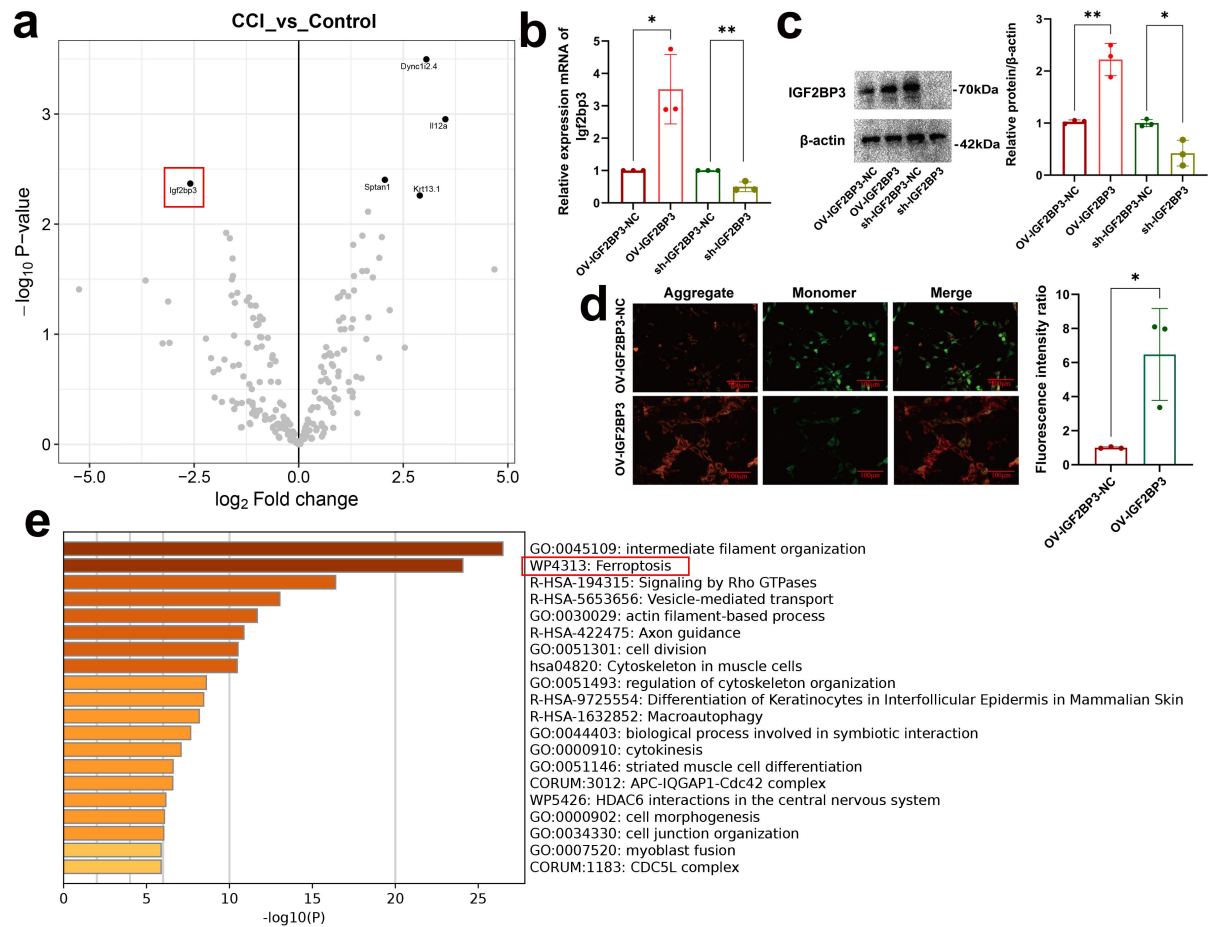
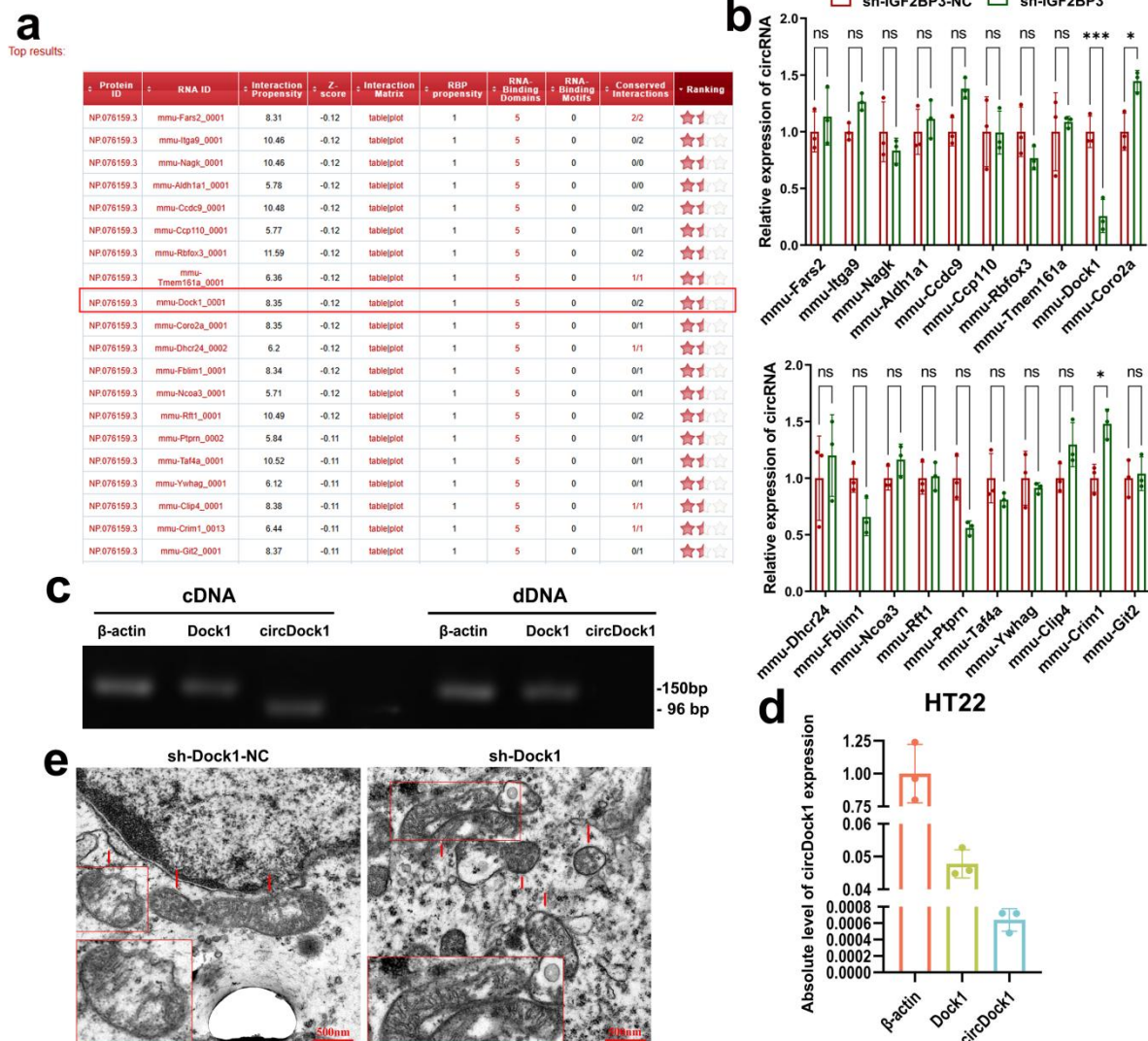


