SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Validation of multiplex SNuPE assays for the *H19/Igf2* region.

A representative mixing experiment is shown. Fourteen control DNA samples were processed in replicates along with the ChIP samples. Sonicated 129 and CS genomic DNA were mixed in different % ratios (100:0, 95:5, 90:10, 80:20, 70:30, 60:40 and 50:50, 40:60, 30:70, 20:80, 10:90, 5:95, 0:100) for the standard curves. 129 X CS DNA was used for skew correction. The components of the 7-plex and *H19* promoter assays, quantitating DNA alleles at the *H19/Igf2* imprinted domain (positions depicted graphically in Figure 1) are indicated at the right. Average measured ratios were plotted against the input ratios with standard deviations. The assays were rigorously quantitative using small amount, (25 ng) of total DNA.

Supplementary Figure 2. Validation of the novel DMR 16-plex SNuPE assays.

The 16 components of the multiplex assay, quantitating DNA alleles at 11 DMRs of interest, are indicated on the right. The components of the multiplex assay, the different DMRs are indicated at the right. Alternative SNPs are included for the *H19/Igf2* ICR, IG-DMR, *Peg1-Mest* and *Zac1* DMRs. Control 129 and JF1 genomic DNA were mixed in different % ratios. 129 X JF1 heterozygous DNA was used for skew correction..

Supplementary Figure 3. Example for the Sequenom SNuPE assays.

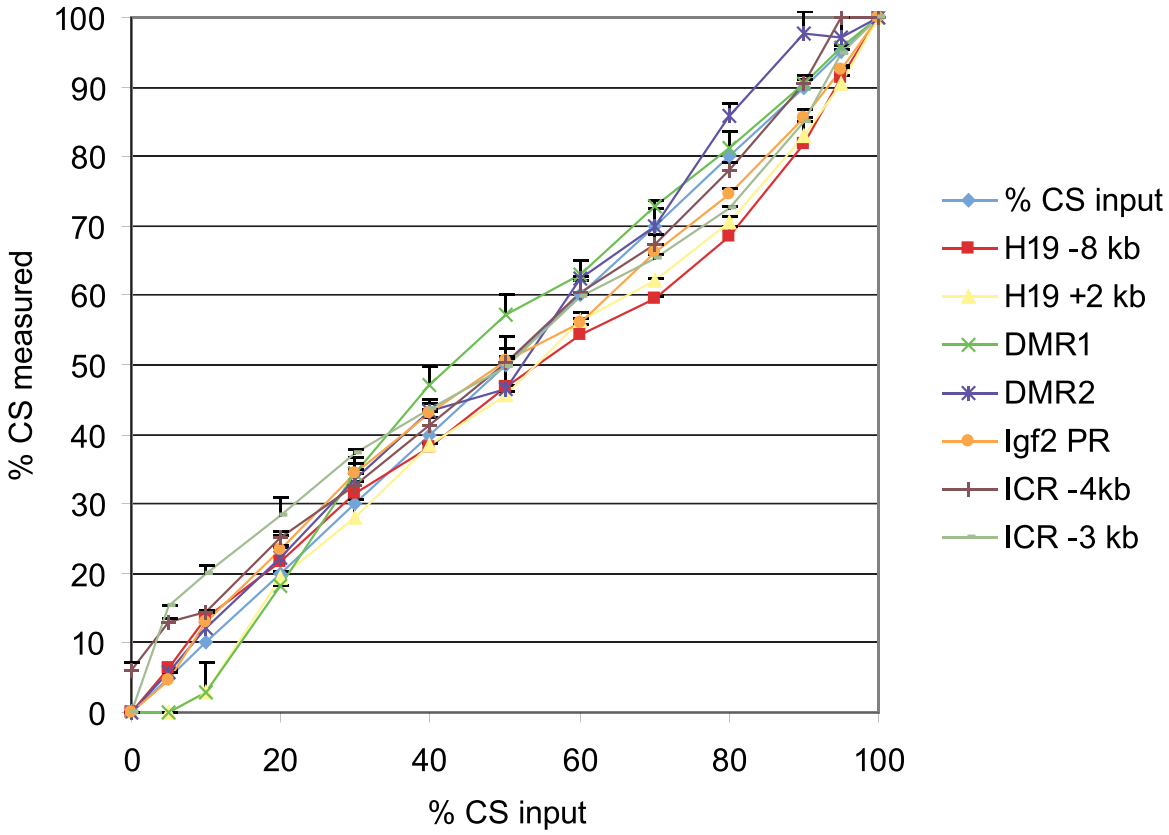
The *Zac1*#1 SNuPE assay is shown from the DMR multiplex reaction. (A) Standards. Sonicated 129 and JF1 genomic DNA were mixed in different % ratios. MALDI-TOF peaks of four data points (0:100, 100:0, 50:50 and 10:90) are shown to illustrate the accuracy of the allele-specific nucleotide incorporation (A versus G) into the unextended primer (UEP) on 129 and JF1 DNA as indicated. An asterisk indicates the peaks of the extended UEP *Peg1-Mest*#2 in the 16-plex assay. All of the data points from the full mass spectrum are plotted in Figure S2. (B) Measurement of allele-specific chromatin composition at the Zac1 DMR. Parental alleles indicated on top in the reciprocal crosses. The H3K4me2 and H3K9me3 antibodies were used to precipitate 129 X JF1 and JF1 X 129 MEF chromatin as marked below each chart. These measurements are shown in Figure 7C and 8B.

