtrl and siSUN treated cells indicating the intracellular localization of Nesprin-2 (red) and βcatenin (green) in relation to the nucleus (blue). White arrows indicate the perinuclear distribution of Nesprin-2 and βcatenin in siSUN treated cells.

Figure S2. Nuclear βcatenin accumulation but not βcatenin-nucleoskeleton association increase under sustained GSK3 β inhibition a) SB415 induced inhibition of GSK3β resulted in a stable increase of soluble nuclear βcatenin at 20, 140 and 160 minute time points. **b)** SB415 induced inhibition of GSK3β resulted in an increased βcatenin-Nsk association as soon as 20 minutes and upon keeping SB415 constraint, βcatenin-Nsk association remained elevated at 140 and 160 minute time points but signal intensity was not changed.

Figure S3. Depletion of Emerin or LaminA/C is not sufficient to inhibit LIV-induced βcatenin-nucleoskeleton association a) siRNA against Emerin (siEMD) did not change the basal and LIV-induced βcatenin-Nsk association between siCtrl and siEMD MSCs. **b)** Similarly, siRNA against LaminA/C (siLmna) did not inhibit LIV-induced βcatenin-Nsk association. LIV was applied at 90 Hz, 0.7g for 20 minutes. Samples were collected immediately after second LIV application.

Figure S4. LINC elements Sun-1 and Sun-2 are necessary for strain-induced βcatenin nuclear entry. Application of strain results in **a)** Akt activation as well as **b)** GSK3β in both siCtrl and siSUN treated cells suggesting an increased βcatenin pool in the cytoplasm (n=3). **c)** Separating soluble nuclear and cytoplasmic fractions 160 min post-strain showed that in siCtrl cells βcatenin entered the nucleus while in siSUN cells there was no increase in nuclear βcatenin and βcatenin in the cytoplasmic fraction was increased. Group comparisons were made using One-way ANOVA followed by a Newman-Keuls post-hoc test (Fig.S4a-b). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, against control and each other.

Figure S5. Nesprin-2 Immunoprecipitation. Supplementary file for Fig.4f displaying full size blots and other control lanes.

Figure S6. Comparison of LIV and strain induced changes of Lamin A/C and Lamin B1. Changes in Lamin A/C and Lamin B1 protein amounts were reported as ratio between **a)** control and LIV samples (n=8) and **b)** control and strain samples (n=6). 120 minutes after the first mechanical challenge, no differences between LaminA/C and LaminB1 was detected in response to either LIV or Strain. Group comparisons were made using unpaired T-test (Figure S6a-b).

Figure S7. Total βcatenin levels did not change with LIV. Whole cell lysates 160min post-LIV were probed via total βcatenin (BD, 610154) and β-Tubulin (Santa Cruz, SC-9104)

Table.S1 : Cell Culture and Pharmacological Reagents

Cell Culture and Pharmacological Reagents		Final Concentration
IMDM	GIBCO	-
FBS	Atlanta Biologicals	10% v/v
Penicillin/streptomycin	GIBCO	1% v/v
SB415286	Sigma Aldrich	20μM

Table.S2 : Antibodies used and their final concentrations for western blots

Antibodies		Final Concentration
Akt (#4685)	Cell Signaling	1/2000
p-Akt Ser473 (#4058L)	Cell Signaling	1/1000
LaminA/C (#4C11)	Cell Signaling	1/1000
p-FAK Tyr397 (#328 3)	Cell Signaling	1/1000
FAK (sc-558)	Santa Cruz Biotechnology	1/500
RhoA (sc-418)	Santa Cruz Biotechnology	1/1000
Emerin (ab40725)	Abcam	1/1000
Nesprin-2 (IQ565).	Immunoquest	1/500
βcatenin (#610051)	Milipore	1/2000

