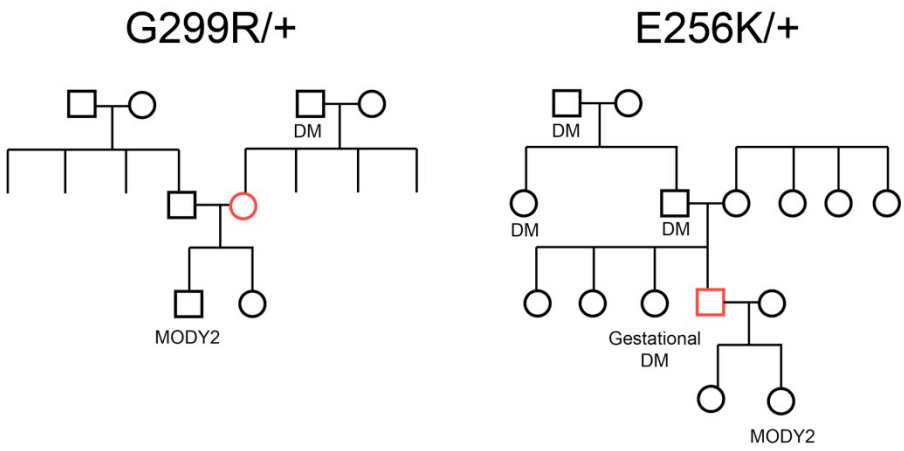
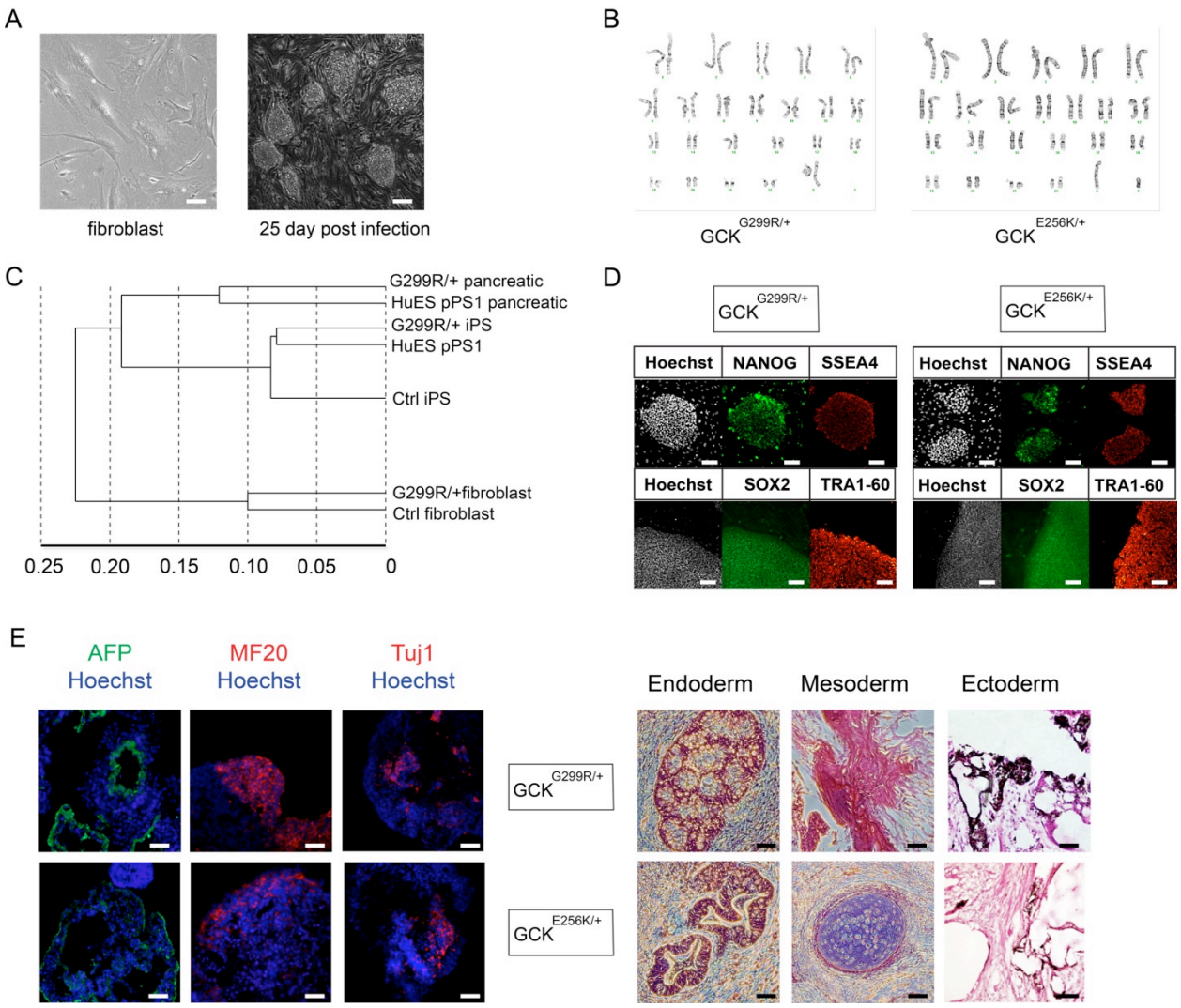
