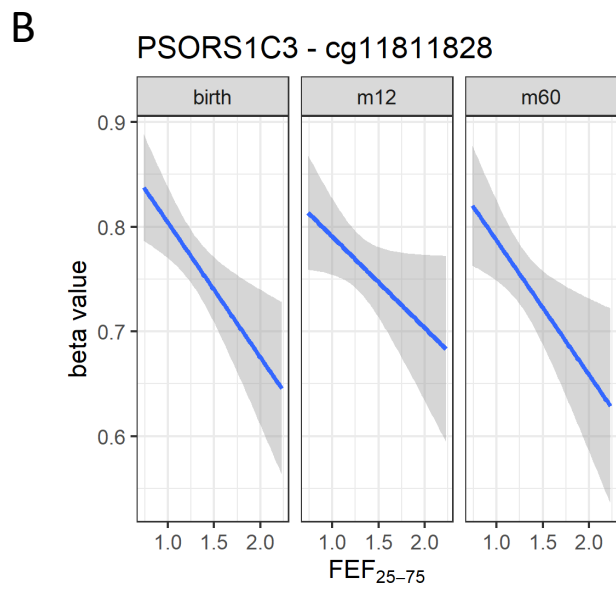
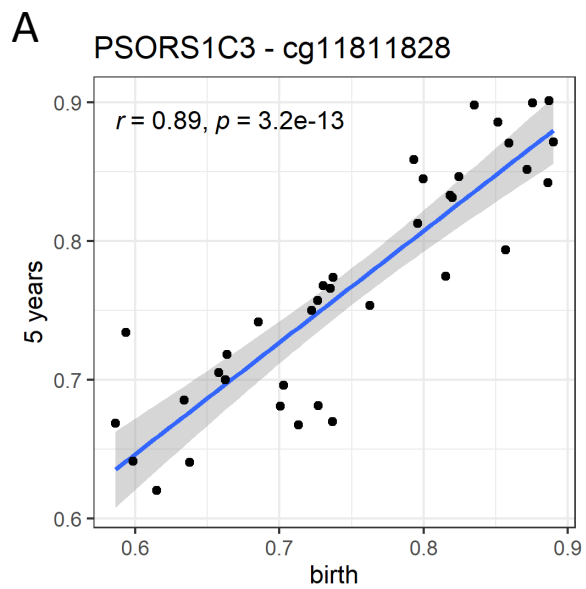
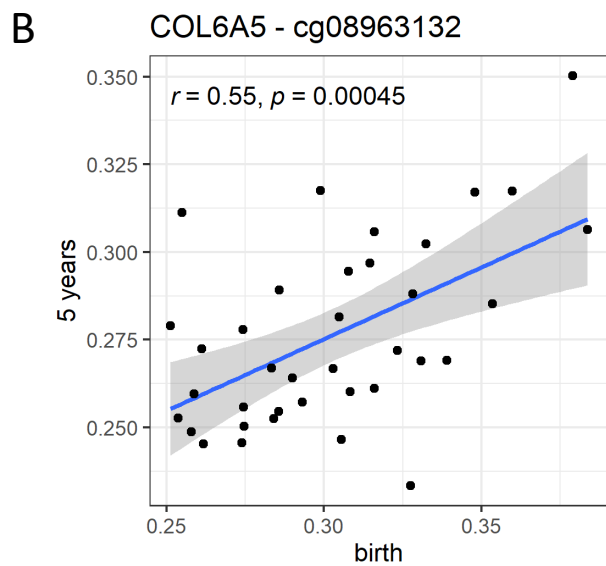
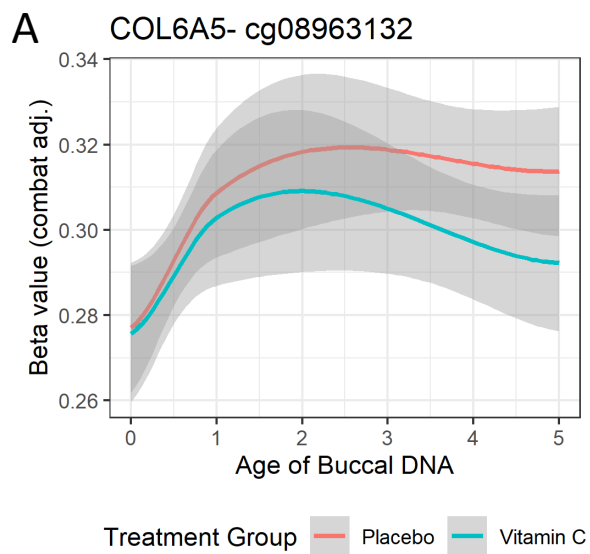


Example of genotype inspection







Supplemental Methods:

Collection of buccal samples

Patients were instructed not to eat or drink at least 30 minutes before buccal sample collection. Buccal DNA was collected using 4 Sterile HydraFlock® Large Tip Flocked Swabs (Puritan 253406H). Each swab was applied to the inside of the child's cheek and rolled up and down for 30-60 seconds, making certain to roll the swab over the entire inner cheek and upper gum. Swabs were placed together in a 15 ml conical tube containing 1600ul of 1X Saliva Lysis Buffer (SLB) before capping tube and vortexing for 10 seconds. The swabs were frozen upright in SLB at -20°C within 1 week from sampling and stored until DNA extraction.

2X Saliva Lysis Buffer

0.3 M TRIS-HCl

0.67 M urea

0.67 M NaOAc

0.6% sodium dodecyl sulfate

3.3 mM EDTA

30% ethanol

Extraction of buccal DNA

Buccal DNA was extracted using the Maxwell® 16 Instrument (Promega AS3000) and Maxwell® 16 Blood DNA Extraction Kit (Promega AS1010). Proteinase K (Promega 20mg/ml) was added to 15ml conicals containing 4 swabs and ~1.6ml SLB. Tubes were vortexed to collect SLB at bottom. Tubes were incubated in a 56°C water bath for 1 hr and then vortexed. Lysate (800ul) was added to well #7 of the Maxwell® 16 Blood DNA cartridge. The remaining lysate was stored for a second DNA extraction. Alcohol Wash Buffer (2 parts Milli-Q water, 1 part 100% EtOH, 1 part 100% isopropanol) was added to wells #4, #5 and #6. Cartridges were processed using the Buffy Coat Protocol on the

Maxwell® 16 Instrument and eluted in 400ul elution buffer. Purity was measured by spectrophotometer and concentration measured with the Quant-iT PicoGreen dsDNA Assay (Invitrogen® P7589).

Quantification of DNAm

Buccal DNAm was assessed on the MethylationEPIC BeadChips, measuring over 850,000 CpGs at a nucleotide resolution, at the Fred Hutchinson Cancer Research Center Genomics Resource. In brief, up to 500ng of DNA was bisulfite converted using the EZ DNA Methylation Kit (Zymo Research) following the Illumina-specified instructions. Converted DNAs were applied to Illumina Infinium Methylation EPIC 8-Sample Beadchips following the Infinium HD Methylation 15019521v01 protocol. Processed BeadChips were scanned using the Illumina iScan+ with ICS v3.3.28 and intensity data was extracted with Illumina GenomeStudio software (GenomeStudio v2011.1 with Methylation Analysis Module v1.9.0).