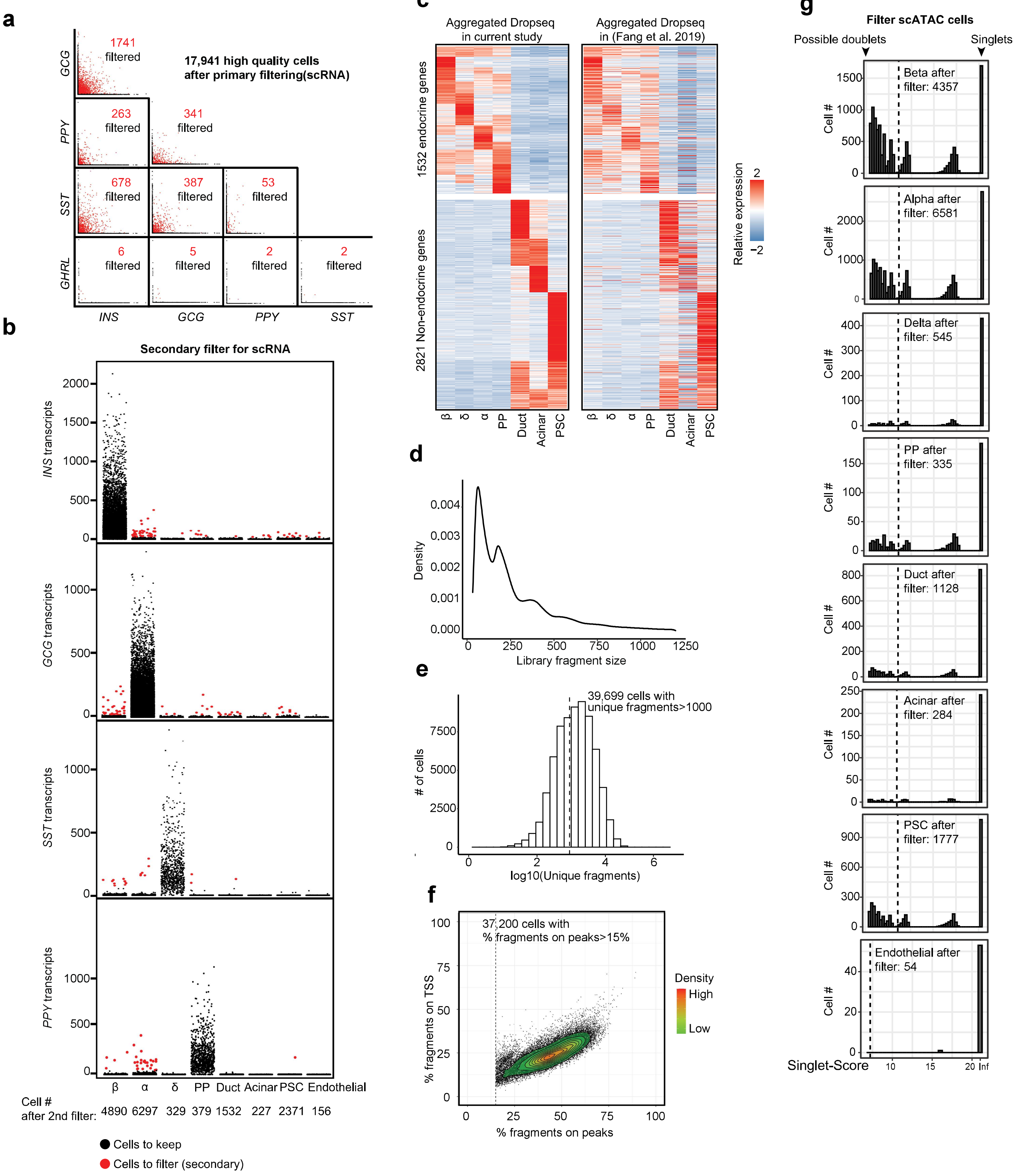
Supplementary Information

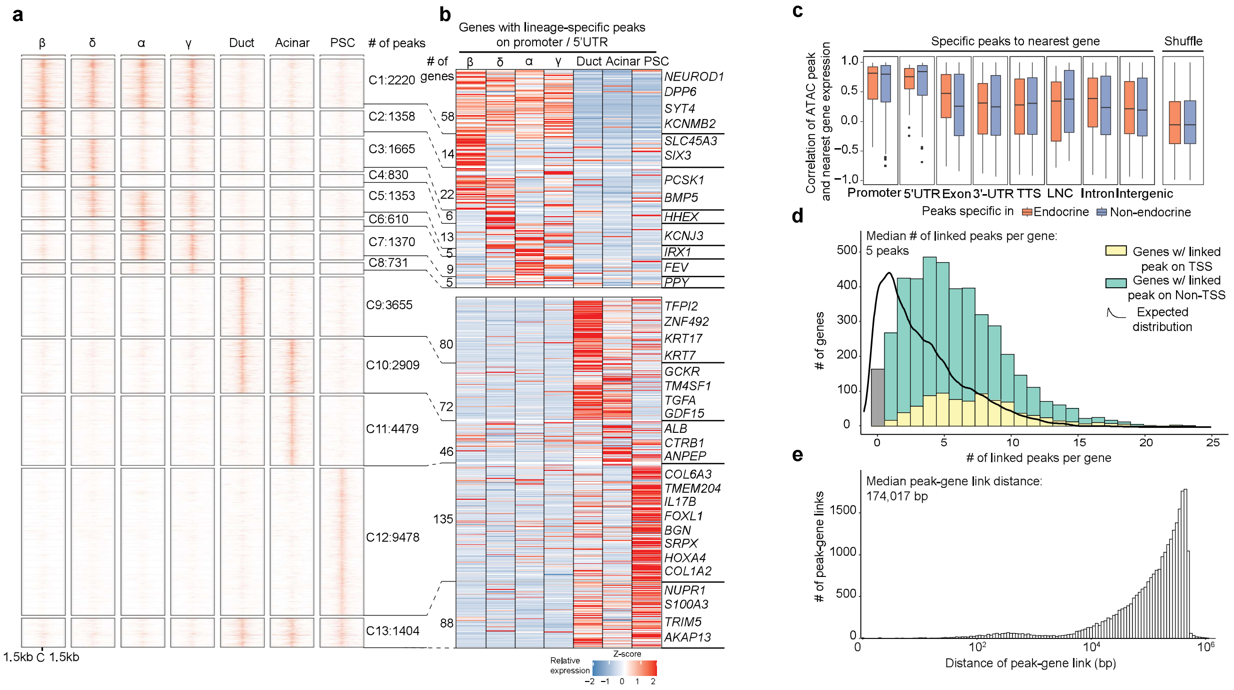
Single cell multiomic analysis reveals diabetes-associated β-cell heterogeneity driven by *HNF1A*

