

Supplementary Information

A Pair of Maternal Chromosomes Derived from Meiotic Nondisjunction in Trisomy 21 Affects Nuclear Architecture and Transcriptional Regulation

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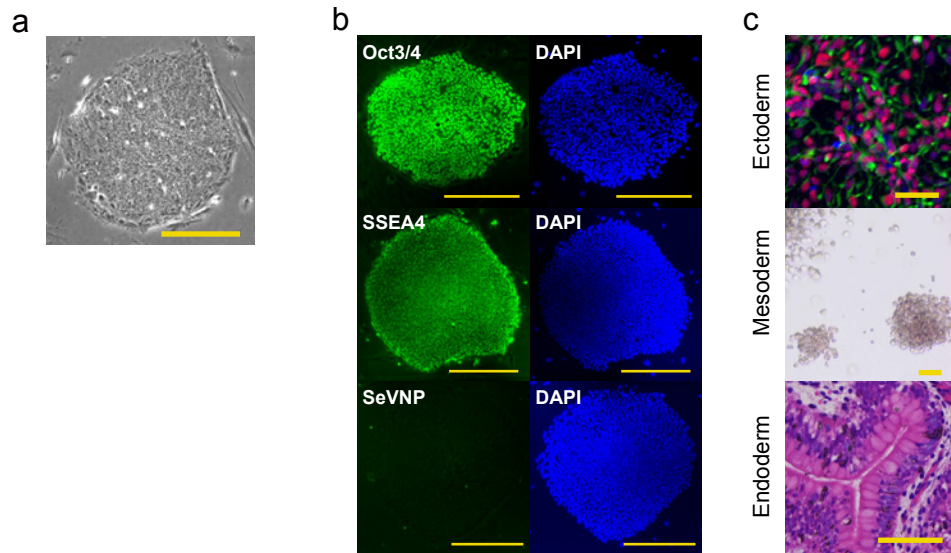
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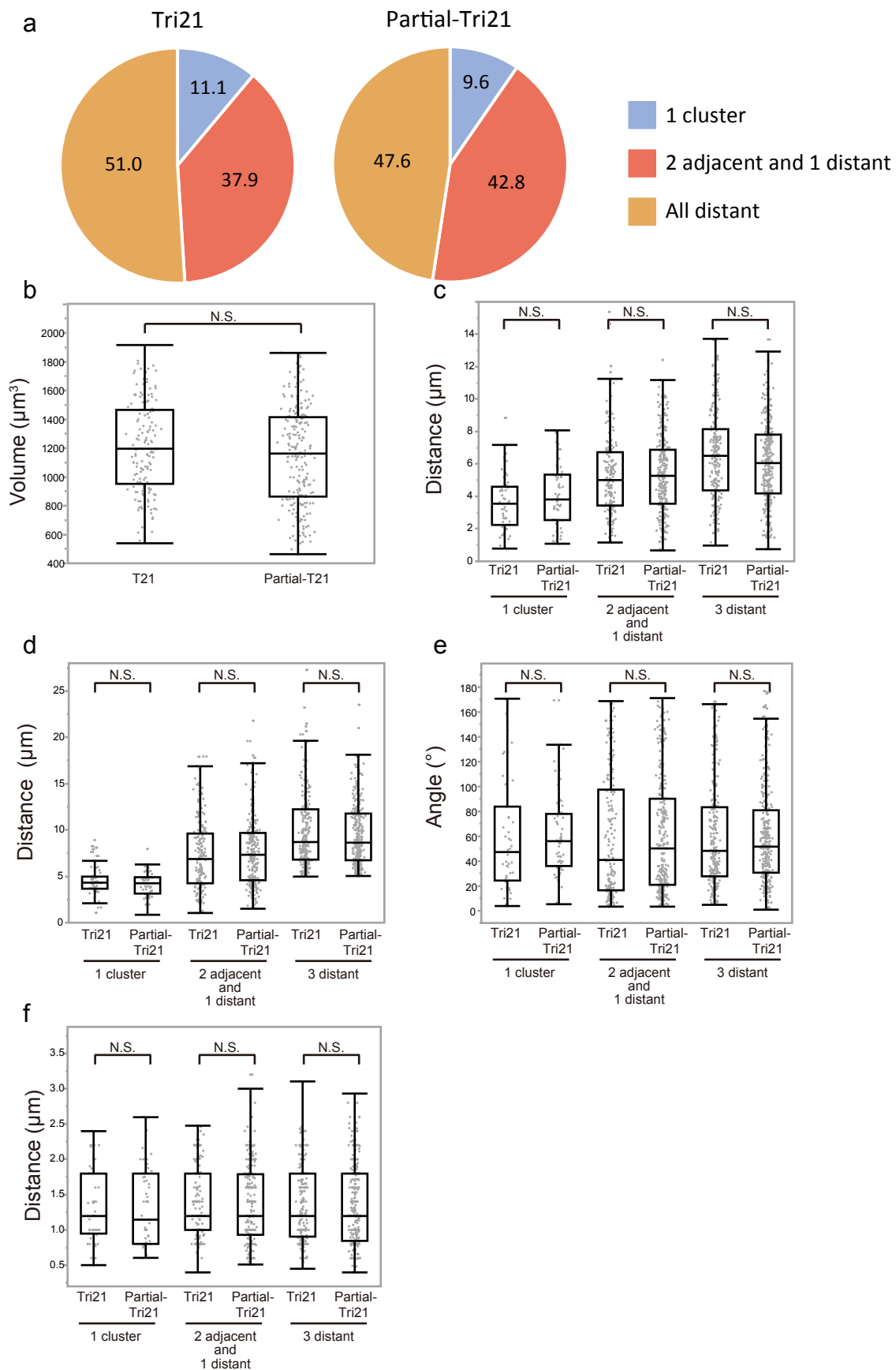


