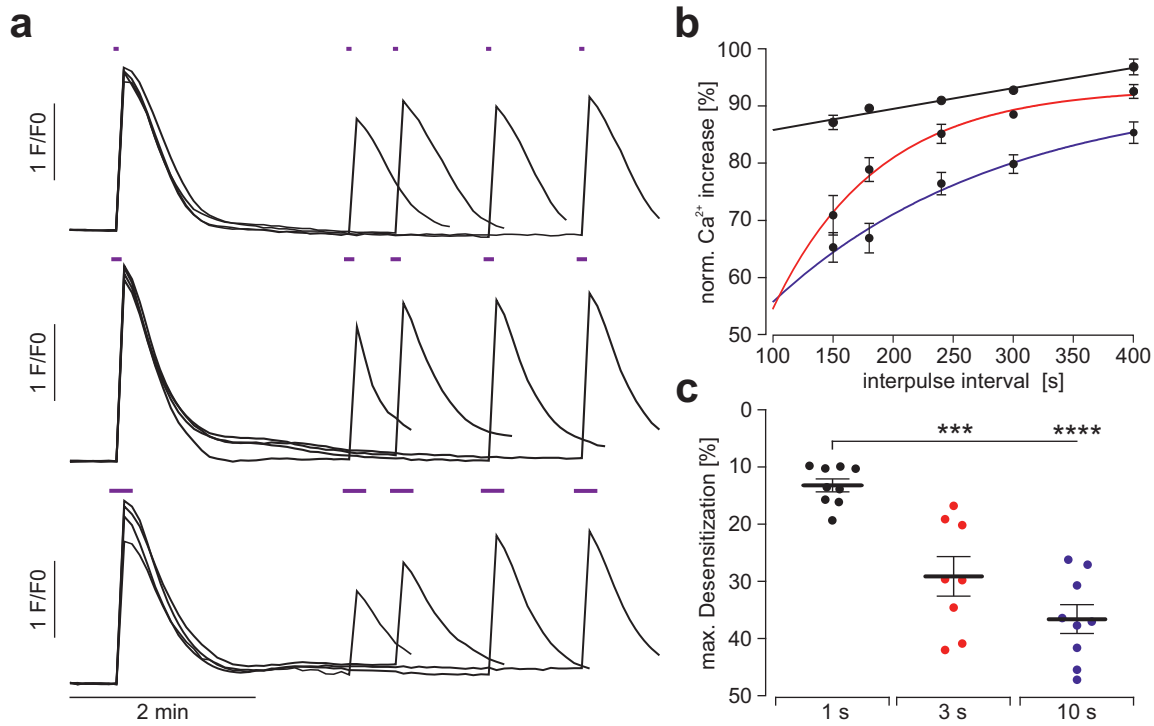
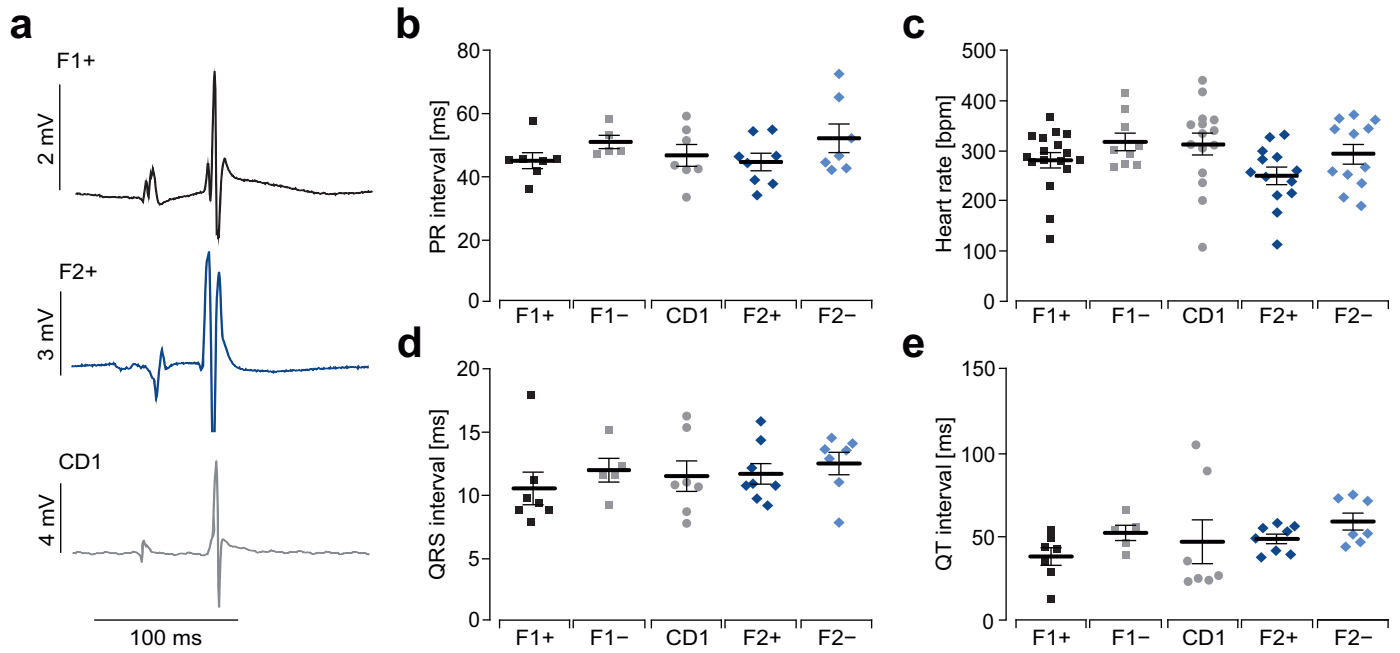


