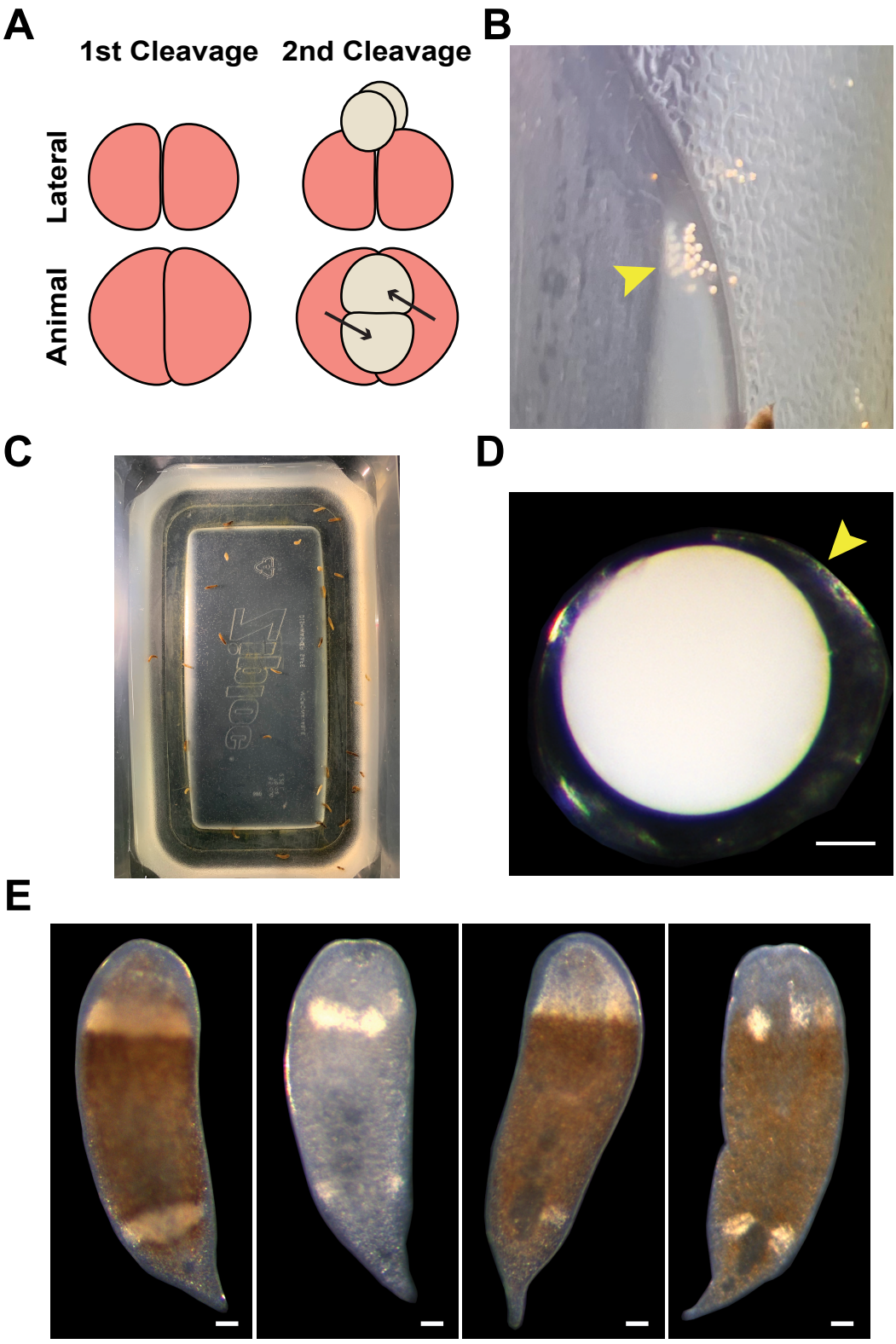


Fig. S1. Culturing *Hofstenia* embryos. (A) Schematic illustrating the first two cleavages of duet cleavage. Arrows denote the counter-clockwise direction of cleavage when viewed from the animal pole. The micromeres (yellow) are situated at the cell junction of the macromeres (red). (B) A representative image of a clutch of *Hofstenia* embryos. (C) Inside of a tupperware box used for culturing *Hofstenia*. (D) Darkfield image of a zygote inside of a clear egg shell (yellow arrow). Scale bar, 100μm. (E) The phenotypic diversity in coloration among *Hofstenia* juveniles.

