**Supplementary Materials and Methods**

**Primer sequences**

The sequences of primers used for qRT-PCR are indicated below.

*CXCR4* forward: 5’-CCTATGCAAGGCAGTCCATGT-3’

*CXCR4* reverse: 5’-GGTAGCGGTCCAGACTGATGA-3’

*GLUT1* forward: 5’-CGGGCCAAGAGTGTGCTAAA-3’

*GLUT1* reverse: 5’-TGACGATACCGGAGCCAATG-3’

*HIF-1A* forward: 5’-CTGCCACCACTGATGAATTA-3’

*HIF-1A* reverse: 5’-GTATGTGGGTAGGAGATGGA-3’

*HIF-2A* forward: 5’-TGCTCCCACGGCCTGTAC-3’

*HIF-2A* reverse: 5’-TTGTCACACCTATGGCATATCACA-3’

*KLF4* forward: 5’-CCCACATGAAGCGACTTCCC-3’

*KLF4* reverse: 5’-CAGGTCCAGGAGATCGTTGAA-3’

*LEF1* forward: 5’-GATCTTCGCCGAGATCAGTC-3’

*LEF1* reverse: 5’-GCCTCCATCTGGATGCTTTC-3’

*MYC* forward: 5’-GGAGACACCGCCCACCA-3’

*MYC* reverse: 5’-GCGCTGCGTAGTTGTGCTG-3’

*OCT4* forward: 5’-GAGAACCGAGTGAGAGGCAACC-3’

*OCT4* reverse: 5’-CATAGTCGCTGCTTGATCGCTTG-3’

*TBP* forward: 5’-GGGCATTATTTGTGCACTGAGA-3’

*TBP* reverse: 5’-TAGCAGCACGGTATGAGCAACT-3’

*18S* forward: 5’-AACCCGTTGAACCCCATT-3’

*18S* reverse: 5’-CCATCCAATCGGTAGTAGCG-3’

**Fluorescent western blot analysis**

Cells were washed once with cold PBS then lysed in RIPA buffer (Thermo Fisher) supplemented with protease inhibitors (Roche). Clarified cell lysates were quantified then boiled in Laemmli buffer for 5 min (Bio-Rad). For analysis of HIF proteins, cells were washed once with cold PBS then lysed directly in Laemmli buffer. Cell lysates were passed through a 23-gauge needle 10 times then boiled for 5 min. Proteins were separated by SDS-PAGE and transferred onto polyvinylidene fluoride membrane. Membranes were blocked for 1 h in Odyssey Blocking Buffer (LI-COR), and probed overnight at 4°C with primary antibodies for c-Myc (1:1000, Cell Signaling Technology), LEF1 (1:500, Cell Signaling Technology), HIF-1α (1:500, BD Transduction Laboratories), HIF-2α (1:500, Novus Biologicals), ERK2 (1:1000, Santa Cruz Biotechnology), or β-tubulin (1:20,000, Thermo Fisher). Bound antibodies were visualized using IRDye secondary antibodies (LI-COR) and an Odyssey Classic Infrared Imaging System (LI-COR).

**CD133 antibody staining and flow cytometry**

Cells were stained with APC-conjugated antibodies for human CD133/1 (1:50; Miltenyi), as well as SYTOX Blue (Thermo Fisher) to exclude dead cells, and samples were analyzed using a BD LSR II flow cytometer.

**Assessment of the prognostic value of hypoxia gene sets in colon cancer patients**

Hypoxia gene sets were obtained from Winter *et al.* ([1](#_ENREF_1)), Eustace *et al.* ([2](#_ENREF_2)), and Dekerval *et al.* ([3](#_ENREF_3)), consisting of 156, 26, and 923 genes, respectively, that exhibited differential regulation in hypoxia versus normoxia. RNAseq2 normalized count expression datasets for primary colon adenocarcinoma samples were downloaded from The Cancer Genome Atlas portal in R using the RTCGAToolbox ([4](#_ENREF_4)) package (version 2.2.2). From this dataset, a total of 190 samples for which survival information was annotated were selected for further analysis. For each gene set, a signature score was assigned to each sample by calculating the signed average of the signature gene expression using the *sig.score* function of the genefu package (version 2.4.2) ([5](#_ENREF_5)) in R. Samples were subsequently median-dichotomized into ‘high’ and ‘low’ groups based on their signature scores. Kaplan-Meier curves were generated using the *km.coxph.plot* function of the R package survcomp ([6](#_ENREF_6)) (version 1.22.0), to estimate the prognostic value of the binary hypoxia classification based on overall survival censored at 5 years.

**Supplementary References**

1. Winter SC, Buffa FM, Silva P, Miller C, Valentine HR, Turley H*, et al.* Relation of a hypoxia metagene derived from head and neck cancer to prognosis of multiple cancers. Cancer Res **2007**;67(7):3441-9 doi 10.1158/0008-5472.CAN-06-3322.

2. Eustace A, Mani N, Span PN, Irlam JJ, Taylor J, Betts GN*, et al.* A 26-gene hypoxia signature predicts benefit from hypoxia-modifying therapy in laryngeal cancer but not bladder cancer. Clin Cancer Res **2013**;19(17):4879-88 doi 10.1158/1078-0432.CCR-13-0542.

3. Dekervel J, Hompes D, van Malenstein H, Popovic D, Sagaert X, De Moor B*, et al.* Hypoxia-driven gene expression is an independent prognostic factor in stage II and III colon cancer patients. Clin Cancer Res **2014**;20(8):2159-68 doi 10.1158/1078-0432.CCR-13-2958.

4. Samur MK. RTCGAToolbox: a new tool for exporting TCGA Firehose data. PLoS One **2014**;9(9):e106397 doi 10.1371/journal.pone.0106397.

5. Gendoo DM, Ratanasirigulchai N, Schroder MS, Pare L, Parker JS, Prat A*, et al.* Genefu: an R/Bioconductor package for computation of gene expression-based signatures in breast cancer. Bioinformatics **2016**;32(7):1097-9 doi 10.1093/bioinformatics/btv693.

6. Schroder MS, Culhane AC, Quackenbush J, Haibe-Kains B. survcomp: an R/Bioconductor package for performance assessment and comparison of survival models. Bioinformatics **2011**;27(22):3206-8 doi 10.1093/bioinformatics/btr511.