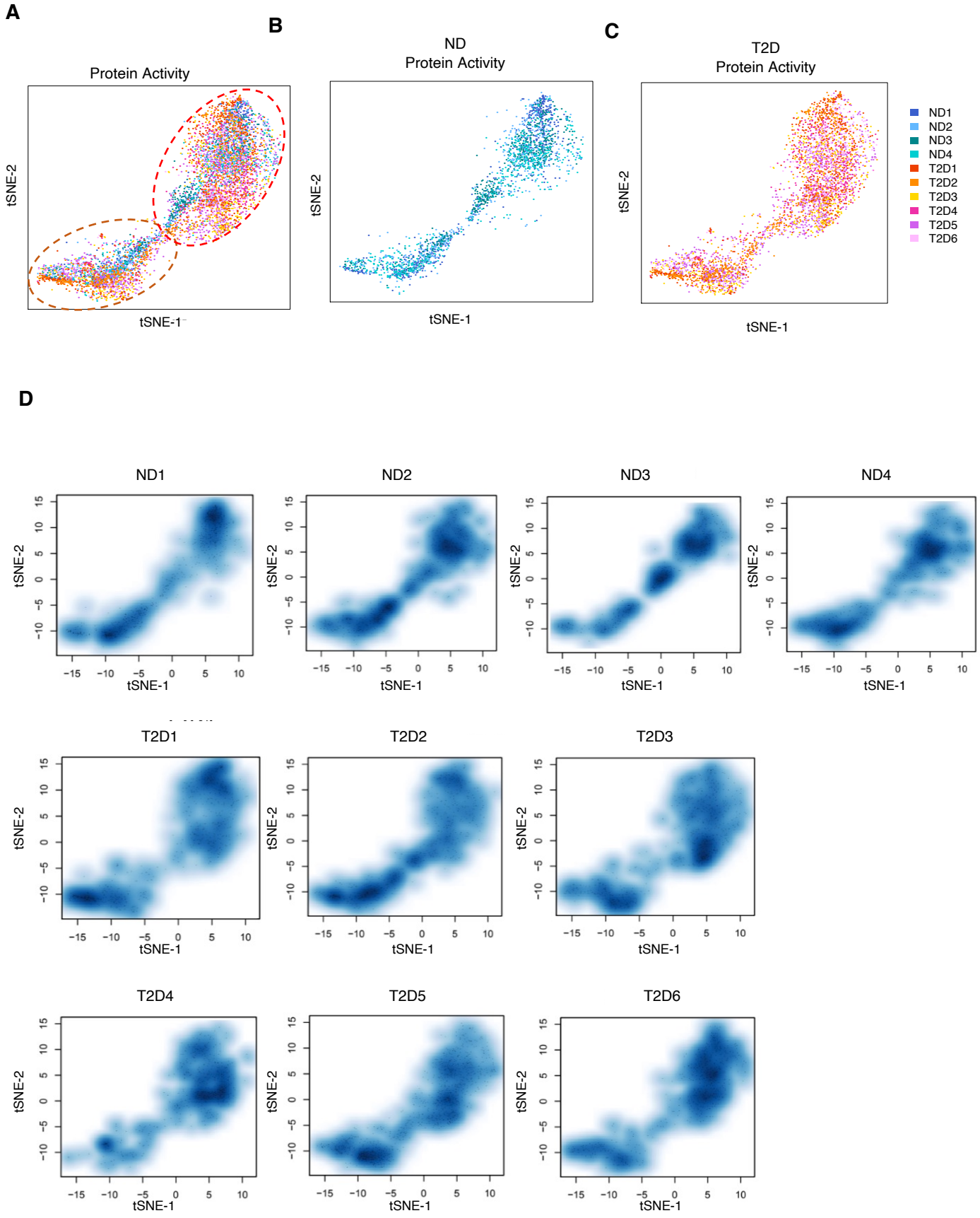
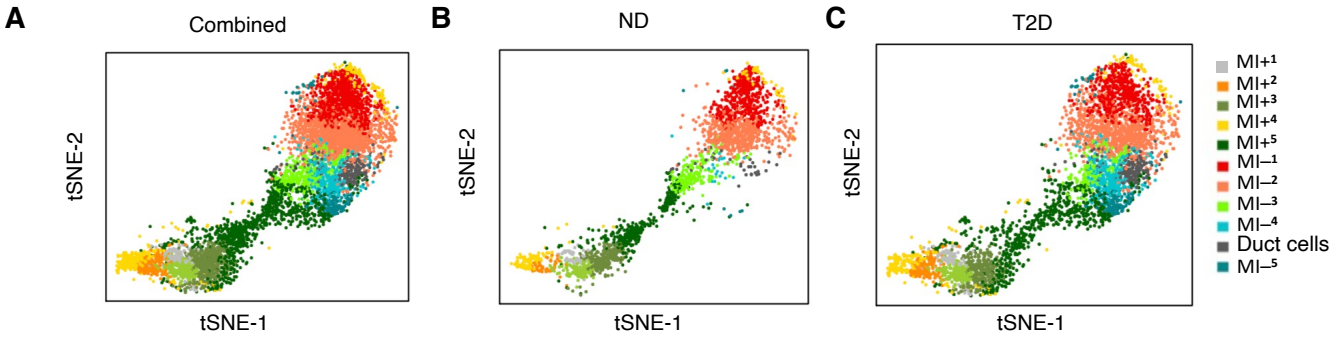
Supplemental Figure 1

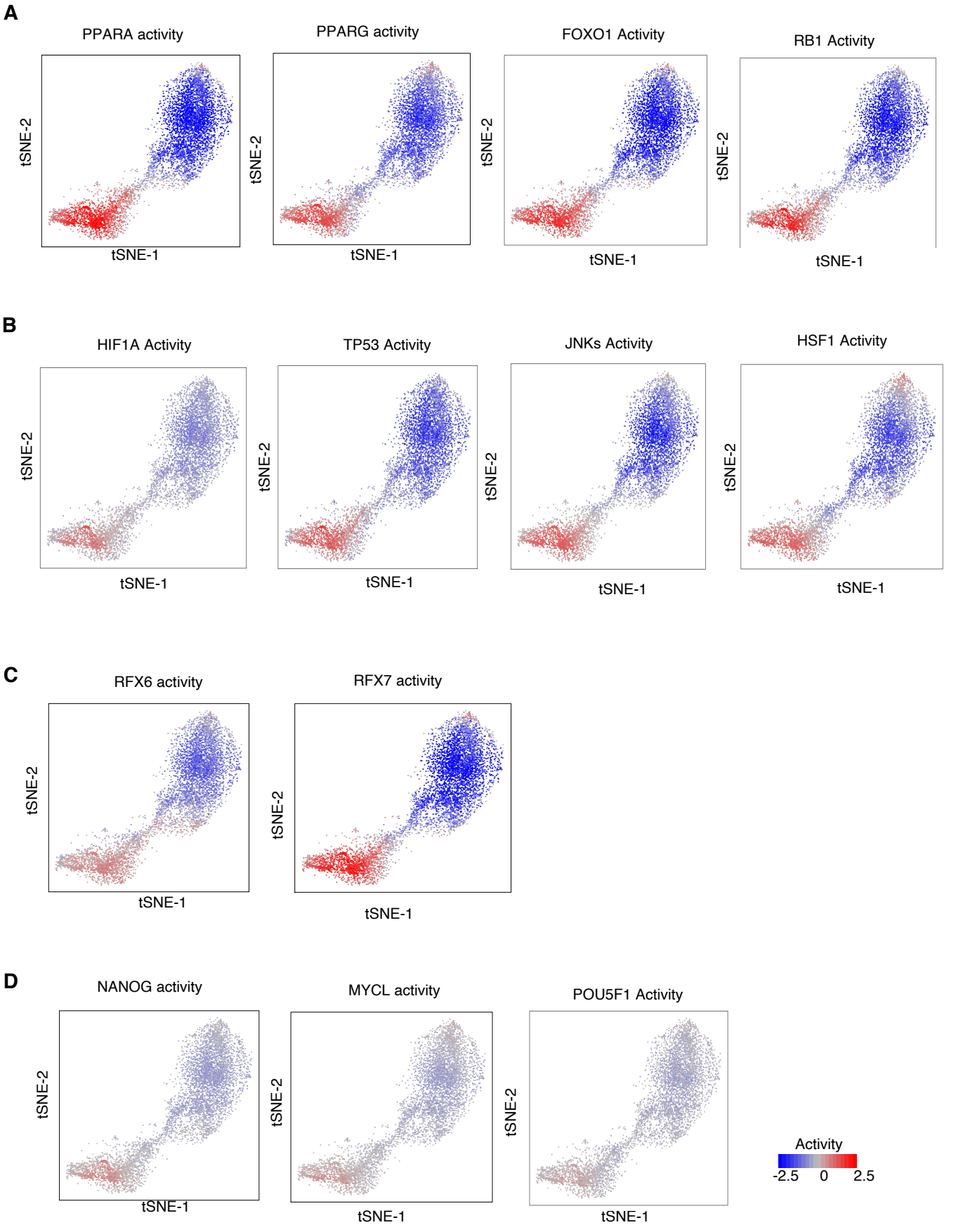


Supplemental Figure 1. T-SNE visualization of human islet cells
T-SNE visualization of human islets including both non-diabetic and T2D (A), human non-diabetic only (B) or T2D islets (C) based on metaVIPER-inferred transcriptional regulator activity profiles. (D) Single cells from individual donors were projected onto 2-D t-SNE space based on metaVIPER-inferred transcriptional regulator activity profiles.



