

Supporting Online Material for

Optogenetic Control of Cardiac Function

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Materials and Methods

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Additional supplemental material includes the following:

Movies S1 to S11

Supporting Online Material

Materials and Methods

Transgenic fish

Zebrafish used for this study were in the TL background and transgenic for a combination of *Tg(E1b:Gal4)s1101t*, *Tg(UAS:NpHR-mCherry)s1989t*, *Tg(UAS:eNpHR-mCherry)s1988t*, *Tg(UAS:ChR2(H134R)-eYFP)s1990t*, *Tg(UAS:Kaede)s1999t*.

Transgenic lines were generated using the tol2 transposase system as described elsewhere (*SI*). Animals were raised in the dark and treated with PTU on the first day post fertilization to prevent pigment synthesis. Since transgene expression varied between fish of the same clutch and between clutches, animals were sorted about two hours prior to imaging. For sorting, the animals were placed under an epifluorescence dissecting microscope and the magnification changer dial was used to concentrate the light and assess how much light was needed to stop the heartbeat (in the case of NpHR fish). Only hearts that responded at relatively low light levels were used in the study. As an estimate, we used the best 20-33 % of the fish. We did not observe any long-term effects on cardiac function from these manipulations, even after repeated photostimulation over several hours.

DMD stimulation

DMD setup. For patterned illumination, a digital micromirror device (DMD) was coupled into the SPIM microscope (Fig. S3). We used the DMD and light bulb of a consumer market video projector (IBM iLM300). The video projector was partially disassembled to remove optical elements in front of the DMD. The DMD was driven via VGA signals from the computer, and the VGA signals were specified from within the custom Labview microscope software.

DMD pattern generation. Patterns could be generated in two ways. First, arbitrary regions of interest (ROIs) could be drawn on the live image within LabVIEW, which allowed instantaneous illumination of the sample under visual inspection. Second, a sequence of pre-programmed ROIs could be displayed automatically at pre-defined time

points during a recording. For some hearts we did not use automated techniques to localize pacemaker cells. Instead, we exploited the fact that in mSPIM the embryo can be illuminated and imaged from different angles by moving (3 axes) and rotating (1 axis) it in between the three objective lenses. We used the rotational axis to find both i) the optimal angle at which stimulation on one side of the SAR but not the other blocked heartbeats and ii) the angle at which stimulation on either side had the same effect (orthogonal to the optimal angle). With this protocol the location of the pacemaker cells was determined with a precision of 15°.

Cell numbers. The reported cell numbers in the main text correspond to the number of cells contained in the illuminated ROI. It is likely that adjacent cells were also hyperpolarized to a certain degree due to electrical coupling.

Light intensities. At full power (intensity=255), the light intensities were 150 mW/mm² (without filter), 60 mW/mm² (red filter, NpHR) and 16 mW/mm² (blue filter, ChR2). Light intensities used in experiments with NpHR expressing hearts were typically around 30 mW/mm².

Kinetics. The kinetics of the NpHR induced cardiac arrest (Fig. S7) was in agreement with reported time constants for NpHR ($\tau_{on} = 36$ ms (S2)). The pacemaker's susceptibility for membrane potential manipulation was similar over the time course of the heartbeat at the tested light intensity (30 mW/mm², Fig. S7).

Analysis

Heart function during depolarization (ChR2) or hyperpolarization (NpHR) of the illuminated regions was monitored in real time with high-speed recordings (55-132 frames/s) of optical sections of the zebrafish heart using two Andor EMCCD cameras DV885. Image analysis was performed in Matlab. The intensity fluctuation of an individual pixel over time was plotted. To find a suitable pixel, a kymograph (x-z projection) of the image time stack was visually inspected, and a line was selected from this kymograph. A peak finder algorithm (S3) was used to identify peaks automatically. It proved helpful to adjust a single parameter in the peak finder algorithm for every recording empirically since peak shapes could be different in each recording. The identified systolic time points were then combined with the sequence of ROIs used in the

recording to create heart rate maps. Sound files were generated using the systolic time points.

