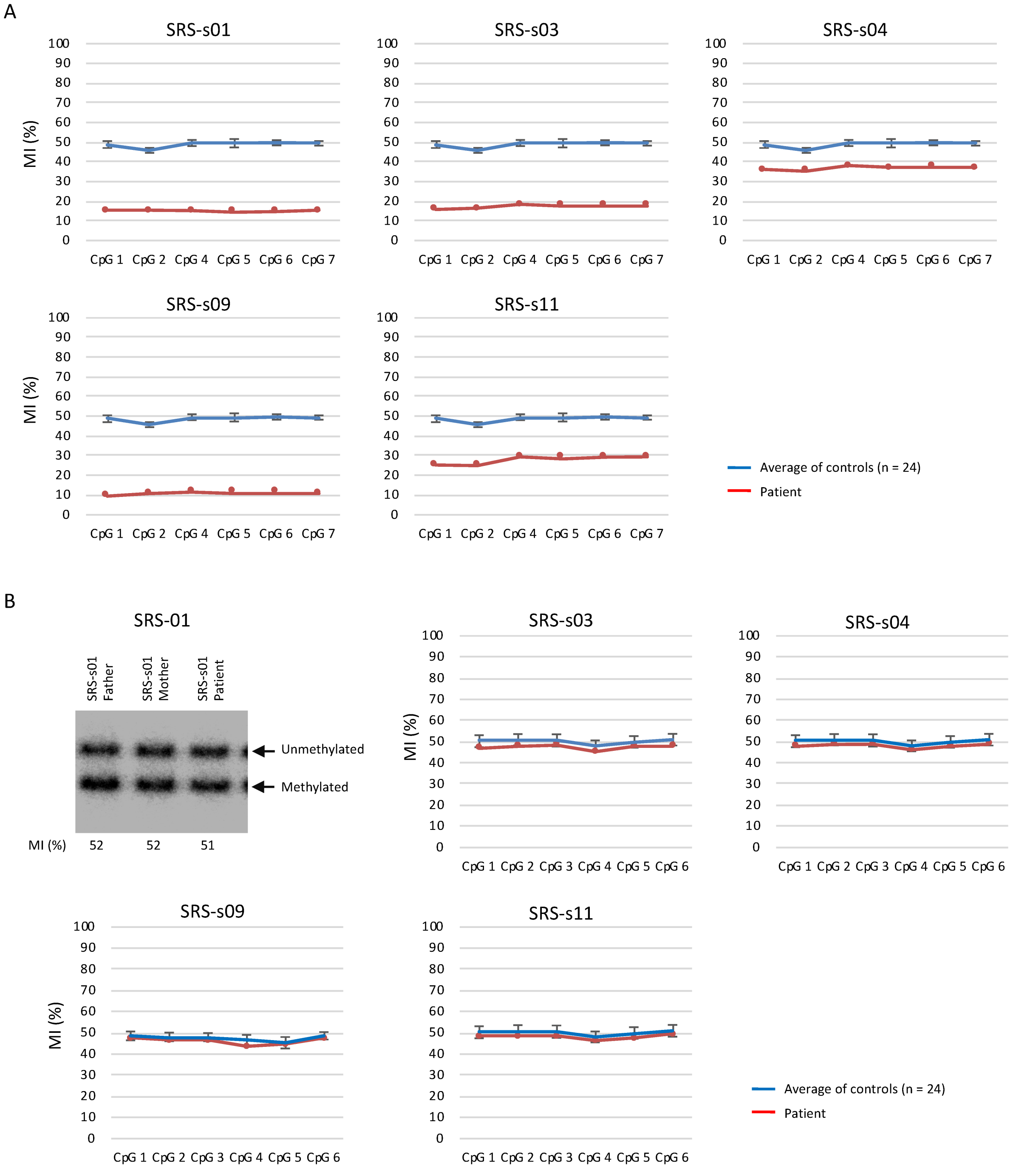
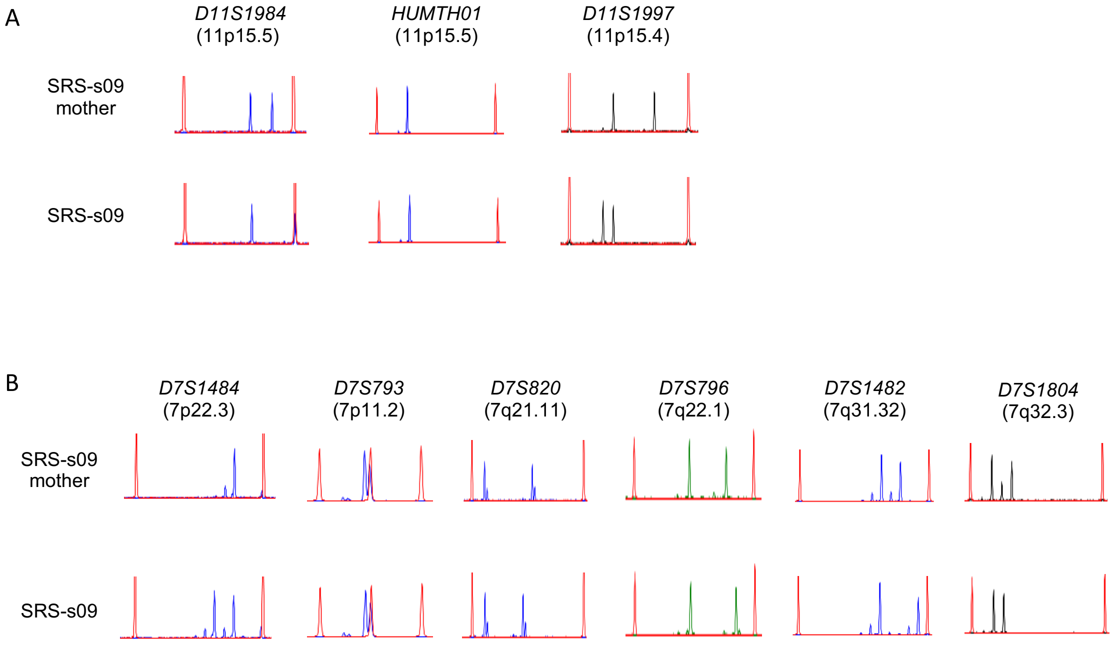


