Cell Reports, Volume *14*

Supplemental Information

MafA-Controlled Nicotinic Receptor Expression Is Essential for Insulin Secretion and Is Impaired in Patients with Type 2 Diabetes

Elvira Ganic, Tania Singh, Cheng Luan, João Fadista, Jenny K. Johansson, Holly Ann Cyphert, Hedvig Bennet, Petter Storm, Gaëlle Prost, Henrik Ahlenius, Erik Renström, Roland Stein, Leif Groop, Malin Fex, and Isabella Artner

Figure S1

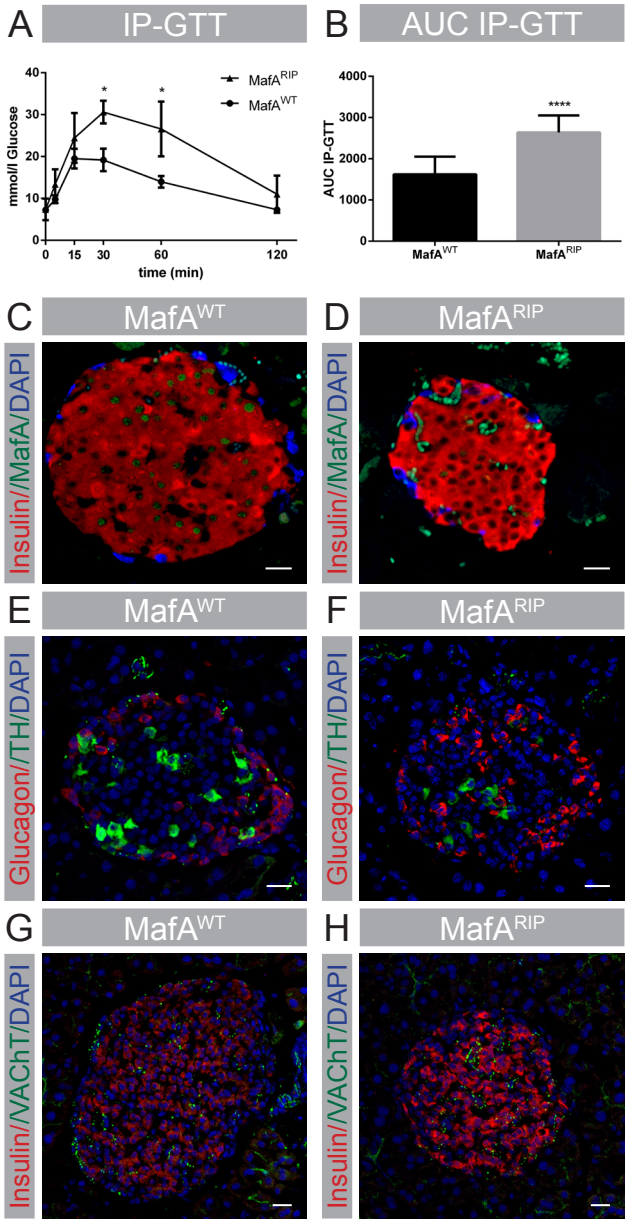

Figure S1. Functional and morphological analysis of MafA^{RIP} and MafA^{WT} mice, related to Figure 1.
A and B: Blood glucose levels after intraperitoneal glucose injection (intraperitoneal glucose tolerance test (IPGTT)) and area under curve (AUC) for the IPGTT in MafA^{RIP} and MafA^{WT} mice, n=8-11. **C and D**: MafA^{WT} and MafA^{RIP} islets are stained for β cells (insulin, red)) and MAFA (green). **E and F**: Tyrosine hydroxylase (TH, green) showing the presence of sympathetic nerve fibers. α cells are stained for glucagon in red. **G and H**: Immunostaining for the vesicular acetylcholine transporter (VAChT, green) visualizes parasympathetic nerve fibers in the MafA^{WT} and MafA^{RIP} pancreata. Scale bars = 20 μm. Nuclei are stained with DAPI (blue).

