# PCSK9 affects expression of key surface proteins in human pancreatic beta cells through intra- and extracellular regulatory circuits.

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## Abstract

**Aims/hypothesis**: Proprotein convertase subtilisin/kexin 9 (PCSK9) is involved in the degradation of LDLR. However, PCSK9 can target other proteins in a cell-type specific manner. While PCSK9 has been detected in pancreatic islets, its expression in insulin-producing pancreatic beta cells is debated. Herein, we studied PCSK9 expression, regulation and function in the human pancreatic beta cell line EndoC-βH1.

**Methods**: We assessed PCSK9 expression in mouse and human pancreatic islets, and in the pancreatic beta cell line EndoC-βH1. We also studied PCSK9 regulation by cholesterol, lipoproteins, Mevastatin, and by SREBPs transcription factors. To evaluate PCSK9 function in pancreatic beta cells, we performed PCSK9 gain-and loss-of-function experiments in EndoC-βH1 using siPCSK9 or recombinant PCSK9 treatments, respectively.

**Results**: We demonstrate that PCSK9 is expressed and secreted by pancreatic beta cells. In EndoC-βH1 cells, PCSK9 expression is regulated by cholesterol and by SREBPs transcription factors. Importantly, PCSK9 knockdown results in multiple transcriptome, proteome and secretome deregulations and impaired insulin secretion. By gain- and loss-of- function experiments, we observed that PCSK9 regulates the expression levels of LDLR and VLDLR through an extracellular mechanism while CD36, PD-L1 and HLA-ABC are regulated through an intracellular mechanism.

**Conclusions/interpretation**: Collectively, these results highlight PCSK9 as an important regulator of CD36, PD-L1 and HLA-ABC cell surface expression in pancreatic beta cells.

**Data availability:** RNA-seq data have been deposited to GEO database with accession number GSE182016. Mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the following identifiers: PXD027921, PXD027911 and PXD027913.

**Keywords** Human pancreatic beta cell, PCSK9, LDLR, CD36, PD-L1, HLA-ABC

## Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is the last discovered member of the proconvertases family [1]. It is mainly expressed in liver, cerebellum, lung and organs of the gastro-intestinal tract including pancreas (https://gtexportal.org/home/gene/PCSK9) [2]. Hepatocytes express the highest levels of PCSK9 and are the source of circulating PCSK9 [3]. PCSK9 is synthesized as an immature zymogen precursor and is autocatalytically cleaved in the endoplasmic reticulum between its N-terminal pro-domain and catalytic domain [4]. The resulting mature PCSK9 is next released into the circulation where it plays a key role in the regulation of cholesterol homeostasis [3–5].

PCSK9 binds extracellularly to the Low Density Lipoprotein (LDL) Receptor (LDLR) and targets it for lysosomal degradation, preventing its recycling to the plasma membrane [4]. In addition, a number of studies suggest that PCSK9 can also decrease LDLR abundance at the cell surface prior to its secretion via an intracellular route. Indeed, PCSK9 and LDLR interact early in the secretory pathway [6] and PCSK9 favors LDLR degradation even when its endocytosis is blocked [7]. Moreover, inhibition of LDLR traffic between the trans Golgi network and the lysosome abolishes PCSK9 induced LDLR degradation [8].

In addition to LDLR, PCSK9 enhances the degradation of other proteins: Very Low Density Lipoprotein Receptor (VLDLR), LDL-related protein-8 (LRP8 also called ApoER2) [9], Cluster of differentiation 81 (CD81) [10], LDL-related protein-1 (LRP1) [11], Cluster of differentiation 36 (CD36) [12], β-secretase 1 (BACE1) [13] as well as the epithelial sodium channel subunits (SCNN1A, SCNN1B and SCNN1G, also called α-, β- and γ-ENaC) [14]. Very recently, it was also demonstrated that PCSK9 disrupts the recycling of major histocompatibility complex (MHC I) to the cell surface, thus positioning PCSK9 inhibitors as a new way to enhance immune checkpoint therapy for cancer [15]. Some of the above-described proteins including the LDLR have been shown to be regulated by PCSK9 both intracellularly and extracellularly [9–12, 15]. For other proteins such as BACE1, intracellular PCSK9-mediated protein degradation has been described as an important route [13]. Moreover, it is the unique route for the epithelial sodium channel ENaC degradation by PCSK9 [14]. Finally, for other proteins, their route of degradation by PCSK9 is far less studied.

Regulation of PCSK9 expression in the liver and hepatocytes has been deeply studied [4]. A Sterol Regulatory Element (SRE) has been identified in the PCSK9 promotor [16] and the Sterol Regulatory Element-Binding Protein (SREBP) transcription factors play a major role in this regulation [17, 18]. In hepatocytes, PCSK9 expression is dependent on both SREBP-1 and SREBP-2 proteins, while the regulation of PCSK9 by cholesterol is predominantly SREBP-2 dependent [16–18]. PCSK9 expression is also modulated by insulin in a SREBP-1c dependent manner [19].

PCSK9 gain-of-function mutations are associated with familial hypercholesterolemia [20] while patients with loss-of-function PCSK9 mutations have reduced circulating LDL-cholesterol levels [21]. These discoveries led to validation of PCSK9 as a therapeutic target for the treatment of hypercholesterolemia. The first anti-PCSK9 monoclonal antibodies were approved for the treatment of hypercholesterolemia in 2015 [22] and recently, small interfering RNAs targeting intracellular PCSK9 were approved by the European Medicines Agency (EMA) for the treatment of hypercholesterolemia or mixed dyslipidemia [23]. Interestingly, PCSK9 loss-of-function mutations are associated with increased prevalence of type 2 diabetes [24]. There is no evidence of an effect of anti-PCSK9 inhibitory

97 antibodies on the transition to new onset diabetes, suggesting that reducing circulating PCSK9 does
98 not impair pancreatic beta cell function [25–27].

99 The islets of Langerhans are micro-organs distributed throughout the pancreas. They contain beta,
100 alpha, delta, PP and epsilon endocrine cells that produce and release insulin, glucagon, somatostatin,
101 pancreatic polypeptide and ghrelin, respectively. PCSK9 is expressed in mouse and human pancreatic
102 islets [28, 29]. However, the precise cell type expressing PCSK9 within islets remains unclear. Some
103 studies demonstrate that PCSK9 is only expressed in somatostatin-producing delta cells [28, 30], while
104 other studies indicate that beta cells express PCSK9 [29, 31, 32]. The function of PCSK9 within islets is
105 also debated. Global Pcsk9-deficient mice were glucose intolerant and insulinopenic [29, 30], though,
106 different studies showed conflicting results [28, 31]. These discrepancies might be due to differences
107 in the genetic background and age of the mice. Finally, PCSK9 function in islets has only been studied
108 through the regulation of LDLR [28–30] without a search for additional targets.

109 The aim of this study was to clarify the role of PCSK9 in human pancreatic beta cells. By using the
110 human pancreatic beta cell line EndoC-βH1 [33], we investigated PCSK9 expression, function and its
111 regulation. We also searched for additional cell surface proteins regulated by PCSK9 in human
112 pancreatic cells by performing gain- and loss-of-function experiments.

113

114 ## Experimental procedure

115 ### Ethical statement

116 This study was performed according to the Declaration of Helsinki and the Declaration of Istanbul. No
117 tissues were procured from prisoners. Human islets were provided by the Leiden University Medical
118 Center (Leiden, The Netherlands) and the human islet core facility of St-Louis Hospital (Paris, France).
119 They were prepared from pancreata of brain-dead donors after informed consent was signed from
120 next-of-kin, and processed for islet isolation according to the procedures approved by the ethics
121 committee of the French Agency of Biomedicine, and the Dutch national law. All of the animal studies
122 complied with the ARRIVE guidelines and were conducted in strict accordance with the EU Directive
123 2010/63/EU for animal experiments and with regard to specific national laws and INSERM guidelines.
124

125 ### Culture of EndoC-βH1 cells and treatment

126 The human pancreatic beta cell line EndoC-βH1 cells (Univercell Biosolutions, Toulouse, France
127 [mycoplasma negative]) was cultured as previously described [33]. Experiments were carried out 24
128 hours after seeding the cells at a density of approximately $10^5$ cells /$cm^2$. The following compounds
129 were used for treatment: Cholesterol-Methyl-β-Cyclodextrin (Sigma-Aldrich, France), Mevastatin
130 (Sigma-Aldrich, Germany), human native LDL and copper oxidized LDL or VLDL (see below for
131 lipoproteins extraction protocol), Human Recombinant Wild Type PCSK9 (Circulex).

132 ### Human hepatocytes, HepG2 and HEK293T culture

133 Human primary hepatocytes were isolated from liver fragments obtained from adult patients
134 undergoing partial hepatectomy as previously described [34] and cultured in William's Medium E (Life
135 Technologies) supplemented with 10% FCS (Perbio), 50 µM hydrocortisone hemisuccinate (SERB
136 laboratory), 5 µg/ml insulin (Sigma-Aldrich), 2 mM L-glutamine and 200 U/ml penicillin, 200 µg/ml
137 streptomycin. The human hepatocellular carcinoma cell line HepG2 and the human embryonic kidney
138 (HEK293T) (mycoplasma free) were cultured in Dulbecco's Modified Eagle's Medium (Gibco) and
139 DMEM/F12 (Gibco), respectively, supplemented with 10 % FCS, 100 units/mL penicillin, and 100 µg/mL
140 streptomycin.

141 ### Human islets

142 Islets were cultured in 12-well plates in CMRL medium supplemented with 10% FCS, Hepes and
143 penicillin/streptomycin (all from Thermo Fisher Scientific). Donor's characteristic and islets details are
144 presented in **Supplementary Table 1**.

145 ### siRNA transfection

146 EndoC-βH1 cells were transfected as described [35] in OptiMEM using Lipofectamine RNAiMAX (Life
147 Technologies) with siRNA SMARTpools (Horizon Discovery LTD, UK). Medium was replaced 2.5 hours
148 later with fresh culture medium and analyses were carried out 3 to 6 days following transfection. siRNA
149 reagents used: Control non-targeted siRNA (siCTRL, D-001810-01-20); siRNA targeting: PCSK9 (siPCSK9)
150 (M-005989-01-0005), SREBF-1 (siSREBF-1) (M-006891-00-0005) and SREBF-2 (siSREBF-2) (M-009549-
151 00-0005) at the final concentration of 80 nM.

152

153 **Cell sorting of mouse alpha, beta and delta cell populations**

154 Alpha, beta and delta cell population were prepared from mouse pancreatic islets as previously
155 described [36].

156 **Lipoproteins preparation and LDL oxidation**

157 LDL and VLDL were extracted from the plasma of healthy volunteers, by sequential
158 ultracentrifugations, as previously described [37]. Protein concentration of the lipoproteins was
159 determined by using BCA assay (Pierce). Copper-oxidation of LDL was done by incubating the native
160 LDL with 5 µmol/l $CuSO_4$ at 37°C for 16 hours followed by dialysis (3 times) against PBS containing 0.1
161 mM ethylenediamine tetraacetic acid and storage at 4°C.

