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Supplemental Information

Cellular IP_6 Levels Limit HIV Production while Viruses that Cannot Efficiently Package IP_6 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 IP_5, IP_6, 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 ± 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) TiO_2-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 IP_6 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 IP_5 and IP_6 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.

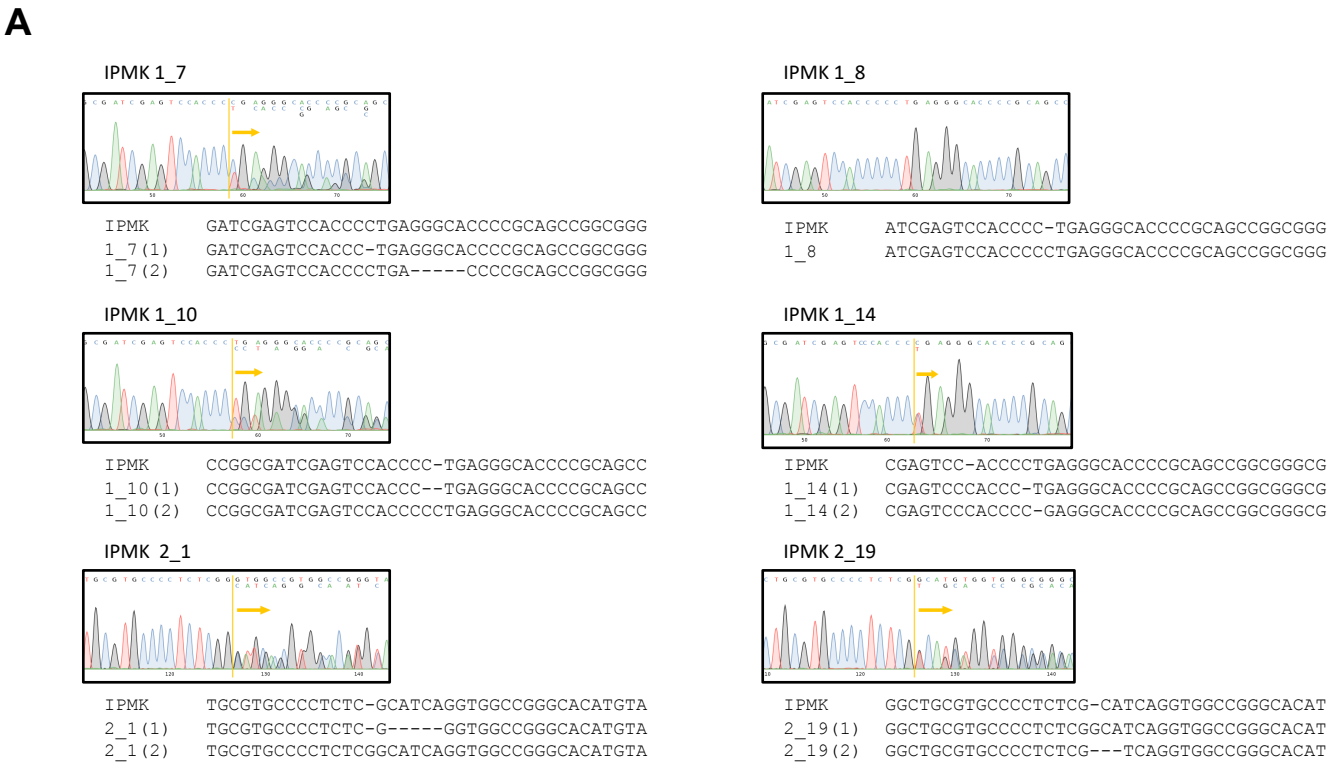
Supplementary Figure 3 related to Figure 4: Alignment of lentiviral gag sequences. Residues are highlighted by conservation, with the mature and immature charged ring residues boxed.

