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Supplemental information

Mechanisms of differential desensitization of metabotropic glutamate receptors

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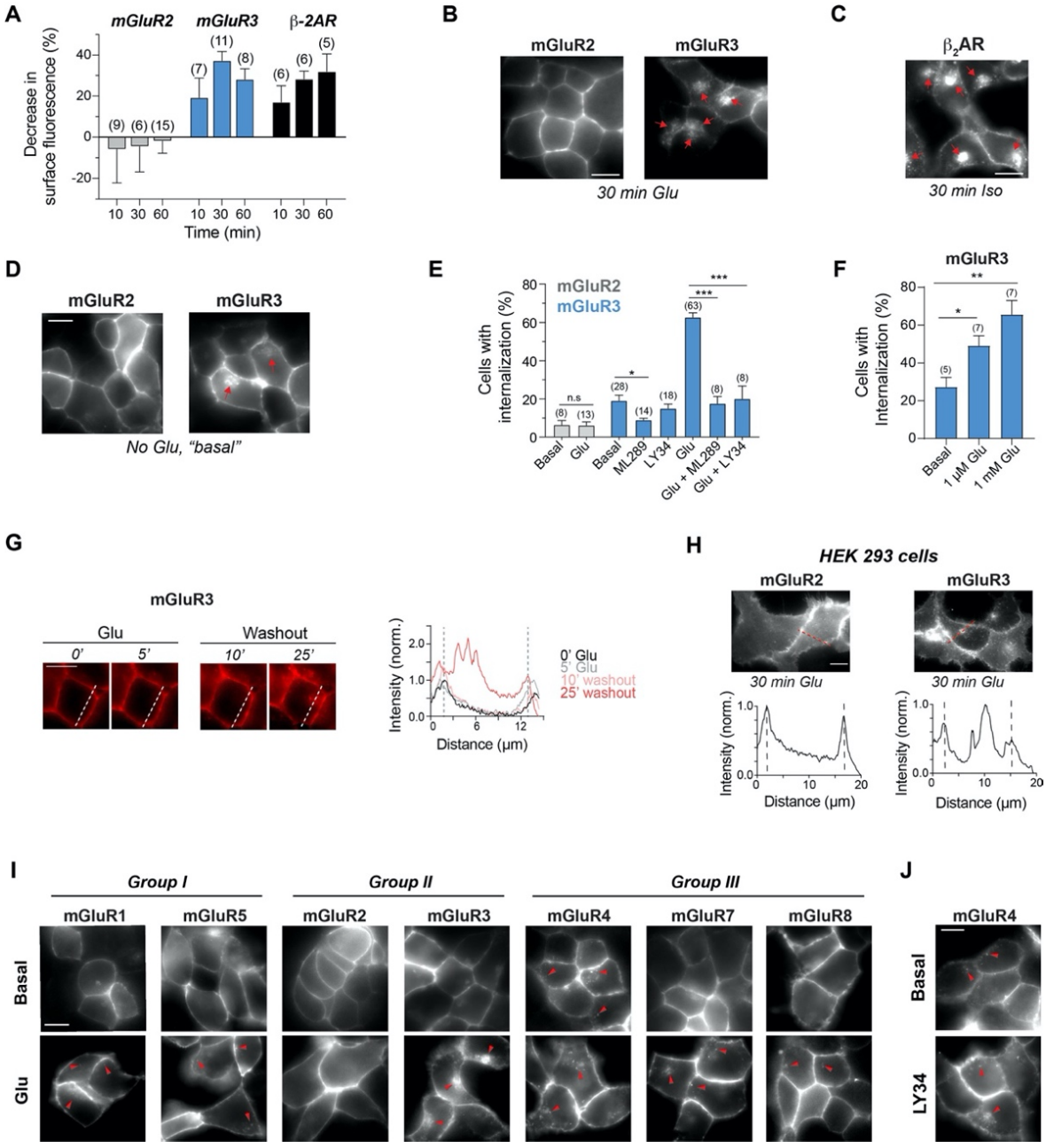


Figure S1. Further analysis of mGluR internalization, Related to Figure 1

(A) HEK 293T cells expressing BG-546-labeled SNAP-mGluR2, -mGluR3, or -β_2AR were stimulated with 1 mM Glu (mGluR) or 10 μM Iso (β_2AR) for the indicated times to measure the percent decrease in surface fluorescence as compared to no agonist control.

(B-C) Representative images of HEK 293T cells expressing either BG-546-labeled mGluR2, mGluR3 (B), or β_2AR (C) following 30 min of agonist treatment. Red arrows point to internalized receptors. Scale bar=10 μm.

(D) Images showing that under basal conditions, mGluR3 but not mGluR2 can internalize. Red arrows point to internalized mGluR3.

(E) Summary bar graph of the percentage of cells that exhibit internalization of either mGluR2 or mGluR3. ML289 partially blocks the basal internalization of mGluR3, and both 100 μM ML289 and 20 μM LY34 block glutamate-mediated internalization of mGluR3. * indicates statistical significance (unpaired t-tests, p= 0.02 for Basal vs ML289, <0.0001 for Glu vs Glu + ML289, and <0.0001 for Glu vs Glu + LY34).
(F) Quantification of the percentage of cells that exhibit mGluR3 internalization following 30 min of either 1 μM or 1 mM glutamate treatment. * indicates statistical significance (unpaired t-tests, p=0.02 for 1 μM, 0.003 for 1 mM).
(G) Left, time-lapse images of a HEK 293T cell expressing BG-546-labeled SNAP-mGluR3. After 5 min in 1 mM glutamate, cells were washed continuously for 25 min. Right, intensity line scan through the white dotted line on the images showing that after 25 min washout (red line), there is clear mGluR3 fluorescence inside the cell. Black dotted lines represent the cell surface.
(H) Top, Representative images of HEK 293 cells showing that mGluR3 but not mGluR2 can internalize in this cell type after 30 min 1 mM glutamate treatment. Bottom, intensity line scans taken from the red dotted lines on the images. The black dotted lines represent the cell surface.
(I) Representative images of BG-546-labeled mGluRs in HEK 293T cells under basal conditions and after 30 min glutamate treatment. Red arrows point to internalized receptors.
(J) Representative images of BG-546-labeled mGluR4 in HEK 293T cells under basal conditions and 45 min treatment with 20 μm LY34 during BG-546 labeling. Red arrows point to internalized receptors.
The number of fields of cells used in the analysis are in parentheses. Scale bars=10 μm. Error bars show s.e.m.

