



### **Figure 3-figure supplement 1. Hypoxia-induced DBC1 degradation is dependent on E3 Ligase SIAH2.**

(A-B) HEK293T cells were transfected with HA-tagged wild-type Ubiquitin or the different lysine-to-arginine mutants together with Myc-DBC1 and Flag-SIAH2 or the empty Flag-vector for 24 h and then treated with MG132 (10  $\mu$ M) for 6 h. Ubiquitylation assays were performed and the ubiquitylated DBC1 was detected using an anti-HA antibody. (C) HEK293T cells were transfected with Myc-DBC1 or the Myc-DBC1 (K287R) mutant and Flag-SIAH2 or the empty Flag-vector for 24 h and then treated with MG132 (10  $\mu$ M) for 6 h. Ubiquitylation assays were performed and the ubiquitylation level of DBC1 was detected using an anti-HA antibody. (D) HeLa cells were transfected with Myc-DBC1 (K287R) mutant and Flag-SIAH2 by quantitative gradient and then protein level of DBC1 were detected by Western blotting. (E) The *SIAH2*-knockout, DBC1-knockout and *SIAH2/DBC1* double knockout MDA-MB-231 cells were detected by Western blotting. (F) The WT and *SIAH2*-knockout MCF7 cells were exposed to hypoxia for the indicated time and then protein level of DBC1 was detected by Western blotting. (G) The *SIAH2*-knockout, DBC1-knockout and *SIAH2* and DBC1 double knockout MCF7 cells were detected by Western blotting. (H) The WT and *SIAH2*-knockout MCF7 cells were exposed to normoxia or hypoxia for 12 h, CHX (10  $\mu$ M) was added for the indicated time, and the cell lysates were subjected to Western blotting analysis of DBC1. (I) MDA-MB-231 cells were cultured under normoxic or hypoxic conditions for 24 h and then treated with Doramapimod (1  $\mu$ M) for 6 h, and the cell lysates were subjected to Western blotting analysis of DBC1. (J) MDA-MB-231 cells were cultured under normoxic or hypoxic conditions for 24 h and then treated with Doramapimod (1  $\mu$ M) for 6 h. Immunoprecipitation was performed with an anti-DBC1 antibody. Co-immunoprecipitated endogenous SIAH2 and OTUD5 were detected by Western blotting with an anti-SIAH2 and anti-OTUD5 antibodies.