Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading

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Studies of cellular mechano-signaling have often utilized static models that do not fully replicate the dynamics of living tissues. Here, we examine the time-dependent response of primary human mesenchymal stem cells (hMSCs) to cyclic tensile strain (CTS). At low-intensity strain (1 h, 4% CTS at 1 Hz), cell characteristics mimic responses to increased substrate stiffness. As the strain regime is intensified (frequency increased to 5 Hz), we characterize rapid establishment of a broad, structured and reversible protein-level response, even as transcription is apparently downregulated. Protein abundance is quantified coincident with changes to protein conformation and post-translational modification (PTM). Furthermore, we characterize changes to the linker of nucleoskeleton and cytoskeleton (LINC) complex that bridges the nuclear envelope, and specifically to levels and PTMs of Sad1/UNC-84 (SUN) domain-containing protein 2 (SUN2). The result of this regulation is to decouple mechano-transmission between the cytoskeleton and the nucleus, thus conferring protection to chromatin.
The structures and integrity of the human body are defined by stiff tissues such as skin, muscle, cartilage and bone. Tissue mechanical properties are determined primarily by the extracellular matrix (ECM), in particular by the identities and concentrations of its constitutive proteins\textsuperscript{1-3}. ECM properties are further modulated by protein cross-linking, post-translational modifications (PTMs), and higher-order organization. Cells resident within tissues maintain mechanical equilibrium with their environments\textsuperscript{4,5}, and the mechanical properties of cells are also regulated by the identities, concentrations, conformations and PTMs of structural intracellular proteins\textsuperscript{4,6,7}. The characteristics of adherent cells can be influenced by physical stimulation from the surrounding ECM. Cellular protein content\textsuperscript{1}, morphology\textsuperscript{1,8}, motility\textsuperscript{9,10} and differentiation potential\textsuperscript{11,12} are amongst behaviors known to be affected by stiffness. Cells in living tissues experience microenvironments of diverse stiffness\textsuperscript{5}, but are also subject to deformation during activity. Cells sense and respond to mechanical signals through pathways of mechanotransduction\textsuperscript{13,14}, but must also maintain integrity and homeostasis within the tissue. A mismatch between mechanical loading and cellular regulation can contribute to pathology, such as in musculoskeletal and connective tissue disorders\textsuperscript{3,4}, with ageing being a significant risk factor\textsuperscript{16}.

Here, we compare responses to stiffness and mechanical loading in primary human mesenchymal stem cells (hMSCs), a cell type with important physiological and reparative roles, that have led to investigations of their therapeutic potential in tissues such as muscle\textsuperscript{17} and heart\textsuperscript{18}. Using mass spectrometry (MS), we identify a rapid, reversible and structured regulation of the proteome following high-intensity mechanical loading. Furthermore, we identify the Sad1/UNC-84 (SUN) domain-containing protein 2 (SUN2) as a strain-induced breakpoint in the linker of nucleoskeleton and cytoskeleton (LINC) complex of proteins that acts as a key mediator of intracellular mechano-transmission\textsuperscript{13,19}, thus enabling the nucleus to decouple from the cytoskeleton in response to intense strain.

**Results**

**Strain cycle unouples cell and nuclear morphologies.** Primary hMSCs were cultured on stiffness-controlled polyacrylamide hydrogels or silicone elastomer sheets that could be subjected to cyclic tensile strain (CTS); both substrates were collagen-I coated. hMSCs cultured for 3 days were found to spread increasingly on stiffer substrates over a physiological range (2–50 kPa; Fig. 1a, Supplementary Fig. 1a), as has been reported previously\textsuperscript{1,20}. Cells subjected to sinusoidal, equiaxial CTS for 1 h at 1 or 2 Hz (change in strain \(= 4\%\)) showed significantly increased spreading immediately after loading (\(p \leq 0.05\), determined by ANOVA testing), returning to initial spread areas after 24 h (Fig. 1b, Supplementary Figs. 1b, c). Earlier reports of cell behavior following strain have described cell alignment relative to the direction of strain\textsuperscript{21} and reorganization of focal adhesion (FA) complexes and the cytoskeleton\textsuperscript{22,23}. As the strain applied in our system had radial symmetry, no overall alignment was observed, but increased cell spreading was consistent with previous reports describing FA activation\textsuperscript{24}. The increase in spreading of hMSCs following dynamic straining at 1 and 2 Hz was thus similar to that observed with changes in static substrate stiffness.

To explore mechanisms that allow cells to endure more challenging mechanical environments (e.g. the mechanical environments encountered within dynamic tissue, including muscle, skin and cartilage), we increased the frequency of CTS to 5 Hz (change in strain \(= 3.6\%\)). The increased cell spreading observed at lower frequencies was not seen following 1 h of 5 Hz CTS (Fig. 1c). Cell spreading was significantly decreased 24 h after

**CTS-induced nuclear contraction requires ion channels.** Stretch activated ion-channels can enable rapid cellular responses to mechanical stimulation\textsuperscript{26}. To investigate the role of ion channels in strain-induced nuclear contraction, we combined 5 Hz CTS with a panel of ion channel inhibitors: GdCl\textsubscript{3}, a broad-spectrum inhibitor of stretch-activated ion channels\textsuperscript{27}; RN9893, an inhibitor of transient receptor potential cation channel subfamily V member 4 (TRPV4)\textsuperscript{28}; amiloride, an inhibitor of acid sensing ion channels (ASICs)\textsuperscript{27}, and GsMTx4, an inhibitor of piezo channels\textsuperscript{29} (Supplementary Figs. 2a, b). GdCl\textsubscript{3}, RN9893 and amiloride inhibited nuclear contraction following CTS. GsMTx4 did not prevent nuclear contraction, although earlier work has shown it to be effective in inhibiting chromatin condensation under milder loading regimes (3% uniaxial strain at 1 Hz)\textsuperscript{29}. This suggested that activation of different ion channels may be specific to the loading regime.

CTS at 1 and 5 Hz significantly increased the texture parameter of nuclear DAPI staining (1 Hz, \(p < 0.0001\) and 5 Hz, \(p = 0.002\), ANOVA; Supplementary Fig. 3a–c), indicative of chromatin condensation\textsuperscript{23,30} (comparable to the effect of divalent ions, Supplementary Fig. 3d, e). Treatment with GdCl\textsubscript{3} at its IC\textsubscript{50} of 10 \(\mu\)M\textsuperscript{31} did not prevent changes to DAPI-stain texture following CTS at 5 Hz (\(p = 0.02\), ANOVA). This contrasted with earlier characterizations of milder loading regimes, where GdCl\textsubscript{3} was found to block chromatin condensation, although the drug concentration was higher in this case\textsuperscript{28,32}. Our finding indicated the robustness of the chromatin condensation response in cells subjected to high-intensity CTS, but also suggested that chromatin condensation and contraction of nuclear area could be caused by different mechanisms.

