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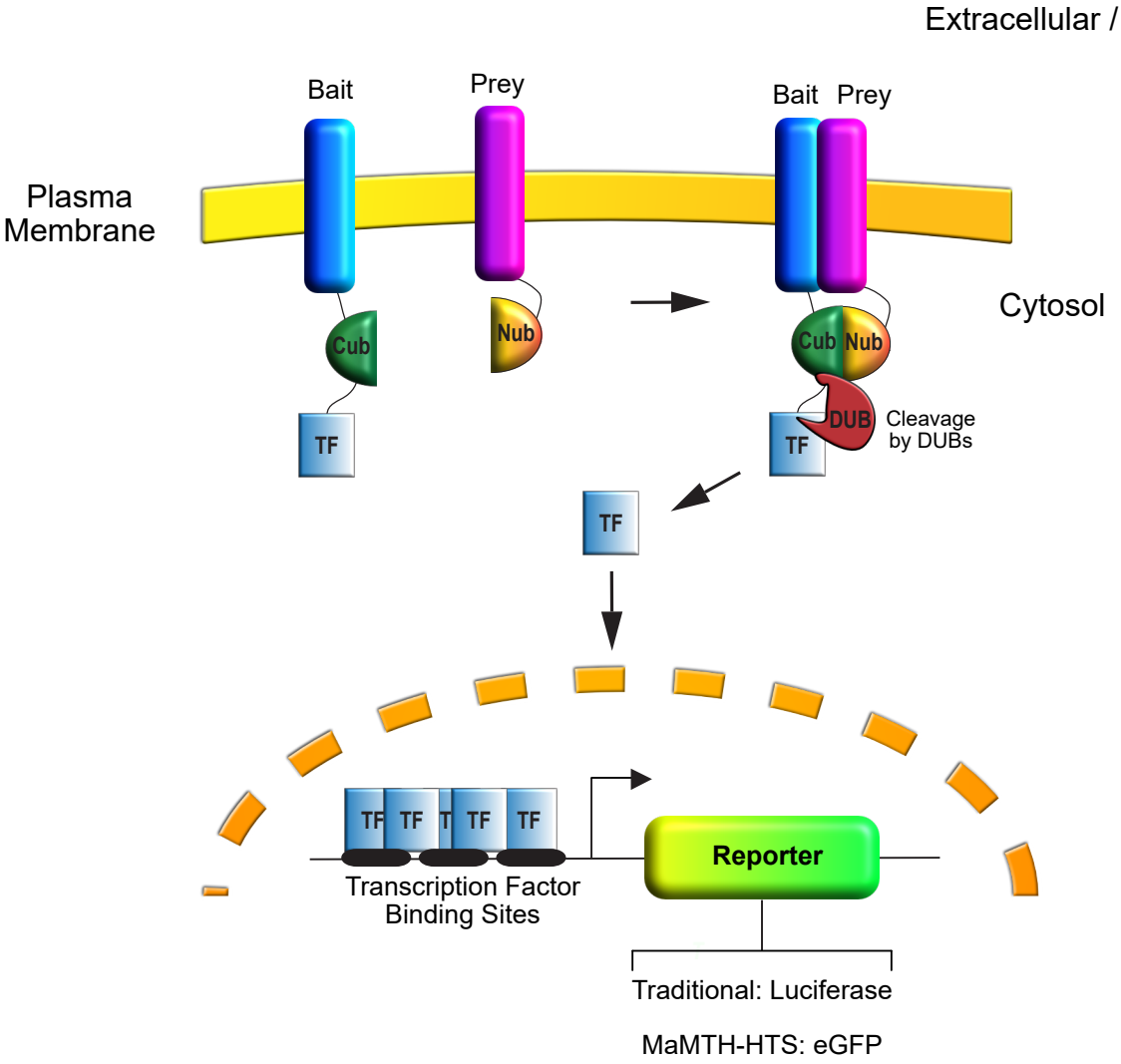
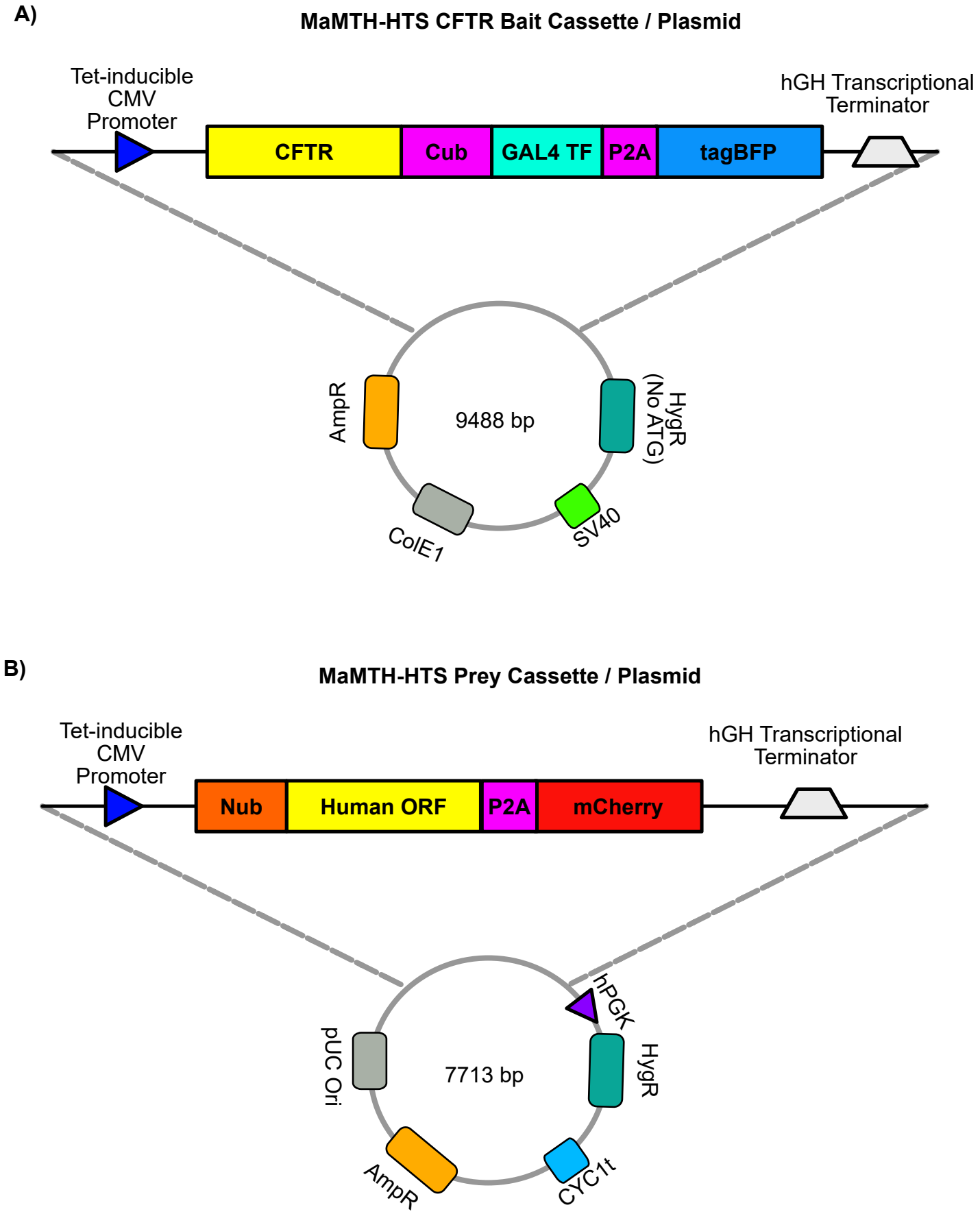
