

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR CT_FiPS1_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: CT_FiPS1_Sv4F_1*

***(CTL1 in the publication)**

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by
Immunofluorescence

Annex 4: Karyotype

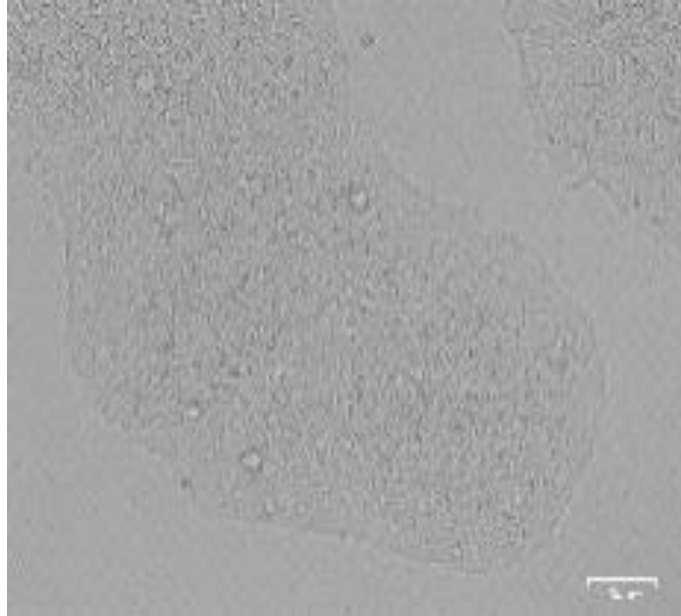
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

Annex 1

Morphology and Alkaline phosphatase staining

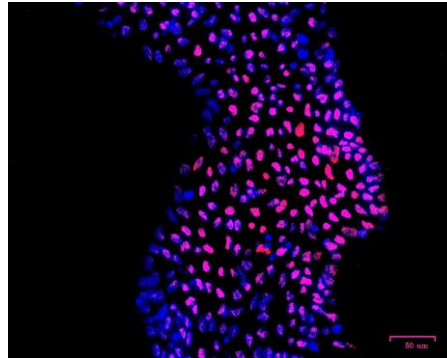


CT_FiPS1_Sv4F_1 Passage 1

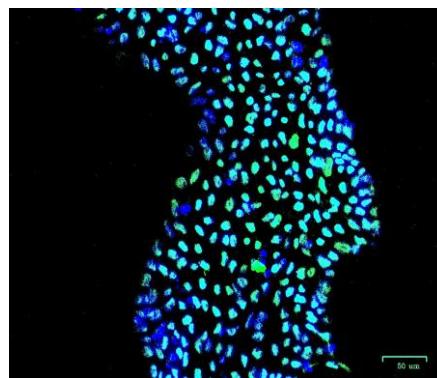
Annex 2

Pluripotency markers

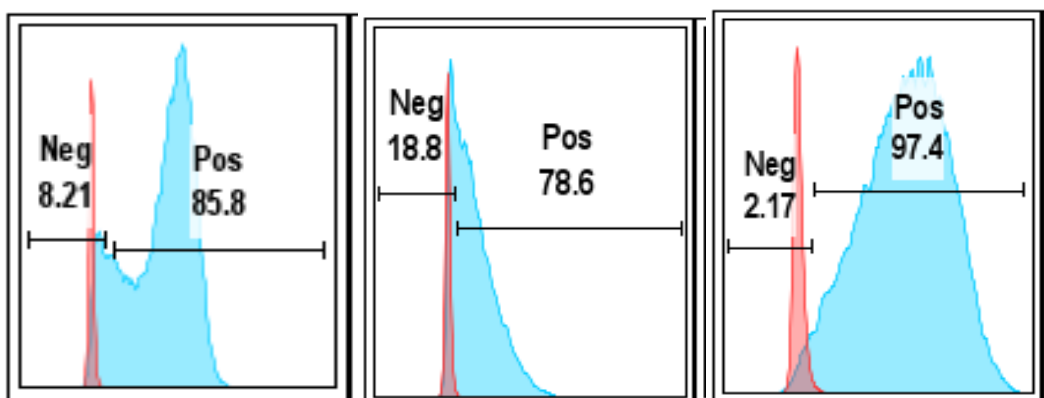
NANOG



OCT4



SSEA-4 / TRA 1-60 / TRA 1-81



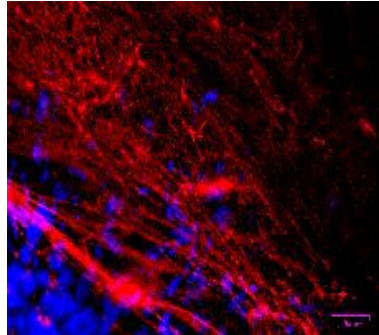
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in CT_FiPS1_Sv4F_1 at passage 10.

