Supplementary data

Acute hypoxia affects P-TEFb through HDAC3 and HEXIM1-dependent mechanism to promote gene-specific transcriptional repression.

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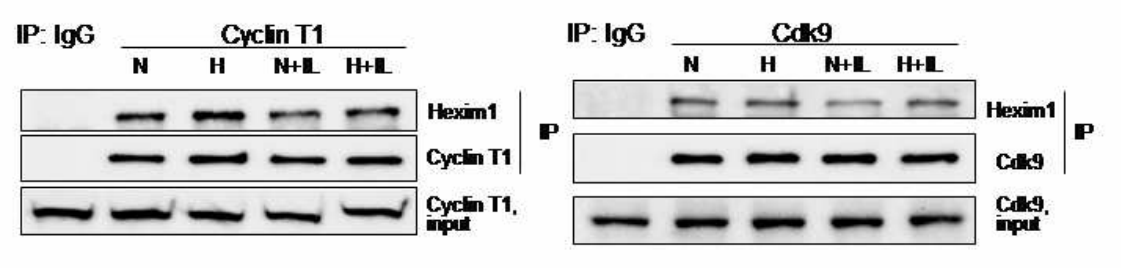
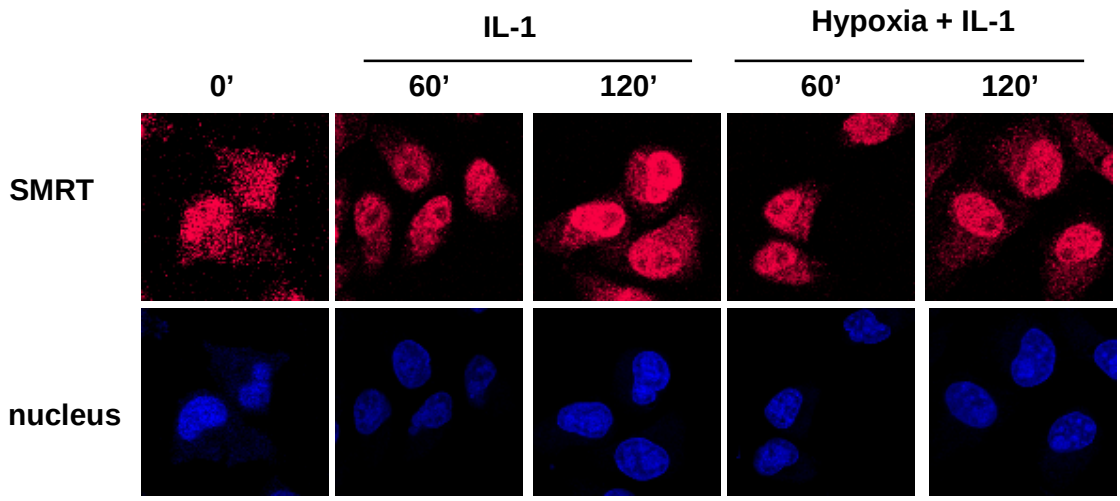
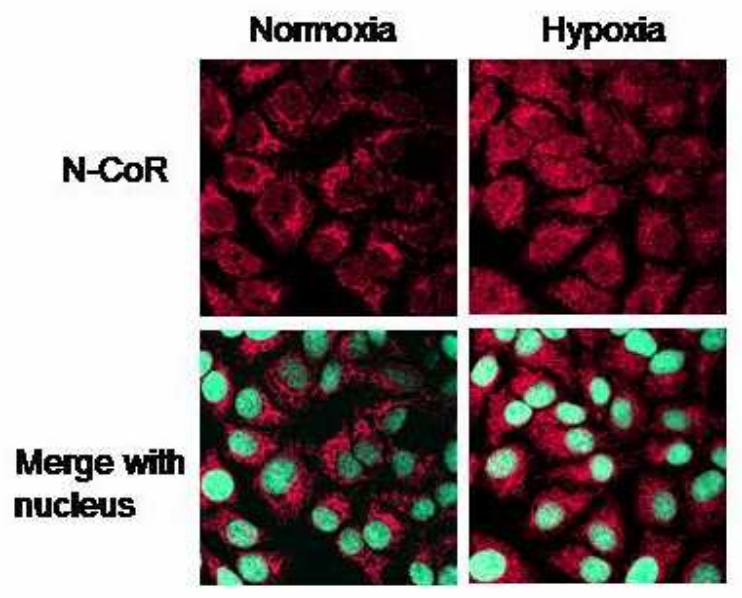


