

Supporting Information

Stach *et al.* 10.1073/pnas.0710196105

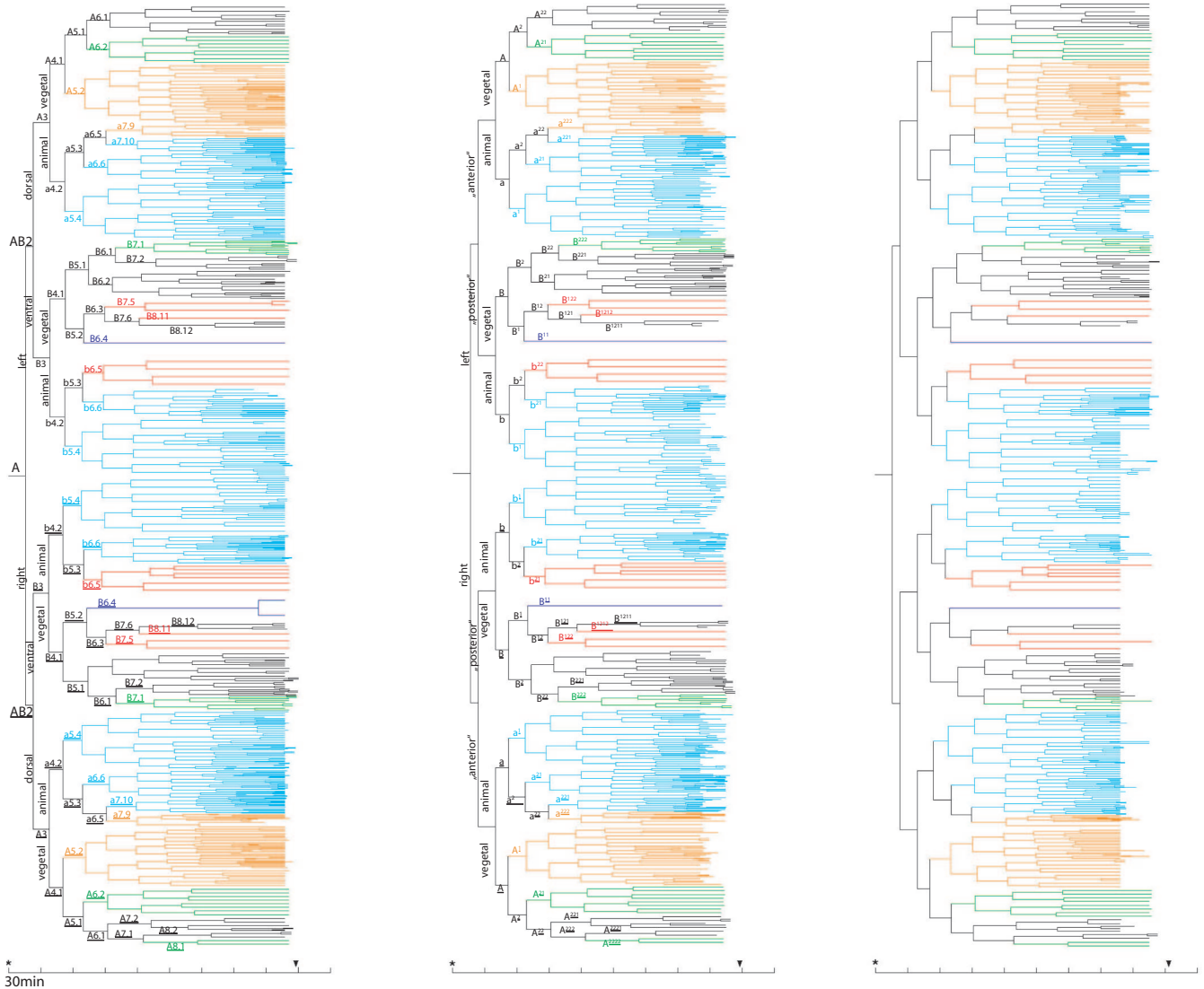


Fig. S1. Tree representations and fate maps of the development of three individuals of the tunicate chordate *Oikopleura dioica*. Colors indicated different tissues identifiable at hatching (see Fig. 1 for details). Left tree with nomenclature according to Conklin (1) and Nishida (2). Middle tree with nomenclature according to Delsman (3). Asterisks indicate times of fertilization, arrowheads mark times of hatching. Distance between two dashes on x axis corresponds to 30 min developmental time at 15°C.

1. Conklin EG (1905) Organization and cell lineage of the ascidian egg. *J Acad Nat Sci Philadelphia Series 2*, 13:1–119, plates I–XII.
2. Nishida H (1987) Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. *Dev Biol* 121:526–541.
3. Delsman HC (1910) Beiträge zur Entwicklungsgeschichte von *Oikopleura dioica*. *Verhandelingen uit het Rijksinstituut voor het Onderzoek der Zee* 3:1–24.

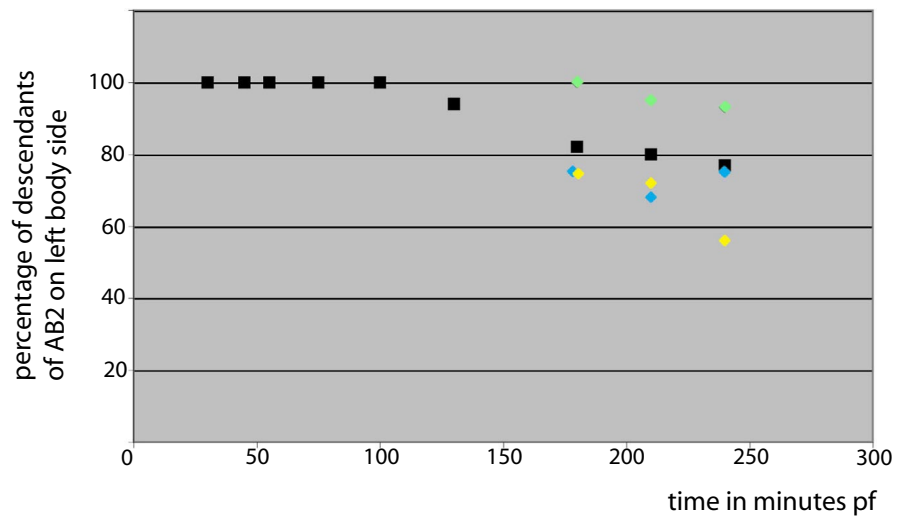


Fig. 52. Percentage of descendant cells of AB2 that remain on the left body side. To facilitate counting, tail cells were not considered. Black squares, total cell number; green, endodermal cells; blue, epidermis cells; yellow, nervous system cells.

