

SUPPLEMENTARY MATERIAL

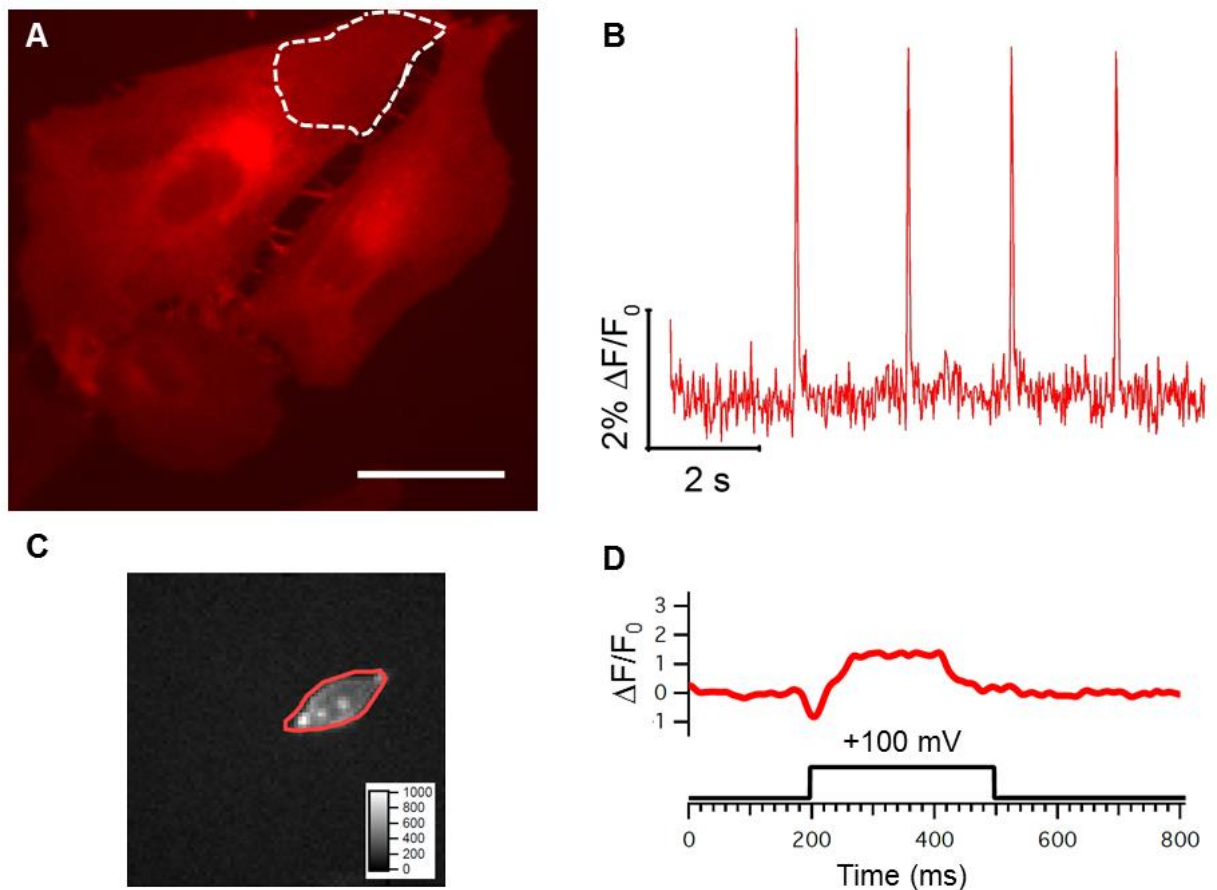


Fig. S1. FlicR0.1 response to electric field stimulation in HeLa cells and membrane potential step in HEK293 cells (A) Image of HeLa cells expressing FlicR0.1. Scale bar 10 μm . (B) FlicR0.1 fluorescence response of regions shown in (A) to electrical field stimulation pulses (~ 50 V/cm, 20 ms). (C) Image of HEK293 cells expressing FlicR0.1 under the CMV promoter. (D) FlicR0.1 fluorescence response (top panel) to a 300 ms square wave in membrane potential (lower panel) from -70 mV to $+30$ mV acquired at 1 ms intervals. Fluorescence imaging for field stimulation measurements was performed at 100 Hz. Illumination intensity was 0.2 W/cm 2 .

