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Supplementary Materials for

TRIM26 is a critical host factor for HCV replication and contributes to host tropism

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The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/2/eabd9732/DC1)

Tables S1 and S2

Figure S1

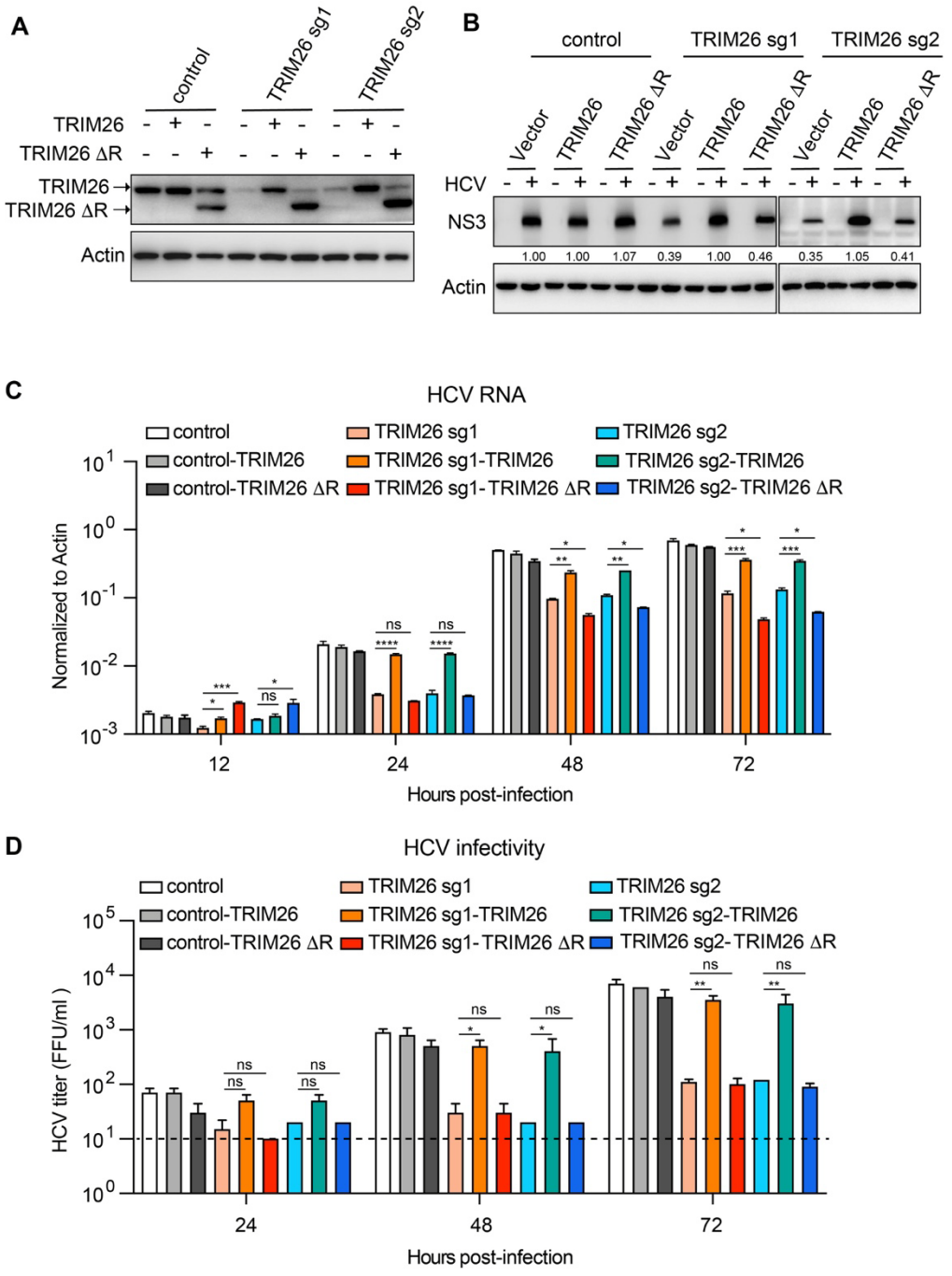

Figure S1. Reconstitution of TRIM26 expression restores HCV infection in *TRIM26*-knockdown cells.

