Supplemental data figure legends

S1: Related to Figure 2B-D. A) Western blot showing the differential binding of full length GST-HAF to in vitro transcribed/translated V5- tagged HIF-1α [298-400} and V5-tagged HIF-2α [298-400]. Binding of HAF to HIF-α was detected using anti-V5 antibody. B, C) Representative Ponceau staining of membranes used for pulldown assays showing equal loading of GST or GST-HAF.

S2: **Related to Fig 4.** Microarray analysis for the identification of HIF-1α or HIF-2α target genes in LN229 GBM cells after 16 hours' hypoxia. A) Western blot showing specific siRNA mediated knockdown of HIF-1α or HIF-2α. B) Lists of selected i) HIF-1α, ii) HIF-2α or iii) HIF-1/2α target genes. Data was obtained in triplicate and normalized to values obtained with scr siRNA transfection in normoxia. The cut-off for hypoxic induction was set at >2 and $p<0.05$. ? indicates no significance difference between treatment versus control.

S3: **Related to Fig 4**. Kinetics of *OCT3/4, MMP9* and *VEGFA* induction in response to increasing hypoxic duration in U87 cells. Data was obtained by Taqman RT-PCR and are the mean of at least 2 experiments ± SE.

S4: **Related to Fig 4E**. Western blot showing the effects of hypoxic duration and intensities of the levels of HIF-1α, HIF-2α and HAF in PANC-1 cells. Data are representative of at least 2 separate experiments.

S5: Related to Fig 5D. Quantitation of U87 colony number and size obtained in normoxia in 3D Nanoculture plates. Cells were transfected with Scr non-targeting control siRNA, siRNA towards HIF-1α, HIF-2α or HAF, 72 hours prior to seeding and allowed to grow for a further 72 hours.

Data are the mean of 4 replicate wells from a representative experiment ± SE. Refer to images in **Fig 5D**.

S6: Related to Fig 6E. Representative images and western blots showing the effect of siRNA knockdown of control (Scr), HIF-1α or HIF-2α on TGF-β induced EMT and associated EMT markers in PANC-1 cells. Cells were transfected wth siRNA for 72 hours, reseeded at 70% confluence, serum starved for 24 hours and treated with normal serum-containing media or serum-free media containing 10ng/ml TGF-β for a further 48 hours.

S7: **Related to Fig 6F**. Representative histograms showing FACs analysis of CD133-APC in HAF-GFP or vector-GFP expressing U87 cells. GFP+ viable cells were gated using an IgG isotype control.

Supplementary experimental procedures

Microarray sample preparations

Samples were processed using Affymetrix GeneChip Whole Transcript (WT) Sense Target Labeling Assay protocol . Starting with 1µg of total RNA, we performed ribosomal RNA (rRNA) reduction to reduce the 28S and 18S rRNA population significantly from the total RNA and thus minimize background and increase array detection sensitivity and specificity.

This rRNA reduced RNA sample was used to generate double-stranded cDNA using random hexamers tagged with a T7 promoter sequence. The double-stranded cDNA was subsequently used as a template and amplified by T7 RNA polymerase to produce antisense cRNA. In the second cycle of cDNA synthesis, we used 10ug of this cRNA and random primers to produce single-stranded DNA in the sense orientation.

We then fragmented 5.5ug of the Single-Stranded DNA into 40-70nt fragments. In order to reproducibly fragment the single-stranded DNA and improve the robustness of the assay, a

novel approach was utilized where dUTP was incorporated in the DNA during the second-cycle, first-strand reverse transcription reaction. This single-stranded DNA sample was then treated with a combination of uracil DNA glycosylase (UDG) and apurinic/apyrimidinic endonuclease 1 (APE 1) that specifically recognizes the unnatural dUTP residues and breaks the DNA strand. Then, the Fragmented Single-Stranded DNA was labeled by terminal deoxynucleotidyl transferase (TdT) with the Affymetrix® proprietary DNA Labeling Reagent covalently linked to biotin.

Following the recommended procedures, we anticipated generating sufficient target for hybridization to a single array. Sample quality analysis (QA) and quality control (QC) were confirmed using the NanoDrop ND-1000 and Bioanalyzer 2100. Hybridizations were performed using a GeneChip® Hybridization Oven, while a array washing and staining was performed using a GeneChip® Fluidics Station 450. Arrays were scanned using a GeneChipScanner 3000 7G.