

Heme oxygenase 1 linked to inactivation of subchondral osteoclasts in osteoarthritis

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Materials and methods

1. DEGs identified by GEO2R

The DEGs of these two profiles were analyzed using data analysis tool NCBI GEO2R (<https://www.ncbi.nlm.nih.gov/geo/geo2r>). Subsequently, Benjamini & Hochberg was chosen for the option of applying adjustment to the *P* values. The significance level cutoff was 0.05. The DEGs of the two expression profiles were analyzed by Venn diagram using Funrich, and 206 identical genes were detected. The intersection of DEGs was selected for further analysis.

2. Functional annotation and pathway enrichment analysis

GO database was employed to determine our target genes, cellular component (CC), molecular function (MF) and biological process (BP). KEGG is another important pathway-related database. We obtained annotations on the function of genes and various pathways by using it. Metascape (<https://metascape.org/gp/index.html#/main/step1>) is an online service tool that integrates GO, KEGG, and UniProt database resources, so we use it to obtain pathway enrichment, biological process annotation, and gene-related PPI network analysis. The intersection of DEGs was selected. Subsequently, GO and KEGG enrichment networks for genetic analysis were obtained on the Metascape website. $P < 0.05$ was chosen as the cutoff value. PathCards (<https://pathcards.genecards.org>) displays each superpathway and provides customers with a complete channel for systems analysis. We searched “oxidative stress” on the Pathcards website

and KEGG PATHWAY database for related pathway genes.

3. PPI network construction

The STRING 11.5 (<https://string-db.org>) online database can integrate functional correlations of all known proteins. We added the intersection of DEGs to the online page for analysis. The results were then imported into Cytoscape software. PPI network images were constructed using cytoHubba function of Cytoscape software to screen out the top hub genes. Furthermore, we used the Molecular Complex Detection (MCODE) plug-in to identify key subnetworks in PPI networks.

4. Prediction of miRNA network construction

The miRWalk 3.0 online web tool (<http://mirwalk.umm.uni-heidelberg.de>) was used to predict the related upstream miRNA network. The miRNA-mRNA regulatory network could also be obtained using the Target Mining page of this tool. The cytoHubba function in Cytoscape software was employed to identify the top 20 interacting miRNAs of each gene and to construct miRNA-mRNA regulatory network images.

Table S1 Primers used in qRT-PCR

| Gene | Primer Sequence (F) | Primer sequence (R) |
|--------------|-------------------------|---------------------------|
| <i>CTSK</i> | CTTCCAATACGTGCAGCAGA | TCTTCAGGGCTTTCTCGTTC |
| <i>MMP9</i> | CGTGTCTGGAGATTCGACTTGA | TTGGAAACTCACACGCCAGA |
| <i>NFAT2</i> | GAGAATCGAGATCACCTCCTAC | TTGCAGCTAGGAAGTACGTCTT |
| <i>HMOX1</i> | GGAAATCATCCCTTGCACGC | TGTTTGAACCTGGTGGGGCT |
| <i>Nrf2</i> | TGCCCCATCAGGCCAGT | GCTCGGCTGGGACTCGTGTT |
| <i>NQO1</i> | TCCAGACTCCGATCATCAAGC | GCTCATGGTGTTGAGAATTGTGT |
| <i>SOD1</i> | GCTGTACCAGTGCAGGACCTCAT | CTCTCCTGAGAGTGAGATCACACGA |
| <i>SOD2</i> | CAGACCTGCCTTACGACTATGG | CTCGGTGGCGTTGAGATTGTT |
| <i>CAT</i> | CAGGAAGGCTTGCTCAGGAA | AGGACGGGTAATTGCCATTG |
| <i>GAPDH</i> | GGTTGTCTCCTGCGACTTCA | TGGTCCAGGGTTTCTTACTCC |

F: forward; R: reverse.

