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Supplementary Materials for

ULK1-mediated metabolic reprogramming regulates Vps34 lipid kinase activity by its lactylation

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This PDF file includes:

Fig.S1

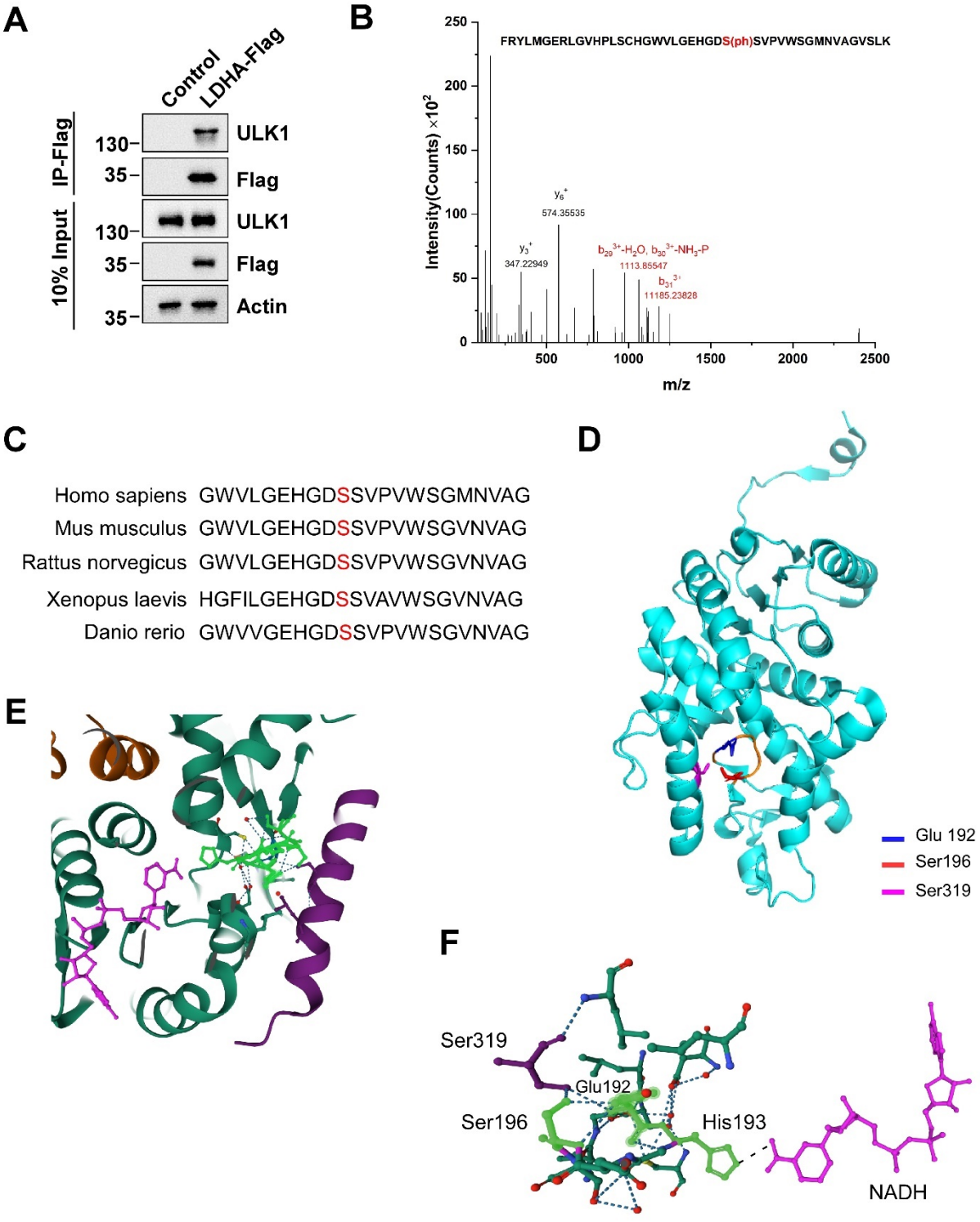

A
Control
LDHA-Flag
IP-Flag
10% Input
130
35
130
35
35
ULK1
Flag
ULK1
Flag
Actin
B
FRYLMGERLGVHPLSCHGWVLGEHGDS(ph)SVPVWSGMNVAGVSLK
Intensity(Counts) ×10²
m/z
y6+ 574.35535
y3+ 347.22949
b29 3+-H2O, b30 3+-NH3-P 1113.85547
b31 3+ 11185.23828
C
Homo sapiens GWVLGEHGDSSVPVWSGMNVAG
Mus musculus GWVLGEHGDSSVPVWSGVNVAG
Rattus norvegicus GWVLGEHGDSSVPVWSGVNVAG
Xenopus laevis HGFILGEHGDSSVAVWSGVNVAG
Danio rerio GWVVGEHGDSSVPVWSGVNVAG
D
Glu 192
Ser196
Ser319
E
F
Ser319
Glu192
Ser196
His193
NADH

Fig.S1. LDHA is a substrate of ULK1.

(A) Interaction assay of endogenous ULK1 and LDHA. LDHA-Flag was expressed in HEK293T cells. Immunoprecipitation was performed using anti-Flag beads and analyzed by western blot with antibody to ULK1. (B) Identification of LDHA phosphorylation at Serine 196 by mass spectrometry. (C) Alignment of sequences of LDHA from various species. Red indicates the identified phosphorylation site of LDHA. (D) Spatial conformation of LDHA. The region Glycine191–Serine197 in LDHA forms a loop. Images were created using Pymol (http://www.pymol.org) and Protein Data Bank (PDB) accession 5ZJD. (E-F) The Serine196 and Glutamate192 in the loop maintain LDHA conformation with Serine 319 by hydrogen bond. The loop (Green) and NADH (purple) are shown. Images were created using Pymol (http://www.pymol.org) and Protein Data Bank (PDB) accession 5ZJD.

Fig.S2.