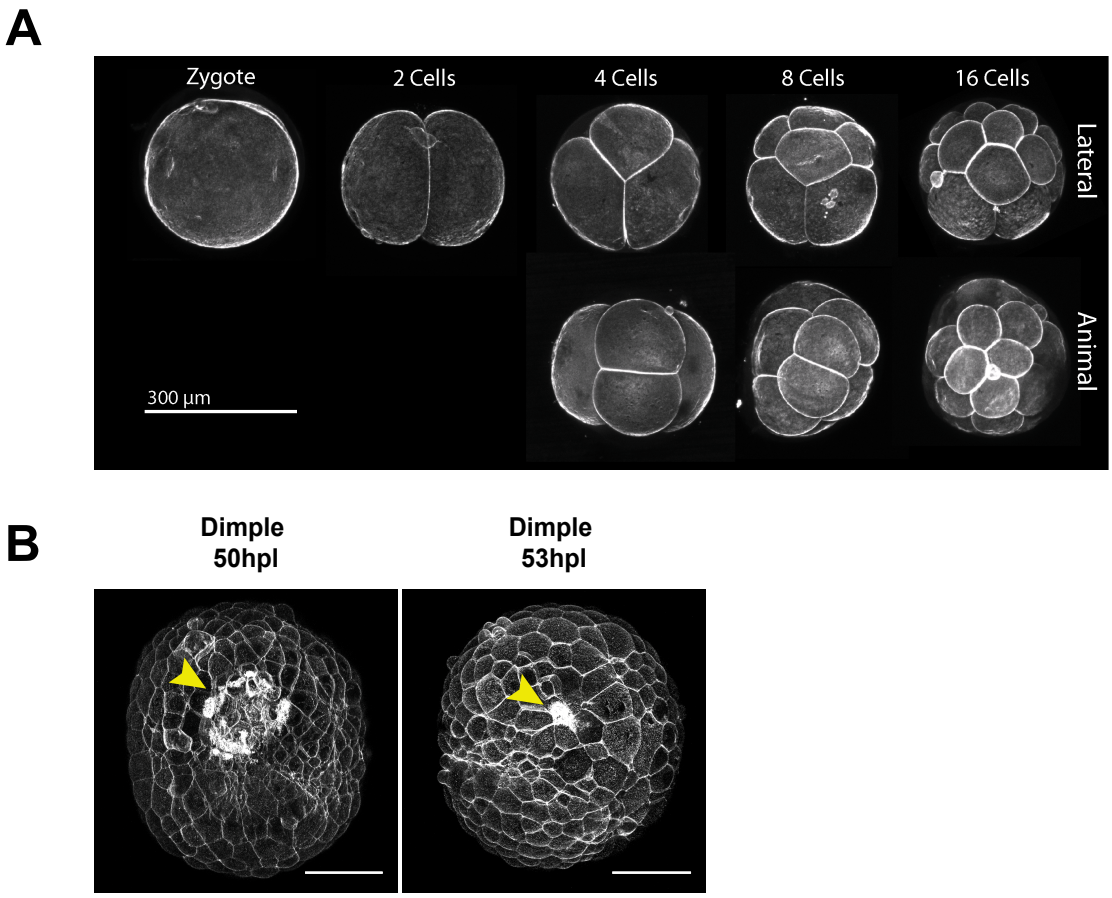


Fig. S2. Phalloidin staining of *Hofstenia* embryos. (A) Representative images of phalloidin stained early cleavage embryos. Scale bar, 300µm (B) Phalloidin stained Dimple stage embryos. A ring of actin is present at the site of cell internalization, or the “dimple”. This ring becomes progressively smaller as the embryo progresses through the Dimple stage. Scale bars, 100µm

