Electronic Supplementary Material (ESI) for Integrative Biology

SUPPLEMENTARY FIGURE LEGENDS

Figure S1-S4: See below.

Supplementary Video 1. Example of a reversal induced by a 1 μN stimulus delivered to the head of a wild type animal. The stimulus was positioned at 0.33 of the length from nose tip to tail spike. In this and all experiments, the stimulus was applied for 100 ms and followed by an observation period of 2 s. The image sequences in Figure 2E (top) are drawn from this movie.

Supplementary Video 2. Small forces often fail to induce reversals. Example of a wild-type animal challenged with a 100 nN force applied for 100 ms to the head (0.28 of the distance from nose tip to tail spike) that continued moving forward for the entire 2 s observation period. The image sequences in Figure 2E (bottom) are drawn from this movie.

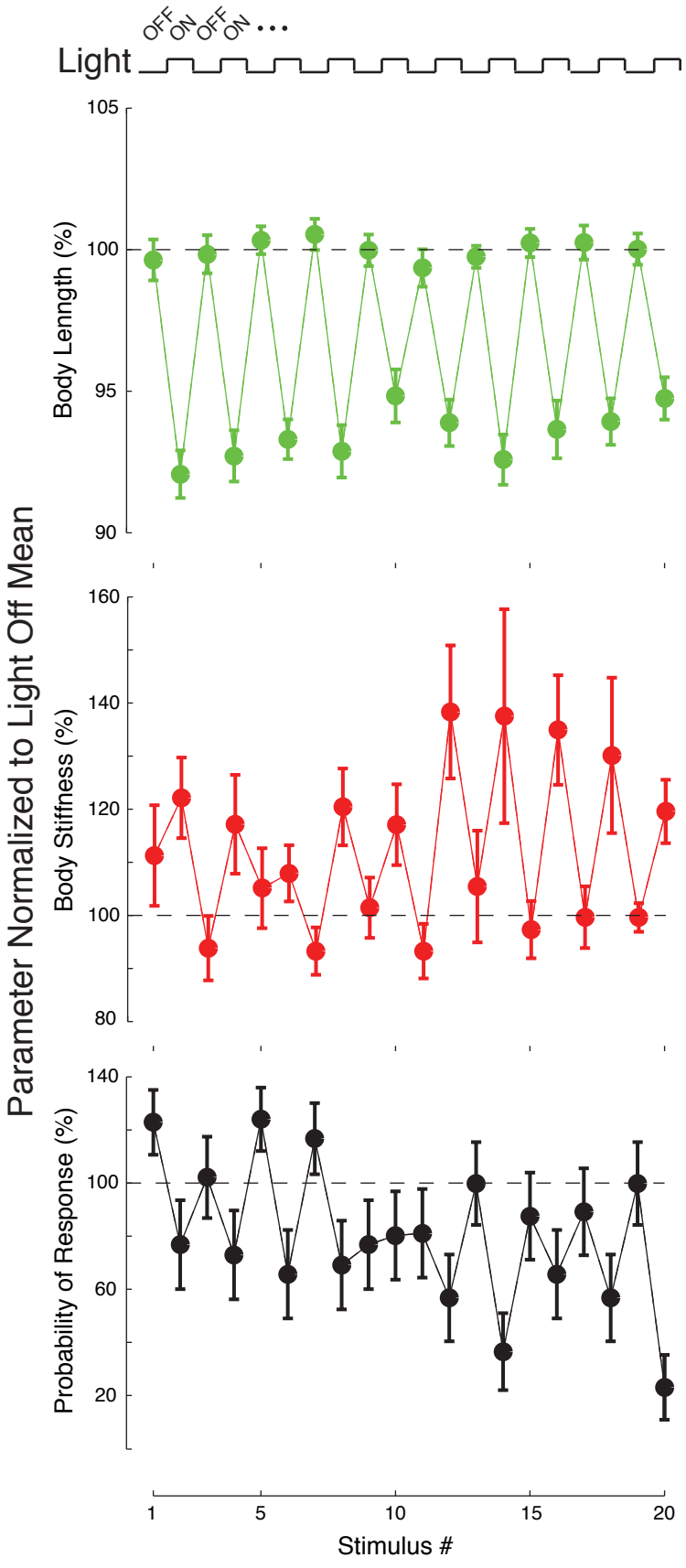

Figure S1. Muscle hypercontraction affects body length, body stiffness, and force sensitivity. Data represent the stimulus # mean ± s.e.m. normalized with respect to the average resting (light off – all even stimulus #'s) value across all animals and force levels. The variation in the probability of worm response is large despite the more modest variation in body length and body stiffness with changing optical state for two primary reasons: (i) The probability of response plotted here is an average across our experiments conducted at all force levels. The fact that animals don't respond in either light state when small forces are applied ($p \sim 0$ for every trial) and the fact that animals are very likely to respond in either light state when large forces are applied ($p \sim 0.8$-1.0) means that data from experiments conducted at low and high forces act to diminish the observed difference between the