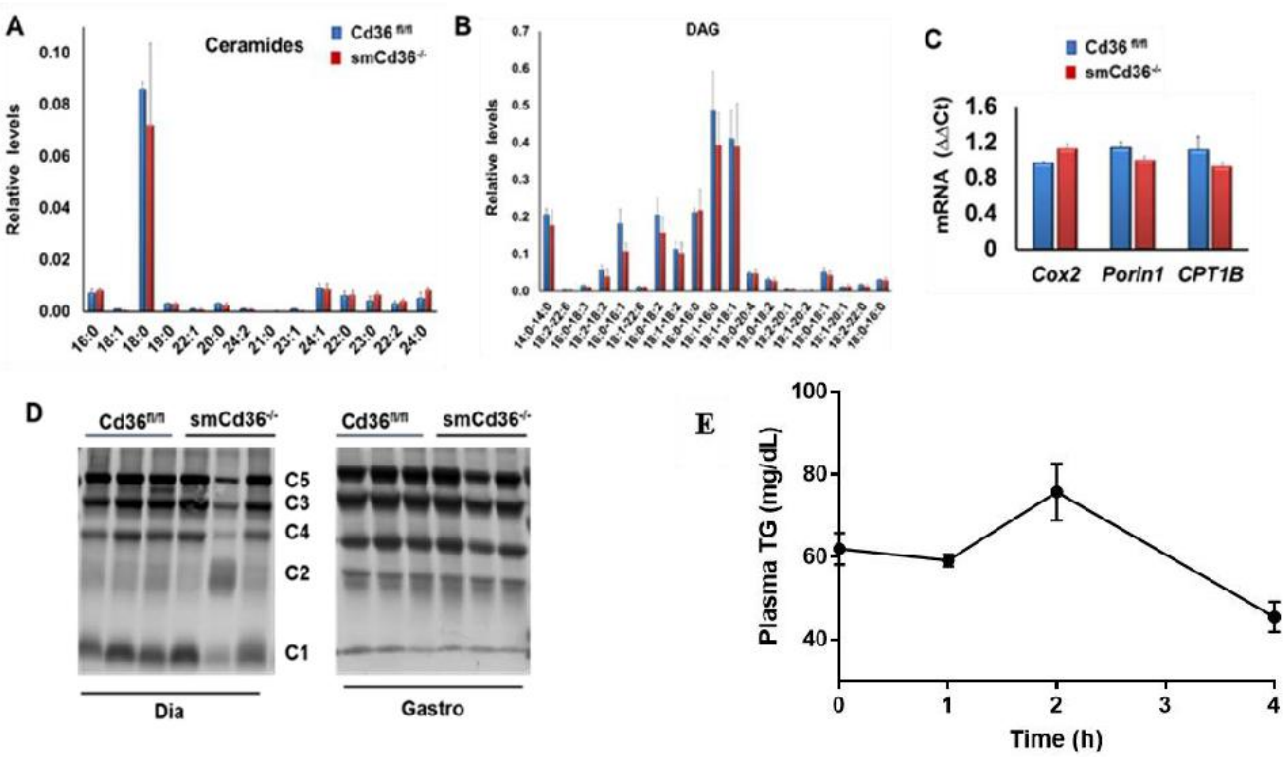
SUPPLEMENTARY DATA

Supplementary Figure S1. Skeletal muscle CD36 deletion affects glucose metabolism *in-vivo* in mice

A, B: Ceramides and DAG content in quadriceps was measured by mass spectrometry as described in Methods. n=4/genotype.
C, D: Mitochondrial gene and protein expression are not altered by smCD36$^{-/-}$: **C.** Expression of mitochondria-specific genes was measured by qPCR and normalized by 36B4. n=4-5/genotype.
D. Expression of representative proteins of electron transfer chain (ETC) complexes (C1-C5) was not changed in diaphragm (Dia) and gastrocnemius (Gastro) of smCD36$^{-/-}$ mice.
E. Plasma triglycerides (TG) in mice, detected after palm oil gavage. Means ±SE are shown. n=3.



