ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR 3PNF_SiPSsv_MM_11 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 3PNF_SiPSsv_MM_11

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by Immunofluorescence

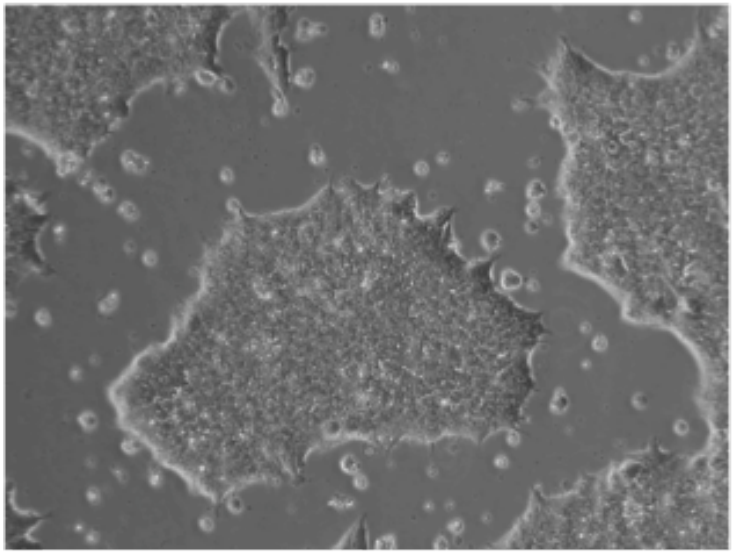
Annex 4: Karyotype

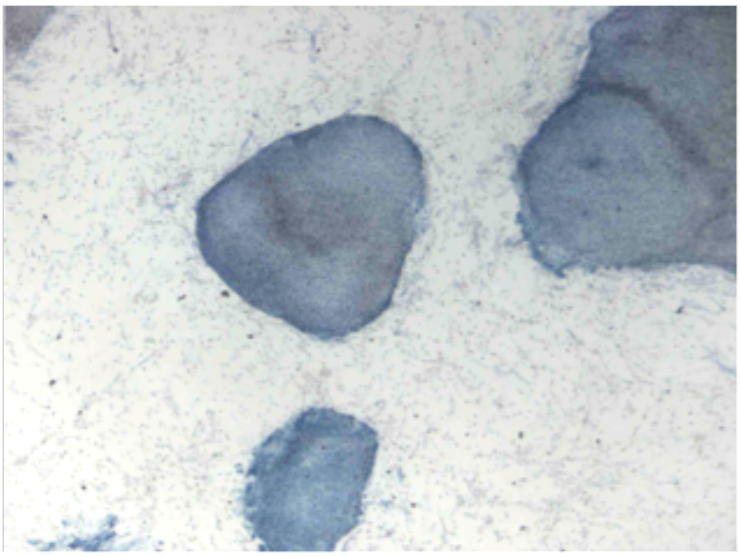
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1
Morphology and Alkaline phosphatase staining

