Supplementary Materials

Supplementary Materials and Methods

Generation of 3D Skin Equivalent

3D skin equivalents were generated on polycarbonate culture inserts (Nunc) according to the previously described protocol [1]. Briefly, neutralized type I collagen (Sigma) containing human dermal fibroblast (Thermo Fisher Scientific, Cat# C0135C) were poured on the insert and incubated at 37 ^{0}C for 2 hrs to polymerize. The dermal equivalent was incubated with the fibroblast medium for 5 days. Then, 1x10^{6} KCs derived from Ctr-iPSCs and PsO-iPSCs at day 20 of differentiation were seeded on the dermal equivalent. The cells were grown in 1:1 of N2 medium and DMEM containing 10% FBS, 1% NEAA, 1% L-Glutamine for 10 days in the submerged condition, following which the cells were exposed to the air and fed from below for 5 days in medium containing 1.3mM $CaCl_2$.

Supplementary Results and Discussion

The PSORS1 locus maps to the Major Histocompatibility Complex (MHC) on chromosome 6p21 and is conclusively validated the major genetic determinant of psoriasis [2]. These investigations defined a consensus 150 kb minimal interval spanning the MHC class I region and encompassing nine genes [2,3]. We found HLA-C localized in this region was overexpressed in our psoriasis iPSCs derived keratinocytes. HLA-C encodes a MHC class I receptor that participates to immune responses through the presentation of antigens to CD8+ T lymphocytes. It is therefore a very plausible candidate gene, especially as serological studies carried out as early as the mid-1970s had identified an association between psoriasis and the HLA-C [4]. Given its very prominent position in the genetic landscape of psoriasis, the MHC

region has been the subject of very detailed genetic studies, carried out in European and Asian populations. These have identified many additional association signals that are independent of HLA-C and map to HLA-A, HLA-B and HLA-DQA1 [5,6]. In our RNA-seq results, HLA-DQA1 and DQB1 were down-regulated in both psoriasis iPSCs derived keratinocytes, while HLA-A was up-regulated in 1PSO and down-regulated in 2PSO.

It is generally accepted that T helper cells including Th1, Th17 and Th22 cells contribute to the development of psoriasis by secreting various cytokines [7,8], which results in the excessive proliferation and aberrant differentiation of keratinocytes. However, recent studies show that keratinocyte produces various kinds of cytokines leading to an amplified immune response [8-10] and thus directly or indirectly causes the major histological features of psoriasis. In our RNA-seq results, we found a number of cytokines deregulated in psoriasis iPSCs derived keratinocytes which consequently result in altered expression of downstream genes. In the RNA-seq data analysis, we found members of interferon-inducible protein with tetratricopeptide repeats (IFITs) family, member of interferon induced transmembrane (IFITM) protein family and members of PYHIN (IFI200/HIN-200) family were upregulated in KCs derived from PsO-iPSCs (Table S4).

