

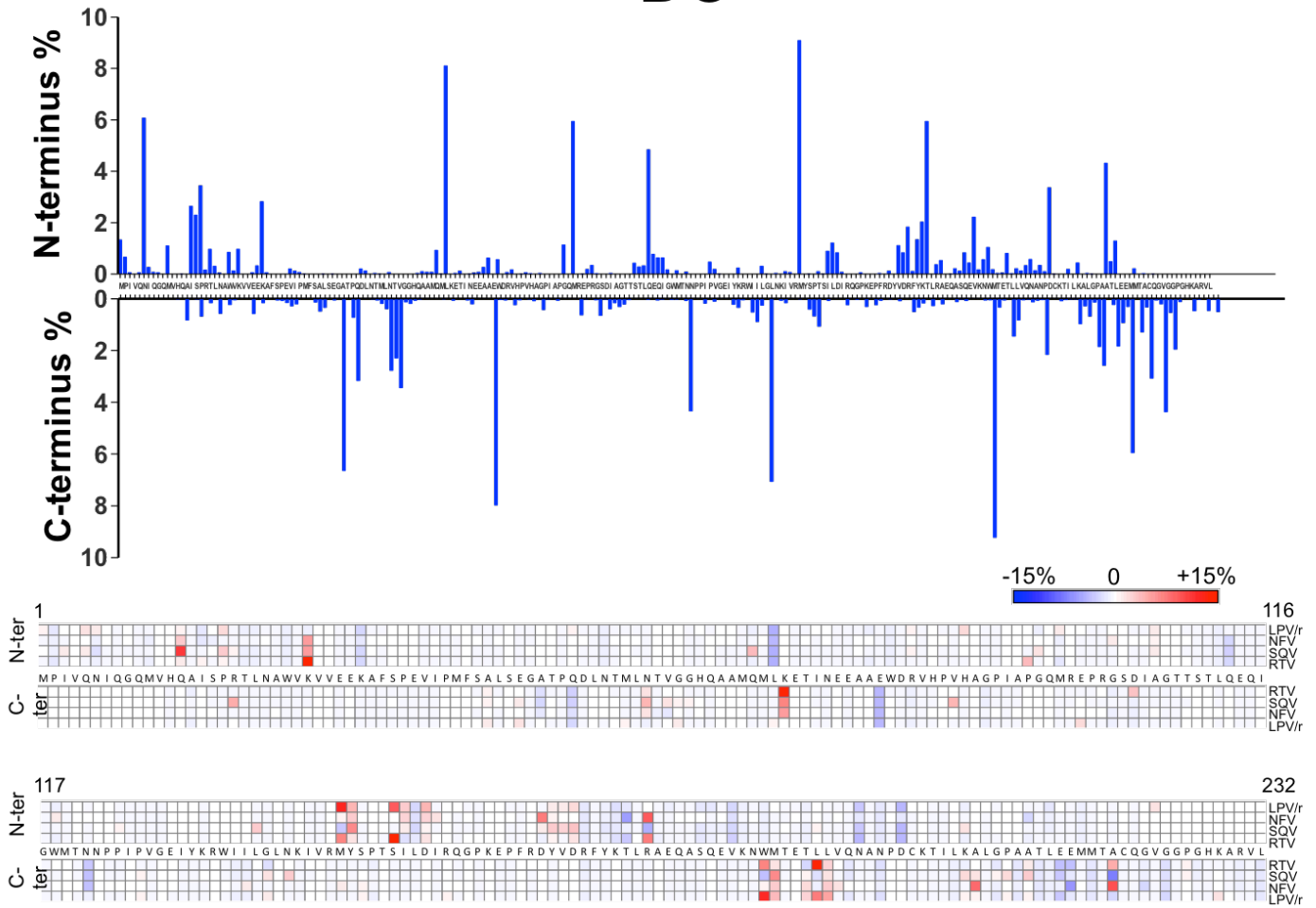
Supplemental Figure 1



Supplemental Figure 1. Variable Degradation Pattern in Dendritic Cells, Macrophages and CD4 T Cells. HIV p24–35mer (MVHQAI SPRTLNAWVKVVEEKAFSPEVIMFAALS, aa 10–44 in Gag p24) was incubated at 37 degrees with DC, macrophage or CD4 T cell extracts for 60 minutes at pH4.0. Degradation products were analyzed by mass spectrometry and aligned to the original p24-35 sequence.

