LEDGIN (ALLINI) Disruption of HIV-1 Assembly Does Not Involve LEDGF/p75

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Abstract

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Background: LEDGF/p75, the product of the *PSIP1* gene, is an HIV-1 integration cofactor that tethers the viral pre-integration complex to the site of its subsequent integration. Allosteric integrase inhibitors or ALLINIs (also known as LEDGINs) disrupt the interaction of LEDGF/p75 with the HIV-1 IN dimer and impair integration. However, a considerably more potent (and apparently main) mechanism of ALLINI action was recently identified: disrupting proper particle assembly. It is not clear whether this antiviral effect involves LEDGF/p75. Some studies suggest that it is LEDGF/p75-independent but others suggest LEDGF/p75-dependence and, consistent with this, that LEDGF/p75 incorporation into HIV-1 particles occurs and is necessary for normal HIV-1 infectivity. RNAi-depleted cells still contain some LEDGF/p75 protein, and a fractionally minute LEDGF/p75 residuum can maintain integration cofactor function. Mouse *PSIP1* gene knockout cell lines exist but cannot be used for HIV assembly studies. Here we definitively answer two questions: does LEDGF/p75 play a role in HIV-1 assembly and does the ALLINI antiviral mechanism involve LEDGF/p75? To do so, we used TAL effector nucleases (TALENs) to delete the *PSIP1* gene from informative human cell lines.

Methodology: We designed and constructed multiple TALEN pairs that site-specifically target *PSIP1*. We used them to generate clonal Jurkat and 293T cell lines that are -/- for the entire 42 kb gene, and separately, -/- for the only the exons that encode the LEDGF/p75 integrase binding domain (IBD). Correct deletion of all *PSIP1* alleles was verified at the DNA and protein levels, precise chromosomal deletion junctions were determined, and comprehensive viral life cycle analyses were conducted in the presence and absence of drug.

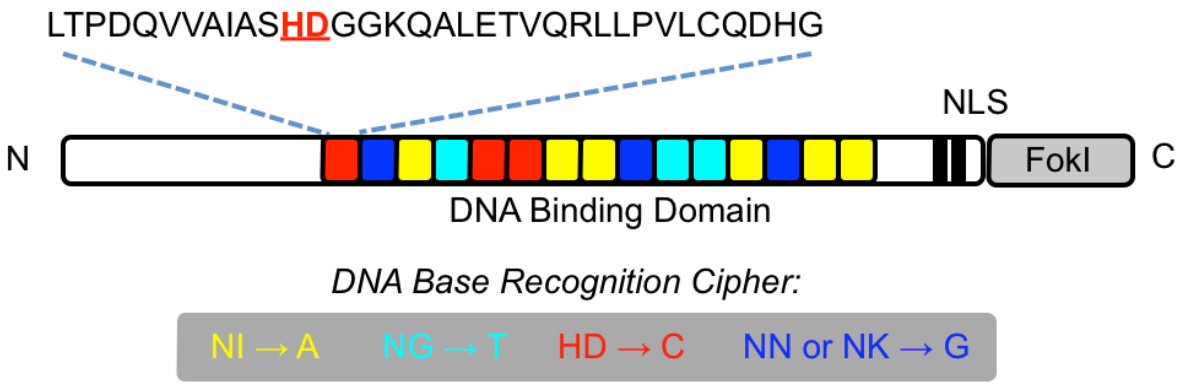
Results: HIV-1 integration is impaired, and spreading viral replication is blocked, but HIV-1 production and infectious particle assembly are normal in *PSIP1* -/- cells. This is the case whether the entire gene is deleted or only the IBD-encoding gene segment is deleted. We further determined that ALLINI potency for blocking HIV-1 infectious particle formation is unaffected by total removal of LEDGF/p75-encoding capacity from the cell. TALEN gene KO was efficient. For example, a third of single cell clones generated from a single transfection were found to contain bi-allelic gene disruptions due to NHEJ-generated indels at the specific target site, suggesting efficacy for gene targeting cure strategies aimed at this and other HIV dependency factor genes such as *CCR5*.

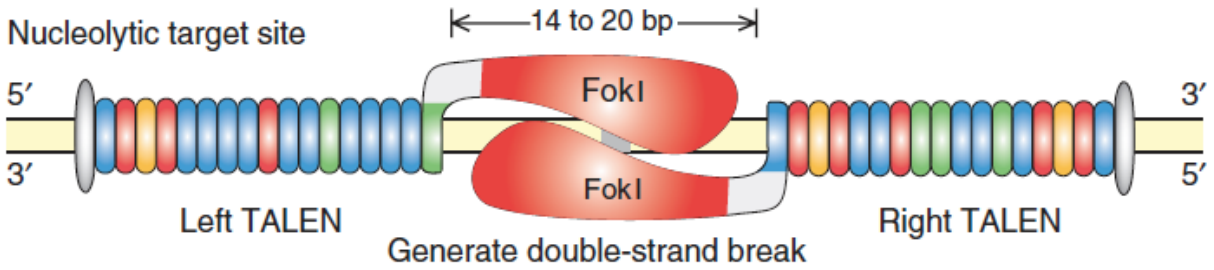
Conclusions: The main ALLINI mechanism is independent of LEDGF/p75. This protein also does not play a detectable role in particle assembly. The LEDGF/p75 cofactor role is confined to the integration step of the viral life cycle.

