Supplementary Files

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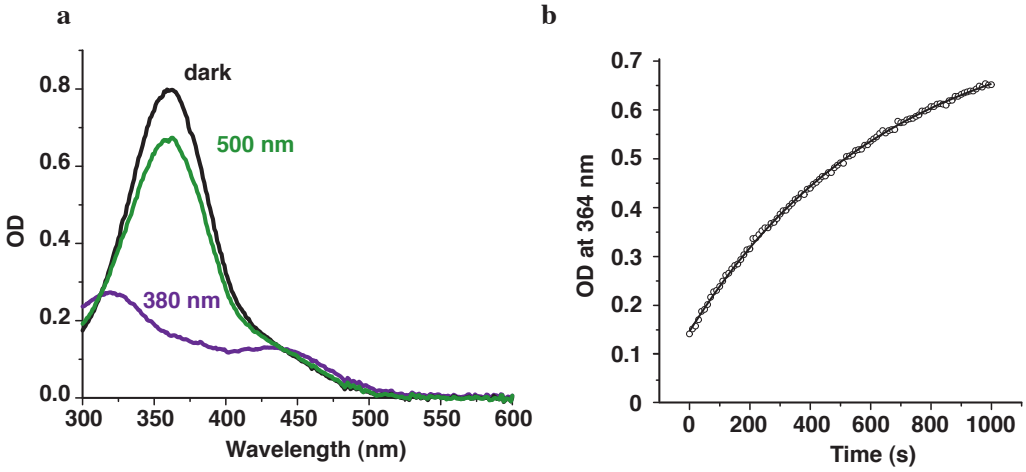
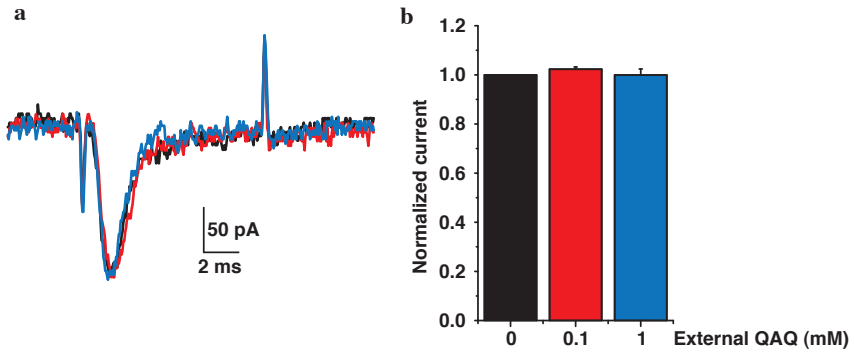
Supplementary Figure 1: Photochemical properties of QAQ.



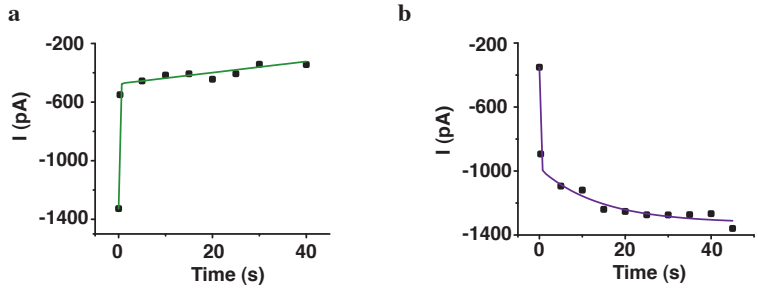
(**a**) UV-Vis spectrum of QAQ in solution (10 mM PBS, pH 7.4) either in the dark, or under illumination with 500 nm or 380 nm light. (**b**) Thermal relaxation of QAQ from *cis* to *trans* in the dark. Data was fitted using a mono-exponential decay ($t_{1/2}$ = 449 +/- 5 s).

Supplementary Figure 2: External QAQ does not block voltage-gated Na^+ channels.



