Supplemental Materials

Molecular Biology of the Cell

Vora *et al*.

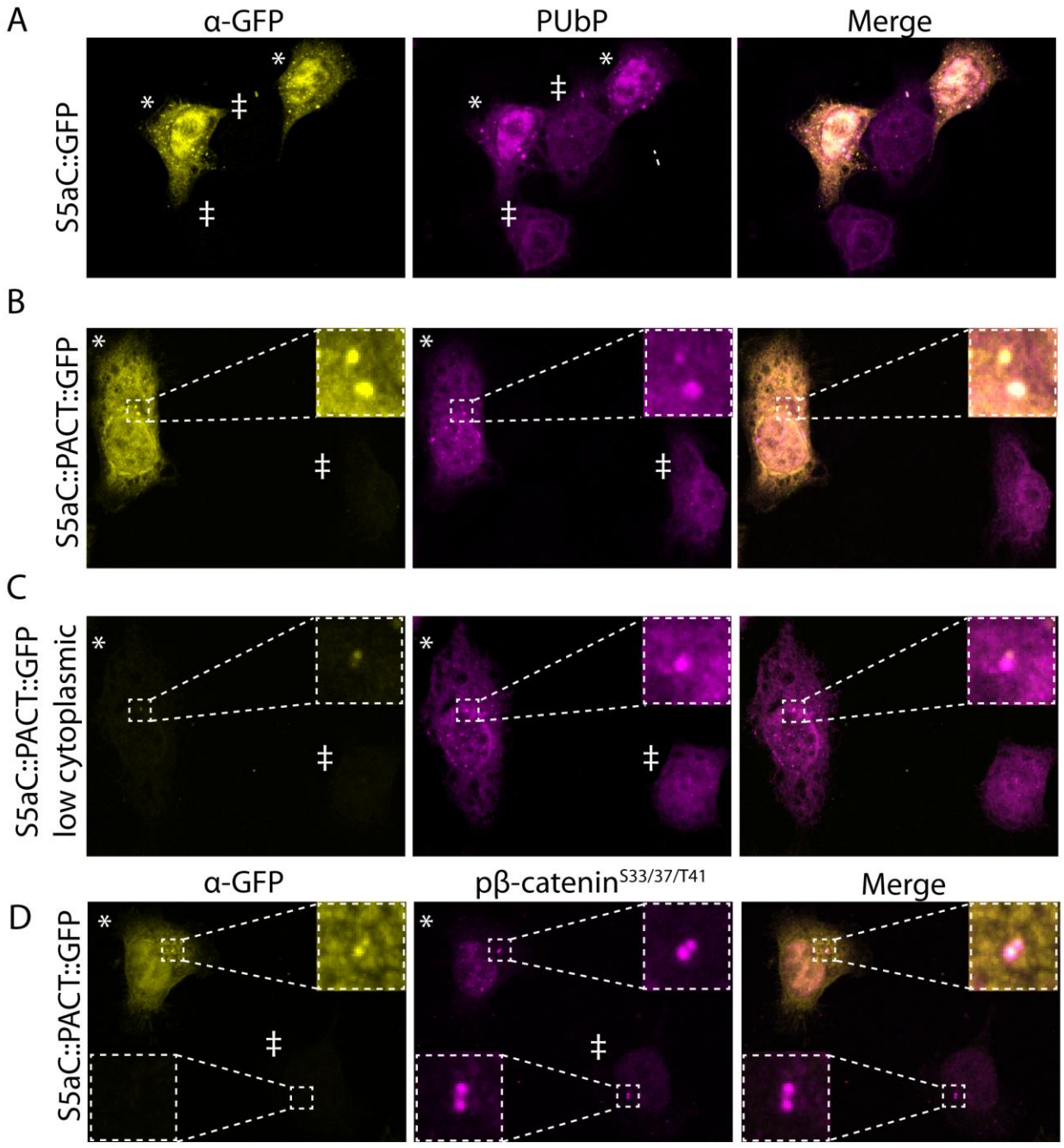

Figure S1. pβ-catenin does not accumulate at centrosomes after inhibition of centrosome-associated degradation. A) Hela cells successfully transfected with the dominant negative proteasome subunit S5aC::GFP (marked with *) show strong expression of the transgene upon immunostain with antibodies against GFP (green), and accumulation of polyubiquitinated proteins (PUbP's; magenta), while untransfected cells (marked with ‡ in the second column) show wild-type levels. B) Cell transfected with S5aC::PACT::GFP (*) shows punctate enrichment of the fusion protein at centrosomes (4μm X 4μm dashed boxes, magnified at top right) that accumulate PUbP's (magenta, 2nd column) while the untransfected cell (‡, 2nd column)