Supplementary Figures and Tables

Figure S1

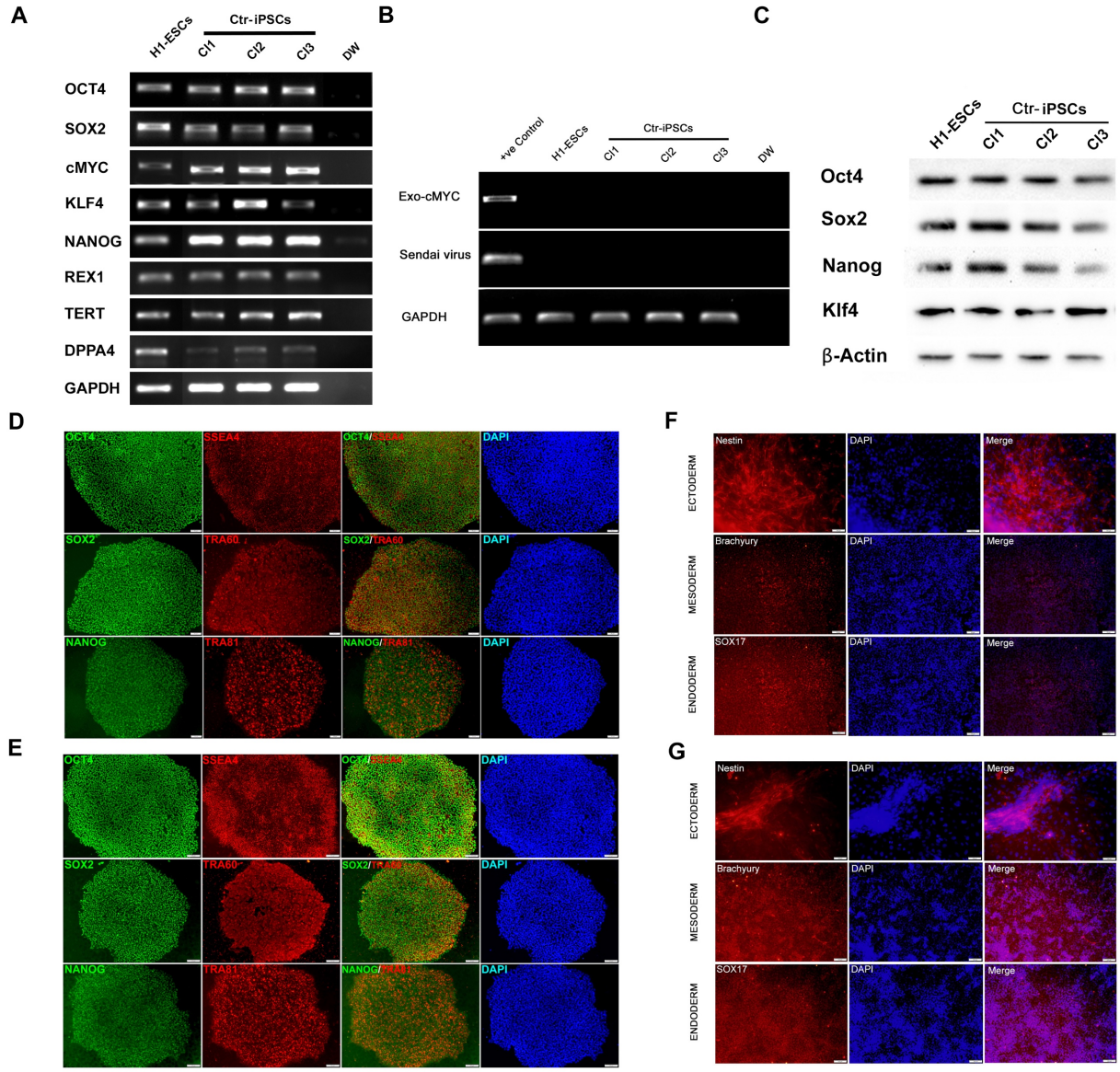

Figure S1: Characterization of the generated iPSC clones from a healthy individual (Ctr1-iPSCs). (A) The three iPSC clones expressed the endogenous pluripotency markers (OCT4, SOX2, CMYC, KLF4, NANOG, REX1, TERT, and DPPA4) by RT-PCR. (B) RT-PCR showing the loss of the exogenous pluripotency factor, c-MYC and the backbone of the Sendai virus used for the transduction. (C) Western blotting showing expression of the self-renewal markers (OCT4, SOX2, NANOG, KLF4). (D) Immunofluorescence images showing the expression of pluripotency markers in Ctr1-iPSC clone 1 (D) and Ctr-iPSCs clone 2 (E). Representative images of the spontaneously differentiated EBs showing the expression of NESTIN (ectoderm), BRACHYURY (mesoderm) and SOX17 (endoderm) in Ctr1-iPSCs clone 1 (F) and Ctr1-iPSCs clone 2 (G). DW, distal water.