## Supplementary Information

|                                                                                                                                                                                                                |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Supplementary Figure 1. The research origins of IGF2BP3, its transfection efficiency into plasmids, and its effects on ferroptosis. ....                                                                       | 2  |
| Supplementary Figure 2. Verify the binding of IGF2BP3 to <i>circDock1</i> and confirm the circular structure formation of <i>circDock1</i> . ....                                                              | 3  |
| Supplementary Figure 3. Localization of <i>circDock1</i> , possibility of binding to IGF2BP3, and effects on mitochondrial membrane potential after overexpression of <i>circDock1</i> in OGD HT22 cells. .... | 4  |
| Supplementary Figure 4. Coding capacity prediction of <i>circDock1</i> and effects of DOCK1-704aa, IGF2BP3 on ferroptosis. ....                                                                                | 6  |
| Supplementary Figure 5. Assess the transfection efficiency of TCF21 knockdown and its effects on mitochondrial membrane potential changes. ....                                                                | 7  |
| Supplementary Figure 6. Tcf21 phosphorylation induced by <i>circDock1</i> -704aa downregulated Tcf21 expression. ....                                                                                          | 8  |
| Supplementary Figure 7. Rescue experiments for ferroptosis and changes in protein expression levels in CCI. ....                                                                                               | 10 |
| Supplementary Table 1. The primers for qRT–PCR assays. ....                                                                                                                                                    | 11 |
| Supplementary Table 2. The primers for RIP assays. ....                                                                                                                                                        | 11 |
| Supplementary Table 3. The primers for ChIP assays. ....                                                                                                                                                       | 11 |
| Uncropped raw images of western blots .....                                                                                                                                                                    | 11 |



**Supplementary Figure 1. The research origins of IGF2BP3, its transfection efficiency into plasmids, and its effects on ferroptosis. (a)** Differential gene analysis between mouse CCI model group and control group. **(b)** The transfection efficiency of IGF2BP3 plasmids in HT22 cells was detected by qRT-PCR. **(c)** Transfection efficiency of IGF2BP3 plasmids in HT22 cells was detected by Western Blot. **(d)** The JC-1 fluorescent probe was used to detect the effects of OV-IGF2BP3-NC and OV-IGF2BP3 on mitochondrial membrane potential levels in OGD HT22 cells. **(e)** Pathway enrichment analysis of mouse CCI model group and control group.



