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SUPPLEMENTARY METHODS

Sample and procedures

Avon Longitudinal Study of Parents and Children (ALSPAC)

Of the initial 14,541 pregnancies in the ALSPAC cohort, there were 14,676 fetuses that resulted in 14,062 live births and 13,988 children alive at 1 year of age. When the children were approximately 7 years old, there was an attempt to increase the study's sample size. Subsequent recruitment efforts added 913 children to the study, making the total sample size 15,454 pregnancies for analyses after age 7, from 15,589 fetuses, of which 14,901 were alive at 1 year of age.[1,2] Further details of the study and available data are provided on the study website through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

All data are available by request from the ALSPAC Executive Committee for researchers who meet the criteria for access to confidential data (http://www.bristol.ac.uk/alspac/researchers/access/). Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees. Consent for biological samples was collected in accordance with the Human Tissue Act of 2004. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee. Secondary analyses of these data were approved with oversight by the Mass General Brigham Institutional Review Boards (IRB) (Protocol 2017P001110).

Short Mood and Feelings Questionnaire (SMFQ) at 10.6 years of age

Children completed the SMFQ survey during a half-day session they attended at the ALSPAC clinic site at age 10.6 years. The 13-item measure uses a 3-point Likert scale with values 0=not true, 1=sometimes true, and 2=true to capture the child's mental state over the previous 2 weeks. Items include: "I felt miserable or unhappy", "I felt lonely", and "I cried a lot".[3] The SMFQ is highly correlated with both questionnaire and interview measures of psychopathology as well as clinician-related diagnoses of depression in children and adolescents.[4-7] Internal consistency reliability for the SMFQ in the Accessible Resource for Integrated Epigenomic Studies (ARIES) sample was very high ($\alpha = 0.78$).

Being a longitudinal study, ALSPAC had repeated measures of depressive symptoms across time. We focused on the age 10.6 timepoint for several reasons. First, it was the next measurement of childhood depressive symptoms that occurred after the DNAm assessment at age 7 years. Thus, we could maintain temporality in the association between exposure to adversity prior to age 7, DNAm at age 7, and subsequent depressive symptoms, reducing concerns about reverse causation. Moreover, in large population-based samples like ALSPAC, reports of child depressive symptoms are uncommon before age 10.6 and diagnosis for major depression before age 7 is rare (0-1.4% prevalence) [8]. Indeed, age 10.6 is the first occasion in ALSPAC when children self-reported their own levels of depressive symptoms. Child self-reports are advantageous, because self-reports of depressive symptoms have been shown to often be more reliable and less biased than parental reports, which typically underreport depressive symptoms.[9,10] Age 10.6 was also advantageous as it allowed us to observe more immediate effects of DNAm changes on depressive symptoms and do so before the onset of puberty. Interpretation of the effects of DNAm on depressive symptoms become more complicated after puberty, when high-risk behaviors like smoking and drinking develop.

DNA methylation (DNAm)

The DNA used for this assay was collected from whole blood and peripheral blood leukocytes when children were approximately 7 years old. Extractions were completed within 3 weeks after DNA collection.[11] The University of Bristol performed the DNAm wet laboratory procedures, preprocessing analyses, and quality control.[12,13] DNAm values were expressed using beta values, which represent the proportion of methylated cells at each observed CpG site. DNAm data were processed using an analytic pipeline based on the *meffil* R package developed by Min and colleagues.[13] The pipeline included background correction and functional normalization to diminish variation due to technical artifacts. Furthermore, samples with more than 10% missing CpG site measurements (detection *P* value > 0.01 or bead count < 3) were removed. Cross-hybridizing probes and polymorphic probes were removed leaving 450,745 probes.

To reduce the impact of potential outliers, we winsorized DNAm values for each CpG site by setting the bottom 5% and top 95% to the values for the 5th and 95th quantiles, respectively.[14] We controlled for cell-type heterogeneity by estimating cell counts from DNAm measurements using the Houseman method[15] and then regressed the cell count estimates from our DNAm measurements as advised by Edgar and colleagues.[16] Finally, we converted these adjusted DNAm measurements, represented as beta values (values between 0 and 1 indicating the ratio of methylated to unmethylated cells), to *M* values (the $\log_2$-transformation) because, unlike beta values, *M* values do not suffer from severe heteroskedasticity (unequal variance of the residuals) and therefore provide a more accurate detection and true positive rate than beta values.[17] Beta values, however, were used in all figures.

Sociodemographic confounders

We controlled for the following sociodemographic confounders measured at child birth: *sex* assigned at birth (0=male, 1=female); *child race/ethnicity,* based on parent self-reported race/ethnic group at birth (0=non-White, 1=White); *birthweight*; *number of previous pregnancies (parity)* (0-3+); *maternal age*; *highest level of maternal education* (1=less than O-level, 2=O-level, 3=A-level, 4=degree or above); *sustained maternal smoking during pregnancy* (0=non-smoker, 1=smoker in 2 or more trimesters, including the third trimester). As noted above, we did not control for prior depression or internalizing symptoms, as incidence in general is rare[8] and self-report measures of depression before age 10.6 were unavailable.

Data analysis

All scripts used to conduct these analyses can be found on our GitHub page: https://github.com/thedunnlab/.

Selecting the best explanatory life course exposure

We used the structured life course modeling approach (SLCMA; pronounced "slick-mah") to identify the single life course hypothesis for each adversity that explained the most variation in early adolescent depressive symptoms. The SLCMA is a two-stage modeling approach that enables researchers to identify the most parsimonious explanation for outcome variation from multiple a priori specified life course hypotheses.[18-21]

In the first stage of the SLCMA, a variable selection tool called least angle regression (LARS) selects the life course model with the highest explanatory power for inclusion in the statistical model. In the second stage of the SLCMA, post-selection inference is applied to calculate the total effect of adversity on depressive symptoms for the selected life course model. The SLCMA provides a distinct advantage over simpler modeling techniques, like multiple linear regression, because it allows for consideration of multiple life course hypotheses *a priori* and bases selection of the life course hypothesis on its explanatory power, rather than on hypotheses developed after observation. The SLCMA also allows for more nuanced life course modeling that considers timing and frequency of exposure, unlike a simplistic ever versus never exposed hypothesis.

Childhood adversity parameterization in the SLCMA

The SLCMA analysis we performed included 3 categories of exposure variables. To test for sensitive periods, a binary variable with a value of 1 for exposed or 0 for unexposed was assigned at each measurement timepoint. To test the accumulation hypothesis, a count variable was created by summing the binary measurement points for each adversity (values ranged from 0 to 6). Finally, to test the recency hypothesis, each binary measurement point was multiplied by the child's age in years at that timepoint, giving greater weight to more recent exposures to adversity. These weighted values then were summed to create the recency hypothesis variable.

Controlling for confounding and scaling

To control for confounding and to help sustain our statistical power, we separately regressed the life course hypothesis selected for each adversity, DNAm data, and age 10.6 SMFQ score on the previously mentioned covariates. We took the residuals from these regressions and standardized them before using them in our mediation analysis. This approach ensured our results could be interpreted in terms of *correlation* between childhood adversity and depressive symptoms after adjusting for confounders.

Benefits of using the Schaid-Sinnwell model for mediation analysis

A full description of the benefits of using the Schaid-Sinnwell model, as well as further details on its specific methodology can be found in Schaid and Sinnwell's 2020 paper on the topic.[22]

High dimensional mediation analysis

Using statistical mediation analyses, we determined the extent to which DNAm, as measured through individual CpG sites, mediated the relationship between each type of

childhood adversity and adolescent depressive symptoms (**Figure S2**).

As described elsewhere[23,24], testing for mediation using DNAm data is particularly challenging due to the high-dimensional nature of possible mediators. Our analytic samples consisted of all children with complete cases (n=627-675), which means that the number of potential mediators (450,745 CpGs) was over 650 times greater than the number of observations. Therefore, before conducting the mediation analyses, we used pruning and sure independence screening (SIS) – a method applied widely for dimension reduction in big data settings[25] – to reduce the number of potential DNAm mediators to a viable number, defined by $q = \lfloor (n - 3 - 1)/2 \rfloor$, where n equals the analytic sample size (**Figure 1**). We chose the value q because it allowed us to consider the maximum number of mediators given our sample size.

We calculated q by subtracting 3 from n to accommodate the estimation of the direct effect of exposure on outcome (∂) as well as the variances of the exposure and outcome. We subtracted an additional 1 to ensure that the final number of parameters estimated was at least 1 less than the sample size. Finally, we divided by 2 to accommodate that each potential mediator required the estimation of both an α and a β parameter.

Pruning

As our analyses were not stratified by sex (due to sample size constraints), we removed CpGs located in sex chromosomes from consideration. We also removed non-variable CpGs (ie, CpGs whose methylation levels varied by less than 5% across the sample), as these CpGs may not provide much insight into the underlying mechanisms of the childhood adversity-adolescent depression relationship.[16,26] After this step, we had 278,586 probes for downstream analyses.

Sure independence screening (SIS)

We then made use of a method proposed by Fan and Lv to reduce our probes to the pre-specified q, selecting the top sites by ranking the remaining 278,586 probes by the absolute value of their marginal correlation with each adversity and SMFQ score.[22,27] This calculation took the form:

$$\left| cor(x, m_j) \cdot cor(m_j, y) \right|, \quad (1)$$

where x is the adversity exposure, m_j is a given CpG site where $j = 1, \ldots, 278{,}586$, and y is SMFQ score. Thus, the assumption is: CpGs that show stronger correlations with both the exposure and the outcome are more likely to be mediators than CpGs with lower or no correlation with either the exposure or the outcome. Therefore, we focused on the CpGs showing the greatest likelihood of being mediators and selected the top q sites to include in our model.

Shrinkage parameter

Once the top q mediation sites were entered into the structural equation model (SEM), the SEM's iterative algorithm identified the CpG sites showing the strongest mediation signal by systematically working through a grid of values for the shrinkage parameter, λ, beginning with 1 (the most stringent penalty) and gradually reducing to zero (no penalty) by 0.1 increments. The iterative selection of a penalty parameter is necessary to identify the number of model parameters that result in the lowest Bayesian Information Criterion (BIC). Due to our relatively limited sample size (compared to a larger-scale clinical or epidemiological study), we balanced false-positive and false-negative results using a uniform modest penalty term of 0.2 across all 7 adversities.

Estimating effect estimates and standard errors

As penalized regression models tend to excessively shrink parameter estimates,[22,28] we used the relaxed lasso approach to calculate our effect sizes. The relaxed lasso approach refits the selected model parameters without constraining them. We used the R package *lavaan* to approximate standard errors using the *sem* function [29].

Monte Carlo for *P* value and confidence interval estimation

We assessed the statistical significance of our results by estimating a *P* value and confidence interval of the indirect effect for each CpG mediator using the Monte Carlo method for assessing mediation (MCMAM)[30].

Annotation of CpGs

CpGs were matched to genes based on two primary annotations: (1) the Illumina annotation, which notes whether the CpG is located within specific genes or near their transcriptional start site (TSS) and (2) the annotation developed by Price et al.[31], which notes the gene with the closest TSS.

Sensitivity and colocalization analyses

Age-related changes in DNA methylation levels

To determine whether our mediating CpGs were enriched for age-dependent changes in DNAm levels, we leveraged a prior analysis of DNAm trajectories from birth to late adolescence [32]. This prior study used data from both ALSPAC and Generation R to detect linear and non-linear changes in DNAm across development (ages 0-18), with potential inflection points at age 6 and 9.

Briefly, we obtained summary statistics for these analyses from the EWAS catalog (https://www.ewascatalog.org/) and performed permutation analyses to determine whether our set of 67 mediating CpGs were enriched for different patterns of DNAm change across time. Specifically, we selected a random subset of 67 CpGs from our analytic sample of 278,586 CpGs across 10,000 permutations. We then assessed whether these CpGs were associated with linear and non-linear changes in DNAm from birth to age 18 ($p<1x10^{-8}$), as reported by Mulder et al.

When contrasting the mediation set to the permutation sets of CpGs, we found little differences, other than a slight enrichment for CpGs with non-linear changes in DNAm from age 9 onwards (p=0.0495; q=0.297; **Figure S9**). These findings suggest mediating CpGs were no more likely to show changes in DNAm over time than by random chance.

Mediation through cell type proportions

Given that cell type plays a major role in driving DNAm differences, we investigated whether cell type proportions could explain the mediation patterns we observed in our primary analyses. To this end, we used the *mediation* package in R [33] to perform mediation analyses of the relationship between each of the seven childhood adversities and depressive symptoms. Rather than use DNAm as the mediator, we used one of six bioinformatically-predicted cell types (B cell, CD4+ T cell, CD8+ T cell, Granulocyte, Monocyte, Natural killer cell) from the Houseman deconvolution [15]. This approach resulted in 42 separate mediation analyses.

We did not identify any evidence of mediation through cell type proportions (**Figure S10**), with the proportion of mediated effects ranging from 0.0016% to 3.88% (mean=0.73%), considerably smaller than those observed for DNAm. The lowest p-value was 0.44. These

findings suggest that our results were not mediated by cell type differences between participants and further point to DNAm as a potential mediator of the relationship between adversity and depression.

Colocalization with genome-wide association studies (GWAS) of major depressive disorder

We assessed whether mediating CpG sites were enriched for genetic risk for major depressive disorder (MDD) using colocalization analyses, a method that estimates the probability of shared variants between two analyses[34-36]. Briefly, we obtained *cis*-mQTLs for mediating CpGs using GoDMC[37] ($p<1x10^{-4}$ within 1Mb from the CpG). We then extracted all available SNP associations within 1Mb of the mQTL, which were intersected with the most recent GWAS of MDD[38]. CpGs with fewer than 10 associated SNPs were removed from the analysis, resulting in a set of 39 CpGs (of 67 total CpGs) for analysis.

We used the default settings of the *coloc.abf* function from the *coloc* package in R[34-36] to estimate the posterior probability of a single shared variant (PP_{H4}) from these data. None of the mediating CpGs showed a $PP_{H4}\geq0.8$ (max $PP_{H4}=0.11$, which is generally considered evidence for colocalization. These findings suggest there were no colocalized signals between the mediating CpGs and genetic liability for MDD.

Replication analyses

Future of Families and Child Wellbeing Study (FFCWS)

Cohort details

FFCWS is a prospective, longitudinal birth cohort of almost 5,000 families in the USA followed to capture a representative sample of families vulnerable to risk factors linked to nonmarital childbearing[39]. From 1998 to 2000, 4,898 children in 75 hospitals were enrolled in the study (76% unmarried parents). FFCWS is an ethnically/racially diverse sample (50% Black; 24% Hispanic; 18% White) enriched for families with fewer socioeconomic resources (65% with ≤ high-school degree; 39% below poverty line at birth). Families were interviewed when children were 1, 3, 5, 9, and 15 years old. Follow-up completion rates are excellent (>75% at all ages).

Childhood adversity

We investigated five measures of childhood adversity in the FFCWS cohort, which are outlined below. For all adversities, we analyzed the presence/absence of the exposure during the specific timepoint that was closest to ALSPAC.

1) Caregiver physical and emotional abuse (N=1,319): The Conflict Tactics Scale was collected from mothers, fathers, and primary caregivers (if not mother or father) at ages 3, 5, and 9. Participants were classified as having been exposed to caregiver physical or emotional abuse exposed if they experienced (1) physical punishment on two or more occasions (e.g., spanking, hitting, slapping) OR (2) verbal aggression on three or more occasions (e.g., shouting/yelling, calling them names/dumb/lazy, threatened to hit, etc.).

2) Maternal psychopathology (N=1,671): Maternal depression was measured at ages 1, 3, 5, and 9 using the CIDI-SF scale for depression[40-43]. Participants were classified as exposed if mothers met a liberal threshold score of ≥3 in the CIDI-SF.

3) One adult in the household (N=1,653): At ages 1, 3, 5, and 9, primary caregivers reported the number of individuals aged 18+ living in the household. Participants were classified as exposed if only one adult lived in the household.

