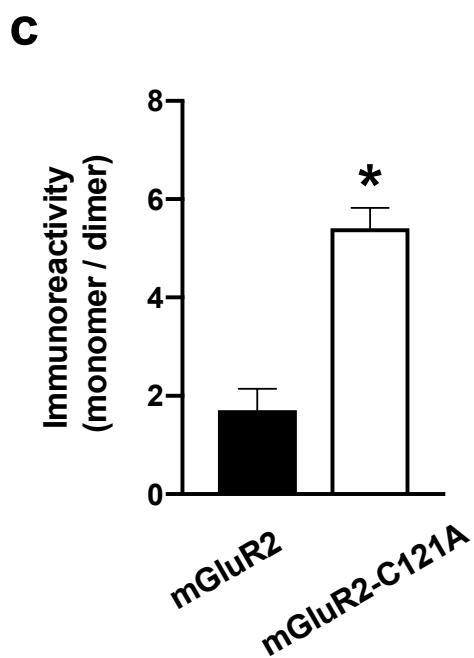
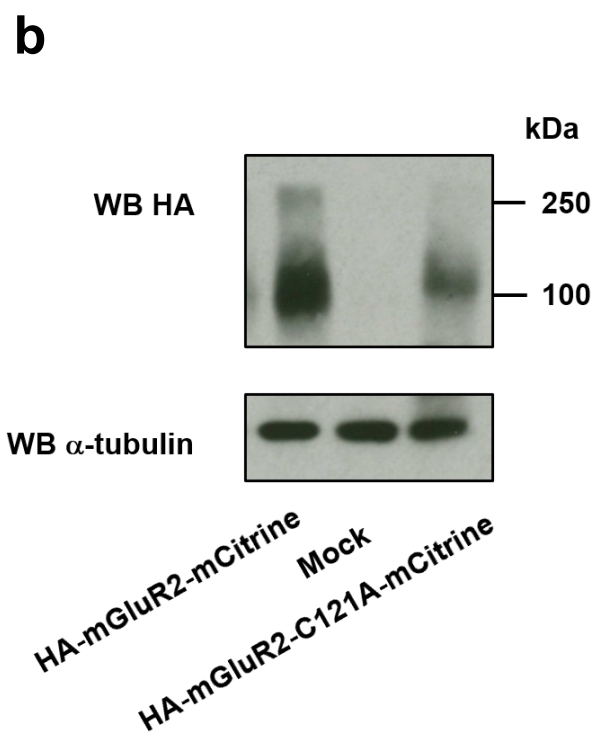
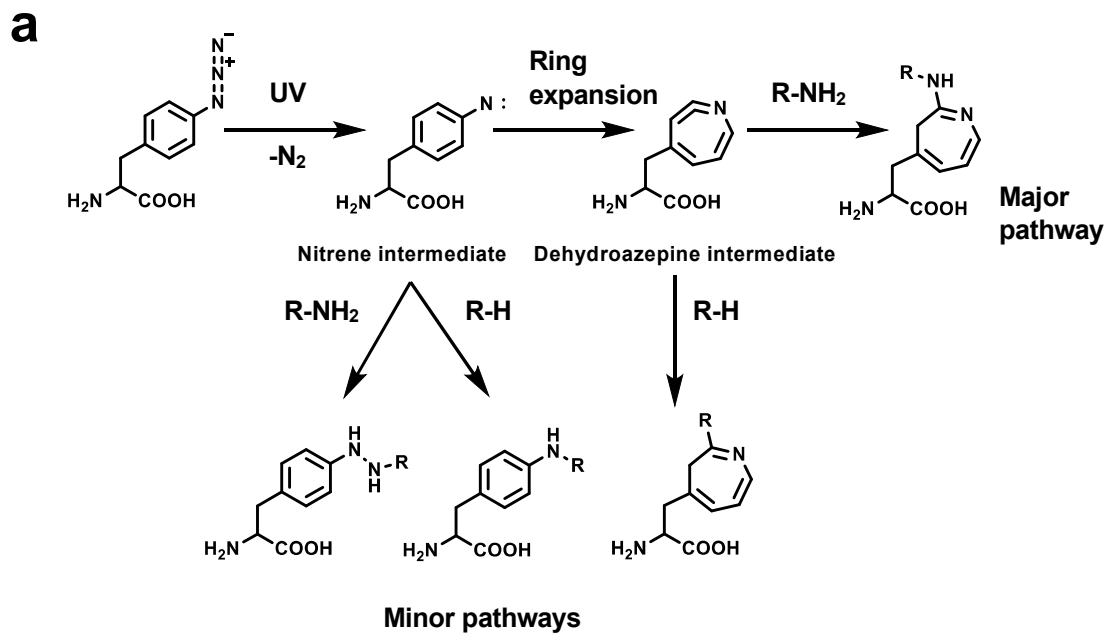


