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Copper affects the binding of HIF-1α to the critical motifs of its target genes

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Supplementary Tables

Table S1 Binding sites of HIF-1α and the coupled genes in promoters

	HIF-1α binding sites in 500 bp of TSS	Overlapped sites with Pol II (promoters)	Genes assigned to promoters
Class 1	931	917	907
Class 2	149	147	147
Class 3_Hypoxia	642	630	626
Class 3_TEPA+Hypoxia	636	628	621
Class 3 (RPs)	-	624	616
Class 3 (DPs)	-	6	6

Table S2 Binding sites of HIF-1α and the coupled genes in putative enhancer regions

	Overlapped HIF-1α binding sites with H3K27ac in 20 kb of TSS	Putative enhancers (except the overlapped sites in promoters)	Genes assigned to putative enhancers
Class 1	1430	490	441
Class 2	190	39	37
Class 3_Hypoxia	1083	425	348
Class 3_TEPA+Hypoxia	1080	433	351
Class 3 (RPs)	-	97	95
Class 3 (DPs)	-	328	273

Table S3 List of 281 Cu-dependent genes and 10 Cu-independent genes

| Cu-dependent genes							
ABHD17A	C1orf35	EEF1D	IMP3	MFSD12	PES1	RPL37	TLCD1
ACAP3	C2orf81	EFNA3	ISG20	MIF	PFKL	RPL41	TLE6
ACSF3	CBX5	EGFL7	JUND	MIR6805	PGLS	RPL6	TMEM134
ALDOA	CCDC106	EIF3F	KMT2E-AS1	MPG	PHPT1	RPP25	TMEM255B
ALYREF	CCDC12	EIF4EBP1P1	LAMTOR5	MRPL11	PIDD1	RPS14	TMEM44
ANGPTL4	CCDC124	ENG	LBHD1	MRPL17	PIH1D1	RPS15	TNFRSF6B
ANKRD37	CCDC85B	ENO1	LIMS2	MRPL23	PINX1	RPS19	TNNT1
ANKS3	CCDC94	ERCC1	LOC100133985	MRPL46	PKP3	RPS21	TPRA1
APITD1	CD320	EXD3	LOC100287036	MRPL53	POC1A	RPS26	TPT1
APOPT1	CDC42EP2	EXOSC4	LOC100289361	MRPS24	POLD2	RPS3	TRMU
APRT	CDKN2AIPNL	EXOSC5	LOC100505771	MT2A	POLR2L	RPS3A	TUBB4B
ARL6IP4	CENPV	FAM173A	LOC100506922	MTFP1	PPIH	RPS7	UCKL1
ARPC4	CFDP1	FAM207A	LOC100996732	MYL6B	PPP1R3G	RWDD1	UPP1
ATAD3A	CHMP6	FAM69B	LOC101927245	NARFL	PPP6R1	S100A10	UROD
ATG101	CITED4	FASTK	LOC101928837	NDUFA13	PRKCSH	S100A16	USE1
ATP5J2	CLPP	FBXL15	LOC101929494	NDUFB8	PRPF31	S100A6	VKORC1
ATP5L	CMC2	FBXW5	LOC102723811	NDUFC1	PRR7	SEC61B	VTI1B
ATP5O	CMTM7	FLOT1	LOC102723897	NDUFS7	PRRT3-AS1	SENCR	WASH7P
ATR	COA6	GADD45GIP1	LOC102724064	NELFB	PSCA	SERTAD1	WDR74
AURKAIP1	COPZ2	GAPDH	LOC102724193	NFKB2	PSMA7	SLC39A3	WISP2
B4GALT7	COQ4	GCAT	LOC105369340	NME1	PTGES2	SNHG11	XPNPEP1
B9D1	COX4I1	GLI4	LOC105369347	NME2	PTRH2	SNHG17	ZBTB45
B9D2	CPNE7	GLTSCR2	LOC105371318	NNMT	R3HCC1	SNHG7	ZNF524
BANF1	CPSF1	GNB2	LOC105372480	NOC2L	RAB11B-AS1	SNRPB	ZNF580
BIN1	CRABP2	GNPTG	LOC105372652	NOL12	RANBP1	SNRPD1	ZNF653
BLOC1S1	DDT	GPX1	LOC105372884	NOL3	REXO1	SNRPE	ZNF771
BOLA2B	DDX54	GUK1	LOC284454	NSMCE1	RFXANK	SPAG4	ZNF837
BOLA3	DHRS3	HAUS8	LOC79160	NTSR1	RHOT2	SPC24	ZNHIT2
C10orf10	DMAP1	HCFC1R1	LOC91450	NUDT18	RIN1	SPSB2	ZSWIM7
C11orf31	DNAJB2	HINT1	LY6K	NUTF2	RNASET2	SRA1	
C14orf80	DNLZ	HMGCL	MACROD1	NVL	RPL13AP20	SSSCA1	
C15orf52	DOHH	HOOK2	MAF1	OCEL1	RPL18	STRA13	
C16orf13	DPCD	HYI	MBD3	P3H3	RPL27	SURF6	
C16orf91	DUS1L	ICT1	MDP1	PARK7	RPL29	TBRG4	
C19orf24	DUS3L	ID1	MEG9	PDLIM4	RPL3	TFB1M	
C19orf48	DYNLRB1	IER2	METTL12	PEMT	RPL32	THOC6	
Cu-independent genes							
ADM	DDIT4	ENO2	LDHD	THAP8	LOC101927512	LOC105372497	MROH8
SLC2A1-AS1	LOC100506691						

