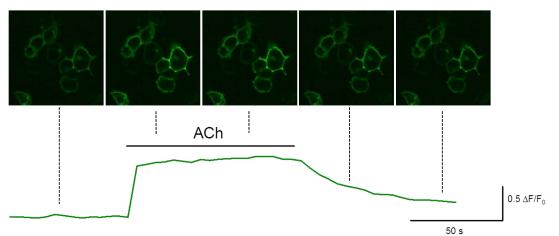
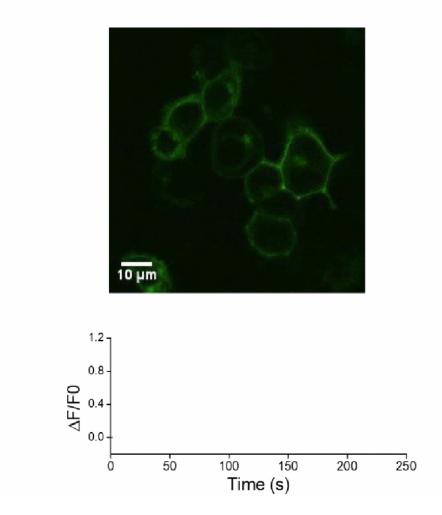
Supplementary movie 1. The fluorescence response of GACh2.0 to ACh in cultured HEK293T cells.

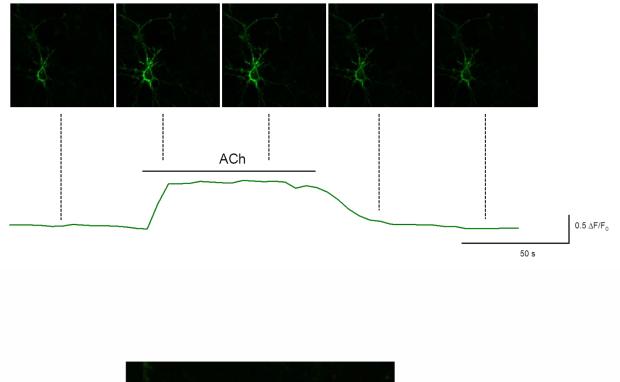
This movie represents the fluorescence response of GACh2.0 expressing HEK293T cells to the application of 100 μ M ACh. Please click the bottom image to view the movie. Experiments were repeated for more than 5 cultures with similar results.

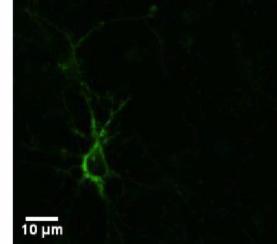


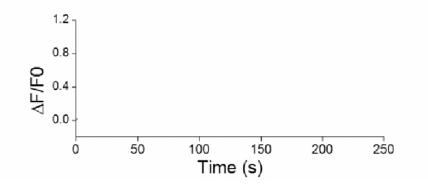


Supplementary movie 2. The fluorescence response of GACh2.0 to ACh in cultured cortical neurons.

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





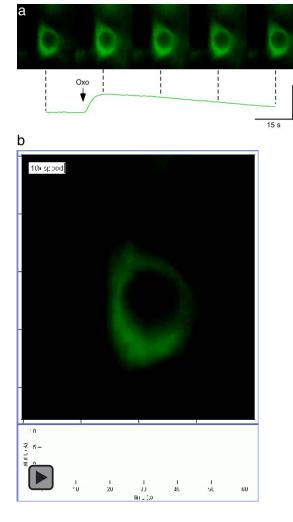


Nature Biotechnology: doi:10.1038/nbt.4184

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

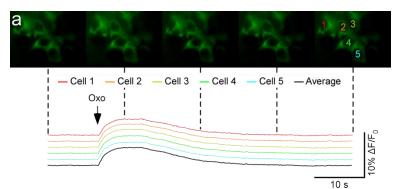
AF/

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.

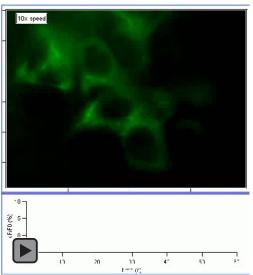


Supplementary movie 4. Imaging cholinergic response of a group of CA1 pyramidal neurons

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.

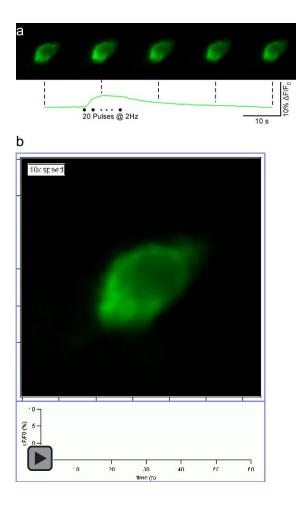


b



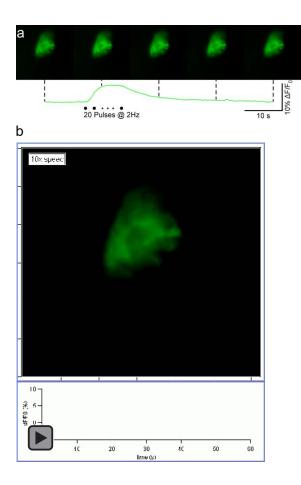
Supplementary movie 5. Imaging cholinergic transmission of an entorhinal stellate neuron

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.



Supplementary movie 6. Imaging cholinergic response of a pancreas cell

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.