Table S2 List of the 206 genes found differentially expressed in GSE41342 and GSE176265

| DEGs of GSE41342 and GSE176265 | | | | | | |
|--------------------------------|--------|---------|-------|----------|--------|-------|
| RNF128 | ACOD1 | PLEKHA1 | PDGFC | SERPING1 | COX6A2 | SMYD5 |
| JKAMP | CYP4V3 | SOX7 | XPR1 | ASB13 | TNNI2 | FXN |
| TLR2 | LARGE | SPG20 | RRBP1 | RUFY3 | SCD1 | GP9 |
| DEPTOR | SOCS2 | P2RX7 | P4HA1 | IL13RA1 | ZWINT | PI16 |

| | | | | | | |
|-----------|---------------|----------|----------|----------|---------|----------|
| ITGB5 | ANXA4 | DST | CPNE8 | EFEMP2 | FAM220A | GPAA1 |
| KLF9 | GBP2 | LY96 | GPX3 | COG7 | DBF4 | IFI27L2A |
| NSMF | MS4A7 | ADAMTS2 | NFKBIZ | P3H3 | UBE2O | HMOX1 |
| CPQ | LPP | RAI14 | FN1 | FBLN2 | CCDC124 | E2F2 |
| RUNX1T1 | GPR137B | RORA | MS4A6D | CYB5R3 | ALAD | RPL3L |
| GPRC5B | LHFPL2 | ITGAV | RHOJ | MAMDC2 | PYCR2 | ASPM |
| NUDT12 | BHLHE40 | LTBP3 | RCAN1 | RRAD | RALGPS2 | BC030867 |
| PROS1 | IGF2R | EPB41L3 | GPX8 | HIF1A | FNDC10 | PAFAH1B3 |
| B3GALNT1 | SHTN1 | MYO1B | TRPS1 | LOX | MYOZ1 | MIS18BP1 |
| CTSK | GNA13 | TMEM204 | ANGPTL4 | SERPINH1 | FCMR | EXOGE |
| LRP11 | SNAP23 | PDGFA | DCN | WISP2 | MRPL33 | STARD10 |
| ZFP532 | NKAP | ARMCX3 | IGFBP7 | MMP14 | GP5 | MGST3 |
| HFE | SLC1A3 | SGCB | SDC1 | AEBP1 | EEF1D | CABLES1 |
| CDKN1C | POGK | LIMA1 | SH3PXD2B | IGF1 | LY6D | RAD51AP1 |
| COL1A1 | GLT8D1 | SEL1L3 | FKBP9 | SLC8A1 | CD79B | ISCA1 |
| PPFIBP1 | TCF4 | RAB34 | CCR5 | THBS2 | KIF18A | POLD1 |
| KIDINS220 | PLTP | EPAS1 | SMARCA2 | BGN | PPP1R3D | SLC6A13 |
| ADCY7 | MYO5A | PGGT1B | COL5A1 | FSTL1 | SUV39H1 | H2AFZ |
| GM2A | PRAMEF8 | TWSG1 | ECM1 | HTRA1 | TESC | TIMELESS |
| SNX33 | SEC23A | CTSL | PKDCC | PDPN | GCH1 | GCLM |
| VIM | ITGB1 | EGR1 | SLC35A1 | DIO2 | CD163 | TOP2A |
| UHRF2 | HTR2B | PRICKLE2 | OLFML3 | COL6A1 | PTP4A3 | TMEM40 |
| MYO1E | SPTBN1 | IFT74 | ENPP1 | MMP2 | SNCA | |
| TIMP2 | FAM160B2 | RHOC | ANPEP | TIMP1 | GFI1B | |
| LOXL1 | MSR1 | MGAT5 | CALU | FANCD2 | MED26 | |
| CYP1B1 | 2510009E07RIK | PDE4DIP | MXRA8 | COX7A1 | RANGAP1 | |

Table S3 Oxidative stress-related genes identified by Pathcards website and KEGG PATHWAY database

| | | | | | | |
|--------|---------|----------|--------|-----------|----------|-------|
| ABCA6 | CYBA | CROT | RING1 | GABARAPL2 | NDUFV3 | GSTA2 |
| AGRP | RPS6KA5 | SCP2 | RNF2 | ATM | NDUFA1 | GSTA3 |
| ATXN3 | RPS6KA4 | ALDH3A2 | RPS27A | PCK2 | NDUFA2 | GSTA4 |
| CAT | TP53 | HACL1 | SCMH1 | G6PC | NDUFA3 | GSTM1 |
| FBXO32 | ATF2 | PECR | SUZ12 | G6PC2 | NDUFA4 | GSTM2 |
| FOXO1 | CREB1 | PHYH | TFDP1 | G6PC3 | NDUFA4L2 | GSTM5 |
| FOXO3 | CYBB | SLC25A17 | TFDP2 | IL7R | NDUFA5 | GSTM6 |
| FOXO4 | NFKB1 | SLC27A2 | TNIK | KLF2 | NDUFA6 | GSTT1 |
| G6PC1 | NOX4 | AGO1 | TNRC6A | S1PR1 | NDUFA7 | GSTO1 |
| GCK | NOX1 | AGO3 | TNRC6B | S1PR4 | NDUFA8 | GSTM3 |
| HDAC1 | GSR | AGO4 | TNRC6C | RAG1 | NDUFA9 | MGST3 |
| HDAC2 | NOX3 | BMI1 | UBA52 | RAG2 | NDUFA10 | GSTA1 |
| IGFBP1 | CYP1A1 | CBX2 | UBC | FBXO25 | NDUFAB1 | GSTO2 |
| INS | XDH | CBX4 | TGFB2 | CHEK2 | NDUFA11 | GSTM7 |