Supplementary Figure S1. Generation of Tri21 iPSCs.

(a) Photomicrograph of Tri21 iPSCs derived from mononuclear cells from a Down syndrome male. Scale bars represent 500 μ m.

(b) Immunocytochemistry for OCT3/4, SSEA4 and SeVNP in Tri21 iPSCs. Nuclei were stained with DAPI. Scale bars represent 500 μ m.

(c) Tri21 iPSCs represented differentiation into three germ layers: ectoderm (neural cells, immunohistochemistry of Sox1 (red) and Nestin (green)), mesoderm (hematopoietic colonies) and endoderm (teratoma formation, H&E staining). Scale bars represent 50 μ m.

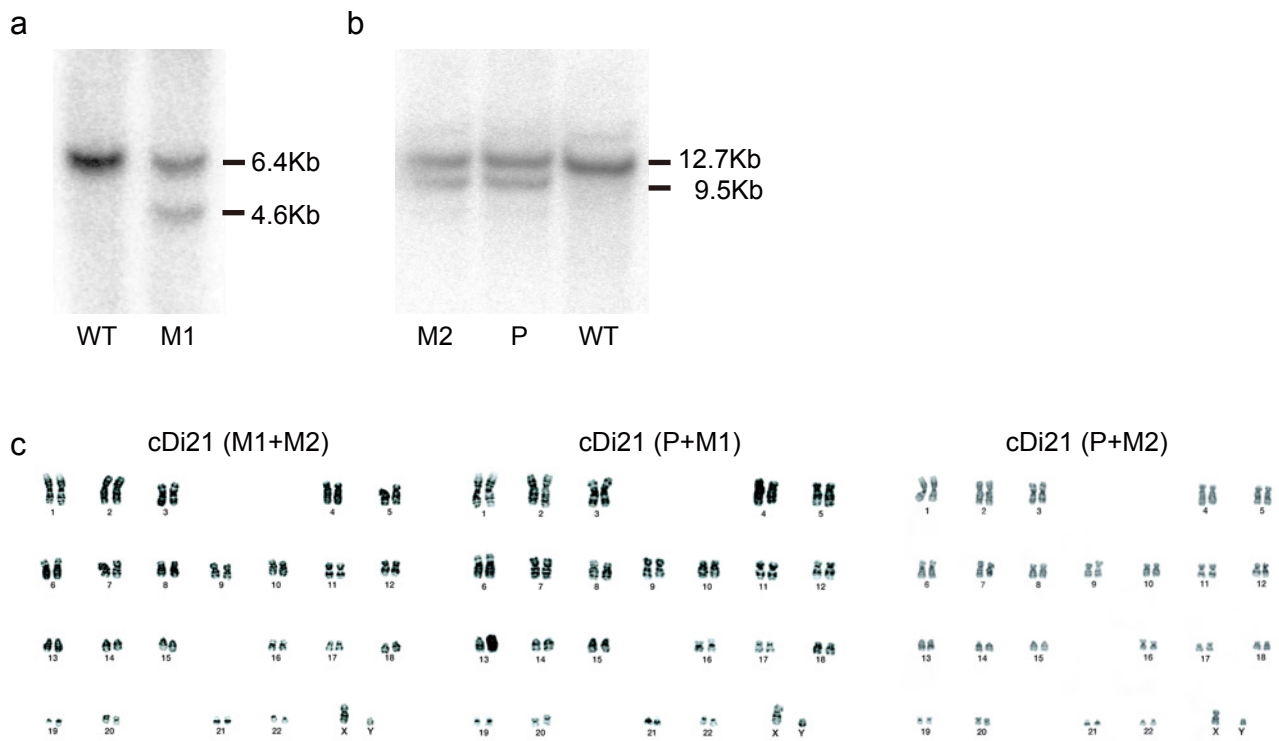


Supplementary Figure S2. Chromosome positioning of chromosome 21 compared Tri21 with Partial-Tri21 iPSCs

(a) Percentage of three localization patterns of chromosome 21 in Tri21 (n=153) and Partial-Tri21 (n=208) iPSCs.

