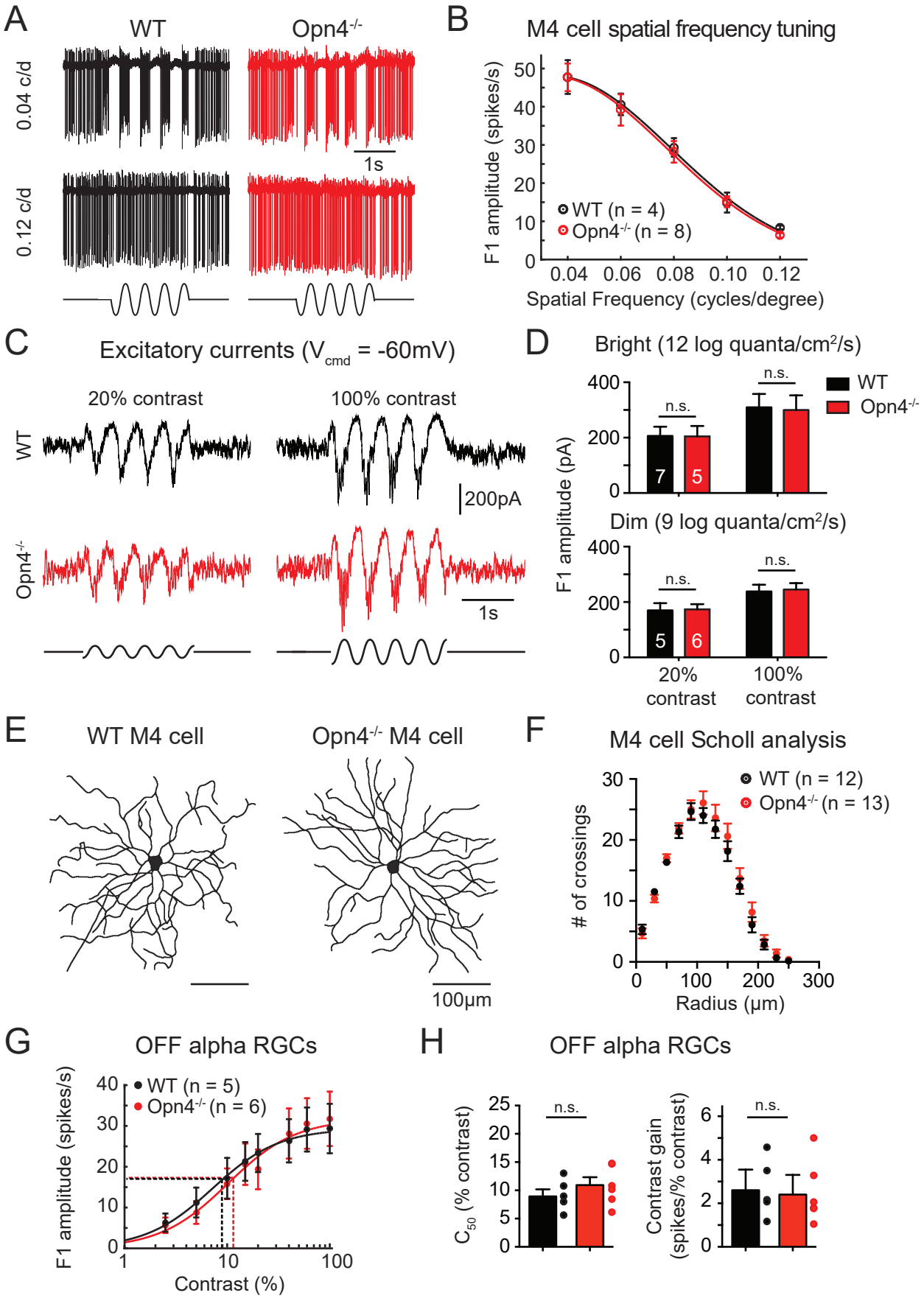

Figure S1. The spatial frequency tuning, excitatory synaptic input, and morphology of M4 cells and the contrast sensitivity of OFF alpha RGCs is normal in Opn4$^{-/-}$ retinas. Related to STAR Methods and Figures 1-2.

(A) Example loose-patch recordings of WT (black) and Opn4$^{-/-}$ (red) M4 cells to drifting sine-wave gratings of 0.04 cycles/degree (top panels) and 0.12 cycles/degree (bottom panels) at 100% contrast. Recordings were made in 10 log quanta/cm^2/s background light.

(B) Mean ± SEM F1 amplitude (spikes/s) of WT (black) and Opn4$^{-/-}$ (red) M4 cells to drifting sine-wave gratings of various spatial frequencies at 100% contrast. No differences were observed in the spatial frequency tuning of WT and Opn4$^{-/-}$ M4 cells.

(C) Representative voltage clamp traces from WT (top, black) and Opn4$^{-/-}$ (bottom, red) M4 cells in response to drifting gratings (0.04 cycles/degree) of 20% (left) and 100% (right) contrast. Cells were held at the chloride equilibrium potential, -60 mV to isolate excitatory currents. Example traces are from recordings made in bright (12 log quanta/cm^2/s) background light. Voltage clamp recordings were made using a cesium based internal solution with 2 mM QX-314.

(D) Group data of the F1 amplitude of excitatory currents measured at bright (top, 12 log quanta/cm^2/s) and dim (bottom, 9 log quanta/cm^2/s) light levels in response to drifting gratings of 20% (left) and 100% (right) contrast. There were no significant differences in excitatory currents elicited in Opn4$^{-/-}$ M4 cells by drifting gratings of either contrast at both light levels.

(E) Representative traced dendritic arbors of M4 cells in WT (left) and Opn4$^{-/-}$ (right) retinas.

(F) Sholl analysis of M4 cells in WT (black) and Opn4$^{-/-}$ (red) retinas. Concentric circles with 20 μm steps from a starting diameter of 10 μm were used. There were no significant differences in the number of crossings at any radii between WT and Opn4$^{-/-}$ M4 cells. Data are mean ± SEM.

