PNAS
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Supplementary Information for

Human iPSC-derived trigeminal neurons lack constitutive TLR3-dependent intrinsic immunity that protects cortical neurons from HSV-1 infection

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1809853115

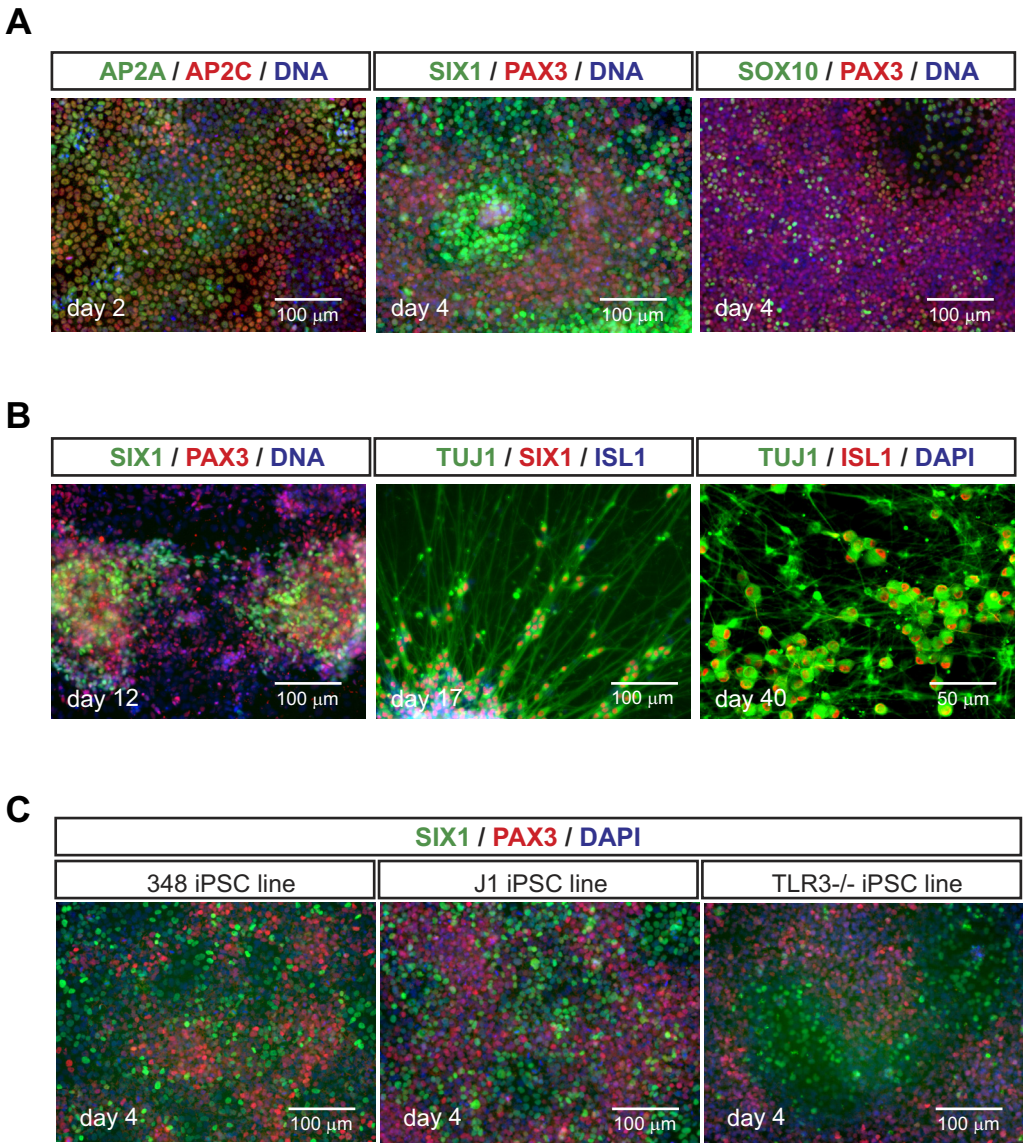

Fig. S1. Immunocytochemical characterization of TG placodes. **(A)** Early differentiation shows induction of AP2A, AP2C (day 2; left panel), SIX1, PAX3 (day 4; middle panel), and SOX10, PAX3 (day 4; right panel). **(B)** Neurogenic step at day 12 of differentiation shows maintenance of SIX1, PAX3 (day 12; left panel), neuronal differentiation by TUJ1, SIX1 (day 17; middle panel) and sensory neuron marker expression by ISL1 (day 40; right panel). **(C)** Validation of placode induction by SIX1, PAX3 expression at day 4 in three additional, independent human iPSC lines (348 iPSC line (1); left panel; J1 iPSC line (2); middle panel; TLR3-/- iPSC line; right panel).