(b–f) Chromosomal localization of chromosome 21 in Tri21 and Partial-Tri21 iPSCs. Three localization patterns of chromosome 21: 1 cluster (Tri21, n=17; Partial-Tri21, n=20), 2 adjacent and 1 distant (Tri21, n=58; Partial-Tri21, n=80) and all distant (Tri21, n=78; Partial-Tri21, n=99). Measurements including (b) the nuclear volume, (c) the distance from the nuclear center to each chromosome 21, (d) the distance between two chromosomes 21, (e) interior angles at the vertex and (f) the distance from chromosome 21 to the nearest nuclear membrane.

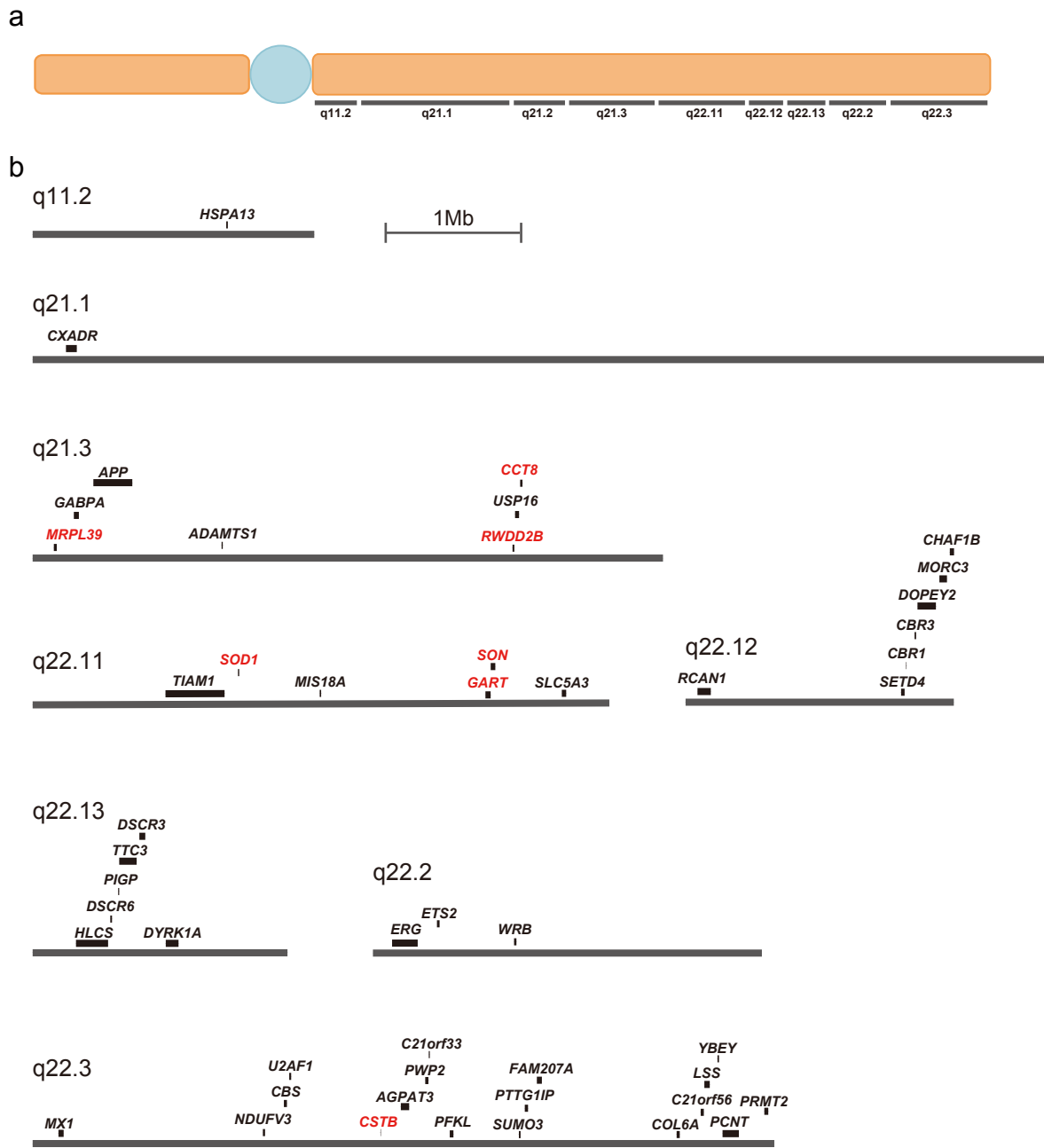
The term “adjacent” here refers to the distance between copies of chromosome 21 being under 5 μm . Box plot represents 25th–75th percentile range \pm min–max. p values were determined by the Mann-Whitney U test.