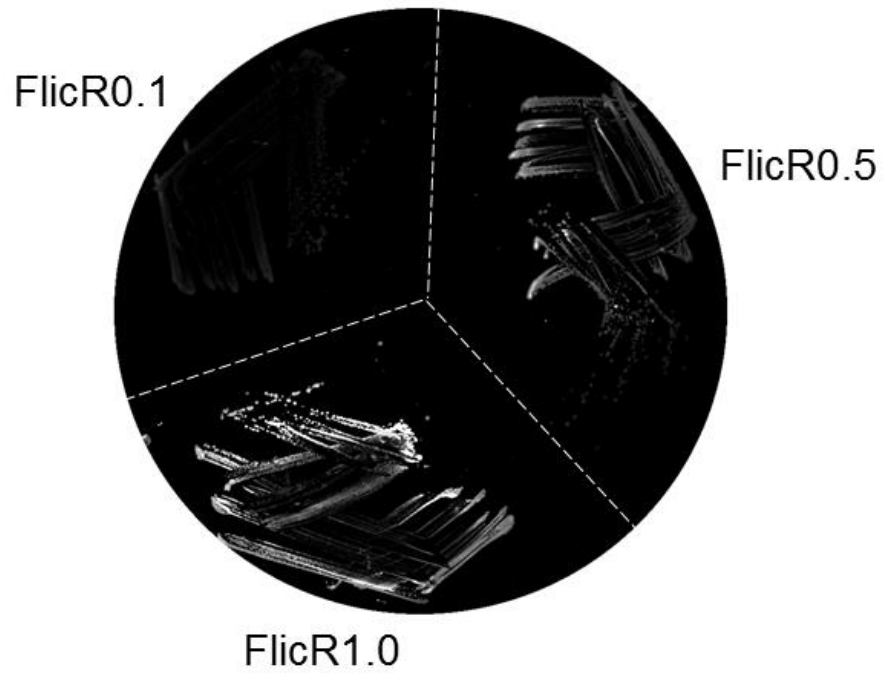


Fig. S2. Evolution of FlicR brightness in *E. coli*. Fluorescence image of *E. coli* expressing various FlicR variants using pcDuEx0.5.

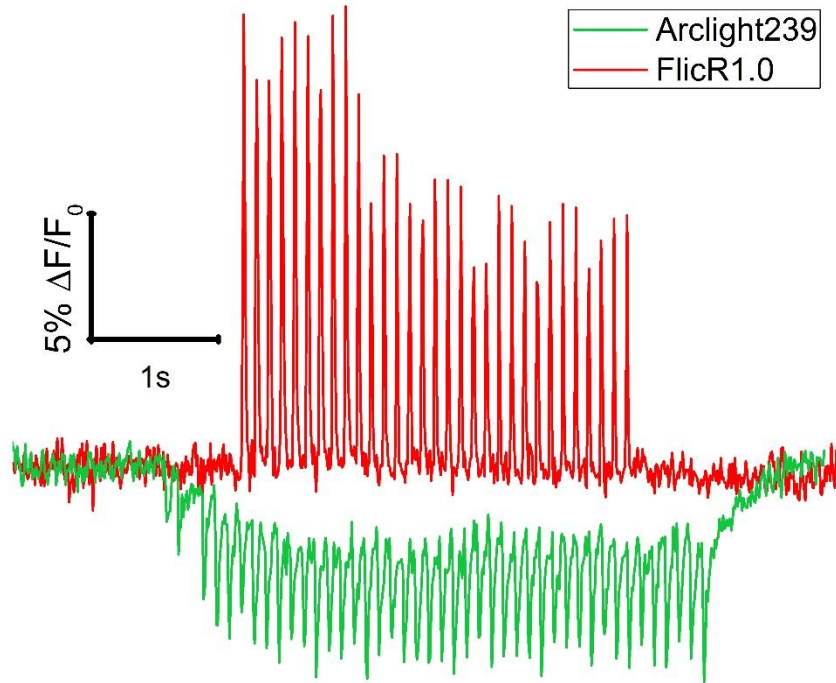


Fig. S3. FlicR1 and ArcLight Q239 response to electric field stimulation in HeLa cells.

