Association between the timing of childhood adversity and epigenetic patterns across childhood and adolescence: findings from the Avon Longitudinal Study of Parents and Children (ALSPAC) prospective cohort

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Summary

Background Childhood adversity is a potent determinant of health across development and is associated with altered DNA methylation signatures, which might be more common in children exposed during sensitive periods in development. However, it remains unclear whether adversity has persistent epigenetic associations across childhood and adolescence. We aimed to examine the relationship between time-varying adversity (defined through sensitive period, accumulation of risk, and recency life course hypotheses) and genome-wide DNA methylation, measured three times from birth to adolescence, using data from a prospective, longitudinal cohort study.

Methods We first investigated the relationship between the timing of exposure to childhood adversity between birth and 11 years and blood DNA methylation at age 15 years in the Avon Longitudinal Study of Parents and Children (ALSPAC) prospective cohort study. Our analytic sample included ALSPAC participants with DNA methylation data and complete childhood adversity data between birth and 11 years. We analysed seven types of adversity (caregiver physical or emotional abuse, sexual or physical abuse [by anyone], maternal psychopathology, one-adult households, family instability, financial hardship, and neighbourhood disadvantage) reported by mothers five to eight times between birth and 11 years. We used the structured life course modelling approach (SLCMA) to identify time-varying associations between childhood adversity and adolescent DNA methylation. Top loci were identified using an R^2 threshold of 0·035 (ie, ≥3·5% of DNA methylation variance explained by adversity). We attempted to replicate these associations using data from the Raine Study and Future of Families and Child Wellbeing Study (FFCWS). We also assessed the persistence of adversity–DNA methylation associations we previously identified from age 7 blood DNA methylation into adolescence and the influence of adversity on DNA methylation trajectories from ages 0–15 years.

Findings Of 13 988 children in the ALSPAC cohort, 609–665 children (311–337 [50–51%] boys and 298–332 [49–50%] girls) had complete data available for at least one of the seven childhood adversities and DNA methylation at 15 years. Exposure to adversity was associated with differences in DNA methylation at 15 years for 41 loci (R^2 ≥0·035). Sensitive periods were the most often selected life course hypothesis by the SLCMA. 20 (49%) of 41 loci were associated with adversities occurring between age 3 and 5 years. Exposure to one-adult households was associated with differences in DNA methylation at 20 [49%] of 41 loci, exposure to financial hardship was associated with changes at nine (22%) loci, and physical or sexual abuse was associated with changes at four (10%) loci. We replicated the direction of associations for 18 (90%) of 20 loci associated with exposure to one-adult household using adolescent blood DNA methylation from the Raine Study and 18 (64%) of 28 loci using saliva DNA methylation from the FFCWS. The directions of effects for 11 one-adult household loci were replicated in both cohorts. Differences in DNA methylation at 15 years were not present at 7 years and differences identified at 7 years were no longer apparent by 15 years. We also identified six distinct DNA methylation trajectories from these patterns of stability and persistence.

Interpretation These findings highlight the time-varying effect of childhood adversity on DNA methylation profiles across development, which might link exposure to adversity to potential adverse health outcomes in children and adolescents. If replicated, these epigenetic signatures could ultimately serve as biological indicators or early warning signs of initiated disease processes, helping identify people at greater risk for the adverse health consequences of childhood adversity.

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Research in context

Evidence before this study

We searched PubMed from database inception to July 2022, with an updated search on May 17, 2023, for articles on childhood adversity and DNA methylation measured during childhood and adolescence in human populations. Search terms were "DNA methylation OR epigenetics", "trauma OR adversity OR abuse", "child OR childhood", and "adolescent OR adolescence". There was no language restriction. We found several studies investigating the relationship between childhood adversity and DNA methylation, including our own that showed that ages 3–5 years were a potential sensitive period for the effects of childhood adversity on DNA methylation measured at 7 years. We also identified two studies that investigated child and adolescent victimisation and young adult DNA methylation at age 18 years. However, our search did not identify any previous studies that investigated time-varying associations between childhood adversity on either adolescent DNA methylation or trajectories of DNA methylation across development.

Added value of this study

To our knowledge, this is the first human study to incorporate time-dependent measures of childhood adversity in the study of longitudinal epigenetic patterns. Our findings 1) highlight an apparent sensitive period between ages 3 to 5 years for the effects of childhood adversity on the epigenome that could be used to guide future interventions; 2) demonstrate the dynamic and temporal effects of adversity exposure on the human epigenome across childhood and adolescence; and 3) identify a biological pathway that may explain why adversity-induced health consequences, such as depression and other physical or mental disorders, unfold over the course of years, rather than immediately after exposure.

Implications of all the available evidence

These analyses extend our previous work that revealed sensitive periods for the association of childhood adversity with epigenetic alterations at age 7 years in the Avon Longitudinal Study of Parents and Children (ALSPAC), highlighting that exposure to adversity between the ages of 3 and 5 years might be more closely linked to biological processes and future health than exposure during other time periods. Our study suggests that epigenetic mechanisms might serve as a biological link between childhood adversity and long-term health. If replicated, these findings could explain why there are both immediate and latent manifestations of disease in people with histories of childhood adversity. Our findings also support the need for future studies investigating the role of DNA methylation trajectories in predicting child and adolescent health, including risk for immune dysfunction, metabolic disorders, and mental health problems.

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Introduction

Children exposed to adversity, such as abuse or maltreatment, family disruption or dysfunction, or poverty, frequently have poor physical and mental health outcomes later in development and across the life course.[1] Epigenetic processes, including DNA methylation, are increasingly recognised as potential underlying mechanisms for poor future health outcomes because DNA methylation is responsive to life experiences[2] and might mediate the link between environmental exposures and health outcomes.[3] A large number of studies in humans, including population-based studies, systematic reviews, and meta-analyses, have shown links between childhood adversity, DNA methylation, and adverse health outcomes across the life course.[4] However, previous studies investigating the epigenomes of children exposed to adversity have not yet explored two key dimensions of the adversity–DNA methylation relationship: 1) the timing of adversity and 2) the timing of changes in DNA methylation and their stability over time. These dimensions are crucial to understand the biological risk posed by childhood adversity, identify children at risk for poor health, and improve intervention targets for health promotion and disease prevention in children and adolescents.

How the timing of childhood adversity might shape DNA methylation remains unclear. Both human and animal studies suggest the existence of sensitive periods for epigenetic programming, when physiological and neurobiological systems are primed for external influences, allowing experiences to impart more enduring effects.[5,6] We have previously identified a potential sensitive period for the effects of adversity on childhood DNA methylation between the ages of 3 and 5 years.[7,8] Briefly, this previous study used the structured life course modelling approach (SLCMA) and prospective data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to identify time-varying associations between childhood adversity and DNA methylation measured in childhood (7 years). Most of the significant epigenetic differences emerged when children were exposed to adversity between ages 3 and 5 years, suggesting this was a potential sensitive period for the effects of childhood adversity on DNA methylation. Evidence for the effects of accumulation or recency of exposure was scarce. However, no previous studies have investigated sensitive periods for epigenetic patterns in adolescence.

Little is known about how DNA methylation profiles of children exposed to adversity vary across development and how DNA methylation variation across time might shape health. Oh and Petronis[9] argued that the dynamic nature of epigenetic mechanisms is best examined through longitudinal studies that model time-dependent epigenetic patterns. Although previous studies have shown that the epigenome is dynamic across development,[10–17] no studies,

to our knowledge, have determined how childhood adversity might influence DNA methylation trajectories.

In this study, we aimed to examine the relationship between time-varying adversity and genome-wide DNA methylation in the ALSPAC cohort study using the SLCMA. Specifically, we aimed to: 1) determine whether childhood adversity has time-dependent associations with adolescent DNA methylation; 2) characterise the developmental trajectories of DNA methylation linked to adversity; and 3) evaluate the persistence of associations between childhood adversity and DNA methylation at 7 years that we previously identified in ALSPAC.[8]

Methods

Study design and participants

For our primary analyses, we used prospective longitudinal data from ALSPAC, an ongoing large population-based birth cohort of children born to mothers who were living in the county of Avon, UK, with expected delivery dates between April 1991 and December 1992.[18,19] 14 451 pregnant women participated in the study and 14 062 of eligible livebirths who were alive at 1 year of age (n=13 988 children) were enrolled in the study. Participants were followed up from before birth to early adulthood (as of 2023, the oldest participant is 32 years).[18,19]

We analysed the blood-based DNA methylation profiles generated as part of the Accessible Resource for Integrated Epigenomic Studies (ARIES) for a subsample of ALSPAC mother–child pairs, which includes cord blood at birth (n=905), whole blood at 7 years (n=970), and peripheral blood leukocytes at 15 years (n=966; appendix p 3).[20]

See Online for appendix

We examined seven types of childhood adversity previously associated with DNA methylation: 1) caregiver physical or emotional abuse, 2) sexual or physical abuse (by anyone), 3) maternal psychopathology, 4) one-adult households, 5) family instability, 6) financial hardship, and 7) neighbourhood disadvantage. These adversities were reported by mothers via mailed questionnaires, collected five to eight times between birth and 11 years (figure 1; appendix pp 16–17). Exposures to adversity were binarised within each timepoint of data collection (see appendix pp 16–17).

Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent 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 (Protocol 2017P001110).

We sought to replicate primary associations between childhood adversity and DNA methylation levels in adolescence using data from the Raine study (n=2868; participants born between 1989 and 1991 followed up until 27 years; appendix p 7)[21,22] and Future of Families and Child Wellbeing Study (FFCWS; n=4898; participants born between 1998 and 2000 followed up until 25 years; appendix pp 7–8).[23]

Procedures

DNA methylation was measured from blood at 485 577 CpG sites using the Infinium HumanMethylation450 BeadChip microarray (Illumina, San Diego, CA, USA). Laboratory procedures, preprocessing, and quality control steps were done as described previously.[20,24] We removed non-variable CpGs (<5% DNA methylation difference between children

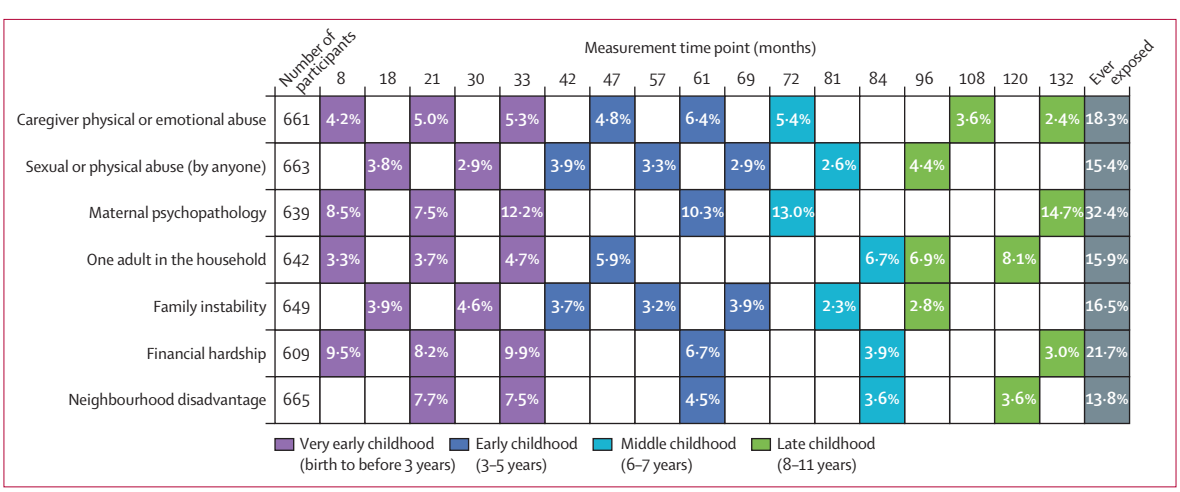

Figure 1: **Summary of exposures and outcomes examined**

Seven types of childhood adversity were assessed five to eight times between birth and 11 years. The effective sample size was based on the availability of complete data for all covariates, all available timepoints of childhood adversity, and DNA methylation at 15 years. Each filled cell represents the timepoint when the adversity was measured, along with the proportion of children exposed to adversity. The additional life course models tested were accumulation and recency, which reflect the total number of exposures across development (accumulation) and exposure to adversity weighted by time (recency). Genome-wide DNA methylation data were collected at birth, 7 years, and 15 years.

in the 10th and 90th percentile), resulting in 302 581 CpGs for analyses (appendix p 3). DNA methylation was analysed as β values, which represent the percent of methylation at each site.

Statistical analysis

We examined time-dependent associations for each adversity separately in children with DNA methylation data from ALSPAC and no missing data for covariates or the adversity timepoints (figure 1). To adjust for known potential confounders,[7] we controlled for age at the time of blood collection, sex, race and ethnicity, maternal age at birth, maternal education at birth, birthweight, number of previous pregnancies, maternal smoking during pregnancy, and cell type proportions estimated using the Houseman method (appendix pp 3–4).[25]

Our primary analyses focused on identifying time-dependent associations between exposure to each childhood adversity and DNA methylation measured in adolescence (age 15 years). We used the SLCMA, a two-stage method that simultaneously compares a priori life course hypotheses explaining exposure–outcome relationships.[26–28] We generated variables corresponding to six separate life course hypotheses, including four sensitive periods hypotheses encoding exposure to each childhood adversity during very early childhood (birth to

	CpG	DNA methylation unexposed*	DNA methylation exposed†	Difference in DNA methylation‡	Effect estimate§ (SE; 95% CI)	R^2¶	p value	FDR-adjusted p value	Nearest gene	Trajectory class
Caregiver physical or emotional abuse										
Early childhood										
5·0 years	cg14855874	0·091	0·121	0·030	0·030 (0·005; 0·019 to 0·041)	0·041	$3·32×10^{-7}$	0·10	*BANK1*	Emergent
5·0 years	cg15454534	0·885	0·868	–0·017	–0·017 (0·003; –0·023 to –0·010)	0·039	$6·76×10^{-7}$	0·10	*OR2T1*	Latent
5·0 years	cg06215562	0·847	0·826	–0·021	–0·021 (0·004; –0·029 to –0·013)	0·035	$2·37×10^{-6}$	0·18	No data‖	Latent
Sexual or physical abuse (by anyone)										
Early childhood										
3·5 years	cg26970800	0·902	0·847	–0·055	–0·055 (0·010; –0·074 to –0·036)	0·044	$8·51×10^{-8}$	0·021	*CBLIF*	Emergent
3·5 years	cg15723468	0·822	0·779	–0·043	–0·045 (0·009; –0·062 to –0·028)	0·041	$1·89×10^{-7}$	0·021	*GALNT2*	Latent
3·5 years	cg17928317	0·681	0·785	0·104	0·076 (0·015; 0·045 to 0·106)	0·041	$2·06×10^{-7}$	0·021	*MAGEC3*	Primed
Late childhood										
8·0 years	cg27558057	0·257	0·289	0·032	0·107 (0·024; 0·059 to 0·155)	0·036	$1·53×10^{-6}$	0·12	*TAF1*	Stable
Family instability										
Very early childhood										
2·5 years	cg02735620	0·877	0·857	–0·021	–0·019 (0·004; –0·027 to –0·012)	0·036	$2·07×10^{-6}$	0·46	*PKD2*	Emergent
Financial hardship										
Very early childhood										
0·7 years	cg14455319	0·289	0·339	0·050	0·052 (0·011; 0·032 to 0·074)	0·036	$3·87×10^{-6}$	0·20	*ANKK1*	Stable
0·7 years	cg13204236	0·861	0·824	–0·037	–0·037 (0·007; –0·051 to –0·023)	0·036	$5·94×10^{-6}$	0·20	*STPG4*	Latent
Early childhood										
5·0 years	cg15037420	0·780	0·746	–0·035	–0·034 (0·007; –0·049 to –0·021)	0·036	$3·04×10^{-6}$	0·20	*BSPH1*	Latent
5·0 years	cg06410970	0·860	0·825	–0·035	–0·033 (0·006; –0·046 to –0·022)	0·036	$5·56×10^{-6}$	0·20	*ANXA11*	Overcompensation
Late childhood										
11·0 years	cg02011706	0·861	0·799	–0·062	–0·064 (0·013; –0·089 to –0·039)	0·036	$5·35×10^{-6}$	0·20	*LMF1*	Emergent
11·0 years	cg04659536	0·901	0·873	–0·029	–0·028 (0·006; –0·039 to –0·017)	0·035	$5·52×10^{-6}$	0·20	*SDK1*	Latent
Recency										
NA	cg17670999	0·817	0·807	–0·010	–0·002 (0·000; –0·003 to –0·001)	0·041	$8·76×10^{-7}$	0·20	*ARHGAP39*	Stable
NA	cg25459301	0·769	0·756	–0·013	–0·003 (0·001; –0·004 to –0·002)	0·036	$4·24×10^{-6}$	0·20	*XKR6*	Overcompensation
NA	cg06812747	0·837	0·825	–0·012	–0·003 (0·001; –0·004 to –0·002)	0·035	$4·98×10^{-6}$	0·20	*FBXL16*	Stable
Maternal psychopathology										
Very early childhood										
2·8 years	cg16813552	0·898	0·883	–0·015	–0·015 (0·003; –0·021 to –0·010)	0·045	$7·11×10^{-8}$	0·22	*OGA*	Stable
Neighbourhood disadvantage										
Very early childhood										
2·8 years	cg04288299	0·914	0·905	–0·009	–0·021 (0·004; –0·029 to –0·013)	0·039	$4·52×10^{-7}$	0·070	*NELFA*	Overcompensation
2·8 years	cg25019631	0·201	0·223	0·023	0·044 (0·009; 0·028 to 0·061)	0·038	$6·16×10^{-7}$	0·070	*CASP9*	Overcompensation
2·8 years	cg04224851	0·907	0·894	–0·013	–0·014 (0·003; –0·020 to –0·009)	0·038	$6·94×10^{-7}$	0·070	*ZFP36L2*	Overcompensation

(Table continues on next page)

	CpG	DNA methylation unexposed*	DNA methylation exposed†	Difference in DNA methylation‡	Effect estimate§ (SE; 95% CI)	R^2¶	p value	FDR-adjusted p value	Nearest gene	Trajectory class
(Continued from previous page)										
One adult in the household										
Very early childhood										
1·8 years	cg05491478	0·908	0·880	−0·028	−0·027 (0·006; −0·039 to −0·016)	0·038	$7·33×10^{-7}$	0·28	*LRRFIP1*	Overcompensation
Early childhood										
3·9 years	cg16907527	0·853	0·824	−0·030	−0·032 (0·005; −0·041 to −0·022)	0·060	$4·17×10^{-10}$	0·0001	*VEGFA*	Flat emergent
3·9 years	cg08818094	0·847	0·798	−0·048	−0·050 (0·008; −0·067 to −0·034)	0·051	$8·79×10^{-9}$	0·0013	*TBC1D19*	Latent
3·9 years	cg01060989	0·824	0·794	−0·031	−0·031 (0·005; −0·042 to −0·021)	0·047	$6·73×10^{-8}$	0·0067	*DUSP10*	Latent
3·9 years	cg15814750	0·723	0·684	−0·039	−0·040 (0·009; −0·058 to −0·025)	0·039	$6·57×10^{-7}$	0·028	*WDR72*	Latent
3·9 years	cg15783822	0·868	0·848	−0·021	−0·021 (0·004; −0·031 to −0·014)	0·039	$8·08×10^{-7}$	0·028	*PRR4*	Latent
3·9 years	cg15864691	0·907	0·889	−0·018	−0·018 (0·004; −0·025 to −0·011)	0·038	$8·36×10^{-7}$	0·028	*HOXA10*	Overcompensation
3·9 years	cg02584161	0·661	0·603	−0·057	−0·058 (0·011; −0·081 to −0·038)	0·038	$1·28×10^{-6}$	0·034	No data‖	Latent
3·9 years	cg02810291	0·840	0·818	−0·022	−0·023 (0·005; −0·033 to −0·014)	0·037	$1·35×10^{-6}$	0·034	*AKAP13*	Overcompensation
3·9 years	cg04036644	0·882	0·855	−0·027	−0·026 (0·005; −0·037 to −0·016)	0·037	$1·36×10^{-6}$	0·034	*LOC286083*	Latent
3·9 years	cg11811897	0·758	0·711	−0·047	−0·047 (0·010; −0·067 to −0·030)	0·037	$1·68×10^{-6}$	0·036	*PKD1L1*	Latent
3·9 years	cg15817130	0·794	0·759	−0·036	−0·038 (0·007; −0·051 to −0·025)	0·037	$1·83×10^{-6}$	0·037	*MYO10*	Latent
3·9 years	cg06711254	0·686	0·631	−0·055	−0·056 (0·012; −0·080 to −0·036)	0·036	$2·15×10^{-6}$	0·040	*FSIP2*	Flat emergent
3·9 years	cg19096460	0·845	0·821	−0·024	−0·024 (0·005; −0·035 to −0·015)	0·035	$2·89×10^{-6}$	0·049	*HERC3*	Latent
3·9 years	cg18980650	0·800	0·760	−0·040	−0·036 (0·007; −0·05 to −0·024)	0·035	$3·31×10^{-6}$	0·051	*NOX1*	Emergent
3·9 years	cg27504269	0·771	0·733	−0·038	−0·040 (0·008; −0·056 to −0·026)	0·036	$3·52×10^{-6}$	0·051	*SLCO1A2*	Latent
Late childhood										
10·0 years	cg12096528	0·890	0·874	−0·016	−0·016 (0·003; −0·023 to −0·010)	0·036	$2·24×10^{-6}$	0·040	*SLC25A41*	Overcompensation
Accumulation										
NA	cg00807464	0·052	0·057	0·006	0·003 (0·001; 0·002 to 0·004)	0·040	$7·56×10^{-7}$	0·028	*CUX2*	Stable
NA	cg10420609	0·559	0·522	−0·037	−0·014 (0·003; −0·020 to −0·009)	0·039	$7·71×10^{-7}$	0·028	*DSP*	Latent
NA	cg14579651	0·634	0·605	−0·028	−0·012 (0·002; −0·018 to −0·008)	0·037	$1·68×10^{-7}$	0·036	*STK38L*	Stable

NA=not applicable. *Mean DNA methylation levels in children with no exposure to adversity from ages 0 to 11. †Mean DNA methylation levels in children exposed to adversity during the selected sensitive period. Accumulation hypotheses show the mean DNA methylation levels in children with at least one exposure to adversity. ‡Difference in mean DNA methylation levels between children exposed to adversity during the selected sensitive period and individuals unexposed to adversity. §Effect estimates were calculated using linear regression of exposure to adversity from the theoretical model and DNA methylation, correcting for the covariates described in the methods. Standard error and confidence intervals are shown for these estimates. ¶Proportion of variation in DNA methylation at this CpG explained by differences in this adversity at this timing, after removing the associations with covariates. ||No genes with 300 kilobases of the CpG.

Table: **Top associations between time-dependent exposure to adversity and DNA methylation at 15 years**

before 3 years), early childhood (3–5 years), middle childhood (6–7 years), and late childhood (8–11 years); and two additive hypotheses: accumulation of risk (total number of time periods exposed to the specific adversity across childhood; appendix p 4), and recency of exposures (accumulation of risk variables, weighted by age to determine whether more recent exposures had a stronger effect than distal exposures). The SLCMA first uses variable selection to identify the life course hypothesis explaining the greatest proportion of outcome variation. Effect estimates, confidence intervals, and p values are then calculated for the selected life course hypothesis using post-selective inference. The SLCMA detects time-varying associations with more statistical power and less bias than traditional epigenome-wide association studies of ever or never-exposed or cross-sectional paradigms.[7,8,29]

We tested associations using selective inference and accounted for multiple-testing using the false-discovery rate (FDR). Consistent with previous work on time-verying exposures to childhood stressors,[7,8] top loci were identified using an R^2 threshold of 0·035 (ie, ≥3·5% of DNA methylation variance explained by adversity exposure); these top loci were assessed in downstream analyses. SLCMA, quantile–quantile plots (appendix p 40), genomic inflation estimates, and functional analyses of top loci are in described in full in the appendix (pp 4–5). Functional analyses included genomic location enrichment, brain–blood correlations using the Blood Brain DNA Methylation Comparison Tool,[30] gene ontology using DAVID[31] or missMethyl,[32] evolutionary conservation using probability of intolerance to loss-of-function mutations,[33] and prior associations determined through the EWAS catalogue.[34]

In sensitivity analyses, we completed internal validation of the SLCMA results using ordinary non-parametric bootstrapping. We investigated the effect of potential confounders or alternative mediators of the association between childhood adversity and DNA methylation at

15 years, including in our regression models exposures to other types of childhood adversity in the same or different sensitive periods, parental socioeconomic position at birth, gestational age in weeks, maternal pre-pregnancy BMI, method of birth (vaginal *vs* Caesarean section), estimated age at pubertal onset, adolescent BMI, adolescent C-reactive protein concentration, and adolescent smoking (appendix pp 5–7, 10–12).

We aimed to replicate observed associations between childhood adversity and DNA methylation levels in adolescence using data from the Raine Study[21,22] and Future of Families and Child Wellbeing Study (FFCWS).[23] Using data from the Raine Study, we analysed the loci linked to one-adult households using blood DNA methylation measured at age 17 years. Using data from the FFCWS, we analysed the loci linked to caregiver physical or emotional abuse, financial hardship, maternal psychopathology, and one-adult households using saliva DNA methylation measured at age 15 years. The timepoint used for adversity exposures was matched to the one identified by SLCMA in ALSPAC (appendix pp 7–10). To provide more accurate comparisons, we corrected for the winner's curse, which refers to the exaggerated effect estimate for a given exposure–outcome relationship present in the sample in which it was first identified, using normalised maximum likelihood estimation (appendix pp 8–9).

Finally, the three waves of longitudinal DNA methylation data available in ALSPAC enabled three subsequent analyses of DNA methylation trajectories across development (appendix pp 12–13). First, we assessed whether DNA methylation differences identified at 15 years emerged earlier in development, using linear regression to test whether exposure to the same type and timing of childhood adversity was associated with DNA methylation at the same top loci at birth or 7 years. Second, we investigated DNA methylation patterns in our top loci before the 15 year timepoint, studying longitudinal change and stability of DNA methylation measured at birth, 7 years, and 15 years among children and adolescents from three distinct exposure groups: 1) children who had adversity exposure during the sensitive period identified from the SLCMA; 2) children who had adversity exposure outside the sensitive period identified from the SLCMA; and 3) children who were never exposed to adversity. Third, we previously identified associations between time-varying exposures to childhood adversity and DNA methylation levels at 7 years for 46 loci across the epigenome.[8] To determine whether these DNA methylation alterations persisted to adolescence, we used linear regressions between the same type and timing of childhood adversity and DNA methylation levels measured at age 15 years for these 46 loci.

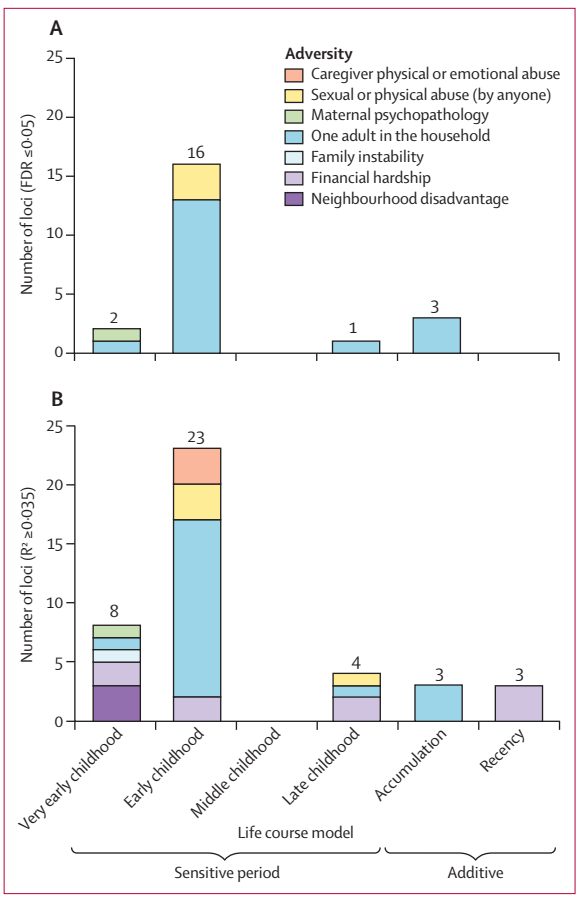

***Figure 2*: Life course theoretical models selected by the SLCMA for top DNA methylation loci at 15 years**

The life course theoretical models were split by sensitive periods (ie, exposure to adversity during specific childhood periods) or additive models (ie, accumulation or recency of exposures). A) Loci identified at an FDR less than 0·05. B) Loci identified at an R^2 of at least 0·035. FDR=false-discovery rate. SLCMA=structured life course modelling approach.

Role of the funding source

The funders of the study played no role in the study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication.

Results

Of 13 988 children in the ALSPAC cohort, 609–665 children (311–337 [50–51%] boys and 298–332 [49–50%] girls) had complete data available for at least one of the seven childhood adversities and DNA methylation assessments. Demographic characteristics did not differ between the ARIES sample and children exposed to any adversity between ages 0–11 years (appendix p 19).

The prevalence of exposure to a given adversity between ages 0–11 years ranged from 100 (15%) of 663 children (for whom data were available) experiencing sexual or physical abuse to 222 (35%) of 639 children (for whom data were available) experiencing maternal psychopathology (appendix p 20). The tetrachoric correlation of exposure within adversity across development ranged from 0·36 (family instability) to 0·79 (one-adult households).

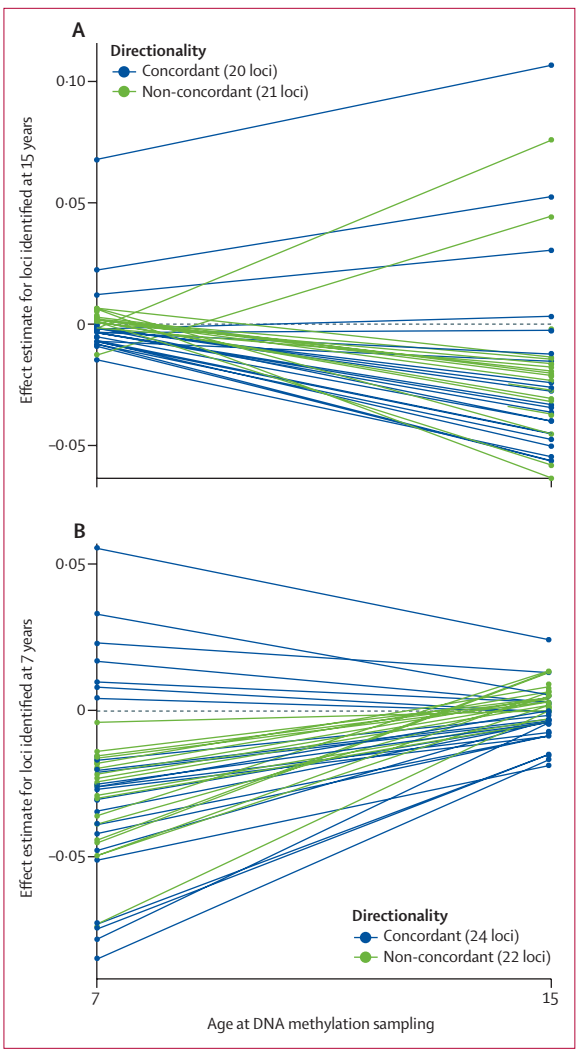

Figure 3: **Persistence and stability of associations between childhood adversity and DNA methylation across development**
A) Estimates of associations and directions of effect between childhood adversity and DNA methylation at 7 years or 15 years for the top 41 loci identified in our study. B) Estimates of associations and directions of effect between childhood adversity and DNA methylation at 7 years or 15 years for the 46 loci identified in our previous study of DNA methylation at 7 years.

Different types of adversity were weakly correlated (r_{avg} ranged from –0·04 to 0·16; appendix p 41).

Across all types of adversity, 41 loci showed associations between exposure to adversity and DNA methylation levels at age 15 years (≥3·5% of DNA methylation variance explained by adversity; table; appendix pp 21–22). 22 (54%) of the 41 loci showed significant associations between exposure to adversity and DNA methylation levels at age 15 years after multiple-test correction (FDR <0·05). Because previous studies have shown that p values are poor metrics of statistical inference on their own,[35,36] particularly in the context of time-varying associations,[8] we focused downstream analyses on the 41 CpGs meeting the R^2 threshold.

Sensitive periods were the most often selected life course hypothesis by the SLCMA, with 35 [85%] of 41 loci showing associations with childhood adversity that occurred during very early childhood (eight [20%] of 41 loci), early childhood (23 [56%] loci), or late childhood (four [10%] loci; figure 2). Only six (15%) loci showed associations with the accumulation or recency of adversity. The highest proportion of loci were associated with exposure to one-adult households (20 [49%] of 41 loci), followed by financial hardship (nine [22%] loci), sexual or physical abuse by anyone (four [10%] loci), caregiver physical or emotional abuse (three [7%] loci), neighbourhood disadvantage (three [7%] loci), family instability (one [2%] locus), and maternal psychopathology (one [2%] locus).

