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Supplementary methods

Genotyping

Genotypes were called using the Genome-studio software and single nucleotide polymorphisms (SNPs) were kept for further analysis according to the following thresholds: 1) minor allele frequency > 0.05 , 2) Hardy-Weinberg equilibrium $> 1 \times 10^{-4}$ and 3) call rate > 0.95 . Imputation was based on the Haplotype Reference Consortium Panel. Following QC, all individuals were suitable for downstream analyses.

A bi-directional MR was performed, first by using T2D or LDL-C as the exposure and CpG methylation as the outcome (referred to as forward MR), then, by using CpG methylation as the exposure and T2D or LDL-C as the outcome (referred to as reverse MR). The Inverse Variance Weighted (IVW) method was reported as the main MR method. IVW requires the validity of all genetic instrument, or a balanced pleiotropy. To validate this assumption, we conducted other complementary MR methods which uses different assumptions, such as simple median, weighted median (more robust to outliers), MR Egger (sensitive to outliers, its intercept is a test to evaluate horizontal pleiotropy).

Mendelian Randomisation (MR):

Forward instrument design (T2D/LDL-C to CpG): We extracted genome-wide significant SNPs from the aforementioned GWAS. For T2D to CpG, we obtained 118 independent signals ($LD\ r^2 < 0.2$) and 241 independent signals ($r^2 < 0.2$) for LDL-C to CpG. We performed a trans mQTL at SNP-CpG pairs of interest as described in the main methods section.

Reverse instrument design (CpG to T2D/LDL-C): To identify SNPs that act as proxies for *PNLIPRPI* methylation, we performed a cis-mQTL for cg15549216, cg06606475, and cg08580014 with a 50kb window. mQTL signals were FDR-corrected, and SNPs in LD were pruned using the `ld_clump()` function of the `ieugwasr` R package. SNPs with an r^2 of < 0.75 and an FDR < 0.75 were considered as viable instruments.

RNA expression of *PNLIPRP1* organ donors

Prior to processing, the samples were stored in OCT at -80°C. OCT was removed and the sample was cut into pieces and incubated in Trizol (15596-026; ThermoFisher), 3% DTT and 5% B-Mercaptoethanol and vortexed vigorously. The supernatant was collected and the RNA extracted using Trizol (15596-026; ThermoFisher). RNA was reverse transcribed using the High-capacity cDNA reverse Transcription Kit (Applied Biosystems; 4368814). The SYBR green reagent mix was used (A25918; ThermoFisher) and qPCRs were performed on the QuantStudio Pro 7 (Applied Biosystems). All primers used in this study are listed in **Supplementary Table 2**. Two-tailed t-tests were performed using GraphPad Prism (GraphPad software Inc). Ten samples were processed (five controls and five T2D individuals), matched for age, sex and BMI.

Functional characterisation

High glucose and insulin treatments: We plated AR42J cells in 6-well plates. Upon confluence, medium was replaced with a 0.1% FBS (26140079; Gibco). AR42J cells (2×10^5 per well) were treated with 20mM glucose or 100nM insulin, or both, for 72 hours and cells were harvested for RT-qPCR. The experiment was performed in three biological replicates. Fold changes were tested using a two-way ANOVA.

Akt response and glucose uptake: AR42J cells were plated and serum starved overnight, washed with PBS and stimulated with or without 200 nM insulin for 1 hour. Protein was harvested in RIPA buffer supplemented with protease and phosphatase inhibitors. The primary antibody used were anti pAKT (S473; Cell Signaling) and anti-Akt (9272; Cell Signaling), and the secondary antibody was goat pAb to Rb igG (Ab205718; Abcam). Details of all antibodies in this study are listed in

Supplementary Table 3.

siRNA knockdown: AR42J cells were transfected in suspension using the AR42J Transfection Reagent kit (1181; Altogen) with *Pnliprp1* siRNA (M-099515-01-0010; Dharmacon) or non-targeting control siRNA (D-001810-10-20; Dharmacon). Following transfection, RNA and protein

from AR42J siRNA were harvested after 48 or 72 hours. This experiment was performed in four biological replicates. *Pnliprp1* expression was tested using a two-tailed t-test in Graphpad Prism. Extracted protein from *Pnliprp1* KD was used for western blotting (method detailed in Supplementary Methods).

Western blotting:

Cells were lysed and protein harvested using a RIPA buffer (89900; ThermoFisher). Protein was quantified using the Pierce BCA Protein Assay Kit (23225; ThermoFisher) and separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane using the iBlot2 Gel Transfer Device (Life Technologies). Membranes were blocked with 5% non-fat dry milk or 5% BSA and incubated overnight at 4°C. Membranes were incubated with secondary antibody for 1 hour at room temperature (RT). Secondary antibody detection was determined using the LI-COR Biosciences imaging system and analysed with ImageJ.

