SUPPLEMENTAL FIGURES

Lagrange strain field during impact

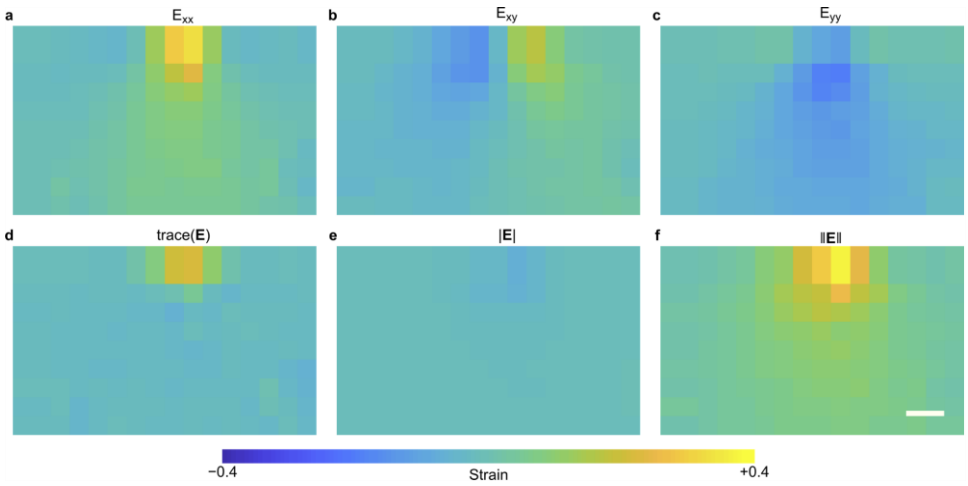

Fig S1. Average components and scalar measures of the Lagrange strain during impact. Strain fields are shown for the various strain measures tested, including: (a) Exx, the axial strain in the lateral direction, (b) Exy, the shear strain, (c) Eyy, the axial strain in the depth direction (parallel to the impact), (d) the trace of the strain tensor (first invariant), (e) the determinant of the strain tensor (second invariant), and (f) the spectral norm of the strain tensor. These strain measures were derived from the average 2D Lagrange strain tensor in each spatial bin at peak indentation during impact. Scale bar indicates 200 μm.

