
Figures and figure supplements

Targeted genome editing by lentiviral protein transduction of zinc-finger and TAL-effector nucleases

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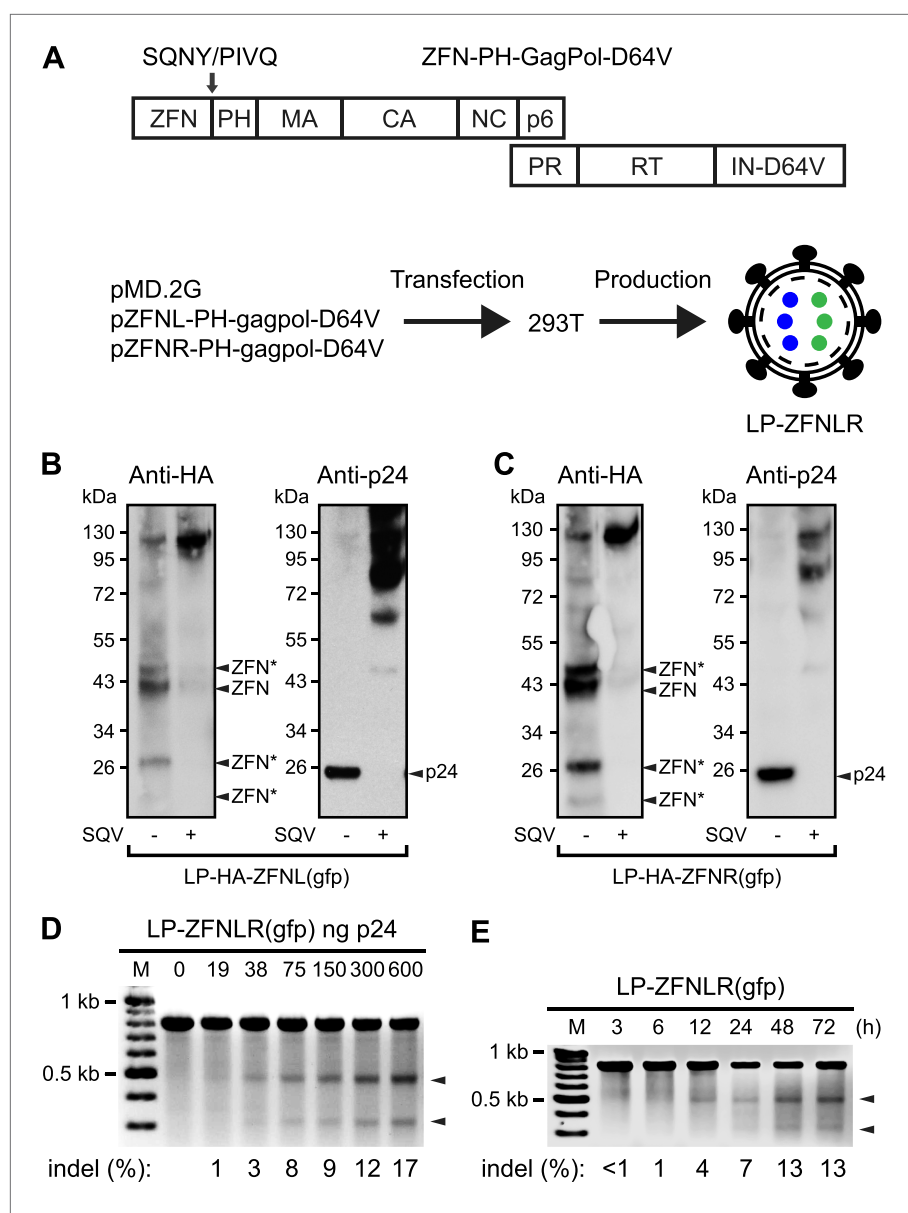


