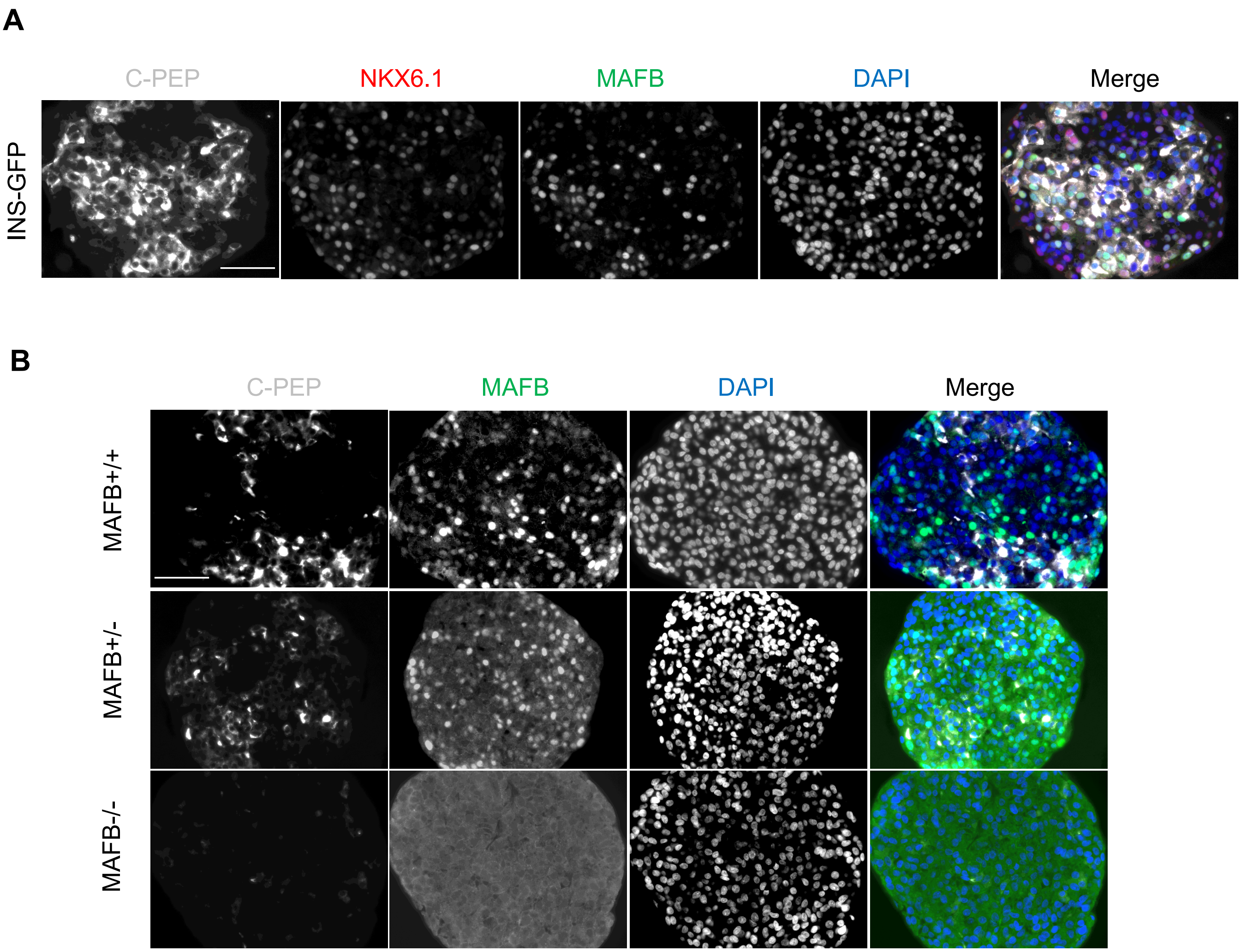
Supplementary Information

Loss of the transcription factor MAFB limits β-cell derivation from human PSCs

Russell et al.

Supplementary Figure 1

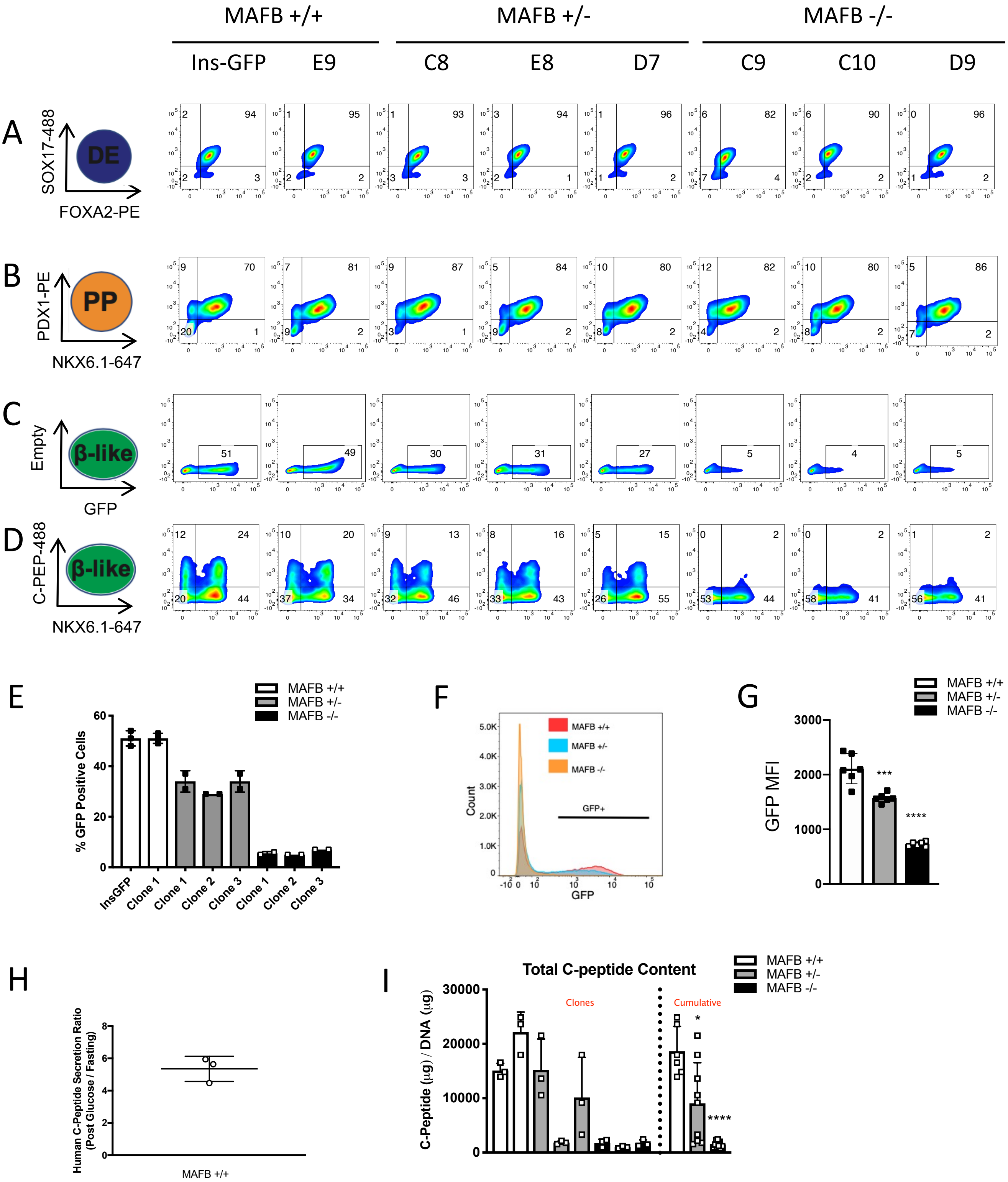


Supplementary Figure 1. MAFB is expressed in hESC derived β-cells.

(A) Representative IF images from 4 independent experiments of INS-GFP cells at the β-cell stage depicting C-PEP, NKX6.1, MAFB and DAPI. Scale bars, 50 μm.

(B) Representative IF images from 3 independent experiments from the β-cell stage of differentiation depicting C-PEP and MAFB expression in MAFB +/+, +/- and -/- cells. DAPI indicates nuclear staining. Scale bars, 50 μm.

Supplementary Figure 2



Supplementary Figure 2. Loss of MAFB inhibits β-cell derivation.

(A) Representative FC plots depicting the percentages of SOX17+ FOXA2+ at the DE stage for individual clones.

(B) Representative FC plots depicting the percentages of PDX1+ NKX6.1+ at the PP stage for individual clones.

(C) Representative FC plots depicting the percentages of GFP+ cells at the β-cell stage for individual clones.

(D) Representative FC plots depicting the percentages of C-PEP+ NKX6.1+ cells at the β-cell stage for individual clones.

(E) Quantification of GFP+ cells for individual clones the β-cell stage.

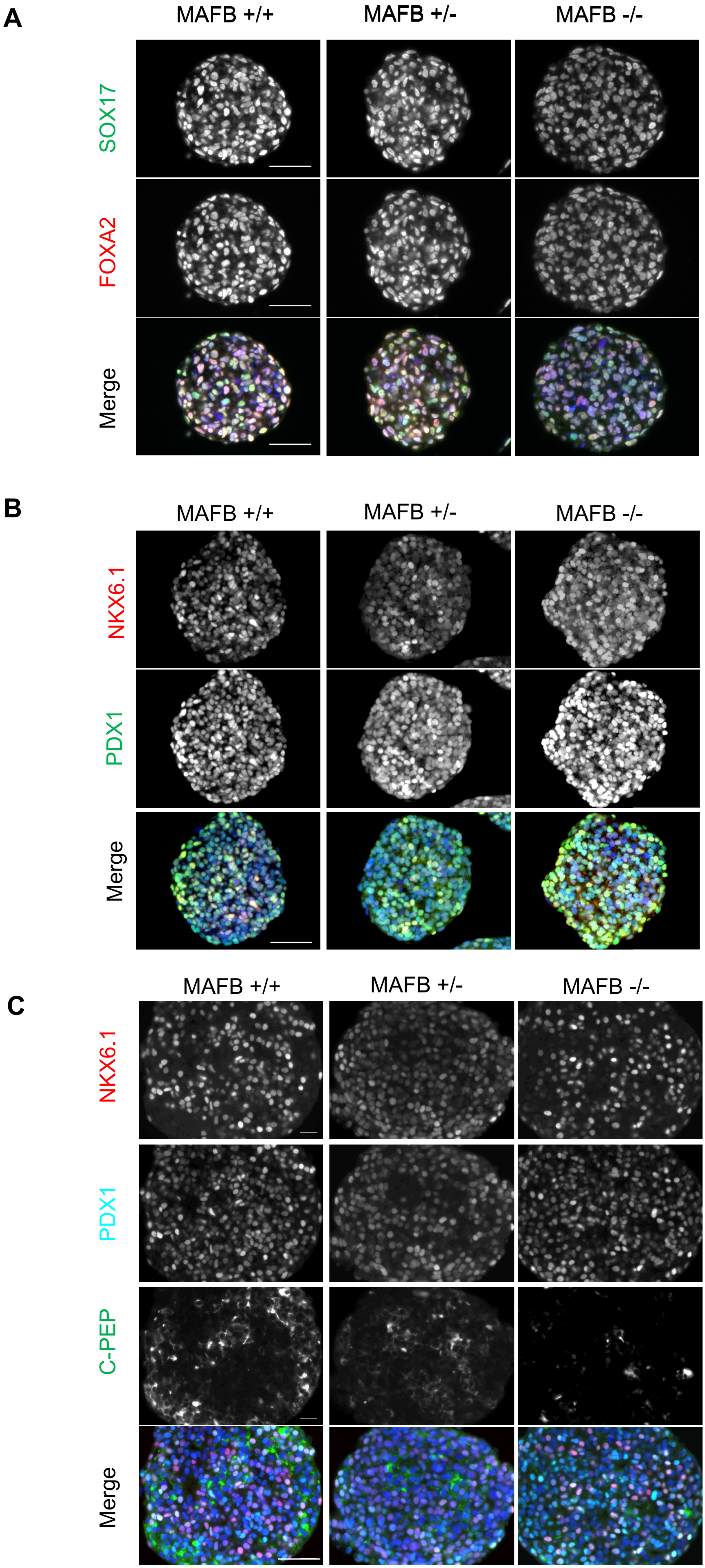
(F) Representative histograms and (G) Mean fluorescence intensity (MFI) of the GFP positive population in MAFB +/+, +/- and -/- cells. P values by one-way ANOVA followed by Dunnett's multiple comparisons test were ***P <0.0004 ****P < 0.0001. N = 6 independent experiments.

