Degradation of the mitochondrial complex I assembly factor TMEM126B under chronic hypoxia

Supplementary Figures

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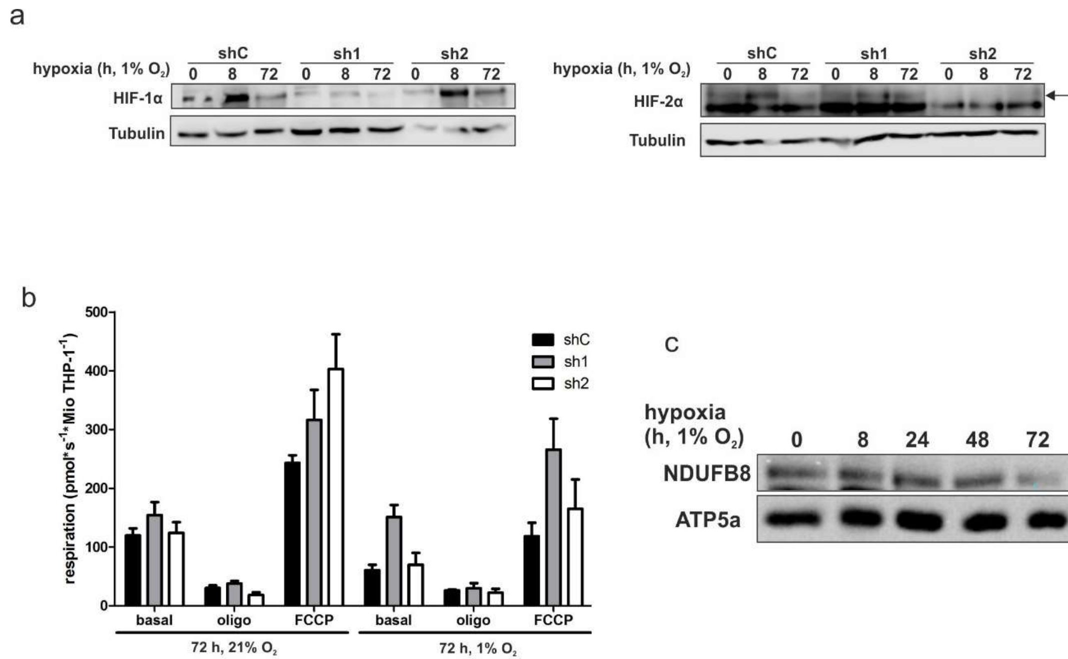

Fig.S1: Validation of the HIF-knockdown and respiration of sh2 cells

(A) THP-1 control (shC), HIF-1α knockdown (sh1), and HIF-2α knockdown (sh2) cells were incubated for indicated time points under hypoxia and expression of HIF-1α and HIF-2α was analyzed by Western blotting. (B) Respiration was measured after culturing THP-1 control (shC), HIF-1α knockdown (sh1), or HIF-2α knockdown (sh2) cells for 72 h in either 21% or 1% O_2. Oxygen consumption with oligomycin (oligo, 2 µg/ml) was compared to the maximal uncoupled respiratory capacity was estimated by subsequently adding carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP, 1 µM). All data are shown as mean values ± SEM. (C) THP-1 cells were time-dependently incubated under hypoxia. Complex I and V were quantified using BNE and expression of NDUFB8 and ATP5a.

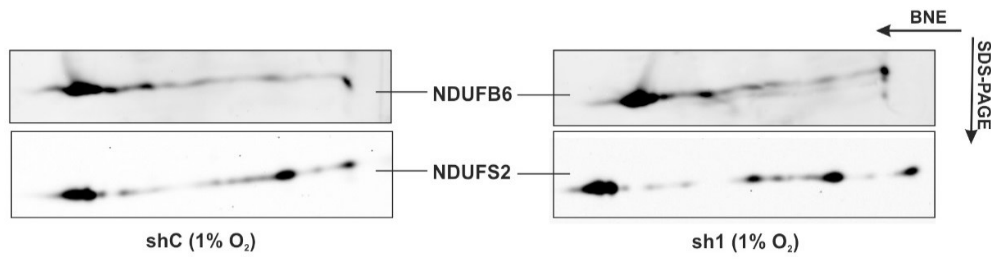

Fig.S2: 2D-BNE of NDUFB6 and NDUFS2

Validation of complexome profiling by 2D-BNE coupled with Western analysis for NDUFB6 and NDUFS2.

