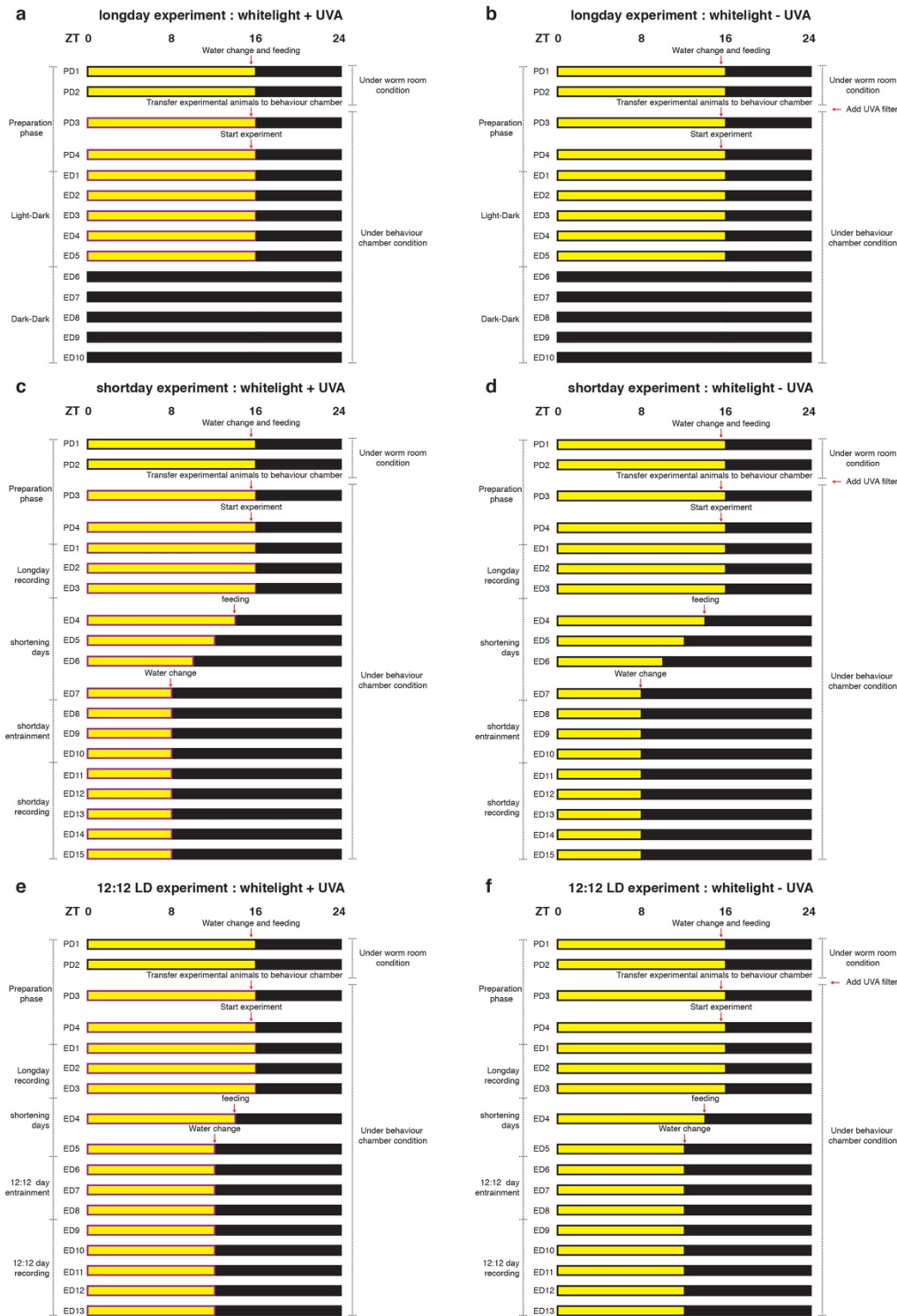


## Supplementary Figures:

# Seasonal variation in UVA light drives hormonal and behavioral changes in a marine annelid via a ciliary opsin

Vinoth Babu Veedin Rajan<sup>1,2</sup>, N. Sören Häfker<sup>1,2</sup>, Enrique Arboleda<sup>1,2,7</sup>, Birgit Poehn<sup>1,2</sup>, Thomas Gossenreiter<sup>1</sup>, Elliot Gerrard<sup>3</sup>, Maximillian Hofbauer<sup>1,2,8</sup>, Christian Mühlestein<sup>6</sup>, Andrea Bileck<sup>4</sup>, Christopher Gerner<sup>4</sup>, Maurizio Ribera d'Alcala<sup>5</sup>, Maria C. Buia<sup>5</sup>, Markus Hartl<sup>1</sup>, Robert J. Lucas<sup>3</sup> and Kristin Tessmar-Raible<sup>1,2, @</sup>



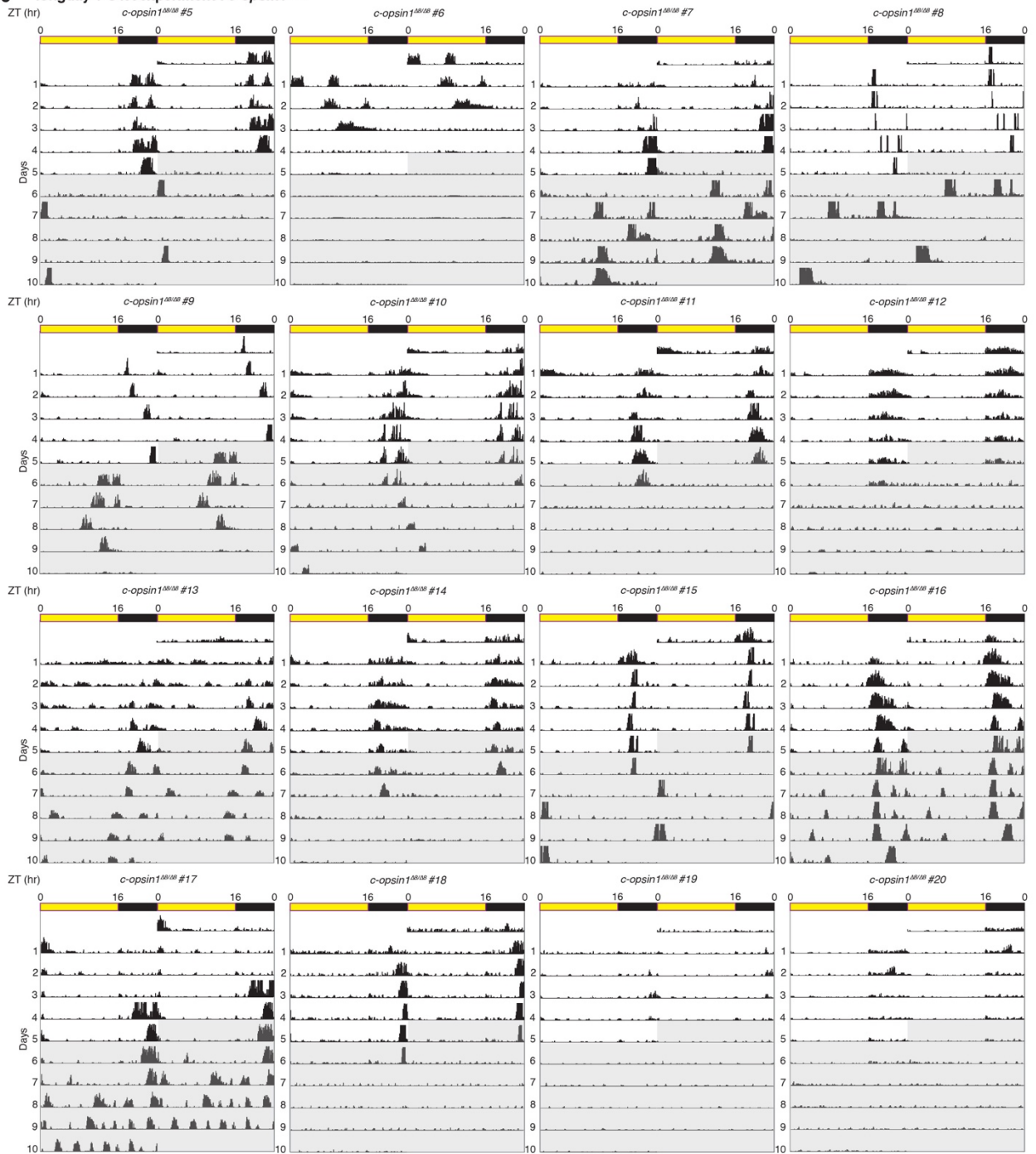
Supplementary Figure 1

**SupplFig. 1. Temporal schematic of illumination for long day and short day experiments.** (a,b) longday (16:8 LD) experiment entrained under “white” light including UVA (a) and “white” light with filter-reduced UVA (B). (c-f) short day (c,d- LD8:16) and equal daylength (e,f- LD12:12) experiments. As worms are normally grown at LD 16:8, shorter daylength are achieved by gradually decreasing daylength from longday. The gradual decrease is performed to avoid confounding the worms’ circalunar entrainment. PD – Preparation day, ED – Experimental day. For light spectra: Extended Data Fig.4.