**Cellular responses to CTS are driven at the protein level.** In order to examine the cellular consequences of high-intensity strain, we applied -omics analyses to hMSCs following 1 h of 5 Hz CTS. Surprisingly, we found few changes to the transcriptome as assessed by RNA-Seq immediately after the treatment (Gaussian width = 0.21; Fig. 2a, Supplementary Data 1 and 2). Furthermore, gene ontology (GO) term analysis\textsuperscript{2,33} of the affected genes suggested a general suppression of transcription and metabolism (Fig. 2b). Downregulation of transcriptional activity is consistent with our observations of chromatin condensation, and previous reports of histone-methylation mediated gene silencing in...
endothelial progenitor cells subjected to low frequency (0.1 Hz) strain cycling. The distribution of changes to gene expression was narrowed 24 h after CTS (Gaussian width = 0.14; Supplementary Fig. 4a).

In contrast to the analysis of transcript levels, analysis of the intracellular proteome by MS, showed greater changes relative to unstrained controls (Fig. 2c, Supplementary Fig. 4b, Supplementary Data 3 and 4). A Gaussian fit to the distribution of protein fold changes had a width of 0.61, indicating a greater perturbation to proteome than transcriptome. Analysis of the Reactome pathways significantly affected by CTS (pathways with over-representation of affected proteins; Bayes-modified t-tests with Benjamini–Hochberg false discovery rate (BH-FDR)-corrected $p < 0.05$) showed that ontologies relating to metabolism of both protein and RNA, signal transduction, and response to external stimuli were downregulated (Fig. 2d). Changes to the transcriptome and proteome following CTS were not correlated (R-squared = 0.002; Fig. 2e), indicating a post-transcriptional regulation of protein levels. The proteome returned towards the control state after 24 h (Gaussian width = 0.25; Supplementary Figs. 4c–e).

The time-resolved proteomic response to 5 Hz CTS was further classified by K-means clustering (Fig. 2f, Supplementary Fig. 4f). Clusters of protein levels were identified with: (cluster 1) an immediate but unsustained decrease, enriched for Reactome annotations associated with translation, protein folding, and mechanisms of actin and tubulin folding; (cluster 2) an initial but unsustained increase, enriched for an annotation of metallothionein binding (associated with the management of oxidative stress); and (cluster 3) an immediate decrease and slow recovery, enriched for annotations for translation and regulation of the Slit/Robo signaling pathway (associated with cell polarity and cytoskeletal dynamics). Taken as a whole, this analysis shows a complex, time-resolved, and structured protein-level response to cellular stress management.
For comparison, we also examined proteomic changes in response to low-intensity CTS (1 h at 1 Hz, change in strain = 4.0%). We found changes to 1 Hz CTS to be less pronounced than those induced at 5 Hz (Gaussian width = 0.30; Supplementary Figs. 5a, b, Supplementary Data 8 and 9), and although Reactome analysis showed similar pathways to be affected (compare Fig. 2d to Supplementary Fig. 5d), we noted that many of the significantly affected proteins were associated with the cytoskeleton. CTS at 1 Hz caused a significant increase in levels of actin (ACTB), vimentin (VIM), tubulin alpha-1B chain (TUBA1B) (all p <
CTS at 5 Hz causes changes to protein conformation and PTMs. As changes in protein conformation are important to mechanotransduction, MS was performed following protein labeling with monobromobimane (mBBr), which by selectively labeling solvent-exposed cysteine residues, acts as an indicator of protein folding (Fig. 3a). MS was used to both identify mBBr-labeled proteins and quantify differential labeling in hMSCs following CTS at 5 Hz, relative to unstrained controls (Fig. 3b, Supplementary Data 5). The histogram of log₂-fold changes in mBBr labeling showed a broad distribution of CTS-induced changes to mBBr reactivity, with labeling increased on average immediately following strain at 1 Hz (Supplementary Fig. 5d, e; Gaussian width = 0.26), although some changes to cysteine proteins persisted (TUBA1B remained elevated, ACTB was decreased; both p < 0.0001, Bayes-modified t-test, BH-FDR correction). Transcripts associated with the cytoskeleton were also affected: vimentin (VIM) was increased immediately following CTS at 1 Hz (p = 0.0003, ANOVA) and alpha-actin-2 (ACTA2) was increased after 24 h (p = 0.004, ANOVA) (Supplementary Fig. 5f, g). These results are consistent with previously observed changes to cell morphology, and earlier characterizations of cellular responses to strain and substrate stiffness, which cellular responses to strain and substrate stiffness, which

Mechano-sensitive phosphosites in SUN2 lamin-binding domain. To understand the regulation of SUN2 and its role in the nuclear decoupling phenomena, we examined the response of hMSCs to shorter durations of CTS at 5 Hz. IF showed that SUN2 was significantly reduced at the NE after 1 min of CTS (p = 0.03, ANOVA). Emerin (EMD), which has a role in the mechanical stimulation of the serum response factor (SRF) pathway, was significantly enriched at the NE (p < 0.0001, ANOVA), consistent with previous reports.

Although phosphorylation may in many cases be mechano-sensitive, it does not necessarily regulate turnover. A similar analysis of oxidized peptides showed that oxidation was increased immediately following CTS (Fig. 3f, Supplementary Data 7), and in a third of detected sites of oxidation, increased oxidation correlated with decreased protein levels (points in the top left quadrant of Fig. 3g). Previous work has established that cyclic stretching can increase levels of reactive oxygen species (ROS) in a range of cell types through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase systems and mitochondria. ROS have an important role in signal transduction, for example during vascularization, but can contribute to oxidative damage to lipids, proteins, and DNA. This potential for oxidative damage is perhaps consistent with the upregulation of protective metallothioneins (Fig. 2f).

Profiles of both phosphorylation and oxidation became similar to controls 24 h after CTS (Fig. 3d, f).
were found that occurred immediately (decrease in pS12, \( p = 0.007 \); increase in pS21, \( p < 0.0001 \); and increase in pS38, \( p < 0.0001 \)) and some persisted 24 h following CTS (increase in pT9, \( p = 0.003 \); decrease in pS12, \( p < 0.0001 \); and increase in pS21, \( p < 0.0001 \); \( p \)-values derived from Bayes-modified \( t \)-tests with BH-FDR-correction; Fig. 5c).

