



Supporting Information

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Mitofusin 1 Drives Preimplantation Development by Enhancing Chromatin Incorporation of Histone H3.3

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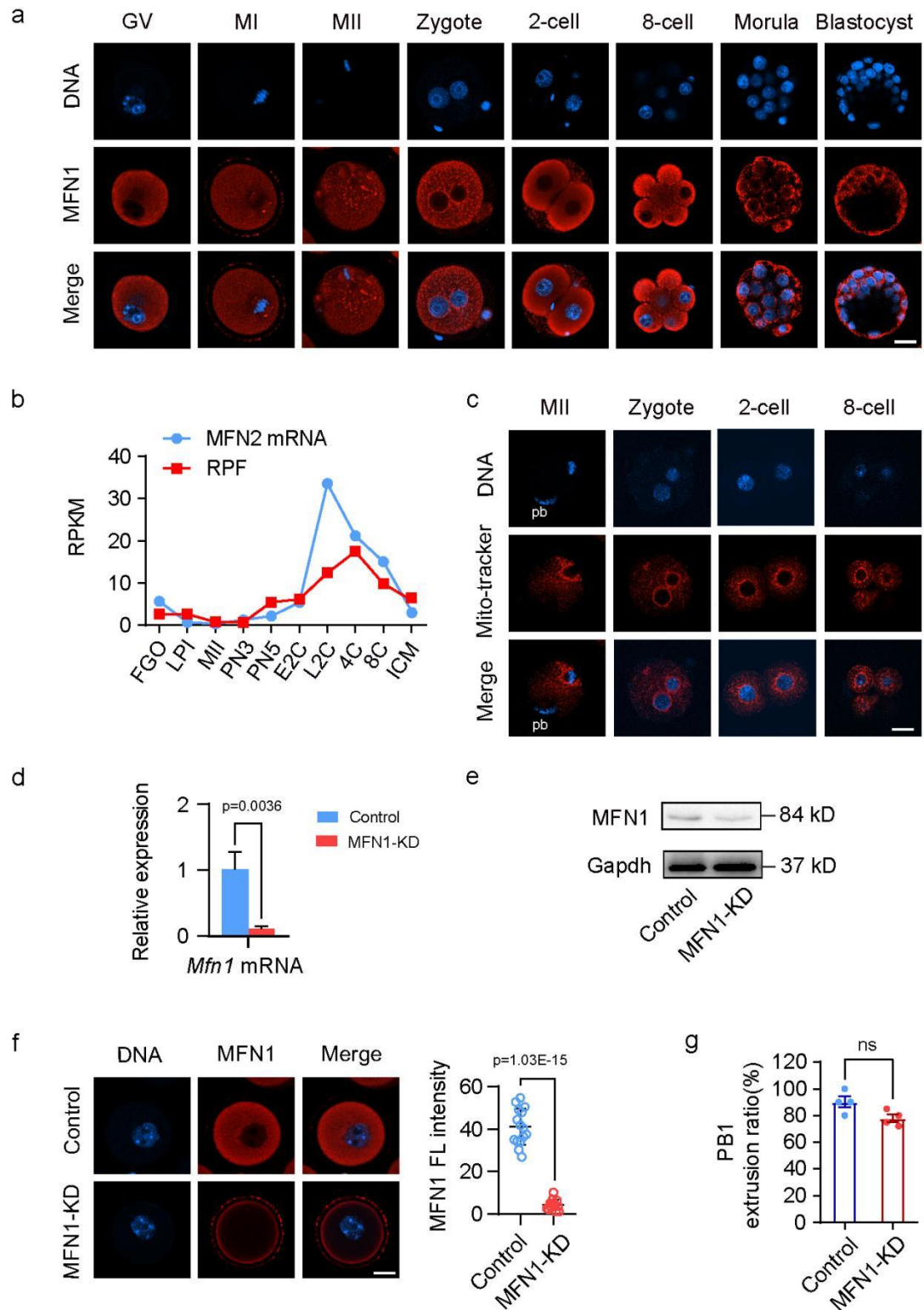


Fig. S1 The expression of MFN1 in mouse oocyte and early embryo and knockdown efficiency.

a Immunostaining of MFN1 at different developmental stages. GV (n = 50); MI (n = 51); MII (n = 55); 1-cell (n = 68); 2-cell (n = 60); 8-cell (n = 70); morula (n = 68); blastocyst (n = 73). Scale bar, 25 μ m.

b The dynamics of *Mfn2* transcript (mRNA-seq signal) and translation (Ribo-seq

signal, RPF, representing the efficiency of translation) levels. Data are expressed as the mean of RPKM from two repeats. RPKM, reads per kilobase of bin per million mapped reads; FGO, LPI, and MII, full-grown, late prometaphase I and MII oocytes; PN3 and PN5, PN3 and PN5 stage of early 1-cell embryos; E2C and L2C, early and late 2-cell embryos; 4C and 8C, 4- and 8-cell embryos; ICM, inner cell mass. Public dataset GSE165782 was used for analysis.

c Immunostaining of mitochondrial distribution in MII, zygotes, 2-cell and 8-cell embryos from control (n = 22, n = 23, n = 20, n = 19) and MFN1-KD (n = 18, n = 25, n = 26, n = 25) groups. Scale bar, 25 μ m.

d qRT-PCR comparing relative expression levels of *Mfn1* mRNA in control and MFN1-KD GV oocytes. The expression levels were normalized to *Gapdh*, which served as an internal control.

e Western blot comparing MFN1 protein levels in control and MFN1-KD GV oocytes. *Gapdh* is the internal control.

f Immunofluorescence staining (left) and the fluorescence intensities (right) of MFN1 in control (n = 15) and MFN1-KD (n = 15) GV oocytes. Scale bar, 25 μ m.

g Quantification of PB1 extrusion rate in control and MFN1-KD oocytes. n = 4 biological replicates. ns, not significant.

Data of **(d)**, **(f)** and **(g)** were presented as mean \pm SEM of at least three independent experiments. P value was calculated using two-tailed Student's t test.

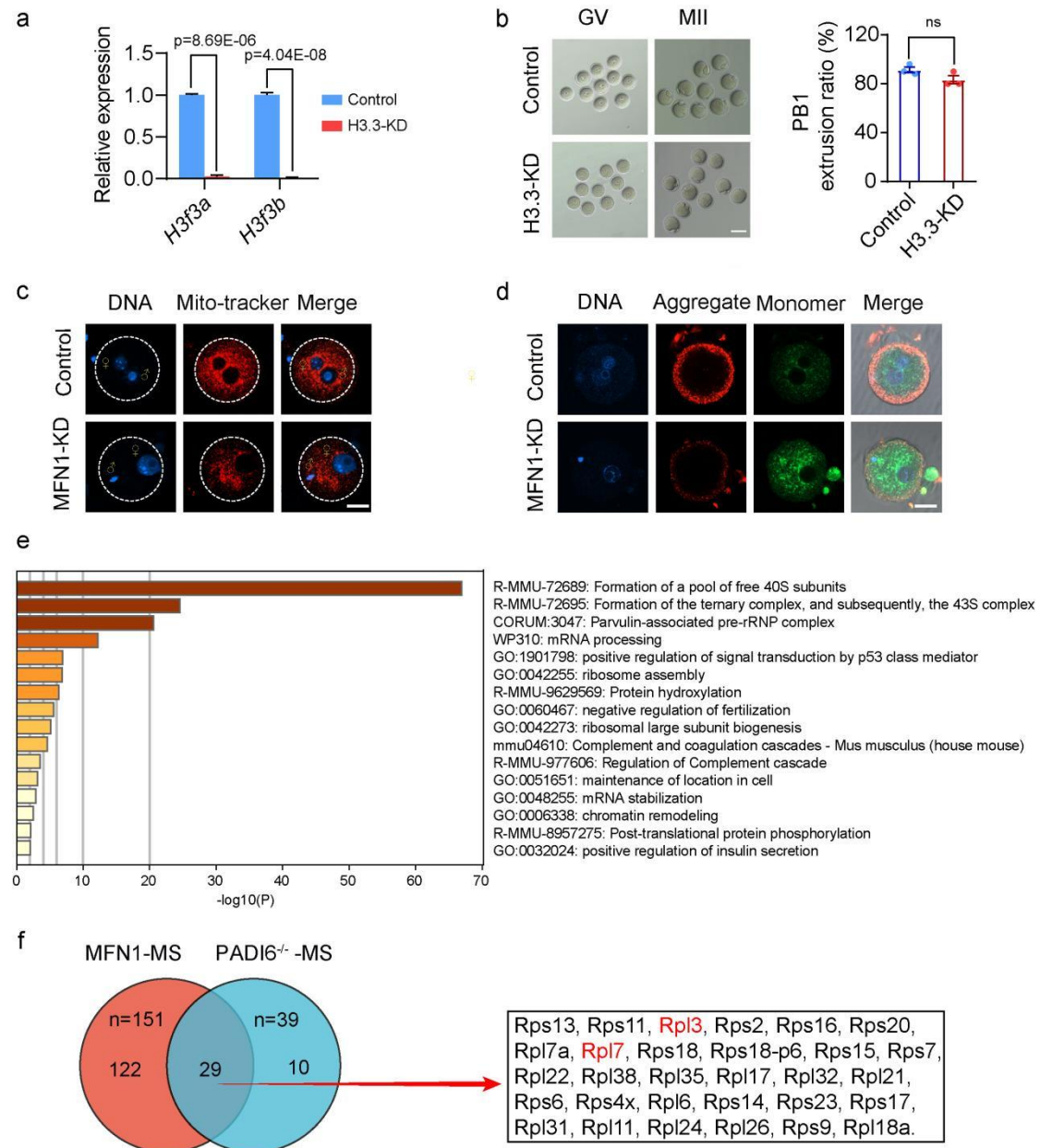


Fig. S2 The impairment of mitochondrial function by MFN1 depletion and the analysis of MFN1 coIP-MS results.

a qRT-PCR comparing relative expression levels of *H3f3a* and *H3f3b* mRNA between control and H3.3-KD GV oocytes. The expression levels were normalized to *Gapdh*, which served as an internal control.

b Representative DIC images (left) of GV and MII oocytes from control (n = 106) and H3.3-KD (n = 117) groups. GV oocytes were microinjected with siRNA against H3f3a and H3f3b. After 14 h, PB1 extrusion rates (right) were analyzed. Scale bar, 25 μm.

c-d Immunostaining of mitochondrial distribution (**c**) and JC1 staining (**d**) in zygotes from control and MFN1-KD groups. Scale bar, 25 μm.

e Gene ontology analysis of MS results using anti-MFN1 antibody in mouse oocytes by Metascape.

f The Venn diagram shows 29 common ribosomal subunits in IP-MS result of MFN1 protein and published MS data from PADI6^{-/-} oocytes. Data of **(a)** and **(b)** were presented as mean \pm SEM from at least three independent experiments. P value was calculated by two-tailed Student's t-test. ns, not significant.

