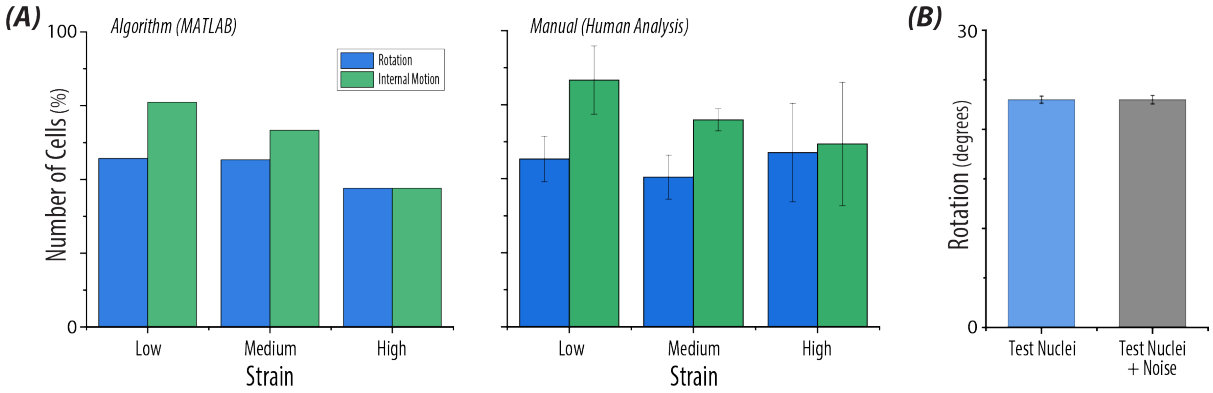
SUPPLEMENTAL INFORMATION

Dynamic Biophysical Responses of Neuronal Cell Nuclei and Cytoskeletal Structure Following High Impulse Loading

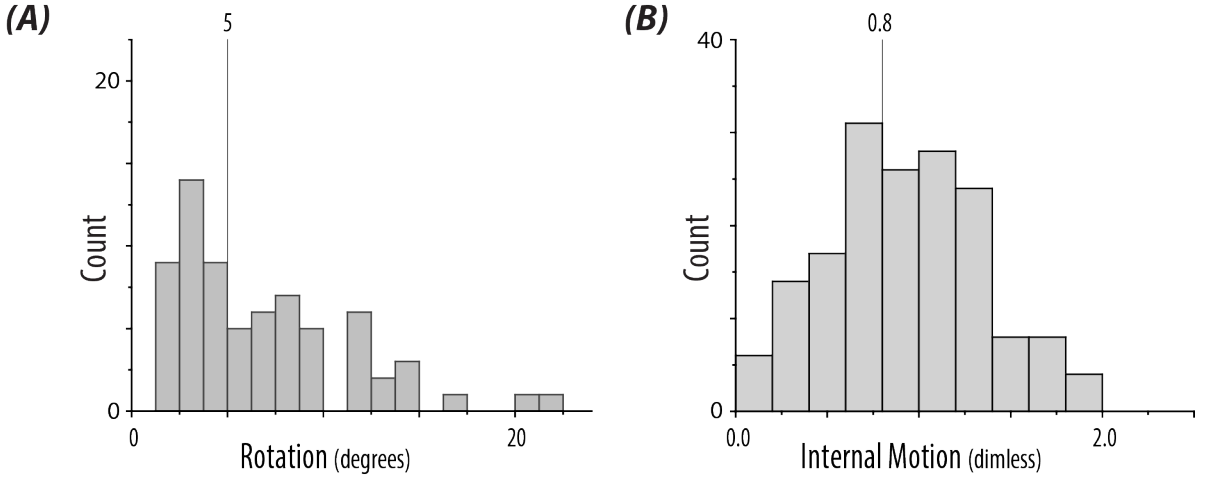
*Stephanie E. Schneider [1], Adrienne K. Scott [1], Benjamin Seelbinder [1], Courtney Van Den Elzen [2], Robert L. Wilson [1], Emily Y. Miller [3], Quinn I. Beato [1], Soham Ghosh [1,4,5], Jeanne E. Barthold [1], Jason Bilyeu [1], Nancy C. Emery [2], David M. Pierce [6,7], Corey P. Neu [1,3,8,]**

