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Supplemental Information

The IS2 Element Improves Transcription Efficiency of Integration-Deficient Lentiviral Vector Episomes

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Supplementary Information

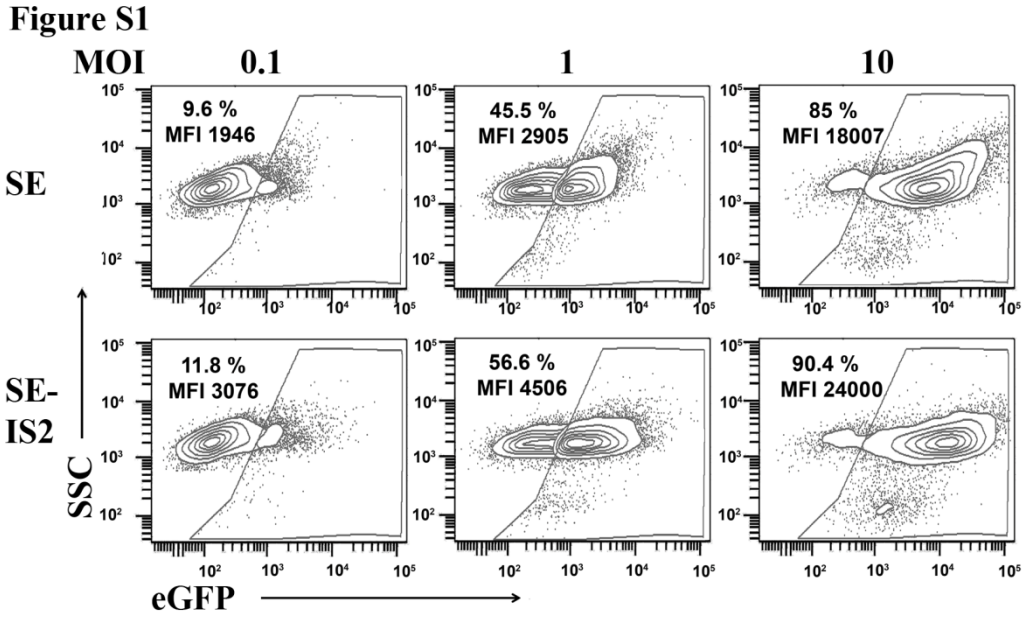

Figure S1. Effects of IS2 elements on IDLVs at different MOIs. Representative plots showing eGFP expression profiles of 293 T cells transduced with increased MOIs of SE (top) and SE-IS2 (bottom) IDLVs. The percentages (%) and expression levels (MFI) of the eGFP+ population are shown in each plot.

