Supplemental Figure Legends Biddlestone et al.

SINHCAF/FAM60A and SIN3A specifically repress HIF 2α expression

**Figure S1: SINHCAF as a HIF 2α repressor.** (A) qPCR validation using HeLa cells, for SINHCAF and SIN3A. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. (B) Quantification of HIF 2α protein levels following SINHCAF depletion compared to control cells exposed to hypoxia. Graph depicts mean and S.E.M. of HIF 2α protein levels normalised to actin of a minimum of 3 independent experiments. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. (C) Quantification of HIF 2α protein levels following SIN3A depletion compared to control cells exposed to hypoxia. Graph depicts mean and S.E.M. of HIF 2α protein levels normalised to actin of a minimum of 3 independent experiments. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. (D) Quantification of HIF 2α protein levels following SINHCAF, PHD2 or SINHCAF and PHD2 depletion compared to control cells exposed to hypoxia. Graph depicts mean and S.E.M. of HIF 2α protein levels normalised to actin of a minimum of 3 independent experiments. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01.

**Figure S2: SINHCAF represses the HIF 2α promoter.** (A) U2OS-HIF 2α promoter cells were transfected with 1µg of control of SINHCAF expressing plasmids prior to lysis and luciferase assay being performed. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

**Figure S3: Validation of SP1 and SP3 knockdown levels.** (A) qPCR and western blot validation for siRNA using HeLa cells. Graphs depict mean + S.E.M. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. HeLa cells were transfected with the indicated siRNA oligonucleotides and exposed where indicated to 1% O2 for 24 hours prior to cell lysis. Whole cell lysates were analysed by immunoblot for the levels of the depicted proteins.
Figure S4: Colony formation assay following SINHCAF and HIF 2α. (A) Colony Formation: HeLa and DLD-1 cells were seeded for colony formation following siRNA transfection. Representative images are displayed.
Sup. Figure S2

A

![Bar graph showing relative luciferase activity for U2OS HIF 2α promoter with EV and SINHCAF conditions](graphimage)

- **U2OS HIF 2α promoter**
- Relative luciferase activity
- **EV**
- **SINHCAF**

**Significance:**
- **EV**
- **SINHCAF** with **,**
Sup. Figure S3

A

Relative mRNA levels

Control    Sp1

Hypoxia Time (Hrs): 0 24 0 24 0 24

siRNA: Control SP1 SP3

SP1

SP3

β-actin

* * *