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Supplemental Information

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Supplementary Material: Mouth Function Determines The Shape Oscillation Pattern In Regenerating *Hydra* Tissue Spheres

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Supplementary Figures

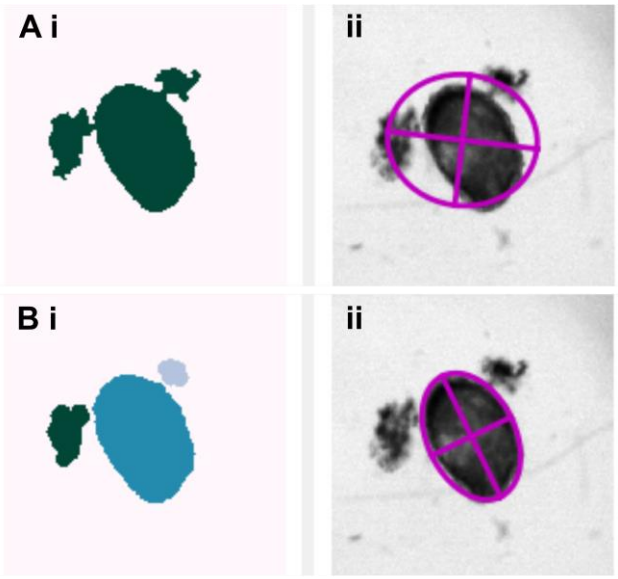

Figure 1. Representative images from image analysis of regenerating tissue spheres. **(A)** Prior to image segmentation using watershedding. i. Debris and tissue piece identified as single object. ii. Raw image with fitted ellipse. **(B)** After image segmentation using watershedding i. Debris and tissue piece identified as separate objects. ii. Raw image with fitted ellipse. The ellipse fit in the bottom right panel was used for analysis.

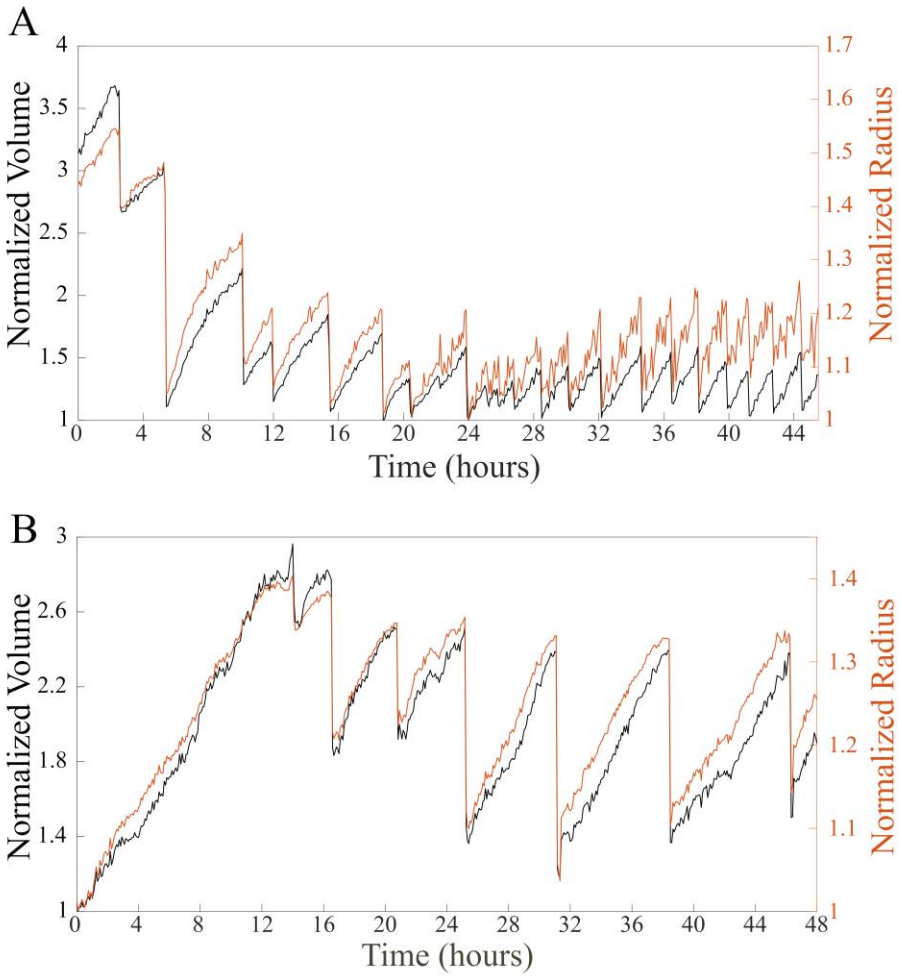

Figure 2. Effective radius and volume dynamics are not qualitatively different. (A) Wild type tissue piece displaying an oscillation pattern shift. Radius plotted in red, calculated volume plotted in black. Both were normalized by dividing by the respective minimum values. **(B)** Tissue piece from a nerve-free animal only displaying LPOs. Normalized radius plotted in red, normalized calculated volume plotted in black.

