### Supplementary Table S1: Phenotypic characteristics of mothers and infants:

### Supplementary Table S1a: Phenotypic assessment of mothers and infants at two time points early in life

| Exposure to early life stress (ELS) | Prenatal / 3rd trimenon                                      | Perinatal / birth                                      |
|-----------------------------------|-------------------------------------------------------------|--------------------------------------------------------|
| Perceived stress (PSS)            | Pre- and perinatal complications                            | Perinatal stressors (e.g. asphyxia, caesarian, preterm birth) |
| Prenatal distress (PDQ)           | Perinatal stressors (e.g. asphyxia, caesarian, preterm birth) |
| Life events (LES)                 | Pregnancy & obstetric history (birth weight, gestational age, birth complications) |
| Social support (Soz-U)            | Maternal health risk behavior (e.g. smoking)                |                                                        |
| Socio-demographic data            | Psychosocial risks                                         |                                                        |
| Maternal health risk behavior (e.g. smoking) | Perinatal stressors (e.g. asphyxia, caesarian, preterm birth) |

| Maternal mental & physical health | Maternity log data                                          | Depression screening (EPDS)                            |
|----------------------------------|-------------------------------------------------------------|--------------------------------------------------------|
|                                  | semi-standardized neuropsychiatric diagnostic interview (MINI) | Anxiety screening (STAI-S, STAI-T, ASQ)                |
|                                  | Depression screening (EPDS)                                 | Anxiety screening (STAI-S, STAI-T, ASQ)                |
|                                  | Anxiety screening (STAI-S, STAI-T, ASQ)                     | Anthropometry                                          |
|                                  | Anthropometry                                              | Individual & family history of metabolic and other medical disorders |

PSS = perceived stress scale; PDQ = prenatal distress questionnaire; LES = life experiences survey; Soz-U = social support questionnaire; M.I.N.I. = Mini-international neuropsychiatric interview; EPDS = Edinburgh postnatal depression scale; STAI-S & STAI-T = state-trait anxiety inventory; ASQ = anxiety screening questionnaire
Supplementary Table S1 b: Demographic characteristics and general medical status of mothers and infants included in the methylome analysis (all data: mean ± SD or percentage)

| Variable                                    | High prenatal ELS (n = 10) | Low prenatal ELS (n = 8) | p value |
|---------------------------------------------|----------------------------|--------------------------|---------|
| Maternal Age (in years)                     | 24.10 ± 5.43               | 34.00 ± 3.30             | 0.000   |
| Smoking during early pregnancy (4th to 12th wpma; %) | 70 %                       | 12.5 %                   | 0.013   |
| Cigarettes in total (4th to 12th wpma)     | 297.70 ± 412.68 0-1092    | 3.00 ± 8.50 0-24         | 0.05    |
| Smoking during late pregnancy (3rd trimenon, %) | 40 %                       | 0 %                      | 0.037   |
| Cigarettes per day (3rd trimenon)          | 3.30 ± 6.31 0-17           | 0                         | ns      |
| Alcohol intake during early pregnancy (4th to 12th wpma; %) | 30 %                       | 75 %                     | ns      |
| Total alcohol intake (4th to 12th wpma in g) | 75.50 ± 187.18             | 23.50 ± 22.62            | ns      |
| Alcohol during late pregnancy (3rd trimenon; %) | 0%                         | 0 %                      | ns      |
| Primiparous                                 | 30 %                       | 37.5 %                   | ns      |
| Number of risk factors in the maternity log | 4.60 ± 2.38                | 3.17 ± 1.51              | ns      |
| Pre-Pregnancy BMI                           | 25.53 ± 7.08               | 21.69 ± 4.57             | ns      |
| Gestational diabetes (%)                   | 20 %                       | 0%                       | ns      |
| Gestational age at birth (wpma)            | 38.70 ± 2.00               | 39.63 ± 1.60             | ns      |
| Infant’s Gender (%), male                  | 50 %                       | 37.5 %                   | ns      |

wpma = weeks postmenstrual age; BMI = body mass index; SD = standard deviation; ns = not significant; % = percentage; g = gram
Supplementary Table S1 c: **Psychopathology, socioeconomic-, psychosocial- and perceived stress of the extreme group mothers** (all data: mean ± SD or percentage)

| Variable                                    | High prenatal ELS (n =10) | Low prenatal ELS (n =8) | p value |
|---------------------------------------------|----------------------------|-------------------------|---------|
| **Maternal psychopathology**                |                            |                         |         |
| EPDS Score¹                                 | 15.40 ± 4.95               | 2.13 ± 1.25             | 0.000   |
| STAI-S Score¹                               | 52.60 ± 13.32              | 32.62 ± 4.81            | 0.001   |
| STAI-T Score¹                               | 50.90 ± 10.50              | 28.75 ± 4.53            | 0.000   |
| ASQ Score¹                                  | 5.40 ± 1.84                | 0.25 ± 0.46             | 0.000   |
| M.I.N.I. Diagnosis²                         |                            |                         |         |
| none                                        | 20 %                       | 100%                    | 0.000   |
| depressive disorders                        | 50 %                       | 0%                      | 0.015   |
| anxiety disorder                            | 10 %                       | 0%                      | ns      |
| **Current psychiatric disorder² (%)**       |                            |                         |         |
| none                                        | 30%                        | 100%                    | 0.001   |
| depressive disorder                         | 50%                        | 0%                      | 0.015   |
| anxiety disorder                            | 10 %                       | 0%                      | ns      |
| **Perceived stress**                        |                            |                         |         |
| PSS Score¹                                  | 32.70 ± 6.93               | 15.25 ± 3.92            | 0.000   |
| PDQ Score¹                                  | 23.70 ± 8.06               | 8.63 ± 4.37             | 0.000   |
| **Socioeconomic and psychosocial stress**   |                            |                         |         |
| LES-negative events Score¹                  | 8.50 ± 6.98                | 1.63 ± 1.06             | 0.013   |
| Soz-U Score¹                                | 37.80 ± 10.68              | 48.00 ± 6.78            | 0.026   |
| Living without a partner² (%)               | 40 %                       | 0%                      | 0.037   |
| Encouragement (Partner)² (%)                | 70 %                       | 100%                    | ns      |
| Separation(s) in the last year²             | 50 %                       | 0%                      | 0.015   |
| Daily arguments²                            | 20 %                       | 0%                      | ns      |
| Physical conflicts within the preceeding 12 months² | 60 %                       | 0%                      | 0.005   |
| Composition of household >one person /room³ | 30 %                       | 0%                      | 0.037   |
| No graduation² (%)                          | 20 %                       | 0%                      | ns      |
| No professional education\(^2\) (%) | 40 % | 0% | 0.037 |
|-------------------------------------|------|----|-------|
| Monthly income per household ≤ 1750 Euro\(^2\) (%) | 70 % | 0% | 0.001 |
| Financial debt\(^2\) (%) | 50 % | 0% | 0.015 |