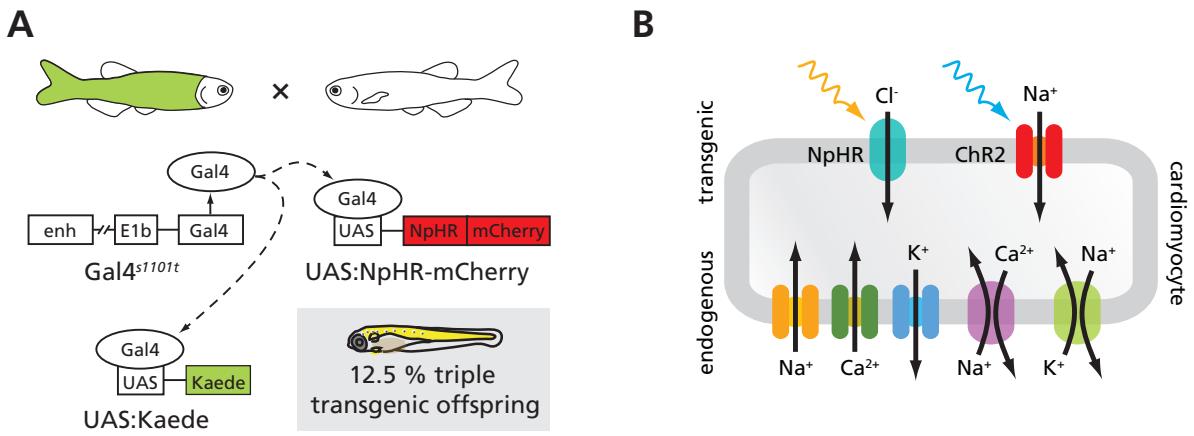


Figure S1: Zebrafish expressing NpHR and ChR2.

(A) The Gal4-UAS system was used to drive tissue specific expression of the fluorophore Kaede as well as NpHR-mCherry. enh, enhancer; E1b, basal promoter. (B) Besides the endogenous ion channels for Na⁺, Ca²⁺ and K⁺, the transgenic hearts also express the photo-activatable Cl⁻-pump NpHR or the photo-activatable cation channel ChR2. Application of orange and blue light hyperpolarizes and depolarizes the cardiomyocytes, respectively.