Table.S3 : sIRNA sequences

siRNA Sequences -Stealth Select siRNAs (Invitrogen)	
siRNA SUN-1 Control	5′- GAAATCGAAGTACCTCGAGTGATAT -3′
siRNA SUN-1	5′- GAAAGGCTATGAATCCAGAGCTTAT-3′
siRNA SUN-2 Control	5′-CACCAGAGGCTAGAACTCTTACTCA-3′
siRNA SUN-2	5′-CACCAGAGGCTAGAACTCTTACTCA-3′
siRNA Emerin Control	5′-CAACCCUUACUCG-GGUAUCUAGGUG-3′
siRNA Emerin	5′-CAACAUCCCUCAUGGGCCUAUUGUG-3′
siRNA Lamin A/C Control	5′-UGGGAGUCGGAAGAAGACUCGAUCA-3′
siRNA Lamin A/C	5′-UGGGAGAGGCUAAGAAGCAGCUUCA-3′

Table.S4: PCR Primers used

PCR primers	
Sun-1 -Forward	5′-CATGAAGTGCGTCTCTCCAA-3′
Sun-1 -Reverse	5′- CCTGCTTTCAGCTTGGTTTC-3′
Sun-2 -Forward	5′-CAGCAACATGAAGCACCTGT-3′
Sun-2 -Reverse	5′-GCTGCCGATGTAGGACTCTC-3′
Axin-2 -Forward	5′- TAGGCGGAATGAAGATGGAC-3′
Axin-2-Reverse	5′- CTGGTCACCCAACAAGGAGT-3′
18S –Forward	5′-GAACGTCTGCCCTATCAACT-3′
18S -Reverse	5′-CCAAGATCCAACTACGAGCT-3′

Table.S5: Immunostaining antibodies and Reagents and their final concentrations

Immunostaining antibodies and Reagents		Final Concentration
Nesprin-2 (IQ565).	Immunoquest	1/200
βcatenin (#610051)	Milipore	1/200
DyLight 649 Donkey Anti-Mouse	Immuno Research Laboratories	1/500
Alexa Flour 555 Donkey Anti-Rabbit	Invitrogen	1/500
Hoechst 33342	Invitrogen	1 µg/mL
Alexa Fluor 488 Phalloidin	Life Technologies	0.1µM

Table.S6: Overexpression sequences and their final concentrations

Overexpression sequences		Final Concentration
PEGFP-C1-βcatenin (#16071)	Addgene	1µg DNA per 100,000 cells

Table.S7: Overexpression and siRNA reagents and their final concentrations

Knockdown and overexpression Reagents	Final Concentration

LipoD293 (overexpress)	SignaGen Laboratories	3µL per mL
PepMute Plus (siRNA)	SignaGen Laboratories	3µL per µg of DNA
Puromycin (overexpress)	Sigma Aldrich	10 µg/mL

Isolation of Nucleoskeleton, soluble nuclear and nuclear envelope proteins

Immediately following the mechanical challenge, cells were washed and pelleted in ice-cold PBS. Cell pellets were then resuspended in 0.33 M sucrose, 10 mM Hepes, pH 7.4, 1 mM MgCl2 and 0.1% Triton X-100 (pellet vs. buffer, 1:5) then placed on ice for 15 minutes. After centrifugation at 3,000 rpm for 5 minutes, the supernatants were collected (cytoplasmic fraction) and same steps were repeated two more times to ensure highly enriched nucleus with minimal cytoplasmic contamination. Nuclear pellets were then resuspended in 0.45 M NaCl and 10mM Hepes, pH 7.4 and placed on ice for 15 minutes to rupture the nucleus and release soluble nuclear protein fraction. After centrifugation at 12,000 rpm for 5 minutes, the soluble nuclear fraction (it will be referred as nuclear fraction) will be collected. Remaining insoluble Lamin A/C rich pellet contains nuclear envelope, nucleoskeleton and chromatin (Fig.2)(Cook, 1988) This fraction was dissolved in 0.2% SDS for further analysis, this fraction will be referred as nucleoskeletal (Nsk) fraction. For the NsK fraction, we have used LaminA/C as a referent. LaminA/C was compared to an alternative referent LaminB1. Our results showed no differences between laminB1 and LaminA/C (**Fig.S6**).