SD = standard deviation; ns = not significant
\(^1\) the first eight main variables of the principal component analysis (PCA)
\(^2\) the twelve prenatal stressors which generate the adversity score as the ninth main variable of the PCA
Supplementary Table S2: Sequences of primers used for validation of enrichment of methylated DNA, for quantitative PCR (QPCR) and pyrosequencing validation of MeDIP data:

| Human: Validation of the enrichment of methylated DNA: | Validation of MORC1 methylation: |
|-----------------------------------------------------|---------------------------------
| methylated control (H19): forward: 5’- GAGCCGCACCAGATCTTCAG-3’ reverse: 5’-TTGGTGGAACACACTGTGATCA-3’ | QPCR: forward: 5’- TGGGTGAGTGGGATGTTTT - 3’ reverse: 5’- CCGGTGCTCTCCGATAGTA - 3’ |
| unmethylated control (β-actin): forward: 5’- CCAACGCCAAAAACTCTCCC-3’ reverse: 5’- AGCCATAAAAGGCAACTTTCG-3’ | Pyrosequencing: Amplification: forward-1: 5’- TGGGTTTTAAAAAAGGTTTGGAAAGTTATTG -3’ reverse-1: 5’- TATTTTAAATTCCCTCCCC - 3’ forward-2: 5’- GTGGTTGTGAGTGTTGGATGTGGTTGATAGTA - 3’ reverse-2: 5’- TCTCACAATAACACCCCTCCCTCTA - 3’ forward-3: 5’- TGGGAATTAGGAGGAAGAAGA - 3’ reverse-3: 5’- CATCACCACATCAACCACTATT - 3’ Sequencing: CpG 1: 5’- TTATTGTATAAGATTATTAAAAATG - 3’ CpG2 and 3: 5’- ATTTTTAGTTTTAATTAAAAATG - 3’ CpG 4: 5’- TTTTTTTTATAAGATTAAAT - 3’ CpG 5: 5’- AATTTTTTTTAAGTAATTTTGAG - 3’ CpG 6: 5’- AAGAGAGAATTTGAATGG - 3’ |

| Primates: Validation of the enrichment of methylated DNA: | Validation of MORC1 methylation: |
|-----------------------------------------------------|---------------------------------
| methylated control (H19): forward: 5’- TTGGTGGAACACGCTGATCA-3’ reverse: 5’- GAGCCGCACCAGGTCTTCAG-3’ | forward – 1: 5’- GGACTCGATGCAAATCCCTG - 3’ reverse – 1: 5’- CTTCGCTTAGTCTCCCCTA - 3’ forward – 2: 5’- AGGGATTGGTGTGGGTAGATAAA - 3’ reverse – 2: 5’- GCTTGTGAGTAGAAGAAACCACAT - 3’ |
| unmethylated control (GAPDH): forward: 5’- TTTCTTTCCTTTCGCGTCTCG-3’ reverse: 5’- CCATTTATTTCCCTTCCGGTT-3’ | |
Rats: Validation of the enrichment of methylated DNA:

methylated control (H19):
forward: 5´- CCAAGACAGAAGGGGACCAT-3´
reverse: 5´- TAGATTTGGGTTCCGCTGT-3´

unmethylated control (β-actin):
forward: 5´- TGGGATAGTGTCACAAAGGG -3´
reverse: 5´- GAAGAGTTTGGCGATGGGTG -3´

Validation of MORC1 methylation:

forward: 5´- TAGTGGTAGACGTGGTCTG - 3´
reverse: 5´- TCAGGTCGAGCTTGAAGACA - 3´

Supplementary Table S3: List of genes associated with the 25 top differentially methylated probes in the ELS extreme group analyses:

Supplementary Table S3a: List of genes associated with the 25 top differentially methylated probes in the ELS extreme group analysis of human CD34+ cells

| Gene names | More methylated in | p value* |
|------------|--------------------|----------|
| KRTAP10-3  | low ELS            | 7.04E-09 |
| (4 probes) |                    |          |
| MIR889     | low ELS            | 0.00062  |
| (2 probes) |                    |          |
| AL591025.1 | low ELS            | 0.00062  |
| (4 probes) |                    |          |
| MIR539     | low ELS            | 0.00062  |
| (3 probes) |                    |          |
| SYNE2      | low ELS            | 0.00062  |
| (3 probes) |                    |          |
| B3GAT2     | high ELS           | 0.00077  |
| (5 probes) |                    |          |
**Supplementary Table S3b: List of genes associated with the 25 top differentially methylated probes in CD3+ T cells observed in the comparison of maternally reared (MR) and surrogate-peer reared (SPR) primates at postnatal days 14 - 30**

| Gene names | More methylated in | p value* |
|------------|--------------------|----------|
| LOC705164  | MR                 | 2.44E-07 |
| (7 probes) |                    |          |
| CALML5     | MR                 | 2.44E-07 |
| (9 probes) |                    |          |
| LOC694416  | MR                 | 2.44E-07 |
| (9 probes) |                    |          |

* FDR corrected

**Supplementary Table S3c: List of genes associated with the 25 top differentially methylated probes in CD3+ T cells observed in the comparison of MR and SPR 2 year old primates**

| Gene names | More methylated in | p value* |
|------------|--------------------|----------|
| UROD       | MR                 | 9.05 E-06|
| (6 probes) |                    |          |
| MYOM2      | MR                 | 1.08E-05 |
| (2 probes) |                    |          |
| U7         | MR                 | 1.08E-05 |
| mml-mir-1240| MR               | 1.13E-05|

* FDR corrected
**Supplementary Table S3d: List of genes associated with the 25 top differentially methylated probes in the prefrontal cortex (PFC) observed in the comparison of control (Ctrl) and prenatal stress exposed (PS) male rats at PND62**

| Gene names | More methylated in | p value* |
|------------|--------------------|----------|
| GNAS3      | PS                 | 3.22E-07 |
| (2 probes) |                    |          |
| Asb10      | PS                 | 3.22E-07 |
| (6 probes) |                    |          |
| Kcnk2      | PS                 | 9.44E-06 |
| (13 probes)|                    |          |
| Fam183b    | Ctrl               | 1.68E-05 |
| (4 probes) |                    |          |

* FDR corrected
Supplementary Table S4: Genes showing differential methylation in response to ELS in human cord blood CD34+ cells and in the CD3+ T cells of 14 - 30 day and 2 year old monkeys:

| Gene names | Human CD34 | Day 14 - 30 old monkey CD3+ T cells | 2 year old monkey CD3+ T cells |
|------------|------------|-------------------------------------|-------------------------------|
|            | More methylated in | More methylated in | More methylated in |
| 7SK        | Mixed       | MR                                 | Mixed                          |
| AADACL4    | low ELS     | MR                                 | MR                            |
| ABI3BP     | high ELS    | MR                                 | MR                            |
| ABLIM1     | low ELS     | MR                                 | MR                            |
| ACESL5     | high ELS    | MR                                 | MR                            |
| ACVRL1     | high ELS    | MR                                 | MR                            |
| ADAM10     | low ELS     | MR                                 | MR                            |
| ADARB2     | low ELS     | MR                                 | MR                            |
| AMPD3      | low ELS     | SPR                                | MR                            |
| ANK1       | Mixed       | MR                                 | MR                            |
| ARHGAP18   | low ELS     | MR                                 | MR                            |
| ARHGAP25   | high ELS    | MR                                 | SPR                           |
| ARHGAP6    | high ELS    | MR                                 | SPR                           |
| ARHGEF10   | low ELS     | MR                                 | MR                            |
| ARHGEF2    | low ELS     | MR                                 | MR                            |
| BIN3       | low ELS     | SPR                                | SPR                           |
| C2orf43    | low ELS     | MR                                 | MR                            |
| C9orf3     | low ELS     | SPR                                | MR                            |
| CACNA1C    | low ELS     | MR                                 | MR                            |
| CAST       | high ELS    | SPR                                | SPR                           |
| CCDC19     | low ELS     | MR                                 | MR                            |
| CD248      | low ELS     | SPR                                | MR                            |
| CDH6       | high ELS    | SPR                                | SPR                           |
| CDK17      | high ELS    | MR                                 | MR                            |
| CELF3      | high ELS    | SPR                                | MR                            |
| CEP350     | low ELS     | MR                                 | MR                            |
| CHMP5      | high ELS    | MR                                 | MR                            |
| CHN2       | low ELS     | MR                                 | SPR                           |
| CMA1       | high ELS    | SPR                                | SPR                           |
| CNIH       | low ELS     | MR                                 | MR                            |
| COL4A6     | high ELS    | SPR                                | MR                            |
| CSRNP3     | high ELS    | SPR                                | SPR                           |
| CTNNBL1    | high ELS    | SPR                                | SPR                           |
| CTNN       | low ELS     | MR                                 | MR                            |
| CYFIP2     | high ELS    | MR                                 | MR                            |
| DAAM1      | high ELS    | SPR                                | MR                            |
| DCAF11     | low ELS     | MR                                 | MR                            |
| DHDDS      | high ELS    | MR                                 | MR                            |
| Gene      | Expression   | Tissue 1 | Tissue 2 |
|-----------|--------------|----------|----------|
| DLGAP2    | low ELS      | MR       | MR       |
| DNAH6     | high ELS     | SPR      | SPR      |
| DNAH8     | low ELS      | MR       | SPR      |
| DOCK5     | low ELS      | MR       | MR       |
| EIF1B2    | low ELS      | MR       | MR       |
| ENSF4E3   | high ELS     | MR       | MR       |
| ENMX2     | high ELS     | SPR      | MR       |
| EPB41L1   | low ELS      | MR       | MR       |
| ER3       | low ELS      | MR       | Mixed    |
| EXOC2     | low ELS      | MR       | MR       |
| EYS       | Mixed        | MR       | SPR      |
| FAM26F    | high ELS     | SPR      | SPR      |
| FBXO48    | low ELS      | MR       | SPR      |
| FGD4      | high ELS     | MR       | Mixed    |
| FGF1      | high ELS     | MR       | MR       |
| FOXA2     | high ELS     | SPR      | SPR      |
| FOXP1     | high ELS     | SPR      | MR       |
| FRMD3     | high ELS     | SPR      | MR       |
| FRMD4A    | Mixed        | MR       | MR       |
| G3BP2     | low ELS      | MR       | MR       |
| GCA       | high ELS     | SPR      | SPR      |
| GLYCTK    | low ELS      | MR       | MR       |
| GORAB     | high ELS     | MR       | MR       |
| GRM2      | high ELS     | SPR      | MR       |
| GUCA1C    | high ELS     | MR       | MR       |
| HAPLN1    | high ELS     | SPR      | SPR      |
| HBXIP     | high ELS     | SPR      | MR       |
| HDGFL1    | low ELS      | MR       | Mixed    |
| HECW1     | high ELS     | MR       | MR       |
| HKDC1     | low ELS      | MR       | MR       |
| HLX       | low ELS      | MR       | MR       |
| HSD17B10  | high ELS     | Mixed    | SPR      |
| IKZF4     | low ELS      | MR       | MR       |
| IL17RE    | low ELS      | MR       | MR       |
| INPPL1    | high ELS     | MR       | MR       |
| IPCST1    | low ELS      | MR       | MR       |
| ITGB8     | high ELS     | SPR      | SPR      |
| KCNH5     | high ELS     | MR       | Mixed    |
| KCNJ1     | low ELS      | SPR      | SPR      |
| KIAA0240  | high ELS     | MR       | MR       |
| KIF6      | low ELS      | MR       | SPR      |
| LIMS2     | low ELS      | MR       | MR       |
| LSMD1     | low ELS      | MR       | MR       |
| LZTS2     | Mixed        | MR       | MR       |
| MARK2     | low ELS      | MR       | MR       |
| MGST3     | high ELS     | SPR      | SPR      |
| Gene        | Low ELS | Mixed | Mixed |
|-------------|---------|-------|-------|
| MORC1       | low ELS |       |       |
| MPDU1       | low ELS | MR    | MR    |
| MRPL23      | high ELS| MR    | MR    |
| MTHFD1L     | low ELS | MR    | MR    |
| MYH4        | high ELS| SPR   | SPR   |
| MYOM2       | low ELS | MR    | MR    |
| NCAM1       | low ELS | MR    | MR    |
| NDUF2F2     | low ELS | SPR   | SPR   |
| NME1        | low ELS | MR    | MR    |
| NOL6        | high ELS| MR    | MR    |
| NUDC        | low ELS | MR    | MR    |
| ORC5        | high ELS| SPR   | SPR   |
| PAN2        | low ELS | MR    | MR    |
| PBD         | high ELS| SPR   | MR    |
| PBXIP1      | low ELS | MR    | MR    |
| PCIIF1      | low ELS | MR    | MR    |
| PCNP        | low ELS | MR    | MR    |
| PDE1A       | high ELS| SPR   | SPR   |
| PDE4D       | high ELS| Mixed | SPR   |
| PDE4DIP     | low ELS | MR    | MR    |
| PE15        | Mixed   | MR    | SPR   |
| PHF7        | high ELS| MR    | SPR   |
| PHLDB1      | low ELS | MR    | MR    |
| PKM2        | low ELS | MR    | MR    |
| PLA2G2C     | low ELS | MR    | MR    |
| PLCL1       | high ELS| MR    | SPR   |
| PLEKHG1     | low ELS | MR    | MR    |
| PLEKHM2     | high ELS| MR    | MR    |
| PPIA4       | low ELS | MR    | MR    |
| PPP2R2B     | high ELS| SPR   | SPR   |
| PRMT5       | high ELS| SPR   | SPR   |
| PRRC2B      | low ELS | MR    | MR    |
| PSE11       | low ELS | MR    | MR    |
| PTCD3       | low ELS | MR    | SPR   |
| PTC1        | high ELS| MR    | MR    |
| RAB13       | low ELS | MR    | MR    |
| RABGAP1L    | Mixed   | MR    | SPR   |
| RAPGEF5     | low ELS | MR    | MR    |
| RBM25       | low ELS | MR    | MR    |
| RBM47       | high ELS| MR    | MR    |
| REPIN1      | low ELS | MR    | MR    |
| RFTN1       | high ELS| MR    | Mixed |
| RGS7BP      | high ELS| SPR   | SPR   |
| RNF26       | low ELS | MR    | MR    |
| RUNX1       | high ELS| MR    | MR    |
| SARDH       | low ELS | MR    | MR    |
| Gene       | ELS Level | Expression | MR Level |
|------------|-----------|------------|----------|
| SERINC1    | low       | MR         | MR       |
| SETD5      | high      | MR         | MR       |
| SH2B2      | low       | MR         | MR       |
| SH2D4B     | low       | MR         | MR       |
| SLC12A6    | low       | SPR        | SPR      |
| SLC25A25   | low       | MR         | MR       |
| SLC01C1    | high      | SPR        | SPR      |
| SNORA49    | low       | MR         | MR       |
| SNORD67    | high      | SPR        | MR       |
| SNTB1      | high      | SPR        | MR       |
| SPACA1     | low       | MR         | MR       |
| SQSTM1     | high      | MR         | MR       |
| SRC        | low       | MR         | MR       |
| ST3GAL5    | Mixed     | MR         | MR       |
| ST6GAL1    | low       | MR         | SPR      |
| STIL       | low       | MR         | SPR      |
| STT3A      | high      | MR         | MR       |
| SULF1      | low       | MR         | SPR      |
| SUN1       | low       | MR         | MR       |
| SUPT6H     | low       | SPR        | SPR      |
| SUV39H1    | low       | MR         | MR       |
| SYNE1      | low       | MR         | MR       |
| SYNE2      | low       | MR         | MR       |
| SYNPO      | low       | MR         | MR       |
| SYNPO2L    | high      | MR         | MR       |
| TACC3      | high      | MR         | MR       |
| THSD4      | high      | Mixed      | SPR      |
| TLE1       | low       | MR         | MR       |
| TMCC2      | low       | Mixed      | SPR      |
| TPK1       | high      | MR         | MR       |
| TSLP       | high      | SPR        | SPR      |
| TTC1       | high      | MR         | MR       |
| TXNRD2     | low       | MR         | SPR      |
| U6         | Mixed     | MR         | Mixed    |
| U7         | low       | MR         | Mixed    |
| UMODL1     | low       | MR         | MR       |
| UNC13C     | low       | SPR        | SPR      |
| USP34      | low       | MR         | MR       |
| VILL       | low       | MR         | MR       |
| VWF        | low       | MR         | MR       |
| WDFY3      | low       | MR         | Mixed    |
| XPO5       | high      | MR         | MR       |
| ZCWPW1     | low       | MR         | MR       |
| ZMAT4      | low       | SPR        | MR       |
| ZMYND8     | low       | MR         | MR       |
| ZNF672     | low       | MR         | MR       |
Supplementary Table S5. Genes showing differential methylation in response to ELS in rat PFC, human cord blood CD34+ cells and in the CD3+ T cells of 14 - 30 day and 2 year old monkeys:

| Gene names | Adult rat PFC | Human CD34+ | Day 14 - 30 old monkey CD3+ T cells | 2 year old monkey CD3+ T cells |
|------------|---------------|-------------|-------------------------------------|-------------------------------|
|            | More methylated in | More methylated in | More methylated in | More methylated in |
| 7SK        | Mixed          | Mixed       | MR                                  | Mixed                         |
| ACVRL1     | PS             | high ELS    | MR                                  | MR                            |
| ADARB2     | Ctrl           | low ELS     | MR                                  | MR                            |
| ARHGEF2    | Ctrl           | low ELS     | MR                                  | MR                            |
| CACNA1C    | PS             | low ELS     | MR                                  | MR                            |
| CAST       | Ctrl           | high ELS    | SPR                                 | SPR                           |
| CSRNP3     | PS             | high ELS    | SPR                                 | SPR                           |
| CTTN       | PS             | low ELS     | MR                                  | MR                            |
| DNAH8      | PS             | low ELS     | MR                                  | SPR                           |
| ERI3       | PS             | low ELS     | MR                                  | Mixed                         |
| HECW1      | Ctrl           | high ELS    | MR                                  | MR                            |
| INPPL1     | PS             | high ELS    | MR                                  | MR                            |
| IPCEF1     | Ctrl           | low ELS     | MR                                  | MR                            |
| KCNH5      | Ctrl           | high ELS    | MR                                  | Mixed                         |
| MORC1      | Ctrl           | low ELS     | Mixed                               | Mixed                         |
| PDE4DIP    | Ctrl           | low ELS     | MR                                  | MR                            |
| PPFIA4     | PS             | low ELS     | MR                                  | MR                            |
| PRMT5      | Mixed          | high ELS    | SPR                                 | SPR                           |
| RAB13      | PS             | low ELS     | MR                                  | MR                            |
| RAPGEF5    | Ctrl           | low ELS     | SPR                                 | MR                            |
| Gene   | Condition | Treatment | Stage 1 | Stage 2 |
|-------|-----------|-----------|---------|---------|
| SARDH | PS        | low ELS   | MR      | MR      |
| SERINC1 | Ctrl | low ELS   | MR      | MR      |
| SETD5 | PS        | high ELS  | MR      | MR      |
| SLC25A25 | PS    | low ELS   | MR      | MR      |
| SUV39H1 | PS     | low ELS   | MR      | MR      |
| SYNPO2L | Ctrl | high ELS  | MR      | MR      |
| U6    | Mixed    | Mixed     | MR      | Mixed   |
| U7    | Mixed    | low ELS   | MR      | Mixed   |
| VILL  | PS        | low ELS   | MR      | MR      |
| ZMYND8 | PS       | low ELS   | MR      | MR      |
Detailed description of Material and Methods:

Subjects

The human cohort has been recruited between 03/2011 and 03/2012 from two obstetric hospitals in the Rhine-Neckar Region of Germany (Mannheim, Ludwigshafen). The mothers were recruited during the third trimester of pregnancy (i.e. 4-8 weeks prior to delivery). Inclusion criteria for mothers were: Caucasian descent; main caregiver; German-speaking; and age 16 – 40 years. Exclusion criteria were: maternal hepatitis B, hepatitis C or HIV-infection; any current psychiatric disorders requiring inpatient treatment; a history, current diagnosis, of schizophrenia / psychotic disorder, or any substance dependency other than nicotine during pregnancy. Exclusion criteria for infants were: birth weight < 1.500 grams; gestational age at birth < 32 week; multiples; or any congenital diseases; malformations; deformations or chromosomal abnormality. The study protocol was approved by the Ethics Committee of the Medical Faculty Mannheim of the University of Heidelberg, and the study was conducted in accordance with the Declaration of Helsinki. All mothers provided written informed consent prior to participation.