Figure S2

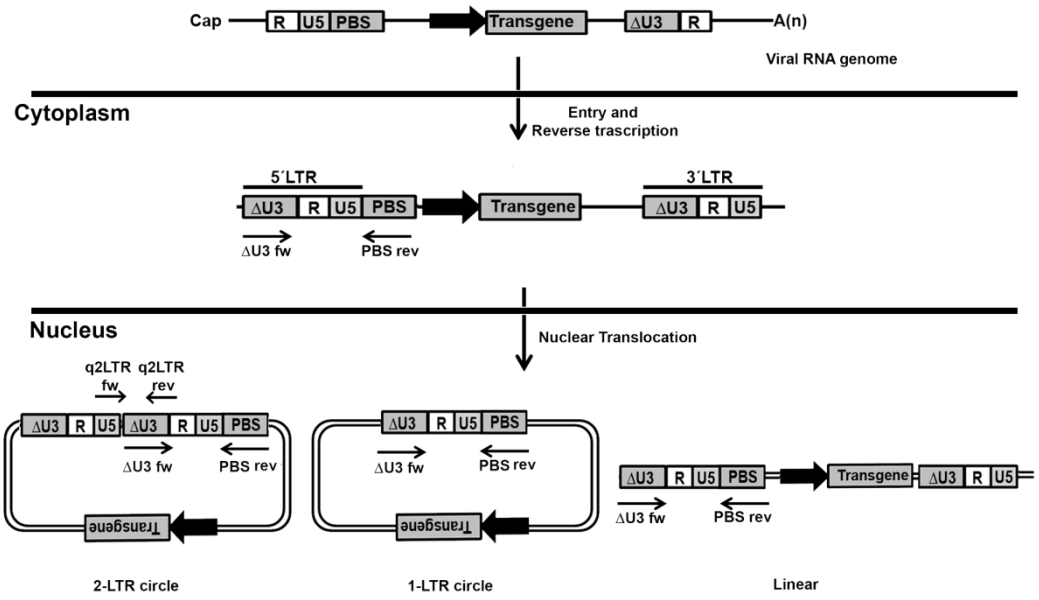

Figure S2. Scheme showing the different forms of the IDLVs genome during transduction of target cells. Vector RNA genome inside IDLVs particles (top) enter the cytoplasm of the target cell (middle) where vector DNA sequences can already be found with complete 5'ΔU3RU5 LTRs. Once the vector pre-integration complex enters the nucleus, IDLVs generates mainly 1-LTR and 2-LTR DNA episomal circles, although some lineal DNAs forms can also be found. Primers used to detect the different forms of vector DNA are indicated with arrows. ΔU3fw and PBSrev primer set were used to detect the amount of total reverse transcribed products (vector DNA genomes). q2LTRfw and q2LTRrev primers were used to assess the relative amounts of 2LTR circles versus total vector DNA genomes.

Figure S3

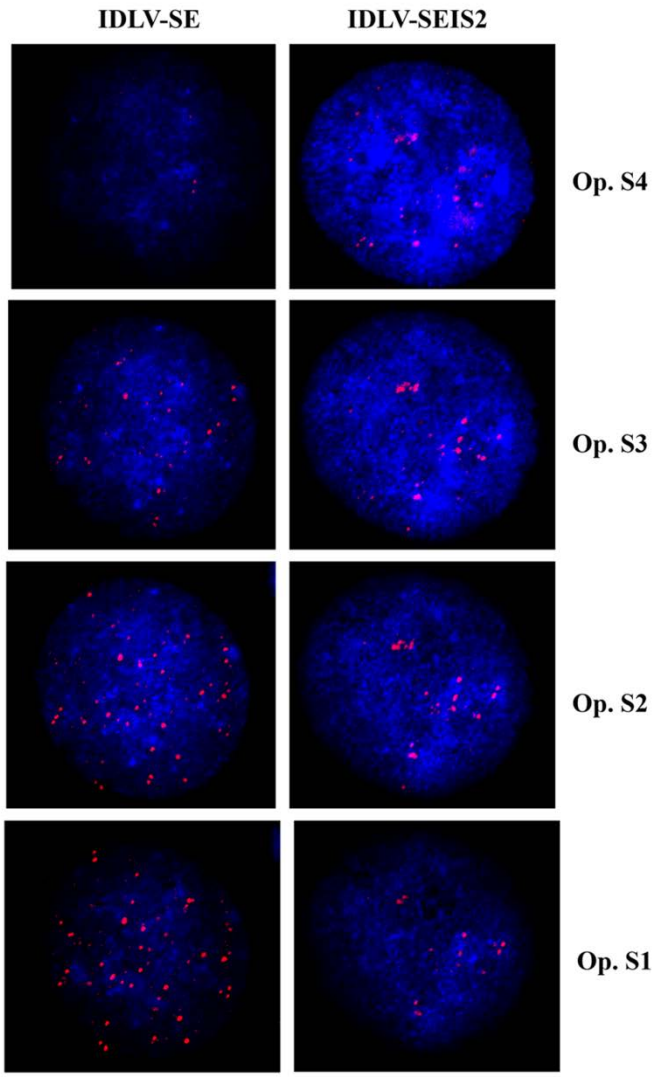

Figure S3 Confocal images showing nuclear localization of Viral Episomes. Nuclear distribution of IDLVs episomes. 293T Cells were transduced at MOI=10. The cells were subsequently fixed, methanol permeabilized, and incubated with Alexa Fluor 555 IDLV-labeled probes Images showing eight continuous optical sections, (Op. S1-6) showing viral episomes localization within the nucleus of the transduced cells.

