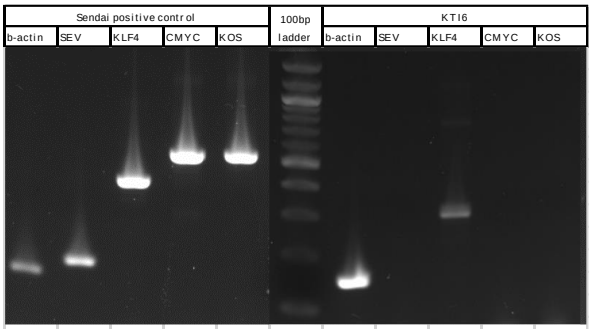
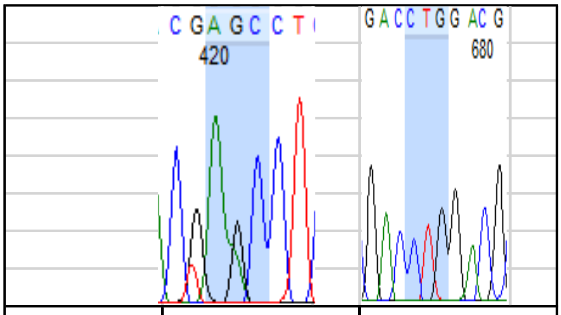
GBA patient PD2 - Cell line ID: 370 (Patient KTI6, age of sampling 55)

This is a Heterozygous N370S IPS line, reprogrammed from Fibroblasts using Sendai virus by University College London (UCL). The line was tested negative for mycoplasma at p8 (by PCR; not shown), and absence of reprogramming factors confirmed by RT-PCR (Fig 1). Genotyping was done by sequencing the RT-PCR product using the primer pair 5'- CTGAGCACAAGTTACAGT TCTGG-3' and 5'- GGAAAGTGAGTCACCCA AACC-3' (Fig 2). Karyotyping was normal 46,XX (Fig 3). The pluripotency of the line was shown by qRT-PCR (Fig 4), and immunostaining (Fig 5). Antibodies used for immunostaining can be found in Table 1. The differentiation potential is supported by EB formation as tested by qRT-PCR (Fig 6).



	b-actin	SEV	KLF4	CMYC	KOS
If positiv	150bp	181bp	410bp	532bp	528bp
If negativ	no band	no band	300bp	no band	no band

Figure 1: Right panel indicates the clearance of Sendai RNA (Sev) as well as ectopic expression reprogramming factors (KLF4, CMYC, KOS) of the IPS line; Left panel shows a sample containing the virus, used as a positive control. B-actin was used as loading control.



Mutation	N370S locus	L444P locus
+/+	AAC	CTG
Δ/Δ	AGC	CCG

Figure 2: Genotyping was done by Sanger sequencing for two common GBA mutations, showing this line carries N370S mutation but not L444P, as indicated by the respective chromatogram.

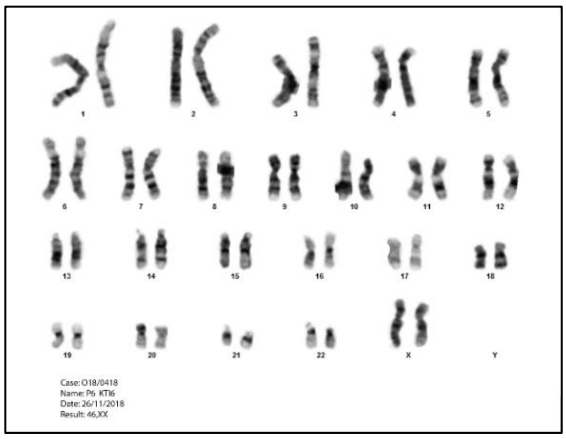


Figure 3: Chromosome analysis of the fixed cell suspension from this stem cell line (p6) showing an apparently normal female karyotype in 20 cells examined, with no abnormalities detected. The preparations obtained from this sample were of sufficient quality

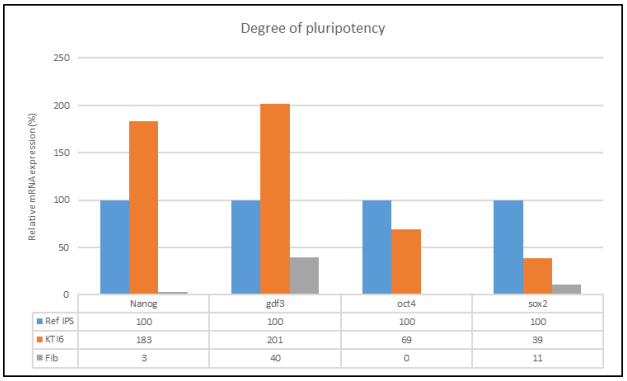


Figure 4: The abundance of pluripotency markers (Nanog; GDF3; OCT4; SOX2) as measured by qRT-PCR. The results are relative to a reference commercial IPS line, and compared to a fibroblast line, as a negative control