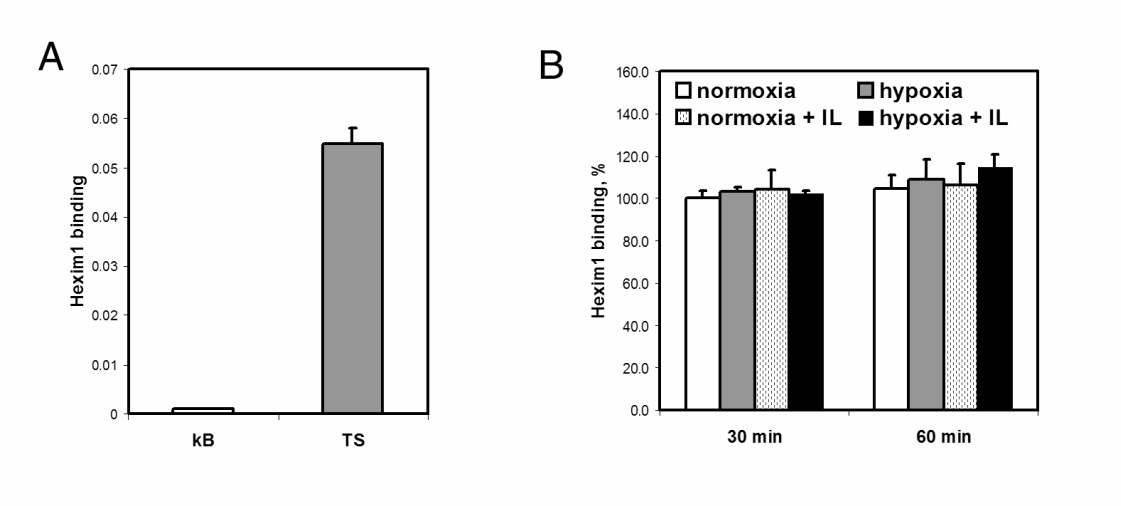
Suppl. Fig. 1. IgG controls for Cyclin T1 and Cdk9 immunoprecipitations. Cells were treated and immunoprecipitated as described in Fig. 1 A. Negative control for immunoprecipitation was performed using normal goat IgG or normal rabbit IgG.



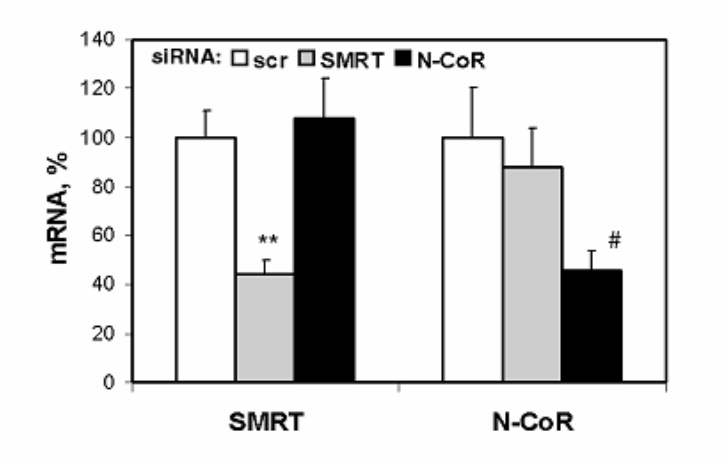
Suppl. Fig. 2. Subcellular localization of SMRT (red) in HeLa cells treated with IL-1β in normoxic and hypoxic environments for the indicated periods of time. Nucleic acids were stained using TOPRO-3 (blue).



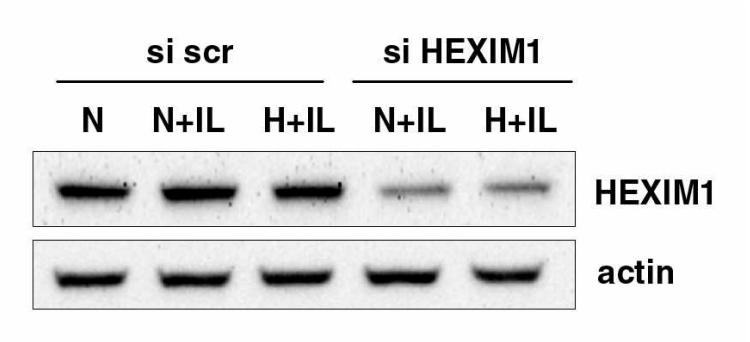
Suppl. Fig. 3. Subcellular localization of N-CoR (red) in HeLa cells incubated under normoxia or hypoxia for 2 hrs. Nuclei stained using SYTO 16 Nucleic Acid Stain (green).



