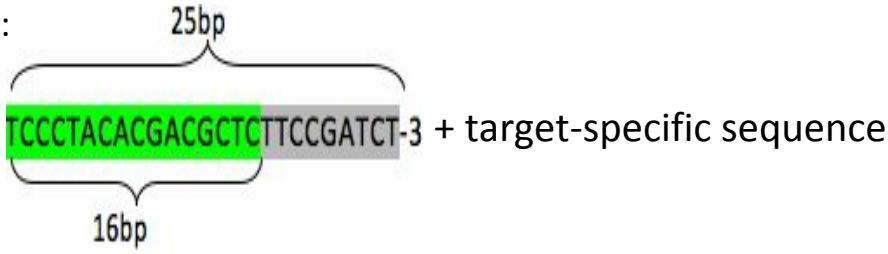
Supplementary Figure S1: primer design for the methylation multiplex PCR method.

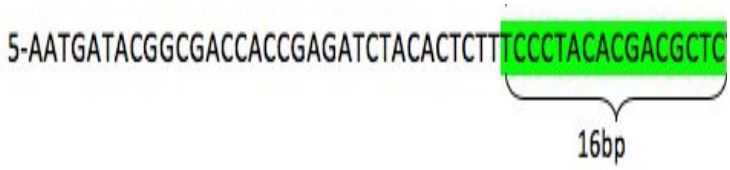
A. Design of forward primers for the 2-step PCR procedure. Scheme shows the sequence of the adaptors used in the forward (left) primers of the 1st PCR reactions, the complete sequence of the primers used in the 2nd step, and the structure of the resulting left flank of the product.
B. Design of reverse primers for the 2-step PCR procedure. Scheme shows the sequence of the adaptors used in the reverse (right) primers of the 1st PCR reactions, and the complete sequence of the primers used in the 2nd step, and the structure of the resulting right flank of the product. The reverse flank contains a 6bp index used as a barcode for distinguishing sequences from different amplifications in the same Nextseq run.

A: design of forward primers

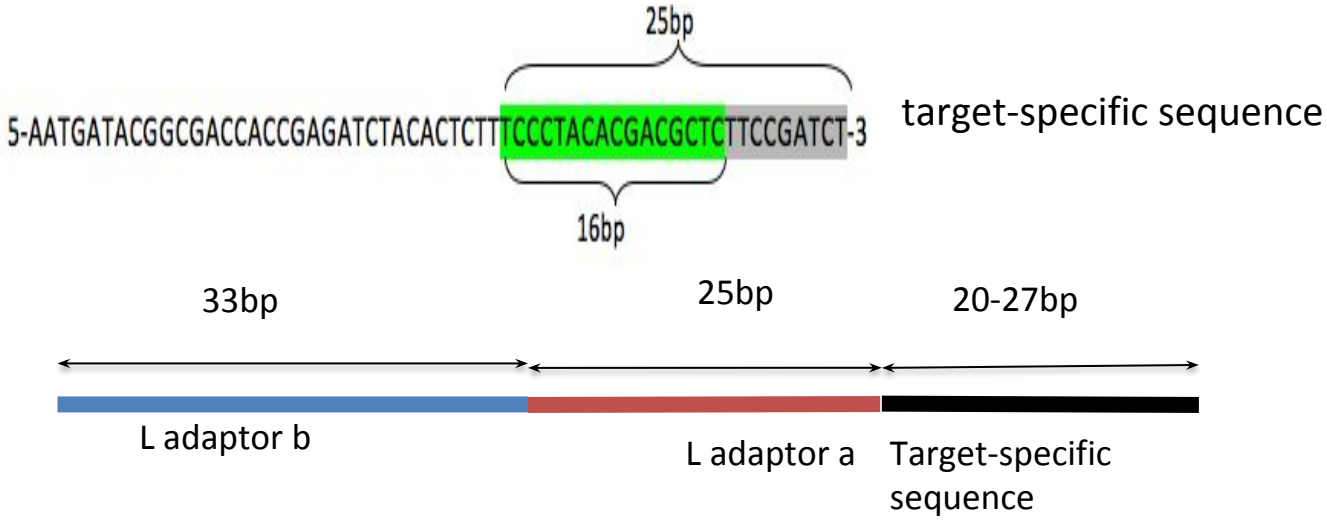
1st step PCR primers:



2nd step PCR primer:



Structure of final forward flank:



Forward primer:

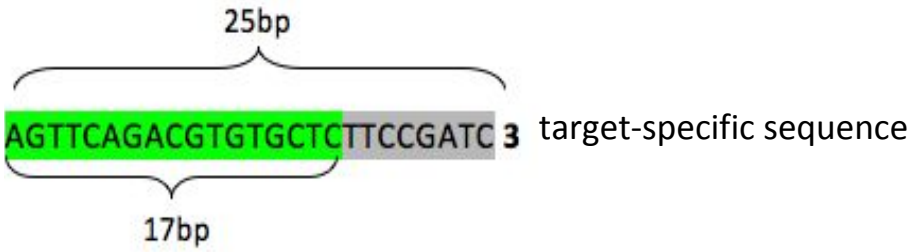
TruSeq Universal Adapter :

