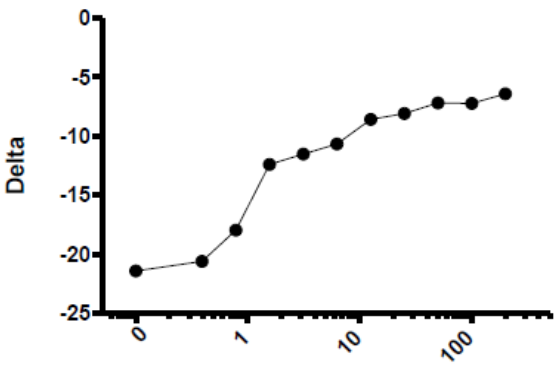
SUPPLEMENTARY DATA

Supplementary Figure 1. *Assay characteristics*. A: Primers and conditions for the PCR reactions. B: Detection limit of the nested PCR reaction: DNA isolated from a patient with long standing T1D, without detectable levels of C-peptide was mixed with dilutions of DNA isolated from human islets, treated with bisulfite, and used for the 2-step PCR reaction. The concentration of total DNA in the serum was between 4-12 ng/µl. Below a concentration of 16 pg/µl, the signal from the unmethylated *INS* DNA was no longer detectable. The deltas for synthetic unmethylated and methylated DNA were -6.04 and < -21. A single experiment, representative of 4 separate experiments is shown. C: Reproducibility of repeated measures: Aliquots of serum from 3 blood draws from the same non-diabetic individual, up to 1 week apart, were analyzed independently. The CVs ranged between 1.1% and 15.8%. We also analyzed the interassay CV with samples from patients with new onset T1D. The interassay CV was 6.43% (range 1.1%-20.1%.)

A.

Group	Primer Orientation	Primer Sequence	PCR Condition
First step PCR	Forward	TTAGGGGTTTTAAGGTAGGGT ATTTGGT	40 cycles, melting temp 54°C
	Reverse	ACCAAAAACAACAATAAACA ATTAACTCACCCTACAA	
Real Time methylation specific nested PCR	Methylated Forward	TAGTCGTAGTTTTTGTGAATTA ATATTTGTGC	50 cycles, melting temp 64°C
	Methylated Reverse	CACCCTACAAATCCTCTACCT CCCG	
	Unmethylated Forward	TTAGTTGTAGTTTTTGTGAATT AATATTTGTGT	
	Unmethylated Reverse	CACCCTACAAATCCTCTACCT CCCA	

B.



C.