



CCKBR signalling controls human definitive endoderm specification and pancreatic lineage differentiation

Lama Saber^{1,2,3} · Kei Kozawa^{1,2} · Christos Karampelias^{1,2} · Changying Jing^{1,2,4} · Jessica Jaki^{1,2} · Melis Akgün Canan^{1,2,5} · Michael Sterr^{1,2} · Osvaldo Rodrigues Pereira^{1,2,3} · Oliver Czarnecki^{1,2,3} · Gregory S. Korbitt^{6,7} · Andrew R. Pepper^{6,7} · Noel Moya^{1,2,8} · Ingo Bartscher^{1,2} · Heiko Lickert^{1,2,3} · Mostafa Bakhti^{1,2}

Received: 10 April 2026 / Accepted: 1 September 2026
© The Author(s) 2026

Abstract

Aims/hypothesis Paracrine signalling by hormone-producing endocrine cells potentially influences pancreatic development, but this feedback regulation remains poorly understood. Here, we investigated the roles of gastrin (GAST), cholecystokinin (CCK) and their receptor cholecystokinin B receptor (CCKBR) during human pancreatic development.

Methods Human iPSCs were differentiated stepwise into definitive endoderm and then the pancreatic endocrine lineage. To assess CCKBR function, CRISPR/Cas9 was used to generate CCKBR-knockout iPSC lines. We profiled transcriptional states by bulk and single-cell RNA-seq with computational analyses, and complemented these with biochemical assays, flow cytometry (FACS) and confocal microscopy.

Results *CCKBR* expression peaked during definitive endoderm formation and progressively declined during differentiation, remaining detectable in pancreatic progenitors but largely absent from the endocrine lineage. By contrast, GAST and CCK were expressed only transiently during pancreatic progenitor and endocrine lineage formation. Pharmacological activation/inhibition and genetic knockout implicated CCKBR signalling during definitive endoderm formation. Consistently, bulk RNA-seq indicated that CCKBR deletion triggered ectodermal- and mesodermal-associated transcriptional programs and diminished pancreatic lineage specification. During pancreatic induction and lineage formation, sustained CCKBR activation with GAST or CCK attenuated endocrinogenesis and promoted epithelial structure formation. Further analysis revealed the emergence of a pancreatic ductal-like cell formation under prolonged CCKBR stimulation.

Conclusions/interpretation This study provides mechanistic evidence that the GAST/CCK–CCKBR axis is functionally important during human endoderm and pancreas development. This pathway is repeatedly required during in vitro pluripotent stem cell differentiation, regulating germ-layer formation as well as ductal vs endocrine fate decisions. These findings support the optimisation of pancreatic differentiation protocols and inform future studies on whether this axis contributes to human pancreas development and disease.

Keywords Beta cells · CCK · CCKBR · Definitive endoderm · Diabetes · Ductal · Endocrinogenesis · Gastrin · Islets · Stem cell differentiation

Abbreviations

ANOVA	Analysis of variance
CCK	Cholecystokinin
CCKAR	Cholecystokinin A receptor
CCKBR	Cholecystokinin B receptor
DE	Definitive endoderm

EP	Endocrine progenitor
GAST	Gastrin
GPCR	G-protein-coupled receptor
H2B	Histone 2B
hPSC	Human pluripotent stem cell
MPC	Multipotent progenitor cell
NGN3	Neurogenin-3
PCA	Principal component analysis
PCW	Post-conception weeks
PGT	Posterior gut tube
PP	Pancreatic progenitor

Kei Kozawa and Christos Karampelias contributed equally to this study.

Extended author information available on the last page of the article

Research in context

What is already known about this subject?

- Gastrin and cholecystokinin (CCK) are transiently expressed during embryonic pancreatic development but are largely absent from the healthy adult pancreas
- In the adult pancreas, gastrin and CCK can reappear in obesity and diabetes and have been linked to exocrine-to-beta cell reprogramming and pancreatic tumourigenesis in mice
- Cholecystokinin A receptor (CCKAR) and cholecystokinin B receptor (CCKBR) are the well-characterised receptors mediating gastrin- and CCK-dependent signalling

What is the key question?

- Do CCKBR and its ligands, gastrin and CCK, play a functional role in human pancreatic induction and lineage fate decisions?

What are the new findings?

- CCKBR signalling is required for efficient definitive endoderm induction
- Sustained CCKBR activation impairs endocrine lineage formation
- CCKBR activation in pancreatic progenitors induces a ductal-like epithelial program

How might this impact on clinical practice in the foreseeable future?

- These findings may help refine stem cell differentiation protocols for diabetes cell-replacement therapies and provide a framework for modelling endocrine, exocrine and ductal pancreatic diseases, including pancreatic ductal adenocarcinoma

SC-islet Stem cell-derived islet
 scRNA-seq Single-cell RNA-seq
 YSE Yolk sac endoderm

Introduction

Replacing lost insulin-producing beta cells with exogenous sources is a promising strategy for treating type 1 diabetes. Transplantation of human cadaveric islets restores glycaemic management, but is constrained by limited donor availability, immune rejection and cellular damage during islet isolation [1]. Generating functional islet cells from human pluripotent stem cells (hPSCs) has emerged as a compelling alternative [2]. Although recent clinical trials have demonstrated the therapeutic potential of stem cell-derived islet (SC-islet) transplantation [3], primary engraftment remains markedly delayed compared with cadaveric islets, often requiring more than 100 days to achieve functional insulin secretion *in vivo*. This highlights the need to refine current protocols and deepen understanding of mechanisms underlying human islet formation.

Multiple protocols exist to direct hPSC differentiation into islet cells [4–10]. Initially built on insights from

mouse pancreatic organogenesis, these protocols have since been refined using human model systems [11–13]. Broadly, they aim to mimic embryonic development, in which the definitive endoderm (DE), which gives rise to organs such as lung, liver, pancreas and gastrointestinal tract, is guided towards pancreatic differentiation (Fig. 1a). In this multi-step procedure, DE gives rise to foregut progenitors, from which pancreatic progenitors (PPs) form and then develop into endocrine progenitors (EPs) marked by neurogenin-3 (NEUROG3 or NGN3). EPs ultimately differentiate into hormone-producing cell types, including insulin-producing beta cells, glucagon-secreting alpha cells and somatostatin-generating delta cells (Fig. 1b) [14, 15]. Efficient SC-islet generation *in vitro* depends on how efficiently cells are directed towards the endocrine lineage. Thus, identifying the molecular factors governing endocrine vs non-endocrine fate decisions is essential for improving functional SC-islet production.

The adult pancreas comprises ~95% exocrine tissue (acinar and ductal cells) and ~5% endocrine islet cells, both arising from multipotent progenitor cells (MPCs) during early organogenesis. Lineage decisions in MPCs are governed by gene regulatory networks and upstream signalling pathways [14, 15]. Hormones produced by emerging endocrine cells

may represent a largely unexplored source of upstream signals. Insulin receptor signalling, for instance, can restrain excessive beta cell formation, helping maintain balance with alpha cells [16, 17], supporting the existence of hormone-mediated feedback mechanisms that coordinate islet cell composition. Whether pancreatic hormones influence the balance between endocrine and exocrine differentiation remains unclear.

Several hormones are transiently expressed during pancreatic development but largely absent from the adult organ, including ghrelin, gastrin (GAST) and cholecystokinin (CCK) [18, 19]. GAST- and CCK-positive cells can re-emerge in pathological conditions such as diabetes [20], where beta cell dedifferentiation occurs, suggesting roles in cellular plasticity [21]. GAST and CCK share a conserved C-terminal α -amidated pentapeptide (Gly-Trp-Met-Asp-Phe-NH₂) essential for their activity [22], and signal through two closely related G-protein-coupled receptors (GPCRs), cholecystokinin A receptor (CCKAR), which preferentially binds sulphated CCK, and cholecystokinin B receptor (CCKBR), which binds GAST and non-sulphated CCK with high affinity [23]. In adult mice, GAST administration reprograms pancreatic exocrine cells into beta cells [24], and cerulein, a CCK analogue, strongly stimulates exocrine secretion and is widely used to induce experimental acute pancreatitis [25]. Despite these established roles in the adult pancreas, the functional significance of the GAST/CCK–receptor axis during human pancreatic development remains unknown.

To address this gap, we investigated the role of CCKBR in human endocrinogenesis, using an in vitro stem cell differentiation system combined with pharmacological and genetic perturbations and RNA-seq. We characterised the expression of GAST, CCK and their receptors across endoderm and pancreatic lineage formation, and assessed the functional consequences of modulating CCKBR activity at distinct differentiation stages, to define the contribution of the GAST/CCK–CCKBR axis to human pancreatic lineage formation.

Methods

Generation and characterisation of CCKBR^{KO} iPSC reporter cell line The CCKBR locus was targeted by homologous recombination and CRISPR/Cas9 using a histone 2B (*H2B*)-*Venus*-3xHA-tag targeting vector with 897 bp upstream of the start codon and 833 bp downstream of the stop codon as 5' and 3' homology arms (HA), respectively (electronic supplementary material [ESM] Table 1a). A pair of gRNAs (ESM Table 1b) introducing dsDNA breaks 8 bp downstream of the start codon and 2 bp upstream of the stop codon of *CCKBR* were cloned into

the *pu6-sgRNA-CAG-Cas9-VenusbpA* expression vector that allowed FACS sorting. This vector and the targeting vector were transfected into HMGUi001 iPSCs using the standard Lipofectamine 3000 protocols. Cas9-Venus⁺ cells were FACS-sorted, and colonies were picked, expanded, screened by PCR and confirmed by Sanger sequencing (ESM Table 1c, d). Karyotyping was performed by the Institute of Human Genetics, Technical University of Munich, using 10–30% confluent hiPSCs. Cells were treated with colcemid, trypsinised, hypotonised and fixed, then placed on slides, dried overnight and stained with Giemsa. At least 20 metaphases were acquired and analysed using GenASIs software (version 8.3.1.60377; Applied Spectral Imaging, www.spectral-imaging.com). Clones were negative for mycoplasma. The obtained clones (HMGUi001-A-65; CCKBR^{KO/Δ12} and HMGUi001-A-67; CCKBR^{KO/KO}) were registered at the European Human Pluripotent Stem Cell Registry (hPSCreg). The Ethics Committee of the Technical University of Munich positively voted on non-commercial research on hiPSCs (219/20 S).

