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ULisboa** Faculdade
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Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway

Simpson *et al.*, 2012
Nature Cell Biology

Andreia Henriques

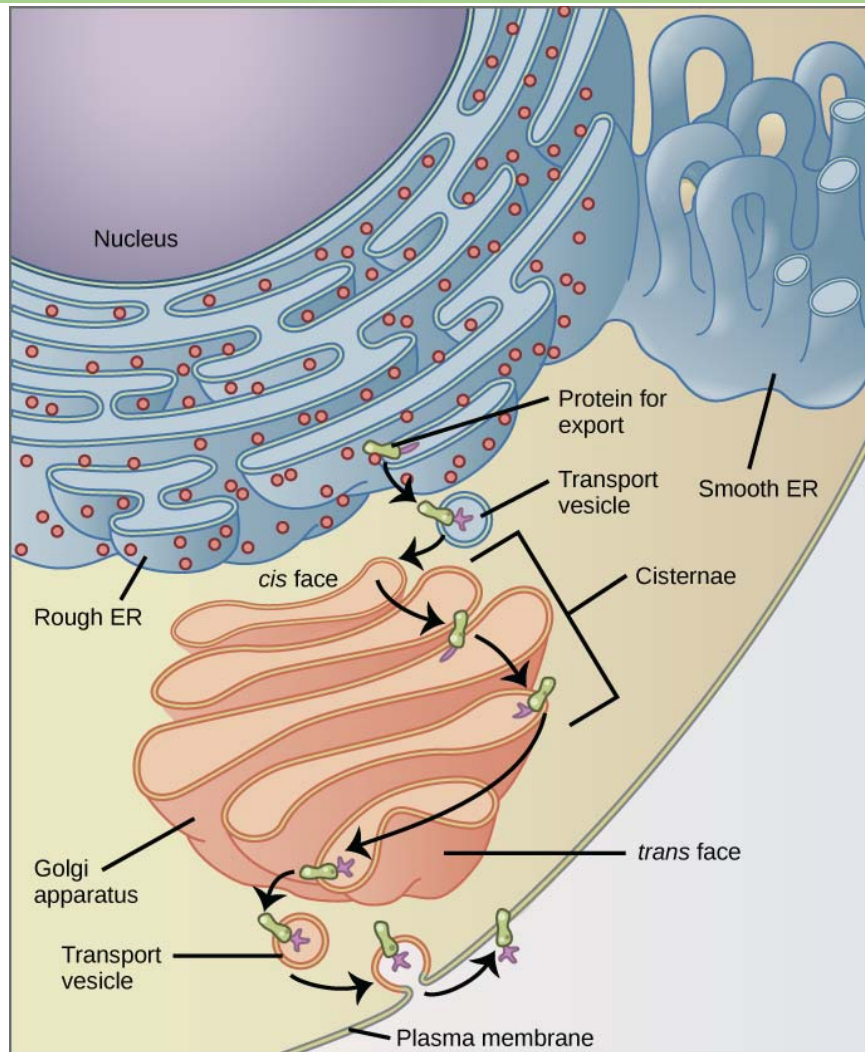
Nuno Domingues

Rute Teixeira



Lisbon, 2015

Secretory pathway



Important to maintain cell homeostasis

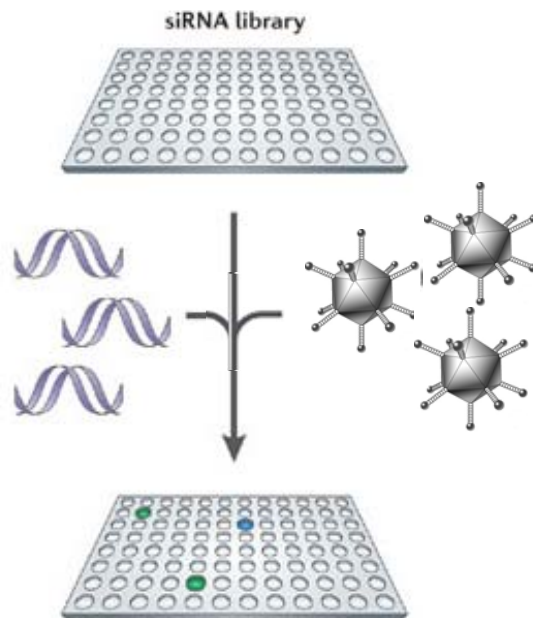
The paradigm of secretory pathway:

Ensures that newly synthesized material originating at the **endoplasmic reticulum (ER)** is correctly modified as it passes through the **Golgi** complex and ultimately out to the **cell surface**.

RNA interference(RNAi)-mediated gene knockdown using a human cell system

tsO45G and Assay design

Cargo protein tsO45G a **temperature sensitive viral transmembrane protein** used as a model in studies of membrane trafficking in the secretory pathway



Preparation of siRNA-spotted chambers
(22,627 genes/51,758 siRNAs/149 chambers)



Plate HeLa Kyoto cells—reverse transfection
(1.0×10^6 cells per chamber) 33 h at 37 °C



Infect with recombinant adenoviruses
(express CFP-tsO45G) 16 h at 39.5 °C

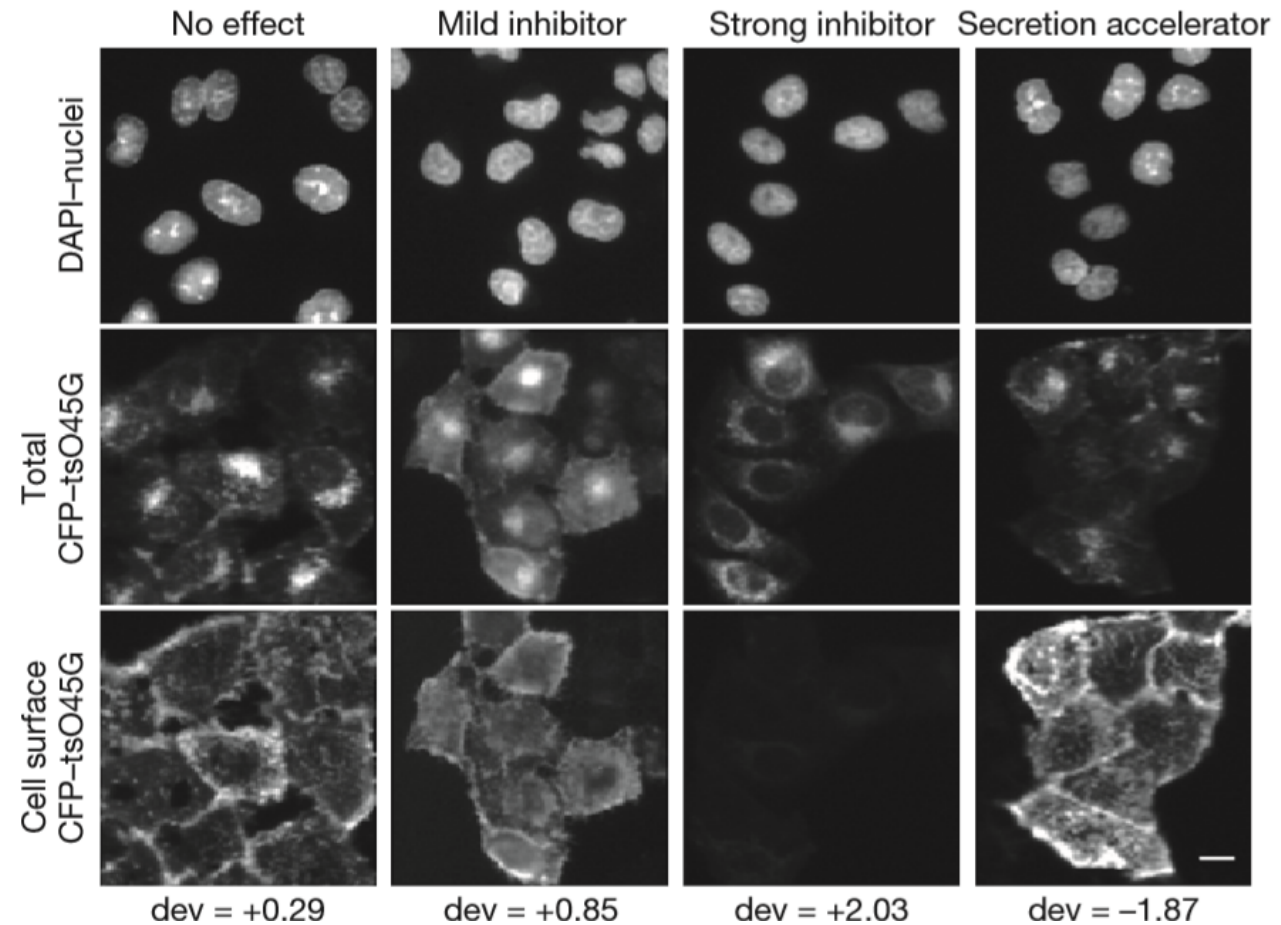
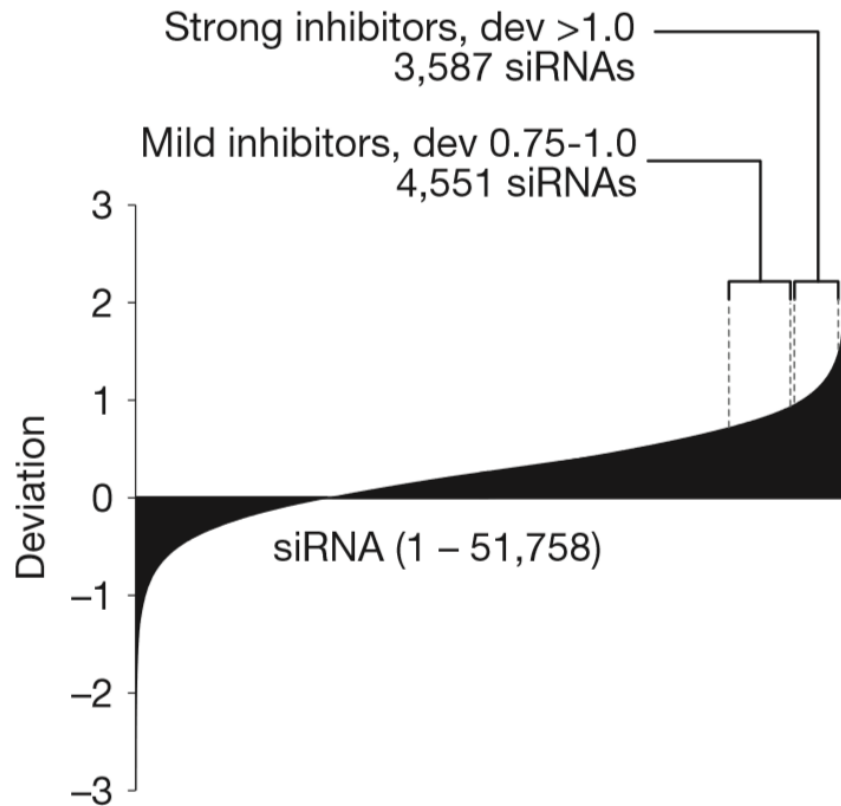