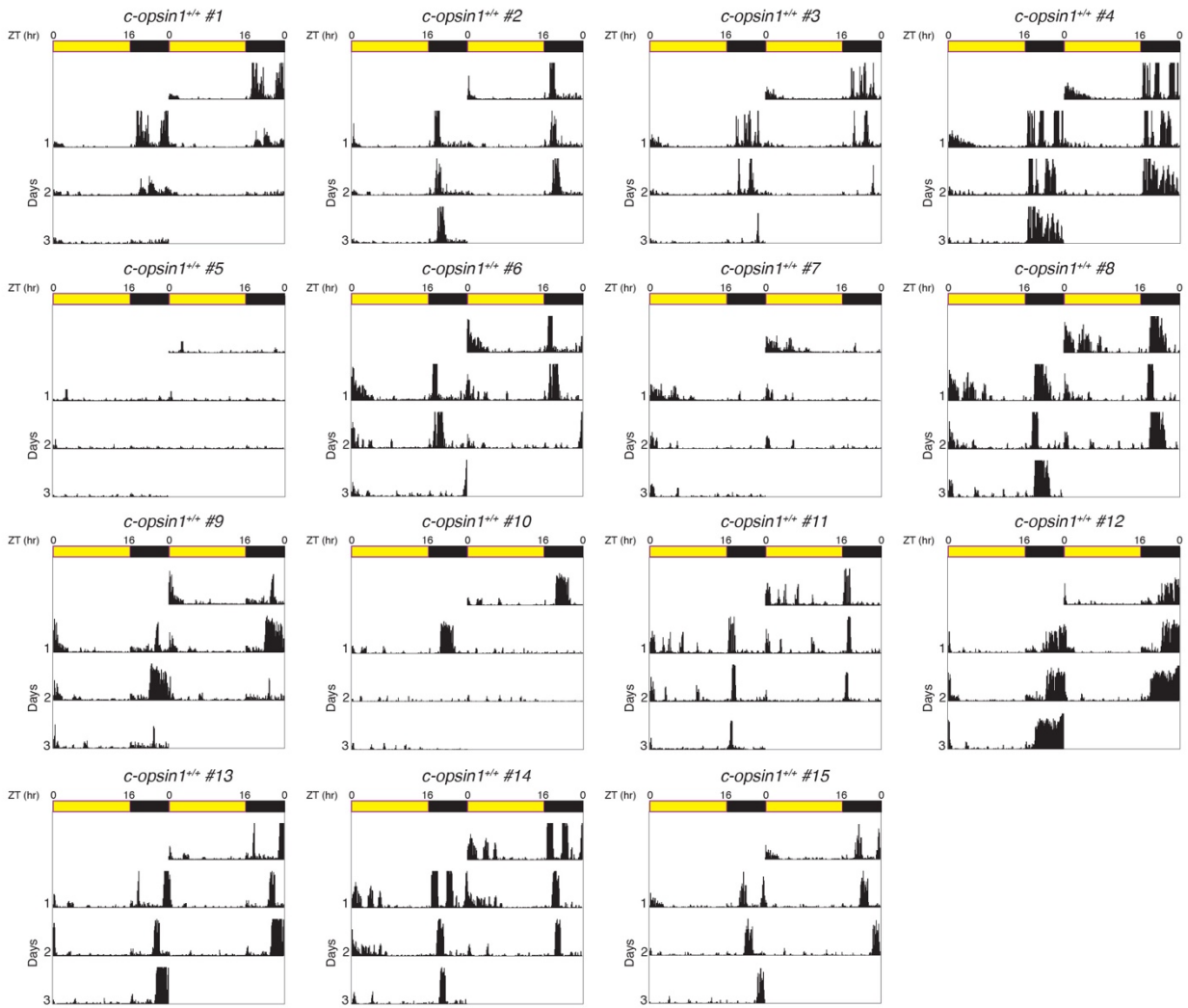
**c longday + UVA experiment : *c-opsin1<sup>Δ8/Δ8</sup>***



Supplementary Figure 2  
Page 3

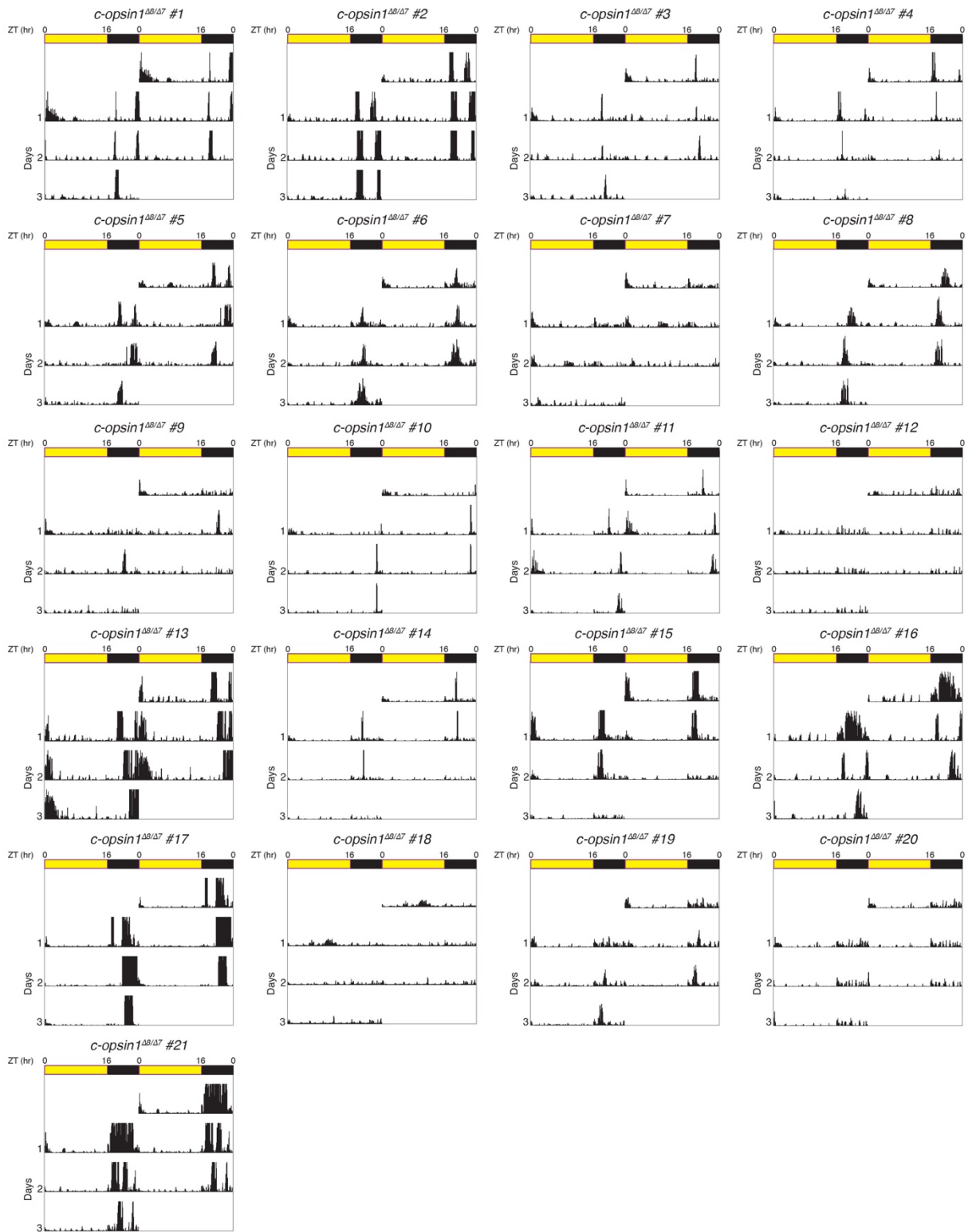
SupplFig. 2. Individual actograms of *c-opsin1<sup>Δ8</sup>* homozygous, heterozygous and wildtype siblings under long day, including strong UVA. Individual double-plotted actograms. 5 days of LD followed by 5 days of DD (shaded). Genotypes as indicated. Y-axis: days.

