Supplemental Material to:

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http://www.landesbioscience.com/journals/epigenetics/article/22141/

Supplementary Table 1. RT-qPCR analsys of gene expression in week 3: ΔCt values

Gene	control		GLP		PLP	
	mean	st.error	mean	st.error	mean	st.error
Igf2	-9.2	0.51	-8.8	1.22	-7.3	0.65
Gnas	-4.7	0.27	-5.7	0.31	-3.4	0.47
Grb10	-9.16	0.17	-9.27	0.31	-8.48	0.32
Pparα	-5.4	0.13	-5.4	0.14	-5.0	0.23
Cdkn1c	-8.2	0.17	-8.5	0.40	-8.0	0.12
Phlda2	-14.3	0.43	-15.6	0.55	-14.8	0.42

ΔCt values for each gene are given as the difference from the mean Ct values of the three housekeeping genes.

Supplementary Table 2. RT-qPCR analsys of gene expression in week 12: ΔCt values

Gene	control		GLP		PLP	
	mean	st.error	mean	st.error	mean	st.error
Gnas	-2.3	0.14	-2.9	0.13	-2.8	0.17
Pparα	-3.6	0.14	-4.0	0.19	-4.5	0.21

ΔCt values for each gene are given as the difference from the mean Ct values of the three housekeeping genes. *Igf2* and *Grb10* were deemed to be undetectable in expression, as their Ct values >35.

Supplementary Table 3. Details of regions analysed and PCR primers for amplification of bisulphite-treated DNA for Sequenom EpiTYPER.

Gene name/DMR	Gene location (region analysed)	CpGs analysed[1]	Primer sequence
Nespas/Gnasxl (E)	chr 2: 174122669 - 174122979	2, [3+4], 5, [7+8], 10, 11, 13, 14, 15, 16, 17, 18, 19, 20	F 5’-GGTGATAAAGTTTTTTTGTAG-3’ R 5’-ACRCAAATCTCAATACTAAA-3’
Nespas/Gnasxl (H)	chr 2: 174124549 - 174124803	1, 2, [4+5], 7, 8, 9, 11, [12-14], 16, 17, 18	F 5’-TTAAGTTAGTTTTTGTTGTTTAAGT-3’ R 5 -CTAAATCTCTACTACCTTCAAACTC-3’
Gnas exon 1A	chr 2: 174153180 - 174153516	1, 2, [3-6], [7-9], [10+11], 12, [13-15], 18, [20-22], 23, [24+25], 26, 27, [28+29], 30, 31, 32	F 5’-GTTTATGGGTYGGTTTTTTGAGAGGTT-3’ R 5’-TCTACCCTATCCCRACTCTTA-3’
Igf2 (DMR1)	chr 7: 149851432 - 149851614	1, 2, 3	F 5’-GGTGAAGGTTTTGTGGGTAGTTAT-3’ R 5’-TCTACCCTCCAAAAATCTCTAAAA-3’
Igf2 (DMR2)	chr 7: 149839814 - 149840078	3, [4+5], [6+7]	F 5’- ATTTTAGGGAAGTTGTTTTGAGGTG-3’ R 5’-TCTAAACTCTTTAAACTCTTTAACAAACA-3’
H19	chr 7: 149765940 - 149766258	[2+3], 4, 6, 7, 8, 9, 10, [11+12], 13	F 5’-GATTTGGTTATAGTTAAATGGATAG-3 R 5’-CATTACAATAATTAAACCCCAAC-3
Grb10	chr 11: 11925732 - 11926044	1, 3, 4, 5, 6, 7, 8, [9-13], 14, 15, 16, [17+18], 19, 26, 27	F 5’-GTAGATTTYGGGAGGTGAATT-3’ R 5’-AACTCCAAAACCCTTTTTCTA-3’
Kcnq1ot1 (KvDMR)	chr 7: 150481596- 150481801	1, [4+5], [7+8], 11, 14, [16+17], [18+19], 21, [22+23], [24+25], [26+27], 28, 29, [30+31], 32, 33	F 5’-GGTTTTAAGATTATTTTTGTTTTTGTAA-3’ R 5’-TTTTCTATTCAACTTAATTCCCAAC-3’
PPARα promoter	chr 15: 85565799- 85565986	[2-4], [8-10], 11,	F 5’-GGGGTGTGTTTAGTTTTGAAT-3’