Co-localization Analysis

MSCs were simultaneously immunostained against Nesprin-2, βcatenin and DAPI and were imaged using Olympus IX-60 inverted microscope using a 40X magnification. Nesprin-2 was labeled via Nesprin-2 (IQ565) primary and Alexa Flour 555 Donkey Anti-Rabbit secondary

antibodies using indicated concentrations (**Fig. S5**). Nesprin-2 was visualized using Texas Red filter (Excitation 542-582, Emission 604-644). βcatenin was labeled via βcatenin (#610051) primary and DyLight 649 Donkey Anti-Mouse secondary antibodies using indicated concentrations (**Fig. S5**). βcatenin was visualized using Cy5 filter (Excitation 604-644, Emission 672-712). Image stacks were divided to individual channels and converted to 8-bit grayscale. Nuclear area of interest has been selected using DAPI stain as a mask where the region inside of the selected area was designated as "Intra-nuclear" and outside was designated as "Extra-nuclear". Using the restore selection function, same areas of interests were extracted for Nesprin-2 and βcatenin channels. Colocalization pixel map was generated using standard "colocalization threshold" option and average colocalization was plotted across the horizontal axis against nuclear position within a rectangular region of interest (Fig.3e and Fig.S1). Using nuclear position as a reference, extra-nuclear to intra-nuclear colocalization ratio was reported for each cell.

Fig.S1- Co-depletion of LINC elements Sun-1 and Sun-2 displaces both Nesprin-2 and βcatenin from nuclear envelope