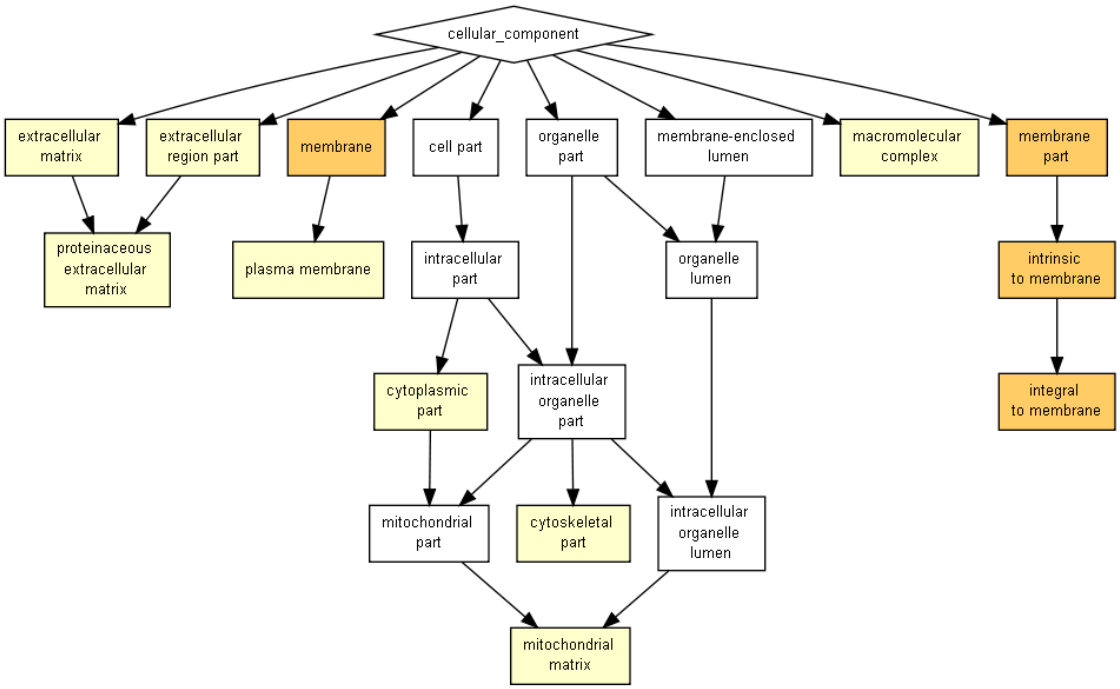
Suppl. Fig. 4. ChIP assay analysis of HEXIM1 occupancy of the MCP-1 gene regulatory region. **(A)** HEXIM1 occupancy of the NF-κB binding site within distal enhancer (κB) and of the transcription start site (TS) of the MCP-1 gene regulatory region in normoxic untreated cells. Immunoprecipitated DNA copy numbers were calculated using calibration curve and normalized by 10% input copy numbers. **(B)** HEXIM1 occupancy of the transcription start site within the MCP-1 gene regulatory region under the treatment with IL-1β and/or hypoxia for the indicated periods of time. Data expressed as a percentage of binding in normoxic cells at 30 min time point after normalization by 10% input.