Supplementary Figure 4. Validation of the H3K79 antibodies.

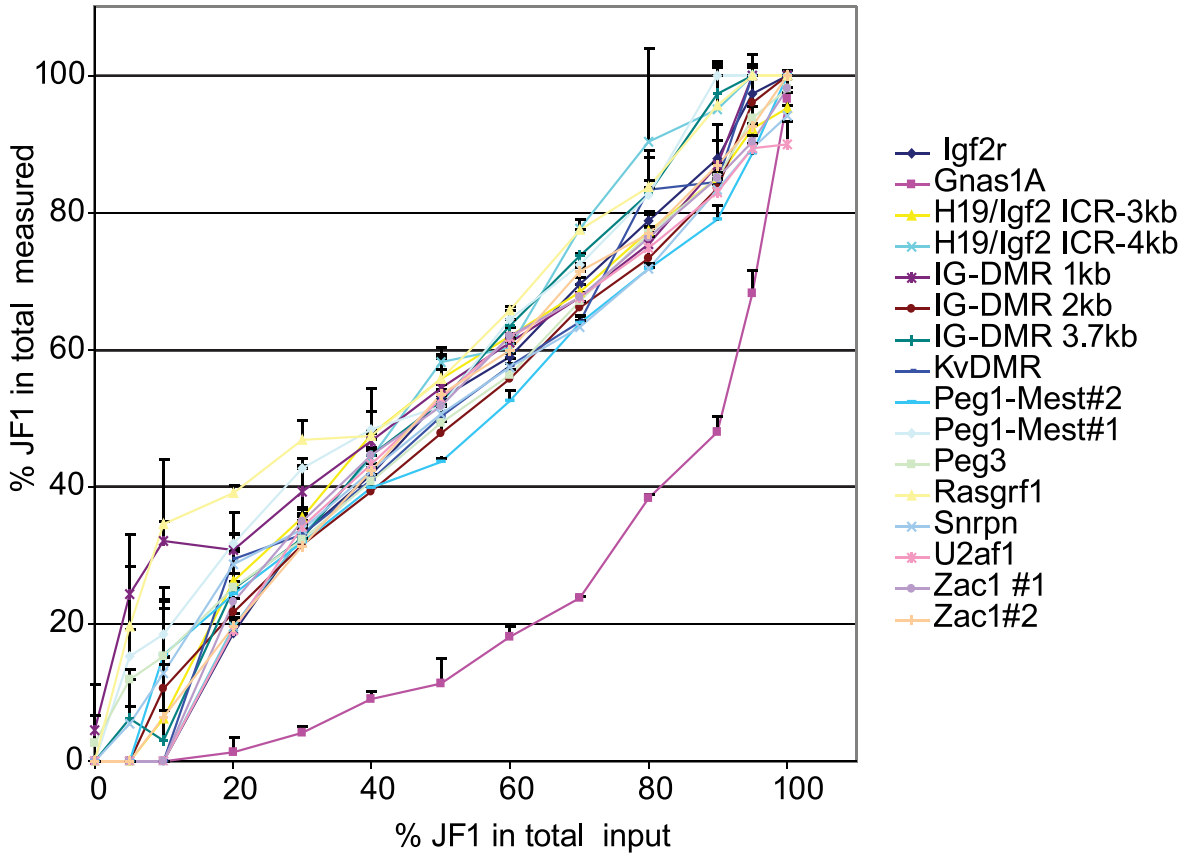
Peptide immuno-dot blot assays are shown. A dilution series was blotted from each modified histone H3 peptide and probed with the specific antibodies indicated at the top. Each antibody specifically recognized its peptide but not any of the other peptides. H3 peptides were the following: monomethyl -lysine 79 (ab4555), dimethyl-lysine 79 (07-366), trimethyl-lysine 79 (ab4557), trimethyl-lysine 9 (ab1773). Specific antibodies were the following: anti-monomethyl-Histone (Lys79) ab2886 (1:170 dilution), anti-dimethyl-Histone (Lys79) 07-366 (1:300 dilution), anti-trimethyl-Histone (Lys79) ab2621 (1:300 dilution), anti-trimethyl-Histone (Lys9) 17-625 (1:300 dilution).