**a longday + UVA : *c-opsin1<sup>+/+</sup>***



Supplementary Figure 3 *cont.*

**b longday + UVA : *c-opsin1<sup>Δ8/Δ7</sup>***

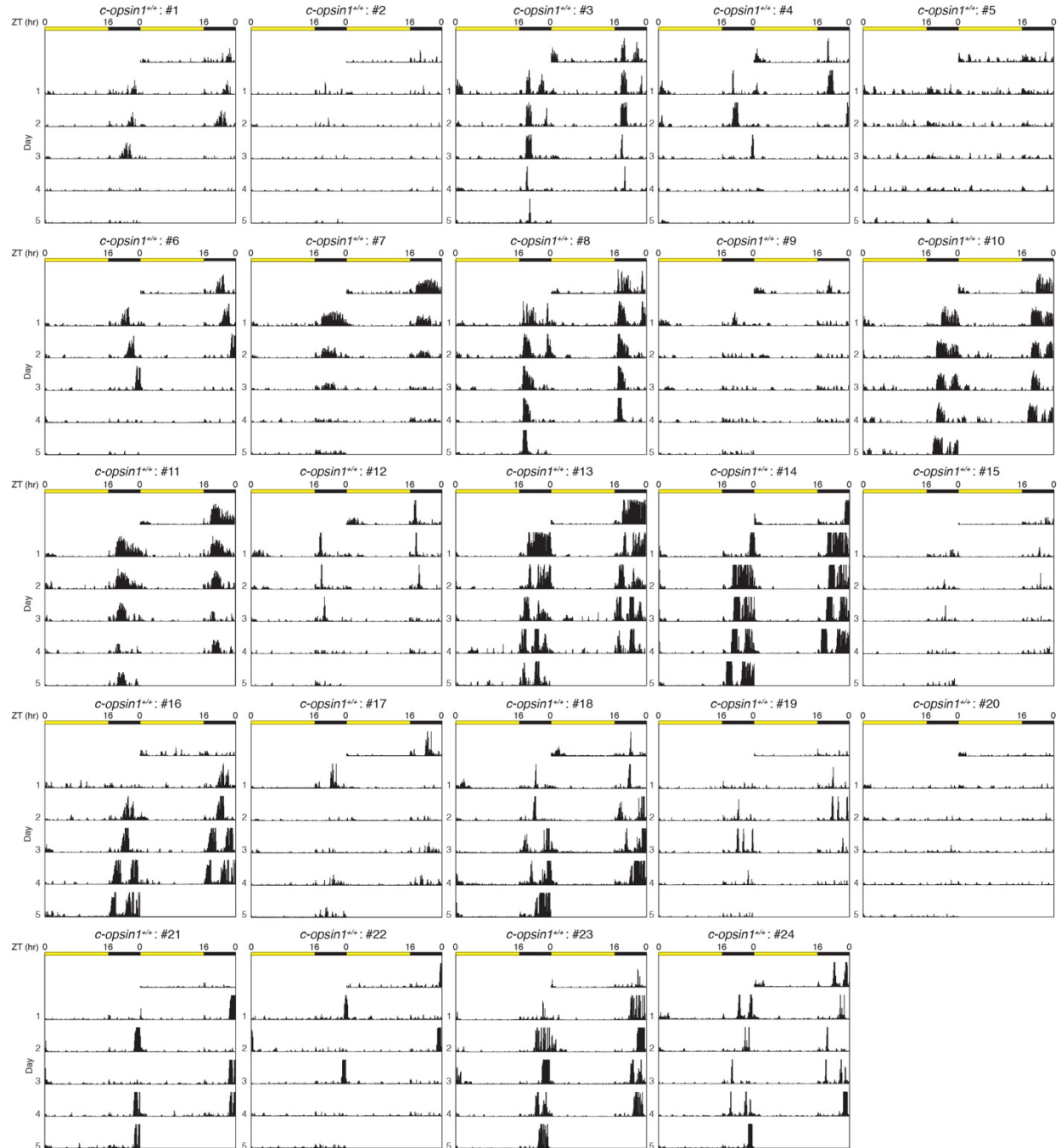


Supplementary Figure 3 cont.

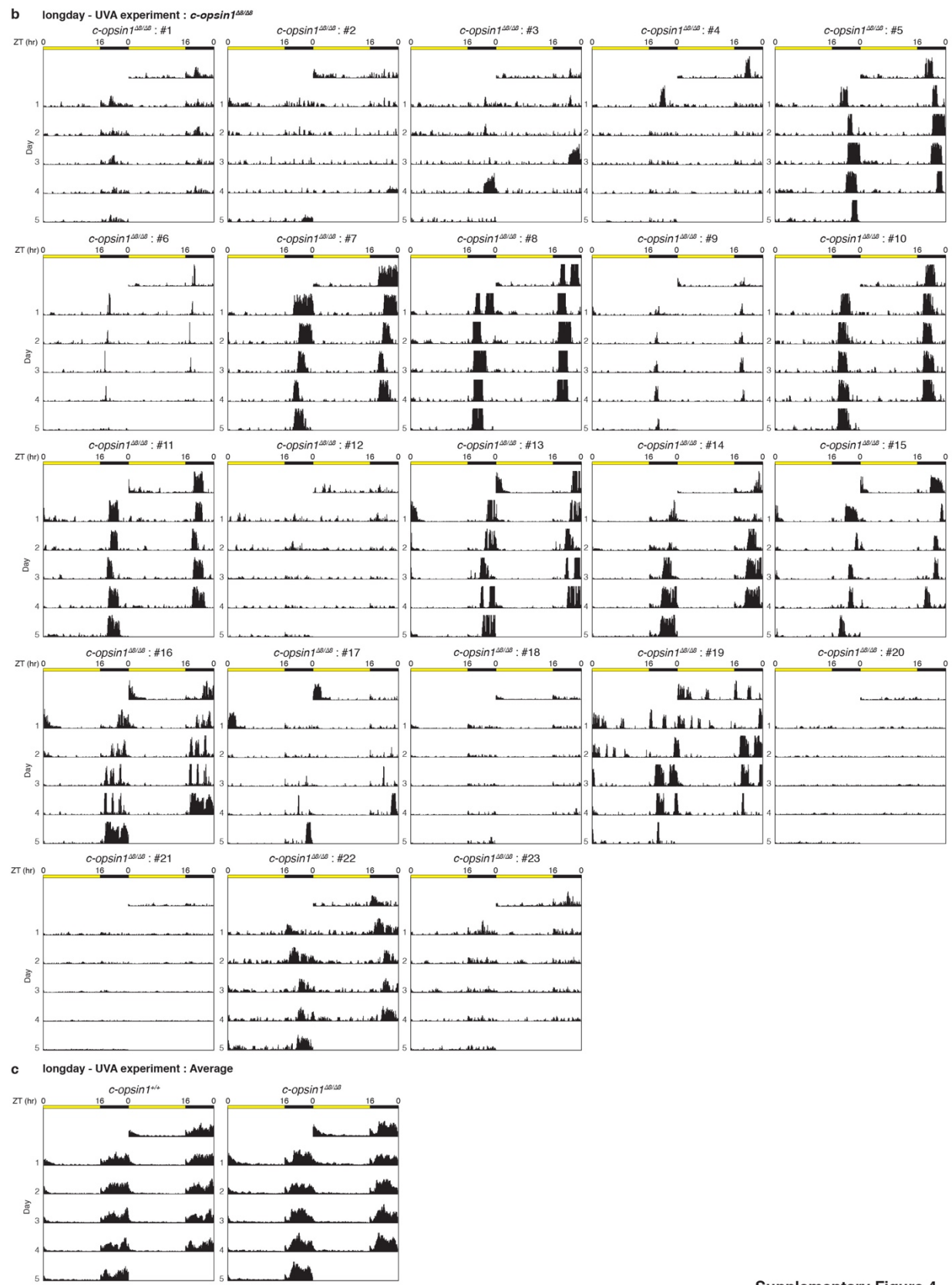
Page 2

SupplFig.3. Individual actograms of *c-opsin1<sup>Δ8/Δ7</sup>* transheterozygous and wildtype under longday, including strong UVA. 3 days of LD. Individual double-plotted actograms plot of *c-opsin1<sup>+/+</sup>* (A) and *c-opsin1<sup>Δ8/Δ7</sup>* (B). Genotypes as indicated. Y-axis: days.