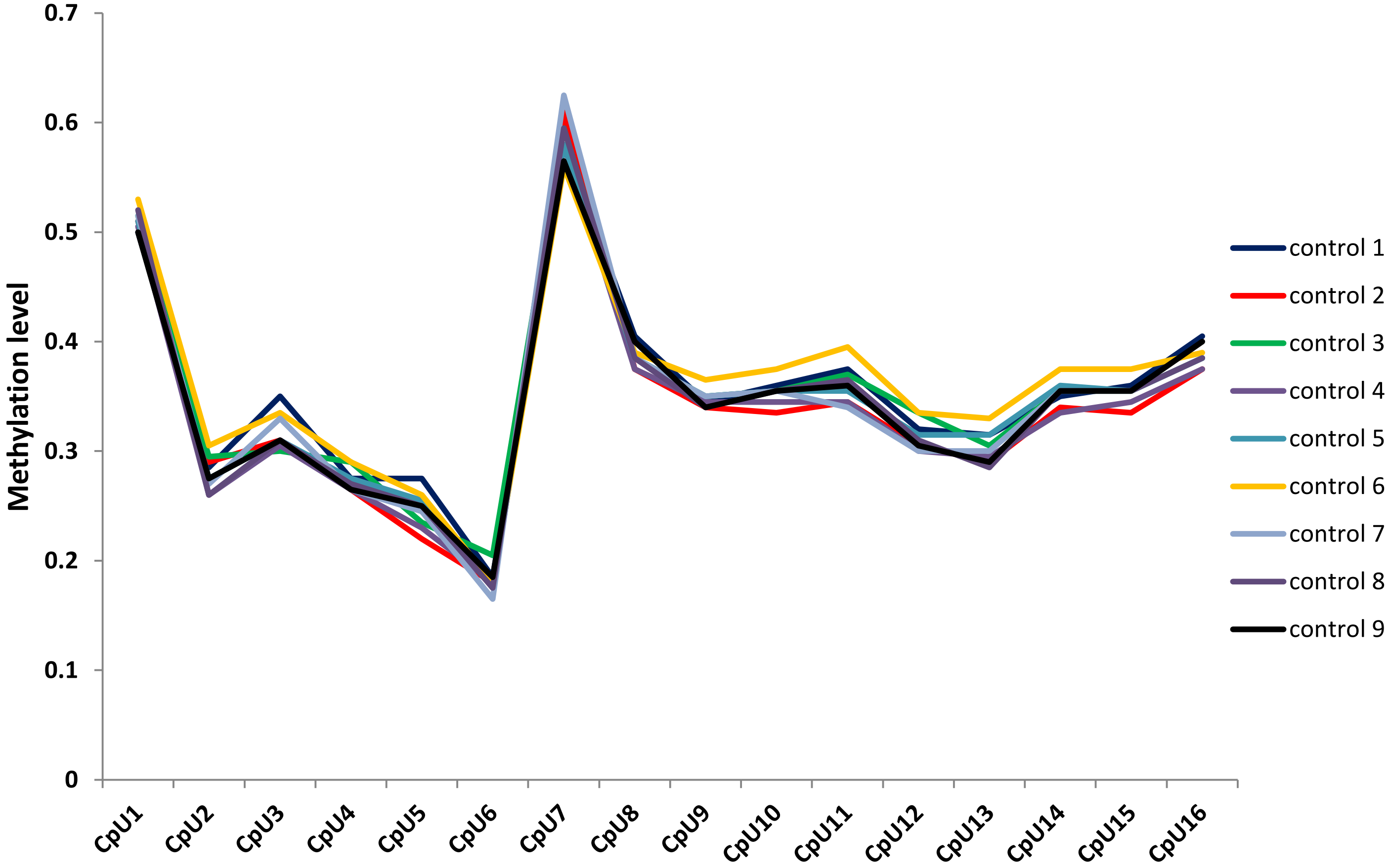
		[12+13], [20-23], [24+25], [27+28]	R 5’-TCACCCCTATCCTAAAACC-3’
PPARα enhancer	chr 15: 85514715 - 85514920	1, 2, 3, 4, [5+6], 7, 8, 9	F 5’-GGTTATTTGTAGATTTGGAAGAATGA-3’ R 5’-ACCCAACCCTTTTAATAACTCCTTA-3
B1 repetitive elements		1, 2	F 5’-GGTGGTGGTGGTGGTTGAGATAG-3’ R 5’-AATAACACACACCTTTAATCCCAACACT-3’