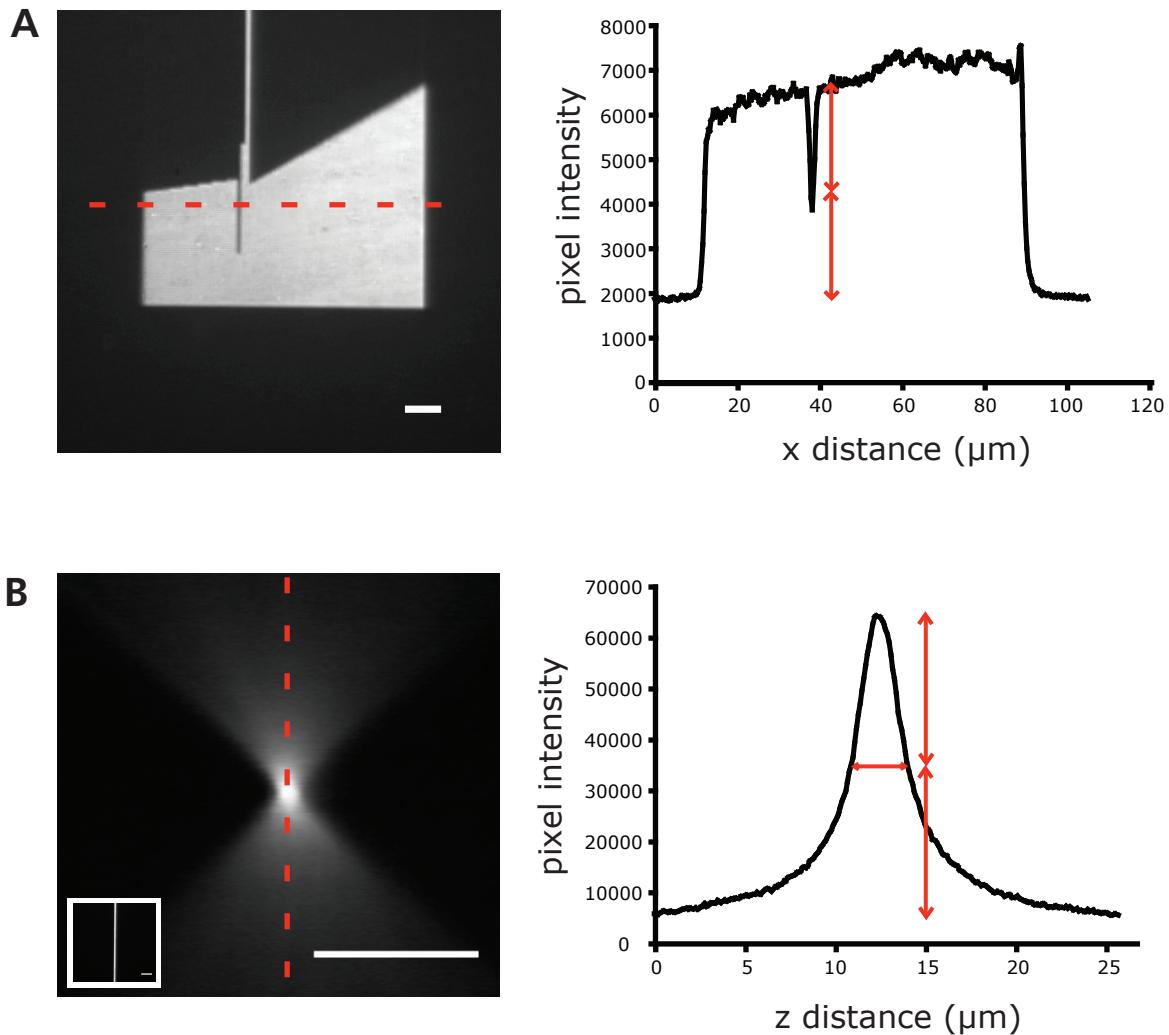


Figure S2: Resolution of the DMD illumination.

(A) A region of interest containing a $2.3 \mu\text{m}$ slit was illuminated with the DMD. The pixel intensity along the red dashed line is plotted to the right. The pixel intensity dropped more than 50 % at the slit (red arrows), indicating that the x-y resolution of the DMD was $2.3 \mu\text{m}$ or better. (B) A vertical bar was generated with the