Supplementary Information

Analysis and molecular determinants of HIV RNase H cleavage specificity at the PPT/U3 junction

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Supplementary Table S1. DNA oligonucleotides used in mutagenesis reactions.[a]

RT	Mutants	Sequences
HIV-1_{BH10}	M184I	5′ TATCTATCAATACATCGATGATTTGT 3′ 5′ ACAAATCATCGATGTATTGATAGATA 3′
	M184V	5′ TATCTATCAATACGTGGATGATTTGT 3′ 5′ ACAAATCATCCACGTATTGATAGATA 3′
	E399D	5′ GGAAACATGGGATACATGGTGGACAGAGTATTGG 3′ 5′ CCAATACTCTGTCCACCATGTATCCCATGTTTCC 3′
	E399G	5′ CAAAAGGAAACATGGGGAACATGGTGGACAGAG 3′ 5′ CTCTGTCCACCATGTTCCCCATGTTTCCTTTTG 3′
	T400A	5′ GGAAACATGGGAAGCATGGTGGACAGAGTATTGG 3′ 5′ CCAATACTCTGTCCACCATGCTTCCCATGTTTCC 3′
	T400S	5′ GGAAACATGGGAATCATGGTGGACAGAGTATTGG 3′ 5′ CCAATACTCTGTCCACCATGATTCCCATGTTTCC 3′
HIV-2_{EHO}	H342Y	5′ GACCTATAAAATTTATCAGGAGCCTTTC 3′ 5′ GAAAGGCTCCTGATAAATTTTATAGGTC 3′
	G344E/D345P	5′ AAAATTCATCAGGAGCCTTTCAAAATCCTGA 3′ 5′ TCAGGATTTTGAAAGGCTCCTGATGAATTTT 3′
	Δ346F	5′ CATCAGGGCGATTTCAAAATCCTGAAAGTGGGT 3′ 5′ ACCCACTTTCAGGATTTTGAAATCGCCCTGATG 3′
	Δ346H	5′ CATCAGGGCGATCATAAAATCCTGAAAGTGGGT 3′ 5′ ACCCACTTTCAGGATTTTATGATCGCCCTGATG 3′
	V351T	5′ CAAAATCCTGAAAACGGGTAAATACGCAA 3′ 5′ TTGCGTATTTACCCGTTTTCAGGATTTTG 3′

[a] Left column indicates the mutation(s) or insertions introduced in the RT.

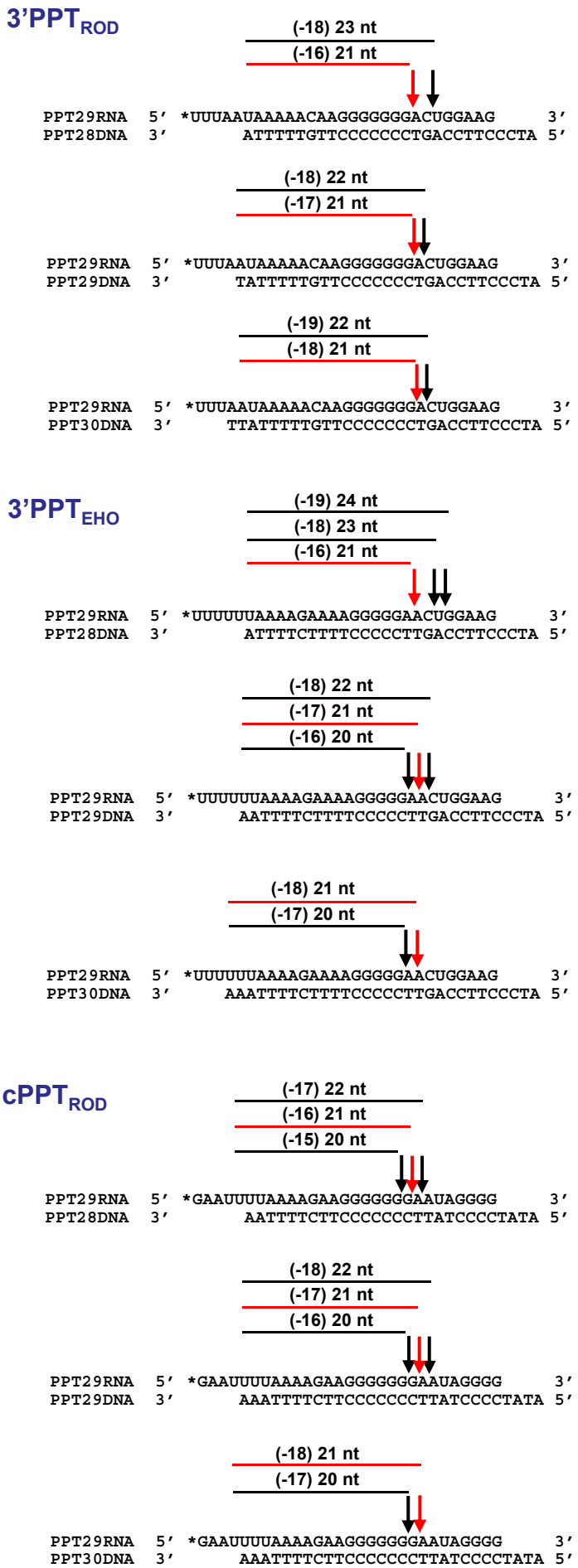

Figure S1. Template-primers used in RNase H cleavage window assays representing PPTs found in HIV-2 ROD and EHO strains. Major cleavage sites observed are indicated above the RNA sequences.

