**Supplementary materials**

**Sex Differences in the Intergenerational Link Between Maternal and Neonatal Whole Blood DNA Methylation: A Genome-wide Analysis in 2 Birth Cohorts**

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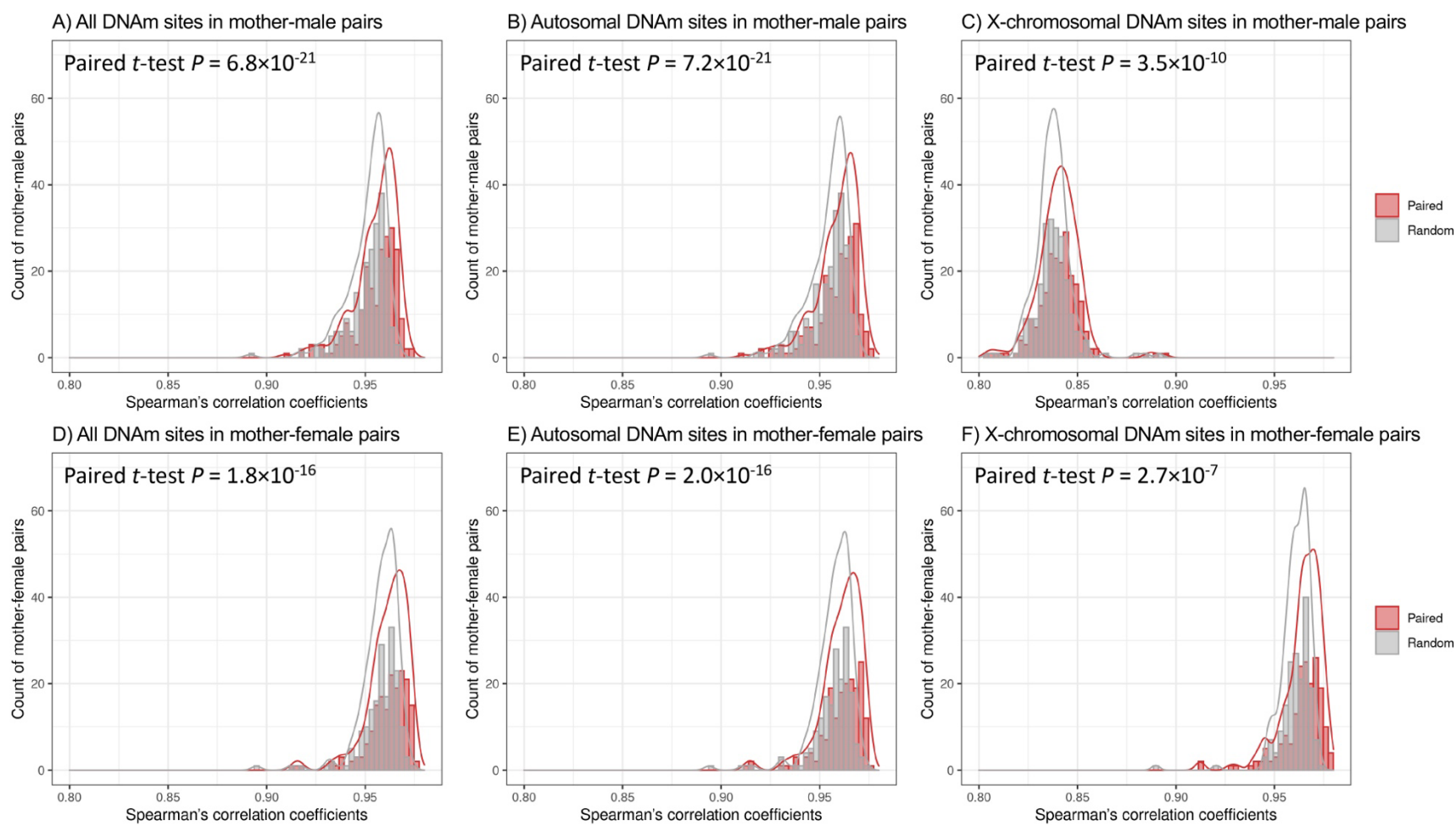
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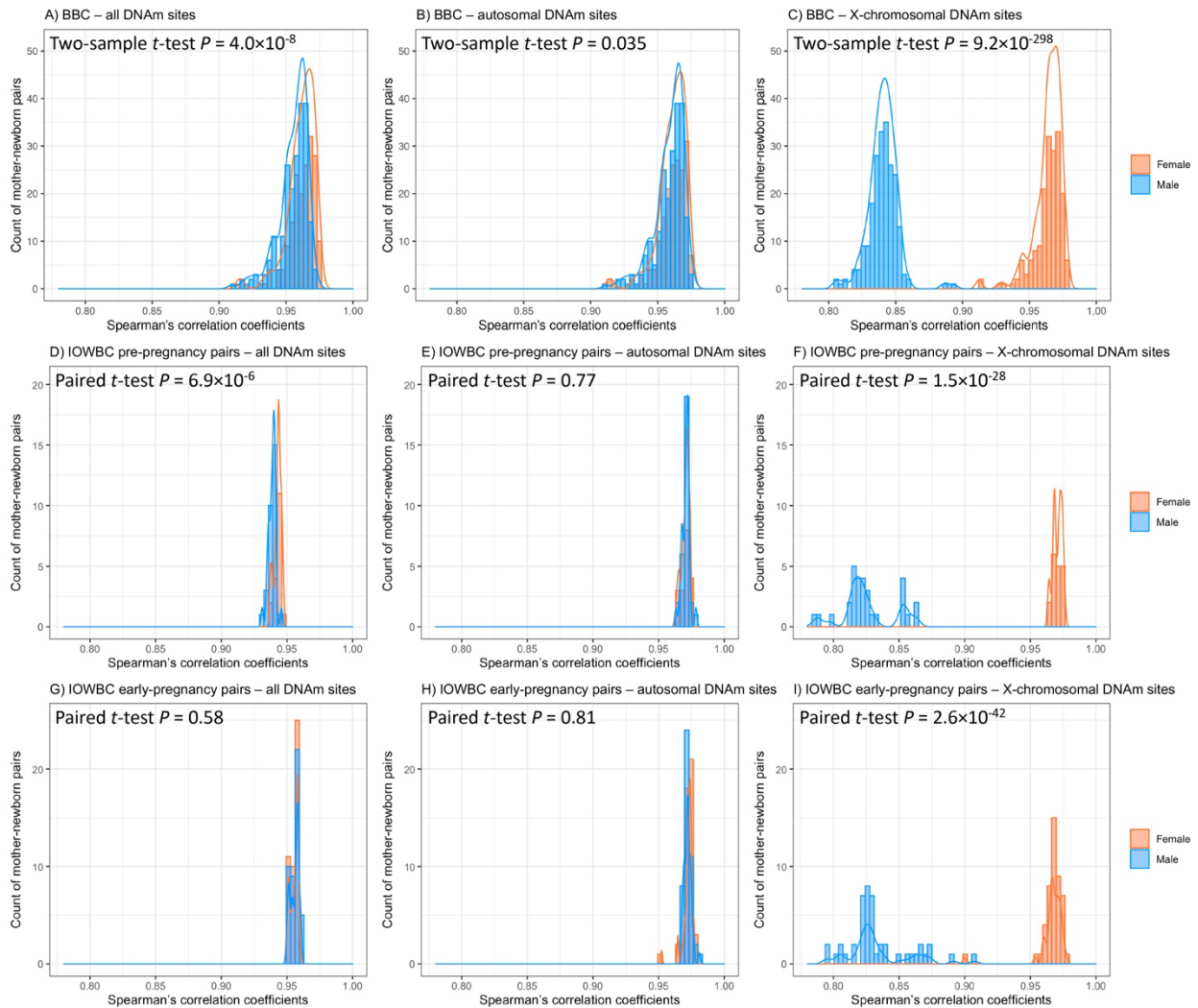
**Supplementary Figure S1.** Distributions of overall maternal-newborn correlations in DNAm, stratified by newborn sex.

