SARS-CoV-2 main protease, 3CL^{pro} (nsp5), regulates the formation of tunneling nanotubes by coordinating cytoskeletal reorganization

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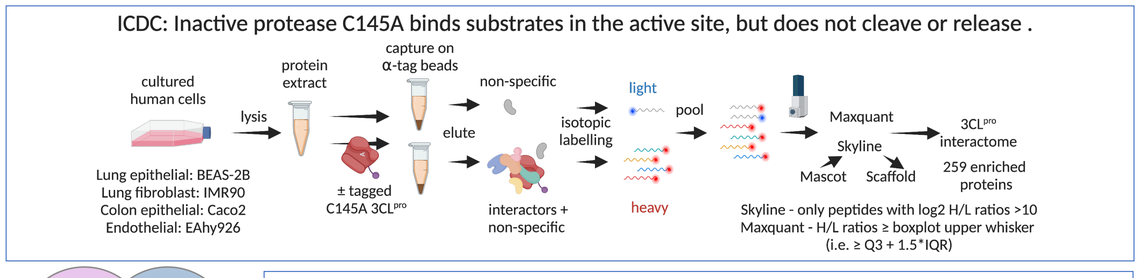
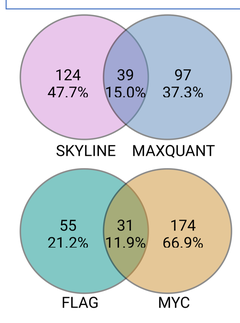
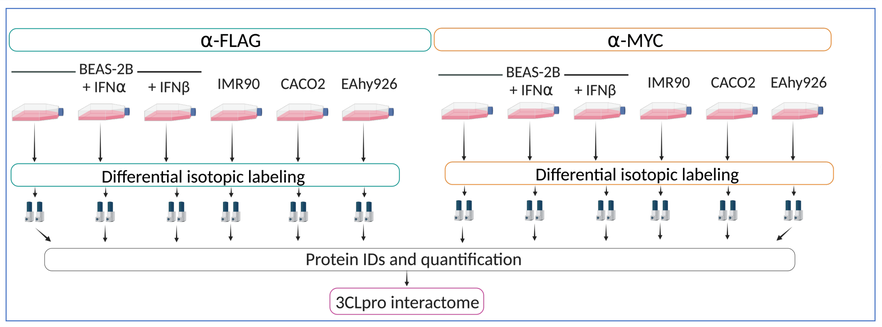
1. Background

The coronavirus SARS-CoV-2 and resulting disease COVID-19 continue to pose a significant threat, despite the availability of vaccines. The approval the SARS-CoV-2 main protease (3CL^{pro}, nsp5) inhibitor PAXLOVID^{TM} to treat COVID-19 emphasises the importance of this viral protease in SARS-CoV-2 pathology. To evaluate how 3CL^{pro} modulates the human proteome to promote infection, we used ICDC (inactive catalytic domain capture) to identify interactors of 3CL^{pro}, and further characterised cleavage of a subset of interactors that contain candidate 3CL^{pro} cleavage sites.

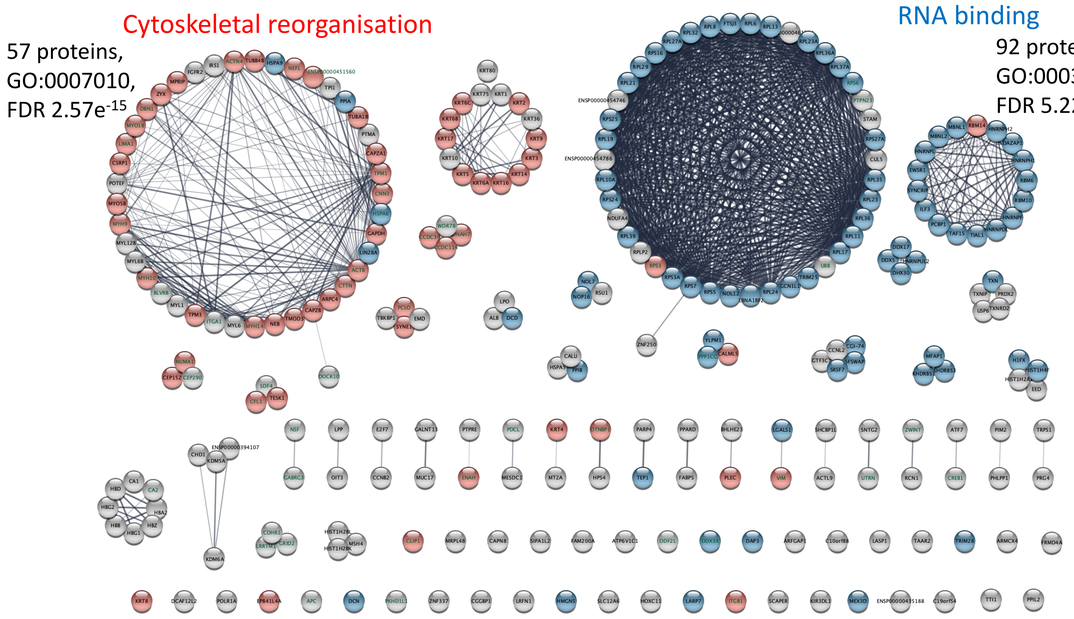
2. Inactive catalytic domain capture (ICDC) strategy



