

Supplementary information

Recovery of stem cell proliferation by low intensity vibration under simulated microgravity requires intact LINC complex

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Figure S1. Comparison of sMG effect on proliferation between confluent and non-confluent cultures.

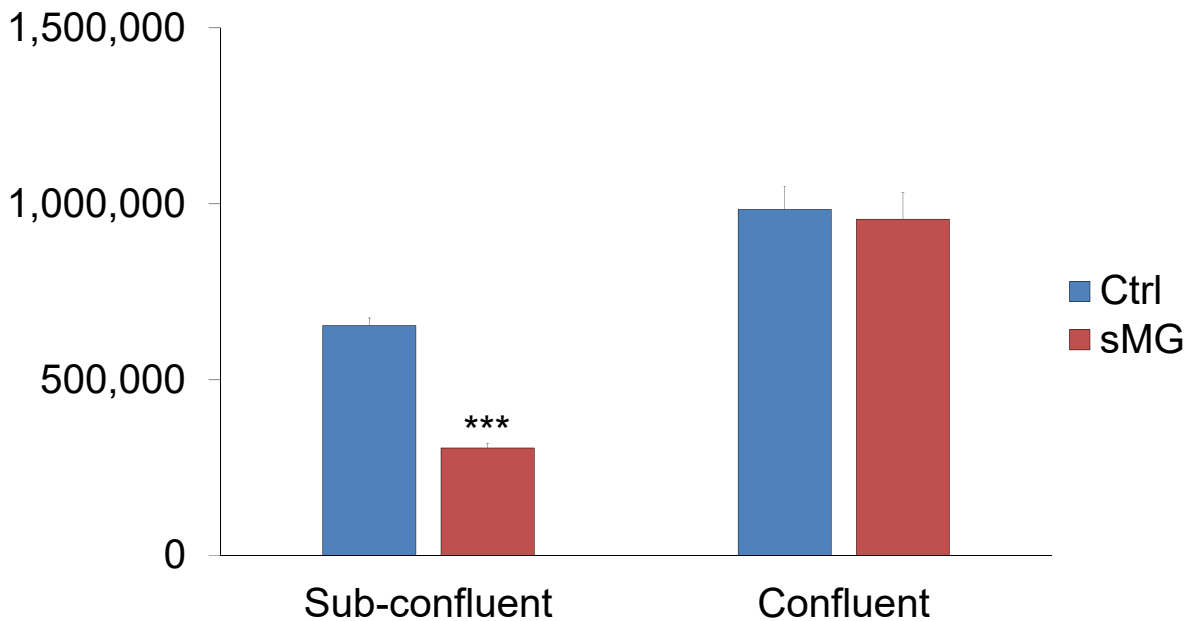


Figure S1. Comparison of sMG effect on proliferation between confluent and non-confluent cultures. 3 days of sMG decreased cell proliferation by 53% in non-confluent cultures (1,800cell/cm², $p < 0.001$, $n = 3/\text{grp}$). There was no difference in cell proliferation when cells plated into confluent cultures (18,000 cell/cm²). Group comparisons were made using unpaired T-test (Figure 1b) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, against control and each other.

Figure S2. F-actin morphology of sMG treated MSCs

F-actin (Phalloidin)

Nucleus (Hoechst 33342)

