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online data

SUPPLEMENTARY ONLINE DATA

Endoplasmic reticulum factor ERLIN2 regulates cytosolic lipid content in cancer cells

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Origins and culture conditions for the cancer cell lines used in the present study

The SUM44 cell line was established from pleural effusion-derived breast cancer cells [1]. SUM44 cells were cultured in Ham's F12 medium supplemented with 0.1 % BSA, fungizone (0.5 μg/ml), gentamicin (5 μg/ml), ethanolamine (5 mmol/l), Hepes (10 mmol/l), transferrin (5 μg/ml), T3 (3,3′,5-triiodo-L-thyronine; 10 μmol/l), selenium (50 μmol/l), hydrocortisone (1 μg/ml) and insulin. The SUM225 cell line was established from a chest wall recurrence of ductal carcinoma *in situ* of breast [2]. SUM225 cells were cultured with 5 % FBS, fungizone (0.5 μg/ml), gentamicin (5 μg/ml), hydrocortisone (1 μg/ml) and insulin (5 μg/ml). Each of these cell lines, from a single human patient, represents a different subtype of breast cancer [1, 2]. All of the currently known oncogenes with altered expression patterns in breast cancer are well represented and have been characterized in the SUM lines. These cell lines have been described in over 50 peer-reviewed publications in cancer research.

MCF10A is a spontaneously immortalized, but non-transformed, human mammary epithelial cell line derived from the breast tissue of a 36-year-old patient with fibrocystic changes [3]. MCF10A cells were cultured in Ham's F12 medium supplemented with 0.1 % BSA, fungizone (0.5 μg/ml), gentamicin (5 μg/ml), ethanolamine (5 mmol/l), Hepes (10 mmol/l), transferrin (5 μg/ml), T3 (10 μmol/l), selenium (50 μmol/l), hydrocortisone (1 μg/ml), insulin (5 μg/ml) and 10 ng/ml epidermal growth factor.

Huh-7 is a hepatocellular carcinoma cell line that was originally derived from a liver tumour in a Japanese male [4]. This cell line was cultured in DMEM containing 10 % FBS, L-glutamine and antibiotics at 37 °C in a 5 % CO_2 environment.

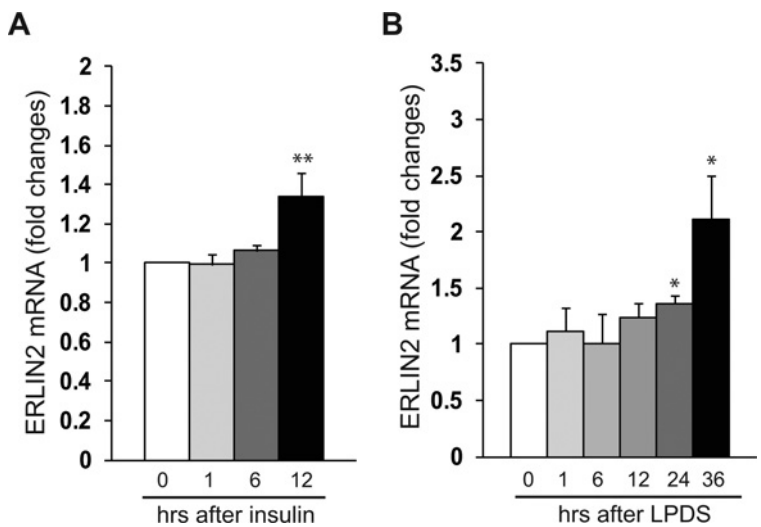

Figure S1 Quantitative real-time RT–PCR analysis of *ERLIN2* mRNA expression in murine primary hepatocytes in response to insulin or LPDS challenge

(**A**) Murine primary hepatocytes were challenged with insulin (100 nM) for 1, 6 or 12 h. Murine primary hepatocytes were cultured in normal medium with vehicle buffer PBS added as a control (0 h under insulin). (**B**) Murine primary hepatocytes were cultured in medium containing LPDS for 1, 6, 12, 24 or 36 h. As a control, murine primary hepatocytes were cultured in normal medium containing 10 % FBS (0 h under LPDS). For (**A** and **B**), total RNAs were isolated from the primary hepatocytes after the treatment, and quantitative real-time RT–PCR was performed to determine *ERLIN2* mRNA expression levels in the primary hepatocytes after insulin or LPDS challenge. The mRNA expression values were determined after normalization to internal control *GAPDH* mRNA levels. The baseline of the *ERLIN2* mRNA level in the hepatocytes challenged with insulin or LPDS at 0 h was set to 1. After the treatments, fold changes of *ERLIN2* mRNA levels in the hepatocytes were calculated by comparison to the baseline mRNA level. Results are means ± S.E.M. ($n = 3$ experiments). *$P < 0.05$; **$P < 0.01$.