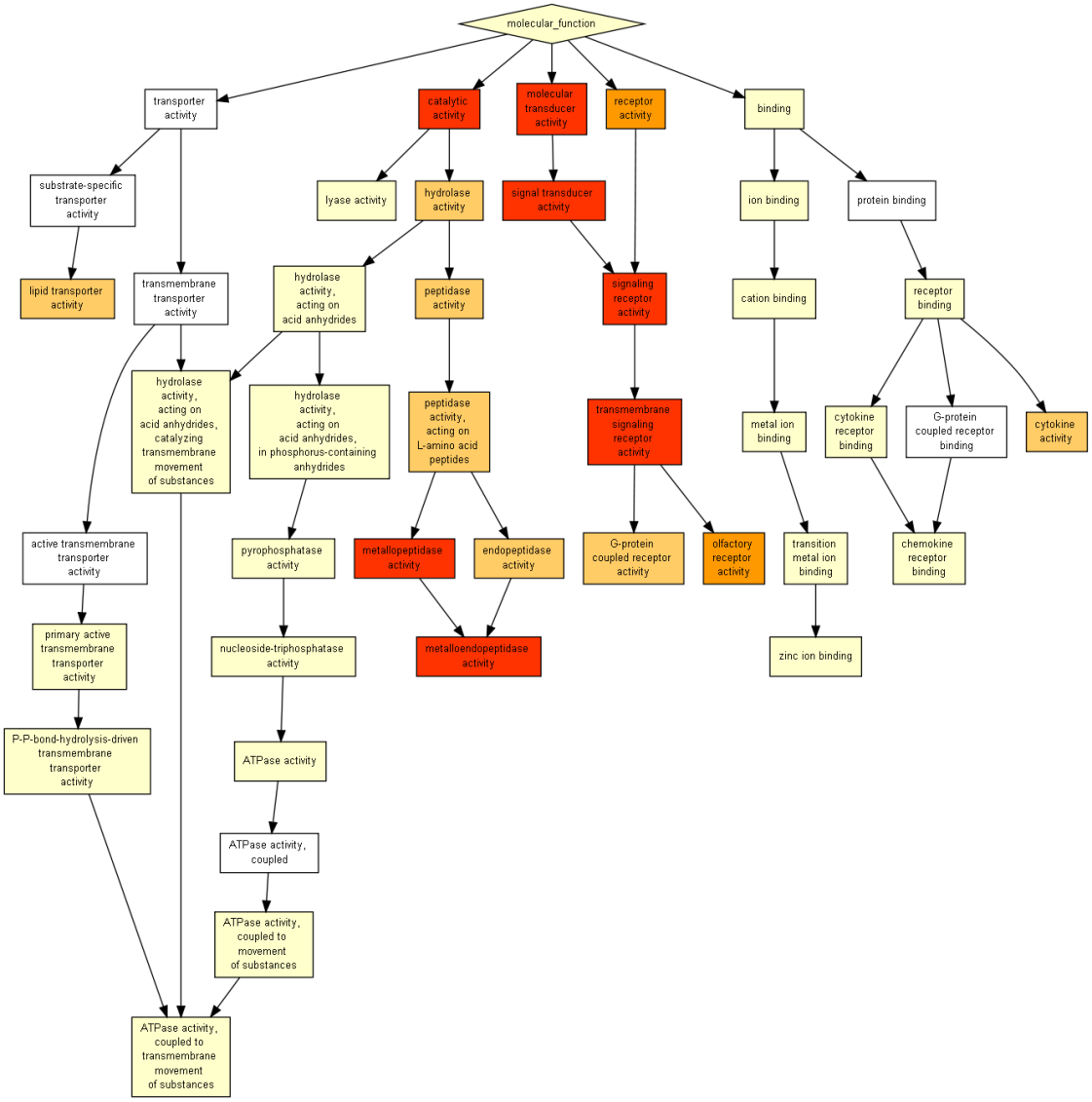
Suppl. Fig. 5. siRNAs directed to either SMRT or N-CoR specifically knocked-down targeted mRNA expression in HeLa cells. Total mRNA was isolated 48 hrs after transfection and analyzed by Q-PCR (n=6). ** $P<0.01$, as compared with siScr for SMRT; # $P<0.05$, as compared with siScr for N-CoR.



