Supporting Materials and Methods

Genetic analyses. Genomic DNA was extracted from peripheral blood leukocytes, saliva, or formalin-fixed archival tissue using commercially available kits (blood: Illustra DNA Extraction Kit BACC2, GE Healthcare, Little Chalfont, UK; saliva: Oragene-DNA for sample collection and prepIT-L2P for DNA extraction, DNA Genotek, Ontario, Canada; formalin-fixed paraffin-embedded tissue: QIAamp DNA FFPE Tissue Kit, Qiagen, Hilden, Germany).

Haplotype analysis was investigated in two individuals (IV/5 from Family 1 and III/1 from Family 2) by analysis of three microsatellite markers (D8S1751, D8S1836, D8S373) centromeric to *MAFA* and three single nucleotide polymorphisms (SNPs) (rs11774714, rs117083418, rs118022661) telomeric to *MAFA*. The three microsatellite markers were amplified by PCR and the fluorescently tagged PCR products were run on an ABI3730 (Applied Biosystems, Warrington, UK). Allele peak heights were compared using the GeneMarker software v2.20 (Soft Genetics, State College, PA, USA). Regions of DNA encompassing the three SNPs were amplified by PCR and Sanger sequenced using standard methods. Details of the PCR primers used for the haplotype analysis are available upon request.

Pathological assessment and MAFA immunohistochemistry. MAFA expression was assessed using an anti-MAFA antibody (Abcam, Cambridge, UK; ab26405). Islets from normal human pancreas served as positive control, while reactions with omission of the primary antibody were run as negative controls. Quantification of immunoreactions was performed on images taken at the magnification of x20 with a Pannoramic Scanner (3DHISTECH, Budapest, Hungary).

Supporting Figures

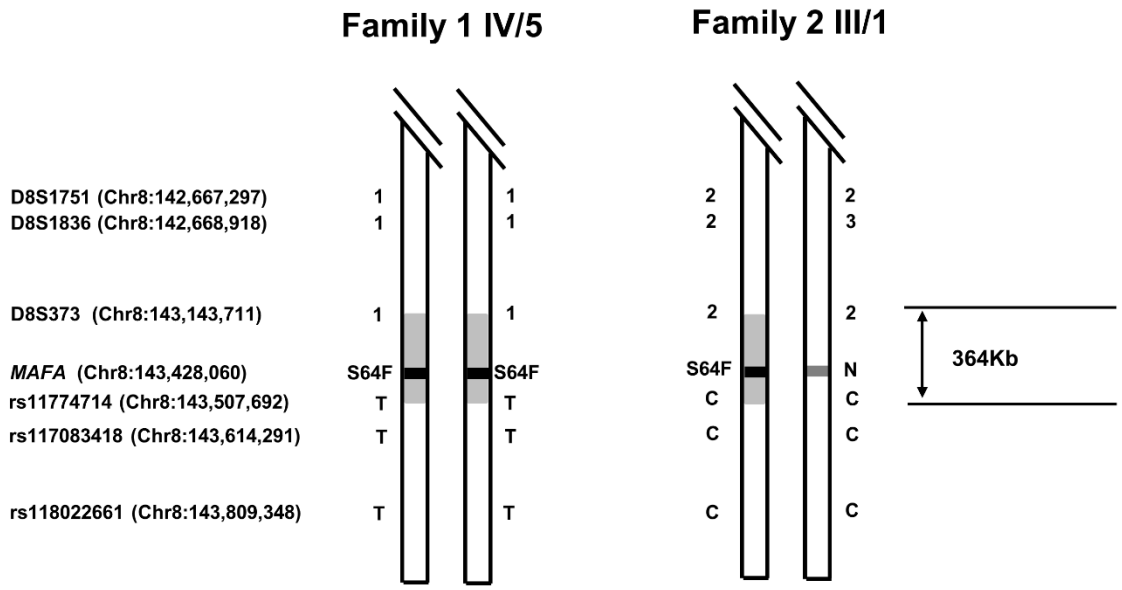
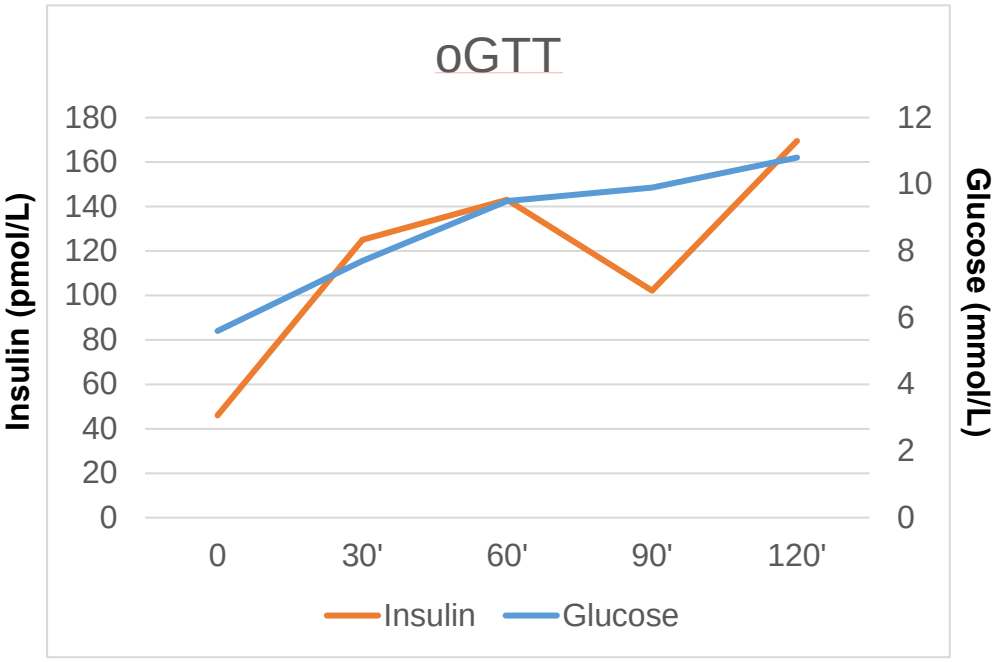


