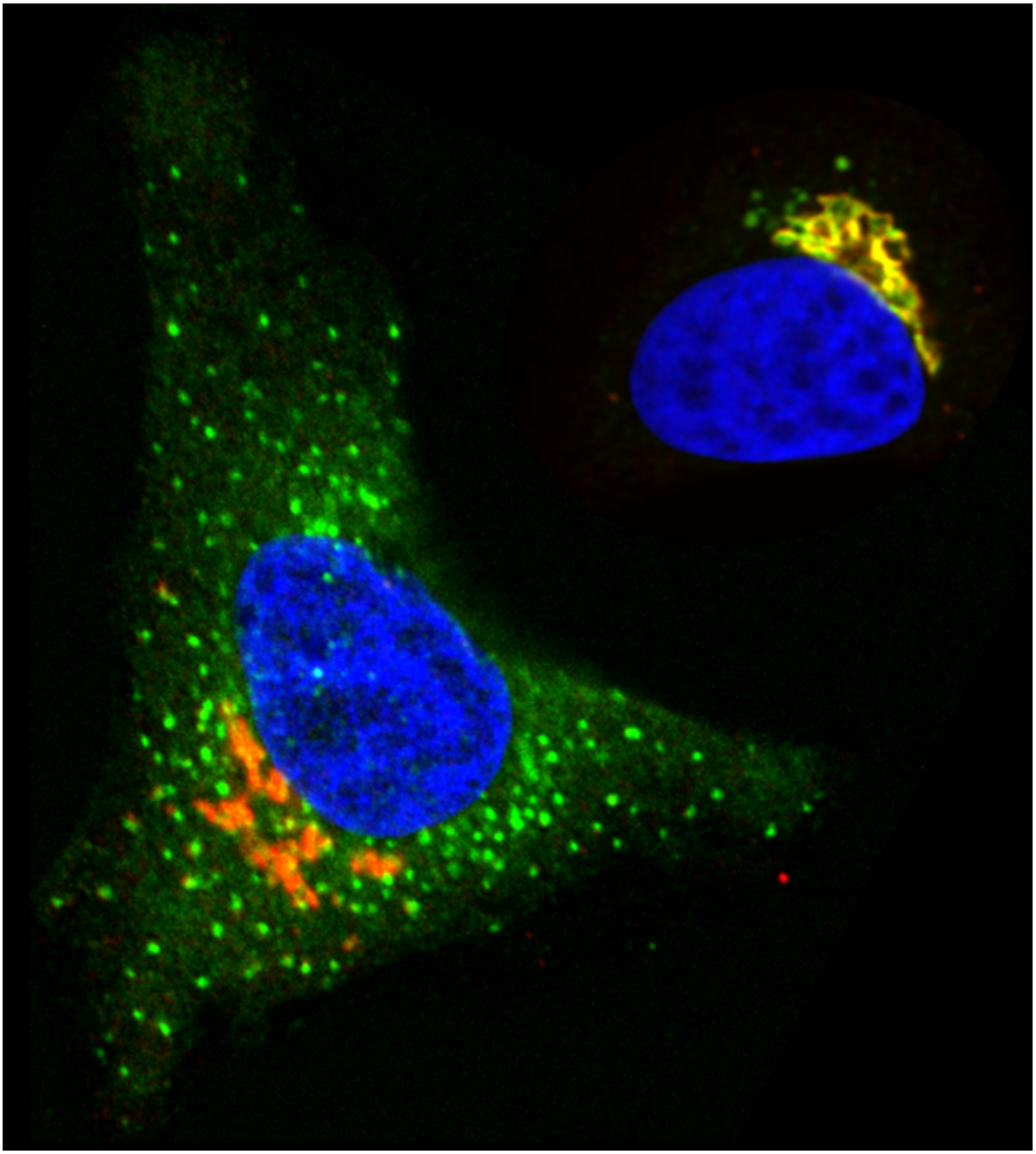
Research

Lab projects:

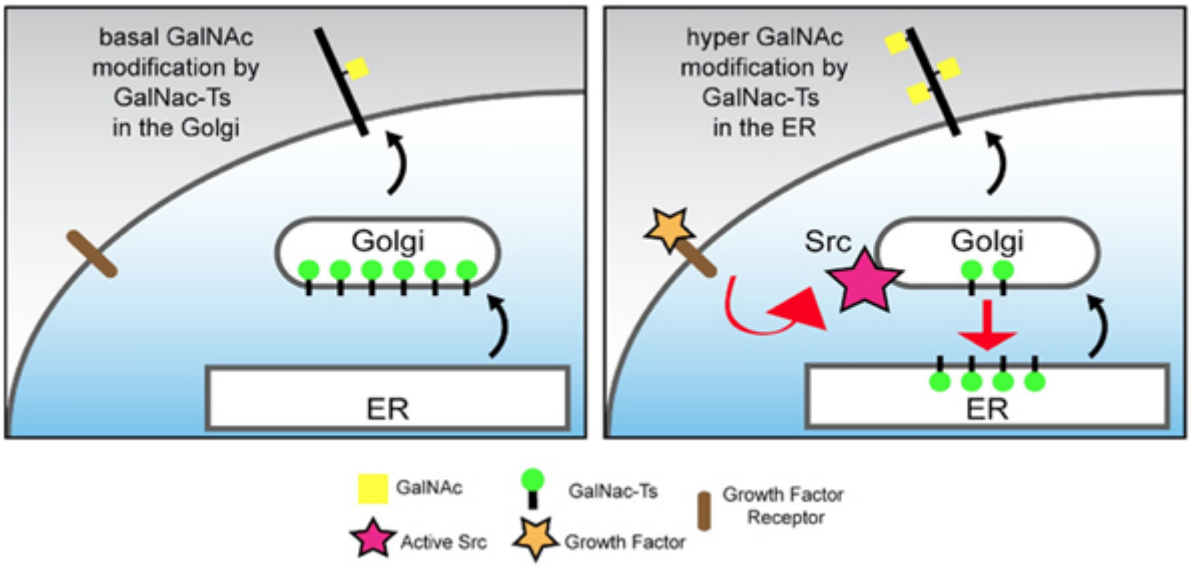
1) Regulation of glycosylation through membrane traffic

The Golgi apparatus in human cells is an example of extensive compartmentalization, with multiple compartments- the cisternae- stacked together and multiple stacks connected together into a network. This complex structure is responsible for the synthesis of the various glycans presented at the cell surface.

In a **published report,** we have described how the organization of the Golgi apparatus can be altered to enhance the O-GalNac glycosylation of proteins upon activation of the known proto-oncogene Src. Interestingly, enhanced O-GalNac glycosylation is a known hallmark of cancerous transformation, with the up-regulation of the Tn antigen. We are now exploring the physiological significance of this O-glycosylation up-regulation.



Activation of Src at the Golgi apparatus promotes redistribution of GalNAc-Ts to the ER. HPL staining (marking the subcellular localization of GalNAc-Ts activity) is redistributed from the Golgi to the ER upon activation of endogenous Src in HeLa cells through treatment with EGF (100 ng/ml) for 4 h. After EGF stimulation, HPL staining revealed that GalNAc-Ts are active both in the ER (from the apparition of novel diffuse HPL-stained structures that co-localize with ER markers) and the ER-Golgi intermediate compartment (ERGIC) (from the apparition of novel punctate HPL-stained structures that co-localize with ERGIC markers). Golgi membranes are stained using giantin antibodies. Nuclei are stained blue with Hoescht. The scale bar represents 10mm.