5 AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 3

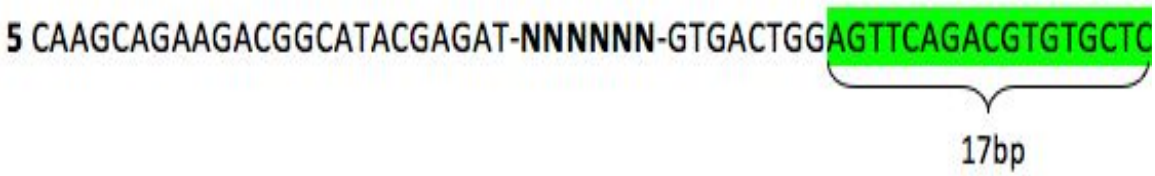
Supplementary Figure S1 continued

B: design of reverse primers

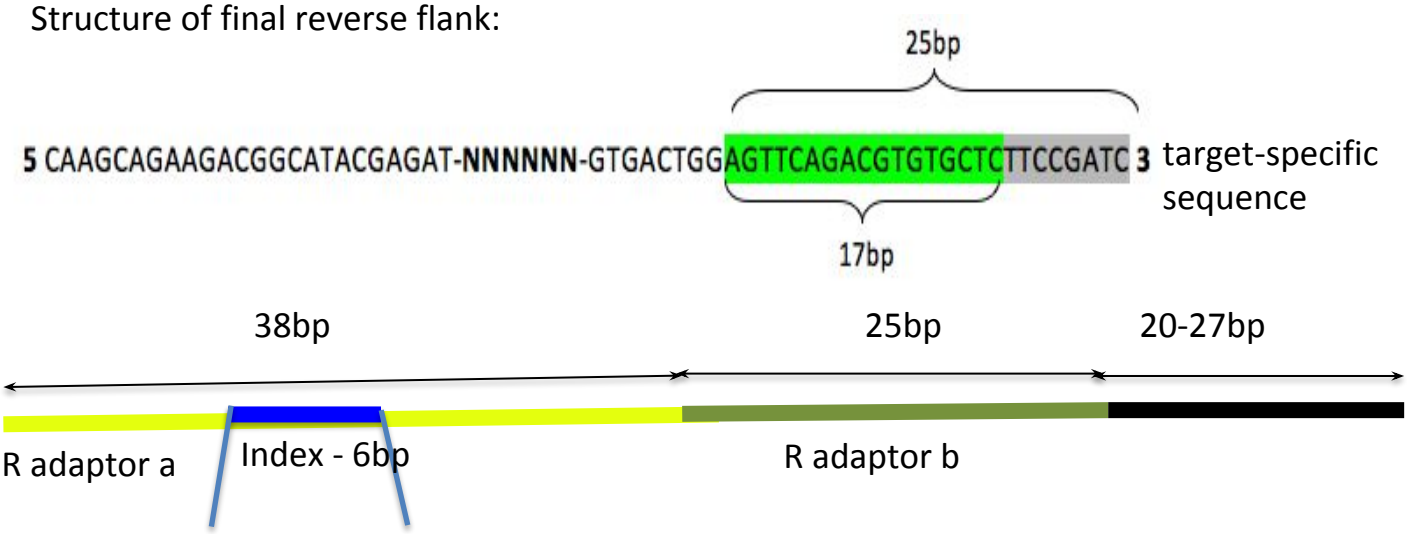
1st step PCR primers:



2nd step PCR primer:



Structure of final reverse flank:



Reverse Primer:

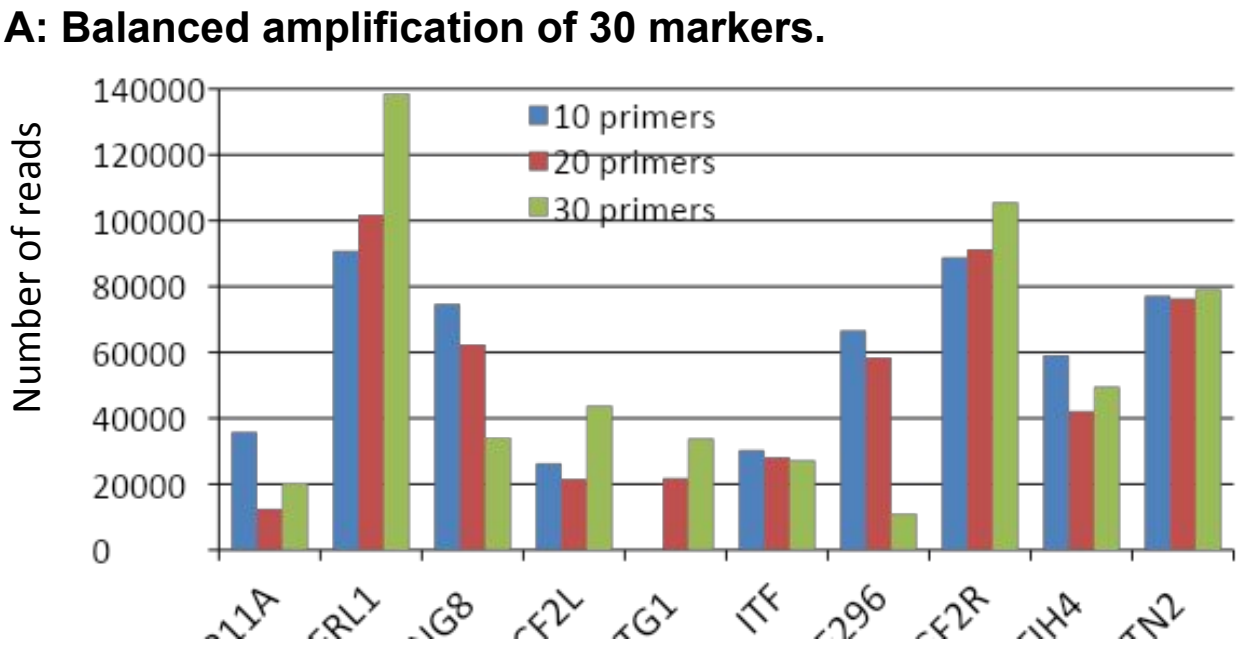
Truseq indexed adapter reverse complement follows:

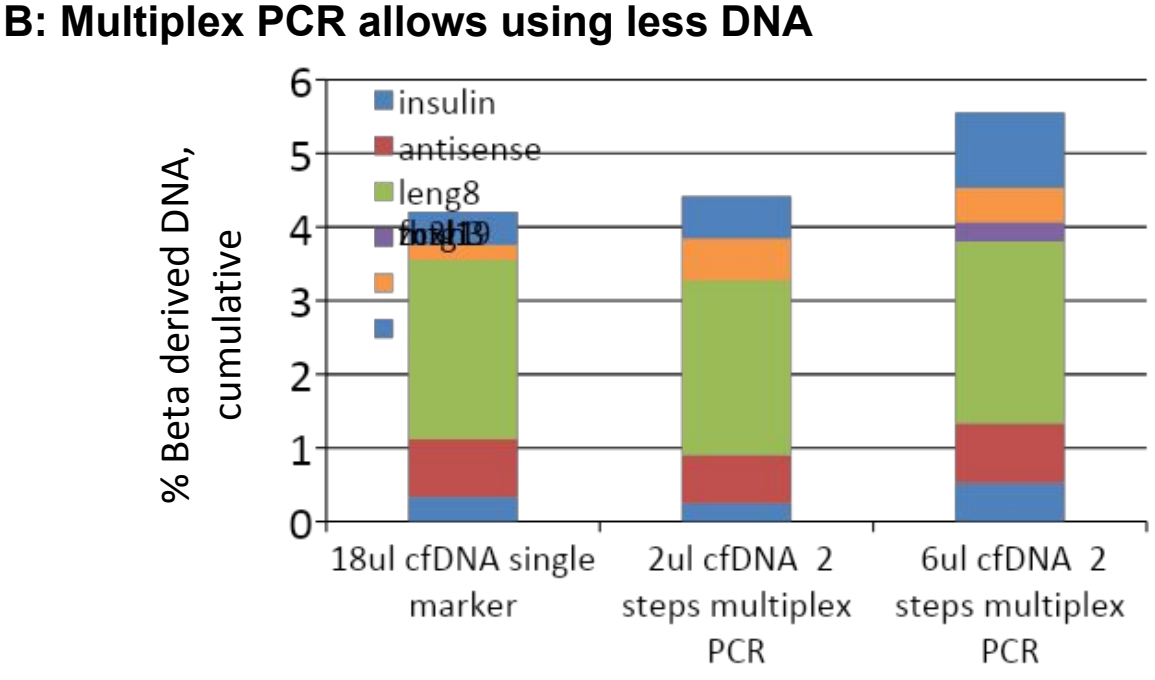
5 CAAGCAGAAGACGGCATACGAGAT-**NNNNNN**-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC **3**