Supplementary Fig 1



Supplementary Fig. 1 scRNA-seq and snATAC-seq filtering strategies and quality control. (**a**) Hormone-based doublets filtering strategy for scRNA-seq data. In principle, any cell that expresses two or more hormone gene is labeled as a doublet and filtered. (**b**) Secondary doublets filtering for scRNA-seq data. After clustering and cell-type annotation using the global transcriptome landscapes, if a classified cell is highly expressing a hormone gene not consistent with the defined cell type, this cell is filtered. (**c**) Cell type specific gene expression profile comparison between current cohort and our previously published cohort. Aggregated RPKM is used for visualization and comparison. (**d-f**) snATAC-seq quality control. (**d**) snATAC data fragment size distribution. (**e**) Distribution of the number of unique fragments per cell. (**f**) snATAC-seq data filtering using the percentage on peaks versus the percentage on TSS. (**g**) snATAC-seq doublets filtering based on the KNN distance ratio (**Methods**).

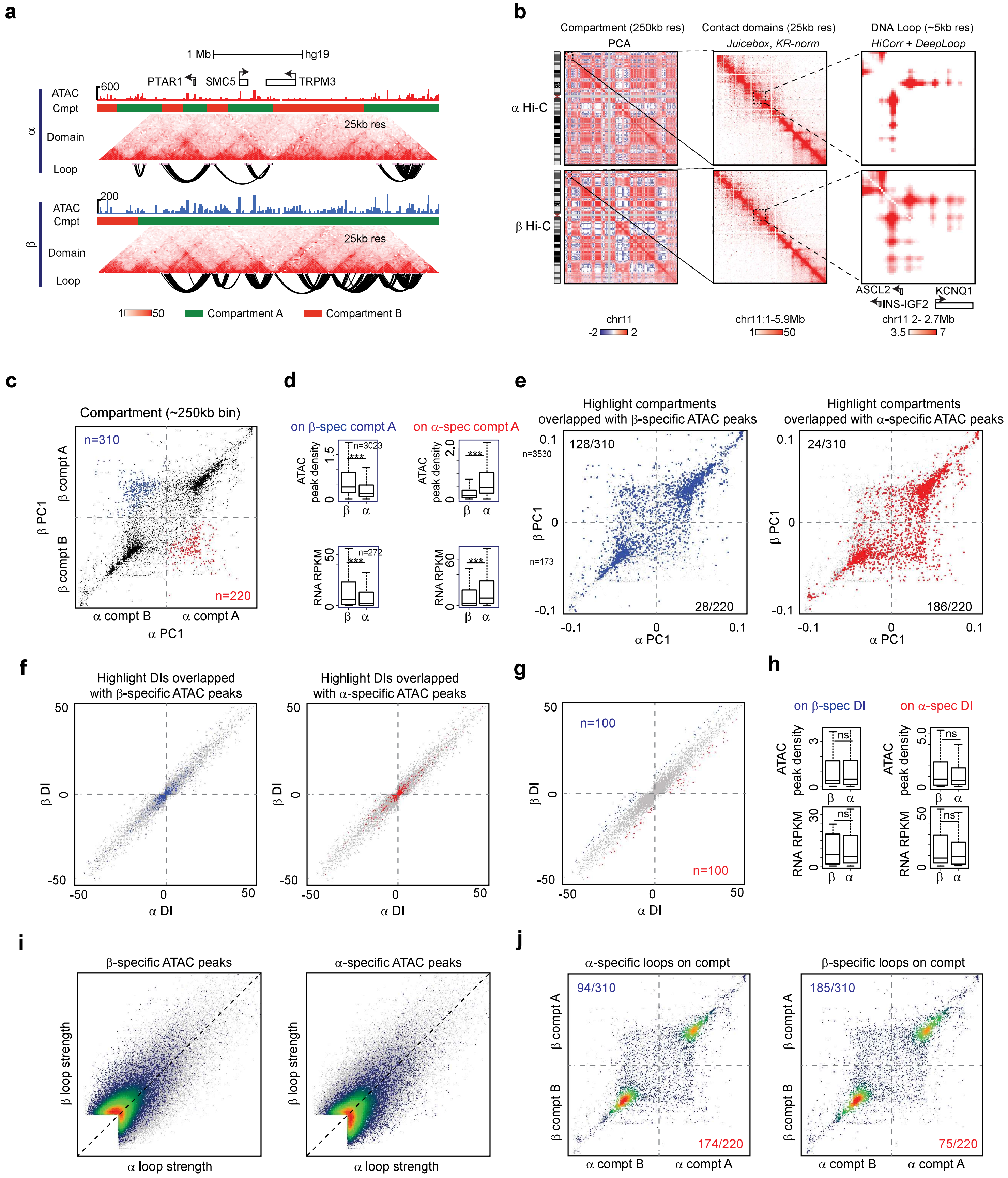
Supplementary Fig 2



Supplementary Fig. 2. Peak-Gene pair analysis.

(**a-b**) Identify thirteen categories of cell type specific peaks (**a**) and their corresponding genes (**b**) if the peaks are located at promoter (-1kb to +100bp) or 5' UTRs. (**c**) Correlation (Pearson's r) between cell-type specific ATAC peaks and their nearest genes stratified by the peak locations. (**d-e**) Peak-gene link analysis based on correlation instead of proximity (**Methods**). (**d**) Summary of the number of linked peaks for each gene. (**e**) Summary of the genomic distance between the peak-gene pairs.

