Supplement for:

Primate immunodeficiency virus Vpx and Vpr counteract transcriptional repression of proviruses by the HUSH complex

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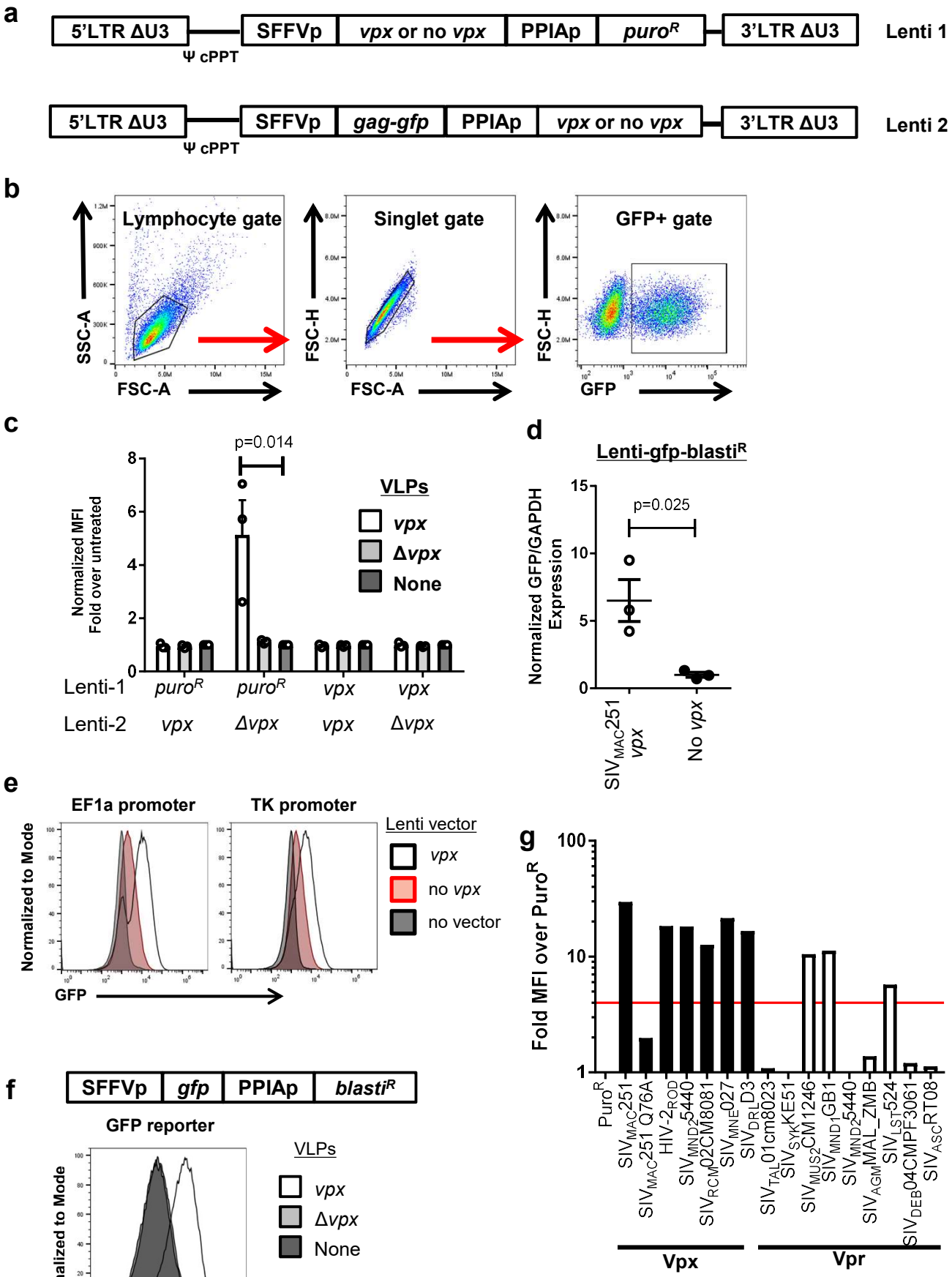
Including:

Supplementary Figures 1, 2, 3, 4, and 5, with corresponding Figure Legends.

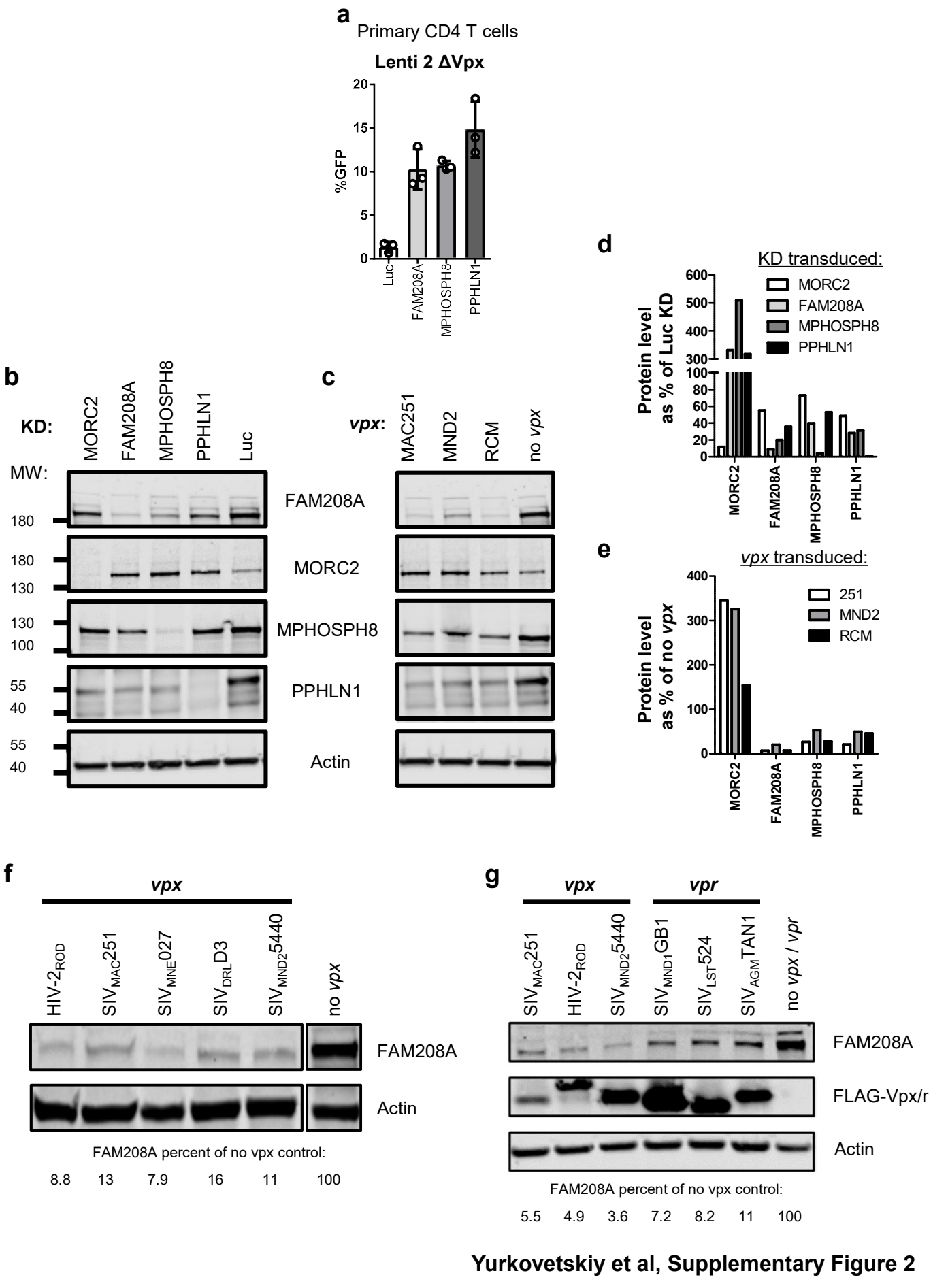
Supplementary Table 1



Yurkovetskiy et al, Supplementary Figure 1

Supplementary Figure 1. Transcriptional activation of lentivector reporter genes by *vpx* and *vpr*.

