

**Title: Maternal Inflammatory Biomarkers During Pregnancy and Early Life
Neurodevelopment in Offspring: Results from the VDAART Study**

Supplementary Table S1: Characteristics of the inflammatory biomarkers subpopulation compared to the full VDAART population

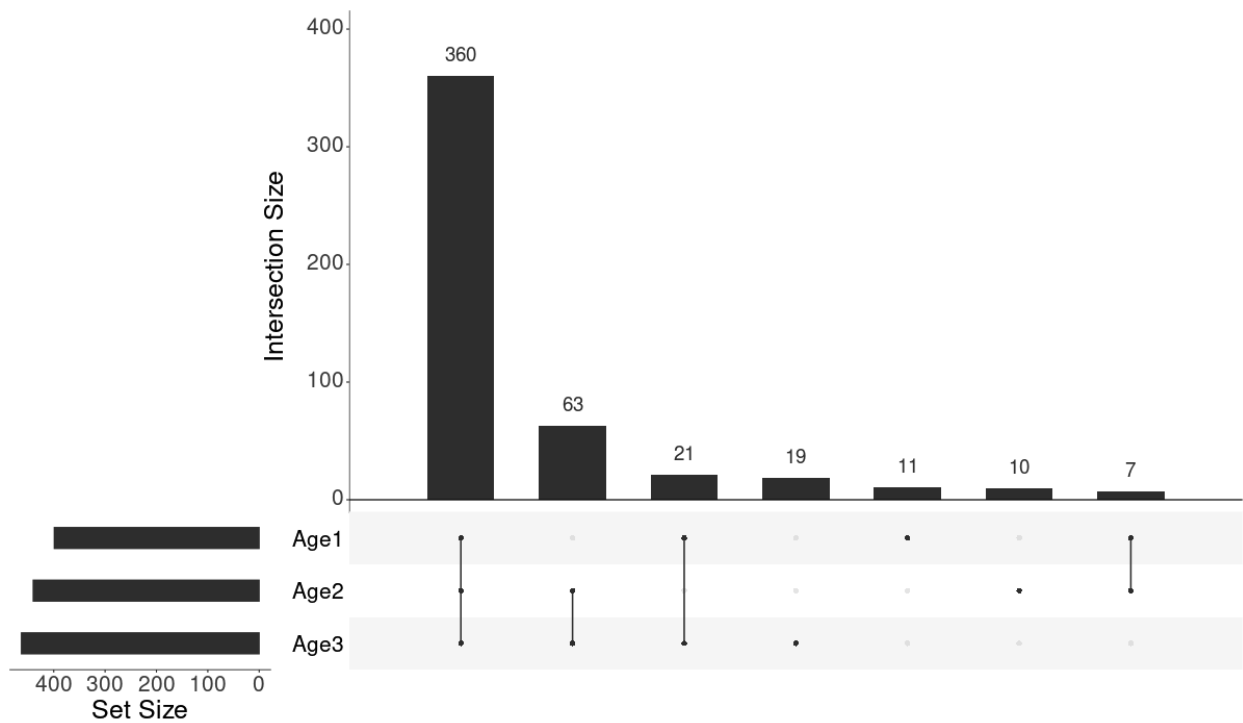
| Variable | | | Inflammatory Biomarker Subpopulation (n=491 mother-offspring pairs) | | Total VDAART population (mothers n=881; Offspring n=816) | | p-value |
|---------------------------|---|------------------------|---|--------|--|--------|---------|
| | | | mean/n | SD/% | mean/n | SD/% | |
| Maternal Characteristics | Age at recruitment | (yrs) | 27.7 | 5.4 | 27.4 | 5.6 | 0.489 |
| | Race/Ethnicity | Black - Hispanic | 21 | 4.30% | 43 | 4.90% | 0.968 |
| | | Black - Non-Hispanic | 187 | 38.10% | 341 | 38.70% | |
| | | Other- Hispanic | 42 | 8.60% | 81 | 9.20% | |
| | | Other - Non-Hispanic | 38 | 7.70% | 65 | 73.80% | 0.971 |
| | | White- Hispanic | 65 | 13.20% | 120 | 13.60% | |
| | | White - Non-Hispanic | 138 | 28.10% | 231 | 26.20% | |
| | Study Site | Boston | 143 | 29.10% | 262 | 29.70% | |
| | | San Diego | 169 | 34.40% | 300 | 34.10% | |
| | | St Louis | 179 | 36.50% | 319 | 36.20% | |
| | Education | Less than college grad | 307 | 62.50% | 590 | 67.00% | 0.110 |
| | | College grad or higher | 184 | 37.50% | 291 | 33.00% | |
| | Marital Status | Not living with Father | 126 | 25.70% | 245 | 27.80% | 0.427 |
| | | Married/Cohabiting | 365 | 74.30% | 636 | 72.20% | |
| | Number previous pregnancies at enrollment | 0 | 173 | 35.23% | 305 | 34.62% | 0.989 |
| | | 1 | 125 | 25.46% | 230 | 26.11% | |
| | | 2 | 91 | 18.53% | 174 | 19.75% | |
| | | 3 | 50 | 10.18% | 83 | 9.42% | |
| | | 4 | 27 | 5.50% | 46 | 5.22% | |
| | | >5 | 25 | 5.09% | 43 | 4.88% | 0.888 |
| | Number living children at enrollment | 0 | 227 | 46.23% | 403 | 45.74% | |
| | | 1 | 152 | 30.96% | 272 | 30.87% | |
| | | 2 | 78 | 15.89% | 140 | 15.89% | |
| | | 3 | 20 | 4.07% | 41 | 4.65% | |
| | | 4 | 13 | 2.65% | 19 | 2.16% | |
| | | >5 | 1 | 0.20% | 6 | 0.68% | |
| | Treatment Arm | Placebo | 254 | 51.70% | 439 | 49.80% | 0.536 |
| | | Vitamin D | 237 | 48.30% | 442 | 50.20% | |
| Offspring Characteristics | Gestational age at delivery | (weeks) | 39.2 | 1.59 | 38.9 | 2.27 | 0.007 |
| | Gestational Age <37 weeks | Yes | 34 | 6.90% | 76 | 9.3% | 0.159 |
| | Sex | Female | 242 | 49.30% | 388 | 47.5% | 0.615 |
| | | Male | 249 | 50.70% | 428 | 52.5% | |
| | Race/Ethnicity ^a | Black - Hispanic | 40 | 8.10% | 52 | 6.4% | 0.179 |
| | | Black - Non-Hispanic | 193 | 39.30% | 303 | 37.1% | |
| | | White- Hispanic | 62 | 12.60% | 142 | 17.4% | |
| | | White - Non-Hispanic | 104 | 21.20% | 186 | 22.8% | |
| | | Other- Hispanic | 54 | 11.00% | 81 | 9.9% | |
| | | Other - Non-Hispanic | 38 | 7.70% | 53 | 6.5% | 0.090 |
| | Weight at birth | (grams) | 3311.2 | 504 | 3258.7 | 596.9 | |
| | Length at birth | (cm) | 50.8 | 2.9 | 50.6 | 3.5 | |
| | Head circumference at birth | (cm) | 34.1 | 1.9 | 34 | 2.1 | |
| | Exclusive breast feeding for first 4 months of life | Yes | 157 | 32.00% | 247 | 30.3% | 0.801 |
| | | No | 300 | 61.10% | 491 | 60.2% | |
| | | Missing | 34 | 6.90% | 78 | 9.6% | |