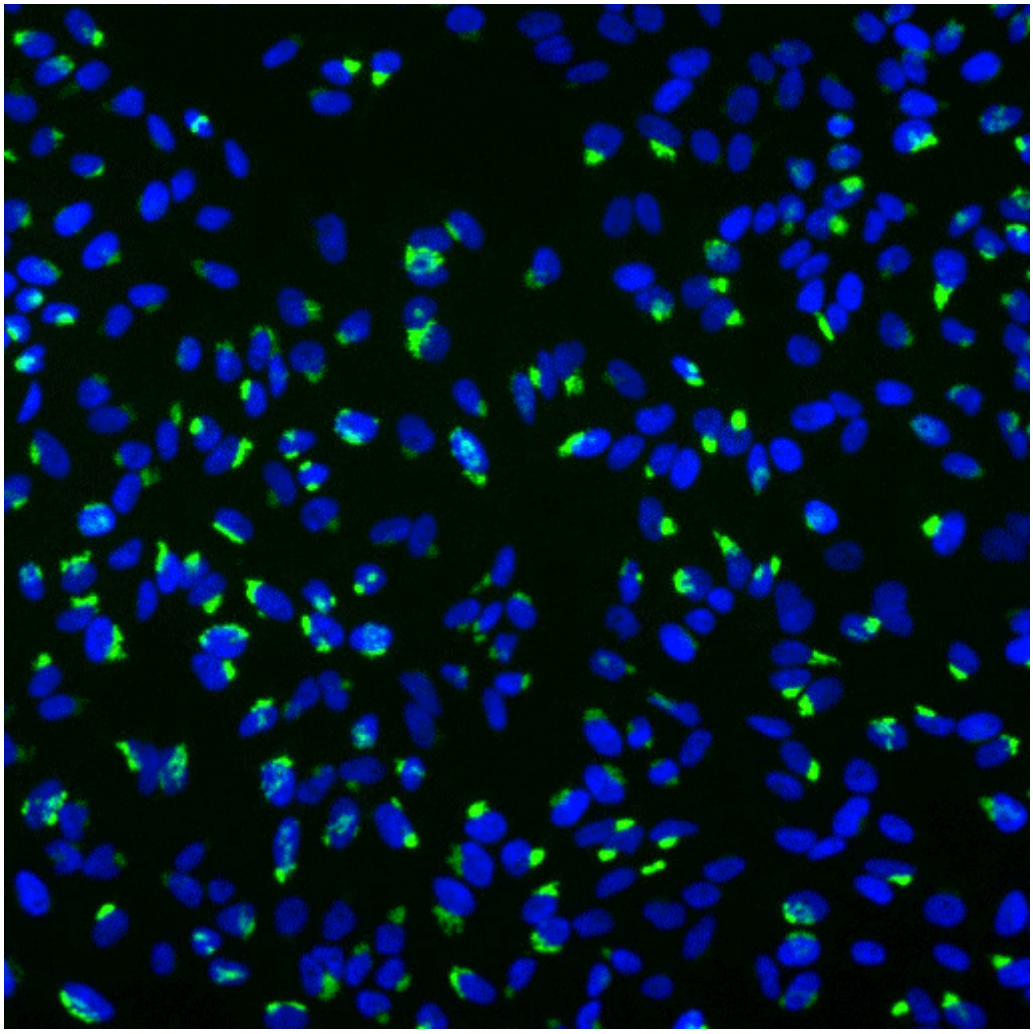


Src activation results in increased O-glycosylation initiation.

A model for Src-induced Golgi-to-ER retrograde trafficking of GalNac-T enzymes. Under normal cellular conditions, GalNac-Ts are predominantly localized to the cis-face of the Golgi apparatus and initiation of O-glycosylation occurs in the Golgi apparatus. Upon activation of Src, GalNac-Ts are selectively trafficked to the ER. This increases the efficiency of O-glycosylation initiation and results in a greater density of GalNAc added onto mucin-like proteins (further O-glycan modifications are not included for sake of clarity).

To test if other mechanisms are also regulating Golgi organization, we have undertaken, in another project, a large-scale RNAi screen to understand the genetic basis of Golgi organization.

