Literature Report

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Covalently Tethered Rhodamine Voltage Reporters for High Speed Functional Imaging in Brain Tissue

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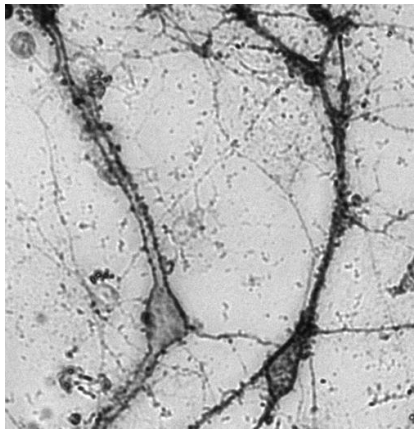
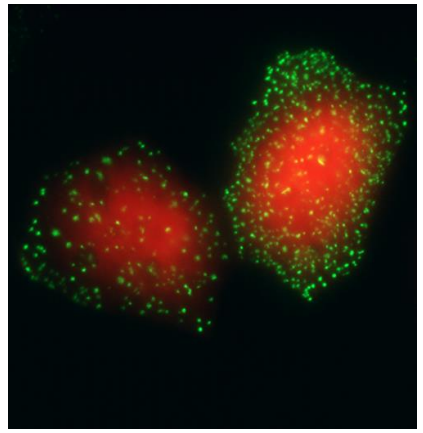
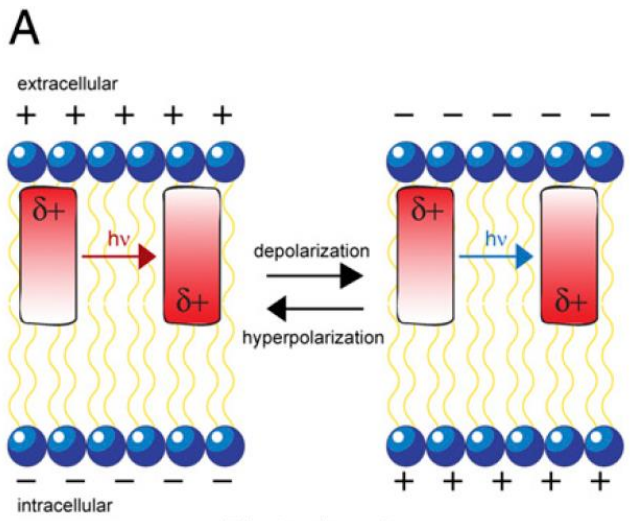
Voltage Imaging