Immunohistochemistry:

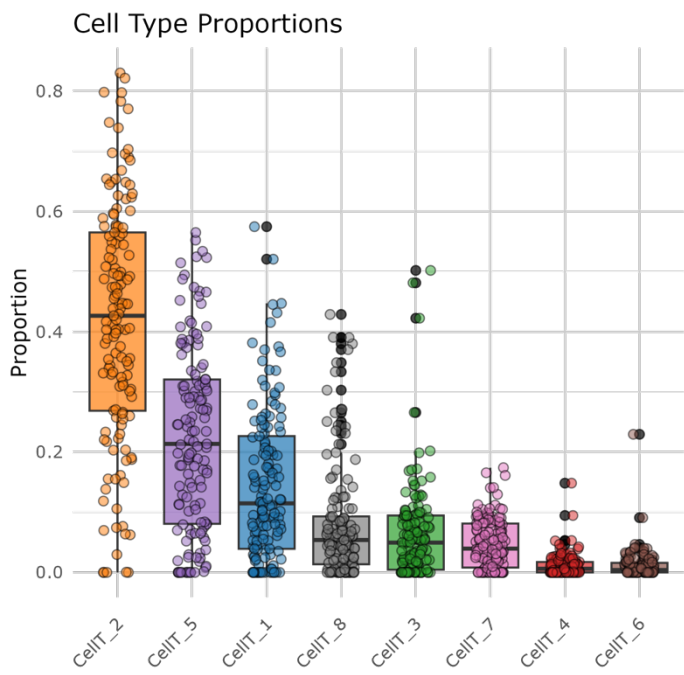
Paraffin was removed from the slides with 100% xylene. Gradually decreasing concentrations of ethanol (100-50%) were used to rehydrate the tissue slides. Antigens were retrieved via incubation in sodium citrate buffer (pH 6). Non-specific site blocking was performed by incubating the slides in a 1x PBS, 0.01% triton, 5% goat serum solution for 30 min at RT. Primary antibodies were incubated overnight at 4°C and secondary antibodies for 1 hour at RT. Images were captured using Zeiss LSM 710 NLO confocal laser scanning microscope.

RNA sequencing:

Mean sequencing depth was of 100 million 100 bp paired-end reads per sample. Illumina Raw data were demultiplexed using bcl2fastq v2.20.0.422 (Illumina) and adapters trimming step has been executed using cutadapt version 3.2. Mapping was done using STAR version 2.7.1a with Rattus

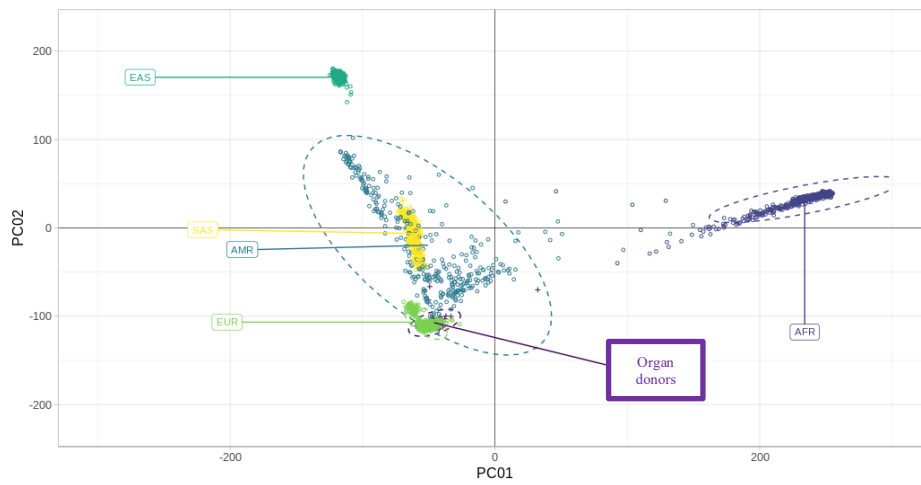
norvegicus.Rnor_6 genome. Raw and normalised counting steps were done using RSEM v1.3.0 using a GTF from Ensembl version 102, and Ensembl v.102 for genes names annotations.

Supplementary Fig. 1: Cell type proportions



Supplementary Fig. 1: Cell types were estimated from DNA methylation values using the RefFreeEWAS R package. Eight cell types were identified, the y-axis indicates the proportion of the cell types.