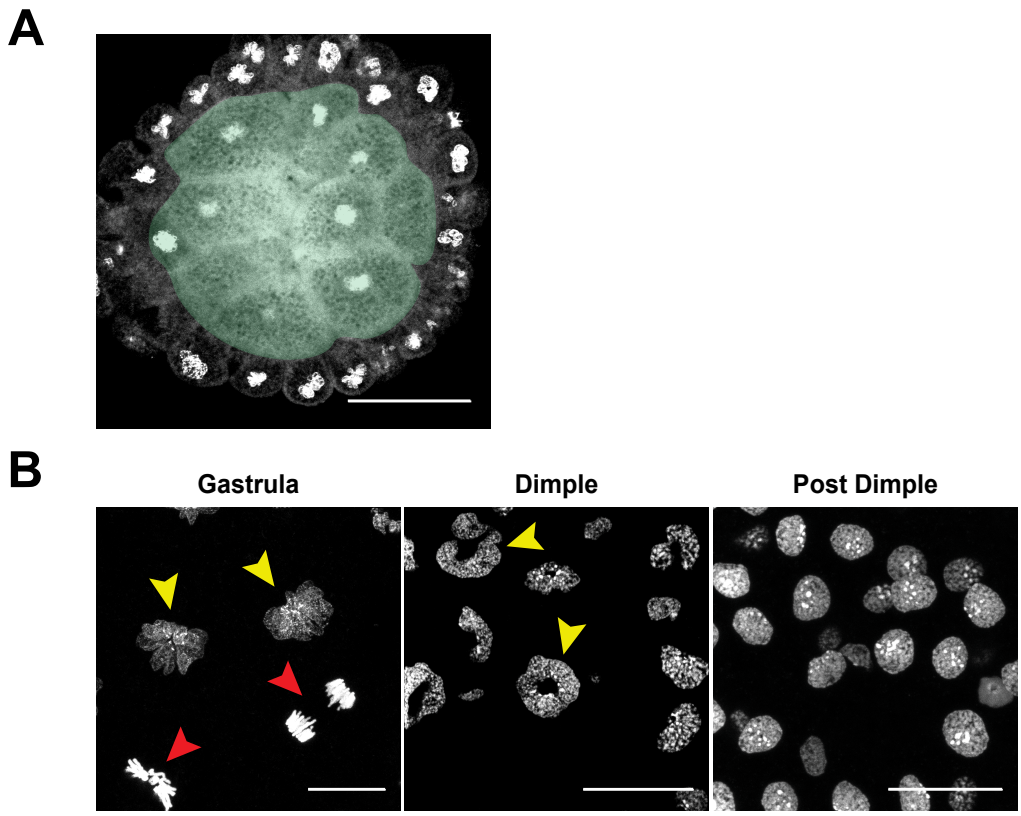


Fig. S3. Nuclear staining of *Hofstenia* embryos. (A) Cross section of a DAPI stained Gastrula stage embryo shows the lack of a blastocoel-like cavity, with the internal space of the embryo being occupied by large cells (shaded green). (B) 63x imaging of Topro3 stained nuclei in Gastrula, Dimple, and Post Dimple stages. A rosette-like shape is present in the majority of Gastrula embryos (yellow arrow), with the exception of cells in anaphase (red arrow). Dimple stage embryos possess ring-shaped nuclei with an empty space at their centers (yellow arrow). Rosette- or ring-shaped nuclei were not detected at the Post Dimple stage. Scale bars, 25μm

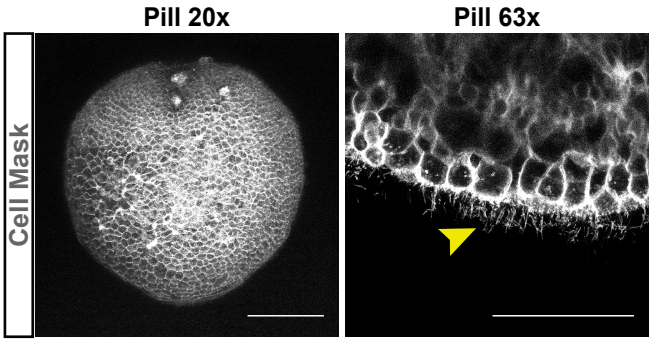


Fig. S4. Cell mask staining reveal the presence of cilia at the Pill stage. Left: 20x magnification image of a Pill stage embryo stained with a Cell Mask plasma membrane dye. Scale bar, 100µm. Right: 63x optical cross section of a stained Pill stage embryo shows the presence of cilia at its surface (yellow arrow). Scale bar, 50µm