Figure S1

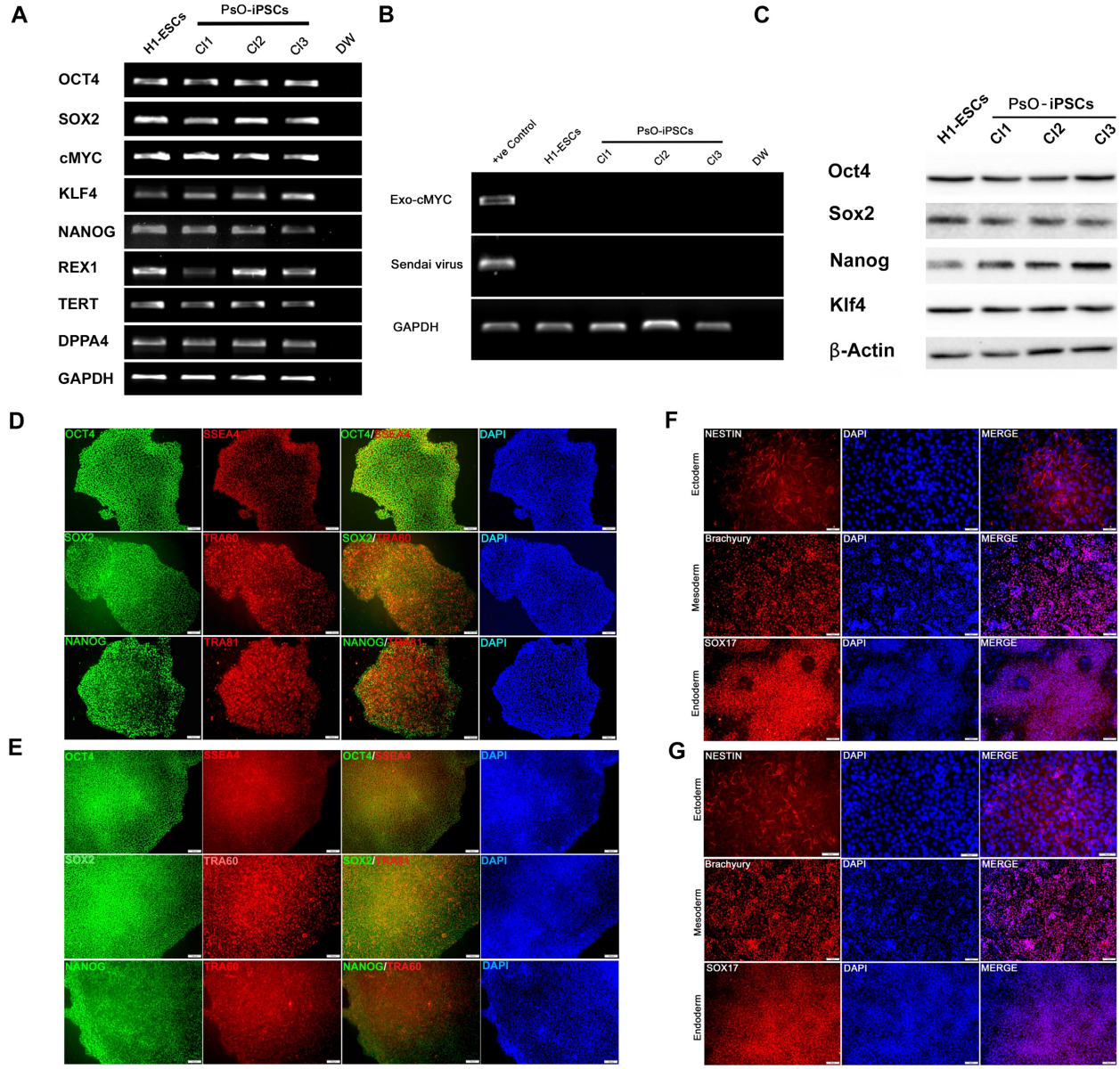

Figure S2: Characterization of the generated iPSCs from a psoriatic patient (PsO2-iPSCs). (A) RT-PCR analysis of iPSC clones generated from a psoriatic patient (PsO2-iPSCs) showing the expression of the endogenous pluripotency markers (OCT4, SOX2, C-MYC, KLF4, NANOG, REX1, TERT, and DPPA4) (A) and the loss of the exogenous expression of pluripotency factor, c-MYC and the backbone of the Sendai virus itself (B). The protein expression of pluripotency markers, including OCT4, SOX2, NANOG, KLF4, SSEA4, TRA81, and TRA60 were confirmed using Western blotting (C) and immunostaining (D & E). Representative images of the spontaneously differentiated EBs showing the expression of NESTIN (ectoderm), BRACHYURY (mesoderm) and SOX17 (endoderm) in clone 1 (F) and clone 2 (G). DW, distal water.

Figure S3

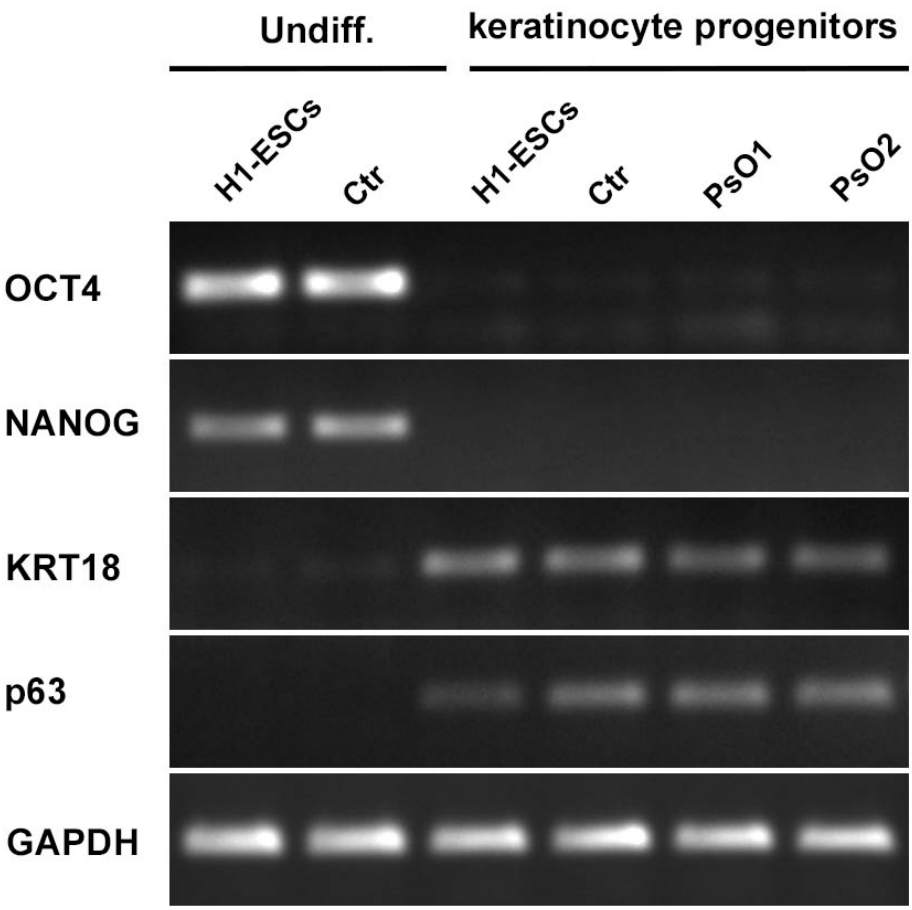

Figure S3: RT-PCR for keratinocytes progenitors at day 14 of differentiation. RT-PCR for keratinocytes progenitor's markers (KRT18 and p63) and pluripotency markers (OCT4 and NANOG) at day 14 of differentiation. The PCR products were analyzed on 1% agarose gel. The differentiation showed marked increase in the keratinocyte progenitors' markers (KRT 18 and p63) and loss of the pluripotency markers (OCT4 and NANOG) at day 14 of differentiation both in control and psoriatic. The expression was compared with H1-ESCs and ctrl- iPSCs undifferentiated cells.