Annex 3

In vitro differentiation

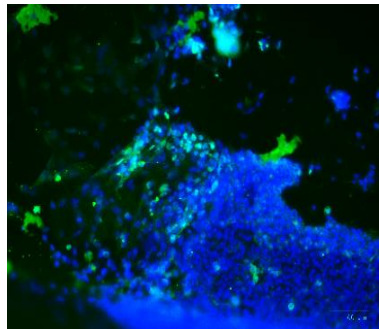
ECTODERM

TUJ1



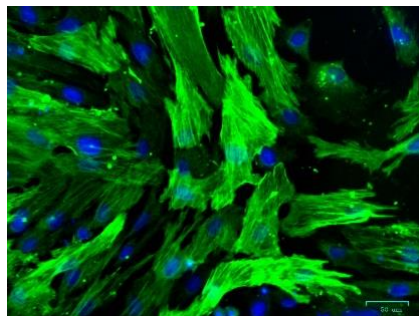
ENDODERM

SOX17



MESODERM

SMA

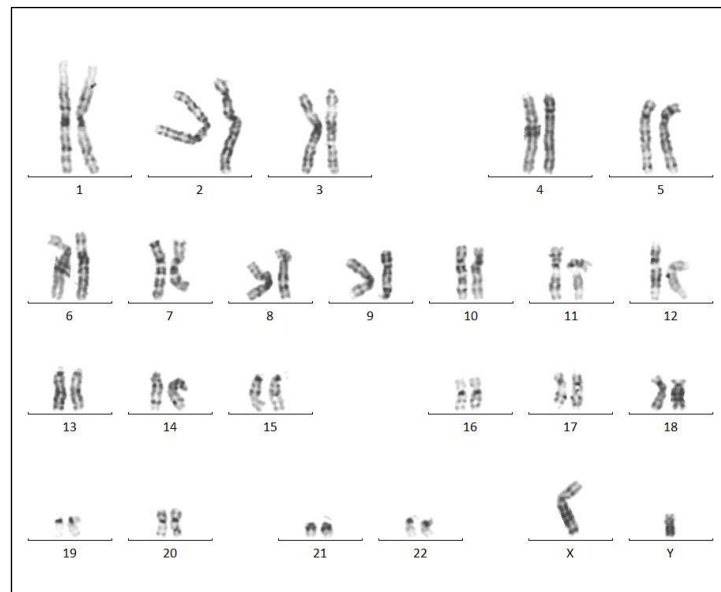


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in CT_FiPS1_Sv4F_1 at passage 12.

Annex 4

Karyotype

Cytogenetic analysis



Patient name: CT_FiPS1_Sv4F_1 passage 10

Result: 46, XY

Specimen type: iPSC

Annex 5

Authentication

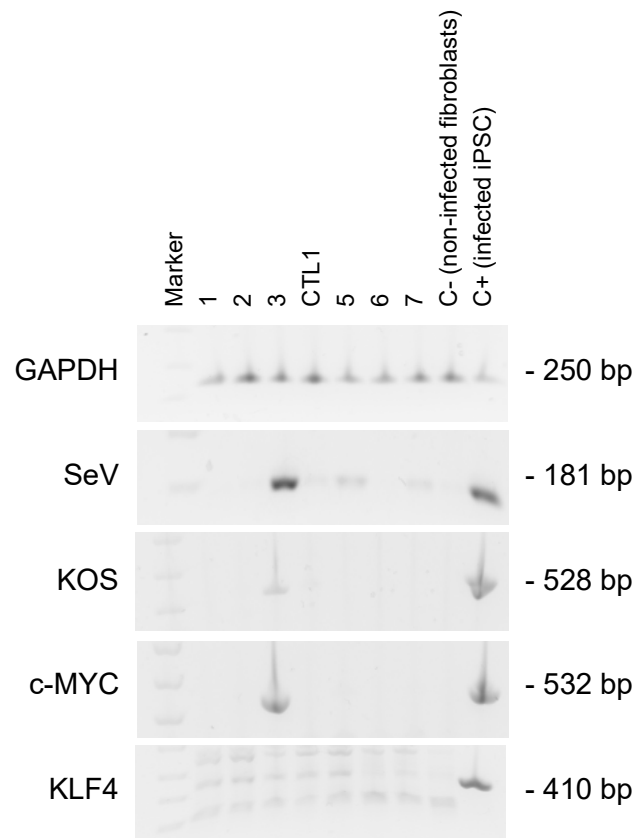
AmpFISTR Identifier Loci	CTL1 fibroblasts	CTL1 iPSC
CSF1PO	10,12	10,12
D2S1338	20,23	20,23
D3S1358	14,17	14,17
D5S818	10,12	10,12
D7S820	10,11	10,11
D8S1179	13	13
D13S317	9,12	9,12
D16S539	10,12	10,12
D18S51	12,13	12,13
D19S433	13,14	13,14
D21S11	29	29
FGA	17,25	17,25
TH01	7	7
TPOX	8,11	8,11
vWA	14,19	14,16
Amelogenin (gender)	X,Y	X,Y

Microsatellite analysis results. Method used: AmpFISTR® Identifier® Plus PCR Amplification Kit (Applied Biosystems, cat #: 4427368).

Parental reprogrammed fibroblasts cells: CTL1 fibroblasts
iPS generated: CTL1 iPSC (CT_FiPS1_Sv4F_1)

Annex 6

Integration / silencing test



RT-PCR analysis showing the absence of Sendai virus and the silencing of the transgenes KOS (KLF4, OCT4 and Sox2), c-MYC and KLF4 in the CT_FiPS1_Sv4F_1 (CTL1) iPS line.

Annex 7

Mycoplasma test



PCR analysis showing the absence of mycoplasma in the CT_FiPS1_Sv4F_1 (CTL1) iPS line.