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Supplementary Video 1 | Cryo-EM tomographic reconstruction of a released EPN. Shown is the raw charge density in different slices of the tomogram (first pass) and isosurface renderings (second pass) of individual protein nanocages (gold) and the limiting membrane of the EPN (green).

Supplementary Video 2 | Single particle cryo-EM reconstruction of released EPN-01* protein nanocage. Shown are the raw charge density (grey, contoured at 4.5 σ) and a rigid body fitting of the I3-01 design model (dark green) plus one N-terminal amino acid (yellow) added to I3-01.

Supplementary Table 1 | Description and experimental results of the 43 EPN constructs and 8 negative control constructs surveyed.

Construct	Membrane-binding element	Assembly element	ESCRT-recruiting element	Architecture*	Release**
EPN-01	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-I3-01-p6	Yes †
EPN-02 (negative control)	Mutated myristoylation motif from HIV-1_{NL4-3} Gag(G_2A)	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-I3-01-p6	No
EPN-03	Myristoylation motif from MARCKS‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-I3-01-p6	Yes †
EPN-04	Myristoylation motif from Src kinase‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-I3-01-p6	No
EPN-05	Myristoylation motif from neurocalcin‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-I3-01-p6	No
EPN-06	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	Myr-p6-I3-01	No
EPN-07	Myristoylation/palmitoylation motif from Lyn kinase‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	MyrPalm-I3-01-p6	Yes †
EPN-08	Myristoylation/palmitoylation motif from Gαo‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	MyrPalm-I3-01-p6	Yes †
EPN-09	Dual palmitoylation motif from GAP43‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	PalmPalm-I3-01-p6	Yes †
EPN-10	Dual palmitoylation motif from PSD-95‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	PalmPalm-I3-01-p6	No
EPN-11	Myristoylation/palmitoylation motif from Lyn kinase‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	MyrPalm-p6-I3-01	Yes †
EPN-12	Prenylation motif from K-Ras4B	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	I3-01-p6-Pren	No

EPN-13	Prenylation motif from K-Ras4B	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-Pren	No
EPN-14	Prenylation motif from paralemmin‡	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-Pren	No
EPN-15	Prenylation motif from RhoF	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-Pren	No
EPN-16	Prenylation motif from Type II inositol 1,4,5-trisphosphate 5-phosphatase	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-Pren	No
EPN-17 (negative control)	PH domain from rat phospholipase Cδ	I3-01	-	PH-I3-01	No
EPN-18	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	PH-I3-01-p6	Yes †
EPN-19	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-pH	No
EPN-20	PH domain from rat phospholipase Cδ	I3-01	Ebola VP40 (partial)	VP40-I3-01-pH	Yes †
EPN-21 (negative control)	PH domain from rat phospholipase Cδ	I3-01	Mutated HIV-1_{NL4-3} $p6^{Gag}$ ($PTAP_{10}AAAA$)	p6-I3-01-PH	No
EPN-22 (negative control)	PH domain from rat phospholipase Cδ	I3-01	Mutated HIV-1_{NL4-3} $p6^{Gag}$ ($YP_{37}AA$)	p6-I3-01-PH	No
EPN-23	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-PH-I3-01	Yes †
EPN-24	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	PH-p6-I3-01	Yes †
EPN-25	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	I3-01-p6-PH	Yes †
EPN-26	PH domain from rat phospholipase Cδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	I3-01-PH-p6	No
EPN-27 (negative control)	C1 domain from PCKδ	I3-01	-	C1-I3-01	No
EPN-28	C1 domain from PCKδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	C1-I3-01-p6	No
EPN-29	C1 domain from PCKδ	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-C1	No
EPN-30 (negative control)	C2 domain from mouse PI3K	I3-01	-	C2-I3-01	No
EPN-31	C2 domain from mouse PI3K	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	C2-I3-01-p6	No
EPN-32	C2 domain from mouse PI3K	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-C2	No
EPN-33 (negative control)	PX domain from p40phox	I3-01	-	PX-I3-01	No
EPN-34	PX domain from p40phox	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	PX-I3-01-p6	No
EPN-35	PX domain from p40phox	I3-01	HIV-1_{NL4-3} $p6^{Gag}$	p6-I3-01-PX	No
EPN-36	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	VP40 (partial)	Myr-I3-01-VP40	Yes †
EPN-37	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	EIAV $p9^{Gag}$	Myr-I3-01-p9	Yes †
EPN-38	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	SV5 M protein (partial)	Myr-I3-01-M	Weak
EPN-39	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	HIV-1 p6 preceded by a 22-residue flexible	Myr-I3-01-linker-p6	Yes †