Figure S4

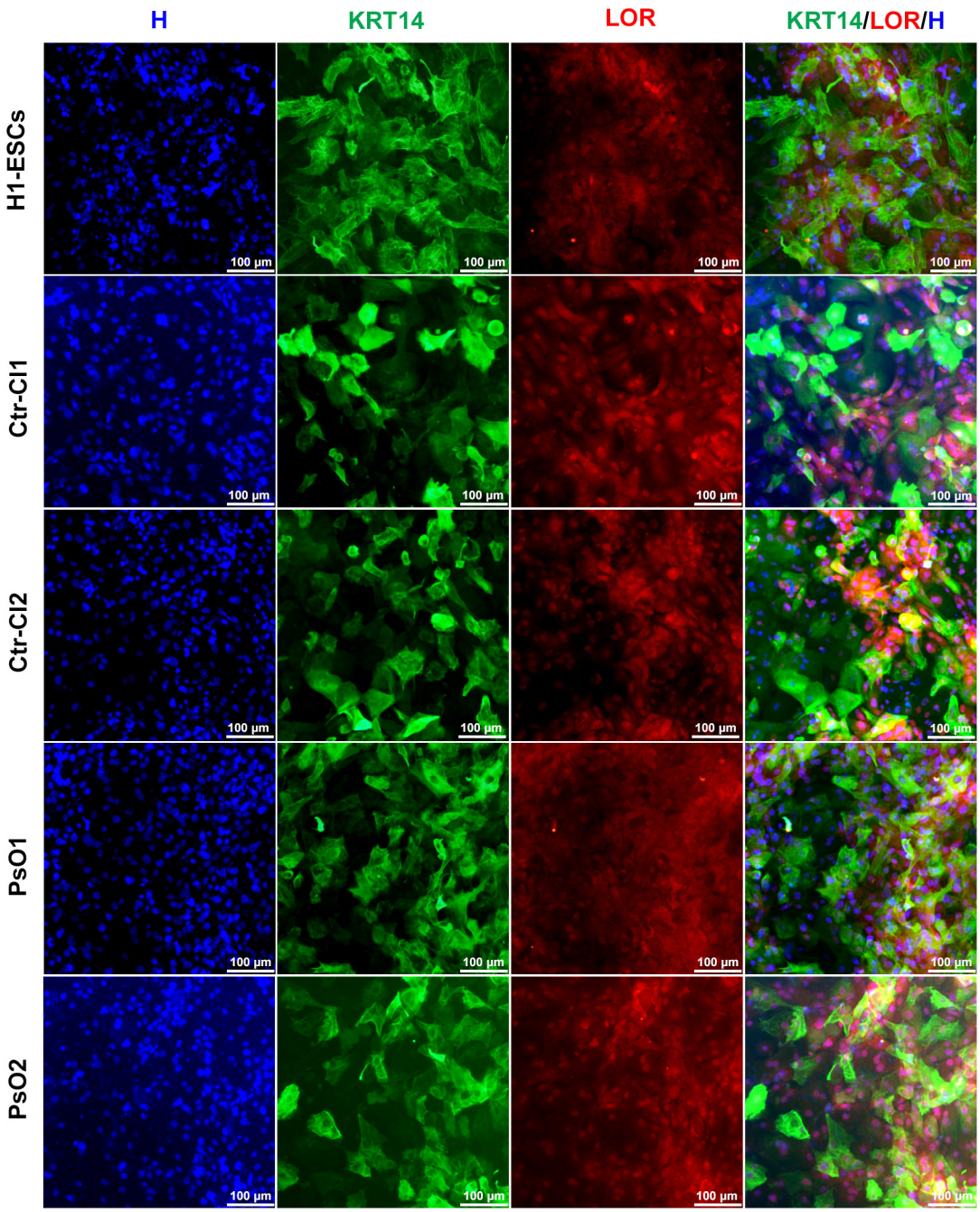

Figure S4: Immunostaining of the mature iPSC-derived keratinocytes (KCs) at day 30 of differentiation. The KCs differentiated from Ctr-iPSCs and PsO-iPSCs expressed KRT14 and Loricrin (LOR) proteins, markers of mature KCs. Scale bar = 100 µm.