Childhood adversity was mainly associated with a decrease in DNA methylation (35 [85%] loci). Exposure to childhood adversity was associated with a mean absolute difference in DNA methylation of 3·5% (SD 1·8%). For loci associated with accumulated time living in one-adult households, each additional exposure timepoint (figure 1) was associated with a mean difference in DNA methylation of 1% (SD 0·6%). For loci associated with the recency of financial hardship, one additional timepoint of exposure was associated with a –1·3% to 2·3% difference in DNA methylation per year of age at exposure (table).

The top 41 loci showed higher representation in low CpG density regions, such as enhancers (p=0·008) and Open Seas (p=0·018; appendix p 42), compared to regions of high CpG density, such as CpG islands. 28 (68%) loci had weak, positive brain–blood correlations in individuals without exposure to adversity (r_{avg} 0·10; appendix pp 23–24, 43), suggesting adversity-associated differences in blood DNA methylation could be reflected in the CNS. No biological processes were significantly enriched in top loci using the DAVID or missMethyl gene ontology tools (appendix pp 44–45). Seven genes, one linked to sexual and physical abuse (*TAF1*), one linked to family instability (*PKD2*), two linked to financial hardship (*FBXL16* and *XKR6*), and three linked to one-adult households (*DSP*, *CUX2*, and *STK38L*), showed evidence of strong functional constraint through analyses of probability of intolerance to loss-of-function mutations (appendix pp 21–22, 46). Finally, several loci were previously associated with gestational age (seven [17%] loci), sex (six [15%] loci), smoking (one [2%] locus), inflammatory bowel disease (one [2%] locus), and rheumatoid arthritis (four [10%] loci). Together, these findings suggest that different types of childhood adversity might act through diverse biological processes (appendix p 5).

Sensitivity analyses of the top associations yielded nearly identical results to the initial analyses: the largest difference in effect estimates between the primary analysis and the bootstrap was 2·03% (appendix pp 25–26, 47). Our results remained stable when

correcting for exposure to other adversities during the sensitive period or across childhood, suggesting that they were not influenced by co-occurring exposure to adversity (appendix pp 48–50).

We attempted to replicate these associations in two independent datasets: the Raine Study and FFCWS (appendix p 51). Using data from the Raine Study (blood DNA methylation), we tested associations for the 20 loci associated with one-adult households (appendix pp 27, 52). 18 (90%) of the 20 loci showed the same direction of effects as in our study, which was more likely than random chance (p=0·0002; appendix p 52). Three of the 20 CpGs were nominally significant (p<0·05; appendix p 27) in the Raine Study; none of their effect estimate confidence intervals crossed zero and all had the same direction as in the ALSPAC cohort. Effect estimates in the Raine Study were smaller than those in the ALSPAC cohort. These differences were mitigated when correcting for winner's curse effects (appendix p 52).

Using data from FFCWS (saliva DNA methylation), we attempted to replicate associations for the 28 loci associated with caregiver abuse, financial hardship, maternal psychopathology, and one-adult households). 18 (64%) loci showed the same direction of effects in the FFCWS as in our study (p=0·092); 11 (73%) of 15 one-adult household-associated loci showed concordant directions (p=0·059; appendix pp 28–29, 53). All 11 of these one-adult household-associated loci also showed the same direction of effects in the Raine Study. Although the magnitudes of effects were smaller in FFCWS than in ALSPAC, one locus associated with the accumulation of one-adult household exposures (cg00807464; *CUX2*) showed nearly identical effect estimates between cohorts.

None of the 41 loci identified for DNA methylation at 15 years showed associations between adversity and DNA methylation at birth (appendix pp 30–31) or at 7 years (appendix pp 32–33). 7-year effect estimates were smaller than the 15 year estimates, with consistent directions of effect in 20 (49%) loci (figure 3A). Irrespective of adversity exposure, correlations in DNA methylation levels across ages were low at the individual level (r_{avg} 0·11; appendix p 54). The emergence of these associations was not explained by early-life confounders (<10% differecne in effect estimates when correcting for parental socioeconomic position, maternal BMI, or gestational age) or biological mediators during adolescence (<5% of the association mediated through age at pubertal onset, adolescent BMI, C-reactive protein concentrations, or smoking), suggesting that some adolescent differences might emerge later in development and become stronger with time (appendix pp 57–62).

Moving beyond adolescent DNA methylation, 34 (83%) loci had significant adversity exposure group-by-age interactions (FDR <0·05), suggestive of more complex patterns of change and stability across development. From these loci, we identified five additional types of longitudinal DNA methylation trajectories (figure 4), which showed distinct DNA methylation patterns across ages and adversity exposure groups (table, appendix pp 63–66), but not between the FDR and R^2 subsets of loci (appendix p 67).

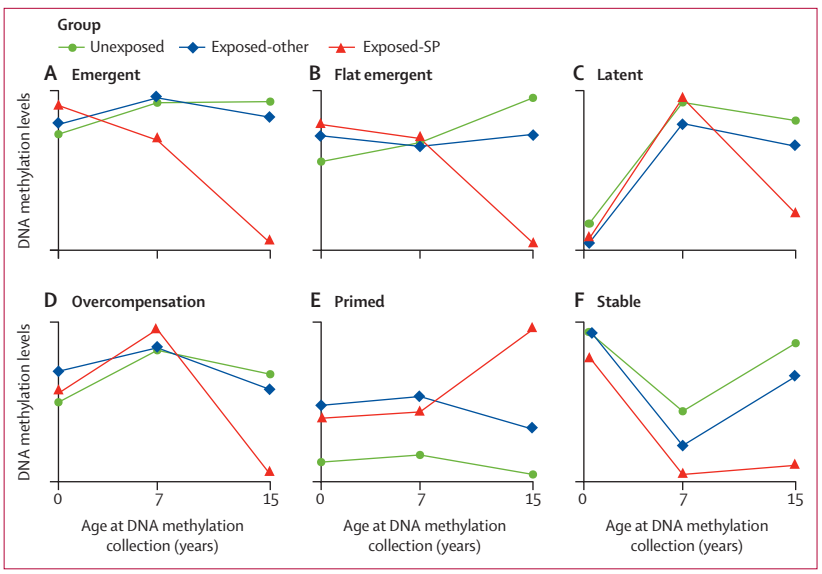

Figure 4: **DNA methylation trajectories across development**
A) Emergent trajectory (five loci): differences in the exposed-SP group appeared in childhood but did not fully emerge until 15 years. B) Flat emergent trajectory (two loci): differences in the exposed-SP group were modest throughout childhood and fully emerged by 15 years. C) Latent trajectory (17 loci): differences for the exposed-SP group emerged at 15 years, with no differences observed from exposure at other times; some CpGs in this cluster showed graded differences between childhood exposed in sensitive periods versus other times. D) Overcompensation trajectory (nine loci): cross-over of DNA methylation differences in the exposed-SP group were present from 7 to 15 years, along with differences in DNA methylation level between ages. E) Primed trajectory (one locus): differences in the exposed groups were apparent from birth but were magnified in the exposed-SP group at 15 years. F) Stable trajectory (seven loci): differences in the exposed-SP group were present at 7 years and remained stable until 15 years. Exposed-SP=exposed during a sensitive period. Exposed-other=exposed to adversity at a different developmental stage.

Of the 46 loci for which we previously identified time-varying associations between adversity and DNA methylation at 7 years,[8] only one [2%] showed an association at 15 years (p<0·05; appendix pp 37–38), which did not pass multiple-test correction. 24 (52%) of the 46 loci identified in our previous study showed consistent directions of effect between 7 years and 15 years (figure 3B).

Discussion

In this analysis of data from the ALSPAC prospective longitudinal cohort study, we found that associations between childhood adversity and DNA methylation vary across the life course, manifesting at different developmental stages through distinct patterns of persistence and latency. To our knowledge, this is the first study to incorporate time-dependent measures of childhood adversity when assessing longitudinal epigenetic patterns.

Our findings suggest that early childhood—the period between ages 3 and 5 years—is a possible sensitive period for the biological embedding of childhood adversity that

manifests in adolescence. These findings are consistent with the findings of previous human and animal studies showing that exposures earlier in life might have greater influence on epigenetic patterns measured in childhood[7,8] or adolescence.[34] Because early childhood is a time of rapid cognitive, social, emotional, and regulatory development,[37] epigenetic processes might be more malleable,[12] resulting in increased sensitivity to life experiences that shape DNA methylation levels and trajectories across development. Our findings suggest that early childhood might be a period for focused interventions to limit or prevent the long-term sequelae of childhood adversity.

Of the seven types of adversity examined, exposure to one-adult households was associated with the highest number of loci having altered DNA methylation at 15 years. By contrast, previous research on DNA methylation from the same children at 7 years identified no associations with one-adult households,[8] suggesting that these associations are specific to adolescence. Previous studies have shown that the effects of single-parent households begin to emerge around puberty, manifesting through shifts in puberty timing,[38] poor self-esteem,[39] and increased depressive symptoms and externalising behaviours.[40] We did not detect any mediation between the associations between one-adult households and DNA methylation through the age of pubertal onset, nor were any identified loci previously linked to pubertal onset or sex hormone concentrations, or confounded by socioeconomic factors (appendix pp 10–12). We also replicated the direction of associations for 11 loci associated with one-adult households in two independent cohorts. These results are particularly salient given the differences in the socio-demographic contexts and in the tissue assessed between studies (saliva in the FFCWS *vs* blood in ALSPAC and the Raine Study). Beyond broad tissue differences, saliva is more heterogenous across individuals than blood,[41] which increases the stringency of the replicated effects and highlights the potential relevance of these top loci. Overall, these findings suggest a latency to the effects of one-adult households on biological processes and health outcomes, which might not become apparent until the rapid developmental changes that occur during puberty.

We identified fewer loci associated with other adversities, such as maternal psychopathology and experiences of sexual, physical, or emotional abuse. These adversities might have subtle influences on the adolescent epigenome, requiring larger sample sizes or meta-analyses to uncover. None of the top 41 loci identified in our study overlapped between different types of childhood adversity, nor were they present among top loci from previous studies of child and adolescent victimisation and DNA methylation at age 18 years.[11,42] As discussed in ongoing debates surrounding the so-called lumping or splitting of childhood adversities in clinical research,[43] different dimensions of adversity could result in distinct epigenetic signatures, a hypothesis supported by the finding that adjusting for other types of adversity only modestly influenced associations.

Most DNA methylation trajectories showed primarily latent associations with adversity, meaning that they did not emerge until the individual reached 15 years. These findings align with those of previous longitudinal studies of genome-wide DNA methylation from ALSPAC[13,14] and Project Viva,[10] which showed that early-life stressors, such as prenatal maternal smoking[13] and socioeconomic disadvantage during childhood,[10,14] can have both immediate and latent associations with DNA methylation during childhood and adolescence. Subtle desynchronisation of DNA methylation levels might appear earlier in development, while evading immediate detection until later in life. These so-called sleeper patterns might explain why complex diseases unfold over years of development, rather than immediately after exposures or risk factors.[9] We also note that most of our top loci showed little individual-level stability over time, suggesting that these latent effects might be located within regions of the epigenome that change across development. More research is needed to determine whether latent associations between childhood adversity and the epigenome persist into adulthood and whether they are more likely than alterations arising earlier in development to influence physical and mental health.

Similarly, the DNA methylation differences that we previously observed at age 7 years did not persist into adolescence.[8] Studies on early-life stressors,[10,14] birthweight and gestational age,[16] and maternal weight before and during pregnancy[15] parallel these findings, showing that DNA methylation differences linked to early-life environments rarely persist across time. Whether these patterns resolve naturally or due to active intervention is unknown and should be investigated to determine whether interventions can be beneficial in reversing the epigenetic effects of early-life stressors. Nevertheless, even short-term alterations that eventually fade over time might alter the developmental trajectories of downstream cellular pathways to influence future health.

Several of the differentially methylated genes that we identified have previously been implicated in processes that could influence downstream disease. For instance, *CUX2* encodes a transcription factor involved in dendrite and synapse formation;[44] alterations to CUX2 could influence neurodevelopment and vulnerability to mental disorders. Several of the top genes identified in our study, including *DUSP10*, *DSP*, and *VEGFA*, are also linked to cardiac function, and might partly reflect mechanisms linking childhood adversity to heart disease.[45] However, findings from epigenome-wide and genome-wide association studies have different interpretations and have not yet converged on common mechanisms underlying human health and disease. Because alterations in DNA methylation might not reflect concomitant changes in gene function or expression, experimental studies are needed to identify the true functional and health consequences of these epigenetic

differences and determine whether short-term or long-term DNA methylation changes, or both, could link childhood adversity to adverse health outcomes across the lifespan.

If replicated, our results might reveal how the biological embedding of early-life exposures through DNA methylation contributes to disease risk across development, which could have important clinical implications for early risk prediction, disease prognosis, and therapeutic guides for individuals and populations exposed to adversity. Several studies have shown that DNA methylation can predict risk and progression of diseases, such as cancer[46] and depression.[47] Some adversity-associated DNA methylation trajectories might be able to predict concomitant trajectories of disease risk. If true, repeated measures of DNA methylation could serve as a biological indicator or early warning sign of initiated disease processes, helping to identify people at increased risk for future disease. Moreover, these adversity-associated DNA methylation trajectories might also act as biological measures of treatment response (eg, to interventions or protective factors designed to buffer the effects of adversity). Studies have shown that, compared with people who do not have post-traumatic stress disorder, DNA methylation differences in adults with post-traumatic stress disorder resolved after psychotherapy treatment, including DNA methylation changes associated with a reduction in symptom severity.[48] Therefore, repeated measures of DNA methylation could be used as a marker of therapeutic effectiveness, tracking possible disease progress and resolution.

Our study has several limitations. First, DNA methylation data for the ARIES subsample of the ALSPAC cohort were generated from slightly different tissue types at each wave. Although we corrected for cell type composition using established methods, differences in the stability of DNA methylation differences between waves might have been partly driven by tissue-based differences and variability. Second, we could not replicate all findings, partly due to the absence of available data from the Raine Study and FFCWS. Additionally, differences in associations between cohorts could reflect differences in the socioeconomic environment or the specific timing and tissue used for DNA methylation measurements, among other factors. Future studies should confirm these longitudinal epigenetic responses to childhood adversity and triangulate the socio-biological factors that modulate adversity-induced epigenetic differences and health outcomes. Third, we cannot rule out the possibility that unmeasured confounding or technical factors influenced our findings. However, our results were robust in internal validation analyses and when controlling for 11 potential confounders and investigating four potential mediators. Similarly, we could not assess the effect of time-varying confounding, which could have influenced our results.[49] Fourth, our analytic subsample was mainly composed of children of European descent. This poor diversity limits the generalisability of our findings, emphasising the importance of replicating this work in more diverse cohorts. Finally, the differences in DNA methylation observed in children and adolescents exposed to adversity might not reflect concomitant phenotypic alterations because epigenetic alterations in peripheral tissues might only partly reflect the causal mechanisms that drive health and disease. Studies that combine both model systems and human populations are necessary to fully delineate the relationships between adversity, DNA methylation, and health.

In summary, this study highlights developmental variability in the relationship between adversity and DNA methylation trajectories across childhood and adolescence. Future studies should continue to investigate longitudinal measures of DNA methylation to identify the potential role of latent and persistent epigenetic alterations in driving the short-term and long-term health outcomes that result from childhood adversity. Ultimately, this research will help to guide intervention strategies and identify individuals at increased risk for physical and mental disorders arising from exposure to childhood adversity.

Contributors

AAL designed the study, did all primary analyses using the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort data, led the replication analyses, interpreted the results, and wrote the manuscript. YZ, BJS, JC, AJS, ADACS, MJS, EW, CLR, and KJR assisted in the design of the study and interpretation of the results and provided critical input during writing of the manuscript. PEM, NMW, SC-W, and R-CH did the Raine Study analyses and provided critical input on the manuscript. JF, CM, LS, and DAN did the FFCWS analyses and provided critical input on the manuscript. ECD obtained grant support for this work, designed the study, interpreted the results, and helped write the manuscript. AAL and YZ directly accessed and verified the ALSPAC data reported in the manuscript. PM and NMW directly accessed and verified the Raine Study data reported in the manuscript. JF and CM verified the FFCWS data reported in the manuscript. All authors had access to the data. AAL reviewed and compiled the scripts and results for the Raine Study and FFCWS analyses. All authors took the final decision to submit the manuscript for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Summary statistics from the current study are available upon request to the corresponding authors. All original code can be found on github.com/thedunnlab/. ALSPAC data are available by request from the ALSPAC Executive Committee for researchers who meet the criteria for access to confidential data (bristol.ac.uk/alspac/researchers/access/). Data from the Raine Study are available with the permission of the Raine Study. Restrictions apply to the availability of these data, which were used under license for this study. The FFCWS data analysed in this study are available with permission from the Future of Families and Childhood Wellbeing Study repository (fragilefamilies.princeton.edu/documentation).

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lussier AA, Zhu Y, Smith BJ, et al. Association between the timing of childhood adversity and epigenetic patterns across childhood and adolescence: findings from the Avon Longitudinal Study of Parents and Children (ALSPAC) prospective cohort. *Lancet Child Adolesc Health* 2023; published online June 13. https://doi.org/10.1016/S2352-4642(23)00127-X.

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SUPPLEMENTAL METHODS

Discovery cohort – the Avon Longitudinal Study of Parents and Children

Sample description

Data came from the Avon Longitudinal Study of Parents and Children (ALSPAC), a longitudinal birth cohort of children born to mothers who were living in the county of Avon, England, with expected delivery dates between April 1991 and December 1992(1, 2). The main goal of the ALSPAC study is to increase knowledge of the pathways influencing lifelong health, with a focus on the genetic and environmental determinants of health and disease. A total of 14,451 pregnant women participated in the study and of 14,062 of eligible live births who were alive at one year of age (n=13,988 children) were enrolled in the study. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/.

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (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 at the time. 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/). Secondary analyses of ALSPAC data were approved with oversight by the Mass General Brigham Institutional Review Boards (IRB) (Protocol 2017P001110).

DNA methylation profiling

The analytic sample came from a subsample of ALSPAC, the Accessible Resource for Integrated Epigenomics Studies (ARIES). The subsample consisted of 1,018 mother-child pairs from whom blood-based DNA methylation data were collected. Participants in the ARIES subsample were randomly selected from ALSPAC participants with complete data across at least five timepoints of data collection (3). Three timepoints of DNAm were collected, including cord blood at birth (n=905), whole blood at age 7 (n=970), and peripheral blood mononuclear cells at age 15 (n=966). 846 individuals had DNAm collected at all three timepoints. Number of samples are based on the number of samples with available data after the pre-processing procedures described in the main text.

DNA methylation pre-processing and normalization

DNAm data were processed using the *meffil* package in R, which performs background correction and functional normalization of DNAm data (4). Twins and samples with >10% of CpG sites with a detection p-value >0.01 or a bead count <3 were removed, as were cross-hybridizing probes and polymorphic probes. To remove possible outliers, we winsorized the beta values (i.e., values that represent the percent of methylation at each CpG site), setting the bottom 5% and top 5% of values to the 5th and 95th quantile, respectively (5). Finally, we removed probes showing little variability across individuals, defined as CpGs with <5% difference in DNAm between the 10^{th} and 90^{th} percentile of values. The final analytic sample after pre-processing consisted of 966 youths and 302,581 CpGs with DNAm data measured at age 15. DNAm measured at age 0 and 7 were similarly pre-processed and normalized.

Covariates

Across all ALSPAC analyses, we controlled for the following covariates, which were measured at birth and coded as follows. We have extensively investigated and discussed the topic of covariates in our prior manuscript on time-varying adversity and childhood DNAm (6). These were selected based on their inclusion in prior studies of early-life exposures and DNAm using data from ALSPAC (6-9).

1. *Sex* – coded as a binary variable, as reported at birth, and confirmed from epigenetic data.
2. *Race/ethnicity* – coded as a binary variable corresponding to white or non-white, as our analytic sample was predominantly white and previous work in the ARIES subsample found no strong evidence of population stratification(6). Race/ethnicity was determined based on parent self-reports at birth; any response other than "White" from either parent resulted in the child received a code of "non-White".
3. *Maternal age at birth* – coded as a categorical variable with three categories of response, ages 15-19, ages 20-35, and age 36+. We categorized this variable because maternal age does not have a linear relationship with health outcomes. Rather, children born to young (age <20) or older (age>35) mothers may be more likely to have deleterious health outcomes (10, 11). As such, using a continuous scale of maternal age is not appropriate for these types of analyses, in spite of the potential increase in power.
4. *Number of previous pregnancies* – coded as a categorical variable, with response categories of 1, 2, and 3+.

5. *Maternal smoking during pregnancy* – coded as an exposure if the mother smoked during at least two trimesters of pregnancy, as previously described (9).
6. *Child birthweight* – coded as a continuous variable.
7. *Maternal education* – coded as a categorical variable with four categories of response, less than O-level, O-level, A-level, and degree or above.
8. *Age at DNAm collection* – continuous measure of the age (in years) at which the blood sample for DNAm was collected from the participant.

We also estimated cell type composition using the Houseman method for all three ages as part of the *meffil* pipeline (4, 12). All estimated cell type proportions were included in downstream analyses and regressions that used DNAm data.

Structured Life Course Modeling Approach (SLCMA)

We tested time-dependent associations for each adversity using the timepoints shown in **Fig. 1**. In the first step, the SLCMA selected the timepoint or additive hypothesis (accumulation; recency) that explained the most variation in a given CpG for each type of adversity (seven separate analyses of 302,581 CpGs). We interpreted the model selected by the SLCMA through six separate life course hypotheses, including four sensitive periods hypotheses that encoded exposure to each childhood adversity during:

1. *very early childhood* – hypothesis selected by the SLCMA fell within the ages of 0-3 (before 36 months);
2. *early childhood* – hypothesis selected by the SLCMA fell within the ages of 3-5 (69 months or before);
3. *middle childhood* – hypothesis selected by the SLCMA fell within the ages of 6-7 (84 months or before);
4. *late childhood* – hypothesis selected by the SLCMA fell within the ages of 8-11 (after 84 months);
5. *accumulation* – total number exposures across childhood, ranging from 0-8 total exposures, depending on the adversity analyzed;
6. *recency* – total number of exposures weighted by age when the adversity was measured.

In the second stage of the SCLMA, we used selective inference to perform post-selection inference(13) and adjusted for covariates using the Frisch-Waugh-Lovell theorem(14), shown to improve statistical power in penalized regression analyses(15, 16). Only complete cases (i.e., individuals with non-missing covariate and exposure data from ages 0-11) were analyzed for each adversity (**Fig. 1**).

Biological implications of loci associated with childhood adversity identified from SLCMA

To further understand the biological implications of significant loci, we investigated the biological implications of findings from SLCMA in four different ways (**Table S5**).

First, we assessed the enrichment of regulatory elements in top loci compared to all analyzed loci using chi-squared tests. Both FDR-significant and R^2-threshold loci were overrepresented in enhancers (**FDR**: p=0.034; **R^2**: p=0.008), but not gene promoters (**FDR**: p=0.17; **R^2**: p=0.17; **Fig. S5A**). These loci were also enriched for regions away from CpG islands ('Open Sea'), rather than CpG Islands, shores, or shelves (**FDR**: p=0.021; **R^2**: p=0.018; **Fig. S2B**). Overall, top loci showed higher representation in regions of lower CpG density, suggesting these genomic regions may be more responsive to childhood adversity.

Second, we examined the correlation of DNAm at the top loci in blood and four different brain regions using the Blood Brain DNA Methylation Comparison Tool(17). Most FDR-significant loci (17/22) had weak, but positive correlations between brain and blood (prefrontal cortex r_{avg}=0.05, range=-0.19-0.65; entorhinal cortex r_{avg}=0.06, range=-0.24-0.60; superior temporal gyrus r_{avg} =0.05, range=-0.18-0.61; cerebellum r_{avg}=0.06, range=-0.14-0.54)(**Table S6**; **Fig. S6**)(17). Similarly, most R^2-threshold loci (28/41) also had weak, but positive correlations, which were, on average, larger than those for the FDR loci (prefrontal cortex r_{avg}=0.11, range= -0.19-0.95; entorhinal cortex r_{avg}=0.11, range=-0.24-0.95; superior temporal gyrus r_{avg} =0.09, range=-0.21-0.94; cerebellum r_{avg}=0.09, range=-0.20-0.97). Thus, adversity-induced alterations to blood DNAm levels may reflect similar changes in the central nervous system.

Third, we analyzed the enrichment of biological processes in top loci using gene ontology (GO) terms from the DAVID tool (18, 19). Although none reached significance, eight distinct clusters of biological processes were overrepresented in FDR-significant loci (n=21 genes)(18, 19). These clusters were implicated in abiotic stimulus, development, ion transport, and cellular regulation of biosynthetic processes (**Fig. S7**). By contrast, 18 clusters were identified for R^2-threshold loci, which were involved in development, MAPK activity, muscle development, and immunity. These results suggest that different types of childhood adversity may act through diverse biological processes, rather than a concerted network of pathways.

We also assessed the enrichment of GO terms in top loci using the *missMethyl* package in R, which accounts for the number of CpG measured in each gene(20). Again, no significant enrichment was detected for

KEGG pathways, biological processes, molecular functions, or cellular components at an FDR<0.05. Among the top 10 processes from KEGG, biological processes, cellular component, and molecular function categories, several pathways and processes were related to immune function, apoptosis, and development (**Fig. S8**).

Fourth, we assessed the evolutionary constraint of genes linked to top loci using data from the Exome Aggregation Consortium (21), which estimated the probability of intolerance to loss-of-function (pLI) mutations using genetic and evolutionary data. In other words, genes with intolerance to loss-of-function are thought to have more functional constraint and thus, may potentially have played a role in human survival and evolution. Genes linked to top loci showed no evidence of enrichment for functionally-constrained genes (**Table S5**; **Fig. S9**). However, 3 FDR-significant genes linked to the accumulation of exposure to one-adult households showed evidence of strong evolutionary constraint (pLI>0.9; *DSP*, *CUX2*, and *STK38L*). Four additional genes with high evolutionary constraint were identified in the R^2-threshold loci (*FBXL16, PKD2*, *TAF1*, and *XKR6*). Five of the seven loci in genes with high functional constraint showed decreased DNAm in participants exposure to childhood adversity (*DSP, STK38L, FBXL16, PKD2, XKR6)*. Together, these findings highlight a potential role for genes influenced by parental and social environment in human survival and evolution.

Finally, we used the EWAS catalog to identify traits previously associated with our top CpGs. All of our top 41 loci showed prior associations in the literature, including 29 that had been previously linked to age. We also found seven CpGs previously associated with gestational age, which had little effect on the strength of associations when we included it in our analysis of additional confounders (**Fig. S19**; see below for details). Similarly, six CpGs were linked to sex differences, though only the three located on chromosome X showed after removing sex as a covariate. One CpG was previously linked to smoking (cg02810291) and one to maternal BMI (cg13204236); additional confounding and mediation analyses for these CpGs again found no differences. Finally, we identified four CpGs previously associated with rheumatoid arthritis, which showed no mediation through CRP levels. However, these findings may point to further relationships between childhood adversity, inflammation, and future health outcomes.

Internal validation of age 15 loci using non-parametric bootstrapping

The ALSPAC cohort is unique; no longitudinal birth cohorts at present have collected comparable measures of childhood adversity and DNAm. At best, other birth cohort studies with repeated measures of childhood adversity have only collected one timepoint of DNAm during childhood or adolescence, but not both. By contrast, studies with repeated DNAm measures do not have repeated and prospective measures of childhood adversity. As such, we could not complete external replication analyses of the associations we detected between time-varying childhood adversity and DNAm at age 15. In the absence of a cohort in which to replicate our findings, we performed internal validation analyses of our associations using ordinary nonparametric bootstrapping(22).

In brief, the bootstrap involves resampling data with replacement from a given sample(23). Unlike parametric methods, such as t-test and linear regressions, the bootstrap does not require assumptions of normality nor rely on parameter estimation (e.g., regression coefficients) from the original sample. Rather, the bootstrap relies on the approximations of test statistics, generated by drawing repeated resamples from a given sample – at random – across thousands of iterations. By resampling with replacement, the original sample size is maintained, with some rows of data omitted and others repeated; this process creates multiple random (re)samples of data from the same underlying population. Since the original sample is drawn from the population of interest, each bootstrap resample can be thought of as a new sample of data drawn from the population. In other words, the bootstrap sample differs from the original sample in each iteration at random, while also remaining similar to the general population from which the original sample was collected. As such, bootstrapping can provide insight into whether findings might be replicated in an independent cohort sampled from the same general population.

Here, we performed a random-x bootstrap resampling using the *boot* package in R(24). For each CpG identified in the analyses of childhood adversity and DNAm at age 15, we performed 10,000 bootstrapped linear regressions of the selected hypothesis (**Table 1**) and DNAm. We included the same covariates as the SLCMA analyses in the bootstrapped models. Effect estimates across the 10,000 bootstraps were averaged to obtain the "bootstrapped effect estimate". 95% confidence intervals were calculated using the normal-theory interval(24). As we used the bootstrap for inference, rather than prediction, we did not estimate the bootstrap optimism. In inference, the optimism would be 0 and therefore, is not informative. Instead, we report the "bootstrap bias", which is the difference between the bootstrapped estimate and the estimate in the full sample.

The results from the bootstrap analyses were nearly identical to those identified in the initial SLCMA analyses (**Table S7**), both in terms of average effect estimates and confidence intervals. The mean difference between effect estimates from the bootstrap and original analyses (i.e., bootstrap bias) across all top loci was 4.57×10^{-5} (2.52×10^{-5} for FDR-significant loci), with the largest absolute magnitude of difference being 2.03%

(comparing the bootstrap to the original effect estimate). In addition, all effect estimates were significant at the 5% level, judged by bootstrap confidence intervals (**Fig. S10**). Confidence intervals were narrower in all but two of the original analyses (linear regression) compared to the bootstrap, suggesting the bootstrap could more precisely assess the effect estimate.

Together, these findings show that our initial results were robust to different analytic subsamples and populations, as well as nonparametric approaches that make fewer distributional assumptions. Thus, our findings may be likely to replicate in independent cohorts.

Adjusting for exposure to other childhood adversities

To further determine the specificity of our associations between subtypes of childhood adversity and DNAm patterns at age 15, we performed a set of mutually-adjusted regression analyses. Specifically, we investigated the impact of correcting for exposure to the other six types of childhood adversity on the strength of association between a given measure of childhood adversity and DNAm.

Children in this analytic sample could have been exposed to adversity before, during, or after the sensitive periods we identified. We therefore coded exposure to other types of childhood adversity in five ways, as outlined below. We investigated these five different ways of coding co-occurring adversities to facilitate future replication of our work in datasets that may not be as fine-grained as ALSPAC, as well as narrow down the periods when co-occurring adversities may have the greatest impact on our results.

1. *Exposed to any other childhood adversity between age 1-11* – the full window of potential exposures to childhood adversity;
2. *Exposed to any other childhood adversity between age 1-7* – the window of potential exposures to childhood adversity that would influence age 7 and age 15 DNAm;
3. *Exposed to any other childhood adversity between age 8-11* – the window of potential exposures to childhood adversity that would only influence age 15 DNAm;
4. *Exposed to any other childhood adversity before the SLCMA-selected sensitive period;*
5. *Exposed to any other childhood adversity during the SLCMA-selected sensitive period;*

NB: for loci with accumulation hypotheses – #4 and #5 were calculated using the accumulation of all exposures to other adversities from age 1-11.

For each of the 41 adolescent-specific loci, we ran five separate regressions that included the base model (no mutual adjustment; i.e., the model we presented in primary text) and one of the five above variables. The strength of associations for the mutually-adjusted models were compared to the base model associations between the specific childhood adversity and DNAm at age 15. We found that all associations remained significant when correcting for other types of childhood adversity, no matter which mutual-adjustment strategy was employed (FDR<0.05 when correcting for testing 41 loci) (**Fig. S11**).

Associations between the accumulation of exposure to one-adult households and DNAm at age 15 were most attenuated in the mutually-adjusted model, showing between a 1 to 39% reduction in the size of the effect estimate per CpG; the average attenuation for these three CpGs was 9.0% (**Fig. S12**). Similarly, the three loci linked to the recency of exposures to financial hardship also showed stronger effect shifts in mutually adjusted models (range = -28% to 27%, mean = 2.4%). These results are perhaps unsurprising, given that accumulation and recency scores across childhood may be more highly correlated with other exposures to childhood adversity.

By contrast, we observed smaller alterations to the effect of exposures during sensitive period hypotheses when performing these mutual-adjustment analyses, suggesting our sensitive period findings were less prone to the influence of other types of childhood adversity. Of note, mutual-adjustment for other adversities reported during the same sensitive period identified by the SLCMA generally had the greatest effect on the strength of associations (**Fig. S12**). In particular, almost all associations between exposure to one adult households during early childhood, and DNAm at age 15 were attenuated when controlling for co-occurring adversities during the same sensitive period (mean = 8.6% reduction in effect estimate, range = -20.6% to 4.0%; **Fig. S13**). This finding suggests one-adult households may co-occur with other adversities more frequently, particularly during early childhood. Nevertheless, the strength of associations remained fairly stable even when controlling for these co-occurring exposures, indicating that associations remained specific to one-adult households.