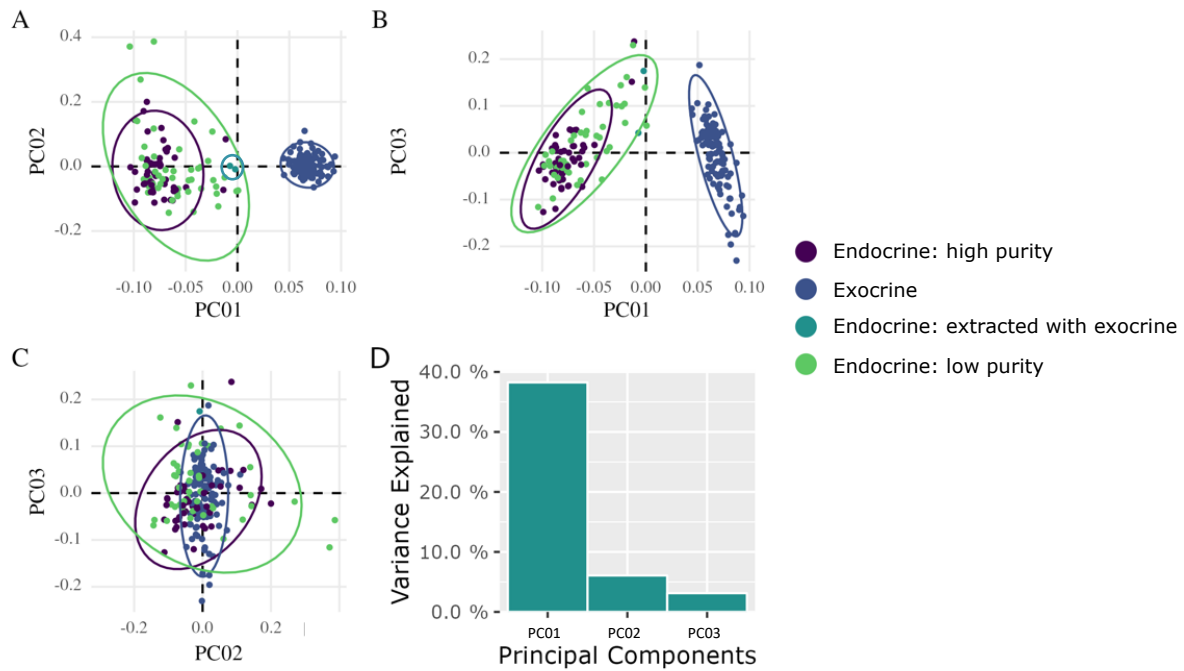
Supplementary Fig. 2: Ancestry clustering of organ donors.



Supplementary Fig. 2: Ancestry clustering of samples from our samples (IMIDIA) confirmed European descent of subjects, compared to the 1,000 genomes. Organ donor samples are depicted as circled in purple. AMR = Ad Mixed American (dark green), EAS = East Asian (teal), SAS = South Asian (yellow), EUR = European (green), AFR = African (dark blue).

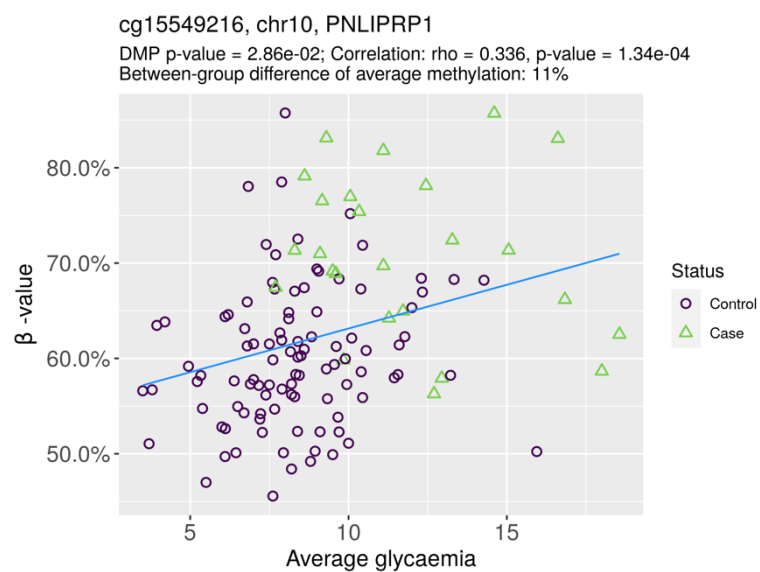
Supplementary Fig. 3: PCA of exocrine and endocrine pancreas

Structure Detection For: 'tissue'



Supplementary Fig. 3. Principal component analysis (PCA) from methylation of pancreas. A) The PC01 and PC02 of the exocrine (dark blue), endocrine with high purity (>2) (purple), endocrine with low purity (< 2) (green) and endocrine samples extracted at the same time with exocrine (teal) pancreas from organ donors, B) PC01 vs PC03, C) PC02 vs PC03, and D) the variation explained by the three PCs.