162 **RNA isolation, reverse transcription, and qPCR**

163 A RNeasy Micro Kit (Qiagen) was used to extract total RNA from EndoC-βH1 cells [38]. Genomic DNA
164 was removed by DNAse treatment following the RNeasy Micro Kit protocol. RNAs were reverse
165 transcribed by using the Maxima First Strand cDNA kit (Thermo Fisher Scientific). RT-qPCR was
166 performed using Power SYBR Green mix (Applied Biosystems) with a QuantStudio 3 analyzer (Thermo
167 Fisher Scientific). Custom primers were designed with Primer-Blast online, and their efficiency and
168 specificity were determined for each pair by RT-qPCR on a serial dilution of cDNA samples. The list of
169 primers is presented in the **Supplementary Table 2**. Relative quantification ($2^{-dCt}$) was used to
170 calculate the expression levels of each target gene, normalized to *CYCLOPHILIN-A* transcripts.

171 **Western blot analysis**

172 Western blot experiments were performed as described [38]. For VLDLR immunoblot, cell extracts
173 were loaded in 8 % Bis-Tris polyacrylamide gels in absence of heating and 2-beta-mercaptoethanol.
174 The following antibodies were used: PCSK9 (1/200; AF3888; Bio-Techne), diluted in TBS 0.1 % Tween
175 with 0,5% BSA; LDLR (1/1,000; ab52818; Abcam), VLDLR (1/1,000; ab75591; Abcam), alpha-Tubulin
176 (1/2,000, T9026; Sigma-Aldrich), beta-actin (1/2,000; A5441; Sigma-Aldrich). Species-specific HRP-
177 linked secondary antibodies (1/1,000; 7074 and 7076; Cell Signaling Technology) were used. Antibodies
178 were validated by knockdown experiments (PCSK9) or or have passed application specific testing
179 standards.

180 **PCSK9 secretion**

181 EndoC-βH1, HepG2, HEK293T and human islets were starved for one hour in their respective culture
182 media (free from BSA or FCS) and then cultivated for 24 hours in fresh BSA/FCS-free culture medium.
183 Conditioned media were collected, centrifugated to eliminate cell debris, and used for Western blot
184 analyses. In the case of human islets, secreted proteins were enriched following precipitation with
185 acetone before Western blot analysis.

186 **Flow cytometry**

187 EndoC-βH1 cells were trypsinized, washed three times in HBSS/1% BSA and incubated at 4 °C for 15
188 minutes in the dark with the following antibodies: LDLR (1/100; MAB CL 472413; Fisher scientific), CD36
189 (1/10, 555455, BD Pharmingen), PD-L1 (1/100, 329714, Biolegend), HLA-ABC (1/100, 3114410,
190 Biolegend) diluted in HBSS medium/1% BSA (Gibco/Roche Diagnostics). Fluorochrome-conjugated
191 primary antibodies were used with the exception of LDLR detection, for which a step of incubation

192 with Alexa-fluor 488 (1/400, Invitrogen) secondary antibody was performed. Following rinsing in
193 HBSS/1% BSA and incubation with FACS medium containing propidium iodide (1/4,000, Sigma-Aldrich),
194 analysis was carried out using a FACS Aria III (BD Biosciences, San Jose, CA, USA). Dead cells were
195 excluded from analyses by using propidium iodide. Data were analyzed using FlowJo 10.7 software
196 (RRID: RRID:SCR_008520). Results are expressed in mean fluorescence intensities fold changes relative
197 to the control condition.

198 **Insulin secretion**

199 EndoC-βH1 cells were transfected with siControl (siCTRL) or siPCSK9 (siPCSK9). Two days later, cells
200 were starved in DMEM containing 0.5 mM glucose for 24 hours. Then, cells were washed twice and
201 then preincubated in Krebs-Ringer bicarbonate HEPES buffer (KRBH) containing 0.2% fatty acid–free
202 BSA in absence of glucose for 1 hour. Insulin secretion was measured following a 1-hour incubation
203 with KRBH containing 0.2% fatty acid–free BSA and 0 mM or 20 mM glucose, or 50 mM KCl. Insulin
204 secretion and intracellular insulin were measured by ELISA as previously described [33].

205 **Fatty acid uptake**

206 Fatty acid uptake in EndoC-βH1 was studied using the Fatty Acid Uptake Kit (Sigma-Aldrich). Cells were
207 transfected with siControl (siCTRL) or siPCSK9 (siPCSK9). Six days later, cells were starved for 1 hour in
208 cell culture medium free from BSA. TF2-C12 fluorescent fatty acid was added to the culture medium
209 for 1 hour. Fluorescence signal was measured at λexit=485nm/λem=520nm and normalized to cell
210 count. Fatty acid uptake was calculated by subtracting the fluorescence intensity measured at 1 hour
211 incubation by that measured at time point zero, following the manufacturer's instructions. Results
212 were expressed in fatty acid uptake fold changes relative to control.

213 **RNA-seq and proteomics analyses**

214 Acquisition of proteomics and RNA-seq data and sample size calculation for omics experiments have
215 been previously described [39]. Benjamini-Yekutielli method [40] was used to adjust for multiple
216 testing for IPA Pathway terms due to non-ignorable overlap between genes underlying top pathways.
217 Gene set enrichment analysis was conducted with GSEA v4.0.3 software (RRID: SCR_003199).

218 **Statistical analysis of RT-qPCR, Western blot and FACS experiments**

219 Each *n* represents an independent experiment. All of the graphs show means +/- Standard Error of the
220 Mean (SEM) and the statistical analysis were conducted using Prism (GraphPad, San Diego, CA, USA)
221 either with t-test if the normality condition was respected or with the non-parametric equivalent
222 Mann-Whitney test. A *p-value* less than 0.05 was considered significant and Symbols for indicating *p-*
223 *values* are *$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$.

224

225 ## Results

226 **PCSK9 is expressed and secreted by pancreatic beta cells**

227 The cell types expressing PCSK9 in mouse and human pancreatic islets remain debated [28–31]. Here,
228 we first analyzed *PCSK9* expression in mouse pancreatic islet cell types by using a FACS-based strategy
229 to purify beta, alpha and delta cell populations (**Fig. S1a**) [36]. RT-qPCR analyses indicated that alpha
230 ($CD71^-$), beta ($CD71^+$) and delta ($CD24^+$) populations were highly enriched in *glucagon*, *insulin-1* and
231 *somatostatin* mRNA, respectively, validating the efficiency of the sorting (**Fig. S1b**). *PCSK9* mRNA
232 analyses indicated that its expression was 3-fold higher in beta than in the delta and alpha cell
233 populations (**Fig. 1a**). PCSK9 mRNA level was also twofold lower in beta cells than in mouse liver (**Fig.**
234 **1a**). We then compared *PCSK9* mRNA expression by RT-qPCR in human islet preparations versus human
235 primary hepatocytes and HEK293T cells (used as positive and negative controls, respectively). *PCSK9*
236 mRNA were detected in human islets, although at a level fivefold lower than in human primary
237 hepatocytes. As expected, *PCSK9* was almost undetectable in HEK293T cells (**Fig. 1b**). We next
238 investigated PCSK9 expression in human islet cell types. Since PCSK9 expression in bulk islets was low,
239 it may be difficult to capture with single-cell sequencing due to limitations of the technology.
240 Therefore, we used a combination of scRNA-seq (E-MTAB-5061 [41], GSE81608 [42], GSE86469 [43])
241 and RNA-seq on highly purified FACS sorted human beta and alpha cells (GSE67543 [44]) to analyze
242 PCSK9 expression. PCSK9 transcripts were enriched in human adult beta cells (**Fig. S1c**). We next
243 analyzed *PCSK9* expression in the human pancreatic beta cell line EndoC-βH1 [33]. There, *PCSK9* mRNA
244 level was higher than in the human hepatoma cell line HepG2 (**Fig. 1c**). Western blot analysis revealed
245 the presence of two major PCSK9 bands at ~75 kDa and ~60 kDa. These bands corresponded to the
246 expected sizes for pro-PCSK9 and mature PCSK9. Quantification of mature PCSK9 level showed a 3-fold
247 higher expression in EndoC-βH1 compared to HepG2 cells, and no expression in HEK293T cells (**Fig. 1d**
248 **and e**). Noteworthy, PCSK9 maturation rate (as measured by the mature PCSK9/total PCSK9 ratio) was
249 higher in HepG2 cells than in the EndoC-βH1 (**Fig. S1d**). Given the fact that PCSK9 is a secreted protein,
250 we also compared by Western blot PCSK9 levels in conditioned media from EndoC-βH1, HepG2 and
251 HEK293T cells. In EndoC-βH1 and HepG2 cells, we detected a single band at approximately 60 kDa (as
252 expected for the secreted mature form of PCSK9) and no signal in conditioned media from the HEK293T
253 cells (**Fig. 1f**). PCSK9 secretion was twice lower in EndoC-βH1 when compared to HepG2 cells (**Fig. 1f**
254 **and g**). It was also the case when PCSK9 secretion was normalized to PCSK9 cell content (**Fig. S1e**).
255 Finally, as *PCSK9* transcripts were present in human islet preparations (**Fig. 1b**), we evaluated whether
256 PCSK9 was secreted by human islets. By Western blot, we detected in conditioned media the presence
257 of a band at ~60 kDa, the expected size for the secreted form of PCSK9 (**Fig. 1h**). Omics data analysis
258 confirmed that EndoC-βH1 cells are a representative *in vitro* model for human beta cells [39]. Thus,
259 mouse and human beta cells express PCSK9, and EndoC-βH1 cells can be used as a model to study
260 PCSK9 regulation and function in human beta cells.

261

262 **Cholesterol, LDL and mevastatin modulate PCSK9 expression and secretion**

263 In mouse and human hepatocytes, PCSK9 expression is regulated by cholesterol and by lipid-lowering
264 drugs of the statin class [16, 45]. Here, we evaluated the effects on PCSK9 expression of cholesterol,
265 LDL and mevastatin in EndoC-βH1 cells. Cholesterol and LDL treatments reduced the mRNA expression
266 of both SREBP2 (*HMGCR* and *LDLR)* and SREBP1 (*FASN* and *SCD*) transcriptional targets by ~50%.
267 Mevastatin treatment increased the expression of these genes by at least two-fold, demonstrating the

268 functionality of the LDL-cholesterol pathway in EndoC-βH1 cells (**Fig. 2a**). *PCSK9* mRNA followed a
269 similar pattern as expression was reduced by cholesterol and LDL treatment and increased following
270 incubation with Mevastatin (**Fig. 2b).** The effects of Mevastatin were reverted by cholesterol or LDL
271 treatments and were thus "cholesterol specific "(**Fig. 2a**, **b**). Similar effects of cholesterol, LDL and
272 Mevastatin were also observed at protein level. Indeed, Western blot analyses indicated that
273 cholesterol and LDL treatments decreased, while Mevastatin increased intracellular PCSK9 levels (**Fig.**
274 **2c and d**). Similar results were also obtained when secreted PCSK9 was analyzed (**Fig. 2e and f**). Finally,
275 we tested whether other classes of human lipoproteins modulate *PCSK9* expression in EndoC-βH1 cells.
276 Treatment with Triglyceride-Rich Lipoproteins (TRL) mimicked the effects of *LDL* on *PCSK9* mRNA
277 expression and on *SREBP-1* and *SREBP-2* transcriptional targets while oxidized LDL had no effect (**Fig.**
278 **2g, h**).