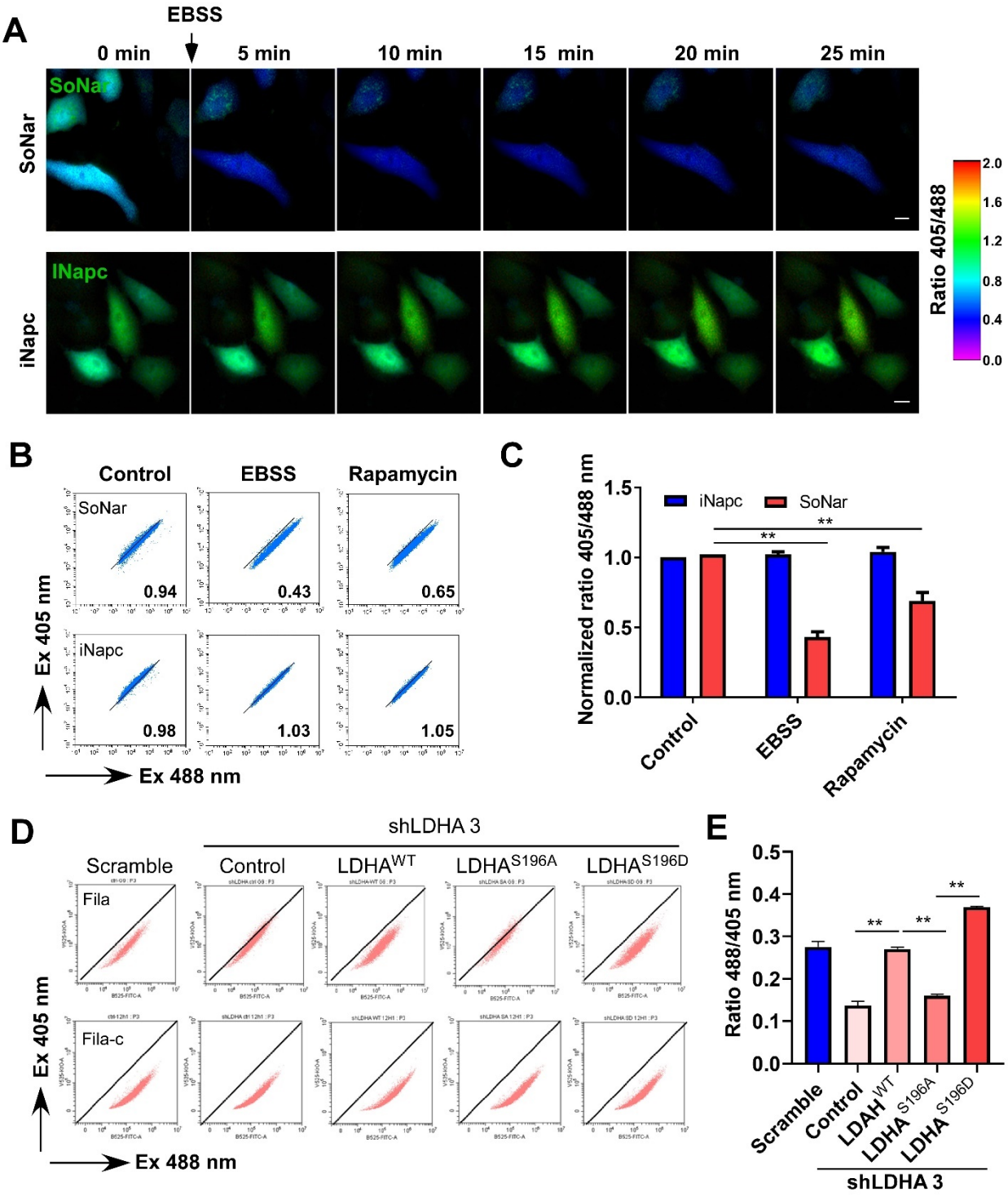

A
EBSS
0 min
5 min
10 min
15 min
20 min
25 min
SoNar
iNapc
Ratio 405/488
2.0
1.6
1.2
0.8
0.4
0.0
B
Control
EBSS
Rapamycin
SoNar
0.94
0.43
0.65
iNapc
0.98
1.03
1.05
Ex 405 nm
Ex 488 nm
C
iNapc
SoNar
Normalized ratio 405/488 nm
1.5
1.0
0.5
0.0
**
**
Control
EBSS
Rapamycin
D
shLDHA 3
Scramble
Control
LDHA^WT
LDHA^S196A
LDHA^S196D
Fila
Fila-c
Ex 405 nm
Ex 488 nm
E
Ratio 488/405 nm
0.5
0.4
0.3
0.2
0.1
0.0
**
**
**
Scramble
Control
LDAH^WT
LDHA S196A
LDHA S196D
shLDHA 3

Fig.S2. ULK1 kinase activity regulates LDHA enzyme activity.

(A) Fluorescence images of SoNar and iNapc in HeLa cells treated with EBSS medium, scale bar, 10 μm. iNapc was used as control sensor. (B) Representative flow-cytometric analyses of the SoNar and iNapc fluorescence upon EBSS medium and Rapamycin. iNapc was used as control sensor. (C) Quantification of (B). n=3. SoNar fluorescence ratio was corrected by iNapc. (D) Representative flow-cytometric analyses of the lactate sensor FiLa and FiLa-C in H1299 cells (control shRNA, LDHA KD/rescued by vector, $LDHA^{WT}$, $LDHA^{S196A}$ and $LDHA^{S196D}$). (E) Quantification of (D). Data are shown as mean ± SD; **p<0.01, n=3. FiLa fluorescence ratio was corrected by FiLa-C.

Fig.S3.

