Supplementary Material

Table S1: Demographics. Demographic data, developmental and behavioral diagnostics, stratified by time point and case-control status. Mean and standard deviation is given for the quantitative measures.

	T0	T1	T2	T3	T4
sample size	173	146	130	111	90
sex maltreated[1]	m=45, f=41	m=36, f=30	m=30, f=30	m=25, f=22	m=21, f=17
sex non-maltreated	m=46, f=41	m=42, f=38	m=36, f=34	m=34, f=30	m=27, f=25
age maltreated[1]	4.36±0.83	4.84±0.85	5.33± 0.81	5.85±0.83	6.39±0.82
age non-maltreated	4.11±0.73	4.60±0.73	5.03±0.74	5.54±0.72	4.04±0.71
number of maltreated[1]	86 (49.7%)	66 (45.2%)	60 (46.2%)	47 (42.3 %)	38 (42.2 %)
and non-maltreated	87 (50.3%)	80 (54.8%)	70 (43.8%)	64 (57.7 %)	52 (57.8 %)
SES[2] maltreated`[1]	9.38±4.46	9.88±4.70	9.75±4.85	9.76±4.63	10.18±4.45
SES non-maltreated	16.10±3.60	16.01±3.50	16.41±3.55	16.40±3.72	15.94±3.83
SON_IQ[3] maltreated[1]	90.09±17.33	-	93.45±17.40	-	95.5±16.74
SON_IQ maltreated	106.32±12.22		109.14±13.00		113.73±13.30
WPPSI[4] maltreated[1]	90.42±13.33	-	92.46±14.24	-	93.37±15.29
WPPSI non-maltreated	105.09±11.96		106.19±9.36		107.69±8.24
CBCL[5] maltreated[1]	54.82±11.39	53.31±10.82	50.53±11.24	51.66±11.18	50.70±12.48
CBCL non-maltreated	41.37±8.97	41.51±9.59	39.1±7.55	41.30±7.76	40.37±8.17
PAPA[6] maltreated[1]	49 (28.3%)	-	36 (20.5%)	-	17 (18.9%)
PAPA[2] non-maltreated	18 (10.4%)		12 (8.2%)		10 (11.1%)

[1] any subject which experienced a maltreatment event of sufficient severity was included in the maltreated group

[2] socio-economic status

[3] SON_IQ is a non-verbal intelligence test, which was used as developmental measure

[4] WPPSI Wechsler preschool and primary scale of intelligence, developmental measure with spoken component

[5] Child behavioral check list, a caregiver report form for problematic behavior

[6] Preschool age psychiatric assessment (PAPA), at least one symptom in the PAPA questionnaire

Table S2: Differentially methylated regions aggregated from linear mixed models (Model 1-3) using all time points (T0-T5). Only regions with more than 5 CpGs and passing multiple testing correction are reported with nominal p-value. No significant (n.s.) hits were found for models 2 (additive model) and 3 (interaction model).

Models	DMRs	# CpGs	p-value	genes	annotation
Time (Model 1)	chr5:126409006-126409311	8	$2.97*10^{-4}$	C5orf63 exon 1	Prenatal phtale exposure
	chr5:178986130-178986831	13	$4.23*10^{-6}$	RUFY1 intron 1	Prenatal lead exposure
	chr6:30095135-30095496	17	$9.83*10^{-9}$	Multiple HLA-REs*	Immune related
	chr7:24323674-24323940	7	$1.24*10^{-3}$	NPY exon 1	Maternal stress, neuroendocrine function
	chr7:63386225-63387148	7	$1.43*10^{-10}$	RP11-73B2.6	Angiogenesis
	chr7:130125763-130125985	7	$3.21*10^{-2}$	MESTIT1 exon 1	Maternal stress
	chr8:22132562-22133357	13	$1.23*10^{-8}$	PIWIL2 exon 1	Prenatal lead exposure
	chr14:24780550-24780891	9	$1.18*10^{-2}$	CIDEB exon 1	Cell death & inflammation
	chr21:45705617-45705743	7	$1.70*10^{-2}$	AIRE exon 1	Autoimmune regulator
Time + Adversity (Model 2)	n.s	-	n.s.		
Time + Maltreatment (Model 2)	n.s	-	n.s.		
Time x Adversity (Model 3)	n.s.	-	n.s		
Time x Maltreatment (Model 3)	n.s.	-	n.s.		

