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## Supplemental Information

# Defining the Homo- and Heterodimerization Propensities of Metabotropic Glutamate Receptors

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# SUPPLEMENTAL INFORMATION

# Defining the homo- and hetero-dimerization propensities of metabotropic glutamate receptors

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# Figure S1

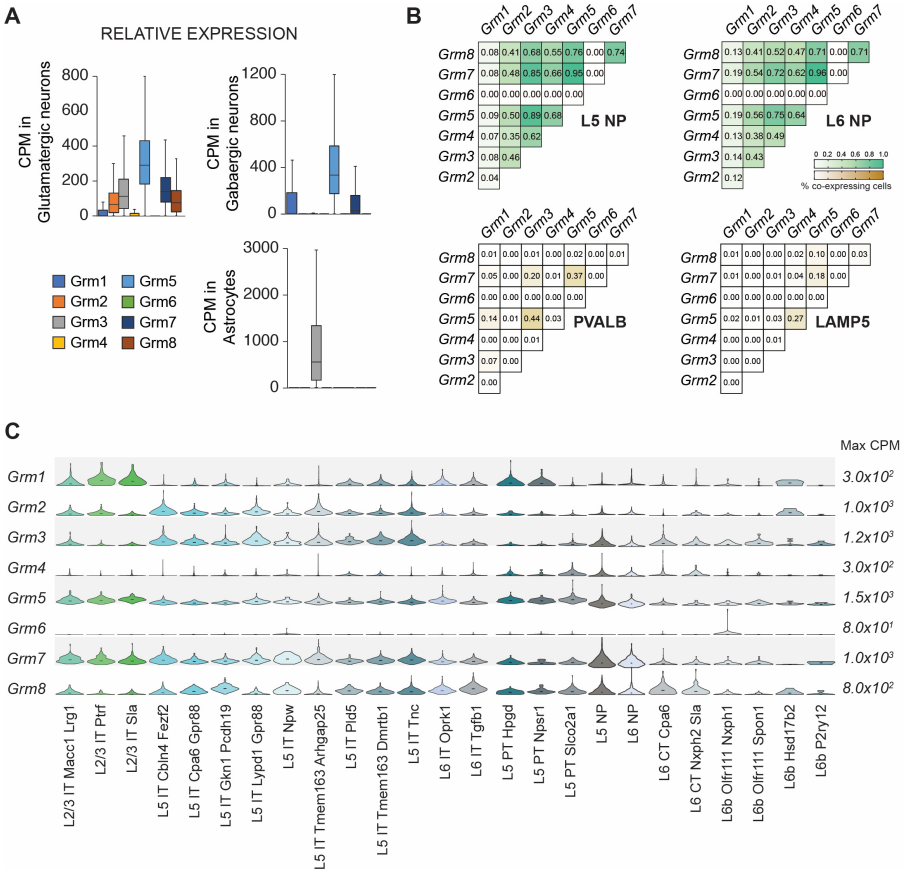


**Figure S1. Further scRNAseq Analysis of Cortical mGluR Expression, Related to Figure 1.**
(A) Box and whisker plots showing expression of the eight different *Grm* subtypes in glutamatergic neurons, GABAergic neurons and astrocytes.
(B) Co-expression analysis (cutoff minimum 5 CPM) shown as heatmaps, in which color range represent proportion of cells within that subclass co-expressing each individual *Grm* pair (see also Fig. 1C).
(C) Violin plots show expression for each of the eight members of the *Grm* across glutamatergic subtypes, dot represents median value in each subclass.
L: layer; CT: corticothalamic; IT: intratelencephalic; PT: pyramidal tract (thalamus, tectum and pons); CPM: counts per million.

**Figure S2**

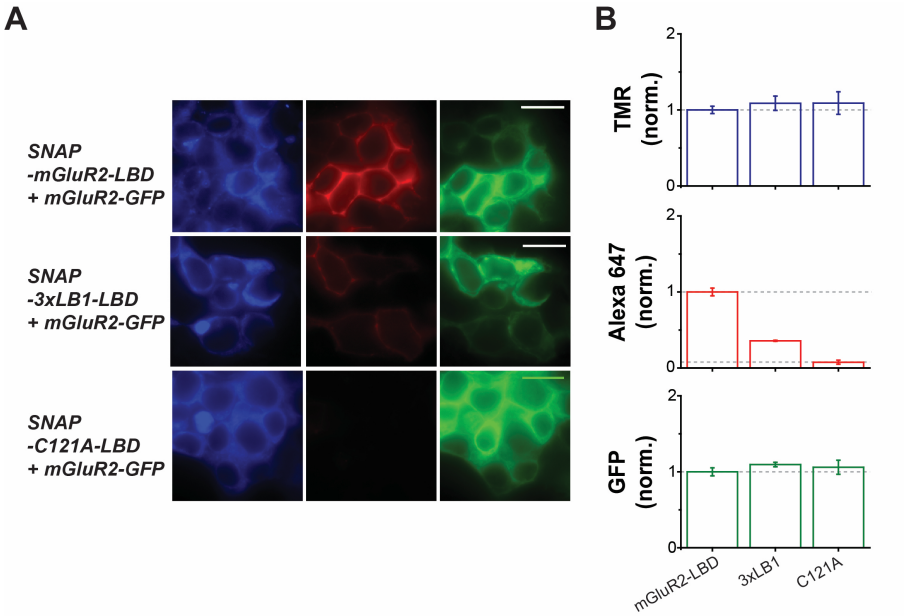


**Figure S2. Validation of the LBD Complementation Assay with mGluR2 Mutations, Related to Figure 2.**

(A-B) Introducing the 3xLB1 mutant (L103A, L154A, and F158A) in the intersubunit hydrophobic interface of the LBD decreases dimerization of SNAP-mGluR2-LBD with mGluR2-GFP as shown in weak Alexa-647 fluorescence image (A) and summary bar graph (B). Introduction of the C121A mutation, which prevents formation an intersubunit covalent disulfide bond, shows background levels of Alexa-647 fluorescence indicating that no interaction exists between SNAP-mGluR2-C121A-LBD and mGluR2-GFP. Fluorescence levels for all three channels are normalized to the homodimer condition expressing SNAP-mGluR2-LBD and mGluR2-GFP. Scale bar is 10 µm.