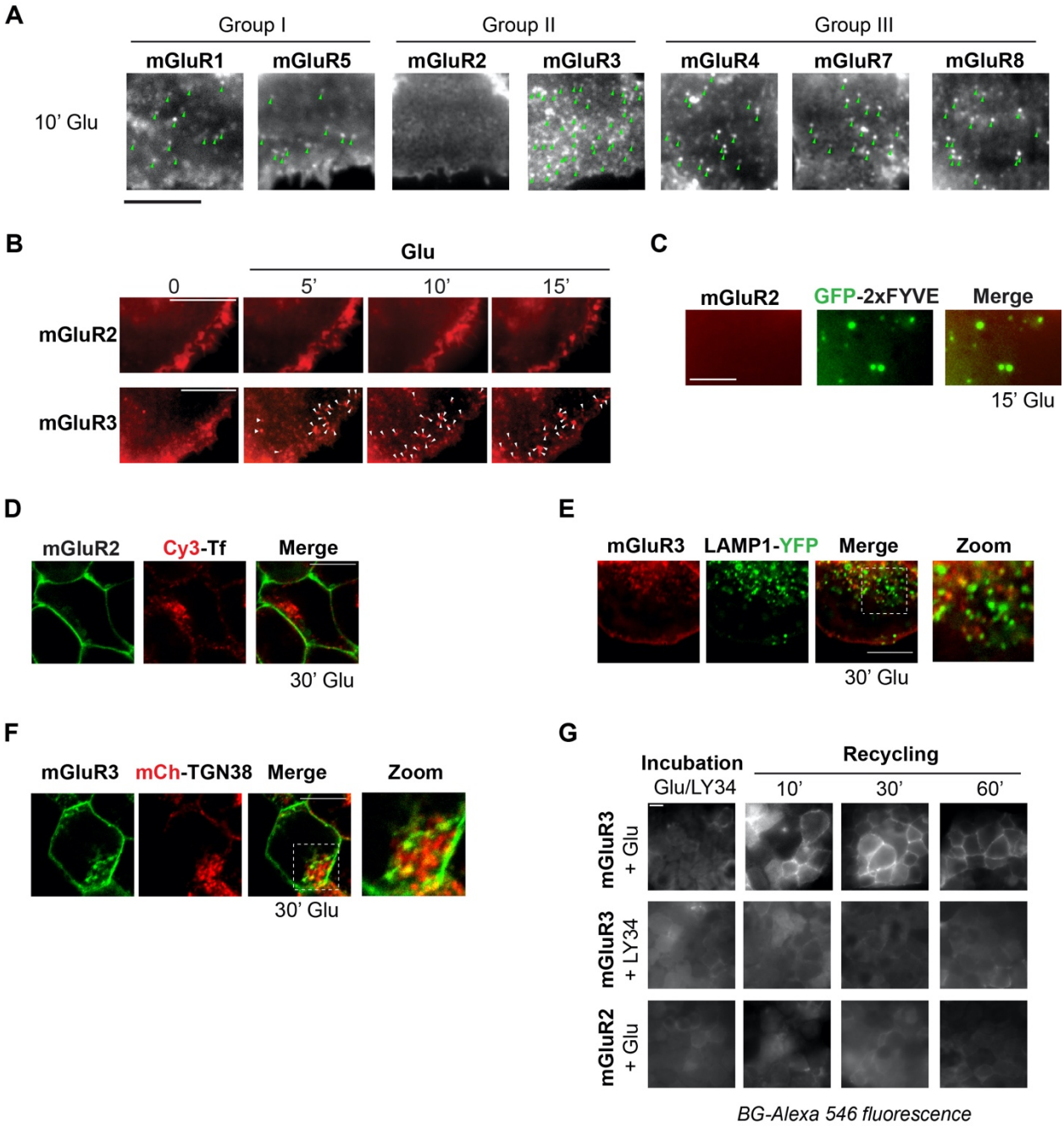


Figure S2. Further characterization of the sub-cellular localization of mGluRs, Related to Figure 1
(A) TIRF images of BG-Alexa546-labeled SNAP-mGluRs in HEK 293T cells following 10 min glutamate treatment. Green arrows highlight examples of receptor puncta. Scale bar= 5 µm.
(B) Time-lapse TIRF images of HEK 293T cells showing that after 5 min treatment in 1 mM glutamate, new mGluR3 puncta emerge and continue to emerge for 15 min. White arrowheads mark newly formed puncta. Scale bar= 10 µm.
(C) TIRF image showing the absence of mGluR2 puncta at GFP-2xFYVE puncta following 15 min glutamate treatment. Scale bar= 5 µm.

(D-F) Confocal images of cells showing a lack of colocalization between BG-488-labeled mGluR2 and Cy3-Tf (D), BG-546-labeled mGluR3 and LAMP1-YFP (E), and BG-488-labeled mGluR3 and mCherry-TGN38 (F) following 30 min in glutamate.
(G) Representative images from the recycling assay. mGluR3-expressing cells, following 30 min in 1 mM glutamate and not 10 μM LY34, show a gain of surface fluorescence following labeling with BG-546 after 10, 30, or 60 min of recycling. mGluR2 does not show surface labeling following incubation in glutamate. Scale bar= 10 μm.

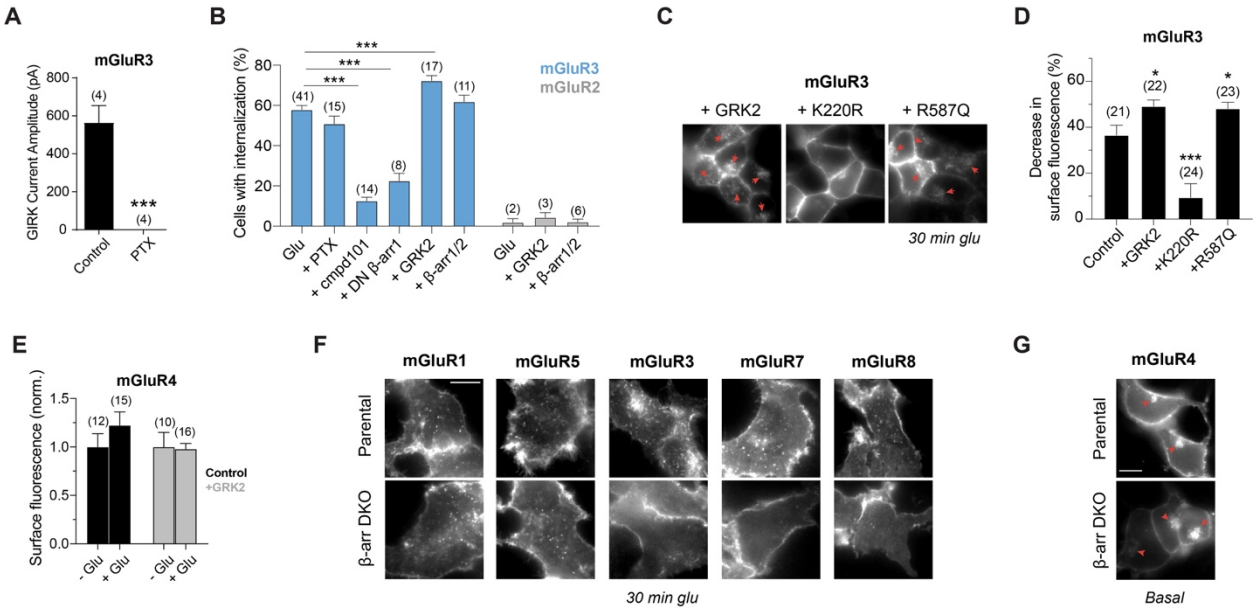


Figure S3. Further characterization of GRK and arrestin dependence of mGluR internalization, Related to Figure 2

(A) Quantification of the amplitudes of mGluR3-mediated GIRK currents following 100 μM glutamate application, without (control) and with co-expression of PTX. *** indicates statistical significance (unpaired t-test, p=0.0008). Number of cells recorded from are shown in parentheses.