Figure S4

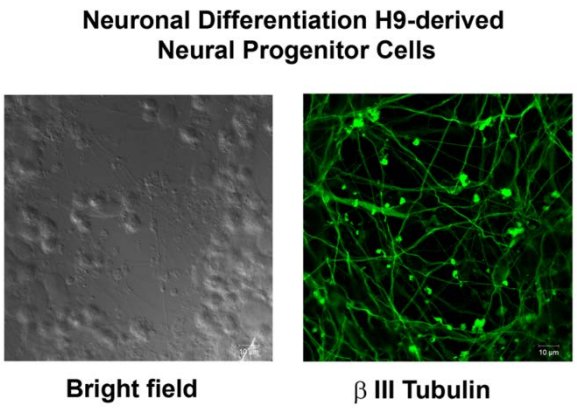

Figure S4. Images of NPCs differentiated into neuron-like cells. NPCs were cultured in neural differentiation media (See Material and Methods for details) and maintained at 37ºC in 5% CO_2 for 28 days. Neural-like cells were fixed and permeabilized for 30 min and stained with anti-β-tubulin (β-tubulin (TUJ1) mouse monoclonal antibody and Goat anti-mouse IgG Alexa Fluor 488. Optical images were captured using a Zeiss LSM 710 confocal microscope and an Axio Imager A1 microscope.

