Supplementary Materials

Molecular Biology of the Cell

Bodzęta *et al*.

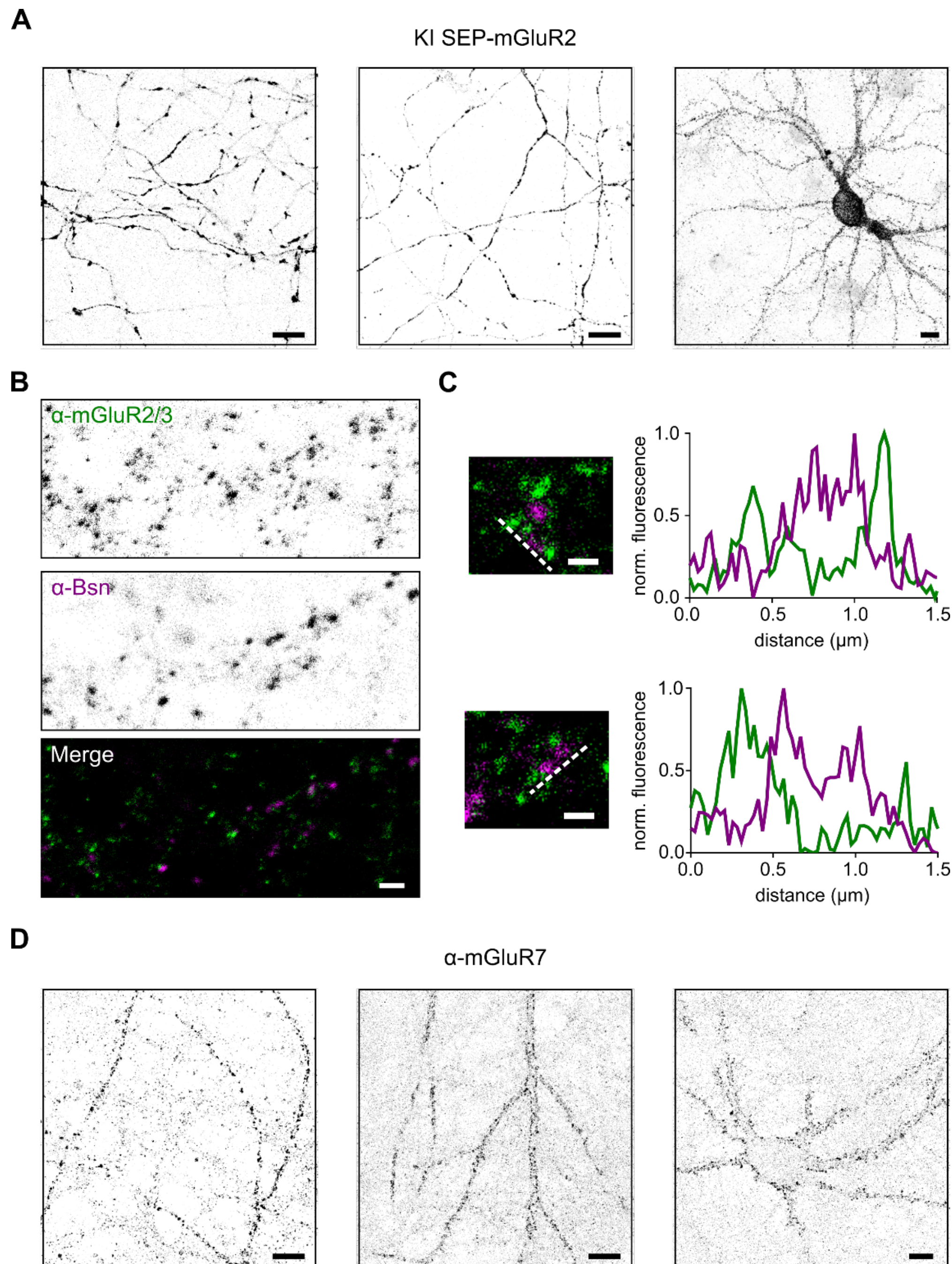


Figure S1 (Related to Figure 1). Distribution of presynaptic mGluRs.
(A) Example confocal images of SEP-mGluR2 CRISPR/Cas9 knock-in neurons. SEP signal was enhanced with anti-GFP (STAR580) staining. Scale bar 10 µm
(B) gSTED image of neuron co-stained with anti-mGluR2/3 (STAR580) and anti-Bassoon (STAR635P) (Bsn). Scale bar, 2 µm.
(C) Example images and intensity profiles of individual mGluR2/3 positive synapses from (B). Scale bar, 500 nm.
(D) Example confocal images of neurons stained with anti-mGluR7 (STAR580). Scale bar, 10 µm.