Figure S2

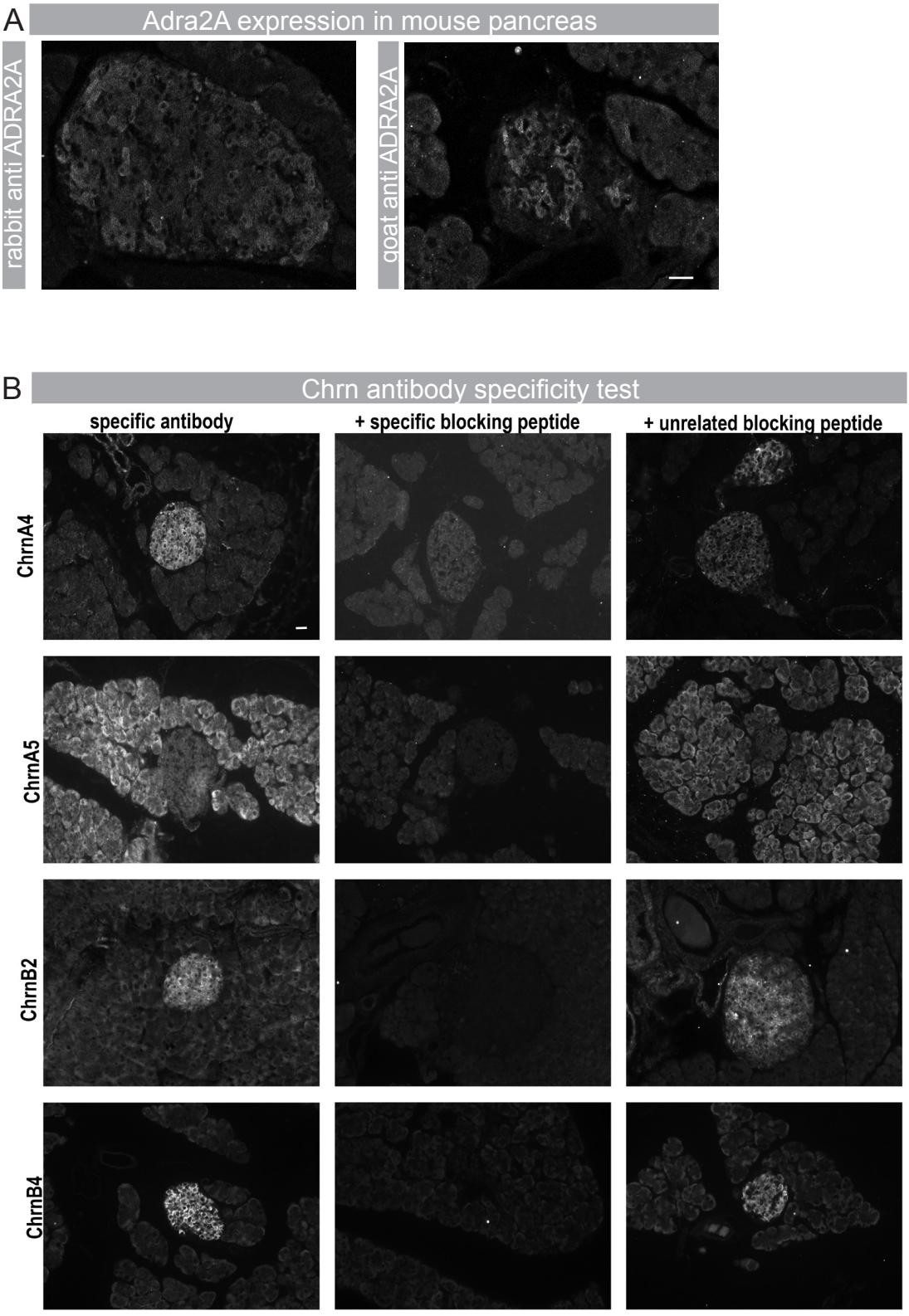

Figure S2 Assessment of adrenergic and nicotinic receptor antibody specificity, related to Figures 2 and 3.
A: MafA^{WT} islets were stained for ADRA2A using antibodies raised in different species and against different ADRA2A peptides as indicated. **B:** MafA^{WT} islets were stained for nicotinic receptor antibodies and antibodies preabsorbed with specific and unrelated peptides as indicated. Scale bars = 20 μm

