Expanded View Figures

Figure EV1. LINC00839 promotes the proliferation, migration, invasion, and EMT of CRC cells.

A Knockdown of LINC00839 was confirmed in SW620 and HCT116 cells by qPCR (mean ± SD, Student's *t*-test, $n = 3$ technical replicates).
B, C Cell proliferation was measured by CCK8 assay in LINC00839-knockdown SW620 and HCT116 cells (mean ± SD, ANOVA, $n = 3$ biological replicates).
D Colony formation was assessed in LINC00839-knockdown SW620 and HCT116 cells.
E Wound-healing assay was performed with LINC00839-knockdown cells and control cells. Scale bars, 100 μm.
F Matrigel invasion assay was performed with LINC00839-knockdown HCT116 and SW620 cells. Scale bars, 100 μm.
G The CDX model revealed the effect of LINC00839 on tumor growth. Representative image of tumors harvested from the mouse model (left panel), quantitative analysis of tumor weight and tumor volume (medium panel), and representative images and quantification of IHC staining of Ki-67 in tumor tissues (left panel; $n = 6$ for each cohort; the results are presented as the mean ± SD and were analyzed by Student's *t*-test (medium and right panel) and ANOVA (left panel)). Scale bars, 100 μm ($n = 6$ biological replicates).
H Cells overexpressing LINC00839 exhibited EMT-like cell morphology: most cells lost their epithelial characteristics and exhibited a spindle-shaped and fibroblast-like morphology. Scale bars, 10 μm.
I GSEA showed the enrichment of epithelial–mesenchymal transition (EMT) signatures in the high HCT116 and SW620 cells expression group (TCGA cohort).
J IF staining of E-cadherin and Vimentin in LINC00839-overexpressing and LINC00839-knockdown cells. Scale bars, 10 μm.
K Epithelial and mesenchymal marker expression in LINC00839-overexpressing and LINC00839-knockdown cells was measured by WB.
L Representative images of IHC staining of Vimentin and E-cadherin in tumors from the orthotopic implantation model. Scale bars, 200 μm.

Data information: In (D–F), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ biological replicates. Across experiments, the *P*-values are as follows: ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.
Source data are available online for this figure.