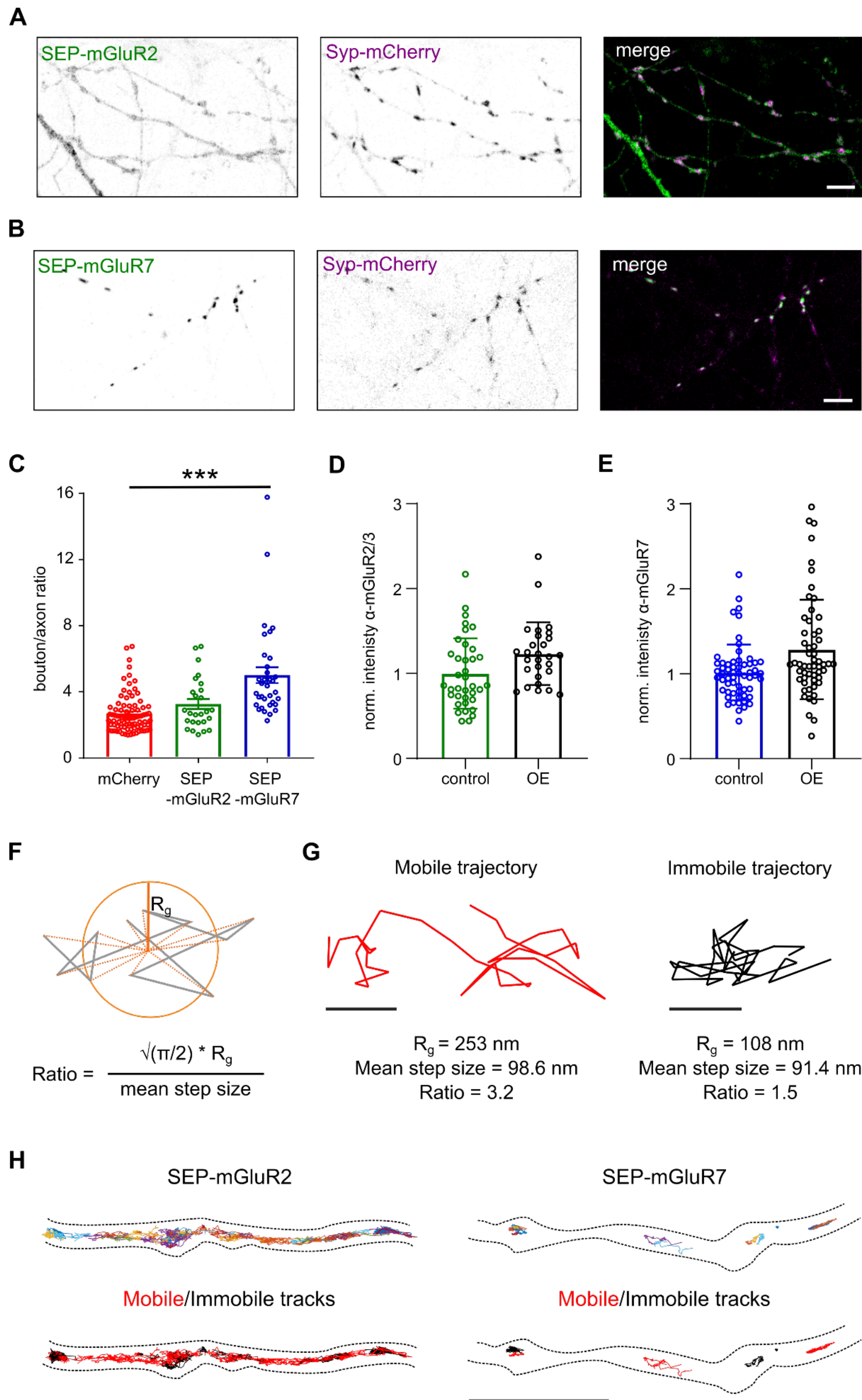