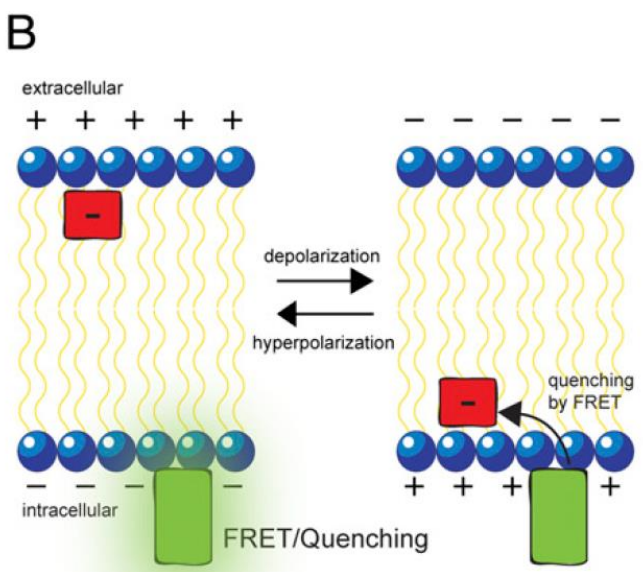


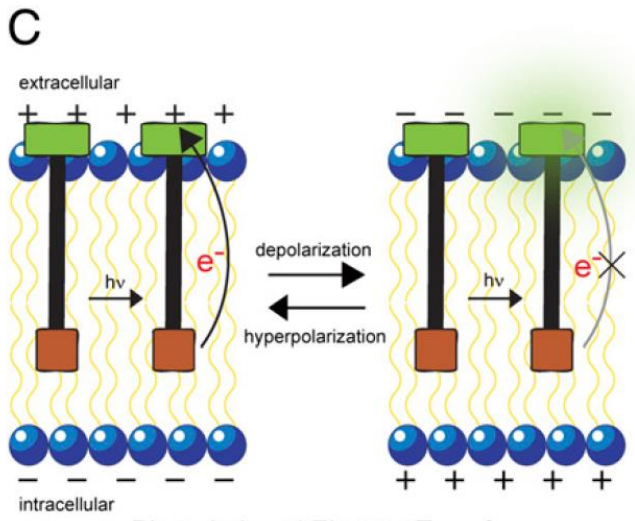
Activity Tracing

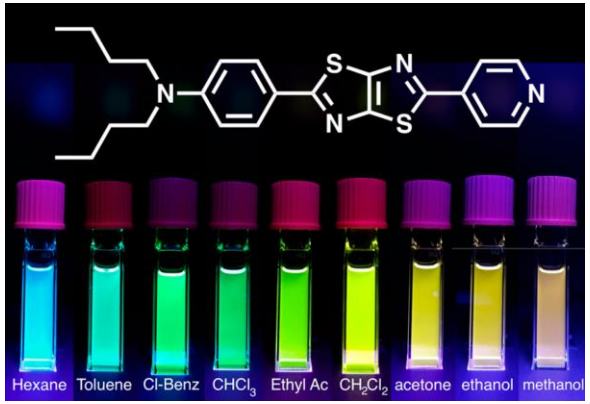


Mechanisms of fluorescent voltage sensing

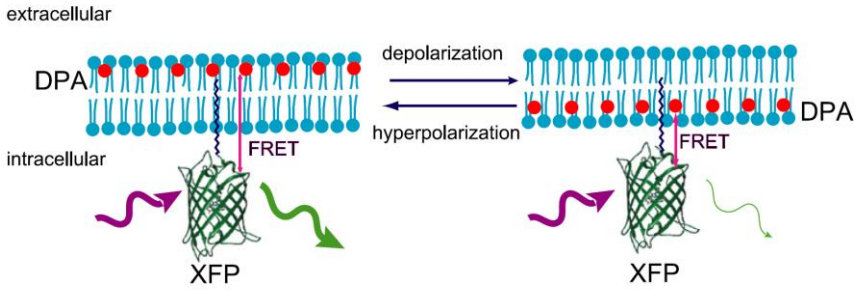




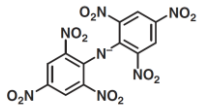




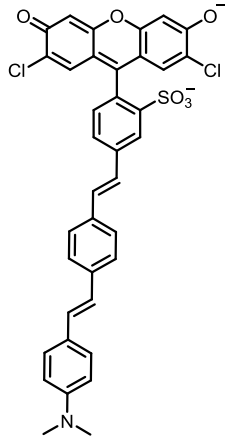
J. Am. Chem. Soc. 2019, 141, 18780−18790



Biophysical Journal 99(7) 2355–2365



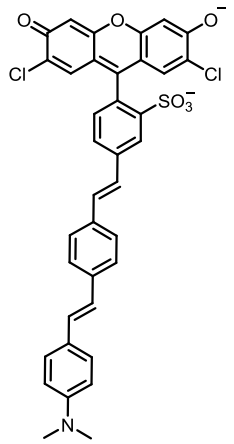
dipicrylamine (DPA)