Together, these results suggest our observed associations between childhood adversity and DNAm at age 15 were mostly specific to each type of childhood adversity and were not the result of other possible co-occurring exposures across childhood. Future studies should further investigate these findings in other cohorts to confirm their robustness and specificity to subtypes of childhood adversity, especially because ALSPAC is a sample where few children were simultaneously exposed to multiple types of adversity (see correlations in **Table S4** and **Fig. S4**).

Replication cohort – The Raine Study

Sample description

The Raine Study, formerly known as the Western Australian Pregnancy Cohort Study, is a continuing longitudinal study based in Perth, Western Australia. Between 1989-1991, 2,979 Generation 1 participants (primary caregivers) were recruited at approximately 18 weeks of pregnancy(25, 26). At birth, 2,868 participants were available for follow-up. Generation 2 participant (offspring) follow-ups were conducted at 34 weeks' gestation, and ages 1, 2, 3, 5, 8, 10, 13, 17, 20, 22, and 27 years; and labelled to reflect average age of participants at each follow-up. Follow-ups were approved by Human Ethics Committee at King Edward Memorial Hospital, Princess Margaret Hospital for Children, and the University of Western Australia in Perth. Generation 2 participants with epigenetic data were included in the present analysis (n=1,190). Local Flinders ethics was ratified by their Human Research Ethics Committee approval number: HEL4641-2.

DNA methylation profiling

Primary caregivers (Gen1) provided written informed consent to participate in the study at each follow-up and participants (Gen2) provided consent when they were old enough. Clinical assessments were performed at multiple follow-ups, including at age 17 years where a blood sample was taken with consent. DNA methylation profiles were generated from whole blood for 1,192 (58 technical replicates) participants. DNA was first extracted from the blood sample via the Puregene DNA Isolation kit (Qiagen, Germany). Genomic DNA was treated with sodium bisulphite with the Zymo EZ DNA Methylation-Gold kit. Processing of the Human Methylation 450K array was performed by the Centre for Molecular Medicine and Therapeutics (The University of British Columbia, Vancouver, Canada).

Quality control was performed via R and Bioconductor packages *shinyMethyl*, *MethylAid*, and *RnBeads*. Four participants were identified as outliers and removed; three additional participants with poor probe quality were also removed, as was one participant with a sex misclassification. CpG sites were removed for the following reasons: CpG with a common SNP disrupted the site leading to genotypic specific DNAm levels; sex chromosome CpGs; CpGs with a detection $p > 0.05$ in any sample; probes with bead counts < 3 in more than 5% of samples. Normalization was performed using beta-mixture quantile normalization (BMIQ) (27).

Childhood adversity (one-adult households)

Gen2 participants were followed-up at 34 weeks' gestation, and ages 1, 2, 3, 5, 8, 10, 13, 17, 20, 22, and 27 years. A variety of clinical and demographic information was collected from Gen1 and Gen2 participants at each follow up. Using these data, we could harmonize one type of time-varying childhood adversity between ALSPAC and the Raine Study cohorts: exposure to one-adult household.

Specifically, information on number of adults (i.e., those aged 18 or older) that the income supported in the Gen2 participant household was collected as a continuous numeric value at ages 1, 2, 3, 5, 8, 10. Data were recoded to reflect a binary in terms of a one-adult household (exposed/unexposed) at each period.

Covariates

The following covariates were included in the analyses of data from the Raine Study:

1. Biological sex assigned at birth
2. First 10 principal components of genetic variance (calculated using SNP data)
3. Maternal education level at birth
4. Maternal age at birth
5. Mother's number of previous pregnancies
6. Child birthweight
7. Maternal smoking during pregnancy
8. Cell type proportions estimated using the Houseman method.

Replication cohort – Future of Families and Child Wellbeing Study (FFCWS)

Sample description

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(28). 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 >75% at all ages.

DNA methylation profiling

DNAm was measured from children's saliva samples at age 15 (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(29). 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.

Childhood adversity

We investigated four 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 closest to ALSPAC.

1. Caregiver physical and emotional abuse (N_{DNAm}=662-1,527): 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_{DNAm} =1,846): Maternal depression was measured at ages 1, 3, 5, and 9 using the CIDI-SF scale for depression(30-33). 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_{DNAm} =799-1842): 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_{DNAm} =722-1,859): Mothers reported material hardship at ages 1, 3, 5, and 9 (34-37) . 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.

Covariates

The following covariates were included in replication analyses using FFCWS data:

1. child sex
2. child birthweight
3. mother's number of prior pregnancies
4. maternal education
5. maternal age at birth
6. maternal smoking during pregnancy
7. city of data collection
8. array type (450K or EPIC)
9. leukocyte proportion estimated using a childhood saliva reference panel(38).

Replication analyses

Winner's curse correction of top ALSPAC loci

Winner's curse is the terms evoked in genome-wide studies to explain why top associations identified from discovery analyses may fail to replicate when tested again in independent data sets(39). In other words, the first identification of a given exposure-outcome relationship may be an exaggerated estimate for a given exposure-outcome relationship in the sample in which it was first identified.

To reduce concerns that our discovery results were biased by Winner's curse, we accounted for Winner's curse when attempting to replicate our findings in the Raine Study and the FFCWS. We used the *winnerscurse* package in R (github.com/amandaforde/winnerscurse), which performs a normalized maximum likelihood estimation (MLE) on our top 41 loci, leveraging the effect estimates and standard errors of these loci to calculate a bias-corrected estimate and 95% confidence intervals(40). As expected, relative to our original discovery results, we found the Winner's curse corrected estimates were smaller and had wider confidence intervals, but remained significantly associated with exposure to childhood adversity (**Table S8**). We use these estimates in downstream replication analyses to assess potential replication more reliably in the Raine Study and FFCWS.

Replication in the Raine Study Generation 2

We focused our replication analyses on the CpGs identified in the primary analyses. Due to the availability of childhood adversity data, we could only investigate the 20 CpGs associated with one-adult households (**Table S8; Fig. S14**). In other words, we could not adequately match the other types of childhood adversity measured in the ALSPAC cohort using data from the Raine Study. We also note that participants from the Raine Study had blood DNAm profiles measured later in development (starting at age 17), meaning that differences in DNAm present earlier in development (i.e., age 15) may have resolved by this timepoint.

As we have previously shown, p-values are an unstable metric for the replication of time-varying associations within and between studies(7). Thus, we focused primarily on replicating the direction and magnitude of associations observed using ALSPAC data.

In the Raine Study (N=382-529), we performed linear regressions of exposures to one-adult households, matched as closely as possible to the time point identified in ALSPAC (**Table S8**) and DNAm measured at age 17, adjusting for covariates. Across all CpGs, the magnitude of effects between adversity and DNAm were smaller in Raine than ALSPAC, even with our Winner's curse bias-corrected estimates (**Fig. S15**). However, 90% of CpGs (18/20) showed the same direction of associations, which is higher than would have been expected under the null ($p=0.000201$) (**Fig. S15**). Three CpGs showed nominal associations in the Raine Study ($p<0.05$), though none passed multiple-test correction. However, their 95% confidence intervals did not overlap with zero; their confidence intervals also overlapped with the winner's curse-correction estimates from the ALSPAC cohort. Prior studies have used both criteria as a metric for replication(41).

Together, these that the associations between one-adult household and DNAm identified in the ALSPAC cohort are partially recapitulated in the Raine Study. Although the replicated effects were smaller in the Raine Study, key differences in the socioeconomic context and age at DNAm measurement could have influenced these findings. These findings further highlight the importance of investigating sensitive periods for childhood adversity and DNAm across sociobiological contexts and across time.

Replication in the FFCWS cohort

We focused our replication analyses on the CpGs identified in the primary analyses, again attempting to replicated the direction and magnitude of associations. Due to the availability of childhood adversity and DNAm data, we could only investigate 28 CpGs associated with caregiver abuse (3 CpGs), maternal psychopathology (1 CpG), one-adult households (15 CpGs), and financial hardship (9 CpGs) (**Table S9; Fig. S14**). Of these loci, five were only measured on the 450K array (not the EPIC array), resulting in a smaller sample size.

We could not adequately match the other types of childhood adversity measured in the ALSPAC cohort (neighborhood disadvantage and physical/sexual abuse) using data from FFCWS, and the loci associated with family instability were not available for analysis. We also note that all participants from FFCWS had DNAm profiles measured from saliva, with a subset having data generated from the EPIC array (N=865-1,043). FFCWS is also demographically distinct from the ALSPAC cohort, having higher prevalence of socioeconomic adversity and more racial/ethnic diversity. These differences may have influenced our ability to replicate associations in FFCWS.

In FFCWS (N=662-1,859), we performed linear regressions of exposures to childhood adversity, matched as closely as possible to the time point identified in ALSPAC (**Table S9**) and DNAm measured from saliva at age 15, adjusting for covariates. Across all CpGs, the magnitude of effects between adversity and DNAm were smaller in FFCWS than ALSPAC, even with our Winner's curse bias-corrected estimates (**Fig. S16**). However, 64% of CpGs (18/28) showed the same direction of associations, which is slightly higher than would have been expected under the null ($p=0.092$)(**Fig. S16**). We also note that 73% of the CpGs associated with one-adult households (11/15) showed the same direction of effects between cohorts, again slightly higher than random chance ($p=0.059$). Importantly, all 11 of these one-adult household CpGs showed the same direction of effects in the Raine Study, which further point to the replication of one-adult household effects across cohorts. In addition, one CpG associated with the accumulation of one-adult household exposures (cg00807464) showed nearly identical effect estimate between cohorts. Although no loci met a nominal $p<0.05$ threshold, several CpGs had confidence intervals that overlapped with those in ALSPAC.

Overall, the directions of associations between childhood adversity and DNAm were largely replicated in the FFCWS cohort, particularly for exposures to one-adult households. Given the clear differences between FFCWS and ALSPAC, it is perhaps unsurprising that the magnitude of associations was smaller in replication analyses. Further studies using large-scale, longitudinal birth cohorts are needed to triangulate these results across cohorts and determine the extent to which differences in the sociodemographic environment might influence the relationship between childhood adversity and adolescent DNAm.

Testing for potential confounding effects of the relationship between childhood adversity and DNA methylation levels at age 7 and 15

Given that our observed associations between childhood adversity and DNAm at age 15 were not present at age 7, we hypothesized that these emergent effects could be influenced by confounding structures of the data, whereby other factors might be driving these adolescent-specific associations. As such, we further investigated whether the associations we observed between time-varying childhood adversity and DNA methylation patterns across development were influenced by confounding factors or methodological artifacts that were not included in our models. We approached the issue of confounders using two approaches, outlined in **Fig. S2** and **Fig. S18**, focusing on the 41 associations that were identified in age 15 DNAm.

Early-life confounders of childhood adversity and DNAm at age 7 and 15

First, we tested whether early-life factors could influence the strength of associations between childhood adversity and DNAm levels at age 7 and 15. To this end, we assessed the impact of removing covariates from our base model (described above) on the estimated effect from a linear regression of time-varying adversity and DNAm levels. When removing individual covariates from the base model, we did not observe any large changes in the effect estimates of the associations between childhood adversity and DNAm at age 15 (**Fig. S19**) or age 7 (**Fig. S20**), except for two CpGs (cg17928317: 37.5% increase; cg27558057: 72.8% decrease). The effect estimates of these two loci changed substantially upon removal of sex as a covariate (**cg17928317**: age 15 β_{base}=0.079, $\beta_{no\ sex}$=0.108; age 7 β_{base} = 0.001, $\beta_{no\ sex}$=0.029; **cg27558057**: age 15 β_{base}=0.106, $\beta_{no\ sex}$=0.029; age 7 β_{base} =0.066, $\beta_{no\ sex}$=-0.024), though we note that both CpGs are located on chromosome X. As such, some amount of sex-dependent variability is expected due to differences in X chromosome dosage between males and females.

Beyond the covariates included in our base model, we also investigated whether other common confounders may have influenced our observed associations. Here, we assessed the impact of adding the following confounding factors known to influence childhood adversity or DNAm patterns to our base regression model: 1) parental socio-economic position (parent SEP) measured at birth, 2) gestational age in weeks, 3) maternal pre-pregnancy BMI, and 4) delivery type (caesarean or non-caesarean birth). We investigated these potential confounding factors due to their influence on risk for childhood adversity, as well as their prior associations with longitudinal DNAm patterns (42, 43). Of note, these factors were omitted from our initial analyses due to their high correlation with other covariates within our base model that are more robust predictors of longitudinal outcomes, such as maternal education, birthweight, maternal age, etc.

In general, the inclusion of parent SEP, gestational age, or maternal BMI did not substantially influence the strength of associations between childhood adversity and DNAm levels at age 15 (**Fig. S19**) or age 7 (**Fig. S20**). Indeed, only four loci showed a >10% change in their effect estimates upon the inclusion of these new covariates, all of which were influenced by the inclusion of maternal pre-pregnancy BMI (two from one-adult households and FDR-significant; two from financial hardship and passing the R^2-threshold). All associations remained significant at a Bonferroni-adjusted $p<0.05$ (for 41 loci). Changes less than 10% are generally thought to reflect factors that have little confounding effects (44), although more recent studies suggest that this threshold may be overly conservative and that thresholds for confounding could reach up 40% (45).

By contrast, we observed more variance in the effect estimates and p-values when including delivery method as a covariate. Indeed, 22 of 41 loci showed >10% change in effect estimates in age 15 DNAm, of which nine were no longer significant at a Bonferroni-adjusted $p<0.05$ (for 41 loci). Only one locus had >40% change in the effect, which was associated with financial hardship during late childhood (cg04659536; effect change = -83.3%). These findings are reflective of potential residual confounding from the method of delivery for a subset of loci at age 15. However, we note that including this covariate substantially reduced our sample size due to higher missingness than other variables, which could potentially introduce issues of selection bias. Associations in age 7 DNAm showed little to no impact of caesarean births on effect estimates.

Taken together, these findings suggest the specific associations between time-varying childhood adversity and DNAm at age 15 may not be due to the effects of common confounders or methodological artifacts arising from our current covariates. Furthermore, the associations between adversity and DNAm at age 7 remained null for all but one of these 41 loci ($p>0.0012$), further suggesting that the latent effects we observed were unlikely due to common confounders. Nevertheless, it is possible that other unmeasured confounders may influence the relationship between childhood adversity and DNAm at age 15, and thus, our findings should be replicated in other longitudinal birth cohorts with repeated measured of childhood adversity and DNAm.

Adolescent-specific factors mediating the relationship between childhood adversity and DNAm

Second, we tested the influence of adolescent-specific factors that could have possibly explained our observed associations. These adolescent-specific factors occurred after childhood adversity and DNAm collection at age 7, but before DNAm collection at age 15 (**Fig. S18**). Because our associations maintained the temporal ordering of exposures preceding the outcome, adolescent-specific confounders should not influence associations with DNAm at age 7. Moreover, confounders are, by definition, linked to both the exposure (adversity) and outcome (DNAm levels at age 15). In the present situation, we could assume that adolescent-specific factors land in the causal path between adversity and DNAm, given that they would occur after adversity and before DNAm. Given this causal path, potential adolescent-specific confounders could be considered mediators, rather than confounders that can be adjusted in a regression model. As such, we performed causal mediation analyses using the R package *mediation* (version 4.5.0) to determine whether our adolescent-specific association were explained, in part, by potential factors on the causal path. To this end, we assessed whether four biological outcomes previously linked to childhood adversity and/or DNAm patterns significantly mediated our observed associations; our rationale for testing these variables is described below. We corrected for the same covariates as previously described in mediation analyses.

Pubertal onset: Exposure to childhood adversity has been associated with earlier pubertal onset in some studies, including ALSPAC (46). Puberty is a time of rapid change and development, with concomitant alterations in epigenetic pathways (47). As such, age at pubertal onset is a plausible candidate to mediate the association between childhood adversity and DNAm levels in adolescence. To estimate pubertal timing, we analyzed the age at peak height velocity, calculated by a method called superimposition by translation and rotation (SITAR), which analyzes height measurements between age 5 and 16 (N=605-654) to identify the age at pubertal onset(48).

We did not identify significant mediation effects for pubertal onset for any of our top 41 loci (lowest p-value = 0.268, cg14455319; **Fig. S21**). Furthermore, when we contrasted our findings to a previous epigenome-wide association study of puberty and gonadal hormone levels, we did not find any overlaps with our 41 adolescent-specific loci(49). These findings suggest pubertal onset was unlikely to explain adolescent-specific associations.

Body mass index (BMI): We next analyzed BMI measured at age 15 (N=569-618). Prior studies have shown that childhood adversity is linked to obesity and changes in metabolic function(50, 51). In addition, a recent study of BMI in the ARIES cohort has shown a strong relationship between DNAm and BMI(52). Although the majority of loci in our analysis showed no significant mediation through BMI at age 15 (**Fig. S22**), 2.67% of the association between exposure to a one adult household in early childhood and DNAm levels at cg16907527 was explained by BMI (p=0.050). Although this association did not survive multiple-test correction, we note this locus is located in *VEGFA*, a gene linked to hyperglycemia and diabetes(53). Together, these finding suggest BMI was not likely to have substantial confounding effects on our findings.

C-reactive protein (CRP): Childhood adversity has been associated with alterations in inflammatory pathways (54), which, in turn, have been linked to genome-wide DNAm differences (55, 56). As such, we assessed the potential role of CRP levels, measured at age 15, as a mediator between childhood adversity and DNAm levels at age 15 (N=491-542). Again, we did not identify any significant mediation effects (**Fig. S23**). Two loci, located in *VEGFA* (cg16907527) and *SLC25A41 (*cg12096528), showed a causal mediation effect with $p<0.05$, suggesting that CRP levels may have slight effects on our associations. Again, these did not survive multiple-test correction for the analysis of 41 loci. Overall, these findings suggest that CRP may not have been an important confounding factor in our analyses.

Adolescent smoking: Smoking and exposure to cigarette smoke is one of the strongest and best-replicated associations with DNAm patterns(57). In addition, smoking in early adolescence may reflect increased risk-taking behaviors, which are linked to a higher likelihood of exposure to some types of childhood adversity(58). As such, we investigated daily smoking at age 15 (meaning whether the adolescent smoked every day or not) explained the relationship between childhood adversity and DNAm levels at age 15 (N=566-613). At age 15, adolescents were asked if they smoked every day during their clinic visit, reported as yes/no (adolescents who reported "not applicable" were coded as "no"). In the subsets of adolescents with childhood adversity, DNAm, and covariates, the prevalence of smoking at age 15 ranged from 3.9 to 4.3% (mean = 4.0%) across the adversities analyzed (prevalence varied due to the varying completeness of each type of adversity). Again, we did not observe any significant mediation effects of smoking on the association between childhood adversity and DNAm at age 15 (**Fig. S24**), suggesting that smoking may not have confounded our findings.

All taken together, these results suggest that our findings were not influenced by these four biological and environmental factors linked to childhood adversity and known to influence DNAm levels. Although we cannot rule out that other pathways may be involved in our adolescent-specific associations, these analyses provide additional support for the direct and latent effects of childhood adversity on the adolescent epigenome.

Types of DNAm trajectories across development for age 15 loci

To further refine the patterns of change and stability in DNAm responses to childhood adversity, we identified the different types of longitudinal DNAm trajectories present in the 41 R^2-threshold loci identified from the SCLMA of age 15 DNAm. We first performed a two-way mixed analysis of variance (ANOVA) of the statistical interaction between age at DNAm collection and exposure group, controlling for the timing of repeated measures of DNAm (i.e., age 0, 7, 15) of each individual as fixed effects. Based on this ANOVA, we split trajectory types based on the statistical significance of exposure group-by-age interactions, finding two sets of loci: 1) 7 loci that did not show any group-by-age interactions (i.e., stable cluster) and 2) 34 loci with significant group-by-age interactions (FDR<0.05).

Focusing on the second subset, we characterized the patterns of DNAm that could be used to distinguish between different types of DNAm trajectories across development. To this end, we applied a Tukey *post-hoc* test to identify the significant contrasts from the ANOVA of exposure group-by-age interactions for each locus, which included exposure group differences, mean age differences, and exposure group differences *within* and *between* each age. As we were interested in changes across time and age 15-specific patterns, we focused our analyses on a subset of these Tukey contrasts, which included:

1. *mean exposure group differences across all age* – meaning comparisons between individuals exposed during the period selected by the SLCMA (exposed-SP), individuals exposed outside the period selected by the SLCMA (exposed-other), and individuals with no exposure (unexposed);
    a. Exposed-SP versus Exposed-other
    b. Exposed-SP versus Unexposed
    c. Exposed-other versus Unexposed
2. *mean age differences across exposure groups for neighboring ages* – meaning mean differences between age 7 and 0, as well as mean differences between age 15 and 7;
    a. Age 7 versus Age 0
    b. Age 15 versus Age 7
3. *exposure group differences within each age* – meaning differences between exposure groups at age 0, age 7, or age 15.
    a. Age 0-specific differences
        i. Exposed-SP versus Exposed-other
        ii. Exposed-SP versus Unexposed
        iii. Exposed-other versus Unexposed
    b. Age 7- specific differences
        i. Exposed-SP versus Exposed-other
        ii. Exposed-SP versus Unexposed
        iii. Exposed-other versus Unexposed
    c. Age 15-specific differences
        i. Exposed-SP versus Exposed-other
        ii. Exposed-SP versus Unexposed
        iii. Exposed-other versus Unexposed

We recoded these contrasts as categorical variables to reflect whether the differences from the Tukey were significant (0 = p>0.05; 1 = p<0.05). We then performed divisive hierarchical clustering using a dissimilarity matrix for these categorical patterns (i.e., 0/1 based on significance) using the *cluster* package in R (59). We selected the number of distinct types of trajectories based on the inflection point of the sum of squares (lowest without meaningful decrease), with no more than one trajectory type with one CpG (**Fig. S25**). This step resulted in six distinct types of DNAm trajectories (**Fig. S26**), which showed distinct profiles of age, group, and group-by-age differences (**7**). Trajectories were plotted using cell-type corrected DNAm values and complete cases for covariates measured at birth (age 0: N = 559-616; age 7: N = 613-668; age 15: N= 609-665; sample sizes varied by adversity; **Fig. S28**).

For the seven loci without exposure group-by-age interactions, we identified slight differences between youths exposed during a sensitive period and those who were unexposed at age 7, which fully emerged by age 15 (i.e., stable).

Finally, we did not identify any differences in the enrichment of DNAm trajectories between loci in the FDR-significant and R^2-threshold subsets (χ^2=1.92, p =0.86; **Fig. S29**). These findings further emphasize that p-values do not show the whole picture, though additional differences may emerge when thresholds are relaxed further.

Investigating adversity-DNAm relationships within a threat and deprivation paradigm

To investigate potential differences between in sensitive period enrichment among our top loci in the context of threat versus deprivation-type exposures(60-62), we used the following definitions to classify our adversities into this established paradigm:

A. *Threat:* Threat exposures are defined as "experiences that represent a threat to one's physical integrity"(60). Based on this definition, exposures to 1) caregiver physical or emotional abuse, and/or 2) physical or sexual abuse (by anyone) were categorized as threat-type exposures.
B. *Deprivation:* Deprivation exposures are defined as the "absence of expected environmental inputs and complexity"(60). Based on this definition, exposures to 1) family instability, 2) financial hardship, 3) maternal psychopathology, 4) neighborhood disadvantage, and/or 5) one-adult households were categorized as deprivation-type exposures.

Following the classification of adversities into these paradigms, we investigated differential patterns of sensitive period enrichment for the 41 top loci identified at age 15 and 22 loci that passed an FDR<0.05 threshold (**Fig. S30**). Although there were differences in the number of adversities contributing to these two exposure paradigms, we observed more loci associated with a deprivation paradigm (34 loci) than a threat paradigm (7 loci). Furthermore, both exposure paradigms had more associations with exposure during early childhood than other exposure periods or models. However, loci associated with threat exposures were clustered mainly within early childhood, while loci associated with deprivation exposures were more distributed across time periods ($p = 0.32$). Together, these findings suggest that deprivation-type exposures during early childhood may have greater impacts on adolescent DNAm profiles, but these effects can be further refined by investigating specific types of childhood adversity.

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SUPPLEMENTAL TABLES

Table S1. Summary of the childhood adversity variables analyzed in the present study.

Adversity	Respondent	Questionnaire items	Exposure classification	Assessment timepoints (question range)
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.	Exposed: mother, the partner, or both, endorsed any of the items. Unexposed: any negative response and no positive response. Missing: all questions unanswered.	8 months (since birth) 1.75 years (since age 8 months) 2.75 years (since age 18 months) 4 years (since age 2.5) 5 years (in past year) 6 years (since age 5) 9 years (since age 8) 11 years (since age 9)
Sexual or physical abuse	Mother	1) an item asking if the child was exposed to either sexual or physical abuse from anyone.	Exposed: an affirmative response was provided to either item. Unexposed: any negative response was available and no positive response was provided. Missing: both questions unanswered.	1.5 years (since age 6 months) 2.5 years (since age 18 months) 3.5 years (in past year) 4.75 years (since age 3) 5.75 years (in past 15 months) 6.75 years (since age 5) 8 years (since age 7)
Maternal psychopathology	Mother	1) the Crown-Crisp Experiential Index (CCEI), assessing anxiety and depression, 2) the Edinburgh Postnatal Depression Scale (EPDS), 3) a question asking about suicide attempts	Exposed: one or more of the following criteria was met: 1) CCEI depression score > 9 2) CCEI anxiety score > 10 3) EPDS score > 12 4) a suicide attempt since the time of the last interview Unexposed: none of the above criteria above were met and none of the scores were missing. Missing: Any of the prorated scales or questions were missing.	8 months (11,2=current; 3=since birth) 1.75 years (1,2=current; 3=since age 8 months) 2.75 years (1,2=current; 3=since age 18 months) 5 years (1,2=current; 3=in past year) 6 years (1,2=current; 3=since age 5) 11 years(1,2=current; 3=since age 9)
One adult in the household	Mother	1) an item asking about the number of adults (>18 years of age) living in the household.	Exposed: fewer than two adults were residing in the household. Unexposed: two adults or more were residing in the household. Missing: question unanswered.	8 months (current) 1.75 years (current) 2.75 years (current) 4 years (current) 7 years (current) 8 years (current) 10 years (current)
Family instability	Mother	Child 1) taken into care, 2) separated from their mother for two or more weeks, 3) separated from their father for two or more weeks, 4) acquired a new parent.	Exposed: at least two of these events occurred at a single time point. Unexposed: none of the events occurred at a single time point and no questions were missing. Missing: any question was unanswered.	1.5 years (since age 6 months) 2.5 years (since age 18 months) 3.5 years (in past year) 4.75 years (since age 3) 5.75 years (in past 15 months) 6.75 years (since age 5) 8 years (since age 7)

Financial hardship	Mother	Family had difficulty affording the following items, coded on a Likert-type scale (1=not difficult; 2=slightly difficult; 3=fairly difficult; 4=very difficult): 1) items for the child, 2) rent or mortgage, 3) heating, 4) clothing, 5) food.	Exposed: mothers reported at least fair difficulty for three or more items at each time point. Unexposed: mothers reported on all five items, but the above criterion was not met. Missing: any question unanswered.	8 months (current) 1.75 years (current) 2.75 years (current) 5 years (current) 7 years (current) 11 years (current)
Neighborhood disadvantage	Mother	The following problems happened in the neighborhood (2=serious problem, 1=minor problem, 0=not a problem or no opinion): 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, 8) disturbance from youth.	Exposed: scores >=8 of the total sum of questions, corresponding to the 95th percentile of exposure. Unexposed: scores were <8 and no questions were missing. Missing: any question unanswered.	1.75 years (current) 2.75 years (current) 5 years (current) 7 years (current) 10 years (current)

Table S2. Distribution of the accumulation score for each type of adversity

Adversity	N	Mean	SD	Accumulation score (% of participants)								
				0	1	2	3	4	5	6	7	8
Caregiver physical or emotional abuse	661	0.37	1.01	81.7	9.2	5.1	1.2	0.5	1.7	0.5	-	0.2
Sexual or physical abuse (by anyone)	663	0.24	0.65	84.6	10.3	2.6	2.0	0.6	-	-	-	-
Family instability	649	0.24	0.66	83.5	11.9	2.5	1.2	0.8	0.2	-	-	-
Financial hardship	609	0.41	0.96	78.3	11.3	4.8	3.0	1.8	0.7	0.2	-	-
Maternal psychopathology	639	0.66	1.23	67.6	16.0	7.2	4.5	2.4	1.3	1.1	-	-
Neighborhood disadvantage	642	0.39	1.18	84.1	7.6	2.7	1.7	1.1	0.9	0.9	0.9	-
One adult in the household	665	0.27	0.81	86.2	7.2	3.0	1.7	1.1	0.9	-	-	-

Table S3. Distribution of covariates in the total ALSPAC sample, ARIES subsample, and among those exposed to any adversity between age 0-11.

	ALSPAC (N=14,885)	ARIES* (N=966)	Exposed to any adversity (N=647)	ALSPAC vs. ARIES	ALSPAC vs. Exposed	ARIES vs. Exposed
	N (%)	N (%)	N (%)			$\chi 2$ test p-value
Sex				0.068	0.11	0.99
Male	7535 (51.3)	466 (48.2)	311 (48.1)			
Female	7148 (48.7)	500 (51.8)	336 (51.9)			
Race/Ethnicity				0.007	0.38	0.28
White	11468 (95.0)	900 (97)	596 (95.8)			
Non-white	609 (5)	28 (3)	26 (4.2)			
Maternal education				<0.001	<0.001	0.49
less than O-level	3728 (30)	152 (16.1)	118 (18.6)			
O-level	4294 (34.6)	321 (34)	202 (31.8)			
A-level	2791 (22.5)	279 (29.5)	194 (30.6)			
Degree or above	1599 (12.9)	193 (20.4)	121 (19.1)			
Maternal age at birth				<0.001	<0.001	0.68
Ages 15-19	650 (4.7)	9 (0.9)	9 (1.4)			
Ages 20-35	12354 (88.4)	858 (89.4)	572 (88.7)			
Age 36+	968 (6.9)	93 (9.7)	64 (9.9)			
Smoking during pregnancy				<0.001	<0.001	0.19
Smoker	2557 (21.1)	98 (10.7)	80 (13.1)			
Non-smoker	9536 (78.9)	814 (89.3)	532 (86.9)			
Previous pregnancies				0.004	0.1	0.96
0	5770 (44.6)	439 (47.1)	295 (47.0)			
1	4539 (35)	346 (37.1)	229 (36.5)			
2	1848 (14.3)	113 (12.1)	77 (12.3)			
3+	767 (5.9)	34 (3.6)	26 (4.1)			
Birthweight				<0.001	<0.001	0.98
< 3000	3646 (24.8)	149 (15.4)	101 (15.6)			
3000 - 3499	4922 (33.5)	339 (35.1)	228 (35.2)			
3500 - 3999	4378 (29.8)	331 (34.3)	216 (33.4)			
>= 4000	1734 (11.8)	147 (15.2)	102 (15.8)			

*The ARIES subsample with DNA methylation data collected at age 15-17, without twins.
P-values, used to evaluate whether distributions differed across each sample comparison, were determined by chi-square tests. Maternal education values are presented from lowest level of education (less than O-level) to highest (degree or above). Differences between the total sample number and each variable are due to missing values (not shown in table).

Table S4. Prevalence and correlations between adversities occurring from age 0-11.

Adversity	Prevalence (% any exposure)[1]	Average within adversity correlation[2]	Average correlation with other adversities[3]
Caregiver physical or emotional abuse	18.1	0.562	0.137
Sexual or physical abuse (by anyone)	15.1	0.402	0.090
Family instability	24.4	0.597	0.153
Financial hardship	15.9	0.357	-0.035
Maternal psychopathology	34.8	0.611	0.161
Neighborhood disadvantage	16.1	0.741	0.112
One adult in the household	17.9	0.786	0.127

[1]Prevalence of any exposure to adversity between the ages of 0 and 11.
[2]Average tetrachoric correlation of exposure to adversity between different timepoints across development.
[3]Average tetrachoric correlation of exposure to different types of adversity across development.

Table S5. Annotated loci identified at age 15.

