Supplementary Information

Photochemical activation of TRPA1 channels in neurons and animals

AUTHORS
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SUPPLEMENTARY RESULTS

FIGURES

Supplementary Figure 1. **Optovin action spectrum and phenotypic similarity to known neuroactive compounds.** (a) Behavior excitation scores of zebrafish treated with optovin and the indicated stimulus wavelengths (n=10). At 387nm, the difference between the treated and untreated groups is significant, $p<0.001$. (b) Dendrogram showing phenotypic distances between optovin and 700 annotated neuroactive compounds. Lower branches of the tree are collapsed for clarity. For all plots of the behavioral excitation score, values are means ± standard deviation.

Supplementary Figure 2. **Stimulus and response duration are proportional.** (a) Line plot showing the duration of motor excitation relative to stimulus duration (n=5). Differences between treated and untreated groups are significant, $p<0.001$. (b-d) Examples of optovin-treated wells treated with different stimulus durations. Red vertical lines indicate the beginning and end of the stimulus pulse. The pink horizontal lines indicate the magnitude and duration of the behavioral excitation score.

Supplementary Figure 3. **Optovin treatment does not affect survival rates in larval or adult zebrabra fish.** Barplots showing the percentage survival of adult (n=2) (a) and larval (n=150) (b) zebrafish after the indicated duration of optovin treatment (10 μM). Differences between groups were not significant, $p=0.7$.

Supplementary Figure 4. **Optovin does not affect the activity of the hERG potassium channel**. Optovin (PDSP 22594) was tested for hERG inhibition using the FluxOR system with cisapride as a positive control. Optovin showed no effect on hERG activity with or without prior exposure to light (microscope light source, maximum illumination, 30 seconds).

Supplementary Figure 5. **Optovin-treated spinalized preparations respond to light**. (a) Behavioral excitation scores of intact and spinalized zebrafish embryos (30 hpf). Differences between treated and untreated groups are significant, $p<0.001$. Photographs showing zebrafish embryos at 35hpf before (b) and after (c) transection posterior to the hindbrain as indicated by the dashed line. Scale bar, 150 μm. (d,e) Examples of how DMSO-treated (c) and optovin-treated (d) spinalized animals respond to light stimuli.

Supplementary Figure 6 Mustard oil and optovin act on the same subset of DRG sensory neurons. (a-c) Calcium imaging of mouse DRG neurons in response to vehicle (a), optovin and

light (b), and optovin without light (c). Black traces indicate calcium indicator fluorescence readings. No readings were recorded during the 1min optovin treatment period in c) when lights were turned off. (d) Fuorescence traces from 10 example cells showing their response to the indicated treatments. (e)Venn diagram showing that 28/35 mustard oil responsive cells also respond to optovin. (f) After Fura-2, AM loading and washing, neurons were loaded with optovin (100 μM) and with either the TRPA1 antagonist, HC-030031 (26 μM, Sigma) (McNamara et al., 2007) or vehicle control (DMSO, 0.025%). After a 10-minute incubation period, the neurons were exposed to a 5-second laser pulse (405 nm), washed, and then activated with mustard oil (100 μM). Responses are presented normalized to the response elicited by mustard oil per mustard oil-responsive cell. Error bars are standard error.

Supplementary Figure 7. The TrpA1 homolog **TrpA1a is not necessary for the optovin response.** (a) Scatter plot showing the behavioral excitation score (calculated after the second stimulus) for individual WT, heterozygous and homozygous mutant animals treated with optovin (n=14, 22 and 20, respectively). (b) Bar chart showing the behavioral excitation scores for the indicated genotypes. Values are means +/- the standard deviation. The differences between group means are not statistically significant, p=0.18. Plots of the motion index are shown for a wildtype (c), heterozygous (d), and homozygous mutant (e) Trpa1a animal. All genotypes respond to the stimulus, indicating that TrpA1a is not necessary for the response. Red bars indicate timing of a continuous 20s light stimulus (wavelength = 387nm).

Supplementary Figure 8. **TrpA1b mutant animals are not generally defective in photosensation or motor activity.** (a) Scatter plot showing the behavioral excitation score calculated during the PMR excitation phase (after the first stimulus) for individual WT, heterozygous and homozygous mutant animals (n=21, 23 and 16 respectively). (b) Barchart showing the mean behavioral excitation scores for the indicated genotypes. Values are means +/- the standard deviation. The differences between group means are not statistically significant, p=0.02. Representative plots of the motion index are shown for a wildtype (c), heterozygous (d), and homozygous mutant (e) Trpa1b animal. All animals respond to the first pulse of light, indicating that TrpA1b mutant animals are not generally defective for photosensation or motor activity. Note that the overall shape of the motion index peak following the first stimulus is different in WT versus mutant animals. This is because optovin affects the normal photomotor response in WT animals, but not in homozygous mutant animals.

Supplementary Figure 9. **Optovin specifically activates TRPA1, but not TRPV1 or TRPM8.** (a-c) Calcium imaging of cells transfected with the indicated TRP channel, and stimulated with the indicated agonist. (d-e) Quantification of the responses shown above (**p<0.01, ***p<0.0001).

Supplementary Figure 10. **TrpA1 is sufficient for the optovin response in transfected HEK cells.** (a) Representative series from optovin bathed (10μM) HEK cells transfected with human TrpA1 (hTrpA1) as measured by whole-cell patch clamp, plotting the slope of the current-voltage relation during voltage ramps from 0 to +70mV just prior to and following 405 nm illumination. (b) Bar plot showing the average current density of cells transfected with hTrpA1 and treated with optovin and/or light as indicated (n=9, *p<0.0085).

Supplementary Figure 11. **Photoactivated optovin generates singlet oxygen, but negligible amounts of hydroxyl radicals and superoxide.** (a) Line plots showing the fluorescence of singlet oxygen sensor green (SOG) and with the indicated compounds and light. Compared to a known photosensitizer (PEI-ce6) that absorbs blue light and produces singlet oxygen with a quantum yield of about 0.6, optovin could be estimated to have a quantum yield of about 0.04. (b) Line plots showing NBT absorbance (a readout of superoxide generation) at increasing intensity of blue light with optovin and vehicle (b), ascorbate (c), and NADH (d). Increased absorbance would be expected only in presence of electron donor such ascorbate or NADH. We did not see evidence of hydroxyl radical production (data not shown).

Supplementary Figure 12. **Catalase and superoxide dismutase do not significantly reduce optovin activity in HEK cells transfected with hTRPA1.** (a) Calcium imaging of HEK cells transfected with hTRPA1 and exposed to the indicated treatments. (b) Barplot quantifying and summarizing the responses shown above (**p<0.01, ***p<0.0001).

Supplementary Figure 13. **DABCO completely suppresses the optovin response *in vivo*, but does not affect other light elicited behaviors, like the PMR.** (a-c) Examples of behavioral responses of animals treated with DMSO (a), optovin (b), and optovin with DABCO (c). Data quantifying and summarizing the full experiment are shown in Fig. 4c. Two light pulses were presented in each assay and are indicated by vertical bars at 10s and 24s. All animals were dark adapted for > 10min prior to the assay. DMSO treated animals respond to only the first pulse of light. This motor response to the first pulse is the PMR excitation phase. In optovin treated animals, animals respond to both pulses of light. The response to the first pulse can be interpreted as a combination of the PMR excitation phases the response to optovin. The response to the second pulse is due to optovin. In animals treated with optovin and DABCO, the PMR excitation phase can still be seen, but the response to optovin is completely suppressed.

Supplementary Figure 14. Optovin has no activity on the hTrpA1 3CK triple cysteine mutant. (a) 3CK mutant TRPA1 plasmid (provided by Christian von Hehn) was transfected into HEK-293 cells and imaged using Fura-2, AM (Life Technologies) the mutations replace the residues on C619, C639, C663, and K708 to serine residues (Escalera et al., 2008). Laser alone, MO alone (100 μM) and optovin (100 μM) and laser combined were used to elicit activation. Carvacrol (100 μM, Sigma-Aldrich) was used as a positive control as it is an agonist of TRPA1 which does not depend on the cysteine residues otherwise critical for MO-based activation (Hinman et al., 2006) (n= 41 cells).

Supplementary Figure 15. **Optovin is phenotypically unique among 171 rhodanine-containing compounds**. (A) Scatter plot showing the behavioral excitation score for 12,500 treated wells (in black). 171 rhodanine containing compounds are marked in red. 2,500 DMSO treated control wells are marked in blue.

Supplementary Figure 16. Model: **Optovin is rapidly and reversibly photo-converted into a potent TrpA1 agonist.** In the dark, optovin (gray star) does not activate TRPA1 channels (green) which remain closed to calcium ions (yellow). In the light, optovin (purple star) is photo-activated to an excited form that activates TRPA1 causing it to open and allow calcium ions to enter the cell.

MOVIES

Supplementary Movie 1. Movie of DMSO treated control animals. Eight zebrafish embryos (at 30 hpf) in a well of a 96 well plate. Animals are too young to have hatched and are still in their egg shells. Light stimuli are seen at 10 and 20s as bright white flashes. DMSO treated animals respond to the first pulse of light, but not the second pulse.

Supplementary Movie 2. Movie of optovin treated animals. Unlike DMSO treated animals, optovin treated animals respond to both pulses of light.

