Regulation of the expression and biologic activity of BAFF through the Interferon-a/ autophagy axis in Systemic Lupus Erythematosus

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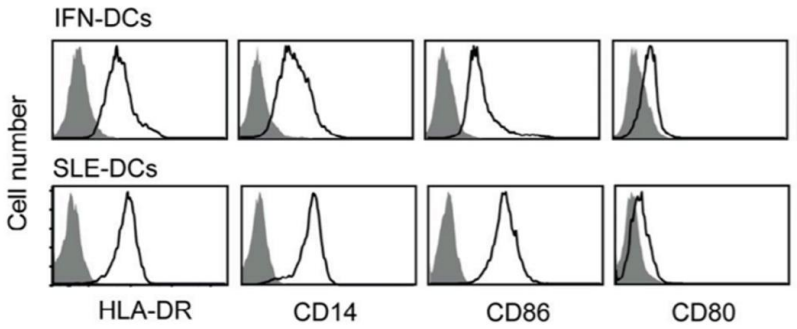
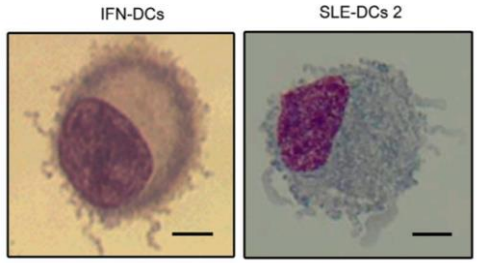
ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

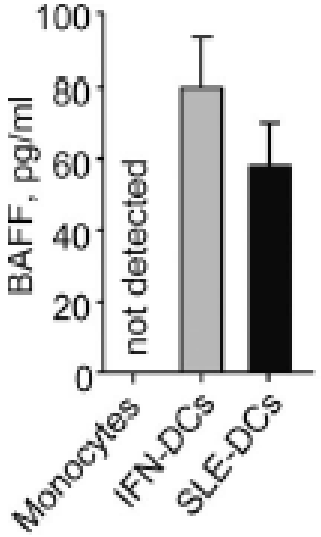
Heraklion 10.10.2021

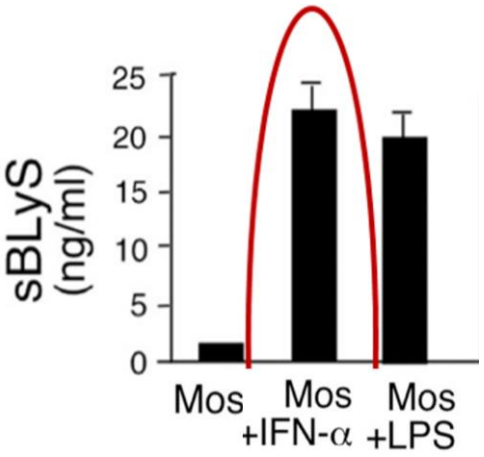
Monocytes play a central role in SLE pathogenesis and behave as dendritic cells under the effect of type I IFN

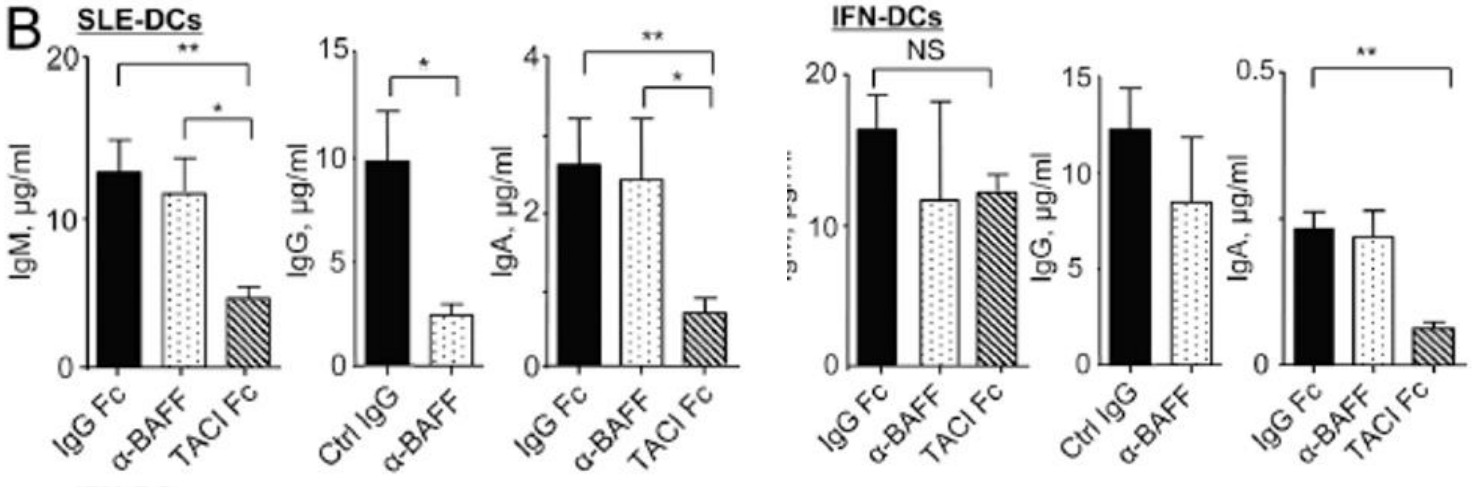
DC-like phenotype of CD14+ Monocytes in lupus









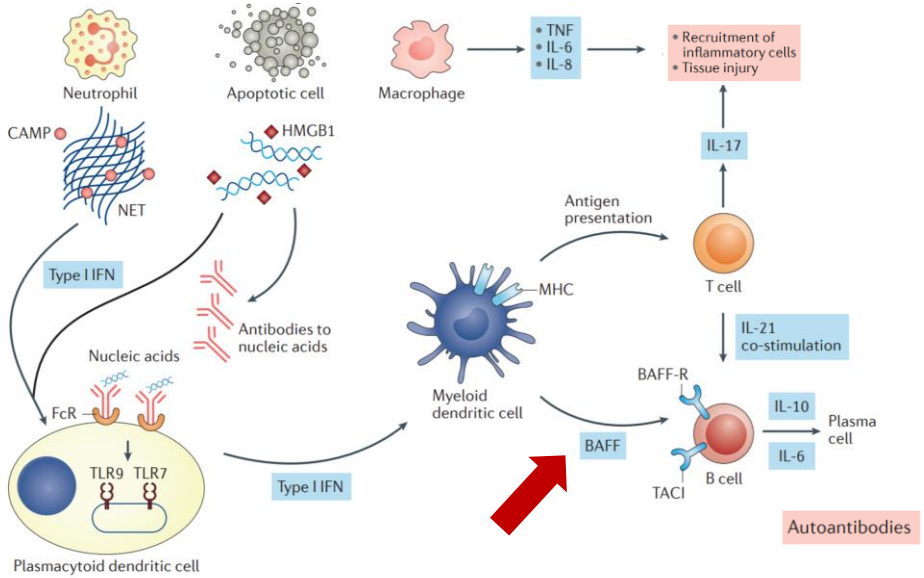


A key feature of SLE (DC-like) monocytes is that they secrete high amounts of BAFF

contributing to maturation of B-cells to IgG-secreting plasmablasts

(Blanco et al. 2001; Zhang et al.2001; Litinskiy et al. 2002; Yihong et al. 2009; Lopez et al. 2014;Lopez et al. 2016).

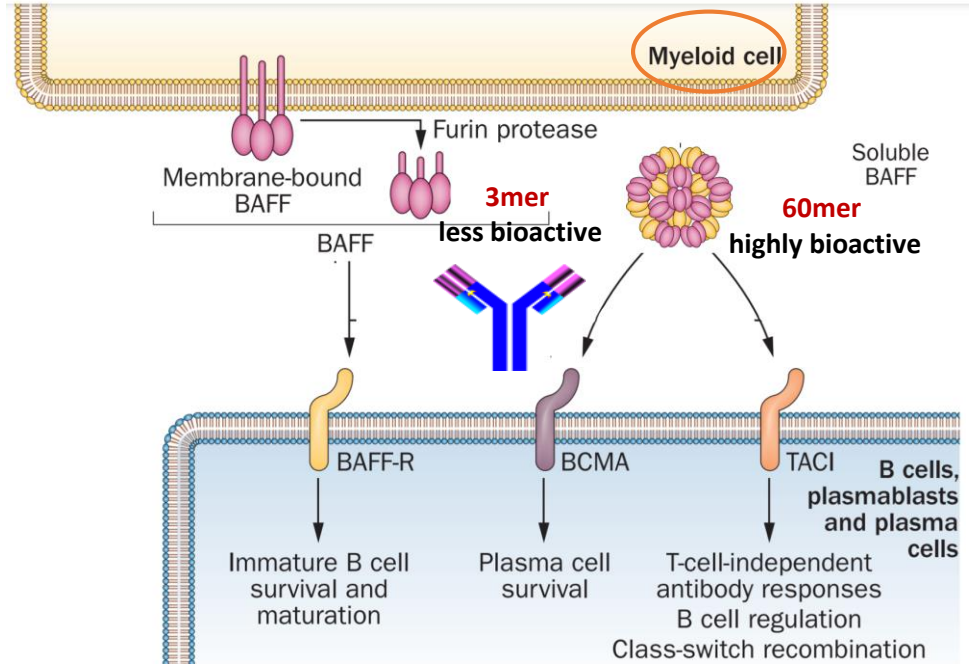
Strategies to block BAFF secretion have significant therapeutic potential in autoantibody-driven pathologies such as SLE