(G) Contrast response function of OFF alpha RGCs recorded in WT (black) and Opn4$^{-/-}$ (red) retinas. Recordings were made at bright background light levels (12 log quanta/cm^2/s).

(H) Mean ± SEM of C_{50} and contrast gain of OFF alpha RGCs in WT (black) and Opn4$^{-/-}$ (red) retinas recorded at 12 log quanta/cm^2/s background light. n.s. not significant.

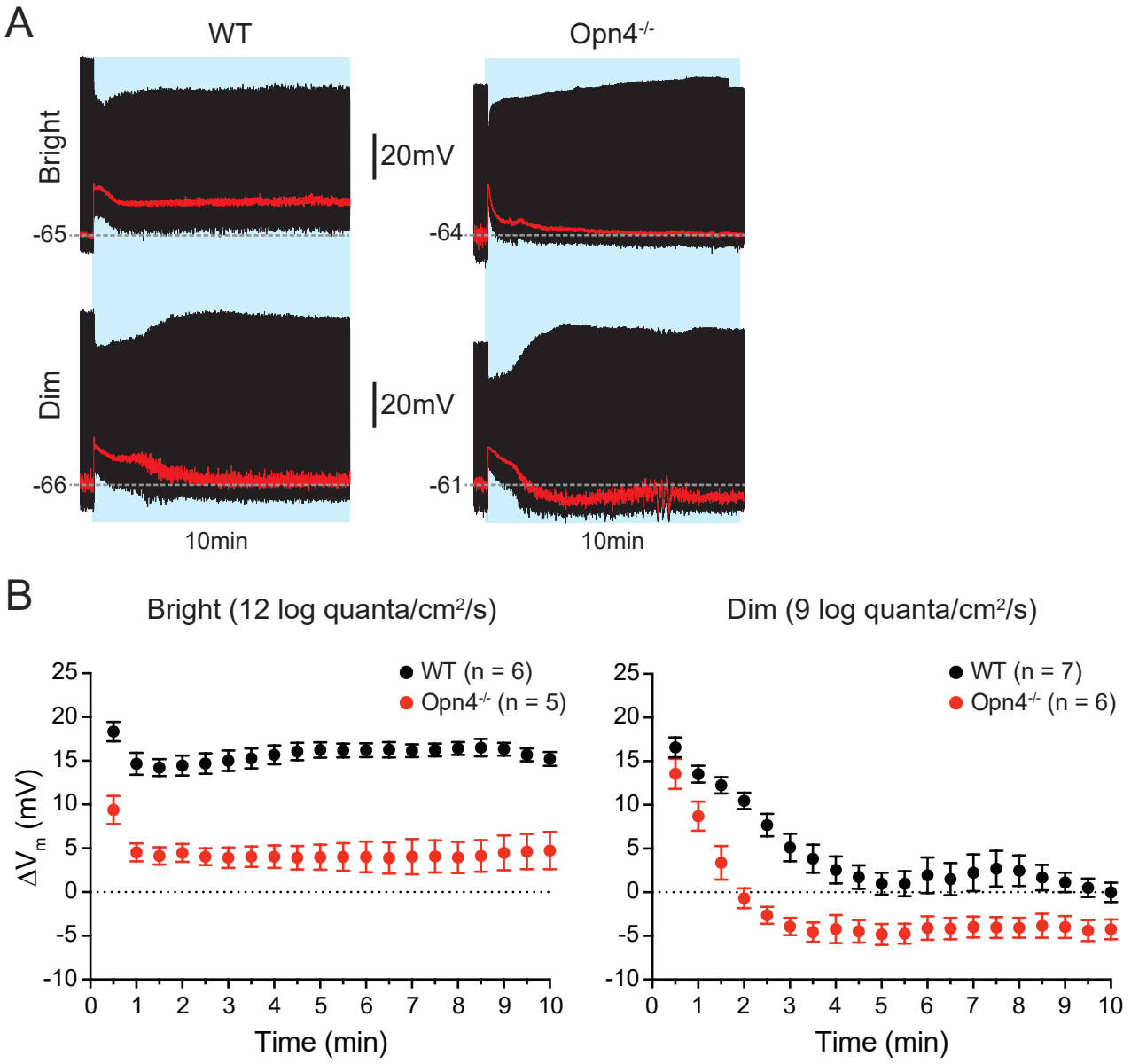

Figure S2. Time course of M4 cell responses to tonic background light exposure. Related to STAR Methods and Figures 3-4.

(A) Whole-cell current clamp recordings of WT (left panels) and Opn4$^{-/-}$ (right panels) M4 cells exposed to 10 min of bright (12 log quanta/cm^2/s, top panels) or dim (9 log quanta/cm^2/s, bottom panels) background light from darkness. Red line is the trace filtered using a 1s moving average. Gray dotted line indicates baseline membrane potential in darkness. Synaptic blockers were omitted from the extracellular solution in this set of experiments.

(B) Mean ± SEM change in membrane potential (ΔV_m) from baseline of WT (black) or Opn4$^{-/-}$ (red) M4 cells in 30s bins over 10 min of exposure to bright (12 log quanta/cm^2/s, left panel) or dim (9 log quanta/cm^2/s, right panel) background light. Dotted line indicates 0 mV change from baseline. WT M4 cells were more depolarized than Opn4$^{-/-}$ M4 cells, and reached a steady-state V_m within 5 minutes of background light exposure.