Supplementary Figure 5. Validation of the 7-plex RNA SNuPE assays.

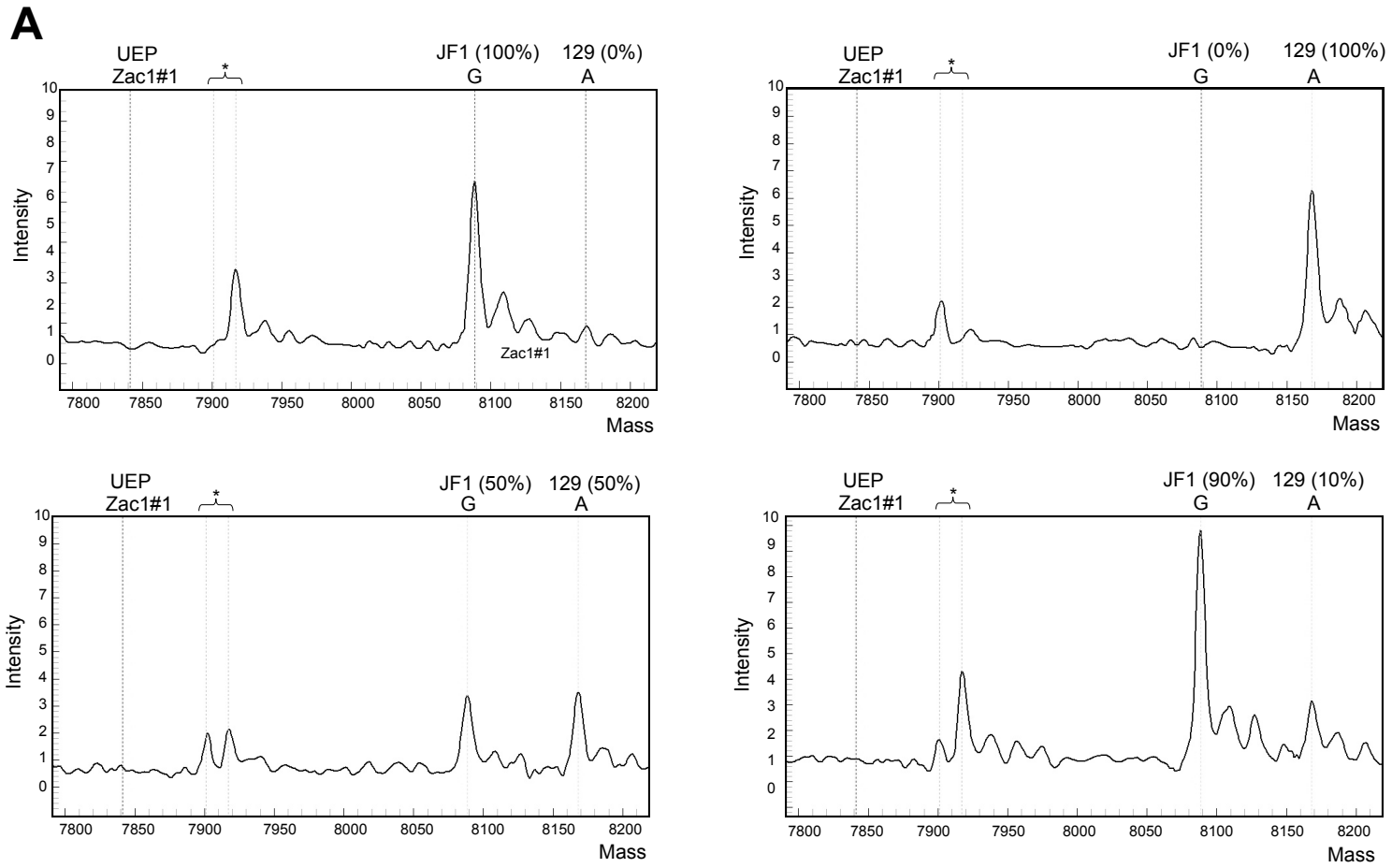
The components of the 7-plex RNA assays are indicated at the right. SNPs were obtained between 129 and JF1 RNA for *Gtl2*, *H19*, *Igf2*, *Snrpn* and *Zac1 imprinted genes* by DNA sequencing. Alternative SNPS (A and B) in the same transcript gave identical results. Allele-specific expression analysis was performed on the SEQUENOM allelotyping platform similar to multiplex ChIP-SNuPE assays. For quantitation controls, RNA was prepared form 13.5 dpc inbred 129S1 and JF1 embryos. 129 and JF1 RNAs were mixed in different ratios (100:0, 95:5, 90:10, 80:20, 70:30, 60:40 and 50:50, 40:60, 30:70, 20:80, 10:90, 5:95, 0:100) and 200 ng of the mixes were used to prepare cDNA using Superscript III Random Primer Synthesis kit. 2 μl of first strand cDNA was used for the sequenom reaction. The control mixing experiment is shown. 129 X JF1 DNA was used for skew correction. These fourteen control DNA samples were processed in replicates along with the experimental samples. Average measured ratios were plotted against the input ratios with standard deviations. The *Igf2* transcript quantitation required curve fitting. The assays were rigorously quantitative.