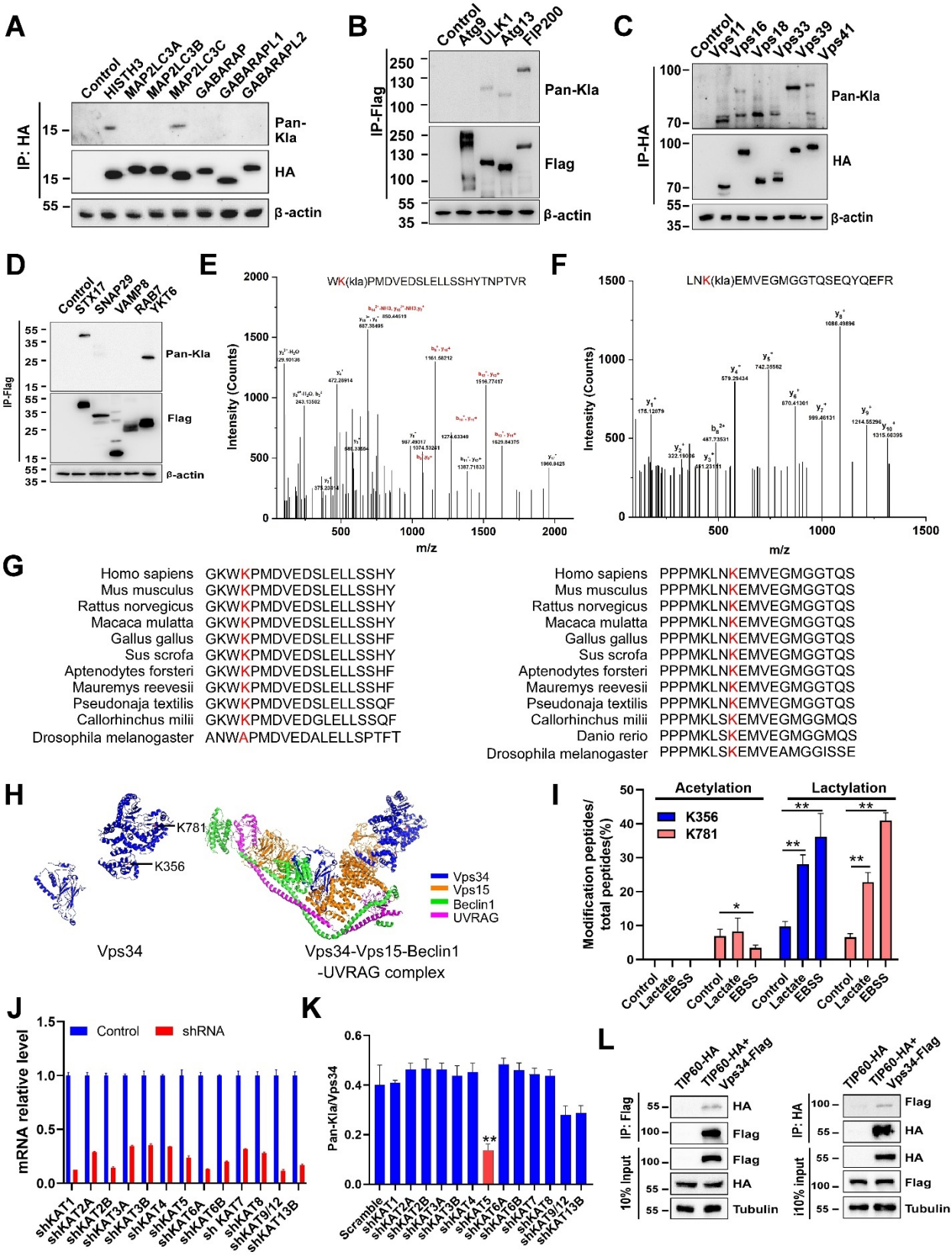

A
Control
HIST1H3
MAP2LC3A
MAP2LC3B
MAP2LC3C
GABARAP
GABARAPL1
GABARAPL2
IP: HA
Pan-Kla
HA
β-actin
B
Control
Atg9
ULK1
Atg13
FIP200
IP-Flag
Pan-Kla
Flag
β-actin
C
Control
Vps11
Vps16
Vps18
Vps33
Vps39
Vps41
IP-HA
Pan-Kla
HA
β-actin
D
Control
STX17
SNAP29
VAMP8
RAB7
YKT6
IP-Flag
Pan-Kla
Flag
β-actin
E
WK(kla)PMDVEDSLELLSSHYTNPTVR
Intensity (Counts)
m/z
F
LNK(kla)EMVEGMGGTQSEQYQEFR
Intensity (Counts)
m/z
G
Homo sapiens GKWKPMDVEDSLELLSSHY
Mus musculus GKWKPMDVEDSLELLSSHY
Rattus norvegicus GKWKPMDVEDSLELLSSHY
Macaca mulatta GKWKPMDVEDSLELLSSHY
Gallus gallus GKWKPMDVEDSLELLSSHF
Sus scrofa GKWKPMDVEDSLELLSSHY
Aptenodytes forsteri GKWKPMDVEDSLELLSSHF
Mauremys reevesii GKWKPMDVEDSLELLSSHF
Pseudonaja textilis GKWKPMDVEDSLELLSSQF
Callorhinchus milii GKWKPMDVEDGLELLSSQF
Drosophila melanogaster ANWAPMDVEDALELLSPTFT
Homo sapiens PPPMKLNKEMVEGMGGTQS
Mus musculus PPPMKLNKEMVEGMGGTQS
Rattus norvegicus PPPMKLNKEMVEGMGGTQS
Macaca mulatta PPPMKLNKEMVEGMGGTQS
Gallus gallus PPPMKLNKEMVEGMGGTQS
Sus scrofa PPPMKLNKEMVEGMGGTQS
Aptenodytes forsteri PPPMKLNKEMVEGMGGTQS
Mauremys reevesii PPPMKLNKEMVEGMGGTQS
Pseudonaja textilis PPPMKLNKEMVEGMGGTQS
Callorhinchus milii PPPMKLSKEMVEGMGGMQS
Danio rerio PPPMKLSKEMVEGMGGMQS
Drosophila melanogaster PPPMKLSKEMVEAMGGISSE
H
K781
K356
Vps34
Vps34
Vps15
Beclin1
UVRAG
Vps34-Vps15-Beclin1
-UVRAG complex
I
Acetylation
Lactylation
K356
K781
Modification peptides/
total peptides(%)
Control
Lactate
EBSS
J
Control
shRNA
mRNA relative level
shKAT1
shKAT2A
shKAT2B
shKAT3A
shKAT3B
shKAT4
shKAT5
shKAT6A
shKAT6B
sh KAT7
shKAT8
shKAT9/12
shKAT13B
K
Pan-Kla/Vps34
Scramble
shKAT1
shKAT2A
shKAT2B
shKAT3A
shKAT3B
shKAT4
shKAT5
shKAT6A
shKAT6B
shKAT7
shKAT8
shKAT9/12
shKAT13B
L
TIP60-HA
TIP60-HA+
Vps34-Flag
IP: Flag
IP: HA
10% Input
HA
Flag
Tubulin

Fig.S3. TIP60 mediates Vps34 lactylation.

(A-D) Lactylation screening of autophagy core proteins in HEK293T cells. HA tagged or Flag-tagged autophagy core proteins were expressed individually in HEK293T cells and purified by Immunoprecipitation. Lactylation was analyzed by western blot with Pan-Kla antibody. (E) Identification of Vps34 lactylation at Lysine 356 by mass spectrometry. (F) Identification of Vps34 lactylation at Lysine 781 by mass spectrometry. (G) Alignment of sequences of Vps34 from various species. Red indicates the identified lactylation site of Vps34. (H) K356 and K781 sites in structure of Vps34 and Vps34-Vps15-Beclin1-UVRAG complex. Images were created using Pymol (http://www.pymol.org) and Protein Data Bank (PDB) accession 7BL1. (I) The acetylation and lactylation percentage of Vps34 K356 and K781 in normal medium, EBSS medium (3 h) and normal medium containing lactate (1 mM,12 h) by mass spectrometry. Data are shown as mean ± SD, ** $p < 0.01$, $n = 3$. (J) Relative mRNA expression level in acyltransferase knockdown HEK293T by fluorescence quantitative PCR. Data are shown as mean ± SD, ** $p < 0.01$, $n = 3$. (K) Quantification of Figure 3F. Data are shown as mean ± SD, ** $p < 0.01$, $n = 3$. (L) Vps34 interacts with TIP60. HA tagged TIP60 and Flag tagged Vps34 were co-expressed in HEK293T cells, Immunoprecipitation was performed and analyzed by western blot.

Fig.S4.

