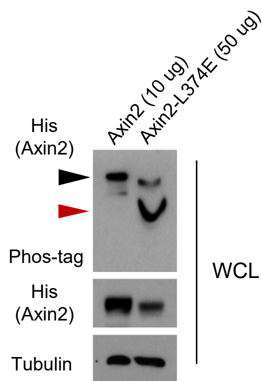
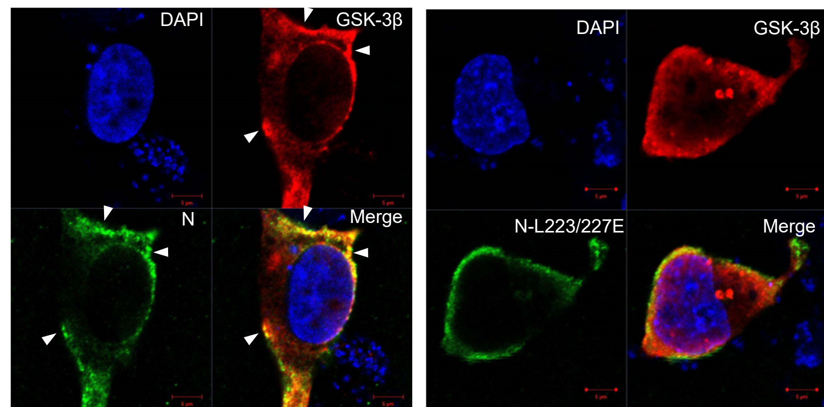
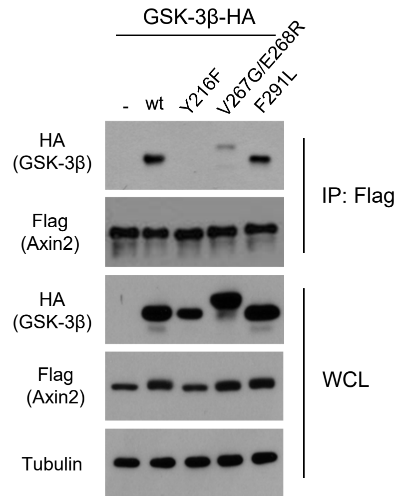
Molecules and Cells

