



Certificate of analysis

SFC840-03-03 LRRK2-/- C11

(V2: corrected typos M.W. and KO sequence)

Operator: Sally Cowley Date: 22/04/2015

Supervisor: Sally Cowley Date: 22/04/2015

Signature:

SACowley

Source of parental iPSc line and reprogramming information

- SF840 from UOXF 04/04/2011
- Reprogrammed at UOXF S [JMSCF]
- Reprogrammed on 13/12/2012 at passage 5
- Cytotune v1 WP3 SOP10
- SFC840-03-03 StemBANCC QC passed
22/04/2015

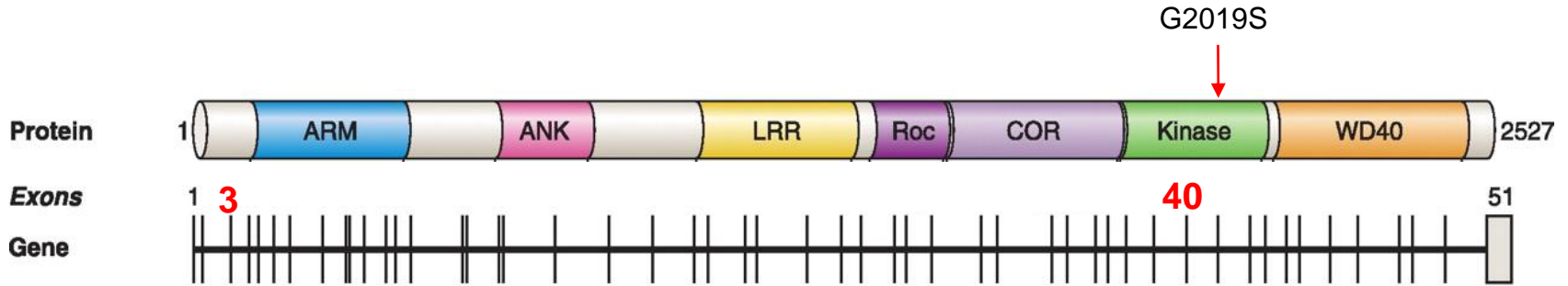
Gene editing information

- Strategy: CRISPR/Cas9-mediated disruption of exon 3 and clone-screening for bi-allelic out-of-frame repair, to create Lrrk2 knockout line
- Neon transfection of SFC840-03-03 p20 20.06.2014 SC UOXF S [JMSCF]
- CRISPR/Cas9 px462 (nickase, Zhang lab) plasmids encoding gRNA20 and gRNA21

RF128 CACCGATTAAGCTTTGCATTGTACC
RF129 AAACGGTACAATGCAAAGCTTAATC
RF130 CACCGCCCAGGATGTTGGAAATGAT
RF131 AAACATCATTTC AACATCCTGGGC

gRNA20 oligo 1
gRNA20 oligo 2
gRNA21 oligo 1
gRNA21 oligo 2

Sequence confirmation of gene editing: out of frame deletion on both alleles



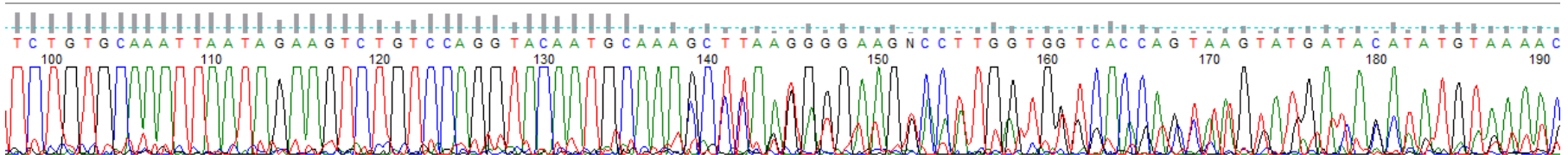
gRNA21
 CCAGGTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAAATGATTGG
 GGTCCATGTTACGTTTCGAATTACCCTGGGGTCCTACAACCTTTACATACC
 gRNA20

WT TCTGT**CCA**GGTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAAATGAT**TGG**GAAGT

