

Supplementary Table 1 – Causal framework for testing various explanations for the association between methylation at *HIF3A* and body mass index

	Cross-sectional analysis		Longitudinal analysis		Mendelian randomization analysis		Inter-generational analysis			
	Basic model	Adjusted model	<i>HIF3A</i> baseline – BMI follow-up	BMI baseline – <i>HIF3A</i> follow-up	IV for methylation	IV for BMI	IV for maternal BMI	IV adjusted for offspring genotype	Independent maternal association	Independent paternal association
Analysis confounded by socio-economic and lifestyle factors										
<i>HIF3A</i> with causal effect on BMI										
BMI with causal effect on <i>HIF3A</i>										
Bidirectional causal effect										
BMI – <i>HIF3A</i> association confounded by intra-uterine effect										

Unfilled boxes represent no associations observed in the outlined analyses, dark filled boxes represent strong evidence of association and light filled boxes represent weak evidence of association.

Supplementary Table 2 - Associations between methylation at cg27146050 in adolescence and potential confounding factors

Confounder	Group	Methylation at cg27146050 (N=974) <sup>†</sup>	
		Mean	P-value for trend
Adolescent age	<17 years	0.172	0.006
	≥17 years	0.165	
Sex	Male	0.171	<0.001
	Female	0.162	
Birthweight	Low	0.168	0.001
	Normal	0.165	
	Macrosomia	0.176	
Gestational age	Pre-term	0.165	0.88
	Term	0.167	
	Post-term	0.162	
Maternal education	No degree	0.168	0.08
	Degree	0.163	
Household social class	Manual	0.170	0.22
	Not manual	0.166	
Adolescent smoking status	Never/less than weekly	0.167	0.77
	Weekly	0.171	
	Daily	0.166	
Adolescent alcohol consumption	Less than weekly	0.166	0.79
	At least weekly	0.167	
Maternal smoking during pregnancy	Never	0.168	0.03
	Any	0.162	
Maternal alcohol during first trimester	Never	0.168	0.28
	Any	0.166	

<sup>†</sup>N varies from 792 to 974 depending on completeness of data.

Supplementary Table 3 – Associations between methylation at cg27146050 and BMI in adolescence with adjustment for cell composition

Model	N	Without adjustment for cell composition		With adjustment for cell composition	
		Percentage change in BMI (95% CI) <sup>c</sup>	p-value	Percentage change in BMI (95% CI) <sup>c</sup>	p-value
Basic model <sup>a</sup>	845	4.66 (1.04, 8.29)	0.012	5.27 (1.62, 8.92)	0.005
Adjusted model <sup>b</sup>	804	3.49 (-0.12, 7.10)	0.058	4.01 (0.37, 7.64)	0.031

<sup>a</sup> adjusted for age, sex, smoking and batch

<sup>b</sup> adjusted for age, sex, smoking, batch, alcohol, maternal education, social class, maternal smoking, maternal alcohol, birthweight and gestational age

<sup>c</sup> Coefficients have been converted into percentage change in BMI for every 0.1 unit increase in methylation  $\beta$  value.

Supplementary Table 4- Associations between methylation at three CpG sites at *HIF3A* and FMI in adolescence

	Basic model (N=829) <sup>a</sup>		Adjusted model (N=789) <sup>b</sup>	
	Percentage change in BMI (95% CI) <sup>c</sup>	p-value	Percentage change in BMI (95% CI) <sup>c</sup>	p-value
cg22890170	2.84 (-0.37, 6.05)	0.08	1.99 (-1.28, 5.25)	0.23
cg27146050	11.79 (-0.15, 23.72)	0.05	8.86 (-3.27, 20.98)	0.15
cg16672562	1.87 (-0.77, 4.52)	0.17	1.55 (-1.16, 4.26)	0.26

<sup>a</sup> Adjusted for age, sex, smoking and batch.

