

Supplemental Information

Mutations in the C1 element of the insulin promoter lead to diabetic phenotypes in homozygous mice

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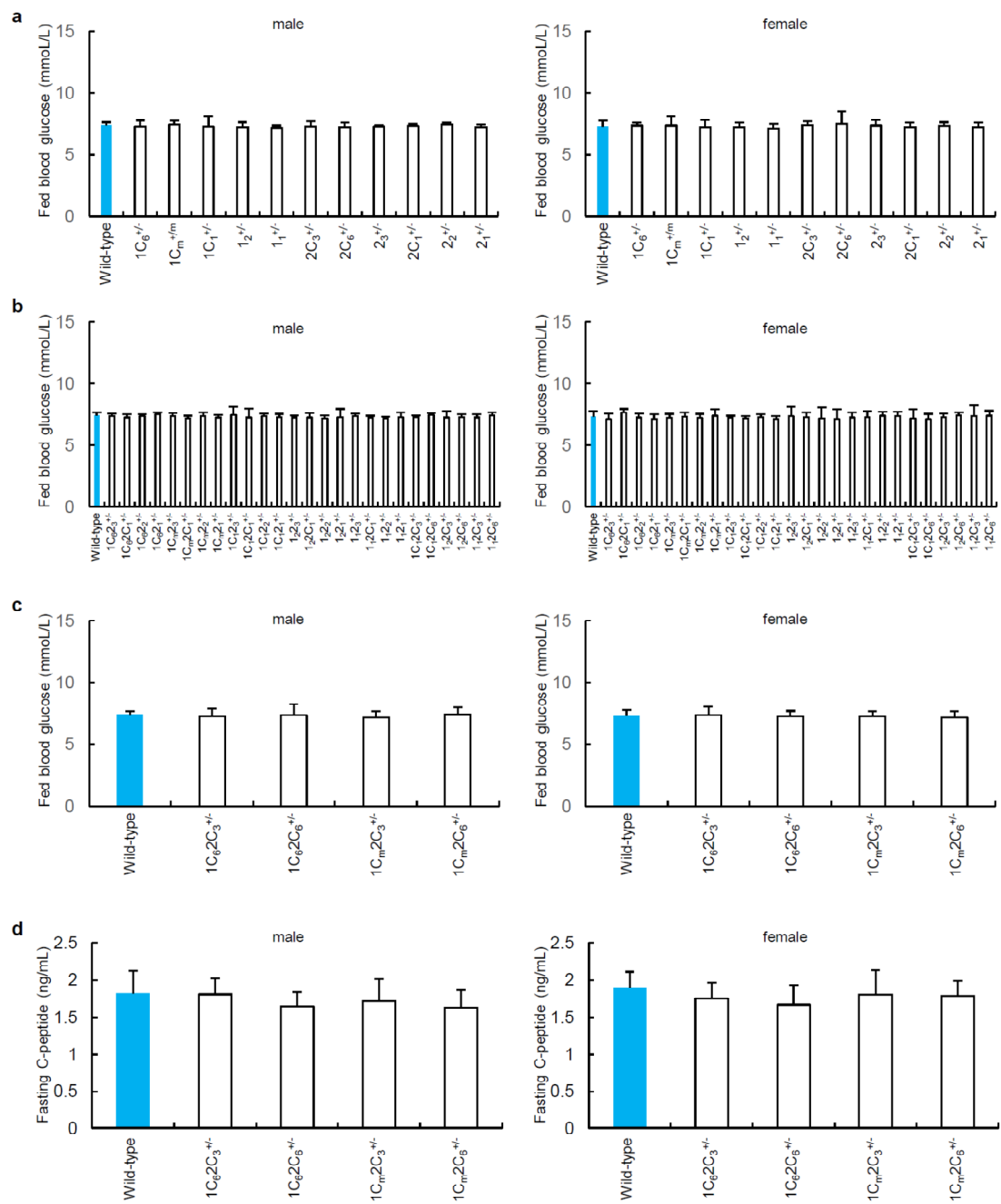
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Supplemental tables/figures count: tables 2 / figures 4