Supplementary Figure S2: performance of the multiplex methylation PCR assay.

A. Balanced multiplex amplification of up to 30 methylation markers. A cocktail of 10 hepatocyte-specific methylation markers (unmethylated specifically in hepatocytes and methylated elsewhere; unpublished data) was used in a 2-step multiplex reaction on cfDNA from a healthy donor (known to contain about 1% liver cfDNA). The reaction mix contained only these 10 primers, or an additional 10 unrelated primers, or an additional 20 unrelated markers. The similar number of sequence reads in all 3 reactions indicates that the 10 hepatocyte markers were amplified with similar efficiency even in the background of 30 different reactions in the same tube.

B. Multiplex PCR increases sensitivity of the assay, and allows using less cfDNA. Five loci with a pancreas-specific methylation pattern were probed. Left bar, we performed 5 independent PCR reactions, each using 2 ul of bisulfite converted cfDNA (a total of 10 ul needed). Right bar, multiplex PCR was performed using all 5 primer pairs and only 2 ul template.



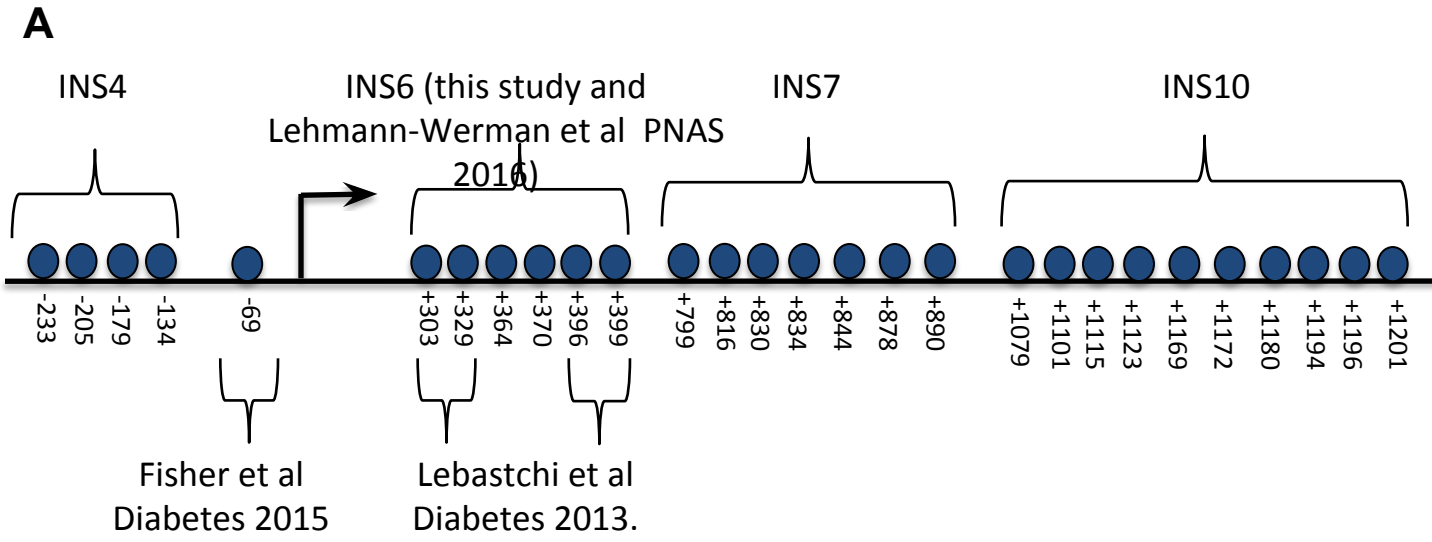


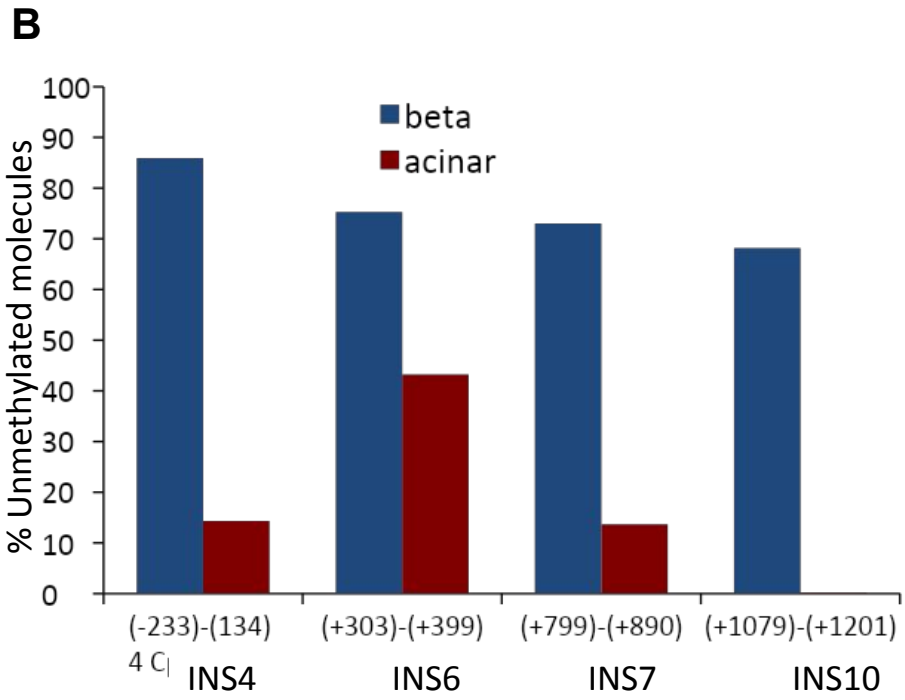
Supplementary Figure S3: Analysis of insulin gene methylation.