Fluorescence trace of FlicR1 (red) and ArcLight Q239 (green) to a train of electrical pulses (50V/cm, 10 ms) at 10 Hz. Traces were recorded consecutively in the same cell co-expressing FlicR1 and ArcLight Q239. Fluorescence traces were collected at 100 Hz using an EMCCD camera. Illumination intensities were 0.2 W/cm² for FlicR1 and 0.1 W/cm² for ArcLight Q239. Although ArcLight Q239 has a larger full response amplitude (35% $\Delta F/F$ per 100 mV) (Jin et al., 2012) than FlicR1 (6.6% $\Delta F/F$ per 100 mV), the slower kinetics of ArcLight Q239 prevent it from reaching its full response amplitude during a 10 ms pulse.

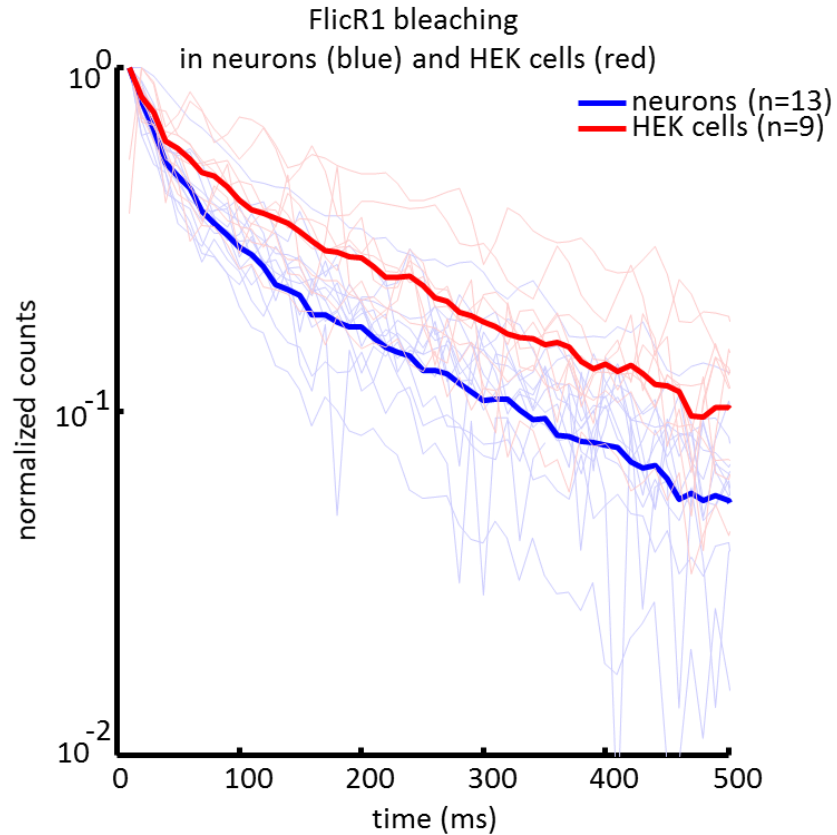


Fig. S4. Comparison of photobleaching rates of FlicR1 in neurons and HEK cells. Under 2-photon excitation, FlicR1 bleaches faster in neurons than in HEK cells ($n = 13$ neurons, $n = 9$ HEK cells) all recorded with excitation at 1120 nm with ~ 10 mW power. Thick lines are averages of their kind. Traces taken as point recordings without scanning the 2-photon excitation beam.