279

280 **PCSK9 is regulated by the SREBPs-1 and 2 transcription factors**

281 We first tested whether *PCSK9* mRNA levels were dependent on SREBPs transcription factors. Co-
282 transfection of the EndoC-βH1 cells with siSREBF-1+siSREBF-2 efficiently reduced mRNA expression of
283 *SREBF-1*, *SREBF-2* and their respective target genes: *FASN*, *SCD* (SREBP-1 targets) and *HMGCR*, *LDLR*
284 (SREBP-2 targets). *PCSK9* mRNA levels were downregulated indicating dependence of SREBPs activity
285 (**Fig. 3a**). Single siSREBF-2 downregulated *SREBF-2* and its target *HMGCR*, but not *SREBF-1*. It increased
286 PCSK9 mRNA levels (**Fig. 3b**). Single siSREBF-1 transfection specifically reduced *SREBF-1* expression.
287 The expression of canonic SREBP-1 liver targets *FASN* and *SCD* was not modulated, indicating the
288 existence of compensatory mechanism that maintain *FASN*, *SCD* and *LDLR* expression when SREBF-1
289 or SREBF-2 are reduced following knockdown (**compare Fig. 3a to 3b and 3c**). On the other hand,
290 *SREBF-1* knockdown was sufficient to decrease *PCSK9* expression, indicating that SREBF-1 is essential
291 to maintain PCSK9 expression (**Fig. 3c**).

292 We next assessed the SREBPs dependence of PCSK9 upon Mevastatin treatment. As expected,
293 Mevastatin treatment increased the mRNA expression of SREBP-1 targets, SREBP-2 targets and also
294 *PCSK9* (**Fig. 3d**). This induction was reverted upon SREBF-1+2 double knockdown (**Fig. 3e**). Single
295 SREBF-2 knockdown reduced the expression of *SREBF-2* and its target genes *HMGCR* and *LDLR*. PCSK9
296 expression was also down-regulated suggesting a SREBP-2 dependence (**Fig. 3f**). However, we noted
297 that upon Mevastatin treatment, siSREBF-2 decreased *SREBF-1* levels (**Fig. 3f**). We thus performed
298 single siSREBF1 knockdown that did not modulate *PCSK9* expression (**Fig. 3g**). Taken together, our data
299 indicate that SREBP-2, but not SREBP-1 is involved in the induction of PCSK9 by Mevastatin in human
300 beta cells.

301

302 **Insights from hypothesis-generating omics analysis**

303 We investigated the effect of siRNA-mediated PCSK9 knockdown in EndoC-βH1 cells in transcriptomics
304 and proteomics experiments. PCSK9 knockdown was confirmed in all omics experiments (**Fig. S2**).
305 Changes in gene and protein expression levels were generally consistent and had the same
306 directionality (60.3% for RNA-seq vs DIA cell lysate proteomics and 59.7% for RNA-seq vs TMT cell
307 lysate proteomics) (**Fig. S3**). IPA Pathway enrichment analysis indicated impaired mitochondrial
308 function (**Fig. 4a**) which was confirmed by GSEA analysis (**Fig 4b**). Normal mitochondrial function is

309 crucial for insulin secretion by beta cells [46]. We investigated the expression of markers associated
310 with glucose-stimulated insulin secretion [47]. Out of 147 markers, 45 (31%) were differentially
311 expressed **(Fig. 4c**). We also observed consistent up-regulation of PCSK1 levels, an enzyme involved in
312 pro-insulin to insulin processing, in the transcriptome and proteome experiments (**Fig. S4**).
313 Interestingly, we observed an up-regulation of insulin in DIA secretome proteomic at 72 hours after
314 PCSK9 knockdown (**Fig. S4a**). Following PCSK9 knockdown, insulin secretion was impaired: basal and
315 glucose-stimulated insulin secretion increased, while KCl-induced insulin secretion was reduced
316 **(Fig.4d).** Gene Ontology Cellular Component enrichment analysis indicated overrepresentation of
317 MHC-I complex and plasma membrane proteins among genes and proteins up-regulated after PCSK9
318 knockdown (**Fig. 4e**). MHC I complex enrichment was stronger on protein level than on mRNA level.
319 Golgi and endoplasmic reticulum proteins were also up-regulated, which is consistent with the
320 reported role of PCSK9 in intracellular degradation of proteins in the ER-to-Golgi intermediate
321 compartment [13]. CD36, CD274 (PD-L1) and MHC I complex proteins (particularly HLA-A, B, C and E)
322 were top findings in proteomics experiments **(Fig. 4f, g, h)**. ENaC channel subunits were not expressed
323 in Endoc-βH1 cells. LDLR, VLDL, LRP1 and LRP8 levels were not altered (**Fig. S5**). CD81 was up-regulated
324 on mRNA but not protein level (**Fig. S5**). mRNA and whole-cell protein expression of APP and APLP2
325 was increased after PCSK9 knockdown (**Fig. S5**). Up-regulation of APP and APLP2 mRNA was confirmed
326 by RT-qPCR together with the other top differentially expressed genes (**Fig. 4i**)

327

328 **PCSK9 knockdown does not affect LDLR levels**

329 In hepatocytes, PCSK9 interacts with LDLR and favors its lysosomal degradation [4, 5, 48]. Lack of LDLR
330 dysregulation after PCSK9 knockdown in the omics experiments were unexpected, so we evaluated
331 the effects of exogenously added human recombinant PCSK9 on LDLR expression. Incubating EndoC-
332 βH1 cells with recombinant PCSK9 reduced LDLR protein levels (**Fig. 5a and b**). We also performed a
333 confirmatory siRNA experiment to test whether endogenously produced PCSK9 regulated LDLR
334 expression. PCSK9 knockdown was efficient with a ~80% decrease at the mRNA level (**Fig. 5c**), at the
335 intracellular protein level (for both Pro-PCSK9 and mature PCSK9) (**Fig. 5d, f**) and at the secreted
336 protein level (**Fig. 5e, f**). We next looked at LDLR levels. As expected, PCSK9 knockdown did not
337 modulate LDLR mRNA levels (**Fig. 5g)**. Moreover, despite the sharp decrease in PCSK9 protein
338 expression and secretion, PCSK9 knockdown did not impair LDLR protein levels as measured by
339 Western blot (**Fig. 5h and i**). To determine whether PCSK9 specifically modulates LDLR at the cell
340 surface, we performed FACS analyses. Again, PCSK9 knockdown did not modulate cell surface LDLR
341 levels (**Fig. 5j, k**). Taken together, these results indicate that LDLR can be targeted by extracellular
342 PCSK9 but is not regulated by intracellular PCSK9 in EndoC-βH1 cells.

343

344 **PCSK9 knockdown potentiates the induction of PD-L1 and HLA-ABC by interferon-γ**

345 MHC-I and PD-L1 are increased in pancreatic islets from type I diabetics and in response to Interferon
346 class I stimulation [49–51]. While characterizing the effect of PCSK9 knockdown on the proteome of
347 EndoC-βH1, we observed up-regulation of MHC-I (particularly HLA-A, B, C and E) and PD-L1 (**Fig. 4f, g**).
348 We thus asked whether PCSK9 regulates PD-L1 and/or HLA-ABC cell surface expression in basal
349 conditions and following interferon-γ treatment. PCSK9 knockdown consistently reduced *PCSK9* mRNA
350 levels in basal or Interferon-γ stimulated conditions (**Fig. 6a**) with a slight increase of *MHC-I mRNA*

351 under basal conditions (**Fig. 6b**). On the other hand, upon Interferon-γ treatment, *MHC-I* and *PD-L1*
352 mRNA levels were unchanged following PCSK9 knockdown (**Fig. 6b**). We next measured by FACS cell
353 surface expression of PD-L1 following siRNA-mediated PCSK9 knockdown or following treatment with
354 recombinant PCSK9. It revealed that while siRNA-mediated PCSK9 knockdown increased cell surface
355 expression of PD-L1 (**Fig. 6c, d**), recombinant PCSK9 did not have any effects (**Fig. 6e, f**). We performed
356 a similar experiment, measuring cell surface expression of HLA-ABC. Again, while siRNA-mediated
357 PCSK9 knockdown increased cell surface expression of HLA-ABC (**Fig. 6g, h**), recombinant PCSK9 did
358 not have any effects (**Fig. 6i, j**). This data indicates that PD-L1 and HLA-ABC cell surface expression is
359 regulated by intracellular PCSK9.

360

361 **PCSK9 regulates the expression of VLDLR and CD36, 2 actors involved in fatty acid homeostasis**

362 Fatty acid homeostasis is important for proper beta cell function (Lytrivi et al., 2020). Interestingly,
363 VLDLR and CD36 are involved in the uptake of triglyceride-rich lipoproteins and fatty acids,
364 respectively, and are PCSK9 degradation targets in liver cells [9, 12]. We further investigated their
365 expression by RT-qPCR and Western blot. PCSK9 knockdown slightly decreased VLDLR mRNA (**Fig. S6a**)
366 but did not impact VLDLR protein expression (**Fig. 7a, b**). Incubating EndoC-βH1 cells with recombinant
367 PCSK9 reduced VLDLR protein expression (**Fig. 7c, d)**. PCSK9 knockdown cells exhibited a clear
368 reduction in PCSK9 mRNA level (**Fig. S6b**) and a slight, but non-statistically significant, increase in CD36
369 mRNA level (**Fig. S6c)**. FACS analysis revealed a greater than two-fold increase of CD36 cell surface
370 expression (**Fig. 7e, f**). On the other hand, incubating EndoC-βH1 cells with recombinant PCSK9 did not
371 affect CD36 cell surface expression (**Fig. 7g-h)**. Finally, upon PCSK9 knockdown, fatty acid uptake
372 increased by 50% (**Fig. 7i**).

373 In conclusion, both extracellular and intracellular PCSK9 can modulate beta cell fatty acid metabolism
374 through the regulation of VLDLR and CD36 expression respectively.