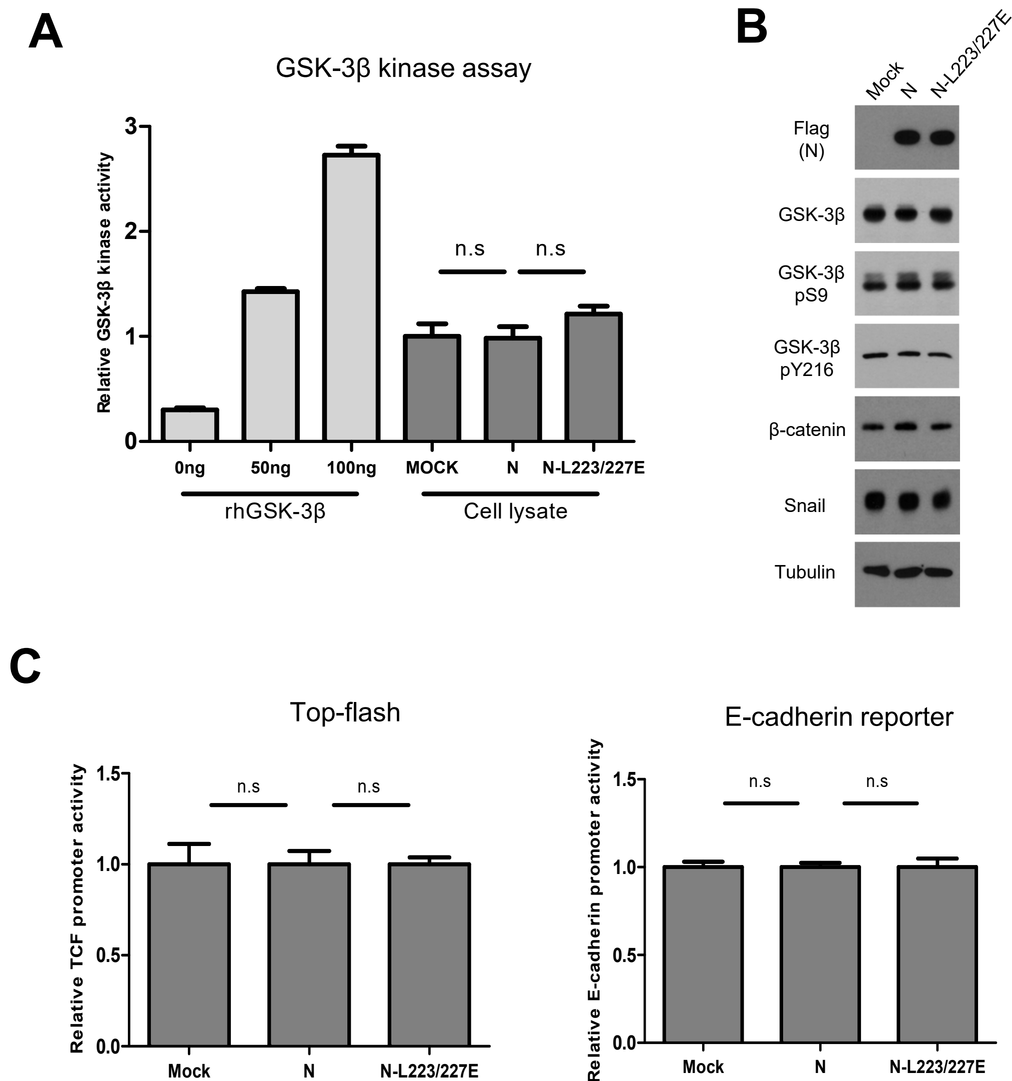




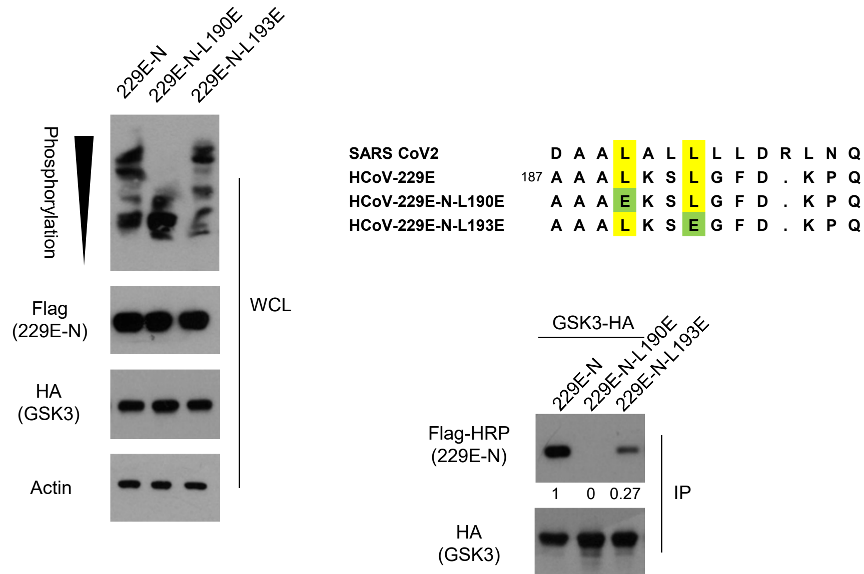
Supplementary Fig. S1. Axin2 share GSK-3β site with N. (A) Wt or GID mutant Axin2 expression vectors were transfected into 293 cells, and the lysates were subjected to western blot and Phos-tag gel analysis to determine protein abundance and mobility shift by phosphorylation status of Axin2, respectively. Black arrowhead indicates phosphorylated band of Axin2, and red arrowhead represents dephosphorylated Axin2. (B) Confocal images of ancestral N or L223/227E mutant (green) and GSK-3β (red) in 293 cells. Arrowheads indicate co-localized foci of N and GSK-3β in condensate-like structures. Nuclear staining with DAPI and (blue). Scale bars = 5 μm.



Supplementary Fig. S2. Axin2 share GSK-3β site with N. Flag-tagged Axin2 was co-transfected with wt (wild type) or Axin-binding mutants (Y216F, V267G/E268R, F291L) GSK-3β expression vectors in 293 cells. Following immunoprecipitation (IP) with anti-flag, GSK-3β binding was determined using western blot analysis. WCL, whole cell lysate.



Supplementary Fig. S3. N protein does not affect endogenous GSK-3 activity and subsequent Wnt signaling and Snail abundance. (A) 293 cells were transfected with N or L223/227E mutant N, and endogenous GSK-3 kinase activity was determined. rhGSK-3 in the commercial kit served as the positive control. (B) N or L223/227E mutant N was overexpressed in 293 cells, and the lysates were subjected to western blot analysis to determine the phosphorylation status of GSK-3β and protein abundance of β-catenin and Snail. Tubulin served as the loading control. (C) Canonical Wnt and E-cadherin transcriptional activity by N or L223/227E mutants were assessed with the Top-flash having 7× TCF/LEF binding sites (left) or Ecad(-108)-Luc, having the E-cadherin proximal promoter sequence from nt -108 to +125 (right). The reporter activity was normalized with co-transfected SV40-renilla (1 ng) with a dual-luciferase assay system. n.s, not significant.



Supplementary Fig. S4. Substitution of Leu to Gly in putative GSK-3 binding region of 229E-N decreased GSK-3 binding and N phosphorylation. The 229E-N or 229E-N-L190E or 229E-N-L193E were transfected into 293 cells and the lysates were subjected to Phos-tag gel analysis to determine N phosphorylation status (left). Black arrowhead indicates number of phosphorylation residues of 229E-N on a Phos-tag gel analysis. Interaction between 229E-N or its mutants and GSK-3β were determined following immunoprecipitation (IP) with anti-HA beads (right). Whole cell lysate (WCL) serves as input abundance for IP.