**Supplementary Figure 2. Verify the binding of IGF2BP3 to *circDock1* and confirm the circular structure formation of *circDock1*.**

**(a)** CircRNAs potentially interacting with IGF2BP3 predicted by catRAPID (<https://catRAPID>). **(b)** The expression mRNA of circRNAs in sh-IGF2BP3 HT22 cells. **(c)**

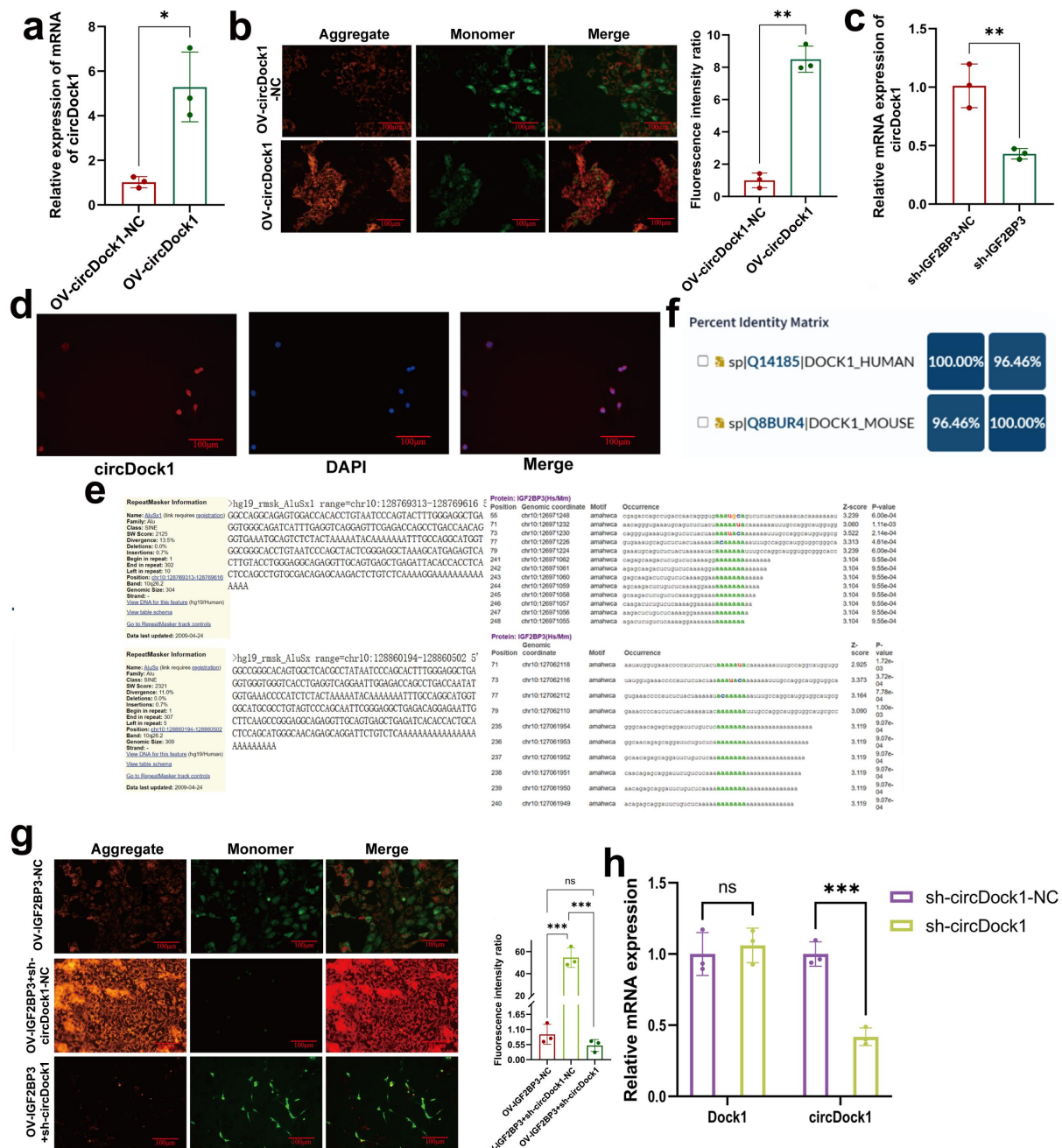
Agarose gel electrophoresis was used to detect the ring formation of *circDock1*. **(d)**

The absolute expression levels of *circDock1* and *Dock1* in HT22 cells. **(e)**

Transmission electron microscopy was used to examine the morphological changes

in mitochondria after knockdown of *Dock1*. Data are presented as the mean  $\pm$  SD (n

= 3, each group). \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001.