DMD (inset). A z-stack was recorded and an x-z projection is plotted on the left. The z-resolution was $8.9 \mu\text{m}$, which corresponds to the peak width at half maximum (red arrows). Scale bars, $10 \mu\text{m}$.

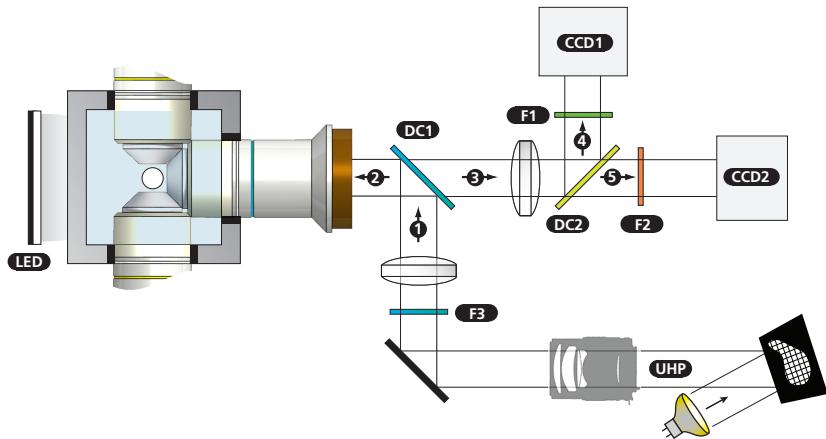
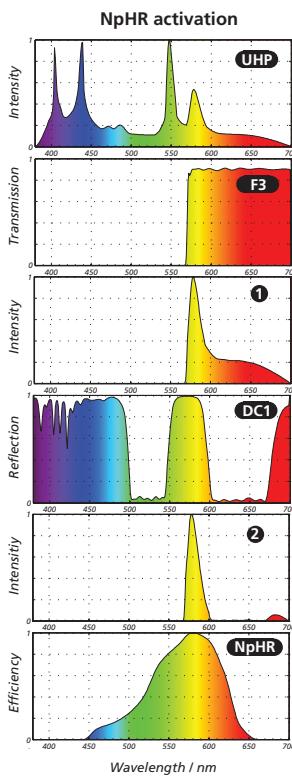
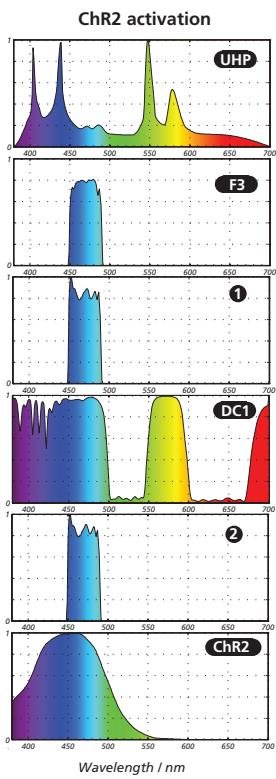
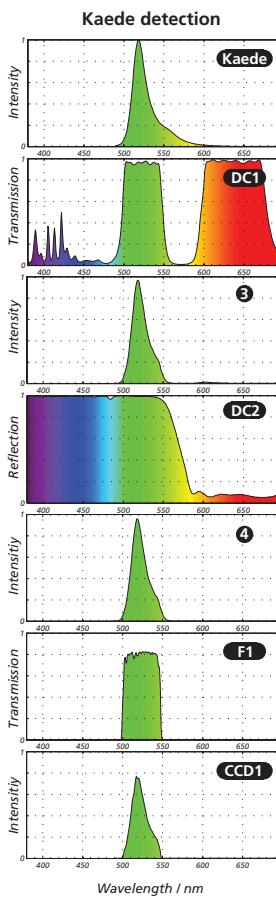
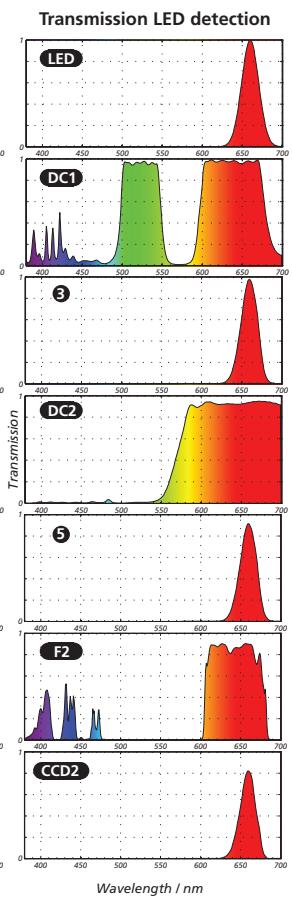
A**B****C****D****E**