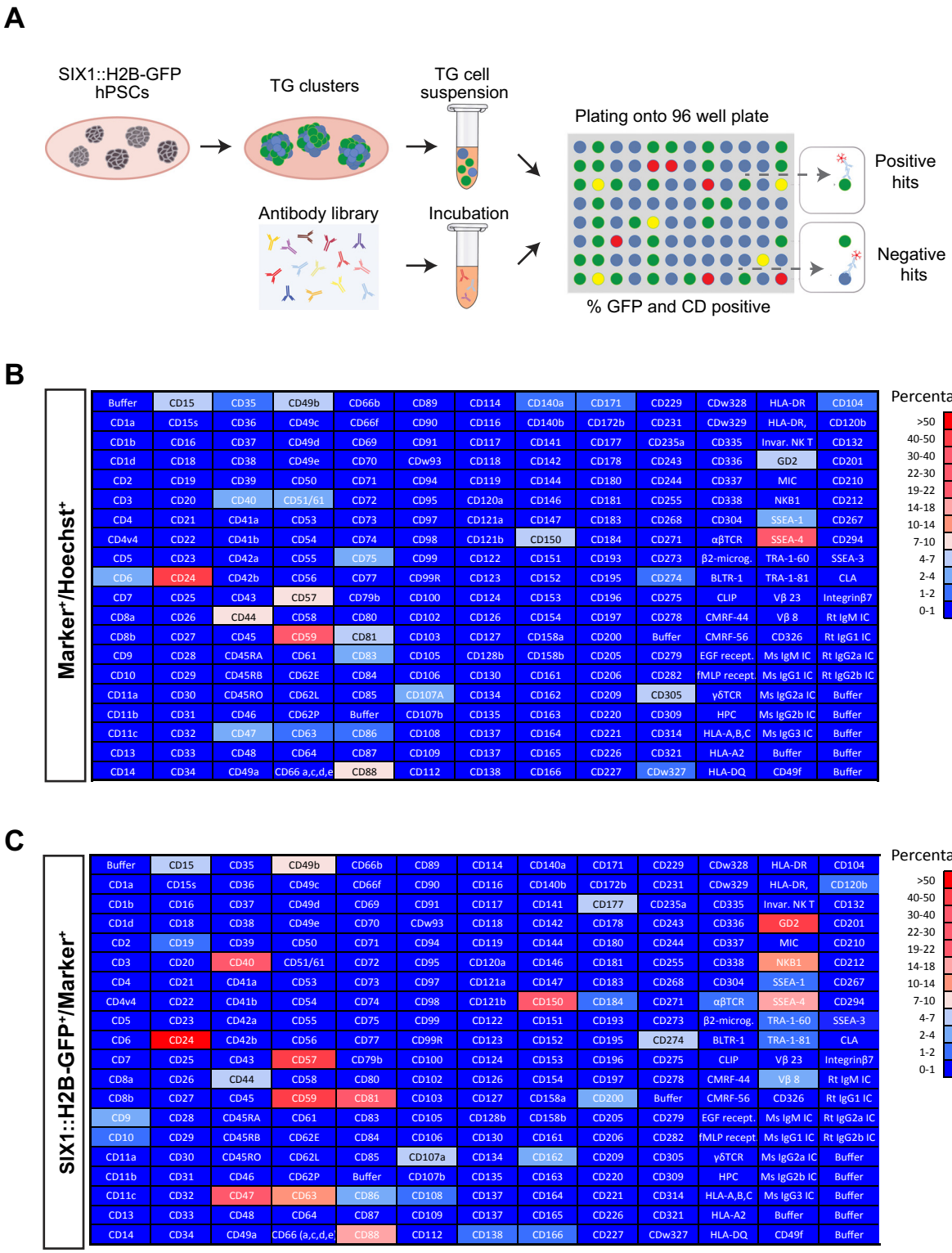

Fig. S2. Cell surface marker screen on day 12 trigeminal neuron clusters. **(A)** Schematic illustration of the experimental setup and readout. **(B)** Heat map of surface marker positive cells. Data is presented as percent of total cells (DAPI+). **(C)** Heat map of SIX1::H2B-GFP positive cells over surface marker positive cells. Data is presented as percent of marker positive cells.