**a** longday - UVA experiment : *c-opsin1<sup>+/+</sup>*

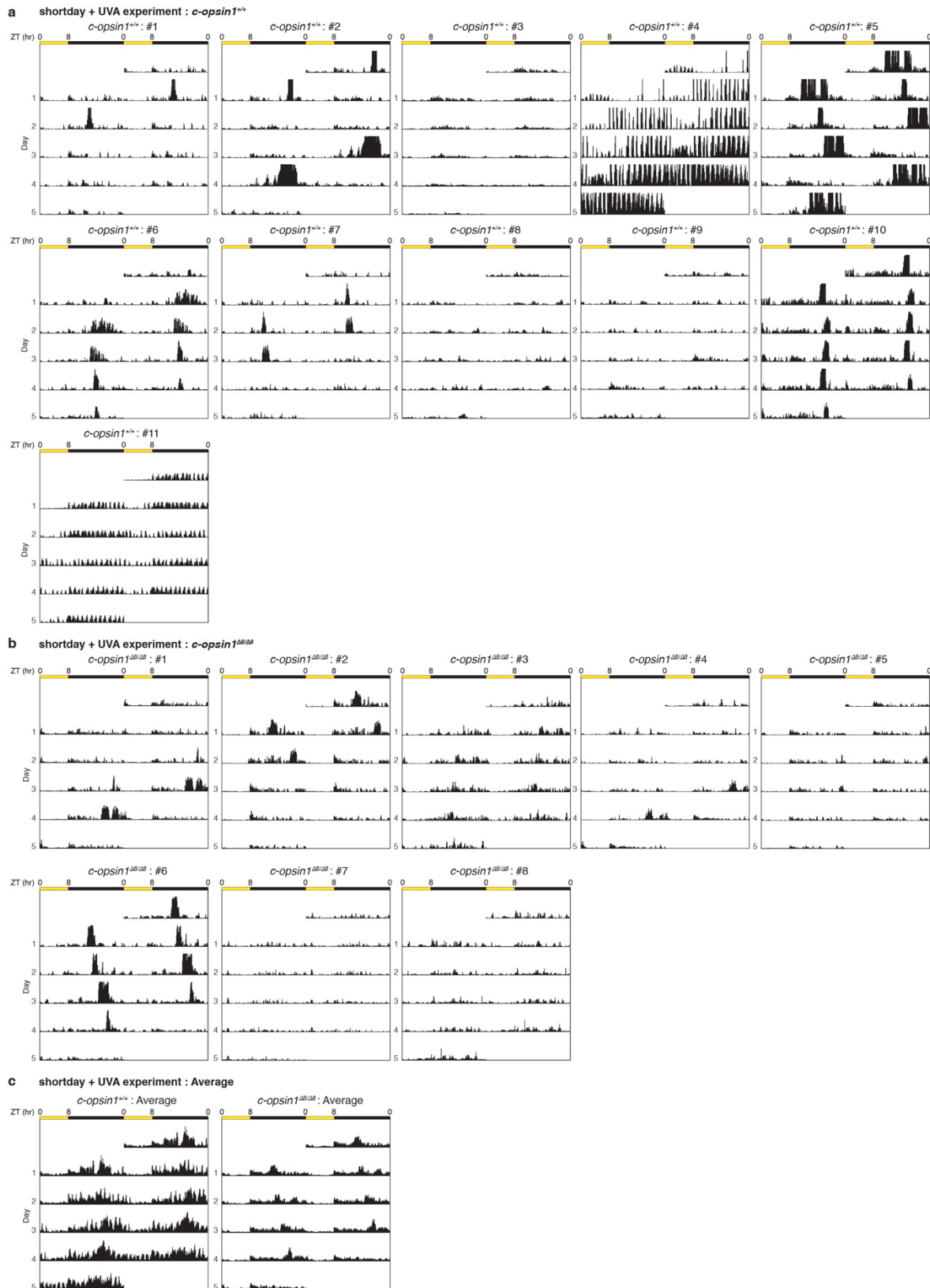






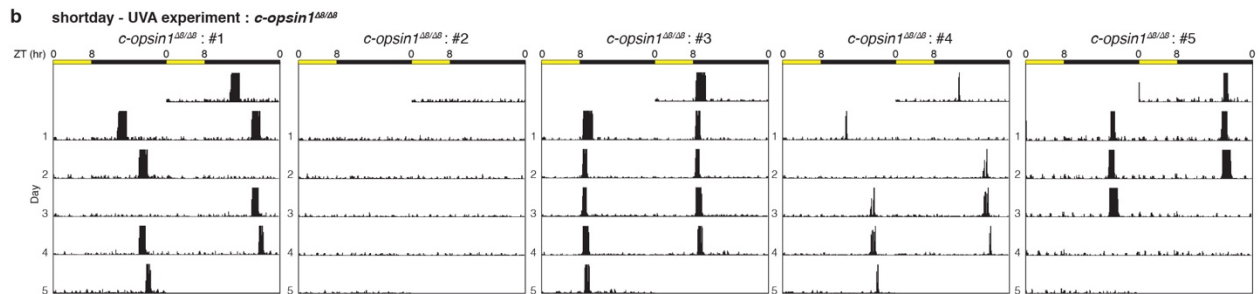
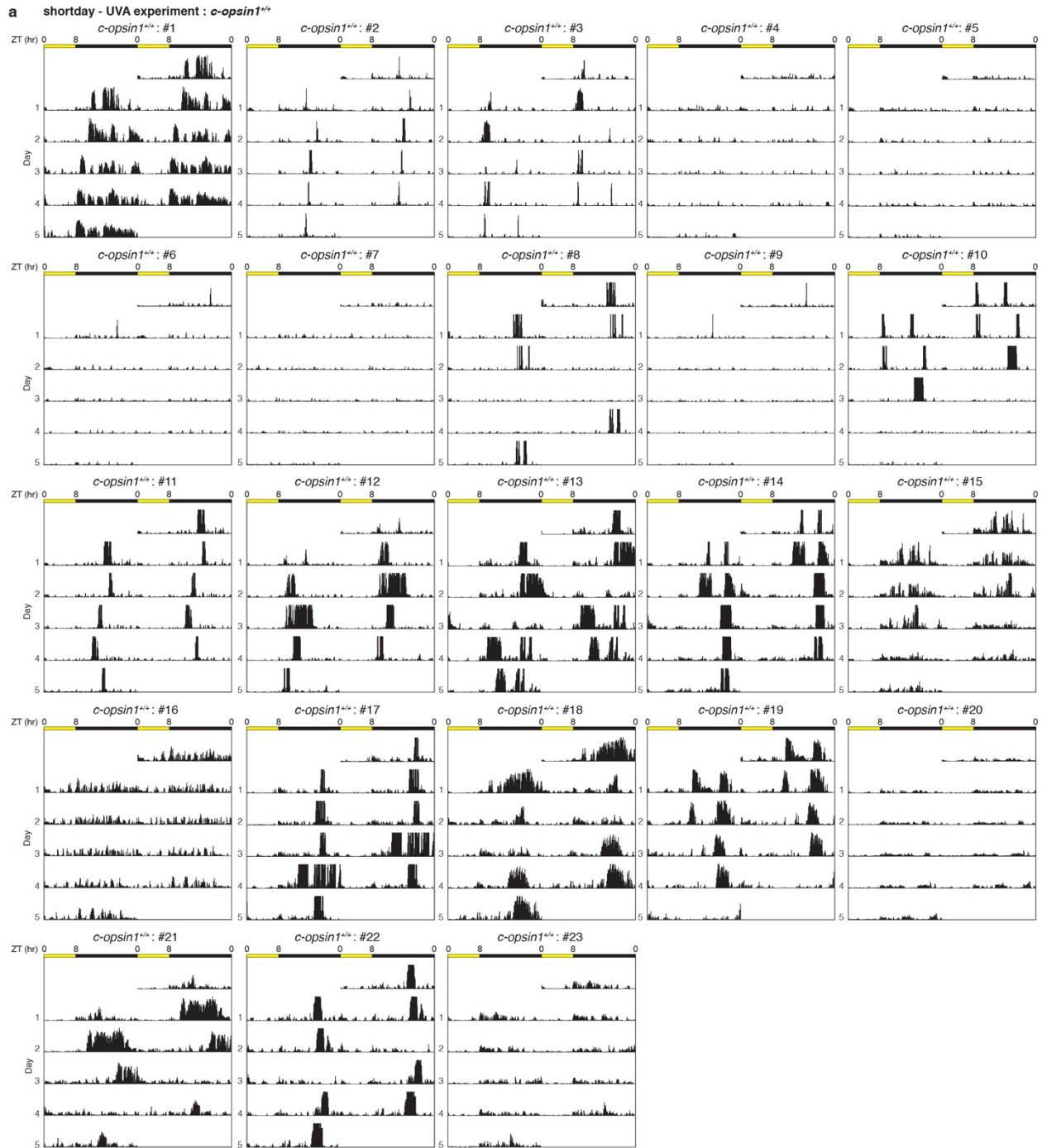
Supplementary Figure 4  
Page. 2

