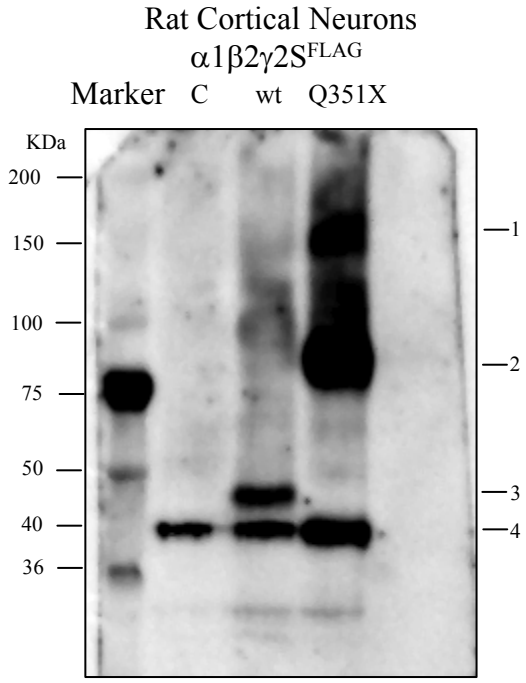
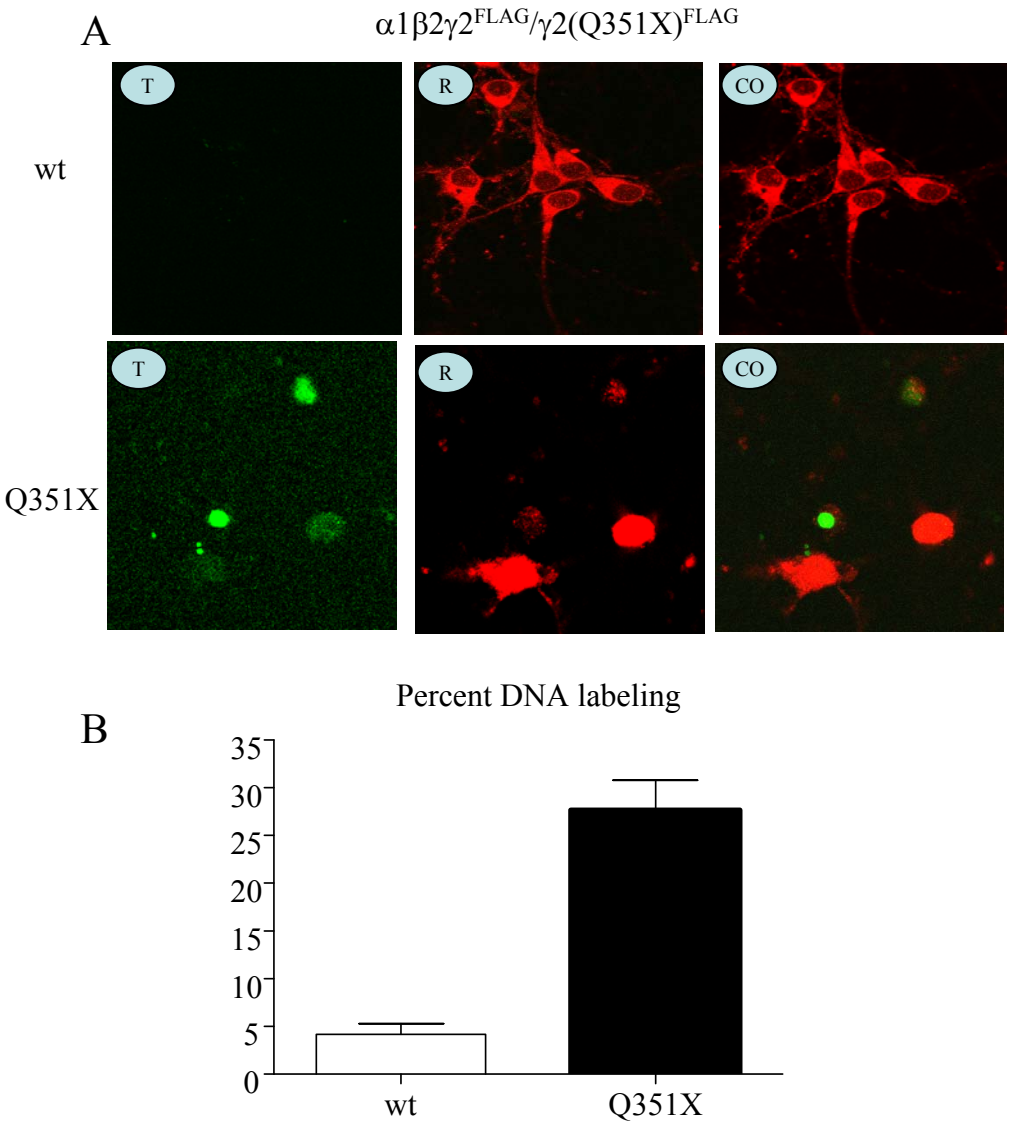