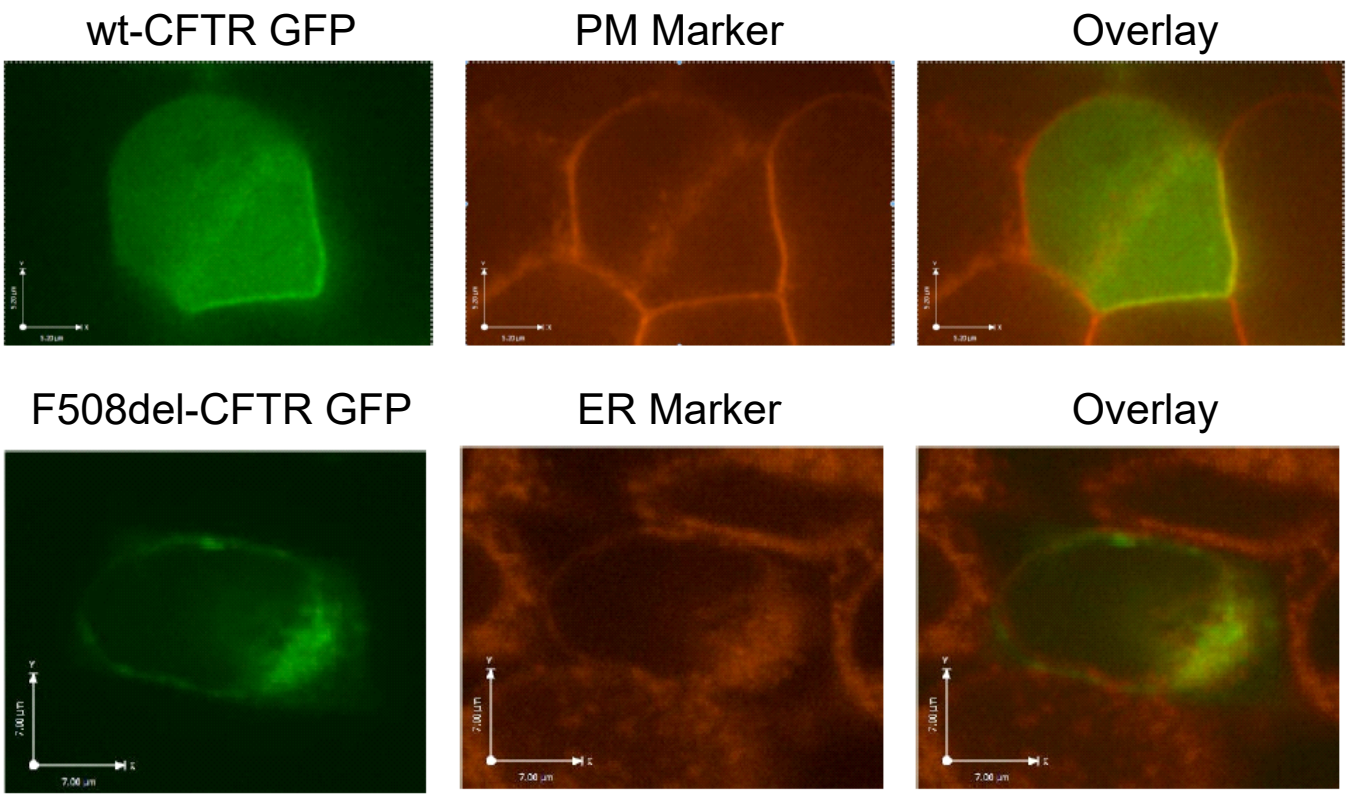
Appendix Figure S10 – Overview of the validation strategy used in this work. Created with BioRender.com.



