Supplementary Figures

The non-structural NS1 protein of influenza viruses modulates *TP53* splicing through the host factor CPSF4

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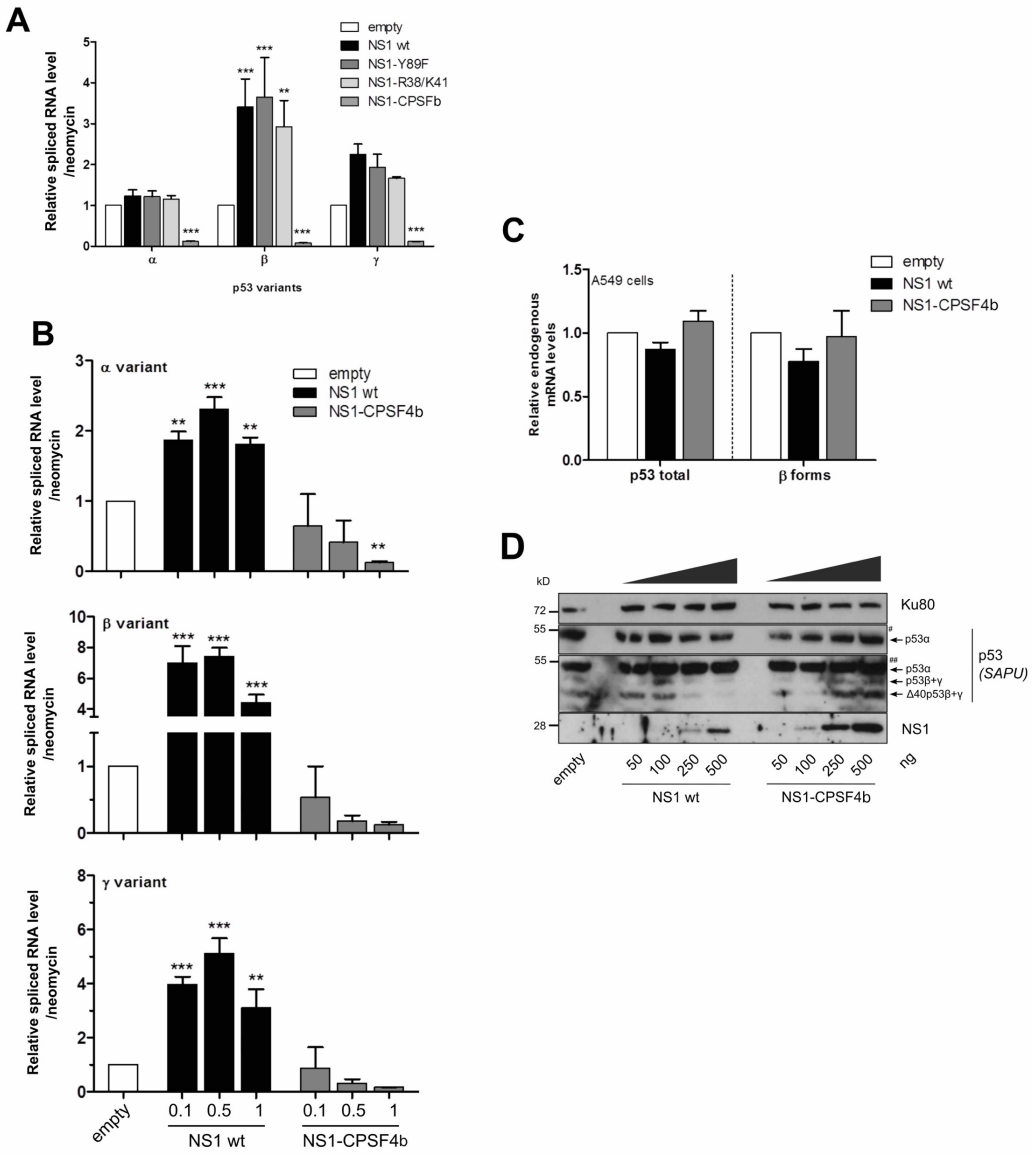
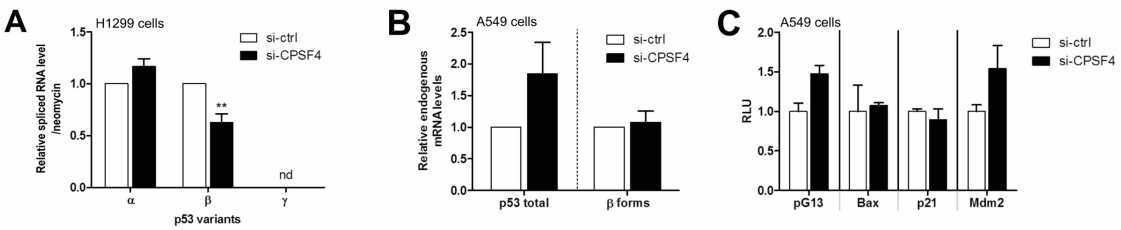


