Supporting Information

Reprogramming the transcriptional response to hypoxia with a chromosomally encoded cyclic peptide HIF-1 inhibitor.

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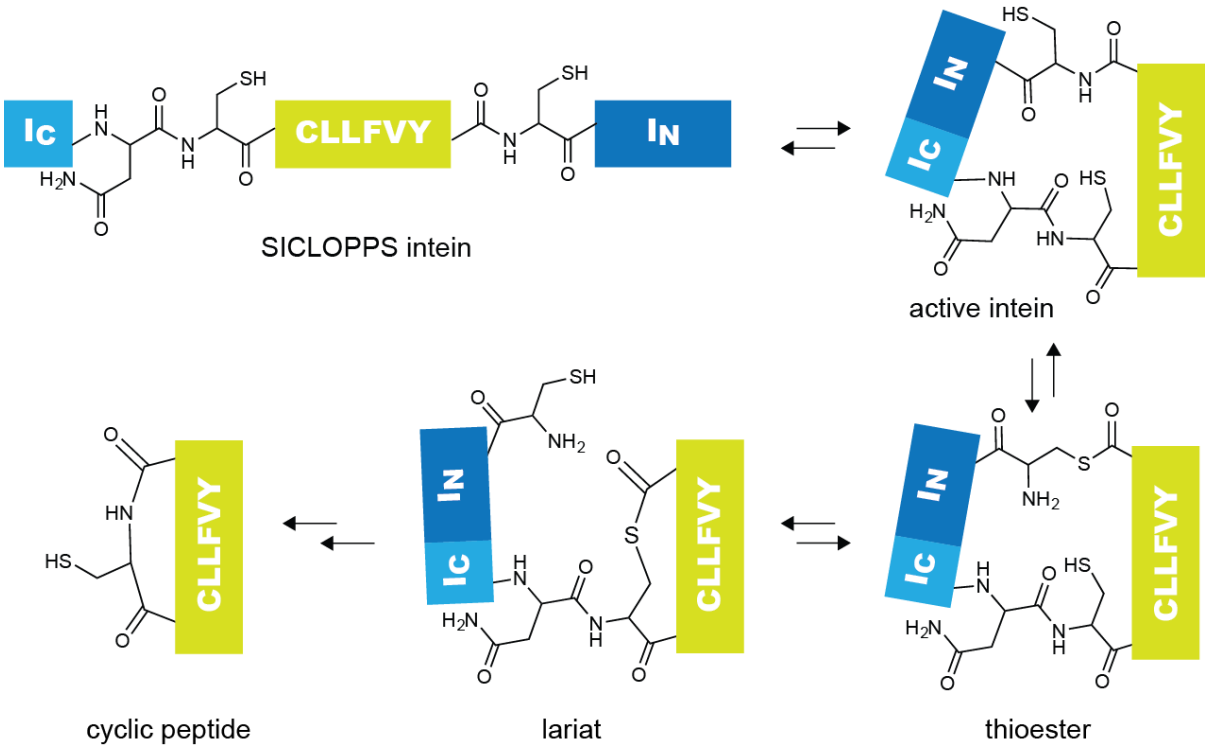

Figure S1. Mechanism of SICLOPPS intein splicing. Splicing is initiated by nucleophilic attack by the Cys residue at the N-intein/extein splice junction causing an N-S acyl shift of the peptide bond at the N-terminus and forming a thioester. Next, a nucleophilic attack by the Cys residue of the extein on the thioester bond results in cyclization of the extein, forming a lariat intermediate. Cyclization of the conserved C-terminal Asn cleaves the peptide bond, releasing the cyclized extein. The final step is a spontaneous N-S acyl shift resulting in the rearrangement of the thioester to an amide bond.