Figure S5

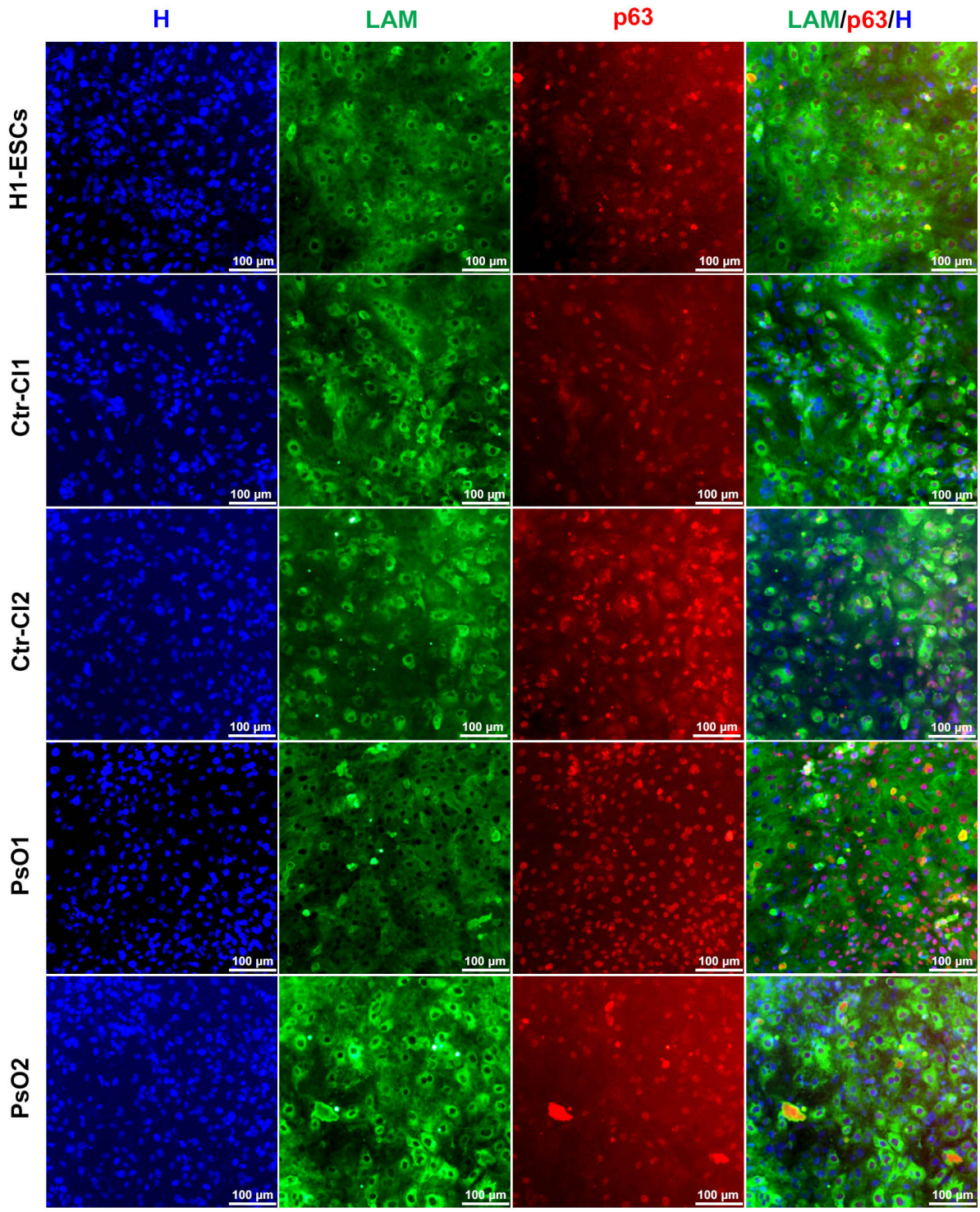

Figure S5: Immunostaining of the mature iPSC-derived keratinocytes (KCs) at day 30 of differentiation. The KCs differentiated from Ctr-iPSCs and PsO-iPSCs expressed nuclear p63 and cytosolic Laminin (LAM), markers of mature KCs. Scale bar = 100 µm.