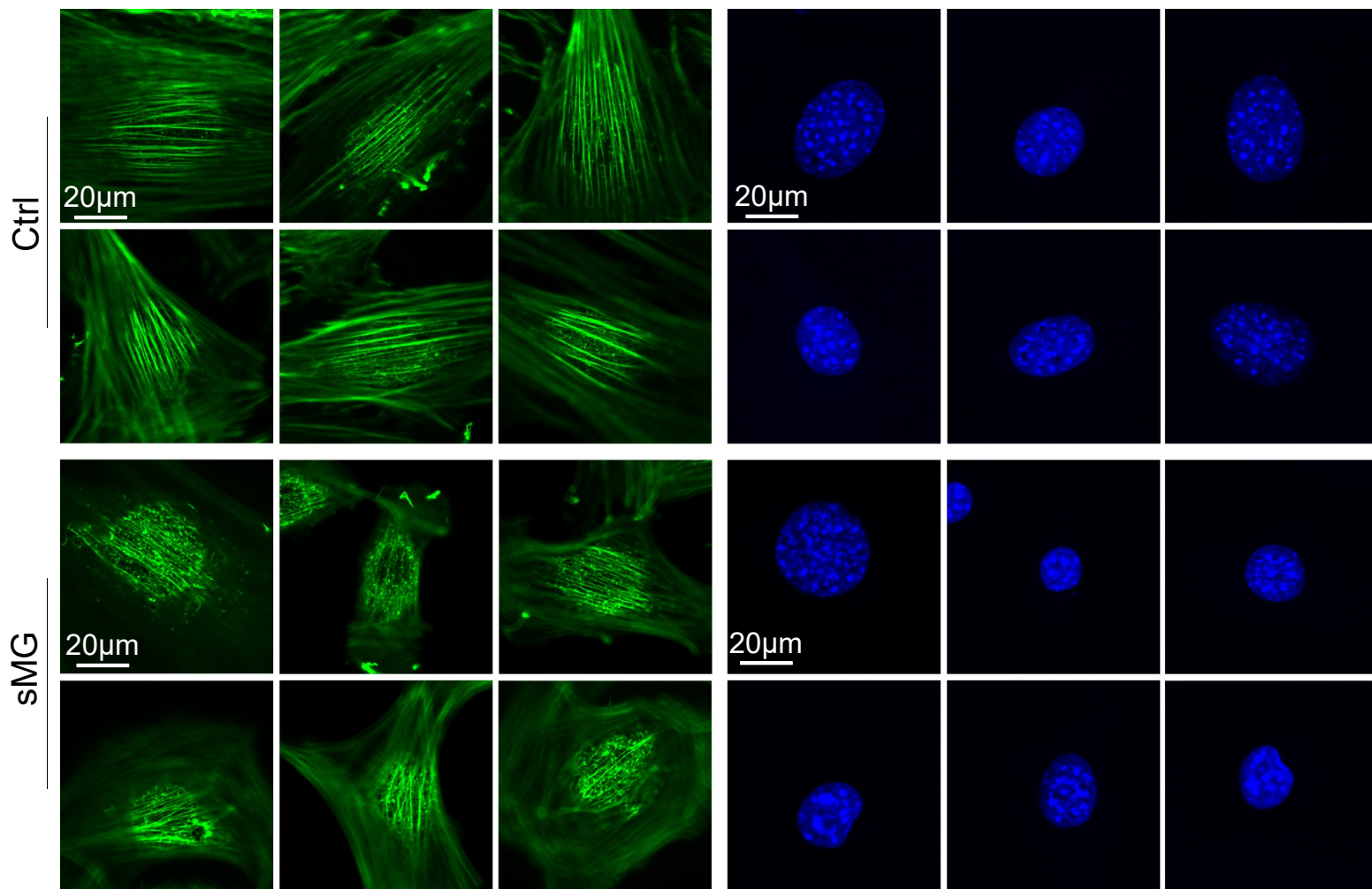


Figure S2. Representative images of actin morphology between control and sMG treated MSCs. Following sMG treatment, F-actin and nucleus were stained via Phalloidin (Green) and Hoechst 33342 (Blue) and nuclear height was measured via confocal microscopy. Apical actin structure appeared disorganized in sMG groups.