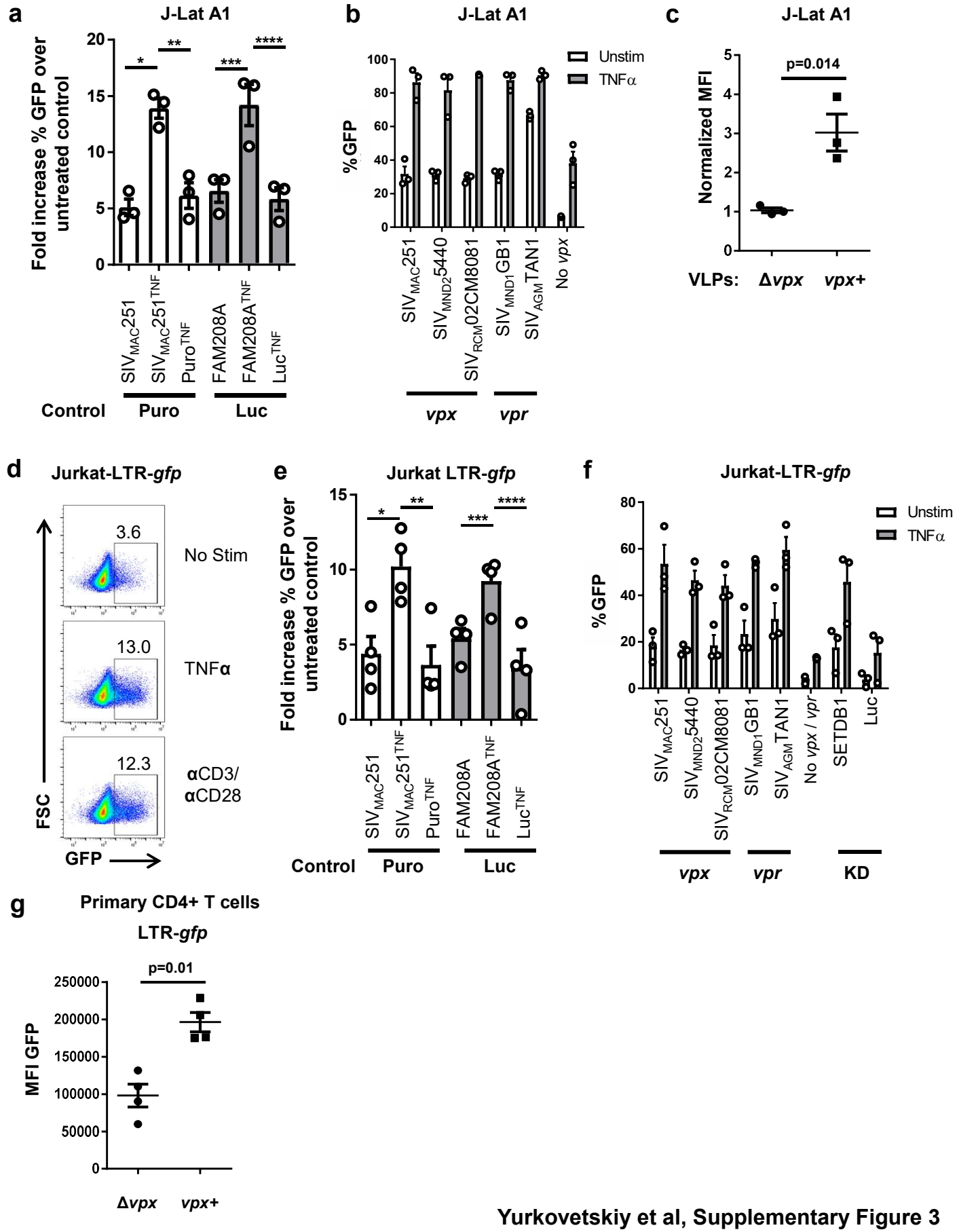
a, Schematic of *vpx*$^+$ and no *vpx* versions of Lenti 1 and Lenti 2 vectors used in Figs 1 and 2. **b**, Representative live, singlet, lymphoid, GFP$^+$ flow cytometry gating strategy. **c,** Quantification of results from Fig 1b. Jurkat-*vpx* or Jurkat-*puro*^{R} transduced with Lenti-2-*vpx*, or Lenti-2-no *vpx,* were treated with Vpx$^+$ VLPs, ΔVpx VLPs, or no VLPs, and analyzed three days later. MFI was normalized for each group of VLP treated cells to untreated samples; (mean ± S.E.M., n=3 independent experiments), significance determined by 1-way ANOVA with Dunnett's post-test comparing treated to untreated samples in each group. **d**, Representative qPCR analysis of *gfp* expression after Lenti-gfp-*blasti*^{R} cells were transduced with SIV_{MAC}251 Vpx or empty vectors (mean ± S.E.M., n=3 replicates), significance determined by unpaired two-tailed *t* test **e,** Jurkat cells were transduced with Lenti-gfp-*blasti*^{R} with gag-GFP driven by EF1α or TK promoters and Blasti^R driven by CypA promoter. Three days after selection cells were transduced with SIV_{MAC}251 Vpx (white) or control puro^R (red) vectors and selected with blasticidin. Untransduced cells are shown in grey (n=3). **f,** Jurkat cells were transduced with Lenti-gfp-*blasti*^{R} with untagged GFP driven by SFFV promoter. Three days after selection cells were treated with Vpx$^+$ VLPs, ΔVpx VLPs, or no VLPs and analyzed by FACS for GFP expression (n=3). **g,** Transactivation of Lenti-gfp-blasti^R reporter cells by the indicated *vpx* and *vpr* expression lentivectors. Red line indicates 4-fold transactivation, which was used as a cutoff for activity.



Yurkovetskiy et al, Supplementary Figure 2

Supplementary Figure 2. HUSH components inhibit provirus expression in primary CD4+ T cells; Vpx and Vpr from multiple lentiviral species deplete FAM208A.

a, Quantification of results from Fig. 2d. $CD4^+$ T cells were positively selected with magnetic beads, activated for 3 days with PHA, transduced with the indicated shRNA-$puro^R$ knockdown or control vectors, and selected with puromycin. Cells were then transduced with a lenti-gfp vector in the absence of *vpx*, and analyzed for GFP expression two weeks later (mean ± S.E.M., n=3 donors). **b,** Immunoblot analysis for components of the HUSH complex in Jurkat cells expressing shRNA constructs against HUSH complex and MORC2 (n=3). **c,** Immunoblot analysis of Jurkat lines transduced to express *vpx* from SIV_{MAC}251, SIV_{RCM}NG411, SIV_{MND2}5440, or control (n=3). **d,** Levels of HUSH components and MORC2 in (**b**) presented as shRNA treated condition relative to control (n=3). **e,** Levels of HUSH components and MORC2 in (**c**) presented as *vpx* treated condition relative to control (n=3). **f,** Immunoblotting for FAM208A and Actin using lysate from Jurkat cells stably transduced with lentivectors producing the indicated Vpx proteins (n=3). **g,** Immunoblotting for FAM208A, FLAG-Vpx, and FLAG-Vpr in Jurkat cells stably transduced with lentivectors expressing the indicated 3xFLAG tagged Vpx and Vpr constructs.

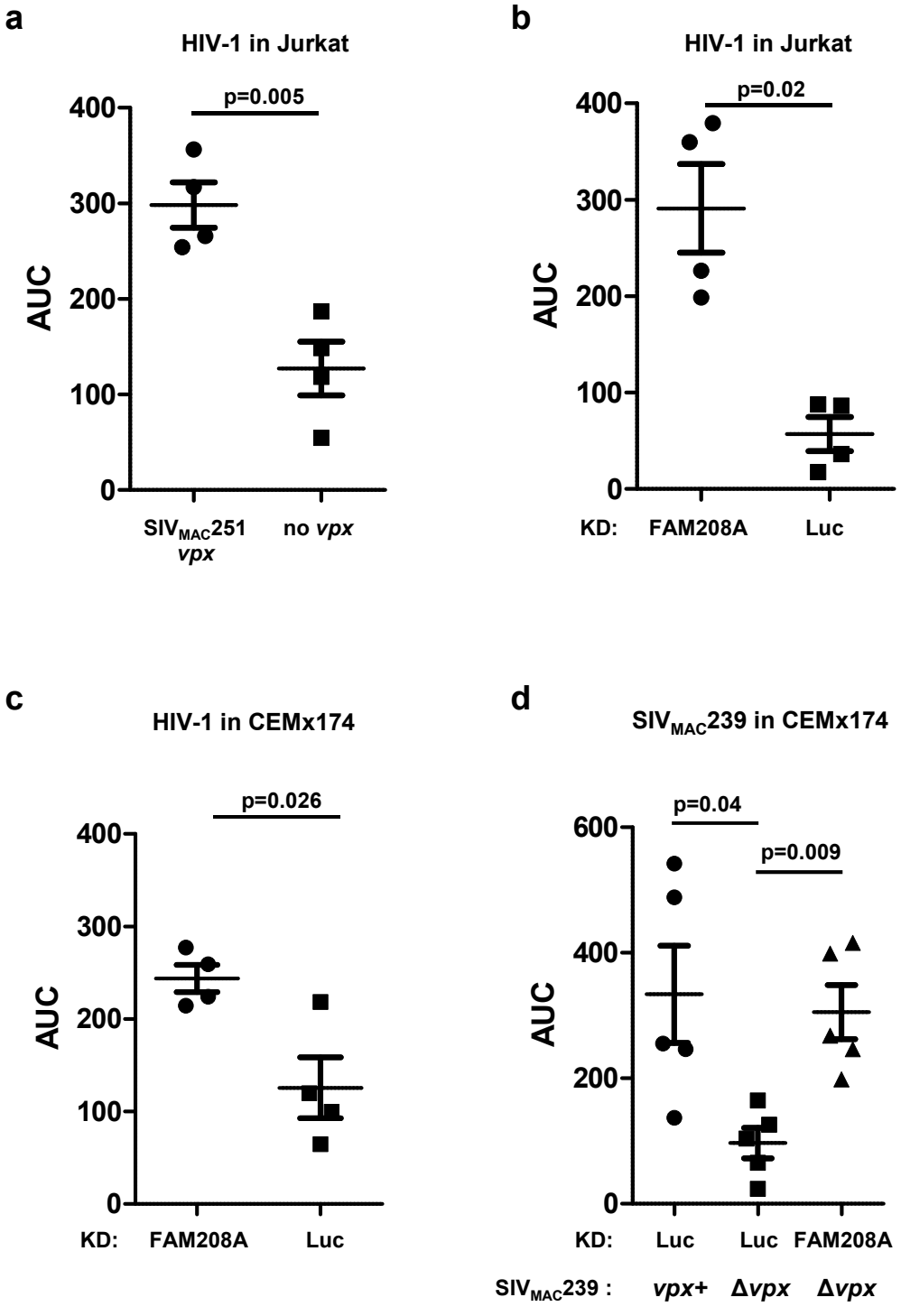


Yurkovetskiy et al, Supplementary Figure 3

Supplementary Figure 3. Expression from the HIV-1 LTR is activated by diverse Vpx and Vpr proteins