Taken together, this evidence suggests a putative mechanism (Fig. 5d) whereby high-intensity CTS causes rapid loss of SUN2 from the NE – potentially mediated by phosphorylation of the lamin-binding domain – followed by a slower remodeling of cell and nuclear morphology and the cellular proteome (including turnover of SUN2). The composition of the nuclear lamina has been used previously as a readout of nuclear adaption to the mechanical properties of the cellular microenvironment, with an increased ratio of A-type to B-type lamins being indicative of nuclear stiffening in response to stiffness1.44. We used IF to quantify the ratio of total LMNA to lamin-B1 after 1 h of CTS at 5 Hz, finding it to be significantly decreased (\( p = 0.007 \), ANOVA; Supplementary Fig. 7a). Our findings contrast earlier reports1 of responses within the nuclear lamina to increasing substrate stiffness (summarized in Supplementary Fig. 7b) and suggest that SUN2-mediated nuclear decoupling could desensitize the lamina to mechanical stimulation. Lastly in our investigations into causation, we found that treatment with GdCl\(_3\) – shown to
Fig. 3 Post-translational modification (PTM) states respond to CTS. **a** Schematic diagram of differential labeling of sulfhydryl groups by monobromobimane (mBBr)1, 40. Exposed cysteine residues are rapidly labeled, but those sequestered within folded proteins are unreactive; changes to the extent of labeling are indicative of altered protein conformation in response to a stress condition. Changes in protein quantity are determined by rates of synthesis versus turnover. **b** Histogram showing log2-fold changes to mBBr labeling site occupancy immediately and 24 h after hMSCs were subjected to CTS (1 h at 5 Hz, 2.6–6.2% strain; n = 3 donors), relative to unstrained controls. The distribution immediately following CTS was broad and displaced to the right. After 24 h, the distribution was narrowed and centered around zero. Annotations indicate two sites within LMNA with significantly increased modification (p < 0.05). **c** Correlation between changes to mBBr labeling site occupancy and the quantity of the parent protein (i.e. source of the labeled peptide), immediately following CTS. **d** Histogram showing changes to phosphosite occupancy immediately and 24 h after CTS (1 h at 5 Hz, 2.6–6.2% strain; n = 3 donors), relative to unstrained controls. All phosphorylation sites shown have been previously in the PhosphoSitePlus database70. The distribution was shifted to the right immediately following CTS. **e** Correlation between phosphorylation and protein quantity. **f** Histogram showing changes to oxidation-site occupancy immediately and 24 h after CTS (1 h at 5 Hz, 2.6–6.2% strain; n = 3 donors), relative to unstrained controls. **g** Correlation between oxidation and protein quantity; in 33% of cases, increased oxidation correlated with decreased protein levels (points in the top left quadrant). In **c**, **e**, and **g**, data points are annotated as indicated in the legend if log2-fold change > 0.5 and p < 0.05, otherwise they are shown in gray; labels indicate modified sites within proteins. p-values were calculated using empirical Bayes-modified t-tests with Benjamini-Hochberg correction. See Supplementary Data 3–7.

![Fig. 3](https://www.nature.com/naturecommunications)

Fig. 4 CTS causes loss from the nuclear envelope (NE) and turnover of SUN2 protein. **a** Changes to linker of nucleoskeleton and cytoskeleton (LINC) complex and NE proteins in primary hMSCs, detected and quantified by MS immediately following CTS (1 h at 5 Hz, 2.6–6.2% strain; n = 3 donors), relative to unstrained controls. SUN2 was found to be significantly down-regulated, but was recovered 24 h after CTS (Supplemental Fig. 3d). Numbers in blue indicate the number of peptides detected per protein identity. p-values were calculated using empirical Bayes-modified t-tests with Benjamini-Hochberg correction. See Supplementary Data 4. **b** Immunofluorescence (IF) quantification of proteins localized at the NE immediately following CTS (1 h at 5 Hz, 2.6–6.2% strain; n = 3 donors) for SUN1, SUN2, LMNA, and LMNB1; n = 4 donors for EMD; see Supplementary Figs. 6a–e for data distributions and donor-to-donor variation). **c** IF quantification of SUN2 at the NE following 1 and 10 min of CTS at 5 Hz (2.6–6.2% strain; n = 3 donors). Red line indicates SUN2 levels following 1 h of CTS (p = 0.03). Significant loss of SUN2 at the NE occurred within 1 min (min, p = 0.002; 10 min, p < 0.0001). **d** Nuclear to cytoplasmic area ratios quantified following 1 and 10 min of 5 Hz CTS. Red line indicates ratio following 1 h of CTS (p = 0.003; Fig. 1f). These results indicate that loss of SUN2 from the NE precedes changes to cellular morphology. p-values in **b–d** determined from linear models (ANOVA tests). All plots show mean ± s.e.m.; see Supplementary Table 1 for sample sizes.

![Fig. 4](https://www.nature.com/naturecommunications)

SUN2 acts upstream of chromatin and cytoskeletal regulation. As the level of SUN2 was decreased in response to CTS, we sought to investigate how this could affect cellular function. We quantified the effects of siRNA-mediated knockdown (KD) of SUN2 on primary hMSCs by MS, comparing two siRNAs to increase confidence of identifying on-target effects (Fig. 6a, Supplementary Data 10 and 11). The siRNAs depleted SUN2 to 35% of scrambled controls (Supplementary Fig. 9a, b). A Reactome pathway analysis of both KDS (Fig. 6b) identified significant perturbations to processes with the following annotations: polycomb repressive complex 2 (PRC2) methylates histones and DNA; protein lysine methyltransferases (PKMTs) methylate histone lysines; and, Rho GTPases activate IQ motif containing GTPase activating proteins (IQGAPs) (all p-values < 0.05, Bayes-modified t-tests, BH-FDR correction). These annotations suggested that SUN2 could be upstream of chromatin regulation and nucleus-to-cytoskeleton (inside-to-outside) signaling, consistent with our earlier observations. A scatter plot of protein fold changes following SUN2 KD versus CTS showed correlation in cytoskeletal proteins (Fig. 6c), suggesting SUN2 regulation following CTS could be upstream of cytoskeletal remodeling.

