Supporting Information

Structural insights into the mechanism of human T-cell leukemia virus type 1 Gag targeting to the plasma membrane for assembly

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Table S1. Statistics for the NMR structures of HTLV-1 myr(–)MA_{99}

Completeness of resonance assignments (%)[a]	
backbone	98
side chains	95
NOE restraints	1005
intraresidual (|i−j| = 0)	286 (28.4 %)
sequential (|i−j| = 1)	256 (25.5 %)
medium-range (1 <|i−j| <5)	263 (26.2 %)
long-range (|i−j| ≥ 5)	200 (19.9 %)
NOE restraints per residue	10.15
Dihedral angle restraints	144
Number of models	20
Target function [$Å^2$]	0.63 ± 0.05
RMS NOE restraint violation [Å]	0.0064
RMS dihedral restraint violation [º]	0.192
Atom position RMSD (residues 21–93, [Å])	
backbone	0.44 ± 0.1
all heavy atoms	0.72 ± 0.1
Ramachandran statistics [%]	
most favored	86
allowed	12
disallowed	2

[a] Excluding the C-terminal 6xHis-tag, terminal NH_3^+ and $COO^–$, Pro N, Lys NH_3^+, Arg NH_2, Cys SH, Met S, OH groups, carboxyl and aromatic quaternary carbons.

Table S2. Fluorescence particle analysis used to determine relative particle production

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Gag Variant (Replicate)		VLPs	Area imaged (μm^2)	VLP/μm^2	Transfection efficiency (%)	VLP/μm^2 adjusted
WT	(1)	32	338.7	0.094	0.54	0.094
	(2)	19	213.4	0.089	0.62	0.089
	(3)	11	213.4	0.052	0.22	0.052
G2A	(1)	0	338.7	0.000	0.75	0.000
	(2)	4	213.4	0.019	0.99	0.006
	(3)	0	213.4	0.000	0.46	0.000
PTRP	(1)	17	304.83	0.056	0.60	0.050
	(2)	11	213.4	0.052	0.31	0.052
	(3)	7	213.4	0.033	0.33	0.022

Raji/CD4

Gag Variant (Replicate)		VLPs	Area imaged (μm^2)	VLP/μm^2	Transfection efficiency (%)	VLP/μm^2 adjusted
WT	(1)	173	338.7	0.511	16.13	0.511
	(2)	120	338.7	0.354	23.81	0.354
	(3)	13	213.4	0.061	1.49	0.061
	(4)	7	128.04	0.055	2.09	0.055
G2A	(1)	25	338.7	0.074	16.18	0.074
	(2)	21	338.7	0.062	28.13	0.052
	(3)	8	213.4	0.037	2.47	0.023
	(4)	9	213.4	0.042	2.74	0.032
PTRP	(1)	106	338.7	0.313	27.50	0.184
	(2)	9	213.4	0.042	1.95	0.032
	(3)	11	213.4	0.052	3.97	0.027