| | | | | | | |
|----------|--------|-------------|--------|-----------|---------|----------|
| NPY | HMOX1 | CBX6 | TGFB3 | ATR | NDUFA12 | GSTM4 |
| NR3C1 | SP1 | CBX8 | TGFBR1 | CHEK1 | NDUFA13 | MGST2 |
| PCK1 | SOD1 | CDKN2B | TGFBR2 | GORAB | NDUFB2 | GM3776 |
| PLXNA4 | GPX1 | CDKN2C | STK11 | TRP53 | NDUFB3 | GSTA5 |
| POMC | SOD3 | CDKN2D | PRKAA1 | CCND3 | NDUFB4 | GSTT3 |
| PPARGC1A | GSTT2 | COMMD3-BMI1 | PRKAA2 | CCNE1 | NDUFB4B | GSTT4 |
| RETN | MGST1 | E2F1 | PRKAB1 | CCNE2 | NDUFB4C | ND1 |
| SIN3A | NQO1 | E2F2 | PRKAB2 | RPRM | NDUFB5 | ND2 |
| SIRT3 | UGT1A6 | E2F3 | PRKAG1 | CDK1 | NDUFB6 | ND3 |
| SMAD2 | MAOA | EED | PRKAG3 | GTSE1 | NDUFB7 | ND4 |
| SMAD3 | GPX3 | EZH2 | PRKAG2 | FAS | NDUFB8 | ND4L |
| SMAD4 | GCLC | H2AB1 | NLK | PIDD1 | NDUFB9 | ND5 |
| SOD2 | MT1X | H2AC14 | SKP2 | TNFRSF10B | NDUFB10 | ND6 |
| SREBF1 | NFIX | H2AC18 | SETD7 | CASP8 | NDUFB11 | ATP5A1 |
| TRIM63 | TXNRD1 | H2AC19 | CREBBP | BID | NDUFS1 | ATP5B |
| PLCG1 | NOX5 | H2AC20 | EP300 | PMAIP1 | NDUFS2 | ATP5C1 |
| PLCG2 | TXNRD2 | H2AC4 | USP7 | BBC3 | NDUFS3 | ATP5D |
| CAMKK1 | GPX2 | H2AC6 | SLC2A4 | SIVA1 | NDUFS4 | ATP5E |
| CAMK1 | PRDX2 | H2AC7 | STK4 | BCL2 | NDUFS5 | ATP6 |
| CAMK2A | PRDX3 | H2AC8 | IGF1 | EI24 | NDUFS6 | ATP5PB |
| CAMK2D | GPX6 | H2AJ | IGF1R | SHISA5 | NDUFS7 | ATP5G1 |
| CAMK2G | PRDX5 | H2AX | INS2 | PERP | NDUFS8 | ATP5G2 |
| KRAS | GPX5 | H2AZ1 | INS1 | ZMAT3 | NDUFC1 | ATP5G3 |
| MRAS | GPX7 | H2AZ2 | INSR | SIAH1A | NDUFC2 | ATP5H |
| PRKCA | GSTP1 | H2BC1 | IRS1 | SIAH1B | SDHA | ATP5O |
| PRKCB | ERO1A | H2BC10 | IRS2 | CYCT | SDHB | ATP5J |
| PRKCG | NUDT2 | H2BC11 | IRS3 | APAF1 | SDHC | ATP8 |
| PRKCE | AQP8 | H2BC12 | IRS4 | CASP9 | SDHD | VDAC1 |
| PRKCD | NCF2 | H2BC13 | PIK3CA | CASP3 | UQCRC1 | VDAC2 |
| PRKCQ | NCF4 | H2BC14 | PIK3CD | AIFM2 | CYTB | VDAC3 |
| PRKCZ | ATOX1 | H2BC15 | PIK3CB | IGFBP3 | CYC1 | SLC25A4 |
| SRC | GPX8 | H2BC17 | PIK3R2 | SERPINE1 | UQCRC1 | SLC25A5 |
| RAF1 | P4HB | H2BC21 | PIK3R1 | ADGRB1 | UQCRC2 | SLC25A31 |
| ARAF | PRDX1 | H2BC26 | PIK3R3 | CD82 | UQCRH | PPIF |
| BRAF | ATP7A | H2BC3 | PDPK1 | THBS1 | UQCRB | SLC26A1 |
| MAP2K1 | NCF1 | H2BC4 | CHUK | SERPINB5 | UQCRCQ | SLC26A2 |
| MAP2K2 | PRDX6 | H2BC5 | IKBKB | DDB2 | UQCR10 | SLC26A6 |
| MAPK3 | CCS | H2BC6 | PTEN | RRM2B | UQCR11 | SLC26A9 |
| MAPK1 | CDK4 | H2BC7 | AKT1 | RRM2 | COX3 | CYP1A2 |
| CYCS | CDK6 | H2BC8 | AKT2 | SESN1 | COX1 | CYP2F2 |
| BAX | CDKN2A | H2BC9 | AKT3 | SESN3 | COX2 | CYP1B1 |
| BAK1 | TGFB1 | H3-3A | SGK1 | SESN2 | COX4I1 | EPHX1 |
| PPM1L | DDR1 | H3-3B | SGK2 | TSC2 | COX4I2 | EPHX2 |