(B) Summary bar graph of the percentage of cells that exhibit glutamate-dependent internalization of mGluR3 or mGluR2, with co-expression of either PTX, DN β-arr1, GRK2, GRK2-K220R, GRK2-R587Q, β-arrestin1 and β-arrestin2, or treatment with cmpd101. *** indicates statistical significance (unpaired t-test, p<0.0001 for cmpd101, p<0.0001 for DN β-arr1, p=0.0002 for GRK2). Number of fields of cells analyzed are in parentheses.

(C) Representative images of BG-546-labeled mGluR3 following incubation in 1 mM Glu for 30 min, with overexpression of either GRK2, GRK2-K220R, or GRK2-R587Q. Red arrows point at internalized receptors.

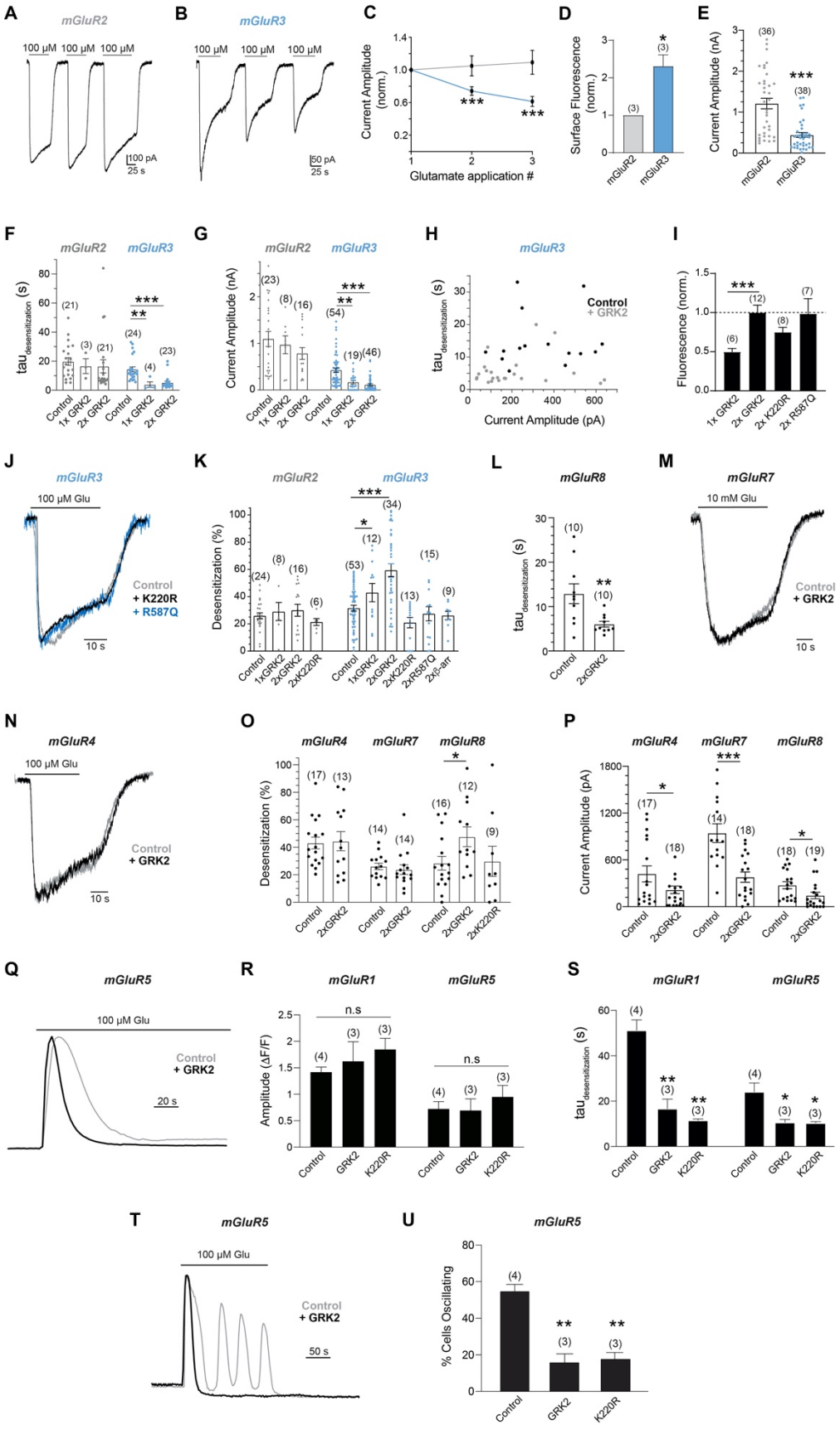
(D) Quantification of BG-Alexa-546-labeled mGluR3 fluorescence following incubation in 1 mM Glu for 60 min, with overexpression of either GRK2, GRK2-K220R, or GRK2-R587Q. Unpaired t tests compared to Glu, * p=0.01 for GRK2, *** p=0.0009 for K220R, * p= 0.03 for R587Q. Number of fields of cells analyzed are in parentheses.

(E) Quantification of BG-Alexa-546-labeled mGluR4 fluorescence before and after 60 min 1 mM Glu treatment, with and without overexpression of GRK2. Data is normalized to -Glu in the respective condition. Number of fields of cells analyzed are in parentheses.

(F) Images of BG-546-labeled mGluRs in CRISPR β-arrestin DKO and parental cells following incubation in Glu for 30 min.

(G) Representative images of BG-546-labeled mGluR4 under basal conditions in CRISPR β-arr DKO and parental cells. Red arrows point at internalized receptors.

Scale bars= 10 μm. Error bars show s.e.m.


A
mGluR2
100 µM 100 µM 100 µM
100 pA
25 s
B
mGluR3
100 µM 100 µM 100 µM
50 pA
25 s
C
Current Amplitude (norm.)
Glutamate application #
D
Surface Fluorescence (norm.)
mGluR2
mGluR3
E
Current Amplitude (nA)
F
tau desensitization (s)
Control
1x GRK2
2x GRK2
G
Current Amplitude (nA)
H
tau desensitization (s)
Control
+ GRK2
Current Amplitude (pA)
I
Fluorescence (norm.)
1x GRK2
2x GRK2
2x K220R
2x R587Q
J
mGluR3
100 µM Glu
Control
+ K220R
+ R587Q
10 s
K
Desensitization (%)
Control
1xGRK2
2xGRK2
2xK220R
2xR587Q
2xβ-arr
L
mGluR8
tau desensitization (s)
Control
2xGRK2
M
mGluR7
10 mM Glu
Control
+ GRK2
10 s
N
mGluR4
100 µM Glu
Control
+ GRK2
10 s
O
mGluR4
mGluR7
mGluR8
Desensitization (%)
Control
2xGRK2
2xK220R
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mGluR5
100 µM Glu
Control
+ GRK2
20 s
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T
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100 µM Glu
Control
+ GRK2
50 s
U
mGluR5
% Cells Oscillating
Control
GRK2
K220R

Figure S4. Further analysis of GRK-mediated rapid desensitization of mGluR signaling, Related to Figure 3

(A-B) Representative whole cell patch clamp recordings in HEK 293T cells of GIRK currents following repeated 1 min activation of mGluR2 (A) or mGluR3 (B) by glutamate.

