

Supplementary Information

HIF-1-dependent lipin1 induction prevents excessive lipid accumulation in choline-deficient diet-induced fatty liver

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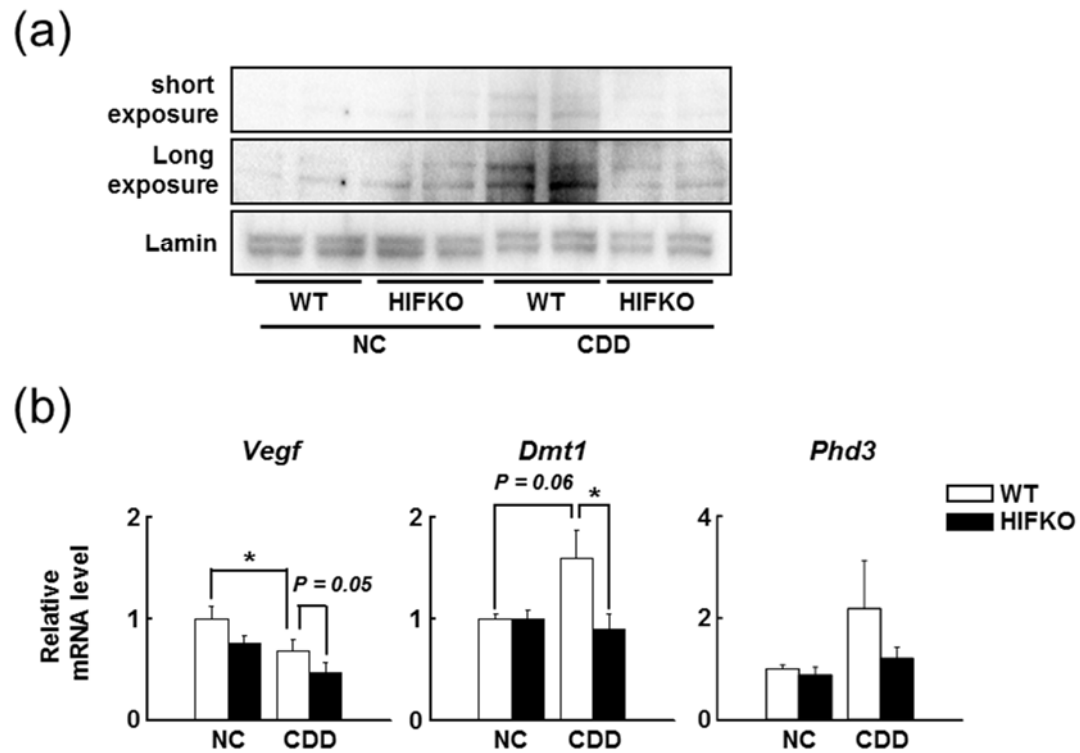
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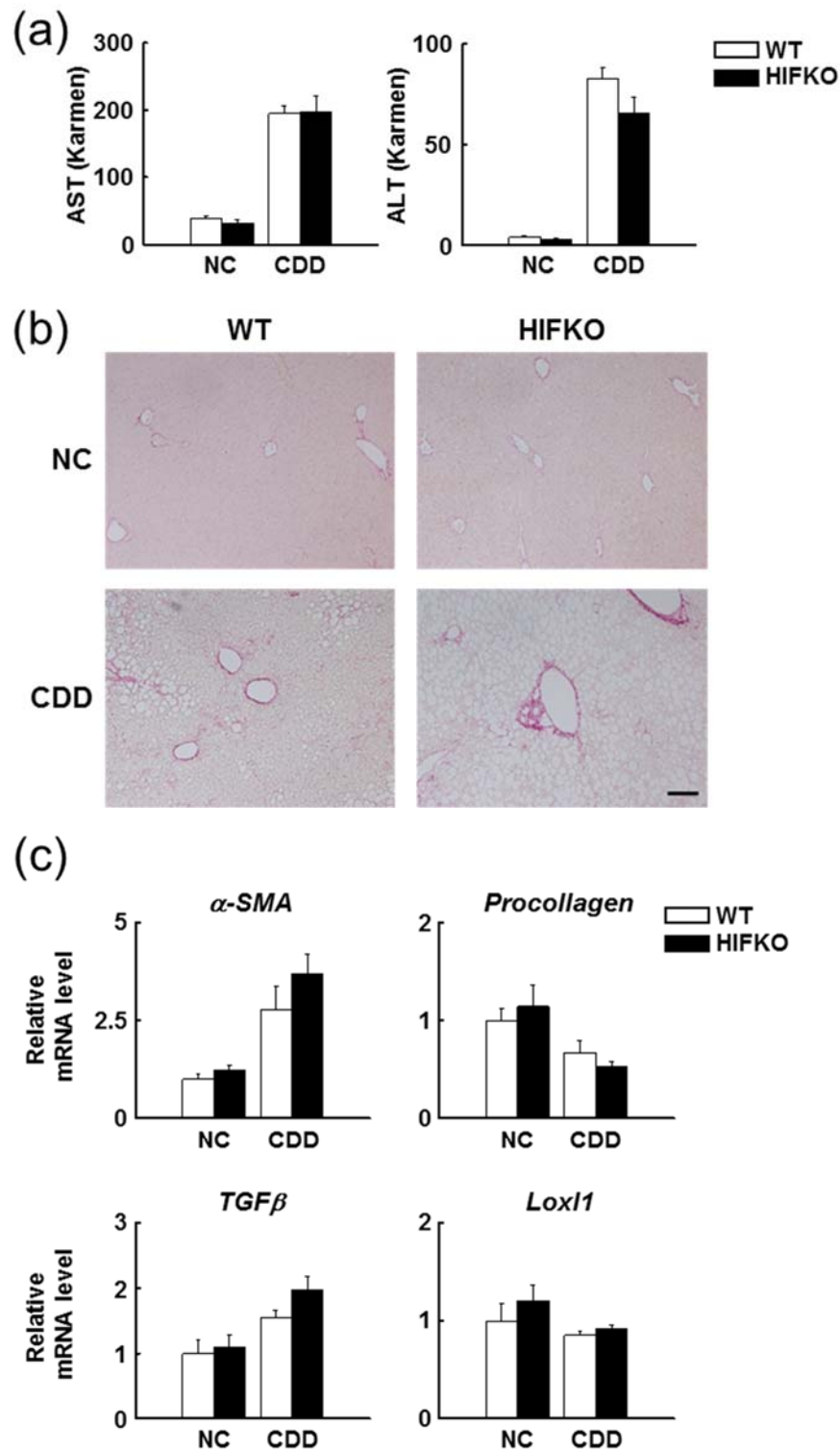
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Supplementary Figure S1. Exposure to a CDD activates HIF-1 α in mouse liver.

