Fig. S1

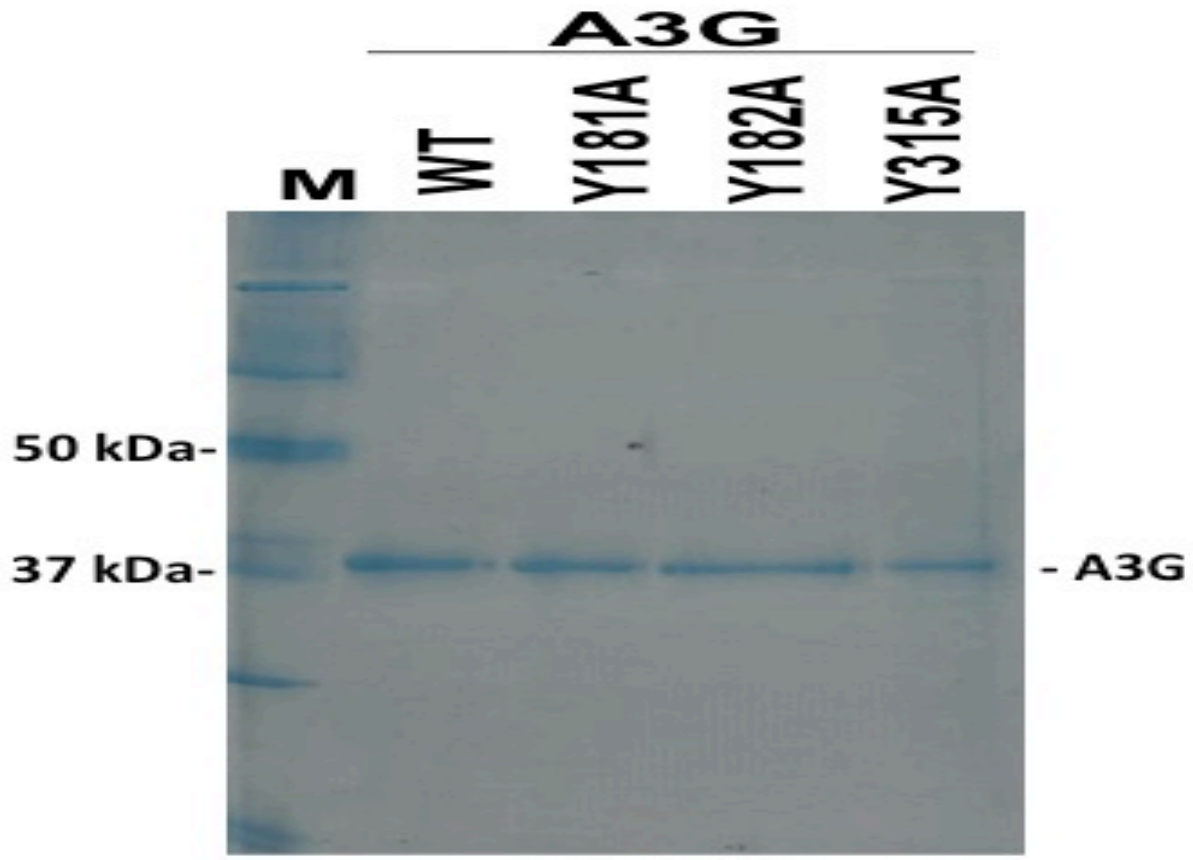

Fig. S2

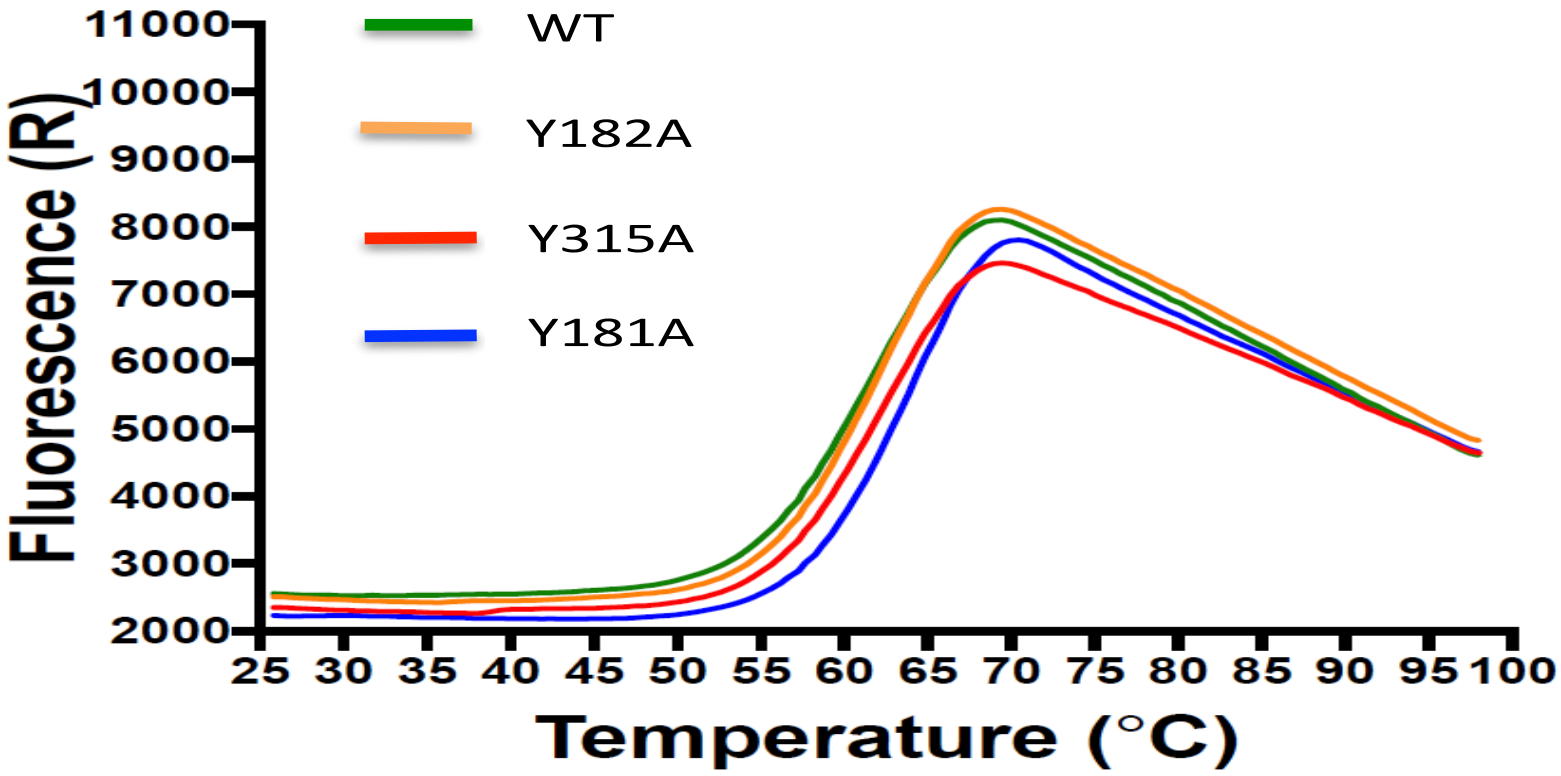
WT
Y182A
Y315A
Y181A
Fluorescence (R)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Temperature (°C)