Table.S1: Differences between Control, sMG, sMG+LIV as determined by proteomics

Accession	Description	Group Comparisons (fold of change)					
		Normalized PSMs					
		Ctrl	sMG	sMG +LIV	sMG vs Ctrl	sMG+LIV vs sMG	sMG+LIV vs Ctrl
P46061	Ran GTPase-activating protein	8.79	1.75	5.17	0.20	2.95	0.59
P11440	Cyclin-dependent kinase 1	7.33	1.75	1.72	0.24	0.98	0.24
Q91YQ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	7.33	1.75	8.61	0.24	4.92	1.18
Q99LT0	Protein dpy-30 homolog	7.33	1.75	3.44	0.24	1.97	0.47
Q99LX0	Protein/nucleic acid deglycase DJ-1	7.33	1.75	3.44	0.24	1.97	0.47
O35639	Annexin A3	5.86	1.75	6.89	0.30	3.94	1.18
P61089	Ubiquitin-conjugating enzyme E2 N	5.86	1.75	3.44	0.30	1.97	0.59
Q61699	Isoform HSP105-beta of Heat shock protein 105 kDa	5.86	1.75	3.44	0.30	1.97	0.59
Q8R1F1	Niban-like protein 1	5.86	1.75	6.89	0.30	3.94	1.18
Q9CPY7	Isoform 2 of Cytosol aminopeptidase	5.86	1.75	6.89	0.30	3.94	1.18
P99029	Isoform Cytoplasmic+peroxisomal of Peroxiredoxin-5, mitochondrial	10.26	3.50	8.61	0.34	2.46	0.84
P62960	Nuclease-sensitive element-binding protein 1	13.19	5.25	10.33	0.40	1.97	0.78
P43275	Histone H1.1	8.79	3.50	10.33	0.40	2.95	1.18
Q64152	Isoform 2 of Transcription factor BTF3	8.79	3.50	3.44	0.40	0.98	0.39
Q8BMS1	Trifunctional enzyme subunit alpha, mitochondrial	8.79	3.50	5.17	0.40	1.48	0.59
Q9WTI7	Isoform 2 of Unconventional myosin-1c	4.40	1.75	5.17	0.40	2.95	1.18
Q9DCL9	Multifunctional protein ADE2 OS=Mus musculus	11.73	5.25	3.44	0.45	0.66	0.29
P08228	Superoxide dismutase [Cu-Zn] OS=Mus musculus	7.33	3.50	1.72	0.48	0.49	0.24
P56959	RNA-binding protein FUS	7.33	3.50	5.17	0.48	1.48	0.71
Q6NZJ6	Isoform 2 of Eukaryotic translation initiation factor 4 gamma 1	7.33	3.50	3.44	0.48	0.98	0.47
Q921F2	TAR DNA-binding protein 43	7.33	3.50	6.89	0.48	1.97	0.94
Q9EQU5	Isoform 2 of Protein SET	7.33	3.50	5.17	0.48	1.48	0.71
Q6ZQ38	Cullin-associated NEDD8-dissociated protein 1	17.59	10.49	5.17	0.60	0.49	0.29
P17427	AP-2 complex subunit alpha-2	2.93	1.75	5.17	0.60	2.95	1.76
P52293	Importin subunit alpha-1	2.93	1.75	6.89	0.60	3.94	2.35
P61161	Actin-related protein 2	10.26	7.00	3.44	0.68	0.49	0.34
Q60865	Caprin-1	8.79	7.00	1.72	0.80	0.25	0.20
P34022	Ran-specific GTPase-activating protein	4.40	3.50	8.61	0.80	2.46	1.96
O35344	Importin subunit alpha-4	5.86	5.25	1.72	0.89	0.33	0.29
Q9CQE8	RNA transcription, translation and transport factor protein	5.86	5.25	1.72	0.89	0.33	0.29
Q9D6Z1	Nucleolar protein 56	5.86	5.25	1.72	0.89	0.33	0.29
P46935	E3 ubiquitin-protein ligase NEDD4	7.33	7.00	3.44	0.95	0.49	0.47
Q9ERK4	Exportin-2	8.79	10.49	5.17	1.19	0.49	0.59
P23116	Eukaryotic translation initiation factor 3 subunit A	5.86	7.00	1.72	1.19	0.25	0.29
P24369	Peptidyl-prolyl cis-trans isomerase B	5.86	7.00	3.44	1.19	0.49	0.59
Q9Z1Q5	Chloride intracellular channel protein 1	5.86	7.00	3.44	1.19	0.49	0.59
P08003	Protein disulfide-isomerase A4	4.40	5.25	1.72	1.19	0.33	0.39
P14733	Lamin-B1	4.40	5.25	1.72	1.19	0.33	0.39
P62849	Isoform 2 of 40S ribosomal protein S24	4.40	5.25	1.72	1.19	0.33	0.39
P63158	High mobility group protein B1	4.40	5.25	1.72	1.19	0.33	0.39
Q64514	Isoform Short of Tripeptidyl-peptidase 2	4.40	5.25	1.72	1.19	0.33	0.39
Q99L47	Hsc70-interacting protein	4.40	5.25	1.72	1.19	0.33	0.39
O35737	Heterogeneous nuclear ribonucleoprotein H	7.33	10.49	5.17	1.43	0.49	0.71
Q60817	Nascent polypeptide-associated complex subunit alpha	7.33	10.49	5.17	1.43	0.49	0.71
Q99PL5	Ribosome-binding protein 1	7.33	10.49	5.17	1.43	0.49	0.71
Q9R0N0	Galactokinase	7.33	10.49	5.17	1.43	0.49	0.71
Q9JMH6	Isoform 2 of Thioredoxin reductase 1, cytoplasmic	14.66	20.99	10.33	1.43	0.49	0.71
P11087	Isoform 2 of Collagen alpha-1(I) chain	4.40	7.00	1.72	1.59	0.25	0.39
Q9D819	Inorganic pyrophosphatase	4.40	7.00	3.44	1.59	0.49	0.78
P37889	Isoform 2 of Fibulin-2	5.86	10.49	5.17	1.79	0.49	0.88
Q3THK7	GMP synthase [glutamine-hydrolyzing]	2.93	5.25	1.72	1.79	0.33	0.59
Q9QZE7	Translin-associated protein X	2.93	5.25	1.72	1.79	0.33	0.59
P47757	Isoform 2 of F-actin-capping protein subunit beta	10.26	19.24	8.61	1.88	0.45	0.84
O09061	Proteasome subunit beta type-1	4.40	8.74	1.72	1.99	0.20	0.39
Q60716	Isoform IIa of Prolyl 4-hydroxylase subunit alpha-2	4.40	8.74	3.44	1.99	0.39	0.78
Q9Z1Q9	Valine--tRNA ligase	11.73	24.48	10.33	2.09	0.42	0.88
Q9CZD3	Glycine--tRNA ligase	5.86	13.99	6.89	2.39	0.49	1.18
P62874	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	2.93	7.00	3.44	2.39	0.49	1.18
P10852	4F2 cell-surface antigen heavy chain	1.47	5.25	1.72	3.58	0.33	1.18
P56655	Cytochrome P450 2C38	1.47	5.25	1.72	3.58	0.33	1.18
Q9WUM4	Coronin-1C	1.47	5.25	1.72	3.58	0.33	1.18
Q60715	Prolyl 4-hydroxylase subunit alpha-1	2.93	13.99	6.89	4.77	0.49	2.35