Figure S1. Results of haplotype analysis for two individuals. Subjects IV/5 from Family 1 and III/1 from Family 2 are shown. The genomic positions (hg38) of *MAFA*, the informative microsatellite markers, and the single nucleotide polymorphisms (SNPs) analyzed are provided. For microsatellite analysis, the different alleles for each marker are denoted by arbitrary values whilst the SNP genotypes and the *MAFA* mutation status are provided. N denotes no mutation. The grey box represents a 364kb region encompassing *MAFA* in which a shared haplotype could not be excluded.



	0	**+30'**	**+60'**	**+90'**	**+120'**	**+150'**	**+180'**
Glucose (mmol/L)	5.6	7.7	9.5	9.9	10.8	10.7	7.4
Insulin (pmol/L)	46	125	143.1	102.1	169.5	160.4	88.9
C-peptide (nmol/L)	0.66	0.89	1.11	1.04	1.45	1.63	1.36

Figure S2. Results of the oral glucose tolerance test (75g) in subject IV/2 (Family 2).

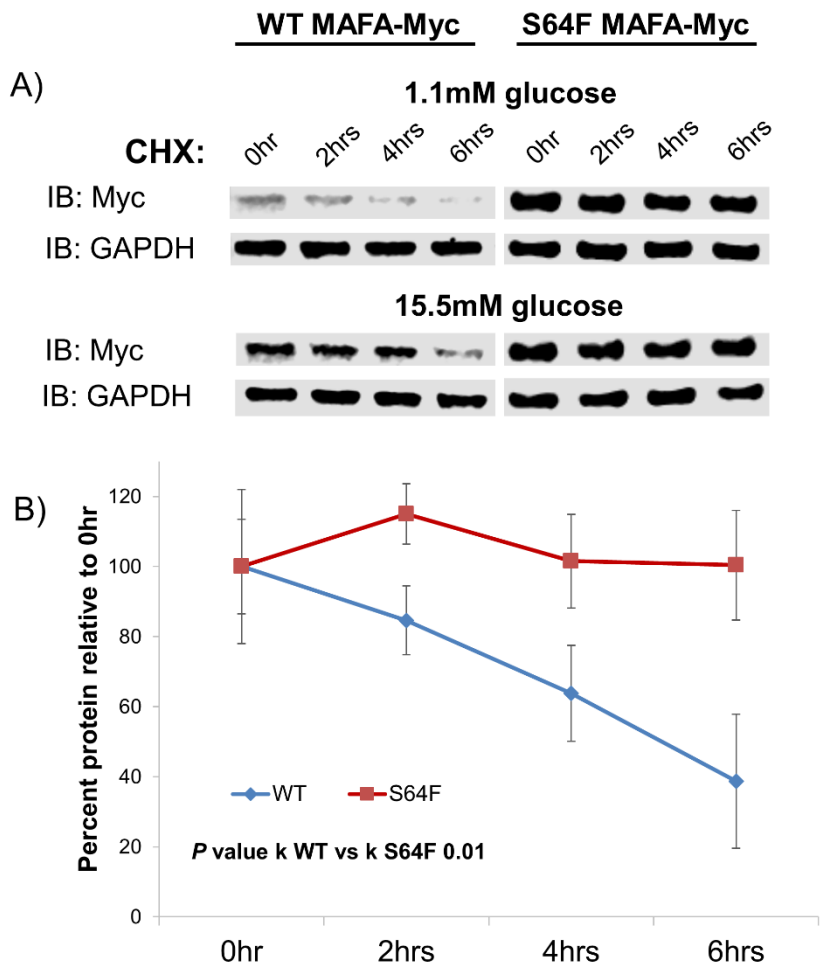


Figure S3. The p.SerS64Phe (S64F) mutation increases the stability of MAFA in MIN6 cells. Wild type (WT) and p.Ser64Phe MAFA-Myc were expressed in MIN6 cells and, after 24 hours, incubated with medium containing 1.1mM or 15.5mM glucose for an additional 12 hours. The transfected cells were then incubated with 50µg/mL cycloheximide (CHX) for the indicated time. Transfected MAFA-Myc and endogenous GAPDH protein levels were determined by immunoblotting (IB) using anti-Myc and anti-GAPDH antibodies, respectively. The Myc protein band intensity in 1.1mM glucose was normalized to the housekeeping gene and plotted as a percentage of the initial band intensity. k, degradation rate constant. Extra sum-of-squares F test. n = 3. Error bars represent SEM.

