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Supplementary Materials for

Receptor-Selective Diffusion Barrier Enhances Sensitivity of Astrocytic Processes to Metabotropic Glutamate Receptor Stimulation

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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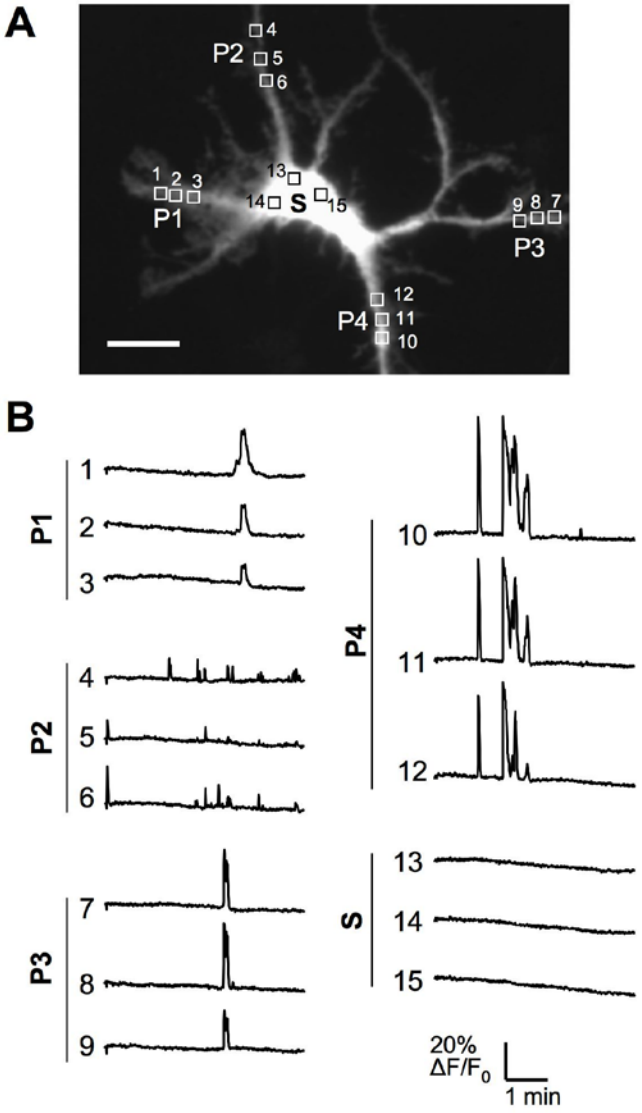

Fig. S1. Spontaneous Ca^{2+} transients in astrocytic processes.

(**A**) Image of an astrocyte transfected with GCaMP2, which distributed throughout the astrocyte and into the fine processes. Representative of N=10 astrocytes. Squares indicate regions of interest (ROI) where $[Ca^{2+}]_i$ was monitored in the four processes (P1: 1–3, P2: 4–6, P3: 7–9, P4: 10–12) or in the soma (S: 13–15). Scale bar: 20 µm. (**B**) Spontaneous Ca^{2+} transient patterns for each of the observed ROI indicated in (**A**). Each process showed an independent pattern of Ca^{2+} transients and the soma did not display any Ca^{2+} transients.

