Supplemental files to

The SARS-coronavirus nsp7+nsp8 complex is a unique multimeric RNA polymerase capable of both *de novo* initiation and primer extension

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Supplemental figures and legends

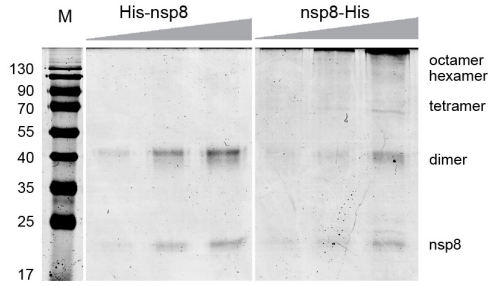

Figure S1: Analysis of nsp8 multimerization via chemical cross-linking. Cross-linking analysis of N-terminally His_6-tagged nsp8 and C-terminally His_6-tagged nsp8. Increasing concentrations of nsp8 were incubated in Hepes buffer (pH 7.5) in the presence of 0.12% glutaraldehyde for 5 min. Subsequent SDS-PAGE analysis and staining with Coomassie G-250 dye shows that only the C-terminally tagged protein with native N-terminus forms higher order multimers, whereas the N-terminally tagged protein reveals solely mono- and dimers.

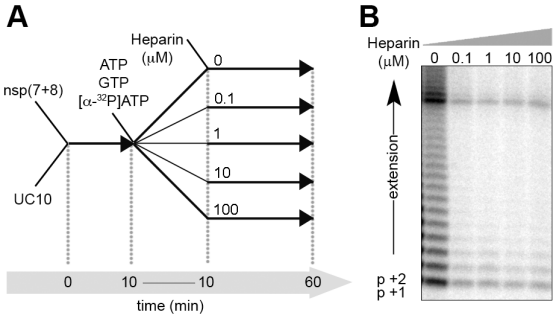

Figure S2: Inhibition of nsp(7+8) activity with heparin. (**A**) Schematic presentation of the single-cycle reaction. Template and nsp(7+8) complex were pre-incubated for 10 min before nucleotides were added. The mixture was then rapidly split into equal aliquots that were immediately mixed with different concentrations of heparin. (**B**) Samples were taken after 60 min and resolved on 20% PAGE/7M Urea.

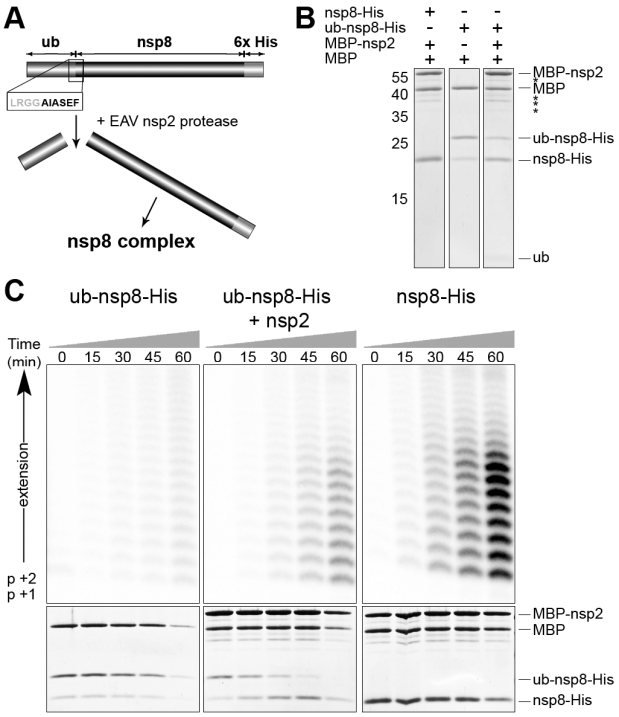

Figure S3: Activation of nsp8 RdRp activity by removal of an N-terminal fusion partner. (**A**) To study whether the interference by non-nsp7 N-terminal extensions was reversible, we purified nsp8 with an N-terminal ubiquitin extension. Addition of either purified EAV nsp2 or Ubp1 would subsequently result in hydrolysis of the fusion protein C-terminal of the LRGG site. (**B**) Analysis of ub-nsp8-His cleavage products by SDS-PAGE and Coomassie G-250 staining. Asterisks indicate unspecific bands (**C**) Time-course of [α-^{32}P]AMP incorporation of ub-nsp8-His, ub-nsp8-His in the presence of EAV nsp2, or nsp8-His that was *in vivo* cleaved by Ubp1. Lower panels demonstrate the stability or cleavage of ub-nsp7-8-his over time through SDS-PAGE analysis. In all reactions, MBP was added an independent loading control.