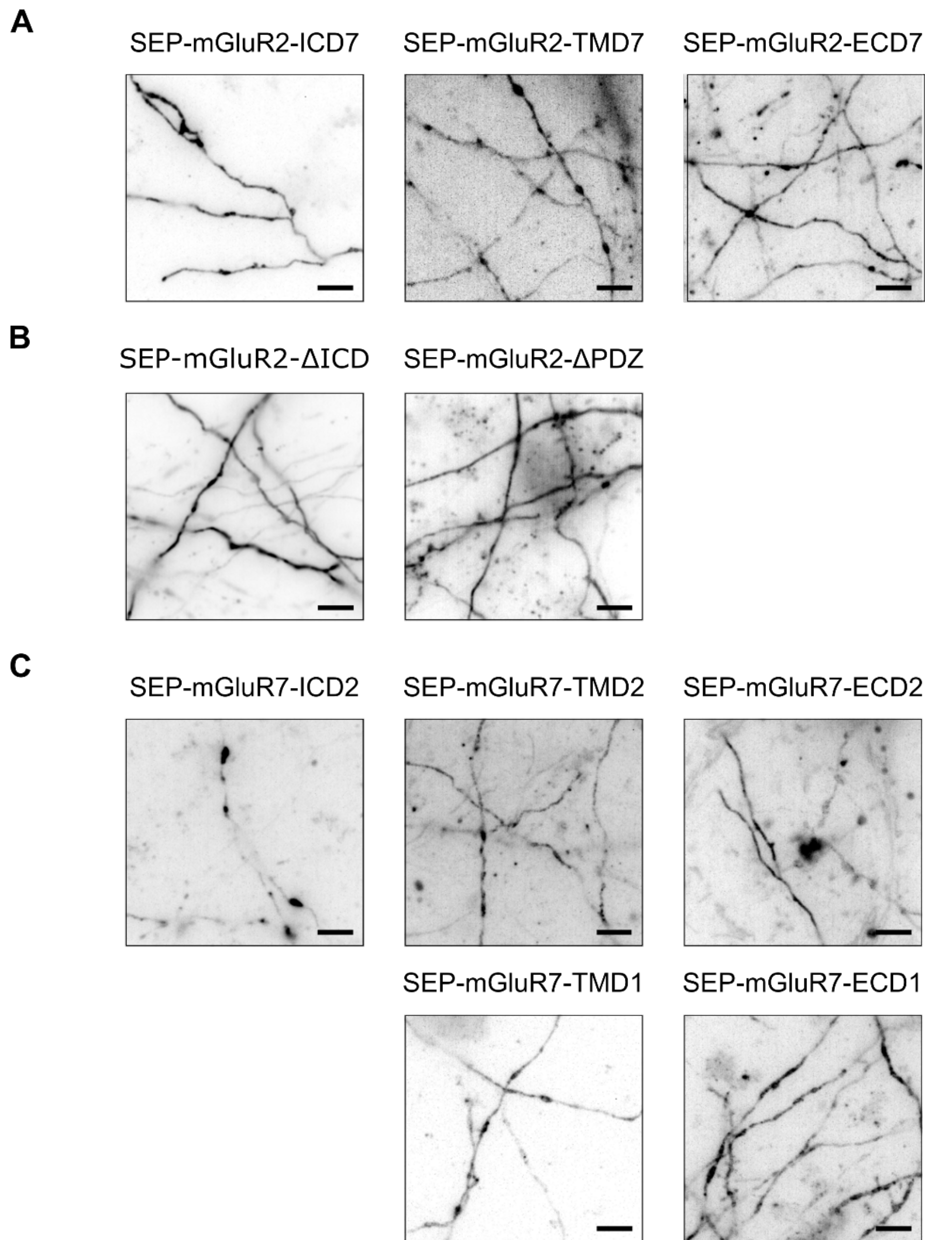