Supplementary Figures
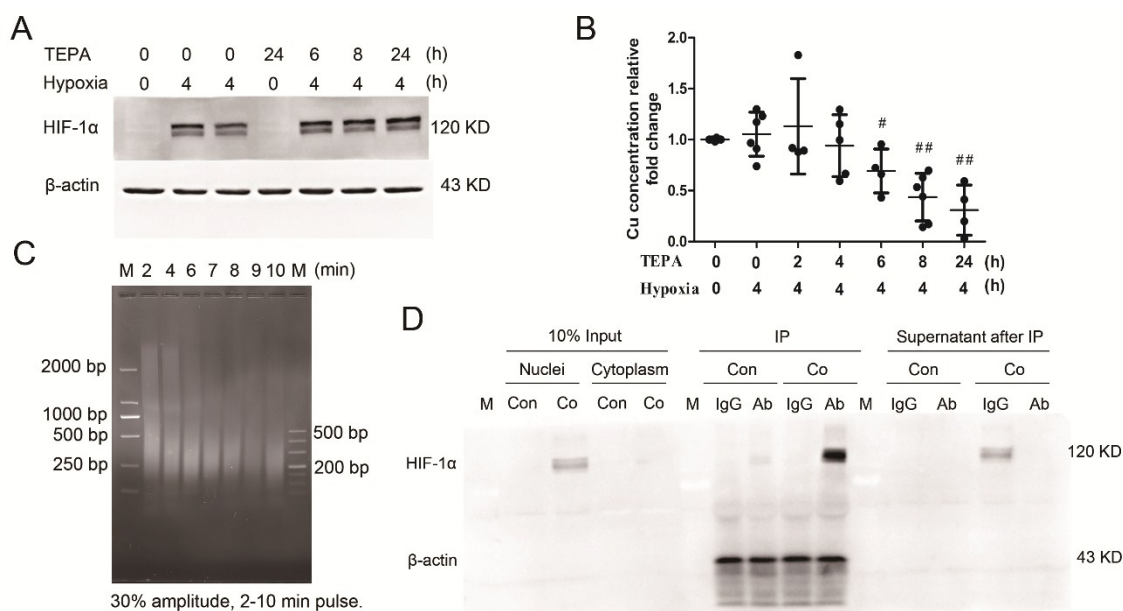
Fig S1. Identification of TEPA treatment conditions, ultrasonic time and specific HIF-1α antibody for ChIP-seq. (A) Western blot analysis of HIF-1α protein accumulation and (B) AAS analysis of Cu concentration under TEPA pretreated hypoxic condition. (C) Analysis of interrupted DNA fragments by ultrasonic treatment of the hypoxic cells, cultured and crosslinked by 1% formaldehyde. (D) Verifying the specificity of HIF-1α antibody (NB100-134) for our ChIP-seq by IP assay and Western blot. Cells were treated with 100 μM $CoCl_2$ for 16 h to induce HIF-1α accumulation. Then HIF-1α-bound proteins were captured by IP assay with the antibody NB100-134 and analyzed by Western blot.