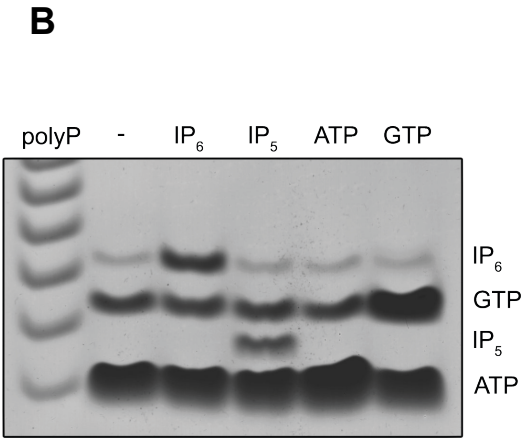
Supplementary Figure 4 related to Figure 5: (A) Western blot showing Gag expression levels of lysine mutants in producer cells. Gag precursor Pr55Gag (pr55), p41 and mature capsid

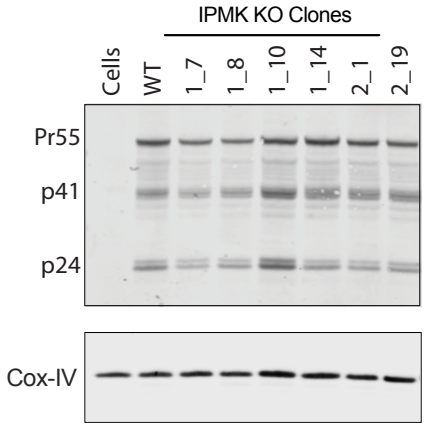
protein (p24) are indicated. (B) Quantification of viral production from 293T, IPMK KO and IPPK KO cells as determined by RT levels in viral supernatants. Error bars depict mean ± SD of two independent experiments. Values are expressed as fold change from levels of RT produced in WT virus. (C) Western 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.

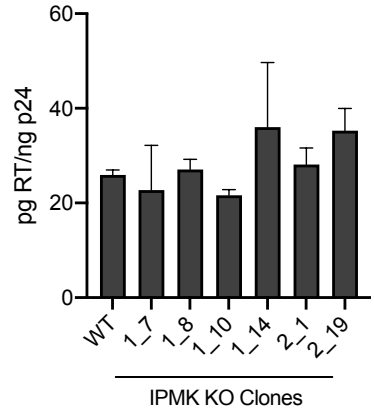
Supplementary Table 1 related to Figure 3: Data collection and refinement statistics. Crystallographic statistics for the HIV-1 hexamer structure complexed with IP_5.

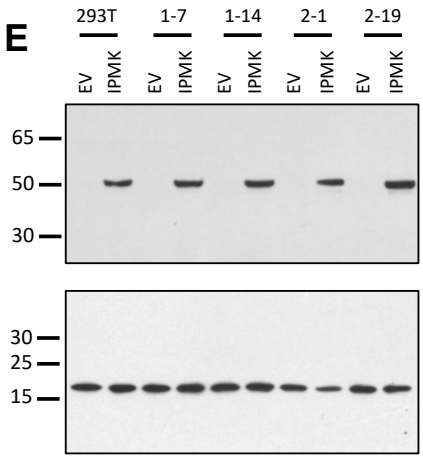
Supplementary Figure 1 related to Figure 1