Release tsO45G from ER
1 h at 32 °C

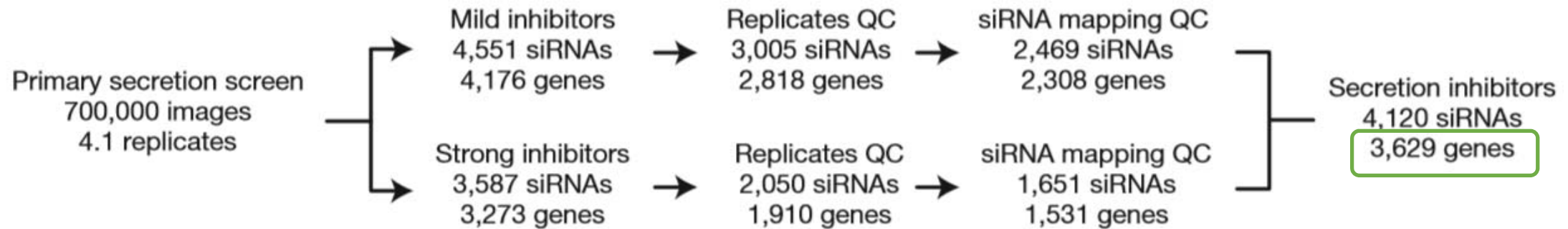


Fix cells, immunostain, image acquisition
(stain cell surface tsO45G, DAPI-stain nuclei)

Genome-wide cell-based secretion screening



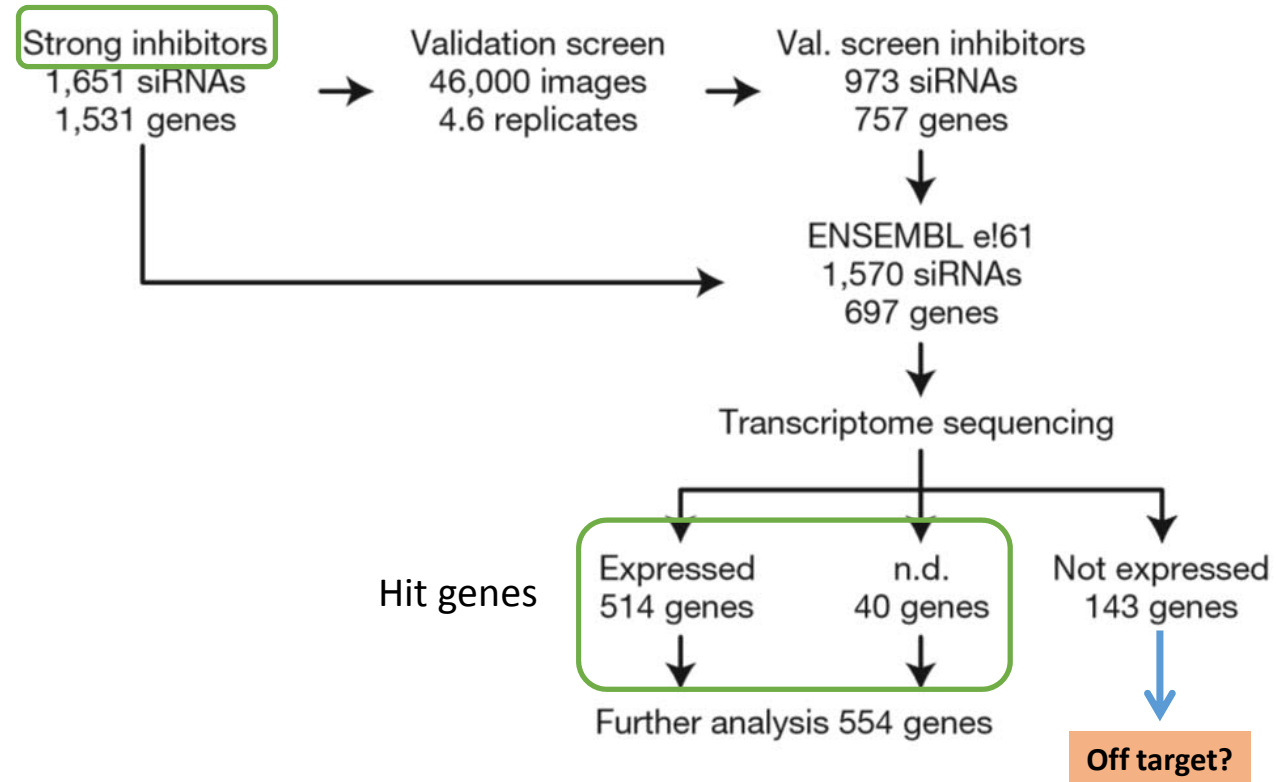
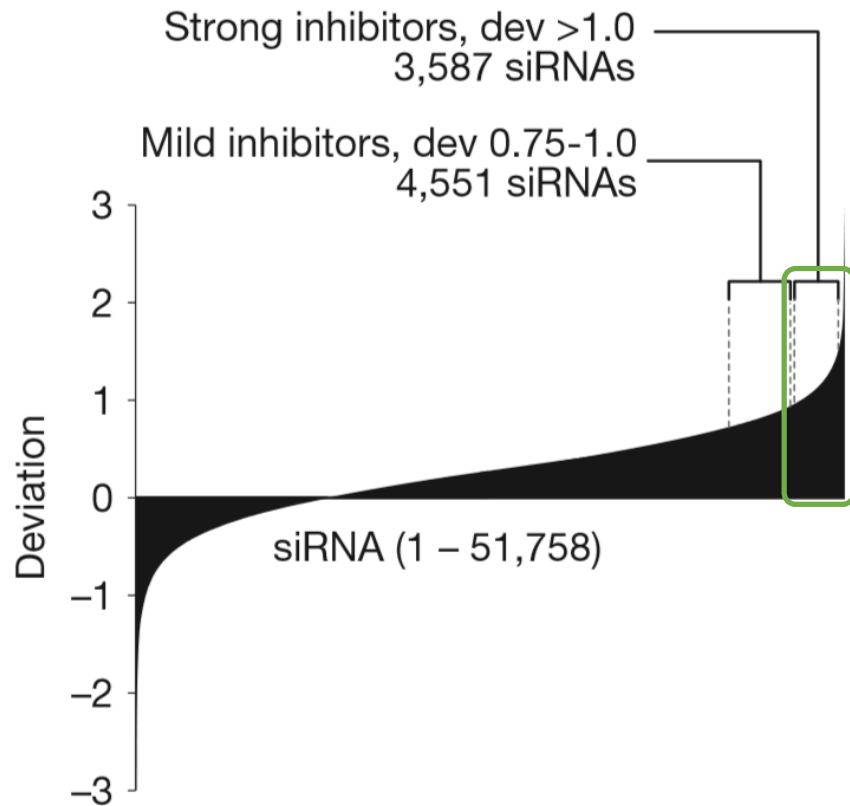
Screen preliminary results



Results pipeline - From 22,627 genes a total of 3,629 were found to inhibit secretion on knockdown

Validation screens

HeLa cells



Known membrane traffic machinery

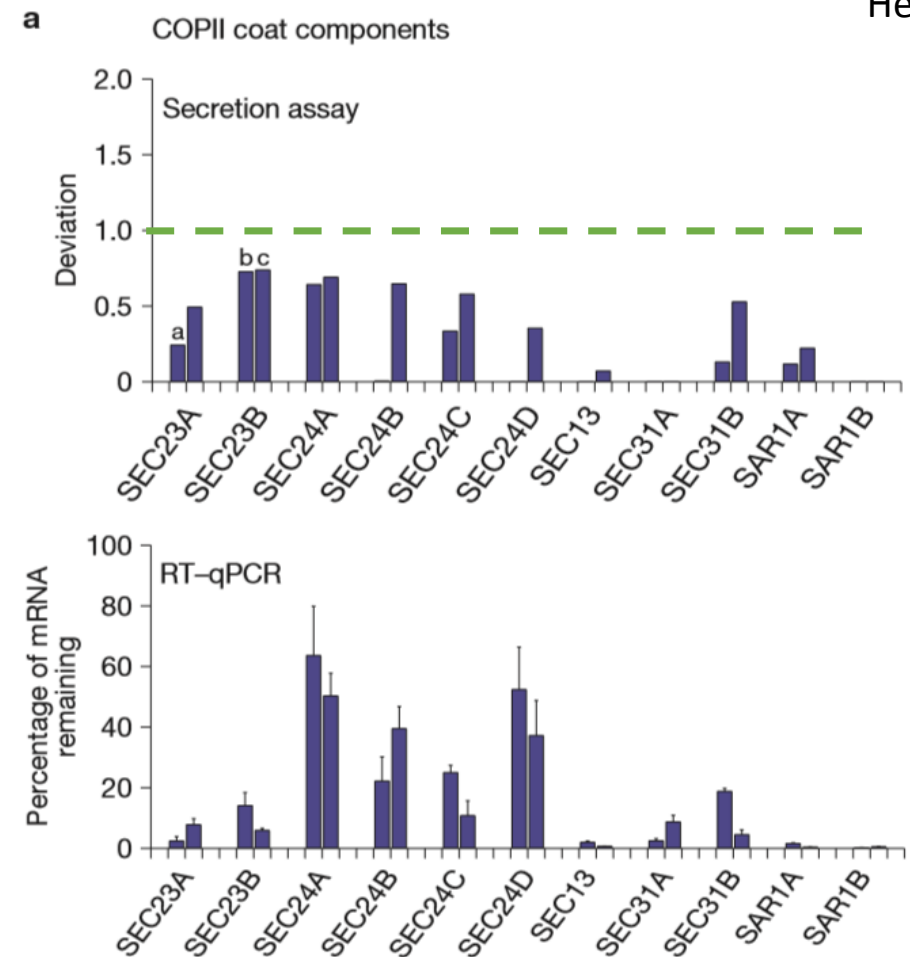
Cytoplasmic coat protein complexes – COPI and COPII coordinate the trafficking process, Identify cargo, reshape the membrane and provide links to accessory proteins