Clone C11

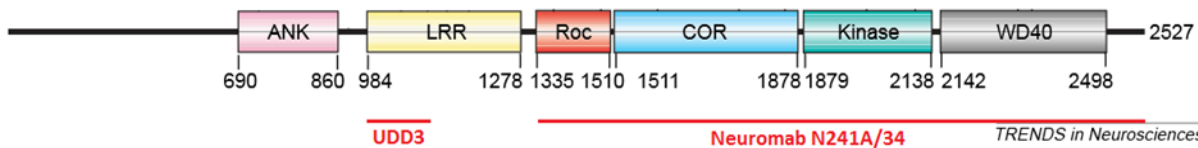
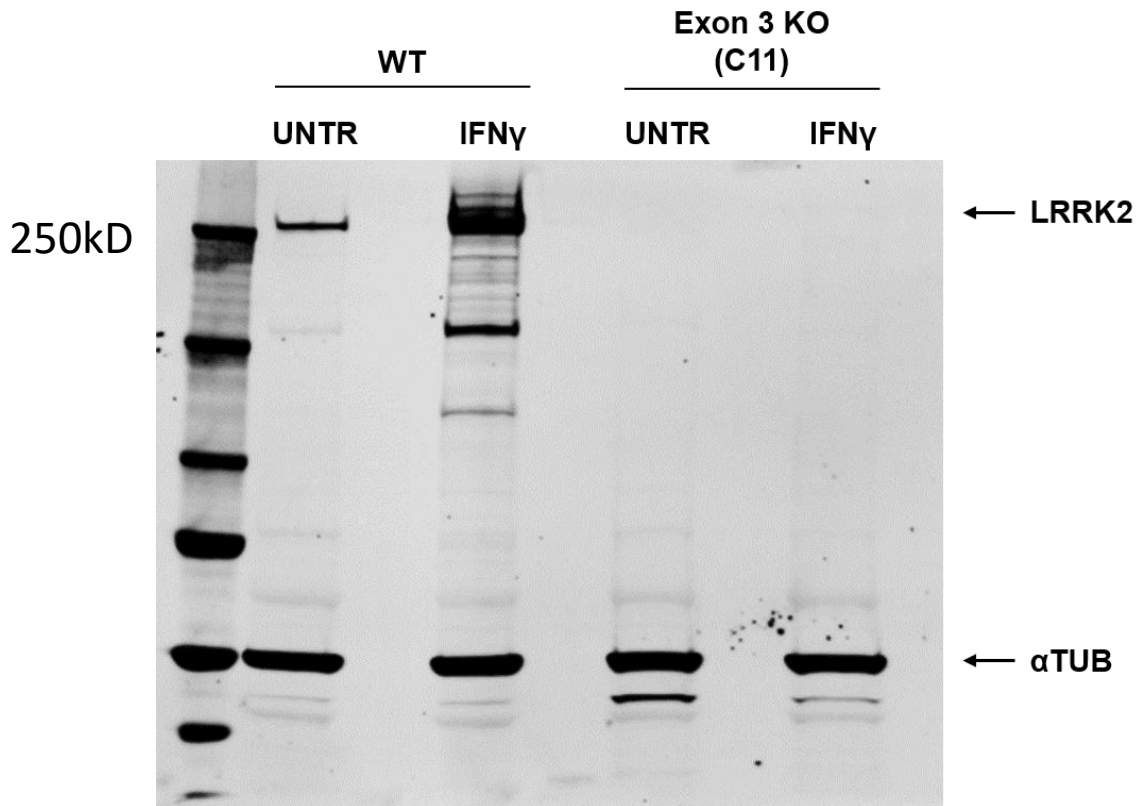
Δ11bp TCTGT**CCA**GGTACAATGCAA-----CCCAGGATGTTGGAAATGAT**TGG**GAAGT

Δ26bp TCTGT**CCA**GGTACAATGCAAAGCTTAATGGGA-----AGT



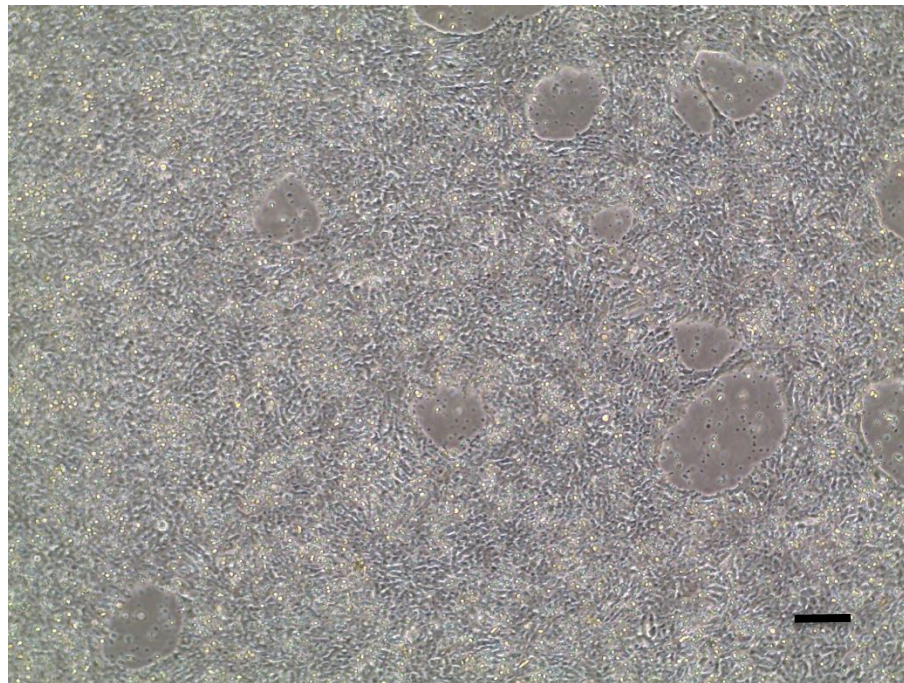
Protein expression confirmation of gene editing:

Differentiation to macrophages for Lrrk2 expression confirms KO by Western Blot [neuromab antibody] vs wt cells + maximal activation of expression by IFN γ [Lrrk2 286kD]



Viability post-thaw and Morphology according to SOP19 passage 29

- Cell count immediately post-thaw 2.75×10^6
- Viability immediately post-thaw 85.7%
- Photo at 24h post-thaw (scale bar = $100\mu\text{m}$):



Mycoplasma test:

According to MycoAlert Lonza LT07-318

Undetectable at passage 29

Sample	Clone	Passage number	Initial	Reading 1	Reading 2	Ratio/Status
+ve control				4.589	75.28	16.40
-ve control				3.879	0.165	0.04
4	SFC840-03-03 Lrrk2 -/- C11	p29	SC	2.194	0.96	0.44

Results mean

Ratio **0 - 0.999** negative for mycoplasma

Ratio **1 – 1.3** Borderline Result (retest required)

Ratio above **1.3** positive for mycoplasma

SNP analysis

according to WP3 SOP Preparation of DNA and RNA samples for Illumina arrays

- Passage 29
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: 2 very small regions of change noted vs SFC840-03-03 (not on Chr12)
- For details and raw data see StemDB