Figure S3

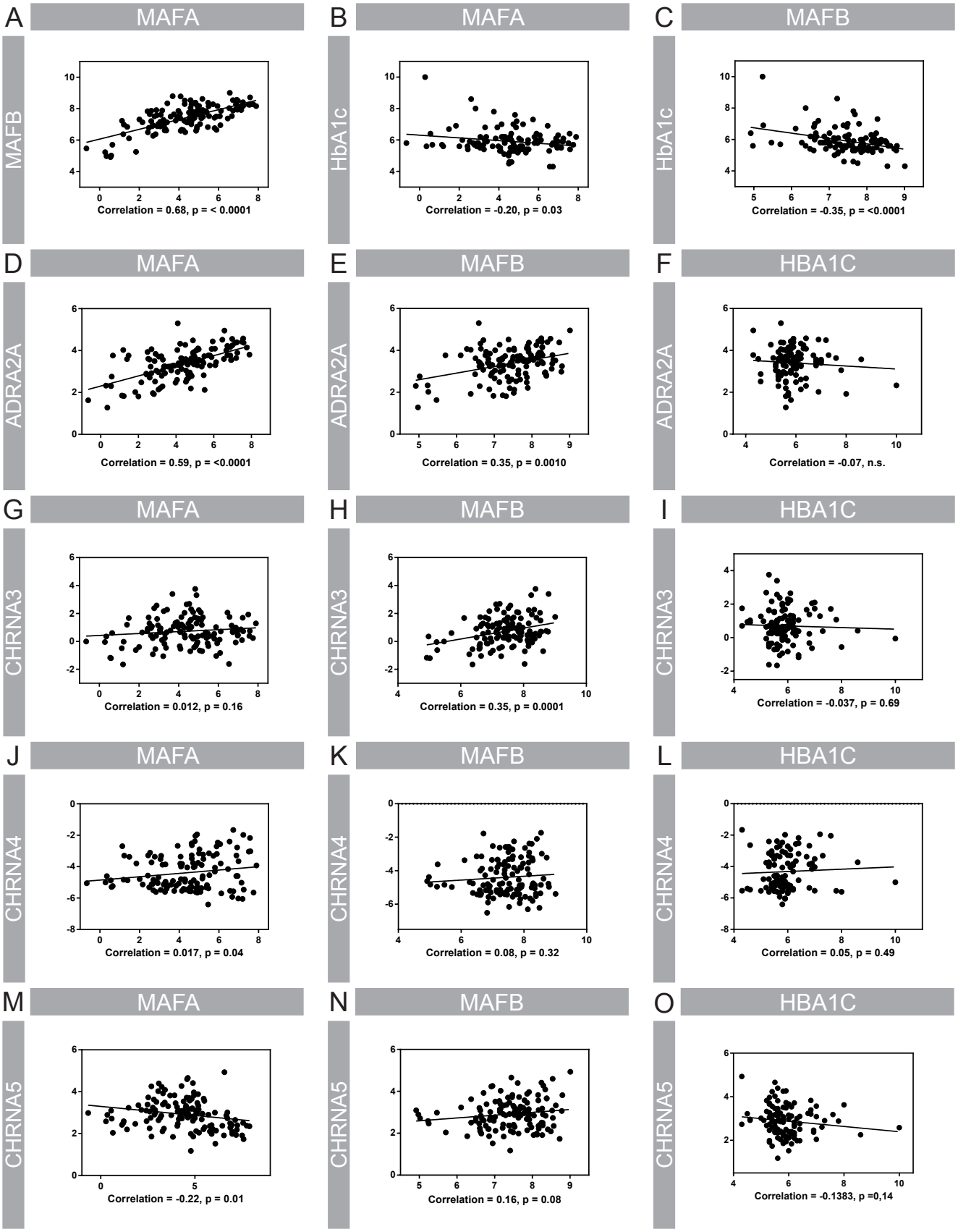

Figure S3 Gene expression correlations of adrenergic and nicotinic receptors with MAF transcription factors and HbA1c, related to Figure 4:.

Correlation analysis of transcript levels of nicotinic (CHRN) and adrenergic (ADRA) genes with MAFA, MAFB or glycated hemoglobin (HbA1C) levels in islets from human donors (n=131). Linear regression and Pearson's correlation analyses were performed using GraphPad Prism. P values and correlation coefficients are indicated under each graph.

Figure S4

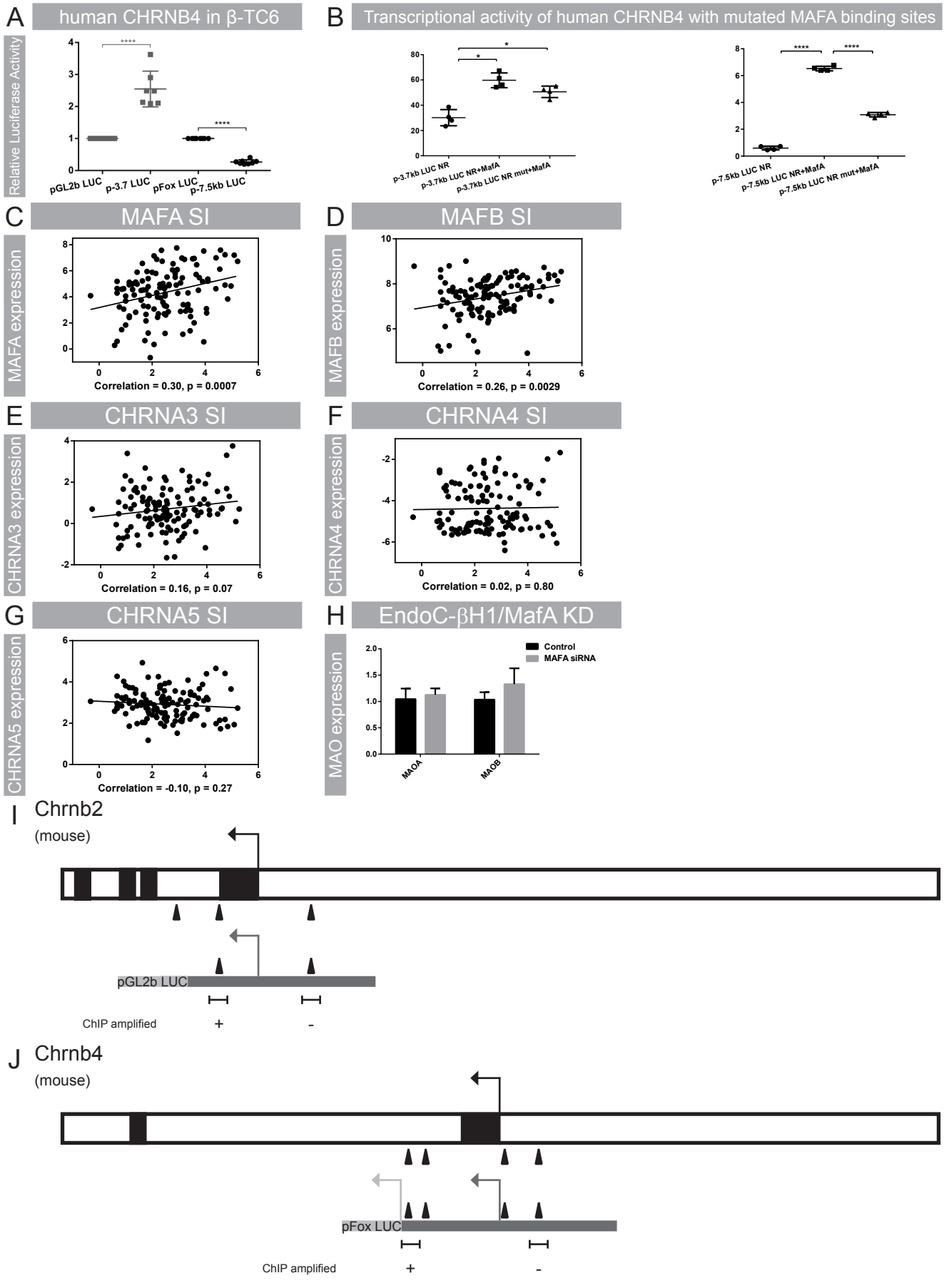
A
human CHRNB4 in β-TC6
Relative Luciferase Activity
pGL2b LUC
p-3.7 LUC
pFox LUC
p-7.5kb LUC
B
Transcriptional activity of human CHRNB4 with mutated MAFA binding sites
p-3.7kb LUC NR
p-3.7kb LUC NR+MafA
p-3.7kb LUC NR mut+MafA
p-7.5kb LUC NR
p-7.5kb LUC NR+MafA
p-7.5kb LUC NR mut+MafA
C
MAFA SI
MAFA expression
Correlation = 0.30, p = 0.0007
D
MAFB SI
MAFB expression
Correlation = 0.26, p = 0.0029
E
CHRNA3 SI
CHRNA3 expression
Correlation = 0.16, p = 0.07
F
CHRNA4 SI
CHRNA4 expression
Correlation = 0.02, p = 0.80
G
CHRNA5 SI
CHRNA5 expression
Correlation = -0.10, p = 0.27
H
EndoC-βH1/MafA KD
MAO expression
Control
MAFA siRNA
MAOA
MAOB
I
Chrnb2
(mouse)
pGL2b LUC
ChIP amplified
+
-
J
Chrnb4
(mouse)
pFox LUC
ChIP amplified
+
-