			linker		
EPN-40	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Ebola VP40 (partial) preceded by a 22-residue flexible linker	Myr-I3-01-linker-VP40	No
EPN-41	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	EIAV p9 preceded by a 22-residue flexible linker	Myr-I3-01-linker-p9	Yes †
EPN-42	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	SV5 M protein (partial) preceded by a 22-residue flexible linker	Myr-I3-01-linker-M	Weak
EPN-43	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif PTAPPEY from Ebola VP40	Myr-I3-01-PTAPPEY	Weak
EPN-44	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif PTAP from HIV-1 p6/Ebola VP40	Myr-I3-01-PTAP	Weak
EPN-45	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif PPEY from Ebola VP40	Myr-I3-01-PPEY	No
EPN-46	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif YPLTSL from HIV-1 p6	Myr-I3-01-YPLTSL	Weak
EPN-47	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif YPDL from EIAV $p9^{Gag}$	Myr-I3-01-YPDL	Weak
EPN-48	Myristoylation motif from HIV-1_{NL4-3} Gag	I3-01	Peptide motif FPIV from SV5 M protein	Myr-I3-01-FPIV	Weak
EPN-49 (negative control)	Mutated myristoylation motif from HIV-1_{NL4-3} Gag(G_2A)	O3-33	HIV-1_{NL4-3} $p6^{Gag}$	Myr-O3-33-p6	No
EPN-50	Myristoylation motif from HIV-1_{NL4-3} Gag	O3-33	HIV-1_{NL4-3} $p6^{Gag}$	Myr-O3-33-p6	No
EPN-51	PH domain from rat phospholipase Cδ	O3-33	HIV-1_{NL4-3} $p6^{Gag}$	p6-O3-33-PH	Yes †

Note: Of the 20 constructs that failed to produce EPNs, the following 11 carried membrane-binding domains that failed in all constructs tested: Five constructs that included four different CaaX boxes as membrane-binding elements (EPNs 12-16) and pairs of constructs that each carried C1 domains (EPNs 28 and 29), C2 domains (EPNs 31 and 32), or PX domains (EPNs 34 and 35). The consistent failure of constructs that carried these membrane-binding elements suggests either that these proteins did not interact with membranes at all, or that they were targeted to internal membranes that did not lead to productive release of extracellular EPNs.

* Architecture refers to the order in the protein primary structure of the three functional elements required for EPN release. For clarity, linkers and epitope tags (i.e., Myc) are not shown.

** Protease and aldolase assays (for I3-01 based constructs) indicate release within membrane-enclosed vesicles

† Results shown in Extended Data Fig. 8

‡ Taken from ref. 1

Supplementary Table 2 | Amino acid sequences of the 43 EPN constructs and 8 negative control constructs surveyed.

Construct	Amino acid sequence
EPN-01	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVE TTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-02	MAARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL

(negative control)	GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVE TTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-03	MGAQFSGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVE TTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-04	MGSSKSGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVE TTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-05	MGKQNSGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVE TTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-06	MGARASLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSL FGNDPSSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITF TVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLD EEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKA MKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKA KAFVEKIRGCTEQKLISEEDL
EPN-07	MGCIKSKRKDNLNGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQC RKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHT ILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVG VGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEE SFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-08	MGCTLSAEERAALGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQC RKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHT ILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVG VGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEE SFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-09	MLCCMRRTKQVEKGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQC RKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHT ILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVG VGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEE SFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-10	MDCLCIVTTKKYRGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQC RKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHT ILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVG VGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEE SFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-11	MGCIKSKRKDNLNLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYP LTSLRSLFGNDPSSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGV HLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEF IVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVV GPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTP VEVAEKAKAFVEKIRGCTEQKLISEEDL
EPN-12	MKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADT VIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFC KEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPN

	VKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKI RGCTEQKLISEEDLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYP LTSLRSLFGNDPSSQKKKKKSKTKCVIM
EPN-13	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGGSSGKKKKKSKTKCVI M
EPN-14	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGGSSGDMKKHRCKCCSI M
EPN-15	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGGSSGAQRQKKRRLCLL L
EPN-16	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGGSSGAQEFIHQFLCNP L
EPN-17 (negative control)	MHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRKVM RSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNTLD LIAPSPADAQHWVQGLRKIIHHSGSMDQRQKGSGSKIEELFKKHKIVAVL RANSVEEAKKKALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIG AGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTE LVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCE WFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDL
EPN-18	MHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRKVM RSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNTLD LIAPSPADAQHWVQGLRKIIHHSGSMDQRQKGSGSKIEELFKKHKIVAVL RANSVEEAKKKALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIG AGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTE LVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCE WFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLQS RPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-19	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKL QEDCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPED RCFSIVFKDQRNTLDLIAPSPADAQHWVQGLRKIIHHSGSMDQRQK
EPN-20	MRRVILPTAPPEYMEAIYPVRGSGSKIEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQC RKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHT ILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVG VGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDLHGLQDDPDLQAL LKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRKVMRSPESQLFSIEDI QEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNTLDLIAPSPADAQHWV QGLRKIIHHSGSMDQRQK

EPN-21 (negative control)	MLQSRPEAAAAPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKL QEDCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPED RCFSIVFKDQRNTLDLIAPSPADAQHWVQGLRKIIHHSGSMDQRQK
EPN-22 (negative control)	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELAALTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKL QEDCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPED RCFSIVFKDQRNTLDLIAPSPADAQHWVQGLRKIIHHSGSMDQRQK
EPN-23	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRK VMRSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNT LDLIAPSPADAQHWVQGLRKIIHHSGSMDQRQKGSGSKIEELFKKHKIVA VLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAI IGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTP TELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNV CEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDL
EPN-24	MHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRKVM RSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNTLD LIAPSPADAQHWVQGLRKIIHHSGSMDQRQKLQSRPEPTAPPEESFRSGV ETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQKIEELFKKHKIVAVLRA NSVEEAKKKALAVFLGGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAG TVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELV KAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWF KAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRGCTEQKLISEEDL
EPN-25	MKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADT VIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFC KEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPN VKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKI RGCTEQKLISEEDLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYP LTSLRSLFGNDPSSQHGLQDDPDLQALLKGSQLLKVKSSSWRRERFYKLQ EDCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEKFARDIPEDR CFSIVFKDQRNTLDLIAPSPADAQHWVQGLRKIIHHSGSMDQRQK
EPN-26	MKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADT VIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFC KEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPN VKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKI RGCTEQKLISEEDLGSDGSGRSGSHGLQDDPDLQALLKGSQLLKVKSSSW RRERFYKLQEDCKTIWQESRKVMRSPESQLFSIEDIQEVRMGHRTEGLEK FARDIPEDRCFSIVFKDQRNTLDLIAPSPADAQHWVQGLRKIIHHSGSMD QRQKLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFG NDPSSQ
EPN-27 (negative control)	MPHRFKVHNYMSPTFCDHCGSLLWGLVKQGLKCEDCGMNVHHKCREKVAN LCGGSDGSGRSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHL IEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIV SPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGP QFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVE VAEKAKAFVEKIRGCTEQKLISEEDL
EPN-28	MPHRFKVHNYMSPTFCDHCGSLLWGLVKQGLKCEDCGMNVHHKCREKVAN LCGGSDGSGRSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHL IEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIV SPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGP QFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVE VAEKAKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVETTTPP QKQEPIDKELYPLTSLRSLFGNDPSSQ
EPN-29	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP

	SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSDGSGRSGSPHRFKVHNYMSPTFCDHCGSLLWG LVKQGLKCEDCGMNVHHKCREKVANLCG
EPN-30 (negative control)	MGAVKLSVSYRNGTLFIMVMHIKDLVTEDGADPNPYVKTYLLPDTHKTSK RKTKISRKTRNPTFNEMLVYSGYSKETLRQRELQLSVLSAESLRENFFLG GITLPLKDFNLSKETVKWYQLTAATYLGSGSGDGGRGSRGGDGSGGSSGK IEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKE KGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVK FVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRG CTEQKLISEEDL
EPN-31	MGAVKLSVSYRNGTLFIMVMHIKDLVTEDGADPNPYVKTYLLPDTHKTSK RKTKISRKTRNPTFNEMLVYSGYSKETLRQRELQLSVLSAESLRENFFLG GITLPLKDFNLSKETVKWYQLTAATYLGSGSGDGGRGSRGGDGSGGSSGK IEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKE KGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVK FVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVEKIRG CTEQKLISEEDLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLT SLRSLFGNDPSSQ
EPN-32	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGGSSGGAVKLSVSYRNG TLFIMVMHIKDLVTEDGADPNPYVKTYLLPDTHKTSKRKTKISRKTRNPT FNEMLVYSGYSKETLRQRELQLSVLSAESLRENFFLGGITLPLKDFNLSK ETVKWYQLTAATYL
EPN-33 (negative control)	MAVAQQLRAESDFEQLPDDVAISANIADIEEKRGFTSHFVFVIEVKTKGG SKYLIYRRYRQFHALQSKLEERFGPDSKSSALACTLPTLPAKVYVGVKQE IAEMRIPALNAYMKSLLSLPVWVLMDEDVRIFFYQSPYDSEQVPQALRRG SDGSGRSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEIT FTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHL DEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVK AMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEK AKAFVEKIRGCTEQKLISEEDL
EPN-34	MAVAQQLRAESDFEQLPDDVAISANIADIEEKRGFTSHFVFVIEVKTKGG SKYLIYRRYRQFHALQSKLEERFGPDSKSSALACTLPTLPAKVYVGVKQE IAEMRIPALNAYMKSLLSLPVWVLMDEDVRIFFYQSPYDSEQVPQALRRG SDGSGRSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEIT FTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHL DEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVK AMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEK AKAFVEKIRGCTEQKLISEEDLQSRPEPTAPPEESFRSGVETTTPPQKQE PIDKELYPLTSLRSLFGNDPSSQ
EPN-35	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQKIEELFKKHKIVAVLRANSVEEAKKKALAVFLGGVHLIEITFTVPDA DTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPF PNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPVEVAEKAKAFVE KIRGCTEQKLISEEDLGSGSAVAQQLRAESDFEQLPDDVAISANIADIEE KRGFTSHFVFVIEVKTKGGSKYLIYRRYRQFHALQSKLEERFGPDSKSSA LACTLPTLPAKVYVGVKQEIAEMRIPALNAYMKSLLSLPVWVLMDEDVRI FFYQSPYDSEQVPQALRR
EPN-36	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLRRVILPTAPPEYMEAIYPV

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EPN-37	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLPIQQKSQHNKSVVQETPQT QNLYPDLSEIKKEYNVKEKDQVEDLNLDSLWE
EPN-38	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLNPRQSIKAFPIVINSDGGE K
EPN-39	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGN DPSSQ
EPN-40	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGRRVILPTAPPEYMEAIYPVR
EPN-41	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGPIQQKSQHNKSVVQETPQTQNLYPDLSEIKKEYNVKEKDQVEDLNLD SLWE
EPN-42	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGNPRQSIKAFPIVINSDGGEK
EPN-43	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGPTAPPEYGGS
EPN-44	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGPTAPGGS
EPN-45	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGPPEYGGS
EPN-46	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK

	GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGYPLTSLGGS
EPN-47	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGYPDLGGS
EPN-48	MGARASGSKSGSGSDSGSKIEELFKKHKIVAVLRANSVEEAKKKALAVFL GGVHLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQCRKAVESG AEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPG EVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVK GTPVEVAEKAKAFVEKIRGCTEQKLISEEDLGSGSGDGGRGSRGGDGSGG SSGFPIVGGS
EPN-49 (negative control)	MAARASGSGSSQAIGILELTSIAAGMELGDAMLKSANVDLLVSKTISPGK FLLMLGGDIGAIQQAIETGTSQAGELLVDSLVLANIHPSVLPAISGLNSV DKRQAVGIVETWSVAACISAADRAVKGSNVTLVRVHMAFGIGGKCYMVVA GDVSDVALAVTVASSSAGAYGLLVYASLIPRPHEAMWRQMVEGQKLISEE DLLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGND PSSQ
EPN-50	MGARASGSGSSQAIGILELTSIAAGMELGDAMLKSANVDLLVSKTISPGK FLLMLGGDIGAIQQAIETGTSQAGELLVDSLVLANIHPSVLPAISGLNSV DKRQAVGIVETWSVAACISAADRAVKGSNVTLVRVHMAFGIGGKCYMVVA GDVSDVALAVTVASSSAGAYGLLVYASLIPRPHEAMWRQMVEGQKLISEE DLLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGND PSSQ
EPN-51	MLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDP SSQSQAIGILELTSIAAGMELGDAMLKSANVDLLVSKTISPGKFLLMLGG DIGAIQQAIETGTSQAGELLVDSLVLANIHPSVLPAISGLNSVDKRQAVG IVETWSVAACISAADRAVKGSNVTLVRVHMAFGIGGKCYMVVAGDVSDVA LAVTVASSSAGAYGLLVYASLIPRPHEAMWRQMVEGQKLISEEDLHGLQD DPDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESRKVMRSPESQ LFSIEDIQEVRMGHRTEGLEKFARDIPEDRCFSIVFKDQRNTLDLIAPSP ADAQHWVQGLRKIIHHSGSMDQRQK

Supplementary Table 3 | Expression constructs and antibodies used in this this study.
3A Mammalian Expression constructs

Plasmid Name	Source	Mutation	Epitope Tags	Addgene ID
pCMV-I3-01-Myc			Myc	80038
pCMV-EPN-01*			Myc	80039
pCMV-EPN-01*(ΔM)		G_1A	Myc	80040
pCMV-EPN-01*(ΔI)		$L_{33}R$	Myc	80041
pCMV-EPN-01*(ΔL1)		$PTAP_{10}AAAA$	Myc	80042
pCMV-EPN-01*(ΔL2)		$YP_{36}AA$	Myc	80043
pCMV-EPN-01*(ΔL1+L2)		$PTAP_{10}AAAA$, $YP_{36}AA$	Myc	80044
pCMV-EPN-01*(ΔLF)		$LF_{45}AA$	Myc	80045
pCMV-EPN-01		M_3I	Myc	80046
pCMV-EPN-01(ΔM)		G_1A, M_3I	Myc	80047
pCMV-EPN-01(ΔI)		M_3I, $L_{33}R$	Myc	80048
pCMV-EPN-01(ΔL1)		M_3I, $PTAP_{10}AAAA$	Myc	80049
pCMV-EPN-01(ΔL2)		M_3I, $YP_{36}AA$	Myc	80050
pCMV-EPN-01(ΔL1+L2)		M_3I, $PTAP_{10}AAAA$, $YP_{36}AA$	Myc	80051
pCMV-EPN-01-(ΔLF)		M_3I, $LF_{45}AA$	Myc	80052
pMM310-Myc	Refs. 2,3		Myc	80053
pCMV-VSV-G-Myc	Ref. 4		Myc	80054
pCMV-VSV-G($P_{127}D$)-Myc	Ref. 4		Myc	80055
pEGFP-VPS4A($E_{228}Q$)	Ref. 5		Myc	80351
pCMV-EPN-01†			Myc	79916
pCMV-EPN-02		G_2A	Myc	79917
pCMV-EPN-03			Myc	79918

pCMV-EPN-04			Myc	79919
pCMV-EPN-05			Myc	79920
pCMV-EPN-06			Myc	79921
pCMV-EPN-07			Myc	79922
pCMV-EPN-08			Myc	79923
pCMV-EPN-09			Myc	79924
pCMV-EPN-10			Myc	79925
pCMV-EPN-11			Myc	79926
pCMV-EPN-12			Myc	79927
pCMV-EPN-13			Myc	79928
pCMV-EPN-14			Myc	79929
pCMV-EPN-15			Myc	79930
pCMV-EPN-16			Myc	79931
pCMV-EPN-17		No late domain	Myc	79932
pCMV-EPN-18			Myc	79933
pCMV-EPN-19			Myc	79934
pCMV-EPN-20			Myc	79935
pCMV-EPN-21			Myc	79936
pCMV-EPN-22			Myc	79937
pCMV-EPN-23			Myc	79938

