

Supplemental Material and Methods:**Antibodies:**

Antibodies for immunoblot and IP were as follows: murine HIF-1 α (Mansfield et al., 2005), human HIF-1 α (BD Biosciences), murine and human HIF-2 α (Novus NB 100-122 and 100-132), Actin (Sigma AC-15), Creb (Cell Signaling), p21 (Santa Cruz C-19), p27 (Santa Cruz F-8), E2F1 (Santa Cruz C-20), Cyclin D2 (Santa Cruz C-17), CDK2 (Santa Cruz M2), Akt (Cell Signaling), c-Myc (Santa Cruz N-262 and C-33), Sp1 (Santa Cruz H-225 and E-3), Miz1 (Santa Cruz H-190 and G-18), Max (Santa Cruz C-17 and H-2) and Mad1 (Santa Cruz C-19 and F-1).

Chromatin IP

Chromatin IP was performed as has been described with some minor modifications (Letting et al., 2003). Cells were fixed with 1% formaldehyde for 10 minutes at 37°C, and nuclear extracts were obtained and sonicated to produce DNA of approximately 600 bp. Specific IP was performed with antibodies against HIF-1 α (Novus NB 100-105), HIF-2 α (Novus NB 100-122), c-Myc (Santa Cruz N-262), p53 (Oncogene Ab-6) or Myctag (Santa Cruz A-14), as well as isotype matched controls (for mouse IgG2a and IgG2b from Becton Dickinson; for rabbit IgG from Upstate). Analysis was performed with SYBR green, with input DNA as the endogenous control.

Transformation Assays

Primary MEFs were generated from day 14 C57-BL/6 embryos. At passage 4, cells were transfected with pBabe-puro, pBabe-puro-RasV12G and pBabe-puro-Myc, pCDNA3.1-Flag, pCDNA3.1-DPM-HIF-1 α -FLAG, and pCDNA-HIF-2 α -FLAG, and MSCV-GFP (the kind gift of Russell Jones) with Lipofectamine 2000 (Invitrogen). They were allowed to grow to confluence, and maintained in culture for 30 days with media changes every 3 days (for this period 5% FBS was used instead of 10% FBS). Transfections were performed in sextuplicate, with four plates stained with Wright-Giemsa and foci counted and two plates used to isolate foci to establish cell lines.

Supplemental Figure 1. Hypoxic HCT116 and WT8 cells induce HIF target genes consistent with their HIF- α subunit expression. Expression of common HIF target VEGF (A), and HIF-1 α and HIF-2 α specific gene targets PGK (B) and Oct4 respectively (C) following 24, 48 or 72 hrs. at 0.5% O₂. Results measured by QRT-PCR and averaged from 4 experiments are shown, error bars \pm 1 SEM.