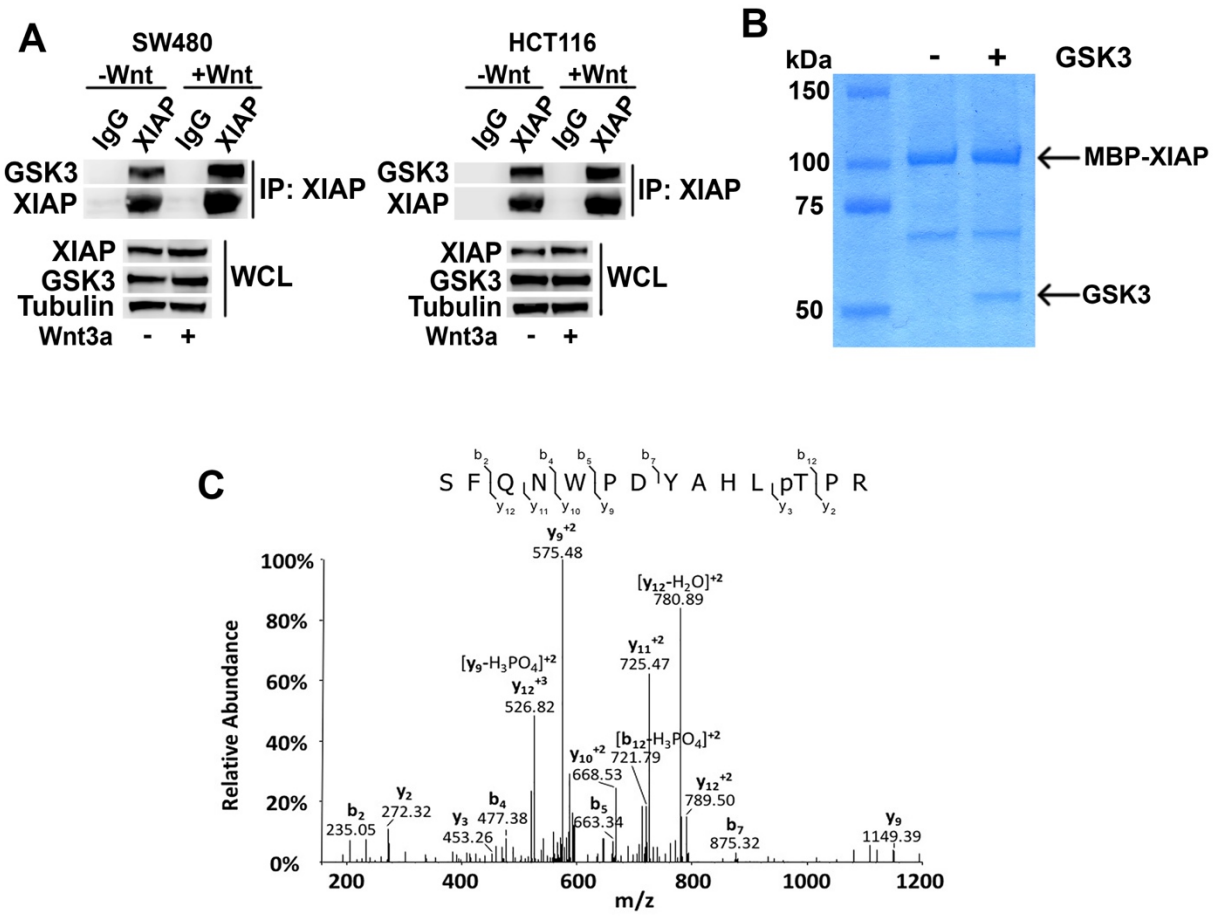
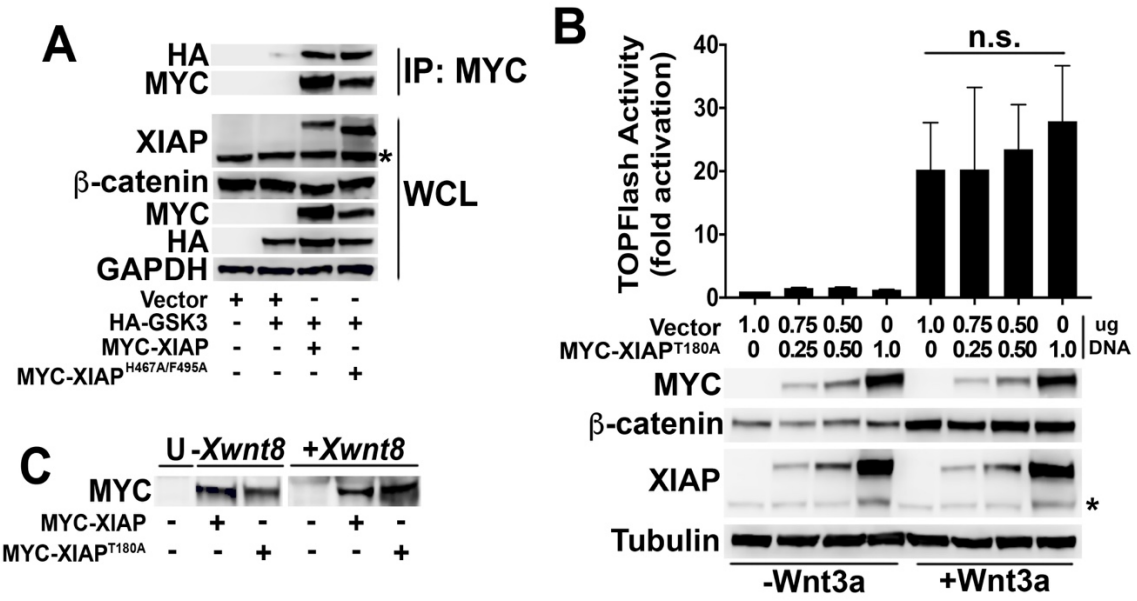
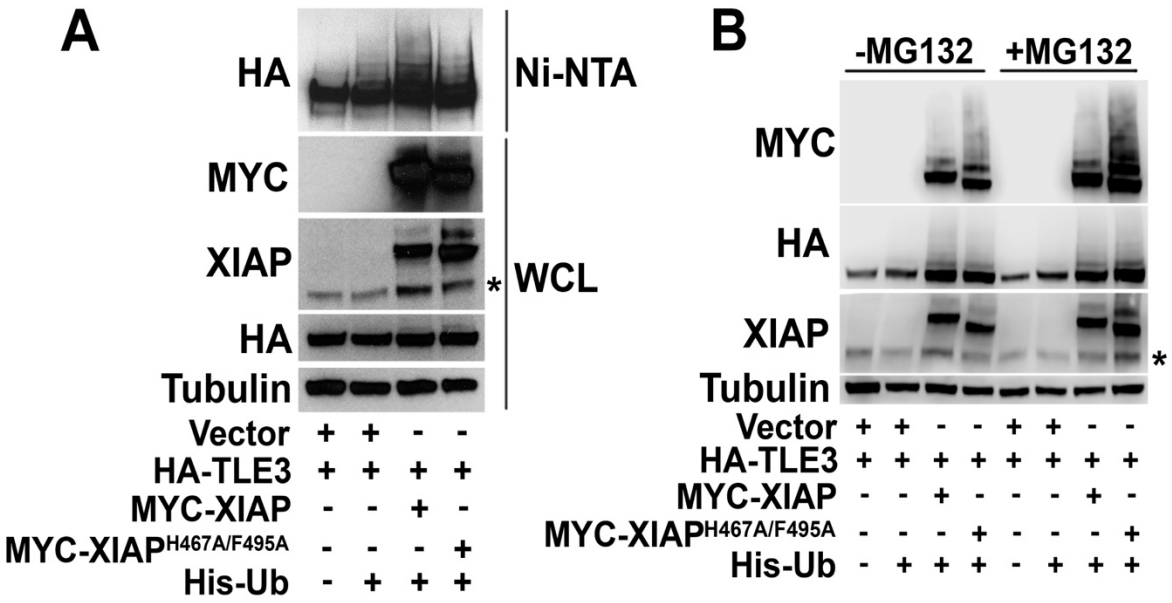