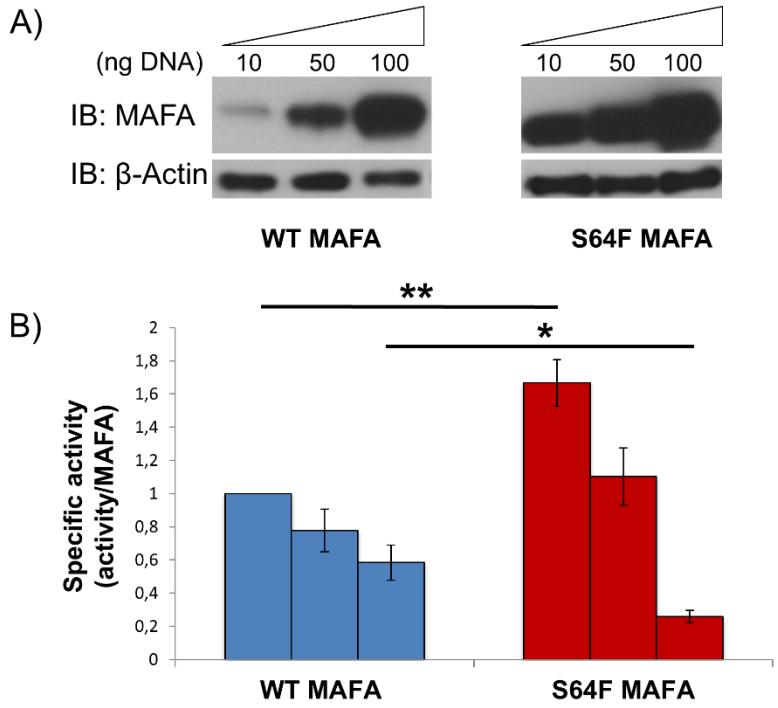


Figure S4. Comparing the activity of full-length wild type (WT) and p.Ser64Phe (S64F) MAFA in insulin II -228-driven luciferase reporter assays in HeLa cells. A) A representative immunoblot (IB) illustrating the amount of MAFA produced in HeLa cells transfected with varying amounts of plasmid DNA (ng). B) The specific activity was calculated as the normalized stimulation of -228-luciferase activity divided by the amount of produced immunodetected MAFA. Student's two-tailed t-test. * = *P* value S64F vs WT <0.05. ** = *P* value S64F vs WT <0.01. n = 3. Error bars represent SEM.