B cell activating factor - BAFF

monoclonal antibody BAFF - Belimumab

~ 40% of SLE patients **un**responsive to the drug



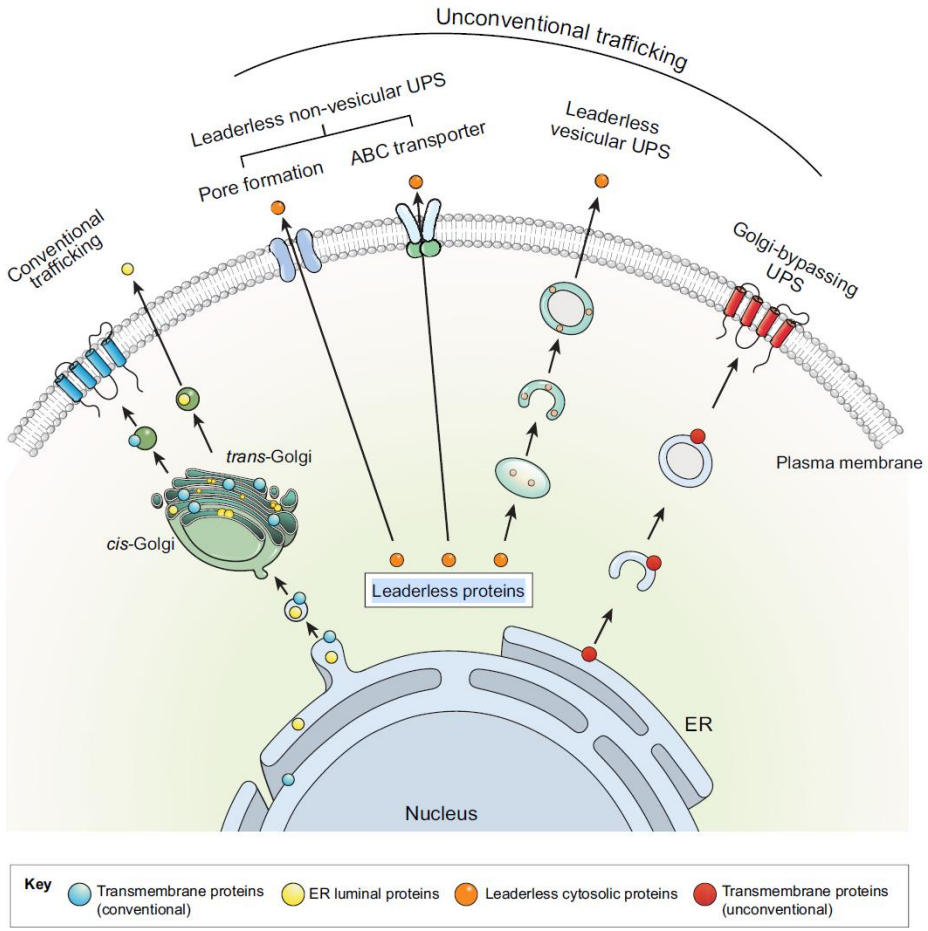
Belimumab targets successfully only the soluble trimeric BAFF

(Vigolo et al.2018)

BAFF lacks the classical signal peptide and might follow 'unconventional' secretion pathways (ie, bypassing the ER-Golgi)

Unconventional protein Secretion-UPS:

→**leaderless cytosolic protein**
secreted from the cells via

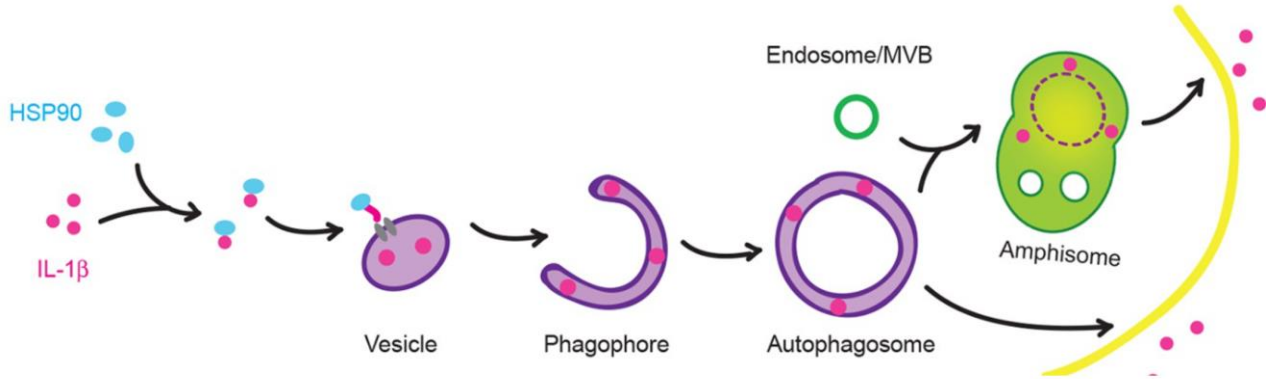


(Lee et al.2018)

«**secretory autophagy**»
→ process in which autophagy **is involved** in **extracellular secretion** of proteins and cytokines

(Cavalli et al.2020)

IL-1β as a prototype



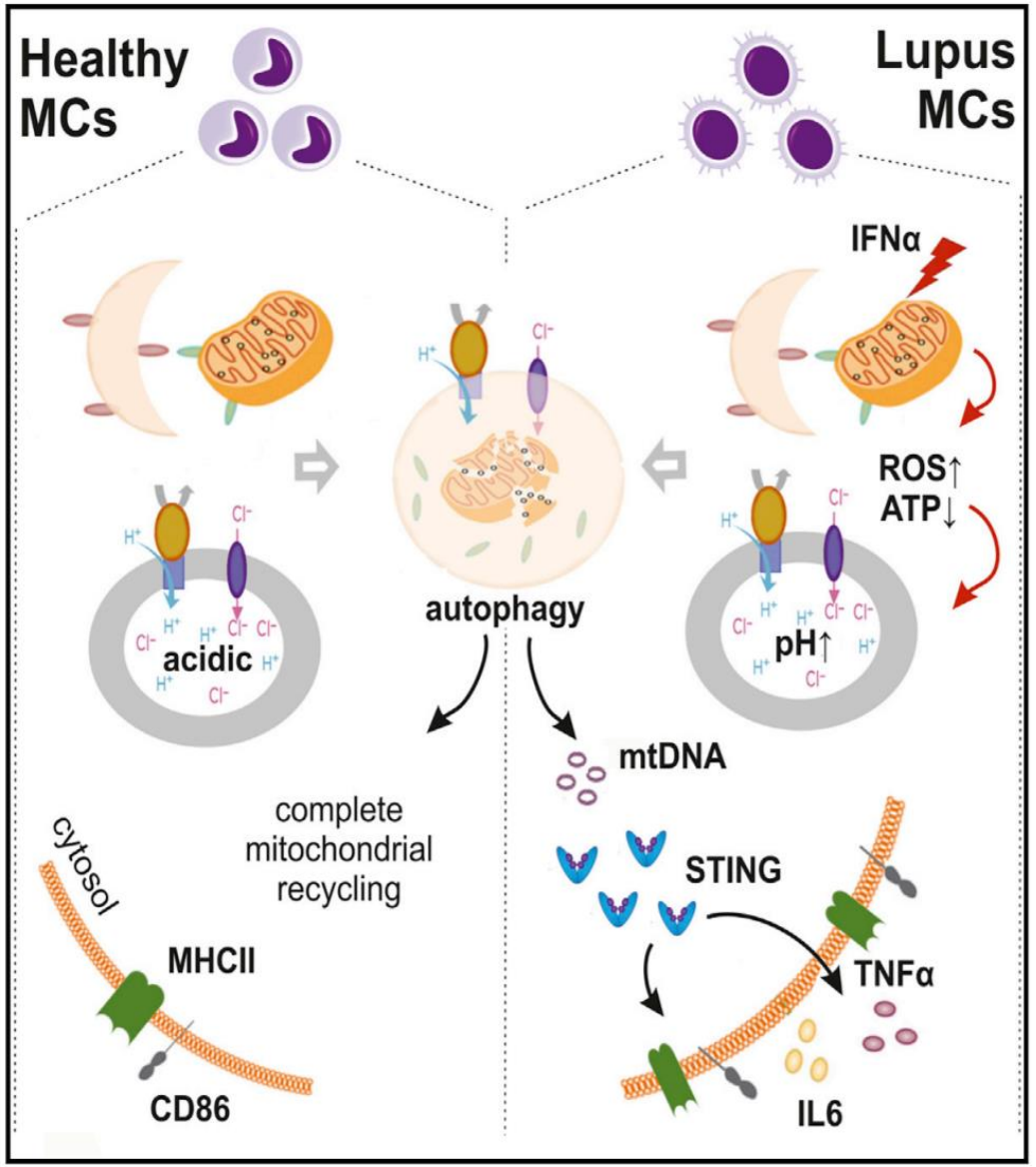
Autophagy is implicated in interferon-mediated monocyte hyperactivity in SLE

✓ **In SLE monocytes autophagy is employed with:**

Enhanced autophagosome formation
Unaffected autophagolysosomal fusion
Defective autolysosomal degradation
(lysosomes not properly acidified)

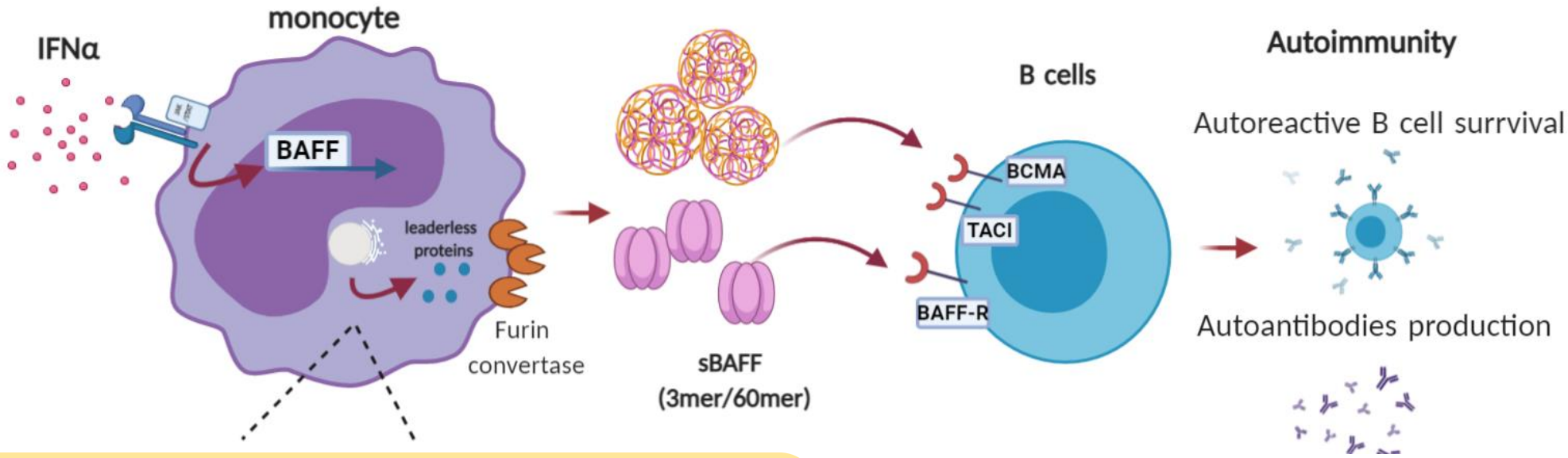
✓ **Upon IFNα signaling in monocytes autophagy is employed with:**