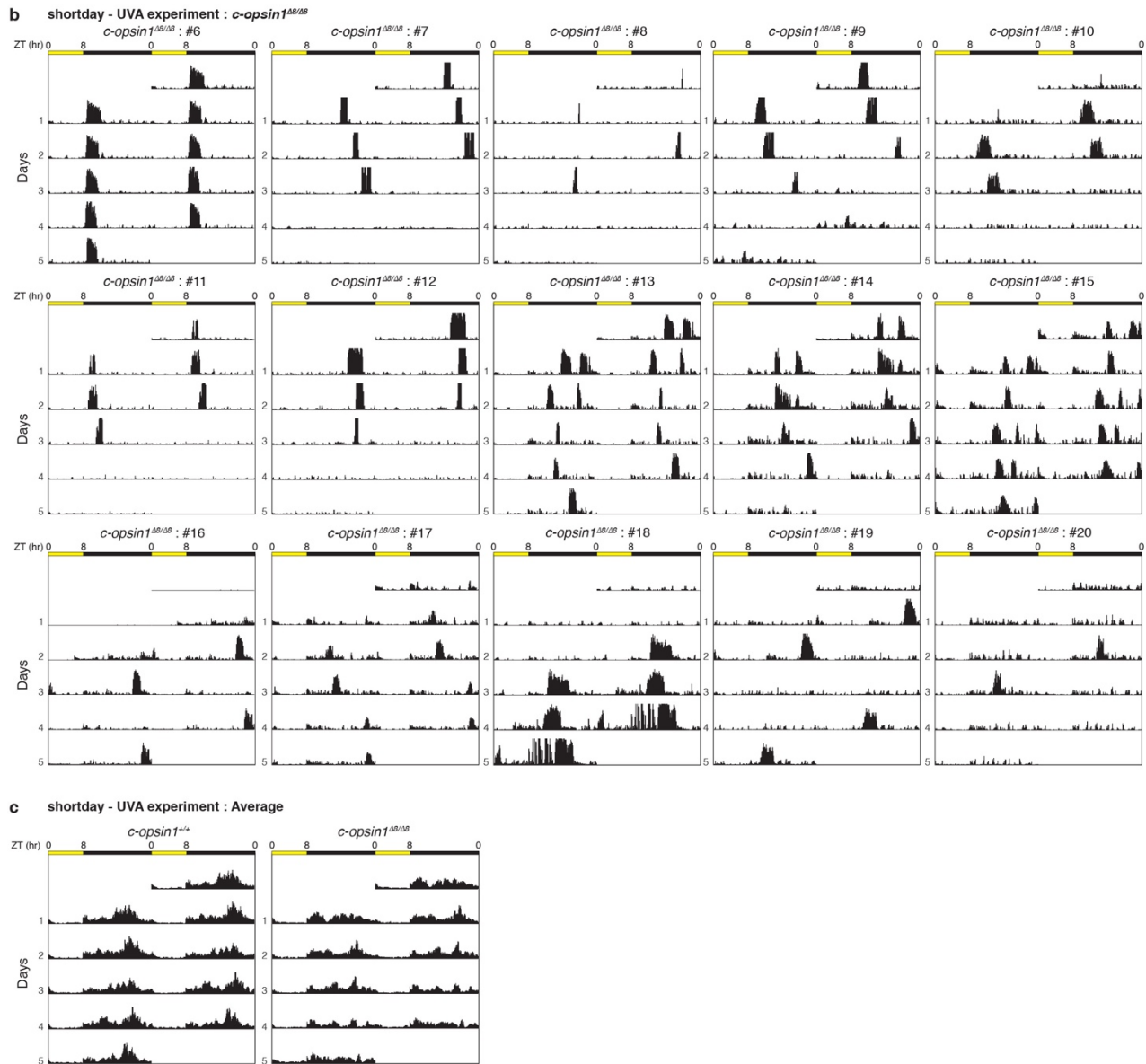
SupplFig.4. Individual double-plotted actograms of *c-opsin1<sup>Δ8</sup>* and corresponding wt under long day, with filter-reduced UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.



Supplementary Figure 5

SupplFig.5. Individual double-plotted actograms of *c-opsin1<sup>Δ8</sup>* and corresponding wt under short day, including strong UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.





Supplementary Figure 6  
Page. 2

SupplFig.6. Individual double-plotted actograms of *c-opsin1<sup>Δ8</sup>* and corresponding wt under shortday, with filter-reduced UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.

| Longday experiment - 16:8LD  |   |              |         |                  |
|--|---|--------------|---------|------------------|
| Comparison   | One-way ANOVA (Sidak's multiple correction test)  | Significant? | Summary | Adjusted P-value |
| <b>c-opsin1<sup>+/+</sup></b><br><b>LD+UVA vs LD-UVA</b>                 | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-8hrs)     | No           | ns      | 0.9837           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(8-16hrs)   | No           | ns      | 0.9998           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(16-24hrs) | Yes          | ****    | <0.0001          |
| <b>c-opsin1<sup>-/-</sup></b><br><b>LD+UVA vs LD-UVA</b>                 | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-8hrs)     | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(8-16hrs)   | No           | ns      | 0.9957           |
|  | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(16-24hrs) | No           | ns      | 0.9761           |
| <b>LD+UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-8hrs)     | No           | ns      | 0.9898           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(8-16hrs)   | No           | ns      | 0.9616           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(16-24hrs) | Yes          | ****    | <0.0001          |
| <b>LD-UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-8hrs)     | No           | ns      | 0.9111           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(8-16hrs)   | No           | ns      | 0.9859           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(16-24hrs) | No           | ns      | 0.2644           |

| Shortday experiment – 8:16LD   |   |              |         |                  |
|--|---|--------------|---------|------------------|
| Comparison   | One-way ANOVA (Sidak's multiple correction test)  | Significant? | Summary | Adjusted P-value |
| <b>c-opsin1<sup>+/+</sup></b><br><b>SD+UVA vs SD-UVA</b>                 | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(0-8hrs) vs. <i>c-opsin1<sup>+/+</sup></i> SD-UVA(0-8hrs)     | No           | ns      | 0.7789           |
|  | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(8-16hrs) vs. <i>c-opsin1<sup>+/+</sup></i> SD-UVA(8-16hrs)   | No           | ns      | 0.9328           |
|  | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(16-24hrs) vs. <i>c-opsin1<sup>+/+</sup></i> SD-UVA(16-24hrs) | No           | ns      | 0.9863           |
| <b>c-opsin1<sup>-/-</sup></b><br><b>SD+UVA vs SD-UVA</b>                 | <i>c-opsin1<sup>-/-</sup></i> SD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(0-8hrs)     | No           | ns      | 0.9989           |
|  | <i>c-opsin1<sup>-/-</sup></i> SD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(8-16hrs)   | No           | ns      | 0.1658           |
|  | <i>c-opsin1<sup>-/-</sup></i> SD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(16-24hrs) | No           | ns      | 0.2459           |
| <b>SD+UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD+UVA(0-8hrs)     | No           | ns      | 0.8137           |
|  | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD+UVA(8-16hrs)   | No           | ns      | 0.3913           |
|  | <i>c-opsin1<sup>+/+</sup></i> SD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD+UVA(16-24hrs) | No           | ns      | 0.0525           |
| <b>SD-UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> SD-UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(0-8hrs)     | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>+/+</sup></i> SD-UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(8-16hrs)   | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>+/+</sup></i> SD-UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> SD-UVA(16-24hrs) | No           | ns      | 0.0603           |

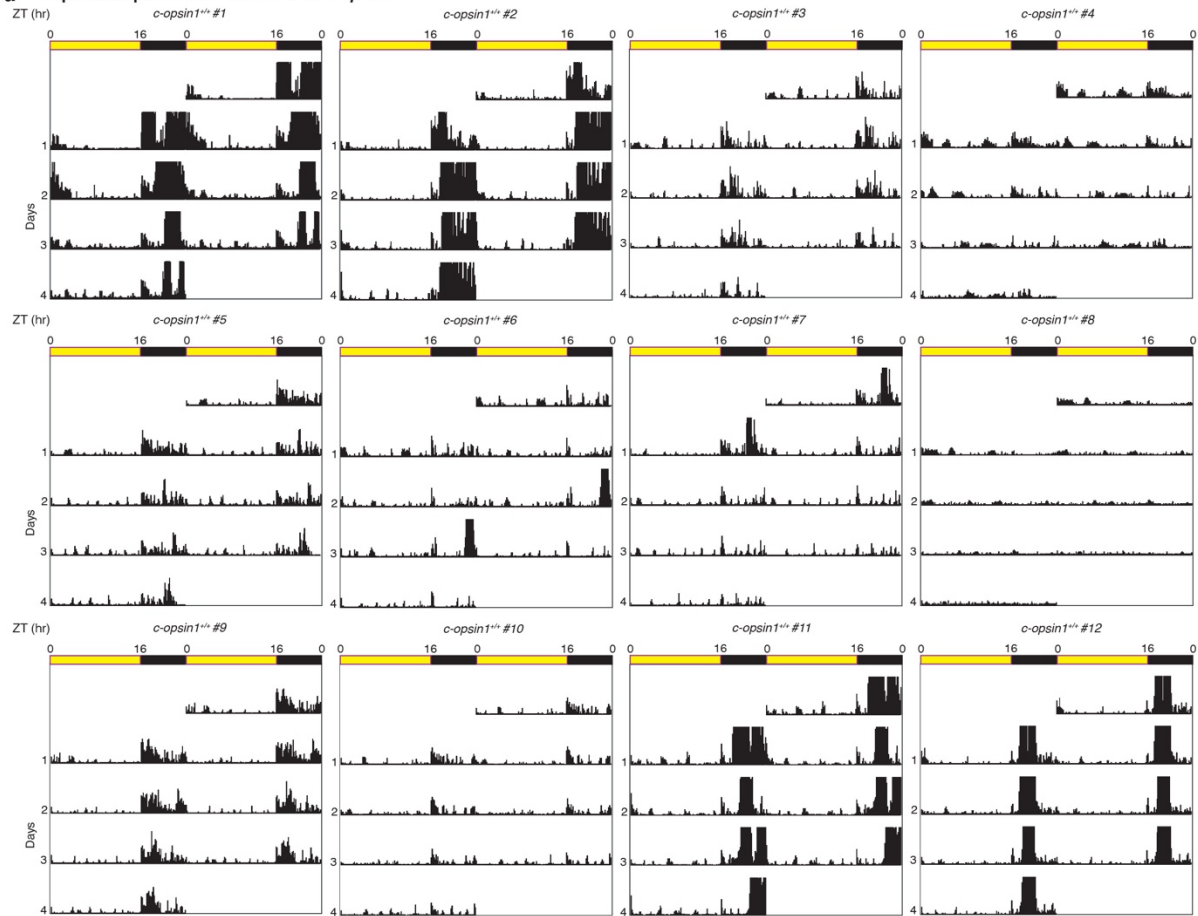
| Equinox experiment – 16:8LD  |   |              |         |                  |
|--|---|--------------|---------|------------------|
| Comparison   | One-way ANOVA (Sidak's multiple correction test)  | Significant? | Summary | Adjusted P-value |
| <b>c-opsin1<sup>+/+</sup></b><br><b>LD+UVA vs LD-UVA</b>                 | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-8hrs)     | No           | ns      | 0.9981           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(8-16hrs)   | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(16-24hrs) | Yes          | **      | 0.0056           |
| <b>c-opsin1<sup>-/-</sup></b><br><b>LD+UVA vs LD-UVA</b>                 | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-8hrs)     | No           | ns      | 0.8926           |
|  | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(8-16hrs)   | No           | ns      | 0.9927           |
|  | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(16-24hrs) | No           | ns      | 0.3264           |
| <b>LD+UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-8hrs)     | No           | ns      | 0.9969           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(8-16hrs)   | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(16-24hrs) | Yes          | **      | 0.0045           |
| <b>LD-UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-8hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-8hrs)     | No           | ns      | 0.9647           |
|  | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(8-16hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(8-16hrs)   | No           | ns      | >0.9999          |
|  | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(16-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(16-24hrs) | No           | ns      | 0.3128           |