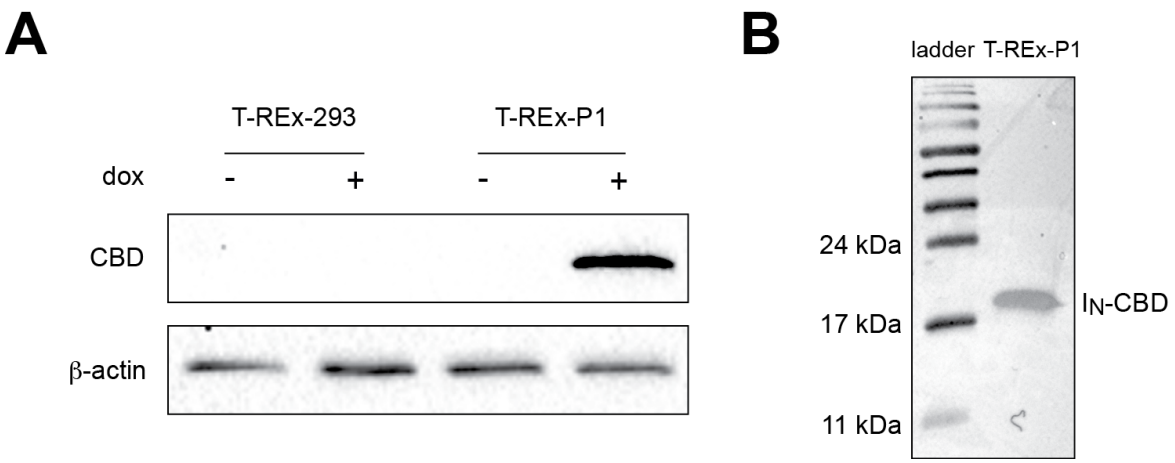

Figure S2. Western blot analysis of SICLOPPS protein expression in T-REx-P1 cells. **(A)** SICLOPPS protein was detected in T-REx-P1 cells treated with 1 µg/mL dox but not in the unintegrated parent cell line T-REx-293, demonstrating successful integration and inducible expression of SICLOPPS inteins in T-REx-P1 cells. **(B)** Following treatment of T-REx-P1 cells with 1 µg/mL dox for 24 h, a band corresponding to the N-intein (18 kDa) was detected, indicating full splicing of the SICLOPPS protein.