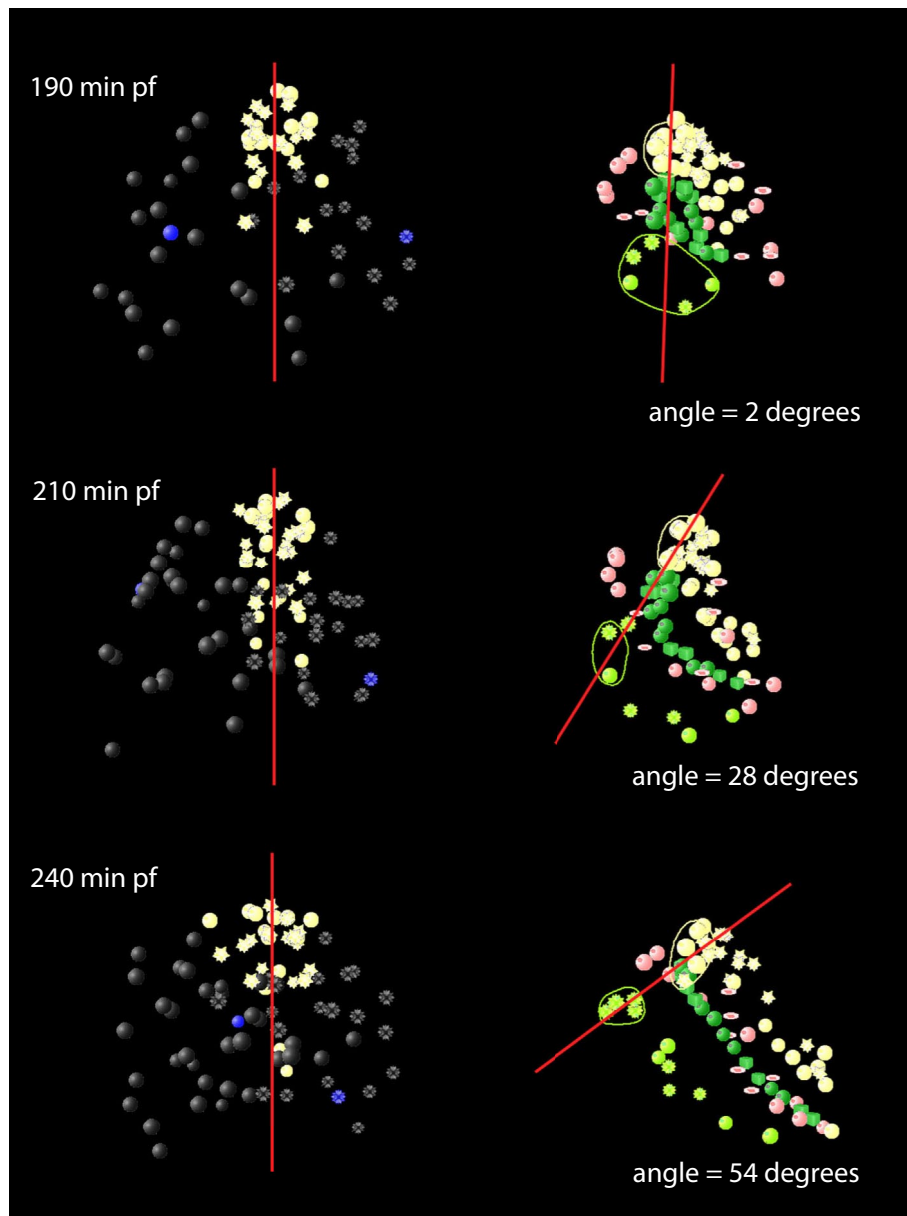


Fig. S3. Tail rotation observed during the development of *Oikopleura dioica* detected by using the 3D representation of the positions of the nuclei of each cell implemented in the 4D microscopy software SIMI^oBioCell. Spheres derive from the right side (AB2). Black, endodermal and meso-/endodermal cells in the trunk; blue, prospective germ cells, green, (prospective) notochordal cells; light green, (prospective) endodermal strand cells; yellow, (prospective) nervous system cells (epidermis cells omitted for clarity). (Left) Anterior view of trunk, with axis of bilateral symmetry indicated by red line. (Right) Anterior view of tail in same angle of view, with anatomical axis of bilateral symmetry indicated by red line.

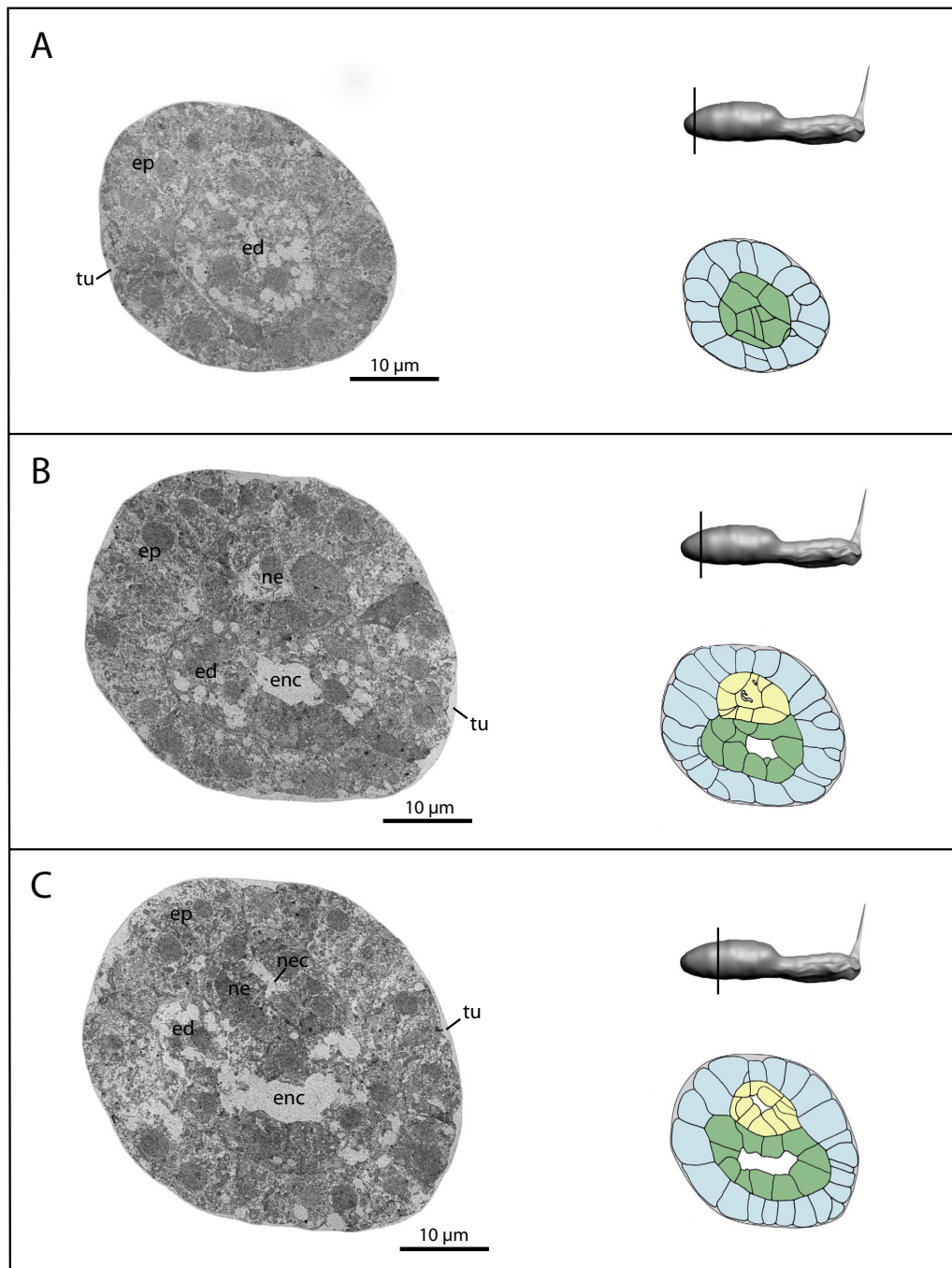


Fig. S5. Selected transmission electron micrographs from a complete series of cross sections of a hatched larva of *Oikopleura dioica*. Planes of sections indicated by black line in schematic in *Upper Right*; schematic representation of cross section in *Bottom Right*. Colors correspond to fates as in Fig. 1. Abbreviations: a, anterior; ax, axon; br, brain; brc, brain cell; c1/2/3/... , cell 1/2/3/... ; cj, cell junction; cm, cell membrane; d, dorsal; dc, dorsal cell; dmb, dorsal muscle band; dmc, dorsal muscle cell; ecm, extracellular matrix; ed - endoderm; edc, endodermal cell; eds, endodermal strand; ems, undifferentiated meso-/endoderm; enc, endodermal cavity; ep, epidermis; epc, epidermis cell; er, endoplasmic reticulum; fl, finlet; gr, granules; l, left; mi, mitochondrion; my, myofibrillae; nc, notochord; ne, nervous system; nec, cavity in the nervous system; nm, nuclear membrane; no, nucleolus; nt, neural tube; nu, nucleus; nu 1, 2, 3, etc., nucleus 1, 2, 3, etc.; nup, nuclear pore; p, posterior; r, right; ta, tail; tr, trunk; tu, tunic; tu 1/2/3, tunic layer 1/2/3; v, ventral; vcb, ventral cellular band; ve, vesicle; vmb, ventral muscle band; vmc, ventral muscle cell.

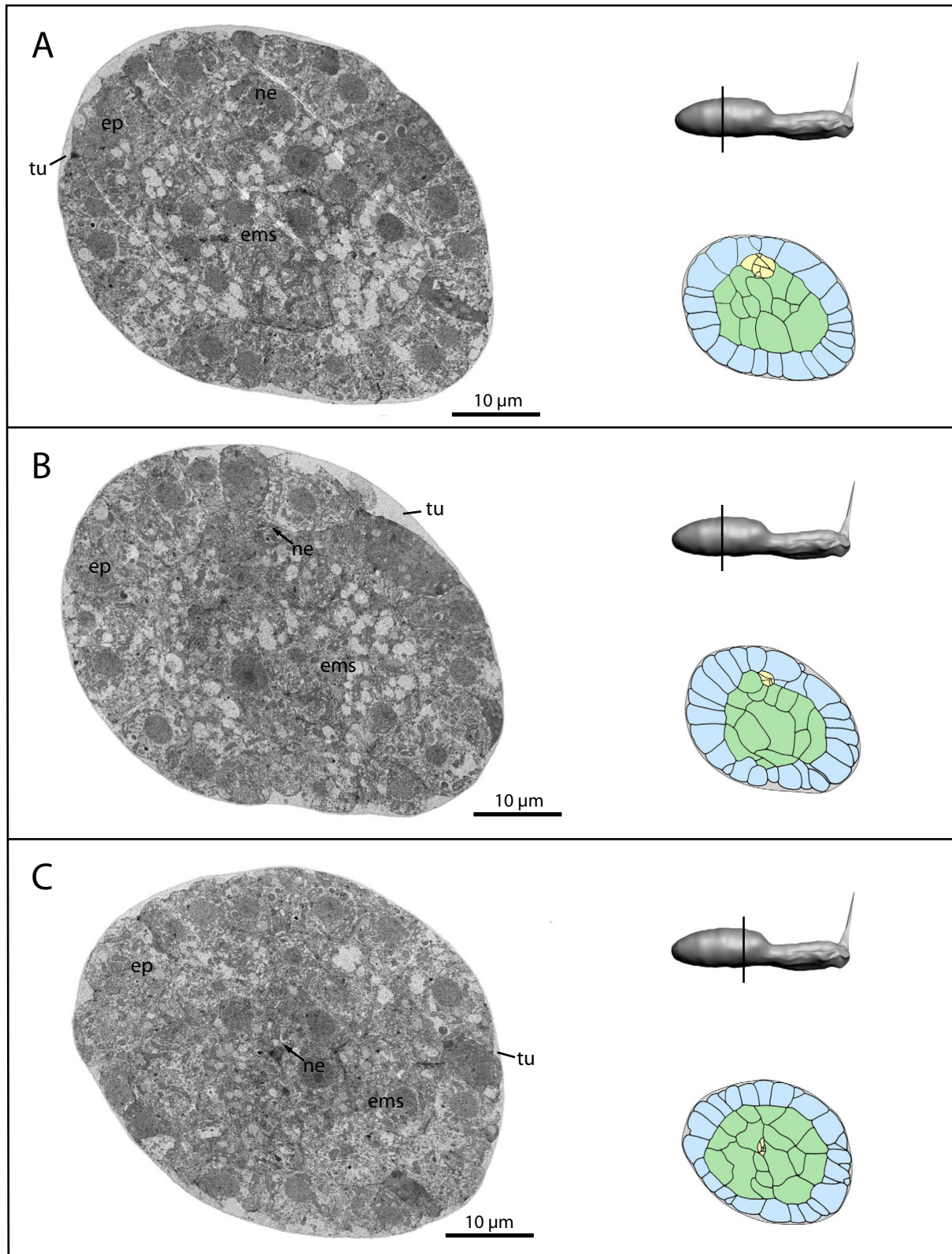


Fig. S6. Selected transmission electron micrograph from a complete series of cross sections of a hatched larva of *Oikopleura dioica*. For details and abbreviations, see Fig. S5 legend.