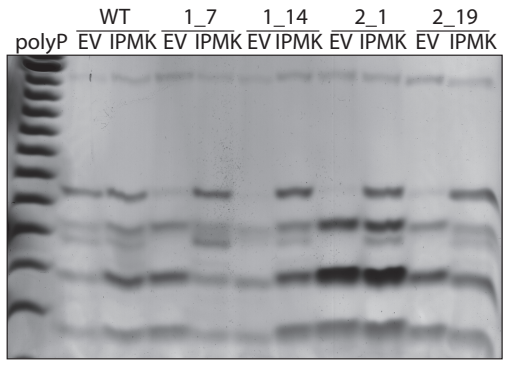


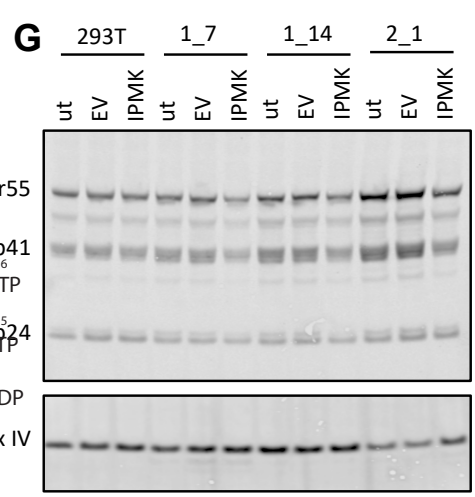




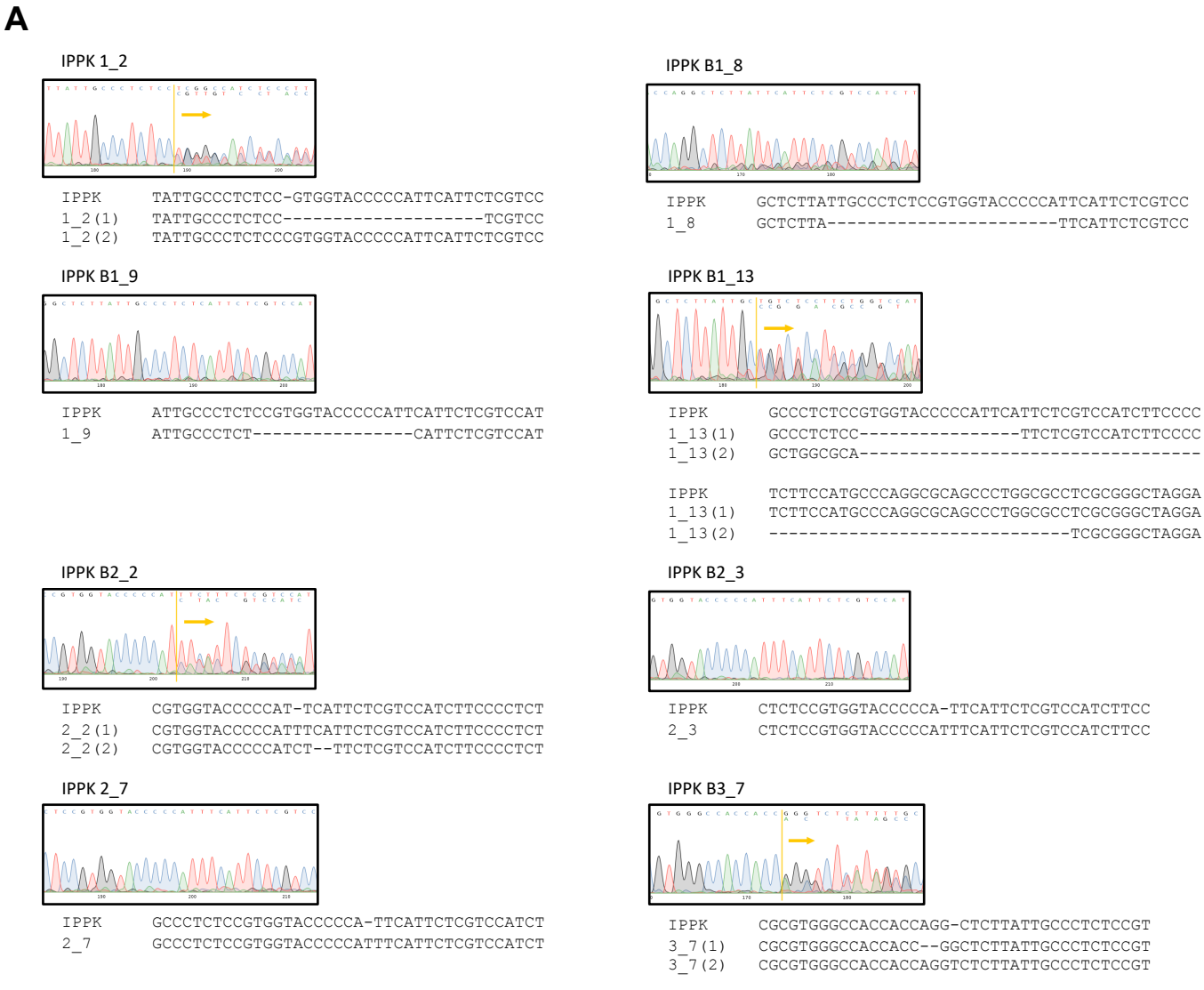




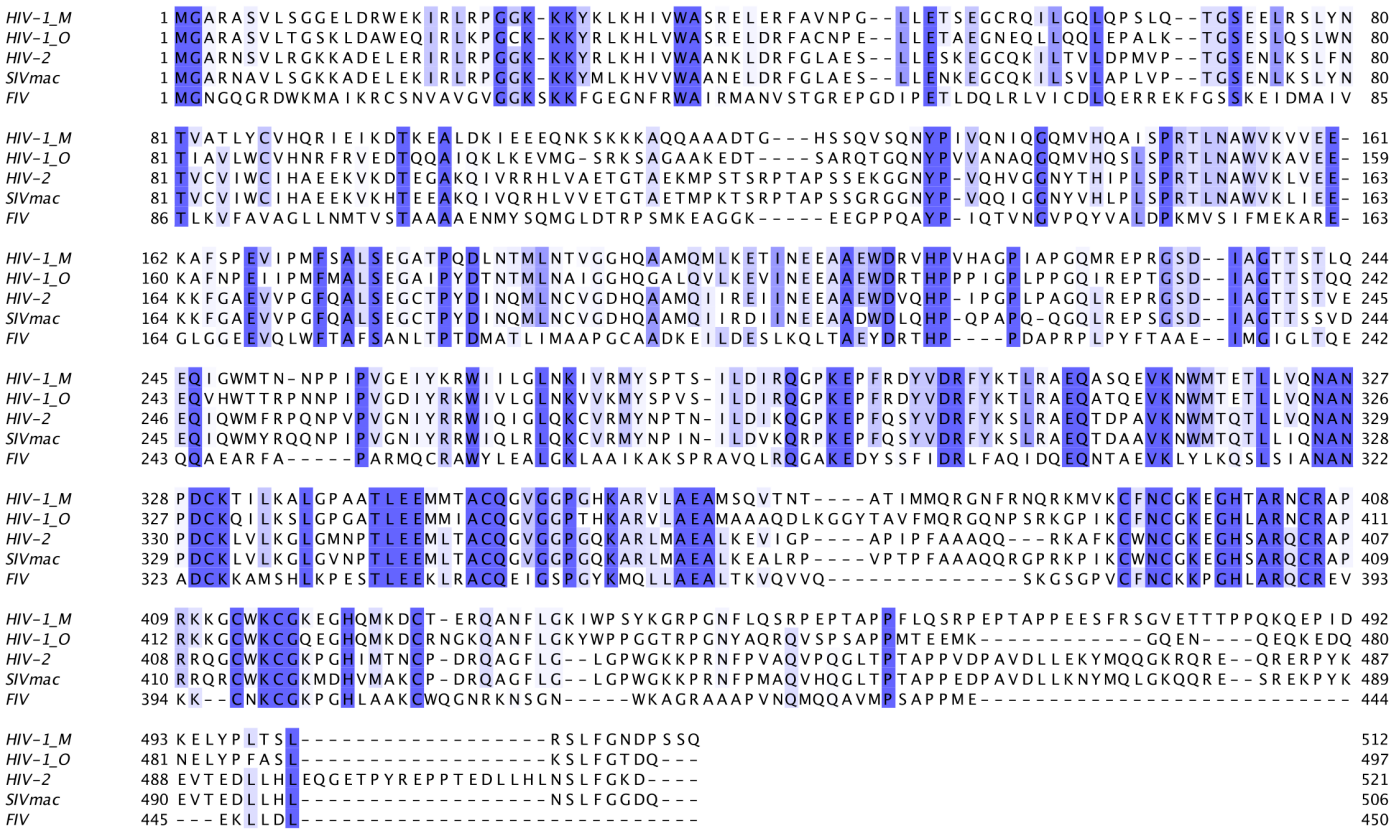