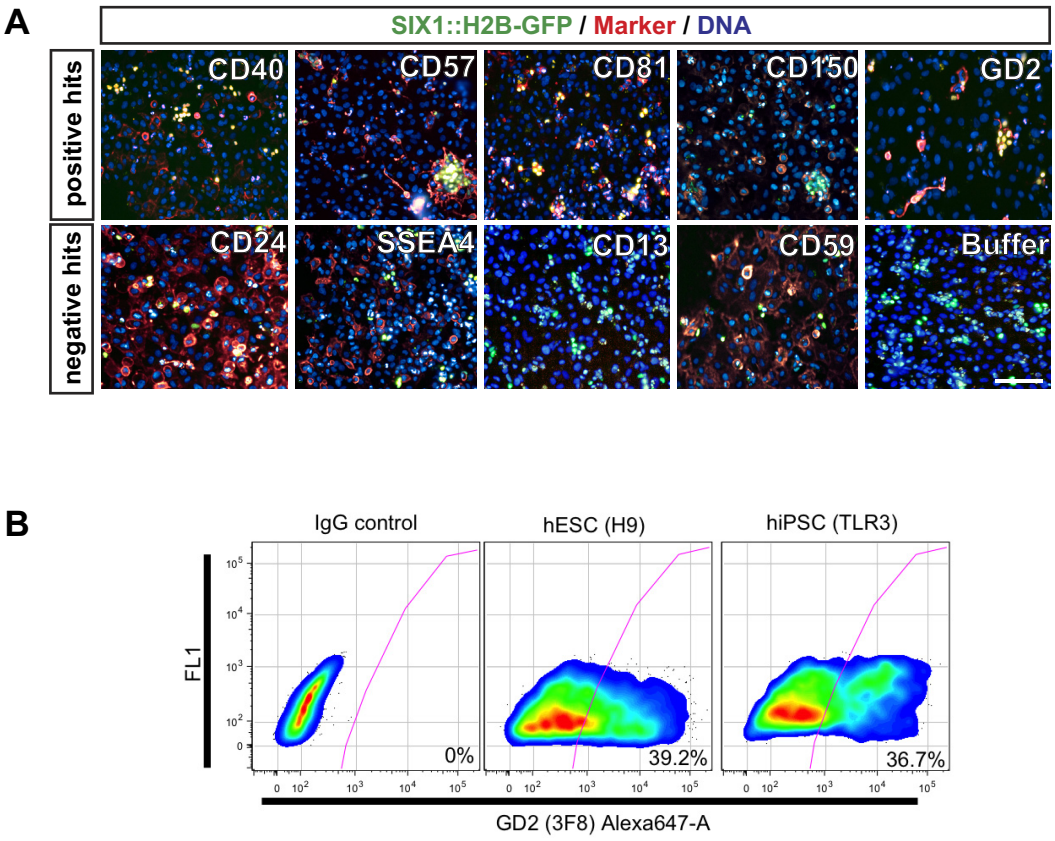

Fig. S3. Cell surface marker screen on day 12 trigeminal neuron clusters. **(A)** Representative images of 5 "positive" and 5 "negative" hits. Scale bar: 50 µm. **(B)** Quantification of GD2 surface expression in human ESC (H9)- and iPSC (TLR3)-derived trigeminal neurons after 12 days of differentiation by flow cytometry.