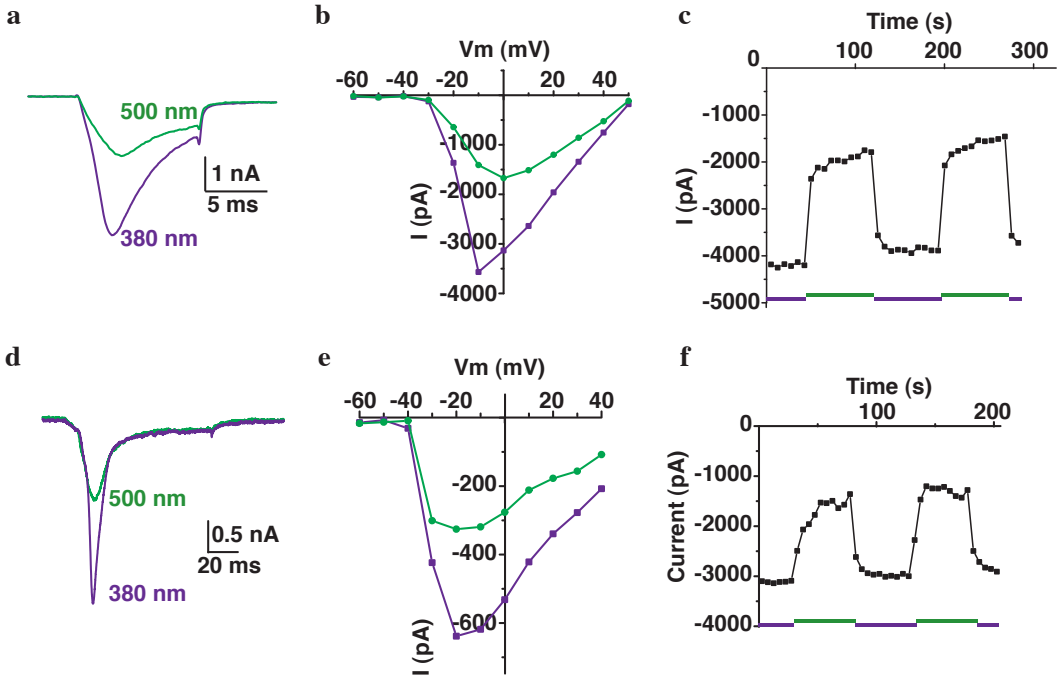
(**a**) Na^+ current recorded from an NG108-15 cell upon depolarization from -70 to -10 mV. Perfusion with QAQ 100 μM (red) or 1 mM (blue) did not alter Na^+ current (black). (**b**) Normalized current (0.1 ± 2.5 % block ± s.e.m., n = 3 cells, p = 0.97 Student t-test).

Supplementary Figure 3: Intracellular QAQ quickly blocks and unblocks Na^+ channels.



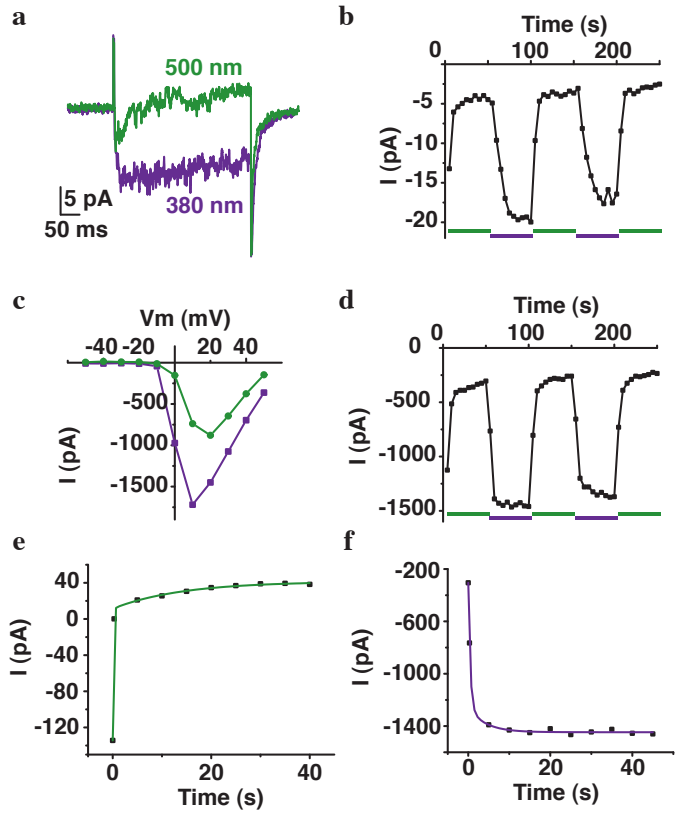
(**a**) Onset of block after switching light to 500 nm. Current was fitted with a bi-exponential decay equation. The fast process ($t_{1/2}$ = 88 ± 5 ms, n = 3 cells) reflects block of Na^+ channels, whereas the slow process may reflect slow inactivation of Na^+ channels. (**b**) Kinetics of unblock after switching light to 380 nm. Current was fitted with a bi-exponential decay equation, with $t_{1/2}$ = 58 ± 30 ms (n = 3 cells). The slow process may reflect slow recovery from inactivation of Na^+ channels.

