

Expanded View Figures

Figure EV1. Expression analysis of vf-Chrimson-eYFP and f-Chrimson-eYFP expressed in NG108-15 cells.

- A–F Immunofluorescence of f-Chrimson-eYFP (A, C, E) and vf-Chrimson-eYFP (B, D, F) plotted across the plasma membrane and the membrane proximal intracellular regions. The line profiles are obtained from single-stack confocal images of NG cells transfected with either construct ($n = 30$ cells, $N = 4$ transfections per construct). Line profiles are grouped according to the cellular distribution of the fluorescence. (A + B): intracellular fluorescence weaker than plasma membrane fluorescence, (C + D): intracellular fluorescence similar to plasma membrane fluorescence (E + F): gradual increase of fluorescence from plasma membrane to cell interior.
- G Exemplary live cells, single-stack confocal image of NG cells expressing f-Chrimson-eYFP. Scale bar = 10 μm .
- H Bar graph summarizing the line profiles, grouped according to the cellular distribution of the fluorescence shown in (A–F).

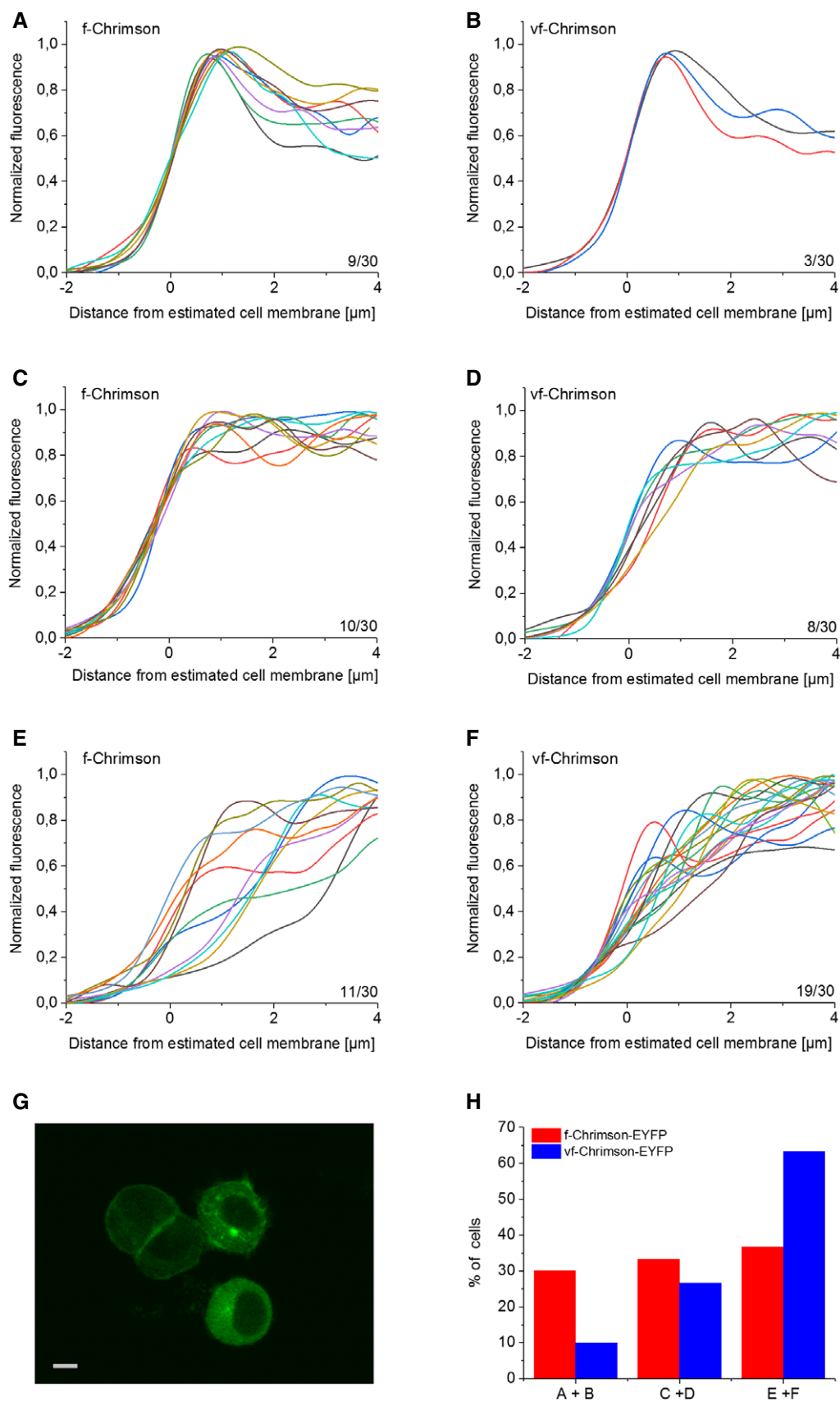


Figure EV1.

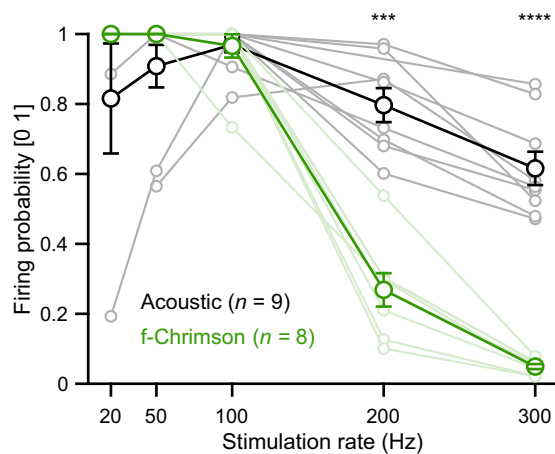


Figure EV2. Firing probability (i.e., probability of observing an action potential evoked by the click/light pulse) decreases with increasing stimulation rates of acoustic and optogenetic stimulation (f-Chrimson).

Firing probability as a function of the stimulation rate (mean $\pm$ SEM) measured acoustically from naïve putative SGNs (black, 100 dB SPL (pe), 300 μ s acoustic click, $n = 9$ units, $N = 3$ mice) or optically from f-Chrimson expressing SGNs (green, 18.3 mW, 1-ms pulse length; $n = 8$, $N = 2$ mice). Stimulation was composed of a 400 ms click/light pulse trains followed by 100-ms recovery in silence/dark. Wilcoxon rank-sum test (***; P -value $\leq 10^{-3}$, ****; P -value $\leq 10^{-4}$).