Figure S4 MAFA controls nicotinic expression, related to Figures 3 and 4**.**

A: Dual luciferase (LUC) reporter assay showing human pCHRNB4 luciferase reporter construct activity of p-3.7LUC and p-7.5kbLUC compared to empty vector control (pGL2bLUC, pFOXLUC) in βTC6 cells (n=7-8), ****p value <0.0001, analyzed by Student's t test. **B:** Dual luciferase reporter assay showing human pCHRNB4 NR luciferase construct activity upon mutation of predicted MAFA binding sites (mut) compared to control constructs. Constructs were co-transfected with pCMV-driven MafA as indicated. Experiments were performed in HEK293 cells n=4; *p value<0.05, ****p value <0.0001, analyzed by Student's t test. **C to G**: Correlation analysis of *MAFA*, *MAFB*, *CHRNA3*, *CHRNA4, CHRNA5* mRNA expression with insulin stimulatory index (SI) in islets from 131 human donors. Correlation coefficients and p values are indicated under each graph. Linear regression and Pearson's correlation analyses were performed using GraphPad Prism. **H**: Effect of siRNA-mediated knockdown of *MAFA* in the human EndoC-βH1 cell line on *MAOA* and *MAOB* mRNA levels, n=3-4. Data are mean ± SEM, analyzed using Student's t-test. **I and J:** Schematic overview of *ChrnB2* and *ChrnB4* genomic regions in the mouse. Potential MafA binding sites are indicated as triangles, the location of specific luciferase reporter constructs and genomic sequences amplified by chromatin immunoprecipitation (ChIP) are indicated for each gene.