We also investigated the effects of SUN2 overexpression (OE), using immortalized hMSCs (Y201 line) that maintain the multipotency and mechano-responsiveness of primary MSCs49,50. The proteomes of Y201 cells were quantified with doxycycline-induced OE of SUN2 to 160% and 410% of control levels (Fig. 6d, Supplementary Fig. 9c, d, Supplementary Data 12 and 13). While the SUN2 KD caused an increase in a number of cytoskeletal proteins, SUN2 OE caused a corresponding decrease in many of the same proteins (including filamin-A, plectin and vimentin), suggestive of a compensatory mechanism within the cytoskeleton. Reactome analysis following SUN2 OE was less specific in its effects than the KD, but interestingly suggested an up-regulation of pathways associated with DNA repair (Fig. 6e). MS also revealed changes to the phosphorylation state of SUN2 following OE (Fig. 6f): consistent with OE driving removal of excess protein, we found the same sites to be affected as when SUN2 was lost following CTS (Fig. 5c).

SUN2 modulates nuclear mechano-transmission and DNA damage. To investigate the role of SUN2 in mechano-transmission to the nucleus, we examined the relationship...
between SUN2 levels and cellular morphology. Y201 cells were cultured on plastic for three days following KD, OE and rescue of SUN2 levels, and compared to controls (Fig. 7a); modulation of SUN2 level at the NE was confirmed in all cases by IF (Supplementary Fig. 10a, b). Previous reports have linked SUN2 OE with abnormally shaped nuclei51 and we found a significant reduction in nuclear form factor in SUN2 OE in immortalised hMSCs (Fig. 8a, b, Supplementary Fig. 10d, e). This contrast between the change in nuclear to cytoplasmic area ratio following CTS (1h at 5 Hz, 2.6–6.2% strain; n = 3 donors). SUN2 KD and rescue of SUN2 expression levels restored the capacity to decouple nuclei from the cytoskeleton following CTS (p < 0.001, ANOVA and Dunnett’s multiple comparison tests; Fig. 8d, e), confirming the importance of correct SUN2 expression levels for this phenomenon to occur. SUN2 OE was also found to prevent the increase in nuclear texture, associated with chromatin condensation, that was caused by CTS (p = 0.01, ANOVA and Dunnett’s multiple comparison tests; Fig. 8f).

Mechanical strain has been shown to cause DNA damage, inducing apoptosis in vascular smooth muscle cells, and causing the accumulation of damage to DNA and chromatin in nuclei subjected to extreme deformation as cells migrate through constricted environments33–35. We were surprised, therefore, to find that CTS here resulted in a small but significant decrease in the intensity of γH2AX staining in primary (p = 0.03, ANOVA) and immortalised MSCs (p = 0.0002, ANOVA), suggestive of a protective effect (Fig. 9a, b, Supplementary Fig. 12a, b). However, we found that the OE of SUN2 in immortalised hMSCs showed to override the decoupling response to CTS raised the baseline level of γH2AX staining (p < 0.0001, ANOVA and Dunnett’s multiple comparison tests) and caused staining to be further increased immediately following CTS (p < 0.0001, ANOVA and Dunnett’s...
**Fig. 6** Knockdown (KD) and overexpression (OE) of SUN2 affect the cellular proteome. a MS characterization of SUN2 KD on protein levels in primary hMSCs. Plot shows correlation between log$_2$-fold changes to the proteome following SUN2 KD with two siRNAs, siRNA1 and siRNA2, each measured relative to scrambled controls ($n = 3$ donors; see Supplementary Figs. 9a, b for histograms). Data points annotated as indicated in the legend where significant changes occurred ($p < 0.05$), otherwise they are shown in gray. b Pathways identified in the Reactome database as significantly affected by both SUN2 KDs, relative to scrambled controls, shown with fold changes to constitutive proteins. c Correlation between changes to protein levels following SUN2 KD (mean of both siRNA treatments, relative to scrambled controls) and immediately following CTS (1 h, 2.6–6.2% strain at 5 Hz relative to unstrained controls). Correlated and anti-correlated data annotated as indicated in the legend where significant changes occurred ($p < 0.05$; $n = 3$ donors), otherwise they are shown in gray. d MS characterization of SUN2 OE in an immortalized human MSC line. Plot shows correlation between log$_2$-fold changes to the proteome following SUN2 OE with two separate expression levels, low (160% of control following 3 days of DOX treatment) and high (410% of control following 4 days of DOX treatment), measured relative to vehicle-only controls ($n = 3$ replicates; see Supplementary Figs. 9c, d for histograms). Data points annotated as indicated in the legend where significant changes occurred ($p < 0.05$), otherwise they are shown in gray. e Analysis of Reactome pathways significantly affected at the protein level following SUN2 low-OE ($p < 0.05$). Protein quantification in a-e based on ≥3 peptides-per-protein. f Quantification of SUN2 phosphosites following low and high OE, as characterized in d. pS21 and pS38 were significantly affected by low SUN2 OE ($p < 0.0001$); high OE significantly affected pT9 ($p = 0.001$), pS12, pS21, and pS38 ($p < 0.0001$). $n = 3$ technical replicates. Data displayed as mean and s.e.m. All $p$-values were calculated using empirical Bayes-modified $t$-tests with Benjamini–Hochberg correction. See Supplementary Data 10–13.