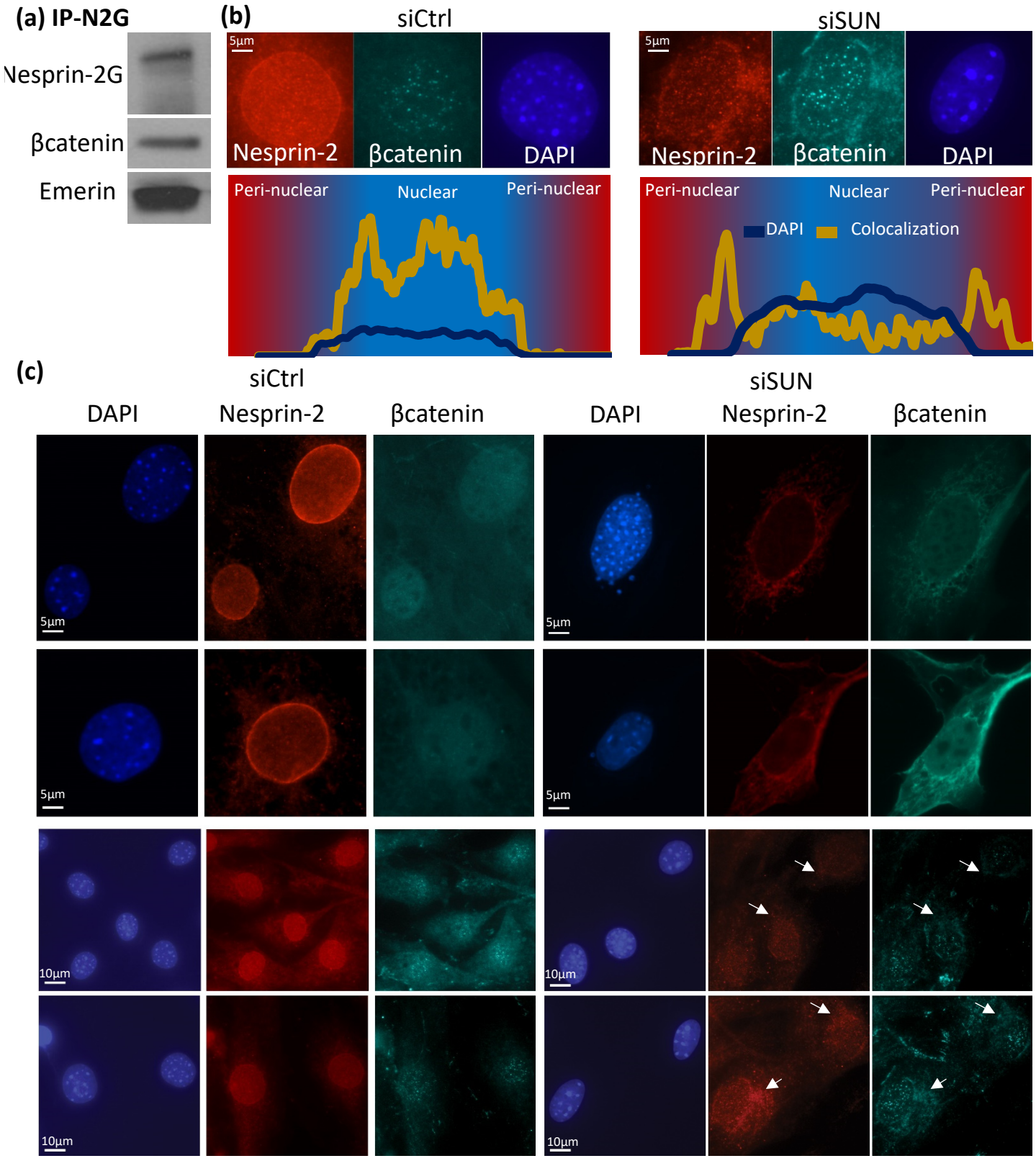


Figure S1 a) Immunoprecipitation of Nesprin-2 showed associations between βcatenin and Emerin. **b)** Representative images of siCtrl and siSUN treated cells showing Nesprin-2 (red) and βcatenin (green) distribution. In siCtrl treated cells, colocalization between Nesprin-2 and βcatenin (represented as yellow line) remains intranuclear while in siSUN treated cells colocalization was shifted into perinuclear area and remains outside of the nuclear boundary (DAPI, blue line). Representative images of **c)** siCtrl and siSUN treated cells indicating the intracellular localization of Nesprin-2 (red) and βcatenin (green) in relation to the nucleus (blue). White arrows indicate the perinuclear distribution of Nesprin-2 and βcatenin in siSUN treated cells. **.**

Fig.S2- Nuclear βcatenin accumulation but not βcatenin-nucleoskeleton association increase under sustained GSK3 β inhibition

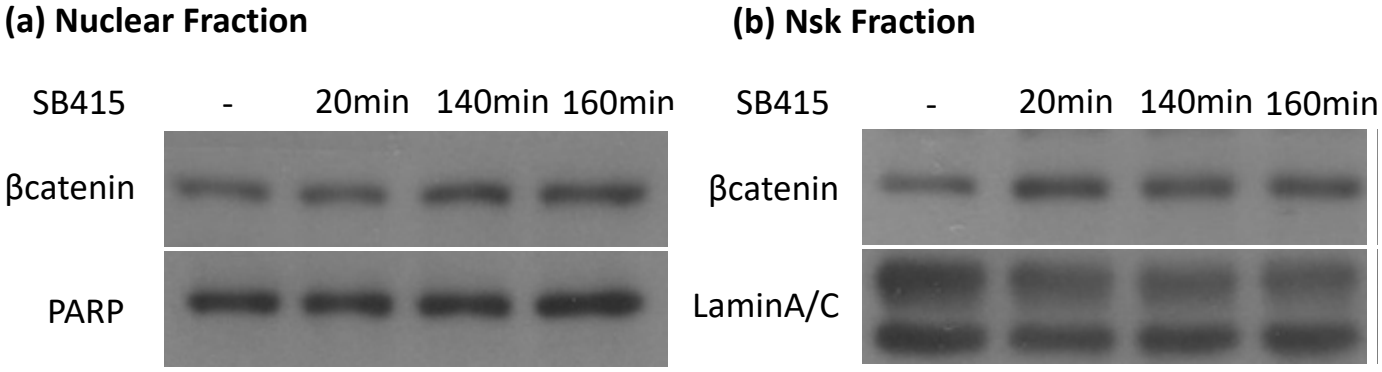
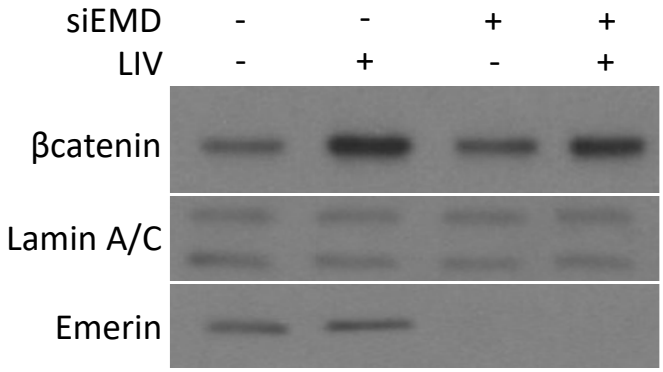