Enhanced autophagosome formation
Unaffected autophagolysosomal fusion
Defective autolysosomal degradation
(lysosomes not properly acidified)
Enhanced immunogenic potential



(Gkirtzimanaki et al.2018)

Hypothesis /Aim of the study



Molecular mechanism of BAFF production/secretion upon IFNα
in autoimmunity remains unspecified

Investigation of the role of autophagy in IFNα-mediated regulation/secretion of bioactive BAFF
thus
contributing to differentiation/activation of B lymphocytes in the context of SLE

Research questions

1. **Is autophagy implicated in monocyte secretion of BAFF in the context of type I IFN signaling and lupus?**

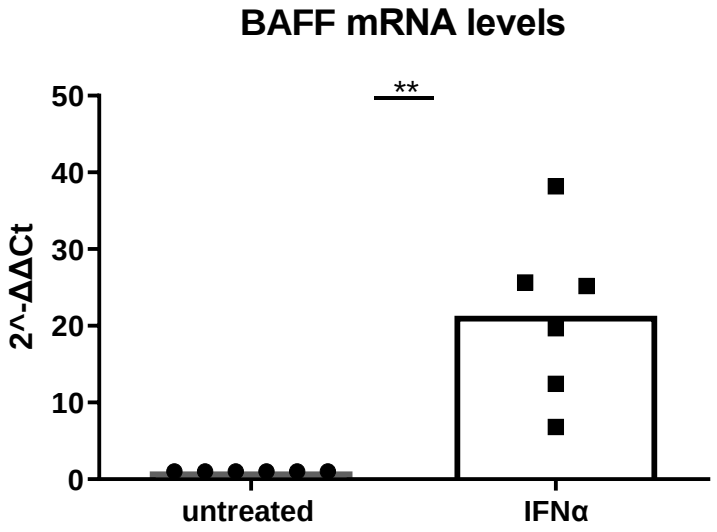
2. Does the IFNα/autophagy axis regulate the secretion of BAFF isoforms with differential bioactivity?

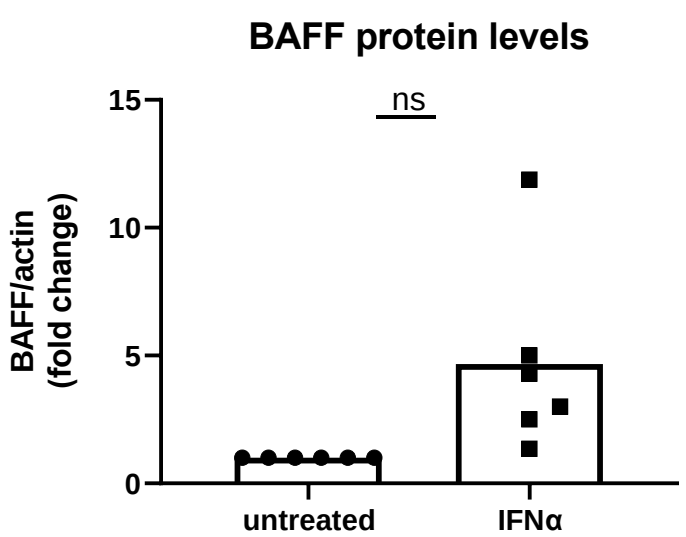
3. Could autophagy be exploited as a novel target to block BAFF secretion of lupus monocytes?

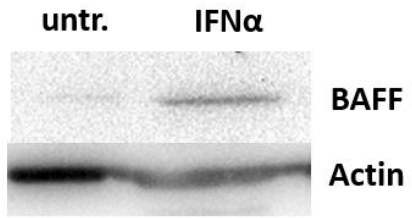
Results

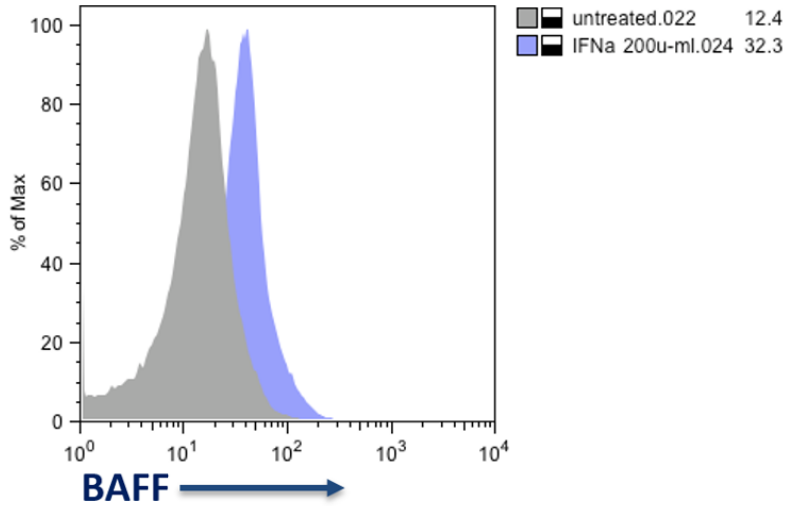
IFNα induces BAFF transcriptional , protein expression and secretion by monocytes

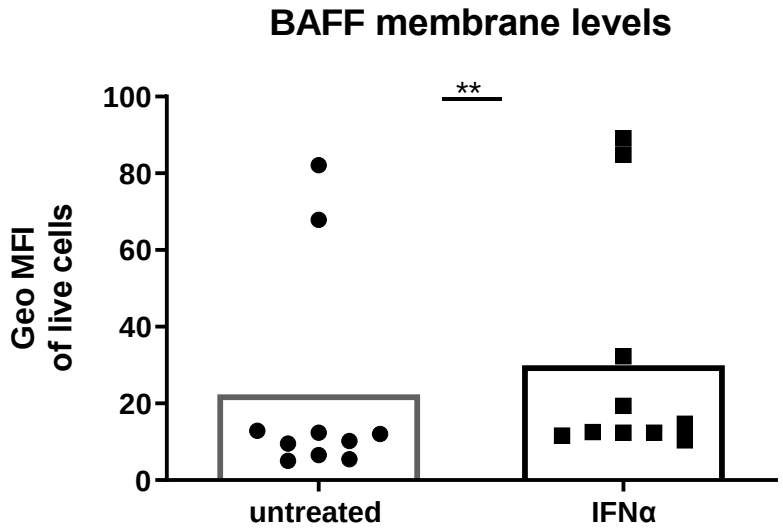
- CD14+ monocytes (healthy donors) treated 18hrs stimulation with 200u/ml IFNα

