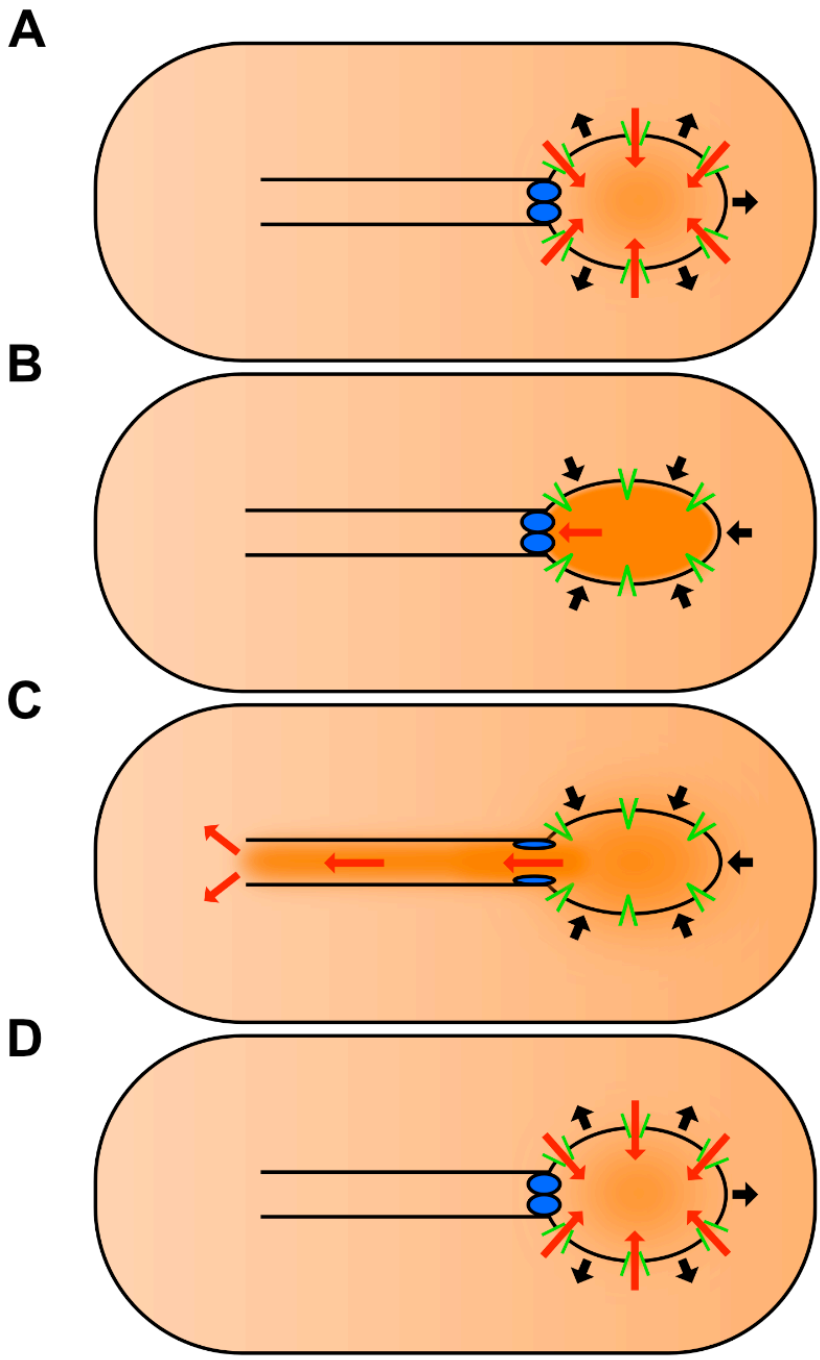


Fig. S1. Model of a contraction cycle of the larval heart. (A) Heart beat initiates by widening of the heart chamber, sucking in haemolymph (red arrows) via open ostia (green lines). Muscles of valve cells are relaxed, the valvosomal compartment defines the roundish shape of the cell (blue ellipses), allowing mechanical contact to the opposing valve cell and thereby an effective closing of the heart lumen. (B) Contraction wave starts posteriorly with contraction of the heart chamber leading to a closing of the ostia cells and an increasing pressure of the haemolymph inside the heart chamber against the still closed valve cells. (C) Contraction wave reaches valves cells leading to a deformation of these cells from roundish to an elongated shape due to the presence of the valvosomal compartment and a dense myofiber network in these cells. Haemolymph is pumped towards the anterior end and valves close the heart lumen directly after the contraction wave proceeds. (D) A new contraction cycle starts with widening of the heart chamber and haemolymph entrance.