Table S1. mRNA expression of islet-specific genes in *MafA*^{WT} and *MafA*^{RIP} isolated islets.

	WT	**MafA^{RIP}**	**p value**	
MafA	0.134	0.0035	0.0009	*
MafB	0.189	0.129	0.32	
Insulin 1	2953.35	1854.27	0.155	
Insulin 2	7099.33	4103.86	0.033	
Glucagon	161.19	166.19	0.907	
Slc2a2	12.58	0.24	0.007	*
glucokinase	4.69	0.79	0.79	
Slc30a8	3.90	0.50	0.008	*
IAPP	1035.9	686.10	0.31	
Pdx1	2.78	0.82	0.005	*
NeuroD1	0.47	0.33	0.25	
Neuronatin	0.002	0.003	0.37	

Table S1. Quantitative expression analysis of β cell genes in isolated islets from *MafA*^{WT} and *MafA*^{RIP} mice, related to Figure 1**.** Data were normalized to the geomean of *Hprt* and β-*actin* mRNA levels and the mean value is shown. Significance was assessed using multiple t-test. ***** indicates genes that were significantly down-regulated in *MafA*^{RIP} compared with *MafA*^{WT} islets.$p \leq 0.01$, $n \geq 3$

Table S2. Primer sequences for luciferase reporter constructs and ChIP analysis

Gene	Primer Sequences-Luciferase constructs	Restriction enzymes
CHRNB4	*F* accaagggtctgctagttcc	NheI G ˇCTAG ˆC SalI G ˇTCGA ˆC
	R agtgtctctctgtgtgtccag	
CHRNB4 *NheI/SalI*	*F NheI* ctgctag accaagggtctgctagttcc	
	R SalI ctgtcgac agtgtctctctgtgtgtccag	
CHRNB2	*F* aatggtcccgagtgtgtcat	XhoI C ˇTCGA ˆG HindIII A ˇAGCT ˆT
	R atccctcgtttcgcttttt	
CHRNB2 *XhoI/HindIII*	*F XhoI* cactcgag aatggtcccgagtgtgtcat	
	R HindIII gcaagctt atccctcgtttcgcttttt	
Human CHRNB4	*F* tcagcctctctcccattctg	NheI G ˇCTAG ˆC BamHI G ˇGATC ˆC
	R ggatcc gacctgcagcattagcatca	
Human CHRNB4 *NheI/Ba*	*F NheI* gctagc tcagcctctctcccattctg	
	R BamHI ggatcc gacctgcagcattagcatca	
	R BamHI ggatcc gacctgcagcattagcatca	**Position**
mouse ChnrA3	*F* CACCCACTTGCCTTGAATCT	intron 1
	R AGGGACTCAGTGGTAGGGAC	
mouse ChrnA4	*F* CACCCTACAGCTTGTCTCCA	upstream
	R AAAGCAGTTCCCTACCCCAG	
mouse ChrnB2	*F* TGAAGCGAAGCCTTTTGTCT	upstream
	R GAGATCAGGGGACCATTTCA	
	F GAGGGCTAGGAGGGGTTAAA	intron
	R TCGCTTCATCTCGTCAATGC	
mouse ChrnB4	*F* CCAAGGGTCTGCTAGTTCCA	upstream
	R ATGAAATGCCTTGAGGATGG	
	F CACAGGTCACAGGCTTTGG	intron 1
	R TGCGGCAATAACAGATTCACA	
human CHRNB4	F..CCCCAGACACCTTGCATTTT	upstream
	R TATTCCGCCCTTTTCCCACA	

Table S2 List of primers used for construction of dual luciferase constructs and chromatin immunoprecipitation analysis, and their location in the respective genes, related to Figures 3 and 4.