lacks any discernable perinuclear punctae corresponding to centrosomes. C) Low-expressing S5aC::PACT::GFP cell (*) with centrosomal localization but undetectable expression in the cytoplasm still over-accumulates centrosomal PUbP's. D) Cell transfected with S5aC::PACT::GFP (*) similar levels of centrosomal pβ-catenin compared to untransfected cell (‡).

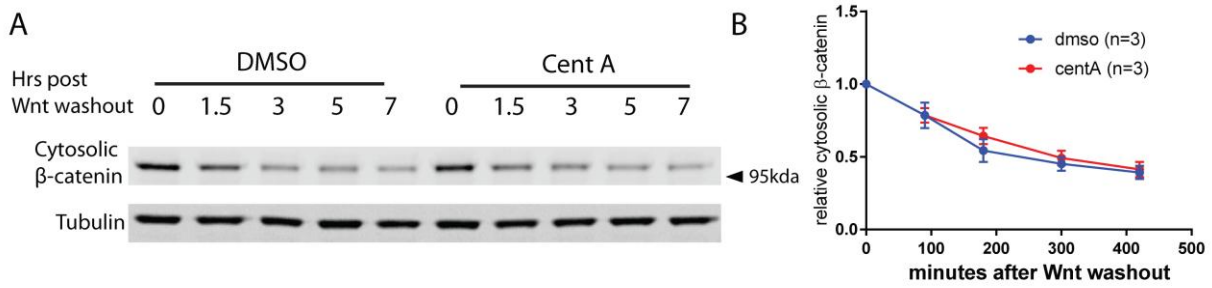

Figure S2. β-catenin degradation is intact in cells without centrosomes. A) Cells were treated with Wnt for 12 hours and allowed to recover without Wnt for indicated timespans and cytosolic extracts were blotted for β-catenin. B) Quantification of β-catenin degradation during recovery from Wnt showing identical degradation rates for cells with and without centrosomes.