VF2.1.Cl

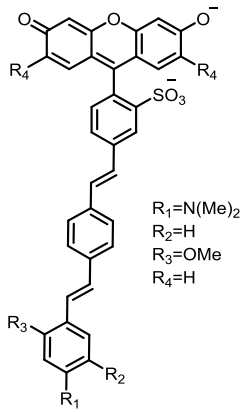
PNAS. 2012, 109, 2114–2119

Introduction



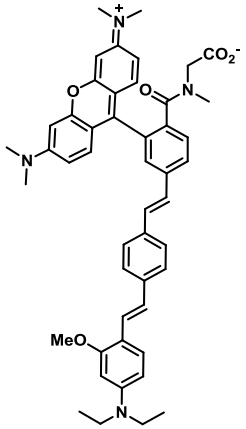
VF2.1.Cl

PNAS. 2012, 109, 2114–2119



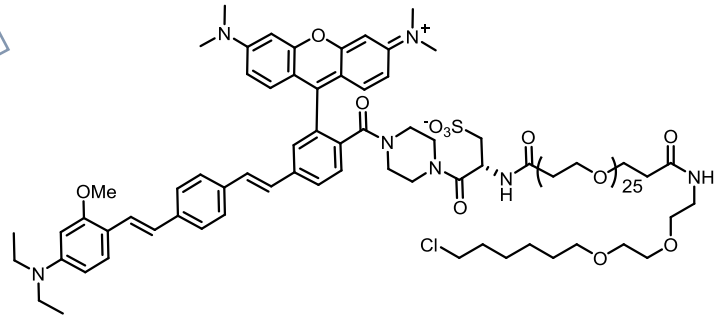
VF2.1(OMe).H

J. Am. Chem. Soc. 2015, 137, 1817−1824

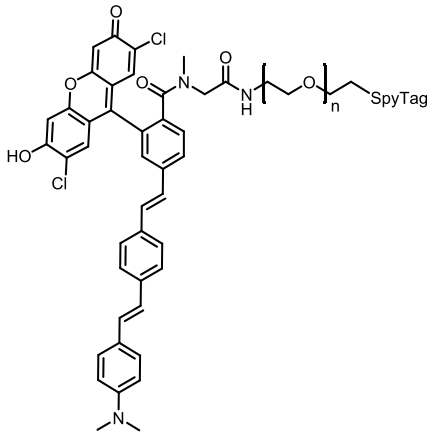


RhoVR1

J. Am. Chem. Soc. 2016, 138, 9085−9088



RhoVR1-PEG$_{25}$-Halo

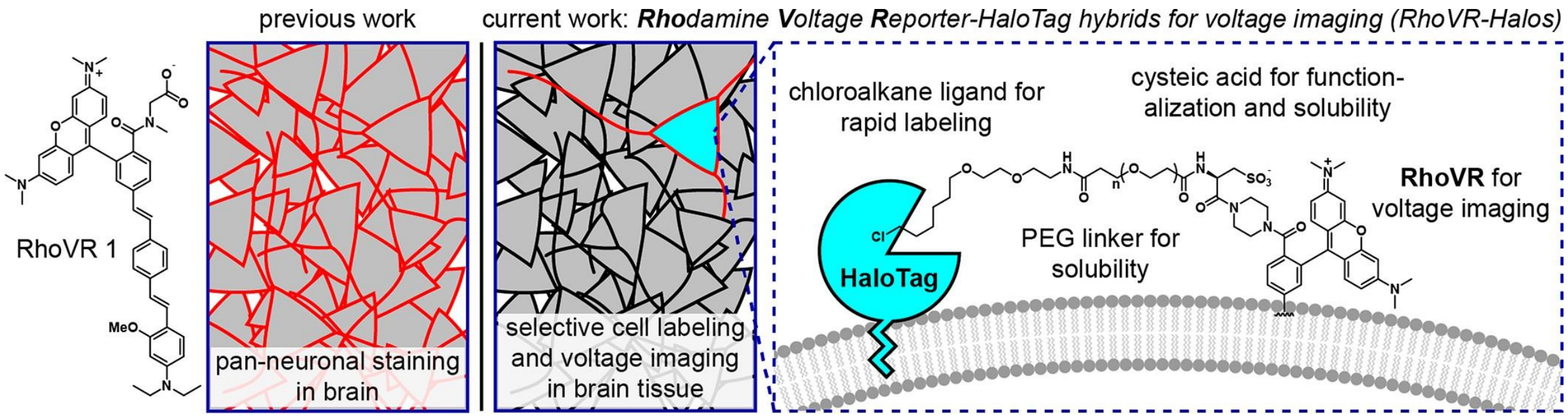


VoltageSpy

J. Am. Chem. Soc. 2019, 141, 1349−1358

compound	λ_{abs}(nm)	λ_{em}(nm)	ΦF	ΔF/F(100mV)
VF2.1.Cl	522	536	0.05	27%
VF2.1(OMe).H	504	524	0.04	48%
RhoVR 1	564	588	0.045	47%
VoltageSpy	526	546	0.087	12.70%
RhoVR1-PEG$_{25}$-Halo	565	585	0.05	34%

Rhodamine Voltage Reporter-HaloTag Hybrids



Characterization of RhoVR 7

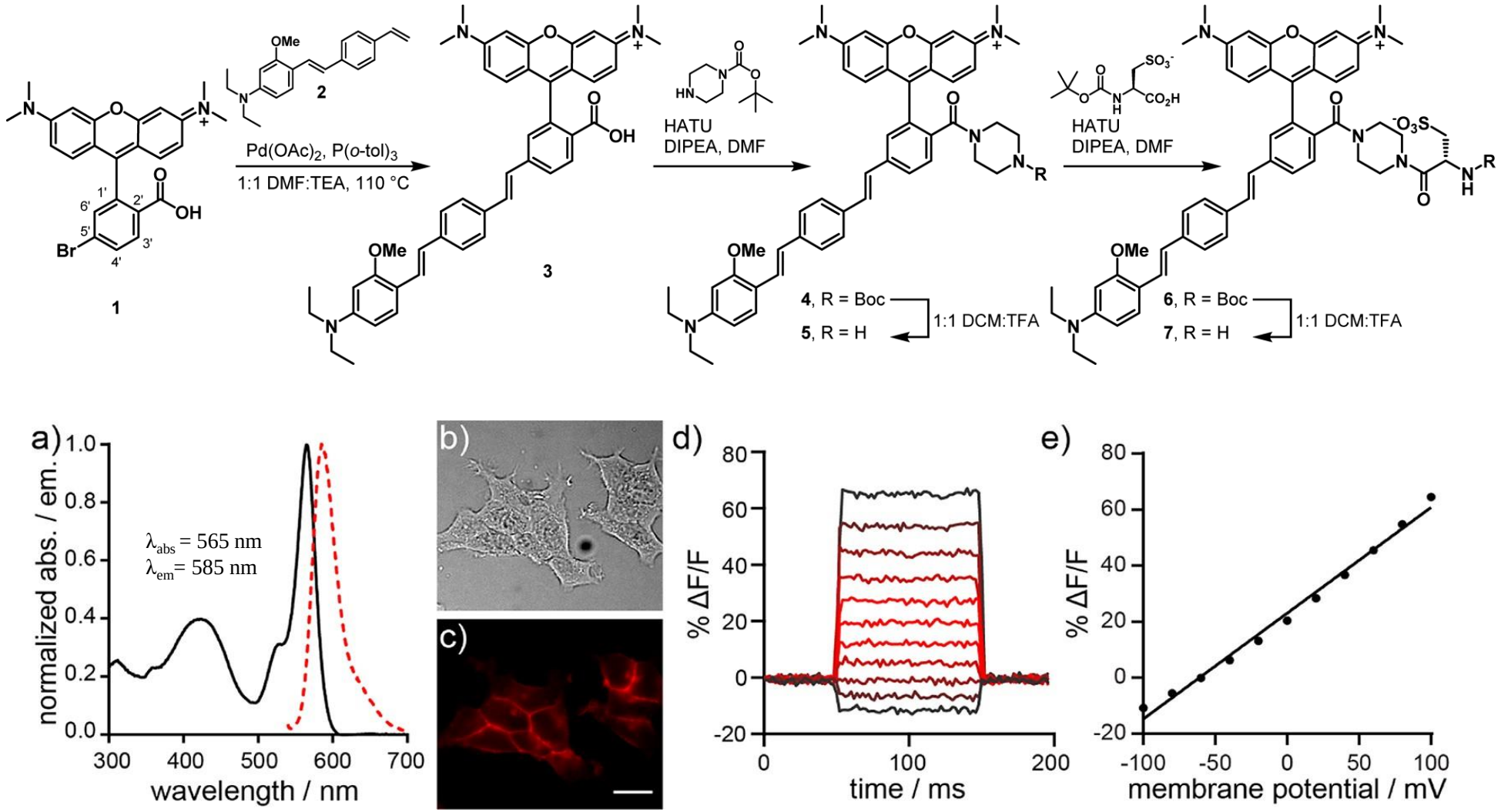
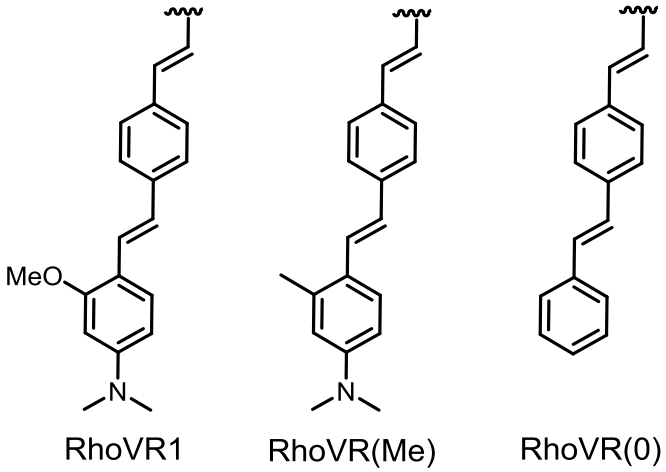