* regulatory element

Table S3: Differentially methylated regions aggregated from linear mixed models (Model 1-3) using the first three time points (T0-T2). Only regions with more than 5 CpGs and passing multiple testing correction are reported with nominal p-value. No significant (n.s.) hits were found for the model Time x Maltreatment.

Models	DMRs	# CpGs	adjusted p-value	gene	annotation
Time (Model 1)	chr1:110254678-110254920	8	$3.18*10^{-3}$	GSTM3/5 enhancer	maternal smoking, FAS[1]
	chr3:182817189-182817627	12	$6.40*10^{-9}$	MCCC1 exon 1	brain volume in schizophrenia
	chr8:144635259-144635611	10	$8.15*10^{-6}$	GSDMS exon 1	pyroptotic cell-death
	chr11:2721242-2721633	12	$2.71*10^{-8}$	KCNQ1 intron 11	neuronal excitability, prenatal arsen exposure
	chr14:106938233-106938452	6	$8.09*10^{-3}$	LINC22001	-
	chr19:57742259-57742445	7	$1.51*10^{-3}$	AURKC exon 1	Prenatal lead & tobacco exposure
	chr20:36148603-36148780	11	$1.59*10^{-3}$	BLCAP intron 1	FAS
Time + Adversity (Model 2)	chr6:31275147-31275808	10	$7.02*10^{-7}$	HLA-B intron 2	Immune related gene
	chr10:42862977-42863595	8	$9.83*10^{-9}$	ZFP91 pseudogene	Cell proliferation / migration
Time + Maltreatment (Model 2)	chr6:31275147-31275808	10	$1.35*10^{-5}$	HLA-B intron 2	Immune related gene
	chr10:42862977-42863595	8	$2.25*10^{-9}$	ZFP91 pseudogene	Cell proliferation / migration
Time x Adversity (Model 3)	chr2:26401597-26402319	10	$1.88*10^{-8}$	GAREML intron 1	FAS, childhood abuse
	chr3:189839037-189839358	7	$3.13*10^{-4}$	P3H2 intron 1	Childhood abuse
	chr19:9785646-9786078	8	$9.32*10^{-4}$	ZNF562 exon 1	Early onset MDD[2]
	chr22:24384104-24384401	8	$1.28*10^{-4}$	GSTT1 exon 1	FAS
Time x Maltreatment (Model 3)	-	-	n.s.		