A. Schematic of CpG sites in the insulin gene, relative to the transcription start site, along with indication of publications that used each site or sites for cfDNA analysis.

B. Percentage of unmethylated molecules from each region of the insulin gene, scoring molecules that are unmethylated in all cytosines, in sorted human beta cells and acinar cells. The downstream area of the gene appears to have a beta-cell-specific demethylation.

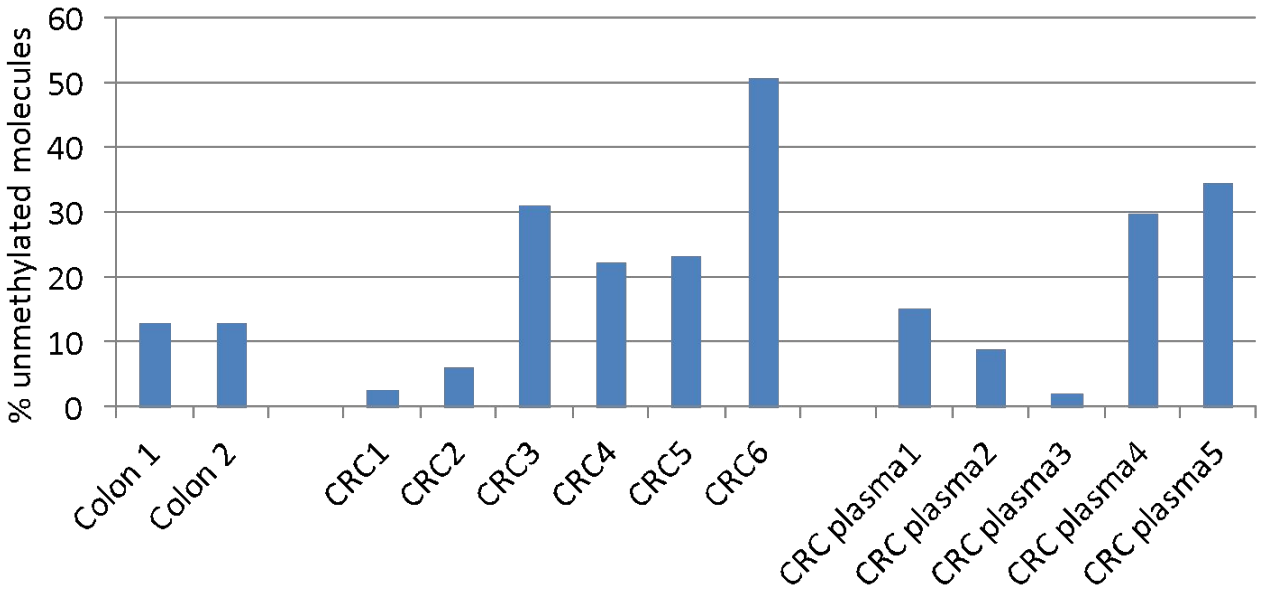
C. Unmethylated insulin in intestinal tissue, colorectal cancer and plasma of patients with colorectal cancer. Graph shows the percentage of unmethylated insulin molecules that are present in DNA from a normal intestine (samples from 2 individuals), colorectal cancer (CRC, n=6), and cfDNA from patients with advanced CRC (n=5).