4) Financial hardship (N=1,667): Mothers reported material hardship at ages 1, 3, 5, and 9 [44-47] . Participants were coded as exposed to financial hardship if mothers reported difficulties paying for the following three items in the past year: (1) food (2) rent, and (3) utilities.

5) Family instability (N=1,677): Parents reported family instability since the prior wave. Instability is experiencing a permanent separation for either parent or the introduction of a new partner for the biological parent with whom the child is living. Experiencing two or more of these events since the prior wave is coded as 1 and less is coded as 0 at ages 1, 3, 5, and 9.

DNA methylation

DNAm was measured from children's saliva samples at age 9 (N=2,020). DNA was collected using the DNA Genotek Oragene kits and purified according to the manufacturer's protocol. DNA was then bisulphite converted using the EZ-96 DNA kit (Zymo Research) and methylation was assessed using the Illumina 450 K array (n=880). A secondary sample was analysed using the Illumina EPIC array (n=1,140).

DNA methylation data were initially processed with the *minfi* R package[48]. Stratified quantile normalization was undertaken to remove bad samples. Probes on sex chromosomes, problems with a SNP within nucleotide of the CpG site, probes with >20% failed samples, and CpG sites with >50% failed samples were removed.

Depressive symptoms

Depressive symptoms were measured at age 15 through self-reported symptoms on the Center for Epidemiological Studies of Depression Scale (CES-D)[49,50], including "Youth worries", "Youth feels too guilty", "Youth is too fearful or anxious", "Youth is nervous, highstrung, or tense", "Youth feels worthless or inferior", and "Youth cries a lot". Continuous measures of depressive symptoms were used in the mediation analyses (0-18 score).

Covariates

The following covariates were included in replication analyses using FFCWS data: child sex; child birthweight; mother's number of prior pregnancies; maternal education; maternal age at birth; maternal smoking during pregnancy; city of data collection as a proxy for race/ethnicity (Detroit or Toledo versus other city); and leukocyte proportion estimated using a childhood saliva reference panel[51].

SLCMA of repeated exposures

We performed a SLCMA of all five available adversities predicting depressive symptoms to determine whether the same period was the most predictive in FFCWS as in ALSPAC (**Table S5**). As described above, we included all timepoints available as sensitive periods, as well as a recency and accumulation hypotheses.

Of note, maternal psychopathology showed the same sensitive period in FFCWS as in ALSPAC (36 months in FFCWS and 33 months in ALSPAC; β=1.27; 95% CI=0.90-1.64; $p=5.21x10^{-11}$). The SLCMA financial stress revealed accumulation as the most predictive of depressive symptoms (β=0.23; 95% CI=0.03-38; p=0.0099). We identified different sensitive periods than ALSPAC for caregiver abuse (60 months; β=-0.05; 95% CI=-0.46-0.37; p=0.96),

one-adult households (12 months; β=0.09; 95% CI=-0.33-0.46; p=0.96), and family instability (12 months; β=0.23; 95% CI=-0.14-0.66; p=0.32). However, these results were largely null.

Generation R Study (GenR)

Cohort details

Data for the current study were drawn from a European subsample of the Generation R Study (GenR). The Generation R study is a population-based prospective cohort study from early fetal life onwards, based in Rotterdam, the Netherlands[52-56]. Pregnant women with an expected delivery date between April 2002 and January 2006 residing in the municipality of Rotterdam, the Netherlands, were invited to enroll in the study. In total, 9,778 pregnant women had 9,749 live-born children. DNA methylation was assessed in 464 children at age 10 years. The Generation R Study was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained for all participants.

Adversity

1) Caregiver physical/emotional abuse (N=315): At age 3, caregivers reported corporal punishment and verbal aggression using the Parent-Child Conflict Tactics Scale. Children were coded as exposed when parents reported ≥2 corporal punishments or ≥3 verbal aggressions.

2) Maternal psychopathology (N=316): At age 3, mothers reported depressive and anxiety symptoms using the Brief Symptom Inventory. Children were coded as exposed when the mother reported a score greater than 0.71, the established cutoff for Dutch samples.

3) Family instability (N=350): At ages 3 and 6, mother reported their marital status. Children were coded as exposed based on their parents' marital status of separated or divorced.

4) Financial hardship (N=313): At age 2 and 3, mothers reported financial difficulties in affording rent, electricity, or food. Children were coded as exposed when the mother reported any financial difficulty in these items.

DNA methylation

DNA was extracted from whole peripheral blood at 10 years using the salting-out method. Five hundred nanograms of DNA per sample underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Shallow) (Zymo Research Corporation, Irvine, CA, USA). Samples were plated randomly onto 96-well plates. Samples were processed with the Illumina Infinium HumanMethylation450 (450k) BeadChip (Illumina Inc., San Diego, CA, USA). Quality control of analyzed samples was performed using standardized criteria. Quality control and normalization of the HumanMethylation450 BeadChip array data was performed according to the Control Probe Adjustment and reduction of global CORrelation (CPACOR) workflow using R [57]. Probes that had a detection p value ≥ 1E− 16 were set to missing per array. Next, the intensity values were quantile normalized for each of the six probe-type categories separately: type II red/green, type I methylated red/green, and type I unmethylated red/green. Arrays with observed technical problems such as failed bisulfite conversion, hybridization or extension, as well as arrays with a sex mismatch were removed from subsequent analyses. Additionally, only arrays with a call rate > 95% per sample were processed further. Probes on the X and Y chromosomes were excluded from the analyses.

Depressive symptoms

Depressive symptoms were measured at age 13-14 using the T-scores of the "Depressive problems" scale of the Youth Self Report (YSR),[58] with higher scores indicating more problems. The items referred to the preceding 6 months and were rated on a three-point scale: "not at all," "a bit," or "clearly."

Covariates

The following covariates were included in replication analyses using GenR data: child sex; child birthweight; mother's number of prior pregnancies; maternal education; maternal age at birth; maternal smoking during pregnancy; 450K array sample plate. Cell types were corrected prior to analysis using the cell type proportions estimated using the Houseman method.

SLCMA of repeated exposures

We performed a SLCMA of exposures measured at multiple timepoints (family instability and financial hardship) predicting depressive symptoms to determine whether the same period was the most predictive in GenR as in ALSPAC (**Table S5**).

The SLCMA of family instability with four life course models (age 3, age 6, accumulation, recency; N=316) revealed age 3 exposure as the most predictive of depressive symptoms (β=-1.34; 95% CI=-4.19-1.66; p=0.47). By contrast, the SLCMA of financial hardship with four life course models (age 3, age 2, accumulation, recency; N=344) revealed recency as the most predictive of depressive symptoms (β=0.576; 95% CI=-0.0054-1.04; p=0.033). These findings contrast those from ALSPAC, which identified a sensitive period around 5 years of age for both family instability (57 months) and financial hardship (61 months).

Comparability of cohorts

The discrepancies in findings from the SLCMA between cohorts could be due to several factors, including the timing of depressive symptom measurements, limited number or granularity of adversity timepoints, or differences in sociodemographic characteristics and adversity prevalence between cohorts. Further, we note that the SLCMA was performed in the subset of participants with epigenetic data, which limited the sample size and may not fully reflect the relationship between childhood adversity and adolescent depressive symptoms.

Replication approach

Replication analyses were performed at the CpG-level for the 70 CpGs identified in the main ALSPAC analyses. Specifically, we performed mediation analyses using the *mediation* package in R[33], analyzing DNAm levels at each CpG as a mediator of the relationship between a given type of childhood adversity and the continuous outcome of depressive symptoms.

Each adversity was investigated separately and only CpGs associated with that specific adversity were investigated. Rather than use the timepoint selected by the SLCMA, we attempted to match the timing of adversity as closely as possible to the sensitive period identified in ALSPAC. We used this approach for two primary reasons: (1) we have previously observed that different timing of exposures may influence different sets of CpGs in studies of childhood adversity and DNAm[59], and (2) we attempted to limit differences as best as possible to improve our ability to replicate our primary results. See **Figure S9** for a summary of the adversities, DNAm timepoint, and depressive symptom measures in each cohort.

We first repeated these analyses of top loci in ALSPAC to obtain mediated effects for each CpG that could be directly compared to the estimates in FFCWS and GenR. These were comparable to those identified in the main analyses, and thus, are shown only in the replication tables and figures for FFCWS and GenR.

For FFCWS (N=1,319-1,677), we analyzed exposures to adversity during the time period that was most closely matched to the sensitive period identified in ALSPAC (**Tables S5, S6; Figures S10**). Beta values of DNAm were used in these analyses due to data availability constraints.

For GenR (N=312-350), we analyzed exposures to adversity during the time period that was most closely matched to the sensitive period identified in ALSPAC (**Tables S7, S8; Figures S11**). M-values were used in these analyses, as these were available and more closely matched to the primary analyses in ALSPAC.

CpG-level results

To determine whether specific CpGs showed more replication across cohort, we investigated CpG-level indirect (or mediated) effects in the FFCWS and GenR cohorts compared to those identified in the primary analyses of ALSPAC. Here, we focused on identifying CpGs that showed the same direction and magnitude of indirect effect, when accounting for the direction of the relationship between adversity and depressive symptoms. In other words, we adjusted the direction of the mediated effect for the sign of the adversity-depression association to determine whether the former was risk-increasing (positive) or protective (negative).

Using data from the FFCWS, we identified four CpGs with similar or larger magnitudes of adjusted indirect effect compared to ALSPAC (**Figure S10**; Tables S5-S6**). Three of these CpGs showed protective effects for one adult households (cg09191574**: -11.0% in ALSPAC versus -10.1% in FFCWS; **cg21079003**: -14.9% in ALSPAC versus -72.5% in FFCWS; **cg03269218**: -6.4% in ALSPAC versus -81.1% in FFCWS). One CpG showed risk-increasing effects for caregiver abuse (**cg12343929**: 22.4% in ALSPAC versus 27.0% in FFCWS).

Using GenR data, we identified five CpGs with similar magnitudes of indirect effect to ALSPAC (**Figure S11**; **Tables S7-S8**), all of which were different from those identified in FFCWS. Four of these CpGs were linked to maternal psychopathology, with one showing risk-increasing effects (**cg19642007**: 8.4% in ALSPAC versus 23.8% in GenR) and three showing protective effects (**cg21665774**: -7.0% in ALSPAC versus -11.6% in GenR; **cg16292933**: -9.2% in ALSPAC versus -76.9% in GenR; **cg00300275**: -6.9% in ALSPAC versus -411.6% in GenR). One CpG was protective for the effects of family instability (cg269299079: -23.7% in ALSPAC versus -14.2% in GenR).

Together, these findings suggest DNAm can partially mediate the link between childhood adversity and depressive symptoms, while further emphasizing potential protective effects of DNAm in these relationships.

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SUPPLEMENTARY TABLES

Table S1. Description of seven childhood adversity measures included in ALSPAC as exposures.

Adversity	Respondent	Instrument or questionnaire items	Exposure definition	Time points of assessment
Caregiver physical or emotional abuse	Mother and partner	1) your partner was physically cruel to your children; 2) you were physically cruel to your children; 3) your partner was emotionally cruel to your children; 4) you were emotionally cruel to your children	Children were coded as having been exposed if the mother, partner, or both responded affirmatively to at least 1 item. Children were considered unexposed if none of these items were positively endorsed by either partner. An observation was considered missing if all questions were left unanswered.	8 months, 1.75 years, 2.75 years, 4 years, 5 years, and 6 years
Sexual or physical abuse	Mother	An item asking whether the child had been exposed to either sexual or physical abuse from anyone	Children were coded as exposed if the mother responded affirmatively and unexposed if the mother did not endorse this question. An observation was considered missing if both questions were left unanswered.	1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, and 6.75 years
Maternal psychopathology	Mother	1) the Crown-Crisp Experiential Index (CCEI), assessing anxiety and depression; 2) the Edinburgh Postnatal Depression Scale (EPDS); and 3) a question asking about suicide attempts in the past 1.5 years	Consistent with cut-offs used in prior ALSPAC studies and elsewhere, children were coded as exposed if 1 or more of the following criteria was met: 1) CCEI depression score > 9 and/or anxiety score > 10; 2) EPDS score > 12; or 3) a suicide attempt reported since the last interview. They were considered unexposed if none of those criteria were met and no prorated scores were missing. An observation was considered missing if a prorated score or the suicidality question was missing.	8 months, 1.75 years, 2.75 years, 5 years, and 6 years of age
1-adult in the household	Mother	An item asking about the number of adults over the age of 18 years living in the household	Children were coded as exposed if only 1 adult lived in the household and coded as unexposed if 2 or more adults lived in the household. An observation was considered missing if this question was left unanswered.	8 months, 1.75 years, 2.75 years, 4 years, and 7 years

Adversity	Respondent	Instrument or questionnaire items	Exposure definition	Time points of assessment
Family instability	Mother	Child had 1) been taken into care; 2) been separated from their mother for 2 or more weeks; 3) been separated from their father for 2 or more weeks; or 4) acquired a new parent.	Children were coded as exposed if mothers responded affirmatively to at least 2 of these events at a given time point and were coded as unexposed if mothers responded affirmatively to 0 or 1 of these events and no questions were unanswered. An observation was considered missing if any of these questions were left unanswered.	1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, and 6.75 years
Financial stress	Mother	The family had difficulty affording the following: 1) items for the child; 2) rent or mortgage; 3) heating; 4) clothing; 5) food. Each of the 5 items was coded on a Likert-type scale (1=not difficult; 2=slightly difficult; 3=fairly difficult; 4=very difficult)	Children were coded as exposed if mothers reported at least "fairly difficult" (corresponding to 3 or higher) for 3 or more items at each time point. Children were considered unexposed if all questions were answered but the exposed criteria were not met. An observation was considered missing if any of these questions were left unanswered.	8 months, 1.75 years, 2.75 years, 5 years, and 7 years
Neighborhood disadvantage	Mother	There were problems in the neighborhood: 1) noise from other homes; 2) noise from the street; 3) garbage on the street; 4) dog dirt; 5) vandalism; 6) worry about burglary; 7) mugging; and 8) disturbance from youth. Response options to each item were: 2=serious problem, 1=minor problem, 0=not a problem or no opinion.	A sum score ranging from 0-16 was created based on responses to each item. Children were coded as exposed if they had scores greater than or equal to 8 and unexposed if their score was less than 8 and no question was left unanswered. An observation was considered missing if any question was left unanswered.	1.75 years, 2.75 years, 5 years, and 7 years

Table S2. Descriptive statistics of the largest analytic sample.

Variable	Level	Analytic Sample (AS) N = 675	ARIES N = 970	*P* value (AS to ARIES)	ALSPAC N = 15,646	*P* value (ARIES to ALSPAC)
Sex (%)	Female	325 (48.1)	490 (50.5)	0.371	7152 (48.7)	0.281
	Male	350 (51.9)	480 (49.5)		7542 (51.3)	
Race (%)	Non-white	22 (3.3)	27 (2.9)	0.784	611 (5.1)	0.004
	White	653 (96.7)	906 (97.1)		11488 (94.9)	
Birthweight (g) (%)	< 3000	94 (13.9)	128 (13.5)	0.943	2760 (20.0)	<0.001
	3000 - 3499	243 (36.0)	342 (36.0)		4924 (35.7)	
	3500 - 3999	229 (33.9)	334 (35.2)		4382 (31.8)	
	4000	109 (16.1)	146 (15.4)		1735 (12.6)	
Mother's birth age (mean (SD))		30.01 (4.20)	29.57 (4.46)	0.045	28.00 (4.96)	<0.001
Previous number of pregnancies (%)	0	308 (45.6)	435 (46.5)	0.96	5800 (44.7)	0.015
	1	259 (38.4)	348 (37.2)		4550 (35.0)	
	2	84 (12.4)	117 (12.5)		1860 (14.3)	
	3+	24 (3.6)	36 (3.8)		772 (5.9)	
Education (%)	CSE/Vocational/None	82 (12.1)	148 (15.6)	0.235	3735 (30.0)	<0.001
	O Level	230 (34.1)	324 (34.1)		4303 (34.6)	
	A level	213 (31.6)	281 (29.6)		2795 (22.5)	
	University Degree	150 (22.2)	196 (20.7)		1603 (12.9)	
Smoked during pregnancy (%)	No	612 (90.7)	818 (89.2)	0.384	9565 (78.8)	<0.001
	Yes	63 (9.3)	99 (10.8)		2577 (21.2)	
Short Mood and Feelings Questionnaire (SMFQ), mean age 10.6 (mean (SD))		3.70 (3.27)	3.83 (3.32)	0.431	4.04 (3.51)	0.086

ARIES, Accessible Resource for Integrated Epigenomic Studies; ALSPAC, Avon Longitudinal Study of Parents and Children
The caregiver physical/emotional abuse analytic sample was used as an example because it had the largest sample size of all 7 adversities studied. The analytic sample did not statistically ($\alpha = 0.05$) differ from the total ARIES sample except for a slight discrepancy in SMFQ scores. *P* values are from the chi-squared test (categorical variables) or a two-sample t-test (continuous variables).