Adversity	Timing	Age (years)	CpG	Chr	Coordinate	Nearest Gene	Distance to gene	Relation to CGI	Enhancer	Promoter	pLI
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	4	102712397	BANK1	0	S_Shore	1	0	1.8E-10
			cg15454534	1	248569605	OR2T1	0	OpenSea	0	0	7.3E-07
			cg06215562	13	82344645			OpenSea	1	0	
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**11**	**59614212**	**CBLIF**	**1237**	**OpenSea**	**0**	**0**	
			cg15723468	**1**	**230387268**	**GALNT2**	**0**	**OpenSea**	**0**	**0**	**8.8E-01**
			cg17928317	**X**	**140982278**	**MAGEC3**	**0**	**OpenSea**	**0**	**0**	**3.2E-08**
	Late childhood	8	cg27558057	X	70712724	TAF1	0	Island	0	1	1.00
Family instability	Very early childhood	2.5	cg02735620	4	88950514	PKD2	0	OpenSea	1	0	1.00
Financial hardship	Very early childhood	0.66	cg14455319	11	113258908	ANKK1	0	S_Shore	1	0	2.5E-08
			cg13204236	2	47476732	STPG4	72991	OpenSea	1	0	
	Early childhood	5	cg15037420	19	48474386	BSPH1	0	OpenSea	0	0	
			cg06410970	10	81921424	ANXA11	0	OpenSea	1	0	3.5E-06
	Late childhood	11	cg02011706	16	891283	LMF1	12350	N_Shelf	0	0	1.1E-14
			cg04659536	7	4218154	SDK1	0	OpenSea	0	0	5.0E-03
	Recency		cg17670999	8	145928398	ARHGAP39	17203	S_Shelf	0	0	1.7E-03
			cg25459301	8	10941183	XKR6	0	OpenSea	1	0	9.6E-01
			cg06812747	16	742426	FBXL16	72	N_Shore	0	0	9.5E-01
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**10**	**103544649**	**OGA**	**0**	**S_Shore**	**0**	**0**	
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	4	1988825	NELFA	0	S_Shore	0	0	1.7E-01
			cg25019631	1	15850977	CASP9	0	N_Shore	0	1	3.2E-03
			cg04224851	2	43304158	ZFP36L2	145381	OpenSea	1	0	4.6E-01
One adult in the household	Very early childhood	1.75	**cg05491478**	**2**	**238621313**	**LRRFIP1**	**0**	**OpenSea**	**0**	**0**	**3.7E-01**
	Early childhood	3.9	**cg16907527**	**6**	**43744388**	**VEGFA**	**0**	**OpenSea**	**1**	**0**	
			cg08818094	**4**	**26806047**	**TBC1D19**	**49128**	**OpenSea**	**1**	**0**	**1.6E-02**
			cg01060989	**1**	**221945814**	**DUSP10**	**30297**	**OpenSea**	**1**	**0**	**5.0E-01**
			cg15814750	**15**	**53880678**	**WDR72**	**0**	**OpenSea**	**1**	**0**	**1.6E-16**
			cg15783822	**12**	**10999279**	**PRR4**	**0**	**OpenSea**	**0**	**0**	**1.0E-05**
			cg15864691	**7**	**27217606**	**HOXA10**	**0**	**N_Shore**	**0**	**0**	**6.8E-01**
			cg02584161	**6**	**156086665**			**OpenSea**	**1**	**0**	

		cg02810291	**15**	**85973746**	**AKAP13**	**0**	**OpenSea**	**1**	**0**	**8.5E-01**
		cg04036644	**8**	**1200583**	**LOC286083**	**43709**	**OpenSea**	**0**	**0**	
		cg11811897	**7**	**47811084**	**PKD1L1**	**3164**	**OpenSea**	**1**	**0**	**1.7E-23**
		cg15817130	**5**	**16742179**	**MYO10**	**0**	**OpenSea**	**0**	**0**	**4.0E-03**
		cg06711254	**2**	**186924071**	**FSIP2**	**226054**	**OpenSea**	**1**	**0**	**3.2E-08**
		cg19096460	**4**	**89490818**	**HERC3**	**22754**	**OpenSea**	**1**	**0**	**7.2E-01**
		cg18980650	X	100130547	NOX1	1212	OpenSea	0	0	3.9E-04
		cg27504269	12	21524305	SLCO1A2	0	OpenSea	0	0	5.6E-15
Late childhood	10	**cg12096528**	**19**	**6427642**	**SLC25A41**	**0**	**S_Shore**	**0**	**0**	**6.0E-05**
Accumulation		**cg00807464**	**12**	**111618977**	**CUX2**	**0**	**OpenSea**	**0**	**0**	**1.00**
		cg10420609	**6**	**7538349**	**DSP**	**3519**	**N_Shelf**	**0**	**0**	**1.00**
		cg14579651	**12**	**27429400**	**STK38L**	**0**	**OpenSea**	**1**	**0**	**9.7E-01**

* CGI = CpG Island; Chr = chromosome; pLI = probability of intolerance to loss of function (Exome Aggregation Consortium). Bolded loci passed a 5% FDR threshold of in the original analysis.

Table S6. Correlation of DNAm in brain and blood for age 15 loci.

Adversity	Timing	Age (years)	CpG	PFC		EC		STG		CER	
				r	p-value	r	p-value	r	p-value	r	p-value
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	0.213	6.78E-02	0.269	2.35E-02	0.444	6.61E-05	0.239	4.46E-02
			cg15454534	0.059	6.16E-01	0.072	5.51E-01	-0.033	7.81E-01	0.145	2.28E-01
			cg06215562	0.068	5.65E-01	0.014	9.07E-01	-0.023	8.46E-01	-0.067	5.80E-01
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**-0.097**	**4.09E-01**	**0.016**	**8.94E-01**	**0.067**	**5.67E-01**	**-0.029**	**8.11E-01**
			cg15723468	**0.035**	**7.69E-01**	**-0.106**	**3.81E-01**	**-0.024**	**8.38E-01**	**0.103**	**3.94E-01**
			cg17928317	**0.649**	**4.08E-10**	**0.6**	**3.10E-08**	**0.61**	**6.23E-09**	**0.538**	**1.34E-06**
	Late childhood	8	cg27558057	0.95	4.40E-38	0.947	9.22E-36	0.914	2.91E-30	0.882	2.80E-24
Family instability	Very early childhood	2.5	cg02735620	-0.061	6.03E-01	-0.027	8.24E-01	0.119	3.09E-01	-0.071	5.56E-01
Financial hardship	Very early childhood	0.66	cg14455319	0.318	5.71E-03	0.246	3.88E-02	0.406	3.04E-04	0.074	5.41E-01
			cg13204236	-0.025	8.30E-01	0.091	4.48E-01	0.001	9.92E-01	-0.101	4.00E-01
	Early childhood	5	cg15037420	0.065	5.81E-01	-0.002	9.88E-01	-0.069	5.58E-01	0.057	6.36E-01
			cg06410970	-0.083	4.80E-01	-0.003	9.78E-01	0.112	3.37E-01	-0.024	8.43E-01
	Late childhood	11	cg02011706	0.062	5.99E-01	0.141	2.42E-01	0.084	4.72E-01	0.207	8.35E-02
			cg04659536	0.952	8.82E-39	0.953	1.17E-37	0.935	1.41E-34	0.968	4.84E-43
	Recency		cg17670999	-0.039	7.42E-01	0.089	4.60E-01	0.139	2.36E-01	-0.199	9.87E-02
			cg25459301	0.39	5.83E-04	0.228	5.62E-02	0.059	6.14E-01	0.211	7.78E-02
			cg06812747	0.075	5.26E-01	-0.107	3.74E-01	-0.037	7.53E-01	-0.158	1.89E-01
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**0.02**	**8.69E-01**	**-0.035**	**7.72E-01**	**0.019**	**8.74E-01**	**0.173**	**1.50E-01**
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	-0.137	2.44E-01	-0.007	9.52E-01	-0.213	6.67E-02	-0.192	1.09E-01
			cg25019631	-0.043	7.17E-01	0.017	8.88E-01	-0.037	7.50E-01	-0.047	6.96E-01
			cg04224851	0.201	8.62E-02	0.092	4.46E-01	-0.147	2.09E-01	0.05	6.81E-01
One adult in the household	Very early childhood	1.75	**cg05491478**	**-0.085**	**4.70E-01**	**0.112**	**3.51E-01**	**-0.058**	**6.22E-01**	**0.057**	**6.36E-01**
	Early childhood	3.9	**cg16907527**	**0.066**	**5.74E-01**	**-0.008**	**9.48E-01**	**-0.062**	**6.00E-01**	**0.051**	**6.74E-01**
			cg08818094	**0.088**	**4.54E-01**	**0.041**	**7.35E-01**	**0.151**	**1.97E-01**	**-0.086**	**4.77E-01**
			cg01060989	**0.034**	**7.75E-01**	**-0.038**	**7.55E-01**	**0.076**	**5.15E-01**	**-0.033**	**7.86E-01**
			cg15814750	**-0.02**	**8.63E-01**	**-0.241**	**4.27E-02**	**0.004**	**7.21E-01**	**-0.029**	**8.12E-01**
			cg15783822	**0.041**	**7.31E-01**	**-0.005**	**9.70E-01**	**0.151**	**1.96E-01**	**0.027**	**8.26E-01**
			cg15864691	**0.034**	**7.72E-01**	**0.085**	**4.80E-01**	**-0.074**	**5.29E-01**	**-0.138**	**2.51E-01**
			cg02584161	**0.166**	**1.58E-01**	**-0.024**	**8.45E-01**	**0.115**	**3.25E-01**	**0.065**	**5.89E-01**

		cg02810291	**-0.185**	**1.15E-01**	**0.187**	**1.18E-01**	**0.134**	**2.50E-01**	**0.058**	**6.32E-01**
		cg04036644	**-0.081**	**4.92E-01**	**0.353**	**2.53E-03**	**0.14**	**2.33E-01**	**0.069**	**5.66E-01**
		cg11811897	**0.054**	**6.46E-01**	**-0.034**	**7.76E-01**	**0.031**	**7.90E-01**	**0.106**	**3.79E-01**
		cg15817130	**0.09**	**4.48E-01**	**0.064**	**5.95E-01**	**-0.036**	**7.57E-01**	**0.167**	**1.63E-01**
		cg06711254	**0.081**	**4.95E-01**	**0.058**	**6.30E-01**	**-0.133**	**2.54E-01**	**0.152**	**2.07E-01**
		cg19096460	**0.044**	**7.13E-01**	**0.139**	**1.39E-01**	**-0.036**	**7.61E-01**	**0.002**	**9.88E-01**
		cg18980650	0.375	9.81E-04	0.352	2.62E-03	0.177	1.28E-01	0.255	3.15E-02
		cg27504269	-0.001	9.96E-01	-0.066	5.82E-01	-0.072	5.42E-01	0.072	5.51E-01
Late childhood	10	**cg12096528**	**0.135**	**2.50E-01**	**0.1**	**4.06E-01**	**-0.181**	**1.21E-01**	**-0.118**	**3.26E-01**
Accumulation		**cg00807464**	**-0.07**	**5.52E-01**	**0.27**	**2.25E-02**	**0.008**	**9.43E-01**	**0.093**	**4.43E-01**
		cg10420609	**0.064**	**5.89E-01**	**-0.095**	**4.29E-01**	**0.039**	**7.41E-01**	**-0.023**	**8.50E-01**
		cg14579651	**0.032**	**7.84E-01**	**-0.117**	**3.29E-01**	**0.097**	**4.09E-01**	**0.043**	**7.24E-01**

PFC = prefrontal cortex; EC = entorhinal cortex; STG = superior temporal gyrus; CER = cerebellum. Values represent the correlation between DNA methylation levels in blood and the specified brain regions, as reported by Hannon et al., 2015. Bolded loci passed a 5% FDR in the original analysis.

Table S7. Associations between childhood adversity and age 15 DNAm using non-parametric bootstrap

Adversity	Timing	Age (years)	CpG	Original effect estimate[1]	Bootstrap effect estimate[2]	Bootstrap bias[3]	% difference[4]
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	3.01E-02	3.02E-02	6.81E-05	-0.23%
			cg15454534	-1.64E-02	-1.64E-02	3.21E-06	0.02%
			cg06215562	-2.11E-02	-2.10E-02	2.27E-05	0.11%
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**-5.47E-02**	**-5.45E-02**	**1.90E-04**	**0.35%**
			cg15723468	**-4.52E-02**	**-4.51E-02**	**1.10E-04**	**0.24%**
			cg17928317	**7.56E-02**	**7.54E-02**	**-2.07E-04**	**0.27%**
	Late childhood	8	cg27558057	1.07E-01	1.05E-01	-1.86E-03	1.74%
Family instability	Very early childhood	2.5	cg02735620	-1.97E-02	-1.97E-02	-2.86E-05	-0.14%
Financial hardship	Very early childhood	0.66	cg14455319	5.29E-02	5.26E-02	-3.25E-04	0.61%
			cg13204236	-3.73E-02	-3.73E-02	-1.96E-05	-0.05%
	Early childhood	5	cg15037420	-3.50E-02	-3.50E-02	6.23E-05	0.18%
			cg06410970	-3.41E-02	-3.41E-02	-2.45E-05	-0.07%
	Late childhood	11	cg02011706	-6.39E-02	-6.44E-02	-4.63E-04	-0.72%
			cg04659536	-2.78E-02	-2.78E-02	3.09E-06	0.01%
	Recency		cg17670999	-2.10E-03	-2.06E-03	4.27E-05	2.03%
			cg25459301	-2.81E-03	-2.76E-03	5.18E-05	1.84%
			cg06812747	-2.75E-03	-2.75E-03	3.38E-06	0.12%
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**-1.52E-02**	**-1.52E-02**	**6.59E-06**	**0.04%**
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	-2.06E-02	-2.07E-02	-5.12E-05	-0.25%
			cg25019631	4.43E-02	4.44E-02	1.29E-04	-0.29%
			cg04224851	-1.43E-02	-1.43E-02	-2.02E-05	-0.14%
One adult in the household	Very early childhood	1.75	**cg05491478**	**-2.76E-02**	**-2.75E-02**	**5.17E-05**	**0.19%**
	Early childhood	3.9	**cg16907527**	**-3.16E-02**	**-3.17E-02**	**-1.40E-04**	**-0.44%**
			cg08818094	**-5.03E-02**	**-5.02E-02**	**4.75E-05**	**0.09%**
			cg01060989	**-3.15E-02**	**-3.15E-02**	**-3.71E-05**	**-0.12%**
			cg15814750	**-4.14E-02**	**-4.13E-02**	**8.80E-05**	**0.21%**
			cg15783822	**-2.21E-02**	**-2.21E-02**	**3.63E-05**	**0.16%**
			cg15864691	**-1.81E-02**	**-1.80E-02**	**5.27E-05**	**0.29%**
			cg02584161	**-5.93E-02**	**-5.93E-02**	**-4.89E-05**	**-0.08%**
			cg02810291	**-2.34E-02**	**-2.33E-02**	**7.06E-05**	**0.30%**

		cg04036644	**-2.62E-02**	**-2.61E-02**	**6.66E-05**	**0.25%**
		cg11811897	**-4.83E-02**	**-4.81E-02**	**2.23E-04**	**0.46%**
		cg15817130	**-3.81E-02**	**-3.81E-02**	**-3.42E-06**	**-0.01%**
		cg06711254	**-5.80E-02**	**-5.80E-02**	**4.05E-05**	**0.07%**
		cg19096460	**-2.49E-02**	**-2.50E-02**	**-8.09E-05**	**-0.32%**
		cg18980650	-3.68E-02	-3.69E-02	-1.77E-04	-0.48%
		cg27504269	-4.06E-02	-4.05E-02	1.55E-04	0.38%
Late childhood	10	**cg12096528**	**-1.66E-02**	**-1.65E-02**	**5.63E-05**	**0.34%**
Accumulation		**cg00807464**	**3.21E-03**	**3.18E-03**	**-3.37E-05**	**1.05%**
		cg10420609	**-1.45E-02**	**-1.45E-02**	**1.36E-05**	**0.09%**
		cg14579651	**-1.29E-02**	**-1.28E-02**	**5.16E-05**	**0.40%**

1 Effect estimate from the original linear regression of childhood adversity and DNAm at age 15 in the full ALSPAC sample.
2 Average of effect estimates from the 10,000 bootstrapped analyses of childhood adversity and DNAm at age 15.
3 Difference in effect estimates between the bootstrapped and original sample.
4 Percent change in absolute effect estimate between the original and bootstrapped analyses.
*Bolded loci passed a 5% FDR threshold in the original analysis.

Table S8. Replication of one-adult household associations in the Raine Study.

		ALSPAC (discovery results)				ALSPAC (Winner's curse corrected)[§]			The Raine Study			
CpG	**Timing**	**Age (years)**	**Effect estimate**	**95% CI**	**P-value**	**Effect estimate**	**95% CI**	**P-value**	**Age (years)**	**Effect estimate**	**95% CI**	**P-value**
cg05491478	Very early childhood	1.75	-0.027	-0.18; -0.09	7.33E-07	-0.022	-0.036; -0.003	2.08E-06	2y (N=448)	-0.0011	-0.004; 0.0018	4.57E-01
cg16907527	Early childhood	3.9	-0.032	-0.23; -0.138	4.17E-10	-0.031	-0.041; -0.021	5.26E-11	3y (N=510)	-0.0026	-0.0086; 0.0034	4.00E-01
cg08818094			-0.05	-0.37; -0.212	8.79E-09	-0.049	-0.066; -0.028	3.49E-09		-0.0031	-0.008; 0.0018	2.08E-01
cg01060989			-0.031	-0.24; -0.135	6.73E-08	-0.030	-0.041; -0.016	1.19E-08		-0.0005	-0.0046; 0.0036	8.12E-01
cg15814750			-0.04	-0.33; -0.166	6.57E-07	-0.031	-0.053; -0.003	3.87E-06		0.0031	-0.0043; 0.0104	4.09E-01
cg15783822			**-0.021**	**-0.17; -0.088**	**8.08E-07**	**-0.018**	**-0.029; -0.003**	**1.47E-06**		**-0.0038**	**-0.0071; -0.0004**	**2.91E-02**
cg15864691			-0.018	-0.14; -0.071	8.36E-07	-0.016	-0.024; -0.004	4.80E-07		-0.0021	-0.0053; 0.0011	2.06E-01
cg02584161			**-0.058**	**-0.45; -0.236**	**1.28E-06**	**-0.053**	**-0.078; -0.016**	**2.85E-07**		**-0.0072**	**-0.0142; -0.0002**	**4.46E-02**
cg02810291			-0.023	-0.18; -0.092	1.35E-06	-0.020	-0.031; -0.004	9.18E-07		-0.0014	-0.0114; 0.0085	7.79E-01
cg04036644			-0.026	-0.21; -0.105	1.36E-06	-0.023	-0.035; -0.005	8.27E-07		-0.0024	-0.0065; 0.0016	2.37E-01
cg11811897			**-0.047**	**-0.37; -0.191**	**1.68E-06**	**-0.041**	**-0.064; -0.008**	**8.98E-07**		**-0.0074**	**-0.0126; -0.0021**	**6.39E-03**
cg15817130			-0.038	-0.29; -0.155	1.83E-06	-0.036	-0.05; -0.017	3.49E-08		-0.0057	-0.0133; 0.0019	1.41E-01
cg06711254			-0.056	-0.45; -0.227	2.15E-06	-0.047	-0.075; -0.007	1.57E-06		-0.0036	-0.0124; 0.0052	4.22E-01
cg19096460			-0.024	-0.2; -0.099	2.89E-06	-0.019	-0.032; -0.002	3.82E-06		-0.0015	-0.0066; 0.0036	5.62E-01
cg18980650			-0.036	-0.26; -0.131	3.31E-06	-0.034	-0.049; -0.014	8.07E-08		-0.0035	-0.0114; 0.0043	3.78E-01
cg27504269			-0.04	-0.31; -0.161	3.52E-06	-0.036	-0.053; -0.011	3.21E-07		-0.0041	-0.0107; 0.0025	2.25E-01
cg12096528	Late childhood	10	-0.016	-0.15; -0.076	2.24E-06	-0.014	-0.022; -0.002	1.19E-06	10y (N=529)	0.0003	-0.0034; 0.004	8.72E-01
cg00807464	Accumulation		0.003	0.07; 0.12	7.56E-07	0.003	0.001; 0.004	6.88E-08	Accumulation	0.0004	-0.0008; 0.0017	4.97E-01
cg10420609			-0.014	-0.53; -0.278	7.71E-07	-0.012	-0.018; -0.004	3.46E-07	(N=381)	-0.0004	-0.0025; 0.0016	6.75E-01
cg14579651			-0.012	-0.49; -0.257	1.68E-06	-0.010	-0.016; -0.002	1.40E-06		-0.0015	-0.0036; 0.0005	1.46E-01

*Bolded CpGs passed a nominal p<0.05 in the Raine Study with 95% confidence intervals (CI) that did not overlap with zero.

[§] Estimates and confidence intervals corrected for winner's curse effects.

Table S9. Replication of childhood adversity associations in the FFCWS cohort.

			ALSPAC (discovery results)				ALSPAC (Winner's curse corrected) [§]			FFCWS				
Adversity	**CpG**	**Timing**	**Age (years)**	**Effect estimate**	**95% CI**	**P-value**	**Effect estimate**	**95% CI**	**P-value**	**Age (years)**	**N***	**Effect estimate**	**95% CI**	**P-value**
Caregiver physical or emotional abuse	cg14855874	Early childhood	5	0.030	0.02; 0.041	4.42E-08	0.029	0.013; 0.041	4.42E-08	5	1527	-0.0005	-0.006; 0.005	8.67E-01
	cg15454534			-0.017	-0.023; -0.01	1.71E-07	-0.015	-0.022; -0.005	1.71E-07		662	0.0014	-0.002; 0.005	3.83E-01
	cg06215562			-0.021	-0.029; -0.013	4.46E-07	-0.019	-0.029; -0.005	4.46E-07		1527	-0.00004	-0.003; 0.002	9.76E-01
Financial hardship	cg14455319	Very early childhood	0.66	0.052	0.031; 0.074	1.94E-06	0.043	0.006; 0.07	1.94E-06	1	1859	-0.0015	-0.009; 0.006	7.17E-01
	cg13204236			-0.037	-0.051; -0.023	2.04E-07	-0.034	-0.05; -0.012	2.04E-07		1859	-0.0029	-0.007; 0.001	1.42E-01
	cg15037420	Early childhood	5	-0.034	-0.048; -0.02	1.89E-06	-0.028	-0.046; -0.004	1.89E-06	5	1845	-0.0024	-0.006; 0.001	1.31E-01
	cg06410970			-0.033	-0.046; -0.021	1.80E-07	-0.031	-0.045; -0.011	1.80E-07		1845	-0.00064	-0.002; 0.001	3.59E-01
	cg02011706	Late childhood	11	-0.064	-0.089; -0.038	9.99E-07	-0.055	-0.085; -0.011	9.99E-07	9	1859	-0.0041	-0.011; 0.003	2.64E-01
	cg04659536			-0.028	-0.039; -0.016	1.70E-06	-0.023	-0.037; -0.004	1.70E-06		1859	-0.0014	-0.004; 0.001	2.90E-01
	cg17670999	Recency		-0.0020	-0.003; -0.001	1.03E-06	-0.0017	-0.003; -0.0003	1.03E-06		722	0.00003	-0.0001; 0.0002	7.32E-01
	cg25459301			-0.0027	-0.004; -0.002	5.54E-06	-0.0020	-0.004; -0.0002	5.54E-06		1661	-0.00012	-0.0004; 0.0001	3.74E-01
	cg06812747			-0.0027	-0.004; -0.002	2.81E-06	-0.0021	-0.004; -0.0003	2.81E-06		1661	0.00020	-0.0001; 0.0005	1.46E-01
Maternal psychopathology	cg16813552	Very early childhood	2.75	-0.015	-0.021; -0.01	5.06E-08	-0.014	-0.02; -0.006	5.06E-08	1	1846	0.0015	-0.001; 0.004	2.69E-01
One-adult household	cg05491478	Very early childhood	1.75	-0.027	-0.038; -0.016	2.08E-06	-0.022	-0.036; -0.003	2.08E-06	1	1842	-0.0007	-0.002; 0.0001	7.26E-02
	cg16907527	Early childhood	3.9	-0.032	-0.041; -0.022	5.26E-11	-0.031	-0.041; -0.021	5.26E-11	3	799	-0.0010	-0.004; 0.002	5.10E-01
	cg08818094			-0.050	-0.067; -0.034	3.49E-09	-0.049	-0.066; -0.028	3.49E-09		1842	-0.0004	-0.002; 0.001	5.58E-01
	cg01060989			-0.031	-0.041; -0.02	1.19E-08	-0.030	-0.041; -0.016	1.19E-08		799	-0.0003	-0.003; 0.002	8.36E-01
	cg15783822			-0.021	-0.03; -0.013	1.47E-06	-0.018	-0.029; -0.003	1.47E-06		1842	0.00000	-0.002; 0.002	9.99E-01
	cg15864691			-0.018	-0.025; -0.011	4.80E-07	-0.016	-0.024; -0.004	4.80E-07		1842	0.0005	-0.003; 0.004	7.99E-01
	cg02810291			-0.023	-0.032; -0.014	9.18E-07	-0.020	-0.031; -0.004	9.18E-07		1842	-0.0016	-0.005; 0.002	4.04E-01

cg04036644		-0.026	-0.037; -0.016	8.27E-07	-0.023	-0.035; -0.005	8.27E-07	1842	-0.0006	-0.003; 0.002	6.10E-01
cg11811897		-0.047	-0.066; -0.029	8.98E-07	-0.041	-0.064; -0.008	8.98E-07	1842	0.0004	-0.003; 0.004	8.20E-01
cg15817130		-0.038	-0.051; -0.024	3.49E-08	-0.036	-0.05; -0.017	3.49E-08	1842	0.0008	-0.002; 0.004	5.42E-01
cg06711254		-0.056	-0.079; -0.034	1.57E-06	-0.047	-0.075; -0.007	1.57E-06	1842	-0.0068	-0.017; 0.003	1.89E-01
cg19096460		-0.024	-0.034; -0.014	3.82E-06	-0.019	-0.032; -0.002	3.82E-06	1842	-0.00005	-0.002; 0.002	9.67E-01
cg18980650		-0.036	-0.049; -0.023	8.07E-08	-0.034	-0.049; -0.014	8.07E-08	799	0.0023	-0.002; 0.007	3.33E-01
cg27504269		-0.040	-0.055; -0.025	3.21E-07	-0.036	-0.053; -0.011	3.21E-07	1842	-0.0046	-0.018; 0.008	4.84E-01
cg00807464	Accumulation	0.0031	0.002; 0.004	6.88E-08	0.0029	0.001; 0.004	6.88E-08	1659	0.0032	-0.002; 0.009	2.45E-01

*CpGs with lower N (<800) were measured on the 450K array only, resulting in a smaller sample size.
§ Estimates and confidence intervals corrected for winner's curse effects.

Table S10. Sensitivity analysis of DNA methylation at birth (cord blood) for loci identified at age 15.

Adversity	Timing	Age (years)	CpG	DNAm unexposed[1]	DNAm exp. SP[2]	Δ DNAm[3]	Effect estimate[4]	SE*	95% CI	P-value	FDR-adjusted p-value
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	0.099	0.112	0.013	0.014	0.007	-0.0004; 0.028	5.60E-02	3.95E-01
			cg15454534	0.866	0.864	-0.003	-0.003	0.005	-0.0124; 0.0072	6.06E-01	8.78E-01
			cg06215562	0.830	0.825	-0.005	-0.005	0.005	-0.0154; 0.0055	3.52E-01	7.10E-01
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**0.890**	**0.901**	**0.011**	**0.012**	**0.013**	**-0.0141; 0.0384**	**3.65E-01**	**7.10E-01**
			cg15723468	**0.849**	**0.835**	**-0.014**	**-0.015**	**0.008**	**-0.03; 0.0005**	**5.80E-02**	**3.95E-01**
			cg17928317	**0.690**	**0.721**	**0.032**	**-0.019**	**0.020**	**-0.0575; 0.0203**	**3.49E-01**	**7.10E-01**
	Late childhood	8	cg27558057	0.242	0.231	-0.012	0.076	0.024	0.0276; 0.1238	2.09E-03	8.56E-02
Family instability	Very early childhood	2.5	cg02735620	0.880	0.881	0.001	0.000	0.005	-0.0093; 0.0092	9.86E-01	9.86E-01
Financial hardship	Very early childhood	0.66	cg14455319	0.254	0.281	0.027	0.028	0.012	0.0055; 0.0513	1.54E-02	3.15E-01
			cg13204236	0.858	0.866	0.007	0.008	0.007	-0.0066; 0.0224	2.83E-01	7.10E-01
	Early childhood	5	cg15037420	0.774	0.763	-0.012	-0.012	0.008	-0.028; 0.0039	1.39E-01	5.39E-01
			cg06410970	0.843	0.857	0.015	0.015	0.009	-0.0024; 0.0319	9.08E-02	4.65E-01
	Late childhood	11	cg02011706	0.837	0.822	-0.014	-0.016	0.019	-0.053; 0.0211	3.99E-01	7.11E-01
			cg04659536	0.898	0.892	-0.005	-0.007	0.007	-0.0204; 0.0073	3.53E-01	7.10E-01
	Recency		cg17670999	0.807	0.807	0.000	0.000	0.000	-0.001; 0.0006	6.21E-01	8.78E-01
			cg25459301	0.757	0.765	0.009	0.001	0.001	-0.0003; 0.0023	1.27E-01	5.39E-01
			cg06812747	0.819	0.817	-0.003	-0.001	0.001	-0.002; 0.0006	3.01E-01	7.10E-01
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**0.899**	**0.896**	**-0.003**	**-0.004**	**0.003**	**-0.0088; 0.0017**	**1.83E-01**	**6.25E-01**
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	0.912	0.921	0.010	0.002	0.005	-0.008; 0.0115	7.23E-01	8.84E-01
			cg25019631	0.227	0.228	0.001	0.004	0.011	-0.018; 0.0258	7.28E-01	8.84E-01
			cg04224851	0.905	0.903	-0.002	-0.001	0.003	-0.0071; 0.0052	7.58E-01	8.88E-01
One adult in the household	Very early childhood	1.75	**cg05491478**	**0.900**	**0.903**	**0.003**	**0.002**	**0.008**	**-0.0131; 0.0167**	**8.16E-01**	**9.24E-01**
	Early childhood	3.9	**cg16907527**	**0.840**	**0.848**	**0.008**	**0.006**	**0.006**	**-0.0066; 0.0179**	**3.68E-01**	**7.10E-01**
			cg08818094	**0.832**	**0.834**	**0.001**	**-0.001**	**0.011**	**-0.0221; 0.0208**	**9.50E-01**	**9.86E-01**
			cg01060989	**0.809**	**0.814**	**0.005**	**0.005**	**0.007**	**-0.0083; 0.0183**	**4.64E-01**	**7.61E-01**
			cg15814750	**0.738**	**0.755**	**0.018**	**0.016**	**0.008**	**0.0006; 0.0324**	**4.25E-02**	**3.95E-01**
			cg15783822	**0.859**	**0.858**	**-0.001**	**0.001**	**0.005**	**-0.0098; 0.0114**	**8.77E-01**	**9.46E-01**
			cg15864691	**0.899**	**0.903**	**0.004**	**0.004**	**0.005**	**-0.0053; 0.0138**	**3.81E-01**	**7.10E-01**

		cg02584161	**0.650**	**0.654**	**0.004**	**0.003**	**0.014**	**-0.024; 0.0297**	**8.34E-01**	**9.24E-01**
		cg02810291	**0.849**	**0.858**	**0.009**	**0.010**	**0.005**	**0.0008; 0.0195**	**3.38E-02**	**3.95E-01**
		cg04036644	**0.889**	**0.889**	**0.001**	**-0.002**	**0.006**	**-0.0137; 0.0096**	**7.31E-01**	**8.84E-01**
		cg11811897	**0.737**	**0.728**	**-0.010**	**-0.011**	**0.011**	**-0.0329; 0.0106**	**3.14E-01**	**7.10E-01**
		cg15817130	**0.787**	**0.782**	**-0.004**	**-0.006**	**0.007**	**-0.0207; 0.0087**	**4.23E-01**	**7.22E-01**
		cg06711254	**0.711**	**0.698**	**-0.013**	**-0.015**	**0.010**	**-0.0352; 0.0052**	**1.45E-01**	**5.39E-01**
		cg19096460	**0.843**	**0.841**	**-0.003**	**-0.003**	**0.006**	**-0.0146; 0.0087**	**6.16E-01**	**8.78E-01**
		cg18980650	0.795	0.791	-0.004	0.003	0.008	-0.0121; 0.0175	7.21E-01	8.84E-01
		cg27504269	0.748	0.752	0.004	0.003	0.008	-0.0133; 0.0189	7.33E-01	8.84E-01
Late childhood	10	**cg12096528**	**0.877**	**0.886**	**0.009**	**0.009**	**0.005**	**-0.0007; 0.0189**	**6.74E-02**	**3.95E-01**
Accumulation		**cg00807464**	**0.052**	**0.052**	**0.001**	**0.000**	**0.001**	**-0.0013; 0.0014**	**9.86E-01**	**9.86E-01**
		cg10420609	**0.555**	**0.559**	**0.004**	**0.001**	**0.002**	**-0.0035; 0.0063**	**5.81E-01**	**8.78E-01**
		cg14579651	**0.615**	**0.611**	**-0.004**	**-0.002**	**0.002**	**-0.0066; 0.0019**	**2.85E-01**	**7.10E-01**

[1]DNAm unexp. = mean DNA methylation levels in individuals with no exposure to adversity from age 0 to 11.
[2]DNAm exp. SP = mean DNA methylation levels in individuals with exposure to adversity that occurred during the selected sensitive period (SP).
[3]ΔDNAm= difference in mean DNA methylation levels between individuals exposed to adversity during the selected sensitive period and individuals unexposed to adversity (i.e., DNAm exp. SP – DNAm unexp.)
[4]Effect estimates were calculated using linear regression of exposure to adversity from the theoretical model and DNA methylation, correcting for the covariates described in the methods.
* SE = standard error; bolded loci passed a 5% FDR threshold in the original age 15 analysis.