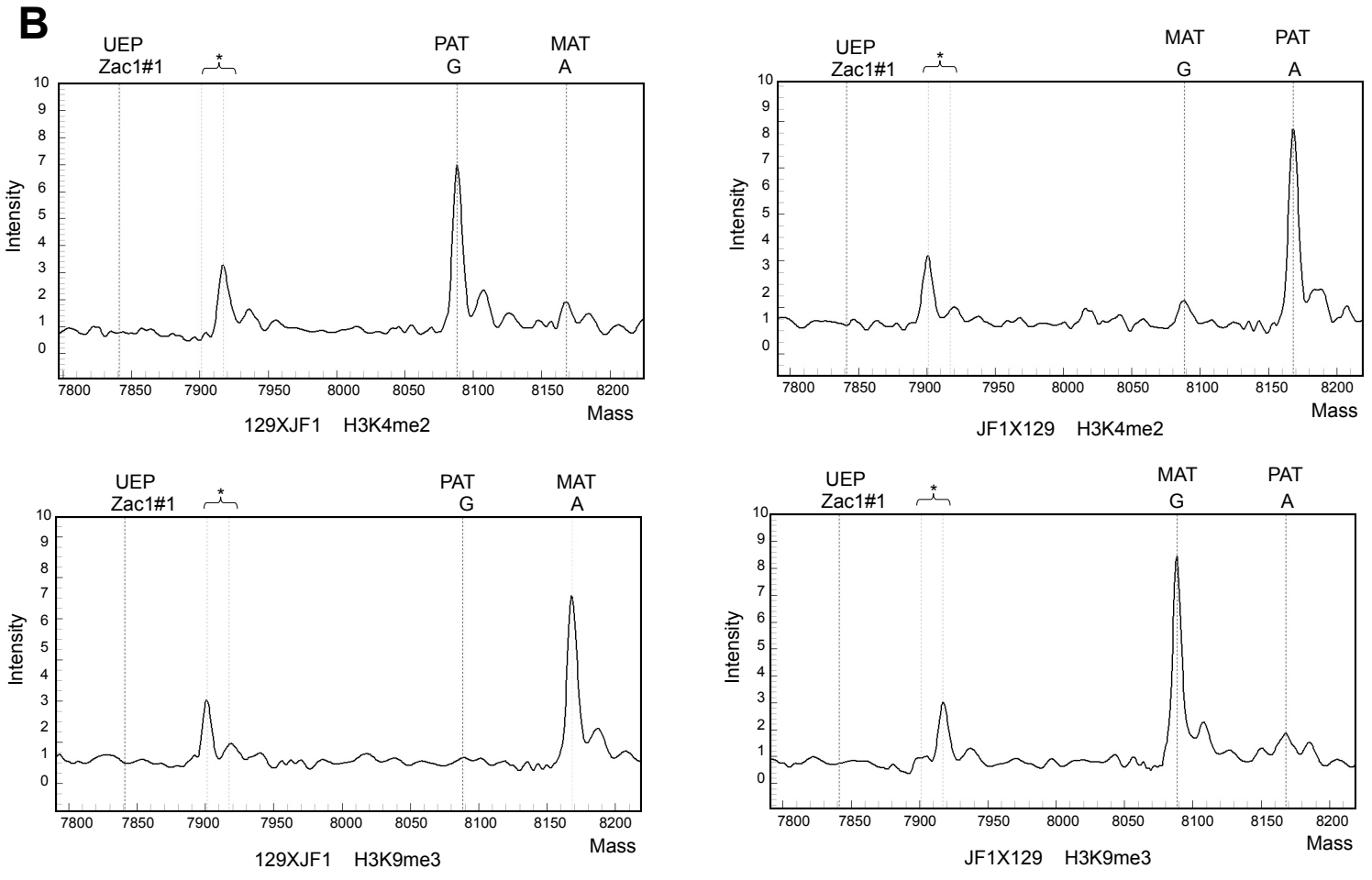


Singh et al., Fig.S1

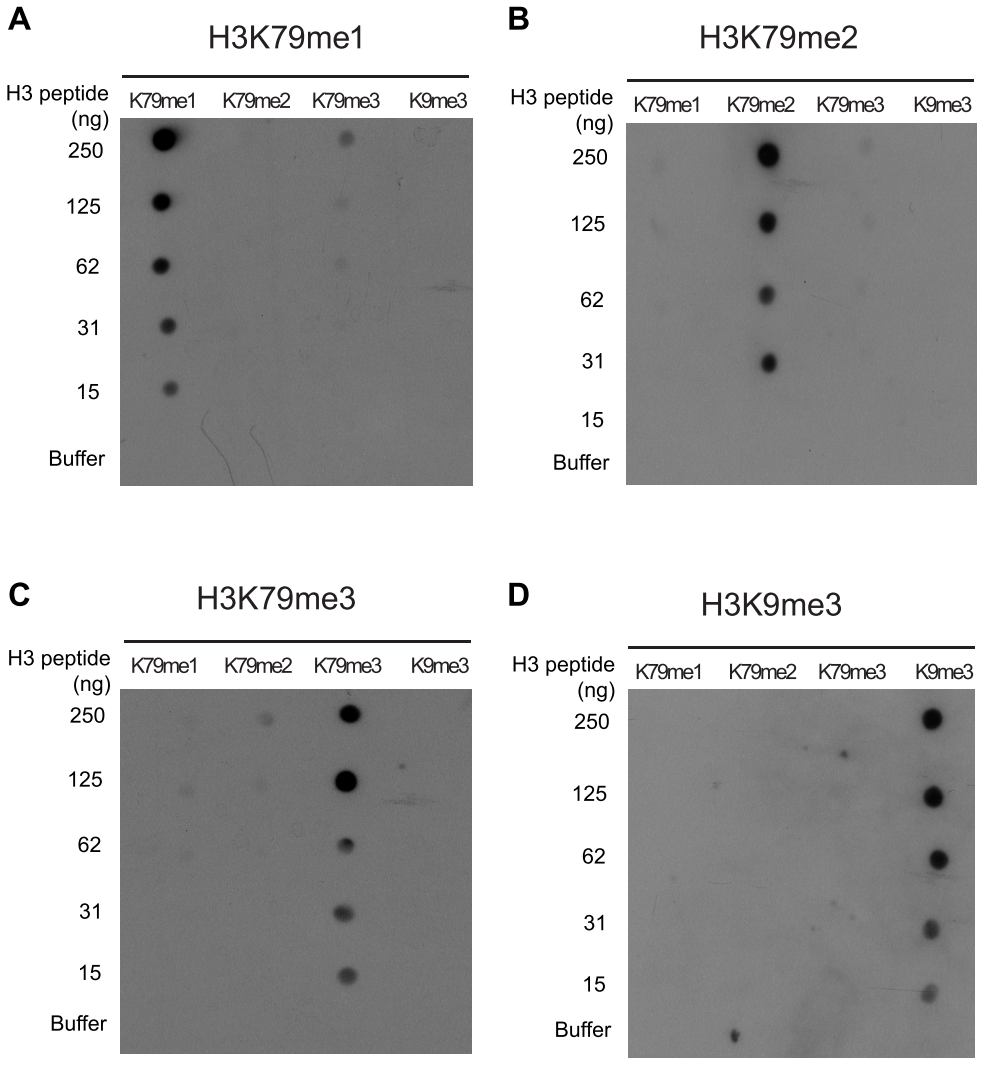


Singh et al., Fig.S2