**Fig. 7** Cell and nuclear areas are both affected by perturbation to SUN2 levels. a Representative images of immortalized MSC nuclei stained against DAPI and SUN2 following control, SUN2 KD (siRNA2), OE and rescue (OE + siRNA2) treatments. Scatter plots showing log$_2$-fold change to nuclear area, cytoplasmic area, and nuclear to cytoplasmic area ratio vs. log$_2$-fold change to SUN2 expression, in immortalized MSC nuclei (control, SUN2 KD, OE, and rescue), relative to control levels. Data points indicate individual nuclei ($n$ ≥11 analysed per condition); annotated points show mean ± s.e.m. for each condition; lines show linear regression analysis (with annotation of gradient, $b$ ± standard deviation). See Supplementary Fig. 10b–e for data distributions and statistics. Nuclear area showed weak positive power law scaling with SUN2 expression ($b = 0.08 ± 0.04$). However, as the cytoplasmic area showed stronger scaling with SUN2 expression ($b = 0.38 ± 0.08$), the nuclear to cytoplasmic area ratio had overall negative scaling ($b = −0.38 ± 0.06$). These results suggest a pronounced inside-to-outside influence of SUN2 levels on the cytoskeleton.
condition. Box-whisker plot center lines show medians, bounds of box show quartiles, whiskers show data spread and outliers determined by the Tukey method; significant differences were determined from linear modeling (ANOVA). See Supplementary Figs. 11a-d for data distributions and variation between donors. Ratios of nuclear to cytoplasmic areas in immortalised hMSCs with inducible SUN2 OE (–) CTS SUN2 OE (+) CTS controls; were normalized to (1 h @ 5 Hz) vs. (control) and induced rescue of SUN2 KD, immediately following 5 Hz CTS. Ratios of nuclear to cytoplasmic areas in immortalised hMSCs with SUN2 OE, SUN2 KD (siRNA2) and SUN2 rescue, immediately following CTS. Ratios were significantly decreased in control cells following CTS (p = 0.03 and p < 0.0001), but not after OE or KD of SUN2. Rescue of SUN2 levels restored the response (p < 0.0001) (see Supplementary Figs. 11g–i). d and e were normalized to (–/−/−) controls; ≥35 cells analysed per condition. f Changes to nuclear texture in immortalised hMSCs following SUN2 OE and CTS (p = 0.04 for CTS vs. unstrained), ≥17 cells analysed per condition. Box-whisker plot center lines show medians, bounds of box show quartiles, whiskers show data spread and outliers determined by the Tukey method; significance determined by Dunnett’s multiple comparison tests. See Supplementary Table 1 for sample sizes.

Fig. 8 Responses to CTS are blocked by KD or OE of SUN2. a SUN2 levels quantified by IF at the NE in primary hMSCs, following SUN2 KD (comparing two siRNA sequences) and in combination with CTS (1 h, 2.6–6.2% strain at 5 Hz; n = 3 donors). Both siRNAs were effective against SUN2 (p < 0.0001). SUN2 levels in the less potent KD (siRNA1) was further decreased by CTS (p < 0.0001); the more efficient KD (siRNA2) showed a small increase (p = 0.04). b Ratios of nuclear to cytoplasmic areas in primary hMSCs following SUN2 KD and CTS (n = 3 donors). SUN2 KD blocked the CTS-induced decrease to ratio (p < 0.0001 for scrambled vs. CTS). c Changes to nuclear texture in primary hMSCs following SUN2 KD and CTS (n = 4 donors). SUN2 KD significantly reduced strain-induced changes in nuclear texture (p < 0.0001 for SUN2 KD vs. scrambled and CTS). Plots show mean ± s.e.m.; p-values determined from linear modeling (ANOVA). See Supplementary Figs. 11a-f for data distributions and variation between donors. d Quantification of SUN2 at the NE in immortalised hMSCs with inducible SUN2 OE (p < 0.0001), SUN2 KD (siRNA2) (p < 0.0001) and induced rescue of SUN2 KD, immediately following 5 Hz CTS. e Ratios of nuclear to cytoplasmic areas in immortalised hMSCs with SUN2 OE, SUN2 KD (siRNA2) and SUN2 rescue, immediately following CTS. Ratios were significantly decreased in control cells following CTS (p = 0.03 and p < 0.0001), but not after OE or KD of SUN2. Rescue of SUN2 levels restored the response (p < 0.0001) (see Supplementary Figs. 11g–i). d and e were normalized to (–/−/−) controls; ≥35 cells analysed per condition. f Changes to nuclear texture in immortalised hMSCs following SUN2 OE and CTS (p = 0.04 for CTS vs. unstrained), ≥17 cells analysed per condition. Box-whisker plot center lines show medians, bounds of box show quartiles, whiskers show data spread and outliers determined by the Tukey method; significance determined by Dunnett’s multiple comparison tests. See Supplementary Table 1 for sample sizes.

Fig. 9 γH2AX staining is increased in SUN2 OE hMSCs subjected to CTS. a The integrated intensity of γH2AX stained foci was significantly decreased in primary hMSCs immediately following CTS (p = 0.03) (2 h, 2.6–6.2% strain at 5 Hz; n = 4 donors). See Supplementary Fig. 12a for data distributions, donor-to-donor variation and representative images. Plot shows mean ± s.e.m. p-values were determined from linear modeling (ANOVA). b γH2AX staining was also significantly decreased in immortalised hMSCs 1 h after CTS (p = 0.0002) (1 h, 2.6–6.2% strain at 5 Hz; ≥29 cells analysed per condition, p-value from two-tailed t-test). c Quantification of γH2AX staining in immortalised hMSCs with inducible SUN2 OE, immediately following CTS (1 h, 2.6–6.2% strain at 5 Hz). CTS significantly increased DNA damage in cells with SUN2 OE (p < 0.0001), ≥27 nuclei analysed per condition. p-values determined by Dunnett’s multiple comparison tests. Box-whisker plot center lines show medians, bounds of box show quartiles, whiskers show data spread and outliers determined by the Tukey method. See Supplementary Table 1 for sample sizes.
multiple comparison tests; Fig. 9c). These results indicated that appropriate levels of SUN2 were essential for the mediation of nuclear decoupling in response to dynamic loading and therefore to afford protection to DNA.

Discussion

We have demonstrated in primary cells from multiple donors that hMSCs have a rapid, structured and reversible response to CTS regulated at the protein level. This response was dependent on both functional ion channels and appropriate levels of the LINC complex protein SUN2 (Fig. 10a). Furthermore, CTS was shown to cause changes within the LINC complex (Fig. 10b), in particular to the regulation of SUN2, enabling cells to decouple nuclear and cellular morphological behaviors and conferring protection to DNA. Robustness is increased through cytoskeletal and nucleoskeletal remodelling in cells that have reached a mechanical equilibrium state on increasingly stiff substrates. However, remodeling of the nuclear lamina seemed less important in the rapid response to high-intensity CTS. Mechano-transmission to the nucleus is an important mode of mechanical transmission to the nucleus is an important mode of mechanical equilibrium state on increasingly stiff substrates.56. Informed written consent was obtained from donors. Experiments followed guidelines and regulations in accordance with the WMA Declaration of Helsinki and the UK Human Tissue Authority. All work was performed with approval from the NHS Health Research Authority National Research Ethics Service (approval number 10/H11013/27) and the University of Manchester. hMSCs were cultured on tissue culture treated polyethylene (TCP) in 1 or 2% FBS DMEM with pyruvate (Thermo Fisher Scientific) supplemented with 10% foetal bovine serum (FBS, Labtech.com) and 1% penicillin/streptomycin cocktail (PS, Sigma-Aldrich). For investigations into the effects of substrate stiffness, hMSCs were seeded onto type-1 collagen coated polyacrylamide gels (2–50 kPa, Matrigen), and cultured in standard medium for three days.