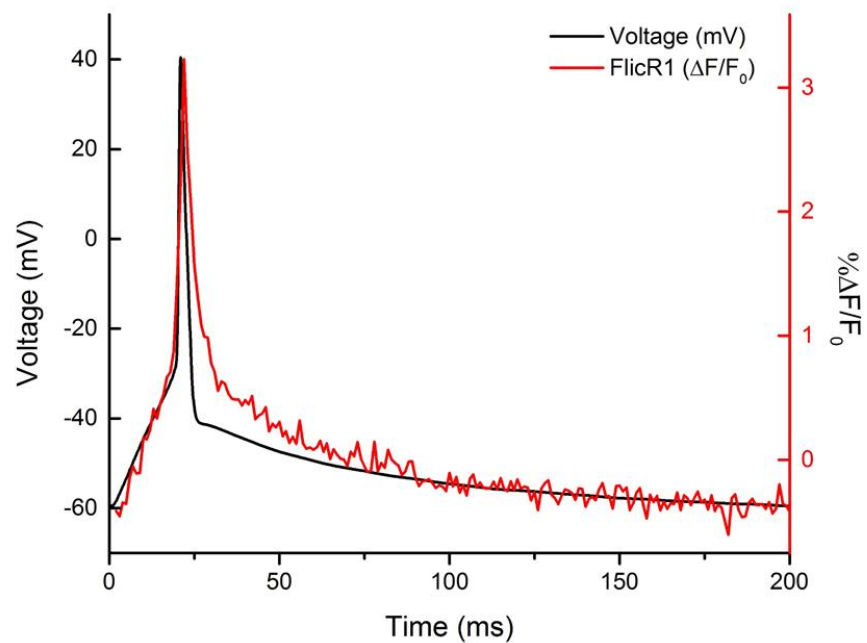


Fig. S5. Overlay of electrically recorded action potential with mean optical recording using FlicR1. Acquired at a frame rate of 1 kHz and an illumination intensity of 10 W/cm².