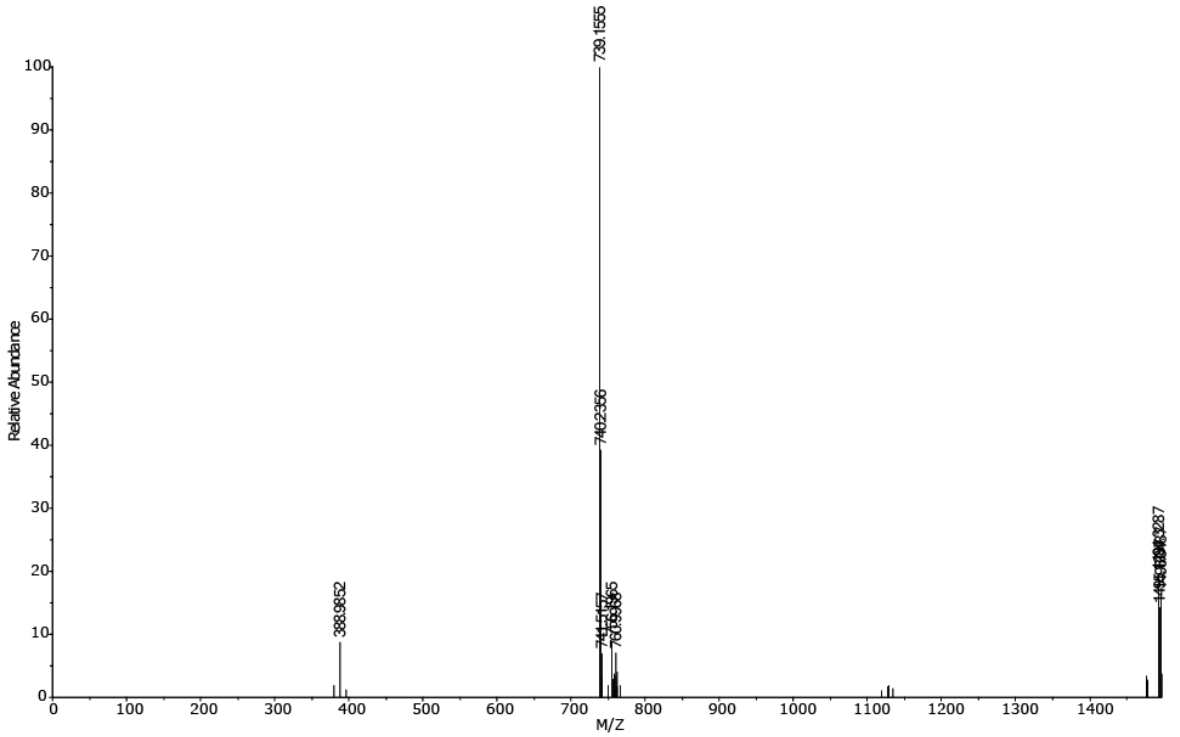

Figure S3. ESI+ MS trace of synthetic P1. LC-ESI-MS (ESI^+, AcCN, 200 nm) RT 2.99 minutes shows: 739.2 ($[M+H]^+$).

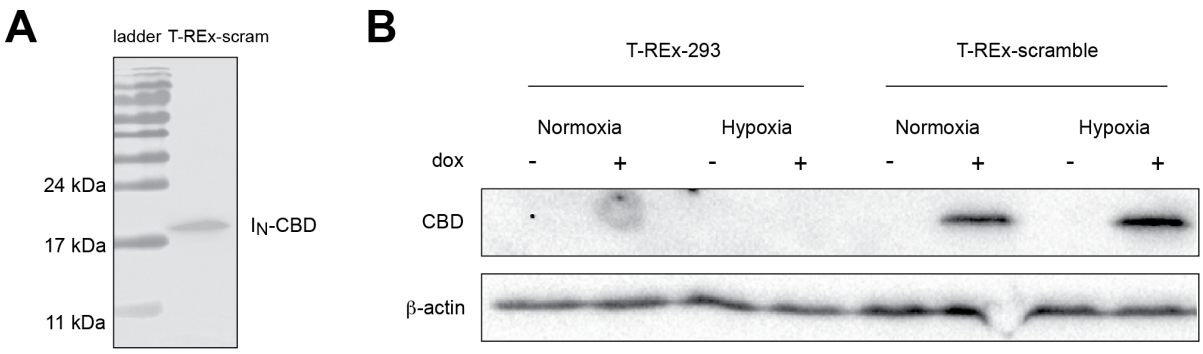

Figure S4. Western blot analysis of SICLOPPS protein in T-REx-Scramble cells (A) Following treatment of T-REx-scramble cells with 1 µg/mL dox for 24 h, a band corresponding to the N-intein (18 kDa) was detected, indicating full splicing of the SICLOPPS inteins. (B) SICLOPPS protein was detected in T-REx-Scramble cells treated with 1 µg/mL dox but not in the unintegrated parent T-REx-293 cells, demonstrating successful integration and inducible expression of the SICLOPPS construct in T-REx-Scramble cells.

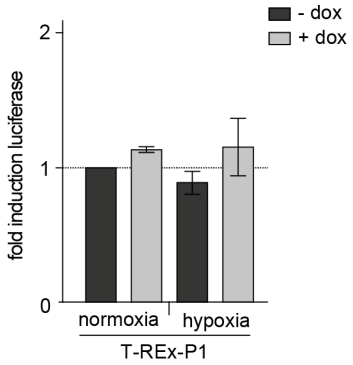

Figure S5. Production of P1 in T-REx-P1 cells does not significantly impact SV40-controlled luciferase expression. T-REx-P1 cells were transfected with pGL3-SV40-Luc. Cells were incubated with vehicle (-) or 1 µg/mL dox (+) for 16 h in normoxia or hypoxia. Experiments were performed in triplicate (n=3). Error bars represent ± SEM.