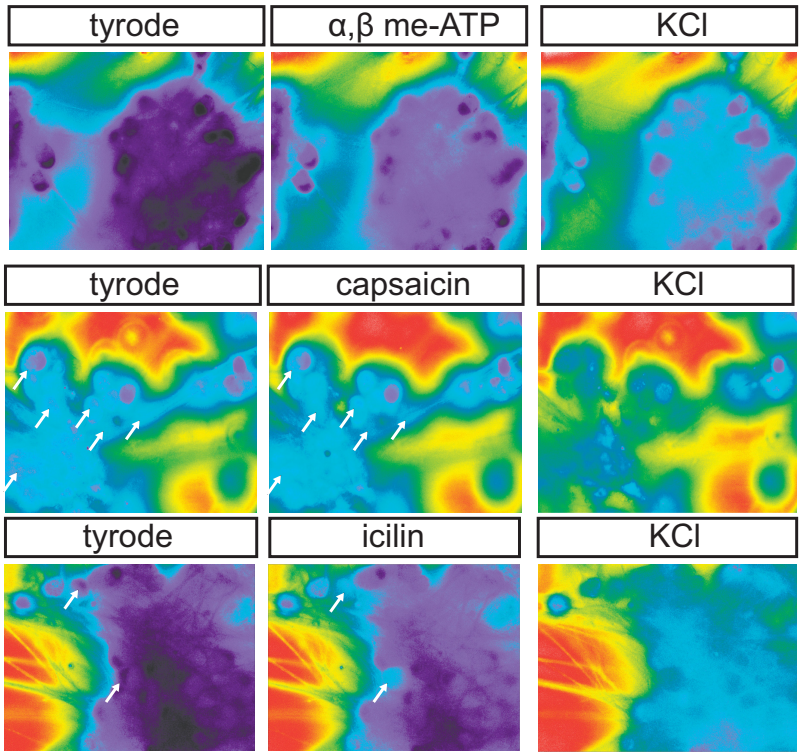

Fig. S4. Calcium response to tyrode, capsaicin, icilin, ATP, and KCl. Representative ratiometric image of a day 60, GD2 sorted TG neuron culture after incubation with the calcium indicator Fura-2. Images correspond to the frame of the time course analysis of the peak response to the respective molecule. Arrows indicate responding cells.

