Figure S1.

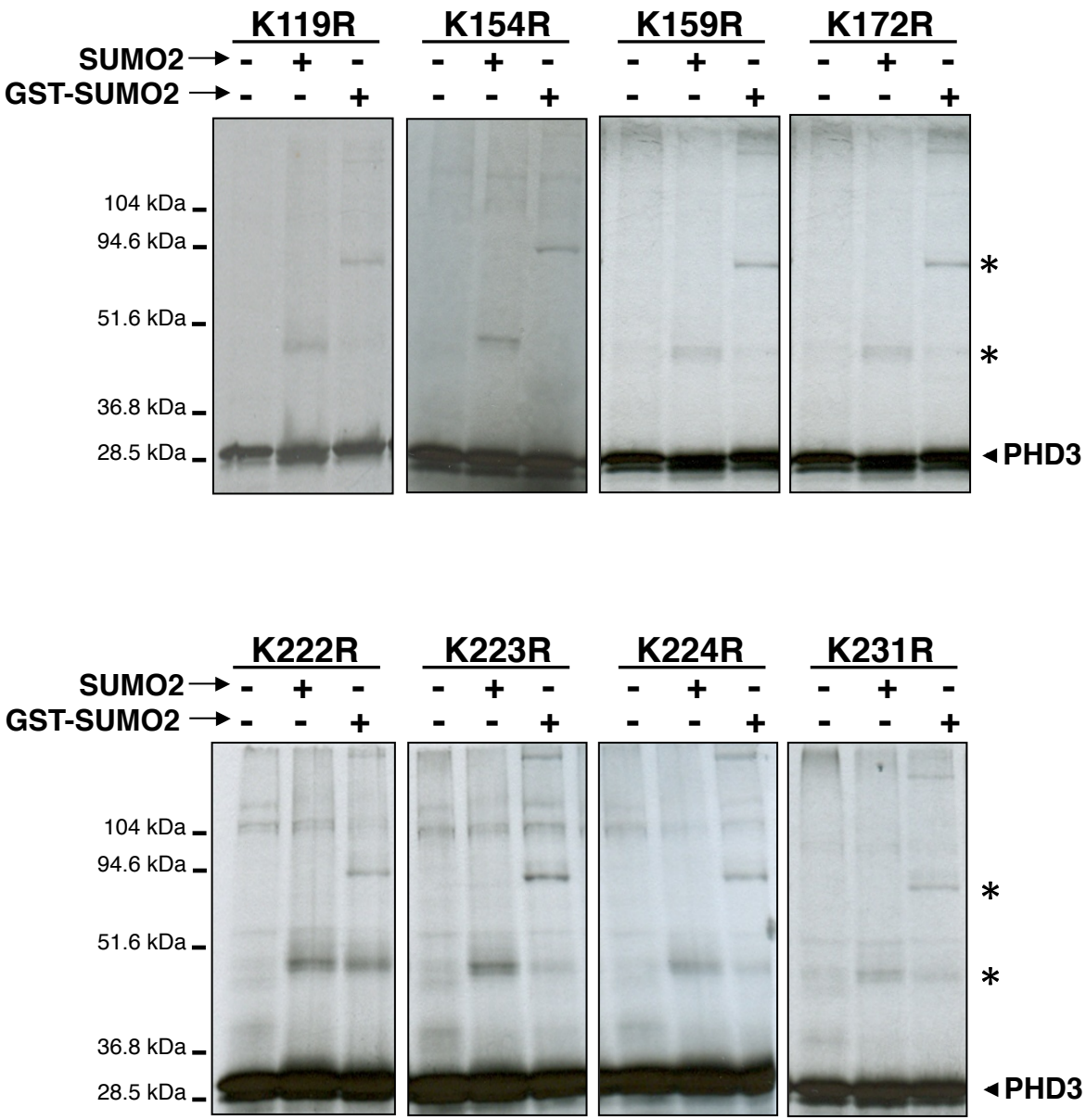

K119R
K154R
K159R
K172R
SUMO2
GST-SUMO2
104 kDa
94.6 kDa
51.6 kDa
36.8 kDa
28.5 kDa
*
*
PHD3
K222R
K223R
K224R
K231R
SUMO2
GST-SUMO2
104 kDa
94.6 kDa
51.6 kDa
36.8 kDa
28.5 kDa
*
*
PHD3

Figure S2.

