SUPPLEMENTARY DATA

Elevated Medium Chain-Acylcarnitines are Associated with Gestational Diabetes and Early Progression to Type-2 Diabetes and Induce Pancreatic β-Cell Dysfunction

Short title: Elevated M-acylCs Impair β-Cell Function

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Supplementary Table 1. Characteristics of SWIFT cohort women with recent GDM who developed IGT, T2D or returned to normoglycemia during 2-year follow up.

Characteristics	NGT (n=11)	IGT (n=13)	T2D (n=12)	p-Value
Age (years)	35.45±1.37	34.92±1.77	35.66±1.6	0.94
BMI (kg/m^2)	33.6±1.24	35.65±1.25	37.17±1.65	0.22
FPG (mg/dl)	91.18±1.81	107.79±2.88	124.75±6.06	<0.001
2hPG, mg/dl	97.9±7.15	155.3±6.68	205.91±14.86	<0.001
Insulin (mU/ml)	30.12±6.68	44.21±5.92	51.77±6.47	0.07
Triglycerides (mg/dl)	89.9±9.85	165.69±28.98	154.25±29.34	0.09
HDL-C (mg/dl)	50.18±3.24	44.23±3.2	41.5±2.33	0.13
HOMA-IR	6.96±1.65	11.93±1.68	16.41±2.31	0.006
HOMA-B	357.87±64.33	359.56±48.56	322.75±59.28	0.95

Supplementary Table 2. Primers used for qPCR

Gene name	Forward (5'-3')	Reverse (5'-3')
mGlut2	CCTTGGGCCTTACGTGTTCT	TTGTACAGCAGCTTTGCGTG
mGCK	ATGCTGGATGACAGAGCCAG	CCACGGTCCATCTCCTTCTG
mUcp1	CACGGGGACCTACAATGCTT	ACAGTAAATGGCAGGGGACG
mUcp2	AAGTGTTTCGTCTCCCAGCC	GGGACCTTCAATCGGCAAGA
mUcp3	GACCCACGGCCTTCTACAAA	TCAAAACGGAGATTCCCGCA
mB-actin	ATGTGGATCAGCAAGCAGGA	AGCTCAGTAACAGTCCGCCTA

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SUPPLEMENTARY DATA

Supplementary Table 3. Antibodies used for western blotting

Antibody	**Dilution**	**Supplier**	**Cat.Number**
Anti-GLUT2	1:1000	Millipore	07-1402
Anti-UCP3	1:1000	Abcam	ab180643
Anti-Total Oxphos	1:250	Mitosciences	ab110413
Anti- β Actin	1:1000	Cell Signaling	4967S

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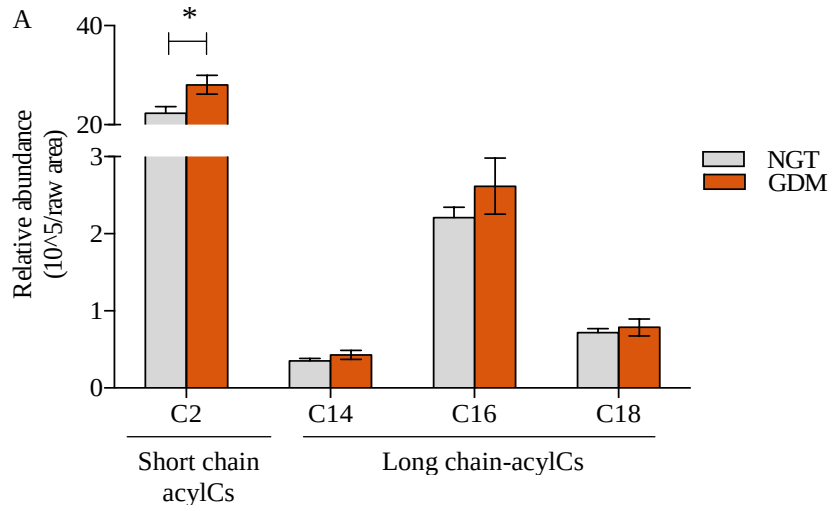
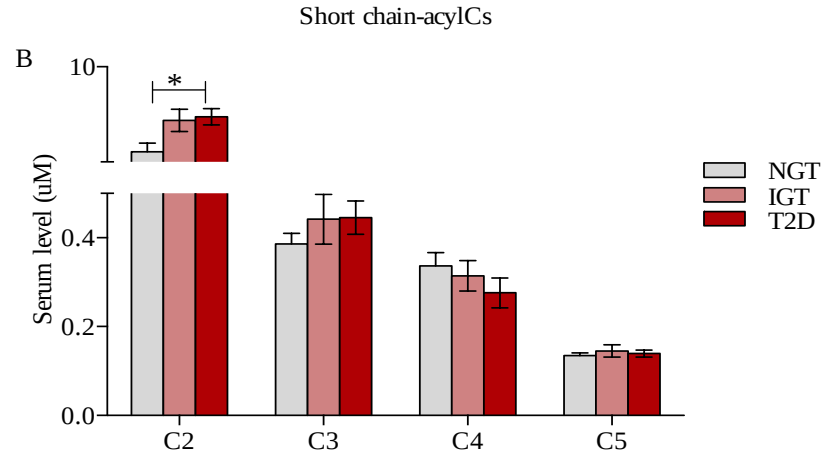
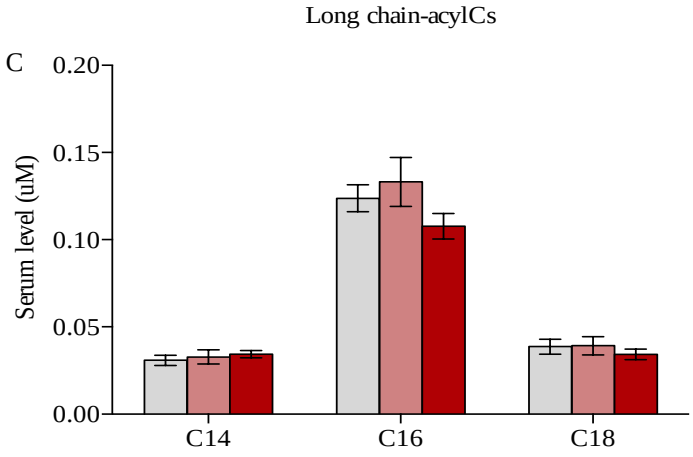
SUPPLEMENTARY DATA