# Table S1

| Condition | Alexa-647 Fluorescence | P-value (unpaired 1-tailed T-test vs. background) | P-value (unpaired 2-tailed T-test vs. homodimer) |
|---|---|---|---|
| **SNAP-mGluR2-LBD** | 0.09 ± 0.01 (n=6) | - | - |
| **+ mGluR1-GFP** | 0.10 ± 0.01 (n=6) | 0.40 (vs. SNAP-mGluR2-LBD alone) | $1.5 \times 10^{-8}$ (vs. mGluR2/2) |
| **+ mGluR2-GFP** | 1.0 ± 0.02 (n=20) | $2.9 \times 10^{-7}$ (vs. SNAP-mGluR2-LBD alone) | - |
| **+ mGluR3-GFP** | 2.1 ± 0.07 (n=10) | $2.7 \times 10^{-12}$ (vs. SNAP-mGluR2-LBD alone) | $5.1 \times 10^{-8}$ (vs. mGluR2/2) |
| **+ mGluR4-GFP** | 1.1 ± 0.03 (n=4) | $3.1 \times 10^{-8}$ (vs. SNAP-mGluR2-LBD alone) | 0.063 (vs. mGluR2/2) |
| **+ mGluR5-GFP** | 0.14 ± 0.04 (n=3) | 0.21 (vs. SNAP-mGluR2-LBD alone) | 0.0028 (vs. mGluR2/2) |
| **+ mGluR7-GFP** | 0.19 ± 0.01 (n=3) | 0.019 (vs. SNAP-mGluR2-LBD alone) | 0.00042 (vs. mGluR2/2) |
| **SNAP-mGluR1-LBD + mGluR2-GFP** | 0.04 ± 0.01 (n=5) | - | 0.000000024 (vs. mGluR2/2) |
| **SNAP-mGluR3-LBD + mGluR2-GFP** | 2.3 ± 0.03 (n=3) | 0.00084 (vs. mGluR1/2) | 0.00088 (vs. mGluR2/2) |
| **SNAP-mGluR4-LBD + mGluR2-GFP** | 0.92 ± 0.02 (n=3) | 0.00011 (vs. mGluR1/2) | 0.084 (vs. mGluR2/2) |
| **SNAP-mGluR5-LBD + mGluR2-GFP** | 0.14 ± 0.01 (n=3) | 0.001 (vs. mGluR1/2) | 0.00011 (vs. mGluR2/2) |
| **SNAP-mGluR7-LBD + mGluR2-GFP** | 0.23 ± 0.06 (n=3) | 0.067 (vs. mGluR1/2) | 0.010097 (vs. mGluR2/2) |
| **SNAP-mGluR2-LBD + CLIP-mGluR1** | 0.19 ± 0.01 (n=3) | 0.007 (vs. SNAP-mGluR2-LBD alone) | $7.6 \times 10^{-5}$ (vs. mGluR2/2) |
| **SNAP-mGluR2-LBD + CLIP-mGluR2** | 1.0 ± 0.05 (n=3) | $2.9 \times 10^{-7}$ (vs. SNAP-mGluR2-LBD alone) | - |
| **SNAP-mGluR2-LBD + CLIP-mGluR3** | 2.08 ± 0.03 (n=3) | $2.0 \times 10^{-7}$ (vs. SNAP-mGluR2-LBD alone) | 0.0011 (vs. mGluR2/2) |