SUN domain-containing protein 2 (SUN2) protein knockdown. hMSCs were incubated in complete medium containing RNAiMax Lipofectamine (Thermo Fisher Scientific) in the presence of short interfering double stranded RNA (siRNA: Thermo Fisher Scientific; final concentration of 10 nM): SUN2 siRNA1 (s24467); sense: GGAAUUCGCAACUAGAAt, antisense: UUCAUGUUGCGGAU UCUCU; SUN2 siRNA2 (s24467); sense: CUCUAGAGCAUGUCCCAAAt, antisense: UUGGCGCAUAGCUCUAAAGAt. A scrambled control was provided by the manufacturer (Thermo Fisher Scientific). Following 24 h of culture, the siRNA and Lipofectamine were removed. Cells were cultured for a further three days in complete media prior to further experimentation or analysis.

Cyclic tensile strain (CTS). CTS was administered to cells using a FlexCell Tension Plus System (FX-4000T or FX-5000T; FlexCell International). Cells (primary hMSCs or transformed immortalised hMSCs, see following descriptions) were cultured for 48 h in the presence of short interfering double stranded RNA (siRNA; Thermo Fisher Scientific; final concentration of 10 nM): - SUN2 siRNA1 (s24467); sense: GGAAUUCGCAACUAGAAt, antisense: UUCAUGUUGCGGAU UCUCU; siRNA 2 (s24467); sense: CUCUAGAGCAUGUCCCAAAt, antisense: UUGGCGCAUAGCUCUAAAGAt. A scrambled control was provided by the manufacturer (Thermo Fisher Scientific). Following 24 h of culture, the siRNA and Lipofectamine were removed. Cells were cultured for a further three days in complete media prior to further experimentation or analysis.

Microscopy and image analysis. Cells were fixed with 4% paraformaldehyde (PFA, VWR International) in PBS for 10 min at RT, followed by 2 × 5 min washes in PBS. Cells were permeabilized using 1% Triton-X (Sigma-Aldrich) in PBS and blocked with 2% bovine serum albumin (RSA, Sigma-Aldrich), 0.25% Triton-X in PBS at RT for 30 mins. Cells were incubated overnight at 4°C with primary antibodies against SUN1 (1:1000; Sigma, HPA008461), SUN2 (1:300; Sigma, HPA001209), LMANA/C (1:200; Santa Cruz Biotechnology, sc-7292), LMNB1 (1:2000; Abcam, ab16048), emerin (1:200; Leica Microsystems, NCL) and phospho-histone H2AX (S139) (γH2AX; 1:300; Merck, 05-636). Following 3 × 5 min PBS washes, cells were incubated with secondary antibodies, specific for mouse or rabbit IgG as appropriate, for 1 h at RT: AlexaFluor-488 goat anti-mouse (1:2000; ThermoFisher Scientific, A11029), AlexaFluor-594 donkey anti-rabbit (1:2000; ThermoFisher Scientific, A21207). Following further 3 × 5 min PBS washes, DAPI (1:1000; Sigma Aldrich, D9542) was used to stain cells at RT for 20 min; when used, AlexaFluor-488 Phalloidin (1:100; Cell Signaling Technology, #8878) was added with the DAPI stain. Samples were washed in PBS for 3 × 5 mins prior to imaging.

Fig. 10 Effects of cyclic tensile strain (CTS) on mesenchymal stem cell (MSC) behavior. a Schematic summarizing the responses of MSCs to low and high intensity CTS, and how these pathways depend on ion channel activity and SUN domain-containing protein 2 (SUN2). b Cartoon summarizing strain-induced changes to proteins in the linker of nucleoskeleton and cytoskeleton (LINC) complex and nuclear envelope (NE). ONM outer nuclear membrane; INM inner nuclear membrane.
Images were captured on a Leica TCS SP5 or SP8 confocal microscope using HCX Apo U-100×/0.5 or HCX Apo U V 63×/0.95 dipping lenses. Images were collected in both channels simultaneous with the same excitation filters and with equal gains. The white light source was filtered for excitation at 488 and 543 nm and a UV laser for excitation at 405 nm. The microscope used LAS X software (version 3.5, Leica); images were processed in ImageJ (version 2.0.0, National Institutes of Health, USA); CellProfiler (version 2.1.1, Broad Institute, USA) was used to characterize cell morphometric parameters: cell area; cell perimeter; nuclear area; ratio of cytoplasmic to nuclear area. For quantification of proteins at the nuclear envelope (NE), the integrated fluorescence intensity was measured at the nuclear periphery, as identified by DAPI staining. Telomerase activity was assessed by examining images at 6x. Telomerase activity was described above and CellProfiler was used to quantify nuclear texture (Haralick texture features; sum variance scale 3; offset set to zero). For quantification of DNA damage, nuclei were imaged at x63 and the integrated fluorescence intensity of nuclear phospho-histone H2AX foci was quantified for each nucleus. Images were corrected for background fluorescence by subtracting the mean intensity of a cell-free area from each pixel; all images under comparison in the same experiment had matched exposure and contrast settings.

Cell proliferation and viability assays. Cell culture medium was removed from hMSCs immediately or 24 h after CTS (1 h at 1 or 5 Hz) and cells washed with PBS. Cells were lysed using 3× freeze/thaw cycles in PBS and lysates cleared using centrifugation at 1,2000 × g for 10 min. Double-strand DNA concentration from each well was quantified using Quant-It PicoGreen Assay (Thermo Fisher Scientific), as described in the manufacturer’s instructions. Telomerase activity was assessed by examining images at 6x. Cell proliferation and viability assays were imaged at 6x. hMSCs were then labelled by incubation with 2 mL of 400 µM monobromobimane (mBBr; Sigma-Aldrich) in PBS at 37 °C for 10 min. Following labelling, 50 µL of 0.4 M glutathione in PBS was added to each well to quench the mBBr reaction. The quenched mBBr solution was removed and cells washed with PBS. Cells were detached from the substrate by incubating with 1 mL of trypsin at 37 °C for 10 min. Trypsin activity was neutralized using serum-containing culture medium and cells pelleted using centrifugation at 400 × g for 5 min. Cells were resuspended in cold PBS, re-pelleted in 1.5 mL tubes (LoBind, Eppendorf) at 400 × g for 5 min and cell pellets stored at −20 °C prior to proteomic analysis.