Table S11. Associations between adversity and DNA methylation at age 7 (whole blood) for loci identified at age 15.

Adversity	Timing	Age (years)	CpG	DNAm unexposed[1]	DNAm exp. SP[2]	Δ DNAm[3]	Effect estimate[4]	SE*	95% CI	P-value	FDR-adjusted p-value
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	0.089	0.102	0.013	0.012	0.006	0.0011; 0.0228	3.06E-02	2.51E-01
			cg15454534	0.888	0.889	0.001	0.001	0.003	-0.0043; 0.0069	6.51E-01	9.60E-01
			cg06215562	0.839	0.843	0.004	0.004	0.005	-0.006; 0.0132	4.58E-01	9.60E-01
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**0.902**	**0.887**	**-0.015**	**-0.015**	**0.010**	**-0.0336; 0.0042**	**1.27E-01**	**6.51E-01**
			cg15723468	**0.799**	**0.807**	**0.008**	**0.006**	**0.009**	**-0.0108; 0.0237**	**4.63E-01**	**9.60E-01**
			cg17928317	**0.695**	**0.726**	**0.031**	**-0.002**	**0.016**	**-0.0326; 0.0285**	**8.97E-01**	**9.60E-01**
	Late childhood	8	cg27558057	0.248	0.224	-0.024	0.068	0.021	0.0257; 0.1097	1.63E-03	6.67E-02
Family instability	Very early childhood	2.5	cg02735620	0.877	0.880	0.002	0.003	0.004	-0.0047; 0.0102	4.72E-01	9.60E-01
Financial hardship	Very early childhood	0.66	cg14455319	0.266	0.288	0.021	0.022	0.009	0.0045; 0.0403	1.43E-02	2.27E-01
			cg13204236	0.867	0.868	0.001	0.002	0.006	-0.0103; 0.0143	7.44E-01	9.60E-01
	Early childhood	5	cg15037420	0.795	0.792	-0.003	-0.003	0.007	-0.0157; 0.0106	7.06E-01	9.60E-01
			cg06410970	0.870	0.868	-0.003	-0.002	0.006	-0.0134; 0.0089	6.89E-01	9.60E-01
	Late childhood	11	cg02011706	0.860	0.863	0.003	0.006	0.012	-0.018; 0.0308	6.05E-01	9.60E-01
			cg04659536	0.906	0.905	-0.001	-0.002	0.005	-0.0106; 0.0075	7.38E-01	9.60E-01
	Recency		cg17670999	0.836	0.836	0.000	0.000	0.000	-0.0004; 0.0009	4.15E-01	9.60E-01
			cg25459301	0.791	0.788	-0.002	0.000	0.000	-0.0011; 0.0007	6.66E-01	9.60E-01
			cg06812747	0.847	0.843	-0.004	0.000	0.000	-0.0009; 0.0008	8.49E-01	9.60E-01
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**0.890**	**0.882**	**-0.008**	**-0.007**	**0.003**	**-0.0134; -0.0009**	**2.47E-02**	**2.51E-01**
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	0.932	0.935	0.003	0.006	0.003	-0.0006; 0.0121	7.41E-02	5.06E-01
			cg25019631	0.194	0.173	-0.021	-0.013	0.009	-0.0296; 0.0044	1.46E-01	6.66E-01
			cg04224851	0.903	0.915	0.012	0.006	0.003	0.0011; 0.0114	1.66E-02	2.27E-01
One adult in the household	Very early childhood	1.75	**cg05491478**	**0.915**	**0.920**	**0.006**	**0.005**	**0.005**	**-0.0046; 0.0151**	**2.94E-01**	**9.60E-01**
	Early childhood	3.9	**cg16907527**	**0.844**	**0.845**	**0.001**	**0.001**	**0.005**	**-0.0083; 0.011**	**7.86E-01**	**9.60E-01**
			cg08818094	**0.858**	**0.851**	**-0.007**	**-0.005**	**0.007**	**-0.0191; 0.0088**	**4.68E-01**	**9.60E-01**
			cg01060989	**0.834**	**0.835**	**0.001**	**0.001**	**0.006**	**-0.0105; 0.0118**	**9.13E-01**	**9.60E-01**
			cg15814750	**0.752**	**0.747**	**-0.006**	**-0.005**	**0.008**	**-0.0207; 0.0098**	**4.81E-01**	**9.60E-01**
			cg15783822	**0.878**	**0.880**	**0.002**	**0.002**	**0.004**	**-0.0054; 0.0101**	**5.46E-01**	**9.60E-01**
			cg15864691	**0.911**	**0.913**	**0.002**	**0.002**	**0.003**	**-0.0035; 0.0085**	**4.11E-01**	**9.60E-01**

		cg02584161	**0.688**	**0.690**	**0.002**	**0.000**	**0.013**	**-0.025; 0.0254**	**9.88E-01**	**9.88E-01**
		cg02810291	**0.833**	**0.836**	**0.003**	**0.003**	**0.005**	**-0.0071; 0.0131**	**5.66E-01**	**9.60E-01**
		cg04036644	**0.903**	**0.903**	**0.000**	**-0.001**	**0.005**	**-0.0096; 0.0085**	**8.99E-01**	**9.60E-01**
		cg11811897	**0.778**	**0.772**	**-0.006**	**-0.008**	**0.009**	**-0.0256; 0.0089**	**3.43E-01**	**9.60E-01**
		cg15817130	**0.822**	**0.824**	**0.002**	**0.000**	**0.006**	**-0.0123; 0.013**	**9.57E-01**	**9.80E-01**
		cg06711254	**0.713**	**0.704**	**-0.008**	**-0.009**	**0.011**	**-0.0316; 0.0134**	**4.27E-01**	**9.60E-01**
		cg19096460	**0.853**	**0.850**	**-0.003**	**-0.002**	**0.005**	**-0.0112; 0.0065**	**6.05E-01**	**9.60E-01**
		cg18980650	0.795	0.788	-0.007	-0.002	0.007	-0.0154; 0.0113	7.62E-01	9.60E-01
		cg27504269	0.783	0.781	-0.001	-0.001	0.008	-0.0163; 0.0141	8.90E-01	9.60E-01
Late childhood	10	**cg12096528**	**0.885**	**0.886**	**0.001**	**0.002**	**0.004**	**-0.0065; 0.0098**	**6.85E-01**	**9.60E-01**
Accumulation		**cg00807464**	**0.050**	**0.051**	**0.001**	**0.001**	**0.001**	**-0.0002; 0.0018**	**1.12E-01**	**6.51E-01**
		cg10420609	**0.603**	**0.602**	**-0.001**	**0.001**	**0.003**	**-0.0047; 0.0058**	**8.34E-01**	**9.60E-01**
		cg14579651	**0.663**	**0.653**	**-0.010**	**-0.003**	**0.003**	**-0.0083; 0.0018**	**2.03E-01**	**8.30E-01**

[1]DNAm unexp. = mean DNA methylation levels at age 7 in individuals with no exposure to adversity from age 0 to 11.
[2]DNAm exp. SP = mean DNA methylation levels at age 7 in individuals with exposure to adversity that occurred during the selected sensitive period (SP).
[3]ΔDNAm= difference in mean DNA methylation levels between individuals exposed to adversity during the selected sensitive period and individuals unexposed to adversity (i.e., DNAm exp. SP – DNAm unexp.)
[4]Effect estimates were calculated using linear regression of exposure to adversity from the theoretical model and DNA methylation, correcting for the covariates described in the methods.
* SE = standard error; bolded loci passed a 5% FDR threshold in the original age 15 analysis.

Table S12. Types of longitudinal DNAm trajectories in response to childhood adversity for top adolescent loci.

Adversity	Timing	Age (years)	CpG	Trajectory name
Caregiver physical or emotional abuse	Early childhood	5	cg14855874	Emergent
			cg15454534	Latent
			cg06215562	Latent
Sexual or physical abuse (by anyone)	Early childhood	3.5	**cg26970800**	**Emergent**
			cg15723468	**Latent**
			cg17928317	**Primed**
	Late childhood	8	cg27558057	**Stable**
Family instability	Very early childhood	2.5	cg02735620	Emergent
Financial hardship	Very early childhood	0.66	cg14455319	Time-stable
			cg13204236	Latent
	Early childhood	5	cg15037420	Latent
			cg06410970	Overcompensation
	Late childhood	11	cg02011706	Emergent
			cg04659536	Latent
	Recency		cg17670999	Stable
			cg25459301	Overcompensation
			cg06812747	Stable
Maternal psychopathology	Very early childhood	2.75	**cg16813552**	**Stable**
Neighborhood disadvantage	Very early childhood	2.75	cg04288299	Overcompensation
			cg25019631	Overcompensation
			cg04224851	Overcompensation
One adult in the household	Very early childhood	1.75	**cg05491478**	**Overcompensation**
	Early childhood	3.9	**cg16907527**	**Flat emergent**
			cg08818094	**Latent**
			cg01060989	**Latent**
			cg15814750	**Latent**
			cg15783822	**Latent**
			cg15864691	**Overcompensation**
			cg02584161	**Latent**
			cg02810291	**Overcompensation**

		cg04036644	**Latent**
		cg11811897	**Latent**
		cg15817130	**Latent**
		cg06711254	**Flat emergent**
		cg19096460	**Latent**
		cg18980650	Emergent
		cg27504269	Latent
Late childhood	10	**cg12096528**	**Overcompensation**
Accumulation		**cg00807464**	**Stable**
		cg10420609	**Latent**
		cg14579651	**Stable**

*Bolded loci passed a 5% FDR threshold in the original analysis.

Table S13. Persistence of differential DNA methylation patterns identified at age 7 (whole blood) into adolescence (age 15).

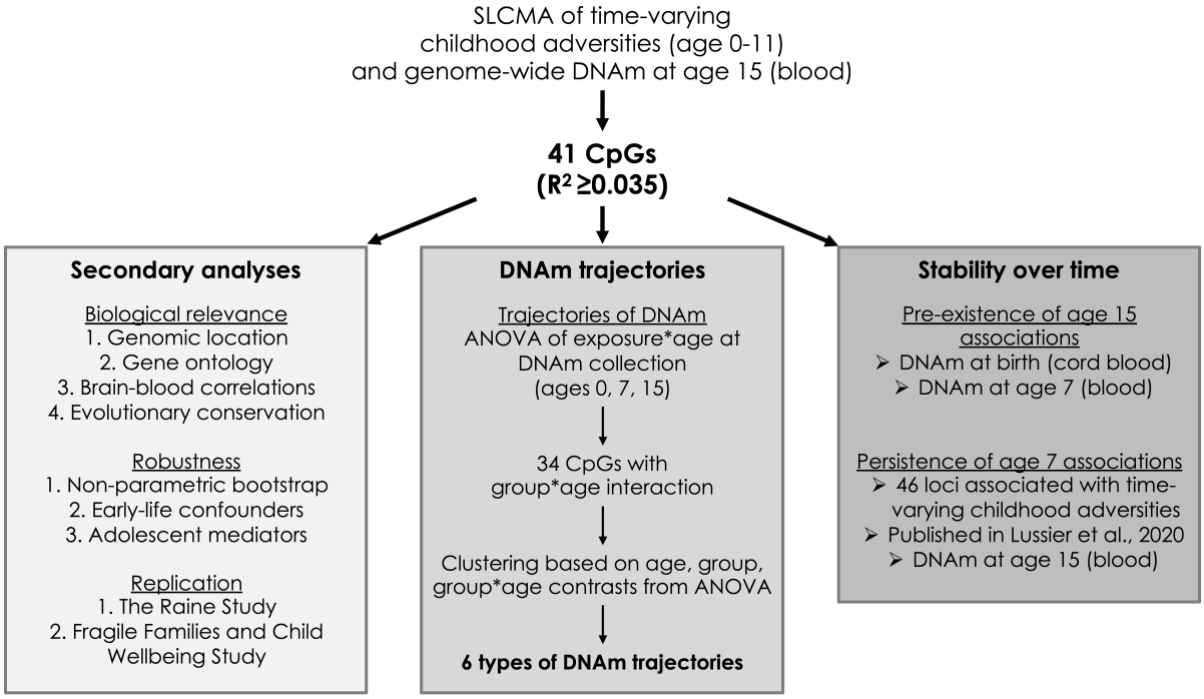
Adversity	Timing	Age (years)	CpG	DNAm unexposed[1]	DNAm exp. SP[2]	ΔDNAm[3]	Effect estimate[4]	SE*	95% CI	P-value	FDR-adjusted p-value
Caregiver physical or emotional abuse	Middle childhood	6	cg12023170	0.098	0.105	0.008	0.006	0.007	-0.0077; 0.0191	4.02E-01	8.56E-01
Sexual or physical abuse (by anyone)	Early childhood	4.75	cg20369299	0.682	0.662	-0.02	-0.016	0.018	-0.0523; 0.0196	3.72E-01	8.56E-01
			cg13817046	0.425	0.424	-0.001	0.001	0.014	-0.0257; 0.0285	9.18E-01	9.61E-01
Family instability	Very early childhood	2.5	cg04079399	0.885	0.883	-0.002	-0.002	0.004	-0.0112; 0.0063	5.90E-01	8.75E-01
	Early childhood	4.75	cg01407460	0.023	0.024	0.000	0.001	0.001	-0.0009; 0.002	4.22E-01	8.56E-01
			cg17134302	0.835	0.836	0.001	0.001	0.006	-0.0099; 0.0118	8.63E-01	9.61E-01
			cg13706680	0.875	0.883	0.008	0.008	0.005	-0.0005; 0.0173	6.30E-02	7.16E-01
			cg27457457	0.664	0.646	-0.017	-0.015	0.016	-0.0469; 0.0176	3.74E-01	8.56E-01
			cg01504589	0.836	0.828	-0.008	-0.007	0.009	-0.0247; 0.0105	4.28E-01	8.56E-01
			cg13876553	0.801	0.805	0.004	0.006	0.009	-0.0128; 0.0243	5.43E-01	8.75E-01
			cg01841772	0.810	0.825	0.014	0.014	0.009	-0.0028; 0.0315	1.02E-01	7.16E-01
			cg16231917	0.214	0.242	0.028	0.025	0.015	-0.0037; 0.0542	8.66E-02	7.16E-01
			cg26997966	0.860	0.854	-0.006	-0.007	0.005	-0.0174; 0.0041	2.24E-01	8.56E-01
			cg14401897	0.799	0.808	0.009	0.010	0.010	-0.0103; 0.0299	3.37E-01	8.56E-01
			cg27639644	0.854	0.851	-0.003	-0.003	0.006	-0.0151; 0.0098	6.78E-01	8.75E-01
			cg02886132	0.878	0.885	0.007	0.007	0.004	-0.0012; 0.0162	9.28E-02	7.16E-01
			cg27061903	0.051	0.054	0.003	0.003	0.003	-0.0025; 0.0085	2.84E-01	8.56E-01
			cg10571837	0.897	0.903	0.006	0.006	0.004	-0.0014; 0.0129	1.15E-01	7.16E-01
			cg12188526	0.883	0.885	0.001	0.002	0.004	-0.007; 0.0104	6.95E-01	8.75E-01
			cg21172807	0.109	0.124	0.014	0.014	0.005	0.0033; 0.0245	9.90E-03	4.55E-01
			cg01267076	0.846	0.847	0.002	0.003	0.007	-0.01; 0.0164	6.36E-01	8.75E-01
			cg22346081	0.858	0.860	0.002	0.002	0.005	-0.0073; 0.0119	6.40E-01	8.75E-01
			cg16338178	0.825	0.821	-0.004	-0.003	0.007	-0.0174; 0.0113	6.75E-01	8.75E-01
			cg08971940	0.772	0.785	0.013	0.014	0.011	-0.0074; 0.0357	1.97E-01	8.56E-01
			cg14948379	0.851	0.848	-0.003	-0.003	0.007	-0.0159; 0.0103	6.79E-01	8.75E-01
			cg01654242	0.810	0.817	0.007	0.007	0.010	-0.013; 0.0272	4.88E-01	8.75E-01

		cg11438065	0.901	0.902	0.002	0.002	0.004	-0.0053; 0.0089	6.12E-01	8.75E-01
		cg22011436	0.840	0.846	0.006	0.007	0.008	-0.0085; 0.0225	3.75E-01	8.56E-01
		cg01587190	0.058	0.061	0.003	0.003	0.002	-0.0003; 0.0072	7.05E-02	7.16E-01
		cg01023798	0.854	0.853	-0.002	0.000	0.006	-0.0123; 0.0122	9.92E-01	9.92E-01
		cg09305491	0.910	0.909	-0.001	-0.001	0.004	-0.008; 0.006	7.80E-01	9.38E-01
		cg22060367	0.880	0.880	0.000	0.000	0.005	-0.0084; 0.0093	9.20E-01	9.61E-01
		cg05353659	0.892	0.888	-0.004	-0.004	0.004	-0.0118; 0.0041	3.41E-01	8.56E-01
		cg27567416	0.882	0.887	0.005	0.006	0.004	-0.002; 0.014	1.42E-01	7.24E-01
		cg07206497	0.876	0.876	0.001	0.002	0.005	-0.0072; 0.0106	7.04E-01	8.75E-01
		cg05886789	0.839	0.841	0.002	0.003	0.006	-0.0086; 0.0155	5.77E-01	8.75E-01
		cg14637285	0.858	0.851	-0.007	-0.007	0.006	-0.0185; 0.0043	2.23E-01	8.56E-01
		cg00967695	0.883	0.875	-0.008	-0.008	0.007	-0.0225; 0.0061	2.62E-01	8.56E-01
		cg01100868	0.892	0.894	0.002	0.003	0.004	-0.0056; 0.0111	5.20E-01	8.75E-01
		cg23184756	0.834	0.835	0.001	0.000	0.006	-0.0121; 0.0131	9.41E-01	9.61E-01
		cg00943585	0.828	0.824	-0.005	-0.003	0.011	-0.0246; 0.0194	8.16E-01	9.38E-01
	5.75	cg17719337	0.040	0.040	0.000	0.000	0.002	-0.0031; 0.0033	9.39E-01	9.61E-01
		cg26848593	0.027	0.028	0.001	0.000	0.001	-0.0015; 0.0025	6.23E-01	8.75E-01
		cg06770536	0.733	0.718	-0.015	-0.018	0.012	-0.042; 0.0051	1.24E-01	7.16E-01
Middle Childhood	6.75	cg19569074	0.677	0.668	-0.009	-0.004	0.016	-0.0356; 0.0274	7.98E-01	9.38E-01
		cg10940545	0.807	0.796	-0.011	-0.015	0.015	-0.0443; 0.0143	3.14E-01	8.56E-01

[1]DNAm unexp. = mean DNA methylation levels at age 15 in individuals with no exposure to adversity from age 0 to 11.
[2]DNAm exp. SP = mean DNA methylation levels at age 15 in individuals with exposure to adversity that occurred during the selected sensitive period (SP).
[3]ΔDNAm= difference in mean DNA methylation levels between individuals exposed to adversity during the selected sensitive period and individuals unexposed to adversity (i.e., DNAm exp. SP – DNAm unexp.)
[4]Effect estimates were calculated using linear regression of exposure to adversity during the selected sensitive period from the theoretical model and DNA methylation, correcting for the covariates described in the methods.
* SE = standard error.

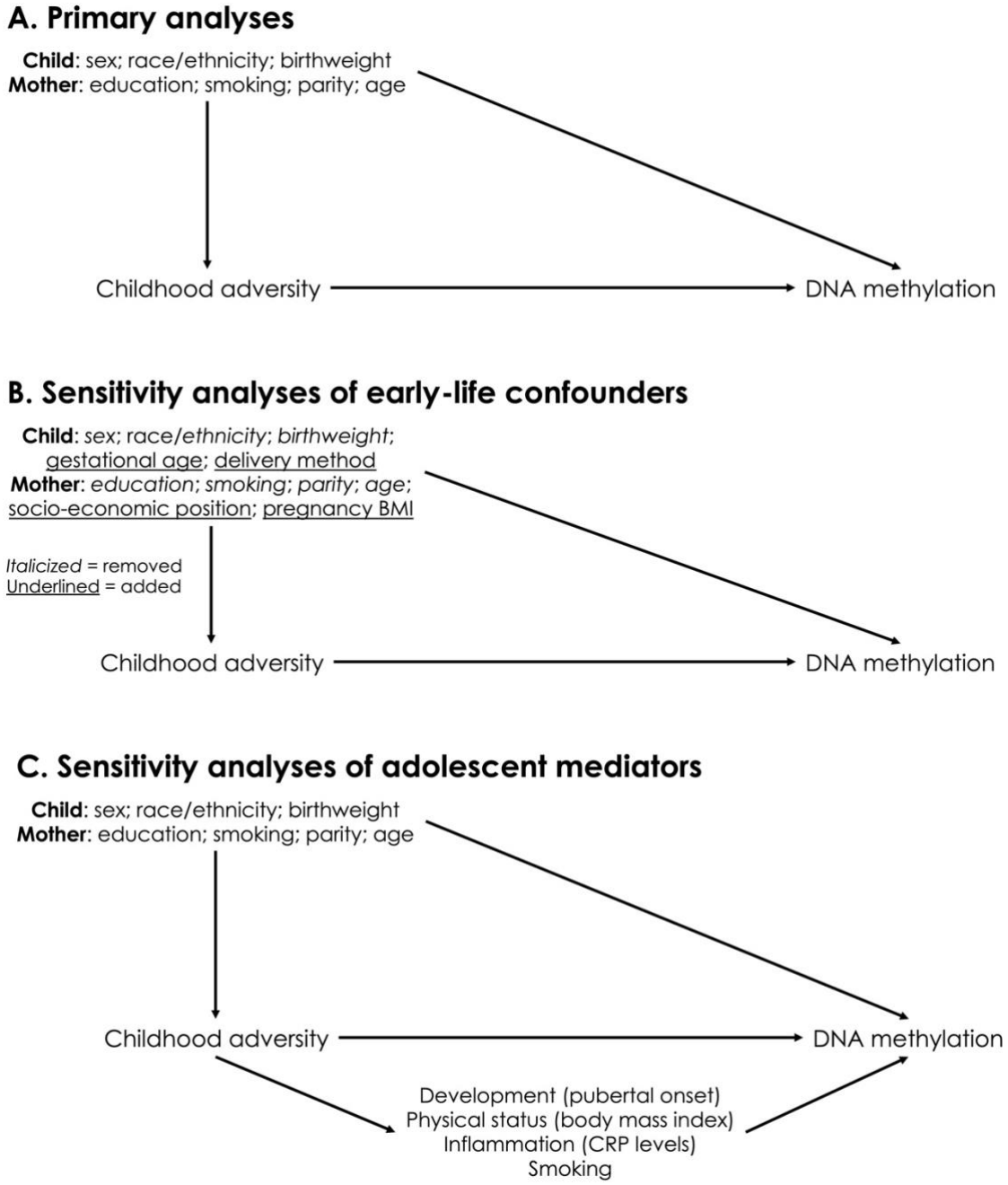
SUPPLEMENTAL FIGURES

Figure S1. Flow-chart of analyses



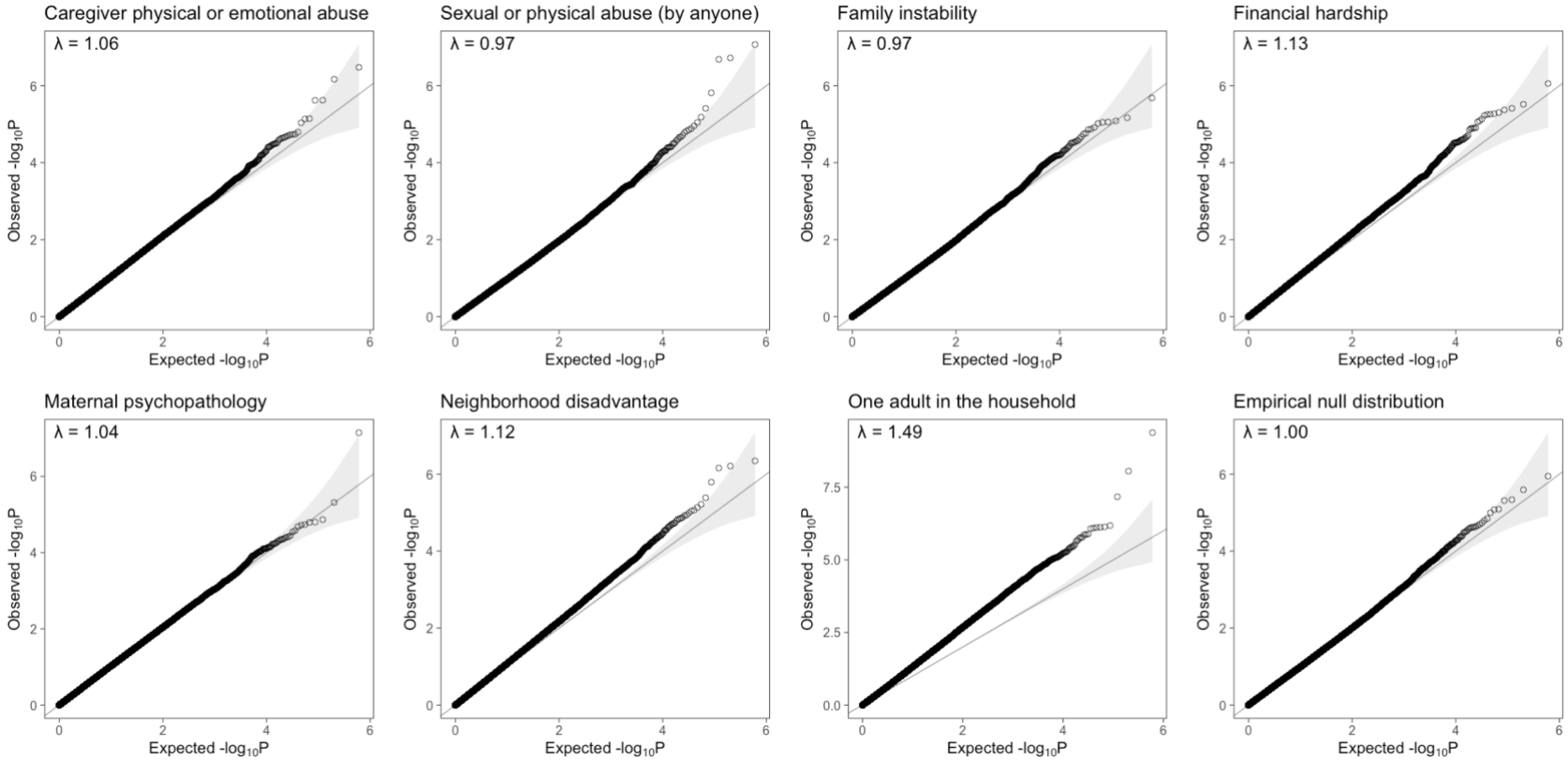
Summary of primary and secondary analyses included in the present manuscript.

Figure S2. Directed acyclic graphs (DAGs) of primary and sensitivity analyses



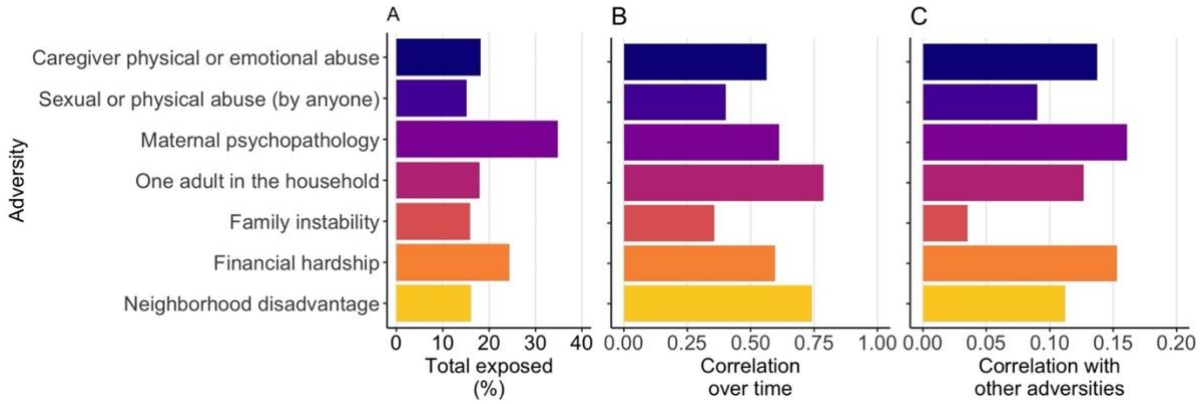
A) Confounders were selected based on prior analyses in the ALSPAC cohorts, which have shown that these child- and mother-based factors are confounders of the relationship between childhood adversity and DNAm.
B) In our sensitivity analyses of early-life confounders, we assessed the impact of removing (italics) or adding (underlined) confounders to our primary model in A. These confounders were added/removed individually.
C) In our sensitivity analyses of adolescent mediators, we investigated mediation through factors related to adolescent development and behaviors, each assessed individually in our primary model.

Figure S3. Quantile-Quantile plots of the epigenome-wide analyses



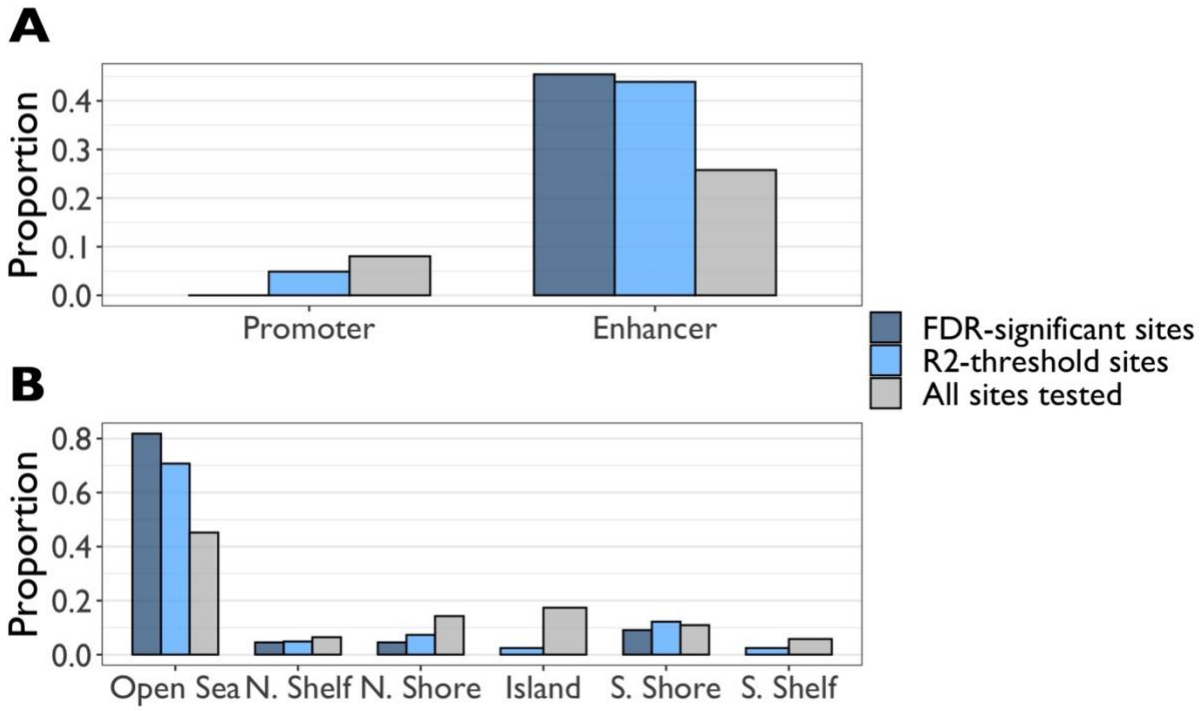
Quantile-quantile (QQ) plots of the expected versus observed p-value distributions for the 302,581 CpGs analyzed for each adversity. The genomic inflation factor (λ) is shown for each adversity and ranged from 0.97 to 1.49, with the one-adult household analysis showing the most inflation (1.49). To determine whether the inflation observed in some of these analyses was due to issues with the method of statistical inference or the assumptions upon which the model relies, we also show a QQ plot of an empirical null distribution, generated using scrambled one-adult household exposure data from ALSPAC with the same covariates as the other analyses. We did not observe any inflation in this model, suggesting that inflation was not due to inference, but instead may represent stronger associations between the exposures and DNAm, which are further amplified due to the non-independence of CpGs (i.e., correlations across the epigenome).