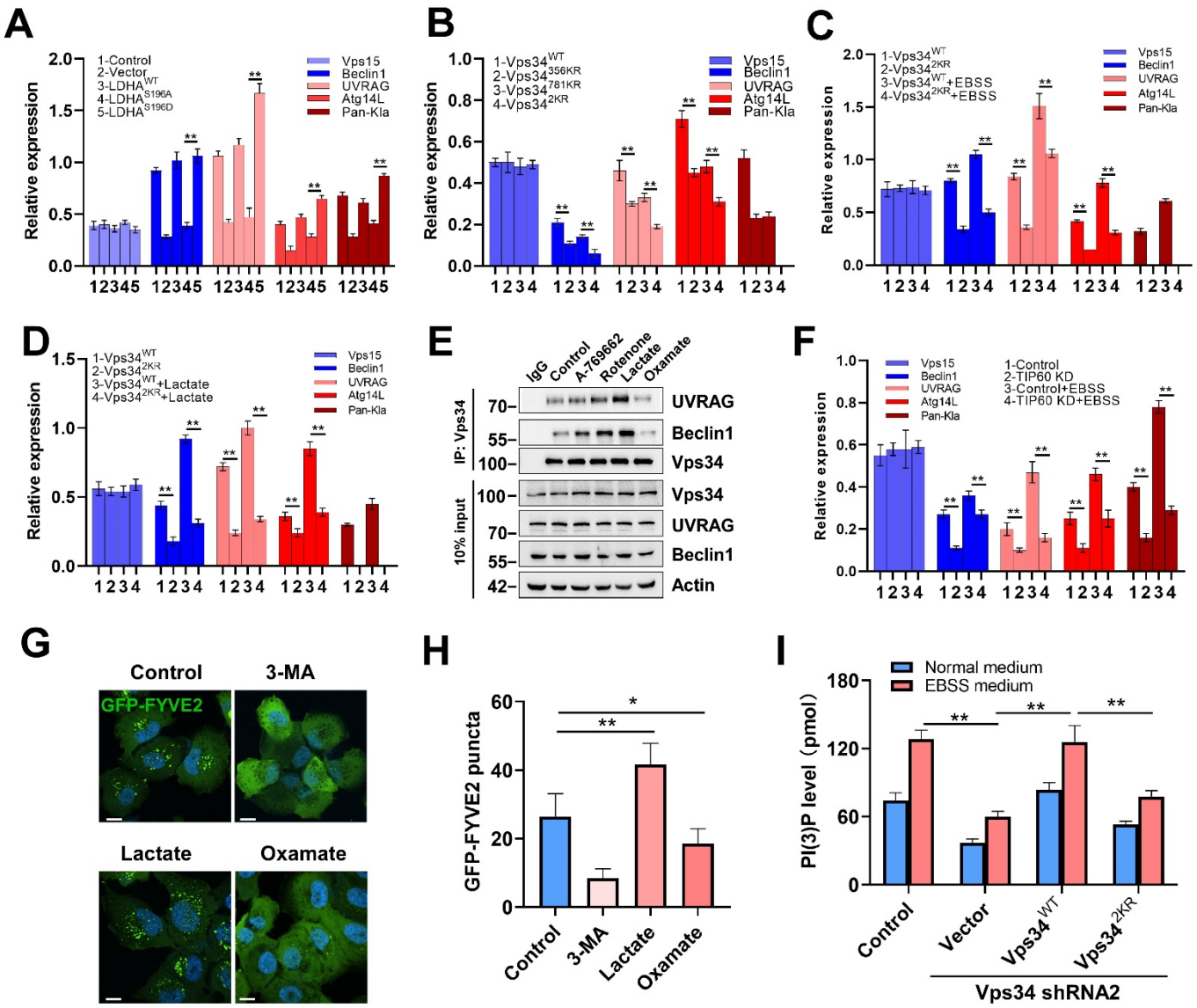

A
B
C
D
E
F
G
H
I
Relative expression
1-Control
2-Vector
3-LDHA WT
4-LDHA S196A
5-LDHA S196D
1-Vps34 WT
2-Vps34 356KR
3-Vps34 781KR
4-Vps34 2KR
1-Vps34 WT
2-Vps34 2KR
3-Vps34 WT+EBSS
4-Vps34 2KR+EBSS
1-Vps34 WT
2-Vps34 2KR
3-Vps34 WT+Lactate
4-Vps34 2KR+Lactate
Vps15
Beclin1
UVRAG
Atg14L
Pan-Kla
IgG
Control
A-769662
Rotenone
Lactate
Oxamate
IP: Vps34
10% input
UVRAG
Beclin1
Vps34
Actin
1-Control
2-TIP60 KD
3-Control+EBSS
4-TIP60 KD+EBSS
Control
3-MA
Lactate
Oxamate
GFP-FYVE2
GFP-FYVE2 puncta
Normal medium
EBSS medium
PI(3)P level (pmol)
Vector
Vps34 WT
Vps34 2KR
Vps34 shRNA2

Fig.S4. Vps34 lactylation enhanced its lipid kinase activity by promoting interaction PI3KC3 subunits.

(A-D) Quantification of western blot in Figure 4A-4D. Data are shown as mean ± SD, ** $p < 0.01$, n=3. (E) Interaction assay of Vps34 with Beclin1 and UVRAG in HEK293T cells that treated with A-769662 (100 nM), rotenone (10 nM), lactate (1 mM) and oxamate (10 mM). Immunoprecipitation was performed using Vps34 antibody and analyzed by western blot with anti-Beclin1 and UVRAG antibody. (F) Quantification of western blot in Figure 4E. Data are shown as mean ± SD, ** $p < 0.01$, n=3. (G) Vps34 kinase activity assay by GFP-FYVE2. U2OS GFP-FYVE2 stable cell line was treated with 3-MA (2 mM), lactate (1 mM) and oxamate (10 mM) and analyzed by confocal microscopy. Scale bar, 10 μm. (H) Quantification of GFP-FYVE2 puncta in (G). Data are shown as mean ± SD; **$p<0.01$, n=30. (I) PI(3)P level determined by PI(3)P ELISA kit in HEK293T cells (control shRNA, Vps34 shRNA2/rescued by vector, $Vps34^{WT}$ and $Vps34^{2KR}$). Data are shown as mean ± SD; **$p<0.01$, n=5.

Fig.S5.