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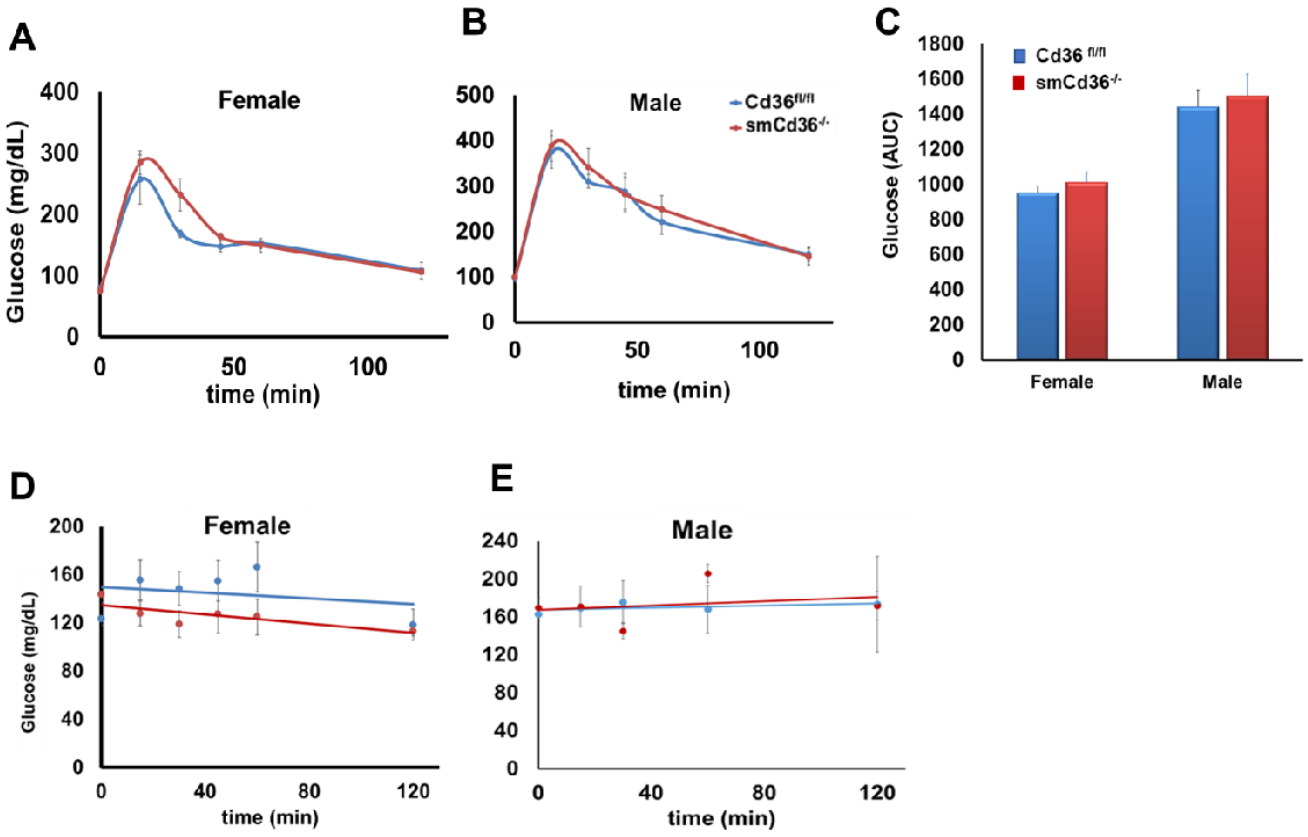
SUPPLEMENTARY DATA

Supplementary Figure S2. Lack of differences in glucose tolerance *in-vivo* between male and female smCd36-/- mice

A-C: No sex-related differences in glucose disposal in smCd36$^{-/-}$ mice: Male and female Cd36$^{fl/fl}$ and smCd36$^{-/-}$ mice were fasted for 16h and glucose and intraperitoneal glucose tolerance test (ipGTT) was carried out (n=5-7/genotype/sex). Blood glucose levels were assayed at indicated times. (A, B) Graphs show means ± SE of blood glucose concentrations. (C) Graph shows means ± SE of area under curve (AUC) of graphs in A and B.

D, E: No sex-related differences in insulin-stimulated glucose disposal in smCd36$^{-/-}$ mice on high fat diet (HFD): Intraperitoneal insulin tolerance test (ITT) for Cd36$^{fl/fl}$ and smCd36$^{-/-}$ male and female mice. Mice were kept on high fat diet for 5 weeks, fasted for 16h and ITT was carried out (n=4-5/genotype/sex). Blood glucose levels were assayed at indicated times. Graphs show means ± SE of blood glucose concentrations.