Supplementary Figure 1. (A) Relative abundance of short chain- and long chain-acylCs in GDM patients (n=12/group); Circulating (B) short chain- and (C) long chain-acylCs in IGT and T2D patients (n=11 for NGT, 13 for IGT and 12 for T2D) (n=11 for NGT, 13 for IGT and 12 for T2D)







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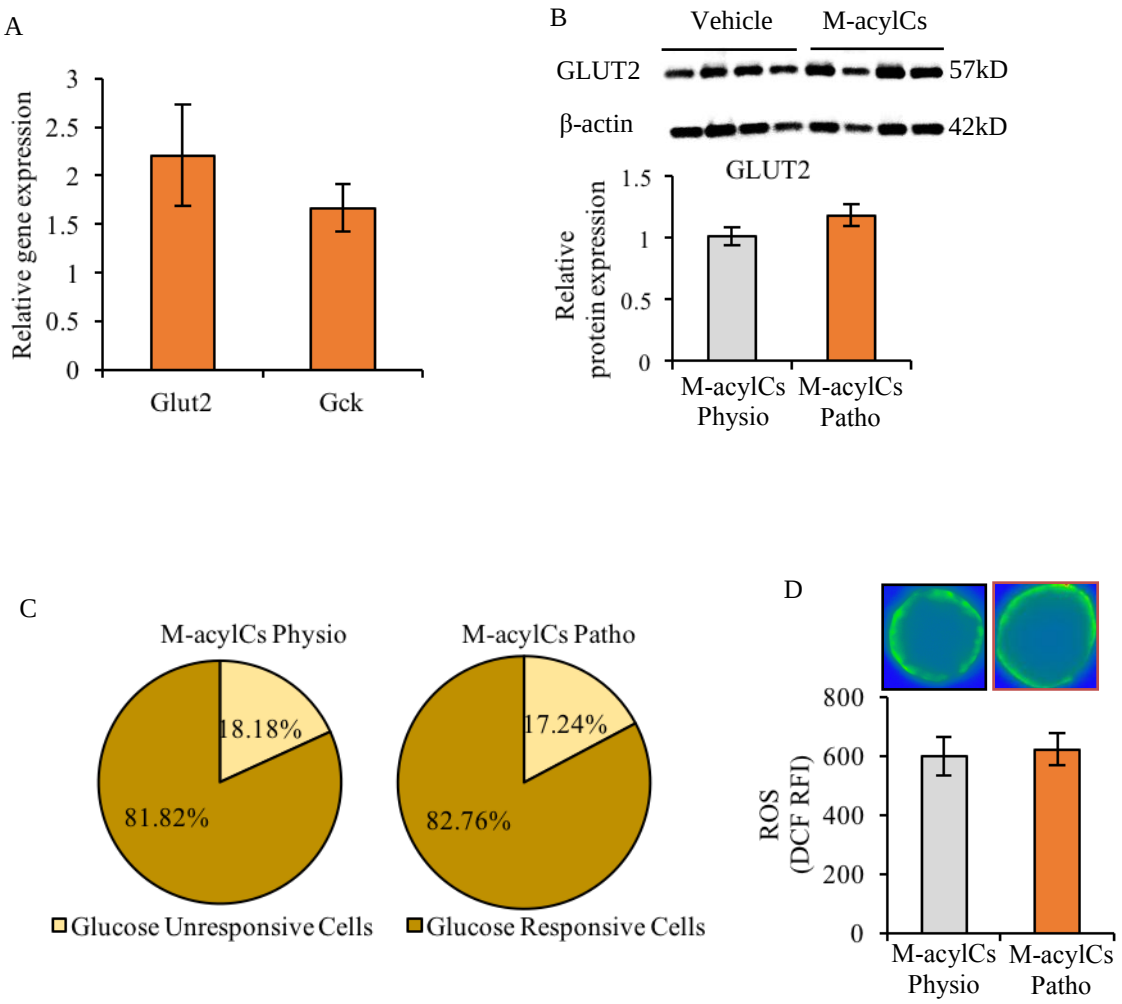
SUPPLEMENTARY DATA

