Supplemental Table and Figure Legends and Methods

Supplemental Figure 1

GSK-3 C-terminal deletion mutants exhibit impaired ability to suppress gene expression of Wnt target Axin2 - GSK-3$\alpha^{-/-}$ GSK-3$\beta^{-/-}$ embryonic stem cells (obtained from Dr. Brad Doble, McMaster University, and Dr. James Woodgett, University of Toronto), which display constitutive active Wnt signaling (42), were transfected with 1 μg GSK-3 construct. After 48 hours, RNA was isolated and subjected to reverse transcription. Resulting cDNA was then analyzed by quantitative PCR. Expression of WT GSK-3α (Figure S1) suppressed transcriptional activation of Wnt target gene Axin2 compared to empty vector (Vec). However, GSK-3 C-terminal deletion, αΔCT-4 was impaired in its ability to suppress Axin2 transcription. As expected, inactive point mutant αK148R was unable to suppress Wnt signaling, while αY279F retained activity and suppressed Wnt activation comparable to WT GSK-3α. A similar trend was observed with GSK-3β (data not shown). (RQ, relative quantity; WT, wild type; Δ, deleted; CT, C-terminus)

Supplemental Figure 2

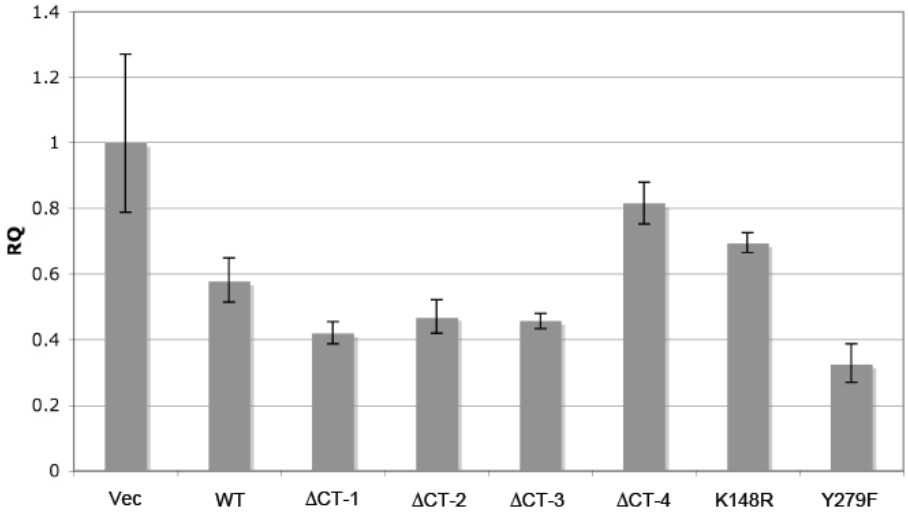
GSK-3 C-terminal deletion mutants mislocalize - GSK-3 constructs were directionally cloned into Gateway destination vector pCSCherry DEST (obtained from Dr. Nathan Lawson, University of Massachusetts Medical School), which generated N-terminal Cherry-tagged proteins. HeLa cells were transiently transfected with 1 μg of an N-terminal Cherry-tagged GSK-3 construct in a 6-well plate. Transfected cells were transferred to coverslips in a 24-well plate and were subsequently fixed with 4% paraformaldehyde (Polysciences, Inc.) followed by permeabilization with 0.1% Triton-X100 (Sigma). Coverslips were mounted using VECTASHIELD mounting media with DAPI (Vector Laboratories). Localization was visualized at 63X magnification with a Leica DMIRB microscope (Leica Microsystems). WT GSK-3α (Figure S2A) and WT GSK-3β (Figure S2B) localized largely in a diffuse pattern in the cytoplasm. Localization of C-terminal deletion mutants αΔCT-4 and βΔCT-4 differed dramatically and exhibited aggregate-like inclusions, which often localized to the perinuclear region. The mislocalization of αΔCT-4 and βΔCT-4 did not appear to be due to the loss of catalytic activity or the lack of $Tyr^{279/216}$ phosphorylation as point mutants αK148R and βK85R and αY279F and βY216F point mutants localized similar to WT GSK-3 (data not shown). (WT, wild type; Δ, deleted; CT, C-terminus)