Supplementary Figure S3. Southern blot and karyotype analysis in targeted chromosome elimination

(a and b) Southern blot analysis of iPSC lines before (WT) and after (P, M1 and M2) targeting using 3' probes in (a) *RUNXI* (probe; Figure 1b) and (b) *ETS2* (probe; Figure 1c) locus. P, M1 and M2 indicate targeted allele in *RUNXI* (M1) and *ETS2* (P or M2). Parental origin of targeted allele was analyzed by STR or SNP genotyping. Expected fragment sizes: *RUNXI* probe (NcoI digest) wild-type allele, 4.6 kb; targeted allele, 6.4 kb. *ETS2* Probe (SpeI digest) wild-type allele, 9.5 Kb; targeted allele, 12.7 kb.

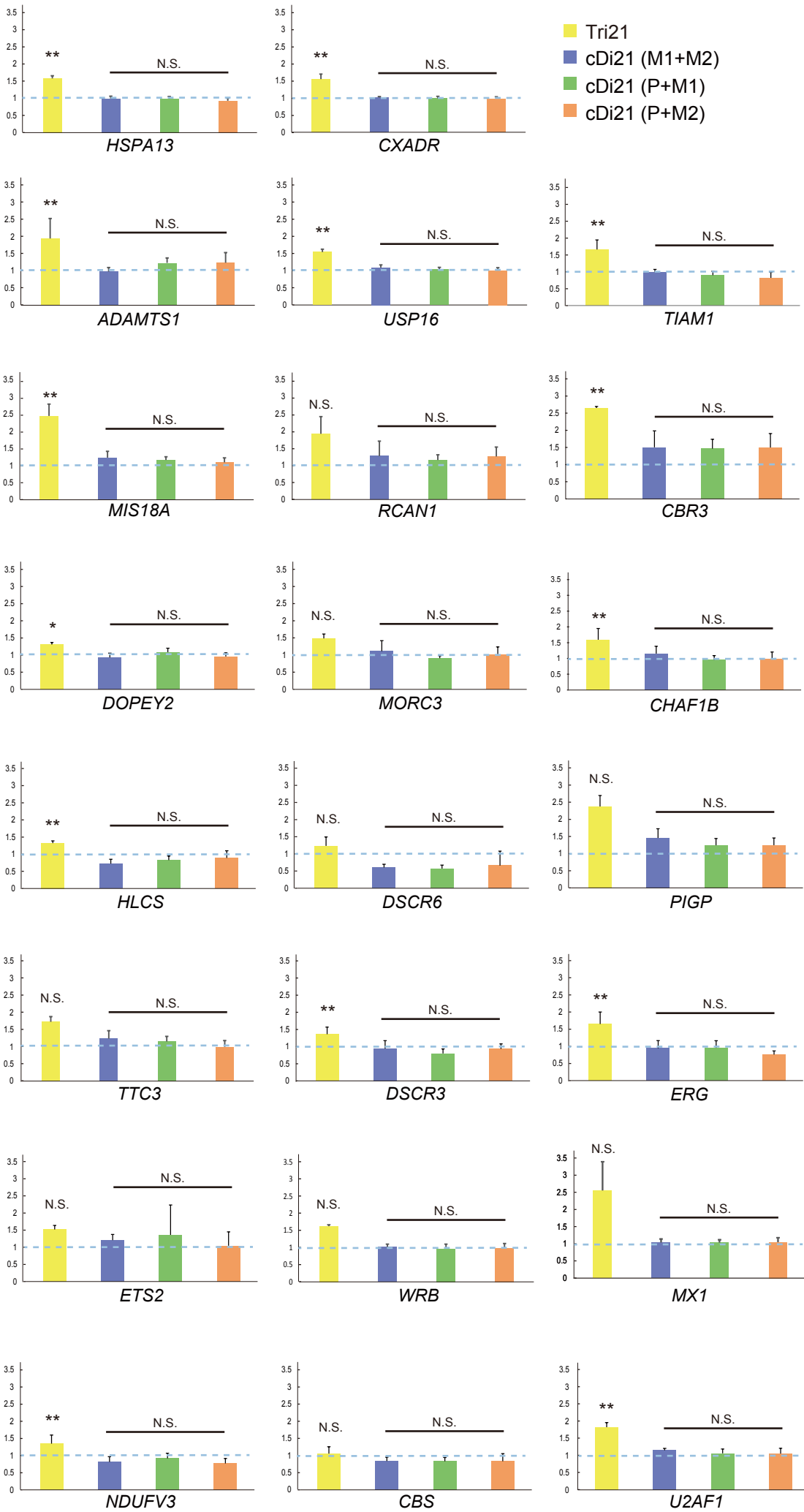
(c) Karyotype analysis in each cDi21 iPSC line.

