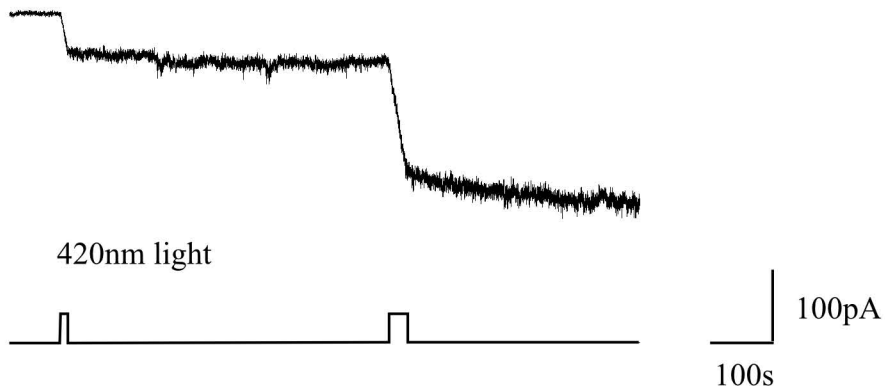
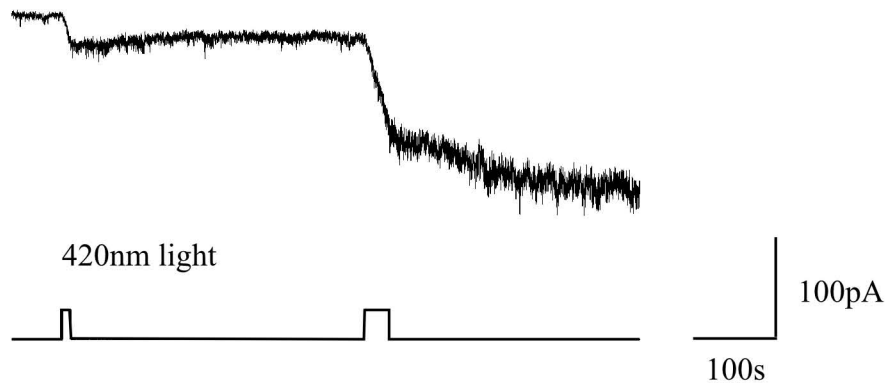


Supplementary Figure 1. Comparison of expression levels of Opn4 isoforms in the mouse retina by qPCR. Specificity and comparable amplification of Opn4L and Opn4S isoforms was confirmed by product melting and standard curve analysis (A) Differentiation plot derived from melting curves of Sybr green I fluorescence against temperature demonstrating that both Opn4S and Opn4L primers amplify a single specific product. For quantification purposes, fluorescence was measured at 82°C to prevent any potential contribution of primer-dimers to fluorescence. (B) Amplification of Opn4S (red) and Opn4L (green) dilution series from 10^5 - 10^7 copies ($R^2 = 0.999$). Both transcripts show comparable amplification kinetics. 10^7 copies of each isoform was tested with the opposite primers, producing a signal $>10^6$ times lower, confirming no crossover amplification. (C) Comparison of Opn4S and Opn4L expression levels in the retina shows significantly less Opn4L expression in comparison with Opn4S (Unpaired t-test, $P < 0.001$, $n = 5$).

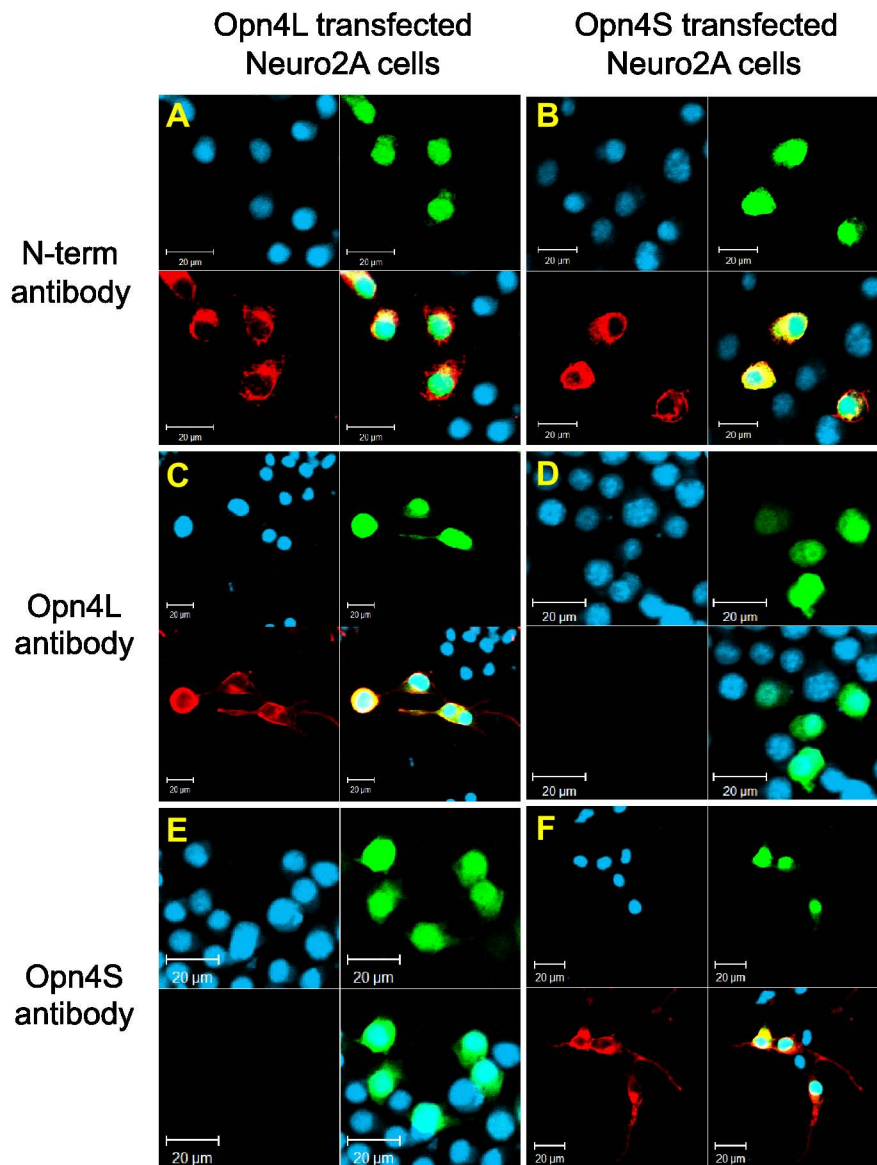
Opn4 Long



Opn4 Short



Supplementary Figure 2. Whole cell currents recorded from Opn4L (A) and Opn4S (B) expressing Neuro-2A cells in response to increasing durations of 420nm light stimuli (10s and 30s as indicated by lower panel). Increasing durations of light stimulation resulted in increasing amplitudes of inward current. Holding potential -50mV.



Supplementary Figure 3. Validation of isoform specific antibodies.

Neuro-2A cells transfected with Opn4L.IRES.GFP (left images) or Opn4S.IRES.GFP (right images) were immuno-labelled with an N-terminal melanopsin antibody (top images), Opn4L antibody (middle images) or Opn4S antibody (bottom images). For each image; top left panel = DAPI (blue), top right panel = GFP (green), bottom left panel = antibody labelling (red), bottom right = merged image. N-terminal antibody labelled both Opn4L and Opn4S transfected cells, whereas both Opn4L and Opn4S antibodies were isoform specific and only labelled Opn4L or Opn4S respectively. Similar levels of performance were observed for both Opn4L and Opn4S antibodies