[1] Each CpG scored on the EpiTYPER for each amplicon is listed. CpGs given together in square brackets are not resolved on separate cleavage fragments but are scored together on a single CpG Unit. Some CpG containing fragments are outside of the size range for effective mass spectrometry analysis and are therefore excluded in the column.

Supplementary Table 4. Primers used for RT-qPCR analysis.

Gene name	Accession number	Primer sequences
Peroxisome proliferative activated receptor, alpha (*Pparα*)	NM_011144.6	F 5’-GGGCTCTCCCCACATCCTT-3’ R 5’-CCCATTTCGGTAGCAGGTAGTC-3’
Insulin-like growth factor 2 (*Igf2*)	NM_001122736.1	F 5’-AGCTTGTTGACACGCTTCAG -3’ R 5’-AAGCAGCACTCTTCCACGAT -3’
Growth factor receptor bound protein 10 (*Grb10*)	AF022072	F 5’-TGCACCACTTCTTGAGGATG-3’ R 5’-GCCGGAAGAGATGCAGTT-3’
Guanine nucleotide binding protein, alpha stimulating (*Gnas*)	NM_010309	F 5’-GGAGAAGGCGCAGCGCGAGGCCAA-3’ R 5’-CTCCGTTAAACCCATTAACATGCA-3’
Cyclin-dependent kinase inhibitor 1C (*Cdkn1c*)	NM_009876	F 5’-GGAGCAGGACGAGAATCAAG-3’ R 5’-GTTCTCCTGCGCAGTTCTCT-3’
Pleckstrin homology-like domain family A member 2 (*Phlda2*)	NM_009434	F 5’-TCAGCGCTCTGAGTCTGAAA-3’ R 5’-CTCCTGGGCTCCTGTCTGAT-3’
β-actin	AK145196.1	F 5’-GACGGCCAGGTCATCACTAT-3’ R 5’-CGGATGTCAACGTCACACTT-3’
β-2 microglobulin	NM_009735	F 5’-TGACCGGCCTGTATGCTATCC-3’ R 5’-GCAGTTCAGTATGTTCGGCTTC-3’
Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*)	M32599.1	F 5’-AGGTTGTCTCCTGCGACTTC-3’ R 5’-TGTTGCTGTAGCCGTATTCATTG-3’

SUPPLEMENTARY FIG. 1



Supplementary Figure 1

EpiTYPER analysis of *Gnas* exon 1A DMR in liver from a control group at week 12. Methylation values for each CpG unit assessed in each of 9 control individuals are plotted, each line representing the mean of the two technical replicates of each sample. The plot indicates the consistency of the methylation profile of this DMR as assessed by EpiTYPER analysis, as well as showing that each CpG unit has a characteristic methylation value that may depart from the 50% level expected for a germline DMR in a somatic tissue

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