Figure 1. Targeted *egfp* gene disruption by lentiviral delivery of ZFN proteins. **(A)** Schematic representation of the composition of Gag and GagPol polypeptides encoded by ZFN-encoding packaging constructs (top) and the production of ZFN-loaded LPs (bottom). Gag is composed of the N-terminal ZFN domain, an HIV-1 cleavage site (SQNY/PIVQ), the phospholipase C- δ 1 pleckstrin homology (PH) domain, matrix (MA), capsid (CA), nucleocapsid (NC), and p6, whereas Pol consists of protease (PR), reverse transcriptase (RT), and integrase harboring the D64V mutation (IN-D64V). LPs harboring two types of ZFNs (indicated by blue and green dots inside the virion) are produced by co-transfecting 293T cells with pMD.2G (encoding VSV-G surface protein) and pZFNL-PH-gagpol-D64V and pZFNR-PH-gagpol-D64V encoding ZFNL and ZFNR, respectively. **(B)** and **(C)** Analysis of the contents of LPs by Western blot using HA- and p24-specific antibodies. HA-tagged ZFNs were incorporated in this LP batch, allowing detection of ZFNs and ZFN derivatives (left panel). ZFNs originating from non-intentional processing or cleavage at cryptic HIV-1 cleavage sites are indicated by arrows labeled with ZFN*. The same membrane was stripped and re-used for detection of p24 (right panels). It is indicated below each panel whether 0.2 μ M of the protease inhibitor Saquinavir (SQV) was included during LP production. **(D)** *egfp* gene disruption by protein transduction of ZFNs in HEK293-eGFPmut reporter cells as measured by Surveyor nuclease-based detection of DNA mismatches. Cells were harvested for analysis 24 hr posttransduction. Arrowheads point to the specific cleavage products. Quantified locus modification rates (indel %) are indicated below relevant lanes. **(E)** *egfp* gene disruption by ZFN proteins at different time points after transduction. HEK293-eGFPmut reporter cells were transduced with 300 ng p24 LP-ZFNLR(gfp). Locus modification rates (indel %) are provided below the gel.

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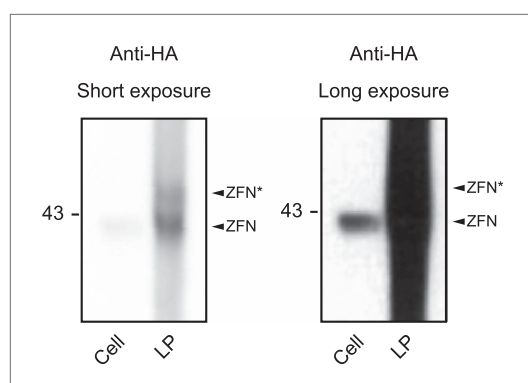


Figure 1—figure supplement 1. Validation of the release of full-length ZFN protein within ZFN-loaded LPs.