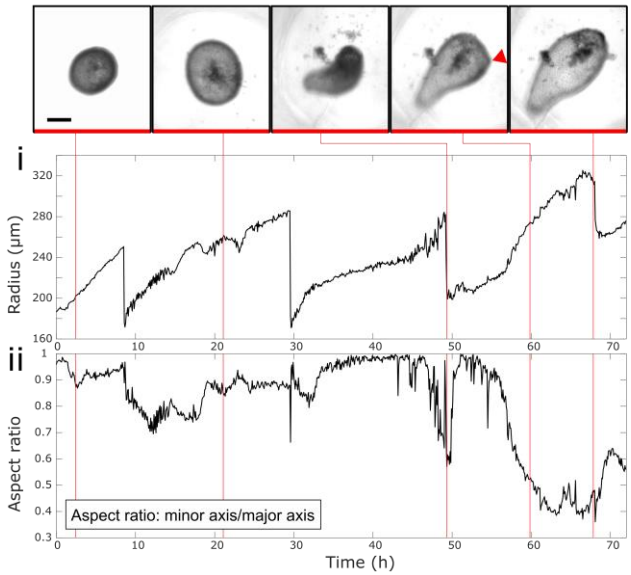

Figure 3. Regeneration of head structures in nerve-free tissue piece over the course of 72 h. i. Radius and ii. aspect ratio plots for regeneration of nerve-free tissue piece, with representative images indicated by red lines. Shape symmetry is broken before 48 h and the appearance of tentacle buds is observed around 60h. Red arrowhead indicates first visible tentacle bud. Scale bar 200 µm.