Correlations between cellular dysfunction and strain over time

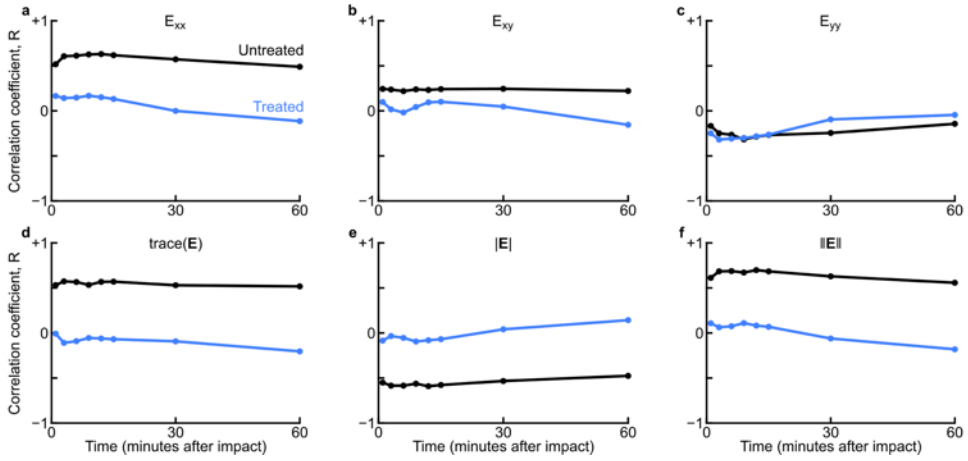

Fig S2. Correlations between fractional depolarization and strain are much weaker after treatment but are consistent over time. Each plot shows the correlation coefficient between the average fraction of depolarized cells in each spatial bin over time and a corresponding strain measure, including: (a) Exx, the axial strain in the lateral direction, (b) Exy, the shear strain, (c) Eyy, the axial strain in the depth direction (parallel to the impact), (d) the trace of the strain tensor (first invariant), (e) the determinant of the strain tensor (second invariant), and (f) the spectral norm of the strain tensor. These strain measures were derived from the average 2D Lagrange strain tensor in each spatial bin at peak indentation during impact and were correlated with the average fraction of depolarized cells in that same spatial bin. In general, the correlation is consistent over time and treatment reduces the correlation (R closer to zero). Additionally, the correlation is strongest for the strain norm, which represents the overall magnitude of strain at a given location.

Validation of experimental method and mitochondrial polarity assay

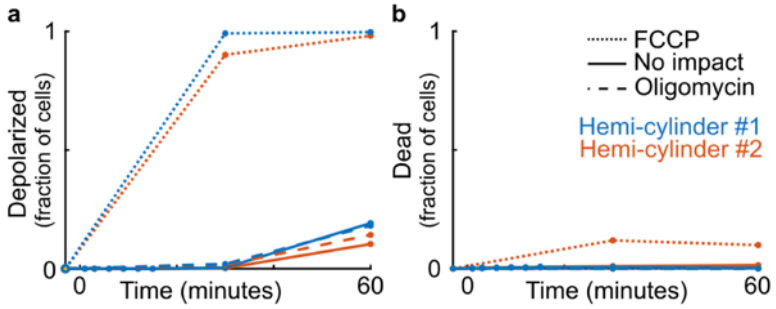

Fig S3. Validation of experimental method and mitochondrial polarity assay. (a) Repeating the experimental method with no treatment and no impact on either hemi-cylinder showed low, but non-zero mitochondrial dysfunction over time, reflecting systematic effects inherent to the method (solid lines). The mitochondrial polarity imaging assay was validated by repeating the experiment (no impact and no treatment) with the addition of either FCCP (dotted lines) or oligomycin (dashed lines) at time zero. (a) As expected, nearly all observed chondrocytes showed depolarization when exposed to FCCP, while chondrocytes exposed to oligomycin retained full mitochondria polarity, paralleling the results in for the no-impact group. (b) In all cases, there was negligible cell death, as expected. The three treatments (no impact, FCCP, and oligomycin) reflect results from three explants taken from three separate animals. For each treatment (i.e. for each animal), lines are shown for both the first and second halves of the associated cylindrical explant, in blue and orange, respectively.

SUPPLEMENTAL METHODS

Data availability

The data and associated analysis code that support the findings of this study are available in figshare: doi.org/10.6084/m9.figshare.6349205.v1.

Image Analysis of Cellular Function

Confocal images at all locations and time points were analyzed to extract individual-cell behavior over time. This analysis included three steps: registration, segmentation, and classification, all of which were performed using MATLAB (The MathWorks, Waltham, MA). Density estimates were then used to compute local variations in the fraction of cells in each state.

Registration. Confocal images at each imaging location were registered over time to correct for drift in the microscope stage. Intensity-based registration was performed between adjacent time points, allowing for rigid-body translation only. All images were then transformed back to the first time point's frame of reference and regions in the newly-transformed space that no longer had complete time information were discarded (i.e. regions of the sample that move out of the field of view).

Segmentation. Images at the first time point were segmented to locate individual cells. Segmentation was performed using a previously-described algorithm based on the watershed transform [74], using parameters that were chosen to match the manually-counted cell density in a test image: equalization clip limit: 0.01; background size: 23 px; median size: 7 px; Gaussian radius: 9 px; minimum area: 300 px^2; maximum area: 5000 px^2; minimum signal: 0.1, i.e. 10% of

full range. The resulting segmentation defines regions (i.e. cells) at the first time point. These same regions were also valid at all subsequent time points, since images had been transformed back to the first time point and chondrocytes are not mobile in their native tissue environment.

