

SUPPLEMENTARY MATERIAL

Nash et al., Daily blue-light exposure shortens lifespan and causes brain neurodegeneration in *Drosophila*.

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Supplementary Fig 5. Genetic manipulation of *cry* or *Rh7* expression does not alter lifespan of flies in B:D versus D:D compared to their respective controls.

Supplementary Table 1. Fly stocks used in this study.

Stock	Genotype	Source	Reference
CS	Canton S	Paul Hardin	
<i>w</i> ¹¹¹⁸ (iso31)	<i>w</i> ¹¹¹⁸ ;;	BDSC stock #5905	1
<i>eya</i> ²	; <i>eya</i> ² ;	BDSC stock #2285	2
<i>so</i> ¹	; <i>so</i> ¹ ;	Doris Kretzschmar	3
<i>cry</i> ⁰²	<i>w</i> ¹¹¹⁸ ;; <i>cry</i> ⁰²	Jeff Hall	4
<i>cry</i> -GFP*	<i>w</i> ¹¹¹⁸ ; <i>cry</i> -GFP; <i>cry</i> ⁰²	Paul Hardin	5
<i>tim</i> -Gal4	<i>w</i> ¹¹¹⁸ ; { <i>tim</i> -Gal4};	Jeff Hall	6
UAS- <i>cry</i>	<i>w</i> ¹¹¹⁸ ; {UAS- <i>cry</i> -24};	Patrick Emery	7
UAS- <i>cry</i> ^b	<i>w</i> ¹¹¹⁸ ; {UAS- <i>cry</i> ^b -31};	Patrick Emery	7
<i>ninaE</i> ⁷	;; <i>ninaE</i> ⁷	BDSC stock #2103	8
<i>ninaE</i> ⁸	<i>w</i> ¹¹¹⁸ ;; <i>ninaE</i> ⁸	BDSC stock #2001	8
<i>per</i> ⁰¹	<i>w</i> ¹¹¹⁸ <i>per</i> ⁰¹ ;;	Jeff Hall	9
<i>Tub</i> -GS	{αTub84B-Switch}10	Subhash Katewa	10
<i>Rh7</i> ¹	<i>w</i> ¹¹¹⁸ ; Tl{Tl} <i>Rh7</i> ¹ ;	BDSC stock #76022	11
UAS- <i>Rh7</i> **	<i>w</i> ¹¹¹⁸ ; P{UAS- <i>Rh7</i> .N}	BDSC stock #76027	11

*We exchanged the *cry*⁰³ allele, originally in this stock, with *cry*⁰².

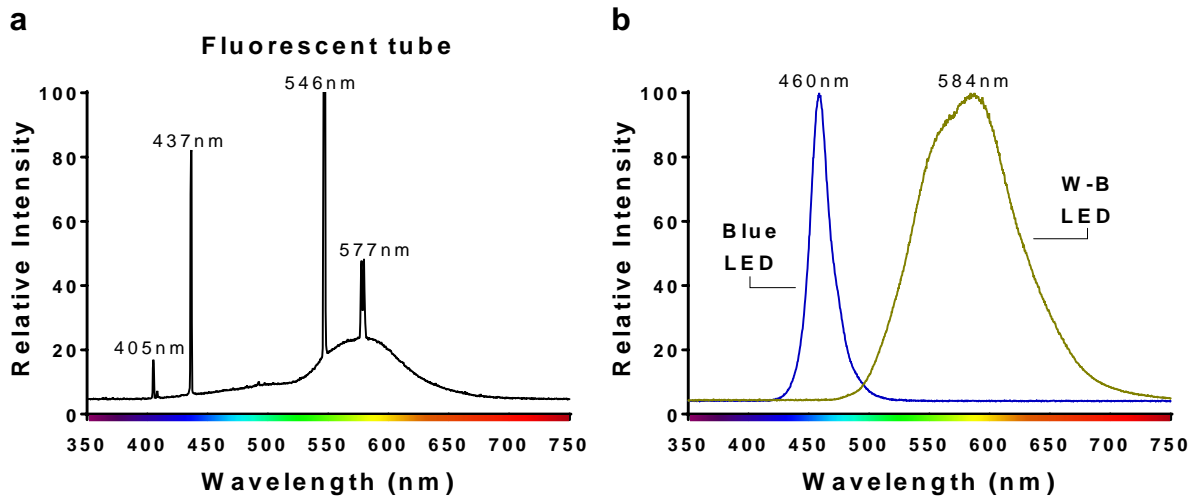
**We removed *Rh7*¹ allele that was present in this stock.