<sup>b</sup> Basic model additionally adjusted for smoking, alcohol, maternal education, social class, maternal smoking, maternal smoking, maternal alcohol, birthweight and gestational age.

<sup>c</sup> Coefficients have been converted into percentage change in BMI for every 0.1 unit increase in methylation  $\beta$  value.

Supplementary Table 5 - Prospective associations between childhood BMI and adolescent methylation, and between childhood methylation and adolescent BMI, adjusted for potential confounding factors

Exposure	Outcome	CpG site	N	Association without adjustment for the outcome at baseline *		N	Association with adjustment for the outcome at baseline *	
				$\beta$ (95% CI)	p-value		$\beta$ (95% CI)	p-value
Birth methylation	Childhood BMI	cg22891070	825	-1.74 (-3.69, 0.25) <sup>a</sup>	0.09	814	-1.54 (-3.46, 0.43) <sup>a</sup>	0.09
		cg27146050	825	-0.06 (-1.10, 0.99) <sup>a</sup>	0.91	814	0.22 (-0.82, 1.27) <sup>a</sup>	0.67
		cg16672562	825	-2.54 (-4.95, -0.06) <sup>a</sup>	0.04	814	-2.50 (-4.87, -0.07) <sup>a</sup>	0.07
Birth weight	Childhood methylation	cg22891070	892	0.02 (0.003, 0.04) <sup>b</sup>	0.02	811	0.02 (0.001, 0.034) <sup>b</sup>	0.02
		cg27146050	892	0.01 (0.0003, 0.01) <sup>b</sup>	0.04	811	0.01 (0.002, 0.013) <sup>b</sup>	0.04
		cg16672562	892	0.03 (0.01, 0.05) <sup>b</sup>	0.01	811	0.02 (0.003, 0.045) <sup>b</sup>	0.01
Childhood methylation	Adolescent BMI	cg22891070	872	0.53 (-0.55, 1.60) <sup>a</sup>	0.34	869	0.03 (-0.82, 0.77) <sup>a</sup>	0.95
		cg27146050	872	1.60 (-1.47, 4.68) <sup>a</sup>	0.31	869	0.78 (-1.50, 3.06) <sup>a</sup>	0.50
		cg16672562	872	0.16 (-0.68, 1.00) <sup>a</sup>	0.70	869	-0.21 (-0.83, 0.42) <sup>a</sup>	0.51
Childhood BMI	Adolescent methylation	cg22891070	919	0.005 (-0.002, 0.013) <sup>c</sup>	0.15	886	0.001 (-0.005, 0.006) <sup>c</sup>	0.83
		cg27146050	919	0.003 (0.001, 0.005) <sup>c</sup>	0.001	886	0.003 (0.001, 0.004) <sup>c</sup>	0.001
		cg16672562	919	0.007 (-0.002, 0.016) <sup>c</sup>	0.12	886	0.003 (-0.004, 0.009) <sup>c</sup>	0.39

\* Also adjusted for age in childhood/adolescence, sex, batch, maternal education, social class, maternal smoking, maternal alcohol, birthweight and gestational age

<sup>a</sup> Coefficients have been converted into percentage change in BMI for every 0.1 unit increase in methylation  $\beta$  value.

<sup>b</sup> Coefficients are change in methylation per 1kg increase in birthweight.

<sup>c</sup> Coefficients are change in methylation per 10% increase in BMI.

Supplementary Table 6 - Association between SNPs at the HIF3A locus and methylation level at cg27146050 in adolescence

Exposure	N	Frequency of effect allele <sup>a</sup>	b (95% CI)	p-value	R <sup>2</sup> <sup>c</sup>	F-statistic
rs8102595	849	0.099	0.023 (0.018, 0.028) <sup>b</sup>	<10 <sup>-10</sup>	8.0%	73.4
rs3826795	849	0.780	0.007 (0.003, 0.011) <sup>b</sup>	0.001	1.4%	12.0
cis SNP score <sup>†</sup>	849	-	0.204 (0.155, 0.252)	<10 <sup>-10</sup>	7.4%	67.6

These models were adjusted for batch, except when calculating R<sup>2</sup> where no covariates were included.

