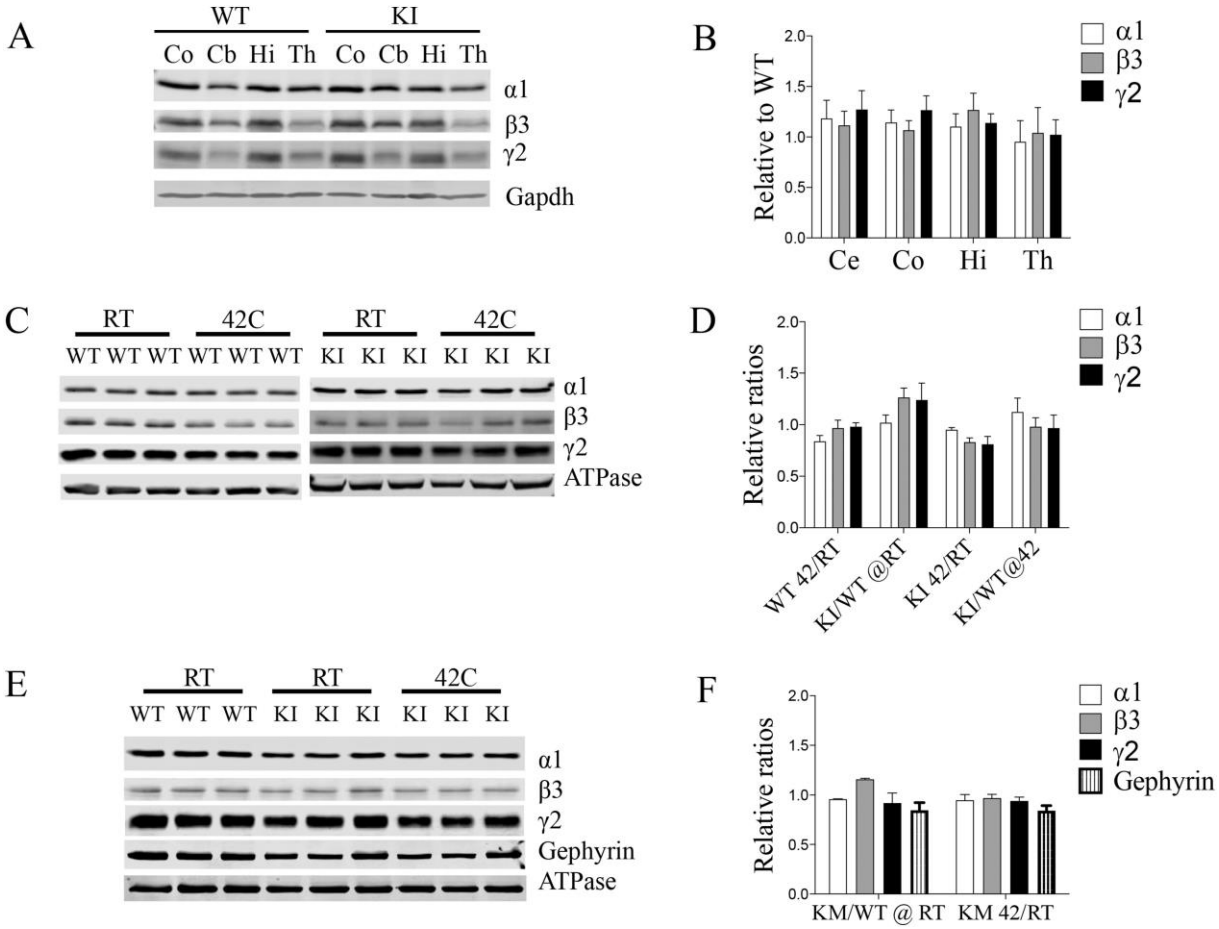

Supplemental Fig. 2. Biogenesis and trafficking of receptors containing mutant γ2(K328M) subunits and normal partnering subunits in *Gabrg2*$^{+/K328M}$ KI mice were not affected by the K328M substitution. The K328M amino acid substitution did not alter $GABA_A$ receptor subunit expression, surface trafficking, or synaptosomal distribution. **A.** Whole cell lysates from different brain regions [cortex (co), cerebellum (ce), hippocampus (hi) and thalamus (Th)] were collected from KI and WT littermates and subjected to SDS-PAGE and immunoblotted with anti-α1, −β3, and -γ2 subunit antibodies. GAPDH served as a loading control. **B.** Band intensities of α1, β3, and γ2 subunits were normalized to the GAPDH signal, then KI was compared to WT (*n* = 3 pairs). **C.** Brain plasma membrane proteins were isolated from mouse brain at room temperature (RT) or 42 °C incubator were analyzed by SDS-PAGE and immunoblotted with anti-α1, −β3, and -γ2 subunit antibodies. Na^+/K^+ ATPase served as a loading control. **D.** Band intensities of α1, β3, and γ2 subunits were normalized to the ATPase signal, then did relative comparison (n = 3 pairs). Relative expression levels of α1, β3, and γ2 subunits from left to right: WT at 42 °C to room temperature (WT 42C/RT); KI to WT at room temperature (KI/WT@RT); KI at 42 °C to room temperature (KI 42C/RT); KI to WT at 42 °C (KI/WT@42C). **E.** Brain synaptosomal proteins isolated from mice at room temperature or 42 °C were analyzed by SDS-PAGE and immunoblotted with anti-α1, −β3, and -γ2 subunit and anti-gephyrin antibodies. Na^+/K^+ ATPase served as a loading control. **F.** Band intensities of α1, β3, and γ2 subunits and

gephyrin were normalized to the ATPase signal, then presented as a relative comparison (n = 3 pairs). Relative expression levels of α1, β3, and γ2 subunits from KI to WT at room temperature (KM/WT@RT) and KI at room temperature and 42 °C (KM 42C/RT). Data were presented as mean ± SEM. Student's *t*-tests were used in each panel.