375

376 ## Discussion

377 Here, we demonstrate that beta cells produce and secrete PCSK9 in a tightly controlled manner. We
378 also observe that PCSK9 regulates the expression of a number of proteins, including LDLR and VLDLR
379 through extracellular mechanisms, and CD36, HLA-ABC and PD-L1 through intracellular mechanisms.

380 In liver cells, PCSK9 is expressed as a pro-enzyme, processed intracellularly into its mature form and
381 finally secreted [1]. By using EndoC-βH1 cells [33], we demonstrated here that it is also the case in
382 human beta cells. Indeed, Western blot analyses detected both Pro-PCSK9 and mature PCSK9 in cell
383 extracts, while only mature PCSK9 was detected in conditioned medium. We noted that the mature
384 PCSK9/PCSK9 total ratio and secreted/intracellular PCSK9 ratio was lower in beta cells compared to
385 liver cells. This suggest that Pro-PCSK9 processing is more efficient in liver cells compared to beta cells
386 and that intracellular PCSK9 pool is higher in beta cells. This may explain the preferential intracellular
387 regulation of some proteins by PCSK9. The mechanisms that regulate Pro-PCSK9 processing and
388 secretion have only been partially characterized [4]. Our results also indicate that PCSK9 processing
389 and secretion might be differentially regulated in liver and beta cells. Beta cells might thus be used as
390 an additional model to study this complex processing.

391 In liver cells, the transcriptional regulation of PCSK9 has been studied in great detail [4]. It has been
392 shown that SREBP2 regulates both basal and Mevastatin-induced expression of PCSK9 [18, 53, 54].
393 Interestingly, we demonstrate that in beta cells, physiological PCSK9 levels are regulated by SREBP1,
394 while pharmacological regulation of PCSK9 by Mevastatin is SREBP2-dependent. This is intriguing as in
395 the case of other SREBP1 targets such as FASN and SCD, we observe a SREBP2 mechanism that
396 compensates for SREBP1-mediated downregulation. This compensatory mechanism is not evident for
397 PCSK9, indicating that physiological levels of PCSK9 in beta cells are highly SREBP1-dependent.

398 The role of PCSK9 in pancreatic islet function is currently debated. Some studies indicate that PCSK9-
399 deficient mice develop hyperglycemia and insulinopenia [29, 30], while others do not observe this
400 phenotype [28, 31]. The reasons for these discrepancies might be linked to the age and/or genetic
401 background of the mice. Here, we observed a sharp decrease in several beta cell enriched transcription
402 factors upon PCSK9 knockdown in EndoC-βH1 cells. It is for example the case for *PDX1* and *NKX6-1* that
403 are known to play major roles in beta cell development and function [55–57]. We also detected some
404 perturbation in glucose-stimulated insulin secretion with a specific increase in basal insulin secretion,
405 which was not addressed by Ramin-Mangata *et al.* [32]. Taken together, the phenotype observed upon
406 PCSK9 knockdown (decreased expression of specific transcription factors and increased basal insulin
407 secretion) resembles that observed in immature fetal beta cells [58, 59] and in insulin-producing beta
408 cells differentiated *in vitro* from multipotent stem cells [60, 61]. It suggests that PCSK9 might be
409 important to maintain a mature phenotype in pancreatic beta cells.

410 In mouse islets, loss of beta-cell PCSK9 results in unchanged LDLR protein levels [31]. Unexpectedly,
411 data from a recently published study [32] indicate that PCSK9 knockdown in EndoC-βH1 cells increased
412 LDLR cell surface expression by 20%. Our data do not support this last claim. We observed, as expected,
413 that exogenously added PCSK9 decreased the expression of LDLR at the cell surface. On the other hand,
414 efficient PCSK9 knockdown did not modify neither *LDLR* mRNA, nor the total amounts of LDLR protein
415 measured either by Western blot or by global mass spectrometry, nor cell surface LDLR levels
416 measured by FACS. Moreover, we observed similar results when looking at VLDLR, yet another PCSK9
417 target [9]: VLDLR protein levels decreased upon treatment with exogenous PCSK9, without any effect

upon PCSK9 knockdown. Our data is consistent with observations in mouse islets [31]. The reasons for the discrepancy with Ramin-Mangata et al. [32] are difficult to discuss, as this study presents FACS data in arbitrary units without supporting FACS plots. Taken together, we propose that in pancreatic beta cells, LDLR and VLDLR levels are regulated by PCSK9 extracellularly rather than through intracellular degradation.

CD36 has been described as another PCSK9 target both in mouse liver and in adipocytes [12]. There, PCSK9 increases CD36 degradation by a mechanism involving both lysosomes and proteasomes [12]. Interestingly, in pancreatic beta cells, inhibiting CD36 has been described as a protective mechanism to prevent excessive lipid accumulation [62], while increased CD36 expression was found deleterious for proper beta cell function [63]. Here, we observe that PCSK9 knockdown in EndoC-βH1 increased cell surface CD36 expression and fatty acid uptake. Based on RNA-seq and proteomics in PCSK9 knockdown cells and the insensitivity of cell surface CD36 expression to recombinant PCSK9, we postulate that PCSK9 regulates CD36 by an intracellular mechanism. The precise mechanism of this regulation was not identified, but likely involves both proteasomal and lysosomal degradation [12]. Further experiments, in particular by using proteasome or lysosome inhibitors are needed to elucidate the nature of CD36 regulation by PCSK9 in the pancreatic beta cell.

In tumoral cells, MHC-I was recently described as a new PCSK9 target, as PCSK9 down regulates MHC-I cell surface expression by increasing its lysosomal degradation and disrupting its recycling [15]. In this model, the authors observed marginal effects of PCSK9 on the immune regulatory protein PDL-1. Interestingly, MHC-I and PDL-1 play major roles in beta cells. Their expression increases in pancreatic beta cell during inflammatory responses [49, 50, 64] and is also induced in islets from type 1 diabetic patients [50, 64]. There, it has been proposed that autoreactive CD8 T cells interact with beta cells and kill them through an MHC-I mediated activation while PD-L1 would be protective against autoimmune injuries [65]. Here, we found that PCSK9 knockdown increases both PD-L1 and HLA-ABC cell surface expression, particularly upon Interferon-γ stimulation. On the other hand, recombinant PCSK9 did not modulate HLA-ABC and PD-L1 levels. We thus propose that PCSK9 regulates the expression of PD-L1 and HLA-ABC in human beta cells by an intracellular mechanism.

In conclusion, we find that PCSK9 is expressed and secreted by human pancreatic beta cells in a tightly regulated manner, which is altered in the presence of statins. In turn, we find that PCSK9 regulates cell surface expression of CD36, PD-L1 and HLA-ABC through intracellular mechanisms, but regulates LDLR and VLDLR expression through an extracellular mechanism in pancreatic beta cells. It will be interesting to determine if PCSK9 monoclonal antibodies or siRNA-based therapies against PCSK9 affect expression levels of LDLR and VLDLR, or CD36, PD-L1, and HLA-ABC respectively in pancreatic beta cells, and whether co-treatment with statins interferes with the effect of PCSK9 siRNA in the pancreas.

458 **Data availability**

459 The datasets generated during and/or analyzed during the current study are available from the
460 corresponding authors.

461 Data sets accession numbers: RNA-seq GSE182016, cell lysate TMT proteomics experiment
462 PXD027921, cell lysate DIA experiment PXD027911, secretome DIA proteomics PXD027913.

463

464 **Author contribution statements**

465 K.S., G.H., I.G. and C.B. performed experiments. M.A. and F.C. provided human islets. K.S., R.S., M.R.
466 and A.J. analyzed data and drafted the manuscript with contributions from S.A., W.L.G., K.SG, C.R.U.

467 All authors approved the final draft.

468

469 **Conflict of interest**

470 S.A., K.Se., M.R., A.J., C.R.U and G.H. are employed by AstraZeneca. R.S. is a shareholder in and
471 consultant for Univercell-Biosolutions.

472

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662 Commun 495(3):2221–2226. https://doi.org/10.1016/j.bbrc.2017.12.111

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664 Obesity Through Lower CD36 Expression and Improved Exocytosis in β-Cells. Diabetes
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670 disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol 17(3):150–161.
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672

673

674 **Figure legends**

675 **Figure 1: PCSK9 is expressed and secreted by pancreatic beta cells**

676 (**a-c**) *PCSK9* mRNA expression in: (a) mouse liver compared to FACS-enriched pancreatic beta, delta
677 and alpha endocrine fractions (*n=4-5*); (b) human islets, hepatocytes and HEK293T cells (*n=5-8*); (c)
678 EndoC-βH1, HepG2 and HEK293T cells (*n=6-7*). **(d, e)** Detection by Western blot and quantification of
679 intracellular PCSK9 in EndoC-βH1, HepG2 and HEK293T cells (*n=5*). (**f, g**) Detection by Western blot and
680 quantification of secreted PCSK9 in EndoC-βH1, HepG2 and HEK293T cells (*n=5-6*). (**h**) Western blot
681 analysis of PCSK9 secretion by human islets, compared to HEK293T cells (*n=7*). Data represent the
682 means ± SEM. *p < 0.05, **p < 0.01

683

684 **Figure 2: Cholesterol, LDL and mevastatin modulate PCSK9 expression and secretion**

685 (**a-f**) EndoC-βH1 cells were exposed to the indicated treatments for 24 hours. (a, b) SREBP-2
686 transcriptional targets (*HMGCR* and *LDLR*), SREBP-1 transcriptional targets (*FASN*, and *SCD*) and PCSK9
687 mRNA were measured by RT-qPCR (*n=5*). Intracellular (c, d) and secreted (e, f) PCSK9 were analyzed
688 and quantified by Western blot (*n=4*). (**g, h**) EndoC-βH1 cells were exposed to native LDL, oxidized LDL
689 or Triglyceride-Rich Lipoproteins (TRL) for 24 hours. *PCSK9*, SREBP1 and SREBP2 targets were measured
690 by RT-qPCR. (*n=4*) Data represent the means ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001

691

692 **Figure 3: PCSK9 is regulated by the SREBPs-1 and 2 transcription factors**

693 (**a-c**) EndoC-βH1 cells were transfected with control nontarget siRNA (siCtrl), siRNA targeting SREBP-
694 1+SREBP-2 (siSREBP-1+2), SREBP-2 (siSREBP-2), or SREBP-1 (siSREBP-1). RT-qPCR for SREBP's and their
695 targets were performed 3 days later (*n=5*). (**d-g**) EndoC-βH1 cells were transfected with siRNA, cultured
696 for 72h and next treated for 24 hours with DMSO (control) or mevastatin before analyses by RT-qPCR
697 (*n=5*). Data represent the means ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001

