

## **Appendix files Garvanska et al.**

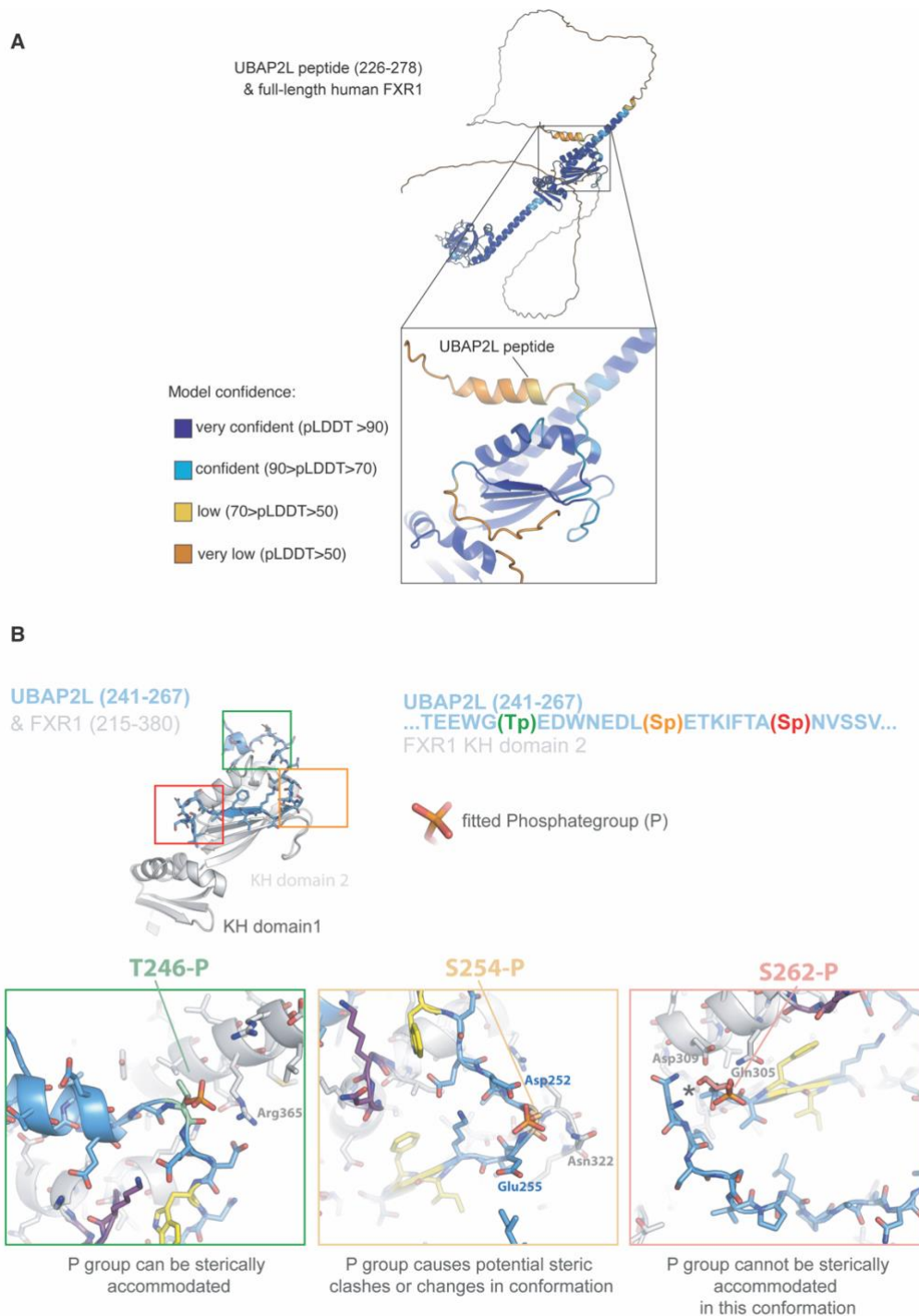
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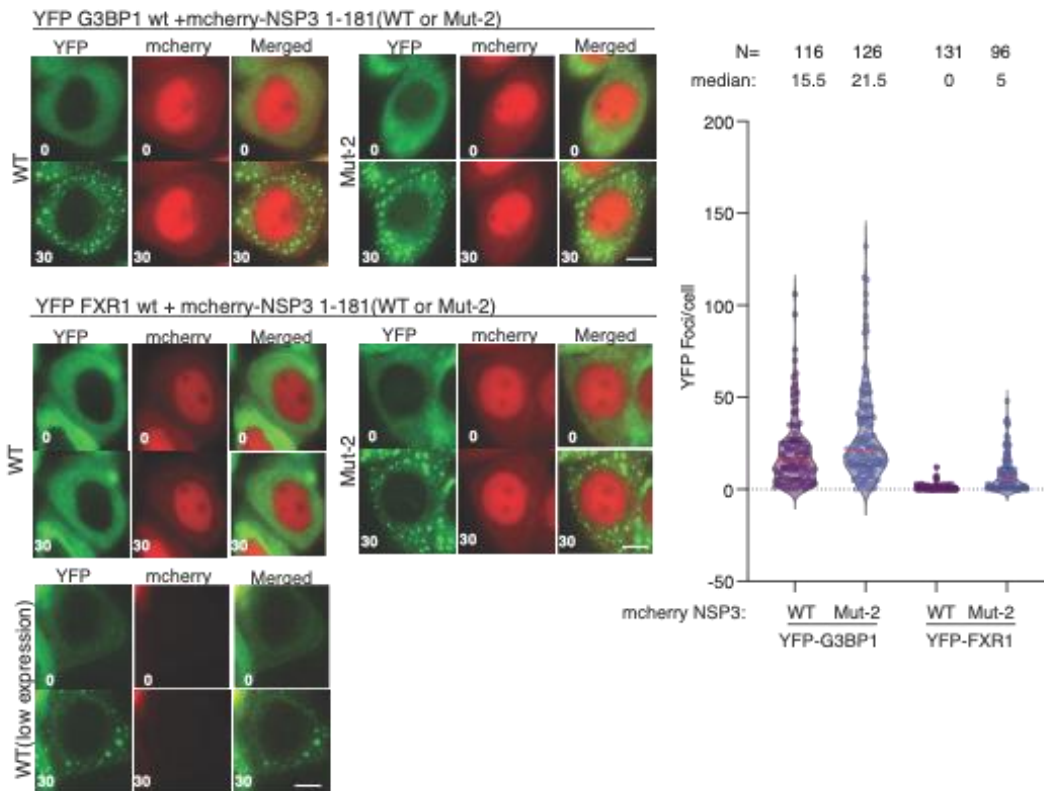
### Appendix Figure S1. Model of interaction of FXR1 to UBAP2L.