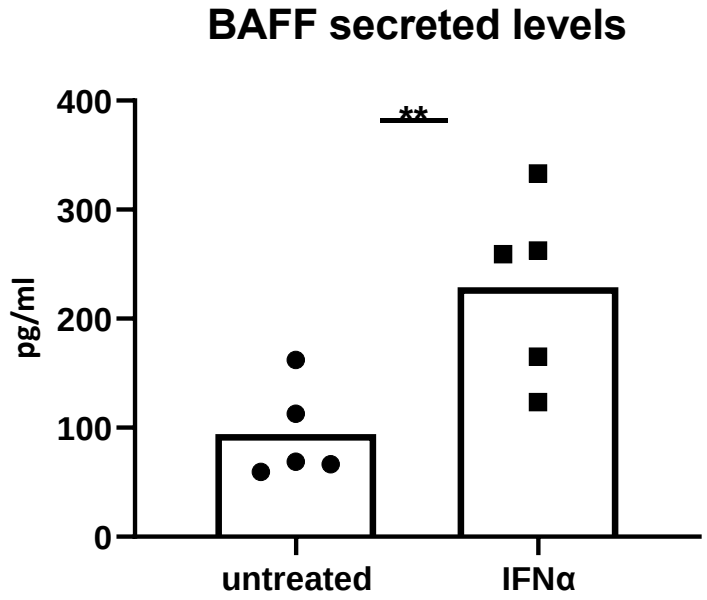










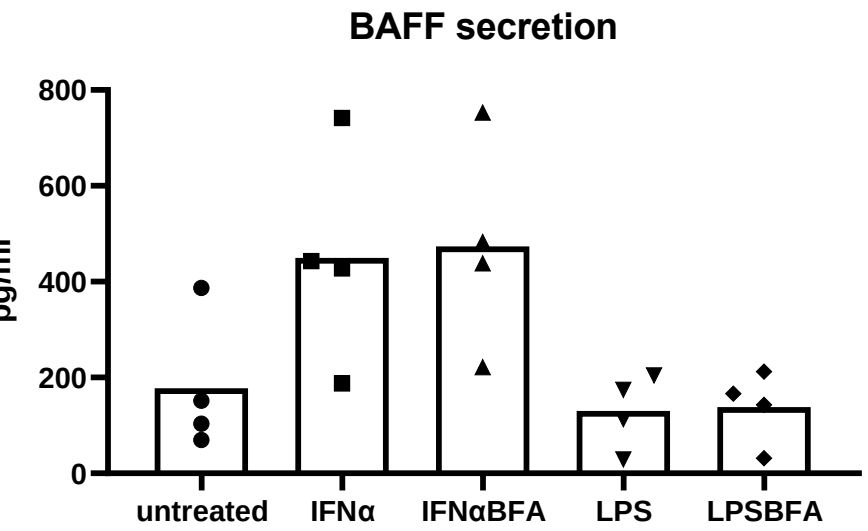


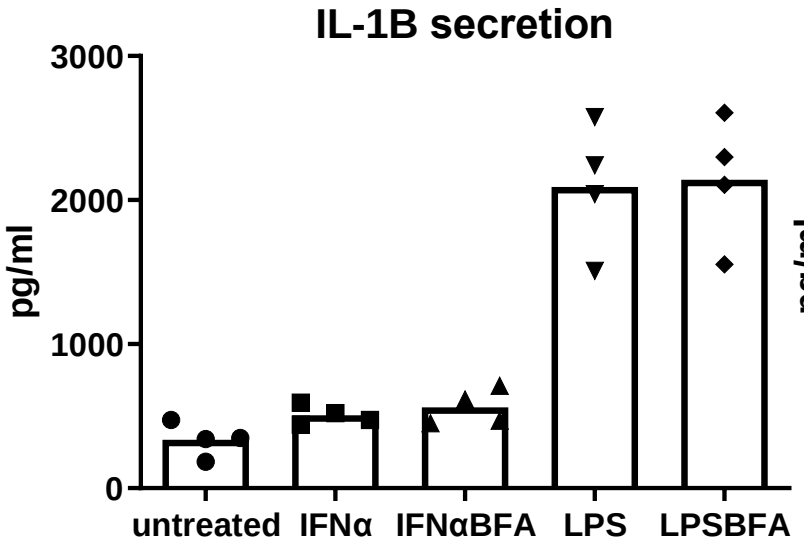
Results

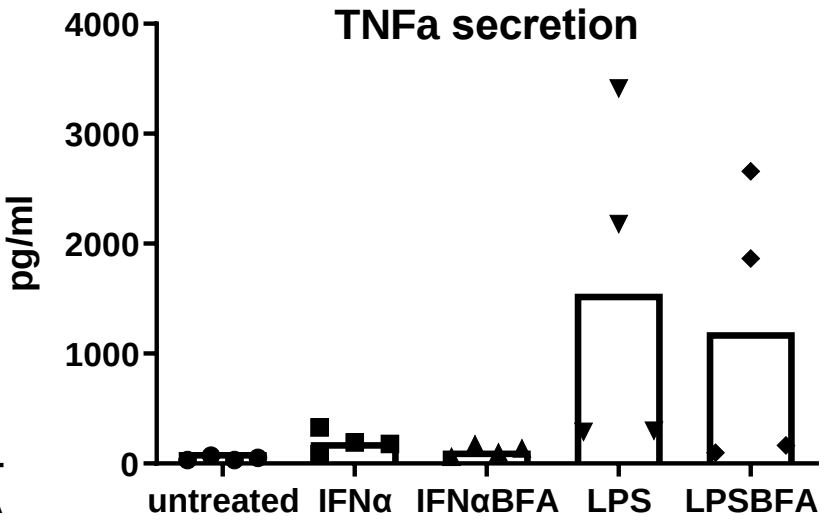
BAFF follows 'unconventional' secretion?

- BAFF **lacks** a signal peptide
- Bioinformatics Analysis (OutCyte tool) → Good score for UPS

❑ CD14+ monocytes (healthy donors) treated 24hr IFNα,LPS with **last 3hr Brefeldin** treatment







❑ Brefeldin <u>failed to inhibit</u> BAFF secretion

→ first direct evidence that

BAFF might follow

non-conventional route of protein secretion

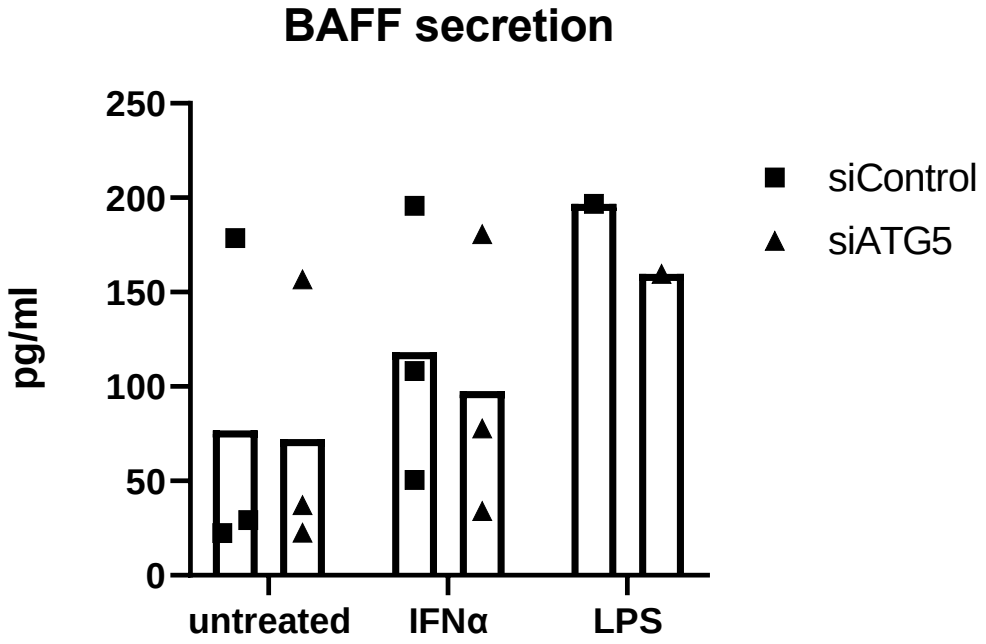
Autophagy affects IFNα- induced BAFF secretion?

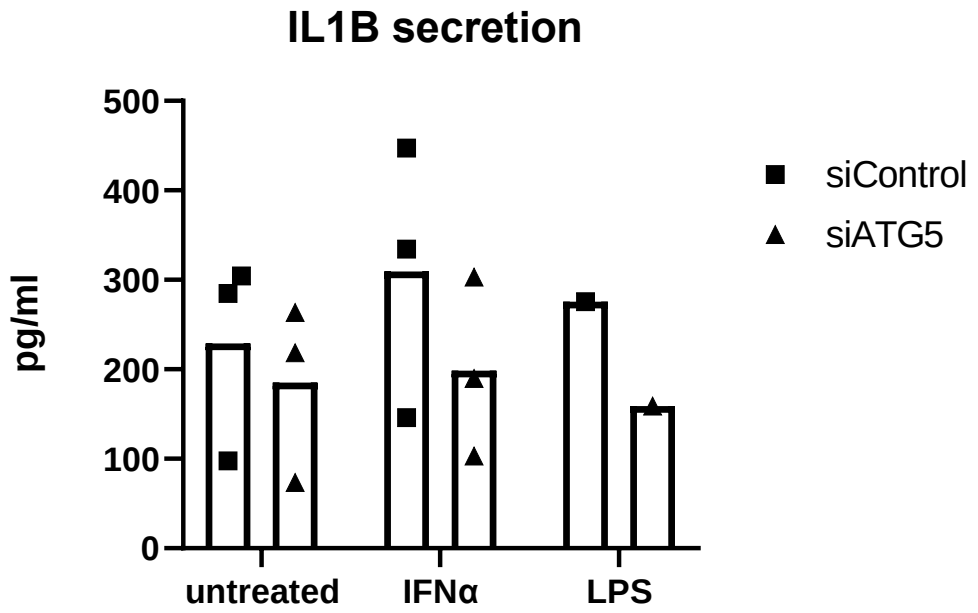
- CD14+ monocytes transfected with siATG5 and stimulated with IFNα for 24hr

Evidence of IFNa-induced BAFF secretion in an autophagy/ATG5-dependent manner

Need to examine:

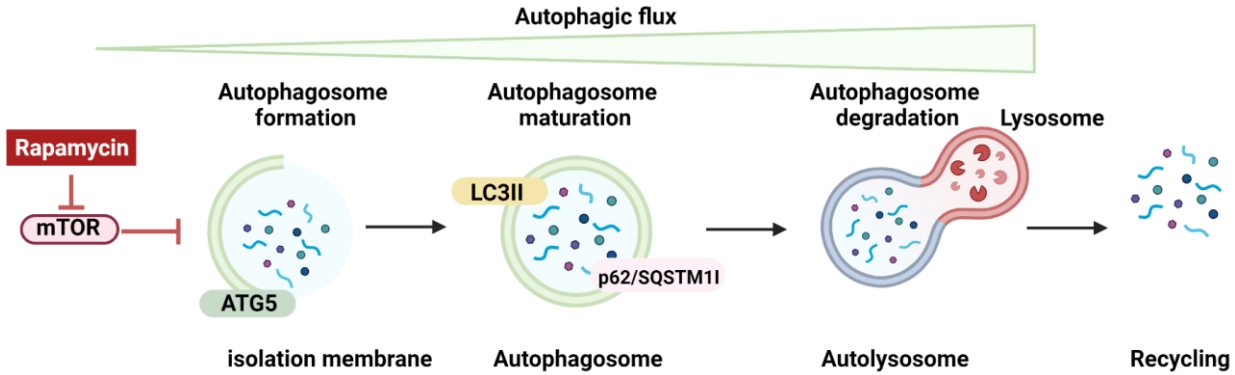
- Evaluation of siATG5
- More replicates/use of THP1
- Examine TNFα secretion

