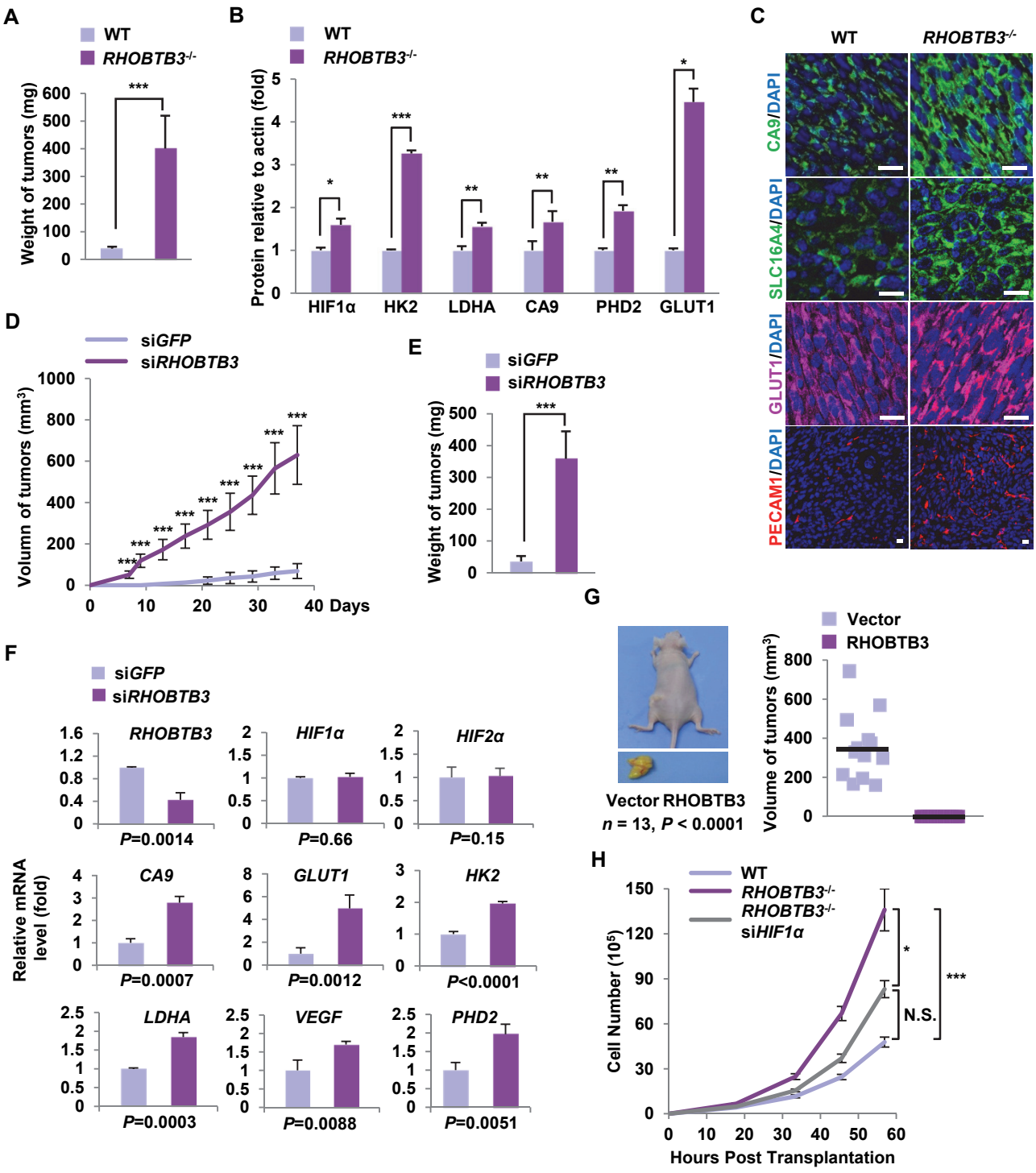
A
WT
RHOBTB3-/-
Weight of tumors (mg)
B
Protein relative to actin (fold)
HIF1α
HK2
LDHA
CA9
PHD2
GLUT1
C
WT
RHOBTB3-/-
CA9/DAPI
SLC16A4/DAPI
GLUT1/DAPI
PECAM1/DAPI
D
siGFP
siRHOBTB3
Volumn of tumors (mm3)
Days
E
siGFP
siRHOBTB3
Weight of tumors (mg)
F
siGFP
siRHOBTB3
Relative mRNA level (fold)
RHOBTB3
HIF1α
HIF2α
CA9
GLUT1
HK2
LDHA
VEGF
PHD2
P=0.0014
P=0.66
P=0.15
P=0.0007
P=0.0012
P<0.0001
P=0.0003
P=0.0088
P=0.0051
G
Vector RHOBTB3
n = 13, P < 0.0001
Vector
RHOBTB3
Volume of tumors (mm3)
H
WT
RHOBTB3-/-
RHOBTB3-/-
siHIF1α
Cell Number (10^5)
N.S.
Hours Post Transplantation

