# **Supporting Information**

### Borowiec et al. 10.1073/pnas.0906474106

#### SI Text

Primers Used to Amplify the DNA Fragments for Luciferase Reported Studies. The fragment spanning positions 11,369,157 (960 bp) was amplified with primers GCTTACGGTACCCACCTTCTTTC-CCATCCCATC and CCATTGCTCGAGGAAACCAAGTCT-CAATCCTAAGATGTG, the fragments spanning positions 11,459,364 and 11,459,531 (527 bp) with primers GGTTTAGGATCCGTAAGGTGTTCAGGACTGGTAAGCGAC and CCATTGTCGACGCGCCCCATCGTCACCCG, and the fragment spanning position 11,468,050 (707 bp) with primers GGTTTAGGATCCGAGGCTGACAGGGGCTGATGG and CCATTACAGCTGGTCCCCATTCCTTTGCGTATTTG.

Antibodies for Western Blotting. Rabbit polyclonal anti-Akt, antiphospho Akt (S473) and anti-BLK antibodies were purchased from Cell Signaling Technology; rabbit polyclonal anti-HNF4 $\alpha$ , anti-HNF1 $\alpha$ , HNF3 $\beta$ , anti-Glut2, anti-GCK, anti-IR $\beta$ , and anti-IGF1R $\beta$  as well as goat polyclonal anti-Kir6.2 and anti-SUR1 were obtained from Santa Cruz Biotechnology; rabbit polyclonal anti-Pdx1 was from Chemicon International; rabbit polyclonal anti-IRS1 and anti-IRS2 were obtained from Upstate; mouse monoclonal anti-Nkx6.1 was obtained from the Developmental Studies Hybridoma Bank (University of Iowa); mouse monoclonal anti- $\alpha$ -tubulin, anti-laminB1, and anti-SOD4 were purchased from Abcam.

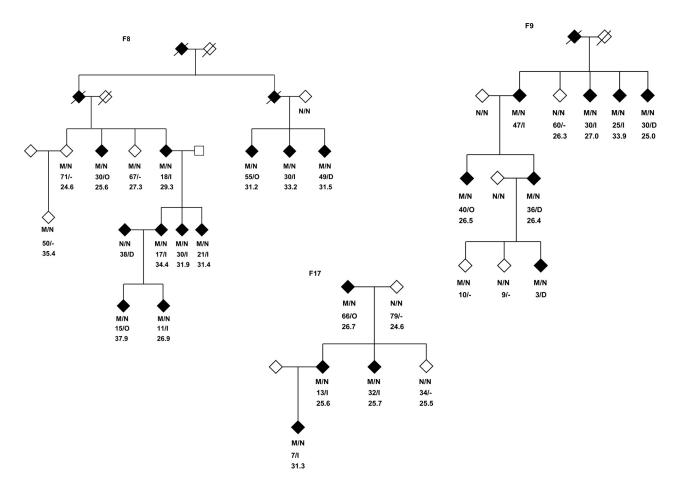


Fig. S1. Cosegregation of mutations at the 8p23 locus and MODY in 3 families showing linkage at 8p23. Filled symbols represent diabetic individuals; empty symbols correspond to nondiabetic family members. The presence (M) or absence (N) of mutations is indicated on the first line under each symbol; age at diagnosis (for diabetic subjects) or at examination (for nondiabetic subjects) and current anti-diabetic therapy (D = Diet Only; O = Oral Agents; I = Insulin) are reported on the second line; BMI is indicated on the third line.

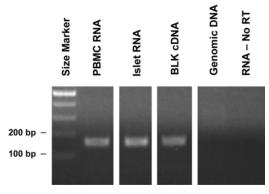


Fig. S2. RT-PCR amplification of BLK from pancreatic islets. Reverse transcription-PCR was performed starting from total RNA extracted from purified peripheral blood mononuclear cells (PBMC) and pancreatic islets. cDNA was synthesized from 1  $\mu$ g total RNA by reverse transcription using random hexamers as primers and standard conditions. PCR was performed from cDNA using primers GCAGATGCTGAAGGGGGAGAAG (exon 4) and GCAGATGCTGAAGGGGGAGAAG (exon 6) for 35 cycles with an annealing temperature of 61°C. The expected PCR product size was 177 bp. BLK cDNA served as a positive control; genomic DNA and an RT preparation from which the reverse transcriptase was omitted from the reaction served as negative controls.

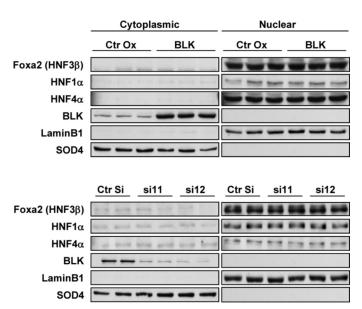
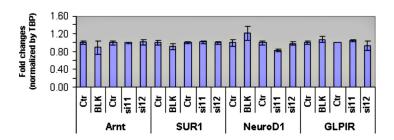
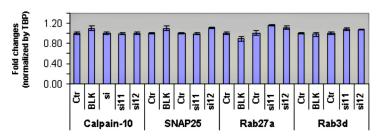


Fig. S3. β-cell expression of HNF-3β, HNF-1α, and HNF-4α in MIN6 β-cells in relation to BLK overexpression or knock-down. Western blot analysis was performed on cytosolic and nuclear fractions prepared from MIN6 β-cells using antibodies specific for each factor. Lamin B1 and SOD4 were used as markers of the nuclear and cytosolic fractions, respectively, to control for purity and gel loading.