(A) Western blot analysis of TRIM26 or TRIM26ΔR expression in the reconstituted cells. (B-D) The reconstituted cells were infected with HCVcc at MOI of 0.1 for the indicated time points. NS3 protein (B), intracellular HCV

RNA (C) and extracellular HCV titer (D) were analyzed. The error bars represent standard deviations from two independent experiments. One-way ANOVA was used for statistical analysis. ns, $P>0.05$;*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, ****, $P<0.0001$.

Figure S2

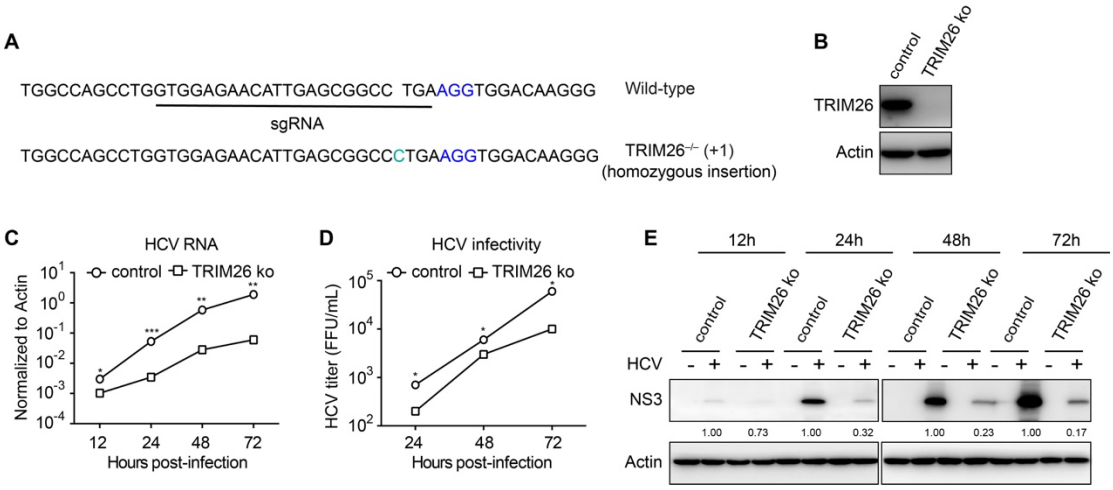

Figure S2. HCV infection is reduced in *TRIM26*-knockout Huh7.5.1 cells.

(A) Alignment of *TRIM26* sequence of wild-type and knockout clone. The designed sgRNA sequence is underlined. (B) Western blot analysis of TRIM26 expression in Huh7.5.1-*TRIM26* knockout monoclonal cells. (C-E) Control cells and Huh7.5.1-*TRIM26* knockout cells were infected with HCVcc at MOI of 0.1 for the indicated time points. Intracellular HCV RNA (C) and extracellular HCV titer (D) as well as NS3 protein (E) were analyzed. The error bars represent standard deviations from two independent experiments. T test was used for statistical analysis. ns, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.001, ****, P<0.0001.The protein levels were quantified by Image J, normalized against internal Actin and expressed as values relative to control cells.