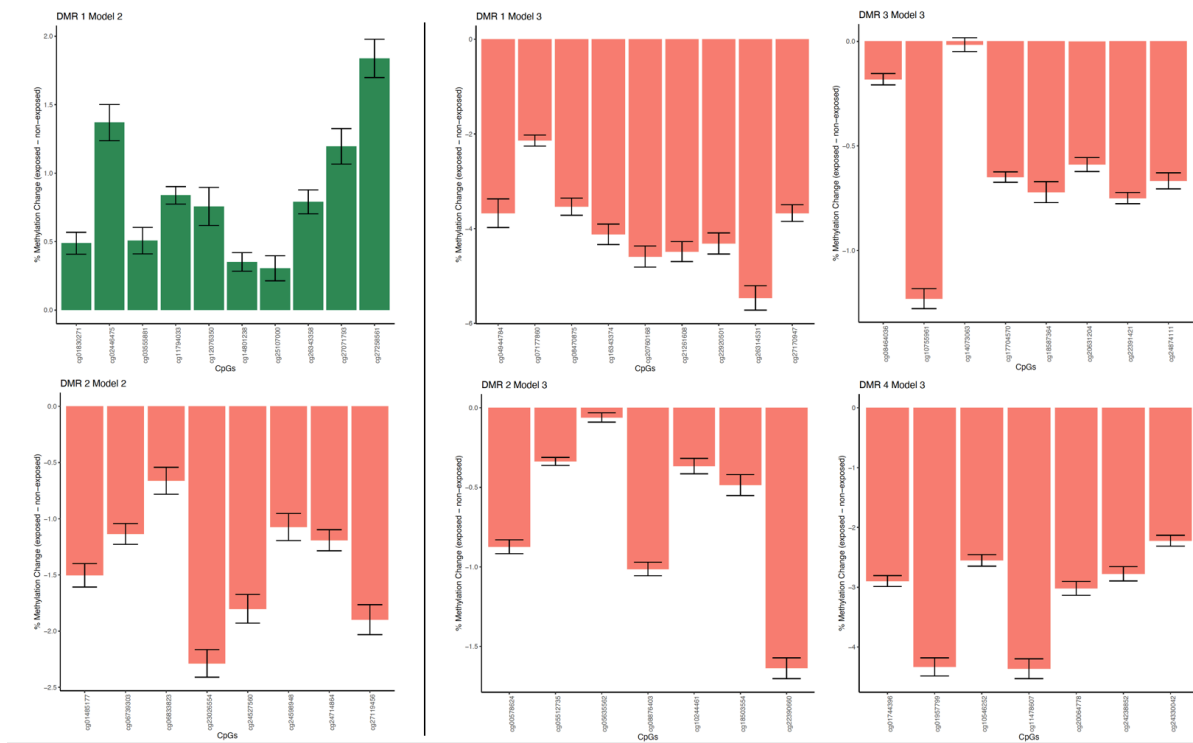[1] Fetal alcohol syndrome
[2] Major depressive disorder

Figure S4: Significantly differentially methylated region by adversity from models 2 and 3 for T0-T2. DMRs obtained from model 2 are shown in the left panel, DMRs from model 3 in the right. Most of the regions are hypomethylated in subjects with high adversity score.

Table S5: Overview of WGCNA modules

Detailed information on associations with exposure variables are given in Figure 5, information on GWAS overlap and enrichment for pathways (KEGG, GO and Reactome) can be found in supplementary table S6 andS7.

Module	#CpGs / genes	Associations	Enrichment
Brown	659 / 637	-	-
Pink	64 / 49	-	-
Blue	22,531 / 8.371	prenatal scores	cell signaling / immune related
Black	91/ 81	-	diabetes type I
Yellow	317 / 143	sex	-
Turquoise	56.344 / 14.736	prenatal scores	-
Green	268 / 164	maltreatment, sex	-
Red	107 / 87	-	-
Grey	2.575 / 1.778	prenatal scores	drug metabolism / immune related

Table S6: Module annotation. CpGs within a module were mapped to genes based on the annotation of the EPIC array and then used with FUMA. Shown here are the top five overlapping GWAS hits per module, where enrichment passed multiple testing correction.

Module*	GWAS	#genes (overlap)	Adjusted p-value
Black	-	-	n.s.
Blue	Heel bone mineral density	389/767	$1.56*10^{-46}$
	Systolic blood pressure	349//746	$2.78*10^{-32}$
	Body-Mass-Index	492/1209	$1.92*10^{-27}$
	Monocyte percentage white cells	100/147	$5.57*10^{-25}$
	Mean platelet volume	132/233	$5.18*10^{-21}$
Brown	Type II diabetes	248/458	$6.94*10^{-5}$
	Systolic blood pressure	36/746	$2.44*10^{-4}$
	Modic change	4/7	$1.92*10^{-3}$
	Atrial fibrillation	16/221	$1.92*10^{-3}$
	Asthma	18/311	$6.15*10^{-3}$
Green	-	-	n.s.
Grey	Pneumonia	8/8	$3.01*10^{-8}$
	Drug induced liver injury	9/13	$5.48*10^{-7}$
	IgE levels	10/18	$9.39*10^{-7}$
	Asthma	43/311	$9.39*10^{-7}$
	Neuromyelitis optica	7/9	$4.43*10^{-6}$
Pink	-	-	n.s.
Red	-	-	n.s.
Yellow	-	-	n.s.