Supplementary Table S2: Correlation between maternal inflammatory biomarkers

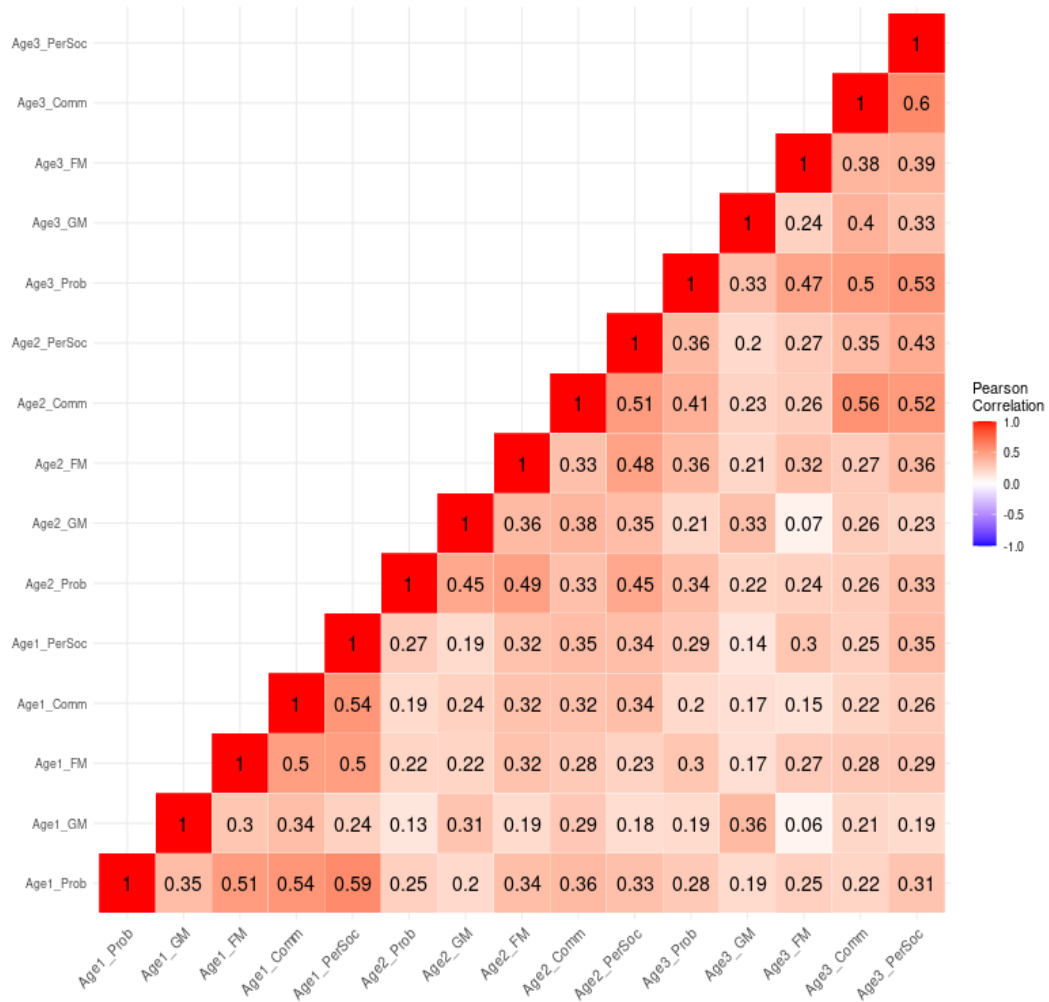
| | Early Pregnancy CRP | Late Pregnancy CRP | Early Pregnancy IL8 | Late Pregnancy IL8 |
|---------------------|---------------------------------|--------------------|--------------------------------|--------------------|
| | r (p-value) | r (p-value) | r (p-value) | r (p-value) |
| Early Pregnancy CRP | 1.000 | | | |
| Late Pregnancy CRP | 0.677 (<2.2x10 ⁻¹⁶) | 1.000 | | |
| Early Pregnancy IL8 | 0.055 (0.220) | −0.045 (0.315) | 1.000 | |
| Late Pregnancy IL8 | 0.191 (2.08x10 ⁻⁵) | 0.124 (0.006) | 0.160 (3.62x10 ⁻⁴) | 1.000 |

Pearson Correlation was used to define rho, p-values are shown in brackets

Supplementary Figure S1: Upset plot showing the timepoints for which 491 offspring had a completed Ages and Stages Questionnaire



Supplementary Figure S2: Correlation between Ages and Stages Questionnaire domains across ages one, two and three