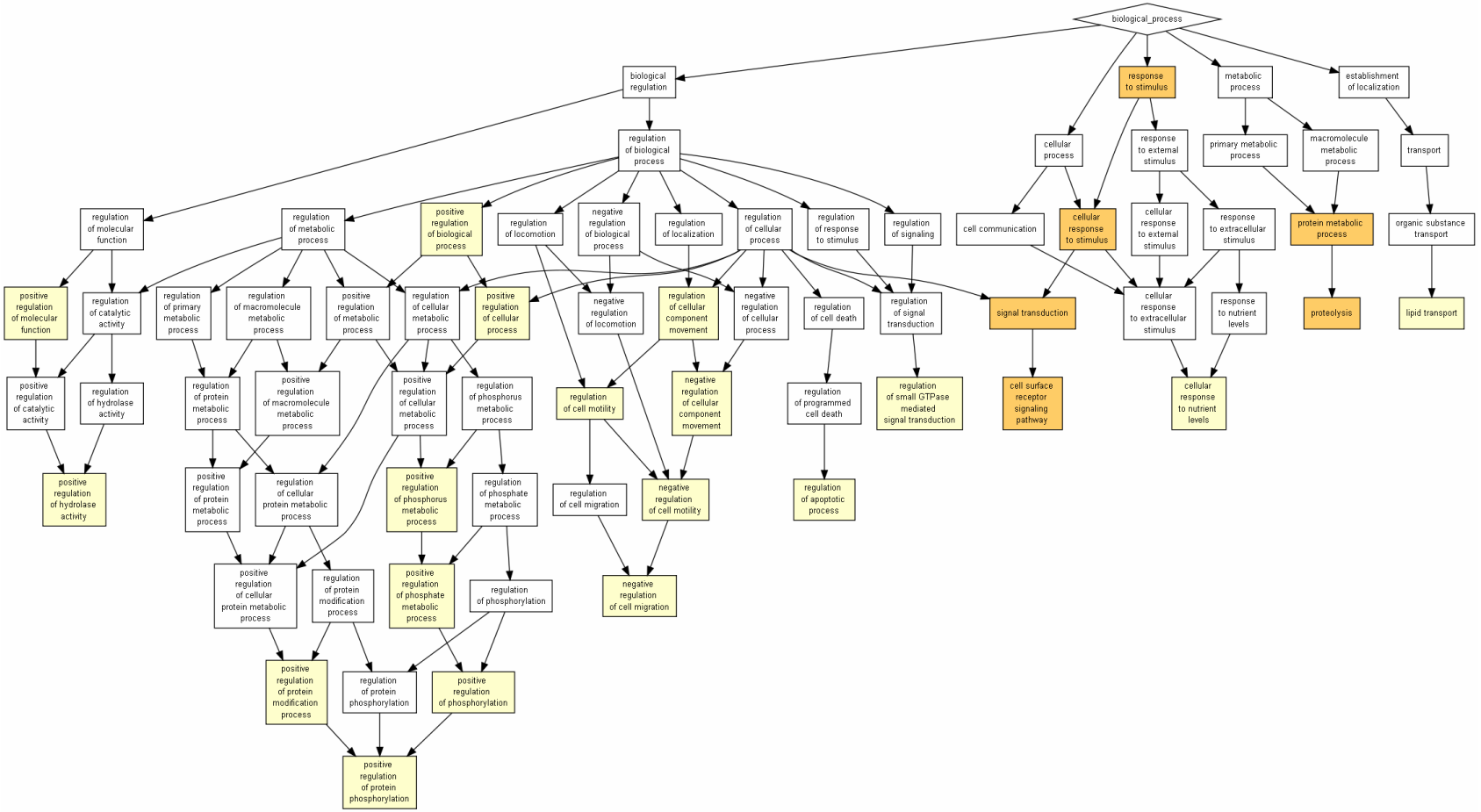
Suppl. Fig. 6. Analysis of specific Hexim1 knock-down in samples used for Agilent mRNA microarray analysis. HeLa cells were transfected with control (siScr) and HEXIM1-targeted (siHEXIM) double-strand siRNA and 48 hr after were left untreated under normoxic condition (N) or treated with IL-1β in combination with normoxia (N+IL) or hypoxia (H+IL) for 1 hr. HEXIM1 protein expression was strongly suppressed in HeLa cells transfected with siRNA directed to HEXIM1, but not in control samples, as analyzed by Western Blot.



Suppl. Fig. 7. Gene ontology's hierarchical annotation tree structure of terms related to cellular component. P-value color scale: white - > 10^{-3}, yellow - 10^{-3} to 10^{-5}, orange – 10^{-5} to 10^{-7}.



Suppl. Fig. 8. Gene ontology's hierarchical annotation tree structure of terms related to molecular function. P-value color scale: white - > 10^{-3}, yellow - 10^{-3} to 10^{-5}, orange – 10^{-5} to 10^{-7}, dark orange – 10^{-7} to 10^{-9}, red - < 10^{-9}.