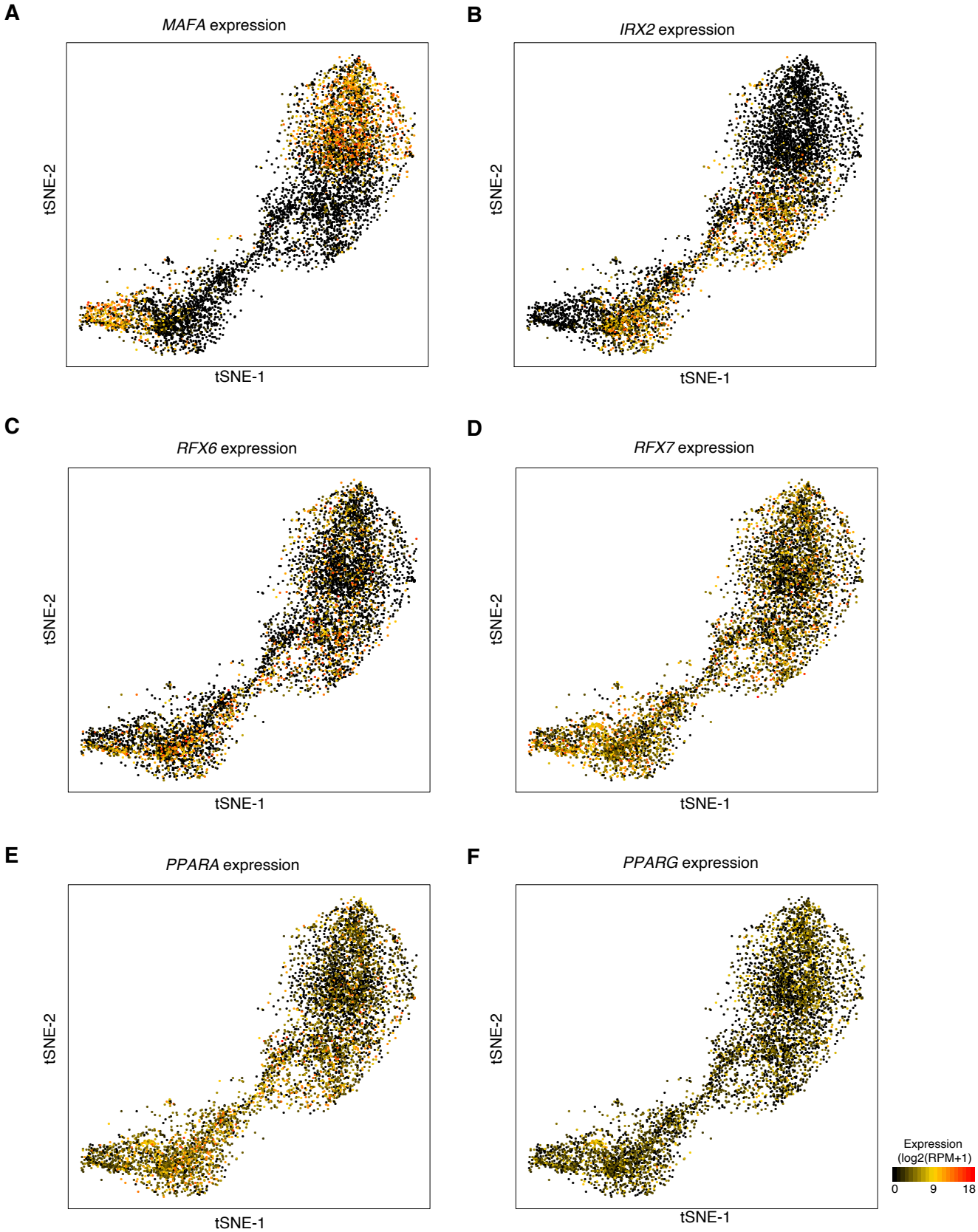
Supplemental Figure 2. iterClust clustering analysis on ND and T2D islet cells.
(A-C) iterClust analyses performed using ND and T2D islet cells. For illustration purpose, sub-clusters were projected onto 2D t-SNE space according to metaVIPER inference as ND and T2D combined (A), or as ND only (B) and T2D only (C).

Supplemental Figure 3

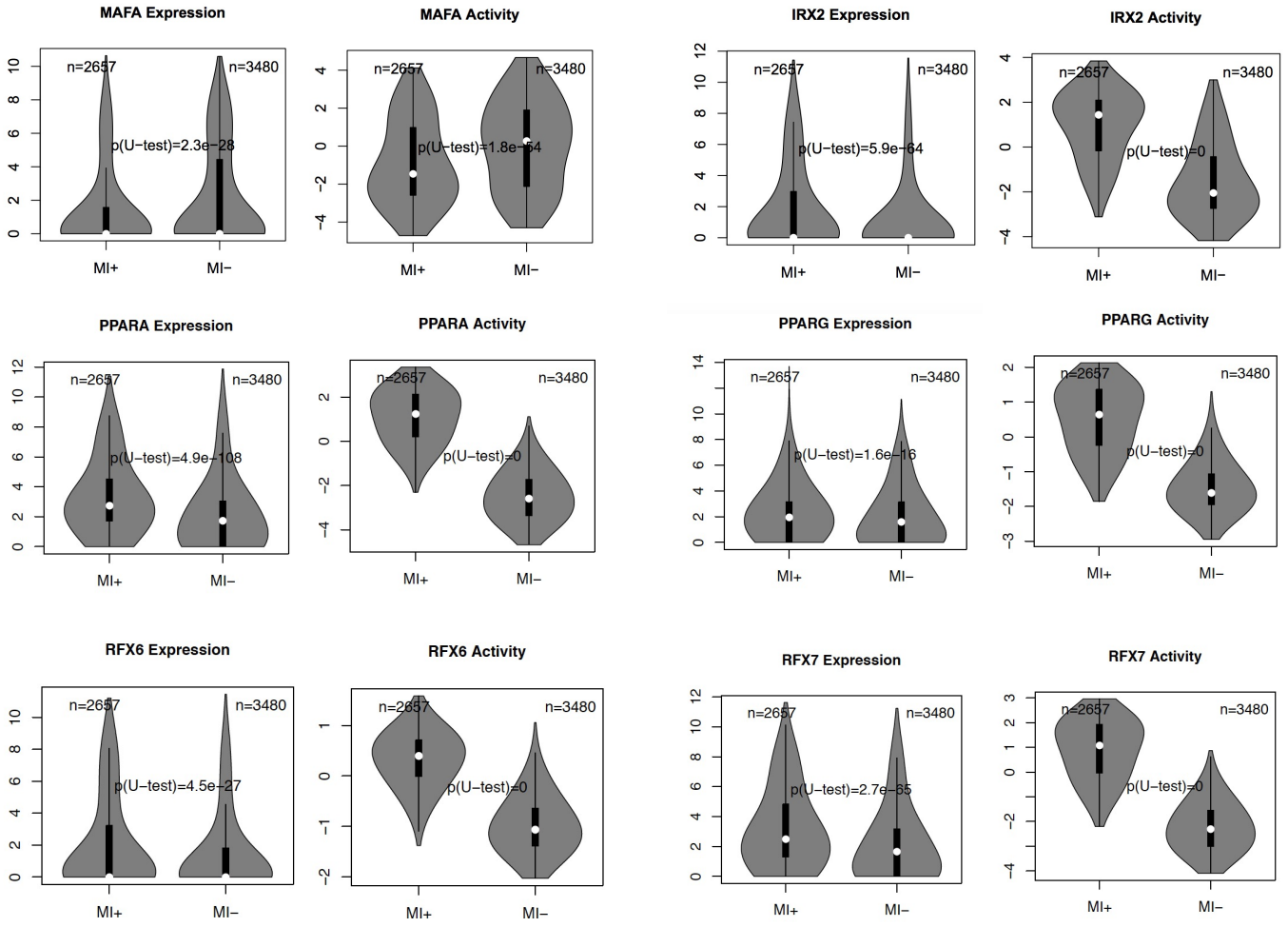


Supplemental Figure 3. MetaVIPER-inferred activity of metabolic-inflexibility/stress-response and stem-like cell markers.