| | | | | | | |
|---------|--------------|--------------|-----------|----------|----------|---------|
| PPP3CA | FN1 | H3C1 | SGK3 | STEAP3 | COX5A | EPHX3 |
| PPP3R1 | SUV39H1 | H3C10 | FOXO6 | COP1 | COX5B | EPHX4 |
| TXN | CDC37 | H3C11 | GRM1 | RCHY1 | COX6A1 | AKR1A1 |
| TXN2 | COL2A1 | H3C12 | HOMER2 | CCNG1 | COX6A2 | CBR1 |
| YWHAB | ANXA5 | H3C13 | HOMER1 | PPM1D | COX6B1 | AHR |
| YWHAG | BMP2 | H3C14 | HOMER3 | TRP73 | COX6B2 | LPO |
| YWHAE | FGF7 | H3C15 | AGAP2 | IL6RA | COX6C | HGF |
| YWHAH | PKD1 | H3C2 | CSNK1E | SOCS3 | COX7A2 | MET |
| SFN | IGFBP7 | H3C3 | IL10 | TNF | COX7A1 | PTPN1 |
| YWHAQ | NRF1 | H3C4 | STAT3 | TNFRSF1A | COX7A2L | IKBKG |
| YWHAZ | EXOC6 | H3C6 | EGF | RELA | COX7B | NFKBIA |
| MAP3K5 | LOXL4 | H3C7 | EGFR | GSK3A | COX7B2 | PTPRJ |
| TRAF2 | ECSIT | H3C8 | GRB2 | GSK3B | COX7C | ACP1 |
| TRAF6 | NLRP3 | H4C1 | SOS1 | NR1H3 | COX8A | PTPN11 |
| USP9X | SIRT6 | H4C11 | SOS2 | RXRA | COX8B | ABL1 |
| UBB | TOMM70 | H4C12 | HRAS | MLX | COX8C | ABL2 |
| STUB1 | IL6 | H4C13 | NRAS | MLXIP | ADCY1 | PRKD1 |
| ARRB1 | TRAF3 | H4C14 | CDK2 | MLXIPL | ADCY2 | PRKD3 |
| ARRB2 | IL18 | H4C15 | PRMT1 | PKLR | ADCY3 | PRKD2 |
| BCL2L1 | TERF2 | H4C16 | FOXP1 | LEP | ADCY4 | PTK2 |
| BCL2L11 | MAVS | H4C2 | CCNB1 | LEPR | ADCY5 | PLD2 |
| BMF | NLRX1 | H4C3 | CCNB2 | ADIPOQ | ADCY6 | PLD1 |
| BAD | NDUFAF2 | H4C4 | CCNB3 | ADIPOR1 | ADCY7 | MAP3K14 |
| MAP2K3 | ACAD9 | H4C5 | CCND1 | ADIPOR2 | ADCY8 | HIF1A |
| MAP2K6 | NDUFAF1 | H4C6 | CCND2 | PPARA | ADCY9 | ARNT |
| MAP2K4 | LOC105371348 | H4C8 | CCNG2 | PPARG | PRKACA | VEGFA |
| MAP2K7 | BCS1L | H4C9 | CDKN1A | CDC42 | PRKACB | KEAP1 |
| MAPK8 | ABCD1 | IFNB1 | CDKN1B | RAC1 | ATG5 | |
| MAPK9 | ACAA1 | KDM6B | RBL2 | MAP3K11 | MTOR | |
| MAPK10 | ACOT4 | LOC102724334 | PLK1 | ITCH | RPTOR | |
| MAPK14 | ACOT6 | MAP4K4 | PLK2 | ERN1 | AKT1S1 | |
| MAPK11 | ACOT8 | MAPKAPK2 | PLK3 | IL1A | RPS6KB2 | |
| MAPK12 | ACOX1 | MAPKAPK3 | PLK4 | IL1B | RPS6KB1 | |
| MAPK13 | DECR2 | MAPKAPK5 | GADD45A | XBP1 | EIF4EBP2 | |
| SHC1 | ECI2 | MDM2 | GADD45B | CEBPA | HSPA8 | |
| NFE2L2 | EHHADH | MDM4 | GADD45G | CYP2E1 | HSPA1L | |
| SIRT1 | HSD17B4 | MINK1 | FASL | EIF2AK3 | HSPA1A | |
| JUN | MLYCD | MOV10 | TNFSF10 | EIF2S1 | HSPA2 | |
| JUNB | ACOX2 | PHC1 | BCL6 | ATF4 | HSPA1B | |
| JUND | ACOX3 | PHC2 | BNIP3 | DDIT3 | CRYAB | |
| FOS | ACOXL | PHC3 | ATG12 | CASP7 | FOXA2 | |
| FOSB | AMACR | RBBP4 | GABARAPL1 | NDUFV1 | CLPB | |
| FOSL1 | CRAT | RBBP7 | GABARAP | NDUFV2 | AS3MT | |