- (a) Representative immunoblots of HIF-1 α protein in livers of mice fed a CDD. NC, mice fed normal chow.
- (b) Expression of well-known HIF-1 target genes in livers treated with a CDD. NC and CDD denote samples collected from mice fed normal chow and a CDD, respectively; $n = 5-10$ mice per group. * $P < 0.05$.

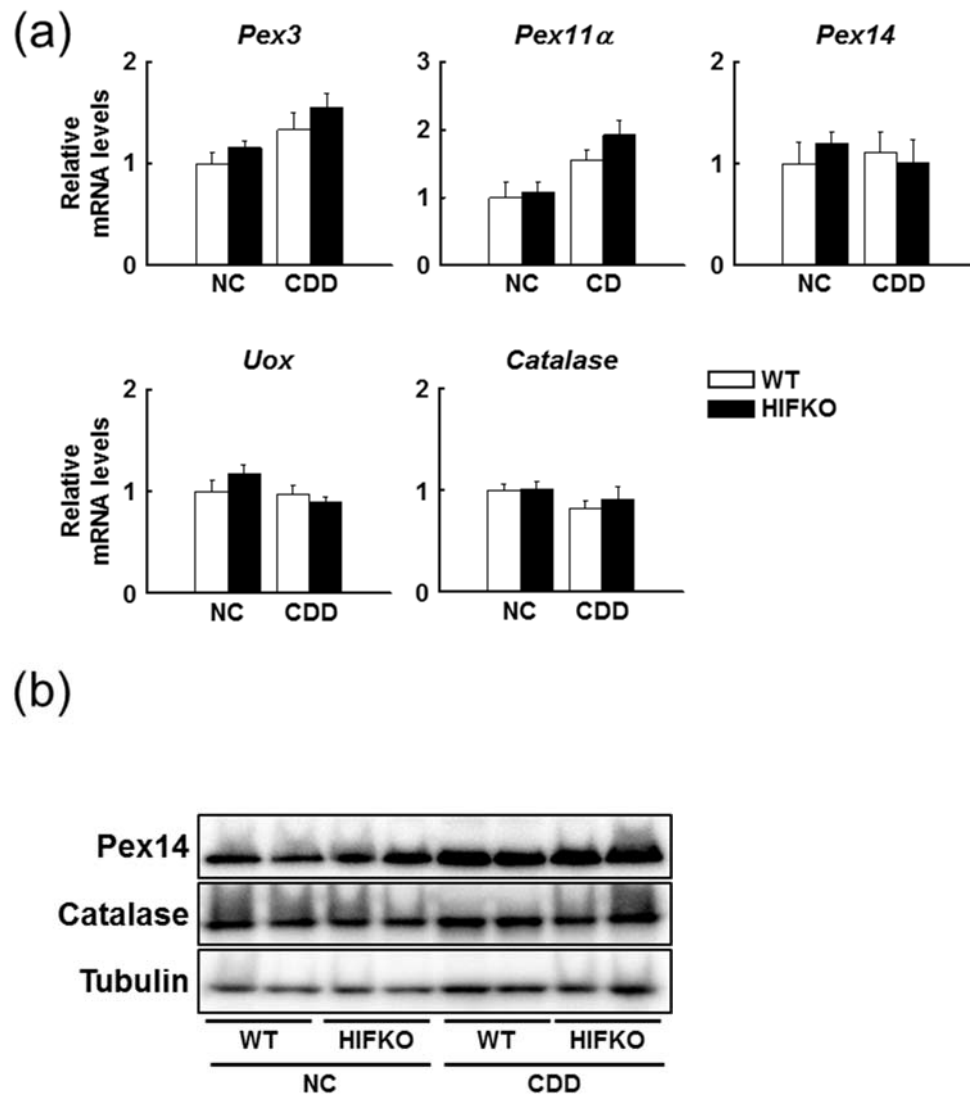


Supplementary Figure S2. Loss of *Hif-1 α* gene shows little effects on liver fibrosis of mice fed a CDD.

(a) Serum levels of AST and ALT in mice fed a CDD. n = 5 mice per group.

(b) Representative sirius red staining image of livers of WT and HIFKO mice fed a CDD. Scale bar is 20 μ m.

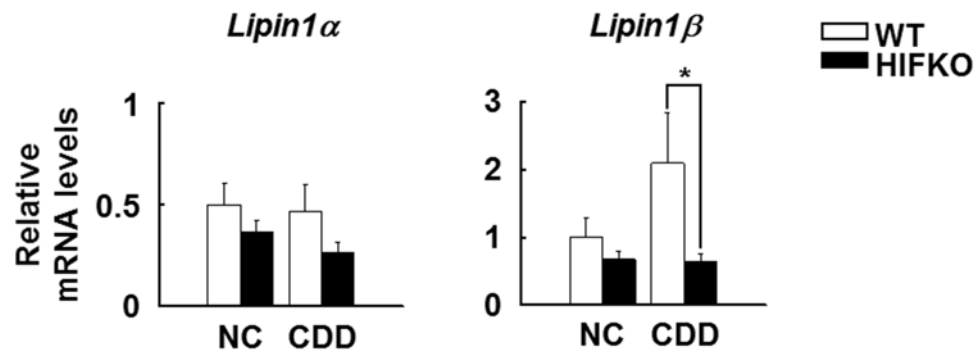
(c) Analysis of mRNA of liver fibrosis-related genes. NC and CDD denote samples collected from mice fed normal chow and a CDD, respectively; n = 5 mice per group.



Supplementary Figure S3. Liver expression of peroxisome-related genes is comparable between WT and HIFKO mice fed a CDD.