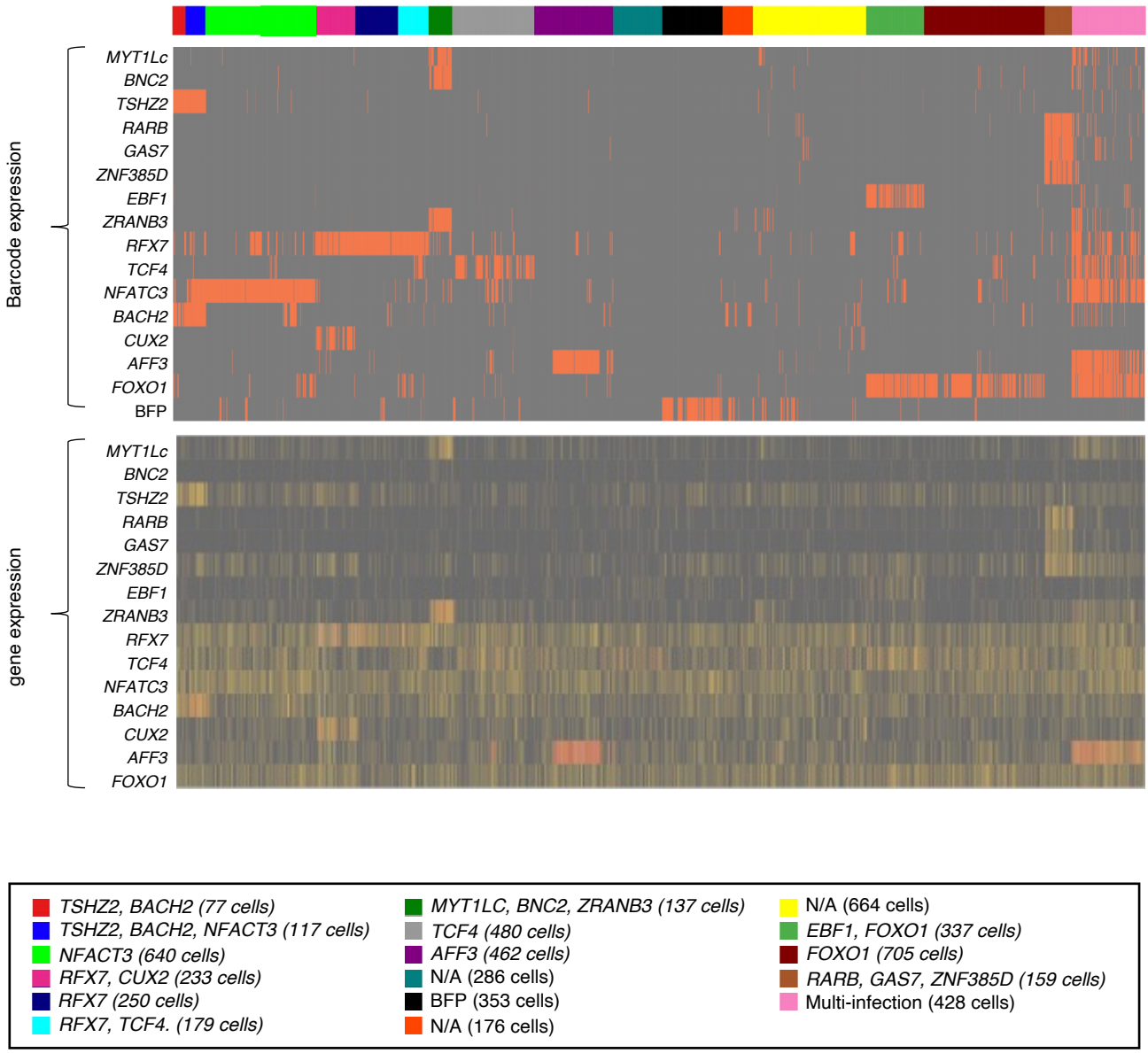
(A) MetaVIPER-inferred activities of metabolic-inflexibility regulators were color-coded on t-SNE plots. (B) MetaVIPER-inferred activities of metabolic stress-related transcriptional regulators, including hypoxic stress-related HIF1A, oxidative stress-related TP53, JNK family, and HSF1 were color-coded on t-SNE plots. (C, D) MetaVIPER-inferred activities of endocrine progenitor markers (C) and stemness markers (D) were color-coded on t-SNE plots.



Supplemental Figure 4. Expression of endocrine, metabolic inflexibility/progenitor markers. The expression levels of MAFA (A), IRX2 (B), RFX6 (C), RFX7 (D), PPARa (E) or PPARg (F) were color-coded on t-SNE plots.



Supplemental Figure 5. Violin plots showing the distribution of cells in MI+ and MI- according to MAFA, IRX2, PPARA, PPARG, RFX6 and RFX7 gene expression or protein activity.

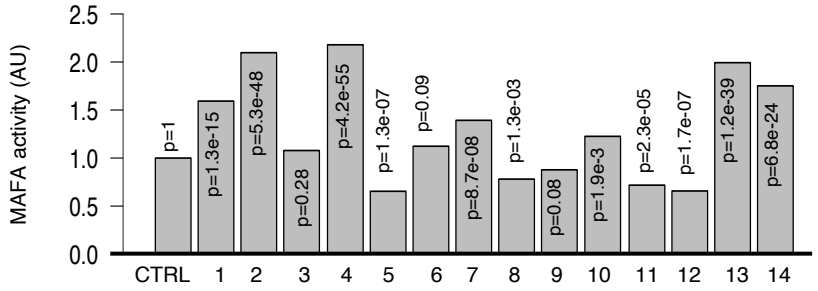


Supplemental Figure 6. Barcode abundance heatmap to determine cell identity after scGOF-seq.

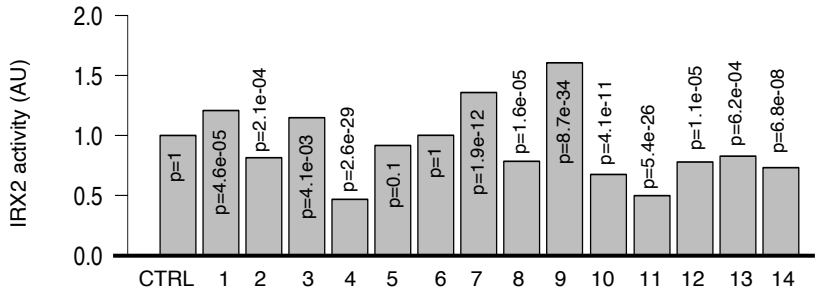
Cell annotation of scGOF-seq is presented for each group, color-coded for single candidate or combinatorial candidate transduction.