Overall mother-newborn correlations comparing paired maternal-newborn samples (marked as red) and randomly paired maternal-newborn samples (computed from 10 permutation; marked as grey), stratified by A-C) mother-male and B-D) mother-female pairs.



A) All DNAm sites in mother-male pairs
Paired *t*-test $P = 6.8 \times 10^{-21}$

B) Autosomal DNAm sites in mother-male pairs
Paired *t*-test $P = 7.2 \times 10^{-21}$

C) X-chromosomal DNAm sites in mother-male pairs
Paired *t*-test $P = 3.5 \times 10^{-10}$

D) All DNAm sites in mother-female pairs
Paired *t*-test $P = 1.8 \times 10^{-16}$

E) Autosomal DNAm sites in mother-female pairs
Paired *t*-test $P = 2.0 \times 10^{-16}$

F) X-chromosomal DNAm sites in mother-female pairs
Paired *t*-test $P = 2.7 \times 10^{-7}$

**Supplementary Figure S2.** Distributions of overall maternal-newborn correlations in DNAm by newborn sex in the BBC and IOWBC.

Overall mother-newborn correlations comparing between newborn sex for all, autosomal, and X-chromosomal DNAm sites in A-C) mother-newborn pairs in the BBC, D-F) pre-pregnancy mother-newborn pairs in the IOWBC, and G-I) early-pregnancy mother-newborn pairs in the IOWBC.
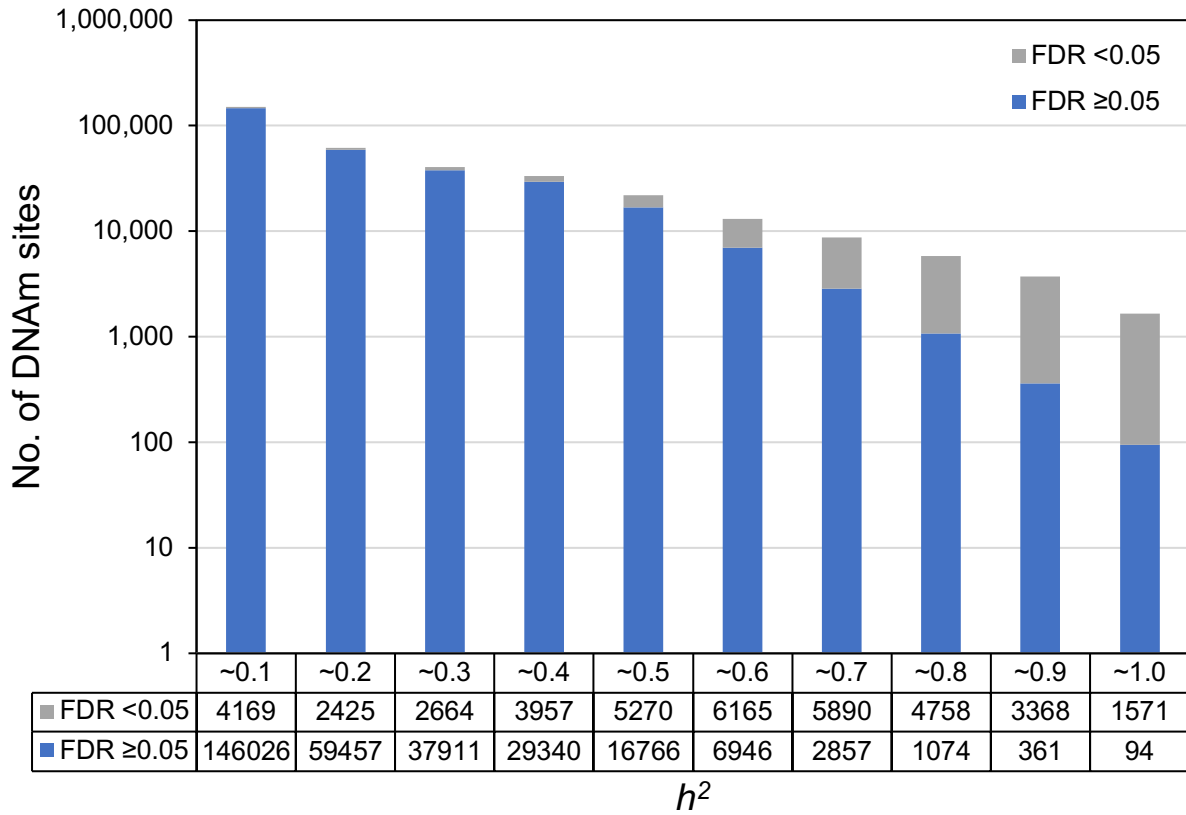
**Supplementary Figure S3.** Distribution of p-values from Likelihood Ratio Test that estimated mother-newborn associations in methylation levels at individual DNAm sites, by the $h^2$ of each DNAm site.