| Equinox experiment - 12:12LD  |   |              |         |                  |
|---|---|--------------|---------|------------------|
| Comparison  | One-way ANOVA (Sidak's multiple correction test)  | Significant? | Summary | Adjusted P-value |
| <b>c-opsin1<sup>+/+</sup></b><br><b>12:12LD+UVA vs 12:12LD-UVA</b>            | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-12hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-12hrs)   | No           | ns      | 0.9234           |
|   | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(12-24hrs) vs. <i>c-opsin1<sup>+/+</sup></i> LD-UVA(12-24hrs) | No           | ns      | 0.0828           |
| <b>c-opsin1<sup>-/-</sup></b><br><b>12:12LD+UVA vs 12:12LD-UVA</b>            | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-12hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-12hrs)   | No           | ns      | 0.9970           |
|   | <i>c-opsin1<sup>-/-</sup></i> LD+UVA(12-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(12-24hrs) | No           | ns      | 0.5743           |
| <b>12:12LD+UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(0-12hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(0-12hrs)   | No           | ns      | 0.9975           |
|   | <i>c-opsin1<sup>+/+</sup></i> LD+UVA(12-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD+UVA(12-24hrs) | No           | ns      | 0.4974           |
| <b>12:12LD-UVA</b><br><b>c-opsin1<sup>+/+</sup> vs c-opsin1<sup>-/-</sup></b> | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(0-12hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(0-12hrs)   | No           | ns      | 0.8849           |
|   | <i>c-opsin1<sup>+/+</sup></i> LD-UVA(12-24hrs) vs. <i>c-opsin1<sup>-/-</sup></i> LD-UVA(12-24hrs) | No           | ns      | 0.8163           |

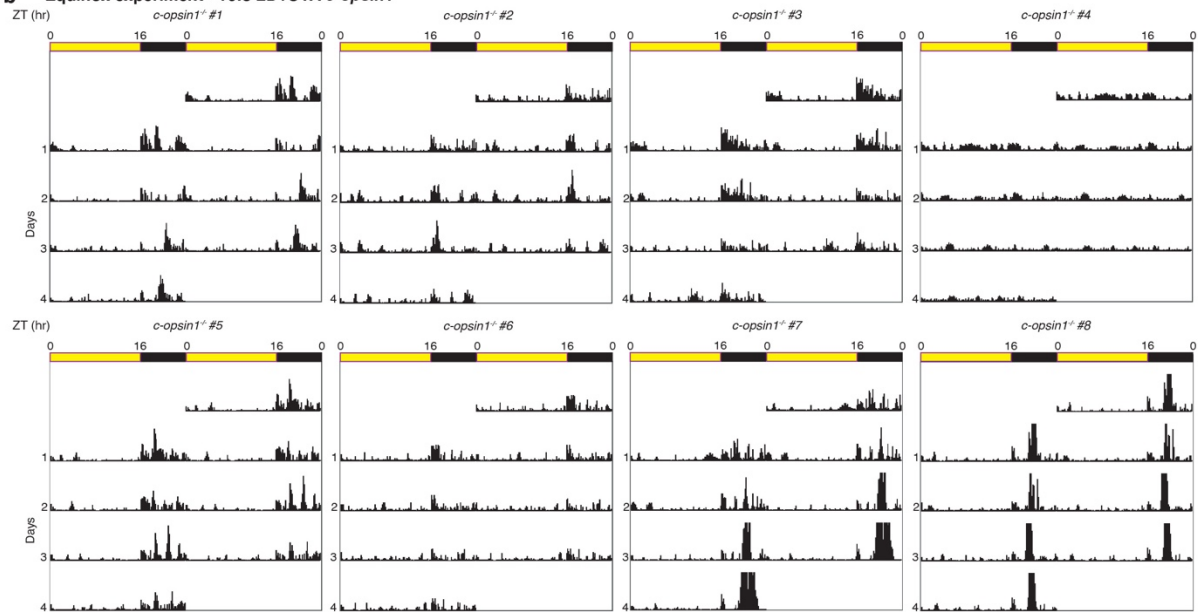
Supplementary Figure 7

SupplFig.7. Statistical analyses and comparisons for different photoperiods, UVA intensities and *c-opsin1* wildtype/mutant.

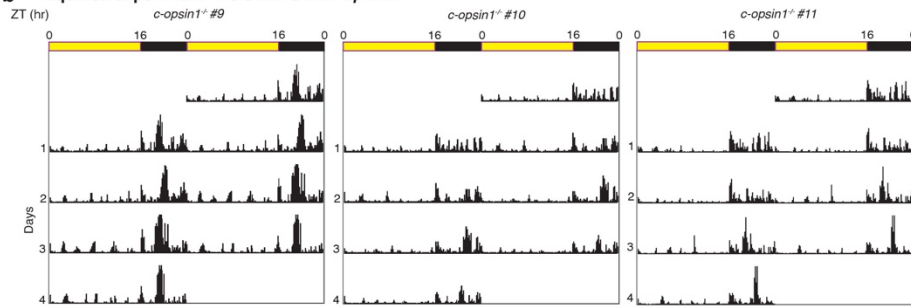
**a Equinox experiment - 16:8 LD+UVA *c-opsin1<sup>+/+</sup>***