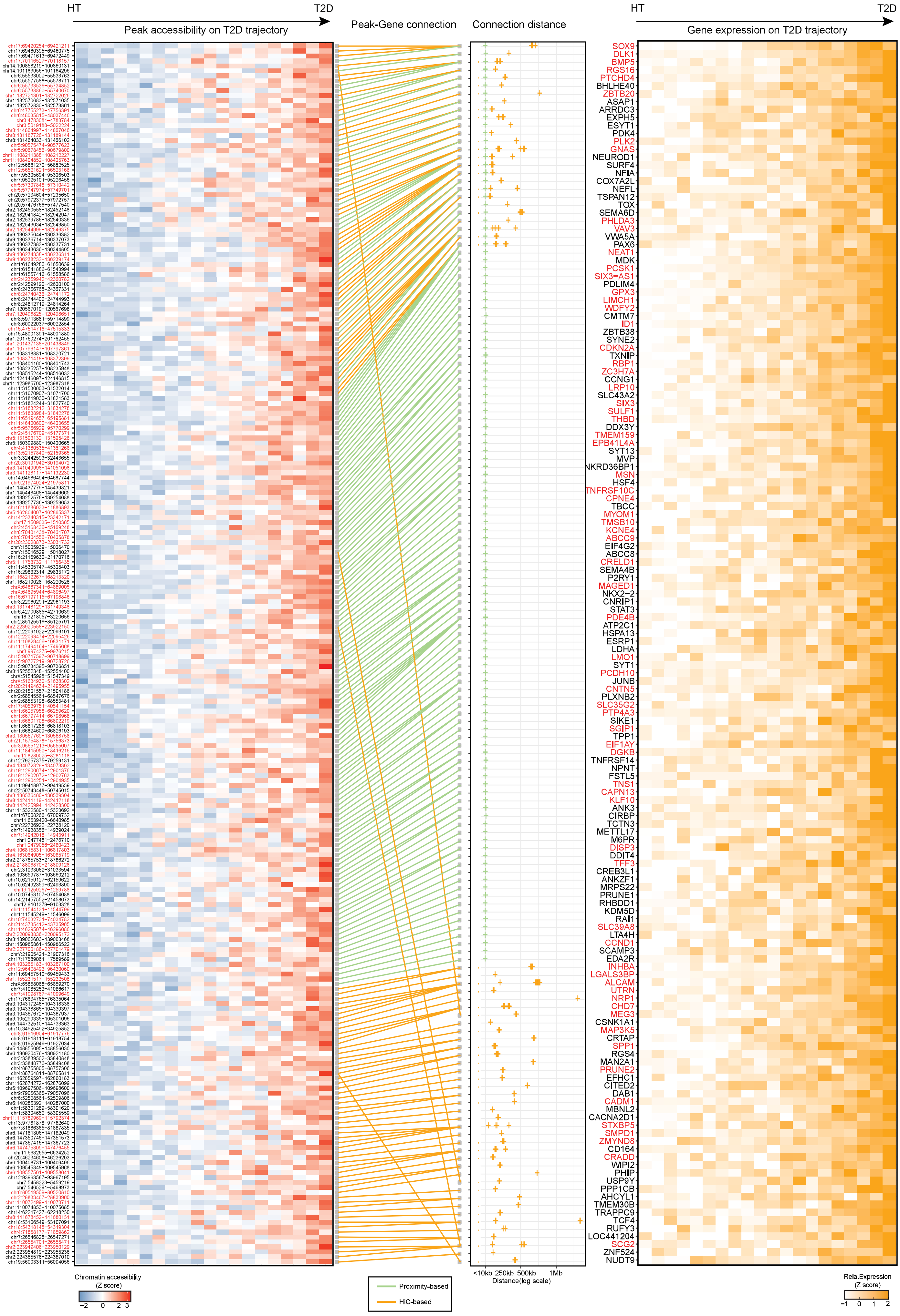
Supplementary Fig 3



Supplementary Fig. 3. 3D genome analysis in α- and β-cells.

(**a**) Genomic tracks showing ATAC, compartment A/B, contact heatmaps, and chromatin loop curves in α-cell and β-cell at locus chr9:73129495-74491882. (**b**) Hierarchical genome architecture (compartment, contact domains and DNA Loops) near *INS-IGF2* locus in both α- and β-cells. (**c**) Scatterplot comparing PC1 values from compartment analyses in α- and β-cells. Highlighted dots are the α-cell or β-cell specific compartment A bins (250kb resolution). (**d**) Boxplots comparing the ATAC intensity (top two panels) and gene expression (bottom two panels) with the 220 α-cell or β-cell specific compartment A regions from (**c**). (**e**) The scatterplots compare the PC1 values of α-cell and β-cell Hi-C data in compartment-level analyses (each dot represents a 250kb bin). Blue highlights bins overlapping 5,000 β-cell specific ATAC peaks. Red highlights bins are overlapping 5,000 α-cell specific ATAC peaks. (**f**) The scatterplots compare the DI (direction index) values of α-cell and β-cell Hi-C data in TAD-level analysis (each dot represents a 25kb bin). Left panel highlights the bins overlapping β-cell specific ATAC peaks; Right panel highlights the bins overlapping α-cell specific ATAC peaks. (**g**) Define a small number of bins with α-cell specific (red) and β-cell specific (blue) DI values. (**h**) Compare the ATAC peak strength and gene expression within the bins with α-cell or β-cell specific DI values. (**i**) Scatterplot comparing the strength of loops between α-cells and β-cells. Heat colored dots are loops overlapping 5,000 β-cell specific ATAC peaks (left panel) or 5,000 α-cell specific ATAC peaks (right panel). (**j**) Scatter plots highlighting the α-cell or β-cell specific loops from **Fig. 3b** to examine their correlation with compartment switching events.