Differentiation towards SC-islets hiPSCs were differentiated into SC-islets following a six-stage protocol [7, 26]. Cells were passaged 2–3 times in 2D culture prior to aggregation, then seeded into six-well ULA plates at 5×10^6 cells per well in StemMACS iPS-Brew XF with 10 μ mol/l Y-27632, and placed on an incubator-based rotational shaker at 100 rev/min. Aggregates formed after 24 h. The next day, differentiation was started and CCKBR agonist/antagonist (ESM Table 1e) was subsequently added according to the stage throughout the differentiation protocol. The detailed differentiation protocol is provided in the ESM Methods.

Bulk RNA-seq analysis Approximately 150–200 aggregates per treatment were collected, washed with PBS and centrifuged to remove the supernatant. The pellet was snap-frozen on dry ice and stored at -80°C until further processing. RNA was isolated with the RNeasy mini kit (Qiagen). RNA integrity number was measured on the Agilent TapeStation 4200 system. Samples with an RNA integrity number >7 were selected for poly-A-selected mRNA sequencing. Libraries were prepared with the Illumina Stranded mRNA Prep, Ligation kit per the manufacturer's instructions. After final quality control, libraries were sequenced paired-end (2 $\times$ 100 bases) on the NovaseqX+ (Illumina) at ≥ 30 million paired-reads per sample. Raw FASTQ files were processed with the nf-core/rnaseq pipeline (v3.21.0; <https://nf-co.re/rnaseq/3.21.0/>) [27] to generate gene counts after alignment to GRCh38. Differential gene expression used raw counts from the Salmon tool (v1.10.1; <https://github.com/COMBINE-lab/salmon>) [28], with differentially expressed genes calculated using DESeq2 (v1.52.0; <https://bioconductor.org/packages/release/bioc/html/DESeq2.html>) [29, 30]. GO

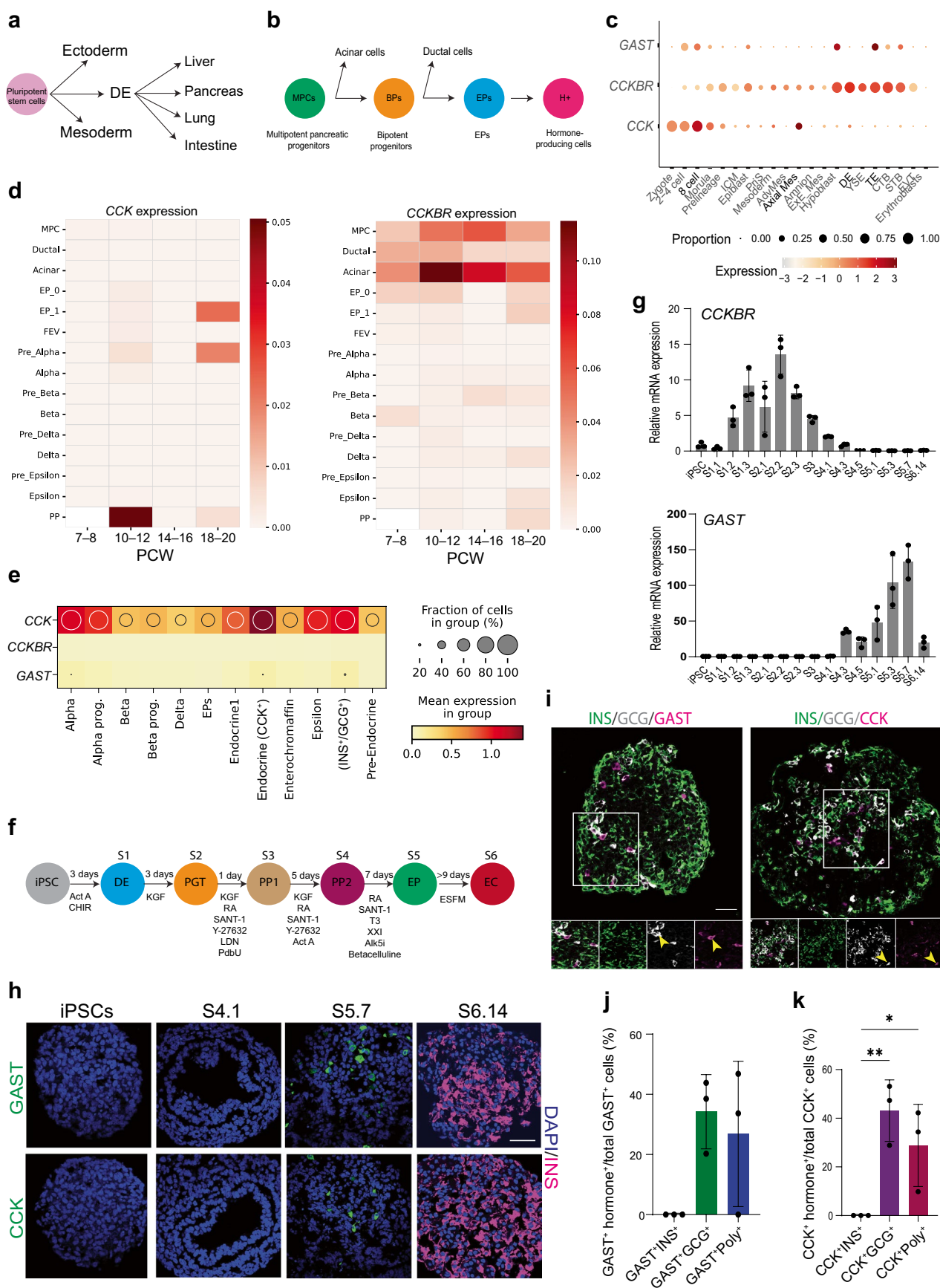


Fig. 1 Expression patterns of *GAST*, *CCK* and their receptors during early human development and pancreatic organogenesis. **(a)** Schematic overview illustrating the major cellular lineages arising from pluripotent cells during early developmental specification. **(b)** Schematic showing the stepwise differentiation of MPCs into distinct pancreatic lineages. **(c)** Dot plot showing expression levels of *GAST*, *CCKBR* and *CCK* during early human embryogenesis, derived from integrated analysis of six embryonic reference scRNA-seq datasets. **(d)** Heat map illustrating the expression of *CCK* and *CCKBR* during human pancreatic development during 7–20 PCW. **(e)** Expression patterns of *CCK*, *CCKBR* and *GAST* in EP-enriched SC-islets at stage 5 (S5). **(f)** Schematic representation of the in vitro human endocrine differentiation protocol and growth factors added at each stage. **(g)** qPCR analysis of *CCKBR* and *GAST* expression levels across sequential stages of differentiation. $n=3$. **(h)** Representative confocal micrographs showing *GAST* and *CCK* expression at distinct stages of differentiation. Scale bar, 50 μm . **(i–k)** Confocal images demonstrating *GAST* and *CCK* expression within *INS*⁺ and *GCG*⁺ cells at stage 5.7 (S5.7), along with corresponding quantification. Arrowheads indicate colocalisation of *GAST* or *CCK* with *GCG*⁺ cells. $n=3$. Scale bar, 50 μm . All statistics were done using one-way ANOVA ($*p\leq 0.05$, $**p\leq 0.01$). Data are represented as mean $\pm$ SD. Schematics **(a, b, f)** were created with BioRender.com. EC, endocrine cells

analysis was performed with the Metascape online platform (<https://metascape.org/gp/#/main/step1>) [31].

Single-cell RNA-seq data generation and analysis Around 100–150 aggregates per treatment were collected and dissociated into single cells through three cycles of 10 min Accutase incubation at 37°C. Cells were then centrifuged, washed with PBS containing 2% FCS and diluted to ~1000 cells/ μl in the same buffer. After quality control and counting, the suspension was immediately used for single-cell RNA-seq (scRNA-seq) library preparation targeting 10,000 cells. Library preparation and data analysis details are provided in the ESM Methods.

Statistical analysis Data are expressed as mean $\pm$ SD. Comparison of two groups used an unpaired two-tailed *t* test; comparison of three or more groups used ordinary one-way analysis of variance (ANOVA). A *p* value ≤ 0.05 was considered significant. All statistics were performed using GraphPad Prism software (RRID:SCR_002798), version 10.1.0 (316); GraphPad Software, Boston, MA, USA. Each experiment was performed in a minimum of three independent biological replicates (independent differentiations), as stated in the figure legends. Samples were not randomised and experimenters were not masked to group assignment. Beyond the scRNA-seq quality-control filtering described below, no data were excluded. Detailed information on hiPSC maintenance, generation of stable cell lines (HEK293T cells), flow cytometry staining, qPCR, western blotting, immunostaining, confocal microscopy and imaging, assessment of proliferation by EdU pulse and reanalysis of scRNA-seq data is provided in the ESM Methods. The list of used primary and secondary antibodies and qPCR probes are provided in ESM Table 1f–h.