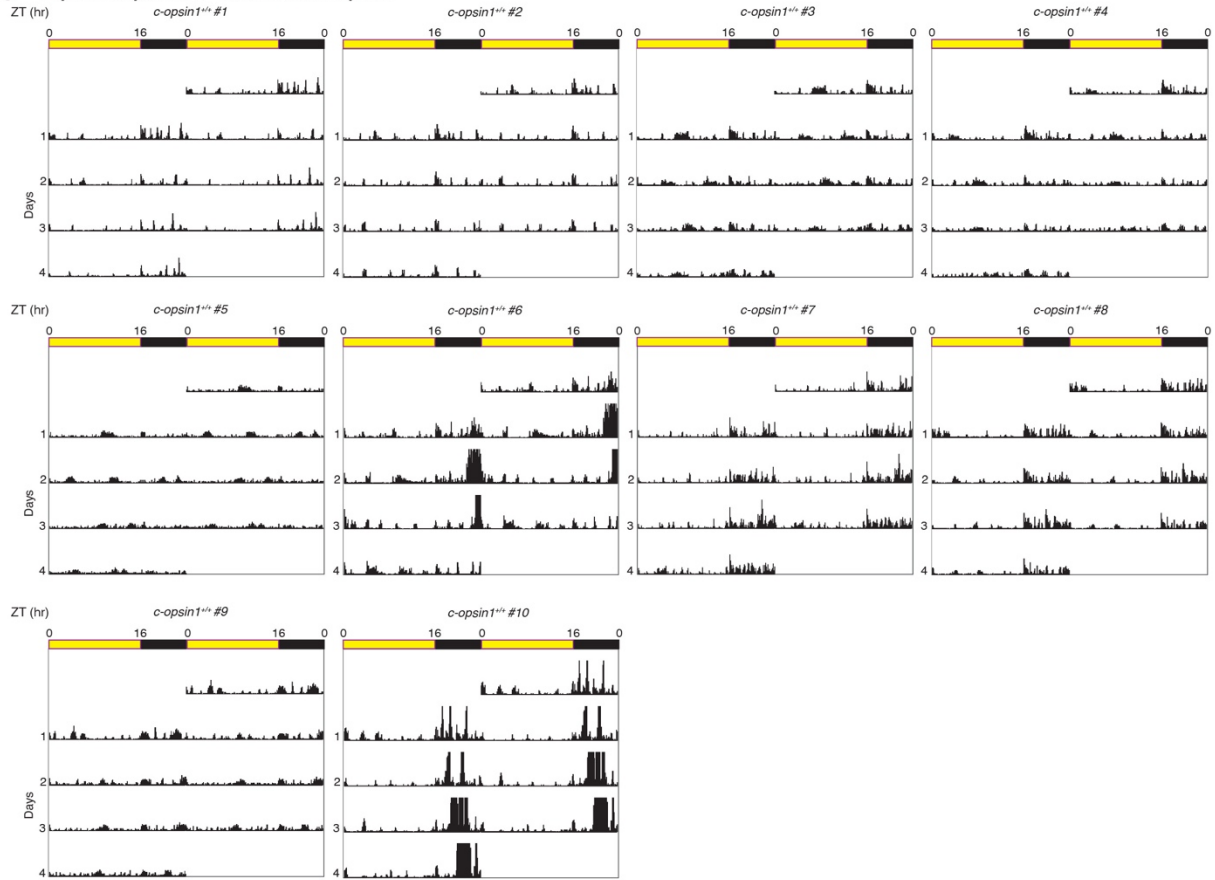
**b Equinox experiment - 16:8 LD+UVA *c-opsin1<sup>-/-</sup>***



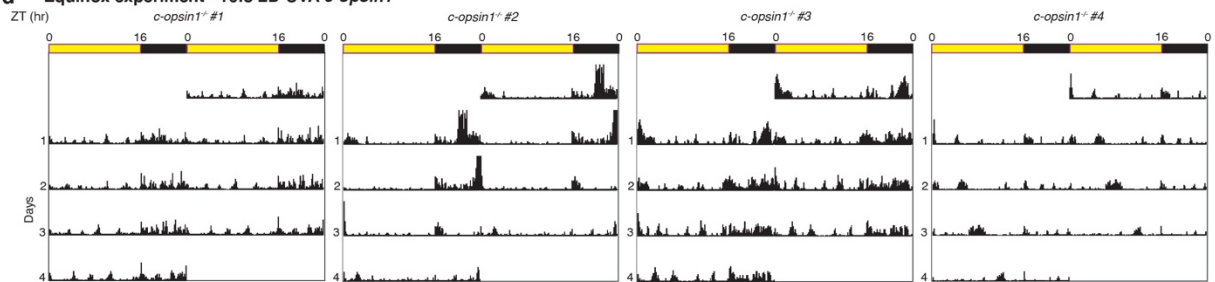
**b Equinox experiment - 16:8 LD+UVA *c-opsin1<sup>-/-</sup>***

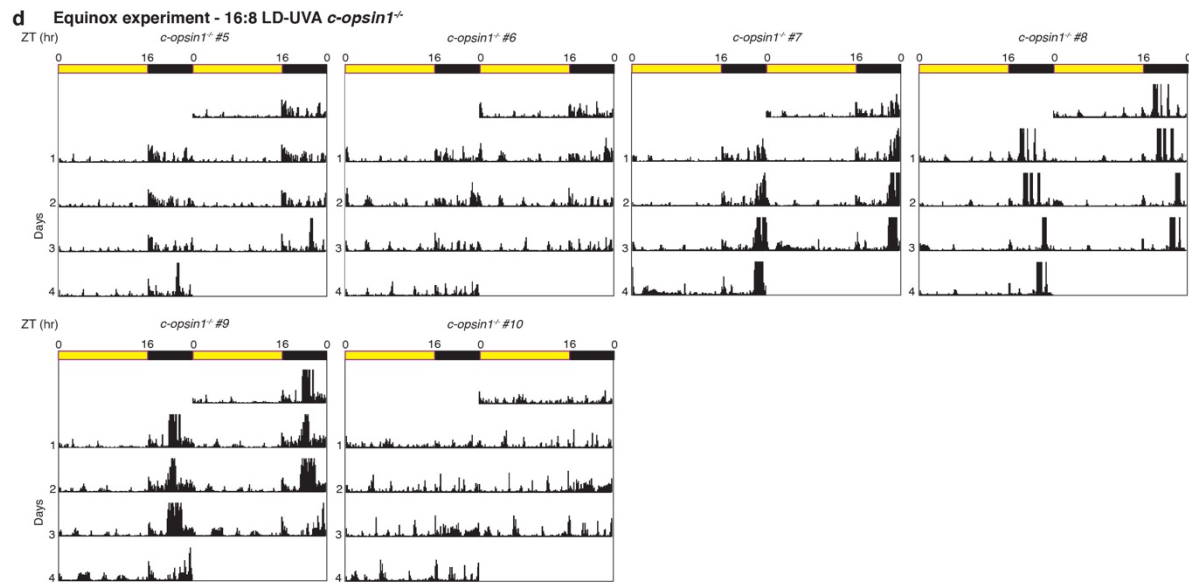


**c Equinox experiment - 16:8 LD+UVA *c-opsin1<sup>+/-</sup>***



**d Equinox experiment - 16:8 LD+UVA *c-opsin1<sup>-/-</sup>***



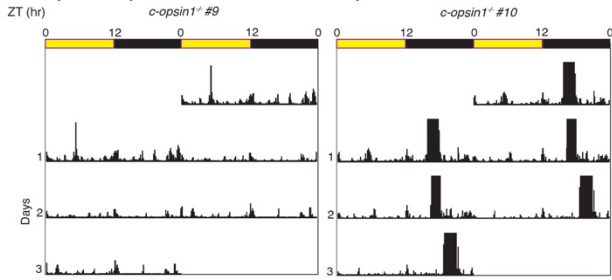


**SupplFig.8. Individual double-plotted actograms of *c-opsin1*<sup>A8</sup> and corresponding wt under long day, strong versus filter-reduced UVA.** The same worms were subsequently analyzed under LD12:12 (see Suppl.Fig. 1e,f and 9), and hence part of the “equinox experiment”.

