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## Supplemental Information

# Time-Restricted PiggyBac DNA Transposition by Transposase Protein Delivery Using Lentivirus-Derived Nanoparticles

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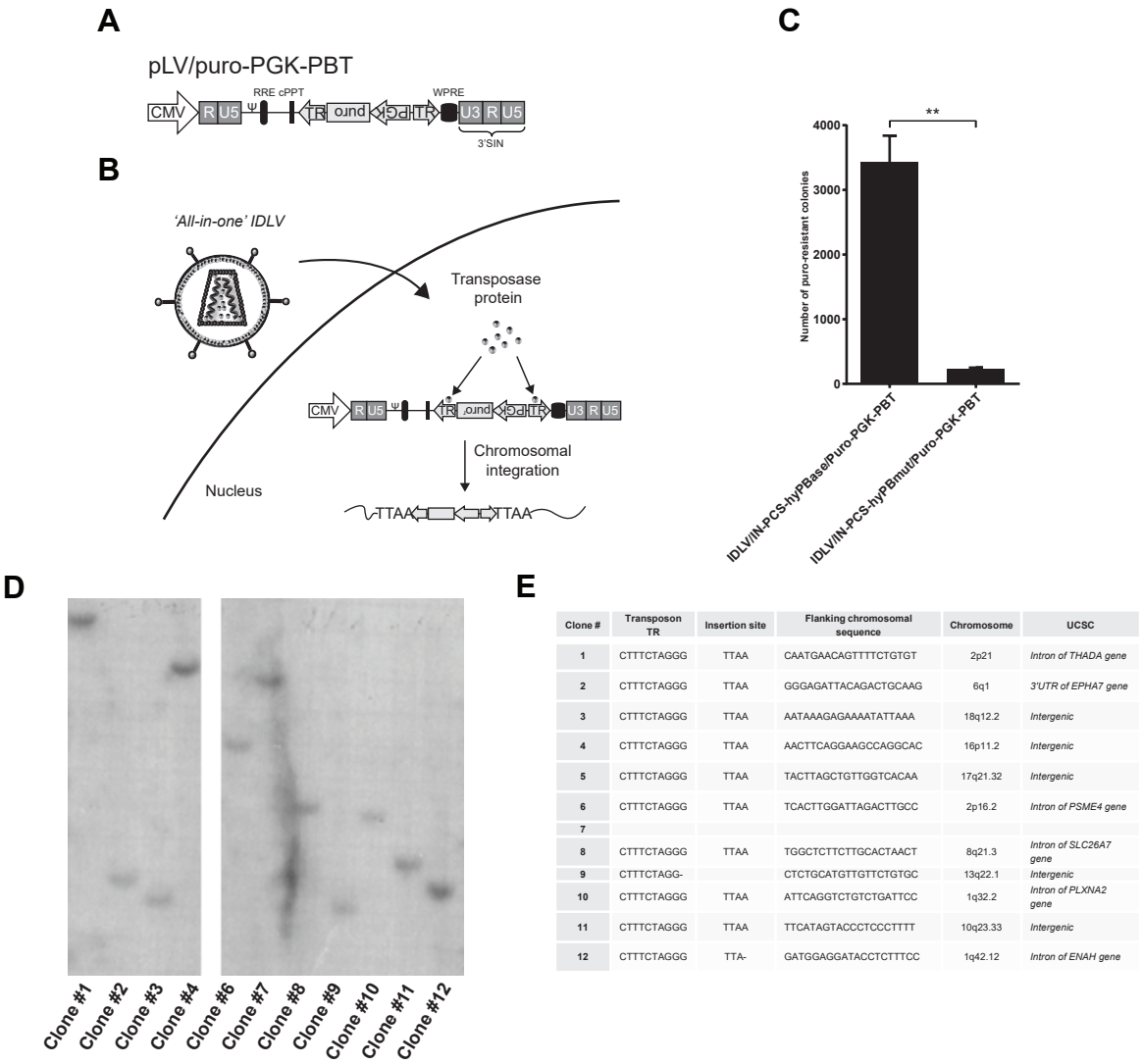


| Clone # | Transposon TR | Insertion site | Flanking chromosomal sequence | Chromosome | UCSC |
|---|---|---|---|---|---|
| 1 | CTTTCTAGGG | TTAA | CAATGAACAGTTTTCTGTGT | 2p21 | *Intron of THADA gene* |
| 2 | CTTTCTAGGG | TTAA | GGGAGATTACAGACTGCAAG | 6q1 | *3'UTR of EPHA7 gene* |
| 3 | CTTTCTAGGG | TTAA | AATAAAGAGAAAATATTAAA | 18q12.2 | *Intergenic* |
| 4 | CTTTCTAGGG | TTAA | AACTTCAGGAAGCCAGGCAC | 16p11.2 | *Intergenic* |
| 5 | CTTTCTAGGG | TTAA | TACTTAGCTGTTGGTCACAA | 17q21.32 | *Intergenic* |
| 6 | CTTTCTAGGG | TTAA | TCACTTGGATTAGACTTGCC | 2p16.2 | *Intron of PSME4 gene* |
| 7 | | | | | |
| 8 | CTTTCTAGGG | TTAA | TGGCTCTTCTTGCACTAACT | 8q21.3 | *Intron of SLC26A7 gene* |
| 9 | CTTTCTAGG- | | CTCTGCATGTTGTTCTGTGC | 13q22.1 | *Intergenic* |
| 10 | CTTTCTAGGG | TTAA | ATTCAGGTCTGTCTGATTCC | 1q32.2 | *Intron of PLXNA2 gene* |
| 11 | CTTTCTAGGG | TTAA | TTCATAGTACCCTCCCTTTT | 10q23.33 | *Intergenic* |
| 12 | CTTTCTAGGG | TTA- | GATGGAGGATACCTCTTTCC | 1q42.12 | *Intron of ENAH gene* |