Figure . (a) Normalized absorption and emission spectra for 7. Dye concentration was 500 nM. (b) Transmitted light (DIC) and (c) widefield fluorescence microscopy image of HEK293T cells stained with 7. Scale bar is 20 μm. (d) Plot of fractional change in fluorescence (ΔF/ F) vs time for a single HEK293T cell stained with 7 and subjected to 100 ms hyper- and depolarizing steps (±100 mV, 20 mV increments) from a holding potential of −60 mV under whole-cell voltage-clamp mode. (e) A plot of fractional change in fluorescence (ΔF/F)vs final membrane potential (mV).

Design of Cell-Surface Expressed HaloTag Enzymes

RhoVR-Halo derivative	ε_{565}[a] (M^{-1} cm^{-1})	Φ_{Fl}	$\Delta F/F$[c] (100 mV)	rel. brightness[c]	SNR[c] (100 mV)
RhoVR 1 (500 nM)	87 000	0.045[a]	47%	100%	90
7	82 000	0.026[a]	38%	18%	30
RhoVR1-PEG_{25}-Halo, **15**	74 000	0.050[a], 0.017[b]	34%	30%	34
RhoVR1-PEG_{13}-Halo, **14**			26%	29%	30
RhoVR1-PEG_{9}-Halo, **13**			20%	34%	14
RhoVR1-PEG_{5}-Halo, **12**			11%	32%	11
RhoVR1-PEG_{0}-Halo, **16**			0%	61%	
RhoVR(Me) (500 nM), **21**	81 000	0.022[a]	13%	220%	77
RhoVR(Me)-PEG_{25}-Halo, **27**	85 000	0.038[a], 0.009[b]	16%	118%	40
RhoVR(0), **32**	71 000	0.219[a]	0%		
RhoVR(0)-PEG_{25}-Halo, **38**	83 000	0.214[a]	0%		

[a]PBS, pH 7.2, 0.1% SDS. [b]HBSS. [c]voltage-clamped HEK cells.