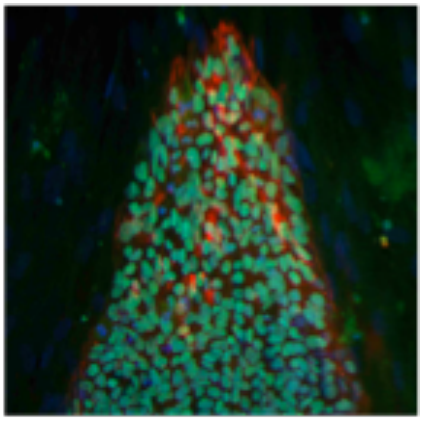




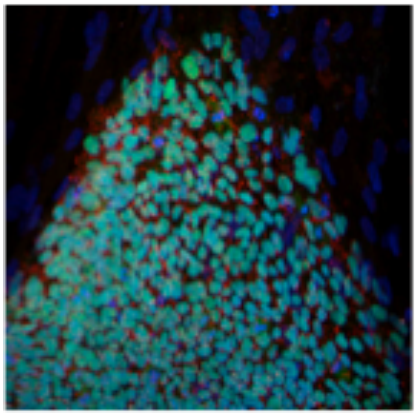
3PNF_SiPSsv_MM_11 Passage 1

Annex 2
Pluripotency markers

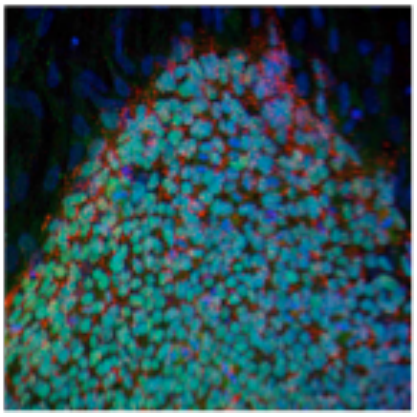
NANOG/TRA181



OCT4/SSEA3



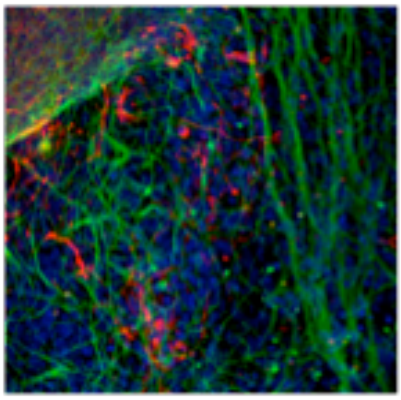
SOX2/ SSEA4



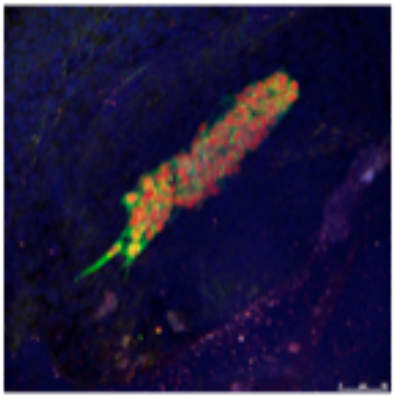
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 3PNF_SiPSsv_MM_11 iPS at passage 8.