Figure S3

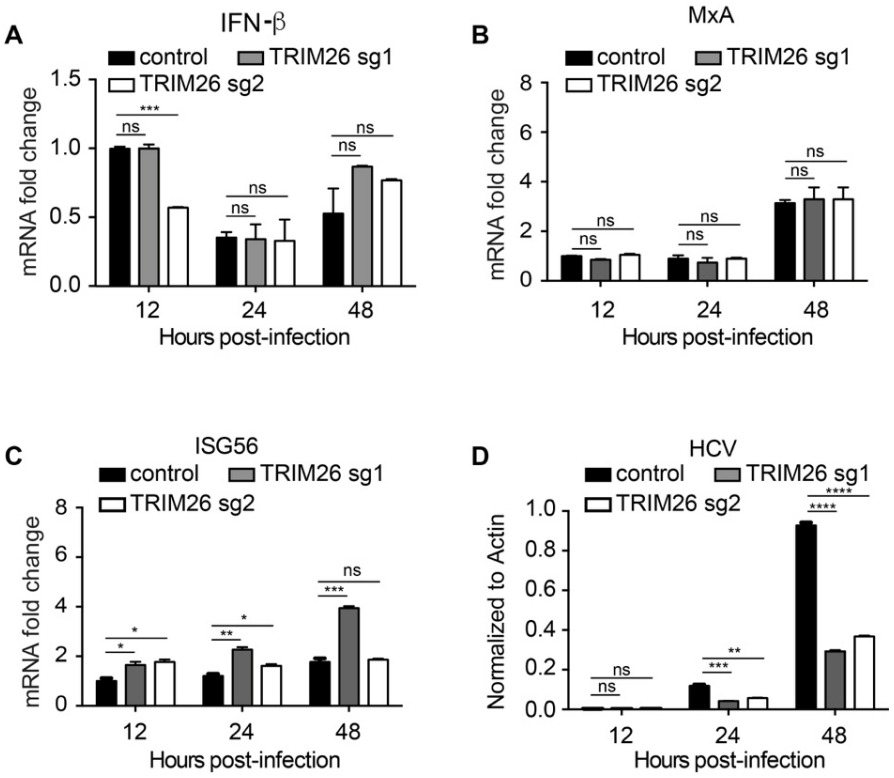

Figure S3. Role of TRIM26 in HCV infection is not related to IFN signaling. Control cells and Huh7-*TRIM26* knockdown cells were infected with HCVcc at MOI of 0.1 for the indicated time points and analyzed by RT-qPCR to detect the mRNA abundance of IFN-β (A), MxA (B), ISG56 (C) and HCV RNA (D). The error bars represent standard deviations from two independent experiments. One-way ANOVA was used for statistical analysis. ns, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.001, ****, P<0.0001.

Figure S4

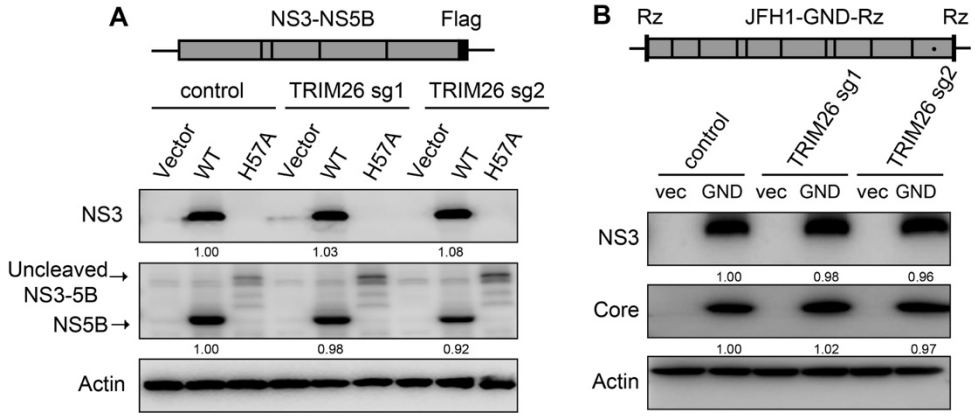

Figure S4. *TRIM26* knockdown has no effect on HCV polyprotein cleavage or translation.

(A)Control cells and Huh7-*TRIM26* knockdown cells were transfected with plasmids expressing NS3-5B-3×FLAG or NS3-5B-3×FLAG H57A, which serves as control. The cell lysates were immunoblotted with indicated antibodies. (B) Control cells and Huh7-*TRIM26* knockdown cells were transfected with plasmids expressing JFH1-GND-Rz and then the cell lysates were analyzed with indicated antibodies. The protein levels were quantified by Image J, normalized against internal Actin and expressed as values relative to control cells.