a, Reanalysis of J-Lat A1 cells from Fig. 3c. J-Lat A1 cells were transduced with $SIV_{MAC}251$ Vpx or control, or with lentivectors expressing shRNA targeting FAM208A or Luc control and stimulated with 10 ng/ml TNFα. Data is presented as fold increase of GFP^+ cells compared to untreated control cells. Expression of Vpx or KD of FAM208A alone led to comparable increase in GFP expression and addition of TNFα led to an additive effect compared to single treatments. (mean ± S.E.M., n=3) *, *P*=0.0042, **, *P*=0.0080, ***, *P*=0.0073, ****, *P*=0.047, significance was determined by 1-way ANOVA with Tukey's post-test. **b,** J-Lat A1 cells were transduced with Lenti 1 encoding Vpx from $SIV_{MAC}251$, $SIV_{RCM}02CM8081$, or $SIV_{MND2}5440$, Vpr from $SIV_{MND1}GB1$, or $SIV_{AGM}TAN1$, or control no *vpx* Lenti 1. Transduced cells were selected with puromycin, activated for 24 hrs with 10 ng/ml of TNFα, and GFP was assessed by flow cytometry (mean ± S.E.M., n=3) **c,** J-Lat A1 cells were treated with vpx^+ or Δvpx SIV3+ vlps for three days and analyzed for GFP expression. Fold MFI change relative to Δvpx treated cells is presented (mean ± S.E.M., n=3), significance was determined by two-tailed unpaired *t* test. **d,** Jurkat LTR-*gfp* cells were activated for 24 hrs with either 10 ng/ml TNFα or 1 μg/ml each of soluble α-CD3 and α-CD28 antibodies. GFP was then assessed by flow cytometry. Representative of three independent experiments. **e,** Reanalysis of Jurkat-LTR-*gfp* cells from Fig. 3f. J-Lat A1 cells were transduced with $SIV_{MAC}251$ Vpx or control, or with lentivectors expressing shRNA targeting FAM208A or Luc control and stimulated with 10 ng/ml TNFα. Data is presented as fold increase of GFP^+ cells compared to untreated control cells. Expression of Vpx or KD of FAM208A alone led to comparable increase in GFP expression and addition of TNFα led to an additive effect compared to single treatments (mean ± S.E.M., n=4). *, *P*=0.018, **, *P*=0.0092, ***, *P*=0.045, ****, *P*=0.0049, significance was determined by 1-way ANOVA with Tukey's post-test. **f,** Jurkat LTR-*gfp* cells were transduced with Lenti 1 vector

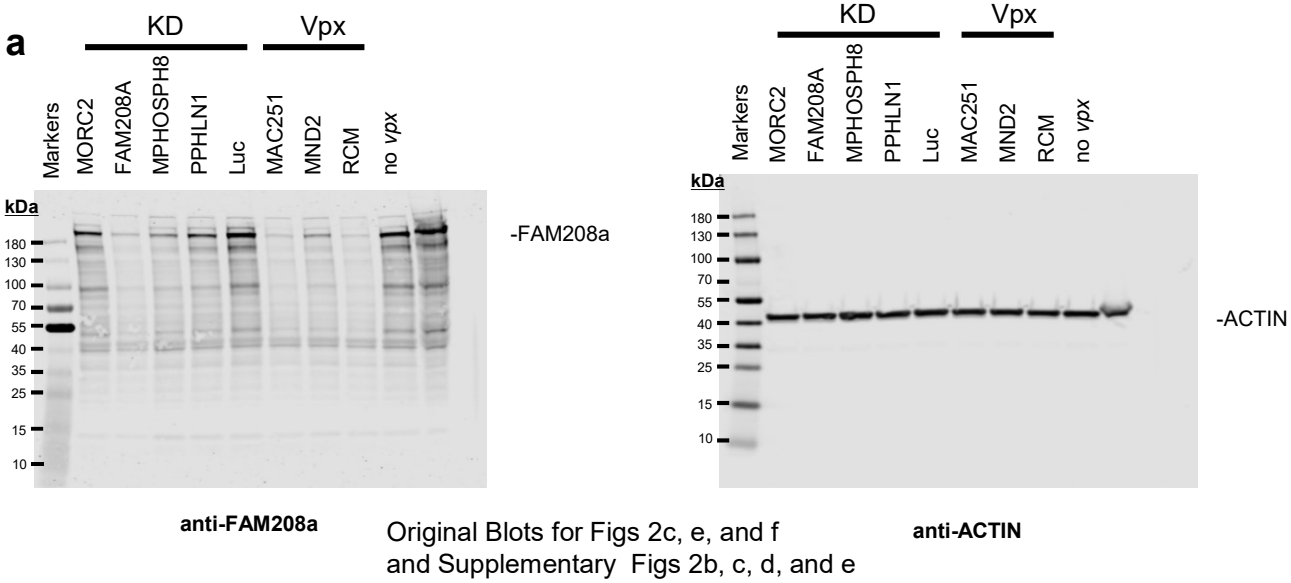
encoding Vpx from $SIV_{MAC}251$, or $SIV_{RCM}02CM808$, or $SIV_{MND}25440$, Vpr from $SIV_{MND1}GB1$, or $SIV_{AGM}TAN1$, control no *vpx* Lenti 1. Jurkat LTR-*gfp* cells were also transduced with lentivectors expressing shRNA targeting SETDB1 or Luc control. Cells were then selected with puromycin, and activated for 24 hrs with 10 ng/ml TNFα. GFP expression was assessed by flow cytometry (mean ± S.E.M., n=3) **g,** Primary $CD4^+$ T cells were activated for 3 days with PHA and then transduced with LTR-*gfp* construct. Cells were cultured for one week after initial transduction followed by transduction with either Δvpr Δvpx or Δvpr vpx^+ VSV-g psuedotyped SIV-luc virus and MFI of the GFP^+ population was assessed by flow cytometry 3 days later (mean ± S.E.M., n=4 independent donors), significance was determined by two-tailed paired *t* test.



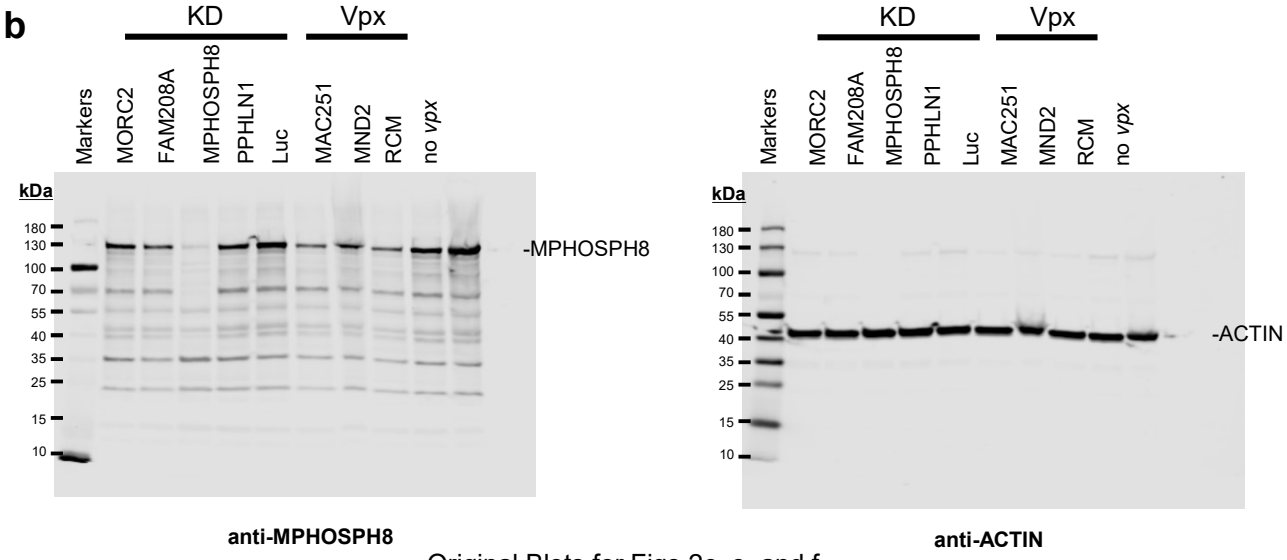
Yurkovetskiy et al, Supplementary Figure 4

Supplementary Figure 4. Quantification of HIV-1 and SIV spreading infections in Jurkat and CEMx174 cells.

a,b, Quantification of results from Figs 4a,b. Replication of HIV-1-ZsGreen in Jurkat cells transduced with SIV_{MAC}251 vpx or control (**a**), or with lentivectors expressing shRNA targeting FAM208A or Luc control (**b**). Replication kinetics was measured by flow cytometry for $ZsGreen^{+}$ cells. Data from biological replicates was normalized to maximum infection observed in that experiment and the area under the curve was calculated for statistical comparison (mean ± S.E.M., n=4 independent experiments), significance was determined by two-tailed paired *t* test. **c,d,** Quantification of results from Figs 4c,d. Spreading infection of HIV-1-ZsGreen (**c**) and SIV_{MAC}239 or SIV_{MAC}239Δvpx (**d**) in CEMx174 cells transduced with FAM208A or Luc control shRNA. Spread of HIV-1-ZsGreen was assessed by flow cytometry as in (**a**), while spread of SIVmac239 (**d**) was assessed by measuring the accumulation of reverse transcriptase (RT) activity in the supernatant. Data from individual replicates was normalized to maximum infection observed in that experiment and the area under the curve was calculated for statistical comparison (mean ± S.E.M., n=4 (**c**) n=5 (**d**) independent experiments). For (**c**), significance was determined by two-tailed paired *t* test. For (**d**), significance was determined by 1-way ANOVA with Dunnett's post-test compared to Luc KD cells infected with SIV_{MAC}239Δvpx.



