SUPPLEMENTAL FIGURE LEGENDS

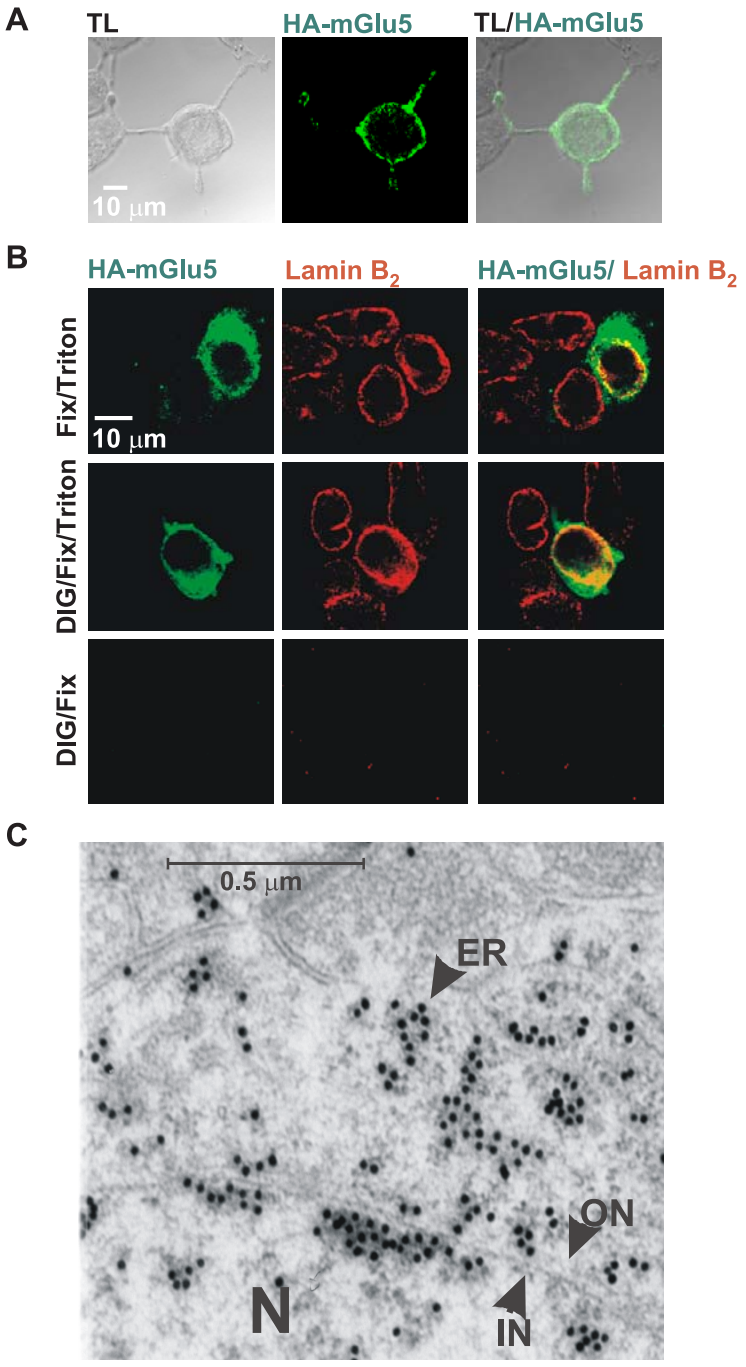
Fig. S1. Plasma membrane and nuclear localization of mGlu5. HEK-293 cells were transiently transfected with N-terminus HA-tagged mGlu5. *A*, *1st panel,* transmitted light (*TL*) image of transfected cell, *2nd panel*, HA staining (*green*) of same cell under non-permeabilized conditions, *3rd panel*, merged image. *B*, brief digitonin permeabilization of plasma membrane revealed presence of mGlu5 receptors on inner nuclear membrane. *Top panel, Fix/Triton*, cells were fixed and permeabilized with Triton-X-100 and processed for immunocytochemistry using the HA epitope-specific antibody (*green*) as well as an antibody directed against the inner nuclear membrane marker, lamin B_2 (*red*), merged images in yellow. *Middle panel, Dig/Fix/Triton*, brief plasma membrane permeabilization followed by fixation and then Triton-X-100 permeabilization to reveal inner nuclear membrane associated proteins. *Bottom panel, DIG-Fix*, digitonin permeabilization followed by fixation only. The lack of anti-lamin B_2 staining under Digitonin-Fix conditions shows the lack of antibody accessibility to the nucleus in the absence of Triton-X-100. All images represent single optical sections of 0.4 μm. *C*, electron micrograph of mGlu5 receptor immunogold labeling in rat visual cortex. Immunogold particles (*black dots*) are associated with inner (*IN*) and outer nuclear (*ON*) membrane as well as endoplasmic reticulum (*ER*). Electron microscopy was performed exactly as previously described (18).