Figure S6

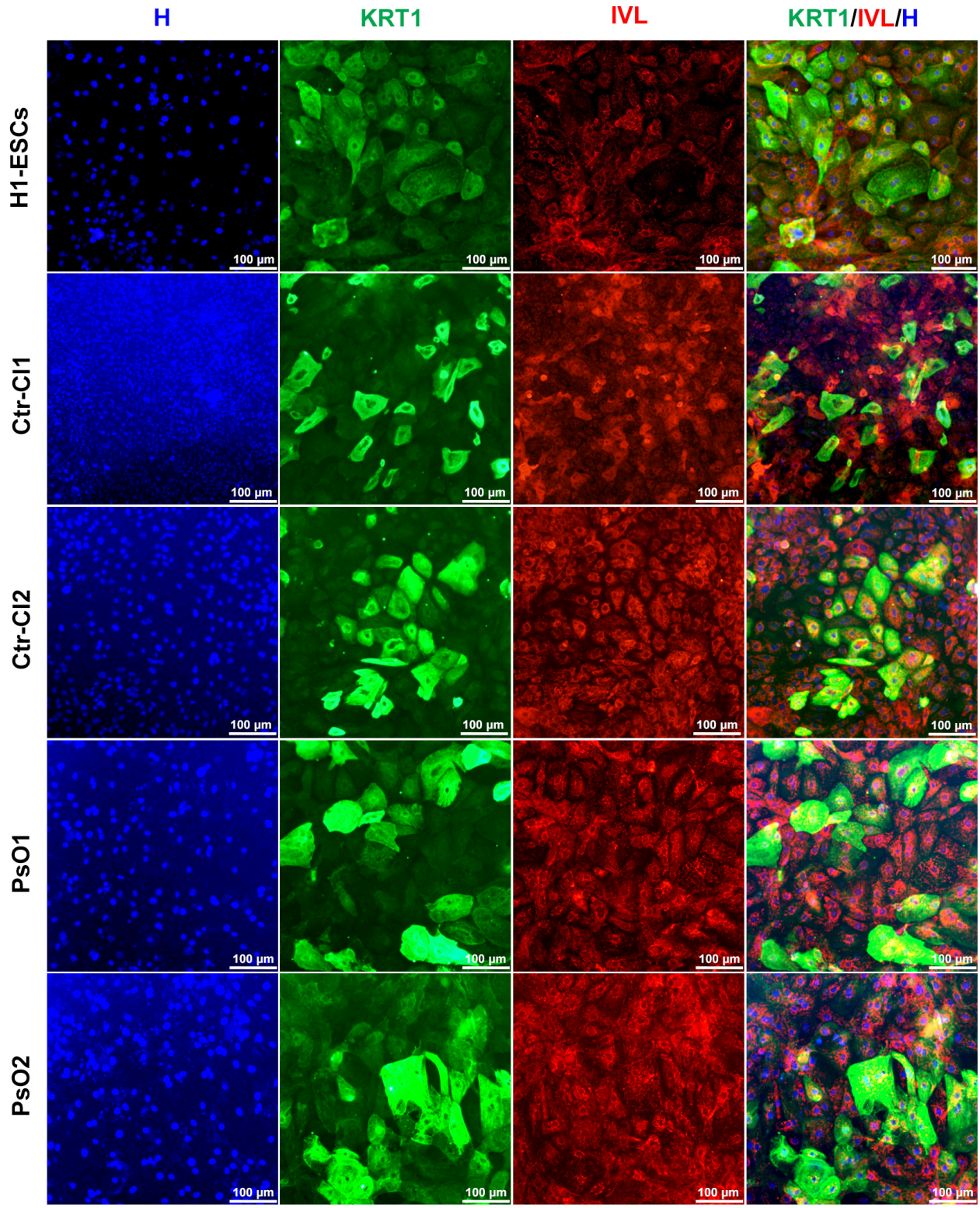

Figure S6: Immunostaining of the mature iPSC-derived keratinocytes (KCs) at day 30 of differentiation. The KCs differentiated from Ctr-iPSCs and PsO-iPSCs expressed KRT1 and Involucrin (IVL), markers of mature KCs. Scale bar = 100 µm.