Mass spectrometry (MS) sample preparation and analysis. Six 1.6 mm beads (Next Advance) were added to the cell pellet tube with 30 µL of SL-DOC (1.1% sodium dodecyl sulfate (Sigma), 0.3% glycerol (Sigma), 350 µM ammonium bicarbonate (AB, Fluka), protease inhibitor cocktail (Sigma), sodium fluoride (Sigma), and sodium orthovanadate (Sigma) in de-ionized (DI) water). Cells were homogenized in a Bullet Blender (Next Advance) at maximum speed for 2 min. Homogenates were cleared by centrifugation (12 °C, 10,000 × g, 5 min). Cells were washed with 350 µL of RW1 buffer using centrifugation (12,000 × g, 5 min). Cells were then re-suspended in 150 µL of digest buffer (1.33 mM CaCl2, Sigma in 25 mM AB) and 50 µL of protein lysate and shaken overnight at 37 °C. The resulting digest was then reduced (addition of 4 µL of 500 mM dithiothreitol (Sigma) in 25 mM AB; 10 min. shaking at 60 °C) and alkylated (addition of 12 µL of 500 mM iodoacetamide (Sigma) in 25 mM AB; 30 min at 37 °C). Peptides were acidified by adding trifluoroacetic acid (Riedel-de Haen) in DI water, and cleaned by two-phase extraction (2 × addition of 200 µL ethyl acetate (Sigma) followed by vortexing and aspiration of the organic layer). Peptides were desalted, in accordance with the manufacturer’s protocol, using POROS R3 beads (Thermo Fisher) and hichloroform/Dichloromethane (9 : 1, v/v) in injection buffer (5% HPLC grade acetonitrile (ACN, Fisher Scientific) 0.1% trifluoroacetic acid (TFA) in DI water) were adjusted to 300 ng/µL. For HMSCs subjected to 5.0 Hz CTS, digested samples were analysed by LC-MS/ MS using an UltiMate 3000 Rapid Separation LC (RSLC; Dionex Corporation, Sunnyvale, CA) coupled to an Orbitrap Elite (Thermo Fisher Scientific, Waltham, MA) mass spectrometer. Peptide mixtures were separated using a gradient from 92% A (0.1% formic acid, FA (Sigma) in deionized water) and 8% B (0.1% FA in ACN) to 33% B, in 104 min at 300 nL per min, using a 75 mm × 250 µm inner diameter 1.7 µm CSH C18, analytical column (Waters). For analysis of SUN2 knockdown samples, LC-MS/MS analysis was performed by the Genomic Technologies Core (Illumina). Data produced by an Illumina HiSeq4000 system was analysed with TopHat (version 2.0.0, Broad Institute, USA) and only matches with the best score were reported for each read. The mapped reads were counted with genes with HTSeq87 with count method set to “union”. Log-transformed transcript fold changes were normalized under the assumption that the majority of genes were not perturbed by any of the experimental conditions.

**Proteomics data processing.** MS spectra from multiple samples were aligned using Progenesis QI (version 4.1, Nonlinear Dynamics) and searched using Mascot (Matrix Science UK), against the SWISS-Prot and TREMBL human databases. Samples were not enriched for PTMs prior to MS (e.g. by affinity column), but MS spectra of samples from a SUN2 over-expressing immortalised cell line and primary MSCs subjected to TS were enriched with 10 µM phosphopeptide enrichment reagent (PTEMS to SUN2. The peptide database was modified to search for alkylated cysteine residues (monosotopic mass change, 57.021 Da), oxidized methionine (15.995 Da).
hydroxylation of asparagine, aspartic acid, proline or lysine (15.995 Da) and phosphorylation of serine, tyrosine, threonine, histidine or aspartate (79.966 Da). In experiments in which these sites were included with mBRB, the modification was searched for in two possible oxidation states (133.053 and 150.056 Da). A maximum of 2 missed cleavages was allowed. Peptide detection intensities were exported from Progenesis QI as Excel spreadsheets (Microsoft) for further processing.

Proteomics datasets were analysed using code written in-house in Matlab with the bioinformatics toolbox (R2015a, The MathWorks, USA). Raw ion intensities from peptides with fewer than 3 unique peptides per protein were excluded from quantification. Peptide lists were filtered leaving only those peptides with a Mascot score corresponding to a Benjamini–Hochberg false discovery rate (BH-FDR) of ≤0.2. Normalization was performed as follows: raw peptide ion intensities were log-transformed to ensure a normal distribution and normalized within-sample by equalizing sample medians (subtracting sample median). Fold-change differences in the quantity of proteins detected in different samples were calculated by fitting a linear regression model that considers donor variability at both the peptide and protein levels. For each protein the following model was fit:

$$y_{\text{gd}} = \beta_0 + X_p \beta_1 + X_p \beta_2 + X_p \beta_3 + X_p \beta_4 + X_p \beta_5 + t_{\text{gd}}$$

(1)

Where $y_{\text{gd}}$ represents the logged peptide intensity for peptide $i$, obtained under experimental treatment $g$, from donor $d$. $\beta$ represents the parameters to be estimated by the model fit, with $\beta_2$ and $\beta_3$ representing the logged fold change in a given protein and peptide $i$, caused by experimental treatment $g$. Where peptide $i$ had been subject to post-translational modification (e.g., phosphorylation or reaction with mBRB), $\beta_2$ would therefore give a measure of the fold-change in site occupancy (i.e. the fraction of the protein present that has been modified). $\beta_0$ is the intercept term that correlates with mean peptide intensity and $t_{\text{gd}}$ corresponds to a Gaussian error term centered on 0 with a width equal to the residual variance, $\sigma^2$. The model was fit using a Bayesian elastic net implemented using a Monte Carlo Markov Chain Gibbs sampler. Additionally, weights were weighted according to the Mascot score, each iteration of the Gibbs’s sampler such that outlier observations would not have their contribution to the model diminished if confidence in their identification was high. The full formulation and implementation are described in Mallikarjun et al.36. Standard error estimates were modified according to empirical Bayes correction of variances and empirical Bayes-modified t-tests were used to compare different experimental conditions. Linear modeling for Reactome pathway analysis was performed as described above using logged fold changes as the response variable according to the model for a given Reactome pathway:

$$y_{\text{gd}} = \beta_0 + X_p \beta_1 + X_p \beta_2 + \epsilon_{\text{gd}}$$

(2)

Where $\beta_1$ and $\beta_2$ denote effect sizes due to experimental treatment $g$ and protein $p$. False positive correction of $\pi$-values for differential abundance was performed using Benjamini–Hochberg correction. Gene Ontology (GO) analysis was performed using Gorilla32. Resulting GO term lists were cleaned of redundant terms using REVIGO35. For cluster analysis, pathway enrichment was detected using the ReactomeFDR tool with a false discovery rate (BH-FDR) <0.05 and ≥2 entities associated. For analysis of phospho-proteomics, highlighted sites are those significantly differentially regulated (Benjamini–Hochberg FDR < 0.05) and observed previously by comparison to the PhosphoSitePlus database70.

