SUPPLEMENTARY DATA:

A new function of copper zinc superoxide dismutase: as a regulatory DNA-binding protein in gene expression in response to intracellular hydrogen peroxide

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SUPPLEMENTARY METHODS:

Supplementary Methods 1. ChIP-Seq and bioinformatic analyses.

To obtain DNA-occupied sites of SOD1 in the genome, ChIP-Seq was performed. HeLa cells were seeded in 25 cm² cell culture flask for ChIP-Seq at a density of 1×10⁶ cells/flask in the DMEM culture medium. After washing with PBS, cells were first incubated for 4 h in the medium mixed respectively with or without 0.4 mM H₂O₂, 2 mM GSH, and 50 μM LD100. Then, ChIP was performed using Pierce Agarose ChIP Kit (Thermo Scientific; 26156). All experimental procedures were performed in strict accordance with the instructions. Briefly, the cells were first incubated with 1% formaldehyde for 10 min, and then incubated with 0.125 M glycine (final concentration) for 5 min. The nuclei from the cells was incubated with 0.6 μL micrococcal nuclease in MNase digestion buffer working solution for 5 min. The input DNA was prepared by treating aliquots of chromatin with NaCl, proteinase K and heating for de-crosslinking. The supernatants of experimental groups were incubated with 8 μL immunoprecipitation grade anti-human SOD1 primary antibody (Abcam; ab16831), anti-RNA polymerase II antibody or normal rabbit IgG overnight at 4 °C on a rocking platform. Following capturing the immune complex using Protein A/G Plus Agarose, the immunocomplex was treated with NaCl, proteinase K and heated for de-crosslinking. Finally, DNA was purified using Agencourt AMPure XP (Beckman Coulter; 15770600).

Illumina sequencing libraries were prepared from ChIP and Input DNAs by the standard consecutive enzymatic steps of end-polishing, dA-addition, and adaptor ligation. After amplification by PCR, the resulting DNA libraries were quantified and sequenced on the Illumina HiSeq platform according to the manufacturer’s instructions. Alignment and downstream processing of data were performed. After removed reads containing adapter, the reads containing poly-N and the low-quality reads from raw data, the clean reads all were introduced into the downstream analyses. Using a Burrows-Wheeler Alignment tool, the clean reads were aligned to the reference genome (1). MACS2 (model-based analysis of ChIP-Seq) was used to identify regions of IP enrichment over background (2), and then MEME was used to detect the sequence motif (3). After motif detection, Tomtom was used to annotate the motifs according to the sequence similarity (4). PeakAnnotator was used...
to identify the nearest TSS of every peak and to obtain the distribution of peak summits on different function regions (5). Gene Ontology (GO) analysis was performed to identify the function enrichment results using GOseq R package. The corrected P value of GO terms less than 0.05 was considered significantly enriched by peak related genes. KOBAS was used to test the statistical enrichment of peak related genes in KEGG pathways (6). The genes regulated by SOD1 at the transcriptional phase were obtained through counting the reads from -3000 to +3000 bp relative to the transcription start site under normal conditions, and should also be differentially expressed following SOD1 knockdown. The public dataset of RNA-Seq (GEO: GSE112007) published in our previous work (7) was used for the data analysis, which provided differentially expressed genes of SOD1 knockdown and inhibition (using 50 μM LD100) in HeLa cells.

**Supplementary Methods 2. RT-qPCR.**
RT-qPCR was performed using commercial kits according to the manufacturer's instructions. Isolation of total RNA from cells was performed using High Pure RNA Isolation Kit (Roche; 11828665001), and cDNA was synthesized from 1 μg of total RNAs using Transcriptor First Strand cDNA Synthesis Kit (Roche; 04897030001). Real-time quantitative PCR was performed using FastStart Essential DNA Green Master (Roche; 06402712001) in 20 μL final volume per well on a LightCycler 96 (Roche). Melt curve analysis was performed to verify the specificity of PCR amplification. All reactions were performed as triplet, and the quantity of mRNA was calculated by $2^{-\Delta\Delta Ct}$ method (8). Quantitative values were obtained from the cycle number (Cq or Ct value). Relative quantities were determined as $Q_{\text{sample}}=2^{-\Delta\Delta Ct}$, where the $\Delta\Delta Ct$ was determined by subtracting the $\Delta Ct$ value of the target gene in experimental groups from the $\Delta Ct$ value of the same target gene in control groups. Data were normalized by the β-actin (ACTB) mRNA level. All the primers were shown in Supplementary Table S1.