Background

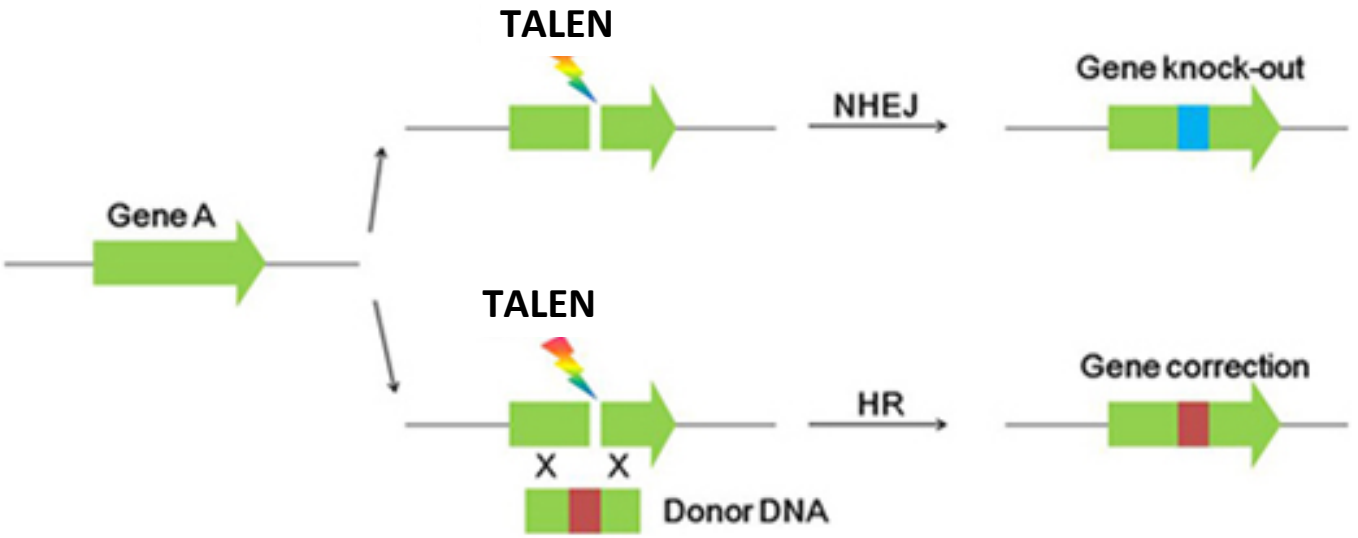
TAL Effector Nucleases

- Bacterial plant pathogens of the genus *Xanthomonas* inject TAL effector proteins that bind specific DNA sequences and alter plant host cell transcription.
- They contain a conserved tandem of 33-35 amino acid repeats that differ mainly at positions 12 and 13. These are termed repeat variable diresidues (RVDs).
- Different RVDs recognize different DNA base pairs, which creates a simple code: the order of RVD repeats in the TALE corresponds to the nucleotide sequence of the target DNA.
- Customized TALENs are designable with TALEs that recognize a specific DNA sequence at the N-terminus; these are fused to an NLS and FokI at the C terminus. A pair of TALENs are built to bind sequences to the right and the left of the target DNA, causing a DSB when the two FokI domains dimerize. Error-prone NHEJ leads to gene-disrupting indels at the target.
- Use of two TALEN pairs can produce full deletion of the intervening chromosome DNA and provision of a template in trans can lead, via HR, to gene replacement, editing or knock-in.



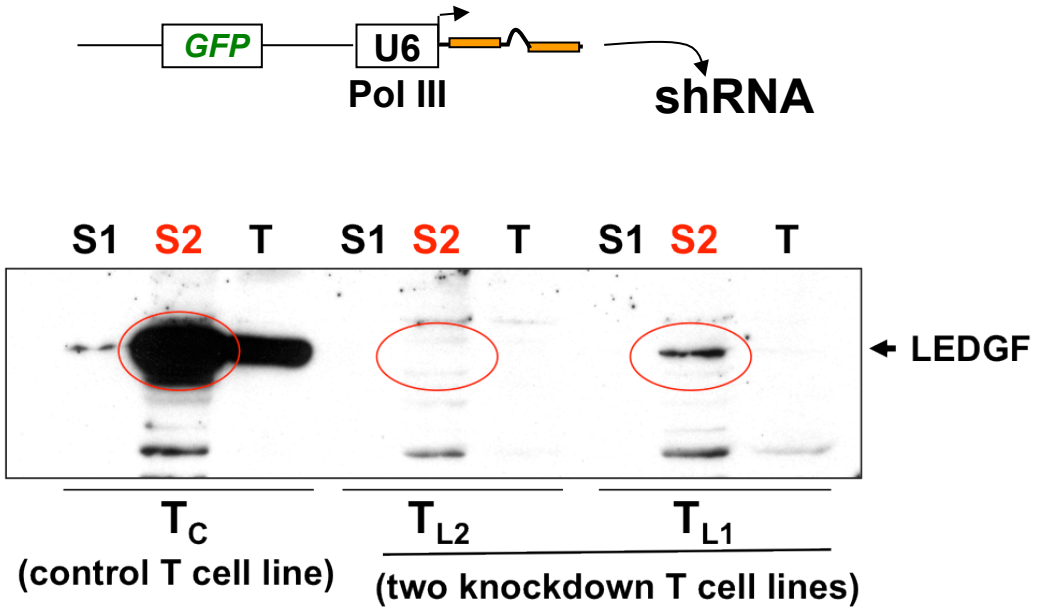


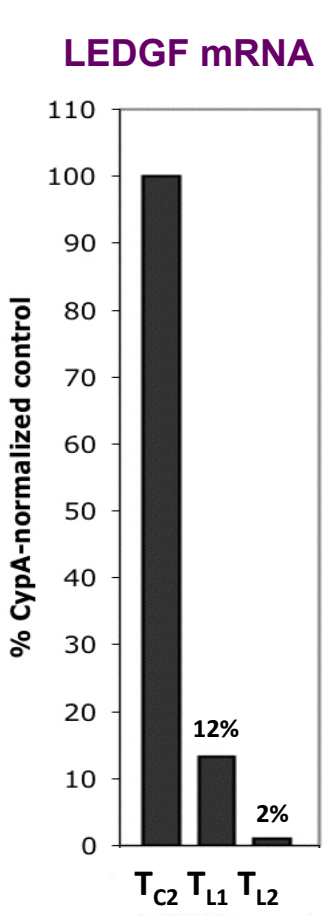
In vivo TALEN-induced genome editing outcomes

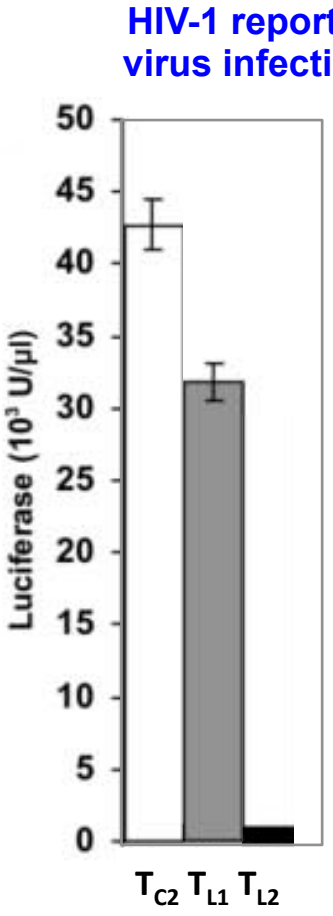


A very small fractional chromatin-bound residuum of LEDGF can maintain cofactor function: strip it from chromatin in CD4+ T cells and HIV-1 is impaired