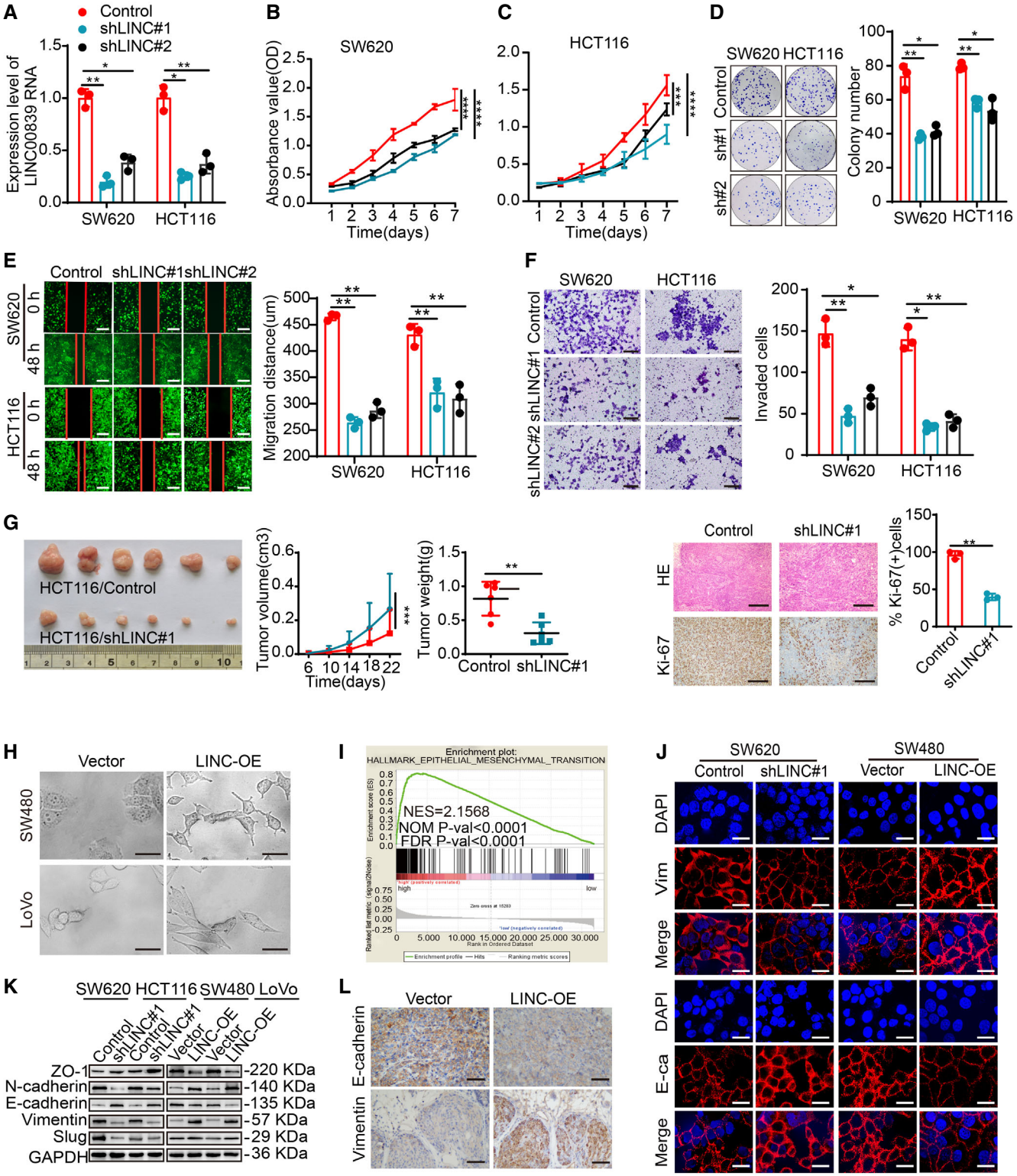
A
Control
shLINC#1
shLINC#2
Expression level of LINC00839 RNA
SW620
HCT116
B
SW620
Absorbance value(OD)
Time(days)
C
HCT116
Time(days)
D
SW620 HCT116
Control
sh#1
sh#2
Colony number
E
Control shLINC#1 shLINC#2
SW620
HCT116
0 h
48 h
Migration distance(um)
F
SW620
HCT116
Control
shLINC#1
shLINC#2
Invaded cells
G
HCT116/Control
HCT116/shLINC#1
Tumor volume(cm3)
Time(days)
Tumor weight(g)
Control shLINC#1
HE
Ki-67
% Ki-67(+)cells
H
Vector
LINC-OE
SW480
LoVo
I
Enrichment plot:
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
NES=2.1568
NOM P-val<0.0001
FDR P-val<0.0001
J
SW620
SW480
Control
shLINC#1
Vector
LINC-OE
DAPI
Vim
Merge
E-ca
K
SW620 HCT116 SW480 LoVo
ZO-1 -220 KDa
N-cadherin -140 KDa
E-cadherin -135 KDa
Vimentin -57 KDa
Slug -29 KDa
GAPDH -36 KDa
L
Vector
LINC-OE
E-cadherin
Vimentin

Figure EV1.

Figure EV2. LINC00839 interacts with the Ruvb1/Tip60 complex to promote OXHPOS and mitochondrial biogenesis.

A Ruvb1 was identified by LC–MS.
B The peak diagram of the centroid structure of LINC00839 was predicted by RNAfold.
C Pattern of the structure of LINC00839 and Ruvb1 and their binding.
D RNA pull-down and WB assays were performed to confirm the specific interaction between some large multiprotein complexes and LINC00839. Dot blot of RNA–protein binding samples indicating that equal amounts of RNA were used in the assay.
E The Ruvb1 knockdown efficiency was confirmed by WB.
F GO enrichment analysis of the DEGs identified in the RNA-seq.
G Glucose intake by LINC00839-overexpressing cells and LINC00839-KD cells was determined.
H Lactate secretion by LINC00839-overexpressing cells and LINC00839-KD cells was measured.
I, J The number of mitochondria in LINC00839-knockdown cells and LINC00839-overexpressing cells as confirmed by TEM. Scale bars, 500 nm.