Figure S3: Optical layout of the microscope with spectra for illumination of NpHR and ChR2 and detection of Kaede and transmission light.

(A) The fluorescence from the sample and the transmitted light are imaged onto two cameras, CCD1 (green channel) and CCD2 (red channel). DMD stimulation patterns are imaged onto the sample at the same time. Dichroic mirrors (DC), mirrors and filters (F) fold the beam paths and define the spectrum of illumination and detection. The locations of the three filters F1-F3, the two dichroics DC1-DC2, and five sample points are indicated. (B) Spectrum of the ultra high pressure (UHP) lamp as it passes through filter F3 (HQ572LP) used for the activation of NpHR. The dominantly yellow light is reflected by the dichroic DC1 towards OL3. NpHR is efficiently activated around its absorption maximum at 580nm. (C) For activation of ChR2, the bandpass HQ470/40x was chosen for F3. DC1 reflects all of the blue light to activate ChR2 around its maximum at 460nm. (D) The emission spectrum of Kaede passes through DC1 and is reflected off DC2 before it reaches F1 and CCD1. (E) The light from the transmission LED passes through DC1, DC2, and F2 before it reaches CCD2. Both Kaede and the LED are well detected on CCD1 and CCD2, respectively.

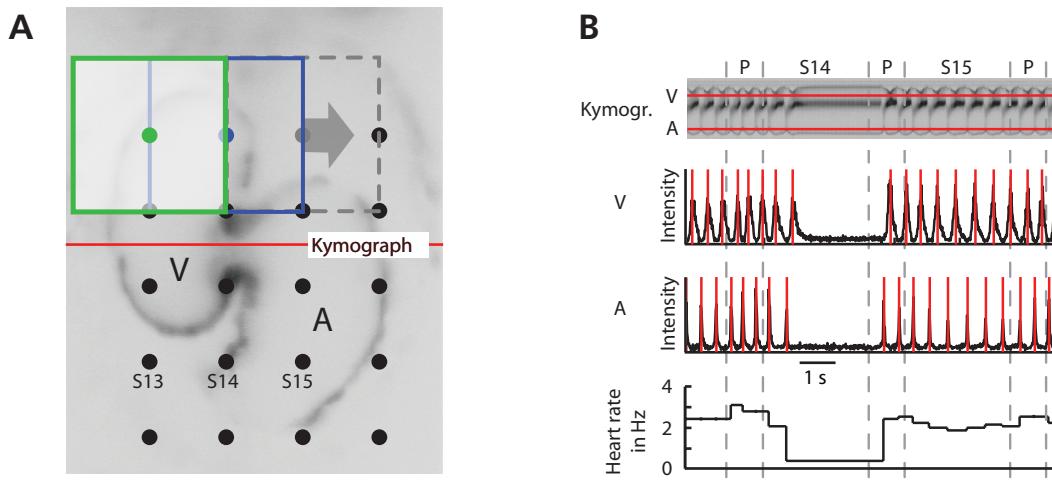


Figure S4: Automated mapping of the cardiac pacemaker using patterned illumination and image analysis.

(A) The whole heart was sequentially illuminated with overlapping squares (see movie S2). (B) The intensities in two lines of the kymograph were analyzed to extract the beat rate of the atrium (A) and ventricle (V). A short section of the kymograph, the intensities along the two lines and the heart rate are shown for samples S14-S15, taken at the locations shown in (A); P, illumination pause.