Table S4 Description and function of the 14 intersection genes

| Description and function of the 14 intersection genes | | |
|-------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gene Symbol | Description | Function |
| <i>HMOX1</i> | Heme oxygenase 1 | Heme oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. |
| <i>IGF1</i> | Insulin-like growth factor 1 | The insulin-like growth factors, isolated from plasma, are structurally and functionally related to insulin but have a much higher growth-promoting activity. |
| <i>FN1</i> | Fibronectin 1 | Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. |
| <i>HIF1A</i> | Hypoxia inducible factor 1 subunit alpha | <i>HIF1A</i> functions as a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions, <i>HIF1A</i> activates the transcription of over 40 genes, including erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, HILPDA, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. |
| <i>MGST3</i> | Microsomal glutathione S-transferase 3 | <i>MGST3</i> also functions as a glutathione peroxidase. |
| <i>CYP1B1</i> | Cytochrome P450 family 1 subfamily B member 1 | Cytochromes P450 are a group of heme-thiolate monooxygenases. |
| <i>GPX8</i> | Glutathione peroxidase 8 | <i>GPX8</i> enables peroxidase activity. <i>GPX8</i> is predicted to be involved in cellular response to oxidative stress. |
| <i>GPX3</i> | Glutathione peroxidase 3 | <i>GPX3</i> protects cells and enzymes from oxidative damage, by catalyzing the reduction of hydrogen peroxide, lipid peroxides, and organic hydroperoxide by glutathione. |
| <i>IGFBP7</i> | Insulin-like growth factor binding protein 7 | <i>IGFBP7</i> binds IGF-I and IGF-II with a relatively low affinity. <i>IGFBP7</i> stimulates prostacyclin production. <i>IGFBP7</i> stimulates cell adhesion. |
| <i>E2F2</i> | E2F transcription factor 2 | Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site |
| <i>COX7A1</i> | Cytochrome C oxidase subunit VIIa polypeptide 1 | This protein is one of the nuclear-coded polypeptide chains of cytochrome C oxidase, the terminal oxidase in mitochondrial electron transport. This nuclear gene encodes polypeptide 1 (muscle isoform) of subunit VIIa. |
| <i>COX6A2</i> | Cytochrome C oxidase subunit 6A2 | This protein is one of the nuclear-coded polypeptide chains of cytochrome C oxidase, the terminal oxidase in mitochondrial electron transport. This nuclear gene encodes polypeptide 2 (heart/muscle isoform) of subunit VIa. |
| <i>SUV39H1</i> | SUV39H1 histone lysine methyltransferase | Histone methyltransferase that specifically trimethylates 'Lys-9' of histone H3 using monomethylated H3 'Lys-9' as substrate. |
| <i>ADCY7</i> | Adenylate cyclase 7 | This is a membrane-bound, calcium-inhibitable adenylyl cyclase, and belongs to the adenylyl cyclase class-4/guanylyl cyclase family. |