(H) Stimulation index of MAFB +/+ cells at 5 weeks post-transplantation. N = 3 independent mice.

(I) Total C-PEP content from individual clones at the β-like cell stage. N = 6 independent experiments.

P values by one-way ANOVA followed by Dunnett's multiple comparisons test were *P < 0.05, **P < 0.01, ***P <0.001, ****P < 0.0001. Data are presented as individual biological replicates and represent the mean ± SD.

Supplementary Figure 3



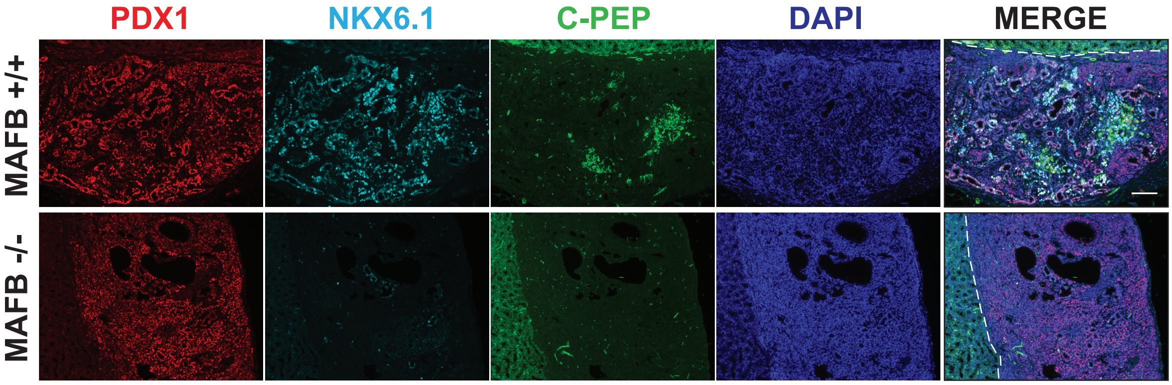
Supplementary Figure 3. MAFB is important for β-cell derivation.

(A) Representative images from 6 independent experiments at the DE stage of differentiation depicting SOX17 and FOXA2 staining. Scale bars, 50 μm.

(B) Representative images from 6 independent experiments at the PP stage of differentiation depicting PDX1 and NKX6.1 staining. Scale bars, 50 μm.

(C) Representative images from 6 independent experiments at the β-like cell stage of differentiation depicting C-PEP co-staining with PDX1 and NKX6.1. Scale bars, 50 μm.

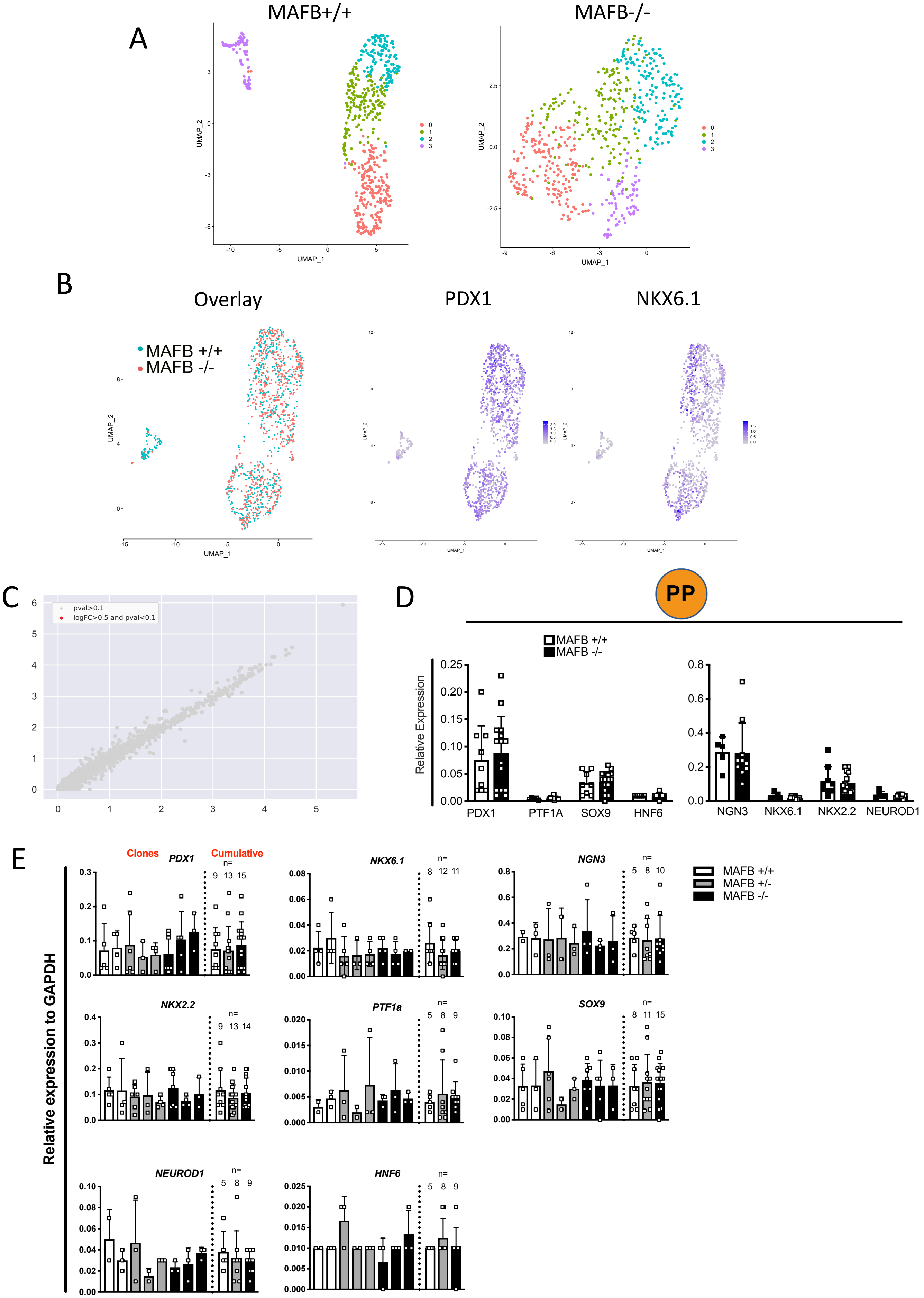
Supplementary Figure 4



Supplementary Figure 4. MAFB is required to generate functional β-cells *in vivo*.

Representative IF images from 6 independent animals (3 MAFB+/+ and 3 MAFB-/-) for C-PEP co-staining with PDX1 and NKX6.1 of grafts removed from kidney capsules 8 weeks post-transplantation. The graft is delineated and marked from the kidney using dashed lines. Scale bars, 100 μm.

Supplementary Figure 5



Supplementary Figure 5. scRNA sequencing reveals MAFB-/- generate Pancreatic Progentior cells in equivalent manner to MAFB+/+ cells.

(A) UMAP plot of MAFB +/+ and -/- cells at the PP stage of differentiation, color-coded by cell populations.

(B) UMAP projections of the integrated analysis of MAFB +/+ and -/- cells at the PP stage, color-coded by genotype (left) and normalized PDX1 and NKX6.1 expression (middle, right).

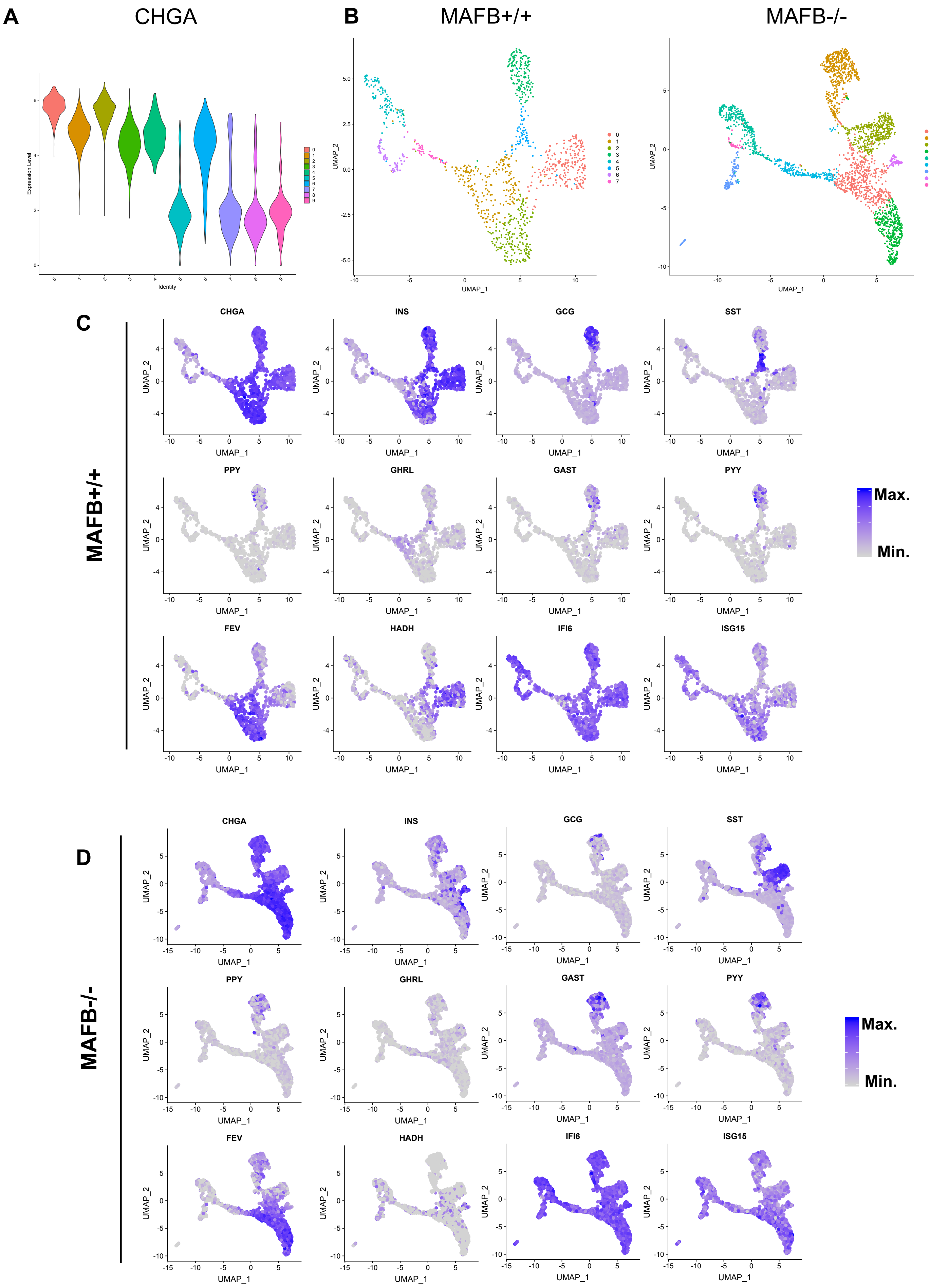
(C) Scatter plot showing the average gene expression (log scale) for MAFB +/+ and -/- cells at the PP cell stage. No genes are significantly differentially expressed (log fold change >0.5, adjusted p value < 0.1) between MAFB +/+ and -/- cells. P value calculated with MAST test.

(D) The relative mRNA expression patterns in MAFB +/+ and -/- cells of designated genes for pan-pancreatic (*PDX1, PTF1A, SOX9* and *HNF6*) or pro-endocrine (*NGN3, NKX6.1, NKX2.2* and *NEUROD1*) at the PP stage as measured by quantitative PCR analysis. Each data point represents an independent biological sample.