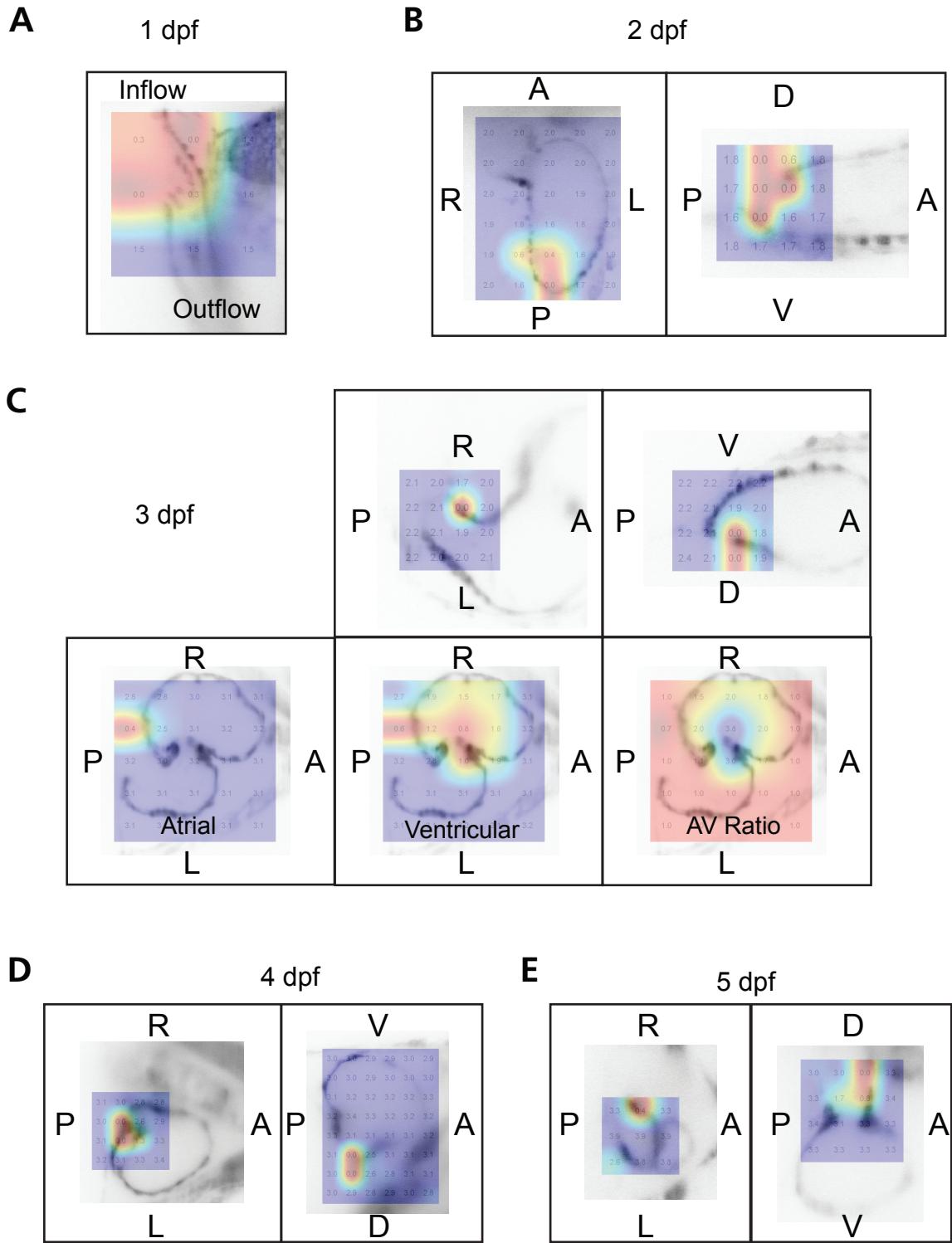


Figure S5: Heart rate maps from animals ranging from 1 dpf to 5 dpf.

(A) At 1 dpf the heartbeat was blocked by illumination of the inflow region. **(B)** In a 2 dpf heart the pacemaker region was slightly biased to the right (left image) and more strongly biased towards the dorsal side of the SAR (right image). **(C)** Top: The center of the pacemaker region was in the dorsal-right quadrant of the SAR in this 3 dpf heart. Bottom: Heartbeats were blocked when the right side of the SAR was illuminated (left image). In addition, the heart developed AV blocks when the AV canal was illuminated (middle and right images). Also see movie S2 of this heart. **(D, E)** The pacemaker region remained in the dorsal-right quadrant of the SAR at 4 and 5 dpf. A=anterior, P=posterior, R=right, L=left, D=dorsal, V=ventral.