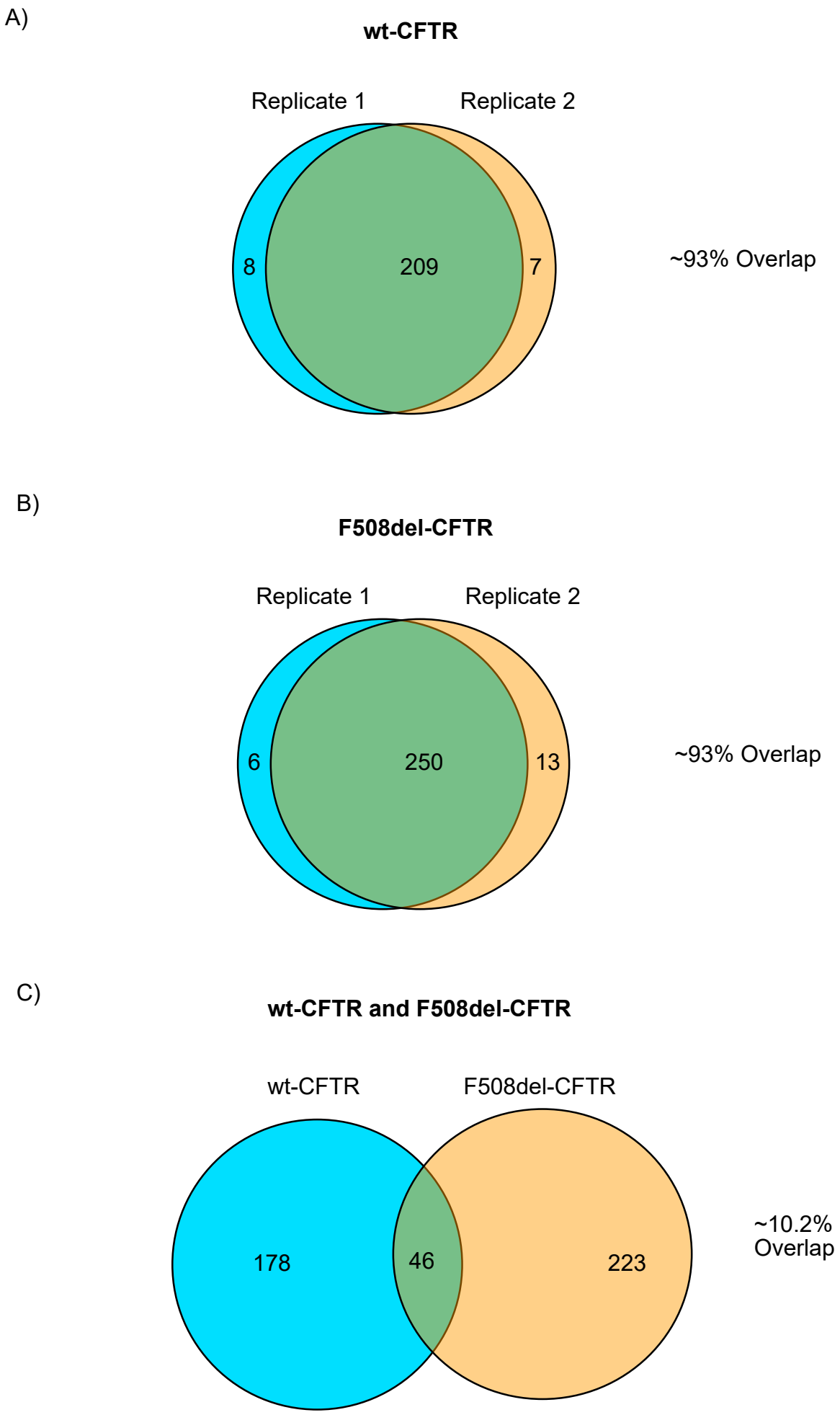
Appendix Figure S1– Overview of the MaMTH system. Integral membrane 'bait', fused to the C-terminus of ubiquitin (Cub) and an artificial transcription factor (TF), is expressed alongside cytosolic or membrane-bound 'prey' fused to the N-terminus of ubiquitin (Nub) in cells carrying a reporter expression system. Interaction of bait and prey leads to ubiquitin reconstitution, proteolytic cleavage by deubiquitinating enzymes (DUBs) and subsequent release of the TF, which then enters the nucleus of the cell and activates reporter (luciferase in the traditional MaMTH system or eGFP in MaMTH-HTS).



