Cell Reports

Supplemental Information

Identification of the HIV-1 Vif and human APOBEC3G protein interface

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SUPPLEMENTAL DATA

Supplemental figures and legends

Figure S1

Patient Vif residues		Counters A3G-125R?	
19	22	✗	✓
R	K	40 (76.9%)	
K	K	7 (13.7%)	
R	N	2 (3.9%)	2 (14.2%)
R	H	3 (5.9%)	1 (7.1%)
S	K		9 (64.3%)
R	V		1 (7.1%)
K	N		1 (7.1%)
Total		52	14

Figure S1. Amino acid identities of Vif at positions 19 and 22 (Related to Figure 3).

Our Amsterdam Cohort Vif panel comprises 66 Vif alleles from 35 chronically infected, untreated HIV+ patients. The table shows the amino acids at positions 19 and 22 of each Vif grouped by whether they do or do counteract A3G-125R. The absolute number and % of 19+22 amino acid pairs are indicated.

Figure S2

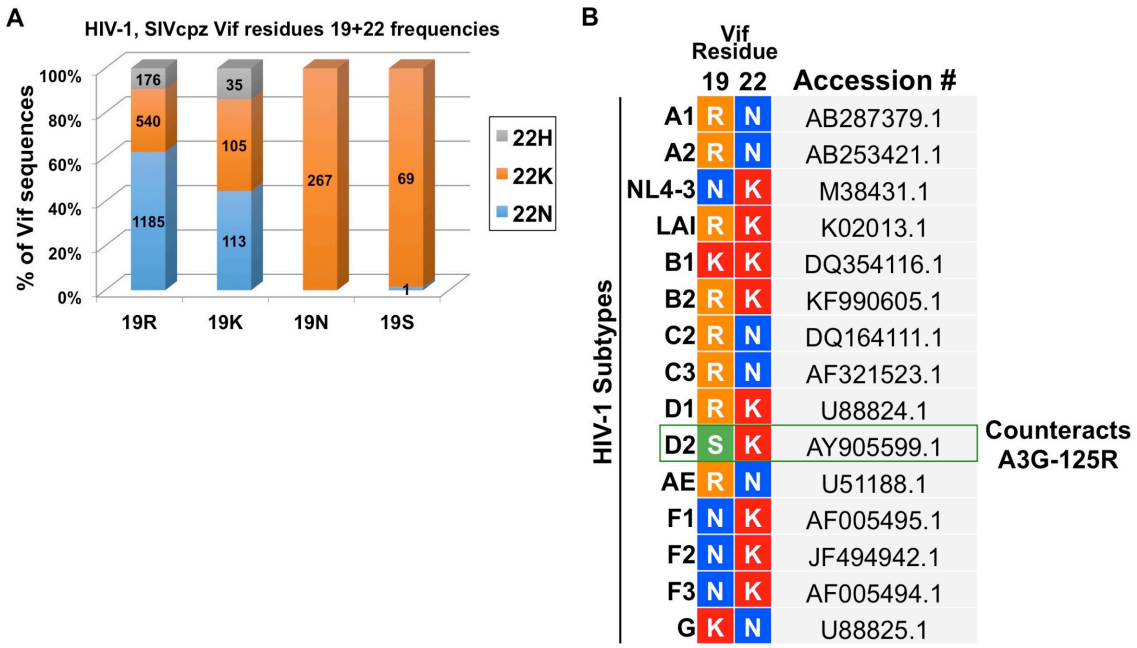

Figure S2. Amino acid identities of Vif at positions 19 and 22 (Related to Figure 4).

(**A**) HIV-1 and SIVcpz Vif aligments were obtained from the Los Alamos HIV sequence database. Sequences containing premature stop codons (*) or other characters (# and X) were excluded. Amino acid frequencies at positions 19 and 22 of a total of 2527 Vif sequences were calculated using BioEdit Sequence Alignment Editor. The relative and absolute frequencies of amino acids found at positions 19 and 22 of Vif are shown grouped by the amino acid identity at position 19.

(**B**) The amino acids at positions 19 and 22 of Vif are shown for the subtype Vif panel used in experiments depicted in Figure 2C. Vif D2 of subtype D is the only Vif that displays partial activity against A3G-125R.