Llano et al., *Science*, 2006

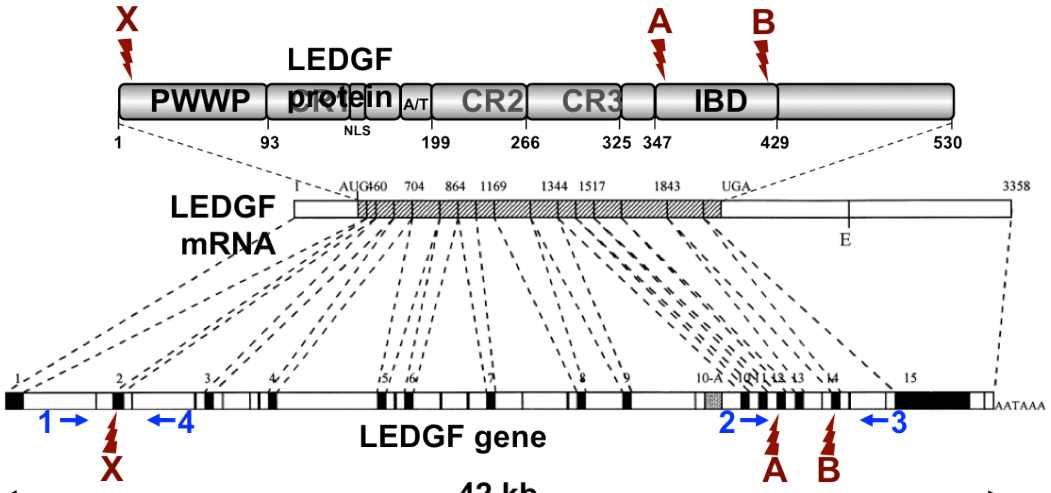


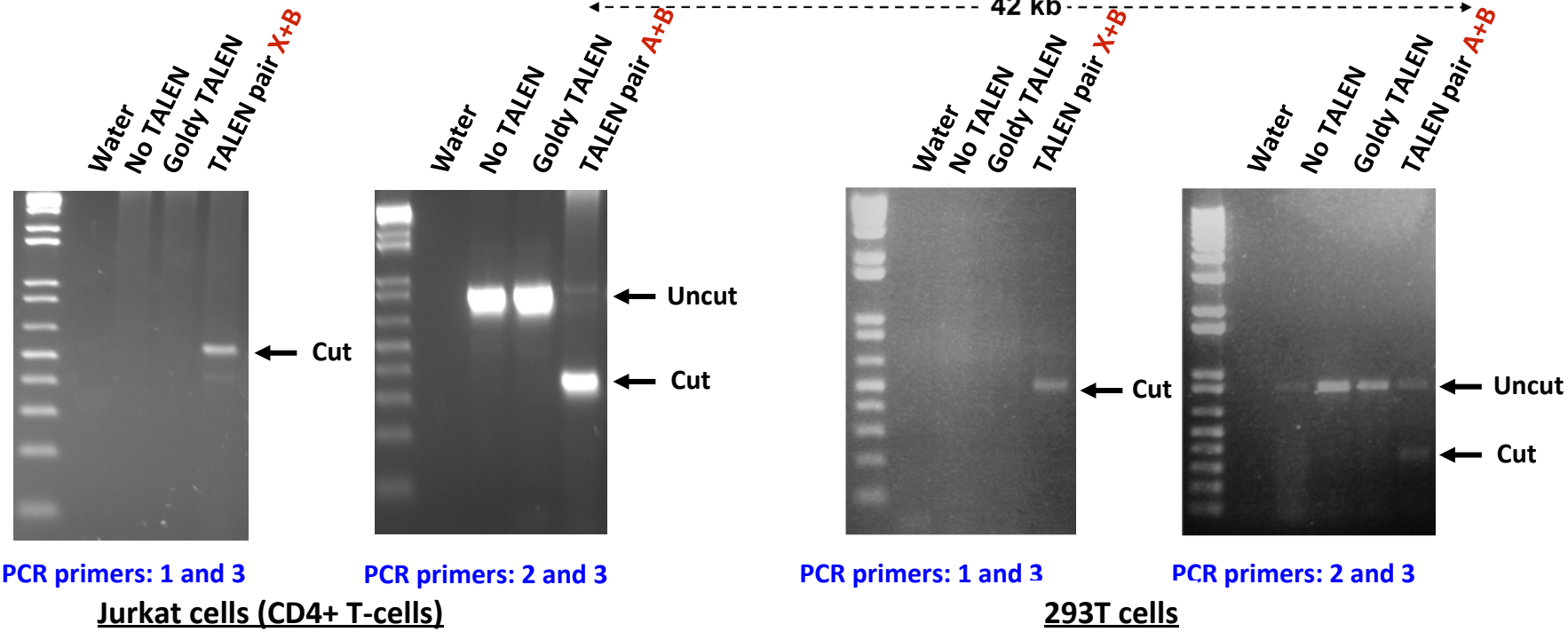




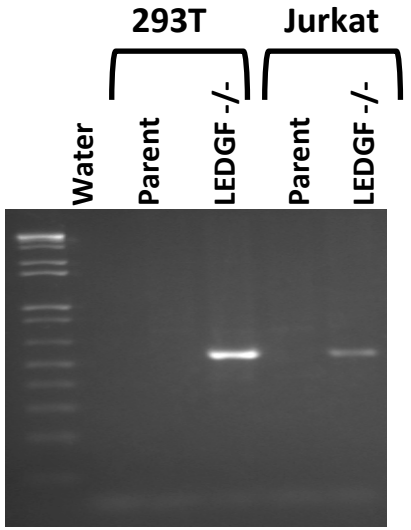
Results

Efficient TALEN mediated gene targeting of *PSIP1* gene

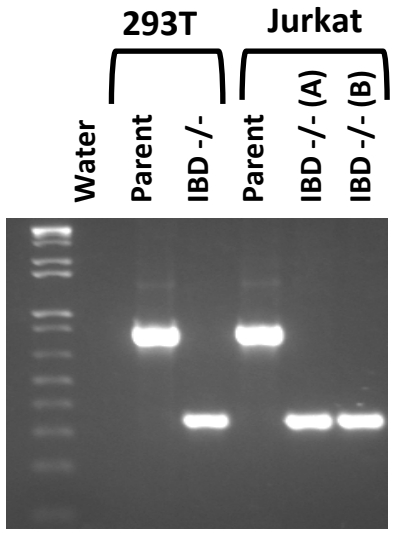




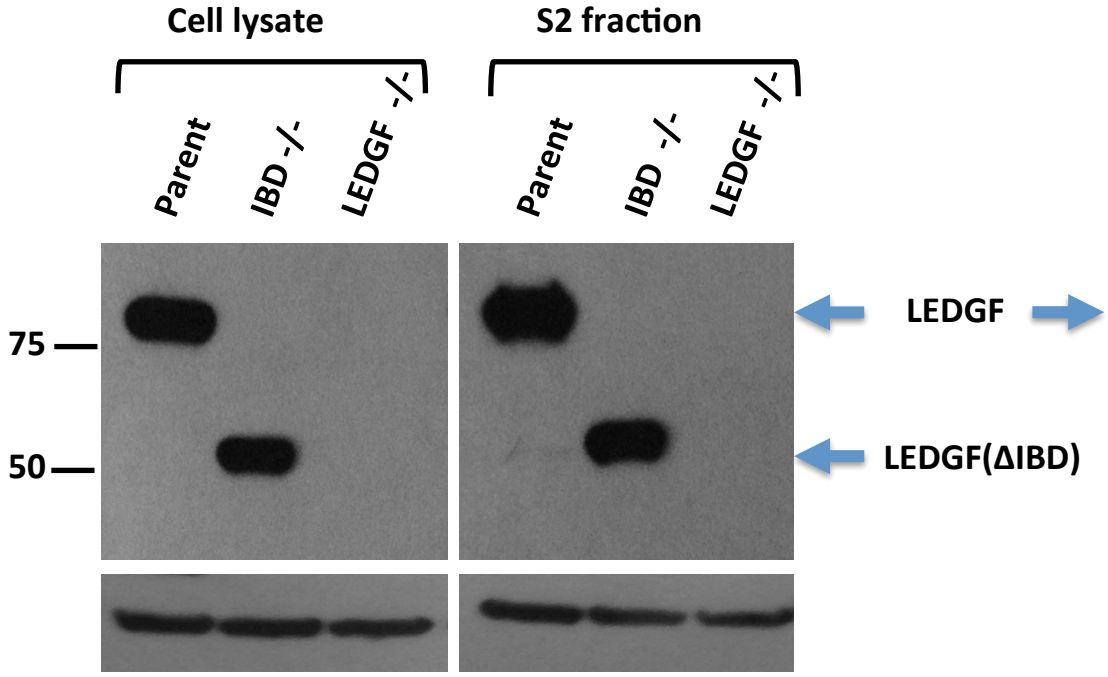
TALEN mediated deletion of PSIP1 or IBD exons in 293T and Jurkat cell lines



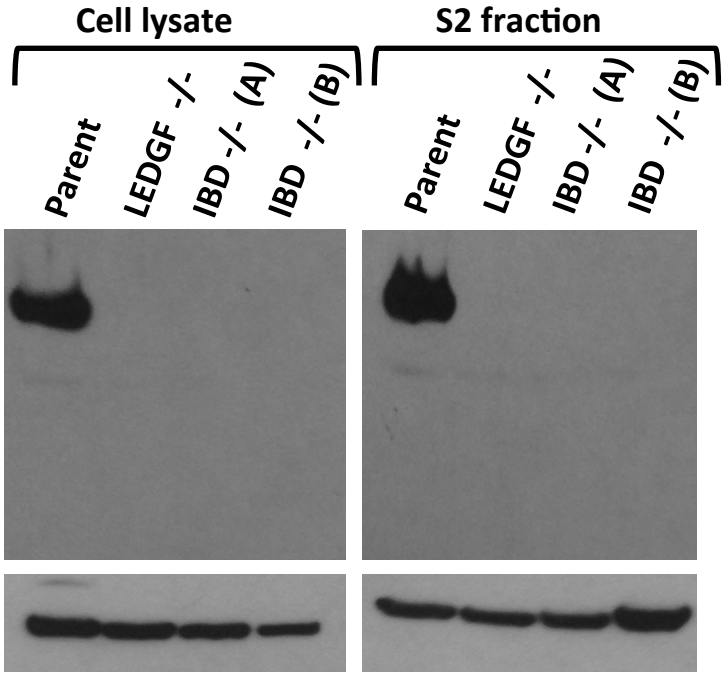
PCR primers: 1 and 3



PCR primers: 2 and 3



293T cells



Jurkat cells

Clones indels sequencing

TALEN pair X and B

Gene sequence

TALEN X spacer — TALEN B spacer