Wagdi et al., 2022: Supplementary Figure 1



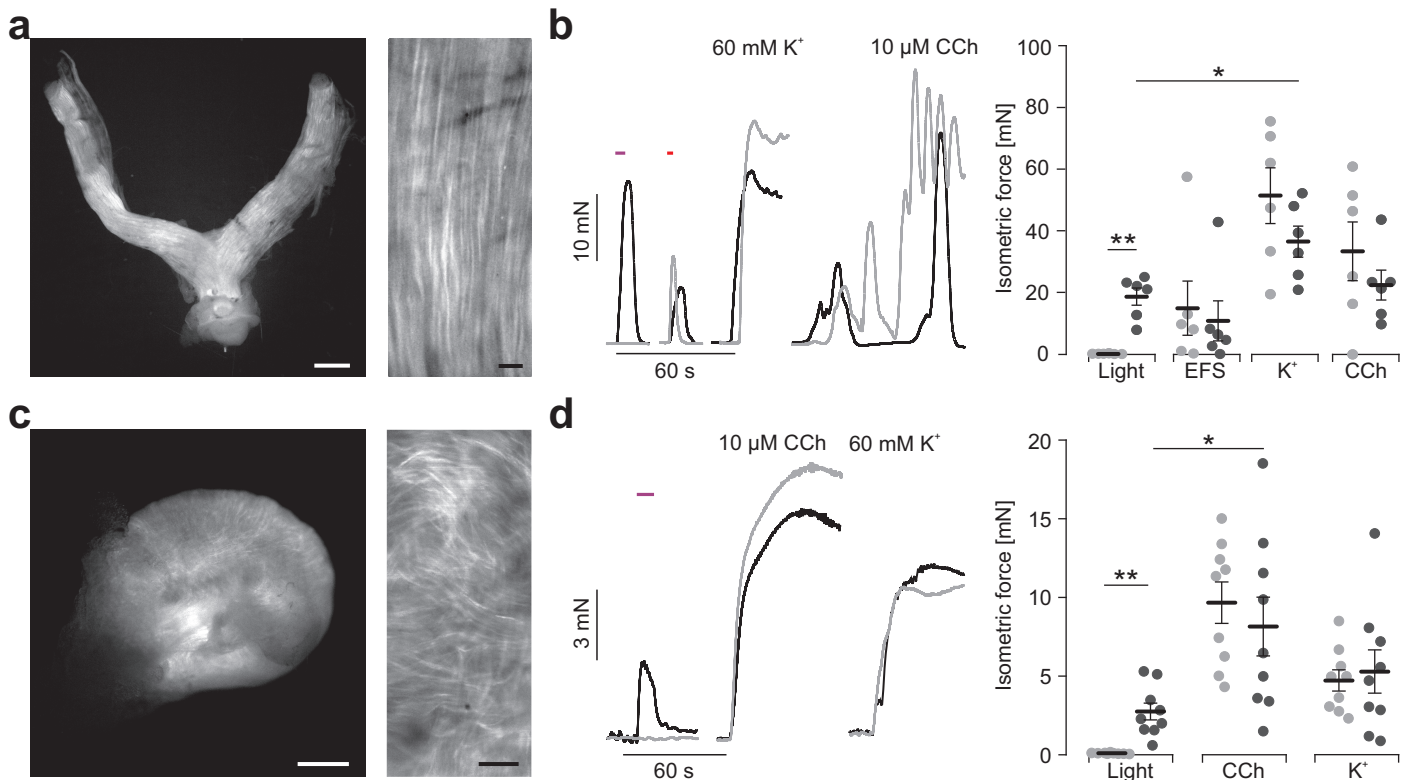
Supplementary Figure 1: Desensitization and recovery from desensitization of light induced Ca^{2+} transients in HEK cells. **a**, Representative traces of Ca^{2+} transients induced by paired pulses of 1, 3 and 10 s long light pulses (violet bars, 1 mW/mm^2) with increasing interpulse intervals (150, 180, 240, 300 s). **b**, Average normalized Ca^{2+} transient amplitudes in dependence to the interpulse interval and the respective one phase decay fits (1 s: black fit, $n = 9$; 3 s: red fit, $n = 8$; 10 s: blue fit, $n = 9$). **c**, Statistical analysis of the effect of the pulse duration on the maximal desensitization (relative decrease) with an ordinary one-way ANOVA with Tukey's multiple comparison test ($p(1 \text{ vs. } 3 \text{ s}) < 0.001$; $p(1 \text{ vs. } 10 \text{ s}) < 0.0001$; $p(3 \text{ vs. } 10 \text{ s}) = 0.11$). Each dot represents the mean of one coverslip. Data are presented as mean values $\pm$ SEM.

