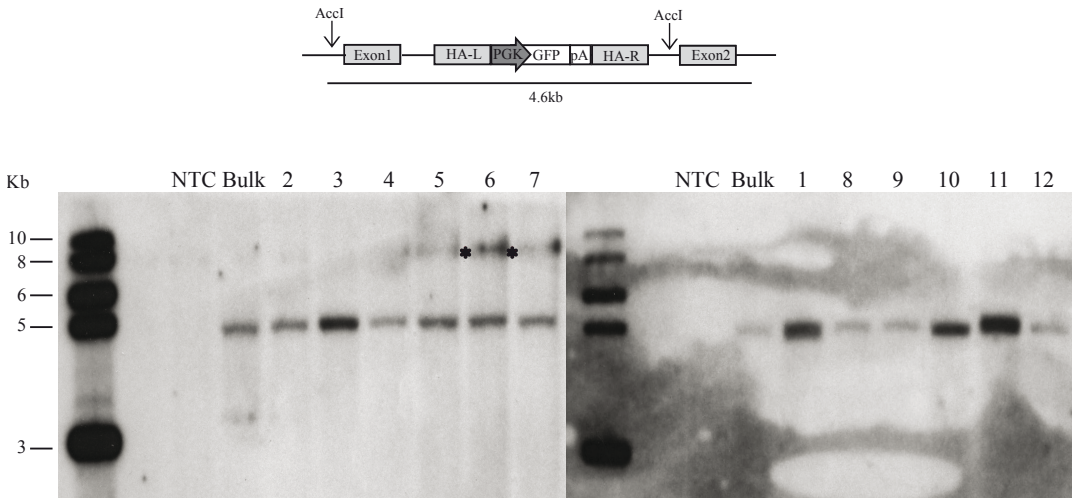
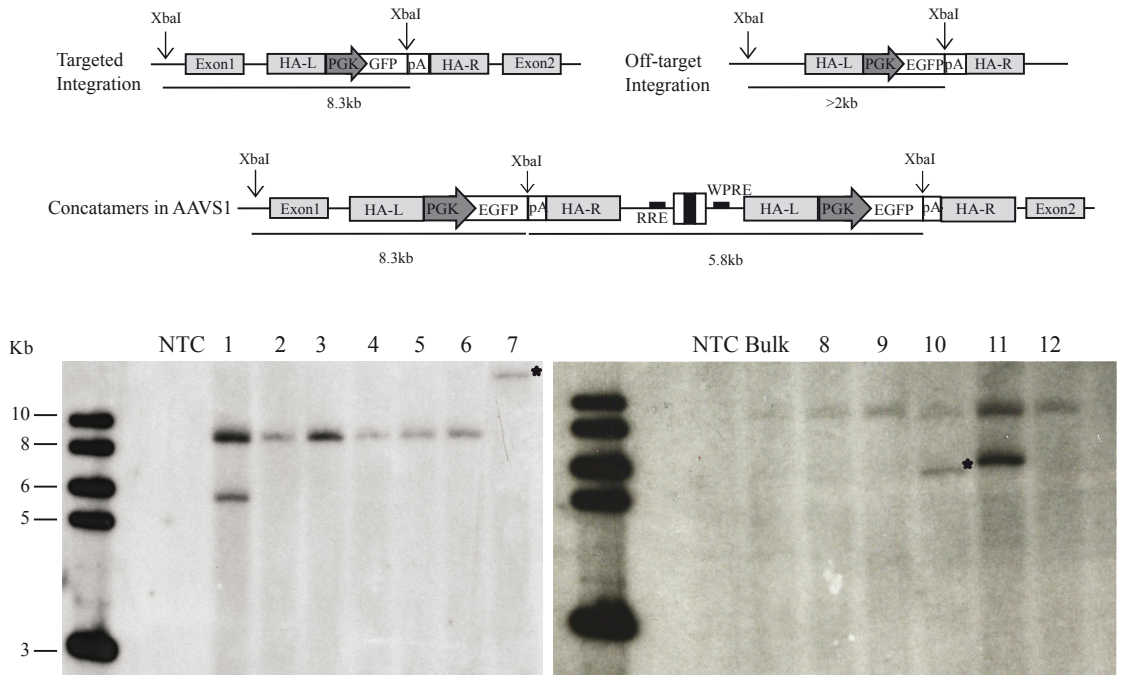
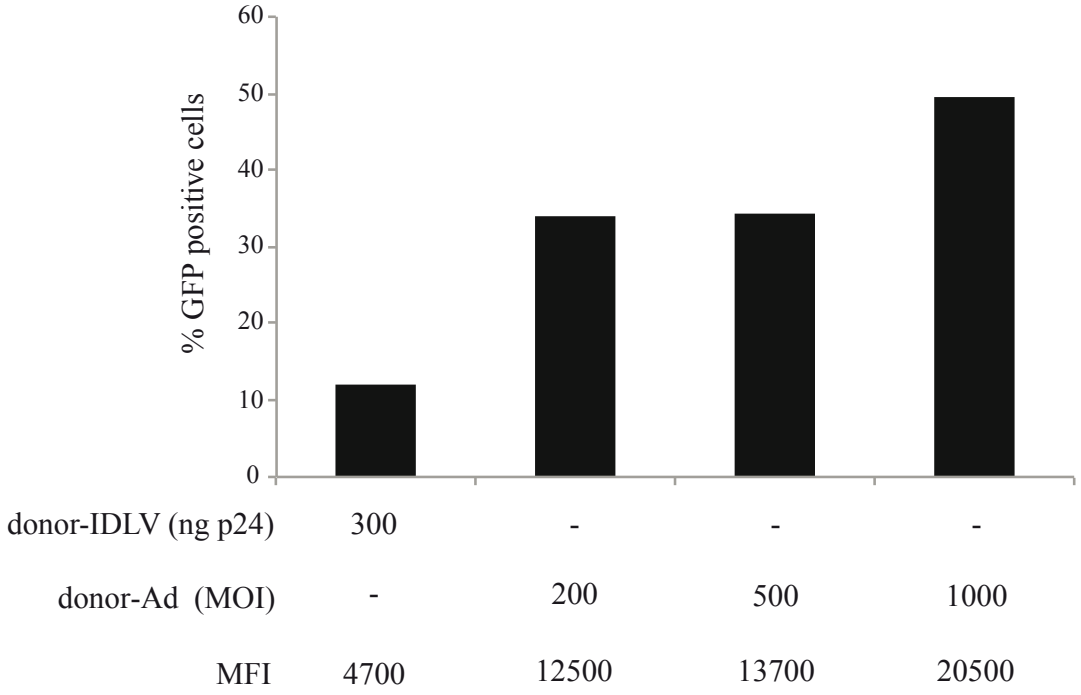