gactcgcgatttcaaACCTGGAGACCTCAT----------/ 42Kb /----------AAGTCTACAATGTTGtataacaagtttaag

Jurkat *PSIP1* -/- (X to A): gactcgcgatttcaaACCAGGttaag

gactcgcgatttAACTAGgttaag

293T *PSIP1* -/- (X to A): gactcgcgatttcaaACCTGGAGGAGAATGTTGtataacaagtttaag

TALEN pair A and B

Gene sequence

TALEN A spacer — TALEN B spacer

caatggattctcgacTTCAAAGGATACATGC----------/ 0.5 kb /---------AAGTCTACAATGTTGtataacaagtttaag

Jurkat IBD -/- Clone A: caatggattctcgacTTTTGtataacaagtttaag

catggattctcgaccTTCAAAGAAGTCTACAATGTTGtataacaagtttaag

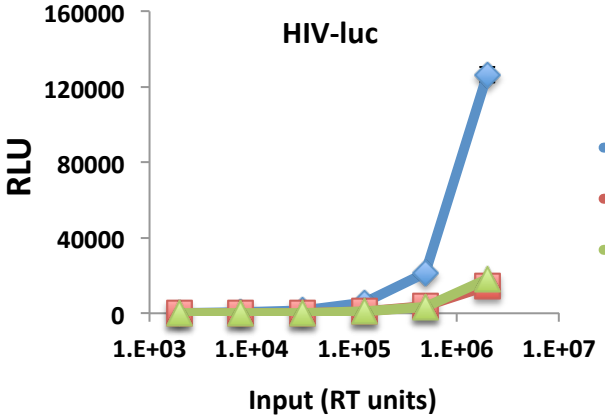
Jurkat IBD -/- Clone B: caatggattctcgacTTCAAAGGATTTACCACAATGTTGtataacaagtttaag

