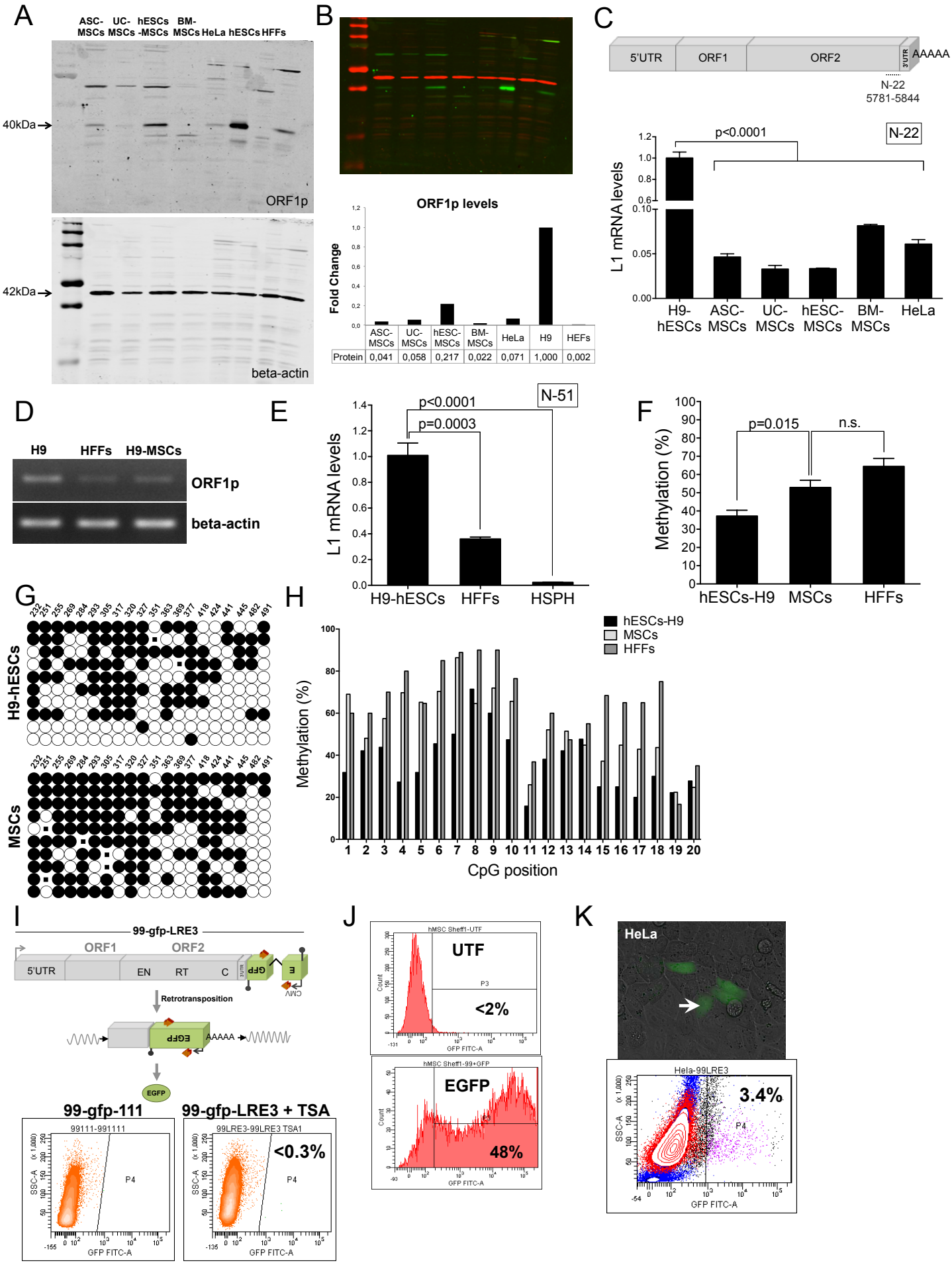
A
ASC-MSCs
UC-MSCs
hESCs-MSCs
BM-MSCs
HeLa
hESCs
HFFs
40kDa
ORF1p
42kDa
beta-actin
B
ORF1p levels
Fold Change
ASC-MSCs
UC-MSCs
hESC-MSCs
BM-MSCs
HeLa
H9
HEFs
Protein
0,041
0,058
0,217
0,022
0,071
1,000
0,002
C
5'UTR
ORF1
ORF2
3'UTR
AAAAA
N-22
5781-5844
p<0.0001
N-22
L1 mRNA levels
H9-hESCs
ASC-MSCs
UC-MSCs
hESC-MSCs
BM-MSCs
HeLa
D
H9
HFFs
H9-MSCs
ORF1p
beta-actin
E
p<0.0001
p=0.0003
N-51
L1 mRNA levels
H9-hESCs
HFFs
HSPH
F
p=0.015
n.s.