Table S3. Results of analysis examining age 7 DNAm as a mediator of the relationship between childhood adversity and depressive symptoms at mean age 10.6.

Adversity	Life Course Model	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
Caregiver Physical/ Emotional Abuse	Very Early Childhood										
		cg23751110 [2]	**-0.101**	**0.038**	**-0.152**	**0.038**	**0.015**	**(0.003, 0.031)**	**0.006**	**SLIT2**	
		cg06804625 [2]	**-0.102**	**0.038**	**0.115**	**0.038**	**-0.012**	**(0.002, 0.025)**	**0.008**	**PSRC1**	**CELSR2**
		cg21089584 [2]	**0.099**	**0.037**	**-0.117**	**0.037**	**-0.012**	**(-0.025, -0.002)**	**0.009**	**JARID2**	**JARID2**
		cg12343929	**0.096**	**0.039**	**0.121**	**0.039**	**0.012**	**(0.002, 0.025)**	**0.013**	**WDR90**	**WDR90**
		cg03965496	**0.084**	**0.037**	**0.115**	**0.037**	**0.01**	**(0.001, 0.022)**	**0.023**	**TRNA_Asn**	
Sexual/Physical Abuse (by anyone)	Very Early Childhood										
		cg24622544 [2]	**-0.113**	**0.039**	**0.141**	**0.037**	**-0.016**	**(0.004, 0.032)**	**0.003**	**POP5**	
		cg10310274	**-0.112**	**0.039**	**0.116**	**0.037**	**-0.013**	**(0.003, 0.028)**	**0.005**	**UCK1**	
		cg26518628 [2]	**0.105**	**0.039**	**-0.111**	**0.037**	**-0.012**	**(-0.025, -0.002)**	**0.008**	**7SK**	
		cg00958217	**-0.097**	**0.039**	**0.127**	**0.037**	**-0.012**	**(0.002, 0.027)**	**0.013**	**THAP3**	**PHF13**
		cg26786980	**-0.087**	**0.039**	**0.101**	**0.037**	**-0.009**	**(0.001, 0.021)**	**0.028**	**RARB**	**RARB**
		cg01973483	**0.077**	**0.039**	**-0.145**	**0.037**	**-0.011**	**(-0.026, 0)**	**0.045**	**PITPNM2**	**PITPNM2**
Maternal Psychopathology	Very Early Childhood										
		cg24059871 [3]	**0.096**	**0.039**	**0.122**	**0.037**	**0.012**	**(0.002, 0.025)**	**0.016**	**POP4**	**POP4**
		cg16292933 [2]	**-0.089**	**0.039**	**0.097**	**0.037**	**-0.009**	**(-0.02, -0.001)**	**0.03**	**SLC4A8**	
		cg19642007	**0.087**	**0.039**	**0.109**	**0.037**	**0.009**	**(0.001, 0.022)**	**0.031**	**TNNT3**	**TNNT3**
		cg10953317 [3]	0.064	0.039	-0.158	0.037	-0.01	(-0.025, 0.002)	0.105	CD300A	CD300A
		cg00300275	0.062	0.039	-0.138	0.037	-0.009	(-0.022, 0.002)	0.111	U80764	CACNB2
		cg06451157	0.067	0.039	-0.081	0.039	-0.005	(-0.016, 0.001)	0.118	HLA-H	HCG2P7
		cg07308232	0.086	0.039	-0.059	0.038	-0.005	(-0.015, 0.001)	0.148	C7orf50	C7orf50
		cg21665774	0.071	0.039	-0.069	0.05	-0.005	(-0.016, 0.002)	0.225	KIAA0355	KIAA0355
		cg18571112	-0.083	0.039	0.058	0.05	-0.005	(-0.017, 0.003)	0.275	SFMBT1	SFMBT1
1-Adult in Household	Early Childhood										
		cg22239534 [3]	**-0.093**	**0.039**	**0.174**	**0.037**	**-0.016**	**(-0.032, -0.003)**	**0.015**	**AK123632**	
		cg06456365	**0.086**	**0.039**	**-0.131**	**0.036**	**-0.011**	**(-0.025, -0.001)**	**0.026**	**RFPL4B**	
		cg21079003 [2]	**-0.084**	**0.039**	**0.119**	**0.036**	**-0.01**	**(-0.023, -0.001)**	**0.03**	**RGMA**	**RGMA**
		cg20930329	**0.078**	**0.039**	**0.122**	**0.036**	**0.009**	**(0, 0.022)**	**0.043**	**AY748447**	

Adversity	Life Course Model	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
		cg03269218 [2]	-0.076	0.039	0.114	0.036	-0.009	(-0.021, 0)	0.051	BC043227	
		cg26078436	-0.08	0.039	-0.1	0.041	0.008	(0, 0.02)	0.054	HBBP1	HBBP1
		cg22255773	0.082	0.039	-0.081	0.037	-0.007	(-0.017, 0)	0.06	LOC100132707	DPP6
		cg00901198	0.078	0.039	-0.084	0.037	-0.007	(-0.017, 0)	0.064	BAI2	BAI2
		cg09191574	0.048	0.039	-0.098	0.04	-0.005	(-0.015, 0.003)	0.229	DIO2	DIO2
		cg03800296	-0.075	0.039	-0.027	0.041	0.002	(-0.005, 0.01)	0.542	TRNA_Gln	
Family Instability	Early Childhood										
		cg27200630	**0.124**	**0.039**	**-0.171**	**0.038**	**-0.021**	**(-0.039, -0.007)**	**0.002**	**PIK3CB**	
		cg21011883	**0.125**	**0.039**	**0.116**	**0.037**	**0.014**	**(0.004, 0.029)**	**0.003**	**L1TD1**	**INADL**
		cg26299079[2]	**0.114**	**0.039**	**-0.124**	**0.037**	**-0.014**	**(-0.029, -0.003)**	**0.003**	**BTBD16**	**BTBD16**
		cg26389281	**-0.107**	**0.039**	**0.148**	**0.037**	**-0.016**	**(0.004, 0.032)**	**0.006**	**ABR**	**ABR**
		cg21305041	**-0.105**	**0.039**	**-0.136**	**0.048**	**0.014**	**(0.002, 0.031)**	**0.011**	**SH3BGRL2**	**SH3BGRL2**
		cg16087263	**0.119**	**0.039**	**0.091**	**0.037**	**0.011**	**(0.001, 0.024)**	**0.014**	**PLA2G2F**	**PLA2G2F**
		cg25513610	0.156	0.039	0.08	0.043	0.013	(0, 0.029)	0.061	CD83	
		cg22839587	-0.113	0.039	-0.054	0.051	0.006	(-0.005, 0.02)	0.29	DPYSL3	DPYSL3
Financial Stress	Early Childhood										
		cg10953317 [2,3]	**-0.159**	**0.039**	**-0.115**	**0.036**	**0.018**	**(0.006, 0.035)**	**0.001**	**CD300A**	**CD300A**
		cg02674870 [2]	**-0.123**	**0.04**	**-0.136**	**0.036**	**0.017**	**(0.005, 0.033)**	**0.0019**	**Mir_598**	
		cg21202551 [2]	**-0.105**	**0.04**	**0.109**	**0.035**	**-0.011**	**(-0.025, -0.002)**	**0.009**	**MIR4710**	
		cg20777315	**0.101**	**0.04**	**0.124**	**0.036**	**0.012**	**(0.002, 0.026)**	**0.0134**	**LOC100505536**	**ISM1**
		cg23462687	**0.088**	**0.04**	**-0.15**	**0.035**	**-0.013**	**(-0.028, -0.001)**	**0.0281**	**HDAC4**	**HDAC4**
		cg00188315	**-0.085**	**0.04**	**-0.124**	**0.036**	**0.011**	**(0.001, 0.024)**	**0.0335**	**LOC285501**	
		cg11293312	**-0.082**	**0.04**	**0.101**	**0.036**	**-0.008**	**(-0.02, 0)**	**0.0437**	**IZUMO1**	**FUT1**
		cg22239534 [2,3]	-0.077	0.04	0.114	0.036	-0.009	(-0.021, 0)	0.0567	AK123632	
		cg11738723	0.078	0.04	-0.101	0.04	-0.008	(-0.02, 0)	0.0611	AX747193	SGK269
		cg07118000 [2]	-0.078	0.04	-0.088	0.035	0.007	(0, 0.018)	0.0624	GPR124	GPR124
		cg04347379 [2]	0.07	0.04	0.144	0.037	0.01	(-0.001, 0.024)	0.077	HEATR2	HEATR2
		cg16515600	0.123	0.04	0.064	0.037	0.008	(-0.001, 0.02)	0.0843	PHTF1	RSBN1
		cg02389555	-0.068	0.04	0.128	0.036	-0.009	(-0.022, 0.001)	0.0872	TRIM27	TRIM27
		cg06820822 [2]	0.07	0.04	-0.083	0.037	-0.006	(-0.016, 0.001)	0.1033	C7orf62	ZNF804B
		cg03033975	0.057	0.04	0.134	0.037	0.008	(-0.003, 0.021)	0.1568	GTPBP5	GTPBP5
		cg16701559	-0.075	0.04	-0.048	0.039	0.004	(-0.002, 0.013)	0.2726	RPP21	TRIM39

Adversity	Life Course Model	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
		cg23798471	0.071	0.04	-0.027	0.041	-0.002	(-0.01, 0.004)	0.5347	SNORA27	
Neighborhood Disadvantage	Very Early Childhood										
		cg18604823	**0.12**	**0.039**	**-0.192**	**0.036**	**-0.023**	**(-0.042, -0.008)**	**0.002**	**GALP**	**GALP**
		cg13003513	**-0.095**	**0.039**	**0.107**	**0.036**	**-0.01**	**(-0.023, -0.001)**	**0.016**	**CARD11**	**CARD11**
		cg11611320	**0.104**	**0.039**	**-0.099**	**0.04**	**-0.01**	**(-0.024, -0.001)**	**0.021**	**LOC401242**	
		cg15027300	**0.086**	**0.039**	**-0.115**	**0.035**	**-0.01**	**(-0.022, -0.001)**	**0.029**	**GGN**	**GGN**
		cg08470892 [2]	**0.088**	**0.039**	**0.105**	**0.036**	**0.009**	**(0.001, 0.021)**	**0.029**	**TPSD1**	**TPSD1**
		cg20262683	**0.085**	**0.039**	**0.09**	**0.035**	**0.008**	**(0, 0.019)**	**0.039**	**NPTX2**	**NPTX2**
		cg14223671 [2]	0.074	0.039	-0.081	0.035	-0.006	(-0.016, 0)	0.075	PRR25	PRR25
		cg24059871 [3]	0.069	0.039	0.121	0.035	0.008	(-0.001, 0.02)	0.078	POP4	POP4
		cg27423959	0.066	0.039	-0.134	0.035	-0.009	(-0.022, 0.001)	0.087	C3orf56	
		cg24738171	0.059	0.039	0.129	0.038	0.008	(-0.002, 0.02)	0.127	HGS	HGS
		cg05931366	-0.057	0.039	-0.137	0.037	0.008	(-0.003, 0.021)	0.149	LINC00266-1	
		cg08073133	0.122	0.039	0.051	0.037	0.006	(-0.002, 0.018)	0.167	SERPINE2	SERPINE2
		cg23119063 [2]	0.099	0.039	0.041	0.037	0.004	(-0.003, 0.014)	0.272	Mir_320	
		cg26595256	0.083	0.039	0.04	0.037	0.003	(-0.002, 0.012)	0.294	TRIO	TRIO
		cg01439119	-0.075	0.039	-0.039	0.039	0.003	(-0.003, 0.011)	0.36	POLR3B	TCP11L2

[a]α= effect of adversity on DNAm at given CpG.
[b]SE = standard error.
[c]β= effect of DNAm at CpG on depressive symptoms.
[d]CI = confidence interval, **bolded** rows indicate statistically significant results at p<0.05 level.
[e]The nearest transcriptional start site (TSS) was identified using the annotation developed by Price et al. (2013).
[f]The gene in which the CpG is located, based on the Illumina annotation.
[2]CpG site is a methylation quantitative trait locus (mQTL), meaning a locus where DNA methylation levels are influenced by common genetic polymorphisms (or SNPs) (19 mQTLs in total). mQTLs were identified using the mQTLdb [60].
[3]Duplicate CpG sites (cg10953317, cg22239534, cg24059871) appearing in 2 different adversities each.

Table S4. Results from sensitivity analysis examining age 7 DNAm as a mediator of the relationship between ever being exposed to childhood adversity and depressive symptoms at mean age 10.6.

Adversity	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
Caregiver Physical/ Emotional Abuse										
	cg08310224	0.071	0.038	0.142	0.038	0.01	(-0.001, 0.024)	0.068	AK058177	PACRG
	cg05401069	0.078	0.038	-0.077	0.039	-0.006	(-0.016, 0.001)	0.084	TTC39C	TTC39C
	cg23037265	-0.062	0.038	-0.096	0.045	0.006	(-0.002, 0.017)	0.138	IFIT1B	IFIT1L
	cg11736524	-0.112	0.038	-0.052	0.043	0.006	(-0.004, 0.018)	0.226	PCAT1	
	cg26345401	0.075	0.038	-0.039	0.042	-0.003	(-0.012, 0.003)	0.376	TATDN1	TATDN1
Sexual/Physical Abuse (by anyone)										
	cg03681057	**-0.107**	**0.039**	**-0.131**	**0.038**	**0.014**	**(0.003, 0.029)**	**0.006**	**HMHB1**	
	cg25858655	**-0.109**	**0.039**	**0.123**	**0.037**	**-0.013**	**(-0.028, -0.003)**	**0.006**	**C2CD2**	**C2CD2**
	cg04595200	**-0.124**	**0.039**	**0.1**	**0.037**	**-0.012**	**(-0.026, -0.003)**	**0.007**	**ALG10**	
	cg07061298	**0.117**	**0.039**	**0.096**	**0.037**	**0.011**	**(0.002, 0.024)**	**0.011**	**HOXA3**	**HOXA3**
	cg26631505	**0.091**	**0.039**	**-0.116**	**0.037**	**-0.011**	**(-0.024, -0.001)**	**0.022**	**MSI2**	**MSI2**
	cg01973483[2]	**0.087**	**0.039**	**-0.136**	**0.036**	**-0.012**	**(-0.026, -0.001)**	**0.026**	**PITPNM2**	**PITPNM2**
	cg03171465	**-0.1**	**0.039**	**0.088**	**0.037**	**-0.009**	**(-0.021, -0.001)**	**0.026**	**C2orf55**	**C2orf55**
	cg21428348	**0.106**	**0.039**	**-0.085**	**0.037**	**-0.009**	**(-0.021, -0.001)**	**0.029**	**AX746653**	**PREX1**
	cg21895593	0.07	0.039	0.134	0.041	0.009	(-0.001, 0.023)	0.073	UBN2	UBN2
	cg23380397	-0.068	0.039	-0.085	0.046	0.006	(-0.001, 0.017)	0.137	Prion_pknot	
	cg13041470	-0.061	0.039	-0.044	0.052	0.003	(-0.004, 0.013)	0.477	RAD18	RAD18
	cg20006618	-0.074	0.039	0.02	0.051	-0.001	(-0.012, 0.007)	0.711	SORBS2	SORBS2
	cg05470328	-0.064	0.039	-0.014	0.054	0.001	(-0.007, 0.01)	0.807	ANGPT1	ANGPT1
Maternal Psychopathology										
	cg07533249	**0.115**	**0.039**	**0.115**	**0.037**	**0.013**	**(0.003, 0.027)**	**0.004**	**CCL4**	
	cg18170581	**-0.094**	**0.039**	**-0.089**	**0.039**	**0.008**	**(0, 0.021)**	**0.035**	**CPT2**	**CPT2**
	cg14423045	0.1	0.039	0.074	0.038	0.007	(0, 0.019)	0.062	DENND3	DENND3
	ch.5.2607995R	0.073	0.039	0.152	0.039	0.011	(-0.001, 0.026)	0.063	DND1	HARS
	cg15223579	-0.072	0.039	0.114	0.039	-0.008	(-0.021, 0)	0.069	ACP2	NR1H3
	cg02203680	0.08	0.039	0.08	0.038	0.006	(0, 0.017)	0.071	LOC641298	RRN3P3
	cg17783213	0.092	0.039	0.072	0.038	0.007	(0, 0.018)	0.075	COL22A1	COL22A1
	cg03107917	0.071	0.039	0.096	0.037	0.007	(-0.001, 0.018)	0.075	ADARB2	
	cg01556514	-0.067	0.039	-0.111	0.038	0.008	(-0.001, 0.02)	0.087	HEY2	
	cg02503527	0.086	0.039	0.068	0.037	0.006	(-0.001, 0.016)	0.095	LOC493754	
	cg24967487	-0.094	0.039	-0.06	0.038	0.006	(-0.001, 0.016)	0.128	DQ584669	
	cg06953703	0.101	0.039	0.056	0.039	0.006	(-0.002, 0.017)	0.159	ATP13A2	ATP13A2

