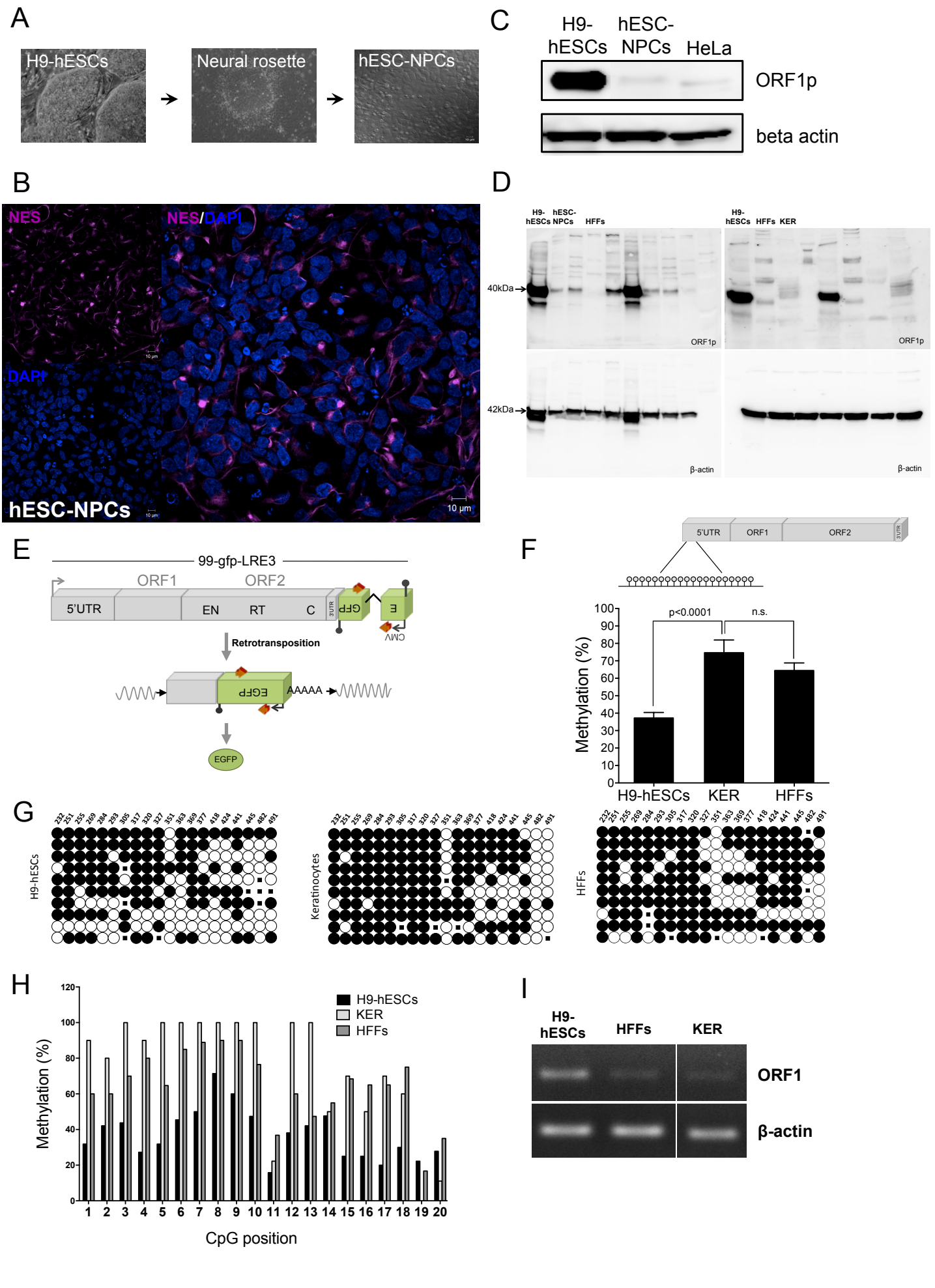
A
H9-hESCs
Neural rosette
hESC-NPCs
C
H9-hESCs
hESC-NPCs
HeLa
ORF1p
beta actin
B
NES
NES/DAPI
DAPI
hESC-NPCs
10 μm
D
H9-hESCs
hESC-NPCs
HFFs
H9-hESCs
HFFs
KER
40kDa
42kDa
ORF1p
β-actin
E
99-gfp-LRE3
ORF1
ORF2
5'UTR
EN
RT
C
3'UTR
GFP
E
CMV
Retrotransposition
EGFP
AAAAA
F
5'UTR
ORF1
ORF2
3'UTR
p<0.0001
n.s.
Methylation (%)
H9-hESCs
KER
HFFs
G
H9-hESCs
Keratinocytes
HFFs
H
H9-hESCs
KER
HFFs
Methylation (%)
CpG position
I
H9-hESCs
HFFs
KER
ORF1
β-actin

Supplemental Figure 1. **LINE-1 expression and methylation analyses in ectodermal cells.** (**A**) Schematic of the differentiation process used to obtain NPCs from hESCs. (**B**) Representative quality controls on hESC-derived NPCs. Shown is a merged image of hESC-derived NPCs stained with an antibody against *NES* (pink); nuclear DNA was stained with DAPI (blue). (**C&D**) Western-blot analyses of L1-ORF1p in the indicated cell type (above each lane). Beta actin was used as a loading control. Full-size Western blot scans of those shown in panel C and in Figure 2B. The right side contains the captured images used in the merged image. White bars, 20 mm. (**E**) Rationale of the LINE-1 retrotransposition assay. The cartoon shows the structure of an active L1Hs element tagged with the *megfpI* retrotransposition indicator cassette (backwards EGFP: green box). Only after a round of L1-retrotransposition EGFP can be expressed (green EGFP: labelled oval). Also shown is the relative position of the primers used in the PCR-intron assay (red arrows). (**F**) Methylation analyses in ectodermal cells. A cartoon of an active L1Hs element, in which the relative positions of 5´UTR, ORF1, ORF2 and 3´UTR sequences are indicated, is shown. Within the 5´UTR, the relative positions of the 20 CpG positions analyzed are indicated (lollipops). Below the cartoon, the graph indicates the percentage of methylated CpG residues in each sample as measured by bisulfite-PCR assays. Also indicated is the p value of the comparisons (<0.0001 and non significant (n.s.), One-way ANOVA with Tukey). (**G&H**) L1Hs promoter methylation analyses in H9-hESCs, Keratinocytes and HFFs. In panel d, each lane corresponds to a sequenced clone (shown are the 10 clones with the highest sequence similarities to L1.3, accession number L19088.1). In panel e, the percentage of methylation for each CpG residue is indicated in the graph as measured by bisulfite conversion assays. Black bars, H9-hESCs; light grey bars, keratinocytes (KER); dark grey bars, HFFs. In panels d &e, the relative position of each CpG is indicated using the sequence of L1.3 as a reference. (**I**) L1Hs mRNA expression analysis as measured by semi quantitative RT-PCR using a set of primers to the ORF1 sequence of a consensus L1Hs element. The gel shows representative data for the indicated sample and amplification of beta actin was included as a control of RNA integrity.