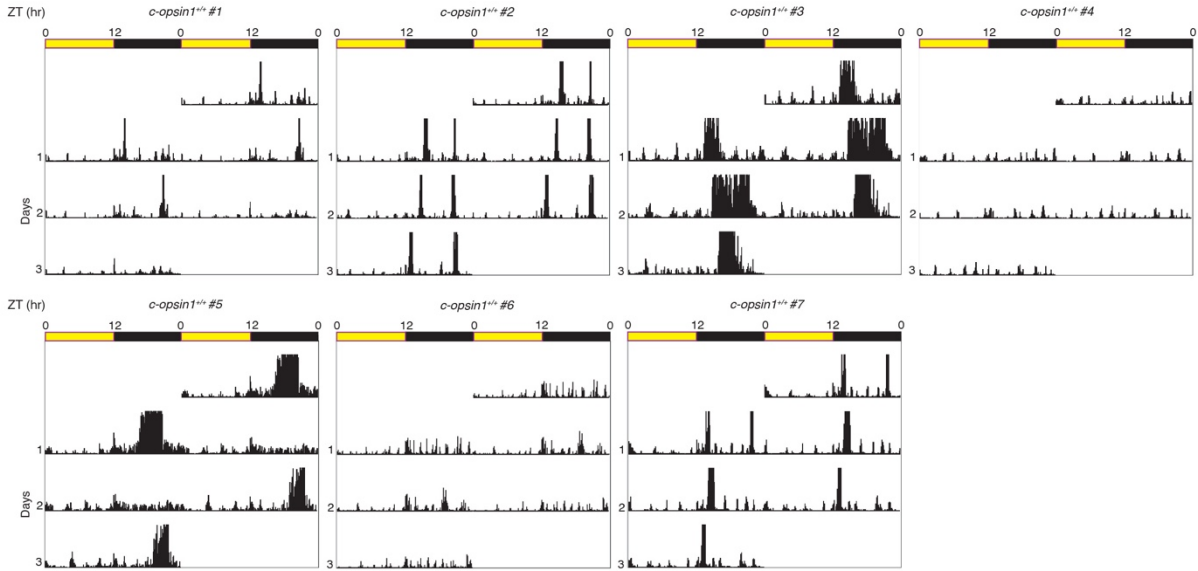




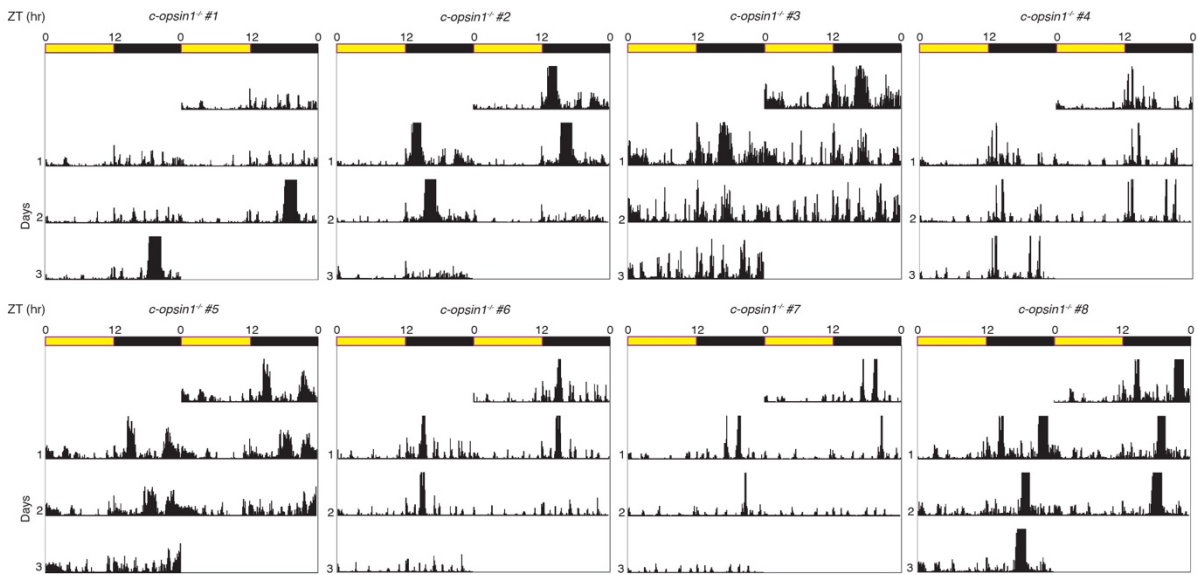
**b Equinox experiment - 12:12 LD+UVA *c-opsin1<sup>-/-</sup>***



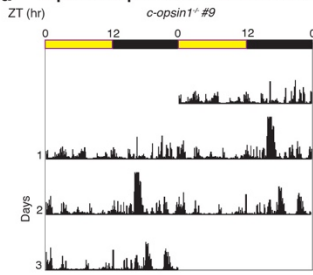
**c Equinox experiment - 12:12 LD+UVA *c-opsin1<sup>+/-</sup>***



**d Equinox experiment - 12:12 LD+UVA *c-opsin1<sup>-/-</sup>***



**d** Equinox experiment - 12:12 LD-UVA *c-opsin1<sup>Δ8</sup>*



# RAMSES-ACC-VIS

Hyperspectral UV-VIS Irradiance Sensor

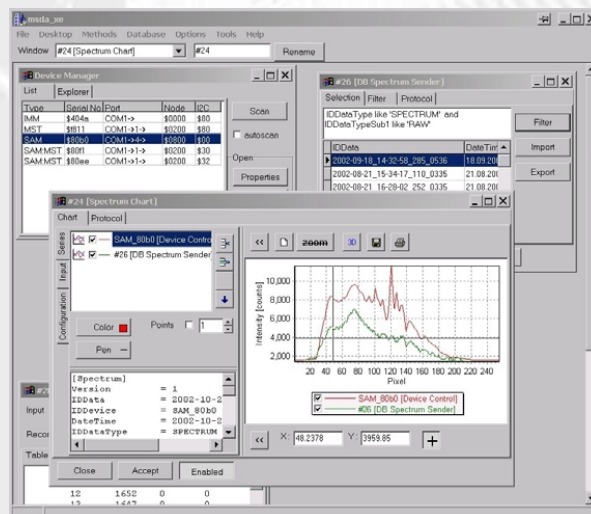
320..950 nm

## Features:

**RAMSES-ACC** is a stand-alone highly integrated hyperspectral radiometer for the UV and/or VIS spectral range. Small size and very low power consumption make it suitable for hand-held and autonomous applications. The sensor is part of the **RAMSES radiometer family**, which is especially designed for combining precision hyperspectral light measurements with a maximum of flexibility.

## Applications:

- monitoring
- water quality
- field measurements
- satellite data validation
- biology
- photosynthesis
- climatology



Sensors are delivered with our free graphical easy-to-use software for measurement, data display and storage.

TriOS GmbH  
Werftweg 15  
D-26135 Oldenburg  
Germany, info@trios.de  
fon +49 (0) 441 - 4 85 98-0  
fax +49 (0) 441 - 4 85 98-20

TriOS Optical Sensors

# RAMSES-ACC

## Features:

- stand-alone hyperspectral radiometer
- low power consumption
- small size
- autorange function
- free Windows-based acquisition and control software

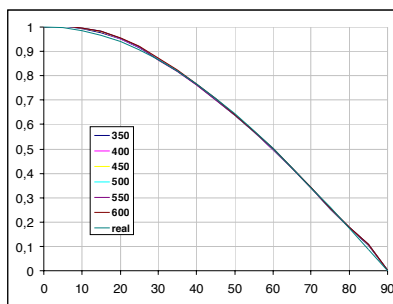
## Accessories:

- power supply
- interface for simultaneous operation of up to 4 radiometers
- data logger units
- sensor frames

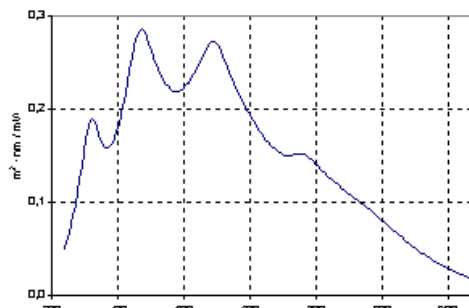
| Technical specifications                  |  |
|---|--|
| VIS                                       |  |
| <b>optical</b>                            |  |
| wavelength range*                         | 320 – 950 nm   |
| detector type*                            | channel silicon photodiode array   |
| spectral sampling*                        | 3.3 nm/pixel   |
| spectral accuracy*                        | 0.3 nm   |
| usable channels                           | 190  |
| typical saturation (4ms integration time) | 10 W m <sup>-2</sup> nm <sup>-1</sup> (at 400nm)<br>8 W m <sup>-2</sup> nm <sup>-1</sup> (at 500nm)<br>14 W m <sup>-2</sup> nm <sup>-1</sup> (at 700nm)        |
| typical NEI (8s integration time)         | 0.4 μW m <sup>-2</sup> nm <sup>-1</sup> (at 400nm)<br>0.4 μW m <sup>-2</sup> nm <sup>-1</sup> (at 500nm)<br>0.6 μW m <sup>-2</sup> nm <sup>-1</sup> (at 700nm) |
| <b>detection</b>                          |  |
| collector type                            | cosine response  |
| accuracy                                  | better then 6 – 10% (depending on spectral range)  |
| <b>electrical</b>                         |  |
| integration time                          | 4 ms – 8 sec.  |
| telemetry data interface                  | RS-232   |
| data rate (RS-232)                        | 1,200 – 19,200 baud  |
| power requirements                        | 1.5 – 11 VDC<br>0.85 W (data acquisition active)<br>100 mW (interface active)<br>0.5 mW (stand-by modus)   |
| connector                                 | SUBCONN-Micro 5 pins, male connector   |
| <b>physical</b>                           |  |
| size                                      | ∅ 4.7 cm x 26 cm (without connector)   |
| weight in air                             | < 1.0 kg (stainless steel/POM housing)   |
| depth rating                              | 300 m  |
| operating temperature                     | -10 to +50°C   |

\* specifications from Carl ZEISS, Germany

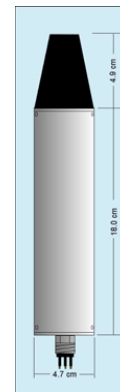
Specifications may change due to technical improvements without notification.



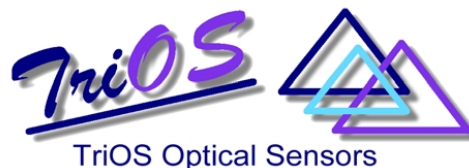
typ. cosine response at different wavelengths



typ. spectral sensitivity of RAMSES-ACC-VIS



TriOS Optical Sensors  
 Wertweg 15  
 D-26135 Oldenburg  
 Germany  
 info@trios.de  
 fon +49 (0) 441 - 4 85 98-0  
 fax +49 (0) 441 - 4 85 98-20



SupplFig.10. Information data sheet of the Ramses hyperspectral radiometers used in the study (TriOS GmbH), new address: TriOS Mess- und Datentechnik GmbH, Buergermeister-Broetjje-Str.25, D-26180, Rastede, Germany