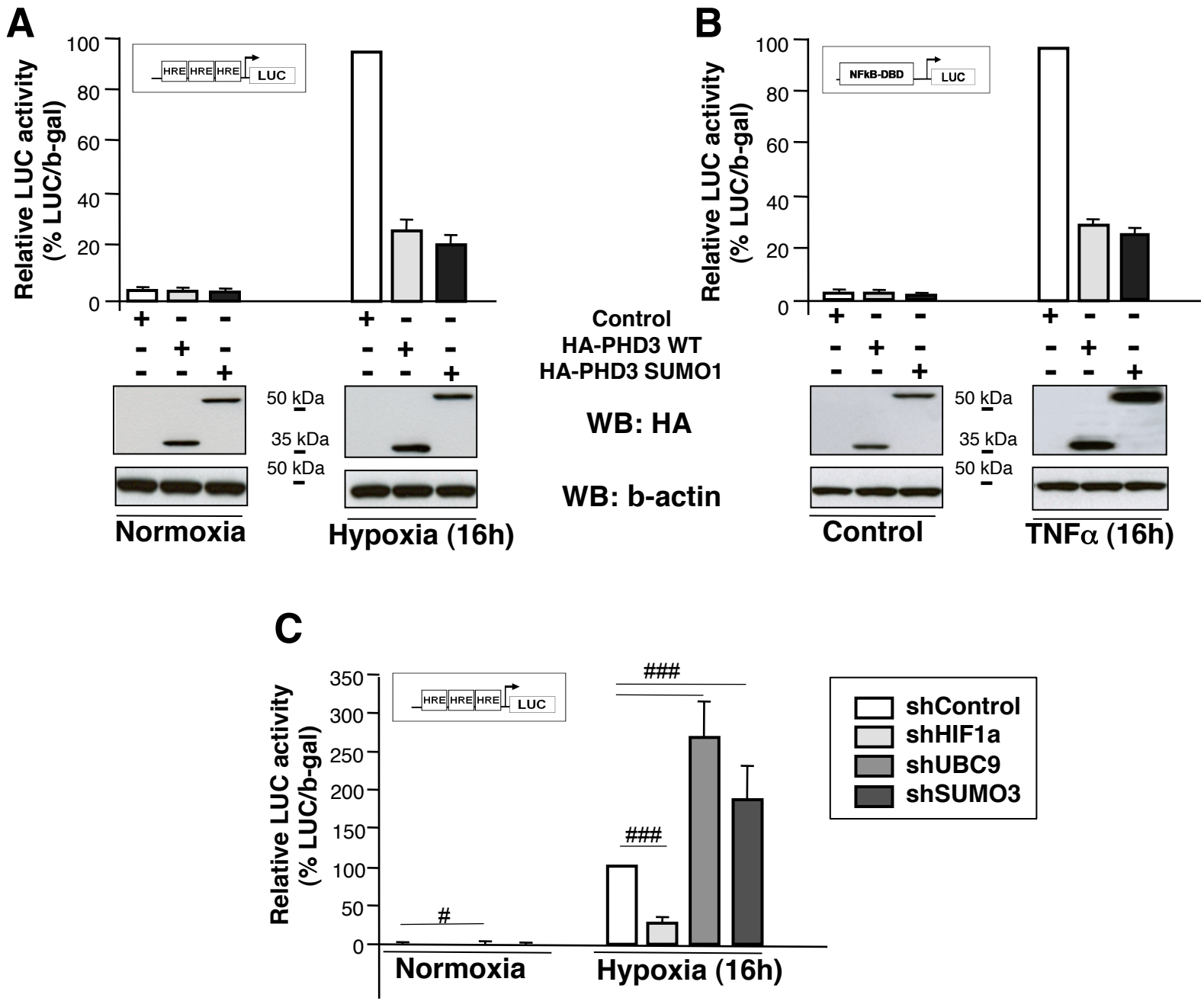

A
Relative LUC activity (% LUC/b-gal)
100
80
60
40
20
0
HRE HRE HRE LUC
+ - -
- + -
- - +
+ - -
- + -
- - +
50 kDa
35 kDa
50 kDa
Normoxia
Hypoxia (16h)
Control
HA-PHD3 WT
HA-PHD3 SUMO1
WB: HA
WB: b-actin
B
Relative LUC activity (% LUC/b-gal)
NFkB-DBD LUC
50 kDa
35 kDa
50 kDa
Control
TNFα (16h)
C
Relative LUC activity (% LUC/b-gal)
350
300
250
200
150
100
50
0
HRE HRE HRE LUC
###
###
#
Normoxia
Hypoxia (16h)
shControl
shHIF1a
shUBC9
shSUMO3

Figure S3.