Supplementary Fig. S1

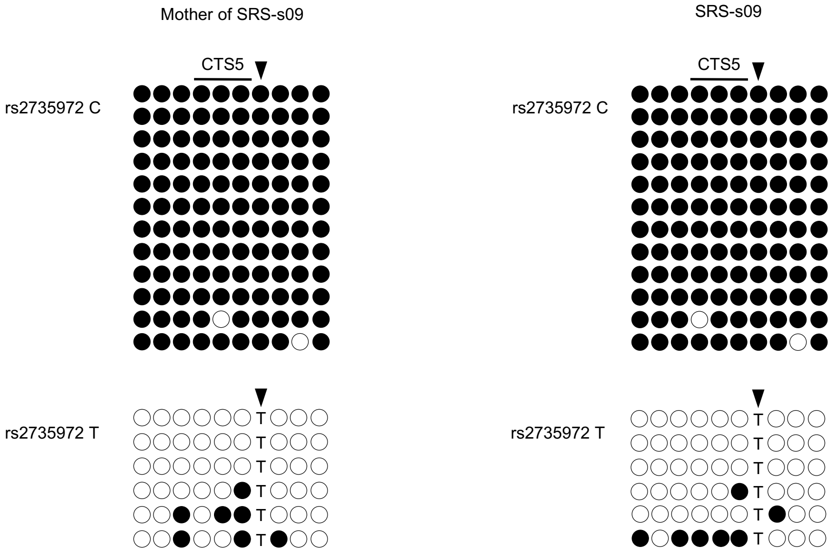
The DNA methylation status of CTS3 in ICR1 and ICR2 in the patients in this study. (A) Methylation status of CTS3, located in the B5 repeat in the centromeric block within ICR1. All patients showed LOM at CTS3. The methylation statuses of all patients were analyzed with bisulfite-pyrosequencing. (B) Methylation status of ICR2. ICR2 is normally methylated on the maternal allele, but not on the paternal allele, resulting in approximately 50% of MI. All patients showed normal differential methylation of ICR2. Methylation statuses of all patients except for SRS-s01 were analyzed with bisulfite-pyrosequencing. SRS-s01 and her parents were analyzed by quantitative hot-stop Combined Bisulfite Restriction Analysis (COBRA). The bisulfite PCR product was digested with *Acc*II, as previously described.[1]

1. Soejima H, Nakagawachi T, Zhao W, et al. Silencing of imprinted CDKN1C gene expression is associated with loss of CpG and histone H3 lysine 9 methylation at DMR-LIT1 in esophageal cancer. *Oncogene* 2004;23:4380-88.