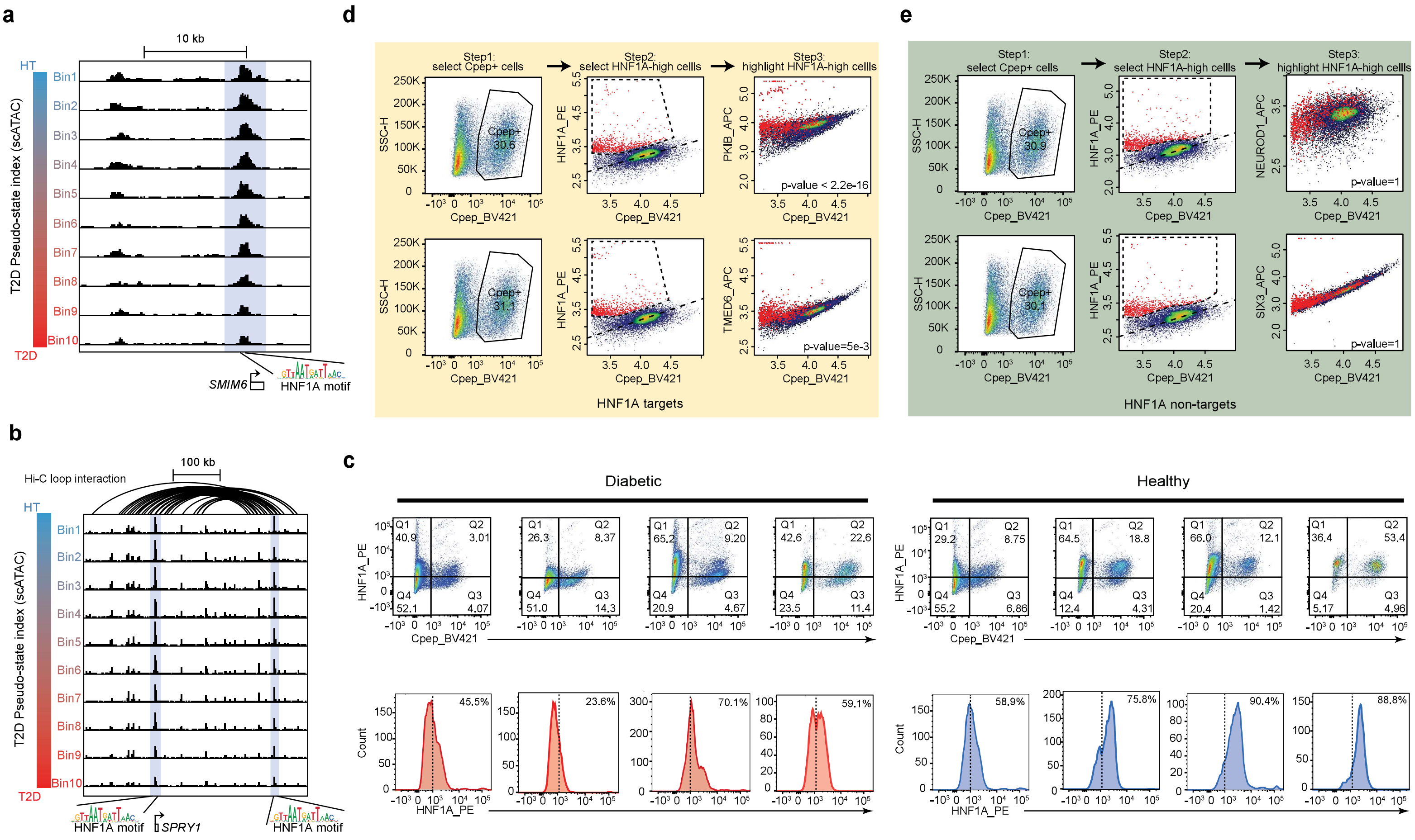
Supplementary Fig 4



Supplementary Fig. 4. A network of T2D upregulated peak-gene pairs.

Connect T2D up-regulated ATAC peaks to up-regulated genes from RePACT analyses through multiomic integration (similar as **Fig. 6a**). Leftmost panel: heatmap of peaks gaining accessibility along T2D trajectory; peak locations (hg19) are on the left of each row. Peaks highlighted in red show intra-donor heterogeneity. Second panel shows the peak-gene connections. Green lines: peaks within 10kb of the TSS; orange lines: distal peak-gene pairs supported by Hi-C loops. Third panel: the distance of each peak-TSS connection. Rightmost panel: heatmap of T2D up-regulated genes. Red-highlighted gene names indicate intra-donor heterogeneous genes.

