

Figure S1. Survival, the body weight of CASK^{floxed/floxed} mice. **a**, Survival of CASK floxed mice obtained from interbreeding with heterozygote CASK floxed mice (CASK floxed/Y and CASK floxed/X). **b**, Plot of the body weight (means \pm SEMs) of littermate male WT (X/Y), heterozygous female (floxed/X), and fCASK (floxed/Y, floxed/floxed).

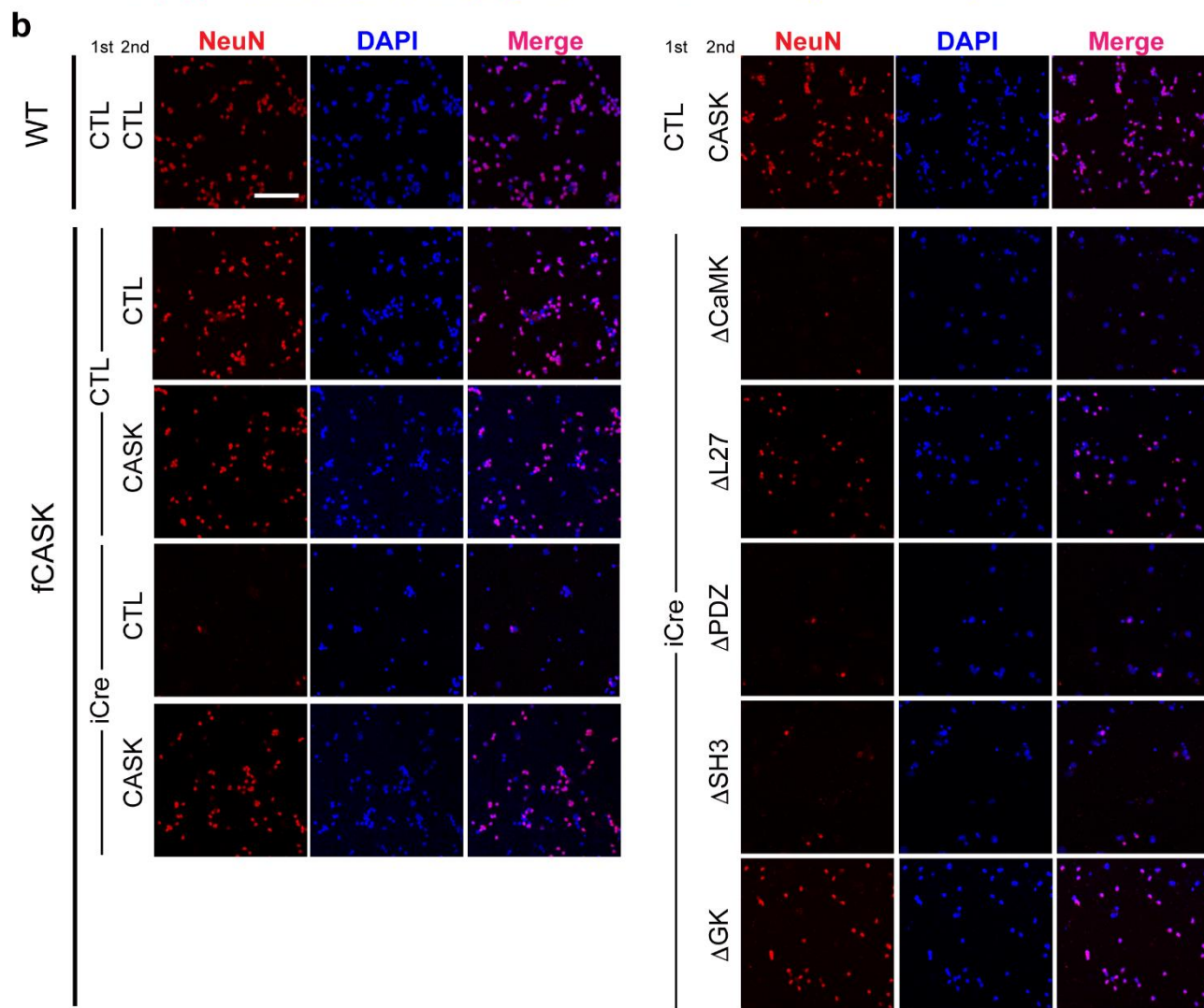
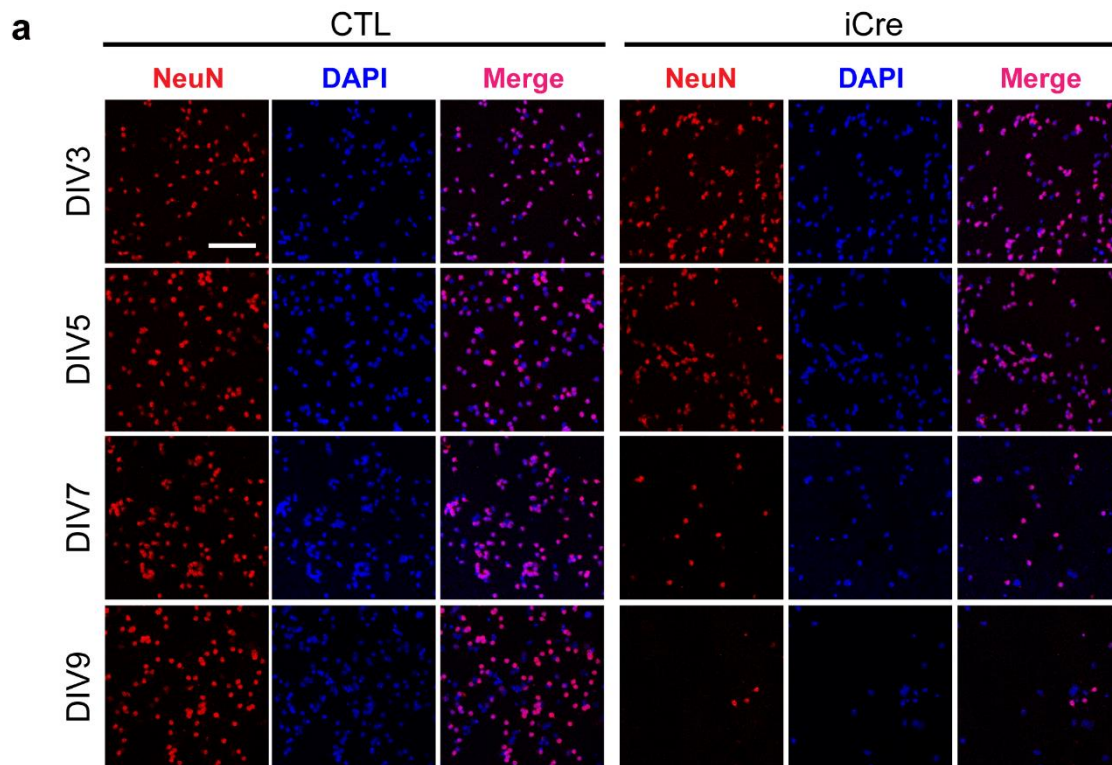


