CHUA et al. Supplementary Material

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. HIF-1α protein stabilization is not altered by modulating intracellular ROS

(a) 143B cells were incubated at either 21% O_2 or 1% O_2 for 4 hours with glucose oxidase at concentration of 25, 100 and 200 mU/ml followed by analysis of HIF-1α by Western blotting.

(b) 143B control cells (open bar) and cytoplasmic catalase (CYTO-CAT) (filled bar) overexpressing cells were stained with 20 μM 2'-7'-Dichlorodihydrofluorescein diacetate (DCFDA) dye for 15 min prior to H_2O_2 (200 μM) treatment for 5 min.
The difference in fluorescence before and after the H_2O_2 treatment was determined in control and CYTO-CAT cells. (n = 3, mean ± SEM). 143B cells that overexpressed with cytoplasmic or mitochondrial catalase (CYTO- CAT or MITO-CAT) or control vector cells were subjected to 21% O_2 or 1% O_2 and HIF-1α was detected by Western blotting.

Fig. S2. Superoxide is not involved in the regulation PHD activity *in vivo*

HEK293 cells treated with diethyldithiocarbamate (DCC) (1 mM), MnTBAP (100 μM), N-acetylcysteine (NAC) (10 mM) and ascorbate (2.5 mM) were exposed to either 21% or 1% O_2. HIF-1α protein abundance was determined by Western blotting.

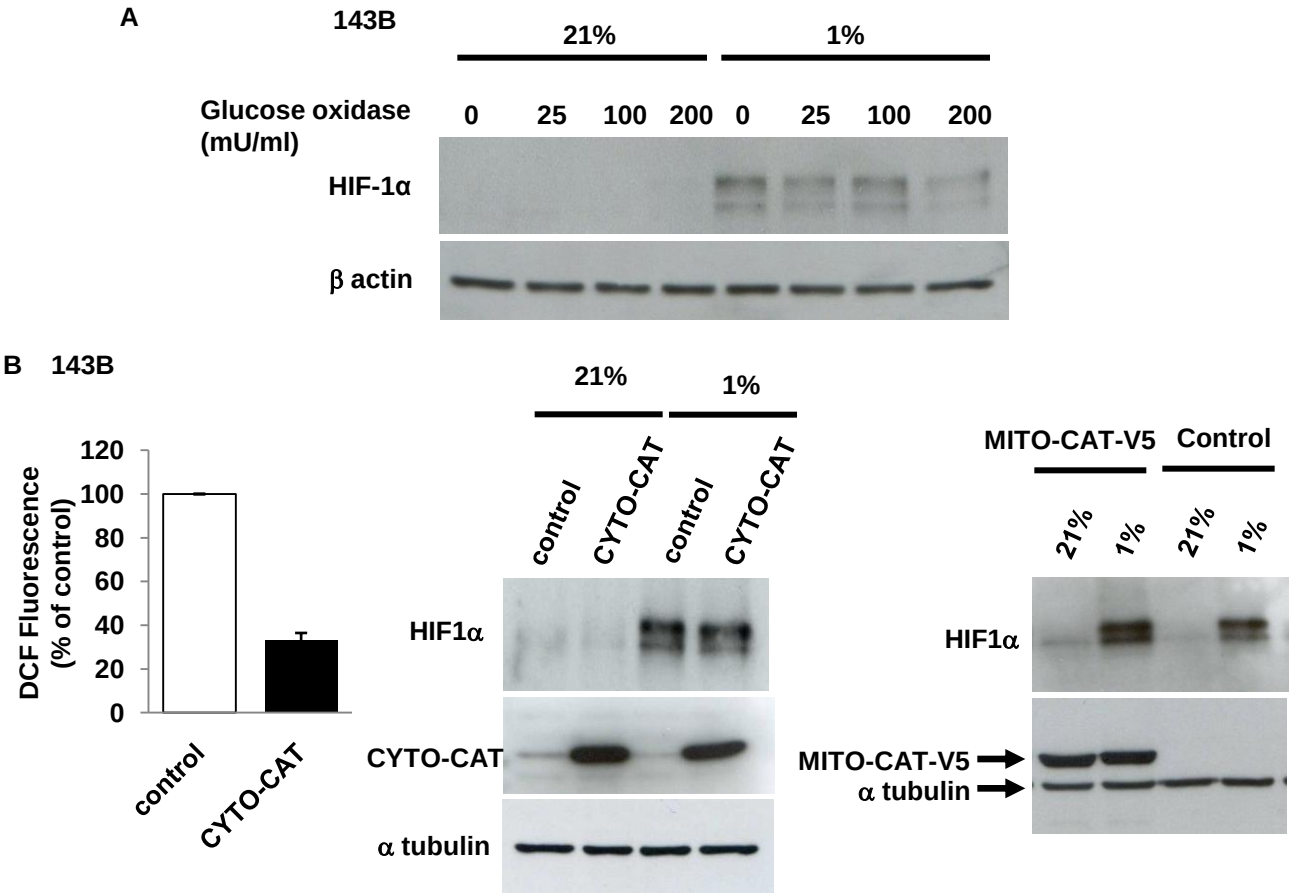
A
143B
21%
1%
Glucose oxidase (mU/ml)
0
25
100
200
0
25
100
200
HIF-1α
β actin
B
143B
21%
1%
control
CYTO-CAT
control
CYTO-CAT
DCF Fluorescence (% of control)
120
100
80
60
40
20
0
control
CYTO-CAT
HIF1α
CYTO-CAT
α tubulin
MITO-CAT-V5
Control
21%
1%
21%
1%
HIF1α
MITO-CAT-V5
α tubulin

Fig. S1

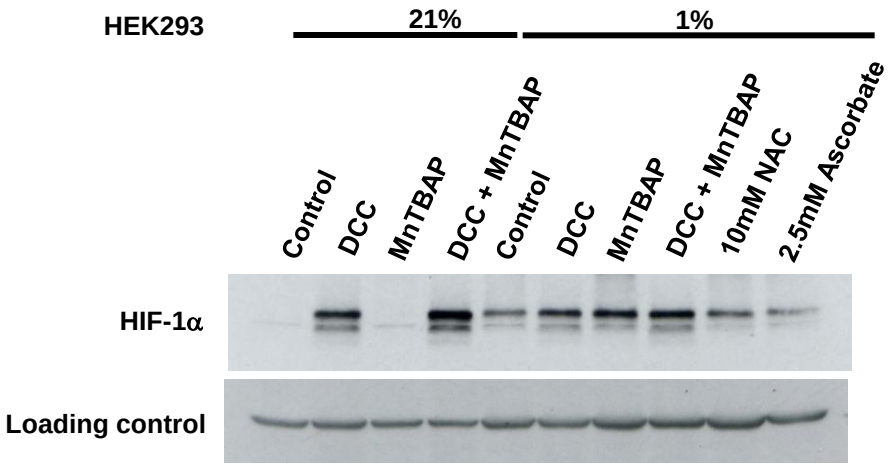
HEK293
21%
1%
Control
DCC
MnTBAP
DCC + MnTBAP
Control
DCC
MnTBAP
DCC + MnTBAP
10mM NAC
2.5mM Ascorbate
HIF-1α
Loading control

Fig.S2