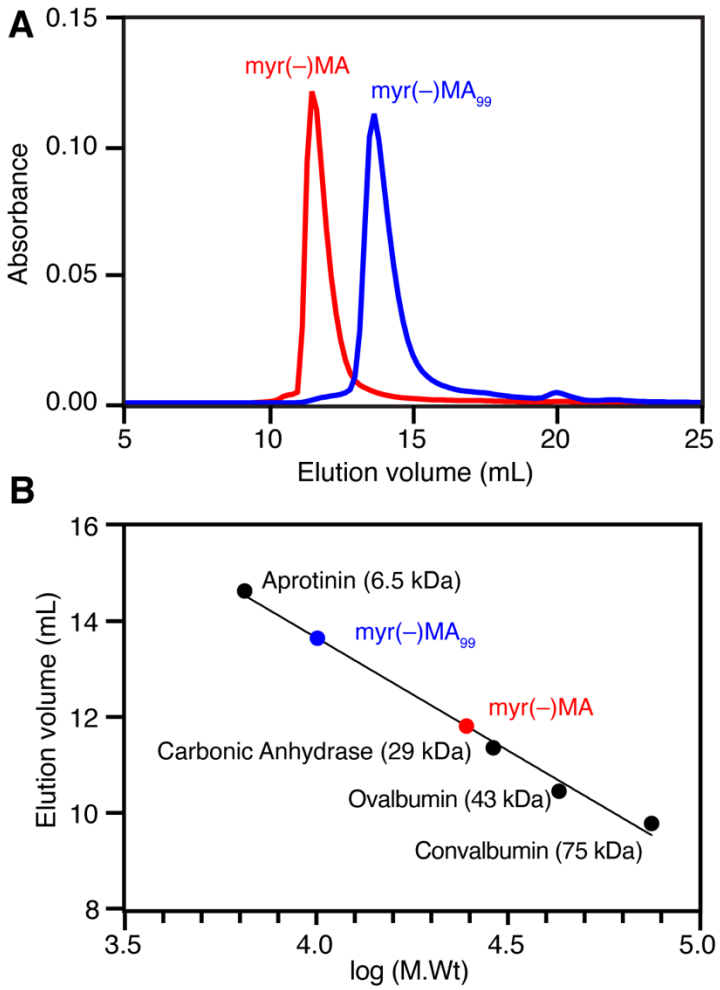

Figure S1. Gel filtration assay of HTLV-1 myr(–)MA proteins. (a) Elution profiles of myr(–)MA and myr(–)MA$_{99}$ using ENrich SEC70 column (BioRad). (b) A molecular weight calibration kit was used to determine the approximate molecular weight of the proteins.