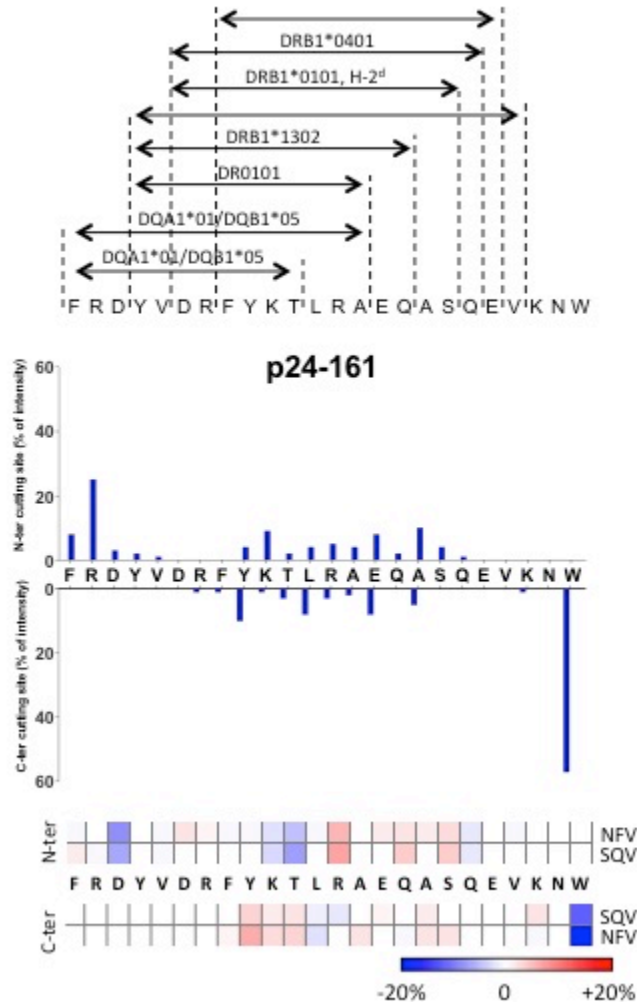
Supplemental Figure 2

DC



Supplemental Figure 2. HIV PIs Alter HIV p24 Degradation Pattern. Full HIV p24 protein was incubated at 37 degrees with DC extracts for 60 minutes at pH4.0. Degradation products were analyzed by LC-MS/MS and the contribution of the cleavage of each amino acid position (N-ter and C-ter) to the total intensity of all degradation products was quantified. The upper panel represents the cutting site intensity at each amino acid location of full HIV p24 protein by DC without PI treatment. The lower panel shows a heat map representing the difference in cutting intensity at each amino acid location of HIV full p24 protein by DCs treated with 5 μ M of different PIs compared to control.

Supplemental Figure 3



Supplemental Figure 3. HIV PIs change p24-161 degradation patterns and epitope production in lysosomal compartments. (Top panel) HIV p24-161 sequence with the known MHC-II epitope locations in this region. (Middle panel) Cleavage pattern of p24-161 incubated with DC cell extracts for 60 minutes at pH4.0. (Lower panel) Heat map representing the difference in cutting intensity at each amino acid location of p24-161 by DCs treated with 5 μ M of SQV or NFV compared to control. Data are representative of three independent experiments with three different donors' DCs analyzed by MS twice each.