Supplemental Figure 7

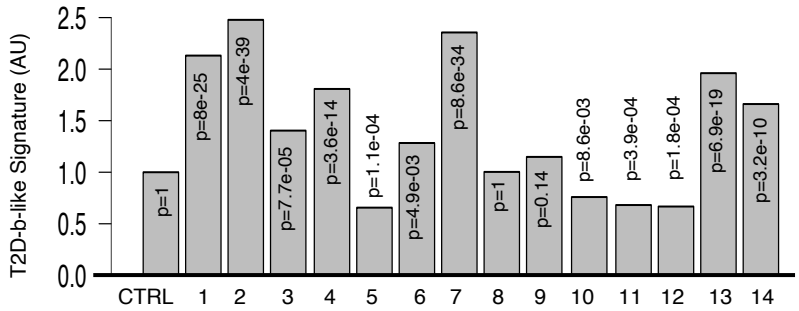
A



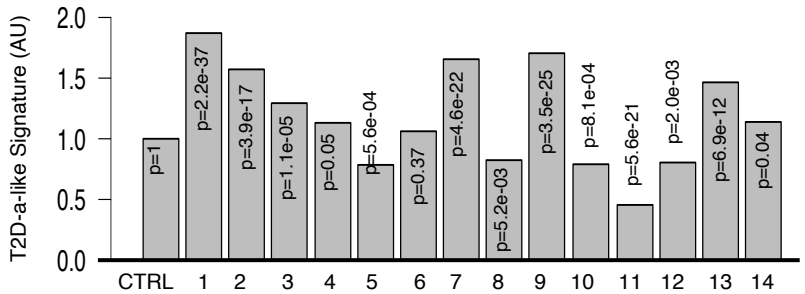
B



C



D

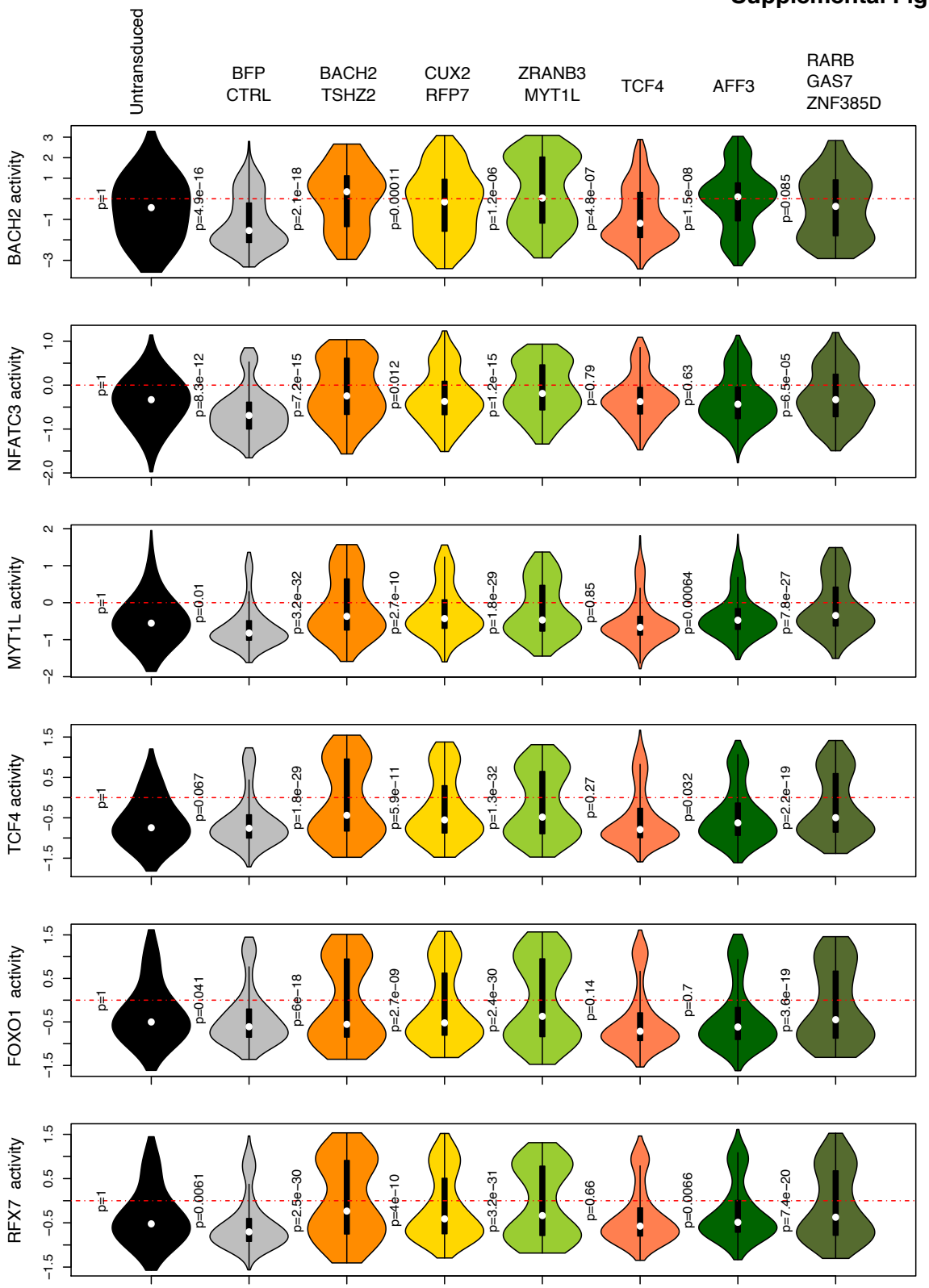


1. TSHZ2, BACH2	*6. RFX7, TCF4*	*11. EBF1, FOXO1*
2. TSHZ2, BACH2, NFATC3	*7. MYT1LC, BNC2, ZRANB3*	*12. FOXO1*
3. NFATC3	*8. TCF4*	*13. RARB, GAS7, ZNF385D*
4. RFX7, CUX2	*9. AFF3*	14. Multi-infection
5.RFX7	10. BFP	

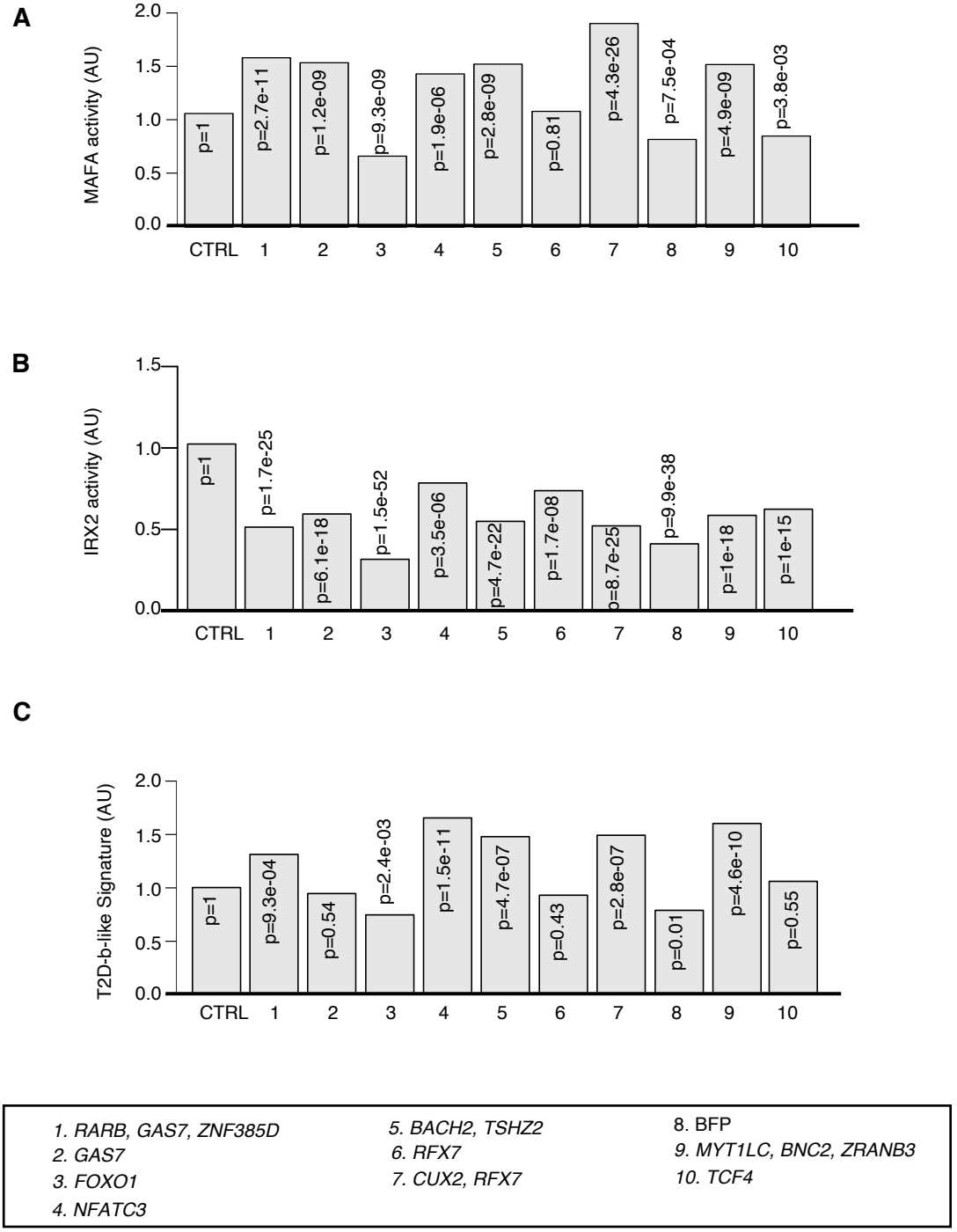
Supplemental Figure 7. scGOF-seq analyses using ND islets.