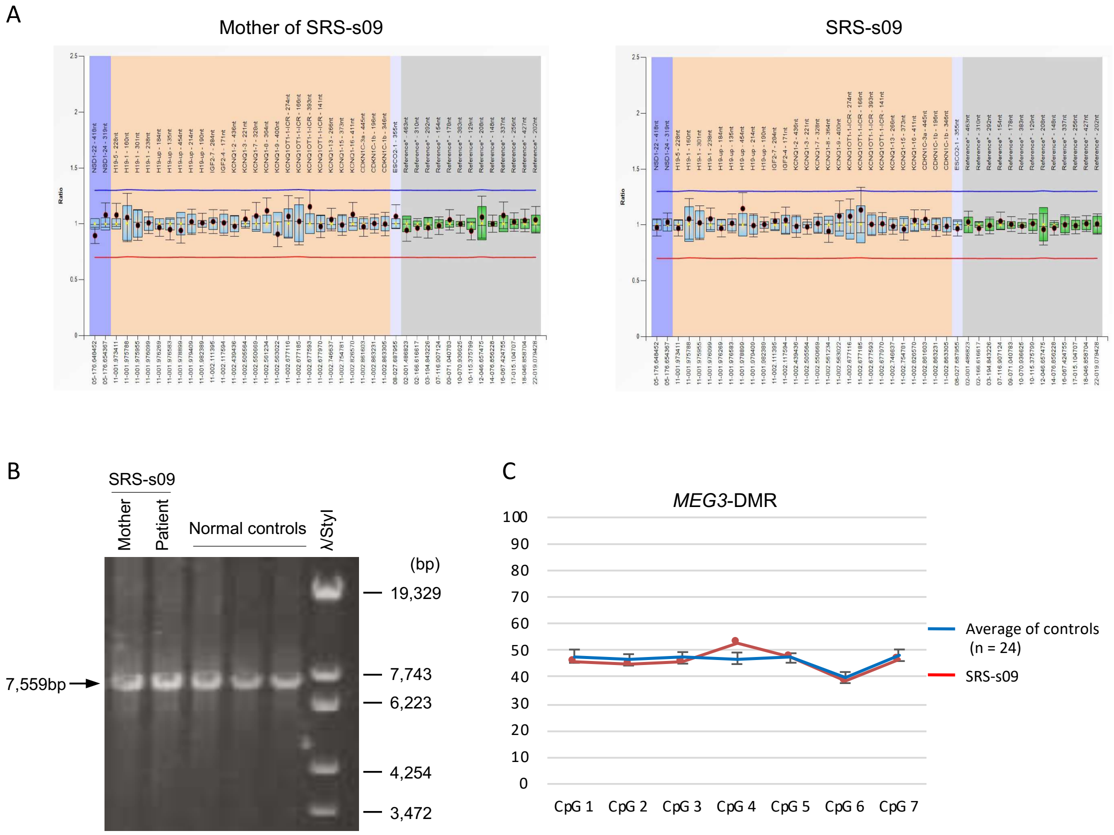
Supplementary Fig. S2

Short tandem repeat analyses (tetranucleotide repeats) for SRS-s09 and his mother did not detect maternal uniparental disomy in SRS-s09. The results for (A) chromosome 11, and (B) chromosome 7 are shown. Red peaks are size markers. A sample from the father of SRS-s09 was not available.



