SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

Human pluripotent stem cell culture. hESCs lines SA001 (XY, passages 20-41, Cellartis AB, Götegorg, Sweden), H9 (XX, passages 64-70, WiCell Research Institue Madison, WI, USA), VUB01 (1) (XY, passages 94-100), VUB03-DM1 (1) (DM1 line, XY, passages 63-67 AZ-VUB, Brussels, Belgium), VUB19-DM1 (2) (DM1 line, XY, passages 72-73 AZ-VUB, Belgium) and VUB05-HD (1) (Huntington line, XY, passage 103-120, AZ-VUB, Belgium) were maintained on a layer of mitotically inactivated murine embryonic STO fibroblasts. The hESCs were cultured in DMEM/F12 glutamax supplemented with 20% knockout serum replacement, 1 mM nonessential amino acids, 1% penicillin/streptomycin, 0.55 mM β-mercaptoethanol and 10 ng/ml recombinant human FGF2 (fibroblast growth factor 2) (all from Invitrogen). Human iPS cells were generated from fetal lung fibroblasts IMR-90 and grown as previously described (3). Cultures were fed daily and manually passaged every 5-7 days.

Immunohistochemistry. Experiments were performed as previously described (4). Briefly, cells were incubated with MAP2 primary antibody (Sigma Aldrich, M1406; 1/1000) and Nestin (NES) (human specific) primary antibody (Millipore, AB5922; 1/2000) overnight at 4°C.

Immunocytochemistry. Immunocytochemistry experiments were performed as previously described (5). Cells were fixed with 4% PFA for 20 min at room temperature, rinsed with PBS and blocked with 1% BSA, 5% normal goat serum and 0.1% triton in PBS solution for 1 hour. Thereafter cells were incubated with beta III-tubulin (TUJ1) primary antibody (mouse IgG, MMS-435P, Covance, dilution 1/500e), SOX2 primary antibody (rabbit, polyclonal, Ab5603MI, Millipore, dilution 1/500e), HuCD primary antibody (mouse, monoclonal, A21271, Invitrogen, dilution 1/1000e) overnight at 4°C. Alexa-488 and alexa-555 labeled secondary antibodies were used at the dilution 1/1000e (Molecular probes). Then DAPI counterstain (2%g/mL, sigma) was applied.

Assessment of copy number variation. Genomic DNA was isolated from NSC using the Wizard® Genomic DNA Purification Kit (Promega, Charbonnieres, France). IntegraChip genome-wide BAC arrays of 5,245 BAC clones (526-kb median spacing) were hybridized by the manufacturer, IntegraGen as previously described (6, 7). The genomic positions of all clones on the array have been determined by BAC end sequencing. A genomic DNA labeling kit (Enzo) was used to label the sample DNA with Cy5 and the reference DNAwith Cy3 [3]. The labeled products were purified with QiaQuick polymerase chain reaction (PCR) purification kits (Qiagen). After addition of tRNA (48 mg) plus a quantity of human Cot DNA (Invitrogen) equal to 50 times the mass of the labeled DNAs, the resulting products were concentrated by vacuum centrifugation, resuspended in 43 mL of hybridization solution (Ambion) and deposited on the array under a coverslip. Hybridization in a sealed chamber (Corning Life Sciences) was for 66 hours at 55°C, and posthybridization treatment was with minor modifications of a published protocol [4]. Slides were scanned (Agilent Technologies), data were extracted with GenePix 6 (Axon InstrumentseMolecular Devices), and copy number analysis was performed with GenoCensus (IntegraGen), which includes block Loess normalization.

Harvest of hESCs, iPS cells and their progenies for chromosome analysis.

Preparation of hESCs, iPS cells and their progenies were done as previously described (8). Actively growing hESC colonies or NSC were treated with colchicine at 1 μg/mL (Eurobio) for 90 min at 37°C. After washing, cells were incubated in trypsin–EDTA 0.05% (Eurobio) for 2–3 min and then harvested. Cells were incubated in 0.075 M KCl (Sigma) for 10–18 min at 37°C, followed by fixation with 3:1 methyl alcohol/glacial acetic acid. Fixed cells were dropped on wet slides and dried at 37°C for G-banding and at room temperature for mFISH, FISH with centromeric-specific probes and arm-specific chromosome painting. **G banding** was performed by immersing slides in 0.05% trypsin–EDTA with two drops of 0.4 M Na2HPO4 for 2 to 10 s, rinsed in distilled water with 2% SVF and stained with Giemsa (Merck) for 5 min, rinsed in distilled water and airdried. Twenty to 50 metaphases were captured using a Zeiss Z1 microscope equipped with an AxioCam camera and ×20 and ×63 plan-apo objectives. Images were analysed, and at least 10 metaphases were fully karyotyped using the MetaSystems Ikaros software (MetaSystems). **For mFISH**, fixed and denatured metaphase chromosomes were hybridised overnight at 37°C with a denatured “cocktail painting mFISH” probe (MetaSystems). Slides were washed in successive baths of 0.4 × SSC and 2 x SSC, 0.05% Tween20, and nuclei were stained with DAPI. Seventeen to 55 metaphases were captured using a Zeiss Z1 fluorescence microscope equipped with a