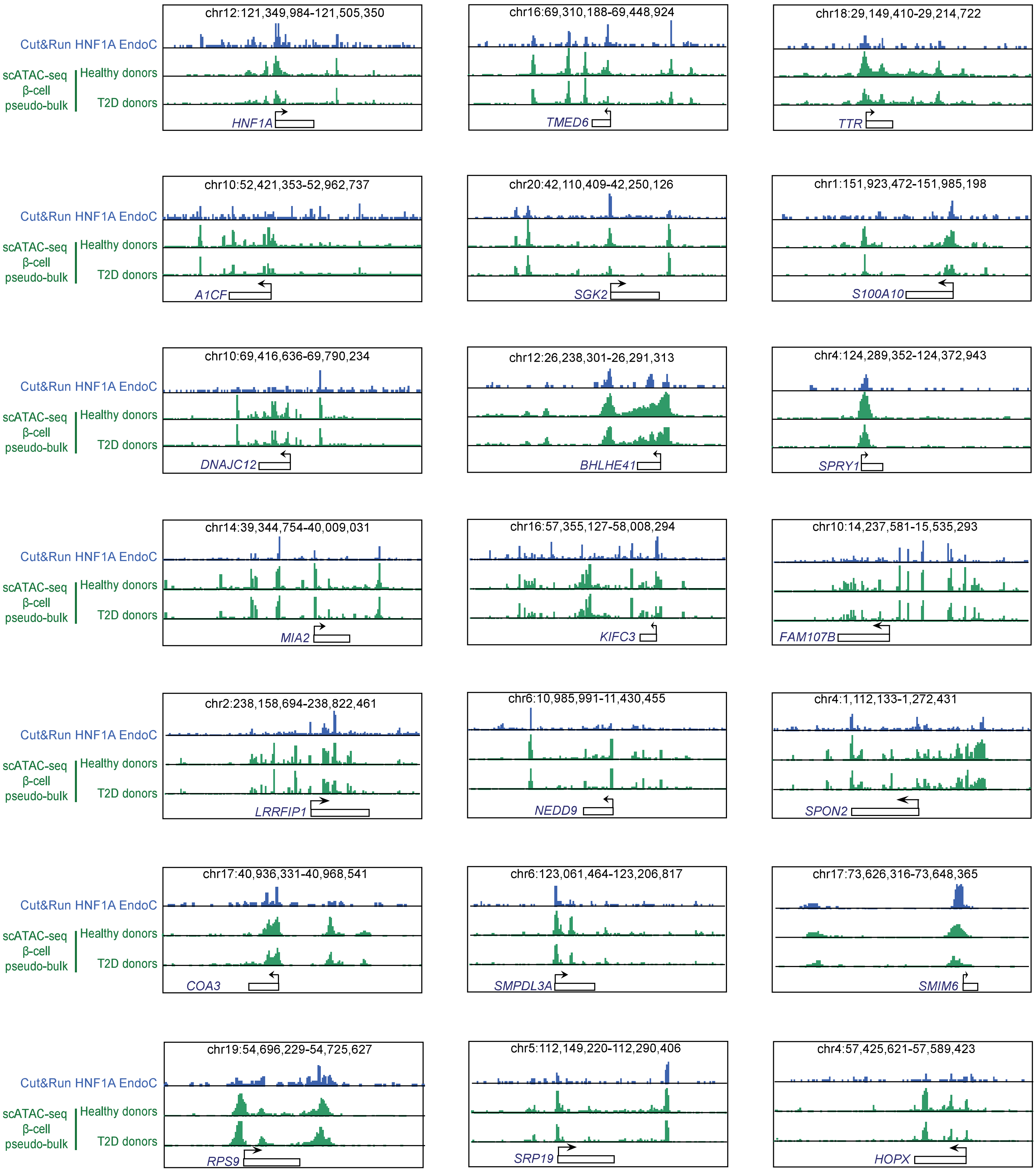
Supplementary Fig 5



Supplementary Fig. 5. Role of HNF1A in Type II Diabetes

Similar as **Fig. 6d**, genome browser snapshots of putative *HNF1A* target genes *SMIM6* (**a**) and *SPRY1* (**b**). (**c**) Flow cytometry scatterplot (top row) of HNF1A and C-pep islet cells from 4 healthy and 4 diabetic donors. The histogram (bottom row) shows the distribution of HNF1A signal in the β-cells (gated with C-peptide) from 4 healthy (blue) and 4 diabetic (red) donors. (**d-e**) Similar as **Fig. 7c-d**, additional β-cell three-way flow cytometry results for (**d**) *HNF1A* target genes PKIB and TMED6 in non-diabetic islets, and (**e**) control genes NEUROD1 and SIX3 in non-diabetic islets. P values from one sided *t.test*.