Annex 3
In vitro differentiation

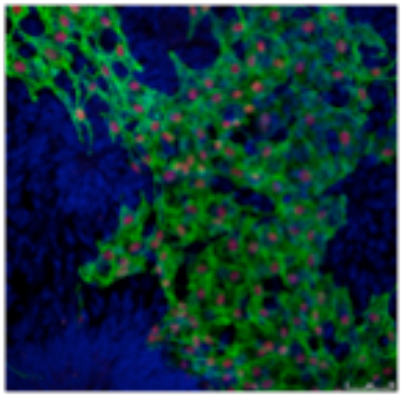
ECTODERM TUJ1/TH



ENDODERM a-FETO FOXA2



MESODERM SMA/GATA4

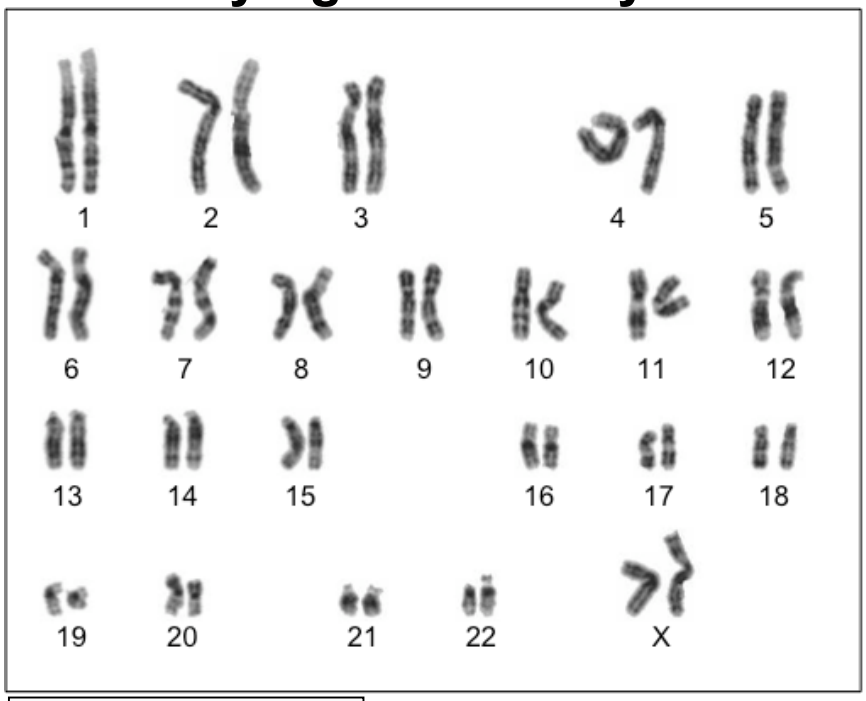


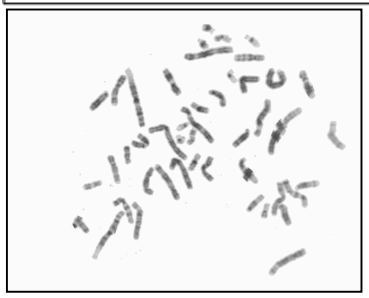
Immunofluorescence of differentiation associated markers TUJ1, TH for ectoderm; a-FETO, FOXA2 for endoderm and SMA, GATA4 for mesoderm in 3PNF_SiPSsv_MM_11 iPS at passage 12.

Annex 4
Karyotype

Ambar
ANÀLISIS MÈDIQUES
BARCELONA

Cytogenetic analysis





Case name: A157473
Patient name: 3PNF_SiPSsv_MM_11 passage 16
Result: 46,XX
Specimen type: stem cells

Annex 5
Authentication

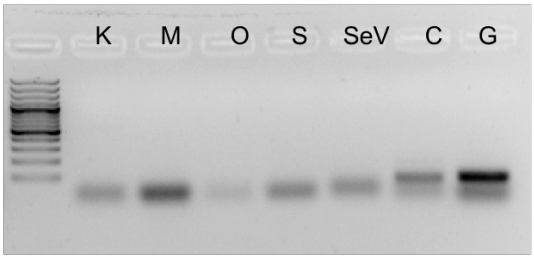
AmpFISTR Identifiler loci	3PNF	3PNF-SC	3PNF_FiPSsv_MM_11
CSF1PO	11,14	11,14	11,14
D2S1338	17,24	17,24	17,24
D3S1358	15,16	15,16	15,16
D5S818	10,11	10,11	10,11
D7S820	10	10	10
D8S1179	10,14	10,14	10,14
D13S317	8,13	8,13	8,13
D16S539	9,12	9,12	9,12
D18S51	13,15	13,15	13,15
D19S433	12,14.2	12,14.2	12,14.2
D21S11	30,32.2	30,32.2	30,32.2
FGA	21,24	21,24	21,24
THO1	8,9.3	8,9.3	8,9.3
TPOX	8	8	8
vWA	16	16	16
Amelogenin (gender)	X	X	X

Microsatellite analysis results. Method used: AmpFISTR Identifiler Plus PCR Amplification Kit (Life Technologies, cat #: 4427368, lot #: 1212014).