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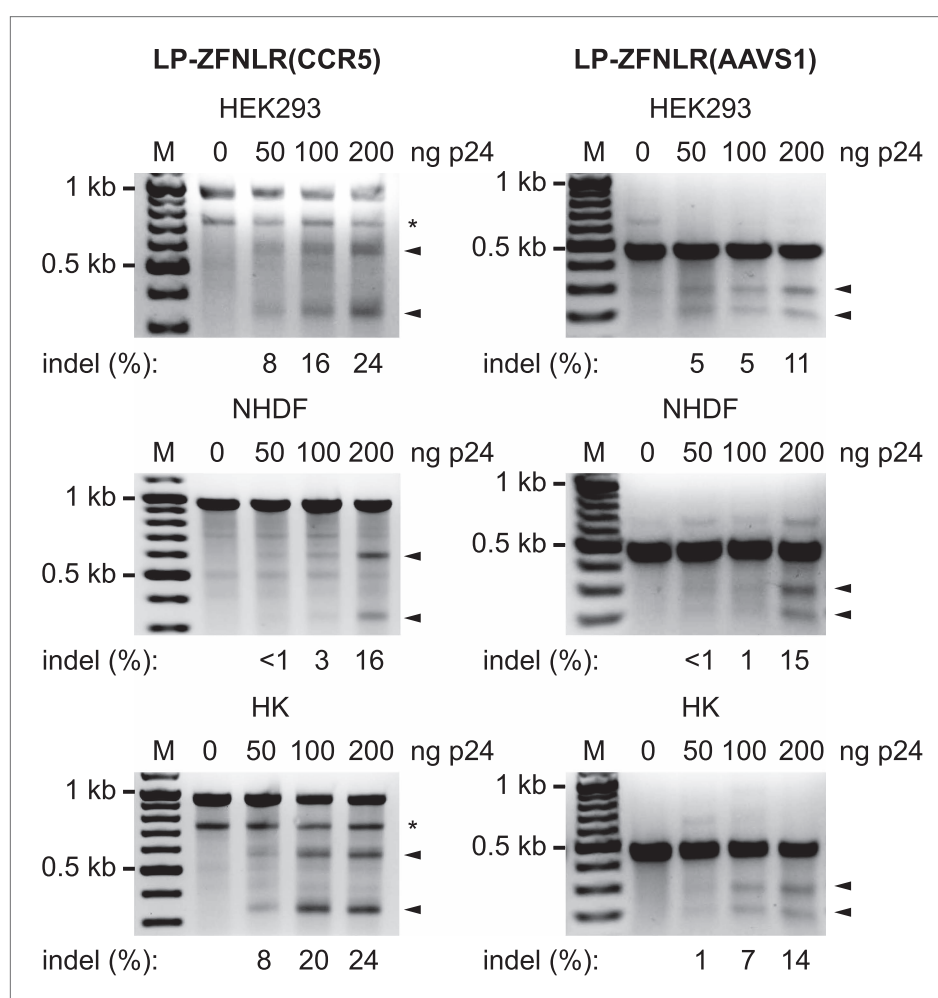


Figure 2. Targeted disruption of endogenous genes by protein transduction of ZFNs. HEK293, normal human dermal fibroblasts (NHDFs) and primary human keratinocytes (HKs) were transduced with increasing amounts of LPs containing ZFNs targeting the CCR5 (left) and AAVS1 (right) loci. Cells were harvested for analysis 24 hr posttransduction. Arrowheads indicate specific cleavage products, whereas fragments marked with * were generated due to the presence of the CCR5 Δ 32 allele in the analyzed cells. Quantified locus modification rates are indicated below relevant lanes.