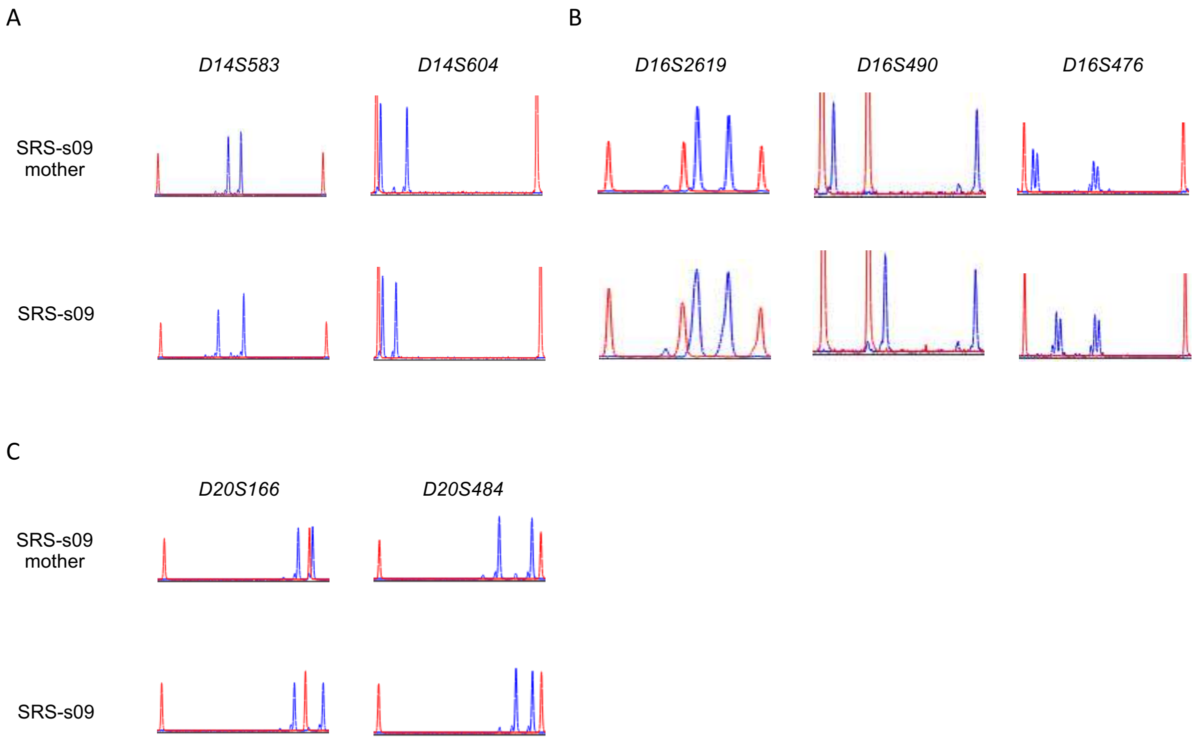
Supplementary Fig. S3

Methylation status at CTS5 in SRS-s09 and his mother. The bisulfite PCR products encompassing a single-nucleotide variant (rs2735972) were cloned and sequenced. The position of the SNV is indicated by arrow heads. Complementary bases of the SNV are shown. CTS5 showed normal differential methylation in both the patient and his mother. A sample from the father of SRS-s09 was not available.



Supplementary Fig. S4

Results of the search for other causative SRS alterations in the SRS-s09 patient. (A) Multiplex ligation-dependent probe amplification (MLPA) detected normal copy numbers of 11p15 in SRS-s09 and his mother. A sample from the father of SRS-s09 was not available. (B) Long-range PCR encompassing the entire ICR1. Deletions or insertions were not found in SRS-s09 or his mother. (C) Methylation status of *MEG3*-DMR at 14q32.2. Bisufite-pyrosequencing of SRS-s09 showed normal methylation.



Supplementary Fig. S5

Short tandem repeat analyses (tetranucleotide repeats) for SRS-s09 and his mother did not detect maternal uniparental disomy in SRS-s09. The results for (A) chromosome 14, (B) chromosome 16, and (C) chromosome 20 are shown. Red peaks are size markers. A sample from the father of SRS-s09 was not available.

Table S1 Methylation indices (MIs) at each differentially methylated site within the *IGF2/H19* domain in control subjects