UV HBO 100-W lamp coupled to an AxioCam camera and ×20 and ×63 objectives. At least 10 analysed metaphases were karyotyped using the MetaSystems Isis software. **FISH with centromeric specific probes for chromosome 1 and chromosome 15:** The centromeric probe specific for human chromosome 1 corresponds to region 1q12 (Locus D1Z1, DNA class: satellite III, reference : LPE 01G (Cytocell) and the centromeric probe specific for human chromosome 15 corresponds to region 15p11.1-q11.1 (Locus D15Z4, DNA class: a satellite, reference: CEP15, Vysis® Abbott Molecular Inc). The centromeric probe for chromosome 1 is labelled with FITC fluorochrome and the centromeric probe for chromosome 15 is labelled with Spectrum Orange. Fixed and denatured metaphase chromosomes and interphase nuclei were hybridised overnight at 37°C with a denatured fluorescently labelled chromosome 1 DNA probe (and fluorescently labelled chromosome 15 DNA probe for NSC-VUB05-HD passage 53). Slides were washed in successive baths of 0.25 xSSC and 2 xSSC, 0.05% Tween20 and nuclei were stained with DAPI. 50 interphase nuclei and 10 metaphases were captured using a Zeiss Z1 fluorescence microscope equipped with a UV HBO 100-W lamp coupled to an AxioCam camera and x20 and x63 objectives. Interphase nuclei and metaphases were analysed using the Metasystems Isis software. **Arm-specific chromosome painting:** Fixed and denatured metaphase chromosomes were hybridised overnight at 37°C with denatured painting probes specific for short arm 1p directly labelled with FITC and long arm 1q directly labelled with TexasRed® (Metasystems’probes). Slides were washed in successive baths of 1xSSC and 2 x SSC, 0.05% Tween20 and nuclei were stained with DAPI. Ten to 20 metaphases were captured using a Zeiss Z1 fluorescence microscope equipped with a UV HBO 100-W lamp coupled to an AxioCam camera and x20 and x63 objectives. Interphase nuclei and metaphases were analysed using the Metasystems Isis software.

SUPPLEMENTAL TABLE AND FIGURE LEGENDS

Supplemental Table 1: Summary of cytogenetic findings.

cell lines	passage	batch	Karyotype [number of metaphases]
VUB03-DM1	hES P67		46, XX [20]
	NSC P18	#a	46,XX [25]
			46, XX, der(4)t(1;4) [2]
			46, XX, der(1)t(1;17) [1]
	NSC P34	#a	46, XX, der(13)t(1;13)[12]
			45, X, der(13)t(1;13)[6] onto 13q
			45, X, der(5)t(1;5) [4]
			45, X, der(13)t(1;13) [2]
			45, X, der(8)t(1;8) [1]
			45, X [1]
	NSC P44	#a	46, X, del(Xq) der(13)t(1;13) [10]
			46, XX, der(13)t(1;13) [6]
			47, XX, +12, der(13)t(1;13) [1]
			47, XX, +11, der(13)t(1;13) [1]
			46, XX, der(13;1), der(6)t(3;6) [1]
			46, X, del(Xq), der(13)t(13;1), der(8)t(8;11) [1]
			60~68, XXXX, +1, +3, +4, +5, +6, +8, +10, +12, +14, +15, +16, +17, +18, +20, +21, +22, der(13)t(1;13) [cp2]

			92, XXXX, der(13)t(1;13) [4]
			92, XX, del(Xq)(x2), der(13)t(1;13) [5]
SA001	hES P20		46, XY [25]
	NSC P10	#a	46, XY [50]
	NSC P15	#a	46, XY [53]
			46, XY, der(22)t(1;22) [2]
	NSC P31	#a	46, XY [34]
			92, XXYY [9]
	hES P41*		46, XY, .arr (1-22)x2,(XY)x1
	NSC P34*	#b	46, XY, .arr (1-22)x2,(XY)x1
	NS P51	#b	46, XY [27]
			46, XY, der(1)t(1;17) [3]
			46, XY, der(Y)t(Y;1),t(9;13) [cp2]
VUB05-HD	hES P103*		46, XY, dup(20)(q11.21).arr 20q11.21
	NSC P38*	#a	46,XY, dup(1)(q11.21q44).arr 1q11.21q44, dup(20)(q11.21).arr 20q11.21
	NSC P53	#a	46, XY, der(15)t(1;15) [20]
	hES P103*		46, XY, dup(20)(q11.21).arr 20q11.21
	NSC P32*	#b	46,XY, dup(1)(q11.21q32.21).arr 1q11.21q32.21, dup(20)(q11.21).arr 20q11.21
	NSC P59	#b	46, XY, der(10)t(1;10) [28]
			46, XY, +5, + der(5), +7, der(10)t(1;10) [cp3]
			46, XY, der(Y)t(Y;1),t(9;13) [cp2]
VUB19-DM1	hES P73		46, XX [25]

	NSC P22	#a	46, XX [22]
			47, XXX, ins(1; 11) [cp2]
	NSC P50	#a	46, XX, der(1)t(1;1) [13]
			46, XX, der(5)t(1;5) [1]
			46, XX, der(18)t(1;18), dup(12) [1]
			51~56, XX, +2, +3, -4, +5, +6, +8, +11, +12, +14, +15, +17, +19, +20, der(1)t(1;1) [cp3]
			78, XX, der(1)t(1;1) [1]
			80, XXX, +der(13)t(1;13) [1]
			70~78, XXXX [4]
H9	hES P64		46, XX [28]
	NSC P52	#a	46, XX, der(17)t(1;17) [14]
			46, XX, der(22)t(1;22) [2]
			46, XX, der(21)t(1;21) [1]
VUB01	hES P94*	#a	46, XY, .arr (1-22)x2,(XY)x1
	NSC P21		46, XY [20]
	NSC P65		46, XY, der(22)t(1;22) [20]
IMR90	iPS P23	#a	46, XX [21]
	NSC P24		46, XX [13]
			46, XX, der(17)t(1 ;17) [6]

* karyotype perfomed using BAC-aCGH. NSC: neural stem cells

Supplemental Table 2: Acquired 1q duplications observed in vivo cited in the article

Acquired 1q duplications	Reference
Hematologic malignancies	Lejeune, J., *Ann Genet,* 1979. (9)
Acute lymphoblastic leukemia	Seghezzi, L. et al, *Cancer Genet Cytogenet*, 1995. (10)
Non-Hodgkin's lymphoma	Sawyer, J.R., *Cancer*, 1995. (11)
Multiple myeloma	Sawyer, J.R., *Blood,* 1998. (12)
Acute lymphoblastic leukemia	Jarvis, A., *Cancer Genet Cytogenet,* 1999. (13)
Non-metastasising primary breast carcinomas	Adeyinka, A., *Int J Cancer* 1999. (14)
Chordomas	Sawyer, J.R., *Neurosurg Focus,* 2001. (15)
Hepato cellular carcinomas	Wong, N., *Am J Pathol* 2001. (16)
Retinoblastoma	Mairal, A., *Genes Chromosomes Cancer* 2000. (17)
Acute lymphoblastic leukaemia	Wan, T.S.K*., Leukemia Research* 2004. (18)
Pediatric brain tumors	Faria, C., *J Neurosurg Pediatr*, 2010. (19)
Pediatric brain tumors	Miwa, T., *Neurosurgery,* 2010. (20)
B cell lymphoma	Fournier, A., *EMBO Mol Med*, 2010. (21)