Figure S5

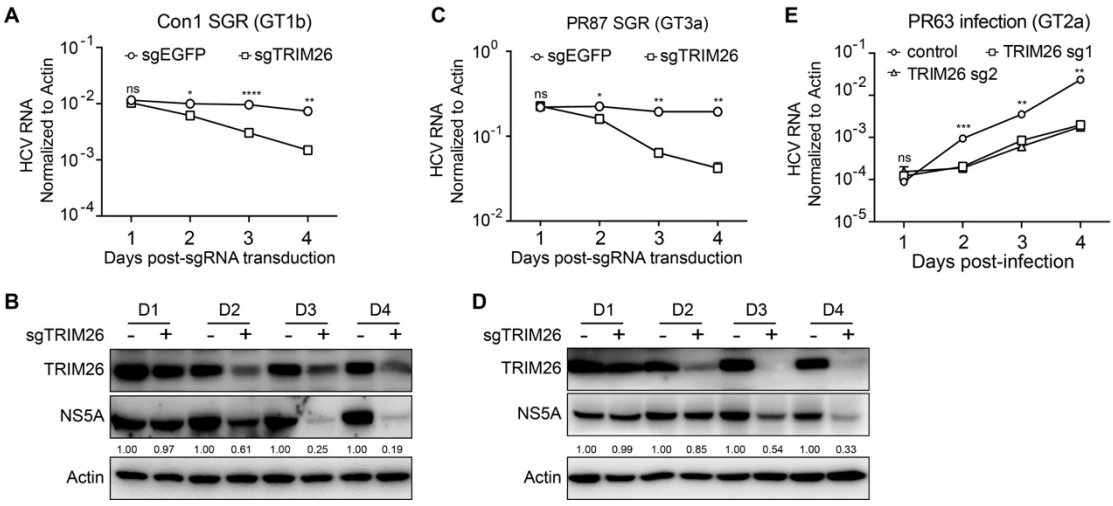

Figure S5. *TRIM26* knockdown reduces replication of multiple HCV strains of different genotypes.

(A-D) HCV Con1-SGR (genotype 1b) and PR87-SGR (genotype 3) cells were transduced with sgEGFP or sgTRIM26 for the indicated time points. Con1 RNA level (A), Con1 NS5A protein (B), PR87 RNA level (C) and PR87 NS5A protein (D) were analyzed by RT-qPCR and Western blot. (E) Control cells and Huh7-*TRIM26* knockdown cells were infected with PR63cc for the indicated time points. PR63 RNA level was analyzed. The error bars represent standard deviations from two independent experiments. T test (A and C) and One-way ANOVA (E) were used for statistical analysis. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001, ****, P<0.0001.The protein levels were quantified by Image J, normalized against internal Actin and expressed as values relative to control.