**Supplementary Methods 3. SOD1 expression and purification.**
We constructed the bacterial expression plasmid pET-28B-WT-SOD1 encoding the human wild-type (WT) SOD1 fused to an N-terminal 6×histidine tag. The recombinant proteins
were expressed in *E. Coli* Rosetta (DE3) pLysS (Novagen; 70956-3). The *E. coli* was grown in LB media with kanamycin sulfate (Sigma; E004000) at 37 °C until reaching an optical density of 0.6–0.8 at 600 nm. The expression of WT SOD1 was induced respectively at 20 °C for 27 h and 20 h with 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma; I6758) in the presence of 0.15 M Cu^{2+} and 0.15 M Zn^{2+}. After harvesting the *E. coli* cells, they were suspended in pH 7.3 50 mM Tris and 0.15 M NaCl buffer. The cells were first disrupted by sonication, and then cell debris was separated by centrifugation at 5,500×g for 3 h. The recombinant protein was purified respectively by a nickel-charged column (GE healthcare; 17-5248-01) under denaturing conditions, and desalting column (GE healthcare; 17-1408-01). Similarly, we also expressed and purified the SOD1 mutants A4V and H46R/H48Q according to the previously reported program (9, 10).

**Supplementary Methods 4. Fluorescence anisotropy.**

To quantify SOD1 binding to DNA, fluorescence anisotropy assay was performed. According to the motifs obtained in ChIP-Seq, the double-stranded DNA oligomers S1 and S7 were designed as a high preference sequence of SOD1 binding and as a control sequence. The 6-FAM labelled probe sequences of S1 and S7 were used into fluorescence anisotropy titrations. For direct fluorescence anisotropy binding assays, increased concentrations of SOD1 or its mutants were titrated into 1 µM DNA probes in pH 7.4 PBS at 37 °C. SOD1 binding to DNAs slows the tumbling of the labelled species, which would be detected by fluorescence anisotropy. Measurements and data analysis were performed as described previously (11). The anisotropy \( r \) was calculated as classically reported (12):

\[
r = \frac{I_{vv} - G I_{vh}}{I_{vv} + 2G I_{vh}} \tag{1}
\]

\( I_{vv} \) and \( I_{vh} \) are vertically and horizontally polarized components of the emission by vertically polarized light excitation. The instrumental correction factor G is determined from standard solutions. Anisotropy \( r \) is linked to the apparent binding constant \( K \) via the following equations (13,14):

\[
K = \frac{C_{L}f_{B}/n}{[C_{L}(1-f_{B})]^{n}(C_{P}-C_{L}f_{B}/n)} \tag{2}
\]

\[
\frac{f_{B}}{f_{F}} = \frac{n[C_{P}]}{n[C_{P}]+[C]} = \frac{r-r_{L}}{r_{L}+r-r_{L}} R = \frac{f_{B}}{f_{F}} = \frac{F_{B}}{F_{F}} \tag{3}
\]
\[ \lg f_B - n \lg(1 - f_B) = \lg K + (n - 1)\lg c_L + \lg n + \lg(C_p - C_L \cdot f_B/n) \] (4)

where \( c_L \) is the concentration of ligands (DNA), \( C_p \) is the concentration of proteins (SOD1 or its mutants), \( r \) is the average anisotropy of reaction systems, \( r_{\text{max}} \) is the anisotropy of maximally ligand-associated proteins, \( r_L \) is the anisotropy of free ligands, \( F_F \) and \( F_B \) are the fluorescence intensity of free and protein-bound ligands, respectively. Dissociation constant \((K_d)\) is the reciprocal of a binding constant \((K_a)\).

**Supplementary Methods 5. Measurements of intracellular H₂O₂ and O₂⁻⁻ levels.**

After incubating cells with different drugs for 4 h, the cells were washed to evaluate the effects of different drugs on intracellular H₂O₂ and O₂⁻⁻ levels. For H₂O₂ measurements, cells were incubated with 5 μM 2',7'-dichlorofluorescein diacetate (DCFH-DA) (Sigma; 287810) for 20 min at 37 °C in the dark. For O₂⁻⁻ measurements, cells were incubated with 5 μM dihydroethidium (DHE) (Sigma; 37291) for 20 min at 37 °C in the dark. Then, the cells were washed with PBS three times and resuspended in 500 μL PBS. Flow cytometry (BD, AccuriTM C6) was used to collect the fluorescence signals. For the samples of LD100, the fluorescence signals of DHE and DCFH-DA were obtained through subtracting the background fluorescence of LD100.