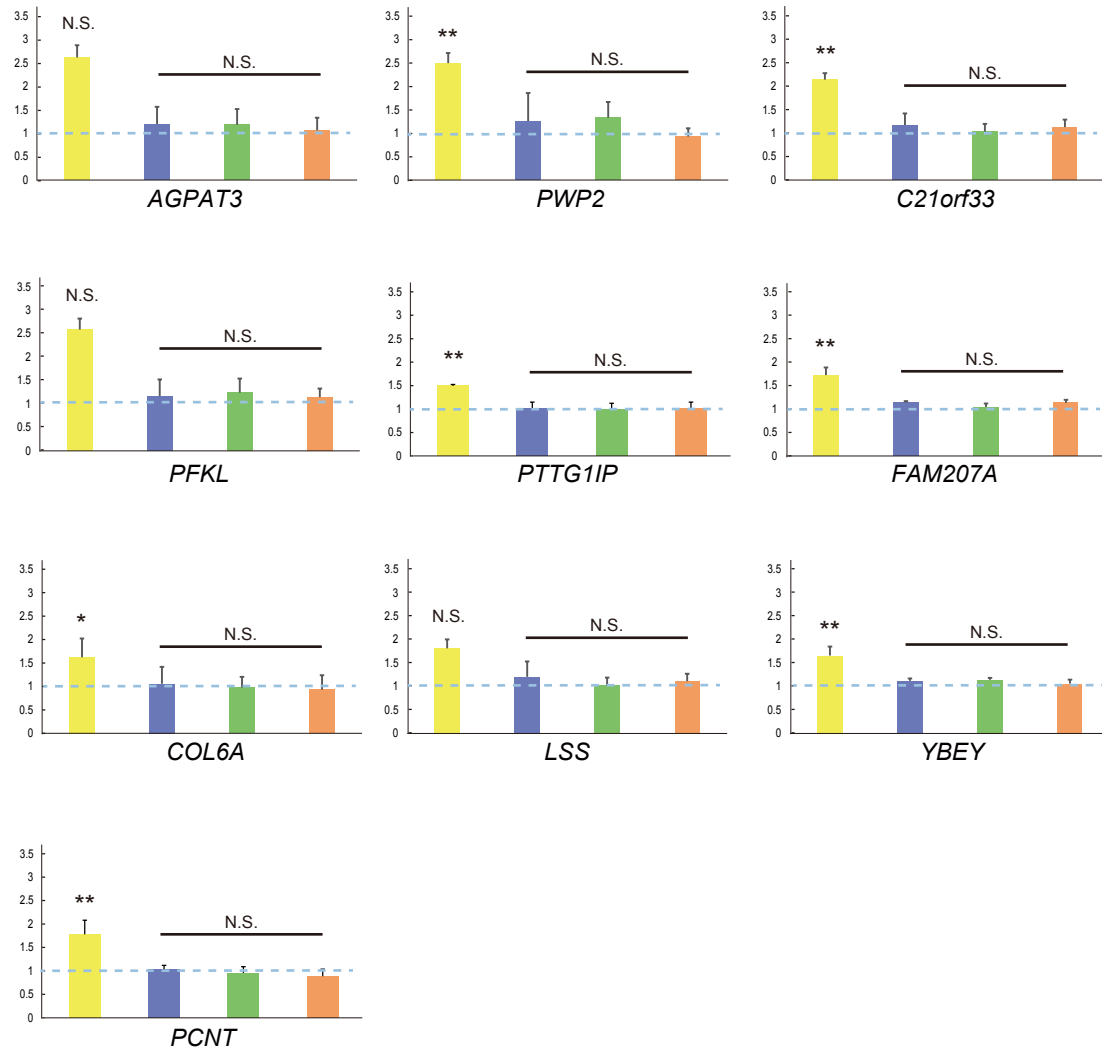


Supplementary Figure S4. Gene positions on long arm of chromosome 21

(a) Chromosome regions of long arm of chromosome 21.

(b) Gene location in each region of chromosome 21. Black boxes indicate gene bodies. Genes in Figure 4c are shown in red.

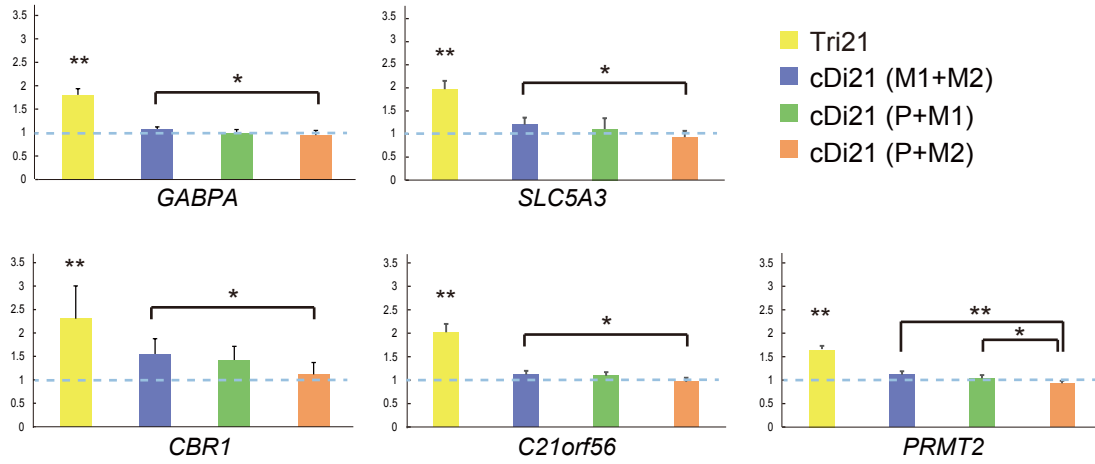




Supplementary Figure S5. Genes whose expression levels were not much different between cDi21 iPSCs

Relative expression levels of genes on chromosome 21 in Tri21 (yellow, n=3), cDi21 (M1+M2) (blue, n=9), cDi21 (P+M1) (green, n=9) and cDi21 (P+M2) (orange, n=9) iPSCs. Gene expression was normalized to that of Di21.

Error bars represent SEM. p values were determined by the Student's t test or Mann-Whitney U test. *p < 0.05, **p < 0.01.



