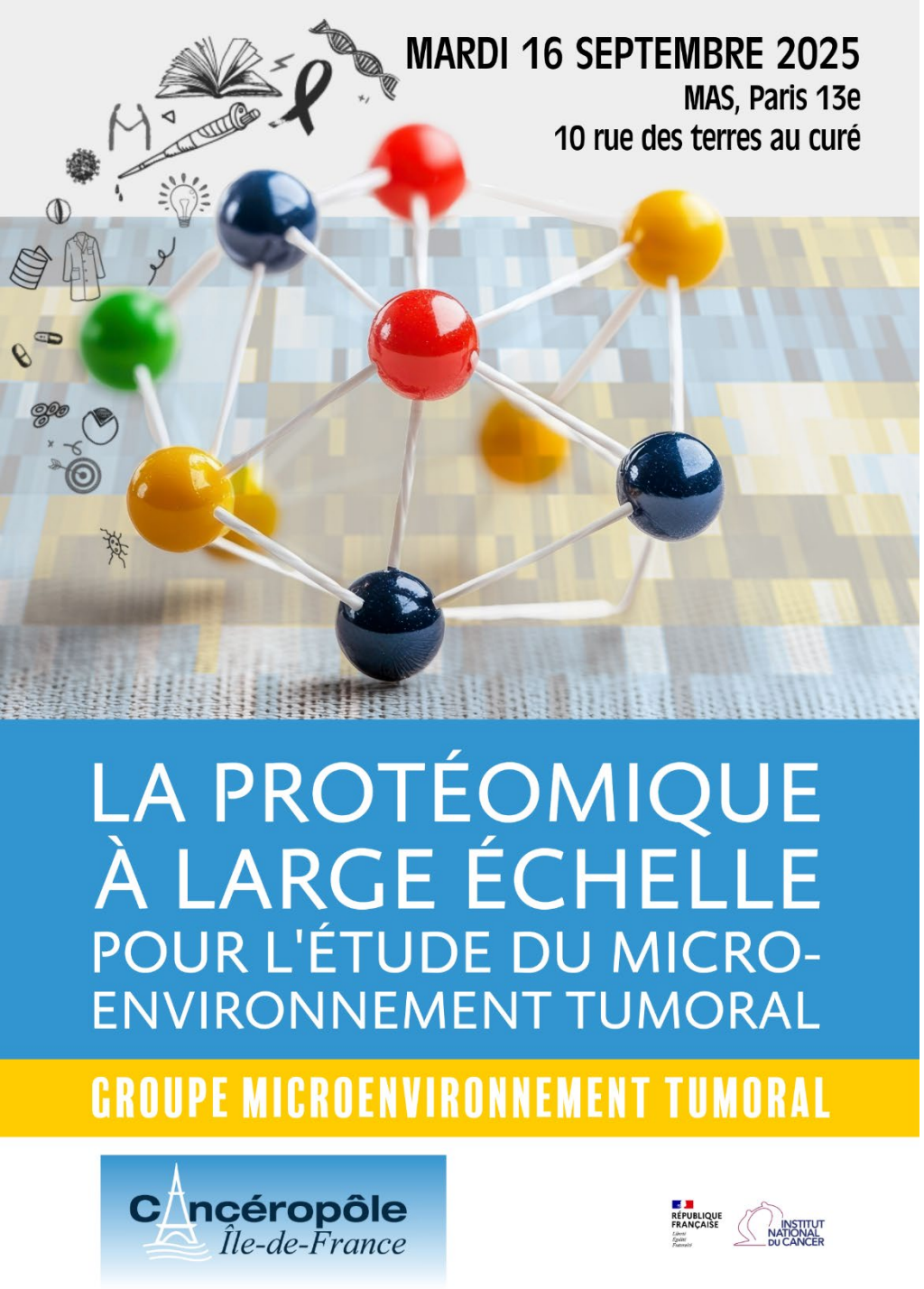
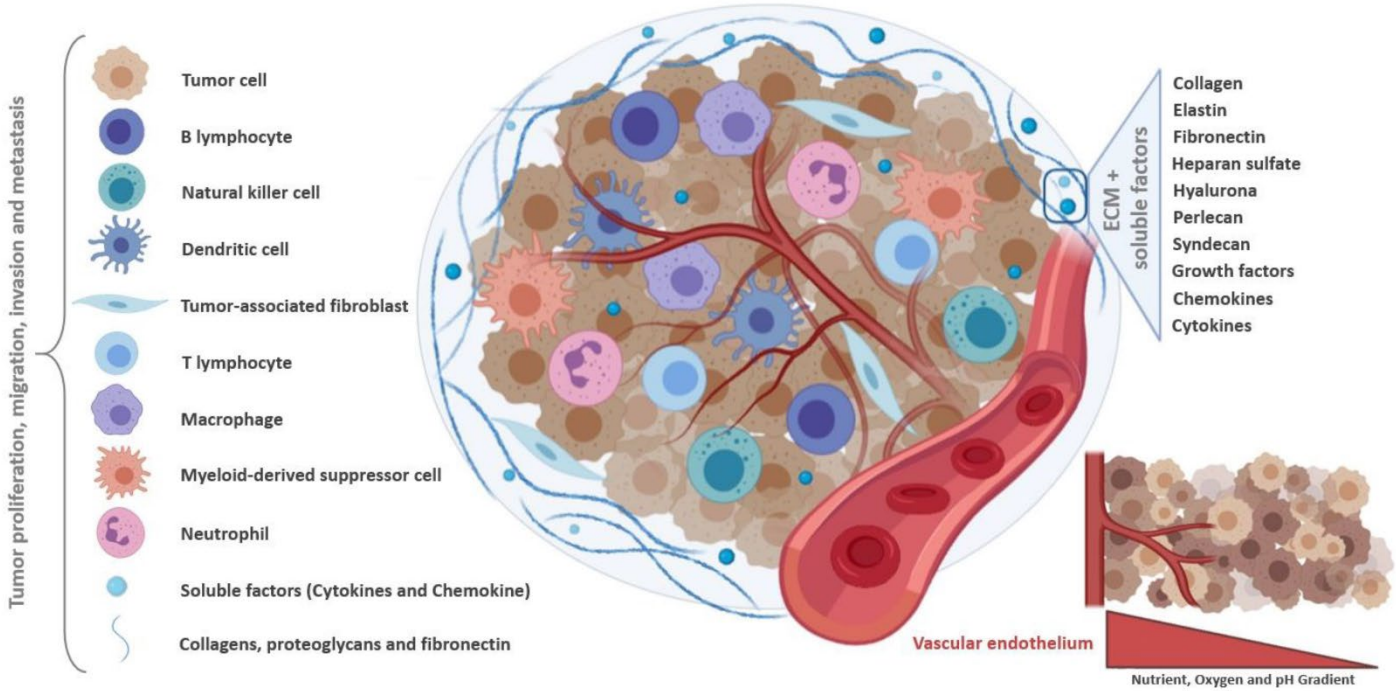