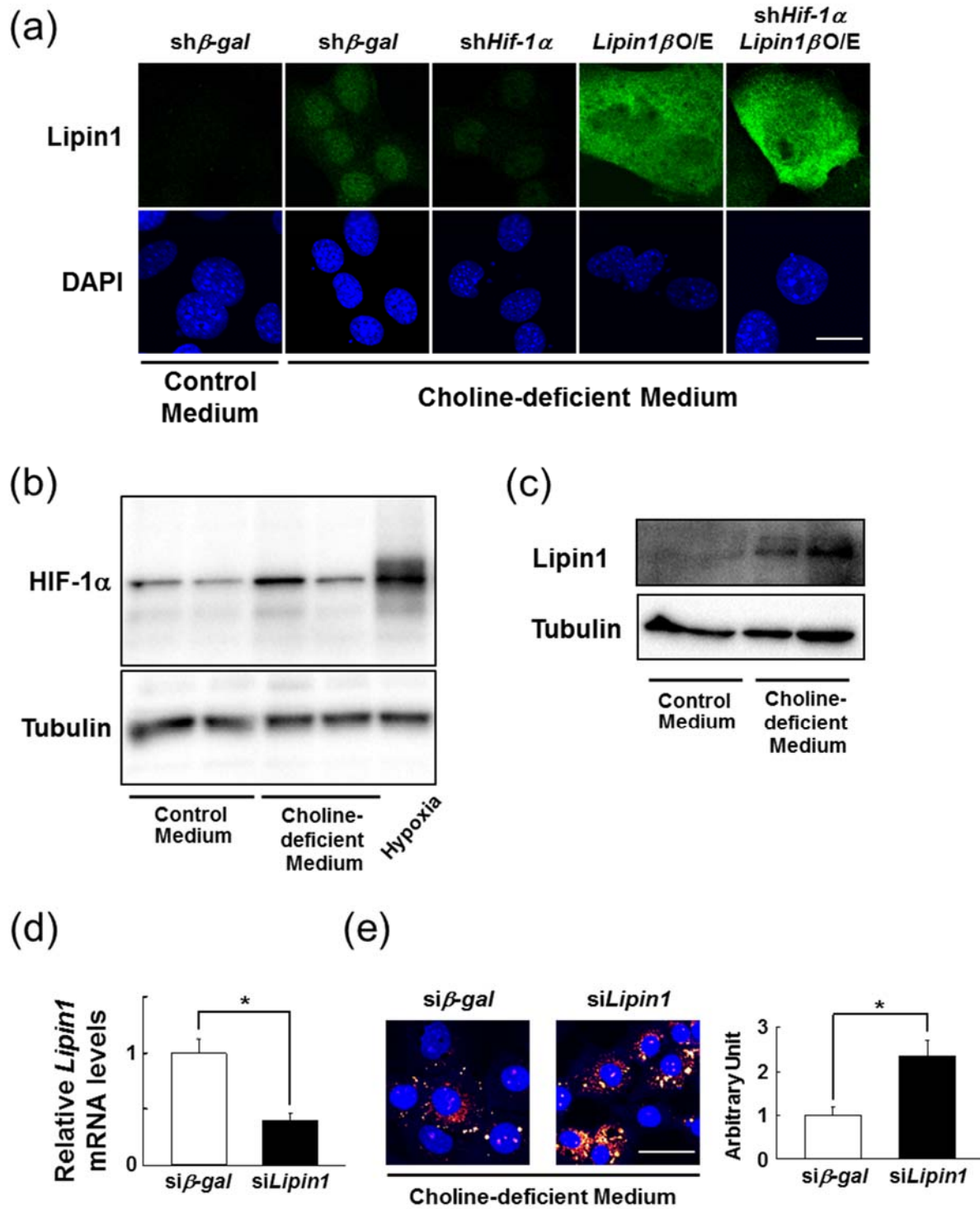
(a) Analysis of mRNA of peroxisomal biogenesis and functions-related genes. NC and CDD denote samples collected from mice fed normal chow and a CDD, respectively; n = 5 mice per group

(b) Representative images of protein expression of Pex14 and Catalase in CDD-treated liver.



Supplementary Figure S4. *Lipin1β* is induced in a HIF-1-dependent manner in mice fed a CDD.

The mRNA was quantified. NC and CDD denote samples collected from mice fed normal chow and a CDD, respectively; n = 5 mice per group. * $P < 0.05$.



Supplementary Figure S5. Suppression of *Lipin1* exacerbates lipid accumulation caused by choline deprivation.