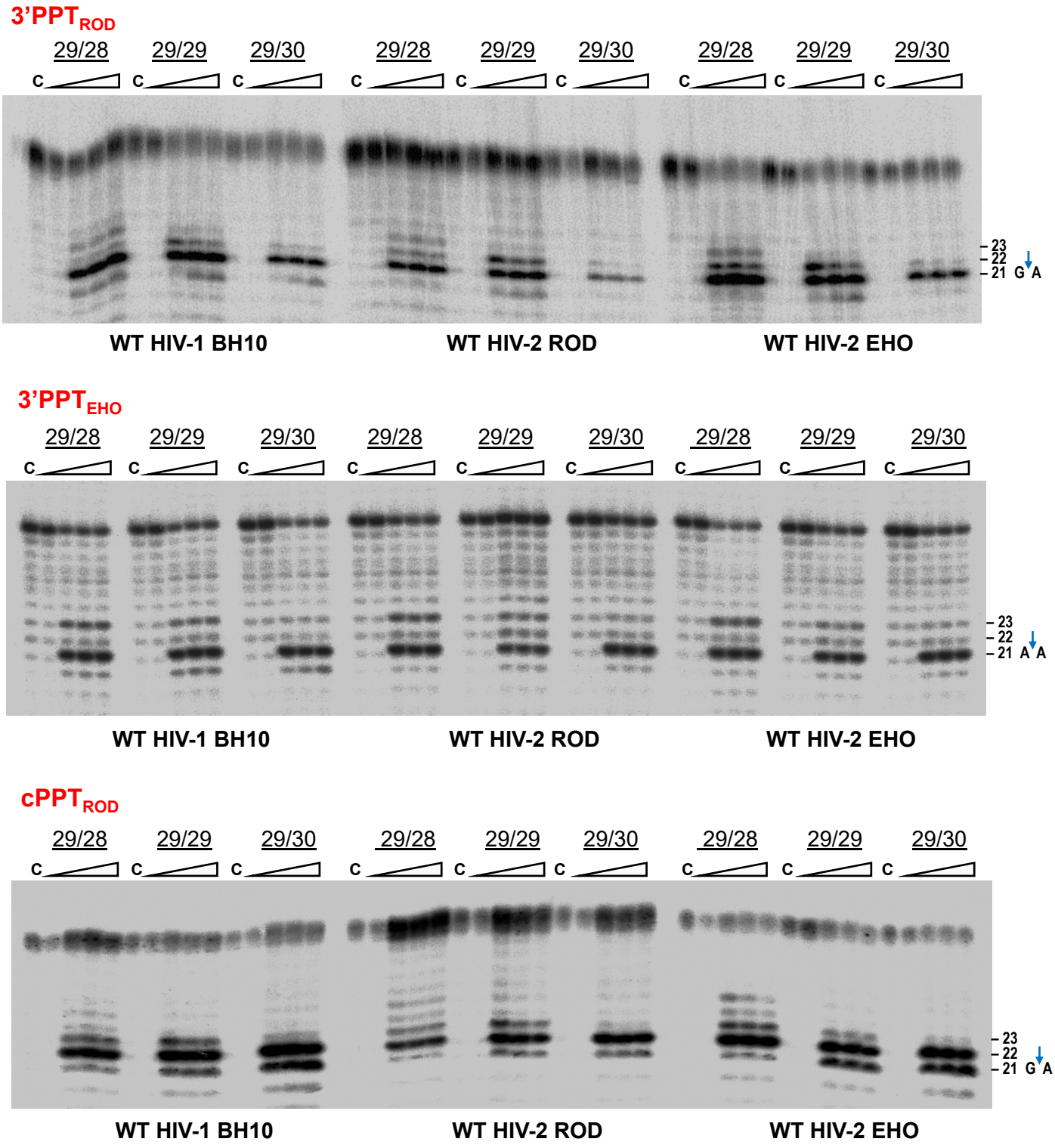

Figure S2. RNase H cleavage specificity of WT HIV-1_{BH10}, HIV-2_{ROD} and HIV-2_{EHO} on representative HIV-2 PPTs. Reactions were carried out in the presence of enzyme and template-primer at concentrations of 125 nM and 25 nM, respectively. RT and template-primer were preincubated during 5 min at 37 ºC, and aliquots were withdrawn at 0, 20, 40 and 180 s after the addition of magnesium and heparin. Lane C shows control experiments including heparin in the preincubation buffer. Numbers above indicate the template-primer used in each experiment: 29/28, 29/29 and 29/30 for 29RNA/28DNA, 29RNA/29DNA and 29RNA/30DNA hybrids, respectively (see Supplementary Figure S1 for sequence details).