Pearson Rho is shown within the heatmap for each pairwise comparison

PerSoc; Personal-social score

Comm; Communication score

FM; Fine-Motor score

GM; Gross-Motor score

Prob; Problem-solving score

Supplementary Table S3: Summary of five Ages and Stages Questionnaire domains at ages one, two and three

| Age (n children) | Domain | Mean (SD) | On Schedule | | Requires Monitoring | | Requires Follow-up | |
|-------------------|------------------------|-------------|-------------|-------|---------------------|-------|--------------------|------|
| | | | n | % | n | % | n | % |
| Age One (n=399) | Personal Social Skills | 48.2 (12.0) | 351 | 88.0% | 29 | 7.3% | 19 | 4.8% |
| | Communication Skills | 51.6 (9.8) | 390 | 97.7% | 5 | 1.3% | 4 | 1.0% |
| | Fine Motor Skills | 54.0 (8.4) | 350 | 87.7% | 43 | 10.8% | 6 | 1.5% |
| | Gross Motor Skills | 52.7 (10.7) | 362 | 90.7% | 30 | 7.5% | 7 | 1.8% |
| | Problem Solving Skills | 48.6 (11.6) | 333 | 83.5% | 43 | 10.8% | 23 | 5.8% |
| Age Two (n=441) | Personal Social Skills | 52.0 (9.0) | 369 | 83.9% | 50 | 11.4% | 21 | 4.8% |
| | Communication Skills | 54.1 (10.3) | 387 | 88.0% | 33 | 7.5% | 20 | 4.5% |
| | Fine Motor Skills | 50.9 (7.3) | 391 | 88.9% | 25 | 5.7% | 24 | 5.5% |
| | Gross Motor Skills | 55.7 (7.1) | 393 | 89.3% | 33 | 7.5% | 14 | 3.2% |
| | Problem Solving Skills | 50.7 (10.0) | 390 | 88.6% | 40 | 9.1% | 10 | 2.3% |
| Age three (n=464) | Personal Social Skills | 54.3 (7.6) | 424 | 91.6% | 19 | 4.1% | 20 | 4.3% |
| | Communication Skills | 53.2 (7.9) | 422 | 91.1% | 28 | 6.0% | 13 | 2.8% |
| | Fine Motor Skills | 44.3 (14.7) | 367 | 79.3% | 71 | 15.3% | 25 | 5.4% |
| | Gross Motor Skills | 56.1 (6.8) | 405 | 87.5% | 50 | 10.8% | 8 | 1.7% |
| | Problem Solving Skills | 53.0 (9.8) | 399 | 86.2% | 41 | 8.9% | 23 | 5.0% |

Each domain consists of six age-specific questions concerning a child's ability to perform a task, with the parent responding "Yes" (10 points), "Sometimes" (5 points) or "Not yet" (0 points) which are summed to generate a domain score.

SD- Standard deviation

To determine categories, domain specific scores are compared to the expected mean score obtained from a reference distribution of scores for that age group and categorized as: (i) "On Schedule for developing normally" (above the mean); (ii) "Requires Monitoring" (1–2 standard deviations below the mean); (iii) "Needs further evaluation" (>2 standard deviations below the mean).

Supplementary Table S4: Associations between 1st and 3rd trimester levels of CRP and IL3 with skills scores in the five domains of the ASQ at ages 1,2, and 3