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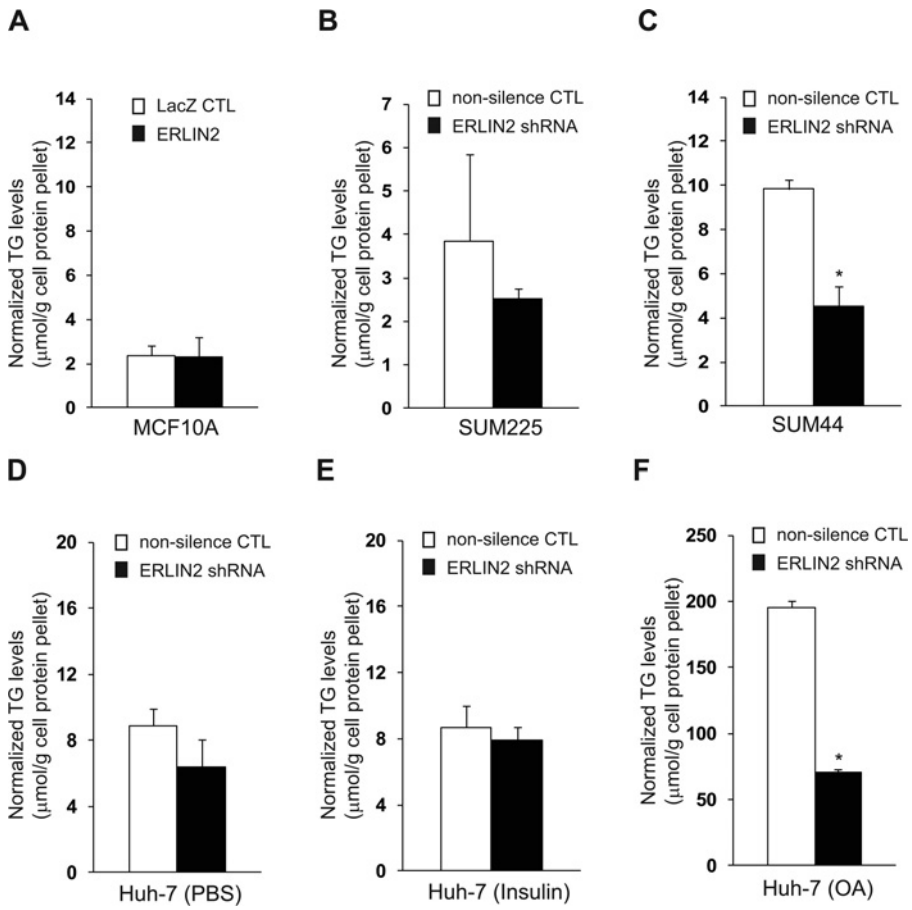

Figure S2 Biochemical quantification of cellular TG levels in MCF10A, SUM225, SUM44 and Huh-7 cells

Levels of total cellular TG were determined using a TG measurement kit from BioAssay System. TG levels were presented after normalization to net weights of cellular pellets. For Huh-7 cells, ERLIN2 knockdown or control (CTL) stable cell lines were incubated with vehicle PBS, insulin (100 nM), or oleic acid (OA) (0.5 mM) for 12 h. (**A**) Cellular TG levels in MCF10A cells expressing exogenous LacZ or ERLIN2. (**B**) Cellular TG levels in ERLIN2 knockdown or control SUM225 cells. (**C**) Cellular TG levels in ERLIN2 knockdown or control SUM44 cells. (**D**) Cellular TG levels in ERLIN2 knockdown or control Huh-7 cells treated with PBS vehicle. (**E**) Cellular TG levels in ERLIN2 knockdown or control Huh 7 cells treated with insulin. (**F**) Cellular TG levels in ERLIN2-knockdown or control Huh-7 cells incubated with oleic acid. Results are means $\pm$ S.E.M. ($n = 3$). *$P < 0.05$.