Figure S2. a) SB415 induced inhibition of GSK3β resulted in a stable increase of soluble nuclear βcatenin at 20, 140 and 160 minute time points. **b)** SB415 induced inhibition of GSK3β resulted in an increased βcatenin-Nsk association as soon as 20 minutes and upon keeping SB415 constraint, βcatenin-Nsk association remained elevated at 140 and 160 minute time points but signal intensity was not changed.

Fig.S3- Depletion of Emerin or LaminA/C are not sufficient to inhibit LIV-induced βcatenin-nucleoskeleton association

(a) siRNA against Emerin (siEMD)



(b) siRNA against Lmna (siLmna)

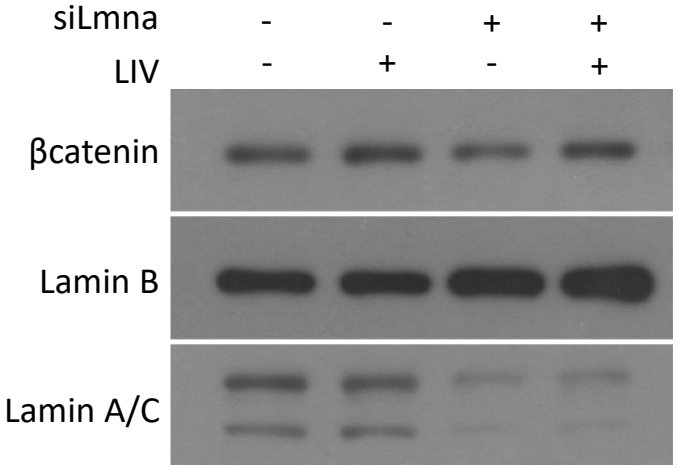


Figure S3. a) siRNA against Emerin (siEMD) did not change the basal and LIV-induced βcatenin-Nsk association between siCtrl and siEMD MSCs. **b)** Similarly, siRNA against LaminA/C (siLmna) did not inhibit LIV-induced βcatenin-Nsk association. LIV was applied at 90 Hz, 0.7g for 20 minutes. Samples were collected immediately after LIV application.

Fig.S4- LINC elements Sun-1 and Sun-2 are necessary for strain-induced βcatenin nuclear entry