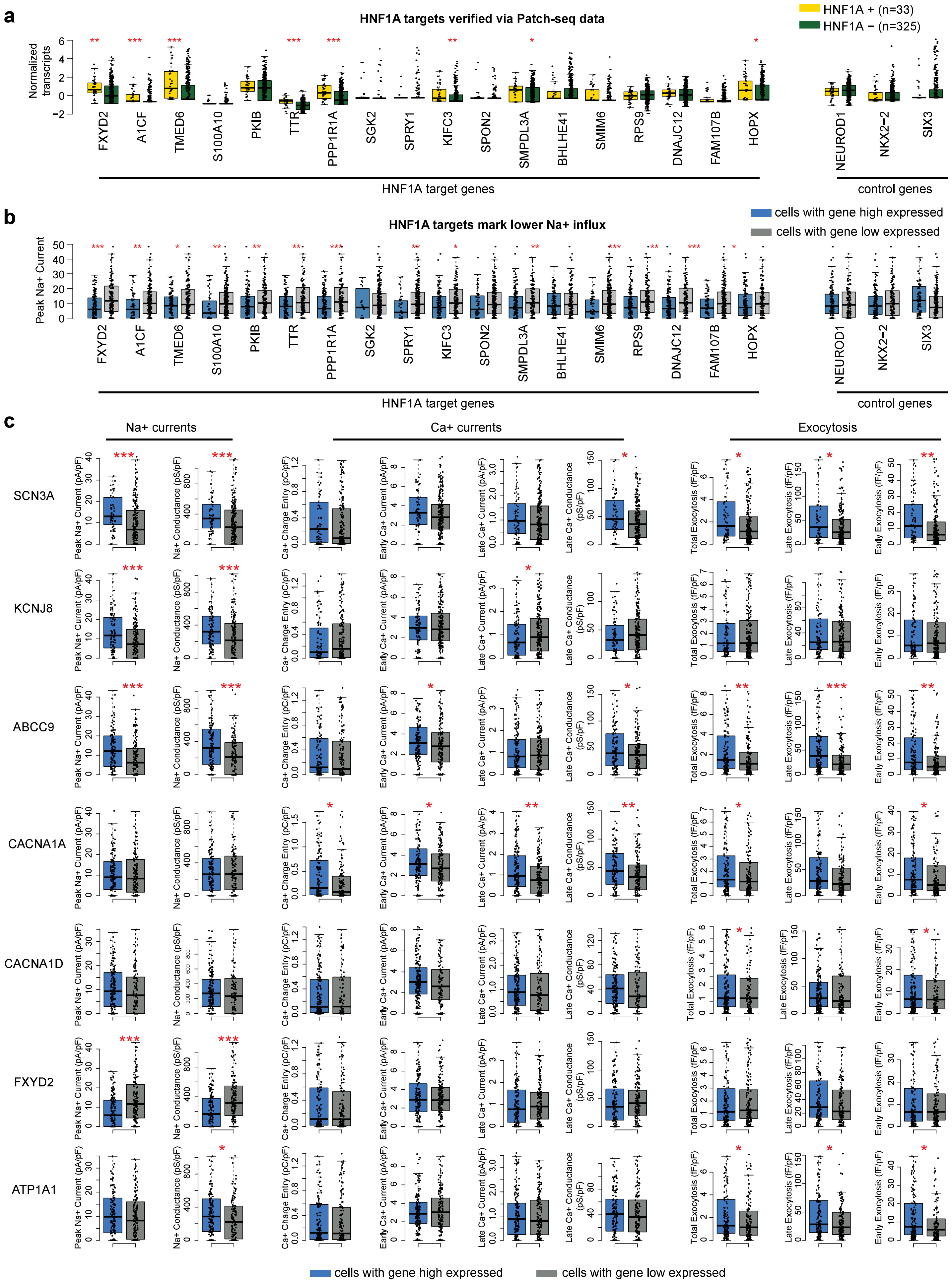
Supplementary Figure 6



Supplementary Fig. 6. HNF1A Cut&Run in EndoC-βH3

Genome browser snapshots of select *HNF1A* target gene loci. Each track has HNF1A Cut&Run (blue) and pseudo-bulk ATAC tracks from T2D and healthy β cells (green).

Supplementary Fig 7



Supplementary Fig. 7. Additional Patch-seq data analysis related to *HNF1A* functions.

(**a**) Expression of *HNF1A* target genes and three non-target control genes in *HNF1A*+ β cells (yellow, n=33) and *HNF1A*- β cells (green, n=325) from Patch-seq data (**Methods**). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ (Mann-Whitney U test). (**b**) Boxplots showing the association between *HNF1A* target genes and Na+ current measurement from Patch-seq data. For each gene of interest, β-cells are divided into high expression group (blue) and low expression group (grey) (**Methods**). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ (Mann-Whitney U test). (**c**) For every denoted channel gene, cells are divided into high- and low-expression groups (**Methods**). The boxplots show if the gene expression is associated with a variety of electrophysiological measurements from Patch-seq data. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ (Mann-Whitney U test).

Supplementary Table 1. eHi-C reads summary

Cell type	Sample	Total reads	Uniquely mapped	Non-redundant	Cis	Trans	Cis-2Mb
Alpha	Hi-C.alpha1	405,505,134	211,513,369	165,434,689	265,909,112	129,064,344	153,872,881
	Hi-C.alpha2	350,990,532	170,669,624	148,809,750			
	Hi-C.alpha3	398,703,410	72,194,498	60,303,123			
	Hi-C.alpha4	368,176,579	219,650,622	187,385,773			
	Hi-C.alpha5	417,280,536	272,466,837	231,279,903			
	Hi-C.alpha6	437,437,508	229,231,601	192,318,080			
	Hi-C.alpha7	430,008,729	194,518,534	172,247,820			
Beta	Hi-C.beta1	397,634,633	184,709,869	147,274,958	195,737,809	87,367,515	120,099,961
	Hi-C.beta2	385,862,924	57,501,715	50,498,651			
	Hi-C.beta3	387,875,392	131,038,273	105,789,167			
	Hi-C.beta4	404,610,467	254,126,546	210,718,872			
	Hi-C.beta5	461,766,459	253,441,320	215,582,251			
	Hi-C.beta6	476,561,356	219,768,827	173,865,774			

Supplementary Table 2. Tn5 oligos

Oligo name	Sequence
pMENTS	5Phos/CTGTCTCTTATACACATCT
Tn5_p7_1	GTCTCGTGGGCTCGG**AAACAC**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_2	GTCTCGTGGGCTCGG**AAAGGG**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_3	GTCTCGTGGGCTCGG**AACCGA**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_4	GTCTCGTGGGCTCGG**CCAATA**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_5	GTCTCGTGGGCTCGG**CCTCTT**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_6	GTCTCGTGGGCTCGG**CGTGAT**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_7	GTCTCGTGGGCTCGG**GCTGAA**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_8	GTCTCGTGGGCTCGG**GGACAA**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_9	GTCTCGTGGGCTCGG**GGCATT**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_10	GTCTCGTGGGCTCGG**TGTCTA**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_11	GTCTCGTGGGCTCGG**TTACCC**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p7_12	GTCTCGTGGGCTCGG**TTTCAG**GTCTCCGCCTCAGATGTGTATAAGAGACAG
Tn5_p5_a	TCGTCGGCAGCGTC**AAGATC**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_b	TCGTCGGCAGCGTC**AATTCC**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_c	TCGTCGGCAGCGTC**CTAAGC**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_d	TCGTCGGCAGCGTC**CTTGTG**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_e	TCGTCGGCAGCGTC**GAATAG**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_f	TCGTCGGCAGCGTC**GATGCT**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_g	TCGTCGGCAGCGTC**TCCAAG**TCGAGGACGGCAGATGTGTATAAGAGACAG
Tn5_p5_h	TCGTCGGCAGCGTC**TGGGTT**TCGAGGACGGCAGATGTGTATAAGAGACAG