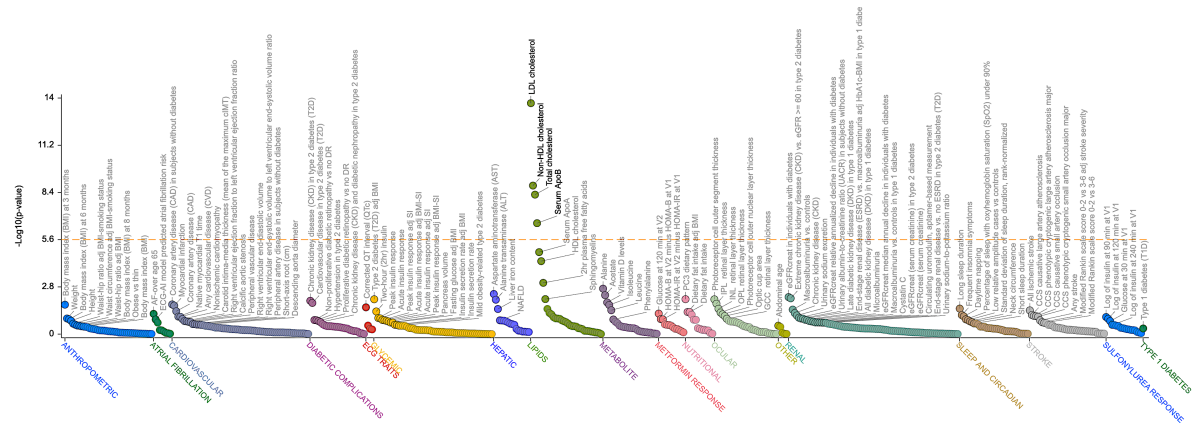
Supplementary Fig. 4: *PNLIPRP1* methylation and glucose levels in cohort



Supplementary Fig. 4: Scatterplot depicting the association between methylation levels of cg15549216 (y-axis) and average glucose levels (x-axis) in non-diabetic (purple) and T2D individuals (green). The correlation rho values were determined by Spearman's correlation coefficient. DMP: differentially methylated position.

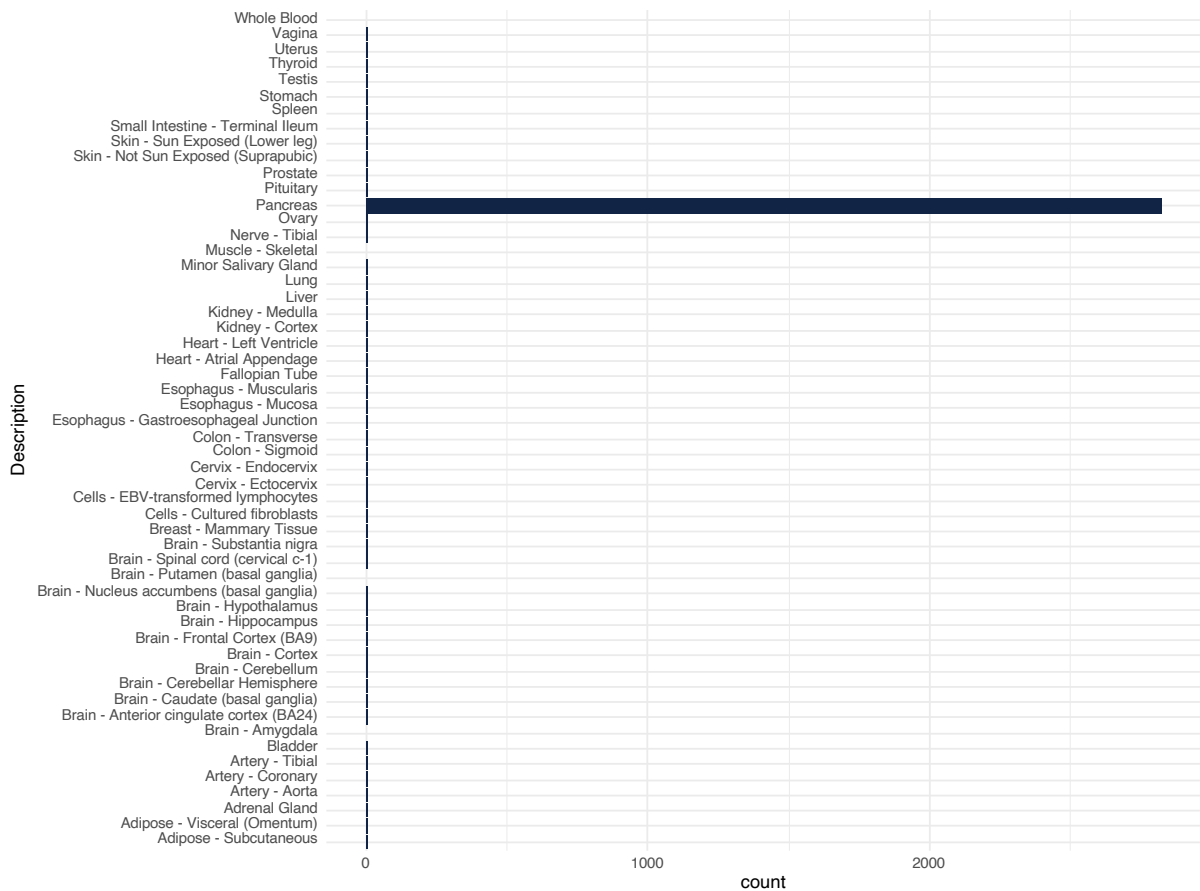
Supplementary Fig. 5: Common variant gene-level associations for *PNLIPRP1*