Figure S2. NeuN staining of cultured CGCs from wild-type and CASK floxed mice infected with lentivirus-iCre and/or CASK rescue constructs. a, Cultured fCASK CGCs are infected with lentivirus-control (CTL) or lentivirus-iCre (iCre) fixed and stained at DIV3, 5, 7, and 9. b, Cultured CGCs from WT and fCASK mice infected with lentivirus-control (CTL) or lentivirus-iCre (iCre) together with lentivirus-CASK (full-length or deletion mutants) stained with an antibody against NeuN (red) and DAPI (blue) at DIV9. Scale bar = 100 μ m.

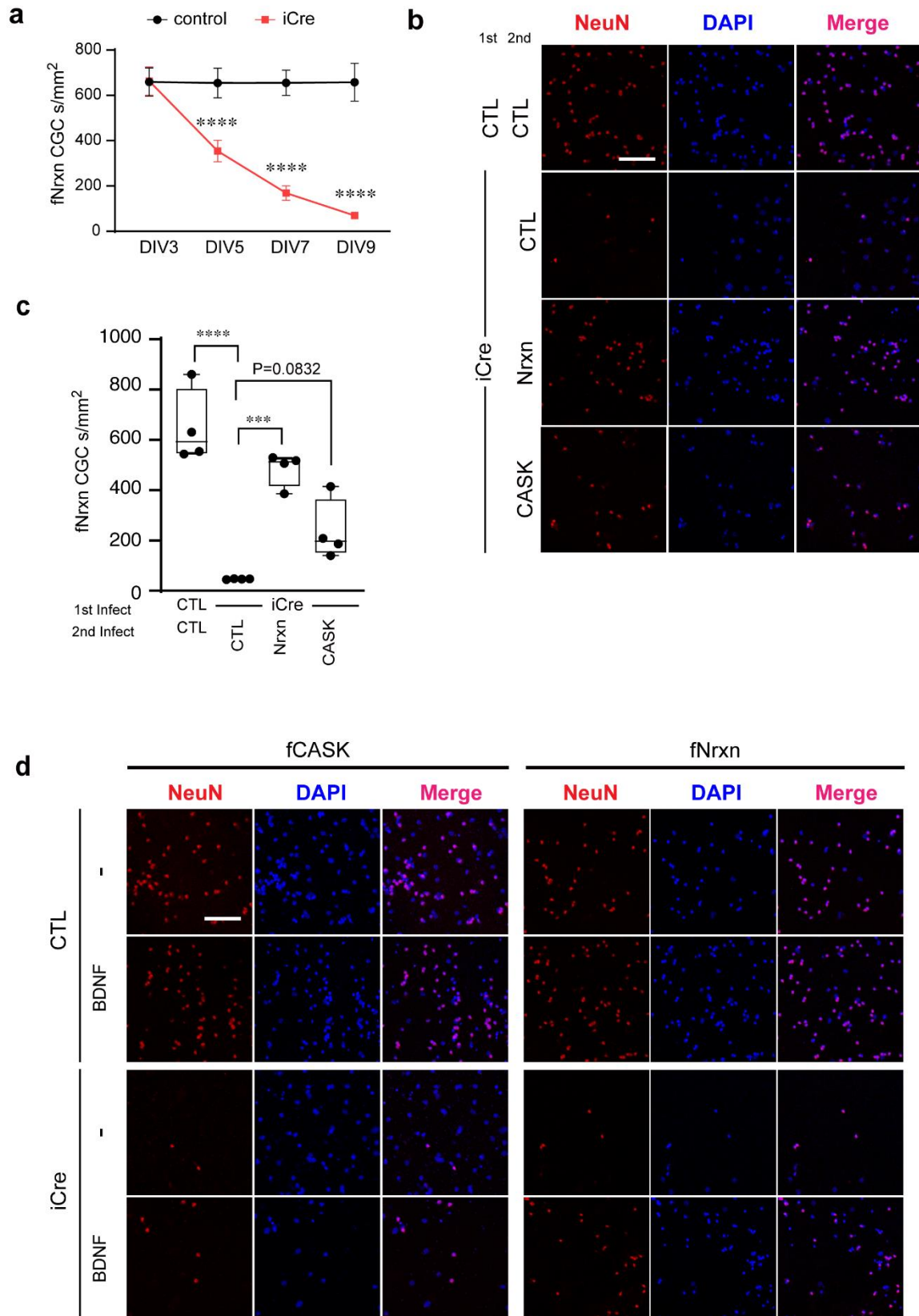


Figure S3. BDNF does not rescue the cell survival defects in cultured CASK KO CGCs. **a**, Cultured fNrxn CGCs are infected with lentivirus-iCre. The cells are stained with an antibody against NeuN and DAPI. The number of NeuN-positive CGCs is quantified. The values are averages of 3 points for each well and 6 wells for each sample. **** $P < 0.0001$; two-way ANOVA. **b**, Cultured fNrxn CGCs are infected with lentivirus-iCre together with lentivirus-Nrxn or lentivirus-CASK. The cells are stained with an antibody against NeuN (red) and DAPI (blue). Scale bar = 100 μm . **c**, Quantification of the number of NeuN-positive CGCs in **b**. The values are averages of 3 points for each well and 4 wells for each sample. Co-infection with lentivirus-CASK fails to rescue the CGC cell-death. **d**, BDNF is added to cultured CGCs prepared from fCASK or fNrxn mice infected with lentivirus-control (CTL) or lentivirus-iCre (iCre). The cells are stained with the antibody against NeuN (red) and DAPI (blue). Scale bar = 100 μm .