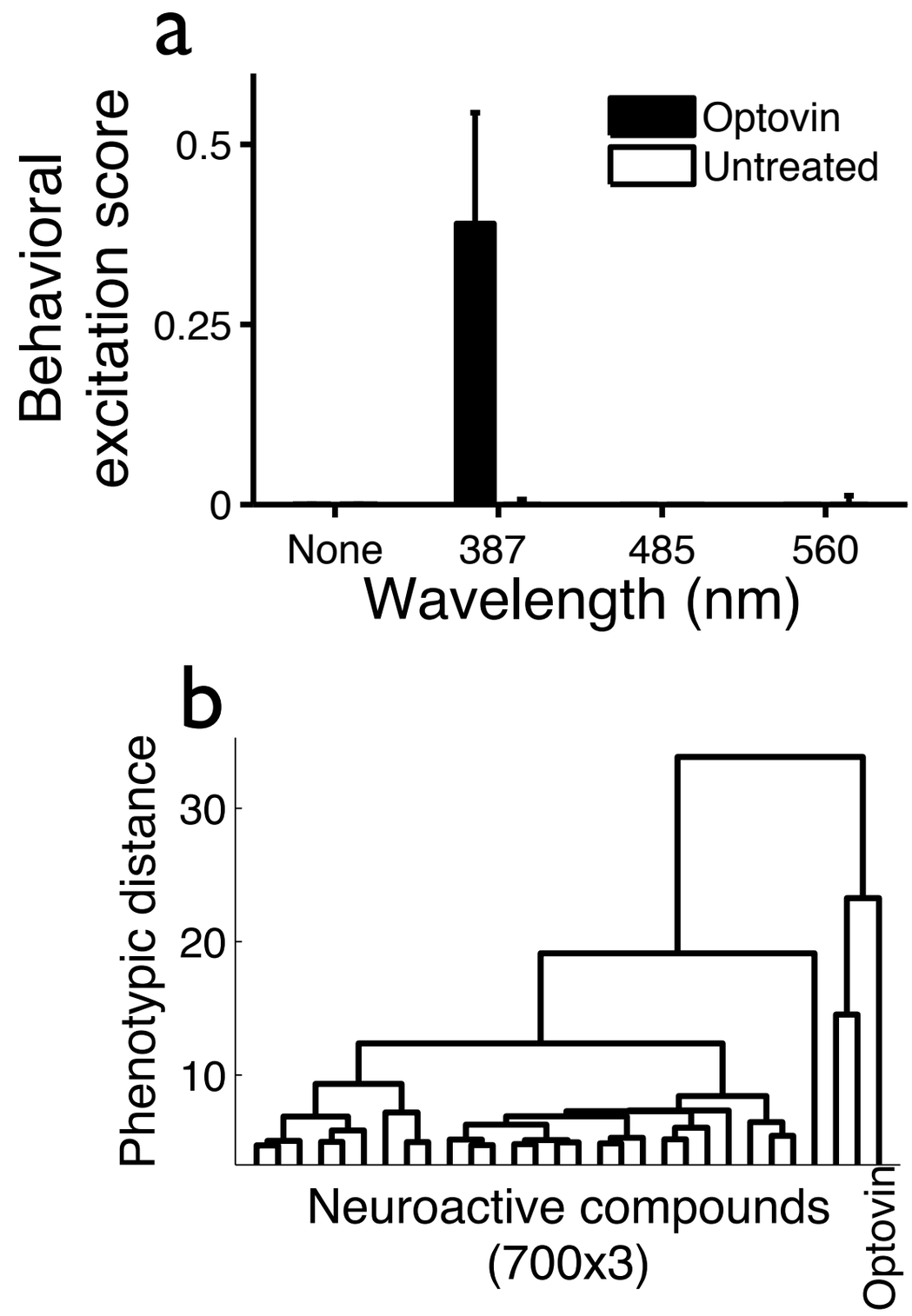
Supplementary Movie 3. Movie showing that optovin-treated spinalized preparations also respond to light. These preparations have transected posterior to the hindbrain, as shown in Supplemental Figure 1.

Supplementary Movie 4. Movie of optovin treated adult zebrafish with repeated 405 nm light point stimulus directed at the dorsal fin. The dorsal fin retracts every time it is touched by the light. It re-extends a few seconds after illumination. Reflected light from the laser pointer can be seen.

Supplementary Movie 5. Movie of DMSO-treated spinalized adult zebrafish with repeated 405 nm light stimuli directed at trunk. The DMSO-treated animal does not respond.

Supplementary Movie 6. Movie of optovin-treated spinalized adult zebrafish with repeated 405 nm light stimuli directed at trunk. The optovin-treated animal responds with vigorous swimming movements.

Supplementary Figure 1. **Optovin action spectrum and phenotypic similarity to known neuroactive compounds.**



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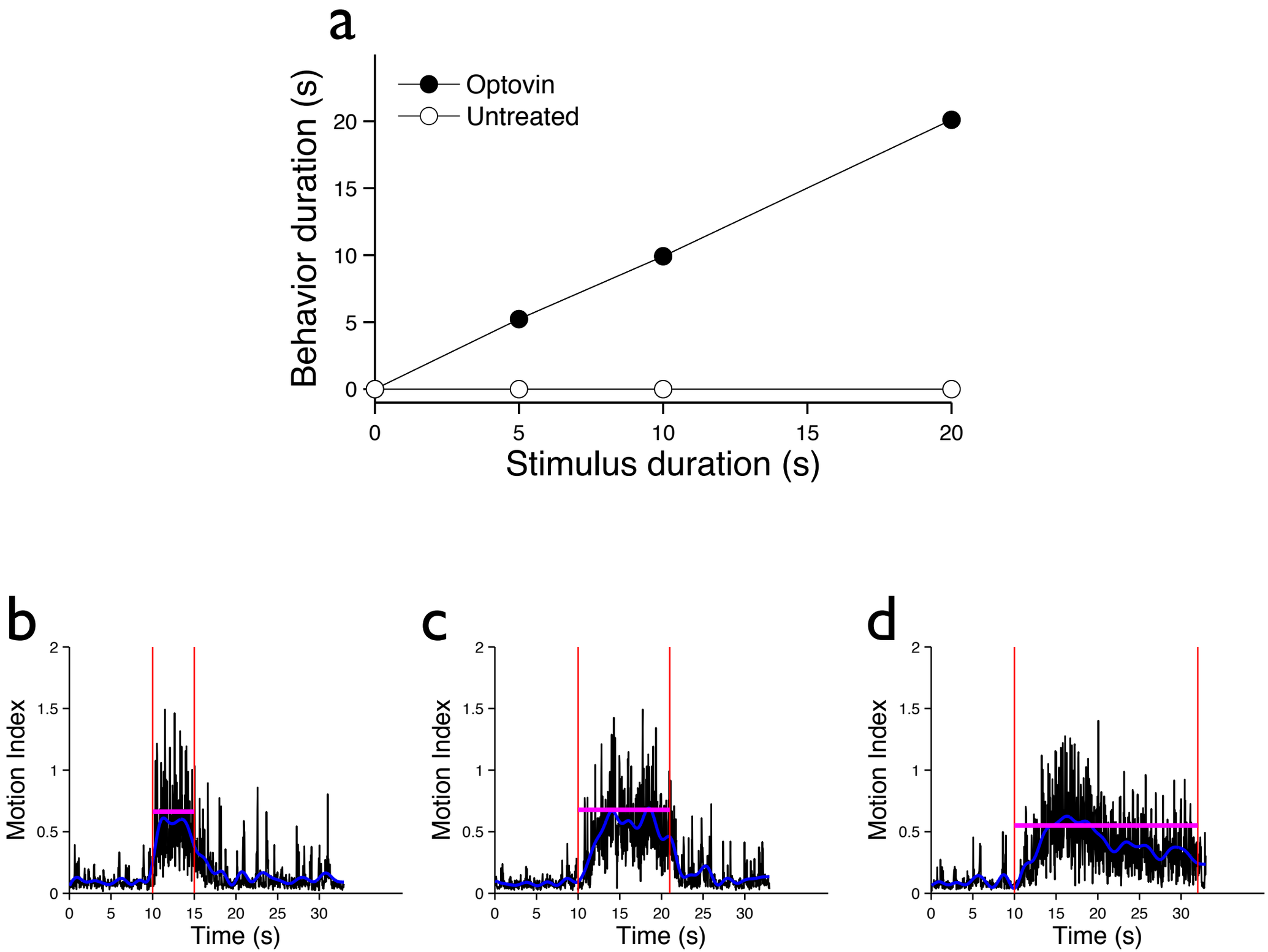

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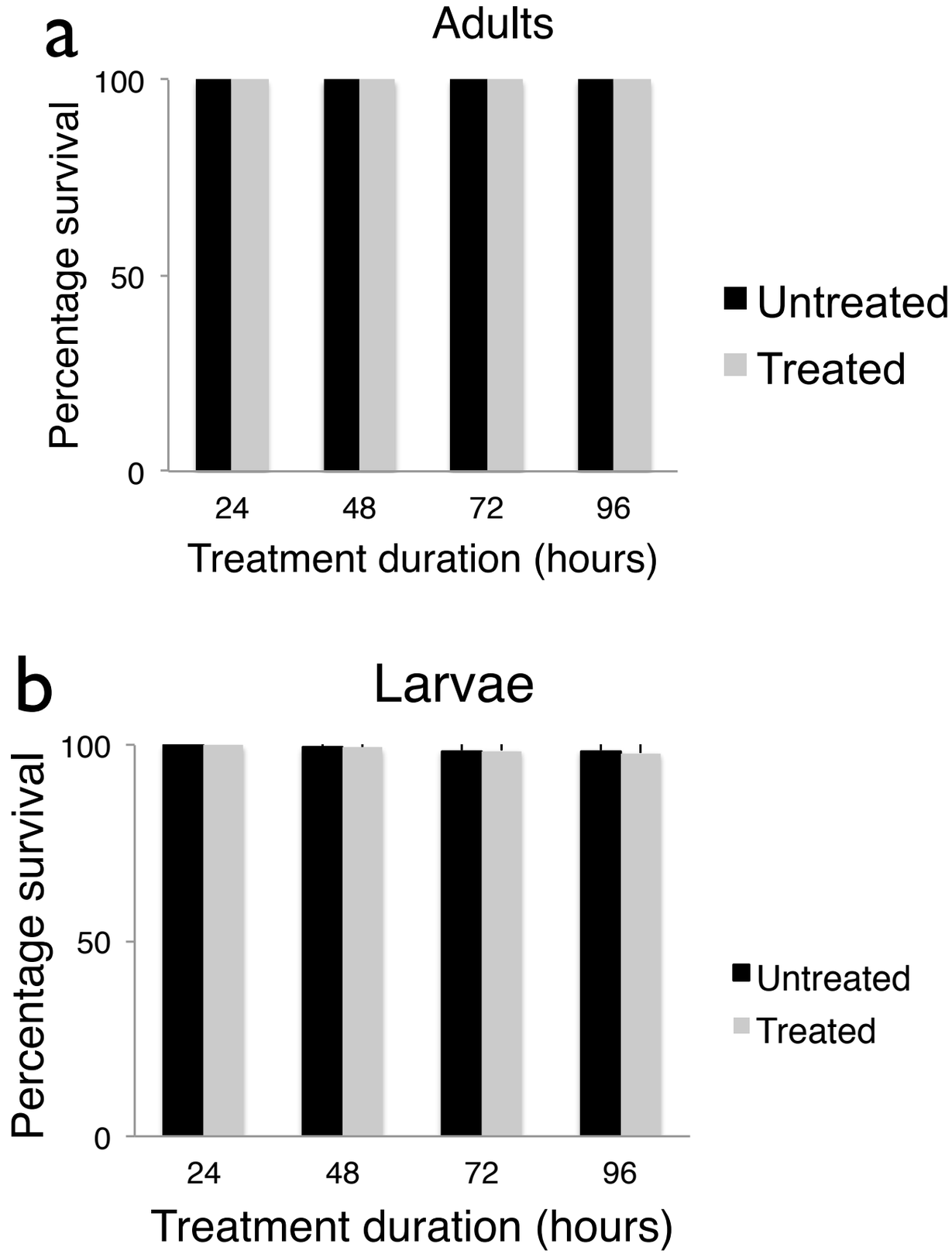