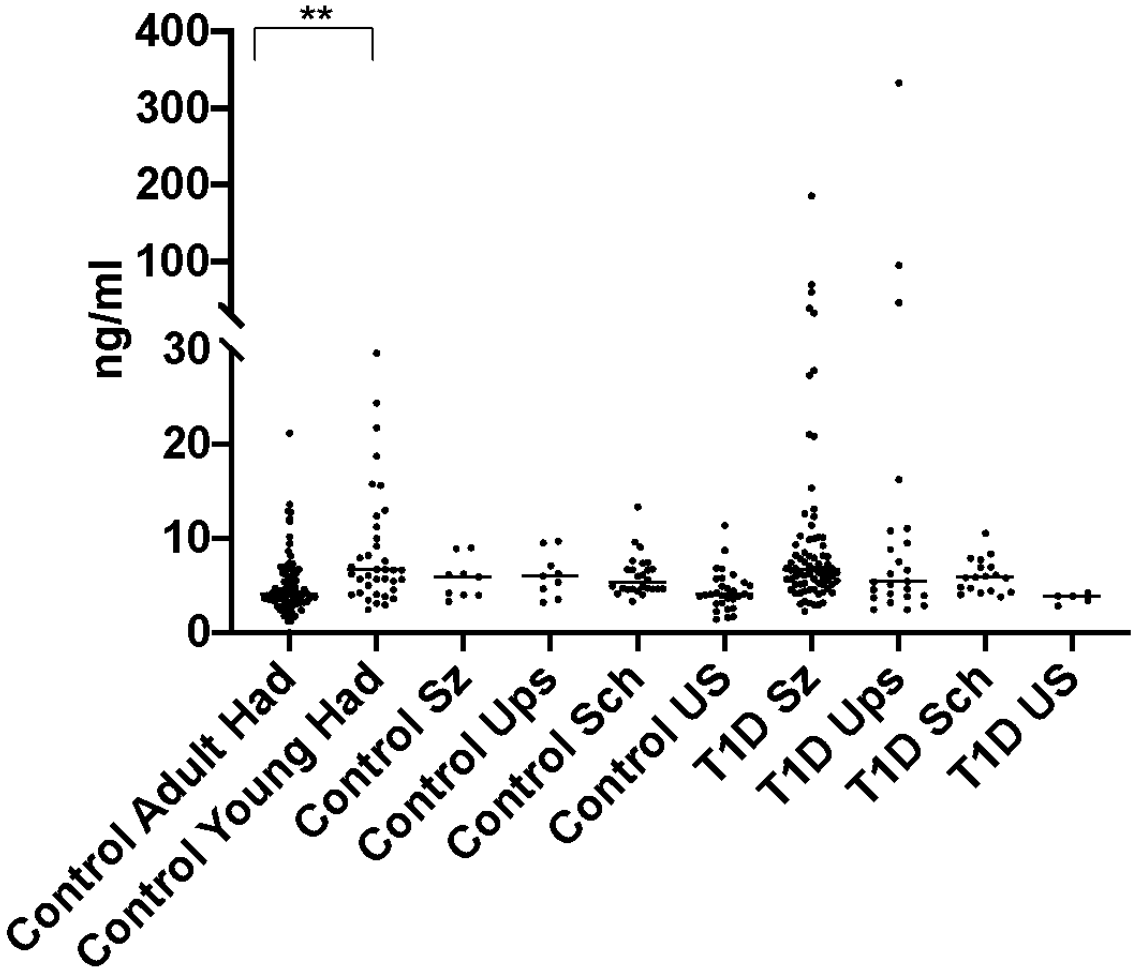


Supplementary Figure S3 continued

C



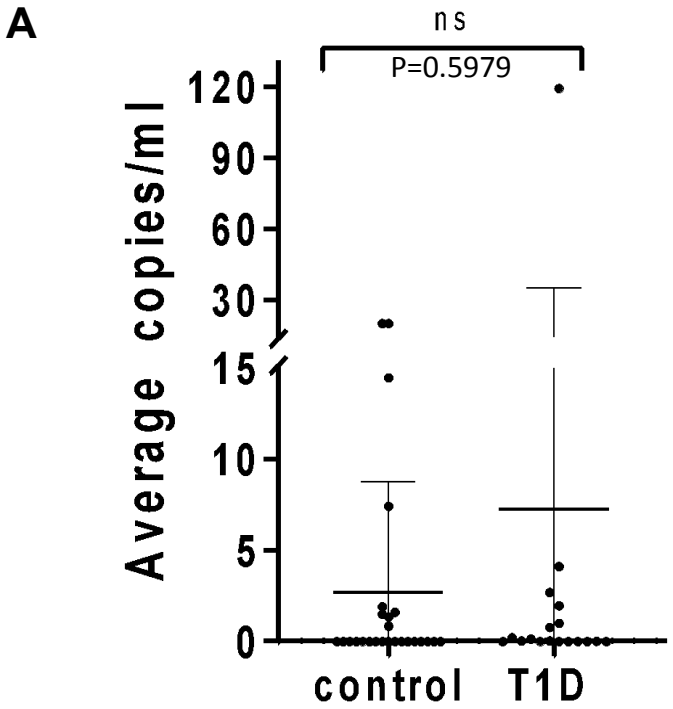
Supplementary Figure S4: Concentration of cfDNA in plasma samples of healthy controls and T1D patients from 4 different cohorts

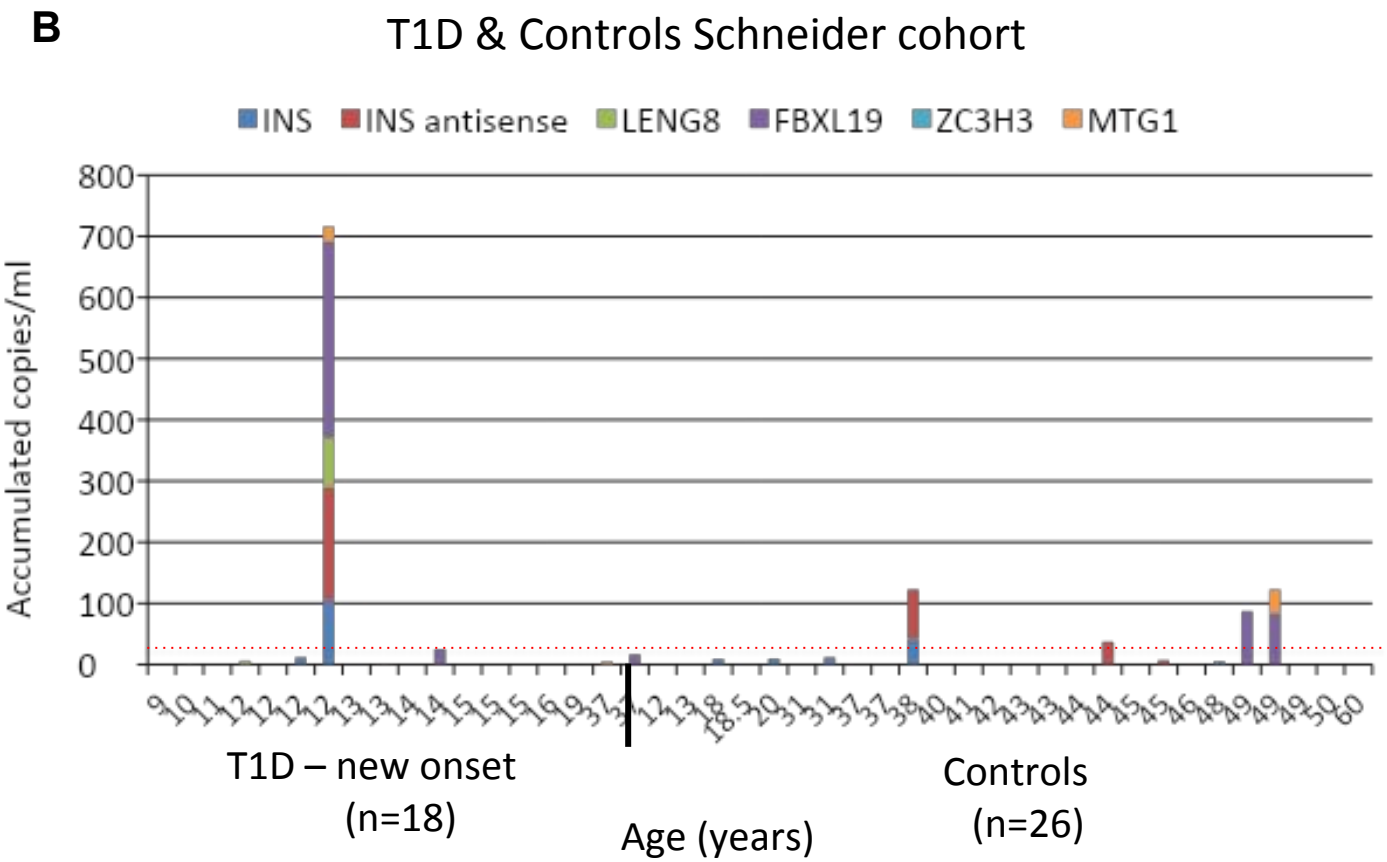


Supplementary Figure S5: Methylation patterns in plasma samples obtained from Schneider Children's hospital.