			MI (%)										
Sample ID	Sex	Age, years	*IGF2*-DMR0	*IGF2*-DMR2	CTS1	CTS2	CTS3	CTS4	CTS5	CTS6	CTS7	*H19*-promoter	Δme
C-1	F	2	51.0	62.7	47.0	45.7	47.3	36.9	56.8	47.7	71.2	44.4	34.3
C-2	F	4	54.9	58.3	46.0	43.9	45.3	42.9	53.7	42.8	69.8	44.0	27.0
C-3	F	4	52.7	64.3	50.5	49.1	45.9	44.1	46.6	47.4	62.2	49.3	18.1
C-4	F	4	49.5	59.1	46.4	44.1	45.0	44.5	50.9	49.4	64.8	46.2	20.7
C-5	F	4	52.3	63.1	48.8	44.7	46.3	43.5	48.0	46.0	61.5	42.8	18.7
C-6	F	3	53.2	58.4	49.1	42.4	45.8	46.3	57.4	52.3	69.8	44.3	27.4
C-7	F	0	53.2	51.0	50.6	44.7	46.6	42.8	59.4	48.3	66.6	47.0	23.8
C-8	F	1	52.6	56.3	47.5	46.2	45.6	45.7	52.7	43.8	61.1	43.0	18.1
C-9	F	1	51.3	57.7	47.2	45.0	48.6	42.2	59.5	49.6	71.3	44.8	29.1
C-10	F	0	53.2	61.2	48.2	46.0	44.3	45.0	58.4	45.8	63.6	42.0	21.6
C-11	F	7	51.7	58.1	48.6	43.3	47.7	44.1	59.9	45.0	59.9	48.4	16.6
C-12	F	7	51.9	57.5	44.7	45.2	47.8	47.5	50.0	48.2	59.4	42.3	17.1
C-13	M	4	54.3	62.3	52.5	47.7	44.7	41.8	52.3	50.4	64.8	47.6	23.0
C-14	M	1	57.3	58.1	48.6	45.4	46.2	43.1	61.2	45.4	69.2	42.6	26.6
C-15	M	4	53.1	60.8	46.4	48.3	43.4	44.9	57.6	47.6	69.4	41.8	27.6
C-16	M	4	47.3	63.2	53.1	48.2	48.9	45.9	49.2	49.8	64.0	44.3	19.7
C-17	M	4	56.8	63.7	46.9	46.2	46.0	44.6	56.5	52.2	68.1	42.8	25.3
C-18	M	3	45.8	60.0	48.5	44.7	45.7	43.4	48.7	49.5	60.1	42.4	17.7
C-19	M	1	51.1	63.3	59.8	42.0	48.1	42.1	47.9	46.8	61.4	42.9	19.4
C-20	M	8	47.0	56.3	47.6	48.6	48.8	41.5	49.6	47.5	61.1	43.9	19.6
C-21	M	6	53.3	56.9	49.0	45.4	49.1	42.4	50.5	47.4	58.6	46.2	16.2
C-22	M	7	51.5	57.2	51.3	47.3	50.3	42.9	58.1	51.3	67.5	43.6	24.6
C-23	M	6	48.8	57.3	47.1	43.1	51.4	42.7	58.7	47.0	65.8	44.5	23.1
C-24	M	7	47.9	53.6	48.7	46.3	48.8	48.6	54.2	47.1	64.4	42.3	22.1
Mean		3.8	51.7	59.2	48.9	45.6	47.0	43.7	54.1	47.8	64.8	44.3	22.4
SD		2.4	2.8	3.3	3.0	1.9	1.9	2.3	4.5	2.4	3.9	2.1	4.5

F, female; M, male; SD, standard deviation

Table S2 Primers for CTS5 bisulfite sequencing, *IGF2* sequencing, *ZFP57* sequencing, and short tandem repeat markers