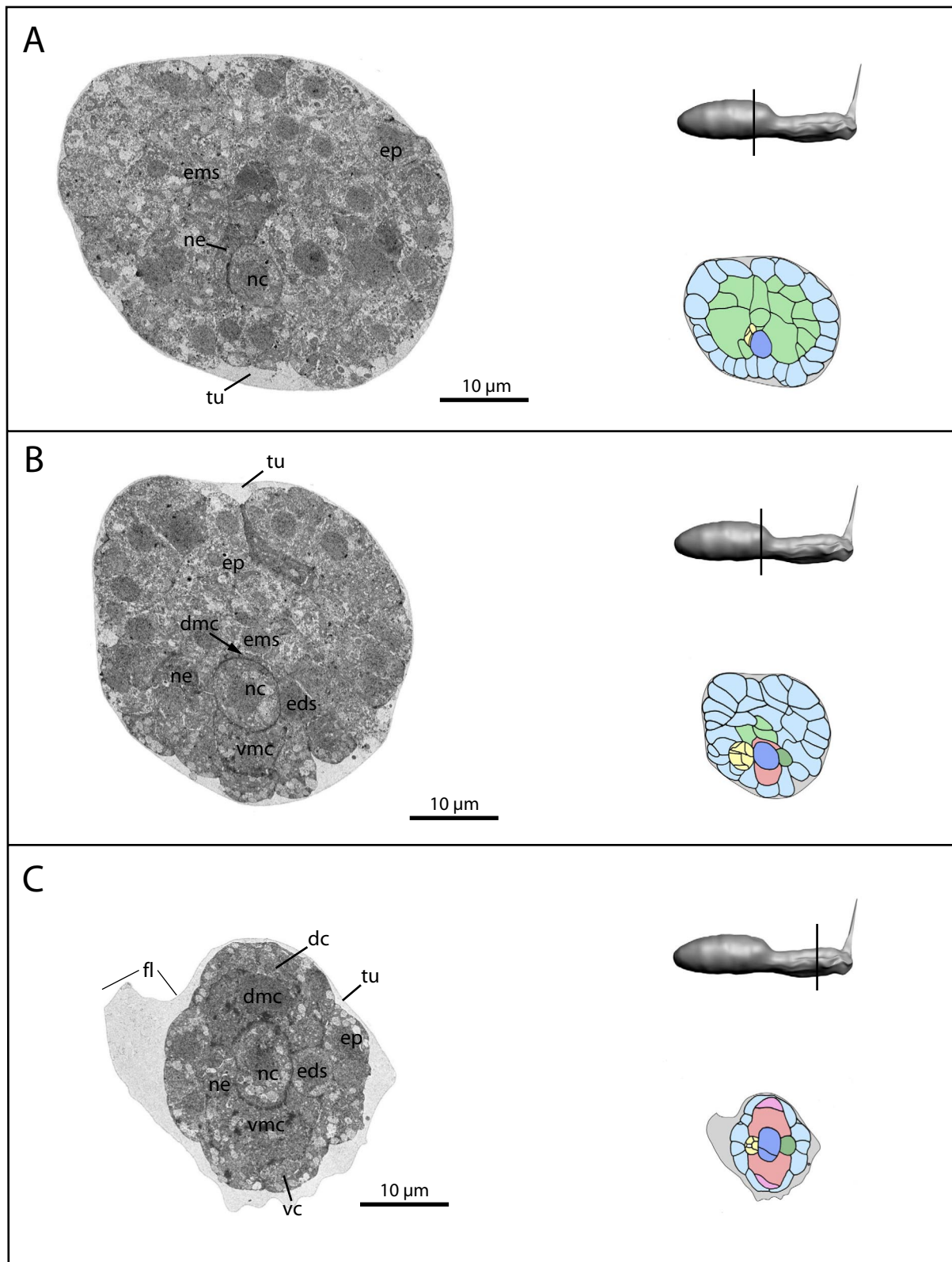


Fig. S7. Selected transmission electron micrograph from a complete series of cross sections of a hatched larva of *Oikopleura dioica*. For details and abbreviations, see Fig. S5 legend.

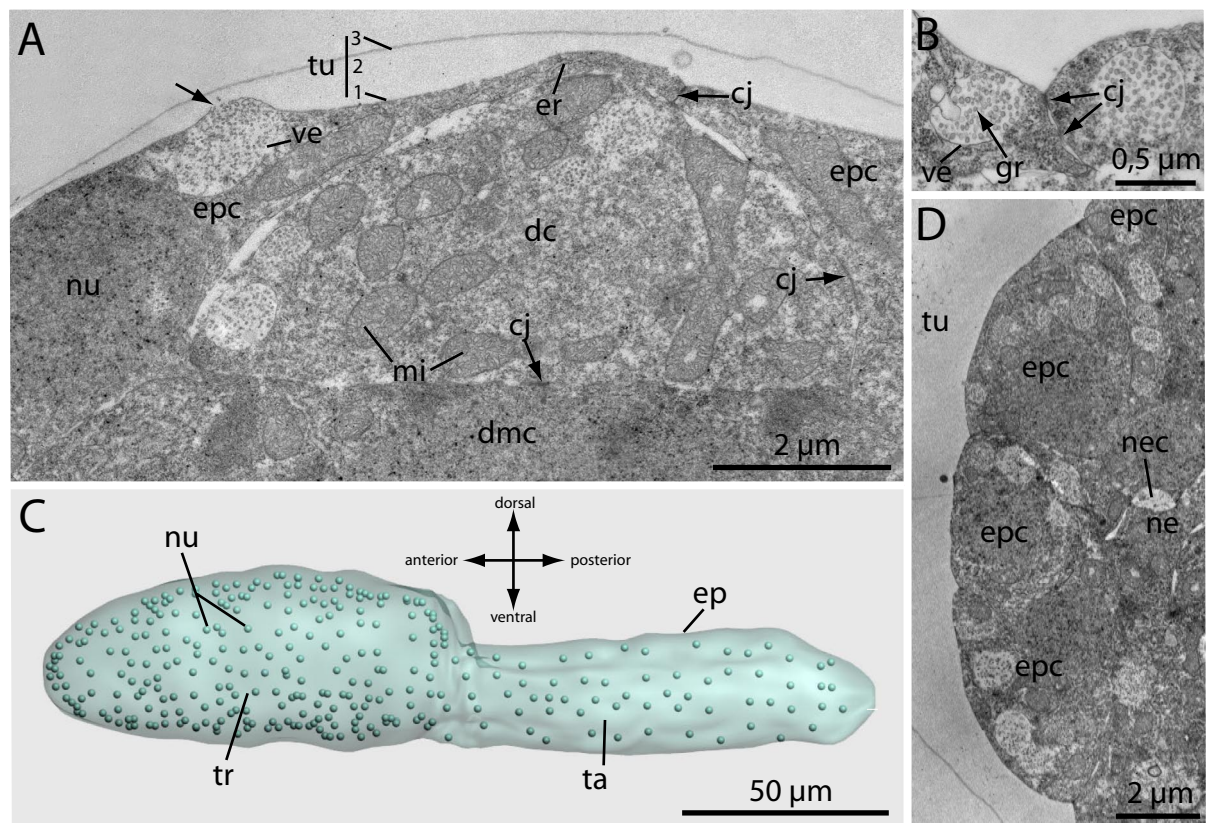


Fig. 58. Microscopic anatomy of epidermis in the hatched larva of *Oikopleura dioica*. 3D reconstruction and transmission electron micrographs at higher magnification. (A) Upper part of tail. (B) Junction between two neighboring epidermis cells. (C) 3D representation of epidermis of entire larva with nuclei of epidermis indicated by spheres. (D) Left part of tail with three cells that are probably prospective fin forming epidermis cells. For abbreviations, see Fig. S5 legend.

