

## Life Sciences Reporting Summary

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### ► Experimental design

#### 1. Sample size

Describe how sample size was determined.

P32. No statistical methods were used to estimate sample size for animal studies throughout.

P37. We did not perform a power analysis, since our goal was to create a new technology; as noted in ref. 61, and recommended by the NIH, "In experiments based on the success or failure of a desired goal, the number of animals required is difficult to estimate..." As noted in the aforementioned paper, "The number of animals required is usually estimated by experience instead of by any formal statistical calculation, although the procedures will be terminated [when the goal is achieved]." These numbers reflect our past experience in developing neurotechnologies.

#### 2. Data exclusions

Describe any data exclusions.

P 26. cells with ratio  $\leq 1$  were excluded from further analysis for Pareto front identification, since that would mean zero fluorescence enrichment on the membrane vs. cytoplasm

P 34. Larvae were screened for green fluorescence in the brain and spinal cord at 2-3 days post fertilization (dpf; animals were used without regard to sex) and subsequently imaged at 3-4 dpf.

P34. SWF4 (flvEx3[rig-3::wArchon1-KGC-EGFP-ER2, sra-6::Chr2-GFP, elt-2::nGFP]) and SWF5 (flvEx4[rig-3::wArchon1-KGC-EGFP-ER2, sra-6::Chr2-GFP, elt-2::nGFP]) were two independent lines generated by injecting the indicated plasmids into N2 background worms and picking those with strongest expression of the wArchon1-EGFP fusion.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

P 37. All attempts at replication were successful.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

P 31. No randomization or blinding were used for animal studies throughout.

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

P 31. No randomization or blinding were used for animal studies throughout.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

## 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g.  $P$  values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

## ► Software

Policy information about [availability of computer code](#)

## 7. Software

Describe the software used to analyze the data in this study.

NIS-Elements AR software, BDFACS Diva software, CellSorter4.0, micro-manager, ScanImage, Zeiss ZEN, Origin, FlowJo v10, Excel, ImageJ, Igor Pro, BoxPlotR, and MATLAB with custom codes.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

## 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

## 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

## 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

P 27. HEK293FT (Invitrogen) and HeLa (ATCC CCL-2)

b. Describe the method of cell line authentication used.

P 27. Cells were authenticated by the manufacturer and tested for mycoplasma contamination to their standard levels of stringency and were here used because they are common cell lines for testing new tools.

c. Report whether the cell lines were tested for mycoplasma contamination.

P 27. Cells were authenticated by the manufacturer and tested for mycoplasma contamination to their standard levels of stringency and were here used because they are common cell lines for testing new tools.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

P 24. HEK293FT cells were selected as an expression host due to the several reasons: i) they are suitable for calcium phosphate transfection; ii) they are widely regarded as high expressors for a variety of protein payloads; iii) they are known to have the lowest mutation rate among commonly used mammalian cell lines towards exogenous DNA46; iv) they are robust and easy to work with. Cells were authenticated by the manufacturer and tested for mycoplasma contamination to their standard levels of stringency and were here used because they are common cell lines for testing new tools.

## ► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

### 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

P30. Hippocampal neurons were prepared from postnatal day 0 or 1 Swiss Webster (Taconic) mice (both male and female mice were used) as previously described. P31. Embryonic day (E) 15.5 timed-pregnant female C57BL/6 and CD1 (Charles River; for Fig. 3, Supplementary Figs. 20-22, 24, and 25) or Swiss Webster (Taconic; Supplementary Fig. 7, 23) mice were deeply anesthetized with 2% isoflurane. P31. Acute brain slices were obtained from C57BL/6 and CD1 (Charles River) mice at P20 – P30, using standard techniques. Mice were used without regard for sex. P33. Zebrafish, *Danio rerio* species, were raised and bred at 28°C according to standard methods. DNA plasmids encoding zArchon1-KGC-GFP-ER2 or miRFP were co-injected with Tol2 transposase mRNA into embryos of the pan-neuronal expressing Gal4 line, *tg(elavl3:GAL4-VP16)nns6(ref52)*. Larvae were screened for green fluorescence in the brain and spinal cord at 2-3 days post fertilization (dpf; animals were used without regard to sex) and subsequently imaged at 3-4 dpf. P34. SWF4 (*flvEx3[rig-3::wArchon1-KGC-EGFP-ER2, sra-6::ChR2-GFP, elt-2::nGFP]*) and SWF5 (*flvEx4[rig-3::wArchon1-KGC-EGFP-ER2, sra-6::ChR2-GFP, elt-2::nGFP]*) were two independent lines generated by injecting the indicated plasmids into N2 background worms and picking those with strongest expression of the wArchon1-EGFP fusion. Results from these two lines were indistinguishable. SWF7 (*flvEx5[rig-3::wArchon1-KGC-EGFP-ER2, elt-2::nGFP]*), generated by injecting the indicated plasmid into N2 background worms, was used for control experiments examining the action of wArchon1 in the absence of channelrhodopsin-2. The transgenic worms (used without regard to sex) at L4 stage of development were put onto NGM plates with OP50 lawns supplemented with 100 µM all-trans-retinal (Sigma-Aldrich, USA) no less than 16 hours prior to experiments.

Policy information about [studies involving human research participants](#)

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.