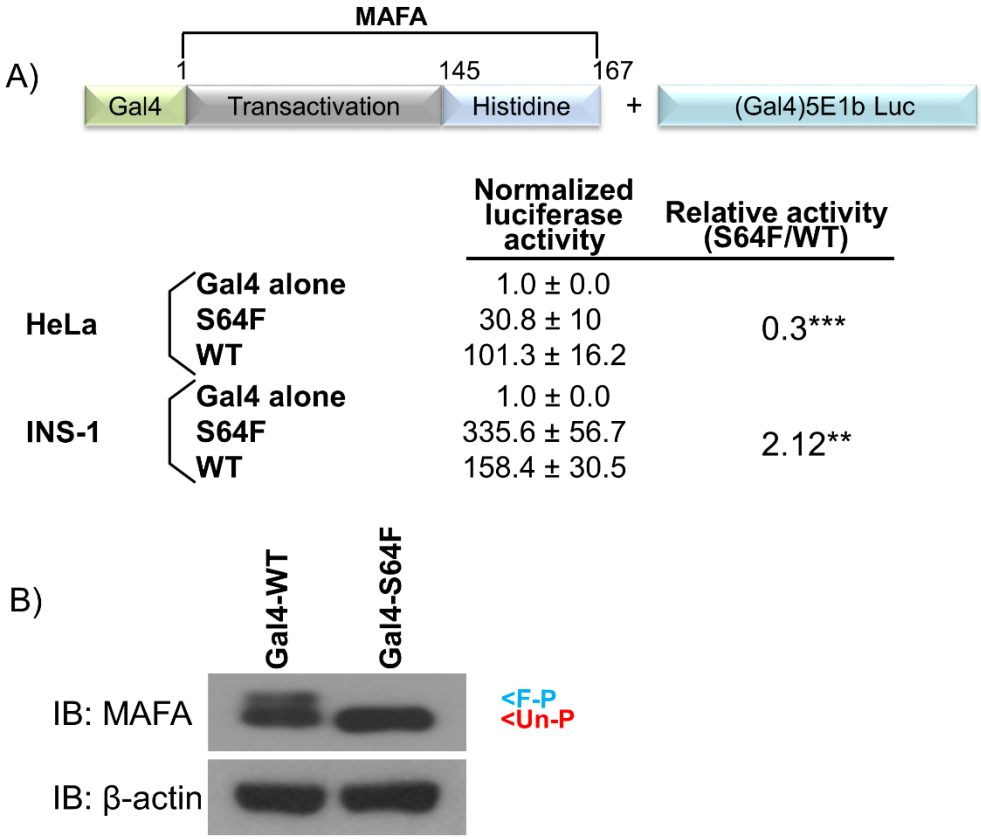


Figure S5. The p.SerS64Phe (S64F) mutation enhances the transcriptional activation potential of MAFA in β cells. A) Top: schematic of the Gal4-MAFA chimera and the Gal4 binding site-driven reporter. Bottom: the luciferase activity in each sample was normalized to a cotransfected internal control expression plasmid. Results are presented as relative to $(Gal4)_5$E1b Luc cotransfected with the Gal4 DNA binding domain vector alone ± SD. Student's two-tailed t-test. ** = *P* value S654F vs wild type (WT) <0.01. *** = *P* value S654F vs WT <0.001. n = 4. Error bars represent SEM. B) The steady state level of Gal4-MAFA(1-167) was unaffected by the p.Ser64Phe mutation in HeLa cells. The arrowheads denote the location of fully phosphorylated MAFA (F-P, blue) and the form lacking Ser65 and GSK3-mediated phosphorylation (Un-P, red).

Supporting Tables

Table S1. The number of novel potentially pathogenic heterozygous variants shared between different combinations of the four samples sequenced using exome sequencing. For example, 85 variants were identified by inspecting only sample 2, but only one variant (*MAFA* c.191C>T; NM_201589.3) was found in all four samples.

		Sample 1	**Sample 2**	**Samples 1 and 2**
		59	85	7
Sample 3	80	17	10	2
Sample 4	84	13	53	4
Samples 3 and 4	7	3	2	1

Table S2. Genes in which novel variants were identified in at least three of the four sequenced individuals. The read depth for the reference and variant are provided for each sample.