Classification. Each identified cell was analyzed at each time point to classify it into one of three possible states: alive with polarized (i.e. functional) mitochondria, alive with depolarized mitochondria, and dead. For classification, scalar features were calculated for each cell at each time point and these features were then thresholded. Given the distribution of pixel intensities inside each cell region at a given time point, the following features were calculated: the 96th percentile of blue pixel values ($B_t^{(96)}$), the 88th percentile of red pixel values ($R_t^{(88)}$), and the relative change in the 90th percentile of the red pixel values ($\bar{R}_t^{(90)} = \left(R_t^{(90)} - R_0^{(90)}\right)/R_0^{(90)}$), where $A_j^{(i)}$ is the i^{th} percentile of pixel values in channel A at time j. The corresponding thresholds were $T_1 = 63$, $T_2 = 27$, and $T_3 = -0.4$ (i.e. 40% loss), respectively, where pixel values were 8-bit (i.e. ranging from 0 to 255). At the first time point, cells with $B_0^{(96)} \leq T_1$ and $R_0^{(88)} > T_2$ (i.e. without Sytox Blue staining and with strong TMRM staining) were marked as alive with polarized mitochondria. All cells not meeting this criterion at the first time point (i.e. cells that were dead or depolarized at the start of the experiment) were marked as invalid and not analyzed further. Within the cells that were alive and polarized at the start of the experiment, those with $B_t^{(96)} > T_1$ were marked as dead. Within the remaining cells that were alive and polarized at the start of the experiment and also not dead at time *t*, those with $\bar{R}_t^{(90)} < T_3$ (i.e. those that had lost more than 40% of their original TMRM signal) were marked as alive with depolarized mitochondria. These features, the percentile levels, and their thresholds were all chosen to optimize the classification accuracy, as calculated from the confusion matrix produced

by applying the classification to a subset of 546 cells that had been manually classified. Notably, the relative change in red signal (i.e. TMRM signal) over time was a better classification metric for loss of mitochondrial polarity than measures of the absolute intensity. This is intuitive, since even normal chondrocytes may have varying number of mitochondria with varying levels of polarity. As such, the relative loss of TMRM signal would be a better indicator of within-cell mitochondrial changes than the absolute intensity at a given time point. This observation reinforces the importance of following individual cells over time, rather than measuring population-averaged metrics at each time point.

Density estimates. The fraction of cells in each state was computed on a 100 μm spatial grid relative to the impact location. For each sample, Gaussian kernel density estimates were computed at each grid point, to determine the local number of cells in each state (polarized, depolarized, dead), as well as the total number of cells. Fractional state occupancy was computed by dividing by the total cell density, and areas that had less than 30 cells/mm^2 total were discarded, in order to ensure adequate counting statistics. To compute average temporal trends, as shown in Figure 3, cell state data was averaged using cells within 212 μm of the impact laterally and 1000 μm deep.

Validation of Experimental Setup and Cellular Function Assay

In order to test the baseline level of cellular dysfunction arising due to systematic and environmental conditions, a no-impact control experiment was performed. For this variant, one pair of hemi-cylindrical samples was prepared, imaged, and analyzed as described in *Methods*, except neither sample was impacted (from N=1 additional animal). In this situation, results showed negligible cell death and minimal depolarization (Supplemental Fig S3, solid lines). This

confirms that the experimental conditions induce minimal cellular dysfunction and thus the main results of this study are not masked by environmentally-induced changes.