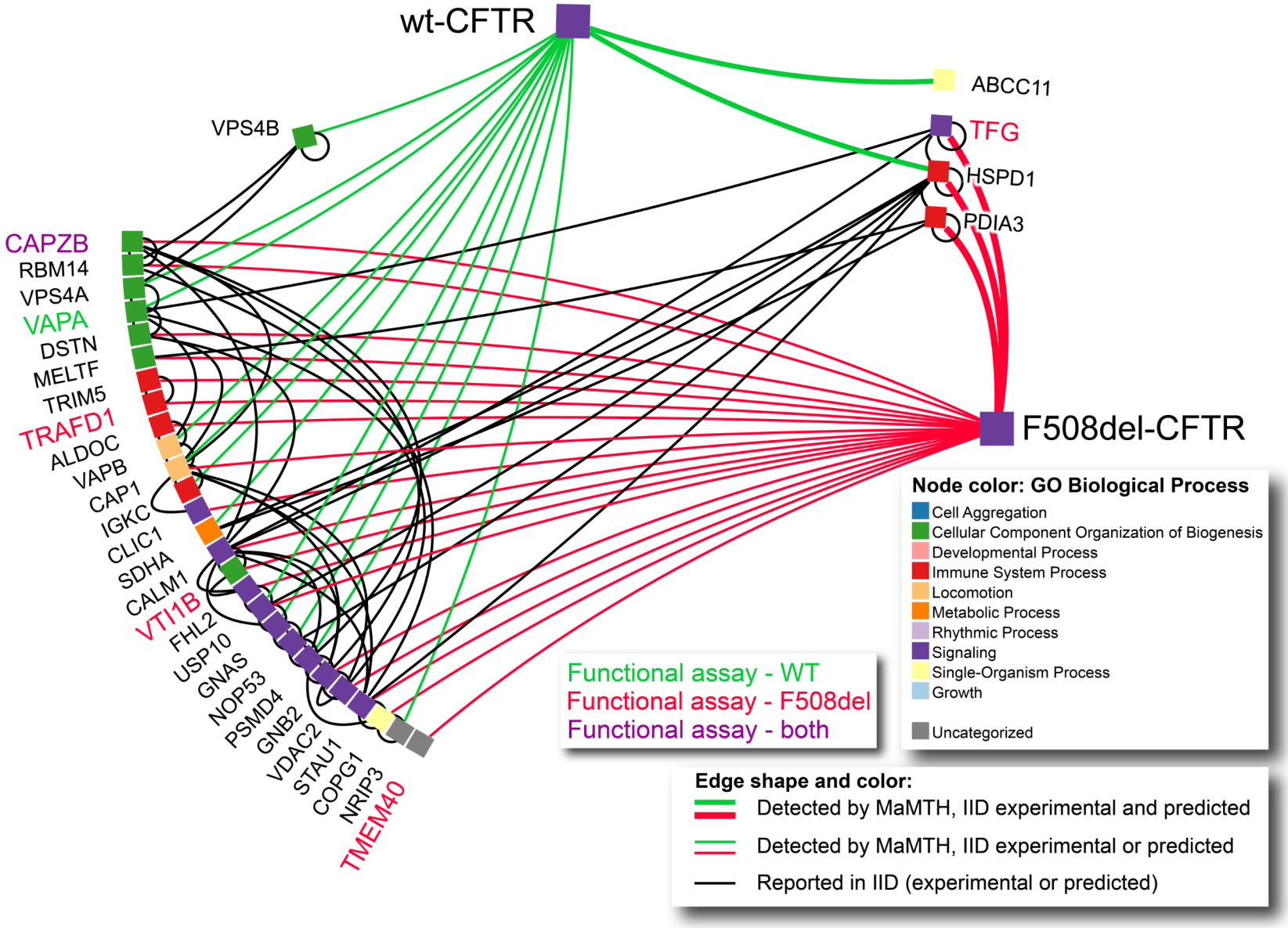
Appendix Figure S2 – MaMTH bait and prey cassettes/plasmids. A) Schematic overview of MaMTH bait cassette and other major plasmid features. B) Schematic overview of MaMTH prey cassette and other major plasmid features.