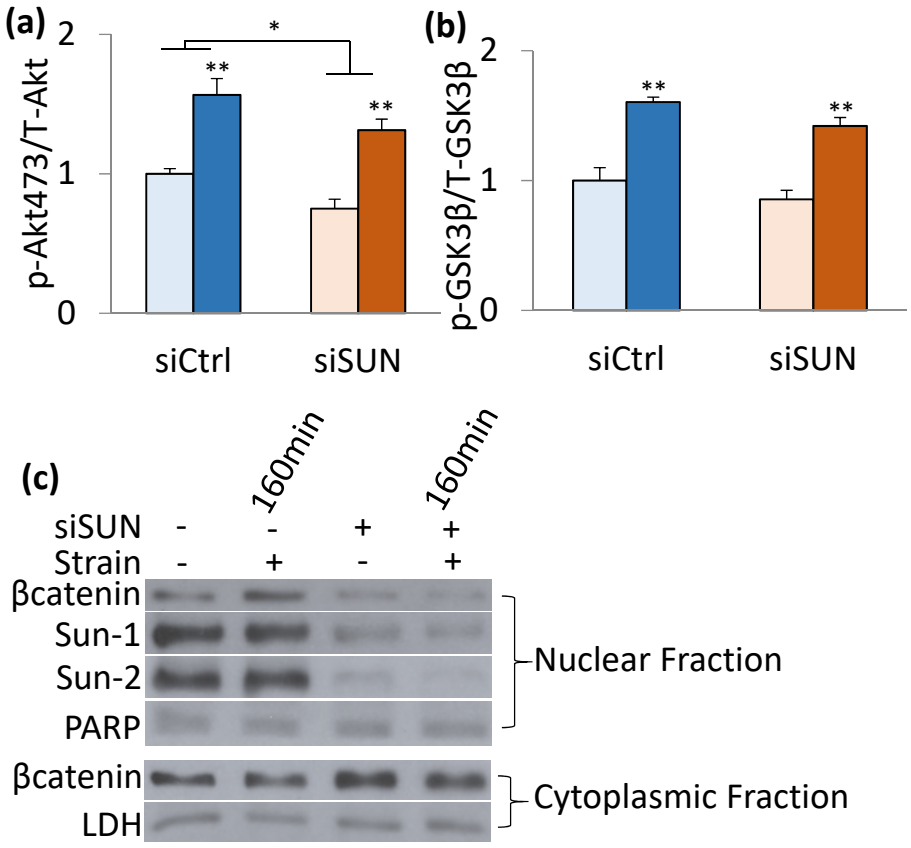


Figure S4. Application of strain results in **a)** Akt activation as well as **b)** GSK3β in both siCtrl and siSUN treated cells suggesting an increased βcatenin pool in the cytoplasm (n=3). **c)** Separating soluble nuclear and cytoplasmic fractions 160 min post-strain showed that in siCtrl cells βcatenin entered the nucleus while in siSUN cells there was no increase in nuclear βcatenin and βcatenin in the cytoplasmic fraction was increased. Group comparisons were made using One-way ANOVA followed by a Newman-Keuls post-hoc test (Fig.S4a-b). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, against control and each other.

Fig.S5- Nesprin-2 Immunoprecipitation

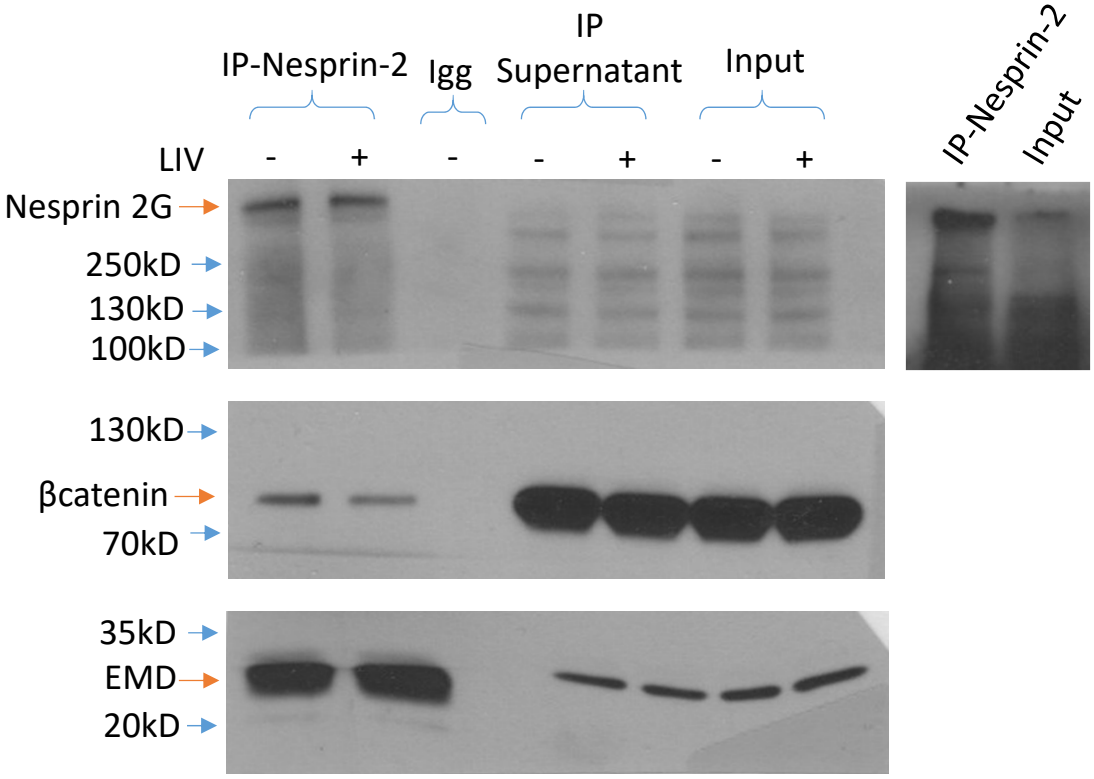


Figure S5. Supplementary file for Fig.4f displaying full size blots and other control lanes.