In order to validate the three-stain assay used to assess cellular function, two comparison experiments were performed. For each of these comparisons, a pair of hemi-cylinders was prepared as described above, except neither sample was impacted (from N=2 additional animals). Images were collected at 0, 30, and 60 minutes and analyzed as described in *Methods*. In the first comparison, 4 µM FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Sigma Aldrich, St. Louis, MO) was added to the impactor's fluid bath immediately after the first imaging time point. FCCP is an ionophore that causes mitochondrial depolarization by dissipating the proton gradient (transporting protons) across the inner mitochondrial membrane. For the second comparison, 2 µM oligomycin (Sigma Aldrich) was added immediately after the first imaging time point. Oligomycin causes hyperpolarization of the inner mitochondrial membrane by blocking the proton channel subunit of ATP synthase. As expected, after adding FCCP to the bath, nearly all of the cells were classified as alive with depolarized mitochondria (Supplemental Fig S3, dotted lines). After oligomycin, nearly all cells remained alive and polarized (Supplemental Fig S3, dashed lines), following the same trend observed in the no-impact control. In both cases, there was negligible cell death throughout the experiment, as expected. These comparisons confirm that the staining assay and analysis procedure used in this study can appropriately capture changes in mitochondrial polarity over time.

Transmission Electron Microscopy

Millimeter-sized cubes of cartilage were micro-dissected from approximately 500 µm below the impact location and 1-2 mm away from the hemicylinder's cut surface. These samples were

transferred to a petri dish on ice containing 2% Gluteraldehyde in 0.05M cacodylate buffer and fixed for 2 hours. Samples were then rinsed in cacodylate buffer and placed in 1% Osmium Tetraoxide for one hour on ice. Samples were rinsed and dehydrated in graded (increasing) concentrations of Ethanol (25%, 50%, 70%, 95%, 100%), stained in 2% Uranyl Acetate in ethanol for 2 days, then placed in anhydrous acetone prior to stepwise embedding in 25%, 50%, 75% then 100% epoxy (EMbed 812, Electron Microscopy Sciences, Hatfield, PA). Finally, samples were sectioned and imaged on an FEI Tecnai 12 BioTwin Transmission Electron Microscope (ThermoFisher Scientific, Waltham, MA) at 120kV, and 4008×2672 digital images were captured using a Gatan Orius® 1000 dual-scan CCD camera (Roper Technologies, Sarasota, FL), using Digital Micrograph (Gatan, Inc., Pleasanton, CA) software.

Statistical Models

Various linear mixed-effects models were fit to the data to test for significant trends and difference between testing conditions. In all cases, models were fit and reduced in MATLAB using `fitlme` and further statistical contrasts and associated p-values were computed using the method `coefTest`. All models included a random effect of source animal on the intercept. Residuals were checked for normality and homogeneity and response variables were transformed to ensure normal residuals. Sample treatment condition and impact condition were encoded as binary variables. Time was also treated as a categorical variable, including time points #2-9, corresponding to 0 through 60 minutes after impact. The time point collected before impact was excluded because all tracked cells were alive and functional at that time, by definition. The details of each model are specified below.

Mixed-effects model of mitochondrial dysfunction over time, impact, and treatment

A mixed-effects linear model was fit to test for significant differences in fractional mitochondrial dysfunction over time, impact condition, and treatment condition, as shown in Figure 3a. This mixed-effects model is detailed in Supplementary Table 1 and was fit by the equation:

$$(fraction\ depolarized)^{\frac{1}{5}} = 1 + treated \times impacted + treated \times time + impacted \times time + treated \times impacted \times time + (1|animal)$$

