

## Regulation of molecular clock oscillations and phagocytic activity via muscarinic Ca<sup>2+</sup> signaling in human retinal pigment epithelial cells

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SUPPLEMENTARY INFORMATION

## Supplementary methods

### The PCR primers

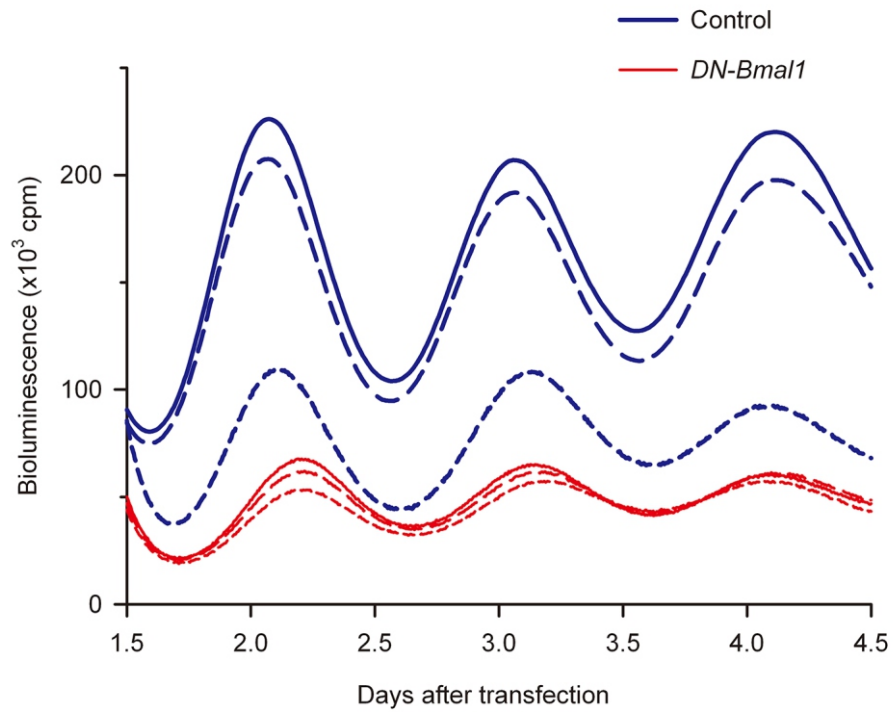
<i>human M1 receptor</i> forward	5'-AGACGCCAGGCAAAGGGGGTGG-3'
<i>human M1 receptor</i> reverse	5'-CACGGGGCTTCTGGCCCTTGCC-3'
<i>human M3 receptor</i> forward	5'-GACAGAAAACCTTTGTCCACCCAC-3'
<i>human M3 receptor</i> reverse	5'-AGAAGTCTTAGCTGTGTCCACGGC-3'
<i>human M5 receptor</i> forward	5'-CTCACCACCTGTAGCAGCTACCC-3'
<i>human M5 receptor</i> reverse	5'-CTCTCTTTCGTTTGGTCATTGATG-3'
<i>human Cry1</i> forward	5'-CTCCATGGGCACTGGTCTCAGTG-3'
<i>human Cry1</i> reverse	5'-TCCCCACCAATTCAGCTGCAAC-3'
<i>mouse Cry1</i> forward	5'-CCCAGGCTTTTCAAGGAATGGAACA-3'
<i>mouse Cry1</i> reverse	5'-TCTCATCATGGTCATCAGACAGAGG-3'
<i>human Cry2</i> forward	5'-CCAAGAGGGAAGGGCAGGGTAGAG-3'
<i>human Cry2</i> reverse	5'-AGGATTTGAGGCACTGTTCCGAGG-3'
<i>mouse Cry2</i> forward	5'-GGGACTCTGTCTATTGGCATCTG-3'
<i>mouse Cry2</i> reverse	5'-GTCACTCTAGCCCGCTTGGT-3'
<i>human Opn4</i> forward	5'-GGACCGCTACCTGGTAATCA-3'
<i>human Opn4</i> reverse	5'-GGAGGAAGAACACGAAGCAG-3'
<i>mouse Opn4</i> forward	5'-CCAGCTTCACAACCAGTCCT-3'
<i>mouse Opn4</i> reverse	5'-CAGCCTGATGTGCAGATGTC-3'
<i>human <math>\beta</math>-actin</i> forward	5'-ATGGCCACGGCTGCTTCCAGC-3'
<i>human <math>\beta</math>-actin</i> reverse	5'-CATGGTGGTGCCGCCAGACAG-3'
<i>mouse <math>\beta</math>-actin</i> forward	5'-AGTGTGACGTTGACATCCGTA-3'
<i>mouse <math>\beta</math>-actin</i> reverse	5'-GCCAGAGCAGTAATCTCCTTCT-3'

### Supplementary figure 1. Effects of dominant-negative *Bmal1* (*DN-Bmal1*) transfections on *Bmal1* transcriptional rhythms

The *Bmal1* transcriptional rhythms were analyzed by *Bmal1-luciferase* activities using the Kronos-Dio luminometer system. Significant amplitude reductions were observed following *DN-Bmal1* transfections 1 day prior to the regular recording schedule.

### Supplementary movie 1. Time-lapse virtual color $\text{Ca}^{2+}$ imaging of hRPE-YC cells

This video demonstrates the entire process of 3-day changes in cytosolic  $\text{Ca}^{2+}$  concentrations shown in Fig. 3A. Warmer color corresponds to higher  $\text{Ca}^{2+}$  concentrations.



Supplementary Fig. 1