Gene	Genomic location	Read depths for reference/variant			
		Family 1 IV/4	Family 1 III/2	Family 1 III/8	Family 1 III/1
MAFA	Chr8(GRCh37):g.144512386G>A	35/22	21/27	14/18	6/19
GBA2	Chr9(GRCh37):g.35739067T>C	45/36	50/30	33/39	34/0
FAM179A	Chr2(GRCh37):g.29258502G>A	37/39	43/33	35/0	21/17
FLYWCH1	Chr16(GRCh37):g.2979993T>G	24/29	21/21	34/0	23/17
SOX8	Chr16(GRCh37):g.1033899_1033901dup	53/43	48/44	38/0	30/24
NT5DC2	Chr3(GRCh37):g.52558614A>G	66/47	34/0	46/46	39/38
ZNF292	Chr6(GRCh37):g.87968733C>T	41/38	52/0	29/38	36/32
RADIL	Chr7(GRCh37):g.4841484dup	44/0	21/28	38/32	23/21

Table S3. *In silico* prediction scores for the *MAFA* p.Ser64Phe (c.191C>T; NM_201589.3) variant.

SIFT	PolyPhen-2	Align GVGD
0.04 – not tolerated[a]	0.969 – probably damaging[b]	class C65-not tolerated[c]

[a]Prediction score from 0.00 to 1.0: 0.00–0.05, not tolerated; 0.051–0.10, potentially not tolerated; 0.101–0.20, borderline; 0.201–1.00, tolerated. http://sift.jcvi.org/

[b]Prediction score from 0 (probably benign) to 1 (probably damaging). http://genetics.bwh.harvard.edu/pph2/

[c]Prediction score from class C0 to class C65: C0–C15, tolerated; C35–C45, variant of unknown significance; C55–C65, not tolerated. http://agvgd.hci.utah.edu/

Table S4. Clinical features of patients with familial insulinomatosis. F1 = Family 1, F2 = Family 2, M = male, F = female, glu = glucose (mmol/L), ins = insulin (pmol/L), C-pep = C-peptide (nmol/L), n/a = not available. [a]previously diagnosed with gestational diabetes (see Table S4).