A) Confidence plots of AlphaFold model of UBAP2L peptide 226-278 binding to full length FXR1.

B) Reported phosphorylation sites in UBAP2L were fitted into the AlphaFold of the FXR1-UBAP2L complex.

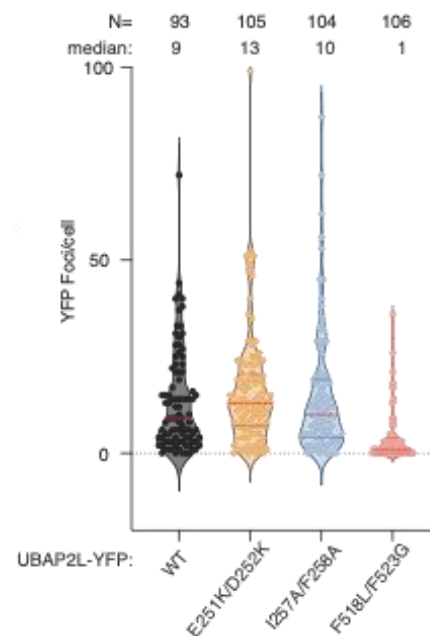
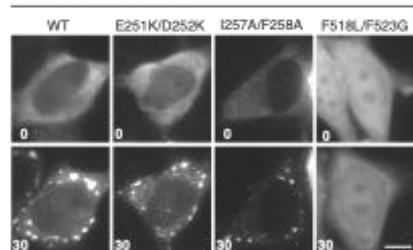
**A**

HeLa



**B**

HeLa-FRT UBAP2L KO  
UBAP2L-YFP



Appendix Figure 2, Garvanska et al

Appendix Figure S2. Analysis of stress granule components by live cell microscopy

A) The indicated YFP tagged stress granule proteins were expressed in HeLa cells in the presence of mCherry tagged NSP3 constructs and monitored by live cell microscopy. Stress granule formation was induced by arsenite and number of stress granules determined after 30 min. A pool of 3 biological replicates is shown in the graph.

B) The indicated UBAP2L-YFP constructs were expressed in HeLa-FRT UBAP2L KO cells and monitored by live cell microscopy. Arsenite was added and localization determined after 30 minutes. In A-B representative stills from the immunofluorescence is shown. The combined data from 2 biological replicates is shown in the graphs.

Data information: Violin plots with the median indicated with red line. The median and number of cells (N) analyzed per condition is indicated above the plot. Scale bar of 10  $\mu$ m indicated in lower right corner.