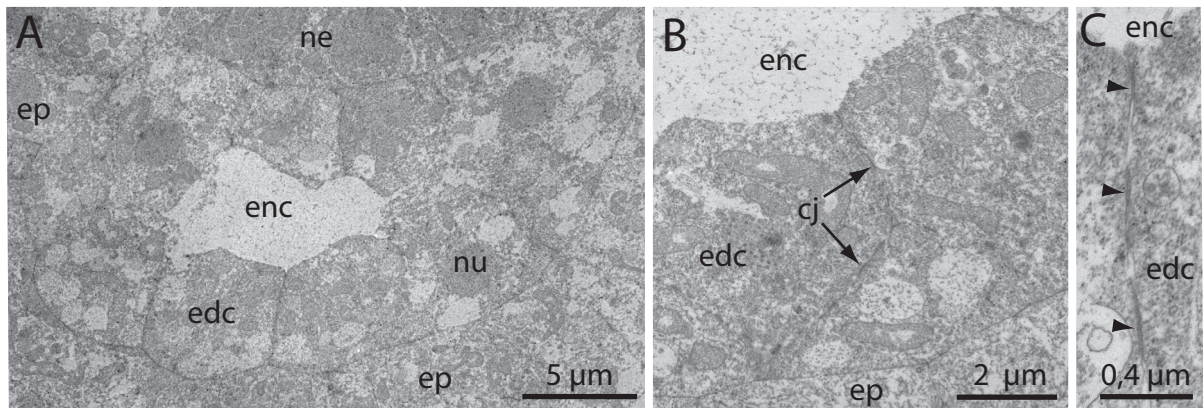


Fig. S9. Transmission electron micrographs of anterior endoderm in the hatched larva of *Oikopleura dioica*. For abbreviations, see Fig. S5 legend.

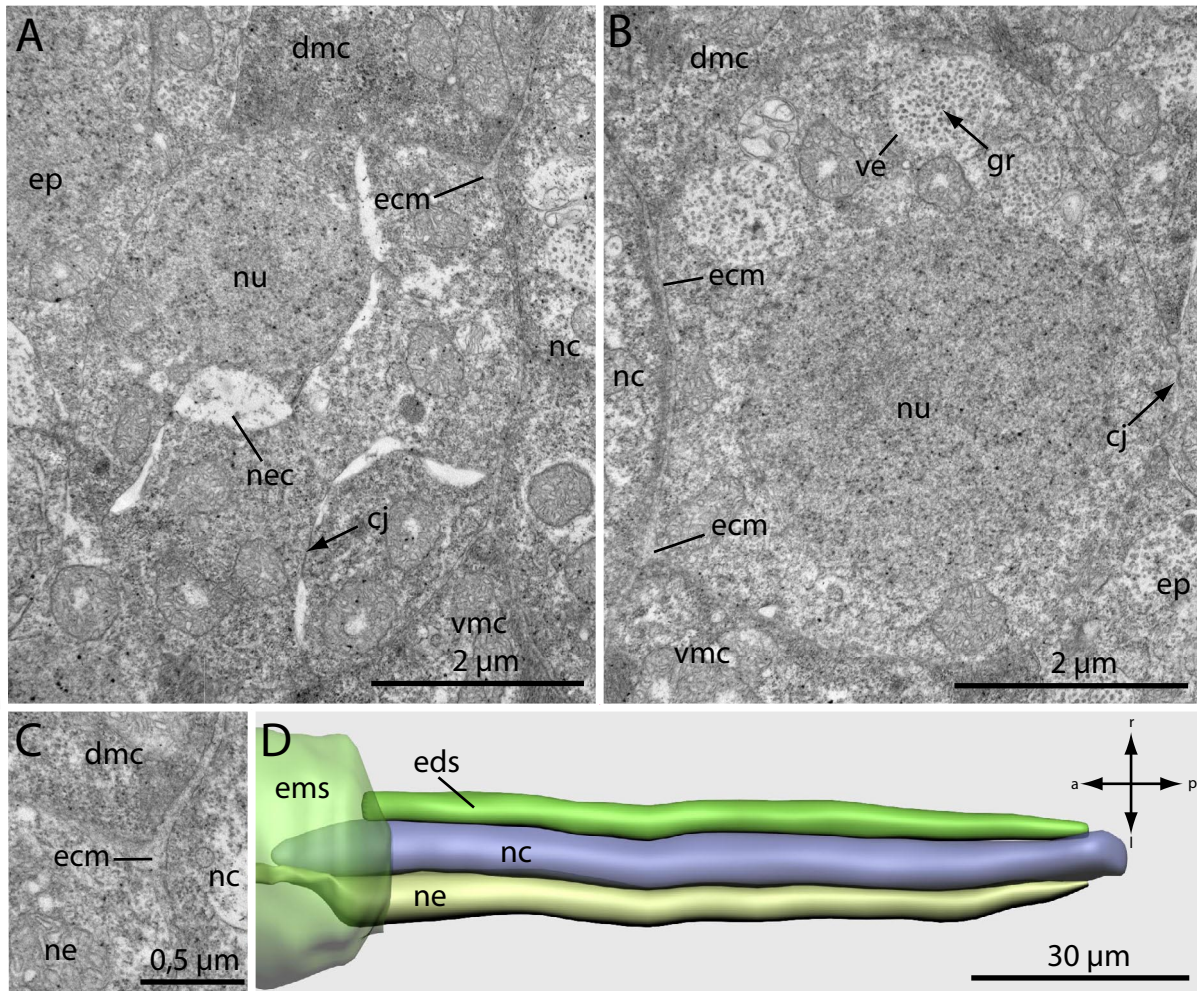


Fig. 510. Microscopic anatomy of the tail in the hatched larva of *Oikopleura dioica*. 3D reconstruction and transmission electron micrographs at higher magnification. (A) Nerve cord. (B) Endodermal strand. (C) Extracellular matrix surrounding the notochord at higher magnification. (D) 3D representation of the tail in dorsal aspect. For abbreviations, see Fig. S5 legend.

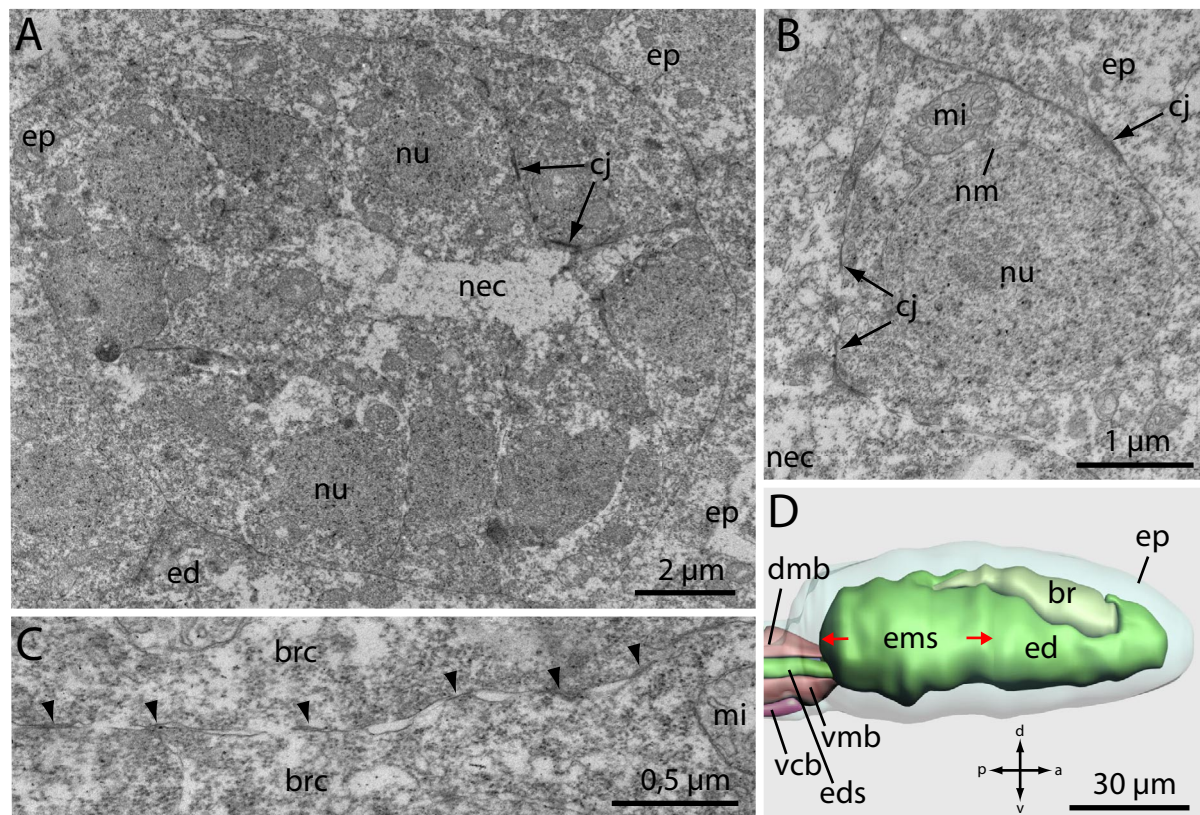


Fig. 511. Microscopic anatomy of the nervous system in the trunk of a hatched larva of *Oikopleura dioica*. 3D reconstruction and transmission electron micrographs at higher magnification. (A) Nervous system in anterior trunk. (B) Posterior brain region at the transition to the nerve connecting the brain with the nerve cord in the tail. (C) Cell junctions between nerve cells in anterior brain. (D), 3D representation of trunk. For abbreviations, see Fig. S5 legend.