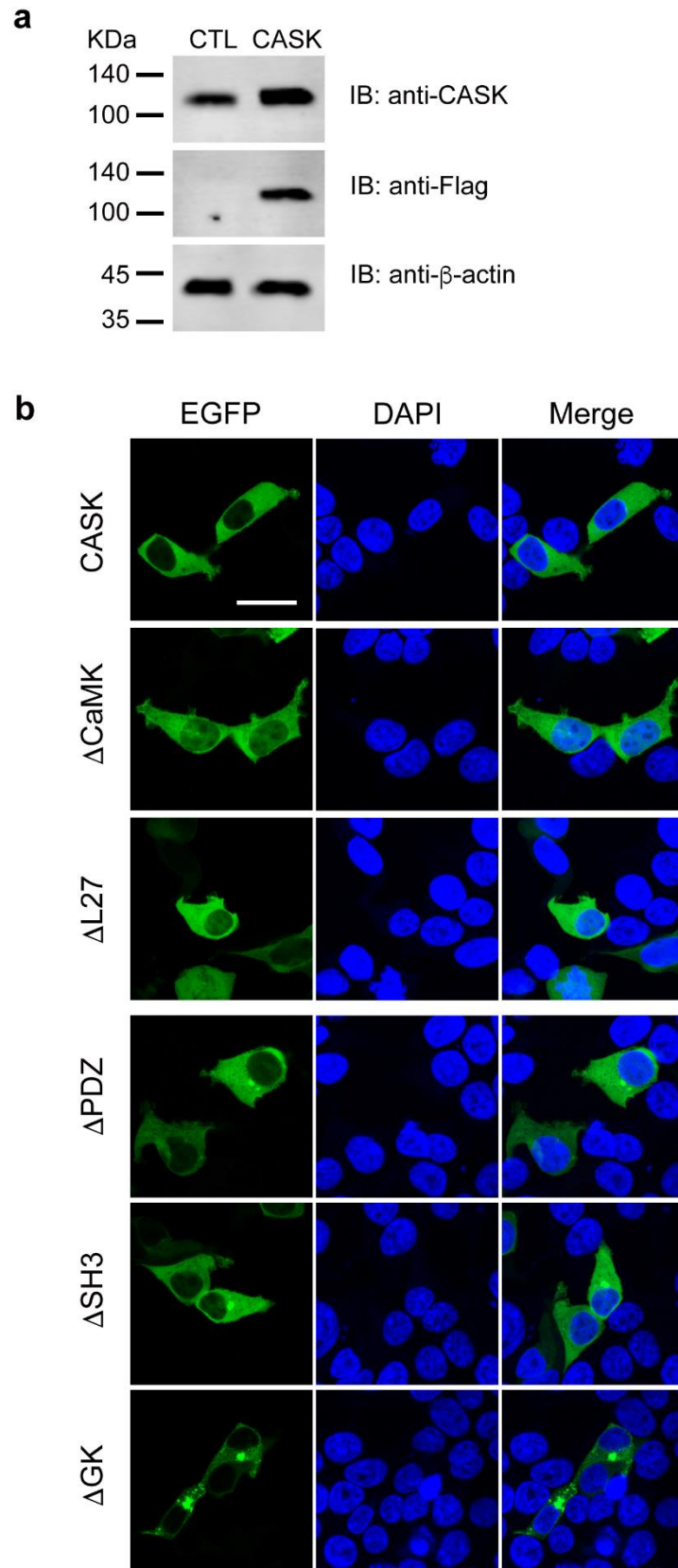


Figure S4. Expression of the deletion mutant of CASK. **a**, Western blot images for examining the expression of Flag-tagged full-length CASK protein in cultured CGCs introduced with lentiviral infection. Cell lysates from lentivirus-control (CTL) or lentivirus-Flag-CASK (CASK) infected CGCs are blotted by antibodies against CASK, Flag, and β -actin. **b**, Expression of full-length and deletion mutants of CASK in HEK293T. HEK293T cells transfected with EGFP-fused full-length or deletion mutants of CASK expression vectors are fixed 1 day after transfection and stained with DAPI. Scale bar = 20 μ m.