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**SUPPLEMENTARY FIGURES:**

**Supplementary Figure S1:**

(A) LD100, a specific SOD1 inhibitor. (B) Immunofluorescence using the antibody against SOD1 (green) and DAPI (blue, a nucleus-staining dye). After treating HeLa cells with or without 1 mM paraquat, 5 μg/mL 4NQO and 10 mM AT for 4 h, immunofluorescence staining was performed. Scale bars, 25 μm. Finally, we quantified the fluorescence intensity of SOD1 in the nucleus and cytoplasm, and then obtained the percentage of SOD1 in the nucleus (n > 20). Data are mean ± SD. (C-D) After treating HeLa cells with or without 2 mM GSH, 0.4 mM H$_2$O$_2$, 50 μM LD100, 1 mM paraquat, 5 μg/mL 4NQO and 10 mM AT for 4 h, the relative H$_2$O$_2$ and O$_2^•$ levels were measured using DCFH-DA (C) and DHE (D), respectively. Data are mean of triplicate samples ± SD (*P < 0.05, **P < 0.01, ***P < 0.001; unpaired Student’s t test), and all error bars are SD.
Supplementary Figure S2. (A) ChIP assays of positive and negative controls. The positive and negative controls of ChIP were performed using the anti-RNA polymerase II antibody and normal Rabbit IgG, respectively. (B) Total number of peaks in ChIP-Seq samples. (C) Boxplots of ChIP-Seq levels for SOD1 under different conditions. Fold enrichment values of total peaks and peaks in the vicinity of TSS (within 3000 bp) were shown separately. (D) Top 6 SOD1 binding motifs enriched in ChIP-Seq samples. Data are mean of triplicate samples ± SD (*P < 0.05, **P < 0.01, ***P < 0.001; unpaired Student’s t test), and all error bars are SD.
Supplementary Figure S3. Distributions of the central positions of SOD1 binding sites located within gene components. (A) The percentage of SOD1 binding sites falling within specific gene components in control, H₂O₂ treated, and LD100 treated groups. U2000, upstream of genes (2000 bp); D2000, downstream of genes (2000 bp); TSS100, TSS ± 100 bp; TTS100, TTS ± 100 bp; and so on. (B) Distances of all peaks to the TSS.
Supplementary Figure S4. The specific inhibitor, LD100, alters the conformation of SOD1. (A) The circular dichroism (CD) spectrum of LD100 treated SOD1. After incubating 5 μM SOD1 with increasing concentrations of LD100 (0, 2.5, 5, 10, 20, 50 and 100 μM) in 10 mM PBS (pH 7.4) at 37 °C for 2 h, the CD spectrums were recorded. (B) Comparison of the SAXS results for SOD1 respectively treated with or without EDTA and LD100. After incubating SOD1 (150 μM) with or without EDTA (60 mM) and LD100 (60 mM) at 37 °C for 12 h, the real-space P(r) distribution and radius of gyration (Rg) were obtained. (C) Space-filling models of LD100 treated and untreated SOD1. Based on SAXS results, space-filling models of LD100 treated and untreated SOD1 were constructed. Space-filling models of SOD1 derived from P(r) data are depicted in grey, with crystal structure docked into mesh envelope.
Supplementary Figure S5. SOD1 and its mutants bind to DNA in vitro. Fluorescence anisotropies of S1 or S7 titrated with wild type human SOD1 or its mutants (A4V or H46R/H48Q). Fluorescence anisotropies of 5'-FAM-labeled double-stranded S1 were obtained through titrating with WT SOD1 (A) and its mutants (C, D) in 10 mM PBS (pH 7.4) at 37 °C. Fluorescence anisotropies of 5'-FAM-labeled double-stranded S7 (B) were obtained through titrating with WT SOD1 in 10 mM PBS (pH 7.4) at 37 °C. r is the fluorescence anisotropy of the SOD1-DNA system (λ_{ex} = 492 nm, and slit = 5 nm). Data are mean of triplicate samples ± SD, and all error bars are SD.
Supplementary Figure S6:

Supplementary Figure S6. The affinity of SOD1 for ssS1 is much stronger than its complementary. After incubating 6 μM SOD1 with 3 μM single-stranded S1 (band 1), or 3 μM DNA complementary to S1 (band 2), or mixture of 3 μM S1 and 3 μM DNA complementary to S1 (band 3), or 3 μM ds S1 (band 4), EMSA assays were performed.

