

Figure 4-figure supplement 1. The stability of DBC1 was regulated by OTUD5.

(A) HEK293T cells were co-transfected with Myc-DBC1 and a series of Flag-Dubs, and immunoprecipitation was performed with an anti-Flag beads. Co-immunoprecipitated DBC1 was detected by Western blotting with an anti-myc antibody. (B) HEK293T cells were co-transfected with Myc-DBC1 and several Flag-Dubs, and immunoprecipitation was performed with an anti-Flag antibody. Co-immunoprecipitated DBC1 was detected by Western blotting with anti-myc antibody. (C) HEK293T cells were transfected with Myc-DBC1 and several Flag-Dubs, and cultured under hypoxia for 24 h and then treated with MG132 (10 μ M) for 6 h. Cells were harvested, denatured and lysed for immunoprecipitation with anti-Myc antibody. The ubiquitination level of DBC1 was assessed by immunoblotting with anti-Ub antibody (FK2). (D) HEK293T cells were transfected with Myc-DBC1 and Flag-OTUD5 for 24 h. Cells were collected for immunoprecipitation with anti-Flag antibody. (E) HEK293T cells were transfected with Myc-DBC1 and Flag-OTUD5 for 24 h. Cells were collected for immunoprecipitation with anti-Myc antibody. (F) MCF7 Cells were collected for immunoprecipitation with anti-DBC1 antibody and co-immunoprecipitated endogenous OTUD5 was detected by Western blotting with an anti-OTUD5 antibody. (G) Purified His-tagged DBC1 proteins were used for His affinity isolation of endogenous OTUD5 of MCF7 cells, and blotted with an anti-OTUD5 antibody. (H) HEK293T cells were co-transfected with Myc-DBC1, Flag-OTUD5 and Flag-SIAH2^{RM} at the indicated dosages for 24 h. Cells were collected for immunoprecipitation with anti-Myc antibody and co-immunoprecipitated SIAH2 and OTUD5 were detected by Western blotting with an anti-Flag antibody.