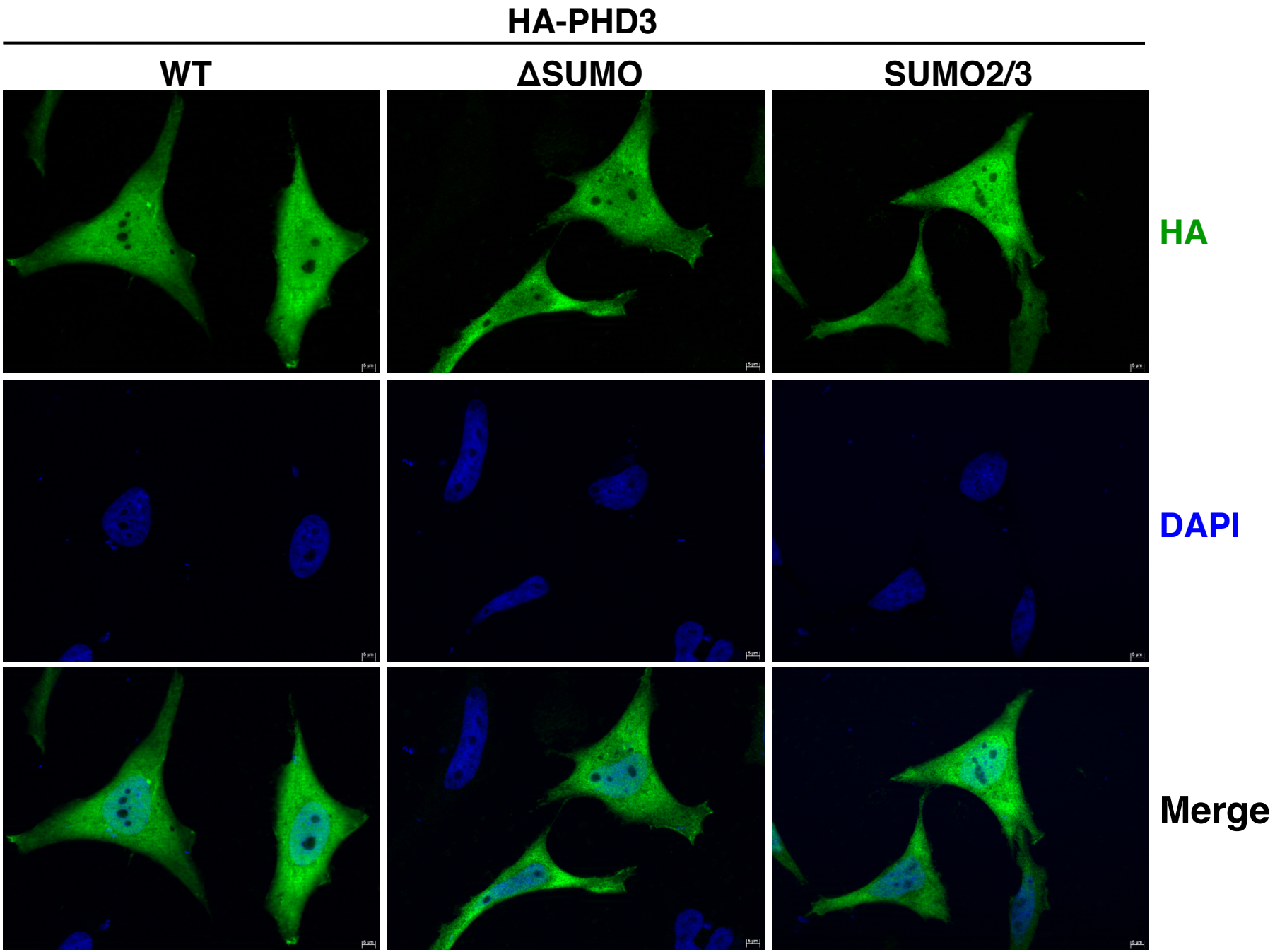
