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 Accl, 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. Bisulfite-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

| Sample ID | Sex | Age, years | IGF2 - DMR0 | IGF2 - DMR2 | CTS1 | CTS2 | CTS3 | CTS4 | CTS5 | CTS6 | CTS7 | H19 - promoter | %me |
|-----------|-----|------------|-------------|-------------|------|------|------|------|------|------|------|----------------|-----|
| C-1       | F   | 2          | 51.0        | 62.7        | 47.0 | 45.7 | 47.3 | 47.3 | 36.9 | 56.8 | 47.7 | 71.2           | 44.4 |
| 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           | 27.8 |
| 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           | 18.2 |
| 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 | 46.8 | 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
| Experiment      | Target region | Primer sequence (5’– 3’) | Location (GRCh37/hg19) | PCR annealing temperature (°C) | Number of analyzed CpG sites |
|-----------------|--------------|--------------------------|------------------------|--------------------------------|-----------------------------|
| Bisulfite       | CTS5         | H19DMR CTCFS BS-F: TTCTAAAAACTTCCCCTTCA | Chr 11 : 2,021,531–2,021,690 | 62                             | 10                          |
|                 |              | H19DMR CTCFS BS-R: TTGGATGATTTGGGATGTTT |                         |                                |                             |
|                 |              | S5: CTAAATAAGCTGCTACTATAGG |                         |                                |                             |
|                 | exon 7       | IGF2-F1: CCCTCACCTCGGCATTATGA | Chr 11 : 2,156,460–2,156,939 | 60                             |                             |
|                 | exon 8 and 9 | IGF2-R1: GCCTGAAAGCTTCAAAGTCTCA |                         |                                |                             |
|                 |              | IGF2-R2: TTGGGAGCCACTCGTGTCT | Chr 11 : 2,154,113–2,155,170 | 60                             |                             |
|                 |              | S: AGGGGACAGGGAGCCCTCGTACGT |                         |                                |                             |
|                 | exon 2       | ZFP57 exon 5: TGCATATTCTTCCCTATGCTTCTTT | Chr 6 : 29,649,649–29,649,861 | 62                             |                             |
|                 | exon 3       | ZFP57 exon 4: CTGCATTTTCCTCGAGGCG | Chr 6 : 29,649,361–29,649,381 | 62                             |                             |
|                 |              | ZFP57 exon 2: TTGTGGGACCAAGAGCTTGT |Chr 6 : 29,640,601–29,641,191 | 62                             |                             |
|                 | exon 5       | ZFP57 exon 1: TGTGTTATTCTTCCTATGCTTCT |Chr 6 : 29,640,866–29,641,562 | 62                             |                             |
|                 | STR markers  | D11S1984 FAM-GCTGAGAAGGACGAAACTC |                         | 60                             |                             |
|                 | (tetranucleotide repeats) | D7S93 FAM-GCTGCCTCTGACAGACTCCCA |                         | 55                             |                             |
|                 |              | D7S820 FAM-CTCTATAGTTAGAAGACTACGG |                         | 55                             |                             |
|                 |              | D7S796 VIC-TCTGGATATTCCCTTGACTTCTAT |                         | 55                             |                             |
|                 |              | D7S1482 FAM-AAGGGACAGGAGCCCTCGTACGT |                         | 55                             |                             |
|                 |              | D7S1804 FAM-CATGCTGGAGGTGTCCTCA |                         | 55                             |                             |
|                 |              | HUMTH01 FAM-CGTCGCCTCTGACTGACAC |                         | 65                             |                             |
|                 |              | D14583 FAM-GCTTACCTCTTATCTTTCCTCA |                         | 55                             |                             |
|                 |              | D145604 FAM-ACACCTGCTGCAAGCGGACGC |                         | 60                             |                             |
|                 |              | D1652619 FAM-CAAGCTGCAGGTAACTTGGGA |                         | 55                             |                             |
|                 |              | D165490 FAM-ACACGTGAAAGGGCTTACAG |                         | 65                             |                             |
|                 |              | D165476 FAM-TGCGAGACTCTCGCTGACG |                         | 60                             |                             |
|                 |              | D205166 FAM-ACAGCTGCTCATTATGAGTCGTG |                         | 56                             |                             |
|                 |              | D205484 FAM-TATCGGCCCAGCTCCCTGAT |                         | 55                             |                             |

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

| 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 | 19.7 | 22.8 | 37.7 | 20.2 | 33.9 | 16.2          | 22.9 |
| 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 | 17.2 | 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 | 38.5          | 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  | 0.8           | 0.6  |
| SRS-s11 #1 | 34.2      | 51.7      | 31.2 | 22.4 | 27.1 | 29.6 | 40.1 | 27.7 | 44.6 | 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  | 3.2  | 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  |

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
| rs number   | Alleles | SRS-s09 moth | 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                       |