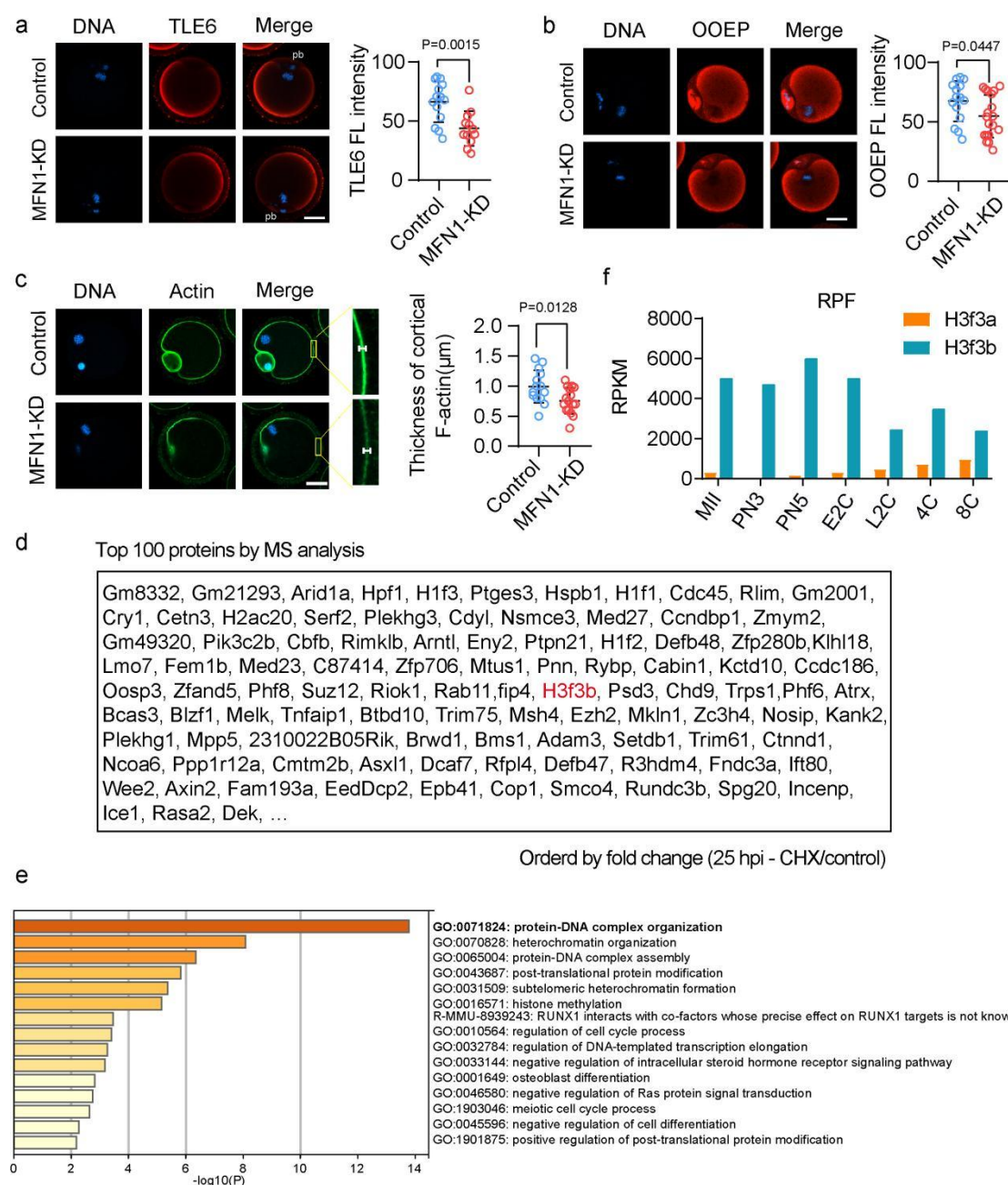


Fig. S3 The impairment of MFN1 on components of cytoplasmic lattice and the proteins sensitive to translation inhibition.

a Representative image of immunofluorescence staining for TLE6 in control and MFN1-KD MII oocytes (left). Scale bar, 25 μ m. Quantification signals of TLE6 in control and MFN1-KD MII oocytes (right). Control (n = 14), MFN1-KD (n = 12).

b Representative image of immunofluorescence staining for OOEP in control and

MFN1-KD MII oocytes (left). Scale bar, 25 μ m. Quantification signals of OOEP in control and MFN1-KD MII oocytes (right). Control (n = 15), MFN1-KD (n = 19).

c Representative image of immunofluorescence staining for F-actin in control (n = 14) and MFN1-KD (n = 16) MII oocytes (left). The right is an enlarged inset image. Scale bar, 25 μ m. Quantification of cortical F-actin width in control and MFN1-KD MII oocytes (right).

d The top 100 proteins with high translation activity, ordered by fold change (25hpi-CHX/control, hpi, hours post-insemination).

e Gene ontology analysis of (**d**).

f The dynamics of *H3f3a* and *H3f3b* translation activity (Ribo-seq signal, RPF, representing the efficiency of translation). Public dataset GSE165782 was used for analysis.

Data of (**a**), (**b**) and (**c**) were presented as mean \pm SEM from at least three independent experiments. P value was calculated by two-tailed Student's t-test. ns, not significant.

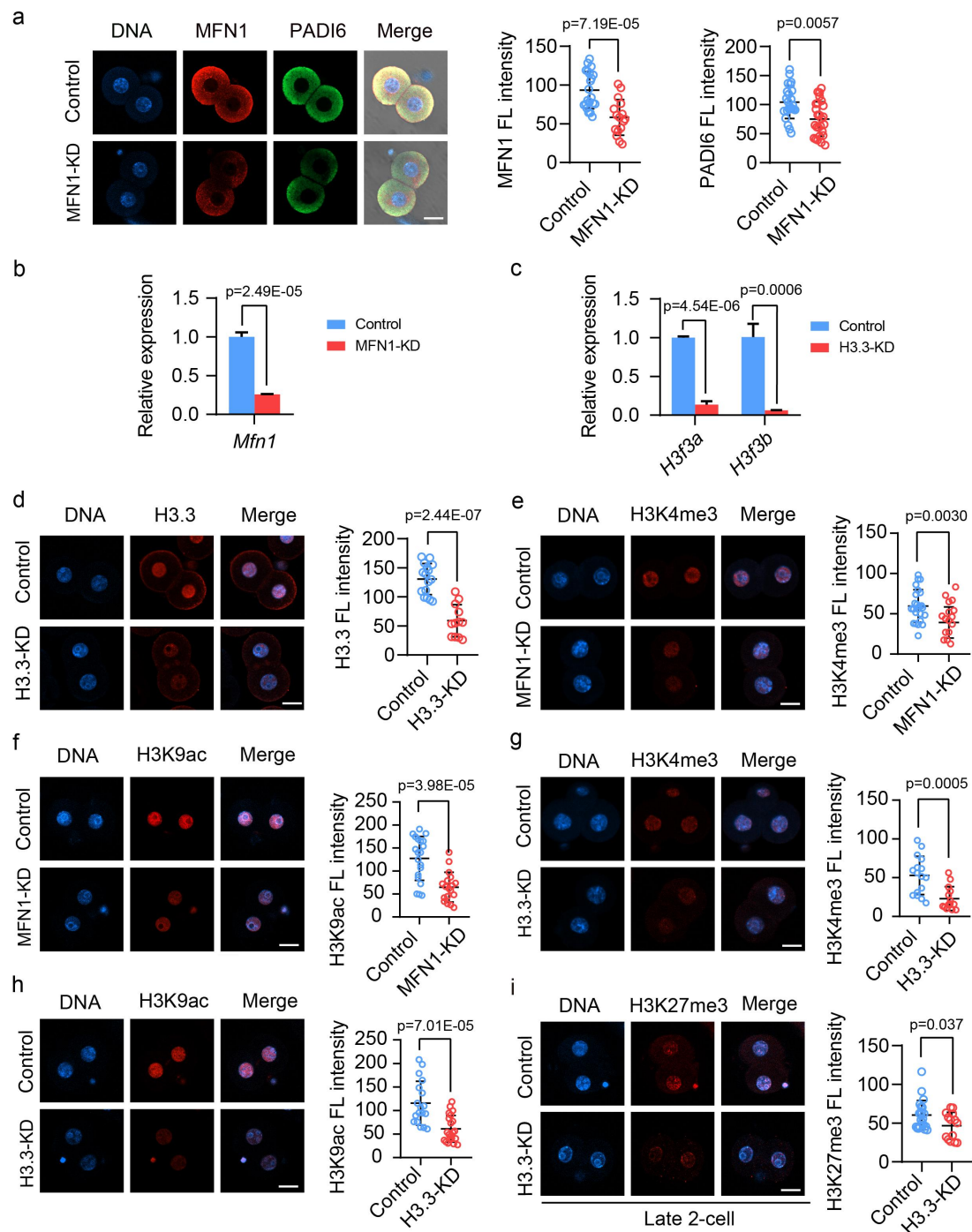


Fig. S4 The effect of MFN1 or H3.3 knockdown on histone modifications in 2-cell embryos.