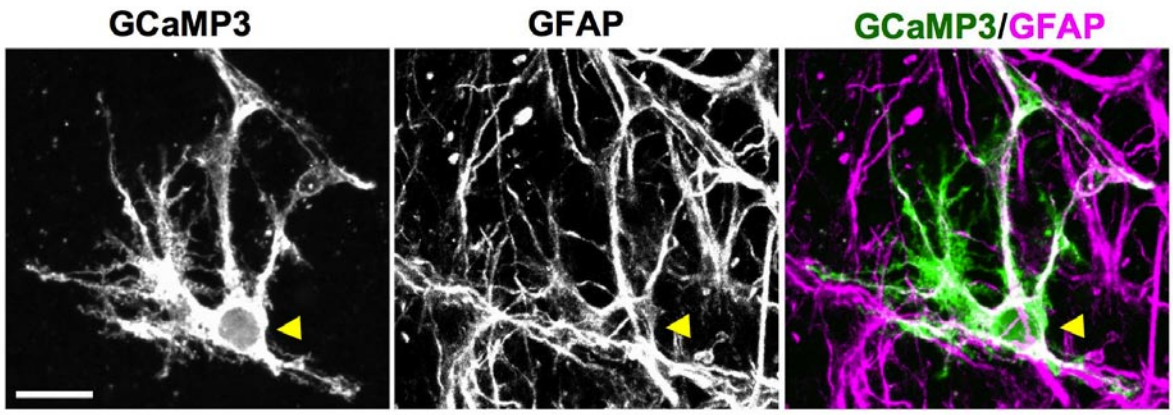

Fig. S2. GFAP expression in a GCaMP3-transfected cell.

Astrocyte in hippocampal slice transfected with GCaMP3 and stained with anti-GFAP antibody. Representative of N=12 astrocytes. The yellow arrowheads indicate the soma. Scale bar: 20 μm.

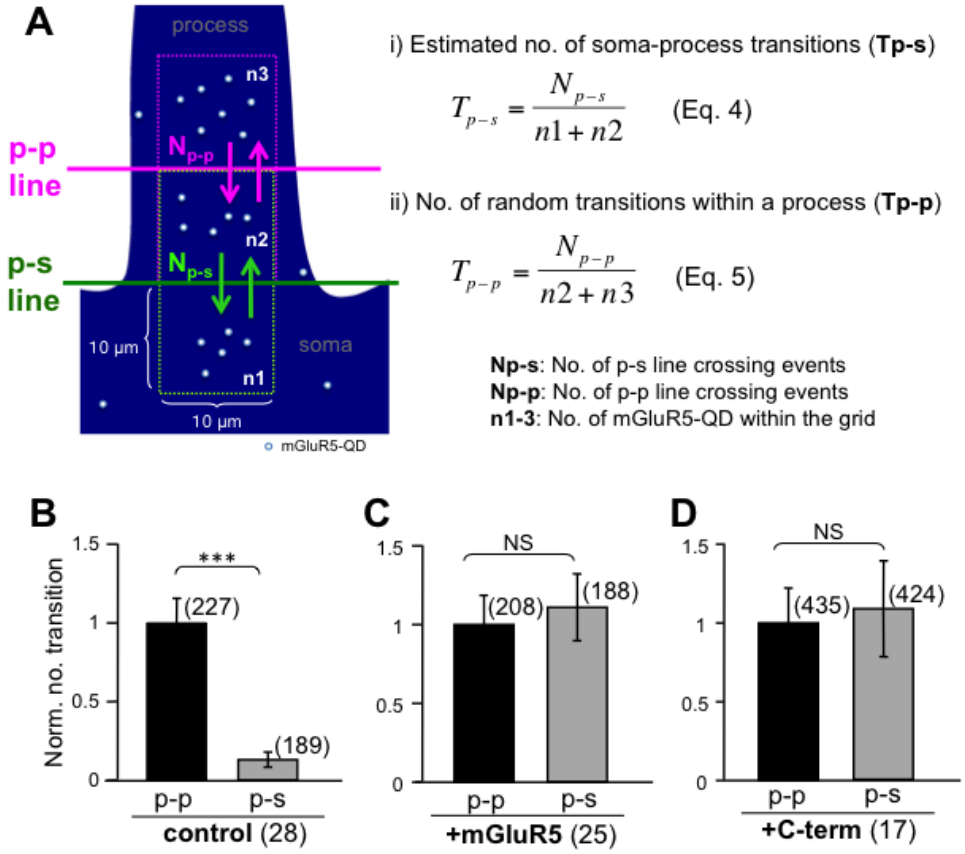

Fig. S3. Quantification of the likelihood of mGluR5-QD soma-process transition.
(**A**) The schematic diagram and the equations for quantification of the likelihood of mGluR5-QD soma-process transition, which was represented by the number of process-soma line (p-s line)-crossing events per one mGluR5-QD within 10 minute-recording time (T_{p-s}). The p-s line (green) was defined as a straight line in contact with the soma at the base of a process. A 10 μm × 10 μm square grid (green, dashed line) was placed on both sides of the p-s line, and the mGluR5-QD within these grids in the first 5 frames were used for the analysis. T_{p-s} was obtained by dividing the number of p-s line crossing events (N_{p-s}) with the number of mGluR5-QD in the grids (n1+n2), (Eq. 4). The number of transitions within a process per mGluR5-QD in 10 min (T_{p-p}) was calculated