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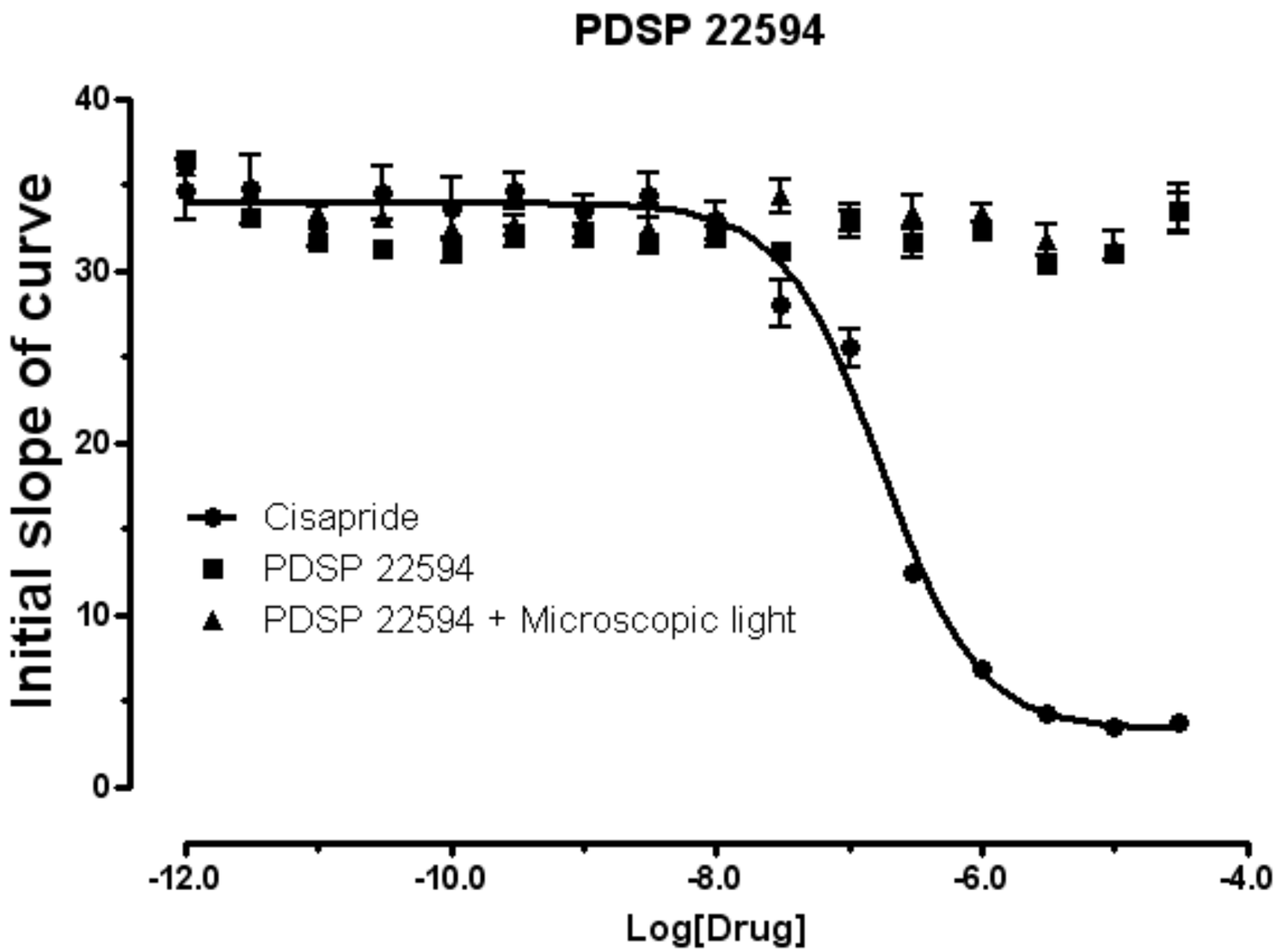
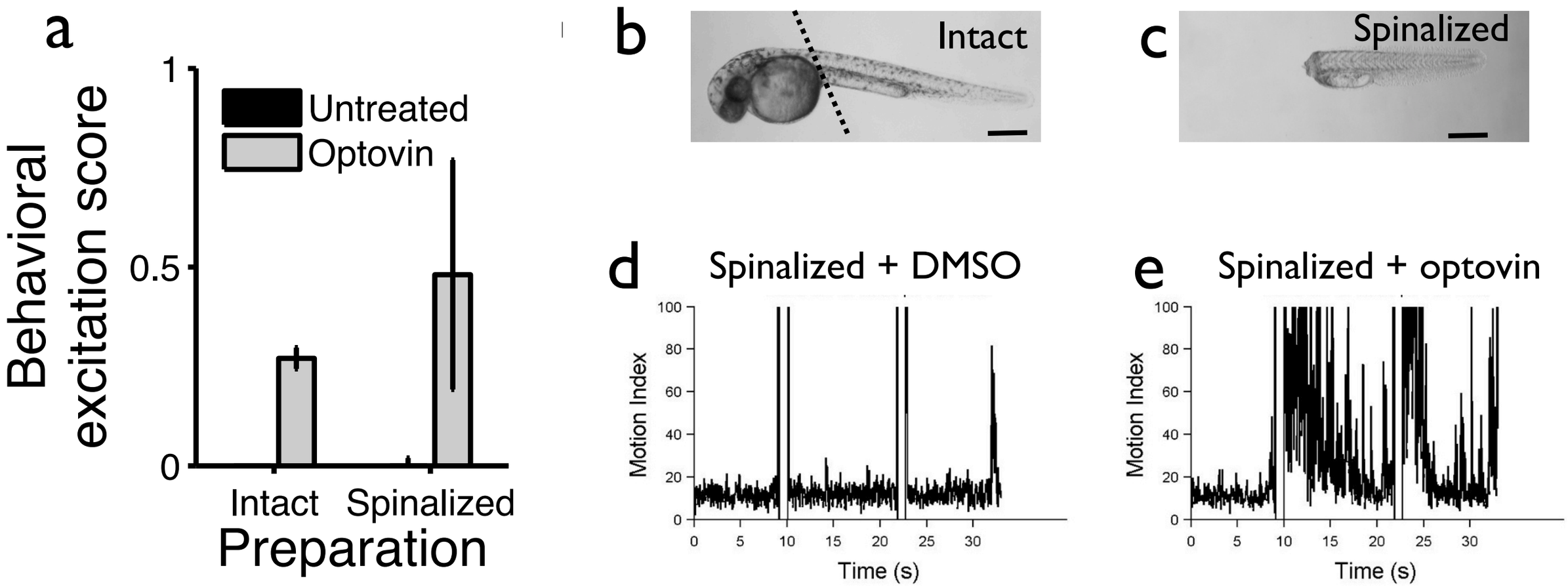
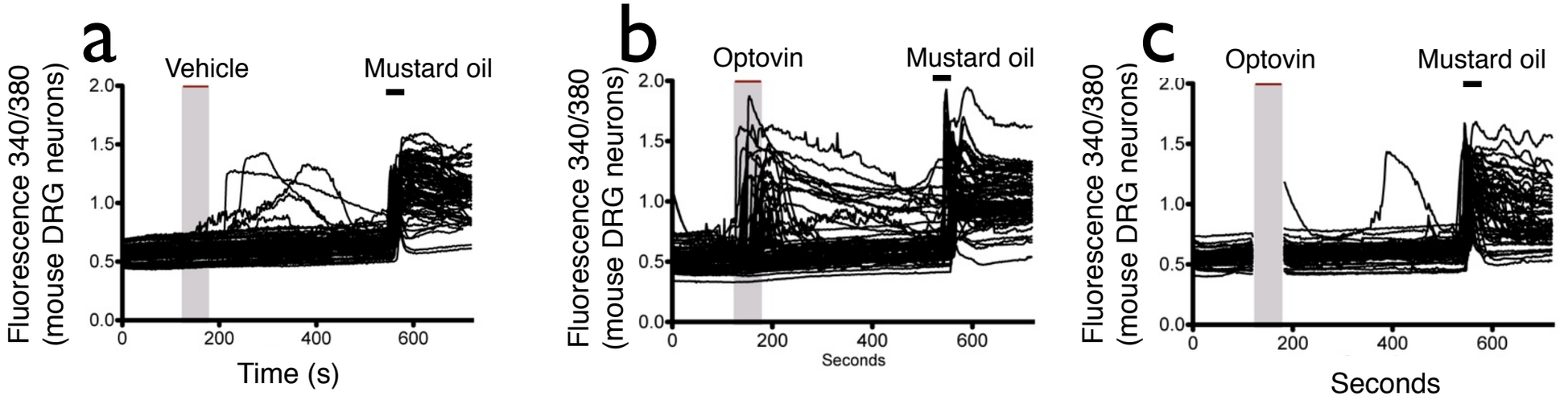
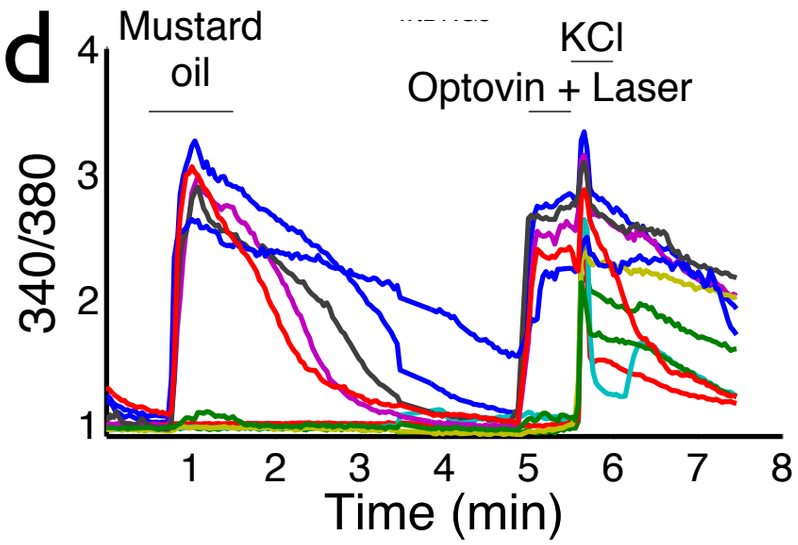