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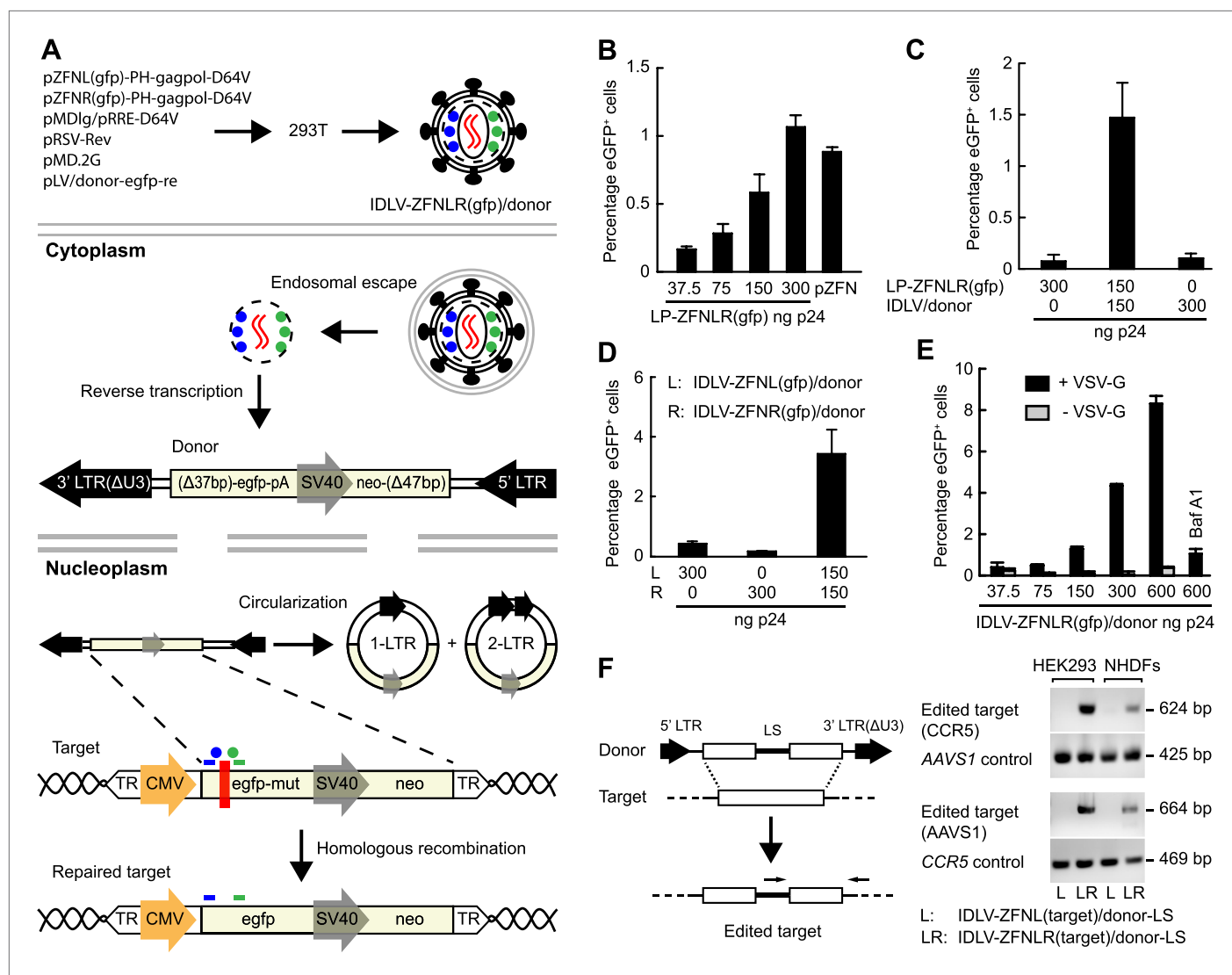


Figure 3. Targeted gene editing by ‘all-in-one’ IDLVs. **(A)** Schematic representation of the production and intracellular action of IDLVs carrying two ZFNs (indicated by blue and green dots) and a vector with the donor sequence for HR-directed repair (indicated in red as an RNA homodimer). Upon endosomal escape and uncoating, the donor sequence is reverse-transcribed to double-stranded DNA that is imported to the nucleus, where it serves as a donor for repair either in the form of linear DNA or as 1-LTR or 2-LTR circles (only HR between linear DNA and the target is shown). Homologous sequences are highlighted in light yellow. The *egfp* gene harboring internal mutations (indicated by a red box) is repaired through ZFN-mediated cleavage and HR using the reverse-transcribed vector as a recombination donor. **(B–E)** Correction of the *egfp* gene by lentiviral delivery of ZFN proteins. Flow cytometric analysis was performed 4 days after transduction or transfection. In **(B)**, donor plasmid (pLV/egfp-donor-fw) was transfected 6 hr prior to ZFN protein transduction. Co-transfection of donor plasmid and ZFN-encoding plasmid DNA (pZFN) served as a positive control. In **(C)**, the donor sequence was provided by IDLV/donor (MOI of 46) co-transduced with LP-ZFNLR(gfp), whereas in **(D)** correction was achieved by co-transduction with two IDLVs (IDLV-ZFNLR(gfp)/donor and IDLV-ZFNLR(gfp)/donor, respectively), both at an MOI of 9, loaded each with one of the two *egfp*-targeting ZFNs. In **(E)**, ‘all-in-one’ IDLVs (IDLV-ZFNLR(gfp)/donor) induced potent gene correction. Gene editing was measured with virus loads ranging from an MOI of 2 (corresponding to 37 ng p24) to an MOI of 34 (corresponding to 600 ng p24). IDLVs without the VSV-G surface protein as well as reporter cells pretreated with of 1 μ M Bafilomycin A1 (Baf A1) served as negative controls. **(F)** Targeted editing at endogenous *CCR5* and *AAVS1* loci. Schematic representation of PCR-based assay used for detection of gene editing at *CCR5* and *AAVS1* loci (left panel). Primers are indicated above the edited target sequence. LS, linker sequence. Gene editing at *CCR5* and *AAVS1* loci in HEK293 cells and NHDFs, as confirmed by PCR (right panel), was obtained with an MOI of 34. PCR fragments amplified from the *AAVS1* locus and the *CCR5* locus served as controls for *CCR5*- and *AAVS1*-directed LS insertion, respectively. The error bars represent \pm SD from three independent replicates of the experiment.