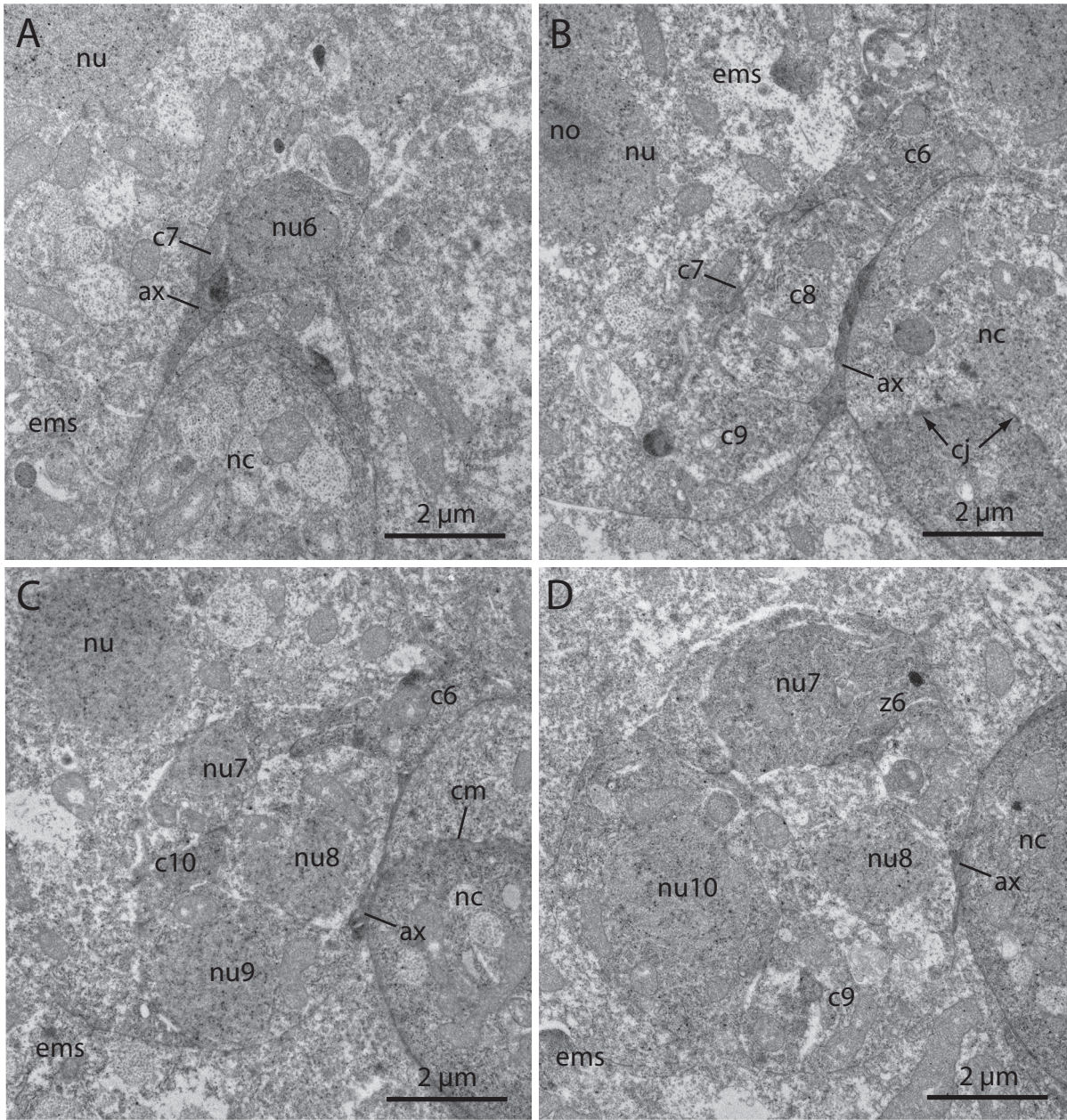


Fig. S13. Transmission electron micrographs of the nervous system of a hatched larva of *Oikopleura dioica*. (A–D) Consecutive sections from anterior to posterior; planes of section are indicated on the schematic 3D representation in Fig. S4G by the downward pointing arrows. For abbreviations, see Fig. S5 legend.

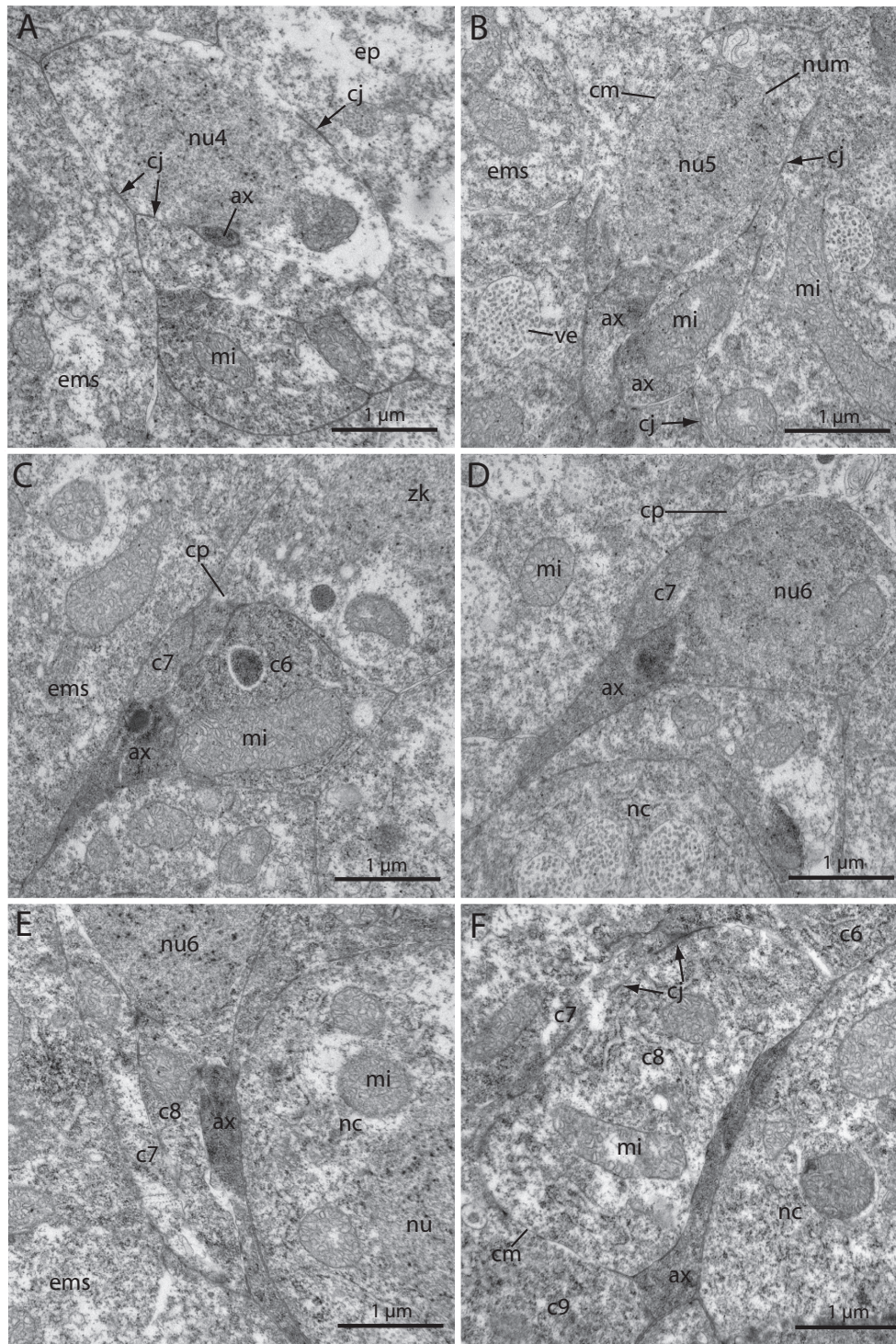


Fig. 514. Transmission electron micrographs of the nervous system of a hatched larva of *Oikopleura dioica* at the transition to the tail region. (A–F) Consecutive sections from anterior to posterior. Numbering of cells according to Fig. S4 G and H. For abbreviations, see Fig. S5 legend.