Common variant gene-level associations for PNLIPRP1 (Ancestry: All) 



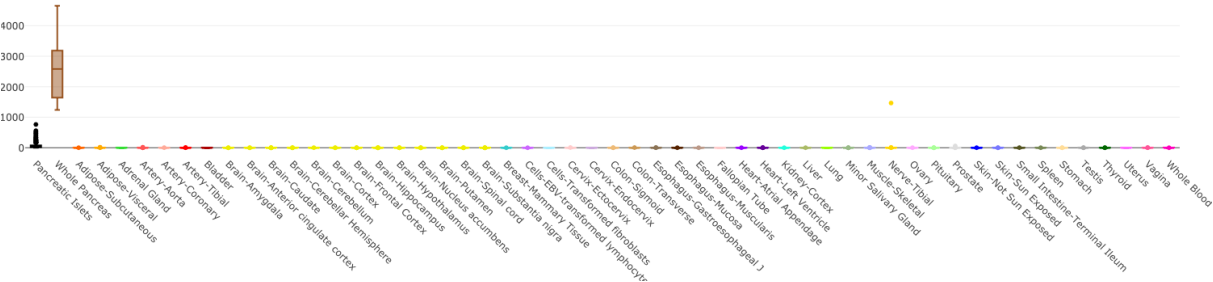
Supplementary Fig. 5: Common variant gene-level associations for *PNLIPRP1* using the Type 2 Diabetes Knowledge Portal, showing all the traits tested. The y-axis represents the $-\log_{10} P$ -value and the red line represents multiple testing correction.

Supplementary Fig. 6: Expression of *PNLIPRP1* in different tissues using GTEx



Supplementary Fig. 6: The gene expression of *PNLIPRP1* all tissues using data obtained from the GTEx public database. The count in the x-axis represents the Transcript per Million (TPM) median.

Supplementary Fig. 7: Expression of *PNLIPRP1* in different tissues using Tiger database.



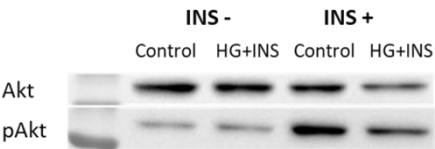
Supplementary Fig. 7: The expression of *PNLIPRP1* in pancreatic islets (TPM = 36) and pancreas (TPM = 2581), compared to all other tissues. Data was obtained from the Tiger database, which differentiates whole pancreas from pancreatic islets (endocrine pancreas), compared to all other gene expression data from GTEx. The count in the X-axis represents the Transcript per Million (TPM) median.

Supplementary Fig. 8: Western blot of Pnliprp1 and beta-actin following Pnliprp1 KD in AR42J.



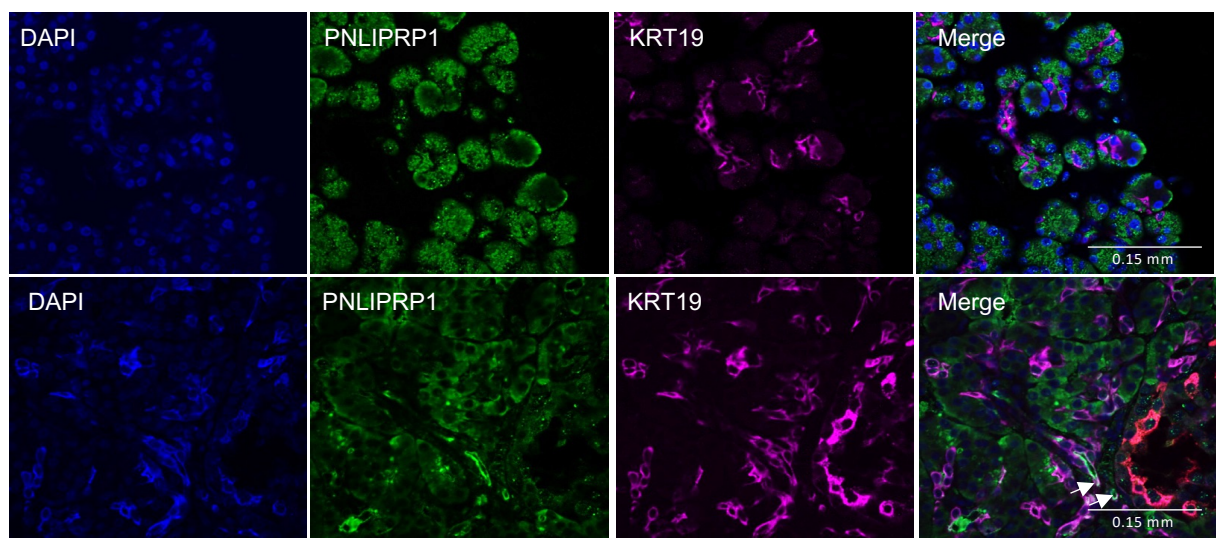
Supplementary Fig. 8: Western blot of Pnliprp1 and housekeeping gene beta-actin following *Pnliprp1* KD (right). Quantification of the protein revealed a decreased expression of *Pnliprp1* in the KD compared to the non-targeting control (left). The x-axis represents the fold-change. The difference was determined based on a two-tailed t-test. * $p < 0.05$.