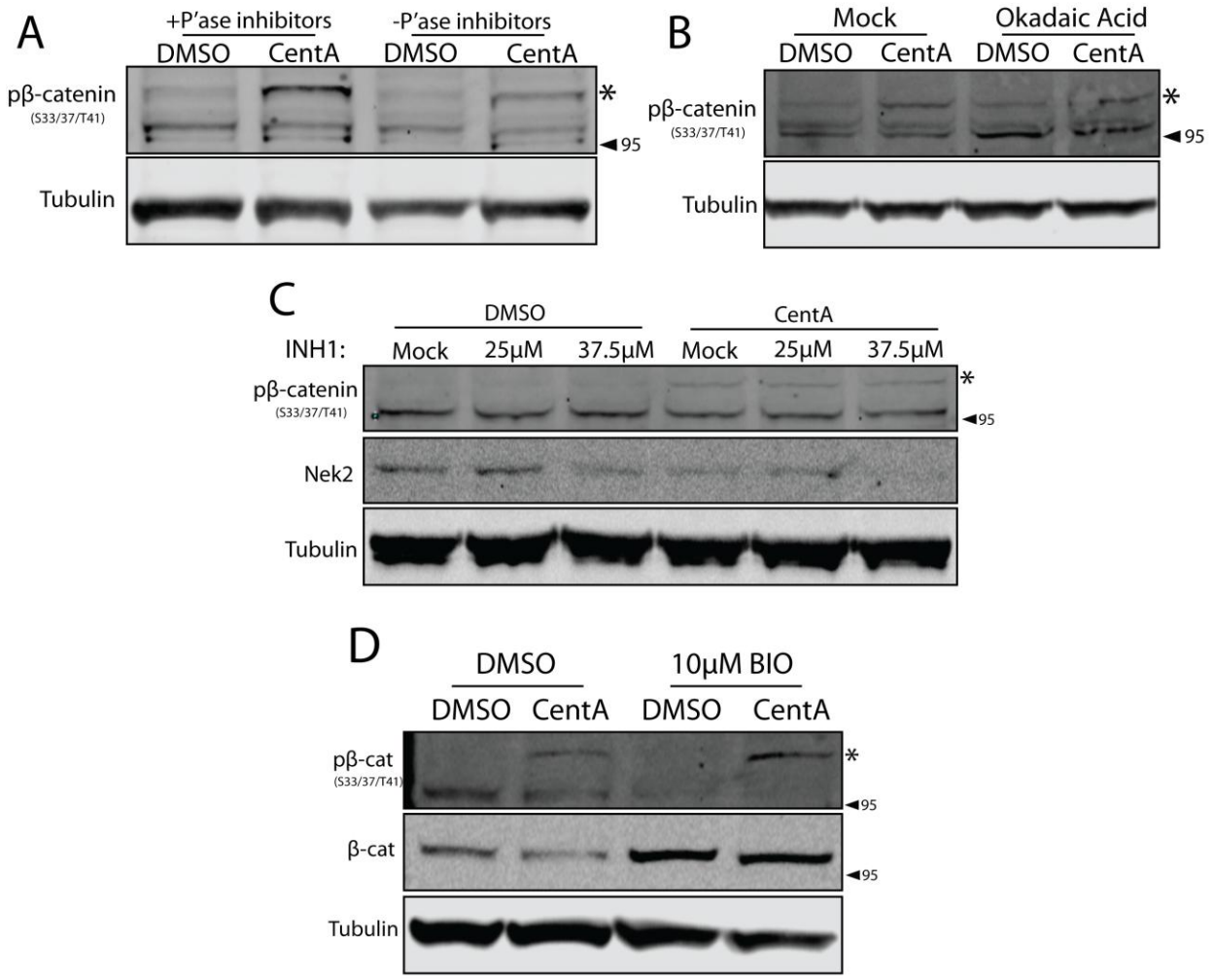

Figure S3. Phosphorylation of CentA-dependent high molecular weight β-catenin is independent of GSK3 and Nek2. A) Lysates from control and CentA cells were harvested in the presence or absence of phosphatase inhibitors showing reduction in both β-catenin^{LowMW} and β-catenin^{HiMW}. B) Control and CentA-treated cells were treated with Okadaic Acid or control for 4 hours and blotted for pβ-catenin showing increase in both β-catenin^{LowMW} and β-catenin^{HiMW}. C) Control and CentA cells treated with INH1 overnight showed severely decreased Nek2 levels yet no change in pβ-catenin^{LowMW} or pβ-catenin^{HiMW}. D) Treatment with BIO for 4 hours followed by blotting for pβ-catenin shows that GSK3 inhibition results in loss of pβ-catenin^{LowMW} in both control and CentA cells, but fails to reduce pβ-catenin^{HiMW} levels.