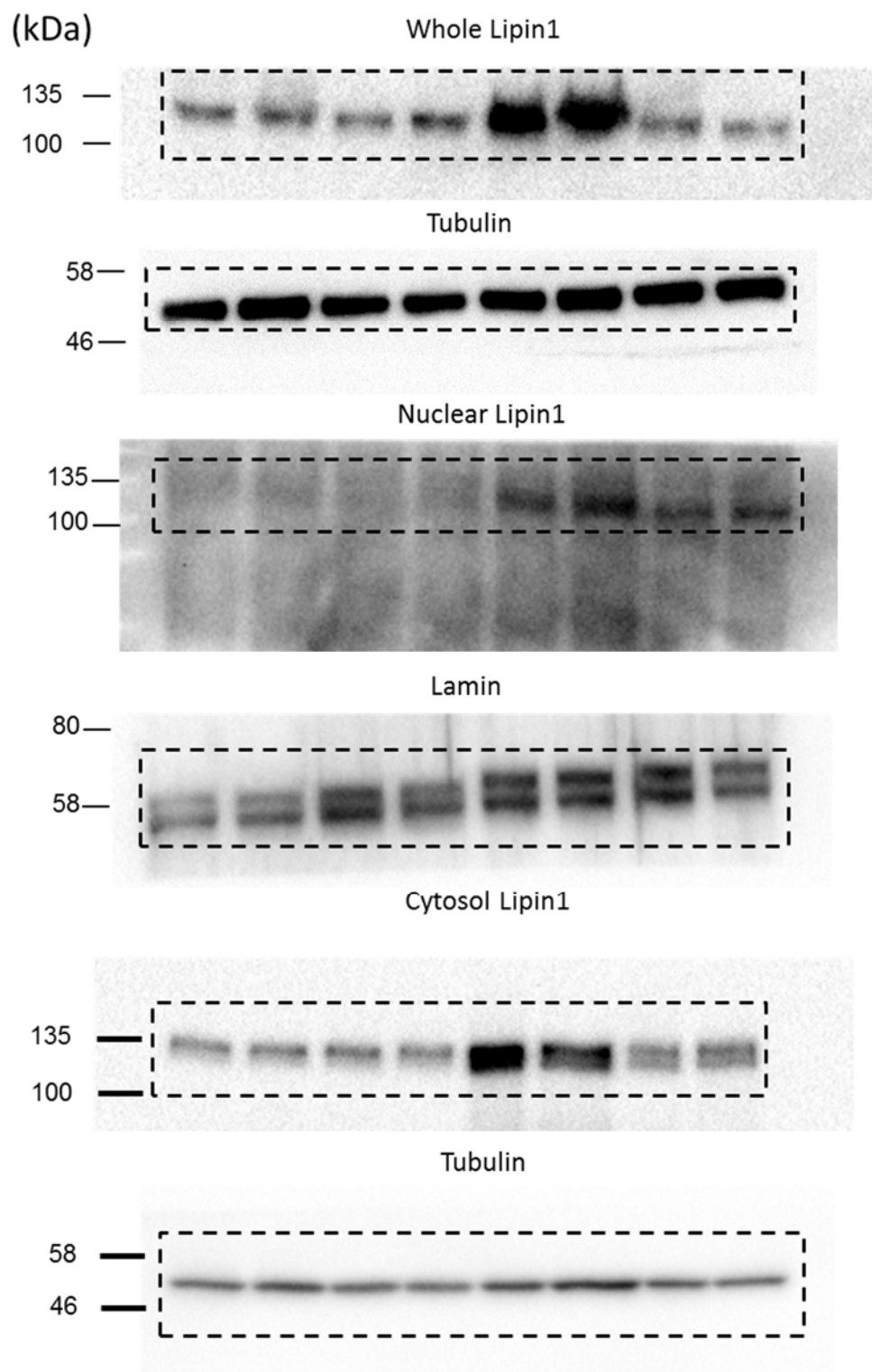
(a) Representative images of lipin1 (top) and DAPI (bottom) staining in AML12 cells treated with choline deficient medium. Scale bar = 20 μ m.

(b), (c) Representative immunoblots of HIF-1 α (b) and lipin1 (c) in AML12 cells exposed to choline deficient medium.

