



**Supplementary Figure 1.** Light stimulation using single-photon and two-photon excitation. **(a)** Hippocampal neurons expressing ChR2-tdimer2. **(b)** Schematic drawing of the shutter system used to combine light stimulation with two-photon imaging. **(c)** Top: Voltage trace showing subthreshold depolarization and action potential generation in a ChR2 expressing neuron evoked by 470 nm light pulses (5 ms) at different intensities (stimulus trace below). Bottom: Responses to repetitive stimulation by 5 ms light pulses at 10, 20, and 50 Hz. **(d)** In response to a burst of 10 light pulses, action potentials were reliably induced up to 10 Hz. At higher frequencies, cells became permanently depolarized and failed to spike reliably. **(e)** Voltage trace showing membrane depolarization of a ChR2 expressing cell in response to laser scanning across the soma at 810 nm (2 frame scans, 500 ms each) at 30, 80, and 140 mW average laser power. Only at high laser intensities, small depolarizations were detectable. **(f)** Membrane depolarization depends on laser power (810 nm). Typical intensities used for calcium imaging were < 30 mW.