Supplementary Figure 2. (A) Glut2 relative gene expression in murine islets isolated from mice injected with and without M-acylCs for 2 weeks (n=4/group); (B) western blot of GLUT2 expression and its quantification in islets isolated from mice injected with M-acylCs (n=4/group).(C) percentage of glucose responsive cells during calcium flux analysis in murine islets treated with and without M-acylCs in vitro (n=68, 84 individual cells). (D)ROS level were evaluated in murine islets treated in vitro (n=6/group). Data are presented as mean±SEM; *p<0.05, **p<0.01.



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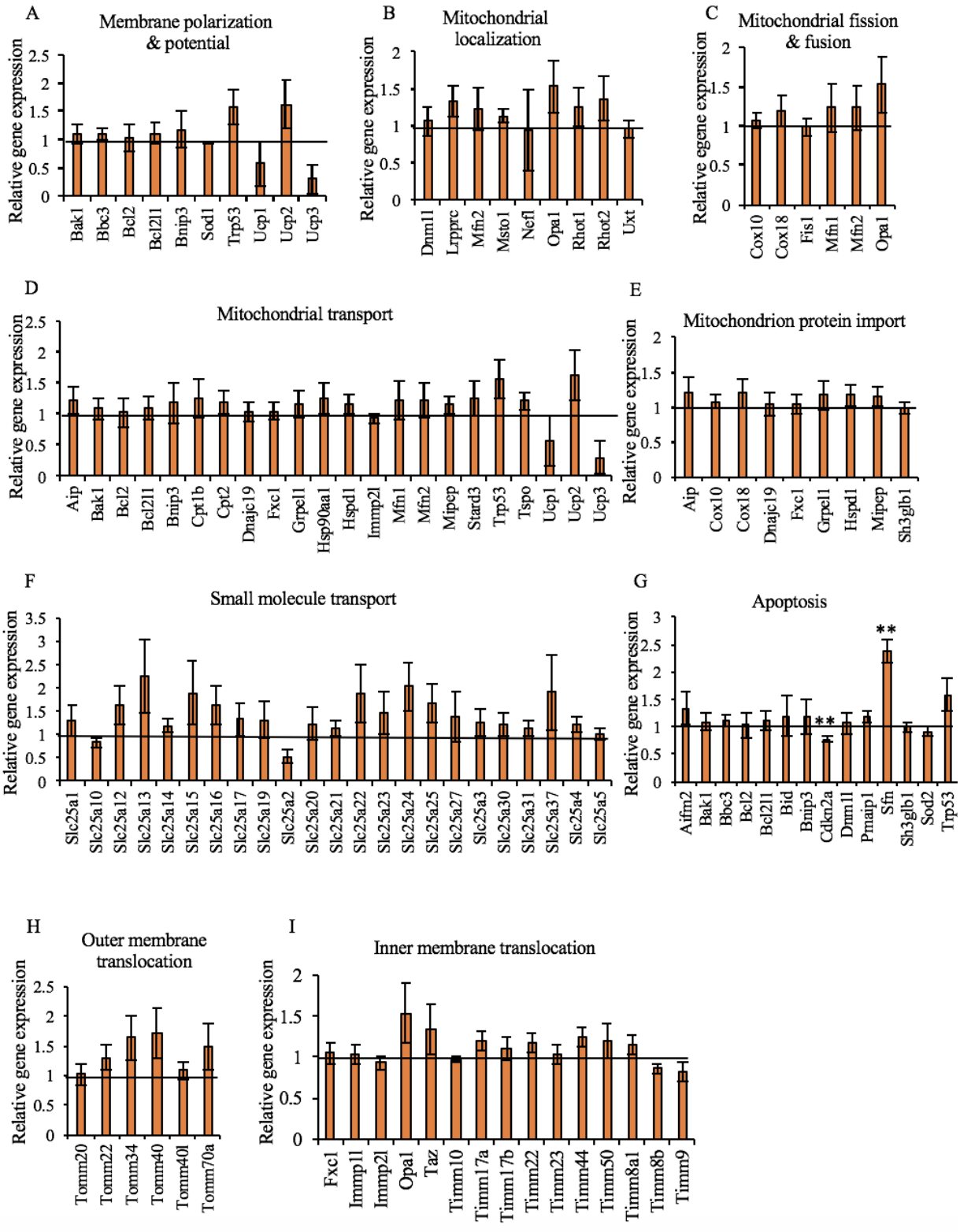
SUPPLEMENTARY DATA