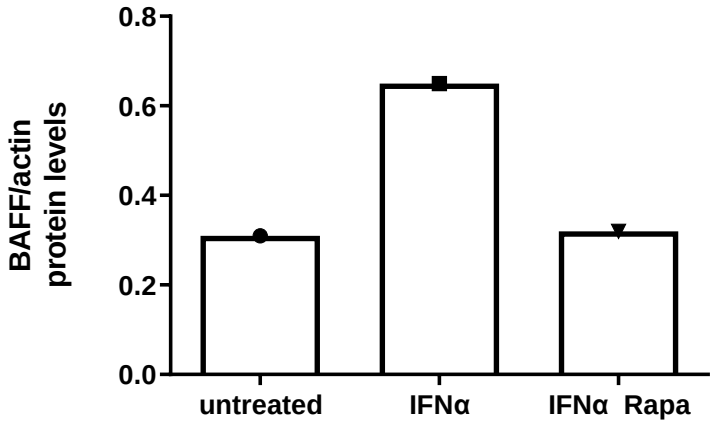


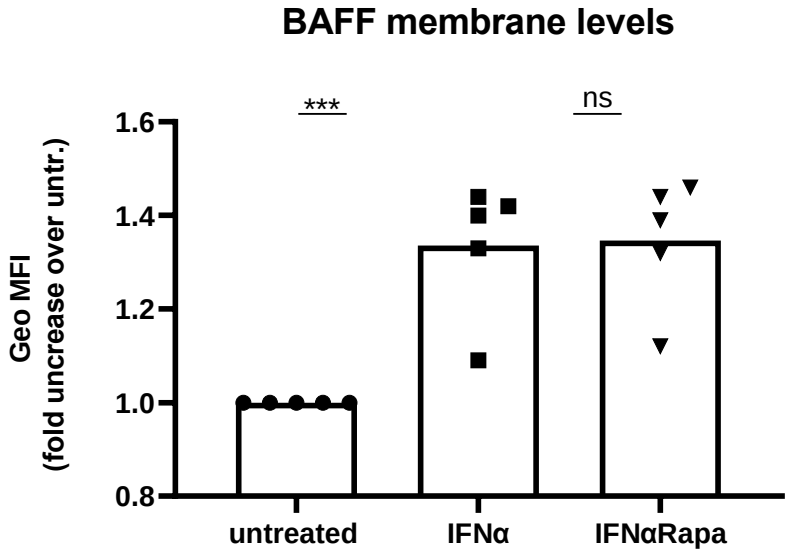


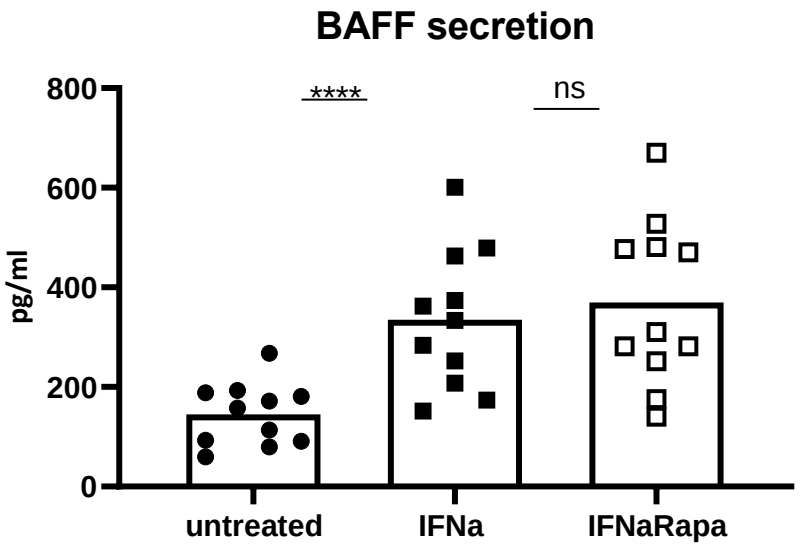
Autophagy affects IFNα-induced BAFF secretion?

- **Pharmacological induction of autophagy with Rapamycin**
  in CD14+ monocytes treated 18hrs with 200u/ml IFNα





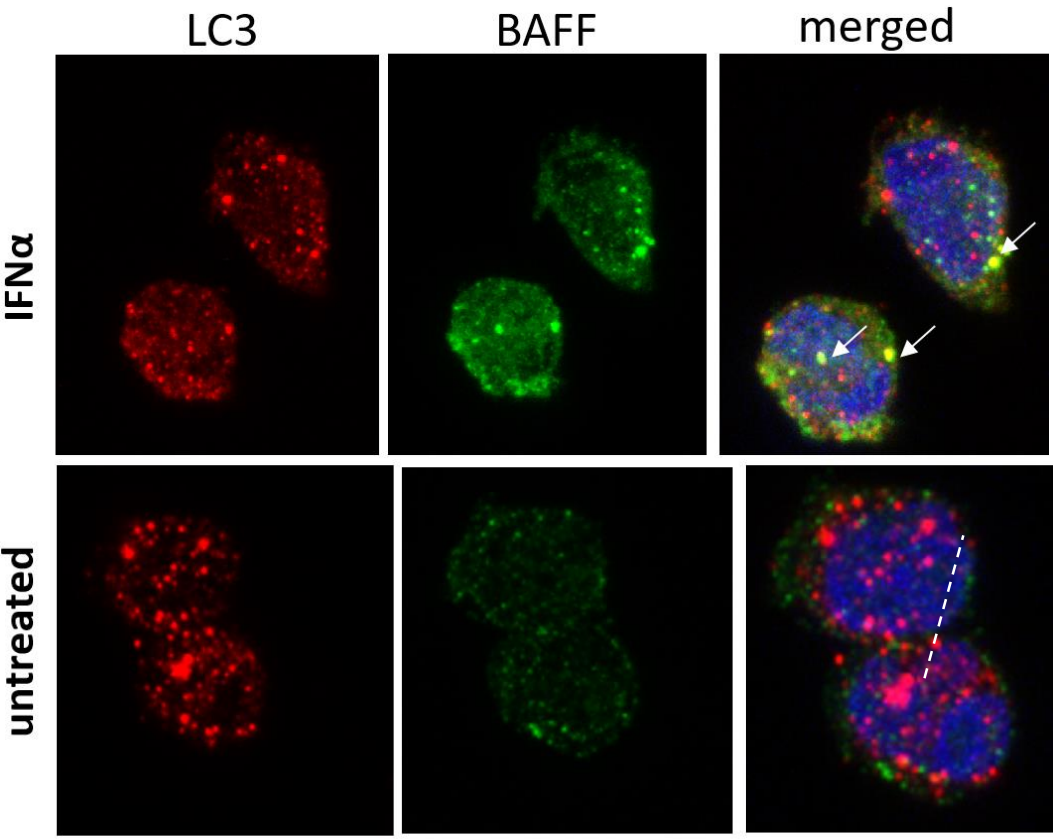


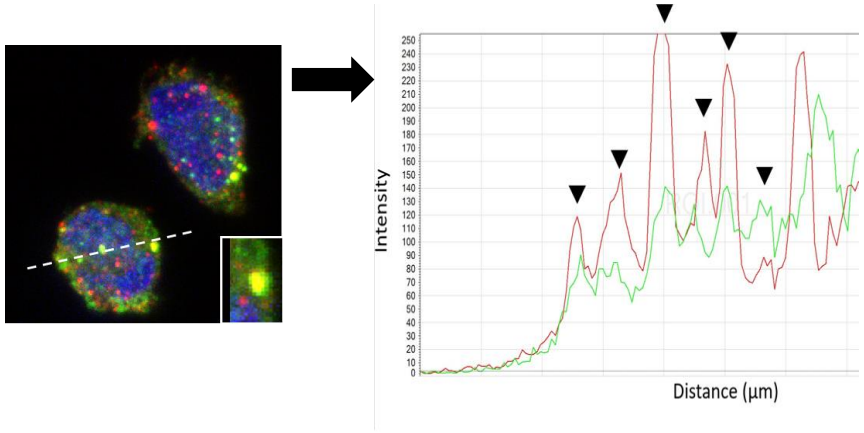


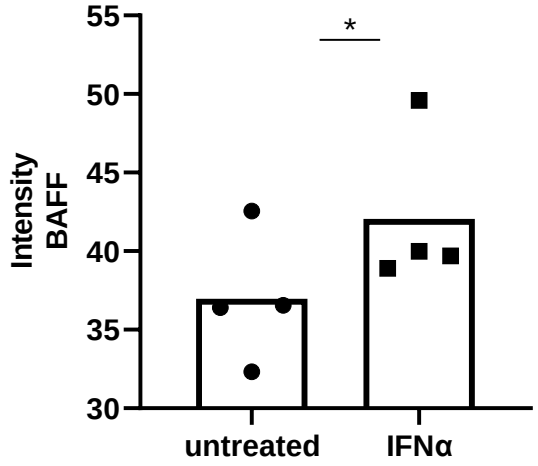
Induction of autophagy with Rapamycin balances/enhances the IFNα-induced BAFF secretion

How autophagy affects IFNα-induced BAFF secretion?

- Confocal microscopy

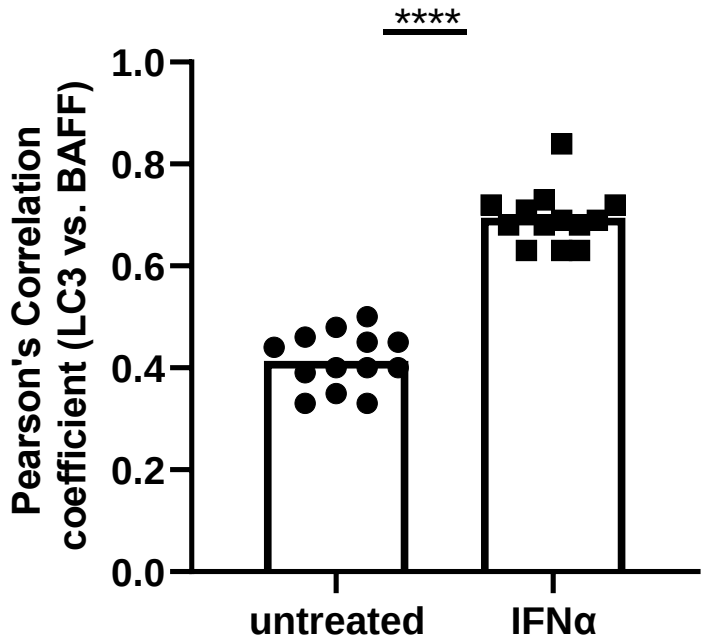






BAFF and autophagic protein LC3 colocalize in the cytoplasm upon IFNα

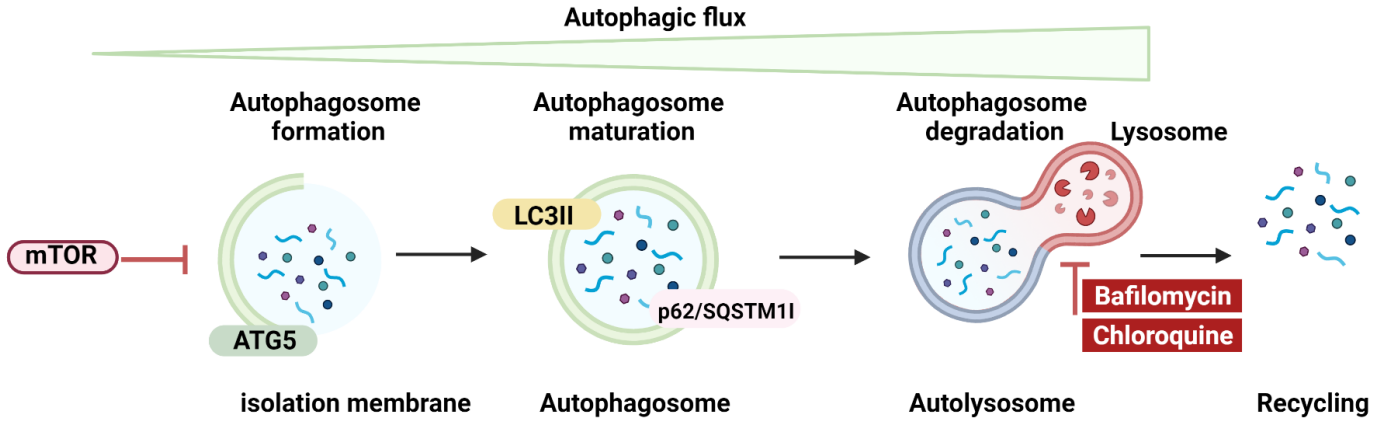
→autophagic organelles and BAFF intersect