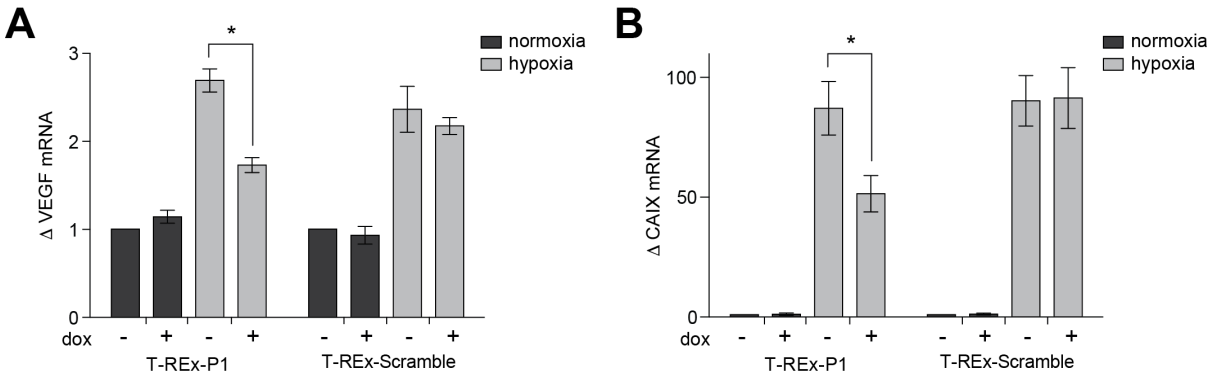

Figure S6. Hypoxic induction of VEGF and CAIX expression is reduced in cells producing P1 but not by those expressing a scrambled variant. T-REx-P1 and T-REx-Scramble cells were treated with vehicle (-) or dox (+) in normoxia or hypoxia for 24 h. **(A)** VEGF and **(B)** CAIX expression analysed by RT-qPCR normalizd to expression of β-actin. Data are means (n=3) ±SEM, *$p < 0.05$.

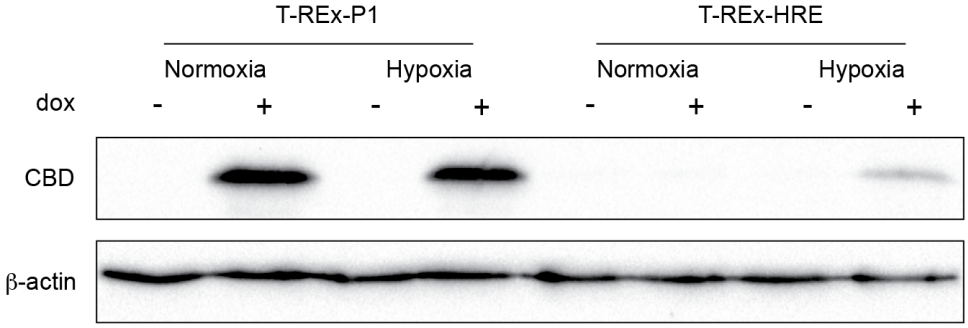

Figure S7. Western blot of SICLOPPS protein in T-REx-P1 and T-REx-HRE cells. Anti-CBD was used to detect integrated construct expression under the control of a CMV promoter (in T-REx-CLLFVY cells) or a HRE promoter (in T-REx-HRE cells). Cells were incubated with vehicle (-) or 1 µg/mL dox (+) in normoxia or hypoxia for 16 h, with β-actin used as a loading control.