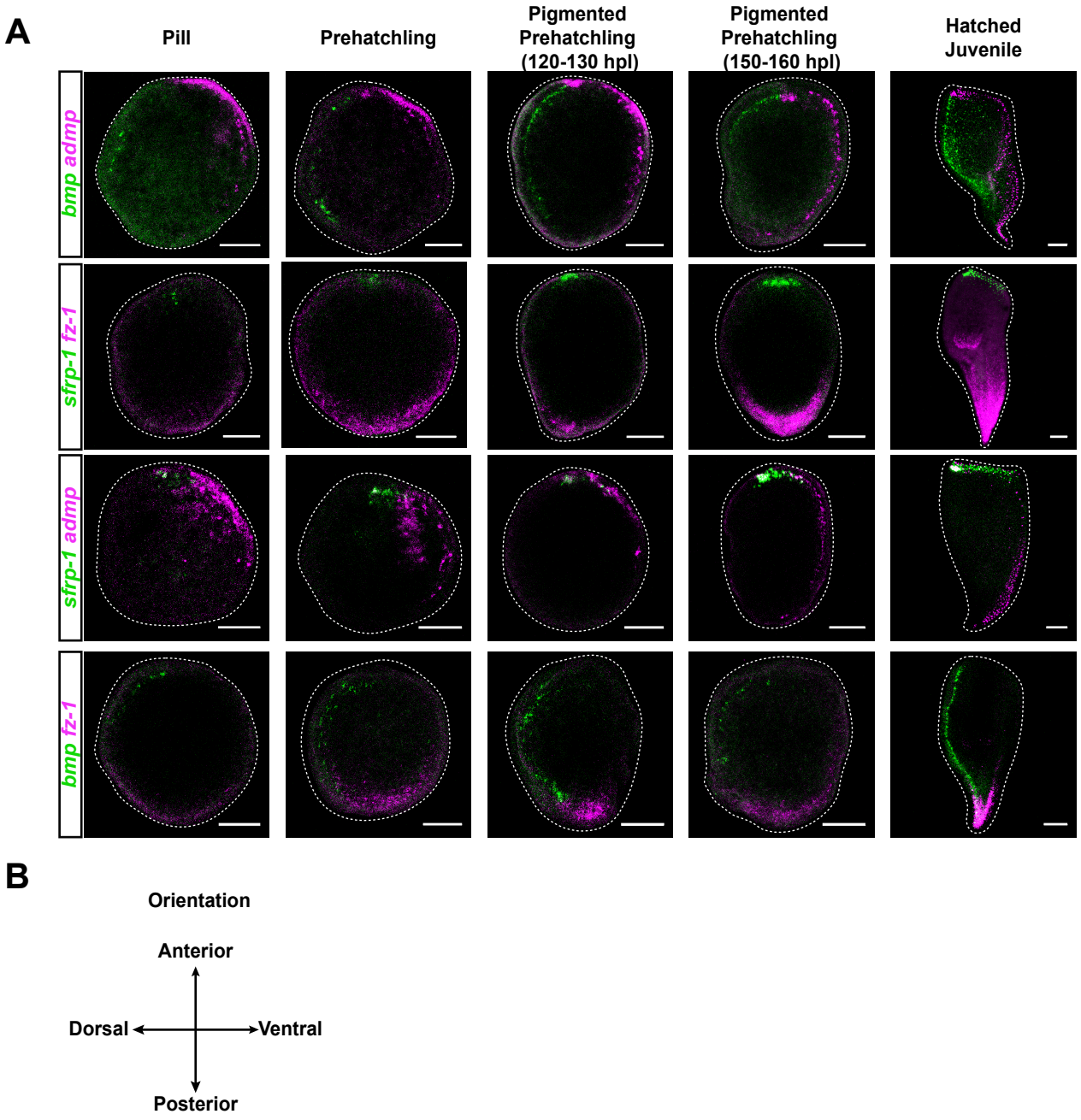


Fig. S5. Expression of axis polarity markers are polarized to opposite poles at the Pill stage. (A) Double *in situ* hybridization of dorsal/ventral and anterior/posterior markers. The dorsal marker *bmp* and ventral marker *admp* occupy opposite regions of the embryo starting at the Pill stage. The anterior marker *sfrp-1* and posterior marker *fz-1* also mark regions that are opposite to each other at the Pill stage. The correct spatial relationship of these axis markers were detected at the Pill stage. (B) Orientation of the embryos shown above. Scale bars, 100μm