Appendix Figure S3 – Live HEK293 cell fluorescence microscopy of transiently expressed wt- and F508-del CFTR proteins fused to MaMTH bait tag (C-terminus of ubiquitin and artificial transcription factor) and GFP showing that expected localization is unaffected. Plasma membrane (PM Marker) was stained using CellMask (Invitrogen). Endoplasmic reticulum (ER marker) was stained using ER-Tracker (Invitrogen).

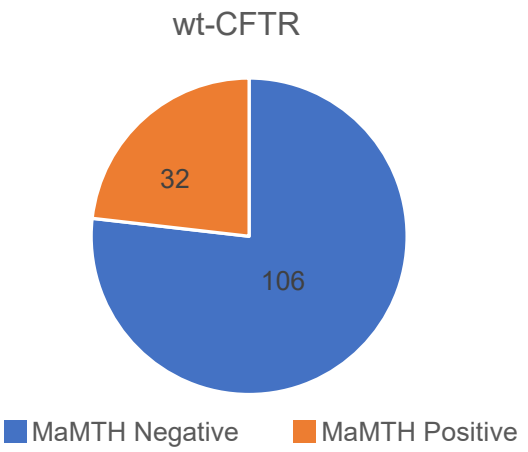


Appendix Figure S4 – Graphical summary of shared and differential interaction numbers across technical replicates and WT vs. F508del. A) Comparison of wt-CFTR interactions across technical replicates 1 and 2. B) Comparison of F508del-CFTR interactions across technical replicates 1 and 2. C) Comparison of interactions between wt-CFTR and F508del-CFTR.

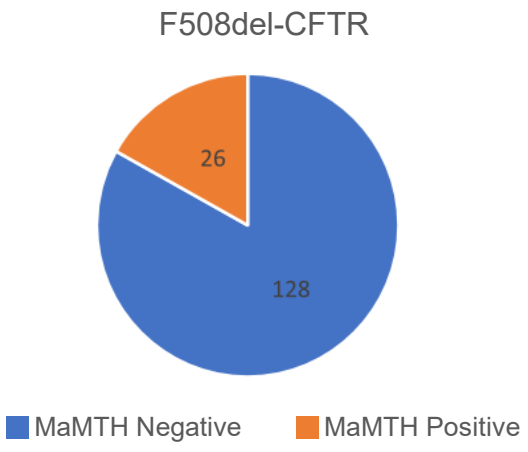


Appendix Figure S5 – Annotated MaMTH-HTS interactome for wt- and F508del- CFTR overlapping with known experimental and predicted interactions from IID v 2021-05. Edge color signifies source of interactions (wt, del or IID), and thickness shows type of overlap with known interactions, according to legend. Node color represents Gene Ontology Biological process. Node label colouring highlights interactors functionally validated in this work.

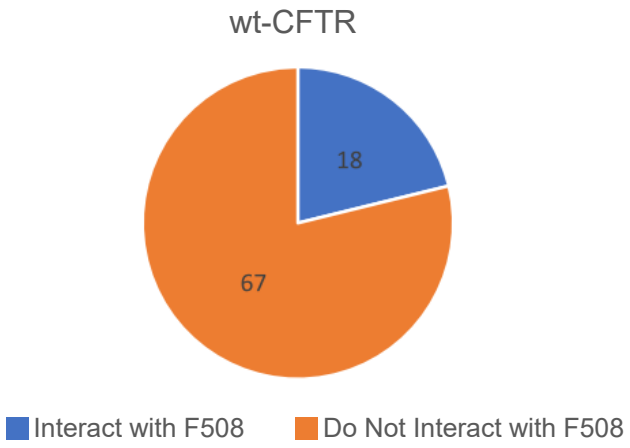
A)



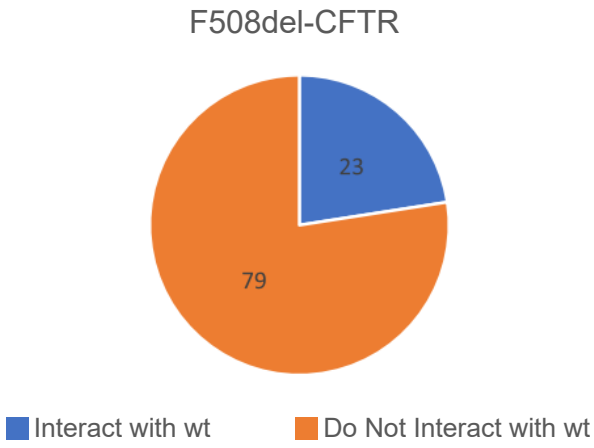
B)