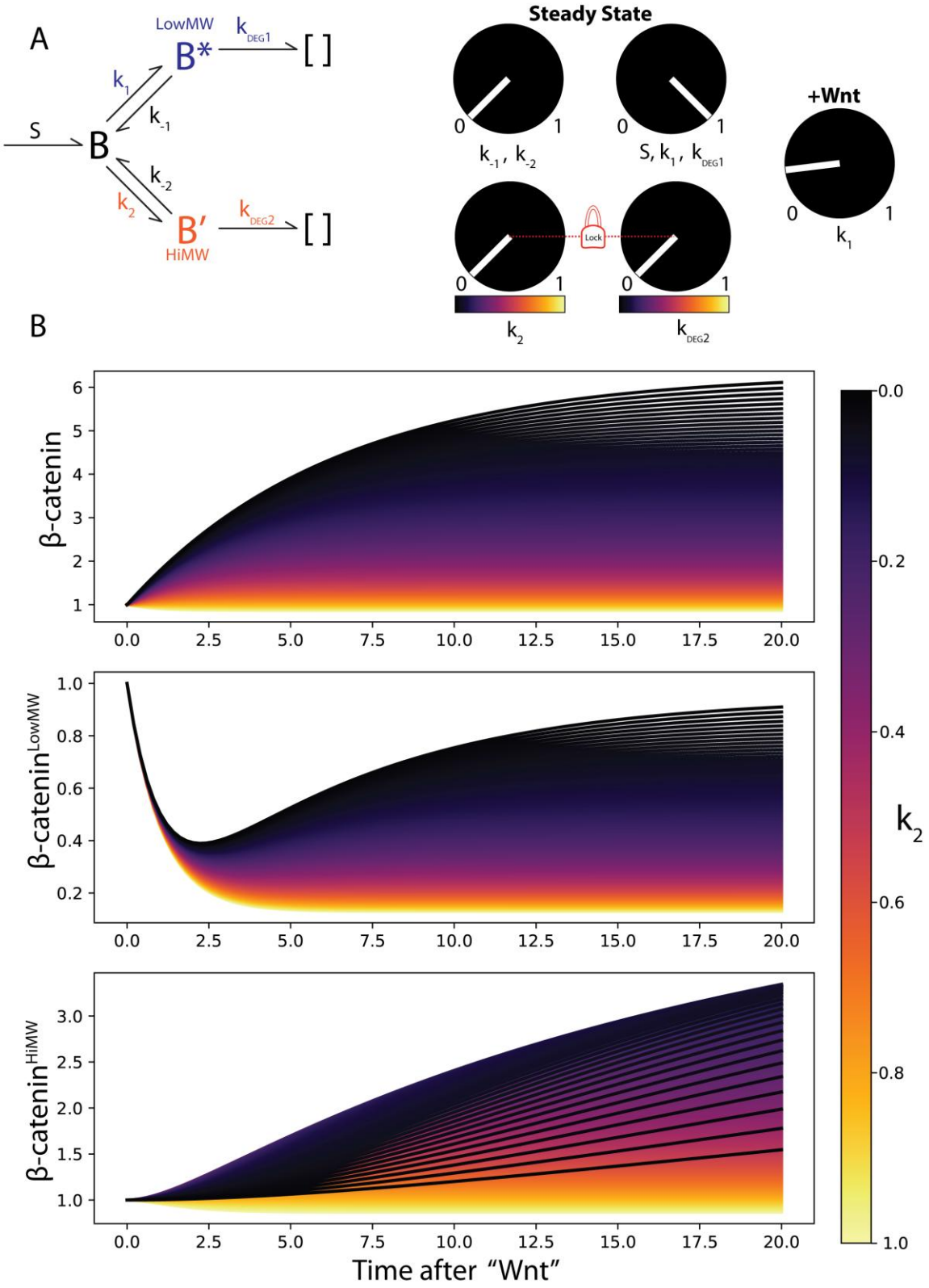

Figure S4. A robust parameter range of Wnt-insensitive β-catenin degradation rates permits moderate β-catenin^{LowMW} recovery while dampening β-catenin increase after Wnt