**NSP3 primers:**

NSP3 FW BglII GTCAAGATCTATGGCCCTACCAAGGTG  
NSP3 Rev NotI GTCAGCGGCCGCTTACTTGCTGAAGTTTGGAG  
NSP3 995 rev GTACGCGGCCGCTCAATCATCTAACCAATCTTCTTC  
NSP3 996 For GTACGGATCCGATAGTCAACAACTGTTGG  
NSP3 1324 Rev GTACGCGGCCGCTCATTAGTAGTGCCACCAGCC  
NSP3 1325 Fw GTACGGATCCATGCTAGCGAAAGCTTTGAG  
NSP3 1875 Rev GTACGCGGCCGCTCAAGGACCTTTGTATTCTGAGG  
NSP3 1876 FW GTACGGATCCATTACGGATGTTTTCTACAAAG

**FXR1 primers:**

FXR1 FW BamHI GTAC GGATCC ATGGCGGAGCTGACGGTGG  
FXR1 Rev 118 GTAC GCGGCCGC TCAATTTTGATTGACAGGCCG  
FXR1 Rev 215 GTAC GCGGCCGC TCAAAGTTGTTTTGTGCATTC  
FXR1 Rev 360 GTAC GCGGCCGC TCATTCCTTTAGATAGGCAATATG  
FXR1 Rev 460 GTAC GCGGCCGC TCAACCACGTGGTCCACCACGAC  
FXR1 Rev FL GTAC GCGGCCGC TCAATCACATCTTTTGCCTAG  
FXR1\_I304/N\_Frw GGAAAAAATGGCAAAGTTAATCAAGAAATAGTGG  
FXR1\_I304/N\_Rev CCACTATTTCTTGATTAACCTTGCCATTTTTTCC  
FXR1 L351P FW GTGCAGGTTCTTCCAGAGTATCATATTGCC  
FXR1 L351P Rev GGCAATATGATACTCTGGAAGAACCTGCAC

**UBAP2L primers:**

UBAP2L\_Frw GATCGCGGCCGCATGATGACATCGGTGGGCACTAACC  
UBAP2L\_200\_rev CATGGCGGCCGCTTAGTTAAAGGTTCCCATTCC  
UBAP2L\_400\_rev CATGGCGGCCGCTTATGATGGGGATTGTGTCGTCG  
UBAP2L\_600\_rev CATGGCGGCCGCTTACCGAGTCTGTGTGGAT  
UBAP2L\_800\_rev CATGGCGGCCGCTTAAGGCAACAACGGCGGGAC  
UBAP2L\_FL\_rev GATCGCGGCCGCTAGCTAGCGGAACCGTTGCC  
UBAP2L\_Fw\_BamHI GATCGGATCCATGATGTCGGTGGGCACTAACC  
UBAP2L\_225\_rev CATGGCGGCCGCTTAAGTGTGTTCCACGTATTGC  
UBAP2L\_250\_rev CATGGCGGCCGCTTAATTCCAATCTTCAGTCCC  
UBAP2L\_275\_rev CATGGCGGCCGCTTATGTCACATTCTCCGAGG  
UBAP2L\_300\_rev CATGGCGGCCGCTTAAGATGAATCATTCTCC  
UBAP2L\_325\_rev CATGGCGGCCGCTTATGATATGGCAGTCTGC  
UBAP2L\_350\_rev CATGGCGGCCGCTTAACCGACATCACCAAATCC  
UBAP2L\_375\_rev CATGGCGGCCGCTTACAACCTGAGCCAGGGC  
UBAP2L EE251-252KK F CTGAAGATTGGAATAAGAAGCTTTCTGAGACCAAG  
UBAP2L EE251-252KK R CTTGGTCTCAGAAAGCTTCTATTCCAATCTTCAG  
UBAP2L I257A/F258A\_FW: CTT TCT GAG ACC AAG GCC GCC ACT GCC TCT AAT GTG  
UBAP2L I257A/F258A\_Rev: CAC ATT AGA GGC AGT GGC GGC CTT GGT CTC AGA AAG

**Appendix Table S1.**

Overview of primers used to clone NSP3, FXR1 and UBAP2L constructs.

**Appendix Table S2.** Affinities and thermodynamic values of NSP3 1-181 binding events inferred from ITC measurements performed at 25 °C. Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ), entropy ( $-T\Delta S$ ), equilibrium dissociation constant ( $K_D$ ) and reaction stoichiometry ( $n$ ) are shown. The protein-protein interaction affinity is defined by the Gibbs energy for binding  $\Delta G = -RT \ln K_A = RT \ln K_D$ .