Figure S5

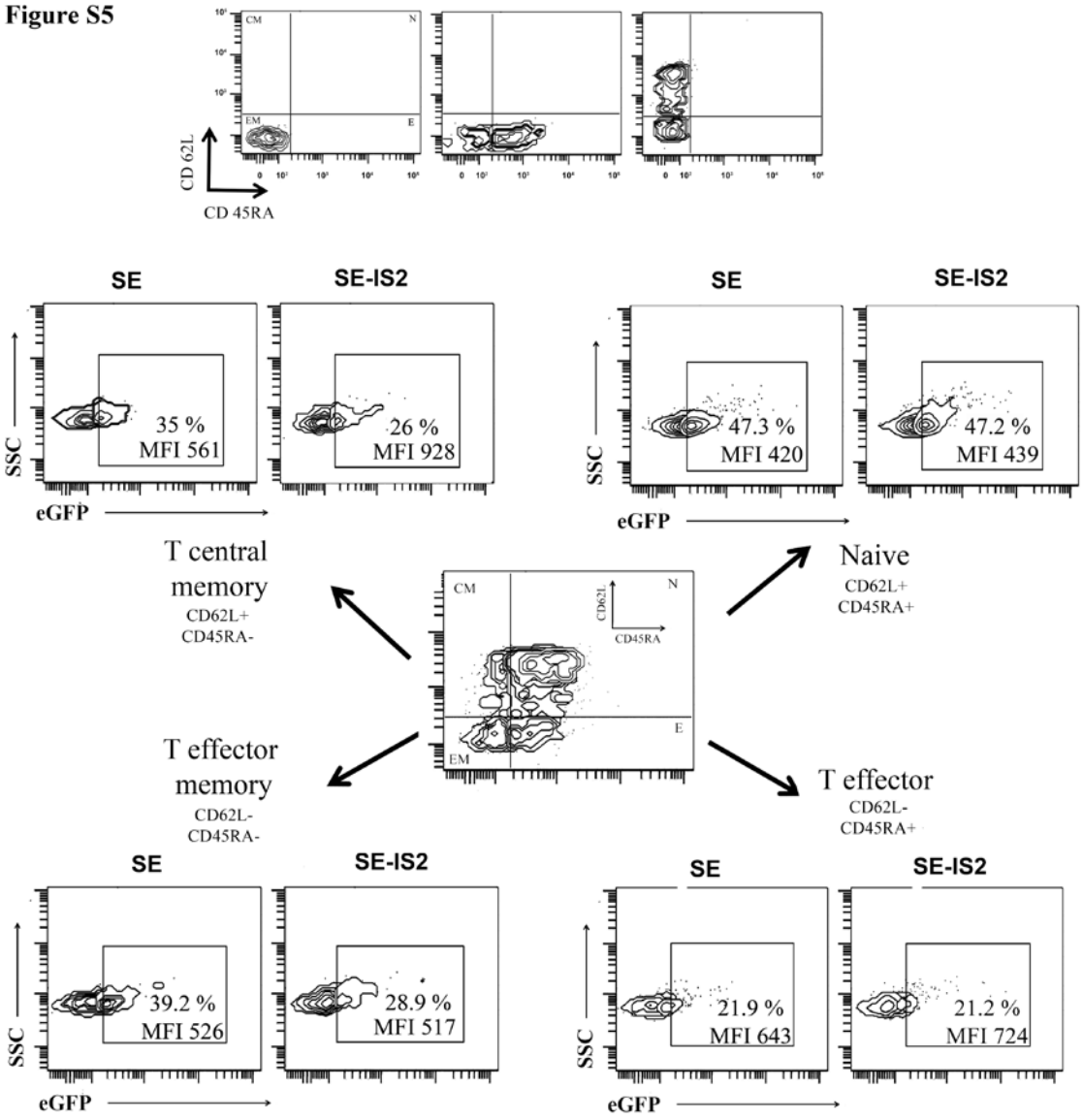

Figure S5. Performance of the different IDLVs in various T cells subpopulations. Top plots show isotype controls (Left), single staining for CD45RA-PE (HI100 clone - Middle) and single staining for CD62L-PE-Cy7 (DREG56 clone - Right). T cells were transduced with the SE and the SE-IS2 IDLVs at MOI=5 and 72h later analyzed. The different transduced cells were stained with CD45RA-PE and CD62L-PE-Cy7 and the different sub-populations (effector memory (CD62L-CD45RA-), effector (CD62L-CD45RA+), central memory (CD62L+CD45RA-) and naïve/stem cell memory (CD62L+CD45RA+)) analyzed for eGFP expression.