Original Blots for Figs 2c, e, and f
and Supplementary Figs 2b, c, d, and e



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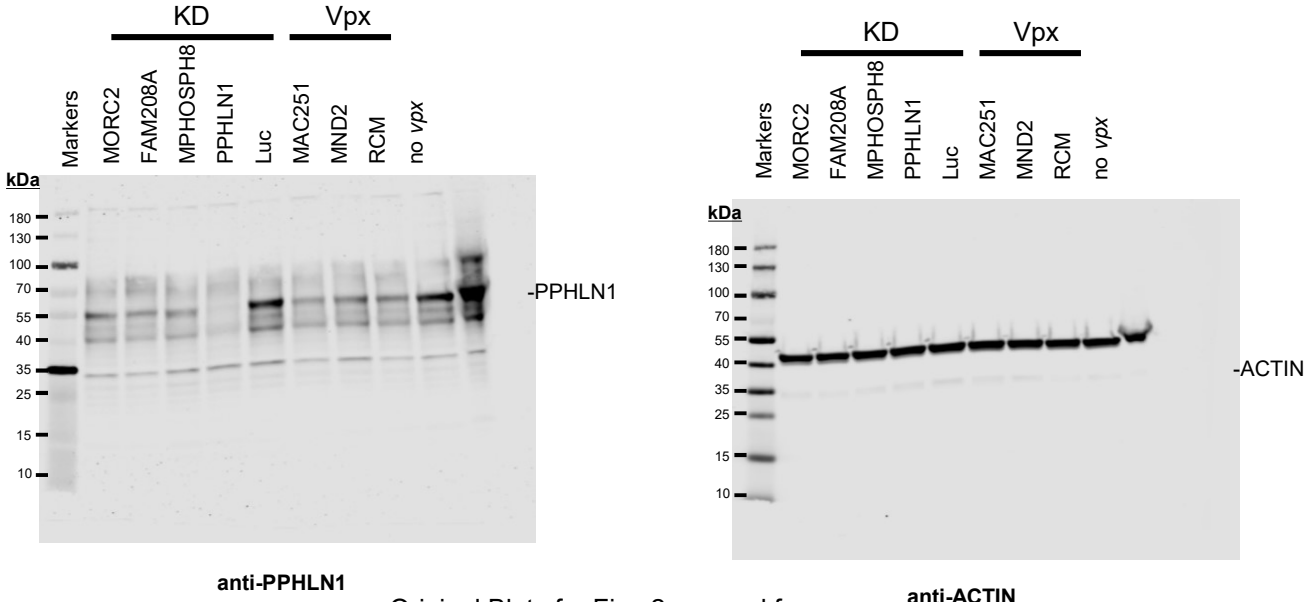
Yurkovetskiy et al, Supplementary Figure 5

c



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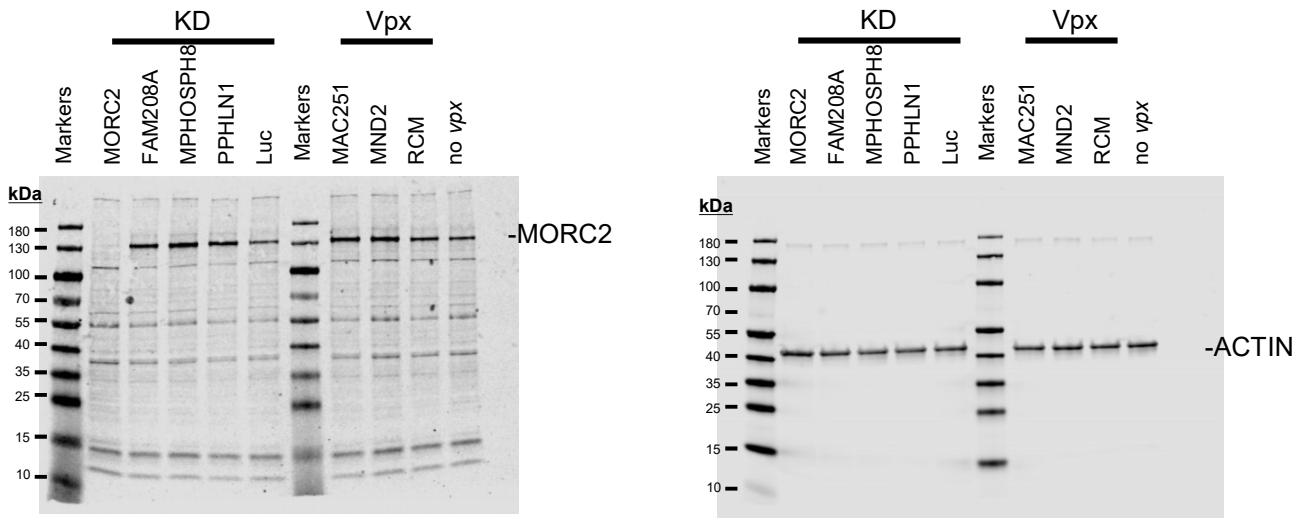
d



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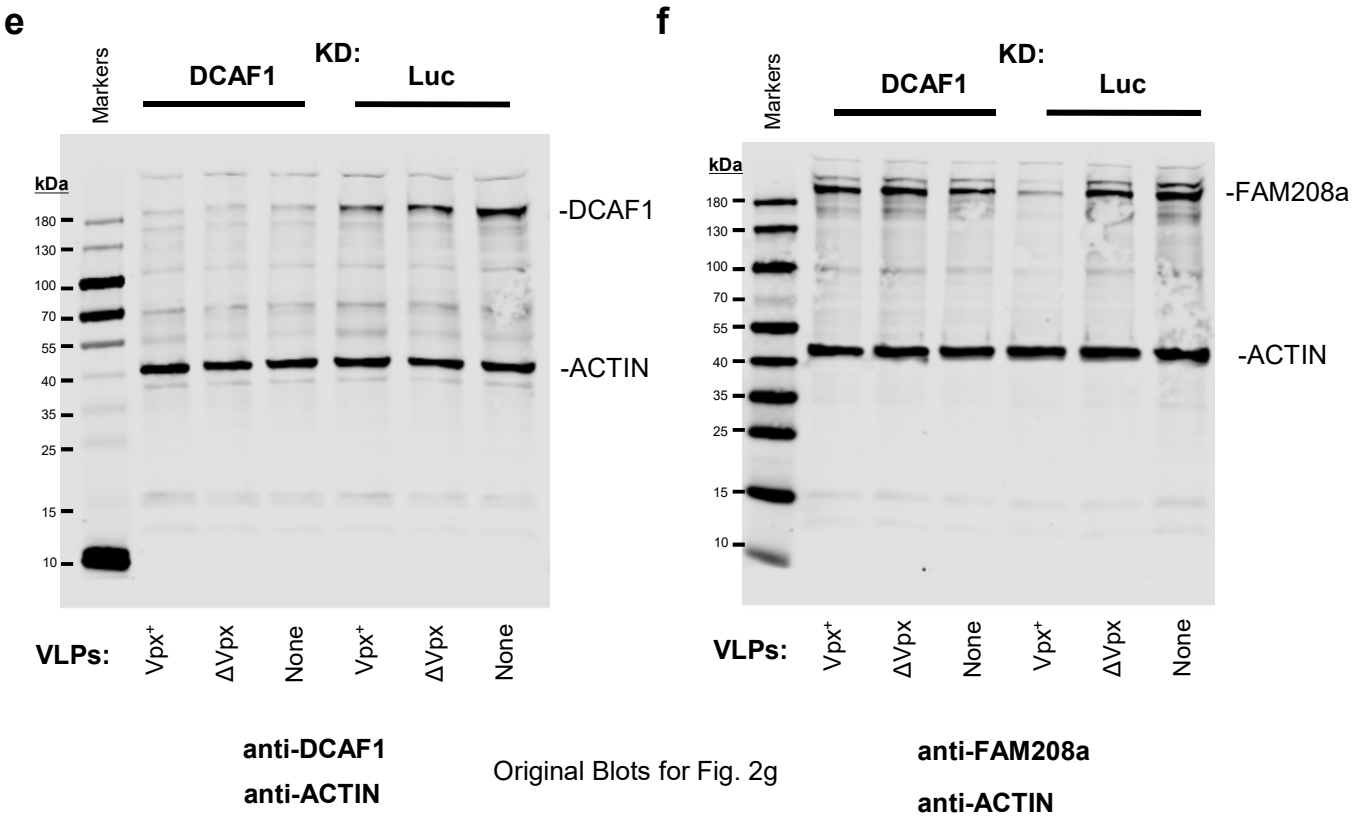