Data information: In (G–J), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ biological replicates. Across experiments, the *P*-values are as follows: ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.
Source data are available online for this figure.

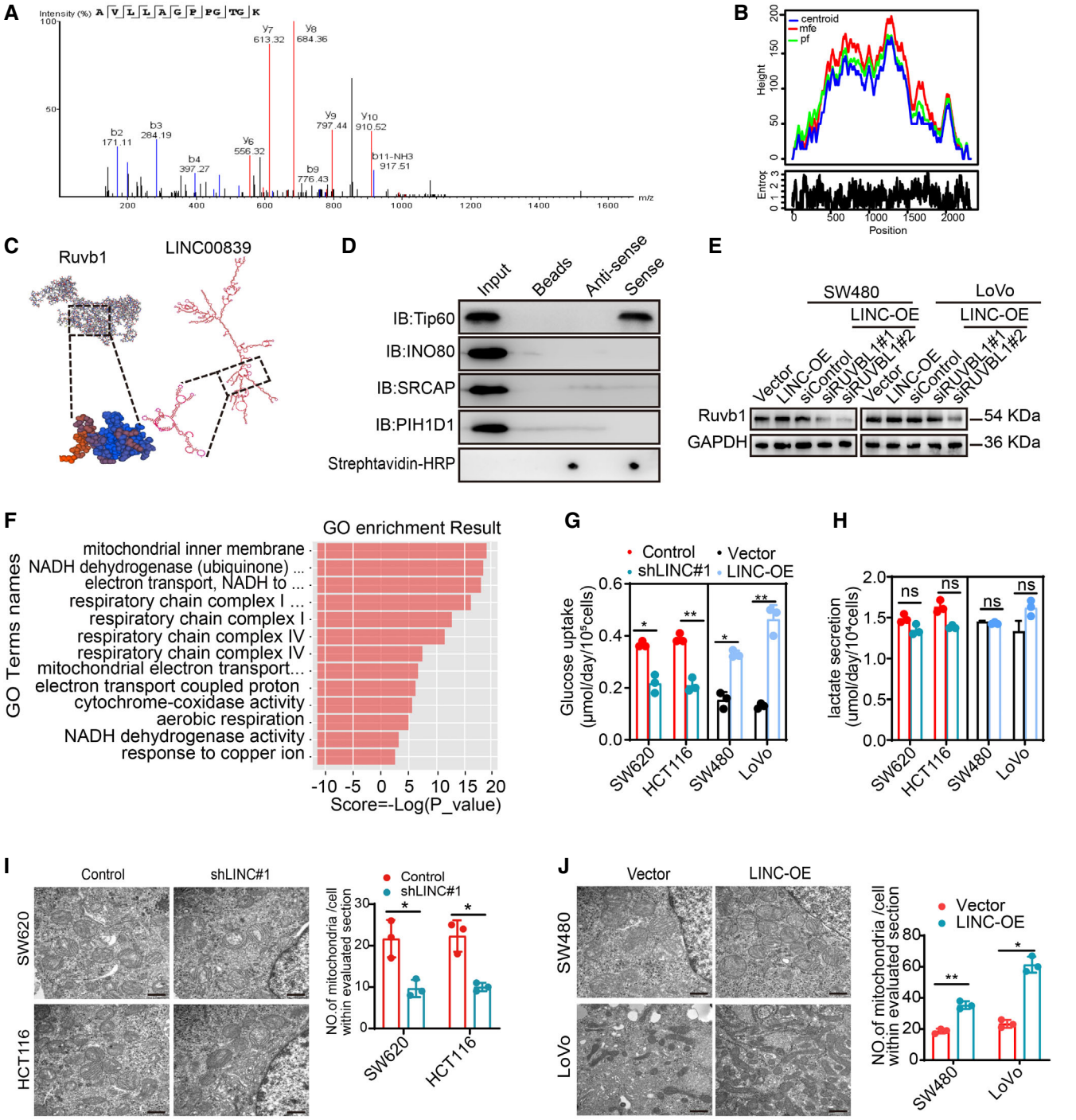
A
B
centroid
mfe
pf
Height
Position
C
Ruvb1
LINC00839
D
Input
Beads
Anti-sense
Sense
IB:Tip60
IB:INO80
IB:SRCAP
IB:PIH1D1
Strephtavidin-HRP
E
SW480
LoVo
LINC-OE
Vector
siControl
siRUVBL1#1
siRUVBL1#2
Ruvb1
GAPDH
54 KDa
36 KDa
F
GO enrichment Result
GO Terms names
mitochondrial inner membrane
NADH dehydrogenase (ubiquinone) ...
electron transport, NADH to ...
respiratory chain complex I ...
respiratory chain complex I
respiratory chain complex IV
respiratory chain complex IV
mitochondrial electron transport...
electron transport coupled proton
cytochrome-coxidase activity
aerobic respiration
NADH dehydrogenase activity
response to copper ion
Score=-Log(P_value)
G
Control
shLINC#1
Vector
LINC-OE
Glucose uptake (μmol/day/10⁵cells)
SW620
HCT116
SW480
LoVo
H
lactate secretion (umol/day/10⁴cells)
ns
I
Control
shLINC#1
SW620
HCT116
NO.of mitochondria /cell within evaluated section
J
Vector
LINC-OE
SW480
LoVo