Supplemental Table 3: Impact of long-term cell culture on chromosomal changes and differentiation potential (from the literature)

NSC: neural stem cells; ND: not determined

Cell type	derived from	Chromosome abnormalities	Differentiation potential *in* vitro	Overgrowth or tumor formation	ref
Neurospheres	Mouse adult cells	Chromosome marker (mostly1)	Yes	No tumor growth	(22)
Neurospheres	Mouse fetal cells	Trisomies (mostly 1)	Declined ability to give rise to neurons	nd	(23)
NSC	Mouse ES or fetal cells	Trisomies (mostly 1 and 3)	Yes	No tumor growth	(24)
NSC	Human fetal cells	No	Yes	nd	(25)
Neural progenitors	Human ES cells carrying 20q11.21 duplication	20q11.21	nd	Tumor growth (subcutaneously injection)	(26)
NSC	Human ES cells	No	Yes	No tumor growth	(27)
NSC	Human fetal cells	Trisomies (mostly 7 and 19)	Yes	No tumor growth	(28)
NSC	Human iPS cells	No	Yes	nd	(29)
NSC	Human fetal cells	yes	nd	Tumor in 2/15 nude mice	(30)

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Supplemental Figure legends

Supplemental Figure 1: Karyotypic analysis of neural derivatives of VUB03-DM1 cell line. (A) Multicolor FISH analysis of neural derivatives of VUB03-DM1 at passage 44 showing a segment of chromosome 1 translocated onto chromosome 13p (der(13)t(1;13)). (B) In some mitoses it was accompanied by additional chromosomal changes. (C and D) In situ hybridization of a juxtacentromeric-specific probe which detects the 1q12 region, showing two types of hybridization signal in the nuclei: condensed spots (C) and dispersed spots (green arrow, D). Nuclei are counterstained with DAPI (blue fluorescence). (E) mFISH analysis of neural derivatives of the VUB03-DM1 cell line. (F) At passage 18, a segment of chromosome 1 is translocated onto chromosome 4q in 7% of the mitoses (VUB03-DM1 NSC P18, 46, XX, der(4)t(1;4)).

Supplemental Figure 2: Genomic integrity control of pluripotent stem cells at the undifferentiated stage. (A-D) G-banding analysis of SA001 P20 (A), VUB19-DM1 P73 (B), H9 P64 (C), IMR90 iPS P23 (D). (E-G) BAC aCGH analysis of SA001 P41 (E), VUB05-HD P103 (F) and VUB01 P94 (G). Note the absence of chromosomal abnormalities in cell cultures except for VUB05-HD showing duplication at 20q11.21.

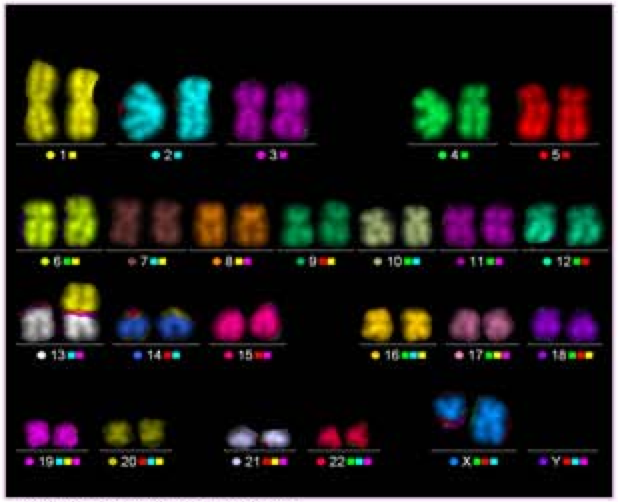
Supplemental Figure 3: Karyotypic analysis of neural derivatives of SA001 cell line. mFISH analysis of neural derivatives of SA001 cell line (batch #a) at passage 10 (A), 15 (B) and 31 (C). At passage 10, there are no chromosomal abnormalities (A) whereas at passage 15 a translocation of a part of chromosome 1 onto chromosome 22p (SA001 NSC P15 der(22)t(1;22)) was observed in 3.6% of the mitoses (B). At passage 31 there were no more translocations of a segment of chromosome 1 onto chromosome 22 but around 20 % of the mitoses were tetrapolyploid (C). (D, E) BAC aCGH and mFISH analysis of neural derivatives of SA001 cell line (batch #b). At passage 34 no chromosomal abnormalities are detected using BAC aCGH technology. Chromosome 1 is depicted (D). At passage 51, 10% of the mitoses exhibited a translocation of a part of chromosome 17 onto chromosome 1p (der(1)t(1;17)) (E).

Supplemental Figure 4: Karyotypic analysis of neural derivatives of VUB05-HD cell line. At passage 38 (batch #a) and 32 (batch #b), BAC aCGH revealed a duplication of the whole arm of chromosome 1q (46,XY,dup(1)(q11.21q44).arr 1q11.21q44) (A: chromosome 1 is depicted) and a duplication of a part of chromosome 1q (46,XY,dup(1)(q11.21q32.21).arr 1q11.21q32.21), (B: chromosome 1 is depicted). At passage 53 (batch #a) and 59 (batch #b), mFISH analysis showed that the duplicated region translocated onto chromosome 15p (VUB05-HD NSC P53, 46, XY, der(15)t(1;15)) (C) and chromosome 10q (VUB05-HD NSC P59, 46, XY, der(10)t(1;10)) (D)