as a control. A parallel line on a process 10 μm from the p-s line was defined as a process-process line (p-p line, magenta), and $T_{p\text{-}p}$ was similarly calculated by counting the p-p line crossing events ($N_{p\text{-}p}$) and the number of mGluR5-QD in the 10 μm × 10 μm grid (magenta, dashed line) contacting the p-p line (n2+n3), using Eq. 5.

(**B-D**) Normalized number of soma-process transitions and line-crossings in the process, measured in control astrocytes (**B**), mGluR5 overexpressing astrocytes (**C**), or mGluR5 C-terminus-expressing astrocytes (**D**). Values are normalized to the average of $T_{p\text{-}p}$. ***: $p<0.005$, NS: not significant, paired t-test. Numbers in parentheses next to each bar represent n2+n3 for p-p and n1+n2 for p-s respectively. Numbers in parentheses at the bottom of the graph represent the number of processes analyzed.

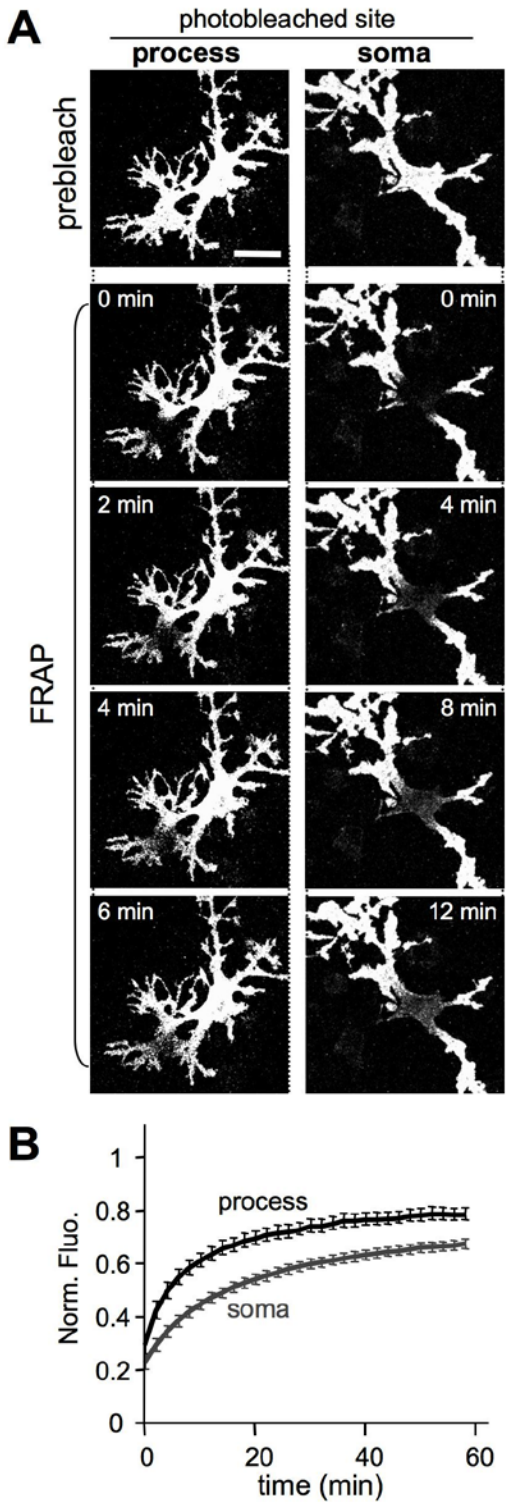

Figure S4. Visualization of lateral diffusion of overexpressed mGluR5 on the cell surface using FRAP.