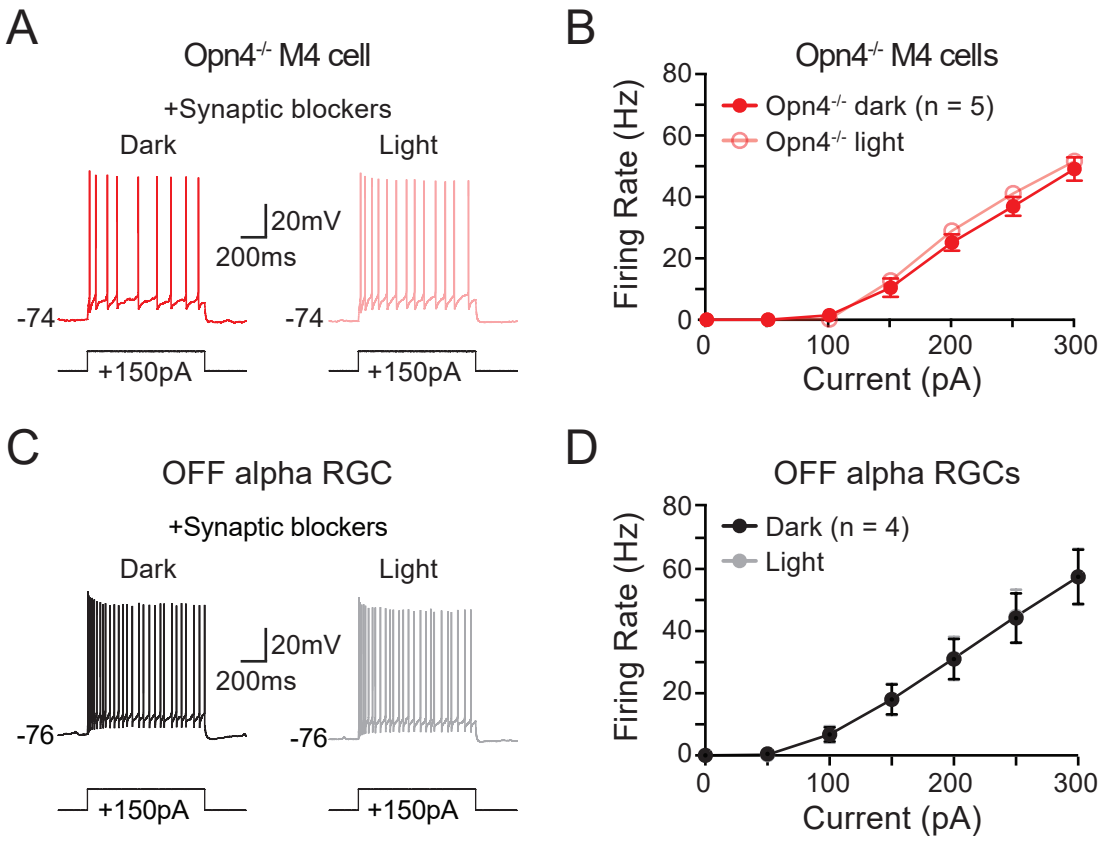

Figure S3. Melanopsin is required for light-mediated increases in excitability in M4 cells and light does not affect the excitability of OFF alpha RGCs. Related to Figure 5.

(A) Example current clamp recording of an M4 cell in response to a 150 pA current injection in Opn4$^{-/-}$ retinas in darkness (dark red) and in 12 log quanta/cm^2/s background light (light red). Recordings were made in synaptic blockers to isolate effects of melanopsin and cells were held at a subthreshold membrane potential of ~-75 mV prior to applying current steps. Synaptic blockers were included in the bath to eliminate rod/cone mediated light input.
(B) Mean ± SEM firing rate plotted as a function of current injected in Opn4$^{-/-}$ M4 cells in dark (dark red) and 12 log quanta/cm^2/s (light red) background light. The current-firing rate relationship of Opn4$^{-/-}$ M4 cells was unchanged in background light.
(C) Example current clamp recording of an OFF alpha RGC in response to a 150 pA current injection in WT retinas in darkness (black) and in 12 log quanta/cm^2/s background light (gray). Synaptic blockers were included in the bath to eliminate rod/cone mediated light input. Recordings were made in synaptic blockers and cells were held at a subthreshold membrane potential of ~-75 mV prior to applying current steps.
(D) Mean ± SEM firing rate plotted as a function of current injected in WT OFF alpha RGCs in dark (black) and 12 log quanta/cm^2/s (gray) background light. The current-firing rate relationship of OFF alpha RGCs was identical in the dark and in background light. Data points in light condition are obscured by those from the dark condition.