Methylation (%)
hESCs-H9
MSCs
HFFs
G
H9-hESCs
MSCs
H
hESCs-H9
MSCs
HFFs
Methylation (%)
CpG position
I
99-gfp-LRE3
ORF1
ORF2
5'UTR
EN
RT
C
3'UTR
GFP
E
CMV
Retrotransposition
EGFP
AAAAA
EGFP
99-gfp-111
99-gfp-LRE3 + TSA
99111-991111
99LRE3-99LRE3 TSA1
P4
<0.3%
SSC-A
GFP FITC-A
J
hMSC Sheff1-UTF
UTF
P3
<2%
hMSC Sheff1-99+GFP
EGFP
48%
Count
GFP FITC-A
K
HeLa
Hela-99LRE3
3.4%
P4
SSC-A
GFP FITC-A

Supplemental Fig. 3. Engineered L1 retrotransposition in MSCs. (**A**) Full-size Western blot scans of those shown in Figure 3C. (**B**) Quantification of L1-ORF1p expression in MSCs by Western-blot; the expression level of L1-ORF1p in hESCs was designated 1 for comparisons. In the fluorescent Western-blot analysis, β-actin (red signal) was used to normalize L1-ORF1p expression (green signal). (**C**) L1Hs mRNA expression analyses by RT-qPCR. A cartoon of an active L1Hs element, where the relative position of the 5´UTR, ORF1, ORF2 and 3´UTR sequences is indicated. Also indicated is the relative position of the primer pair used to analyze L1 expression (N-22 pair). The numbering refers to the nucleotide sequence of L1.3. Below the cartoon, the graph shows expression data in the indicated sample (n=duplicate biological). In the analysis, the expression level detected in H9-hESCs was designated 1 for comparison. Also indicated is the significance of the statistical method applied (see Methods) and p value (0.0001). (**D**) 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 in the indicated sample and amplification of βeta actin was included as a control of RNA integrity. (**E**) L1Hs mRNA expression analyses by RT-qPCR in HSPHs (n=2 biological replicas). The graph shows expression data in the indicated sample. In the analysis, the expression level of H9-hESCs was designated 1 for comparison. Also indicated is the p value of the comparison (<0.001 and <0.0003). (**F**) DNA methylation analyses in MSCs. The graph indicates the percentage of methylated CpG residues within L1Hs 5´UTR sequences as measured by bisulfite-PCR assays in the indicated sample. Also indicated is the p value of the comparisons (0.015); n.s., not significant. (**G&H**) In panel g, each lane corresponds to a sequenced clone (shown are the 10 clones with the highest sequence similarity to L1.3, accession number L19088.1) in the indicated cell type (left black letters). In each lane, white and black circles represent unmethylated and methylated CpG residues, respectively. In panel h, 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, MSCs; dark grey bars, HFFs. In panels G&H, the relative position of each CpG is indicated using the sequence of L1.3 as a reference. (**I**) Rationale of the engineered L1 retrotransposition assay using DNA plasmids, and representative data obtained in MSCs. The right side contains two representative histograms (SSC vs EGFP) of MSCs transfected with an inactive L1Hs clone (99-gfp-JM111) or an active L1Hs element (99-gfp-LRE3). Also, as described, a portion of 99-gfp-LRE3 transfected cells was treated with 500nM TSA for 18h prior to FACS analyses. The percentage of EGFP-expressing cells as determined in triplicate is shown in the histogram. (**J**) Transfection efficiency controls of MSCs. The two histograms (SSC vs EGFP) show the percentage of EGFP-expressing cells after co-transfecting MSCs with plasmids 99-gfp-LRE3 and pCEP-EGFP (bottom histogram). A fraction of MSCs was not transfected (top panel) and were used as an untransfected control. (**K**) Retrotransposition control assays in HeLa cells. Shown are representative retrotransposition results obtained in HeLa cells transfected with plasmid 99-gfp-LRE3, and a picture of HeLa cells expressing L1-EGFP after retrotransposition, along with a representative histogram (SSC vs EGFP) of transfected HeLa cells analyzed 7 days after transfection. The percentage of L1-EGFP expressing cells is also indicated. In panels C, E and F, One-way ANOVA with Tukey was used as a statistical method.