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Supplemental Information

Cellular IP₆ Levels Limit HIV Production

while Viruses that Cannot Efficiently Package IP₆

Are Attenuated for Infection and Replication

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Supplementary Figure 1 related to Figure 1: (A) Analysis of CRISPR knockout clones. Chromatograms and alignments for sequencing of CRISPR/Cas9 knockout clones of IPMK. Indels were identified using the program outlined in Dehairs, J. et al. (Dehairs et al., 2016), as well as manual decoding. (B) PAGE and toluidine blue staining of standard cell extracts including standard controls to demonstrate migration of IP₅, IP₆, ATP and GTP. Standards were run in the presence of cell extract to allow equivalent migration. (C) Western blot for Gag expression levels in transfected IPMK KO clones. Gag precursor Pr55Gag (Pr55), p41 and mature capsid protein (p24) are indicated. Lower panel shows loading control COX-IV. (D) Graph showing pg RT per ng p24 in virions from viral supernatants obtained from transfection of IPMK KO clones. Error bars depict mean \pm SD of three independent experiments with no statistical difference to WT. (E) Western blots showing overexpression of IPMK in CRISPR knockout clones. Clones were blotted for IPMK and lower panel shows Cox-IV loading control. (F) TiO₂-PAGE and toluidine blue staining of cell extracts from IPMK KO clones stably transduced with empty vector control (EV) or a CMV-driven IPMK gene. Synthetic polyP was used for gel orientation. Gels show knockout clones are successfully reconstituted and IP₆ levels restored. (G) Western blot for Gag expression levels in IPMK KO parental clones and cells stably transduced with either EV or IPMK. Gag precursor Pr55Gag (Pr55), p41 and mature capsid protein (p24) are indicated. Lower panel shows loading control COX-IV.

Supplementary Figure 2 related to Figure 2: (A) Analysis of CRISPR knockout clones. Chromatograms and alignments for sequencing of CRISPR/Cas9 knockout clones of IPPK. Indels were identified using the program outlined in Dehairs, J. *et al.* (Dehairs et al., 2016), as well as manual decoding. (B) Comparison of total IP₅ and IP₆ incorporation in virions from IPPK KO clones shown in Figure 2C. (C) Western blot for Gag expression levels in transfected IPPK KO clones. Gag precursor Pr55Gag (pr55), p41 and mature capsid protein (p24) are indicated. Lower panel shows loading control COX-IV.

Supplementary Figure 3 related to Figure 4: Alignment of lentiviral gag sequences. Residues are highlighted by conservation, with the mature and immature charged ring residues boxed.

Supplementary Figure 4 related to Figure 5: (A) Western blot showing Gag expression levels of lysine mutants in producer cells. Gag precursor Pr55Gag (pr55), p41 and mature capsid

protein (p24) are indicated. (B) Quantification of viral production from 293T, IPMK KO and IPPK KO cells as determined by RT levels in viral supernatants. Error bars depict mean ± SD of two independent experiments. Values are expressed as fold change from levels of RT produced in WT virus. (C) Western blot for Gag expression levels in cell lysates from WT and IPMK and IPPK KO cells during WT and K227I virus production. Gag precursor Pr55Gag (pr55), p41 and mature capsid protein (p24) are indicated. (D) Western blots of cell extracts showing cell extracts from stably transduced cells, showing depletion of TNPO3 or NUP153 by expression of short hairpin RNAs.

Supplementary Table 1 related to Figure 3: Data collection and refinement statistics. Crystallographic statistics for the HIV-1 hexamer structure complexed with IP₅.

Supplementary Figure 1 related to Figure 1



B













IPMK 1_8





IPMK 1_14







ATCGAGTCCACCCC-TGAGGGCACCCCGCAGCCGGCGGG

ATCGAGTCCACCCCTGAGGGCACCCCGCAGCCGGCGG

IPMK 2_19



 IPMK
 GGCTGCGTGCCCTCTGG-CATCAGGTGGCCGGGCACAT

 2_19(1)
 GGCTGCGTGCCCCTCTGGGCATCAGGTGGCCGGGCACAT

2_19(2) GGCTGCGTGCCCCTCTCG---TCAGGTGGCCGGGCACAT

D

Supplementary Figure 2 related to Figure 2

Α



IPPK B1_8





IPPK B1 13



IPPK	GCCCTCTCCGTGGTACCCCCATTCATTCTCGTCCATCTTCCCC
$1 \ 13(1)$	GCCCTCTCCTTCTCGTCCATCTTCCCC
1 13(2)	GCTGGCGCA
IPPK	TCTTCCATGCCCAGGCGCAGCCCTGGCGCCTCGCGGGCTAGGA
1_13(1)	TCTTCCATGCCCAGGCGCAGCCCTGGCGCCTCGCGGGCTAGGA
1 13(2)	TCGCGGGCTAGGA