Supplementary Table 2. Light sources used in this study.

Referred to as	Light source	Mfr. and model no.	Peak wavelength(s)	Photon flux density
L:D	White fluorescent tubes (cool white, 4100K)	Philips F96T12/CW/VHO/EW	405 nm, 437 nm, 546 nm, 577 nm	20-30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
B:D	Blue LED	MarsAqua Dimmable 165W LED Aquarium Light (Channel 1)	460 nm	20-30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
B+O:D	Blue LED + orange LED	NovoLux LED blue 40 Orange LED Masterled	460 nm, 600 nm	20-30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
W-B:D	White LEDs with yellow long-pass filter	MarsAqua Dimmable 165W LED Aquarium Light (Channel 2); Rosco color filter #4590 CalColor 90 Yellow	584 nm	20-30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

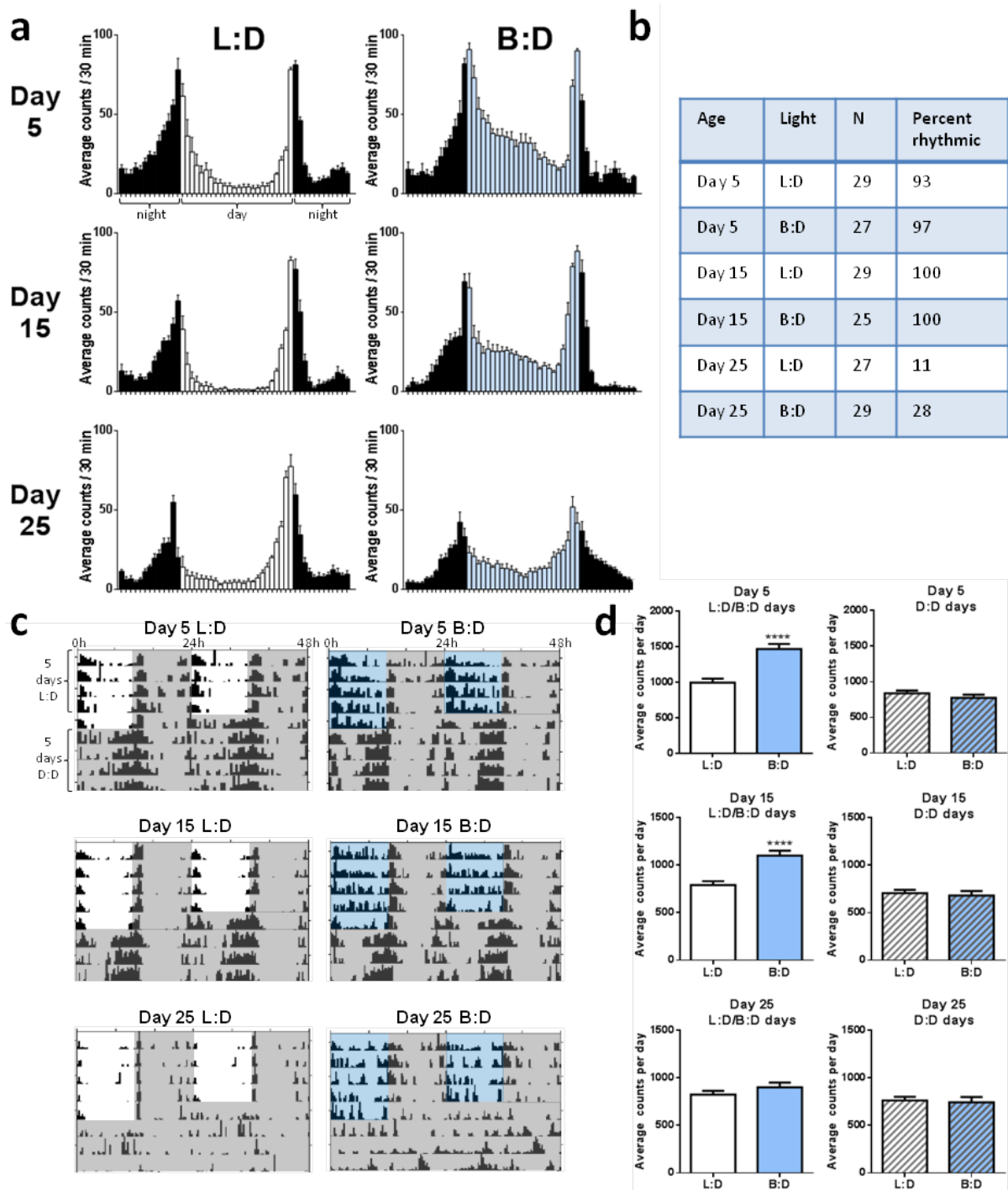
Supplementary Table 3. Primers used in this study.