**Supplementary Figure 3. Localization of circDock1, possibility of binding to**

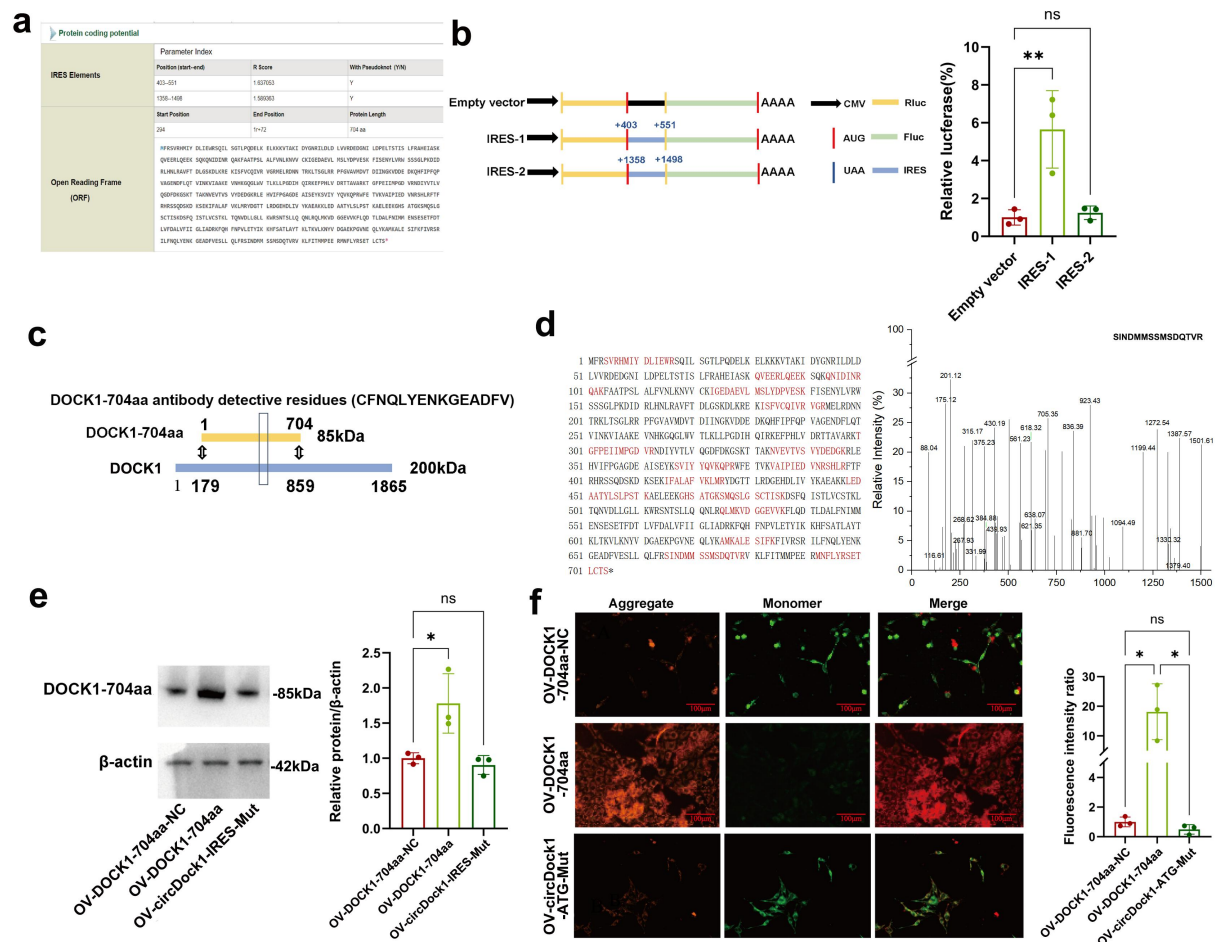
**IGF2BP3, and effects on mitochondrial membrane potential after**

**overexpression of circDock1 in OGD HT22 cells. (a) Transfection efficiency of**

**circDock1 plasmids in HT22 detected by qRT-PCR. (b) The JC-1 fluorescent probe**

**was used to detect the effects of circDock1 plasmids transfection on mitochondrial**

membrane potential levels in OGD HT22 cells. **(c)** The expression of circDock1 mRNA in response to IGF2BP3 knockdown was detected by qRT-PCR. **(d)** FISH assays were used to detect the localization of circDock1 in HT22 cells. Red, circDock1; blue, DAPI nuclear staining. **(e)** Left panel, the sequence of ALU elements. Right panel, the binding sites of IGF2BP3 to flanking sequence at both ends of circDock1 predicted by RBPmap server (<http://rbpmap.technion.ac.il>). **(f)** NCBI predicts homology between mouse dock1 and human DOCK1. **(g)** The JC-1 fluorescent probe was used to detect the effects of IGF2BP3 and circDock1 plasmids transfection on mitochondrial membrane potential levels in OGD HT22 cells. **(h)** Assess the off-target effects of sh-circDock1. Data are presented as the mean  $\pm$  SD (n = 3, each group). \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001.



