



Supplementary information, Figure S3

(A, C) Ectopically expressed RHOBTB3 interacts with VHL (A) and PHD2 (C). HEK293T cells were transfected with RHOBTB3, VHL, and PHD2 carrying different tags as indicated. IP was performed, followed by immunoblotting with antibodies indicated.

(B) VHL is colocalized with RHOBTB3. *RHOBTB3^{-/-}* MEFs were infected with lentivirus expressing HA-RHOBTB3. At 36 h post-infection, cells were maintained in normoxia or exposed to hypoxia (1% O₂) for 12 h. HA-RHOBTB3 and VHL were stained with mouse anti-HA antibody (green) and rabbit anti-VHL antibody (red), respectively. Representative cells are shown and the boxed areas are enlarged on the right side; after superimposition of the two stainings, the yellow color indicates overlapping of the two proteins.

(D) RHOBTB3 does not directly interact with HSP90. HEK293T cells were transfected with different combinations of HIF1 α , MYC-VHL, FLAG-PHD2 and HA-RHOBTB3. At 16 h post-transfection, cells were lysed. The protein extracts were immunoprecipitated with antibody against HA for RHOBTB3, followed by immunoblotting with antibodies indicated.

(E) Less HSP90 was co-precipitated with RHOBTB3 under hypoxic condition. HEK293T cells were transfected with HIF1 α , MYC-VHL, FLAG-PHD2 and HA-RHOBTB3. At 16 h post-transfection, cells were treated with 10 μ M MG-132 and maintained in normoxia or exposed to hypoxia for another 10 h, and were lysed and analyzed as described in (D).