Small molecule inhibition of ion channel activity. hMSCs were cultured in complete medium containing inhibitors against stretch-activated ion channels 10 min prior to, and throughout, strain treatment, with vehicle only used as a control. Gadolinium chloride (GdCl3; Sigma), dissolved in water and used at a final concentration of 10 µM (determined from a dose response experiment assessing its effects on nuclear morphology), was used as a broad-spectrum inhibitor against all stretch-activated ion channels27. Amlodipine (Sigma), dissolved in DMSO and used at a final concentration of 100 µM (determined from a dose response experiment assessing its effects on nuclear morphology), was used as a broad-spectrum inhibitor against all channel types using REVIGO35. For cluster analysis, pathway enrichment was detected using the ReactomeFDR tool with a false discovery rate (BH-FDR) <0.05 and ≥2 entities associated. For analysis of phospho-proteomics, highlighted sites are those significantly differentially regulated (Benjamini–Hochberg FDR < 0.05) and observed previously by comparison to the PhosphoSitePlus database36.

Overexpression of SUN2 in an immortalised hMSC line. The open reading frame of human SUN2 (isoform 2) was cloned into pCMN_HetOn, which contains a Tet response element enabling doxycycline-controlled expression of SUN2 protein. SUN2 (open reading frame); sense: AGACCTACGGCGACATTTCCA, antisense: AACTCCACATTTCCGCAACGCCGCTCACGGCGGCTAC, sequence GGCTACGTAGGCGGGCG GCTCCCATGCTCTTCA; Lentivirus was made by transfecting HEK 293 T cells in a 75 flask, with a complete 4.5 µg of PAP2 packaging vector, 3 µg of pMD2G packaging vector and 6 µg of pCDH_TetOn_Sun2 combined, with polyethylenimine (PEI) (1 µg mL−1) at a ratio of 1:2 DNA:PEI in DMEM medium and incubated at 37 °C overnight. The transfection media was removed and replaced with complete medium containing sodium butyrate 10 mM (HDAC inhibitor) for 8 h, followed by replacement with complete medium. Following an additional 24 h culture in complete medium the virus containing media was removed, filtered through a Millipore Membrane 0.45 µm syringe filter (Merck) and stored at 4 °C, with fresh complete medium added to the HEK 293 T cells. Following an additional 24 h culture period, the second harvest of virus containing media was collected, filtered through a Millipore Membrane 0.45 µm syringe filter, combined with the first viral harvest and concentrated using a VacuPrep 20, 100 kDa MWCO PES column (Sartorius). Concentrated virus was aliquoted into 4× vials and stored at −80 °C until required.

The virus was used to transform immortalised hMSCs (line Y20149). The virus was used to add 2 mL of complete medium containing polybrene 8 µg/mL (Sigma) and added to 40% confluent Y201 hMSCs seeded in a 10 cm² dish. After 48 h of culture the virus containing media was removed and cells washed in complete medium twice. Cells were grown in culture for two weeks to allow transient viral expression to subside, then FACS sorted for blue fluorescent protein positive cells containing stable viral insertions.

Cells containing the inducible SUN2 expression vector were cultured for 4 days in complete medium containing 50 ngmL−1 doxycycline (DOX, Sigma). Following DOX treatment, cells were cultured on either standard polystyrene tissue culture plastic, or type-I collagen coated BioFlex plates, prior to treatments and morphometric characterizations as described above. The length of time cells were cultured following DOX treatment was used to modulate levels of SUN2 expression. Non-transformed cells treated with 50 ngmL−1 DOX were used as controls. For SUN2 rescue experiments, cells containing the inducible SUN2 expression vector were first treated with siRNA against SUN2 (siRNA2) as described above, with DOX then added for a further 4 days of culture. SUN2 rescue cells were then cultured on either standard polystyrene tissue culture plastic, or type-I collagen coated BioFlex plates, and morphometric analysis conducted.

Chromatin condensation assay. Primary hMSCs were cultured on type-I collagen coated BioFlex plates. They were treated for 30 min with complete medium containing MgCl2 and CaCl2 (both Sigma) at 2 mM final concentrations. Cells were fixed in 4% PFA, stained with DAPI (Sigma) for 20 min and imaged as described above.

Statistical treatments and linear modeling. Statistical tests were performed in Mathematica (version 11, Wolfram Research), MatLab (version R2015a, MathWorks) and GraphPad (version 8, Prism). All tests were two-tailed. Evaluations of R-squared and graphical analyses were performed using Igor Pro (version 6.37, WaveMetrics). Linear regression analysis was performed on imaging data where indicated, using the formula:

$$y_{\text{gd}} = \beta_0 + X_p \beta_1 + X_p \beta_2 + \epsilon_{\text{gd}}$$

(3)

Where $y_{\text{gd}}$ represents a vector of normalized intensity data for each cell from experimental treatment $g$ and donor $d$. $\beta$ represent fold change due to experimental treatment group $g$ and donor $d$ to be estimated by the model fit. $\beta_0$ represents the intercept term determined by the mean of $y$. Linear modeling was performed in Matlab using the fitlm function.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the identifiers: PXD013863, PXD012873, PXD012948, PXD012949 and PXD003287. RNA-Seq data is available via EMBL-EBI ArrayExpress with identifier E-MTAB-7925.

Code availability

The BayesENproteomics code used to process MS data36 is available to download from GitHub [https://www.github.com/VenkMallikarjun/BayesENproteomics].

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Author contributions

Investigation, H.T.J.G., V.M., O.D., R.P., M.R.J and J.S.; Formal Analysis, H.T.J.G., V.M., and J.S.; Writing – Original Draft, H.T.J.G.; Writing – Visualization, Review & Editing, H.T.J.G., V.M., O.D., M.R.J, R.P., A.P.G., SM.R and J.S.; Project Administration and Funding Acquisition, J.S.

Additional information

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