## Supplementary Figure 4. Coding capacity prediction of circDock1 and effects

of DOCK1-704aa, IGF2BP3 on ferroptosis. (a) The translation regulation of

circDock1 was predicted using the circRNADb database

(<http://reprod.njmu.edu.cn/circrandb>). (b) Upper panel: Schematic of plasmids with

IRES inserted between the RLuc stop codon and the Fluc start codon. Lower panel:

Luciferase activity of Fluc/RLuc was detected by dual-luciferase reporter assays. (c)

Schematic of the antigen design for the custom circDock1-704aa antibody. (d)

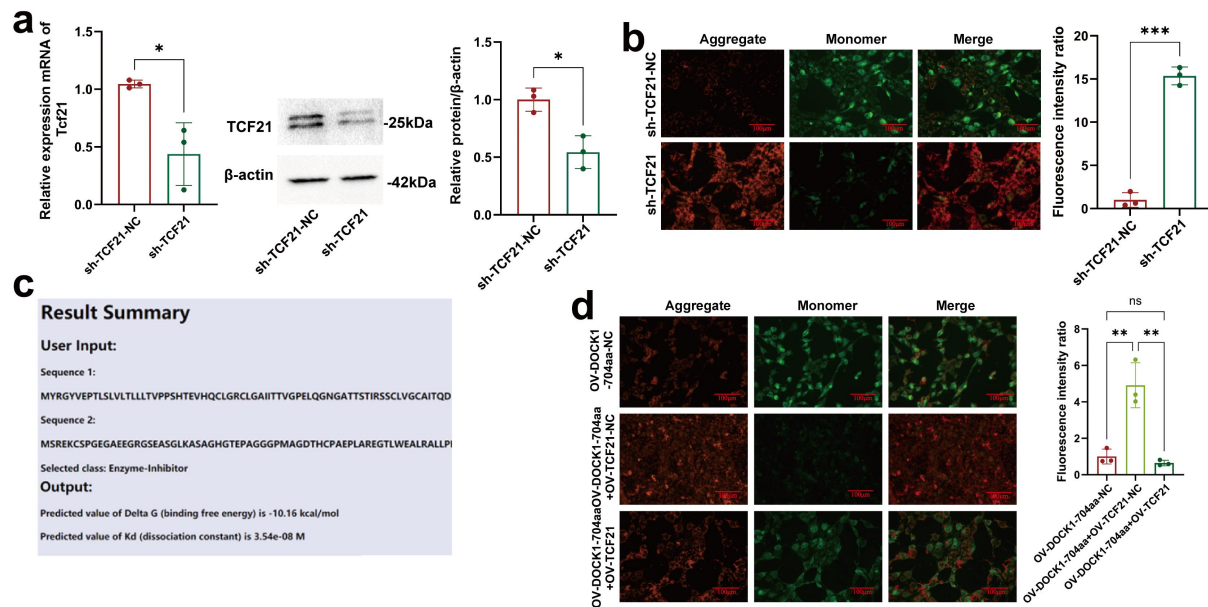
Protein sequence coverage map of ABCC1-677aa (30%) and representative tandem

mass spectrometry spectra of identified peptides. (e) Transfection efficiency of

circDock1-IRES-Mut plasmid in HT22 cells. Left panel: Detected by qRT-PCR. Right

panel: Detected by Western blot. (f) The JC-1 fluorescent probe was used to detect

the effects of circDock1-IRES-Mut plasmid transfection on mitochondrial membrane potential levels in OGD HT22 cells. Data are presented as the mean  $\pm$  SD ( $n = 3$ , each group).  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ .



## Supplementary Figure 5. Assess the transfection efficiency of TCF21

### knockdown and its effects on mitochondrial membrane potential changes. (a)

Transfection efficiency of sh-TCF21 plasmid in HT22 cells. Left panel: Detected by

qRT-PCR. Right panel: Detected by Western blot. **(b)** The JC-1 fluorescent probe

was used to detect the effects of sh-TCF21 plasmid transfection on mitochondrial

membrane potential levels in OGD HT22 cells. **(c)** The binding affinity of circDock1-