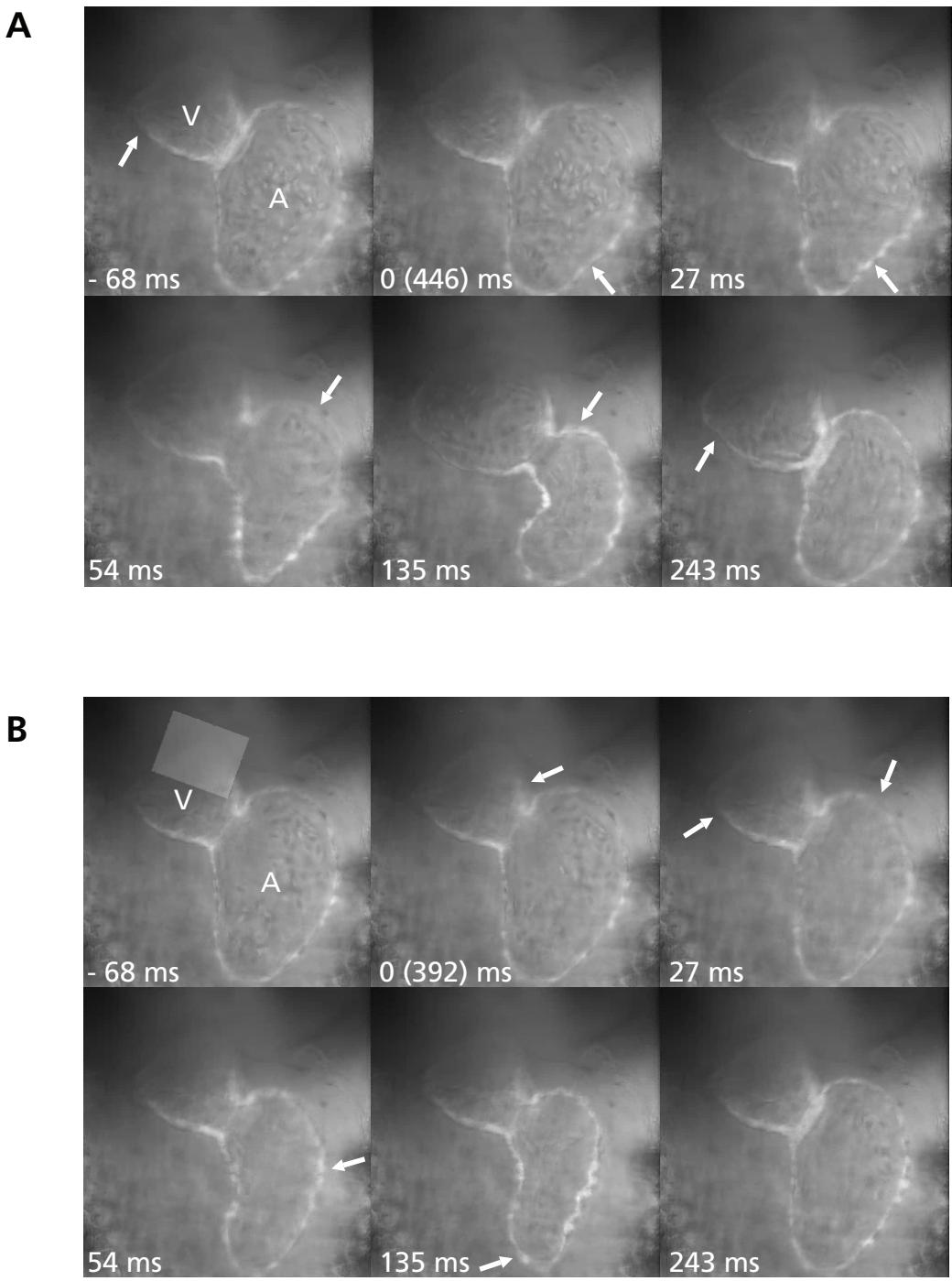


Figure S6: ChR2 stimulation in the distal ventricle reverses cardiac conduction.

(A) Still images from movie S11 show that in the absence of stimulation cardiac conduction progresses from inflow (0 ms) to atrium (54 ms) and ventricle (243 ms). White arrows indicate the location and direction of contraction. Each heartbeat cycle took ≈ 446 ms. (B) The same analysis as in (A) for the ChR2 stimulated case. The heart was stimulated at the outflow region (-68 ms, shaded rectangle). Cardiac conduction progressed from ventricle (0 ms) to atrium (27-54 ms). The SAR contracted last (135 ms). A, atrium; V, ventricle.

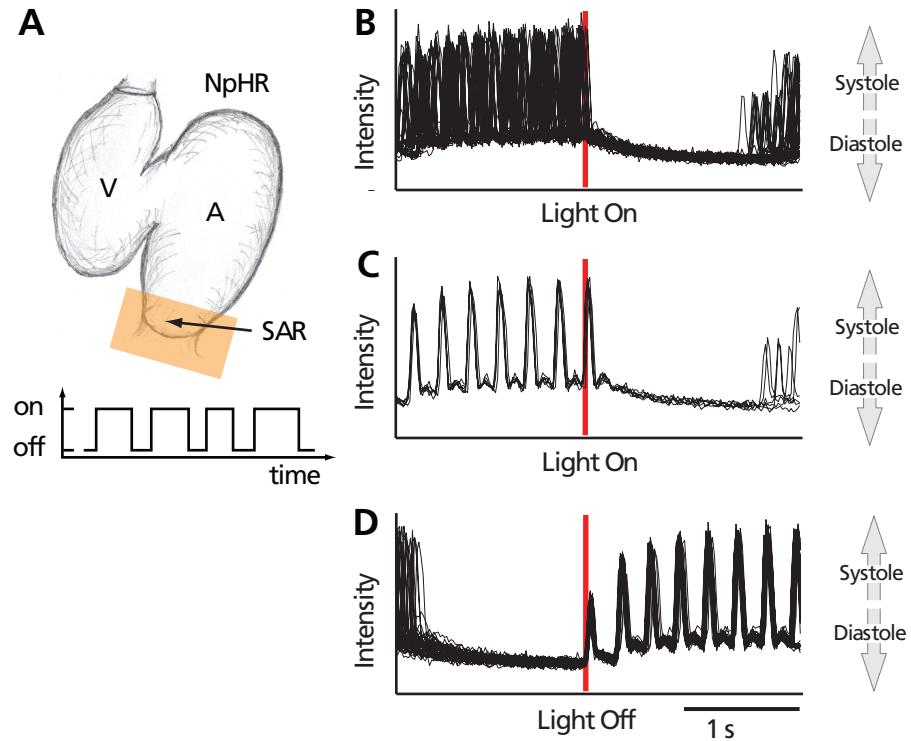


Figure S7: The heart stops instantaneously upon illumination and the recovery is precisely timed.

(A) Schematic of the spatial and temporal pattern of illumination: The sinoatrial ring (SAR) was illuminated with random timing. (B-D) Overlays of motion cardiograms. High fluorescence intensities on the y-axis correspond to atrial systoles and low intensities to atrial diastoles. (B-C) Traces aligned to the onset of illumination. (B) In most cases the heart stopped instantaneously upon illumination. (C) When illuminated during the onset of the systole, one escape beat occurred, since the heart completed the systole. (D) After the light was turned off, the heart started beating instantaneously and was synchronized across trials. A, atrium; V, ventricle; SAR, sinoatrial ring.