COPII - Multi subunit complex, depletion of COP subunits

Deviation in ts045G protein secretion was not significant

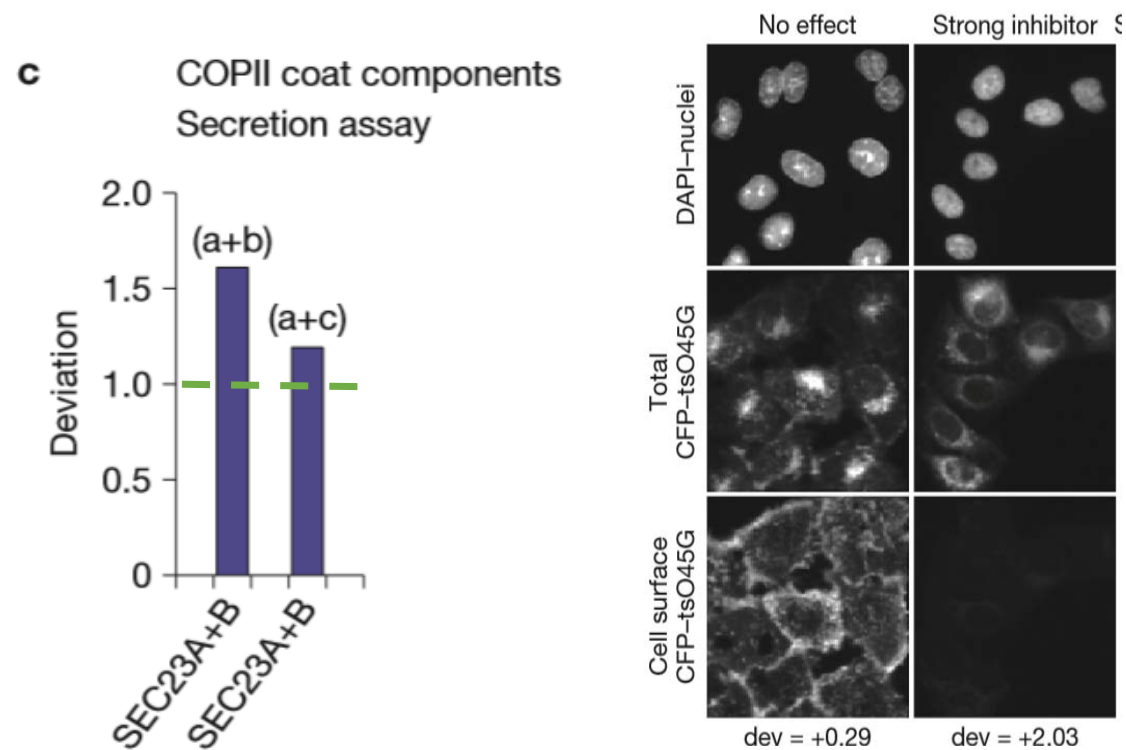
Most siRNAs depleted mRNA levels lower than 20%

HeLa cells



Combined knockdown

When combined knockdown of COPII coat subunits was preformed tsO45G protein secretion was **strongly inhibited**



High degree of redundancy ensures robustness of secretory pathway

Known membrane traffic machinery

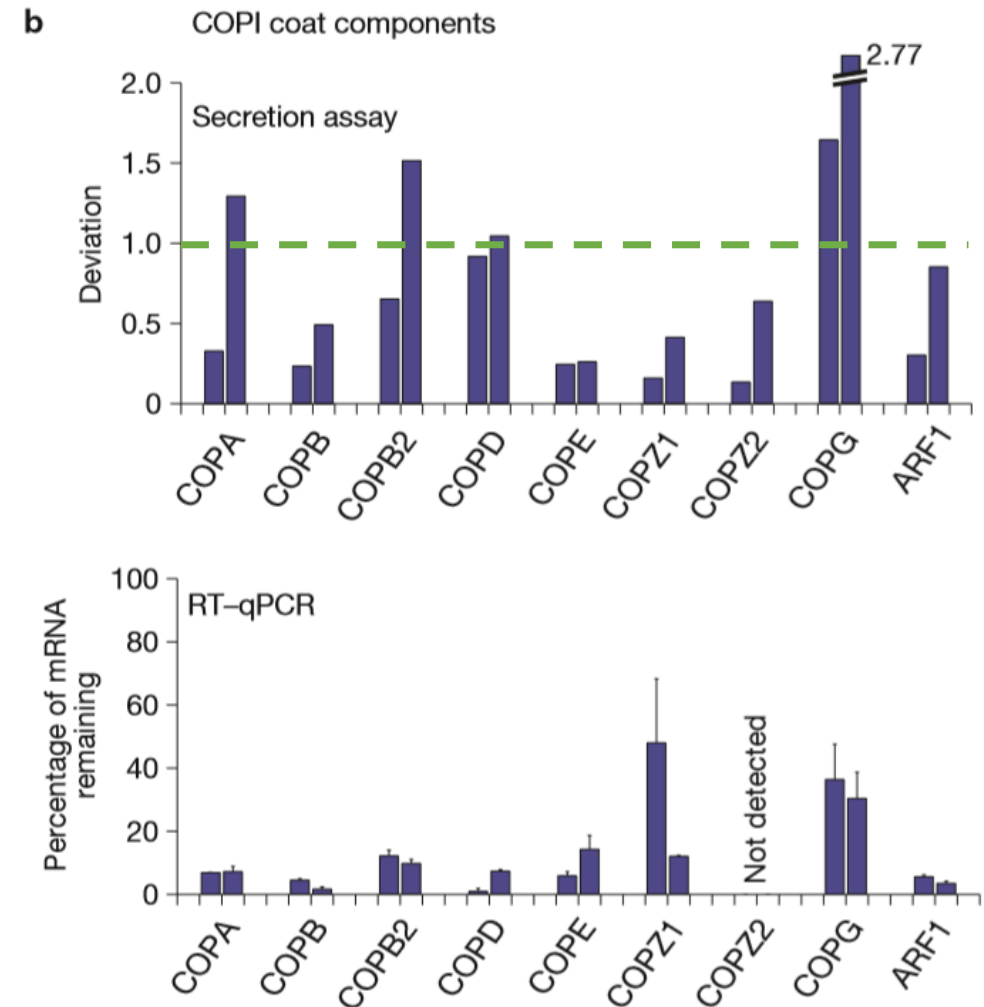
COPI – coat secretory transport carriers going to Golgi

Marked inhibition of ts045G protein secretion with COPI subunit depletion



Isoforms exist in only two of the COPI subunits

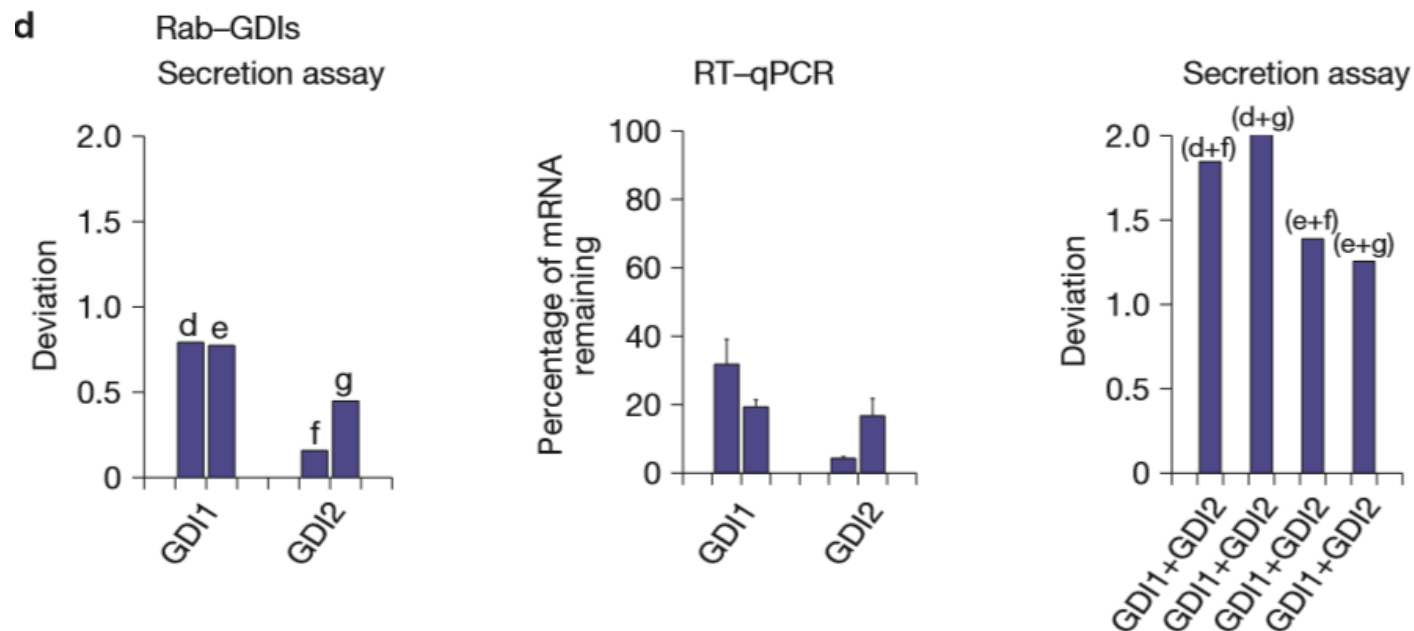
All conventional secretory cargo must pass through the Golgi complex



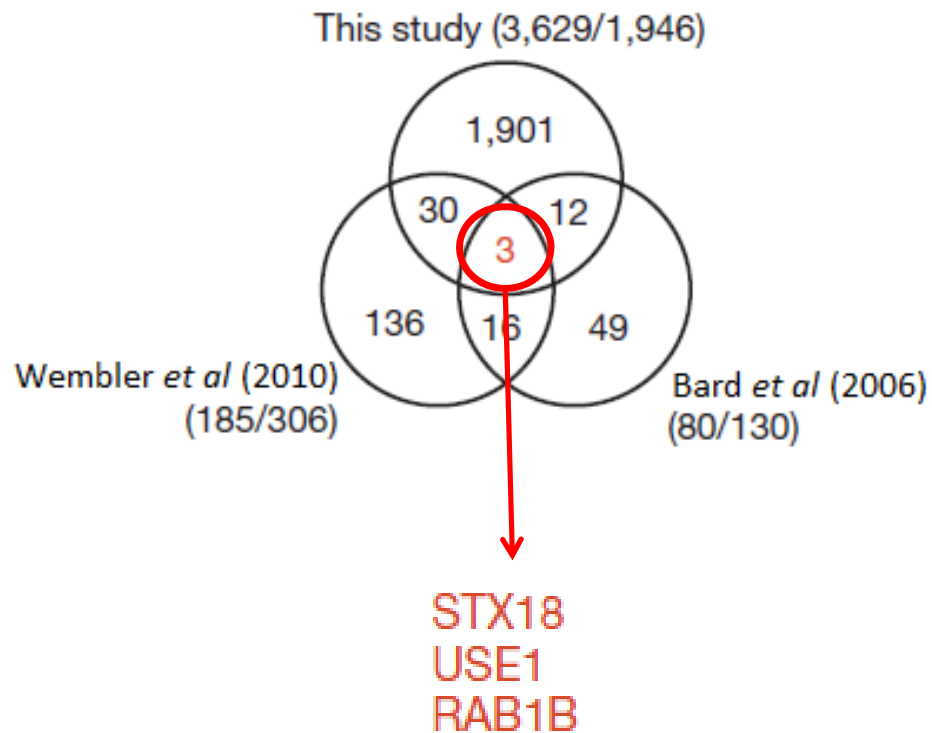
Known membrane traffic machinery

ARF and Rab families of small GTPases also regulate cellular trafficking.
Some elements from these 3 families were found as strong or mild inhibitors.