Supplementary Fig. 9: Phosphorylated Akt following insulin and glucose exposure.



Supplementary Fig. 9: Western blot of Akt and phosphorylated Akt (pAkt) in AR42J cell lines stimulated with or without 200 nM insulin for 1 hour. The insulin treatment was performed following 48 hours of high glucose and insulin treatment.

Supplementary Fig. 10: Immuno-fluoresence of human pancreatic tissue in healthy and T2D



Supplementary Fig. 10: Immunofluorescence of healthy human whole pancreas tissue sample (above) and T2D sample (below) stained for PNLIPRP1 and KRT19, a ductal marker, and INS (insulin – a marker for pancreatic islets). Nuclei were stained with DAPI. This was performed in n=1.

Supplementary Table 1: Exocrine and endocrine organ donor clinical characteristics.

Characteristic	Endocrine, N = 125 ¹	Exocrine N = 141 ¹	p-value ²
Age	73 (60, 78)	69 (58, 77)	0.067
Sex			0.230
Female	52 (42%)	69 (49%)	
Male	73 (58%)	72 (51%)	
BMI	24.5 (22.9, 27.0)	25.1 (23.4, 27.3)	0.256
T2D status			0.122
Non-diabetic	109 (87%)	113 (80%)	
T2D	16 (13%)	28 (20%)	

¹ Median (IQR); n (%)

² Welch Two Sample t-test; Pearson's Chi-squared test

Supplementary Table 2: List of primers used

Gene	Species	Sequence (5' to 3')
AMY2 forward	Rat	GGAGCATCCATCCTGACATT
AMY2 reverse	Rat	AGACCCAGTCATTGCCACA
RPLP0 forward	Rat	ACCTCCTTCTTCCAGGCTTT
RPLP0 reverse	Rat	CCCACCTTGTCTCCAGTCTTT
CTRL forward	Rat	CCTGCACTGAGCTACAACCA
CTRL reverse	Rat	GTGTGTGATCGCCTTCGAGA
CPA2 reverse	Rat	TTATTGGCTGCCCTACTTGGGT
CPA2 reverse	Rat	TGATCTCAAGAACTTGATCTCCAAC
PRSS1 forward	Rat	ACACCCTCAGCAATGGTGTGAA
PRSS1 reverse	Rat	AATCATGCTGCTGGTGATTTC
PNLIPRP1 forward	Rat	ATCAACACTCGCTTCCTGCT
PNLIPRP1 reverse	Rat	TACCTGGGCACCCACTACTC
PNLIPRP1 forward	Human	CCAGATGCTCGACATCCTCT
PNLIPRP1 reverse	Human	ACCCATCTGTTGGTTCGTTC
PNLIPRP2 forward	Human	GGGCAATGTACACCCAAGC
PNLIPRP2 reverse	Human	CTCAGGTTTCATCCTGGAAGC
PNLIP forward	Human	GTGTGGAACCTGACGGAAC
PNLIP reverse	Human	AAGCAGCCGAGTCTTTCGTA
RPLP0 forward	Human	ACCTCCTTTTCCAGGCTTT
RPLP0 reverse	Human	CCCACCTTGTCTCCAGTCTTG

Supplementary Table 3: List of antibodies used

Antibody	Species	Antibody class	Company	Reference	Dilution (western blot)	Dilution (immunofluorescence)
PNLIPRP1	Rabbit	Polyclonal	Invitrogen	PA5-110186	1/1000	1/50
KRT19	Mouse	Monoclonal	Abcam	ab220193	N/A	1/200
INS	Guinea pig	Polyclonal	Dako	IR002	1/3	N/A
B-actin	Rabbit	Polyclonal	Cell Signalling	4967	1/1000	N/A
AKT	Rabbit	Polyclonal	Cell Signalling	9272	1/5000	N/A
pAKT	Rabbit	Polyclonal	Cell Signalling	S473	1/1000	N/A

Supplementary Table 4: Null variants in the *PNLIPRP1* gene

[See Excel file](#)

Supplementary Table 5: GWAS common variants associations using the T2D Knowledge Portal

Gene	Ancestry	P-value	Phenotype	Subjects	Z-Stat	Chr	Start position	End position	Type
PNLIPRP1	Mixed	2.0E-14	Low density lipoprotein (LDL)	2322809	7.56016375	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	1.6E-09	non-High density lipoprotein (non-HDL)	1001197	5.92588709	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	5.4E-09	Cholesterol	2073852	5.71924116	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	2.6E-07	ApoB	437547	5.016	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	6.0E-01	T2D	1249977	-0.2622505	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	2.8E-01	Fasting_glucose_metformin_adjusted_baseline_glucose	807	0.57358	10	118349897	118368687	protein_coding
PNLIPRP1	Mixed	3.1E-01	Change_in_fasting_insulin_after_metformin_adjusted_baseline_insulin	778	0.48225	10	118349897	118368687	protein_coding