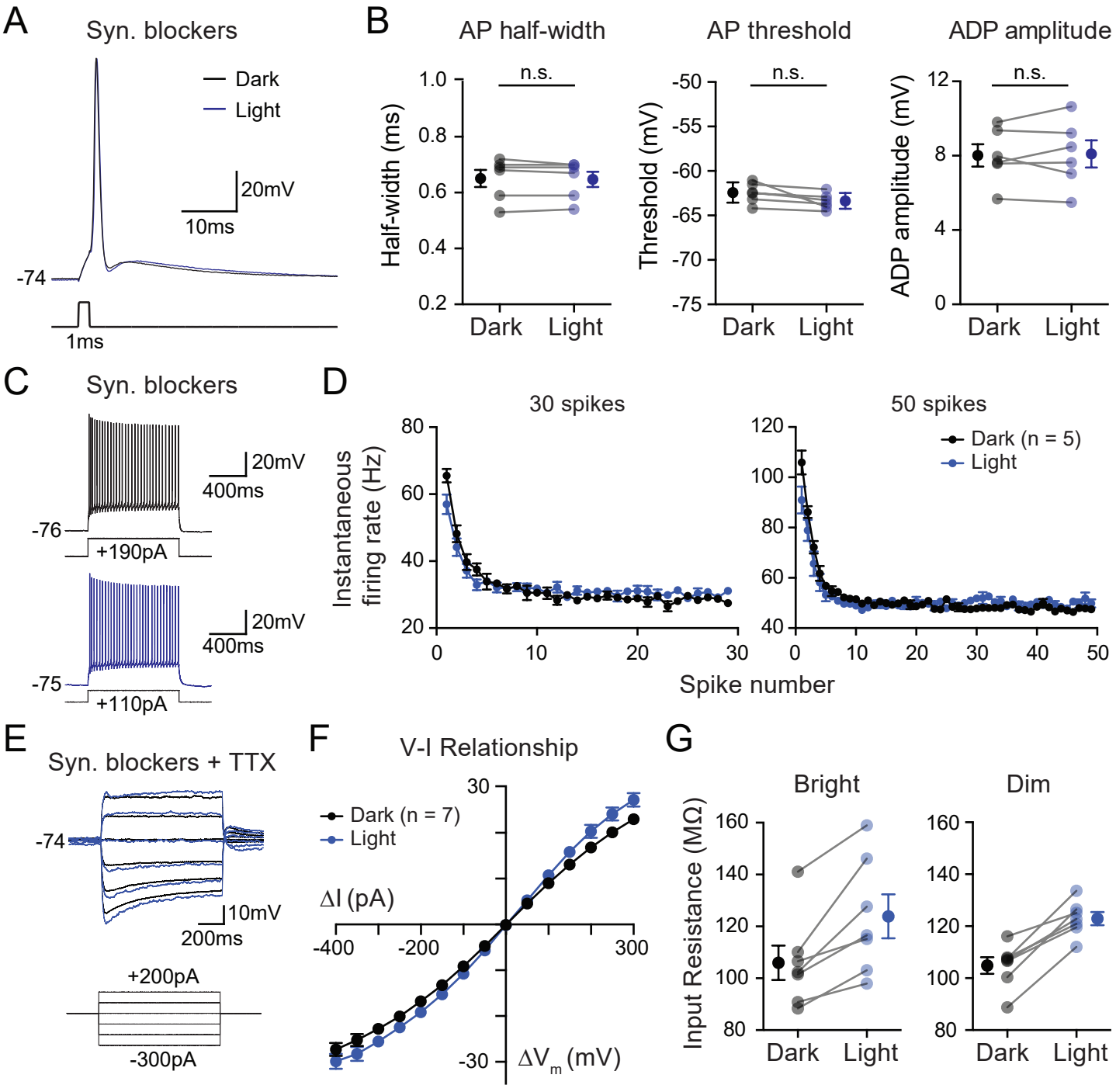

Figure S4. Background light increases M4 cell input resistance (R_{inp}) but not action potential properties or firing dynamics. Related to Figure 5.

(A) Example traces from experiments eliciting single action potentials in M4 cells using a 1ms current injection. Recordings were made in the dark (black) and in 12 log quanta/cm^2/s background light (blue). Action potential waveforms looked identical when cells were exposed to background light.

(B) Grouped data showing action potential (AP) half-width, threshold, and after-depolarization (ADP) amplitude before (black) and after (blue) exposure to 12 log quanta/cm^2/s background light. Solid black and blue points with error bars represent the mean ± SEM. No significant differences were observed in any measurements when cells were exposed to background light.

(C) Example current clamp recordings from an M4 cell in response to a 1s current injection that elicited 30 spikes. Recordings were made in the dark (black) and in bright background light (blue, 12 log quanta/cm^2/s). As expected, less current was required to elicit 30 spikes in background light, indicating light-mediated increases in intrinsic excitability.

(D) Instantaneous firing rate plotted as a function of spike number in spike trains with 30 (left) or 50 (right) spikes. The rate of decline in the instantaneous firing rate during the spike train was unchanged by background light. Solid black and blue points with error bars represent the mean ± SEM.

(E) Example voltage responses from an M4 cell to depolarizing and hyperpolarizing square current injections. Recordings were made in the dark (black) and in bright background light (blue, 12 log quanta/cm^2/s).

(F) Changes in steady-state membrane potential (ΔV_m) plotted as a function of current injected (ΔI) in the dark (black) and in bright background light (blue). Recordings were made in synaptic blockers and TTX. Identical current injections elicited larger voltage responses in background light to both hyperpolarizing and depolarizing current injections, indicating a decrease in leak conductance. Steady-state ΔV_m was calculated by averaging the voltage response during the last 200ms of the 1s current injection.

(G) Grouped data showing the R_{inp} of M4 cells in the dark (black) and after exposure to bright (12 log quanta/cm^2/s, blue, left) or dim (9 log quanta/cm^2/s, blue, right) background light. M4 cells exhibited a ~20% increase in R_{inp} at both light levels. R_{inp} was calculated from Ohm's Law ($\Delta V_m = \Delta I R_m$) using the steady-state ΔV_m in response to a 50 pA hyperpolarizing current injection. Individual cells were analyzed before (black transparent dots) and after (blue transparent dots) light exposure. Solid black and blue points with error bars represent the mean ± SEM of the group.

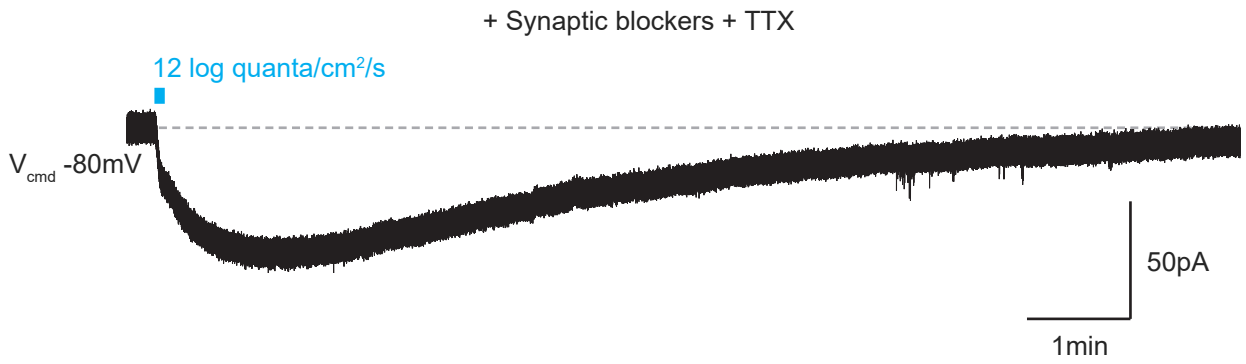

Figure S5. Deactivation kinetics of the M4 cell intrinsic light response. Related to STAR Methods and Figure 7.

