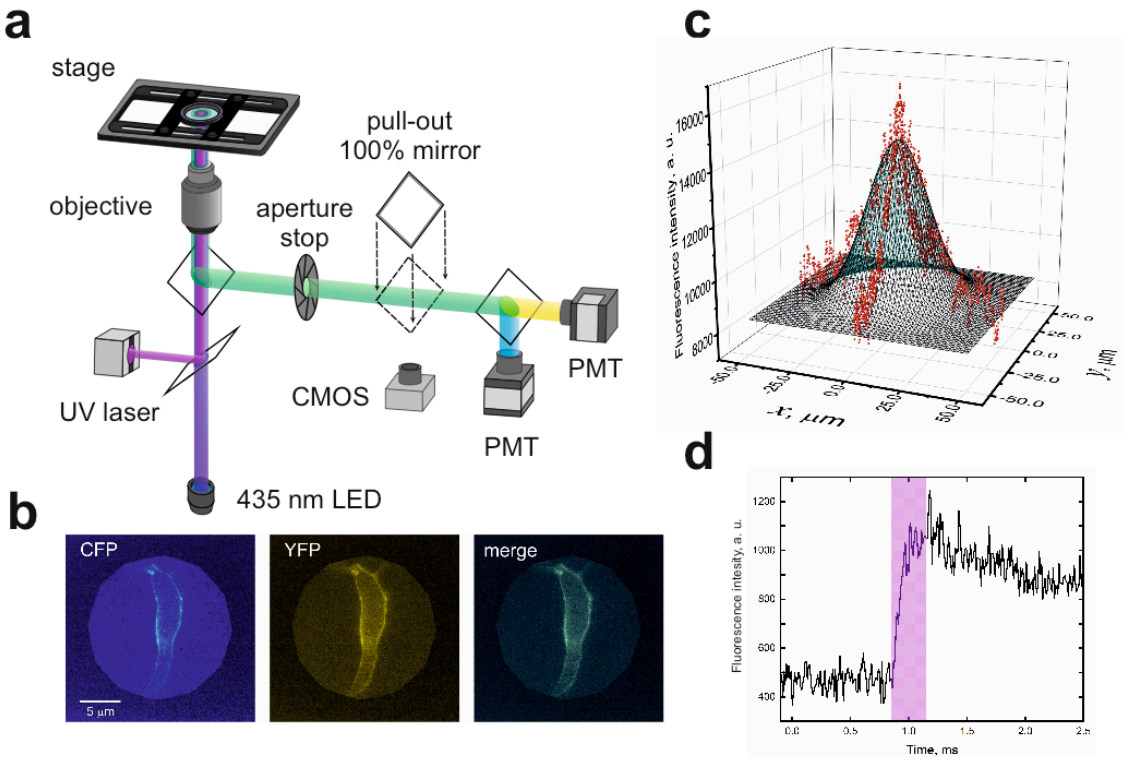
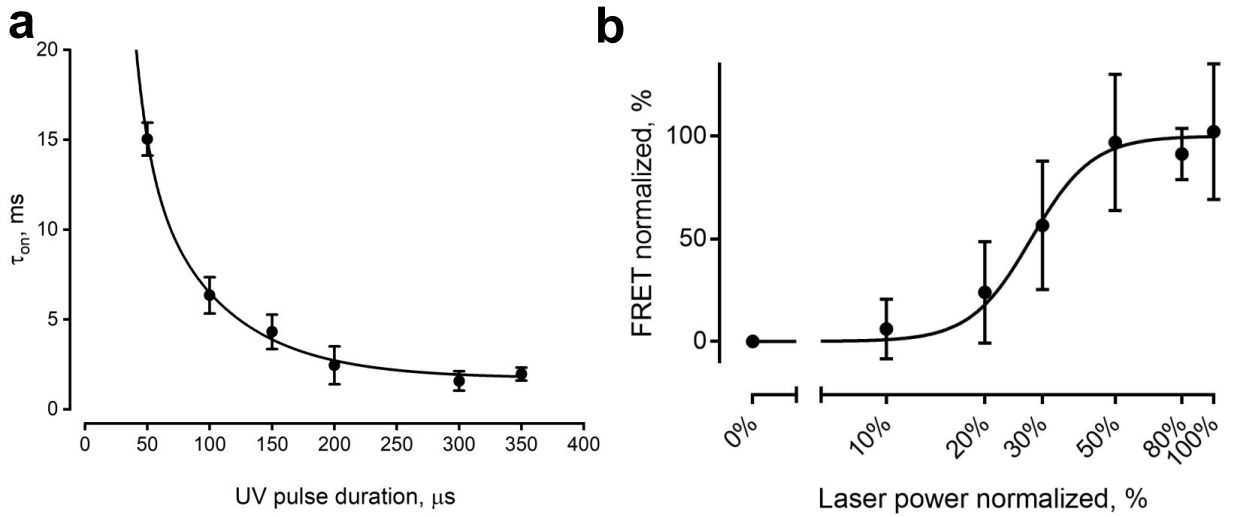
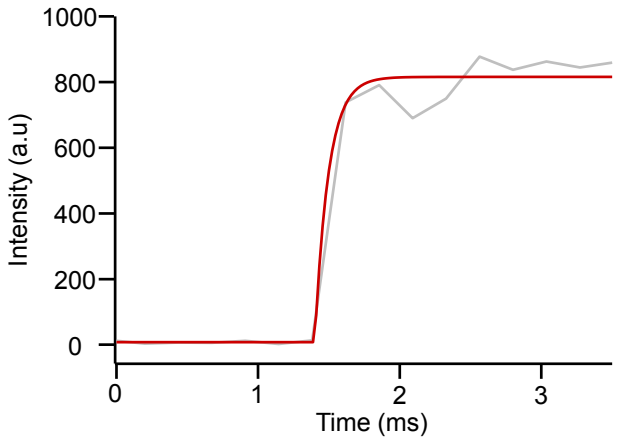