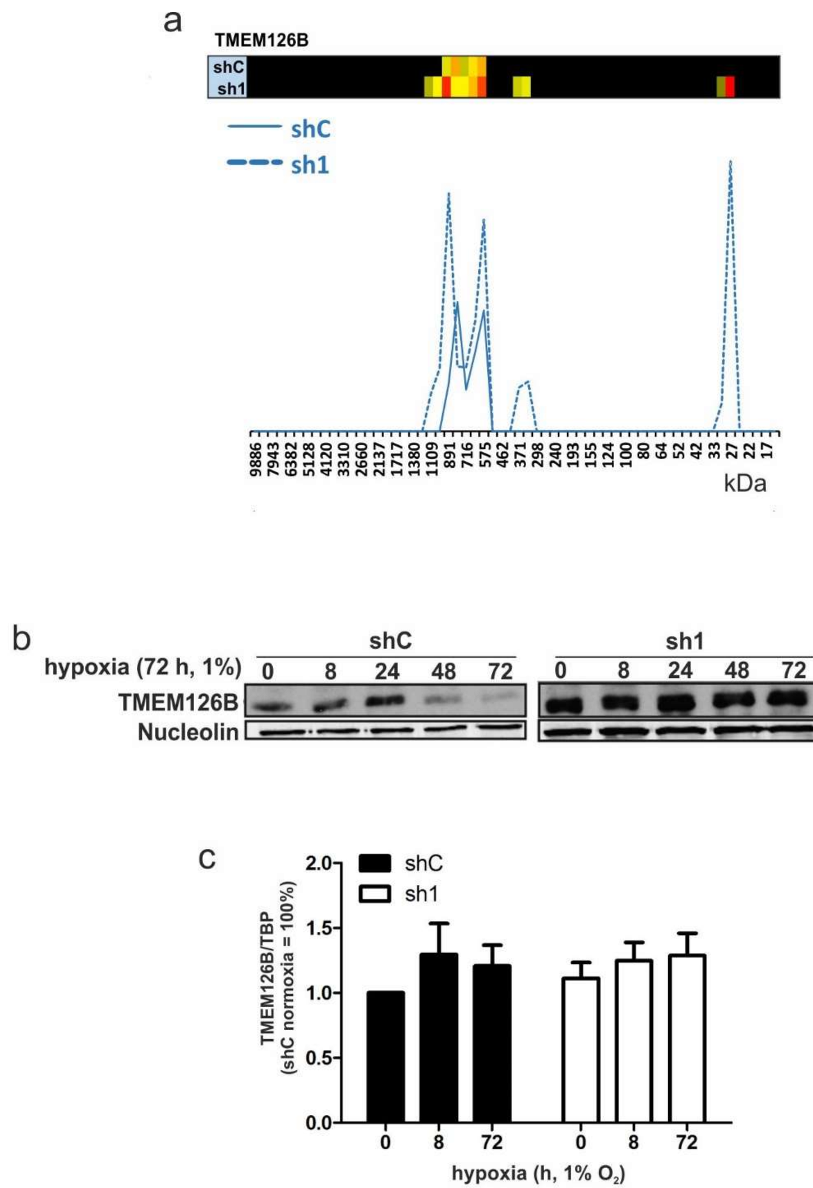

Fig.S3: Profile and time course of TMEM126B

(a) Complexome profile of TMEM126B in THP-1 control (shC) and HIF-1α knockdown cells (sh1). (b) Western analysis of TMEM126B in control (shC) and HIF-1α knockdown cells (sh1). (c) qPCR analysis of TMEM126B in THP-1 cells (n=5).