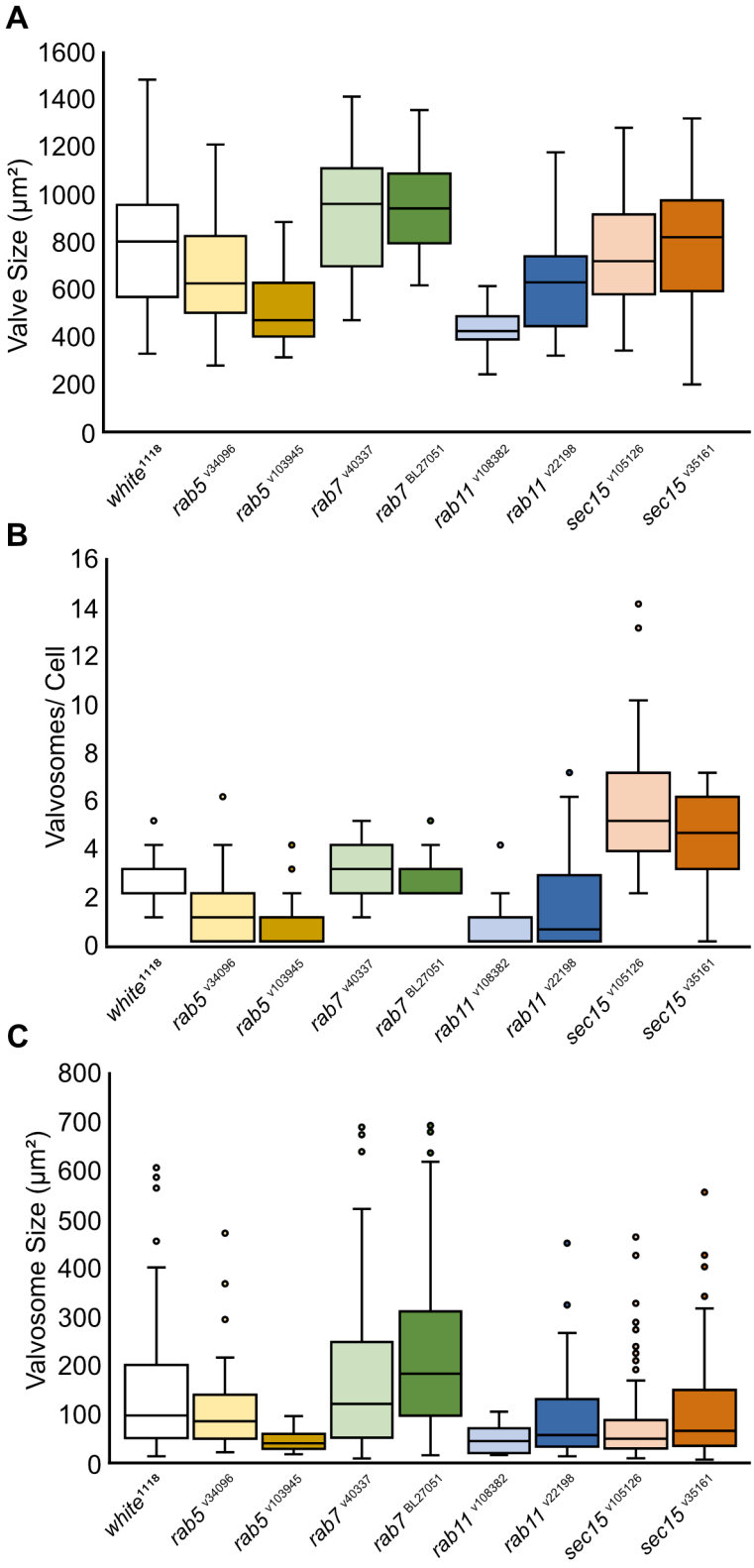


Fig. S2. Effect of heart specific knockdown of different Rab GTPases on valve cells. (A) Valve cell size, (B) Number of valvosomes per cell and valvosome size (C). Cells analysed: *white*^{1118} 38 cells, *rab5*^{v34096} 30 cells, *rab5*^{v103945} 40 cells, *rab7*^{v40337} 38 cells, *rab7*^{BL27051} 38 cells, *rab11*^{v108382} 38 cells, *rab11*^{v22198} 40 cells, *sec15*^{v105126} 