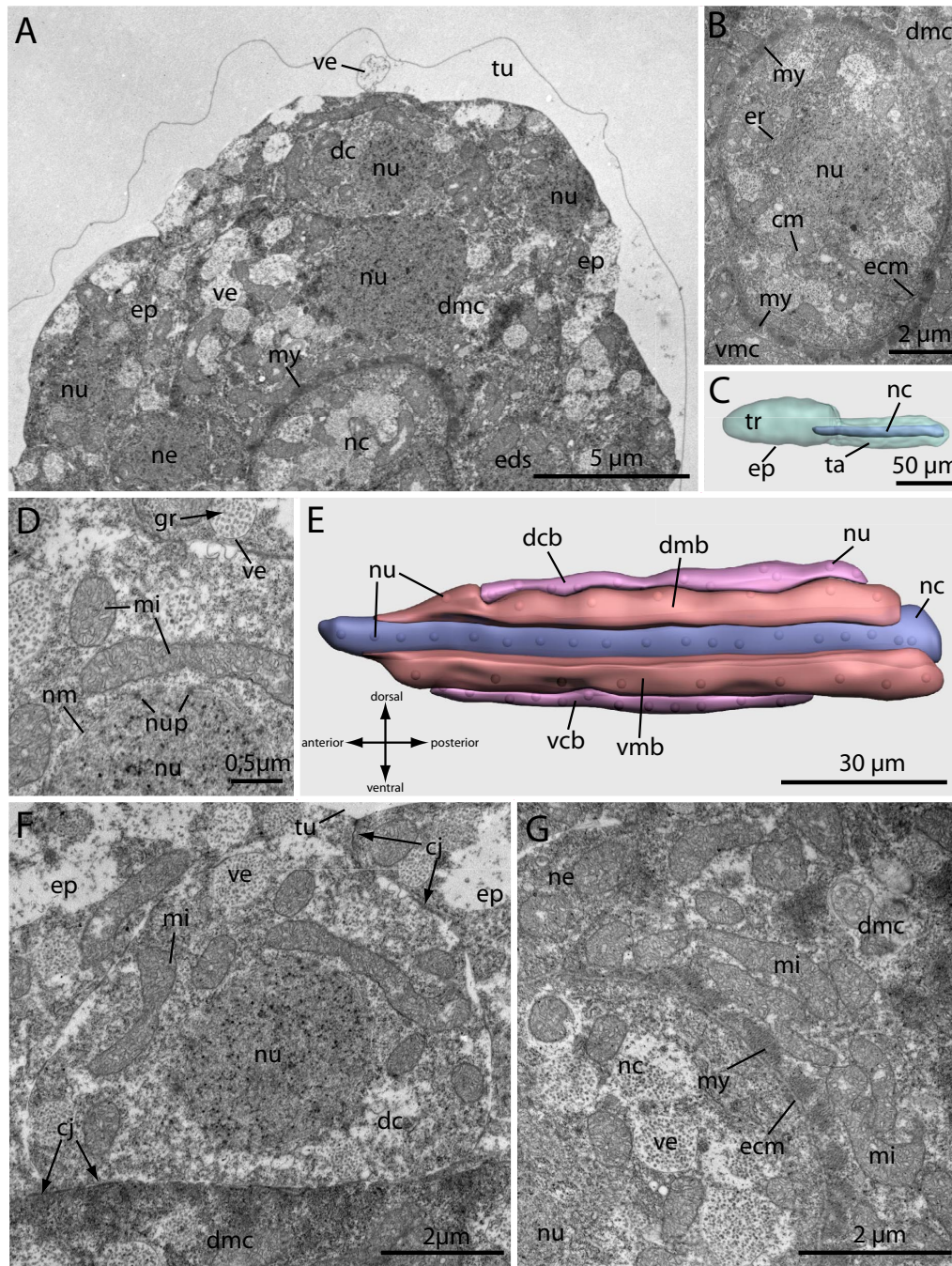
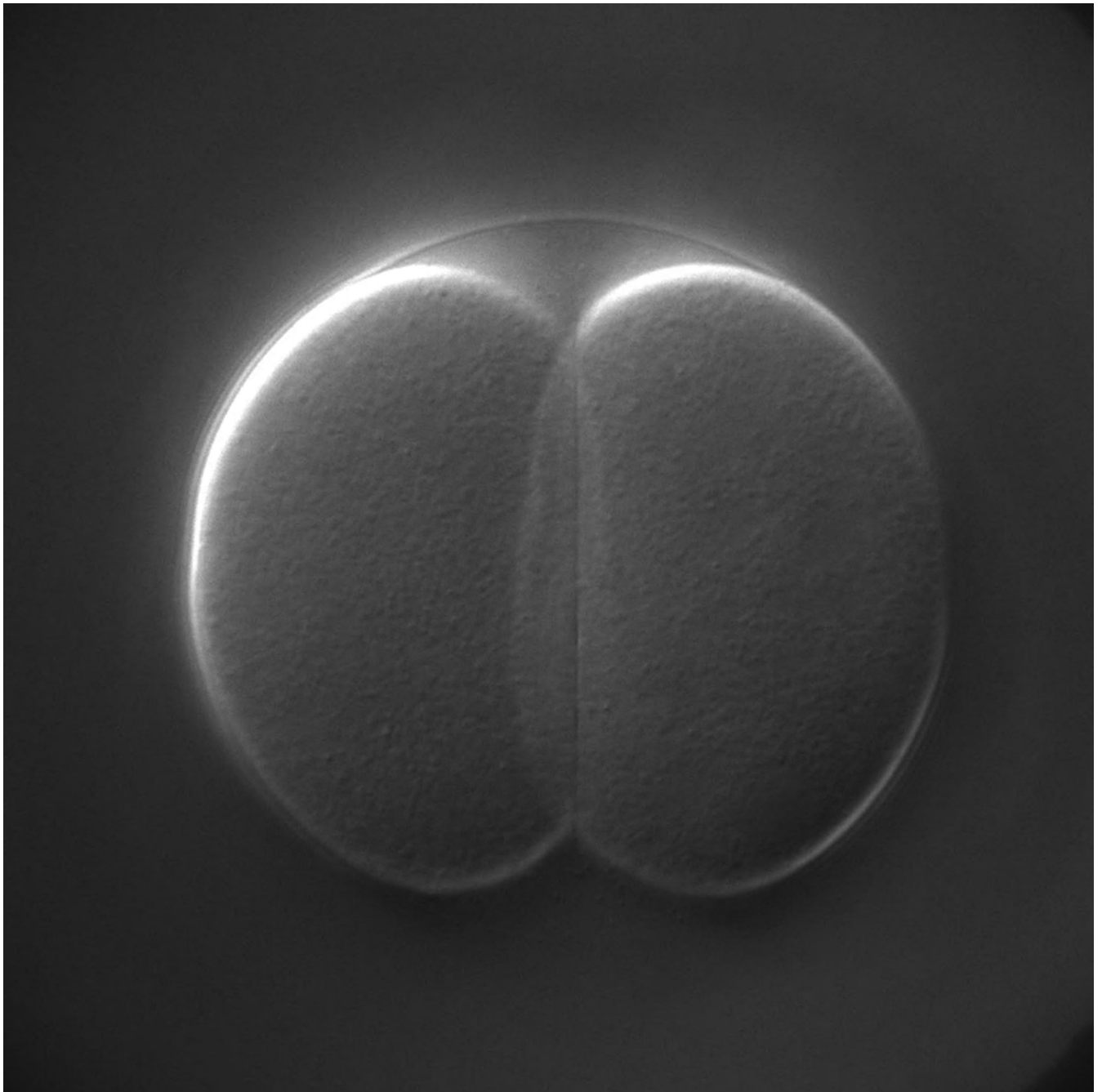
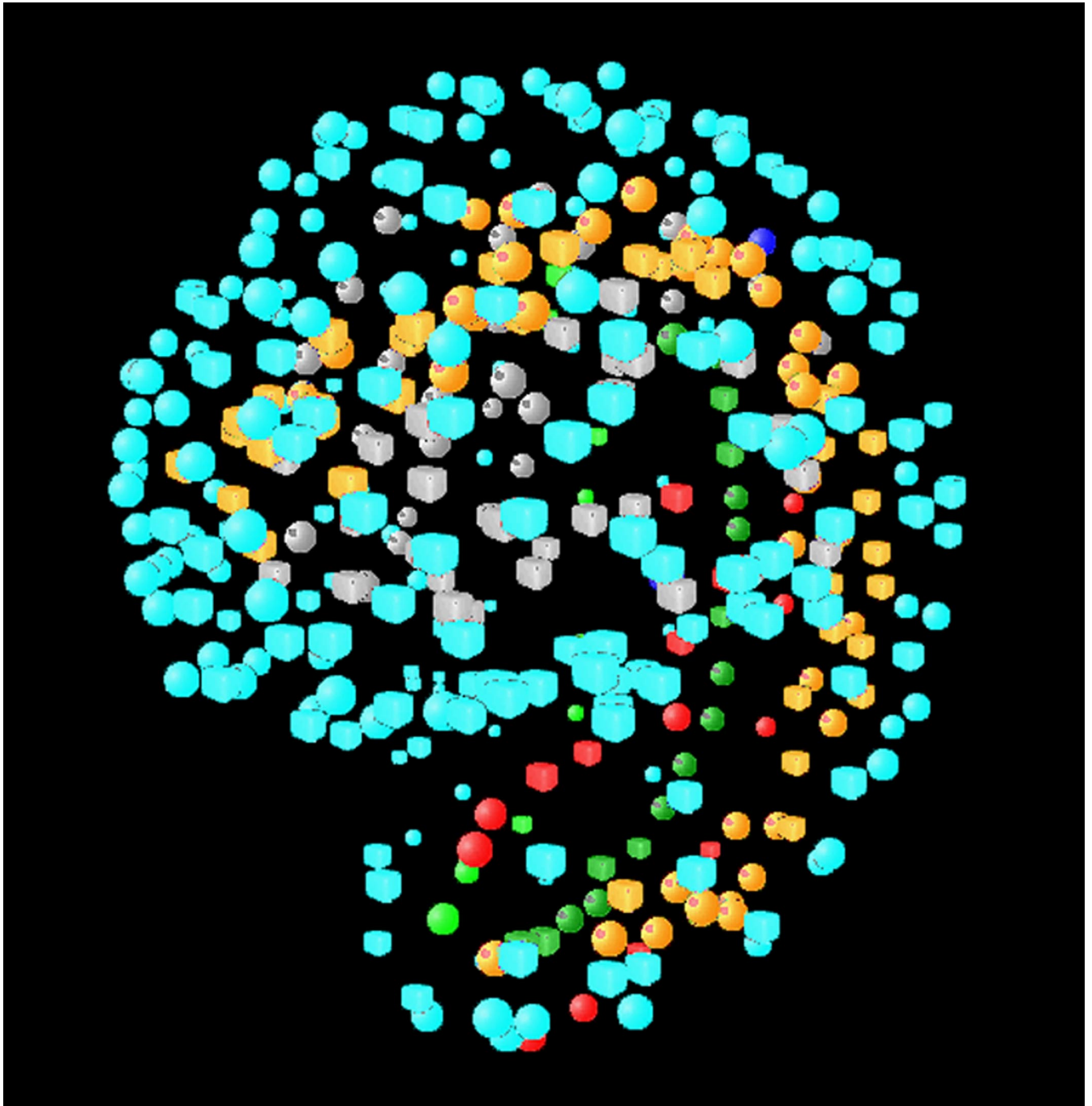