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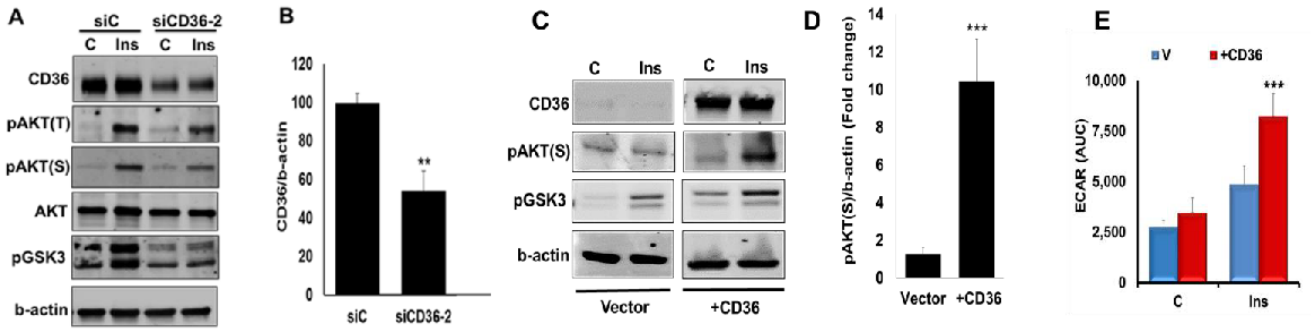
SUPPLEMENTARY DATA

Supplementary Figure S3. siRNA-mediated CD36 depletion in HSMMs results in suppression of insulin signal transduction

A: CD36-depleted and control HSMMs were serum-starved for 16h and incubated with or without insulin (100nM,5 min). Cell lysates were resolved by SDS-PAGE and immunoblotted for CD36, phospho-AKT threonine 308 (pAKT T), phospho-AKT serine 473 (pAKT S), total AKT, phospho-GSK3 α/β serine 21/9 (pGSK3) and b-actin. **B:** Graph shows the quantification by densitometry of CD36 immunoblots normalized to b-actin. Data are reported as mean ± SE of three experiments and expressed as % of siC, **p<0.01, compared to siC.
C, D: CD36 expression enhances insulin signal transduction. HEK 293 cells with stable CD36 expression HEK293 cells were transfected with pcDNA3 containing human CD36 with a C-terminal FLAG tag (1), selected with Geneticin (250 g/mL) and single clones were selected and tested for CD36 expression by western blot and flow cytometry. Cells were incubated with or without insulin (as in A), lysates resolved by SDS-PAGE and immunoblotted as indicated. **C.** Representative immunoblots. **D.** Graph shows quantification of insulin-induced pAKT (S)/b-actin, ***p<0.001, compared to vector controls. Data are means ± SE from three experiments.
E: ECAR for measured in CHO/IR +CD36 cells or an empty vector control (V) in the presence or absence of insulin. Graph shows means ±SE of ECAR area under the curve (AUC) from 32 wells from two representative experiments. ***-p<0.001, compared to unstimulated vector controls.