Adversity	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
1-Adult in Household										
	cg04820159	**-0.096**	**0.039**	**0.117**	**0.037**	**-0.011**	**(-0.025, -0.002)**	**0.014**	**AKT1**	**AKT1**
	cg00936722	**0.094**	**0.039**	**-0.119**	**0.038**	**-0.011**	**(-0.025, -0.002)**	**0.017**	**AK024243**	
	cg06720017	**0.096**	**0.039**	**0.111**	**0.037**	**0.011**	**(0.001, 0.024)**	**0.017**	**LGALS3BP**	**LGALS3BP**
	cg21079003[2]	**-0.089**	**0.039**	**0.109**	**0.037**	**-0.01**	**(-0.022, -0.001)**	**0.024**	**RGMA**	**RGMA**
	cg23921338	**0.088**	**0.039**	**-0.099**	**0.039**	**-0.009**	**(-0.021, -0.001)**	**0.034**	**PCBP1-AS1**	
	cg10539191	-0.082	0.039	-0.073	0.043	0.006	(-0.001, 0.017)	0.12	PPM1L	PPM1L
	cg09610766	-0.078	0.039	-0.05	0.048	0.004	(-0.004, 0.015)	0.327	ALCAM	ALCAM
	cg20320823	-0.076	0.039	-0.017	0.048	0.001	(-0.007, 0.011)	0.732	OR2C3	OR2C3
Family Instability										
	cg01572549	**0.115**	**0.039**	**0.114**	**0.038**	**0.013**	**(0.003, 0.027)**	**0.005**	**LAMA5**	**LAMA5**
	cg22613968	**0.106**	**0.039**	**-0.114**	**0.038**	**-0.012**	**(-0.026, -0.002)**	**0.008**	**MDS2**	**MDS2**
	cg15393937	**-0.111**	**0.039**	**-0.101**	**0.037**	**0.011**	**(0.002, 0.025)**	**0.009**	**IGF2-AS1**	**INS-IGF2**
	cg01256165	**-0.105**	**0.039**	**-0.089**	**0.037**	**0.009**	**(0.001, 0.022)**	**0.023**	**OPRL1**	**OPRL1**
	cg21011883[2]	**0.084**	**0.039**	**0.152**	**0.038**	**0.013**	**(0.001, 0.028)**	**0.03**	**L1TD1**	**INADL**
	cg12899614	-0.087	0.039	-0.109	0.049	0.009	(0, 0.024)	0.053	MIR218-1	SLIT2
	cg25539074	-0.083	0.039	-0.091	0.049	0.008	(-0.001, 0.021)	0.097	GSX2	
Financial Stress										
	cg05831728	**-0.137**	**0.04**	**0.158**	**0.037**	**-0.022**	**(-0.04, -0.008)**	**<0.001**	**UCP1**	**TBC1D9**
	cg04782002	**0.141**	**0.04**	**0.124**	**0.038**	**0.017**	**(0.005, 0.034)**	**0.002**	**AK057887**	**KLHL36**
	cg08072171	**-0.127**	**0.04**	**0.095**	**0.037**	**-0.012**	**(-0.026, -0.002)**	**0.011**	**HGC6.3**	**HGC6.3**
	cg06542559	**0.094**	**0.04**	**-0.109**	**0.038**	**-0.01**	**(-0.023, -0.001)**	**0.024**	**PGCP**	**PGCP**
	cg18976418	**0.108**	**0.04**	**0.085**	**0.038**	**0.009**	**(0.001, 0.022)**	**0.032**	**CTAGE5**	**CTAGE5**
	cg26893605	**0.113**	**0.04**	**-0.082**	**0.038**	**-0.009**	**(-0.022, 0)**	**0.037**	**GALNT2**	**GALNT2**
	cg00357551	**0.079**	**0.04**	**-0.121**	**0.037**	**-0.01**	**(-0.023, 0)**	**0.048**	**FAM196B**	**FAM196B**
	cg07895437	**-0.087**	**0.04**	**-0.087**	**0.037**	**0.008**	**(0, 0.019)**	**0.048**	**TAPBP**	**ZBTB22**
	cg16407947	0.077	0.04	0.127	0.037	0.01	(0, 0.023)	0.054	FBXO18	ANKRD16
	cg13463167	-0.076	0.04	-0.088	0.042	0.007	(-0.001, 0.018)	0.091	DENND1B	DENND1B
	cg02484307	-0.076	0.04	-0.081	0.038	0.006	(-0.001, 0.017)	0.091	JAZF1	
	cg23363031	0.076	0.04	-0.075	0.038	-0.006	(-0.016, 0.001)	0.104	NTM	NTM
	cg25016635	0.07	0.04	-0.073	0.038	-0.005	(-0.015, 0.001)	0.131	DHX37	DHX37
	cg08335618	-0.07	0.04	0.055	0.038	-0.004	(-0.013, 0.002)	0.222	HADH	HADH
	cg16900604	0.083	0.04	-0.017	0.04	-0.001	(-0.01, 0.006)	0.692	CREB1	
	cg15670005	0.063	0.04	0.005	0.042	0	(-0.006, 0.007)	0.931	CALD1	
	cg18391127	-0.098	0.04	0	0.04	0	(-0.009, 0.009)	0.997	MFGE8	
Neighborhood Disadvantage										
	cg12078154	**-0.113**	**0.039**	**0.094**	**0.036**	**-0.011**	**(-0.023, -0.002)**	**0.012**	**RPTOR**	**RPTOR**
	cg27423959[2]	**0.093**	**0.039**	**-0.13**	**0.036**	**-0.012**	**(-0.026, -0.002)**	**0.018**	**C3orf56**	

Adversity	CpG	α[a]	SE[b](α)	β[c]	SE(β)	Indirect Effect	CI[d]	*P* Value	Nearest TSS[e]	UCSC gene[f]
	cg09671837	**-0.091**	**0.039**	**0.148**	**0.036**	**-0.013**	**(-0.028, -0.002)**	**0.019**	**FAM115A**	**FAM115A**
	cg06077224	**-0.092**	**0.039**	**-0.099**	**0.036**	**0.009**	**(0.001, 0.021)**	**0.021**	**ADAP1**	**ADAP1**
	cg04091563	**-0.156**	**0.038**	**-0.079**	**0.036**	**0.012**	**(0.001, 0.027)**	**0.032**	**HTR4**	**HTR4**
	ch.5.2119577R	**0.082**	**0.039**	**-0.115**	**0.036**	**-0.009**	**(-0.022, -0.001)**	**0.034**	**SEMA6A**	
	cg07061298	**-0.122**	**0.039**	**0.077**	**0.037**	**-0.009**	**(-0.022, -0.001)**	**0.036**	**HOXA3**	**HOXA3**
	cg24059871[2]	**0.079**	**0.039**	**0.103**	**0.036**	**0.008**	**(0, 0.02)**	**0.044**	**POP4**	**POP4**
	cg22861548	**0.093**	**0.039**	**0.079**	**0.036**	**0.007**	**(0, 0.018)**	**0.046**	**LYNX1**	**LYNX1**
	cg19934735	-0.076	0.039	0.103	0.037	-0.008	(-0.019, 0)	0.058	MYEOV2	
	cg01454947	0.073	0.039	0.086	0.036	0.006	(0, 0.017)	0.076	COA5	C2orf64
	cg26595256[2]	0.066	0.039	0.074	0.037	0.005	(-0.001, 0.014)	0.126	TRIO	TRIO

[a]α= effect of adversity on DNA methylation (DNAm) at a given CpG.
[b]SE = standard error.
[c]β= effect of DNAm at a CpG on depressive symptoms.
[d]CI = confidence interval, **bolded** rows indicate statistically significant results at p<0.05 level.
[e]The nearest transcriptional start site (TSS) was identified using the annotation developed by Price et al. (2013).
[f]The gene in which the CpG is located, based on the Illumina annotation.
[2]Represents a CpG that was also identified as a mediator in the primary analysis.

Table S5. Results of structured life course modeling approach (SLCMA) of childhood adversity and depression in replication cohorts.

Cohort	Adversity	Sample Size	Selcted timepoint	ALSPAC timepoint	Total Effect	*P* Value	CI[a]
FFCWS	Caregiver physical or emotional abuse	1317	60 months	8 months	-0.05	0.96	(-0.46, 0.37)
	Maternal psychopathology	1711	36 months	33 months	1.27	5.21E-11	(0.90, 1.64)
	One-adult in the household	1699	12 months	47 months	0.09	0.96	(-0.33, 0.46)
	Family instability	1716	12 months	57 months	0.3	0.32	(-0.14, 0.66)
	Financial stress	1702	Accumulation	61 months	0.23	0.0099	(0.03, 0.38)
GenR	Caregiver physical or emotional abuse*	NA	NA	8 months	NA	NA	NA
	Maternal psychopathology*	NA	NA	33 months	NA	NA	NA
	Family instability	316	36 months	57 months	-1.34	0.47	(-4.18, 1.66)
	Financial stress	344	Recency	61 months	0.58	0.033	(-0.01, 1.04)

[a]CI, confidence interval

*only one timepoint was available for caregiver abuse and maternal psychopathology in the Generation R (GenR) cohort.

Results from the structured life course modeling approach (SLCMA), which identified the theoretical life course model with the best explanatory power between each adversity and depressive symptoms in the replication cohorts. The timepoint selected is shown here, along with its corresponding life course model, the effect estimate (total effect) for the sensitive period exposure on SMFQ score, *P* value, and CI. Post-selective inference performed using the max-|*t*|-test.

Table S6. Replication of CpG-level mediation in the FFCWS cohort.

			ALSPAC				FFCWS			
Adversity	**CpG**	**Gene**	**Indirect effect[1]**	**Confidence intervals[2]**	**Indirect effect adj.[3]**	**P-value[4]**	**Indirect effect[1]**	**Confidence intervals[2]**	**Indirect effect adj.[3]**	**P-value[4]**
Caregiver physical or emotional abuse	cg23751110	SLIT2	-24.7%	(-158%; 6.1%)	24.7%	0.056	4.7%	(-38.9%; 33.6%)	-4.7%	0.986
	cg12343929	WDR90	-22.4%	(-113.6%; 4.1%)	22.4%	0.064	-27.0%	(-67.3%; 65.4%)	27.0%	0.912
	cg03965496	TRNA_Asn	-21.7%	(-130.1%; 8.4%)	21.7%	0.058	21.4%	(-53%; 39.8%)	-21.4%	0.886
	cg21089584	JARID2	18.5%	(-13.3%; 107.3%)	-18.5%	0.094	-3.5%	(-48.7%; 56.8%)	3.5%	0.9
	cg06804625	PSRC1	23.1%	(-4.9%; 131.8%)	-23.1%	0.066	-3.5%	(-31.1%; 35%)	3.5%	0.984
Maternal psychopathology	cg07308232	C7orf50	-9.3%	(-39.3%; -0.9%)	-9.3%	0.026	0.7%	(-0.5%; 2.5%)	0.7%	0.3
	cg16292933	SLC4A8	-9.2%	(-44.2%; -0.5%)	-9.2%	0.04	-0.2%	(-1.6%; 0.7%)	-0.2%	0.66
	cg06451157	HLA-H	-7.7%	(-36%; -0.1%)	-7.7%	0.044	-0.2%	(-1.7%; 2.7%)	-0.2%	0.964
	cg18571112	SFMBT1	-7.2%	(-34.9%; -0.1%)	-7.2%	0.044	-0.2%	(-1.1%; 0.5%)	-0.2%	0.51
	cg21665774	KIAA0355	-7.0%	(-31.8%; 0.8%)	-7.0%	0.092	-0.3%	(-4%; 2.9%)	-0.3%	0.812
	cg00300275	U80764	-6.9%	(-38.2%; 2.7%)	-6.9%	0.166	0.0%	(-1.6%; 1.4%)	0.0%	0.95
	cg10953317	CD300A	-5.0%	(-22.2%; 1.4%)	-5.0%	0.124	0.6%	(-0.4%; 2.6%)	0.6%	0.348
	cg19642007	TNNT3	8.4%	(-1.1%; 25.9%)	8.4%	0.086	0.1%	(-1.8%; 1.5%)	0.1%	0.938
	cg24059871	POP4	9.2%	(0.8%; 40.8%)	9.2%	0.03	-0.1%	(-1.9%; 1.1%)	-0.1%	0.866
1-adult household	cg06456365	RFPL4B	-17.7%	(-161.5%; 211.4%)	-17.7%	0.222	-10.0%	(-71.8%; 55.1%)	10.0%	0.992
	cg22239534	AK123632	-17.6%	(-178.8%; 284.3%)	-17.6%	0.186	3.2%	(-34.2%; 26.2%)	-3.2%	0.938
	cg00901198	BAI2	-16.6%	(-182.1%; 213.3%)	-16.6%	0.232	-11.8%	(-42.1%; 33.1%)	11.8%	0.99
	cg22255773	LOC100132707	-15.3%	(-155.8%; 151.6%)	-15.3%	0.194	-3.2%	(-55.9%; 47.4%)	3.2%	0.984
	cg21079003	RGMA	-14.9%	(-152.9%; 187.1%)	-14.9%	0.242	72.5%	(-129.3%; 123.6%)	-72.5%	0.942
	cg09191574	DIO2	-11.0%	(-168.8%; 124.2%)	-11.0%	0.432	-10.1%	(-72.4%; 65.9%)	-10.1%*	0.952
	cg03269218	BC043227	-6.4%	(-70.6%; 71.8%)	-6.4%	0.38	81.1%	(-174.3%; 99.4%)	-81.1%	0.92
	cg03800296	TRNA_Gln	13.9%	(-160.4%; 120.1%)	13.9%	0.21	0.3%	(-32.7%; 28.9%)	-0.3%	0.91
	cg20930329	AY748447	14.2%	(-113.2%; 127.5%)	14.2%	0.192	-0.1%	(-31.3%; 38.9%)	0.1%	0.984
	cg26078436	HBBP1	16.7%	(-209.4%; 156.3%)	16.7%	0.196	-0.4%	(-30.5%; 20%)	0.4%	0.968
Family instability	cg27200630	PIK3CB	-27.3%	(-415.3%; 193.5%)	-27.3%	0.2	-3.2%	(-26.3%; 47.1%)	-3.2%	0.884
	cg26389281	ABR	-23.9%	(-303.4%; 231.4%)	-23.9%	0.196	-3.0%	(-67.1%; 48%)	-3.0%	0.952