Supplementary Figure 1.

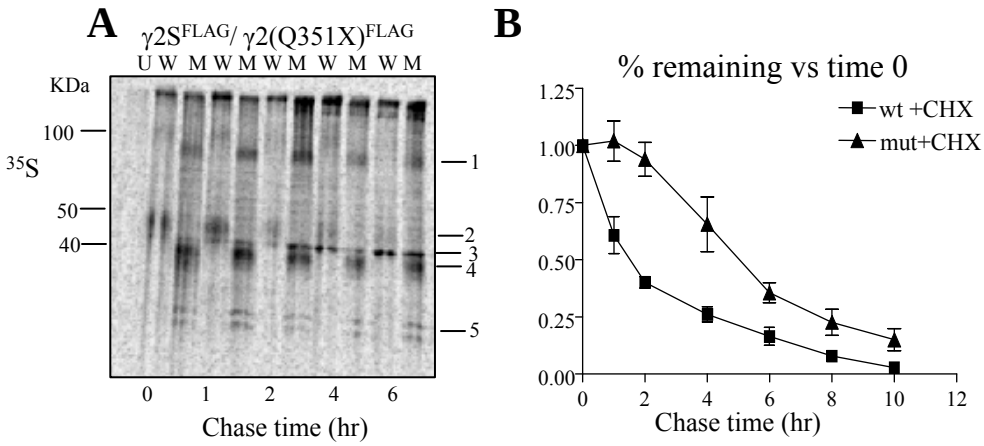
This is anenlarged view of Figure 1C. Rat cortical neurons were transfected with $\gamma 2S^{FLAG}$ and $\gamma 2S(Q351X)^{FLAG}$ subunits for 8 days. The immunopurified subunits were analyzed by immunoblot that was probed with an antibody against FLAG. Wild-type subunits only showed a single distinct band at the predicted mass of a $\gamma 2S^{FLAG}$ subunit (band 3). Mutant subunits formed a substantial amount of high molecular mass protein complex at ~80 KDa (band 2) and ~ 160 KDa (band 1) in addition to a distinct band at the predicted mass of a $\gamma 2S(Q351X)^{FLAG}$ subunit (band 4), which overlapped the nonspecific band seen in untransfected neurons in the 4-10% gradient gel.

Supplementary Figure 1



Supplementary Figure 2.
Rat cortical neurons were transfected with $\alpha1\beta2\gamma2S^{FLAG}$ and $\gamma2S(Q351X)^{FLAG}$ subunits for 8 days. The neurons were then fixed, permeabilized and immunostained with anti-FLAG polyclonal antibody and TUNEL (TdT-mediated dUTP Nick-End Labeling) assay with the DeadEnd Fluorometric TUNEL system (Promega). The fluorescein-12-dUTP labeled DNA and Cy5 conjugated FLAG antibody were then visualized directly under confocal microscopy. DNA was labeled by FITC (green), and FLAG was visualized by Cy5 (red). (A) Representative images of TUNEL and FLAG staining in neurons transfected with wt or mutant receptors are presented. T stands for TUNEL labeling, R stands for receptor with FLAG labeling, and CO stands for colocalized images of T and R. (B) The total TUNEL (FITC) positive neurons that were co-registered with Cy5 were quantified (4.2±1.1 vs 27.6 ± 3.1 ($p < 0.0001$) (F). Data were represented as mean ± SEM from 6 experiments.

