

Supplementary Information

Generation of human induced pluripotent stem cell lines derived from Wolf-Hirschhorn syndrome patients with chromosomal 4p deletion

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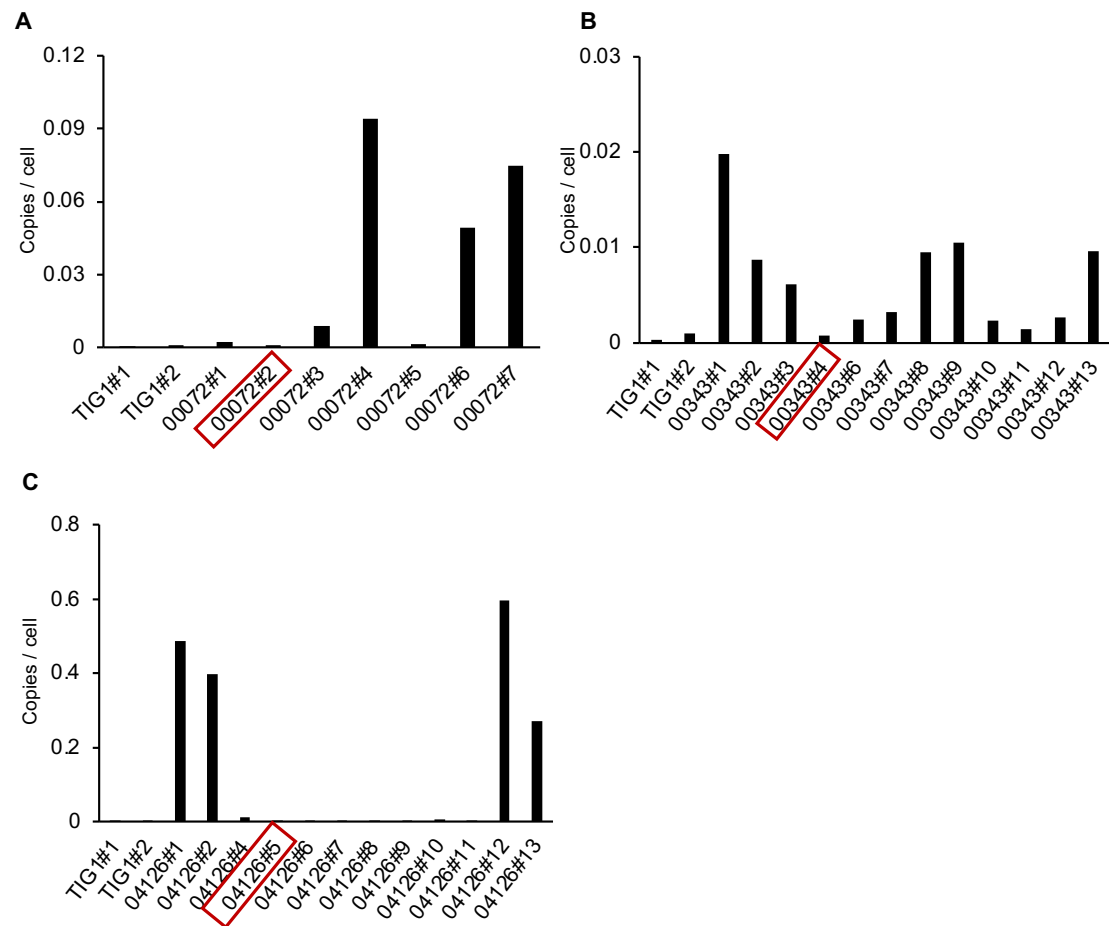
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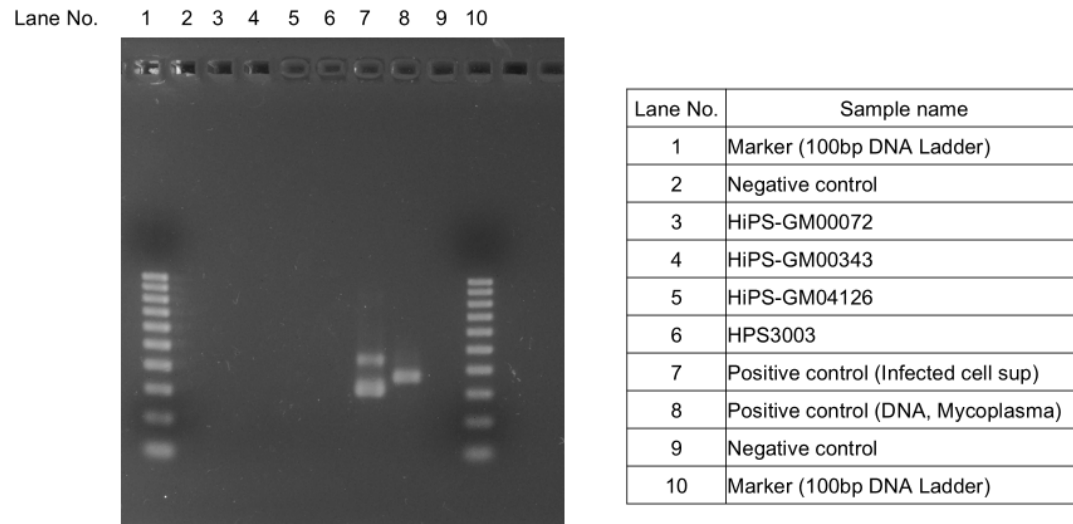
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(Supplementary Table 4 is provided as a spreadsheet)