Wagdi et al., 2022: Supplementary Figure 2



Supplementary Fig. 2: Basal electrocardiogram (ECG) parameters in hOPN5 mice recorded *ex vivo*. **a**, Representative electrogram recordings from hearts of hOPN5 founder line F1 (black), founder line F2 (blue) and CD1 wild type control mice (gray). **b-e**, Statistical analysis of basal ECG parameters PR interval (b), heart rate (c), QRS interval (d) and QT interval (e) in F1 and F2 hOPN5 expressing hearts (F1+, F2+), from their negative littermates (F1- and F2-) and CD1 wild type controls. To exclude rate dependent effects, only electrogram recordings with a heart rate between 250 and 350 bpm were taken into account in b,d and e. Each dot represents the average from one individual heart. Statistical analysis was performed with ordinary one-way ANOVA test (all p values ≥ 0.096) and Tukey's multiple comparison test (all p values ≥ 0.11). Data are presented as mean values $\pm$ SEM.

Wagdi et al., 2022: Supplementary Figure 3



Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	All commercially available softwares used are clearly stated in the methods. The custom made software for contraction analysis of single cardiomyocytes will be made available on an open source software platform: https://github.com/awagdi0/MOCA/
Data analysis	Statistical data are shown as mean $\pm$ standard error of the mean and were analyzed with the GraphPad Prism 8 software. The ecg signal and force measurements were amplified with an animal bio-amplifier (Animal Bio Amp 223 FE136, AD Instruments) and a PowerLab 16/35 recording system and analyzed with LabChart 8.1.16 software.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data will be provided upon personal and reasonable request to the corresponding authors. Data can only be made available upon request since results from the high throughput screen are proprietary to Bayer company. Importantly, we do not have any data which has to be published like sequencing, structures or proteomic/transcriptomics screens.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We could not determine effect sizes before since these were completely new experiments with non-foreseeable results. Whenever possible we had sample sizes clearly proving very significant p values or high n numbers allowing to exclude any effects by classical statistical tests. All n and p values are given in the legends or the text.
Data exclusions	No data were excluded.
Replication	Experiments were performed in three different labs (WG Sasse, WG Bruegmann, Bayer) and all showed comparable and reproducible results.
Randomization	This is not relevant to our study since phenotypes were obvious due to fluorescence expression and light effects. Furthermore we did not look into effects of treatments within the same kind of cells or groups. Used mice were equally male and female and of various ages.
Blinding	Blinding was not possible due to clear light effects but also not necessary since all analysis was objective with clearly pre-defined criteria.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies used are clearly described in the methods parts. aActinin: Sigma (A7811, 1:400) asmooth muscle actinin: Sigma-Aldrich (A2547) βIIITubulin: Biozym/BioLegend (802001, 1:800) cKit: Linaris (MAK5302, 1:400) Oct3/4: SantaCruz Biotechnology (sc-9081, 1:100) HCN4: Alomone (APC-052, 1:200) eGFP: Chromotek (3H9-100, 1:800; 1:400 and 1:100) and Sigma-Aldrich (11.814.460.001, 1:800) All secondary antibodies are clearly identified in the methods section.
Validation	All antibodies used have been often validated and reported before by other scientist. None of the quantitative analysis are depending on antibodies. Relevant citations are: Vogt, Theranostics 2021; Bruegmann, Nature methods 2010; Vogt, Cardiovascular Research 2015, Roell, Nature 2007; Kozasa, Y. et al. (2018) J. Physiol. 596, 809;