on and off states, and also that such data increases the error bars for each trial. A small fraction of trials at selected force levels that could not be included due to failure to deliver the stimulus properly further increases variability. (ii) For each trial, the animal response is scored discretely as either positive (a '1') or negative (a '0'), whereas animal length and body stiffness are continuous variables that have a high probability of changing as a result of light stimulation, but vary in degree of change. There is significant variation from measurement to measurement (worm-worm) in terms of the trials that an animal did or didn't respond to in trials at both light states. For example, if the overall probability of response with the light off is 0.5 for two animals, one animal may have data 0011100011, while another animal may have data 0101010101. This measurement-to-measurement variation of discretized animal responses introduces additional variation in the population average probability of response at each trial in both the light on and the light off states.

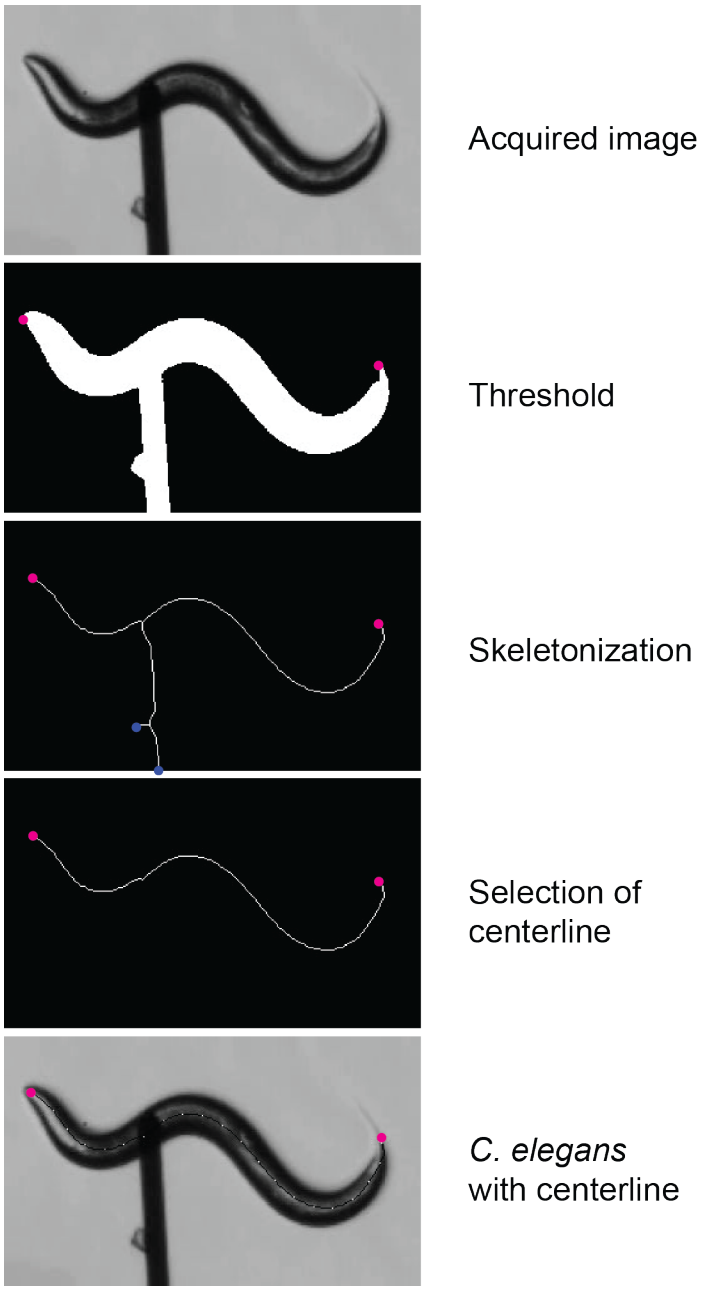

Figure S2. Image post-processing of *C. elegans* under a cantilever. First, an image of *C. elegans* with an overlapping cantilever is acquired by a CCD camera and converted into black and white to extract the boundary of the image. Second, the binary image is converted into a connected set of thin digital curves by a skeletonization process. Last, the centerline of *C. elegans* is calculated by selecting the digital curve that directly connects the animal's head and tail (fuscia dots) and

by removing the digital curve associated with the cantilever shadow or any other endpoints (blue dots). The algorithm and software used here was adapted from Leifer*, et al*.[1]