Supplemental Figure 5: mFISH analysis of neural derivatives of pluripotent stem cell lines. VUB19-DM1 cell line (A-B). (A) At passage 50, a segment of chromosome 1 translocated onto the short arm of chromosome 1 (VUB19-DM1 NSC P50, 46, XX, der(1)t(1;1)). (B) Additional abnormalities were observed in mitoses carrying the chromosome 1 arm duplication. **H9 cell line (C-E).** (C) NSC at passage 52 showing a translocation of a part of chromosome 1 onto chromosome 17q (H9 NSC P52, 46, XX, der(17)t(1;17)), (D) chromosome 21p (H9 NSC P52, 46, XX, der(21)t(1;21)) and (E) chromosome 22p (H9 NSC P52, 46, XX, der(22)t(1;22)). **VUB01 cell line (F).** At passage 65, a segment of chromosome 1 translocated onto the long arm of chromosome 22 (VUB01 NSC P65, 46, XY, der(22)t(1;22)). **IMR90 cell line (G).** At passage 24, a segment of chromosome 1 translocated onto chromosome 17q (IMR90-NSC P24, 46, XX, der(17)t(1;17))

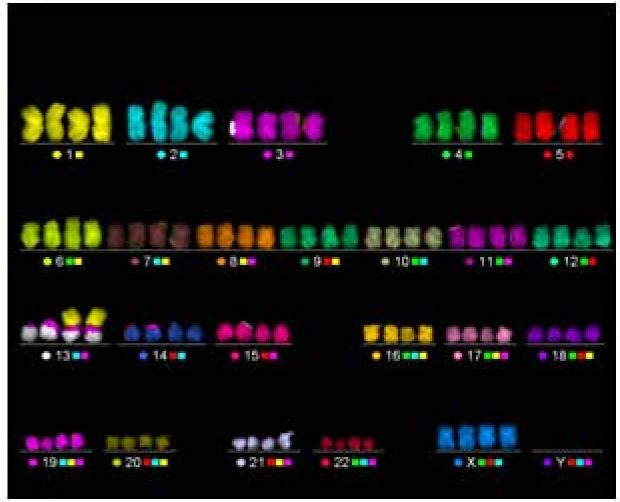
Supplemental Figure 6: NSC analysis with specific probes. Arm-specific chromosome painting (A-C). Chromosome 1 painting using long arm (q, in red) or short arm (p, in green) specific probes. (A) NSC VUB19-DM1 at passage 50 showing two copies of the short arm (p, in green) and three copies of the long arm (q, in red). The third copy of chromosome 1q is translocated onto chromosome 1p. (B) NSC H9 at passage P52 showing two copies of the short arm (in green) and three copies of the long arm (in red). The third copy of chromosome 1q is translocated onto chromosome 17q. (C) NSC VUB01 at passage P65 showing two copies of the short arm (in green) and three copies of the long arm (in red). The third copy of chromosome 1q is translocated onto chromosome 22q. **FISH with centromeric probe specific for chromosome 1q12 region (D-I)**. (D) NSC-VUB19-DM1 at passage 50 showing chromosome 1q arm translocated onto telomeric ends of chromosome 1p. There are 2 centromeres of chromosome 1 (the first one on chromosome 1 and the second one on der (1) chromosome) (metaphases analysed using inverted DAPI). (E) In situ hybridization on interphase nuclei from VUB19-DM1 cell line at passage 50. The depicted figure shows a polyploïd nucleus as revealed by the 4 green spots. All nuclei analysed revealed two types of spots: condensed spots (corresponding to condensed heterochromatin) and dispersed spots corresponding to decondensed heterochromarin (green arrows). (F) NSC-H9 at passage 52 showing chromosome 1q arm translocated onto centromeric region of chromosome 22p. There are 3 centromeres of chromosome 1. (G) NSC-H9 at passage 52 showing chromosome 1q arm translocated onto telomeric ends of chromosome 17q. There are 2 centromeres of chromosome 1. (H) NSC-VUB01 at passage 65 showing chromosome 1q arm translocated onto telomeric ends of chromosome 22q. There are 3 centromeres of chromosome 1. (I) NSC-VUB05-HD (batch #b) at passage 59 showing 1q arm translocated onto telomeric ends of chromosome 10q. There are 3 centromeres of chromosome 1. **Centromeric probes specific for chromosome 1q12 region (in green) and for chromosome 15p11.1-q11.1 region (in red) (J-K)**. (J) NSC-VUB05-HD (batch #a) at passage 53 showing 1q arm translocated onto centromeric region of chromosome 15p. There are 3 centromeres of chromosome 1 (in green) and 2 centromeres of chromosome 15 (in red), one of them colocalized with one of the chromosome 1 centromeres. (K) FISH on NSC-VUB05-HD P53 (batch #a) interphase nuclei with centromeric probes for chromosomes 1 and 15. There are 3 centromeres of chromosome 1 (in green) and 2 centromeres of chromosome 15 (in red), one of them is colocalized with one of the chromosome 1 centromere.

A



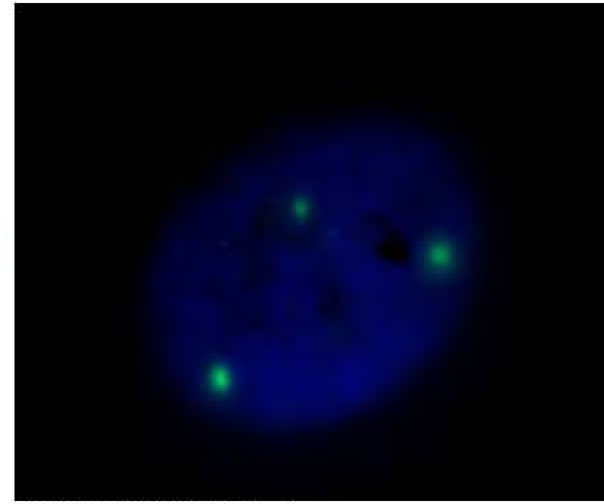
VUB03-DM1 NSC P44:
46 X, del(Xq), der(13)t(1;13)