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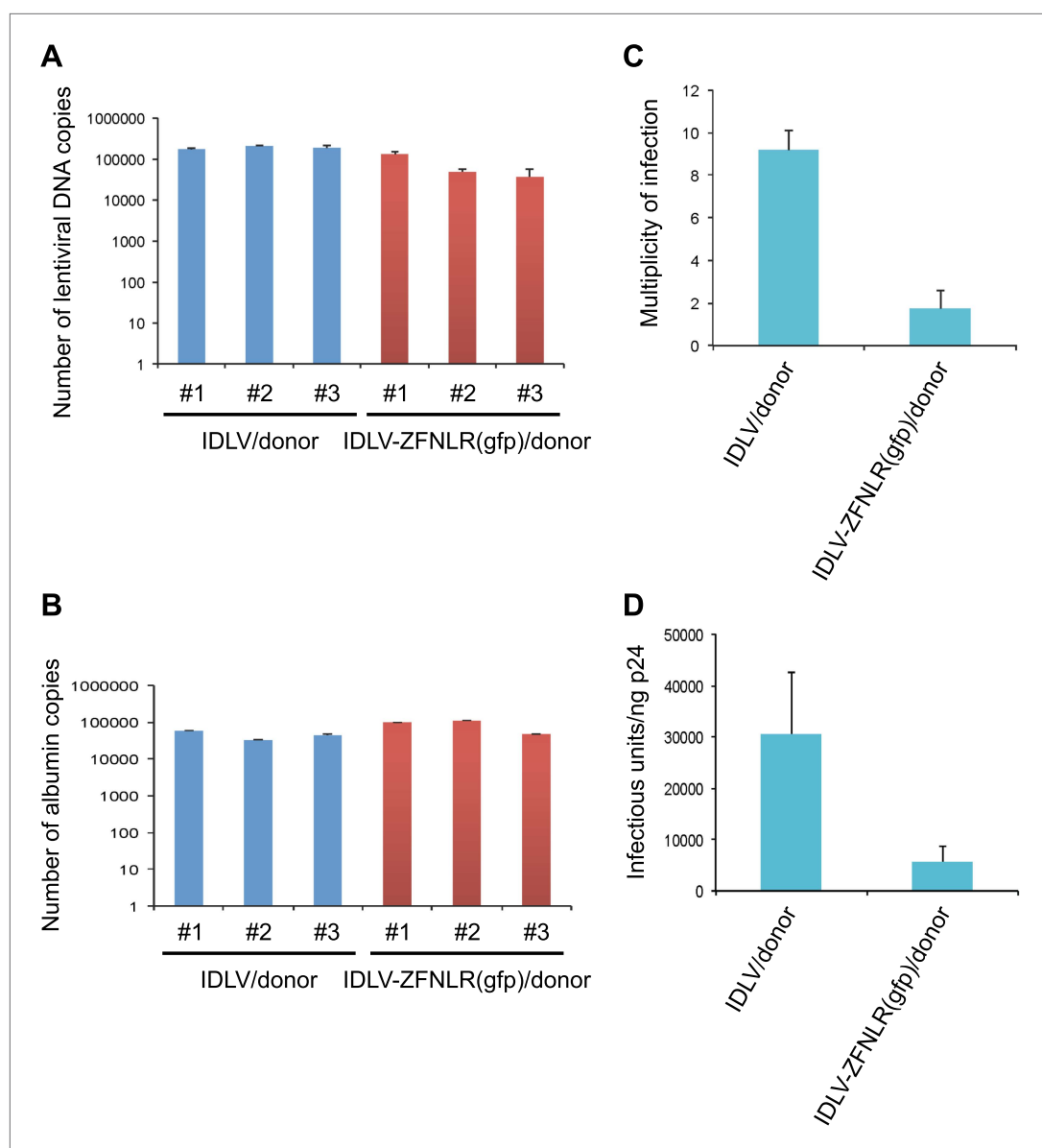