Constructs used in HEK cells

CMV	IgK	HaloTag	HA	DAF	IRES	EGFP
CMV	IgK	HaloTag	HA	pDisplay	IRES	EGFP

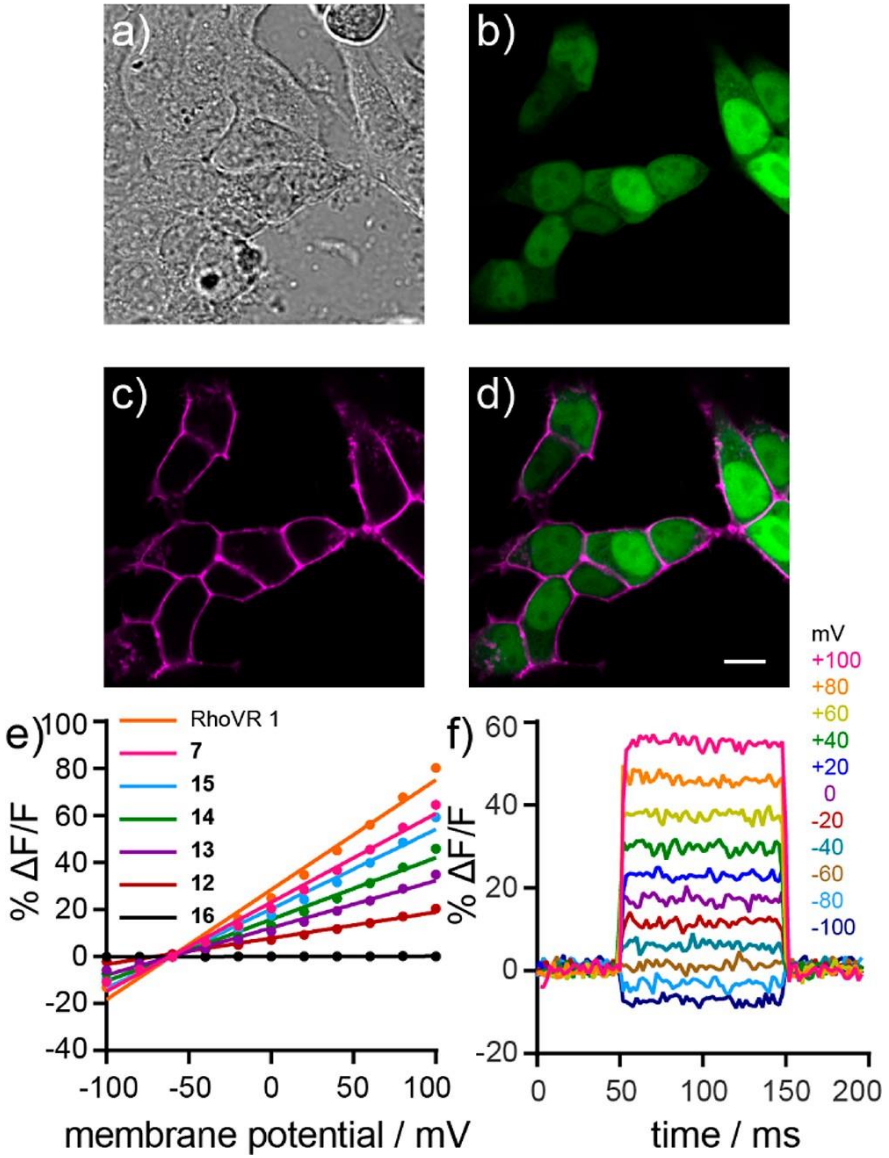
Constructs used in neurons

Synapsin	IgK	HaloTag	HA	pDisplay	IRES	EGFP	WPRE
Synapsin	IgK	HaloTag	HA	DAF	IRES	EGFP	WPRE
Synapsin	IgK	HaloTag	HA	pDisplay	IRES	GCaMP6s	WPRE