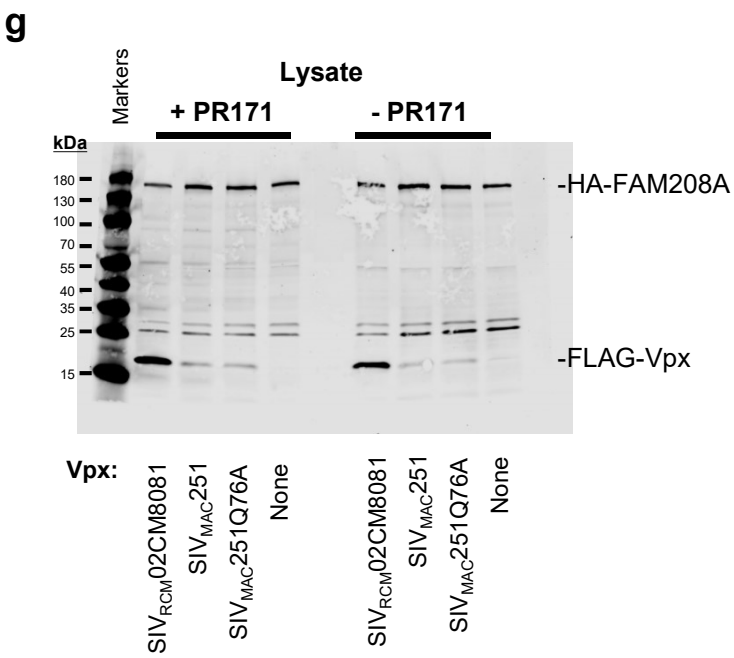
anti-DCAF1

anti-ACTIN

Original Blots for Fig. 2g

anti-FAM208a

anti-ACTIN



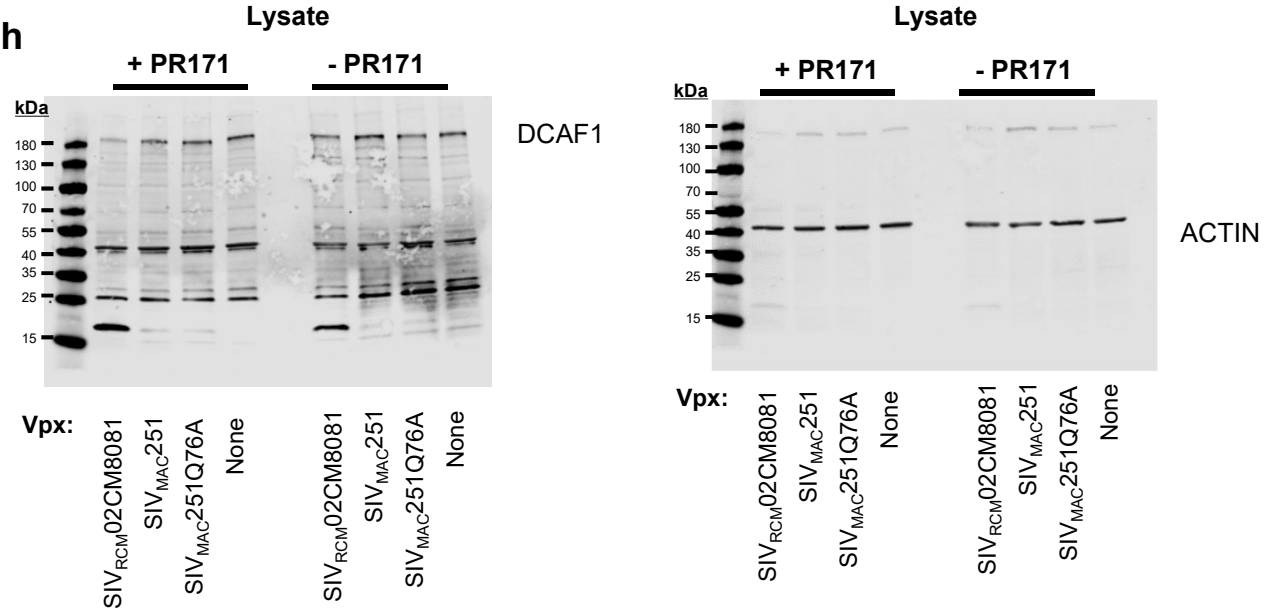
anti-HA-FAM208A

anti-FLAG-Vpx

Original Blots for Fig. 2h

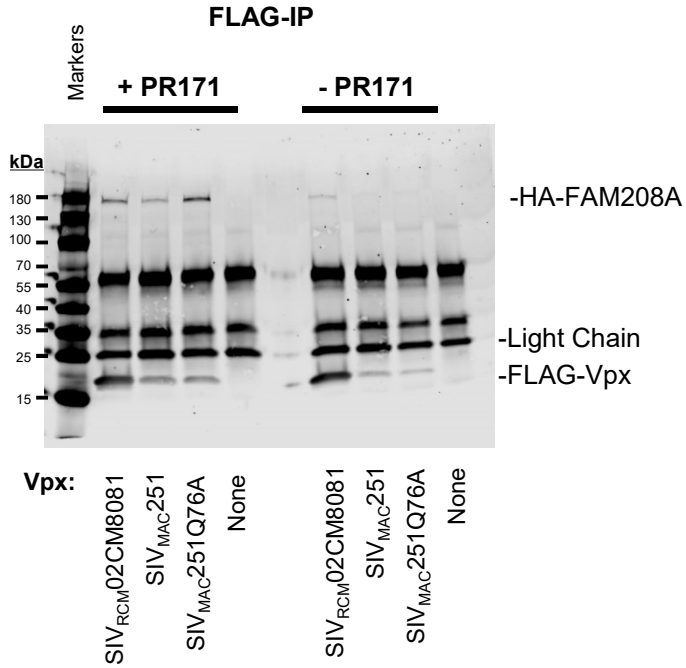
Yurkovetskiy et al, Supplementary Figure 5