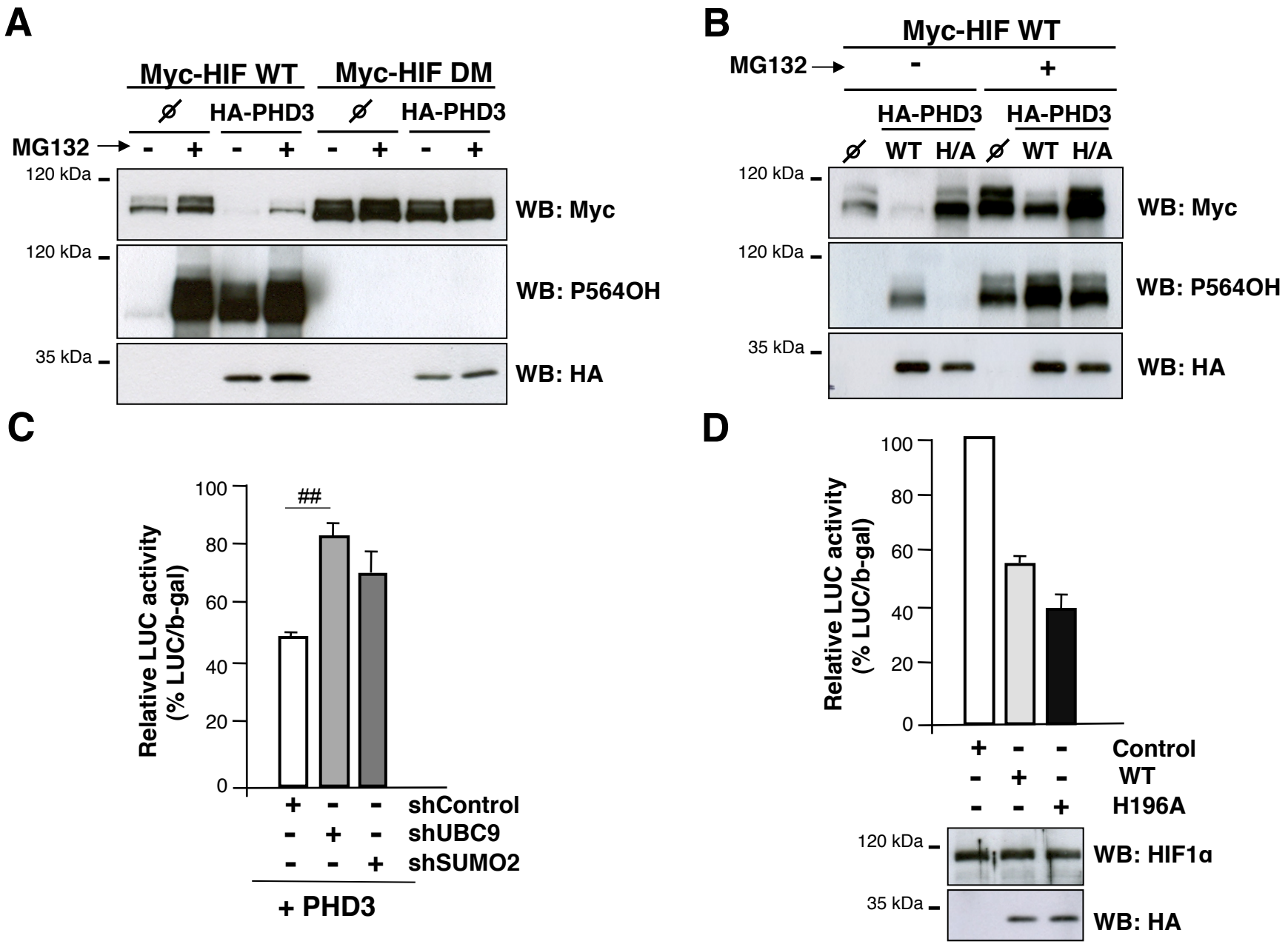