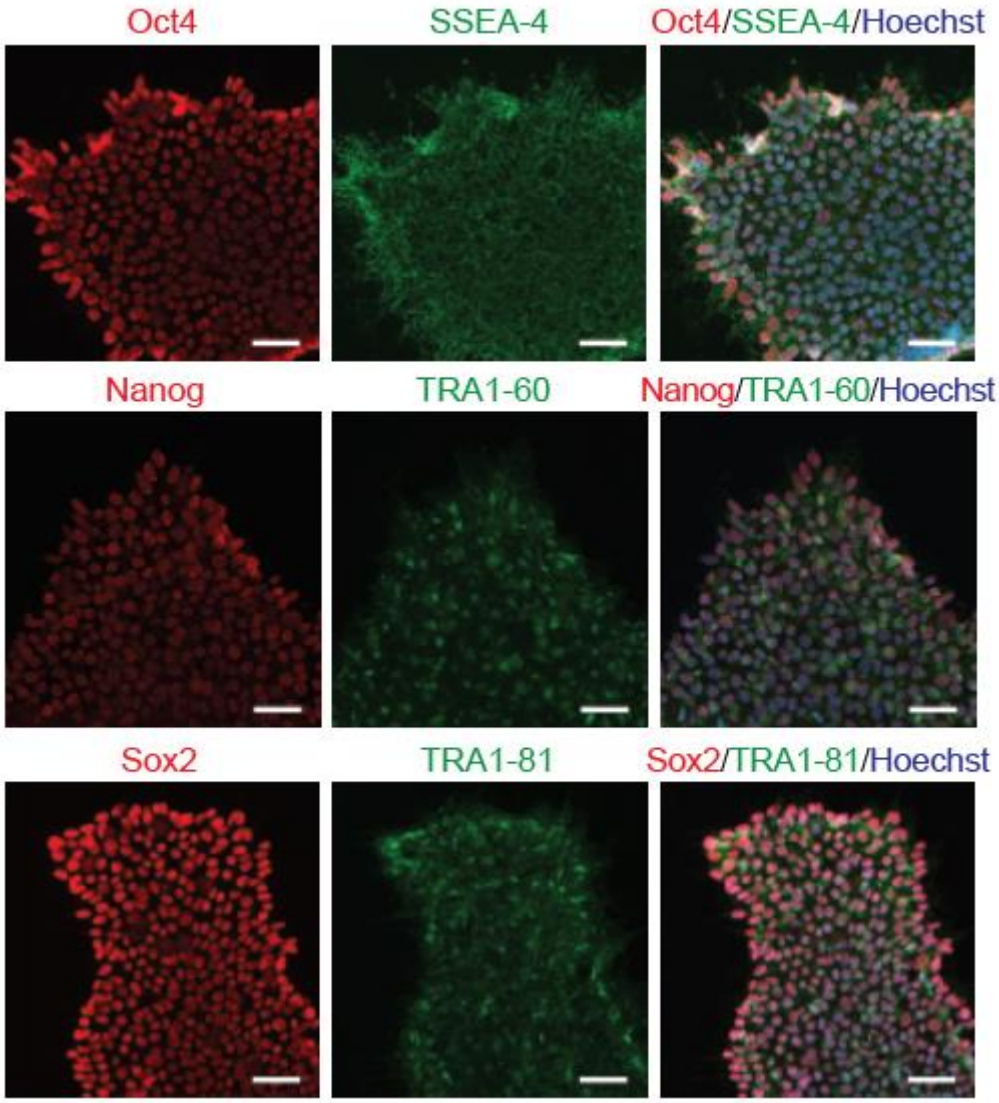


Figure 3: Immunofluorescence staining showed high expression of six pluripotency markers: Oct4 (Red), SSEA-4 (Green), Nanog (Red), TRA-1-60 (Green), Sox2 (Red) and TRA1-81 (Green). Nuclei were counterstained with Hoechst (blue), (scalebar 50 µm).