Supplementary Figure S7:

Supplementary Figure S7. The interaction with DNA does not impact the activity of SOD1. After incubating SOD1 (1 μM) with increasing concentrations of S1 (A) or S7 (B) (0, 0.5, 1 and 2 μM) at 37 °C for 12 h in 10 mM PBS (pH 7.4), the activity of SOD1 was determined using superoxide dismutase assay kit. Data are mean of triplicate samples ± SD, and all error bars are SD.
Supplementary Figure S8: (A) A Venn diagram showing the overlaps between all identified genes in ChIP-Seq under normal conditions, and the differentially expressed genes (DEGs) in LD100-treated and SOD1 knockdown HeLa cells. (B) A Venn diagram showing the overlaps between the potential genes regulated by SOD1 at the transcriptional phase and DEGs in SOD1 knockdown HeLa cells.
Supplementary Figure S9. A heat map of genes in nervous system development derived from the differentially expressed genes caused by DNA binding of SOD1.
Supplementary Figure S10: (A) A heat map of SOD1 and several specific genes regulated by SOD1 binding in prostate normal (RWPE-1) and cancer (DU145) cells. The heat map was drawn using normalized log_{10}(FPKM+1) of the genes (GEO: GSE112007). (B) After incubating HeLa cells with H_2O_2 (0, 0.01, 0.05, 0.40 and 1.00 mM) for 4 h, the expression of CRTC3 and FGFR4 was determined by RT-qPCR. Data are mean of triplicate samples ± SD (*P < 0.05, **P < 0.01, ***P < 0.001; unpaired Student’s t test), and all error bars are SD.
### SUPPLEMENTARY TABLES:

