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## Supplementary information

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# **In vivo and ex vivo electrophysiological study of the mouse heart to characterize the cardiac conduction system, including atrial and ventricular vulnerability**

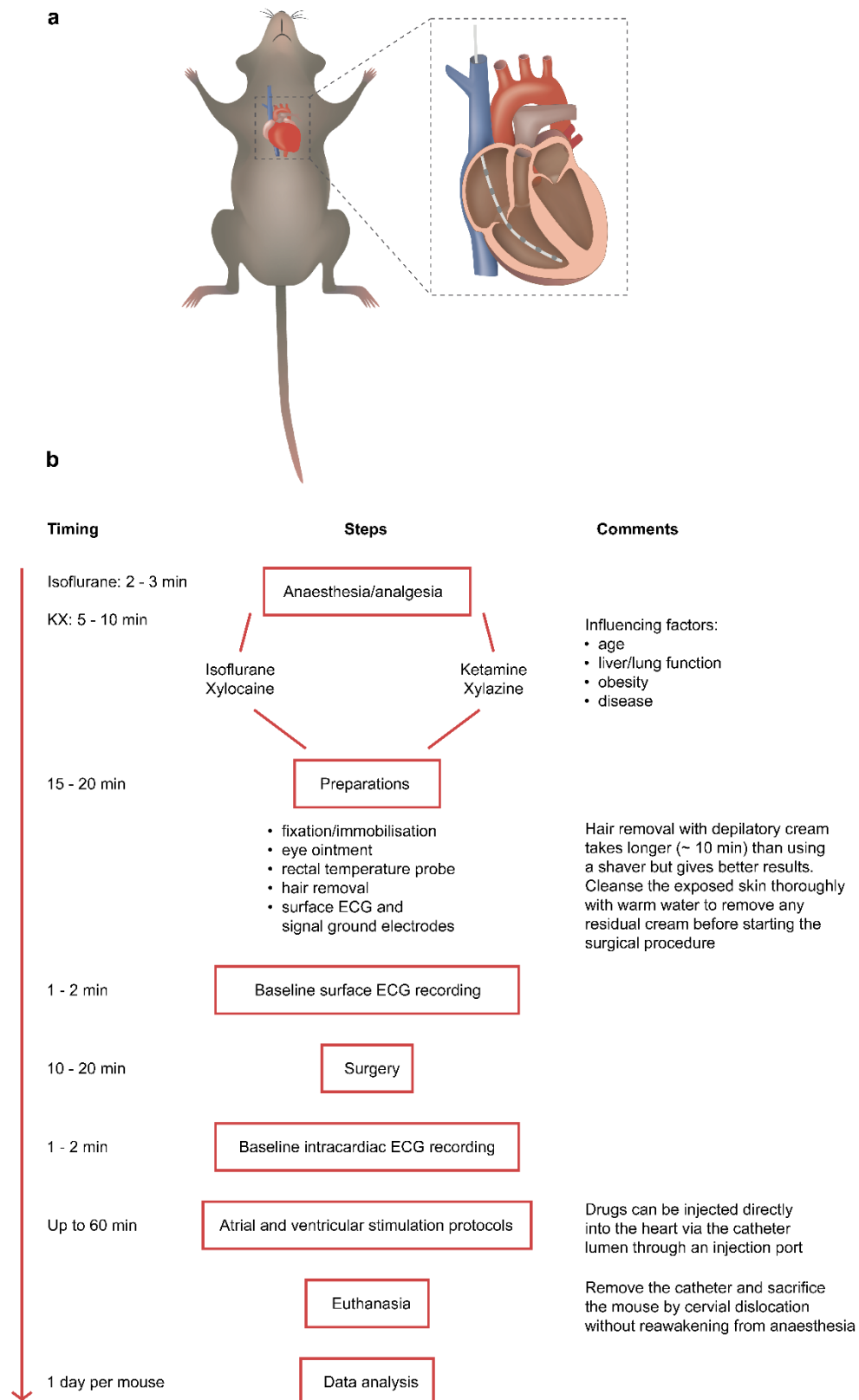
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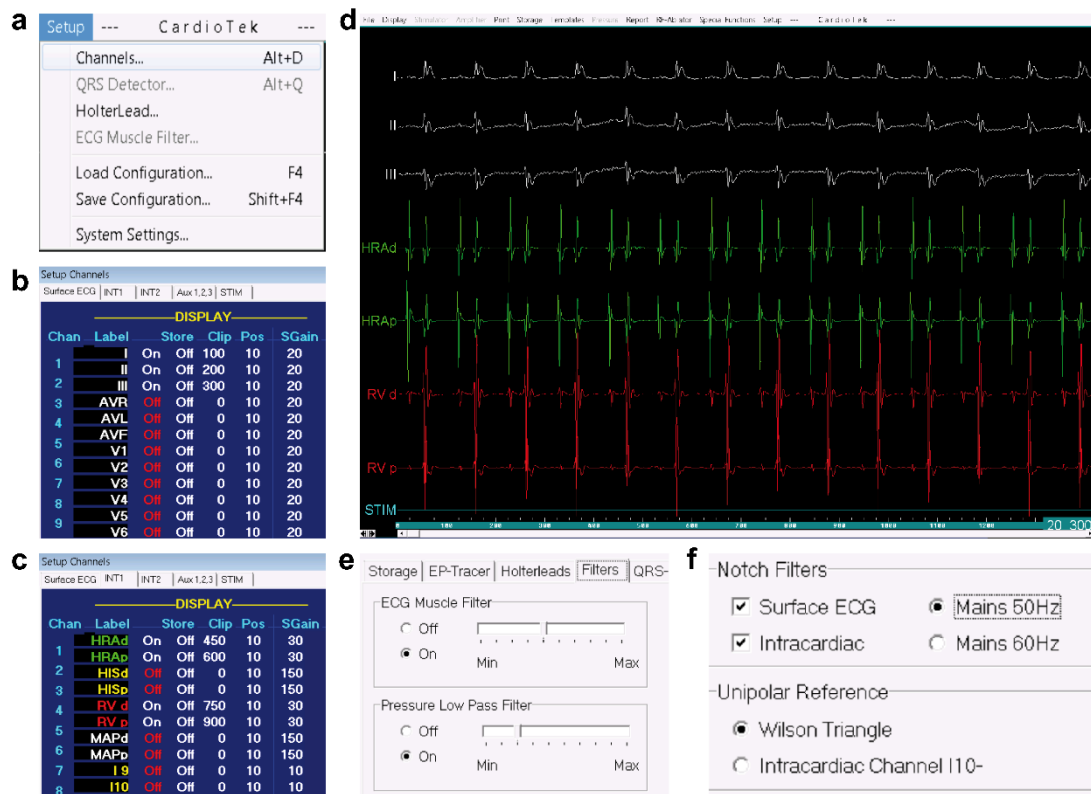
## **Supplementary Information**