Example voltage clamp recording from an intact M4 cell in response to a 5s bright light (12 log quanta/cm^2/s) stimulus. The recording was made in the presence of synaptic blockers and TTX. The gray dotted line indicates the baseline holding current in the dark prior to light stimulation.

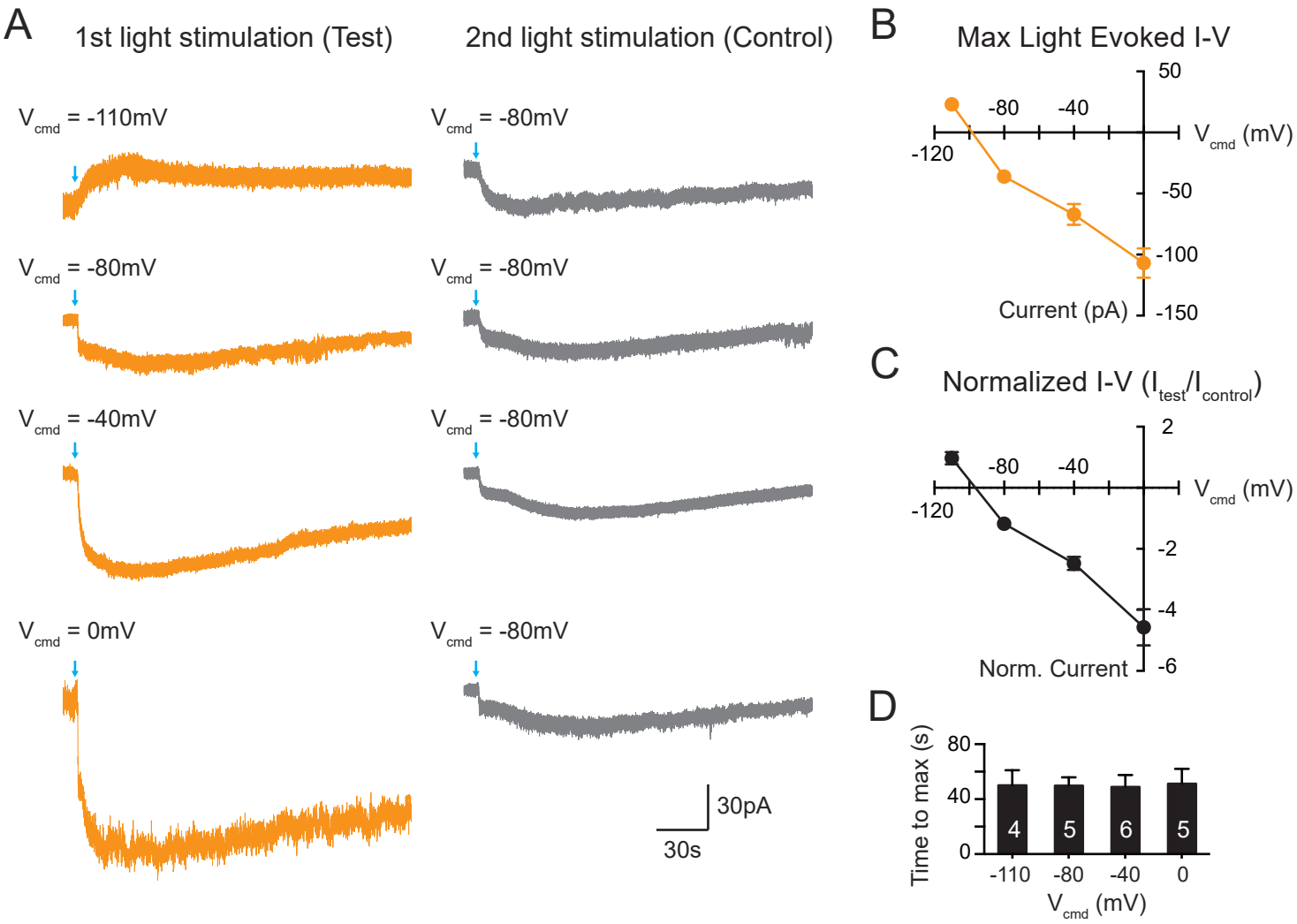

Figure S6. Normalized I-V relationship of the M4 cell photocurrent. Related to Figure 7.

(A) Example voltage clamp recordings from M4 cells in TRPC 3/6/7 KO retinas in response to 2 consecutive 100ms light pulses of 12 log quanta/cms^2/s. Cells were bathed in a cocktail of synaptic blockers and TTX to isolate the melanopsin-mediated photocurrent. M4 cells were first voltage clamped between -110 mV and 0 mV prior to the first light pulse (orange traces). A second light pulse was delivered to the same cell voltage clamped at -80 mV (gray traces) after the holding current returned to baseline (5-7 minutes after the first light pulse). Photocurrents measured after the second light pulse were used to normalize photocurrents measured after the first light pulse. The blue arrow indicates when the 100ms light stimulus was delivered.
(B) I-V relationship of the maximum light evoked current recorded from M4 cells in TRPC 3/6/7 KO retinas in response to the first light pulse. Currents were measured by subtracting the baseline holding current in darkness from the maximum light evoked current.
(C) I-V relationship of the normalized light evoked current recorded from M4 cells in TRPC 3/6/7 KO retinas. The maximum light evoked currents in panel B (I_{test}) were divided by the amplitude of the maximum light evoked current in response to the second light pulse ($I_{control}$). Note that the normalized I-V relationship is identical to the I-V relationship of the maximum light evoked current in panel B. This demonstrates that differences in current amplitudes measured at different voltages cannot be explained by differences in current density between cells.
(D) Times at which maximum light evoked currents were measured at different holding potentials (V_{cmd}). The times are measured relative to the onset of the first 100ms light pulse. Note that the average latency was similar across different holding potentials.