----TTCATTCTCGTCC

IPPK B2_3





CTCTCCGTGGTACCCCCA-TTCATTCTCGTCCATCTTCC CTCTCCGTGGTACCCCCATTTCATTCTCGTCCATCTTCC





IPPK CGCGTGGGCCACCACCAGG-CTCTTATTGCCCTCTCCGT 3 7 (1) CGCGTGGGCCACCACC--GGCTCTTATTGCCCTCTCCGT 3 7 (2) CGCGTGGGCCACCACCAGGTCTCTTATTGCCCTCTCCGT





В

GCCCTCTCCGTGGTACCCCCA-TTCATTCTCGTCCATCT 2_7 GCCCTCTCCGTGGTACCCCCATTTCATTCTCGTCCATCT

1500 IP5 IP6 (normalized CPM) IP incorporation 1000 500 0 N' ~? $\sqrt{2}$ 22 **IPPK KO Clones**





Supplementary Figure 3 related to Figure 4

HIV-1_M HIV-1_O HIV-2 SIVmac FIV	1 MGARAS V LSGGELDRWEK I R LRP GGK – KKYK LKHI VWASR ELER FAVNPG – LLETSEGCRQILGQLQP SLQ – TGSEELR SLYN 1 MGARAS V LTGSK LDAWEQ I R LKP GCK – KKYR LKHLVWASR ELDR FACNPE – LLETAEGNEQLLQQLEPALK – TGSESLQSLWN 1 MGARNS V LRGKKADELER I R LRP GGK – KKYR LKHI VWAANKLDR FGLAES – LLESKEGCQK I LTVLDPMVP – TGSENLKSLFN 1 MGARNAV LSGKKADELEK I R LRP GGK – KKYNLKHV VWAANELDR FGLAES – LLENKEGCQK I LSVLAPLVP – TGSENLKSLFN 1 MGARNAV LSGKKADELEK I R LRP GGK – KKYNLKHV VWAANELDR FGLAES – LLENKEGCQK I LSVLAPLVP – TGSENLKSLYN 1 MGNGQGRDWKMAI KRCSNVAVGVGGKSKKFGEGNFRWAI RMANV STGREP GDI PETLDQLRLVI CDLQERREK FGSSK EI DMAI V	80 80 80 80 85
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	81 TVAT LYCVHQR I E I K DT K EA LDK I E E EQNK SKKK AQQAAADTG HS SQV SQNYP I VQN I QGQMVHQA I SPRT LNAWVK VVE E 81 T I AV LWCVHNR FR V E DTQQA I QK LK EVMG - SRK SAGAAK EDT SARQT GQNYP VVANAQ GQMVHQS L SPRT LNAWVK AV E E 81 T VCV I WC I HA E EKVK DT E GAKQ I VRRHLVA ET GTA EKMP ST SRPTAP S S EK GGNYP - VQHVGG NYTH I P L SPRT LNAWVK LVE E 81 T VCV I WC I HA E EKVK DT E GAKQ I VRRHLVA ET GTA EKMP ST SRPTAP S S EK GGNYP - VQHVGG NYTH I P L SPRT LNAWVK LVE E 81 T VCV I WC I HA E EKVK HT E EAKQ I VQRHLVVET GTA ETMPKT SRPTAP S S GRGGNYP - VQQ I G G NYVHLP L SPRT LNAWVK LI E E 86 T LKVFAVAG L LNMTV STAAAA ENMY SQMG LDT RP SMK EAGGK E EG P P QAYP - I Q T N GVPQ V A LDP KMV S I FMEKAR E -	161 159 163 163 163
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	162 KAFSP EVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQ 160 KAFNPEIIPMFMALSEGAIPYDTNTMLNAIGGHQGALQVLKEVINEEAAEWDRTHPPPIGPLPPGQIREPTGSDIAGTTSTQ 164 KKFGAEVVPGFQALSEGCTPYDINQMLNCVGDHQAAMQIIREIINEEAAEWDVQHP-IPGPLPAGQLREPRGSDIAGTTSTVE 164 KKFGAEVVPGFQALSEGCTPYDINQMLNCVGDHQAAMQIIREIINEEAAEWDVQHP-QPAPQ-QGQLREPSGSDIAGTTSSVD 164 KKFGAEVVPGFQALSEGCTPYDINQMLNCVGDHQAAMQIIRDINEEAADWDLQHP-QPAPQ-QGQLREPSGSDIAGTTSSVD 164 GLGGEEVQLWFTAFSANLTPTDMATLIMAAPGCAADKEILDESLKQLTAEYDRTHPPDAPRPLPYFTAAEIMGIGLTQE	244 242 245 244 244 242
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	245 EQ I GWMT N – NP P I P VG E I Y K RW I I LG L NK I V RMY SPT S – I LD I RQ GP K EP F R DY VDR FY KT L RA EQ A SQ E V K NWMT ET L L VQ NAN 243 EQ V HWT T R P NNP I P VG D I Y R KW I V LG L NK V V KMY SP V S – I LD I RQ GP K EP F R DY VDR FY KT L RA EQ A TQ E V K NWMT ET L L VQ NAN 246 EQ I QWM F R PQ NP V P VG N I Y R RW I Q I G LQ K C V R MY NP T N – I LD I KQ GP K EP F Q SY VDR FY K S L RA EQ T DP A V K NWMT QT L L VQ NAN 245 EQ I QWMY RQQNP I P VG N I Y R RW I Q L R LQ K C V R MY NP T N – I LD I KQ GP K EP F Q SY VDR FY K S L RA EQ T DP A V K NWMT QT L L VQ NAN 245 EQ I QWMY RQQNP I P VG N I Y R RW I Q L R LQ K C V R MY NP I N – I LD V KQ RP K EP F Q SY VDR FY K S L RA EQ T DA A V K NWMT QT L L I Q NAN 243 QQ A E A R F A – – – – P A R MQ C R AWY L E A LG K LA A I K A K S P R A VQ L R Q G A K ED Y S S F I DR L F AQ I DQ EQ NT A E V K LY L KQ S L S I A NAN	327 326 329 328 322