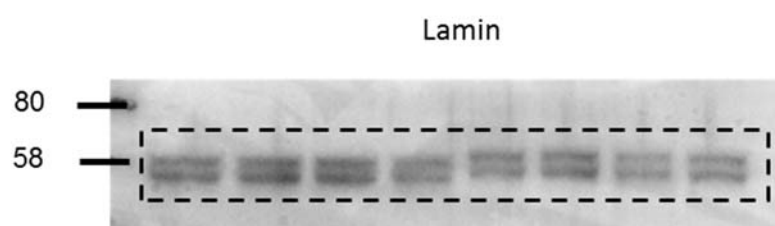
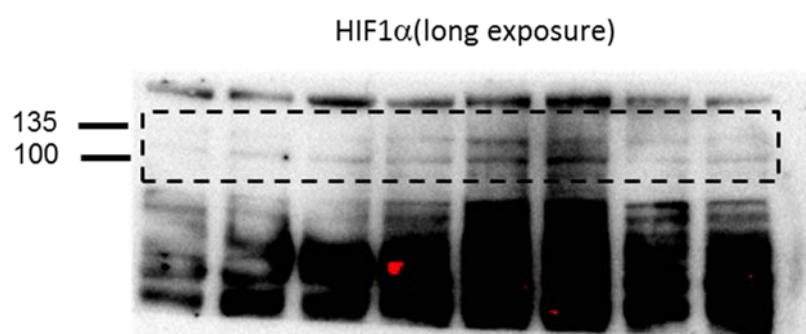
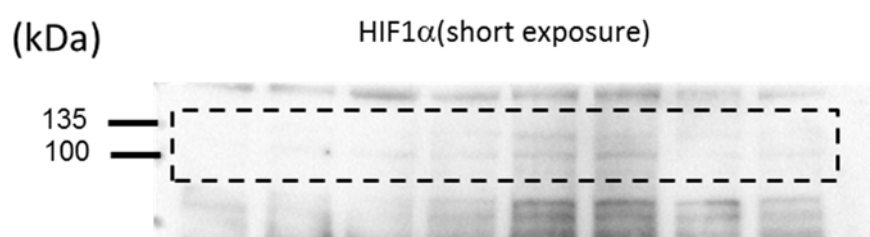
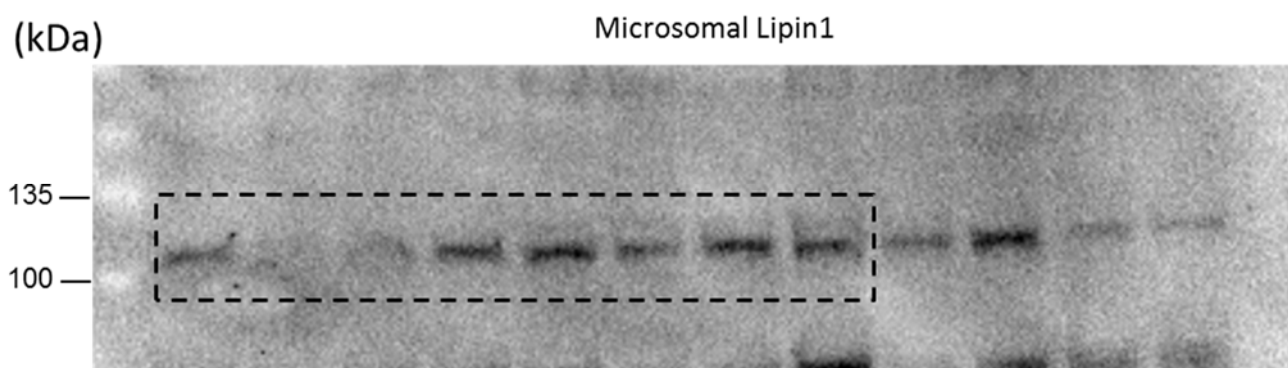
(d) Quantification of Lipin1 mRNA by qPCR in AML12 cells infected with adenovirus expressing shLipin1; n = 3 per group. * $P < 0.05$