**Figure S1. IDLV-mediated co-delivery of IN-fused hyPBase and a transposon donor facilitates efficient transposition in HeLa cells. (A)** Schematic presentation of the PBT/PGK-Puro transposon carried by a lentiviral transfer construct **(B)** Basic concept of PB transposition mediated by co-delivery of transposase protein and transposon donor in 'all-in-one' IDLVs. **(C)** Colony formation by 'all-in-one' IDLVs. HeLa cells were transduced with IDLVs co-packaged with hyPBase protein and a transposon donor incorporating a puromycin selection cassette. The transduced cells were subjected to selection with puromycin. The resulting colonies were fixed, stained and counted. **(D)** Southern blot of single, isolated clones from the colony-forming assay in (C) demonstrating robust production of single-copy clones by transduction of IDLVs carrying both transposase protein as well as transposon donor. **(E)** Mapping of integration sites of clones from (D) by long-distance inverse PCR. Data are presented as mean ± SEM and $n \geq 3$.

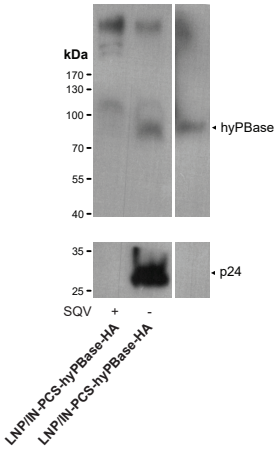


**Figure S2. Protease-dependent release of IN-fused transposase after LNP maturation.** HyPBase and p24 release from the GagPol polypeptide was evaluated by Western blotting of LNP/IN-hyPBase-HA lysates produced either with or without the protease inhibitor saquinavir (SQV).

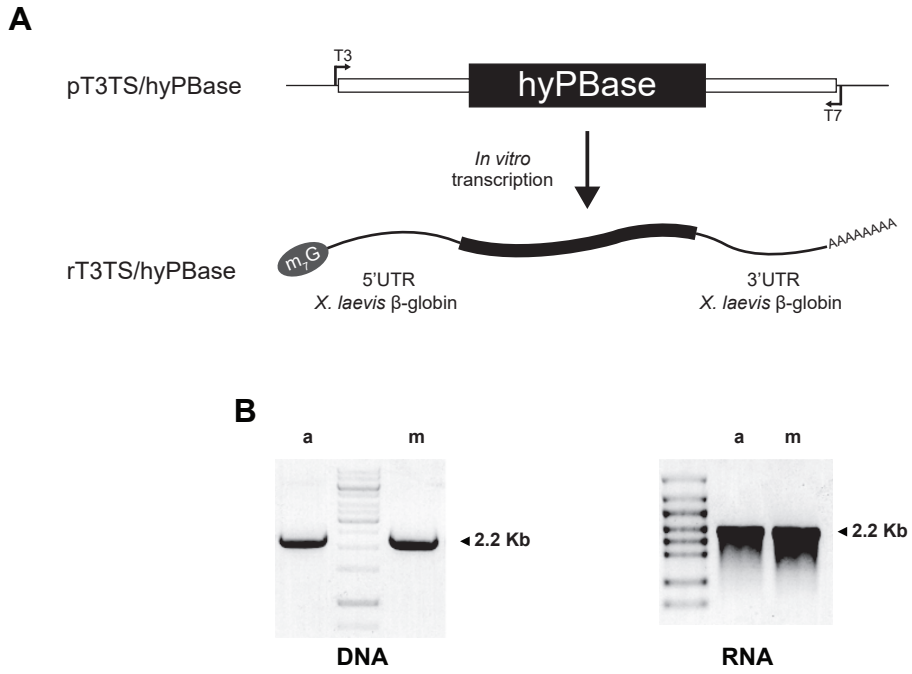


**Figure S3. Production of in vitro-transcribed hyPBase mRNA. (A)** Schematic presentation of the DNA template and mRNA produced by in vitro transcription **(B)** Representative gels of the PCR amplified hyPBase fragment (left) and the corresponding in vitro-transcribed mRNA (right). a: T3TS/hyPBase, m: T3TS/hyPBmut.