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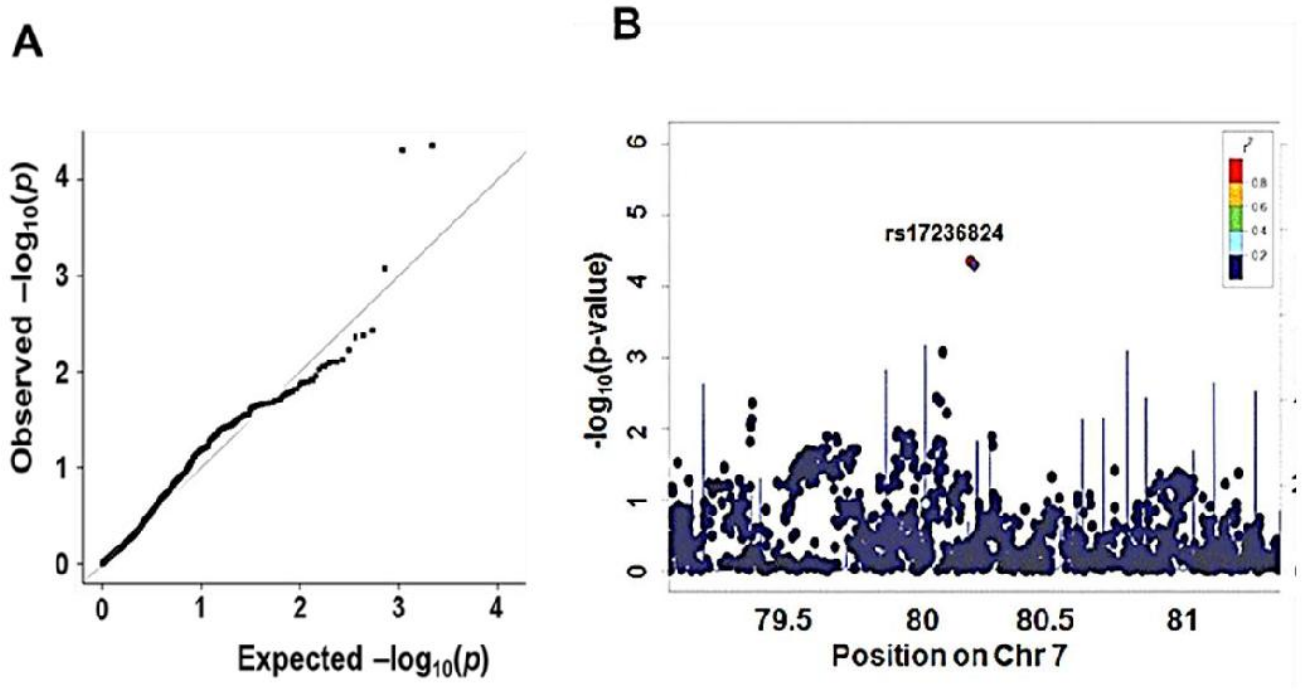
Supplementary Figure S4. Association of low CD36 expression with HOMA-IR

Genome-wide association study (GWAS) analysis was performed using publicly available data generated by the MAGIC Consortium.
A: Decreased CD36 expression associated with higher HOMA-IR or insulin resistance (p=0.03). Single nucleotide polymorphisms (SNPs) within cis region of CD36 (+/- 1MB of transcription start site) showed that distribution of p-values significantly differs from that expected by chance.
B: This gene region contains a variant highly significant for association with HOMA-IR.



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Supplementary Table S1. Primary Antibodies

Name of Antibody	**Vendor**	**Cat. Number**
Goat anti-human CD36	R&D Systems	AF1955
Goat anti-mouse CD36	R&D Systems	AF2519
mouse anti-human CD36 [FA6-152]	Abcam	ab17044
Rabbit anti-Insulin Receptor Beta	Cell Signaling	3025
Mouse anti-Insulin Receptor Beta	Cell Signaling	3020
Mouse anti-pTyr 100	Cell Signaling	94115
Rabbit anti-Src	Santa Cruz Biotechnology	sc-2
Rabbit anti-pSrc (Y416)	Cell Signaling	6943
Mouse anti-b-actin	Santa Cruz Biotechnology	sc-47778
Mouse anti-AKT(pan) (40D4)	Cell Signaling	2920
Rabbit anti-pAKT(S473)	Cell Signaling	4060
Rabbit anti-pAKT(T308)	Cell Signaling	13038
Rabbit anti- pATP-Citrate Lyase (S455)	Cell Signaling	4331
Rabbit anti-PI3 Kinase (P85)	Cell Signaling	4292
Rabbit anti-pGSK3 α/β (S21/9)	Cell Signaling	9331
Mitochondrial Antibody Cocktail	Abcam	ab110413

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Supplementary Table S2. Primers