Results

GAST, *CCK* and *CCKBR* exhibit distinct dynamic expression patterns during human endoderm and pancreatic development in vitro

To characterise the expression patterns of *GAST* and *CCK* and their receptors during early human embryogenesis, we leveraged a reference platform built from six published human embryonic datasets covering the zygote stage, embryonic days E1–E10, E12 and E14, and Carnegie stage 7 (<https://petropoulos-lanner-labs.clintec.ki.se/shinys/app/ShinyEmbryoRef>). *CCKBR* mRNA expression peaked during DE and yolk sac endoderm (YSE) formation, consistent with prior reports in mouse in vivo [32]. *GAST* transcripts were largely confined to the hypoblast and trophoctoderm (TE) lineages, while *CCK* was predominantly expressed around the eight-cell pre-implantation stage and, notably, in axial mesoderm (Fig. 1c), which organises germ-layer formation and specification [33, 34]. *CCKAR* transcripts were not detected at any of these stages. We next examined the expression of *GAST*, *CCK* and their receptors during human pancreatic development across post-conception weeks (PCW) 7–20 (ESM Fig. 1a) [35]. While *GAST* was not detected in this dataset, *CCK* expression remained low overall, with transcripts largely restricted to PP cells at 10–12 PCW and to EPs and pre-alpha populations at 18–20 PCW (Fig. 1d). Both receptors were mainly expressed in MPC, ductal and acinar compartments, with only weak, sporadic signal across endocrine and pre-endocrine populations. *CCKBR* was broadly expressed across all three compartments, peaking in MPCs and acinar cells at 10–12 PCW before gradually declining (Fig. 1d). *CCKAR* showed a more exocrine-restricted pattern, with strong, sustained acinar expression and moderate MPC expression, but became largely restricted away from ductal and endocrine lineages at later stages (ESM Fig. 1b). Analysis of an EP-enriched SC-islet differentiation at stage S5 [36] revealed only minimal expression of *CCKBR*, and no detectable *CCKAR*. *GAST* and *CCK*, by contrast, were expressed in hormone-positive cells, with higher expression in alpha, epsilon and polyhormonal cells than in delta and beta lineages, and higher *CCK* than *GAST* levels overall (Fig. 1e). Together, these analyses demonstrate distinct, stage- and lineage-specific expression patterns of *GAST*, *CCK* and their receptors during early human development and pancreatic organogenesis.

We next experimentally validated these findings using an established 3D iPSC differentiation system (Fig. 1f) [7]. *CCKBR* mRNA was highly expressed at the DE stage (S1) and posterior foregut stage (posterior gut tube [PGT]; S2) but declined towards the PP stage (PP1 and PP2) (Fig. 1g). *CCKAR* expression, in contrast, became detectable at S4 (PP2) and gradually decreased thereafter, with a small secondary peak at the SC-islet stage (S6) (ESM Fig. 1c).

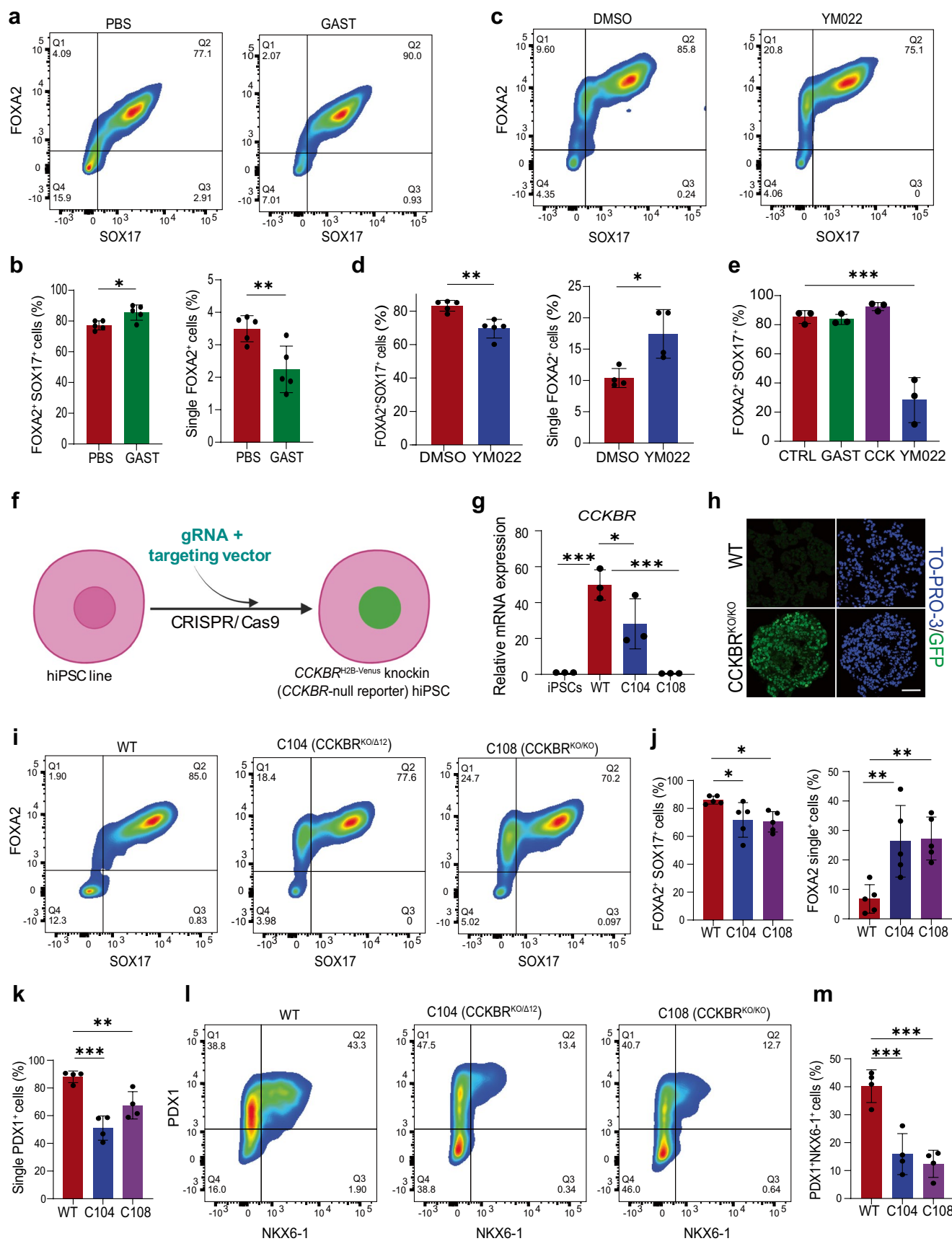


Fig. 2 CCKBR plays an essential role in DE specification. (a, b) Representative FACS plots of FOXA2⁺/SOX17⁺ cell populations at S1.3 (DE), with corresponding quantification (b), following treatment with PBS (control) or GAST. *n*=5. (c, d) Representative FACS plots of FOXA2⁺/SOX17⁺ cells at S1.3 (DE), with quantification (d), following treatment with DMSO (control) or CCKBR antagonist (YM022). *n*=4–5. (e) Quantification of FOXA2⁺/SOX17⁺ cells at S2 upon treatment with CCKBR agonists and antagonist. *n*=3. (f) Schematic illustrating CRISPR/Cas9-mediated knockin of an H2B-Venus reporter into the *CCKBR* locus, replacing the coding region to generate a *CCKBR*^{H2B-Venus} knockin (*CCKBR*-null reporter) allele. Homozygous clone C108 (*CCKBR*^{KO/KO}) and compound-heterozygous clone C104 (*CCKBR*^{KO/Δ12}) were derived. Created with BioRender.com. (g) qPCR analysis of *CCKBR* mRNA expression in iPSC, WT, C104 and C108 cells at S1.3 (DE). *n*=3. (h) Representative confocal image showing H2B-Venus expression in *CCKBR*^{KO/KO} cells at S1.3 (DE). Scale bar, 50 μm. (i, j) Representative FACS plots of FOXA2⁺/SOX17⁺ cell populations in *CCKBR*-targeted cells at S1.3 (DE), with corresponding quantification. *n*=5. (k) FACS analysis of PDX1⁺ cells at S3 (PP1) in *CCKBR*-targeted sample clones C104 and C108. *n*=4. (l, m) Representative FACS plots of PDX1⁺/NKX6-1⁺ cells in *CCKBR*-targeted clones at S4.5 (PP2) and quantification. *n*=4. All statistics were done using two-sided *t* test (b, d) and one-way ANOVA (e, g, j, k, m) (**p*≤0.05, ***p*≤0.01, ****p*≤0.001). Data are represented as mean ± SD

Consistent with prior mouse studies [18, 19], *GAST* and *CCK* mRNAs appeared at the PP2 (S4) and EP stages (S5) but declined at the hormone-producing cell stage (S6) (Fig. 1g, ESM Fig. 1c). Immunostaining confirmed *GAST* and *CCK* at the protein level. Neither peptide was detected in iPSCs, but *GAST*- or *CCK*-expressing cells emerged at S5 stage and declined at S6, confirming their transient appearance at the endocrine stage (Fig. 1h). We further examined the identity of these cells using co-immunostaining for *GAST* and *CCK* with insulin, glucagon, somatostatin and ghrelin in SC-islets at stage S5.7. Both peptides were highly co-expressed with glucagon-producing alpha and polyhormonal cells, with only rare overlap with insulin-, somatostatin- or ghrelin-expressing cells (Fig. 1i–k, ESM Fig. 1d). Collectively, these analyses indicate distinct expression patterns of *GAST*, *CCK* and *CCKBR* in neighbouring tissues and cell types, with an overlap between the peptides and *CCKBR* during pancreatic lineage formation.