Figure S4. Summary of prevalence and correlations between adversities from age 0-11.



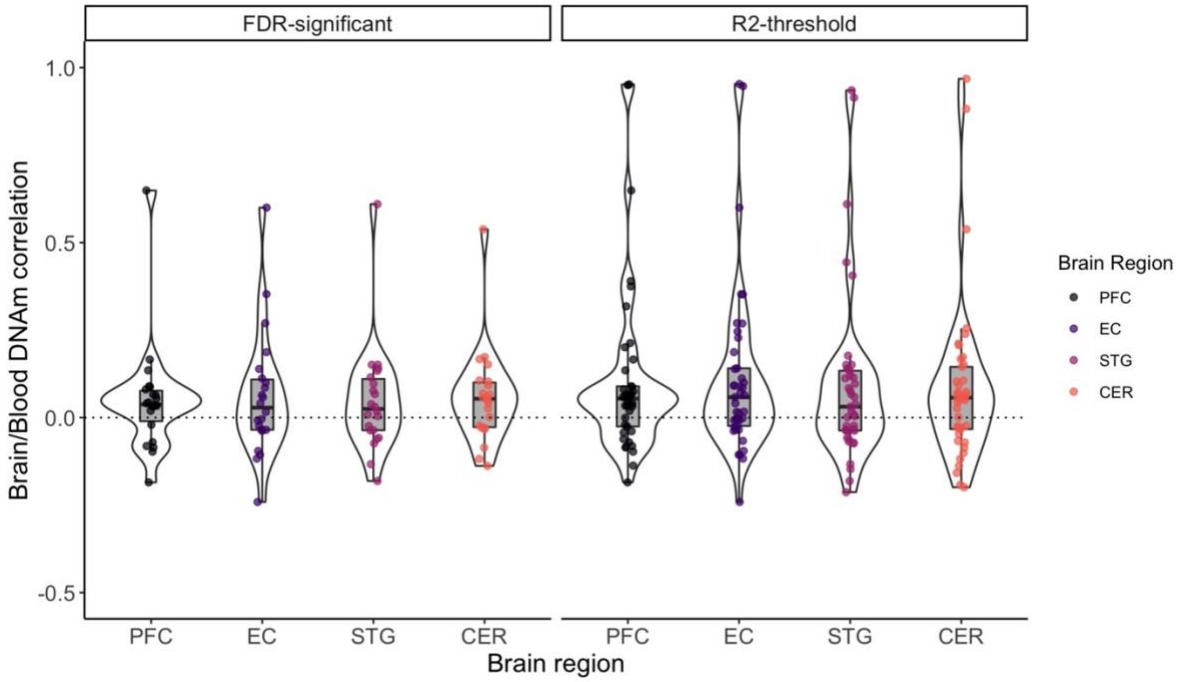
A) The prevalence of each adversity from age 0-11 varied by type, ranging from 15.1% (sexual or physical abuse by anyone) to 34.8% (maternal psychopathology).
B) Exposures within each type of adversity were generally correlated over time, ranging from 0.357 (family instability) to 0.786 (one adult in the household). Closer timepoints tended to be more related than more distant timepoints.
C) On average, the absolute correlation of exposures to different adversities was modest, ranging from -0.035 (family instability; shown here on absolute scale) to 0.161 (maternal psychopathology), which may reflect various dimensions of childhood adversity.
Correlations were assessed using tetrachoric correlations.

Figure S5. Genomic locations of top age 15 loci compared to all sites tested (n=302,581).



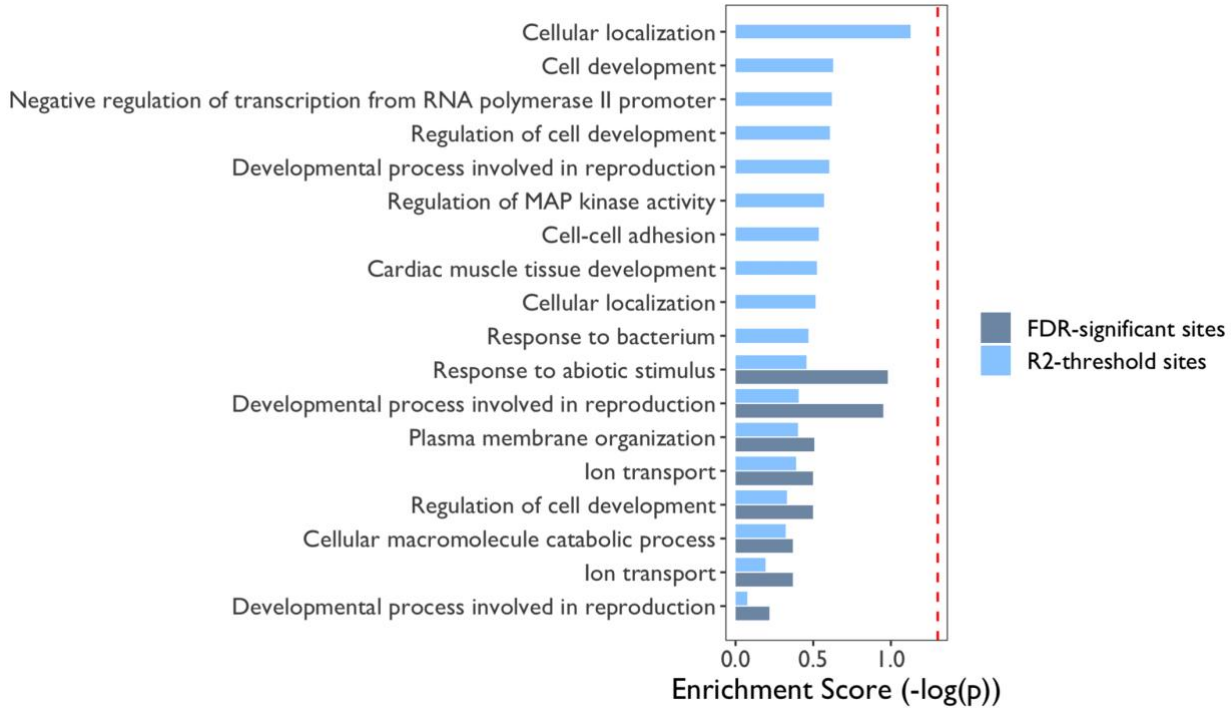
A) Compared to all tested sites, FDR-significant loci showed more enrichment in enhancer regions (χ^2=4.5; p=0.034) and no presence in promoter regions (χ^2=1.9; p=0.17). R^2-threshold loci also showed higher enrichment in enhancers (χ^2=7.1; p=0.0079) and no differences in promoter enrichment (χ^2=0.55; p=0.46).
B) FDR-significant loci also differed in terms of their location relation to CpG islands, showing higher enrichment in Open Sea regions and decreased enrichment in CpG islands compared to all sites (χ^2=13.3; p=0.021). R^2-threshold loci also higher enrichment in Open Sea regions and decreased enrichment in CpG islands compared to all sites (χ^2=13.6; p=0.018).

Figure S6. Brain-blood correlations for top loci identified at age 15.



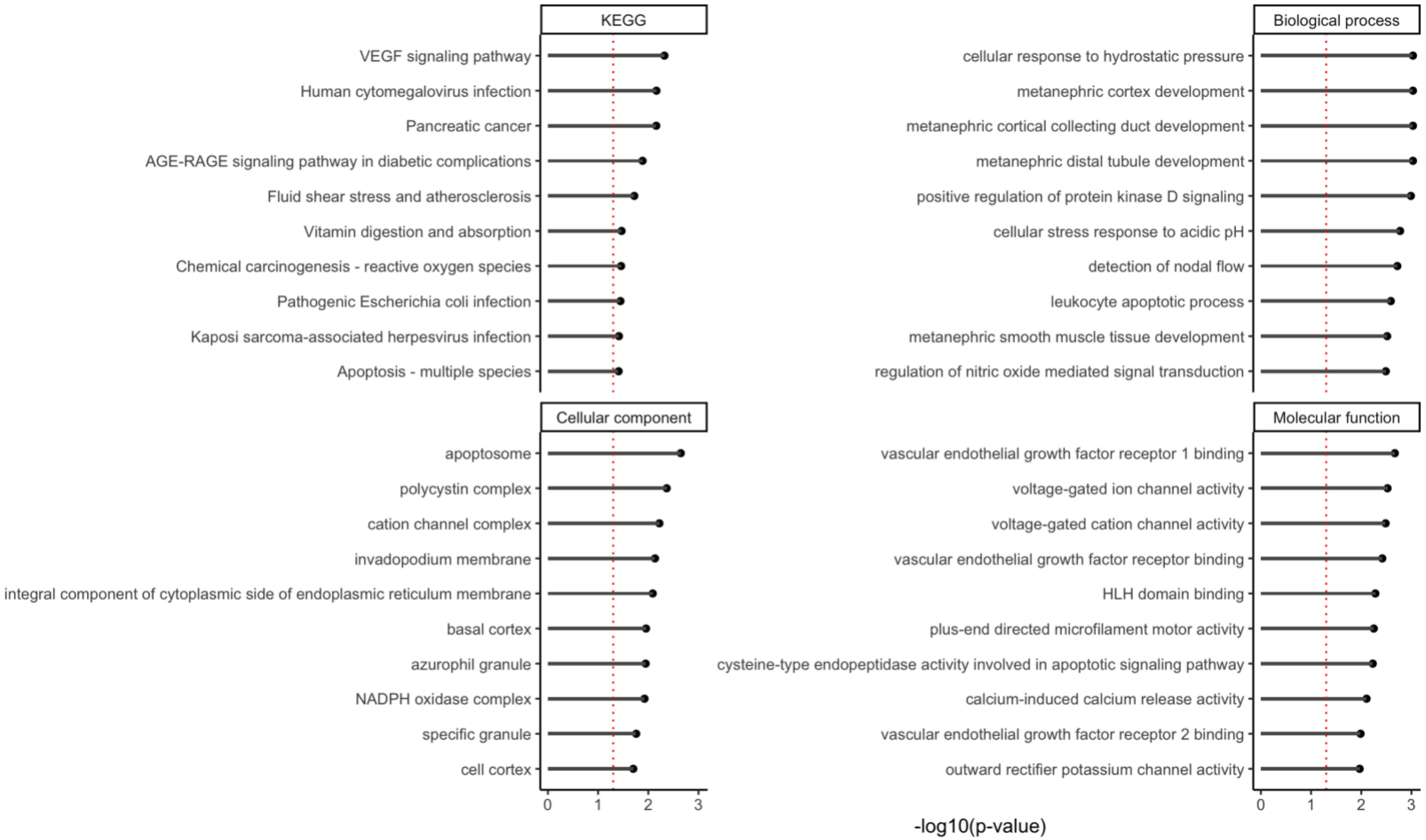
Correlations between DNA methylation measured in blood and specific brain regions are shown for the 22 FDR-significant loci identified at age 15, as well as the 41 loci that passed an R^2 threshold of 0.035. Data were obtained from Hannon et al., 2015. PFC = prefrontal cortex; EC = entorhinal cortex; STG = superior temporal gyrus; CER = cerebellum.

Figure S7. Enrichment of Gene Ontology (GO) term clusters for top loci at age 15 using DAVID.



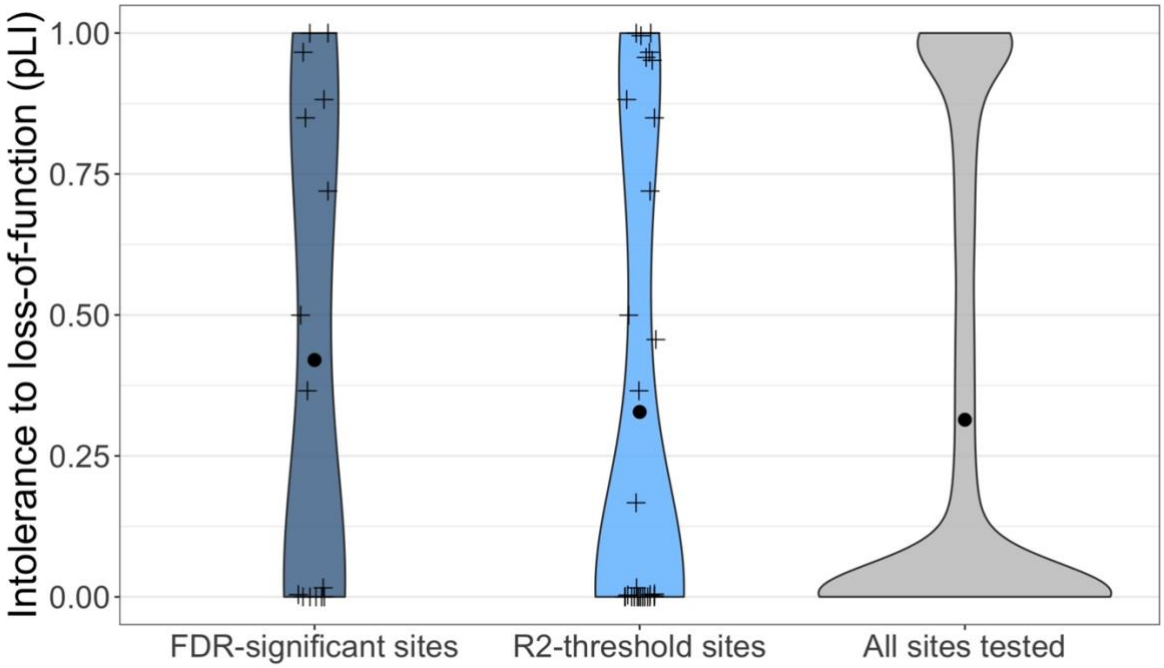
The 22 FDR-significant loci were annotated to 21 unique genes, while the 41 R^2-threshold loci were annotated to 39 genes. The plot shows the clusters of GO biological processes that emerged from these genes, as analyzed using DAVID (4,5). No clusters were significant at $p<0.05$, shown here as the dotted red line corresponding to an enrichment score of 1.3.

Figure S8. Enrichment of Gene Ontology (GO) term for top loci at age 15 using missMethyl.



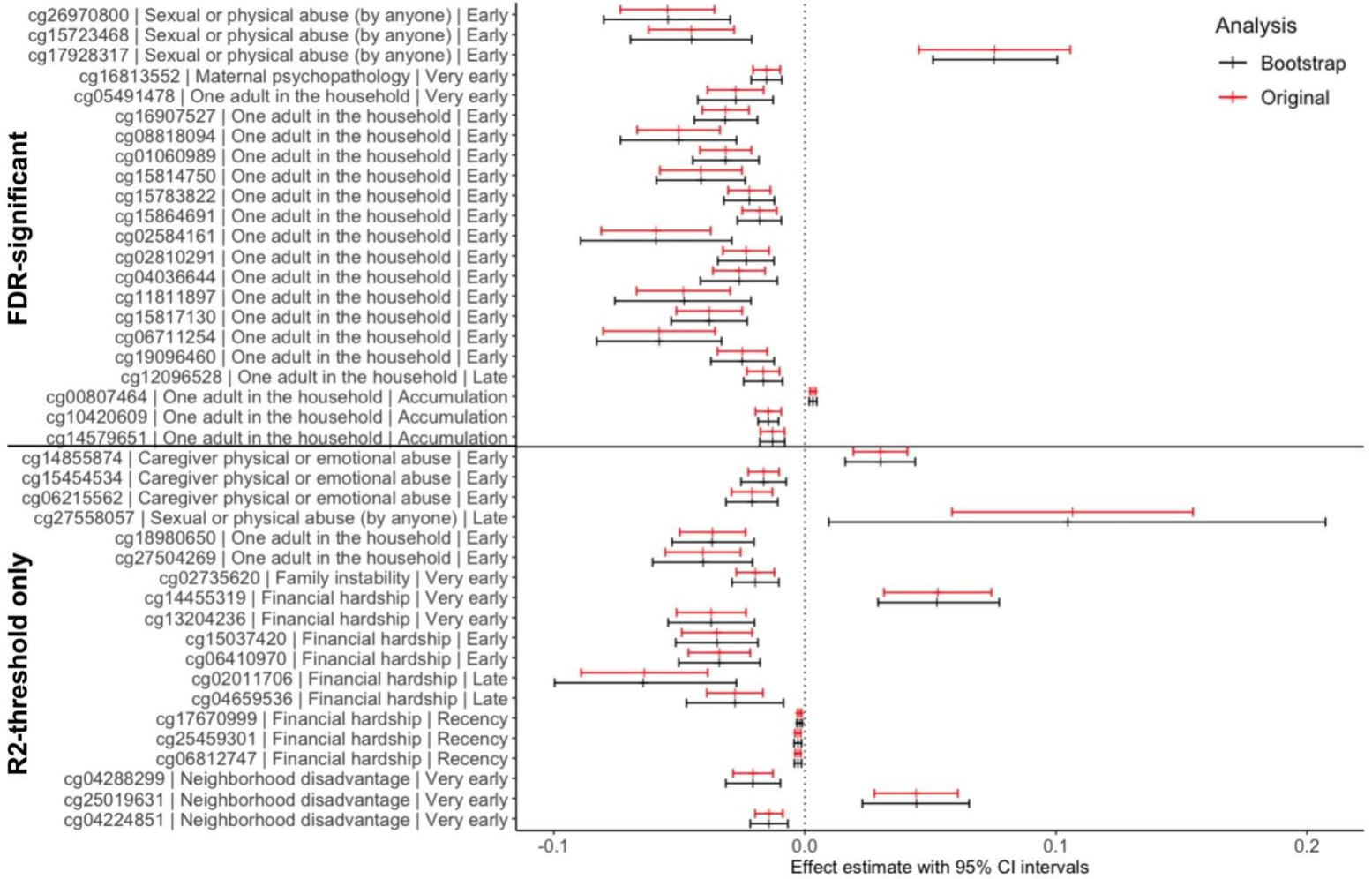
Gene ontology enrichment was completed using the *missMethyl* package on the 41 loci that passed an R2≥0.035. The approach accounts for the total number of CpGs measured in each gene from the 302,581 CpGs analyzed. No clusters were significant at FDR<0.05, shown here as the dotted red line corresponding to an $-\log_{10}(0.05)$. The top 10 processes from KEGG, biological processes, cellular component, and molecular function categories are shown. Top pathways and processes were related to immune function, apoptosis, and development.

Figure S9. Genes annotated to top age 15 loci were no more highly constrained than all sites.



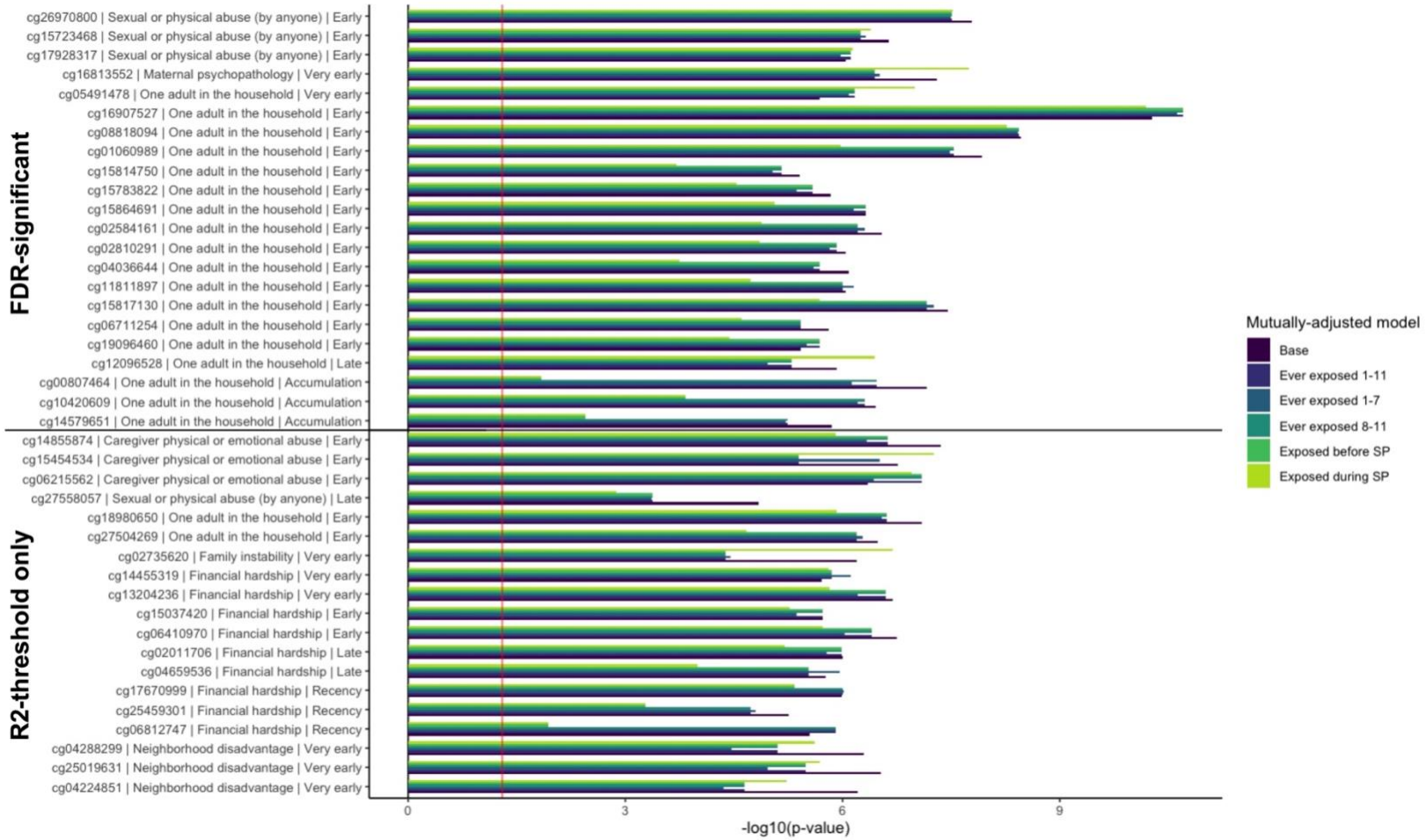
Violin plots show the distribution of gene constraint scores (pLI) for FDR-significant (n=17 annotated genes from 22 loci), R^2-threshold loci (n=33 annotated genes from 41 loci), and genome-wide loci (n=16,114), where higher values represent increased probability of a gene being intolerant to Loss-of-Function variation. Genes annotated to FDR-significant sites were no more highly constrained than the rest of genes tested (permutation p=0.27 for FDR-significant subset; p=0.51 for R^2-threshold subset). Black points represent mean pLI values for the two sets of genes. Three genes in the set of FDR-significant loci showed a pLI>0.9 (*DSP*, *CUX2*, and *STK38L*), with four more in the R^2-threshold subset (*FBXL16*, *PKD2*, *TAF1*, and *XKR6*).

Figure S10. Non-parametric bootstrapping of associations between childhood adversity and DNAm at age 15.



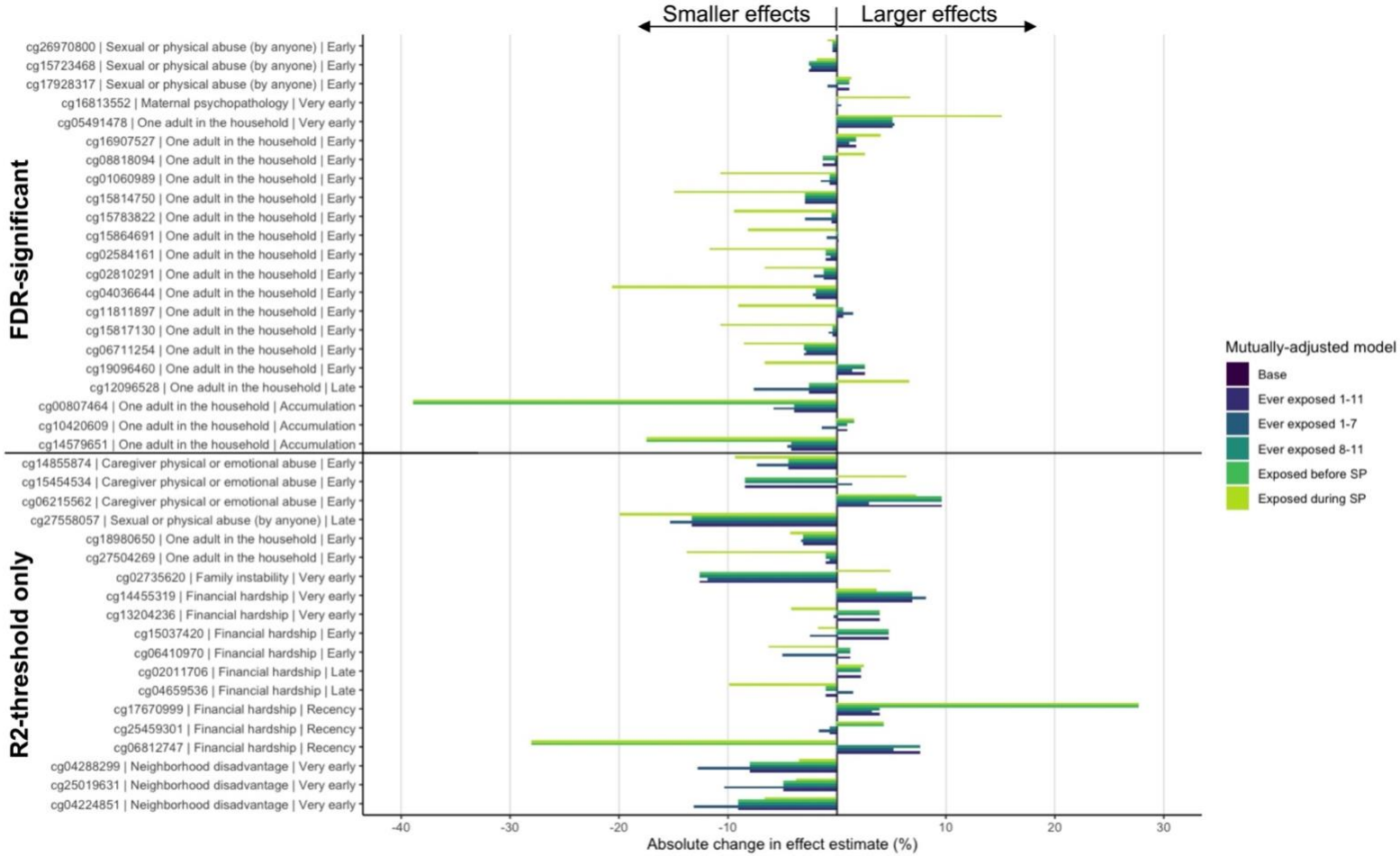
The 41 R^2-threshold associations (of which 22 passed a 5% FDR cutoff) between childhood adversity and DNA methylation at age 15 were internally validated using non-parametric bootstrap analyses. The average effect estimates for the 10,000 bootstraps (black) showed only minor differences from the effects estimates generated in the original analyses of childhood adversity and DNAm (red). 95% confidence intervals are shown.

Figure S11. Significance levels for mutually-adjusted models of adversity and age 15 DNAm.



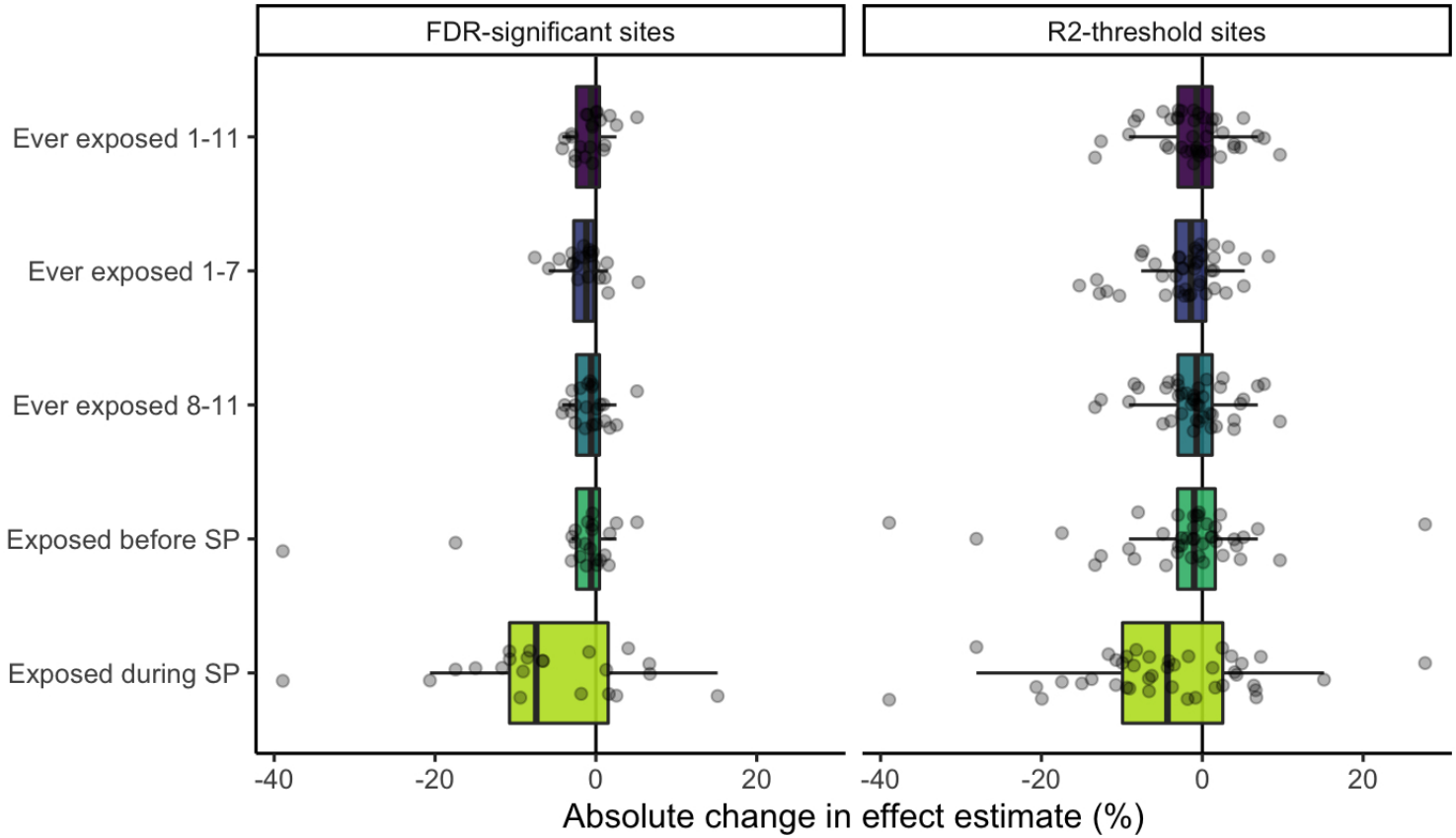
We compared the significance of associations between childhood adversity and DNA methylation (DNAm) at age 15 between the base model and "mutually-adjusted" models, which additionally included other types of childhood adversity. These five mutually-adjusted models included a variable of exposure to any other adversity between age 1-11, age 1-7, or age 8-11. We also tested the effects of exposures to adversity before or during the SLCMA-selected sensitive period; accumulation hypotheses were corrected using the total number of exposures from age 1-11. Significance levels are represented by the $-\log_{10}$ of p-values, whereby larger values represent smaller p-values (higher significance) and smaller values represent larger p-values (lower significance). The red line shows the $-\log_{10}$ of p=0.05. All associated passed a false-discovery rate of 0.05 when correcting for the testing of 22 FDR-significant loci.

Figure S12. Change in effects estimates for mutually-adjusted models of adversity and age 15 DNAm.



The strength of associations between childhood adversity and DNA methylation (DNAm) at age 15 from the base model were compared to mutually-adjusted models, which additionally included other types of childhood adversity. These five "mutually-adjusted" models included a variable of exposure to any other adversity between age 1-11, age 1-7, or age 8-11. We also tested the effects of exposures to adversity before or during the SLCMA-selected sensitive period (SP); accumulation hypotheses were corrected using the total number of exposures from age 1-11. The majority of associations showed little change in the strength of associations between a given childhood adversity and DNAm when accounting for other exposures, shown as the absolute percent change in effect estimate. However, associations between the accumulation of exposures to one-adult households and DNAm at age 15 were most attenuated in the mutually-adjusted models, showing a 1-40% reduction in the size of the effect estimate. Accounting for exposure that co-occurred during the SLCMA-selected sensitive period also resulted in smaller effect estimates for exposures to one-adult households during early childhood.

Figure S13. Average differences across mutually-adjusted models of exposure to childhood adversity and DNA methylation at age 15.