698

699 **Figure 4: Insights from omics experiments**

700 EndoC-βH1 cells were transfected with control nontarget siRNA (siControl) or siRNA targeting PCSK9
701 (siPCSK9). Three days later, cells were profiled by RNA-seq and proteomics. RNA-seq data were
702 analyzed with: (**a**) Ingenuity Pathway Analysis (IPA) software, for the top enriched canonical pathways,
703 and (**b**) Gene Set Enrichment Analysis (GSEA) software with the Hallmark_Oxidative_Phosphorylation
704 gene set. (**C**) GSIS markers deregulated upon PCSK9 knockdown with FDR <0.05 in RNA-seq experiment.
705 (**d**) Basal (0.5 mM), glucose-stimulated (20 mM) and KCl-stimulated (50 mM) insulin secretion was
706 measured in EndoC-βH1 cells, three days following PCSK9 knockdown (*n=6*). (**e**) Gene Ontology analysis
707 of cellular components terms enriched among genes and proteins up-regulated following PCSK9
708 knockdown. (**f-h**) Boxplot showing protein expression of top candidate PCSK9 degradation targets,
709 measured on cell protein extracts by DIA and TMT proteomic. (**i**) RT-qPCR validation of selected top
710 differentially expressed genes (*n=10*). For GSIS and RT-qPCR analyses, data are presented as means ±
711 SEM. For proteomics experiments, median expression and interquartile ranges are shown. + FDR <
712 0.05, *p < 0.05, **p < 0.01 and ***p < 0.001

713

714 **Figure 5: PCSK9 knockdown does not affect LDLR levels**

715 (**a, b**): EndoC-βH1 cells were exposed for 16 hours to human recombinant PCSK9 (2.5 µg/ml). LDLR
716 protein expression was studied by Western blot and quantified (*n=4*). (**c-k**) EndoC-βH1 cells were
717 transfected with control nontarget siRNA (siCtrl) or siRNA targeting PCSK9 (siPCSK9). Analyses were
718 performed 3 days later. (c-f) PCSK9 knockdown efficiency was analyzed by RT-qPCR (*n=9*) (c) and
719 Western blot for intracellular and secreted PCSK9 (*n=5-7*) (d-f). (g-k) The effect of PCSK9 knockdown
720 on LDLR was measured by RT-qPCR, (*n=7*), Western blot (n=5) and by FACS for cell surface expression
721 (*n=6*). Data represent the means ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001

722

723 **Figure 6: PCSK9 knockdown potentiates the induction of PD-L1 and HLA-ABC by interferon-γ**

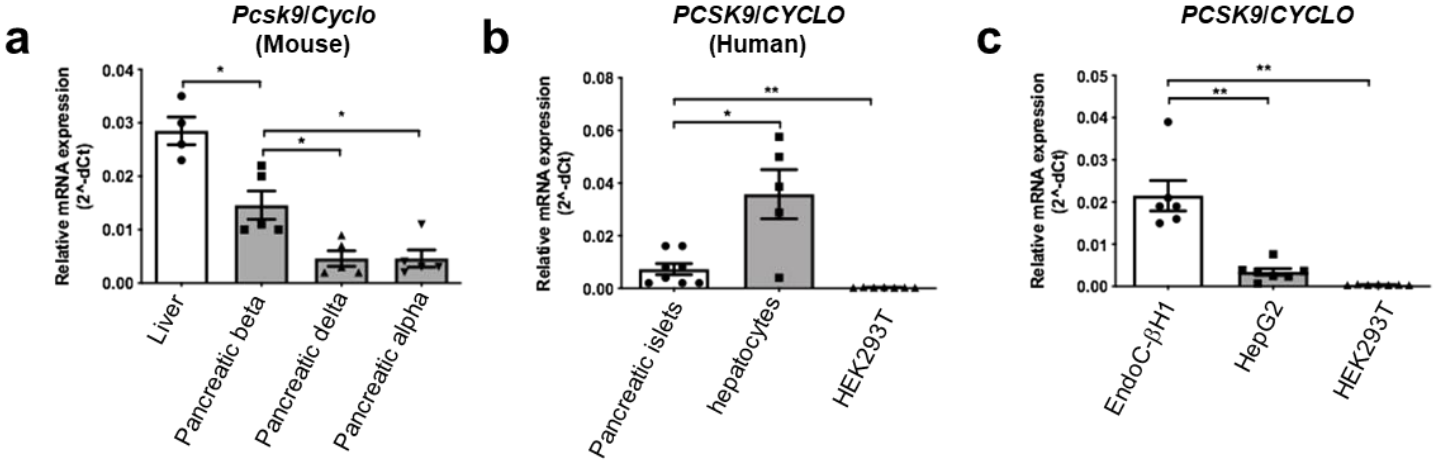
724 (**a-d**) EndoC-βH1 cells were transfected with control nontarget siRNA (siCtrl) or siRNA targeting PCSK9
725 (siPCSK9). Five days later, the cells were incubated for 24 hours with interferon-γ (IFN–γ). (a, b), RT-
726 qPCR analysis of PCSK9, PD-L1 and MHC- I (*n=6-7*) (c, d) Cell surface PD-L1 expression analyzed by
727 FACS (*n=8-9*). (**e, f**) EndoC-βH1 cells were treated with Interferon- γ (IFN-γ). Twenty-four hours later,
728 human recombinant PCSK9 (rPCSK9) (2.5 µg/ml) was added to the culture medium for 16 hours. Cell
729 surface PD-L1 was studied by FACS (*n=4*). (**g-j**) Cell surface HLA-ABC expression analyzed by FACS under
730 the same experimental conditions as in (a-f) (*n=4-7*). Data represent the means ± SEM. *p < 0.05, **p
731 < 0.01

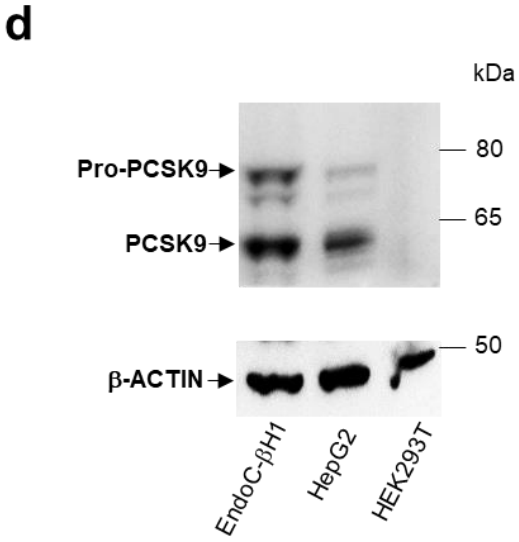
732

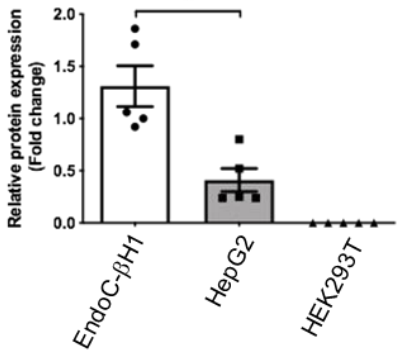
733 **Figure 7: PCSK9 regulates the expression of VLDLR and CD36, 2 actors involved in fatty acid**
734 **homeostasis**

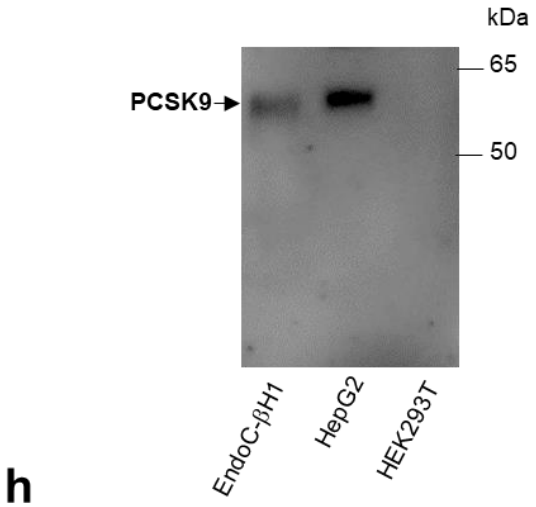
735 (**a, b**) EndoC-βH1 cells were transfected with control nontarget siRNA (siCtrl) or siRNA targeting PCSK9.
736 Three days later, VLDLR expression was studied by Western blot and quantified *(n=5-6*). (**c, d**) EndoC-
737 βH1 cells were exposed for 16 hours to human recombinant PCSK9 (2.5 µg/ml) and VLDLR protein
738 expression was studied by Western blot (*n=4*). (**e, f**): EndoC-βH1 were transfected with siRNA (siCtrl)
739 or siRNA targeting PCSK9. Six days later, cell surface CD36 was analyzed by FACS (*n=4*). (**g, h**) EndoC-
740 βH1 cells were exposed for 16 hours to 2.5 µg/ml human recombinant PCSK9 and cell surface CD36
741 was analyzed by FACS (*n=4*). (**i**) Fatty acid uptake was evaluated six days following PCSK9 knockdown
742 (*n =10*). Data represent the means ± SEM. *p < 0.05, ***p < 0.001

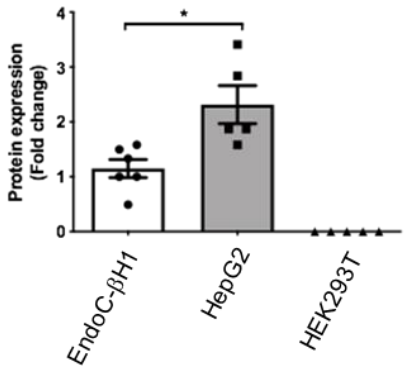
Fig. 1


a
Pcsk9/Cyclo
(Mouse)
Relative mRNA expression
(2^-dCt)
0.04
0.03
0.02
0.01
0.00
*
*
*
Liver
Pancreatic beta
Pancreatic delta
Pancreatic alpha
b
PCSK9/CYCLO
(Human)
Relative mRNA expression
(2^-dCt)
0.08
0.06
0.04
0.02
0.00
**
*
Pancreatic islets
hepatocytes
HEK293T
c
PCSK9/CYCLO
Relative mRNA expression
(2^-dCt)
0.05
0.04
0.03
0.02
0.01
0.00
**
**
EndoC-βH1
HepG2
HEK293T


d
kDa
Pro-PCSK9
PCSK9
β-ACTIN
80
65
50
EndoC-βH1
HepG2
HEK293T


e
Relative protein expression
(Fold change)
2.0
1.5
1.0
0.5
0.0
**
EndoC-βH1
HepG2
HEK293T