The X-axis is the heritability ($h^2$) of individual DNAm sites based on a published study on DNAm quantitative trait loci (van Dongen et al. 2016; PMID: 27051996), but only the heritability of autosomal DNAm sites was available. The Y-axis is the $-\log_{10}$(p-values) from Likelihood Ratio Test that estimated the associations in methylation levels at individual DNAm sites between mothers and their newborns. The red horizontal lines marks FDR=0.05.
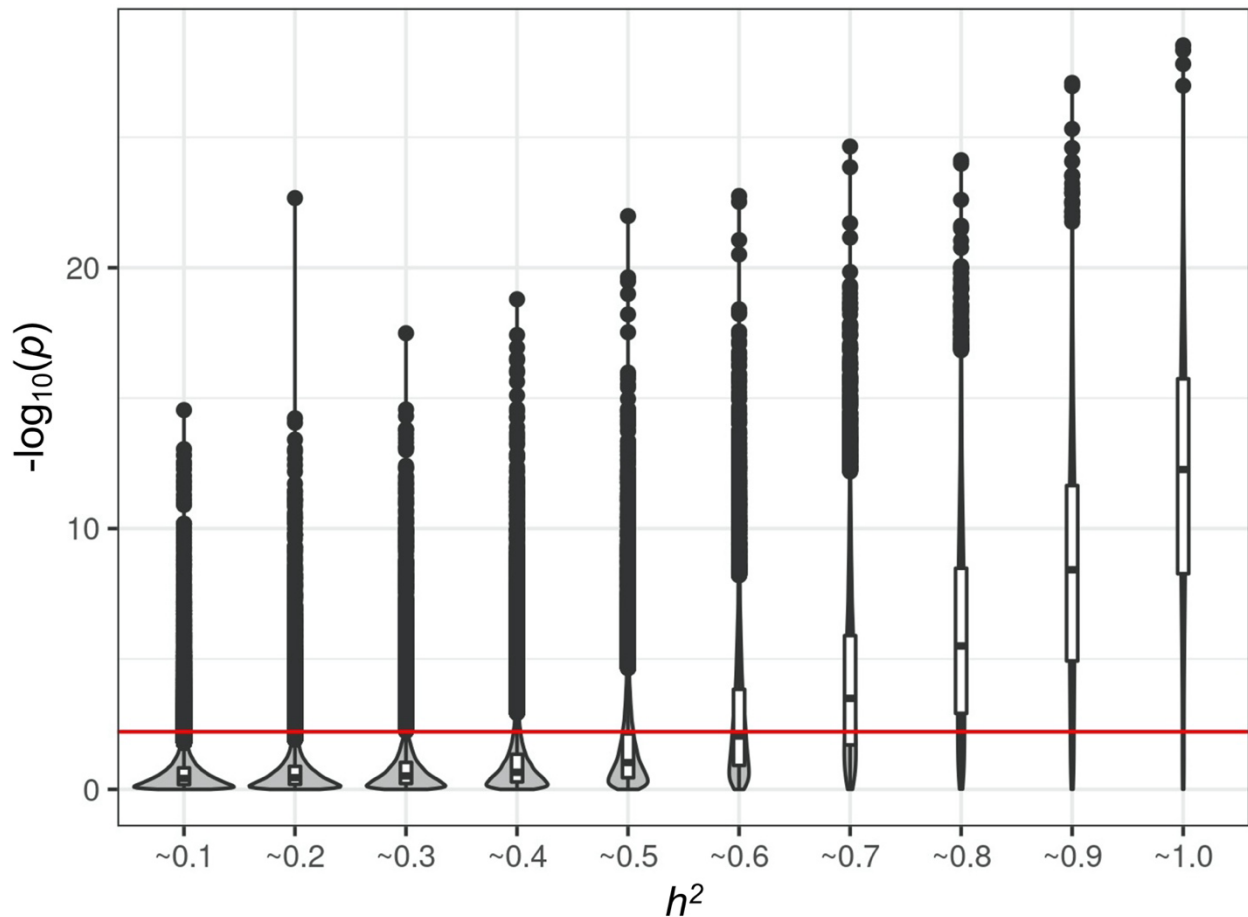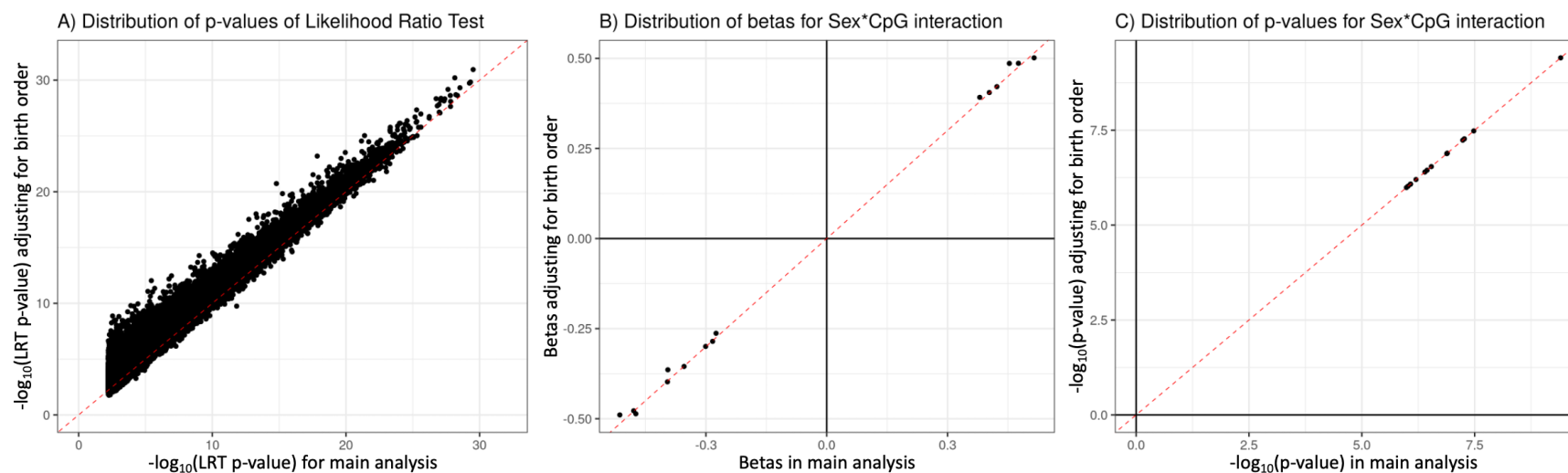
**Supplementary Figure S4.** Numbers of DNAm sites showing significant mother-newborn associations in methylation levels from Likelihood Ratio Test, by the $h^2$ of each DNAm site.