B



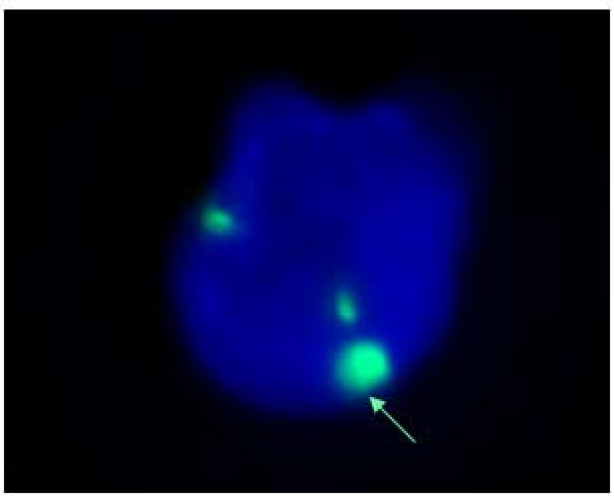
VUB03-DM1 NSC P44: 92 XXXX, der(13)t(1;13)

C



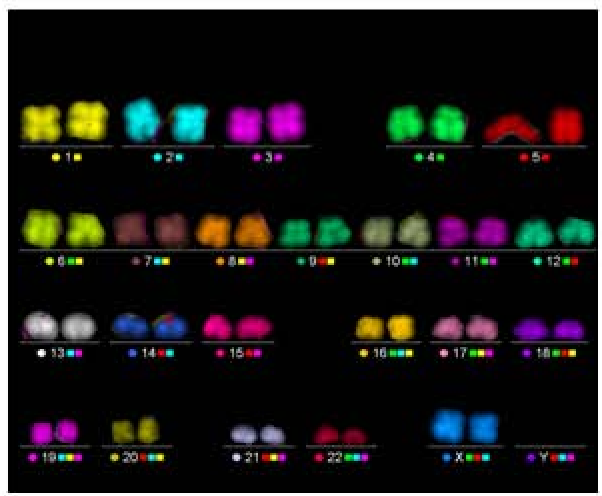
VUB03-DM1 NSC P44

D



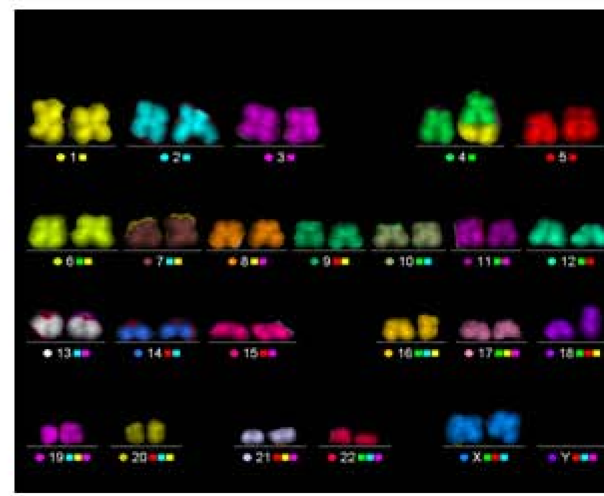
VUB03-DM1 NSC P44

E



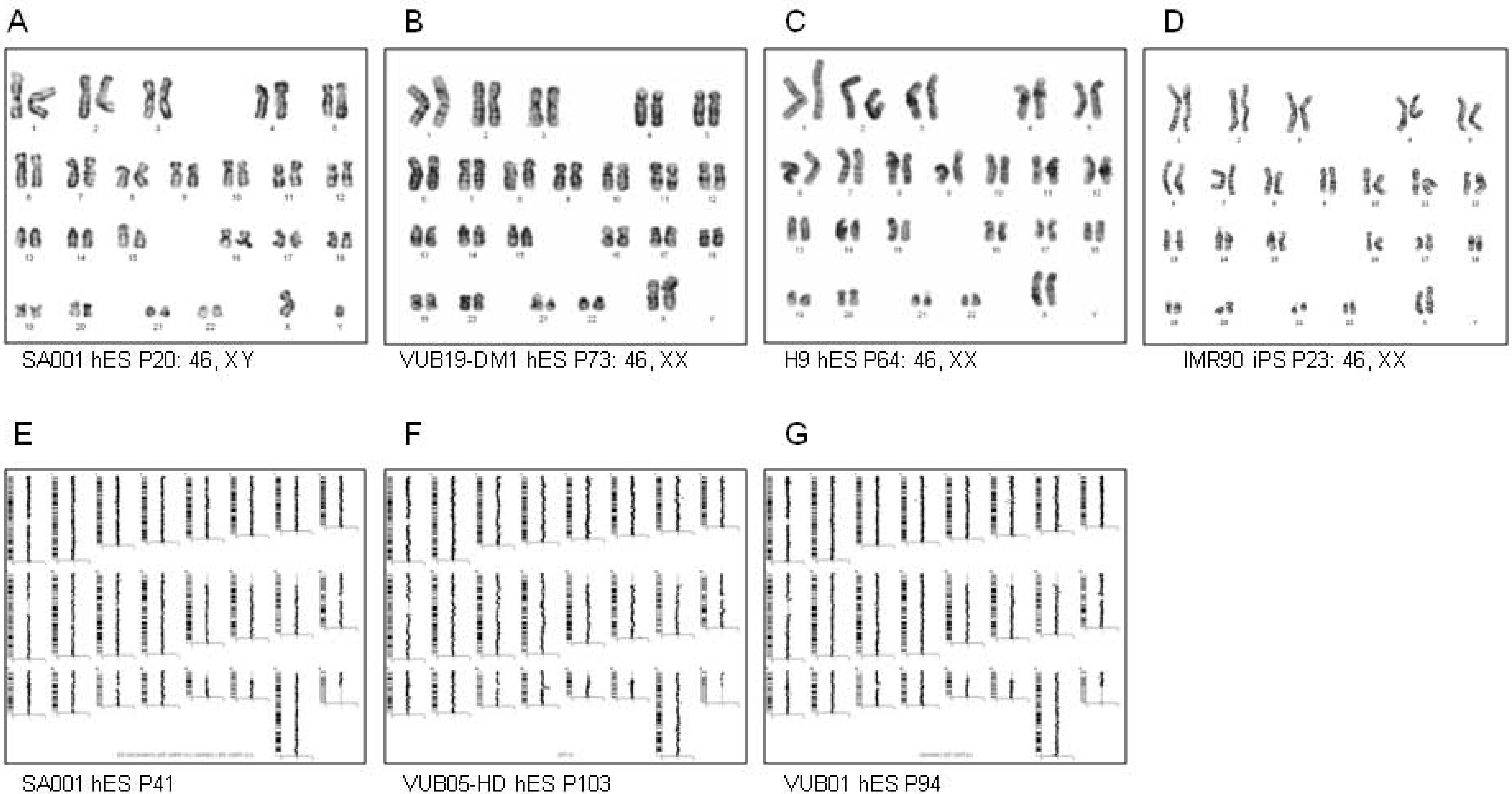