(C) Summary plot showing that repeated glutamate applications reduce the peak amplitudes of mGluR3-mediated currents, but not mGluR2. Values are normalized to the average amplitude of the 1st glutamate application for each receptor. Measurements were taken from individual cells, n=5 for mGluR3, n=12 for mGluR2. Repeated measures one-way ANOVA, *** p=0.0006.

(D) Quantification of the surface fluorescence of BG-546-labeled mGluR2 and mGluR3 in HEK 293T cells following 24 hr expression. Fluorescence is normalized to the average value of mGluR2. Number of independent experiments are in parentheses. Unpaired t-test, * p=0.01.

(E) Quantification of the average GIRK current amplitudes mediated by either mGluR2 or mGluR3. Activation was induced with 100 μM glutamate. Unpaired t-test, *** p<0.0001.

(F) Summary bar graph comparing the tau of desensitization of GIRK currents mediated by mGluR2 or mGluR3 during 30 s 100 μm glutamate application. Unpaired t-tests compared to mGluR3 control, * p=0.02 for 1xGRK2, *** p<0.0001 for 2xGRK2.

(G) Summary bar graph showing GIRK current amplitudes with and without GRK2 co-expression. Unpaired t-tests compared to mGluR3 control, ** p=0.002 for 1x GRK2, *** p<0.0001 for 2x GRK2.

(H) Scatter plot showing the relationship between desensitization kinetics and current amplitude of mGluR3-mediated GIRK currents, with and without 2x overexpression of GRK2.

(I) Quantification of the fluorescence of GFP-tagged WT GRK2 and mutants expressed in cells. The number of fields of cells analyzed are in parentheses. Unpaired t-test, *** p<0.0001.

(J) Representative whole cell patch clamp recordings of GIRK currents mediated by mGluR3 activation with and without overexpression of either GRK2-K220R or GRK2-R587Q.

(K) Summary bar graph of the percent desensitization of mGluR2 or mGluR3-mediated GIRK currents during 30 s glutamate application, with and without overexpression of WT or mutant GRK2, or β-arrestin1 and β-arrestin2. Unpaired t-tests compared to mGluR3 control, * p=0.04 for 1xGRK2, *** p<0.0001 for 2xGRK2.

(L) Quantification of the tau of desensitization of mGluR8-mediated GIRK currents over 30 s 100 μM glutamate application, without (control) and with GRK2 overexpression. Unpaired t-test, ** p=0.009.

(M and N) Representative whole cell patch clamp recordings of GIRK currents mediated by mGluR7 (M) and mGluR4 (N), without (control) and with GRK2 overexpression.

(O) Summary scatter bar graph of the percent desensitization of group III mGluR-mediated GIRK currents over 30 s 100 uM glutamate application for mGluR4 and mGluR8, and 40 s 1-10 mM glutamate application for mGluR7. Unpaired t-test, * p=0.03.

(P) Summary scatter bar graph of the peak GIRK current amplitudes following activation of group III mGluRs. mGluR4 and mGluR8 were activated with 100 uM glutamate while mGluR7 was activated with either 1 mM or 10 mM glutamate. Unpaired t-tests, * p=0.02 for mGluR4, *** p<0.0001 for mGluR8, * p=0.04 for mGluR8.

(Q) Representative average traces of the first calcium transient mediated by activation of mGluR5 without (control) and with GRK2 overexpression.

(R) Average fluorescence intensities of the peak calcium transient mediated by activation of either mGluR1 or mGluR5, without (control) or with overexpression of GRK2 or GRK2-K220R. In parentheses is the number of experiments analyzed per condition.

(S) Quantification of the tau of desensitization from the peak to the end of the first calcium transient mediated by activation of mGluR1 or mGluR5, without (control) or with overexpression of either GRK2 or GRK2-K220R. Unpaired t-tests, p=0.006 for mGluR1 control vs GRK2, p=0.004 for control vs K220R; p=0.05 for mGluR5 control vs GRK2, p=0.05 for control vs K220R. In parentheses is the number of experiments analyzed per condition.

(T) Representative calcium transients mediated by the activation of mGluR5, without (control) or with overexpression of GRK2.

(U) Summary bar graph of the percentage of cells that exhibit calcium oscillations following activation of mGluR5 without (control) or with overexpression of either GRK2 or GRK2-K220R. Unpaired t-tests, ** p=0.008 for control vs GRK2; ** p=0.005 for control vs K220R. In parentheses is the number of experiments analyzed per condition.
In parentheses is the number of individual cells that measurements were taken from, unless otherwise stated. Error bars show s.e.m.