h



Original Blots for Fig. 2h

i



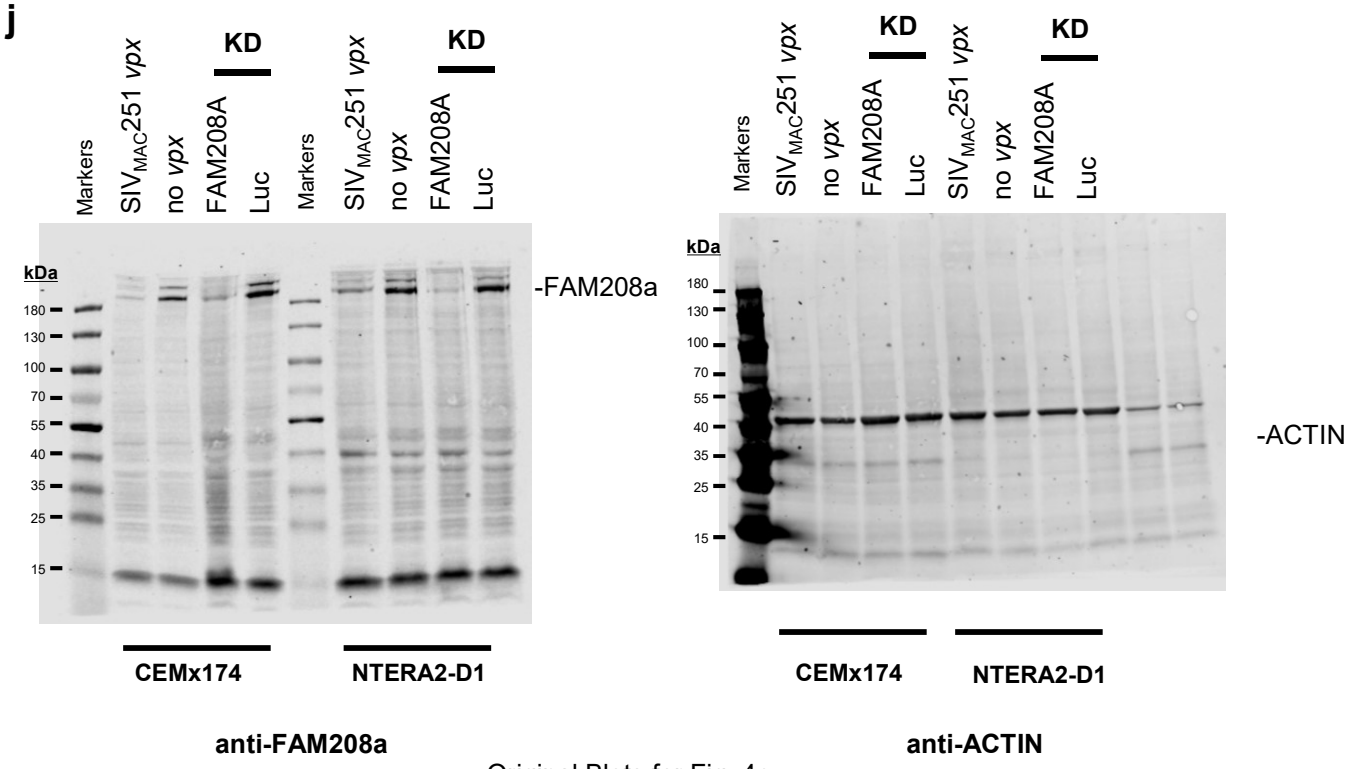
anti-HA-FAM208A

anti-FLAG-Vpx

anti-Light Chain

Original Blots for Fig. 2h

Yurkovetskiy et al, Supplementary Figure 5



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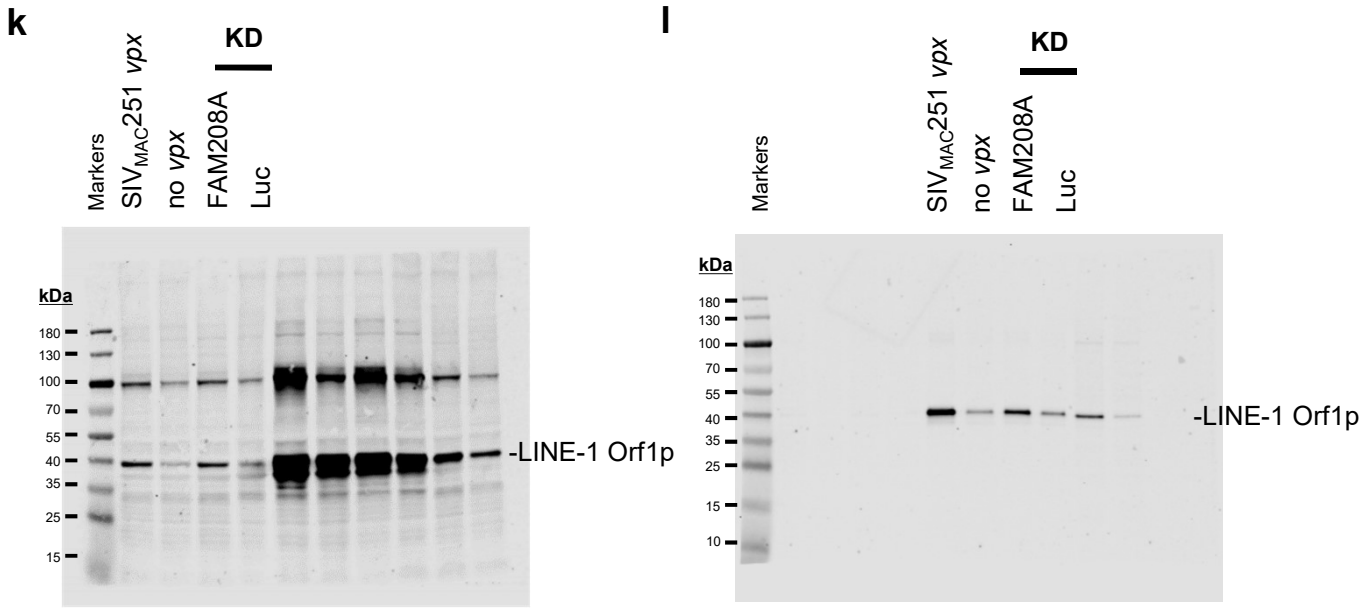
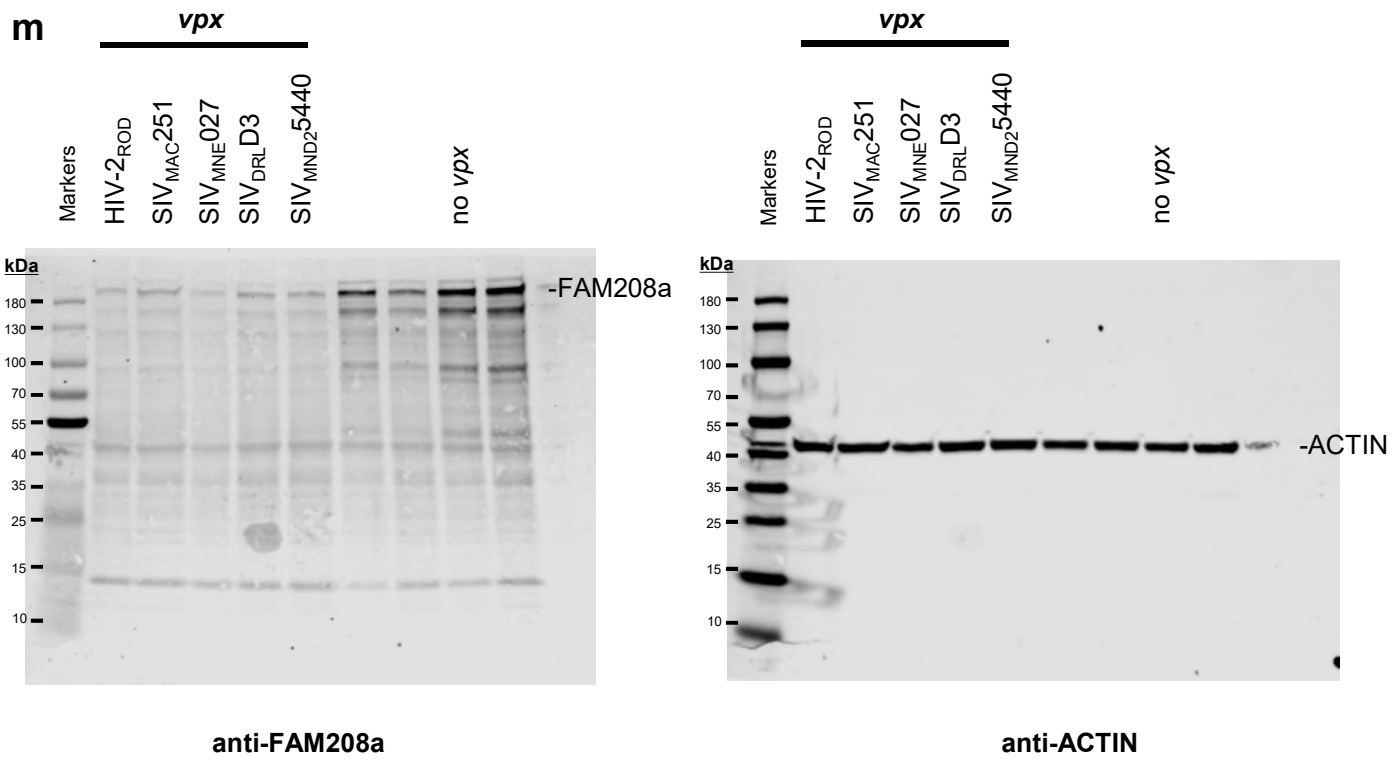


Original Blots for Fig. 4e

Yurkovetskiy et al, Supplementary Figure 5



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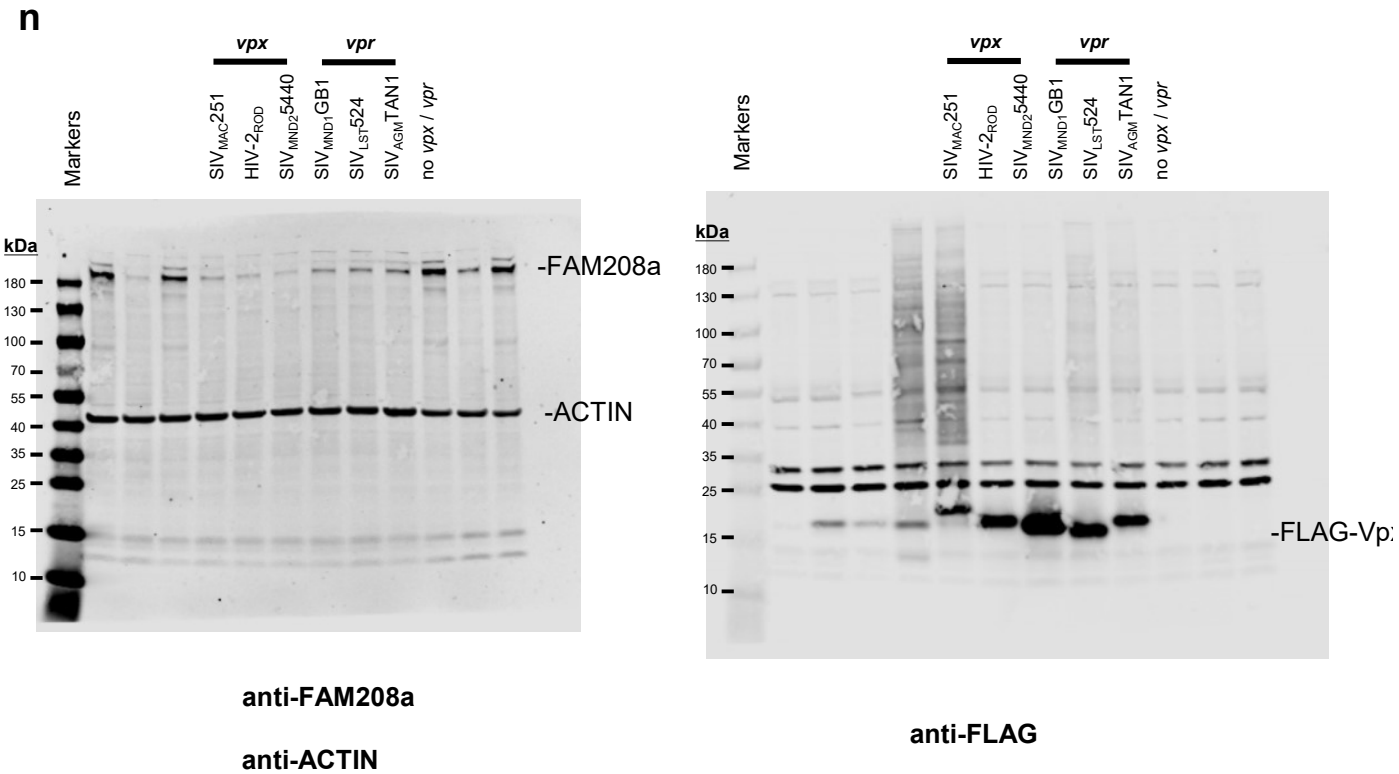

Original Blots for Supplementary Fig. 2g

Yurkovetskiy et al, Supplementary Figure 5

Supplementary Figure 5. Original Western blots.