Supplementary Figure S6. Genes whose expression levels were higher in cDi21 (M1+M2) than in cDi21 (P+M2). Relative expression levels of genes on chromosome 21 in Tri21 (yellow, n=3), cDi21 (M1+M2) (blue, n=9), cDi21 (P+M1) (green, n=9) and cDi21 (P+M2) (orange, n=9) iPSCs. Gene expression was normalized to that of Di21. Error bars represent SEM. p values were determined by the Student's t test or Mann-Whitney U test. *p < 0.05, **p < 0.01.

Supplementary Table S1. Primers for RNA FISH

Gene	No.	Forward primer (5'-3')	Reverse primer (5'-3')
<i>GART</i>	1	CCCTTGTTTATACAGGAAGC	GTAATGGCGCCTGCGTCAGA
	2	TCTGACGCAGGCGCCATTAC	GCATCAGCTTTGGGGCCATT
	3	AATGGCCCCAAAGCTGATGC	AATCCAAGACCACGACAGGC
	4	GCCTGTTCGTGGTCTTGGATT	CATGAGAACTTGGGCGAGAG
	5	CTCTCGCCCAAGTTCTCATG	ATTGTGCCACTGCACTCCAG
	6	CTGGAGTGCAGTGGCACAAT	GGAATCTACGATTATGGGATC
	7	GATCCCATAATCGTAGATTCC	GTCTGGAACTCCTGACATCAGG
	8	CCTGATGTCAGGAGTTCCAGAC	TTCAGCTTCCAGAGTAGCTG
<i>MRPL39</i>	1	CCAGTGATTTGCATTCTCC	AAATGCCAGAGTGAAGGAAG
	2	TAATCTTCCTTCACTCTGGC	TGTATATACAGGGACCTCAC
	3	GTTGCATACAGTGAGGTCCC	AGAAACCACAAGTGTTCTTG
	4	TCAGGAACACTTGTGGTTTC	CCTAAGTAAAAGAGGCACAC
	5	GTGTGCCTCTTTACTTAGG	GCACAAGGATATTTTCTGCG

