Supplemental Methods

**Generation of shRNA expressing MDA-MB-231 cells.** Briefly, expression vectors encoding shRNA sequences targeting HIF-1α and HIF-2α driven by the U6 promoter in a pLKO.1-puro plasmid were purchased from Sigma. Several independent shRNA targeting sequences (at least four per gene) were validated for efficiency of knockdown in breast cancer cell lines (MCF-7 and MDA-MB-231) by qPCR and by western blotting of cells cultured at 6h or 24h of hypoxia (1% O₂), when HIF-1α and HIF-2α expression levels peak, respectively. Sequence NM_001530.x-1048s1c1 to HIF-1α and sequence NM_001430.x-1694s1c1 to HIF-2α achieved the most efficient knockdown for each gene. For generation of stable HIF-1α/2α double knockdown cell lines the original puromycin resistance cassette in shHIF-2α pLKO.1 was replaced by a hygromycin resistance marker. Viral particles were produced in HEK293T cells using the Vira-Power lentiviral expression system according to the manufacturer’s instructions (Invitrogen).

**Transgenic mice and generation of tumors in NOD/SCID/gamma recipients.** At harvest, all tumors were measured with calipers ex vivo, were weighed to determine wet weight and tumors were divided and one portion was processed for histology by fixing 6-8h in 10% neutral-buffered formalin. Visibly necrotic areas were discarded from the remainder of the tumor material, which was cubed and flash frozen in liquid nitrogen. All experiments involving mice were conducted under University of Minnesota or University of Tennessee Health Science Center IACUC approved protocols and followed NIH guidelines for animal care.

**Supplemental figure legends**

**Supplemental Figure 1.**
A. MCF7 cells were cultured in normoxia (20% O₂) or hypoxia (1% O₂) for the indicated amounts of time and lysates were subjected to Western blotting with antibodies for Brk, HIF-1α, and AKT (loading control). B. MCF7 cells were cultured at normoxia or hypoxia (1% O₂) for 24 hours and Brk, VEGF, and MET mRNA expression was assessed via qPCR after normalization to *TBP*. C. MCF7 shControl shHIF1A, shHIF2A and DKD
cells were cultured at normoxia or hypoxia (1% O₂) for 24 hours and *Brk* mRNA was determined via qPCR (normalized to *TBP*). Data are represented as fold *Brk* mRNA relative to each cell line's normoxic condition.

**Supplemental Figure 2.**

HREs 2-5 were assessed via ChIP for HIF-1α, HIF-2α, and RNA pol II recruitment in MDA-MB-231 cells following 24 hours at normoxia or hypoxia (1% O₂). Negative control isotype matched IgG controls were performed on MDA-MB-231 cells cultured at hypoxia for 24 hours.

**Supplemental Figure 3.**

A. MDA-MB-231 shControl, DKD, and DKD + Brk cells were cultured at hypoxia (1% O₂) for 24 hours prior to Western blotting to confirm knockdown with antibodies to HIF-1α, HIF-2α or ERK1/2 (loading control). B. MDA-MB-231 DKD and DKD + Brk cells were cultured at normoxia or hypoxia (1% O₂) for 24 hours and lysates subjected to Western blotting using Brk and β-Actin (loading control) antibodies. *Brk* mRNA expression levels were determined via qPCR after normalization to *TBP*. Asterisks indicate significant comparisons between matched normoxic and hypoxic samples.