Supplementary Table 6: SNPs associated with LDL-C in *PNLIPRP1* region

[See Excel file](#)

Supplementary Table 7: Proxy SNPs for CpGs in T2D MR analysis

Exposure	SNP	Effect_allele .exposure	Other_allele .exposure	Beta.exposure	Standard error.exposure	Outcome	Effect_allele. outcome	Other_allele .outcome	Beta.outcome	Standard error.outcome
CpG	kgp12306719	T	C	0.1133	0.0110	T2D	T	C	0.0122	0.0098
CpG	rs10885990	A	G	0.0929	0.0110	T2D	A	G	0.0012	0.0082
CpG	rs11197753	G	T	0.0637	0.0101	T2D	G	T	-0.0192	0.0267
CpG	rs2301178	A	G	0.0588	0.0116	T2D	A	G	0.0127	0.0085
CpG	rs67322546	A	C	0.1407	0.0108	T2D	A	C	0.0099	0.0099
CpG	rs7906926	T	C	0.0660	0.0098	T2D	T	C	1.00E-04	0.0083

Supplementary Table 8: Proxy SNPs for T2D in MR analysis

[See Excel file](#)

Supplementary Table 9: Proxy SNPs for LDL-C in MR analysis

[See Excel file](#)

Supplementary Table 10: Proxy SNPs for CpGs in LDL-C MR analysis

Exposure	SNP	Effect_allele. exposure	Other_allele .exposure	Beta.exposure	Standard error.exposure	Outcome	Effect_allele .outcome	Other_allele .outcome	Beta.outcome	Standard error.outcome
CpG	kgp12306719	T	C	0.1133	0.0098	LDL_C	T	C	0.0110	0.0017
CpG	rs10885990	A	G	0.0929	0.0098	LDL_C	A	G	0.0005	0.0015
CpG	rs11197753	G	T	0.0637	0.0114	LDL_C	G	T	-0.0095	0.0046
CpG	rs145248244	T	C	0.0659	0.0102	LDL_C	T	C	0.0136	0.0058
CpG	rs17735613	G	A	0.0674	0.0106	LDL_C	G	A	0.0077	0.0021
CpG	rs1867991	A	C	0.0571	0.0099	LDL_C	A	C	0.0006	0.0017
CpG	rs2301178	A	G	0.0588	0.0101	LDL_C	A	G	0.0119	0.0015
CpG	rs67322546	A	C	0.1407	0.0092	LDL_C	A	C	0.0100	0.0018
CpG	rs7906926	T	C	0.0660	0.0091	LDL_C	T	C	0.0004	0.0015

Supplementary Table 11: T2D Leave_one_out_analysis

[See Excel file](#)

Supplementary Table 12: LDL_C reverse Leave_one_out_analysis

Exposure	outcome	SNP	Beta	Standard error	P-value
CpG	LDL_C	kgp12306719	0.0559	0.0239	0.0192
CpG	LDL_C	rs10885990	0.0787	0.0215	0.0002
CpG	LDL_C	rs11197753	0.0664	0.0208	0.0014
CpG	LDL_C	rs145248244	0.0634	0.0218	0.0036
CpG	LDL_C	rs17735613	0.0616	0.0223	0.0058
CpG	LDL_C	rs1867991	0.0676	0.0223	0.0024
CpG	LDL_C	rs2301178	0.0538	0.0174	0.0020
CpG	LDL_C	rs67322546	0.0613	0.0265	0.0207
CpG	LDL_C	rs7906926	0.0704	0.0221	0.0014
CpG	LDL_C	All	0.0643	0.0207	0.0019

Supplementary Table 13: *PNLIPRP1* expression: individuals summary statistics

	Control	T2D	P-value
Age (SD)	64.67 (14.61)	78.2 (5.02)	0.081
Male (%)	4 (66.66)	3 (60)	0.84
Female (%)	2 (33.33)	2 (40)	0.84
Glycaemia (SD)	7.59 (1.62)	13.31 (2.82)	0.0022
Methylation (SD)	0.48 (0.052)	0.674 (0.041)	0.0001
BMI (SD)	24.91 (2.50)	27.028 (4.31)	0.33

Supplementary Table 14: Ranking of *PNLIPRP1* gene in pancreatic cell-types

Ensemble gene ID	Gene name	All versus all						Assigned cell type	Assigned rank
		acinar	alpha	beta	delta	duct	pp		
ENSG00000187021	PNLIPRP1	52	39128	38713	39102	39165	39121	acinar	52
		Exocrine versus endocrine						Assigned cell type	Assigned rank
		acinar	alpha	beta	delta	duct	pp		
		73	39226	39071	39213	29065	39224	acinar	73