### Gene expression analysis on genes affecting insulin secretion



## Gene expression analysis on genes mediating insulin exocytosis



**Fig. S4.** β-cell mRNA levels for proteins involved in insulin biosynthesis and β-cell function in relation to BLK overexpression or knockdown. Real-time quantitative RT-PCR analysis of genes involved in insulin biosynthesis (NeuroD1), β-cell function (Arnt, SUR1, and GLP1R), and insulin exocytosis (Calpain-10, SNAP25, Rab27a, and Rab3d) was performed in MIN6 β-cells expressing control, BLK (overexpression), BLK si11, and BLKsi12 (knockdown). Data are normalized to TBP expression and expressed as fold-change relative to control cells (n = 3).

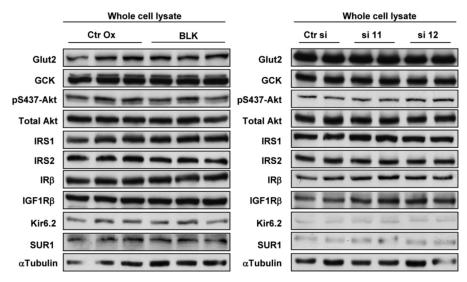


Fig. S5. β-cell expression of proteins involved in insulin signaling and glucose sensing in MIN6 β-cells in relation to BLK overexpression or knockdown. Western blotting analysis of proteins involved in insulin signaling and glucose sensing was performed on whole-cell lysates prepared from MIN6 β-cells expressing control, BLK (overexpression), BLK si11, and BLKsi12 (knockdown).

BLK	Heterozygosity								
Exons	F4	F6	F8	F9	F15	F17			
1	+	+	+	+	+	+			
2	+	+	+	+	+	+			
3	+	+	+	-	-	+			
4	+	+	+	+	+	-			
5	+	+	+	+	+	+			
6	-	+	+	+	+	+			
7	+	+	+	-	-	+			
8	+	+	+	-	-	+			
9	+	+	+	+	+	+			
10	+	+	+	+	+	+			
11	+	+	+	+	+	+			
12	+	+	+	+	+	+			
13	+	+	+	+	+	+			

**Fig. S6.** Detection of heterozygous sites during sequencing. The symbol '+' denotes the presence of at least 1 heterozygous site, the symbol '-' denotes the absence. Out of a total of 68 exons+exon/intron boundaries that were screened (13 exons × 6 families), lack of heterozygosis was observed in only 8 instances. Large deletions at these sites were excluded by long-range PCR.

Table S1. Summary of mutation screening at 8p23

	Symbol	Size, Kb	Exons	Kb sequenced		
Gene				Coding	Noncoding	Sequence differences
RefSeq						
Acyl-malonyl condensing enzyme	AMAC1L2	2	1	1.0	2.1	19
B lymphoid tyrosine kinase	BLK	71	13	4.0	80.0	31
Cathepsin B	CTSB	35	12	1.0	21.0	29
Deubiquitinating enzyme 3	DUB3	3	1	2.7	1.9	10
Farnesyl-diphosphate farnesyltransferase 1	FDFT1	38	8	2.5	16.0	19
GATA-binding protein 4	GATA4	67	8	1.3	77.0	29
L-threonine 3-dehydrogenase	TDH	31	8	2.3	12.0	12
Methionine sulfoxide reductase A	MSRA	400	7	2.6	48.0	3
Myotubularin-related protein 9	MTMR9	46	10	7.4	12.0	23
Nei-like 2	NEIL2	28	5	3.1	9.2	5
PIN2-interacting protein 1	PINX	81	7	1.8	9.0	11
Protein phosphatase 1 regulatory subunit 3b*	PPP1R3B	12	3	0.9	4.1	20
Retinitis pigmentosa 1-like 1	RP1L1	114	6	8.7	24.0	6
SRY-box 7	SOX7	6	2	3.3	3.8	13
Tankyrase 1*	TNKS	220	27	4.0	176.0	22
Other mRNAs						
AA909882	-	5	3	1.1	1.4	3
AJ291676	-	3	1	0.8	1.2	-
AJ301563	_	3	5	0.8	1.2	2
AK055863	-	10	3	1.4	2.8	13
AK124536	_	4	1	2.3	3.4	3
BC043573	_	3	3	1.2	1.3	4
BC065837	_	26	6	1.5	2.7	8
BM803853	_	1	1	1.0	1.2	1
C8orf12	_	50	4	1.2	1.8	12
C8orf13	_	42	3	2.1	1.2	8
C8orf14	_	6	3	1.9	3.0	9
C8orf15		8	2	1.7	2.4	8
C8orf16	_	12	2	1.2	4.7	2
C8orf21	_	300	4	1.9	23.0	11
C8orf49	_	4	1	1.9	1.3	3
C8orf5		6	1	1.8	2.4	5
C8orf6		6	1	1.4	1.6	9
C8orf7		6	2	1.4	2.4	-
C8017 C8orf8	-	20	2	1.4	3.4	4
UNQ9391	_	16	5	2.1	7.8	16
Intergenetic regions	-	10	3	۷.۱	7.0	10
BLK-C8orf14	_	11	_	_	9.0	15
C8orf14-GATA4	-	110	-	-	80.0	22
	-	1,696	- 171	- 76.6	655.3	410
Total		ספס,ו	171	70.0	000.5	410

<sup>\*</sup>Functional candidate genes placed outside the critical interval.