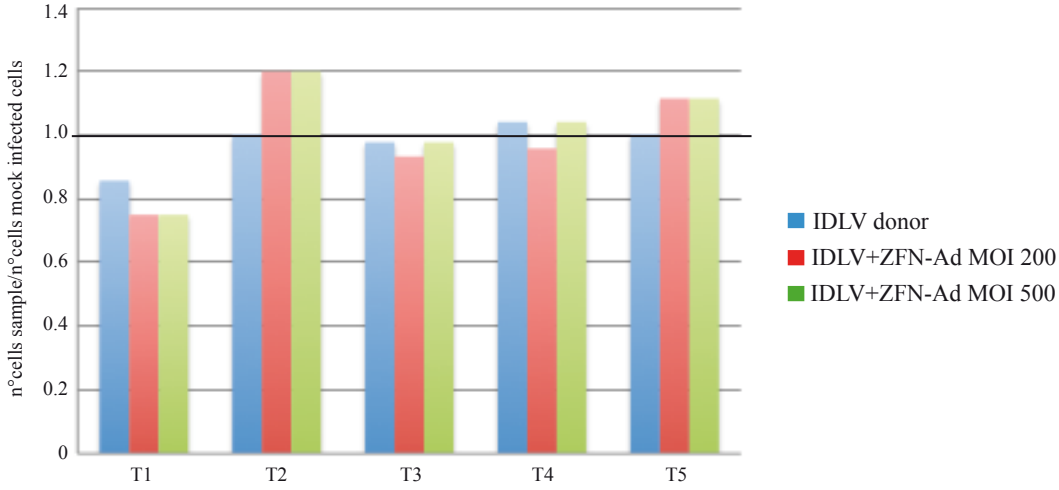
Supplementary figure 1. Southern blot analysis of genomic DNA extracted from HaCaT cell clones, digested with *AccI* that encompasses the AVVS1 target locus, and hybridized to a green fluorescent protein (GFP) probe. The expected band, corresponding to 4.6 kb indicates the presence of a GFP expression cassette correctly integrated into the target locus. Clones showing rearrangements or off-target integrations are highlighted by a black asterisks. The positions of restriction sites used for analysis are indicated. NTC: non treated cells. Molecular weight marker is indicated on the left.