**Table S1. Fluorescence Intensities and P values Related to Figure 3.**

# Figure S3

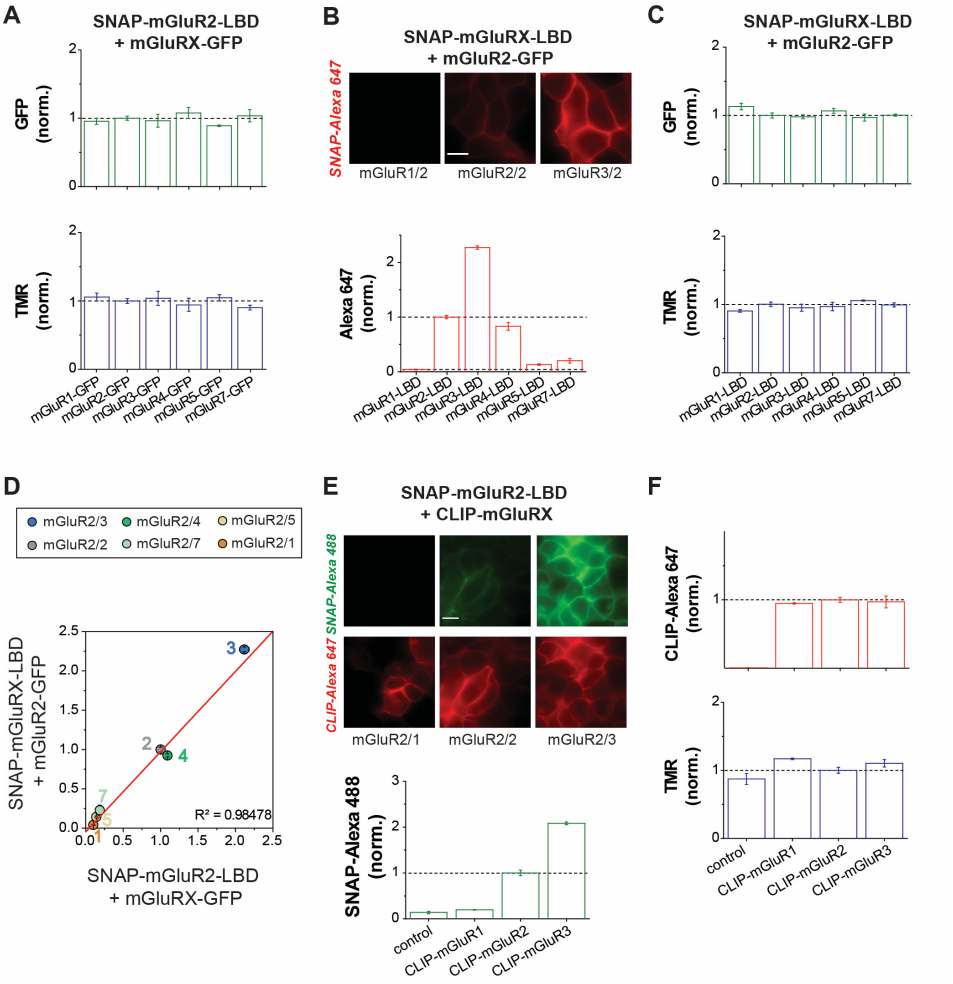


**Figure S3. Further Analysis of mGluR2 Homo- and Hetero-dimerization using LBD Complementation, Related to Figure 3.**

(A) Fluorescence intensity quantification plots of GFP (top) and TMR (bottom) for SNAP-mGluR2-LBD homo- and hetero-dimerization screening with other mGluR subtypes (see Fig. 3C).

(B) Top, representative cell images showing SNAP-mGluR1-LBD, SNAP-mGluR2-LBD, or SNAP-mGluR3-LBD labeled with Alexa-647 when co-expressed with mGluR2-GFP. Bottom, Alexa-647 fluorescence intensity plot for the LBD complementation assay using mGluR2-GFP and SNAP-mGluR-LBD constructs across other subtypes.

(C) Fluorescence intensity quantification plots for GFP (top) and TMR (bottom) for SNAP-mGluRx-LBD homo- and hetero-dimerization screening with mGluR2- other mGluR subtypes.

(D) Scatter plot showing dimerization propensity values for SNAP-mGluR2-LBD with GFP-tagged full length constructs (SNAP-mGluR2-LBD + mGluRX-GFP; x-axis) and the revers experiment (SNAP-mGluRX-LBD + mGluR2-GFP; y-axis).

(E-F) LBD complementation assay using CLIP-tagged full-length mGluR constructs instead of GFP-tagged constructs. Representative cell images show relative dimerization of SNAP-mGluR2-LBD with CLIP-mGluR1, 2, and 3 (E, top). Quantification of fluorescence intensity for BG-Alexa-488 (E, bottom), BC-Alexa-647 (F, top) and SNAP-TMD (F, bottom) is plotted and normalized to the SNAP-mGluR2-LBD with CLIP-mGluR2 condition.

Data are represented as mean ± SEM. All scale bars are 10 µm.