<sup>a</sup> Effect allele for rs8102595 is G; effect allele for rs3826795 is C.

<sup>b</sup> Coefficients are from an additive model and are a unit change in methylation per copy of the effect allele

<sup>c</sup> R<sup>2</sup> is the percentage of variation in methylation that is explained by each SNP.

<sup>†</sup> cis SNP score = 0.075 x rs8102595 + 0.047 x rs3826795 (using weights from a meta-analysis of the discovery and replication cohorts in Dick et al (6)).

Supplementary Table 7 – Associations between BMI allele scores and BMI in adolescence

	Mean allele score (s.d.)	b (95% CI) <sup>a</sup>	p-value	R <sup>2</sup> <sup>b</sup>	F-statistic	N
Adolescent standardised 97 SNP allele score	88.7 (6.2) <sup>c</sup>	0.036 (0.025, 0.046)	<10 <sup>-10</sup>	5.2%	45.7	831

<sup>a</sup> Coefficients are change in log(BMI) per unit increase in the standardised allele score.

<sup>b</sup> R<sup>2</sup> is the percentage of variation in methylation that is explained by the allele score.

<sup>c</sup> BMI allele scores are always standardised except when reporting the mean and s.d. of the allele score.

Supplementary Table 8 - Associations between body mass index, fat mass index, and genetic variants and possible confounding factors<sup>†</sup>

Confounder	Group	Adolescent BMI (kg/m <sup>2</sup> ) (N=952)		Adolescent FMI (kg/m <sup>2</sup> ) (N=935)		Cis-SNP score (N=849)		97-SNP score (N=849)	
		Mean	P-value for difference	Mean	P-value for difference	Mean	P-value for difference	Mean	P-value for difference
Adolescent age	<17 years	21.9		5.90		0.087		0.00	
	≥17 years	22.5	0.04	5.87	0.92	0.089	0.58	0.00	0.98
Sex	Male	22.2		4.17		0.089		0.04	
	Female	22.5	0.21	7.50	<0.001	0.087	0.39	-0.04	0.24
Birth weight	Low	20.5		4.52		0.098		0.30	
	Normal	22.2	<0.001	5.84	0.08	0.086	0.24	-0.02	0.20
	High	23.6		6.28		0.093		0.09	
Gestational age	Pre-term	21.5		4.81		0.010		0.03	
	Term	22.3	0.34	5.90	0.19	0.088	0.17	0.00	0.83
	Post-term	23.6		7.10		0.074		0.20	
Maternal education	No degree	22.4		6.00		0.089		0.00	
	Degree	21.6	0.006	5.24	0.007	0.084	0.13	0.00	0.97
Household social class	Manual	22.5		6.27		0.089		0.01	
	Not manual	22.2	0.43	5.77	0.16	0.089	0.86	-0.01	0.81
Adolescent smoking status	Never/less than weekly	22.2		5.77		0.087		-0.03	
	Weekly	22.9	0.05	6.45	0.12	0.090	0.59	0.45	0.04
	Daily	23.1		6.50		0.093		0.11	
Adolescent alcohol consumption	Less than weekly	22.2		5.89		0.089		-0.04	
	At least weekly	22.7	0.13	5.91	0.95	0.083	0.05	0.11	0.08
	Never	22.2		5.75		0.088		-0.02	

Maternal smoking during pregnancy	Any	22.8	0.05	6.37	0.03	0.089	0.73	0.07	0.27
Maternal alcohol during first trimester	Never	22.5		6.13		0.089		-0.04	
	Any	22.2	0.22	5.68	0.06	0.088	0.57	0.03	0.34

† N varies from 694 to 952 depending on completeness of data on confounding factors

Supplementary Table 9 – Mendelian randomization analysis for the association between maternal BMI and offspring methylation at *HIF3A*.