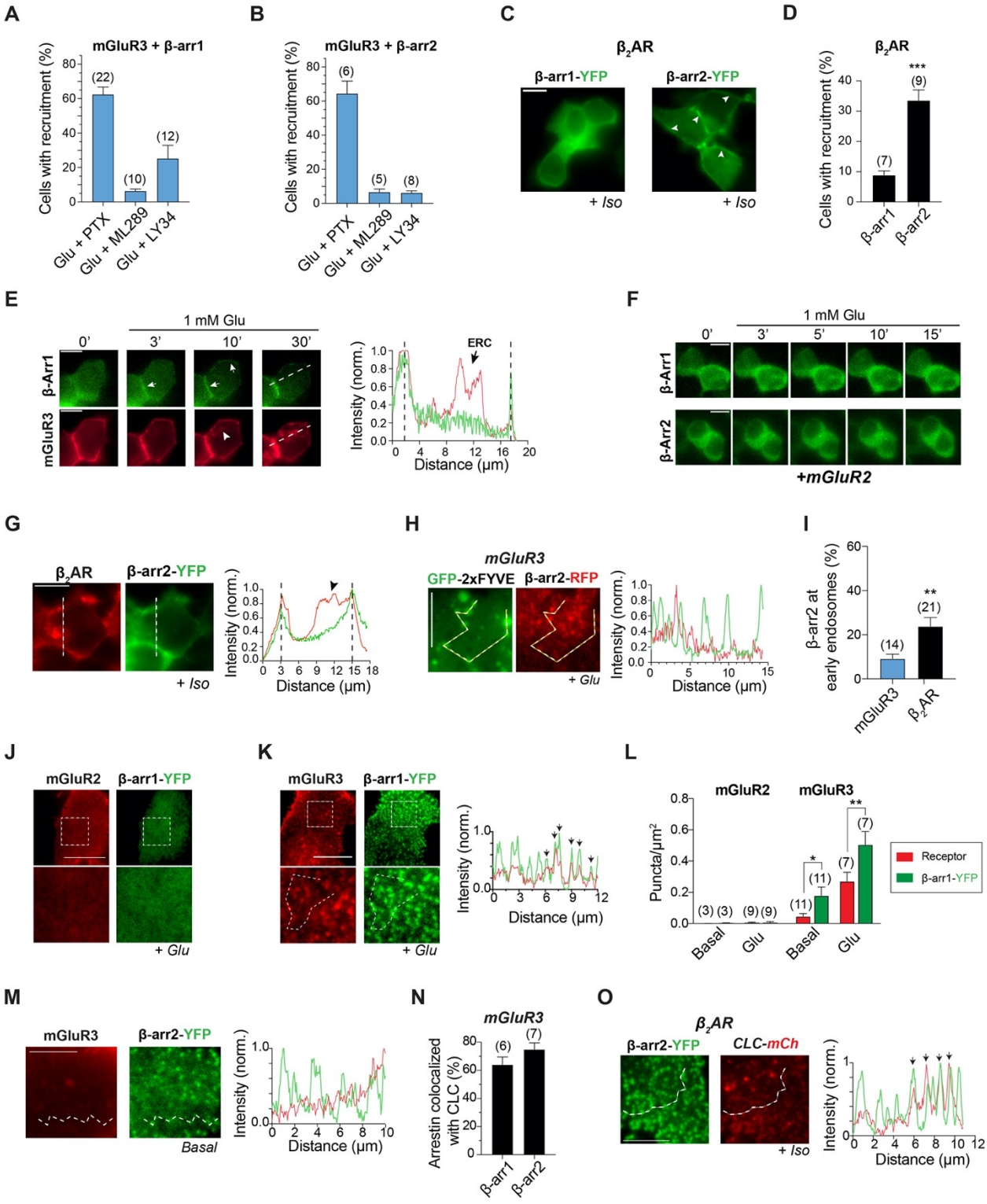

Figure S5. Further analysis of arrestin coupling to mGluR3, Related to Figure 4

(A-B) Summary bar graphs of the percentage of mGluR3-expressing HEK 293T cells that exhibit surface recruitment of β-arr1 (A) or β-arr2 (B) following 15 min glutamate treatment and either co-expression of PTX, treatment with 100 μM ML289, or 20 μM LY34. Number of fields of cells analyzed are shown in parentheses.

(C) Representative images of cells expressing β-arr1-YFP or β-arr2-YFP with β_2AR following 15 min in 10 μM Iso. White arrowheads point at β-arr2-YFP on the cell surface. Scale bar= 10 μm.
(D) Quantification of the percentage of cells that exhibit surface recruitment of β-arr1 or β-arr2 following activation of β_2AR with isoproterenol. Experiment was conducted 48 hr post-transfection. Unpaired t-test, *** $p<0.0001$. Number of fields of cells analyzed are in parentheses.
(E) Left, time-lapse images of a HEK 293T cell expressing BG-546-labeled mGluR3 and β-arr1-YFP. Following treatment with glutamate, surface recruitment of β-arr1 is observed within 3 min and remains on the surface for 30 min. Colocalization between β-arr1-YFP and internalized mGluR3 is not observed at 30 min. White arrows point at β-arr1-YFP on the cell surface. White arrowhead marks internalized mGluR3. Right, intensity line scan along the white dotted line on the 30' images, showing that β-arr1 (green line) and mGluR3 (red line) are present at the cell surface (black dotted lines), but β-arr1 does not accumulate at the ERC with mGluR3.
(F) Time-lapse images showing the lack of β-arr1 and β-arr2 surface recruitment following activation of mGluR2 with glutamate.
(G) Left, representative image of a cell expressing BG-546-labeled β_2AR and β-arr2-YFP, following incubation in isoproterenol for 20 min. β_2AR shows internalization while β-arr2-YFP remains on the surface and does not co-internalize. Right, line scan along the dotted white lines on the images showing the intensity profiles of β-arr1 (green line) and β_2AR (red line). Black dotted lines present the cell surface. The black arrowhead points to internalized β_2AR.
(H) Left, TIRF images showing GFP-2xFYVE-positive early endosomes and β-arr2-RFP puncta following activation of mGluR3 with 1 mM glutamate for 15 min. Right, intensity line scan through the white dotted lines in the images, showing that 2xFYVE peaks (green) do not contain β-arr2 peaks (red).
(I) Quantification of the percentage of β-arr2 puncta that colocalize with GFP-2xFYVE puncta following 15 min activation of either mGluR3 or β_2AR. Unpaired t-test, ** $p=0.008$. Number of cells analyzed are in parentheses.
(J and K) Representative TIRF images of HEK 293T cells expressing β-arr1-YFP and either BG-546-labeled mGluR2 (J) or mGluR3 (K). Activation of mGluR2 with glutamate for 15 min does not generate β-arr1 puncta (J), but mGluR3 activation leads to the formation of both mGluR3 and β-arr1 puncta (K). Intensity line scan through the white dotted line reveals some co-localizing mGluR3 (red line) and β-arr1 (green line) puncta as indicated by the black arrows. Scale bar= 10 μm.
(L) Quantification of the density of receptor (red bars) and β-arr1 (green bars) puncta under basal and glutamate conditions. Number of cells analyzed are in parentheses. Paired t-tests, * $p=0.03$ for mGluR3 basal, ** $p= 0.008$ for mGluR3 + Glu.
(M) Left, TIRF image showing negligible mGluR3 puncta and clear β-arr2 puncta generated under basal conditions. Bottom, intensity line scan through the white dotted line on the image, showing background mGluR3 fluorescence (red line) and clear β-arr1 peaks (green line).
(N) Quantification of the percentage of β-arr-YFP puncta that colocalize with CLC-mCherry puncta following activation of mGluR3 with glutamate for 15 min. Number of cells analyzed are in parentheses.
(O) Left, representative TIRF images of cells expressing β-arr2-YFP and CLC-mCherry with co-expression of β_2AR. Right, Line scan through the white dotted lines on the images show β-arr2 peaks (green line) and CLC peaks (red line). The black arrows denote overlapping peaks.
Scale bar= 10 μm in C, E, F, G, J, K; 5 μm in H, M, N. Error bars show s.e.m.

