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## Supplemental information

# Characterization and functional interrogation of the SARS-CoV-2 RNA interactome

**Athéna Labeau, Luc Fery-Simonian, Alain Lefevre-Utile, Marie Pourcelot, Lucie Bonnet-Madin, Vassili Soumelis, Vincent Lotteau, Pierre-Olivier Vidalain, Ali Amara, and Laurent Meertens**

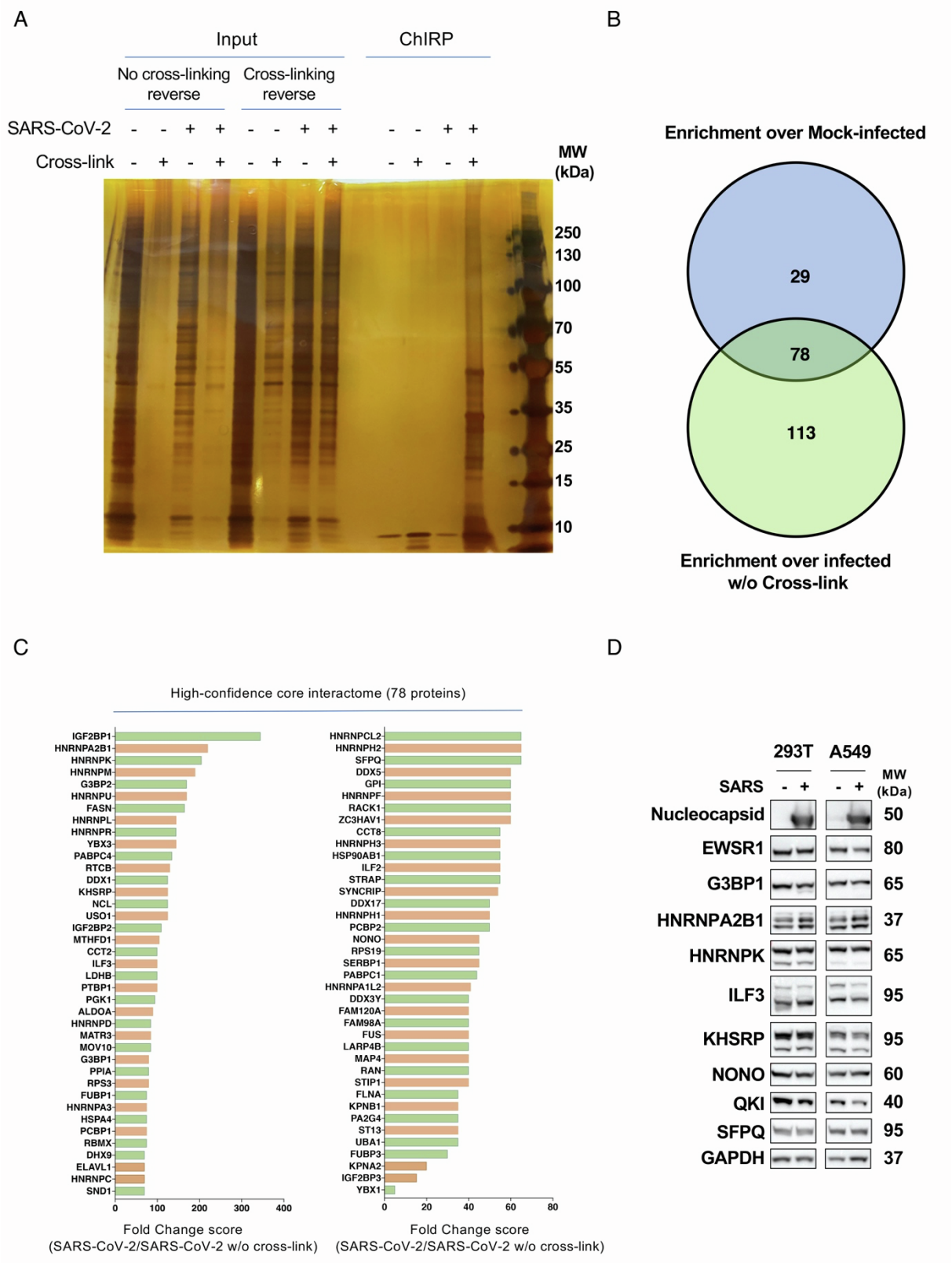


**Figure S1. vRNA-interacting protein enrichment is not affected by an obvious bias resulting from host proteome change following infection. Related to Figure 1. (A)** Proteins enriched by ChIRP from uninfected and SARS-CoV-2-infected cells, treated or not with paraformaldehyde cross-linker, were resolved by SDS-PAGE and visualized by silver staining. Data shown are representative of two biological replicates. Input from all the samples, which were not heated (no cross-linking reverse) or heated to reverse the cross-linking (cross-linking reverse), are