**Figure S4**

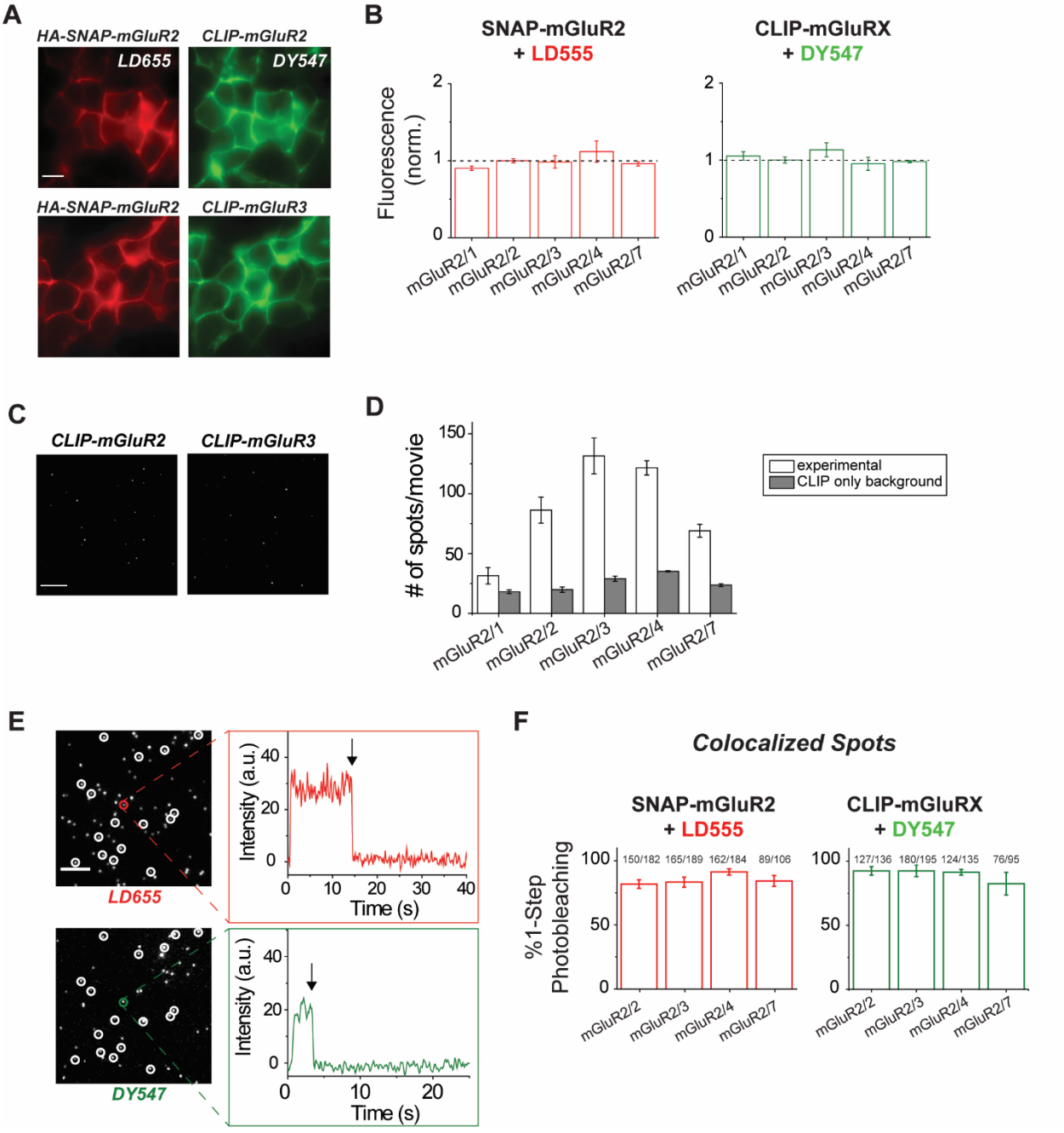


**Figure S4. Further Analysis of mGluR2 Homo- and Hetero-dimerization using SiMPull, Related to Figure 4.**

(A-B) Representative images (A) and quantification (B) of cells with HA-SNAP-mGluR2 co-expressed with CLIP-tagged mGluRs. Cell are labeled with SNAP-LD655 (left column) and CLIP-DY-547 (right column). Fluorescence intensity (B) is normalized to the homodimer condition expressing HA-SNAP-mGluR2 and CLIP-mGluR2.

(C) Representative single molecule images showing minimal non-specific binding of CLIP-mGluR2 (left) and CLIP-mGluR3 (right) when expressed alone and applied to a passivated coverslip coated in anti-HA antibodies.

(D) Quantification of the number of spots isolated per movie for each condition. The number of background spots isolated with the CLIP-construct alone at the same dilution as the associated heterodimer is shown as a gray bar.
(E) Single molecule images of HA-SNAP-mGluR2 (top) with CLIP-mGluR2 (bottom). Co-localized spots are circled and a representative fluorescence intensity is shown for the spot in the red (top) and green circle (bottom) showing 1-step photobleaching in each channel.
(F) Summary of bleaching step analysis across all co-localized spots for each condition. Numbers on each of the bar graphs represents the proportion of spots showing 1-step photobleaching out of the total number of co-localized spots analyzed.
Data are represented as mean ± SEM. All scale bars are 10 μm.

# Table S2

| Condition | Alexa-647 Fluorescence | P-value (unpaired 1-tailed T-test vs. background) | P-value (unpaired 2-tailed T-test vs. homodimer) |
|---|---|---|---|
| **SNAP-mGluR3-LBD** | 0.05 ± 0.00 (n=3) | - | 0.000025 (vs. mGluR3/3) |
| **+ mGluR1-GFP** | 0.17 ± 0.01 (n=4) | 0.0006 (vs. SNAP-mGluR3-LBD alone) | 0.0000085 (vs. mGluR3/3) |
| **+ mGluR2-GFP** | 0.97 ± 0.03 (n=3) | 0.00081 (vs. SNAP-mGluR3-LBD alone) | 0.52 (vs. mGluR3/3) |
| **+ mGluR3-GFP** | 1.00 ± 0.01 (n=12) | 0.000012 (vs. SNAP-mGluR3-LBD alone) | - |
| **+ mGluR4-GFP** | 1.06 ± 0.04 (n=3) | 0.0013 (vs. SNAP-mGluR3-LBD alone) | 0.37 (vs. mGluR3/3) |
| **+ mGluR5-GFP** | 0.13 ± 0.03 (n=7) | 0.054 (vs. SNAP-mGluR3-LBD alone) | 0.00017 (vs. mGluR3/3) |
| **+ mGluR7-GFP** | 0.32 ± 0.03 (n=6) | 0.006 (vs. SNAP-mGluR3-LBD alone) | 0.0022 (vs. mGluR3/3) |