Tumor: 3PNF
Parental reprogrammed Schwann cells: 3PNF_SC
iPS genrerated: 3PNF_SiPSsv_MM_11

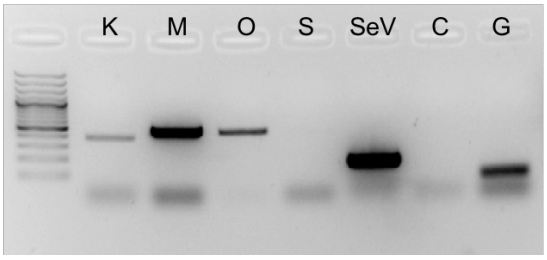
Annex 6
Integration/silencing test

3PNF_FiPSsv_MM_11

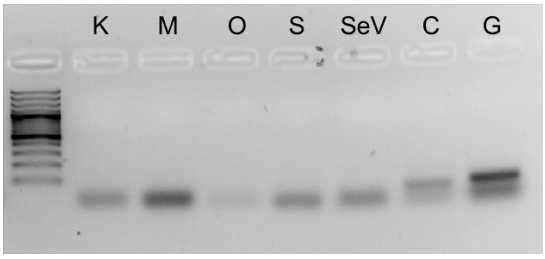


K: KLF4 (Transgene)
M: c-MYC (Transgene)
O: OCT4 (Transgene)
S: SOX2 (Transgene)
SeV: Sendai virus
C: CRIPTO
G: GAPDH

Infected fibroblasts (positive control)



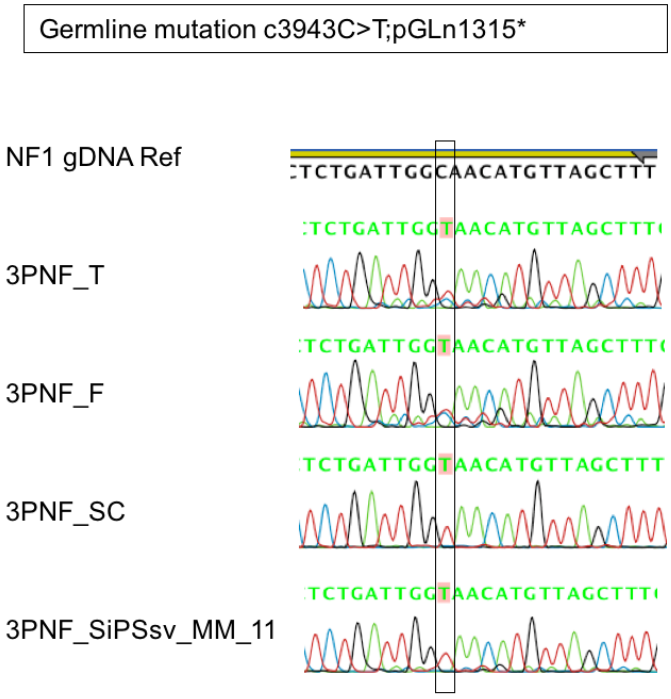
Non-infected iPS (negative control)



RT-PCR analysis showing silencing of the transgenes KLF4, c-MYC, OCT4 and Sox2 and the absence of Sendai virus in the 3PNF_SiPSsv_MM_11 iPS line.