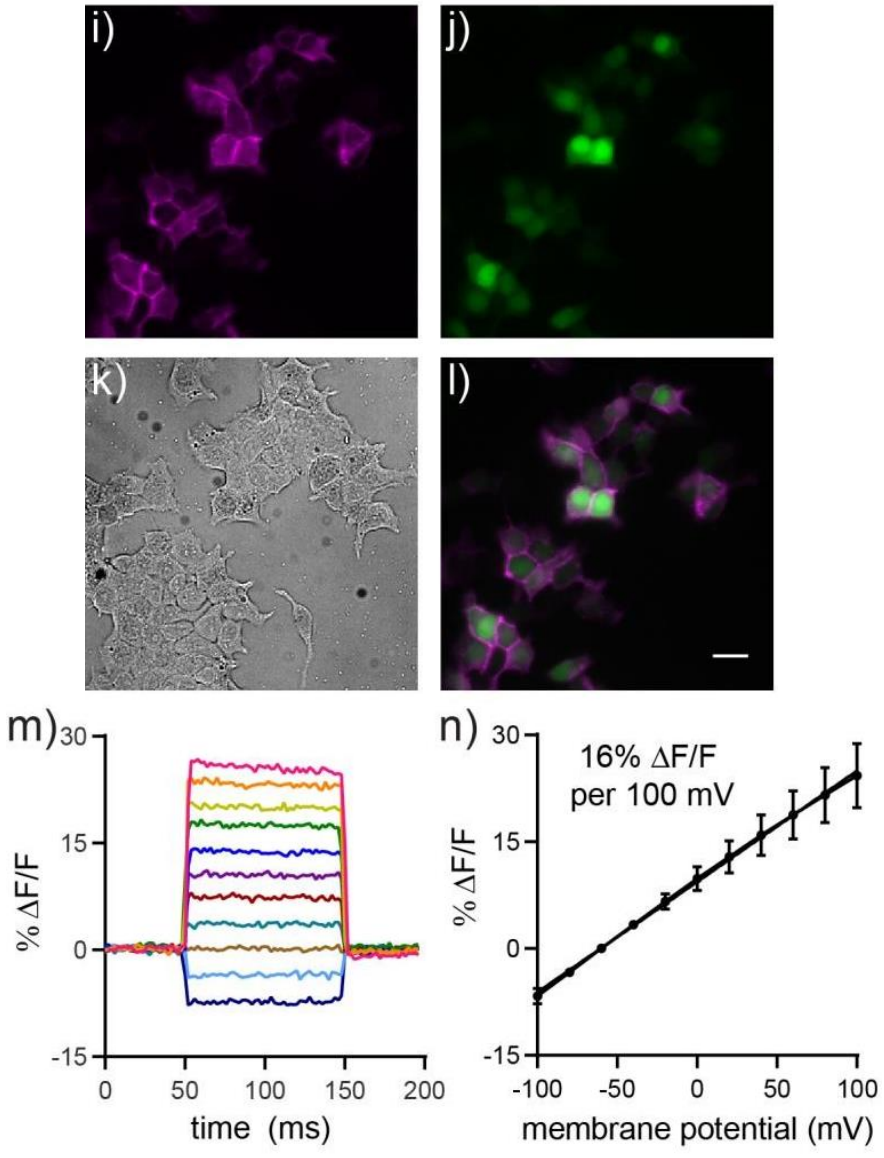
Constructs used in acute brain slices

Synapsin	IgK	HaloTag	HA	pDisplay	WPRE

Cellular labeling and voltage sensitivity

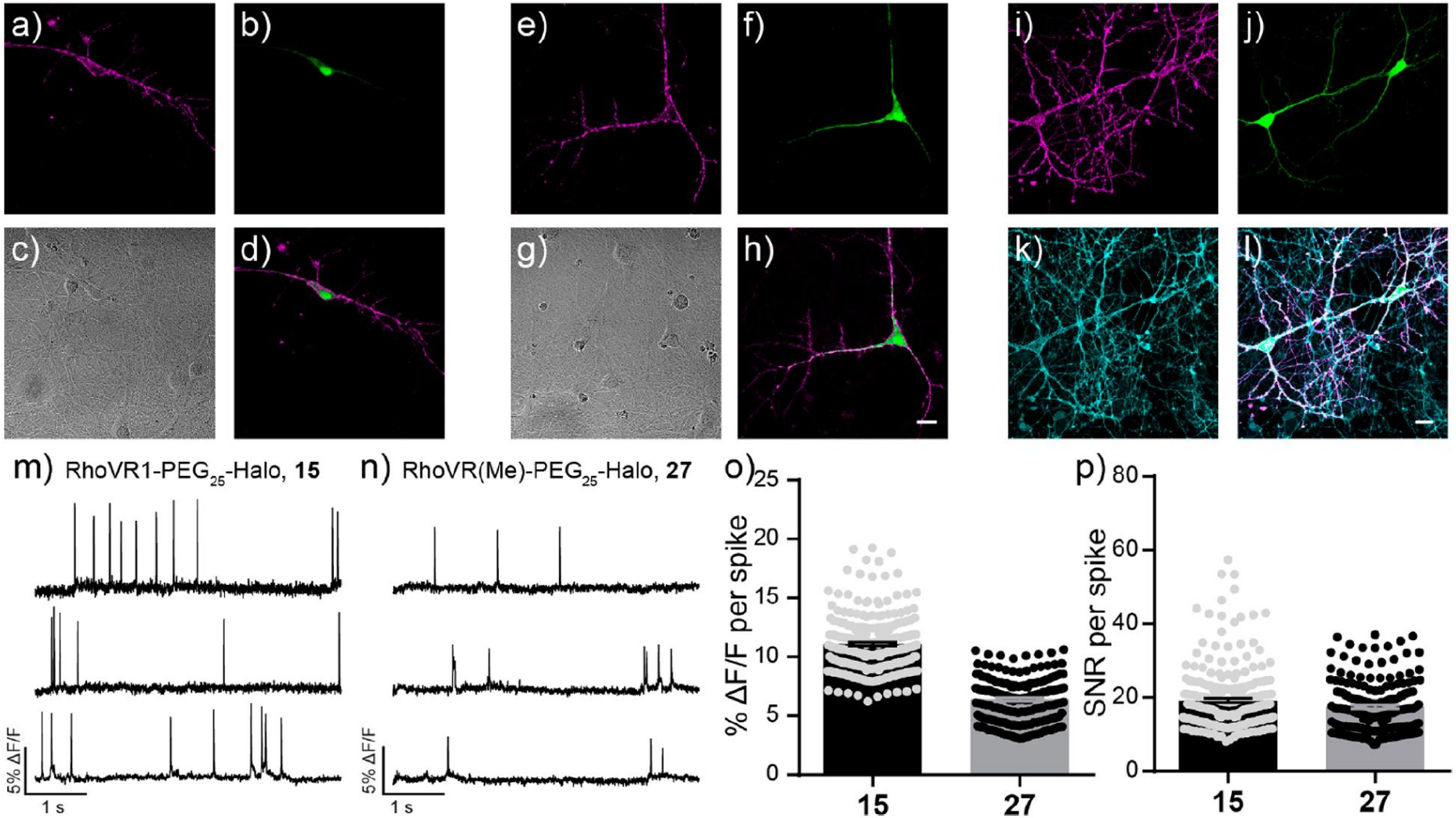


RhoVR1-PEG$_x$-Halo compounds



RhoVR（Me）-PEG $_{25}$ -Halos

Characterization in neurons



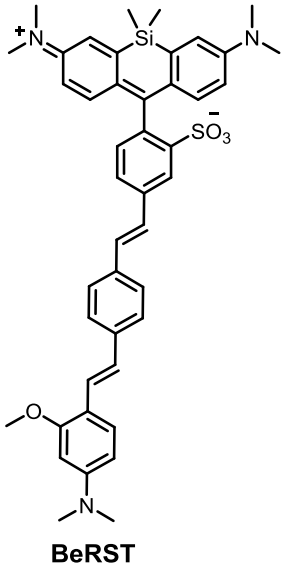


Figure . (a and e) RhoVR fluorescence is localized to the plasma membrane of the HaloTag expressing neurons. (b and f) EGFP fluorescence indicates expression of HaloTag (pDisplay). (c and g) DIC image and (d and h) merged image of neurons in panels (a/b) and (e/f). Scale bar is 20 µm. (i) Maximum projection of RhoVR-Halo 27 fluorescence in (j) HaloTag and EGFP-expressing neurons. (k) Counterstaining with silicon-rhodamine BeRST reveals pan-membrane staining. (l) Merged image showing RhoVR (magenta), EGFP (green), and BeRST (cyan) fluorescence. Scale bar is 20 µm. Plots of fractional change in fluorescence (ΔF/F) vs time in hippocampal neurons stained with (m) 15 or (n) 27. (o) Average ΔF/F and (p) SNR per spike for evoked action potentials recorded with RhoVR-Halos. Error bars are ± SEM for n = 256 or 186 spikes from 30 or 21 neurons for RhoVR1-PEG25-Halo (15)or RhoVR(Me)-PEG25-Halo (27), respectively.