Figure EV2.

Figure EV3. LINC00839 mediates the acetylation of the NRF1 promoter by the Ruvb1/Tip60 complex to regulate OXPHOS, mitochondrial biogenesis, and CRC progression.

A The pattern of 10 NRF1 promoter fragments (upper panel) and ChIP-seq uniform peaks in the NRF1 promoter region was analyzed by ENCODE projects and visualized by UCSC Genome Browser (bottom panel).

B–E The enrichment of the NRF1 promoter (P1-P10) by Tip60 (B), Ruvb1 (C), H4K12ac (D), and H3K14ac (E) antibodies in LINC00839-overexpressing and control LoVo cells as determined by CHIP assay. Normal IgG was used as a negative control.

F, G ATP levels and NAD/NADH ratios in SW480 and LoVo cells were measured.

H Expression of genes related to mitochondrial biogenesis and EMT in SW480 and LoVo cells as determined by WB.

I The number of mitochondria in SW480 and LoVo cells was confirmed by TEM. Scale bars, 500 nm.

J Invasion of SW480 and LoVo cells was assessed by Matrigel invasion assay. Scale bars, 100 μm.

Data information: In (B–G), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ technical replicates. In (I, J), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ biological replicates. Across experiments, the *P*-values are as follows: ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Source data are available online for this figure.

