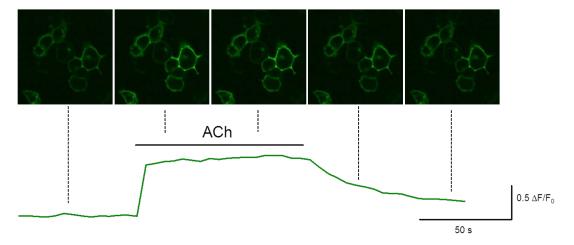
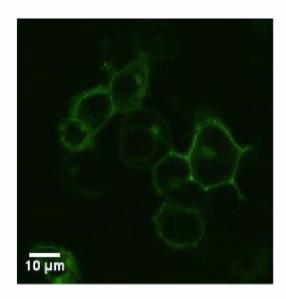
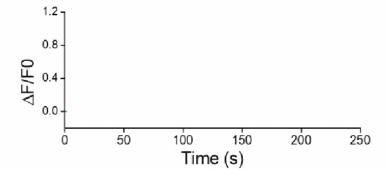
### Supplementary movie 1. The fluorescence response of GACh2.0 to ACh in cultured HEK293T cells.

This movie represents the fluorescence response of GACh2.0 expressing HEK293T cells to the application of 100  $\mu$ M ACh. Please click the bottom image to view the movie. Experiments were repeated for more than 5 cultures with similar results.



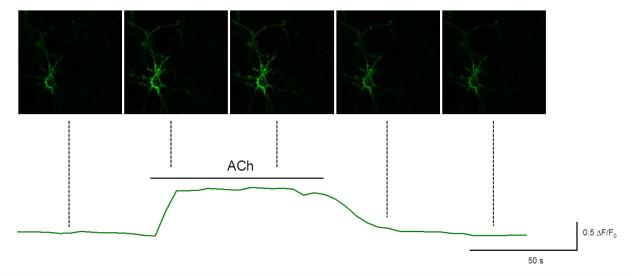


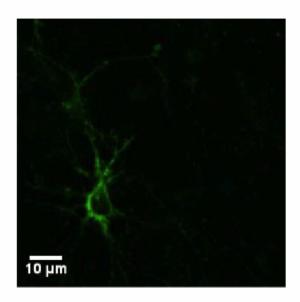


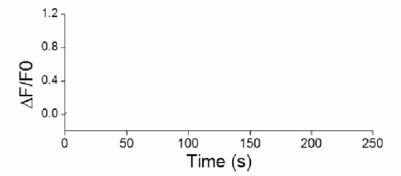


## Supplementary movie 2. The fluorescence response of GACh2.0 to ACh in cultured cortical neurons.

This movie represents the fluorescence response of GACh2.0 expressing cultured neurons to the application of 100  $\mu$ M ACh. Please click the bottom image to view the movie. Experiments were repeated for more than 10 cultures with similar results.



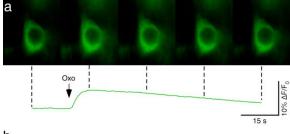


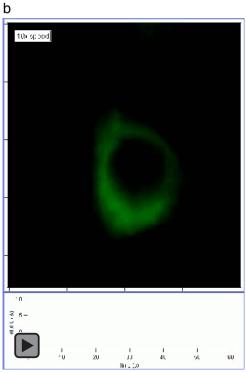




# Supplementary movie 3. Imaging cholinergic response of a CA1 pyramidal neuron

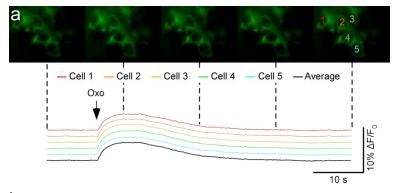
This movie represents the fluorescence response of a GACh2.0 expressing CA1 pyramidal neurons in acute brain slice to the application of Oxo. Please click the start button in **b** to view the video clip. Experiments were repeated for more than 8 animals with similar results.

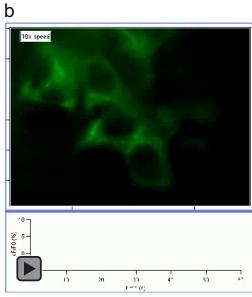




### Supplementary movie 4. Imaging cholinergic response of a group of CA1 pyramidal neurons

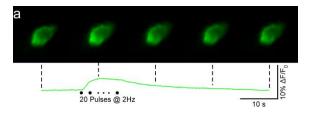
This movie represents the fluorescence response of a group of GACh2.0 expressing CA1 pyramidal neurons to the application of Oxo. Please click the start button in **b** to view the video clip. Experiments were repeated for 8 animals with similar results.

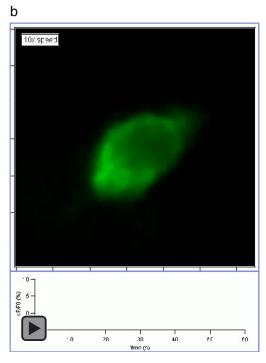




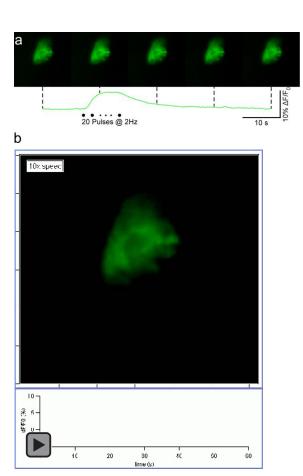
### Supplementary movie 5. Imaging cholinergic transmission of an entorhinal stellate neuron

This movie represents the fluorescence response of a GACh2.0 expressing stellate neuron to 20 pulses of electrical stimulation delivered at 2Hz. Please click the start button in **b** to view the video clip. Experiments were repeated for more than 6 animals with similar results.





This movie represents the fluorescence response of a GACh2.0 expressing pancreas cell to 20 pulses of electrical stimulation delivered at 2Hz. Please click the start button in **b** to view the video clip. Experiments were repeated for more than 5 animals with similar results.



### Supplementary movie 7. Imaging cholinergic transmission of an adrenal cell

This movie represents the fluorescence response of a GACh2.0 expressing adrenal cell to 20 pulses of electrical stimulation delivered at 2Hz. Please click the start button in **b** to view the video clip. Experiments were repeated for more than 4 animals with similar results.