CCKBR loss of function compromises human DE differentiation Due to the high expression levels of *CCKBR* during the DE stage (S1), we next asked whether altering its activity affects DE formation. We thus treated iPSCs from the onset of differentiation with *GAST* as *CCKBR* agonist and assessed DE differentiation at S1.3. The stimulatory activity of *GAST* was first validated in the HEK293T cell line over-expressing the human *CCKBR* by measuring downstream ERK1/2 signalling (ESM Fig. 2a–c). Flow cytometry using the DE markers FOXA2 and SOX17 revealed that *CCKBR* stimulation slightly but significantly increased the proportion of FOXA2/SOX17 double-positive cells while reducing

the frequency of FOXA2 single-positive cells (Fig. 2a,b). Conversely, the selective *CCKBR* antagonist YM022 increased FOXA2 single-positive cells at the expense of FOXA2/SOX17 double-positive cells (Fig. 2c,d). Notably, *CCKBR* activation no longer had a prominent effect at the foregut stage (S2), whereas the impact of receptor inhibition was further increased at this stage (Fig. 2e).

To corroborate these pharmacological results genetically, we generated *CCKBR*-knockout (KO) reporter iPSC lines. Using CRISPR/Cas9-mediated homologous recombination, we targeted the *CCKBR* locus in the HMGU001-A iPSC line [37] to replace the coding region with a nuclear-localised *H2B-Venus-3×HA* cassette (Fig. 2f, ESM Fig. 3a). Genomic PCR identified two independent *CCKBR*-targeted clones carrying the knockin sequence (ESM Fig. 3b). Sanger sequencing showed clone C108 harboured homozygous loss of *CCKBR* (*CCKBR*^{KO/KO}), consistent with biallelic targeting. In clone C104, one allele was replaced by the *H2B-Venus* cassette, while the second carried a 12-bp deletion after the starting ATG (*CCKBR*^{KO/Δ12}), causing loss of four amino acids and probably producing a hypomorphic or non-functional *CCKBR* protein (ESM Fig. 3c,d). As expected, qPCR showed complete loss of *CCKBR* transcripts in C108 and ~50% reduction in C104 (Fig. 2g). Both clones retained a normal 46, XX karyotype (ESM Fig. 3e).

We then differentiated *CCKBR*-targeted clones into DE. Both clones successfully expressed H2B-Venus protein, confirming correct targeting and reflecting high activity of the *CCKBR* promoter at this stage (Fig. 2h). Notably, *CCKBR*-targeted clones showed a reduced proportion of FOXA2/SOX17 double-positive DE cells and increased FOXA2 single-positive mesendoderm progenitors (Fig. 2i,j). This early DE induction defect propagated forward, causing a marked reduction in PP1 (PDX1⁺) formation (Fig. 2k, ESM Fig. 3f) and a >60% decrease in PP2 cell (PDX1⁺/NKX6-1⁺) differentiation (Fig. 2l,m). Further differentiation towards the endocrine lineage was thus strongly impaired.

CCKBR deletion promotes non-DE differentiation programs To further dissect the impact of *CCKBR* loss of function on DE formation, we performed bulk RNA-seq during DE induction at S1.3. Principal component analysis (PCA) revealed a clear separation between WT and *CCKBR*-targeted samples (Fig. 3a). Differential gene expression analysis identified 672 downregulated and 246 upregulated genes in C104 (*CCKBR*^{KO/Δ12}) vs WT (ESM Table 2a–c), and 577 downregulated and 238 upregulated genes in C108 (*CCKBR*^{KO/KO}) vs WT (Fig. 3b, ESM Table 2d–f). Intersecting both comparisons revealed 419 commonly downregulated and 154 commonly upregulated genes across the two clones relative to WT (Fig. 3c, ESM Table 2g, h). *CCKBR* transcript levels matched the qPCR data, being almost absent in C108 and higher in C104.

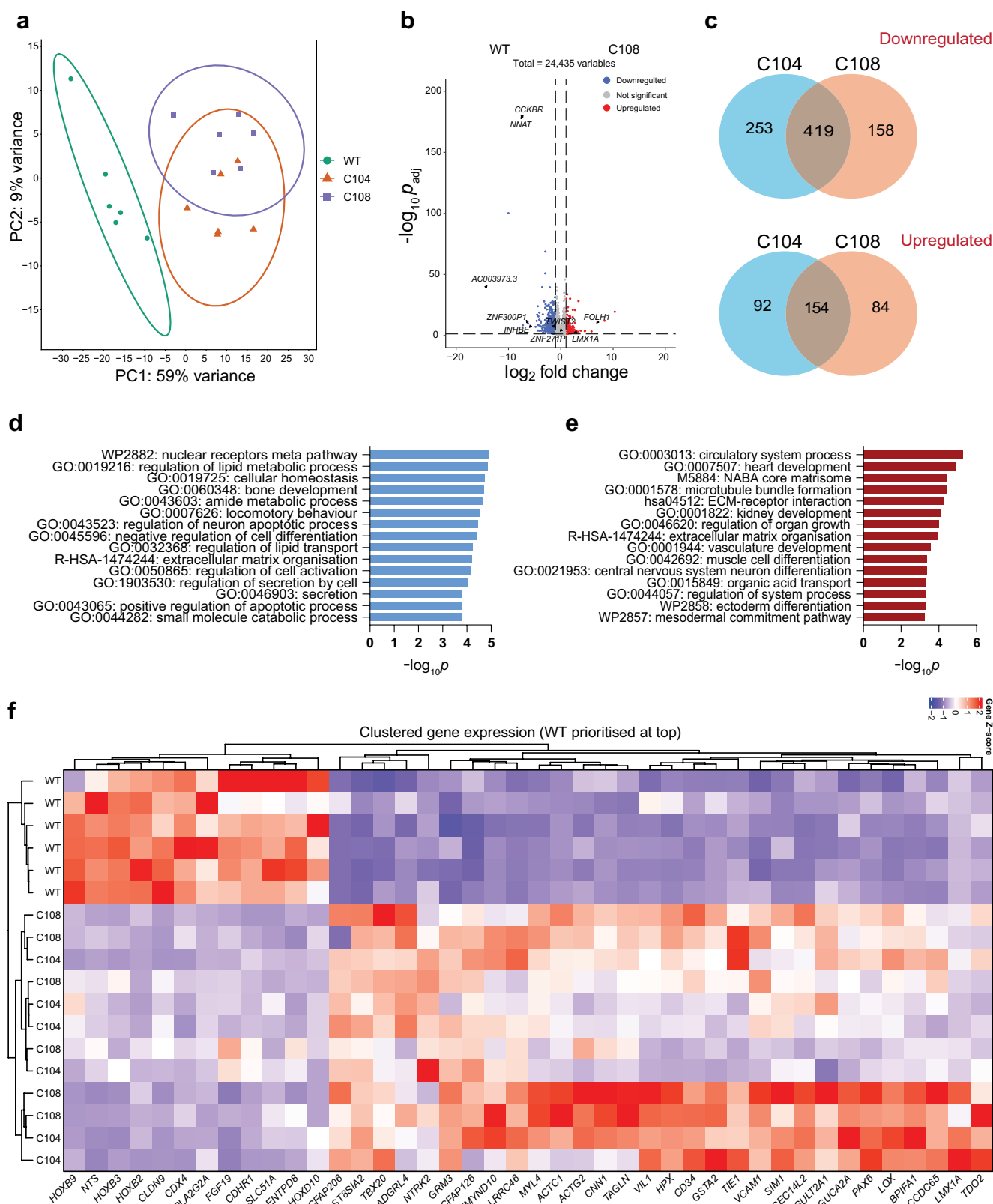


Fig. 3 CCKBR deletion disrupts endodermal differentiation and patterning programs. **(a)** PCA demonstrating clear segregation between WT and CCKBR-targeted samples, including clones C104 and C108. **(b)** Volcano plot illustrating differentially expressed genes in clone C108 relative to WT controls. Significantly upregulated genes are highlighted in red, whereas significantly downregulated genes are shown in blue. **(c)** Venn diagrams representing the overlap of downregulated and upregulated genes shared between CCKBR-targeted samples C108 and C104 compared with WT samples. **(d, e)** Pathway enrichment analyses of significantly downregulated **(d)** and upregulated **(e)** genes in targeted clones relative to WT controls, identifying affected biological processes and signalling pathways. Only 15 selected terms are presented. The list of all identified terms is provided in ESM Table 2. **(f)** Heatmap displaying the expression patterns of selected differentially expressed genes across WT ($n=6$) and C108 ($n=6$) samples

Pathway enrichment analysis of differentially expressed genes showed that downregulated genes were enriched for cell differentiation, ECM/cell–cell adhesion and secretory/hormone-response programs, suggesting a shift in differentiation state and/or lineage identity (Fig. 3d, ESM Table 2i). Upregulated genes, in contrast, were enriched for mesoderm- and ectoderm-derived tissue formation, including mesodermal commitment, muscle/vasculature/heart development and ectoderm/neurodevelopmental differentiation, highlighting a shift away from DE towards alternative mesoderm and ectoderm lineage identities (Fig. 3e, ESM Table 2j).