Gene	Description	Primer sequences
CD36	Cluster of differentiation 36	**F**: GTTATTGGTGCAGTCCTGGC **R**: GGTTCCTTCTTCAAGGACAACTT
HK2	Hexokinase 2	**F**: CTGGTTTCAAAGCGGT **R**:ACTGGTCAACCTTCTG
PFK2	6-phosphofructo-2-kinase	**F**: TTTCGCCCAGACAACAT **R**: CAAAAACCGCAACGT GA
PDHB1	Pyruvate dehydrogenase E1 component, beta subunit	**F**: AAA GGC AAG GGA CCC A **R**: TGG CTT CGA TGT CCA TT
PDK4	Pyruvate Dehydrogenase Kinase 4	**F**: CTG CCTGACCGCTTAG **R**: TGTCTACAAACTCTGAC
ALDOA	Aldolase A	**F**: GGCAGTGGGAGGCAA **R**: GCAGTGCTTTCCGGTC
Glut1	Glucose transporter 1	**F**: GGTGTCGCTGTTTGTT **R**: ATGGCCACGATGCTCA
Glut4	Glucose transporter 4	**F**: AGCGAGTGACTGGAAC **R**: TCAATCACCTTCTGTGG
IRS1	Insulin receptor substrate 1	**F**: AGACGCTCCAGTGAGGATTT **R**: GGTCCTGGTTGTGAATTGTG
Pi3kr1	Phosphatidylinositol 3-kinase regulatory subunit alpha	**F**: GCCATTGAGAAGAAAG **R**: ATCTCCAAGTCCACTGA
IR	Insulin receptor	**F**: TACTGCTATGGGCTTCG **R**: TACCAGGGCACACCTC
Foxo1	Forkhead Box O1	**F**: CGAGTGGATGGTGAAG **R**: AATTGAATTCTTCCAGC
CPT2	carnitine palmitoyltransferase 2	**F**: ATCGTACCCACCATGCA **R**: CTTCTGTGTTCCTGAAC
FABP3	Fatty Acid Binding Protein 3	**F**: GGAAGCTAGTGGACAGCAAGA **R**: CTGTCACCTCGTCGAACTCTAT
ACLy	ATP Citrate Lyase	**F**: ATGCCCCAAGATTCAGT **R**: ACGATGGCCTTGGTATG
FATP1	Fatty Acid Transport Protein 1	**F**: GAACAGAGAGGCCAA **R**: ACGATGTTTCCTGCTG
COX2	Cytochrome c oxidase subunit 2	**F:** CTGGTGAACTACGACTGCTAGA **R:** GGCCATAGAATAACCCTGGTC
Porin1	mitochondrial outer membrane protein Porin1	**F:** GAGTATGGGCTGACGTTTACAG **R:** CCCTCTTGTACCCTGTCTTGAT
CPT1B	carnitine palmitoyltransferase 1B	**F:** GAAAGCCTCCGAAAAGCAC **R:** CTCCAGCACCCAGATGATT

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Supplementary Table S3. Association of low CD36 expression with incidence of diabetes

Effect	p-value	Diabetes type 2 (T2D) and complications	Tissue
-0.92143	1.22E-07	T2D with renal manifestations	Heart-Left Ventricle
-0.76008	0.000524	T2D with ophthalmic manifestations	Heart-Left Ventricle
-0.44804	0.000508	T2D	Heart-Left Ventricle
-0.69329	0.000924	Diabetes mellitus	Heart-Left Ventricle
-0.48817	0.001836	T2D with renal manifestations	Muscle-Skeletal
-0.55167	0.002477	T2D with neurological manifestations	Heart-Left Ventricle

Low muscle CD36 expression associates with increased risk of type 2 diabetes. Analysis of the relationship between muscle and heart CD36 mRNA level and incidence of T2D in the Vanderbilt University's BioVU genomic resource. Significant associations, p-values ranging from 10^{-3} to 10^{-7}, were observed between low CD36 expression and T2D status or with T2D plus associated complications; renal, ophthalmic and neurological.

PrediXcan analysis: PrediXcan (2; 3) was used to evaluate *CD36* contribution to etiology of T2D risk. The genetically-determined expression component was estimated using gene expression imputation trained with reference transcriptome data (N = 44 tissues, 449 donors, version 6p) from the Genotype-Tissue Expression (GTEx) Consortium. PrediXcan was applied to 4702 patients of European ancestry, 1484 patients with T2D, in the BioVU database (4),Vanderbilt University's electronic health records tied to DNA biobank. Samples were genotyped, Illumina 660K, and genotype imputation used 1000 Genomes Project as reference (5).
Gene-level association of *CD36* with T2D risk was replicated using GWAS meta-analysis data from MAGIC Consortium on HOMA-IR (6) and by applying PrediXcan on GWAS summary statistics. MAGIC Consortium "replication" data set had 37037 European ancestry participants.

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