The X-axis is the heritability ($h^2$) of individual DNAm sites based on a published study on DNAm quantitative trait loci (van Dongen et al. 2016; PMID: 27051996), but only the heritability of autosomal DNAm sites was available. The Y-axis is the numbers of DNAm sites. Blue columns are DNAm sites showing nonsignificant mother-newborn associations (FDR≥0.05) in Likelihood Ratio Test, and grey columns are DNAm sites showing significant mother-newborn associations (FDR<0.05). The table below showed the numbers for DNAm showing significant or nonsignificant mother-newborn associations in each $h^2$ category.



| | ~0.1 | ~0.2 | ~0.3 | ~0.4 | ~0.5 | ~0.6 | ~0.7 | ~0.8 | ~0.9 | ~1.0 |
|---|---|---|---|---|---|---|---|---|---|---|
| FDR <0.05 | 4169 | 2425 | 2664 | 3957 | 5270 | 6165 | 5890 | 4758 | 3368 | 1571 |
| FDR ≥0.05 | 146026 | 59457 | 37911 | 29340 | 16766 | 6946 | 2857 | 1074 | 361 | 94 |

$h^2$

**Supplementary Figure S5.** Concordance of results from the main and sensitivity analyses estimating mother-newborn associations in methylation levels for individual DNAm sites.

Distributions of A) p-values from the Likelihood Ratio Test, B) regression coefficients for the interaction term between maternal methylation levels and newborn sex, and C) p-values for the interaction term between maternal methylation levels and newborn sex from both main and sensitivity analyses were shown. The X-axis represented results for main analysis (i.e., models were adjusted for maternal age at delivery, maternal race/ethnicity, maternal smoking, preterm birth, type of delivery, and surrogate variables). The Y-axis represented results from the sensitivity analysis additionally adjusting for birth order.

**Supplementary Figure S6.** Distribution of A) p-values and B) regression coefficients for associations between ΔDNAm and newborn sex at individual DNAm sites, by the $h^2$ of each DNAm site.

ΔDNAm represents differences in maternal-neonatal methylation levels of individual DNAm sites. The X-axis is the heritability ($h^2$) of individual DNAm sites based on a published study on DNAm quantitative trait loci (van Dongen et al. 2016; PMID: 27051996), but only the heritability of autosomal DNAm sites was available. The Y-axis is the A) -$\log_{10}$(p-values) and B) regression coefficients (betas) for associations between ΔDNAm and newborn sex. The red horizontal lines marks FDR=0.05.