Instrumental variable (IV)	Exposure (E)	Outcome (O)	Observed association between IV and O (c)		Expected association between IV and O (a x b)	Difference between observed (c) and expected (a x b) estimates
			N	$\beta$ (95% CI)	$\beta$ (95% CI)	P-value
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at birth at cg27146050	624	-0.0039 (-0.0118, 0.0039)	-0.0011 (-0.0023, 0.0002)	0.46
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at birth at cg25196389	624	0.0132 (0.0002, 0.0263)	0.0029 (0.0005, 0.0054)	0.11
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at birth at cg23548163	624	0.0025 (-0.0074, 0.0124)	0.0015 (0.0000, 0.0030)	0.84
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at birth at cg26749414	624	0.0095 (-0.0110, 0.0301)	0.0064 (0.0022, 0.0105)	0.75
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at birth at cg20667364	624	0.0018 (-0.0051, 0.0087)	0.0008 (-0.0003, 0.0019)	0.77
Maternal standardised 97 SNP allele score*	Maternal pre-pregnancy log BMI	Methylation at adolescence at cg27146050	665	-0.0013 (-0.0042, 0.0015)	0.0007 (0.0002, 0.0013)	0.16

\* Analyses adjusted for offspring allele score.



Supplementary Table 10 – Association analysis of *HIF3A* cis-SNPs with body mass index in GIANT and two-sample Mendelian randomization results

Instrumental variable (IV)	Effect allele	Effect allele frequency	Direct-genotype associations		Wald-ratio estimate*
			N	$\beta$ (SE)	$\beta$ (95% CI)
rs382679	C	0.78	224,403	0.002 (0.005)	0.33 (-1.03, 1.69)
rs8102595	G	0.10	223,534	-0.002 (0.007)	-0.08 (-0.65, 0.49)
Inverse-variance fixed effects meta-analysis	-	-	-	-	-0.02 (-0.55, 0.51)

\*Estimates for SNP-methylation association taken from ARIES; estimates for SNP-BMI association taken from GIANT meta-analysis results of body mass index. Standard errors for the Wald-ratio estimates were calculated using the delta-method (reference 52 in main paper).