Figure S2 (Related to Figure 2). Targeting of SEP-tagged mGluRs and quantification of mobile and immobile fraction in single-molecule tracking experiments.

(A – B) An example confocal image of neurons expressing marker of presynaptic boutons Synaptophysin1-mCherry (Syp-mCherry) and SEP-mGluR2 (A) or SEP-mGluR7 (B). Scale bar, 5 μm.
(C) Quantification of ratios of fluorescence intensity in bouton over axon (n = 84 boutons for mCherry, 26 boutons for SEP-mGluR2, 34 boutons for SEP-mGluR7 from 2 independent experiments). The apparent increase in the bouton/axon ratio of cytosolic mCherry likely results from larger bouton volume compared to the axon. One-way ANOVA followed by Dunnett's multiple comparisons test, *** $P<0.001$.
(D - E) Quantification of overexpression (OE) levels of SEP-tagged presynaptic mGluRs compare to endogenous levels of mGluR2 (D) (n = 37 boutons for control, 28 boutons for SEP-mGluR2 overexpression, from 2 independent experiments) and mGluR7 (E) (n = 59 boutons for control, 56 boutons for SEP-mGluR7 overexpression, from 2 independent experiments)
(F) Geometry criterion of mobile/immobile classification of trajectories in single-molecule experiments is based on the ratio between the radius of gyration (Rg) and the mean step size. For an immobile trajectory this ratio is expected to approach 1, while for diffusion particles this ratio is >1. We experimentally validated that the ratio of 2.11 is a valid criterion to differentiate between immobile and mobile tracks. Grey – theoretical trajectory, orange – radius of gyration.
(G) Examples of mobile and immobile trajectories of mGluR2. Scale bar, 200 nm.
(H) Trajectory maps of SEP-mGluR2 and SEP-mGluR7 from Figure 2E colored coded for mobile and immobile tracks. Red – mobile trajectories, black – immobile trajectories. Scale bar, 5 μm.


A
SEP-mGluR2-ICD7
SEP-mGluR2-TMD7
SEP-mGluR2-ECD7
B
SEP-mGluR2-ΔICD
SEP-mGluR2-ΔPDZ
C
SEP-mGluR7-ICD2
SEP-mGluR7-TMD2
SEP-mGluR7-ECD2
SEP-mGluR7-TMD1
SEP-mGluR7-ECD1