VUB03-DM1 NSC P18: 46, XX

F

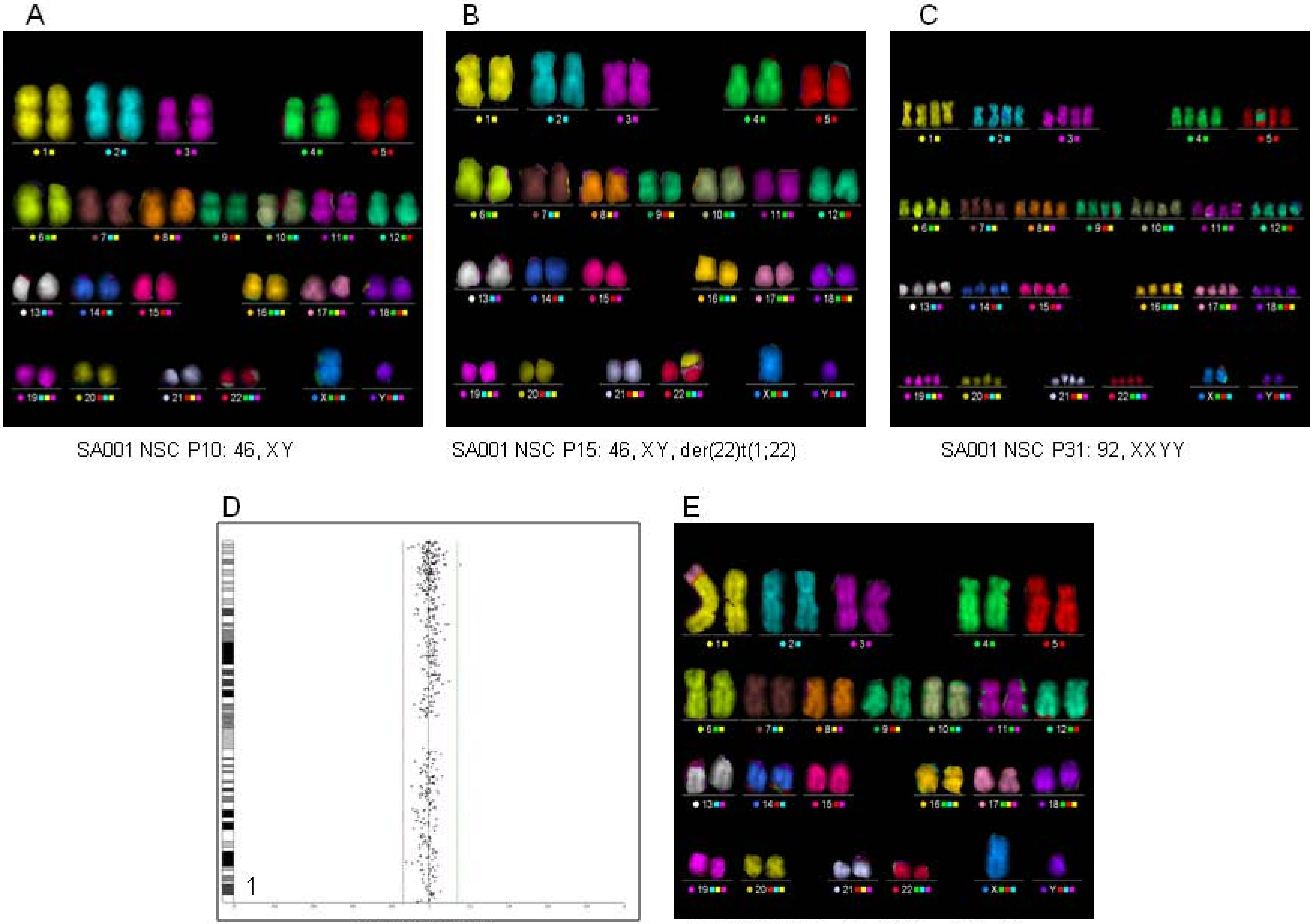


VUB03-DM1 NSC P18: 46, XX, der(4)t(1;4)

Varela & al Supplemental Figure 1



Varela & al Supplemental Figure 2



SA001 NSC P10: 46, XY

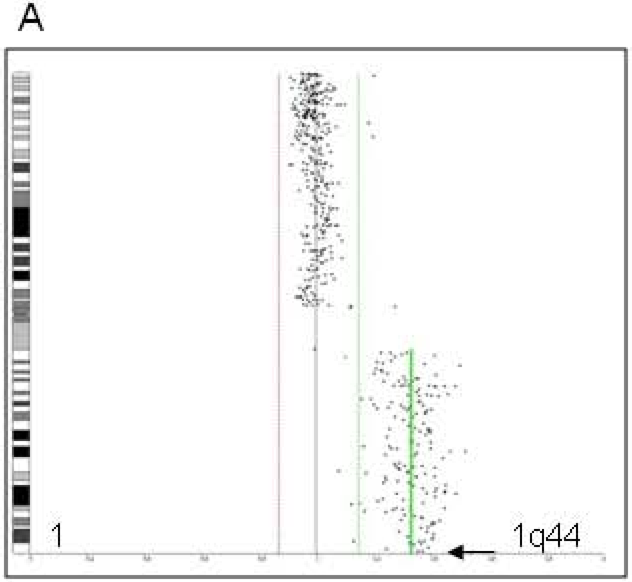
SA001 NSC P15: 46, XY, der(22)t(1;22)