Supplementary Figure 1. Detection of episomal vector copy numbers in hiPSCs

(A-C) qPCR analysis of EBNA1 to detect residual episomal plasmid vector in HiPS-GM00072 (A), HiPS-GM00343 (B), and HiPS-GM04126 (C). # = clone number of each cell line. Red boxes indicate clones used in subsequent analyses. TIG1#1 and #2, established using SeV vector, were used as negative controls for the residual episomal plasmid vector assay.



Supplementary Figure 2. Mycoplasma contamination test

Left panel shows the electrophoresis result of nested PCR products. A summary of each lane's sample is presented in the table on the right. Nuclease free water used in the PCR mixture was loaded as negative control (Lane 2 and Lane 9). DNA extracted from the culture supernatant of mycoplasma-contaminated cells was used as positive control (Lane 7). Purified mycoplasma genomic DNA was used as positive control (Lane 8).

Supplementary Table 1: Reagent information

	Antibodies used for immunocytochemistry and flow-cytometry			
	Antibody	Dilution	Company cat #	RRID
Pluripotency marker	Goat anti-OCT3/4	1:200	R and D systems, Cat# AF1759	AB_354975
Pluripotency marker	Rabbit anti-NANOG	1:500	ReproCELL Incorporated, Cat# RCAB004P-F	AB_1560380
Pluripotency marker	APC anti-human SSEA-4 antibody	1:20	Biolegend, Cat# 330418	AB_2616819
Pluripotency marker	BV421 Mouse Anti-Human TRA-1-60	1:20	BD Biosciences, Cat# 562711	AB_2737738
Differentiation marker (Ectoderm)	Mouse anti-TUJ1	1:250	R and D systems, Cat# MAB1195	AB_357520
Differentiation marker (Mesoderm)	Mouse anti-SMA	1:250	R and D systems, Cat# MAB1420	AB_262054
Differentiation marker (Endoderm)	Mouse anti-AFP	1:200	R and D systems, Cat# MAB1368	AB_357658
Secondary antibody for immunocytochemistry	Donkey anti-Goat IgG Alexa Fluor 488	1:200	Thermo Fisher Scientific, Cat# A-32814	AB_2762838
Secondary antibody for immunocytochemistry	DyLight 649 Donley anti-rabbit IgG	1:500	Biolegend, Cat# 406406	AB_1575135
Secondary antibody for immunocytochemistry	Donkey anti-Mouse IgG Alexa Fluor 488	1:500	Thermo Fisher Scientific, Cat# A-21202	AB_141607
	TaqMan probes			
	Target		TaqMan assay ID	
Housekeeping	GAPDH		Hs02786624_g1	
Chromosome 4	NSD2 (WHSC1)		Hs00370212_m1	
Chromosome 4	NELFA (WHSC2)		Hs00171805_m1	
Chromosome 4	LETM1		Hs00360061_m1	
Chromosome 4	FGFR3		Hs00179829_m1	
	Primers			
	Target	Size of band	Sequence (Forward primer)	Sequence (Reverse primer)
Episomal vector detection	EBNA1	61	ATCAGGGCCAAGA CATAGAGATG	GCCAATGC AACTTGGA CGTT
Nested-PCR, 1st step PCR (MCGpF11/MCGpR1)	Mycoplasma detection	350- 850	ACACCATGGGAG(C/T)TGGTAAT	CTTC(A/T)T CGACTT(C/T))CAGACCCA AGGCAT
Nested-PCR, 2nd step PCR (R16-2/MCGpR21)	Mycoplasma detection	200- 750	GTG(C/G)GG(A/C)T GGATCACCTCCT	GCATCCAC CA(A/T)A(A/ T)AC(C/T)CT T

Supplementary Table 2: Summary of general clinical information for donors of WHS-specific hiPSCs