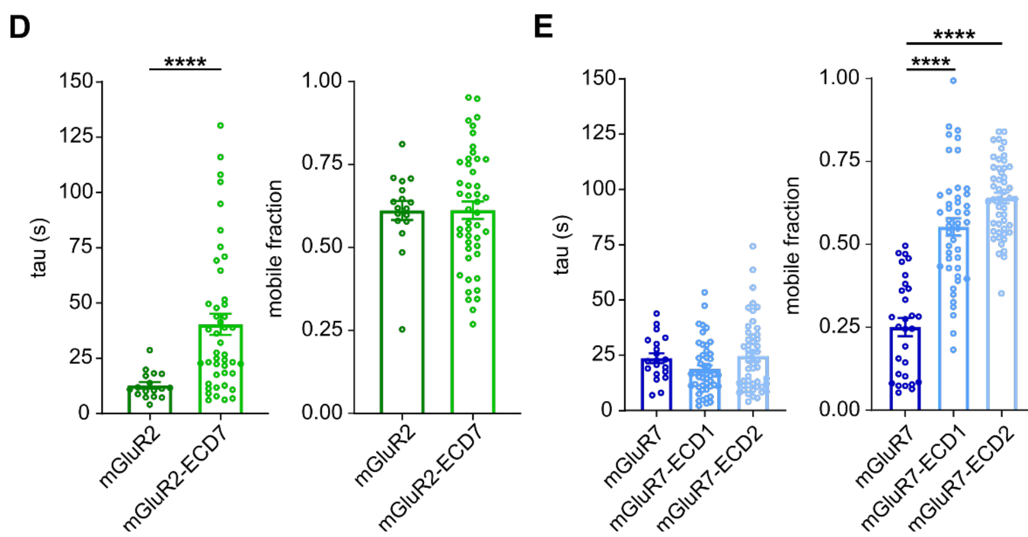

D
E
tau (s)
mobile fraction
****
mGluR2
mGluR2-ECD7
mGluR7
mGluR7-ECD1
mGluR7-ECD2

Figure S3 (Related to Figure 3 and 4). Expression of mGluRs variants and FRAP experiments of extracellular chimeric receptors.
(A) Example images of neurons expressing SEP-tagged chimeric mGluR2 variants. Scale bar, 5 μm.
(B) Example images of neurons expressing SEP-tagged C-terminal deletion mGluR2 variants Scale bar, 5 μm.
(C) Example images of neurons expressing SEP-tagged chimeric mGluR7 variants. Scale bar, 5 μm.
(D and E) Quantification of half time of fluorescence recovery and mobile fraction from FRAP experiments of SEP-tagged extracellular chimeric variants of mGluR2 (D) and mGluR7 (E) (n = 10 - 45 boutons from 2 - 3 independent experiments). Note that the data set of half time and mobile fraction for mGluR2 and mGluR7 are the same as in main Figure 2C and D. One-way ANOVA followed by Tukey's multiple comparisons test; **** $P<0.0001$. Error bars represent SEM.