A. Average of beta-cell DNA signals in all samples.

B. Individual samples.

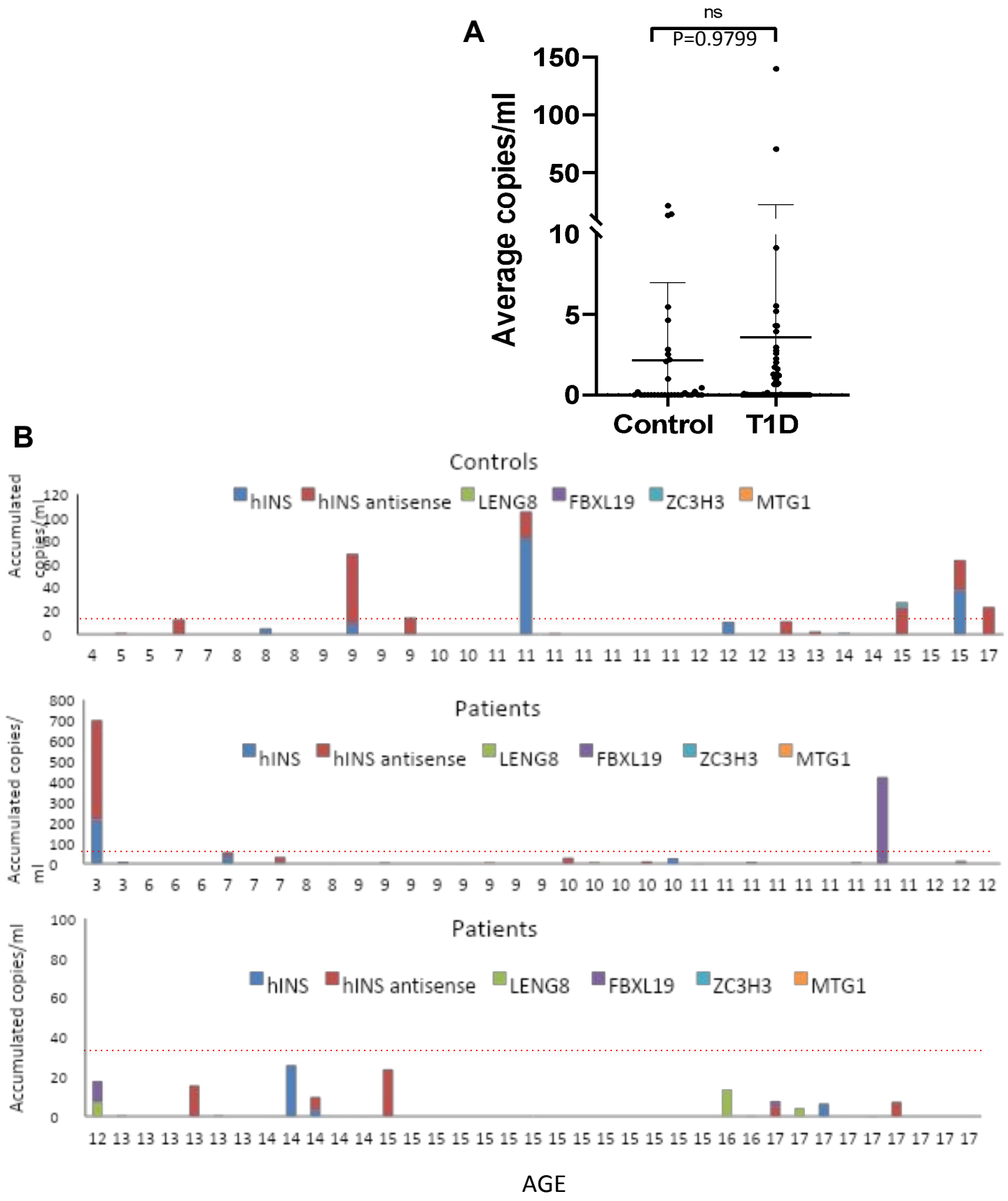




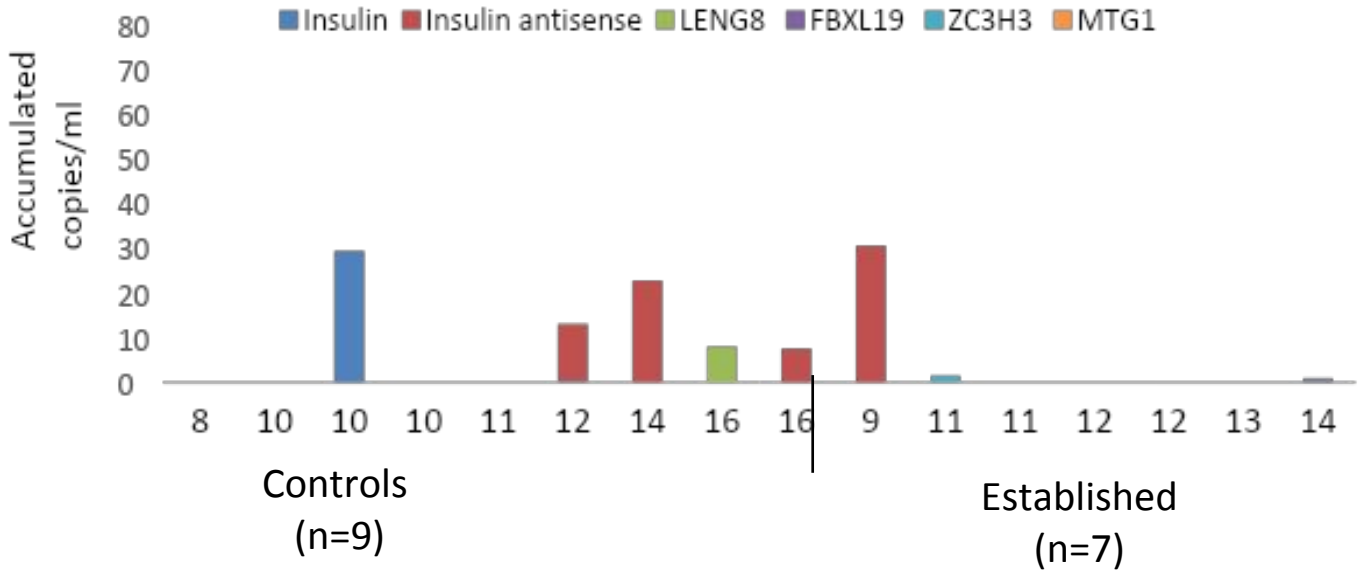
Supplementary Figure S6: Methylation patterns in plasma samples obtained from Shaare Zedek Medical Center.