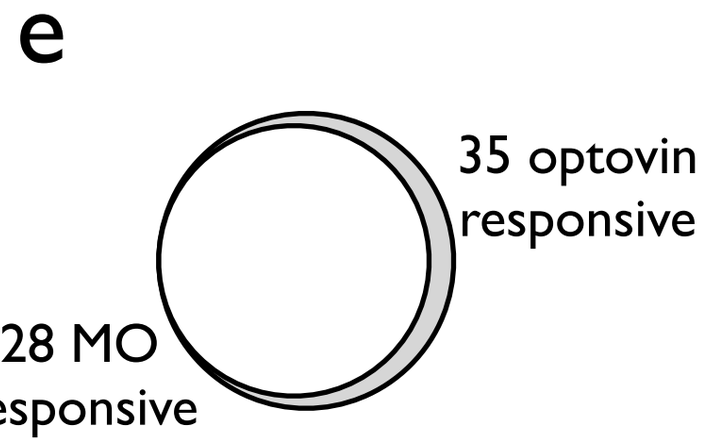
Supplementary Figure 5. Optovin-treated spinalized preparations respond to light

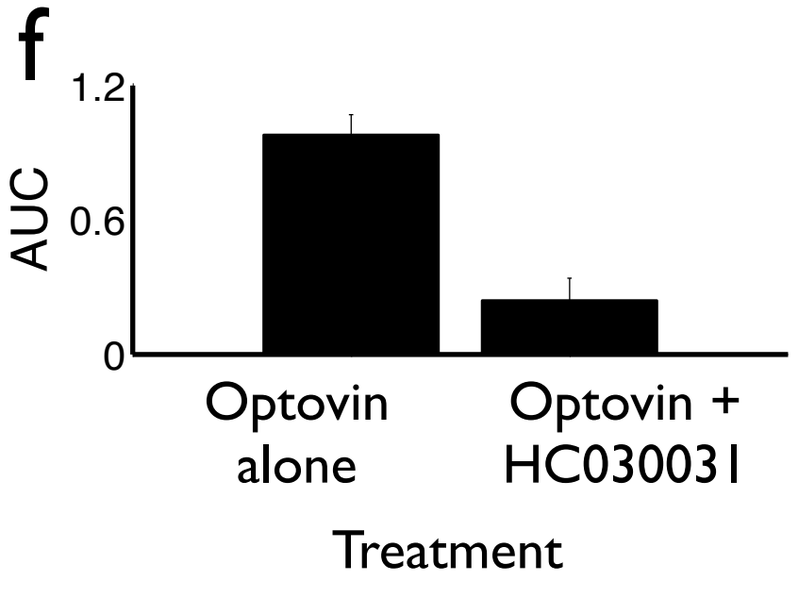


Supplementary Figure 6. **Mustard oil and optovin act on the same subset of DRG sensory neurons**