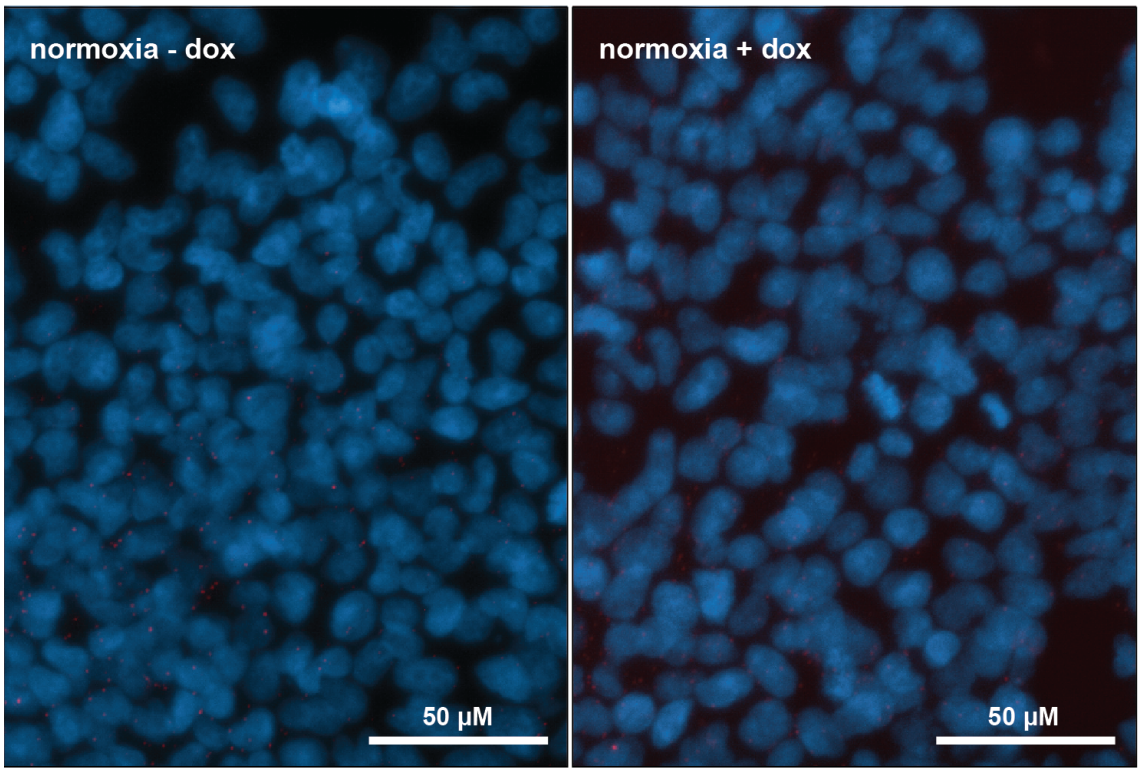

Figure S8. PLA signal is not observed in T-REx-HRE cells in normoxia. T-REx-HRE cells were treated with vehicle (-dox) or 1 µg/mL dox (+dox) and incubated in normoxia for 24 h. Cells were fixed, permeablized and incubated with primary antibodies. Cells were then incubated with Duolink reagents and visualised by fluorescence microscopy.