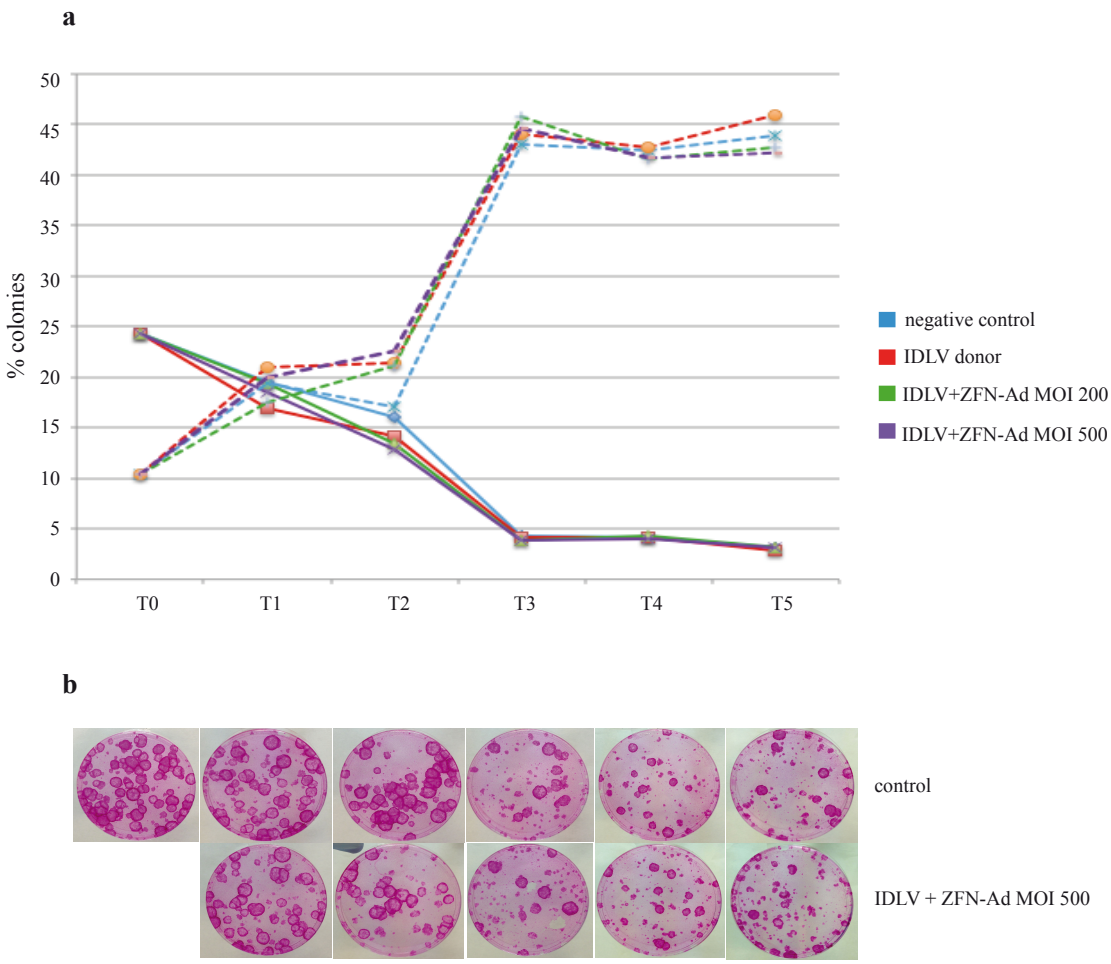
Supplementary figure 2. Southern blot analysis of genomic DNA from HaCaT cell clones, digested with *XbaI* single cutter in the donor cassette and hybridized to a GFP probe. A band corresponding to 8.3 kb indicates integration of one copy of GFP expression cassette into AAVS1 target locus. A band higher than 2kb indicates random integration of one copy of GFP cassette (black stars). Finally, 5.8kb band indicates the presence of integrated head-to-tail vector concatemers. The positions of restriction sites used for analysis are indicated. NTC: non treated cells. Molecular weight marker is indicated on the left.