Fig. S15. Microscopic anatomy of the tail in the hatched larva of *Oikopleura dioica*. 3D reconstruction and transmission electron micrographs at higher magnification. (A) Cross section through tail. (B) Notochord. (C) 3D representation of complete larva in lateral aspect. (D) Higher magnification nuclear region of muscle cell. (E) 3D representation of the tail, without epidermis and nerve tube in lateral aspect. (F) Higher magnification of a cross section through a cell situated dorsal to the dorsal muscle cell, yet beneath the epidermis. (G) Higher magnification of myofibrillae in the dorsal muscle cell close to the notochord. For abbreviations, see Fig. S5 legend.



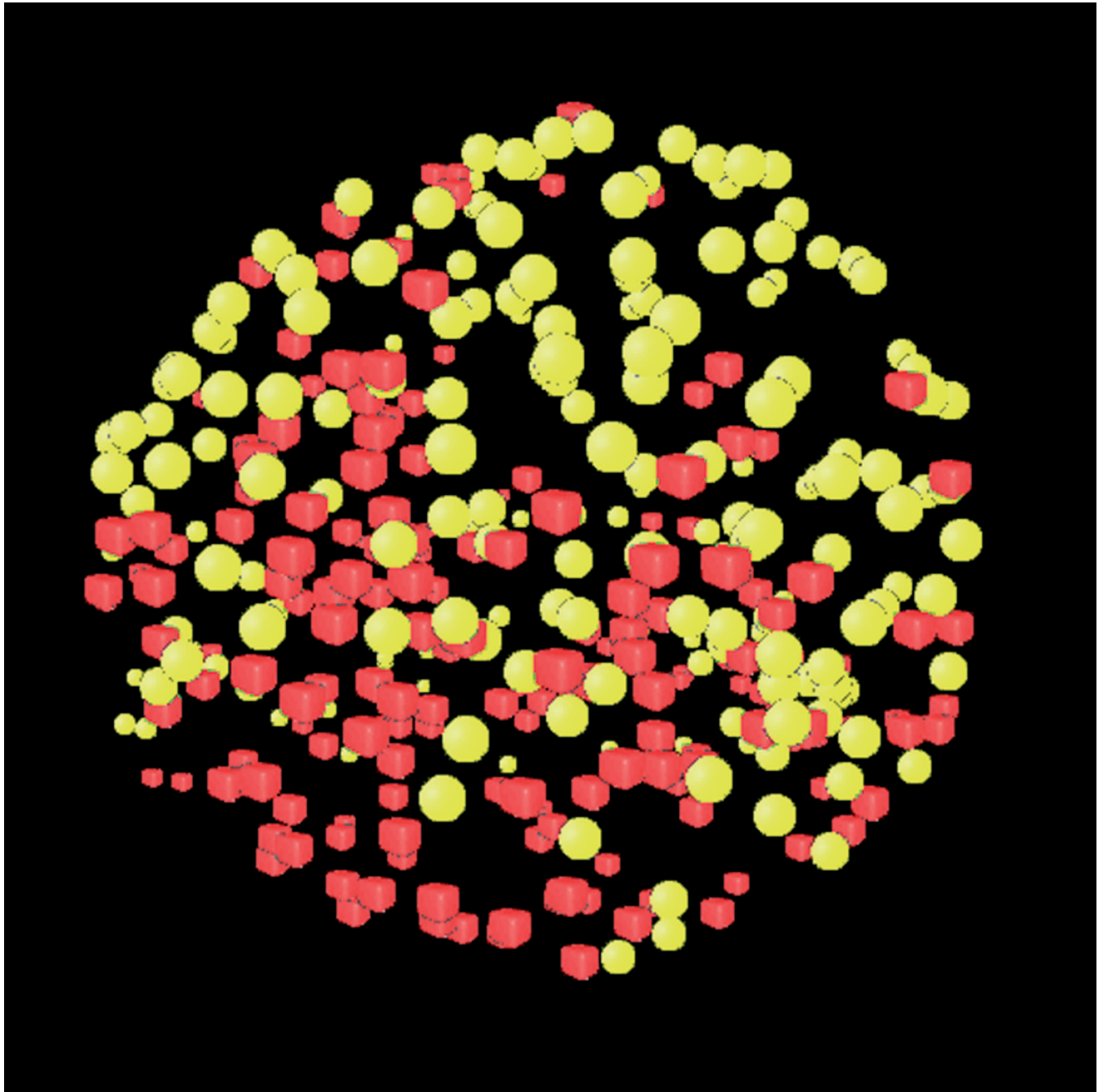
Movie S1. Time-compressed movie of the development of *Oikopleura dioica* in light microscopic aspect.

[Movie S1](#)



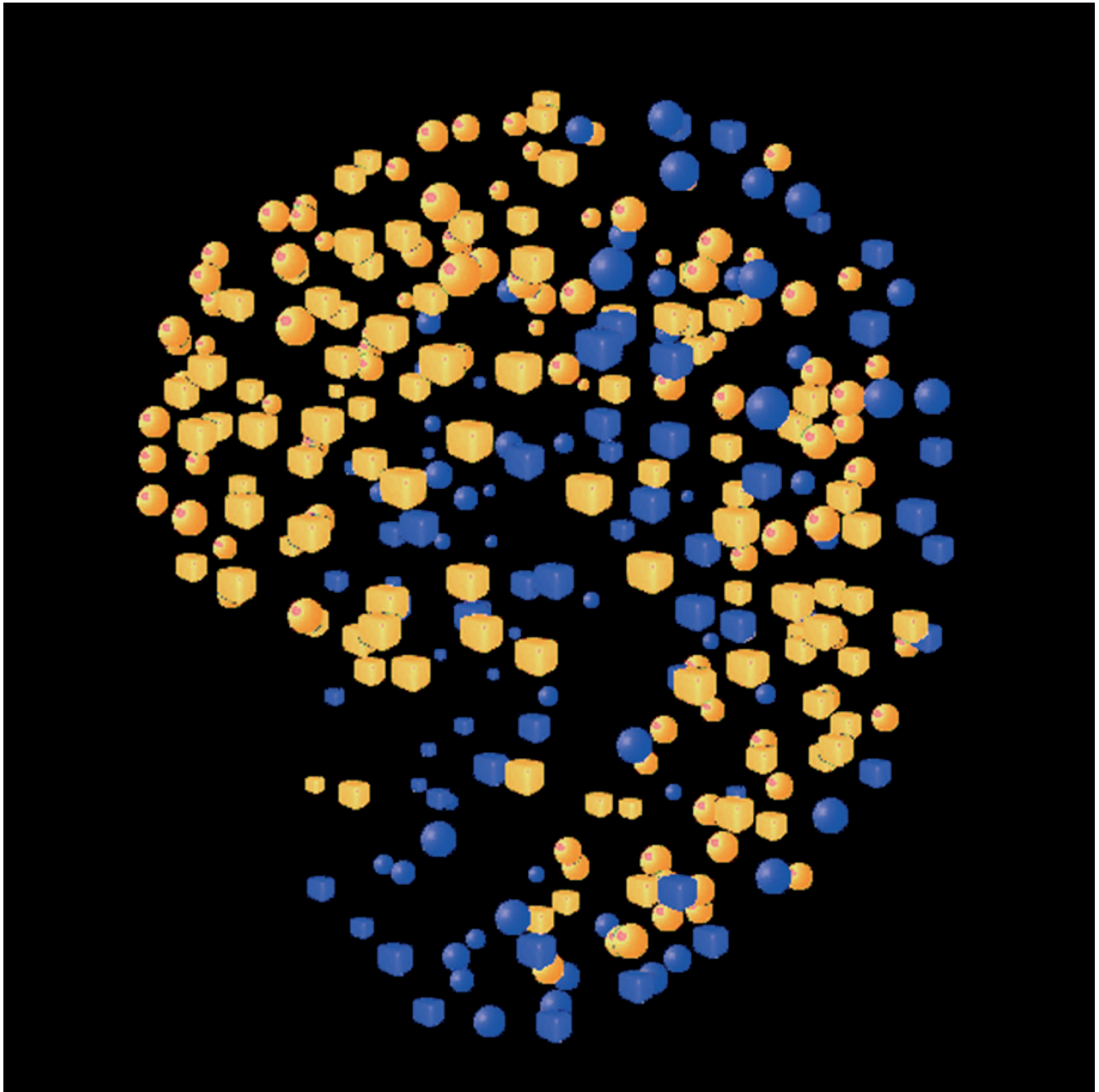
Movie S2. Development of *Oikopleura dioica* using 3D representations of the positions of the nuclei of each cell implemented in the 4D microscopy software SIMI*BioCell. Colors code for tissue fates as seen in Fig. 1. Aspect before hatching as in Fig. 3.