(A) Bar-plots showing the normalized proportion of islet cells with positive MAFA activity in each condition. (B) Same as (A) but for IRX2 activity. (C) Bar-plots showing the normalized proportion of islet cells with a positive T2D-b-like signature (> activity 0) in each condition. (D) Bar-plots showing the normalized proportion of islet cells with a positive T2D-a-like signature (> activity 0) in each condition.

Supplemental Figure 8

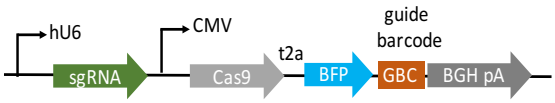


Supplemental Figure 8. Core driver network of ND cell conversion into T2D-β-like signature cells. Violin plots showing the distribution of cells following transduction with each individual candidate or combination thereof analyzed according to core driver network activity, BACH2, NFATC3, MYT1L, TCF4, FOXO1 and RFX7. Non-transduced and BFP-transduced ND islets serve as negative controls.

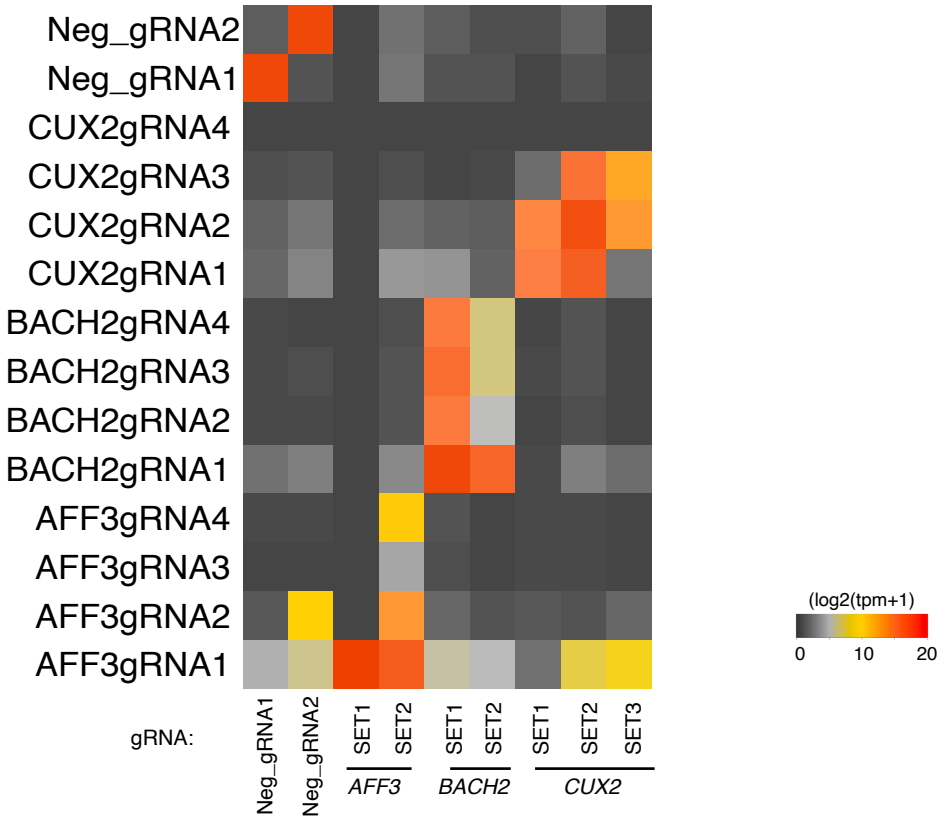


Supplemental Figure 9. Biological scGOF-seq replicate using ND islets (ND6).
(A) Bar-plots showing the normalized proportion of islet cells with positive MAFA activity in each gain-of-function condition. (B) Same as (A) but for IRX2 activity. (C) Bar-plots showing the normalized proportion of islet cells with a positive T2D–β-like signature (> activity 0) in each condition.

A



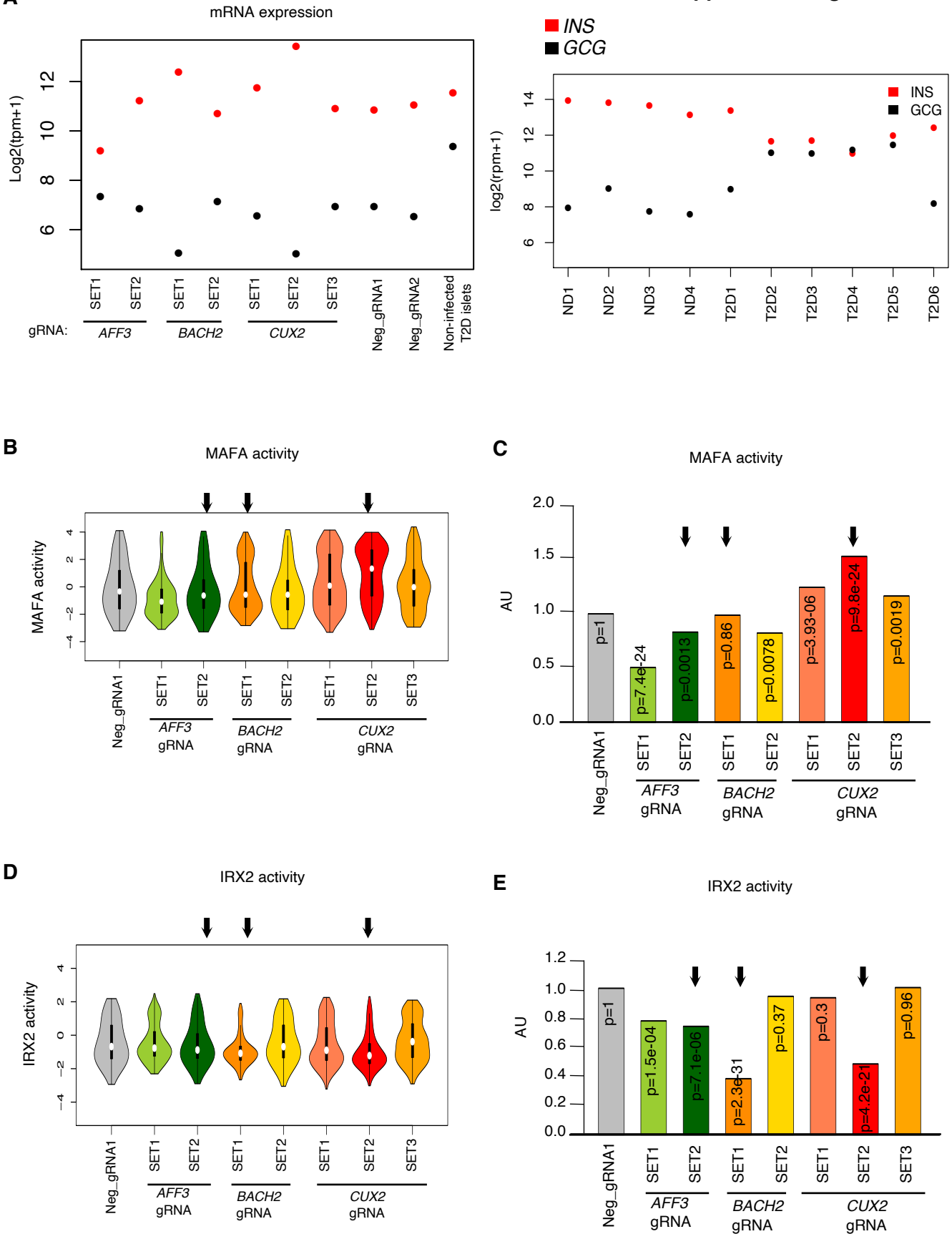
B



Supplemental Figure 10. Heatmap of barcode abundances to determine cell identity after Perturb-seq.