**In vivo and ex vivo electrophysiological study of the mouse heart to characterize the cardiac conduction system, including atrial and ventricular vulnerability.**

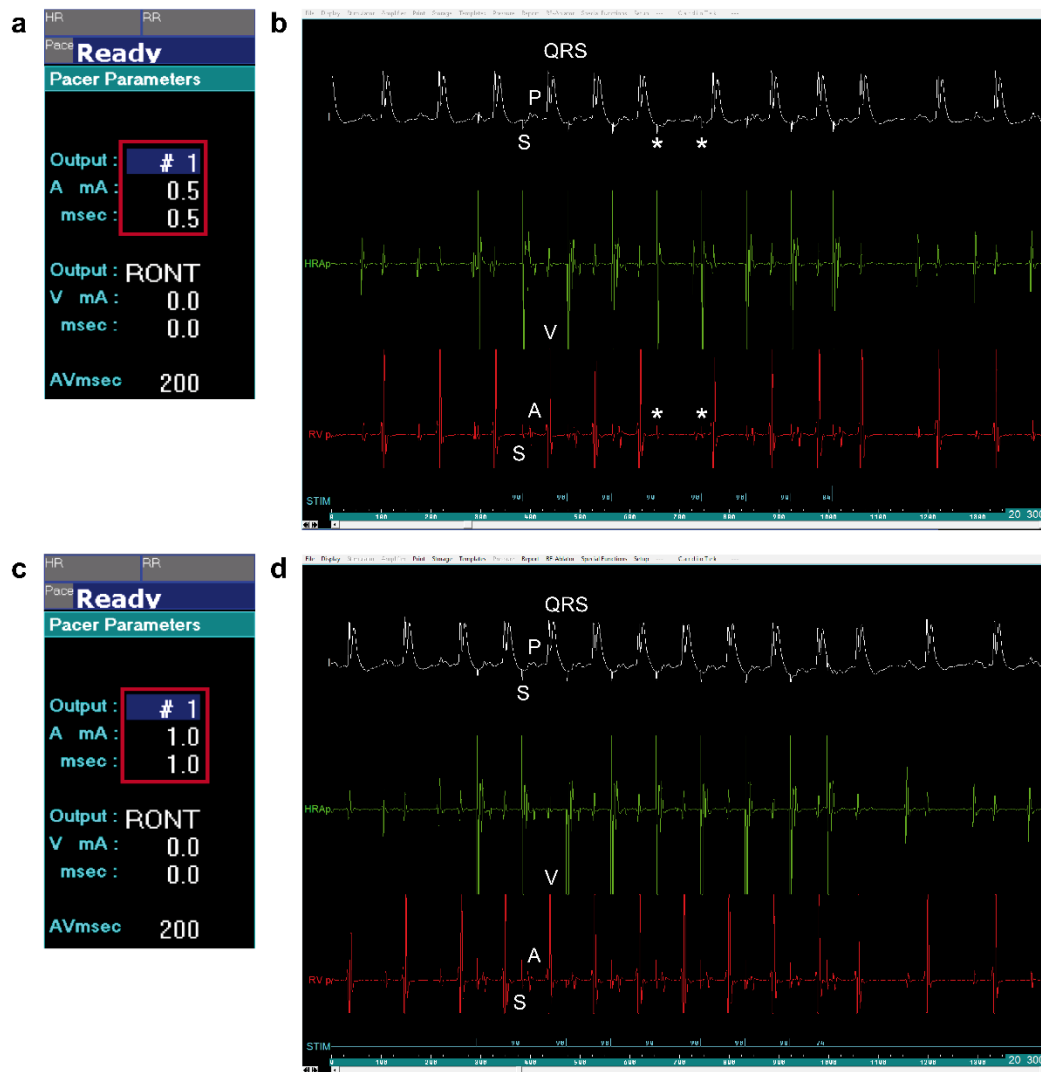
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**Supplementary Fig. 1 | Complete workflow of the protocol including main steps and timing.** **a**, Schematic illustration of an octapolar electrophysiology catheter inserted into the right atrium and ventricle of a mouse heart via the right jugular vein. **b**, Flow chart indicating timing and main steps of the experimental procedure and specific comments.