Suppl. Fig. 9. Gene ontology's hierarchical annotation tree structure of terms related to biological process. P-value color scale: white - > 10^{-3}, yellow - 10^{-3} to 10^{-5}, orange – 10^{-5} to 10^{-7}.

Supplementary Table 1. Functional enrichment of gene ontology (GO) terms in the list of HEXIM1-dependent genes that are differentially expressed in hypoxia.

Gene Ontology for Cellular Component

GO term	Enrichment	No. of genes	P-value
Membrane part	1.06	424	2.11×10^{-7}
Intrinsic to membrane	1.06	385	5.49×10^{-7}
Integral to membrane	1.06	370	2.12×10^{-6}
Membrane	1.09	319	8.15×10^{-6}
Extracellular matrix	2.68	20	2.20×10^{-5}
Plasma membrane	1.10	243	2.87×10^{-5}
Extracellular region part	1.44	77	5.81×10^{-5}
Cytoskeletal part	1.45	54	1.86×10^{-4}
Macromolecular complex	1.29	109	3.71×10^{-4}
Mitochondrial matrix	20.75	3	7.13×10^{-4}
Proteinaceous extracellular matrix	10.21	5	8.26×10^{-4}
Cytoplasmic part	1.73	41	9.76×10^{-4}

Gene Ontology for Molecular Function

GO term	Enrichment	No. of genes	P-value
Signaling receptor activity	1.43	116	3.26×10^{-11}
Transmembrane signaling receptor activity	1.43	109	1.66×10^{-10}
Metallopeptidase activity	20.79	9	1.67×10^{-10}
Metalloendopeptidase activity	26.39	8	2.03×10^{-10}
Signal transducer activity	1.32	131	5.98×10^{-10}
Catalytic activity	3.81	22	6.76×10^{-10}
Receptor activity	1.35	129	1.10×10^{-8}
Olfactory receptor activity	1.61	40	6.63×10^{-8}
G-protein coupled receptor activity	1.44	75	2.17×10^{-7}
Hydrolase activity	5.41	14	4.67×10^{-7}
Lipid transporter activity	12.94	6	1.78×10^{-6}
Cytokine activity	1.96	26	4.62×10^{-6}
Peptidase activity	8.32	9	6.24×10^{-6}
ATPase activity, coupled to transmembrane movement of substances	12.24	5	2.54×10^{-5}
Receptor binding	1.48	70	4.14×10^{-5}
Cytokine receptor binding	1.93	22	6.32×10^{-5}
Metal ion binding	2.92	17	8.99×10^{-5}
ATPase activity	41.23	3	2.11×10^{-4}
Lyase activity	2.73	10	2.49×10^{-4}

Transition metal ion binding	3.87	12	3.13×10^{-4}
Pyrophosphatase activity	1.67	27	3.24×10^{-4}
Zinc ion binding	3.82	12	4.6×10^{-4}
Nucleoside triphosphatase activity	1.66	26	5.04×10^{-4}
Molecular function	1.18	72	5.54×10^{-4}
Chemokine receptor binding	4.11	7	6.38×10^{-4}

Gene Ontology for Biological Process

GO term	Enrichment	No. of genes	P-value
Cellular response to stimulus	1.34	154	9.32×10^{-7}
Signal transduction	1.38	136	9.77×10^{-7}
Proteolysis	8.40	10	1.13×10^{-6}
Response to stimulus	1.27	199	2.24×10^{-6}
Cell surface receptor signaling pathway	1.48	92	3.15×10^{-6}
Protein metabolic process	4.37	15	3.88×10^{-6}
Positive regulation of biological process	1.17	174	2.23×10^{-5}
Positive regulation of cellular process	1.23	144	3.59×10^{-5}
Lipid transport	7.55	7	8.69×10^{-5}
Positive regulation of hydrolase activity	6.40	8	1.25×10^{-4}
Positive regulation of protein phosphorylation	1.70	30	1.84×10^{-4}
Cellular response to nutrient levels	35.73	3	2.7×10^{-4}
Regulation of small GTPase mediated signal transduction	7.66	6	4.21×10^{-4}
Regulation of cellular component movement	5.69	8	4.39×10^{-4}
Positive regulation of protein modification process	1.62	32	5.87×10^{-4}
Positive regulation of molecular function	3.76	12	5.92×10^{-4}
Regulation of apoptotic process	1.65	34	8.84×10^{-4}
Negative regulation of cell migration	11.14	4	9.13×10^{-4}
Regulation of cell motility	5.93	7	9.67×10^{-4}

Supplementary Table 2. Hypoxia down-regulated HEXIM1-dependent genes from EGF and proteolysis groups as analyzed by DAVID functional annotations.