(E) The mRNA expression patterns of designated genes for individual clones for pancreatic (*PDX1, PTF1A, SOX9* and *HNF6*) or pro-endocrine genes (*NGN3, NKX6.1, NKX2.2* and *NEUROD1*) at the PP stage as measured by quantitative PCR analysis. Each data point represents an independent biological sample.

P values by unpaired two-tailed t-test were $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Data are presented as individual biological replicates and represent the mean ± SD.

Supplementary Figure 6



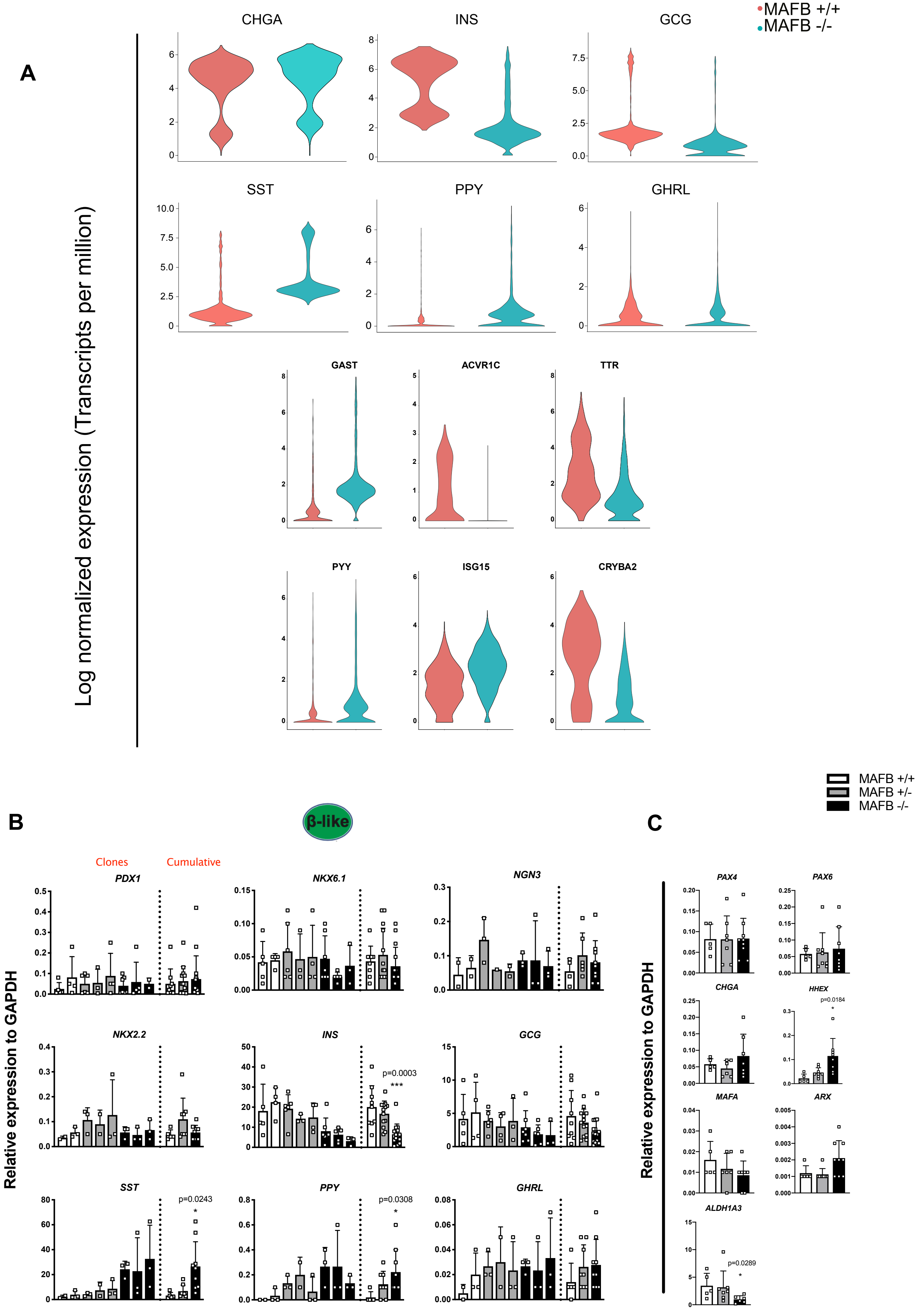
Supplementary Figure 6. scRNA sequencing reveals MAFB regulates endocrine cell lineage commitment.

(A) Violin plot of CHGA gene expression (normalized) in the merged MAFB +/+ and -/- cells from **Figure 4A**, color-coded by cell populations.

(B) UMAP plot of MAFB +/+ and -/- cells at the β-like cell stage of differentiation color-coded by cell identity.

(C,D) UMAP visualization of the log-transformed, normalized expression of selected genes at the β-like cell stage of differentiation in (C) MAFB+/+ and (D) MAFB-/- cells.

Supplementary Figure 7



Supplementary Figure 7. MAFB regulates endocrine hormone producing-cell lineage commitment.

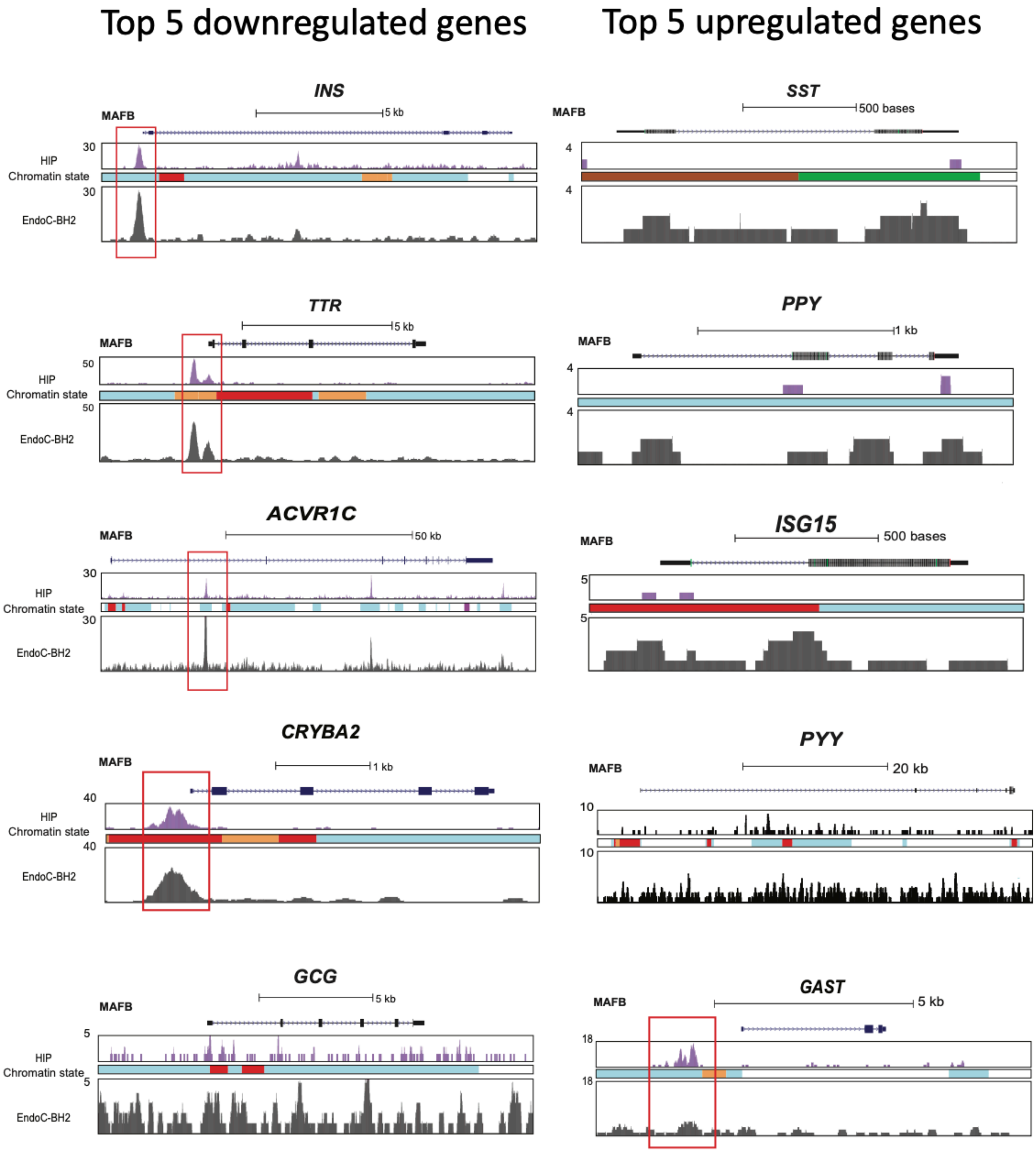
(A) Violin plot showing the expression of the top 5 genes and that are differentially expressed (Up and down regulated; fold change > 0.5, adjusted p value < 0.1) between MAFB +/+ and -/- cells in the β-like cell stage as well as the control endocrine genes *CHGA* and *GHRL*.

(B) The mRNA expression patterns of designated genes for individual clones for pro-endocrine (*NGN3*, *NKX6.1*, *NKX2.2* and *NEUROD1*) stages and islet hormones (*INS*, *GCG*, *SST*, *PPY* and *GHRL*) at the β-like cell stage as measured by quantitative PCR analysis. Each data point represents an independent biological sample.

(C) The mRNA expression patterns of genes implicated in endocrine cell fate (*PAX4*, *PAX6*, *HHEX*, *ARX*, *CHGA* and *MAFA*) as well as the β-cell de-differentiation marker *ALDH1A3* at the the β-like cell stage as measured by quantitative PCR analysis. Each data point represents an independent biological sample.

P values by unpaired two-tailed t-test were *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as individual biological replicates and represent the mean ± SD.

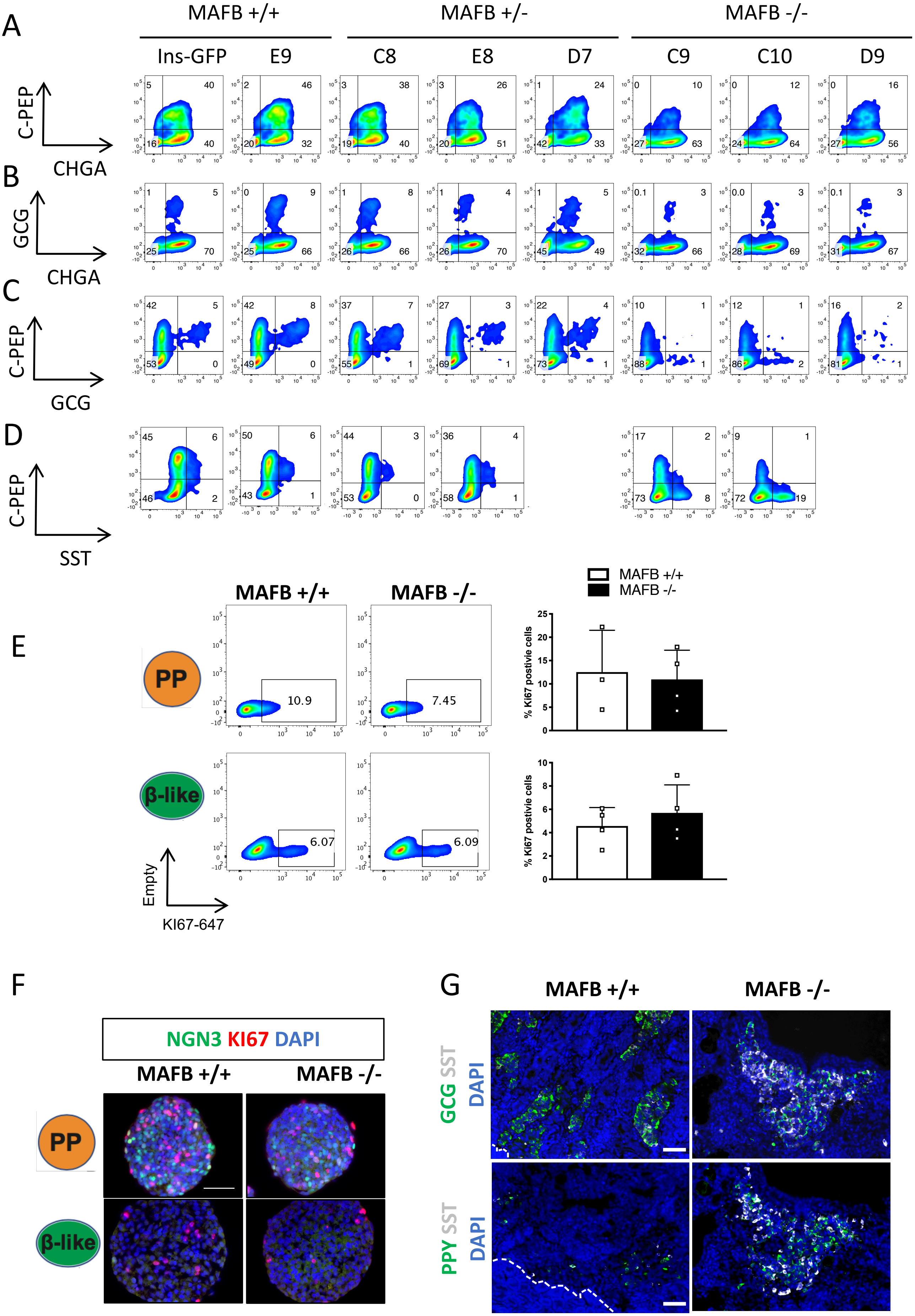
Supplementary Figure 8



Supplementary Figure 8. MAFB occupies α- and β-cell specific genes.