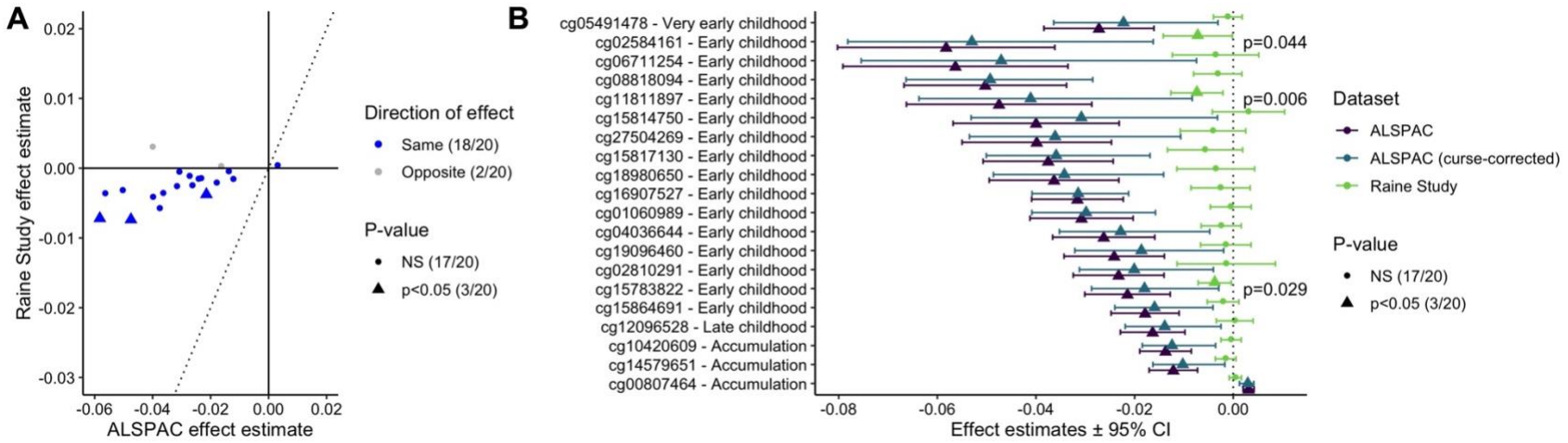
The strength of the associations between childhood adversity and DNA methylation (DNAm) at age 15 from the base model were compared to mutually-adjusted models that accounted for the potential effects of other types of childhood adversity. These "mutually-adjusted" models included a variable of exposure to any other adversity between age 1-11, age 1-7, or age 8-11. We also tested the effects of exposures to adversity before or during the SLCMA-selected sensitive period (SP); accumulation hypotheses were corrected using the total number of exposures from age 1-11. Across all 22 loci FDR-significant, the effects of mutual adjustment were most pronounced when correcting for exposures that occurred during the same sensitive period (mean = -6.3%, range = -38.9% to 15.1%). These effects were similar in the 41 R^2-treshold loci (mean = -4.7%, range = -38.9% to 27.7%). Each point represents one CpG.

Figure S14. Summary of replication cohorts

Cohort	Adversity	Age (years)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ALSPAC	Caregiver physical or emotional abuse	8m	21m	33m	47m	61m	72m			108m		132m				DNAm (blood)		
	Sexual or physical abuse		18m	30m	42m	57m	69m	81m	96m									
	Maternal psychopathology	8m	21m	33m		61m	72m					132m						
	One adult in the household	8m	21m	33m	47m			84m	96m		120m							
	Family instability		18m	30m	42m	57m	69m	81m	96m									
	Financial stress	8m	21m	33m		61m		84m				132m						
	Neighborhood disadvantage	8m	21m	33m		61m		84m			120m							
FFCWS	Caregiver physical or emotional abuse			36m		60m				108m						DNAm (saliva)		
	Maternal psychopathology	12m		36m		60m				108m								
	One adult in the household	12m		36m		60m				108m								
	Family instability	12m		36m		60m				108m								
	Financial stress	12m		36m		60m				108m								
The Raine Study	One adult in the household	12m	24m	36m		60m			96m		120m							DNAm (blood)

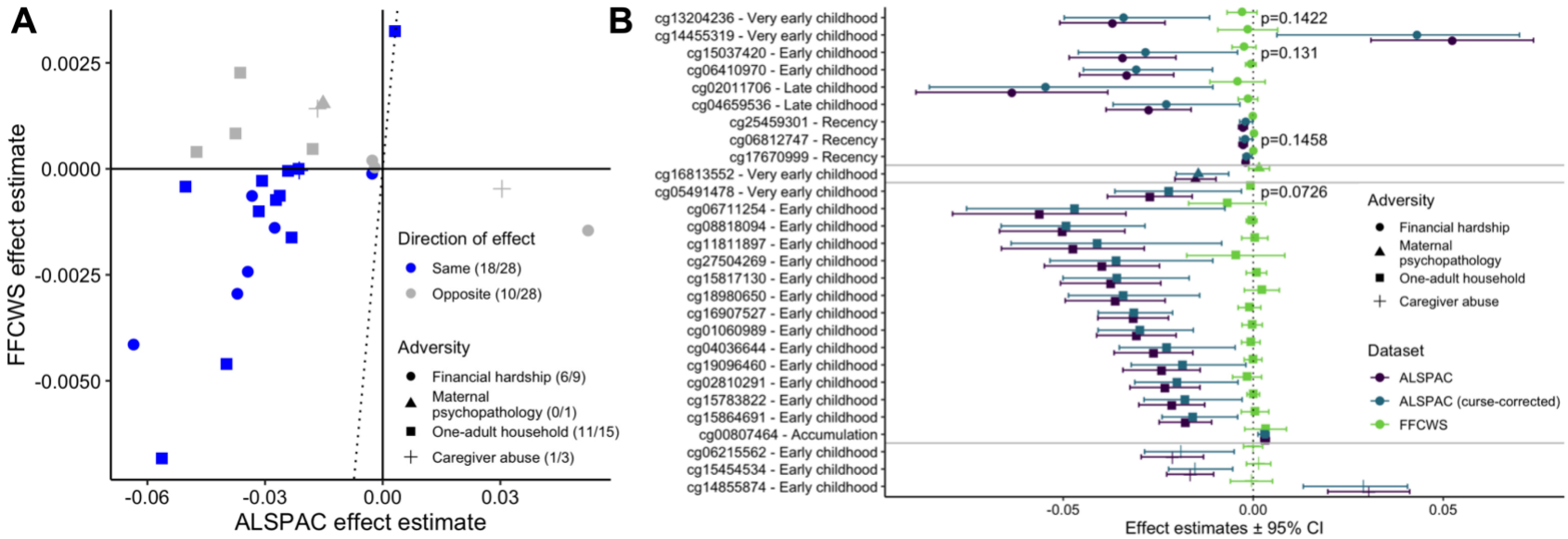
Summary of the childhood adversity measures available in ALSPAC, Future of Families and Child Wellbeing Study (FFCWS), and the Raine Study, as well as the mean age at DNA methylation (DNAm) collection.

Figure S15. Replication of one-adult household associations in the Raine Study



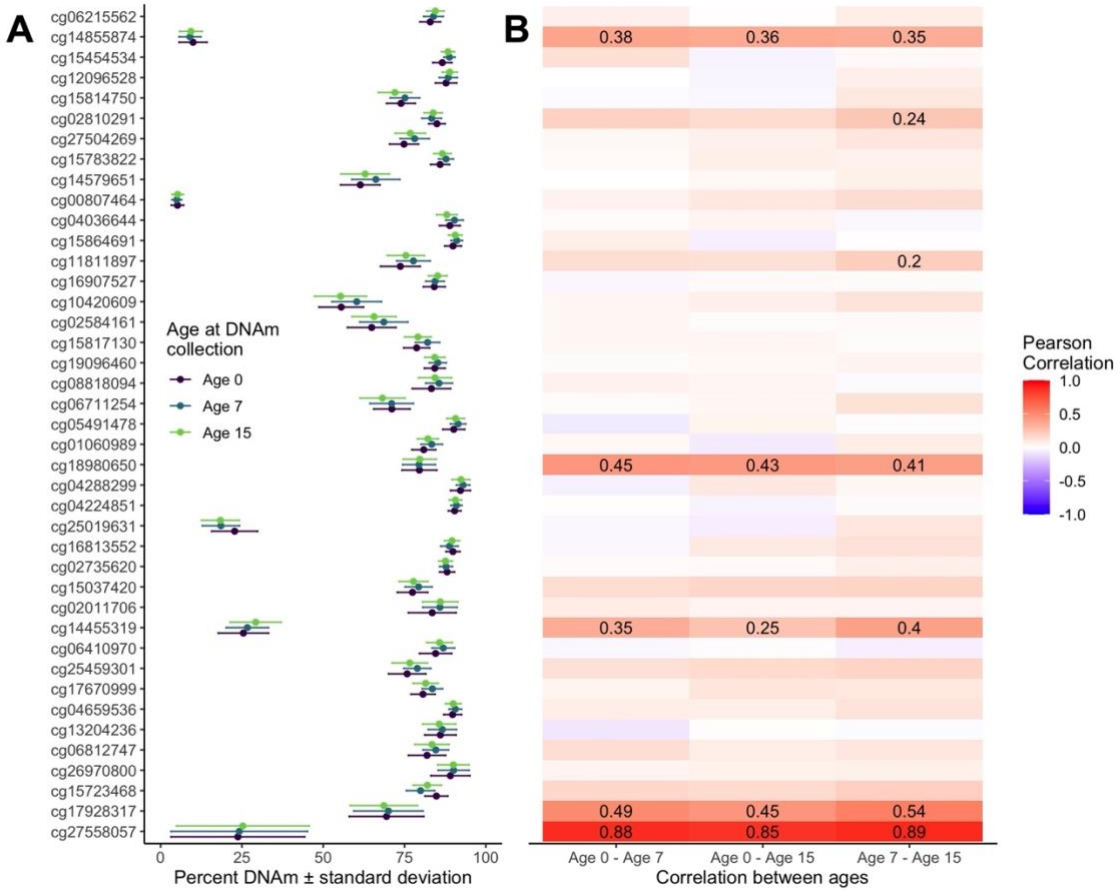
A) For 18 of the 20 CpGs associated with one-adult households, the direction of effect estimates was the same (blue) for ALSPAC (x-axis) and the Raine Study (y-axis), which is a greater number than expected under the null hypothesis (p=0.000201). Three CpGs showed nominally significant associations between exposure to one-adult households and DNAm at age 18 in the Raine Study (p<0.05; triangles).
B) The size of effect estimates was attenuated in the Raine Study (green) compared to the ALSPAC cohort (purple), with only three CpGs in the Raine Study showing 95% confidence intervals (CI) that did not overlap with zero. When correcting for the winner's curse in ALSPAC (blue), these differences were slightly mitigated, and showed some potential overlaps with estimates from the Raine Study (13 of 20 loci with overlapping 95% CI).

Figure S16. Replication of associations in the FFCWS cohort



A) For 18 of the 28 CpGs associated with four types of childhood adversity, the direction of effect estimates was the same (blue) for ALSPAC (x-axis) and the FFCWS cohort (y-axis), which is (p=0.092). Associations with one-adult households showed closer concordance across cohorts, with 11 of 15 CpGs analyzed showing the same direction of effects between cohorts (p=0.059).
B) The size of effect estimates was attenuated in FFCWS (green) compared to the ALSPAC cohort (purple), with only one CpG showing concordant effects between cohorts (cg00807464, one-adult households, accumulation). When correcting for the winner's curse in ALSPAC (blue), these differences were slightly mitigated, and showed some potential overlaps with estimates from the FFCWS cohort (12 of 28 loci with overlapping 95% CI).

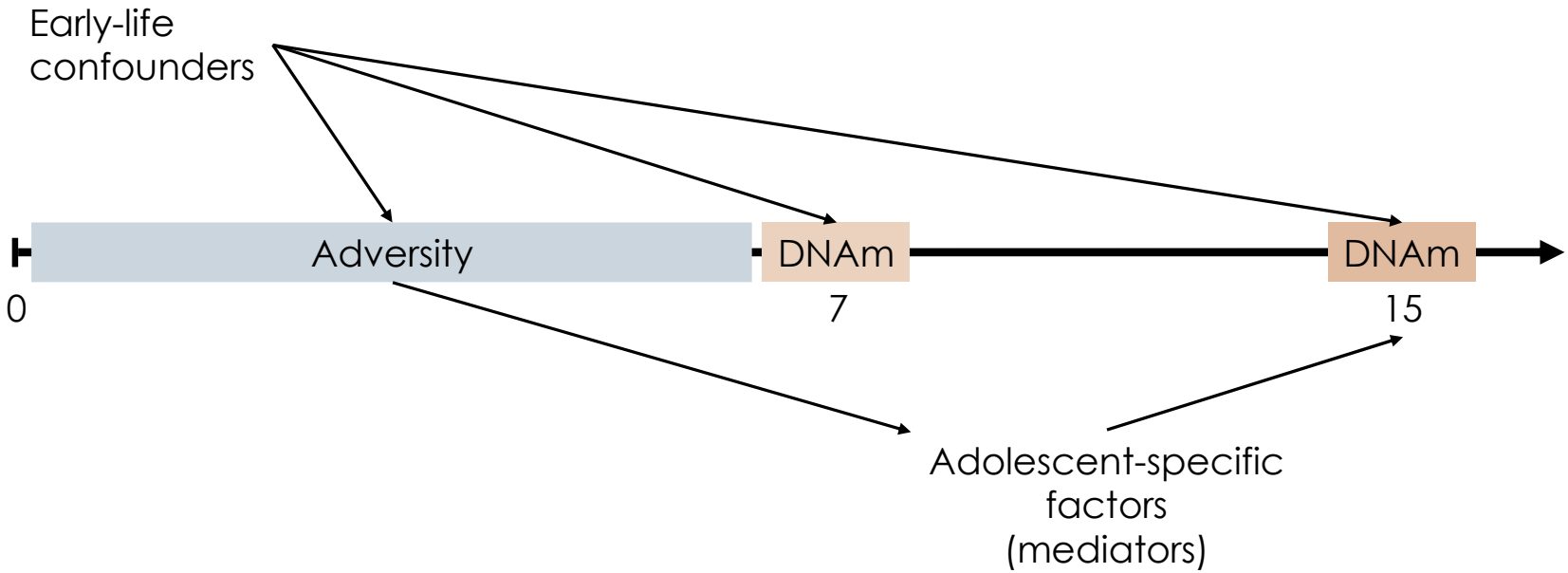
Figure S17. Population and individual-level stability of DNAm from birth to adolescence of top loci



A) Mean DNAm and standard deviation of the top 41 loci at birth, age 7, and age 15. Population-level DNAm levels were similar across ages, as were their distributions.

B) Individual-level Pearson correlations were low across ages, with only five CpGs showing an r >0.2 across all three ages. These findings suggest that top loci may be located in regions of the genome that are more variable across development.

Figure S18. Accounting for potential confounders and mediators of adversity-DNAm relationships.

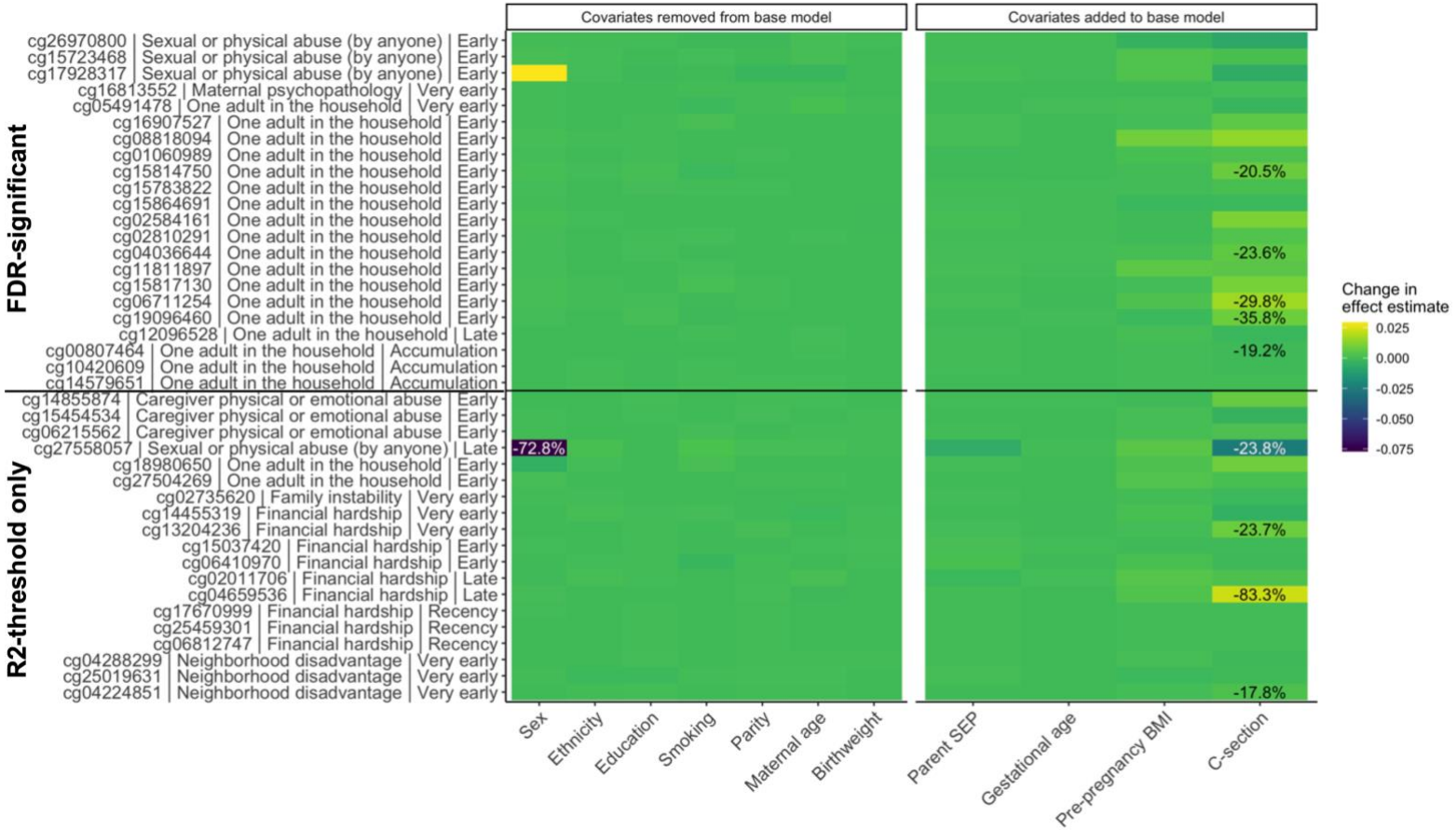


We identified two main types of factors that may have influenced or explain the results of our analyses between time-varying childhood adversity and DNA methylation (DNAm) patterns at age 7 and 15.

First, early-life confounders could have influenced the results of analyses of both age 7 and age 15 DNA methylation levels. These early-life confounders were investigated by including or removing covariates from the regression analyses of the 41 adolescent-specific loci to determine whether they influenced the strength of associations.

Second, adolescent-specific factors, meaning those that occurred after age 7, could only influence associations with age 15 DNA methylation for temporal reasons. Given that confounders must be causally associated with the exposure (adversity) and outcome (DNAm at age 15), adolescent-specific factors were considered as potential mediators of this relationship. In this case, any factors that significantly mediated this relationship would explain why associations between adversity and DNAm were not present at age 7.

Figure S19. Effects of early-life confounders on associations between adversity and DNAm at age 15.



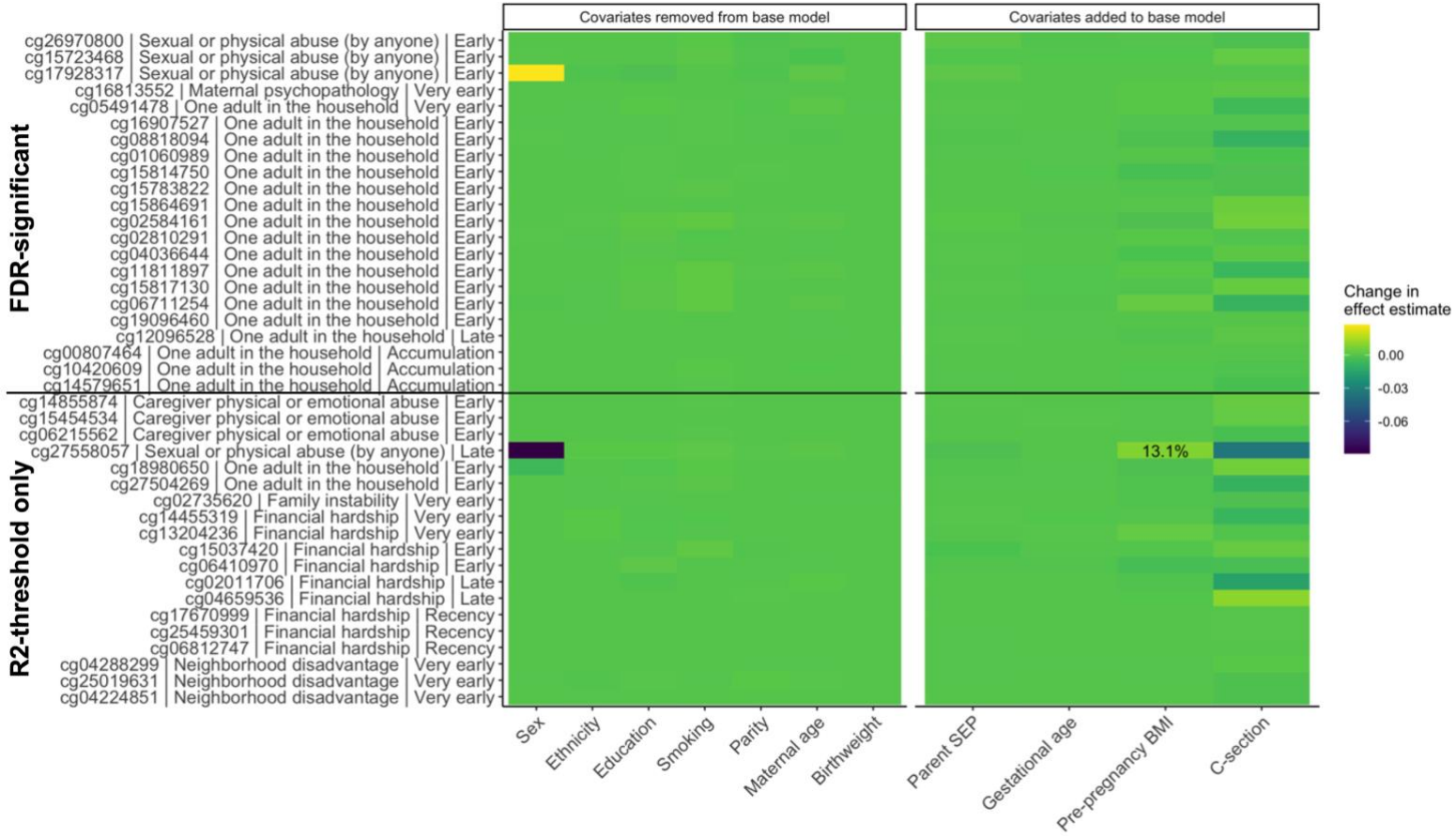
Our base regression model included the following covariates: sex, ethnicity, maternal education at birth, maternal smoking during pregnancy (smoking), parity, maternal age at birth, and birthweight. We investigated the impact of removing these covariates or adding additional ones to our regression analyses, specifically for the CpGs that showed associations between childhood adversity and age 15 DNA methylation.

Removing any one of the main covariates from our analyses resulted in small changes to the effect estimate from the regression model, except for two CpGs on chromosome X (cg17928317; cg27558057), which showed large changes in effect when sex was not included in the model.

When adding potential confounders to the regression model, we again found small changes in effect estimates, with only four CpGs showing a >10% change in effect upon including of maternal pre-pregnancy body mass index (BMI). Parental socio-economic status at birth (SES parent) and gestational age in weeks did not influence the strength of associations. Including delivery method (C-section) as a covariate induced broader changes in effect estimates.

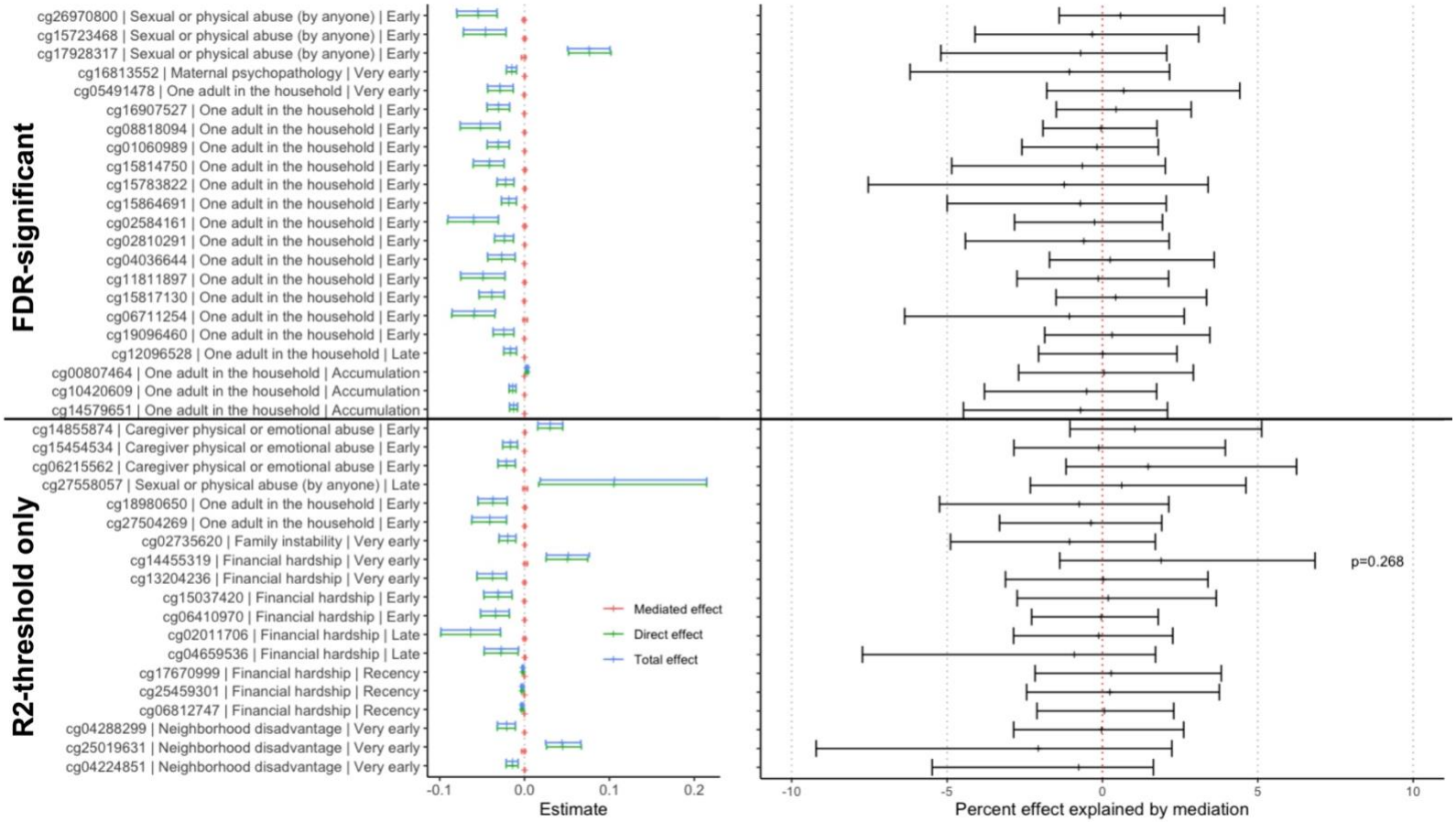
Percent changes in effect estimates are shown for CpGs that no longer met a Bonferroni-adjusted $p<0.05$ (for 41 tests) after covariate removal/addition.

Figure S20. Effects of early-life confounders on associations between adversity and DNAm at age 7.



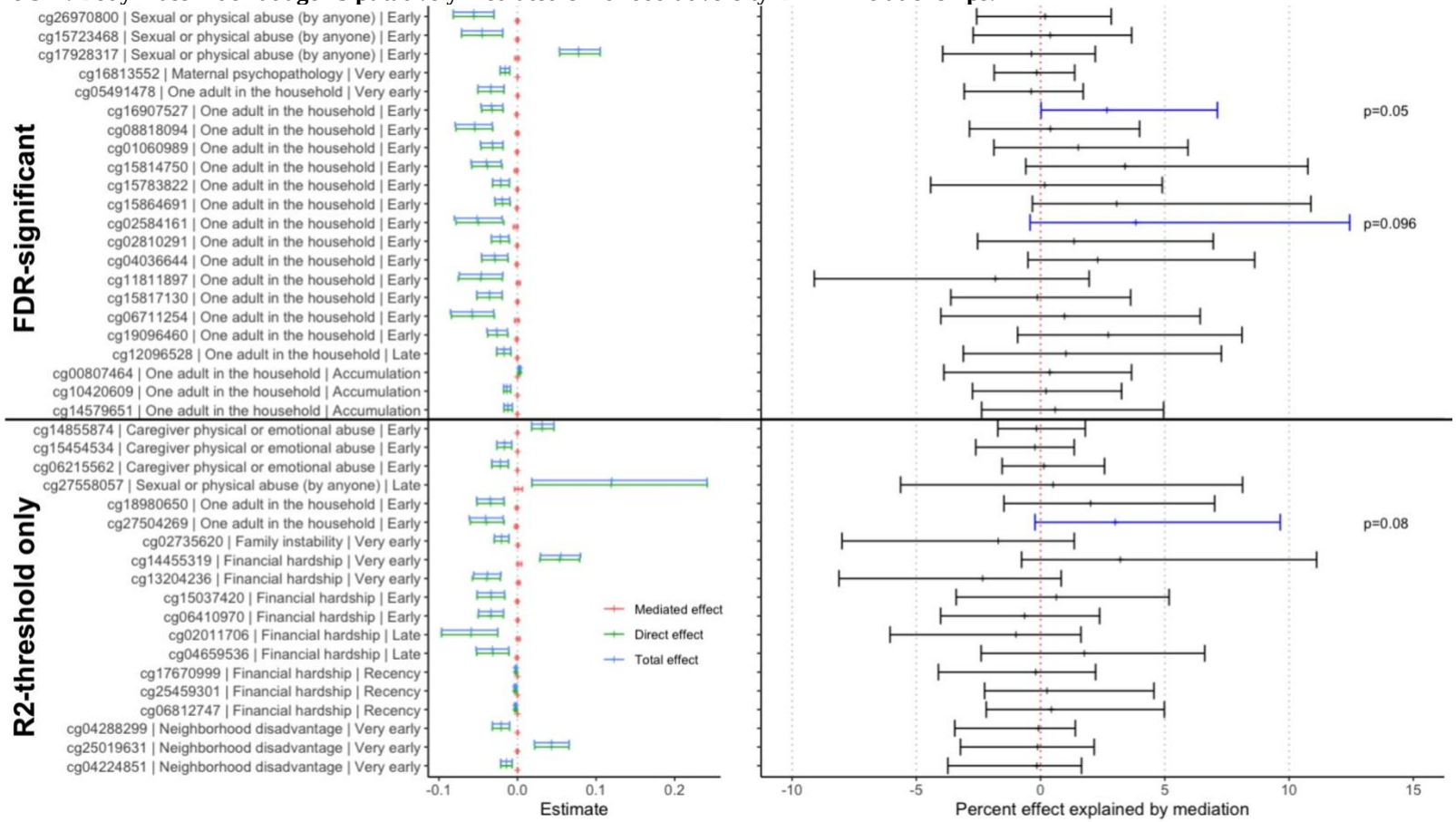
Our base regression model included the following covariates: sex, ethnicity, maternal education at birth, maternal smoking during pregnancy (smoking), parity, maternal age at birth, and birthweight. We investigated the impact of removing or adding confounders to our regression analyses of our 41 top adolescent CpGs. With this base model, none of the loci showed significant associations between childhood adversity and DNA methylation at age 7. Removing covariates from the primary model resulted in small changes to the effect estimate from the regression model, except for two CpGs on chromosome X (cg17928317; cg27558057), which showed a larger change in effect when sex was not included in the model.
When adding parental socio-economic status at birth (SES parent), gestational age in weeks, maternal pre-pregnancy body mass index (BMI), or delivery method (C-section) to the base model, we again found minor fluctuations in the strength of associations, suggestive of little confounding effects on these associations.
Percent changes in effect estimates are shown for CpGs that no longer met a Bonferroni-adjusted $p<0.05$ (for 41 tests) after covariate removal/addition.

Figure S21. Age at pubertal onset did not mediate childhood adversity-DNAm relationships.