Among the downregulated genes were *CDX4* and several *HOX* genes (*HOXB2*, *HOXB3*, *HOXB9*, *HOXD10*), suggesting disrupted anterior–posterior endoderm patterning in CCKBR^{KO} clones. Lower *FGF19*, *NTS*, *SLC51A*, *ENTPD8* and *PLA2G2A*, along with epithelial markers *CLDN9* and *CDH1*, indicated a reduced anterior/foregut-like epithelial signature. The upregulated gene set, by contrast, contained a substantial non-DE component spanning both mesodermal and ectodermal lineages. This mesodermal signature included vascular/endothelial genes (*CD34*, *TIE1*, *VCAM1*, *ADGRL4*), as well as contractile cardiac (*ACTC1*, *MYL4*, *TBX20*) and smooth-muscle genes (*ACTG2*, *CNN1*, *TAGLN*, *LOX*). Ectoderm-associated genes encompassed neuroectoderm/CNS-like identities, including *PAX6*, *SIM1*, *NTRK2*, *LMX1A*, *GRM3* and *ST8SIA2*. The upregulated endoderm-related genes did not reflect a pancreatic program but instead matched other endoderm-derived epithelia. *VILI1* and *GUCA2A* supported an intestinal-like signature, while *BPIFA1* and a strong motile-cilia program (*ZMYND10*, *CCDC65*, *CFAP126/CFAP206*, *LRR46*, *TEKTIP1*) suggested ciliated airway-like lineages. Finally, a hepatic-like metabolic/detox signature (*HPX*, *GSTA2*, *SULT2A1*, *TDO2*, *SEC14L2*) further indicated another non-pancreatic endodermal identity (Fig. 3f).

Sustained CCKBR activation impairs endocrine lineage differentiation To test how prolonged CCKBR stimulation affects pancreatic differentiation, we treated cells with GAST and CCK from the onset of differentiation (S1) and performed FACS analysis at S4.5 to quantify cells co-expressing PDX1 and NKX6-1 (PDX1⁺/NKX6-1⁺) (Fig. 4a). Sustained stimulation of CCKBR with GAST or non-sulphated CCK significantly reduced PDX1⁺/NKX6-1⁺ cells, indicating impaired PP formation (Fig. 4b,c). FACS further revealed a significant reduction in EPs at S5.4 assessed by NGN3 expression (Fig. 4d,e), and immunostaining confirmed fewer NGN3⁺ and NKX2-2⁺ cells upon sustained stimulation (Fig. 4f). In stimulated cells the fraction of hormone-positive endocrine cells at S5.7 was also reduced, largely due to fewer SC-beta cells (Fig. 4g–i), indicating reduced endocrine differentiation. To assess whether this impairment reflects reduced PP specification, we treated cultures with similar PP2 numbers starting at S4.1 (Fig. 4j,k). This later treatment still reduced NGN3⁺ cells (Fig. 4l,m) and hormone-expressing cells (ESM Fig. 4a), although the effect was smaller than when stimulation began at the start of differentiation. Together, these data demonstrate that sustained CCKBR stimulation reduces the rate of endocrine lineage formation.

Prolonged CCKBR signalling drives a non-endocrine cell fate program Reduced endocrine lineage formation upon sustained CCKBR stimulation coincided with an increased fraction of cells lacking endocrine (NGN3, NKX2-2) marker expression (Fig. 4). This non-endocrine fraction expanded more when stimulation was applied from the onset of differentiation than when treatment began at S4.1 (Fig. 5a). Consistent with prior reports of mitogenic effects of GAST and CCK in other tissues (e.g. gastric mucosa and neuronal cells) [38, 39], the non-endocrine population showed a high rate of cell division (ESM Fig. 4b).

To identify this population, we treated cells from the start of differentiation with GAST or non-sulphated CCK and performed scRNA-seq analysis at S5.4. After quality control, we profiled 25,651 single cells via droplet-based scRNA-seq. Unsupervised clustering identified three major cell clusters including an endocrine pancreatic, a fibroblast-like and a non-endocrine pancreatic epithelial cluster (Fig. 5b, ESM Fig. 5a,b). Sub-clustering of the non-endocrine pancreatic epithelial cells identified four clusters with distinct marker genes (Fig. 5c, d, ESM Table 3a). Pathway enrichment analysis of genes highly expressed (log fold ≥ 2) in the endocrine cluster identified terms associated with endocrine differentiation and morphogenesis, regulated hormone secretion and synaptic/cellular signalling (Fig. 5e, ESM Table 3b, c). Among the epithelial cells, sub-cluster 3 exhibited an exocrine/ductal-like identity, with genes enriched for ductal/

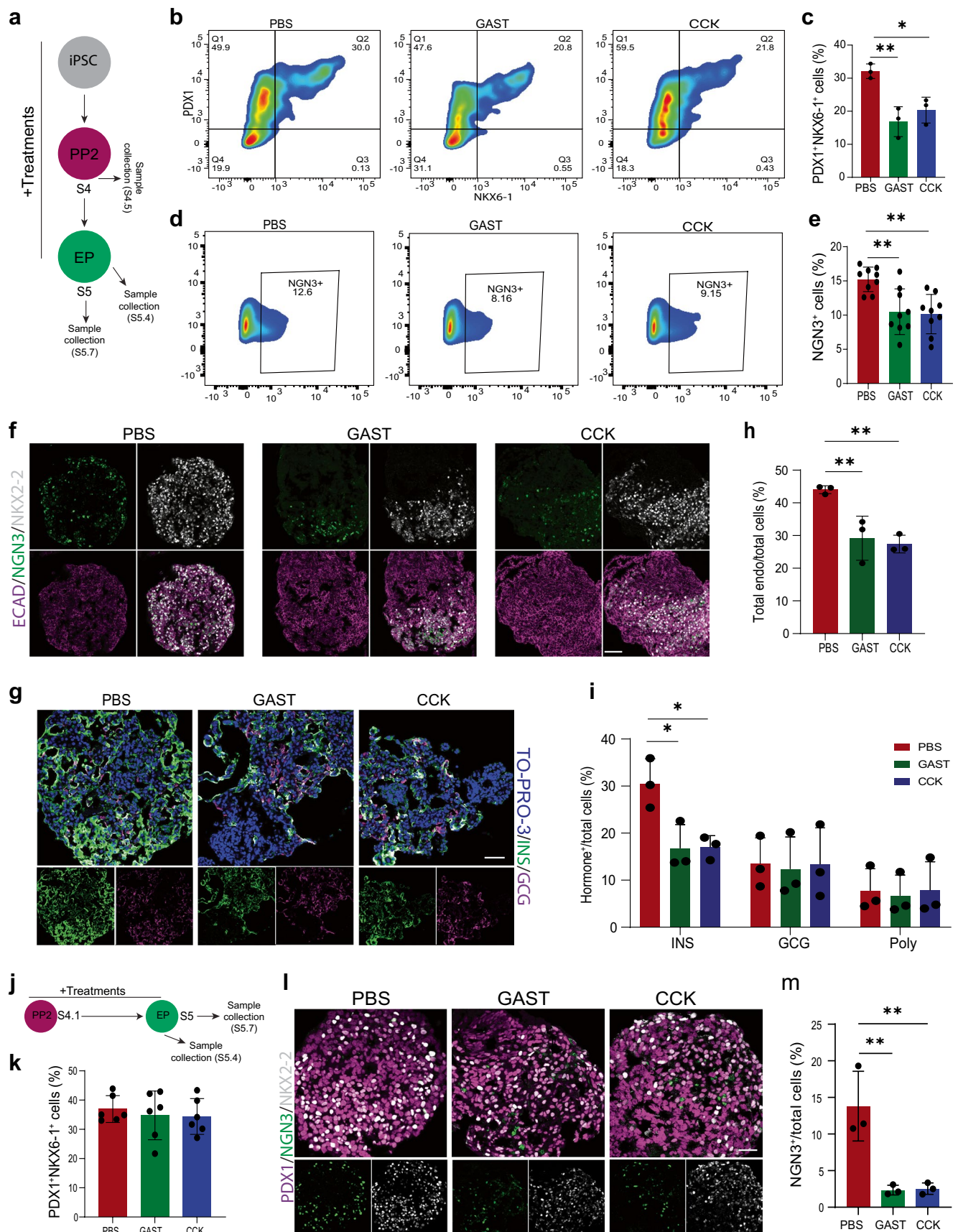


Fig. 4 Prolonged CCKBR signalling hampers endocrine cell formation. **(a)** Schematic diagram illustrating the initiation of treatments at S1.1 in differentiated cells. **(b, c)** Representative FACS plots and corresponding quantification of PDX1⁺/NKX6-1⁺ cells at S4.5 (PP2) following GAST and CCK treatment. $n=3$. **(d, e)** FACS analysis and quantification of NGN3 expression at S5.4 (EP) after stimulation with the indicated treatments. $n=9$. **(f)** Representative confocal micrographs showing NGN3⁺/NKX2-2⁺ cells at S5.4 (EP) upon sustained activation of the CCKBR. Scale bar, 50 μ m. **(g–i)** Representative confocal images of hormone-expressing cells, including INS and GCG, following treatment at S5.7. Scale bar, 50 μ m. Quantifications of the percentage of total endocrine cells (endo) relative to total cells **(h)** and the percentage of hormone-positive cells relative to total cells **(i)** are shown. $n=3$. **(j)** Schematic diagram illustrating the initiation of treatments at S4.1 (PP2) in differentiated cells. **(k)** FACS-based quantification of PDX1⁺/NKX6-1⁺ cells at S4.5 (PP2) following treatment. $n=6$. **(l, m)** Representative confocal images and the respective quantification of NGN3⁺ cells at S5.4 (EP) upon treatment from beginning of PP2 stage. $n=3$. Scale bar, 50 μ m. All statistics were done using one-way ANOVA (* $p\leq 0.05$, ** $p\leq 0.01$). Data are represented as mean $\pm$ SD. Schematics **(a, j)** were created with BioRender.com