Experiment	Target region	Primer sequence (5'– 3')	Location (GRCh37/hg19)	PCR annealing temperature (°C)	Number of analyzed CpG sites
Bisulfite sequencing	CTS5	H19DMR CTCF5 BS-F: TTCTAAAAACTTCCCCTTCA	Chr 11 : 2,021,531–2,021,690	52	10
		H19DMR CTCF5 BS-R: TTGGATGATTTGGGATGTTT			
		S[a]: CTAATACGACTCACTATAGG			
IGF2 sequencing	exon 7	IGF2-F1: CCCTCACCTCGGCATTATGA	Chr 11 : 2,156,460–2,156939	60	
		IGF2-R1: GCGGGAAGGTCAAAGTCTCA			
	exons 8 and 9	IGF2-F2: TTGTGGGACCAAGAGCTTGT	Chr 11 : 2,154,113–2,155,170	60	
		IGF2-R2: CGTGGAACCGAGAGATTTTC			
		S: AGGGACAAGGACCCGTGACT			
ZFP57 sequencing	exon 2	e2-F: TTACCCTCCCTCTTGCTGAT	Chr 6 : 29,644,526–29,645,188	62	
		e2-R: GCTCCTTTTTCTGGATGTCG			
	exon 3	e3-F: TCTTGAGTCTCTCCTGTTCTTCC	Chr 6 : 29,643,640–29,643,861	62	
		e3-R: TGTCCAGGGAAACCAGATGT			
	exon 4	e4-F: TCTCCTTTCCGCATCTACAT	Chr 6 : 29,643,101–29,643,398	62	
		e4-R: TCCATCCTTTCAGGGGTTAT			
	exon 5	e5-F1: TGTGTTATTCTTCCTCATTCACTGT	Chr 6 : 29,640,866–29,641,562	62	
		e5-R1: GCCATAGGACCCTCAGTTCT			
		e5-F2: TGCACGCTCTGTGACAAGAC	Chr 6 : 29,640,601–29,641,191	62	
		e5-R2: TCTGACCAGCCTGGAAAATG			
		e5-F3: CCCCATTGTTCTTTGACTTT	Chr 6 : 29,640,199–29,640,729	62	
		e5-R3: CTACTCCAGCCTCATTACCC			
STR markers (tetranucleotide repeats)	*D7S1484*	FAM-GCTGACAAGAGCGAAACTC		60	
		GAACTGTGAACGAATACACCA			
	D7S793	FAM-GTCTCTCTCACACACACATTCC		55	
		CAACTTAATGGAGGTGTAGTTACA			
	D7S820	FAM-TGTCATAGTTTAGAACGAACTAACG		55	
		CTGAGGTATCAAAAACTCAGAGG			
	D7S796	VIC-TTTTGGTATTGGCCATCCTA		55	
		GAAAGGAACAGAGAGACAGGG			
	D7S1482	FAM-AAAGTGGGGATAAGGCAGC		60	
		AGATGCACAACACATACACG			
	D7S1804	NED-TTCAAGTGGTTGGGTTCACT		55	
		TGGGTCTAGTCCAGTGGTGT			
	D11S1984	FAM-GGGTGACAGAGCAAAATTCT		55	
		ACACCTGGATCTTGGACTCA			
	HUMTH01	FAM-CAGCTGCCCTAGTCAGCAC		65	
		GCTTCCGAGTGCAGGTCACA			
	D11S1997	NED-TTTGTTTTCCTAAGAAAGATAAAGC		60	
		CTGGACAAAATAAAGACCAGC			
	D14S583	FAM-GCTTGCAATCTCTTACTTTTCC		55	
		AAACATGCTTTCAGGTCTGC			
	D14S604	FAM-AGACAGACAAGGGCTCACAG		55	
		TGTTGGATCCTTCCAGAAAA			
	D16S2619	FAM-CAAGTCCAAGGGTAATTGGA		55	
		CCTATCTCTATCCATGTACCACG			
	D16S490	FAM-ACAGGAGTGAGCCACCGTA		60	
		AAACCCAAATAGATGACAGGC			
	D16S476	FAM-TTGCACTCCACTCTGGGCA		60	
		TTGCCTTGGCTTTCTGTTGG			
	D20S166	FAM-CAGCCTCCATTATCAAGTG		56	
		AGCAAAACTGACTCAAGAAG			
	D20S484	FAM-TATCAGGCCTCACCCTGG		55	
		AAAAGAATAAGAAGCTCTAAAAGTG			

F, forward primer; R, reverse primer; S, sequence primer; STR, short tandem repeat; FAM, 6-FAM-labeled; VIC, VIC-labeled ; NED, NED-labeled

[a] The sequence primer is positioned in a pT7Blue T-Vector

Table S3 Methylation indices (MIs) and MI differences at each differentially methylated site within the *IGF2/H19* domain in patients with Silver-Russell syndrome