38 cells, *sec15*^{v35161} 30 cells. All RNAi lines were driven by *hand*C-GFP; *hand*C-Gal4 line.

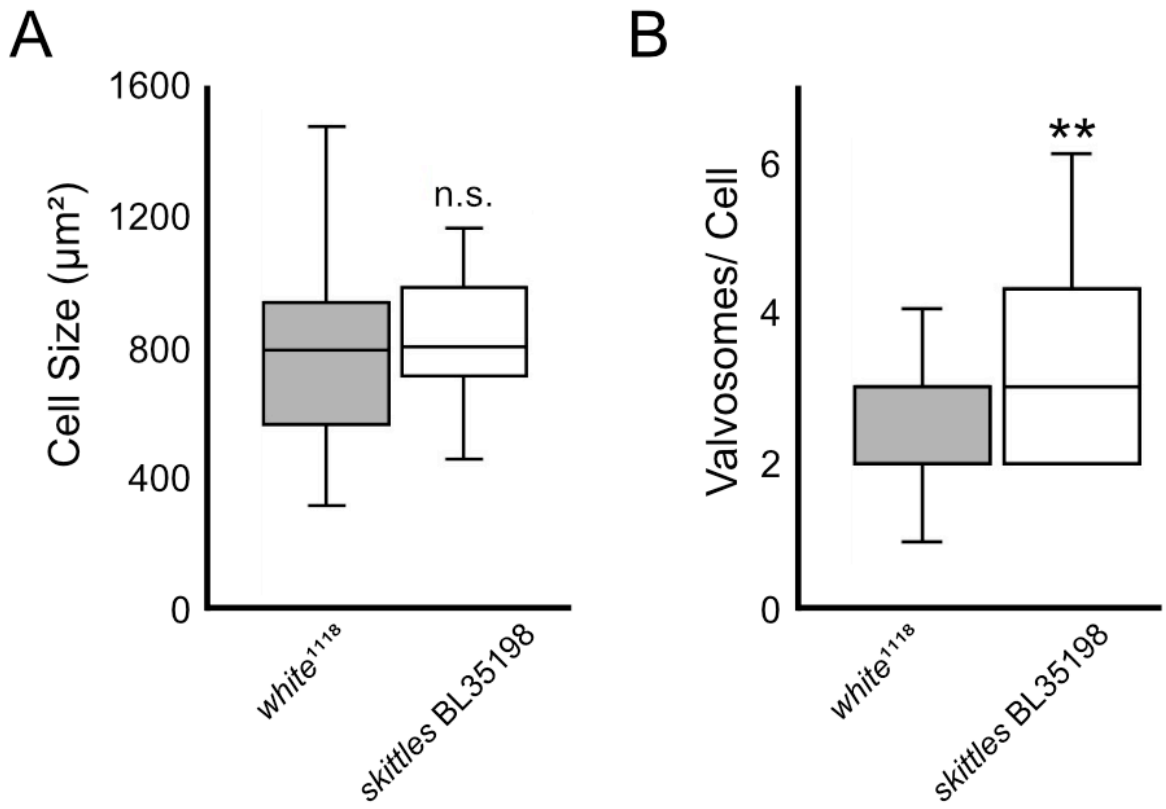


Fig. S3. Heart specific downregulation of *skittles*. Depletion of the PIP5 kinase skittles, has no effect on cell size (A) but leads to an increased number of valvosomes per cell (B). Cells analysed: *white*^{1118} = 38 cells, *skittles* (BL35198) = 40 cells (driven by *hand*C-Gal4).

