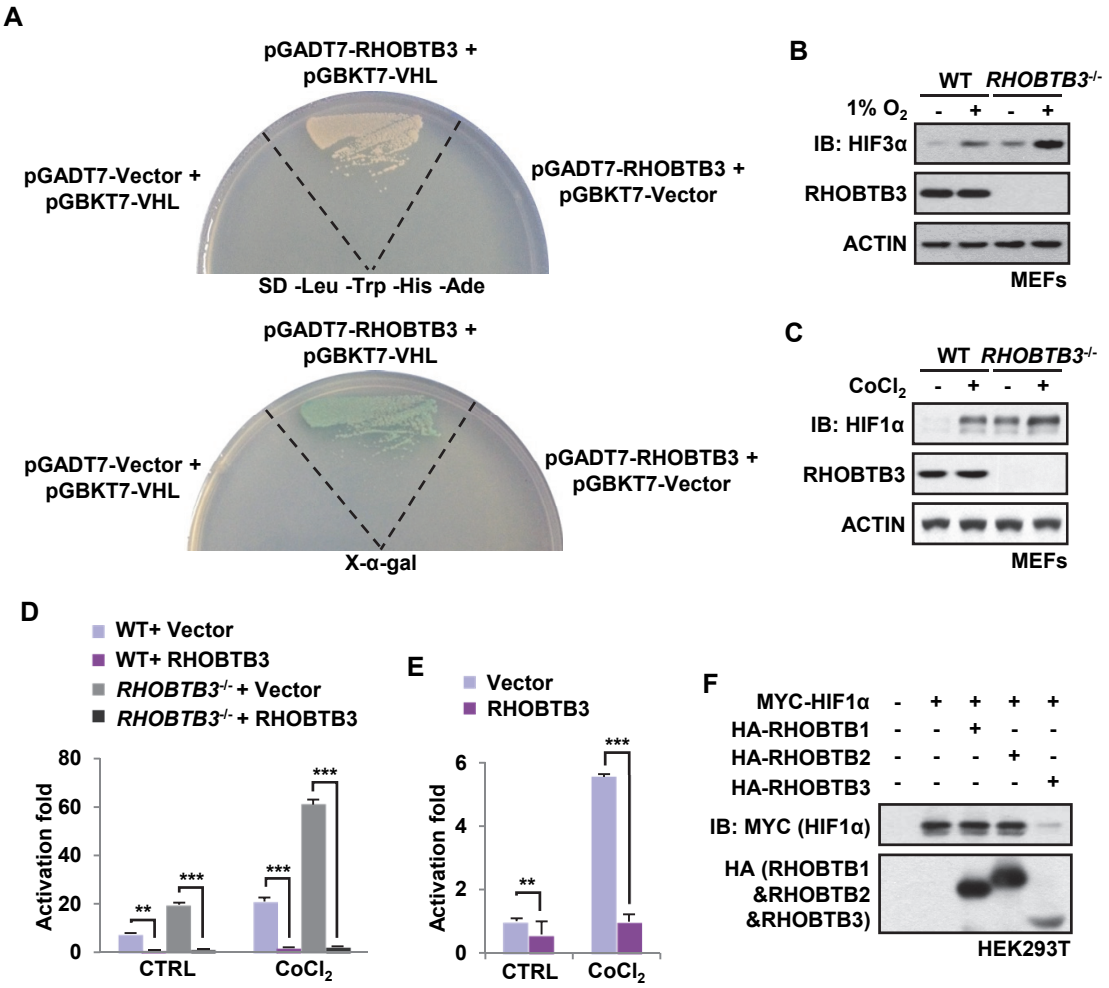

Supplementary information, Figure S1

(**A**) Verification of the interaction between VHL and RHOBTB3 by yeast two-hybrid screen as previously described [81].

(**B**) Protein levels of HIF3α are elevated in *RHOBTB3*$^{-/-}$ MEFs under both normoxic and hypoxic conditions. *RHOBTB3*$^{-/-}$ MEFs and control (WT) MEFs were maintained in normoxia or exposed to hypoxia for 8 h. Cells were then lysed and analyzed by immunoblotting with antibodies indicated.

(**C**) RHOBTB3 deficiency leads to greater accumulation of HIF1α after $CoCl_2$ treatment. *RHOBTB3*$^{-/-}$ MEFs and control (WT) MEFs were treated with $CoCl_2$ for 8 h, followed by immunoblotting with antibodies indicated.

(**D**) The transcriptional activity of HIF1α is enhanced in the absence of RHOBTB3. *RHOBTB3*$^{-/-}$ MEFs and WT MEFs were treated with $CoCl_2$ for 8 h. Cells were then lysed and the transcriptional activities of the reporter were measured by the Dual Luciferase Assay as described in Figure 2E. Data are presented as mean ± SEM, n = 3 for each group, **p < 0.01, ***p < 0.001 (ANOVA followed by Tukey).

(**E**) Ectopically expressed RHOBTB3 suppresses the transcriptional activity of HIF1α. HEK293T cells were transfected with HA-RHOBTB3 or vector as a control. 8 h later, cells were treated with $CoCl_2$ for additional 8 h, and were then lysed and analyzed as described in (**C**). Data are presented as mean ± SEM, n = 9 for each group, **p < 0.01, ***p < 0.001 (ANOVA followed by Tukey).

(**F**) RHOBTB3, but not RHOBTB1 or RHOBTB2, downregulates the protein levels of HIF1α. HEK293T cells were transfected with different combinations of MYC-HIF1α, HA-RHOBTB1, HA-RHOBTB2, and HA-RHOBTB3. At 16 h post-transfection, cells were subjected to immunoblotting with antibodies indicated.