Supplemental Figure 1: Assay reproducibility and age effect. (A) Histogram of pairwise Pearson correlations coefficients (R) for all 96 technical controls from the SSC experiments. The dashed red line represents the mean correlation coefficient (R = 0.990). The controls are made up of 20 replicates of lymphocyte DNA and 76 replicates of a lymphoblast cell line DNA; notably, each replicate type was only compared to itself. (B) Representative plot of two technical replicates shows the correlation of β values (R = 0.990). (C) Quantile-quantile (Q-Q) plot of age effect in the SSC group. Each point represents one CpG locus interrogated by the assay and the age effect (*t*-statistic) is plotted on the y-axis compared to that expected by the null hypothesis on the x-axis. Loci that have a significant age-association (FDR<0.01) are colored red (age-methylated) or green (age-demethylated). The solid blue line represents the distribution under the null hypothesis and the dashed blue lines represent the point-wise significance thresholds. (D) Scatterplot of permuted (1,000 permutations) age-associated *P*-values (x-axis) compared to asymptotic *P*-values (y-axis) calculated using the linear model in the SSC group (Pearson R = 0.999). (E-F) Scatter plots of age effect *t*-statistics published by Teschendorff *et al.* (Teschendorff et al. 2010) in the T1D (Pearson R = 0.981) and OC (R = 0.987) populations, compared to *t*-statistics based on analysis of the same data via our approach. (G) Q-Q plot of age effect in the CHB group, as described for panel C. (H) Venn diagrams of significant (FDR<0.01) age-methylated (red) and age-demethylated (green) loci in the SSC (solid line) and CHB (dashed line) groups. Diagrams include only loci interrogated by both the HumanMethylation27 (SSC) and HumanMethylation450 (CHB) panels (*N*=25,978). Significant loci for each group were determined by independent analysis where correction for multiple hypothesis testing accounted for all

loci on their respective platforms.

Supplemental Figure 2: Validation and replication of age-associated DNA methylation. (A-B) Scatterplots of Infinium DNA methylation levels (β; y-axis) compared to percent methylation determined by pyrosequencing (x-axis) in a subset of 75 SSC individuals at two representative loci. P-value representing significance of correlation between the Infinium methylation level and pyrosequencing is shown in the top right corner, and the linear trend is shown as a blue line. (C-F) Interrogation of the age-effect in surrounding CpG loci located near the two representative age-associated loci chosen for validation by pyrosequencing (Figure 1). Shown are Infinium HumanMethylation27 (dark blue) and pyrosequencing (gray) data for a subset of 75 individuals in the SSC population as well as HumanMethylation450 data for the entire CHB population (light blue). Lines represent the linear regression of each set of data independently and the *P*-values are the significance of age-associated DNA methylation. The y-axis is the methylation level measured by the HumanMethylation27, HumanMethylation450 or pyrosequencing assays. Below each plot is a schematic of the interrogated locus and annotated genes in the region. The CpG locus shown in the above plot is indicated by the black triangle and CpG coverage of each assay is denoted by vertical lines with those colored in red denoting a significant (FDR<0.01) age-methylating effect and those in green a significant age-demethylating effect; those in black are not significantly associated with age. The chromosome (chr), total CpGs in the region (small vertical black lines) and relative genomic coordinates (NCBI build 36.1) are denoted on the x-axis below the gene schematic.

Supplemental Figure 3: Overlap of age-associated DNA methylation in pediatric and adult populations. (A-B) Scatterplots of the DNA methylation age effect (*t*-statistic) in SSC pediatric individuals compared to that published by Teschendorff *et al*. for the T1D (A) and OC (B) adult population. The Pearson correlation coefficients (R) are shown in the bottom right corners and are larger than expected by chance ($P<0.001$). (C) Venn diagrams of the overlap of age-associated genes in the SSC pediatric and adult T1D and OC populations for age-methylated (red) and age-demethylated (green) loci. The overlap between the SSC pediatric cohort and the T1D and OC studies is larger than expected by chance for both studies ($P<0.001$). (D-F) Scatterplots of the age-methylated effect (*t*-statistic) in the SSC population as compared to the published age correlations (ρ) by Rakyan *et al.* for whole blood (D), $CD4^+$ T-cells (E) and $CD14^+$ monocytes. (G) Venn diagram showing the overlap of SSC age-associated loci and those found in $CD34^+$ hematopoietic progenitor cells (HPC) by Bocker *et al*. where a significant overlap exists ($P<0.001$).

Supplemental Figure 4: Preferential location of age-dependent loci in the T1D and OC adult populations relative to CpG islands. (A-B) Proportion of age-methylated (red), age-demethylated (green) and total assay loci (gray) in CpG islands (A) and CpG island "shores" (B) in the T1D and OC populations.

Supplemental Figure 5: Proportion of age-methylated loci reported on the X-chromosome by Teschendorff *et al*. in the T1D and OC adult populations as compared to the total proportion of assay probes on the X-chromosome. *P*-values are calculated using

Fisher's exact test

Supplemental Figure 6: Nucleotide overrepresentation in age-associated loci. (A-B) Nucleotide frequencies of the flanking 60 bp to each side of the CpG interrogated by the HumanMethylation27 (A) and HumanMethylation450 (B) panels. (C-F) Relative proportions of nucleotides at each position for age-demethylated (C-D) and age-demethylated (E-F) loci in the SSC (C, E) and CHB (D, F) pediatric populations. The relative proportion is calculated for each base as the proportion of age-associated loci that are a given nucleotide divided by the proportion of all assay probes that contain that same nucleotide at the given location.