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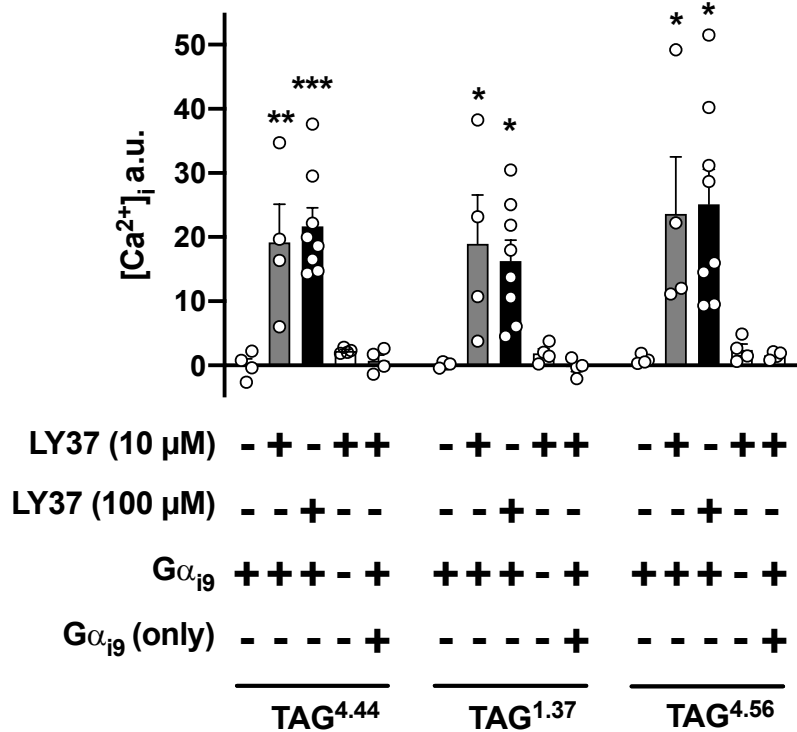
Supplemental Information

**Site-Specific Incorporation of Genetically
Encoded Photo-Crosslinkers Locates the Heteromeric
Interface of a GPCR Complex in Living Cells**

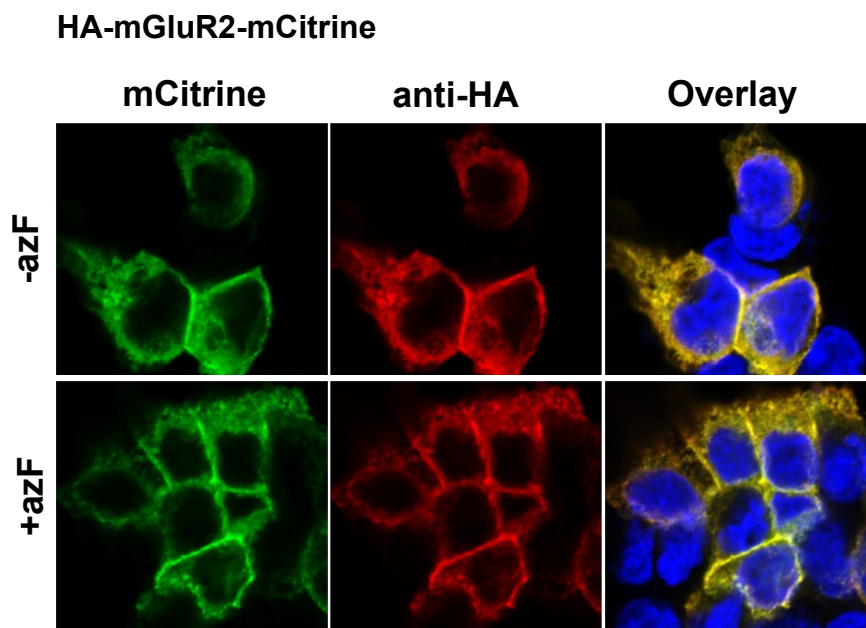
**Urjita H. Shah, Rudy Toneatti, Supriya A. Gaitonde, Jong M. Shin, and Javier González-
Maeso**