Fig. S3

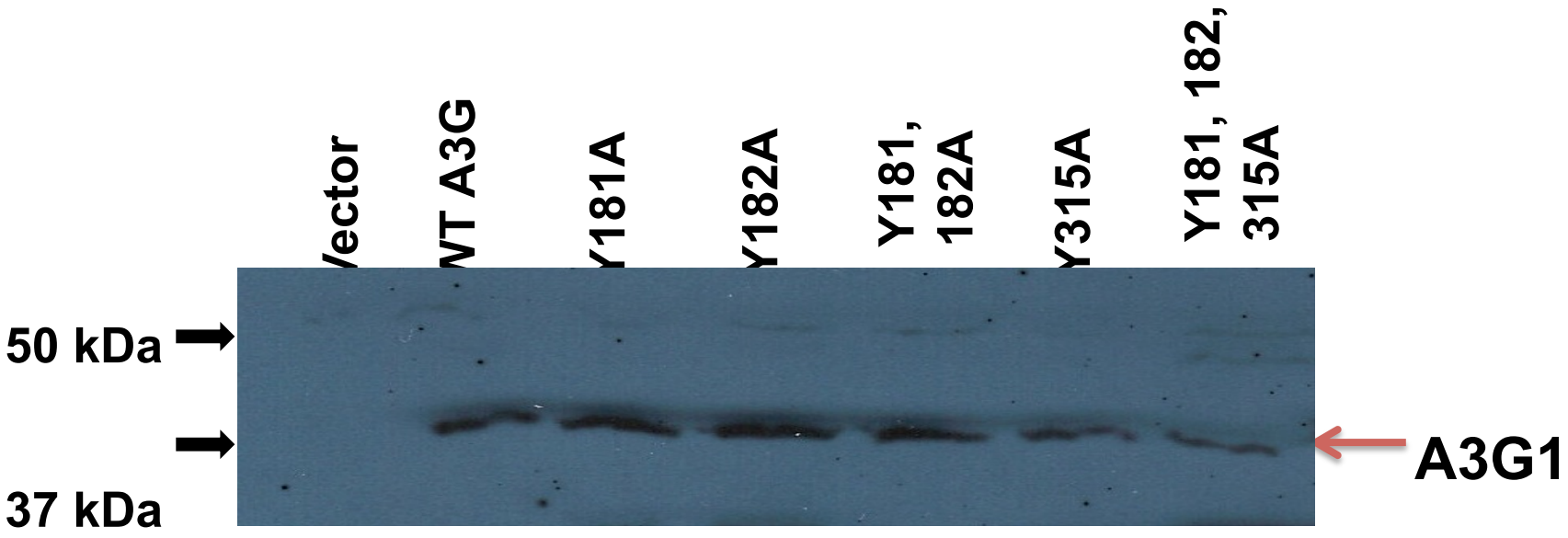
Vector
WT A3G
Y181A
Y182A
Y181, 182A
Y315A
Y181, 182, 315A
50 kDa
37 kDa
A3G1