**Table S2. Fluorescence Intensities and P values Related to Figure 5.**

# Figure S5

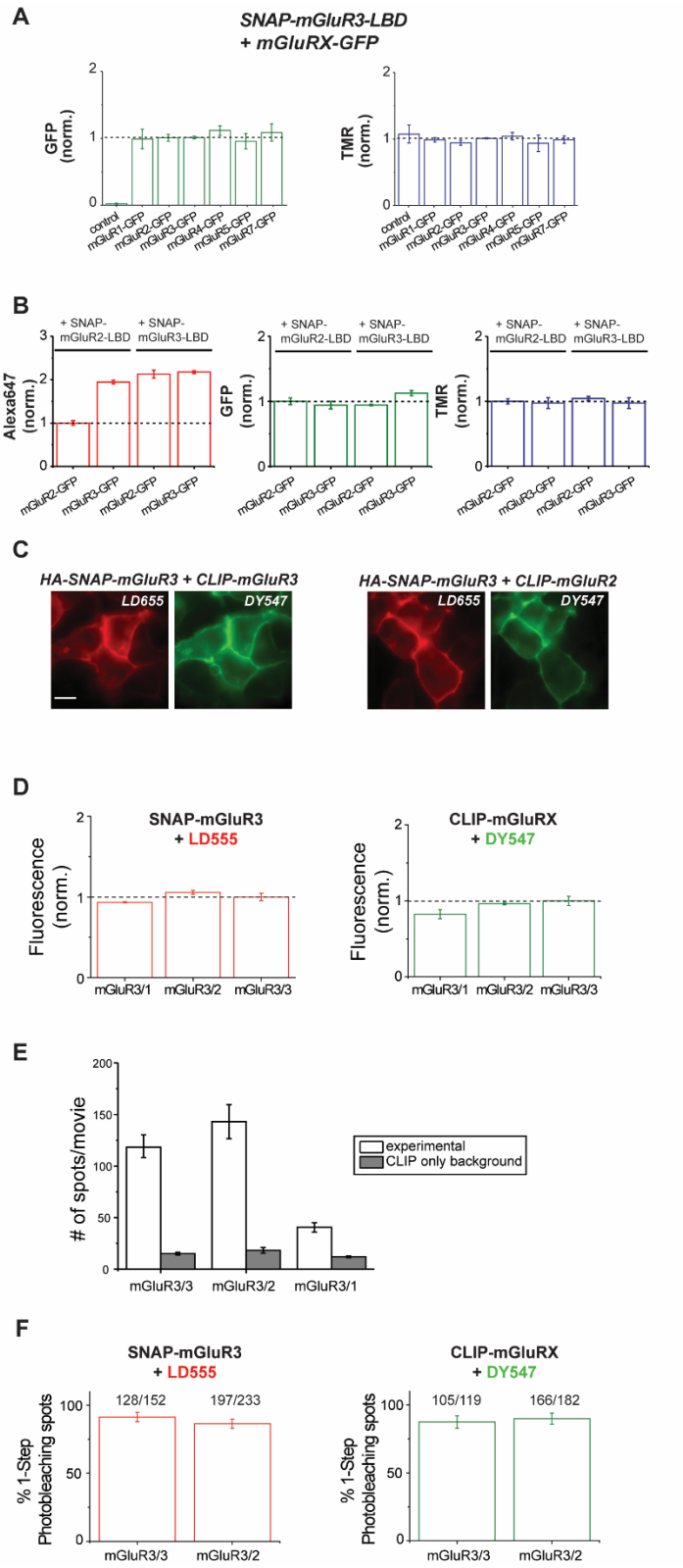


**Figure S5. Further Analysis of mGluR3 Homo- and Hetero-dimerization, Related to Figure 5.** (A) Quantification of GFP (left) and TMR (right) fluorescence intensity used for SNAP-mGluR3-LBD dimerization screening in Fig. 5B.

(B) Global comparison of LBD dimerization propensity for all combinations of mGluR2 and mGluR3. Fluorescence intensity is normalized to the level observed with the SNAP-mGluR2-LBD/mGluR2-GFP condition.
(C-D) Representative images (C) and fluorescence intensity quantification (D) from cells co-expressing HA-SNAP-mGluR3 with CLIP-tagged mGluRs. SNAP- and CLIP tags are labeled with LD655 and DY547, respectively.
(E) Quantification of the number of spots isolated per movie for each condition. The number of background spots isolated with the CLIP-construct alone at the same dilution as the associated heterodimer is shown as a gray bar.
(F) Summary of bleaching step analysis across all co-localized spots for each condition. Numbers on each of the bar graphs represents the proportion of spots showing 1-step photobleaching out of the total number of co-localized spots analyzed.