signaling. (A) Model for β-catenin metabolism with Wnt-insensitive degradation steps (left panel). β-catenin is continually synthesized with rate S and channeled to one of two modification cascades (Wnt-sensitive [at rate k_1] and Wnt-insensitive [at rate k_2]) before being degraded (with rates k_{DEG1} and k_{DEG2} for β-catenin^{LowMW} and β-catenin^{HiMW}, respectively). On the right panel is a schematic depicting all parameter values included in the simulation. To simulate "+Wnt" conditions in k_1 is reduced 5-fold, while K_2 and K_{DEG2} are locked together and varied. (B) Simulations of various β-catenin species over a range of Wnt-insensitive degradation rates. Assuming β-catenin^{HiMW} levels are at steady state under default conditions, k_2 and k_{DEG2} remain fixed to one another. We then varied them from 0.01 to 1 under conditions of Wnt signaling (simulated by setting k_1 to 0.15). Note that the β-catenin^{HiMW} accumulation rate after Wnt increases as k_2 approaches 0.2 (yellow to purple) but then decreases as k_2 goes on to approach 0.01 (purple to black).

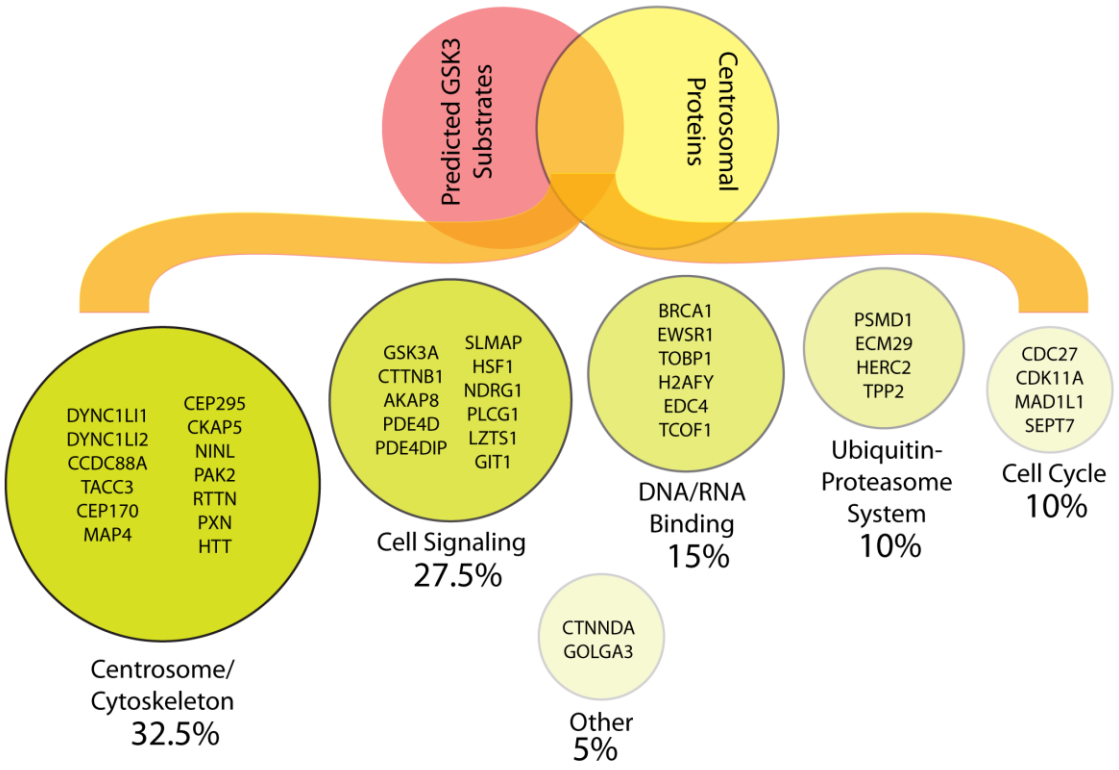

Figure S5. Centrosomal proteins with phosphorylation sites similar to β-catenin. A list of centrosomal proteins from the centrosome proteomics database was screened for sequences with tandem [S/T]XXX[S/T] motifs. Of the 191 proteins screened, a total of 40 (21%) proteins fulfilled this requirement. These 40 proteins were then organized by gene function (e.g. proteins with known function at the centrosome or in cell signaling).