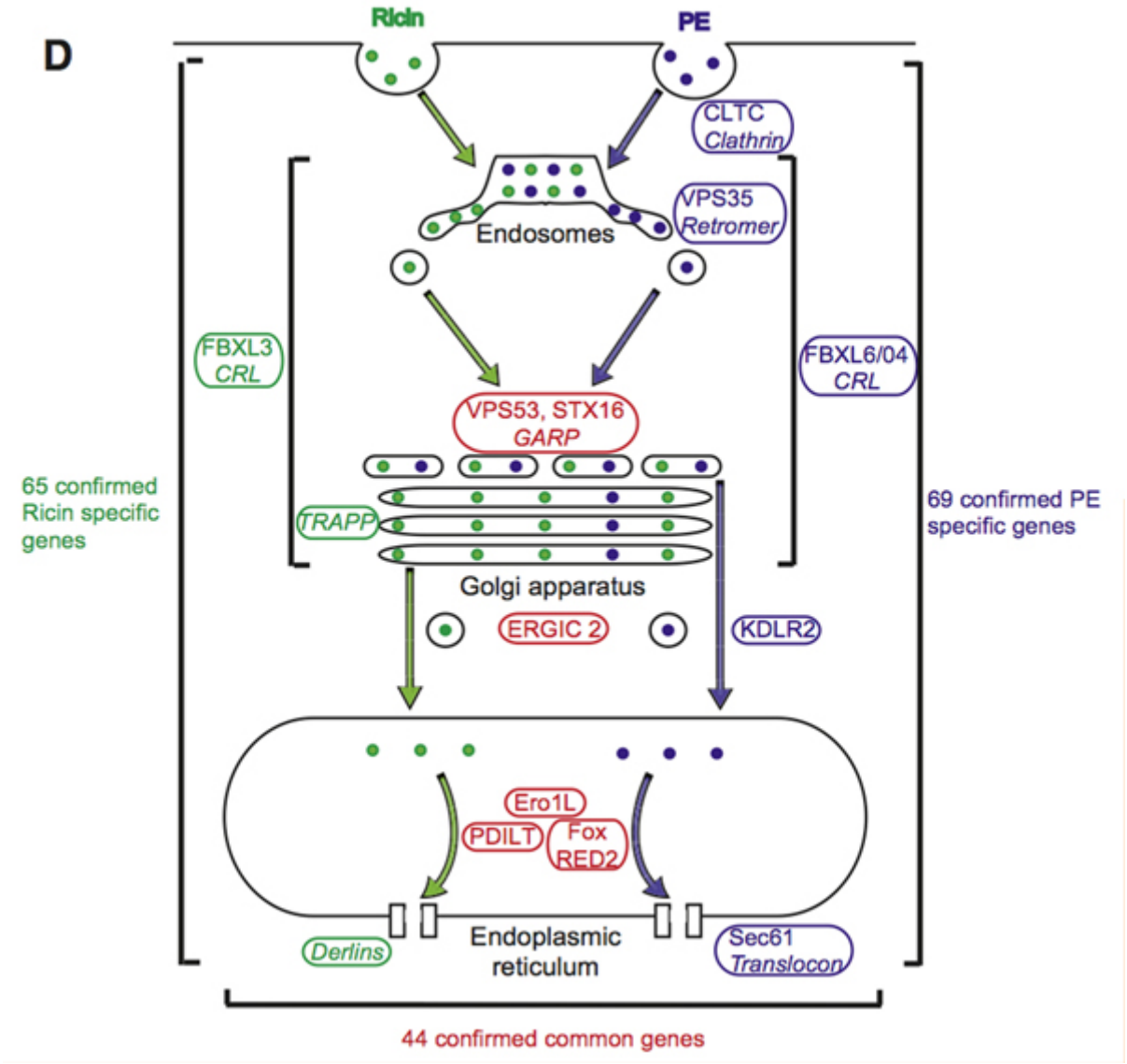
For this, we have developed an automated analysis of Golgi morphological parameters.



We next used large-scale RNAi screening using the IMCB RNAi screening facility, which is embedded within the lab.

2) Regulation of retrograde membrane traffic and hijacking by toxins

In the secretory pathway, most proteins are synthesized in the endoplasmic reticulum (ER), then transported in an anterograde fashion to the Golgi and onwards to the plasma membrane, the endosomes or the extracellular space. By contrast, few proteins follow the opposite route: after endocytosis, they percolate through endosomes, reach the Golgi apparatus then move on to the ER. Various clinically important proteins, such as the Ricin, Cholera, Shiga and Pseudomonas toxins follow this retrograde trafficking pathway. In a **published report**, we have characterized the human genes required for intoxication by two toxins, Ricin and Pseudomonas Exotoxin. Most of these genes are likely to be implicated in the intracellular retrograde traffic. Their discovery offers many new insights into the mechanisms of retrograde membrane traffic.



Hypothetical model depicting how PE and Ricin travel from extracellular space to the ER with some identified genes highlighted