ChIP-seq binding profiles for MAFB at the indicated *gene loci* in Human Islet Pancreas [1,2] and EndoC-BH2 cells. Tracks are set to the same scale for HIP and EndoC-BH2 cells per gene and the lengths of genes and direction of transcription are indicated by blue arrows. The top 5 up- and down-regulated genes identified in our MAFB +/+ and -/- β-cell differentiations in **Figure 4B** are outlined.

Supplementary Figure 9



Supplementary Figure 9. MAFB regulates differentiation of islet-hormone producing cell types.

(A) Representative FC plots depicting the percentages of C-PEP+ CHGA+ cells for individual clones at the β-like cell stage.

(B) Representative FC plots depicting the percentages of CHGA+ GCG+ cells for individual clones at the β-like cell stage.

(C) Representative FC plots depicting the percentages of C-PEP+ GCG+ cells for individual clones at the β-like cell stage.

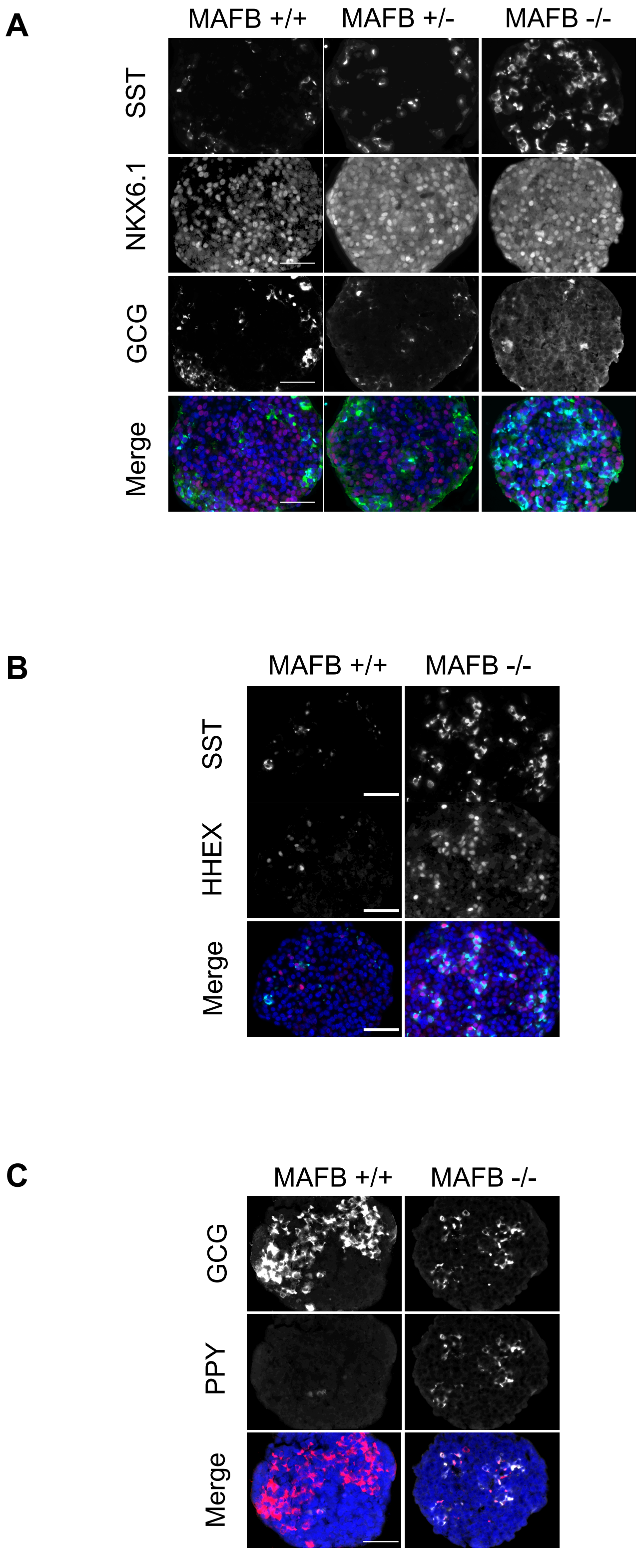
(D) Representative FC plots depicting the percentages of C-PEP+ SST+ cells for individual clones at the β-like cell stage.

(E) Representative FC plots depicting the percentages of KI67+ cells at the PP and β-cell stage and respective quantification (n=3).

(F) Representative IF images from 3 independent experiments of MAFB +/+ and -/- cells at the PP and β-cell stage depicting NGN3, KI-67 and DAPI. Scale bars, 50 μm.

(G) Representative IF images from 6 independent animals (3 MAFB+/+ and 3 MAFB-/-) for SST co-staining with GCG (upper panel) and PPY (lower panel) of grafts removed from kidney capsules 8 weeks post-transplantation of MAFB +/+ and -/- cells. The graft is delineated and marked from the kidney using dashed lines where indicated. Scale bars, 50 μm.

Supplementary Figure 10



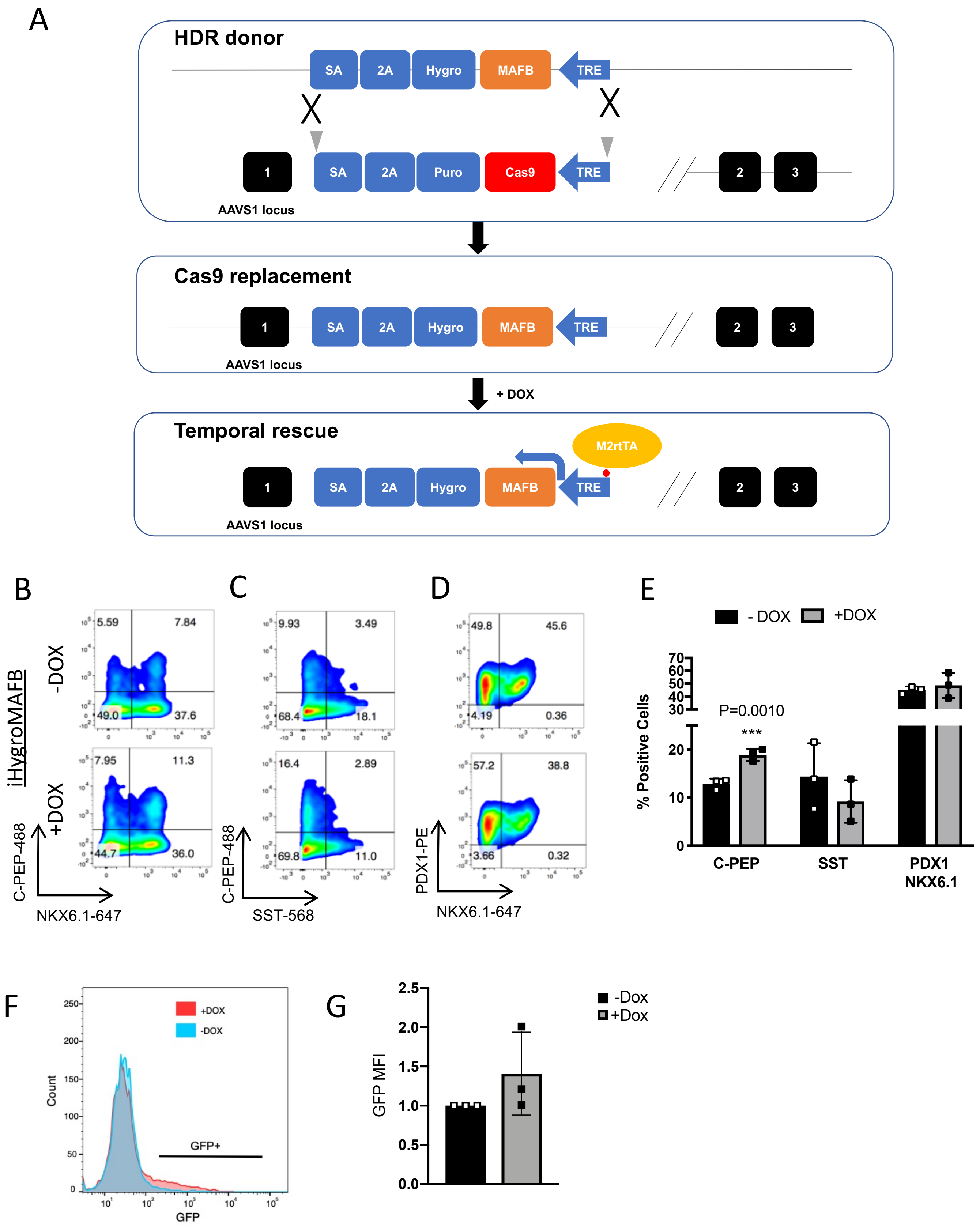
Supplementary Figure 10. MAFB directs Pancreatic Endocrine cell lineage specification.

(A) Representative IF images from 3 independent experiments the β-cell stage of differentiation depicting GCG, SST, NKX6.1 and DAPI. Scale bars, 50 μm.

(B) Representative IF images from 3 independent experiments the β-like cell stage of differentiation depicting SST and HHEX and DAPI. Scale bars, 50 μm.

(C) Representative IF images from 3 independent experiments the β-like cell stage of differentiation depicting PPY and GCG and DAPI. Scale bars, 50 μm.

Supplementary Figure 11



Supplementary Figure 11. Rescue of MAFB in MAFB-/- cells.

(A) Schematic outlining gene rescue and temporal control of *MAFB* expression in *MAFB-/-* hESCs.