SA001 NSC P31: 92, XXYY

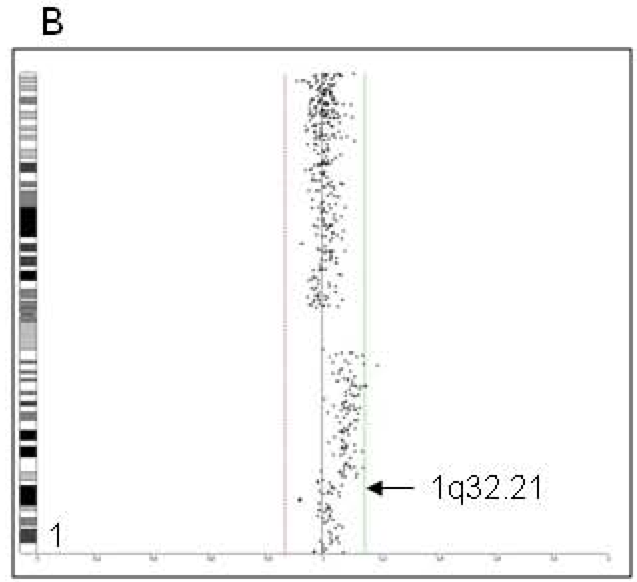
SA001 NSC P34

SA001 NSC P51: 46, XY, der(1)t(1;17)