Supplementary Figure 4: QAQ photosensitizes voltage-gated Na^+ channels from sensory TG neurons.



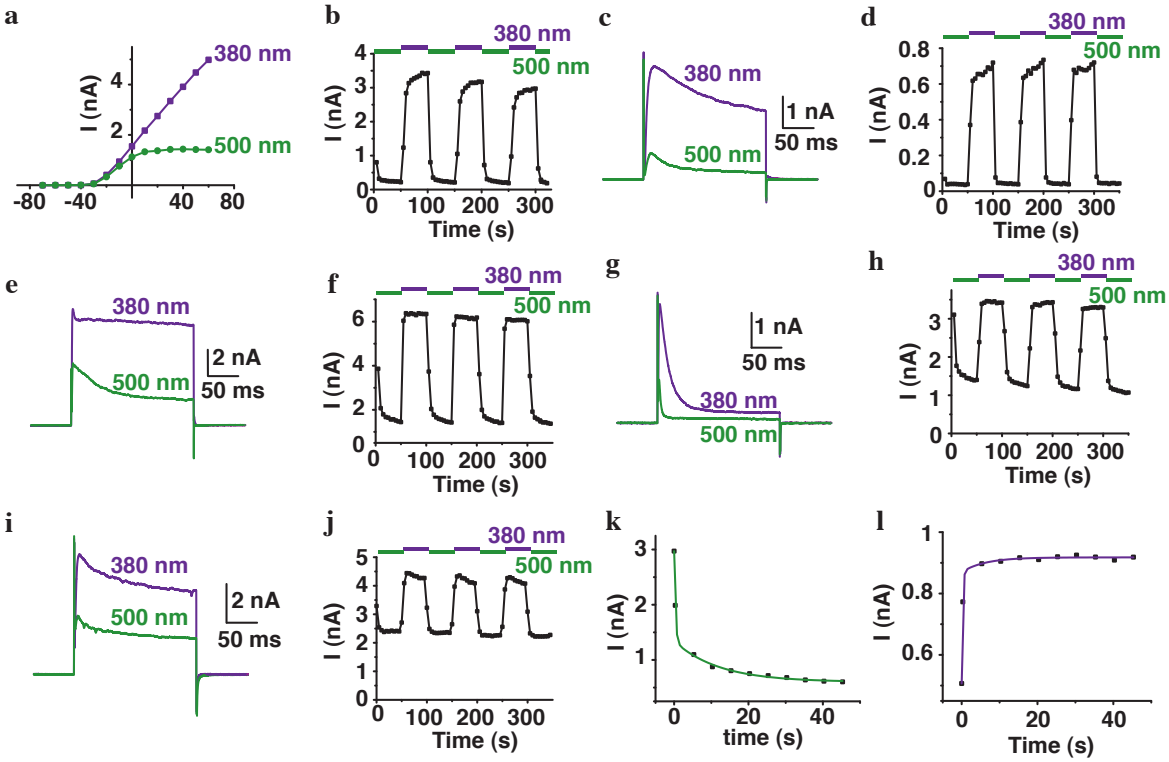
(**a**) Na^+ current recorded from a TG neuron upon depolarization from -70 to -10 mV, using 100 μM QAQ in the pipette and under both wavelengths of light (49.7 ± 5.2 % photoswitching ± s.e.m., n = 6 cells). (**b**) I/V dependency of block and unblock. (**c**) Reversibility of block and unblock. (**d**) TTX-resistant Na^+ current recorded from a TG neuron upon depolarization from -70 to -10 mV, using 100 μM QAQ in the pipette, 0.5 μM TTX in the bath, and under both wavelengths of light (46.3 ± 7.9 % photoswitching ± s.e.m., n = 4 cells). (**e**) I/V dependency of block and unblock. (**f**) Reversibility of block and unblock under both wavelength of light.

Supplementary Figure 5: QAQ photosensitizes various voltage-gated Ca^{2+} channels.