a Representative images of MFN1 and PADI6 localization in control and MFN1-KD 2-cell embryos (left). Scale bar, 25 μ m. The fluorescence intensities of MFN1 and PADI6 signals were examined in control (n = 21) and MFN1-KD (n = 16) early 2-cell embryos (right).

b The depletion efficiency of MFN1 at the levels of mRNA (**b**) and protein (**c**) in early 2-cell embryos by qRT-PCR and immunostaining. The mRNA expression levels were normalized to *Gapdh*, which served as an internal control.

c, d The depletion efficiency of H3.3 at the levels of mRNA (**c**) and protein as shown

with immunostaining (**d**) in early 2-cell embryos. The mRNA expression levels were normalized to *Gapdh*, which served as an internal control. In (**d**), control (n = 16) and MFN1-KD (n = 12). Scale bar, 25 μ m.

e Immunostaining images (left) and quantification signals (right) of H3K4me3 for mouse early 2-cell embryos from control (n = 24) and MFN1-KD (n = 16) groups. Scale bar, 25 μ m.

f Immunostaining images (left) and quantification signals (right) of H3K9ac for mouse early 2-cell embryos from control (n = 20) and MFN1-KD (n = 18) groups. Scale bar, 25 μ m.

g Immunostaining images (left) and quantification signals (right) of H3K4me3 for mouse early 2-cell embryos from control (n = 16) and H3.3-KD (n = 14) groups. Scale bar, 25 μ m.

h Immunostaining images (left) and quantification signals (right) of H3K9ac for mouse early 2-cell embryos from control (n = 18) and H3.3-KD (n = 21) groups. Scale bar, 25 μ m.

i Immunostaining images (left) and quantification signals (right) of H3K27me3 for mouse late 2-cell embryos from control (n = 24) and H3.3-KD (n = 26) groups. Scale bar, 25 μ m.

Data of (**a-i**) were presented as mean \pm SEM of at least three independent experiments. P value was performed by two-tailed Student's t test.

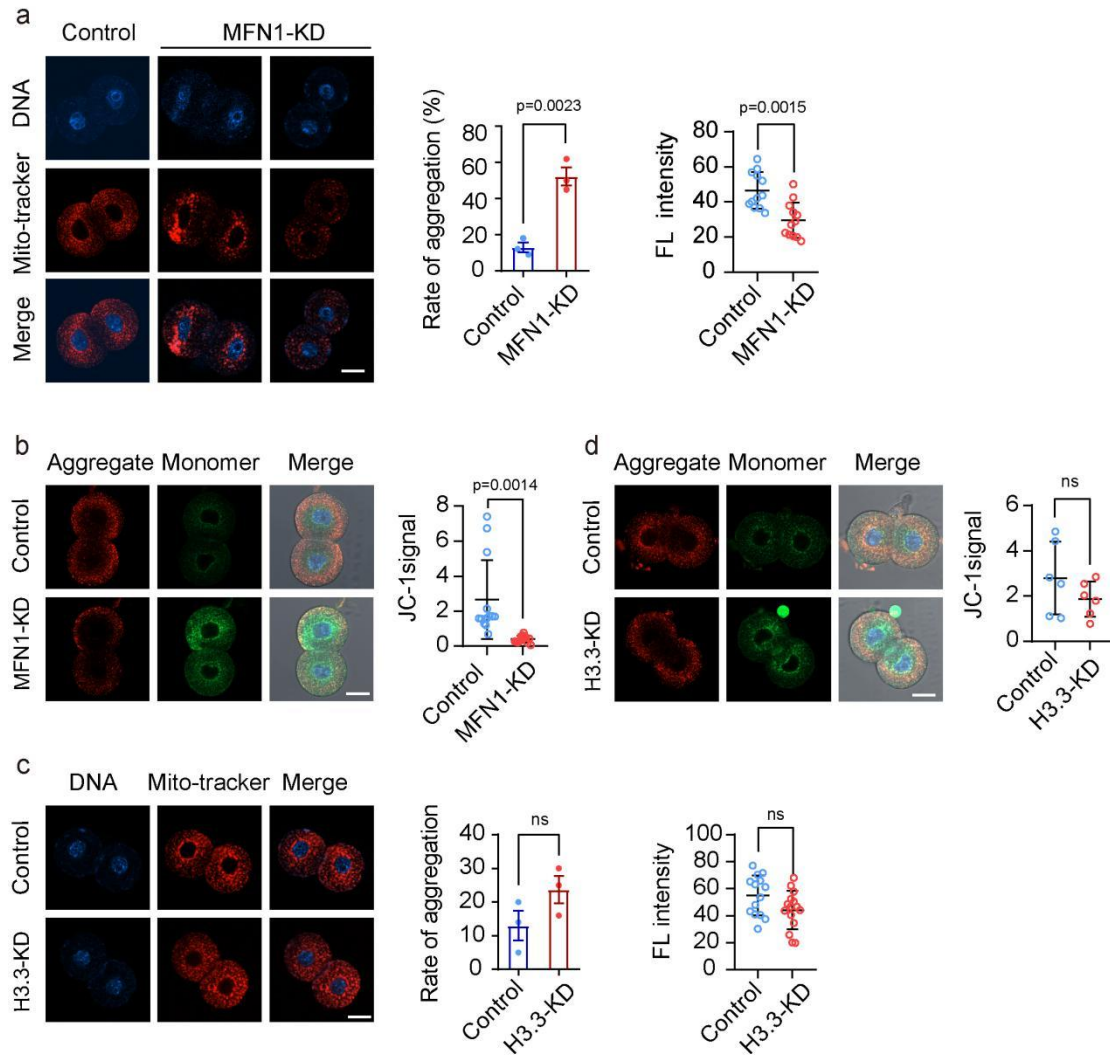


Fig. S5 The impact of MFN1 and H3.3 depletion on mitochondrial distribution and function in early 2-cell embryos.

a Early 2-cell embryos with *Mfn1* depletion were stained with Mito-tracker Red to show mitochondrial distribution (left panel). Scale bar, 25 μ m. Quantification of aggregation rate and fluorescence intensity of Mito-tracker signals in control (n = 12) and MFN1-KD (n = 12) early 2-cell embryos (right panel).

b Representative images (left panel) of MMP in control (n = 13) and MFN1-KD (n = 13) early 2-cell embryos. Red, high membrane potential; green, low membrane potential. Scale bar, 25 μ m. The ratio of red to green fluorescence intensity was quantified in control and MFN1-KD early 2-cell embryos (right panel).

c Early 2-cell embryos with H3.3 depletion were stained with Mito-tracker Red to show mitochondrial distribution (left panel). Scale bar, 25 μ m. Quantification of aggregation rate and fluorescence intensity of Mito-tracker signals in control (n = 14) and H3.3-KD (n = 15) early 2-cell embryos (right panel).

d Representative images (left panel) of MMP in control (n = 6) and H3.3-KD (n = 6) early 2-cell embryos. Red, high membrane potential; green, low membrane potential. Scale bar, 25 μ m. The ratio of red to green fluorescence intensity was quantified in control and H3.3-KD early 2-cell embryos (right panel).

Data of (a-d) were presented as mean \pm SEM of at least three independent experiments. P value was performed by two-tailed Student's t test.