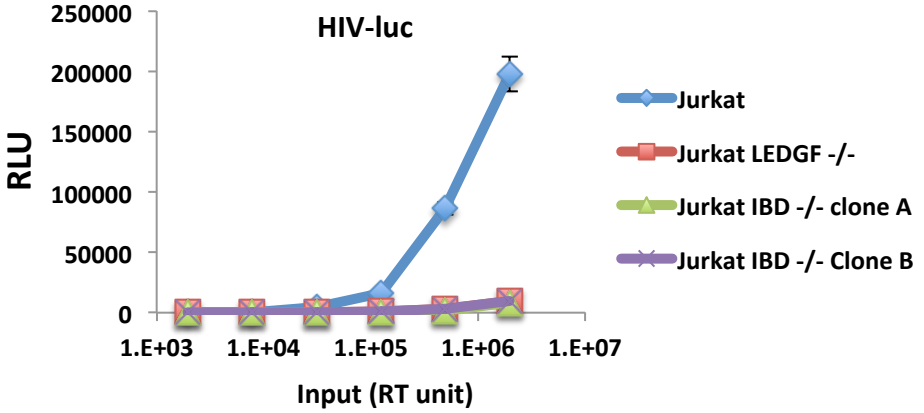
aggaacaaatggaaactgagcaCTTAAATGagtttaag

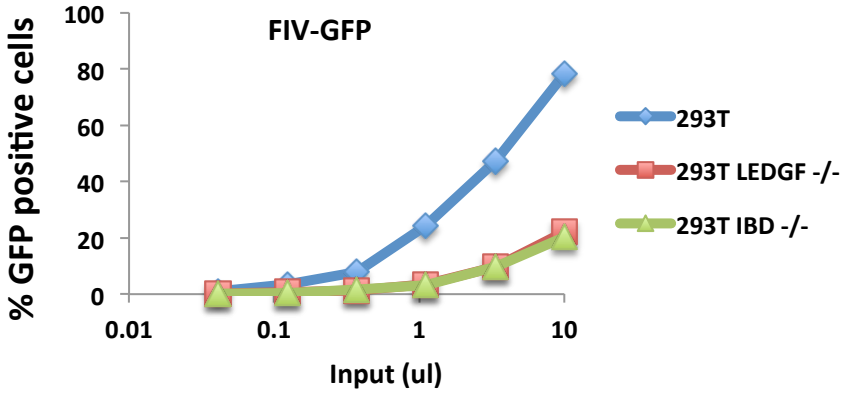
293T IBD -/-: caatggattctcgacTTCAAGTTGtataacaagtttaag

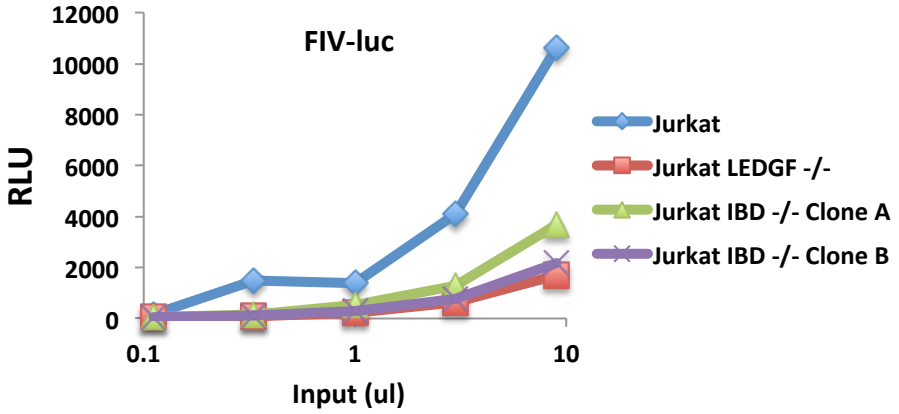
caatggattctcgacTTCAGTGTATAACAAGTTTGagaacatgttcttggtt

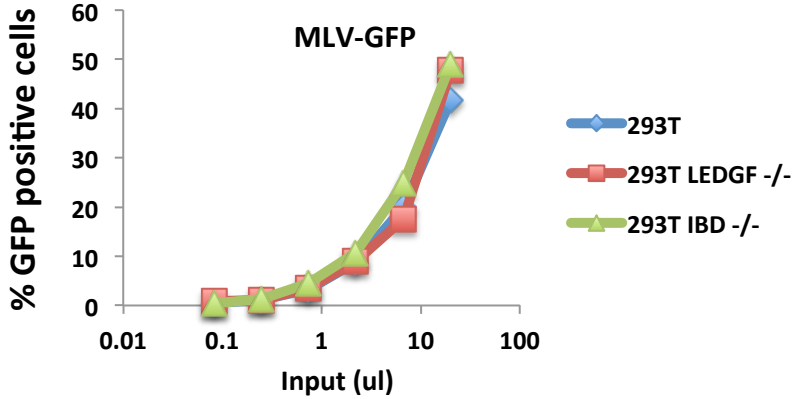
Lentiviral infection is impaired in LEDGF KO cells