[1] Paul M. Rady Department of Mechanical Engineering, University of Colorado Boulder, Boulder, CO
[2] Department of Ecology and Evolutionary Biology, University of Colorado Boulder, Boulder CO
[3] Biomedical Engineering Program, University of Colorado Boulder, Boulder, CO
[4] Department of Mechanical Engineering, Colorado State University, Fort Collins, CO
[5] School of Biomedical Engineering, Colorado State University, Fort Collins, CO
[6] Department of Mechanical Engineering, University of Connecticut, Storrs, CT
[7] Department of Biomedical Engineering, University of Connecticut, Storrs, CT
[8] BioFrontiers Institute, University of Colorado Boulder, Boulder, CO
** Corresponding Author*

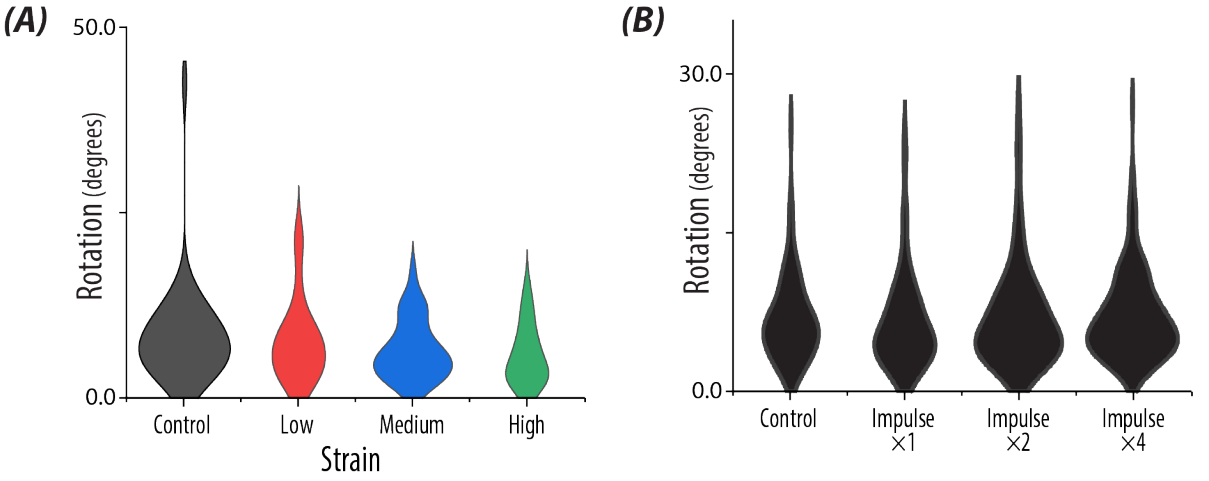
SUPPLEMENTAL FIGURES



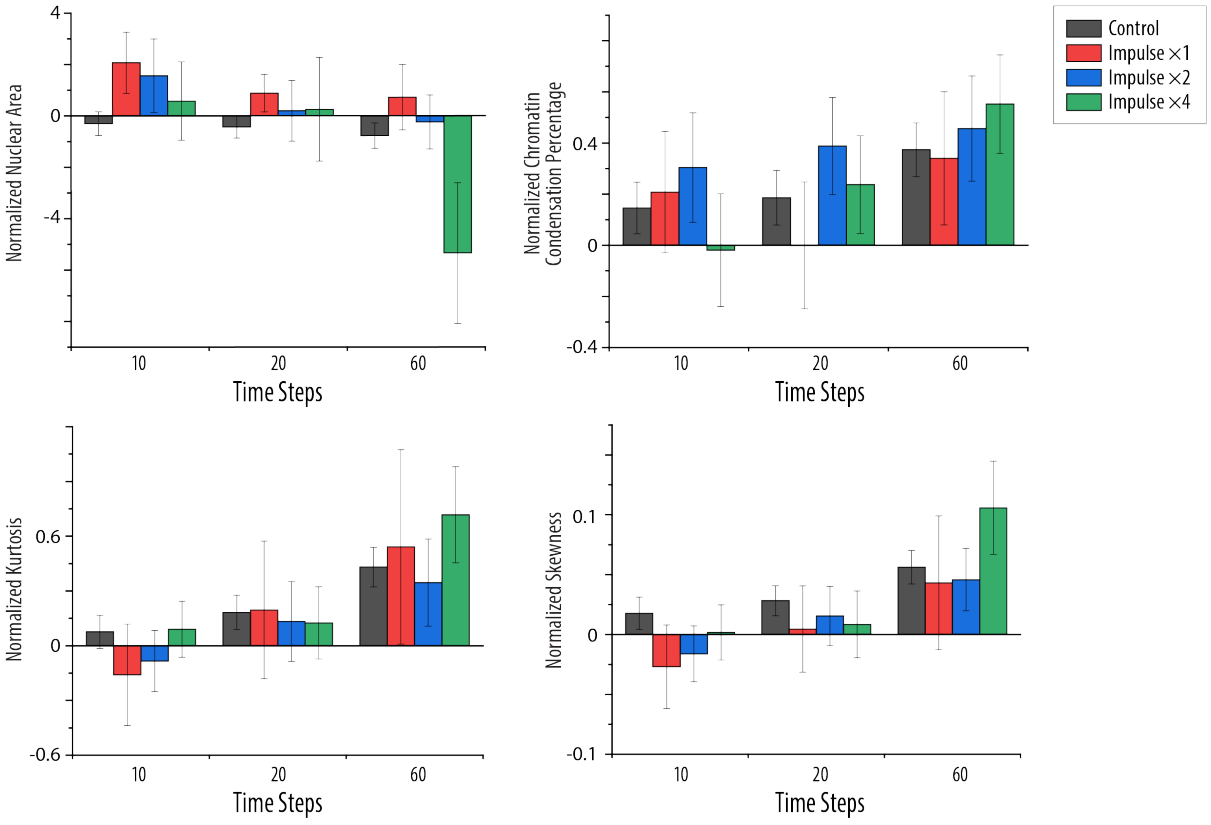
Supplemental Figure 1. Nuclear Movement Quantification compared with Human Analysis. A) 68 nuclei from 5 experiments with strains between 0-18% were analyzed for nuclear movement using our algorithm for calculating rotation and the surrogate marker of β from the power-law equation for internal motion. Using the predetermined cutoff values for rotation and internal motion, nuclei were classified into groups of "Rotation" or "No Rotation" and "Internal Motion" or "No Internal Motion." Comparing the methods of nuclear movement using our MATLAB algorithms with observations from 3 independent persons, we saw similar frequencies of nuclei which had Rotation or Internal Motion at each strain level. **B)** We validated our algorithm for quantifying rotation by rotating 12 different nuclei at various strain magnitudes 23 degrees. Additionally, we added random noise to the image and ran the nuclei through the same algorithm. Plot represents an average of the output value for all 12 nuclei. Error bars = SD.