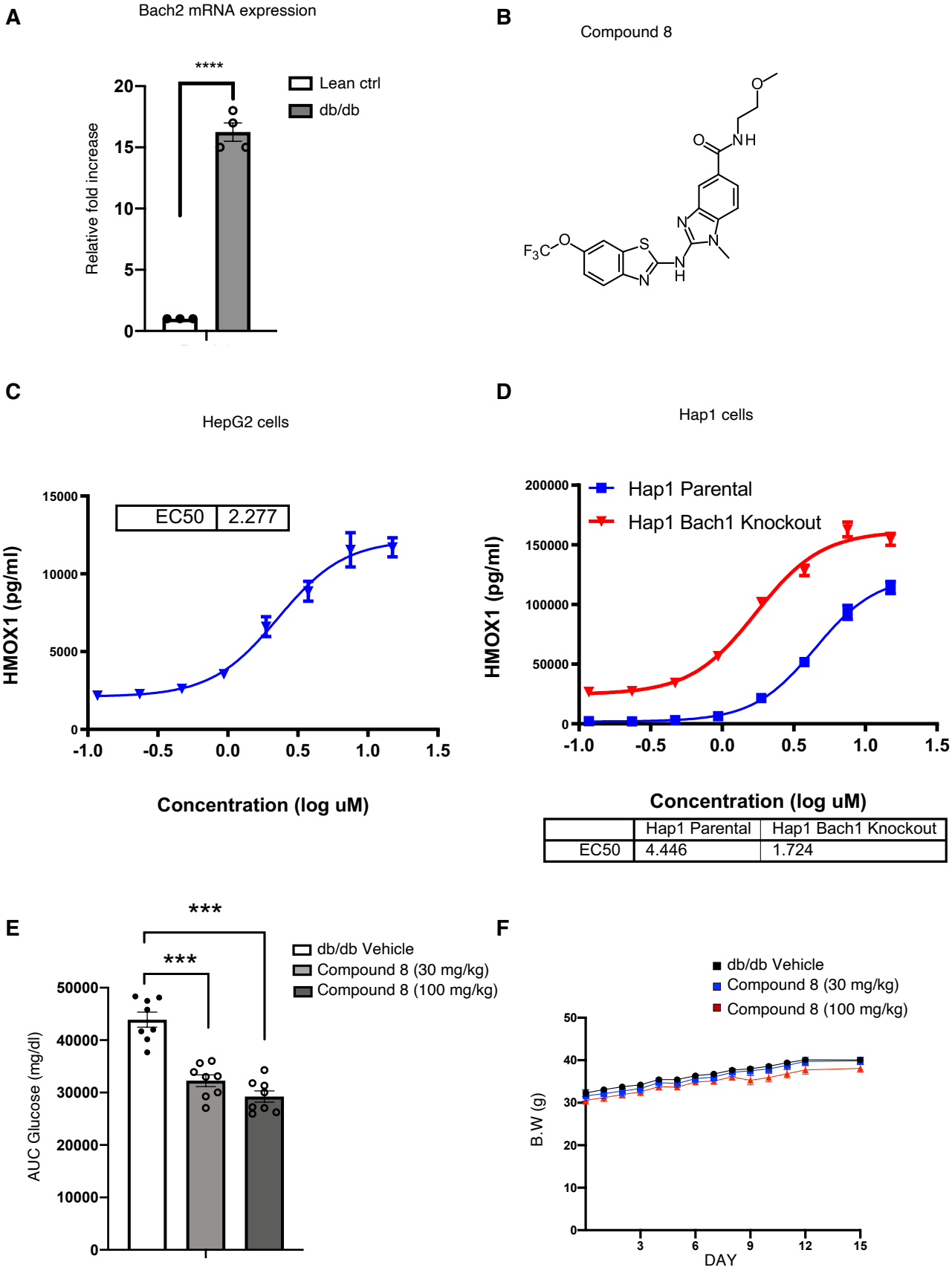
(A) Schematic drawing of modified Perturb-seq plasmids. (B) Heatmap showing the abundance of Perturb-Seq BCs. Cells were ordered according to dendrogram, which was generated based on the abundance of BCs. Clusters were determined by iterClust iterative clustering based on the dendrogram.

Supplemental Figure 11



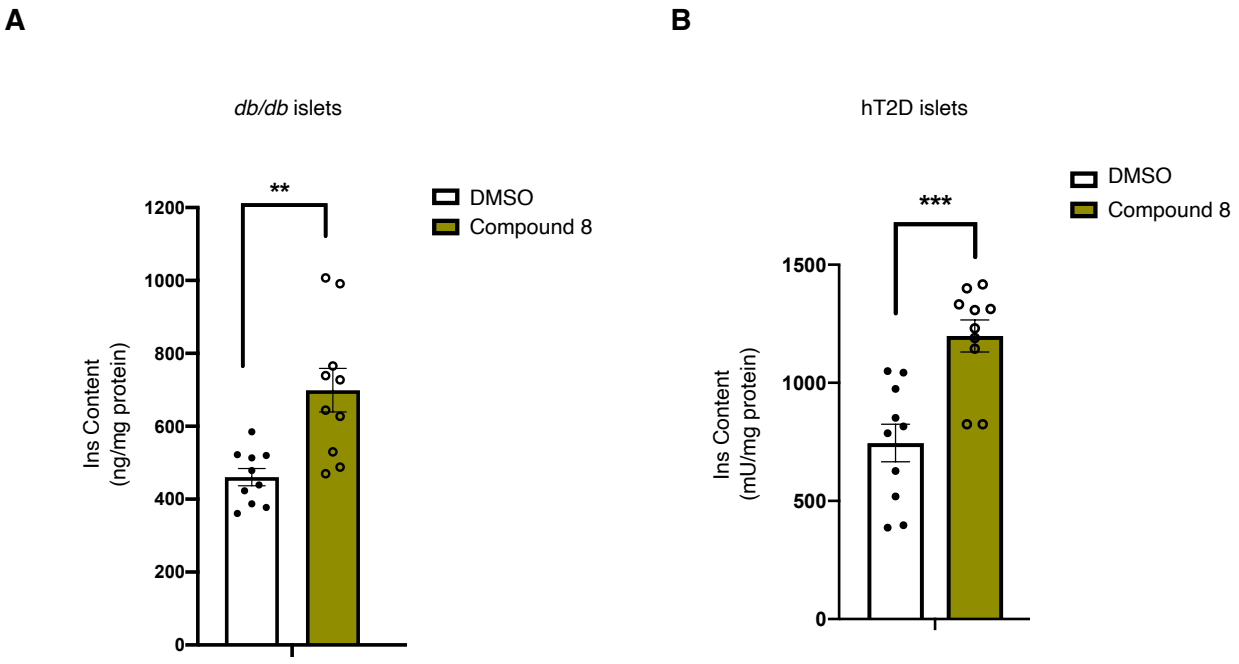
Supplemental Figure 11. Perturb-seq analyses using T2D islets.
(A) *INS* and *GCG* mRNA expression in each group of Perturb-Seq analyses (LEFT) or in each donor (RIGHT). Non-transduced and Neg_RNA-transduced T2D islets serve as negative controls. (B) Violin plots showing the distribution of cells with MAFA activity in each gRNA set condition. Non-transduced and Neg_RNA-transduced T2D islets serve as negative controls. (C) Bar-plots showing the normalized proportion of islet cells with positive MAFA activity in each condition. (D) Violin plots showing the distribution of cells with IRX2 activity as in (B). (E) Bar-plots showing the normalized proportion of islet cells with positive IRX2 activity in each condition.