shown. **(B)** Venn Diagram comparing the significantly enriched RBP from SARS-CoV-2 RNA interactomes. **(C)** Fold change (SAINTexpress FC score) of the high-confidence core interactome identified in our SARS-CoV-2 ChIRP-M/S. **(D)** Immunoblot of a panel of 9 proteins from lysates of 293T-ACE2 and A549-ACE2 cells infected with SARS-CoV-2 or mock-infected (-). Infection was detected using the anti-SARS-CoV-2 nucleocapsid mAb. Data shown are representative of three biological replicates.

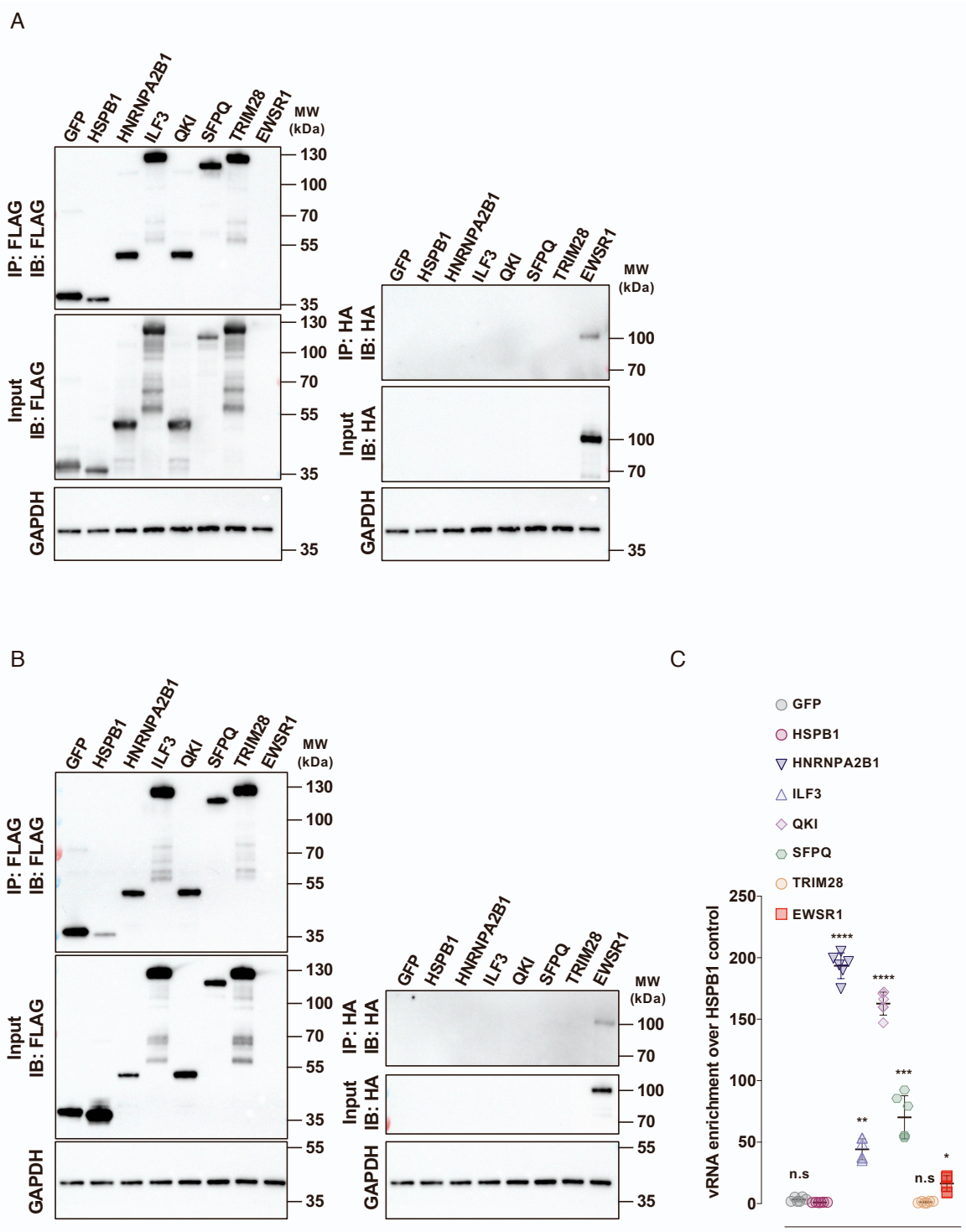


**Figure S2**. **ChIRP enriched proteins that bind to SARS-CoV-2 vRNA. Related to Figure 1. (A)** 293T-ACE2 ectopically expressing the indicated proteins bearing HA or FLAG tag or **(B)** A549-ACE2 cells stably expressing them, were challenged with SARS-CoV-2 at MOI 0.05 (aiming for 50% infected cells). 24 hours post-infection cells were lyzed and proteins were pulled-down with protein G magnetic beads coupled with anti-FLAG or anti-HA antibodies. Proteins contained in the input or IP were resolved by SDS-PAGE and visualized by either anti-HA or anti-FLAG antibodies. Data shown are representative of two biological replicates. **(C)** Enrichment of immunoprecipitated vRNA from A549-ACE2 was quantified by RT-qPCR and calculated as $2^{(-\Delta\Delta Ct\ [\text{normalized RIP/normalized HSPB1}])}$. Data shown are mean ± SD of two biological replicates (with technical triplicates). Adjusted p-

values were calculated by Kruskal-Wallis test with Benjamini, Krieger and Yekutieli correction. (n.s, non-significant; * $p<0.05$; *** $p<0.01$; *** $p<0.001$ and **** $p<0.0001$).