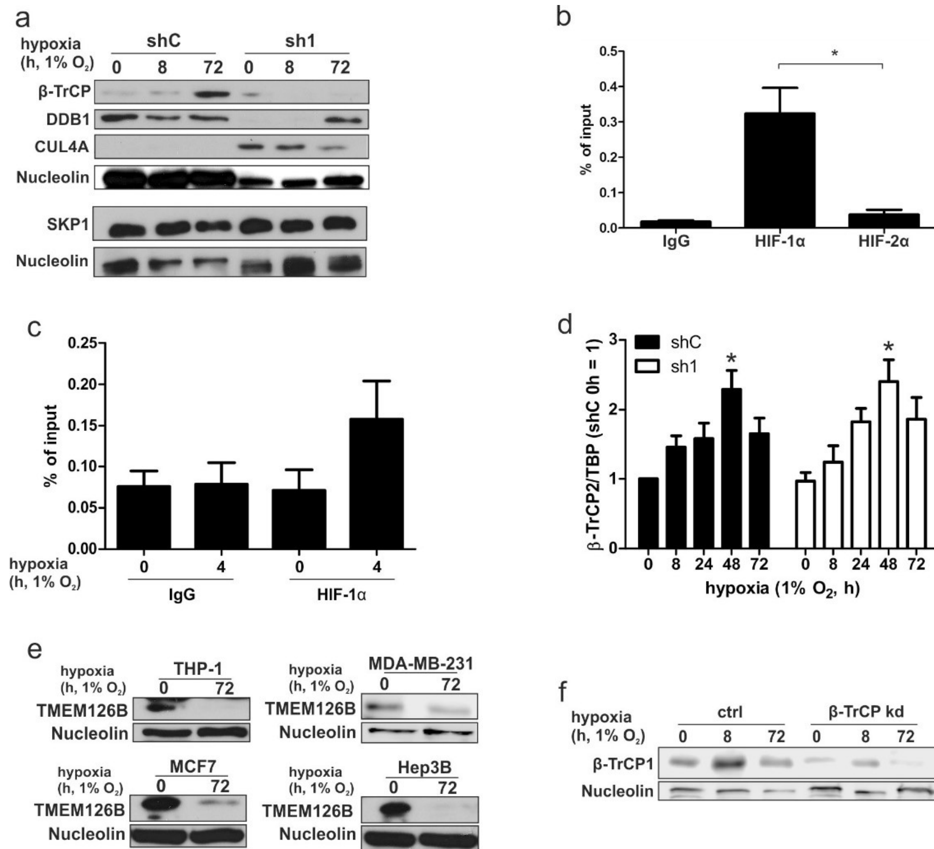

Fig.S4: E3 Ubiquitin ligase complex regulation under hypoxia and verification of hypoxic TMEM126B regulation

(a) Western analysis of the E3 ubiquitin ligase complex members F-box/WD repeat-containing protein 1A (β-TrCP1), DNA damage-binding protein 1 (DDB1), cullin4A (CUL4A), and S-phase kinase-associated protein 1 (SKP1) in THP-1 control (shC) and HIF-1α knockdown cells (sh1). (b) ChIP analysis for HIF-1α and HIF-2α binding to the β-TrCP gene in primary human macrophages incubated for 4 h under hypoxia. (c) ChIP analysis for HIF-1α binding to the β-TrCP gene in MDA-MB-231 cells incubated for 4 h under hypoxia. (d) mRNA analysis of β-TrCP2 in shC and sh1 cells. (e) Western analysis of TMEM126B in THP-1, MDA-MB-231, MCF7, and Hep3B cells. (f) Western analysis of β-TrCP in MDA-MB-231 control and IPTG-inducible β-TrCP knockdown cells.