Fig. S2. Cell-permeable mGlu5 agonist quisqualate activates intracellular receptors. *A*, anti-quisqualate antibody rapidly stains quisqualate (*Quis*)-treated HEK cells following 10 μM quisqualate exposure. *B*, [^{3}H]quisqualate uptake in HEK cells in the presence of indicated agonist and antagonists assayed in quadruplicate in three independent experiments. Study reveals that although quisqualate can be taken up into HEK cells, neither the LY antagonists nor the mGlu5 agonist DHPG compete for quisqualate uptake. In contrast, the sodium-dependent excitatory amino acid transport (EAAT) blocker, threo-β-benzyloxyaspartate (TBOA), inhibited about 70% of radiolabeled ligand uptake. Previously, we showed that the IC_{50} for L-cystine for [^{3}H]quisqualate plasma membrane transport in sodium-free buffer conditions was 112$\pm$29 μM (19). Thus both the cystine/glutamate exchanger and/or EAAT-mediated activity allow for intracellular quisqualate or glutamate uptake. Although without a radiolabeled analog, it is difficult to completely rule out the possibility that LY393053 and LY367366 may be taken up by other types of amino acid/carboxylate transport systems, it would appear at least that the EAATs and the cystine/glutamate exchanger are not involved. Similarly, DHPG did not affect quisqualate or glutamate uptake either (19; this paper), suggesting that it too is a nontransported ligand. *C*, representative traces are shown of cytoplasmic (*blue line*) or nuclear (*red line*) Ca^{2+} responses from wild type mGlu5 expressing HEK cells following bath addition of 100 μM DHPG (impermeable agonist), 40 μM LY393053 (impermeable antagonist), 10 μM quisqualate (*Quis*; permeable agonist) and 1 μM MPEP (permeable antagonist) when indicated (*black line*); $n = 31$. *D,* In the presence of the impermeable antagonist, DHPG cannot activate mGlu5 cell surface receptors as revealed by intracellular Ca^{2+} changes whereas the permeable agonist, quisqualate, can; $n = 24$. Oscillations are represented as the fractional change in fluorescence relative to the basal value.