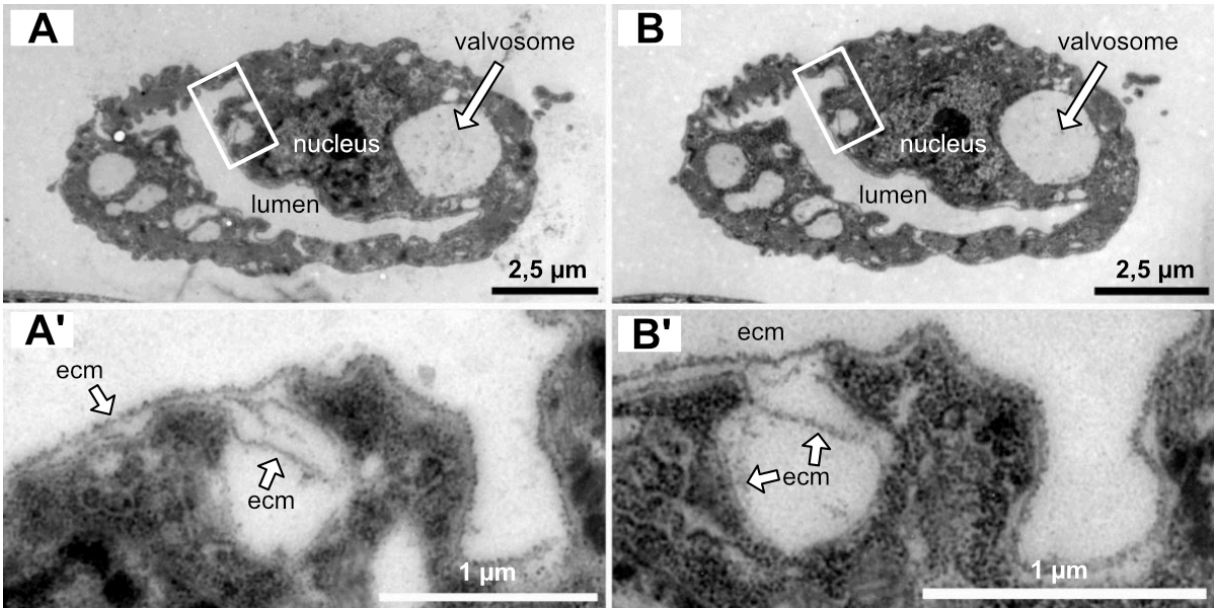


Fig. S4. TEM cross section of 1st instar larva valve cells. (A & B) Overview of larval valve cells with maturing valvosome and plasma membrane invaginations (boxed). (A'-B') Higher magnifications from A & B showing plasma membrane tubulation and presence of ECM on invagination pore.