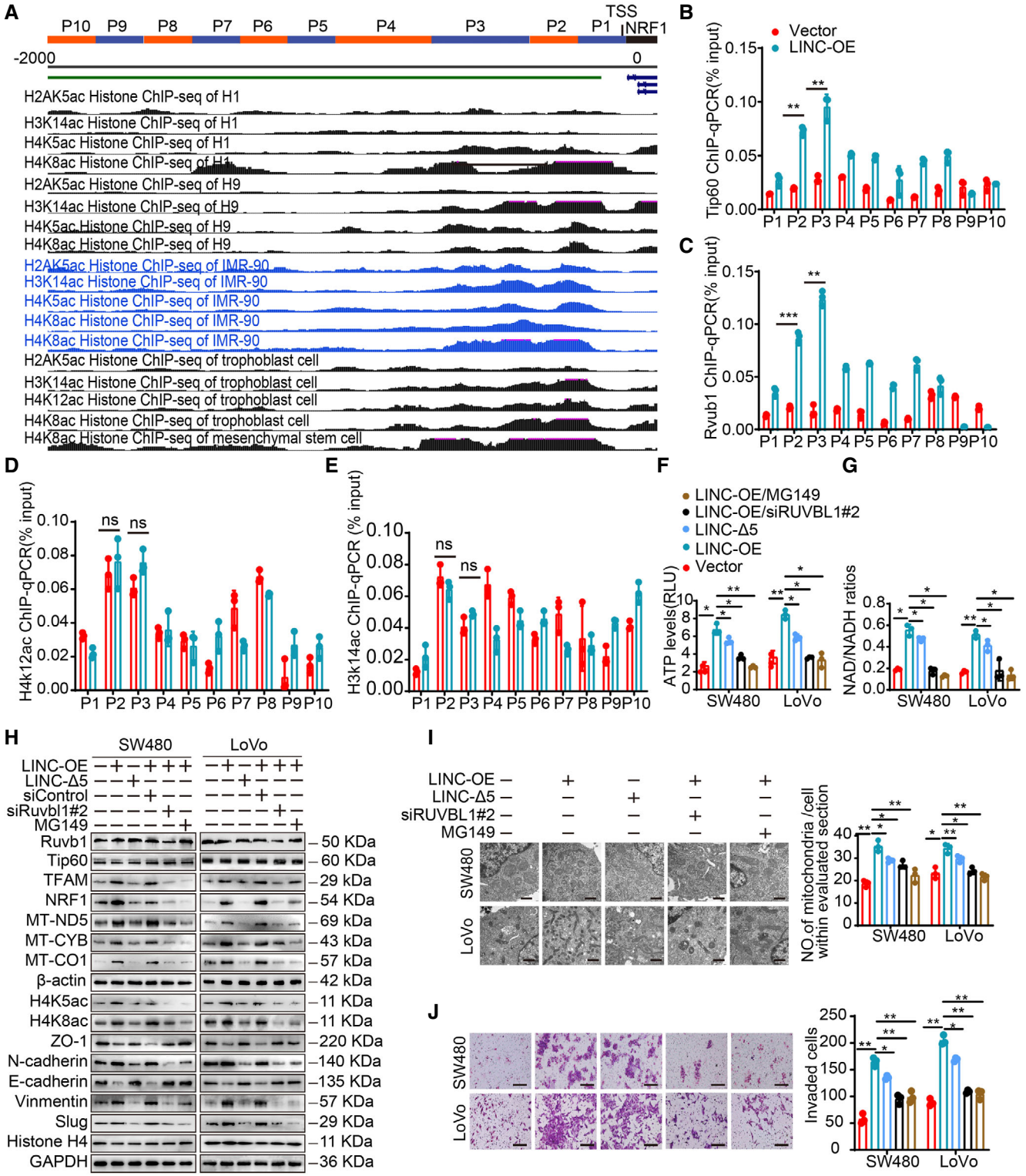
A
P10 P9 P8 P7 P6 P5 P4 P3 P2 P1 TSS INRF1
-2000 0
H2AK5ac Histone ChIP-seq of H1
H3K14ac Histone ChIP-seq of H1
H4K5ac Histone ChIP-seq of H1
H4K8ac Histone ChIP-seq of H1
H2AK5ac Histone ChIP-seq of H9
H3K14ac Histone ChIP-seq of H9
H4K5ac Histone ChIP-seq of H9
H4K8ac Histone ChIP-seq of H9
H2AK5ac Histone ChIP-seq of IMR-90
H3K14ac Histone ChIP-seq of IMR-90
H4K5ac Histone ChIP-seq of IMR-90
H4K8ac Histone ChIP-seq of IMR-90
H4K8ac Histone ChIP-seq of IMR-90
H2AK5ac Histone ChIP-seq of trophoblast cell
H3K14ac Histone ChIP-seq of trophoblast cell
H4K12ac Histone ChIP-seq of trophoblast cell
H4K8ac Histone ChIP-seq of trophoblast cell
H4K8ac Histone ChIP-seq of mesenchymal stem cell
B
Vector
LINC-OE
Tip60 ChIP-qPCR(% input)