A) Distribution of p-values by $h^2$

B) Distribution of regression coefficients by $h^2$
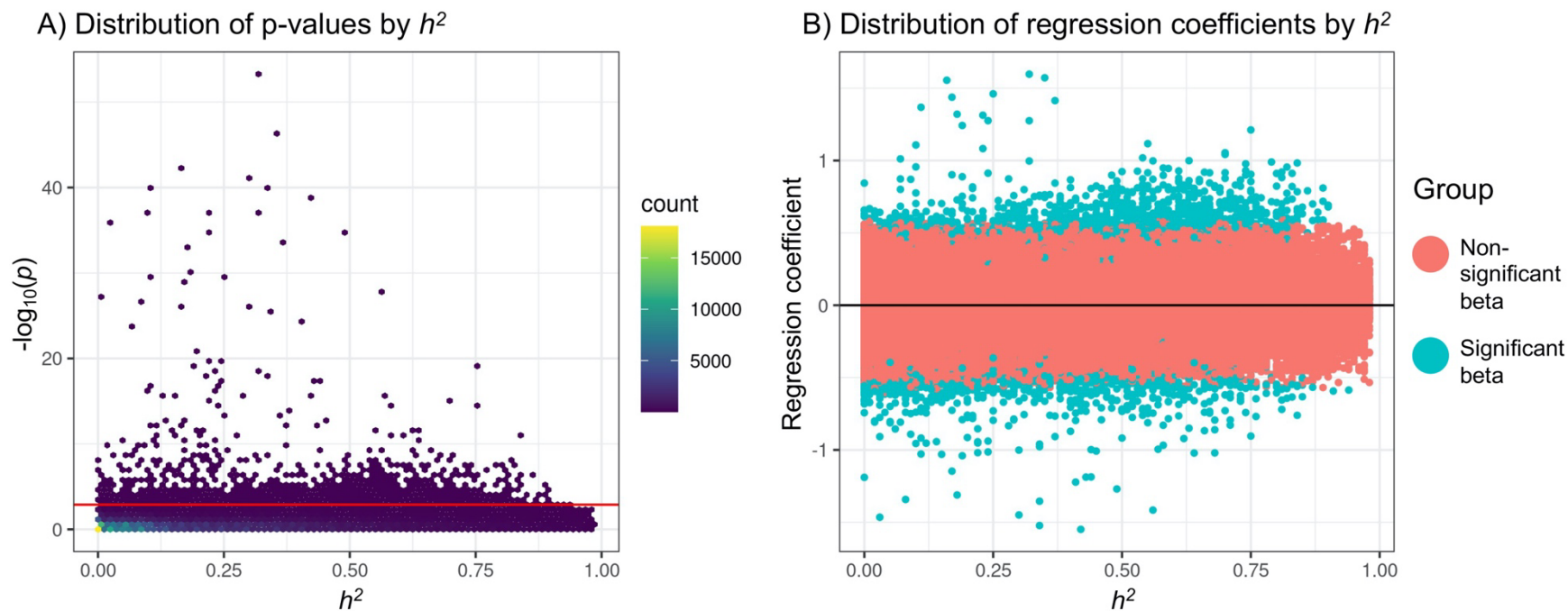
**Supplementary Figure S7.** Concordance of regression coefficients between BBC and IOWBC pre-pregnancy pairs for associations between ΔDNAm and newborn sex.

Regression coefficients for associations between ΔDNAm and newborn sex were compared between the BBC (primary analysis) and pre-pregnancy pairs from the IOWBC (replication) in A) all available 8,896 DNAm sites, and B) 1,245 DNAm sites showed a p<0.05 in the IOWBC. P-values for chi-square test for concordance were presented. C) DNAm sites with significant associations between ΔDNAm and newborn sex (p<0.05) in the IOWBC were enriched, with a p<$2.2\times10^{-16}$ in the Kolmogorov-Smirnov Test.



A) All available DNAm sites in the IOWBC pre-pregnancy mother-newborn pairs (No. of DNAm sites: 8,896)

|  | $\beta_{BBC} > 0$ | $\beta_{BBC} < 0$ |
|---|---|---|
| $\beta_{IOWBC} > 0$ | **1,960 (22.0%)** | 2,069 (23.3%) |
| $\beta_{IOWBC} < 0$ | 1,180 (13.3%) | **3,687 (41.4%)** |

**Chi-square test for concordance $P$ = $5.2\times10^{-127}$**

B) DNAm sites with a p<0.05 in the IOWBC pre-pregnancy mother-newborn pairs (No. of DNAm sites: 1,245)

|  | $\beta_{BBC} > 0$ | $\beta_{BBC} < 0$ |
|---|---|---|
| $\beta_{IOWBC} > 0$ | **253 (20.3%)** | 98 (7.9%) |
| $\beta_{IOWBC} < 0$ | 33 (2.6%) | **861 (69.2%)** |