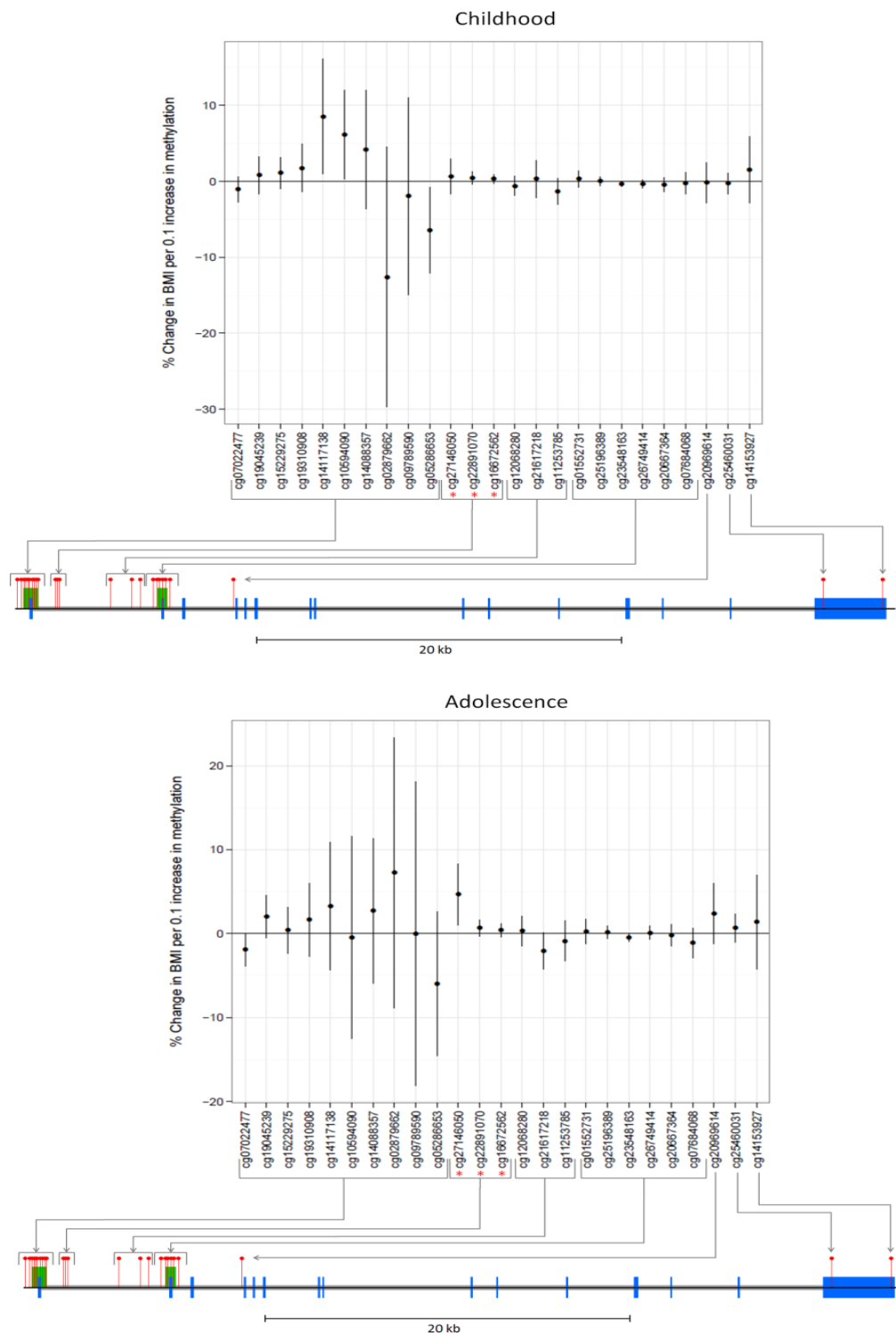
Supplementary Table 11 - Associations between methylation at three CpG sites at *HIF3A* and BMI in adult women

	At pregnancy (mean age 29.2 years) Basic model (N=874)*		At follow up (mean age 47.4 years) Basic model (N=694)*	
	Percentage change in BMI (95% CI)	p-value	Percentage change in BMI (95% CI)	p-value
cg22891070	0.79 (-0.04, 1.61)	0.06	0.87 (-0.27, 2.02)	0.14
cg27146050	2.17 (-1.26, 5.71)	0.22	2.85 (-1.84, 7.76)	0.24
cg16672562	0.75 (0.03, 1.47)	0.04	0.21 (-0.78, 1.21)	0.68

\*Adjusted for age, smoking and batch.