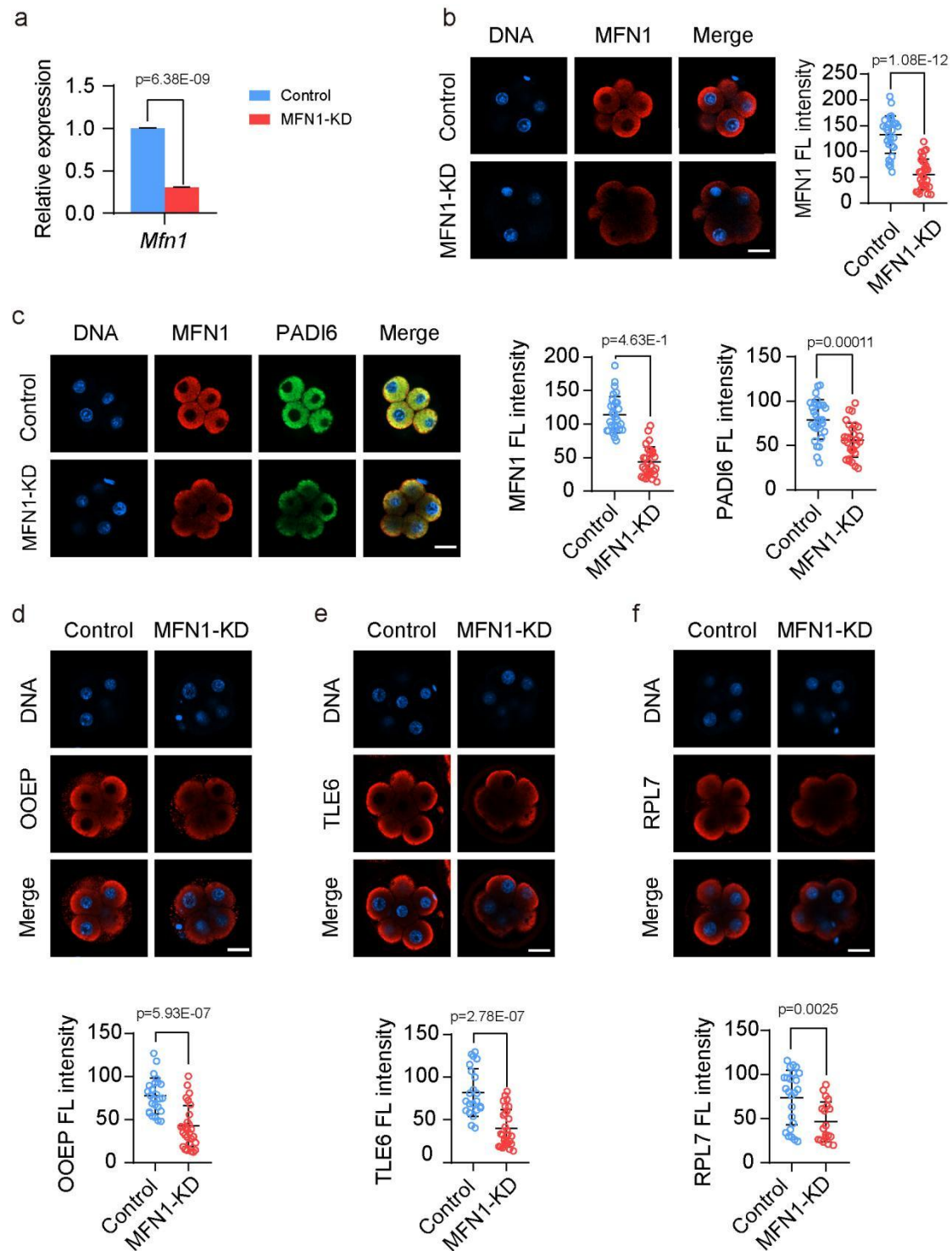


Fig. S6 Effects of MFN1 knockdown on the components of cytoplasmic lattices and ribosomal proteins in 8-cell embryos.

a, b The depletion efficiency of MFN1 at the levels of mRNA (**a**) and protein as shown with immunostaining (**b**) in 8-cell embryos. Scale bar, 25 μ m. In (**b**), control (n = 30) and MFN1-KD (n = 30).

c Representative images of MFN1 and PADI6 localization in control and MFN1-KD 8-cell embryos (left panel). 8-cell embryos were immunostained with anti-MFN1 and anti-PADI6 antibodies and imaged by confocal microscope with identical settings and parameters. Scale bar, 25 μ m. The fluorescence intensity of MFN1 and PADI6 signals was recorded in control (n = 29) and MFN1-KD (n = 28) 8-cell embryos (right panel).

d Representative images of OOEP in control and MFN1-KD 8-cells embryos (upper panel). Scale bar, 25 μ m. The fluorescence intensity of OOEP signals was measured in control (n = 25) and MFN1-KD (n = 28) 8-cell embryos (lower panel).

e Representative images of TLE6 in control and MFN1-KD 8-cells embryos (upper panel). Scale bar, 25 μ m. The fluorescence intensity of TLE6 signals was recorded in control (n = 22) and MFN1-KD (n = 28) 8-cell embryos (lower panel).

f Representative images of RPL7 in control and MFN1-KD 8-cells embryos (upper panel). Scale bar, 25 μ m. The fluorescence intensity of RPL7 signals was measured in control (n = 24) and MFN1-KD (n = 19) 8-cell embryos (lower panel).

Data of (**a-f**) were presented as mean \pm SEM of at least three independent experiments. P value was performed by two-tailed Student's t test.

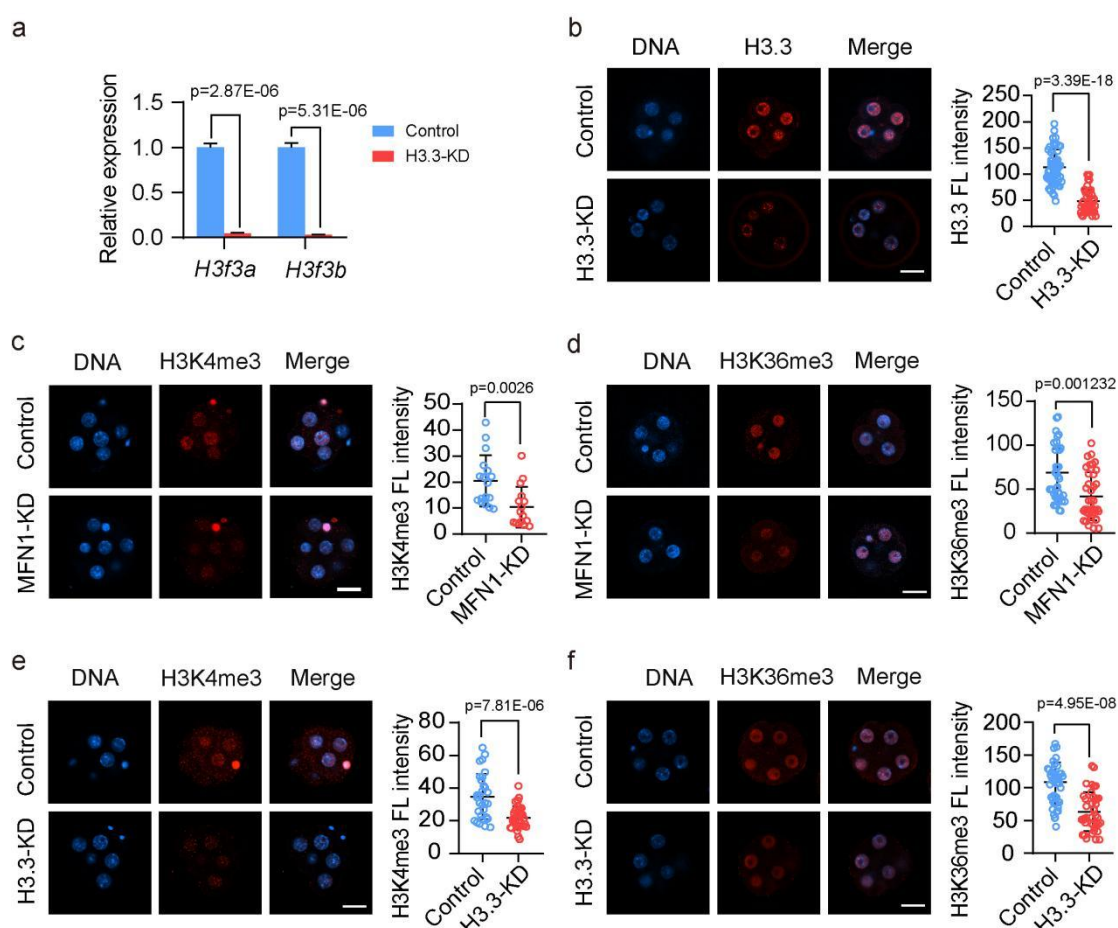


Fig. S7 The effect of MFN1 or H3.3 knockdown on histone modifications in 8-cell embryos.

a, b The depletion efficiency of H3.3 at the levels of mRNA (**a**) and protein as shown with immunostaining (**b**) in 8-cell embryos. Scale bar, 25 μ m. In (**b**), control (n = 54)

and H3.3-KD (n = 43).

c Immunostaining images (left panel) and quantification signals (right panel) of H3K4me3 for 8-cell embryos from control (n = 17) and MFN1-KD (n = 16) groups. Scale bar, 25 μ m.

d Immunostaining images (left panel) and quantification signals (right panel) of H3K36me3 for mouse 8-cell embryos from control (n = 39) and MFN1-KD (n = 39) groups. Scale bar, 25 μ m.

e Immunostaining images (left panel) and quantification signals (right panel) of H3K4me3 for mouse 8-cell embryos from control (n = 30) and H3.3-KD (n = 36) groups. Scale bar, 25 μ m.

f Immunostaining images (left panel) and quantification signals (right panel) of H3K36me3 for mouse 8-cell embryos from control (n = 45) and H3.3-KD (n = 40) groups. Scale bar, 25 μ m.

Data of (a-f) were presented as mean \pm SEM of at least three independent experiments. P value was performed by two-tailed Student's t test.

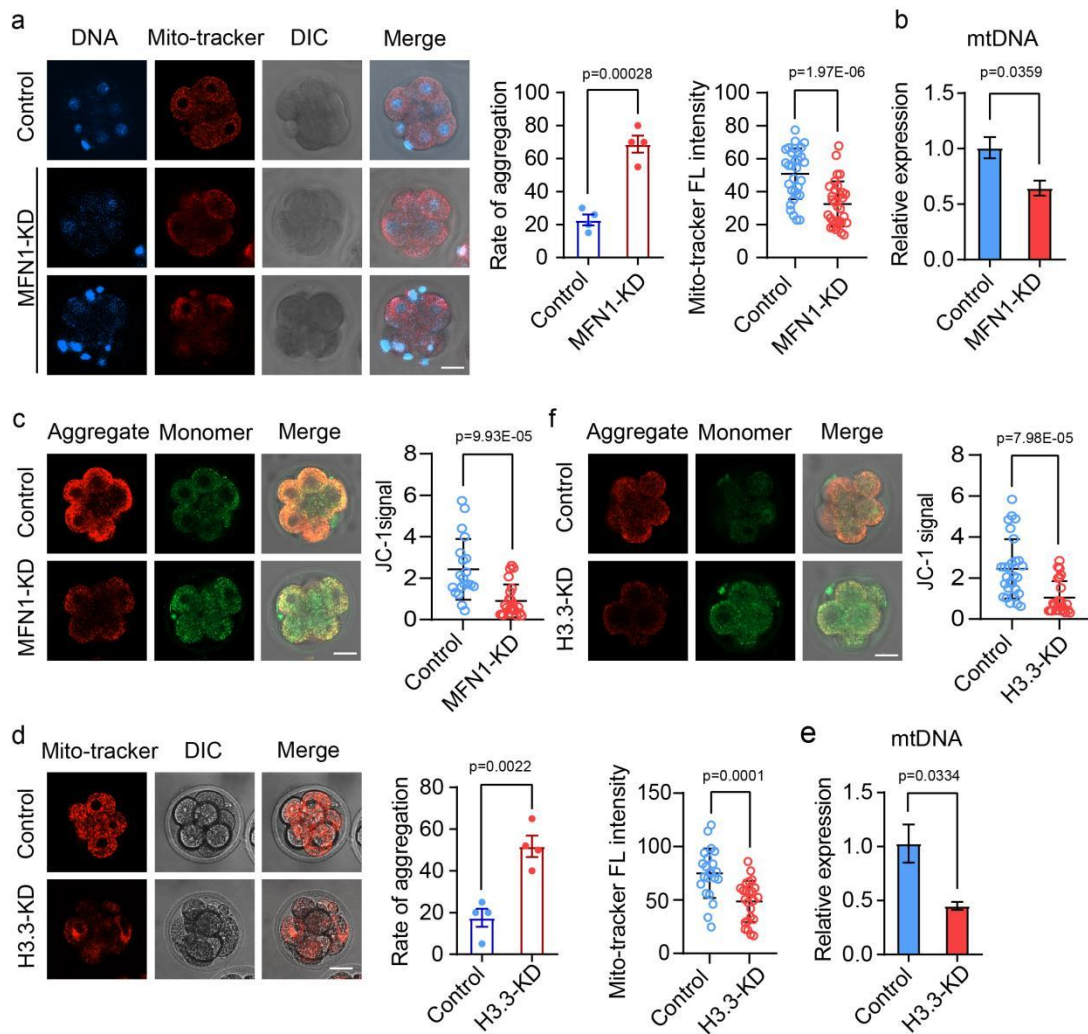


Fig. S8 Effects MFN1 and H3.3 knockdown on mitochondrial distribution and function in 8-cell embryos.