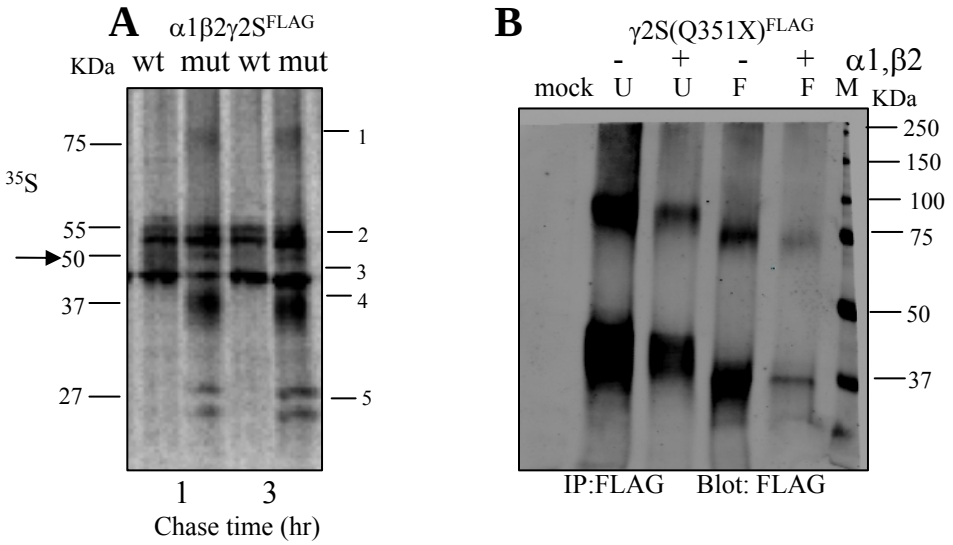
Supplementary Figure 2



Supplementary Figure 3.

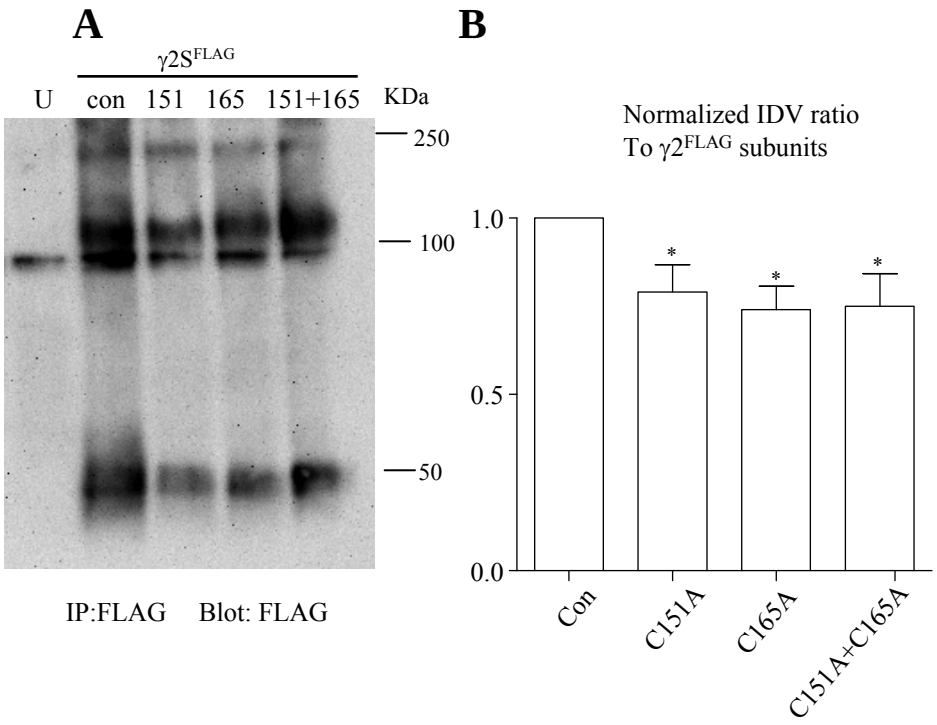
The increased radioactivity of the mutant γ2S(Q351X)FLAG subunit in the early time points was attenuated when protein synthesis was blocked. **[**^{35}S] pulse labeled untransfected cells (U) or cells transfected with γ2S^{FLAG} (W) or γ2S(Q351X)^{FLAG} (M) subunits were lysed, immunopurified and analyzed by SDS-PAGE. **(A)** After 20 min of labeling, cells were chased for the indicated times with the addition of cycloheximide (100 μg/ml) in the chase medium to block protein synthesis. Band 1 indicates the high molecular mass protein complex, band 2 indicates γ2S^{FLAG} monomers, band 3 indicates the nonspecific band, band 4 indicates γ2S(Q351X)^{FLAG} subunit monomers. Band 5 indicates the unknown double bands that are reproducibly pulled down with the mutant γ2S(Q351X)^{FLAG} subunit in our pulse chase experiments. **(B)** The percent radioactivity remaining was plotted versus the amount of radioactivity measured at time 0 for either wild-type or mutant subunits from pooled data (n = 7 for 0, 1, 2, 4, 6 hr of chase time points. N = 4 for 8 and 10 hrs of chase time points.