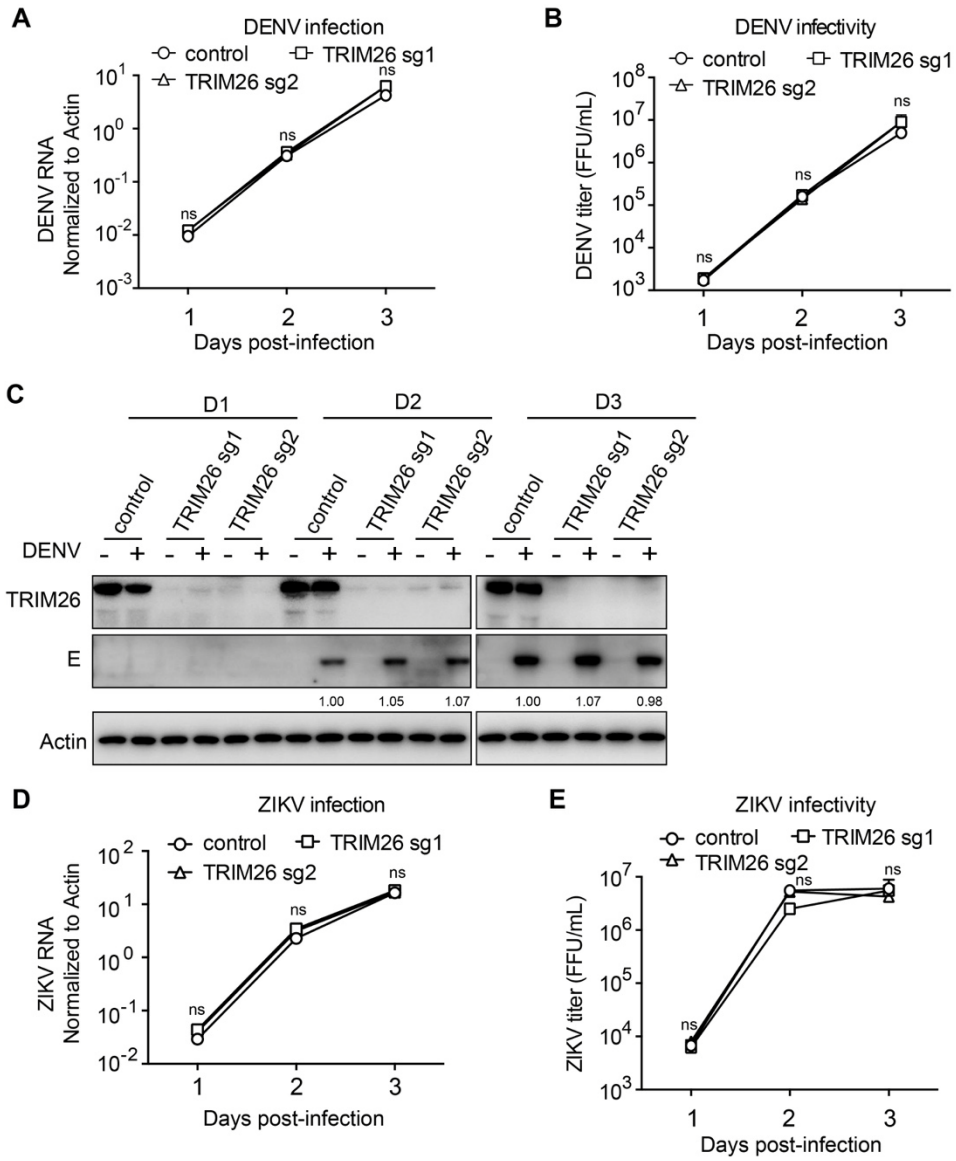

Figure S6. *TRIM26* knockdown has no obvious effect on DENV or ZIKV infection.

(A-C) Control cells and Huh7-*TRIM26* knockdown cells were infected with DENV at MOI of 0.1 for the indicated time points. Intracellular DENV RNA level (A), extracellular DENV titer (B) and E protein expression (C) were analyzed. (D-E) Control cells and Huh7-*TRIM26* knockdown cells were infected with ZIKV at MOI of 0.1 for the indicated time points. Intracellular ZIKV RNA level (D) and extracellular ZIKV titer (E) were analyzed. The error bars represent standard deviations from two independent experiments. One-way ANOVA was used for statistical analysis. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001,

****, P<0.0001.The protein levels were quantified by Image J, normalized against internal Actin and expressed as values relative to control cells.

Figure S7

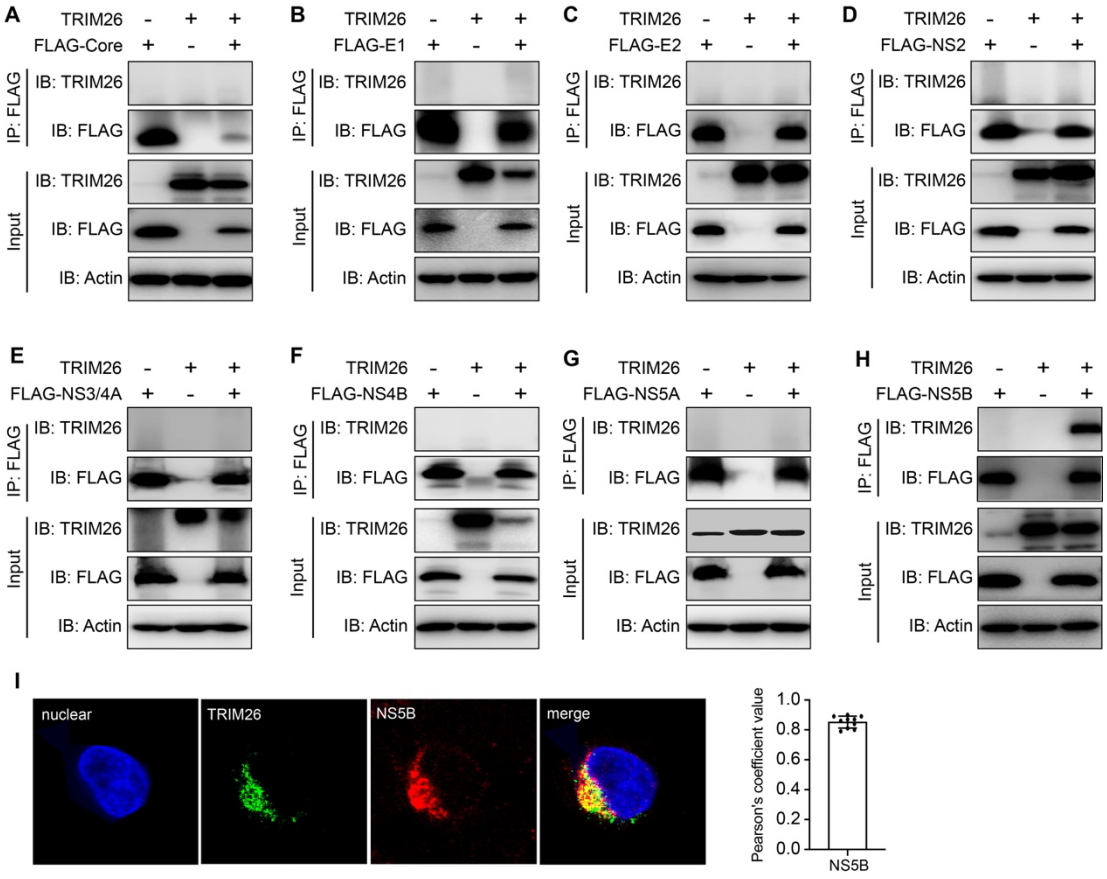

Figure S7. TRIM26 interacts with HCV NS5B.