Supplementary Figure 1. GSK3 binds and phosphorylates XIAP at threonine 180. (A) Endogenous XIAP co-immunoprecipitates with endogenous GSK3 from SW480 and HCT116 colorectal cancer cell lines. Cell were incubated in the absence or presence of Wnt3a, lysed, and immunoprecipitation performed. WCL = whole cell lysates. IP = immunoprecipitation. (B) Coomassie showing equal loading of MBP-XIAP from the *in vitro* kinase assay (Fig. 1B) demonstrating equivalent amounts of XIAP protein in the reactions. (C) Mass spectrometry from an *in vitro* kinase reaction containing purified XIAP and GSK3 identifies phosphorylated threonine 180 on XIAP. The peptide sequence is shown above the spectrum with corresponding b and y ion splits with pT being the phosphorylated T180 site.



Supplementary Figure 2. The XIAP^{T180A} mutant does not potentiate Wnt signaling in contrast to XIAP. (A) The ligase mutant, XIAP$^{H467A/F495A}$, interacts with GSK3 to a similar extent as wild-type XIAP. HEK293STF cells were transfected as indicated, lysates collected, and immunoprecipitation performed. Asterisk indicates endogenous XIAP. WCL = whole cell lysates. IP = immunoprecipitation. (B) Injected XIAP and XIAP^{T180A} mRNAs are expressed at similar levels in *Xenopus* embryos. Sample buffer was added to pooled embryos from each condition and immunoblotting performed. U = uninjected. (C) Overexpression of XIAP^{T180A} fails to alter Wnt signaling. No statistically significant (as assessed by the student's t-test) increase or decrease in TOPFlash activity was detected even when XIAP^{T180A} is expressed at high levels. Lysates were collected and immunoblotted as indicated. Asterisk indicates endogenous XIAP.



Supplementary Figure 3. The ligase mutant, XIAP$^{H467A/F495A}$, shows decreased ubiquitination of TLE3 and is rapidly turned over in cultured mammalian cells. (A) XIAP$^{H467A/F495A}$ is impaired in its capacity to ubiquitinate TLE3. HEK293STF cells were transfected as indicated, lysed under denaturing conditions, and His-Ub modified proteins isolated by nickel affinity chromatography. Transfected XIAP and TLE3 were detected by immunoblotting with anti-MYC and anti-HA antibodies, respectively. Asterisk indicates endogenous XIAP. (B) Treatment with the proteasomal inhibitor, MG132, indicate enhanced ubiquitination of XIAP$^{H467A/F495A}$. HEK293STF cells were transfected as indicated, and cells incubated in the absence or presence of MG132. Cells were then collected and immunoblotting performed. Transfected XIAP and TLE3 were detected by immunoblotting with anti-MYC and anti-HA antibodies, respectively. Asterisk indicates endogenous XIAP.