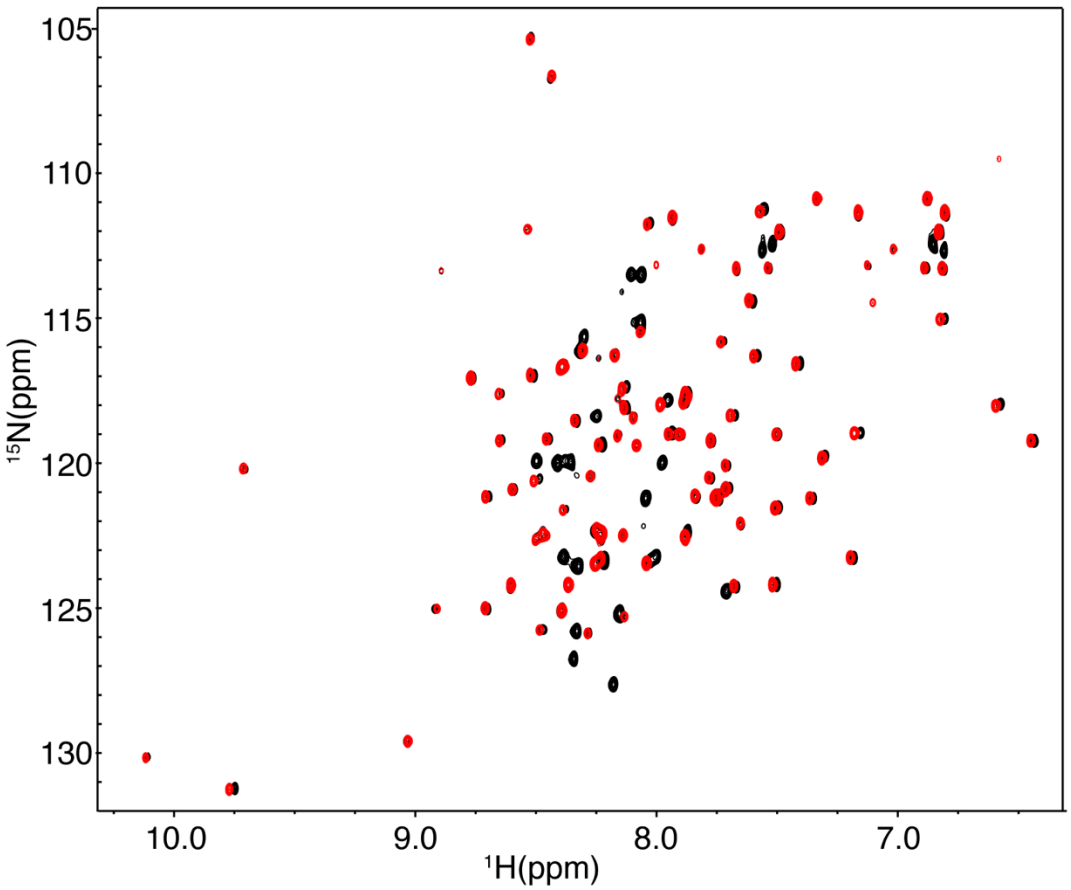

Figure S2. NMR spectra of HTLV-1 myr(–)MA proteins. Overlay of 2D ^{1}H-^{15}N HSQC spectra myr(–)MA (black) and myr(–)MA$_{99}$ (red) proteins.