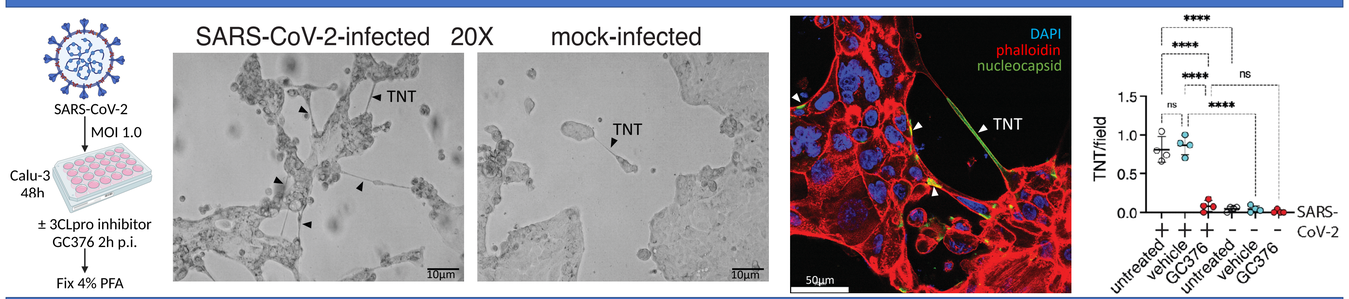




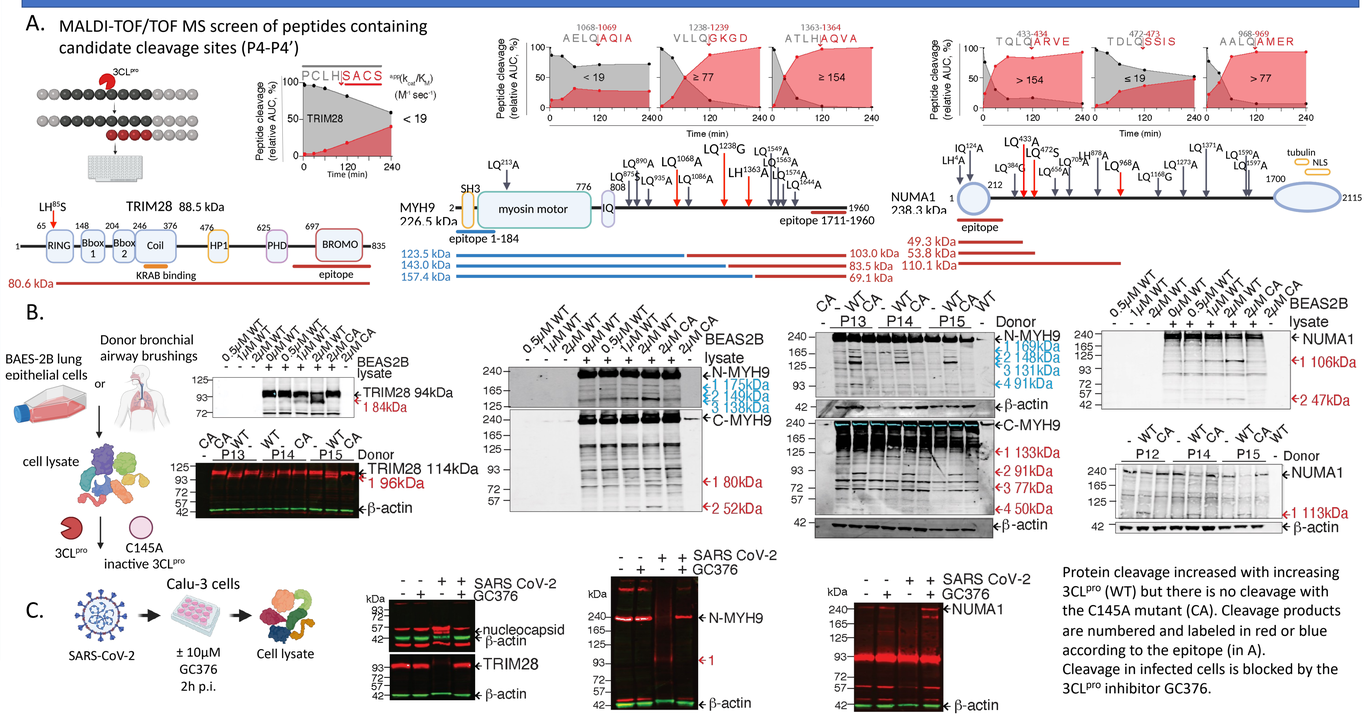
3. 3CL^{pro} interactors are involved in RNA binding and cytoskeletal reorganisation