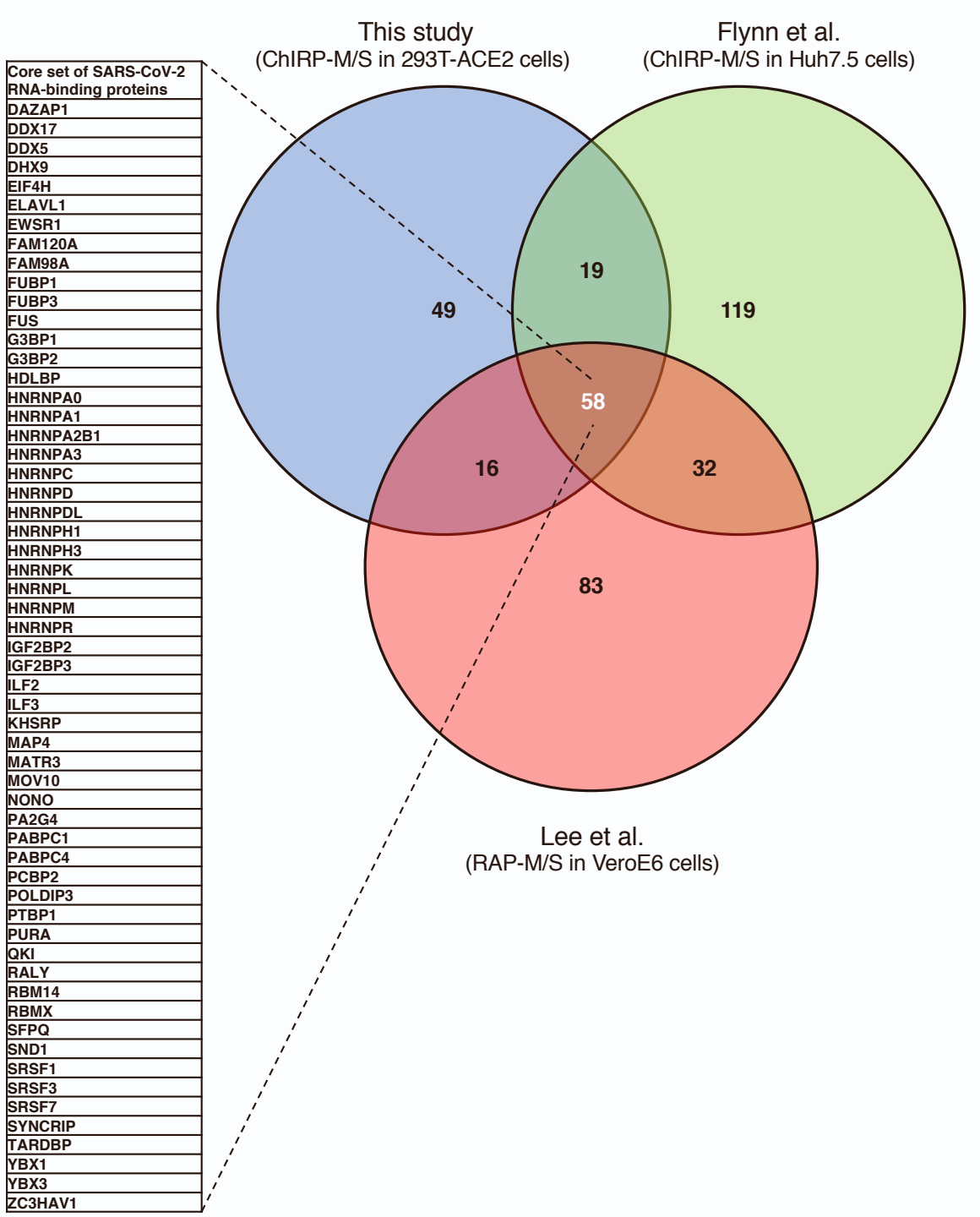


**Figure S3. Intersection of SARS-CoV-2 interactomes identified a set of core SARS-CoV-2 RNA RBP. Related to Figure 1**. Venn Diagram comparing the significantly enriched RBP from SARS-CoV-2 RNA interactomes.

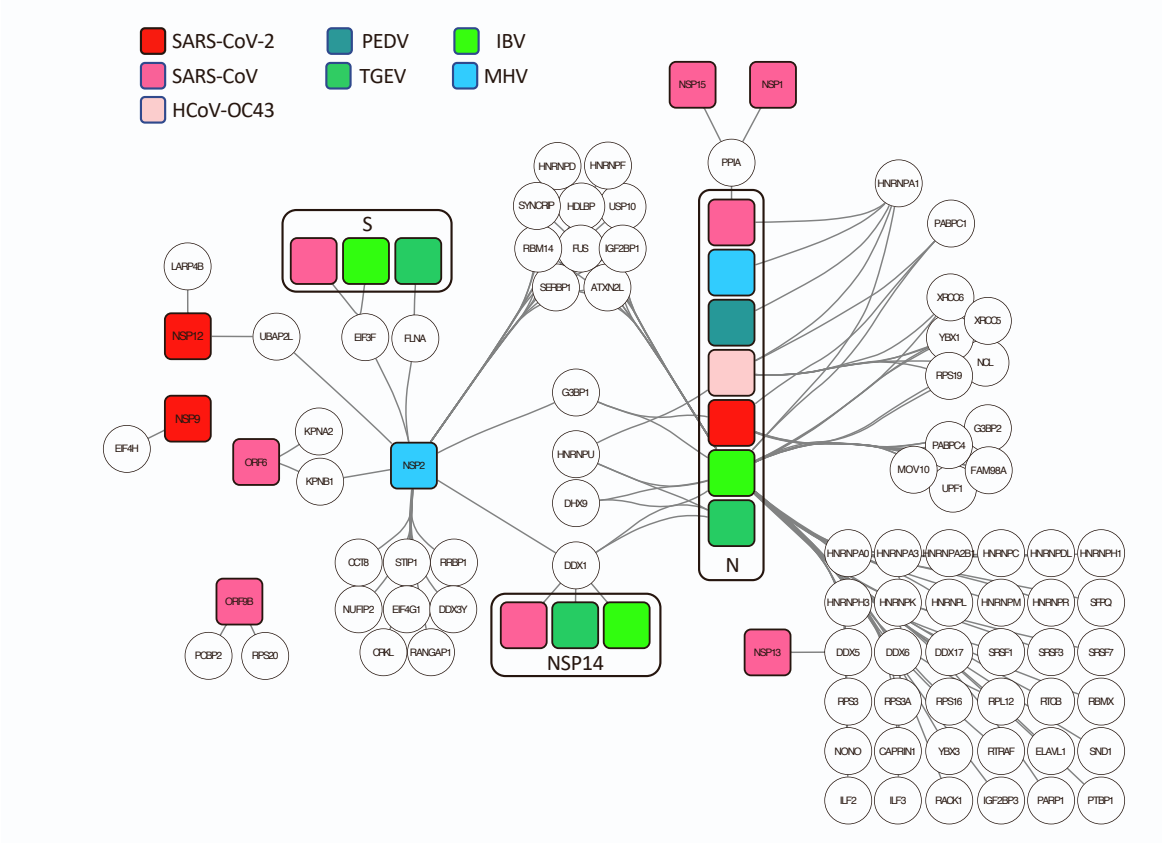


**Figure S4. Intersection of the ChIRP interactome with known coronavirus-host interactions. Related to Figure 1.** Map showing interactions between the cellular proteins of our ChIRP dataset and viral proteins of SARS-CoV-2 or other coronaviruses. This analysis was performed using the reference coronavirus interactome previously published (Perrin-Cocon et al., 2020). Round circles correspond to host proteins. Squares correspond to viral proteins. Human coronavirus OC43 (HCoV-OC43). Porcine epidemic diarrhea virus (PEDV). Infectious bronchitis virus (IBV). Mouse hepatitis virus (MHV). Transmissible gastroenteritis virus (TGEV).