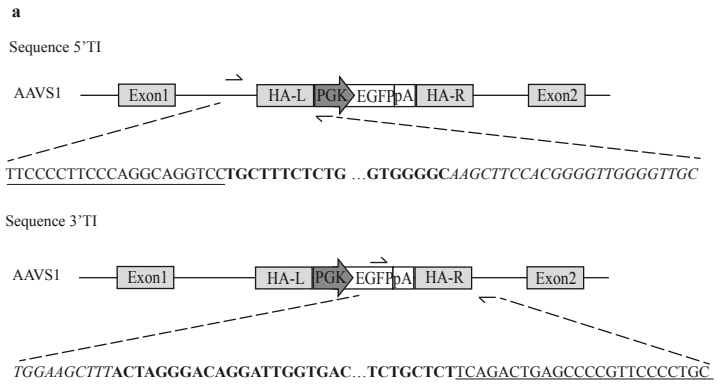
Supplementary figure 3. Human primary keratinocytes were infected with the indicated doses of donor IDLV (ng p24) or adenoviral vector serotype 5/50 (multiplicities of infection, MOI) carrying the donor GFP expression cassette. GFP expression was evaluated by FACS analysis 48h after transduction. MFI: mean fluorescence intensity.



Supplementary figure 4. Cell proliferation assay in untreated and treated human primary keratinocytes. The number of cells was determined at each passage after infection (T1-5) in both uninfected and IDLV donor/ZFN-Ad-coinfected cells (MOI 200 and 500). The ratio between the number of cells of each sample and the number of mock infected cells (reference value) is plotted for each cell passages. The black solid line indicates the reference value.



Supplementary figure 5. Colony-forming efficiency (CFE) assay in untreated and treated human primary keratinocytes. **a)** Quantitative analysis of growing colonies at passage T0 (infection time) and T1-T5 (post infection) in untreated (negative control) and treated cells.
% CFE (solid lines) and % of abortive colonies (dash lines) is calculated as reported in material and method. **b)** Representative images of clonal analysis performed on cells stained with rhodamine B at passage T0 and T1-T5 post infection in control and treated cells.