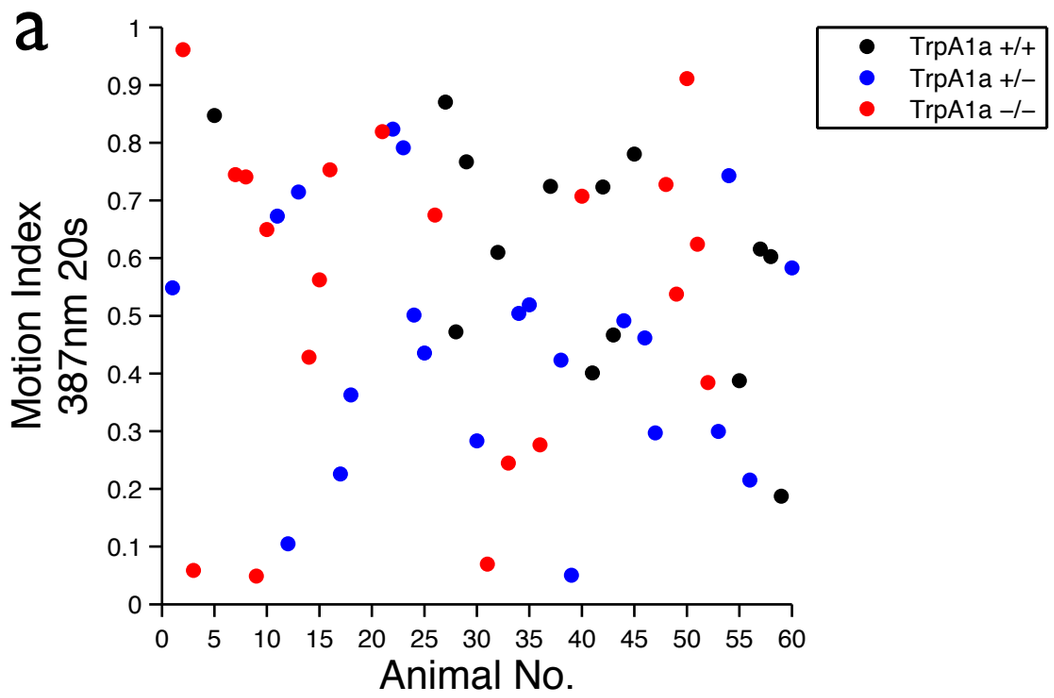


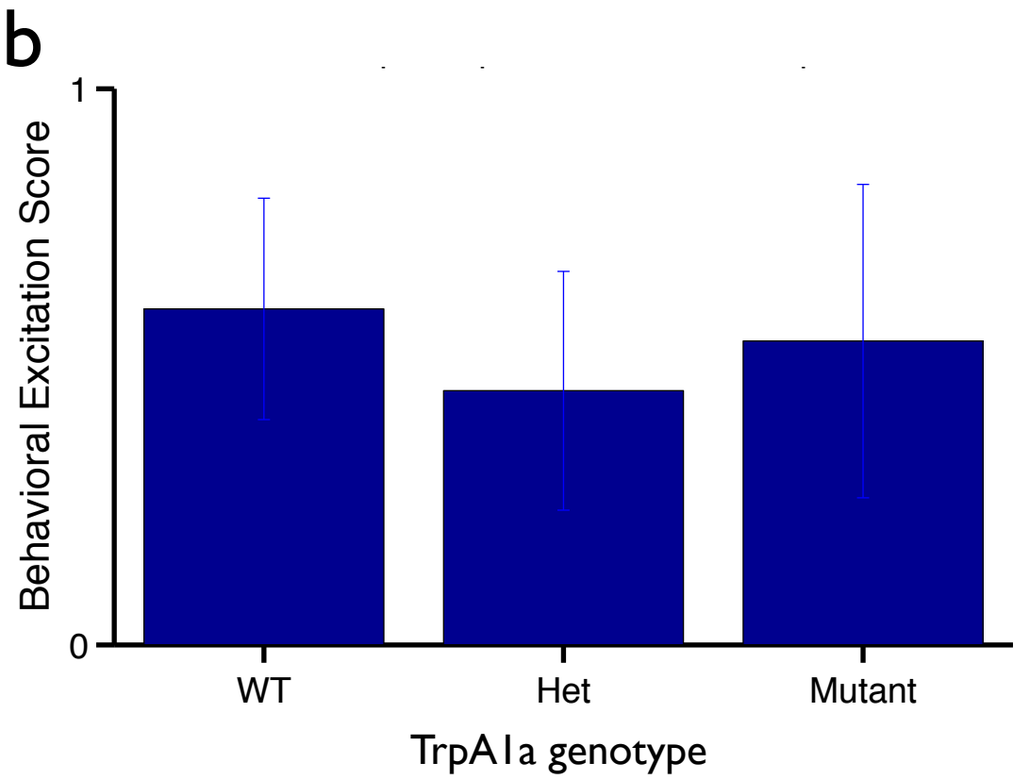


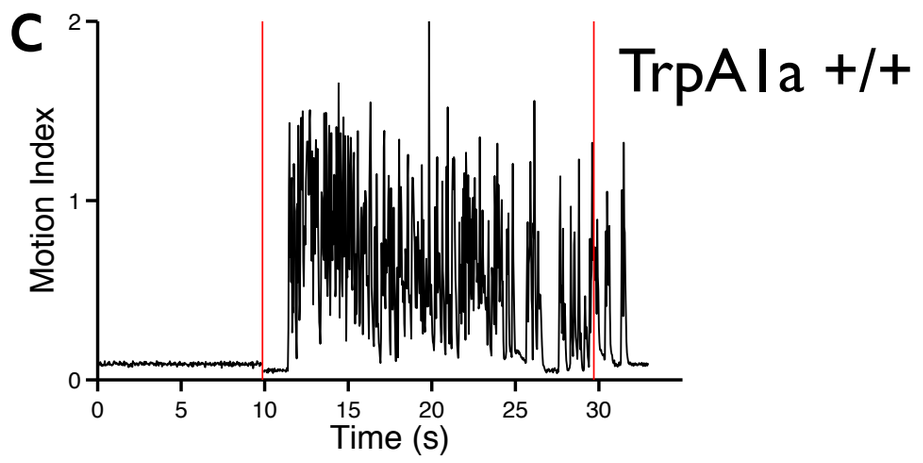


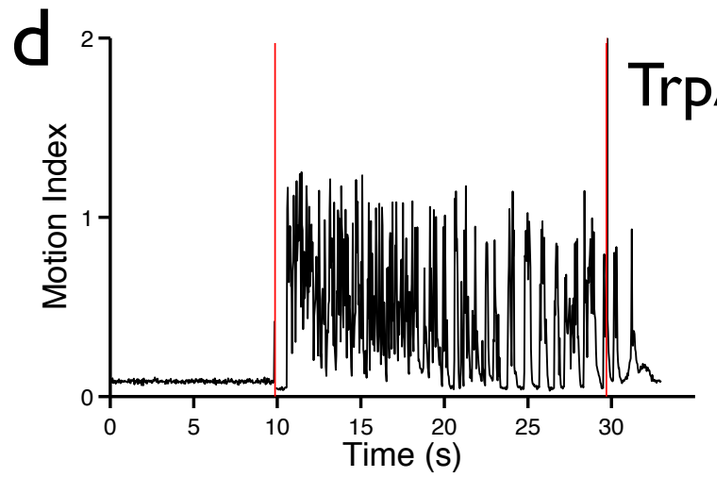


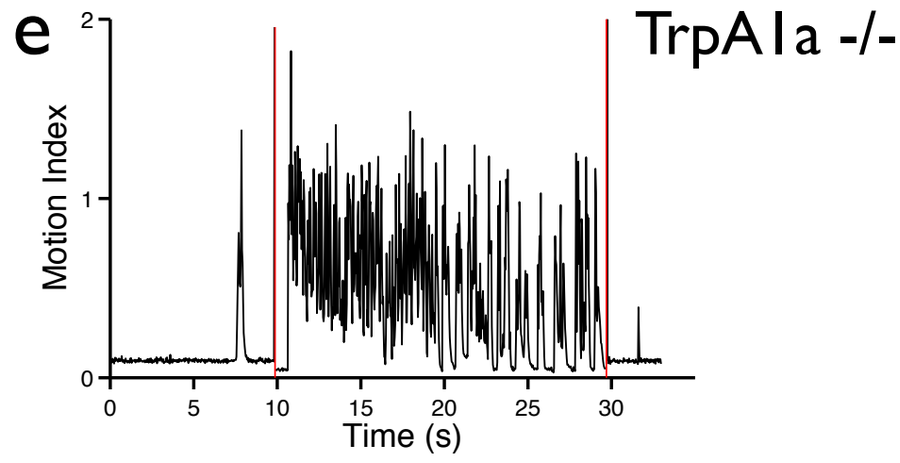
Supplementary Figure 7. **TrpA1a is not necessary for the optovin response**











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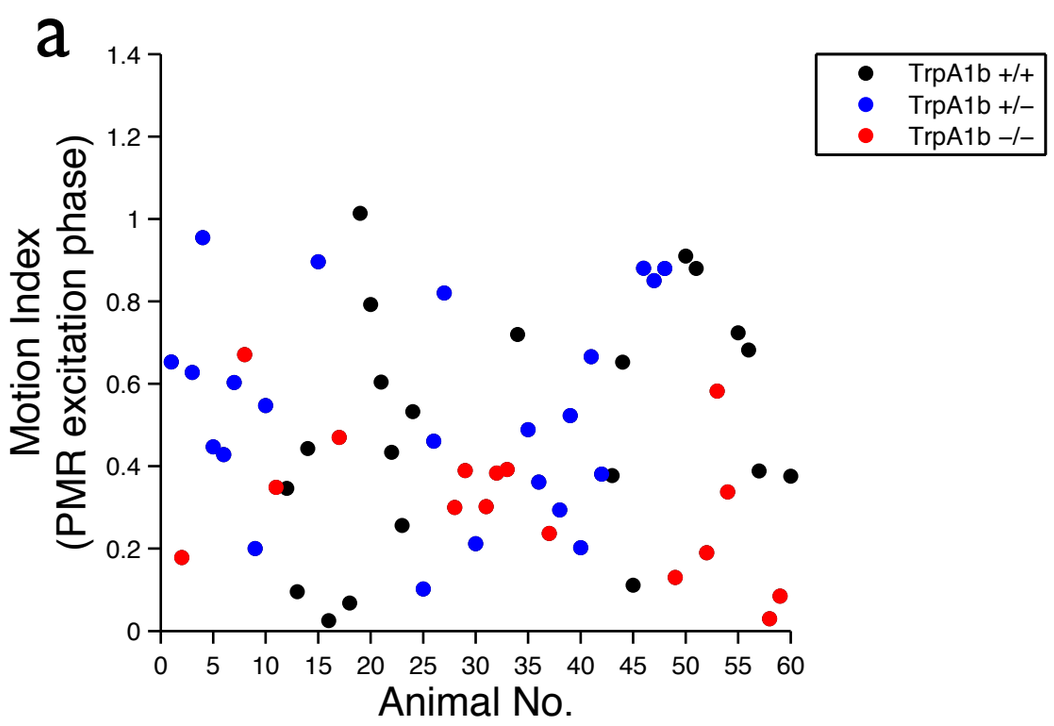
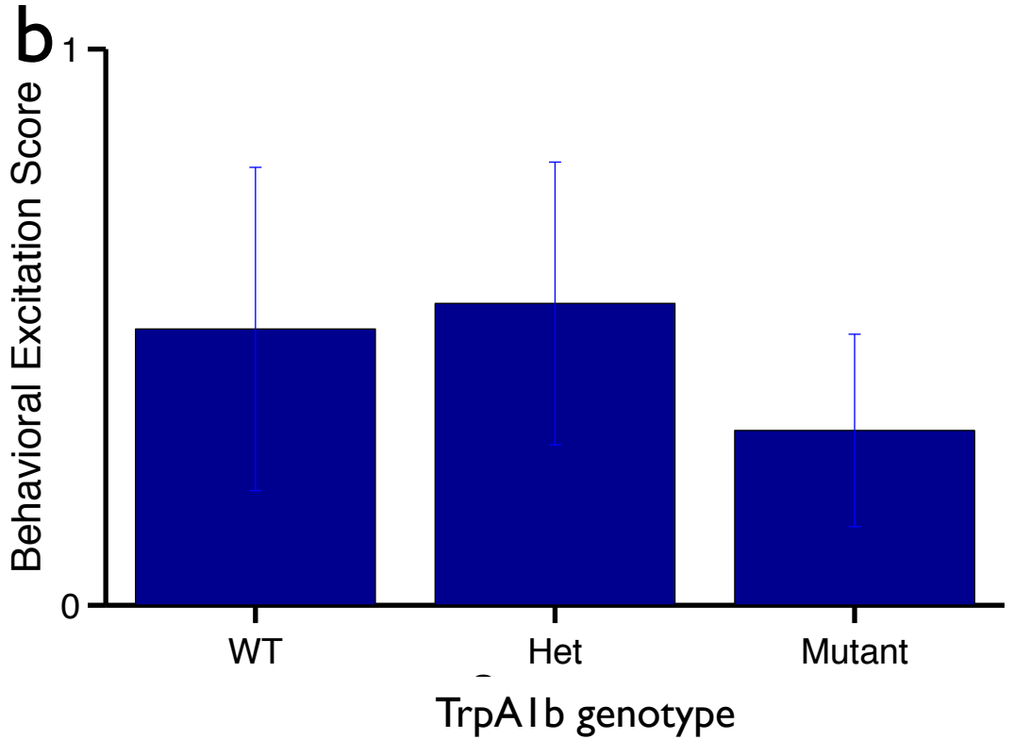
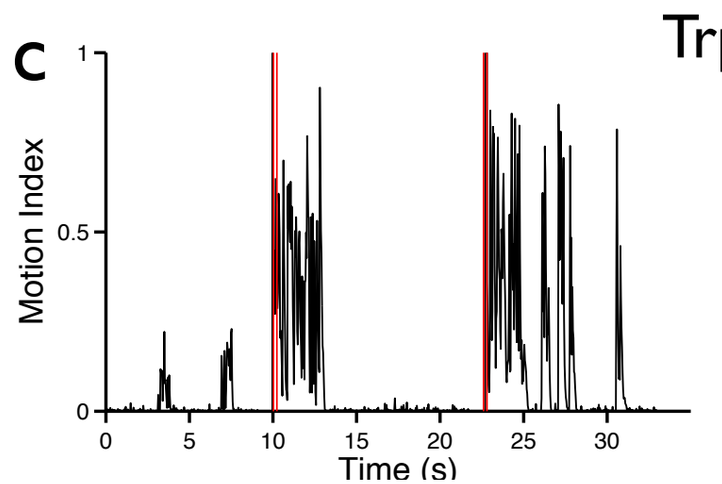
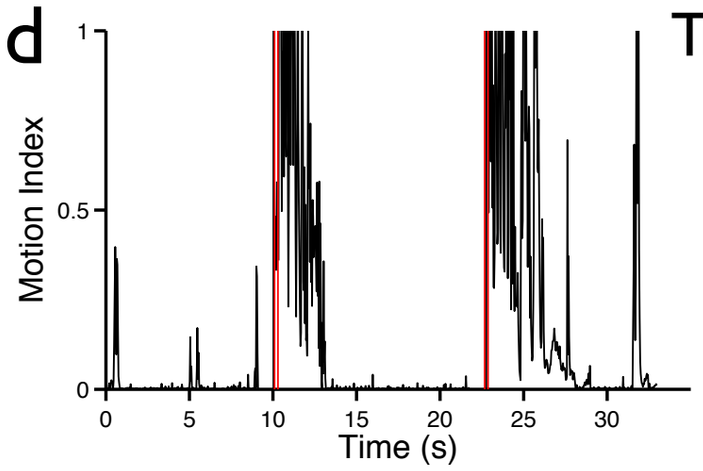
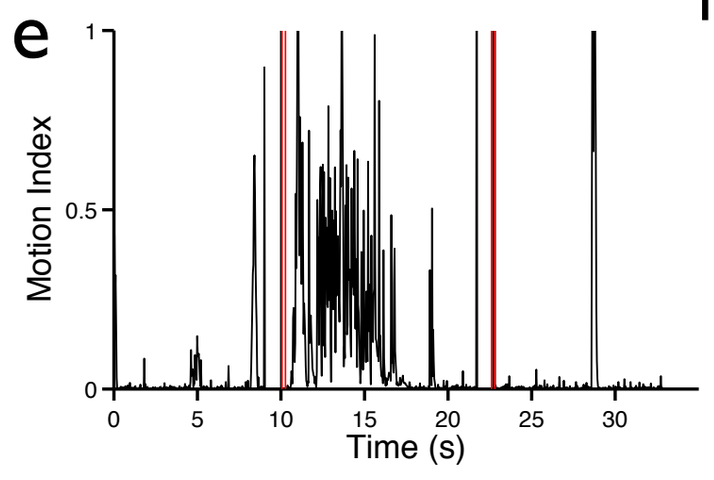