(A-H) HEK293T cells were transfected with plasmids expressing TRIM26 together with FLAG-tagged HCV proteins core (A), E1 (B), E2 (C), NS2 (D), NS3/4A (E), NS4B (F), NS5A (G) and NS5B (H). The co-IP assays were performed with anti-FLAG antibody. (I) HEK293T cells co-transfected with TRIM26 and FLAG-NS5B were analyzed by immunofluorescence microscopy. The colocalization of TRIM26 (green) and FLAG-NS5B (red) was determined by Pearson's coefficient values from ten individual images.

Figure S8

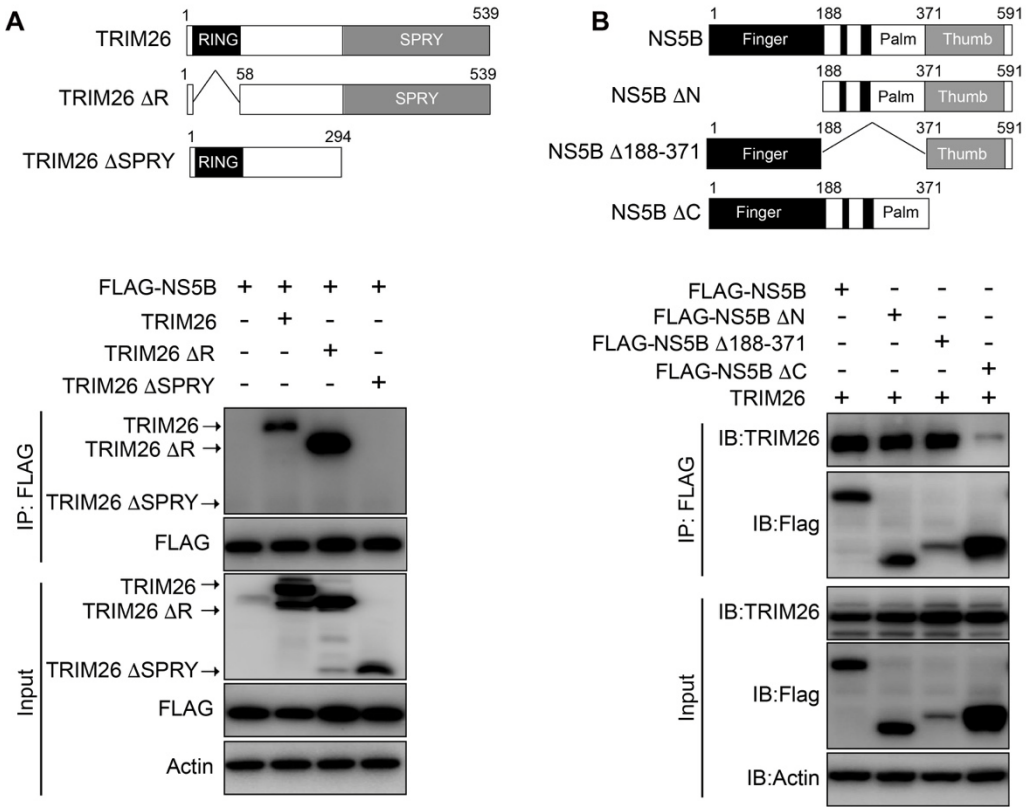

Figure S8. Domain mapping of the TRIM26-NS5B interaction.

(A) Schematic of functional domains of TRIM26 and its mutants. HEK293T cells were transfected with plasmids expressing FLAG-tagged NS5B and either TRIM26, TRIM26ΔR or TRIM26ΔSPRY. The cell lysates were immunoprecipitated with anti-FLAG antibody and then immunoblotted with the indicated antibodies. (B) Schematic of functional domains of NS5B and its mutants. HEK293T cells were transfected with plasmids expressing TRIM26 and FLAG-tagged NS5B, NS5BΔN, NS5BΔ188-371 or NS5BΔC. The cell lysates were immunoprecipitated with anti-FLAG antibody and then analyzed by immunoblot with the indicated antibodies.