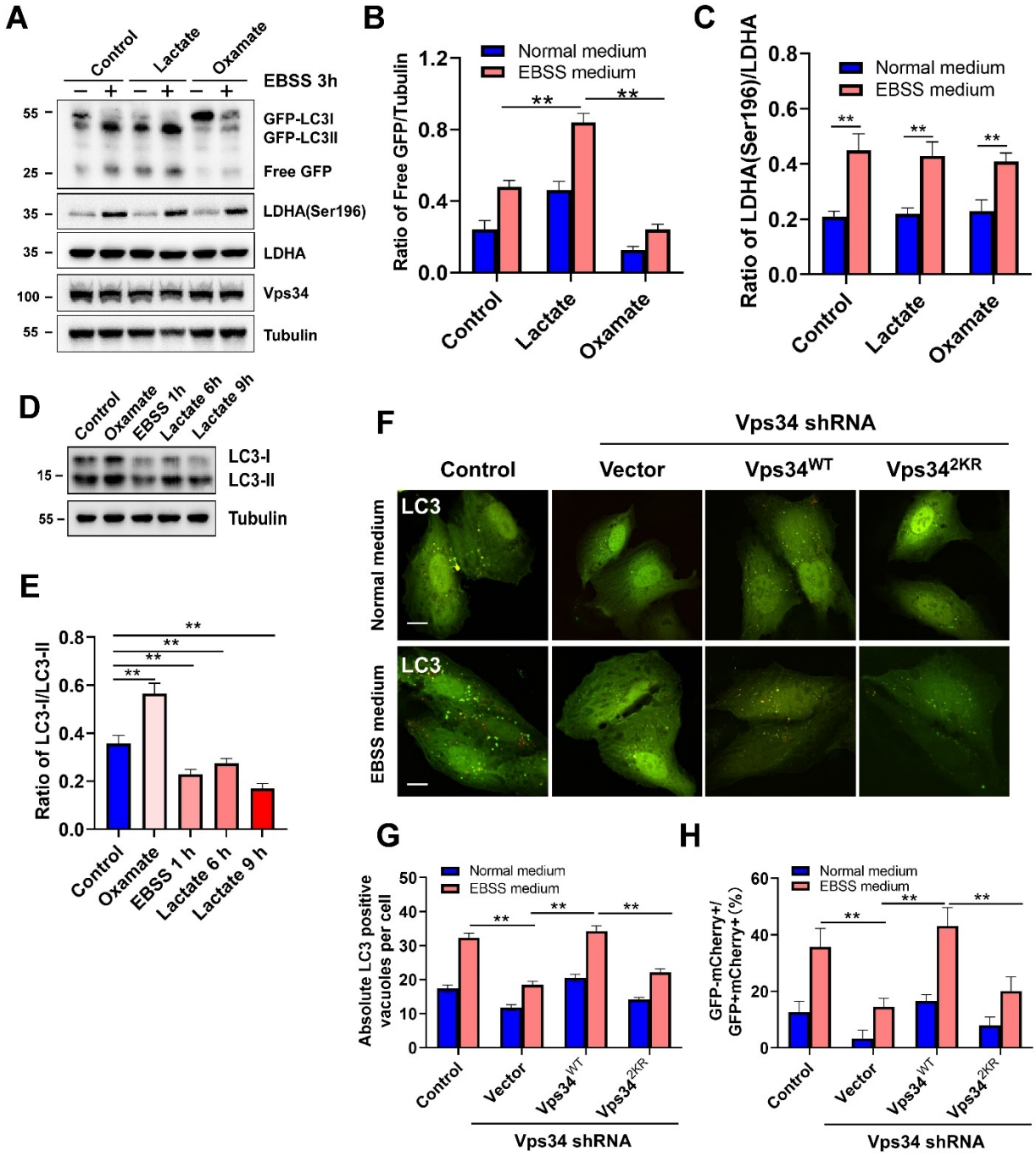

A
Control
Lactate
Oxamate
EBSS 3h
GFP-LC3I
GFP-LC3II
Free GFP
LDHA(Ser196)
LDHA
Vps34
Tubulin
B
Normal medium
EBSS medium
Ratio of Free GFP/Tubulin
C
Ratio of LDHA(Ser196)/LDHA
D
Control
Oxamate
EBSS 1h
Lactate 6h
Lactate 9h
LC3-I
LC3-II
Tubulin
E
Ratio of LC3-I/LC3-II
F
Vps34 shRNA
Control
Vector
Vps34WT
Vps34 2KR
LC3
Normal medium
EBSS medium
G
Absolute LC3 positive vacuoles per cell
H
GFP-mCherry+/ GFP+mCherry+ (%)

Fig.S5. Vps34 lactylation promotes autophagy

(A) GFP-LC3 cleavage assay. HEK293T cells were retreated with lactate (1 mM) and oxamate (10 mM) for 12 h under normal medium or EBSS medium for 2 h and analyzed by western blot with GFP antibody. (B) Quantification of free GFP/Tubulin in (A). Data are shown as mean ± SD; **p<0.01, n=3. (C) Quantification of LDHA (Ser196)/LDHA in (A). Data are shown as mean ± SD; **p<0.01, n=3. (D) Autophagy activity assay. HEK293T cells were treated with oxamate and lactate. Autophagy level was measured by LC3 turnover. (E) The ratio of LC3-I/LC3-II in (D). Data are shown as mean ± SD; **p<0.01, n=3. (F) Autophagosome maturation assay. mCherry-GFP-LC3 was transfected in U2OS cells (control shRNA, Vps34 KD, Vps34 KD/rescued by $Vps34^{WT}$, $Vps34^{2KR}$) under normal medium or EBSS medium for 2 h and analyzed by confocal microscopy. (G) Quantification of the relative $GFP^{-}mCherry^{+}$ LC3 puncta. which indicate autolysosomes in (E), Data are shown as mean ± SD; **p<0.01, n=30. (G) Quantification of the relative $GFP^{-}mCherry^{+}$ LC3 puncta. which indicate autolysosomes in (F), Data are shown as mean ± SD; **p<0.01, n=30. (H) The percentage of $GFP^{-}mCherry^{+}$ and GFP^{+} $mCherry^{+}$ in (F), Data are shown as mean ± SD; **p<0.01, n=30.

Fig.S6.