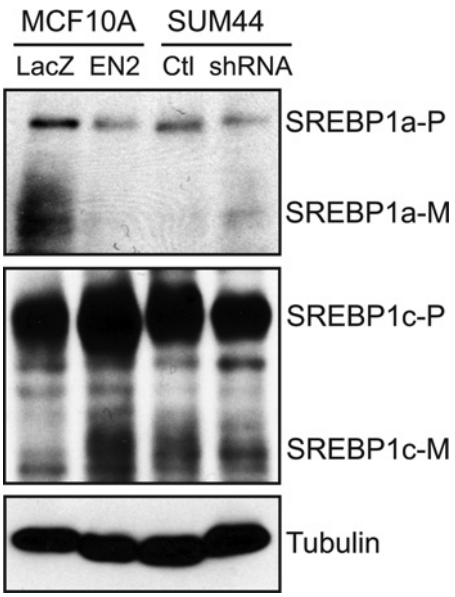

Figure S3 Western blot analysis of SREBP1c and SREBP1a protein levels in MCF10A expressing LacZ control or ERLIN2 and in ERLIN2 knockdown or control SUM44 cells

Tubulin was included as a loading control. LacZ, LacZ overexpression; EN2, ERLIN2 overexpression; Ctl, non-silence control; shRNA, ERLIN2 shRNA knockdown; SREBP1a-P, SREBP1a precursor; SREBP1a-M, mature SREBP1a; SREBP1c-P, SREBP1c precursor; and SREBP1c-M, mature SREBP1c. Note that our data showed that SUM44 and MCF10A cells only express trace levels of SREBP1a, which is consistent with the published conclusion that SREBP1c, but not SREBP1a or SREBP2, is induced in human breast cancer cell lines [5]. Interestingly, our data shows that expression levels of SREBP1a were decreased in MCF10A cells overexpressing ERLIN2, whereas SREBP1a levels were increased in ERLIN2 knockdown SUM44 cells. The correlation of SREBP1a levels with ERLIN2 induction and malignancy states is an interesting question to be elucidated in the future.

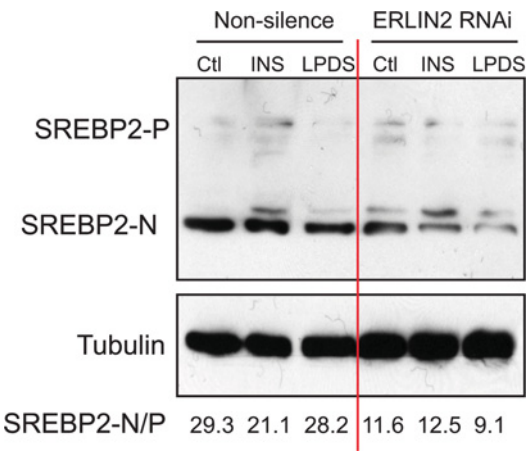

Figure S4 Western blot analysis of cleaved SREBP2 protein levels in Huh-7 cells that were transduced by non-silencing or ERLIN2 shRNAi lentivirus

The Huh-7 cells were treated with vehicle PBS or insulin (100 nM) for 6 h or cultured in LPDS-containing medium for 12 h. Tubulin was included as a loading control. SREBP2 protein signals were detected by using an antibody against the N-terminal SREBP protein fragment (Cayman Chemicals). The values below the gels represent the ratios of mature cleaved SREBP2 to SREBP2 precursor signal intensities. Ctl, control cells treated with vehicle PBS; INS, insulin; SREBP2-N, cleaved SREBP2 (N-terminal); SREBP2-P, SREBP2 precursor.