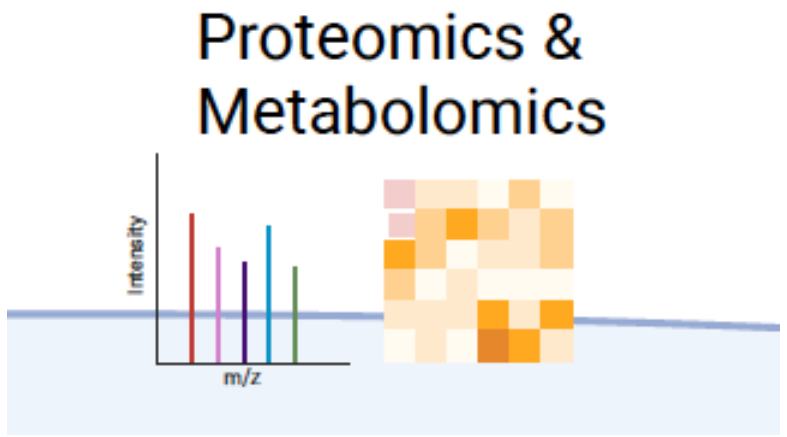
Mass spectrometry –based proteomics (and metabolomics) to elucidate tumor-microenvironment metabolic interactions

Angela Bachi

IFOM – AIRC Institute of Molecular Oncology
angela.bachi@ifom.eu

Tumor MicroEnvironment





Soluble factors

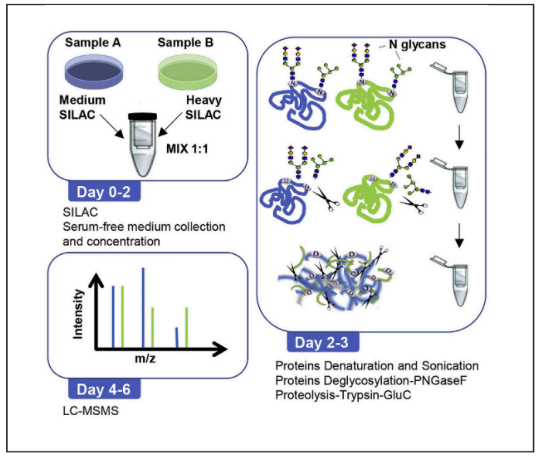
Metastatic melanoma secretome is enriched in amyloid aggregates