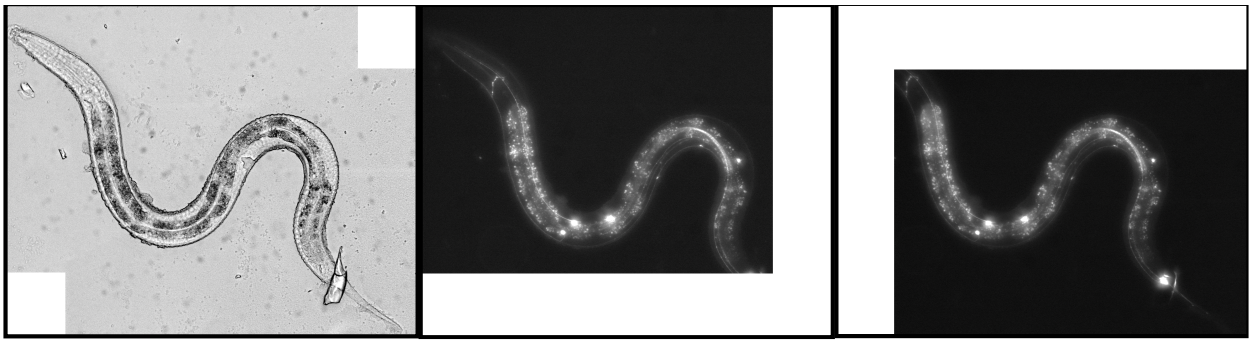

Figure S3. A) The merge of two bright field images to create one image of an entire worm. B) The translation of the anterior portion so that the pixels are mapped to the same coordinates at the anterior portion of the worm in the brightfield image. C) The translation of the posterior portion so that the pixels are mapped to the same coordinates at the posterior portion of the worm in the brightfield image.

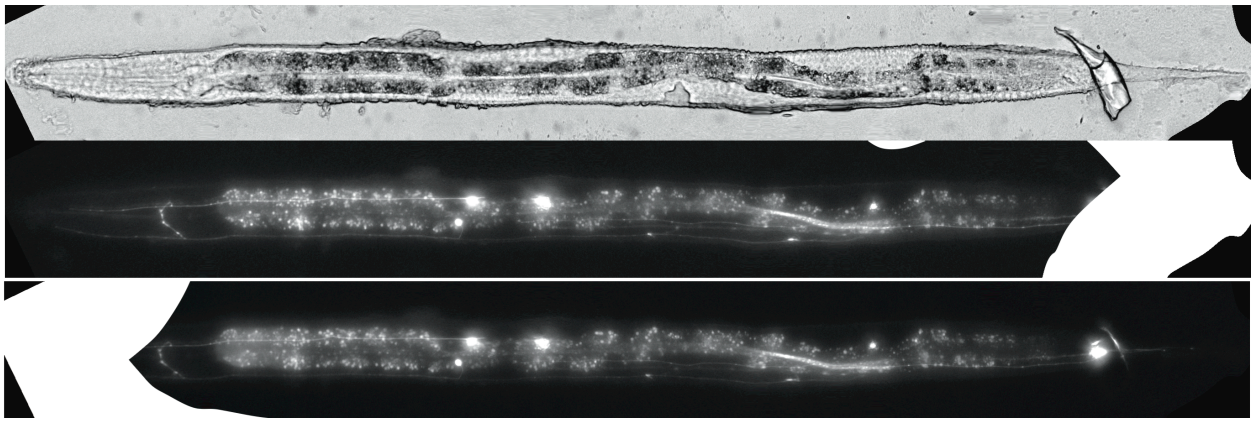

Figure S4. The straightened translated images created using the Straighten tool in ImageJ. Each pixel in each image maps to the same point in real space so the location of features in different images can be compared. A) brightfield, B) Anterior portion, CFP, C) Posterior portion, CFP.

REFERENCES

1. A. M. Leifer, C. Fang-Yen, M. Gershow, M. J. Alkema, and A. D. T. Samuel, Optogenetic manipulation of neural activity in freely moving *Caenorhabditis elegans*, *Nat. Methods*, 2011, **8**(2), 147-152.