P1 P2 P3 P4 P5 P6 P7 P8 P9P10
C
Rvub1 ChIP-qPCR(% input)
P1 P2 P3 P4 P5 P6 P7 P8 P9P10
D
H4k12ac ChIP-qPCR(% input)
ns
P1 P2 P3 P4 P5 P6 P7 P8 P9P10
E
H3k14ac ChIP-qPCR (% input)
ns
P1 P2 P3 P4 P5 P6 P7 P8 P9P10
F
LINC-OE/MG149
LINC-OE/siRUVBL1#2
LINC-Δ5
LINC-OE
Vector
ATP levels(RLU)
SW480 LoVo
G
NAD/NADH ratios
SW480 LoVo
H
SW480 LoVo
LINC-OE
LINC-Δ5
siControl
siRuvbl1#2
MG149
Ruvb1 – 50 KDa
Tip60 – 60 KDa
TFAM – 29 kDa
NRF1 – 54 KDa
MT-ND5 – 69 kDa
MT-CYB – 43 kDa
MT-CO1 – 57 kDa
β-actin – 42 kDa
H4K5ac – 11 KDa
H4K8ac – 11 KDa
ZO-1 – 220 KDa
N-cadherin – 140 KDa
E-cadherin – 135 KDa
Vinmentin – 57 KDa
Slug – 29 KDa
Histone H4 – 11 KDa
GAPDH – 36 KDa
I
LINC-OE
LINC-Δ5
siRUVBL1#2
MG149
SW480
LoVo
NO.of mitochondria /cell within evaluated section
SW480 LoVo
J
SW480
LoVo
Invaded cells
SW480 LoVo