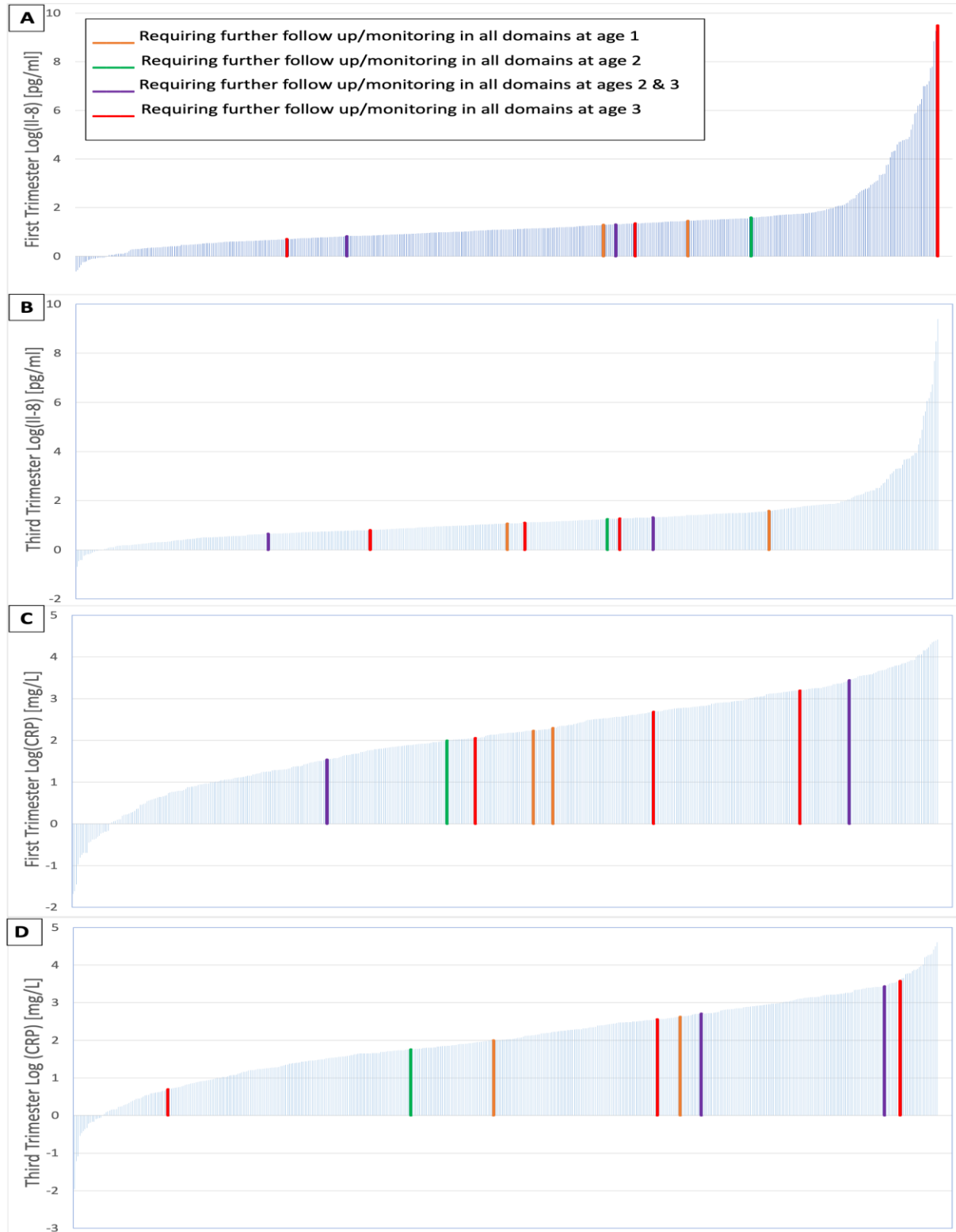
| | | 1stTrimester.CRP β (95% CI) <i>p-value</i> | 3rdTrimester.CRP β (95% CI) <i>p-value</i> | 1stTrimester.IL8 β (95% CI) <i>p-value</i> | 3rdTrimester.IL8 β (95% CI) <i>p-value</i> |
|-------------------------------------|----------------|--|--|--|--|
| Personal-social skills score | Age1yrs | -0.445 (-1.488,0.598) 0.402 | -0.142 (-1.282,0.997) 0.806 | 0.052 (-0.844,0.947) 0.910 | -0.072 (-1.157,1.014) 0.897 |
| | Age2yrs | -0.206 (-0.942,0.529) 0.582 | -0.069 (-0.858,0.719) 0.863 | -0.844 (-1.452,-0.237) 0.007* | -0.272 (-0.988,0.443) 0.455 |
| | Age3yrs | -0.135 (-0.749,0.478) 0.665 | -0.346 (-1.01,0.318) 0.306 | -0.260 (-0.757,0.238) 0.305 | 0.466 (-0.145,1.077) 0.134 |
| | Age3yrs | -0.135 (-0.749,0.478) 0.665 | -0.346 (-1.01,0.318) 0.306 | -0.260 (-0.757,0.238) 0.305 | 0.466 (-0.145,1.077) 0.134 |
| Problem solving skills score | Age1yrs | -1.028 (-2.022,-0.035) 0.043* | -0.914 (-2.001,0.173) 0.099 | -0.311 (-1.168,0.545) 0.475 | 0.157 (-0.882,1.195) 0.767 |
| | Age2yrs | 0.040 (-0.792,0.872) 0.925 | -0.127 (-1.018,0.764) 0.780 | -1.221 (-1.904,-0.538) 4.9x10 ⁻⁴ ** | -0.093 (-0.903,0.716) 0.821 |
| | Age3yrs | -0.083 (-0.889,0.723) 0.840 | 0.009 (-0.864,0.882) 0.984 | -0.410 (-1.063,0.243) 0.218 | 0.291 (-0.512,1.095) 0.477 |
| | Age3yrs | -0.083 (-0.889,0.723) 0.840 | 0.009 (-0.864,0.882) 0.984 | -0.410 (-1.063,0.243) 0.218 | 0.291 (-0.512,1.095) 0.477 |
| Gross motor skills score | Age1yrs | -0.331 (-1.263,0.601) 0.485 | -0.665 (-1.681,0.351) 0.199 | -0.902 (-1.697,-0.107) 0.026* | 0.039 (-0.931,1.009) 0.937 |
| | Age2yrs | 0.174 (-0.423,0.771) 0.568 | -0.073 (-0.712,0.567) 0.824 | -0.194 (-0.691,0.303) 0.444 | 0.025 (-0.556,0.606) 0.932 |
| | Age3yrs | -0.022 (-0.576,0.532) 0.937 | -0.079 (-0.679,0.522) 0.797 | -0.374 (-0.822,0.075) 0.102 | 0.045 (-0.508,0.598) 0.872 |
| | Age3yrs | -0.022 (-0.576,0.532) 0.937 | -0.079 (-0.679,0.522) 0.797 | -0.374 (-0.822,0.075) 0.102 | 0.045 (-0.508,0.598) 0.872 |
| Fine motor skills score | Age1yrs | -0.400 (-1.13,0.329) 0.281 | -0.151 (-0.949,0.646) 0.709 | -0.880 (-1.500,-0.259) 0.006* | -0.162 (-0.921,0.598) 0.676 |
| | Age2yrs | -0.081 (-0.698,0.535) 0.795 | -0.346 (-1.005,0.314) 0.303 | -0.919 (-1.425,-0.414) 3.9x10 ⁻⁴ ** | 0.070 (-0.530,0.670) 0.818 |
| | Age3yrs | -0.489 (-1.659,0.68) 0.411 | -0.798 (-2.064,0.468) 0.216 | 0.221 (-0.729,1.17) 0.648 | 0.299 (-0.869,1.467) 0.615 |
| | Age3yrs | -0.489 (-1.659,0.68) 0.411 | -0.798 (-2.064,0.468) 0.216 | 0.221 (-0.729,1.17) 0.648 | 0.299 (-0.869,1.467) 0.615 |
| Communication skills score | Age1yrs | 0.348 (-0.505,1.2) 0.423 | -7.9x10 ⁻⁵ (-0.932,0.932) 0.999 | -0.564 (-1.293,0.166) 0.130 | 0.058 (-0.829,0.946) 0.897 |
| | Age2yrs | -0.448 (-1.287,0.39) 0.294 | -0.431 (-1.329,0.467) 0.346 | -0.483 (-1.181,0.214) 0.174 | 0.069 (-0.748,0.886) 0.868 |
| | Age3yrs | -0.640 (-1.265,-0.015) 0.045* | -0.725 (-1.401,-0.048) 0.036* | -0.498 (-1.006,0.009) 0.054 | -0.038 (-0.664,0.589) 0.906 |
| | Age3yrs | -0.640 (-1.265,-0.015) 0.045* | -0.725 (-1.401,-0.048) 0.036* | -0.498 (-1.006,0.009) 0.054 | -0.038 (-0.664,0.589) 0.906 |

ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level and gestational age at delivery