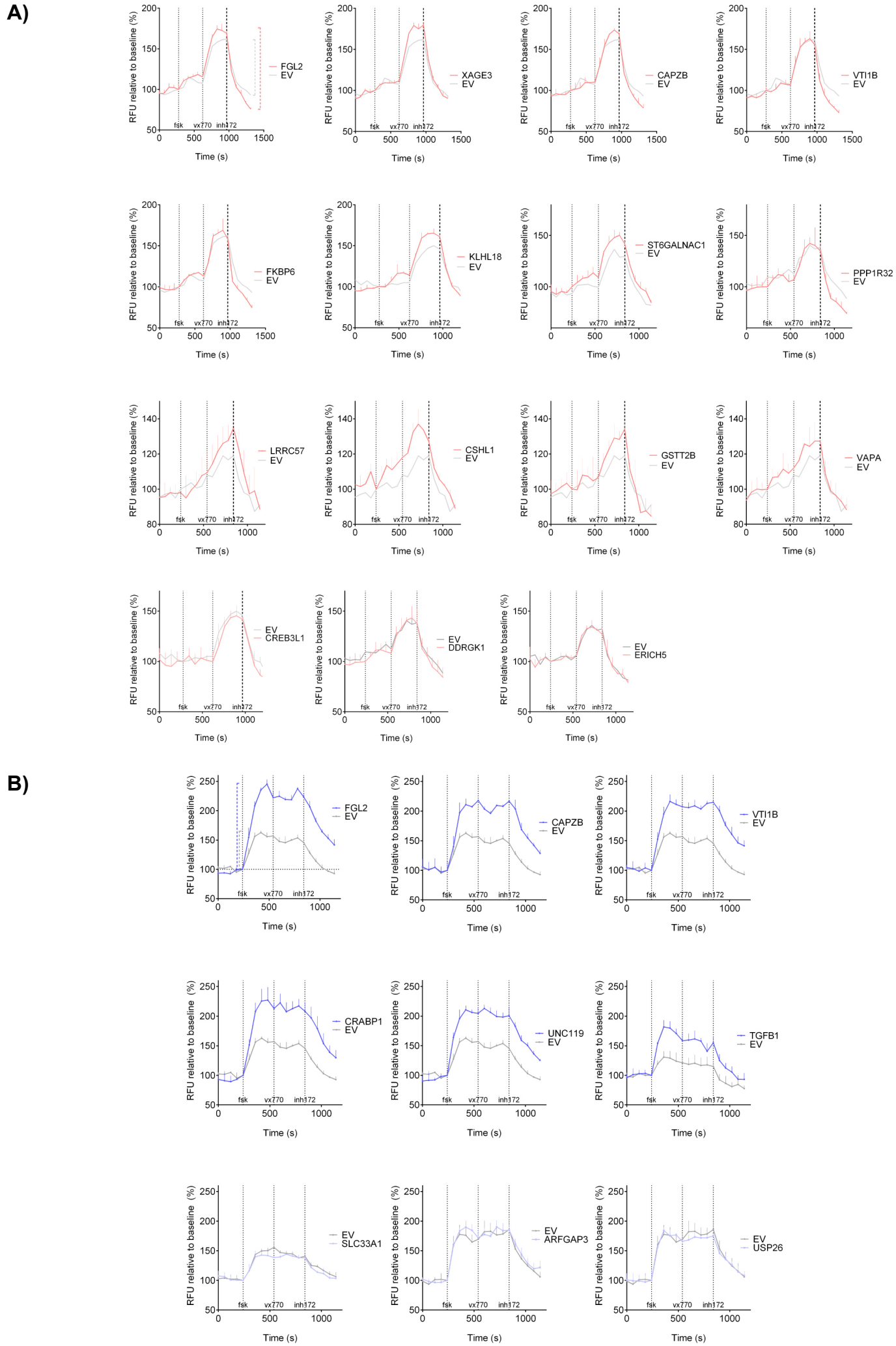
C)



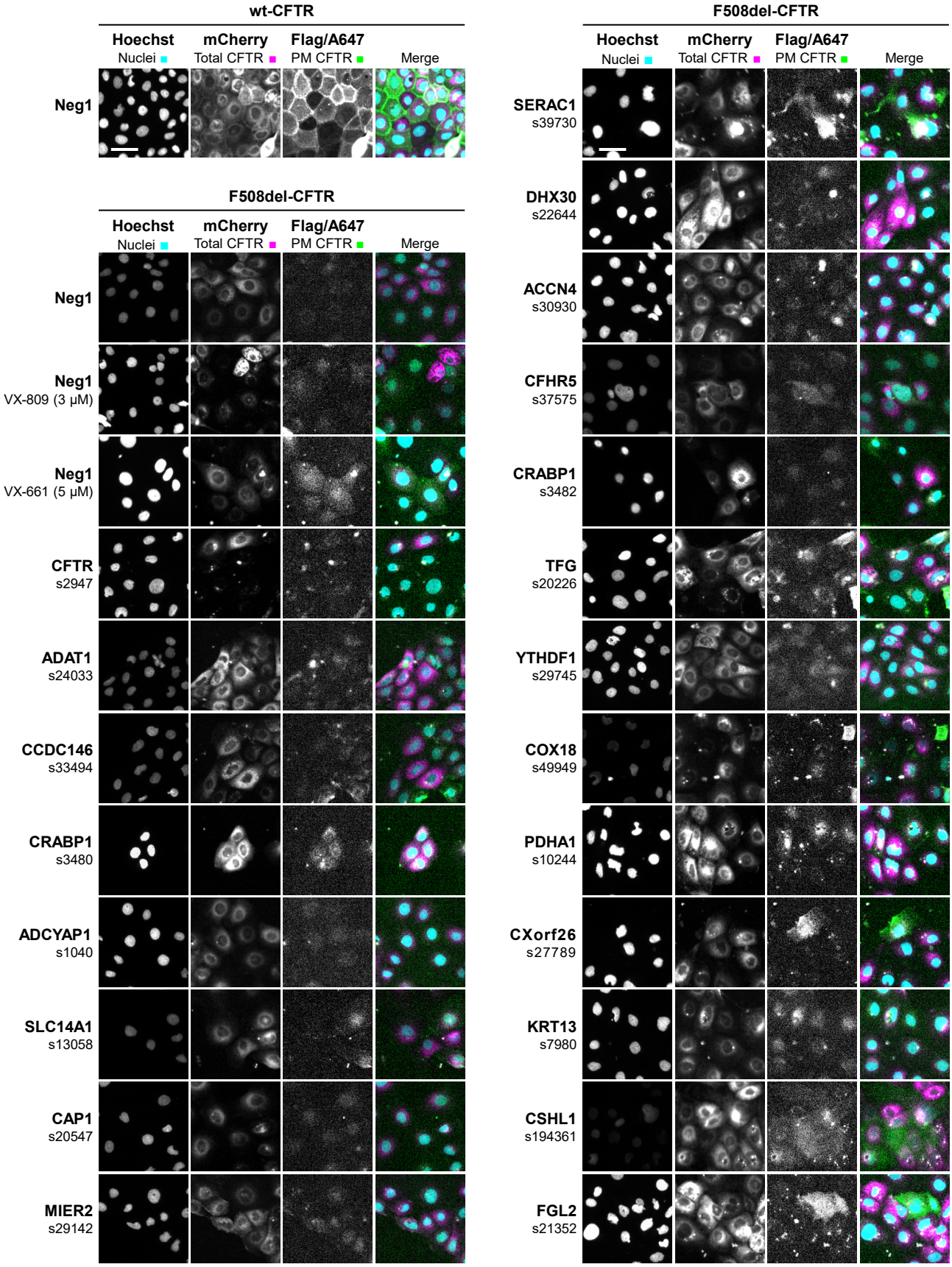
D)