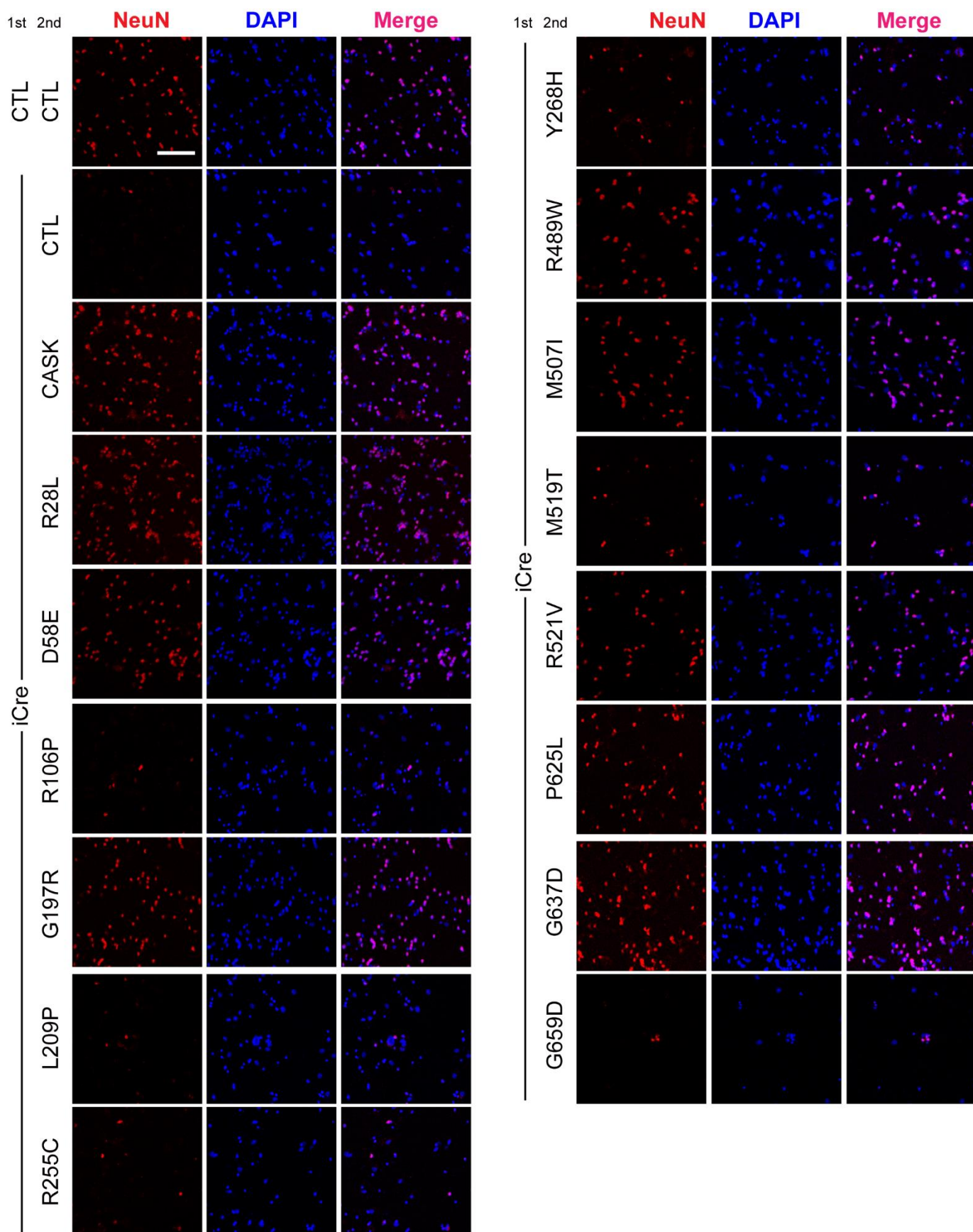


Figure S5. NeuN staining of CGCs infected with lentivirus-iCre and/or CASK missense mutations. Cultured CGCs are infected with lentivirus-iCre together with lentivirus-CASK (WT or missense mutants). The cells are stained with the antibody against NeuN (red) and DAPI (blue). Scale bar = 100 μ m.