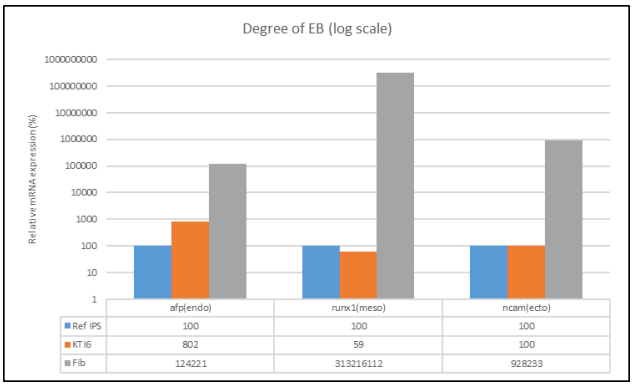
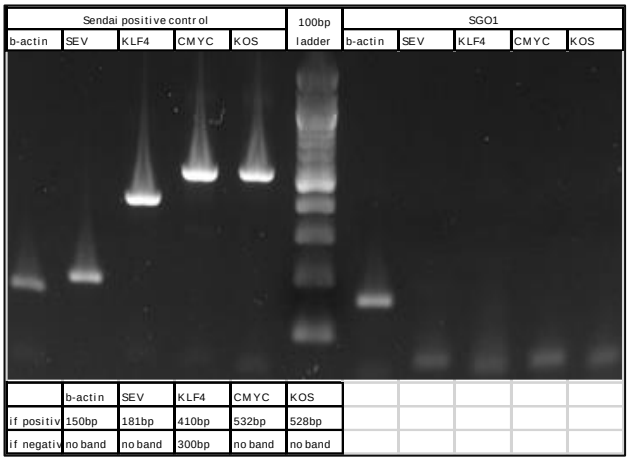


Figure 4: The abundance of Embryoid Body (EB) markers in three germ layers was done by qR-PCR. The results are relative to a reference commercial IPS line, and compared to a fibroblast line, as a negative control.

GBA patient - Cell line ID: 371 (Patient SGO1, age of sampling 75)

This is a Heterozygous N370S IPS line, reprogrammed from Fibroblasts using Sendai virus by University College London (UCL). The line was tested negative for mycoplasma at p18 and p27 (by PCR; not shown), and absence of reprogramming factors by RT-PCR (Fig 1). Karyotyping was normal 46,XX (Fig 2). Genotyping was done by sequencing the RT-PCR product using the primer pair 5'- CTGAGCACAAGTTACAGTTCTGG-3' and 5'- GGAAAGTGAGTCACCCAAACC-3' (Fig 3). Genotyping of the genomic PCR product of the LRRK2 gene using the primer pair 5'-GCATCAGCCATGATGGATA-3' and 5'-GAGTTGCAATCAGCAAGATGA-3' showed presence of a heterozygous LRRK2-G2019S mutation (Fig 4). The pluripotency of the line was shown by qRT-PCR (Fig 5) and immunostaining (Fig 6). Antibodies used for immunostaining can be found in Table 1. The differentiation potential is supported by EB formation as tested by qRT-PCR (Fig 7).



	b-actin	SEV	KLF4	CMYC	KOS
If positiv	150bp	181bp	410bp	532bp	528bp
If negativ	no band	no band	300bp	no band	no band

Figure 1:. Right panel indicates the clearance of Sendai RNA (Sev) as well as ectopic expression reprogramming factors (KLF4, CMYC, KOS) of the IPS line, left panel shows a sample containing the virus, used as a positive control. B-actin was used as loading control