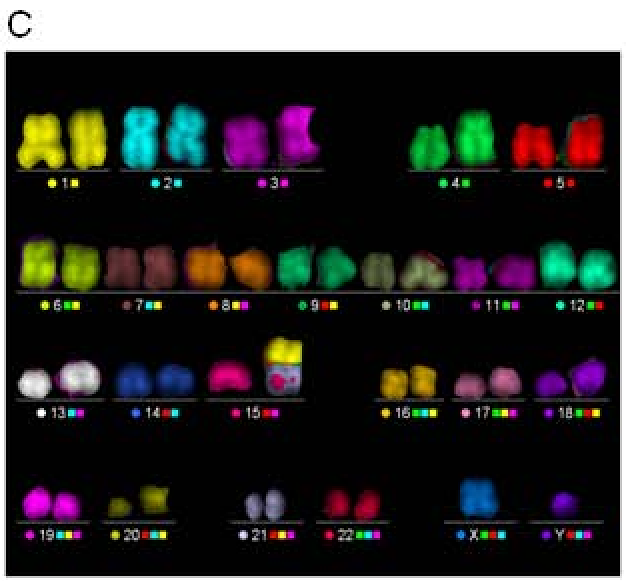
Varela & al Supplemental Figure 3



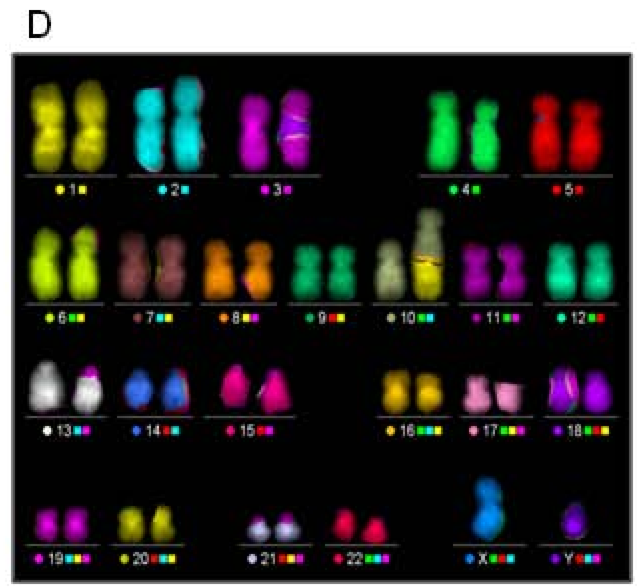
VUB05-HD NSC P38



VUB05-HD NSC P32

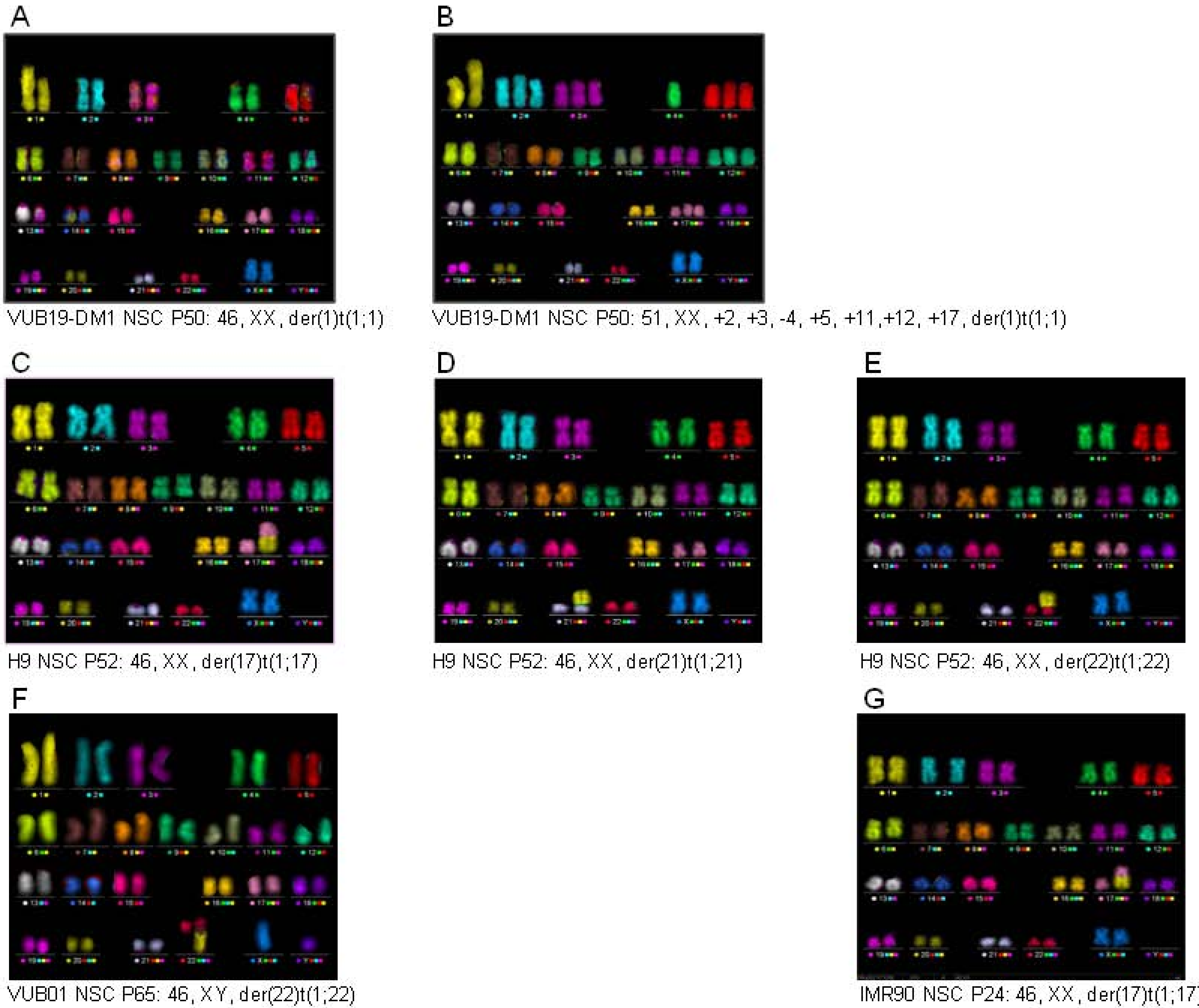


VUB05-HD NSC P53: 46, XY, der(15)t(1;15)

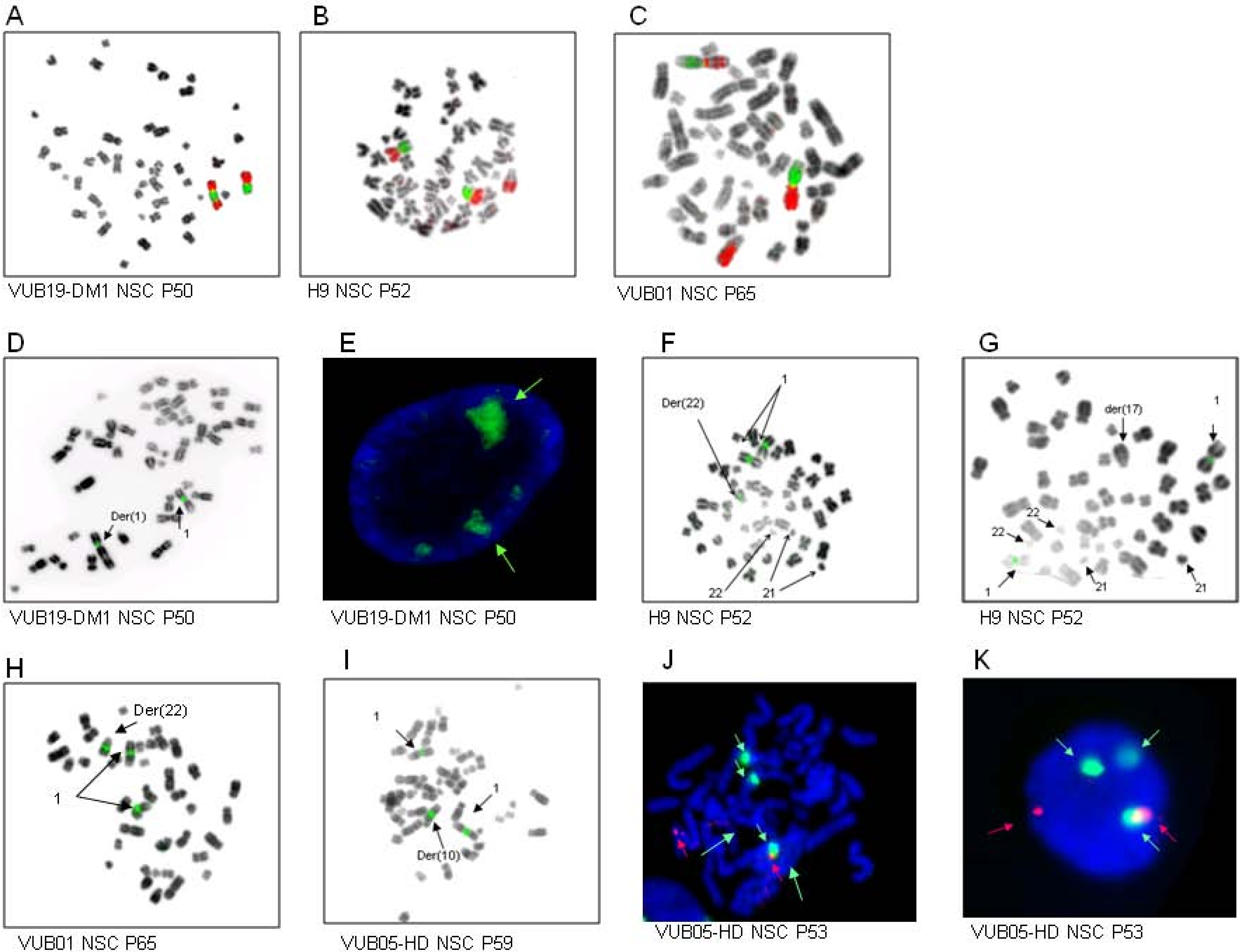


VUB05-HD NSC P59: 46, XY, der(10)t(1;10)

Varela & al Supplemental Figure 4



Varela & al Supplemental Figure 5



Varela & al Supplemental Figure 6