Supporting Information

Gonzalez et al. 10.1073/pnas.0911854107

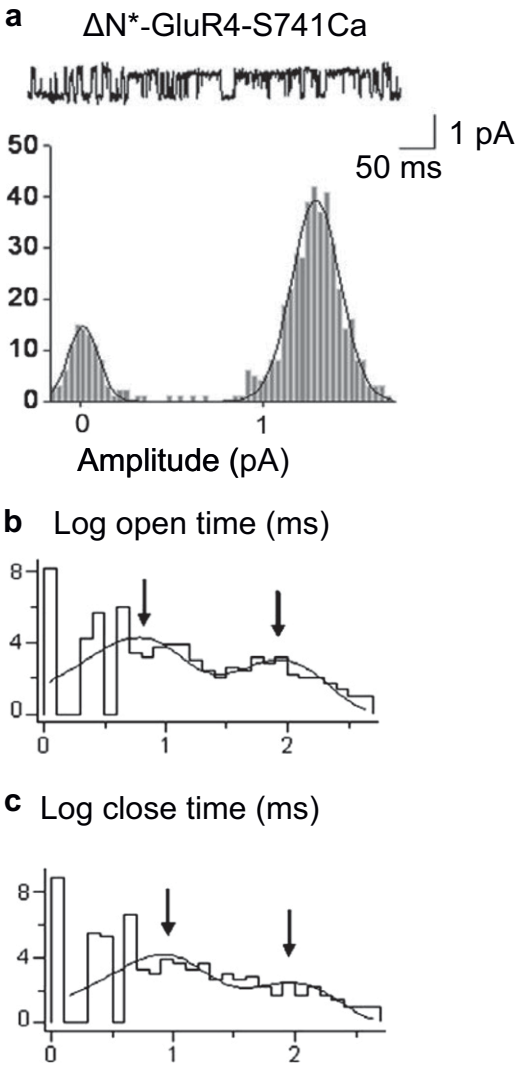

Fig. S1. Single-channel currents from bilayers of membrane preparations of modified glutamate receptors expressed in oocytes obtained using the same protocol as for the LRET investigations. (*A*) Amplitude histograms and representative single-channel traces are shown for terbium chelate and ATTO465-labeled ΔN*-GluR4-S741C receptors. Currents were recorded at 120 mV in the presence of 1 mM glutamate and 100 μM cyclothiazide. The primary conductance state is 9–12 pS. (*B*) Open and (*C*) close time distributions are shown for ΔN*-GluR4-S741C; two distinct close times and two distinct open times were obtained.

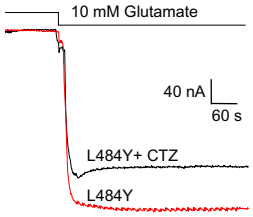

Fig. S2. Currents from GluR4-S742C-L484Y receptors expressed in oocytes. Currents were recorded in saturating concentrations of glutamate (10 mM) and in the presence and absence of cyclothiazide (CTZ) (100 μM).

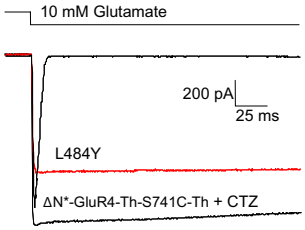

Fig. S3. Currents from ΔN*-GluR4-Th-S741C-Th receptors expressed in HEK-293 cells. Currents were recorded in the presence of saturating concentrations (10 mM) of glutamate, in the presence of 10 mM glutamate and 100 μM cyclothiazide (CTZ), and for the L484Y mutation.

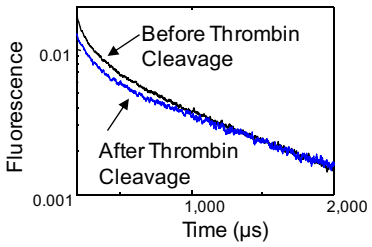

Fig. S4. The LRET lifetimes measured at 510 nm for donor:acceptor–tagged ΔN*-GluR4-Th-S741-Th before and after thrombin cleavage shown in the logarithmic *y* axis.

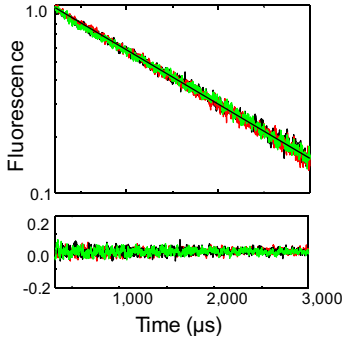

Fig. S5. LRET lifetimes shown in the logarithmic *y* axis for donor-only–tagged ΔN*-GluR4-S741 expressed in oocytes. Lifetimes were measured at 488 nm in the Apo (black), open (10 mM glutamate in the presence of 100 μM cyclothiazide; red), and desensitized (10 mM glutamate; green) states. Residuals for the LRET lifetime fits are shown in linear *y* axis.