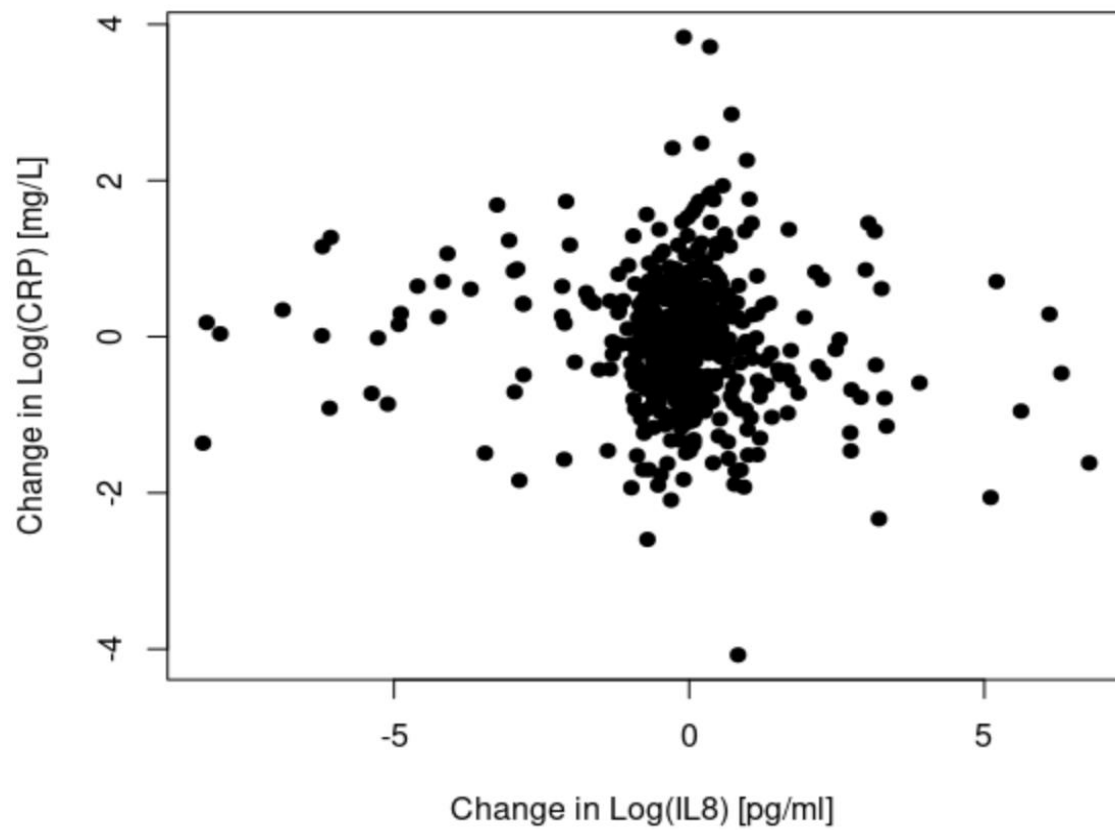
*Significant at $p < 0.05$

**Significant after Benjamini-Hochberg FDR correction

Supplementary Figure S3: (A-D) Distribution of IL8 and CRP in the early and late pregnancy, each mother is represented by a bar, mothers of children who performed poorly across all Ages and Stages Questionnaire domains in any given year are highlighted



Supplementary Figure S4: Correlation between change in IL8 from the 1st to the 3rd trimester and change in CRP during the same period in 491 VDAART mothers



Pearson $\rho = -0.08$, $p = 0.072$

Supplementary Table S5: Interaction effect estimates and p-values for a maternal biomarker*child sex (Male) interaction on Ages and Stages Questionnaire domain scores at ages 1,2, and 3

| | | 1stTrimester.CRP | | 3rdTrimester.CRP | | 1stTrimester.IL8 | | 3rdTrimester.IL8 | |
|------------------------------|---------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value |
| Personal-social skills score | Age1yrs | -0.723 | 0.494 | 0.279 | 0.809 | 1.437 | 0.109 | -1.092 | 0.323 |
| | Age2yrs | 0.059 | 0.935 | 1.073 | 0.166 | -0.391 | 0.513 | 0.610 | 0.393 |
| | Age3yrs | 0.253 | 0.679 | 0.846 | 0.196 | -1.161 | 0.019* | 0.333 | 0.586 |
| Problem solving skills score | Age1yrs | -0.304 | 0.763 | 0.655 | 0.552 | 0.993 | 0.248 | 0.102 | 0.923 |
| | Age2yrs | 0.052 | 0.950 | 0.183 | 0.837 | 0.996 | 0.143 | -0.079 | 0.923 |
| | Age3yrs | 0.576 | 0.478 | 0.634 | 0.467 | -1.395 | 0.033* | 0.332 | 0.684 |
| Gross motor skills score | Age1yrs | -0.029 | 0.976 | 0.882 | 0.390 | 0.348 | 0.662 | 0.125 | 0.899 |
| | Age2yrs | -0.895 | 0.136 | -0.317 | 0.620 | 0.507 | 0.308 | -0.073 | 0.902 |
| | Age3yrs | -0.505 | 0.364 | -0.717 | 0.230 | -0.231 | 0.608 | 0.761 | 0.173 |
| Fine motor skills score | Age1yrs | 0.377 | 0.610 | 0.298 | 0.712 | -0.340 | 0.585 | -0.440 | 0.570 |
| | Age2yrs | 0.145 | 0.815 | 0.307 | 0.641 | 0.174 | 0.731 | 0.401 | 0.510 |
| | Age3yrs | 0.438 | 0.708 | 0.849 | 0.499 | 0.030 | 0.975 | 2.077 | 0.077 |
| Communication skills score | Age1yrs | 1.220 | 0.154 | 1.564 | 0.094 | 0.292 | 0.688 | 0.061 | 0.946 |
| | Age2yrs | -0.944 | 0.260 | 0.251 | 0.778 | 0.217 | 0.754 | 0.770 | 0.350 |
| | Age3yrs | 0.363 | 0.562 | 0.132 | 0.844 | -0.377 | 0.457 | 0.575 | 0.362 |

ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level, gestational age at delivery, child race/ethnicity, and child sex

**p<0.05*

Supplementary Table S6: Interaction effect estimates and p-values for a maternal biomarker*breastfeeding (exclusive breast feeding for four months, Yes) interaction on Ages and Stages Questionnaire domain scores at ages 1,2, and 3

| | | 1stTrimester.CRP | | 3rdTrimester.CRP | | 1stTrimester.IL8 | | 3rdTrimester.IL8 | |
|-------------------------------------|---------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value |
| Personal-social skills score | Age1yrs | -0.527 | 0.627 | 0.029 | 0.981 | -0.591 | 0.500 | -0.862 | 0.480 |
| | Age2yrs | 0.403 | 0.617 | 0.463 | 0.592 | 1.297 | 0.039* | -0.397 | 0.658 |
| | Age3yrs | -0.477 | 0.477 | 0.170 | 0.815 | -0.175 | 0.732 | 0.078 | 0.920 |
| Problem solving skills score | Age1yrs | 0.197 | 0.852 | -0.946 | 0.417 | 0.324 | 0.706 | -0.746 | 0.532 |
| | Age2yrs | 0.550 | 0.538 | -0.272 | 0.776 | 1.183 | 0.088 | 0.530 | 0.593 |
| | Age3yrs | 0.122 | 0.890 | 1.233 | 0.196 | -0.258 | 0.700 | 0.027 | 0.979 |
| Gross motor skills score | Age1yrs | 0.343 | 0.734 | -1.336 | 0.228 | 0.056 | 0.944 | -0.028 | 0.980 |
| | Age2yrs | 0.956 | 0.145 | 0.031 | 0.965 | -0.242 | 0.641 | -0.057 | 0.938 |
| | Age3yrs | 0.964 | 0.111 | -0.198 | 0.762 | 0.276 | 0.548 | -0.410 | 0.557 |
| Fine motor skills score | Age1yrs | 0.697 | 0.367 | 0.200 | 0.815 | -0.723 | 0.242 | -1.054 | 0.225 |
| | Age2yrs | -0.055 | 0.934 | 0.003 | 0.997 | -0.718 | 0.162 | -0.503 | 0.493 |
| | Age3yrs | -0.302 | 0.809 | 1.117 | 0.410 | -1.574 | 0.098 | -1.514 | 0.294 |
| Communication skills score | Age1yrs | 0.984 | 0.273 | 0.060 | 0.952 | -0.534 | 0.461 | -0.378 | 0.709 |
| | Age2yrs | -0.679 | 0.460 | -1.389 | 0.159 | 0.215 | 0.767 | 0.192 | 0.852 |
| | Age3yrs | -0.394 | 0.563 | -0.333 | 0.652 | -0.043 | 0.934 | -0.430 | 0.586 |

ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level, gestational age at delivery, child race/ethnicity, and child sex

**p<0.05*

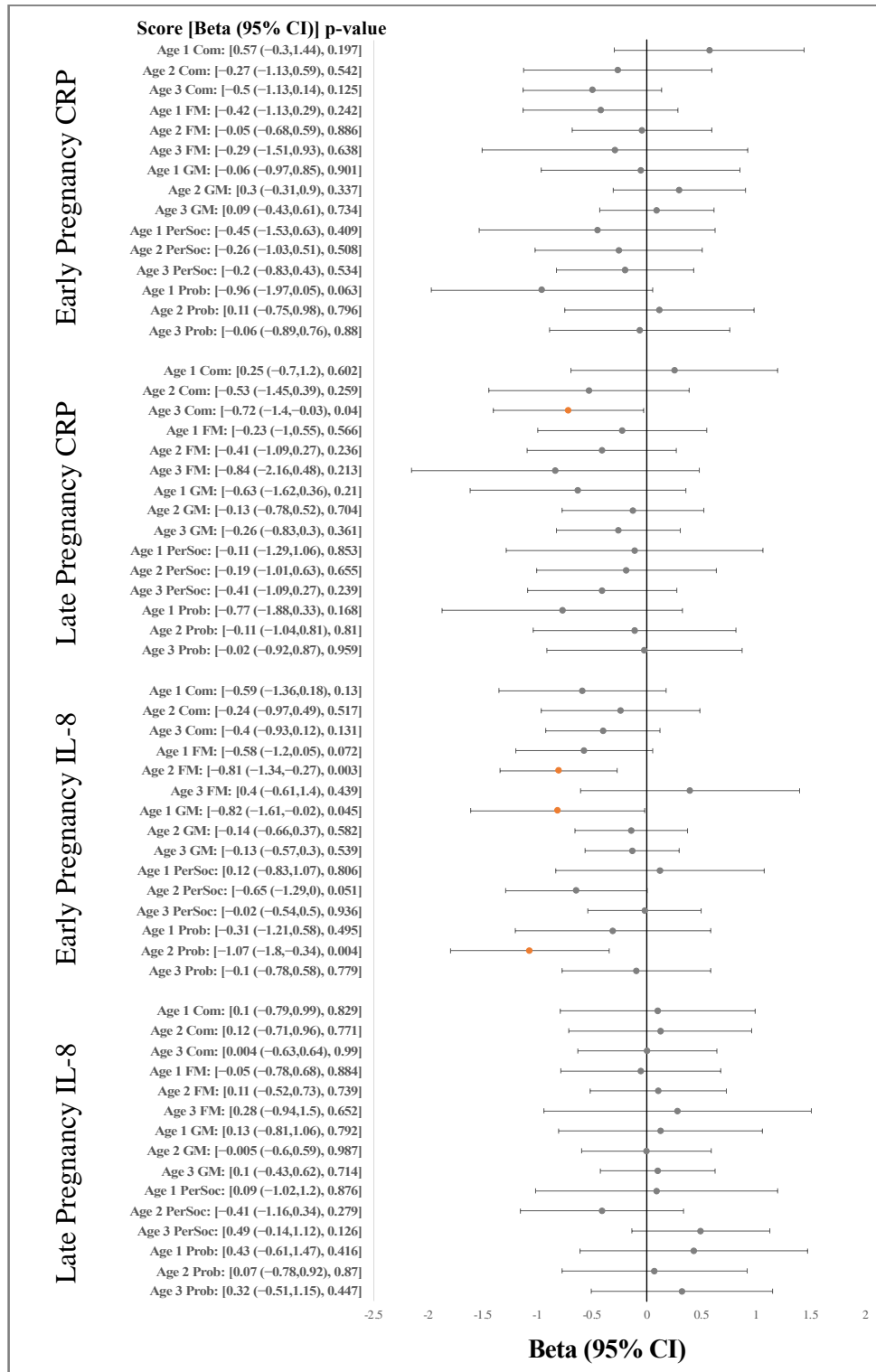
Supplementary Table S7: Interaction effect estimates and p-values for a maternal biomarker*vitamin D supplementation interaction on Ages and Stages Questionnaire domain scores at ages 1,2, and 3