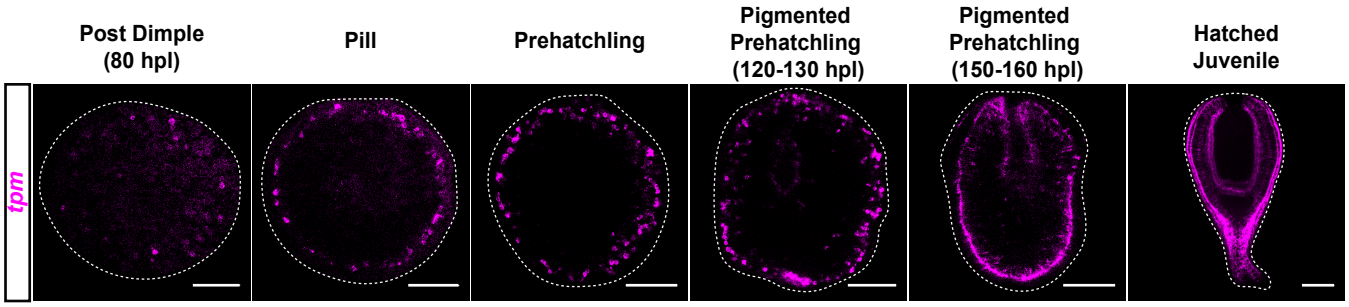
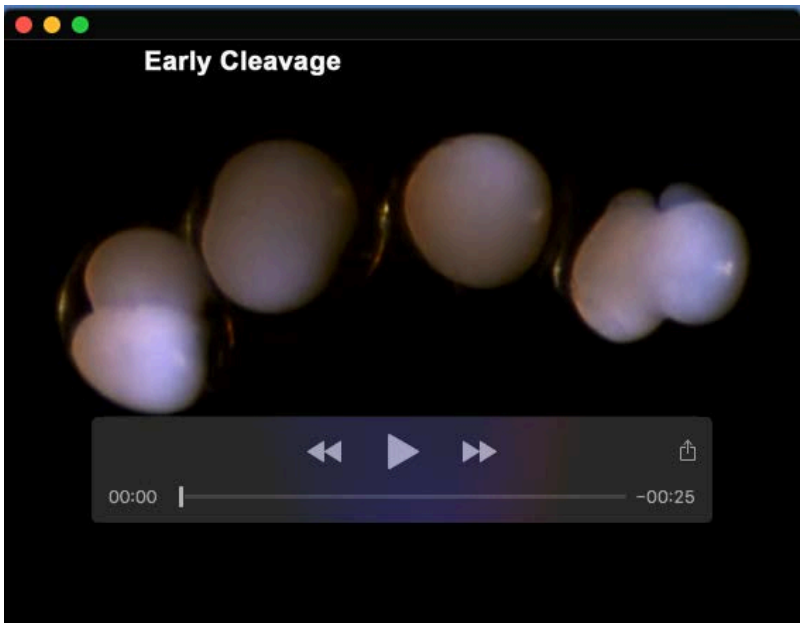
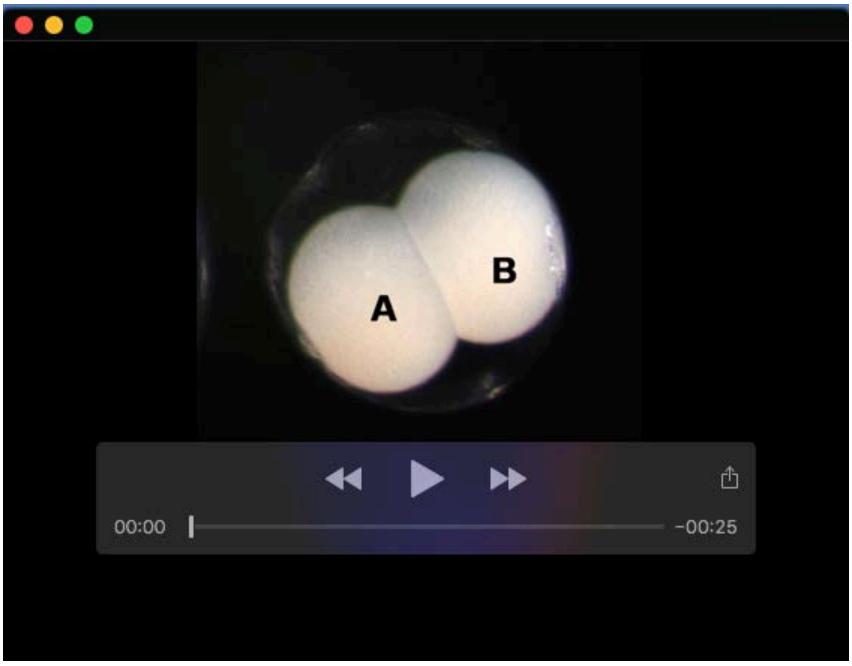