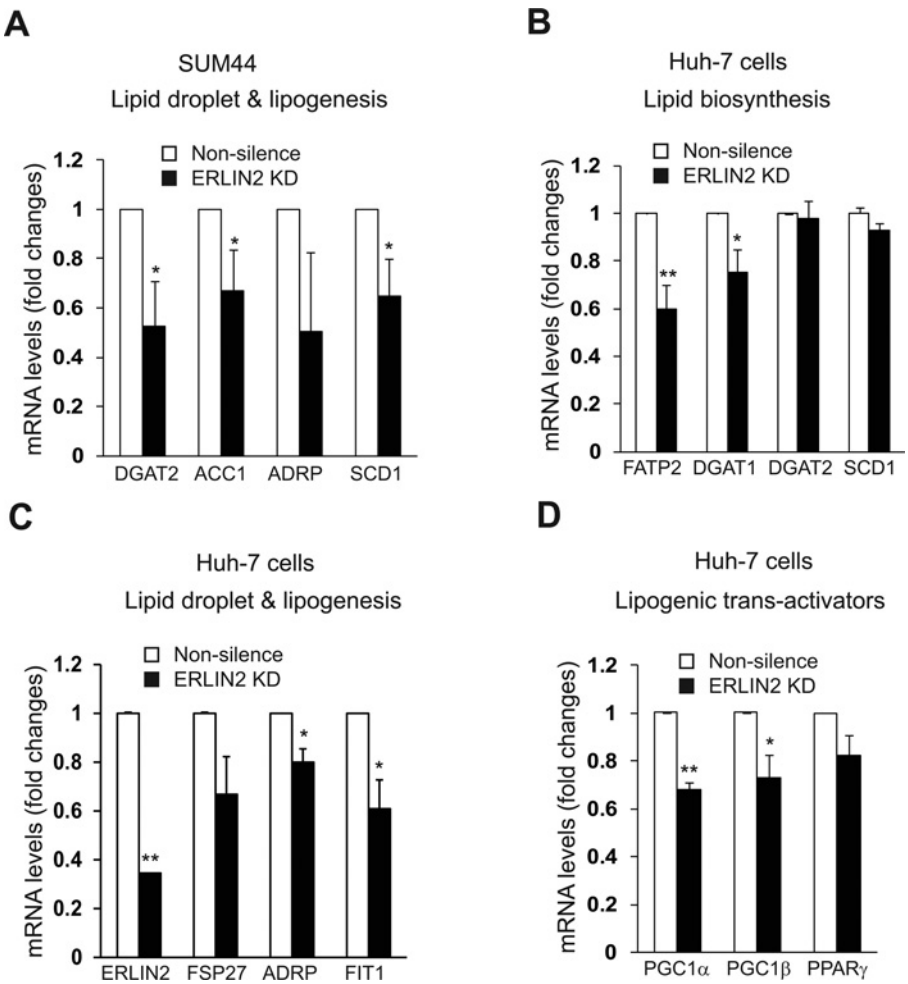

Figure S5 Quantitative real-time RT–PCR analysis of expression of lipogenic genes in ERLIN2 knockdown and non-silenced control Huh-7 or SUM44 cells

(**A**) Expression of the genes involved in lipid droplet formation and lipogenesis, including *DAGT2*, *ACC1*, *ADRP*, and *SCD1*, in ERLIN2-knockdown and control SUM44 cells. (**B**) Expression of the genes involved in lipid biosynthesis, including *FATP2*, *DGAT1*, *DAGT2* and *SCD1*, in ERLIN2 knockdown and control Huh-7 cells. (**C**) Expression of the *ERLIN2* gene and the genes involved in lipid droplet formation and lipogenesis, including *FSP27*, *ADRP* and *FIT1*, in ERLIN2 knockdown and control Huh-7 cells. (**D**) Expression of the genes encoding the lipogenic *trans*-activators PGC1α, PGC1β and PPARγ2 in ERLIN2 knockdown and control Huh-7 cells. For (**A**–**D**), total RNAs were isolated from the cells, and quantitative real-time RT–PCR was performed to determine mRNA expression levels. The mRNA expression values were determined after normalization to internal control *GAPDH* mRNA levels. To determine the expression profile for a particular gene, the baseline mRNA level in control cells was set to 1. Fold changes in the mRNA levels of the ERLIN2-knockdown cells were calculated by comparison with the baseline mRNA level. Results are means $\pm$ S.E.M. ($n = 3$ experimental repeats). $^{*}P < 0.05$; $^{**}P < 0.01$. ACC1, acetyl-CoA carboxylase 1; ADRP, adipose differentiation-related protein; FATP2, fatty acid transport protein 2; FIT1, fat-inducing transcript 1; FSP27, fat-specific protein 27; PPAR, peroxisome-proliferator-activated receptor.

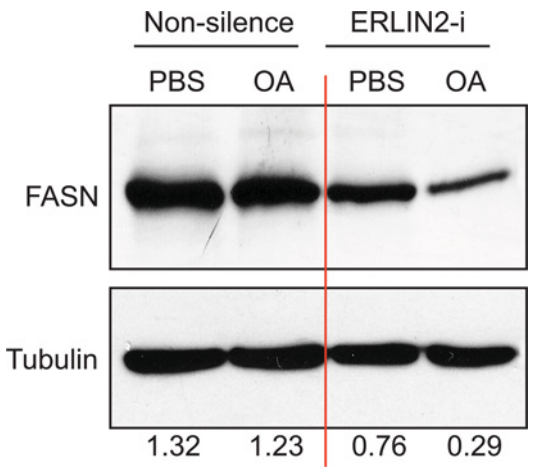

Figure S6 Western blot analysis of FAS protein levels in Huh-7 cells that were transduced by non-silencing or ERLIN2 shRNAi lentivirus in the presence or absence of oleic acid treatment

The non-silencing control or ERLIN2 knockdown Huh–7 cells were treated with vehicle PBS or oleic acid (OA) (0.5 mM) for 12 h. Tubulin was included as a loading control. The values below the gels represent the ratio of FAS to tubulin signal intensities.