* the turquoise module was not annotated as it contained over 50% of the CpGs from the analysis

Table S7: Module annotation. Reported here are the top five pathways per module from the functional annotation analysis

Module*	Pathway	#genes (overlap)	Adjusted p-value
Black	-	-	n.s.
Blue	Chemokine signaling	91/177	$4.31*10^{-11}$
	Focal adhesion	96/195	$1.82*10^{-10}$
	Regulation of actin skeleton	98/205	$7.40*10^{-10}$
	Leukocyte migration	60/111	$1.17*10^{-8}$
	B-cell receptor signaling	42/72	$1.61*10^{-7}$
Brown	Diabetes Mellitus Type I	6/40	$1.93*10^{-2}$
	Allograft rejection	5/34	$2.71*10^{-2}$
Green	-	-	n.s.
Grey	Diabetes Mellitus Type I	11/40	$3.40*10^{-4}$
	Allograft rejection	10/34	$3.40*10^{-4}$
	Autoimmune Thyroid disease	11/49	$1.18*10^{-3}$
	Drug metabolism cytochrome p450	12/59	$1.25*10^{-3}$
	Antigen processing	13/75	$2.97*10^{-3}$
Pink	-	-	n.s.
Red	-	-	n.s.
Yellow	-	-	n.s.

* the turquoise module was not annotated as it contained over 50% of the CpGs from the analysis

Supplementary data S8: List of unique genes in the green module. The 265 CpGs grouped into the green module mapped to 164 genes. Information on the CpGs and the mapping to genes are listed in the file Supplementary_Data_Table_S8.xlsx.
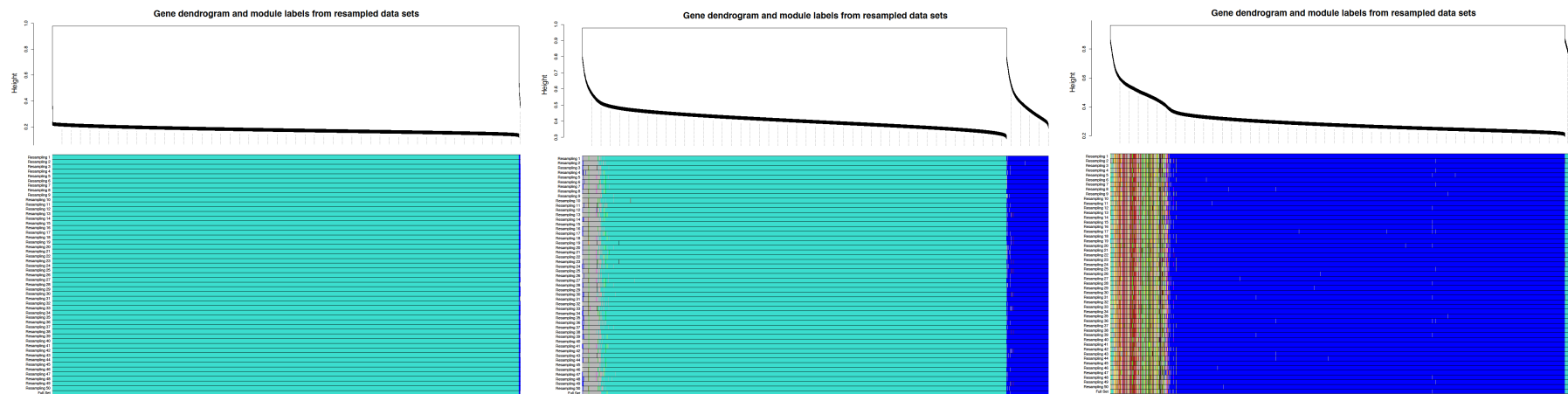
Figure S9: Module stability analysis. Block-wise module construction with maximum module size of 10,000 (due to computational complexity) was repeated (n=50) with 66% of the samples. The full data set was computed using 3 blocks at maxBlockSize of 30,000. Shown here are the dendrograms (blocks1-3) of the full data set with the matched assignments of the resampled networks.