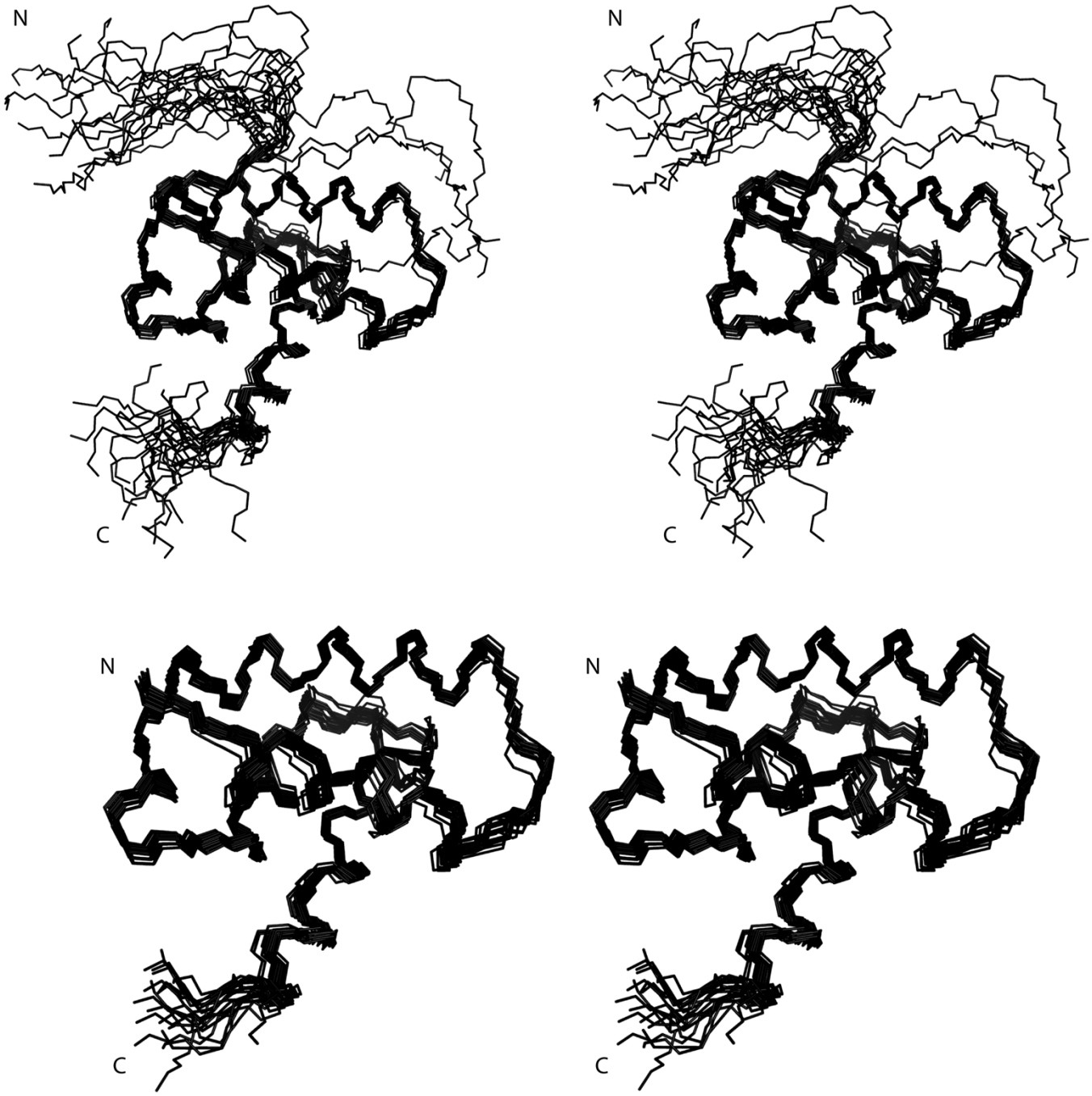

Figure S3. NMR Structure of HTLV-1 myr(–)MA$_{99}$. Stereo-view illustration showing the best-fit backbone superposition of the 20 refined structures calculated for the myr(–)MA$_{99}$ protein (upper panel). Residues 1-17 and 97-99 were omitted in the structures for clarity (lower panel).

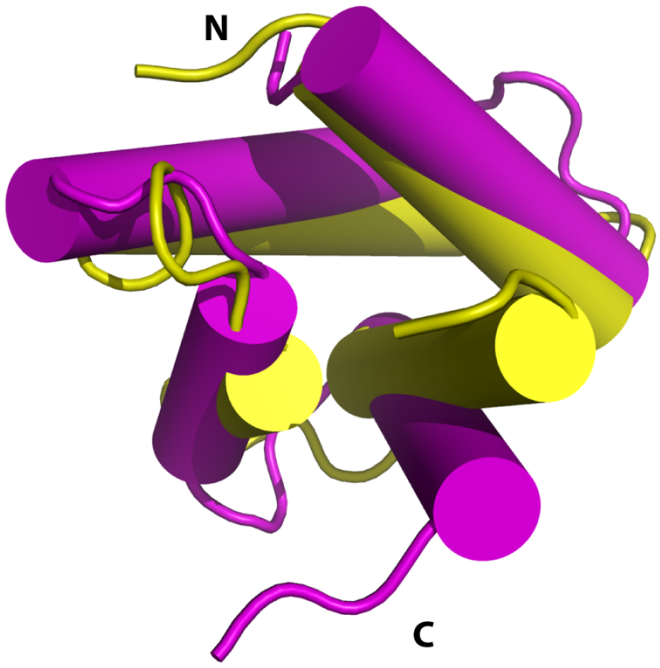

Figure S4. Overlay of residues 18-97 of HTLV-1 myr(–)MA$_{99}$ (yellow) and HTLV-2 myr(–)MA (PDB 1JVR; magenta). Ribbon representations of the structures were generated using the PyMOL molecular graphics system (Schrödinger, LLC).