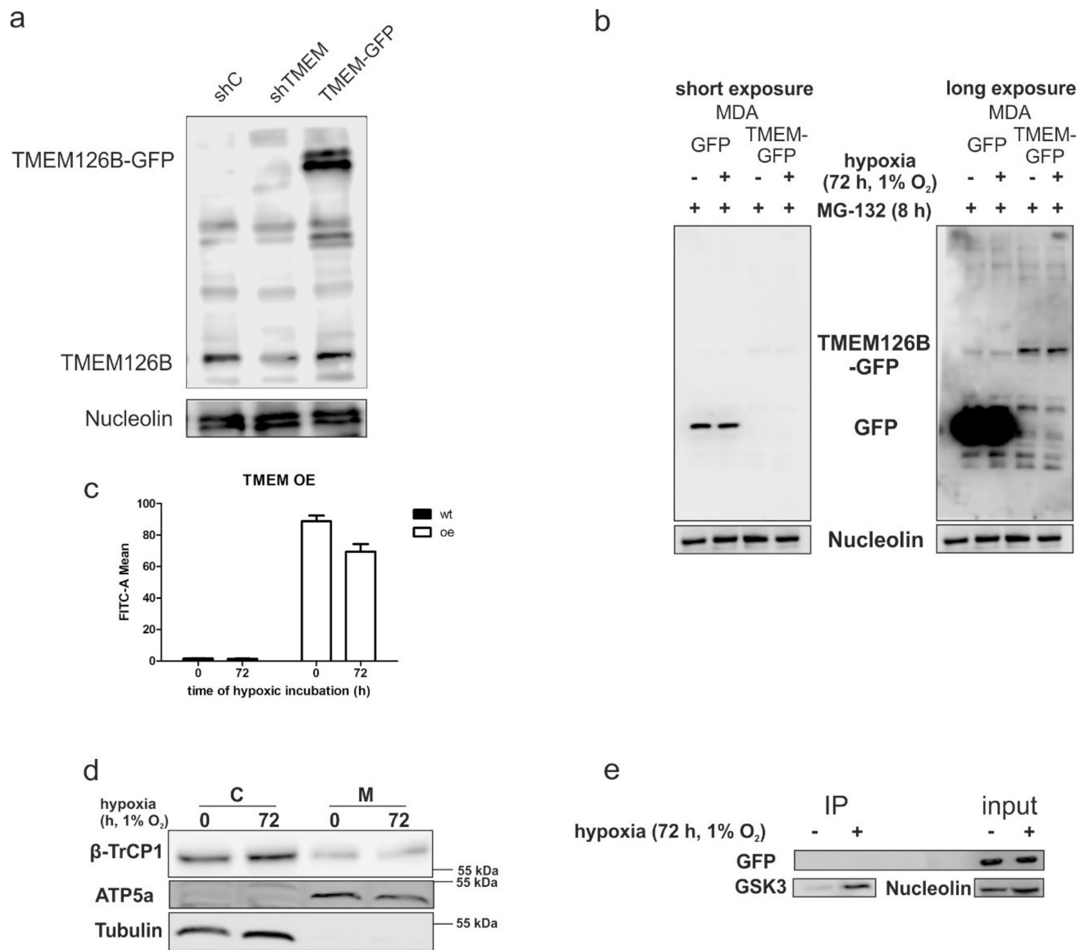

Fig.S5: Validation of TMEM126B overexpression and IP

(a) Western analysis of control (shC), TMEM126B knockdown (shTMEM) and TMEM126B-GFP overexpressing MDA-MB-231 cells stained for TMEM126B. (b) Western analysis of control (shC) and TMEM126B-GFP overexpressing MDA-MB-231 cells stained for GFP. (c) FACS analysis of TMEM-GFP expressing cells compared to wildtype (wt) in normoxia or chronic hypoxia depicted as FITC-A mean fluorescence. (d) Western analysis of β-TrCP1 in mitochondrial (M) and cytosolic (C) fractions of MDA-MB-231 lysate. (e) Western analysis of GFP after IP using an Akt substrate antibody. GSK3 serves as a positive control for the IP and Nucleolin as a loading control for the input.