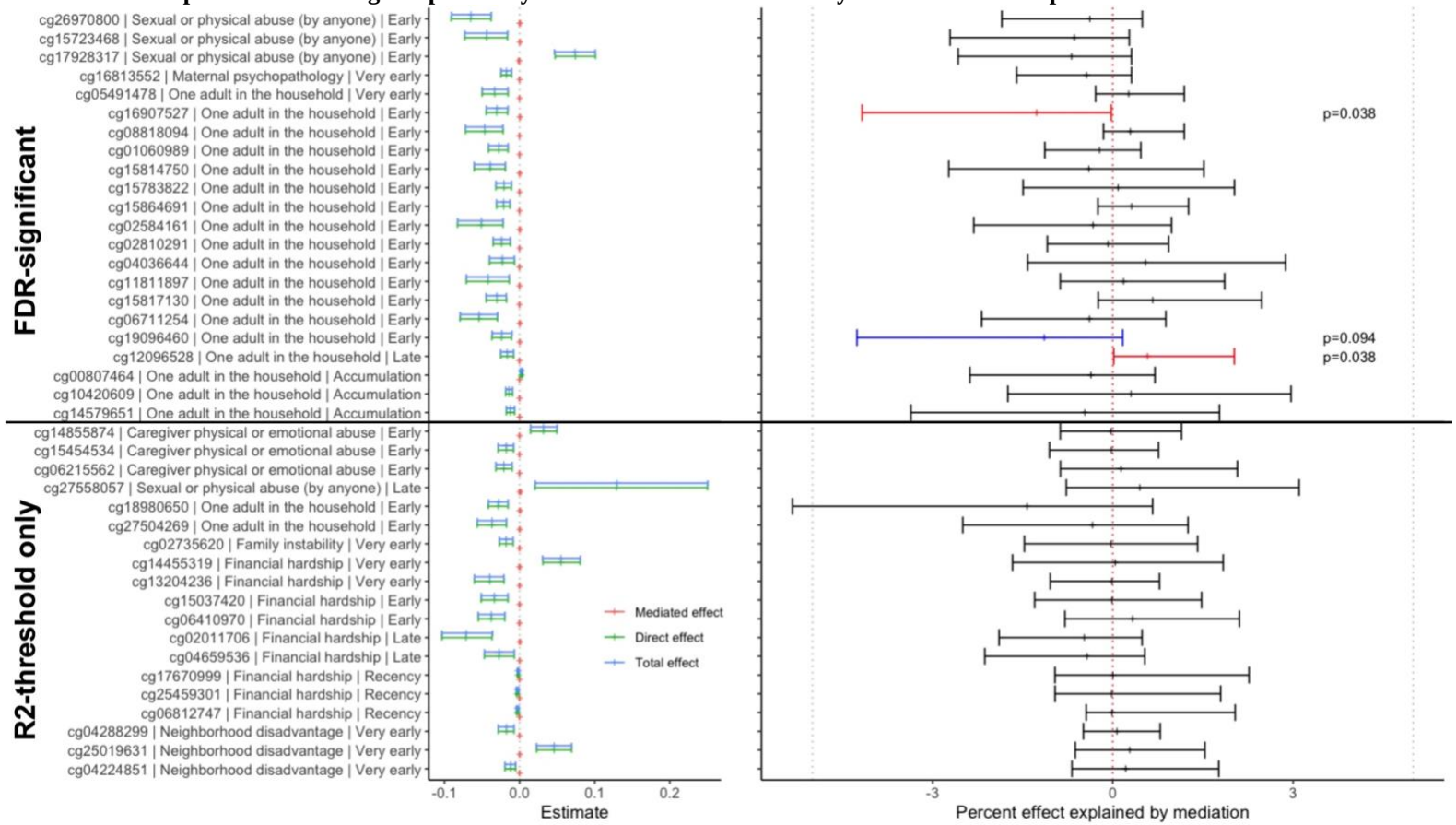
Mediation by the age of pubertal onset, estimated using peak height velocity, was tested for the loci associated with childhood adversity and DNA methylation at age 15. The average causal mediation effect (mediated effect, red; left panel) was close to zero for all CpGs, explaining very little of the association between childhood adversity and DNA methylation levels. None of the estimated mediated effects were significant ($p>0.05$). The lowest p-value belong to cg14455319 ($p=0.268$). Y-axis is noted as "CpG | childhood adversity | SLCMA hypothesis".

Figure S22. Body mass index at age 15 putatively mediated childhood adversity-DNAm relationships.



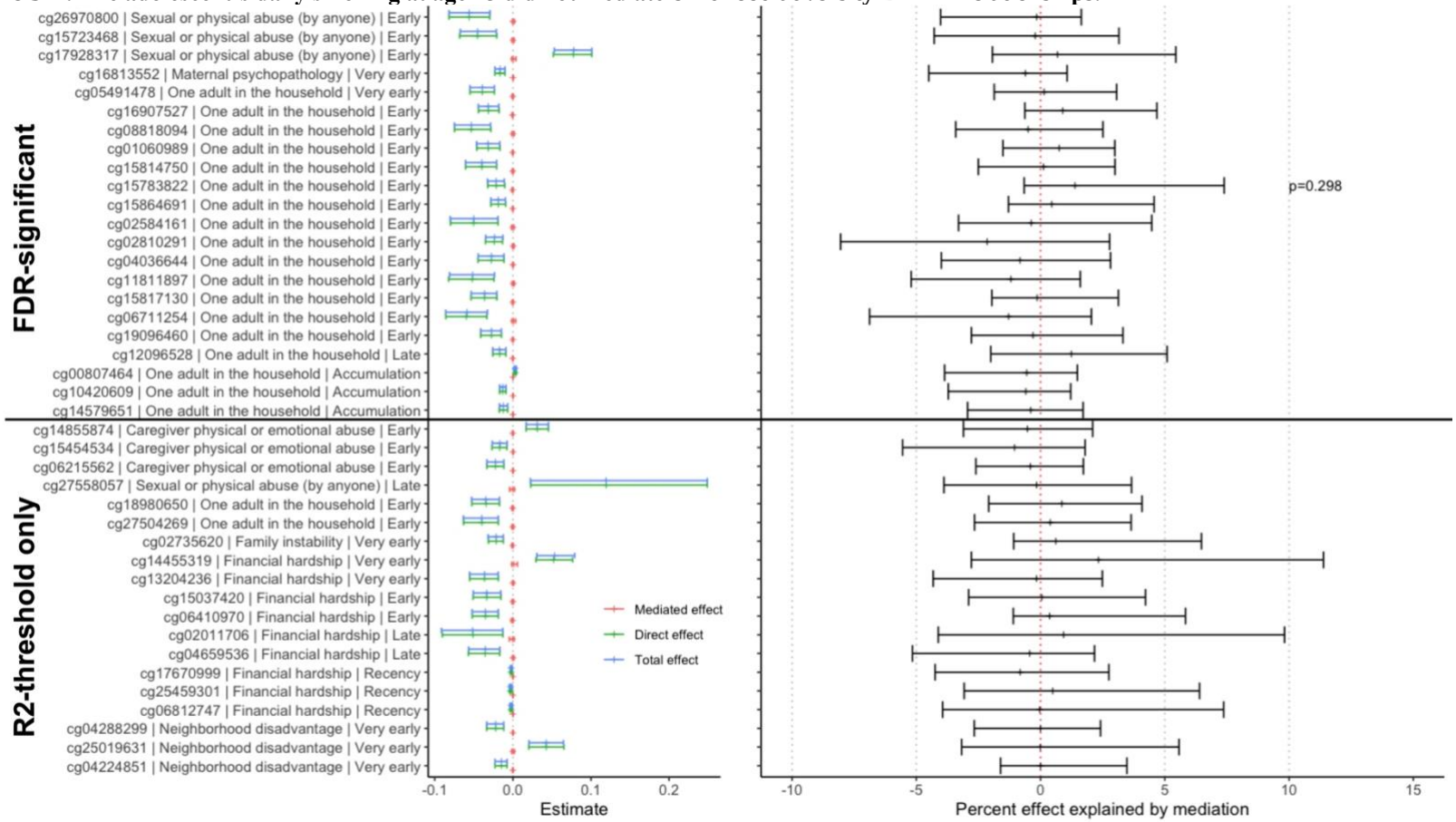
Mediation by body mass index, measured at age 15, was tested for the loci associated with childhood adversity and DNA methylation at age 15. The average causal mediation effect (mediated effect, red; left panel) was near zero for all CpGs, explaining very little of the association between childhood adversity and DNA methylation levels. However, one locus (cg16907527) showed nearly significant mediated effects, explaining 2.67% of the relationship between childhood adversity and DNA methylation (p=0.050). Two other loci showed causal mediation with p<0.1, shown in blue (right panel). No associations were significant after correction for multiple-testing at a false-discovery rate <0.05. Y-axis is noted as "CpG | childhood adversity | SLCMA hypothesis".

Figure S23. C-reactive protein levels at age 15 putatively mediated childhood adversity-DNAm relationships.



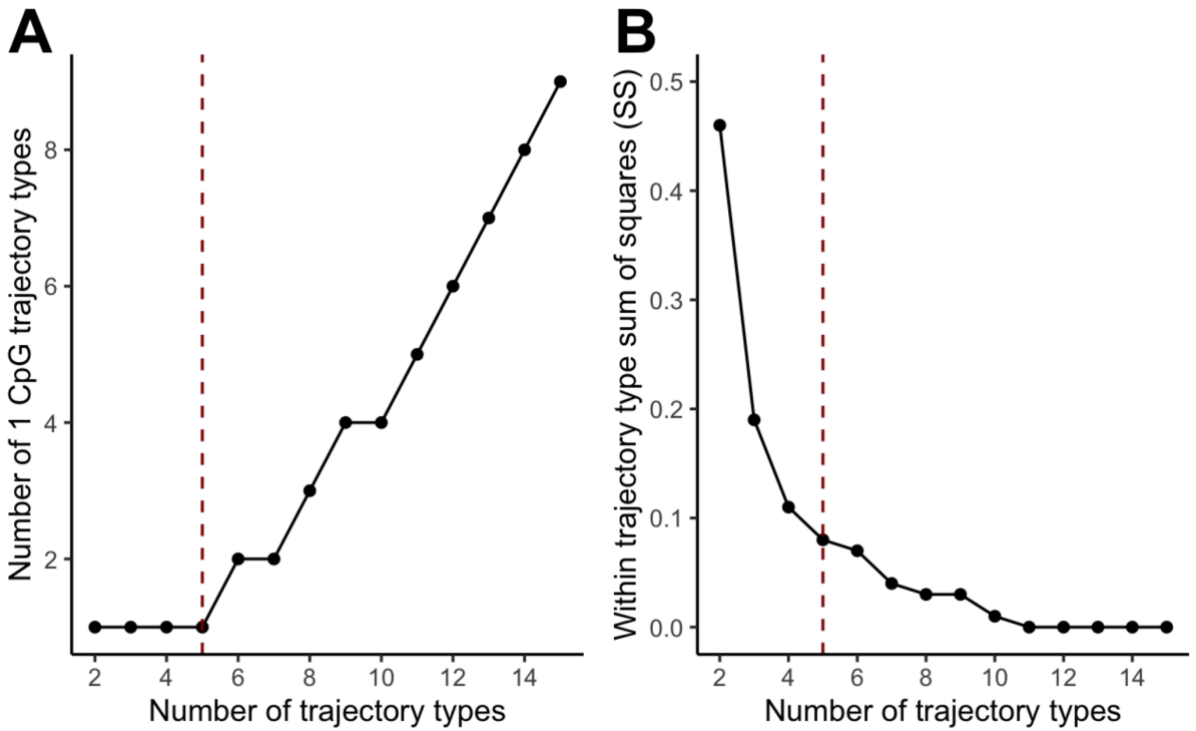
Mediation by the levels of C-reactive protein, measured at age 15, was tested for the loci associated with childhood adversity and DNA methylation at age 15. **A)** The average causal mediation effect (mediated effect, red; left panel) was close to zero for all CpGs, explaining very little of the association between childhood adversity and DNA methylation levels. **B)** Two of the estimated mediated effects were significant ($p<0.05$, red; cg16907527, *VEGFA*, -1.27% relationship explained; cg12096528, *SLC25A41*, -1.14% relationship explained) and one locus showed a putative causal mediation effect with ($p<0.1$, blue; cg19096460, *HERC3*). However, none of these passed multiple-test correction. Y-axis is noted as "CpG | childhood adversity | SLCMA hypothesis".

Figure S24. The adolescent's daily smoking at age 15 did not mediate childhood adversity-DNAm relationships.



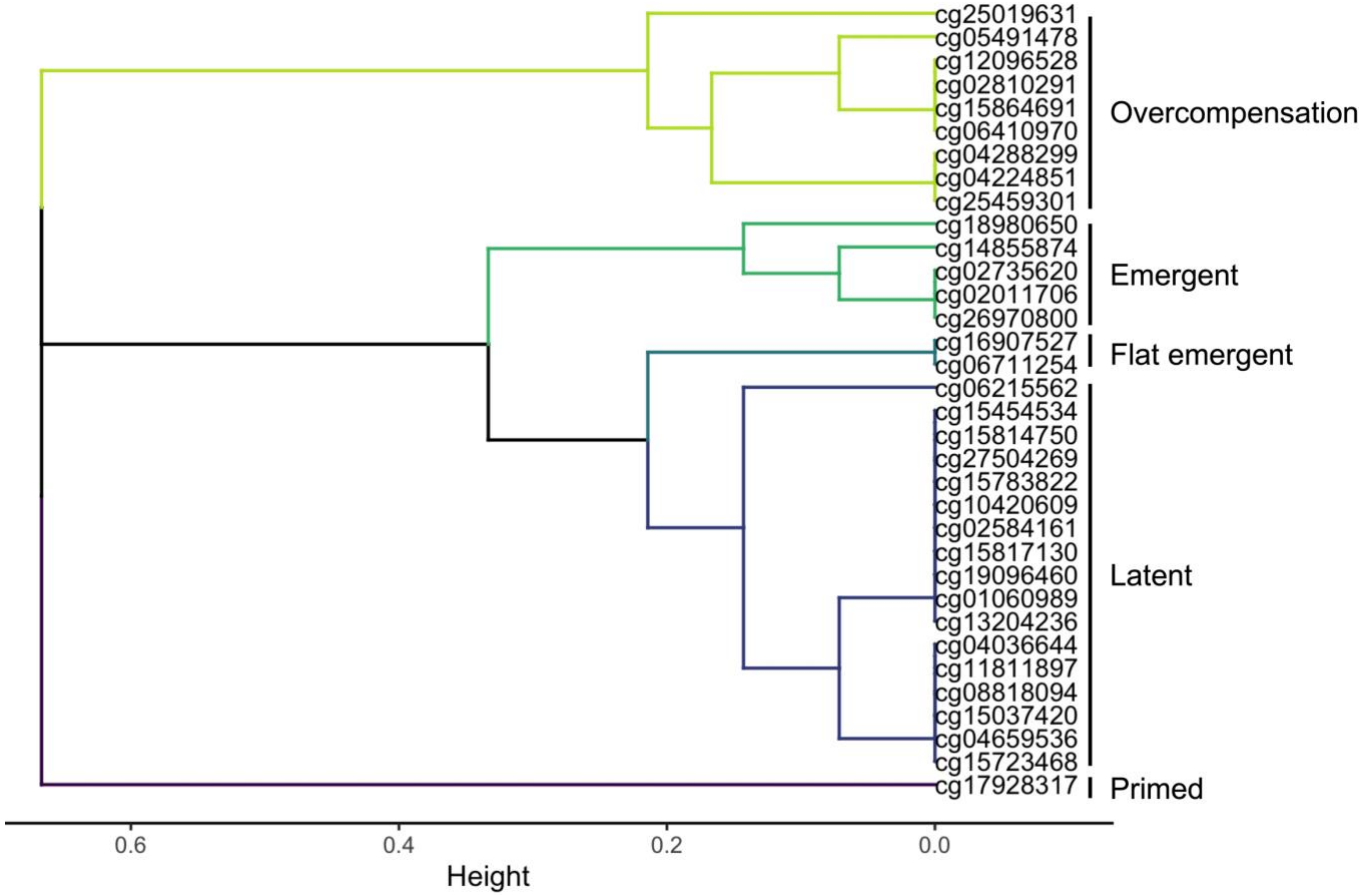
Mediation by smoking behavior at age 15, categorized as the adolescent smoking cigarettes on a daily basis, was tested for the 23 loci significantly associated with childhood adversity and DNA methylation at age 15. The average causal mediation effect (mediated effect, red; left panel) was close to zero for all CpGs, explaining very little of the association between childhood adversity and DNA methylation levels. None of the estimated mediated effects were significant ($p>0.05$). The lowest p-value belonged to cg15783822 ($p=0.298$). Y-axis is noted as "CpG | childhood adversity | SLCMA hypothesis".

Figure S25. Selection metrics for the number of types of DNAm trajectories across development.



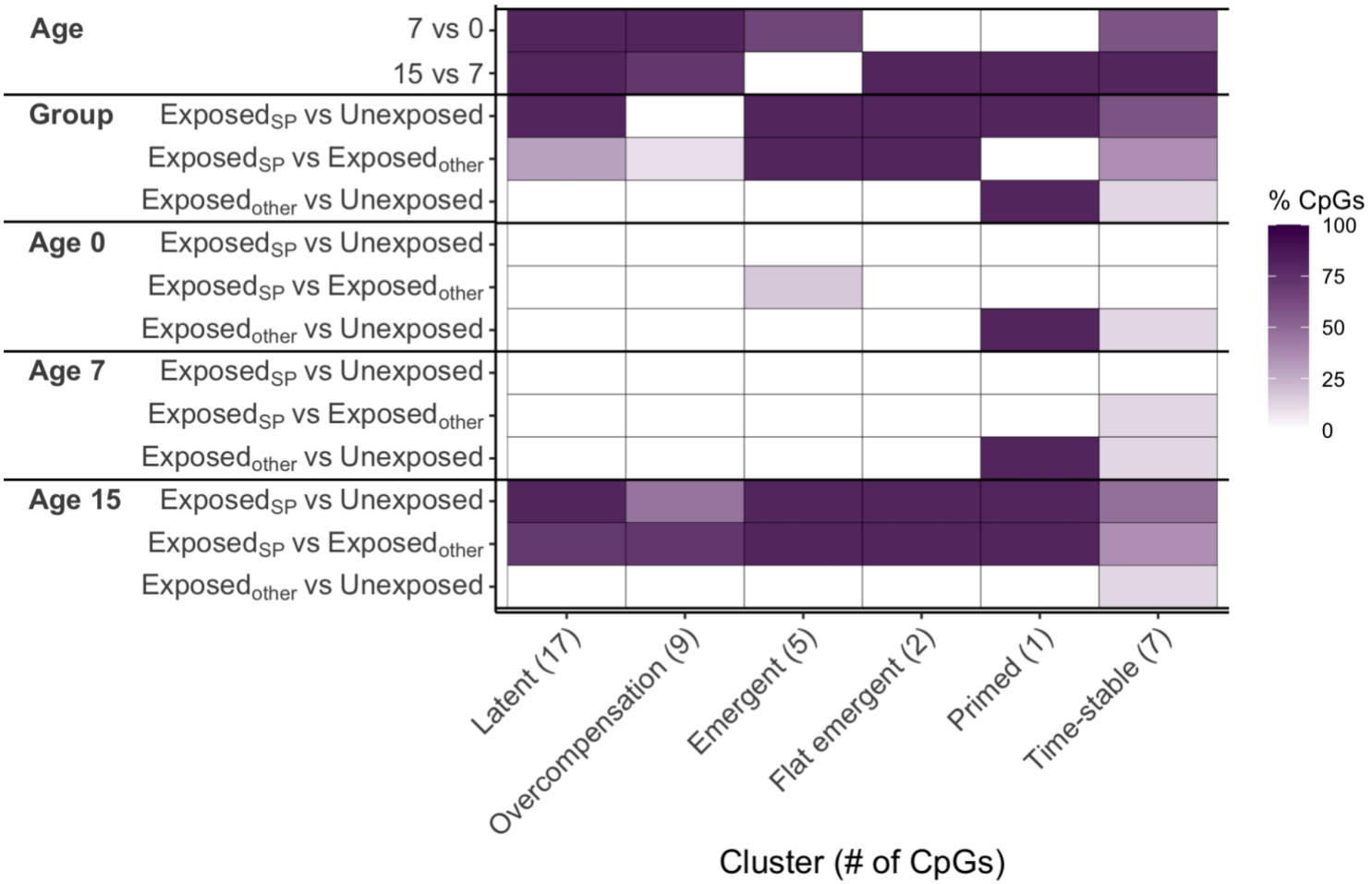
A) Number of trajectory types that were composed of a single CpG, with the x-axis showing the total number of different trajectory types. From the 2 to 5 trajectory solutions, only one trajectory type was composed of a single CpG.
B) The mean within trajectory type sum of squares shown by number of total trajectories, where lower values reflect closer observations within clusters (i.e., more homogenous clusters). This metric showed an almost complete drop-off by the model with 5 trajectory types, suggesting that the good model fit was achieved.
The red dashed line represents the number of total trajectory types selected for final analyses (5), based on the number of trajectory types with single loci and elbow of the minimal sum of squares plot.

Figure S26. Hierarchical clustering of CpGs based on a five-trajectory model.



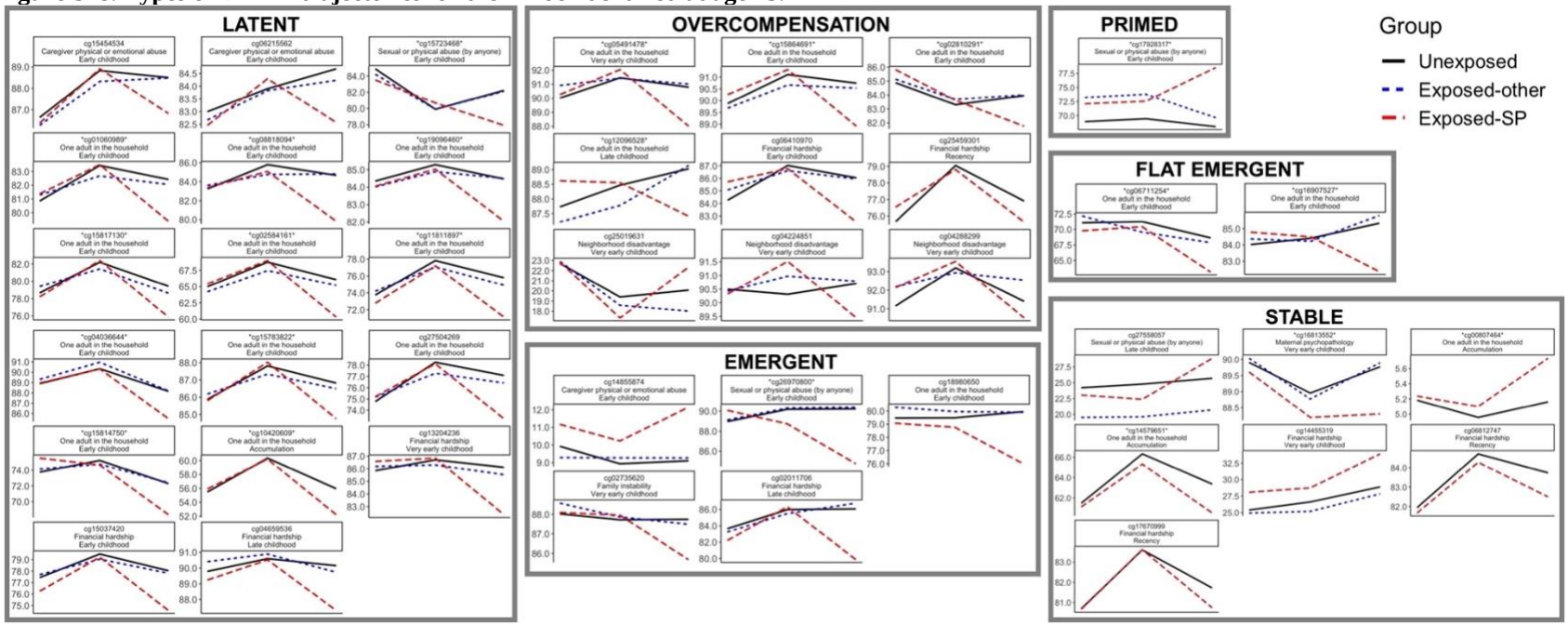
Hierarchical clustering of age 15 loci using Tukey summary statistics for group-by-age interactions revealed five additional types of longitudinal DNAm patterns beyond those that did not show significant group-by-age interactions. These types of trajectories ranged in size from 1 (primed) to 17 CpGs (latent).

Figure S27. Distinguishing features between the six types of DNA methylation trajectories.



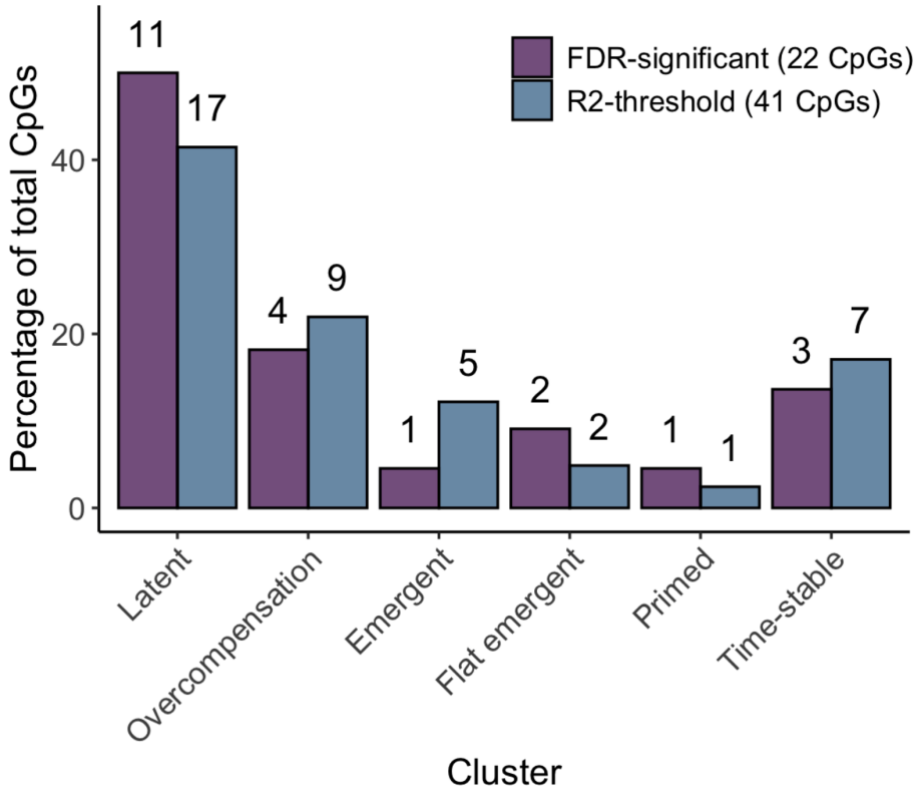
Summary of the significant Tukey summary statistics used to differentiate the six types of DNA methylation trajectories. The fraction of loci with a significant contrast for each type of trajectory is shown (lighter color indicates more loci, or a greater fraction of trajectories). The summary statistics on the y-axis show whether the contrast was significant for: 1) mean differences between ages (age 0, age 7, age 15), 2) mean exposure group differences *across* all ages (exposed during the period identified from the SLCMA [exposed$_{SP}$]; exposed during other period [exposed$_{other}$], or unexposed), and 3) exposure group differences *within* each age.

Figure S28. Types of DNAm trajectories for the 41 loci identified at age 15.



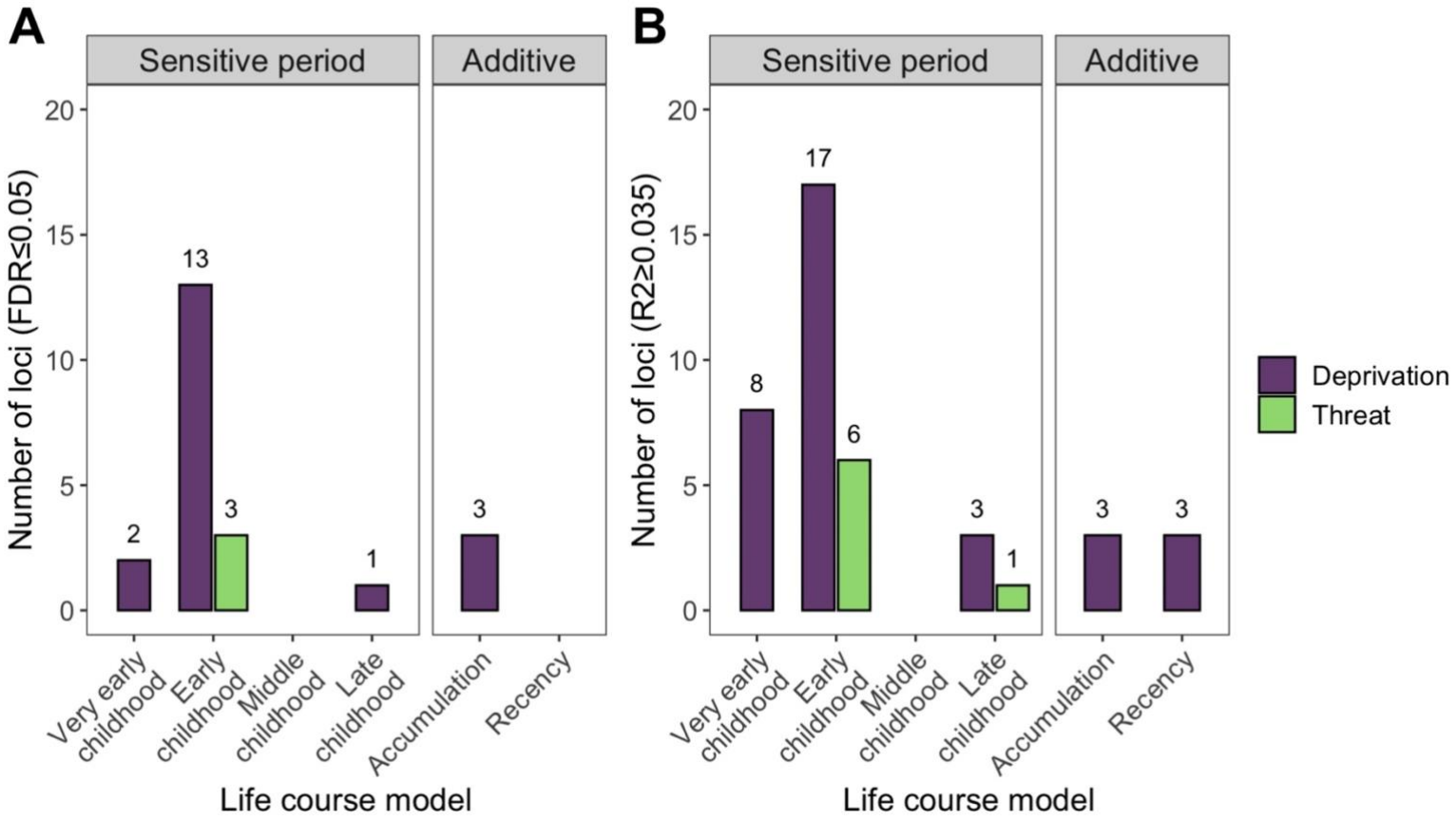
Shown here are the cell-type corrected DNA methylation (DNAm) values on the y-axis and the age at DNAm collection on the x-axis for the 41 loci identified from the SLCMA analyses of age 15 DNAm. Of the 41 loci, seven did not show significant exposure group by age effects (group-by-age effects) and are shown as "Stable". From the 34 loci with significant group by age effects, we identified five distinct types of DNAm trajectories and responses to childhood adversity across development. These DNAm trajectories were identified based on mean exposure group differences *across* ages, mean age differences *across* exposure groups, and exposure group differences at specific ages. Exposure groups were as follows: 1) exposed to adversity *during* the period identified from the SLCMA (exposed-SP; red); 2) exposed to adversity *outside* the period identified from the SCLMA (exposed-other; blue); or 3) unexposed to adversity across development (black). The childhood adversity and hypothesis selected in the SLCMA are shown in the header of each individual plot. Waves of DNAm collection are shown on the x-axis (age 0, 7, and 15 at the inflection points) and percent DNAm is shown on the y-axis.

Figure S29. Types of trajectories based on the significance threshold of top loci.



The fraction of CpGs falling within different types of DNA methylation trajectories across development did both vary based on selection thresholds based on and FDR<0.05 or and $R^2 \geq 0.035$ ($\chi^2=1.92$, p =0.86). However, the were generally more CpGs in the latent class and fewer in the emergent class for the FDR-significant loci compared to the R^2-threshold loci.

Figure S30. Enrichment of top adolescent loci within the threat versus deprivation paradigm.



The life course theoretical models were split by sensitive periods (i.e., exposure to adversity during specific childhood periods) or additive models (i.e., accumulation or recency of exposures). Colors represent the two adversity paradigms, threat versus deprivation. **A)** Of the 22 loci identified at a false-discovery rate (FDR) <0.05, most loci were associated with exposure to deprivation during early childhood. **B)** Of the 41 loci identified at an R2≥0.035 cutoff and $p<1x10^{-5}$ threshold, most associations were again linked to a deprivation exposure, particular during very early and early childhood. Exposures to threat-type adversities were mainly linked to DNAm when they occurred during early childhood.