All data are mean ± SEM.

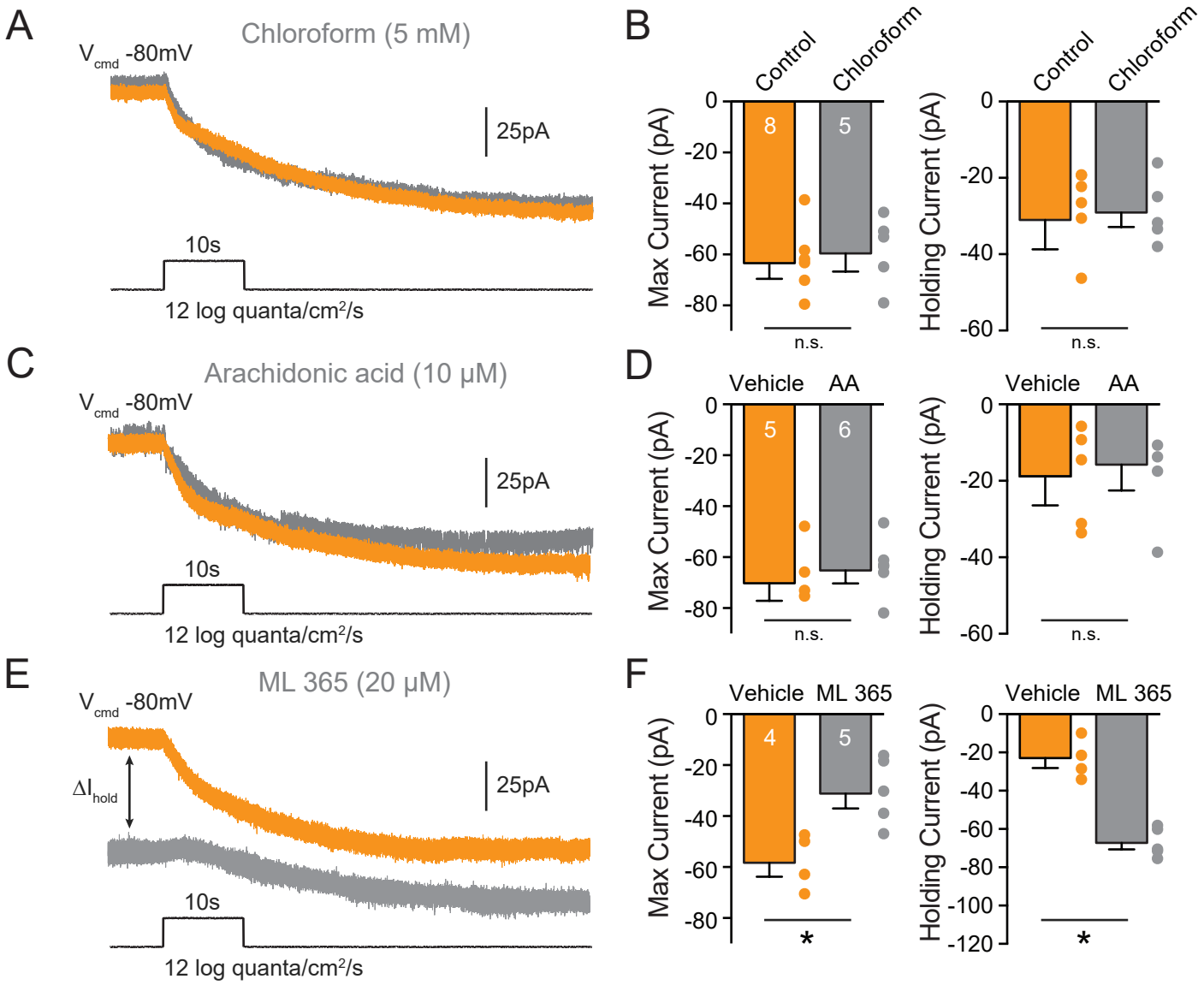

Figure S7. The melanopsin-mediated current in M4 cells is sensitive to ML 365 and insensitive to chloroform and arachidonic acid. Related to Figure 7.

(A) Example whole-cell voltage clamp recordings from M4 cells in TRPC 3/6/7 KO retinas held at -80 mV and exposed to a 10s light step of 12 log quanta/cm^2/s. Recordings were made in control solution (synaptic blockers + TTX, orange), control solution with 5 mM chloroform (gray).

(B) Mean ± SEM of the maximum light evoked current and holding current in TRPC 3/6/7 KO M4 cells bathed in control solution (synaptic blockers + TTX, orange) or control solution with 5 mM chloroform (gray). Chloroform had no effect on the light evoked and holding current.

(C) Example whole-cell voltage clamp recordings from M4 cells in TRPC 3/6/7 KO retinas held at -80 mV and exposed to a 10s light step of 12 log quanta/cm^2/s. Recordings were made in vehicle (synaptic blockers + TTX + Tocrisolve 100, orange) or in vehicle + 10 µM arachidonic acid (gray).

(D) Mean ± SEM of the maximum light evoked current and holding current in TRPC 3/6/7 KO M4 cells bathed in vehicle (synaptic blockers + TTX + Tocrisolve 100, orange) or vehicle + 10 µM arachidonic acid (AA, gray). Arachidonic acid had no effect on both the light evoked and holding current.

(E) Example whole-cell voltage clamp recordings from M4 cells in TRPC 3/6/7 KO retinas held at -80 mV and exposed to a 10s light step of 12 log quanta/cm^2/s. Recordings were made in vehicle (synaptic blockers + TTX + 0.2% DMSO, orange) or in vehicle + 20 µM ML 365 (gray).