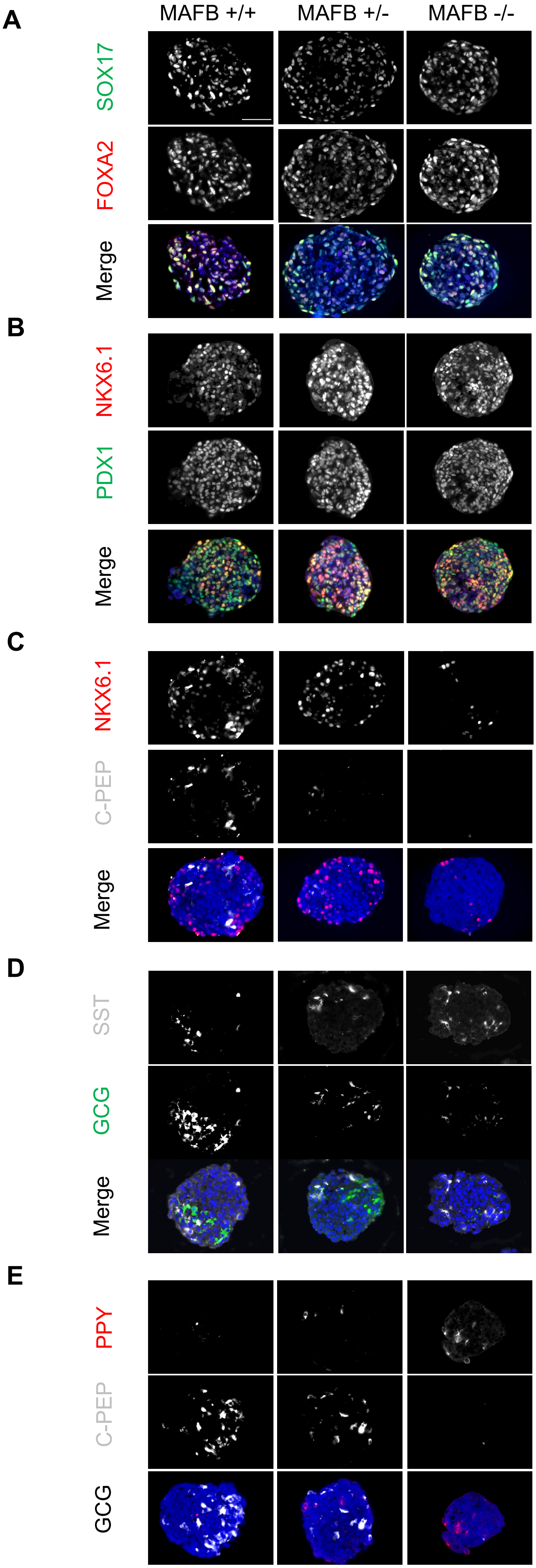
(B) Representative FC plots depicting the percentages of C-PEP+ NKX6.1+ cells at the β-cell stage.
(C) Representative FC plots depicting the percentages of C-PEP+ SST+ cells at the β-cell stage.

(D) Representative FC plots depicting the percentages of PDX1+ NKX6.1+ cells at the β-cell stage.
(E) Quantification of data from B,C,D (n = 3).

(F) Representative histograms and (G) Mean fluorescence intensity (MFI) of the GFP positive population in -DOX and +DOX treated cells (n = 3).

P values by paired two-tailed t-test were *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as individual biological replicates and represent the mean ± SD.

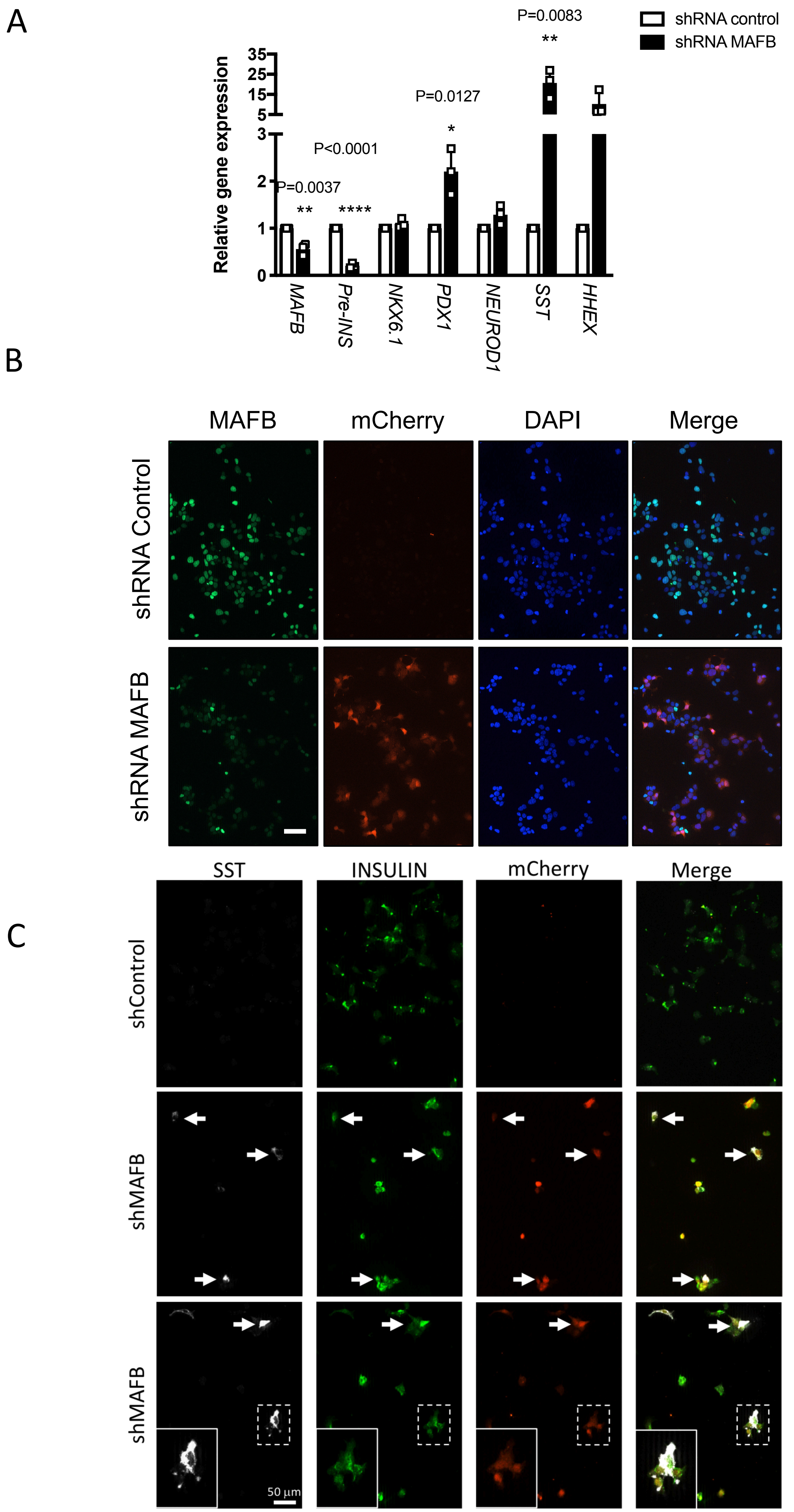
Supplementary Figure 12



Supplementary Figure 12. MAFB is critical to β-cell identity.

(A – E) Representative IF images from 3 independent experiments at the DE, PP and β-like cell stages of differentiation for the respective markers as indicated. Scale bars, 50 µm.

Supplementary Figure 13



Supplementary Figure 13. MAFB is important for β-cell identity in EndoC-βH2 cells.

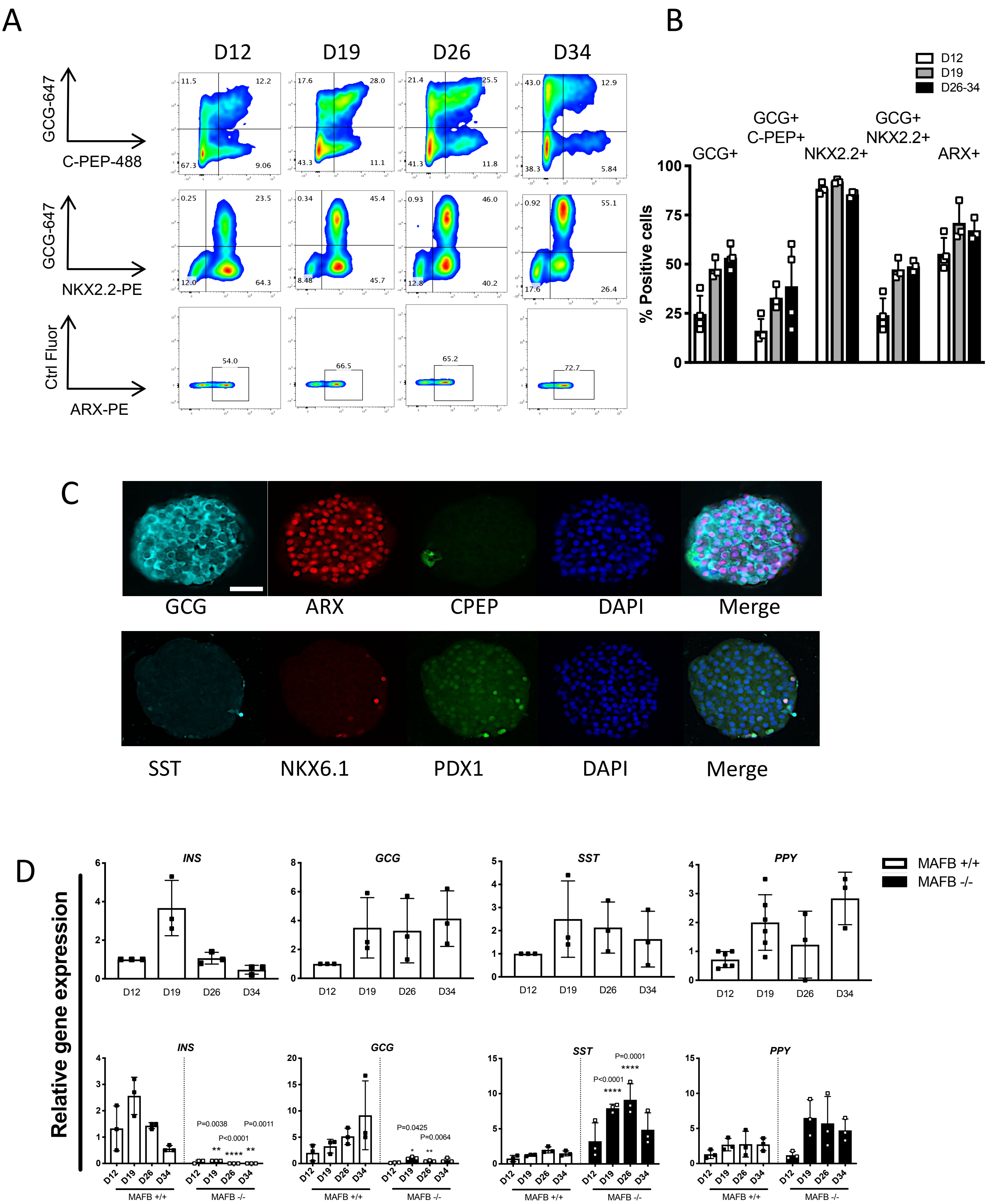
(A) The mRNA expression patterns of indicated genes for pancreatic and islet cell subtypes in EndoC-βH2 cells with shRNA control or MAFB knockdown as measured by quantitative PCR analysis (n=3).

(B) Representative IF images of EndoC-βH2 cells with shRNA control or MAFB depicting MAFB, mCherry and DAPI one week post lentiviral transduction. Scale bars, 50 µm.

(C) Representative IF images of EndoC-βH2 cells with shRNA control or MAFB depicting SST, INS, mCherry/GFP and DAPI one week post lentiviral transduction. Scale bars, 50 µm.

P values by paired two-tailed t-test were *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Data are presented as individual biological replicates and represent the mean ± SD.

Supplementary Figure 14



Supplementary Figure 14. Characterization of human α-cell differentiation from INS-GFP hESCs.

(A) Representative FC plots depicting the percentages of GCG+ C-PEP+ cells, GCG+ NKX2.2+ and ARX+ cell at the indicated stages of α-cell differentiation in INS-GFP cells.