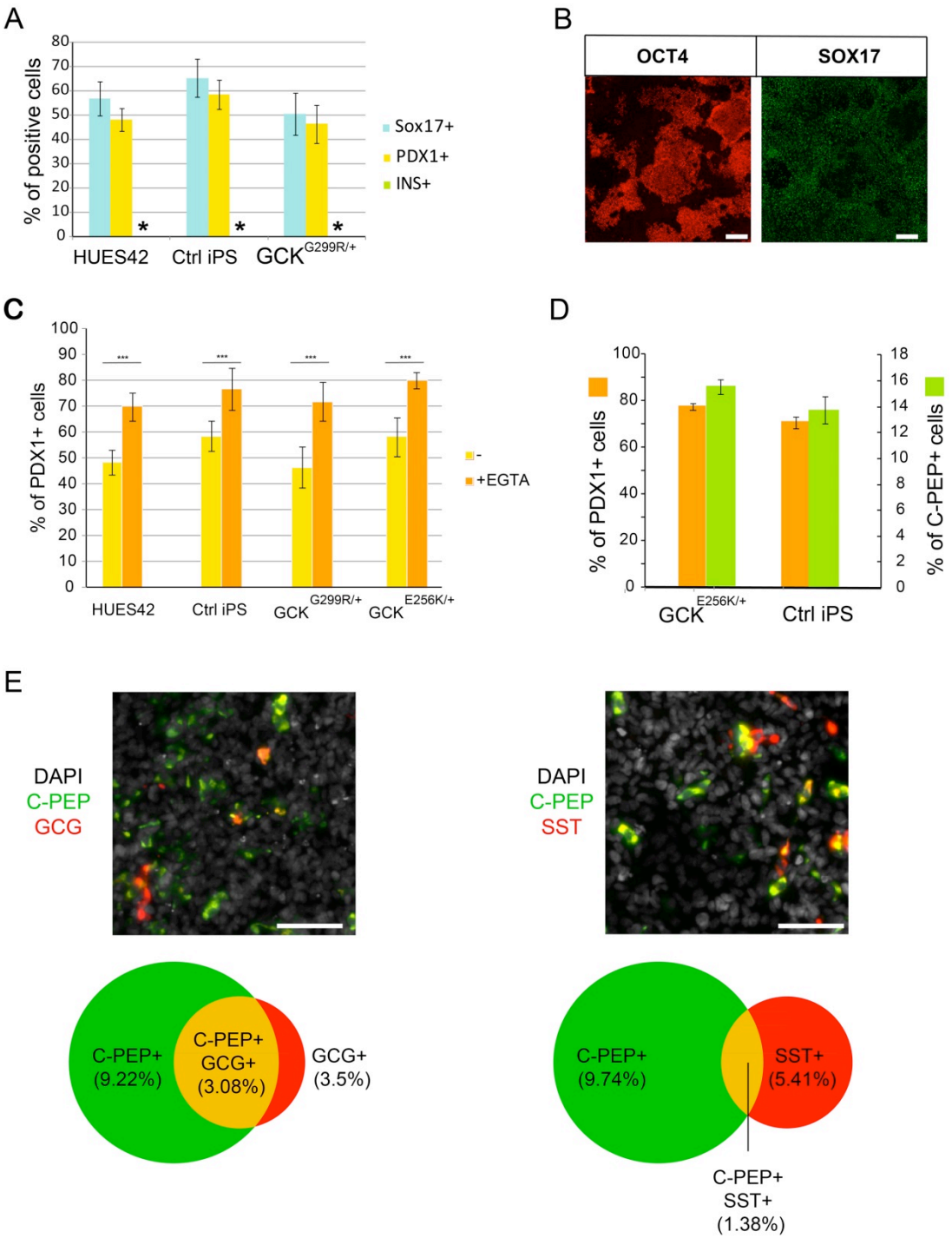