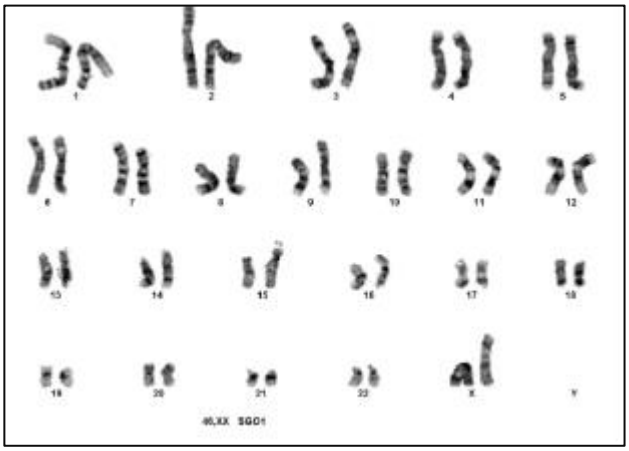
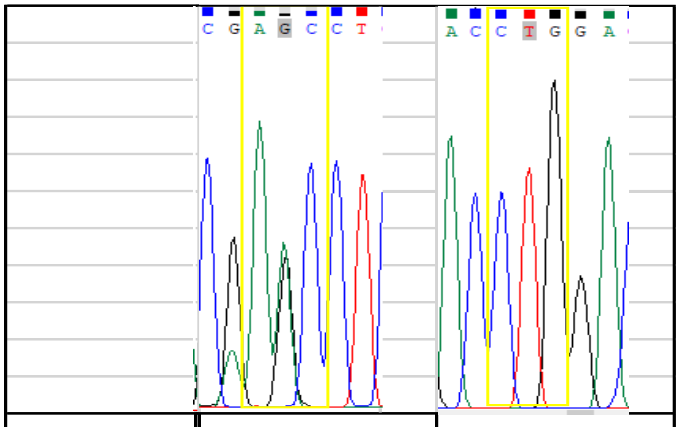
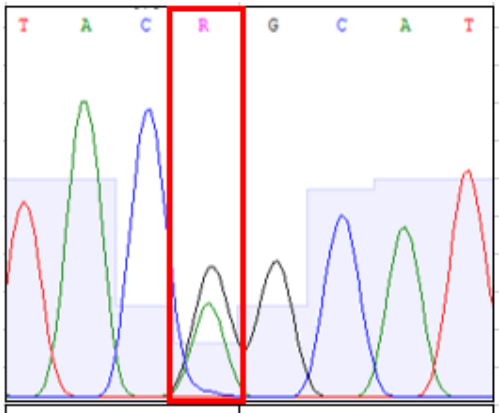


Figure 2: Chromosome analysis of the fixed cell suspension from this stem cell line (P18) showing a female karyotype. No abnormality detected. (20 cells examined, 95% chance of detecting a 14% population).



Mutation	N370S locus	L444P locus
+/+	AAC	CTG
Δ/Δ	AGC	CCG

Figure 3: Genotyping was done by Sanger sequencing for two common GBA mutations, showing this line carries N370S mutation but not L444P, as indicated by the respective chromatogram.



Mutation	LRRK2-G2019S
+/+	ACGGCA
Δ/Δ	ACAGCA

Figure 4: Genotyping was done by Sanger sequencing for the LRRK2 G2019S as indicated by the respective chromatogram

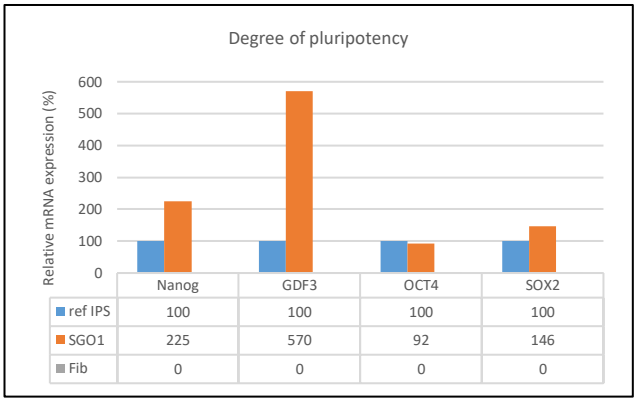


Figure 5: 1The abundance of pluripotency markers (Nanog; GDF3; OCT4; SOX2) as measured by qRT-PCR. The results are relative to a reference commercial IPS line, and compared to a fibroblast line, as a negative control