Appendix Figure S6 – Summary of MaMTH luciferase analysis of selected interactors identified in MaMTH-HTS. A) Proportion of wt-CFTR interactors identified in MaMTH-HTS producing significant signal in traditional MaMTH. B) Proportion of F508del-CFTR interactors identified in MaMTH-HTS producing significant signal in traditional MaMTH. C) Proportion of wt-CFTR interactors identified in MaMTH-HTS that interact with F508del-CFTR in traditional MaMTH. D) Proportion of F508del-CFTR interactors identified in MaMTH-HTS that interact with wt-CFTR in traditional MaMTH.



Appendix Figure S7 – FLIPR membrane potential traces for interactors whose transient expression in HEK293 cells affects CFTR channel activity. A) Interactors affecting F508del-CFTR channel activity. B) Interactors affecting wt-CFTR channel activity. Three select representative traces for interactors whose expression did not affect channel activity are also shown for each.



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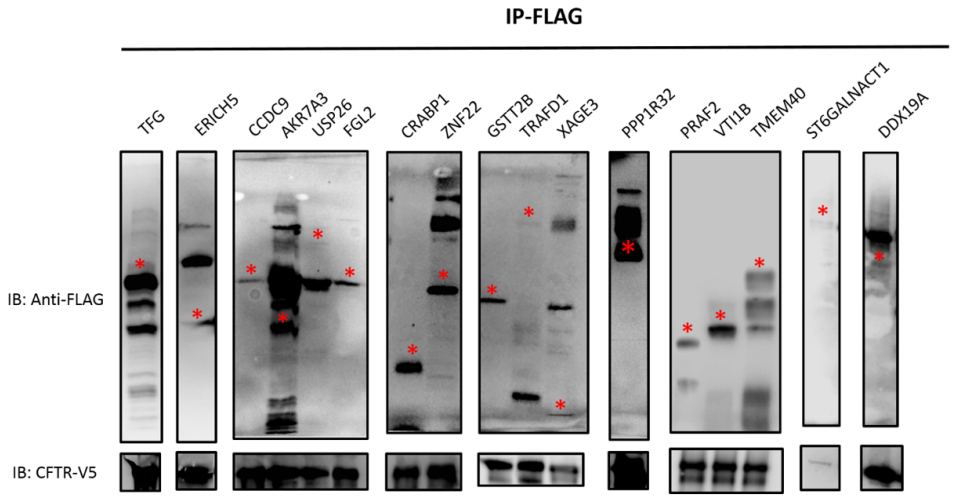
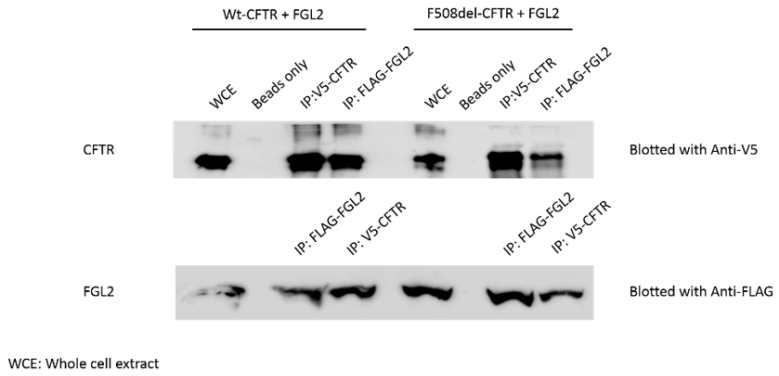
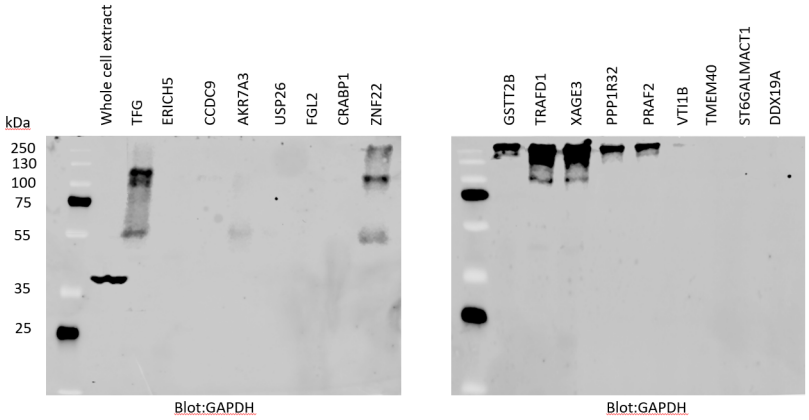