Supplementary Table 3. PCR oligos

Name	Sequence
Nextera_N701	CAAGCAGAAGACGGCATACGAGAT**TCGCCTTA**GTCTCGTGGGCTCGG
Nextera_N702	CAAGCAGAAGACGGCATACGAGAT**CTAGTACG**GTCTCGTGGGCTCGG
Nextera_N703	CAAGCAGAAGACGGCATACGAGAT**TTCTGCCT**GTCTCGTGGGCTCGG
Nextera_N704	CAAGCAGAAGACGGCATACGAGAT**GCTCAGGA**GTCTCGTGGGCTCGG
Nextera_N705	CAAGCAGAAGACGGCATACGAGAT**AGGAGTCC**GTCTCGTGGGCTCGG
Nextera_N706	CAAGCAGAAGACGGCATACGAGAT**CATGCCTA**GTCTCGTGGGCTCGG
Nextera_N707	CAAGCAGAAGACGGCATACGAGAT**GTAGAGAG**GTCTCGTGGGCTCGG
Nextera_N708	CAAGCAGAAGACGGCATACGAGAT**CCTCTCTG**GTCTCGTGGGCTCGG
Nextera_N709	CAAGCAGAAGACGGCATACGAGAT**AGCGTAGC**GTCTCGTGGGCTCGG
Nextera_N710	CAAGCAGAAGACGGCATACGAGAT**CAGCCTCG**GTCTCGTGGGCTCGG
Nextera_N711	CAAGCAGAAGACGGCATACGAGAT**TGCCTCTT**GTCTCGTGGGCTCGG
Nextera_N712	CAAGCAGAAGACGGCATACGAGAT**TCCTCTAC**GTCTCGTGGGCTCGG
Nextera_N713	CAAGCAGAAGACGGCATACGAGAT**TCATGAGC**GTCTCGTGGGCTCGG
Nextera_N714	CAAGCAGAAGACGGCATACGAGAT**CCTGAGAT**GTCTCGTGGGCTCGG
Nextera_N715	CAAGCAGAAGACGGCATACGAGAT**TAGCGAGT**GTCTCGTGGGCTCGG
Nextera_N716	CAAGCAGAAGACGGCATACGAGAT**GTAGCTCC**GTCTCGTGGGCTCGG
Nextera_N717	CAAGCAGAAGACGGCATACGAGAT**TACTACGC**GTCTCGTGGGCTCGG
Nextera_N718	CAAGCAGAAGACGGCATACGAGAT**AGGCTCCG**GTCTCGTGGGCTCGG
Nextera_N719	CAAGCAGAAGACGGCATACGAGAT**GCAGCGTA**GTCTCGTGGGCTCGG
Nextera_N720	CAAGCAGAAGACGGCATACGAGAT**CTGCGCAT**GTCTCGTGGGCTCGG
Nextera_N721	CAAGCAGAAGACGGCATACGAGAT**GAGCGCTA**GTCTCGTGGGCTCGG
Nextera_N722	CAAGCAGAAGACGGCATACGAGAT**CGCTCAGT**GTCTCGTGGGCTCGG
Nextera_N723	CAAGCAGAAGACGGCATACGAGAT**GTCTTAGG**GTCTCGTGGGCTCGG
Nextera_N724	CAAGCAGAAGACGGCATACGAGAT**ACTGATCG**GTCTCGTGGGCTCGG
Nextera_N725	CAAGCAGAAGACGGCATACGAGAT**TAGCTGCA**GTCTCGTGGGCTCGG
Extended_N726	CAAGCAGAAGACGGCATACGAGAT**CTCATTGT**GTCTCGTGGGCTCGG
Extended_N727	CAAGCAGAAGACGGCATACGAGAT**GAGTGTAG**GTCTCGTGGGCTCGG
Extended_N728	CAAGCAGAAGACGGCATACGAGAT**GCTAGGTT**GTCTCGTGGGCTCGG
Extended_N729	CAAGCAGAAGACGGCATACGAGAT**GGAAGGAT**GTCTCGTGGGCTCGG
Extended_N730	CAAGCAGAAGACGGCATACGAGAT**GGTCACTC**GTCTCGTGGGCTCGG
Extended_N731	CAAGCAGAAGACGGCATACGAGAT**GTTTCGTT**GTCTCGTGGGCTCGG
Extended_N732	CAAGCAGAAGACGGCATACGAGAT**TCAAACAT**GTCTCGTGGGCTCGG
Extended_N733	CAAGCAGAAGACGGCATACGAGAT**TCCCATGT**GTCTCGTGGGCTCGG
Extended_N734	CAAGCAGAAGACGGCATACGAGAT**TGTTTCCC**GTCTCGTGGGCTCGG
Extended_N735	CAAGCAGAAGACGGCATACGAGAT**AAACAAAC**GTCTCGTGGGCTCGG
Extended_N736	CAAGCAGAAGACGGCATACGAGAT**TTTACATC**GTCTCGTGGGCTCGG
Nextera_N501	AATGATACGGCGACCACCGAGATCTACAC**TAGATCGC**TCGTCGGCAGCGTC
Nextera_N502	AATGATACGGCGACCACCGAGATCTACAC**CTCTCTAT**TCGTCGGCAGCGTC
Nextera_N503	AATGATACGGCGACCACCGAGATCTACAC**TATCCTCT**TCGTCGGCAGCGTC
Nextera_N504	AATGATACGGCGACCACCGAGATCTACAC**AGAGTAGA**TCGTCGGCAGCGTC
Nextera_N505	AATGATACGGCGACCACCGAGATCTACAC**GTAAGGAG**TCGTCGGCAGCGTC
Nextera_N506	AATGATACGGCGACCACCGAGATCTACAC**ACTGCATA**TCGTCGGCAGCGTC
Nextera_N507	AATGATACGGCGACCACCGAGATCTACAC**AAGGAGTA**TCGTCGGCAGCGTC
Nextera_N508	AATGATACGGCGACCACCGAGATCTACAC**CTAAGCCT**TCGTCGGCAGCGTC
Nextera_N509	AATGATACGGCGACCACCGAGATCTACAC**CGTCTAAT**TCGTCGGCAGCGTC
Nextera_N510	AATGATACGGCGACCACCGAGATCTACAC**TCTCTCCG**TCGTCGGCAGCGTC
Nextera_N511	AATGATACGGCGACCACCGAGATCTACAC**TCGACTAG**TCGTCGGCAGCGTC
Nextera_N512	AATGATACGGCGACCACCGAGATCTACAC**TTCTAGCT**TCGTCGGCAGCGTC
Nextera_N513	AATGATACGGCGACCACCGAGATCTACAC**CCTAGAGT**TCGTCGGCAGCGTC
Nextera_N514	AATGATACGGCGACCACCGAGATCTACAC**GCGTAAGA**TCGTCGGCAGCGTC
Nextera_N515	AATGATACGGCGACCACCGAGATCTACAC**CTATTAAG**TCGTCGGCAGCGTC
Nextera_N516	AATGATACGGCGACCACCGAGATCTACAC**AAGGCTAT**TCGTCGGCAGCGTC