Singh et al., Fig.S3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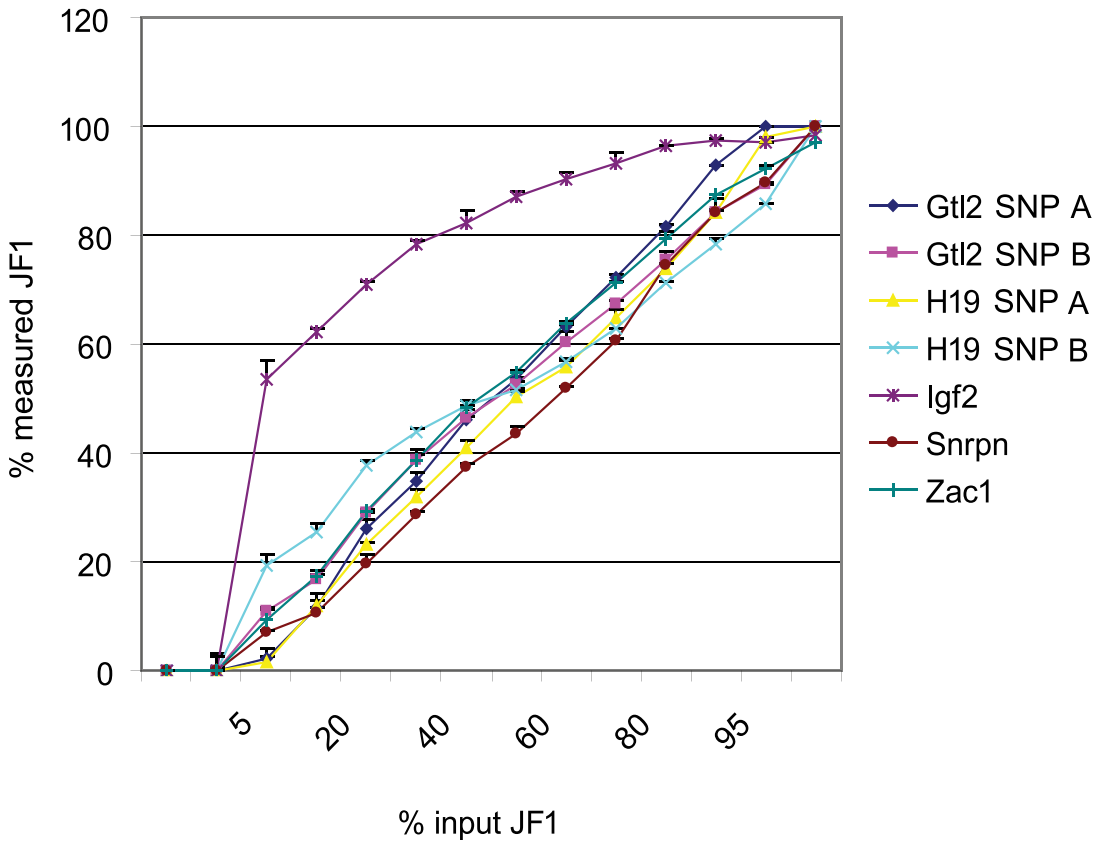