**Supplementary Figure 1. Flash photolysis in single-cell experiments. (A)** Schematic diagram of the custom built microscope setup for UV-uncaging studies. A 435 nm light-emitting diode (LED) excites the FRET donor CFP. Fluorescent light coming from the donor CFP and the acceptor YFP was either imaged with a CMOS camera, or split at 509 nm and collected by photomultipliers (PMT). In addition, the UV laser delivers a beam onto the specimen to uncage the ligand. A 405 nm edge filter blocks UV light in the emission path. (**B**) Images of the mGluR1 *E*-sensor (see Figure 1A) expressed in a HEK-TsA cell. (**C**) Uncaging of fluorescein. *x* and *y* profiles through the fluorescence distribution *F* of uncaged CMNB-fluorescein were fitted with Gaussian functions spreading to half-maximal intensities $FWHM_x$ = 32.4±0.3 µm and $FWHM_y$ = 37.7±0.4 µm. (**D**) Time course of fluorescence intensity of uncaged fluorescein upon laser illumination. Shown in pink is the 300 µs long UV-laser flash.




**Supplementary Figure 2. Dependence of mGluR1 *E*-sensor activation on UV-pulse duration and laser power.** (**a**) Time constant of the *E*-sensor determined as in Fig. 1 as a function of UV flash duration. The data show that the rate constant approached a minimum when the laser power was set to 100% indicating the release of saturating concentrations of glutamate from MNI-glutamate. (**b**) Amplitude of the E-sensor FRET response as a function of UV-laser power with UV pulse duration set to 300 μs. Radiant exposure measured after the objective was 458.6 J/cm$^2$ at 100% UV-laser power. Data points are mean±SEM from at least 4 different cells.




**Supplementary Figure 3**. **Time course of solution change in the piezo-controlled concentration jump system.** Solution exchange was measured optically close to an empty outside-out patch with the fluorescent dye DY-647 in solution. Data points indicate an individual time course of such a solution exchange. The time course was fitted by an exponential function (red curve) yielding for this particular time course a time constant of 137 μs. The mean time constant was 220±60 μs.