(F) Mean ± SEM of the maximum light evoked current and holding current in TRPC 3/6/7 KO M4 cells bathed in vehicle (synaptic blockers + TTX + 0.2% DMSO, orange) or vehicle + 20 µM ML 365 (gray). ML 365 significantly reduced the light evoked current and increased the holding current.

Vehicle and drug recordings were made in separate cells to prevent decrease in responses due to run down or light adaptation. n.s. not significant, * $P < 0.05$. ** $P < 0.01$.

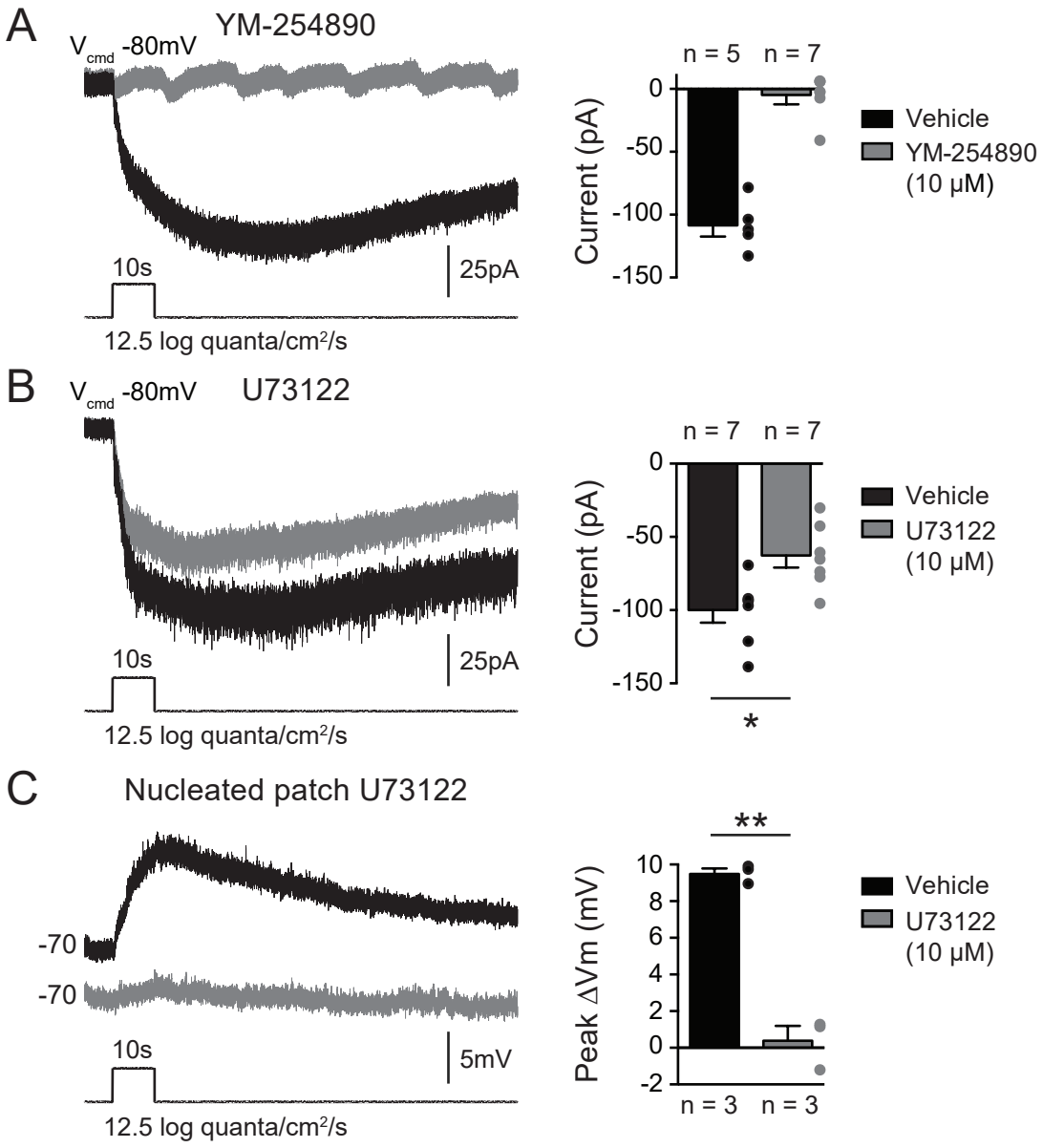

Figure S8. Melanopsin phototransduction in M4 cells acts through the Gq pathway and phospholipase C (PLC). Related to Figure 8.

(A) Left: example whole-cell voltage clamp recordings from M4 cells in WT retinas held at -80 mV and exposed to a 10s light step of 12.5 log quanta/cm^2/s. Vehicle (0.1% DMSO, black) or vehicle + 10 μM YM-254890 (gray) was included in the internal solution. Some cells exhibited background oscillations in holding current when YM-254890 was included in the internal solution.

Right: mean + SEM of the maximum current evoked by light. The Gq inhibitor YM-254890 abolished the melanopsin-mediated current in WT M4 cells.

(B) Left: example whole-cell voltage clamp recordings from M4 cells in WT retinas held at -80 mV and exposed to a 10s light step of 12.5 log quanta/cm^2/s. Vehicle (0.01% chloroform, black) or vehicle + 10 μM U73122 (gray) were added to the bath solution, which contained synaptic blockers and TTX.

Right: mean + SEM of the maximum current evoked by light. The PLC inhibitor U73122 attenuated the melanopsin-mediated current in intact M4 cells.

(C) Left: example current clamp from nucleated M4 cells in WT retinas exposed to a 10s light step of 12.5 log quanta/cm^2/s. Vehicle (0.01% chloroform, black) or vehicle + 10 μM U73122 (gray) were added to the bath solution, which contained synaptic blockers and TTX.