Comparison of RhoVR1-PEG25-Halo to GEVIs

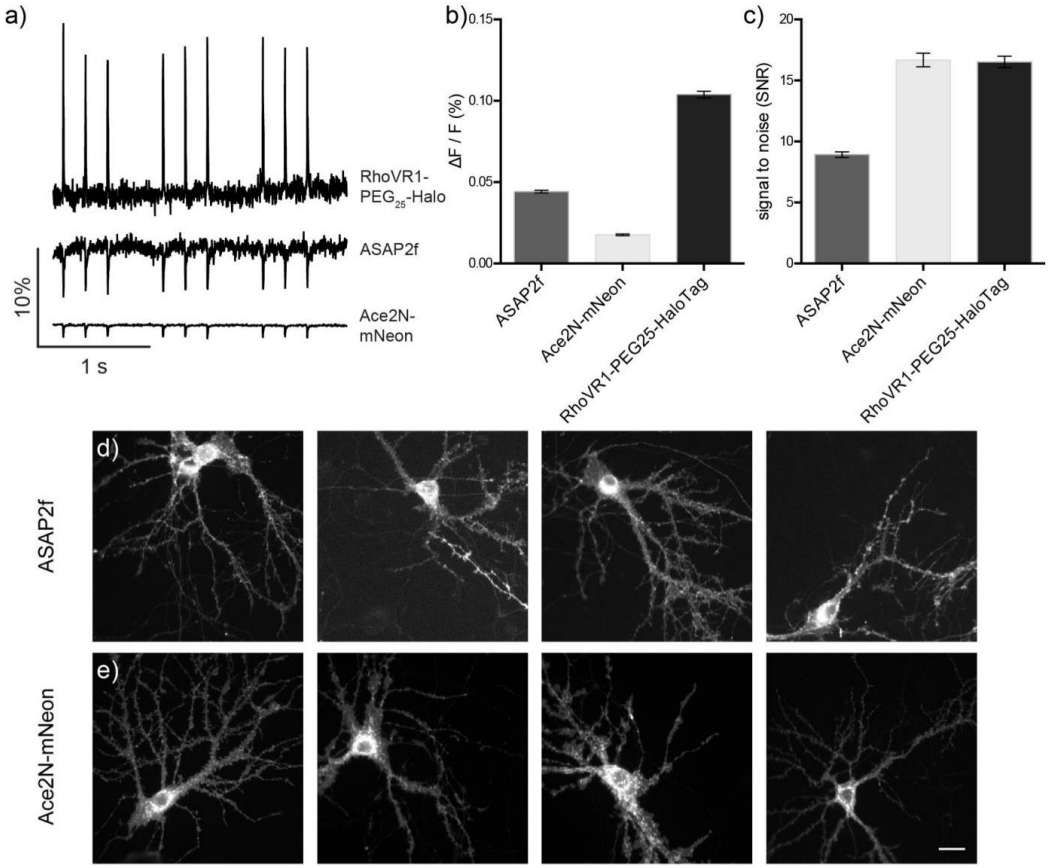


Figure . Comparison of RhoVR1-PEG25-Halo 15 to GEVIs ASAP2f and Ace2N-mNeon. a) Representative voltage recordings of evoked activity measured with 15, ASAP2f and Ace2N-mNeon. Voltage traces were acquired with identical parameters and matched light power. b) Average voltage sensitivities per spike (evoked) for 15, ASAP2f and Ace2N-mNeon (n=101-211). c) Average SNRs for individual spikes (evoked) for 15, ASAP2f and Ace2N-mNeon (n=101-211). Error bars are ±S.E.M. Representative widefield fluorescence images of d) ASAP2f and e) Ace2N-mNeon. Scale bar is 20 µm.