Figure 3—figure supplement 1. Determination of multiplicity of infection (MOI) of IDLVs carrying left and right ZFNs.

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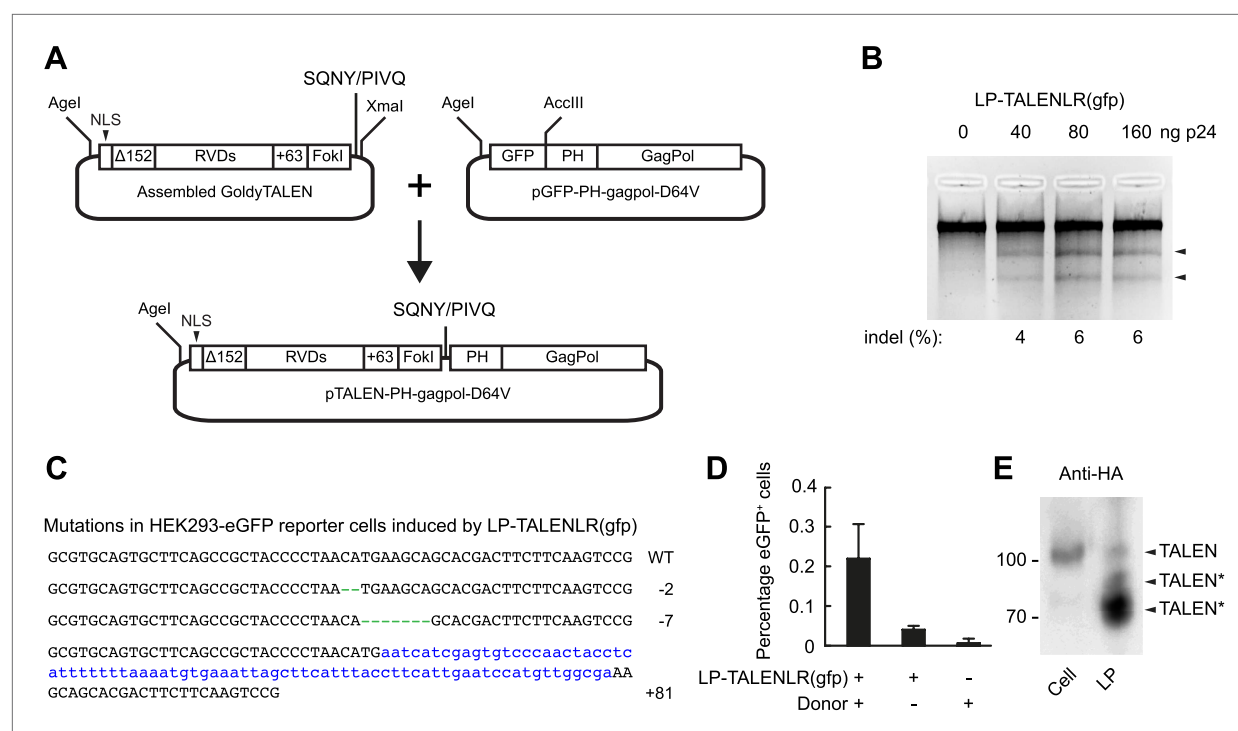