Right: mean + SEM of the maximum current evoked by light. The PLC inhibitor U73122 abolished the intrinsic light response in nucleated M4 cells.

* $P < 0.05$. ** $P < 0.01$. Note the higher light intensity used for these experiments, which explains the larger current amplitudes and faster kinetics in the vehicle controls. Vehicle and drug recordings were made in separate cells to prevent decrease in responses due to run down or light adaptation.

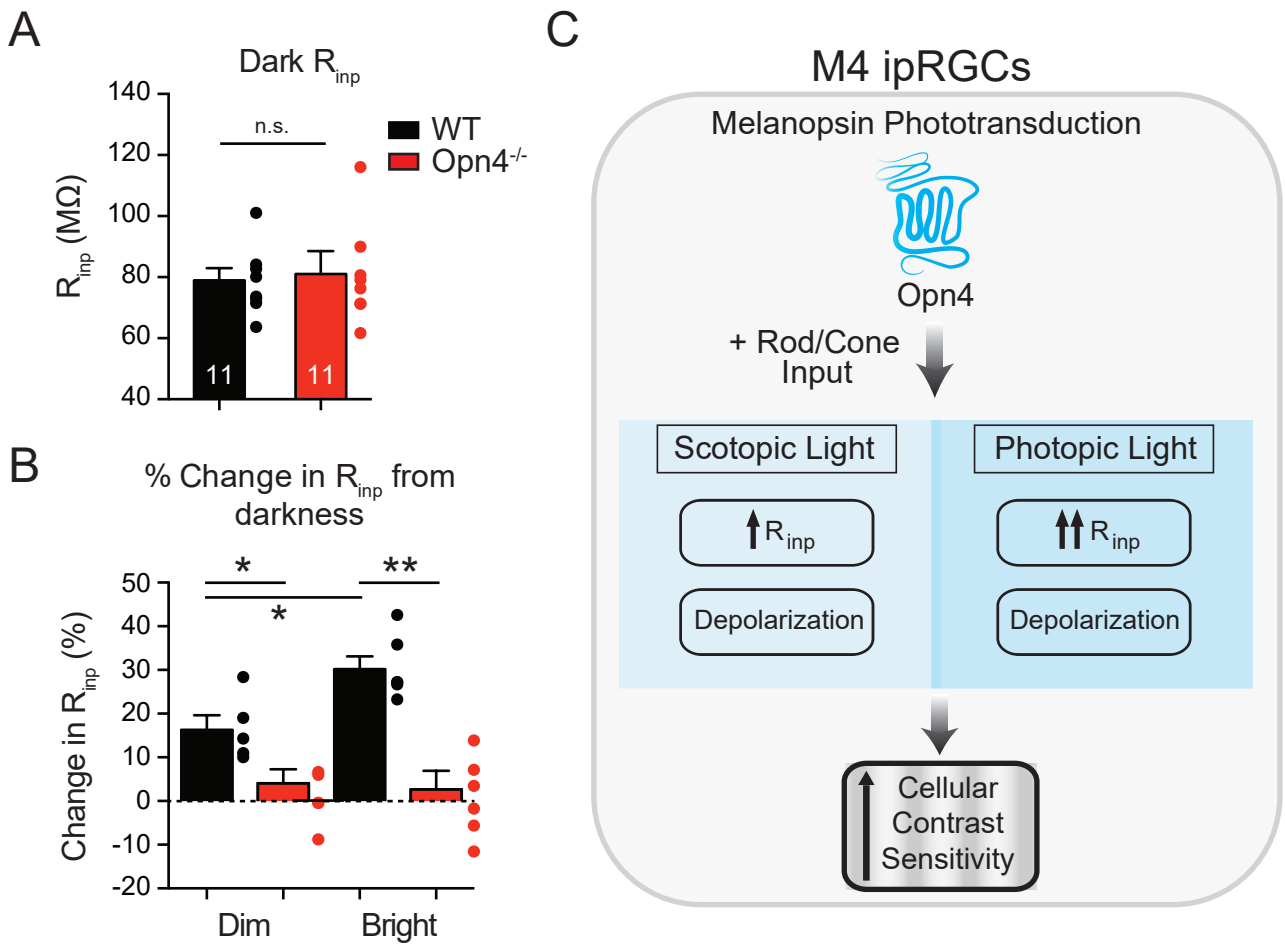

Figure S9. Melanopsin-mediated increases in R_{inp} in the presence of rod/cone input. Related to Figure 8.

(A) Input resistance (R_{inp}) of intact M4 cells in WT (black) and Opn4$^{-/-}$ (red) retinas measured in the dark without synaptic blockers. There were no significant differences in R_{inp} between WT and Opn4$^{-/-}$ M4 cells in the dark. R_{inp} was measured by holding cells at a subthreshold V_m of ~-75mV and applying 1s hyperpolarizing current injections of 50, 100, and 150 pA. The steady-state ΔV_m (measured in the last 200ms of the current injection) was used to calculate R_{inp} from Ohm's law. R_{inp} was calculated by averaging R_{inp} measured in response to the three different current injections.

(B) % increase in R_{inp} in background light from darkness in WT and Opn4$^{-/-}$ M4 cells. Changes in R_{inp} were significantly different between WT (black) and Opn4$^{-/-}$ (red) M4 cells in both dim (9 log quanta/cm^2/s) and bright (12 log quanta/cm^2/s) background light. WT M4 cells also exhibited significantly larger increases in R_{inp} in bright background light compared to dim background light. * $P < 0.05$. ** $P < 0.01$.

(C) A schematic describing melanopsin-dependent changes in V_m and R_{inp} in M4 cells at different light levels in the presence of rod and cone input. In dim light, there is a melanopsin-dependent increase in V_m and R_{inp}. In bright light, there is a melanopsin-dependent increase in V_m and a greater increase in R_{inp} than in dim background light. These effects work to enhance cellular contrast sensitivity at both light intensities.