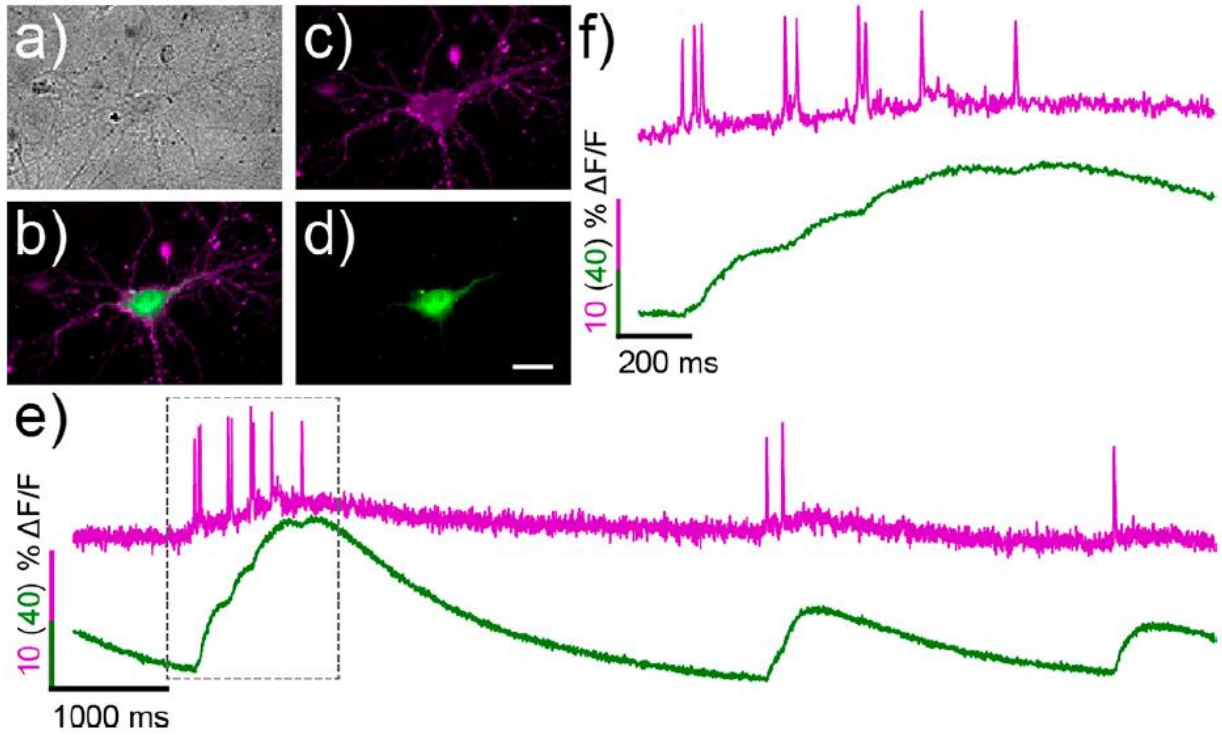


Figure . Simultaneous, two-color voltage and Ca2+ imaging inhippocampal neurons with RhoVR-Halo 15 and GCaMP6s. (a) Transmitted light image of neurons expressing cell-surface HaloTag and cytosolic GCaMP6s. (b) Merged widefield fluorescence microscopy image depicting 15 (magenta) and GCaMP6s (green) staining. Individual channels of the same neuron show (c) membraneassociated 15 and (d) cytosolic GCaMP6s. (e) Plot of ΔF/F vs time for 15 (magenta) and GCaMP6s (green). (f) Expanded time scale of the boxed region in panel (e). Scale bar is 20 µm.

Deploying RhoVRs in Mouse Brain Slice

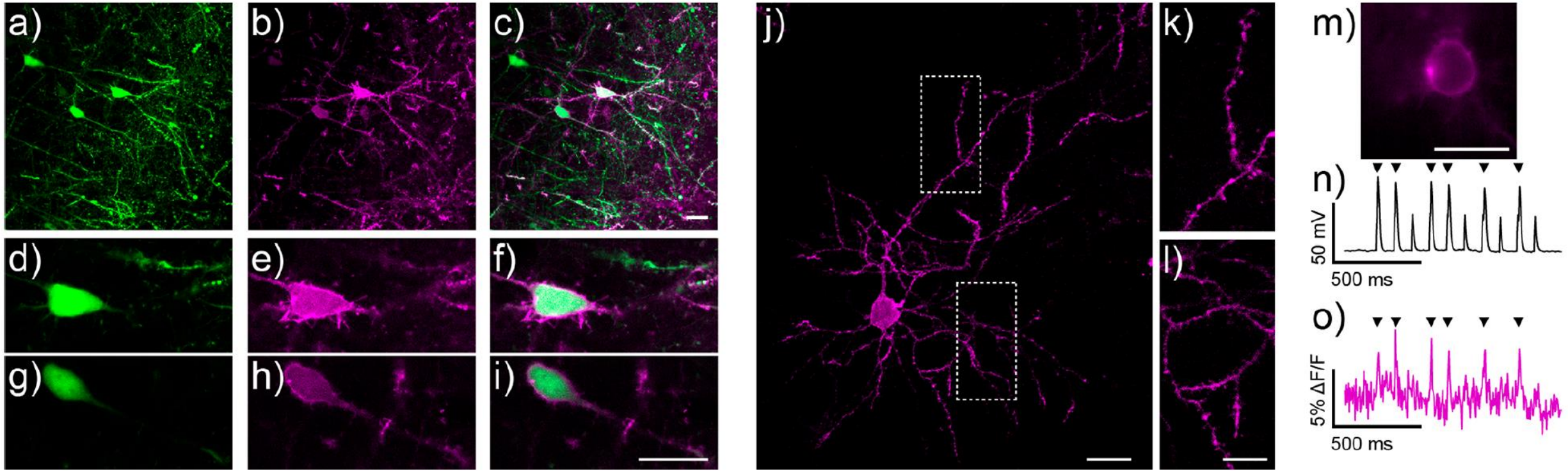


Figure . (a−i) One-photon, confocal microscopy of 15 in mouse brain slice prepped from animals expressing HaloTag-pDisplay and EGFP (introduced via in utero electroporation). Maximum projection of (a) EGFP (green), (b) 15 (magenta), and (c) merged fluorescence in mouse cortical brain slice stained with 15 (250 nM, 15 min loading). Maximum projections are constructed from 25 slices with optical sections of about 0.8 µm. (j) RhoVR fluorescence associated with a slice treated with 250 nM 15 for 15 min at room temperature. Image is a maximum projection of 100 optical slices over 37 µm, with excitation provided at 860 nm. Scale bar is 20 µm. (k and l) Expanded view of boxed regions in panel (j). Scale bar is 10 µm. (m−o) Widefield fluorescence microscopy and dual voltage imaging and electrophysiology of cortical neurons in brain slice stained with 15. (m) Widefield fluorescence image of 15 fluorescence (250 nM, 15 min loading) in a cortical neuron expressing HaloTag-pDisplay. (n) Plot of voltage vs time for the neuron in panel (n) during current injection to evoke action potentials. Electrophysiology is digitized at 20 kHz. (o) Plot ofΔF/F vs time for the same neuron. Fluorescence data were acquired at 500 Hz, and are not corrected for bleaching. Arrows indicate evoked spike. Small spikes are subthreshold current injections. Scale bar is 20 µm.