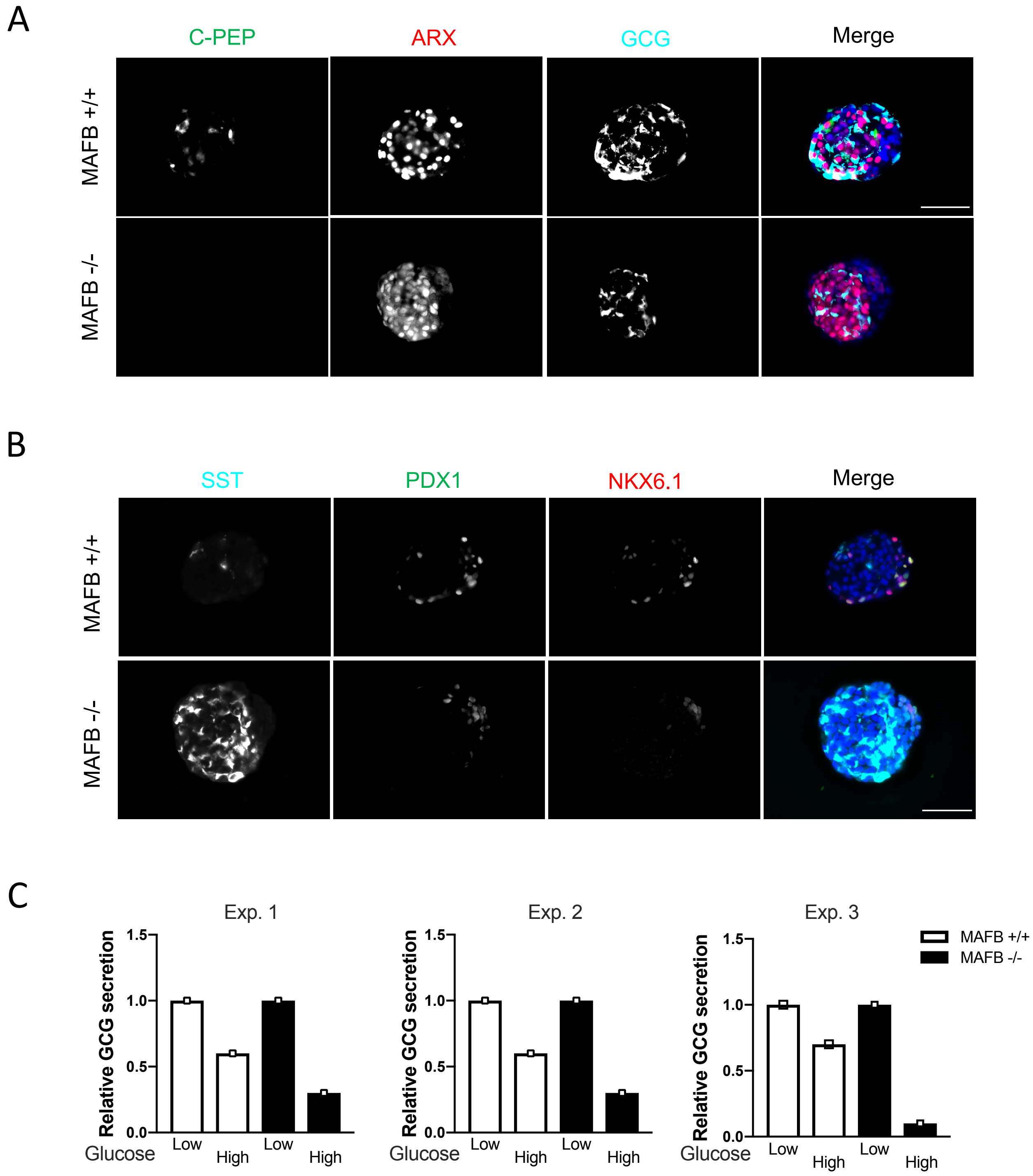
(B) Quantification of FC data from A (n=3).

(C) Representative IF images from 3 independent experiments from differentiating α-cells depicting GCG, INS and ARX (top panel) and SST, NKX6.1 and PDX1 (lower panel) at D26. Scale bars, 50 µm.

(D) The mRNA expression patterns of islet hormones throughout α-cell differentiation at the indicated timepoints as measured by quantitative PCR analysis. Top panel – parental INS-GFP cell line. Lower panel – MAFB +/+ and -/- differentiations. (n=3).

P values by paired two-tailed t-test were *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as individual biological replicates and represent the mean ± SD.

Supplementary Figure 15

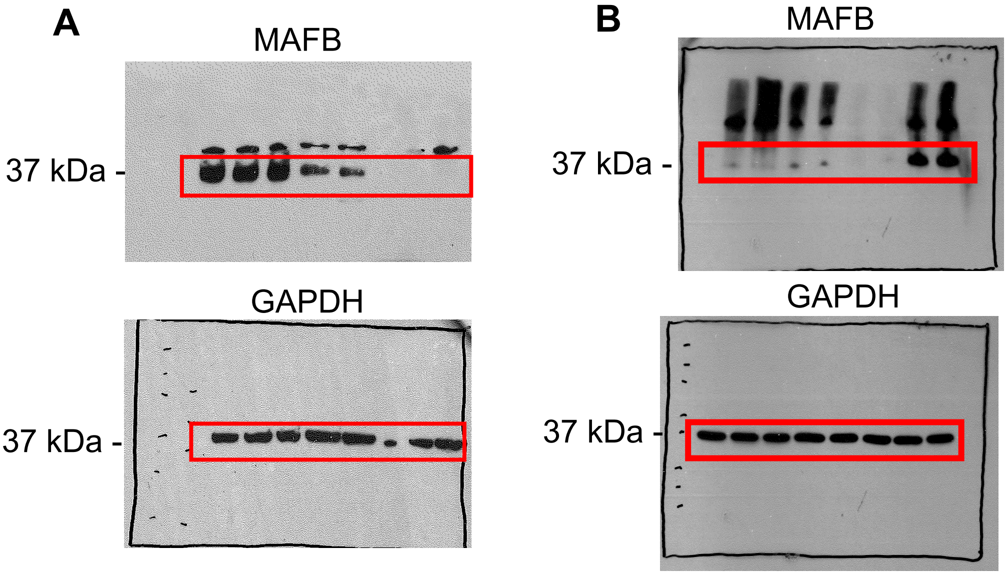


Supplementary Figure 15. Loss of MAFB limits α-cell differentiation.

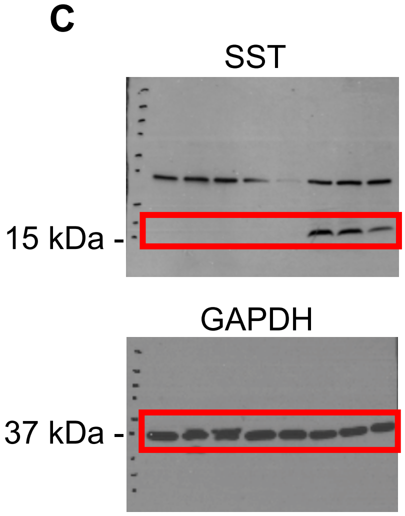
(A,B) Representative IF images from 3 independent experiments at the α-cell stage depicting GCG, INS and ARX and PDX1, SST and NKX6.1 in d26 α-cells from MAFB +/+ and -/- differentiations. Scale bars, 50 µm.

(C) Quantification of GCG secretion in response to low and high glucose from MAFB +/+ and -/- cells at d26-34 *in vitro*. 3 independent experiments are shown.

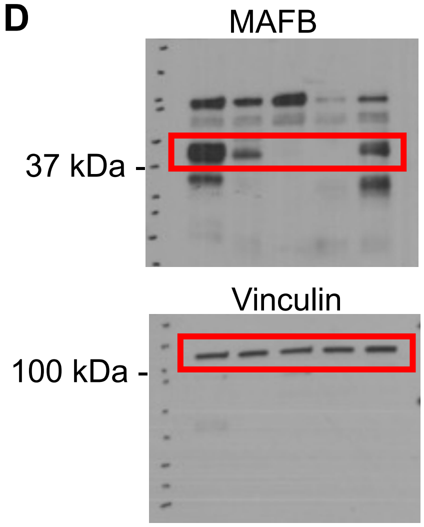
Supplementary Figure 16



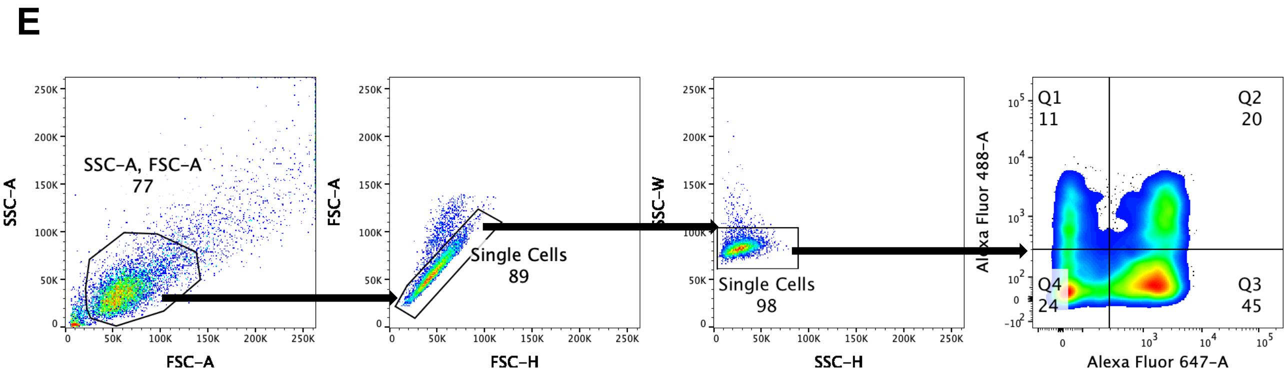
Uncropped immunoblots from Figure 1



Uncropped immunoblots from Figure 4



Uncropped immunoblots from Figure 5



Gating Strategy for Flow Cytometry Analysis

Supplementary Figure 16.

(A-D) Uncropped western blots.

(E) Flow Cytometry gating strategy.

Supplementary References:

1 Pasquali, L. *et al.* Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. *Nat Genet* **46**, 136-143, doi:10.1038/ng.2870 (2014).

2 Mularoni, L., Ramos-Rodriguez, M. & Pasquali, L. The Pancreatic Islet Regulome Browser. *Front Genet* **8**, 13, doi:10.3389/fgene.2017.00013 (2017).

Supplementary Table 1. hESC differentiation into β-like cells – Backbone Media Formulation

Day	Media components				
D0	DMEM/F-12	1xGlutamax	1xMEM-NEAA	1x 2-Mercaptoethanol	4% KSR
D1-5	RPMI	1xGlutamax			
D6-7	DMEM	1xGlutamax			
D8	DMEM	1xGlutamax			
D9-11	DMEM	1xGlutamax			
D12-20	DMEM	1xGlutamax			

Supplementary Table 2. hESC differentiation into β-like cells – Media components

Day	Media	Supplement
D0	Seed hESCs 5.5 million cells/ well in 6 well plates with D0 media	
	Day0	HERβ1 Activin A FGF-2 10ng/ml 10ng/ml 4.16ng/ml
D1	Rinse cells with DPBS (w/o Ca^{2+}, Mg^{2+}) for 5 min and added Day 1 differentiation media	
	Day 1	FBS Activin A ITS Wnt-3a 0.2% 100ng/ml 0.02X 50ng/ml
D2	Day 2	FBS Activin A ITS 0.2% 100ng/ml 0.05X
D3	Day 3	FBS KGF ITS TGF-β IV 0.2% 25ng/ml 0.1X 2.5μM
D4-5	Day 4-5	FBS KGF ITS 0.2% 25ng/ml 0.1X
D6-7	Day 6-7	B27 TTNPB 1X 3nM
D8	Day 8	B27 TTNPB EGF 1X 3nM 50ng/ml
D9-11	Day 9-11	B27 KGF EGF 1X 50ng/ml 50ng/ml
D12-20	Day 12-20	B27 1X N-acetly L-cysteine 1mM NEAA 1X Heparin 10ug/ml Zinc sulfate 10μM Alk5i II 10uM T3 136ng/mL LDN 500nM XXi 2μM Vitamin C 500μM

Supplementary Table 3. CRISPR gRNA target sequences for targeting MAFB.

Gene + guide no	gRNA Target sequence	PAM
MAFB gRNA_1	GGTGTGTCTTCTGTTCGGTC	GGG
MAFB gRNA_2	TCTTCACGTCGAACTTGCGC	AGG
MAFB gRNA_3	ACACGGAGTGCTGAGCGGTG	TGG

Supplementary Table 4. PCR and sequencing primers used for genotyping and sequencing

Primer	Sequence (5’to 3’)
MAFB Seq Forward	GTGGAGAGGCAAGCGGAG
MAFB Seq Reverse	GGGTTCATCTGCTGGTAGT

Supplementary Table 5. Clonal Cell line sequence information.