exocrine lineage specification, epithelial morphogenesis and adhesion, and exocrine secretory function (Fig. 5f, ESM Table 3d, e). More specifically, this cluster showed prominent expression of *SLC4A4*, *SOX9*, *CA2*, *CA12*, *ONECUT1*, *ONECUT2*, *AGR2*, *MUC13*, *TFF1*, *TFF2*, *SULT1C2* and *MBOAT2* (Fig. 5g). Additional expression of *CTSE*, *TCN1*, *CBLIF*, *AOX1*, *HABP2*, *ASAH2*, *SLC47A1*, *UGT2B7*, *UGT2B10* and *UGT2B11* further supported this epithelial exocrine/ductal-like profile. These genes were largely absent or lower in the endocrine pancreatic and fibroblast-like clusters, and more limited across the other non-endocrine epithelial populations (Fig. 5g), with higher expression in GAST-treated and, to a lesser extent, CCK-treated samples compared with controls (ESM Fig. 5c). However, other canonical ductal markers (*CFTR*, *KRT19*, *SPPI1*, *MUC1*, *GP2*) and acinar markers (*CPA1*, *AMY2A*) were absent or low in this cluster (Fig. 5g), indicating only a partial ductal program rather than a fully mature identity. Together, these data support that sustained CCKBR activation biases cell fate towards an exocrine/ductal-like trajectory rather than endocrine commitment.

We then validated the ductal-like identity of the non-endocrine fraction upon GAST and non-sulphated CCK treatment from the S1 stage by immunostaining. Epithelial identity was supported by polarised staining of the apical marker Ezrin, consistent with apico–basal organisation (ESM Fig. 6a). Treated samples also showed strong, specific CK7 (a pancreatic ductal keratin) expression, further supporting a ductal-like identity (Fig. 5h). When treatment began at S4, we still observed Ezrin and CK7 expression upon CCKBR stimulation (Fig. 5i, ESM Fig. 6b), indicating a fate shift of PPs towards ductal-like identity.

Discussion

Here, we identify a previously unrecognised pathway in which CCKBR first promotes DE formation and anterior–posterior patterning, and later, in pancreatic differentiation, restrains the endocrine program and favours an exocrine/ductal-like fate. CCKBR expression peaks during human DE specification in vitro and then progressively declines, becoming nearly absent at the hormone-producing stage. Pharmacological activation of CCKBR promotes DE induction, but when sustained, reduces PP2 cell formation and endocrine lineage specification. These two later effects are mechanistically separable, since stimulating CCKBR in cultures with comparable PP2 cell numbers still reduced NGN3⁺ cell formation, indicating that sustained CCKBR signalling acts directly on endocrine induction independent of its earlier effect on progenitor numbers. In contrast, genetic deletion of CCKBR impairs DE formation, with this early defect propagating to later stages as reduced PP differentiation, consistent with reports that DE patterning perturbations, such as altered WNT signalling, have lasting downstream effects on pancreatic differentiation efficiency [40]. Thus, although pharmacological and genetic perturbations of CCKBR converge on a similar net reduction in endocrine cell formation, they do so through distinct mechanisms. Sustained pharmacological activation directly suppresses the endocrine differentiation program, whereas genetic loss of CCKBR reduces endocrine output indirectly, via impaired earlier DE specification and diminished progenitor supply. Together, our data support a biphasic model of CCKBR action, whereby this receptor facilitates DE formation during germ-layer specification and, at later stages, redirects PPs towards an exocrine/ductal-like rather than endocrine identity, potentially balancing the proportion of these compartments.

GAST and non-sulphated CCK are the best-established ligands for CCKBR [23, 41]. scRNA-seq of early human embryos revealed expression of these ligands in mesodermal tissues, including the primitive streak and axial mesendoderm, raising the possibility that ligand-dependent CCKBR signalling specifies anterior endodermal fates. However, in our in vitro system, neither GAST nor CCK transcripts are detected at the DE stage, nor are these ligands supplied in the culture medium, yet CCKBR deletion still impairs DE specification. This indicates that CCKBR signalling is already active during DE induction, possibly engaged by an unrecognised medium-derived or endogenous ligand, or through ligand-independent constitutive activity, a property common among GPCRs [42]. Supporting this, a constitutively active CCKBR splice variant (CCK-BRi4sv/CCK2i4sv) described in colorectal cancer cells shows spontaneous signalling and increased growth without ligand [43].

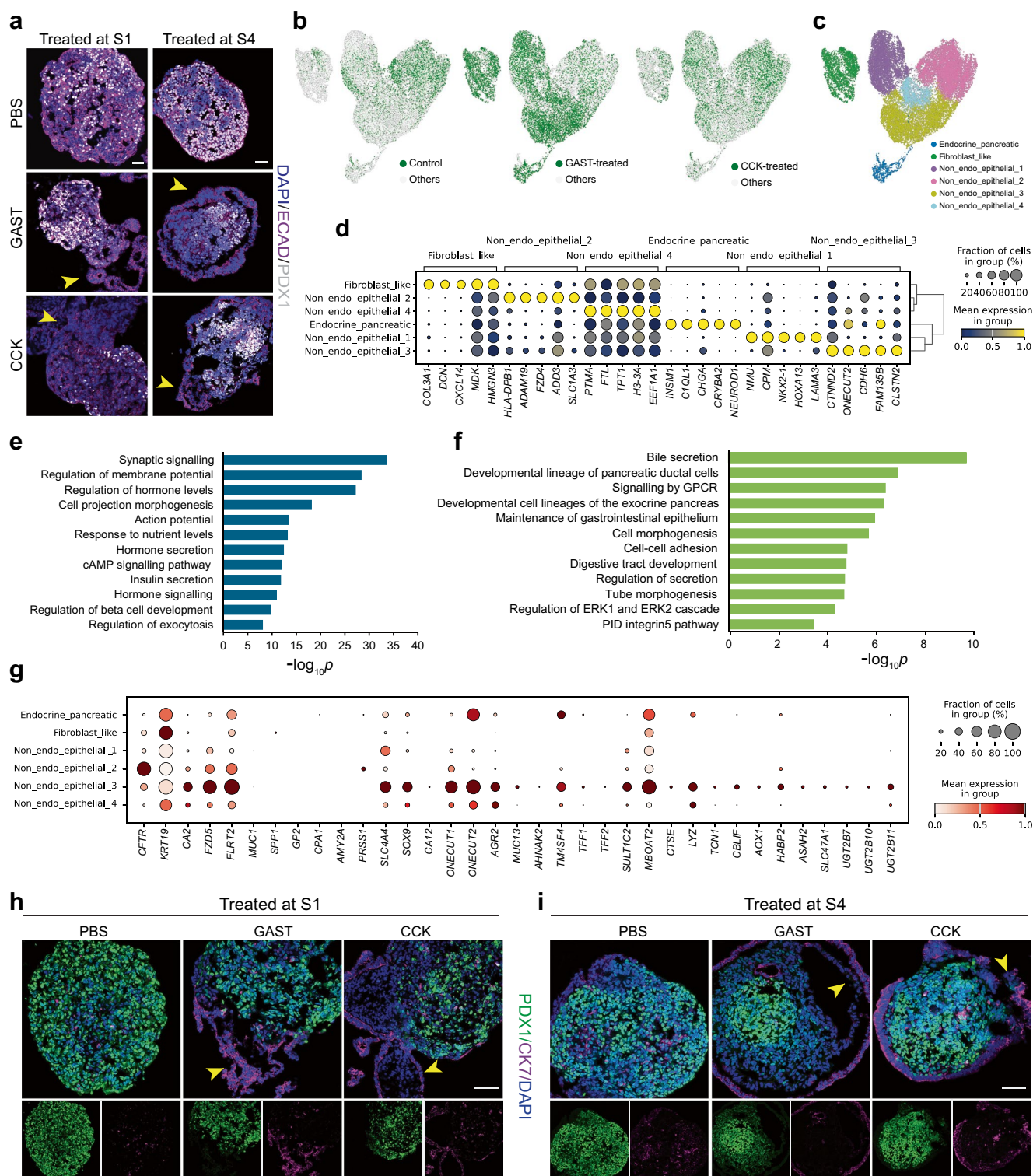


Fig. 5 Prolonged CCKBR activation shifts cells towards secretory ductal-like cell fate. **(a)** Representative confocal images illustrating the effects of GAST and non-sulphated CCK on PDX1⁺ cell populations and E-cadherin expression, initiated at S1 and S4, respectively. Arrow heads denote expansion of the non-endocrine epithelial fraction. Scale bar, 50 μ m. **(b)** UMAP visualisation of scRNA-seq data showing clustering by sample origin. **(c)** UMAP visualisation of scRNA-seq data showing sub-clustering of non-endocrine pancreatic epithelial (non_endo_epithelial) clusters. **(d)** Dot plot displaying the expression of top genes across six distinct cellular clusters identified by unsupervised analysis. **(e, f)** Pathway enrichment analysis of genes significantly upregulated in endocrine **(e)** and non_endo_epithelial_3 **(f)** lineages. Only 12 selected terms are presented. The list of all identified terms is provided in ESM Table 3. **(g)** Dot plot illustrating the expression levels of ductal/exocrine-associated genes across identified lineages. **(h, i)** Representative confocal images showing expression of CK7 in cells treated from S1 **(h)** and S4 **(i)**. Arrowheads indicate CK7 localisation within the expanded non-endocrine cell population. Scale bar, 50 μ m

Because endocrinogenesis is not synchronous, early differentiated endocrine cells may remain in close contact with bipotent progenitors that retain ductal or endocrine potential. GAST and CCK expression, predominantly in (pre-)alpha cells, is therefore consistent with a model in which endocrine cells provide paracrine feedback influencing the endocrine-vs-ductal fate decision in neighbouring progenitors. This is conceptually similar to Notch-mediated lateral inhibition, where emerging EPs restrain further endocrine induction in adjacent bipotent progenitors [44, 45]. This suggests layered feedback mechanisms from EPs or hormone-producing cells may shape pancreatic organogenesis, influencing not only endocrine subtype composition, as reported for insulin receptor signalling [16], but also the endocrine and exocrine balance. Consistent with this, GAST and CCK are transiently expressed during this window and then disappear [18, 19]. Notably, in vivo analysis across fetal pancreas stages showed only very low CCK expression restricted to pre-alpha cells and no detectable GAST, contrasting with the broader and higher expression of both peptides in the stem cell-derived system. This difference may partly reflect off-target generation of intestinal hormone-expressing cells during stem cell differentiation, such as enterochromaffin-like cells [9], for which there is limited evidence in the developing human pancreas in vivo [46].