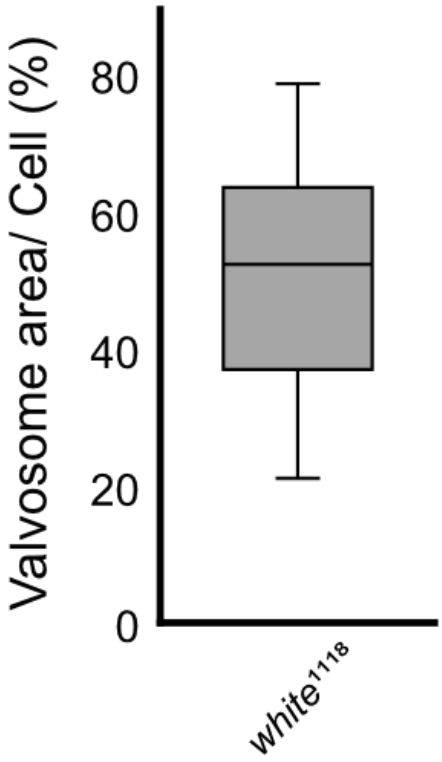


Fig. S5. The valvosomal compartment occupies most of the valve cell area. 40% to 65% of the cells´ area is occupied by the valvosomal compartment. Cells analysed *white*^{1118} = 38 cells. Confocal section through the nuclear plane were used.