ID	**F1 III/1**	**F1 III/2**	**F1 III/8**	**F1 III/11**	**F1 III/12**	**F1 III/19**	**F1 IV/4**	**F2 II/1**	**F2 III/1**	**F2 III/3[a]**
Sex	M	F	F	F	F	F	F	M	F	F
Age at diagnosis	25	n/a	48	44	53	46	18	38	28	55
Biochemistry at diagnosis	glu 2.1 ins 316.7 C-pep 15	n/a	glu 2.6 ins 319.5 C-pep 2.4	glu 2.5 ins 77.8	glu 2.3 ins 84 C-pep 7.21	glu 2.7 ins 105.6	glu 2.8 ins 125 C-pep 0.65	glu 0.9 ins 1389 (after glucagon)	glu <1.1 ins 1111.2 (after tolbutamide)	glu 2.4 ins 18.8
Treatment	enucleation x2, diazoxide + verapamil	n/a	octreotide + verapamil + dexamethasone	partial pancreatectomy, octreotide + diazoxide	verapamil	partial pancreatectomy x2, total pancreatectomy	partial pancreatectomy, octreotide + verapamil + dexamethasone	enucleation, partial pancreatectomy, diazoxide	enucleation, partial pancreatectomy, diazoxide, partial pancreatectomy, completion pancreatectomy	diazoxide
TNM staging (AJCC)	pT1m N0 M0	n/a	n/a	pT1m N0 M0	n/a	pT1m N0 M0	n/a	n/a	n/a	n/a
Persistent or recurrent disease after surgery	yes	n/a	n/a	yes	n/a	yes	yes	yes	yes	n/a

Table S5. Clinical features of *MAFA* mutation carriers affected with diabetes mellitus. F1 = Family 1, F2 = Family 2, M = male, F = female, glu = glucose (mmol/L), ins = insulin (pmol/L), n/a = not available. [a]gestational diabetes, followed by impaired glucose tolerance. Subsequently diagnosed with insulinomatosis (see Table S3).

ID	**F1 III/3**	**F1 III/6**	**F1 III/7**	**F1 III/9**	**F1 III/13**	**F1 III/14**	**F1 III/16**	**F1 III/20**	**F1 IV/2**	**F1 IV/5**	**F1 IV/6**	**F1 IV/9**	**F2 II/2**	**F2 III/3[a]**	**F2 III/5**	**F2 IV/1**
Sex	M	M	M	M	M	F	F	M	F	F	M	M	M	F	M	M
Age at diagnosis	45	62	56	65	42	35	52	n/a	30	11	28	24	18	27	20	41
BMI (kg/m^2)	20	23.9	24	20	26.9	27	30	24	21.9	25.7	27	27	28.3	n/a	obese	24.4
Treatment	diet	diet	metformin + glimepiride	diet	metformin + glibenclamide	metformin + liraglutide	metformin	diet	metformin + gliclazide	glargine	metformin	metformin + saxagliptin	diet	diet (pregnancy), chlorpropamide afterwards (age 33-35)	diet	diet
Biochemistry (fasting)	n/a	glu 8.4 ins 166	glu 8.6 ins 63.2	glu >16.7	n/a	glu 7.4 ins 84	glu 7.9 ins 84.7	glu 16.7	n/a	glu 16.7	glu 7.7 ins 66.7	glu 12.2 ins 68.8	n/a	glu 6.2 (pregnancy)	n/a	n/a
Latest HbA1c mmol/mol (%)	50 (6.7)	58 (7.5)	74 (8.9)	n/a	64 (8)	49 (6.6)	49 (6.6)	37 (5.5)	60 (7.6)	40 (5.8)	37 (5.5)	n/a	n/a	n/a	n/a	51 (6.8)
Congenital cataract/glaucoma	no	no	no	no	no	no	yes	yes	Yes	yes	no	no	no	no	no	no

Table S6. MAFA expression pattern in *MAFA* mutation-positive insulinomatosis, *MAFA* mutation-negative sporadic insulinomatosis and sporadic insulinoma controls. MAFA protein staining was classified as negative (0), weak (1), moderate (2), strong (3) (percentage of positive cells), or patchy. F1 = Family 1, F2 = Family 2.