We need to access the Rab-GDI importance to secretory process
is believed to play a key role
ensure that Rabs are correctly placed at the membrane in the right time



Comparison with other cellular processes



Loss-of-function secretory transport screens

Drosophila S2 cells

- Soluble vs transmembrane cargoes
- ≠ knockdown time

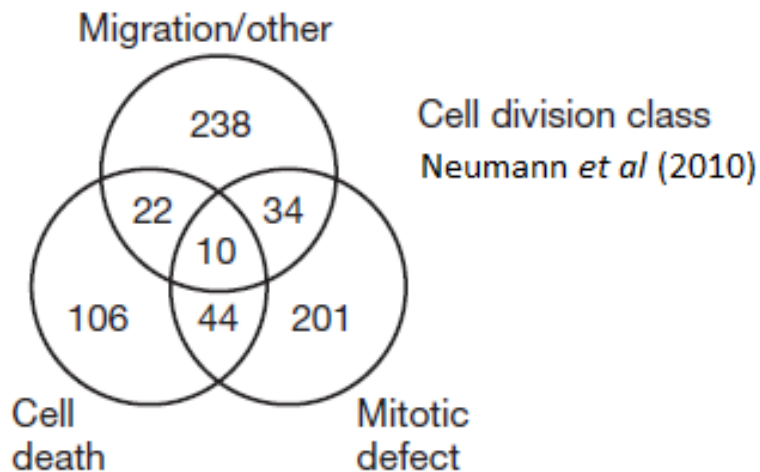


Little overlap

Consistent with Uemura *et al.* (2009):

- USE1 essential for ER morphology
- Interaction with STX18

Comparison with other cellular processes



Live-cell screen for human genes in cell death, mitotic defects and motility

18% (655 genes) overlap

Genes involved in fundamental cellular processes



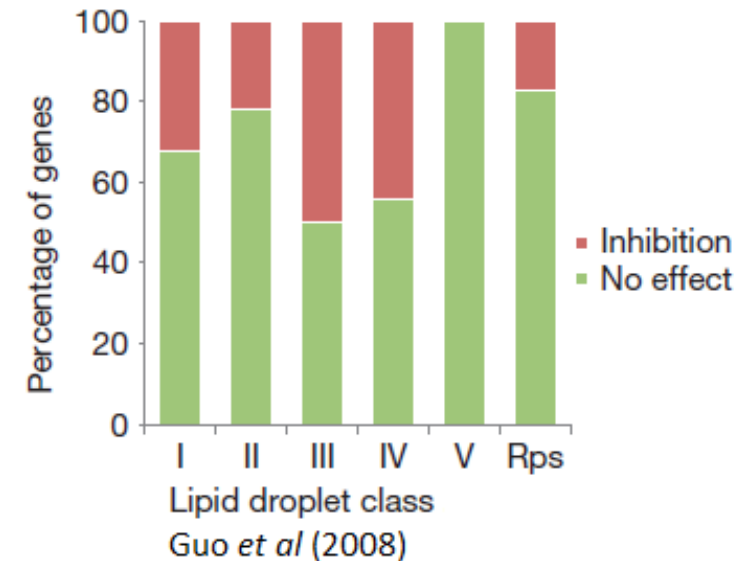
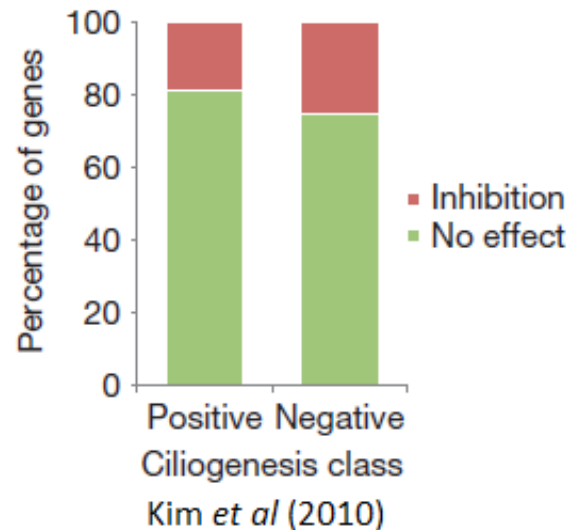
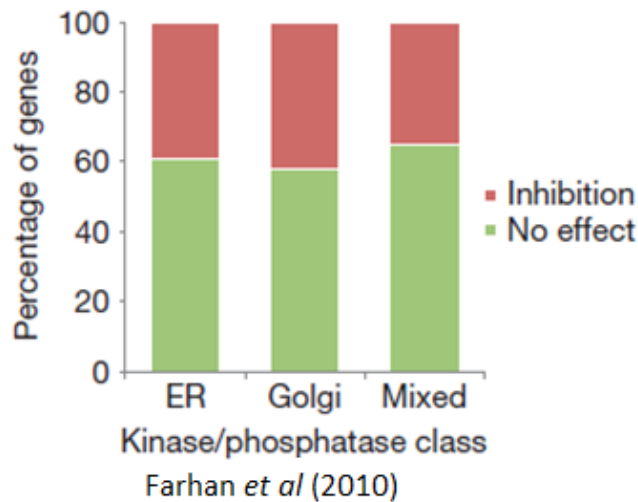
KD causes cell death

KD of membrane traffic regulators caused cell death



Importance of secretory pathway!

Comparison with other cellular processes



Kinase and phosphatases i

39% (48 genes) overlap!



Anterograde vs bi-direction

Ciliogenesis is dependent on membr

21% overlap overall

Small GTPases associated with mem
general and ciliary

Drosophila S2 screen

Link with ER in yeast

22% (40 genes) overlap, phenotype class dependent

Class III contains COPI coat complex components.

Subcellular localization

1531 genes (strong inhibitors)



179 ORFs

GFP variant

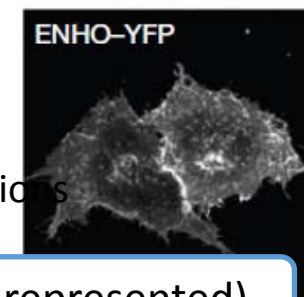
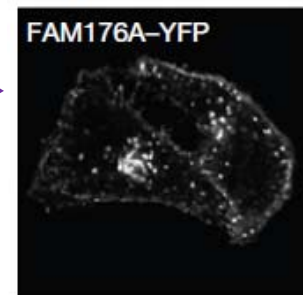
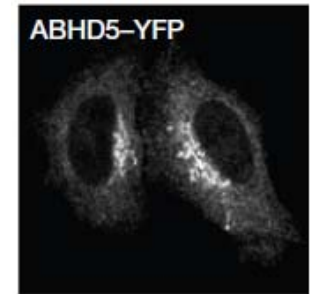
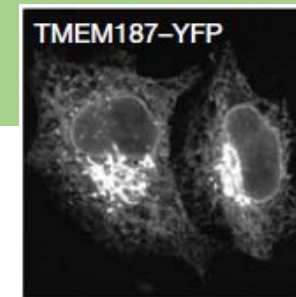
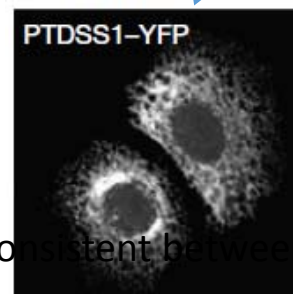
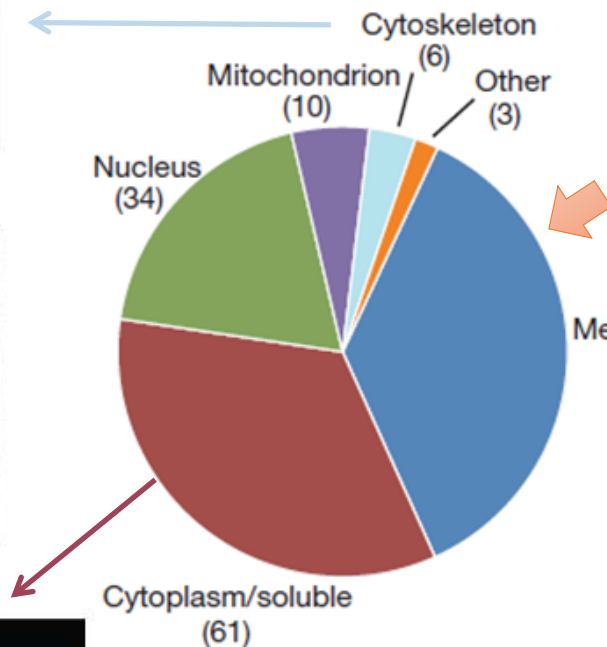
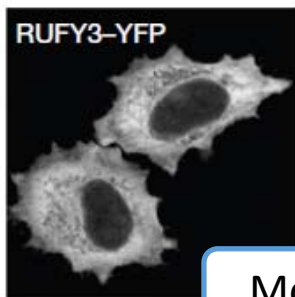
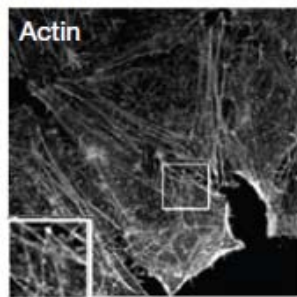
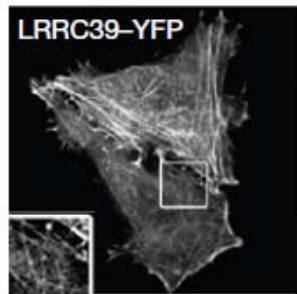
ORF



ORF

GFP variant

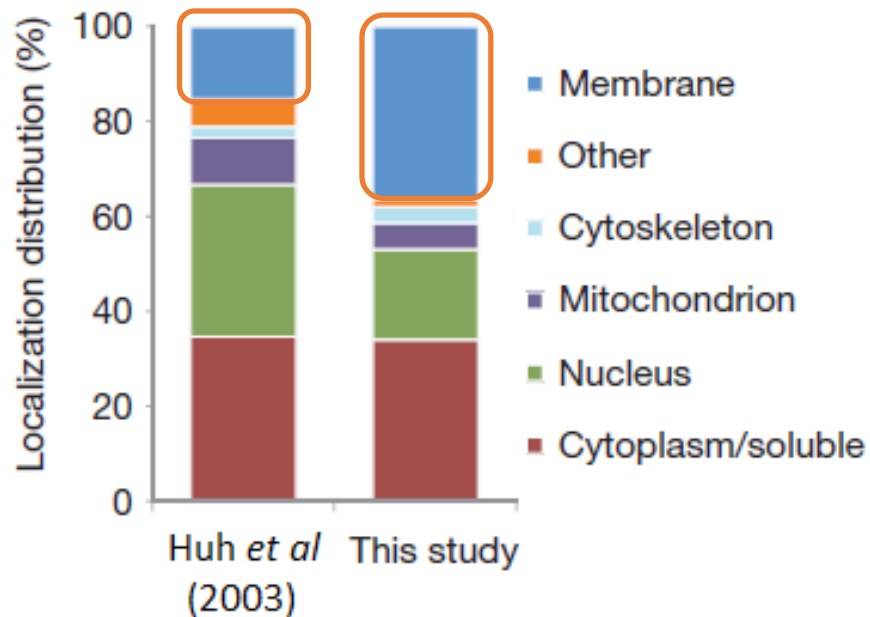
Subcellular localization



20% localizations were inconsistent between the 2 fusion orientations

Most abundant were endomembrane organelles (ER + Golgi most represented)