(e) Representative images of Nile Red staining in Lipin1-knockdown cells treated with choline deficient medium. Red spots indicate lipid accumulation. DAPI staining is shown in blue. Scale bar is 25 μ m.

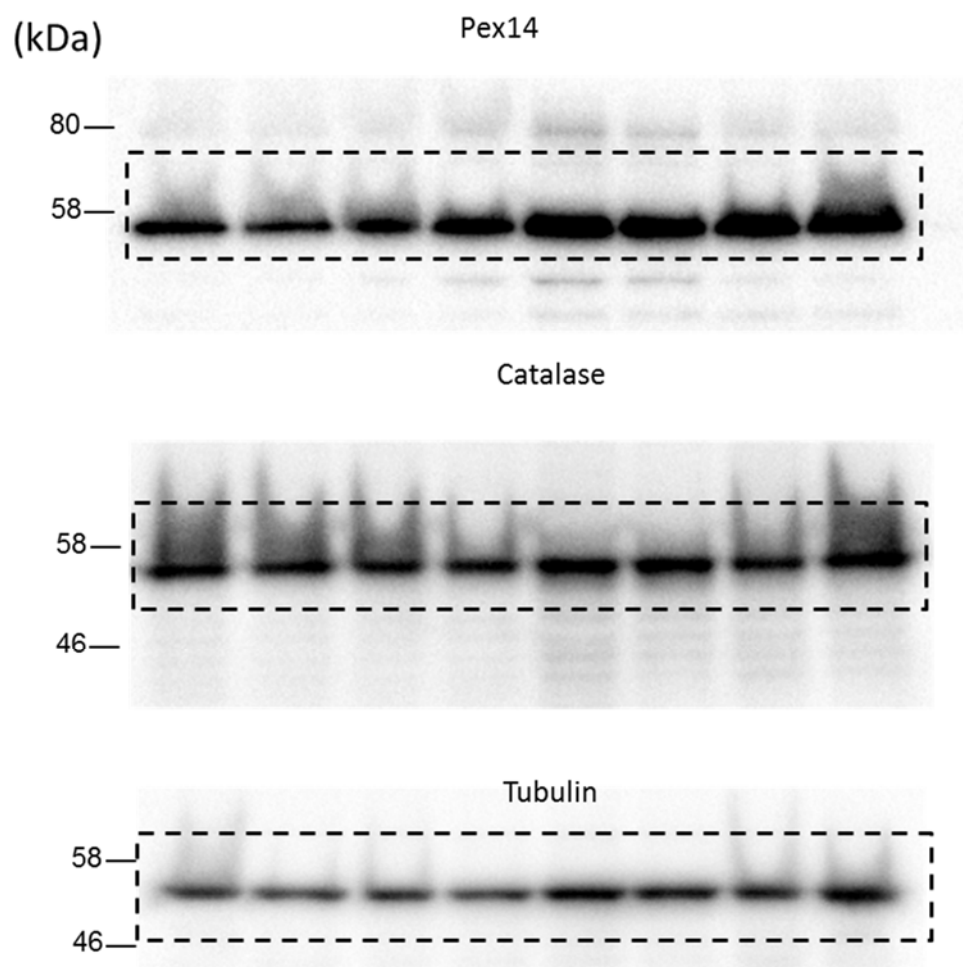
Densitometric quantification of lipid accumulation. n=4 plates per group. * $P < 0.05$.