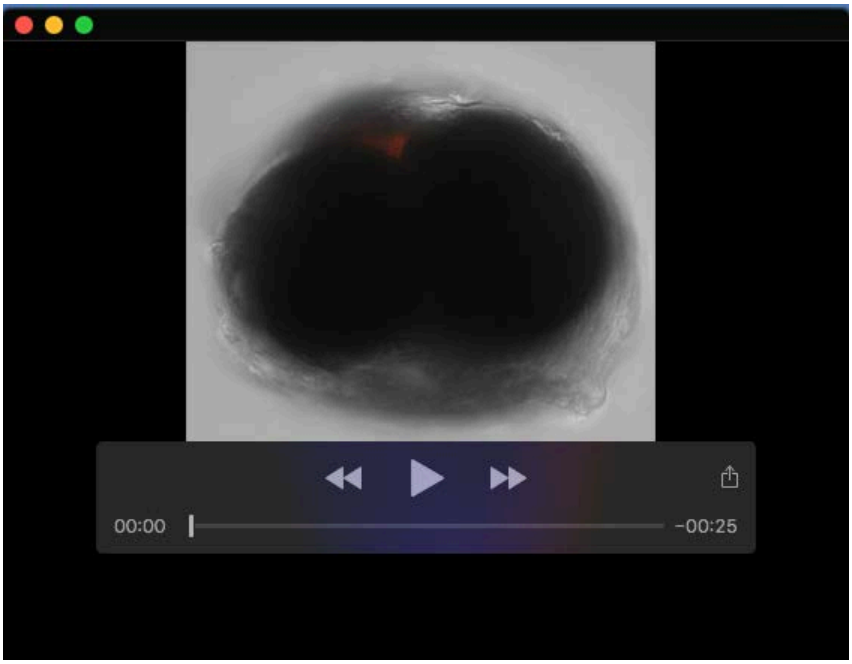
Fig. S6. Cross sections of embryos showing *tropomyosin* expression *in situ*. Optical cross sections of *tpm in situ* hybridizations. Cells expressing *tpm* are situated below the surface of the embryo, but are distributed around the periphery of the cross section. Scale bars, 100μm



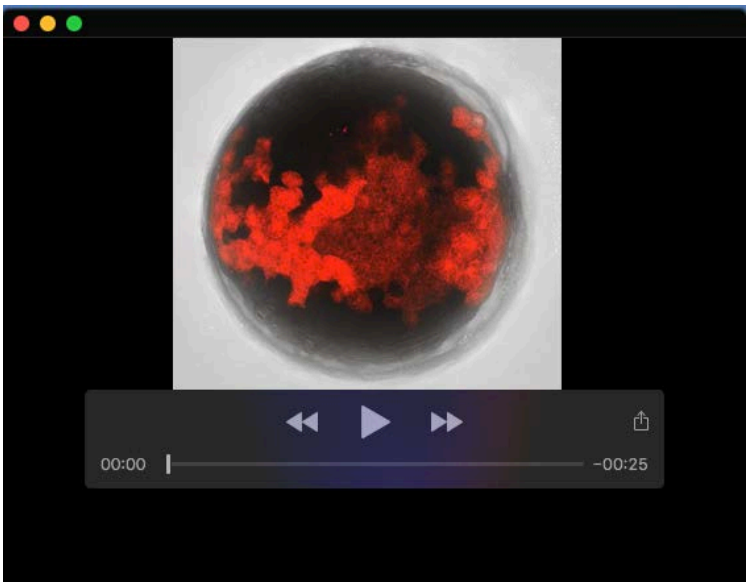
Movie 1. Time-lapse of *Hofstenia* development. Time-lapse recording of one focal plane of embryos from zygote to hatching. This video shows 8 days of development in 2 minutes and 24 seconds. Developmental stages are denoted in the video.