	MI (%)										
Patient ID	*IGF2*-DMR0	*IGF2*-DMR2	CTS1	CTS2	CTS3	CTS4	CTS5	CTS6	CTS7	*H19* -promoter	∆me
SRS-s01 #1	26.0	37.7	27.4	18.7	15.7	22.8	37.7	20.2	33.9	16.2	22.0
SRS-s01 #2	26.4	38.8	28.0	20.3	14.4	25.7	39.0	20.3	34.4	15.1	24.6
Mean	26.2	38.2	27.7	19.5	15.0	24.2	38.3	20.2	34.1	15.7	23.3
SD	0.3	0.7	0.4	1.1	0.9	2.0	0.9	0.0	0.3	0.8	1.9
SRS-s03 #1	21.3	37.8	24.5	17.0	17.8	19.4	42.1	26.4	43.1	23.8	26.2
SRS-s03 #2	20.3	37.7	24.8	17.1	16.7	18.3	45.6	26.1	43.6	24.0	28.9
Mean	20.8	37.7	24.7	17.0	17.2	18.9	43.8	26.3	43.4	23.9	27.5
SD	0.7	0.1	0.2	0.1	0.8	0.8	2.5	0.2	0.4	0.1	1.9
SRS-s04 #1	42.7	51.5	40.9	30.5	36.6	32.9	44.7	38.1	54.2	33.4	23.8
SRS-s04 #2	43.0	51.3	41.4	30.2	36.8	32.3	46.1	37.1	54.1	31.6	23.9
Mean	42.9	51.4	41.1	30.3	36.7	32.6	45.4	37.6	54.2	32.5	23.9
SD	0.2	0.2	0.3	0.2	0.1	0.4	1.0	0.7	0.1	1.3	0.1
SRS-s09 #1	21.9	31.1	21.6	11.3	11.1	42.6	53.6	43.3	66.3	36.5	55.2
SRS-s09 #2	21.5	29.4	20.9	10.3	10.4	40.6	57.4	42.7	66.4	35.6	56.0
Mean	21.7	30.2	21.2	10.8	10.8	41.6	55.5	43.0	66.4	36.0	55.6
SD	0.3	1.2	0.5	0.7	0.5	1.4	2.7	0.4	0.0	0.6	0.6
SRS-s11 #1	34.2	51.7	31.2	22.4	27.1	26.6	40.1	27.7	44.8	28.5	17.7
SRS-s11 #2	34.6	52.1	31.9	23.5	28.1	22.1	38.7	29.1	44.7	27.2	21.2
Mean	34.4	51.9	31.5	23.0	27.6	24.3	39.4	28.4	44.7	27.9	19.4
SD	0.3	0.3	0.5	0.8	0.7	3.2	1.0	1.0	0.0	0.9	2.5
Healthy control (n = 24)	51.7	59.2	48.9	45.6	47.0	43.7	54.1	47.8	64.8	44.3	22.4
SD	2.8	3.3	3.0	1.9	1.9	2.3	4.5	2.4	3.9	2.1	4.5
	MI difference (%)										SD
SRS-s01	25.5	20.9	21.2	26.1	31.9	19.5	15.7	27.6	30.7	28.6	5.3
SRS-s03	30.9	21.4	24.2	28.6	29.7	24.9	10.3	21.6	21.4	20.4	5.9
SRS-s04	8.9	7.8	7.8	15.2	10.3	11.1	8.7	10.2	10.6	11.8	2.2
SRS-s09	30.0	29.0	27.7	34.7	36.2	2.1	-1.4	4.9	-1.5	8.3	15.8
SRS-s11	17.4	7.3	17.4	22.6	19.4	19.4	14.7	19.5	20.1	16.4	4.2

MIs of patients were analyzed by two independent experiments.

MI difference: the difference between the mean MI value derived from the blood of 24 healthy children and that derived from the blood of each patient at each DMS.

∆me, the difference between the maximum and minimum MIs among all DMSs; SD, standard deviation

Table S4 A list of variants in ICR1 found in SRS-09 and his mother

rs number	Alleles	SRS-s09 mother	SRS-s09	Allele frequency in 1000 Genomes
rs2251375	C>A	C/A	C	A = 0.4806
rs2251312	G>C	G/C	G/C	G = 0.1358
rs2107425	C>T	C/T	C	T = 0.4479
rs2735972	A>G	A/G	A/G	A = 0.1324
rs2735971	T>C	T/C	T/C	T = 0.1330
rs2735970	T>C	T/C	T	C = 0.4645
rs2525882	C>T	C/T	C	C = 0.2294
rs12417375	A>T	A/T	A	T = 0.0994
rs2735469	A>G	G	G	A = 0.0803
rs17658052	G>A	G/A	G	A = 0.0621
rs61520309	C>T	C	C/T	T = 0.0911
rs74668776	C>T	C/T	C	T = 0.0621
rs2525885	C>T	C/T	T	C = 0.2007
rs4930103	G>A	G/A	A	A = 0.4145
rs78033535	C>T	C/T	C	T = 0.0469
rs4929983	C>T	C/T	C/T	T = 0.4032
rs4929984	C>A	C	C/A	A = 0.3662
rs77640953	C>T	C	C/T	T = 0.0290
rs2735461	C>G	G	G	C = 0.0327
rs74584156	T>G	T/G	T/G	G = 0.0465