	cg26299079	BTBD16	-23.7%	(-302%; 250.5%)	-23.7%	0.196	-1.8%	(-65%; 62%)	-1.8%	0.922
	cg21011883	L1TD1	-6.1%	(-78.7%; 57.5%)	-6.1%	0.546	3.0%	(-41.4%; 46.5%)	3.0%	0.866
	cg16087263	PLA2G2F	18.5%	(-143.9%; 245%)	18.5%	0.18	-0.1%	(-40.5%; 49%)	-0.1%	0.974
	cg21305041	SH3BGRL2	23.1%	(-186.7%; 282.6%)	23.1%	0.216	0.1%	(-39.4%; 33.1%)	0.1%	0.998
	cg22839587	DPYSL3	23.9%	(-167.4%; 287.2%)	23.9%	0.218	-0.4%	(-24.6%; 20.8%)	-0.4%	0.952
	cg25513610	CD83	29.8%	(-243.1%; 373.6%)	29.8%	0.18	-4.6%	(-84.2%; 95.4%)	-4.6%	0.792
Financial hardship	cg21202551	MIR4710	-12.5%	(-78%; -1.7%)	-12.5%	0.024				
	cg23462687	HDAC4	-11.1%	(-66.5%; -0.5%)	-11.1%	0.046	-0.9%	(-7.3%; 3.8%)	-0.9%	0.574
	cg06820822	C7orf62	-10.0%	(-68.2%; 0%)	-10.0%	0.056	-1.6%	(-10.8%; 3.2%)	-1.6%	0.426
	cg11738723	AX747193	-10.0%	(-65.4%; -1.2%)	-10.0%	0.024				
	cg22239534	AK123632	-9.4%	(-46%; 0.7%)	-9.4%	0.07	0.8%	(-3.5%; 9%)	0.8%	0.714
	cg11293312	IZUMO1	-8.9%	(-54.1%; -0.2%)	-8.9%	0.048	0.2%	(-3.2%; 5.5%)	0.2%	0.85
	cg02389555	TRIM27	-8.3%	(-55.1%; 3.6%)	-8.3%	0.174	-1.1%	(-6.8%; 3.4%)	-1.1%	0.524
	cg23798471	SNORA27	-7.7%	(-57.7%; 4%)	-7.7%	0.184				
	cg10953317	CD300A	5.3%	(-3.6%; 37.4%)	5.3%	0.25	2.1%	(-1.7%; 15%)	2.1%	0.282
	cg07118000	GPR124	8.3%	(-3.6%; 55.3%)	8.3%	0.152	-0.4%	(-5.1%; 2.8%)	-0.4%	0.768
	cg03033975	GTPBP5	8.4%	(-8.1%; 44%)	8.4%	0.194	-0.3%	(-6.3%; 4.5%)	-0.3%	0.858
	cg16701559	RPP21	9.4%	(-1.4%; 51.1%)	9.4%	0.094	-1.1%	(-49.6%; 45.5%)	-1.1%	0.908
	cg00188315	LOC285501	10.6%	(-3.7%; 51.2%)	10.6%	0.112	-1.6%	(-14.1%; 3.2%)	-1.6%	0.48
	cg04347379	HEATR2	10.8%	(-2.5%; 45.6%)	10.8%	0.066	0.7%	(-3.4%; 7.4%)	0.7%	0.688
	cg20777315	LOC100505536	11.2%	(1.7%; 56.2%)	11.2%	0.032	0.1%	(-5%; 6.8%)	0.1%	0.936
	cg16515600	PHTF1	14.0%	(2%; 88%)	14.0%	0.02				
	cg02674870	Mir_598	17.0%	(3.3%; 101.1%)	17.0%	0.02	0.2%	(-2.4%; 4.8%)	0.2%	0.908

[1] Proportion of the total effect of the childhood adversity on depressive symptoms mediated through DNA methylation at that CpG.
[2] 95% confidence intervals.
[3] Proportion of the total effect of the childhood adversity on depressive symptoms mediated through DNA methylation at that CpG, adjusted for the direction of the total effect (whereby positive effects are risk-increasing and negative effects are protective).
[4]P-value of the indirect (mediated) effect.
*The total effect for cg09191574 was 0.0059 (p=0.814) due to sample differences (availability of data on 450K versus EPIC array).

Table S7. Summary of mediation replication across top CpGs for ALSPAC and FFCWS.

Adversity	ALSPAC						FFCWS					
	Timepoint (months)[1]	# CpGs[2]	Indirect (%)[3]	Direct (%)[4]	Total effect[5]	P-value (total)[6]	Timepoint (months)[1]	# CpGs[2]	Indirect (%)[3]	Direct (%)[4]	Total effect[5]	P-value (total)[6]
Caregiver physical or emotional abuse	8	5	-27.1	127.1	-0.049	0.05	36	5	-31.6	131.6	-0.002	0.886
Maternal psychopathology	33	9	-34.7	134.7	0.116	0.002	36	9	0.3	99.7	0.114	<1e-4
1-adult household	47	10	-54.7	154.7	0.062	0.178	36	10	170.5	-70.5	-0.002*	0.94
Family instability	57	8	14.3	85.7	0.069	0.18	60	8	-19.9	119.9	0.007	0.66
Financial hardship	61	17	16.9	83.1	0.093	0.02	60	13	-2.7	102.7	0.036	0.02

[1] Timepoint when adversity was measured. Selected using the SLCMA for ALSPAC and the closest time period for FFCWS.
[2] Number of CpGs included in the total indirect (mediated) effects. Note that not all CpGs for financial hardship were available in FFCWS.
[3] Percent of the relationship between the childhood adversity and depressive symptoms explained by DNA methylation at those CpGs.
[4] Direct effect is the effect of childhood adversity on depressive symptom score unrelated to mediation, listed here in terms percent of the *total* effect.
[5] Total effect of the relationship between the childhood adversity and depressive symptoms. Effects were standardized so that this measure reflects the correlation between adversities.
[6] P-value of the relationship between the childhood adversity and depressive symptoms.
*Due to sample differences, the total effect of childhood adversity on depressive symptoms for this CpG was estimated at 0.0059 (p=0.814).

Table S8. Replication of CpG-level mediation in the Generation R cohort.

			ALSPAC				Generation R			
Adversity	CpG	Gene	Indirect effect[1]	Confidence intervals[2]	Indirect effect adj.[3]	P-value[4]	Indirect effect[1]	Confidence intervals[2]	Indirect effect adj.[3]	P-value[4]
Caregiver physical or emotional abuse	cg23751110	SLIT2	-24.7%	(-158%; 6.1%)	24.7%	0.056	4.4%	(-100.2%; 128.8%)	4.4%	0.726
	cg12343929	WDR90	-22.4%	(-113.6%; 4.1%)	22.4%	0.064	0.3%	(-35.3%; 63.6%)	0.3%	0.952
	cg03965496	TRNA_Asn	-21.7%	(-130.1%; 8.4%)	21.7%	0.058	-5.6%	(-129.4%; 110.3%)	-5.6%	0.756
	cg21089584	JARID2	18.5%	(-13.3%; 107.3%)	-18.5%	0.094	3.8%	(-106.3%; 93.4%)	3.8%	0.896
	cg06804625	PSRC1	23.1%	(-4.9%; 131.8%)	-23.1%	0.066	1.1%	(-64%; 59.4%)	1.1%	0.976
Maternal psychopathology	cg07308232	C7orf50	-9.3%	(-39.3%; -0.9%)	-9.3%	0.026	-59.9%	(-84.9%; 172%)	59.9%	0.972
	cg16292933	SLC4A8	-9.2%	(-44.2%; -0.5%)	-9.2%	0.04	76.9%	(-186%; 242.2%)	-76.9%	0.932
	cg06451157	HLA-H	-7.7%	(-36%; -0.1%)	-7.7%	0.044	-186.5%	(-408.9%; 239.4%)	186.5%	0.958
	cg18571112	SFMBT1	-7.2%	(-34.9%; -0.1%)	-7.2%	0.044	1.6%	(-82.4%; 103.9%)	-1.6%	0.964
	cg21665774	KIAA0355	-7.0%	(-31.8%; 0.8%)	-7.0%	0.092	11.6%	(-107.8%; 98.7%)	-11.6%	0.758
	cg00300275	U80764	-6.9%	(-38.2%; 2.7%)	-6.9%	0.166	411.6%	(-756.9%; 414.9%)	-411.6%	0.932
	cg10953317	CD300A	-5.0%	(-22.2%; 1.4%)	-5.0%	0.124	-1.0%	(-66.8%; 109.7%)	1.0%	0.898
	cg19642007	TNNT3	8.4%	(-1.1%; 25.9%)	8.4%	0.086	-23.8%	(-72.4%; 98.3%)	23.8%	0.878
	cg24059871	POP4	9.2%	(0.8%; 40.8%)	9.2%	0.03	-2.0%	(-103.4%; 80.1%)	2.0%	0.986
Family instability	cg27200630	PIK3CB	-27.3%	(-415.3%; 193.5%)	-27.3%	0.2	-2.1%	(-72.3%; 81.8%)	-2.1%	0.792
	cg26389281	ABR	-23.9%	(-303.4%; 231.4%)	-23.9%	0.196	-1.4%	(-100.3%; 83.3%)	-1.4%	0.96
	cg26299079	BTBD16	-23.7%	(-302%; 250.5%)	-23.7%	0.196	-14.2%	(-180.7%; 169.8%)	-14.2%	0.858
	cg21011883	L1TD1	-6.1%	(-78.7%; 57.5%)	-6.1%	0.546	0.8%	(-154.6%; 92%)	0.8%	0.95
	cg16087263	PLA2G2F	18.5%	(-143.9%; 245%)	18.5%	0.18	0.2%	(-57.7%; 55.2%)	0.2%	0.95
	cg21305041	SH3BGRL2	23.1%	(-186.7%; 282.6%)	23.1%	0.216	-7.9%	(-129.8%; 129.4%)	-7.9%	0.79
	cg22839587	DPYSL3	23.9%	(-167.4%; 287.2%)	23.9%	0.218	-4.2%	(-66.9%; 73.8%)	-4.2%	0.958
	cg25513610	CD83	29.8%	(-243.1%; 373.6%)	29.8%	0.18	-10.1%	(-118.8%; 176.4%)	-10.1%	0.962
Financial hardship	cg21202551	MIR4710	-12.5%	(-78%; -1.7%)	-12.5%	0.024	0.0%	(-12.1%; 15.5%)	0.0%	0.966
	cg23462687	HDAC4	-11.1%	(-66.5%; -0.5%)	-11.1%	0.046	-0.1%	(-9.1%; 9.2%)	-0.1%	0.986
	cg06820822	C7orf62	-10.0%	(-68.2%; 0%)	-10.0%	0.056	0.0%	(-23.2%; 10%)	0.0%	0.722
	cg11738723	AX747193	-10.0%	(-65.4%; -1.2%)	-10.0%	0.024	1.2%	(-9%; 14.4%)	1.2%	0.73
	cg22239534	AK123632	-9.4%	(-46%; 0.7%)	-9.4%	0.07	-2.6%	(-31.6%; 22.7%)	-2.6%	0.434

cg11293312	IZUMO1	-8.9%	(-54.1%; -0.2%)	-8.9%	0.048	2.2%	(-12.4%; 26.2%)	2.2%	0.48
cg02389555	TRIM27	-8.3%	(-55.1%; 3.6%)	-8.3%	0.174	-3.0%	(-37%; 20.8%)	-3.0%	0.394
cg23798471	SNORA27	-7.7%	(-57.7%; 4%)	-7.7%	0.184	2.7%	(-28.8%; 21.6%)	2.7%	0.654
cg10953317	CD300A	5.3%	(-3.6%; 37.4%)	5.3%	0.25	-0.6%	(-42.9%; 28.2%)	-0.6%	0.948
cg07118000	GPR124	8.3%	(-3.6%; 55.3%)	8.3%	0.152	3.8%	(-29.5%; 33.5%)	3.8%	0.562
cg03033975	GTPBP5	8.4%	(-8.1%; 44%)	8.4%	0.194	-0.3%	(-19.8%; 9.2%)	-0.3%	0.946
cg16701559	RPP21	9.4%	(-1.4%; 51.1%)	9.4%	0.094	2.0%	(-29%; 27.7%)	2.0%	0.76
cg00188315	LOC285501	10.6%	(-3.7%; 51.2%)	10.6%	0.112	0.5%	(-11.2%; 22.1%)	0.5%	0.874
cg04347379	HEATR2	10.8%	(-2.5%; 45.6%)	10.8%	0.066	0.2%	(-12.6%; 17.7%)	0.2%	0.908
cg20777315	LOC100505536	11.2%	(1.7%; 56.2%)	11.2%	0.032	1.4%	(-12.2%; 24.6%)	1.4%	0.676
cg16515600	PHTF1	14.0%	(2%; 88%)	14.0%	0.02				
cg02674870	Mir_598	17.0%	(3.3%; 101.1%)	17.0%	0.02	0.0%	(-12.8%; 10.3%)	0.0%	0.974

[1] Proportion of the total effect of the childhood adversity on depressive symptoms mediated through DNA methylation at that CpG.
[2] 95% confidence intervals.
[3] Proportion of the total effect of the childhood adversity on depressive symptoms mediated through DNA methylation at that CpG, adjusted for the direction of the total effect (whereby positive effects are risk-increasing and negative effects are protective).
[4]P-value of the indirect (mediated) effect.

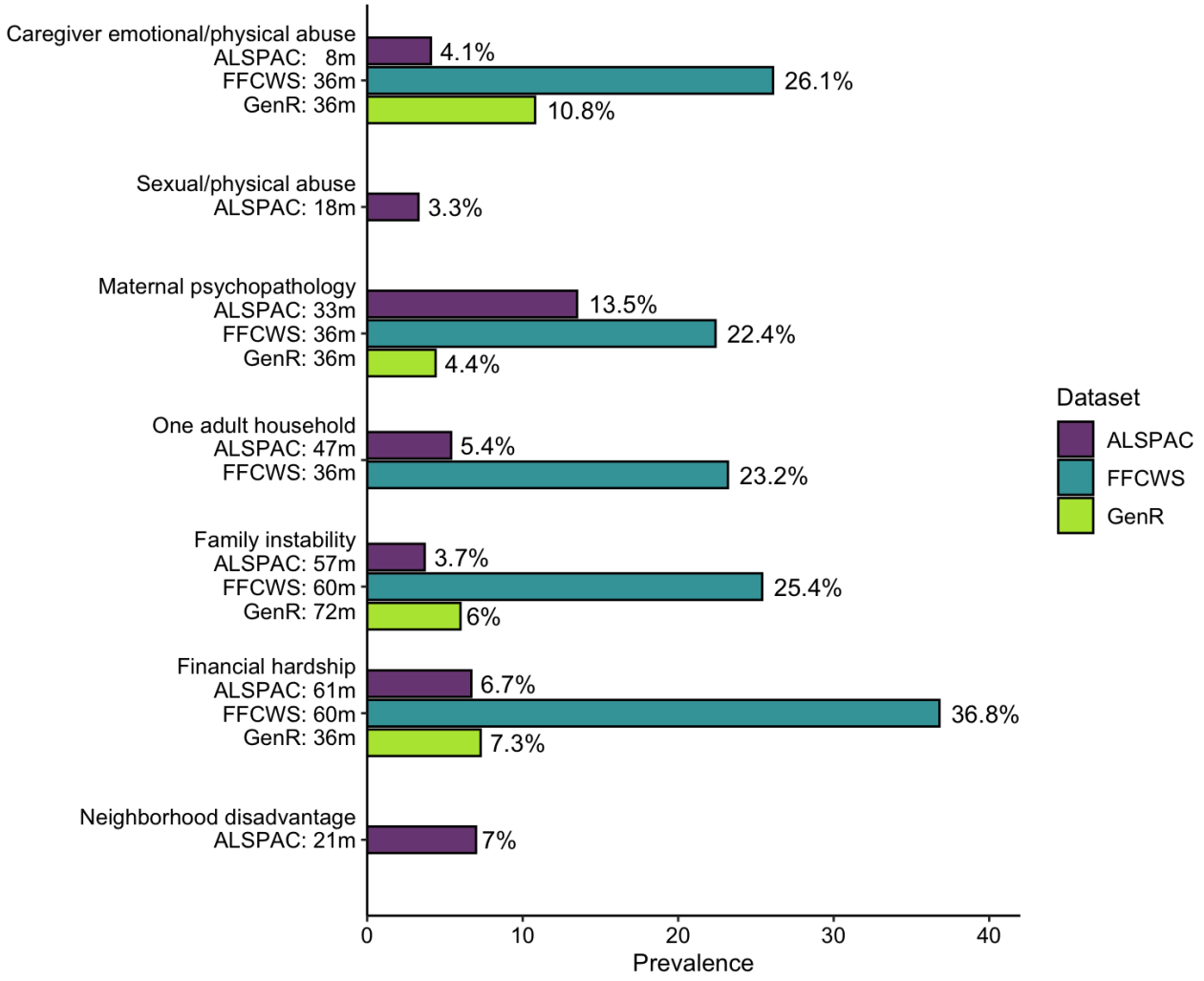
Table S9. Summary of mediation replication across top CpGs for ALSPAC and Generation R.