Gene Name	Symbol	FlyBase ID	Primers (5' – 3')
Decapping protein 2	<i>DCP2</i>	FBgn0036534	Fwd: CCAAGGGCAAGATCAATGAG Rev: GCATCGATTAGGTCCGTGAT
Glutamate-cysteine ligase catalytic subunit	<i>Gclc</i>	FBgn0040319	Fwd: ATGACGAGGAGAATGAGCTG Rev: CCATGGACTGCAAATAGCTG
Glutathione S transferase O1	<i>GstO1</i>	FBgn0035907	Fwd: AGCTGTATTTCGATGCGCTTT Rev: GGTTTGTGCGGAAGATTGAT
Thioredoxin reductase-1	<i>Trxr-1</i>	FBgn0020653	Fwd: AAAAGCAGGATGATGGCAAG Rev: CCACAGAACGGTGTCTGATAA
cap-n-collar	<i>cncC</i> (isoform C)	FBgn0262975	Fwd: TCGGAGATGACGAGGAGGAGAGT Rev: GCATTGATGATCGCCTCCTGGT
Glutathione S transferase D1	<i>GstD1</i>	FBgn0001149	Fwd: TGAAGCCGGAGTTCCTGAAGA Rev: TGTCGAAGTACAGGCGCTGAT
Glutathione S transferase D2	<i>GstD2</i>	FBgn0010038	Fwd: ACATTGCCATCCTGTCCACTG Rev: TCCTGGAGTCACCTTCTTGGC
Heat shock protein 23	<i>Hsp23</i>	FBgn0001224	Fwd: GTGTGCGAAAATCGGAAAGGA Rev: ACGGAGTTGTCTGCACTTT
Heat shock protein 68	<i>Hsp68</i>	FBgn0001230	Fwd: CCATCATGACCAAGATGCAC Rev: ACGGTGGGACCCCTTATAACC
Heat shock protein 70Aa, Ab, Ba, Bb, Bbb, Bc	<i>Hsp70Aa, Ab, Ba, Bb, Bbb, Bc</i>	FBgn0013275, FBgn0013276, FBgn0013277, FBgn0013278, FBgn0051354, FBgn0013279	(common to all listed Hsp70 genes) Fwd: CCCACTTTCATTGGGAATTG Rev: TAGTCTGCTTGCACGGAATG
Lactate dehydrogenase	<i>Ldh</i>	FBgn0001258	Fwd: CGTTTGGTCTGGAGTGAACA Rev: GCAGCTCGTCCACTTCTCT