Fig. S4

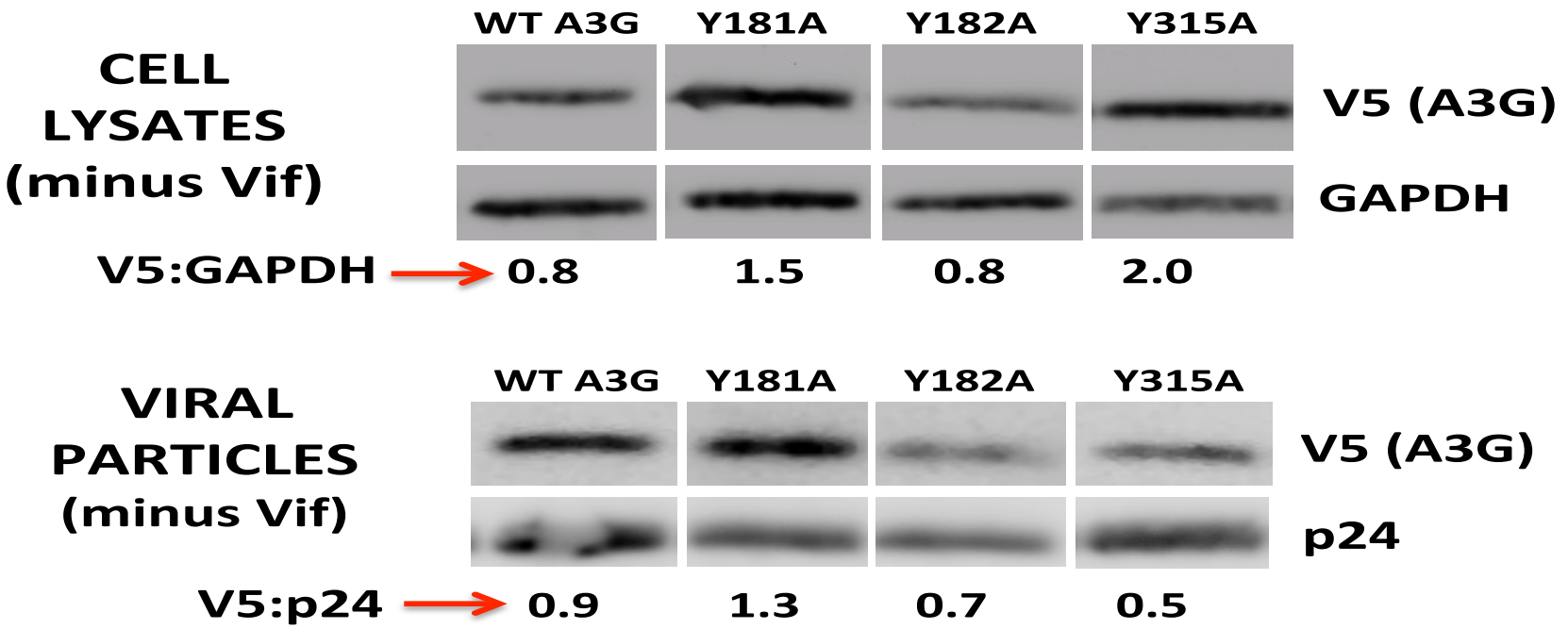
WT A3G
Y181A
Y182A
Y315A
CELL LYSATES (minus Vif)
V5 (A3G)
GAPDH
V5:GAPDH
0.8
1.5
0.8
2.0
WT A3G
Y181A
Y182A
Y315A
VIRAL PARTICLES (minus Vif)
V5 (A3G)
p24
V5:p24
0.9
1.3
0.7
0.5

Table S1. Mass to Charge Ratios for A3G Tryptic Peptide aa 181-194, Sequence YYILLHIMGEILR, Identified by MS in Cross-linked Sample with ssDNA Containing BrdU (Fragmentation Ions for the Same Peptide that is Presented in Fig. 1, top panel).

	Tyr	Tyr	Ile	Leu	Leu	His	Ile	Met	Gly	Glu	Ile	Leu	Arg
Ion	1	2	3	4	5	6	7	8	9	10	11	12	13
a	520.012*	683.075*	796.159*	909.243	1022.32*	1159.38*	1272.47*	1419.505	1476.527	1605.569	1718.654	1831.738	1987.839
a^{2+}	250.66**	342.04*	398.58*	455.12**	511.663*	580.193*	636.735*	702.22**	730.76**	795.28**	851.82**	908.37**	986.42**
b	548.006*	711.07*	824.154*	937.238*	1050.32*	1187.38*	1300.46*	1447.5	1504.522	1633.564	1746.648	1859.732	2015.834
b^{2+}	274.503*	356.035*	402.57**	469.119*	525.661*	594.190*	650.73**	716.25**	744.76**	809.28**	865.82**	922.33**	1000.41
y	175.119	288.203	401.287*	530.33*	587.351*	734.387*	847.471*	984.53*	1097.614	1210.698	1323.782	1486.845	2033.844
	Arg	Leu	Ile	Glu	Gly	Met	Ile	His	Leu	Leu	Ile	Tyr	Tyr

Footnote: Possible 1+ (a, b, y) and 2+ (a^{2+}, b^{2+}) values for mass to charge fragments of the A3G tryptic peptide $m/z = 678.977^{3+}$, aa 181-194, sequence YYILLHIMGEILR, produced after cross-linking/modification of BrdU to Y181. An asterisk (*) indicates MS/MS identified fragments containing BrdU cross-linked nucleotide, a double asterisk (**) indicates the identified MS/MS cross-linked fragments that lost hydroxyl group or water molecule. This peptide was also modified by methionine oxidation.