Subcellular localization



Comparison with localization of the *Saccharomyces cerevisiae* proteome

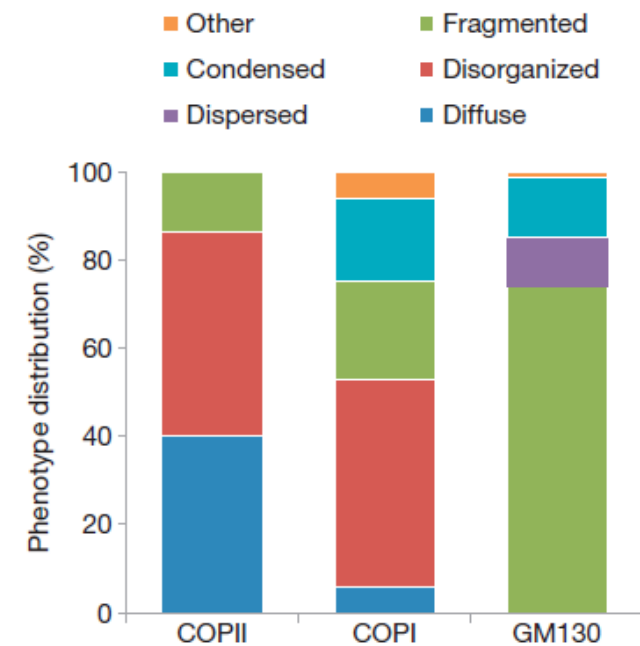
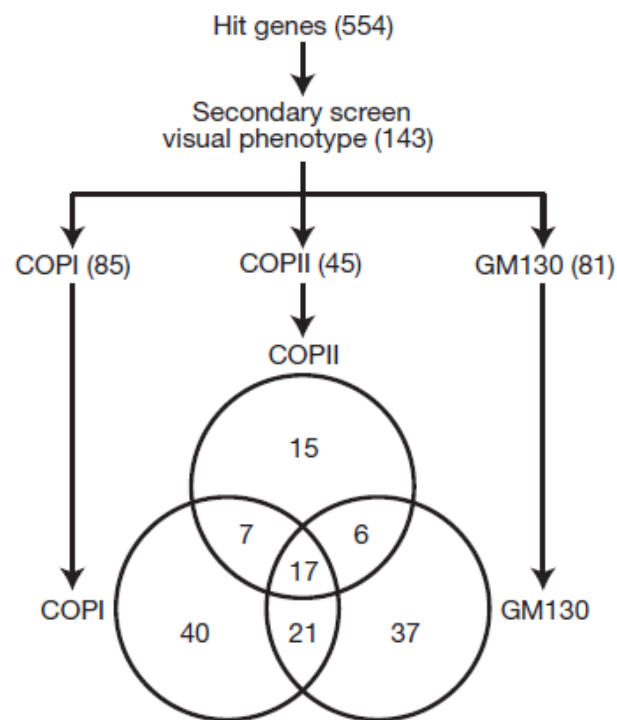
2.4-fold higher fraction of proteins in the endomembrane.

Set from this study enriched in proteins associated and functioning in the secretory pathway

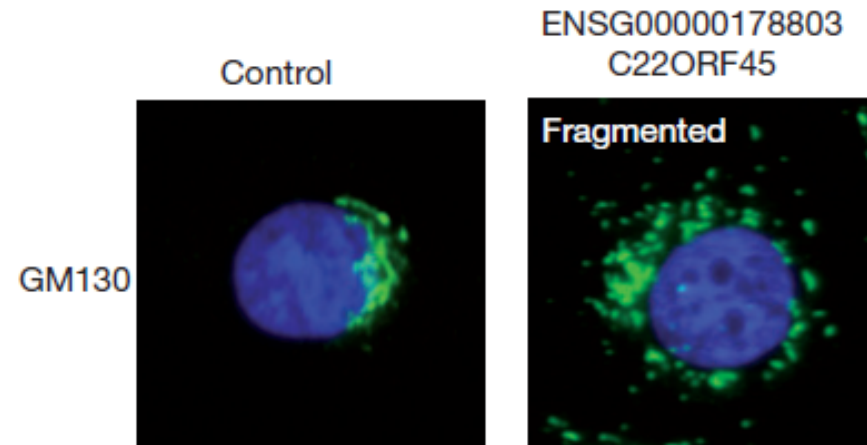
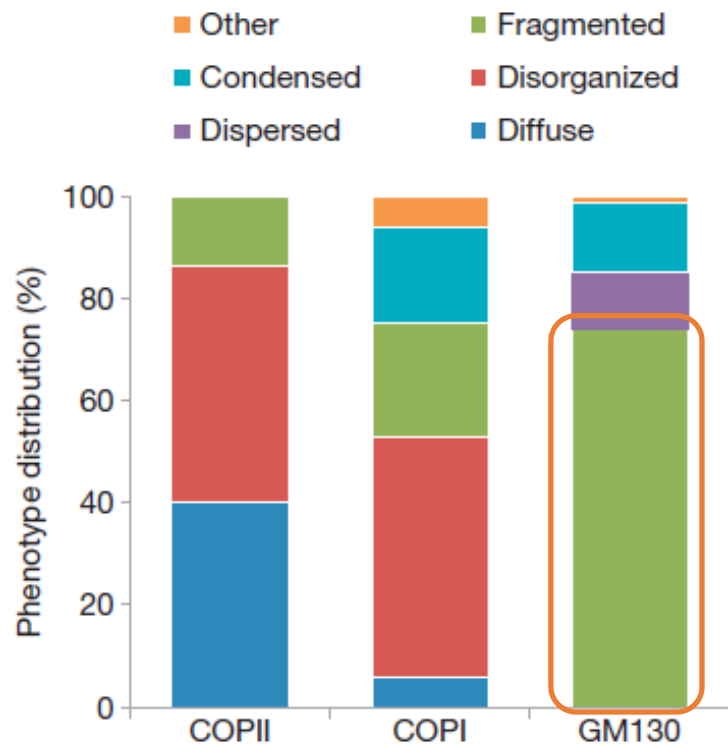
Morphological changes in early secretory pathway

554 validated hits → secondary RNAi-based screen → Immunostaining of:

- COPI coat
- COPII coat
- GM130 (Golgi integrity)



Golgi



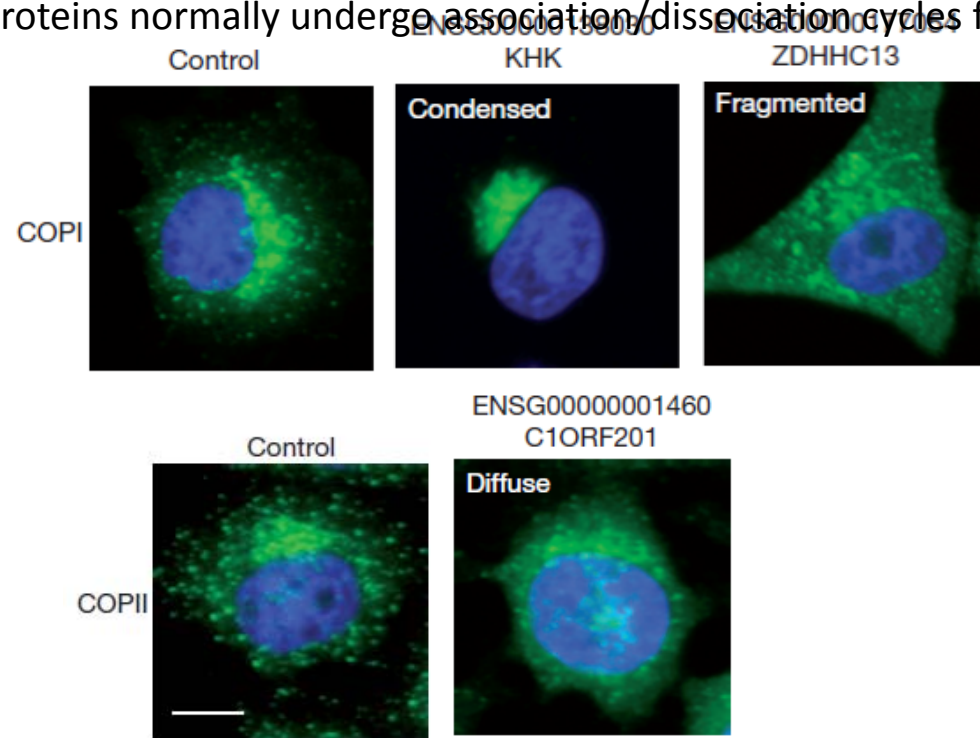
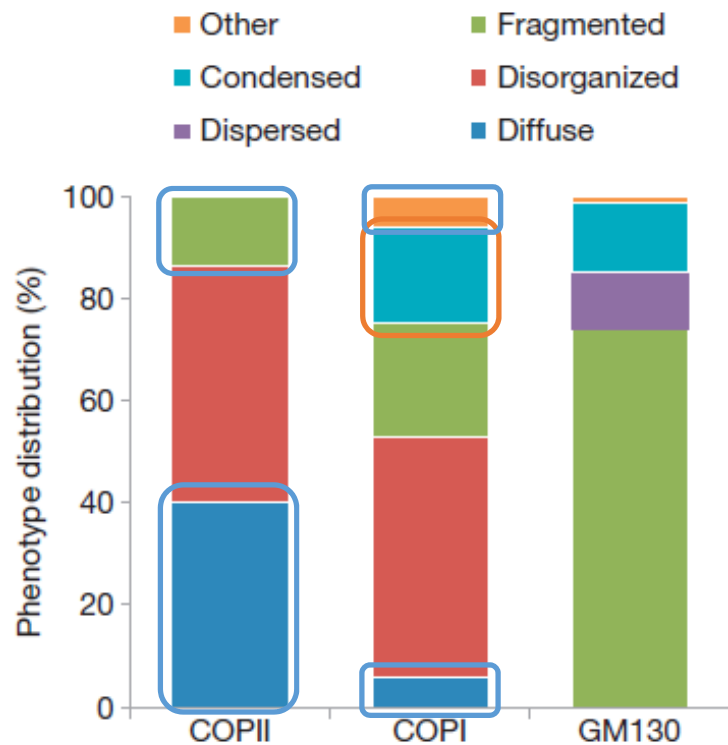
Golgi complex morphology is a result of coat proteins and interactions with cytoskeleton