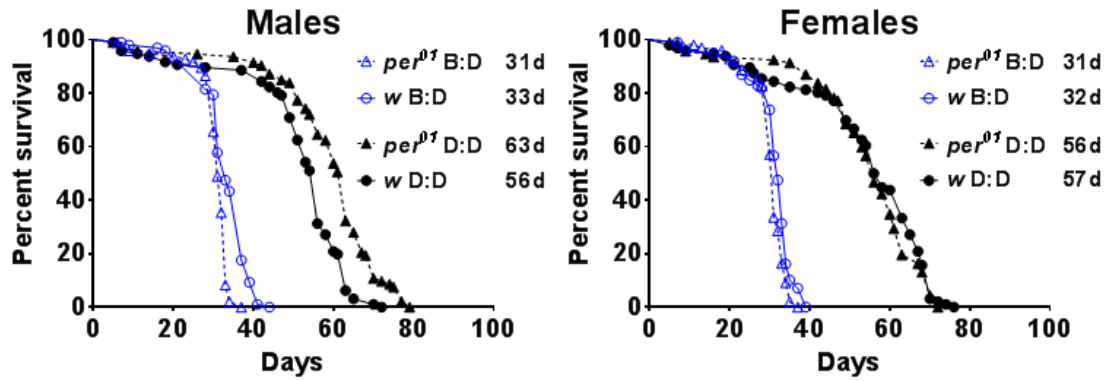


Supplementary Fig 1. Spectral characteristics of light used in this study.

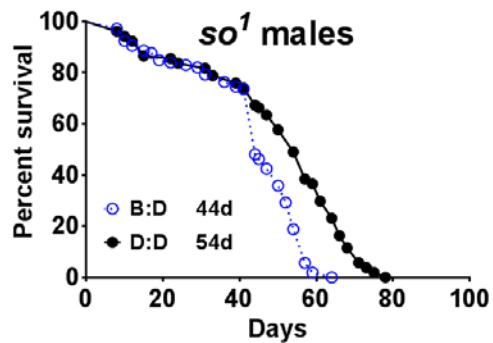
a White fluorescent spectrum (used in L:D conditions). **b** Spectrum of blue LED (used in B:D conditions) with peak wavelength at 460 nm and white LED with blue light blocked by yellow filter (used in W-B:D condition) with peak wavelength at 584nm. Intensity of each light spectrum is normalized to the peak (set as 100%) of the same spectrum.