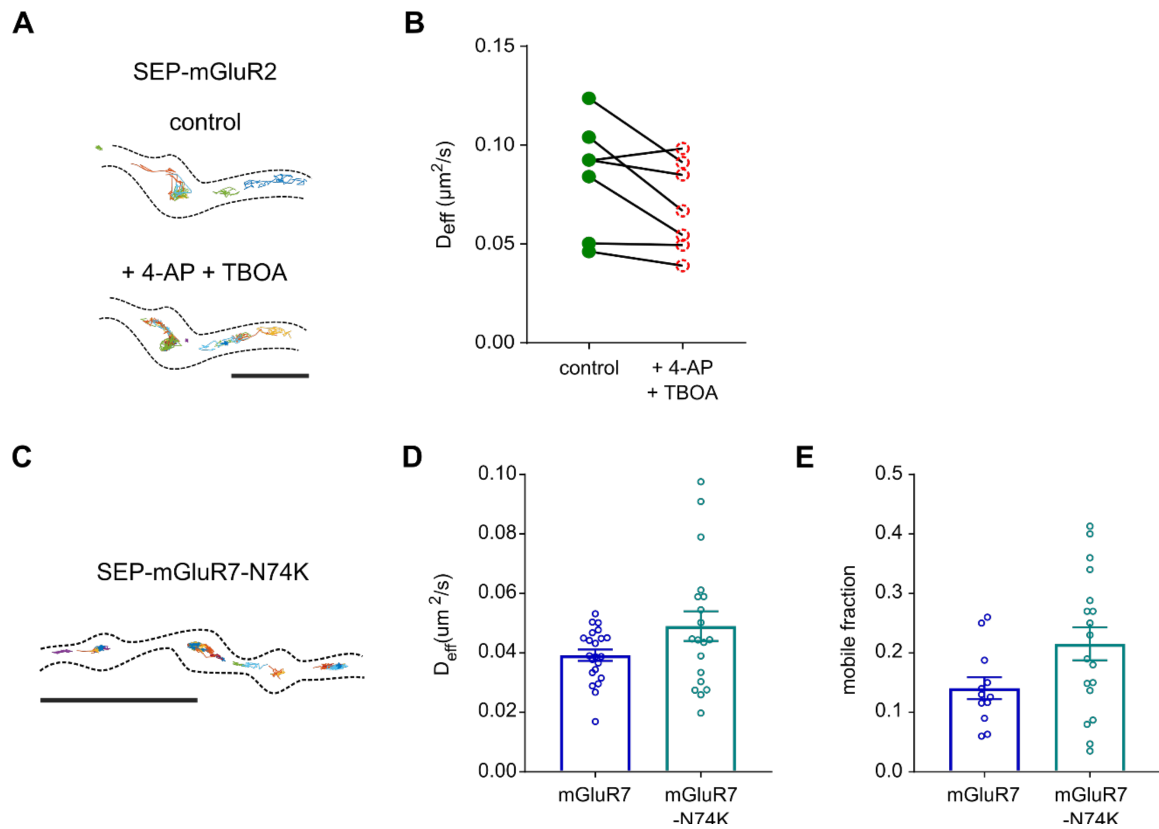


Figure S4 (Related to Figure 5). Mobility of presynaptic mGluR2 does not depend of neuronal activity and high-affinity mutant of mGluR7 displays similar mobility as wild-type receptor.
(A) Example tracks of SEP-mGluR2 before and after incubation with 200 µM 4-AP and 10 µM TBOA. Scale bar, 2 µm.
(B) Quantification of diffusion coefficient (D_{eff}) of SEP-mGluR2 before and after incubation with 4-AP and TBOA (n = 7 fields of view from 2 independent experiments).
(C) Example trajectories of SEP-mGluR7-N74K.. Scale bar, 5 µm.
(D and E) Quantification of average diffusion coefficient (D_{eff}) (D) and mobile fraction (E) of SEP-mGluR7 and mutant SEP-mGluR7-N74K (n = 22 fields of views for SEP-mGluR7, 19 fields of view for SEP-mGluR7-N74K from 2 independent experiments).
Trajectories are displayed with random colors. Outlines of cells is based on TIRF image of SEP signal. Error bars represent SEM.