Assessment of ELS and selection of extreme groups of the sample for the genome-wide analysis

The mothers were assessed using a structured interview and a series of questionnaires in order to collect information concerning a broad range of environmental and sociodemographic risk factors, prenatal medical risk factors, general medical characteristics, and psychosocial risk factors (see Supplementary Table S1a for a summary of this phenotypic assessment). Cord blood was collected immediately after the birth.

Eight main stressor variables derived from eight different questionnaires were selected to represent a variety of prenatal adversities, and to take three different dimensions of stress into account: a) maternal psychopathology (primarily depressive and anxiety symptoms); b) perceived stress; and c) socioeconomic and psychosocial stress (for details see Supplementary Table S1c). In addition, an "adversity score" was calculated by summing up the number of dichotomous stressful prenatal adverse conditions and environmental circumstances (for details see Supplementary Table S1c). To obtain a homogeneous composite measure of prenatal stress, a principal component analysis (PCA)
was performed. This involved the eight main stressor variables and the total adversity score as a ninth main variable. This analysis yielded a first principal component (PC1), which explained around 60% of the common variance. PC1 was then used to determine the following two extreme groups: 10 infants with extremely high levels of prenatal ELS; and 10 infants with extremely low levels of prenatal ELS.

The sociodemographic and medical characteristics of the mothers and infants in the extreme groups are shown in Supplementary Table S1b. The psychopathology, perceived stress, and psychosocial and socioeconomic stress status of the mothers are shown in Supplementary Table S1c. For the comparison of the extreme groups, two-tailed t-tests for independent samples were used (SPSS® Statistics 20). The nominal level of significance was set at α = 0.05. All data and results are expressed as means ± standard deviation (SD) or as a percentage, as appropriate. The data sets of two infants in the low ELS group did not pass our quality control filters, and the group size decreased to n = 8.

Animal rearing and experimental conditions

Rhesus monkeys

Male rhesus monkeys (Macaca mulatta) were reared at the breeding facility of the Animal Center of the Laboratory of Comparative Ethology, National Institute of Child Health and Human Development (NICHD; Head: Stephen Suomi; Poolsville, MD). At birth, the animals were randomly divided into two groups: “mother-reared” (MR); and a “surrogate peer-reared” (SPR). Briefly, MR animals were reared by their biological mothers within a social group. The SPR monkeys were placed in a nursery for the first month of life, until they were able to drink milk independently from a bottle. They were then transferred to a cage with their inanimate surrogate mother. After 37 days of age, the individual housing with the surrogate mother was supplemented by 2 h/day of social interaction in a playroom with age-matched peers. At ~ 7 months of age, the SPR animals were socially housed in a large, mixed-sex peer group and were maintained under identical physical and social conditions. All environmental conditions, procedures and handling of animals were in strict compliance with the requirements of the Institutional Animal Care and Use Committee, and all experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Blood samples were obtained from monkeys aged 14 - 30 days old (born between 2010 and 2011), and from
monkeys aged 2 years (born between 2006 and 2009). All samples were processed and analyzed by experimenters who were blind to rearing conditions.

Rats

Adult female and male Sprague-Dawley rats were used (body weight 230–260g and 400g, respectively; Charles-River, Calco, Italy). The animals were housed in an air conditioned room (temperature 21 ± 1°C, relative humidity 60 ± 10%) under a reversed 12/12 hours light/dark cycle with lights off from 08:00 a.m. to 08:00 p.m. Pellet food and tap-water were available continuously. Two same sex animals were housed per cage (37×21×19 cm), and were left undisturbed for the first 10 days of the experimental period. One female and one male were then mated for 24 hours. Following this step, females were housed individually while males were returned to their home cage with their cage-mates. Females were weighted every 7 days in order to assess pregnancy-related increases in body weight. Female rats were assumed to be pregnant when they showed an increase in body weight of around 20 g. Pregnant rats were randomly assigned to one of the following conditions: Control dams (Ctrl, dams were left undisturbed until parturition, n = 7); or Prenatal Stressed dams (PS, PS rats were subjected to a repeated restraint stress procedure, n = 9). Briefly, the stress procedure involved restraining the pregnant rat within a transparent cylinder (7.5 cm diameter, 19 cm long) under bright light (6,500 lx) for 45 min three times per day from day 14 of pregnancy until delivery. Stress sessions were conducted at differing periods of the day in order to reduce possible habituation (9:00 a.m., 1:00 p.m., and 5:00 p.m. ±2 h; separated by a 2-4 h interval between sessions).

At birth, the Ctrl and PS litters were culled to 5 males and 5 females. On postnatal day (PND) 21, the pups were weaned. Same sex rats were then housed in groups of three per cage. On PND62 one male from each Ctrl and PS litter was sacrificed. The brains were dissected. The PFCs were collected and stored at -80°C until DNA extraction. All animal experiments were conducted according to the authorization from the Health Ministry n. 295/2012-A (20/12/2012), in full accordance with the Italian legislation on animal experimentation (Decreto Legislativo 116/92) and adherent to EU recommendation (EEC Council Directive 86/609).
Molecular methods

Separation of CD34+ cells from human cord blood

Human cord blood was drawn into ethylenediaminetetraacetic (EDTA) coated tubes immediately after birth. CD34+ cells were extracted within 24 h following delivery. Briefly, peripheral blood mononuclear cells (PBMC) were isolated by centrifuging the cord blood with Ficoll-Pague PLUS (GE Healthcare; Munich; Germany) in Leucosep tubes (Greiner Bio-One; Frickenhausen; Germany). CD34+ cells were then isolated from the PBMCs by immunomagnetic isolation using the Dynal CD34 Progenitor Cell Selection System (life technologies; Darmstadt; Germany) in accordance with the manufacturer’s instructions. The CD34+ cells were then stored at −80°C until DNA extraction.