(**a**), Original blot staining FAM208a and ACTIN for Figs 2c, e, and f and Supplementary Figs 2b, c, d, and e. (**b**), Original blot staining MPHOSPH8 and ACTIN for Figs 2c, e, and f and Supplementary Figs 2b, c, d, and e. (**c**), Original blot staining PPHLN1 and ACTIN for Figs 2c, e, and f and Supplementary Figs 2b, c, d, and e. (**d**), Original blot staining MORC2 and ACTIN for Figs 2c, e, and f and Supplementary Figs 2b, c, d, and e. (**e**), Original blot staining DCAF1 and ACTIN for Fig. 2g. (**f**), Original blot staining FAM208a and ACTIN for Fig. 2g. (**g**), Original blot for Fig. 2h staining lysates for HA-FAM208a and FLAG-Vpx. (**h**), Original blot for Fig. 2h staining lysates for DCAF1 and ACTIN. (**i**), Original blot for Fig. 2h staining FLAG-IP for HA-FAM208a, FLAG-Vpx, and Light Chain. (**j**), Original blot staining FAM208a and ACTIN for Fig. 4e. (k), Original blot staining CEMx174 lysates for LINE-1 Orf1p for Fig. 4e. (**l**), Original blot staining NTera2-D1 lysates for LINE-1 Orf1p for Fig. 4e. (**m**), Original blot staining FAM208a and ACTIN for Fig. 4e. (**m**), Original blot staining FAM208a and ACTIN for Supplementary Fig. 2f. (**n**), Original blot staining FAM208a, FLAG-Vpx/r and ACTIN for Supplementary Fig. 2f.

Supplementary Table 1. Plasmids used in this study.

Plasmid Name	Purpose	Notes	Source
HIV1-ZsGreen	Replication competent HIV-1	HIV-1 NL4-3 in pBluescript with flanking host sequences deleted and intact *env*. ZsGreen in place of *nef*	Gift from Dr. Massimo Pizzato
$SIV_{mac}239$ SpX	Replication competent SIV	Molecular clone of $SIV_{MAC}239$ proviral DNA	NIH AIDS Reagent #12249
$SIV_{mac}239$ SpX ΔVpx	SIV Δ*vpx*	Molecular clone of $SIV_{MAC}239$ Δ*vpx* proviral DNA	NIH AIDS Reagent #12252
pMD2.G	VSV-G	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	Addgene #12260
SIV3+	SIV_{MAC251} *gag-pol*/*vpx*	Production of SIV VLPs containing Vpx protein	Gift from Dr. Andrea Cimarelli
SIV3+ Δ*vpx*	SIV_{MAC251} *gag-pol*/Δ*vpx*	Production of SIV VLPs without Vpx protein.	Gift from Dr. Andrea Cimarelli
SIV-Luc Δ*vpx* Δ*vpr* E-	Transduction of primary CD4+ cells	Two part SIV vector used to as control for expression of Vpx in transduced cells	Gift from Dr. Nathaniel Landau
SIV-Luc Δ*vpr* E-	Transduction of primary CD4+ cells	Two part SIV vector used to express Vpx in transduced cells	Gift from Dr. Nathaniel Landau
pscALPS *gag*-gfp/blasti	Lenti-gfp-blasti	SFFV promoter expresses *gag*-gfp fusion with CypA promoter driving blasticidin resistance gene	Addgene #115803
pscALPS gfp/blasti	GFP reporter	SFFV promoter expresses non-fused gfp with CypA promoter driving blasticidin resistance gene	Addgene #115804
pscALPS *gag*-gfp/*vpx*	Lenti-gfp-*vpx*	SFFV promoter expresses *gag*-gfp fusion with CypA promoter driving expression of $SIV_{MAC}251$ *vpx*	Addgene #115807
pscALPS *gag*-gfp/Δ*vpx*	Lenti-gfp-Δ*vpx*	SFFV promoter expresses *gag*-gfp fusion with no ORF after CypA promoter	Addgene #115808
pecALPS *gag*-gfp/blasti	Lenti-gfp-blasti	EIF1a promoter expresses *gag*-gfp fusion and CypA promoter expresses blasticidin resistance gene	Addgene #115805
pkcALPS-*gag*-gfp/blasti	Lenti-gfp-blasti	TK promoter expresses *gag*-gfp fusion and CypA promoter expresses blasticidin resistance gene	Addgene #115806
HIV-1 LTR-gfp	HIV-1 LTR-gfp	HIV-1 LTR driven reporter vector that retains complete LTRs, *tat*, and *rev*, but has a frameshift mutation in *env*, an ngfr reporter gene in place of *nef*, and *gfp* in place of *gag, pol, vif, and vpr*	Addgene #115809

pscALPS-$SIV_{MAC}251$ *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-*Vpx*$_{MAC}$251 and puromycin resistance protein	Addgene #115810
pscALPS SIV_{MND2} *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-SIV_{MND2} Vpx (AY159322) and puromycin resistance protein	Addgene #115811
pscALPS-SIV_{RCM} *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-SIV_{RCM} Vpx (AF349680) and puromycin resistance protein	Addgene #115812
pscALPS-SIV_{MNE} *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-SIV_{MNE} Vpx (U79412) and puromycin resistance protein	Addgene #115813
pscALPS-SIV_{DRL} *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-SIV_{DRL} Vpx (KM378563) and puromycin resistance protein	Addgene #115814
pscALPS-SIV_{AGI} *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-SIV_{AGI} Vpx (HM803690) and puromycin resistance protein	Addgene #115815
pscALPS-$HIV2_{ROD}$ *vpx*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG-$HIV2_{ROD}$ Vpx (M15390) and puromycin resistance protein	Addgene #115816
pscALPS-SIV_{MND1} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{MND1} Vpr (M27470) and puromycin resistance protein	Addgene #115817
pscALPS-SIV_{LST} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{LST} Vpr (AF188116) and puromycin resistance protein	Addgene #115818
pscALPS-SIV_{AGM}TAN1 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{AGM} Vpr (TAN1) (U58991) with repaired premature stop codon and puromycin resistance protein	Addgene #115819
pscALPS-SIV_{TAL} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{TAL} Vpr (AM182197) and puromycin resistance protein	Addgene #115820
pscALPS-SIV_{SYK} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{SYK} Vpr (L06042) and puromycin resistance protein	Addgene #115821
pscALPS-SIV_{SAB}92018 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{SAB} Vpr (HQ378594) and puromycin resistance protein	Addgene #115822
pscALPS-SIV_{DEB} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{DEB} Vpr (FJ919724) and puromycin resistance protein	Addgene #115823
pscALPS-SIV_{ASC} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{ASC} Vpr (KJ461715) and puromycin resistance protein	Addgene #115824
pscALPS-SIV_{AGM}VER9063 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{AGM} Vpr (Ver 9063) (L40990) and Puro resistance marker	Addgene #115825
pscALPS-SIV_{AGM}VERAGM3 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{AGM} Vpr (Ver AGM3) (M30931) and puromycin resistance protein	Addgene #115826
pscALPS-SIV_{GRV} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{GRV} Vpr (M66437) and puromycin resistance protein	Addgene #115827
pscALPS-SIV_{AGM}MAL *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV_{AGM} Vpr (MAL_ZMB) (LC114462) and puromycin resistance protein	Addgene #115828
pscALPS-SIV_{DEN} *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG SIV_{DEN} Vpr (AJ580407) and puromycin resistance protein	Addgene #115829