a Representative images of mitochondrial localization in control and MFN1-KD

8-cell embryos (left panel). Scale bar, 25 μ m. The aggregation rate of mitochondrial distribution and the fluorescence intensity of Mito-tracker signals was measured in control (n = 31) and MFN1-KD (n = 31) 8-cell embryos (right panel).

b The mtDNA levels were measured in control and MFN1-KD 8-cell embryos. n = 4 biological replicates.

c MMP was detected by JC-1 staining (left panel) in control and MFN1-KD 8-cell embryos (Red, high membrane potential; green, low membrane potential). Scale bar, 25 μ m. The ratio of red and green fluorescence intensity was calculated in control (n = 20) and MFN1-KD (n = 23) 8-cell embryos (right panel).

d Representative images of mitochondrial localization in control and H3.3-KD 8-cell embryos (left panel). Scale bar, 25 μ m. The aggregation rate of mitochondrial distribution and the fluorescence intensity of Mito-tracker signals was measured in control (n = 22) and H3.3-KD (n = 24) 8-cell embryos (right panel).

e The mtDNA levels were measured in control and H3.3-KD 8-cell embryos. n = 3 biological replicates.

f MMP was detected by JC-1 staining (left panel) in control and H3.3-KD 8-cell embryos. Scale bar, 25 μ m. The ratio of red and green fluorescence intensity was calculated in control (n = 28) and H3.3-KD (n = 25) 8-cell embryos (right panel).

Data of (a-f) were presented as mean \pm SEM of at least three independent experiments. P value was performed by two-tailed Student's t test.

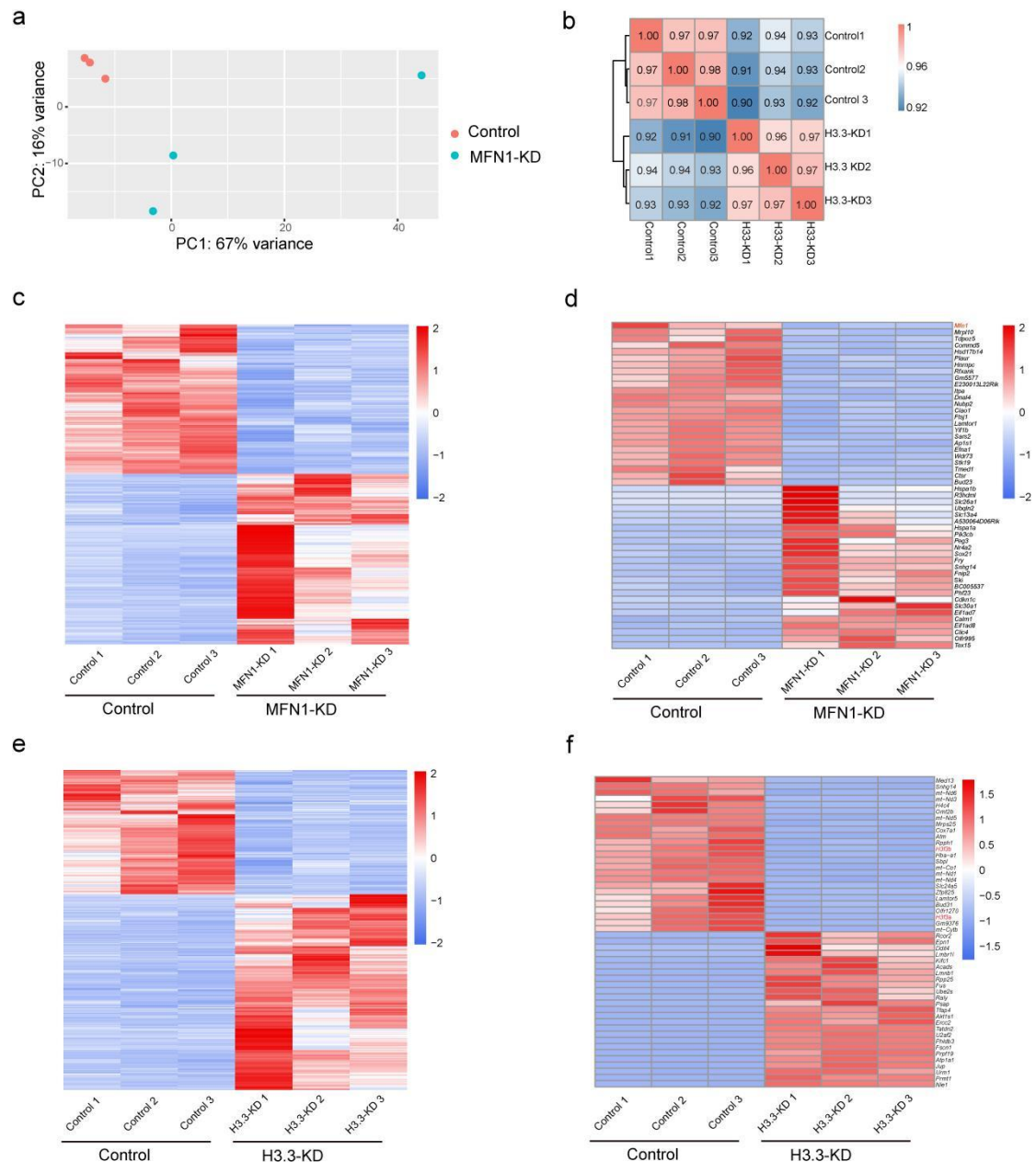


Fig. S9 Effects of MFN1 and H3.3 depletion on the transcriptome of 8-cell embryos.

a Principal Component Analysis (PCA) for transcriptomes of control and MFN1-KD 8-cell embryos.

b Heatmap of Pearson correlation coefficients for transcriptomes of control and H3.3-KD 8-cell embryos.

c Heatmap illustration displayed DEGs between control and MFN1-KD 8-cell embryos.

d Heatmap of top 50 DEGs of MFN1-KD 8-cell embryos by adjusted P values.

e Heatmap illustration showed DEGs between control and H3.3-KD 8-cell embryos.

f Heatmap of top 50 DEGs in H3.3-KD 8-cell embryos by adjusted P values.

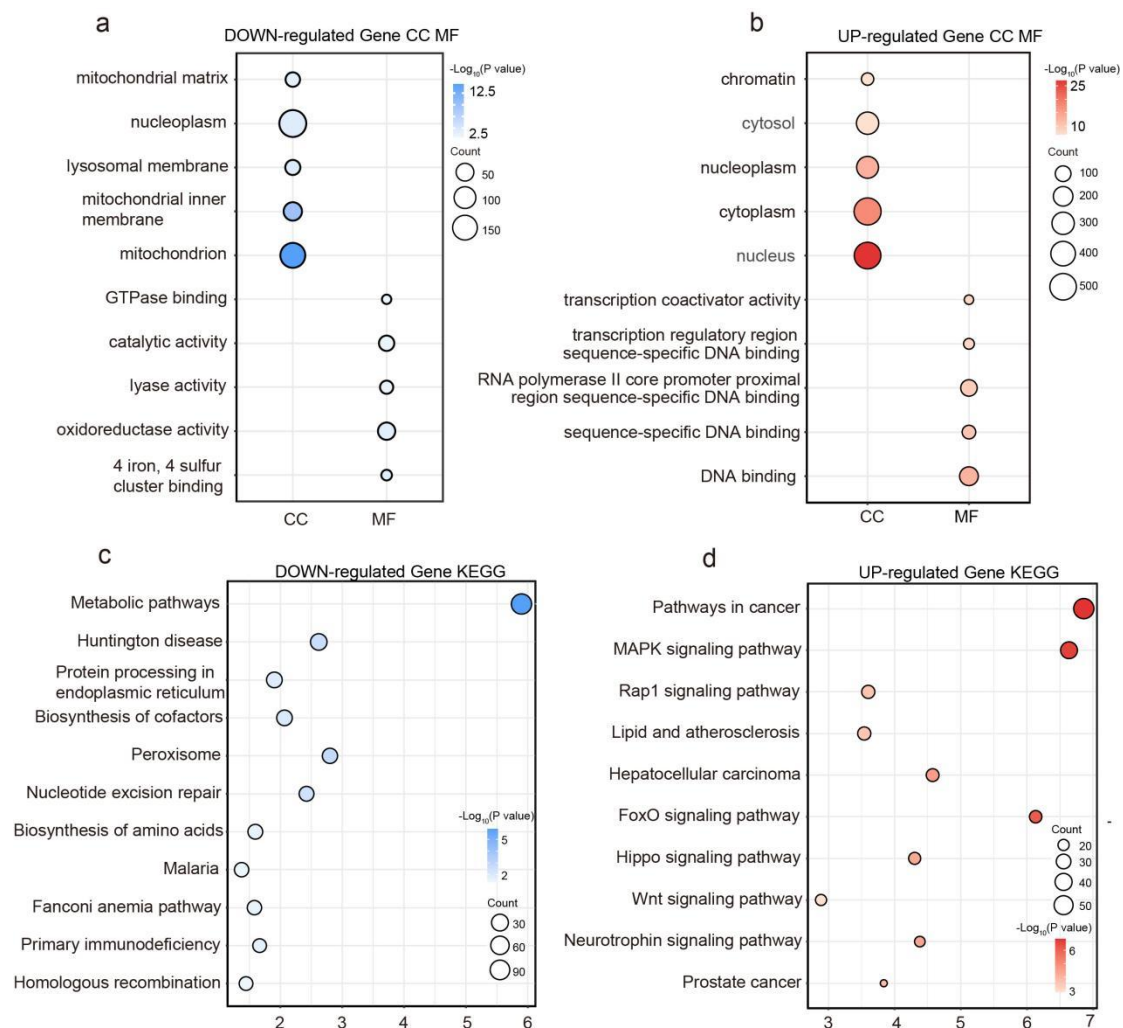


Fig. S10 Effect of MFN1 depletion on transcriptome of 8-cell embryos.