| | | 1stTrimester.CRP | | 3rdTrimester.CRP | | 1stTrimester.IL8 | | 3rdTrimester.IL8 | |
|-------------------------------------|---------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value | Interaction β | Interaction p-value |
| Personal-social skills score | Age1yrs | 0.044 | 0.967 | -1.035 | 0.366 | -0.091 | 0.919 | 1.153 | 0.300 |
| | Age2yrs | 0.514 | 0.487 | 0.809 | 0.305 | 0.090 | 0.883 | 0.622 | 0.396 |
| | Age3yrs | -0.762 | 0.217 | -1.364 | 0.040* | -0.422 | 0.395 | 0.039 | 0.950 |
| Problem solving skills score | Age1yrs | -0.552 | 0.582 | -0.472 | 0.665 | 0.128 | 0.880 | -1.000 | 0.348 |
| | Age2yrs | 0.085 | 0.919 | -0.250 | 0.779 | 0.350 | 0.611 | 0.420 | 0.613 |
| | Age3yrs | 0.635 | 0.434 | -1.270 | 0.145 | -0.111 | 0.865 | -0.057 | 0.945 |
| Gross motor skills score | Age1yrs | -1.115 | 0.235 | -0.723 | 0.479 | 0.148 | 0.851 | -0.527 | 0.597 |
| | Age2yrs | -0.282 | 0.638 | -0.009 | 0.988 | -0.120 | 0.810 | 0.446 | 0.454 |
| | Age3yrs | 0.904 | 0.105 | 0.152 | 0.801 | -0.258 | 0.565 | 0.315 | 0.579 |
| Fine motor skills score | Age1yrs | -0.474 | 0.519 | -0.757 | 0.345 | 0.523 | 0.396 | 1.164 | 0.135 |
| | Age2yrs | -0.061 | 0.922 | -0.154 | 0.815 | -0.851 | 0.095 | -0.332 | 0.590 |
| | Age3yrs | -0.051 | 0.965 | -0.256 | 0.840 | 0.085 | 0.929 | 1.411 | 0.238 |
| Communication skills score | Age1yrs | -0.771 | 0.370 | -1.670 | 0.074 | -0.519 | 0.474 | 0.166 | 0.855 |
| | Age2yrs | -1.285 | 0.127 | -1.372 | 0.127 | 0.339 | 0.630 | -1.002 | 0.231 |
| | Age3yrs | 0.173 | 0.783 | -0.630 | 0.352 | -0.201 | 0.691 | 0.943 | 0.141 |

ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level, gestational age at delivery, child race/ethnicity, and child sex

** $p < 0.05$*

Supplementary Figure S5: Associations between 1st and 3rd trimester levels of CRP and IL8 with skills scores in the five domains of the Ages and Stages Questionnaire at ages 1,2, and 3 excluding 34 mother-child pairs with a gestational age <37 weeks



ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level and gestational age at delivery