Sustained CCKBR activation reduced endocrine lineage formation and shifted cells towards a ductal-like fate. However, this population expressed only a subset of ductal/epithelial markers, while several canonical ductal markers were not detected, indicating that these cells only partially resemble ductal cells and lack a fully established mature ductal program. The differentiation protocol used is optimised for islet cell generation, particularly beta cells. Therefore, the emergence of ductal-like cells under these pro-endocrine conditions suggests that effect on ductal specification could be stronger in a protocol tailored for ductal differentiation. Indeed, GAST has been used in human pancreatic ductal

organoid culture media, perhaps to reinforce ductal identity, but CCKBR expression has not been shown in this model system [47]. It thus remains unclear whether GAST acts via CCKBR here or via other receptors. Future work should assess whether CCKBR activation enhances ductal formation in ductal-directed differentiation protocols, supporting studies of ductal plasticity and disease models such as pancreatitis and pancreatic cancer [48–50].

In summary, our findings demonstrate that CCKBR signalling is functionally required at two distinct developmental windows, first for DE formation and later for pancreatic lineage fate decisions, providing the first evidence for a functional GAST/CCK–CCKBR axis during human endoderm and pancreatic development. However, the downstream signalling pathways through which CCKBR shapes these processes warrant further investigation. Nonetheless, these results may already help refine differentiation protocols to better direct specific pancreatic lineages for distinct applications. Beyond stem cell differentiation, given reports of GAST and CCK re-expression in the adult pancreas in diabetes [20, 21], our work may also inform the role of these hormones in islet regeneration. Finally, our findings may extend to pancreatic ductal adenocarcinoma, where CCK has been implicated in endocrine–exocrine crosstalk and exocrine tumour progression [51].

Supplementary Information The online version contains peer-reviewed but unedited supplementary material available at <https://doi.org/10.1007/s00125-026-06905-0>.

Acknowledgements We thank M. Catani and E. Schlüssel (Helmholtz Munich) for the helpful comments and discussion; A. Bastidas-Ponce, A. Shahryari, P. Cota, K. Diemer, I. Kunze, E. V. Baumgart (all Helmholtz Munich) and K. Seeberger (University of Alberta, Edmonton, AB, Canada) for technical support; and D. M. Thomson (Helmholtz Munich) for administrative support. We acknowledge the technical support of Core Facility Genomics at Helmholtz Munich. The graphical abstract and other schematic figures were generated with BioRender.com. We disclose that ChatGPT and Claude were used only for language editing of the manuscript; all content was reviewed and approved by the authors.

Data availability Raw bulk RNA-seq reads were uploaded to SRA with accession number PRJNA1440183. The raw and processed scRNA-seq data generated in this study have been deposited in the GEO database and are available under accession number GSE327505.

Funding Open Access funding enabled and organized by Projekt DEAL. The work was supported by the Helmholtz Society, Helmholtz Portfolio Theme 'Metabolic Dysfunction and Common Disease'. The work was also funded by research grants from the German Center for Diabetes Research (DZD e.V.) (MB), EFSD and Lilly European Diabetes Research Programme (MB), and Breakthrough T1D (3-SRA-2023-1420-S-B). LS was supported by the Helmholtz Research School for Diabetes (HRD), which is funded by the Helmholtz Association – Initiative and Networking Fund (INF). CK has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement 101108496. CJ was supported by a scholarship under the State Scholarship Fund by the China Scholarship Council (File No. 202208080154).

Authors' relationships and activities NM is a co-founder and Chief Scientific Officer of Delphi Stem, Inc., a company developing stem cell-based therapies for diabetes.

Contribution statement LS conceived the study, performed the experiments, analysed the data and drafted the manuscript. KK contributed to methodology and generation of CCKBR-targeted samples and their characterisation. CK performed bulk and scRNA-seq data analysis and contributed to manuscript preparation. CJ and MS generated scRNA-seq libraries and performed data analysis. JJ, MAC and ORP contributed to methodology and acquisition and analysis of data. OC performed image acquisition and analysis. GSK and ARP contributed to the conception of the study and the interpretation of the data. NM contributed to the conception of the study and generated preliminary data. IB contributed to and supervised the generation of CCKBR-targeted samples. HL contributed to the conception and design of the study, supervised the study, provided resources, acquired funding and critically revised the manuscript for important intellectual content. MB conceived and supervised the study, acquired funding and wrote the manuscript. All authors revised the manuscript critically for important intellectual content and approved the final version to be published. LS, HL and MB are responsible for the integrity of the work as a whole.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Wang X, Zeng Z, Li D et al (2025) Advancements and challenges in immune protection strategies for islet transplantation. *J Diabetes* 17(1):e70048. <https://doi.org/10.1111/1753-0407.70048>
- Chen JT, Dadheech N, Tan EHP et al (2025) Stem cell therapies for diabetes. *Nat Med* 31(7):2147–2160. <https://doi.org/10.1038/s41591-025-03767-8>
- Reichman TW, Markmann JF, Odorico J et al (2025) Stem cell-derived, fully differentiated islets for type 1 diabetes. *N Engl J Med* 393(9):858–868. <https://doi.org/10.1056/nejmoa2506549>
- Pagliuca FW, Millman JR, Gürtler M et al (2014) Generation of functional human pancreatic β cells in vitro. *Cell* 159(2):428–439. <https://doi.org/10.1016/j.cell.2014.09.040>
- Russ HA, Parent AV, Ringler JJ et al (2015) Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *EMBO J* 34(13):1759–1772
- Rezania A, Bruin JE, Arora P et al (2014) Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol* 32(11):1121–1133. <https://doi.org/10.1038/nbt.3033>
- Velazco-Cruz L, Song J, Maxwell KG et al (2019) Acquisition of dynamic function in human stem cell-derived β cells. *Stem Cell Reports* 12:351–365. <https://doi.org/10.1016/j.stemcr.2018.12.012>
- Nair GG, Liu JS, Russ HA et al (2019) Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nat Cell Biol* 21:263–274. <https://doi.org/10.1038/s41556-018-0271-4>
- Balboa D, Barsby T, Lithovius V et al (2022) Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. *Nat Biotechnol* 40(7):1042–1055. <https://doi.org/10.1038/s41587-022-01219-z>
- Meng G, Gu J, Liew SY et al (2025) Reconstruction of endocrine subtype-complete human pluripotent stem cell-derived islets with capacity for hypoglycemia protection in vivo. *Cell Stem Cell* 32(9):1438–1456.e7. <https://doi.org/10.1016/j.stem.2025.07.006>
- Jennings RE, Berry AA, Gerrard DT et al (2017) Laser capture and deep sequencing reveals the transcriptomic programmes regulating the onset of pancreas and liver differentiation in human embryos. *Stem Cell Reports* 9(5):1387–1394. <https://doi.org/10.1016/j.stemcr.2017.09.018>
- Jennings RE, Berry AA, Strutt JP, Gerrard DT, Hanley NA (2015) Human pancreas development. *Development* 142(18):3126–3137. <https://doi.org/10.1242/dev.120063>
- Siehler J, Blöchliger AK, Meier M, Lickert H (2021) Engineering islets from stem cells for advanced therapies of diabetes. *Nat Rev Drug Discov* 20(12):920–940. <https://doi.org/10.1038/s41573-021-00262-w>
- Bastidas-Ponce A, Scheibner K, Lickert H, Bakhti M (2017) Cellular and molecular mechanisms coordinating pancreas development. *Development* 144(16):2873–2888. <https://doi.org/10.1242/dev.140756>
- Larsen HL, Grapin-Botton A (2017) The molecular and morphogenetic basis of pancreas organogenesis. *Semin Cell Dev Biol* 66:51–68. <https://doi.org/10.1016/j.semcdb.2017.01.005>
- Cota P, Caliskan ÖS, Bastidas-Ponce A et al (2024) Insulin regulates human pancreatic endocrine cell differentiation in vitro. *Mol Metab* 79:101853. <https://doi.org/10.1016/j.molmet.2023.101853>
- Duvillier B, Currie C, Chrones T et al (2002) Increased islet cell proliferation, decreased apoptosis, and greater vascularization leading to β -cell hyperplasia in mutant mice lacking insulin. *Endocrinology* 143(4):1530–1537. <https://doi.org/10.1210/endo.143.4.8753>
- Suissa Y, Magenheimer J, Stolovich-Rain M et al (2013) Gastrin: a distinct fate of neurogenin3 positive progenitor cells in the embryonic pancreas. *PLoS One* 8(8):2–11. <https://doi.org/10.1371/journal.pone.0070397>
- Liu G, Pakala SV, Gu D, Krah T, Mocnik L, Sarvetnick N (2001) Cholecystokinin expression in the developing and regenerating pancreas and intestine. *J Endocrinol* 169(2):233–240. <https://doi.org/10.1677/joe.0.1690233>
- Dahan T, Ziv O, Horwitz E et al (2017) Pancreatic β -cells express the fetal islet hormone gastrin in rodent and human diabetes. *Diabetes* 66(2):426–436. <https://doi.org/10.2337/db16-0641>
- Sachs S, Bastidas-Ponce A, Tritschler S et al (2020) Targeted pharmacological therapy restores β -cell function for diabetes remission. *Nat Metab* 2(2):192–209. <https://doi.org/10.1038/s42255-020-0201-1>
- Rehfeld JF (2019) Premises for cholecystokinin and gastrin peptides in diabetes therapy. *Clin Med Insights Endocrinol Diabetes* 12:1179551419883608. <https://doi.org/10.1177/1179551419883608>
- Rehfeld JF, Friis-Hansen L, Goetze JP, Hansen TVO (2007) The biology of cholecystokinin and gastrin peptides. *Curr Top Med Chem* 7(12):1154–1165. <https://doi.org/10.2174/156802607780960483>
- Sasaki S, Miyatsuka T, Matsuoka T et al (2015) Activation of GLP-1 and gastrin signalling induces in vivo reprogramming of pancreatic exocrine cells into beta cells in mice. *Diabetologia* 58(11):2582–2591. <https://doi.org/10.1007/s00125-015-3728-z>