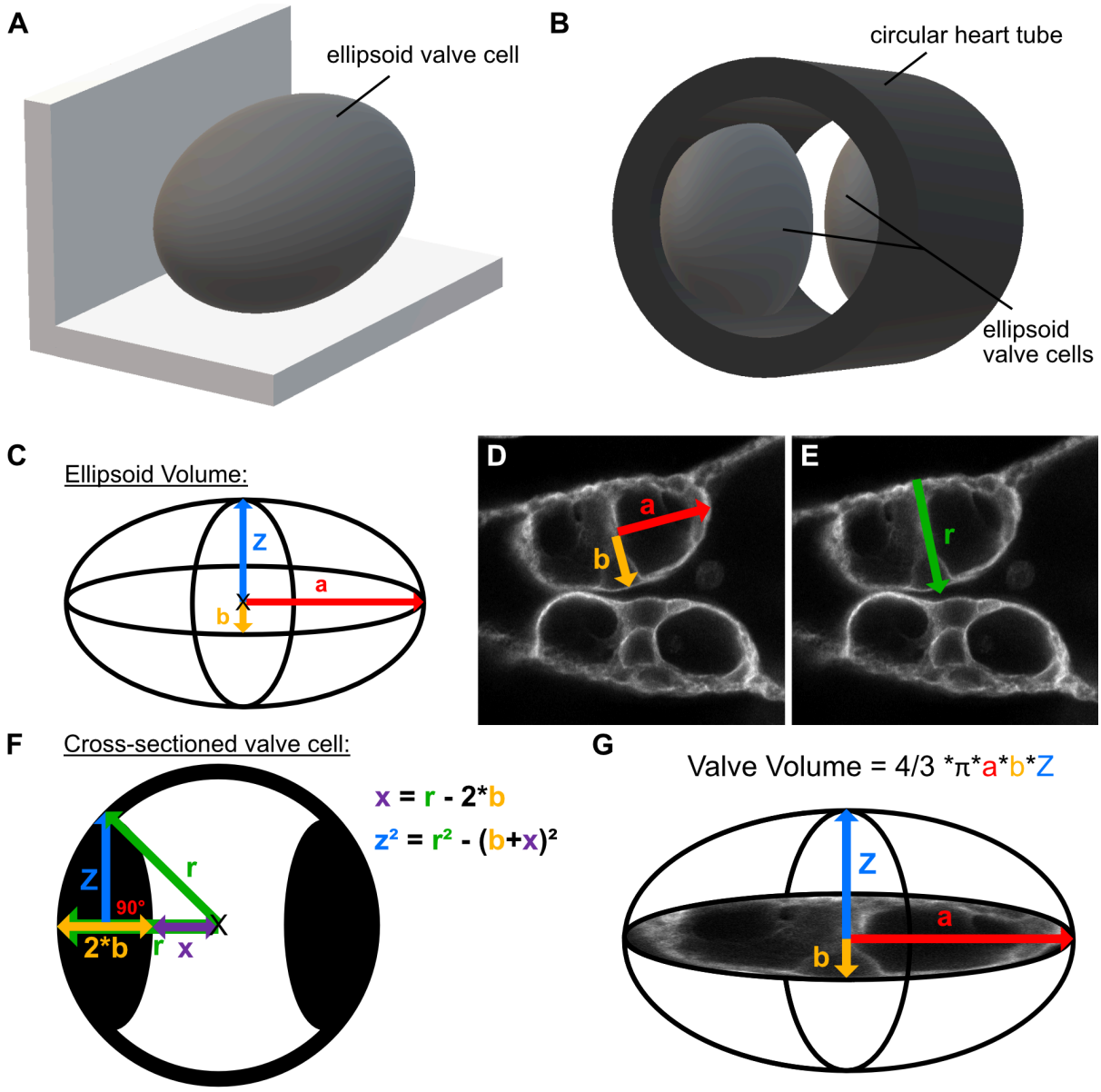
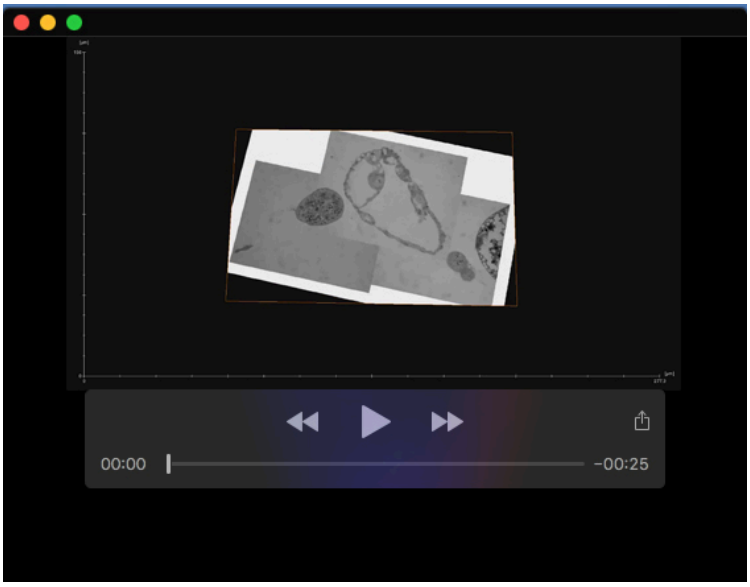
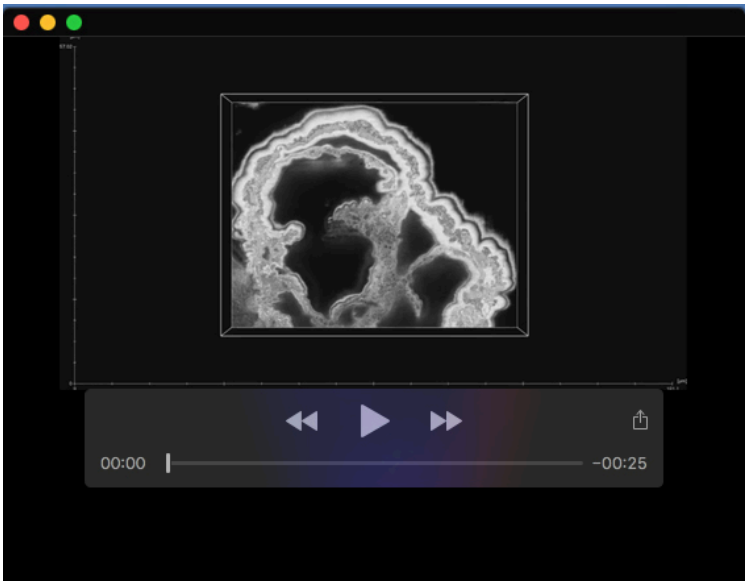