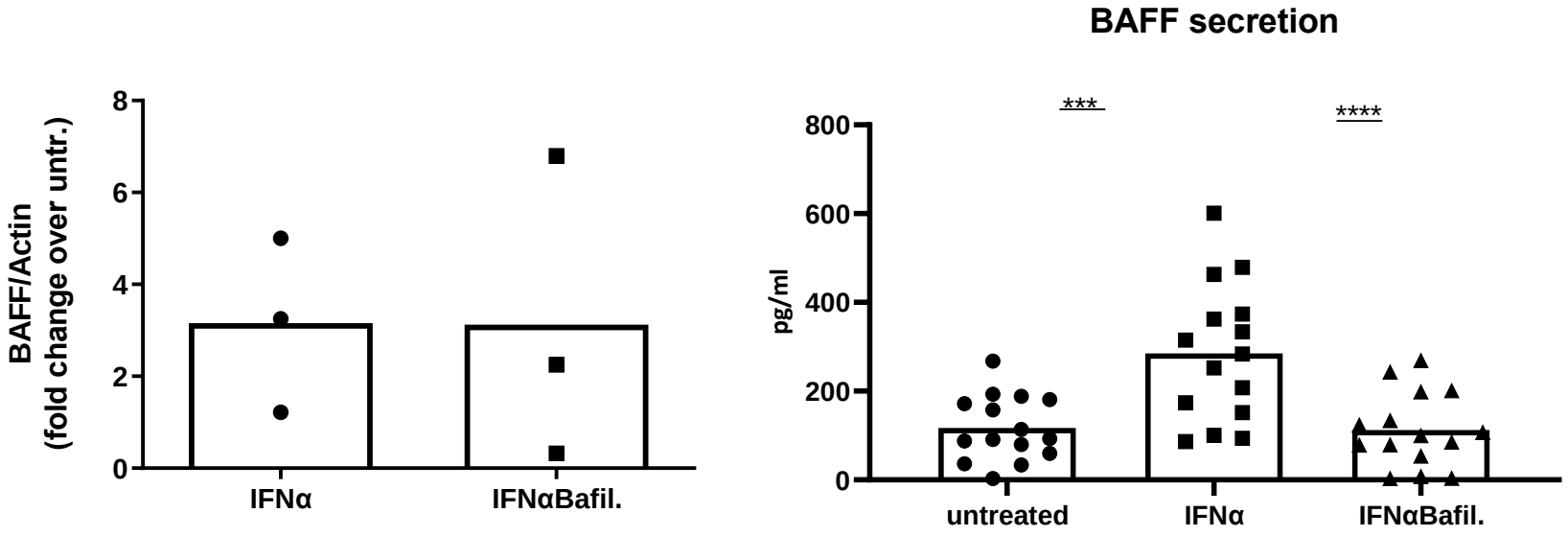


Results

Whether LC3+ organelles containing BAFF are on pathway to degradation or facilitated IFNα-induced BAFF secretion?

❑ **Inhibition** of autophagy with Bafilomycin (3hr pretr.) in CD14+ monocytes treated 18hrs with IFNα

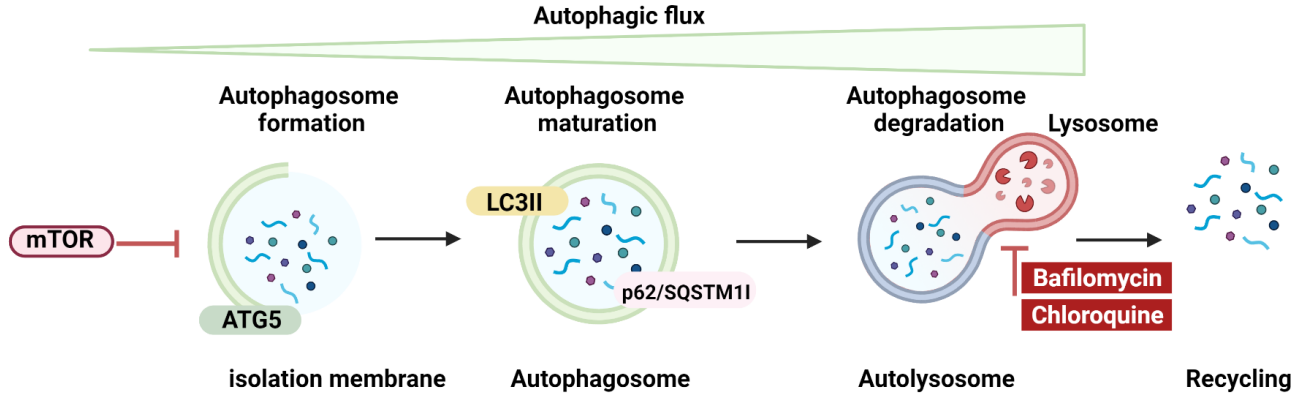




Inhibition of autophagy flux reduces BAFF secretion

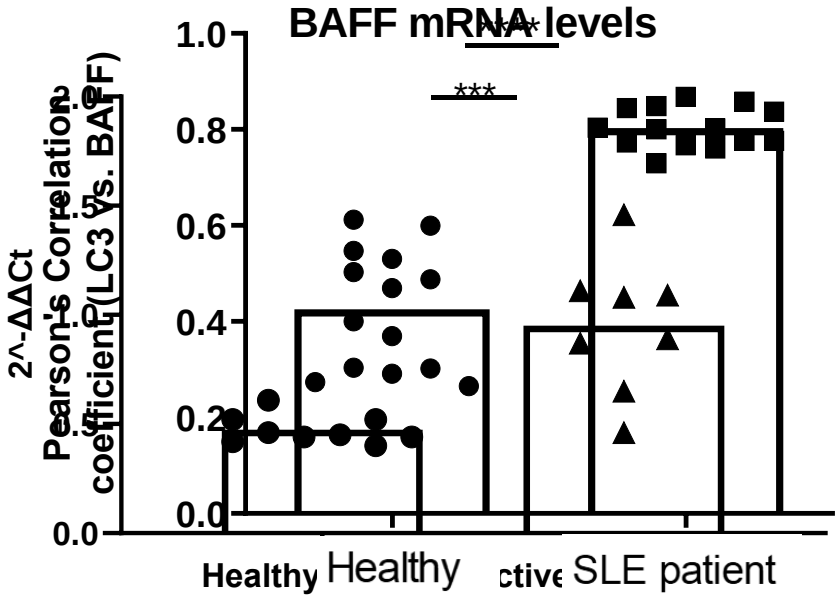
Whether LC3+ organelles containing BAFF are on pathway to degradation or facilitated IFNα-induced BAFF secretion?

- **Inhibition** of autophagy with
in CD14+ monocytes treated 18hrs with l IFNα +/- CQ

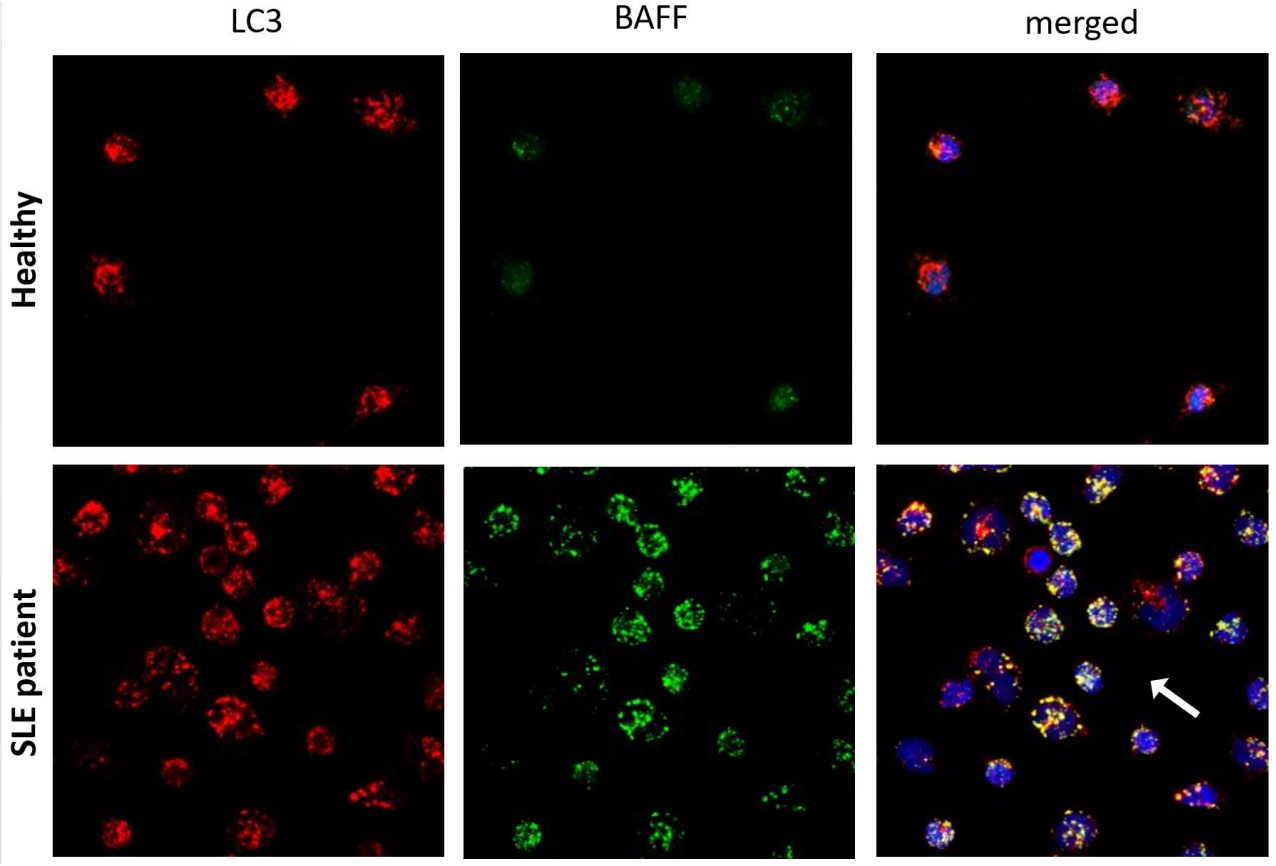


These data may imply that **BAFF is not a degradative substrate** for the autophagy-lysosomal pathway
but that **the secretion of BAFF depends on the autophagy-lysosomal pathway in IFNα-stimulated monocytes**