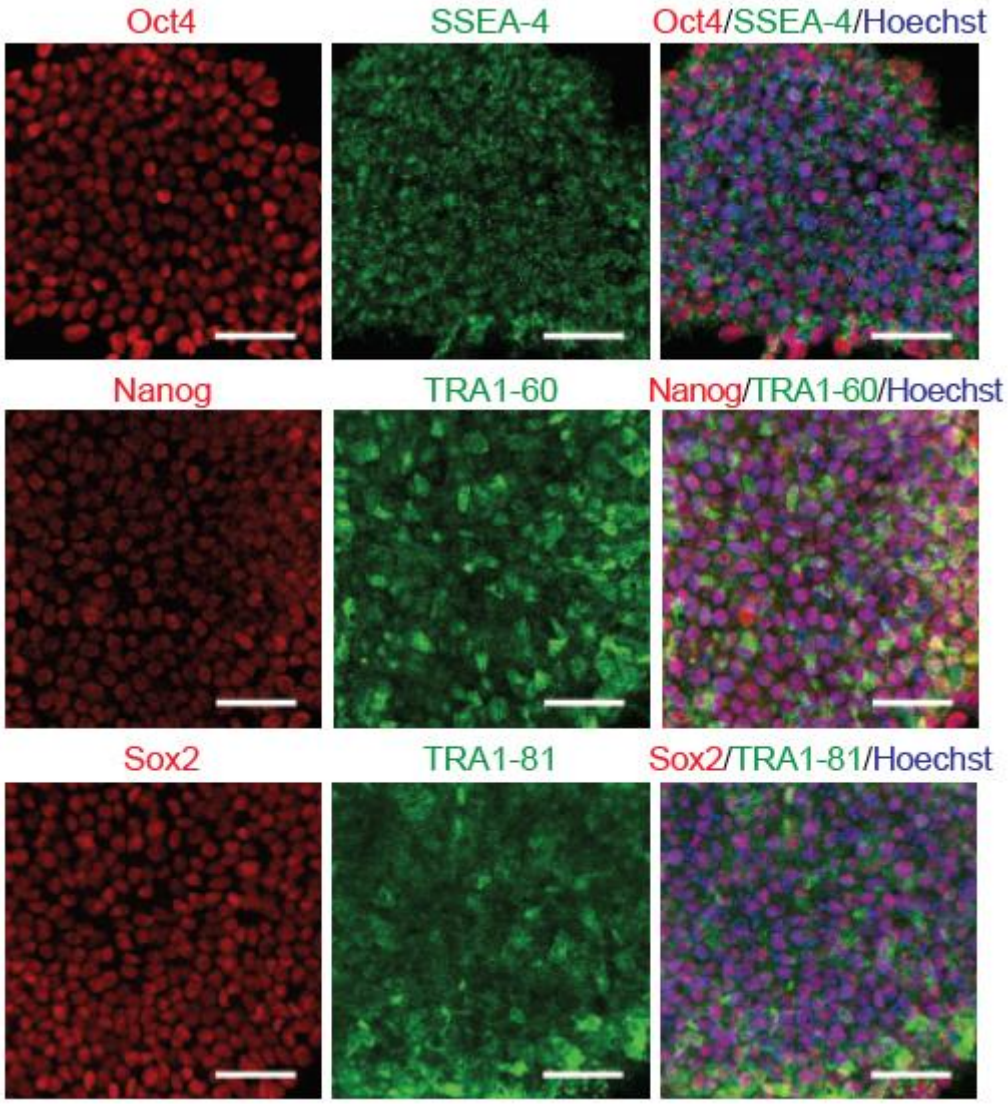


Figure 6: Immunofluorescence staining showed high expression of six pluripotency markers: Oct4 (Red), SSEA-4 (Green), Nanog (Red), TRA-1-60 (Green), Sox2 (Red) and TRA1-81 (Green). Nuclei were counterstained with Hoechst (blue), (scalebar 50 µm).

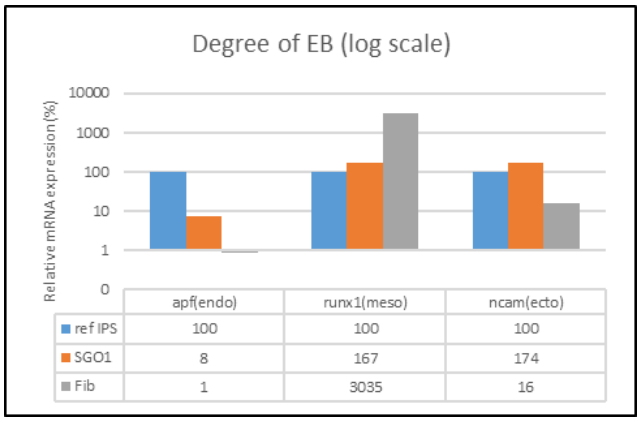


Figure 7: The abundance of Embryoid Body (EB) markers in three germ layers (AFP; RUNX1; NCAM) was done by QRT-PCR, and shown below. The results are relative to a reference commercial IPS line, and compared to a fibroblast line, as a negative control.

Table 1. Antibodies used for Immunocytochemistry of iPSCs

Antibody	**Dilution**	**Source**	**Ref.-No.**
SOX2	1:200	R&D systems	AF2018
OCT4	1:400	Abcam	ab19857
NANOG	1:100	Millipore	AB5731
SEEA4	1:25	Millipore	MAB4304
TRA-1-60	1:25	Millipore	MAB4360
TRA-1-81	1:25	Millipore	MAB4360