Table.S2 : Cell Culture and Pharmacological Reagents

Cell Culture and Pharmacological Reagents		Final Concentration
IMDM	GIBCO	-
FCS	Atlanta Biologicals	10% v/v
Penicillin/streptomycin	GIBCO	1% v/v

Table.S3 : 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
Lamin B1 (OAEB03011)	Aviva Systems Biology	1/1000
Vinculin (4650T)	Cell Signaling	1/500
Sun-1 (HPA008346)	Sigma Aldrich	1/1000
Sun-2 (ab87036)	Abcam	1/1000
GAPDH (5174S)	Cell Signaling	1/1000
YAP (14074S)	Cell Signaling	1/1000

Table.S4 : 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'

Table.S5: 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'
Lmna -Forward	5'- TGAGTACAACCTGCGCTCAC -3
Lmna -Reverse	5'- TGTGACACTGGAGGCAGAAG
Nesprin1G -Forward	5'-AAGCAACTCAAGGCTTTCCA-3'
Nesprin1G -Reverse	5'-GTCTGAGCTCGTCCAGTTCC-3'
Nesprin2G -Forward	5'- ACCTCGCCAACACATACCTC-3',
Nesprin2G -Reverse	5'- TGCTTCTTGCTGCTTCTTCA-3'
18S -Forward	5'-GAACGTCTGCCCTATCAACT-3'
18S -Reverse	5'-CCAAGATCCAACACTACGAGCT-3