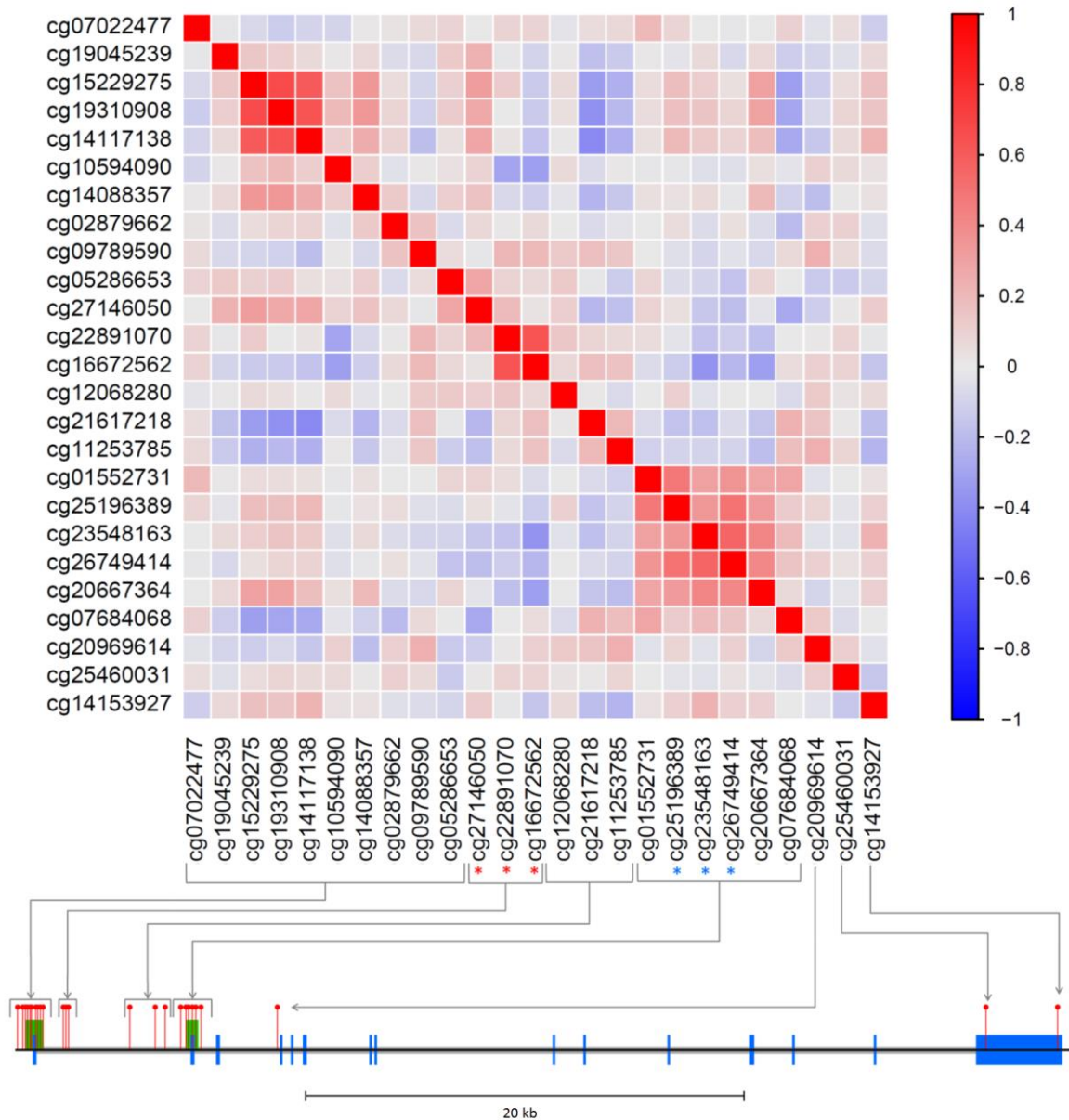
Coefficients have been converted into percentage change in BMI for every 0.1 unit increase in methylation  $\beta$  value.

Supplementary Figure 1 - Associations between methylation at *HIF3A* CpG sites and BMI