Supplementary Figure 3



Supplementary Figure 4. These are the enlarged views of Figure 4C and 4D. **(A)** Cells expressing wild-type and mutant γ2S(Q351X)^{FLAG} subunits with α1 and β2 subunits were pulse chased for 1 and 3 hr and lysed for immunopurification and SDS-PAGE. Band 1 represents the high molecular mass protein complex and band 4 represents unaggregated γ2S(Q351X)^{FLAG} subunit monomers. There were multiple bands in the band 2 area that indicate the wild-type γ2S^{FLAG} and coimmunoprecipitated α1 and β2 subunits. The arrow points to γ2S^{FLAG} subunit band. Band 3 indicates a nonspecific band that was frequently pulled down with γ2S^{FLAG} subunits in our pulse chase experiments. Band 5 indicates the unknown double bands that were reproducibly pulled down with the mutant γ2S(Q351X)^{FLAG} subunit but not with the wild-type γ2S^{FLAG} subunits. Band 5 was not present in Fig 4 C due to their unknown identity. **(B)** Cells expressing mutant γ2S(Q351X)^{FLAG} subunits without (3 μg cDNA) or with α1 and β2 subunits (1 μg cDNA each) were lysed for immunopurification and treated (F) or untreated (U) with PNGase F and fractionated by SDS-PAGE. M stands for marker.

Supplementary Figure 4



Supplementary Figure 5. The cysteines in the signature Cys-loop destabilized the wild-type γ2S subunit but to a lesser extent than to the mutant γ2S(Q351X) subunit. Total lysates of HEK 293T cells untransfected (U) or transfected with the wild-type γ2S^{FLAG} subunits (con) or with γ2S^{FLAG} subunits containing mutations in C151 (151), C165 (165) or both (151+165). Wild-type γ2S subunits without or with mutation of the cysteines were immunopurified using anti-FLAG antibody and analyzed by SDS-PAGE (**A**). **(B)** The total IDVs in A for γ2S^{FLAG} subunits without or with mutation of the cysteines were plotted. The data were normalized to the γ2S^{FLAG} subunit without mutant cysteines. The total IDV of the wild-type γ2S^{FLAG} subunits was arbitrarily taken as 1 (n = 4 from four different transfections, *p < 0.05 vs γ2S^{FLAG} subunit).

Supplementary Figure 5