Coefficient name	Coefficient estimate	Standard Error	t Statistic	DOF	p Value	95% CI Lower	95% CI Upper
(Intercept)	0.022	0.051	0.4	4632	6.7E-01	-0.078	0.122
'treated'	-0.061	0.077	-0.8	4632	4.2E-01	-0.212	0.089
'impacted'	0.635	0.023	27.9	4632	4.9E-158	0.591	0.680
'time_3'	0.016	0.021	0.8	4632	4.5E-01	-0.026	0.058
'time_4'	0.023	0.021	1.1	4632	2.8E-01	-0.019	0.065
'time_5'	0.103	0.021	4.8	4632	1.5E-06	0.061	0.144
'time_6'	0.129	0.021	6.1	4632	1.5E-09	0.087	0.171
'time_7'	0.141	0.021	6.6	4632	3.5E-11	0.100	0.183
'time_8'	0.226	0.021	10.6	4632	5.8E-26	0.184	0.267
'time_9'	0.500	0.021	23.5	4632	1.3E-115	0.459	0.542
'treated:impacted'	-0.457	0.034	-13.5	4632	1.1E-40	-0.524	-0.391
'treated:time_3'	-0.016	0.033	-0.5	4632	6.2E-01	-0.080	0.048
'treated:time_4'	-0.023	0.033	-0.7	4632	4.8E-01	-0.087	0.041
'treated:time_5'	-0.103	0.033	-3.2	4632	1.6E-03	-0.166	-0.039
'treated:time_6'	-0.129	0.033	-4.0	4632	7.6E-05	-0.193	-0.065
'treated:time_7'	-0.097	0.033	-3.0	4632	2.8E-03	-0.161	-0.034
'treated:time_8'	0.031	0.033	1.0	4632	3.4E-01	-0.033	0.095
'treated:time_9'	-0.022	0.033	-0.7	4632	4.9E-01	-0.086	0.042
'impacted:time_3'	0.046	0.032	1.4	4632	1.5E-01	-0.017	0.109
'impacted:time_4'	0.055	0.032	1.7	4632	8.9E-02	-0.008	0.118
'impacted:time_5'	0.010	0.032	0.3	4632	7.6E-01	-0.053	0.073
'impacted:time_6'	-0.025	0.032	-0.8	4632	4.3E-01	-0.088	0.038
'impacted:time_7'	-0.034	0.032	-1.1	4632	2.9E-01	-0.098	0.029
'impacted:time_8'	-0.089	0.032	-2.8	4632	5.6E-03	-0.152	-0.026
'impacted:time_9'	-0.324	0.032	-10.1	4632	1.5E-23	-0.387	-0.261
'treated:impacted:time_3'	0.089	0.048	1.9	4632	6.4E-02	-0.005	0.183
'treated:impacted:time_4'	0.110	0.048	2.3	4632	2.1E-02	0.017	0.204
'treated:impacted:time_5'	0.172	0.048	3.6	4632	3.2E-04	0.078	0.266
'treated:impacted:time_6'	0.217	0.048	4.5	4632	5.8E-06	0.123	0.311
'treated:impacted:time_7'	0.199	0.048	4.2	4632	3.2E-05	0.105	0.293
'treated:impacted:time_8'	0.150	0.048	3.1	4632	1.7E-03	0.056	0.244
'treated:impacted:time_9'	0.288	0.048	6.0	4632	1.9E-09	0.194	0.382

Table S1. Mixed-effects model of the fractional depolarization over time, impact condition, and treatment condition. Each row corresponds to one coefficient in the full model. This model was fit to the data shown in Figure 3a and used compute associated statistical comparisons.

Mixed-effects model of cell death over time, impact, and treatment

A mixed-effects linear model was fit to test for significant differences in fractional cell death over time, impact condition, and treatment condition, as shown in Figure 3b. This mixed-effects model is detailed in Supplementary Table 2 and was fit by the equation:

$$(fraction\ dead)^{\frac{1}{10}} = 1 + treated \times impacted + treated \times time + impacted \times time + (1|animal)$$