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Commercially available, generated by us or given by collaboration partners. Each source is clearly defined and citations are given for the source. HEK cells (AD293, ATCC, U.S.), R1 mouse embryonic stem cells (ES cells)
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Authentication

None of the cell lines were additionally authenticated since all are standard cell lines and have not been newly generated for this study. HEK knockout cell lines were functionally tested by positive controls.

Mycoplasma contamination

All cell lines are regularly tested for possible mycoplasma contamination. Results were always negative.

Commonly misidentified lines
(See [ICLAC](#) register)

None.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

This is how we stated this in the methods: "Mice were kept in 12/12 h dark/light cycles with food and water ad libido at room temperature and standard humidity. [...] hOPN5/eYFP transgenic mice were created by pronuclear injection of FVB/N mice using standard procedures by the core facility of the Max-Planck Institute of Experimental Medicine, Göttingen. [...] Positive founder mice were mated with CD1 wild-type animals to obtain heterozygous offspring and wild type littermate controls. [...] Including all experiments, we used 34 male and 29 female transgenic OPN5 mice
203 (16 ± 1.1 weeks old), 31 male and 15 female wild type siblings (12 ± 0.85 weeks old) and 15 male and 18 female CD1 wild type mice (27 ± 2.5 weeks old) were used as controls. [...] Melanopsin expressing transgenic mice were generated using a previously described G4 embryonic stem cell line stably transfected with the CAG-melanopsin-IRES-GFP plasmid. [...] The mice investigated in this study were back-crossed at least 10 generations. Mice of this transgenic mouse line were in total 2 male and 3 female (51 ± 4.2 weeks old) and 5 female CD1 wild type mice (20 ± 1.1 weeks old) as controls."

Wild animals

No wild animals were used in this study.

Field-collected samples

No field collected samples were used in this study.

Ethics oversight

"All animal work conformed to the European Guideline for animal experiments 2010/63/EU and the mouse generation was approved by the Niedersächsische Landesamt für Verbraucherschutz und Lebensmittelsicherheit (approval number 33.9-42502-04-16/2352) and by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (approval number AZ.84-02.04.2012A146)."

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Title: Supplementary Movie 1:

Description: Representative video of Ca²⁺ imaging to determine the light sensitivity of hOPN5 in polyclonal HEK cells (corresponding to Figure 2a). XRhod-1-AM signal is shown in fire color code (blue to yellow with increasing fluorescence signal). Cyan dots in the top left corner indicate the five UV light pulses (385 nm, 100 ms) with 0, 0.1, 0.3, 1, 3, 10 $\mu\text{W}/\text{mm}^2$, respectively. Video is displayed at 30x speed.

Title: Supplementary Movie 2:

Description: Video showing the expression of hOPN5/eYFP (green) in a 3D rendered volume (normal shading) of light-sheet Z-stack images after tissue clearing. The posterior surface of the heart was cropped to visualize the distribution of hOPN5/eYFP in the endocardium.

Title: Supplementary Movie 3:

Description: 3D rendered volume (blend shading) of light-sheet Z-stack images of cleared hearts with hOPN5/eYFP shown in green and HCN4 staining shown in purple. The posterior wall of the right atrium as well as a rectangular window anterior to the tricuspid valve were cropped to visualize the sinus and atrioventricular nodes.