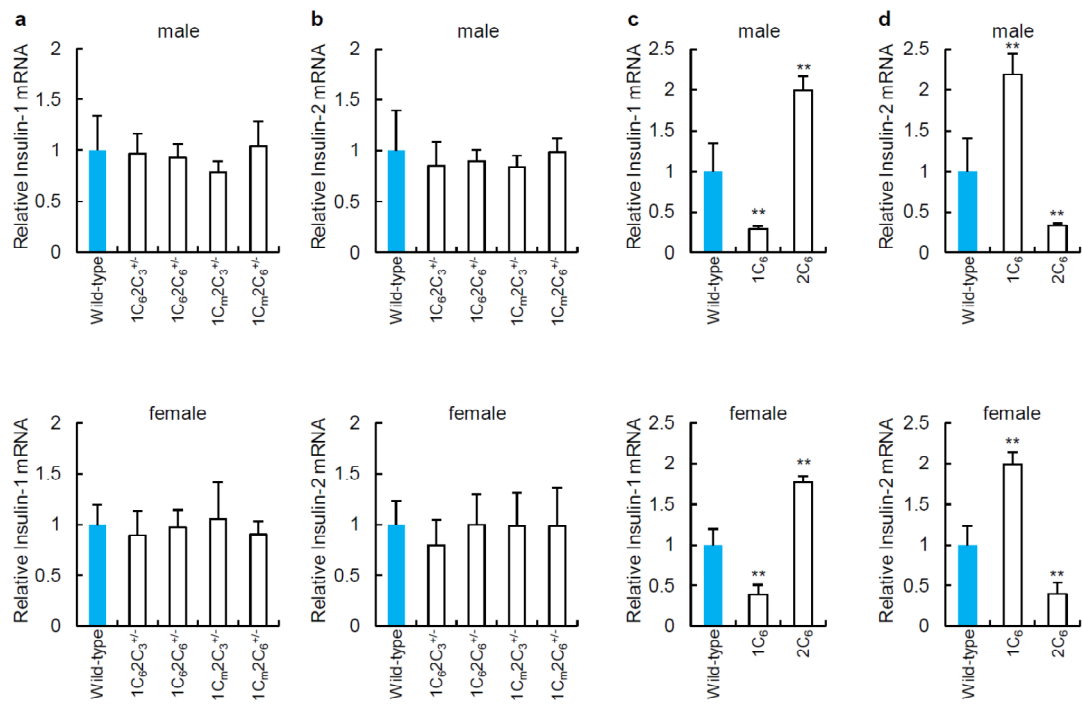
Although the insulin promoter sequence is not as well conserved between species as the transcribed sequences of the insulin gene, most of the functionally critical promoter sequence elements are well conserved. In particular, bases –151 to –103 of the promoters of mouse *Ins1* and *Ins2* and bases –149 to –102 of the human insulin promoter are highly conserved. These sequences include the GG2-A2, C1 and E1 elements.



Supplementary Fig 2 Noguchi et al.

Supplementary Fig. 2 Blood glucose concentrations of heterozygous mice with mutations of the *Ins1* or *Ins2* promoters or both.

a Blood glucose levels of fed wild-type (n=6) and heterozygous mice with deletion or replacement of only the *Ins1* promoter or deletion of only the *Ins2* promoter (n=6 each) at 12 weeks of age. **b** Blood glucose levels of fed wild-type (n=6) and heterozygous mice with any deletion of the *Ins1* promoter along with ≤ 3 -base deletions in the *Ins2* promoter ($1C_62_3^{+/-}$, $1C_62C_1^{+/-}$, $1C_62_2^{+/-}$, $1C_62_1^{+/-}$, $1C_m2_3^{+/-}$, $1C_m2C_1^{+/-}$, $1C_m2_2^{+/-}$, $1C_m2_1^{+/-}$, $1C_12_3^{+/-}$, $1C_12C_1^{+/-}$, $1C_12_2^{+/-}$, $1C_12_1^{+/-}$, $1_22_3^{+/-}$, $1_22C_1^{+/-}$, $1_22_2^{+/-}$, $1_22_1^{+/-}$, $1_12_3^{+/-}$, $1_12C_1^{+/-}$, $1_12_2^{+/-}$, and $1_12_1^{+/-}$; n=6 each) and ≤ 3 -base deletions in the *Ins1* promoter with any deletion in the *Ins2* promoter ($1C_12C_3^{+/-}$, $1C_12C_6^{+/-}$, $1_22C_3^{+/-}$, $1_22C_6^{+/-}$, $1_12C_3^{+/-}$, and $1_12C_6^{+/-}$; n=6 each) at 12 weeks of age. **c** Blood glucose levels of fed wild-type (n=6) and heterozygous mutant mice with >3 -base deletions in the *Ins1* and *Ins2* promoters (n=6 each) at 12 weeks of age. **d** Fasting C-peptide levels of wild-type (n=8) and heterozygous mutant mice with >3 -base deletions in the *Ins1* and *Ins2* promoters (n=8 each) at 12 weeks of age.

