Appendix figure legends

Appendix Figure S1. Hypoxia-inducible factor 1 and its target NIX are induced in response to hypoxia

HeLa cells were exposed to hypoxia (1% O$_2$) for the indicated times. NIX, HIF1 and Actin were detected by the corresponding antibodies.

Appendix Figure S2. FUNDC1 or DRP1 puncta are closely associated with lacunae formed by ER lattices in hypoxic conditions

A. MEFs were transfected by Cherry-ER for 24 h and exposed to hypoxia (1% O$_2$) for 5 h or not. Cells were immunostained by anti-TOM20 and anti-FUNDC1. Bar = 10 µm.

B. MEFs were transfected by Cherry-ER for 24 h and exposed to hypoxia (1% O$_2$) for 5 h or not. Cells were immunostained by anti-TOM20 and anti-DRP1. Bar = 10 µm.

Appendix Figure S3. Identification of the Calnexin binding site in FUNDC1

A. HeLa cells were transfected for 24 h by constructs expressing various deletions of FUNDC1 in the AA 96-138 region. Cell lysates were analyzed by immunoprecipitation using anti-Calnexin antibody and immunoblotted with anti-MYC and anti-Calnexin antibodies.

B. HeLa cells were transfected for 24 h by vector or constructs expressing various mutants of FUNDC1 in the AA 129-138 region. Cell lysates were analyzed by immunoprecipitation using anti-Calnexin antibody and immunoblotted with anti-MYC and anti-Calnexin antibodies.

Appendix Figure S4. The interaction of different truncated CNX with FUNDC1
HeLa cells were co-transfected by FLAG-FUNDC1 and different truncated MYC-CNIX or empty vector. About 24h, cell were harvested and lysed, and then analyzed by immunoprecipitation using anti-FLAG and immunoblotted with anti-MYC and anti-FLAG antibodies.

**Appendix Figure S5. Time course of Calnexin, DRP1 and FUNDC1 distribution in different subcellular fractions under hypoxia**

Immunoblots of subcellular fractions from HeLa cells exposed to hypoxia (1% O₂) for the indicated times. Different proteins were immunoblotted with the corresponding antibodies. PNS: post-nuclear supernatant; CYTO: cytosol; ER: endoplasmic reticulum; MITO: mitochondria; MAM: mitochondrial-associated membrane.

**Appendix Figure S6. Time course of the FUNDC1 binding with DRP1 and CNX**

HeLa cells were exposed to hypoxia (1% O₂) for the indicated times and cell lysates were immunoprecipitated using anti-FUNDC1 antibody. Endogenous DRP1, Calnexin and FUNDC1 were detected by anti-DRP1, anti-Calnexin and anti-FUNDC1 antibodies. The bar charts show the density ratio for the indicated WB bands in the upper panel.

**Appendix Figure S7. The interaction of different truncated FUNDC1 with LC3**

HeLa cells were transfected with different truncated FUNDC1-MYC or empty vector for 24 h. Cell lysates were immunoprecipitated by anti-LC3 antibody and immunoblotted with anti-MYC and anti-LC3 antibodies.
Appendix Figure S8. Reconstitution of CNX in CNX-depleted cells restores mitochondrial fission under hypoxia

CNX-KD HeLa cells were transfected by empty vector or MYC-CNX. About 24 h post-transfection, cells were exposed to hypoxia (1% O$_2$) for 12 h and then immunostained by anti-TOM20. Bar = 25 µm.

Appendix Figure S9. DRP1 has no effect on the FUNDC1 accumulation at MAM under Hypoxia

HeLa cells were transfected using si-DRP1 or Scramble RNAs. After 48h, cells were exposed to Hypoxia for 5h, and then subcellular fraction were isolated using gradient centrifugation. PNS: post-nuclear supernatant; CYTO: cytosol; ER: endoplasmic reticulum; MITO: mitochondria; MAM: mitochondrial-associated membranes.

Appendix Figure S10. ER morphology is not affected by the loss of CNX or FUNDC1 under normoxia

HeLa cells were transfected by scramble RNA, si-CNX or si-FUNDC1 for 36 h, then immunostained by anti-TOM20 and ER-Tracker. Boxed regions are shown enlarged in the “Zoom” panels. Bar = 20 µm.

Appendix Figure S11. Calnexin or FUNDC1 depletion does not affect mitochondrial morphology under normoxic conditions
HeLa cells were transfected by scramble RNA, si-CNX or si-FUND1C1 for 36 h, then immunostained by anti-TOM20. Bar = 20 µm. The bar chart shows the proportion of cells with elongated, intermediate or fragmented mitochondria.

