Supplementary Information for

Dragonfly red opsins share a common tuning mechanism with mammalian red opsins and further enhancement of near-infrared sensitivity

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Figure S1

Fig. S1. Alignment of amino acid sequences of the third intracellular loops of opsin. Third intracellular loops of opsins (black plane letters) were replaced with that of Gs-coupled jellyfish opsin (cyan bold letter). The schematic drawing of chimeric mutants (Dragonfly Gs-RhLW) is also shown.

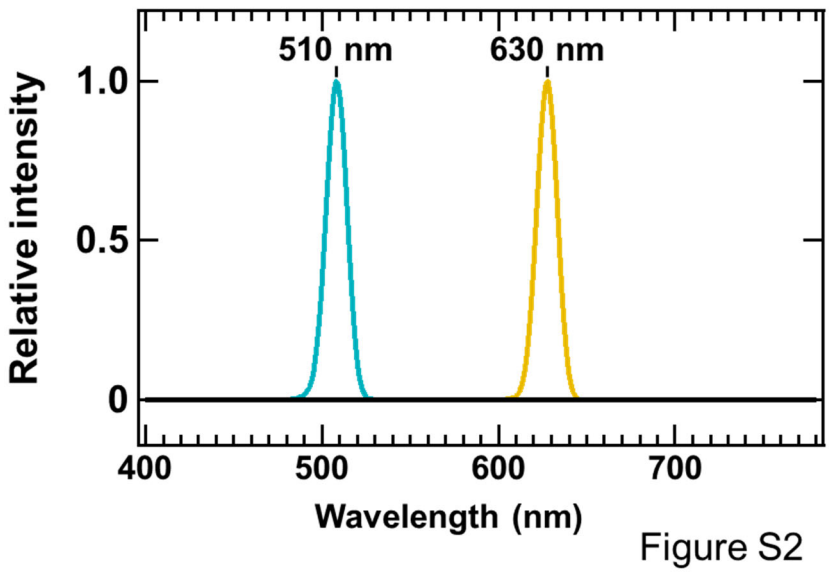

Fig. S2. Spectra of lights for HAS stimulation. Spectra of narrow-band 510 nm and 630 nm lights are shown.