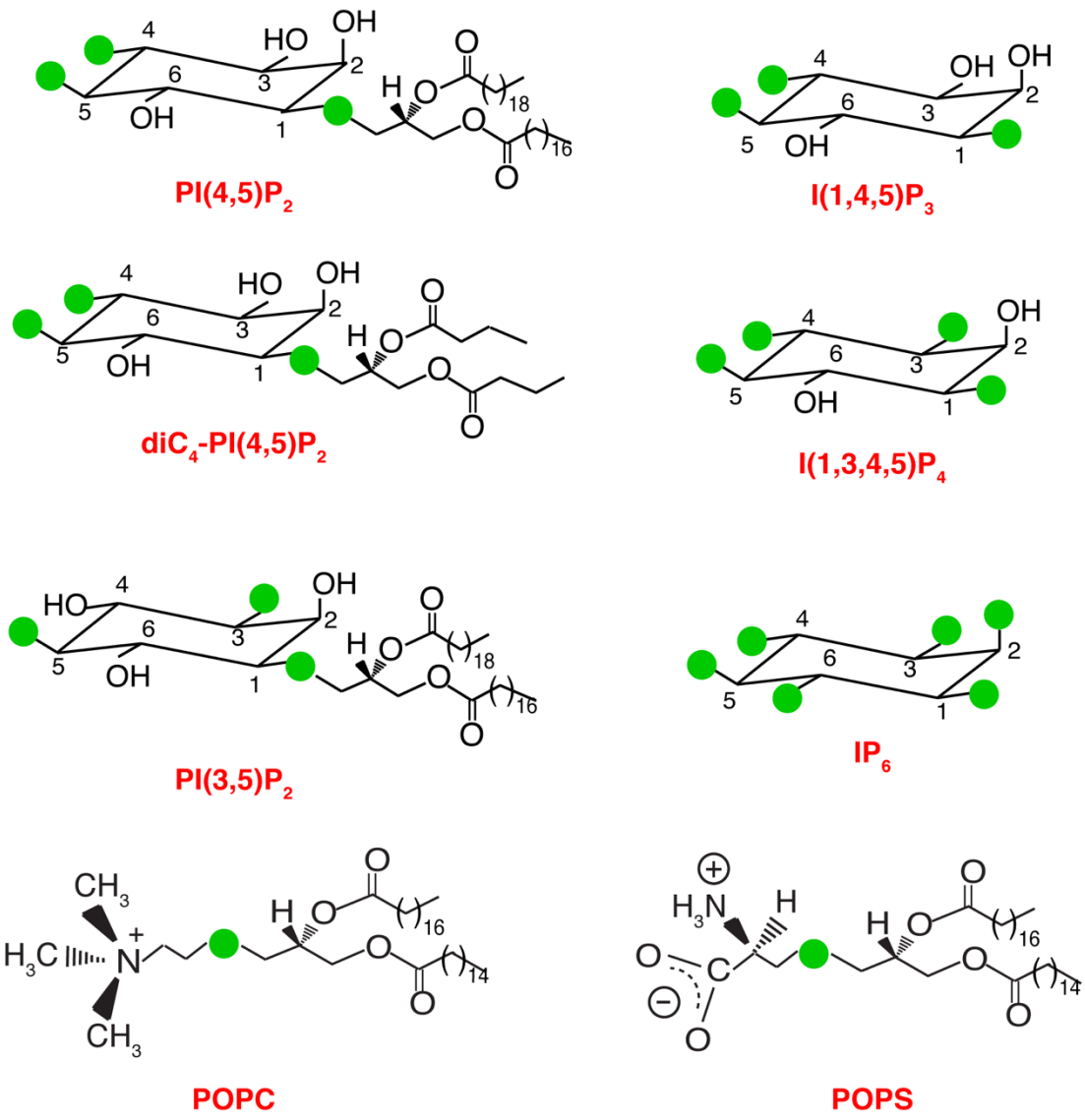

Figure S5. Chemical structures of lipids used in this study. Phosphate groups are shown as green spheres.