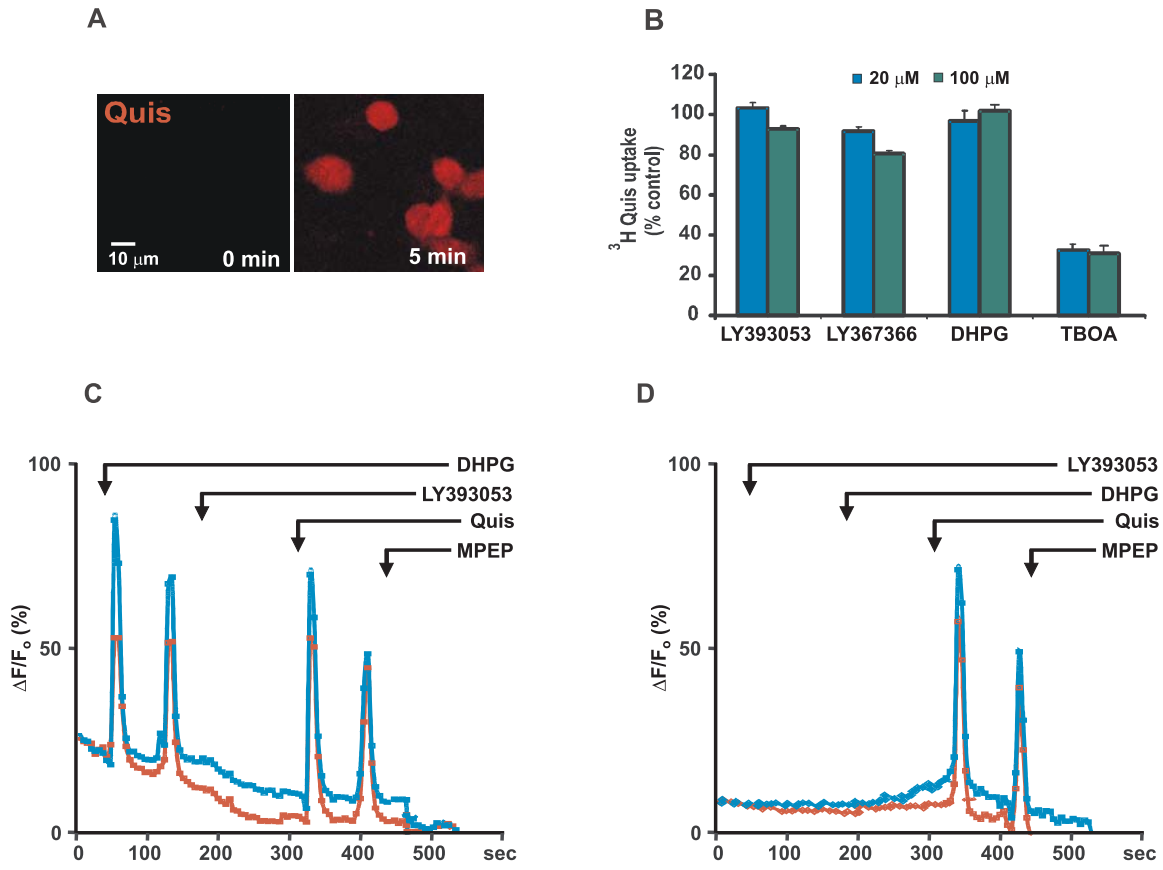
Fig. S3. Purity of nuclear preparations from mGlu5/HEK cells and striatal cells. To assess the purity of nuclear preparations from either the HEK or striatal cell types the technique of Eder and Bading (29) was utilized in which living cells were loaded with MitoTracker Red (*red*) which is excluded from the nucleus, as well as Hoechst (*blue*) which is sequestered in the nucleus *(A,B).* Using the described nuclear preparation protocol, no MitoTracker was seen in purified nuclear preparations stained with Hoechst in either HEK or striatal neurons verifying the purity of these organelles *(C,D).* As further evidence of the purity of the nuclei, addition of the impermeable

agonist, DHPG did not induce Ca^{2+} oscillations in purified mGlu5/HEK *(E,G)* or striatal *(F,H)* nuclei because it could not enter the nuclear lumen whereas it readily activated cell surface receptors (Fig. S2). Representative traces of nuclear (*red line*) Ca^{2+} responses in isolated mGlu5-expressing HEK nuclei (*E*) and endogenous mGlu5 expressing striatal nuclei (*F*) represented as the fractional change in fluorescence relative to the basal level. Isolated nuclei were treated with 100 μM DHPG, 10 μM quisqualate (*Quis*) and 1 μM MPEP when indicated (*black line*). Compiled data from maximum response of initial peak ($\Delta F/F_o$, %) from either mGlu5/HEK nuclei (*G*, $n = 9$) or striatal nuclei (*H*, $n = 11$) from three independent experiments. *, $p < 0.001$ when compared with basal Ca^{2+} responses.

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3