f
kDa
PCSK9
65
50
EndoC-βH1
HepG2
HEK293T


g
Protein expression
(Fold change)
4
3
2
1
0
*
EndoC-βH1
HepG2
HEK293T

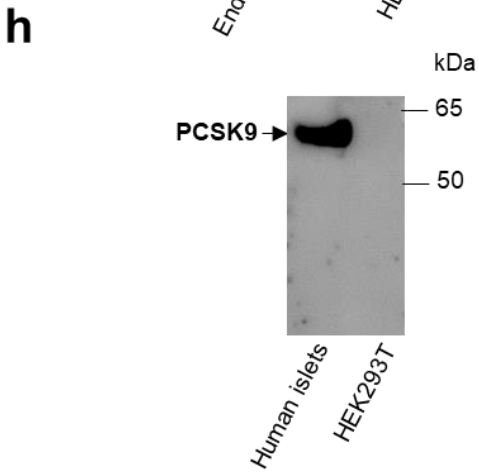

h
kDa
PCSK9
65
50
Human islets
HEK293T

Fig. 2

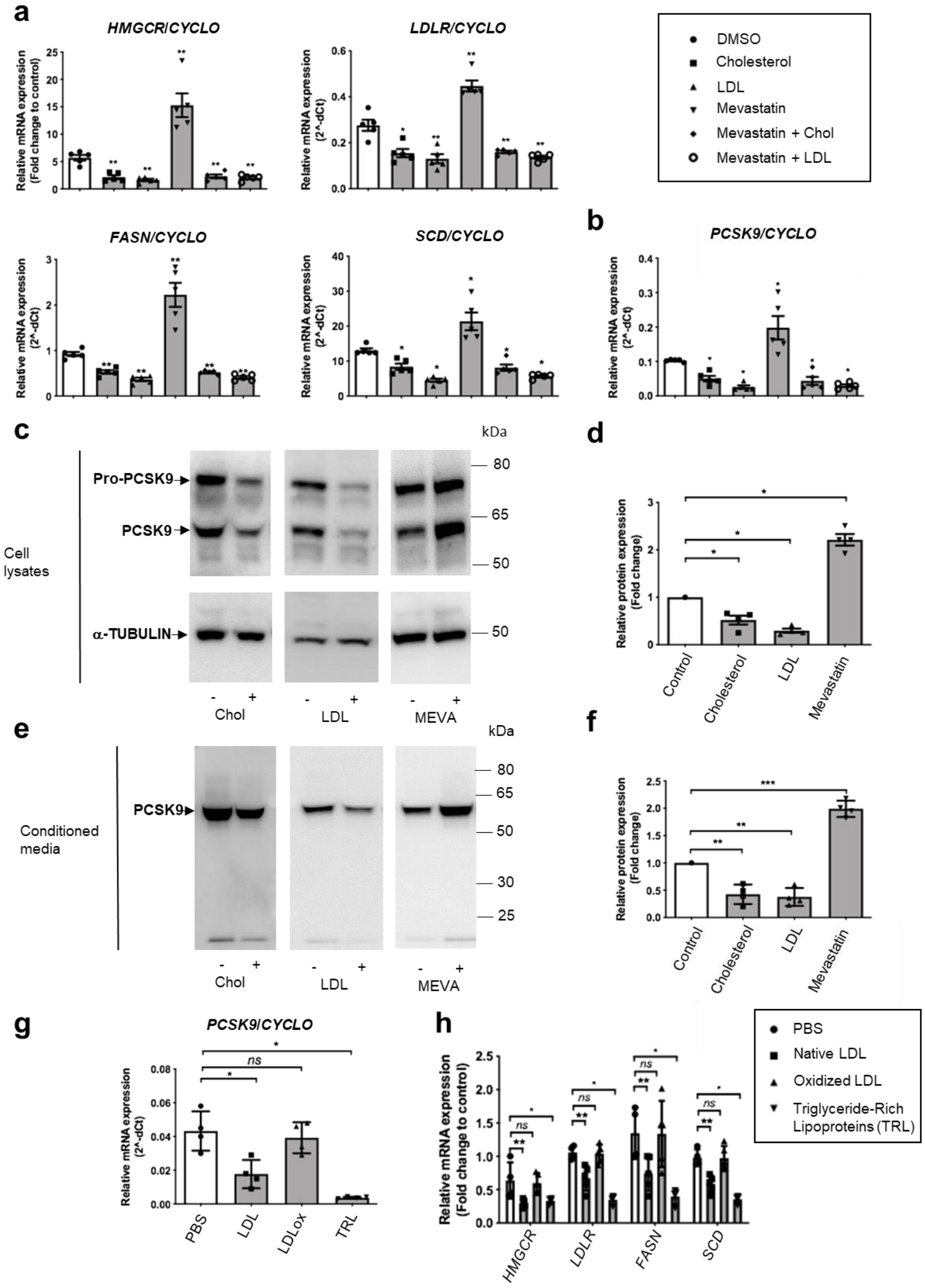
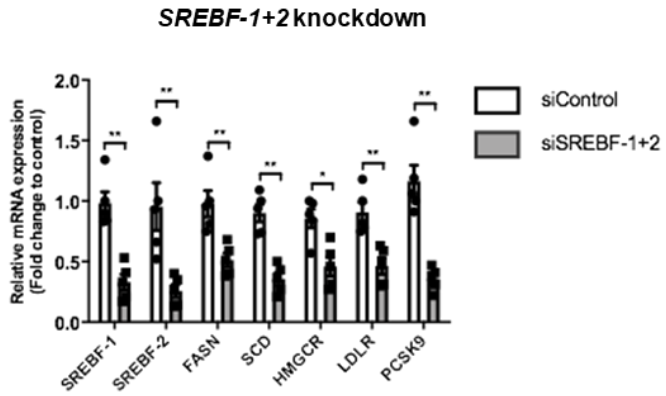
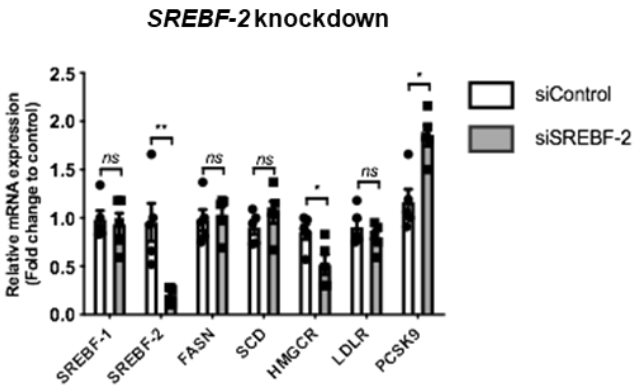

a
HMGCR/CYCLO
LDLR/CYCLO
FASN/CYCLO
SCD/CYCLO
Relative mRNA expression (Fold change to control)
Relative mRNA expression (2^-dCt)
DMSO
Cholesterol
LDL
Mevastatin
Mevastatin + Chol
Mevastatin + LDL
b
PCSK9/CYCLO
c
kDa
Pro-PCSK9
PCSK9
α-TUBULIN
Cell lysates
80
65
50
Chol
LDL
MEVA
d
Relative protein expression (Fold change)
Control
Cholesterol
LDL
Mevastatin
e
Conditioned media
30
25
f
g
PCSK9/CYCLO
PBS
LDL
LDLox
TRL
h
Native LDL
Oxidized LDL
Triglyceride-Rich Lipoproteins (TRL)
HMGCR
FASN
SCD

Fig. 3

a




b

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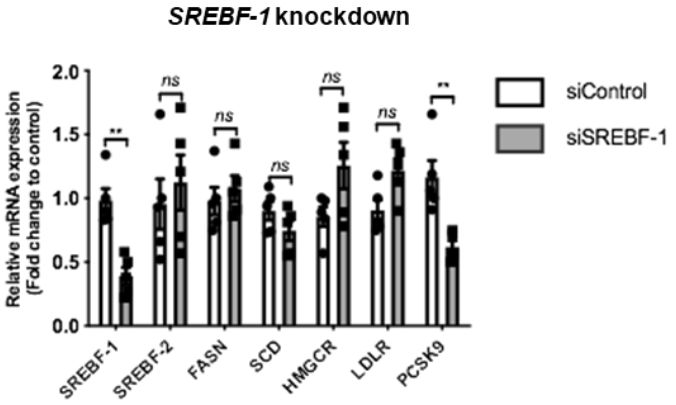


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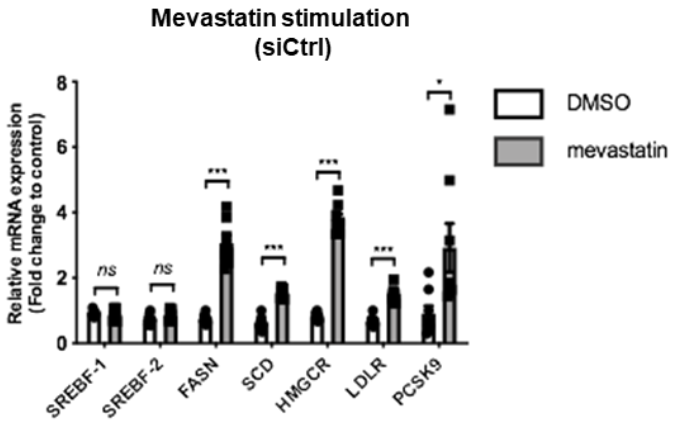


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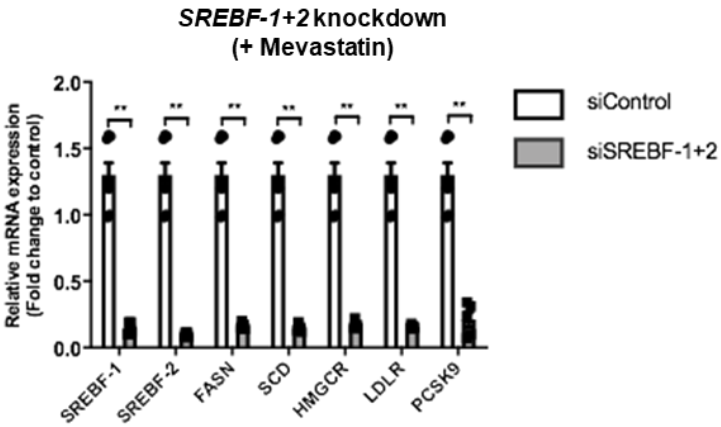