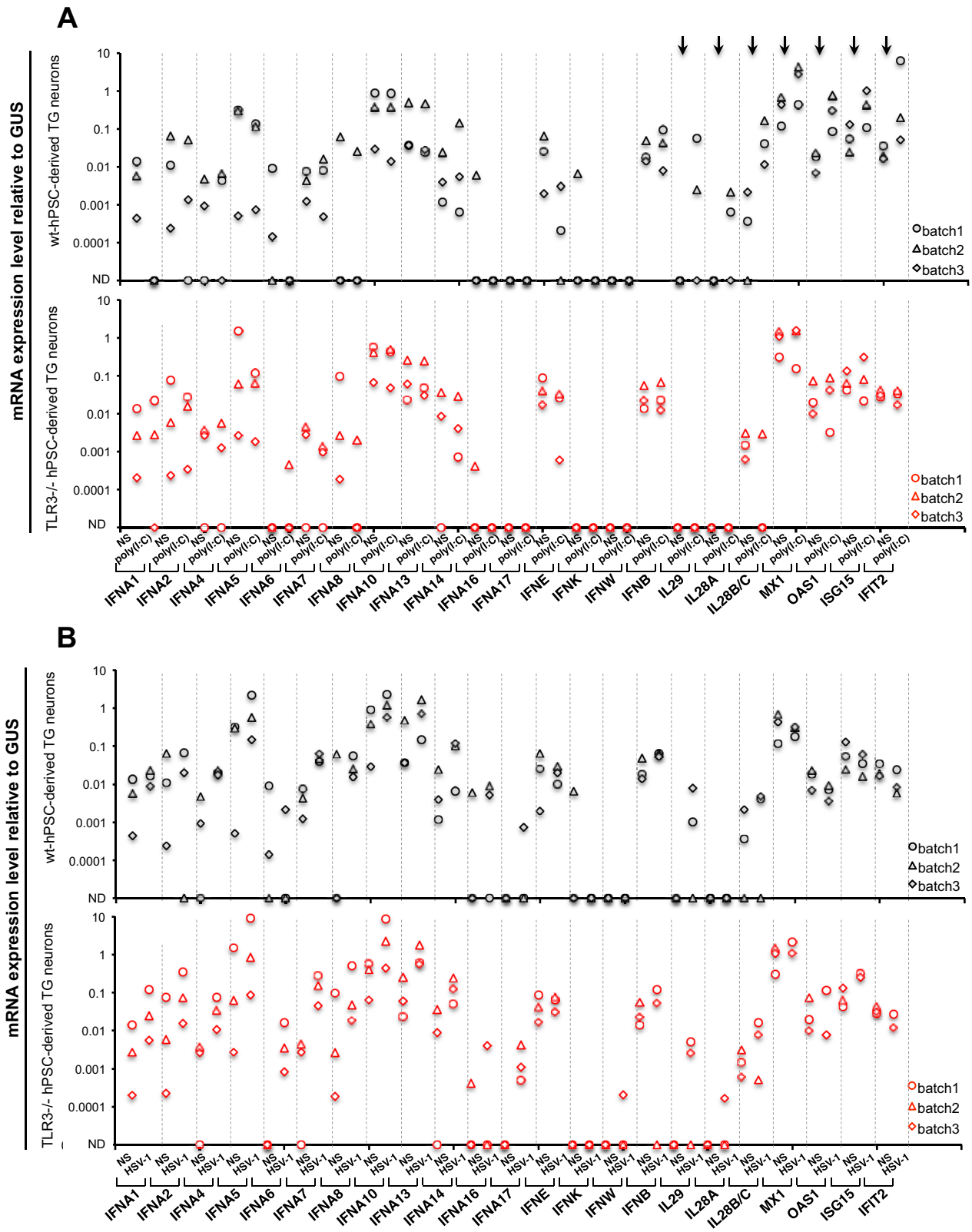

Fig. S5. Gene expression for 19 different IFNs and 4 different ISGs upon poly(I:C) or HSV-1 stimulation. The relative expression levels of IFNs and ISGs in TG neurons derived from TLR3 wild-type control human ESCs versus TLR3-/- iPSCs was assessed under basal conditions and compared to **(A)** stimulation with poly(I:C) or **(B)** following exposure to HSV-1. IFNs that showed a clear increase in gene expression following poly(I:C) treatment in control but not in TLR3-/- TGs are indicated with a black arrow. ND: not detected.