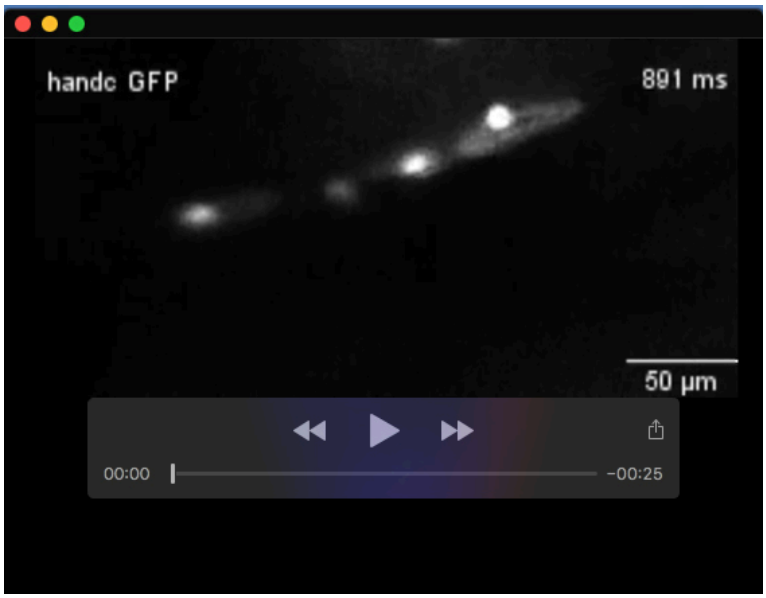
Fig. S6. Calculation of valve cells volume from confocal live recordings of intact 3rd instar larva. Volume calculation is possible assuming an ellipsoid shape of the valve cell (A) and a circular heart tube (B). For valve cell volume calculation three factors (*a*, *b* & *Z* in C) have to be determined. (D & E) Scheme illustrating points of measurements. Factors a and b were determined as half of the width/ length of the valve cell respectively and radius r as half of the total heart tube diameter at the position of the valve cells. (F) Applying the theorem of Pythagoras, the depth of the cell, factor Z, is determined. The three factors a, b, and Z were determined for the cell in the closed and in the open state and volume was calculated with the given formular (G).



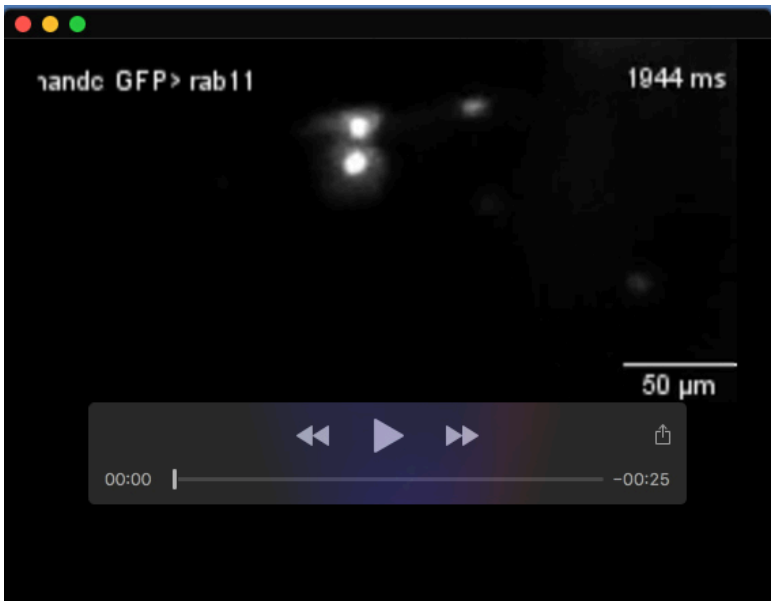
Movie 1. 3D reconstruction of valve cells and valvosome from 700nm semi-thin sections.



Movie 2. Serial Block Face analysis of valve cells and 3D reconstruction reveals connections between valvosomes.



Movie 3. Slow motion video (6fps) of intact *Drosophila* larva valve cells utilising a *hand*C-GFP reporter line captured for 10 sec at ~81ms/ frame (12 fps).



Movie 4. Slow motion video (6fps) of intact *Drosophila* larva valve cells utilising a *hand*C-GFP reporter *hand*C-Gal4 line driving RNAi of *rab11*, captured for 10 sec at ~81ms/ frame (12 fps).