BAFF is transcriptionally upregulated in active SLE CD14+ monocytes



BAFF and LC3 co-localize in CD14+ monocytes derived from active SLE patients



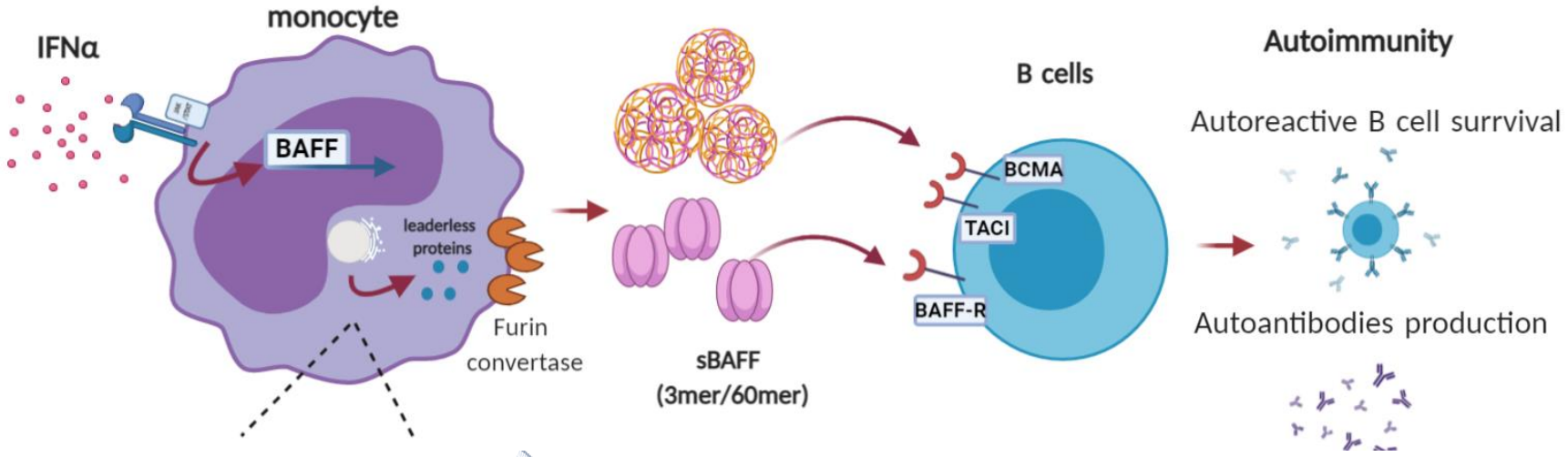
Conclusions:

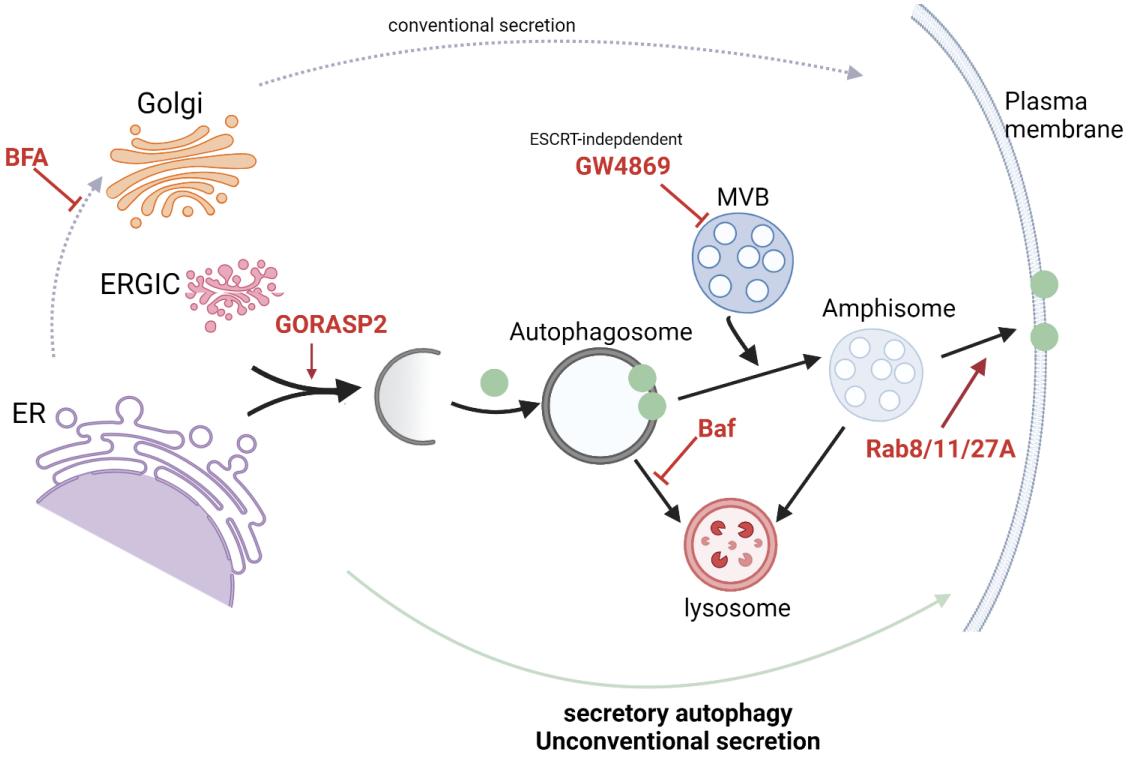
-BAFF is overexpressed and appears to be colocalized with LC3 protein in monocytes from SLE active patients and healthy donors in presence of IFNα

- Autophagic pathway appears to be involved in IFNα-induced production and secretion of BAFF

The potential of our work is to unravel for the **first time** the **impact/role of autophagy** in the **regulation of BAFF production in the context of SLE**, therefore creating the prospect for new therapeutic targets.

Pending/future work





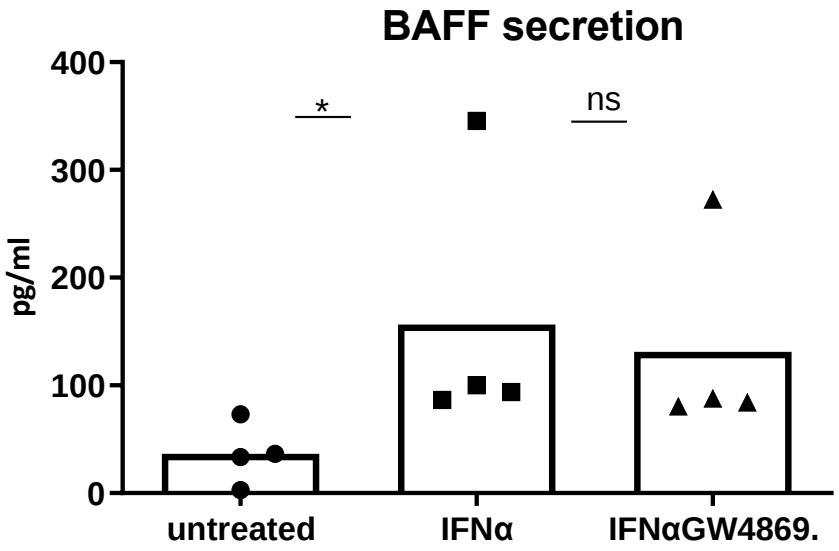
1. **Is autophagy implicated in monocyte secretion of BAFF in the context of type I IFN signaling and lupus?**

2. Does the IFNα/autophagy axis regulate the secretion of BAFF isoforms with differential bioactivity?

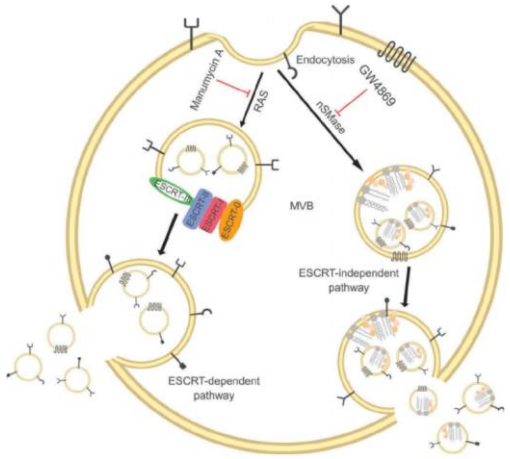
3. Could autophagy be exploited as a novel target to block BAFF secretion of lupus monocytes?

Results

Is IFNα- induced BAFF secretion regulated by MVB formation?

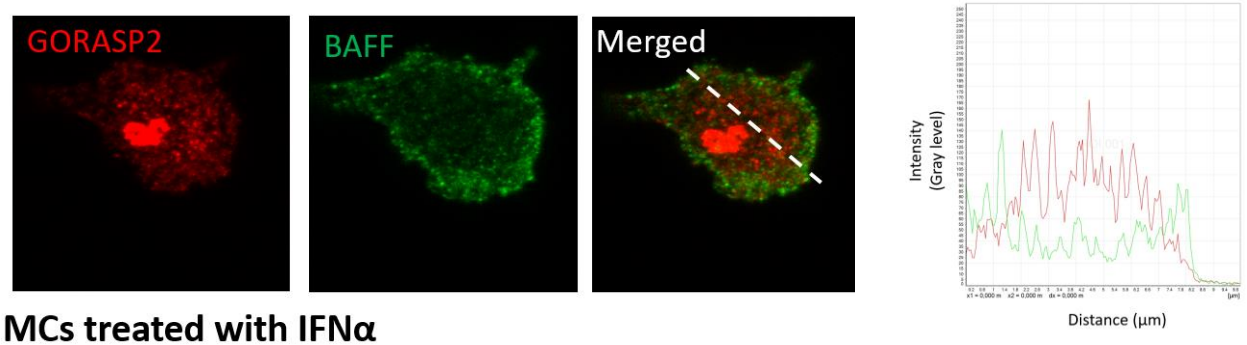


GW4869
Inhibitor of one of the two ways of MVB formation

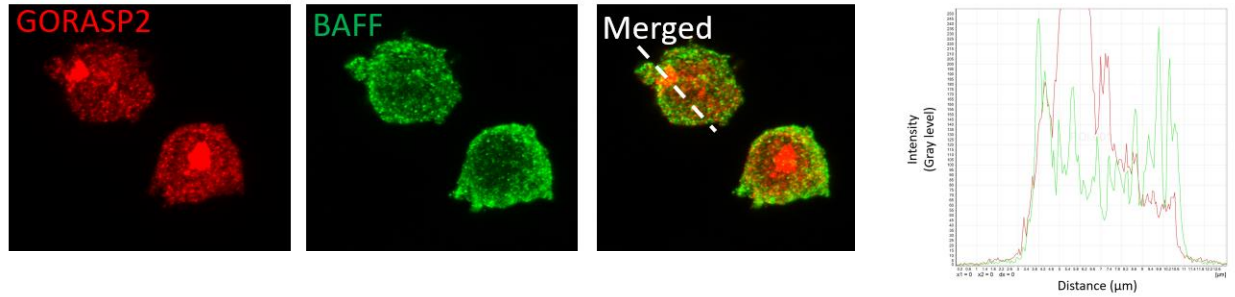


IFNα- induced BAFF and GORASP2 association?