Adversity	ALSPAC						Generation R					
	Timepoint (months)[1]	# CpGs[2]	Indirect (%)[3]	Direct (%)[4]	Total effect[5]	P-value (total)[6]	Timepoint (months)[1]	# CpGs[2]	Indirect (%)[3]	Direct (%)[4]	Total effect[5]	P-value (total)[6]
Caregiver physical or emotional abuse	8	5	-27.1	127.1	-0.049	0.05	3y	5	7.9	92.1	2.50	0.096
Maternal psychopathology	33	9	-34.7	134.7	0.116	0.002	3y	9	251.6	-151.6	-0.08	0.93
Family instability	57	8	14.3	85.7	0.069	0.18	6y	8	-38.9	138.9	0.44	0.69
Financial hardship	61	17	16.9	83.1	0.093	0.02	3y	16	4.0	96.0	0.78	0.44

[1] Timepoint when adversity was measured. Selected using the SLCMA for ALSPAC and the closest time period for Generation R.
[2] Number of CpGs included in the total indirect (mediated) effects. Note that not all CpGs for financial hardship were available in Generation R.
[3] Percent of the relationship between the childhood adversity and depressive symptoms explained by DNA methylation at those CpGs.
[4] Direct effect is the effect of childhood adversity on depressive symptom score unrelated to mediation, listed here in terms percent of the *total* effect.
[5] Total effect of the relationship between the childhood adversity and depressive symptoms. Effects were standardized so that this measure reflects the correlation between adversities.
[6] P-value of the relationship between the childhood adversity and depressive symptoms.

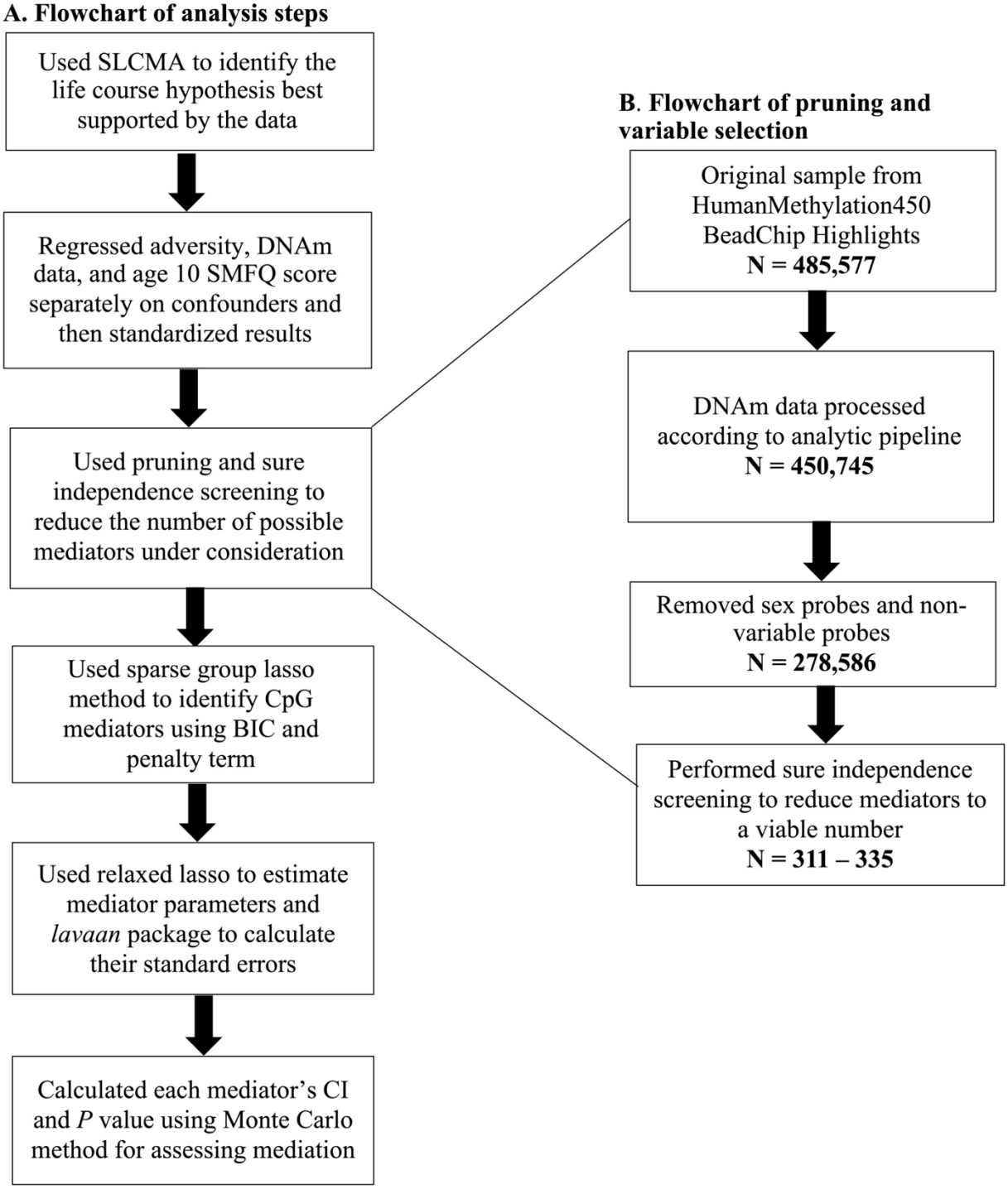
SUPPLEMENTARY FIGURES

Figure S1. Prevalence of exposures within each childhood adversity's analytic sample and across cohorts.



Prevalence of adversity exposure within each analytic sample for the ALSPAC cohort ranged from 3.3% in sexual/physical abuse (by anyone) to 13.5% in maternal psychopathology. These numbers varied in the FFCWS and GenR cohorts, with higher prevalence observed in FFCWS across all adversities and slightly higher prevalence in GenR. The timepoints shown are those selected from the SLCMA in ALSPAC and the best matched timepoint in the replication cohorts.

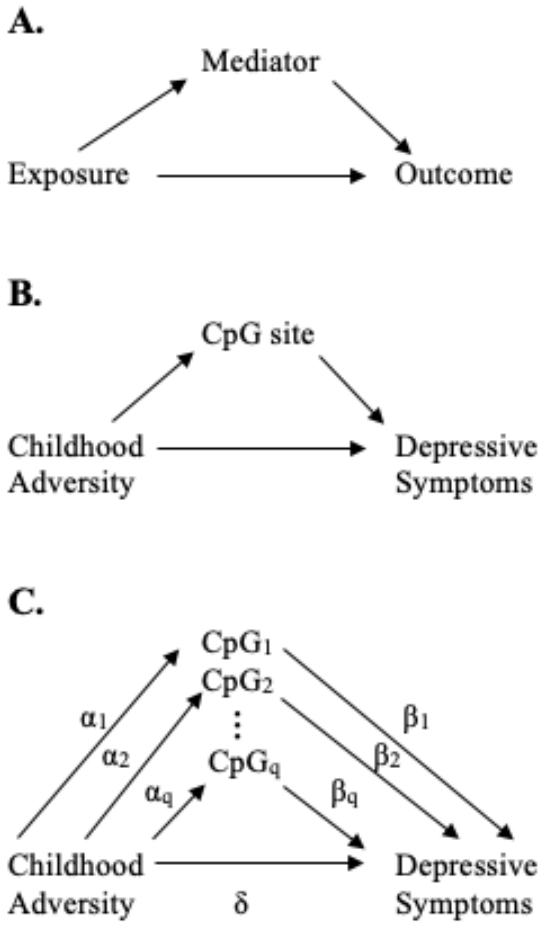
Figure S2. Flowchart of analysis steps for each adversity-outcome relationship.



SLCMA, structured life course modeling approach; DNAm, DNA methylation; SMFQ, Short Mood and Feelings Questionnaire; CpG, cytosines preceding a guanine nucleotide; BIC, Bayesian information criterion; CI, confidence interval.

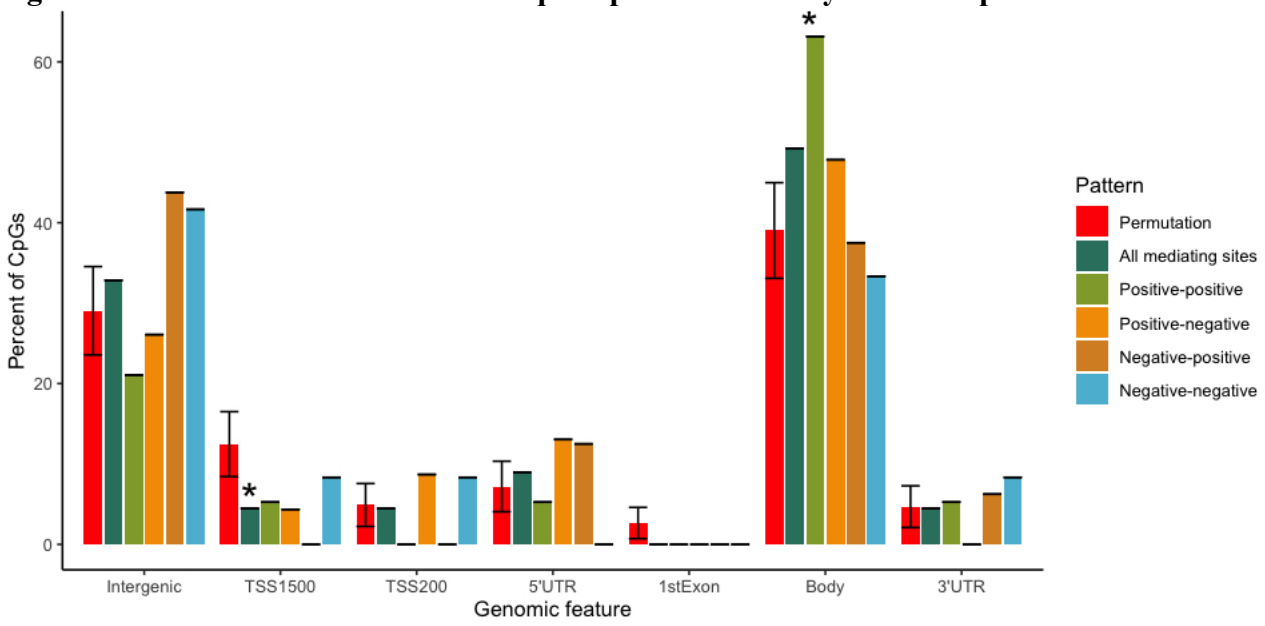
A. depicts the steps of the mediation analysis performed 7 times, once for each of the 7 adversities we studied (N=627-675). **B.** highlights the steps taken to reduce the mediators under consideration to a viable number for each of the 7 adversities.

Figure S3. Single and multiple mediator structures.



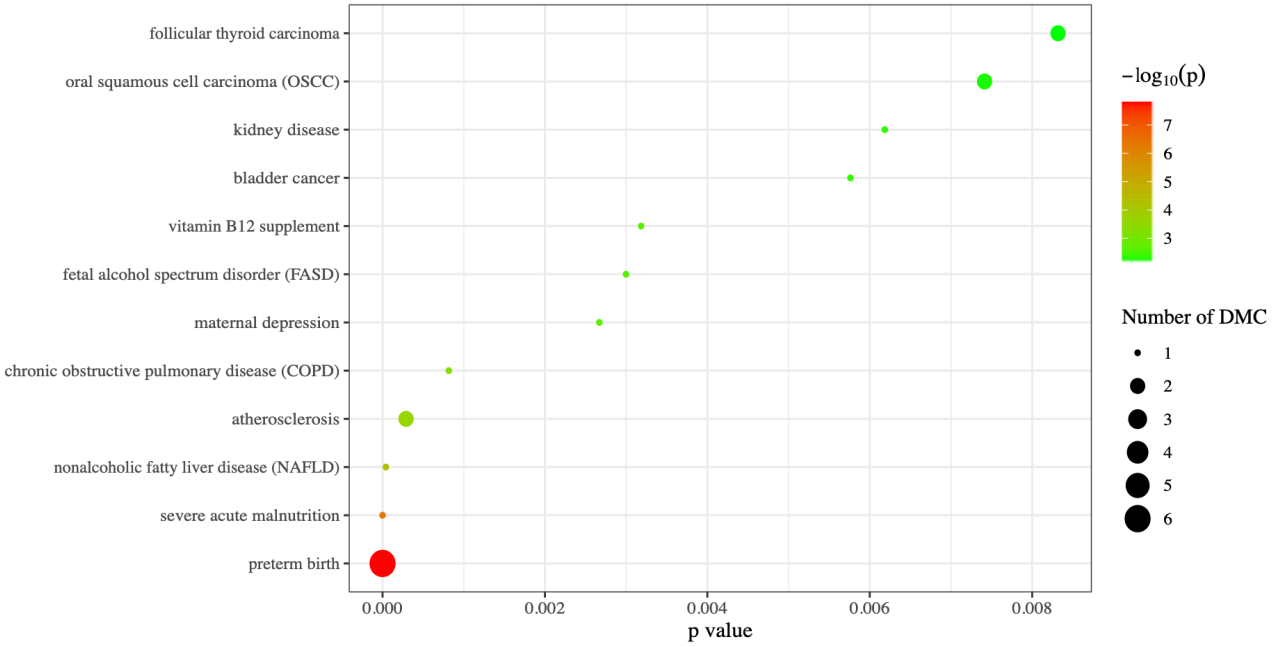
CpG = DNA region where a cytosine nucleotide is followed by a guanine nucleotide;
α_i = effect estimate of childhood adversity on CpG_i;
β_i = effect estimate of CpG_i DNA methylation on depressive symptoms;
Directed acyclic graph (DAG) depicting the relationships between the study variables.
A. Shows a general exposure-mediator-outcome relationship with a single mediator.
B. Shows an exposure-mediator-outcome relationship within the context of our study.
C. Shows a simplified version of our multiple mediator analysis where q represents the total number of mediators considered in the analysis after sure independence screening.

Figure S4. Genomic features for 67 unique CpG mediators by mediator pattern.