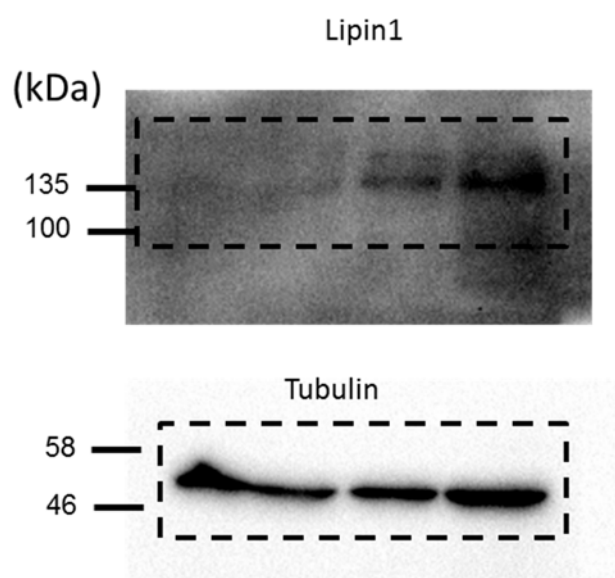
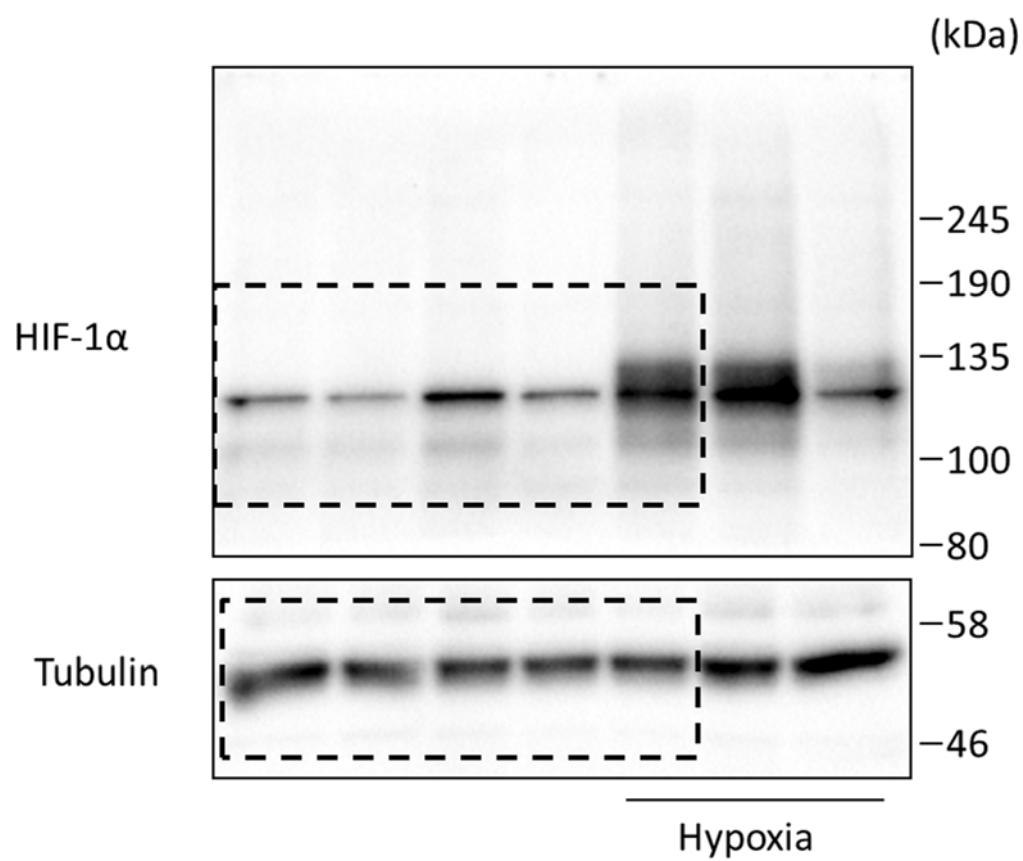
Supplementary Figure S6.