Supplementary Figure 3. Mitochondrial gene-array was performed in murine islets treated with and without acylCs*in vitro* and relative gene expression of mitochondrial genes is shown by their functional group (n=3/group). Data are presented as mean±SEM; **p<0.01.



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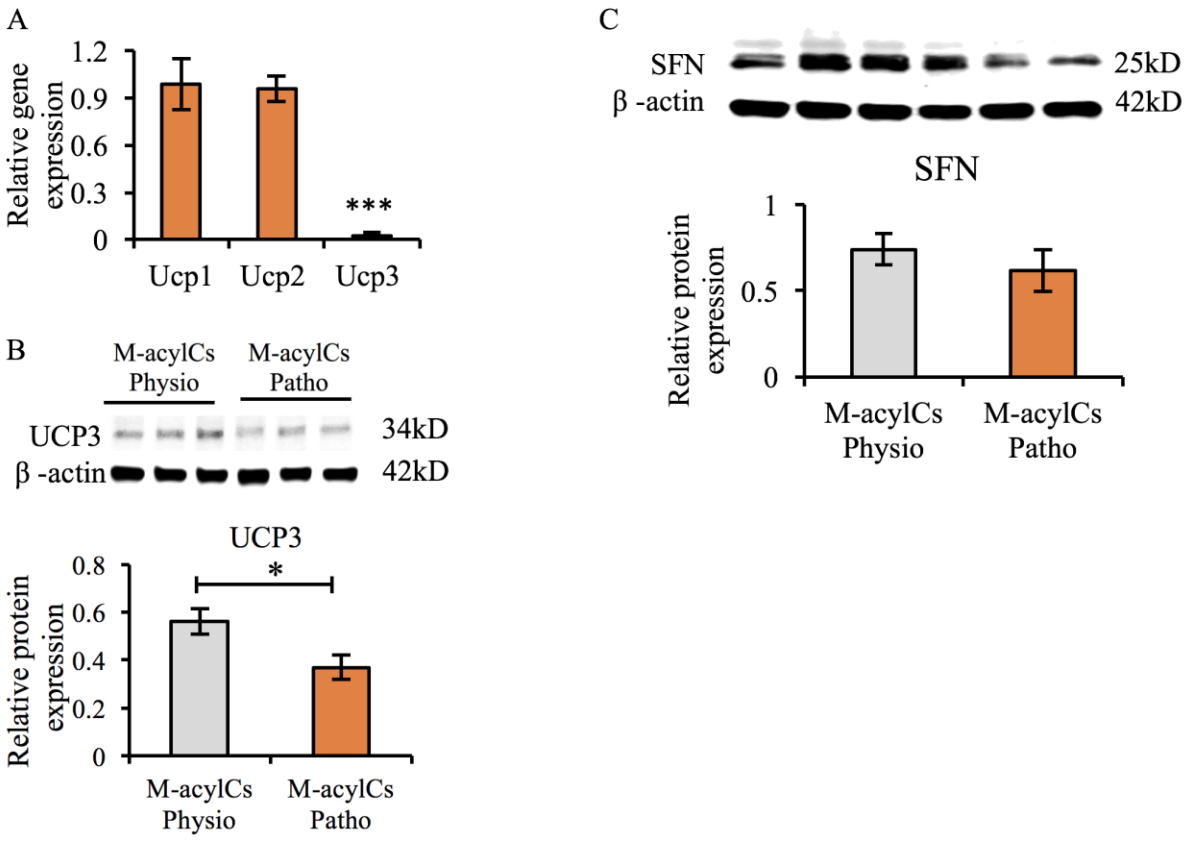
SUPPLEMENTARY DATA

Supplementary Figure 4. (A) Gene expression of uncoupling proteins, (B) a presentative western blot of UCP3 expression and its quantification (n=3/group); (C) A representative western blot of stratifin (SFN) expression and its quantification. Data are presented as mean±SEM; *p<0.05, ***p<0.001 vs M-acylCs Physio group



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