	$\Delta G$ ( <i>kcal mol<sup>-1</sup></i> )	$\Delta H$ ( <i>kcal mol<sup>-1</sup></i> )	$-T\Delta S$ ( <i>kcal mol<sup>-1</sup></i> )	$K_D$ ( $\mu M$ )	$n$
NSP3 1-181/FXR1 <sup>1-122</sup>			No binding		
NSP3 1-181/FXR1 <sup>212-289</sup>			No binding		
NSP3 1-181/FXR1 <sup>215-360</sup>	-7.70	-25.9 ± 0.38	18.2	2.26 ± 0.13	0.90 ± 0.01
NSP3 1-181mut1/FXR1 <sup>215-360</sup>			No binding		
NSP3 1-181/FXR1 <sup>215-360</sup> I304N			No binding		

**Appendix Table S2 continued.** Affinities and thermodynamic values of FXR1<sup>215-360</sup> binding events inferred from ITC measurements performed at 25 °C. Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ), entropy ( $-T\Delta S$ ), equilibrium dissociation constant ( $K_D$ ) and reaction stoichiometry ( $n$ ) are shown. The protein-protein interaction affinity is defined by the Gibbs energy for binding  $\Delta G = -RT \ln K_A = RT \ln K_D$ . <sup>a</sup>The stoichiometry ( $n$ ) was fixed to 1 when not explicitly stated.

FXR1 <sup>215-360</sup> complex	$\Delta G$ ( <i>kcal mol<sup>-1</sup></i> )	$\Delta H$ ( <i>kcal mol<sup>-1</sup></i> )	$-T\Delta S$ ( <i>kcal mol<sup>-1</sup></i> )	$K_D$ ( $\mu M$ )	$n$
FXR1 <sup>215-360</sup> /NSP3 WT peptide	-7.86	-24.20 ± 0.12	16.3	1.74 ± 0.04	0.95 ± 0.01
FXR1 <sup>215-360</sup> /NSP3 M1	-5.49	-18.40 ± 8.67	13.0	95.1 ± 43.2	
FXR1 <sup>215-360</sup> /NSP3 M2			No binding		
FXR1 <sup>215-360</sup> /NSP3 M3			No binding		
FXR1 <sup>215-360</sup> /NSP3 M4			No binding		

**Appendix Table S2 continued.** Affinities and thermodynamic values of NSP3 alphavirus binding events inferred from ITC measurements performed at 25 °C. Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ), entropy ( $-T\Delta S$ ), equilibrium dissociation constant ( $K_D$ ) and reaction stoichiometry ( $n$ ) are shown. The protein-protein interaction affinity is defined by the Gibbs energy for binding  $\Delta G = -RT \ln K_A = RT \ln K_D$ . <sup>a</sup>The stoichiometry ( $n$ ) was fixed to 1 when not explicitly stated.

	$\Delta G$ ( <i>kcal mol<sup>-1</sup></i> )	$\Delta H$ ( <i>kcal mol<sup>-1</sup></i> )	$-T\Delta S$ ( <i>kcal mol<sup>-1</sup></i> )	$K_D$ ( $\mu M$ )	<sup>a</sup> $n$
NSP3 peptide alphavirus/FXR1 <sup>1-122</sup>	-6.43	-6.11 ± 0.65	-0.32	19.4 ± 3.16	
NSP3 peptide alphavirus/FXR1 <sup>212-289</sup>			No binding		
NSP3 peptide alphavirus/FXR1 <sup>215-360</sup>			No binding		

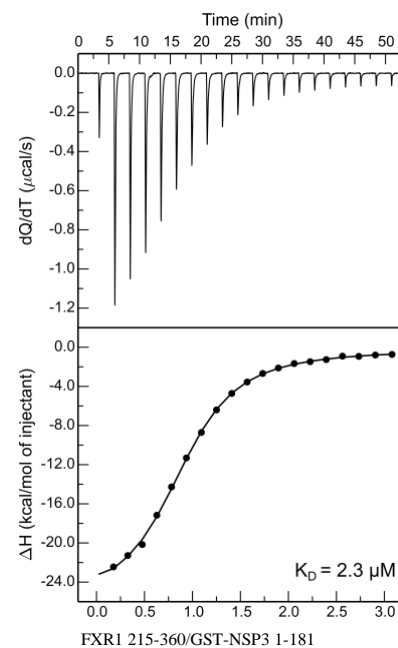
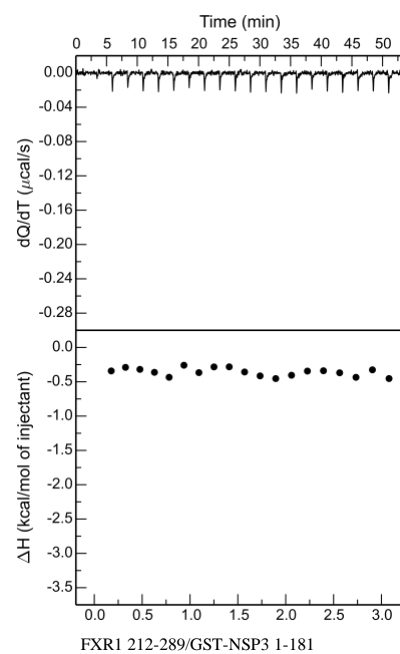
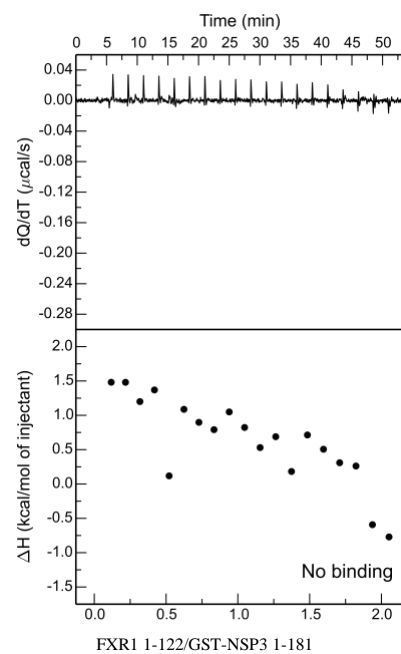