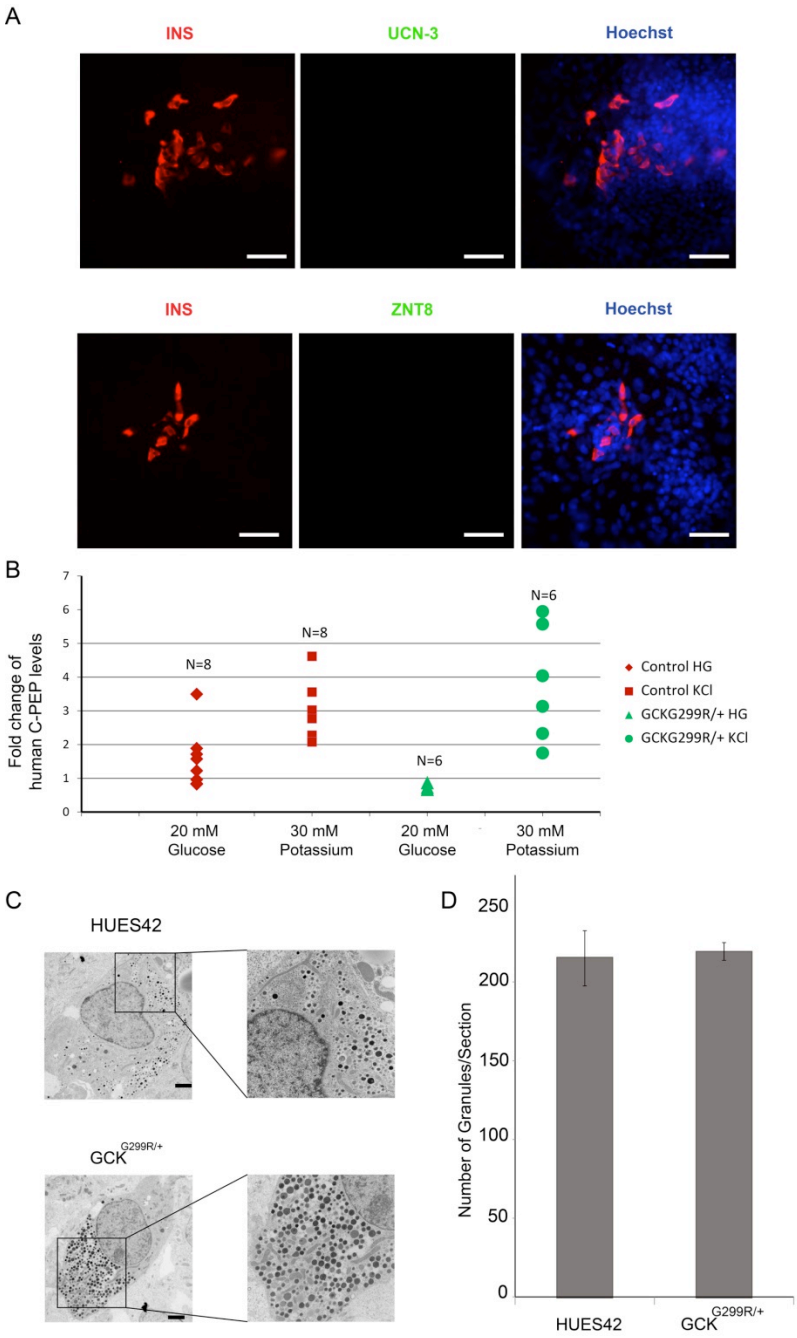
Supplementary Figure 1 Pedigrees of the two MODY2 subjects that were studied (marked in red). DM, diabetes mellitus; MODY, maturity onset diabetes of the young.