# Figure S6

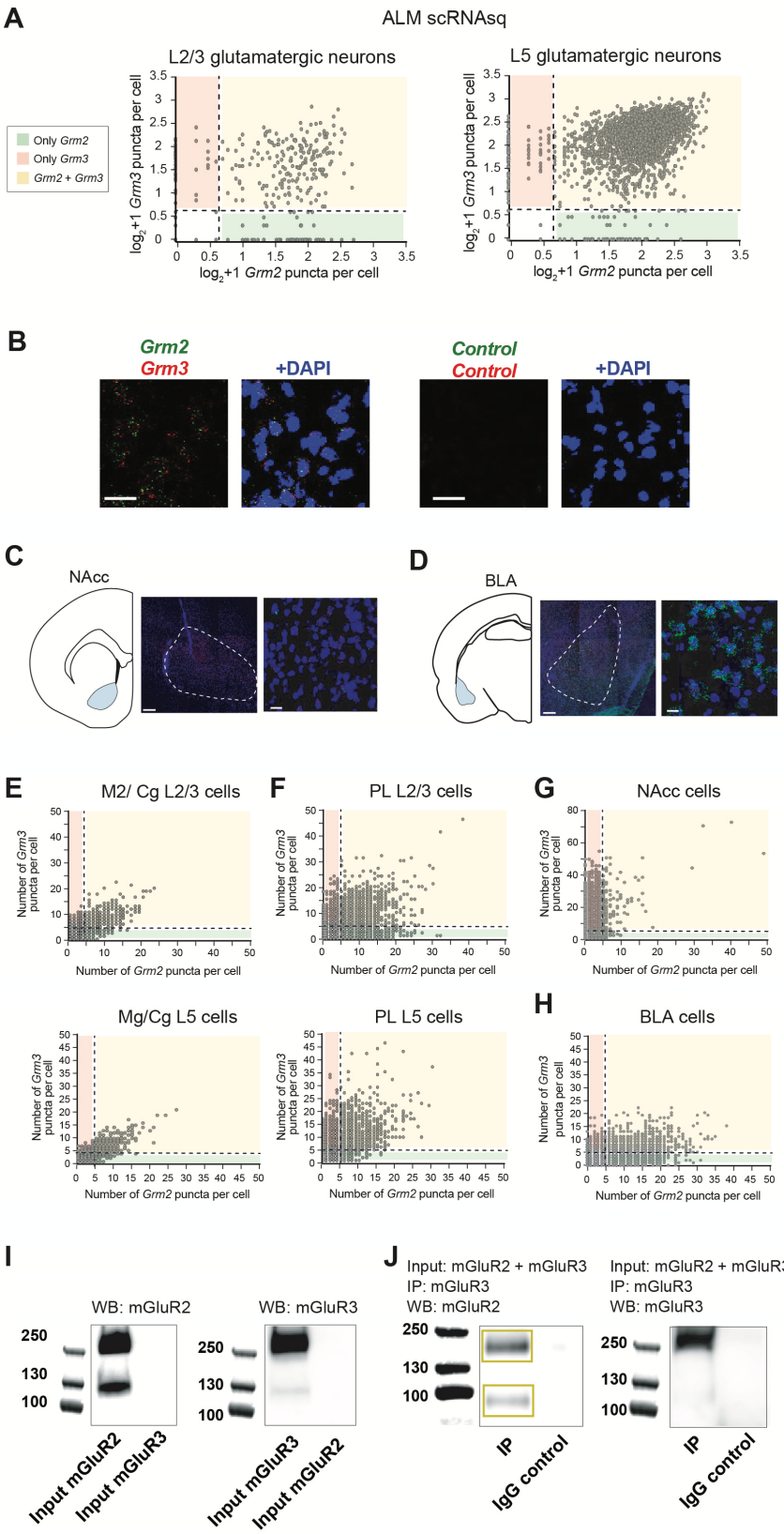
A
ALM scRNAsq
L2/3 glutamatergic neurons
L5 glutamatergic neurons
Only Grm2
Only Grm3
Grm2 + Grm3
log2+1 Grm3 puncta per cell
log2+1 Grm2 puncta per cell
B
Grm2
Grm3
+DAPI
Control
Control
+DAPI
C
NAcc
D
BLA
E
M2/ Cg L2/3 cells
F
PL L2/3 cells
G
NAcc cells
Mg/Cg L5 cells
PL L5 cells
H
BLA cells
Number of Grm3 puncta per cell
Number of Grm2 puncta per cell
I
WB: mGluR2
WB: mGluR3
Input mGluR2
Input mGluR3
J
Input: mGluR2 + mGluR3
IP: mGluR3
WB: mGluR2
WB: mGluR3
IP
IgG control

**Figure S6. Further Analysis of mGluR2/3 Co-expression and Co-assembly, Related to Figure 6.**
(A) Scatter plots for *Grm2* and *Grm3* expression ($log_2$ +1 of puncta per cell) where each dot represents a single cell from the original scRNAseq study (Tasic, et al 2018). Dotted lines denote cutoffs used for classifying cells as positive for Grm2, Grm3 or both.
(B) Representative images showing specificity of Grm2 and Grm3 probes. Control probes, right, provided by the manufacturer do not show any clear, punctate fluorescence.
(C-D) Representative confocal images of FISH experiments in the Nucleus Accumbens (NAcc) and Basolateral Amygdala (BLA).
(E-H) Scatter plots for *Grm2* and *Grm3* expression (puncta per cell) where each dot represents a single cell. Dotted lines denote cutoffs used for classifying cells as positive for Grm2, Grm3 or both.
(I) Western blot controls demonstrating the subtype-specificity of anti-mGluR2 (left) and anti-mGluR3 (right) antibodies used in co-IP studies (see Fig. 6E). Antibodies were tested on lysate from HEK 293T cells transfected with either mGluR2 or mGluR3.
(J) Co-immunoprecipitation of mGluR2 via an mGluR3-specific antibody. Controls using an anti-IgG antibody confirm the specificity of the pulldown.