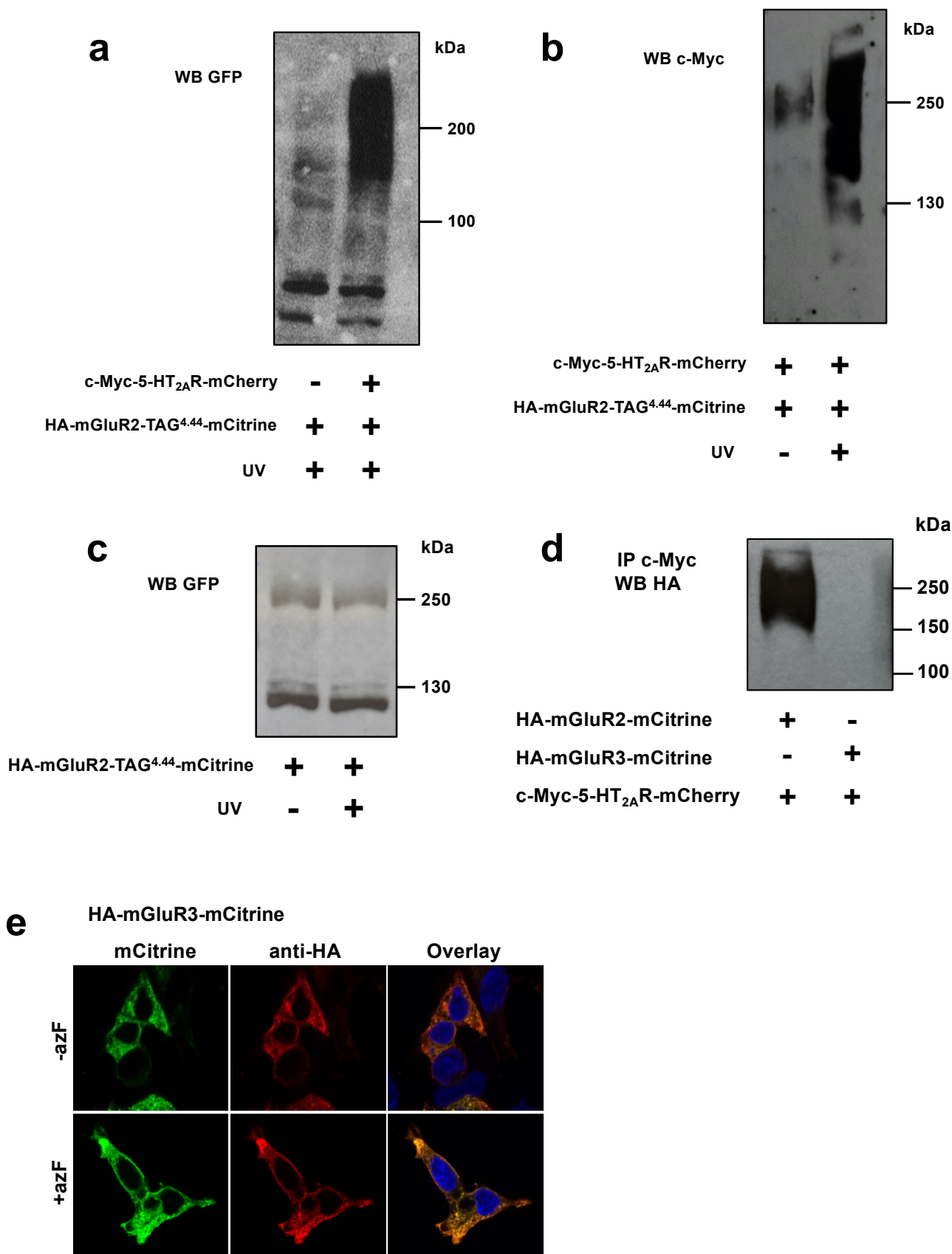


a



b





Supplementary Figure legends

Figure S1. (A) The photoactivatable unnatural amino acid azF upon exposure to UV-A light forms an unstable nitrene intermediate that can either undergo addition reactions with double bonds, insertion into C–H and N–H sites (minor pathways), or ring expansion into a dehydroazepine intermediate that can react with a nucleophile such as a primary amine (major pathway) (Preston and Wilson, 2013). **(B,C)** Immunoblot analysis in membrane preparation of cells stably expressing HA-GluR2-mCitrine, HA-mGluR2-C121A-mCitrine, or mock (n = 2). Representative immunoblots **(B)** and quantification of immunoreactivity **(C)**. Mean \pm s.e.m. * $p < 0.05$ by Student's *t* test. Related to Figures 1 and 3.

