Supplement for:

# Cyclophilin A protects HIV-1 from restriction by human TRIM5α

Kyusik Kim, Ann Dauphin, Sevnur Komurlu, Sean M. McCauley, Leonid A. Yurkovetskiy, Claudia Carbone, William E. Diehl, Caterina Strambio-De-Castillia, Edward M. Campbell, and Jeremy Luban

**Including:**

**Supplementary Tables 1, 2, and 3 with Supplementary References**

**Supplementary Table 1. Plasmids used in this study.**

| Plasmid Name | Purpose | Notes | Source |
|---|---|---|---|
| pAIP-hGMCSF-co[1,2] | Stable 293 cell line expressing human cytokine GM-CSF | SFFV promoter expresses codon-optimized human GM-CSF and puromycin N-acetyltransferase | Addgene #74168 |
| pAIP-hIL4-co[1,2] | Stable 293 cell line expressing human cytokine IL4 | SFFV promoter expresses codon-optimized human IL4 and puromycin N-acetyltransferase | Addgene #74169 |
| pSIV-Δpsi/Δenv/ΔVif/ΔVpr | $SIV_{MAC251}$ *gag-pol/vpx* | Production of SIV-VLPs containing Vpx protein | Addgene #132928 |
| pMD2.G | VSV Glycoprotein | Pseudotype HIV-1 vectors with VSV Glycoprotein | Addgene #12259 |
| psPAX2 | HIV-1 *gag-pol* | Encodes gag structural proteins and pol enzymes to generate virion particles to generate 3-part lentiviral vector | Addgene #12260 |
| p8.9 NΔSB[3] | HIV-1 *gag-pol* | Minimal HIV-1 packaging plasmid for gag and pol expression | Addgene #132929 |
| p8.9 NΔSB P90A[4] | HIV-1 *gag-pol* | p8.9 NΔSB bearing P90A mutant capsid sequence | Addgene #132930 |
| p8.9 NΔSB D185K / D186L[4] | HIV-1 *gag-pol* | p8.9 NΔSB bearing RT-D185K/D186L mutant reverse transcriptase sequence | Addgene #132931 |
| pALPS GFP[2] | GFP reporter of lentiviral vector | SFFV promoter expresses GFP | Addgene #101323 |
| pWPTS GFP[4] | Lentivector for quantification of HIV-1 cDNA | Contains a unique loxP sequence within a region of the 3' LTR U3. EF1α promoter expresses GFP | Addgene #12255 |
| pAPM-D4-miR30-L1221[5] | Lentivector for luciferase (control) knockdown | SFFV promoter expresses puromycin N-acetyltransferase and miR30-based shRNA targeting luciferase. Target sequence: 5′-TACAAACGCTCTCATCGACAAG-3′; negative control for knockdown | Addgene #115846 |
| pAPM-D4-miR30-TRIM5 | Lentivector for TRIM5 knockdown | SFFV promoter expresses puromycin N-acetyltransferase and miR30-based shRNA targeting TRIM5. Target sequence: 5′-TGCCAAGCATGCCTCACTGCAA-3′ | Addgene #132932 |
| pABM-D4-miR30-L1221 | Lentivector for luciferase (control) knockdown | SFFV promoter expresses blasticidin S-deaminase and miR30-based shRNA targeting luciferase. Target sequence: 5′-TACAAACGCTCTCATCGACAAG-3′; negative control for knockdown | Addgene #132933 |

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|---|---|---|---|
| pABM-D4-miR30-CypA | Lentivector for CypA knockdown | SFFV promoter expresses blasticidin S-deaminase and miR30-based shRNA targeting cyclophilin A. Target sequence: 5′-CTGGATTGCAGAGTTAAGTTTA-3′ | Addgene #132934 |
| pPU-Empty-miR30-L1221 | All-in-one knockdown-rescue lentivector for luciferase (control) knockdown | SFFV promoter expresses puromycin N-acetyltransferase and miR30-based shRNA targeting luciferase. Target sequence: 5′-TACAAACGCTCTCATCGACAAG-3′; negative control in knockdown-rescue experiment | Addgene #132935 |
| pPU-Empty-miR30-TRIM5 | All-in-one knockdown-rescue lentivector for TRIM5 knockdown | SFFV promoter expresses puromycin N-acetyltransferase and miR30-based shRNA targeting TRIM5. Target sequence: 5′-TGCCAAGCATGCCTCACTGCAA-3′ | Addgene #132936 |
| pPU-huTRIM5α-miR30-L1221 | All-in-one knockdown-rescue lentivector for huTRIM5α expression and luciferase (control) knockdown | SFFV promoter expresses puromycin N-acetyltransferase, exogenous huTRIM5α protein and miR30-based shRNA targeting luciferase. Target sequence: 5′-TACAAACGCTCTCATCGACAAG-3′ | Addgene #132937 |
| pPU-huTRIM5α-miR30-TRIM5 | All-in-one knockdown-rescue lentivector for huTRIM5α expression and TRIM5 knockdown | SFFV promoter expresses puromycin N-acetyltransferase, exogenous huTRIM5α protein and miR30-based shRNA targeting TRIM5. Target sequence: 5′-TGCCAAGCATGCCTCACTGCAA-3′ | Addgene #132938 |
| pNL4-3/Mac-tropic Env | Full-length infectious HIV-$1_{MAC}$ | Encodes HIV-$1_{NL4-3}$ proviral sequences containing macrophage-tropic JR-FL *env* | Neagu et al., 2009[6] |
| pLXIN GFP | GFP reporter of MLV-based vector | MLV-based transfer vector expressing GFP and neomycin resistance protein | Addgene #132940 |
| pCIG3 N[7] | N-tropic MLV *gag-pol* | MLV packaging plasmid for N-tropic MLV gag-pol expression | Addgene #132941 |
| pCIG3 B[7] | B-tropic MLV *gag-pol* | MLV packaging plasmid for B-tropic MLV gag-pol expression | Addgene #132942 |
| pUC57mini ZM249M | HIV-1 clade C molecular clone | Molecular clone of transmitted/founder virus HIV-$1_{ZM249M}$ | Salazar-Gonzalez et al., 2009[8]; NIH AIDS Reagent #12416 |
| pUC57mini ZM249M | HIV-1 clade C molecular clone | Molecular clone of transmitted/founder virus HIV-$1_{ZM249M}$ bearing P90A capsid | Salazar-Gonzalez et |