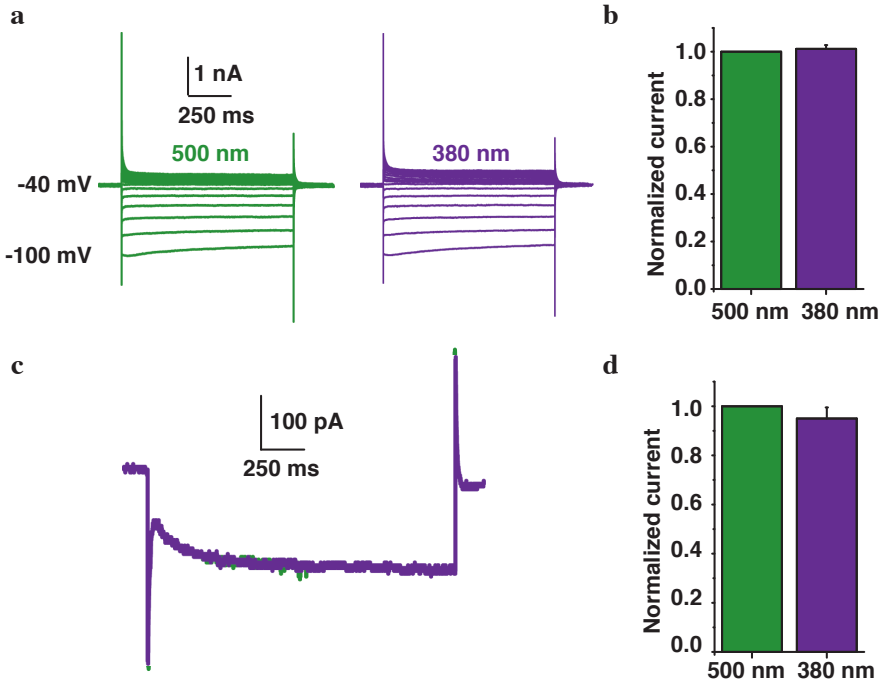
Whole-cell calcium current recording from GH3 cells, using 100 μM QAQ in the pipette, showing (**a**) raw current traces (77.9 ± 2.9 % photoswitching ± s.e.m., n = 5 cells) and (**b**) reversibility of block and unblock under both wavelength of light. Depolarization to +10 mV. Whole cell calcium current recording from HEK-293 cells stably expressing $Ca_v2.2$, using 100 μM QAQ in the pipette, showing (**c**) I/V dependency and (**d**) reversibility of block and unblock. Depolarization to +10 mV. For both voltage-gated Ca^{2+} channels currents were measured using barium instead of calcium, to limit fast inactivation of the current. (**e**) Onset of block on $Ca_v2.2$ after switching light to 500 nm. Current was fitted with a bi-exponential decay equation. For the fast process $t_{1/2}$ = 187 ± 57 ms (n = 3 cells). (**f**) Kinetics of unblock on $Ca_v2.2$ after switching light to 380 nm. Current was fitted with a bi-exponential decay equation. For the fast process $t_{1/2}$ = 270 ± 30 ms (n = 3 cells).

Supplementary Figure 6: QAQ photosensitizes various voltage-gated K^+ channels.



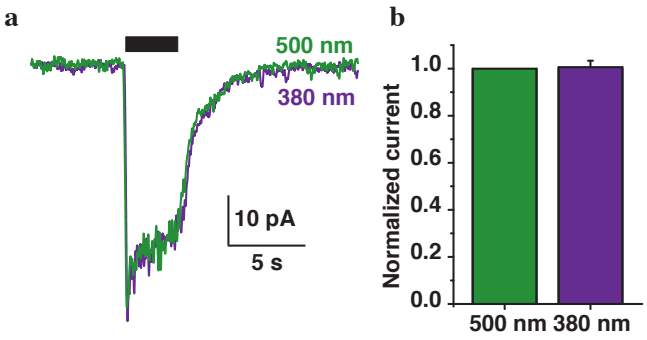
(**a**) I/V relationship for Shaker steady-state current under 380 and 500 nm light illumination, using 100 μM QAQ in the patch pipette. (**b**) Reversibility of QAQ block for Shaker current, using 100 μM QAQ in the pipette. (**c**), (**e**), (**g**) Whole cell current from HEK-293 cells transfected with $K_v2.1$ (86.0 ± 3.5 % photoswitching ± s.e.m., n = 4 cells), $K_v3.1$ (46.6 ± 13.4 % photoswitching s.e.m., n = 4 cells) and $K_v4.2$ (47.1 ± 9.4 % photoswitching s.e.m., n = 4 cells), respectively. (**d**), (**f**), (**h**) Reversibility of QAQ block on $K_v2.1$, $K_v3.1$ and $K_v4.2$, respectively. (**i**) Whole cell potassium current from a hippocampal neuron using 100 μM QAQ in the patch pipette (36.0 ± 5.3 % photoswitching ± s.e.m., n = 13 cells). (**j**) Reversibility of QAQ block in a hippocampal neuron. For all recordings: depolarization from -70 to +40 mV. (**k**) Onset of block on Shaker after switching light to 500 nm. Current was fitted with a bi-exponential decay equation. For the fast process $t_{1/2}$ = 201 ± 20 ms (n = 3 cells). (**l**) Kinetics of unblock on Shaker-IR after switching light to 380 nm. Current was fitted with a bi-exponential decay equation. For the fast process $t_{1/2}$ = 164 ± 15 ms (n = 3 cells).