Supplemental Figure 3

GSK-3 C-terminal deletion mutants exhibit reduced half-lives - HEK 239T cells were transiently transfected with GST-tagged GSK-3 constructs. Twenty-four hours after transfection, cells were pulsed with 85 uCi ^{35}S-methionine labeled media (Perkin Elmer) per well for 15 minutes and subsequently chased for 0 minutes, 6 hours, 12 hours, and 24 hours. Equal amounts of protein lysate from each time point was purified on glutathione sepharose and separated by Tricine-SDS-PAGE. After electrophoresis, the gel was dried and exposed to a phosphor screen (Amersham Biosciences) for 24 - 48 hours. Images were collected from a Typhoon 9400 variable mode imager (Amersham Biosciences) and analyzed using ImageQuant software (Amersham Biosciences). Although C-terminal deletion resulted in a reduction in protein half-lives for both GSK-3α (Figure S3A) and GSK-3β (Figure S3B), constitutive protein expression under the CMV promoter maintained steady-state protein levels. Thus, protein instability is unlikely to account for the loss of activity we observed. Interestingly, αΔCT3 exhibited a slightly longer half-life than WT GSK-3α suggesting that αΔCT3 gains conformational stability. However, this stabilized structure did not result in an active enzyme and appears to be an isoform-specific phenomenon

as it was not noted with GSK-3β. The significance of this observation requires further investigation. (vec, vector; WT, wild type; Δ, deleted; CT, C-terminus)

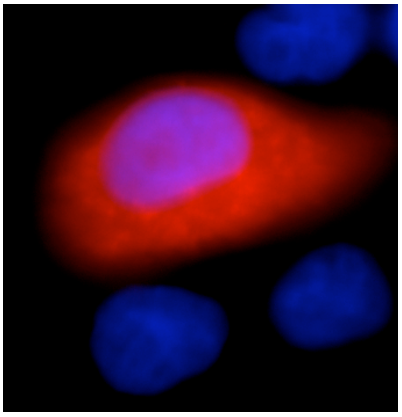
Supplemental Figure 1



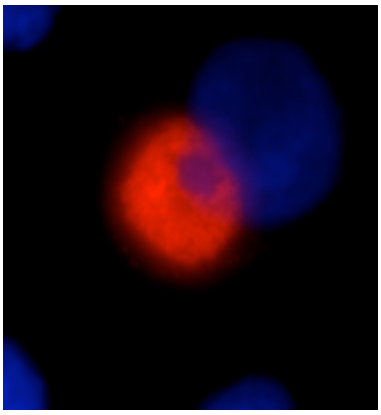
Supplemental Figure 2

A)

GSK-3α WT

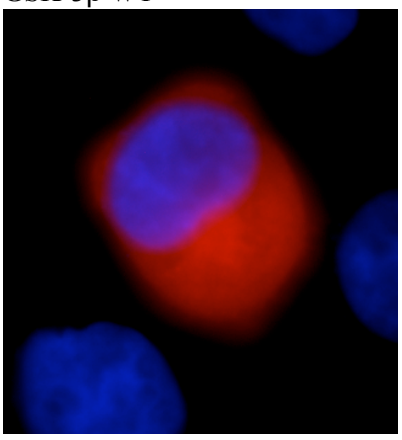


GSK-3α ΔCT-4

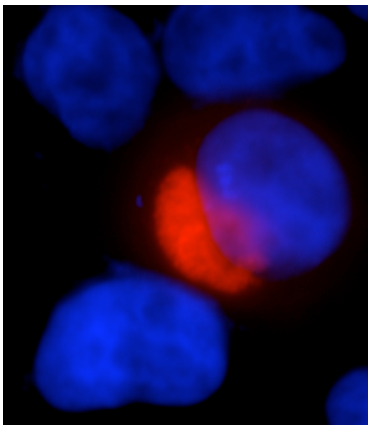


B)