**Chi-square test for concordance $P$ = $6.7\times10^{-147}$**

C) Distribution of p-values from the IOWBC pre-pregnancy mother-newborn pairs

**Kolmogorov-Smirnov test $P < 2.2\times10^{-16}$**
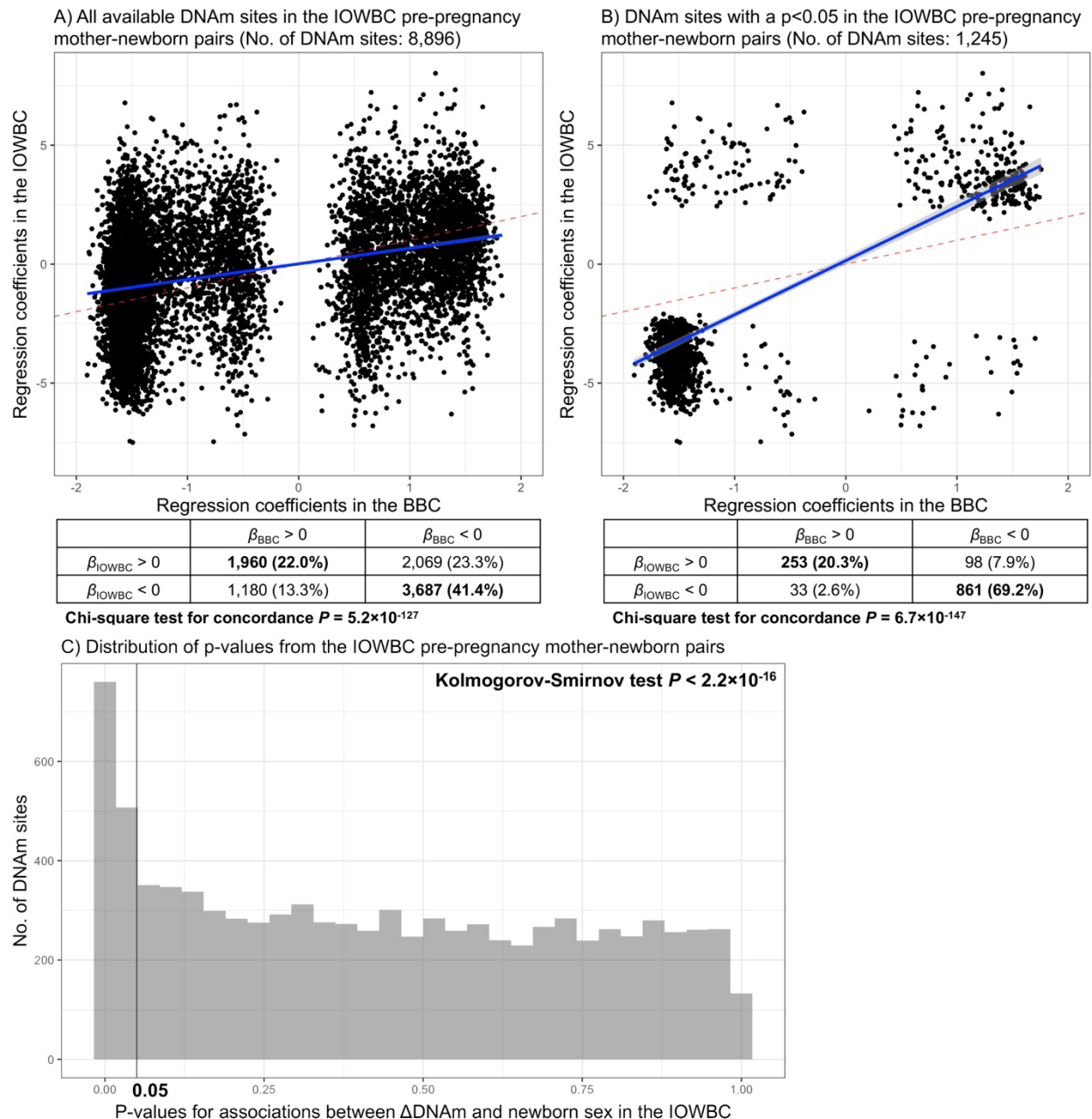
**Supplementary Figure S8.** Concordance of regression coefficients between BBC and IOWBC early-pregnancy pairs for associations between ΔDNAm and newborn sex.
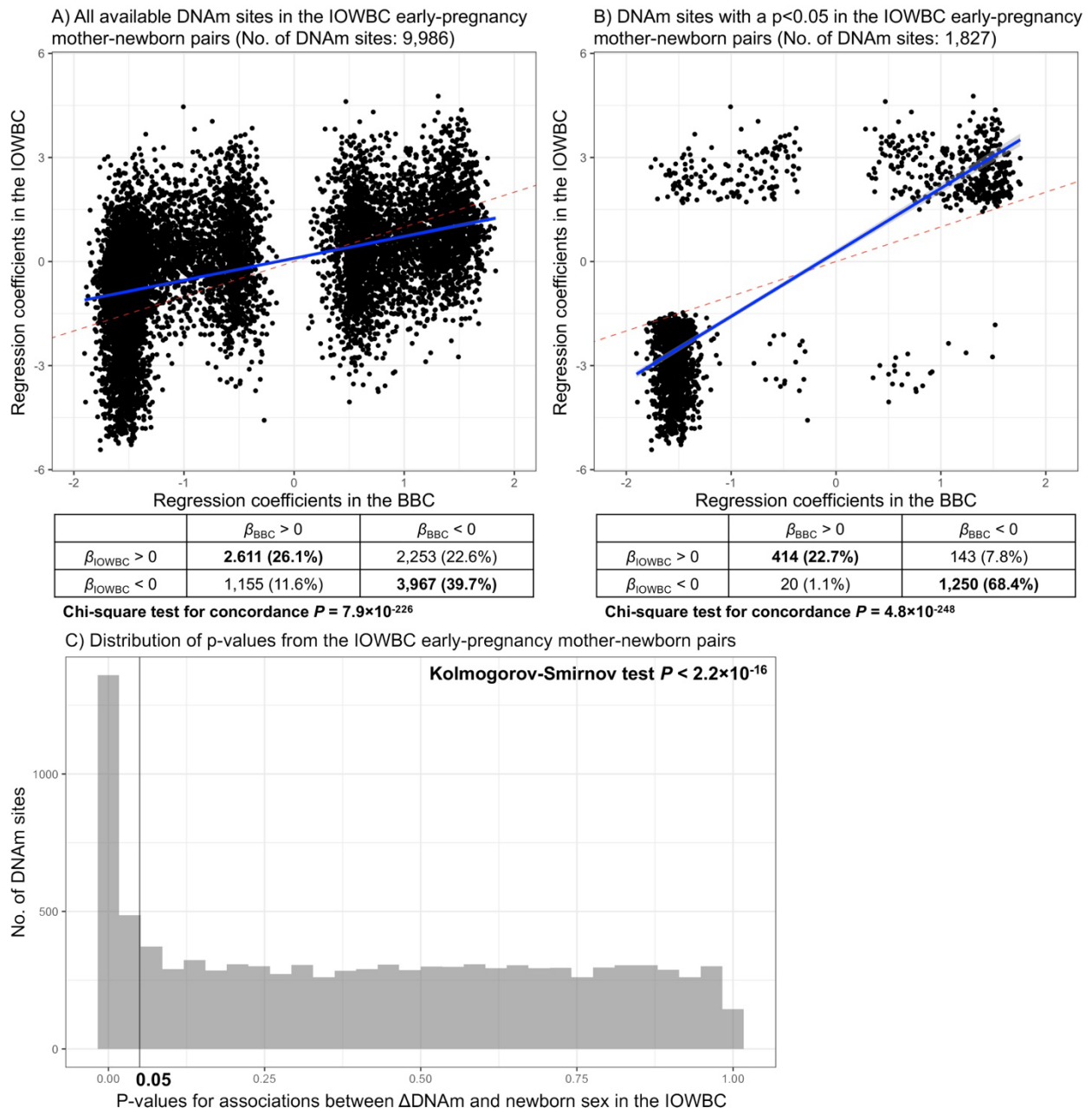
Regression coefficients for associations between ΔDNAm and newborn sex were compared between the BBC (primary analysis) and early-pregnancy pairs from the IOWBC (replication) in A) all available 9,986 DNAm sites, and B) 1,827 DNAm sites showed a p<0.05 in the IOWBC. P-values for chi-square test for concordance were presented. C) DNAm sites with significant associations between ΔDNAm and newborn sex (p<0.05) in the IOWBC were enriched, with a p<2.2×10$^{-16}$ in the Kolmogorov-Smirnov Test.