Supplementary Figure 7: QAQ does not photosensitize K_{ir} or HCN channels.



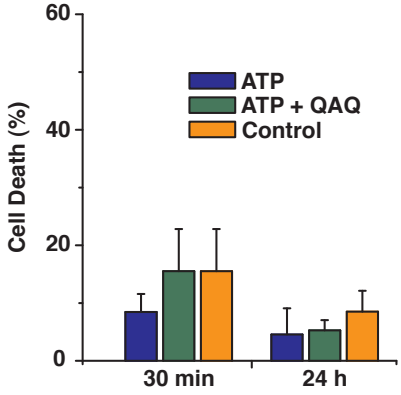
(**a**) K_{ir} currents were measured from HEK-293 cells transfected with K_{ir} 2.1. Currents were elicited by voltage steps from -40 to -100 mV, in 10 mV increments, under illumination with 380 or 500 nm light. (**b**) Normalized current measured at -100 mV under both wavelengths of light (1.2 ± 1.5 % photoswitching, n = 5 cells, p > 0.05). (**c**) HCN currents were measured from acutely dissociated TG neurons upon hyperpolarization to -100 mV under 380 and 500 nm light. (**d**) Normalized current under both wavelengths of light (-5.2 ± 4.7 % photoswitching, n = 3 cells, p > 0.05). Error bars ± s.e.m.; Student t-test.

Supplementary Figure 8: QAQ does not photosensitize glutamate receptors.



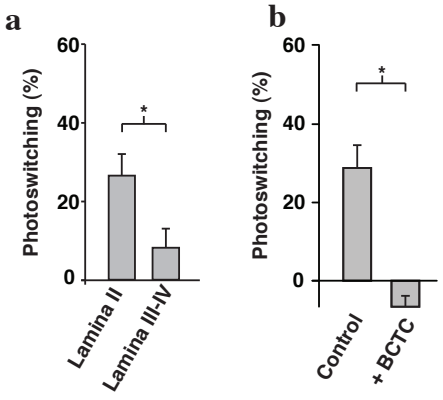
(**a**) Glutamate receptor currents were measured from hippocampal neurons in culture two days after plating. Extracellular solution is similar to K^+ current recording solution without Mg^{2+}. The black bar represents perfusion of extracellular solution containing 100 μM glutamate and 10 μM glycine. Currents were measured under illumination with 380 or 500 nm light. (**b**) Normalized glutamate current under both wavelengths (0.6 ± 2.6 % photoswitching, n = 3 cells, $p > 0.05$ Student t-test). Error bars ± s.e.m.

Supplementary Figure 9: Neuronal survival after ATP and QAQ treatment.



The Live/Dead assay was performed as described by Invitrogen (L-7013). Only dead cells were quantified by using DEAD Red. $P2X_7$ and GFP co-transfected hippocampal neurons were identified by green fluorescence. Cultured hippocampal neurons were transfected with $P2X_7$ (d7) and treated for 30 min with ATP (2.5 mM) +/- QAQ (100 μM), without external calcium, at d9-10. A Live/Dead Assay (Molecular Probes) was used to quantify cell survival among transfected cells, 30 minutes or 24 h after treatment. Both treatments did not cause toxicity compared to control (n = 11-18 fields of view, each field of view containing approximately 1-10 transfected cells; error bars ± s.e.m.).

Supplementary Figure 10: QAQ photosensitizes second order neurons in spinal cord slices.



(**a**) Percent photoswitching for laminae II (26.5 ± 5.0 % photoswitching, n = 22 cells) and III-IV neurons (8.2 ± 4.9 % photoswitching, n = 11 cells, $p<0.05$). (**b**) Percent photoswitching for lamina II neurons with (6.6 ± 3.0 % photosensitization, n = 6 cells) and without 10 μM BCTC (26.5 ± 5.0 % photoswitching, n = 22 cells, $p < 0.05$). Error bars ± s.e.m.; Student t-test.