Supplemental Figure 2. Test set of nuclei used to classify nuclear movement for rotation and internal motion. A) Quantified values of rotation from 91 nuclei that received a single high impulse with strains between 0% to 18% were used to determine a cutoff value of 5 between "Rotation" and "No Rotation." **B)** Derived values of the diffusive exponent, β, from 91 nuclei that received a single high impulse with strains between 0% to 18% were used to determine a cutoff value of 0.8. Nuclei with a value of β greater than 0.8 were classified as having Internal Motion and nuclei with a value less than 0.8 were classified as having "No Internal Motion."



Supplemental Figure 3. Violin plots show distribution of rotation at each strain level or repetitive loading groups. A) Distribution of the rotational values of each nucleus in the respective strain levels. Violin plots represents the same data as in Figure 3B. **B)** Distribution of rotational values for the repetitive loading experiments. Violin plots represents the same data as in Figure 4B.



Supplemental Figure 4. Morphological analysis of High Strain nuclei after repetitive loading. Z-projected images of nuclei which received >18% strain were analyzed for alteration in morphological features including Nuclear Area, Chromatin Condensation Parameter, Kurtosis, and Skewness. No significant differences were observed in the high strain group

which receive repetitive loading. All values were normalized relative to time step, Pre. Time steps 10 min, 20 min, and 60 min were plotted. Control: n =162 nuclei, Impulse ×1: n = 18 nuclei, Impulse ×2: n = 31 nuclei, Impulse ×4: n = 25 nuclei. N > 5 animals. Error bar = SEM.