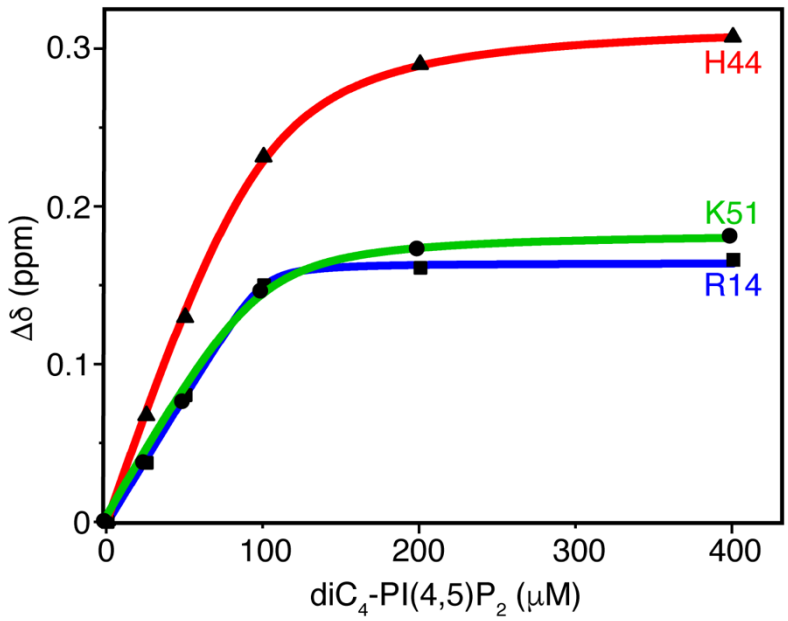

Figure S6. Binding isotherms obtained by fitting ^{1}H and ^{15}N NMR chemical shift data of HTLV-1 myr(–)MA upon titration with diC$_4$-PI(4,5)P$_2$ at 0 M NaCl, which yielded an average K_d of 7.2 ± 2.1 μM.