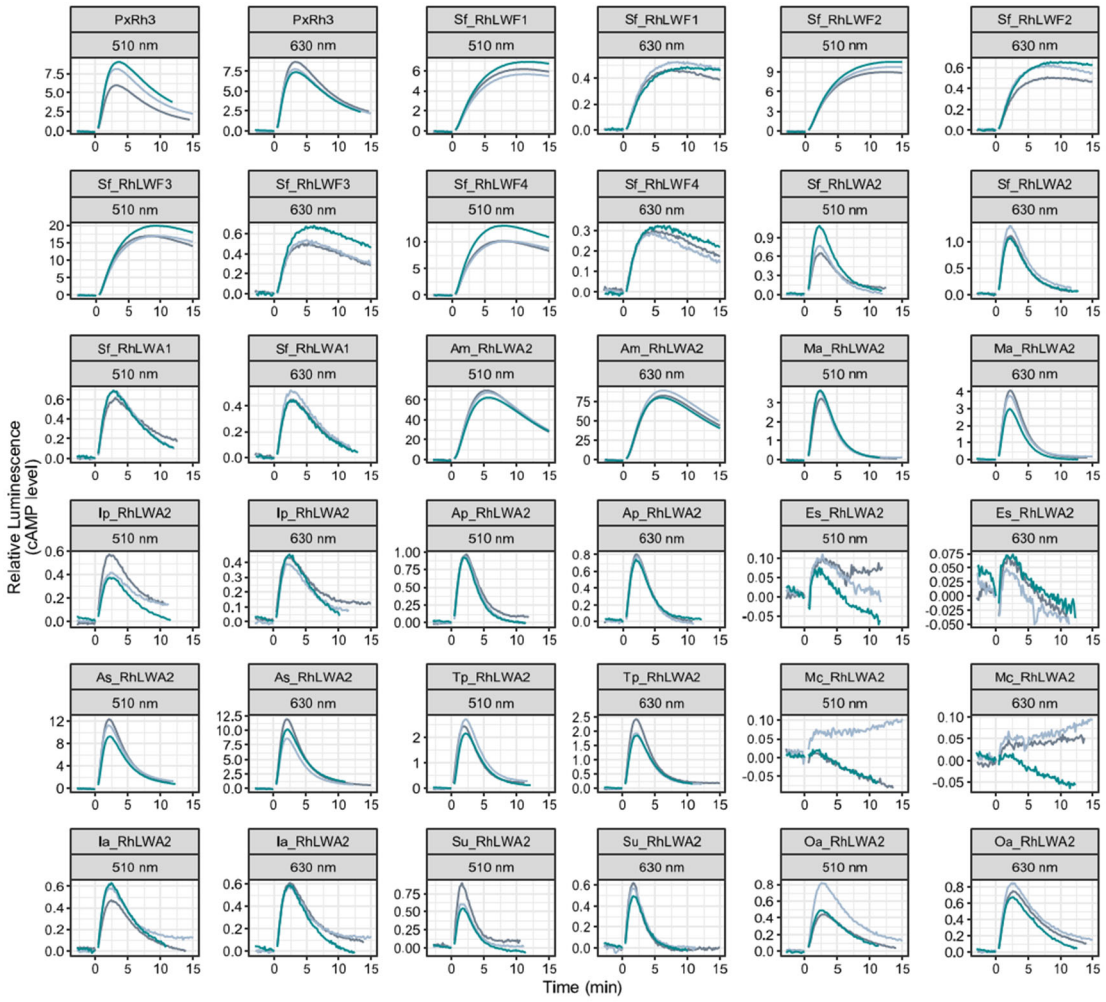

Fig. S3. The datasets of time-dependent luminescence changes for Fig. 1.
Light-induced changes in intracellular cAMP in HEK293 cells expressing Dragonfly Gs-RhLW in response to 630-nm light and 510-nm light measured by GloSensor cAMP assay. At time 0 indicates the timing of light stimulation. The three colored curves correspond to the three experimental replicates. Am, *Asiagomphus melaenops*; Ma, *Macromia amphigena*; Sf, *Sympetrum frequens*; Ip, *Indolestes peregrinus*; Ap, *Anax parthenope*; Es, *Epiophlebia superstes*; As, *Anotogaster sieboldii*; Tp, *Tanypteryx pryeri*; Mc, *Mnais costalis*; Ia, *Ischnura asiatica*; Su, *Somatochlora uchidai*; Oa, *Orthetrum albistylum*.

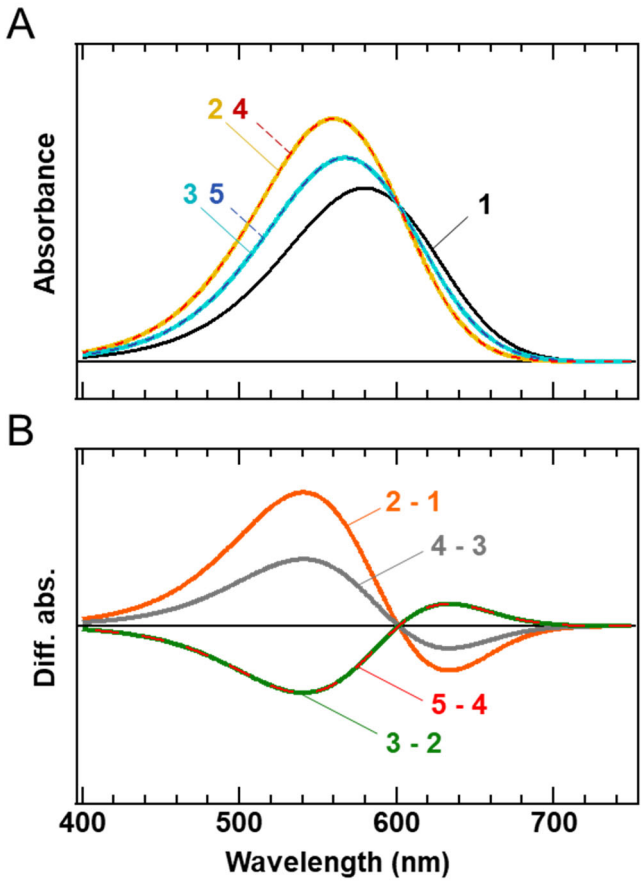

Figure S4

Fig. S4. Schematic representations of the relationship between absolute and difference spectra of bistable pigments.

(A) Diagrams of absolute spectra of opsin-based pigments in the dark (1: black curve), after red-light irradiation (2: yellow curve), after subsequent green-light irradiation (3: cyan curve), after a second red-light irradiation (4: red curve), and after a second green-light irradiation (5: blue curve). (B) The difference spectra are calculated by subtracting two spectra (after minus before irradiation) according to the notations.

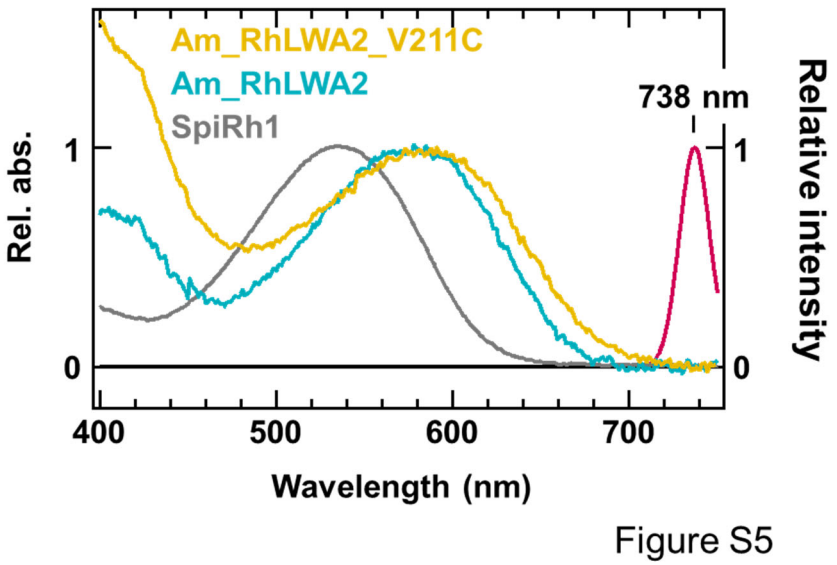

Figure S5

Fig. S5. Absorption spectra of SpiRh1, Am_RhLWA2, and the V211C mutant, along with the NIR light spectrum.

Absorption spectra of SpiRh1 (gray), Am_RhLWA2 (cyan) and the V211C mutant (yellow) are shown for comparison. Each absorption spectrum was normalized to the maximum absorbance in the visible light region. The spectrum of tightly filtered 738 nm light is also shown.