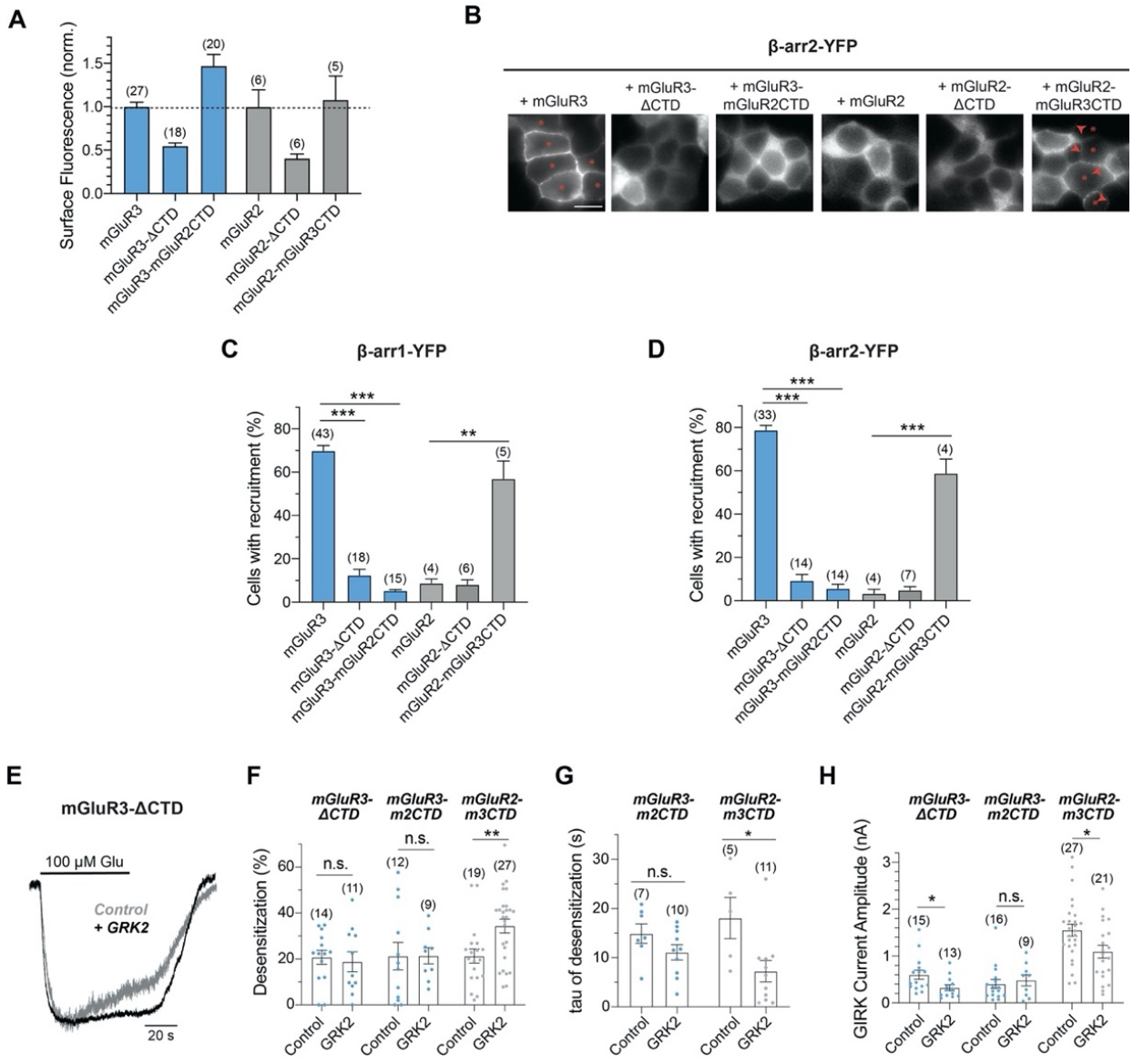

Figure S6. Further characterization of the C-terminal domains of mGluR2 and mGluR3, Related to Figure 5

(A) Surface fluorescence of BG-546-labeled SNAP-tagged CTD constructs in HEK 293T cells. Fluorescence was measured after 24 hr expression for mGluR3 variants, and at 48 hr expression for mGluR2 variants. All values were normalized to the average fluorescence of mGluR3. In parentheses is the number of fields of cells that were analyzed.

(B) Representative images of HEK 293T cells expressing β-arr2-YFP following activation of either mGluR3 or mGluR2 CTD variants with 15 min glutamate. Red asterisks mark cells that exhibit surface recruitment of β-arr2. Red arrowheads point at regions on the cell surface with β-arr2 recruitment. Scale bar=10 μm.

(C and D) Summary bar graphs with quantification of the percentage of cells that exhibit β-arr1 (C) or β-arr2 (D) surface recruitment following glutamate application. In parentheses is the number of fields of cells that were analyzed. Unpaired t-tests, *** $p<0.0001$ for β-arr1 mGluR3 vs mGluR3-ΔCTD, *** $p<0.0001$ for β-arr1 mGluR3 vs mGluR3-mGluR2CTD, ** $p= 0.002$ for β-arr1 mGluR2 vs mGluR2-mGluR3CTD,

*** p<0.0001 for β-arr2 mGluR3 vs mGluR3-ΔCTD, *** p<0.0001 for β-arr2 mGluR3 vs mGluR3-mGluR2CTD, *** p=0.0002 for β-arr2 mGluR2 vs mGluR2-mGluR3CTD.
(E) Representative whole cell patch clamp recordings of GIRK currents mediated by the activation of mGluR3-ΔCTD with and without GRK2 overexpression in HEK 293T cells.
(F) Summary scatter bar graph of the percent desensitization of GIRK currents during activation of the CTD variants with glutamate. Desensitization was measured over 60 s for mGluR3-ΔCTD and over 30 s for mGluR3-mGluR2CTD and mGluR2-mGluR3CTD. Unpaired t-test, ** p=0.005. The number of cells recorded from are in parentheses.
(G) Summary scatter bar graph of the tau of desensitization of GIRK currents over ~30 s glutamate application to activate mGluR3-mGluR2CTD or mGluR2-mGluR3CTD without (control) or with GRK2 overexpression. Unpaired t-test, * p=0.02. The number of cells recorded from are in parentheses.
(H) Summary scatter bar graph of GIRK current amplitudes following activation of the CTD variants, with and without co-expression of GRK2. Unpaired t-tests, * p=0.03 for mGluR3-ΔCTD, * p=0.02 for mGluR2-mGluR3CTD. The number of cells recorded from are in parentheses.
Error bars show s.e.m.