Supplemental Figure 12

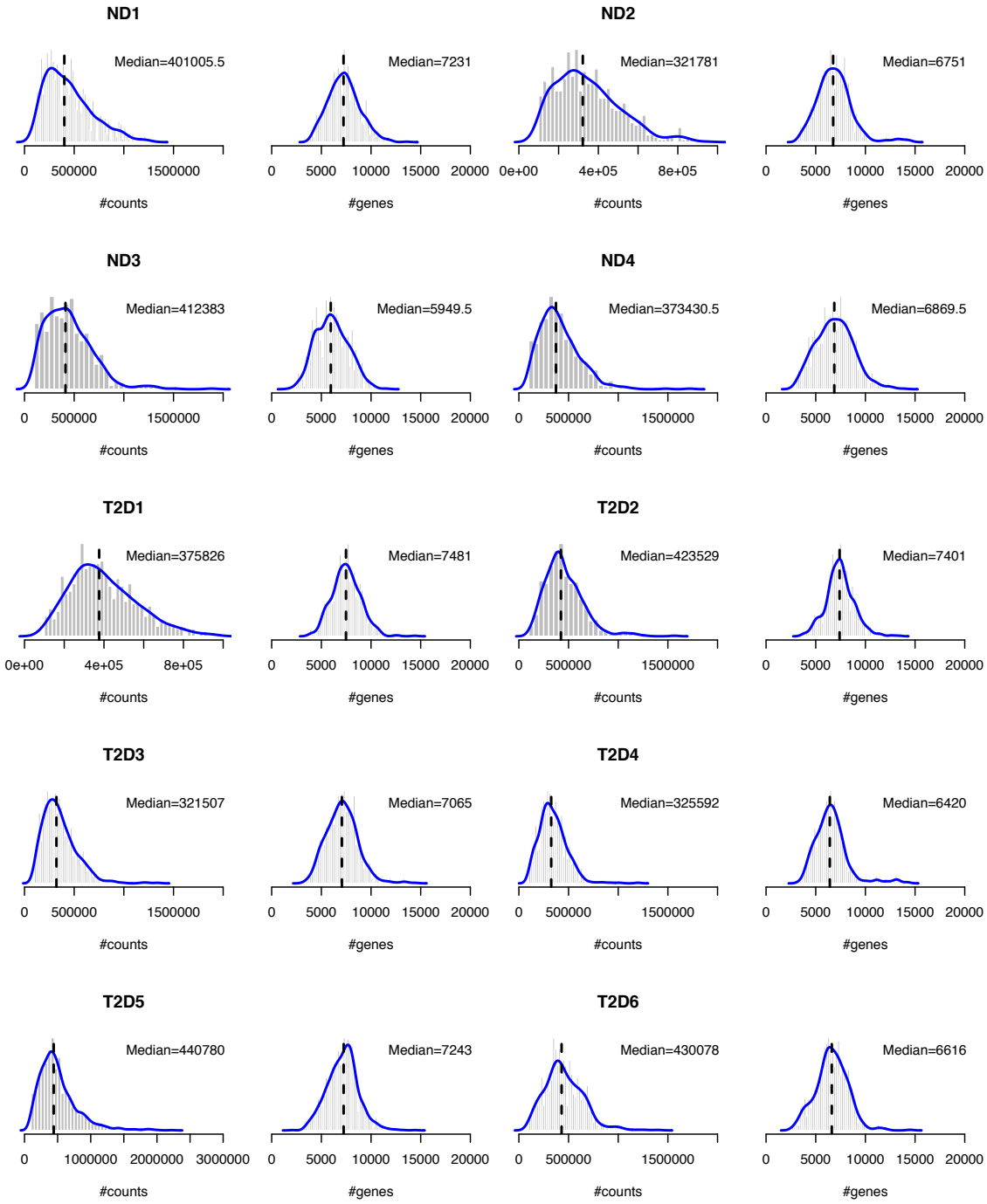


Supplemental Figure 12. Compound 8 inhibits BACH1/2 cellular activity

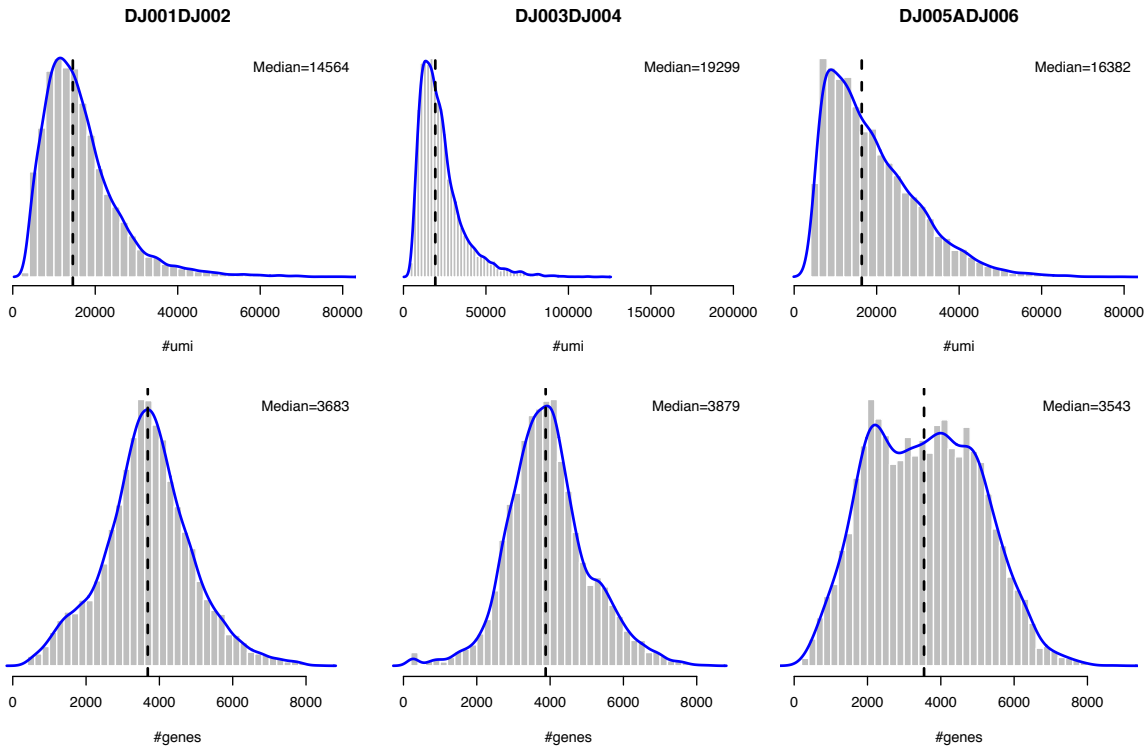
(A) Bach2 mRNA expression in sorted beta-cells from lean control or db/db mice. ANOVA was performed between the two group (n=3 per each group). (B) The structure of compound 8. (C) Compound 8 activity measured by Hmox1 ELISA assay in HepG2 cells, which express Bach1, but no Bach2. (D) Compound 8 activity measured in Bach1 knockout Hap1 cells, which then only express Bach2. (E) Quantification of areas under the curve for the OGTT experiments in db/db mice with compound 8 or vehicle control. All data are expressed as means ± SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Dunnett's method was performed between the two groups (n=8 mice per each group). (F) Body Weight of db/db mice for compound 8 or vehicle treatment.



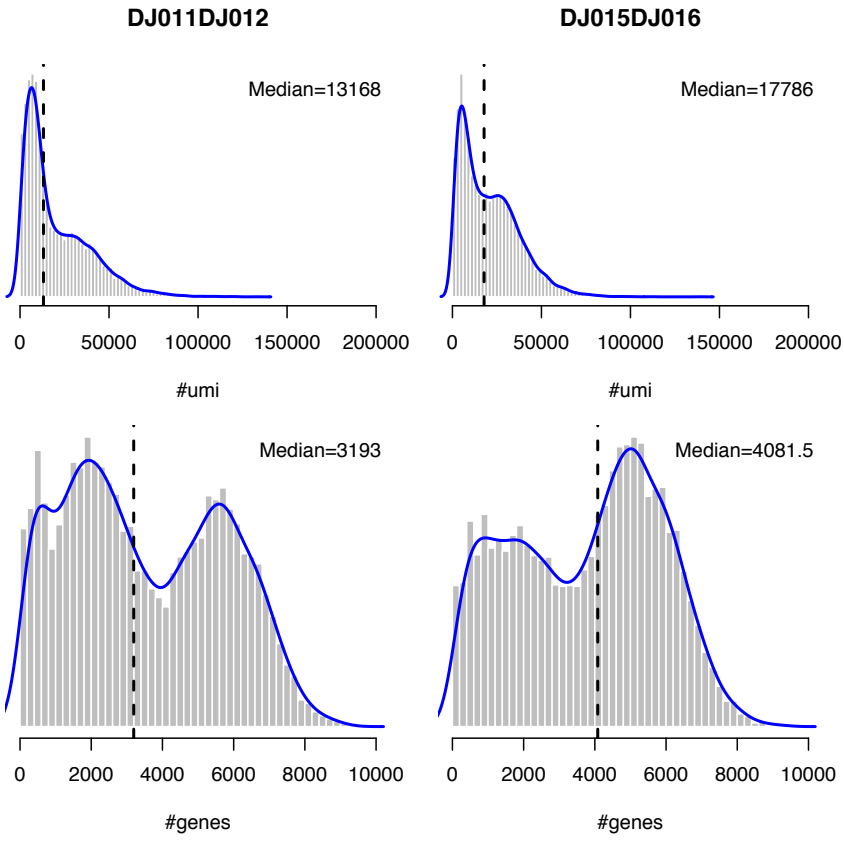
Supplemental Figure 13. Compound 8 increases insulin content.
(A) Insulin Content in in islets isolated from db/db mice following 24 hrs treatment with compound 8. ANOVA was performed between the two group (n=10 per each group). (B) Insulin secretion as in (B) but in hT2D islets.



Supplemental Figure 14. Quality control of scRNA-Seq datasets. Distributions of sequencing depth (#UMIs/counts per cell) and transcriptome complexity (#genes per cell) for each sample were presented.



Supplemental Figure 15. Quality control of scGEF-Seq datasets. Distributions of sequencing depth (#UMIs/counts per cell) and transcriptome complexity (#genes per cell) for each sample were presented.