| | | | |
|---|---|---|---|
| P90A | | mutation | al., 2009[8]; NIH AIDS Reagent #12416 |
| pUC57mini ZM249M Δenv eGFP[2,8] | HIV-1 clade C molecular clone | Molecular clone of transmitted/founder virus HIV-$1_{ZM249M}$[8] with deletion of 79 nucleotides following the env signal peptide and GFP in place of nef | Addgene #101321 |
| pUC57mini ZM249M P90A Δenv eGFP | HIV-1 clade C molecular clone | Molecular clone of transmitted/founder virus HIV-$1_{ZM249M}$[8] containing P90A capsid mutation, with deletion of 79 nucleotides following the env signal peptide and GFP in place of nef | Addgene #132939 |
| pUC57mini Z331M-TF Δenv eGFP[2,9] | HIV-1 clade C molecular clone | Molecular clone of transmitted/founder virus HIV-$1_{Z331M-TF}$[9] with deletion of 79 nucleotides following the env signal peptide and GFP in place of nef | Addgene #101320 |

**Supplementary Table 2. Drugs and reagents.**

| Reagents | Source | Identifier |
|---|---|---|
| DMEM, high glucose | ThermoFisher | 11995073 |
| Fetal Bovine Serum | GE Healthcare Life Sciences | SH30087.03 |
| RPMI-1640 | ThermoFisher | 11875119 |
| Human AB+ Serum | Omega Scientific | HS-20 |
| HEPES | Corning | 25-060-CI |
| MEM Non-Essential Amino Acids | Corning | 25-025-CI |
| Sodium Pyruvate | Corning | 25-000-CI |
| GlutaMAX™-I | ThermoFisher | 35050-061 |
| Opti-MEM | ThermoFisher | 31985-062 |
| TransIT-LT1 | Mirus Bio | MIR2306 |
| BD Cytofix Fixation Buffer | BD Bioscience | 554655 |
| cOmplete mini protease inhibitor | Roche | 11836170001 |
| 2-mercaptoethanol | Invitrogen | 21985-023 |
| Laemmli Buffer | BioRad | 161-0737 |
| 4-20% gradient SDS-PAGE gels, 12 well | BioRad | 456-1095 |
| 0.45 mM nitrocellulose membrane | BioRad | 162-0112 |
| TBS Odyssey Blocking Buffer | Li-Cor | 927-50000 |
| Pierce™ 16% Formaldehyde (w/v), Methanol-free | ThermoFisher | 28906 |
| PIPES | Sigma-Aldrich | P1851 |
| Phalloidin (FITC) | Enzo Life Science | ALX-350-268-MC01 |
| Hoeschst 33342 | Invitrogen | H3570 |
| Cyclosporine A | Sigma-Aldrich | 30024 |
| Cyclosporine H | Sigma-Aldrich | SML1575 |
| GS-CypAi3 | Gilead | Non-commercial |
| GS-CypAi48 | Gilead | Non-commercial |
| MG132 | Sigma-Aldrich | M7449 |

**Supplementary Table 3. qPCR primers and probes for Late RT quantification.**

| Primer or Probe name | Sequence | Note |
|---|---|---|
| pWPT J1B Fwd | 5’-GCATACATTATACGAAGTTATGCTGC-3’ | Primer to detect late RT product |
| pWPT J2 Rev | 5’-GCCGTGCGCGCTTCAGCAAGC-3’ | Primer to detect late RT product |
| LRT-P (Probe) | 5’-(FAM)-CAGTGGCGCCCGAACAGGGA-(TAMRA)-3’ | Probe to detect late RT product |
| MH533 | 5′-ACCCACTCCCTCTTAGCCAATATT-3′ | Primer to detect mitochondrial DNA |
| MH534 | 5′-GTAGGGCTAGGCCCACCG-3′ | Primer to detect mitochondrial DNA |
| Mito-P (Probe) | 5′-(FAM) CTAGTCTTTGCCGCCTGCGAAGCA (TAMRA)-3′ | Probe to detect mitochondrial DNA |

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