**Appendix Table S2 continued.** Affinities and thermodynamic values of FXR1<sup>215-360</sup> binding events inferred from ITC measurements performed at 25 °C. Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ), entropy ( $-T\Delta S$ ), equilibrium dissociation constant ( $K_D$ ) and reaction stoichiometry ( $n$ ) are shown. The protein-protein interaction affinity is defined by the Gibbs energy for binding  $\Delta G = -RT \ln K_A = RT \ln K_D$ . <sup>a</sup>The stoichiometry ( $n$ ) was fixed to 1 when not explicitly stated.

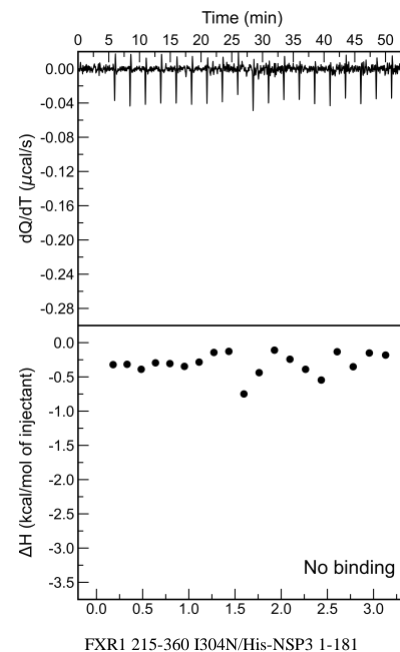
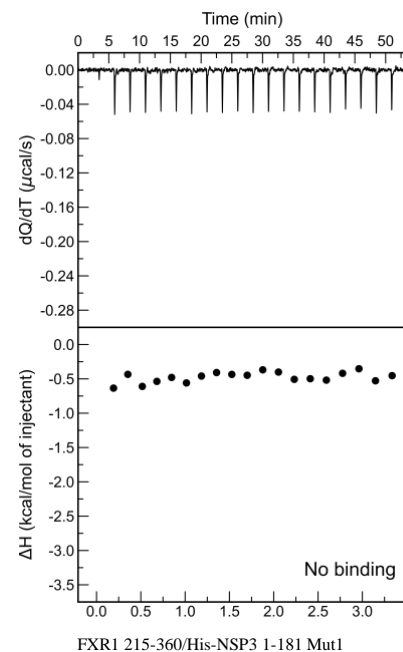
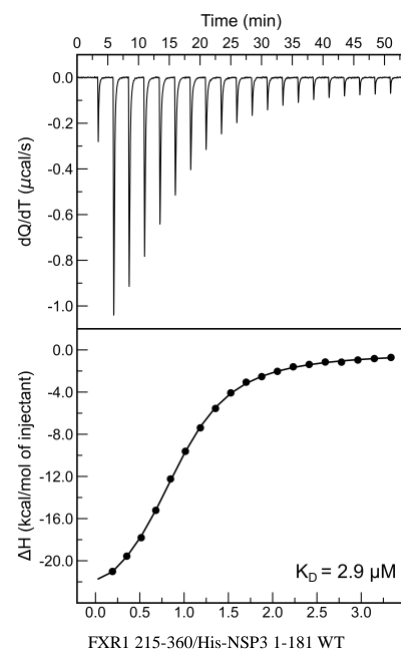
FXR1 <sup>215-360</sup> complex	$\Delta G$ ( <i>kcal mol<sup>-1</sup></i> )	$\Delta H$ ( <i>kcal mol<sup>-1</sup></i> )	$-T\Delta S$ ( <i>kcal mol<sup>-1</sup></i> )	$K_D$ ( $\mu M$ )	$n$
FXR1 <sup>215-360</sup> /UBAP2L <sup>263-290</sup>	-6.92	-13.7 ± 0.54	6.79	8.53 ± 0.69	0.96 ± 0.01
FXR1 <sup>215-360</sup> /UBAP2L <sup>263-290</sup> pS274	-6.27	-8.91 ± 2.38	2.65	25.5 ± 7.34	0.92 ± 0.01
FXR1 <sup>215-360</sup> /UBAP2L <sup>263-290</sup> pT266	-7.42	-12.0 ± 0.26	4.57	3.66 ± 0.24	0.90 ± 0.01
FXR1 <sup>215-360</sup> /UBAP2L <sup>263-290</sup> pS282			No binding		