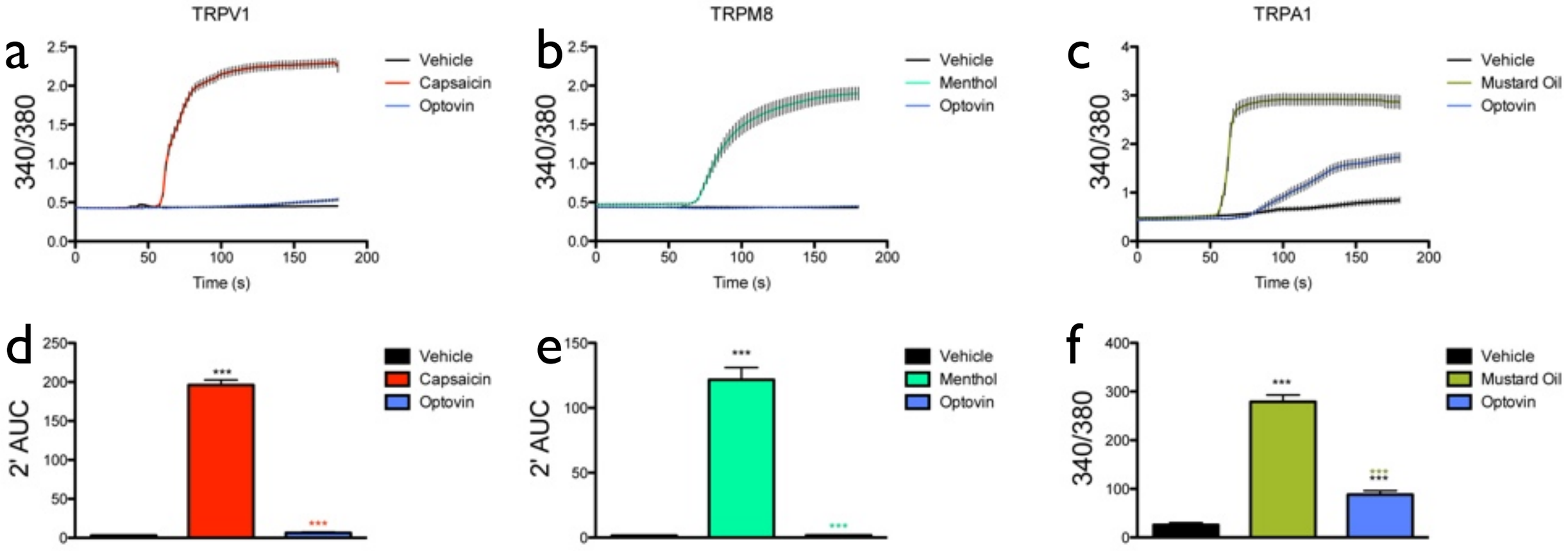








Supplementary Figure 9. **Optovin specifically activates TRPA1, but not TRPV1 or TRPM8**



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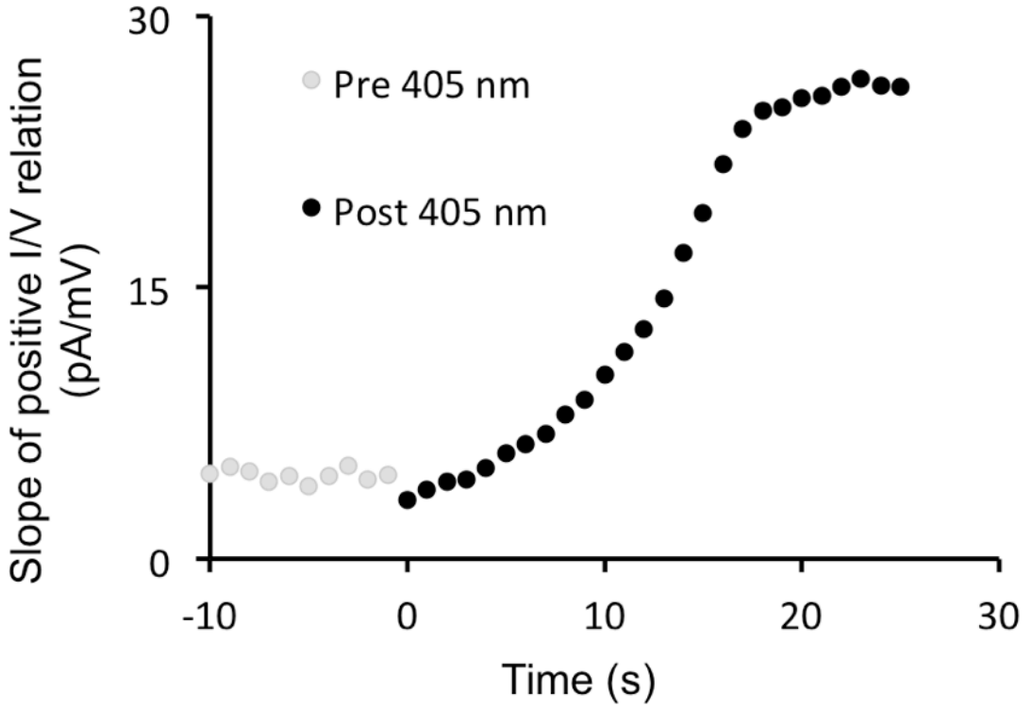
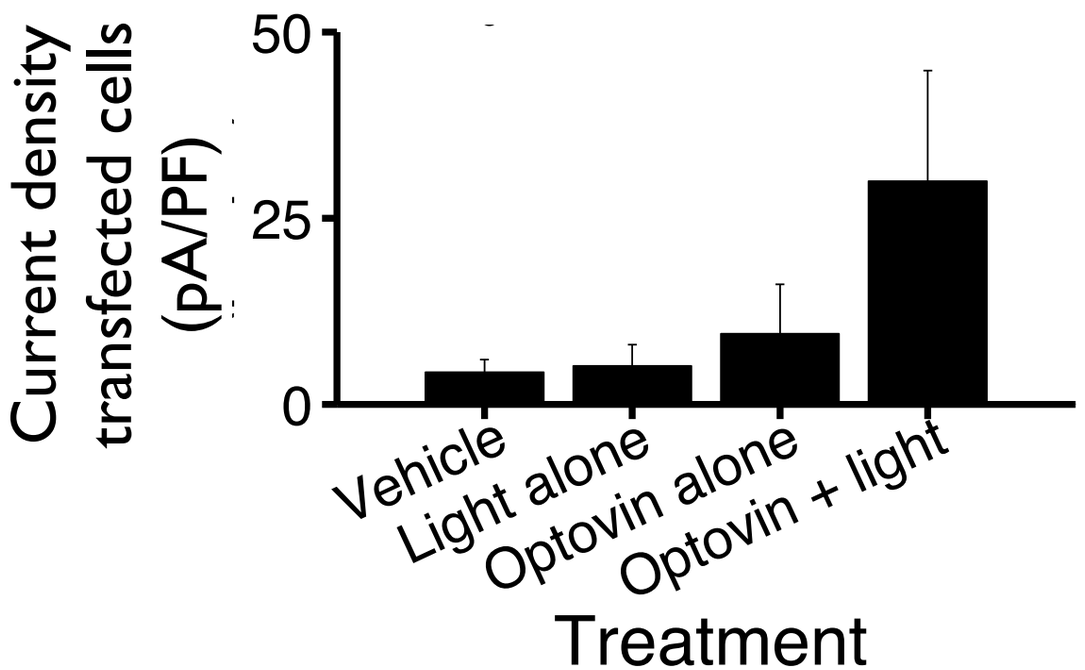
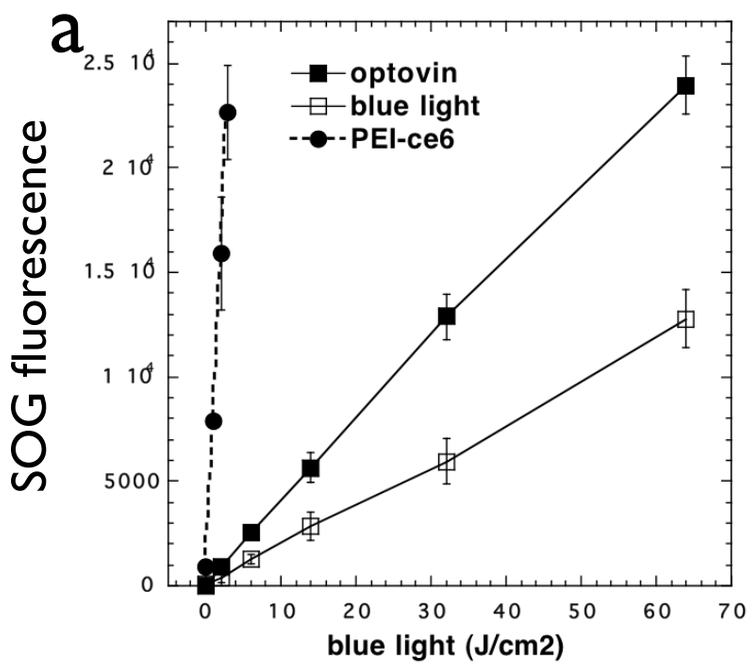
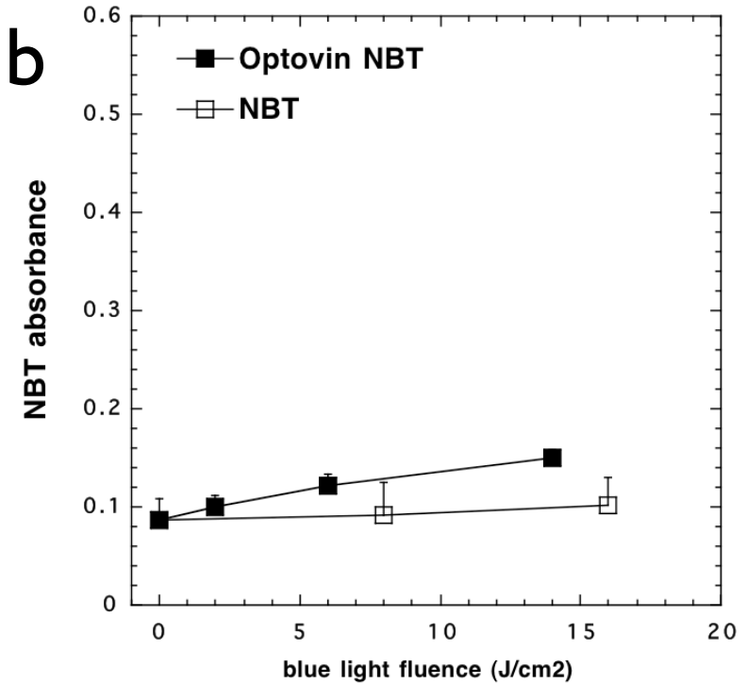
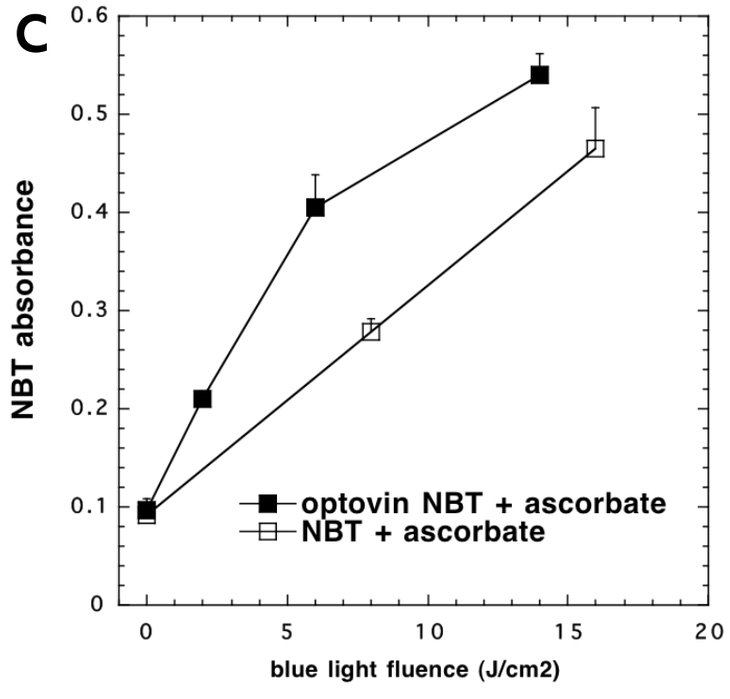


