Figure S1

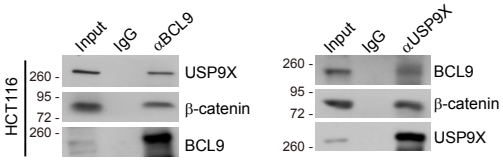

Figure S1. Co-immunoprecipitation Analysis of the Interaction between USP9X and BCL9. Whole cell lysates from HCT116 cells were immunoprecipitated (IP) followed by immunoblotting (IB) with antibodies against the indicated proteins.

Figure S2

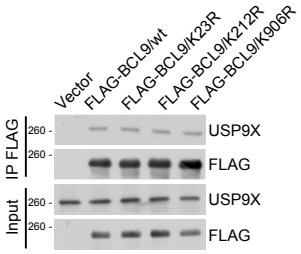

Figure S2. Co-immunoprecipitation Analysis of the Interaction between USP9X and BCL9 Mutants. HeLa cells with doxycycline (Dox)-inducible expression of stably integrated FLAG-BCL9/wt, FLAG-BCL9/K23R, FLAG-BCL9/K212R or FLAG-BCL9/K906R were collected for co-immunoprecipitation assays with anti-FLAG followed by IB using antibodies against the indicated proteins.

Figure S3

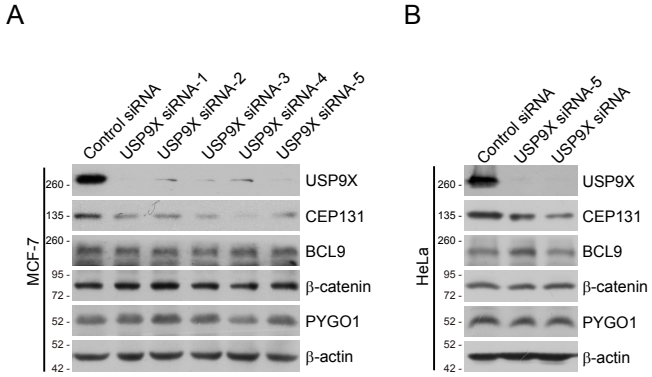

Figure S3. USP9X Depletion Has Little Effect on the Expression of β-catenin. (A) MCF-7 cells were transfected with control siRNA or USP9X siRNAs. Cellular extracts were prepared and analyzed by Western blotting. (B) HeLa cells were transfected with control siRNA or USP9X siRNAs. Cellular extracts were prepared and analyzed by Western blotting.

Figure S4

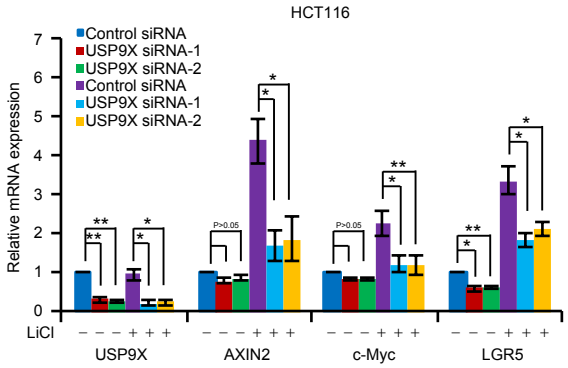

Figure S4. USP9X-promoted BCL9 Deubiquitination Is Required for Transcriptional Wnt Responses in HCT116 cells. HCT116 cells transfected with control siRNA or USP9X siRNAs were cultured in the absence or presence of 25 mM LiCl for 6 hours and collected for qRT-PCR analysis with *USP9X*, *AXIN2*, *c-Myc* and *LGR5* primers. Each bar represents the mean ± S.D. for biological triplicate experiments. $^{*}P<0.05$, $^{**}P<0.01$, one-way ANOVA.

Figure S5

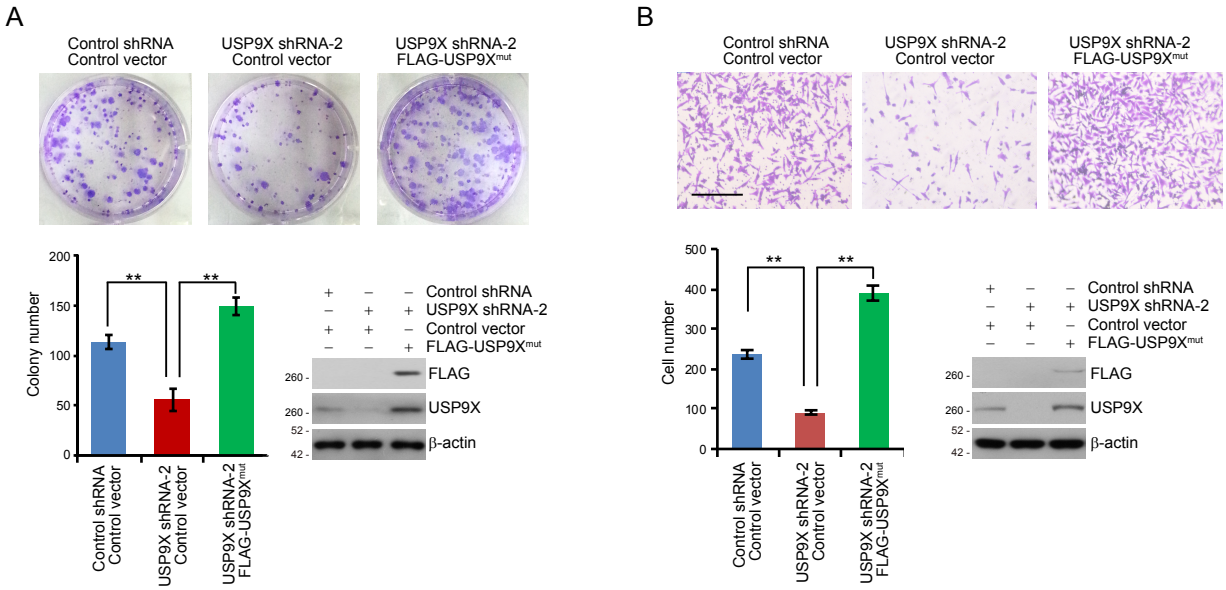

Figure S5. USP9X Is Essentially Required for Breast Cancer Cell Proliferation and Invasion. (A) Colony formation assays with MCF-7 cells stably expressing control shRNA or USP9X shRNA and RNAi-resistant USP9X. Representative images are shown. Each bar represents the mean ± S.D. for biological triplicate experiments. $^{**}P<0.01$, one-way ANOVA. Cellular extracts from these cells were collected and analyzed by Western blotting. USP9X^{mut} represents RNAi-resistant USP9X. (B) Tranwell invasion assays with MDA-MB-231 cells stably expressing control shRNA or USP9X shRNA and RNAi-resistant USP9X. Representative images are shown. Each bar represents the mean ± S.D. for biological triplicate experiments. $^{**}P<0.01$, one-way ANOVA. Scale bar, 50 μm. Cellular extracts from these cells were collected and analyzed by Western blotting.