Clone Name INS-GFP cells	Allele 1	Allele 2	Mutation	MAFB
E9	TACACGGAGTGCTGAGCGGTGTGG	TACACGGAGTGCTGAGCGGTGTGG	WT / WT	+/+
C8	TACACGGAGTGCTGAGCGGTGTGG	TACACGGAGTGCTGAGC TACACGGAGTGTGGAGCTACACGGAGCTACACGGA -GTGTGG	WT / 35bp insertion, 1bp deletion	+/-
D7	TACACGGAGTGCTGAGCGGTGTGG	TACACGGAGTGCTGAG -----------	WT / 11bp deletion	+/-
E8	TACACGGAGTGCTGAGCGGTGTGG	TACACGGAGTGCTGAGCGG GTGTGG	WT / 1bp insertion	+/-
C9	TACACGGAGTGCTGAG -----------	TACACGGAGTGCTGAGCGG GTGTGG	11 bp deletion / 1bp insertion	-/-
C10	TACACGGAGTGCTGAG ----------	TACACGGAGTG---------- TGTGG	10 bp deletion / 10 bp deletion	-/-
D9	TACACGGAGTGCTGAGCGG GTGTGG	TACACGGAGTGCTGAGCGG GTGTGG	1 bp insertion / 1 bp insertion	-/-

iPSCs	Mutation	MAFB
WT	WT / WT	+/+
Het	WT / 1bp insertion	+/-
KO	-83bp / -83bp	-/-

Supplementary Table 6. Differentially Expressed genes from MAFB +/+ and -/- cells at the beta-like stage (P_adj<0.1, FC>0.5)

Upregulated	Gene	avg_logFC	p_val_adj
1	GAST	2.316356715	5.47E-303
2	SST	2.041943535	3.41e-312
3	PPY	1.413802582	2.66E-86
4	PYY	0.855968146	5.21E-40
5	ISG15	0.708019373	2.08E-106
6	LY6E	0.70691153	1.69E-110
7	CGA	0.694610481	5.11E-26
8	TPPP3	0.687042188	2.92E-55
9	RGS4	0.677778669	6.87E-62
10	HSPA1B	0.669118669	6.82E-128
11	KCTD12	0.627579284	4.84E-73
12	FXYD2	0.600230106	2.50E-15
13	APOC1	0.561225169	1.98E-50
14	CTSB	0.554216445	4.72E-66
15	HSPA1A	0.549208538	2.61E-62
16	TIMP3	0.53469464	5.92E-48

Downregulated	Gene	avg_logFC	p_val_adj
1	GCG	-2.014125141	2.97E-196
2	INS	-1.934440177	0
3	CRYBA2	-1.931697312	7.71E-270
4	TTR	-1.227343372	4.19E-141
5	ACVR1C	-1.202830614	8.46E-202
6	SLC30A8	-0.964682775	3.13E-147
7	PPP1R1A	-0.829768652	1.25E-177
8	MT1G	-0.789324756	5.68E-20
9	PCP4	-0.767236561	4.91E-23
10	STMN2	-0.766230297	6.22E-121
11	NPTX2	-0.688057809	6.09E-95
12	COL5A2	-0.680313646	5.13E-49
13	MT2A	-0.655040748	2.85E-09
14	HMGB3	-0.641933516	2.24E-137
15	EPHX1	-0.638270828	1.44E-161
16	HADH	-0.638030748	8.31E-132
17	MT1X	-0.601419028	3.06E-25
18	ERO1LB	-0.596307901	7.24E-46
19	NEUROD1	-0.574711197	3.39E-93
20	CRISPLD1	-0.546023471	1.81E-74
21	NKX6-1	-0.54402969	8.58E-86
22	GAL	-0.537813148	2.19E-28
23	AIF1	-0.536504473	1.62E-127
24	SLC7A8	-0.536149322	1.43E-122
25	SNORA76	-0.509134644	8.98E-136
26	RP11-788H18.1	-0.504352542	5.51E-105

Supplementary Table 7. Differentially Expressed genes from MAFB +/+ and -/- cells in INS NKX6.1 high (Beta-like) cells at D20 (P_adj<0.1, FC>0.5)

Upregulated	**Gene**	**avg_logFC**	**p_val_adj**
	SST	1.7366719	1.96E-60
	GAST	1.66897755	3.46E-35
	TDO2	1.20887123	4.11E-14
	GIP	1.13050186	0.00014082
	DLK1	1.11721996	3.94E-13
	CALB2	1.04430784	3.19E-19
	LY6E	1.03407021	2.42E-23
	DDC	1.01089582	1.35E-25
	KCTD12	0.99970662	4.50E-23
	RGS4	0.9782056	1.94E-13
	WLS	0.9635128	6.22E-30
	APOB	0.94060072	2.25E-10
	PYY	0.90482411	5.19E-08
	RGS2	0.87481822	1.48E-18
	HSPA1B	0.85069924	2.87E-22
	IGFBP3	0.84857646	9.44E-15
	ISG15	0.82724423	5.18E-17
	FEV	0.81770723	3.27E-09
	GUCY2C	0.80399178	4.64E-14
	RAMP1	0.80390259	2.45E-13
	C15orf48	0.79627586	2.56E-30
	HSPA1A	0.79213823	6.01E-11
	CXCL12	0.77664092	4.10E-16
	PAM	0.76879532	3.18E-19
	SEZ6L	0.75968371	4.28E-27
	FXYD2	0.75562301	0.00166728
	CHGA	0.7494752	1.63E-25
	ASCL1	0.69631919	0.00012541
	MAFB	0.68021658	6.96E-14
	RGS9	0.68020767	2.00E-22
	GCH1	0.67314402	2.87E-21
	RP11-279F6.1	0.65388567	6.65E-09
	WFDC2	0.65133335	2.86E-16
	HSPB1	0.64429653	2.87E-12
	IFI6	0.64402879	7.07E-25
	CLDN3	0.63748219	8.09E-21
	AC009014.3	0.61817642	1.89E-16
	C12orf75	0.61341069	8.35E-14
	HLA-B	0.60411727	2.97E-16
	PTPRN	0.59283695	4.94E-23
	LINC00261	0.58999555	1.71E-09
	ETV1	0.58263018	3.27E-13
	CYBA	0.57910221	1.21E-17
	FAM46B	0.57774928	1.81E-14
	LY6H	0.57595111	5.92E-10
	HLA-C	0.5571406	1.29E-17
	HES1	0.5568618	0.01859399
	MT-ND4L	0.55588667	1.66E-14
	SPINK1	0.54124436	2.16E-07
	MDK	0.54081909	1.21E-06
	PPY	0.53417065	1.66E-21
	RAB3B	0.53256072	1.68E-11
	APOE	0.53055919	3.72E-12
	NAV2	0.52619448	1.44E-14
	SCG2	0.51930402	1.50E-05
	TMSB4X	0.51453668	4.90E-05
	DSP	0.50794817	2.14E-10
	LCOR	0.50065824	1.24E-19
Downregulated	TTR	-2.1840245	2.48E-52
	CRYBA2	-2.0521569	4.99E-67
	SLC30A8	-1.7606669	1.60E-42
	MT1G	-1.4277739	0.0097721
	NEFM	-1.3136923	1.40E-11
	GCG	-1.0470691	4.83E-36
	MT1X	-1.0106086	0.03612605
	ACVR1C	-1.0016424	1.10E-46
	NPTX2	-0.9677836	1.55E-11
	RP11-788H18.1	-0.9259451	3.40E-22
	HADH	-0.8920445	3.15E-22
	RTN1	-0.8858712	6.72E-34
	SMC2	-0.8849714	2.72E-16
	ASPH	-0.8531848	9.22E-20
	EPHX1	-0.8363238	8.25E-28
	PCDH7	-0.8161754	6.93E-17
	EGFL7	-0.8028547	1.78E-15
	TLE4	-0.7756815	1.48E-14
	HPCA	-0.7358968	4.32E-28
	AIF1	-0.70121	1.30E-23
	TUBB2B	-0.6797651	2.46E-17
	LBH	-0.676144	1.69E-12
	SLC25A5	-0.6717014	6.83E-20
	HMGB3	-0.6698967	1.85E-18
	SNORA76	-0.6643836	2.98E-19
	STMN2	-0.6572494	7.77E-21
	ROBO2	-0.6440677	5.72E-39
	TNFRSF21	-0.6343054	3.32E-17
	HNRNPA1	-0.6315225	2.04E-27
	ALDH1A1	-0.6303556	4.42E-09
	FTL	-0.6107457	5.93E-26
	CHODL	-0.6075175	6.93E-25
	NME1	-0.6039177	2.97E-21
	ANGPT2	-0.5926007	2.03E-15
	PPP1R1A	-0.5921187	3.13E-23
	SURF4	-0.5745254	1.76E-17
	SVIP	-0.5743591	3.82E-07
	FGF12	-0.5677965	2.57E-21
	PLXNA2	-0.5503125	2.38E-08
	RAN	-0.5452398	4.38E-21
	PCSK2	-0.5311741	1.66E-08
	APOA2	-0.5283145	4.51E-10
	SNHG8	-0.5262266	6.64E-14
	KRT19	-0.5223486	4.34E-09
	MAN1A1	-0.5214265	1.72E-11
	TUBB	-0.5134801	1.90E-13
	LINC00643	-0.5048979	2.74E-15

Supplementary Table 8. Differentially Expressed genes from MAFB +/+ and -/- cells in GCG PPY PYY GAST high (Alpha-like) cells at D20 (P_adj<0.1, FC>0.5)

Upregulated	**gene**	**avg_logFC**	**p_val_adj**
	GAST	2.34845121	4.24E-33
	SST	1.87687227	6.10E-44
	APOC1	1.42598852	2.08E-34
	PPY	1.39317369	9.42E-10
	GHRL	1.20480579	1
	TPPP3	1.19890362	3.37E-28
	CALB2	1.0297454	4.96E-12
	LY6E	1.02849752	9.55E-25
	TIMP3	0.97653856	6.30E-21
	VIM	0.94252217	2.57E-17
	FXYD2	0.90341815	7.48E-06
	MAFB	0.86993285	5.57E-33
	PEG10	0.83442657	0.00310048
	APOE	0.80874644	2.76E-25
	RGS4	0.80821309	5.77E-24
	ISG15	0.76157627	3.34E-21
	LYZ	0.75428174	0.01653335
	AMBP	0.75381568	1.68E-13
	HSPA1B	0.73811796	1.77E-22
	C10orf10	0.72203225	4.91E-07
	NEAT1	0.7071935	7.69E-06
	PYY	0.6740438	1
	MLF1	0.67158036	3.98E-19
	FOXJ1	0.66121597	3.15E-06
	TMOD1	0.65144922	4.25E-22
	BAMBI	0.64792289	6.78E-17
	HSP90AA1	0.63034606	3.50E-29
	C1orf192	0.59054142	8.24E-08
	TDO2	0.58867598	0.00101285
	PIFO	0.57029439	7.96E-13
	TMX4	0.5698137	7.19E-11
	HSPA1A	0.56574268	3.53E-10
	DSP	0.55331361	1.13E-15
	RGS9	0.55011204	3.41E-19
	AGT	0.54569588	1.97E-05
	GC	0.54338691	0.43178532
	MDK	0.54269379	4.40E-12
	SEZ6L	0.5424836	1.97E-19
	RPL17	0.53597572	6.38E-29
	MYO10	0.526973	6.49E-16
	TXNDC12	0.52233686	3.75E-19
	MYH10	0.51451642	1.90E-17
	UBE2L6	0.514254	2.58E-18
	C8orf4	0.51300744	1.17E-07
	HEPACAM2	0.51253835	5.94E-12
	DNAJB1	0.51086168	3.59E-15
	EIF4A2	0.50786082	7.53E-26
	SPTSSB	0.5016538	8.17E-09
Downregulated	INS	-2.6998144	5.21E-122
	GCG	-2.3085079	3.86E-73
	CRYBA2	-2.0366977	2.67E-113
	APOA2	-1.7674806	7.56E-31
	ACVR1C	-1.4741353	2.27E-72
	EPHX1	-1.0894711	9.60E-75
	TTR	-1.0717345	8.43E-31
	SLC30A8	-1.0484865	3.94E-49
	PPP1R1A	-0.8705498	3.16E-51
	HMGB3	-0.8705292	2.63E-31
	ALDH1A1	-0.8406196	3.10E-23
	GAL	-0.8321412	3.24E-07
	PCSK2	-0.8142138	4.87E-39
	RP11-788H1	-0.7751951	2.08E-30
	STMN2	-0.6887093	2.25E-24
	AIF1	-0.6817355	3.21E-35
	SLC7A8	-0.6718028	2.57E-43
	SERPINI1	-0.6313049	7.91E-23
	MAN1A1	-0.5520895	2.80E-16
	C2CD4A	-0.5212733	4.15E-19
	MT-ATP6	-0.5107123	4.85E-24
	NPTX2	-0.5058441	7.00E-27