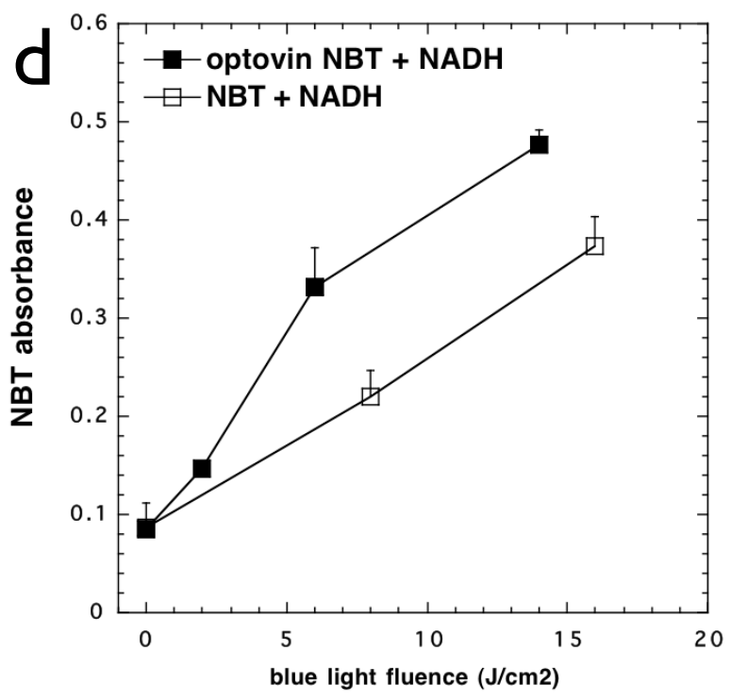


Supplementary Figure 11. **Photoactivated optovin generates negligible amounts of hydroxyl radicals and superoxide**









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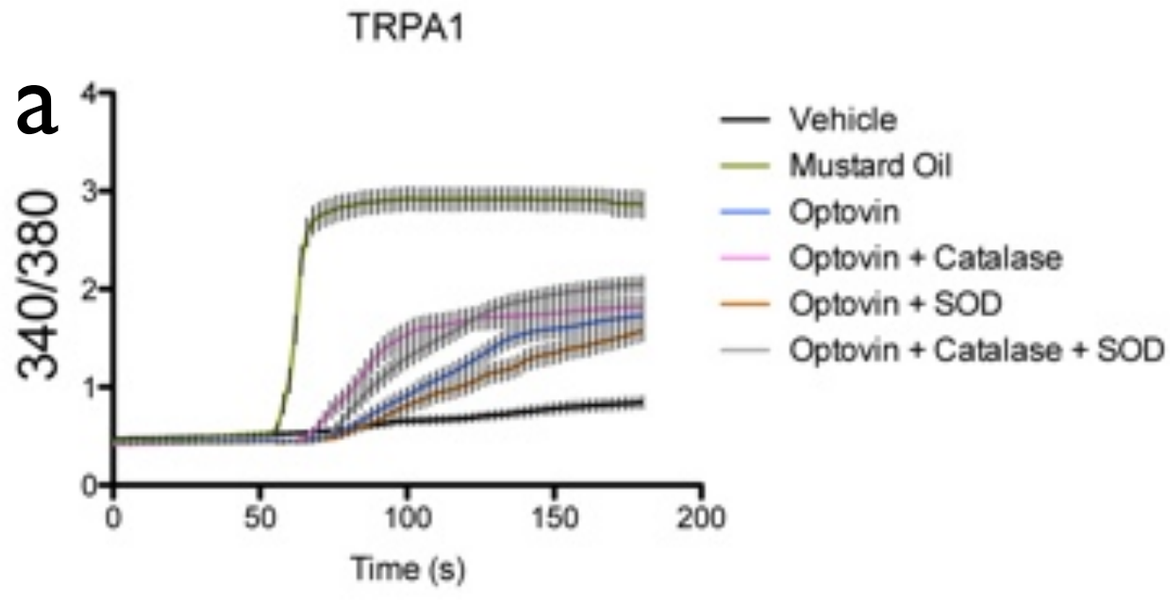

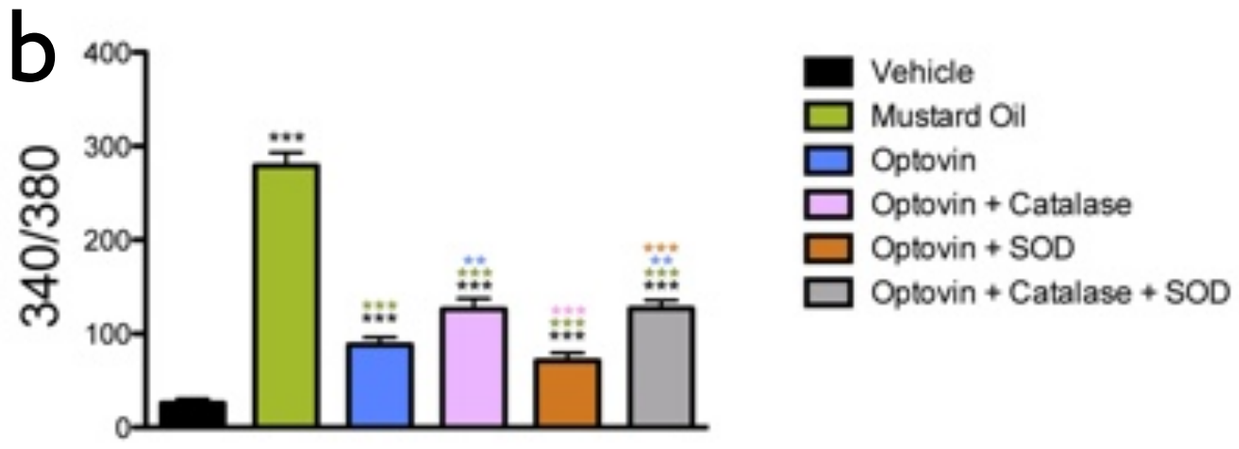

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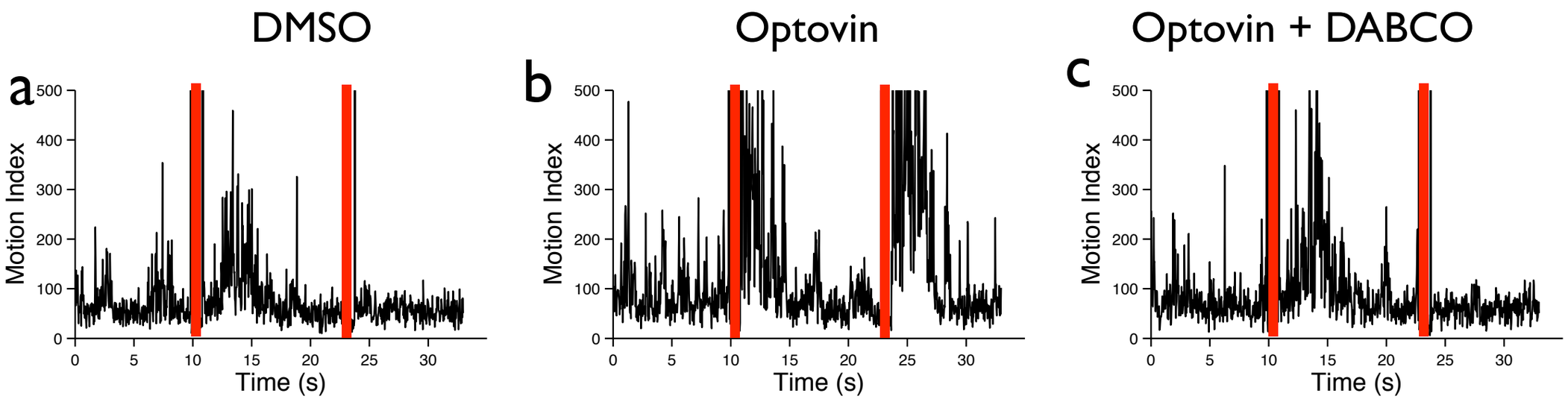