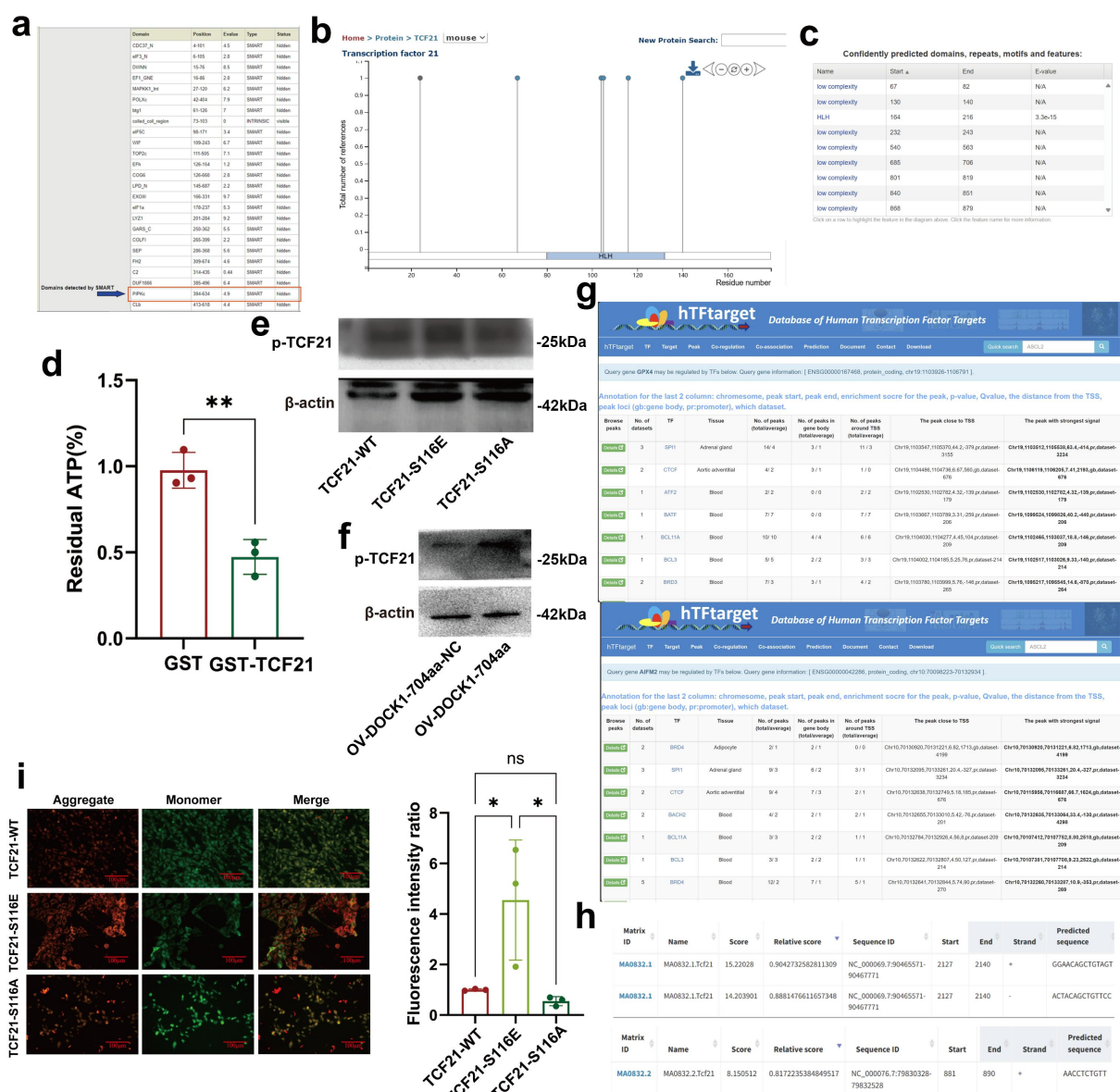
704aa and Tcf21 was predicted by the PPA-Pred2 server ([PPA-Pred \(iitm.ac.in\)](http://PPA-Pred.iitm.ac.in)). **(d)**

