

1 **SUPPLEMENTAL FIGURES**

2

3 **S1. FACS profiles for HeLa cells transduced with AcGFP, CPSF6-358-**

4 **AcGFP, AcGFP-CPSF6(301-358), and AcGFP-CPSF6(301-358)<sub>ala</sub> followed by**

5 **WT HIV or N74D HIV infection.** A) HeLa cells stably expressing AcGFP,

6 CPSF6-358-AcGFP, AcGFP-CPSF6(301-358), and AcGFP-CPSF6(301-358)<sub>ala</sub>

7 after transduction using the non-selectable pMX vector were enriched by FACS

8 twice to adjust protein expression levels. FL-1 axis measures GFP fluorescence

9 levels with arbitrary logarithmic units. FL-2 axis measurements are compared to

10 screen for auto fluorescence characteristic of dead cells. Cell populations are

11 over 99% pure for GFP expression. CPSF6-358-AcGFP population has lower

12 mean fluorescence on the FL-1 channel. B) HeLa cells stably expressing AcGFP,

13 CPSF6-358-AcGFP, AcGFP-CPSF6(301-358), and AcGFP-CPSF6(301-358)<sub>ala</sub>

14 were infected with WT HIV-RFP or N74D HIV-RFP. 2 days later, HIV infection

15 was assayed by FACS by enumerating GFP/RFP double-positive cells (R2).

16

17 **S2. Single tryptophan substitutions of CPSF6-358 residues 316-321 impairs**

18 **antiviral activity.** HeLa cells were stably transduced with LPCX vectors

19 encoding CPSF6-358 with single residue tryptophan mutations. Empty vector

20 control, CPSF6-358 expressing vector, and tryptophan mutant cell lines were

21 infected with WT or N74D HIV-RFP and assayed 2 days later.

22

23 **S3. Analysis of TRIM5-CPSF6 fusion proteins transiently expressed in 293T**

24 **cells.** A) TRIM5-CPSF6 fusion proteins described in Figure 3 were ectopically

25 expressed in 293T cells. As controls, empty vector, rhTRIM5(1-299),

26 CPSF6(261-358), and CPSF6(301-358) constructs were also transiently

27 transfected in 293T cells. The expression of controls and TRIM5-CPSF6 fusion

28 proteins were examined by western analysis at 2 days post-transfection. B)

29 293T cells expressing control protein or TRIM5-CPSF6 fusion proteins were

30 infected with WT HIV-RFP or N74D HIV-RFP at 2 days post-transfection and

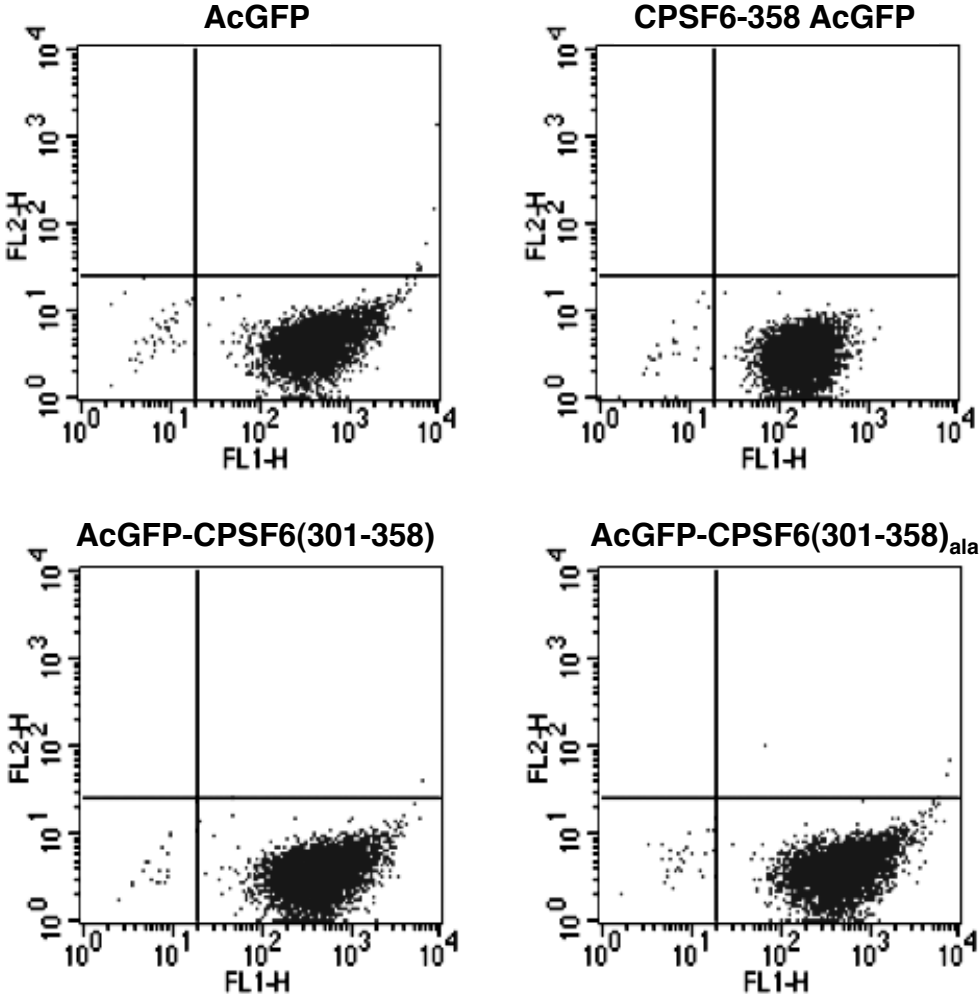
31 then assayed by FACS analysis at 2 days post-infection.