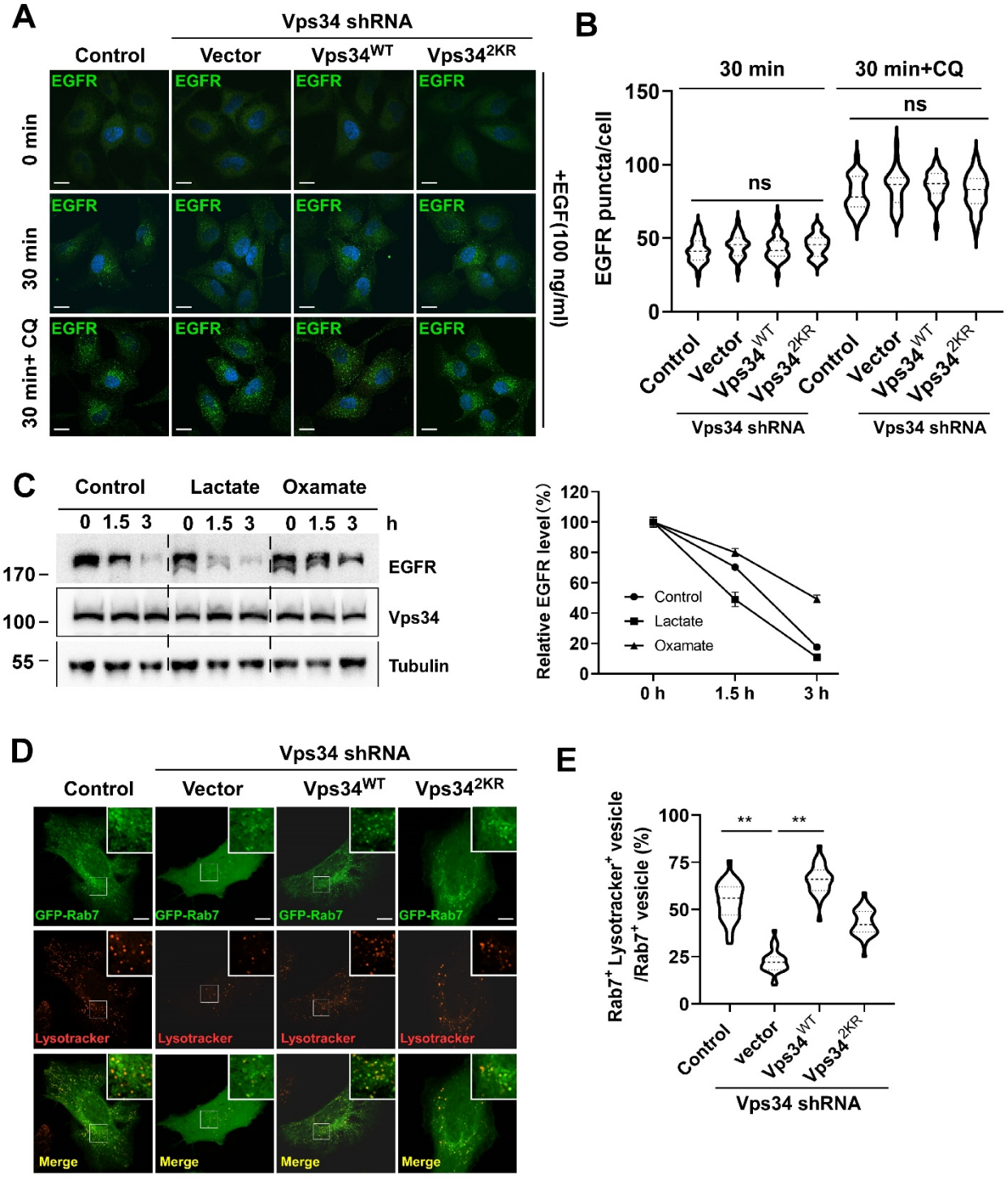
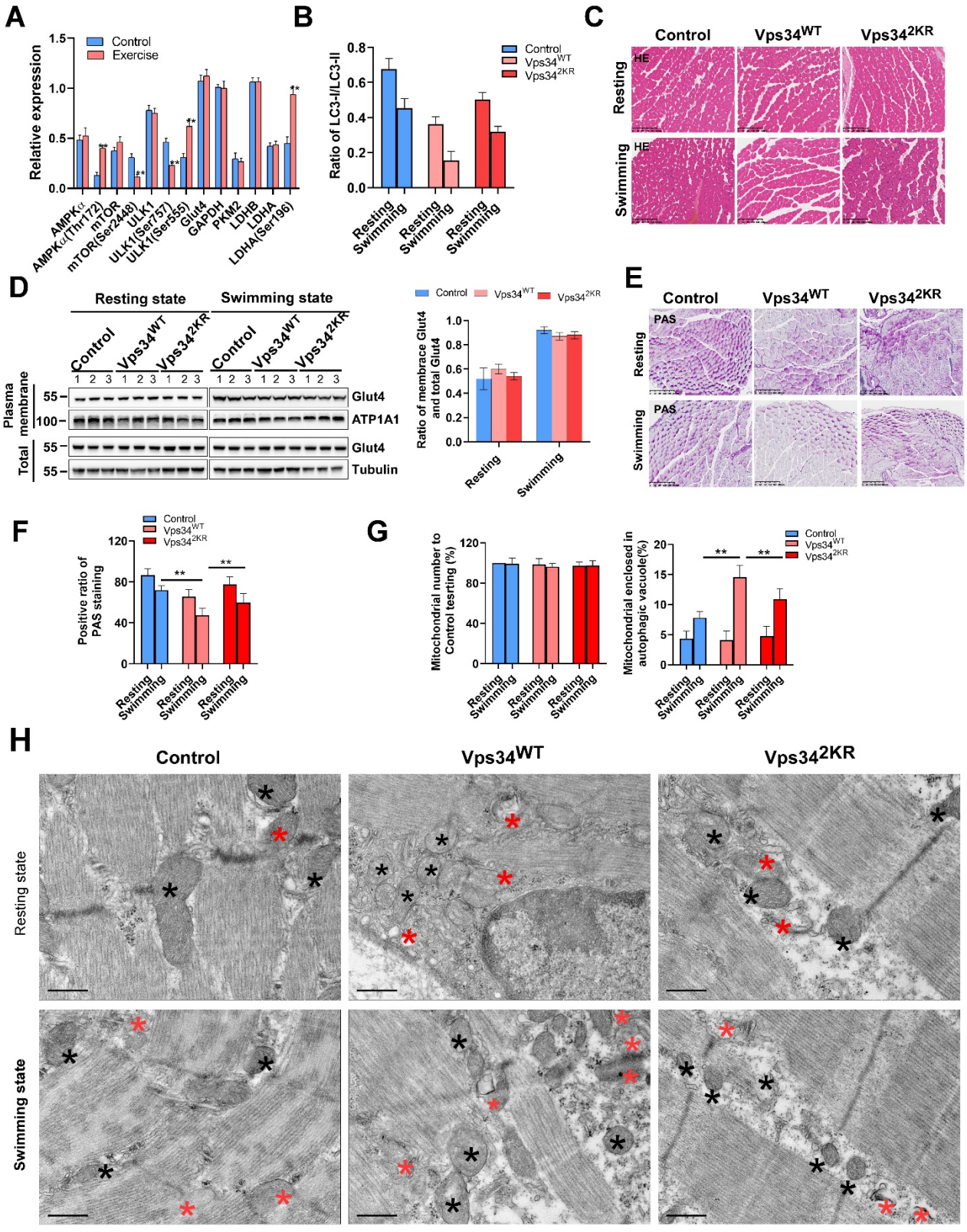

A
Vps34 shRNA
Control
Vector
Vps34WT
Vps34 2KR
0 min
30 min
30 min+ CQ
EGFR
+EGF(100 ng/ml)
B
30 min
30 min+CQ
ns
ns
EGFR puncta/cell
150
100
50
0
Control
Vector
Vps34 WT
Vps34 2KR
Vps34 shRNA
C
Control
Lactate
Oxamate
0 1.5 3
h
170
100
55
EGFR
Vps34
Tubulin
Relative EGFR level (%)
120
100
80
60
40
20
0
Control
Lactate
Oxamate
0 h
1.5 h
3 h
D
Vps34 shRNA
Control
Vector
Vps34WT
Vps34 2KR
GFP-Rab7
Lysotracker
Merge
E
Rab7+ Lysotracker+ vesicle /Rab7+ vesicle (%)
100
75
50
25
0
**
**
Control
vector
Vps34 WT
Vps34 2KR
Vps34 shRNA

Fig.S6. Vps34 lactylation promotes endo-lysosomal trafficking

(A) Suppression of Vps34 expression does not impede internalization of the EGFR. Hela cells (control shRNA, Vps34 KD, Vps34 KD/rescued by $Vps34^{WT}$, $Vps34r^{2KR}$) were stimulated with EGF (100 ng/ml) or EGF (100 ng/ml) and Chloroquine (CQ, 10 μM) after overnight incubation with serum-free medium. Scale bar, 10 μm. (B) Quantification assay of internalization of the EGFR in Hela cells (control shRNA, Vps34 KD, Vps34 KD/rescued by $Vps34^{WT}$, $Vps34r^{2KR}$). Data are shown as mean ±SD; n=30. (C) EGFR degradation assay. HEK293T cells were co-treated with lactate (1 mM) and oxamate (10 mM) with EGF (100 ng/mL) for 0 h,1.5 h and 3 h. (D) EGFR levels were quantified by Image J software and normalized by percentage of 0 h EGFR level. Data are shown as mean ±SD; **p<0.01, n=3. (E) Colocalization of GFP-Rab7 and lysotracker in U2OS cells (control shRNA, Vps34 KD/rescued by vector, Vps34WT and Vps342KR). Colocalization of GFP-Rab7+ lysotracker+ vesicles were analyzed by confocal microscopy. Scale bar, 10 μm. (F) Quantification of GFP-Rab7+ lysotracker+ vesicles per cell. Data are shown as mean ± SD; **p<0.01, n = 30.