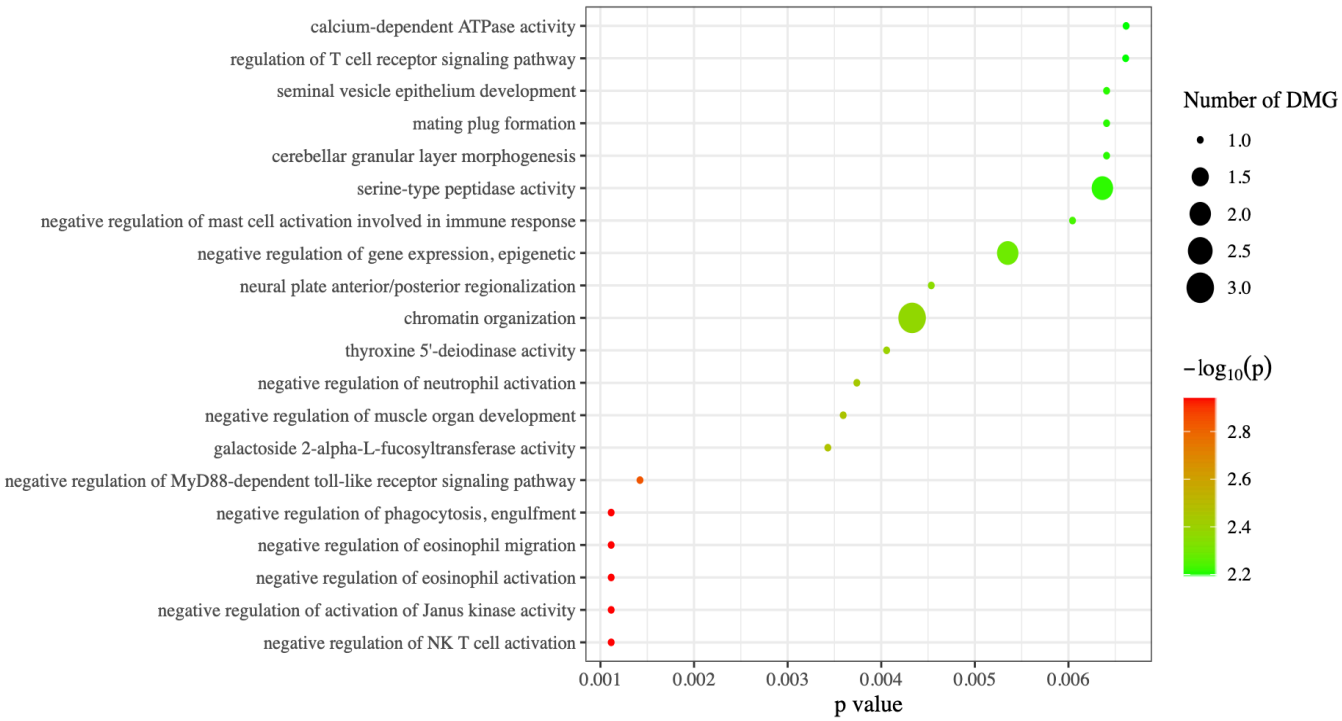
The graph above depicts where the selected mediators fall in the genome. The x-axis represents the 7 categories of genomic features and the y-axis measures the percent of CpGs that fall within each category. Red bars are permuted values, which represent the percent of CpGs for each genomic feature that we would expect by chance. Dark green bars represent the percent of CpGs that fall into each pattern for all unique mediating CpGs (67 total). The remaining bars separate the CpG sites by mediator pattern. A * indicates the amount was different than what would be expected by random chance at $p<0.05$. Error bars show the permutation range.

Figure S5. Trait enrichment of mediating loci (n=70).



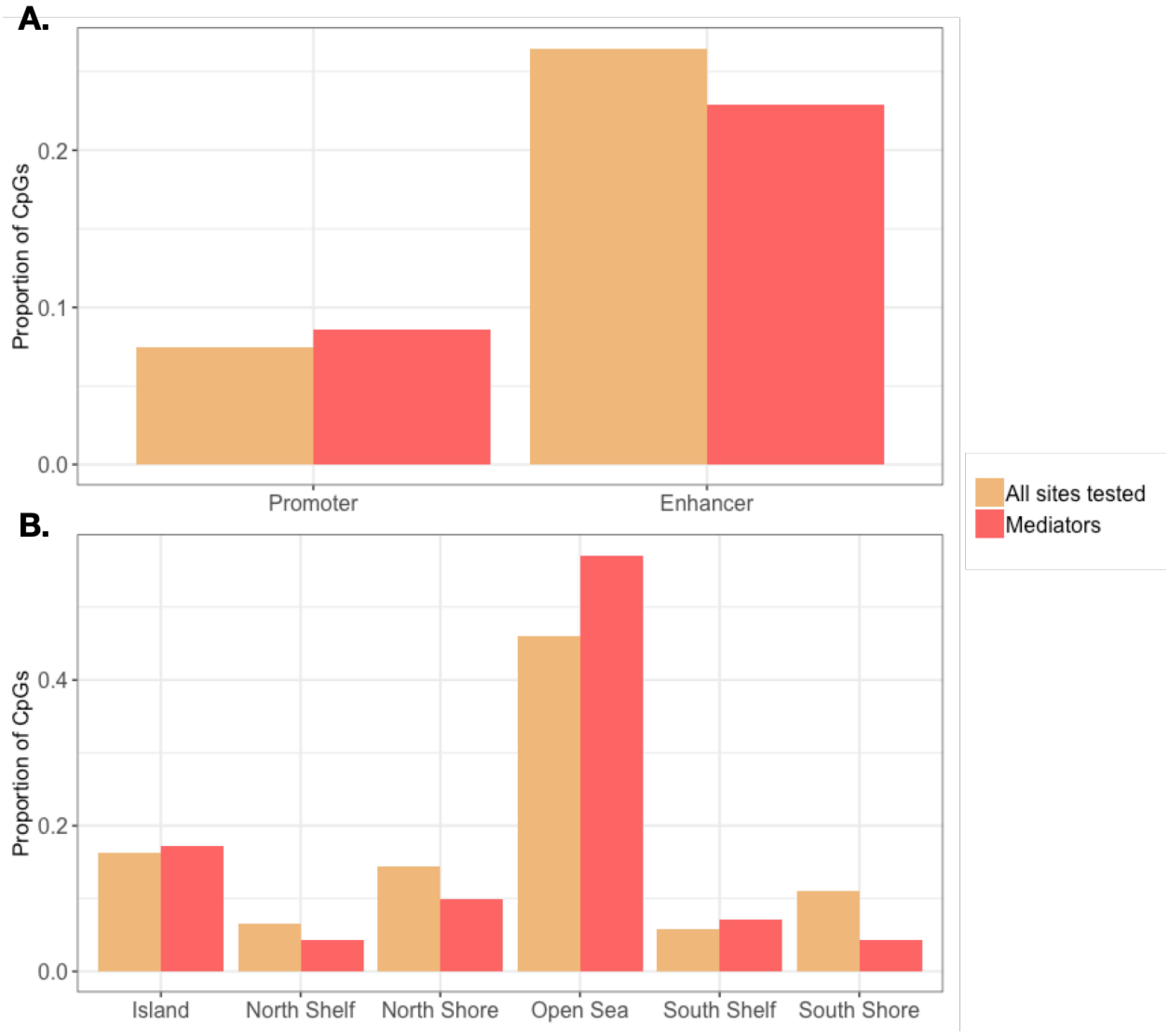
Source: EWAS Atlas. Results of a trait enrichment analysis show 12 traits associated with these 70 mediating loci. The size of the points reflects the number of differentially methylated CpGs (DMCs) associated with each disease process (listed on the y-axis). The color of each point indicates the statistical significance of this association, with red signifying more significant points and green, less significant. Significance was determined using the weighted Fisher's exact test to calculate the co-occurrence probability between the mediating DNA methylation probes and trait-related DNA methylation probes. The weight of each probe equals the number of studies reporting this probe-trait association and is set to equal 1 if absent. This analysis identified enrichment with CpG sites previously observed to be associated with preterm birth (overlap of 6 CpG sites, Bonferroni-adjusted $p<6e-5$), severe acute malnutrition (overlap of 1 CpG site, Bonferroni-adjusted $p<0.006$), and maternal psychopathology (1 CpG site, Bonferroni-adjusted $p<0.003$). The analytic samples contained between 17 and 20 children born preterm.

Figure S6. Gene ontology enrichment of mediating loci (n=70).



Source: EWAS Atlas. Gene ontology (GO) enrichment analysis showed that these 70 sites were linked to genes weakly enriched with 20 biological processes, including chromatin organization and negative epigenetic regulation of gene expression. The size of the points reflects the number of differentially methylated genes (DMGs) associated with each process and the color indicates the statistical significance of the association. GO analysis implementation is based on the gometh function in the missMethyl package which adjusts the number of CpGs associated with each gene.

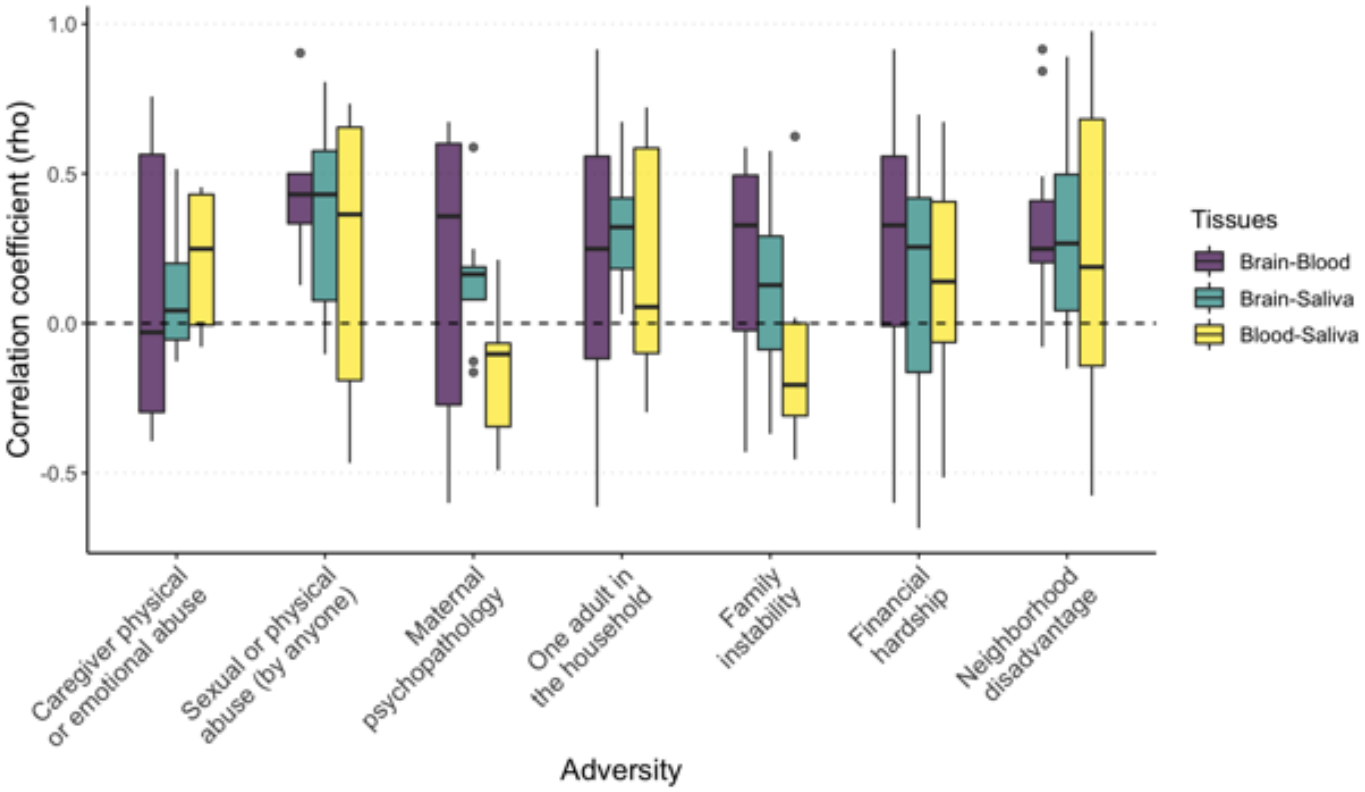
Figure S7. Genomic locations of 70 mediating loci compared to all sites tested (n=278,586).



A. Compared to all tested CpG sites, the 70 mediating loci were no more likely to be in enhancer regions (χ^2=0.307; p=0.58) or promoter regions (χ^2=0.014; p=0.91). However, these results were not statistically significant.

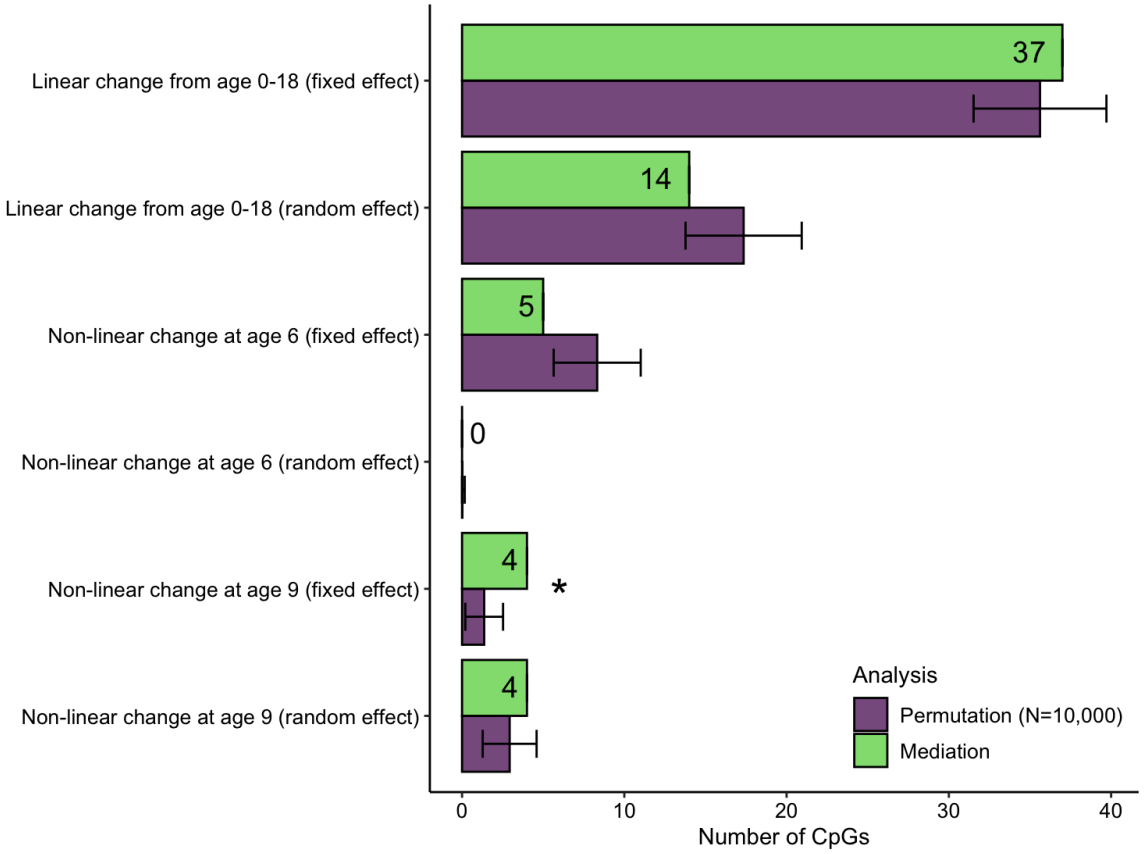
B. Mediating loci differed in terms of their location relation to CpG islands, showing higher enrichment in Open Sea regions and CpG islands, while showing decreased enrichment in southern shore regions compared to all sites, but these differences were non-significant (χ^2=6.503; p=0.26).