a-b GO enrichment analysis of downregulated (**a**) and (**b**) upregulated genes in MFN1-KD 8-cell embryos on cellular component and molecular function compared to control.

c-d KEGG enrichment analysis of downregulated (**c**) and upregulated (**d**) genes in MFN1-KD 8-cell embryos on cellular component and molecular function compared to control.

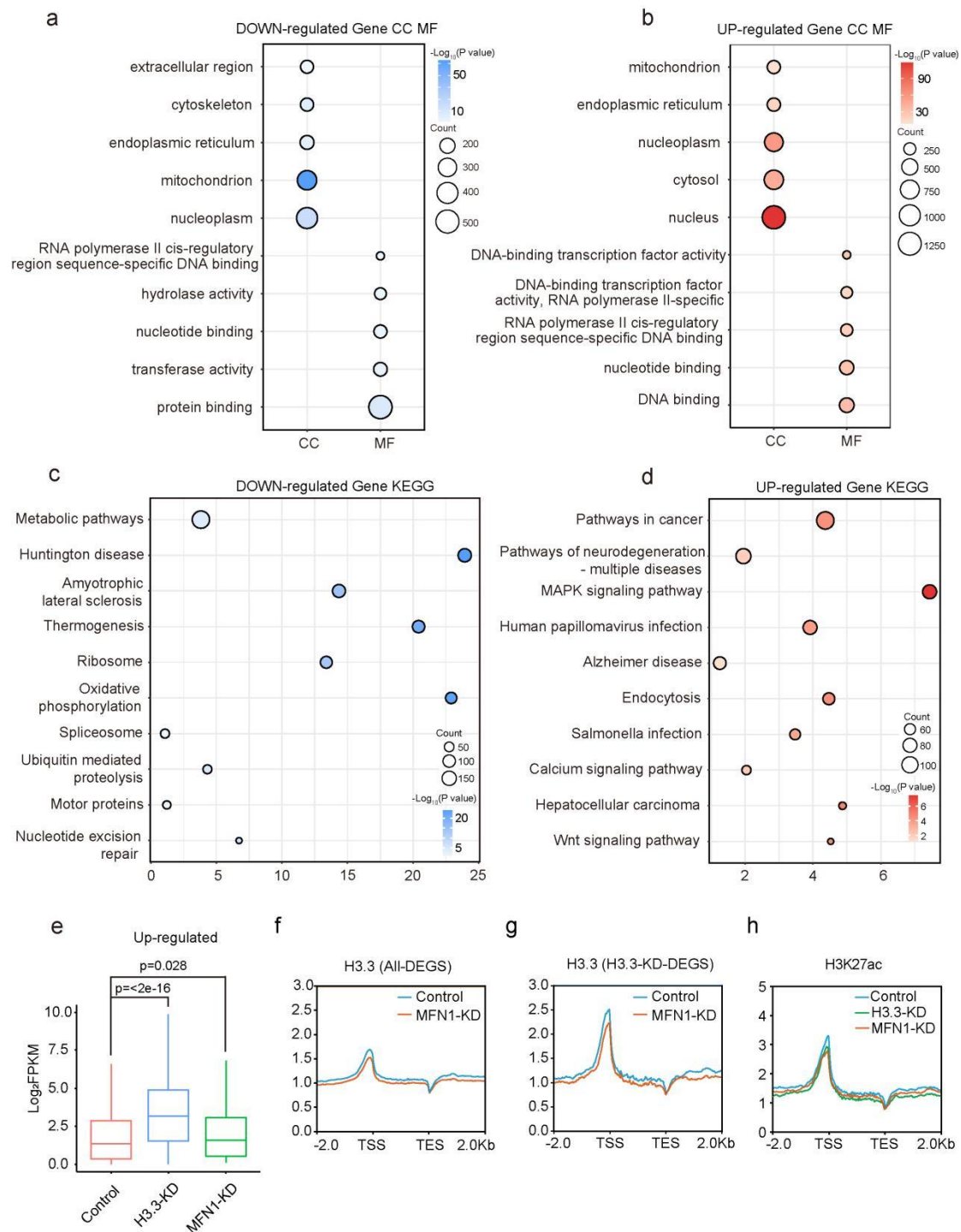


Fig. S11 Effect of H3.3 depletion on transcriptome of 8-cell embryos.

a-b GO enrichment analysis of downregulated (**a**) and (**b**) upregulated genes in H3.3-KD 8-cell embryos on cellular component and molecular function compared to control.

c-d KEGG enrichment analysis of downregulated (**c**) and upregulated (**d**) differentially expressed genes in H3.3-KD 8-cell embryos on cellular component and molecular function compared to control.

e Box plot of mRNA levels by RNA-seq depicting expression of upregulated genes by H3.3 knockdown in H3.3-KD and MFN1-KD 8-cell embryos. Mann-Whitney U test

was used to calculate P values.

f Density plot demonstrated the enrichment of H3.3 at 2 kb upstream/downstream of all genes in control and MFN1-KD 8-cell embryos.

g Density plot demonstrated the enrichment of H3.3 at 2 kb upstream/downstream of all DEGs by H3.3 depletion in control and MFN1-KD 8-cell embryos.

h Density plot of H3K27ac at 2 kb upstream/downstream of all genes in control, H3.3-KD and MFN1-KD 8-cell embryos.

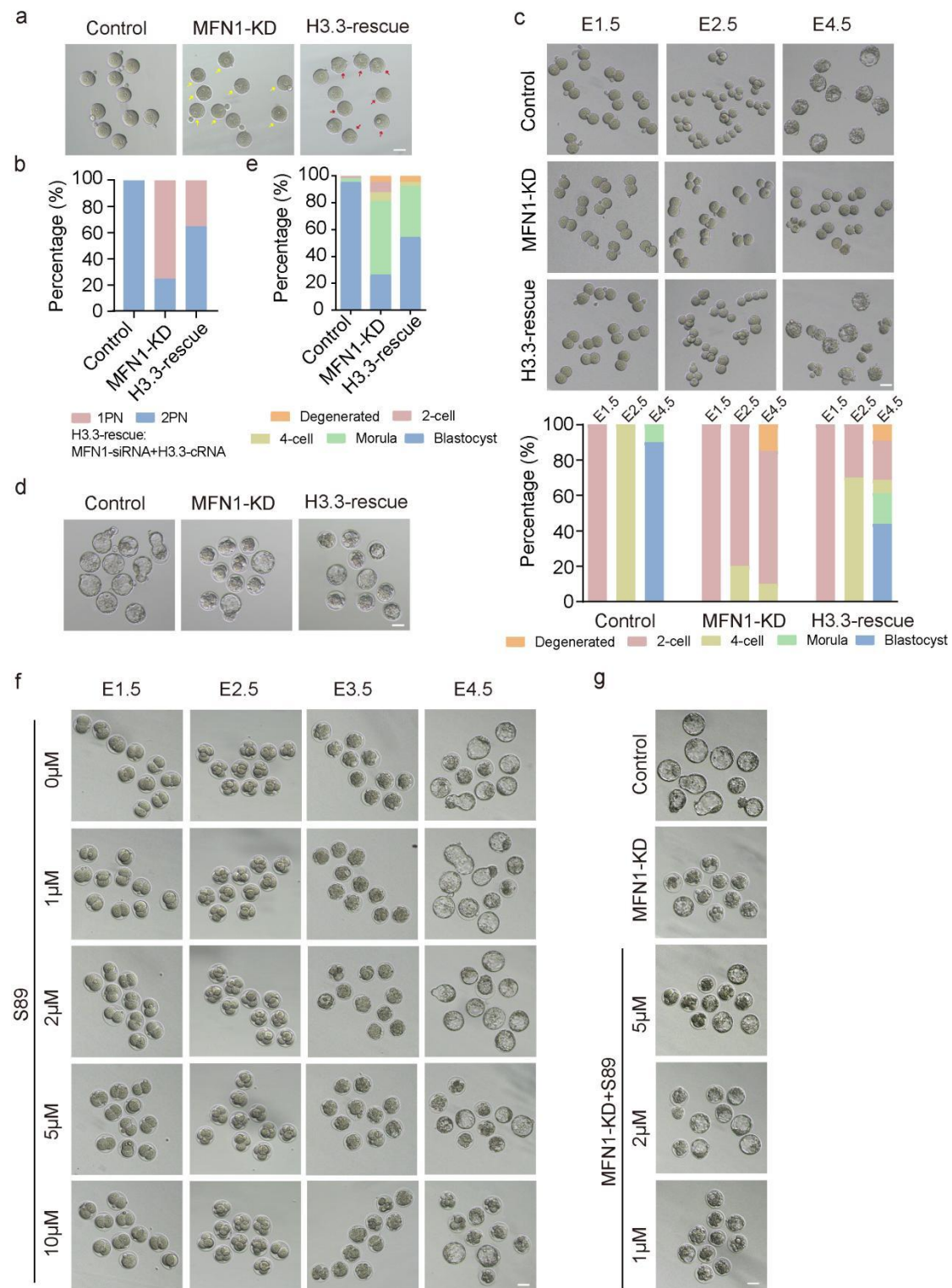


Fig. S12 Exogenous H3.3 and S89 supplementation exert rescuing effect on early embryonic developmental defects.