Function	Gene	Gene name
Epidermal growth factor (EGF)	ADAM11	ADAM metallopeptidase domain 11
	ADAM23	ADAM metallopeptidase domain 23
	ADAM33	ADAM metallopeptidase domain 33
	CD248	CD248 molecule, endosialin
	ELTD1	EGF, latrophilin and severn transmembrane domain containing 1
	EGFL6	EGF-like-domain, multiple 6
	FAT3	FAT tumor suppressor homolog 3 (Drosophila)
	ACAN	Aggrecan
	ATRNL1	Attractin-like 1
	F10	Coagulation factor X
	DLK1	Delta-like 1 homolog (Drosophila)
	EREG	Epiregulin
	EYS	Eyes shut homolog (Drosophila)
	FAM53A	Family with sequence similarity 5, member B
	LDLR	Low density lipoprotein receptor
	MEP1A	Meprin A, alpha (PABA peptide hydrolase)
	NTNG1	Netrin G1
	PAMR1	Peptidase domain containing associated with muscle regeneration 1
	SELP	Selectin P (granule membrane protein 140KDa, antigen Cd62)
	SLIT1	Slit homolog 1 (Drosophila)
	TNXB	Tenascin XB
	THBS2	Thrombospondin 2
	THBS3	Thrombospondin 3
Proteolysis	ADAM11	ADAM metallopeptidase domain 11
	ADAM23	ADAM metallopeptidase domain 23
	ADAM30	ADAM metallopeptidase domain 30
	ADAM33	ADAM metallopeptidase domain 33
	ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5
	ADAMTS6	ADAM metallopeptidase with thrombospondin type 1 motif, 6
	AGBL4	ATP/GTP binding protein-like 4
	WFDC10A	WAP four-disulfide core domain 10A
	WFDC2	WAP four-disulfide core domain 2
	A2ML1	Alpha-2-macroglobulin-like 1
	CAPN11	Calpain 11
	CAPN3	Calpain 3, (p94)
	CPA6	Carboxypeptidase A6
	CASP10	Caspase 10, apoptosis-related cysteine peptidase
	CMA1	Chymase 1, mast cell
	CELA1	Chymotrypsin-like elastase family, member 1
	F10	Coagulation factor X
	F11	Coagulation factor XI
	FAP	Fibroblast activation protein, alpha
	GGT5	Gamma-glutamyltransferase 5
	GGT7	Gamma-glutamyltransferase 7
	KLK13	Kallikrein-related peptidase 13
	MGRN1	Mahogunin, ring finger 1
	MASP2	Mannan-binding lectin serine peptidase 2

	PRSS38	Marapsin 2
	MMP21	Matrix metallopeptidase 21
	MMP3	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)
	MEP1A	Meprin A, alpha (PABA peptide hydrolase)
	PAMR1	Peptidase domain containing associated with muscle regeneration 1
	PCSK5	Proprotein convertase subtilisin/kexin type 5
	RNF148	Ring finger protein 148
	RNF39	Ring finger protein38
	SERPINA12	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12
	SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
	SERPINA5	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5
	SERPINA7	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7
	SERPINA9	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9
	SERPINA1	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
	ADAMTS7	Similar to hCG1991431; similar to COMPase; ADAM metallopeptidase with thrombospondin type 1 motif, 7
	TRIM61	Similar to tripartite motif protein 21; tripartite motif-containing 61
	SERP2	Stress-associated endoplasmic reticulum protein family member 2
	TRIML1	Tripartite motif family-like 1
	TRIM50	Tripartite motif-containing 50
	TRIM64	Tripartite motif-containing 64
	TRIM67	Tripartite motif-containing 67
	TRIM9	Tripartite motif-containing 9
	UBD	Ubiquitin D
	USP26	Ubiquitin specific peptidase 26
	USP49	Ubiquitin specific peptidase 49
	UBA7	Ubiquitin-like modifier activating enzyme 7