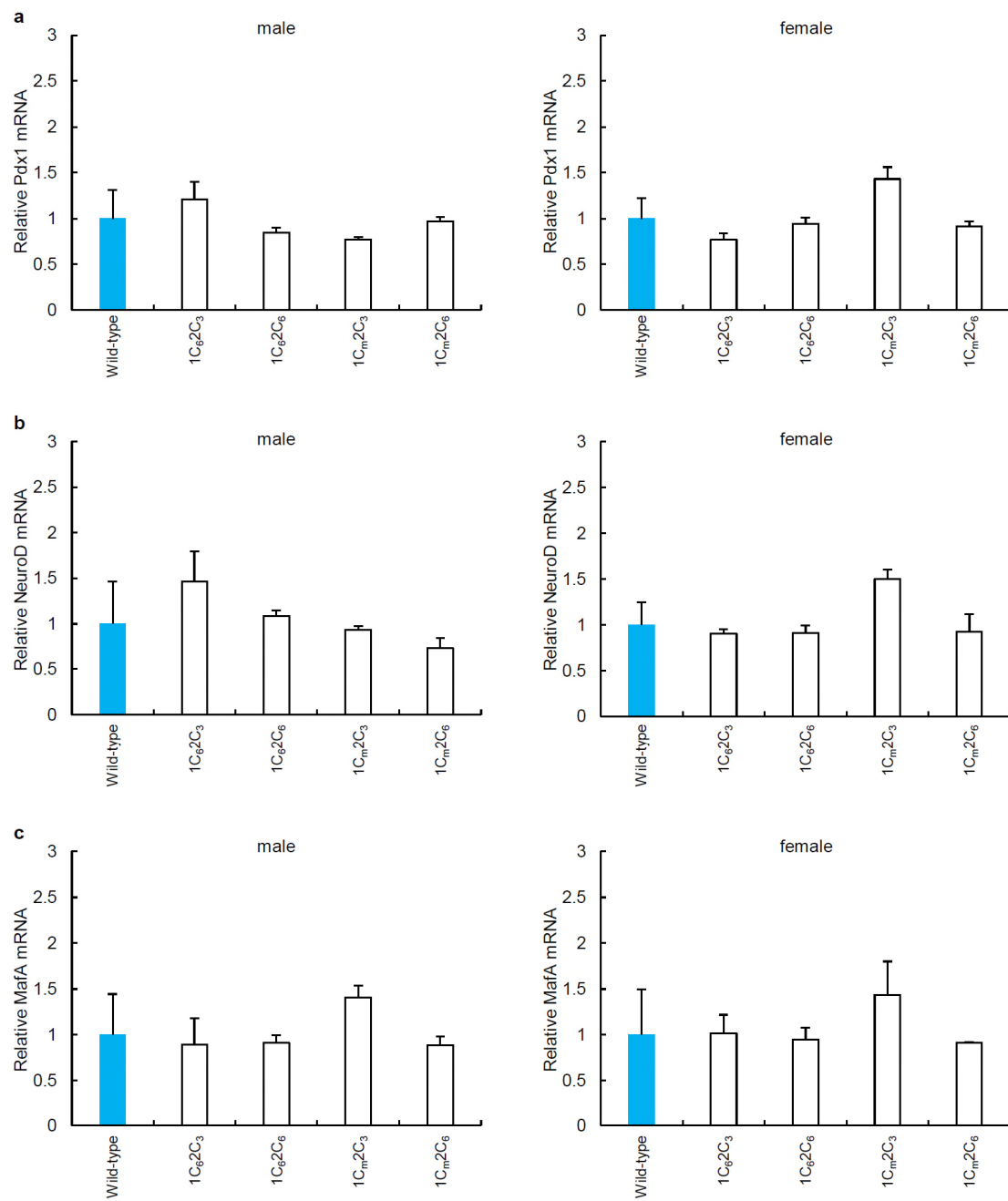


Supplementary Fig 3 Noguchi et al.