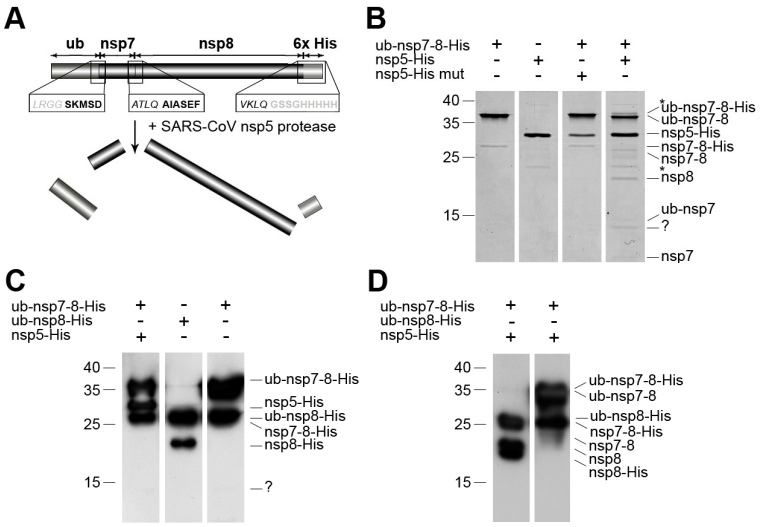

Figure S4: Processing of ub-nsp7-8-His by the SARS-CoV nsp5 main protease. (**A**) Due to the presence of the natural nsp7-8 cleavage sites in the ub-nsp7-nsp8-His construct, the polyprotein could be processed into mature subunits by the SARS-CoV nsp5 main proteinase. The question mark indicates an unidentified cleavage product. (**B**) SDS-PAGE analysis followed by Coomassie G-250 dye staining demonstrates that addition of nsp5 to purified ub-nsp7-nsp8-His results in cleavage at the sites indicated in Fig. 2C. Asterisk indicates non-specific band. The question mark indicates an unidentified cleavage product. (**C**) Western blot analysis of the protein samples used in Fig. 2D using an anti-His_6 monoclonal antibody and (**D**) an anti-nsp8 monoclonal antibody.

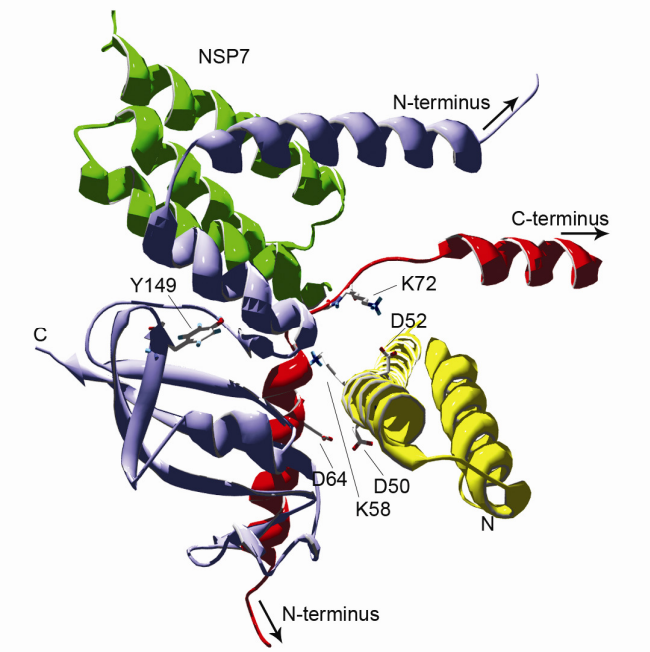

Figure S5: Position of (putative) critical residues in the published nsp7+8 structure. Position of the critical residues, including D50, D52 in the nsp(7+8) hexadecamer. For reference, the conserved residues K58A, D62, K72A, and Y149, and the location of the termini are also indicated. The three nsp8 subunits that are shown in this figure are coloured yellow, red and blue, whereas residues of the different termini are hidden to simplify the image. The nsp7 subunit that is in direct contact with the three nsp8 subunits is shown in green.

Table S1: DNA primers that were used for cloning of nsp8 and nsp7-8

Primer	Sequence
SAV704	5′-GCGGGTACCCCGCGGTGGATCTAAAATGTCTGACGTAAAGTGCACA-3′
SAV429	5′-GCGCGATCGGGATCCCTGTAGTTTAACAGCT-3′
SAV428	5′-GCGGGTACCCCGCGGTGGAGCTATTGCTTCAGAAT-3′

Table S2: DNA primers that were used for mutagenesis of nsp8

nsp8 mutation	PCR primers	Sequence
D50A	SAV574	5′-GCTAAATCTGAGTTTGCCCGTGATGCTGCCATG-3′
	SAV575	5′-CATGGCAGCATCACGGGCAAACTCAGATTTAGC-3′
D52A	SAV590	5′-TCTGAGTTTGACCGTGCTGCTGCCATGCAACGC-3′
	SAV591	5′-GCGTTGCATGGCAGCAGCACGGTCAAACTCAGT-3′
K58A	SAV402	5′-GCCATGCAACGCGCTTTGGAAAAGATGG-3′
	SAV403	5′-CCATCTTTTCCAAAGCGCGTTGCATGGC-3′
K127A	SAV501	5′-GACTACAGCAGCCGCACTCATGGTTGTTG-3′
	SAV502	5′-CAACAACCATGAGTGCGGCTGCTGTAGTC-3′
D161A	SAV503	5′-CCAGCAAGTTGTTGCTGCGGATAGCAAGA-3′
	SAV504	5′-TCTTGCTATCCGCAGCAACAACTTGCTGG-3′
D163A	SAV505	5′-AGTTGTTGATGCGGCTAGCAAGATTGTTC-3′
	SAV506	5′-GAACAATCTTGCTAGCCGCATCAACAACT-3′