Figure S7

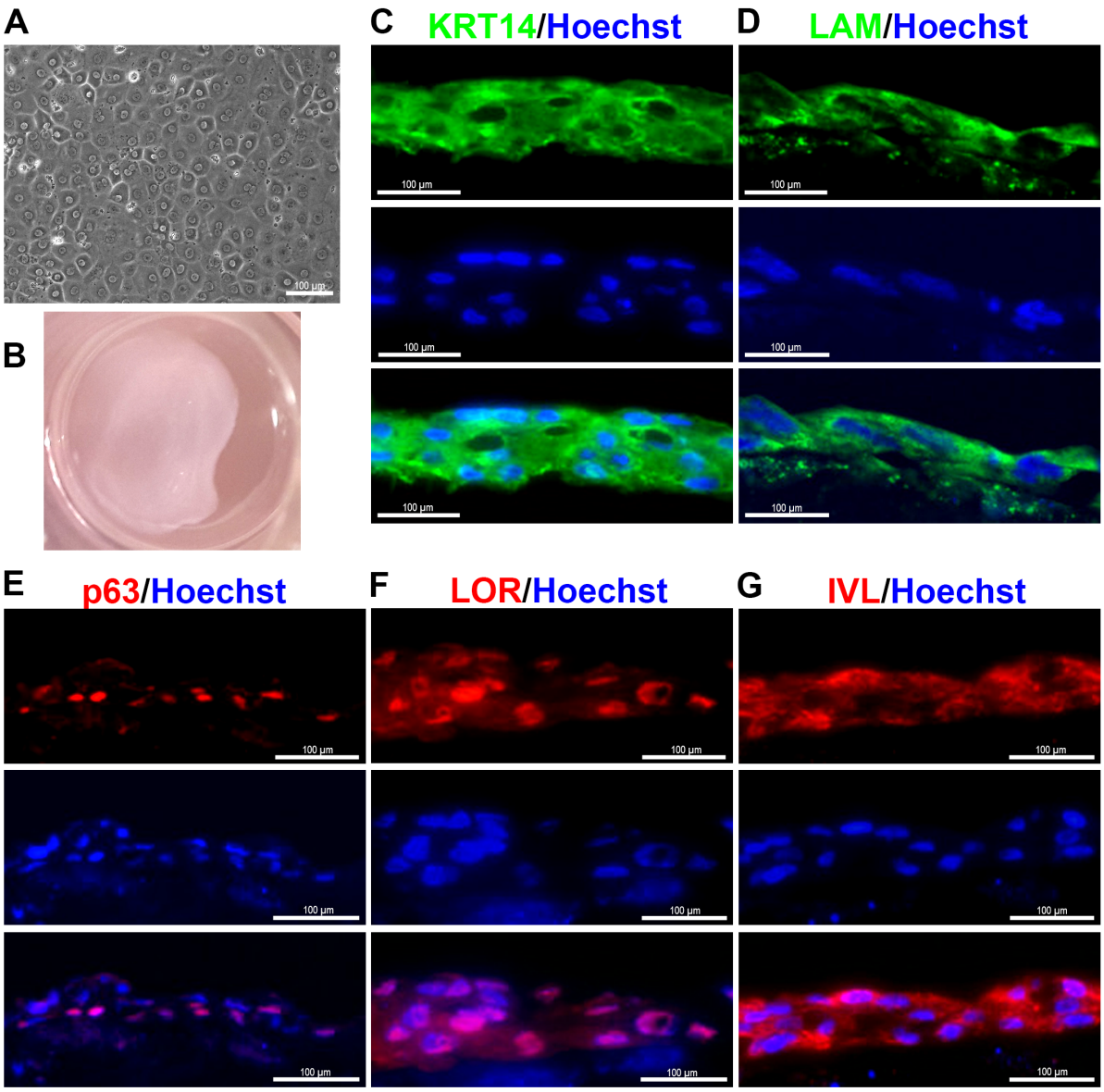

Figure S7. iPSC-derived three-dimensional (3D) skin equivalents. (A) Morphology of keratinocytes generated from Ctr-iPSCs at day 30 of differentiation. (B) Bright field image of iPSC-derived skin equivalents at the end of differentiation protocol. Immunostaining analysis of 3D skin equivalents with antibodies against KRT14 (C), Laminin (LAM) (D), p63 (E), Loricrin (LOR) (F), and Involucrin (IVL) (G). The nuclei were stained with Hoechst. The 3D skin equivalents express markers of epidermal keratinocytes. Scale bars, 100 µm.

Table S1. The details of the antibodies used for immunostaining and Western blotting

For immunostaining			
Antibody	**Company**	**Catalog #**	**Dilution**
Rabbit anti-OCT4	CST	9656S	1:500
Rabbit anti-SOX2	CST	9656S	1:500
Rabbit anti-NANOG	CST	9656S	1:500
Mouse anti-SSEA4	CST	9656S	1:500
Mouse anti-TRA60	CST	9656S	1:500
Mouse anti-TRA81	CST	9656S	1:500
Mouse anti-NESTIN	R&D	MAB1259	1:500
Mouse anti-SOX17	OriGene Technologies	CF500096	1:2000
Mouse anti-Brachyury	Abcam	ab140661	1:1000
Rabbit anti-Laminin	Abcam	ab14509	1:500
Rabbit anti-Loricin	Abcam	ab85679	1:500
Mouse anti-p63	Abcam	ab735	1:500
Rabbit anti-Involucrin	Abcam	ab53112	1:500
Rabbit anti-cytokeratin 18	Abcam	ab181597	1:500
Mouse anti-cytokeratin 1	Abcam	ab81623	1:500
Mouse anti-cytokeratin 14	ThermoFisher Scientific	MAS-11599	1:500
Alexa Fluor 488 anti-rabbit IgG	ThermoFisher Scientific	A-21206	1:500
Alexa Fluor 488 anti-mouse IgG	ThermoFisher Scientific	A-21202	1:500
Alexa Fluor 568 anti-rabbit IgG	ThermoFisher Scientific	A-10042	1:500
Alexa Fluor 568 anti-mouse IgG	ThermoFisher Scientific	A-10037	1:500
For Western blotting			
Rabbit anti-OCT4	CST	9656S	1:5000
Rabbit anti-SOX2	CST	9656S	1:5000
Rabbit anti-NANOG	CST	9656S	1:5000
Rabbit anti-KLF4	Abcam	ab215036	1:1000
Mouse anti-cytokeratin 1	Abcam	ab81623	1:1000
Mouse anti- Cytokeratin 14	ThermoFisher Scientific	MAS-11599	1:1000
Mouse anti-Cytokeratin 19	EMD Millipore	CBL198	1:1000
Rabbit anti-E-Cadherin	CST	3195S	1:1000
Rabbit anti-Filaggrin	Abcam	ab81468	1:1000
Mouse anti-HLA-C	EMD Millipore	MABF233	1:1000
Mouse anti- beta-ACTIN	Santa Cruz Biotechnology	sc-47778	1:10000
Peroxidase AffiniPure Donkey Anti-Mouse IgG	Jackson ImmunoResearch Laboratories	715-035-150	1:10000
Peroxidase AffiniPure Donkey Anti-Rabbit IgG	Jackson ImmunoResearch Laboratories	711-035-152	1:10000