Single cell analysis ranking of PNLIPRP1 gene, compared with all other genes, and their assigned cell type in pancreatic cell-types from Li et al., 2016

Supplementary Table 15: Pnliprp1 KD in AR42J

[See Excel file](#)

Supplementary Table 16: *Pnliprp1* KD pathway analysis: down-regulated genes

Term	P-value	Adjusted P-value	Odds Ratio	Genes
Cell cycle	2.98E-15	2.21E-12	143.60	CEP57;TOP2A;BRCA1;SMC3;PCM1;XPO1;NUF2;CEP290;HSP90AA1;RFC1;UBE2E1;SMC1A;CKAP5;CCNA2;PSMA6;PSMA4;DBF4;CCNE2;POT1;ANAPC4;MCM4;PCNA;PRKDC;TTK;TYMS;SKA1;NSL1;ORC4;DSN1;CDC45;CEP70;ORC2;RBBP7;BUB1;PLK4;RANBP2;NDC80;CENPE;POLA1;CENPF;CENPH;CENPI;ALMS1;TUBGCP5;CDK1;FGFR1OP;ATM;ATR;MAD2L1
Messenger RNA processing	4.56E-14	1.69E-11	191.96	NUP205;CSTF3;SRSF1;NXF1;SNRPD1;PCF11;DHX15;SNRPB2;HNRNPA1;SF3B1;SRSF10;SRSF11;RBM5;RANBP2;SF3A3;RBM39;HNRNPA3;NUP155;PRPF4B;SMC1A;CLK4;PTBP2;CLK1;HNRNPH1;HNRNPA2B1;SRSF3;SNRPE;HNRNPC;SRSF5;SREK1;SRSF7
Capped intron-containing pre-mRNA processing	1.40E-11	2.93E-09	171.32	RANBP2;SF3A3;NUP205;HNRNPA3;NUP155;CSTF3;SRSF1;SMC1A;NXF1;SNRPD1;PCF11;HNRNPH1;HNRNPA2B1;SRSF3;SNRPE;SNRPB2;SRSF5;HNRNPC;HNRNPA1;SRSF7;SF3B1;RBM5;SRSF11
DNA replication	1.59E-11	2.93E-09	134.09	PCNA;SMC3;SKA1;NSL1;ORC4;DSN1;XPO1;CDC45;NUF2;ORC2;BUB1;RANBP2;RFC1;SMC1A;CKAP5;NDC80;CCNA2;POLA1;PSMA6;CENPE;CENPF;PSMA4;DBF4;CENPH;CENPI;MCM4;SSBP1;MAD2L1
Messenger RNA splicing: major pathway	8.91E-10	1.32E-07	199.97	HNRNPA3;CSTF3;SRSF1;SMC1A;PCF11;HNRNPH1;HNRNPA2B1;SRSF3;SNRPB2;SRSF5;HNRNPC;HNRNPA1;SRSF7;RBM5;SRSF11

Supplementary Table 17: *Pnliprp1* KD pathway analysis: up-regulated genes

Term	P-value	Adjusted P-value	Odds Ratio	Genes
Cholesterol biosynthesis	1.01E-05	0.0044	15.1160	SREBF1;MVK;DHCR24;MVD;SREBF2;TM7SF2
SREBP control of lipid biosynthesis	1.47E-05	0.0044	45.1709	SREBF1;SCAP;LDLR;SREBF2
Metabolism	1.83E-05	0.0044	1.8801	ALAS1;COX4I1;HEXA;HS6ST1;TM7SF2;ALAD;LIPE;NDST1;GYS1;ALDH2;IMPA2;PGLS;GLUL;COX8A;BCKDHA;ACSL1;MED9;DDOST;MED25;IVD;ITPKA;CHST1;MVD;TKT;ATP6V0D1;ALDOA;ST6GALNAC4;ATP6VOC;CSNK1G2;CERS1;ALDH9A1;ISYNA1;AHCY;MVK;MRI1;GATA4;ADCY1;AGPAT1;OAZ1;CYB5R3;GNA11;POLR2E;B4GALNT1;LDLR;MPST;MDH2;PYCR1;DHCR24;PYCR2;NR1D1;HS3ST6;CYP8B1;ASS1;GLUD1;GALE;ETNK2;IMPDH1;LPCAT3;GNB2;MGAT4B;ALDH18A1
Arginine and proline metabolism	2.10E-05	0.0044	7.9120	GLUD1;ALDH2;PYCR1;PYCR2;ALDH18A1;GLUL;ASS1;ALDH9A1
Lipid and lipoprotein metabolism	3.09E-05	0.0044	2.6097	SLC25A1;ALAS1;SMARCD3;MVK;HEXA;AGPAT1;PLD3;TM7SF2;LIPE;PSAP;LDLR;SREBF1;ACSL1;MED9;DHCR24;NR1D1;CYP8B1;SREBF2;MED25;ETNK2;LPCAT3;MVD;CSNK1G2;TNFRSF21;CERS1;PNPLA2