Golgi fragmentation may be a result of these perturbations

Coat proteins

Coat proteins normally undergo association/dissociation cycles from membranes

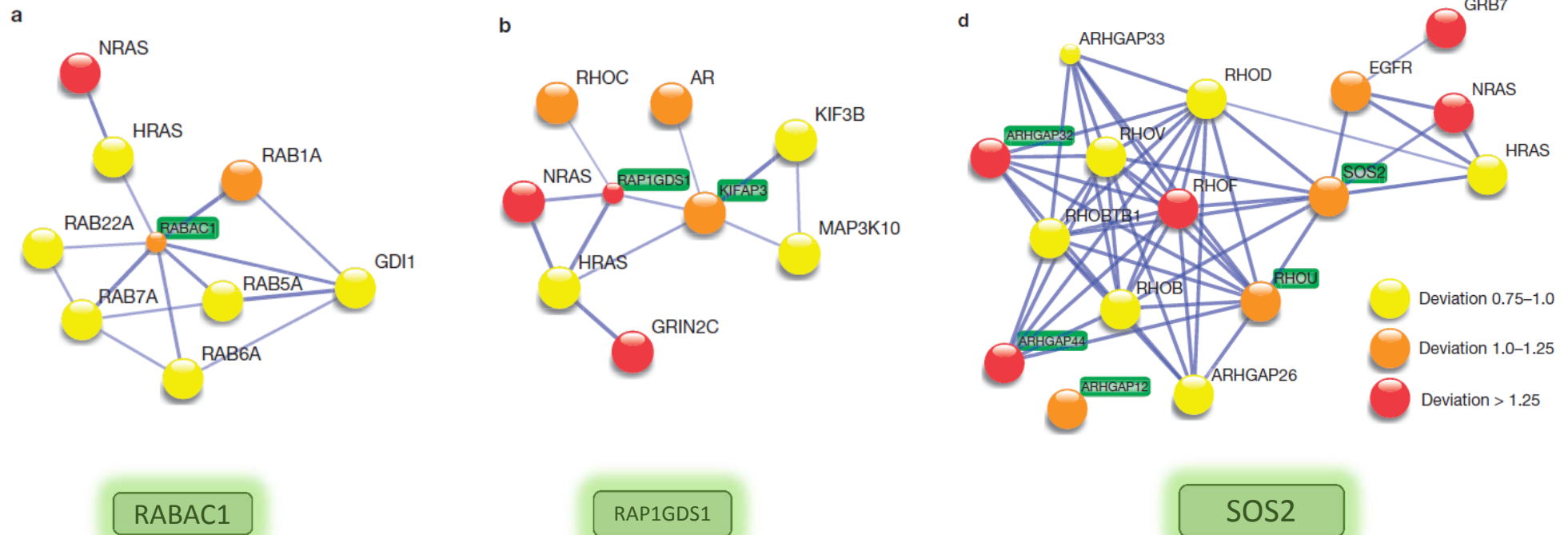


Compaction phenotypes → role in coat dissociation

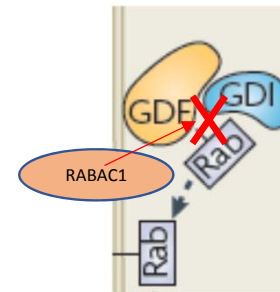
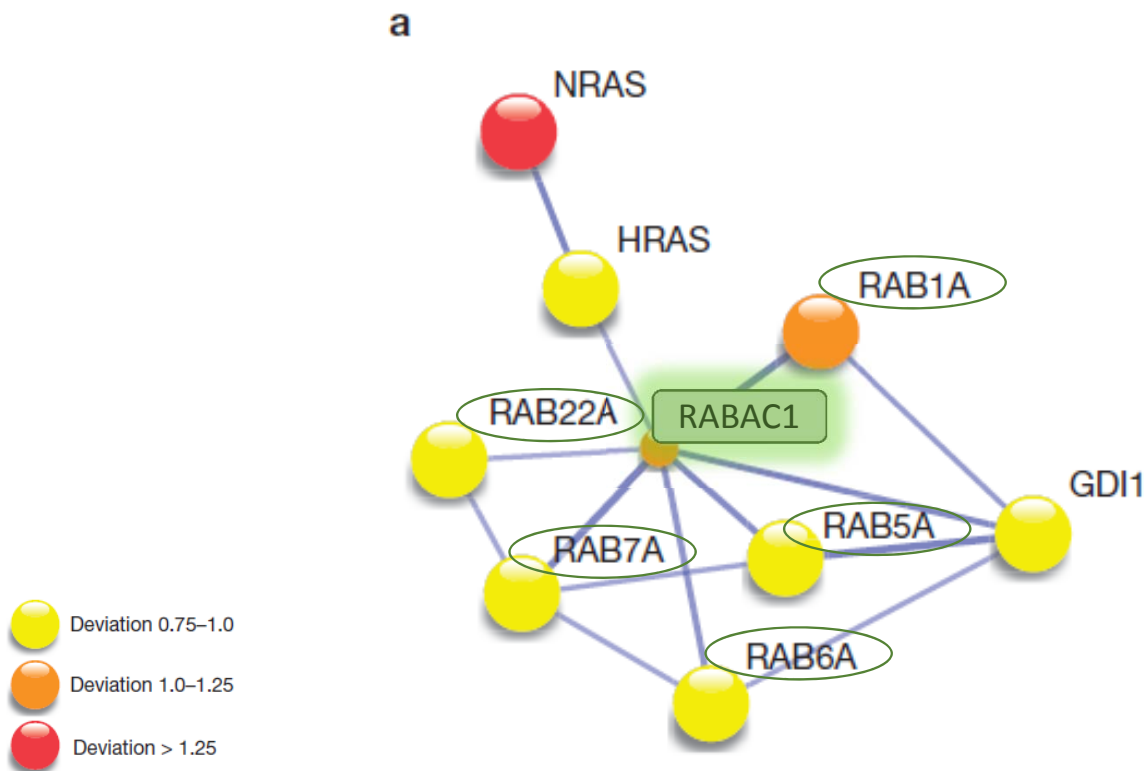
Diffuse phenotypes → role in coat recruitment

Network analysis

STRING – interactions database



Network analysis - RABAC1



Link between membrane traffic and signalling:

- Dissociates GDI-bound endosomal Rabs, delivering them to appropriate membranes

RABAC1

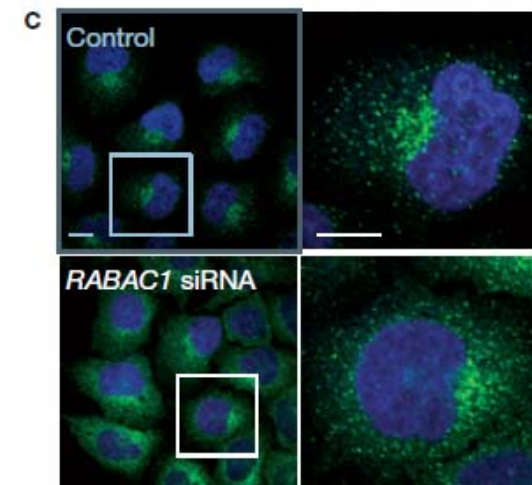
Depletion of RABAC1:

- Mild inhibition of secretion
- Mild morphological changes to COPII and COPI coats

Overexpression:

- Rate of ER-to-Golgi traffic of tsO45G reduced

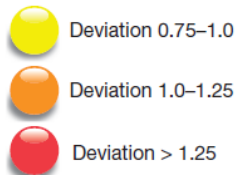
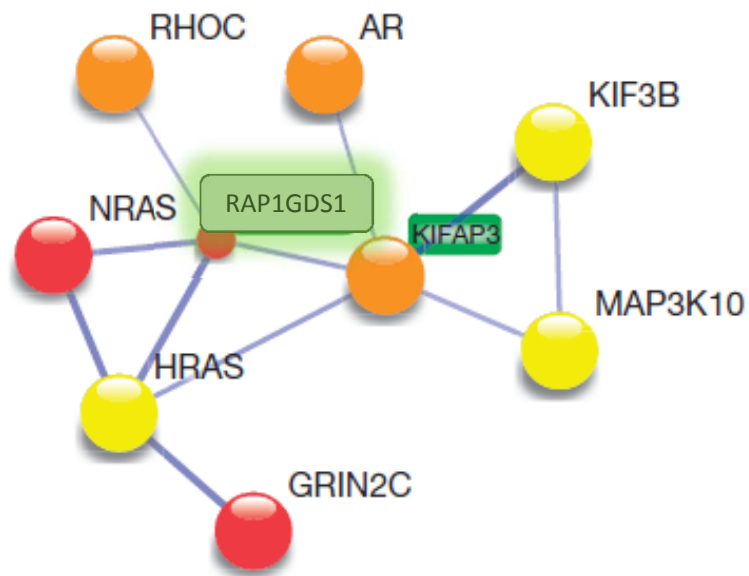
Hub for secretory traffic organization?



Stain: COPII (SEC13)

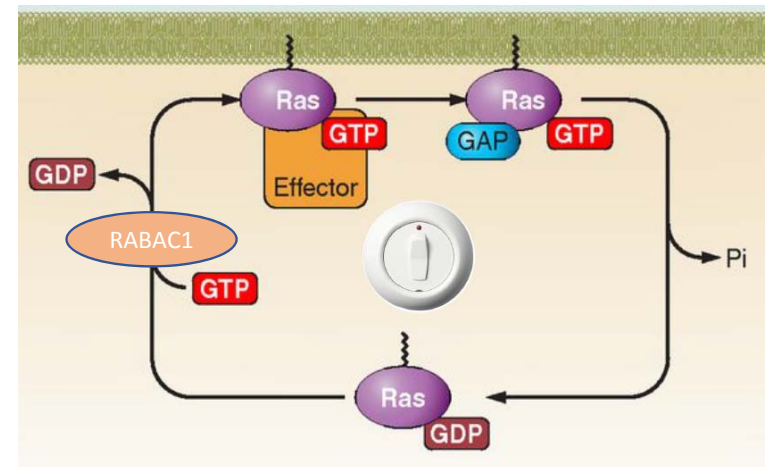
Network analysis – RAP1GDS1

b



Early secretor pathway key regulator?