B)



C)



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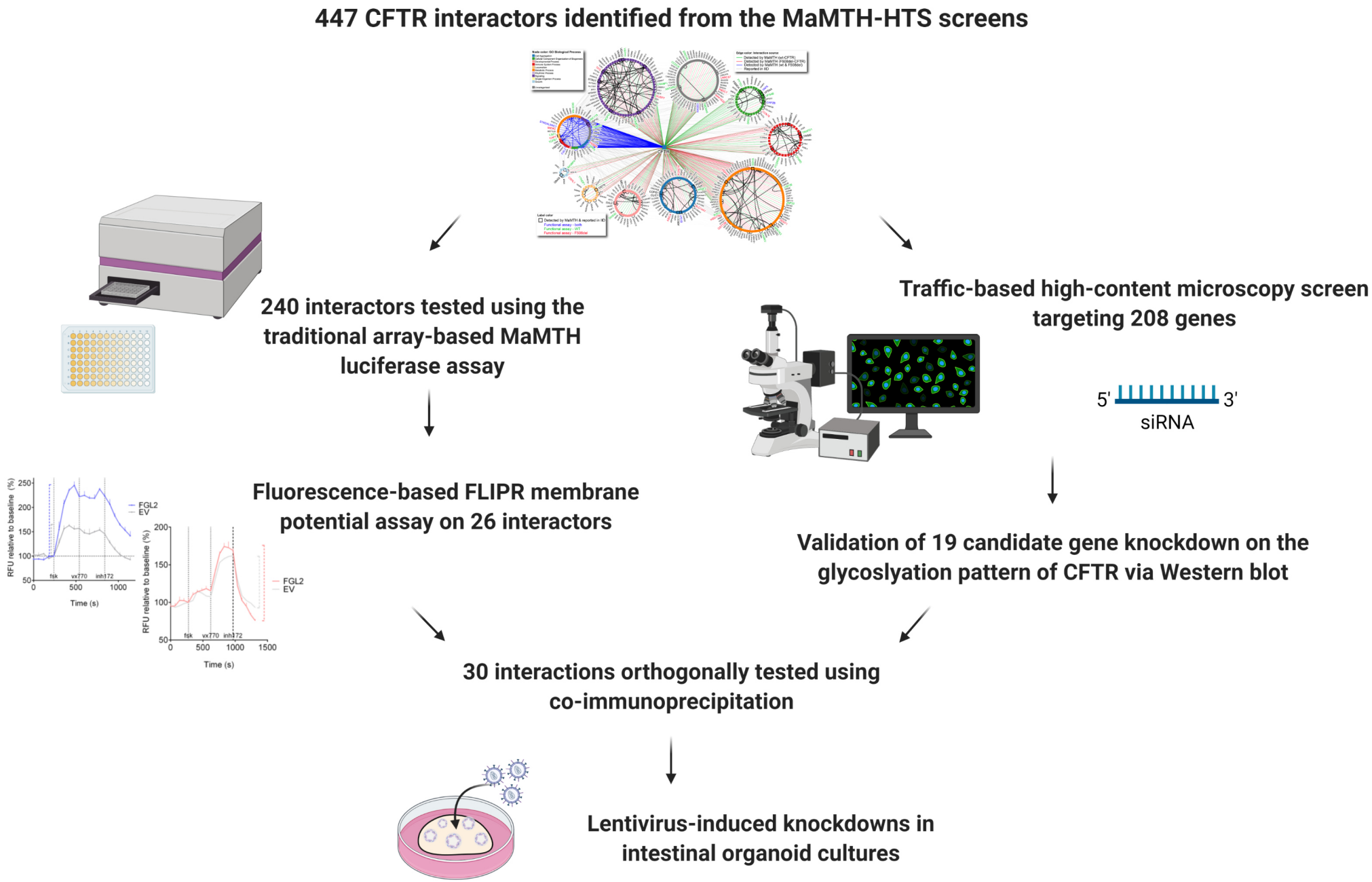

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