Comment S10: Comparison of beta value and M-values as input.

We were concerned that the distribution of beta-values impacted the module detected of WGCNA. We found significant differences between the methylation levels of the different modules ($p < 2.2*10^{-16}$), and differences for the MAD-score ($p=1.95*10^{-15}$) (Figure S15). Therefore, we re-ran WGCNA with z-standardized beta-values. Here, we found no significant differences between the modules (lowest p=0.24). However, the scale-free topology of the M-values was not as good as when using beta-values (Figure S16) and never reached a good fit (r^2 of 0.8 or above). Therefore, we decided to continue the analyses with the modules identified from the beta-values. The comparison was performed with power 6 for both data sets, as this is the recommended power for signed networks.
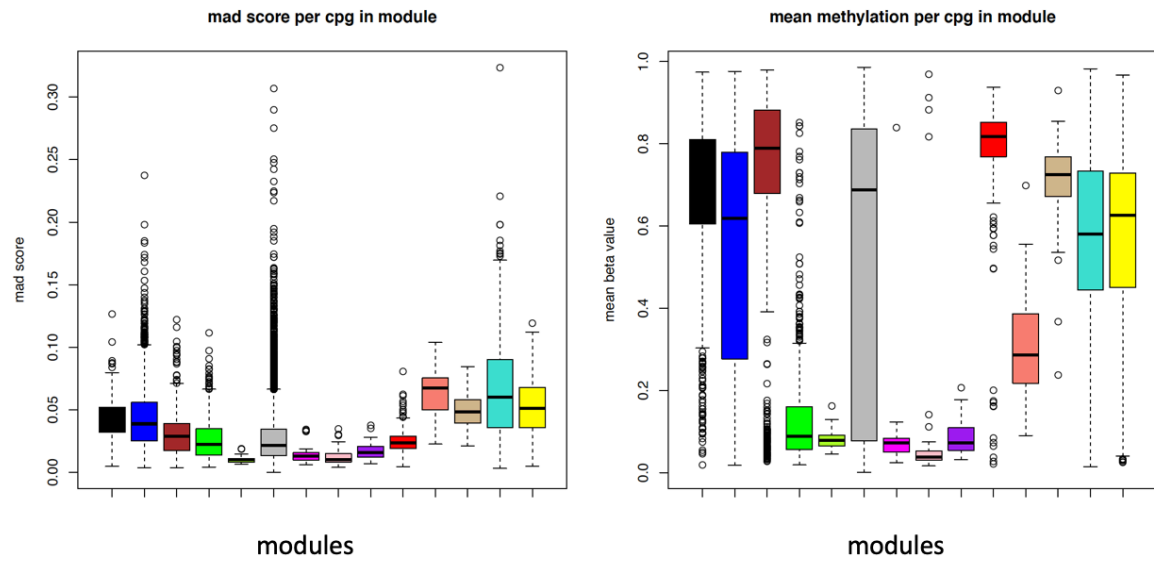
Figure S11: Mean methylation and MAD score by module. In order to investigate if the distribution of beta-values impacted the module detection we checked if there were significant differences in MAD scores (left) and methylation levels (right) between the modules detected.
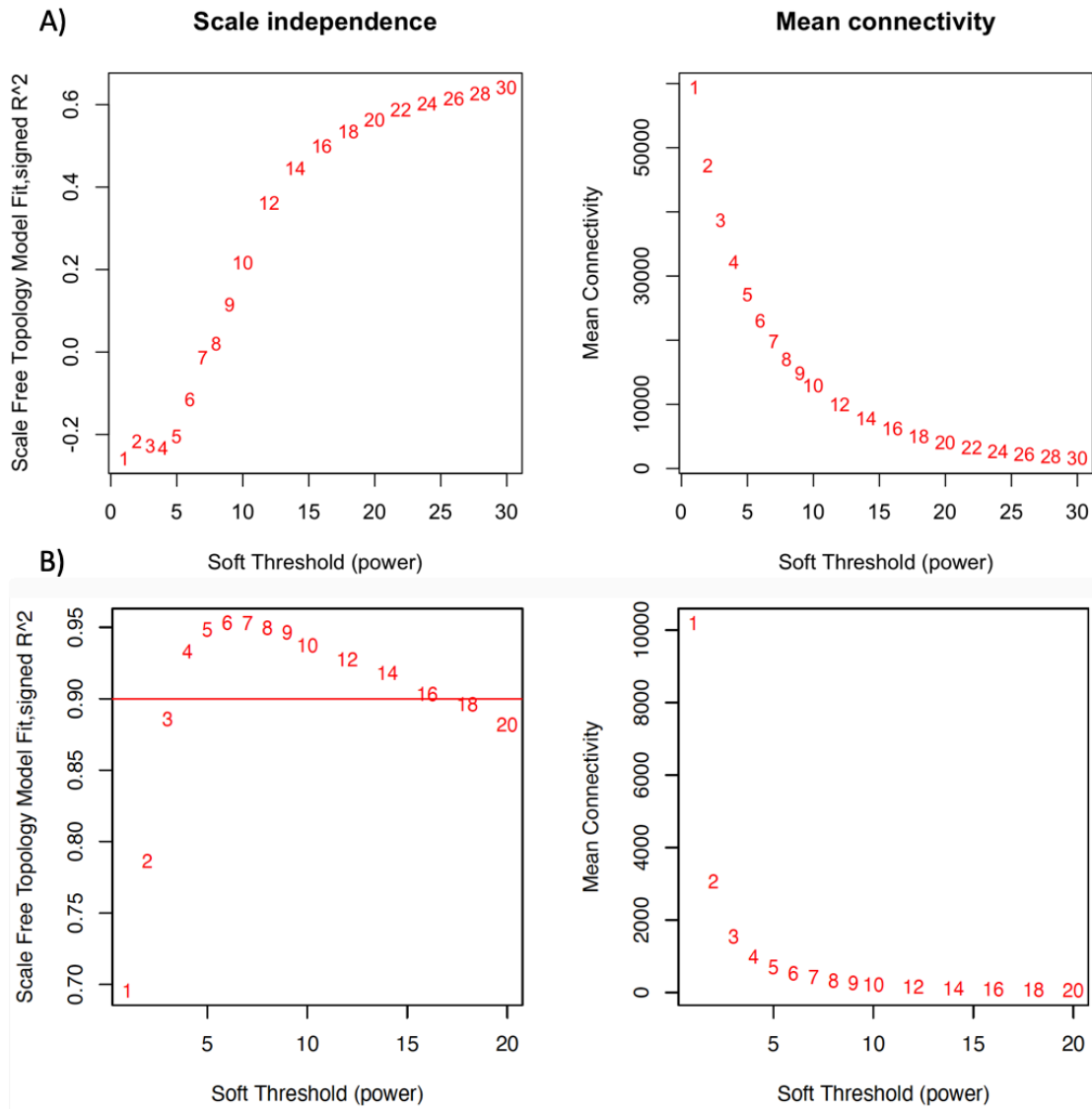
Figure S12: scale-free topology fit. Comparison of scale-free topology fit (left) and mean connectivity (right) for standardized M-values (A) and beta values (B). The first power to reach a r^2 of 0.8 with a mean connectivity in the hundreds are considered good.