Supplemental Table 1

Common Peptides	Protein AC	Proteins	Common Peptides	Protein AC	Proteins	Common Peptides	Protein AC	Proteins
AVIQMTEQELK EQNTLQELK VEAVEQNTLQELK VEQNTLQELK	Q9H299	SH3 domain-binding glutamic acid-rich protein	EVGGEALGRLL KVVNDEVGGEALG KVVNDEVGGEALGRLL KVVNDEVGGEALGRLL LTPFEKSAVYALWG VCLVAHFG			LVSNIKFERVPLQ P26599		Poly(pyrimidine tract-binding protein 1
KGNYAERVGAGAPVYM KGNYAERVGAGAPVY	Q96KRS;Q16777	Histone H2A	VEYVGGGALGRLL VHLTPEEKSAVYALWG VHLTPEEKSAVYALWG	P68871	Hemoglobin subunit beta	ATPFLVVRHQLIKT P15954		Cytochrome c oxidase subunit 7C
LGHLNWFPPF	Q8Z90	Otagelin	WGVNDEVGGEA WGVNDEVGGEALG WGVNDEVGGEALGR WGVNDEVGGEALGRLL	P02042	Hemoglobin subunit delta	INETGDEPFFQYK P15104		Glutamine synthetase
TEENPKM	Q04760	Lactoylglutathione lyase	KVVNDEVGGEALGRLL P02042		Hemoglobin subunit delta	IVNTNVPRAVSPDG P14174		Macrophage migration inhibitory factor
ALKAAHHTHKE	P68511	Protein S100-A12	WGVNDEVGGEA WGVNDEVGGEALG WGVNDEVGGEALGR WGVNDEVGGEALGRLL	P02042	Hemoglobin subunit delta	YDMNMAANVGVNNT P14174		
AAHLPAEFTPAVHASLKD AAHLPAEFTPAVHASL AAHLPAEFTPAVHASL AAHLPAEFTPAVH ASVSTVLSKYR ASVSTVLSKYR FLASVSTVLSKYR KVGAAHAGEVGAELER AAHLPAEFTPAVHASLKD LASVSTVLSKYR LYTLAAHLPAAEFTPAVHASLKD PAEFTPAVHASLKD PAVHASLKD PWNFKLLSH PWNFKLLSH SVSTVLSKYR FPAVHASLKD VDFPWNFKLLSH	P69905	Hemoglobin subunit alpha	WGVNDEVGGEA WGVNDEVGGEALG WGVNDEVGGEALGR WGVNDEVGGEALGRLL KVVNDEVGGEALGRLL P02042		Hemoglobin subunit delta	MAATGLEDQDGRS P07399		Plastin-3
LVVVPWTFQ LVVVPWTFQR LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF VDFPWNFKLLSH VDFPWNFKLLSH LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF	P68871;P02042	Hemoglobin subunit beta/delta	WGVNDEVGGEA WGVNDEVGGEALG WGVNDEVGGEALGR WGVNDEVGGEALGRLL KVVNDEVGGEALGRLL P02042		Hemoglobin subunit delta	MAADGDFRDKC P09382		Galectin-1
						NRPFSGDGNVDF P07996		Thrombospondin-1
						STCQDAANVGVNNT P07737		Profilin-1
						MGKSNVQDQWFS P07399		Cathepsin D
						ALPYNALGK P06703		Protein S100-A6
						GAGIANDHFVK P04406		Glyceraldehyde-3-phosphate dehydrogenase
						SWYDNEFGYSNR P04406		Glyceraldehyde-3-phosphate dehydrogenase
						SWYDNEFGYSNRVVD P04406		Glyceraldehyde-3-phosphate dehydrogenase
						SWYDNEFGYSNRVVDL P04406		Glyceraldehyde-3-phosphate dehydrogenase
						WYDNEFGYSNRVVDL P04406		Glyceraldehyde-3-phosphate dehydrogenase
						YDNEFGYSNRVVDL P04406		Glyceraldehyde-3-phosphate dehydrogenase
						YDNEFGYSNRVVDL P04406		Glyceraldehyde-3-phosphate dehydrogenase
						YDNEFGYSNRVVDL P04406		Glyceraldehyde-3-phosphate dehydrogenase
						ADDVWNPFCGVIL P04075		Fructose-bisphosphate aldolase A
						APPGSAEAEVQAKQQA P07388		SH3 domain-binding glutamic acid-rich-like protein
						IGLTFAPPSAEAEVQAKQQA P07388		SH3 domain-binding glutamic acid-rich-like protein
						ELLNWNVA P04406		Unassigned
						IRGNSCHOTAPG P04406		Unassigned
						PAVAGHTLTA P04406		Unassigned
						VNDKPLDL P04406		Unassigned