Table.S6: Immunostaining antibodies and reagents and their final concentrations

Immunostaining antibodies and Reagents		Final Concentration
Hoechst 33342	Invitrogen	1 µg/mL
Alexa Fluor 488 Phalloidin	Life Technologies	0.1µM

Figure S3 Unprocessed Blots

Fig 5 – Lamin A/C

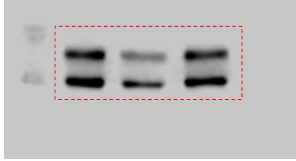


Fig 5 – pFAK

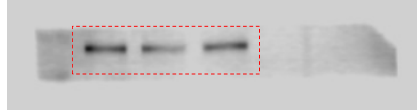


Fig 4 – Lamin A/C



Fig 3 – YAP

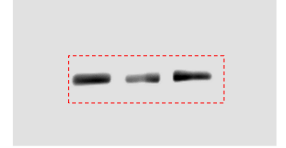


Fig 5 – Sun-1

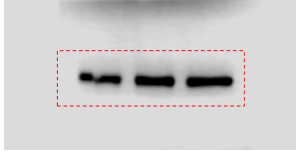


Fig 5 – TFAK

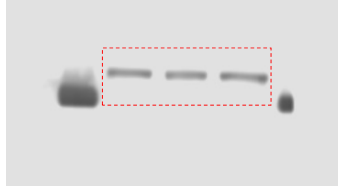


Fig 3 – GAPDH

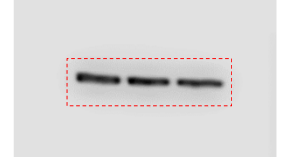


Fig 5 – LmnB

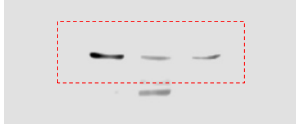


Fig 5 – GAPDH

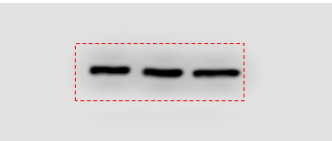


Fig 4 – Sun-1

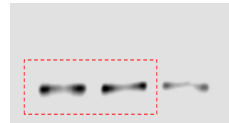


Fig 5 – Sun-2

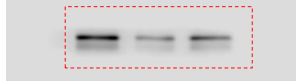


Fig 4 – Sun-2



Fig 5 – GAPDH

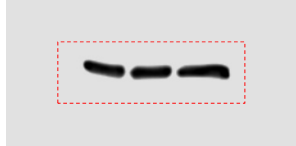


Fig 5 – Vinculin

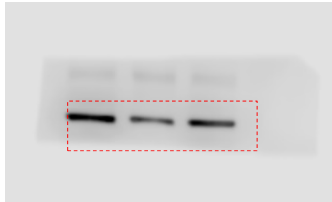


Fig 4 – Sun-1

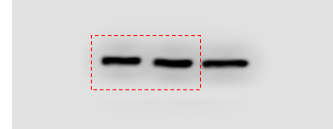


Fig 4 – pAkt

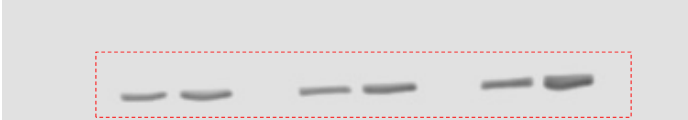


Fig 4 – tAkt

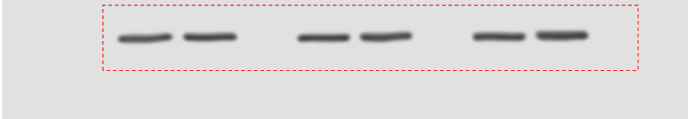


Figure S3. Unprocessed blots as obtained by LiCor C-DiGit blot scanner.