## Table S3

| Condition | Alexa-647 Fluorescence | P-value (unpaired 1-tailed T-test vs. background) | P-value (unpaired 2-tailed T-test vs. homodimer) |
|---|---|---|---|
| **SNAP-mGluR1-LBD** | 0.05 ± 0.01 (n=4) | - | 0.0000063 (vs. mGluR1/1) |
| **+ mGluR1-GFP** | 1.00 ± 0.03 (n=13) | 0.0000032 (vs. SNAP-mGluR1-LBD alone) | - |
| **+ mGluR2-GFP** | 0.12 ± 0.02 (n=5) | 0.01 (vs. SNAP-mGluR1-LBD alone) | 0.0000015 (vs. mGluR1/1) |
| **+ mGluR3-GFP** | 0.42 ± 0.03 (n=4) | 0.00017 (vs. SNAP-mGluR1-LBD alone) | 0.00030 (vs. mGluR1/1) |
| **+ mGluR4-GFP** | 0.09 ± 0.00 (n=3) | 0.019 (vs. SNAP-mGluR1-LBD alone) | 0.000024 (vs. mGluR1/1) |
| **+ mGluR5-GFP** | 1.52 ± 0.02 (n=4) | 0.0000005 (vs. SNAP-mGluR1-LBD alone) | 0.00029 (vs. mGluR1/1) |
| **+ mGluR7-GFP** | 0.06 ± 0.01 (n=3) | 0.31 (vs. SNAP-mGluR1-LBD alone) | 0.00016 (vs. mGluR1/1) |
| **SNAP-mGluR5-LBD** | 0.10 ± 0.02 (n=3) | - | 0.0011 (vs. mGluR5/5) |
| **+ mGluR1-GFP** | 0.95 ± 0.06 (n=4) | 0.00018 (vs. SNAP-mGluR5-LBD alone) | 0.56 (vs. mGluR5/5) |
| **+ mGluR2-GFP** | 0.12 ± 0.06 (n=5) | 0.21 (vs. SNAP-mGluR5-LBD alone) | 0.000087 (vs. mGluR5/5) |
| **+ mGluR3-GFP** | 0.14 ± 0.03 (n=4) | 0.17 (vs. SNAP-mGluR5-LBD alone) | 0.00011 (vs. mGluR5/5) |
| **+ mGluR4-GFP** | 0.11 ± 0.02 (n=3) | 0.39 (vs. SNAP-mGluR5-LBD alone) | 0.00070 (vs. mGluR5/5) |
| **+ mGluR5-GFP** | 1.00 ± 0.04 (n=8) | 0.00055 (vs. SNAP-mGluR5-LBD alone) | - |
| **+ mGluR7-GFP** | 0.05 ± 0.01 (n=4) | 0.13 (vs. SNAP-mGluR5-LBD alone) | 0.0000051 (vs. mGluR5/5) |
| **SNAP-mGluR4-LBD** | 0.15 ± 0.01 (n=3) | - | 0.00019 (vs. mGluR4/4) |
| **+ mGluR1-GFP** | 0.18 ± 0.03 (n=3) | 0.25 (vs. SNAP-mGluR4-LBD alone) | 0.00061 (vs. mGluR4/4) |
| **+ mGluR2-GFP** | 2.16 ± 0.30 (n=3) | 0.01056 (vs. SNAP-mGluR4-LBD alone) | 0.060 (vs. mGluR4/4) |
| **+ mGluR3-GFP** | 3.34 ± 0.15 (n=3) | 0.0011 (vs. SNAP-mGluR4-LBD alone) | 0.0041 (vs. mGluR4/4) |
| **+ mGluR4-GFP** | 1.0 ± 0.05 (n=9) | 0.000095 (vs. SNAP-mGluR4-LBD alone) | - |
| **+ mGluR5-GFP** | 0.16 ± 0.03 (n=3) | 0.38 (vs. SNAP-mGluR4-LBD alone) | 0.00086 (vs. mGluR4/4) |

| | | | |
|---|---|---|---|
| **+ mGluR7-GFP** | 0.60 ± 0.07<br>(n=3) | 0.011<br>(vs. SNAP-mGluR4-LBD alone) | 0.027<br>(vs. mGluR4/4) |
| **SNAP-mGluR7-LBD** | 0.16 ± 0.02<br>(n=3) | - | 0.0006<br>(vs. mGluR7/7) |
| **+ mGluR1-GFP** | 0.14 ± 0.02<br>(n=3) | 0.22<br>(vs. SNAP-mGluR7-LBD alone) | 0.00026<br>(vs. mGluR7/7) |
| **+ mGluR2-GFP** | 0.82 ± 0.06<br>(n=3) | 0.00023<br>(vs. SNAP-mGluR7-LBD alone) | 0.059<br>(vs. mGluR7/7) |
| **+ mGluR3-GFP** | 2.35 ± 0.20<br>(n=3) | 0.00029<br>(vs. SNAP-mGluR7-LBD alone) | 0.0035 (**)<br>(vs. mGluR7/7) |
| **+ mGluR4-GFP** | 1.15 ± 0.11<br>(n=3) | 0.0062<br>(vs. SNAP-mGluR7-LBD alone) | 0.30<br>(vs. mGluR7/7) |
| **+ mGluR5-GFP** | 0.18 ± 0.03<br>(n=3) | 0.36<br>(vs. SNAP-mGluR7-LBD alone) | 0.00085<br>(vs. mGluR7/7) |
| **+ mGluR7-GFP** | 1.0 ± 0.02<br>(n=9) | 0.00030<br>(vs. SNAP-mGluR7-LBD alone) | - |
| **SNAP-mGluR1-LBD + mGluR1-GFP** | 0.52 ± 0.02<br>(n=6) | - | 0.000016<br>(vs. mGluR2/2) |
| **SNAP-mGluR2-LBD + mGluR2-GFP** | 1.00 ± 0.03<br>(n=7) | - | - |
| **SNAP-mGluR3-LBD + mGluR3-GFP** | 2.10 ± 0.03<br>(n=3) | - | 0.001<br>(vs. mGluR2/2) |
| **SNAP-mGluR4-LBD + mGluR4-GFP** | 0.37 ± 0.04<br>(n=4) | - | 0.0011<br>(vs. mGluR2/2) |
| **SNAP-mGluR5-LBD + mGluR5-GFP** | 1.02 ± 0.13<br>(n=2) | - | 0.92<br>(vs. mGluR2/2) |
| **SNAP-mGluR7-LBD + mGluR7-GFP** | 0.19 ± 0.01<br>(n=2) | - | 0.015<br>(vs. mGluR2/2) |

