

Supplementary information, Figure S4 TDU domains and PPXY motifs mediated the

interaction between SdBP and Sd/Yki.

(A) GST pull-down of Sd and SdBP TDU variants. SdBP was unable to bind to Sd when both TDU domains were mutated. Extracts from S2 cells expressing SdBP and TDU variants were incubated with GST-Sd, followed by Western blot using Flag and GST antibodies. (B) Yeast two-hybrid identified that SdBP bound to Yki. (C-D) PPXY motifs of SdBP (C) and WW domains of Yki (D) mediated their interaction. SdBP-PPXY123 cannot bind to Yki (C). Deletion of both Yki C-terminal WW domains (WW12) abolished SdBP-Yki binding (D). Extracts from S2 cells expressing SdBP and PPXY variants were incubated with GST-Yki (C), Extracts from S2 cells expressing Myc-Yki and its WW variants were incubated with GST-SdBP (D), results were analyzed by Western blot using indicated antibodies. (E) GST pulldown assay showed that TDU domains and PPXY motifs mediated the interaction between SdBP and Sd/Yki respectively. Purified SdBP and its variants proteins were incubated with GST-Sd or GST-Yki, results were analyzed by Western blot using indicated antibodies. (F) No interaction between SdBP and other members of the Hpo pathway was detected. SdBP did not interact with indicated upstream components such as Mer, Wts, Mats. Western blot of IP or lysates from S2 cells expressing indicated proteins.