Singh et al., Fig.S5

Supplementary Table 1. Primer sequences

Sequenom-DMR 16-plex assay	Forward	Reverse	UEP
SNuPE-Igf2r	5'-ACGTTGGATGGTTCTGTGATCAGGGCCAAC-3'	5'-ACGTTGGATGTTGCCTCTCTTGCAACGTG-3'	5'-GTGGCACTTTTGAGTTCATC-3'
SNuPE-Gnas1A	5'-ACGTTGGATGTTATTCTAGAGCCCCGTGTG-3'	5'-ACGTTGGATGAACAGCCGAGAAGAAGAGCC-3'	5'-CACCTCTCCCACCTA-3'
SNuPE-H19/Igf2 ICR -3kb	5'-ACGTTGGATGCGATTGCGCCAAACCTAAAG-3'	5'-ACGTTGGATGACCCACAGCATTGCCATTTG-3'	5'-cttaCATTTGTGAATTCCAATACC-3'
SNuPE-H19/Igf2 ICR -4kb	5'-ACGTTGGATGTTTTCTAGGCTGGTACCTCG-3'	5'-ACGTTGGATGCTGGTCTTTACACACAAAGG-3'	5'-cccaGACTCCCAAATCAACAAG-3'
SNuPE-IG-DMR 1kb	5'-ACGTTGGATGGGATGGTAGTAGATAACCTG-3'	5'-ACGTTGGATGCCCGTGATTCACCACAATTC-3'	5'-TGTCTGAGAACAAAGAGC-3'
SNuPE-IG-DMR 2kb	5'-ACGTTGGATGCAATGGAGAATGCCTTGAGC-3'	5'-ACGTTGGATGTTACTCGCGTGATCCCATTC-3'	5'-gtcgGTGAGAATGTTTTAGCCAT-3'
SNuPE-IG-DMR2 3.7kb	5'-ACGTTGGATGGGGTAATTGGCAACCATAGG-3'	5'-ACGTTGGATGATGAGTCGGTGTGAAGTAAC-3'	5'-cTGCCATTTACTGCAGCC-3'
SNuPE-KvDMR	5'-ACGTTGGATGCTCAGTTCTCGTATTTTGCG-3'	5'-ACGTTGGATGTCAAAGGGCCTCAAGACCAC-3'	5'-gCGTATTTTGCGTCCACG-3'
SNuPE-Peg1-Mest#1	5'-ACGTTGGATGAACAACAAAAAGACCCCCCC-3'	5'-ACGTTGGATGCCACTAACGGGTTTTAAGGC-3'	5'-gaaaaCTAATGCTTTTGCTTTTCAA-3'
SNuPE-Peg1-Mest#2	5'-ACGTTGGATGAGAGCTGGTCATTGTTGTGG-3'	5'-ACGTTGGATGTGTGACAATCTCAGCTCCTG-3'	5'-gaTGAATTCCTAAATCACATGC-3'
SNuPE-Peg3	5'-ACGTTGGATGTATCCTTAGCGTCACCACTG-3'	5'-ACGTTGGATGCCAGTCCTGCATCATTCAAG-3'	5'-GGCGGTGTCTGAAGTA-3'
SNuPE-Rasgrf1	5'-ACGTTGGATGATCCGTGGCTACCGCTATTG-3'	5'-ACGTTGGATGGGTAGCGCAACAGTGAAGTG-3'	5'-ggagGTGCGGCAGCCATAGC-3'
SNuPE-Snrpn	5'-ACGTTGGATGTGCCTTTTGGCAGGACATTC-3'	5'-ACGTTGGATGCACATGCGCACATTTTTGCC-3'	5'-CAGGACATTCCGGTCA-3'
SNuPE-U2af1	5'-ACGTTGGATGGGCTTCCCATGTTGTTTTCC-3'	5'-ACGTTGGATGGTACTCTTATGTGTTCTTTG-3'	5'-aatcACTTTACATTGTGATGCTTT-3'
SNUPE-Zac1#1	5'-ACGTTGGATGCCTGAGCCATGTTTCAGGAT-3'	5'-ACGTTGGATGATTTGGGCTTCTTCCCTTTC-3'	5'-ATATACTGATGATGGGGTGGGGTAA-3'
SNUPE-Zac1#2	5'-ACGTTGGATGCATATGCACCAGAGAACAGG-3'	5'-ACGTTGGATGTTATGTTTCCCAGTCAGGCG-3'	5'-actcCACAGTAAGGCCTGTT-3'