Supplementary Table S2. Primers for Quantitative RT-PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>ACTB</i>	TCAAGATCATTGCTCCTCTGAG	ACATCTGCTGGAAGGTGGACA
<i>HSPA13</i>	CCAGAGAGATGACGATCTTAGGA	TCACTTTAGGAGTAGGCAATGGT
<i>CXADR</i>	GTGCTCCTGTGCGGAGTAG	ATGGCAGATAGGCAGTTTCCC
<i>MRPL39</i>	CCGACAGAATTGACAGAAATGCG	CTTCTCAGTTCGGGGAGTTAATG
<i>GABPA</i>	AAAGAGCGCCGAGGATTTTCAG	CCAAGAAATGCAGTCTCGAG
<i>APP</i>	CTTGGAGAGGTGTGCTCTGAA	GGTTCCTGGGTAGTCTTGAGT
<i>ADAMTS1</i>	CAGAGCACTATGACACAGCAA	AGCCATCCCAAGAGTATCACA
<i>RWDD2B</i>	AAATGGCGATGTTTTCTCTGGC	GGCGTGTCTCTAACCCACTC
<i>USP16</i>	AGCCTTCAGTTTGGCTGTGT	GGCTTTGGAGTTGTAATGCTGG
<i>CCT8</i>	TTTTCTGATTCCGGCCATT	AACCATGCCATGCAATACTGA
<i>TIAM1</i>	ATGACGCTACATATTTGGCTGAG	ACCCAAGATTTCTTCGTTGCTT
<i>SOD1</i>	GGTGGGCCAAAGGATGAAGAG	CCACAAGCCAAACGACTTCC
<i>MIS18A</i>	AGCTCCATGAGCGAAGACG	ACGTTTGGATAGCTTCTGTTCC
<i>GART</i>	CTGGGATTGTTGGGAACCTGAG	ACCAAAGCAGGGAAAGTCTGCAC
<i>SON</i>	TTTCTGGAGCCCTCTTTCAA	GCCAGGAGTCTACCAAATGAAG
<i>SLC5A3</i>	TCTTGGAGCAGTCCGTTTGA	GATGAAGCCCGGCCTATTATC
<i>RCAN1</i>	GCTCCGCCAAATCCAGACAA	GCTGCGTGCAATTCATACTTTTC
<i>SETD4</i>	GAAAGCTAGGAAGTTTCAAGATTCAA	GACTCATCAGCCCTCTTCTGTGA
<i>CBR1</i>	CTGATCCCACACCCTTTCAT	TTAAGGGCTCTGACGCTCAT
<i>CBR3</i>	CCGTGCGCTTCAAGAGTGATG	CGGCAGTAACTCGTTGCACA
<i>DOPEY2</i>	TGAATGGGCGGATCTCATATCT	AGCCGTCTTGGCAACAAGG
<i>MORC3</i>	ATGCAGTATTGAGAGGGACCAGTAT	CACACTGTGAACGGATTTGTGA
<i>CHAF1B</i>	CCATCATATGGGATGTCAGCAA	CTTCATGCTGTCGTCGTGAAAC
<i>HLCS</i>	AGGACAAAGGGCCCAACAG	CGCTGCCCAGATGGACTT