Separation of CD3+ T cells from monkey peripheral blood

For the 2-year old monkeys, 20 ml of blood was drawn into EDTA-coated tubes and stored at 4°C overnight. For the 14- to 30-day old monkeys, 3 ml of blood was perfused in EDTA-coated tubes. Briefly, PBMCs were isolated through centrifugation with Ficoll-Paque (GE Healthcare; Burnaby; BC; Canada). The PBMCs were then washed twice with HBSS (life technologies; Burlington; ON; Canada). T cells were isolated from the PBMCs by immunomagnetic isolation using CD3+ Dynabeads (life technologies). The beads were washed three times and incubated with the PBMCs for 45 min on a rotator at 4°C followed by a washing step (five times) with PBS/FBS. CD3+ T cells were then stored at −80°C until DNA extraction.

Extraction of DNA

Genomic DNA was extracted from CD34+ cells using the Qiagen Blood Mini Kit (Qiagen; Hilden; Germany). CD3+ T cell DNA was extracted using the Wizard Genomic DNA Purification kit (Promega; Madison; WI; USA). Isolation of DNA from brain samples was performed using the DNeasy Tissue kit (Qiagen; Toronto; ON; Canada). Genomic DNA was shared by sonication and quantified by fluorometric analysis (Qubit® 2.0 Fluorometer, life technologies). Each of the above procedures was performed in accordance with the instructions of the respective manufacturer.
MeDIP analysis of genome-wide promoter DNA methylation

The procedure used for the MeDIP analysis was adapted from previously published protocols \(^9\), as described in our previous studies \(^{10}\). Briefly, 2µg of DNA were sonicated, and methylated DNA was immunoprecipitated using 10µg of anti-5-methyl-cytosine (Eurogentec; Fremont; CA; USA). The monkey study involved DNA pools from: (i) 6 MR and 5 SPR monkeys sampled twice, i.e. at postnatal days 14 and day 30; and (ii) pools of DNA of 6 MR and 4 SPR monkeys sampled at age 2 years. For the rat study, samples of 4 Ctrl and 4 PS male rats sacrificed on PND62 have been used. The DNA-antibody complex was immunoprecipitated with 5 mg of protein G, and the methylated DNA was resuspended in 250 µl of digestion buffer (50 mM TRisHCl pH8; 10 mM EDTA; 0.5% SDS) and treated with 40 mg of proteinase K overnight at 55°C. The input and bound fractions were purified. Specificity for methylated DNA and the absence of unspecific binding were validated through PCR analysis of an unmethylated and a methylated control gene (for primer sequences see Supplementary Table S2).

The input and bound fraction were purified and then amplified using the Whole Genome Amplification Kit (Sigma-Aldrich; St. Luis; MO; USA). The amplified input and bound fractions were labeled for microarray hybridization with Cy3-dUTP and Cy5-dUTP, respectively, using the CGH Enzymatic Labeling Kit (Agilent Technologies; Mississauga; ON; Canada) in accordance with the manufacturer’s instructions. For the rhesus macaque experiment, DNA samples were hybridized in triplicates.

MeDIP microarray design, hybridization, scanning, and analysis

Custom designed tiling arrays were used (Agilent Technologies). For the human studies, a 400K promoter tiling array was designed in 2009 using the eArray array design platform from Agilent. Probes were selected to tile all known gene promoters, i.e. intervals roughly 1200 bp upstream to 400 bp downstream of the transcription start sites (TSS) of genes described in the Ensembl database (version 55) at 100 bp-spacing. For the analysis of genome-wide DNA methylation in *Macaca mulatta*, custom 244K and 400K promoter tiling array designs were used for the brain and T cells studies respectively (Agilent Technologies). Microarray probe sequences were selected to tile all gene promoter regions defined as the genomic interval from -2000bp upstream to 400bp downstream of
each transcription start site as defined for the Rhesus Macaque by the Ensembl database (version 64.10) [http://www.ensembl.org] as detailed in our previous study 10.

For the rat studies, a custom 400K promoter tiling microarray design was used (Agilent Technologies). Probes were selected to tile all known gene promoters, i.e. intervals from -1000bp upstream to 200bp downstream around each transcription start site defined in the Ensembl database (version 62). Probes were placed approximately every 100bp. All genomic coordinates are given with respect to the rn4 (RGSC 3.4).

All the steps of the hybridization, washing scanning, and feature extraction procedures were performed in accordance with the Agilent Technologies protocol for chip-on-chip analysis. Probe intensities were extracted from scan images using Agilent's Feature Extraction 9.5.3 Image Analysis Software. Extracted microarray intensities were processed and analyzed using the R software environment for statistical computing. Log-ratios of the bound (Cy5) and input (Cy3) microarray channel intensities were computed for each microarray. Microarrays were normalized to one another using quantile-normalization under the assumption that all samples had identical overall methylation levels. Methylation levels were estimated from normalized probe intensities by applying a Bayesian deconvolution algorithm. Promoter methylation levels were obtained by calculating the median estimated methylation level across each promoter. Promoters were defined as the region within 2000 to -400bp of the transcription start sites of each gene. Differential methylation between groups of samples was determined in several stages to ensure both statistical significance and biological relevance. In the first stage, linear models implemented in the 'limma' package of Bioconductor 11 were used to combine the two dye labeling schemes from the dye swaps and to compute a modified t-statistic for each probe. An individual probe was classified as being differentially methylated if the p-value of its t-statistic was a maximum of 0.05 (uncorrected for multiple testing), and the associated difference of means between the groups was at least 0.5. Given that the DNA samples were sonicated prior to hybridization, the assumption was made that probes within 500bp should show approximate agreement. Therefore, the genome was grouped into 1000bp regions and the significance of enrichment for high or low t statistics of probes within each region (containing at least 1 probe) was calculated. Significance was determined using the Wilcoxon rank-sum test comparing t statistics of the probes within the region against those of all the probes on the microarray and then adjusted to obtain false discovery rates for each region. A probe was classified as being differentially methylated if it satisfied each of the following criteria:
1. the significance of its t-statistic was a maximum of 0.05 and the difference of means between the groups was at least 0.5,

2. it belonged to a region whose false discovery rate was a maximum of 0.2

A promoter was classified as being differentially methylated if it contained a differentially methylated probe.