Sample	**ID**	**MAFA expression (tumor)**	**MAFA expression (normal islets)**
Familial insulinomatosis	F1 III/19	2 (100%)	3
Familial insulinomatosis	F2 III/1	patchy (100%)	3
Sporadic insulinomatosis	S1	patchy (100%)	3
Sporadic insulinomatosis	S2	2 (100%)	3
Sporadic insulinomatosis	S3	3 (100%)	3
Sporadic insulinomatosis	S5	3 (100%)	3
Sporadic insulinomatosis	S6	0	0
Sporadic insulinomatosis	S7	3 (100%)	3
Sporadic insulinomatosis	S8	3 (100%)	3
Sporadic insulinomatosis	S9	3 (100%)	3
Sporadic insulinoma	C1	3 (90%)	3
Sporadic insulinoma	C2	patchy (90%)	3
Sporadic insulinoma	C3	patchy (90%)	no islets
Sporadic insulinoma	C4	2 (100%)	2
Sporadic insulinoma	C5	3 (100%)	3
Sporadic insulinoma	C6	patchy (70%)	2

Table S7. Clinical features of patients with sporadic insulinomatosis. M = male, F = female, glu = glucose (mmol/L), ins = insulin (pmol/L), pro-ins = pro-insulin (pmol/L), C-pep = C-peptide (nmol/L), n/a = not available.

ID	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
Sex	F	F	F	F	F	M	F	F	F
Age at diagnosis	17	48	64	47	51	55	28	37	20
Biochemistry at diagnosis	glu 1.9 ins 5016.4 C-pep 0.95	glu 2.3 ins 137.5 C-pep 0.83	ins undetectable pro-ins 20.4 C-pep 0.12 in the presence of hypoglycemia	glu 1.8 ins 27.8 pro-ins 41 C-pep 0.65	glu 1.59 ins 37.5 pro-ins 29.4 C-pep 0.24	n/a	n/a	n/a	n/a
Treatment	enucleation x2, diazoxide + dexamethasone, Whipple	diazoxide + prednisone, partial pancreatectomy, diazoxide, octreotide	partial pancreatectomy x2	partial pancreatectomy	partial pancreatectomy	partial pancreatectomy	partial pancreatectomy	partial pancreatectomy	partial pancreatectomy
TNM staging (AJCC)	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0	pT1m N0 M0
Persistent or recurrent disease after surgery	yes	yes	yes	no	no	n/a	no	n/a	n/a

Table S8. Sequencing metrics for the four samples analyzed using exome sequencing.

Sample ID	% of target intervals covered by ≥15 reads
Family 1 IV/4	98.0%
Family 1 III/2	97.9%
Family 1 III/8	97.4%
Family 1 III/1	97.1%

Table S9. *MAFA* primers used for Sanger sequencing (A: peripheral blood- or saliva-derived DNA; B: formalin-fixed paraffin embedded tissue-derived DNA).

A.

Amplicon	**Forward primer (M13 tailed)** **All primers start 5'** **TGTAAAACGACGGCCAGT**	**Reverse primer (M13 tailed)** **All primers start 5'** **CAGGAAACAGCTATGACC**
1A	CGGAGTTGACCACGTGAAAC	CAGAAGCTGGGCGAGGAG
1B	TCAACGACTTCGACCTGATG	CGCTCATCCAGTACAGATCC
1C	CTCCTCGCCCAGCTTCTG	GGATGACCTCCTCCTTGCTG
1D	GAGCGCTTCTCCGACGAC	TGGTGTCCACGTCCTGTACC

B.

Amplicon	**Forward primer**	**Reverse primer**
1A	CGGAGTTGACCACGTGAAAC	GGCTCCTTCTTCACCTCGAAC
1B	TCGAGGTGAAGAAGGAGCCT	CCAGTACAGATCCTCCAGCG
1C	CTGTACTGGATGAGCGGCTA	CCAGCTGGTCGTCGGAGA
1D	ACCACCACCACCACCATG	TTGTACAGGTCCCGCTCTTT
1E	GCACATTCTGGAGAGCGAGA	CTGGTGTCCACGTCCTGTAC