Figure EV3.

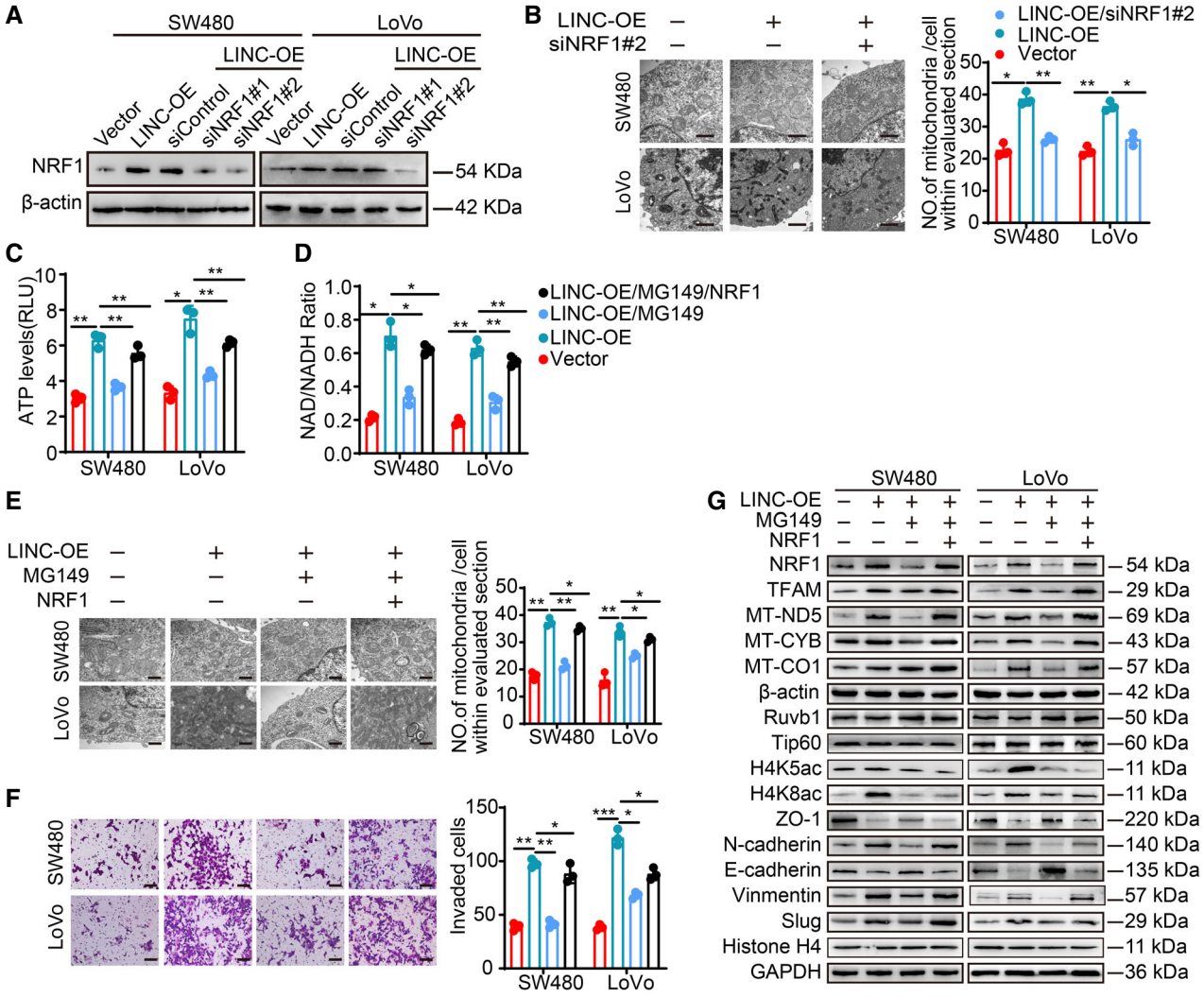

Figure EV4. LINC00839 promotes OXPHOS, mitochondrial biogenesis, and CRC invasion through Ruvb1/Tip60-NRF1 signaling.

A The NRF1 knockdown efficiency was confirmed by WB.

B The number of mitochondria in SW480 and LoVo cells was confirmed by TEM. Scale bars, 500 nm.

C, D ATP levels and NAD/NADH ratios in SW480 and LoVo cells were determined.

E The number of mitochondria in SW480 and LoVo cells was confirmed by TEM. Scale bars, 500 nm.

F Invasion of SW480 and LoVo cells was determined by Matrigel invasion assay. Scale bars, 100 μm.

G Expression of genes related to mitochondrial biogenesis and EMT markers in SW480 and LoVo cells as determined by WB.

Data information: In (B, E, and F), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ biological replicates. In (C and D), the data are presented as the mean ± SD and were analyzed by Student's *t*-test, $n = 3$ technical replicates. Across experiments, the *P*-values are as follows: ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Source data are available online for this figure.