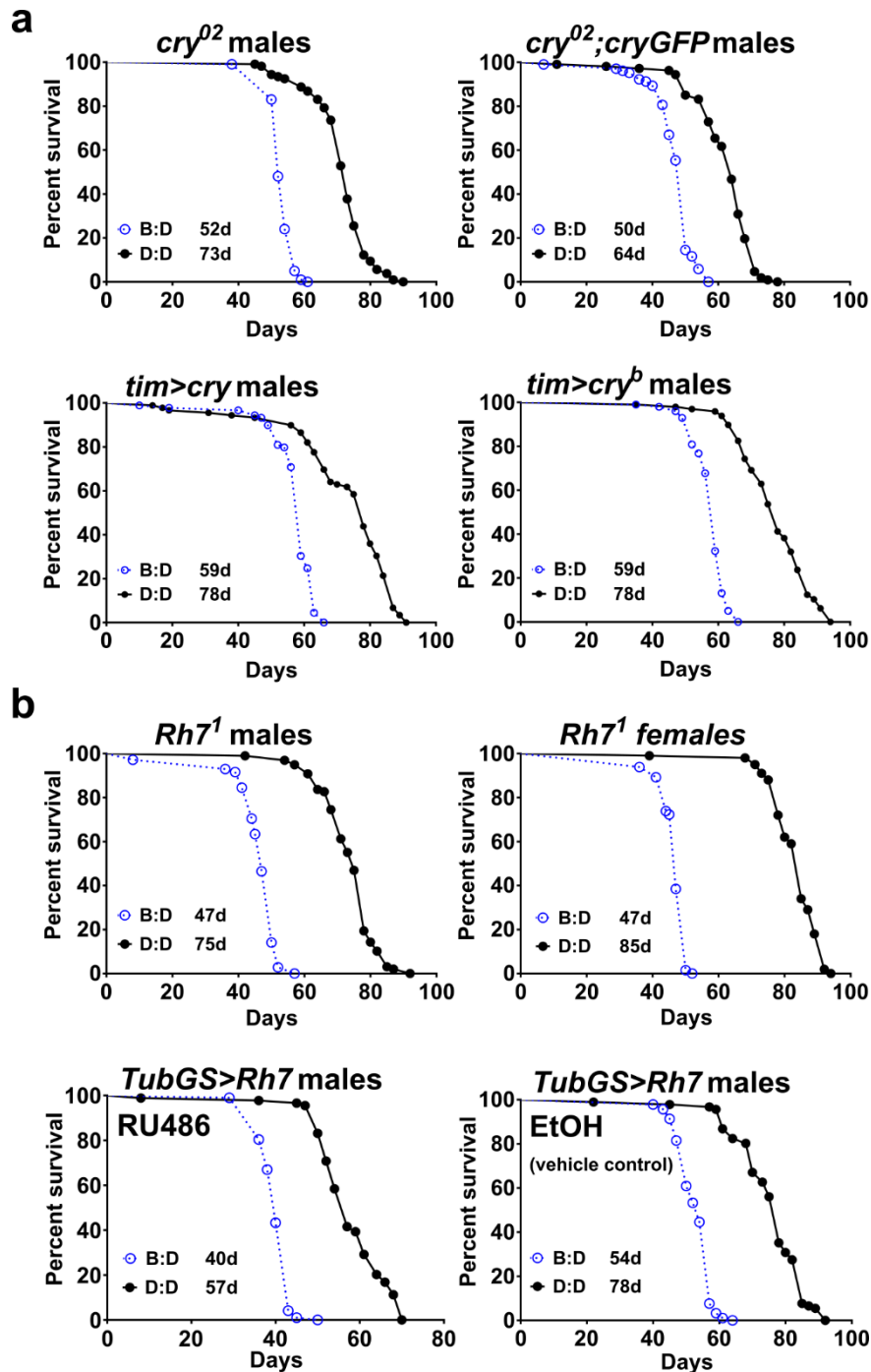
Supplementary Fig 2. Young *w* males under B:D show increased locomotor activity compared to those in L:D. **a** Averaged activity counts of all flies in each group, over five consecutive 24 h cycles of L:D or B:D. **b** Summary of experimental details, including percent of rhythmic calculated from D:D days. **c** Representative actograms of individual flies from each group. Gray shaded areas indicate darkness, and white or blue areas indicate white or blue light, respectively. **d** At day 5 and day 15, flies kept in B:D are significantly more active than flies kept in L:D, during the light:dark days (unpaired t-test; **** $p < 0.0001$). No significant difference in activity levels is observed between the same flies which were previously in L:D or B:D, but switched to D:D.



Supplementary Fig 3. Clock mutants ($w^{1118}per^{01}$) had similarly reduced median lifespan to w^{1118} controls in B:D compared to D:D.



Supplementary Fig 4. Lifespan of *sine oculis* (so^1) mutants, which lack the retina and ocelli, was significantly reduced in B:D compared to D:D. (Log-rank test, $p < 0.0001$)



Supplementary Fig 5. Blue light-sensitive proteins CRY or RH7 are not involved in mediating susceptibility to blue light. **a** Mutants not expressing cryptochrome (*cry⁰²*), or flies with elevated expression of this gene via the *tim*-Gal4>UAS-*cry* driver/responder system (left graphs) show similar lifespan differences between B:D and D:D conditions as their respective controls of a *cry* rescue line and *tim*-Gal4>UAS-*cry^b* flies overexpressing a nonfunctional version of CRY (right graphs). **b** Mutants lacking Rhodopsin7 (*Rh7¹*) and flies overexpressing this gene using the GeneSwitch system induced by the drug RU486 show highly reduced lifespans in B:D compared to D:D. Detailed genotypes are given in Supplementary Table 1.

SUPPLEMENTARY REFERENCES

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