Supplemental Figure 16. Quality control of scGEF-Seq datasets. Distributions of sequencing depth (#UMIs/counts per cell) and transcriptome complexity (#genes per cell) for each sample were presented.

Supplemental Tables.

Supplemental Table 1. Donor information

Donor	Sex	Age	BMI	HbA_{1C} (%)	Treatment	Duration (Yrs)
ND1	M	25	26	N/A		
ND2	F	51	20	N/A		
ND3	F	53	34	5.3		
ND4	F	36	24	5.0		
T2D1	M	59	32	9.6	No Tx	2
T2D2	M	58	39	8.9	Metformin	8
T2D3	M	51	25	6.9	N/A	upon admissoion
T2D4	F	42	28	6.7	Insulin	6
T2D5	M	48	44	6.6	N/A	upon admissoion
T2D6	M	59	33	6.6	Metformin	2

Supplemental Table 1. Human islet donor information with age, sex, BMI, HbA1C and medical history.

Supplemental Table 2. List of factors included in each IterClust parameter

Parameter	Genes
(*a*) hormone *mRNA expression*	*INS*, *GCG, PPY* and *SST*
(*b*) *Activity* of TFs characteristic of either b- or a-cell identity	MAFA, PDX1, NKX2.2, NEUROD1 IRX2, ARX, GLI3, ITGB8, F10, SPOCK3, CLU
(*c*) *Activity* of metabolic-inflexibility/stress-response drivers	PPARa/g, RB1, FOXO1, FOXM1
(*d*) *Activity* of TFs of endocrine progenitors and stem-like cells	POU5F1, MYCL, NANOG

Supplemental Table 3. scGOF-seq barcode dictionary

Gene	Guide Barcode
AFF3	GATTCTGCCACTACTTCG
BACH2	AAGTAGCGCCTAGACGCA
BNC2	TAGAACATCAATCCGGTT
CUX2	TTGCACCGGAAAGTCTGC
EBF1	TACGTGTCCGTATGACAT
FOXO1	CGCAAGTGTAGCATCAGA
GAS7	CTGACCAACCGCAGAAGT
MYT1L	AGGACCACTGGACATCCA
NFATC3	GTTGAATTGTGGAGTTAT
RARB	TAATCCGTACAGGTGTCA
RFX7	GAGTGCTTAATGTACCCA
TCF4	TGACACGTATTTCGGAGG
TSHZ2	AAATGACCAACTTGACGT
ZNF385D	AGCTAGGCCATTTGTATC
ZRANB3	CTAAACTCATAACATAGA
tagBFP	CTCCGGTTGCAGAGGCTA

Supplemental Table 3. List of TFs tested in scGOF-seq experiments and corresponding guide barcode for cell annotation.

The TFs selected for analysis included: AFF3 [5], BACH2 [6], BNC2 [7], GAS7 [8], MYT1L [9], NFATC3 [10], RFX7 [11], TSHZ2 [12], ZRANB3 [13], and ZNF385D [14].

Supplemental Table 4. Donor information used for functional studies

Donor	Sex	Age	BMI	HbA_{1C} (%)	Treatment	Duration (Yrs)	Experiment
ND5	F	49	33	5.9			GOF-seq
ND6	M	25	31	5			GOF-seq
T2D7	F	66	29	7.2	Metformin	6	Perturb-seq
ND7	F	50	23	5.6			scCalcium Image
ND8	F	25	34	5.1			scCalcium Image
T2D8	F	62	29	7.6	Oral medicine	5	in vitro GSIS_Compound8
T2D9	F	38	44	7.0	N/A	Unkown	in vitro GSIS_Compound8

Supplemental Table 4. Human islet donor information with age, sex, BMI, HbA1C, medical history and functional studies used for.

Supplemental Table 5. Perturb-seq Barcode dictionary

Gene	gRNA	Barcode
AFF3 gRNA1	GAGGAATGACTCTCTAGTTG	ACTCTGAACATACCCCGT
AFF3 gRNA2	CAACTAGAGAGTCATTCCTC	GATACAGCCAGCCGGTTG
AFF3 gRNA3	GTGAAGACATCTTAAACCAG	CCTGCCAATTGCAGATTA
AFF3 gRNA4	AGTCATCAGCCAGCAGATGC	GCGAATAGTAAGAACCTC
BACH2 gRNA1	ACGTGACTTTGATCGTGGAG	GTACGAGGAATTGATGCT
BACH2 gRNA2	CAGCAGAGAAAACATCCGCG	GCTTCTCACATCGACAAT
BACH2 gRNA3	AGTTCTCGCAGTCCTCGTGT	CAGCTATGCCTGGTTGCC
BACH2 gRNA4	AATTATGGACAGCCCCACGT	AATCACACGCGGGCGTTG
CUX2 gRNA1	GCTGCGGCGGAAGTACGACG	GTGGAATAATAACATAAT
CUX2 gRNA2	GCGGCGGAAGTACGACGAGG	ACGACCCGCGGGGTACCA
CUX2 gRNA3	ACGAAGTGTGGAGGTCTCGC	TCAGTGGGACTAGTTGAA
CUX2 gRNA4	GGCGAGACCTCCACACTTCG	GAATAACACTGTCGGCGC
NEG CTRL 1	GGTCCATGGGTGGAGTTACG	CTGTGACATCCTGATAAG
NEG CTRL 2	GGACGCTAAACCAACGGTGC	CGGCATGTTCGTATAAGG

Supplemental Table 5. List of TFs tested in Perturb-seq experiments and corresponding gRNA and guided barcode for cell annotation.

Supplemental Table 6	**Clinical Characteristics of ND and T2D individuals**						
ND group							
Case ID	Gender (F/M)	Age (years)	BMI (kg/m²)	FBG (mmol/L)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)
ND936	F	51	21.09	6.06	2.41%	1.63%	16.67%
ND02740	M	58	24.62	5.12	4.46%	39.92%	37.04%
ND09714	M	64	23.03	4.58	2.22%	17.65%	11.11%
ND10095	M	77	21.56	5.5	3.37%	31.07%	45.45%
ND05742	M	31	20.15	4.35	3.47%	16.46%	33.33%
ND05933	F	30	16.27	4.25	6.63%	24.61%	16.67%
ND06658	F	21	18.25	4.39	7.28%	25.68%	37.50%
ND08138	F	68	25.24	4.81	11.11%	54.55%	0.00%
ND963	M	56	20.66	5.25	3.35%	30.36%	0.00%
Mean SEM		50.67 6.40	21.21 0.96	4.92 0.20	4.92 0.97	26.88 5.01	21.97 5.64
T2D group							
Case ID	Gender (F/M)	Age (years)	BMI (kg/m²)	FBG (mmol/L)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)	BACH2⁺ INS⁺-GCG⁻ / INS⁺-GCG⁻ cells (%)
T2D05941	F	54	20.82	4.60	13.98%	72.40%	75.00%
T2D04918	F	49	26.22	5.79	10.34%	47.52%	50.00%
T2D05501	F	58	19.92	6.82	54.05%	80.23%	100.00%
T2D06554	F	69	18.83	5.0	18.06%	83.33%	77.78%
T2D05314	F	46	24.24	5.80	15.65%	81.35%	80.65%
T2D10569	M	59	26.22	4.60	16.07%	82.95%	75.76%
T2D03547	M	71	26.04	5.46	23.16%	83.85%	90.91%
T2D719544	M	63	23.42	9.51	10.81%	85.37%	85.71%
T2D721777	F	69	21.26	5.78	16.59%	73.53%	85.00%
Mean SEM		59.78 3.00	23.00 0.96	5.93 0.50	19.86 4.46	76.73 3.95	80.09 4.60

Supplemental Table 6. All clinical characteristics of human subjects used for immunostaining are summarized.

Supplemental Movie 1. 3D plot showing integrated β-cell factor, α-cell factor, and stemness activity on the X, Y and Z axis, respectively at the single cell level.

Supplemental Movie 2. 3D plot showing integrated β-cell factor, α-cell factor, and stemness activity on the X, Y and Z axis, respectively based on the average cell behaviors of each cluster.

Supplemental Data 1. (A) List of differentially activated master regulators between Cluster MI$_{-1}$ (healthiest β-cells) vs. MI+^{2} (T2D-β-like cells), or MI+^{5} (healthiest α-cells) vs. MI+^{2} (T2D-β-like cells). (B) List of differentially activated master regulators between Cluster MI$_{-1}$ (healthiest β-cells) vs. MI$_{-5}$ (T2D-α-like cells) , or MI+^{5} (healthiest α-cells) vs. MI$_{-5}$ (T2D-α-like cells).