[Movie S2](#)



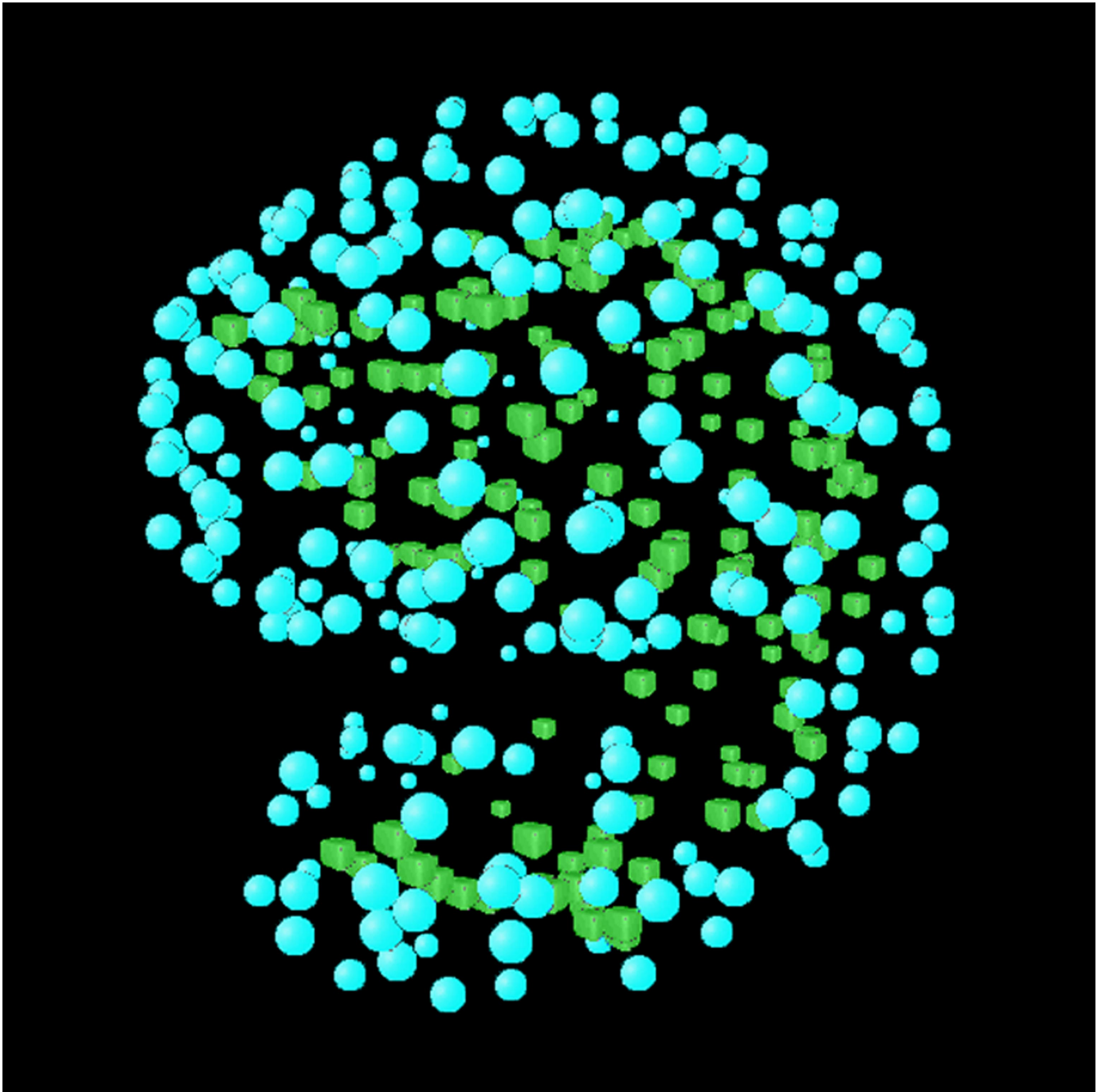
Movie S3. Movie of the development of *Oikopleura dioica*, using 3D representations of the positions of the nuclei of each cell implemented in the 4D microscopy software SIMI[®]BioCell. Virtual cell marking: red cubes, left sided cells derived from AB2; yellow spheres, right sided cells derived from AB2. Aspect before hatching as in Fig. 3, third column.

[Movie S3](#)



Movie S4. Movie of the development of *Oikopleura dioica*, using 3D representations of the positions of the nuclei of each cell implemented in the 4D microscopy software SIMI[®]BioCell. Virtual cell marking: blue, ventral cells (traditional "anterior"); orange, dorsal cells (traditional "posterior"); cubes, left-sided cells derived from AB2; spheres, right-sided cells derived from AB2. Aspect before hatching as in Fig. 3, fourth column.

[Movie S4](#)



Movie S5. Movie of the development of *Oikopleura dioica*, using 3D representations of the positions of the nuclei of each cell implemented in the 4D microscopy software SIMI[®]BioCell. Virtual cell marking: light blue, animal cells; light green, vegetal cells; cubes, left-sided cells derived from AB2; spheres, right-sided cells derived from AB2. Aspect before hatching as in Fig. 3, far right.

[Movie S5](#)

Table S1. Cell lineage characters for phylogenetic analysis and as estimates of heterochrony in chordate evolution.

Animal	Ref.	First cleavage, min	Blastula	Gastrulation	Neurulation, h	Fate restriction of tail musculature	Fate restriction of notochord	Fate restriction of nervous system	Fate restriction of epidermis
<i>Oikopleura</i>	This study	20	3	5	6	6	6	5	5
<i>Ciona</i>	1–5	60	5	7	10	6	7	6	5
<i>Halocynthia</i>	6–8	120	5–6	7–8	9–10	7	7	7	5
<i>Branchiostoma</i>	9–15	75	8	9–10	≈11	na	na	na	na
<i>Lampetra</i>	—	180–240	na	na	na	na	na	na	na
<i>Xenopus</i>	16, 17	90	na	na	24	na	na	na	na
<i>Ambystoma</i>	18	200	11	≈16	na	na	na	na	na
<i>Echinus</i>	19, 20	60–70	10	≈12	ne	ne	na	na	na
<i>Sphaerechinus</i>	20	—	9	—	ne	ne	na	na	na
<i>Patiriella</i>	21	40–60	6–9	30 h–35 h	ne	ne	na	na	na
<i>Saccoglossus</i>	22, 23	100–120	>8	13 h–24 h	44–95	ne	ne	na	na

Figures given without units are cleavage numbers approximately in the middle of the respective event. Fate restrictions are given as the earliest determination of a clonal line dedicated to a single tissue type. na, data not available; ne, not applicable.

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Table S2. Table to facilitate translation between the cell labeling systems used by Conklin (1) in roman and Delsman (2) in italics

Zygote	2-cells	4-cells	8-cells	16-cells	32-cells	62-cells	122-cells		
A	AB2	A3	A4.1 <i>A</i>	A5.1 <i>A²</i>	A6.1 <i>A²²</i>				
					A6.2 <i>A²¹</i>				
				A5.2 <i>A¹</i>	A6.3 <i>A¹²</i>				
					A6.4 <i>A¹¹</i>				
						a7.9 <i>a²²²</i>			
						a7.10 <i>a²²¹</i>			
			a4.2 <i>a</i>	a5.3 <i>a²</i>	a6.5 <i>a²²</i>				
					a6.6 <i>a²¹</i>				
				a5.4 <i>a¹</i>	a6.7 <i>a¹²</i>				
					a6.8 <i>a¹¹</i>				
				B3	B4.1 <i>B</i>	B5.1 <i>B²</i>	B6.1 <i>B²²</i>	B7.1 <i>B²²²</i>	
								B7.2 <i>B²²¹</i>	
		B6.2 <i>B²¹</i>							
		B5.2 <i>B¹</i>	B6.3 <i>B¹²</i>			B7.5 <i>B¹²²</i>			
						B7.6 <i>B¹²¹</i>	B8.11 <i>B¹²¹²</i>		
			B6.4 <i>B¹¹</i>				B8.12 <i>B¹²¹¹</i>		
		b4.2 <i>b</i>	b5.3 <i>b²</i>		b6.5 <i>b²²</i>				
					b6.6 <i>b²¹</i>				
					b6.7 <i>b¹²</i>				
			b5.4 <i>b¹</i>		b6.8 <i>b¹¹</i>				

Cells of the left side of the embryo are tabulated up to the stage where all tissue restrictions identified in the present study occurred. Nomenclature on the right side is the same with denominations (Conklin) or superscripts (Delsman) underlined, see also Fig. S1. Colors indicate tissue restrictions (see Fig. 1).

1. Conklin EG (1905) Organization and cell lineage of the ascidian egg. *J Acad Nat Sci Philadelphia*. 13(2):1–119, plates I–XII.

2. Delsman HC (1910) Beiträge zur Entwicklungsgeschichte von *Oikopleura dioica*. *Verhandelingen uit het Rijksinstituut voor het Onderzoek der Zee* 3:1–24.