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	328 P DCK T I L K A L GP AA T L E EMMT A C G GV G G P G H K A R V L A E A M S Q V T N T A T I MMQ R G N F R N Q R K M V K C F N C G K E G H T A R N C R A P 327 P D C K Q I L K S L GP G A T L E EMM I A C Q G V G G P T H K A R V L A E A MAA A Q D L K G G Y T A V F M Q R G Q N P S R K G P I K C F N C G K E G H L A R N C R A P 330 P D C K L V L K G L G M N P T L E E M L T A C Q G V G G P G Q K A R L MA E A L K E V I G P A P I P F A A A Q Q R K A F K C W N C G K E G H S A R Q C R A P 329 P D C K L V L K G L G V N P T L E E M L T A C Q G V G G P G Q K A R L MA E A L K E V I G P V P T P F A A A Q Q R G P R K P I K C W N C G K E G H S A R Q C R A P 323 A D C K K A M S H L K P E S T L E E K L R A C Q E I G S P G Y K M Q L L A E A L T K V Q V V Q S K G S G P V C F N C K K P G H L A R Q C R E V	408 411 407 409 393
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	409 RK K G CWK C G K E G H Q M K D C T – E R Q A N F L G K I W P S Y K G R P G N F L Q S R P E P T A P P F L Q S R P E P T A P P E E S F R S G V E T T T P P Q K Q E P I D 412 R K K G CWK C G Q E G H Q M K D C R N G K Q A N F L G K Y W P P G G T R P G N Y A Q R Q V S P S A P P M T E E M K – – – – – – – G Q E N – – – – Q E Q K E D Q 408 R R Q G CWK C G K P G H I M T N C P – D R Q A G F L G – – L G P W G K K P R N F P V A Q V P Q G L T P T A P P V D P A V D L L E K Y M Q Q G K R Q R E – – Q R E R P Y K 410 R R Q R CWK C G K M D H V M A K C P – D R Q A G F L G – – L G P W G K K P R N F P M A Q V H Q G L T P T A P P E D P A V D L L E K Y M Q Q G K R Q R E – – S R E K P Y K 410 R R Q R CWK C G K M D H V M A K C P – D R Q A G F L G – – L G P W G K K P R N F P M A Q V H Q G L T P T A P P E D P A V D L L K N Y M Q L G K Q Q R E – – S R E K P Y K 394 K K – – C N K C G K P G H L A A K C W Q G N R K N S G N – – – – W K A G R A A A P V NQ M Q Q A V M P S A P P M E – – – – – – – – – – – – – – – – – –	492 480 487 489 444
HIV-1_M HIV-1_O HIV-2 SIVmac FIV	493 K E L Y P L T S L	512 497 521 506 450

Supplementary Figure 4 related to Figure 5





Supplementary Table 1 related to Figure 3: Data collection and refinement statistics

	6R8C		
Data collection			
Space group	P6		
Cell dimensions			
a, b, c (Å)	90.66, 90.66, 57.00		
α, β, γ (°)	90.0, 90.0, 120.0		
Resolution (Å)	78.52-1.91 (1.98-1.91)		
R _{meas}	6.6 (73.0)		
$CC_{1/2}$ (%)	99.8 (83.3)		
Ι/σΙ	17.7 (2.6)		
Completeness (%)	99.3 (90.0)		
Redundancy	6.5 (6.0)		
Resolution (Å)	1.91		
No. reflections	20758		
$R_{\rm work} / R_{\rm free}$	0.19/0.23		
No. atoms	1855		
Protein	1612		
Ligand/ion	64		
Water	179		
B-factors			
Protein	35.2		
Ligand/ion	85.6		
Water	42.3		
R.m.s. deviations			
Bond lengths (Å)	0.02		
Bond angles (°)	1.90		
*Values in parentheses are for	highest-resolution shell.		