Figure S4.


A
Myc-HIF WT
Myc-HIF DM
HA-PHD3
MG132
120 kDa
WB: Myc
120 kDa
WB: P564OH
35 kDa
WB: HA
B
Myc-HIF WT
MG132
HA-PHD3
WT H/A
120 kDa
WB: Myc
120 kDa
WB: P564OH
35 kDa
WB: HA
C
Relative LUC activity (% LUC/b-gal)
##
shControl
shUBC9
shSUMO2
+ PHD3
D
Relative LUC activity (% LUC/b-gal)
Control
WT
H196A
120 kDa
WB: HIF1α
35 kDa
WB: HA

Supplementary Table1.

MUTANTS	OLIGO SENSE
HA-PHD3 K119R	5´-GTCAAGGAGAGGTCTAGAGCAATGGTGGCTTGCTATCCG -3´
HA-PHD3 K154R	5´-CTACTATCTGAACAGGAATTGGGATGCCAAGCTTCATGGTGGGATC-3´
HA-PHD3 K159R	5´-GAATTGGGATGCCAGGCTACATGGTGGCATCCTGCGGATATTTC-3´
HA-PHD3 K172R	5´-CTGCGGATATTTCCAGAGGGGAGATCTTTCATAGCAGATGTGGAG-3´
HA-PHD3 K222R	5´-GAAAGGGCAGAAGCCAGAAAGAAATTCAGGAATTTAACTAG-3´
HA-PHD3 K223R	5´-GAAAGGGCAGAAGCCAAAAGGAAATTCAGGAATTTAACTAG-3´
HA-PHD3 K224R	5´-GAAAGGGCAGAAGCCAAAAAGAGATTCAGGAATTTAACTAG-3´
HA-PHD3 K231R	5´-CAGGAATTTAACTAGGAGAACTGAATCTGCCCTCACTG-3´
HA-PHD3 K222/223/224R	5´-GAAGAAAGGGCAGAAGCCAGAAGGAGATTCAGGAATTTAACTAGG-3´
HA-PHD3 H196A	5'–CTGGTCAGATCGTAGGAACCCTGCAGAAGTGCAGCCCTCTTACGC-3'

Underlining indicates the enzymatic restriction site modified by the silent mutation.

Fig. S1. Mapping the PHD3 SUMOylation site(s). *In vitro* SUMOylation of the PHD3 constructs corresponding to the individual mutation of the 8 conserved Ks located at the C-ter end of the protein. The non-modified (◄) and the modified (*) forms of PHD3 were detected by autoradiography.

Fig. S2. PHD3 SUMO1 conjugation does not impact on HIF either NF-κB activity. PHD3 WT and PHD3 SUMO1 constructs were transfected together with HRE-Luc (A) or NF-κB-Luc (B). After 16 h of hypoxia (A, 1% O_2) or TNFα (B, 20 ng/ml) treatment, luciferase and β-galactosidase activity were measured. (C) Endogenous SUMOylation impacts on HIF activity. The indicated pools of shRNAs together with HRE-Luc reporter vector were transfected. Cells were incubated in normoxia (20% O_2) or hypoxia (1% O_2) for 16 h and luciferase and β-galactosidase activity were measured. Data show the mean±s.e.m.; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ versus control cells.

Fig. S3. PHD3 SUMO conjugation does not affect PHD3 localisation. Immunofluorescence staining showing HeLa cells transfected with the indicated HA-tagged PHD3 constructs using the anti-HA antibody. Nuclei were visualized by DAPI staining. Scale bar: 5µm.

Fig. S4. Validation of the anti-HIF1αP564OH antibody. (A,B) HEK293T cells were transfected with Myc-HIF1 WT or Myc-HIF1 DM (P402/564A) with or without the indicated HA-PHD3 constructs. Cells were lysed after 4 h of MG132 treatment (10 µM) and proteins visualized by WB. ø, empty vector. (C) Endogenous SUMOylation impacts on PHD3-mediated repression of HIF. HEK293T were transfected with the reporter plasmids, Myc-HIF1 DM (P402/564A), PHD3 and the indicated shRNAs. $^{\#\#}P<0.01$ versus control cells. (D) PHD3-mediated repression is independent of hydroxylase activity. Cells were transfected with the reporters, Myc-HIF1 DM (P402/564A) and the PHD3 constructs. Luciferase and β-galactosidase activity was measured in cells incubated in normoxia (20% O_2). Data show the mean±s.e.m. In parallel, cells were transfected to analyse protein expression levels by WB.

Supplementary Table 1. Sequences of the primers used for mutagenesis. The mutations designed to replace the lysine (K) residue by arginine (R) or histidine (H) by alanine (A) are shown in red. Some oligos contain an additional silent mutation (in blue), which modifies the enzymatic restriction pattern of the mutated construct.