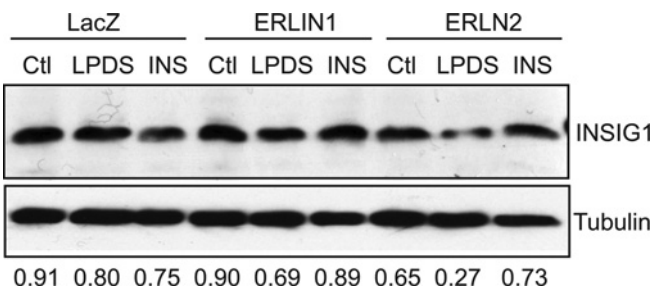

Figure S7 Western blot analysis of INSIG1 protein levels in the Huh-7 cell line that was transduced by lentivirus overexpressing LacZ, ERLIN1 or ERLIN2

Cell lysates were prepared from the Huh-7 cell lines cultured in normal medium (Ctl), LPDS medium for 12 h, or challenged with insulin (INS, 100 nM) for 6 h. Tubulin was included as a loading control. The values below the gels represent INSIG1 signal intensities after normalization to tubulin signal intensities.

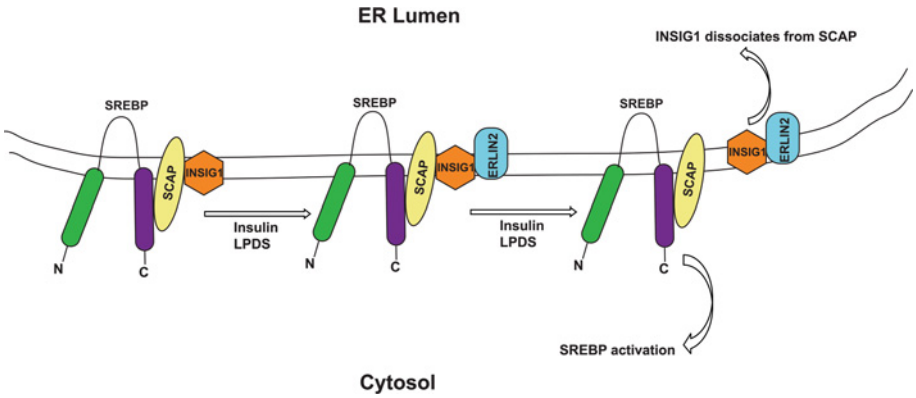

Figure S8 A working model for regulation of SREBP activation by ERLIN2

In response to insulin or LPDS challenge, ERLIN2 interacts with the INSIG1–SCAP binding complex by directly binding to INSIG1. The interaction between ERLIN2 and INSIG1 facilitates the dissociation of SCAP from INSIG1, thus promoting SREBP–SCAP complex release from the ER to Golgi for SREBP processing.

Table S1 Sequence information for the real-time PCR analysis

ACC1, acetyl-CoA carboxylase 1; ACTB, β-actin; ADRP, adipose differentiation-related protein; FATP2, fatty acid transport protein 2; FIT1, fat-inducing transcript 1; FSP27, fat-specific protein 27.

Gene symbol	Forward primer (5′→3′)	Reverse primer (5′→3′)
PGC1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
PGC1β	AGAAGCTCCTCCTGGCCACATCCT	GCCTTCTTGTCTTGGGTGCTGTCC
ERLIN2	GAACCAGTGGTGGTGTGATG	TATGAACGCTGCAGAACTGG
FSP27	GAGTCCAACGCAGTCCAGCTGAC	GCAGCTGCTGGGTCACCACAG
ACC1	AGGGCTAGGTCTTTCTGGAAGTGGA	TCAGCTCCAGAGGTTGGGCCA
DGAT1	CCGTGAGCTACCCGGACAAT	AGGATCCGTCGCAGCAGAA
DGAT2	TTTCGAGACTACTTTCCCATCCA	TGGCCTCTGTGCTGAAGTTG
ADRP	GATGGCAGAGAACGGTGTGAA	TCAATCCTGTCTAGCCCCTTACAG
SCD1	CTGCCCCTACGGCTCTTTCT	ACGTCGGGAATTATGAGGATCA
PPARγ2	CCTATTGACCCAGAAAGCGATT	CATTACGGAGAGATCCACGGA
FIT1	TTCGCCAGCCACGGCAACTT	GCGCCGTGTAGCCAGGAACA
FATP2	CCACAGGTCTTCCAAAAGCAGCCA	GTGCAGCACTGTGGTAAAAGGGCA
ACTB	AGCCTCGCCTTTGCCGATCCG	ACATGCCGGAGCCGTTGTCGA

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