Supplementary Table 9. Differentially Expressed genes from MAFB +/+ and -/- cells in SST HHEX high (Delta-like) cells at D20 (P_adj<0.1, FC>0.5)

Upregulated	**Gene**	**avg_logFC**	**p_val_adj**
	PPY	1.16921263	0.00065802
	TPPP3	0.94230623	4.95E-05
	SST	0.83167127	3.23E-07
	APOC1	0.7776412	0.00014862
	MAFB	0.71361123	2.97E-07
	APOE	0.70040977	1.17E-05
	LY6E	0.6953392	0.00016574
	ISG15	0.69312906	2.74E-05
	PYY	0.6342115	0.00345824
	C1orf192	0.56016347	0.01327972
	IFI6	0.55576006	1.14E-11
Downregulated	INS	-2.8150699	3.58E-59
	CRYBA2	-2.2224018	4.01E-55
	ACVR1C	-1.3812356	3.03E-35
	GCG	-1.143319	4.24E-46
	TTR	-1.1143138	5.29E-20
	EPHX1	-1.0512858	5.57E-33
	PPP1R1A	-1.024554	1.87E-36
	MT1X	-0.9083369	0.00957171
	HADH	-0.8972487	2.75E-21
	STMN2	-0.8549379	5.11E-12
	MAN1A1	-0.8211267	3.64E-19
	NEUROD1	-0.8039444	1.83E-12
	FABP5	-0.7611367	1.93E-13
	IGFBP5	-0.7404403	7.73E-05
	KRT19	-0.7397835	2.47E-09
	HMGB3	-0.7186303	1.31E-11
	ERO1LB	-0.7150173	1.94E-09
	C9orf16	-0.6663357	7.60E-09
	MT-ATP6	-0.6039107	7.24E-15
	PCSK2	-0.5968298	1.43E-09
	SLC30A8	-0.5885128	4.54E-05
	PLCE1	-0.5561556	2.75E-11
	FTL	-0.5540723	2.65E-10
	AIF1	-0.5484026	4.55E-08
	MT-ND2	-0.5467455	3.56E-13
	TUBB2B	-0.5396288	1.38E-05
	RPS21	-0.5078175	6.78E-12
	SNORA76	-0.5072658	2.52E-07
	IGFBP2	-0.5026126	0.0011693
	SLC7A8	-0.5016193	6.41E-07

Supplementary Table 10. hESC differentiation into α-like cells – Media components

Day	Media	Supplement			
D0	Seed hESCs 5.5 million cells/ well in 6 well plates with D0 media				
	Day 0	HERβ1	Activin A	FGF-2	
		10ng/ml	10ng/ml	4.16ng/ml	
D1	Rinse cells with DPBS (w/o Ca^{2+}, Mg^{2+}) for 5 min and added Day 1 differentiation media				
	Day 1	FBS	Activin A	ITS	Wnt-3a
		0.2%	100ng/ml	0.02X	50ng/ml
D2	Day 2	FBS	Activin A	ITS	
		0.2%	100ng/ml	0.05X	
D3	Day 3	FBS	KGF	ITS	TGF-β IV
		0.2%	25ng/ml	0.1X	2.5μM
D4-5	Day 4-5	FBS	KGF	ITS	
		0.2%	25ng/ml	0.1X	
D6-8	Day 6-8	Noggin	TTNPB		
		100 ng/ml	3nM		
D9-11	Day 9-11	Noggin	Alk5i II	DAPT	
		50 ng/ml	1μM	1μM	
D12-18	Day12-18	Alk5i II			
		1μM			
D19-onward	Day 19	Day 19 media on ward until ~ day 55			

Supplementary Table 11. hESC differentiation into α-like cells – Backbone Media Formulation

Day	**Media components**
D0	DMEM/F-12 1xGlutamax 1xMEM-NEAA 1x 2-Mercaptoethanol 4% KSR
D1-5	RPMI 1xGlutamax
D6-8	DMEM 1xGlutamax
D9-11	DMEM/F-12 1xGlutamax 1xMEM-NEAA 1xB27
D11-54	DMEM/F-12 1xGlutamax 1xMEM_NEAA 1xB27 0.2%BSA

Supplementary Table 12. Chemicals and media components

Components	**Vendor**	**Cat. No.**
DMEM	Life Tech	11960-051
DMEM-F12	Life Tech	11320-033
RPMI	Mediatech	15-040-CM
HERβ1(Heregulin-β1)	PeproTech	100-03
Activin A	R&D	338-AC/CF
FGF-2	R&D	233-FB/CF
FBS	Corning	35-011-CV
ITS (Insulin-Transferrin-Selenium)	Gibco	41400045
Wnt-3a	R&D	5036-WN-010/CF
TGF-β IV (TGF-β RI Kinase Inhibitor IV)	CalBiochem	616454-2MG
KGF (FGF-7)	R&D	251-KG-050/CF
Noggin	R&D	3344-NG
TTNPB	Sigma-Aldrich	T3757-10MG
Alk5i II (ALK5 Inhibitor 2)	Axxora	ALX-270-445-M001
DAPT	Sigma-Aldrich	D5942-5MG
Glutamax	Gibco	35050-061
MEM-NEAA	Gibco	11140-050
2-Mercaptoethanol	Emd-Millipore	ES-007-E
KSR (KnockOut^{TM} Serum replacement)	Gibco	10828-028
B27 (NeuroCult^{TM} SM1 Neuronal)	Stemcell Technologies	5711
BSA	Sigma-Aldrich	A4503-50G
Doxycycline	Sigma-Aldrich	D9891
LDN	Fisher	04-0074-10

Supplementary Table 13. CRISPR gRNA target sequences for targeting AAVS1 locus

Primer	Sequence (5’to 3’)
AAVS1-cr1-ex	GATGACCGAGTACAAGCCCA
AAVS1-cr2-ex	GACAGTACTAAGCTTTACTA

Supplementary Table 14. Antibodies used for FACS analysis

Manufacturer	Antibody	Catalogue No.	Dilution	Fluorophore
BD Pharmingen/Fisher	Foxa2	561589	1:100	PE
BD Pharmingen/Fisher	Sox17	562205	1:100	Alexa F. 488
Biolegend	TRA-1-60-R	330605	1:200	Alexa F. 647
BD Pharmingen/Fisher	PDX1	562161	1:50	PE
BD Pharmingen/Fisher	NKX6.1	563338	1:50	Alexa F. 647
BD Pharmingen/Fisher	C-peptide	051109MI	1:200	Alexa F. 488
Fisher	PAX6	562249	1:50	Alexa F. 647
BD Pharmingen/Fisher	NKX2.2	564730	1:50	PE
BD Pharmingen/Fisher	Chromogranin A	BDB564563	1:50	PE
Sigma	Glucagon	G2654	1:1000	Alexa F. 647
BD Pharmingen/Fisher	KI67	550609	1:20	Alexa F. 647
Millipore	ARX	MABN102	1:200	PE
DAKO	SST	A0566	1:250	Alexa F. 568
Peninsula Laboratories International, Inc.	PPY	T-4088	1:500	Alexa F. 647

Supplementary Table 15. Antibodies used for IF analysis

Antibody	Species	IF Dilution	Company	Catalogue No.
Somatostatin	Rabbit	1:250	DAKO	A0566
Somatostatin	Sheep	1:250	Biogenesis	8330-0350
PDX1	Goat	1:100	R&D	AF2419
NKX6.1	Mouse	1:100	Hybridoma Bank, U Iowa	F55A10-c
Sox17	Goat	1:200	R&D	AF1924
FoxA2	Rabbit	1:1000	Millipore	07-633
KI-67	Mouse	1:400	BD	550609
NGN3	Sheep	1:200	R&D	AF3444
Glucagon	Rabbit	1:200	Linco	4030-01F
Glucagon	Mouse	1:250	Sigma	G2654
C-peptide	Rat	1:1000	DSHB	GN-1D4-C
NGN3	Sheep	1:200	R&D	AF3444
ARX	Mouse	1:200	Millipore	MABN102
HHEX	Rabbit	1:200	R&D	MAB83771-100
PPY	Rabbit	1:2000	Peninsula Laboratories International, Inc.	T-4088
MAFB	Rabbit	1:250	Sigma	HPA005653

Supplementary Table 16. Antibodies used for WB analysis

Antibody	**Species**	**IF Dilution**	**Company**	**Catalogue No.**
GAPDH	Mouse	1:3000	Santa Cruz	sc-32233
MAFB	Rabbit	1:300	Sigma	HPA0055653
Somatostatin	Rabbit	1:2000	Dako	A0566
Vinculin	Mouse	1:10,000	Santa Cruz	sc-73614

Supplementary Table 17. qPCR primer sequences

Primer Name	Forward Sequence	Reverse Sequence
ALDH1A3	TCTGAGGGTTCTAATACAGCCC	CCCTGGAGACGATGGATACAG
ARX	GTGCAAGGCTCCCCTAAGAG	CGTTCTCGCGGTACGACTT
CHGA	TGACCTCAACGATGCATTTC	CTGTCCTGGCTCTTCTGCTC
GAPDH	CTCACCGGATGCACCAATGTT	CGCGTTGCTCACAATGTTCAT
GCG	GAGATTTCCCAGAAGAGGTCG	TGGCGGCAAGATTATCAAGAA
GHRL	CAACGCCCCCTTTGATGTTG	CTGCTGGTACTGAACCCCTG
HHEX	GCGAGAGACAGGTCAAAACC	AGGGCGAACATTGAGAGCTA
HNF6	ATGTCCAGCGTCGAACTCTAC	TGCTTTGGTACAAGTGCTTGAT
INS	AGAGGCCATCAAGCAGATCACTGT	ACAGGTGTTGGTTCACAAAGGCTG
MAFA	GAGAGCGAGAAGTGCCAACT	TTCTCCTTGTACAGGTCCCG
MAFB	CATAGAGAACGTGGCAGCAA	ATGCCCGGAACTTTTTCTTT
NEUROD1	ATGACCAAATCGTACAGCGAG	GTTCATGGCTTCGAGGTCGT
NGN3	TAAGAGCGAGTTGGCACTGAGCAA	TTTGAGTCAGCGCCCAGATGTAGT
NKX2.2	GGAGCTTGAGTCCTGAGGG	TCTACGACAGCAGCGACAAC
NKX6.1	CCGAGTCCTGCTTCTTCTTG	ATTCGTTGGGGATGACAGAG
PAX4	CTACCGCACAGGTGTCTTGG	CGTTGGATTTCCCAGGCAA
PAX6	TGGGCAGGTATTACGAGACTG	ACTCCCGCTTATACTGGGCTA
PDX1	TACTGGATTGGCGTTGTTTGTGGC	AGGGAGCCTTCCAATGTGTATGGT
PPY	AGTGTACCCAGGGGACAATGC	CAGCATGTTGAGTGTATCTACGGA
Pre-INS	GTGAACCAACACCTGTGCGG	AGGGGCAGCAATGGGCAGTT
PTF1A	TTCACCGACCAGTCTTCACG	GTGGCTAAGGAACTCCACCT
SOX9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
SST	CGCTGTCCATGGTCCTG	GGGCATCATTCTCCGTCT