Figure S8. Brain-blood-saliva correlations of DNA methylation for mediating CpGs



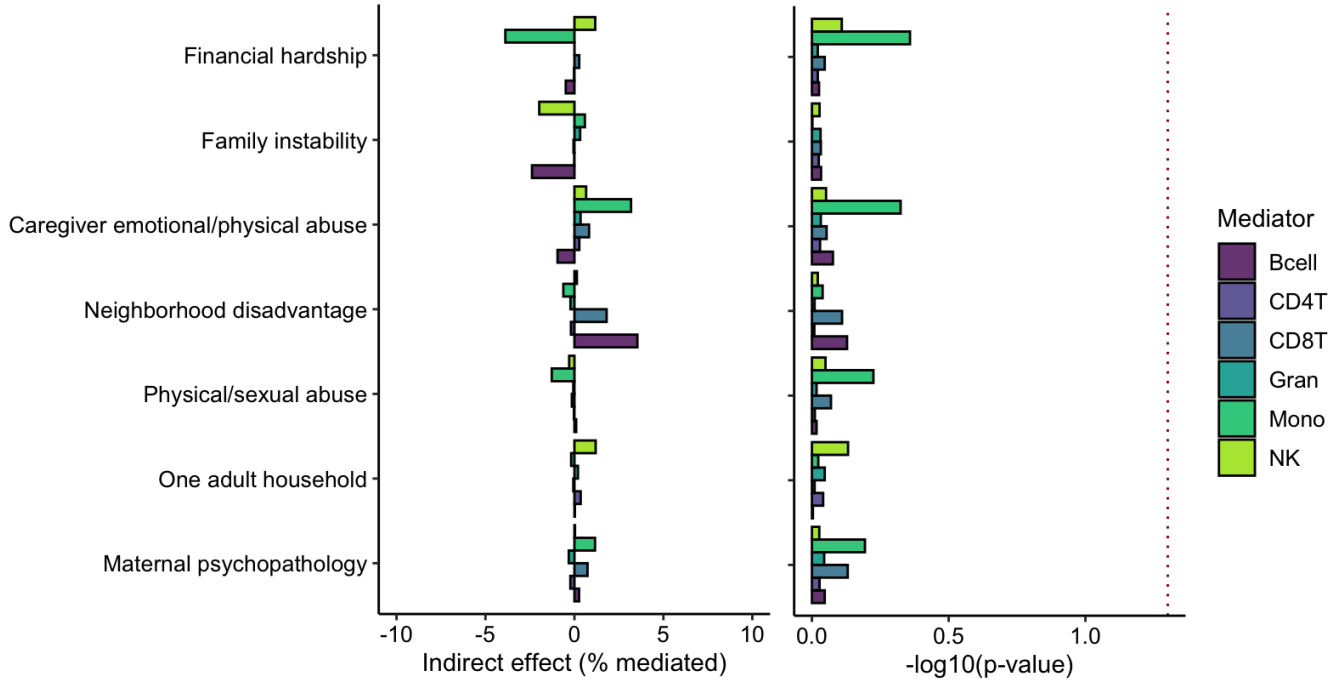
Correlations of DNA methylation levels between tissues were assessed using publicly-available data from Braun et al. (2019). CpGs associated with each type of adversity are shown separately. Boxplots show the median (center line), upper and lower quartiles (box limits), 1.5x interquartile range (whiskers), and outliers (points).

Figure S9. Enrichment of mediating CpGs for age-related changes in DNA methylation.



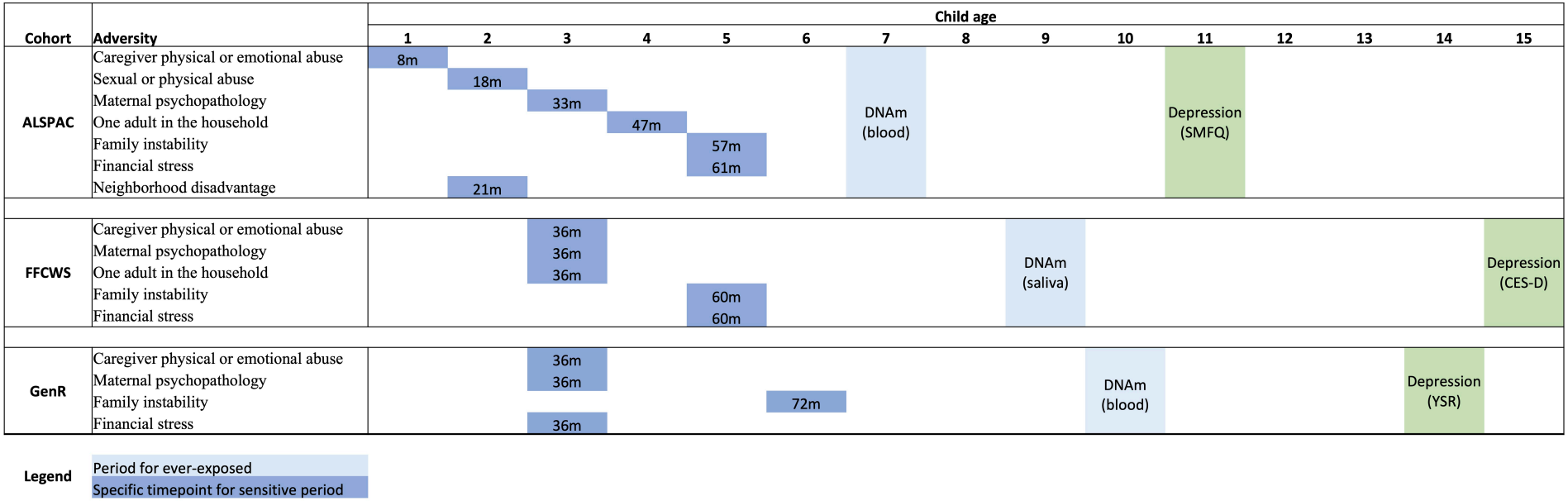
Using data from Mulder et al. (2021, Human Molecular Genetics), we investigated whether mediating CpGs were enriched for six types of age-related changes in DNA methylation. Using a permutation test (N=10,000 permutations), we found that mediating CpGs (green) were no more likely to show age-related changes in DNAm than random chance. The only exception was non-linear change in DNAm starting at age 9, which showed slightly higher chance of occurring in mediating sites (p=-0.0495; q=0.297). Error bars show the permutation range.

Figure S10. Mediation of adversity to depressive symptoms through cell-type proportions.



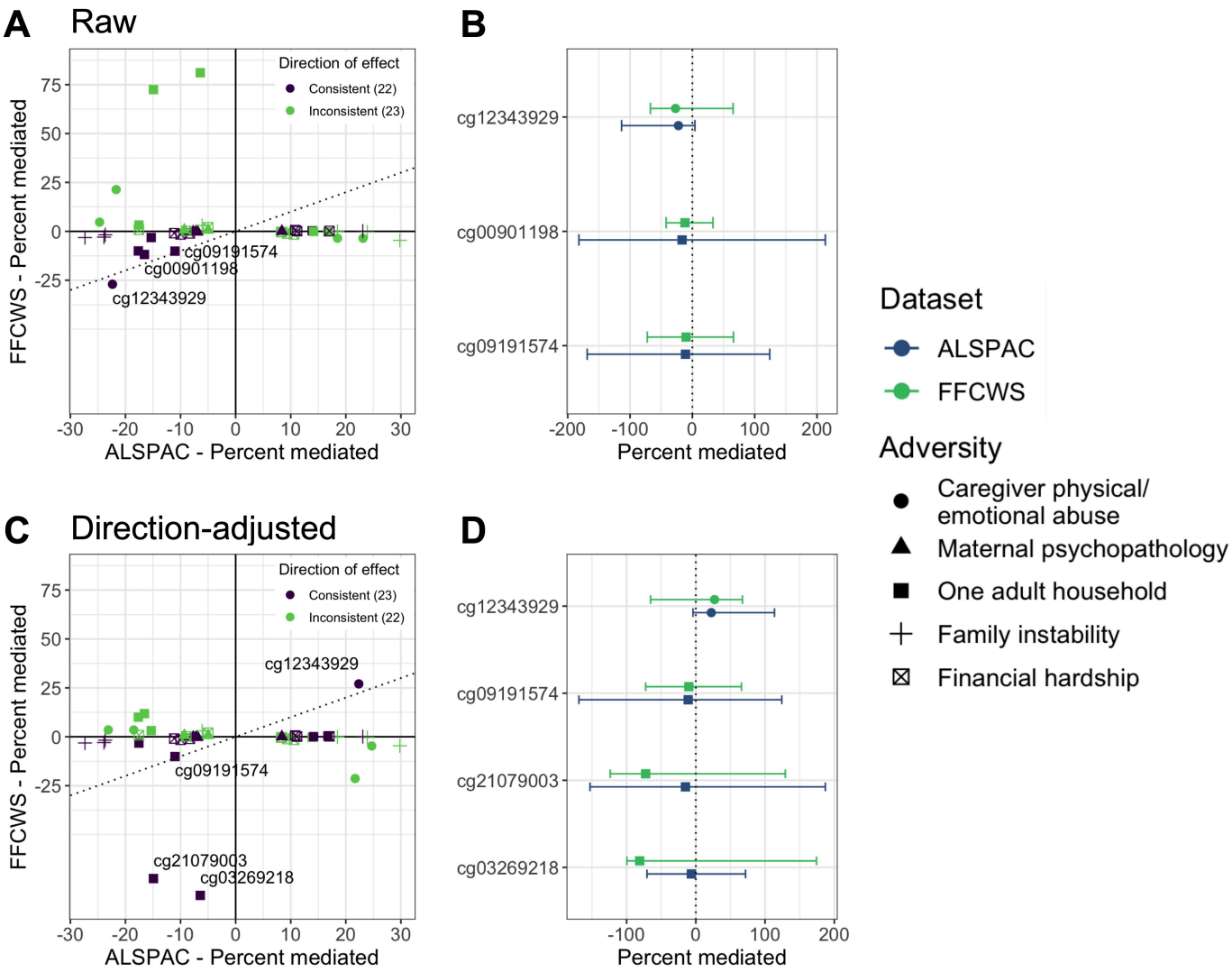
We investigated whether cell-type proportions mediated the relationship between adversity and depressive symptoms. We did not identify any evidence of mediation through the six bioinformatically-predicted cell types from DNAm data. The dashed line in the righthand panel represents a p-value of 0.05.

Figure S11. Summary of variables across ALSPAC, FFCWS, and GenR cohorts.



Primary analyses in ALSPAC focused on exposures to adversity during sensitive periods (dark blue), DNA methylation (DNAm) at age 7, and depressive symptoms at age 10.6.
Replication analyses in the FFCWS cohort focused on exposures to five adversities directly comparable to those in ALSPAC; comparability was based on both the type of adversity measure examined and sensitive period explored. DNAm was measured from saliva at age 9 and depressive symptoms were indexed at age 15.
Replication analyses in the GenR cohort focused on exposures to four adversities directly comparable to those in ALSPAC, both in terms of adversity measurement and sensitive period explored. DNAm was measured in blood at age 10 and depression was assessed at age 13-14.

Figure S12. CpG-level mediation in ALSPAC versus FFCWS.

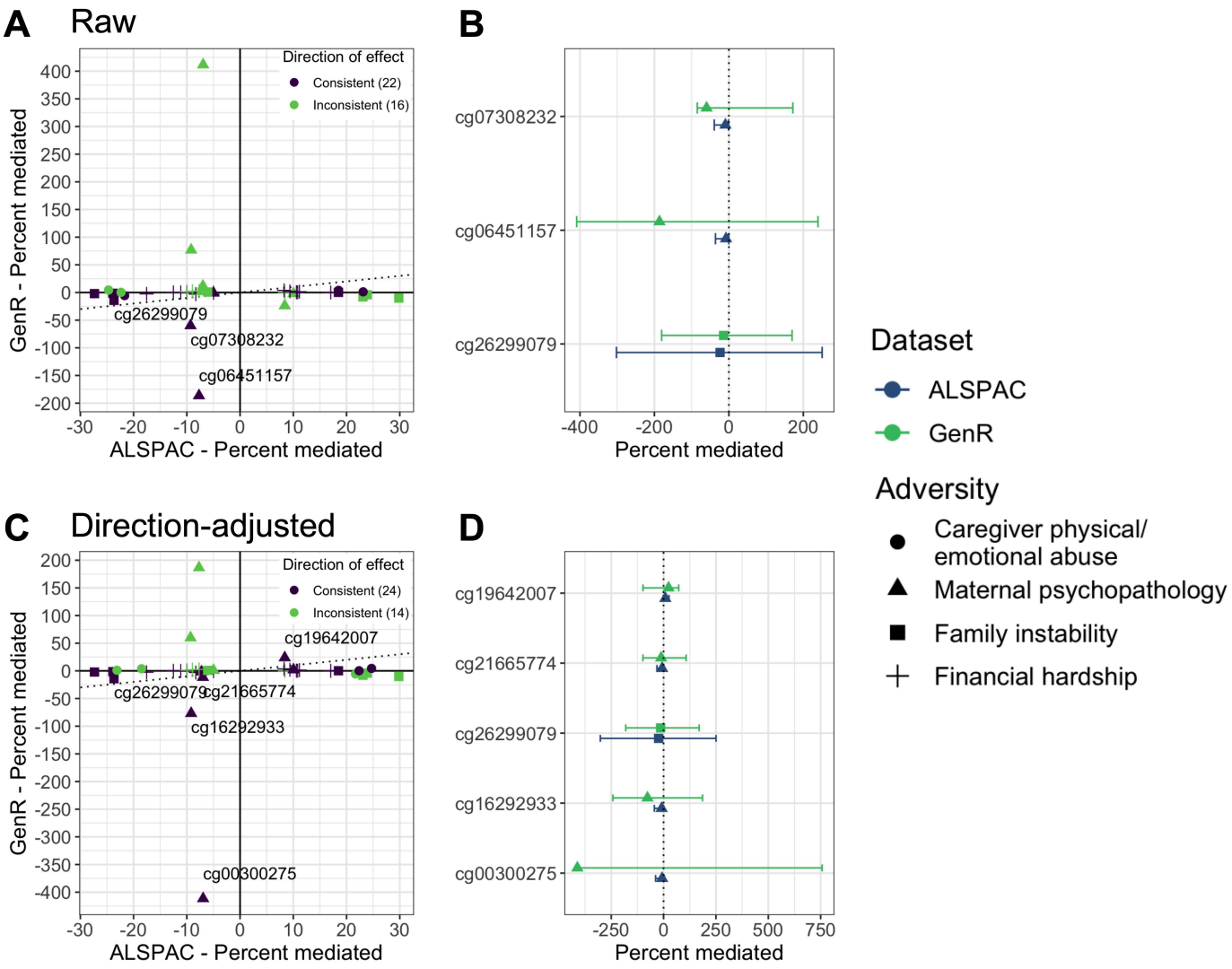


Panels A and B show the raw indirect (mediated) effects, while Panels C and D show the direction-adjusted indirect effects, which account for the effects of adversity on depressive symptoms and reflects the risk-increasing (positive mediated effects) or protective (negative mediated effects) of DNAm on depression. Shapes reflect the adversity associated with each CpG. ALSPAC effects shown here were calculated using the *mediation* package for comparability.

A-C) Approximately half of all analyzed CpGs showed concordant effect directions between cohorts (22/45 CpGs for raw effects, 23/45 CpGs for adjusted effects; purple). CpGs with discordant directions are shown in green. Annotated CpGs showed similar indirect effects (percent mediated) in ALSPAC and FFCWS.

B-D) Forest plot of the CpGs with similar effects between cohorts, showing the 95% confidence intervals for the indirect effect estimate (percent mediated). ALSPAC estimates are shown in blue and FFCWS estimates are shown in green.

Figure S13. CpG-level mediation in ALSPAC versus GenR.



Panels A and B show the raw indirect (mediated) effects, while Panels C and D show the direction-adjusted indirect effects, which account for the effects of adversity on depressive symptoms and reflects the risk-increasing (positive mediated effects) or protective (negative mediated effects) of DNAm on depression. Shapes reflect the adversity associated with each CpG. ALSPAC effects shown here were calculated using the *mediation* package for comparability.

A-C) More than half of all analyzed CpGs showed concordant effect directions between cohorts (22/38 CpGs for raw effects, 24/14 CpGs for adjusted effects; purple). CpGs with discordant directions are shown in green. Annotated CpGs showed similar indirect effects (percent mediated) in ALSPAC and Generation R (GenR).

B-D) Forest plot of the CpGs with similar effects between cohorts, showing the 95% confidence intervals for the indirect effect estimate (percent mediated). ALSPAC estimates are shown in blue and GenR estimates are shown in green.