Supplementary Movie 1 | NpHR activation induces cardiac arrest.

A 3 dpf *Et(E1b:Gal4-VP16)s1101t; Tg(UAS:NpHR-mCherry)s1989t; Tg(UAS:Kaede)s1999t* heart stopped beating when illuminated with orange light and recovered instantaneously when the illumination was stopped. The kymograph is plotted on the bottom. The kymograph was analyzed to generate an audio channel with high pitch tones corresponding to atrial contractions and low pitch tones to ventricular contractions.

Supplementary Movie 2 | Automated mapping of a 3 dpf heart.

The superimposed gray rectangle indicates the illuminated region (*Top*: ventricle; *Bottom*: atrium). The file contains audio information: high pitch tones correspond to atrial contractions and low pitch tones to ventricular contractions. The heartbeat stops at position 6 and AV blocks are induced when the AVC is illuminated (e.g., position 8). The heart rate maps of this experiment are shown in Fig. S5C, bottom row. The movie runs 1.8x faster than real time.

Supplementary Movie 3 | Silencing of the pacemaker using a light sheet.

In this experiment, the green laser of the mSPIM is used at high intensity to illuminate the NpHR expressing heart within the white lines. The brightfield channel (top) and the red channel (bottom) are shown. The heart is mechanically moved through the light sheet and the heart arrests when the pacemaker region at the inflow is within the illuminated stripe.

Supplementary Movie 4 | Hyperpolarization of the atrium does not silence the pacemaker.

The same approach as in movie S3 is used. The heart only arrests when the pacemaker region is in the illuminated stripe. Illumination of very large areas in the ventricle or atrium is not sufficient to induce cardiac arrest.

Supplementary Movie 5 | NpHR induced silencing is constricted to illuminated area.

The SAR was illuminated within a narrow region of interest (ROI). The heart stopped

when the pacemaker region was illuminated (ROI 2) and continued beating when the cells directly adjacent were illuminated (ROI 1 and 3).

Supplementary Movie 6 | Remnant pacing in sinus lips.

The SAR was illuminated within a narrow region of interest (ROI). The sinus lip continued beating while atrial and ventricular contractions were blocked.

Supplementary Movie 7 | Illumination of three cells blocks heartbeat.

NpHR activation in approximately three cells in the right half of the SAR induced cardiac arrest.

Supplementary Movie 8 | Control experiment for movie S7.

NpHR activation in an adjacent region of the same size did not induce cardiac arrest.

Supplementary Movie 9 | Atrioventricular block

Illumination of the AVC region causes AV block. The light intensity was decreased from maximum power (32 mW/mm^2) to 22 % (6.9 mW/mm^2) over the course of the movie. The 2:1 AV block was stable over a range of intensities. The file contains audio information, high pitch tones correspond to atrial contractions and low pitch tones to ventricular contractions.

Supplementary Movie 10 | Precise tuning of heart rate with ChR2.

Blue light pulses are used to activate ChR2. The frequency varied between 2 and 5 Hz. The illuminated region (SAR) is shown as a gray rectangle. The file contains audio information, very high pitch tones correspond to the driving frequency (ChR2 stimulation), high pitch tones correspond to atrial contractions and low pitch tones to ventricular contractions. The instantaneous heart rate is displayed for every beat at the bottom and the length of the gray bar is proportional to the heart rate.

Supplementary Movie 11 | Reversed cardiac conduction induced by ChR2.

A 2 dpf heart was illuminated with blue light pulses to activate ChR2 in a ventricular region close to the bulbus. The conduction wave reversed, starting at the stimulated region and traveling towards the atrium and SAR. Note that the net blood flow stopped (and did not reverse) since the ventricle did not fill with blood from the bulbus during diastoles. The file contains audio information, high pitch tones correspond to atrial contractions and low pitch tones to ventricular contractions. Also see Fig. S6.

Supplementary References

- S1. A. B. Arrenberg, F. Del Bene, H. Baier, *Proc Natl Acad Sci U S A* **106**, 17968 (Oct 20, 2009).
- S2. F. Zhang et al., *Nature* **446**, 633 (Apr 5, 2007).
- S3. T. O'Haver, www.mathworks.com/matlabcentral/fileexchange (2006).