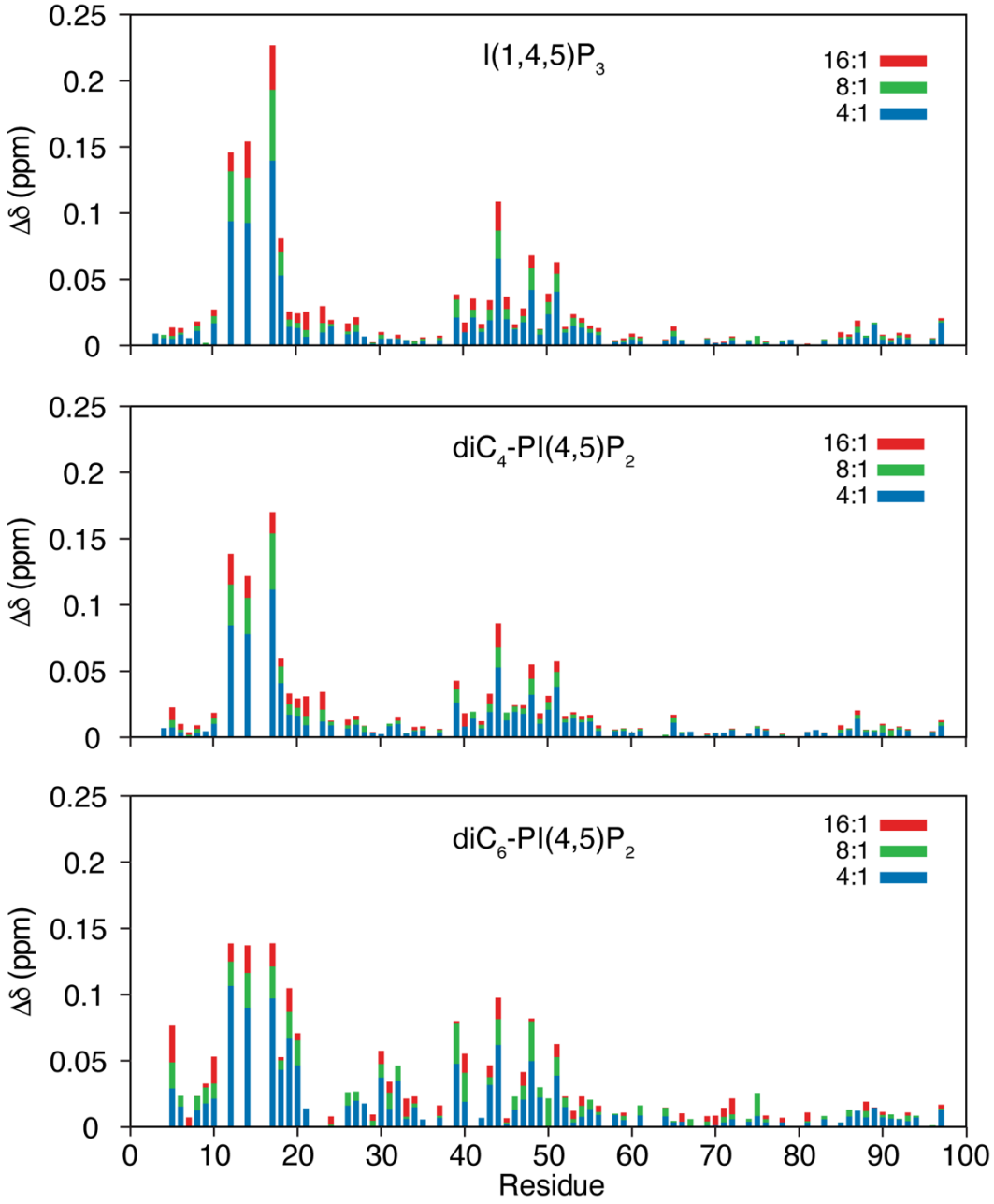

Figure S7. Histograms of normalized ^{1}H-^{15}N chemical shift changes vs. residue number calculated from the HSQC spectra for HTLV-1 myr(–)MA upon titration with $PI(4,5)P_2$ analogs.