(**A**) FRAP experiments on astrocytes overexpressing mGluR5-pHluorin. A part of a process (left) or the entire soma (right) were subjected to the photobleaching. Top: fluorescence image of astrocytes expressing mGluR5-pHluorin before photobleaching. mGluR5-pHluorin overexpressed on the cell surface uniformly distributed across the entire astrocyte. Bottom: time-lapse fluorescence images during FRAP. Time on the images indicates the time after photobleaching. Note that the mGluR5-pHluorin signal gradually recovered from the edge of the photobleached area. Scale bar: 20 μm (**B**) Time course of the recovery of fluorescence intensity normalized by initial fluorescence intensity before bleaching (mean±SEM), measured in the photobleached area located in the soma (gray, n=16) or in the process (black, n=11).

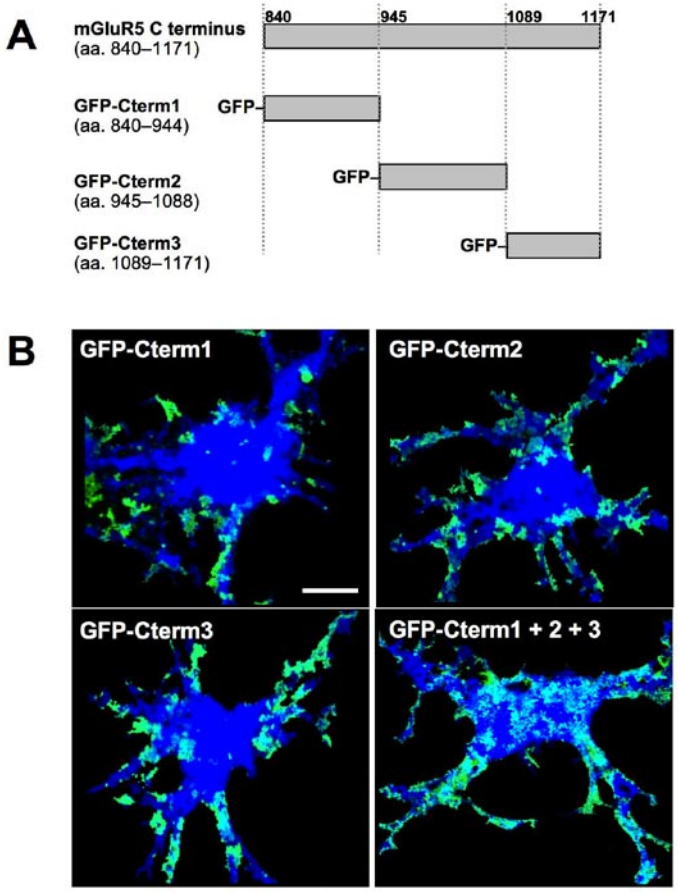

Fig. S5. Effect of mGluR5 C-terminal partial fragment expression on the mGluR5-selective diffusion barrier.

(**A**) Schematic representation of the mGluR5 C terminus fragment (aa. 840-1171) and partial fragments tagged with GFP. GFP-Cterm1 (aa. 849-944), GFP-Cterm2 (aa. 945-1088) and GFP-Cterm3 (aa. 1089-1171) expressing plasmids were constructed by inserting the corresponding PCR fragments into the *EcoRI* site of pEGFP-C2 (Clonetech).

(**B**) Examples of the cell surface exploration by mGluR5-QDs in 10 min (green) on cells expressing mGluR5 C-terminus partial fragments (blue). No mGluR5-QDs diffused beyond the soma-process boundary in cells expressing GFP-Cterm1 (n=25 cells), GFP-Cterm2 (n=24 cells), or GFP-Cterm3 (n=22 cells) alone. mGluR5-QD overcame the mGluR5-selective diffusion barrier in some astrocytes transfected with all of these fragments (n=7 of 20 cells). Scale bar: 10 μm.

Movie S1 (.mov format): Spontaneous Ca^{2+} transients in astrocytes

Spontaneous Ca^{2+} transients in cultured astrocyte transfected with GCaMP2. Representative of N=10 astrocytes. Time-courses of the Ca^{2+} transient observed in each process are shown in Fig. S1B.