Table S2. Mass to Charge Ratios for A3G Tryptic Peptide aa 314-320, Sequence IYDDQGR, Identified by MS in Cross-linked Sample with ssDNA containing BrdU (Fragmentation Ions for the Same Peptide that is Presented in Fig. 1, Bottom Panel).

	Ile	Tyr	Asp	Asp	Gln	Gly	Arg
Ion	1	2	3	4	5	6	7
a	86.096	633.096*	748.123*	863.149*	991.208**	1048.23*	1204.331
a^{2+}	43.547	317.048**	374.562**	432.075*	496.104*	524.615*	602.666*
b	114.091	661.09*	776.117*	891.14**	1019.203	1076.22*	1232.326
b^{2+}	57.546	331.045*	388.559**	446.072*	510.102*	538.61**	616.663*
y	175.119	232.14	360.199	475.226*	590.253*	1137.25*	1250.336
	Arg	Gly	Gln	Asp	Asp	Tyr	Ile

Footnote: Possible 1+ (a, b, y) and 2+ (a^{2+}, b^{2+}) values for mass to charge fragments of the A3G tryptic peptide $m/z = 625.3162^{2+}$, aa 314-320, sequence IYDDQGR, produced after cross-linking/modification of BrdU to Y315. An asterisk (*) indicates MS/MS identified fragments containing BrdU cross-linked nucleotide, a double asterisk (**) indicates the identified MS/MS cross-linked fragments that lost hydroxyl group or water molecule.

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. Purification of A3G WT and tyrosine mutant proteins. Proteins were produced in Baculovirus system and purified with Ni-NTI-agarose resin (Qiagen) as described in SI Materials and Methods. 2.5 μg of each protein was loaded onto 12% SDS-PAGE gel and stained with Coomassie SimpleBlue SafeStain (Thermo Scientific). BioRad Broad Range Markers were used as molecular mass standards.

FIGURE S2. WT A3G and mutant Y181A, Y182A and Y315A showed similar profile of unfolding (dissociation curve) as determined by thermo fluorimetry. WT and mutant A3G proteins were analyzed for dissociation (unfolding) in presence of SYPRO Orange fluorescence dye using Stratagene Mx3000P real-time qPCR system with 2 °C/min temperature increase, from 25 °C to 95 °C. Protein were expressed in Baculovirus system and purified with Ni-NTI resin (Qiagen), final protein concentration was 5 μM, reactions were ran in 1× PBS.

FIGURE S3. A3G WT and tyrosine mutants showed similar protein expression level in bacterial reporter strain BW310 used in DNA mutator assay. A3G WT and tyrosine mutants were subcloned into pTrc99A vector and transformed into *E. coli* BW310 reporter strain. Single colonies were inoculated into liquid LB and protein expression was induced by addition of 0.5 mM IPTG overnight. Cell extracts were prepared from 1 ml cultures, loaded onto 12% SDS-PAGE gel, western blotted and probed with anti-A3G antibody. Five independent colonies for each WT or mutant A3Gs were tested and a typical representative western blot is presented.

FIGURE S4. A3G Y315A mutation affects protein packaging with HIV viral particles. WT and mutant A3G constructs were co-transfected into HEK293T cells with plasmid encoding VSV-G coat protein in the absence of Vif and protein expression were evaluated by western blots with anti-A3G and control anti-GAPDH antibodies (top panel). Isolated viral particles were analyzed by western blotting for A3G content and the corresponding values for each mutant was normalized based on the level of p24 capsid protein in the particles (bottom panel). A3G:control protein ratio values are shown below each lane/sample. While ratios of WT A3G, Y181A and Y182A to control proteins were similar in cell extracts and in samples recovered with viral particles, Y315A mutation markedly reduced the recovery of A3G assembled with virions.