Supplementary Figure 2 *GCK* mutant iPS cells are pluripotent. (A) Fibroblast cell line (scale bar, 10μm) and induced pluripotent cells (scale bar, 100μm) were derived from a MODY2 subject carrying a hypomorphic mutation (G299R) in the glucokinase gene (*GCK*). (B) iPS cells from the two MODY2 subjects had normal karyotypes. (C) A cluster tree showing global gene expression profiles of iPS cells and fibroblast cells of control and MODY2 subjects and pancreatic cells differentiated from MODY2 iPS and human ES cells (pPS1, (Noggle et al., 2011). (D) Pluripotency marker genes expressed in the stem cells generated from two MODY2 subjects. Scale bar, 50 μm. (E) Embryoid bodies formed by *GCK* mutant stem cells contained three germ layers- endoderm (AFP+), mesoderm (MF20+) and ectoderm (Tuj1+) (left panel). *GCK* mutant stem cells formed teratomas that contained tissue structures from three germ layers (right panel). Scale bar, 200 μm.



Supplementary Figure 3 Differentiation of stem cells into beta cells *in vitro*. (A) Efficiency of generating pancreatic progenitors and insulin-producing cells using a published protocol (D'Amour et al., 2006) . * indicates no insulin positive cells. (B) Distribution of SOX17+ and OCT4+ cells after 3 days of differentiation following the published protocol. Scale bar, 50 μm. (C) Quantification of pancreatic progenitor cells (PDX1+) after 8 days of differentiation. ***: P<0.001. (D) Differentiation efficiency of *GCK*$^{E256K/+}$ and control cells. (E) Expression of endocrine hormones after 12 days of differentiation and diagrams showing proportion of insulin and glucagon (left) or insulin and somatostatin (right)-producing cells. Scale bar, 100 μm. C-PEP: C-peptide, GCG: glucagon, SST: somatostatin.



Supplementary Figure 4 Beta cells derived *in vitro* were not fully mature yet displayed insulin secretion defect specific to glucose. (A) Immunostaining of *in vitro* differentiated beta cells. INS: insulin, UCN-3: urocotin-3, ZNT8: zinc transporter 8. Scale bar, 100 μm. (B) Insulin (C-peptide) secretion of *in vitro* derived beta cells in response to glucose (20mM) and potassium (30 mM). The basal condition was 2.5 mM glucose and 4.8 mM potassium. 5 out 8 control replicas showed response to glucose while none of the *GCK* mutant replicates did. All the control and GCK mutant replicates showed response to potassium. (C) Electron microscope (EM) images of insulin producing cells derived from ES cells and *GCK*$^{G299R/+}$ cells. Scale bar, 2 μm (D) Quantification by EM of insulin granule numbers per insulin-producing cell, by genotype. (n=3 per genotype).

Supplementary Table 1 Summary of clinical characteristics of the 2 MODY2 subjects.

Genetic Diagnosis	Age at Clinical Diagnosis	Anti-GAD Antibodies	BMI	Race	Family history of diabetes	Controlled with oral agents
GCK mutation gly229>arg	21	Neg	21	Caucasian	3 generations	yes
GCK mutation glu256>lys	47	Neg	26	Caucasian	2 generations	yes

Supplementary Table 2 Primer sequences.

primer	sequences
GCK-5arm-forward	ccgctcgagcggtgcatcttccagct
GCK-5arm-reverse	cccaagcttgggcaccttccctgcct
GCK-3arm-forward	ccgctcgagcgggctggaatcaatttcccaga
GCK-3arm-reverse	cggaattccgcgtgatgctgttccagagaa
GCK-correction-forward	ccgctcgagcggtccccaagacacttccacat
GCK-correction-reverse	ggactagtccataggcgttccactgacagg
P1	gcatcttccagctcttcgac
P2	ctaaagcgcatgctccagac
P3	aggccctagtttcccatcc
Southern Probe forward	tccagatgctcctgtcagtg
Southern Probe reverse	gagccaaagcaattccacat
INS RTPCR forward	ttctacacacccaagacccg
INS RTPCR reverse	caatgccacgcttctgc
GCK RTPCR forward	ctgaacctcaaaccccaaac
GCK RTPCR reverse	tgccaggatctgctctacct
GLUT2 RTPCR forward	catgtgccacactcacacaa
GLUT2 RTPCR reverse	atccaaactggaaggaaccc

Supplementary Table 3 Beta-cell differentiation medium compositions.

Stage	Day	Basic Medium	Supplement
Stage 1: Definitive Endoderm	1	RPMI	Activin A (100 ng/ml) Wnt3A (25 ng/ml) 75 uM EGTA
	2-3	RPMI	Activin A (100 ng/ml), 0.2% FBS
Stage 2: Primitive Gut Tube	4-5	RPMI	FGF10 (50 ng/ml), KAAD-cyclopamine (0.25 uM) 2% FBS
Stage 3: Posterior Foregut	6-8	DMEM	FGF10 (50 ng/ml), KAAD-cyclopamine (0.25 uM) Retinoic acid (2 uM) LDN-193189 (250 nM) B27
Stage 4: Pancreatic Endoderm	9-12	CMRL	Exendin-4 (50 ng/ml) SB431542 (2uM) B27
Stage 5: Endocrine	13+	CMRL	B27

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D'Amour, K.A., Bang, A.G., Eliazer, S., Kelly, O.G., Agulnick, A.D., Smart, N.G., Moorman, M.A., Kroon, E., Carpenter, M.K., and Baetge, E.E. (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol *24*, 1392-1401.

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