Table S2: The list of primers for RT-PCR and qPCR used in this study

Gene name	Forward	Reverse	Product size
OCT4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC	119
SOX2	GGGAAATGGGAGGGGTGCAAAAGAGG	TTGCGTGAGTGTGGATGGGATTGGTG	126
c-MYC	GCGTCCTGGGAAGGGAGATCCGGAGC	TTGAGGGGCATCGTCGCGGGAGGCTG	328
KLF4	CCCAATTACCCATCCTTCCT	ACGATCGTCTTCCCCTCTTT	108
NANOG	CATGAGTGTGGATCCAGCTTG	CCTGAATAAGCAGATCCATGG	192
REX1	TCACAGTCCAGCAGGTGTTTG	TCTTGTCTTTGCCCGTTTCT	205
TERT	CCTGCTCAAGCTGACTCGACACCGTG	GGAAAAGCTGGCCCTGGGGTGGAGC	446
DPPA4	GGAGCCGCCTGCCCTGGAAAATTC	TTTTTCCTGATATTCTATTCCCAT	384
EXO-cMYC	TAACTGACTAGCAGG CTTGTCG	TCC ACA TAC AGT CCT GGA TGATGATG	532
Sendai-V	GGATCACTAGGTGATATCGAGC	ACCAGACAAGAGTTTAAGAGATATGTATC	181
MAP2	CAGGTGGCGGACGTGTGAAAATTGAGAGTG	CACGCTGGATCTGCCTGGGGACTGTG	187
PAX6	CGAATTCTGCAGGTGTCCAA	ACAGACCCCCTCGGACAGTAAT	207
T	GCCCTCTCCCTCCCCTCCACGCACAG	CGGCGCCGTTGCTCACAGACCACAGG	258
GATA6	AAGCGCGTGCCTTCATCA	TCATAGCAAGTGGTCTGGGC	157
SOX17	TCCTGGAGGAGCTAAGGAAA	GCCACTTCCCAAGGTGTAAA	773
GAPDH	ACGACCACTTTGTCAAGCTCATTTC	GCAGTGAGGGTCTCTCTCTTCCTCT	132
KRT18	GTACTGGTCTCAGCAGATTG	CTGGCCTTCAGATTTCTCAT	150
P63	TTCGGACAGTACAAAGAAC	CCCTCACTGGTAAGTATAAC	128
IRS2	ACTTCTTGTCCCACCACTTG	TGACATCCTGGTGATAAAGCC	78
PSORS1C1	GATGGCATCTAGAAGTAGAC	GATGGTTTCTGTTGCATTT	138
TNFAIP2	CAATGTGAGGGAGTTGATG	GAGGTGATCTGGATGATGT	129
Filaggrin	GGTCACTTTAGTAGTCTTTC	CATAATCTGCACTACCATAG	169
SLC39A4	GTCAGAGAGGTATCTGTACG	GAAGGTCTGCAGGATGTAG	124
HLA-C	TGATGTGTAGGAGGAAGAG	GTCTCAGGCTTTACAAGTG	113
BST2	CAATGTCACCCATCTCCTG	TGTAGTGATCTCTCCCTCAA	175
OAS1	GGATTCTGCTGGCTGAAAG	GCTGGGTCTATGAGAGAAATG	116
OAS3	CTTCAATGTCCTGGGTCAG	GCTTCACCAGCAAGATTAG	176
IFI44	TGATAGATACCAGTTTAATCCC	CATCTGAGAGGAGAAGTATTG	142

IFI44L	TCGAGTTGAAGGAATTAAGG	TGAAACCAAGTCTGCATAG	121
IFIT3	CATGAGTGAGGTCACCAAGA	GTAGGCCAACAAGTTGTACATT	178
IFITM1	GTCCACCGTGATCAACATC	CTTCCTGTCCCTAGACTTCA	139
IFITM3	CTACTCCGTGAAGTCTAGG	GATGACGATGAGCAGAATG	136
PARP12	GGGAAGAACTGTAGGAATAG	TAATGTTGGCAAATTTCTGG	152
PARP14	GAAATCCAAAGTTGACATCC	ATCCTCTCAATCTTCTCTATTC	156
KRTAP19-1	CTTTGGAAGCTACGGATATG	CTCCTCTGCTTCCAATTTC	156

Table S3: The deep coverage of the sequencing experiments per sample

Sample	**Number of input reads**	**Number of uniquely mapped reads (%)**	**Number of multi-mapped reads (%)**
Ctr -replicate1	28,011,217	26,129,902 (93.3%)	1,195,359 (4.3%)
Ctr – replicate2	30,405,493	28,307,585 (93.1%)	1,347,399 (4.4%)
PsO1-replicate1	19,836,988	18,412,476 (92.8%)	954,769 (4.8%)
PsO1-replicate2	19,500,016	17,976,811 (92.2%)	1,047,795 (5.4%)
PsO2-replicate1	45,138,646	41,293,365 (91.5%)	2,112,214 (4.7%)
PsO2-replicate2	32,665,903	30,029,437 (91.9%)	1,434,537 (4.4%)

Table S4. Chromosomal location of some of type I interferon (IFN)-inducible genes significantly upregulated in KCs generated from PsO-iPSCs in comparison to those generated from Ctr-iPSCs.

Gene name	Chromosome location	log2(FC) in KCs-PsO1	log2 (FC) in KCs-PsO2
IFIT1	chr10:89392545-89406487	6.81603	2.02651
IFIT2	chr10:89301948-89309276	5.92189	2.32401
IFIT3	chr10:89327818-89340968	6.73403	3.38887
IFIT5	chr10:89414567-89421002	2.69751	1.4171
IFITM1	chr11:313990-315272	6.81603	3.94693
IFITM2	chr11:308106-309410	4.45095	2.66693
IFITM3	chr11:319672-320914	4.41602	3.46056
IFI6	chr1:27666060-27672213	4.72468	1.73349
IFI16	chr1:159009891-159055155	2.19279	1.08832
IFI44	chr1:78649788-78664078	5.85548	2.66848
IFI44L	chr1:78620381-78646145	5.6387	2.92383

Supplementary References

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