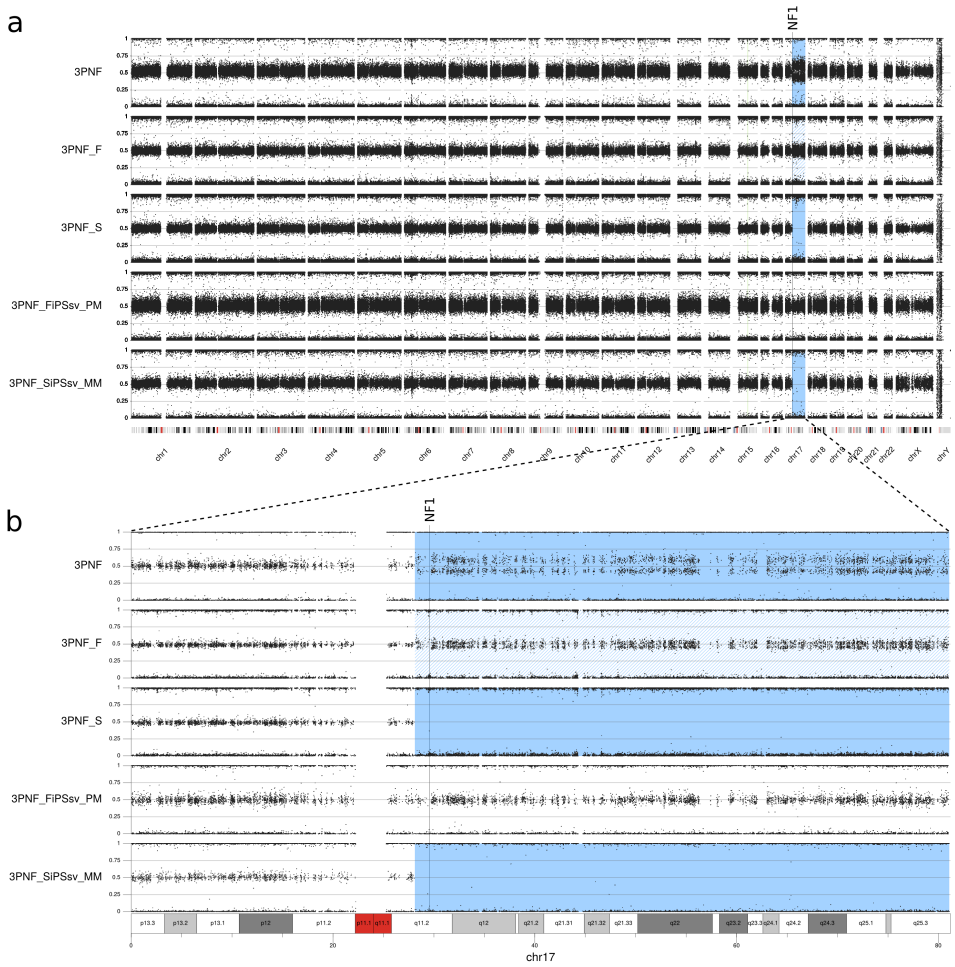
Annex 7
Genotype

Germline mutation c3943C>T;pGLn1315*



Sanger sequencing showing the germline mutation c3943C>T;pGLn1315* in the NF1 gene. The germline mutation was found in the tumor (3PNF_T), and confirmed in primary fibroblasts (3PNF_F), in the reprogrammed Schwann cells (3PNF_SC) and in the iPS line generated 3PNF_SiPSsv_MM_11 at passage 15.

Somatic mutation: LOH due to homologous recombination. Reduction to homozygosity of *NF1* germline mutation.



a) B-allele frequency (BAF) data from SNP-array analysis showing the somatic mutation of the tumor. 3PNF: primary tumor; 3PNF_F: primary fibroblasts from skin; 3PNF_S: primary Schwann cells; 3PNF_FiPSsv_PM: *NF1* (+/-) iPSC generated from skin fibroblasts; 3PNF_SiPSsv_MM: *NF1* (-/-) iPSC generated from primary Schwann cells. The genome of all samples is mostly 2n, denoted by a BAF signal around 0.5. A blue shaded region indicates somatic copy neutral (CN)-LOH on chromosome 17q.

b) A detailed view of BAF for chromosome 17. Somatic *NF1* inactivation is produced by mitotic recombination generating CN-LOH in 17q and the reduction to homozygosity for the constitutional *NF1* mutation. LOH is observed in 3PNF and in 100% of cells in 3PNF-derived primary SCs (3PNF_S) and in the generated *NF1*(-/-) iPSCs (3PNF_SiPSCsv_MM), but not in 3PNF_FiPSsv_PM. Fibroblast primary culture (3PNF_F) is an early passage and still exhibit a residual LOH due to the presence of tumor SCs.