**Table S3. Fluorescence Intensities and P values, Related to Figure 7.**

# Figure S7

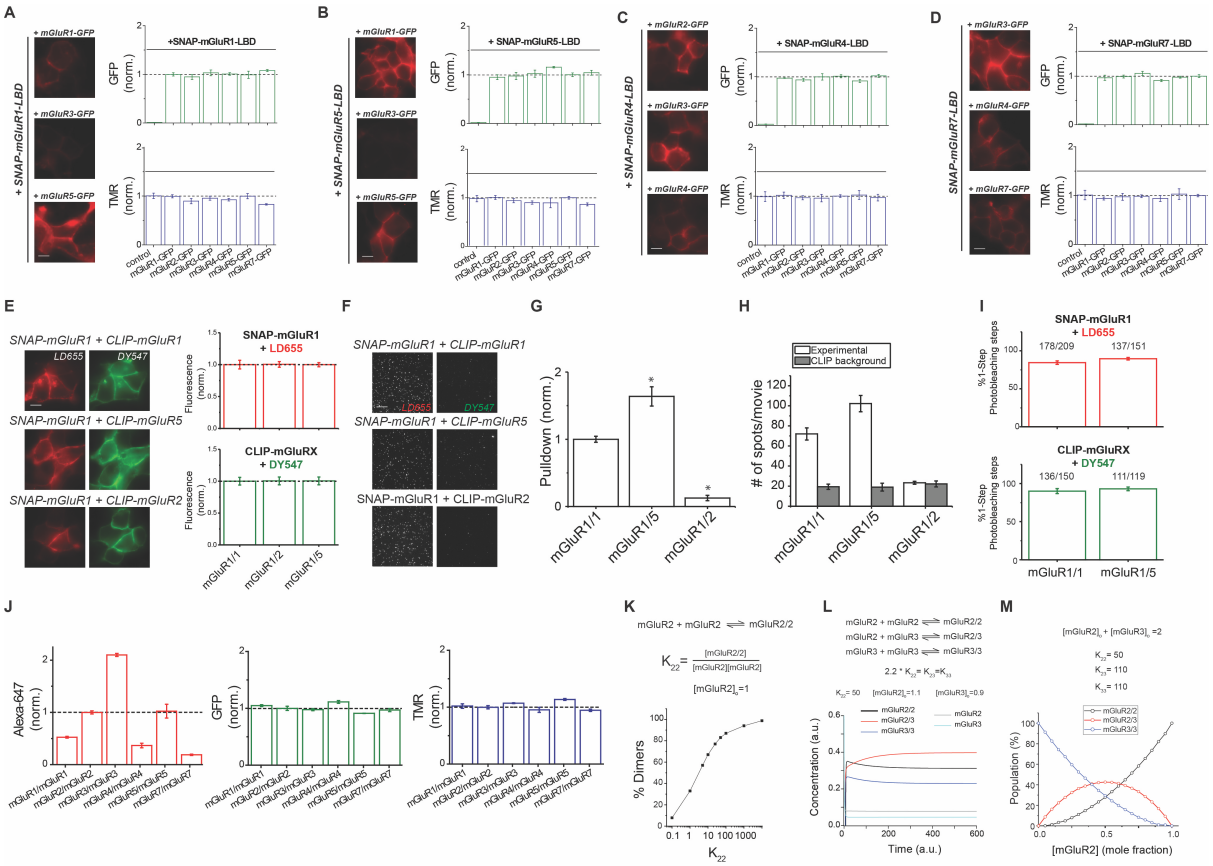


**Figure S7. Further Analysis of Homo- and Hetero-dimerization across All Three mGluR Subgroups, Related to Figure 7.**

(A-D) Representative cell images in Alexa647 channel and quantification of GFP (top) and TMR (bottom) fluorescence intensity used for SNAP-mGluR1-LBD (A), SNAP-mGluR5-LBD (B), SNAP-mGluR4-LBD (C), and SNAP-mGluR7-LBD (D) dimerization screening (see Fig. 7A-D).

(E) Representative cell images (left) and fluorescence intensity quantification (right) from cells co-expressing HA-SNAP-mGluR1 with CLIP-tagged mGluR1, 2, or 5. SNAP- and CLIP tags are labeled with LD655 and DY547, respectively.

(F) Representative single molecule pulldown images of HA-SNAP-mGluR1 with CLIP-mGluR1 (top), CLIP-mGluR5 (middle) or CLIP-mGluR2 (bottom).

(G) Quantification of pulldown via HA-SNAP-mGluR1 normalized to the homodimer condition of HA-SNAP-mGluR1 with CLIP-mGluR1. * indicates statistical significance (unpaired t test versus mGluR1/1: p=0.04 for mGluR1/5; p=0.0009 for mGluR1/2). Data are represented as mean ± SEM.

(H) Quantification of the number of spots isolated per movie for each condition. The number of background spots isolated with the CLIP-construct alone at the same dilution as the associated heterodimer is shown as a gray bar.

(J) Analysis of relative homodimerization strength in the LBD complementation assay across all subtypes tested. Bar graphs show average values in the Alexa-647 (left), GFP (middle) and TMR (right) channels. Data are represented as mean ± SEM. All scale bars are 10 µm.

(K-M) Kinetic modeling of mGluR2 homo- and hetero-dimerization. A simple model of homodimerization (K) shows the dependence of the dimer population on different values of $K_{22}$ at a fixed protein concentration. (L) shows a representative simulation of homo- and hetero-dimerization of mGluR2/2, mGluR2/3 and mGluR3/3 for the stated parameters and (M) shows the distribution of each dimer across a range of expression ratios at a fixed total protein concentration and fixed values for equilibrium constants.