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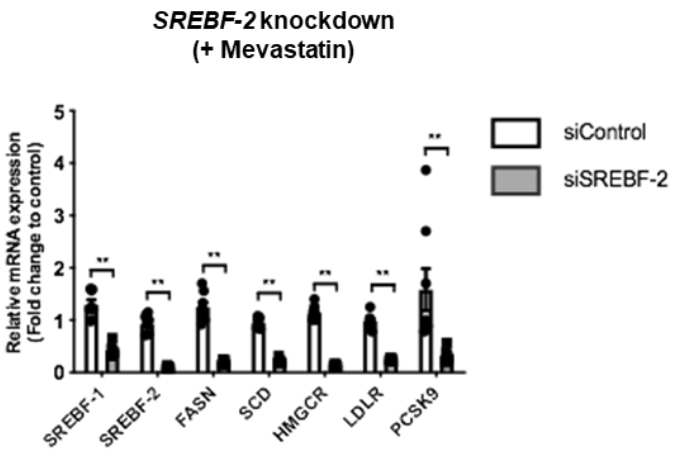



g

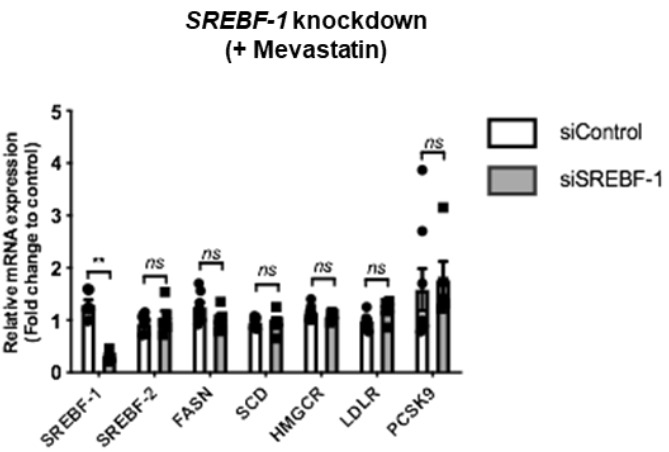


Fig. 4

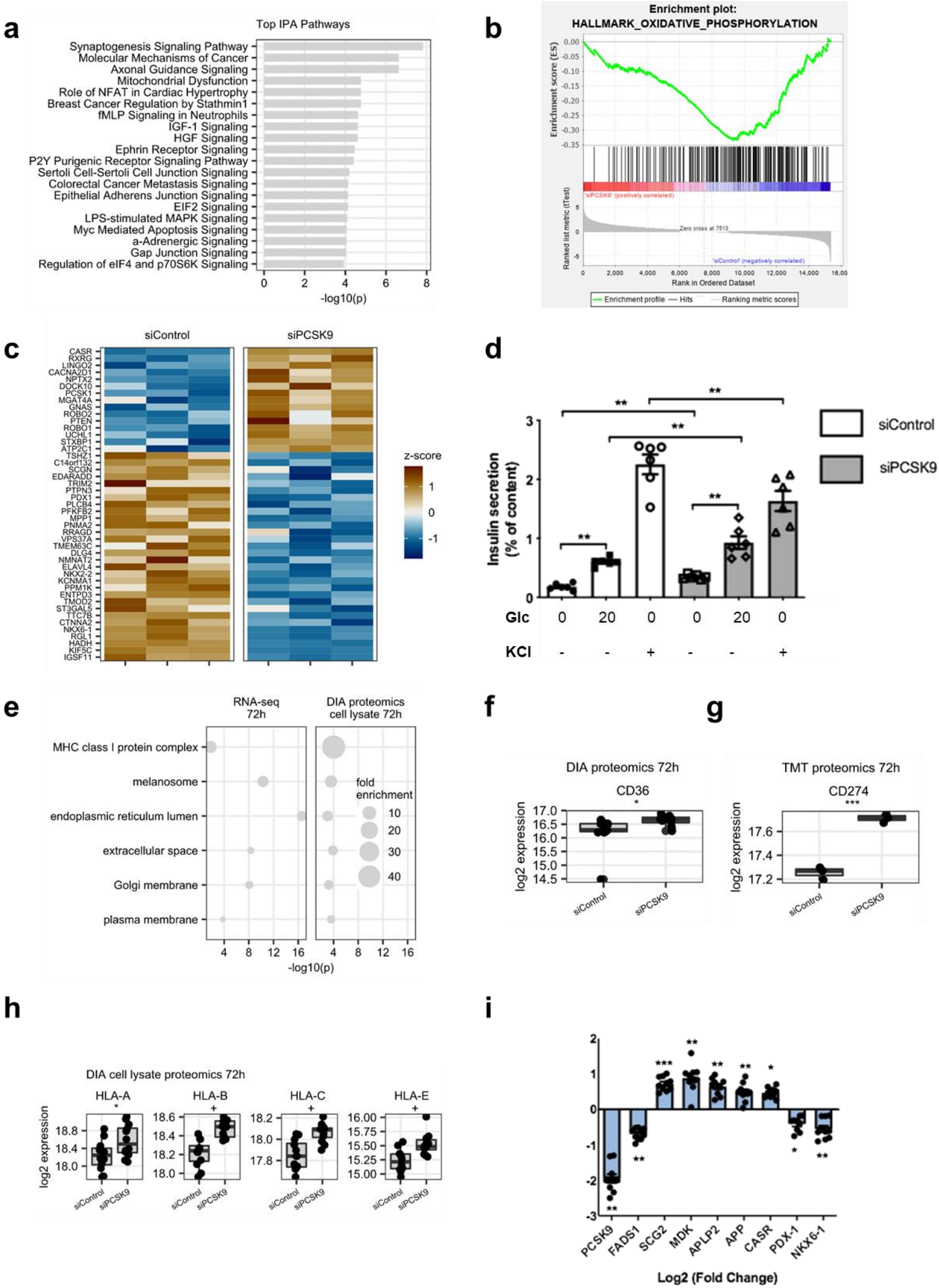

a
Top IPA Pathways
Synaptogenesis Signaling Pathway
Molecular Mechanisms of Cancer
Axonal Guidance Signaling
Mitochondrial Dysfunction
Role of NFAT in Cardiac Hypertrophy
Breast Cancer Regulation by Stathmin1
fMLP Signaling in Neutrophils
IGF-1 Signaling
HGF Signaling
Ephrin Receptor Signaling
P2Y Purigenic Receptor Signaling Pathway
Sertoli Cell-Sertoli Cell Junction Signaling
Colorectal Cancer Metastasis Signaling
Epithelial Adherens Junction Signaling
EIF2 Signaling
LPS-stimulated MAPK Signaling
Myc Mediated Apoptosis Signaling
a-Adrenergic Signaling
Gap Junction Signaling
Regulation of eIF4 and p70S6K Signaling
-log10(p)
b
Enrichment plot:
HALLMARK_OXIDATIVE_PHOSPHORYLATION
Enrichment score (ES)
Ranked list metric (tTest)
Zero cross at 7513
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
c
siControl
siPCSK9
z-score
d
Insulin secretion (% of content)
siControl
siPCSK9
Glc
KCl
e
RNA-seq 72h
DIA proteomics cell lysate 72h
MHC class I protein complex
melanosome
endoplasmic reticulum lumen
extracellular space
Golgi membrane
plasma membrane
fold enrichment
-log10(p)
f
DIA proteomics 72h
CD36
log2 expression
siControl
siPCSK9
g
TMT proteomics 72h
CD274
log2 expression
siControl
siPCSK9
h
DIA cell lysate proteomics 72h
HLA-A
HLA-B
HLA-C
HLA-E
log2 expression
siControl
siPCSK9
i
PCSK9
FADS1
SCG2
MDK
APLP2
APP
CASR
PDX-1
NKX6-1
Log2 (Fold Change)

Fig. 5

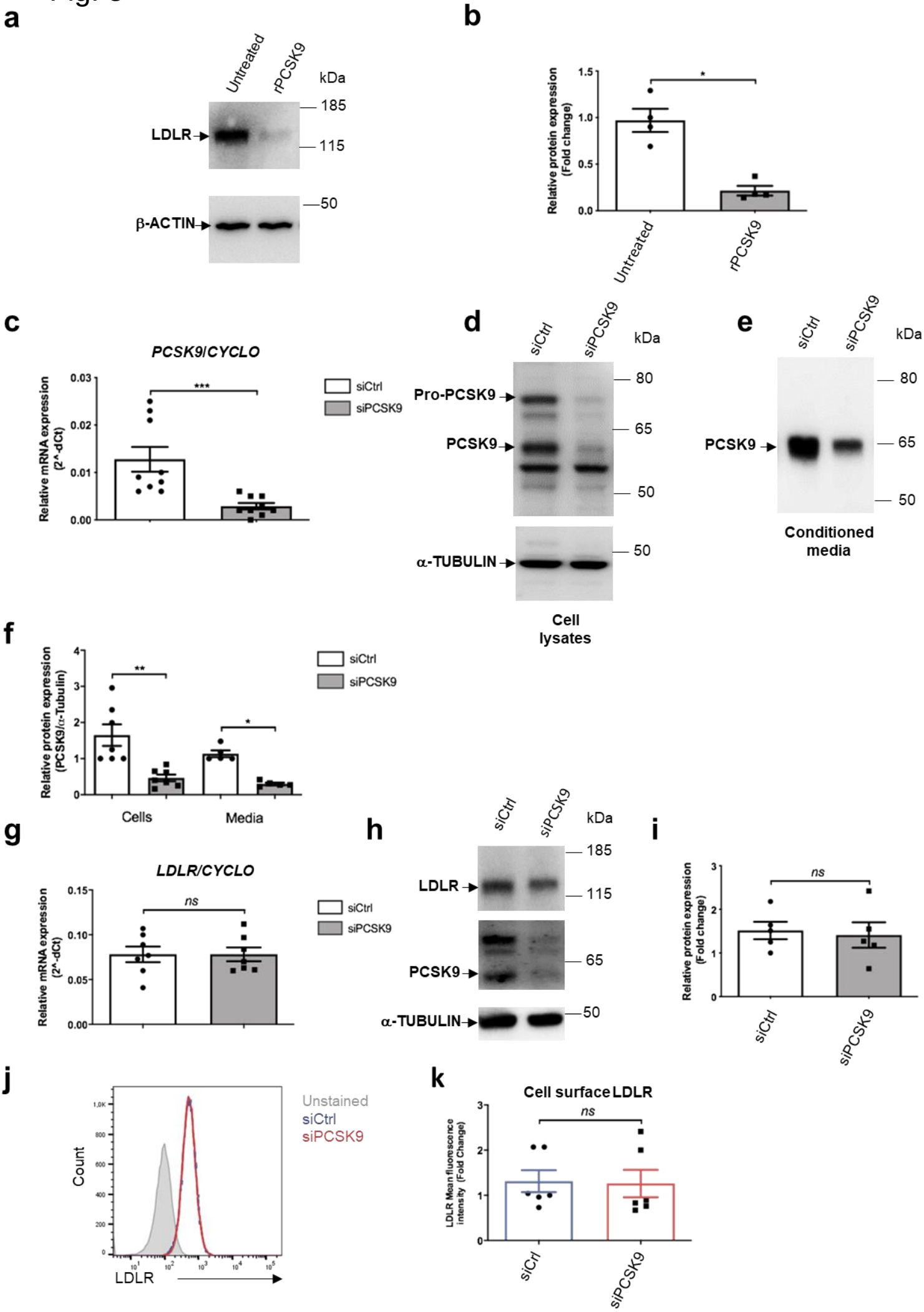

a
Untreated
rPCSK9
kDa
185
115
50
LDLR
β-ACTIN
b
Relative protein expression (Fold change)
1.5
1.0
0.5
0.0
*
Untreated
rPCSK9
c
PCSK9/CYCLO
Relative mRNA expression (2^-dCt)
0.03
0.02
0.01
0.00
***
siCtrl
siPCSK9
d
siCtrl
siPCSK9
kDa
Pro-PCSK9
PCSK9
α-TUBULIN
80
65
50
50
Cell lysates
e
siCtrl
siPCSK9
kDa
PCSK9
80
65
50
Conditioned media
f
Relative protein expression (PCSK9/α-Tubulin)
4
3
2
1
0
**
*
Cells
Media
siCtrl
siPCSK9
g
LDLR/CYCLO
Relative mRNA expression (2^-dCt)
0.15
0.10
0.05
0.00
ns
siCtrl
siPCSK9
h
siCtrl
siPCSK9
kDa
LDLR
PCSK9
α-TUBULIN
185
115
65
50
i
Relative protein expression (Fold change)
3
2
1
0
ns
siCtrl
siPCSK9
j
Count
LDLR
Unstained
siCtrl
siPCSK9
k
Cell surface LDLR
LDLR Mean fluorescence intensity (Fold Change)
3
2
1
0
ns
siCtrl
siPCSK9