**Appendix Table S2 continued.** Affinities and thermodynamic values of NF1/N protein from SARS-CoV2 and FXR1 215-360 binding inferred from ITC measurements performed at 25 °C. Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ), entropy ( $-T\Delta S$ ), equilibrium dissociation constant ( $K_D$ ) and reaction stoichiometry ( $n$ ) are shown. The protein-protein interaction affinity is defined by the Gibbs energy for binding  $\Delta G = -RT \ln K_A = RT \ln K_D$ . <sup>a</sup>The stoichiometry ( $n$ ) was fixed to 1 when not explicitly stated.

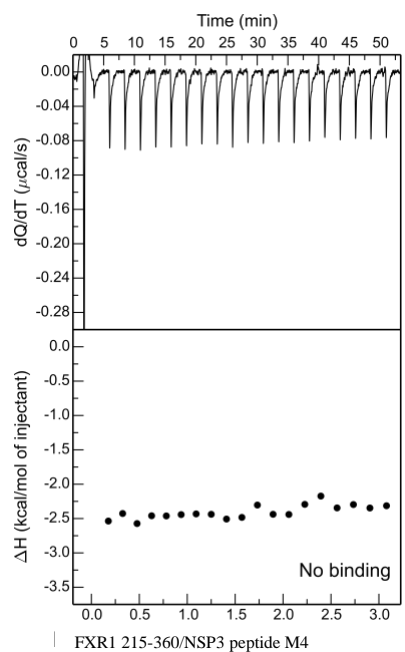
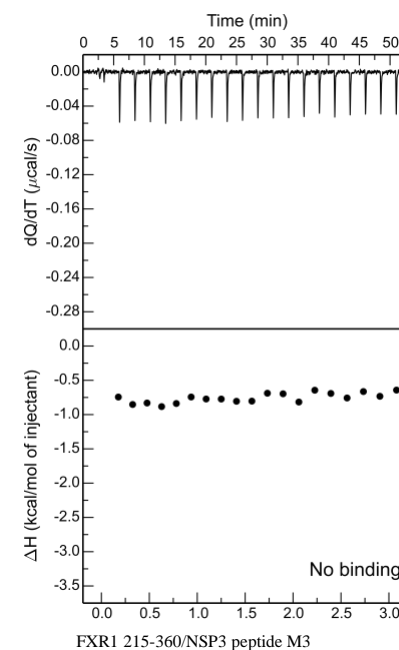
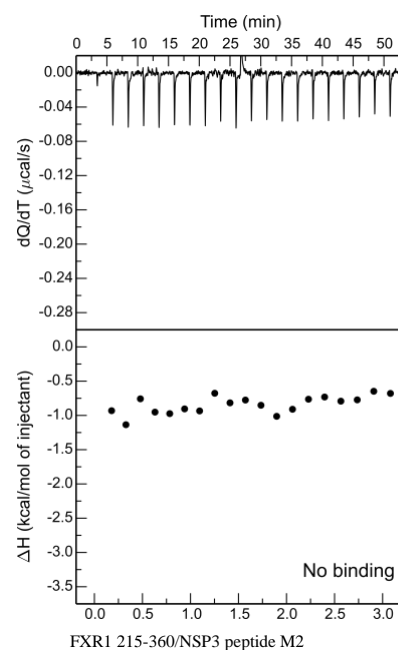
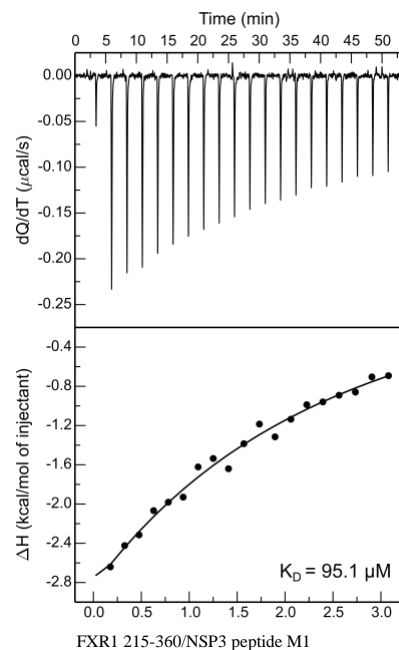
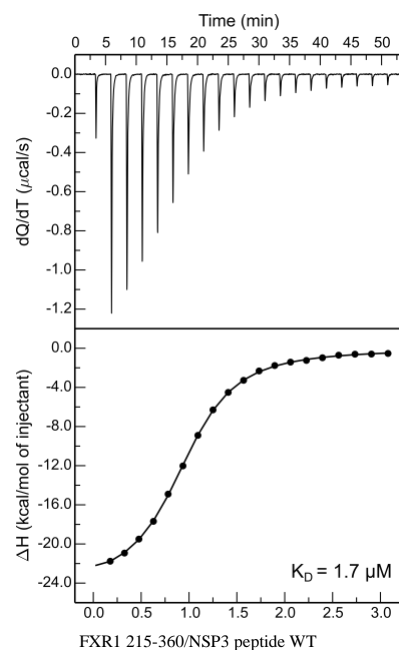
FXR1 <sup>215-360</sup> complex	$\Delta G$ ( <i>kcal mol<sup>-1</sup></i> )	$\Delta H$ ( <i>kcal mol<sup>-1</sup></i> )	$-T\Delta S$ ( <i>kcal mol<sup>-1</sup></i> )	$K_D$ ( $\mu M$ )	$n$
N SARS-CoV-2/NF1 RNA	-8.76	-30.1 ± 0.68	21.3	0.38 ± 0.04	0.82 ± 0.01
FXR1 <sup>215-360</sup> /NF1 RNA	<i>No binding</i>				