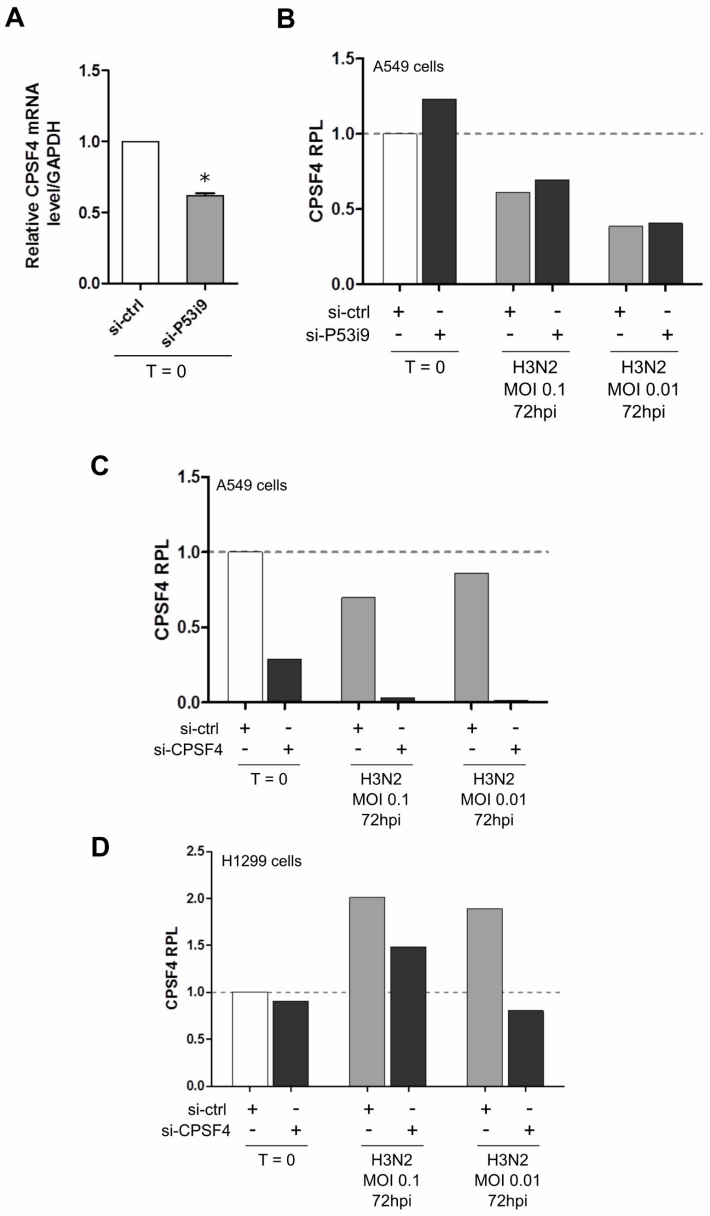
Supplementary Figure 1

Supplementary Figure 1. (A,B) The expression of α, β and γ mRNA variants was measured by RT-qPCR in H1299 cells 48 h after co-transfection of *TP53*-i9 minigene and 1µg of the empty pCI plasmid, pCI NS1wt, pCI NS1-Y89F, pCI NS1-R38A/K41A or pCI NS1-CPSF4b plasmids **(A)** or with increasing amounts (0.1, 0.5 or 1µg) of pCI NS1 wt or pCI NS1-CPSF4b plasmids **(B).** Mean values +/- standard deviation for at least three independent experiments are shown, and statistical tests compared each condition with its control condition using two-way ANOVA (**, $p < 0.01$; ***, $p < 0.001$). **(C)** A549 cells were transfected with 1µg of the empty pCI plasmid, pCI NS1 wt or pCI NS1-CPSF4b for 48 h, and endogenous expression of total p53 or β mRNA variants were measured by RT-qPCR. Levels of mRNA were measured in five independent experiments and normalized against the empty plasmid condition. **(D)** Increasing concentrations of NS1-expressing plasmids (wt or NS1-CPSF4b mutant) were transfected into A549 cells, and p53 isoform expression was detected by western blot using SAPU antibody. # and ## indicate short and long exposure respectively. Based on their size, we identified some p53 isoforms. NS1 was also detected, and Ku80 was used as loading control.



Supplementary Figure 2

Supplementary Figure 2 – The silencing of cellular CPSF4 factor impacts *TP53* splicing and p53 transcriptional activity. CPSF4 silencing was performed by transfecting H1299 (p53-null) or A549 (p53wt) cells with either a non-specific siRNA (negative-control si-ctrl), or a pool of siRNAs targeting cellular CPSF4. **(A)** Twenty-four hours following siRNA treatment, H1299 cells were transfected with the *TP53*-i9 minigene plasmid, and the relative expression of spliced α, β and γ p53 mRNA variants was measured by RT-qPCR at 48 hours post transfection. **(B)** Similar si-RNA treatment was performed on A549 cells, and relative mRNA levels of global p53 or β variants was measured and normalized on actin expression. **(C)** Twenty-four hours following siRNA treatment, A549 cells were transfected with pG13-Luc, Mdm2-Luc, Bax-Luc or p21-Luc reporter plasmids and relative luciferase units were measured 48 h later. Mean values +/- standard deviation of experimental duplicates are shown and statistical tests compared each condition with the si-ctrl condition using two-way ANOVA and Dunnett's post-test (**, $p < 0.01$).



Supplementary Figure 3

Supplementary Figure 3. (A) After being treated with siRNA targeting alternatively spliced p53β and p53γ isoforms (si-P53i9), cell lysates were harvested before (T=0) and after infection with H3N2 influenza, CPSF4 expression levels were quantified by RT-qPCR and normalized against GAPDH expression. The relative level of protein (RPL) for CPSF4 was measured by densitometry from western blots of Figure 5F **(B)**, Figure 6B (**C**) and Figure 6E (**D**).