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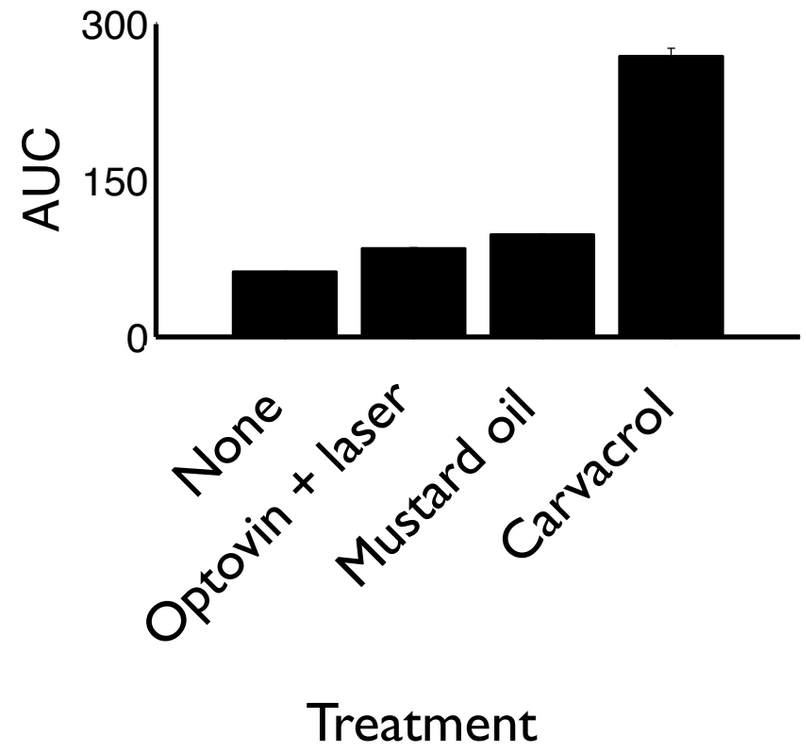

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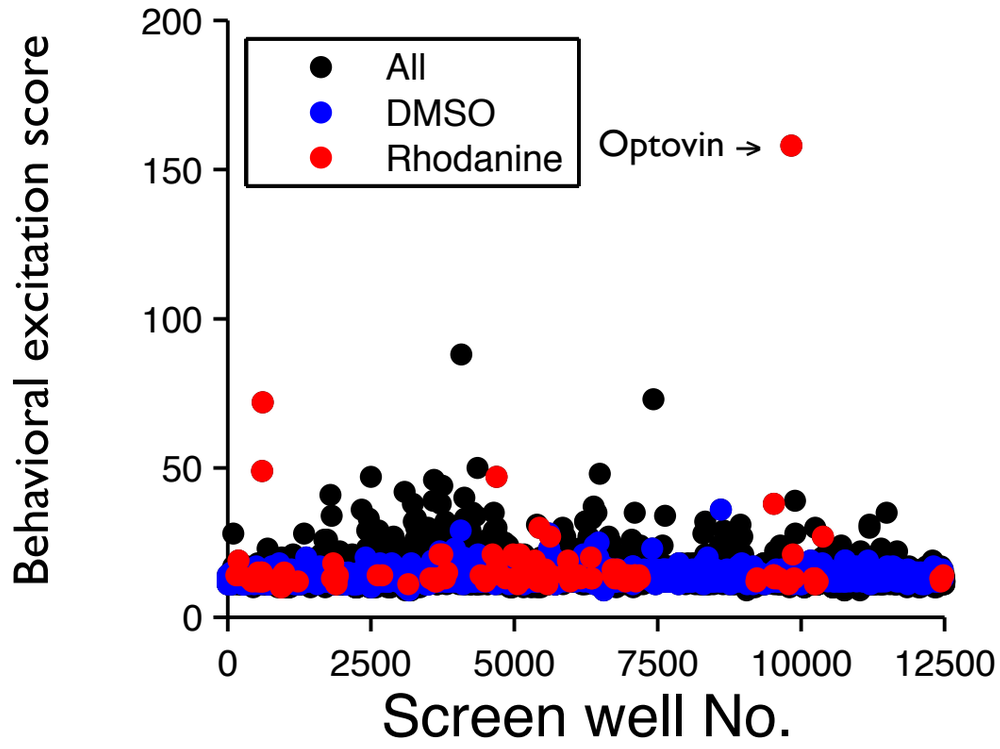

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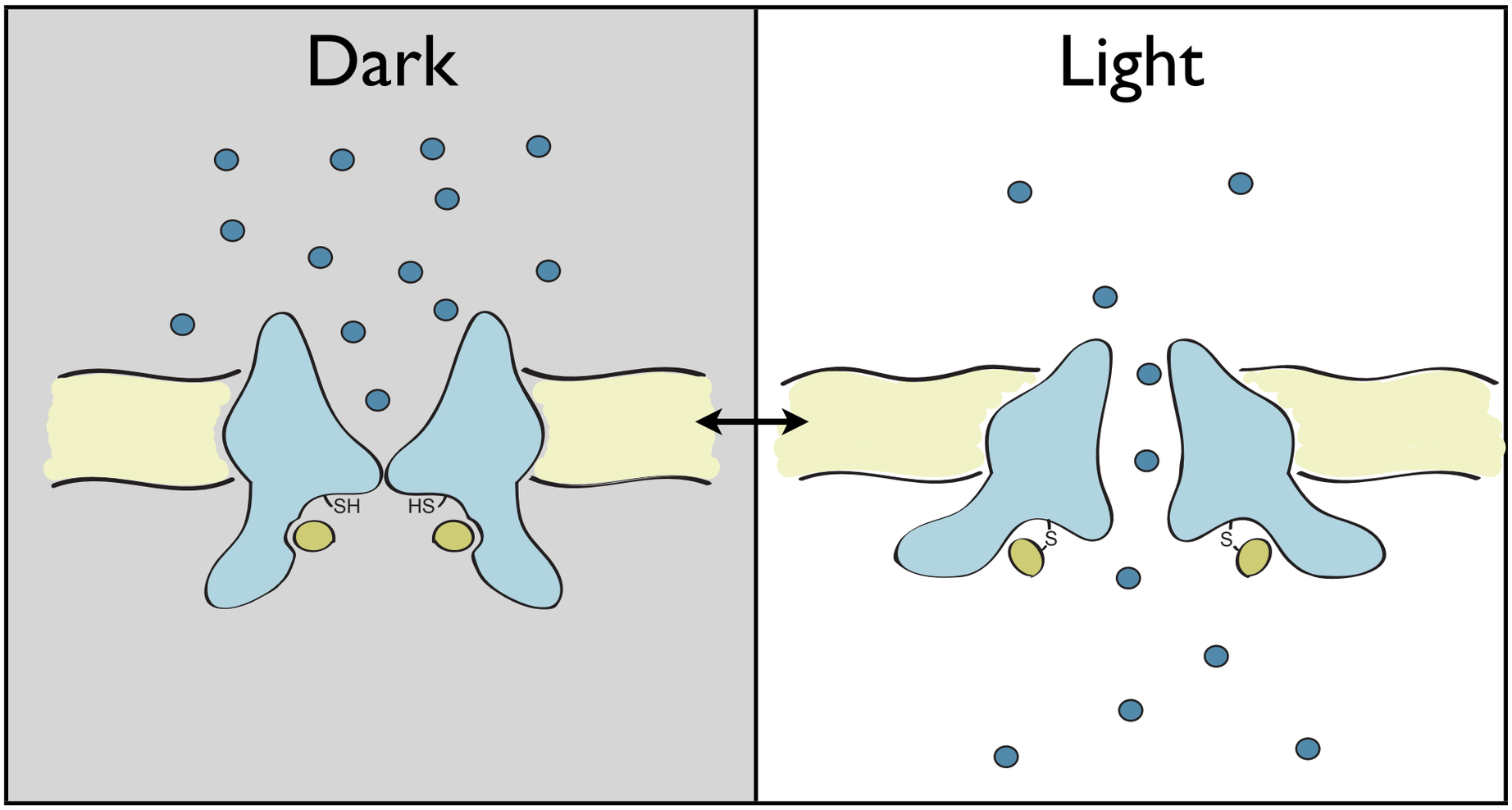

Supplementary Table 1

Receptor	Avg% inhibition	sem
DOR	-7.2	0.7
KOR	10.9	2.6
MOR	-0.7	1.4
Sigma 1	-13.7	6.8
Sigma 2	16.0	14.8
Alpha1D	30.4	4.4
5-HT2B	-2.0	3.0
5-HT2C	7.0	9.1
5-HT6	-16.5	5.3
5-HT7	-0.8	2.5
PBR	-11.3	1.2
Alpha2A	40.6	3.0
Alpha2B	8.9	10.7
Alpha1A	-5.4	2.4
Alpha1B	7.2	2.8
Alpha2C	3.3	4.5
GABAA	-2.2	6.5
A2B2	-19.2	3.6
A2B4	-7.8	6.4
A3B2	-5.6	0.7
A3B4	-12.5	3.0
A4B2	-26.8	1.6
A4B2**	-29.1	3.4
A4B4	-8.8	2.6
Beta1	19.4	5.0
Beta2	-3.8	6.0
Beta3	20.2	3.9
5-HT1D	12.9	17.5
5-HT2A	-1.1	6.3
5-ht1e	-21.5	7.0
D1	9.0	10.4
D2	-6.8	10.3
D3	34.3	11.5
D4	0.2	3.9
D5	13.6	2.7
M1	32.9	4.2
M1	16.9	7.5
M2	6.6	13.6
M3	9.1	3.5
M4	3.5	4.8
M5	3.7	5.2
NET	-1.3	7.5
5-HT1A	6.7	3.7
5-HT1B	11.8	1.2
BZP Rat Brain Site	5.3	1.3
5-HT3	-1.7	5.8

Receptor	Avg% inhibition	sem
5-ht5a	-17.7	4.8
H1	1.1	8.5
H2	41.1	14.2
DAT	1.5	8.7
SERT	75.8	4.2

Supplementary Table 2. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Whole organism, zebrafish behavior-based
	Target	Nervous system and behavior
	Primary measurement	Zebrafish motor activity following a white light stimulus
	Key reagents	None
	Assay protocol	See online methods
	Additional comments	None
Library	Library size	10,000
	Library composition	Uncharacterized, drug-like
	Source	TimTec Corporation
	Additional comments	None
Screen	Format	96-well plates
	Concentration(s) tested	10 μM
	Plate controls	DMSO, >10% control wells per plate
	Reagent/ compound dispensing system	Manual
	Detection instrument and software	Hamamatsu ORCA—ER camera mounted on a microscope (Nikon TE200) with a 1X objective and controlled by Metamorph Software (Molecular Devices)
	Assay validation/QC	Standard deviation of controls
	Correction factors	None
	Normalization	Relative to plate controls
	Additional comments	None
Post-HTS analysis	Hit criteria	Behavioral excitation score > 30 standard deviations above control mean
	Hit rate	1/10,000
	Additional assay(s)	As described in paper
	Confirmation of hit purity and structure	Compounds were repurchased (Tim Tec corporation)
	Additional comments	None