Fig.S7.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Relative expression
Control
Exercise
AMPKα
AMPKα(Thr172)
mTOR
mTOR(Ser2448)
ULK1
ULK1(Ser757)
ULK1(Ser555)
Glut4
GAPDH
PKM2
LDHB
LDHA
LDHA(Ser196)
B
Ratio of LC3-I/LC3-II
Control
Vps34WT
Vps342KR
Resting
Swimming
C
Control
Vps34WT
Vps342KR
HE
Resting
Swimming
D
Resting state
Swimming state
Plasma membrane
Total
Glut4
ATP1A1
Tubulin
Ratio of membrace Glut4 and total Glut4
E
PAS
F
Positive ratio of PAS staining
G
Mitochondrial number to Control testing (%)
Mitochondrial enclosed in autophagic vacuole(%)
H
Resting state
Swimming state

Figure S7. Vps34 lactylation is required for skeletal muscle homeostasis and cancer progress. Related to Figure 7

(A) Quantification assay of signaling pathway protein expression in mouse skeletal muscle of control group and swimming group by western blot in Figure 7A. Data are shown as mean ±SD; **p<0.01, n=3. (B) Quantification of LC3-I/LC3-II in Figure 7H. Data are shown as mean ±SD; **p<0.01, n=3. (C) H&E staining showing mice muscle histopathologic changes in control group, AAV-Vps34^{WT} group and AAV-Vps34^{2KR} group during resting state and swimming state. Scale bars, 200 μm, n=5. (D) Swimming promotes GLUT4 membrance distribution in mice muscle tissues of control group, AAV-Vps34^{WT} group and AAV-Vps34^{2KR} group by Western blot. Plasma Membrane Protein was Isolated by cell fractionation. Data are shown as mean ±SD; **p<0.01, n=5. (E) PAS staining showing glycogen level in mice muscle tissues of control group, AAV-Vps34^{WT} group, and AAV-Vps34^{2KR} group during resting state and swimming state. Scale bars, 50 mm, n=5. (F) Quantification analysis of PAS staining. Data are shown as mean ±SD; **p<0.01, n=5. (G) Quantification analysis of mitochondrial number and the percentage of mitochondria enclosed in an autophagic vacuole. Quantification data are shown as mean ±SD; **p<0.01, n=10. (G) Transmission electron microscopy showing the morphology of mitochondria and lysosome like structures in mice muscle tissues of control group, AAV-Vps34^{WT} group, and AAV-Vps34^{2KR} group during resting state and swimming state. Scale bars, 1 μm. n=10.

Table S1 **Table S1 Lactylation peptides of Vps34 in vitro lactylation by mass spectrometry**

Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	ΔCn	q-Value	PEP
SGPsDHDLKPNAATR	1	3	1	Q8NEB9	S4(Phospho)	0.0000	0	0.01451
YAPSENGPnGISAEVmDTYVK	9	6	1	Q8NEB9	N9(Deamidated)	0.0000	0	5.331E-10
YENFDDIKnGLEPTKK	4	3	1	Q8NEB9	N9(Deamidated)	0.0000	0	0.0000012
YENFDDIKnGLEPTK	4	3	1	Q8NEB9	N9(Deamidated)	0.0000	0	1.523E-07
YAPSENGPnGISAEVMDTYVK	11	6	1	Q8NEB9	N9(Deamidated)	0.0000	0	2.241E-09
KYAPSEnGPnGISAEVMDTYVK	1	6	1	Q8NEB9	N7(Deamidated)	0.0000	0	0.00003609
KYAPSEnGPNGISAEVMDTYVK	9	6	1	Q8NEB9	N7(Deamidated)	0.0000	0	0.03263
YAPSEnGPnGISAEVmDTYVK	1	6	1	Q8NEB9	N6(Deamidated)	0.0000	0	0.00002901
YAPSEnGPnGISAEVMDTYVK	1	6	1	Q8NEB9	N6(Deamidated)	0.0000	0	7.177E-10
SGPSDHDLKPnAATR	4	3	1	Q8NEB9	N11(Deamidated)	0.0000	0	0.00009001
KYAPSENGPnGISAEVmDTYVK	4	6	1	Q8NEB9	N10(Deamidated)	0.0000	0	0.000002893
nGLEPTKK	1	3	1	Q8NEB9	N1(Deamidated)	0.0000	0	0.0116
nGLEPTK	2	3	1	Q8NEB9	N1(Deamidated)	0.0000	0	0.02933
DPKPLPPPmK	5	3	1	Q8NEB9	M9(Oxidation)	0.0000	0	0.000151
LNKEMVEGmGGTQSEQYQEFR	2	3	1	Q8NEB9	M9(Oxidation)	0.0000	0	0.001853
AVLEDPmLK	8	4	1	Q8NEB9	M7(Oxidation)	0.0000	0	0.003613
VPDPQmSmENLVESK	7	3	1	Q8NEB9	M6(Oxidation)	0.0000	0	5.176E-10
VPDPQmSMENLVESK	8	3	1	Q8NEB9	M6(Oxidation)	0.0000	0	3.457E-08
LNKEmVEGmGGTQSEQYQEFR	3	3	1	Q8NEB9	M5(Oxidation)	0.0000	0	0.0009105
SSNFmYLmVEFR	2	3	1	Q8NEB9	M5(Oxidation)	0.0000	0	0.000594
SSNFmYLMVEFR	2	3	1	Q8NEB9	M5(Oxidation)	0.0000	0	0.0004774
THEmYLNVmR	1	3	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.004063
EIEmINESEKR	2	3	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.00006621