Patient number	HiPSC line name (RIKEN BRC ID)	Age at blood collection (Years old)	Sex	Ethnicity	Original donor sample	Clinical symptoms	References
1	HiPS-GM00072 (HPS5146)	11	Male	Caucasian	Fibroblast (GM00072 line)	Multiple abnormalities, including mental retardation, microcephaly, hypertelorism, oblique palpebral fissures, strabismus, and hypospadias.	[30-32]
2	HiPS-GM00343 (HPS5147)	3	Male	Unknown	Fibroblast (GM00343 line)	Multiple congenital anomalies	[31,32]
3	HiPS-GM04126 (HPS5148)	1	Male	Caucasian	Fibroblast (GM04126 line)	Unknown	[32]
4	CiRA-j-1560-c (HPS3003)	30-39	Male	Asian	PBMC	Not available in public	[20]

Supplementary Table 3: Deleted gene list in WHS-specific hiPSCs.

"x" indicates the deletion in the hiPSC line.

	HiPS-GM00072	HiPS-GM00343	HiPS-GM04126	HPS3003
<i>ZNF595</i>		x	x	x
<i>ZNF718</i>		x	x	x
<i>ZNF876P</i>		x	x	x
<i>ZNF732</i>		x	x	x
<i>ZNF141</i>		x	x	x
<i>ABCA11P</i>		x	x	x
<i>ZNF721</i>		x	x	x
<i>PIGG</i>		x	x	x
<i>PDE6B</i>		x	x	x
<i>ATP5I</i>		x	x	x
<i>MYL5</i>		x	x	x
<i>MFSD7</i>		x	x	x
<i>PCGF3</i>		x	x	x
<i>CPLX1</i>		x	x	x
<i>GAK</i>		x	x	x
<i>TMEM175</i>		x	x	x
<i>DGKQ</i>		x	x	x
<i>SLC26A1</i>		x	x	x
<i>IDUA</i>		x	x	x
<i>FGFRL1</i>		x	x	x
<i>RNF212</i>		x	x	x
<i>SPON2</i>		x	x	x
<i>LOC100130872-SPON2</i>		x	x	x
<i>LOC100130872</i>		x	x	x
<i>CTBP1</i>		x	x	x
<i>C4orf42 (CTBP1-AS2)</i>		x	x	x
<i>MAEA</i>		x	x	x
<i>KIAA1530 (UVSSA)</i>		x	x	x
<i>CRIPAK</i>		x	x	x
<i>FAM53A</i>	x	x	x	x
<i>SLBP</i>	x	x	x	x
<i>TMEM129</i>	x	x	x	x
<i>TACC3</i>	x	x	x	x
<i>FGFR3</i>	x	x	x	x
<i>LETM1</i>	x	x	x	x
<i>WHSC1</i>	x	x	x	x
<i>SCARNA22</i>	x	x	x	x
<i>WHSC2 (NELFA)</i>	x	x	x	x
<i>MIR943</i>	x	x	x	x
<i>C4orf48</i>	x	x	x	x
<i>NAT8L</i>	x	x	x	x
<i>POLN</i>	x	x	x	x
<i>HAUS3</i>	x	x	x	x
<i>MXD4</i>	x	x	x	x
<i>ZFYVE28</i>	x	x	x	x
<i>RNF4</i>	x	x	x	x
<i>FAM193A</i>	x	x	x	x
<i>TNIP2</i>	x	x	x	x