Supplementary Fig. 3. *Ins1* and *Ins2* mRNA levels and insulin promoter activities

of heterozygous/homozygous mice with mutations in the *Ins1* and *Ins2* promoters.

a and b qRT-PCR analysis of *Ins1* (**a**) and *Ins2* (**b**) in pancreatic islets of heterozygous mice with mutations in the insulin promoter. **c and d** qRT-PCR analysis of *Ins1* (**c**) and *Ins2* (**d**) in pancreatic islets of homozygous mice with mutations in the insulin promoter. Pancreatic islets (purity >95%) of wild-type mice served as a control. The data are expressed as the target gene-to-*Gapdh* ratio; that of the control cells was arbitrarily defined as 1 (n=8). ** $p < 0.05$



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Supplementary Fig. 4 *Pdx1*, *NeuroD* and *MafA* mRNA levels in mice with insulin promoter mutations.

a qRT-PCR analysis of *Pdx1* expression in pancreatic islets of mice with mutations in the insulin promoter. **b** qRT-PCR analysis of *NeuroD* expression in pancreatic islets of mice with mutations in the insulin promoter. **c** qRT-PCR analysis of *MafA* expression in pancreatic islets of mice with mutations in the insulin promoter. Pancreatic islets (purity >95%) of wild-type mice served as a control. The data are expressed as the target gene-to-*Gapdh* ratio; that of the control cells was arbitrarily defined as 1 (n = 8). The error bars represent the standard error.

Supplementary Table 1. Heterozygous genotypes

Name	Mutated Elements in <i>Ins1</i> Promoter	Mutated Elements in <i>Ins2</i> Promoter	Diabetes	Figure #	Comments
1C ₆ 2C ₃ ^{+/-}	C	C	-	Fig S2C	Mutation of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C ₆ 2C ₆ ^{+/-}	C	C	-	Fig S2C	Deletion of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C ₆ 2 ₃ ^{+/-}	C	none	-	Fig S2B	
1C ₆ 2C ₁ ^{+/-}	C	C (1 base only)	-	Fig S2B	
1C ₆ 2 ₂ ^{+/-}	C	none	-	Fig S2B	
1C ₆ 2 ₁ ^{+/-}	C	none	-	Fig S2B	
1C ₆ ^{+/-}	C	none	-	Fig S2A	
1C _m 2C ₃ ^{+/-}	C (replacement)	C	-	Fig S2C	Mutation of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C _m 2C ₆ ^{+/-}	C (replacement)	C	-	Fig S2C	Mutation of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C _m 2 ₃ ^{+/-}	C (replacement)	none	-	Fig S2B	
1C _m 2C ₁ ^{+/-}	C (replacement)	C (1 base only)	-	Fig S2B	
1C _m 2 ₂ ^{+/-}	C (replacement)	none	-	Fig S2B	
1C _m 2 ₁ ^{+/-}	C (replacement)	none	-	Fig S2B	
1C _m ^{+/-}	C (replacement)	none	-	Fig S2A	
1C ₁ 2C ₃ ^{+/-}	C (1 base only)	C	-	Fig S2B	
1C ₁ 2C ₆ ^{+/-}	C (1 base only)	C	-	Fig S2B	
1C ₁ 2 ₃ ^{+/-}	C (1 base only)	none	-	Fig S2B	
1C ₁ 2C ₁ ^{+/-}	C (1 base only)	C (1 base only)	-	Fig S2B	
1C ₁ 2 ₂ ^{+/-}	C (1 base only)	none	-	Fig S2B	
1C ₁ 2 ₁ ^{+/-}	C (1 base only)	none	-	Fig S2B	
1C ₁ ^{+/-}	C (1 base only)	none	-	Fig S2A	
1 ₂ 2C ₃ ^{+/-}	none	C	-	Fig S2B	
1 ₂ 2C ₆ ^{+/-}	none	C	-	Fig S2B	