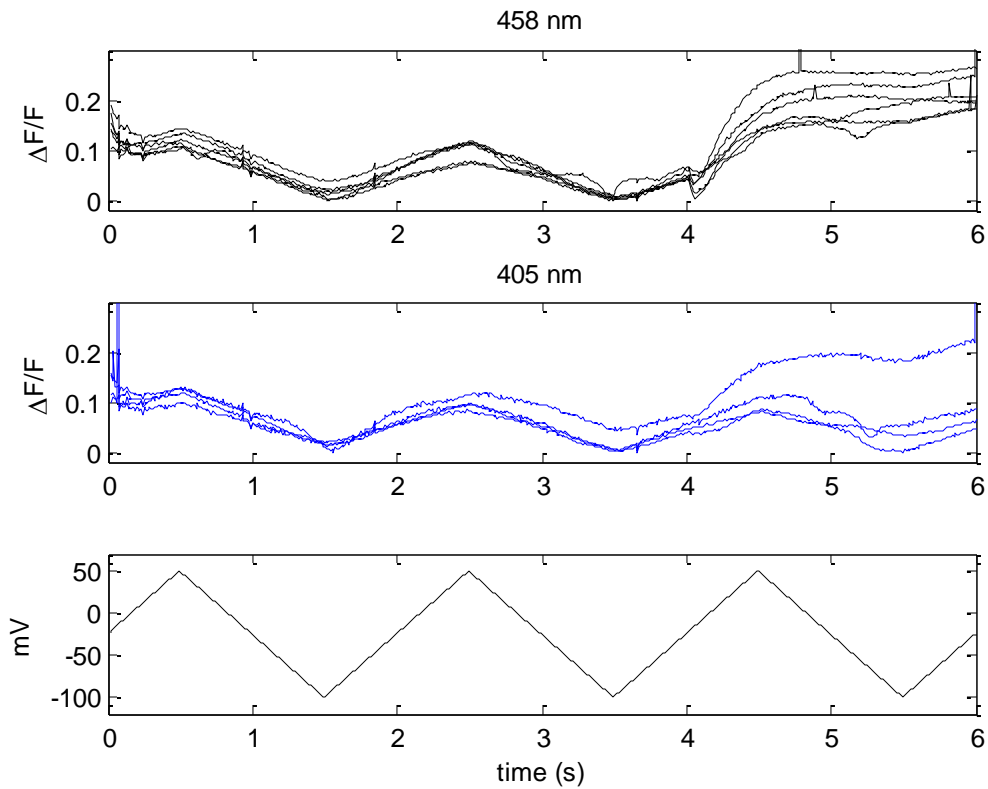


Fig. S6. Photoactivation and voltage sensitivity of FlicR1 during blue light illumination.

Fluorescence intensity of FlicR1 expressed in HEK cells to a triangle wave in membrane potential from -100 mV to $+50$ mV. Blue light at 458 nm (208 mW/cm²) or 405 nm (25 mW/cm²) was turned on for the last two seconds of the run. The traces show three runs each from two cells for 458 nm and two runs each from two cells at 405 nm. The fluorescence trace was acquired at a frame rate of 100 Hz using 561 nm excitation at an intensity of 10 W/cm².

Table S1. Primers used in FlicR1 construction and directed evolution.

Primer	SEQUENCE
FW-BamHI-VSD	CTGAGGATCCACCATGGAGGGATTTCGACGGTTCAGATT TAGTCC
RV-XbaI-cpmApple	CAGGTCTAGACTAACGCGTAGCCTCCCAGCCCATGGTCTT C
FW-VSD242- cpmApple	CAAGAATATTTTATTCCCACCAACAAATGAAGGCTNNKN NKGTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD241- cpmApple	CGATTAGCAAGAATATTTTATTCCCACCAACAAATGAAG NNKNNKGTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD240- cpmApple	CGATTAGCAAGAATATTTTATTCCCACCAACAAATGNNK NNKGTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD239- cpmApple	CGATTAGCAAGAATATTTTATTCCCATCAACAANNKNNK GTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD238- cpmApple	CGTGTGGTTCGATTAGCAAGAATATTTTATTCCCACCAAN NKNNKGTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD237- cpmApple	CGTGTGGTTCGATTAGCAAGAATATTTTATTCCCACNNKN NKGTTTCCGAGCGGATGTACCCCGAGGACGGC
FW-VSD236- cpmApple	CGTGTGGTTCGATTAGCAAGAATATTTTATTCCNNKNNK GTTTCCGAGCGGATGTACCCCGAGGACGGC
RV-cpmApple-VSD242	GCCGTCCTCGGGGTACATCCGCTCGGAAACMNNMNNAG CCTTCAT TTGTTGGTGGGAATAAAAATATTCTTG
RV-cpmApple-VSD241	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN MNN CTT CAT TTG TTG GTG GGA ATA AAA TAT TCT TGC TAA TCG
RV-cpmApple-VSD240	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN MNN CAT TTG TTG GTG GGA ATA AAA TAT TCT TGC TAA TCG
RV-cpmApple-VSD239	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN MNN TTG TTG ATG GGA ATA AAA TAT TCT TGC TAA TCG
RV-cpmApple-VSD238	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN

	MNN TTG GTG GGA ATA AAA TAT TCT TGC TAA TCG AAC CAC ACG
RV-cpmApple-VSD237	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN MNN GTG GGA ATA AAA TAT TCT TGC TAA TCG AAC CAC ACG
RV-cpmApple-VSD236	GCC GTC CTC GGG GTA CAT CCG CTC GGA AAC MNN MNN GGA ATA AAA TAT TCT TGC TAA TCG AAC CAC ACG
FW-FlicR-207X	CACTGTGTTAGATGAATACNNKCAAGAAACAGGAGCCGA TG
RV-HindIII-cpmApple	CAGGAAGCTTCTAACGCGTAGCCTCCCAGCCCATGGTCTT C