Fig.S6- Comparison of LIV and strain induced changes of Lamin A/C and Lamin B1

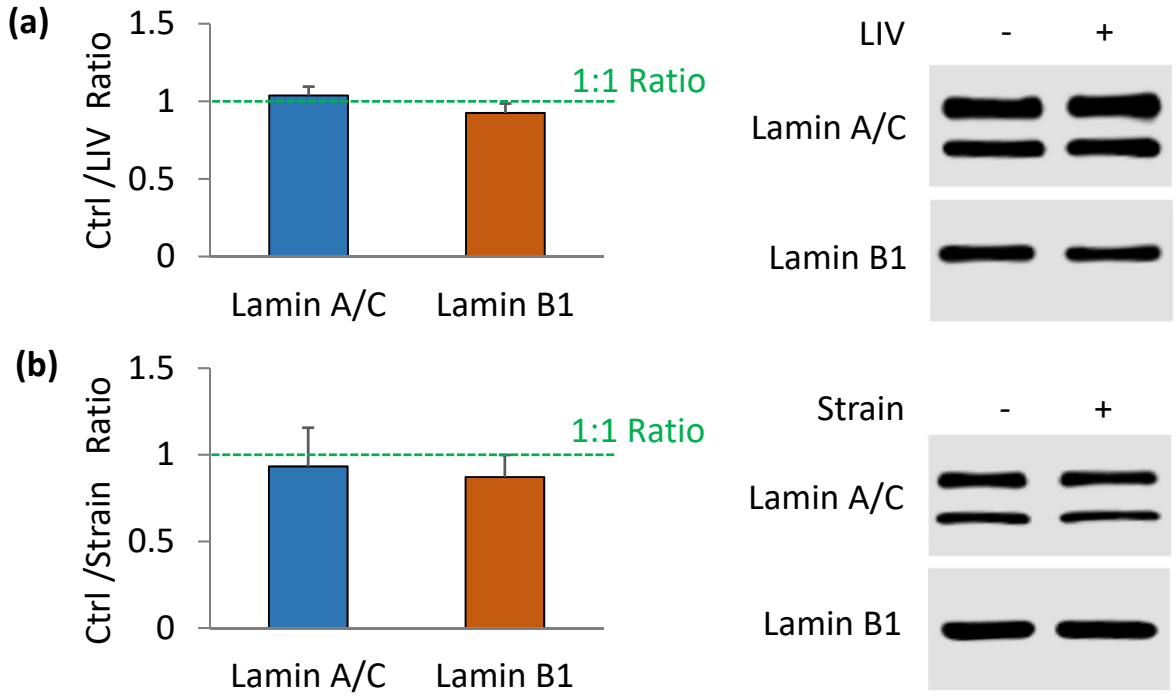


Figure S6. Changes in Lamin A/C and Lamin B1 protein amounts were reported as ratio between **a)** control and LIV samples (n=8) and **b)** control and strain samples (n=6). 120 minutes after the first mechanical challenge, no differences between LaminA/C and LaminB1 was detected in response to either LIV or Strain. Group comparisons were made using unpaired T-test (Figure S6a-b).

Fig.S7- Total βcatenin levels did not change with LIV

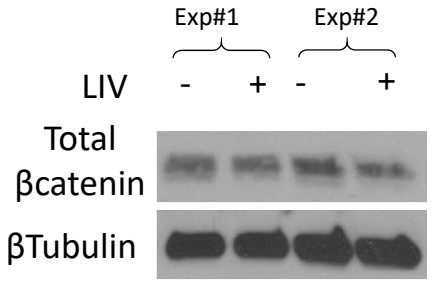


Figure S7. Whole cell lysates 160min post-LIV were probed via total βcatenin(BD, 610154) and β-Tubulin (Santa Cruz, SC-9104)