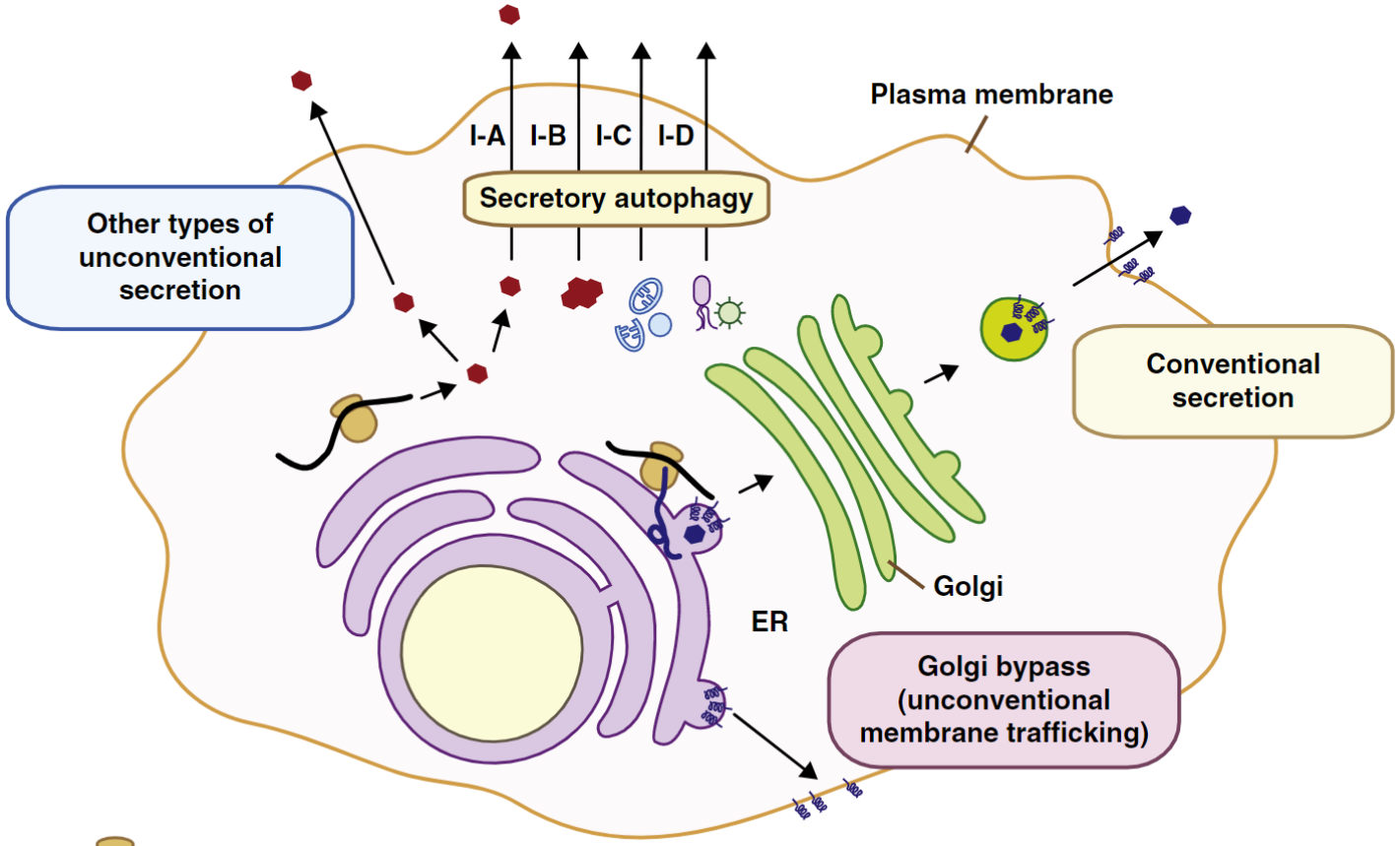
MCs untreated



MCs treated with IFNα



GORASP2/**GRASP55** and BAFF **seem to have some interaction**



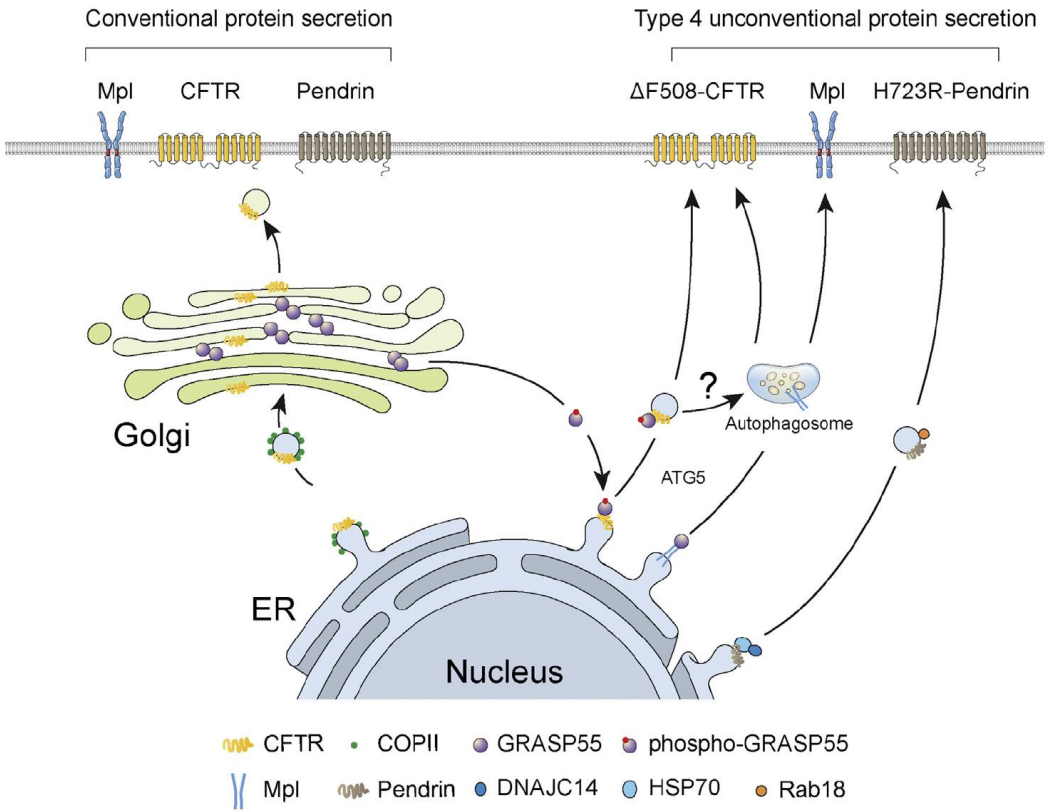
Ribosome
Unconventionally secreted proteins
Conventionally secreted proteins
mRNA
Protein aggregates
Mitochondria and organellar material
Bacteria and viruses

I-A. Unconventional secretion of cytosolic proteins with extracellular functions

I-B. Removal of aggregate-forming proteins

I-C. Extracellular release of cytoplasmic organellar material

I-D. Microbial release from cells for transmission



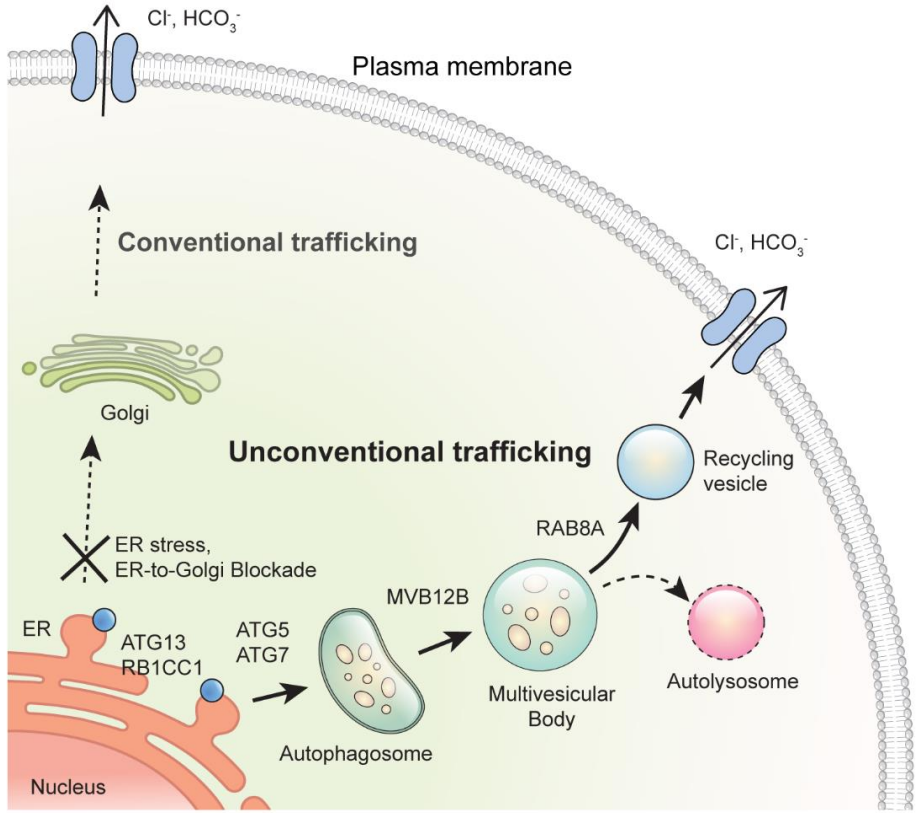


Figure S10. A model of a vesicular trafficking pathway involved in unconventional secretion of CFTR. Under normal conditions, CFTR travels to the cell surface via a conventional Golgi-mediated route. Under ER stress or an ER-to-Golgi blockade, the core-glycosylated CFTR leaves the ER and is subsequently recruited to the autophagic vacuoles and MVBs. Although some CFTRs undergo degradation pathways, other CFTRs travel to recycling vesicles via MVB12B- and RAB8A-mediated mechanisms and eventually travel to the cell surface.