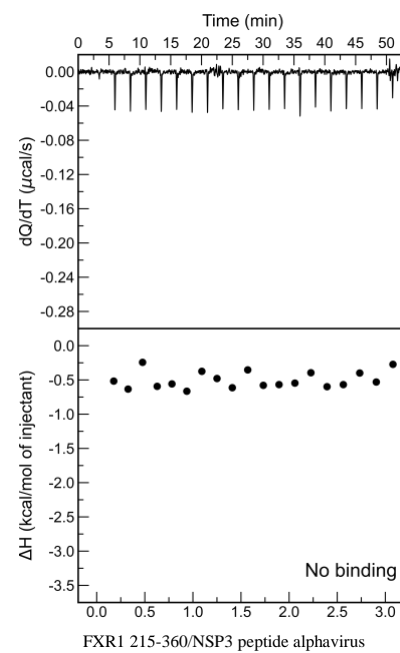
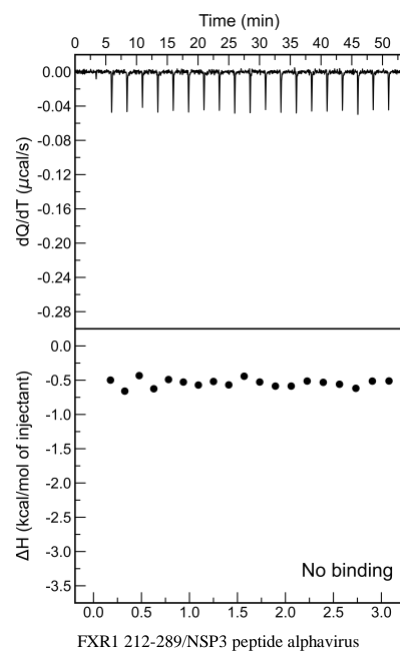
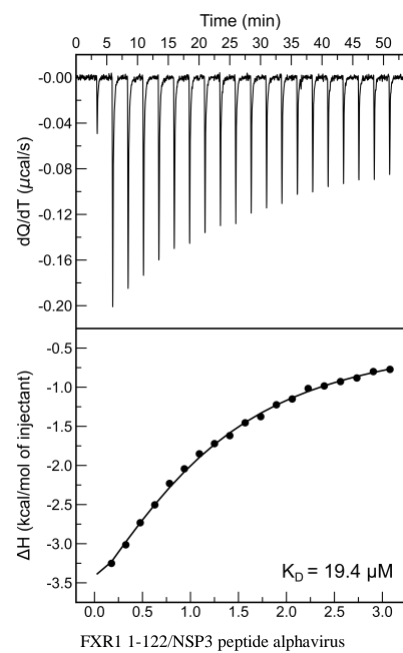
**Appendix Table S2**  
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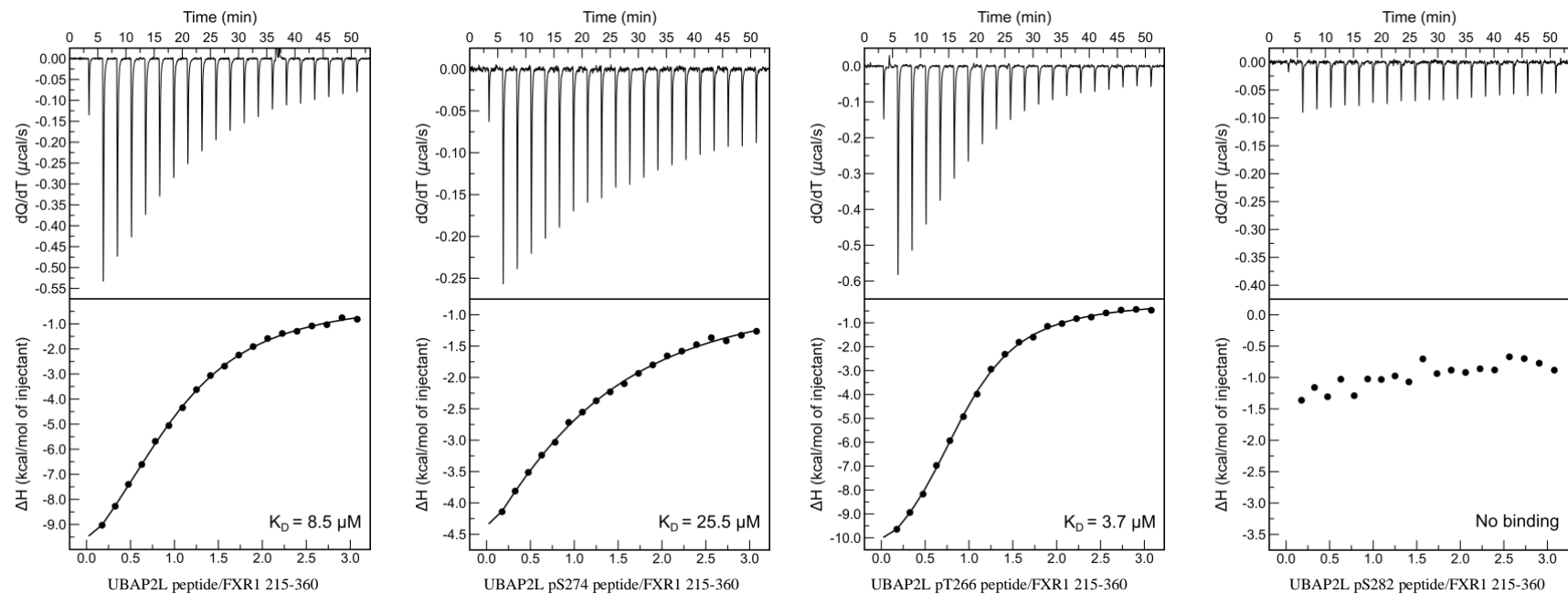
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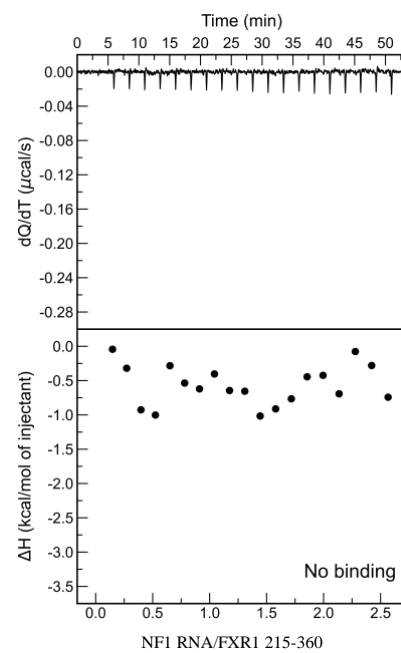
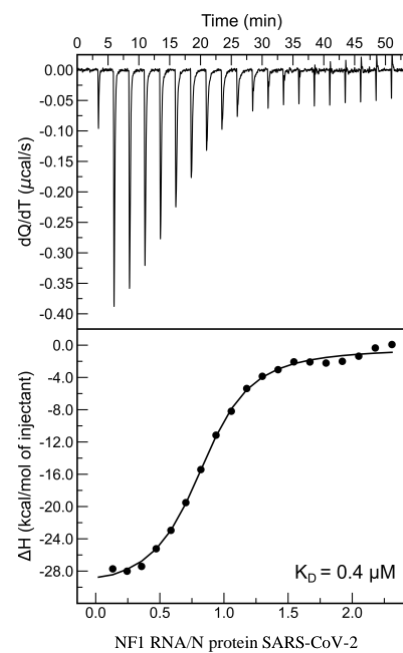
**Appendix Table S2  
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**Appendix Table S2**  
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**Appendix Table S2  
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