a-b Representative images (**a**) and stacked bar plots at the zygotes (**b**) of PN formation after fertilization in control oocytes (n = 96), MFN1-KD oocytes (n = 103), and co-injection of H3.3 cRNA into the MFN1-depleted oocytes (H3.3-rescue oocytes) (n = 105). GV oocytes from control group, MFN1-KD group, and H3.3-rescue group were cultured in M16 medium for *in vitro* maturation. Subsequently, matured oocytes

were selected for IVF, and the PN formation were analyzed. Compared with the MFN1-KD group, the proportion of 2PN formation in the H3.3-rescue group was significantly increased. Scale bar, 80 μ m.

c Representative images (upper panel) and rates (lower bar graph) of early embryos at the indicated time points after fertilization in control (n = 80), MFN1-KD (n = 64), and H3.3-rescue groups (n = 66). Generally, MII oocytes were injected with control siRNA or siRNA against *Mfn1* before IVF. For the rescue experiment, H3.3 cRNA was co-injected with siRNA against *Mfn1*. Then, the developmental progression of early embryos in control, MFN1-KD, and H3.3-rescue groups was determined by recording the rate of early embryos at specific time points. The blastocyst rate in the H3.3-rescue group showed partial restoration. Scale bar, 80 μ m.

d-e Representative images (d) and stacked bar plots (e) of blastocyst formation in control embryos (n = 107), MFN1-KD embryos (n = 112), and H3.3-rescue embryos (n = 110). Generally, after IVF, collected zygotes were injected with control siRNA or siRNA against *Mfn1*, followed by culturing in KSOM medium for early development examination. For the rescue experiment, H3.3 cRNA was co-injected with siRNA against *Mfn1*. The developmental progression of early embryos in control, MFN1-KD, and H3.3-rescue groups was determined by recording the rate of blastocyst at E4.5. The blastocyst rate in the H3.3-rescue group showed partial restoration. Scale bar, 80 μ m.

f Representative images showing the developmental progression of early embryos with different concentrations of S89 (0 μ M, 1 μ M, 2 μ M, 5 μ M, and 10 μ M) at each time point. Scale bar, 80 μ m. 0 μ M, n = 80. 1 μ M, n = 80. 2 μ M, n = 90. 5 μ M, n = 90. and 10 μ M, n = 85.

g Representative images of blastocyst formation in the control group, MFN1-KD group, and S89-rescue group with different concentrations (0 μ M, 1 μ M, 2 μ M, and 5 μ M). Scale bar, 80 μ m. Control, n = 90. MFN1-KD, n = 88. 0 μ M, n = 93. 1 μ M, n = 86. 2 μ M, n = 95. 5 μ M, n = 87.

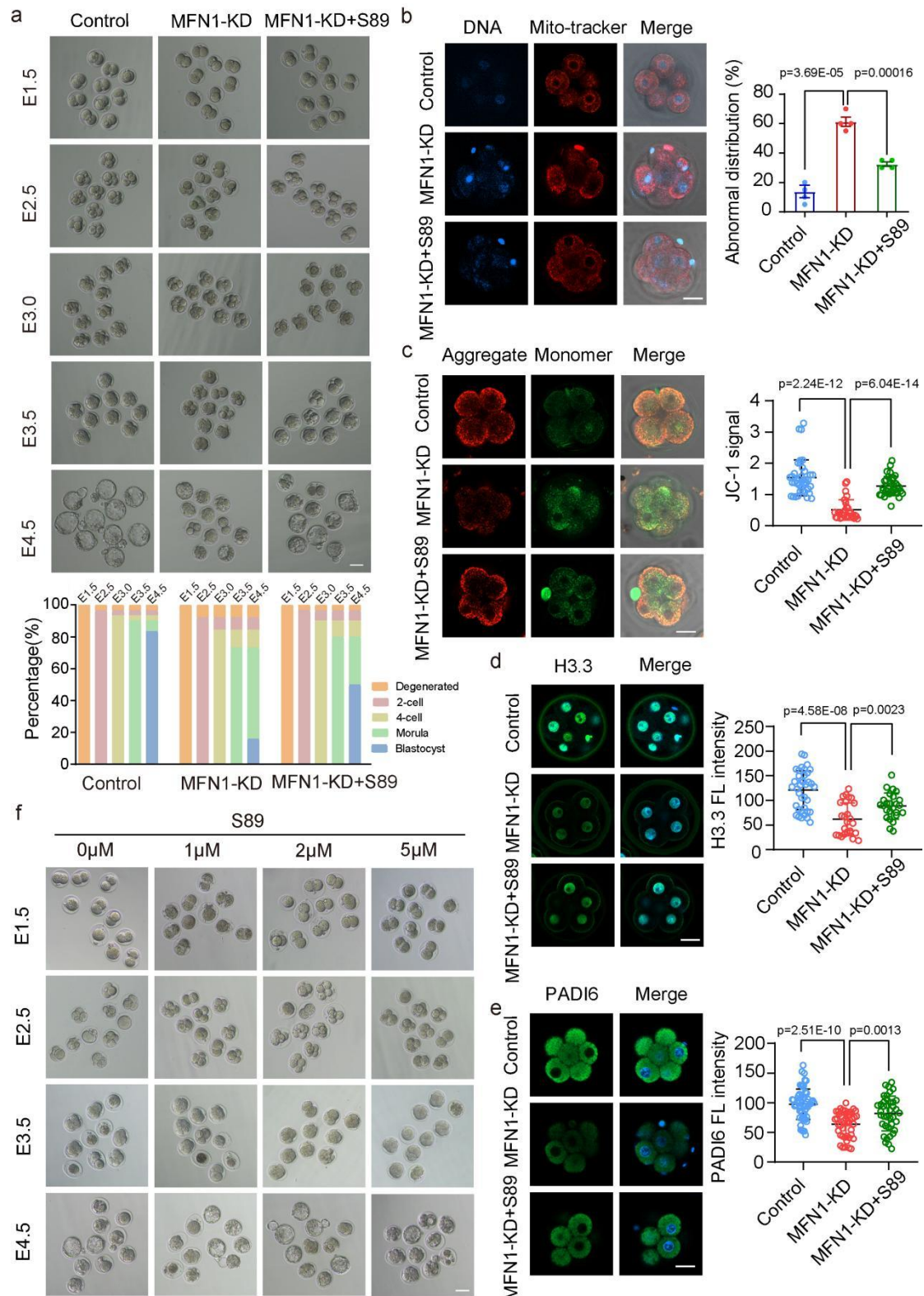


Fig. S13 Effects of S89 supplementation on early embryo development from MFN1-KD embryos and maternally aged embryos.

a Representative images (upper panel) showing the development of control ($n = 90$), MFN1-KD ($n = 90$) and MFN1-KD with S89 supplementation ($n = 90$) embryos at each time point. Scale bar, 80 μ m. Quantification (lower panel) of the developmental rate at the indicated time points from at least three independent experiments.

b Mitochondrial distribution (left panel) and aggregation ratio (right panel) in 8-cell embryos from control (n = 53), MFN1-KD (n = 52) and MFN1-KD with S89 supplementation (n = 52) groups. Scale bar, 25 μ m.

c JC1 staining (left panel) in control (n = 39), MFN1-KD (n = 29) and MFN1-KD with S89 supplementation (n = 35) 8-cell embryos to detect MMP. MMP level was indicated by red/green ratio (right panel). Scale bar, 25 μ m.

d Immunostaining (left panel) and fluorescence intensities (right panel) of H3.3 in control (n = 37), MFN1-KD (n = 26) and MFN1-KD with S89 supplementation (n = 25) 8-cell embryos. Scale bar, 25 μ m.

e Immunofluorescence staining (left panel) and fluorescence intensities (right panel) of PADI6 in control (n = 52), MFN1-KD (n = 55) and MFN1-KD with S89 supplementation (n = 58) 8-cell embryos. Scale bar, 25 μ m.

f Representative images illustrate the development of early embryos from aged female mice treated with different concentrations (0 μ M, 1 μ M, 2 μ M, and 5 μ M) of S89 at each time-point. 0 μ M, n = 63. 1 μ M, n = 65. 2 μ M, n = 72. 5 μ M, n = 69. Scale bar, 80 μ m.

Data of (**b-e**) were presented as mean \pm SEM from at least three independent experiments. P value was calculated by two-tailed Student's t-test.