Figure S6

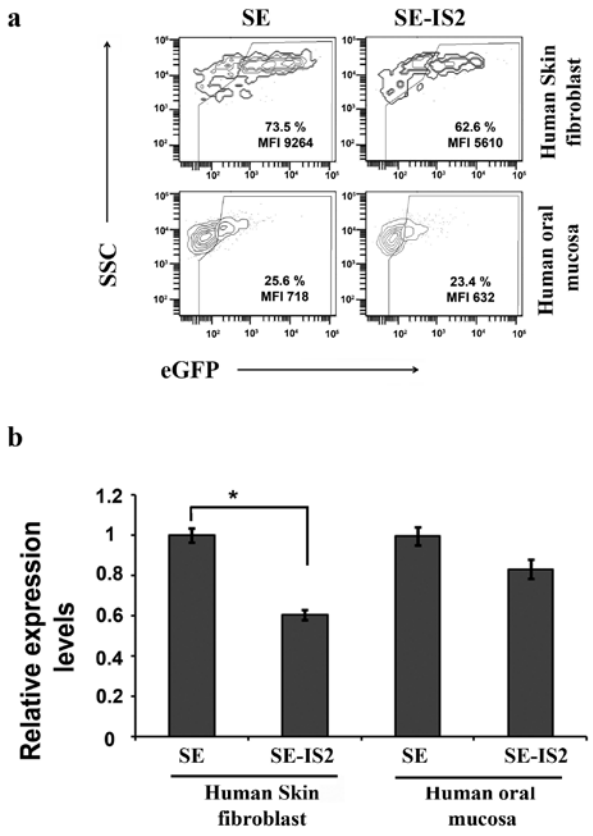

Figure S6. Performance of the different IDLVs in differentiated skin and oral mucosa cells (a). Representative plots showing eGFP expression profiles. Differentiated skin and oral mucosa cells were transduced at MOI=10 with SE- and SEIS2-IDLVs and 72h later analyzed. The percentage (%) of the eGFP+ population and the expression levels (MFI) are shown in each plot. **(b).** Graph showing the relative expression levels (MFI) of the SE-IS2-IDLVs in the different cell types normalized to SE-IDLVs in the same cell lines and using equal MOIs. The values represent means +/− SEM of at least four separate experiments (*$p < 0.05$).

Supplemental Methods

Isolation and culture of primary human T cells

Peripheral blood mononuclear cells (PBMCs) from a healthy donor were isolated by density gradient (Lymphosep, Biowest) and T cells were purified using the Pan T cell Isolation kit (Milteny Biotec) following manufacturer's instructions. T lymphocytes were activated with TransAct T Cell Reagent (Milteny Biotec) in TexMACS medium supplemented with 5% of human AB serum (male HIV tested, Biowest) and 20 $U7ml^{-1}$ of IL-2 (Milteny Biotec) during 48h. Cells were plated at a density of 10^6 cells7ml and incubated at 37ºC, 5% CO_2.

Primary cell cultures of human oral mucosa and skin fibroblasts

Primary cell cultures of human oral mucosa and skin fibroblasts from biopsies of normal oral mucosa and skin were obtained from healthy donors. Human oral mucosa and skin biopsies were washed in 1x PBS and enzymatically digested using 2 mg/ml Clostridium histolyticum collagenase I (Gibco-BRL) at 37°C for 6 hours. Isolated fibroblasts were collected by centrifugation and expanded in culture flasks containing basal culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 0.25 μg /ml amphotericin B, all from Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com under standard cell culture conditions)."

T cells Transduction

Activated T cells were transduced with viral supernatants and spinoculated at 800g during 1h, and cells were washed 4 hours later. Similarly, Jurkat cells resuspended in viral supernatants were spinoculated at 800g at 32ºC during 30 minutes. Four hours after transduction, cells were washed and plated at a density of 100.000 cells/ml.

Immunocytochemistry of mature Neural-like cells

Mature neural-like cells were fixed in 4% paraformaldehyde for 30 min at room temperature. After fixation, the cells were washed three times with PBS. To permeabilize the cells and to avoid non-specific antigens, the fixed cells were incubated in PBS with 2% goat serum and 0,1% Triton-X100 buffer (SigmaAldrich, St. Louis, MO,

http://www.sigmaaldrich.com) for 30 min at room temperature. After two washes with washing solution (PBS containing 0,1% goat serum and 0,05% Triton X-100), cells were incubated with primary antibody anti-β-tubulin (β-tubulin (TUJ1) mouse monoclonal antibody, Covance Inc., http://es.covance.com) diluted 1:1000 in PBS overnight. Slides were then washed three times with washing solution and incubated with the secondary antibody (Goat anti-mouse IgG secondary antibody, Alexa Fluor 488 conjugate, Thermo Fisher Scientific, https://www.thermofisher.com) at 1:1000 dilution for 30 minutes. All incubations were performed in a humidified chamber at 4°C. Lastly, slides were washed twice and mounted in mounting medium (SlowFade Gold Antifade Mountant with DAPI, Thermo Fisher Scientific, Thermo Fisher Scientific, https://www.thermofisher.com) with cover slip. Optical scanning was performed using a Zeiss LSM 710 confocal microscope and an Axio Imager A1 microscope.

Flow cytometry

For T cell phenotypic characterization, cells were stained during 30 min on ice and dark with CD45RA-PE (HI100 clone) and CD62L-PE-Cy7 (DREG56 clone) antibodies, both from eBiosciences.