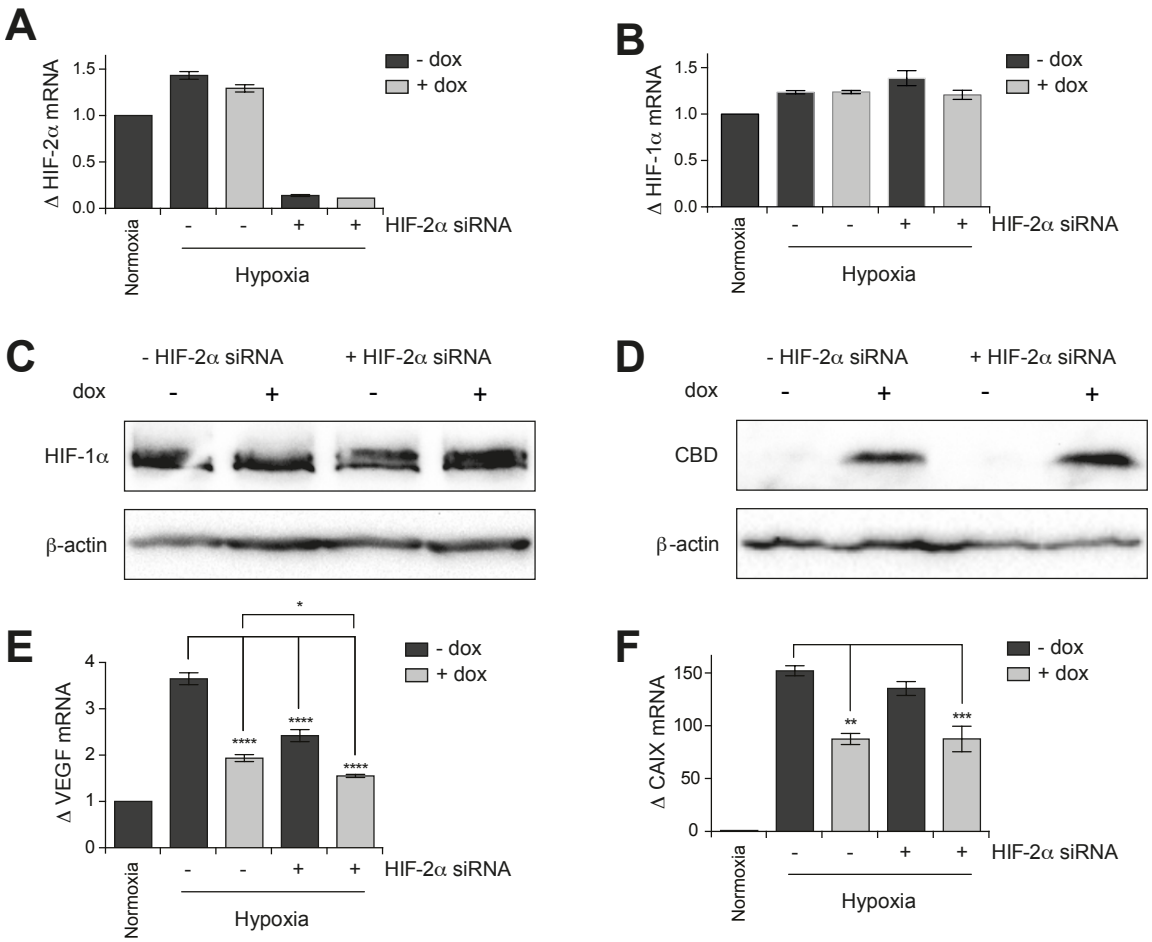

Figure S9. Effect of HIF-2α knockdown on HIF-1 target gene expression.T-REx-HRE cells were transfected with negative control siRNA (-) or HIF-2α siRNA (+) for 24 h then exposed to hypoxia for 24 h with vehicle (- dox) or 1 μg/mL dox (+ dox). (A) RT-qPCR analysis of HIF-2α mRNA shows HIF-2α knockdown by siRNA. (B) RT-qPCR analysis of HIF-1α expression shows it is not affected by dox treatment or HIF-2 knockdown. (C) Immunoblot analysis of HIF-1α protein levels show they are not affected by dox treatment or HIF-2α knockdown. (D) Immunoblot analysis of dox-induced SICLOPPS protein expression shows it is not affected by HIF-2α knockdown. (E) RT-qPCR analysis of VEGF expression shows HIF-2α knockdown inhibits it. (F) RT-qPCR analysis of CAIX expression shows it is not affected by HIF-2α knockdown. Data are means (n=3) ±SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