Fig. 6

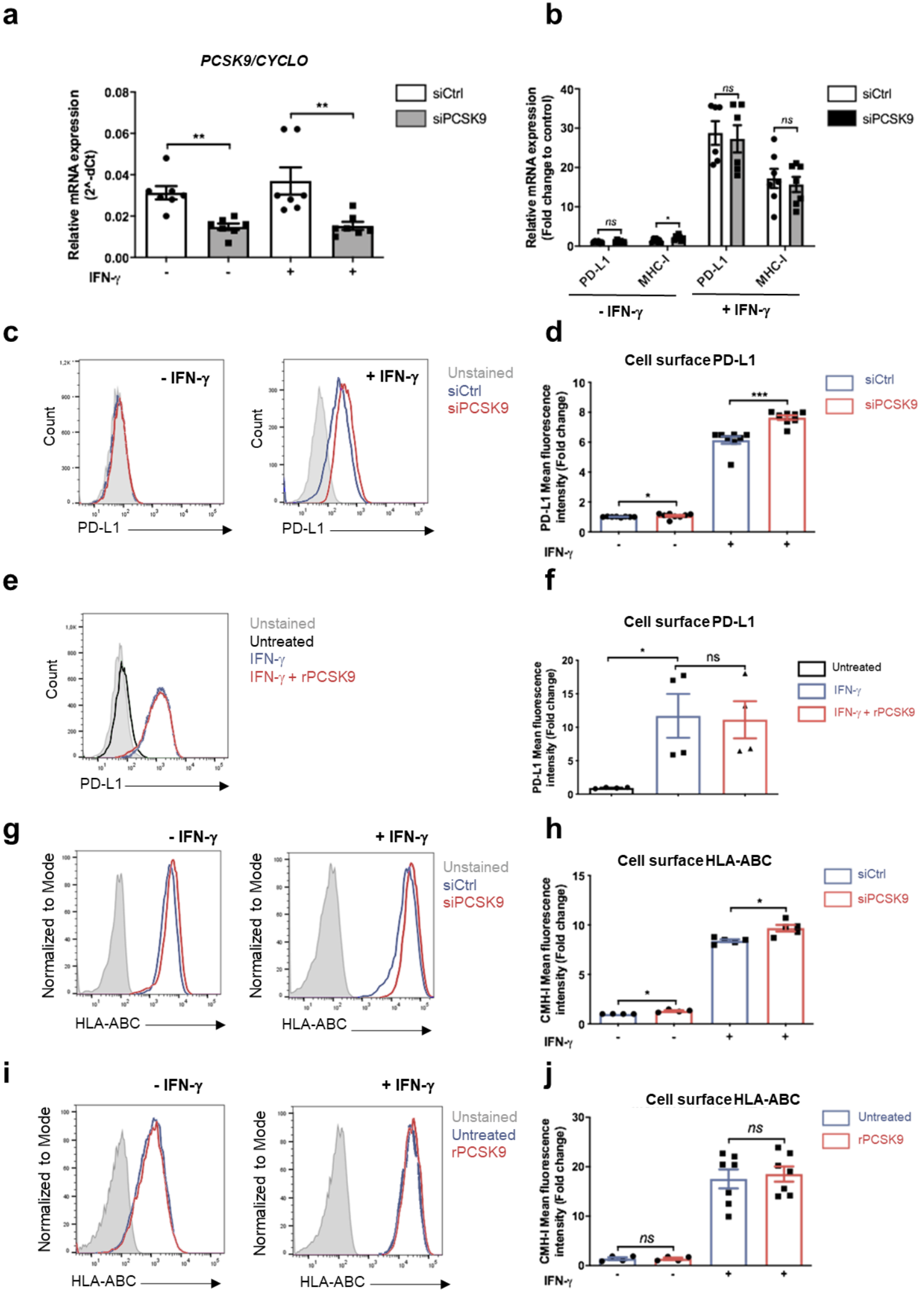
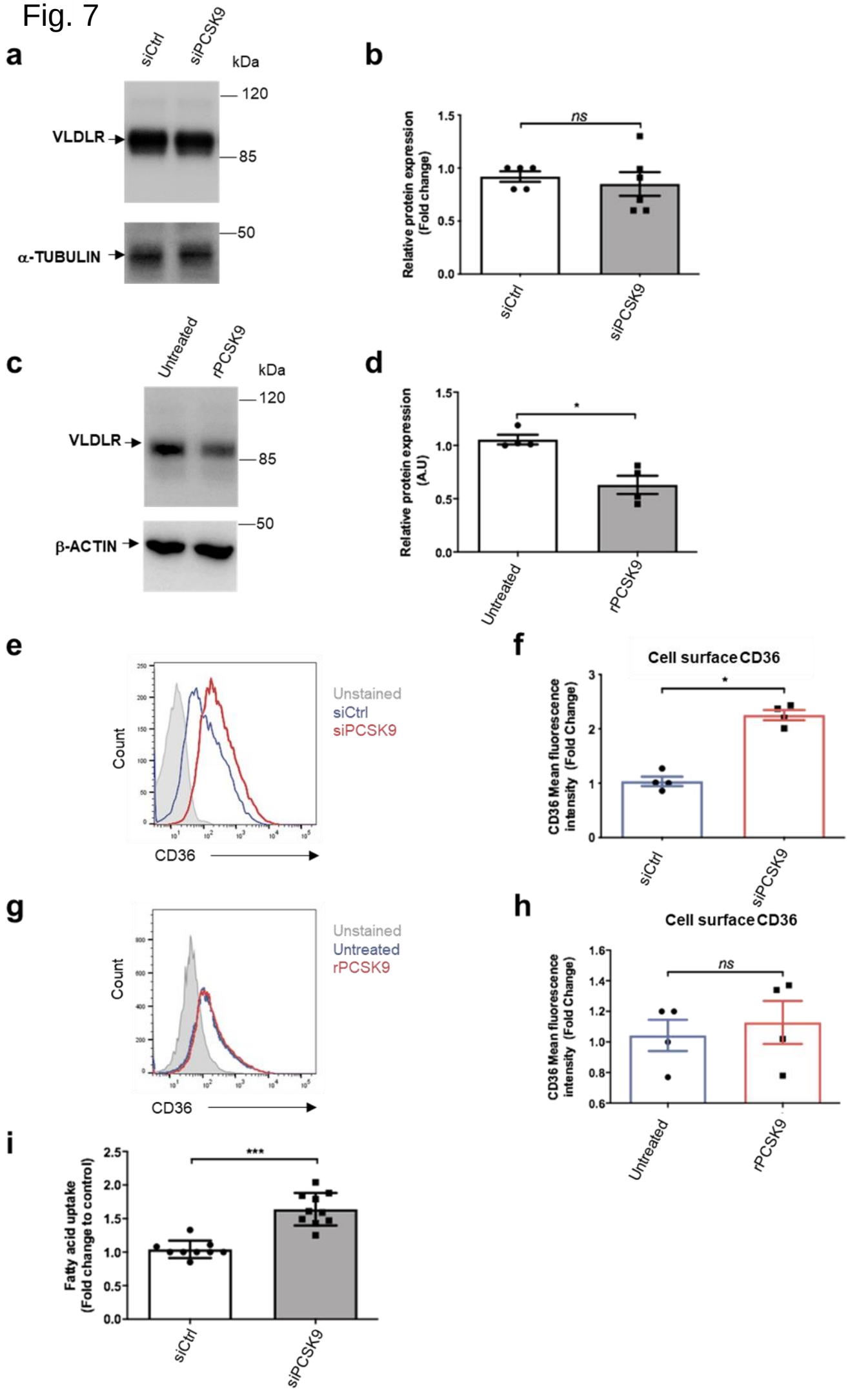

a
PCSK9/CYCLO
Relative mRNA expression (2^-dCt)
siCtrl
siPCSK9
IFN-γ
b
Relative mRNA expression (Fold change to control)
PD-L1
MHC-I
- IFN-γ
+ IFN-γ
c
- IFN-γ
+ IFN-γ
Count
PD-L1
Unstained
siCtrl
siPCSK9
d
Cell surface PD-L1
PD-L1 Mean fluorescence intensity (Fold change)
e
Unstained
Untreated
IFN-γ
IFN-γ + rPCSK9
f
Cell surface PD-L1
g
Normalized to Mode
HLA-ABC
h
Cell surface HLA-ABC
CMH-I Mean fluorescence intensity (Fold change)
i
rPCSK9
j

Fig. 7


a
siCtrl
siPCSK9
kDa
120
VLDLR
85
50
α-TUBULIN
b
Relative protein expression (Fold change)
ns
1.5
1.0
0.5
0.0
siCtrl
siPCSK9
c
Untreated
rPCSK9
kDa
120
VLDLR
85
50
β-ACTIN
d
Relative protein expression (A.U)
*
1.5
1.0
0.5
0.0
Untreated
rPCSK9
e
Count
Unstained
siCtrl
siPCSK9
CD36
f
Cell surface CD36
CD36 Mean fluorescence intensity (Fold Change)
*
3
2
1
0
siCtrl
siPCSK9
g
Count
Unstained
Untreated
rPCSK9
CD36
h
Cell surface CD36
CD36 Mean fluorescence intensity (Fold Change)
ns
1.6
1.4
1.2
1.0
0.8
0.6
Untreated
rPCSK9
i
Fatty acid uptake (Fold change to control)
***
2.5
2.0
1.5
1.0
0.5
0.0
siCtrl
siPCSK9