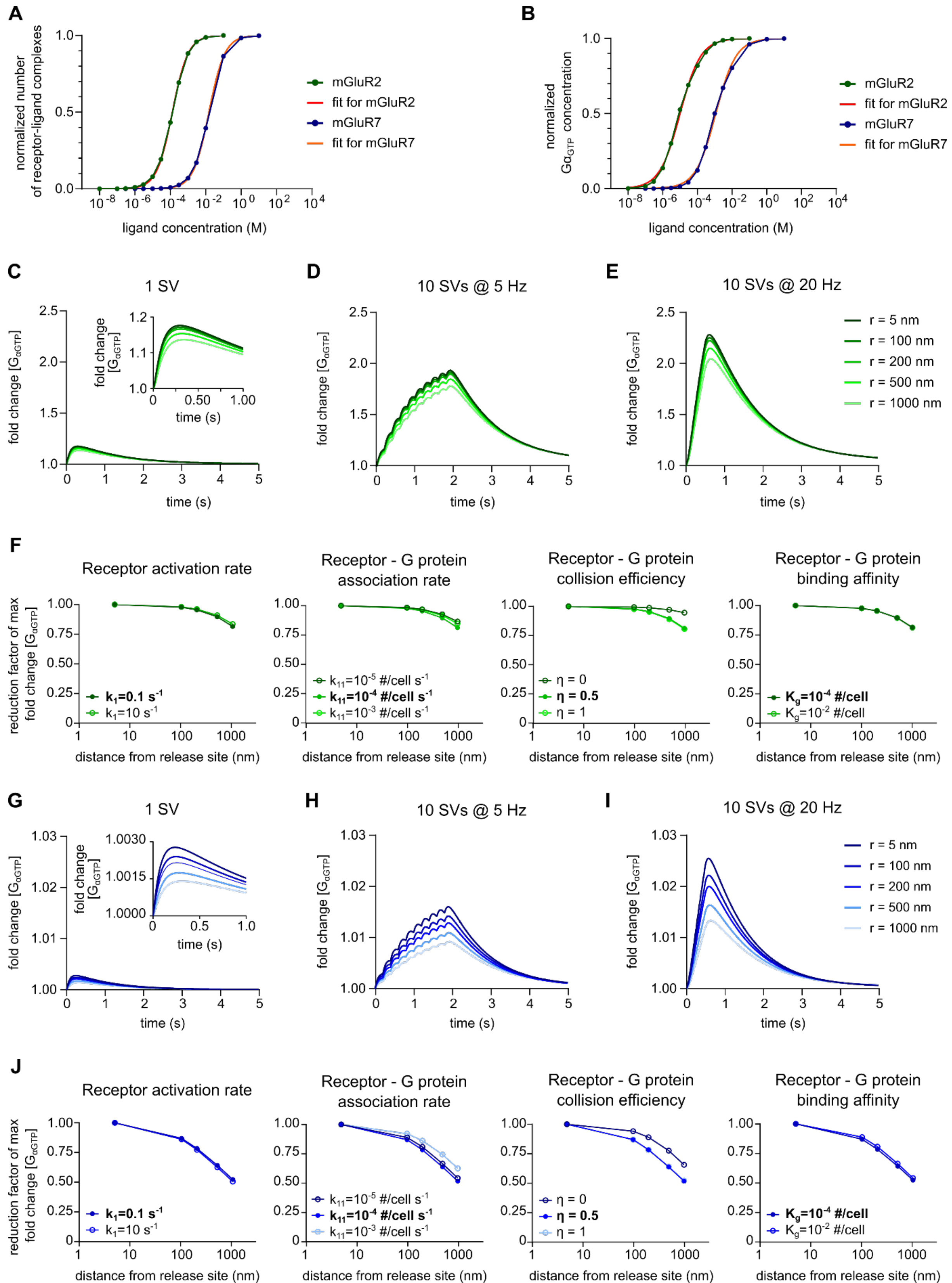


Figure S5 (Related to Figure 6). mGluR2 activation is loosely coupled to the distance to the release site, while mGluR7 activation is most prominent close to the release site.

(A – B) Calibration of the model to match the number of receptor-ligand complexes (A) and $G\alpha_{GTP}$ concentration (B) with reported EC_{50} values for mGluR2 and mGluR7.

(C - E) Time courses of mGluR2 response to glutamate after the release of 1 SV (C), 10 SVs at 5 Hz (D) and 10 SVs at 20 Hz (E) at different distances from the release site.
(F) Reduction factor of mGluR2 activation at different distances from the release site after release of 10 SVs at 20Hz for various values of different parameters in cTCAM. Bold value – values used in the main model. Note that after changing one cell-specific parameters, the model was calibrated to match the reported EC_{50} values for mGluR2.
(G - I) Time courses of mGluR7 response to glutamate after the release of 1 SV (G), 10 SVs at 5 Hz (H) and 10 SVs at 20 Hz (I) at different distances from the release site.
(F) Reduction factor of mGluR7 activation at different distances from the release site after release of 10 SVs at 20Hz for various values of different, cell-specific parameters in cTCAM. Bold value – values used in the main model. Note that after changing one cell-specific parameter, the model was calibrated to match the reported EC_{50} values for mGluR7.