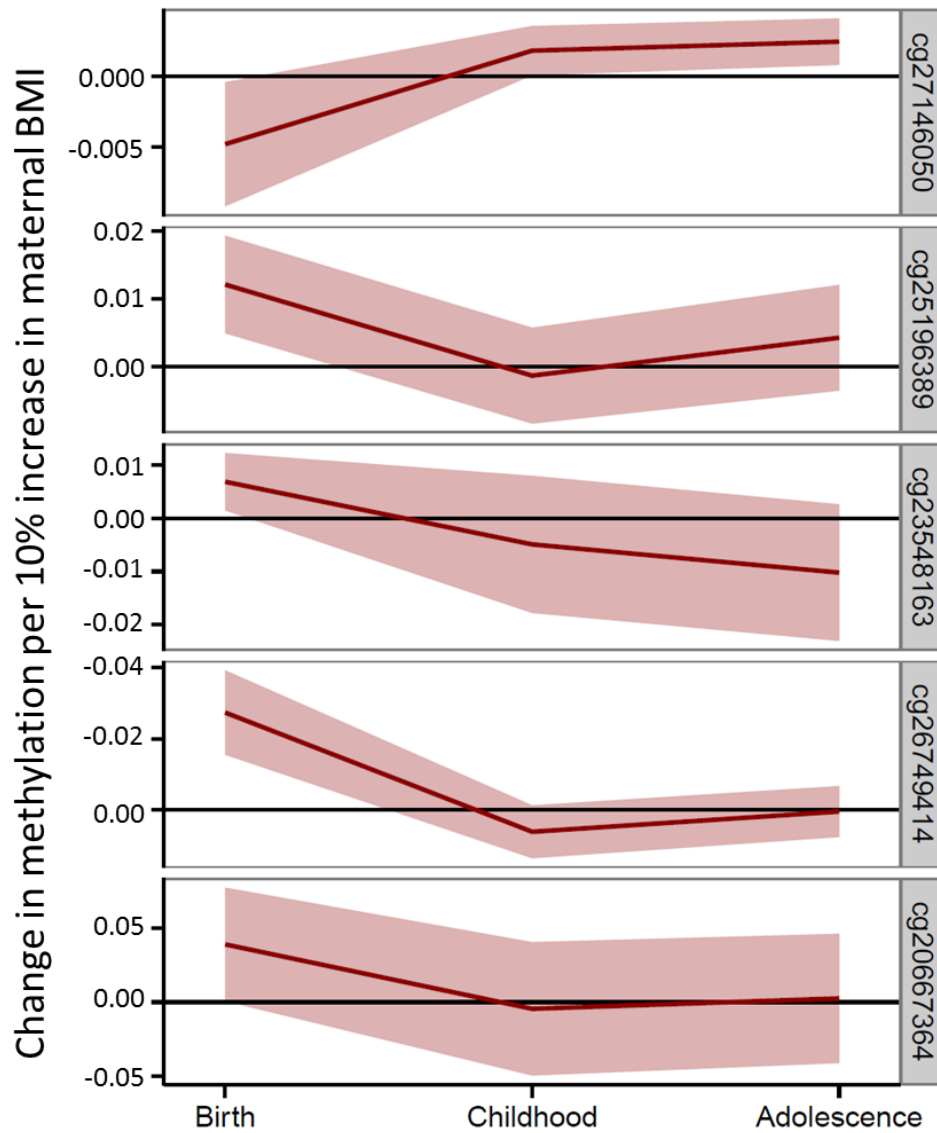
Associations of BMI and peripheral blood methylation at all 25 CpG sites at the *HIF3A* locus in childhood and adolescence. The locations of CpG sites on the *HIF3A* gene are mapped on the diagram below the graph. Blue blocks are exons, grey blocks are introns, green blocks are CpG islands and red pins are CpG sites. The three sites previously identified in adult peripheral blood as associated with own BMI are highlighted with a red asterisk. No other sites survived correction for multiple testing.

Supplementary Figure 2 - A heatmap of the correlation between methylation  $\beta$ -values at each *HIF3A* CpG site at birth.



The locations of CpG sites on the *HIF3A* gene are mapped on the diagram below the graph. Blue blocks are exons, grey blocks are introns, green blocks are CpG islands and red pins are CpG sites. The three sites previously identified in adult peripheral blood as associated with own BMI are highlighted with a red asterisk.

Supplementary Figure 3 –Associations between maternal pre-pregnancy BMI and offspring DNA methylation from birth to adolescence\*



\*birth (cord blood at birth, n=797), childhood (peripheral blood at around 7 years-old, n=846) and adolescence (peripheral blood at around 17 years-old, n=853)  
 Mean change in methylation 10% increase in maternal pre-pregnancy BMI; error bands indicate 95% confidence intervals