<i>SH3BP2</i>	x	x	x	x
<i>ADD1</i>	x	x	x	x
<i>MFSD10</i>	x	x	x	x
<i>C4Orf10 (NOP14-AS1)</i>	x	x	x	x
<i>NOP14</i>	x	x	x	x
<i>GRK4</i>	x	x	x	x
<i>HTT</i>	x	x	x	x
<i>C4Orf44 (MSANTD1)</i>	x	x	x	x
<i>RGS12</i>	x	x	x	x
<i>HGFAC</i>	x	x	x	x
<i>DOK7</i>	x	x	x	x
<i>LRPAP1</i>	x	x	x	x
<i>ADRA2C</i>	x	x	x	x
<i>LOC348926</i>	x	x	x	x
<i>OTOP1</i>	x	x	x	x
<i>TMEM128</i>	x	x	x	x
<i>LYAR</i>	x	x	x	x
<i>ZBTB49</i>	x	x	x	x
<i>D4S234E (NSG1)</i>	x	x	x	x
<i>STX18</i>	x	x	x	x
<i>MSX1</i>	x	x	x	x
<i>CYTL1</i>	x	x	x	x
<i>STK32B</i>	x	x	x	x
<i>C4Orf6</i>	x	x	x	x
<i>EVC2</i>	x	x	x	x
<i>EVC</i>	x	x	x	x
<i>CRMP1</i>	x	x	x	x
<i>C4Orf50</i>	x	x	x	x
<i>JAKMIP1</i>	x	x	x	x
<i>WFS1</i>	x	x	x	x
<i>PPP2R2C</i>	x	x	x	x
<i>MAN2B2</i>	x	x	x	x
<i>MRFAP1</i>	x	x	x	x
<i>LOC93622</i>	x	x	x	x
<i>S100P</i>	x	x	x	x
<i>MRFAP1L1</i>	x	x	x	x
<i>CNO (BLOC1S4)</i>	x	x	x	x
<i>KIAA0232</i>	x	x	x	x
<i>TBC1D14</i>	x	x	x	x
<i>CCDC96</i>	x	x	x	x
<i>TADA2B</i>	x	x	x	x
<i>GRPEL1</i>	x	x	x	x
<i>FLJ36777</i>	x	x	x	x
<i>SORCS2</i>	x	x	x	x
<i>PSAPL1</i>	x	x	x	x
<i>LOC84740</i>	x	x	x	x
<i>AFAP1</i>	x	x	x	x
<i>ABLIM2</i>	x	x	x	x
<i>SH3TC1</i>	x	x	x	x
<i>HTRA3</i>	x	x	x	x
<i>ACOX3</i>	x	x	x	x
<i>C4Orf23 (TRMT44)</i>	x	x	x	x

<i>CPZ</i>	x	x	x	x
<i>HMX1</i>	x	x	x	x
<i>LOC650293</i>	x	x	x	x
<i>USP17</i>	x	x	x	x
<i>USP17L6P</i>	x	x	x	x
<i>DEFB131</i>	x	x	x	x
<i>MIR548I2</i>	x	x	x	x
<i>DRD5</i>	x	x	x	x
<i>SLC2A9</i>	x	x	x	x
<i>WDR1</i>	x	x	x	x
<i>ZNF518B</i>	x	x	x	x
<i>CLNK</i>	x	x	x	x
<i>MIR572</i>	x	x	x	x
<i>HS3ST1</i>	x	x	x	x
<i>HSP90AB2P</i>	x	x	x	x
<i>RAB28</i>	x	x	x	x
<i>NKX3-2</i>	x	x	x	x
<i>LOC285548</i>	x	x	x	x
<i>BOD1L</i>	x	x	x	x
<i>CPEB2</i>	x	x	x	x
<i>C1QTNF7</i>	x	x	x	x
<i>CC2D2A</i>	x	x	x	x
<i>FBXL5</i>	x	x	x	x
<i>LOC285550 (FAM200B)</i>	x	x	x	x
<i>BST1</i>	x	x	x	x
<i>CD38</i>	x	x	x	x
<i>FGFBP1</i>	x	x	x	x
<i>FGFBP2</i>	x	x	x	x
<i>PROM1</i>	x	x	x	x
<i>TAPT1</i>	x	x	x	
<i>FLJ39653 (TAPT1-AS1)</i>	x	x	x	
<i>LDB2</i>	x	x	x	
<i>QDPR</i>	x	x	x	
<i>CLRN2</i>	x	x	x	
<i>LAP3</i>	x	x	x	
<i>MED28</i>	x	x	x	
<i>FAM184B</i>	x	x	x	
<i>DCAF16</i>	x	x	x	
<i>NCAPG</i>	x	x	x	
<i>LCORL</i>	x	x	x	
<i>SLIT2</i>	x	x	x	
<i>MIR218-1</i>	x	x	x	
<i>PACRGL</i>	x	x	x	
<i>KCNIP4</i>	x	x	x	
<i>NCRNA00099</i>	x	x	x	
<i>GPR125</i>	x	x	x	
<i>GBA3</i>	x	x	x	
<i>PPARGC1A</i>	x	x	x	
<i>MIR573</i>	x	x	x	
<i>DHX15</i>	x	x	x	
<i>SOD3</i>	x	x	x	
<i>CCDC149</i>	x	x	x	

<i>LGI2</i>	×	×	×	
<i>SEPSECS</i>	×	×	×	
<i>PI4K2B</i>	×	×	×	
<i>ZCCHC4</i>	×	×	×	
<i>ANAPC4</i>	×	×	×	
<i>SLC34A2</i>	×	×	×	
<i>SEL1L3</i>	×	×	×	
<i>C4orf52 (SMIM20)</i>		×	×	
<i>RBPJ</i>		×	×	
<i>CCKAR</i>		×	×	
<i>TBC1D19</i>		×	×	
<i>STIM2</i>		×	×	