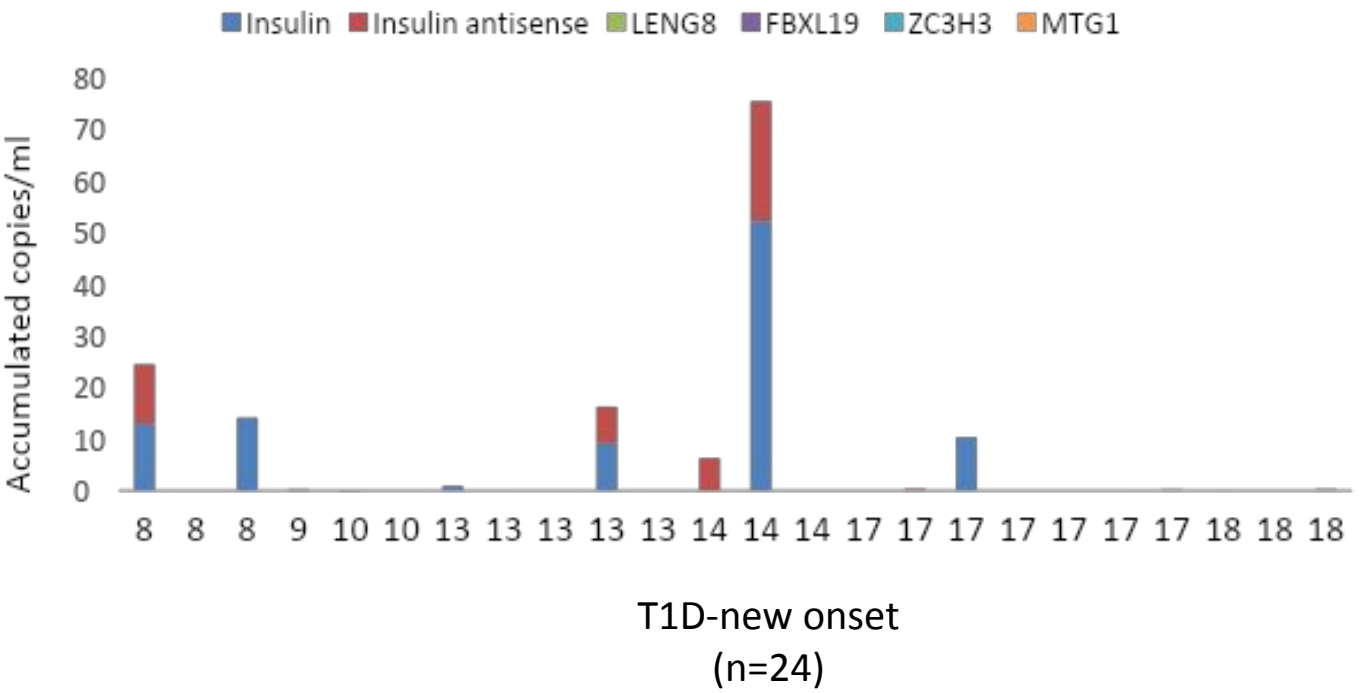
A. Average of beta-cell DNA signals in all samples.