Grey; NS

Orange: Significant at $p < 0.05$

Red: Significant after Benjamini-Hochberg FDR correction

PerSoc; Personal-social score

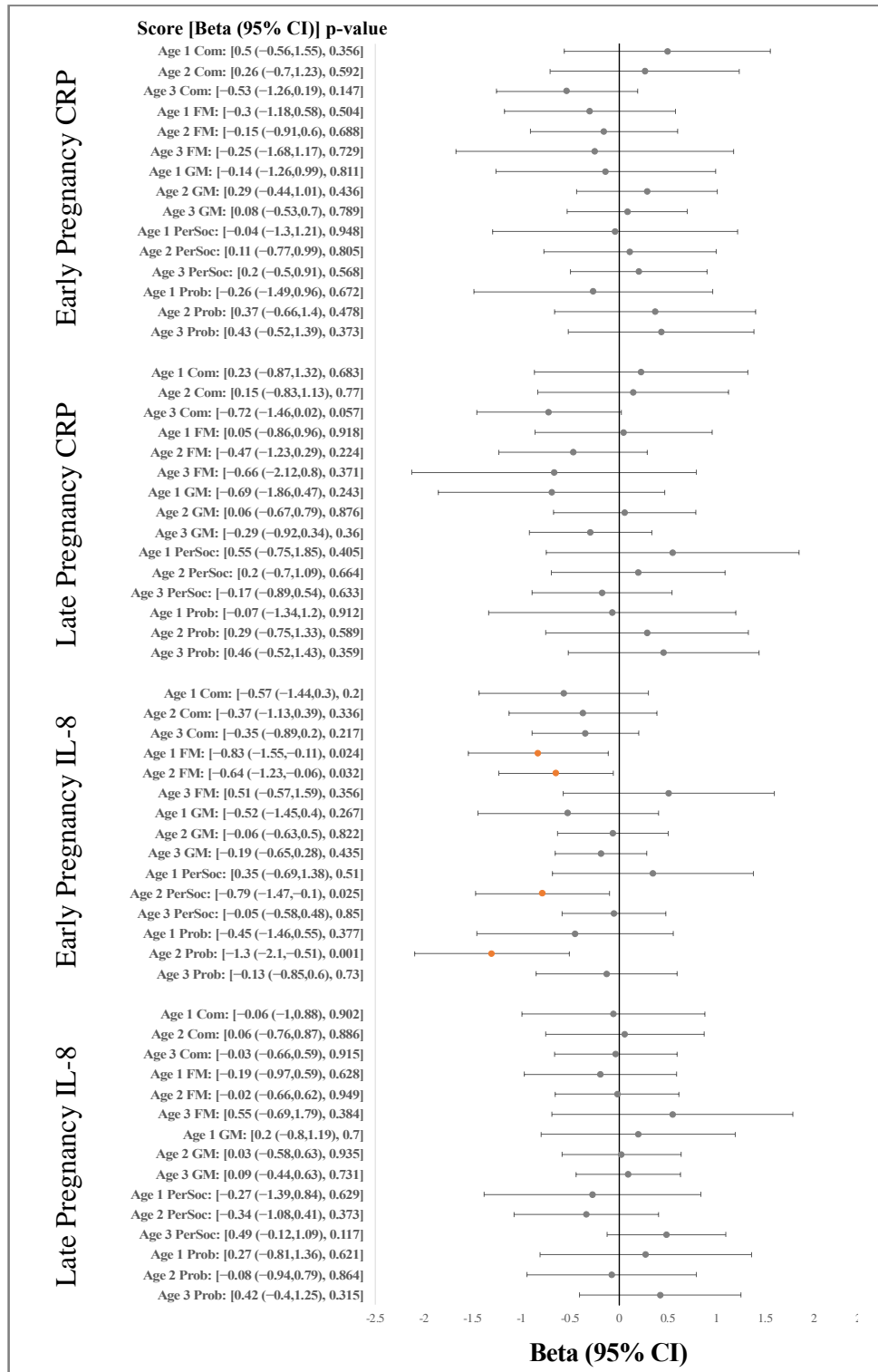
Comm; Communication score

FM; Fine-Motor score

GM; Gross-Motor score

Prob; Problem-solving score

Supplementary Figure S6: Associations between 1st and 3rd trimester levels of CRP and IL8 with skills scores in the five domains of the Ages and Stages Questionnaire at ages 1,2, and 3 with additional adjustment for pre-pregnancy BMI



ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level, gestational age at delivery and prepregnancy BMI

Grey; NS

Orange: Significant at $p < 0.05$

Red: Significant after Benjamini-Hochberg FDR correction

PerSoc; Personal-social score

Comm; Communication score

FM; Fine-Motor score

GM; Gross-Motor score

Prob; Problem-solving score

Supplementary Table S8: Association between prepregnancy BMI and Ages and Stages Questionnaire scores in 426 mother-child pairs

| Domain | Age gp | β | Lower 95% CI | Upper 95% CI | P-value |
|-------------------------------------|----------------|---------|--------------|--------------|---------|
| Personal-social skills score | Age1yrs | -0.120 | -0.286 | 0.046 | 0.158 |
| | Age2yrs | -0.085 | -0.196 | 0.025 | 0.132 |
| | Age3yrs | -0.049 | -0.137 | 0.040 | 0.282 |
| Problem solving skills score | Age1yrs | -0.201 | -0.363 | -0.038 | 0.016* |
| | Age2yrs | -0.056 | -0.185 | 0.073 | 0.395 |
| | Age3yrs | -0.059 | -0.179 | 0.062 | 0.340 |
| Gross motor skills score | Age1yrs | -0.041 | -0.191 | 0.108 | 0.587 |
| | Age2yrs | -0.025 | -0.116 | 0.066 | 0.592 |
| | Age3yrs | 0.024 | -0.054 | 0.102 | 0.545 |
| Fine motor skills score | Age1yrs | -0.055 | -0.171 | 0.062 | 0.358 |
| | Age2yrs | 0.015 | -0.080 | 0.110 | 0.755 |
| | Age3yrs | -0.061 | -0.241 | 0.118 | 0.503 |
| Communication skills score | Age1yrs | 0.013 | -0.127 | 0.154 | 0.851 |
| | Age2yrs | -0.120 | -0.242 | 0.002 | 0.054 |
| | Age3yrs | -0.086 | -0.177 | 0.005 | 0.066 |

ASQ score and biomarker levels were both treated as continuous variables. Analyses were adjusted for study site, treatment group, maternal marital status and educational level, gestational age at delivery

**Significant at $p < 0.05$*

Supplementary Table S9: CRP and IL-8 as non-significant mediators in the association between pre-pregnancy BMI and age one problem solving score

| Independent variable | Mediator | Outcome | Direct Effect ¹ | Indirect Effect ² | Proportion Mediated ³ |
|----------------------|----------------------|-------------------------------|---|--|---|
| Pre-pregnancy BMI | Early pregnancy CRP | Age One problem solving score | -0.183 (-0.417, 0.050) <i>p</i> = 0.120 | -0.022 (-0.129, 0.080) <i>p</i> = 0.680 | 0.098 (-0.569, 1.590) <i>p</i> = 0.700 |
| Pre-pregnancy BMI | Early pregnancy IL-8 | Age One problem solving score | -0.199 (-0.391, -0.020) <i>p</i> = 0.022 | -0.001 (-0.016, 0.010) <i>p</i> =0.814 | 0.003 (-0.112, 0.150) <i>p</i> =0.814 |
| Pre-pregnancy BMI | Late pregnancy CRP | Age One problem solving score | -0.199 (-0.410, 0.010) <i>p</i> = 0.040 | -0.004 (-0.074, 0.060) <i>p</i> =0.874 | 0.021 (-0.569, 1.000) <i>p</i> =0.894 |
| Pre-pregnancy BMI | Late pregnancy IL-8 | Age One problem solving score | -0.206 (-0.405, -0.020) <i>p</i> = 0.036 | 0.006 (-0.015, 0.030) <i>p</i> =0.036 | -0.022 (-0.272, 0.130) <i>p</i> =0.604 |

¹Direct effect = effect of independent variable on outcome, controlling for the mediator, reported as estimate (95% confidence interval) and associated *p*-value

²Indirect effect = average mediator effect, reported as estimate (95% confidence interval) and associated *p*-value

³Proportion mediated = proportion of effect of independent variable on outcome through the mediator (indirect effect/total effect), reported as proportion (95% confidence interval) and associated *p*-value

All models were adjusted for study site, treatment group, maternal marital status and educational level