Figure S9

A

Genotypes	GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	location
sequence number	496	169	46	69	5	86	2	4	
K20	99.6	98.2	95.6	100	100	100	0	100	outside
K36	0	96.4	0	0	0	0	0	0	outside
K43	0	29.6	0	0	0	0	0	100	outside
K50	97.3	100	91.3	100	100	95.4	100	100	outside
K51	99.2	100	95.6	97.1	100	100	100	100	outside
K69	99.8	84.5	95.6	95.6	0	97.7	0	75.0	outside
K72	99.8	98.8	97.8	100	100	98.8	100	100	outside
K77	43.3	91.7	0	0	80.0	0	0	75.0	outside
K100	99.4	85.2	100	98.6	100	93.0	100	100	outside
K106	100	96.5	97.8	98.6	100	75.6	100	100	outside
K120	0	79.3	0	0	0	0	100	0	outside
K124	85.5	26.6	0	0	0	0	100	0	outside
K141	100	100	100	100	100	100	100	100	inside
K151	98.9	98.2	97.8	98.6	100	94.2	100	100	outside
K154	0	96.4	0	0	0	0	100	0	outside
K155	100	100	95.6	100	100	96.5	100	100	outside
K172	100	100	93.5	98.6	100	100	100	100	inside
K181	43.3	97	89.1	0	100	100	0	100	outside
K206	0	76.9	78.3	0	100	62.8	100	100	outside
K211	100	98.8	100	100	100	94.2	100	100	outside
K212	95.4	93.5	100	82.6	100	96.5	50.0	75.0	outside
K270	53.2	99.4	93.5	0	100	55.8	0	75.0	outside
K298	99.6	100	95.7	100	100	100	100	100	inside
K304	0	91.7	50	0	0	0	0	0	outside
K491	99.8	100	95.7	100	100	91.9	100	100	outside
K501	0	96.4	0	0	0	0	0	0	outside
K517	0	13.6	89.1	57.9	0	89.5	0	0	outside
K531	0	85.2	0	0	80.0	59.3	0	100	outside
K533	100	100	100	100	100	100	100	100	outside
K535	98.8	97.1	60.9	0	80.0	97.7	0	100	outside

(red: conserved lysines)

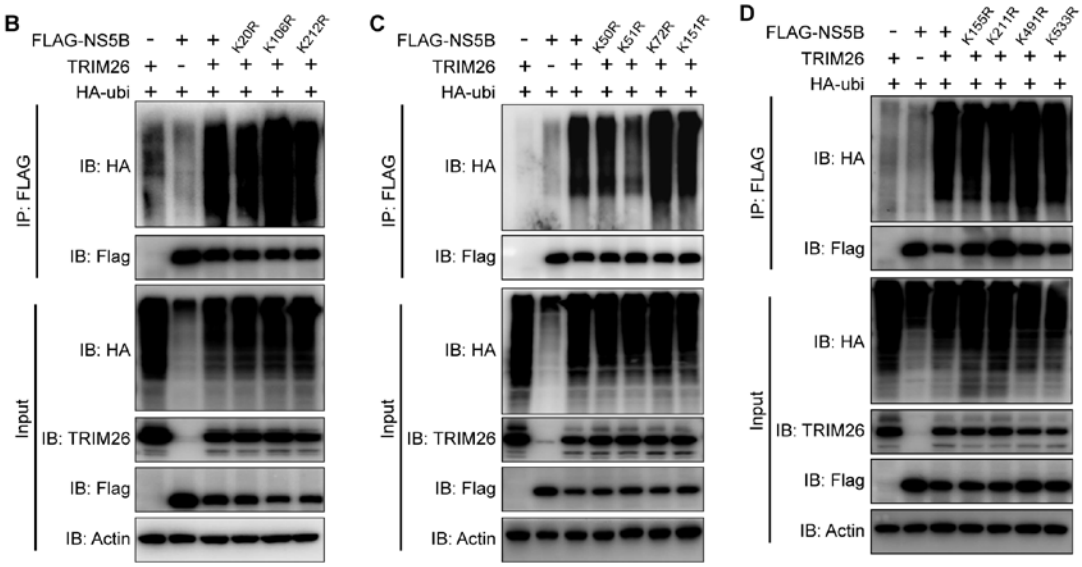

Figure S9. TRIM26 mediates the ubiquitination of NS5B at residue K51.