Figure 4. Targeted *egfp* gene editing by lentiviral delivery of TALEN proteins. **(A)** Schematic representation of the construction of the TALEN-GagPol polypeptide expression construct. GoldyTALEN was assembled as the Golden Gate assembly method into a shuttle plasmid pC-Goldy-TALEN-PH and was then cut out and cloned into pGFP-PH-gagpol-D64V to get the destination construct pTALEN-PH-gagpol-D64V expressing polypeptides composed of GoldyTALEN and GagPol connected by the HIV-1 protease cleavage site SQNY/PIVQ. NLS, SV40 nuclear localization signal, $\Delta 152$, 152 amino acids deletion from the wild-type TALE protein, RVDs, repeat variable di-residues, +63, 63 amino acids following the last repeat, PH, phospholipase C- $\delta 1$ pleckstrin homology domain. **(B)** *egfp* gene disruption by protein transduction of TALENs in HEK293-eGFPmut reporter cells as measured by Surveyor nuclease assay 24 hr posttransduction. **(C)** Sequences of *egfp* gene disruption by TALEN protein transduction in the HEK293-eGFPmut reporter cell line. Genomic DNA of HEK293-eGFPmut reporter cells transduced with 160 ng p24 LP-TALENLR(gfp) was used as PCR template for amplification and subsequent cloning of the part of the *egfp* gene encompassing the region recognized by the two TALENs. The wild-type sequence is shown at the top. The net change of length caused by the indels is indicated to the right of each sequence. Green dashes represent deleted nucleotides, whereas blue lower case letters illustrate inserted nucleotides. Three alleles out of 46 sequenced clones were found to be disrupted (disruption frequency: 6.5%). **(D)** Targeted *egfp* gene repair in HEK293-eGFPmut reporter cells. Cells were transfected with 1.8 μ g donor plasmid (pLV/*egfp*-donor-fw) and transduced with 160 ng p24 of LP-TALENLR(gfp) 6 hr later. Cells treated only with LP-TALENLR(gfp) or donor served as negative controls. eGFP expression was analyzed by flow cytometry 15 days posttransduction. **(E)** Analysis of the contents of LP-HA-TALENLR(gfp) by Western blot using HA-specific antibody. The left lane shows protein derived from 293T cells expressing right HA-TALEN(gfp) from transfected plasmid pcDNA3.1-Goldy-HA-TALENLR(gfp). The expected size of the full-length TALEN is indicated, and truncated TALEN derivatives originating from non-intentional cleavage at cryptic, internal HIV-1 cleavage sites are indicated by an asterisk (*). The error bars represent \pm SD from three independent replicates.