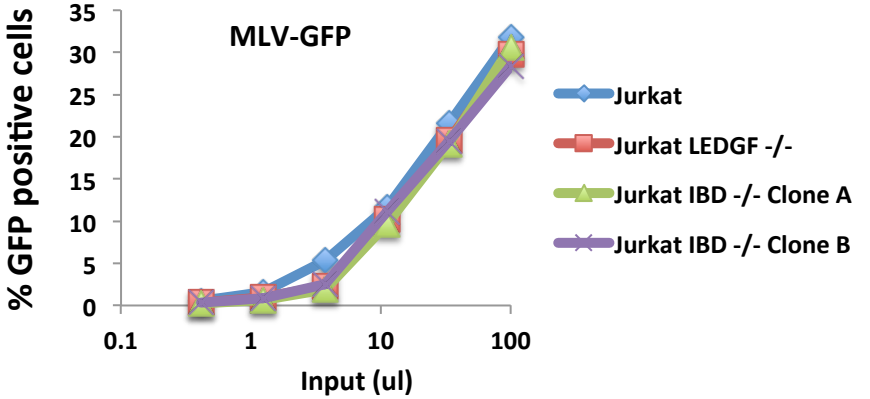




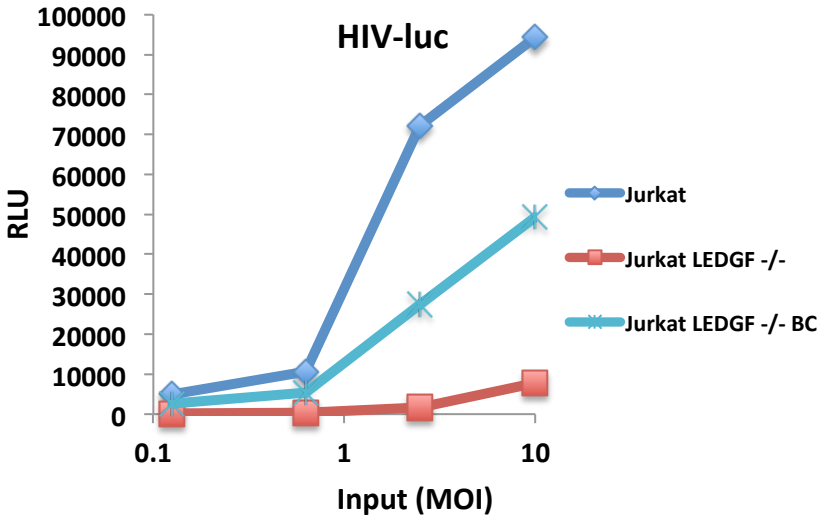


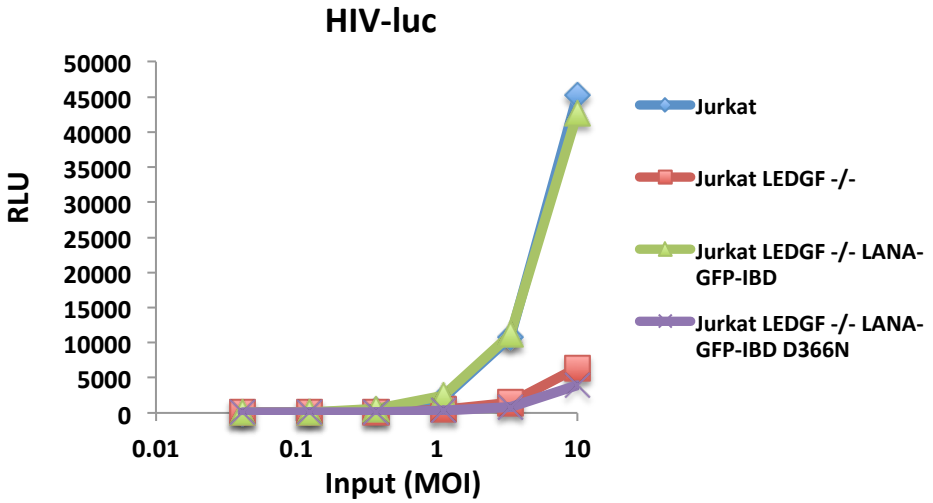




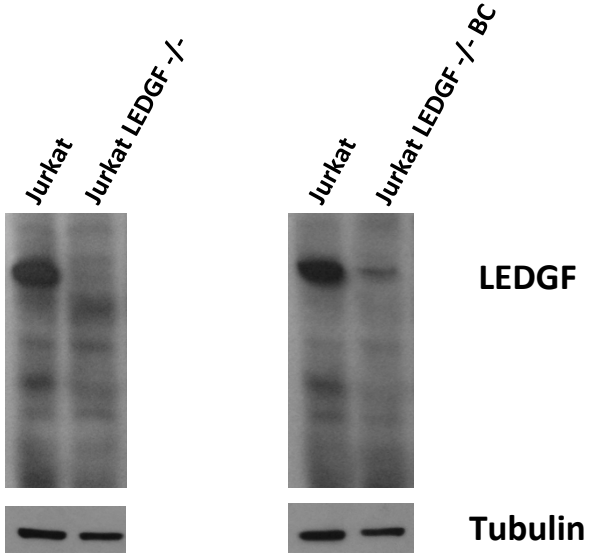


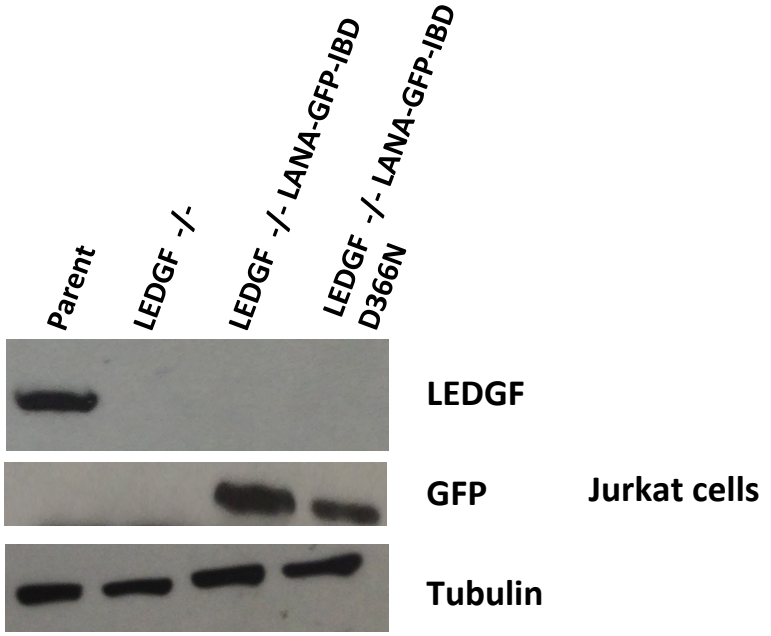
HIV-1 infectivity is rescued in T-cells by re-expression of LEDGF or expression of an alternatively tethered IBD (LANA31-GFP-IBD)