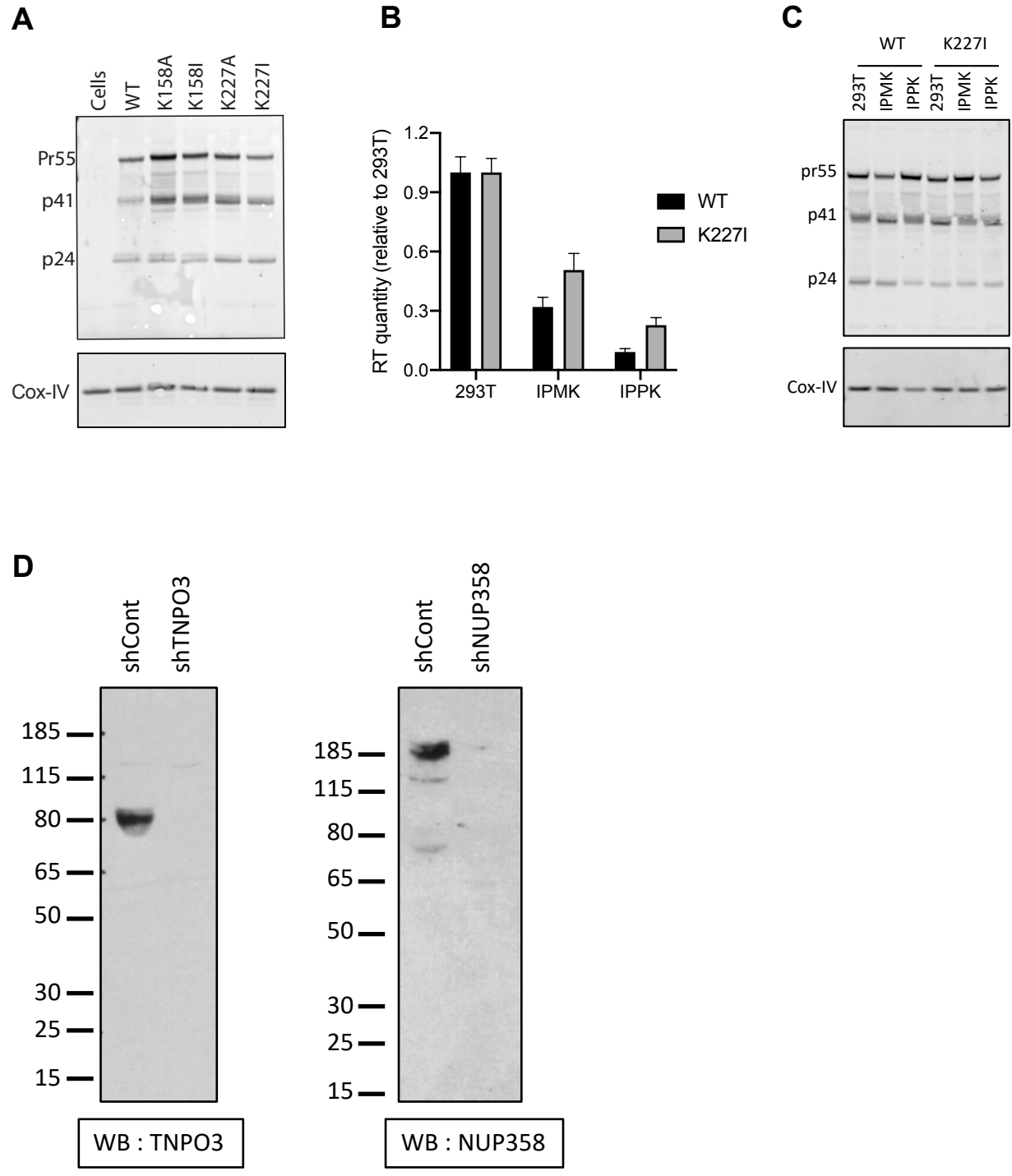
Supplementary Figure 2 related to Figure 2



Supplementary Figure 3 related to Figure 4



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)
$I / \sigma I$	17.7 (2.6)
Completeness (%)	99.3 (90.0)
Redundancy	6.5 (6.0)
Resolution (Å)	1.91
No. reflections	20758
R_{work} / R_{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.