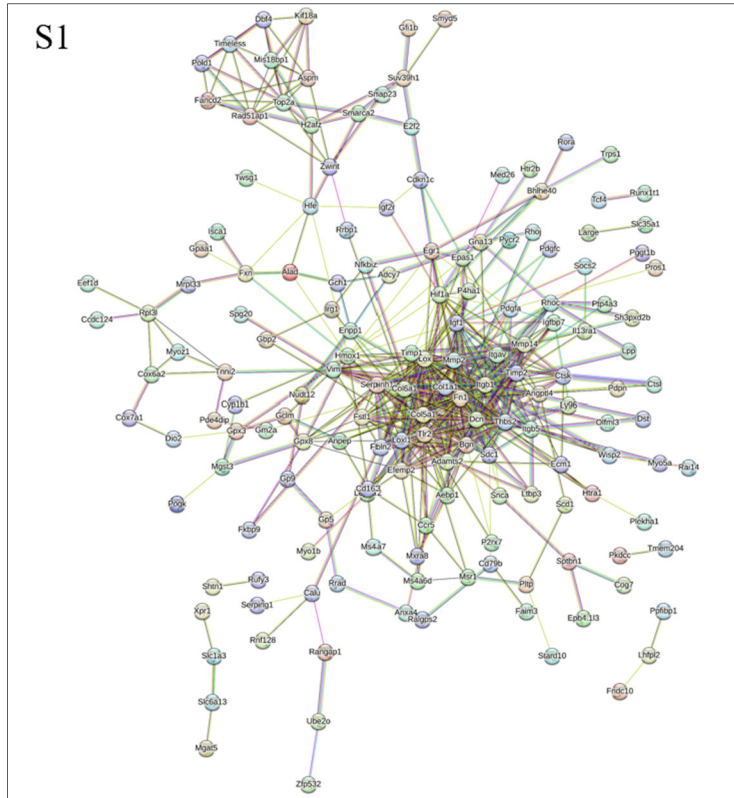


Fig. S1 PPI network of the screened DEGs.

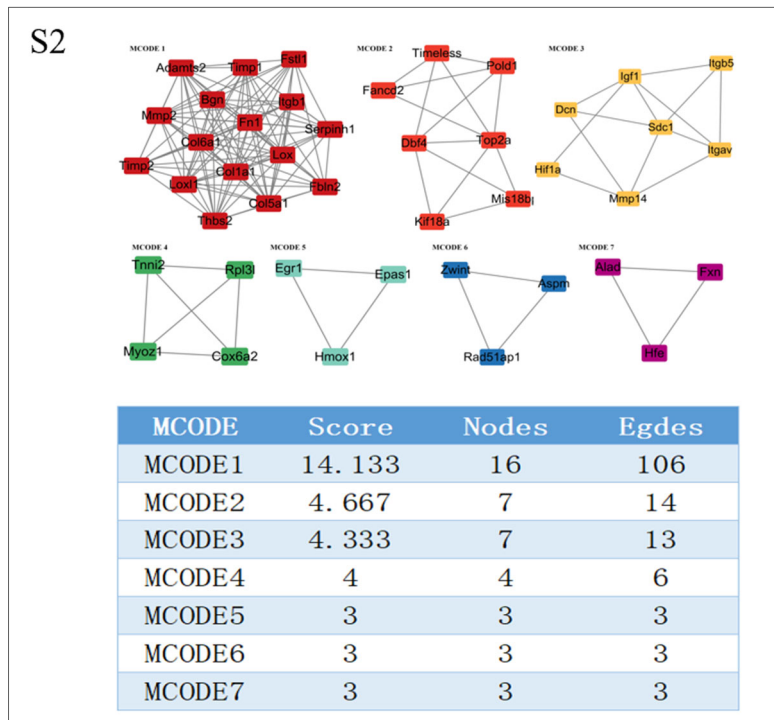


Fig. S2 Seven MCODE components were constructed with the screened DEGs. The score, nodes, and edges of each MCODE.