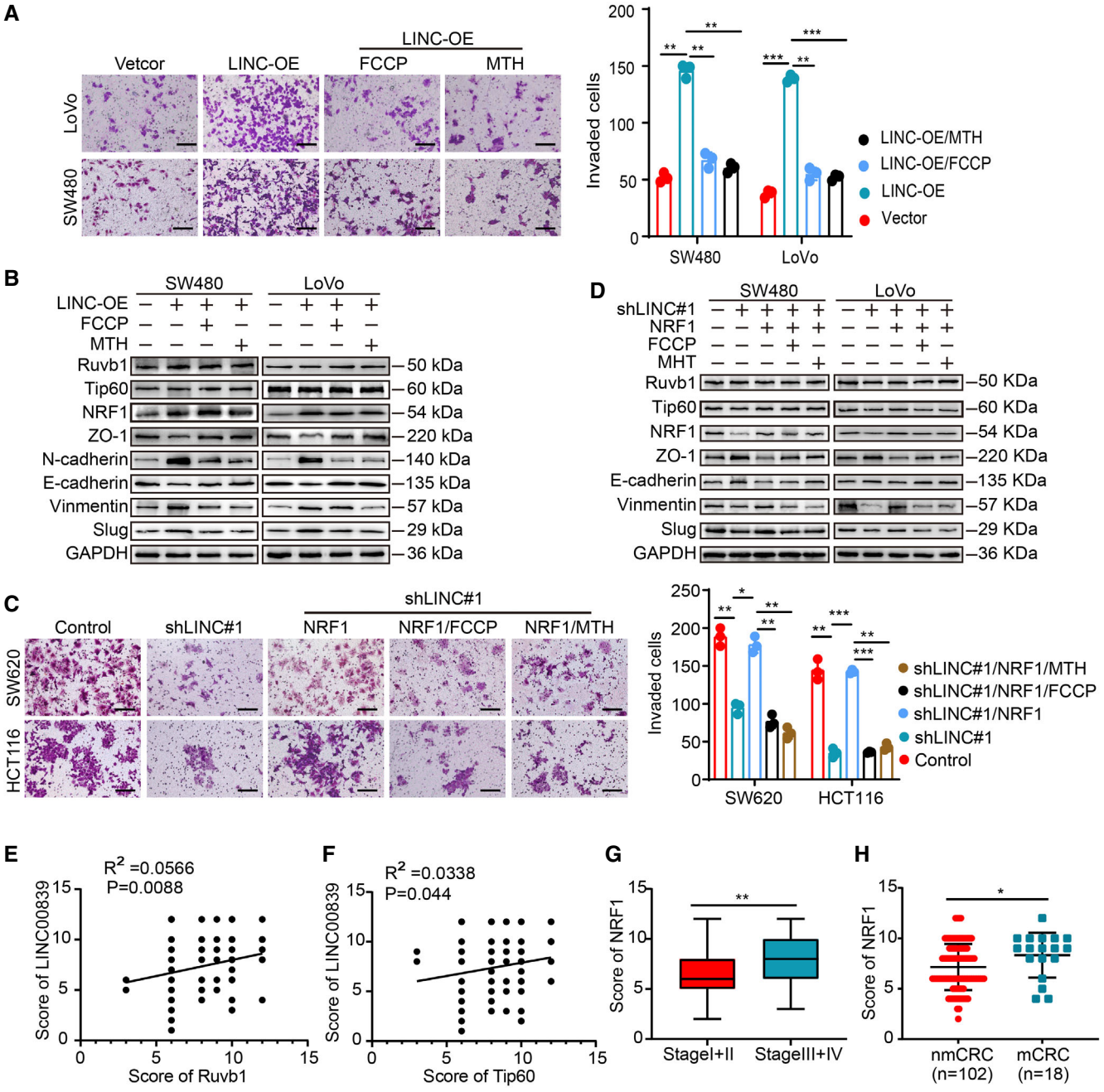

Figure EV5. LINC00839 promotes CRC progression through Ruvb1/Tip60-NRF1-mediated OXPHOS.

A Invasion of LINC00839-overexpressing cells treated with FCCP and MTH was determined by Matrigel invasion assay. Scale bar, 100 μm.

B EMT marker expression levels in LINC00839-overexpressing cells treated with FCCP and MTH were measured by WB.

C Invasion of LINC00839-knockdown cells in which NRF1 was overexpressed and to which FCCP and MTH were added was measured by Matrigel invasion assay. Scale bar, 100 μm.

D EMT marker expression levels in LINC00839-knockdown cells in which NRF1 was overexpressed and to which FCCP and MTH were added were measured by WB.

E The correlation between the expression of LINC00839 and Ruvb1 in the CRC cohort (n = 120, Pearson).

F The correlation between the expression of LINC00839 and Tip60 in the CRC cohort (n = 120, Pearson).

G The expression levels of NRF1 in patients with early-stage (stage I and II) and advanced-stage (stage III and IV) disease.

H Expression of NRF1 in nonmetastatic CRC patients (nmCRC) and metastatic CRC patients (mCRC).

Data information: In (A and C) the data are presented as the mean ± SD and were analyzed by Student's t-test, n = 3 biological replicates. In (G), the middle lines represent the median, and the upper and lower lines represent the upper and lower quartiles. In (H), the data are presented as the mean ± SD and were analyzed by Student's t-test. Across experiments, the P-values are as follows: ns = not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Source data are available online for this figure.