- GEF for Ras and Rho subfamilies

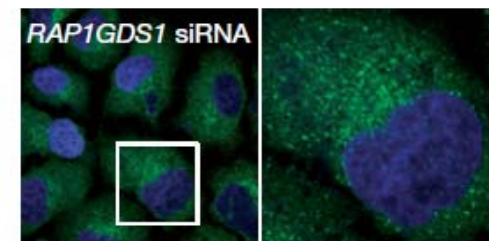
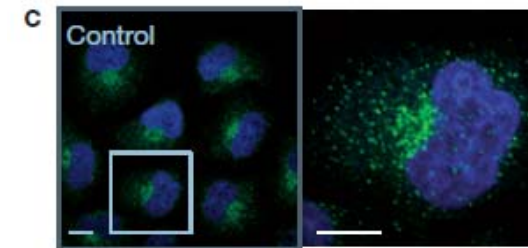


RAP1GDS1

- Resides at or close to PM
- Post prenylation at ER
- Control of prenylation of Rap1, RhoA and Rac1?

RNAi experiments:

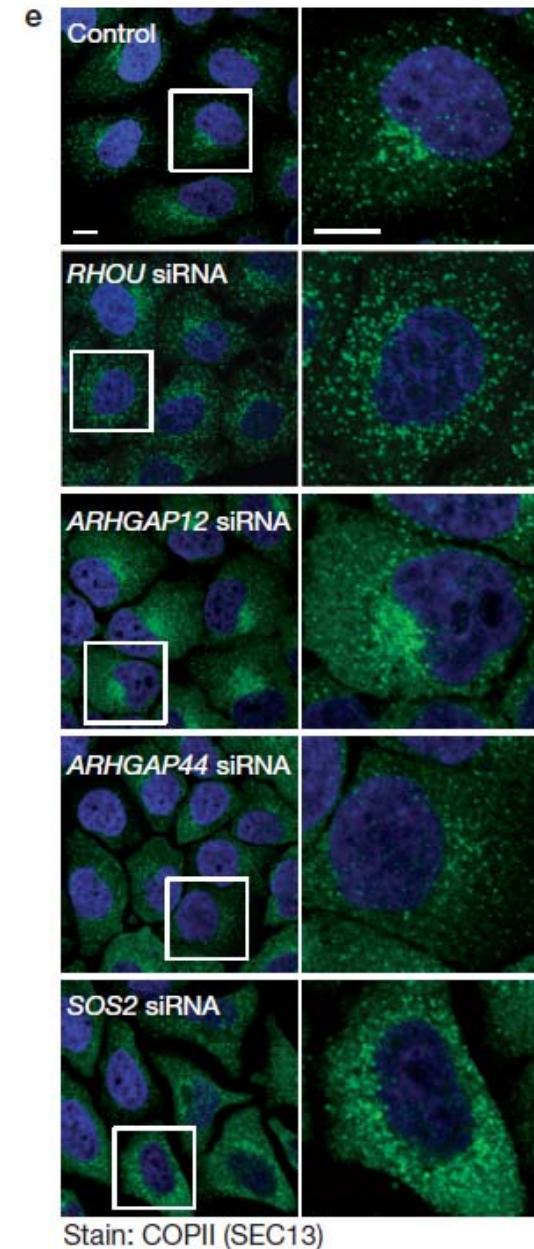
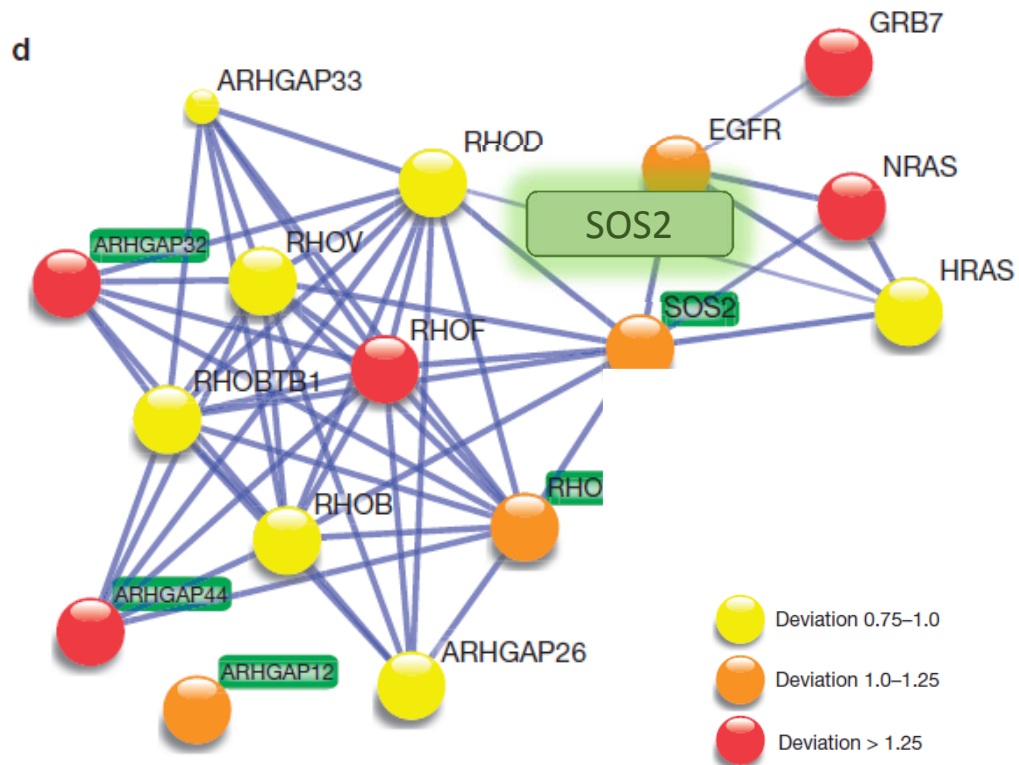
- Decreases secretor activity
- Reorganization of COPII-coated ER exit sites
- Diffuse phenotype



Stain: COPII (SEC13)

Network analysis – SOS2

- GEF for Ras and Rho
- Signalling with receptor tyrosine kinases



SOS2

- GEF for Ras and Rho
- Signalling with receptor tyrosine kinases

RNAi experiments:

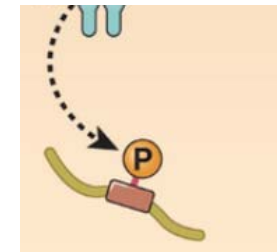
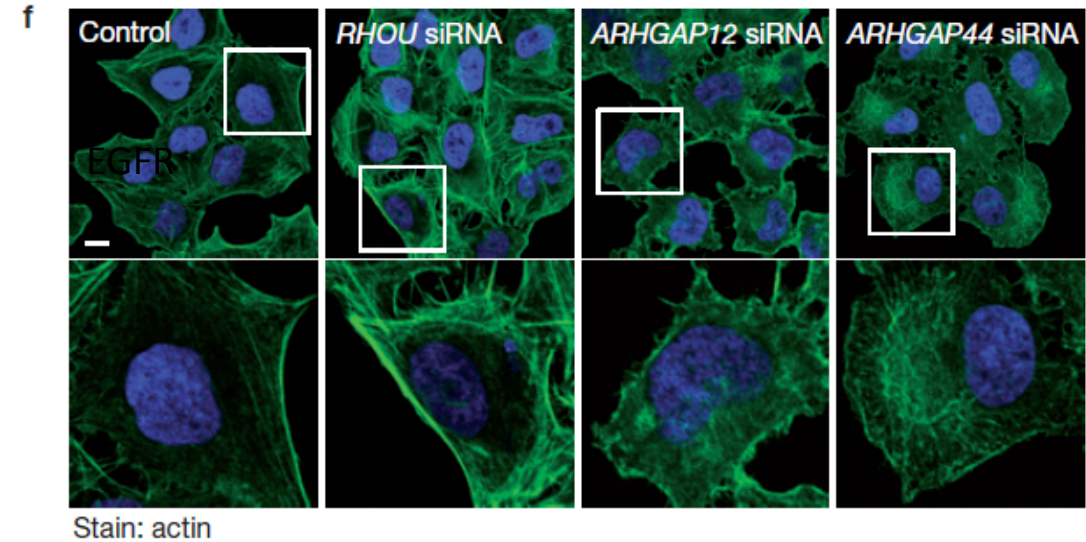
SOS2 + **RHO** → ↑ Stress fibers

SOS2 + 2 GAPs for Rho (ARHGAP12 and ARHGAP44)

Loss of F-actin

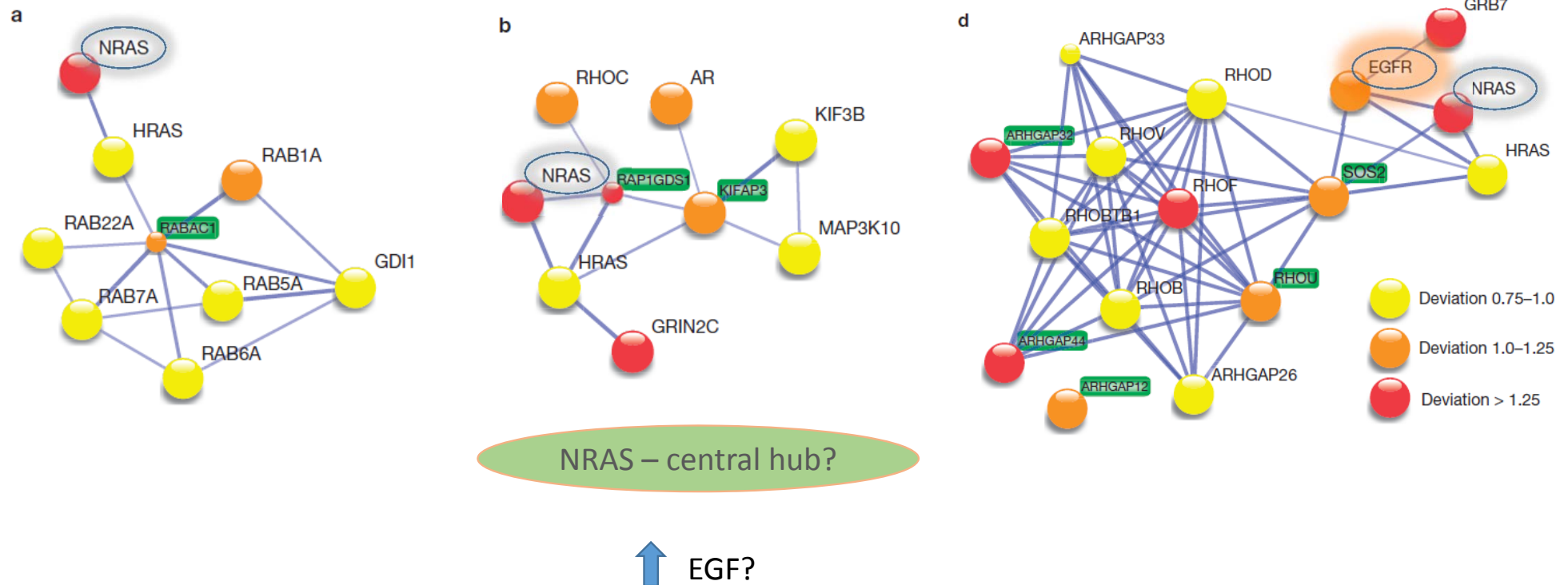
Actin accumulation in bundles

Reorganization of COPII-coated ER exit sites



Early secretor pathway key regulator?