25. Lampel M, Kern HF (1977) Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretagogue. *Virchows Arch A Pathol Anat Histol* 373(2):97–117. <https://doi.org/10.1007/BF00432156>
26. Cota P, Saber L, Taskin D et al (2023) NEUROD2 function is dispensable for human pancreatic β cell specification. *Front Endocrinol (Lausanne)* 14:1286590. <https://doi.org/10.3389/fendo.2023.1286590>
27. Ewels PA, Peltzer A, Fillinger S et al (2020) The nf-core framework for community-curated bioinformatics pipelines. *Nat Biotechnol* 38:276–278. <https://doi.org/10.1038/s41587-020-0439-x>
28. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C (2017) Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 14(4):417–419. <https://doi.org/10.1038/nmeth.4197>
29. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550. <https://doi.org/10.1186/s13059-014-0550-8>
30. Zhu A, Ibrahim JG, Love MI (2019) Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. *Bioinformatics* 35(12):2084–2092. <https://doi.org/10.1093/bioinformatics/bty895>
31. Zhou Y, Zhou B, Pache L et al (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 10(1):1523. <https://doi.org/10.1038/s41467-019-09234-6>
32. Wu F, Liufu Z, Liu Y et al (2022) Species-specific rewiring of definitive endoderm developmental gene activation via endogenous retroviruses through TET1-mediated demethylation. *Cell Rep* 41(11):111791. <https://doi.org/10.1016/j.celrep.2022.111791>
33. Arnold SJ, Robertson EJ (2009) Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo. *Nat Rev Mol Cell Biol* 10:91–103. <https://doi.org/10.1038/nrm2618>
34. Scheibner K, Bakhti M, Bastidas-Ponce A, Lickert H (2019) Wnt signaling: implications in endoderm development and pancreas organogenesis. *Curr Opin Cell Biol* 61:48–55. <https://doi.org/10.1016/j.ceb.2019.07.002>
35. Yang K, Spitzer H, Sterr M et al (2025) A multimodal cross-species comparison of pancreas development. *Nat Commun* 16(1):9355. <https://doi.org/10.1038/s41467-025-64774-4>
36. Akgün Canan M, Cozzitorto C, Sterr M et al (2026) Resolving human α versus β cell fate allocation for the generation of stem cell-derived islets. *Nat Commun* 17:6050. <https://doi.org/10.1038/s41467-026-75255-7>
37. Wang X, Sterr M, Ansarullah et al (2019) Point mutations in the PDX1 transactivation domain impair human β -cell development and function. *Mol Metab* 24:80–97. <https://doi.org/10.1016/j.molmet.2019.03.006>
38. Langmesser S, Cerezo-Guisado MI, Lorenzo MJ, Garcia-Marin LJ, Bragado MJ (2007) CCK1 and 2 receptors are expressed in immortalized rat brain neuroblasts: intracellular signals after cholecystokinin stimulation. *J Cell Biochem* 100(4):851–864. <https://doi.org/10.1002/jcb.21193>
39. Ryberg B, Tielemans Y, Axelson J et al (1990) Gastrin stimulates the self-replication rate of enterochromaffinlike cells in the rat stomach. Effects of omeprazole, ranitidine, and gastrin-17 in intact and antrectomized rats. *Gastroenterology* 99(4):935–942. [https://doi.org/10.1016/0016-5085\(90\)90610-D](https://doi.org/10.1016/0016-5085(90)90610-D)
40. Zhao J, Liang S, Cen HH et al (2024) PDX1+ cell budding morphogenesis in a stem cell-derived islet spheroid system. *Nat Commun* 15(1):5894. <https://doi.org/10.1038/s41467-024-50109-2>
41. Zeng Q, Ou L, Wang W, Guo DY (2020) Gastrin, cholecystokinin, signaling, and biological activities in cellular processes. *Front Endocrinol (Lausanne)* 11:112. <https://doi.org/10.3389/fendo.2020.00112>
42. Seifert R, Wenzel-Seifert K (2002) Constitutive activity of G-proteins-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedeberg Arch Pharmacol* 366:381–416. <https://doi.org/10.1007/s00210-002-0588-0>
43. Hellmich MR, Rui XL, Hellmich HL, Fleming RYD, Evers BM, Townsend CM (2000) Human colorectal cancers express a constitutively active cholecystokinin-B/gastrin receptor that stimulates cell growth. *J Biol Chem* 275(41):32122–32128. <https://doi.org/10.1074/jbc.M005754200>
44. Ahnfelt-Ronne J, Jorgensen MC, Klinck R et al (2012) Ptf1a-mediated control of Dll1 reveals an alternative to the lateral inhibition mechanism. *Development* 139(23):4492. <https://doi.org/10.1242/dev.090597>
45. Apelqvist A (1999) Notch signalling controls pancreatic cell differentiation. *Nature* 400(August):877–881. <https://doi.org/10.1038/23716>
46. Zhu H, Wang G, Nguyen-Ngoc KV et al (2023) Understanding cell fate acquisition in stem-cell-derived pancreatic islets using single-cell multiome-inferred regulomes. *Dev Cell* 58(9):727–743. <https://doi.org/10.1016/j.devcel.2023.03.011>
47. Georgakopoulos N, Prior N, Angres B et al (2020) Long-term expansion, genomic stability and in vivo safety of adult human pancreas organoids. *BMC Dev Biol* 20(1):4. <https://doi.org/10.1186/s12861-020-0209-5>
48. Molnár R, Madácsy T, Varga Á et al (2020) Mouse pancreatic ductal organoid culture as a relevant model to study exocrine pancreatic ion secretion. *Lab Invest* 100(1):84–97. <https://doi.org/10.1038/s41374-019-0300-3>
49. Breunig M, Merkle J, Wagner M et al (2021) Modeling plasticity and dysplasia of pancreatic ductal organoids derived from human pluripotent stem cells. *Cell Stem Cell* 28(6):1105–1124. <https://doi.org/10.1016/j.stem.2021.03.005>
50. Karampelias C, Yang K, Farkas FJ et al (2025) Benchmarking porcine pancreatic ductal organoids for drug screening applications. *EMBO Mol Med* 17(12):3657–3688. <https://doi.org/10.1038/s44321-025-00330-3>
51. Garcia CC, Venkat A, McQuaid DC et al (2026) Beta cell-derived cholecystokinin drives obesity-associated pancreatic adenocarcinoma development. *Nat Commun* 17(1):3292. <https://doi.org/10.1038/s41467-026-69821-2>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Lama Saber^{1,2,3}  · Kei Kozawa^{1,2} · Christos Karampelias^{1,2}  · Changying Jing^{1,2,4}  · Jessica Jaki^{1,2} · Melis Akgün Canan^{1,2,5}  · Michael Sterr^{1,2} · Osvaldo Rodrigues Pereira^{1,2,3}  · Oliver Czarnecki^{1,2,3}  · Gregory S. Korbitt^{6,7}  · Andrew R. Pepper^{6,7}  · Noel Moya^{1,2,8}  · Ingo Bartscher^{1,2} · Heiko Lickert^{1,2,3}  · Mostafa Bakhti^{1,2} 

✉ Heiko Lickert
heiko.lickert@helmholtz-munich.de

✉ Mostafa Bakhti
mostafa.bakhti@helmholtz-munich.de

¹ Institute of Diabetes and Regeneration Research, Helmholtz Munich, Neuherberg, Germany

² German Center for Diabetes Research (DZD), Neuherberg, Germany

³ School of Medicine, Technical University of Munich (TUM), Munich, Germany

⁴ Munich Medical Research School (MMRS), Ludwig Maximilian University (LMU), Munich, Germany

⁵ Present Address: Stem Cell Institute, Ankara University, Ankara, Türkiye

⁶ Alberta Diabetes Institute, Li Ka Shing Centre for Research, Edmonton, AB, Canada

⁷ Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

⁸ Present Address: Regenerative Medicine Division, Delphi Stem, Inc., Atlanta, GA, USA