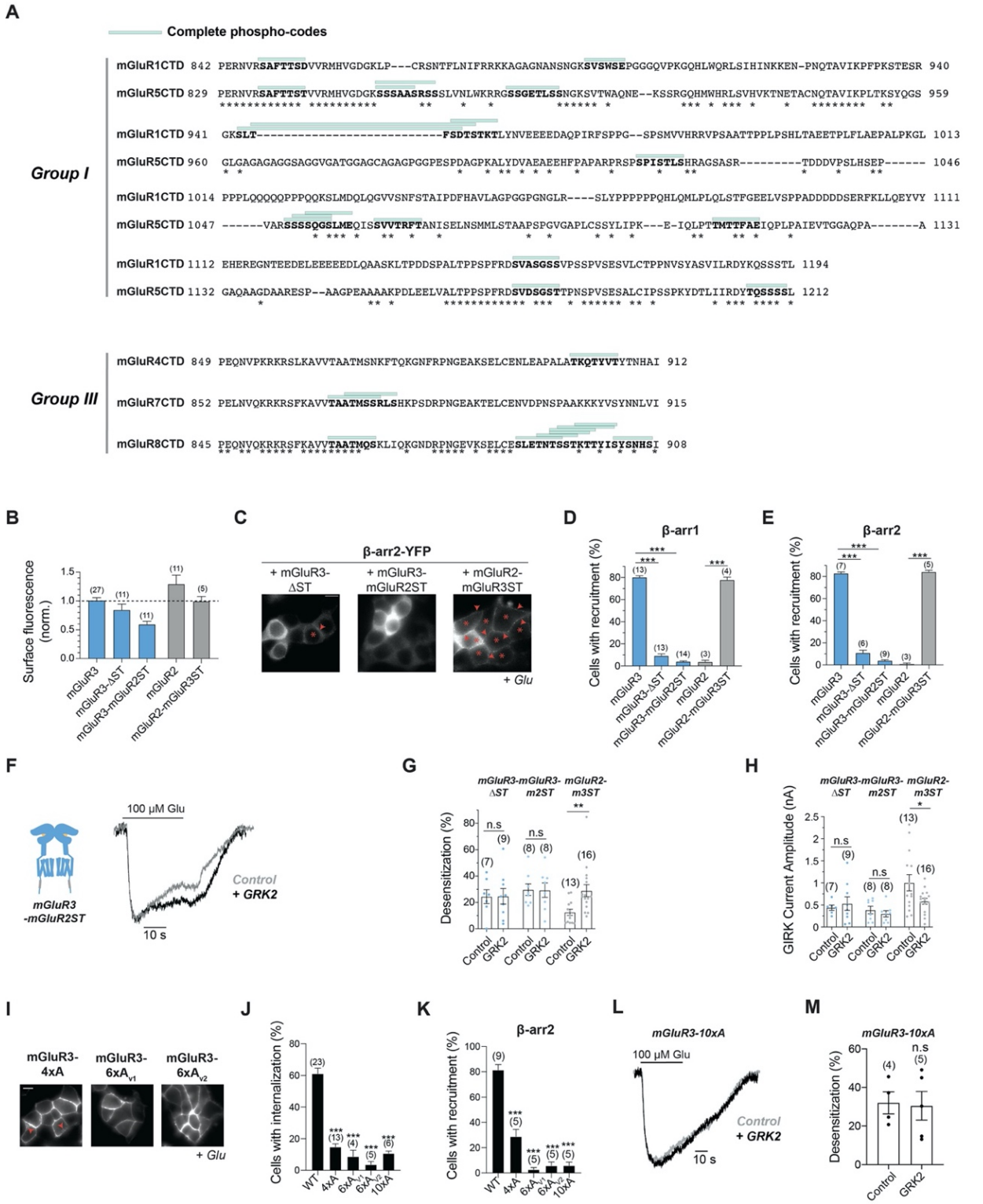

Figure S7. Further analysis of ST-rich sequences and their role in desensitization, Related to Figure 6

(A) The C-terminal domain sequences of group I and group III mGluRs. * indicates residues that are the same between sequences of the same group. Predicted complete phospho-codes are highlighted along the sequences as determined by PhosCoFinder (*Zhou et al., Cell, 2017*).

(B) Quantification of the surface fluorescence of BG-546-labeled SNAP-tagged ST variants in HEK 293T cells. Fluorescence was measured after 24 hr expression for mGluR3 variants, and at 48 hr expression for mGluR2 variants. All values were normalized to the average fluorescence of mGluR3. In parentheses is the number of fields of cells that were analyzed.
(C) Representative images of HEK 293T cells expressing β-arr2-YFP following activation of either ST variants with glutamate for 15 min. Red asterisks mark cells that exhibit surface recruitment of β-arr2. Red arrowheads point at regions on the cell surface with β-arr2 recruitment.
(D and E) Summary bar graphs with quantification of the percentage of cells that exhibit β-arr1 (D) or β-arr2 (E) surface recruitment following glutamate application. In parentheses is the number of fields of cells that were analyzed. Unpaired t-tests, *** $p<0.0001$ for all comparisons shown.
(F) Representative whole cell patch clamp recording of GIRK currents mediated by the activation of mGluR3-mGluR2ST with and without GRK2 overexpression in HEK 293T cells.
(G and H) Summary scatter bar graphs of the percent desensitization (G) and peak amplitudes (H) of GIRK currents mediated by ST variants, without (control) and with GRK2 overexpression. Desensitization was measured over 30 s glutamate application. Unpaired t-tests, ** $p=0.006$ for percent desensitization, * $p=0.03$ for amplitudes. The number of cells recorded from are in parentheses.
(I) Representative images of HEK 293T cells expressing mGluR3 alanine mutants. Cells were treated with 1 mM Glu for 30 min. Red arrows point at internalized receptors.
(J and K) Summary bar graphs showing the percentage of cells that exhibit internalization (J) or surface recruitment of β-arr2 (K) across mGluR3 alanine mutants.
(L) Representative whole cell patch clamp recording of GIRK currents mediated by activation of mGluR3-10xA without (control) and with GRK2 overexpression.
(M) Quantification of the percent desensitization of mGluR3-10xA-mediated GIRK currents over 30 s of glutamate application, without (control) and with GRK2 overexpression. The number of cells recorded from are in parentheses.
Scale bars= 10 μm. Error bars show s.e.m.