Figure S2. (A) Cells co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR2-TAG^{1.37}-mCitrine, HA-mGluR2-TAG^{4.44}-mCitrine or HA-mGluR2-TAG^{4.56}-mCitrine, were loaded with Fura-2 and monitored for intracellular calcium release after administration of LY379268 (LY37; 10 μ M or 100 μ M), or vehicle. Experiments were carried out in cells exposed to azF. Controls included cells untransfected with G α_{i9} , and cells transfected with G α_{i9} only (n = 4 – 8 independent experiments per experimental condition). Mean \pm s.e.m. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ by Dunnett's *post hoc* test of one-way ANOVA. **(B)** Cells exposed to azF (+azF) or mock (-azF) and transfected with the constructs encoding "wild-type" HA-mGluR2-mCitrine were permeabilized to detect the HA epitope and imaged to detect mCitrine fluorescence. Nuclei were stained in blue with Hoechst. Related to Figure 2.

Figure S3. (A) Cells exposed to azF were co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR2-TAG^{4.44}-mCitrine and/or c-Myc-5-HT_{2A}R-mCherry. Cells were then exposed to UV, processed for membrane preparation, and analyzed by Western blotting (WB) with antibody against GFP (which also recognizes mCitrine). **(B)**. Cells exposed to azF were co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR2-TAG^{4.44}-mCitrine and c-Myc-5-HT_{2A}R-mCherry. After this, the same group of cells was separated into two groups that

were either exposed to UV or mock. Cells (UV[+] and UV[-]) were afterwards processed for membrane preparation and analyzed by Western blotting (WB) with antibody against c-Myc. **(C)** Cells exposed to azF were co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR2-TAG^{4.44}-mCitrine. After this, the same group of cells was separated into two groups that were either exposed to UV or mock. Cells (UV[+] and UV[-]) were afterwards processed for membrane preparation and analyzed by Western blotting (WB) with antibody against GFP. **(D)** Cells co-transfected with c-Myc-5-HT_{2A}R-mCherry and either HA-mGluR2-mCitrine or HA-mGluR3-mCitrine were subjected to co-immunoprecipitation (IP) with antibody against the c-Myc tag, and then analyzed by Western blotting with antibody against the HA tag. **(E)** Cells exposed to azF (+azF) or mock (-azF) and transfected with the constructs encoding “wild-type” HA-mGluR3-mCitrine were permeabilized to detect the HA epitope and imaged to detect mCitrine fluorescence. Nuclei were stained in blue with Hoechst. Related to Figures 3 and 4.

Table S1. Binding saturation curves with [³H]LY341495. Related to Figure 2

| | mGluR2 | mGluR2-TAG ^{4.44} | | mGluR2-TAG ^{4.56} | | mGluR2-TAG ^{1.37} | |
|------------------------|--------------|----------------------------|---------------|----------------------------|--------------|----------------------------|--------------|
| | | +azF | -azF | +azF | -azF | +azF | -azF |
| B_{max} | 2030 ± 129.9 | 332.4 ± 115.5 | 123.0 ± 70.77 | 335.1 ± 63.1 | 167.0 ± 9.46 | 246.8 ± 35.9 | 33.52 ± 7.61 |
| K_D | 2.64 ± 0.31 | 4.58 ± 2.13 | 3.84 ± 5.09 | 1.95 ± 0.34 | 2.57 ± 0.38 | 1.98 ± 0.85 | 0.22 ± 0.23 |

B_{max} (fmol/mg prot); K_D (nM)