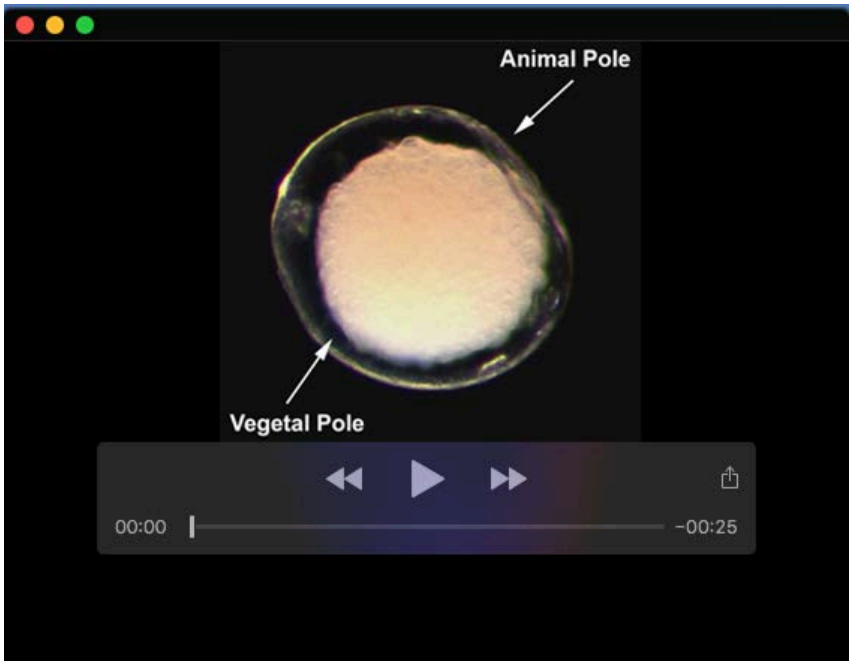
Movie 2. Early cleavage of *Hofstenia*. Time-lapse recording of the first 24 hours of development. The blastomere names that correspond to Fig. 2A are labeled.



Movie 3. Vegetal view of a *Hofstenia* embryo with 1a and 1b blastomeres labeled with fluorescein dextran. Time-lapse recording of a 4-cell embryo viewed from the vegetal pole after both 1a and 1b micromeres were injected with fluorescein dextran. Labeled daughter cells can be seen moving towards the vegetal pole, internalizing the macromeres in the process.



Movie 4. Animal hemisphere view of a Dimple stage *Hofstenia* embryo with 1a and 1b blastomeres labeled with fluorescein dextran. Time-lapse recording of a Dimple stage embryo. All labeled cells are the progeny of the 1a and 1b blastomeres. A patch of labeled cells can be seen being internalized.



Movie 5. The Dimple stage cell internalization occurs on the animal hemisphere. Timelapse recording of an embryo from a lateral view from the Early Cleavage to the completion of the Dimple stage. The Dimple forms on the animal hemisphere, while the internalization of the macromeres occurs on the vegetal pole.

Table S1. Differentiated cell type marker genes and nomenclature. The justifications for the naming conventions for all previously unpublished genes, sequence and primer sequences.

Click here to download Table S1

Table S2. RNA-seq analysis. (A) GO enrichment analysis results listing the top terms enriched for each cluster along with their multiple comparisons adjusted p-values. (B) Differential expression analysis results showing the adjusted p-values (q-values). (C) Transcripts per million (TPM) values for all genes with at least one statistically significant difference in gene expression across development. This table was used to generate the heatmap in Fig. 5B. (D) Average TPM values for all genes in the *Hofstenia miamia* 70 transcriptome.

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