Validation of the MeDIP data

Gene-specific validation of the MeDIP data was performed applying quantitative-real time PCR (QPCR) to the samples used in the MeDIP microarray analysis. QPCR was performed in 1X Power SYBR Green Master Mix (life technologies) for the human analysis, and in Light Cycler 480 SybrGreen I Master (Roche Diagnostics; Laval; QC; Canada) for the rat and monkey analyses using 4 - 10ng of DNA and 1.5 - 4 µM gene specific primers (see Supplementary Table S2) which were designed using Primer 3 (http://frodo.wi.mit.edu/) software. Relative enrichment of triplicate reactions was determined after normalizing from the input fraction in each sample using the $2^{-\Delta\Delta Ct}$ method. All data are expressed as group means ± SEM. To test for statistical significance the Mann-Whitney U test was used (one-tailed), and the alpha level was set at 0.05. The Graphpad 5 software (La Jolla; CA; USA) was used to perform statistical analyses.

Analysis of MORC 1 expression in human cord blood

Cord blood for RNA extraction was collected immediately after birth and drawn into PAXgene™ Blood RNA tubes (PreAnalytiX; Hombrechtikon, Switzerland). RNA was extracted using the PAXgene™ Blood RNA Kit (Qiagen; Hilden; Germany). The quality and quantity of the RNA samples were analyzed using an Agilent 2100 Bioanalyzer (Agilent, Böblingen, Germany). For one of the infants from the high ELS group, no RNA was available for analysis. A total of 1µg of RNA derived from each human cord blood sample was reverse transcribed using the High capacity cDNA Reverse Transcription Kit (life technologies). The level of MORC1 gene expression was analyzed using QPCR and the $2^{-\Delta\Delta Ct}$ method. QPCR was performed in 1X TaqMan Fast Advanced Master Mix (life technologies). The following TaqMan Gene Expression Assays (life technologies) were used: Hs01075271_m1 for MORC1; and Hs99999903_m1 for β-actin, which served as housekeeping gene. PCR reactions were carried out in a total volume of 10 µl containing 25 ng of cDNA. The analysis was run on a 7900HT Fast Real-Time PCR System using a single 384 plate in order to avoid plate effects. All samples were run in triplicates.
All data are expressed as group means ± SEM. To test for statistical significance the Mann-Whitney U test was used.

**Pyrosequencing**

A total of 500 ng DNA isolated from CD34+ cells was bisulfite-treated using the EpiTect Bisulfite Kit (Qiagen) and stored at -20°C until further analysis. Six CpG sites in the MORC1 promoter region (NC_000003.11, assembly: CRCh37/hg19, position: 108,838,104 - 108,833,644) were analyzed by pyrosequencing. Three different DNA fragments were amplified by PCR (HotStar Taq DNA Polymerase, Qiagen) from 2 µl of bisulfite-treated DNA in a PCR volume of 50 µl (primer information: Table S2). The 5´ end of the reverse primers was biotinylated (Eurofins, Ebersberg, Germany). Methylated and unmethylated EpiTect control DNA samples (Qiagen) were used as controls for bisulfite conversion, amplification, and pyrosequencing. Successful amplification and specificity of the PCR products were checked on an agarose gel. Pyrosequencing was performed using a PyroMark Q24 Advanced system (Qiagen; primer information see Table S2) in accordance with the manufacturer’s protocol. The percentage of methylation at each CpG site was quantified using the PyroMark Q24 Advanced software version 3.0.0 (Qiagen, Hilden, Germany). Quality control filtering and statistical analyses of the pyrosequencing results were conducted using R Version 2.15.3 (http://www.r-project.org). All samples were run in triplicate. Measurements marked as unreliable by Pyromark software were removed. Replicate measurements were averaged after outlier removal (values deviating more than 3%). A Mann-Whitney U test was used to compare the mean percentage of methylation of CpG sites for the ELS vs. control groups. Data are presented as the mean ± SEM.

**Gene-based analysis**

Genotypes were investigated using GWAS data of a previous study of MDD 12. The patients were recruited from consecutive admissions to the Department of Psychiatry of the University of Bonn, and the control subjects were drawn from three population-based epidemiological studies in Germany, as described elsewhere 12. All samples were individually genotyped using either Illumina HumanHap 550v3 or Illumina human 610 W quad BeadChips (Illumina Inc., San Diego; CA; USA). After stringent quality control, the data of 593 patients with a DSM-IV diagnosis of MDD and 1307 control subjects were analyzed (for details, see 12). In the present study, dbSNP Build 138 Phase I
(http://www.ncbi.nlm.nih.gov/projects/SNP) was searched for SNPs across the genes. To test for association between MDD and the whole set of genetic variants in the dataset, the set-based test was performed (with default options and 10^5 permutations), as implemented in PLINK (v1.0.7)\textsuperscript{13}

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