Unique to Control	Protein AC	Protein	Unique to SQV	Protein AC	Protein	
AVDGEPLGRVSF	P62937	Peptidyl-prolyl cis-trans isomerase A	AIVEALNGEVAQAQKAPLVLK	Q99497	Protein DJ-1	
QNTLQELK	Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	AIVEALNGEVAQAQKAPLVLDK		Histone H2A	
SYGAGPVVCTQLNS	Q9RF9	RNA-binding protein 14	KGNVYERIGAGAPVY	Q96KRS		
MAVFLCLSP	Q8L0K7	N-acetylaspartylglutamate synthase A	YVYAKDQKMPHPAPQ	Q86AE4	Far upstream element-binding protein 1	
IEILNPKMA IEILNPKMAT IQKCDPPIK FTPAVHASLKD HLPAEFTPAVHASLKD SPADKTNKAAWAG	Q04760 P69905	Lactoylglutathione lyase Hemoglobin subunit alpha	AAHLPAEFTPAVHASLKD DKLASVSTVLSKYR GRVGAHAGEVGAELER PADKTNKAAWAGVGAHAGEVGAELER SPADKTNKAAWAGVGVG	P69905	Hemoglobin subunit alpha	
YVVPWTFQRF EVGGEALGRLL DIAVDGEPLGRVSF DIAVDGEPLGRVSF DIAVDGEPLGRVSF VDFPWNFKLLSH VDFPWNFKLLSH LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF LVVVPWTFQRF	P68871;P02042 P62937 P62899 P62314 P62158 P61604 P51553 P40925 P30101 P27797 P27348 P26447 P23528 P15554 P15104 P14174 P13796 P12544 P06396 P04406 Q043598 Q00754	Hemoglobin subunit beta/delta Peptidyl-prolyl cis-trans isomerase A 60S ribosomal protein L31 Small nuclear ribonucleoprotein Sm D1 Calmodulin 10 kDa heat shock protein, mitochondrial Isocitrate dehydrogenase (NAD) subunit gamma Malate dehydrogenase, cytoplasmic Protein disulfide-isomerase A3 Calreticulin 14-3-3 protein theta Protein S100-A1 Cellin-1 Cytochrome c oxidase subunit 7C Glutamine synthetase Macrophage migration inhibitory factor Plastin-2 Granin A Gelolin Glyceraldehyde-3-phosphate dehydrogenase 2'-deoxyadenosine 5'-phosphate 1H hydrolase 1 Lysosomal alpha-mannosidase	TLAAHLPAEFTPAVH TLAAHLPAEFTPAVHASLKD VLSFADKTNKAAWAGVGAHAGEY VTLAAHLPAEFTPAVHASLKD PDVAVGNPKVKAHGKKVVG PWTQRF YVVPWTFQRF KVVNDEVGGEAL KVVNDEVGGEALGR VDEGGEALGRLL VHLTPEEKSAVTA VHLTPEEKSAVYALWG VTLAVGKVVNDEVGGEA WGVNDEVGGEA PDIAVDGEPLGRVSF VNPVTFDIAVDGEPLGR AIDPDGSDIERSMPEQTG PAGDQAPRAVPL DGPVYTPDKAPNVAVTR VTRPKAPNVAVTR IAVFKYAGKDG LTDQFRESRISDTSAG SDVLELTDQFRESRISDTG SDVLELTDQFRESRISDTSAG PVDLSKWSRSL DVGQTVDFPHTATPK DGPLAKHRESITKAVTKYTSK ATPFLVVRHQLIKT IVNTNVPRAVSPDG MLDLNLI RSPFYVYFINGKQK SFLPVIR FVNWIDSIQR MQQLVQKQIFSP FNSGKVDVAINDFPD NEFGYSNRVVD SWYDNEFGYSNRVVD DAECPVEVETRNA PGSKEAEVQAKQQA IPGSKAEVQAKQ	P68871;P02042 P62937 P62899 P62314 P62158 P61604 P51553 P40925 P30101 P27797 P27348 P26447 P23528 P15554 P15104 P14174 P13796 P12544 P06396 P04406 Q043598 Q00754	P68871;P02042 P62937 P62899 P62314 P62158 P61604 P51553 P40925 P30101 P27797 P27348 P26447 P23528 P15554 P15104 P14174 P13796 P12544 P06396 P04406 Q043598 Q00754	Hemoglobin subunit beta/delta Peptidyl-prolyl cis-trans isomerase A 60S ribosomal protein L30 Actin Rho GDP-dissociation inhibitor 2 Protein S100-A11 Protein disulfide-isomerase A3 Phosphoethanolamine-binding protein 1 Colfin-1 Histone H2B type 1 Cytochrome c oxidase subunit 7C Macrophage migration inhibitory factor Poly (ADP-ribose) polymerase 1 Glutathione S-transferase P Cathepsin G Neutrophil elastase Cathepsin D Glyceraldehyde-3-phosphate dehydrogenase NADH dehydrogenase (ubiquinone) iron-sulfur protein
		Proteins uniquely represented in Control				
					Proteins uniquely represented in SQV	

Supplemental Table 1: Peptides eluted from SQV treated and untreated PBMCs and their protein of origin. Top tables represent the peptides commonly present on treated and untreated PBMCs. Bottom left table presents peptides uniquely present on untreated PBMCs. Bottom middle table represents peptides uniquely present on SQV treated PBMCs. Blue cells are the proteins represented uniquely in control and pink proteins represented uniquely in SQV treatment.