GSK-3β WT

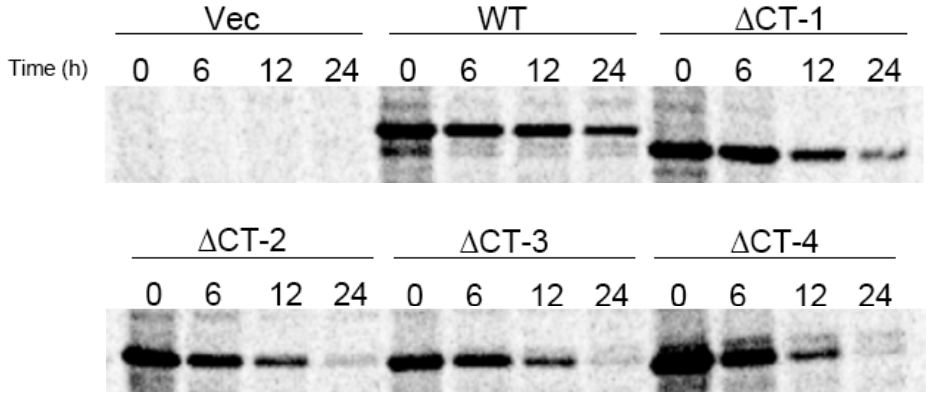


GSK-3β ΔCT-4



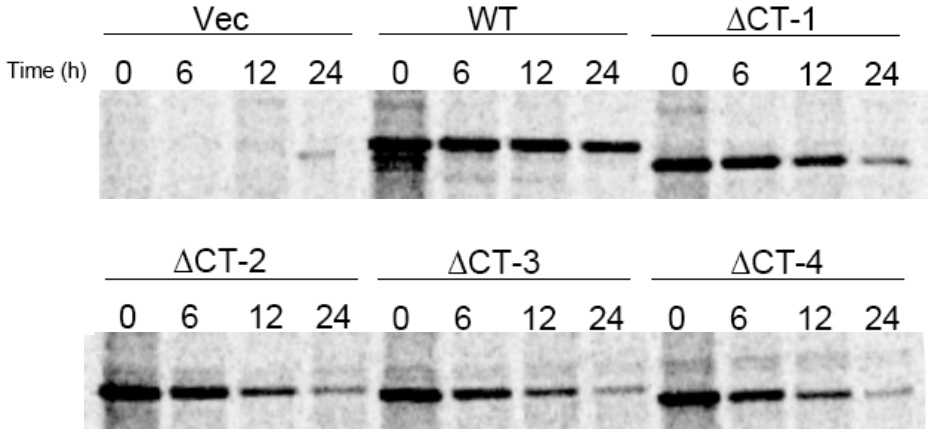
Supplemental Figure 3

A)



	WT	ΔCT-1	ΔCT-2	ΔCT-3	ΔCT-4
Half-life (min)	289 ± 17	224 ± 7	169 ± 12	315 ± 29	173 ± 4

B)



	WT	ΔCT-1	ΔCT-2	ΔCT-3	ΔCT-4
Half-life (min)	630 ± 23	347 ± 17	289 ± 24	224 ± 36	210 ± 38

Supplemental Table 1

Oligonucleotides used for site-directed mutagenesis. Underlined sequences denote the mutated codon(s).

Point Mutation	Sense (5′ → 3′)	Antisense (5′ → 3′)
GSK-3α S21A	CAGGGCGCGGACTAGCGCGTTCGCGGAGCCCG	CGGGCTCCGCGAACGCGCTAGTCCGCGCCCTG
GSK-3α K148R	GAACTAGTCGCCATCAGGAAGGTTCTCCAGGAC	GTCCTGGAGAACCTTCCTGATGGCGACTAGTTC
GSK-3α R159A	ACAAGAGGTTCAAGAACGCAGAGCTGCAGATCATGCG	CGCATGATCTGCAGCTCTGCGTTCTTGAACCTCTTGT
GSK-3α Y279F	GGGGAGCCCAATGTCTCCTTCATCTGTTCTCGCTACTAC	GTAGTAGCGAGAACAGATGAAGGAGACATTGGGCTCCCC
GSK-3α P442/443A	GCCATTCTCATCGCTGCTCACTTGAGGTCC	GGACCTCAAGTGAGCAGCGATGAGAATGGC
GSK-3β S9A	GGCCCAGAACCACCGCCTTTGCGGAG	CTCCGCAAAGGCGGTGGTTCTGGGCC
GSK-3β K85R	AACTGGTCGCCATCAGGAAAGTATTGCAGGACAA	TTGTCCTGCAATACTTTCCTGATGGCGACCAGTT
GSK-3β R96A	AAGAGATTTAAGAATGCAGAGCTCCAGATCATG	CATGATCTGGAGCTCTGCATTCTTAAATCTCTT
GSK-3β Y216F	GGAGAACCCAATGTTTCGTTTATCTGTTCTCGGTACTATAGG	CCTATAGTACCGAGAACAGATAAACGAAACATTGGGTTCTCC
GSK-3β P379/380A	GGCTACCATCCTTATTGCTGCTCATGCTCGGATTCAAG	CTTGAATCCGAGCATGAGCAGCAATAAGGATGGTAGCC