The JC-1 fluorescent probe was used to detect the effects of OV-circDock1-704aa

and OV-Tcf21 plasmid transfection on mitochondrial membrane potential levels in

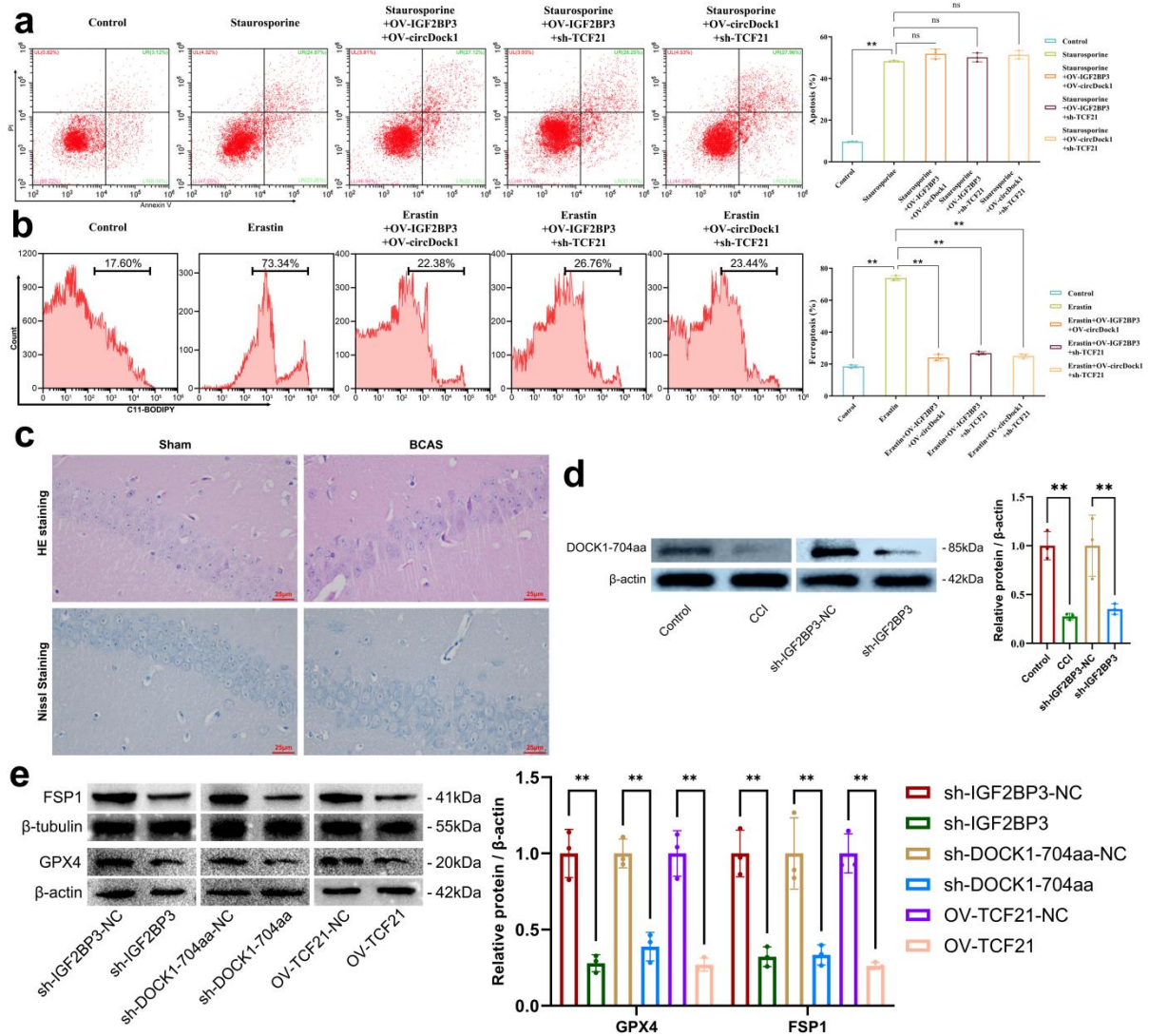
OGD HT22 cells. Data are presented as the mean  $\pm$  SD ( $n = 3$ , each group).  $*P <$

$0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ .



**Supplementary Figure 6. Tcf21 phosphorylation induced by circDock1-704aa downregulated Tcf21 expression. (a)** The phosphorylation domain of circDock1 was predicted by the circRNADb database (<http://reprod.njmu.edu.cn/circrandb>). **(b)** The domain of Tcf21 that could be phosphorylated was predicted by uniprot (<https://www.uniprot.org>). **(c)** The potential phosphorylation sites were predicted by NetPhos 3.1 (<http://www.cbs.dtu.dk/services/NetPhos/>). **(d)** GST-Tcf21 kinase activity was validated by in vitro kinase assay. **(e)** S116 phosphorylation levels were

detected using the custom p-Tcf21-S116 antibody in HT22 cells transfected with Tcf21-WT, Tcf21-S116E, and Tcf21-S116A plasmids. **(f)** S116 phosphorylation levels were also detected in DOCK1-704aa upregulated HT22 cells. **(g)** hTFtarget predicts transcription factors that may bind to both GPX4 and FSP1 (<https://guolab.wchscu.cn/hTFtarget/#!/>). **(h)** The binding sites of Tcf21 to Fsp1 and Gpx4 were predicted by JASPER (<http://jaspar.genereg.net/>). **(i)** The JC-1 fluorescent probe was used to detect the effects of phosphorylation plasmid transfection on mitochondrial membrane potential levels in OGD HT22 cells. Data are presented as the mean  $\pm$  SD (n = 3, each group). \*\* $P < 0.01$ .



**Supplementary Figure 7. Rescue experiments for ferroptosis and changes in protein expression levels in CCI.** (a) Flow cytometry was used to detect apoptosis and the rescue effects on it. One-way ANOVA analyse. (b) Flow cytometry was used to detect ferroptosis and the rescue effects on it. One-way ANOVA analyse. (c) Degree of brain injury in the CCI model. (d) The expression levels of the DOCK1-704aa protein in the CCI model and in cells with reduced IGF2BP3 expression. One-way ANOVA analyse. (e) The expression levels of GPX4 and FSP1 proteins under conditions of IGF2BP3 knockdown, DOCK1-704aa knockdown, and TCF21 overexpression. Two-way ANOVA analyse. Data are presented as the mean  $\pm$  SD (n

= 3, each group). \*\* $P < 0.01$ .