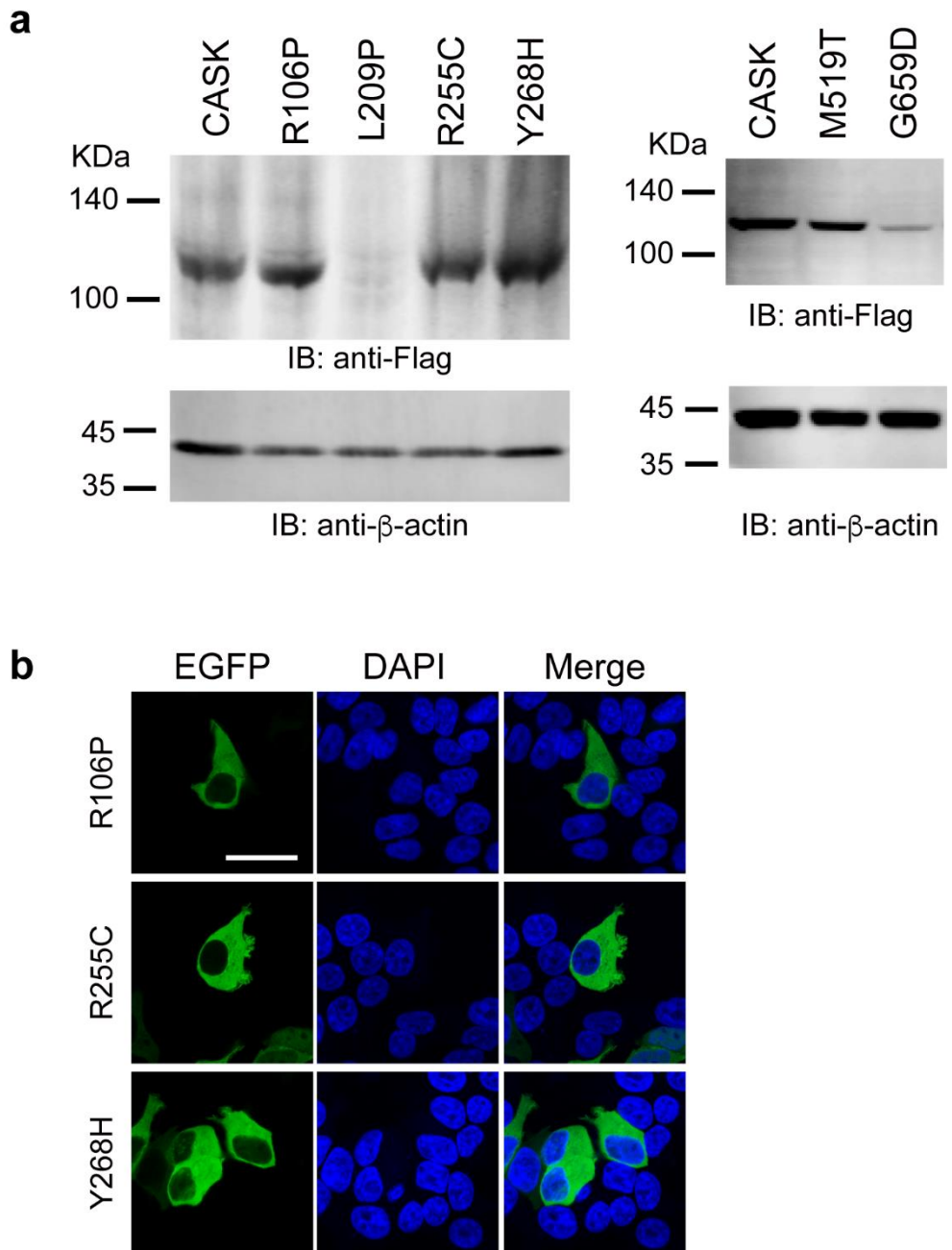


Figure S6. Expression of missense mutant of CASK. **a**, Western blot analysis of the expression of missense mutants of CASK. Cell lysates from WT CGCs infected with lentivirus expressing Flag-tagged CASK variants are blotted with anti-Flag and β -actin antibodies. **b**, Expression of missense mutants of CASK in HEK293T. HEK293T cells expressing EGFP fused