Figure S3

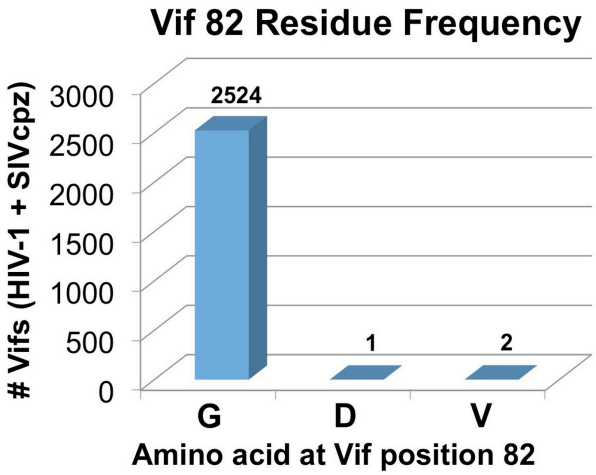

Figure S3. Vif 82G is highly conserved among HIV-1 and SIVcpz Vifs alleles (Related to Figure 5).

HIV-1 and SIVcpz Vif aligments were obtained from the Los Alamos HIV sequence database. Sequences containing premature stop codons (*) or other characters (# and X) were excluded. The amino acids found at position 82 of a total of 2,527 sequences were determined using BioEdit Sequence Alignment Editor. Only three amino acids were found at position 82 and the respective distribution is shown.

Figure S4

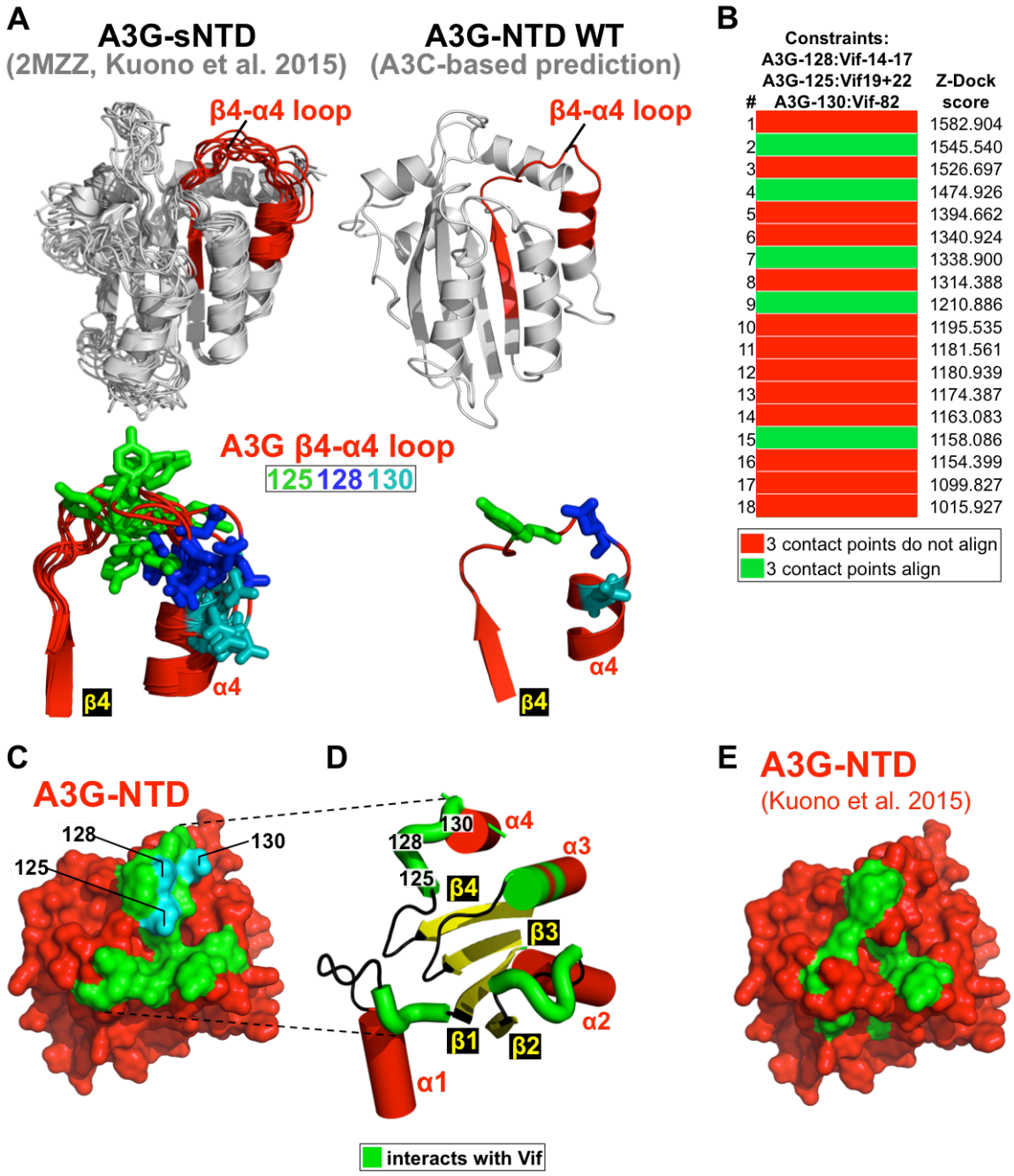

Figure S4. Protein docking of Vif and A3G and comparison of the Vif binding site of Kuono et al. 2015 (Related to Figure 7).