Network analysis – NRAS and EGF

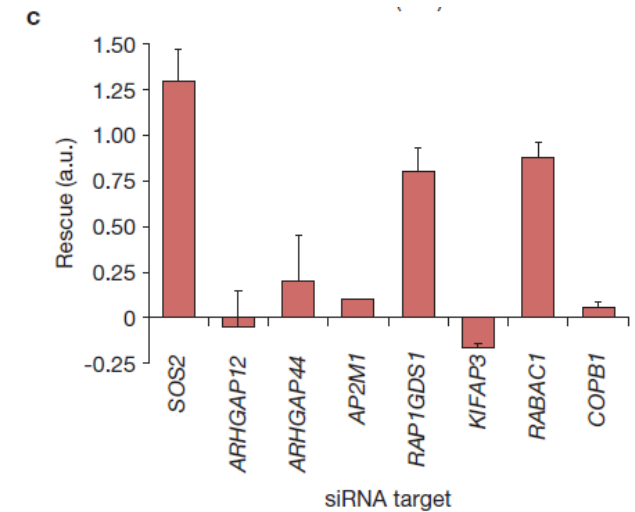
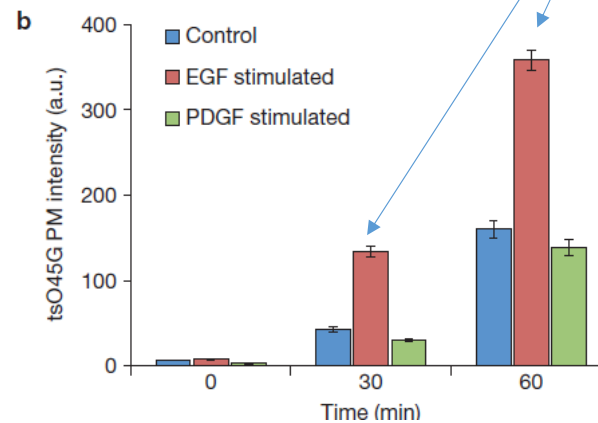
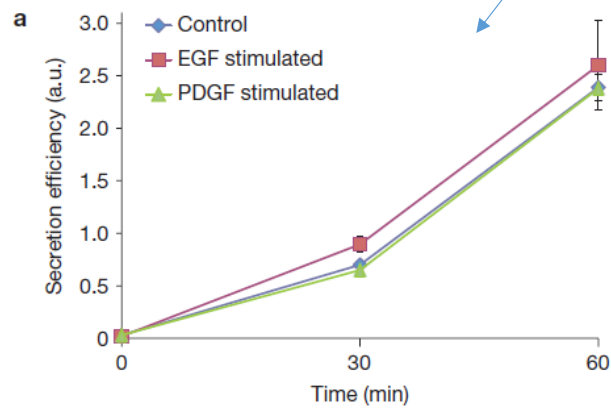


EGF experiments

$$\text{tsO45G efficiency of transport} = \frac{\text{tsO45G cell surface}}{\text{Total tsO45G}} \quad \longleftrightarrow$$

tsO45G cell surface only

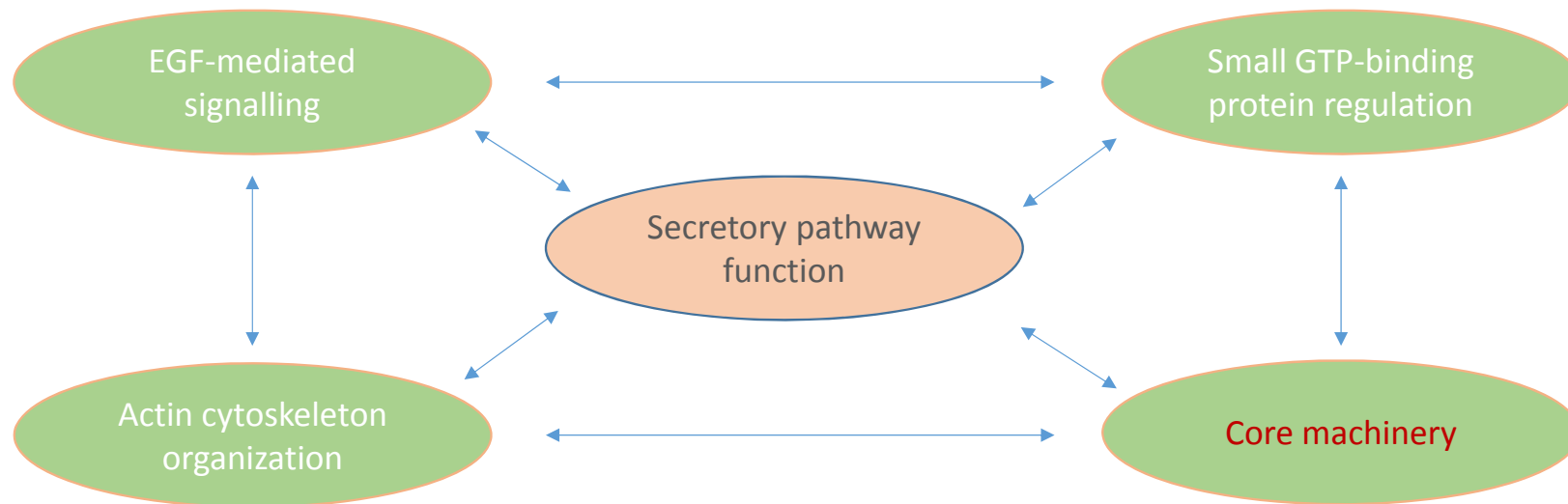
Secretory machinery activated with EGF



Conclusions

GLOBAL ANALYSIS OF GENE FUNCTION IN SECRETION OF THE MODEL TRANSMEMBRANE PROTEIN ts045G

15 % OF GEMONE ENCODES PROTEINS LINKED TO THIS PROCESS



HOW DO EVENTS IN CELL PERIPHERY MODULATE SECRETION MACHINERY IN ER AND GOLGI?



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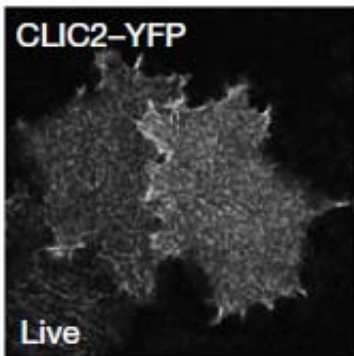
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Subcellular localization



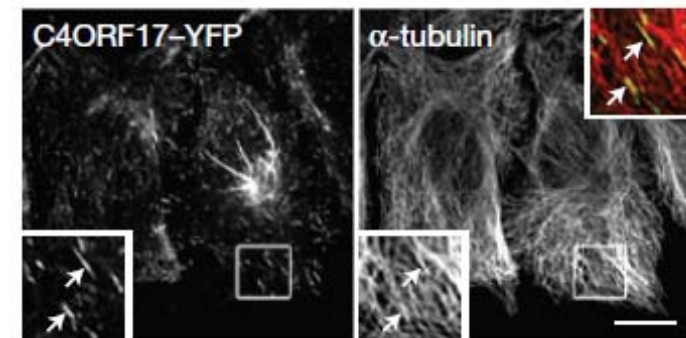
CLIC2

- Short filaments throughout cytoplasm
- Structures lost in fixation
- Minor co-localization with actin
- Intracellular chloride channel
- Possible role in calcium signaling

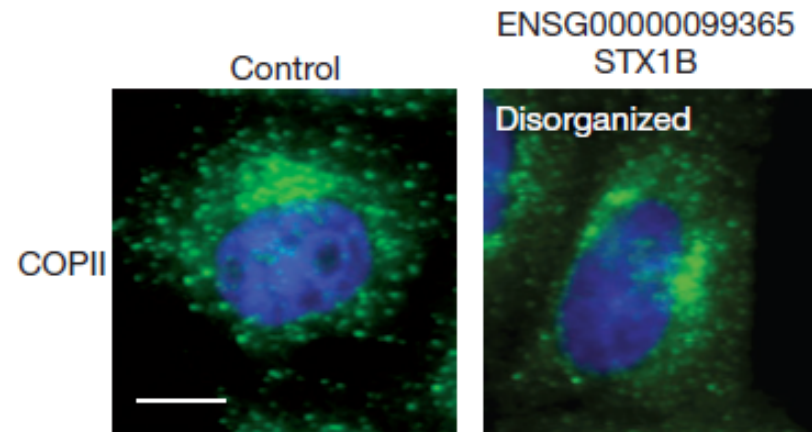
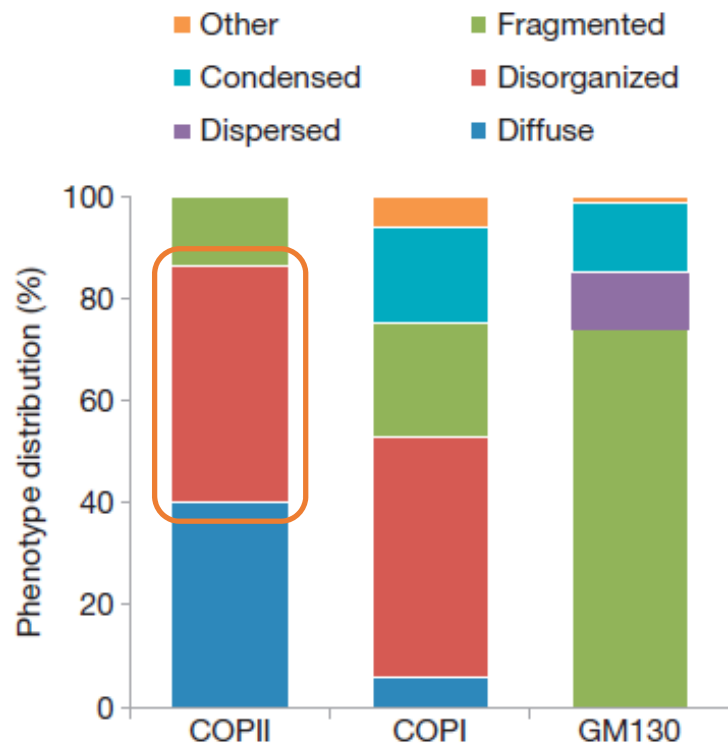


C4ORF17

- Short filaments and intense filamentous bundles
- Same in both live and fixed cells
- Partial co-localization with microtubules



COPII



Punctate ER exit sites can be discriminated,
but their distribution is disturbed