CASK having R106P, R255C, or Y268H mutations are fixed 1 day after transfection and stained with DAPI. Scale bar = 20 μ m.

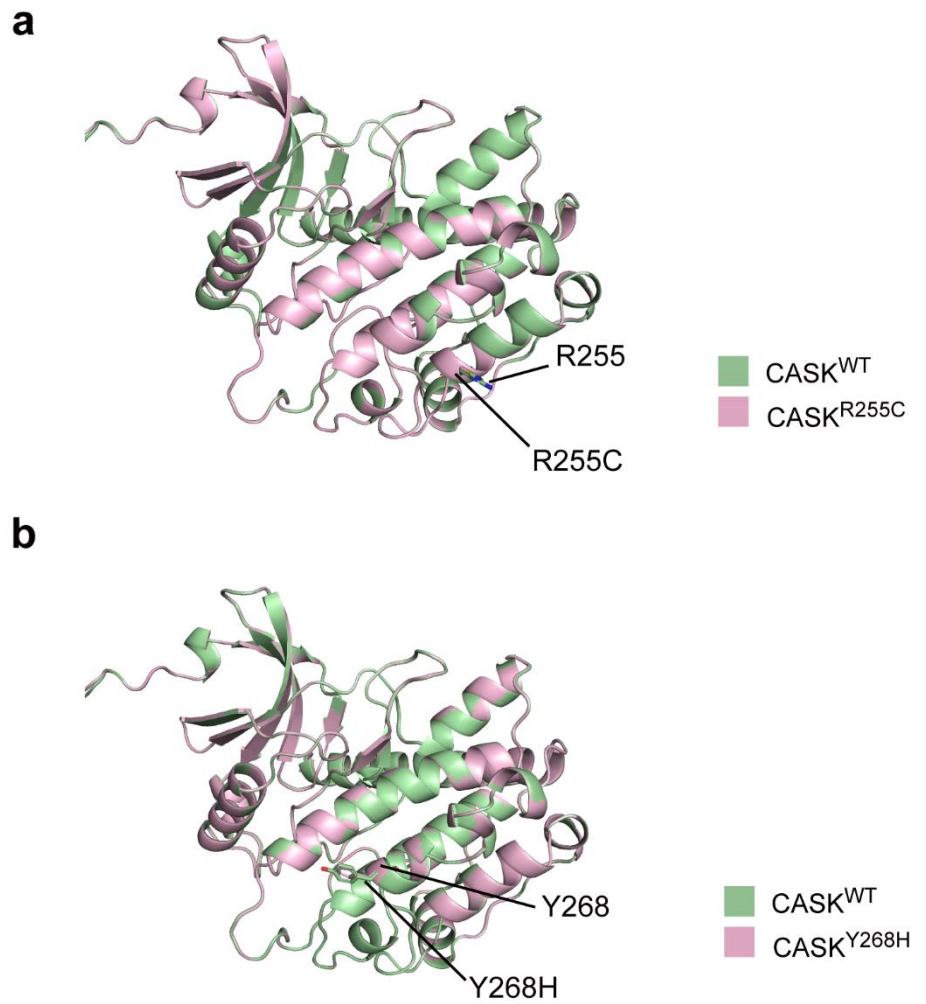


Figure S7. a, Superimposed image of wild-type (Green) and R255C mutant (Pink) CASK CaMK domain modeled by AlphaFold2.2. **b**, Superimposed image of wild-type (Green) and Y268H mutant (Pink) CASK CaMK domain modeled by AlphaFold2.2.