Sequenom-H19 7 plex	Forward	Reverse	UEP
SNuPE-H19-4kb	5'-ACGTTGGATGTTGCGCCAAACCTAAAGAGC-3'	5'-ACGTTGGATGAGGTACTGAACTTGGGTGAC-3'	5'-CATTTGTGAATTCCAATACC-3'
SNuPE-H19-3kb	5'-ACGTTGGATGACACTTGTGTTTCTGGAGGG-3'	5'-ACGTTGGATGATGCCTTCCTATAGTGAGCC-3'	5'-aaGGGGTCCCTTTGGTC-3'
SNuPE-H19+8	5'-ACGTTGGATGCCCTAAGAACCTTCTTCACC-3'	5'-ACGTTGGATGTTGCATCATAGGACCACCAC-3'	5'-taTTCACCTTCCTGGGT-3'
SNuPE-H19+2	5'-ACGTTGGATGGCTTTGAGTCTCTCCGTATG-3'	5'-ACGTTGGATGATGGACGACAGGTGGGTACT-3'	5'-ATGTATACAGCGAGTGTG-3'
SNuPE-Igf2-PR2	5'-ACGTTGGATGAGCCGATCCAGGGGACTGT-3'	5'-ACGTTGGATGCTGAGTTAAGGCGCAGGTAG-3'	5'-CAGGGACCTACTTGC-3'
SNuPE-Igf2-DMR2	5'-ACGTTGGATGACATCAGGCTGTTCCCCTTG-3'	5'-ACGTTGGATGGGGTTGTTTAGAGCCAATCA-3'	5'-CCAATCAAATTTGGTTTTTTAGAA-3'
SNuPE-Igf2-DMR1	5'-ACGTTGGATGAAGAACACATGCATACCCTG-3'	5'-ACGTTGGATGTAGGAAAACGCTGAGGTCAC-3'	5'-CACCTTAAGGTGTCCA-3'
Sequenom-H19 Pr Singleplex	Forward	Reverse	UEP
H19-Promoter	5'-TTTGGAGAATTTCAGGACGGGTGCG-3'	5'-ACCCCACGACTCTCCTCCAGCTCTC-3'	5'-TCTTCCCCAGTTTCCCC-3'
Real time-Exon specific primers for expression analysis (cDNA template)			
	Probe Sequence	Sense Primer	Anti-sense Primer
Peg3 exon 3-4 HEX	CGCTCCAGGTCTCGCTCACTGCCA	TCACGAAGACGACACCAACAG	GGCTCCACATCTCTGCTTCTG
Dot1L exons 5-7 FAM	CCGCAGCAACCTGAAGGACAACCTG	CCCTTCTCCCCTGAGGTGTATG	TCCGCTTTCTCCACTCCGTAG
Zac1 exon1 TEX	CGCCCAGGAGATGCCCGCTTGC	GCGTTCTCCAACCTCACTCG	GCAGCCTAAGCAGCCATGAC
Dlk1 exons 2-3 TAMRA	CGTCCTCTTGCTCCTGCTGGCTT	GGCCGCCCCGAGATGATC	ACAGGGTGGGTCGCATTCAG
Rtl1 exon TEX	ACCGTTGAAGAGACACCAGGCAGCA	GTCCTCCGAGGGCTCATCC	GCCTCCACCTGGGTCTCC
Gtl2 exon 1 HEX	TCCTGCGTGCTCCTTCTGCCTCCAT	CCCTGAAAGGGGCTGATTGG	GATTAACTCAGAGCGGGTCTCC
Snrpn exon 6-7 TEX	CATTGCTCGTGTGCCTCTTGCTGG	GGCCCACCTCCTAAAGATACTG	ACCTGCTGGCACTCCTCTG
Igf2 exon 2-3 HEX	CCTTCAAGCCGTGCCAACCGTCGC	GGACCGCGGCTTCTACTTC	AGCAGCACTCTTCCACGATG
H19 exon 4-5 FAM	TGCCTCAGGAATCTGCTCCAAGGTG	CTGAATCAAGAAGATGCTGCAATC	GGTGCTATGAGTCTGCTCTTTC
Gapdh exon 5-6 Cy5	CGTGCCGCCTGGAGAAACCTGCC	AATGTGTCCGTCGTGGATCTG	CAACCTGGTCCTCAGTGTAGC
Sequenom-allele specific expression for shRNA			