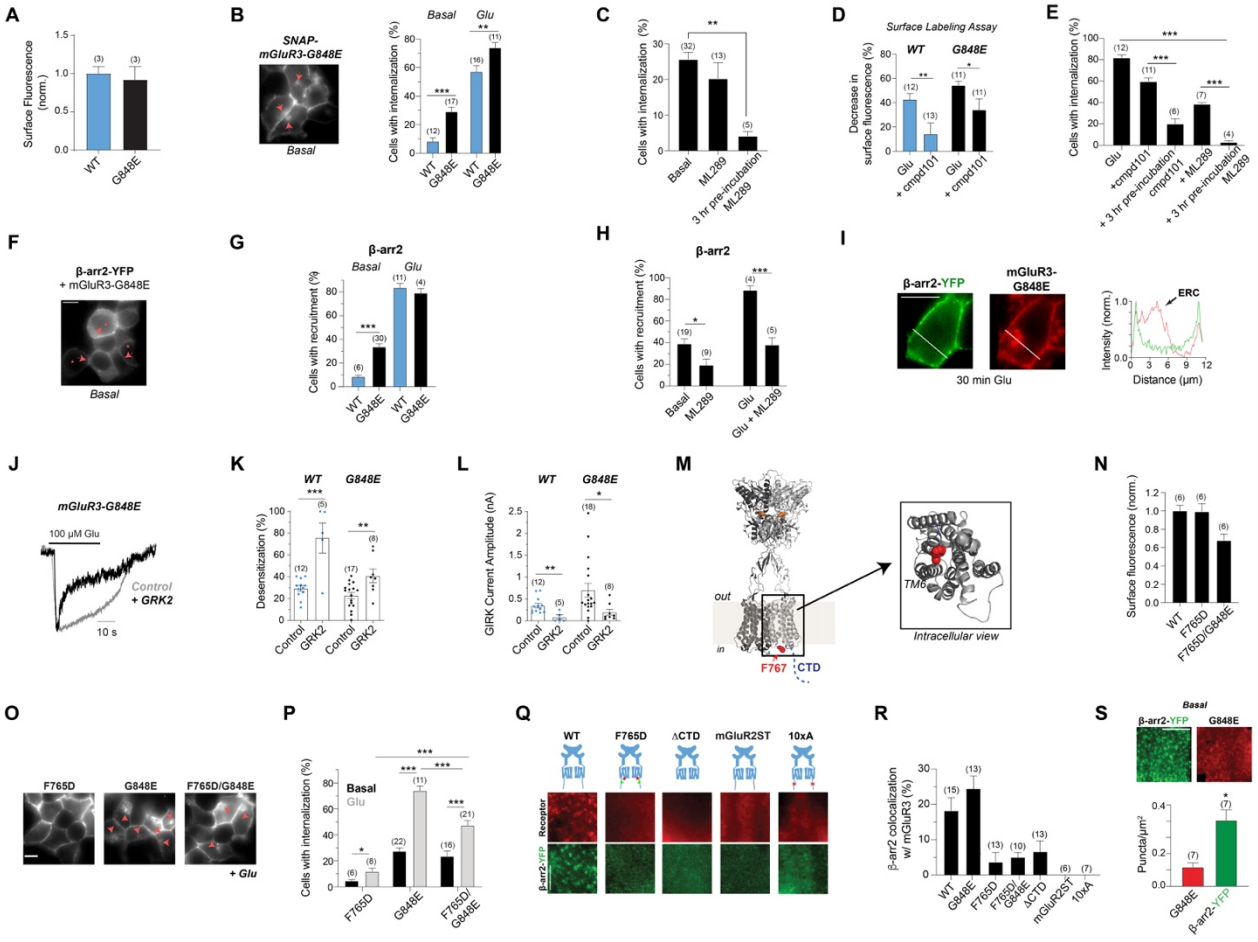

Figure S8. Further analysis of the role of the transmembrane core and CTD in β-arrestin coupling to mGluR3, Related to Figure 7

(A) Quantification of the surface fluorescence of BG-546-labeled SNAP-mGluR3 WT and G848E in HEK 293T cells. The number of independent experiments is shown in parentheses.

(B) Left, representative images of cells showing basal internalization of BG-546-labeled mGluR3-G848E. Red arrows point to internalized receptors. Scale bar= 10 μm. Right, Quantification of the percentage of cells that show internalization of mGluR3 WT or G848E under basal conditions and after 30 min glutamate treatment. Unpaired t-tests, *** p<0.0001 for basal, ** p=0.008 for Glu. The number of fields of cells analyzed is shown in parentheses.

(C) Summary bar graph of the percentage of cells that exhibit basal internalization of mGluR3-G848E following preincubation in ML289. The number of fields of cells analyzed is in parentheses. Unpaired t-test, *** p<0.0001.

(D) Quantification of the percent surface fluorescence decrease as measured using the surface labeling assay. The number of fields of cells analyzed is in parentheses. Unpaired t-tests, ** p=0.009 for WT and * 0.05 for G848E).

(E) Quantification of the percentage of cells that exhibit internalization following 30 min treatment with Glu with either cmpd101 or ML289. Pre-incubation refers to the amount of time cells were incubating in cmpd101 or ML289 prior to Glu treatment. The number of fields of cells analyzed is in parentheses. Unpaired t-tests, *** p<0.0001 for all comparisons made.

(F) Representative image showing surface recruitment of β-arr2-YFP by mGluR3-G848E under basal conditions. Red asterisks denote the cells that show β-arr2 recruitment, the red arrows point to regions on the cell surface with β-arr2. Scale bar=10 μm.

(G) Quantification of the percentage of cells that exhibit β-arr2 recruitment by mGluR3 WT and G848E under basal conditions and after 15 min glutamate. Unpaired t-test, *** p=0.0004. The number of fields of cells analyzed is shown in parentheses.
(H) Quantification of the percentage of cells that show β-arr2 surface recruitment of mGluR3-G848E following 15 min incubation in the indicated drugs. The number of fields of cells analyzed is in parentheses. Unpaired t-tests, * p=0.02 for basal vs ML289, *** p=0.0007 for Glu vs Glu + ML289.
(I) Left, representative images of a cell showing β-arr2-YFP on the cell surface and internalized receptor in the ERC following incubation in glutamate. Right, intensity line scan along the white line on the images, showing the overlap of β-arr2-YFP (green line) and receptor (red) at the cell membrane, but not in the ERC. Scale bar= 10 μm.
(J) Representative whole cell patch clamp recordings of GIRK currents mediated by activation of mGluR3-G848E without (control) and with GRK2 overexpression.
(K) Summary scatter bar graph showing the percent desensitization of mGluR3 WT or G848E evoked GIRK currents, with and without GRK2 overexpression, during 30 s glutamate applications. Unpaired t-tests, *** p=0.0009 for WT, ** p= 0.003 for G848E). The number of cells recorded from are in parentheses.
(L) Summary scatter bar graph showing the peak amplitudes of GIRK currents mediated by mGluR3-G848E activation, without (control) and with GRK2 overexpression. Unpaired t-tests, ** p=0.009 for WT, * p=0.03 for G848E. The number of cells recorded from are in parentheses.
(M) Structural model of ligand-bound (orange) mGluR5, showing the location of the conserved residue, F767 (red), and the CTD (blue).
(N) Quantification of the surface fluorescence BG-546-labeled mGluR3 WT, F765D, and F765D/G848E in HEK 293T cells. Values were normalized to the average surface fluorescence of mGluR3 WT. The number of fields of cells analyzed is in parentheses.
(O) Representative images of cells expressing mGluR3-F765D, G848E, or F765D/G848E, following incubation in glutamate for 30 min. Red arrows point at internalized receptors. Scale bar= 10 μm.
(P) Quantification of the percentage of cells that exhibit internalization of mGluR3 mutants under basal and glutamate conditions. The number of fields of cells analyzed is in parentheses. Unpaired t-tests, * p=0.04 for F765D, *** p<0.0001 for G848E, *** p=0.0001 for F765D/G848E, *** p<0.0001 for G848E vs F765D/G848E, *** p<0.0001 for F765D vs F765D/G848E.
(Q) Representative TIRF images of β-arr2-YFP and mGluR3 variants following glutamate treatment for 15 min. Scale bar= 5 μm.
(R) Summary bar graph of the percent colocalization of β-arr2 puncta with the puncta of mGluR3 variants. The number of cells analyzed are shown in parentheses.
(S) Top, TIRF image showing β-arr2-YFP puncta under basal conditions with co-expression mGluR3-G848E. Bottom, quantification of the density of mGluR3-G848E puncta and β-arr2-YFP puncta under basal conditions. The number of cells analyzed are in parentheses. Paired t-test, * p=0.01. Scale bar= 5 μm.
Error bars show s.e.m.