b

CCCACTGTTTCCCCTTCCCAGGCAGGTCC**TGCTTTCTCTGACCTGCATTCTCTCCCCTGGGCCTGTGCCGCTTT**
CTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCCCAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCA
TCTTCCTCCACTCCCTCTTCCCCTTGCTCTCTGCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCT
TCTCGGCGCTGCACCACGTGATGTCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCT
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TCGTTTCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGCATCA
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AGTCTTCTTCCTCCCAACCCGGGCCCCTATGTCCACTTCAGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGT
CCCCGAGCTGGGACCACCTTATATTCCAGGGCCGGTTAATGTGGCTCTTGGTTCTGGGTACTTTTATCTGTCCC
CTCCACCCCACAGTGGGGGC*AAGCTTCCACGGGGTTGGGGTT*

c

CCCACTGTTTCCCCTTCCCAGGCAGGTCC**TGCTTTCTCTGACCTGCATTCTCTCCCCTGGGCCTGTGCCGCTTT**
CTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCCCAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCA
TCTTCCTCCACTCCCTCTTCCCCTTGCTCTCTGCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCT
TCTCGGCGCTGCACCACGTGATGTCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCT
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CCTTACCTCCCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCATGGCATCTTCTAGGGGTCCGAGAGCTCAGCT
AGTCTTCTTCCTCCCAACCCGGGCCCCTATGTCCACTTCAGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGT
CCCCGAGCTGGGACCACCTTATATTCCAGGGCCGGTTAATGTGGCTCTTGGTTCTGGGTACTTTTATCTGTCCC
CTCCACCCCACAGTGGGGGC*AAGCTTCCACGGGGTTGGGGTT*

Supplementary figure 6. a) Sequencing of the amplified 5' and 3' junctions between the transgene cassette and the AAVS1 locus confirmed that ZFN-mediated targeted integration occurred by homologous recombination in sample #3.2 eight weeks after transplantation. **b**) and **c**) sequences of the 5' transgene-genome junction amplified on #2 and #5 spots. Bold, homology arm; Italic, expression cassette; Underlined, AAVS1 locus.

Supplementary Table 1. Comparison between cleavage efficiency (%indels) and percentage of GFP integration in the cell types tested.

Cell type	**% indels**	**% GFP**
HaCaT cells	29	20
immortalized primary keratinocytes	26	12.3
primary keratinocytes	9	0.3

Supplementary Table 2. List of primers used for targeted integration analyses.

Application	Primer ID	Sequence	Expected amplicon
Targeted integration in the AAVS1 locus (5' junction)			
	TI5 sense	5'-CCCACTGTTTCCCCTTCCCA-3'	0.9kb
	TI5 antisense	5'-AACCCCAACCCCGTGGAAG-3'	
Targeted integration in the AAVS1 locus (3' junction)	TI3 sense	5'-CGACAACCACTACCTGAGCA-3'	
	TI3 antisense	5'-CTGGGGCCTCTGGGGGAT-3'	1.3kb
	TI3Ad antisense	5'-GACCTGCCTGGAGAAGGAT-3'	1.7kb
Head-to-tail vector concatamers	Concatamers sense	5'-GCTCGGCTGTTGGGCACTG-3'	0.7kb
	Concatamers antisense	5'-CGCACCCATCTCTCTCCTTCTA-3'	
Cel-I assay	Cel-I sense	5'-CTTGCTTTCTTTGCCTGGAC-3'	0.6kb
	Cel-I antisense	5'-AGGTTCTGGGAGAGGGTAG-3'	
Deep sequencing of the AAVS1 locus	HS sense	5'-GGAGAGAGATGGCTCCAGGAA-3'	0.2kb
	HS antisense	5'-ATGTGGCTCTGGTTCTGGGTACT-3'	
GFP	GFP sense	5'-TGACCCTGAAGTTCATCTGC-3'	0.49kb
	GFP antisense	5'-GACTGGGTGCTCAGGTAGTG-3'	
GAPDH	GAPDH sense	5'-GACCACAGTCCATGCCATCAC-3'	0.47kb
	GAPDH antisense	5'-TCCACCACCCTGTTGCTGTAG-3'	