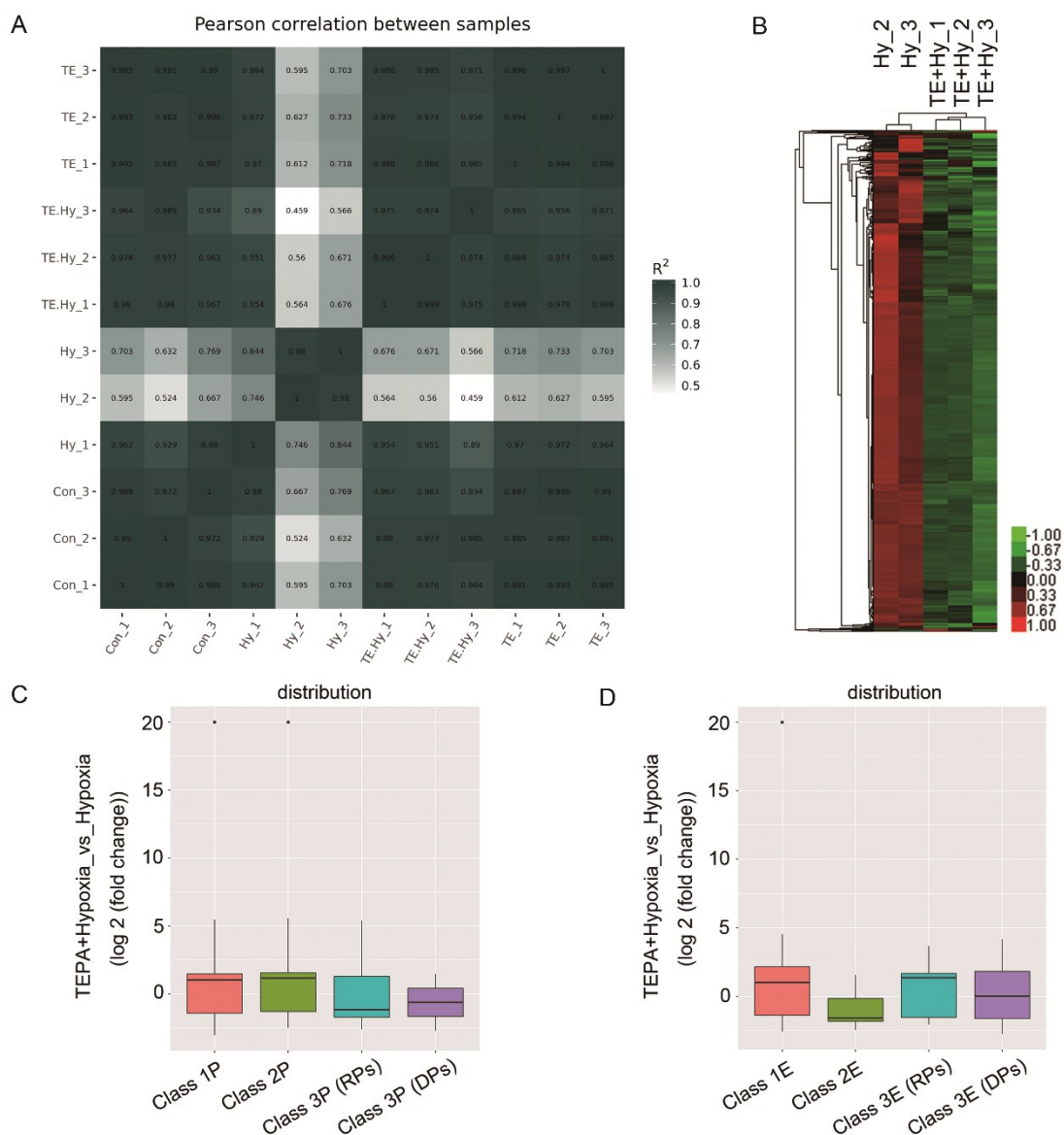
Fig S2. The differential mRNA expression of HIF-1 target genes induced by the selective regulation of Cu on HIF-1α binding pattern. (A) Pearson correlation analysis of RNA-seq data. (B) Heat map of mRNA expression levels of the 2444 genes that upregulated under hypoxia stimulation, among which 2156 genes were significantly downregulated by Cu deprivation induced by TEPA. (C) & (D) Box plots show the distributions of mRNA expression differential data from TEPA-treated hypoxic group compared to TEPA-untreated hypoxic group in each class.
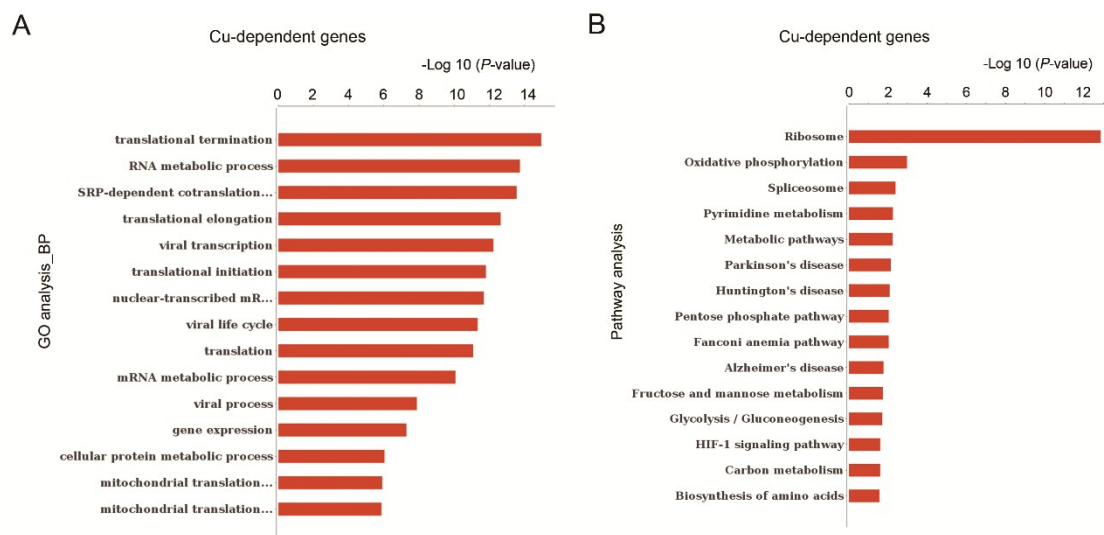
Fig S3. GO and pathway analyses of Cu-dependent genes. (A) GO and (B) pathway analyses of Cu-dependent genes.