**Supplementary Table S1.** Nervous system development-linked genes regulated by DNA-SOD1 interactions

| Gene name | Gene name | Gene name | Gene name | Gene name | Gene name | Gene name | Gene name |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| SEMA3F    | PHGDH     | PPARD     | OLFM1     | CLSTN3    | ERCC3     | SEZ6L2    |
| PLXND1    | DPYSL2    | ADGRG6    | PRDM12    | PDX1      | HIPK1     | SPHK1     |
| ITGA3     | TGFBR2    | EPM2A     | C1QL1     | KATNB1    | IFT122    | ANAPC2    |
| PAX6      | ABL1      | PTK7      | PPARG     | AFG3L2    | CITED2    | PTPN11    |
| TEAD3     | SMARCB1   | SRF       | RAP1GAP2  | MINK1     | SOX17     | GATA2     |
| MAPK8IP2  | DGRG14    | WNT5A     | HRSP12    | CARM1     | MICAL1    | EGR3      |
| NRXN3     | SIX4      | PLXNA1    | MATN2     | PTPRF     | NSMF      | SLITRK4   |
| TIMP2     | PTK6      | ADAM23    | NES       | CELSR2    | SMPD1     | SIAH2     |
| JADE2     | E2F1      | RTN4      | MYH10     | HES6      | NAB2      | TSKU      |
| HSPA5     | CHRD1     | EPHA4     | DCLK1     | BOC       | RRM1      | NKX2-5    |
| FSTL4     | STK24     | ARHGEF2   | SPINK5    | SPATA5    | TRAPPCC9  | IFT140    |
| ATP2B4    | ARHGEF10  | GNPAT     | MEN1      | PLK2      | WDR81     | SEMA4D    |
| MPPED2    | ERCC2     | B4GALT2   | CHL1      | EGFR      | CTNNB1    | WNT7B     |
| ROGDI     | APLP1     | NRP2      | GSTM3     | PRRPS1    | COL3A1    | SLC3A1    |
| PITX1     | NAPA      | NR4A3     | NAV1      | SHC3      | SEMA4C    | WDR5      |
| GAL       | MEGF8     | APAF1     | IL15RA    | PARD3     | ATF5      | MAFK      |
| WDR1      | SCN1B     | FAM126A   | CCDC64    | INA       | PCSK9     | UNC13B    |
| TCF3      | PLOD3     | EGR2      | SERPINE2  | HMGA2     | GPRIN1    | SMURF1    |
| ADGRL1    | TMEM106B  | CIT       | IL6       | ASAP1     | PTK2      | PLXNB3    |
| LLGL2     | LIMK1     | WWP1      | DDX56     | NRG1      | SDC2      | L1CAM     |
| CELSR1    | NCS1      | OBSL1     | DBNL      | SSBP3     | NPAS2     | GD1       |
| RAP1GAP   | RAPGEP1   | PREX1     | HIX       | MMP14     | KCNK3     | MAFB      |
| TP73      | CCL2      | SOX9      | FPGS      | KIT       | LPAR3     | NRAS      |
| DNM2      | MMD       | BMP2      | CTV      | RUNX1     | LAMB2     | CSNK1E    |
| HSP90AA1  | RAPGEP2   | GLIS2     | KIAA0319  | ABR       | QARS      | NME2      |
| SRRT      | PPARC1A   | SMARCA4   | ATAT1     | CALM3     | DAG1      | LYN       |
| BAX       | FOLR1     | LIF       | SORL1     | G6PD      | CSPG4     | CUX1      |
| KDM2B     | PITPN1M   | TWSG1     | SPTBN5    | GPSM1     | CNP       | SOCS7     |
| NRCAM     | RAB35     | PHF10     | SHROOM3   | NTN3      | ADCY6     | ZNF280B   |
| TYRO3     | UST       | UNC13A    | EGF       | NFIA      | LIG4      |
| Target genes | Forward primer sequences 5’->3’ | Reverse primer sequences 5’->3’ |
|--------------|---------------------------------|-------------------------------|
| QRFP         | CAGAAGAGAGCCCACAGACG            | CCTCAGCCAGGTTCCCTAAC          |
| TNFRSF25     | CAGCTCTACGACGTGATGGA            | ACCGTACTTAGGGCTTCTGC          |
| NFIC         | CACTCATCCGGTTTTCTTC             | GCTTTTGCGCTGCTGACTCA          |
| FGFR4        | CAAAGACAACGCTCTGACA             | TGGATACTTCCGGGACTC            |
| MAF1         | ATGGCAGGAGACGACAAACA            | GAGCGTGCAATCAGGTAGA           |
| DNM2         | TGGGGTGCGAGGGGTATATCA           | GTGGTCAAAGTGAGCGAGGA          |
| PTK6         | CAGGCTTATCAAGGAGGACG            | TGCACCCATCACCTCAAGTA          |
| NRG1         | CTACATCTACATCCACCCTGG           | ACTTGCAAAAGTATCCTGAGG         |
| TUSC2        | AATGGCAGAGTTAGCACCACCC         | ACCCAGTCCCACCTCCTATC          |
| SPEG         | AGGAGCTGACTCAGGAGAG             | TTTCCCCAAACCGGGGA             |
| CRTC3        | TCTCTCCACCGCTGGATTA            | ATGGGGAAGCTCGAGGTGCTG         |
| KCNAB2       | TCGGGGTCTAGAAAGGAGGG            | GGAAGCTCGAGGCATAGGAC          |
| ACTB         | CCACACCTTCTCAATGAGC            | TGAGGTAGTCAGTCAGGTGCC         |
| INA          | ACCAAGAGTGAAGTGGGCACG           | AAGTGATAGCCAGTGAGTA           |
| UNC13A       | CACTCAGACGGAACCCAGAT           | TGAGAGCGACAGGGCATAG           |
SEQUENCE TRACKS:

Sequence track of AP001469.7:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20AP001469.7

Sequence track of ARHGEF16:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20ARHGEF16

Sequence track of BEND3:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20BEND3

Sequence track of CBARP:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20CBARP

Sequence track of CRTC3:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20CRTC3

Sequence track of DNM2:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20DNM2

Sequence track of FADS2:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20FADS2

Sequence track of FGFR4:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20FGFR4

Sequence track of KCNAB2:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20KCNAB2

Sequence track of MAF1:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20MAF1

Sequence track of MAP3K14-AS1:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20MAP3K14-AS1

Sequence track of NFIC:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20NFIC

Sequence track of OLFM1:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20OLFM1

Sequence track of PBX2:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20PBX2

Sequence track of PRSS56:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20PRSS56

Sequence track of PTK6:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20PTK6

Sequence track of QRFP:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20QRFP
Sequence track of RP11-334J6.6:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20RP11-334J6.6

Sequence track of SNRNP70:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20SNRNP70

Sequence track of SPEG:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20SPEG

Sequence track of TNFRSF25:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20TNFRSF25

Sequence track of TUSC2:
https://genome.ucsc.edu/s/199284lixiang/Sequence%20track%20of%20TUSC2