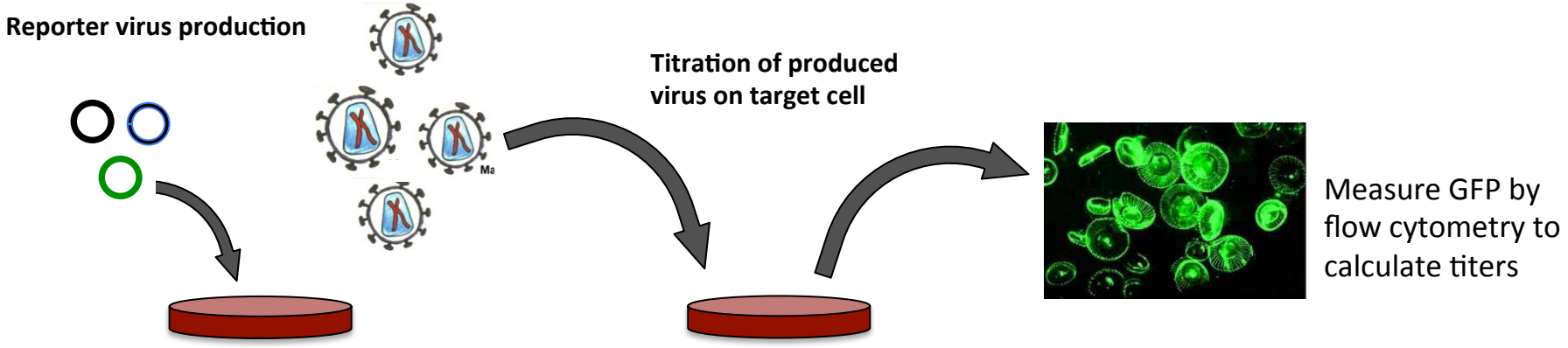


BC= back-complemented





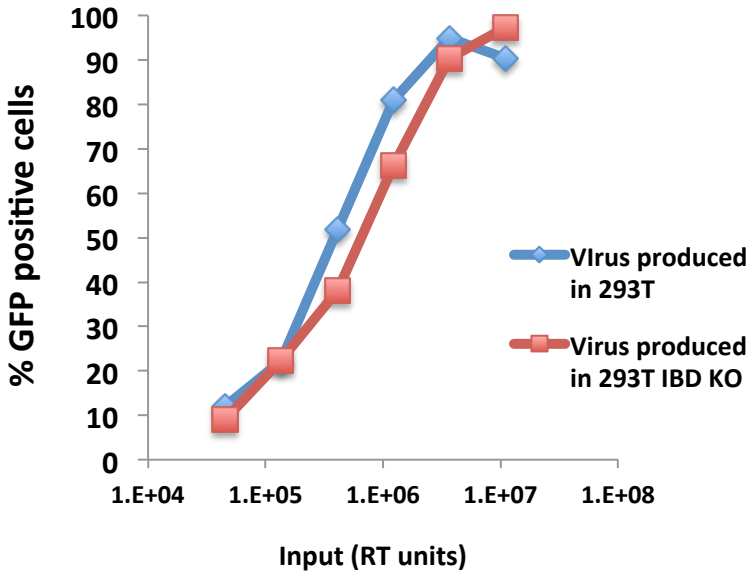
HIV-1 production and infectious particle assembly are normal in *PSIP1* -/- cells

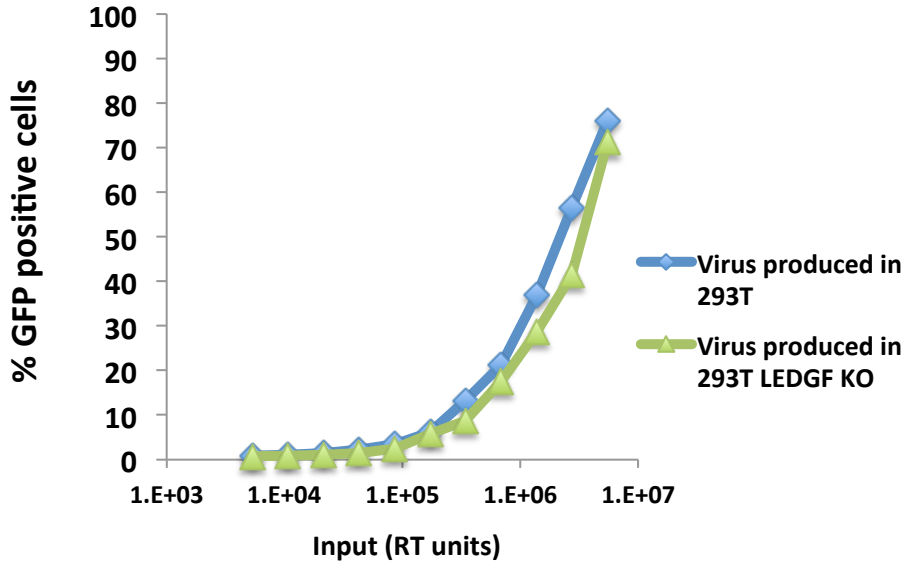


Produce virus by transfection in 6 well plate

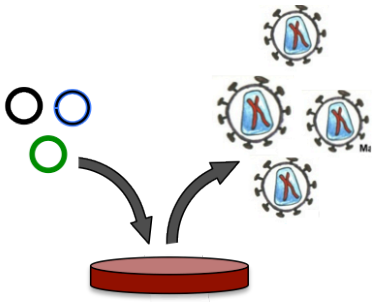
- **293T cells**
- **293T LEDGF -/- cells**
- **293T IBD -/- cells**

Infect target cells with normalized viruses





ALLINI potency for blocking HIV-1 infectious particle formation is unaffected by total removal of LEDGF/p75-encoding capacity from the cell



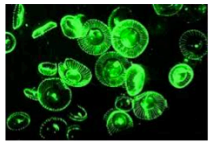
Reporter virus production in the presence of ALLINI-2 or DMSO

Produce virus by transfection in 96 well plates (4 drug dilutions, in triplicate):

- 293T cells
- 293T LEDGF KD cells
- 293T LEDGF -/- cells
- 293T IBD -/- cells

Titration of produced virus on target cell

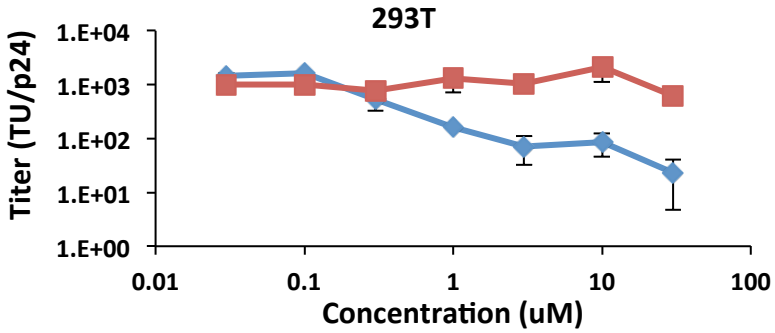
Infect target cells with normalized viruses

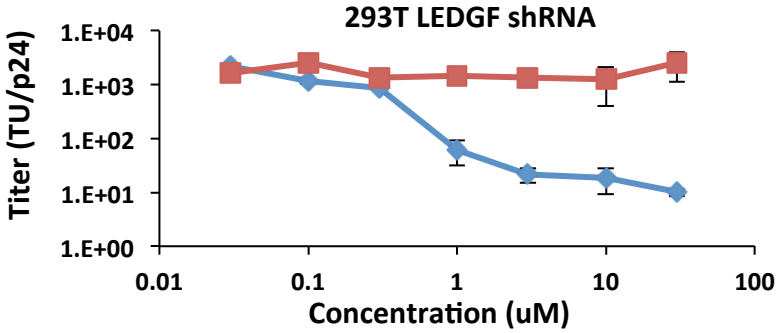


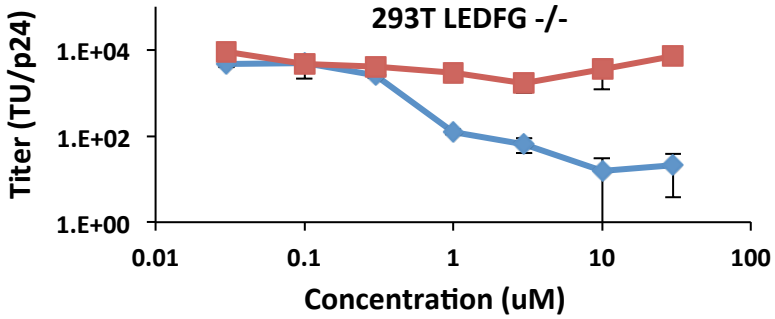
Measure GFP by flow cytometry to calculate titers