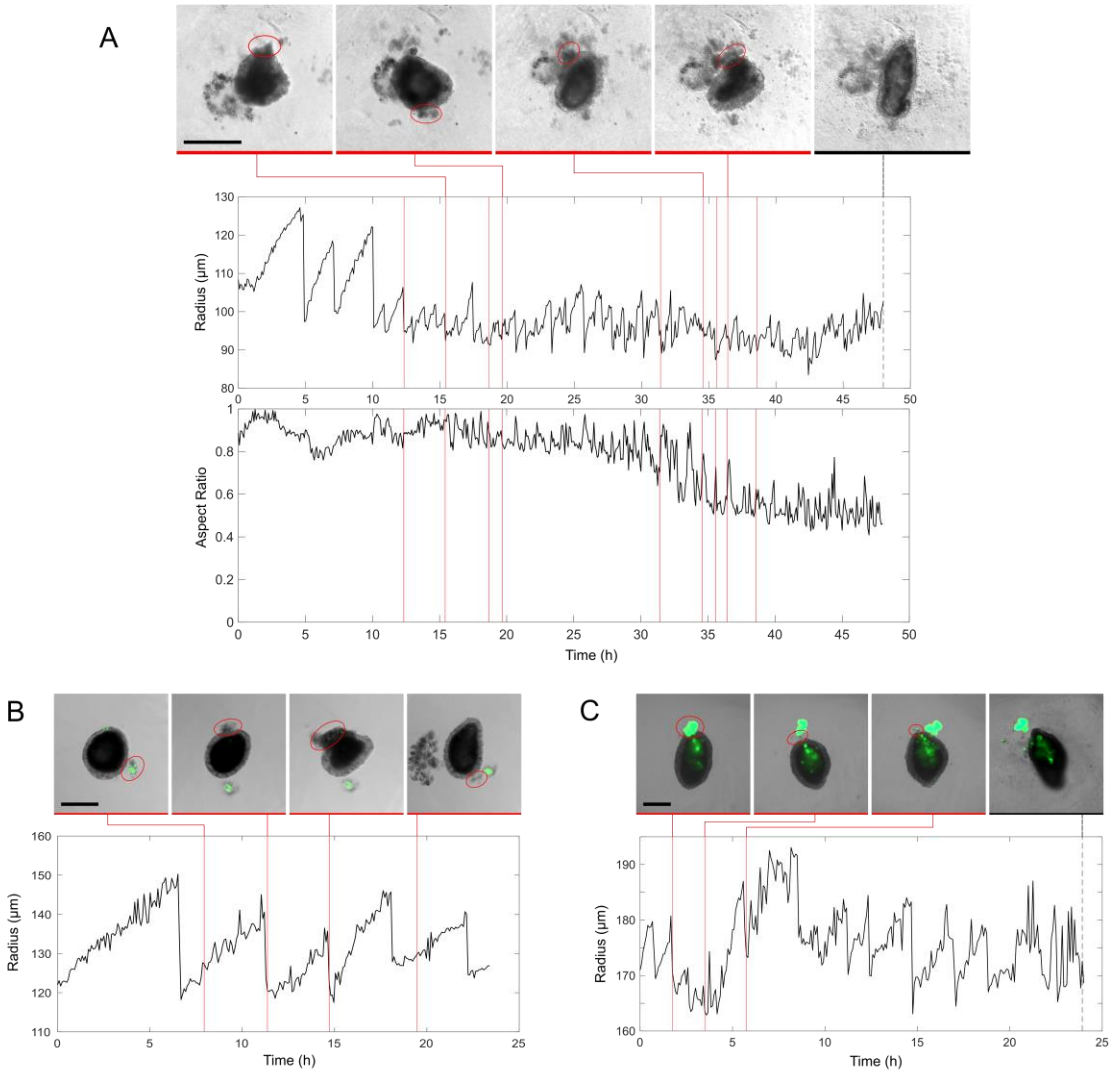

Figure 4. Debris is ejected throughout the regeneration process. (A) Cell debris ejected from an un-injected tissue piece. Red lines on radius and aspect ratio plots indicate the earliest frame in which a new piece of ejected debris can be clearly observed. Image series illustrate representative rupture events, with new debris circled in red. Dashed black line and associated image indicate the last frame of the video, showing the presence of a body axis. **(B)** Tissue piece injected with microbeads 5 h after cutting. 4 trackable rupture events with ejection of both beads and cell debris are observed. Images have been rotated to standardize the orientation of the tissue piece. Oscillations resemble LPOs and rupture site is not conserved. **(C)** Tissue piece injected with microbeads 24 h after cutting. 3 trackable rupture events with ejection of both beads and cell debris are observed. Images have been rotated to standardize the orientation of the tissue piece. Rupture site is conserved, and oscillations resemble SPOs. The last frame of the video shows the tissue piece is oblong with a conical hypostome structure. Scale bars 200 µm.

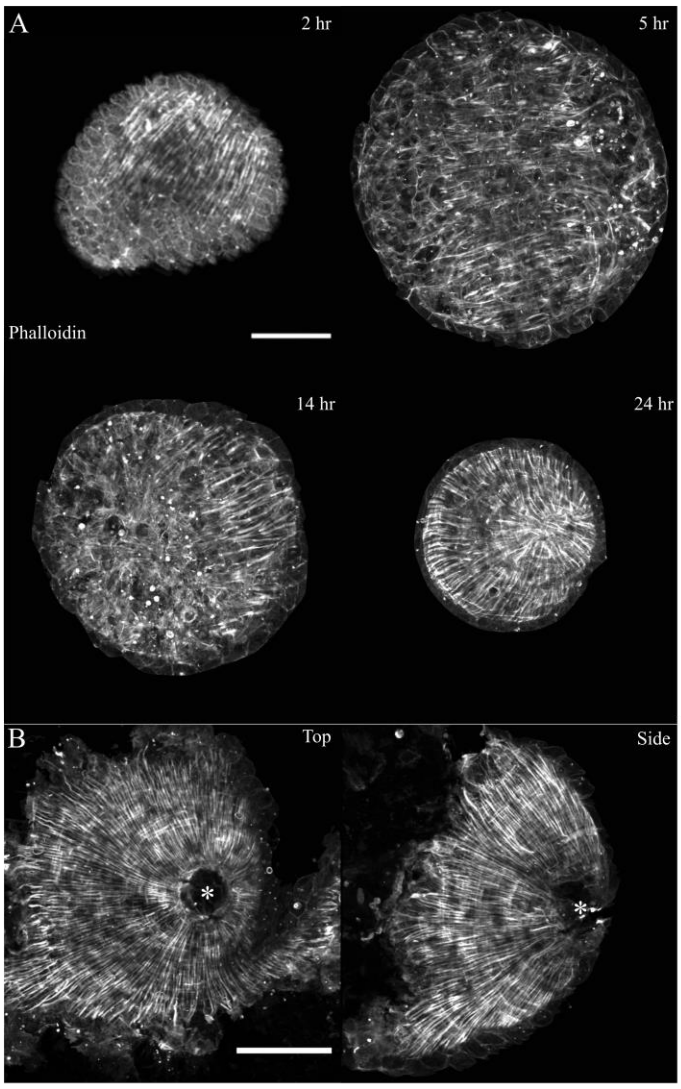

Figure 5. Retention of myoneme structure in tissue pieces. (A) Body column tissue pieces fixed and stained with phalloidin 2, 5, 14 and 24 h after cutting. **(B)** Phalloidin staining of head pieces 5 h after excision showing retention of normal myoneme organization of the mouth. Damage to the aboral side of the piece (on the left in the side view) occurs during mounting due to the conical shape of head pieces and does not accurately represent the live state. Scale bars 100 µm.

Supplementary Movies

Movie 1. Regeneration of wildtype tissue piece. Raw video of the tissue piece represented in Figure S4 A. Scale bar 200 µm, total time 48 h. Recorded at 1 frame every 5 minutes (0.003 fps), playback at 10 fps.

Movie 2. Regeneration of nerve-free tissue piece. Raw video of the tissue piece represented in Figure 3, showing formation of body axis and head structures. Scale bar 200 µm, total time 72h. Recorded at 1 frame every 5 minutes (0.003 fps), playback at 10 fps.