B. Individual samples.

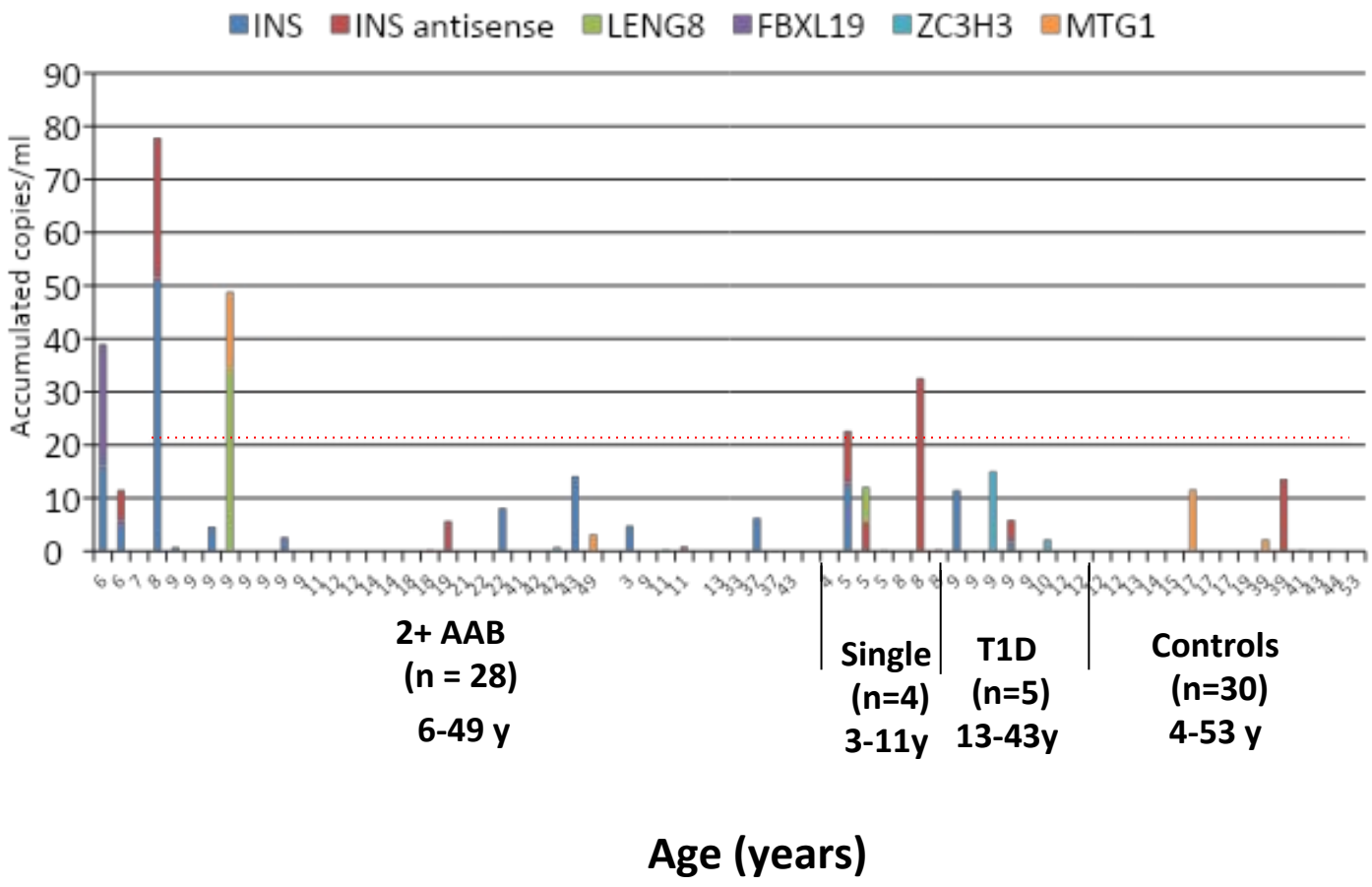


Supplementary Figure S7: Methylation patterns in plasma samples obtained from Uppsala.





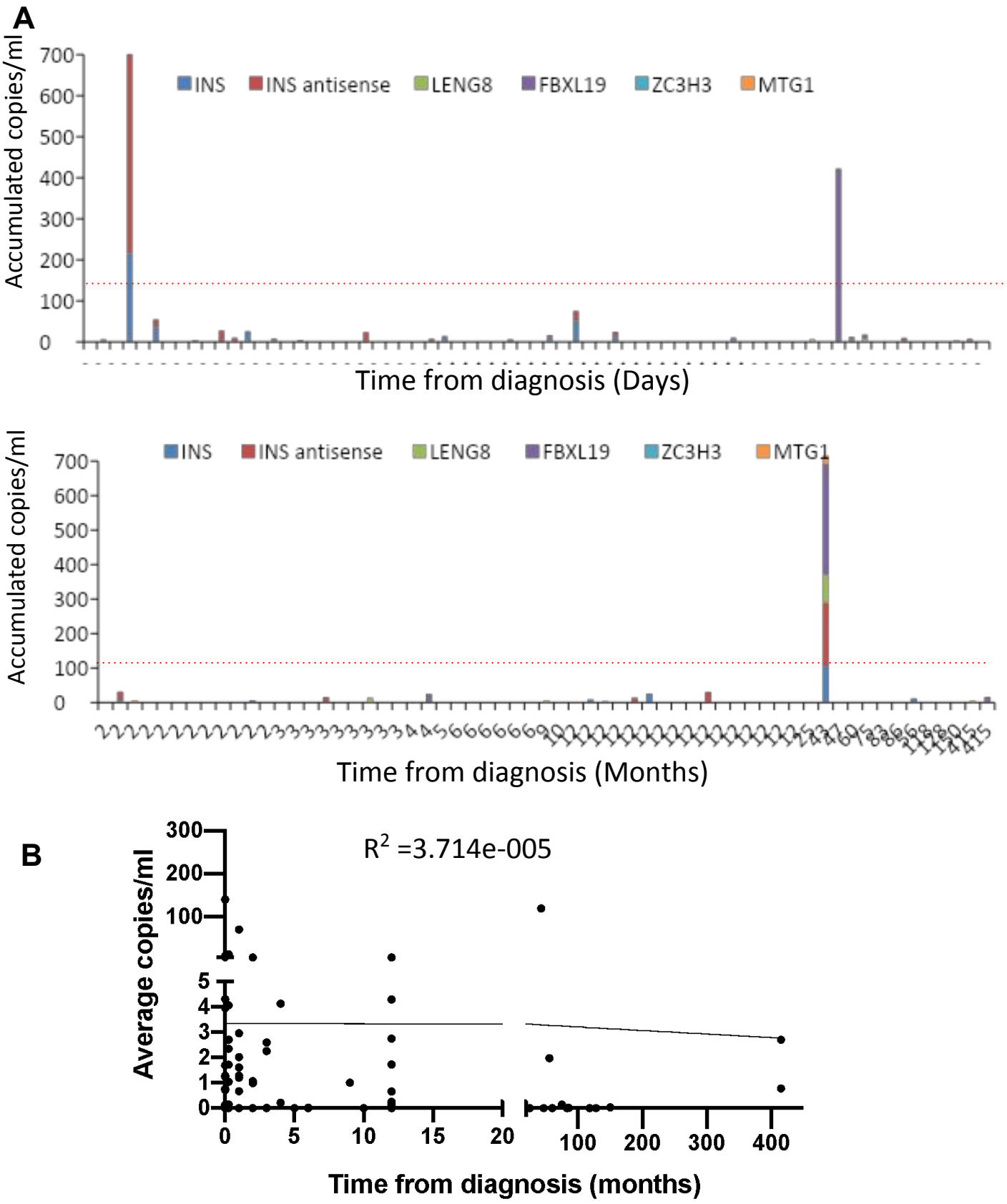
Supplementary Figure S8: Methylation patterns in plasma samples obtained from the US cohort.



Supplementary Figure S9: Beta-cell markers in cfDNA as a function of duration of T1D.

A. Time from diagnosis of T1D, in days (upper panel) and months (lower panel). Each point at the X axis is an individual.

B. Correlation between beta-cell signal and time from diagnosis.



Supplementary Table S1: Target sequences of primers used for multiplex amplification.

Locus	Forward primer	Reverse primer	Position
INS	TTGTTGGTTTTTTGGGGATT	ACCCTACAAATCCTCTACCTCC	Chr11-2182015: 2182157
INS antisense	GTTTATTTTGTAGGTTTTTTGTTT	CTACTAACCCTCTAAAAACCTAAC	11-2182008: 2182157
LENG8	TAGGTTTTTTTAGTATAGTATGGTG	CAACTCCTAACTTACTAATACTAACC	Chr19-54963267: 54963394
FBXL19	TTGGTAGGTTTGGAGTTGATAG	AAAAATAAACACTAAAATCCCC	Chr16-30958098: 30958215
ZC3H3	GTTTTTTTATATATAATTATAAGTTGTT	ATAAAAATACTTACTACTACCTTTCC	Chr8-144546327: 144546436
MTG1	GGAGGTTGTAGTGAGTTAAGATTA	TCAACAACTACTCAATTACACACTA	Chr10-135206679: 135206808