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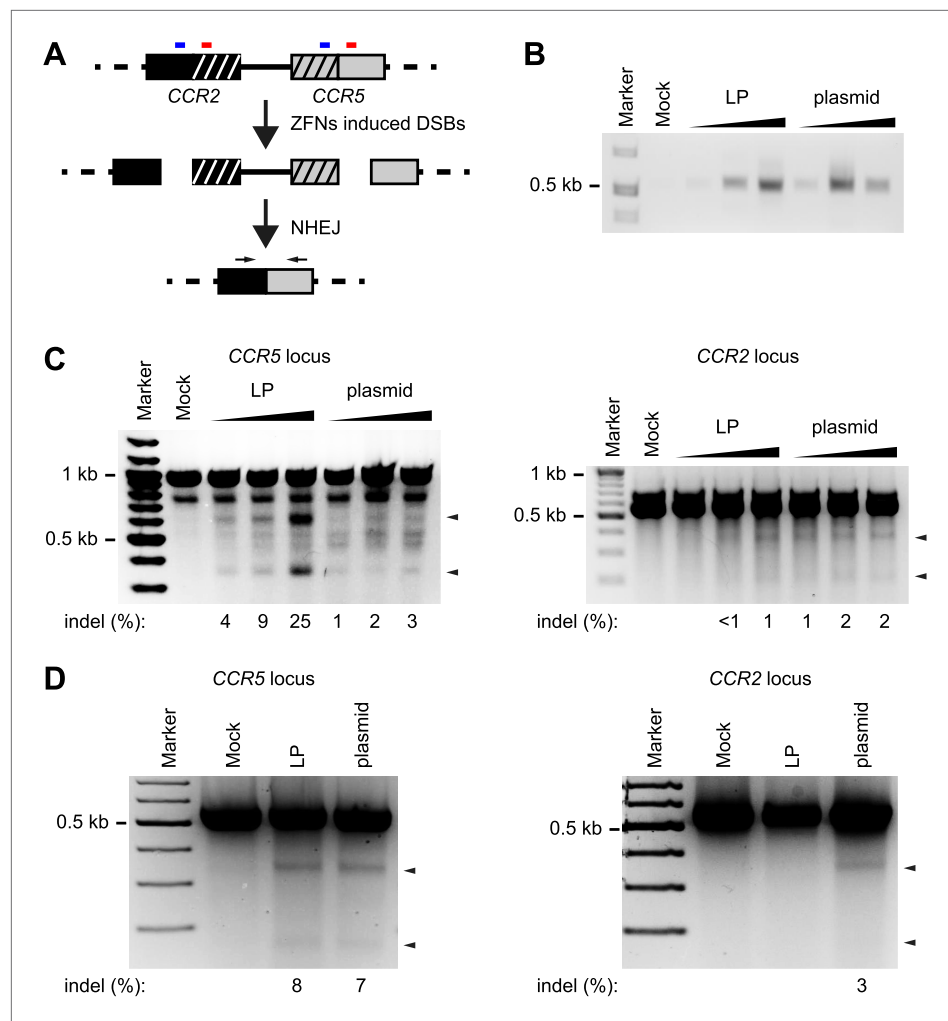


Figure 5. On- and off-target activity by CCR5-targeting ZFNs delivered by LP protein transduction or plasmid transfection. **(A)** Schematic representation of the PCR-based assay used for detection of simultaneous on-target and off-target cleavage at the CCR5 and CCR2 loci, respectively. Small blue and red boxes above each locus indicate the location of the two ZFN recognition sites in the CCR5 locus and the highly homologous sequences located within the CCR2 locus. Horizontal arrows indicate the location of sequences recognized by the primers used. **(B)** Detection of off-target cleavage by PCR-based detection of CCR2-CCR5 fusion fragments. HEK293 cells were seeded at a density of 1×10^5 cells/well on day 1 and were on day 2 either transduced with increasing amounts of LP-ZFNLR(CCR5) (50, 100, 200 ng p24, respectively; indicated by 'LP') or co-transfected with increasing amounts of the two ZFN-encoding plasmids (50 ng + 50 ng, 100 ng + 100 ng, 200 ng + 200 ng, respectively; indicated by 'plasmid'). On day 3, cells were harvested for genomic DNA purification. **(C)** Disruption within the CCR5 and CCR2 loci after LP- and plasmid-directed ZFN delivery. HEK293 cells were treated with increasing amounts of LP-ZFNLR(CCR5) and ZFN-encoding plasmids as in **(B)**. Sequence changes introduced by NHEJ-directed repair were identified by Surveyor nuclease-directed detection of mismatches in re-annealed PCR products. Analyses of CCR5- and CCR2-directed cleavage were performed on the same genomic DNA samples. Arrowheads indicate specific cleavage products. Quantified locus modification rates are indicated below each gel. **(D)** Distinct levels of CCR2 gene disruption using experimental conditions that support similar levels of CCR5 disruption after LP- and plasmid-directed ZFN delivery. HEK293 cells were treated by LP transduction (80 ng p24) or plasmid transfection (300 ng + 300 ng), essentially as described in **(B)**, facilitating similar levels of gene disruption in the CCR5 locus. Surveyor nuclease assays were performed as in **(C)** except for the use of an alternative CCR5 primer set. Quantified locus modification rates are indicated below relevant lanes.

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