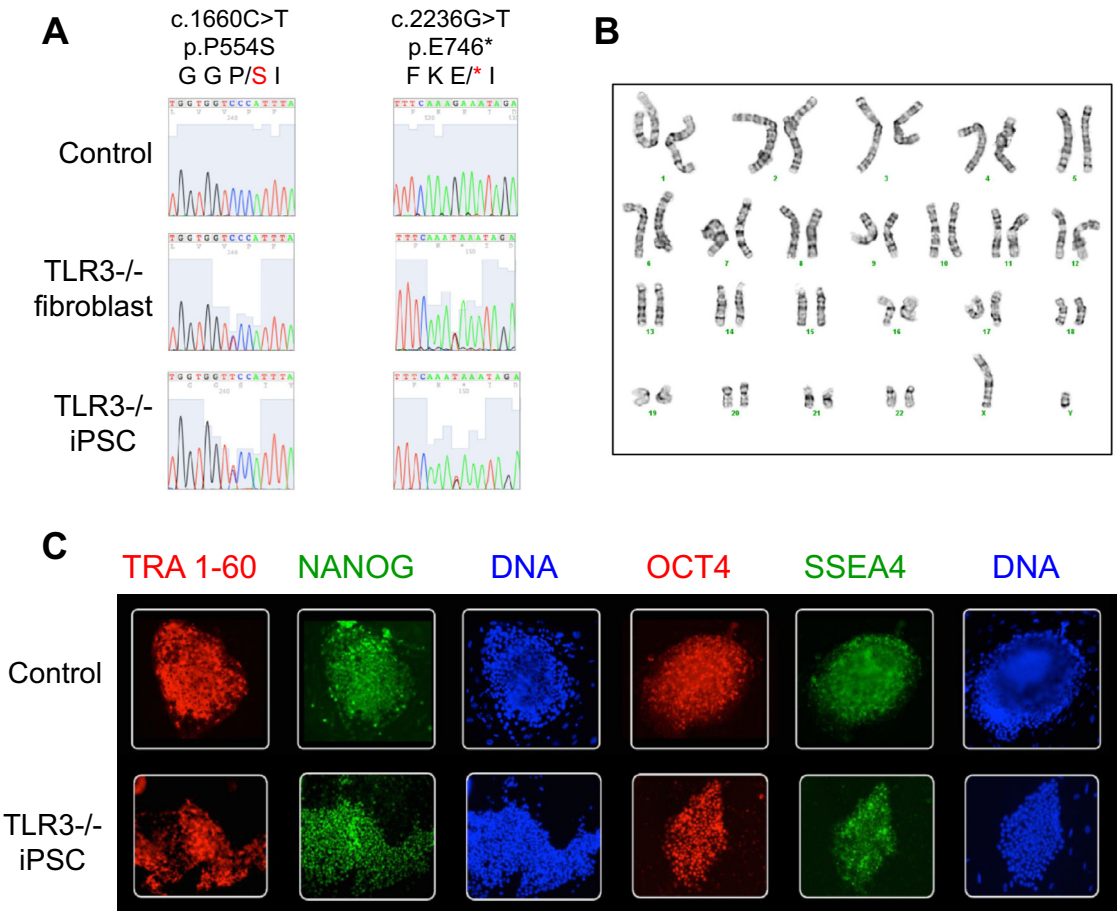

Fig. S6. Characterization of Sendai-vector induced iPSCs from control and TLR3-deficient patient fibroblasts. (A) Sequence analysis of the TLR3 mutation in fibroblasts and iPSCs. **(B)** Normal karyotype of TLR3-/- iPSCs by G-banding. **(C)** Expression of pluripotent markers TRA-1-60, NANOG, POU5F1 (OCT4) and SSEA-4 in TLR3-/- patient-specific iPSCs.

Table S1. List of antibodies used in this study

Name	Company	catalogue #	dilution
Brn-3A	Millipore	MAB1585	1:200
GD2 (3F8)	gift from Nai-Kong Cheung (MSKCC)	not applicable	1:1000
GD2 (14G2a)	BD Pharmigen	554272	1:500
ISL1	Developmental Studies Hybridoma Bank	39.4D5	1:100
NANOG	Cell Signaling	4903	1:500
PAX3	DSHB	Pax3	1:100
Peripherin	Santa Cruz	SC-7604	1:200
POU5F1 (OCT4)	Cell Signaling	2840	1:500
SIX1	Sigma	HPA001893	1:1000
SOX2	Cell Signaling	3579	1:500
SOX10	Cell Signaling	89356S	1:100
SSEA3	Santa Cruz	SC-21703	1:100
SSEA4	Millipore	MAB4304	1:200
AP2A (TFAP2A)	Abcam	Ab108311	1:100
AP2C (TFAP2C)	Sigma	HPA055179	1:250
TRA1-81	Millipore	MAB4381	1:200
TUJ1	Covance	MMS-435P	1:1000

References

1. Miller JD, *et al.* (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* 13(6):691-705.
2. Kriks S, *et al.* (2011) Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* 480(7378):547-551.