pCMV-EPN-24			Myc	79939
pCMV-EPN-25			Myc	79940
pCMV-EPN-26			Myc	79941
pCMV-EPN-27		No late domain	Myc	79942
pCMV-EPN-28			Myc	79943
pCMV-EPN-29			Myc	79944
pCMV-EPN-30		No late domain	Myc	79945
pCMV-EPN-31			Myc	79946
pCMV-EPN-32			Myc	79947
pCMV-EPN-33		No late domain	Myc	79948
pCMV-EPN-34			Myc	79949
pCMV-EPN-35			Myc	79950
pCMV-EPN-36			Myc	79951
pCMV-EPN-37			Myc	79952
pCMV-EPN-38			Myc	79953
pCMV-EPN-39			Myc	79954
pCMV-EPN-40			Myc	79955
pCMV-EPN-41			Myc	79956
pCMV-EPN-42			Myc	79957
pCMV-EPN-43			Myc	79958

pCMV-EPN-44			Myc	79959
pCMV-EPN-45			Myc	79960
pCMV-EPN-46			Myc	79961
pCMV-EPN-47			Myc	79962
pCMV-EPN-48			Myc	79963
pCMV-EPN-51			Myc	79966
pCMV-EPN-03(ΔM)		G_2A	Myc	80104
pCMV-EPN-07(ΔM)		G_2A, C_3A	Myc	80105
pCMV-EPN-08(ΔM)		G_2A, C_3A	Myc	80106
pCMV-EPN-18(ΔM)		$R_{40}A$	Myc	80107
pCMV-EPN-51(ΔL)		$PTAP_{10}AAAA$, $YP_{37}AA$	Myc	80108
pCMV-EPN-51(ΔM)		$R_{40}A$	Myc	80109
pCMV-EPN-51(ΔI)		$A_{167}R$	Myc	80110

† To simplify cloning, the version of EPN-01 used in Extended Data Fig. 8 was cloned into pCMV using the *Kpn*I restriction site at the 5′ end of the insert, while the EPN-01/EPN-01* constructs used in Figures 1-3 were cloned into pCMV using the *Not*I restriction site at the 5′ end.

3B Bacterial expression constructs

Plasmid Name	Source	Mutation	Epitope Tags	Addgene ID
pET29b-EPN-01*			Myc	80423
pET29b-EPN-01*-His			Myc, His	84509
pET29b-EPN-01(ΔM)-His		G_2A	Myc, His	84510
pET29b-EPN-01(ΔL1+ΔL2)-His		$PTAP_{10}AAAA$, $YP_{37}AA$	Myc, His	84511

Supplementary Table 4 | Antibodies

Antigen	Species	Membrane	Blocking	Dilution	Source
Myc	mouse	PVDF/Nitrocellulose	5% milk/0.5% milk	5,000	EMD Millipore (05-724)
GAPDH	mouse	PVDF	5% milk	10,000	EMD Millipore (MAB374)
Actin	goat	Nitrocellulose	0.5% milk	200	Santa Cruz Biotech. (sc-1615)
ALIX	rabbit	Nitrocellulose	0.5% milk	500	Covance (UT324)
Myc-Agarose	rabbit				Sigma (A7470)
IgG-Agarose	rabbit				Sigma (A2909)
Myc	mouse	Nitrocellulose	0.5% milk	20,000	Cell Signaling Technology (2276)

Supplementary References

1 Resh, M. D. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1451**, 1-16, doi:10.1016/s0167-4889(99)00075-0 (1999).

2 Tobiume, M., Lineberger, J. E., Lundquist, C. A., Miller, M. D. & Aiken, C. Nef does not affect the efficiency of human immunodeficiency virus type 1 fusion with target cells. *J Virol* **77**, 10645-10650 (2003).

3 Cavrois, M., De Noronha, C. & Greene, W. C. A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. *Nature biotechnology* **20**, 1151-1154, doi:10.1038/nbt745 (2002).

4 Yee, J. K., Friedmann, T. & Burns, J. C. Generation of high-titer pseudotyped retroviral vectors with very broad host range. *Methods Cell Biol* **43 Pt A**, 99-112 (1994).

5 von Schwedler, U. K. *et al.* The protein network of HIV budding. *Cell* **114**, 701-713 (2003).