Nextera_N517	AATGATACGGCGACCACCGAGATCTACAC**GAGCCTTA**TCGTCGGCAGCGTC
Nextera_N518	AATGATACGGCGACCACCGAGATCTACAC**TTATGCGA**TCGTCGGCAGCGTC
Extended_N519	AATGATACGGCGACCACCGAGATCTACAC**TTTACATC**TCGTCGGCAGCGTC
Extended_N520	AATGATACGGCGACCACCGAGATCTACAC**AAACAAAC**TCGTCGGCAGCGTC
Extended_N521	AATGATACGGCGACCACCGAGATCTACAC**AGCAAATT**TCGTCGGCAGCGTC
Extended_N522	AATGATACGGCGACCACCGAGATCTACAC**CGTTGGTG**TCGTCGGCAGCGTC
Extended_N523	AATGATACGGCGACCACCGAGATCTACAC**GACTTCTT**TCGTCGGCAGCGTC
Extended_N524	AATGATACGGCGACCACCGAGATCTACAC**GGTCACTC**TCGTCGGCAGCGTC

Supplementary Table 4. qPCR primers

Primer	Sequence 5’ to 3’
HNF1A F1	GGTCCTACGTTCACCAACACA
HNF1A R1	CTCTGGGTCACATGGCTCT
S100A10 F	GGCTACTTAACAAAGGAGGACC
S100A10 R	GAGGCCCGCAATTAGGGAAA
A1CF F	TGTGGACAACTGCCGATTATTT
A1CF R	TGACATCGACAACACCTTCAGTA
TTR F	TGGGAGCCATTTGCCTCTG
TTR R	AGCCGTGGTGGAATAGGAGTA
DNAJC12 F	AGCATCCTGAAAACCCCAAAG
DNAJC12 R	GCGAGAAACCCACCGTCTT
BHLHE41 F	AAGGAGCATGAAACGAGACGA
BHLHE41 R	CTCGGTTAAGGCGGTTAAAGC
KIF3 F	GTGGAGCTGCGACTCAAGG
KIF3 R	CTGCTTGGTCTTGGACGACT
PPP1R1A F	CCACGGCAACGGAAGAAGAT
PPP1R1A R	CCCCAGGTGATGTTCAACCA
SGK2 F	ACTCCACCCTTCAACCCAAAT
SGK2 R	GCGCATAAGAAAATCCCAGGAA
TMED6 F	GGGCTGGTCGTTCTGAATCTA
TMED6 R	CGCTGAACCTCGTAACTGAAAT
PKIB F	GAGTCTGGGGTCGCCAATTTT
PKIB R	TGAACTCTGGATGTCTGGTAAGG
GAPDH F	GGAGCGAGATCCCTCCAAAAT
GAPDH R	GGCTGTTGTCATACTTCTCATGG
ACTIN F	CATGTACGTTGCTATCCAGGC
ACTIN R	CTCCTTAATGTCACGCACGAT
FXYD2 F	ATCCTCCTCAGTAAGTGGGGT
FXYD2 R	CTTGGCAACTCCCGAAAGC

Supplementary Table 5. Antibodies used in this study

Flow Cytometry Antibodies

Target	Primary Ab	Secondary Ab
HNF1A	Cat# 89670S, CST	Cat# 406421, Biolegend
CPEP	Cat# GN-ID4-s, DSHB	Cat# 565013, BD Biosciences
TTR	Cat# sc-377517, Santa Cruz	Cat# A865, Invitrogen
SIX3	Cat# sc-398797, Santa Cruz	Cat# A865, Invitrogen
TMED6	Cat# SAB1408365, Sigma	Cat# A865, Invitrogen
PKIB	Cat# SAB1412738, Sigma	Cat# A865, Invitrogen
A1CF	Cat# SAB1400523, Sigma	Cat# A865, Invitrogen
NEUROD1	Cat# 563566, BD Biosciences	--
NKX2.2	Cat# 564729, BD Biosciences	--

Primary Ab

Name	Catalog #	Lot #	Clone #	Dilution	Manufacturer
HNF1A	Cat# 89670S	#1	NA	1:50	CST
CPEP	Cat# GN-ID4-s	NA	NA	1:50	DSHB
TTR	Cat# sc-377517	#J0316	NA	1:50	Santa Cruz
SIX3	Cat# sc-398797	#B0922	NA	1:50	Santa Cruz
TMED6	Cat# SAB1408365	#10027	NA	1:50	Sigma
PKIB	Cat# SAB1412738	#12139-7F8	NA	1:50	Sigma
A1CF	Cat# SAB1400523	#HC271	NA	1:50	Sigma
NEUROD1	Cat# 563566	#8352861	R8-294	1:50	BD Biosciences
NKX2.2	Cat# 564729	#9031625	74.5A5	1:50	BD Biosciences

Secondary Ab

PE Donkey anti-rabbit	Cat# 406421	#B233152	poly4064	1:125	Biolegend
BV421 Goat anti-rat	Cat# 565013	#1085807	NA	1:125	BD Biosciences
APC Goat anti-mouse	Cat# A865	#2384050	NA	1:125	Invitrogen

Cut and Run Ab

HNF1A	Cat# ab204306	#1003158-2	NA	1ug	abcam

Supplementary Table 6. Patient Donor Information used in this study

Islets used in scRNA-seq/scATAC-seq				
Donor	HbA1C	BMI	Age (yrs)	Sex
H1	5.8	31.9	45	M
H2	5.6	24.2	24	M
H3	5.4	30.7	62	F
H4	5.8	35.1	41	M
H5	5.6	28.5	55	M
H6	5.2	23.5	41	M
H7	5.4	23.2	43	F
T2D1	6.7	32.5	58	M
T2D2	5.9	31.7	63	M
T2D3	7.6	24.8	52	M
T2D4	6.7	25.8	53	M

Islets used in Flow Cytometry					
Donor	HbA1C	BMI	Age (yrs)	Sex	Fraction of HNF1A+ β cells
H8	5	31.4	43	F	58.9
H9	4.6	20.8	20	M	75.8
H10	5.4	30.7	62	F	90.4
H7	5.4	23.2	43	F	88.8
T2D1	6.7	32.5	58	M	45.5
T2D5	6.6	31.2	47	M	23.6
T2D3	7.6	24.8	52	M	70.1
T2D4	6.7	25.8	53	M	59.1