Supplementary Figure S6.



Supplementary Figure S6.



Supplementary Figure S6.

For qPCR assay

Gene name	AssayID	Gene name	AssayID	Gene name	AssayID	Gene name	AssayID	Gene name	AssayID
AOX1	Mm00443579_m1	Catalase	Mm01340251_m1	CPT1	Mm00550438_m1	LCAD	Mm00599860_m1	MCAD	Mm00431611_m1
MTP	Mm00435015_m1	PGC1 α	Mm00447183_m1	PPAR α	Mm00440939_m1	RXR α	Mm01332431_m1		

Gene name	Forward primer	Reverse primer	Probe
DGAT2	CTGGCTGATAGCTGTGCTCTACTT	AAAGGTGGCAGGAGATCGC	CTGGCATTGTACTGGAACACGCC
FASN	TCCTGGAACGAGAACACGATCT	GAGACGTGTCACTCTGGACTTG	AGCTCAAGCTGCGGAACTTCAG
SCD1	GGCCTGTACGGGATCATACTG	GGTCATGTAGTAGAAATCCGGAAGA	TTCCCTCTGCAAGCTCTACACCTGC
SREBP-1c	AGCCATGGATTGCACATTTG	GGCCCGGGAAATCACTGT	AGACATGCTCAGCTCATCAACAACAA

Gene name	Forward primer	Reverse primer	Gene name	Forward primer	Reverse primer
Abcd1	TGCGTGAGCATTGATGTG	TGCGATACCAGCATCTTTGG	ApoB	GAAGCGCACCAAGATCAAC	AAGGGTACACTGTTGGTCC
CD36	GATGTGGGACCCCTTCATG	CCGGGCTGAATTCCA	DMT1	AGGGCATGTGGCACTCTATG	CTCAGCAGGACTTTCGAGATG
FATP2	CAACACACCGCAGAAACCAA	CCATTTCOCAGGGCTTTTTT	HIF1	CACCGATTGCGCATGGA	TTGACGTTGAGAACTCATCTTT
Lipin1	OCAAGACCAACATCAGCTCGTA	CAATGGGAAGAGTGATGCA	Lipin1 α	GGTCCCCAGCCCCAGTCTT	GCAGCCTGTGGCAATTCA
Lipin1 β	CAGCCTGGTAGATTGCCAGA	GCAAGCCTGTGGCAATTCA	pex3	CCCTAGGCAACCCACACAAC	GGACTGTGCCAGGAAGATG
pex11 α	CCGAGTGGCCAAACCAAGC	GCCCAGTCTGAACATTTAGC	pex14	AGGCCGAGAGGACAGAAAGC	GGACAGAGGCCAGTGTGTC
PHD3	AATTGGGACGCCAAGTTACAC	GGAGGGCTGGACTTCATGTG	PPAR γ	GGGATGTCTCACAATGCCATC	GGGATGTCTCACAATGCCATC
Uox	GATCAAAACAGTCCATGCATTC	CGGGAGGTCCGTTTCTCATC	VEGF	AGTCCCATGAAGTGATCAAGTTCA	ATCCGCATGATCTGCATGG
β -actin	ACGGCCAGTGCATCACTATTG	CAAGAAGGAAGGCTGAAAAGA	18S	CTTTGCTCTGGTCCGTCTT	TTGTTATTGGCCGCTAGA

For ChIP-qPCR assay

Gene name	Forward primer	Reverse primer
AOX-1	TCTAACGTCAGTCAAGTCGGC	CGGGGTGAGGAGGCTAATAGA
AOX-2	TGAACCTGGAGACGTGAGGT	GGCTCCTCGCCAAAGGAAA
PPAR α	TCAACCATCTCCAGGGTCTCAG	ACTATGCTATTGTGGCGATGAGC
MCAD	GATTTCTTCTCAGTCTCCT	CGGAGAAGAAGACTGTGTGC

Supplementary Table S1. Primer and probe sequences