<i>DSCR6</i>	GTCAAAGCGTCAAGAATACCTGC	AAGTCAATCGTGGCTTGC ACT
<i>PIGP</i>	TGTTTGGGATTAACATGATGAGTACCT	TCTGCTGTTGATTTTTTGCATAGTT
<i>TTC3</i>	TCACTGTGGCGGATTATGCC	GTAAGCTGAGTCACACGAACAT
<i>DSCR3</i>	CCTACACTGGAGACCACCACTT	GATGAGGTGGTCAGGGTGAAG
<i>DYRK1A</i>	ACCACCTCCAGTACATCGTCAAG	CCGGGCTCTCCCACTGTT
<i>ERG</i>	GCTGCTCAACCATCTCCTTC	ACAGGAGCTCCAGGAGGAAC
<i>ETS2</i>	TGGAGACGGATGGGAGTTTA	CGACGTCTTGTGGATGATGT
<i>WRB</i>	CGTGCCGAGTAAATGGATAACC	GAAGCACAATAGCGACAAC TTTG
<i>MX1</i>	GTTTCCGAAGTGGACATCGCA	CTGCACAGGTTGTTCTCAGC
<i>NDUFV3</i>	TCAGCCACAGAATCCAAGAAG	CTGTAGTCATGATGCTGCAGGTT
<i>CBS</i>	GGCCAAGTGTGAGTTCTTCAA	GGCTCGATAATCGTGTCCCC
<i>U2AF1</i>	TGAACGTCTGTGACAACCTG	ATCACAGCCTTTTCCGCATC
<i>CSTB</i>	GCCACCGCCGAGACCCAGCA	TGGCTTTGTTGGTCTGGTAG
<i>AGPAT3</i>	GAGCCAAC TGGTCATGCTG	ACCCACAGAGGAAGTCGATCT
<i>PWP2</i>	CCACTCGGTACAACGTCAAGT	TCAGGGGAGAAGGACACACTG
<i>C21orf33</i>	GATGCGGAGTCTACGATGGG	CCAGGTCTGTGATTTTGCCAC
<i>PFKL</i>	GGCATTATGTGGGTGCCAAAGTC	CAGTTGGCCTGCTTGATGTTCTCA
<i>SUMO3</i>	GAATGACCACATCAACCTGAAGG	GCCCGTCCAACCTGAATCT
<i>PTTG1IP</i>	TGAAGAACGTCTCCTGTCTT	ACTACCGACATGGTGATGAT
<i>FAM207A</i>	GAGCGTCCCTTCCATCAGG	GCTCACGCCTCAGCTTCATT
<i>COL6A</i>	ACACCGACTGCGCTATCAAG	CGGTCACCACAATCAGGACTT
<i>C21orf56</i>	TCCGGCCTAAGAAGGTGTGT	GGTGGAGGTCTGCTCGATCT
<i>LSS</i>	GCACTGGACGGGTGATTATGG	TCTTCTCTGTATCCGGCTG
<i>YBEY</i>	GCGCCACTTCGAGTAAGAT	ACATCGGTTGGGACATTTCTATC
<i>PCNT</i>	AGGAGGAGAGTCCGGTAACC	TCAGGGGTGTCGTCACATGAT
<i>PRMT2</i>	GGATCATCAGTCTCTCTGTGCA	GTGCTGTGCCATCTCACTGG