Coefficient name	Coefficient estimate	Standard Error	t Statistic	DOF	p Value	95% CI Lower	95% CI Upper
(Intercept)	0.079	0.048	1.7	4639	9.9E-02	-0.015	0.173
'treated'	-0.098	0.071	-1.4	4639	1.7E-01	-0.238	0.041
'impacted'	0.095	0.020	4.9	4639	1.2E-06	0.057	0.133
'time_3'	-0.008	0.021	-0.4	4639	7.1E-01	-0.049	0.033
'time_4'	0.001	0.021	0.0	4639	9.7E-01	-0.040	0.042
'time_5'	0.059	0.021	2.8	4639	5.1E-03	0.018	0.100
'time_6'	0.067	0.021	3.2	4639	1.4E-03	0.026	0.108
'time_7'	0.067	0.021	3.2	4639	1.5E-03	0.026	0.108
'time_8'	0.078	0.021	3.7	4639	1.9E-04	0.037	0.119
'time_9'	0.067	0.021	3.2	4639	1.5E-03	0.026	0.108
'treated:impacted'	-0.082	0.013	-6.1	4639	1.4E-09	-0.108	-0.055
'treated:time_3'	0.023	0.026	0.9	4639	3.9E-01	-0.029	0.074
'treated:time_4'	-0.001	0.026	0.0	4639	9.8E-01	-0.052	0.051
'treated:time_5'	-0.057	0.026	-2.2	4639	2.9E-02	-0.109	-0.006
'treated:time_6'	-0.070	0.026	-2.7	4639	7.9E-03	-0.121	-0.018
'treated:time_7'	-0.065	0.026	-2.5	4639	1.3E-02	-0.117	-0.014
'treated:time_8'	-0.023	0.026	-0.9	4639	3.9E-01	-0.074	0.029
'treated:time_9'	0.005	0.026	0.2	4639	8.5E-01	-0.047	0.056
'impacted:time_3'	0.121	0.026	4.6	4639	4.4E-06	0.069	0.172
'impacted:time_4'	0.225	0.026	8.6	4639	1.2E-17	0.174	0.277
'impacted:time_5'	0.238	0.026	9.1	4639	1.5E-19	0.187	0.290
'impacted:time_6'	0.288	0.026	11.0	4639	1.0E-27	0.237	0.339
'impacted:time_7'	0.295	0.026	11.2	4639	6.6E-29	0.243	0.346
'impacted:time_8'	0.325	0.026	12.4	4639	1.0E-34	0.274	0.376
'impacted:time_9'	0.334	0.026	12.7	4639	1.6E-36	0.283	0.385

Table S2. Mixed-effects model of the fractional cell death over time, impact condition, and treatment condition. Each row corresponds to one coefficient in the full model. This model was fit to the data shown in Figure 3b and used compute associated statistical comparisons.

Mixed-effects model of mitochondrial dysfunction over time, impact strain norm, and treatment

A mixed-effects linear model was fit to test for significant differences in fractional mitochondrial dysfunction over time, impact strain norm, and treatment condition, as shown in Figure 4. This model was fit using data from impacted samples only, because no-impact samples have zero strain, by definition, and thus the two variables are not independent. This mixed-effects model is detailed in Supplementary Table 3 and was fit by the equation:

$$(fraction\ depolarized)^{\frac{1}{2}} = 1 + time + treated \times strain + (1|animal)$$

Coefficient name	Coefficient estimate	Standard Error	t Statistic	DOF	p Value	95% CI Lower	95% CI Upper
(Intercept)	0.036	0.048	0.7	5709	4.6E-01	-0.059	0.130
'treated'	-0.002	0.071	0.0	5709	9.8E-01	-0.142	0.138
'strain'	2.440	0.061	39.9	5709	8.0E-308	2.321	2.560
'time_3'	0.056	0.012	4.8	5709	1.3E-06	0.033	0.078
'time_4'	0.071	0.012	6.1	5709	9.1E-10	0.048	0.093
'time_5'	0.094	0.012	8.1	5709	4.5E-16	0.071	0.116
'time_6'	0.100	0.012	8.7	5709	5.1E-18	0.077	0.123
'time_7'	0.113	0.012	9.8	5709	1.9E-22	0.090	0.135
'time_8'	0.195	0.012	17.0	5709	5.4E-63	0.173	0.218
'time_9'	0.316	0.012	27.5	5709	6.3E-156	0.294	0.339
'treated:strain'	-2.371	0.090	-26.2	5709	2.8E-143	-2.548	-2.194

Table S3. Mixed-effects model of the fractional mitochondrial dysfunction over time, impact strain norm, and treatment condition. Each row corresponds to one coefficient in the full model. This model was fit to the data shown in Figure 3 and Figure 4 and used compute associated statistical comparisons.