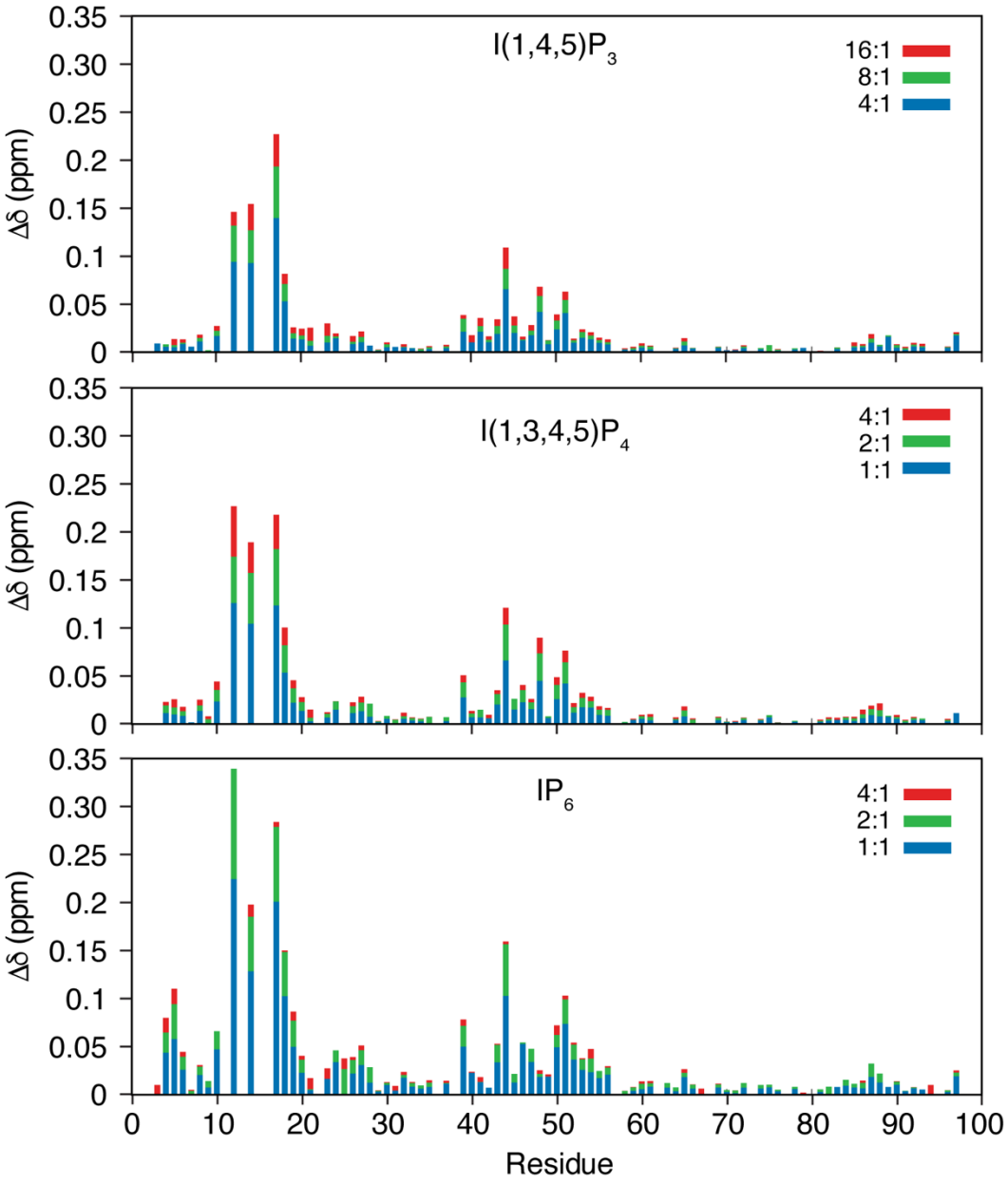

Figure S8. Histograms of normalized ^{1}H-^{15}N chemical shift changes vs. residue number calculated from the HSQC spectra for myr(–)MA upon titration with various inositol phosphates.

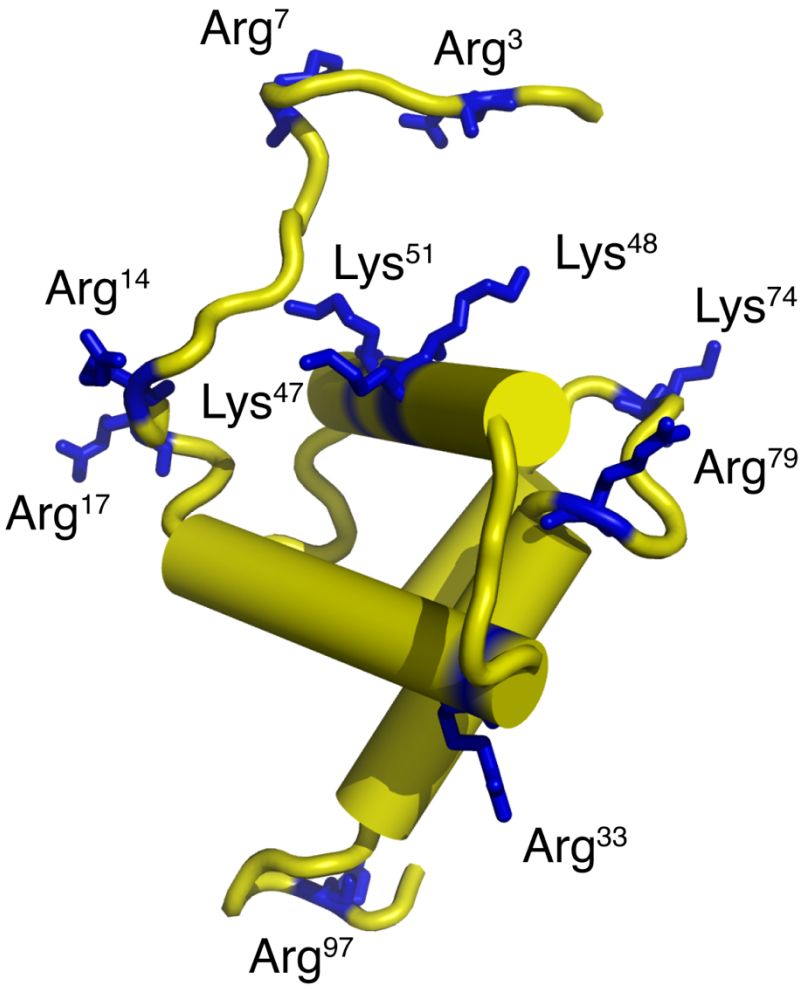

Figure S9. Cartoon representation of the HTLV-1 myr(–)MA_{99} structure with the lysine and arginine residues highlighted as blue sticks.