SNP_ID	Forward	Reverse	UEP
Igf2	ACGTTGGATGACATCAGGCTGTTCCCCTTG	ACGTTGGATGGGGTTGTTTAGAGCCAATCA	TTGCCCCACACCATC
H19 SNP A	ACGTTGGATGGCTTGAGAGACTCAAAGCAC	ACGTTGGATGCCAGTGCAATCGACTTAGTG	CTCTGTTTCCCCATTTAC
H19 SNP B	ACGTTGGATGTTGCCCTCAGACGGAGATG	ACGTTGGATGGCTTTGAGTCTCTCCGTATG	AGCATTGCCAAAGAGG
SNRPN	ACGTTGGATGCACAGATATGACATTTGCTC	ACGTTGGATGAGTGAATGTCAGAAATCAGG	GACATTTGCTCAAGCTAG
GTL2 SNP A	ACGTTGGATGAGGCTGTTGTGTCTTCACTG	ACGTTGGATGCTGTGAGGTAGGAACCTGAG	CACTGTTGAGTCTACATCT
GTL2 SNP B	ACGTTGGATGGAAATTTATTGAAAGCACC	ACGTTGGATGCAGCCGCAATGTGCTTAGAA	AGCACCATGAGCCAC
ZAC1	ACGTTGGATGAAGCCCAGACAGAAAGAAGG	ACGTTGGATGGCTATTGTGCTCTGGATCTC	CAGCCTGACTCCAGAAA
DMRs- Real time	Probe sequence	Sense Primer	Anti-sense Primer
Peg1-Mest DMR-TET	CCTGCCATTGTGAGTCCAGAACCTG	CAGGAGCTGAGATTGTCACAGAG	GCACGATCTAGTCGCACCTATG
Zac1 DMR-Cy5	CGACGCCTTCATGTCCGCCTGACTG	CCTTACTGTGTAGTACCACAAAGC	AACTGGCTTATTTGCTCCTATACTG
Gnas1A DMR-FAM	TGCTGCCTGCTTCTTCTATCCGTGG	GGCTCTTCTTCTCGGCTGTTG	GGTTCGGATCTCCTCACAAGTAG
Peg3 DMR-TAMRA	TTCTGAATGAGAGAGACCACACCCCTTTG	ATCTTGAATGATGCAGGACTGG	GTCAGCAAAGATGGCGTCAG
Snrpn promoter DMR-TAMRA	CATGCGTCCCAGGCAATGGCTGC	TCCTTTTGGTAGCTGCCTTTTGG	CCGCAATGGCTCAGGTTTGTC
KvDMR1 -TEX	CCGCAGTGGCTCCGTATTCGTT	CGGCTGGGCTCCATCTTC	CGACCTCGGGGCTCAAAG
Igf2r DMR2-TAMRA	AAACCCTGCCCTTCCCTCCGTCTCC	CAAGAGAGGCAAGCTCAAAAGTG	CCTCATGCATAGCCAGGATAGC
U2af1 DMR-TEX	AGAACACATAAGAGTACAGAAACACTAATGCTCCTTG	ATTAACATCGTTTTTATATTGTCTGAATCTG	CAGCCAGGGATACATAGTGAGAG
H19-Igf2 ICR-FAM	ACATTCACACGAGCATCCAGGAGGC	CACTTACACCCAGGACTCAAAGG	GCGTATAAACCCCACAACTGATTC
Rasgrf1 DMR-Cy5	CCGTGGCTACCGCTATTGCTGTTGC	GCTGCTGCTCCCACATCC	GGTAGCGCAACAGTGAAGTG
IG-DMR-TET	CGGCACAATTCCACGGCGGTTCG	CGTGTACTAATGCCGCTTCG	GCTACTCCACGGCGAACC
Control regions-Real time PCR	Probe sequence	Sense Primer	Anti-sense Primer
c-myc promoter-Cy5	CTGCCTCGCTCCACACAATACGCCA	AGATAACTCATTCGTTCGTCCTTCC	TGTGTTCTTGCCCTGCGTATATC
IAPLTR2b-TET	CGGTTTGCTGCGTTCTTCCTGGC	TCCTGAAGATGTAAGCAATAAAGC	TCTTACACGCGTTCTCGAC
Maj satellite	Sybr	GACGACTTGAAAAATGACGAAATC	CATATTCCAGGTCCTTCAGTGTGC