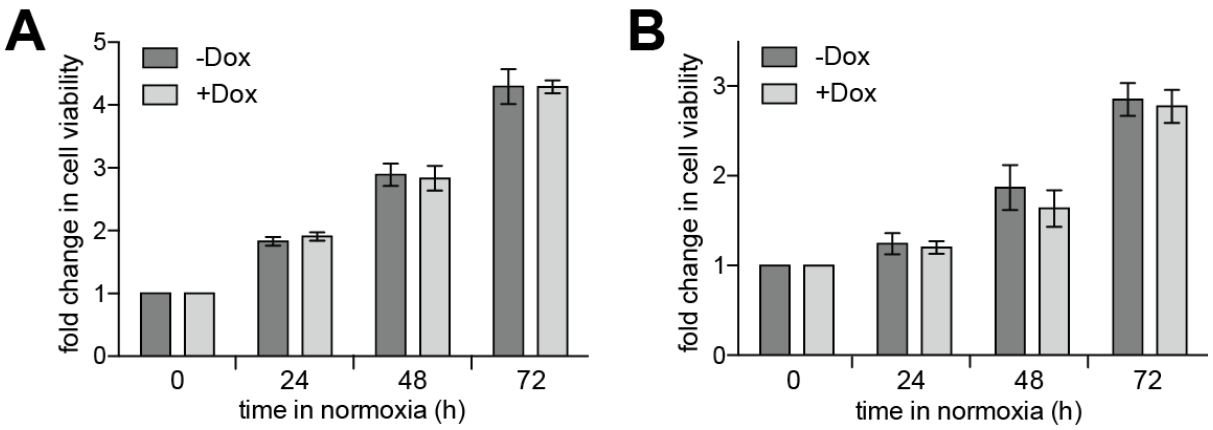

Figure S10. Viability of T-REx-HRE cells cultured for 72 hours in normoxia. **(A)** Cells were cultured in normoxia with or without 1µg/mL dox and their viability assessed by an MTT assay at 0, 24, 48 and 72 h. **(B)** Cells were treated as (A) with the addition of 3 mg/mL 2DG. Data are means (n=3) ±SEM.

Table S1. Genes analysed in hypoxia array.

Gene Symbol	Gene Name	Category	Group	NCBI Gene Reference
ADM	Adrenomedullin	Signaling molecule	Peptide hormone	NM_001124.1
ANGPTL4	Angiopoietin-Like 4	Signaling molecule	Other signaling molecule	NM_139314.1
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator	Transcription factor	Basic helix-loop-helix transcription factor	NM_178426.1
ARNT2	Aryl-Hydrocarbon Receptor Nuclear Translocator 2	Transcription factor	Basic helix-loop-helix transcription factor	NM_014862.3
ATP1B1	Atpase, Na+/K+ Transporting, Beta 1 Polypeptide	Transporter	Cation transporter	NM_001001787.1
BHLHB2	Basic Helix-Loop-Helix Domain Containing, Class B, 2	Nucleic acid binding	Nuclease	NM_003670.1
CREBBP	CREB Binding Protein (Rubinstein-Taybi Syndrome)	Transcription factor	Transcription cofactor	NM_001079846.1
CUL2	Cullin 2	Miscellaneous function	Other miscellaneous function protein	NM_003591.2
DDIT4	DNA-Damage-Inducible Transcript 4	Molecular function unclassified	Molecular function unclassified	NM_019058.2
DDIT4L	DNA-Damage-Inducible Transcript 4-Like	Molecular function unclassified	Molecular function unclassified	NM_145244.2
EGLN1	Egl Nine Homolog 1 (C. Elegans)	Molecular function unclassified	Molecular function unclassified	NM_022051.1
EGLN2	Egl Nine Homolog 2 (C. Elegans)	Molecular function unclassified	Molecular function unclassified	NM_053046.2
EGLN3	Egl Nine Homolog 3 (C. Elegans)	Molecular function unclassified	Molecular function unclassified	NM_022073.3
EP300	E1A Binding Protein P300	Transcription factor	Transcription cofactor	NM_001429.2
EPAS1	Endothelial PAS Domain Protein 1	Transcription factor	Basic helix-loop-helix transcription factor	NM_001430.3
EPO	Erythropoietin	Signaling molecule	Peptide hormone	NM_000799.2
FRAP1	FK506 Binding Protein 12-Rapamycin Associated Protein 1	Kinase	Other kinase	NM_004958.2
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	Oxidoreductase	Dehydrogenase	NM_002046.3
GUSB	Glucuronidase, Beta	Hydrolase	Galactosidase	NM_000181.2
HIF1A	Hypoxia-Inducible Factor 1, Alpha Subunit (Basic Helix-Loop-Helix Transcription Factor)	Transcription factor	Basic helix-loop-helix transcription factor	NM_181054.1
HIF1AN	Hypoxia-Inducible Factor 1, Alpha Subunit Inhibitor	Molecular function unclassified	Molecular function unclassified	NM_017902.2
HIF3A	Hypoxia Inducible Factor 3, Alpha Subunit	Transcription factor	Basic helix-loop-helix transcription factor	NM_152794.2
HIG2	Hypoxia-Inducible Protein 2	Molecular function unclassified	Molecular function	NM_013332.3