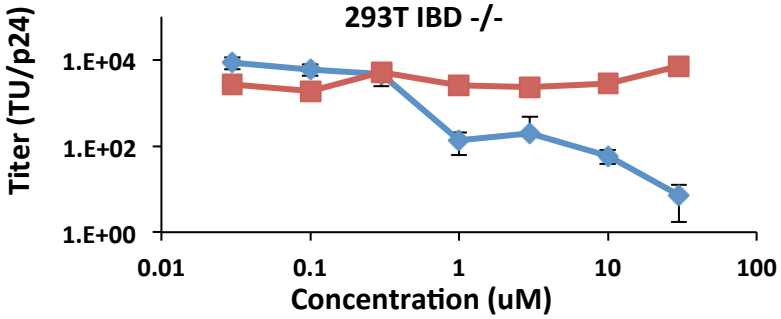
ALLINI-2

DMSO

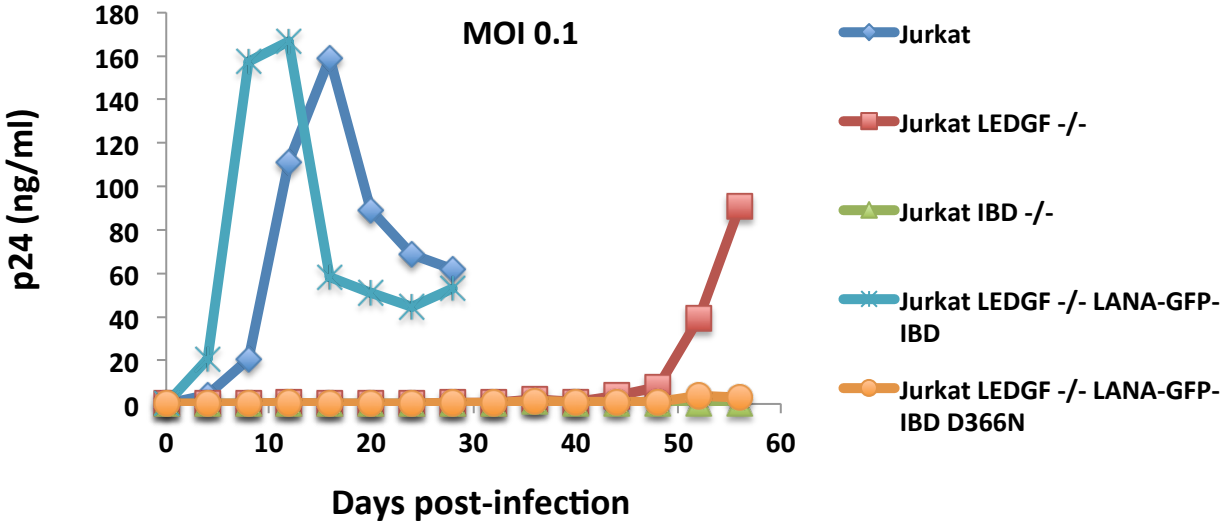


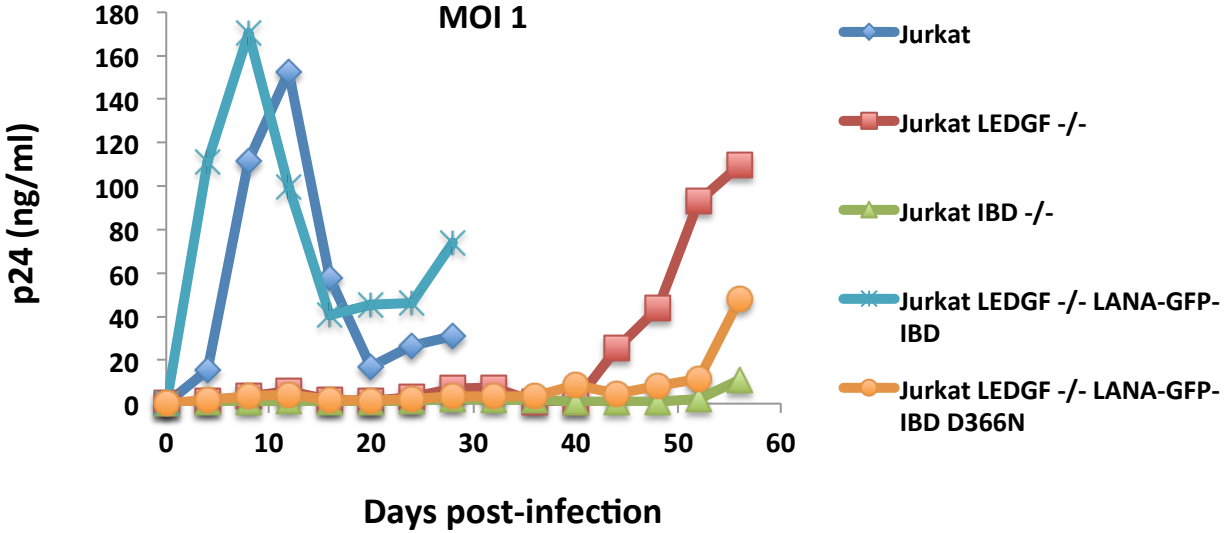






HIV-1 NL4-3 challenge of PSIP1 -/- cells





Conclusions

- *PSIP1* (LEDGF) gene deletions and *PSIP1* IBD exon segment deletions were achieved in human diploid and polyploid cell lines with TALENs.
- Single gene disruptions occurred with high efficiency, with virtually all TALENS we constructed being effective. The predictable cipher provides an advantage over zinc finger nuclease technology.
- Large chromosome segments > 40 kb were excised with two TALEN pairs.
- Both T cells and adherent cells were targetable.
- HIV-1 integration is inhibited in *PSIP1* -/- cells.
- We find that HIV-1 particle production levels and infectious HIV-1 particle assembly are normal in PSIP1 -/- cells. LEDGF does not play a detectable role in particle assembly and its cofactor role depends on its role in early events**.**
- We also find that the potency of a representative allosteric inhibitor of HIV-1 integrase (ALLINI), which binds to the LEDGF IBD binding site on IN, is unaffected by total removal of LEDGF from the cell.
- We conclude that the dominant ALLINI mechanism is entirely independent of LEDGF.

References

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Future Directions

- Integration site analysis
- Knock out HRP2 also.
- CCR5 and other dependency factor KO.
- Determine the importance of LEDGF *in vivo.* A germline knockout of *PSIP1* (LEDGF) is in progress in the domestic cat. This integration cofactor is used by all lentiviruses, including FIV, yet by no other retroviruses. Therefore, gene disruption in the cat will be informative.

Acknowledgments

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  - **Eric Poeschla**
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