Table S1 (Related to Figure 6). Parameters used in the cTCAM model of presynaptic mGluRs activity

Parameter [a]	Symbol	Value (unit)
Receptor activation rate	k_1	0.1 s^{-1}
#active/#inactive receptors	K_{act}	0.01
Receptors - G protein collision efficiency	η	0.5
Receptor - G protein association rate	k_{11}	$1 \cdot 10^{-4}$ #/cell s^{-1}
Receptor - G protein binding affinity	K_g	$1 \cdot 10^{-4}$ #/cell
Ligand - Receptor association constant	k_3	$1 \cdot 10^{7}$ M^{-1} s^{-1}
Ligand - Receptor equilibrium association constant for mGluR2 [b]	K_a	$0.7 \cdot 10^{4}$ M^{-1}
Ligand - Receptor equilibrium association constant for mGluR7 [b]	K_a	60 M^{-1}
Parameter for receptor activation via ligand binding	α	5
Parameter for G protein coupling via receptor activation	β	5
Joint coupling parameter (activation, ligand binding, G protein coupling)	δ	5
Parameter for G protein coupling via ligand binding	γ	5
G protein activation rate	k_{Gact}	5 s^{-1}
GTP hydrolysis rate	k_{GTP}	1 s^{-1}
$G_{\alpha\beta\gamma}$ association constant	k_G	$1 \cdot 10^{-4}$ #/cell s^{-1}
Total numbers of receptors [c]	R_{tot}	100
Total numbers of G proteins [c]	G_{tot}	1000

[a] values used in the simulation are taken from (Kinzer-Ursem and Linderman, 2007) unless indicated otherwise

[b] K_a for mGluR2 and mGluR7 was estimated by calibration the cTCAM model to match the output $G_{\alpha GTP}$ concentration with the published EC_{50} values (Schoepp *et al.*, 1999)
[c] total numbers of presynaptic mGluRs and G-proteins inside presynaptic boutons were estimated based on quantitative mass-spectrometry data published in (Wilhelm *et al.*, 2014)

Table S2 (Related to Figure 6). Parameters tested to validate of cTCAM model of presynaptic mGluRs activity

Tested parameter [a] (symbol)	Value (unit)	K_a value after model calibration for mGluR2	K_a value after model calibration for mGluR7
Receptor activation rate (k_1)	**$0.1\ s^{-1}$ [b]**	**$0.7 \cdot 10^4\ M^{-1}$ [b]**	**$60\ M^{-1}$ [b]**
	$10\ s^{-1}$	$0.6 \cdot 10^4\ M^{-1}$	$54\ M^{-1}$
Receptor - G protein association rate (k_{11})	$1 \cdot 10^{-5}$ #/cell s^{-1}	$1.3 \cdot 10^4\ M^{-1}$	$117\ M^{-1}$
	$1 \cdot 10^{-4}$ #/cell s^{-1} [b]	**$0.7 \cdot 10^4\ M^{-1}$ [b]**	**$60\ M^{-1}$ [b]**
	$1 \cdot 10^{-3}$ #/cell s^{-1}	$1.8 \cdot 10^4\ M^{-1}$	$154\ M^{-1}$
Receptors - G protein collision efficiency (η)	0	$7.8\ 10^4\ M^{-1}$	$681\ M^{-1}$
	0.5 [b]	**$0.7 \cdot 10^4\ M^{-1}$ [b]**	**$60\ M^{-1}$ [b]**
	1	$0.65 \cdot 10^4\ M^{-1}$	$58\ M^{-1}$
Receptor - G protein binding affinity (K_g)	**$1 \cdot 10^{-4}$ #/cel [b]**	**$0.7 \cdot 10^4\ M^{-1}$ [b]**	**$60\ M^{-1}$ [b]**
	$1 \cdot 10^{-2}$ #/cell	$1.2 \cdot 10^4\ M^{-1}$	$113\ M^{-1}$

[a] values used in the simulation are taken from (Kinzer-Ursem and Linderman, 2007) unless indicated otherwise

[b] bold values – values used in the model in main Figure 6 C-E