4. SARS-CoV-2 infection of human lung epithelial cells induces the formation of tunneling nanotubes (TNT) that contain virus

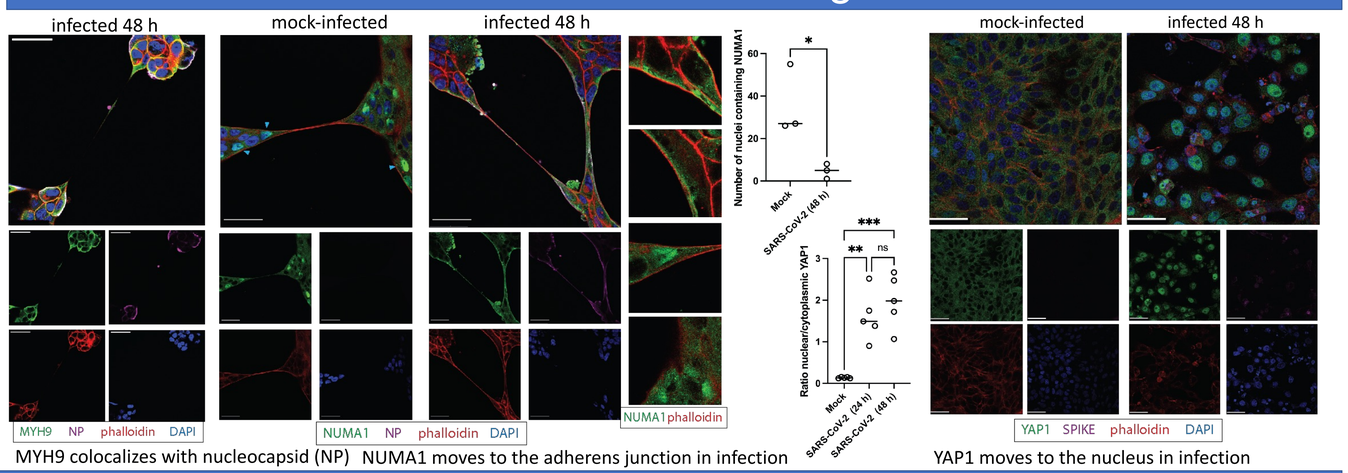


5. Proteins from the interactome are 3CL^{pro} substrates



Protein cleavage increased with increasing 3CL^{pro} (WT) but there is no cleavage with the C145A mutant (CA). Cleavage products are numbered and labeled in red or blue according to the epitope (in A). Cleavage in infected cells is blocked by the 3CL^{pro} inhibitor GC376.

6. 3CL^{pro} cleavage induces protein translocation and activation of the zonular signalosome

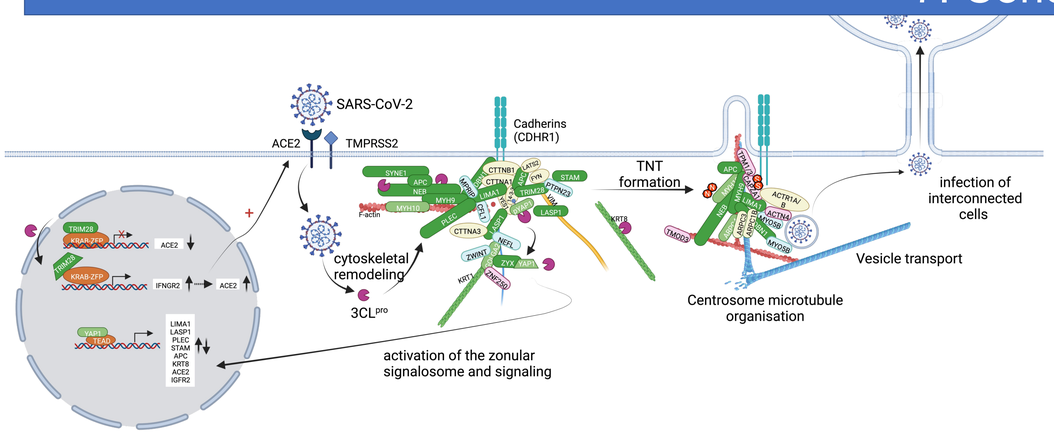


MYH9 colocalizes with nucleocapsid (NP) NUMA1 moves to the adherens junction in infection

YAP1 moves to the nucleus in infection

7. Conclusions

1. ICDC significantly expanded the interactome of 3CL^{pro} (nsp5).
2. Many interactors are involved in cytoskeletal organization.
3. Proteins with candidate 3CL^{pro} cleavage sites were cleaved *in vitro* and in infected cells and cleavage was blocked by the 3CL^{pro} inhibitor GC376.
4. Cleavage of proteins by 3CL^{pro} in the adherens junction of lung epithelial cells stimulates protein translocation and disrupts cytoskeletal networks.
5. In infection of human lung epithelial cells, 3CL^{pro} induces the formation of tunneling nanotubes that enable SARS-CoV-2 to infect distant cells.



Acknowledgements

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