**Supplementary Table 1. The primers for qRT–PCR assays.**

| Primer    | Forward                 | Reverse                  |
|-----------|-------------------------|--------------------------|
| Igf2bp3   | GGTGAAGACGGGCTACGC      | GTAAACTATCCAGCACCTCCCA   |
| CircDOCK1 | ACAGCGAAGAACGTGGAG      | AATCGCTTCATCACCAGCAC     |
| Dock1     | ATGCCAGAAAGTCTCAACAGC   | AACAGTGCATGGAGTAGTGCC    |
| β-actin   | GTGACGTTGACATCCGTAAAGA  | GCCGGACTCATCGTACTCC      |
| Tcf21     | TCTCCAAGCTGGACACTCTCA   | ATAAAGGGCCACGTCAGGTTGA   |
| Gpx4      | ATAAGAACGGCTGCGTGGTGAAG | TAGAGATAGCACGGCAGGTCCTTC |
| Fsp1      | TCCGGAGATGGGCACTAGAA    | CCTGTTGCTGTCCAAGTTGC     |

**Supplementary Table 2. The primers for RIP assays.**

| Primer | Forward              | Reverse              |
|--------|----------------------|----------------------|
| e      | TCCCAGGTACTCAGGATGCT | TTTGAGATGGAGGCTCGCTC |
| d      | TGACCTCGGAAGCAAAGACC | ACCCGGGAAAATCACATGCT |
| b      | AATTGGAGACCAGCCTGACC | AGAATCCTGCTCTGTTGCCC |

**Supplementary Table 3. The primers for ChIP assays.**

| Primer | Forward               | Reverse                     |
|--------|-----------------------|-----------------------------|
| Fsp1   | GGTTTTTTCATTTCCCAGCTG | TAAATTATTCCAGGTCCACATCAAAGG |
|        | CTTGTAAGCT            | GGAGCTATAAGG                |
| Gpx4   | AATTCCTGTGTGGACCA     | CAGGGCTTGACCTTGCTTCTAG      |

**Uncropped raw images of western blots**

|                         |                                                    |  |                     |  |
|-------------------------|----------------------------------------------------|--|---------------------|--|
|                         | β-actin (42kDa)                                    |  | IGF2BP3 (70kDa)     |  |
| Figure 1b               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa) FSP1 (41kDa)                     |  |                     |  |
| Figure 1g               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Figure 1g               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Figure 2g               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Figure 2g               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa) FSP1 (41kDa)                     |  |                     |  |
| Figure 3g               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Figure 3g               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Figure 4c               |                                                    |  |                     |  |
|                         |                                                    |  |                     |  |
|                         | β-actin (42kDa) DOCK1-704aa (85kDa) Dock1 (200kDa) |  |                     |  |
| Figure 4d               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Figure 4e               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Figure 4f               |                                                    |  |                     |  |
|                         | β-actin (42kDa) DOCK1-704aa (85kDa) Dock1 (200kDa) |  |                     |  |
| Figure 5a               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Figure 5e               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Figure 5e               |                                                    |  |                     |  |
|                         | DOCK1-704aa (85kDa)                                |  | TCF21 (25kDa)       |  |
| Figure 6b               |                                                    |  |                     |  |
|                         | TCF21 (25kDa)                                      |  | DOCK1-704aa (85kDa) |  |
| Figure 6b               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | TCF21 (25kDa)       |  |
| Figure 6c               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | TCF21 (25kDa)       |  |
| Figure 6d               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Figure 6h               |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Figure 6h               |                                                    |  |                     |  |
|                         | β-tubulin (55kDa) FSP1 (41kDa)                     |  |                     |  |
| Figure 7f               |                                                    |  |                     |  |
|                         | β-actin (42kDa) GPX4 (20kDa)                       |  |                     |  |
| Figure 7f               |                                                    |  |                     |  |
|                         | β-actin (42kDa) IGF2BP3 (70kDa)                    |  |                     |  |
| Supplementary Figure 1c |                                                    |  |                     |  |
| Supplementary Figure 2c |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Supplementary Figure 4e |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | TCF21 (25kDa)       |  |
| Supplementary Figure 5a |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | p-TCF21 (25kDa)     |  |
| Supplementary Figure 6e |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | p-TCF21 (25kDa)     |  |
| Supplementary Figure 6f |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Supplementary Figure 7d |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | DOCK1-704aa (85kDa) |  |
| Supplementary Figure 7d |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Supplementary Figure 7e |                                                    |  |                     |  |
|                         | β-actin (42kDa)                                    |  | GPX4 (20kDa)        |  |
| Supplementary Figure 7e |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Supplementary Figure 7e |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Supplementary Figure 7e |                                                    |  |                     |  |
|                         | β-tubulin (55kDa)                                  |  | FSP1 (41kDa)        |  |
| Supplementary Figure 7e |                                                    |  |                     |  |