Fig. S2 USP25 stabilizes and deubiquitinates tankyrase, related to Figure 2. (A) H4 cells stably expressing TNKS2 were transfected with increasing amount of Flag-USP25 and HA-TNKS2 levels were detected. (B) A schematic of the Cas9 sgRNA/oligo targeting site in the exon2 of Usp25. The sgRNA coding sequence is underlined and labeled in red. The protospacer-adjacent motif (PAM) sequence is underlined. The number of sequences analyzed is indicated below. (C) Lysates from HEK293T cells expressing USP25 WT or C178S, were subjected to IP/Western analysis using the indicated antibodies. (D) HEK293T cells transfected with the indicated constructs were treated with 10 μM MG132 for 6 h before collection. The whole cell lysate was subjected to pulldown with Ni2+ beads and western blot with anti-TNKS1/2 antibody to detect ubiquitylated tankyrase. (E) Usp25+/+ and Usp25−/− HEK293T cells were transfected with His-Ub. The whole cell lysate was subjected to pulldown with Ni2+ beads and western blot with anti-TNKS1/2 antibody to detect ubiquitylated tankyrase.