1

2 **S4. Lack of evidence for positive selection of *CPSF6*.** A) Seven orthologous  
3 *CPSF6* genes were analysed from the simian primate species shown. B) The  
4 molecular evolution of *CPSF6* is compared to that of two primate genes known to  
5 evolve under positive selection, *TRIM5* and *APOBEC3G*. For each, an alignment  
6 was created of orthologous sequences from the species shown in panel A. These  
7 matched-species datasets were then analysed using the PAML software  
8 package. Twice the difference in the natural logs of the likelihoods ( $2\Delta\ell$ ) of the  
9 two models (M7-M8) being compared is shown. The p-value indicates the  
10 confidence with which the null model (M7; positive selection of codons  
11 disallowed) can be rejected in favor of the model of positive selection (M8;  
12 positive selection of some codons allowed). Positive selection is supported in  
13 only the latter two genes. In contrast, the very low value of  $2\Delta\ell$  for *CPSF6*  
14 suggests that positive selection is not supported. C) A protein alignment of the  
15 seven *CPSF6* orthologs is shown in the region of the CA-binding domain.

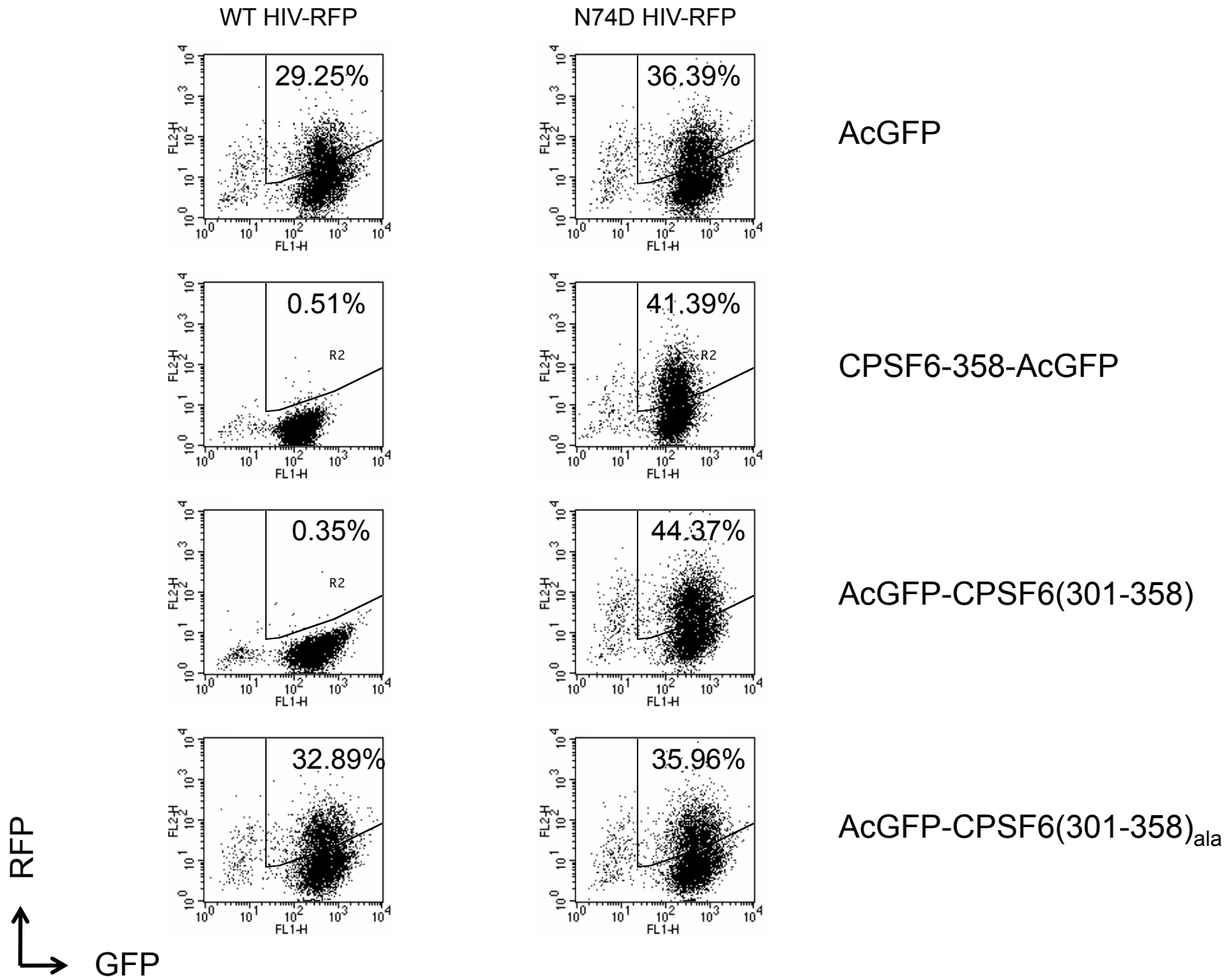
# Supplemental Figure 1

A

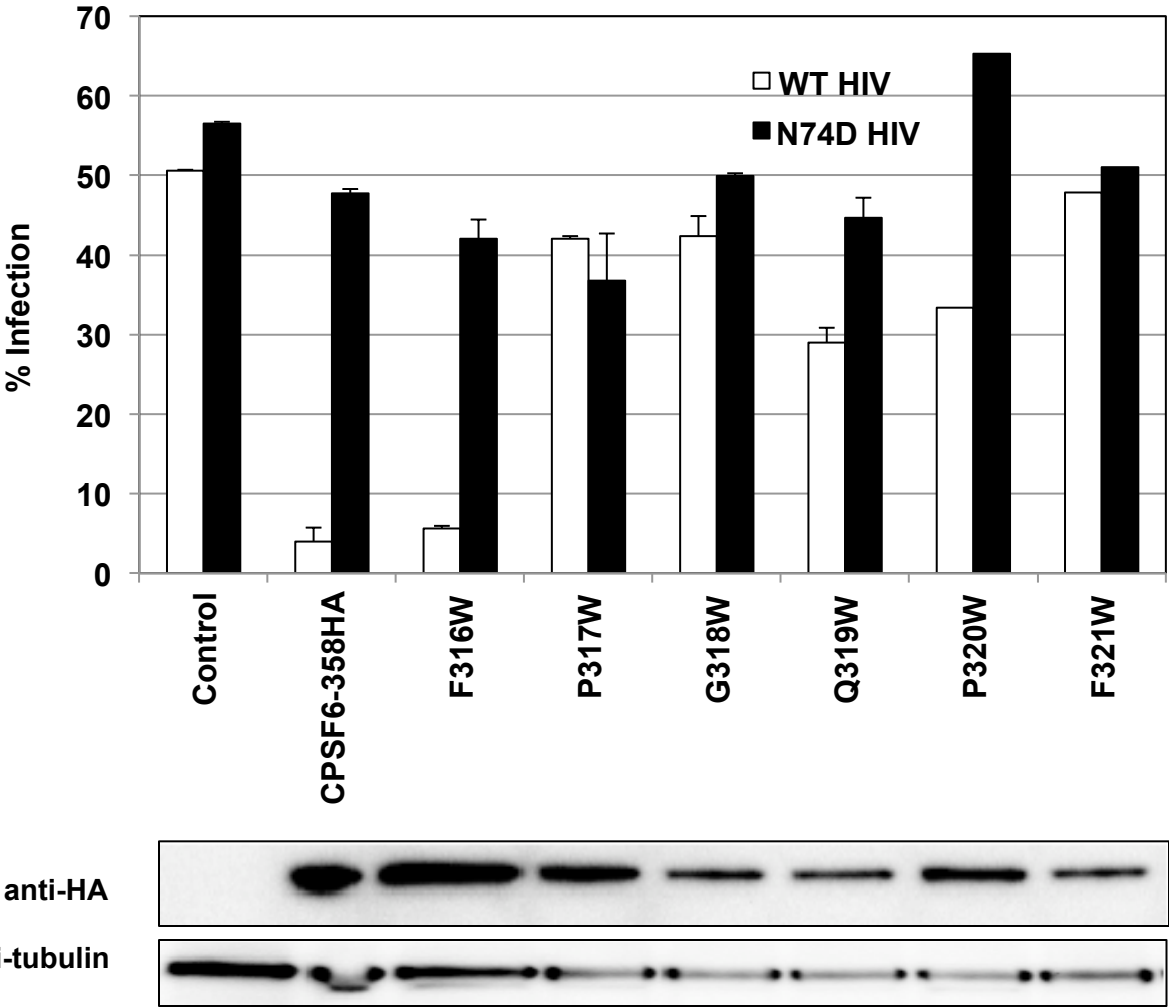


# Supplemental Figure 1

B

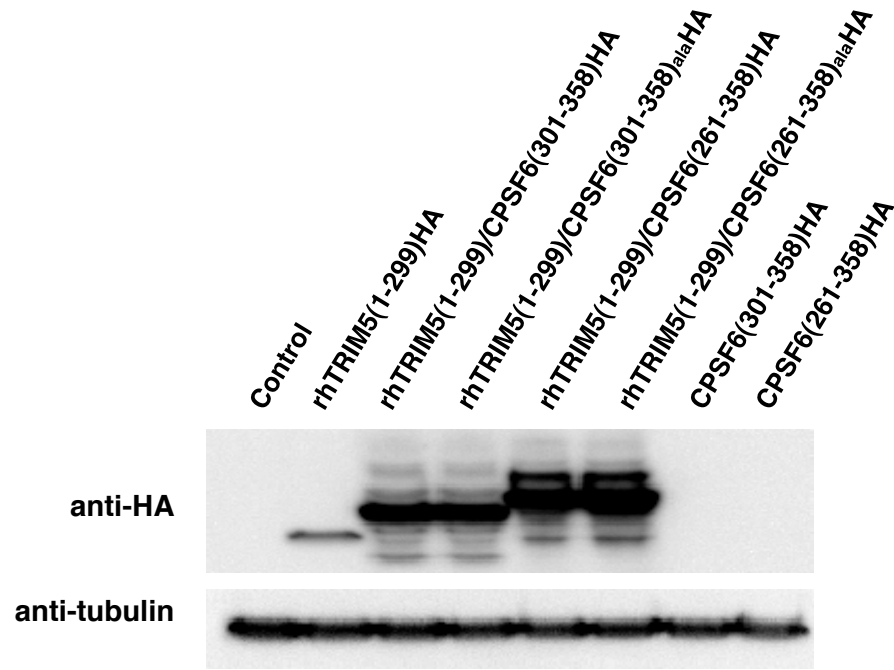


# Supplemental Figure 2

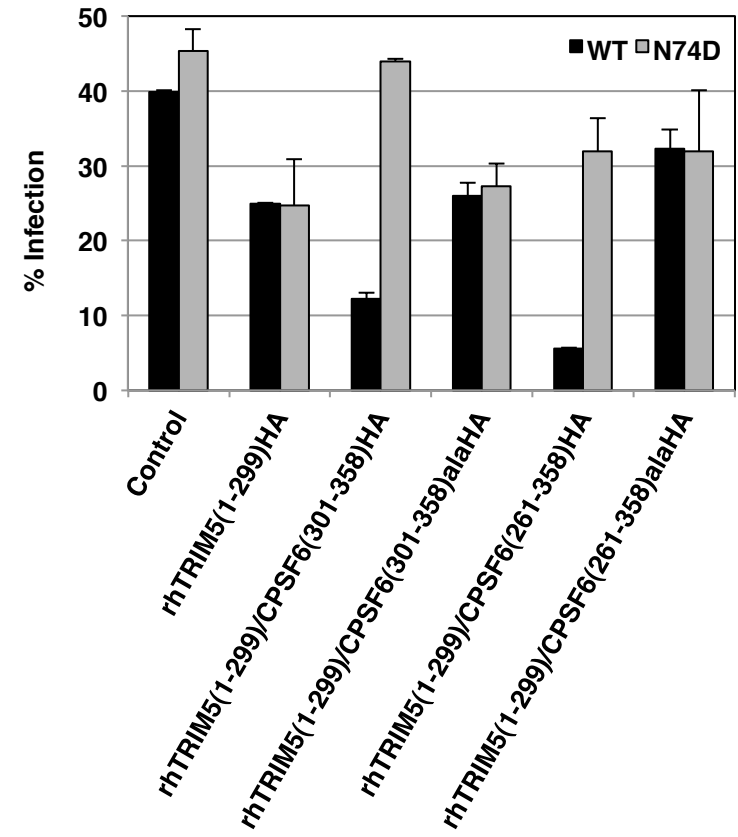


# Supplemental Figure 3

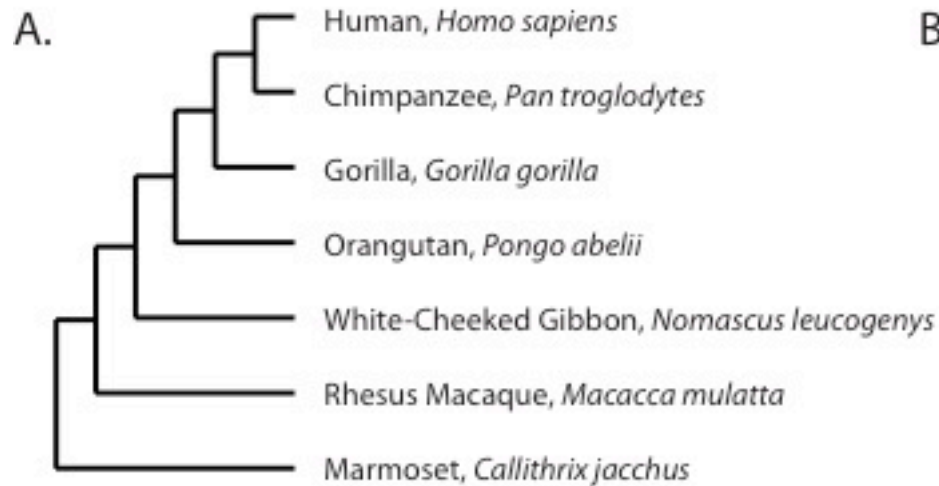
A



B



## Supplemental Figure 4



B.

Gene	2Δ	p-value
<i>CPSF6</i>	0.00027	not sig.
<i>TRIM5</i>	24	p<0.001
<i>APOBEC3G</i>	40	p<0.001

C.

CA binding

Human	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
Chimpanzee	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
Gorilla	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
Rhesus	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
Marmoset	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
WhiteCheekedGibbon	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
Orangutan	AGPPNRGDRPPPPVLFPGQPFQPPPLGPLPPGPPPPVPGYGPPPQGGPPPPPGPF
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