$1_2 2_3^{+/-}$	none	none	-	Fig S2B
$1_2 2C_1^{+/-}$	none	C (1 base only)	-	Fig S2B
$1_2 2_2^{+/-}$	none	none	-	Fig S2B
$1_2 2_1^{+/-}$	none	none	-	Fig S2B
$1_2^{+/-}$	none	none	-	Fig S2A
$1_1 2C_3^{+/-}$	none	C	-	Fig S2B
$1_1 2C_6^{+/-}$	none	C	-	Fig S2B
$1_1 2_3^{+/-}$	none	none	-	Fig S2B
$1_1 2C_1^{+/-}$	none	C (1 base only)	-	Fig S2B
$1_1 2_2^{+/-}$	none	none	-	Fig S2B
$1_1 2_1^{+/-}$	none	none	-	Fig S2B
$1_1^{+/-}$	none	none	-	Fig S2A
$2C_3^{+/-}$	none	C	-	Fig S2A
$2C_6^{+/-}$	none	C	-	Fig S2A
$2_3^{+/-}$	none	none	-	Fig S2A
$2C_1^{+/-}$	none	C (1 base only)	-	Fig S2A
$2_2^{+/-}$	none	none	-	Fig S2A
$2_1^{+/-}$	none	none	-	Fig S2A

Supplementary Table 2. Homozygous genotypes

Name	Mutated Elements in <i>Ins1</i> Promoter	Mutated Elements in <i>Ins2</i> Promoter	Diabetes	Figure #	Comments
1C ₆ 2C ₃	C	C	+	Fig 1E, 2, 5, Fig S3	Deletion of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C ₆ 2C ₆	C	C	+	Fig 1E, 2, 3, Fig S3	Deletion of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C ₆ 2 ₃	C	none	-	Fig 1D	
1C ₆ 2C ₁	C	C (1 base only)	-	Fig 1D	
1C ₆ 2 ₂	C	none	-	Fig 1D	
1C ₆ 2 ₁	C	none	-	Fig 1D	
1C ₆	C	none	-	Fig 1C	
1C _m 2C ₃	C (replacement)	C	+	Fig 1E, 2, 6, Fig S3	Mutation of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C _m 2C ₆	C (replacement)	C	+	Fig 1E, 2, 4, Fig S3	Mutation of elements bound by MafA in <i>Ins1</i> and <i>Ins2</i>
1C _m 2 ₃	C (replacement)	none	-	Fig 1D	
1C _m 2C ₁	C (replacement)	C (1 base only)	-	Fig 1D	
1C _m 2 ₂	C (replacement)	none	-	Fig 1D	
1C _m 2 ₁	C (replacement)	none	-	Fig 1D	
1C _m	C (replacement)	none	-	Fig 1C	
1C ₁ 2C ₃	C (1 base only)	C	-	Fig 1D	
1C ₁ 2C ₆	C (1 base only)	C	-	Fig 1D	
1C ₁ 2 ₃	C (1 base only)	none	-	Fig 1D	
1C ₁ 2C ₁	C (1 base only)	C (1 base only)	-	Fig 1D	
1C ₁ 2 ₂	C (1 base only)	none	-	Fig 1D	
1C ₁ 2 ₁	C (1 base only)	none	-	Fig 1D	
1C ₁	C (1 base only)	none	-	Fig 1C	
1 ₂ 2C ₃	none	C	-	Fig 1D	
1 ₂ 2C ₆	none	C	-	Fig 1D	

$1_2 2_3$	none	none	-	Fig 1D
$1_2 2C_1$	none	C (1 base only)	-	Fig 1D
$1_2 2_2$	none	none	-	Fig 1D
$1_2 2_1$	none	none	-	Fig 1D
1_2	none	none	-	Fig 1C
$1_1 2C_3$	none	C	-	Fig 1D
$1_1 2C_6$	none	C	-	Fig 1D
$1_1 2_3$	none	none	-	Fig 1D
$1_1 2C_1$	none	C (1 base only)	-	Fig 1D
$1_1 2_2$	none	none	-	Fig 1D
$1_1 2_1$	none	none	-	Fig 1D
1_1	none	none	-	Fig 1C
$2C_3$	none	C	-	Fig 1C
$2C_6$	none	C	-	Fig 1C
2_3	none	none	-	Fig 1C
$2C_1$	none	C (1 base only)	-	Fig 1C
2_2	none	none	-	Fig 1C
2_1	none	none	-	Fig 1C