Appendix Figure S12. Colocalization of mitochondria and LC3 is impaired in MFN1/2 double KO and FUNDC1 KD cells under hypoxia

MFN1/2 double KO (DKO) MEF cells were transfected by scramble RNA or si-FUND1C1 for 24 h, and then cells were exposed to hypoxia or not for 12h. Cells were then immunostained by anti-TOM20 and anti-LC3. Bar = 10 µm.

Appendix Figure S13. The knock down efficiency of si-RNA

A. The knockdown efficiency of si-RNA in FIG.3

B. The knockdown efficiency of si-RNA in FIG.4

C. The knockdown efficiency of si-RNA in FIG.6
Appendix Figure S1. Hypoxia-inducible factor 1 and its target NIX are induced in response to hypoxia
Appendix Figure S2. FUNDC1 or DRP1 puncta are closely associated with lacunae formed by ER lattices in hypoxic conditions

A

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Normoxia

Hypoxia 5h

B

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Normoxia

Hypoxia 5h
Appendix Figure S3. Identification of the Calnexin binding site in FUNDC1

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Calnexin

Myc-FUNDC1
Appendix Figure S4. The interaction of different truncated CNX with FUNDC1

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- FLAG-FUNDC1
- MYC-CNX-FL
- MYC-CNX-N
- MYC-CNX-C
- MYC
Appendix Figure S5. Time course of Calnexin, DRP1 and FUNDC1 distribution in different subcellular fractions under hypoxia

0 1 3 6 12 h Hypoxia

PNS

MFN2
MID49
FIS1
Calreticulin
FUNDC1
DRP1
MID51
CNX
VDAC1
MFF
TOM20
FACL4
Actin

0 1 3 6 12 h Hypoxia

ER

MFN2
DRP1
CNX
Calreticulin

0 1 3 6 12 h Hypoxia

MITO

FIS1
MFN2
MID51
FUNDC1
MID49
DRP1
MFF
VDAC1
TOM20

0 1 3 6 12 h Hypoxia

MAM

FUNDC1
DRP1
MFF
CNX
VDAC1
FACL4
Appendix Figure S6. Time course of the FUNDC1 binding with DRP1 and CNX

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h Hypoxia

Endo-Drp1
Endo-CNX
Endo-FUNDC1

Ratio of density

- 0h
- 1h
- 3h
- 6h
Appendix Figure S7. The interaction of different truncated FUNDC1 with LC3

The figure illustrates the interaction of different truncated versions of FUNDC1 with LC3. The blot shows the expression of Light chain and LC3 under various KD concentrations (25, 15, 10) for different truncation points (FL, 1-138, 55-155, 1-80). The image includes bands for IB:MYC and IB:LC3, indicating the presence of MYC and LC3 proteins, respectively, after IP-LC3 and 1/20 Input treatments.
Appendix Figure S8. Reconstitution of CNX in CNX-depleted cells restores mitochondrial fission under hypoxia

Percentage of cells with mitochondrial fragmentation

45±8

88±12
Appendix Figure S9. DRP1 has no effect on the FUNDC1 accumulation at MAM under Hypoxia
Appendix Figure S10. ER morphology is not affected by the loss of CNX or FUNDC1 under normoxia.
Appendix Figure S11. Calnexin or FUNDC1 depletion does not affect mitochondrial morphology under normoxic conditions

Control  Si-CNX  Si-FUNDC1

TOM20

Mitochondrial morphology

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Appendix Figure S12. Colocalization of mitochondria and LC3 is impaired in MFN1/2 double KO and FUNDC1 KD cells under hypoxia

<table>
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<td>TOM20</td>
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**Normoxia**

**Sample Hypoxia 12h**

**Si-FUNDC1 Hypoxia 12h**
Appendix Figure S13. The knock down efficiency of si-RNA

A  Scr  Si-FDC1

Scr  Si-MFF

Scr  Si-FIS1

FDC1  MFF  FIS1

Actin  Actin  Actin

B  Scr  Si-FDC1

Scr  Si-MFF

Scr  Si-FIS1

Scr  Si-MID49

Scr  Si-MID51

FDC1  MFF  FIS1  Mid49  Mid51

Actin  Actin  Actin  Actin  Actin

C  Scr  Si-FDC1

Scr  Si-MFF

Scr  Si-FIS1

FDC1  MFF  FIS1

Actin  Actin  Actin