(A) Conservation and location of NS5B lysine residues among different HCV genotypes. Eleven lysine residues that are highly conserved (more than 90%) among GT1, GT2 and GT3 and located on the surface of NS5B are highlighted in red. (B-D) HEK293T cells were transfected with plasmids expressing TRIM26, HA-tagged ubiquitin and FLAG-tagged wild-type or lysine-mutated NS5B. The cell lysates were immunoprecipitated with anti-FLAG antibody and then immunoblotted by indicated antibodies.

Figure S10

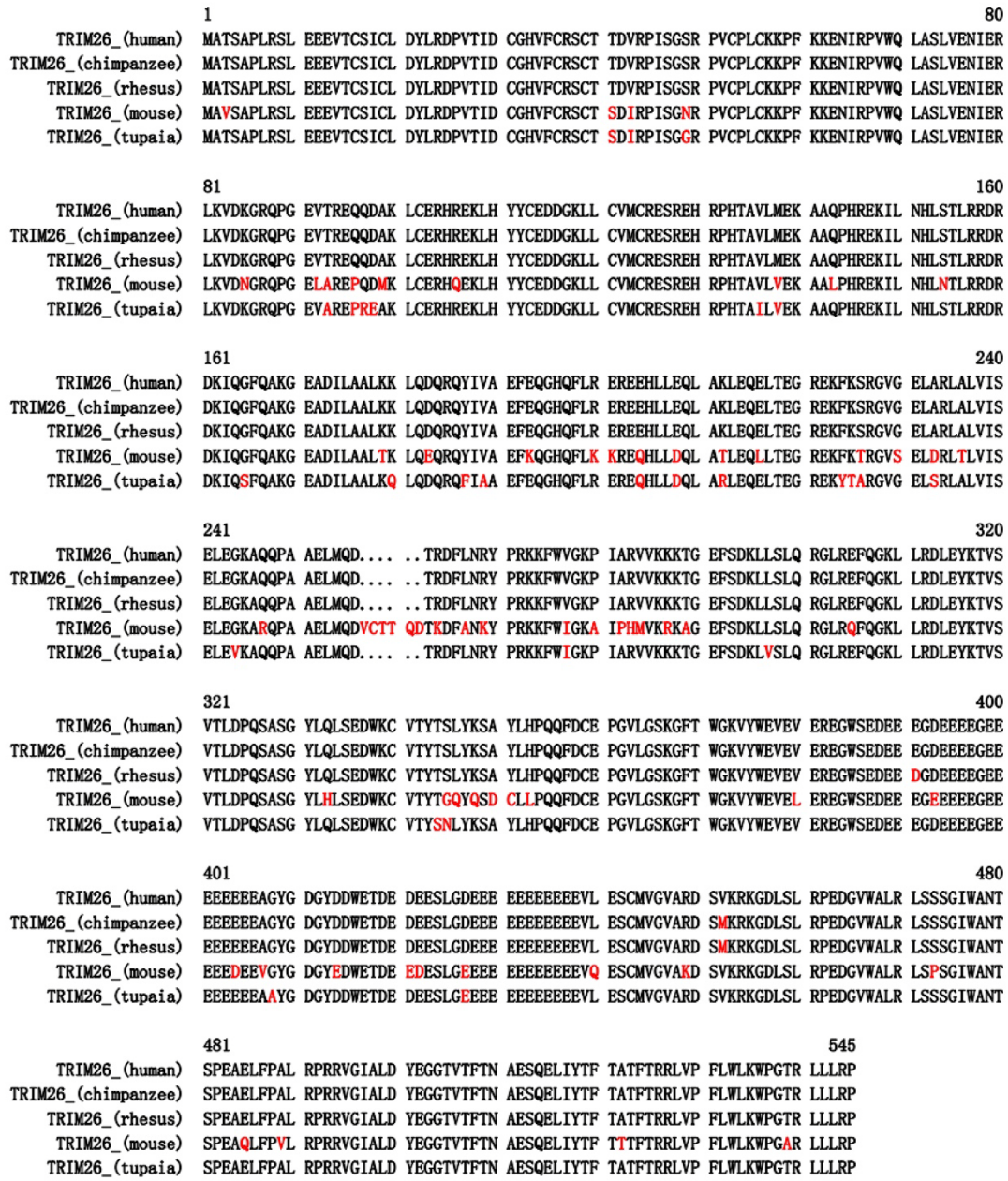

Figure S10. Alignment of TRIM26 amino acid sequence from different species.

Schematic presentation of amino acid sequence alignment of TRIM26 of human, chimpanzees, rhesus, mouse and tupaia.