EIEmINESEK	3	3	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.0003825
THEmYLNVMR	5	3	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.001136
WKPmDVEDSLELLSSHYTNPTVR	10	3	1	Q8NEB9	M4(Oxidation)	0.0000	0	1.55E-11
SALmPAQLFFK	6	4	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.0003004
HGFmQFIQSVPVAEVLDTEGSIQNFFR	1	6	1	Q8NEB9	M4(Oxidation)	0.0000	0	0.0001724
EmVEGmGGTQSEQYQEFRK	1	3	1	Q8NEB9	M2(Oxidation)	0.0000	0	0.003474
EmVEGmGGTQSEQYQEFR	6	3	1	Q8NEB9	M2(Oxidation)	0.0000	0	2.148E-11
EmVEGMGGTQSEQYQEFR	7	3	1	Q8NEB9	M2(Oxidation)	0.0000	0	5.578E-11
HGDDLRQDQLILQIISLmDK	2	4	1	Q8NEB9	M18(Oxidation)	0.0000	0	0.0002385
KYAPSENGPNGISAEVmDTYVK	1	6	1	Q8NEB9	M17(Oxidation)	0.0000	0	0.00003675
QDQLILQIISLmDK	1	4	1	Q8NEB9	M12(Oxidation)	0.0000	0	0.000000412
LDLSDEEAVHYmQSLIDESVHALFAAVVEQIHK	2	3	1	Q8NEB9	M12(Oxidation)	0.0000	0	1.609E-11
LQALLGDNEKmNLSDVELIPLPLEPQVK	1	3	1	Q8NEB9	M11(Oxidation)	0.0000	0.003	0.1538
TSSTLSEDQmSR	2	6	1	Q8NEB9	M10(Oxidation)	0.0000	0	0.000001516
mNLSDVELIPLPLEPQVK	2	4	1	Q8NEB9	M1(Oxidation)	0.0000	0	6.635E-12
LNkEMVEGMGGTQSEQYQEFR	1	3	1	Q8NEB9	K3(Lactylation)	0.0000	0	0.000302
WkPMDVEDSLELLSSHYTNPTVR	3	3	1	Q8NEB9	K2(Lactylation)	0.0000	0	1.032E-09
GIIPETATLFkSALMPAQLFFK	1	4	1	Q8NEB9		0.0000	0.003	0.117
FSGLYQETcSDLYVTcQVFAEGKPLALPVR	4	4	1	Q8NEB9	C9(Carbamidomethyl)	0.0000	0	1.094E-09
FHYIYScDLDINVQLK	6	4	1	Q8NEB9	C7(Carbamidomethyl)	0.0000	0	0.0001072
KQcYTAFLHLR	5	3	1	Q8NEB9	C3(Carbamidomethyl)	0.0000	0	0.00001475
ScAGYcVITYILGVGDR	1	6	1	Q8NEB9	C2(Carbamidomethyl)	0.0000	0	2.46291E-13
QcYTAFLHLR	7	3	1	Q8NEB9	C2(Carbamidomethyl)	0.0000	0	0.0003119
TKEVPDGENLEQDLcTFLISR	3	3	1	Q8NEB9	C15(Carbamidomethyl)	0.0000	0	5.714E-08
EVPDGENLEQDLcTFLISR	4	3	1	Q8NEB9	C13(Carbamidomethyl)	0.0000	0	1.101E-09
cVnWDLPQEAK	1	3	1	Q8NEB9	C1(Carbamidomethyl)	0.0000	0	0.0001585
cDDKEYGIVYYEK	6	3	1	Q8NEB9	C1(Carbamidomethyl)	0.0000	0	0.0002614
cVNWDLPQEAK	5	3	1	Q8NEB9	C1(Carbamidomethyl)	0.0000	0	0.00001451

cDDKEYGIVYYEKDGDESSPILTSFELVK	2	3	1	Q8NEB9	C1(Carbamidomethyl)	0.0000	0	7.541E-11
HGDDLR	1	19	1	Q8NEB9		0.0000	0	0.03664
NGLEPTKK	1	3	1	Q8NEB9		0.0000	0	0.03688
SGPSDHDLKPNAATR	4	3	1	Q8NEB9		0.0000	0	1.411E-07
NGLEPTK	1	3	1	Q8NEB9		0.0000	0	0.05384
IGSLEGK	4	3	1	Q8NEB9		0.0000	0	0.04868
TSSTLSEDQMSR	7	6	1	Q8NEB9		0.0000	0	2.405E-07
DPKPLPPPMK	12	3	1	Q8NEB9		0.0000	0	0.00008636
YYLTNQEK	11	3	1	Q8NEB9		0.0000	0	0.00509
EIEMINESEKR	6	3	1	Q8NEB9		0.0000	0	0.000002995
YPDLPR	15	6	1	Q8NEB9		0.0000	0	0.05416
YENFDDIK	6	3	1	Q8NEB9		0.0000	0	0.006574
EIEMINESEK	4	3	1	Q8NEB9		0.0000	0	0.00002276
LQALLGDNEK	16	3	1	Q8NEB9		0.0000	0	0.001277
FSQALLK	12	3	1	Q8NEB9		0.0000	0	0.07158
VWPNVEADGSEPTKTPGR	1	6	1	Q8NEB9		0.0000	0	0.004616
HLDNLLLTK	9	6	1	Q8NEB9		0.0000	0	0.0004425
TEDGGKYPVIFK	10	4	1	Q8NEB9		0.0000	0	2.193E-07
LPVKYPDLPR	3	6	1	Q8NEB9		0.0000	0	0.00006292
VWPNVEADGSEPTK	15	6	1	Q8NEB9		0.0000	0	7.511E-07
THEMYLNVMR	7	3	1	Q8NEB9		0.0000	0	0.00007128
YENFDDIKNGLEPTKK	2	3	1	Q8NEB9		0.0000	0	2.576E-08
EYGIVYYEK	2	3	1	Q8NEB9		0.0000	0	0.08536
VDWLDR	7	3	1	Q8NEB9		0.0000	0	0.04942
QALELLGK	4	3	1	Q8NEB9		0.0000	0	0.01052
AVLEDPMLK	4	4	1	Q8NEB9		0.0000	0	0.01162
YPVIFK	1	4	1	Q8NEB9		0.0000	0.003	0.1757
EMVEGMGGTQSEQYQEFR	3	3	1	Q8NEB9		0.0000	0	4.054E-11

SLLAAQQTFVDR	19	3	1	Q8NEB9		0.0000	0	0.000002442
YENFDDIKNGLEPTK	2	3	1	Q8NEB9		0.0000	0	0.000001611
KYAPSENGPNGISAEVMDTYVK	2	6	1	Q8NEB9		0.0000	0	0.0001265
DQLNIIVSYPPTK	12	3	1	Q8NEB9		0.0000	0	6.669E-07
AVPVGGTTVSLFGK	14	6	1	Q8NEB9		0.0000	0	0.000003656
QLTYEEQDLVWK	6	3	1	Q8NEB9		0.0000	0	0.000004473
VPDPQMSMENLVESK	5	3	1	Q8NEB9		0.0000	0	7.791E-09
WKPMDVEDSLELLSSHYTNPTVR	7	3	1	Q8NEB9		0.0000	0	4.515E-10
GIIPETATLFK	10	4	1	Q8NEB9		0.0000	0	0.0005927
DGDESSPILTSFELVK	3	3	1	Q8NEB9		0.0000	0	8.786E-09
SALMPAQLFFK	4	4	1	Q8NEB9		0.0000	0	0.00006161
NAQVALTIWDVYGPGK	4	6	1	Q8NEB9		0.0000	0	8.575E-11
WNWNEWLK	2	5	1	Q8NEB9		0.0000	0	0.0008799
LFHIDFGYILGR	2	3	1	Q8NEB9		0.0000	0	0.00005964
MNLSDVELIPLPLEPQVK	3	4	1	Q8NEB9		0.0000	0	3.565E-10
SSNFMYLMVEFR	1	3	1	Q8NEB9		0.0000	0	0.002487
HGDDLRQDQLILQIISLMDK	3	4	1	Q8NEB9		0.0000	0	6.294E-07