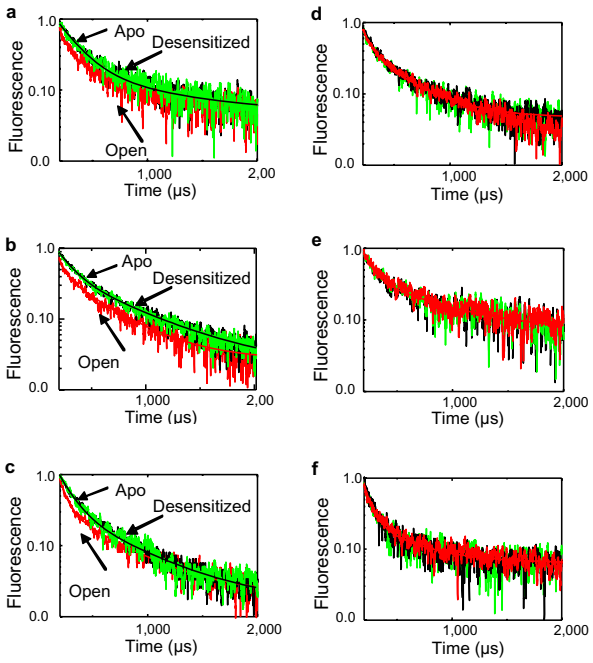

Fig. S6. The LRET lifetimes measured at 510 nm for donor:acceptor–tagged receptors shown in the logarithmic *y* axis. Lifetimes for (*A*) ΔN*-GluR4-G740C, (*B*) ΔN*-GluR4-S741C, (*C*) ΔN*-GluR4-S742C, (*D*) ΔN*-GluR4-G740C-L484Y, (*E*) ΔN*-GluR4-S741C-L484Y, and (*F*) ΔN*-GluR4-S742C-L484Y receptors expressed in oocytes in the absence of agonists (black), with 10 mM glutamate (green), and with 10 mM glutamate in the presence of 100 μM cyclothiazide (red).

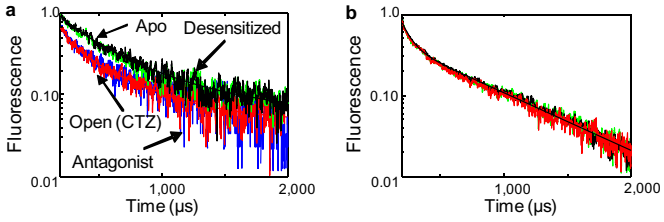

Fig. S7. The LRET lifetimes measured at 510 nm for donor:acceptor–tagged receptors shown in the logarithmic *y* axis. Lifetimes after subtraction of the residual lifetimes upon thrombin digestion are shown for (*A*) ΔN*-GluR4-Th-S741-Th and (*B*) ΔN*-GluR4-Th-S741-Th-L484Y receptors expressed in HEK-293 cells in the absence of agonists (black), with 10 mM glutamate (green), with 10 mM glutamate in the presence of 100 μM cyclothiazide (red), and in the presence of 200 nM antagonist ZK200775 (blue).

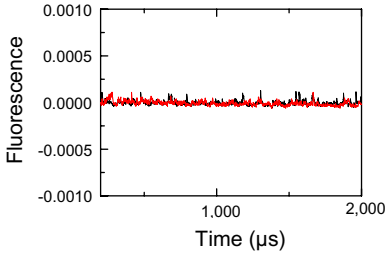

Fig. S8. The donor-only (black) and acceptor-only (red) fluorophore samples were probed at 510 nm.

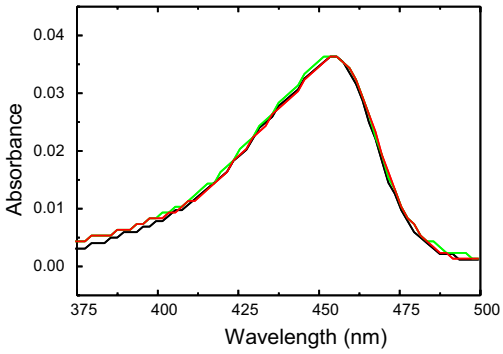

Fig. S9. Absorption spectrum of ATTO 465 (black) is not significantly affected by the addition of 10 mM glutamate (green) or 100 μM cyclothiazide (red).