(**A**) Ten structural states from the recently published NMR structure of a soluble variant of the A3G-NTD (2MZZ) were overlaid (left) and compared with the A3C-

based (PDB 3V0W), predicted structure for the A3G-NTD used in this study (right). The entire A3G-NTD is shown in the upper panels with the β4-α4 loop indicated in red. Shown below, the β4-α4 loop is enlarged with residues 125 (green), 128 (blue) and 130 (cyan) are depicted. (**B**) The top Z-dock output structures ranked by Z-dock scores are shown. Constraints were applied to force docking of the specific three contact points (A3G-128:Vif-14-17, A3G-125:Vif-19+22, A3G-130:Vif-82). Output structures were analyzed for alignment of the three identified amino acid pairs. The model structures in which the three contact points aligned properly are indicated in green and are identical or structurally very similar. (**C, D**) A closer inspection of the interacting surface of A3G based on the Vif-A3G model analyzed with UCSF chimera software (indicated in green) shows that the A3G surface interacting with Vif consists of the loops between a-1 and β-1, α-2 and β-2, β-3 and α-3 and between β-4 and α-4. (**E**) The Vif surface interacting with A3G based on the Vif-A3G model (indicated in green), largely overlaps with the Vif residues that were determined experimentally to be important for counteracting with A3G (Kouno et al., 2015).

Supplemental Tables

Table S1. Literature-derived Vif residues important for A3G counteraction (Related to Figure 6).

Publication	PMID	Vif residues	Affects A3G counteraction	Affects A3C/A3F/A3H counteraction*	A3G Specific?#
(Wichroski et al., 2005)	15537645	9	yes	N.T.	
(Simon et al., 2005)	16201018	22, 40, 45	**yes**	**no**	**yes**
(Santa-Marta et al., 2005)	15611076	73-99, 169-192	yes	N.T.	
(Schröfelbauer et al., 2006)	16731937	14-17	**yes**	**no**	**yes**
(Tian et al., 2006)	16501124	5, 11, 21, 38, 89	yes	yes	no
(Russell and Pathak, 2007)	17522216	40-44, 48	**yes**	**no**	**yes**
(Mehle et al., 2007)	17898068	42, 43	**yes**	**no**	**yes**
(Goila-Gaur et al, 2008)	18023836	23-43	yes	N.T.	
(Donahue et al., 2008)	18499212	161-164	yes	yes	no
(He et al., 2008)	18619467	52-72	yes	yes	no
(Yamashita et al., 2008)	18603011	21, 32, 38, 40, 43, 69	**yes**	**no**	**yes**
(Zhang et al., 2008)	18619467	22, 41, 42	**yes**	**no**	**yes**
(Pery et al., 2009)	19109396	69-72	yes	N.T.	
(Chen et al., 2009)	19535450	22, 26, 30, 40	**yes**	**no**	**yes**
(Dang et al., 2009)	19535447	22, 26	**yes**	**no**	**yes**
(Dang et al., 2010a)	20335268	81-89	yes	yes	no
(Dang et al., 2010b)	20592083	96-107	yes	yes	no
(Reingewertz et al., 2013)	23545135	8-45, 83-99, 154-192	yes	N.T.	

N.T. indicates that the Vif mutants have not been tested with A3C, A3F or A3H
* indicates whether the Vif mutants were also tested against A3C, A3F or A3H
# Vif mutations are regarded A3G specific when the Vif mutant fails to counteract A3G, but remains active against A3C, A3F or A3H

Table S2. Summary of interacting residues (Related to Figure 6).

			A3G Variant			
			WT	**125R**	**128K**	**130R**
Vif Variant	**NL4-3**	**WT**	+	-	-	-
		SEMQ	+/-	+/-	+	+
		15E	+/-	+/-	+	+
		15A	+	+/-	-	+/-
		82D	+/-	-	-	+
		82K	+	N.T.	N.T.	-
	LAI	**WT**	+	-	-	-
		19S	+	+/-	N.T.	N.T.
		22V	+	+/-	N.T.	N.T.
		19S+22V	-	+	-	-

*N.T. = Not tested. “+” indicates Vif rescues infectivity, “-“ indicates restriction, “+/-“ indicates partial rescue.

Supplemental References (Related to Table S1)

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