A) All available DNAm sites in the IOWBC early-pregnancy mother-newborn pairs (No. of DNAm sites: 9,986)

|  | $\beta_{BBC} > 0$ | $\beta_{BBC} < 0$ |
|---|---|---|
| $\beta_{IOWBC} > 0$ | **2.611 (26.1%)** | 2,253 (22.6%) |
| $\beta_{IOWBC} < 0$ | 1,155 (11.6%) | **3,967 (39.7%)** |

**Chi-square test for concordance P = 7.9×10$^{-226}$**

B) DNAm sites with a p<0.05 in the IOWBC early-pregnancy mother-newborn pairs (No. of DNAm sites: 1,827)

|  | $\beta_{BBC} > 0$ | $\beta_{BBC} < 0$ |
|---|---|---|
| $\beta_{IOWBC} > 0$ | **414 (22.7%)** | 143 (7.8%) |
| $\beta_{IOWBC} < 0$ | 20 (1.1%) | **1,250 (68.4%)** |

**Chi-square test for concordance P = 4.8×10$^{-248}$**

C) Distribution of p-values from the IOWBC early-pregnancy mother-newborn pairs

**Kolmogorov-Smirnov test _P_ < 2.2×10$^{-16}$**

**Supplementary Figure S9.** Flow chart for analysis steps in the Boston Birth Cohort (BBC).