pscALPS-SIV$_{GSN}$CN71 *vpr*	Lentivector expressing *vpx*	Encodes codon optimized 3xFLAG SIV$_{GSN-CN71}$ Vpr (AF468658) and puromycin resistance protein	Addgene #115830
pscALPS-SIV$_{GSN}$CN166 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG SIV$_{GSN-CN166}$ Vpr (AF468659) and puromycin resistance protein	Addgene #115831
pscALPS-SIV$_{SUN}$K08 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{SUN}$ Vpr (FR751162) and puromycin resistance protein	Addgene #115832
pscALPS-SIV$_{CPZ}$TAN3 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{CPZ-TAN3}$ Vpr (DQ374658) and puromycin resistance protein	Addgene #115833
pscALPS-SIV$_{CPZ}$LB7 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{CPZ-LB7}$ Vpr (DQ373064) and puromycin resistance protein	Addgene #115834
pscALPS-SIV$_{GOR}$ -*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{GOR}$ Vpr (FJ424871) and puromycin resistance protein	Addgene #115835
pscALPS-HIV-1 Group O-*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-HIV1 Group O Vpr (L20571) and puromycin resistance protein	Addgene #115836
pscALPS-HIV-1 Group P-*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-HIV1 Group P Vpr (HQ179987) and puromycin resistance protein	Addgene #115837
pscALPS-SIV$_{RCM}$-*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{RCM}$ Vpr (HM803689) and puromycin resistance protein	Addgene #115838
pscALPS-SIV$_{WRC}$-*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{WRC}$ Vpr (AM713177) and puromycin resistance protein	Addgene #115839
pscALPS-SIV$_{COL}$BWC01 *vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{COL}$Vpr (KF214240) and puromycin resistance protein	Addgene #115840
pscALPS-SIV$_{MUS2}$1246-*vpr*	Lentivector expressing *vpr*	Encodes codon optimized 3xFLAG-SIV$_{MUS2}$1246 Vpr (EF070329) and puromycin resistance protein	Addgene #115841
pcDNA3.1 FLAG-SIV$_{MAC}$251-*vpx*	Expression plasmid	Encodes codon optimized FLAG tagged SIV$_{MAC}$251-Vpx	Addgene #115842
pcDNA3.1 FLAG-SIV$_{MAC}$251-*vpx*-Q76A	Expression plasmid	Encodes codon optimized FLAG tagged SIV$_{MAC}$251-Vpx-Q76A mutant	Addgene #115843
pcDNA3.1 FLAG-SIV$_{RCM}$-*vpx*	Expression plasmid	Encodes codon optimized FLAG tagged SIV$_{RCM}$-Vpx	Addgene #115844
pcDNA3.1 HA-FAM208A	Expression plasmid	Encodes codon optimized HA tagged FAM208A	Addgene #115845
pAPM-D4-miR30-L1221	Lentivector luciferase knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-CTTGTCGATGAGAGCGTTTGT-3'; negative control for other knockdowns	Addgene #115846
pAPM-D4 miR30-HDAC1 ts1	Lentivector HDAC1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TATGAGTCATGCGGATTCG-3'	Addgene #115847
pAPM-D4 miR30-HDAC1	Lentivector HDAC1	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TAAGAACGGGAAGAATGGG-3'	Addgene #115848

ts2	knockdown		
pAPM-D4 miR30-HDAC1 ts3	Lentivector HDAC1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTAATGTAGTCATCGCTGT-3'	Addgene #115849
pAPM-D4 miR30-AGO1 ts1	Lentivector AGO1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTCTGCTTGAAATACTGTG-3'	Addgene #115850
pAPM-D4 miR30-AGO1 ts2	Lentivector AGO1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGATATCAGAGATTTCTGG-3'	Addgene #115851
pAPM-D4 miR30-AGO1 ts3	Lentivector AGO1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTGACATTGATCTTGAGGC-3'	Addgene #115852
pAPM-D4 miR30-AGO2 ts1	Lentivector AGO2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TAATACATCTTTGTCCTGC-3'	Addgene #115853
pAPM-D4 miR30-AGO2 ts2	Lentivector AGO2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TCATCTGCACGCACTGCGT-3'	Addgene #115854
pAPM-D4 miR30-AGO2 ts3	Lentivector AGO2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTGCTAATCTCTTCTTGCC-3'	Addgene #115855
pAPM-D4 miR30-AGO3 ts1	Lentivector AGO3 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGACTTGAACACATTGTGT-3'	Addgene #115856
pAPM-D4 miR30-AGO3 ts2	Lentivector AGO3 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TCTGAACTACAATGTAGGT-3'	Addgene #115857
pAPM-D4 miR30-AGO3 ts3	Lentivector AGO3 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TAGCTTCTTGATACATCGT-3'	Addgene #115858
pAPM-D4 miR30-SETDB1 ts1	Lentivector SETDB1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTCGCATGCTGACTATCAG-3'	Addgene #115859
pAPM-D4 miR30-SETDB1 ts2	Lentivector SETDB1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-ACACAATCCATCTTCTCCA-3'	Addgene #115860
pAPM-D4 miR30-SETDB1 ts3	Lentivector SETDB1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTGTTGTCAAATTTCACCT-3'	Addgene #115861
pAPM-D4 miR30-TRIM28 ts1	Lentivector TRIM28 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AAGGTTGTAGTCCTCAGTG-3'	Addgene #115862
pAPM-D4 miR30-TRIM28 ts2	Lentivector TRIM28 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TCAATAACAATAAGGTTGT-3'	Addgene #115863
pAPM-D4 miR30-TRIM28 ts3	Lentivector TRIM28 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGAGTAGGGATCATCTCCT-3'	Addgene #115864
pAPM-D4 miR30-DNMT3a ts1	Lentivector DNMT3a knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TAATCTCCTTGACCTTGGG-3'	Addgene #115865
pAPM-D4 miR30-DNMT3a ts2	Lentivector DNMT3a knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TATCATTCACAGTGGATGC-3'	Addgene #115866

pAPM-D4 miR30-DNMT3a ts3	Lentivector DNMT3a knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AGAACTCAAAGAAGAGCCG-3'	Addgene #115867
pAPM-D4 miR30-PIWIL2 ts1	Lentivector PIWIL2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-CGAACATTGACAACCTGGG-3'	Addgene #115868
pAPM-D4 miR30-PIWIL2 ts2	Lentivector PIWIL2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AGCAGACAAGCCTCGACCT-3'	Addgene #115869
pAPM-D4 miR30-PIWIL2 ts3	Lentivector PIWIL2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AGATTAGTACTGATTTTCT-3'	Addgene #115870
pAPM-D4 miR30-FAM208A ts1	Lentivector FAM208A knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTCTTCTACTGGTTCCCGG-3'	Addgene #115871
pAPM-D4 miR30-FAM208A ts2	Lentivector FAM208A knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGAATTGCTGTTCTCTCCT-3' Exhibited best KD activity and utilized in further experiments	Addgene #115872
pAPM-D4 miR30-FAM208A ts3	Lentivector FAM208A knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-ATCTTAGCACCAGAATCGT-3'	Addgene #115873
pAPM-D4 miR30-MPHOSPH8 ts1	Lentivector MPHOSPH8 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AAATCTCTTATTTCACCCT-3'	Addgene #115874
pAPM-D4 miR30-MPHOSPH8 ts2	Lentivector MPHOSPH8 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTGCTTCTGTCTTGATTCC-3' Exhibited best KD activity and utilized in further experiments	Addgene #115875
pAPM-D4 miR30-MPHOSPH8 ts3	Lentivector MPHOSPH8 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTCTCTTCTCTGCTGTCGG-3'	Addgene #115876
pAPM-D4 miR30-PPHLN1 ts1	Lentivector PPHLN1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TCATCTGATTTCTCTAGCT-3'	Addgene #115877
pAPM-D4 miR30-PPHLN1 ts2	Lentivector PPHLN1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTCATATTCATATCGTCCC-3' Exhibited best KD activity and utilized in further experiments	Addgene #115878
pAPM-D4 miR30-PPHLN1 ts3	Lentivector PPHLN1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGAGTTCTTCAACACACCG-3'	Addgene #115879
pAPM-D4 miR30-SUV39h1 ts1	Lentivector SUV39h1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGAGGATACGCACACACTT-3'	Addgene #115880
pAPM-D4 miR30-SUV39h1 ts2	Lentivector SUV39h1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AGAGCAGGTAGGAGCAGGT-3'	Addgene #115881
pAPM-D4 miR30-SUV39h1 ts3	Lentivector SUV39h1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-CATTCTCTACAGTGATGCG-3'	Addgene #115882

pAPM-D4 miR30-SUV39h2 ts1	Lentivector SUV39h2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TCATCAGACTCATAGTCCA-3'	Addgene #115883
pAPM-D4 miR30-SUV39h2 ts2	Lentivector SUV39h2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TAAATTTCTTTATCATTGA-3'	Addgene #115884
pAPM-D4 miR30-SUV39h2 ts3	Lentivector SUV39h2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-ACATTATCAGCTTAACGCT-3'	Addgene #115885
pAPM-D4 miR30-MORC2 ts1	Lentivector MORC2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGAGATTGAAGATGATCAC-3' Exhibited best KD activity and utilized in further experiments	Addgene #115886
pAPM-D4 miR30-MORC2 ts2	Lentivector MORC2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TTTTCCACAGAACTCAGCT-3'	Addgene #115887
pAPM-D4 miR30-MORC2 ts3	Lentivector MORC2 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-TGTCTGTGACAGGTTCCCG-3'	Addgene #115888
pAPM-miR30-DCAF1	Lentivector DCAF1 knockdown	SFFV promoter expressing puromycin resistance protein and miR30-shRNA target site: 5'-AGCACTTCAGATTATCATCAAT-3'	Addgene #115889