Supplementary information, Figure S7

(**A**) The sizes of xenografts derived from *RHOBTB3*$^{-/-}$ MEFs are overwhelmingly larger than those derived from control MEFs. The weight of tumors (Figure 7B) are presented as mean ± SEM, n = 11 for each group, ***p < 0.001 (Student's *t*-test).

(**B**) Relative protein levels of HIF1α, HK2, LDHA, CA9, PHD2 and GLUT1 in xenografts derived from WT or *RHOBTB3*$^{-/-}$ MEFs as shown in Figure 7C. Relative protein levels were calculated from densitometry performed on developed films and normalized to ACTIN. Data are presented as mean ± SEM, n = 5 for each group, *p < 0.05, **p < 0.01, ***p < 0.001 (Student's *t*-test); N.S., not significant.

(**C**) Immunostaining for CA9, solute carrier family 16 member 4 (SLC16A4), GLUT1 and platelet/endothelial cell adhesion molecule 1 (PECAM1) in xenografts derived from *RHOBTB3*$^{-/-}$ MEFs and WT MEFs. Scale bar, 10 μm.

(**D**) Knockdown of *RHOBTB3* accelerates xenograft growth. The volumes of tumors described in Figure 7D were recorded day by day until day 37. Tumor volumes were calculated as described in Figure 7B. Data were presented as mean ± SEM, n = 12 for each group, ***p < 0.001 (ANOVA followed by Tukey).

(**E**) The weights of xenografts derived from *RHOBTB3*-knockdown HeLa cells are increased in comparison with that derived from control MEFs. The weights of tumors described in Figure 7D were presented as mean ± SEM, n = 12 for each group, ***p < 0.001 (Student's *t*-test).

(**F**) The mRNA levels of HIF target genes are up-regulated in xenografts derived from *RHOBTB3*-knockdown HeLa. Xenografts derived from *RHOBTB3*-knockdown HeLa and the control HeLa cells described in Figure 7D were homogenized and total RNAs were purified, followed by real-time PCR analysis. Values are presented as mean ± SEM, n = 6 for each group. Statistical analysis was done by Student's *t*-test.

(**G**) Overexpression of RHOBTB3 strongly suppresses xenograft growth. Suspensions of WT HeLa cells or HeLa cells stably expressing RHOBTB3 (3×10^6 each) were injected intradermally into each flank of nude mice. Tumors were allowed to develop for 35 days and the volumes were calculated as described in Figure 7B. Values of tumor volumes are presented as mean ± SEM, n = 13 for each group, p < 0.0001 (Student's *t*-test).

(**H**) RHOBTB3 suppresses cell proliferation through HIF1α. *RHOBTB3*$^{-/-}$ si*HIF1α* MEFs were established by infecting *RHOBTB3*$^{-/-}$ MEFs with lentivirus carrying siRNA against *HIF1α* for 36h. MEFs were cultured and the numbers of viable cells were determined by trypan blue exclusion assay. Values are presented as mean ± SEM, n = 3 for each group, *p < 0.05, ***p < 0.001 (ANOVA followed by Tukey), N.S., not significant.