Radioligand binding saturation curves with [³H]LY341495 in membrane preparations of cells transfected with HA-mGluR2-mCitrine, or previously exposed to azF or vehicle and co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR2-TAG^{4.44}-mCitrine, HA-mGluR2-TAG^{4.56}-mCitrine, or HA-mGluR2-TAG^{1.37}-mCitrine (n = 4 independent binding assays per experimental condition). Density (B_{max}) was higher with HA-mGluR2-mCitrine, as compared to HA-mGluR2-TAG^{4.44}-mCitrine (+azF), HA-mGluR2-TAG^{4.56}-mCitrine (+azF), or HA-mGluR2-TAG^{1.37}-mCitrine (+azF), (*p* < 0.001 by Dunnett's *post hoc* test of one-way ANOVA). No statistically significant difference between densities of HA-mGluR2-TAG^{4.44}-mCitrine (+azF), HA-mGluR2-TAG^{4.56}-mCitrine (+azF), or HA-mGluR2-TAG^{1.37}-mCitrine (+azF) (*p* > 0.05 by Dunnett's *post hoc* test of one-way ANOVA). Density was higher with HA-mGluR2-TAG^{4.44}-mCitrine (+azF), HA-mGluR2-TAG^{4.56}-mCitrine (+azF) and HA-mGluR2-TAG^{1.37}-mCitrine (+azF), as compared to HA-mGluR2-TAG^{4.44}-mCitrine (-azF), HA-mGluR2-TAG^{4.56}-mCitrine (-azF), or HA-mGluR2-TAG^{1.37}-mCitrine (-azF), respectively (*p* < 0.001 by Student's *t*-test). No statistically significant difference between affinities (K_D) of [³H]LY341495 against HA-mGluR2-mCitrine, HA-mGluR2-TAG^{4.44}-mCitrine (+azF), HA-mGluR2-TAG^{4.56}-mCitrine (+azF), and HA-mGluR2-TAG^{1.37}-mCitrine (+azF) (*p* > 0.05 by Dunnett's *post hoc* test of one-way ANOVA). Data are mean ± s.e.m.

Table S2. Binding saturation curves with [³H]LY341495. Related to Figure 4.

| | mGluR3 | mGluR3-TAG ^{4.44} | |
|------------------------|--------------|----------------------------|------|
| | | +azF | -azF |
| B_{max} | 3255 ± 439.5 | 263.0 ± 47.92 | n.a. |
| K_D | 5.50 ± 0.89 | 3.09 ± 0.85 | n.a. |

B_{max} (fmol/mg prot); K_D (nM)

Radioligand binding saturation curves with [³H]LY341495 in membrane preparations of cells transfected with HA-mGluR3-mCitrine, or previously exposed to azF or vehicle and co-transfected with constructs encoding suppressor tRNA and azF aaRS, along with HA-mGluR3-TAG^{4.44}-mCitrine (n = 4 independent binding assays per experimental condition). Density (B_{max}) was higher with HA-mGluR3-mCitrine, as compared to HA-mGluR3-TAG^{4.44}-mCitrine (+azF), (*p* < 0.001 by Student's *t*-test). Non-linear regression analysis (binding saturation curve) was not applicable (n.a.) in HA-mGluR3-TAG^{4.44}-mCitrine (-azF) cells. No statistically significant difference between affinities (K_D) of [³H]LY341495 against HA-mGluR3-mCitrine and HA-mGluR3-TAG^{4.44}-mCitrine (+azF), (*p* > 0.05 by Student's *t*-test). Data are mean ± s.e.m.

Table S3: Primer Sequences. Related to STAR Methods.

| Mutant | Oligonucleotide Sequence |
|---|---|
| HA-mGluR2-Cys121Ala-mCitrine | Forward: 5'- GGCTCACGCCACATCGCGCCCGACGGCTCTTAT-3' Reverse: 5'- ATAAGAGCCGTCTGGGCGCGATGTGGCGTGAGCC-3' |
| HA-mGluR2-Ala ^{4.44} TAG-mCitrine | Forward: 5'- GATAAGTGCCAGGCAGATCTACACCTGTGAGGCAGGACT- 3' Reverse: 5'-AGTCCTGCCTCACAGGTGTAGATCTGC CTG GCA CTT ATC-3' |
| HA-mGluR2-Val569 ^{1.37} TAG-mCitrine | Forward: 5'-GGTGACAGGTCCCTAAGCCCAGGCATCGC-3' Reverse: 5'-GCGATGCCTGGGCTTAGGGACCTGTCACC-3' |
| HA-mGluR2-Ile693 ^{4.56} TAG-mCitrine | Forward: 5'-CGGGCCAGCTGCTCTAGGTGGTCGCCTGGCT-3' Reverse: 5'-AGCCAGGCGACCACCTAGAGCAGCTGGCCCG-3' |
| HA-mGluR3-Phe690 ^{4.44} TAG-mCitrine | Forward: 5'-CCCCAGTTCTCAGGTTTAGATCTGCCTGGGTCTG-3' Reverse: 5'-CAGACCCAGGCAGATCTAACCTGAGAACTGGGG-3' |