**Supplementary Fig. 2 | CardioTek EP Tracer software settings.** **a**, Drop-down menu for surface and intracardiac ECG settings. **b**, Setup of the surface ECG traces. The *Label* column designates the different surface ECG leads. Storage is activated for surface ECG leads I, II and III according to Einthoven. **c**, Setup of the intracardiac ECG traces. Green: intracardiac, atrial leads (HRAd, distal high right atrium; HRAp, proximal high right atrium). Yellow: Infrahisian leads (HISd, distal His-Bundle; HISp, proximal His-Bundle). Note that due to the small heart size of mice, only an octapolar catheter can be used and therefore His electrograms cannot be recorded. Red: intracardiac, ventricular leads (RVd, distal right ventricle; RVp, proximal right ventricle). Note that for all intracardiac leads, the designations *proximal* and *distal* are related to the tip of the catheter. The *Clip* column indicates whether a trace is clipped when its amplitude interferes with other traces. Clipping is disabled (Off) for all traces. The *Pos* column designates the vertical position of the ECG traces in the display window. If the value is set to 0, the respective trace is not displayed. The value set in column *SGain* controls the amplitude of the trace on screen. SGain does not influence the amplitude of the signal stored on the hard disk. **d**, Display window showing the surface and intracardiac ECG and stimulation trace (Cyan: STIM, Stimulation channel indicates the timing of applied stimuli). **e**, **f**, Filter settings. The filter settings must be adjusted according to the specific electrical disturbances in order to achieve an optimal signal-to-noise ratio. Representative recordings are obtained from a 4-month-old male mouse.



**Supplementary Fig. 3 | Determination of correct stimulus amplitude and duration for atrial pacing.** **a**, Pacer parameters window. HRAd (Output #1) is selected as the stimulation electrode pair. The stimulus amplitude is 0.5 mA, the stimulus duration is 0.5 ms (red rectangle). **b**, ECG recording with the settings from (a). Eight stimuli are applied with a stimulus interval of 90 ms, followed by one extrastimulus with a coupling interval of 84 ms (cyan line, STIM). Only stimuli # 1, 2, 3, 4, 7, 8 induce a sequence of atrial and ventricular excitation. It is best seen in the surface lead that the stimulus artifacts are directly followed by the P-wave, which in turn is followed by the QRS complex. Stimuli # 5 and 6 (marked with \*) do not induce a response. A possible explanation for not eliciting a response could be that the heart is relatively small and the electrode pair is not located close enough to the sinus node. **c**, HRAd (Output #1) is selected as the stimulation electrode pair (red rectangle). The current amplitude and duration are raised to 1.0 mA and 1.0 ms. **d**, ECG recording with the settings from (c). Every stimulus elicits a response indicating that these settings can be used for atrial pacing. Representative recordings are obtained from a 3-month-old male mouse.



**Supplementary Fig. 4 | Determination of time intervals using CardioTek EP Tracer software. a,** Both cursors are positioned exactly above each other on the right edge of the screen. The time interval between cursors is therefore 0 ms (red rectangle, dT0). **b,** The left cursor is positioned directly on the stimulus artifact that is best identified in the surface ECG leads. The right cursor is positioned on the atrial signal, which is best identified in HRap. It corresponds to the beginning of the P wave in the surface ECG leads. The time interval between cursors is 143 ms (red rectangle, dT143). Representative recordings are obtained from a 4-month-old male mouse.