			unclassified	
HMOX1	Heme Oxygenase (Decycling) 1	Oxidoreductase	Oxygenase	NM_002133.1
HYOU1	Hypoxia Up-Regulated 1	Chaperone	Hsp 70 family chaperone	NM_006389.3
IGFBP1	Insulin-Like Growth Factor Binding Protein 1	Miscellaneous function	Other miscellaneous function protein	NM_000596.2
ING4	Inhibitor Of Growth Family, Member 4	Molecular function unclassified	Molecular function unclassified	NM_016162.2
MB	Myoglobin	Transfer/carrier protein	Other transfer/carrier protein	NM_203377.1
MT3	Metallothionein 3	Miscellaneous function	Other miscellaneous function protein	NM_005954.2
NOS1	Nitric Oxide Synthase 1 (Neuronal)	Synthase and synthetase	Synthase	NM_000620.1
NOS2A	Nitric Oxide Synthase 2A (Inducible, Hepatocytes)	Synthase and synthetase	Synthase	NM_000625.3
NOS3	Nitric Oxide Synthase 3 (Endothelial Cell)	Synthase and synthetase	Synthase	NM_000603.3
NOTCH1	Notch Homolog 1, Translocation-Associated (Drosophila)	Signaling molecule	Membrane-bound signaling molecule	NM_017617.3
PIK3CA	Phosphoinositide-3-Kinase, Catalytic, Alpha Polypeptide	Kinase	Other kinase	NM_006218.2
PRKAA1	Protein Kinase, AMP-Activated, Alpha 1 Catalytic Subunit	Kinase	Protein kinase	NM_206907.3
PRKAA2	Protein Kinase, AMP-Activated, Alpha 2 Catalytic Subunit	Kinase	Protein kinase	NM_006252.3
PTEN	Phosphatase And Tensin Homolog (Mutated In Multiple Advanced Cancers 1)	Phosphatase	Protein phosphatase	NM_000314.4
RBX1	Ring-Box 1	Molecular function unclassified	Molecular function unclassified	NM_014248.2
SLC2A8	Solute Carrier Family 2, (Facilitated Glucose Transporter) Member 8	Transporter	Carbohydrate transporter	NM_014580.3
TGFBR2	Transforming Growth Factor, Beta Receptor II (70/80kda)	Receptor	Cytokine receptor	NM_001024847.2
TP53	Tumor Protein P53	Transcription factor	Other transcription factor	NM_000546.3
VEGFA	Vascular Endothelial Growth Factor A	Signaling molecule	Growth factor	NM_001025366.1
VHL	Von Hippel-Lindau Tumor Suppressor	Ligase	Other ligase	NM_198156.1