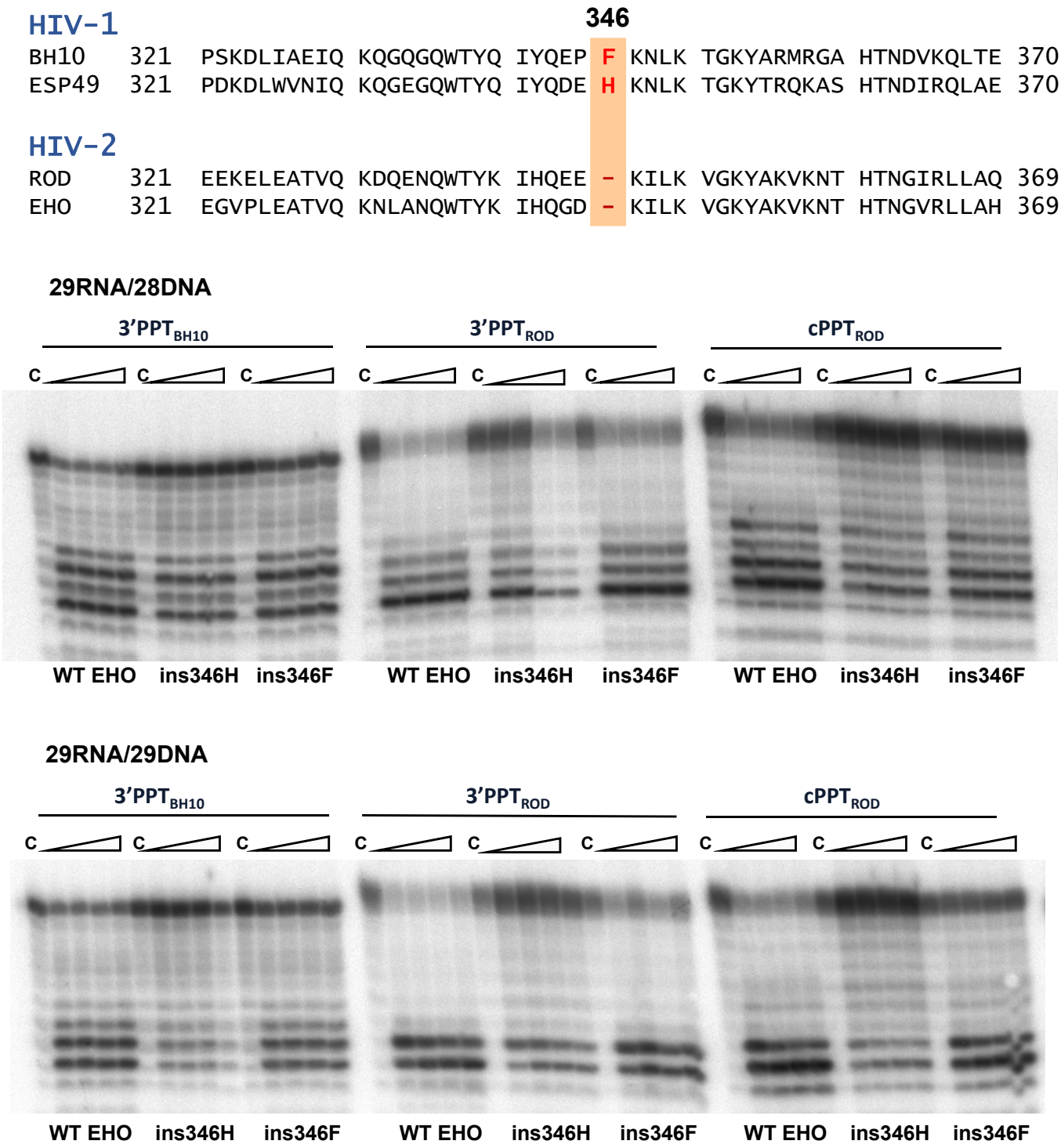

Figure S3. Effect of one-amino-acid insertions in the connection subdomain of HIV-2_{EHO} on the RNase H cleavage window. Amino acid sequence differences between representative HIV-1 and HIV-2 RTs are shown above. Gels below show representative RNase H cleavage assays carried out with 29RNA/28DNA and 29RNA/29DNA hybrids mimicking sequences found in representative PPTs of HIV-1 and HIV-2 strains (see Figure 4A and Supplementary Figure S1 for details). Reactions were carried out in the presence of RT and template-primer at concentrations of 125 nM and 25 nM, respectively. RT and template-primer were preincubated during 5 min at 37 ºC, and aliquots were withdrawn at 0, 20, 40 and 180 s after the addition of magnesium and heparin. Lane C shows control experiments including heparin in the preincubation buffer.