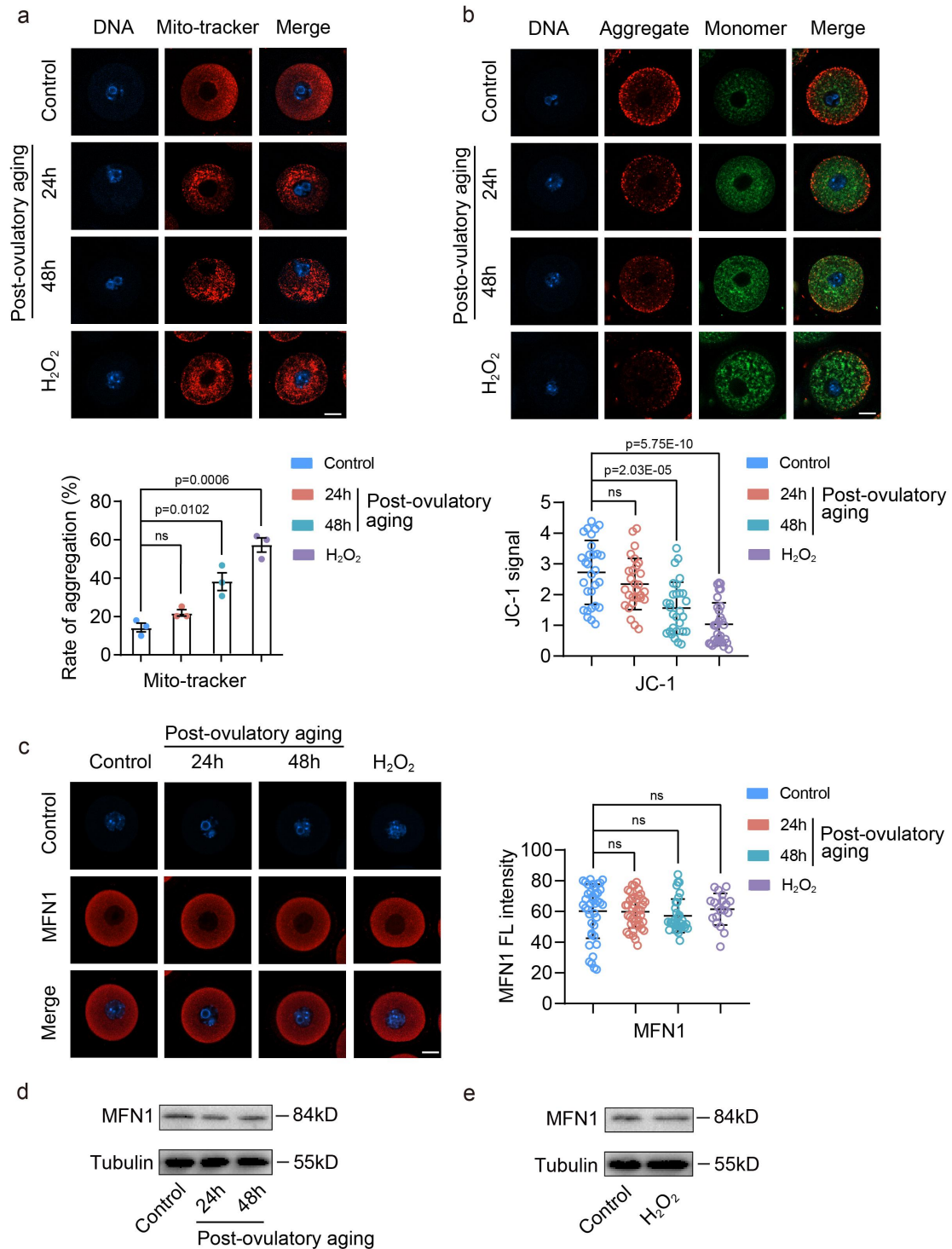


Fig. S14 The impact of *in vitro* simulated aging conditions on mitochondrial integrity and MFN1 proteins in mouse oocytes.

a Mitochondrial distribution (upper panel) and aggregation ratio (lower panel) in GV oocytes from control (n = 50), 24h of post-ovulatory aged (n = 48), 48h of post-ovulatory aged (n = 50) and H₂O₂ treated (n = 55) groups. Generally, GV oocytes were maintained for 24h or 48h in 2.5 μ M milrinone for post-ovulatory aging treatment. For the H₂O₂-treated experiments, GV oocytes were cultured in 100 μ M H₂O₂ for 30 minutes. Scale bar, 20 μ m.

b JC1 staining (upper panel) in GV oocytes from control (n = 58), 24h of post-ovulatory aged (n = 55), 48h of post-ovulatory aged (n = 56) and H₂O₂-treated (n = 61) groups to detect MMP. MMP level was indicated by red/green ratio (lower panel). Scale bar, 20μm.

c Immunostaining (left panel) and fluorescence intensities (right panel) of MFN1 in control (n = 78), 24h of post-ovulatory aged (n = 85), 48h of post-ovulatory aged (n = 82) and H₂O₂-treated (n = 94) GV oocytes. Scale bar, 20 μm.

d Western blot comparing MFN1 protein levels in control and post-ovulatory aged GV oocytes. Tubulin was used as the internal control.

e Western blot comparing MFN1 protein levels in control and H₂O₂-treated GV oocytes. Tubulin was used as the internal control.

Data of (a, b, c) were presented as mean±SEM from at least three independent experiments. P value was calculated by two-tailed Student's t-test.

Supplementary Information

Table S1. The primer sequences used in this study

Primer	Sequence (5'-3')
<i>pVax1-IVT-F</i>	CGTGTACGGTGGGAGGTCTA
<i>pVax1-IVT-R</i>	TTCGCTTGCTGTCCATAAAA
<i>Zscan4-F</i>	GAGATTCATGGAGAGTCTGACTGA
<i>Zscan4-R</i>	GCTGTTGTTTCAAAAGCTTGATGACTTC
<i>Zfp352-F</i>	ACCACCTCAAAGAACACCAG
<i>Zfp352-R</i>	ACAAGGGACAAGCGTAGAAC
<i>MERV-L-F</i>	ATCTCCTGGCACCTGGTATG
<i>MERV-L-R</i>	AGAAGAAGGCATTTGCCAGA
<i>Tcstv1-F</i>	GGATCCCTGAAGGTAAATCCTC
<i>Tcstv1-R</i>	AACCATCCATCCTCAGGAAC
<i>Tdp4-F</i>	ACCCAAGACCTGCAATCAAG
<i>Tdp4-R</i>	ATTCATGGCCAGCTACCAAC
<i>Eifla-F</i>	CCAAAGAATAAAGGCAAAGGAG
<i>Eifla-R</i>	CTCACACCGTCAAAGCACATT
<i>Gapdh-F</i>	TCTTCCAGGAGCGAGACCC
<i>Gapdh-R</i>	CGGAGATGATGACCCTTTT
<i>5.8S F</i>	CTTAGCGGTGGATCACTCGG
<i>5.8S R</i>	ACGCTCAGACAGGCGTAGCC
<i>28S F</i>	CTGTCCCTACCTACTATCCA
<i>28S R</i>	CTCCCACTTATTCTACACCT
<i>18S F</i>	CGGCTACCACATCCAAGGAA
<i>18S R</i>	GCTGGAATTACCGCGGCT
<i>ND1-F</i>	CTAGCAGAAACAAACCGGGC
<i>ND1-R</i>	CCGGCTGCGTATTCTACGTT
<i>β-globin-F</i>	GAAGCGATTCTAGGGAGCAG
<i>β-globin-R</i>	GGAGCAGCGATTCTGAGTAGA

F: forward, R: reverse.

Table S2. siRNA sequences against specific genes used in this study.

Genes	Sequence (5'-3')
<i>Mfn1</i> 1s	CCUUGAUGCUGAUGUCUUUTT
<i>Mfn1</i> 1as	AAAGACAUCAGCAUCAAGGTT
<i>Mfn1</i> 2s	GCAGAAGGAUUUCAAGCAATT
<i>Mfn1</i> 2as	UUGCUUGAAAUCCUUCUGCTT
<i>H3f3a</i> 1s	CGUUCAUUUGUGUGUGAAUUUUU
<i>H3f3a</i> 1as	AAAUUCACACACAAAUGAACGUU
<i>H3f3a</i> 2s	GCGAGAAAUUGCUCAGGACUUUU
<i>H3f3a</i> 2as	AAGUCCUGAGCAAUUUCUCGCUU
<i>H3f3b</i> 1s	UCUGAGAGAGAUCCGUCGUUAUU
<i>H3f3b</i> 1as	UAACGACGGAUCUCUCUCAGAUU
<i>H3f3b</i> 2s	GAAGCUGCCAUUCCAGAGAUUUU
<i>H3f3b</i> 2as	AAUCUCUGGAAUGGCAGCUUCUU
<i>Neg</i> s	UUC UCC GAA CGU GUC ACG UUU
<i>Neg</i> as	ACG UGA CAC GUU CGG AGA AUU

H3.3: H3.3A (*H3f3a*), H3.3B (*H3f3b*); *Neg*: negative control siRNA sequences

Table S3. Information on antibodies used in this study.

Antibodies	Vendor	Catalog number
Anti-MFN1 antibody	Proteintech	13798-1-AP
Anti-H3.3 antibody	Active motif	91191
Anti-H3.3S31p antibody	Active Motif	39637
Anti-RNA Pol II antibody	Active Motif	39097
Anti-Ser2p antibody	Abcam	ab193468
Anti-H4 antibody	Abclonal	A23000
Anti-PADI6 antibody	LSBio	LS-C695606
Anti-TLE6 antibody	Abmart	M034864
Anti-OOEP antibody ¹	N/A	N/A
Phalloidin-FITC (F-actin)	Sigma-Aldrich	P5282
Anti-RPL7 antibody	Proteintech	14583-1-AP
Anti-RPL3 antibody	Proteintech	11005-1-AP
Anti-H3K27me3 antibody	Diagenode	C15410069
Anti-H3K4me3 antibody	CST	9751S
Anti-H3K27ac antibody	Abcam	ab177178
Anti-H3K9ac antibody	Abcam	ab32129
Anti-H3K36me3 antibody	Abclonal	A2366
Anti-Lamin B1 antibody	Santa Cruz	sc-30264
Goat anti-mouse IgG (H+L)	Yeasten	33912ES60
Goat anti-rabbit IgG (H+L)	Yeasten	33112ES60
Anti-rabbit IgG for IP (HRP)	Vazyme	RA1008
Anti-mouse IgG for IP (HRP)	Vazyme	RA1009

Reference

1. Li L, Baibakov B, Dean J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev Cell* 2008, **15**(3): 416-425.