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Secret3D Workflow for Secretome Analysis



Vittoria Matafora, Angela Bachi

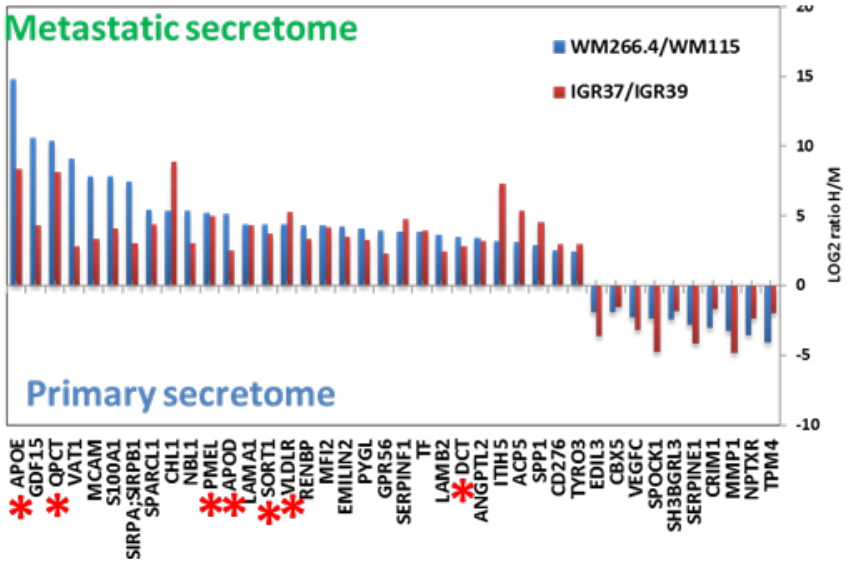
vittoria.matafora@ifom.eu (V.M.)
angela.bachi@ifom.eu (A.B.)

HIGHLIGHTS

Secret3D workflow allows the analysis of secreted proteins from *in vitro* cultured cells

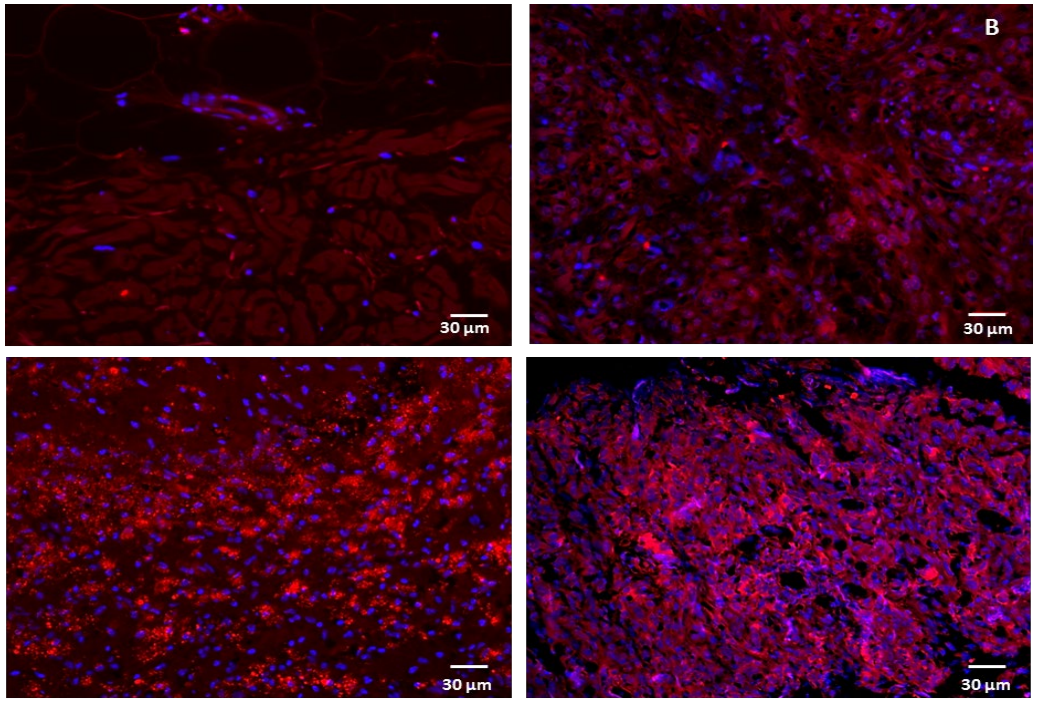
De-glycosylation and double digestion enhance protein identification and quantification

Identification of putative glycosylation sites of the secreted proteins



Healthy skin

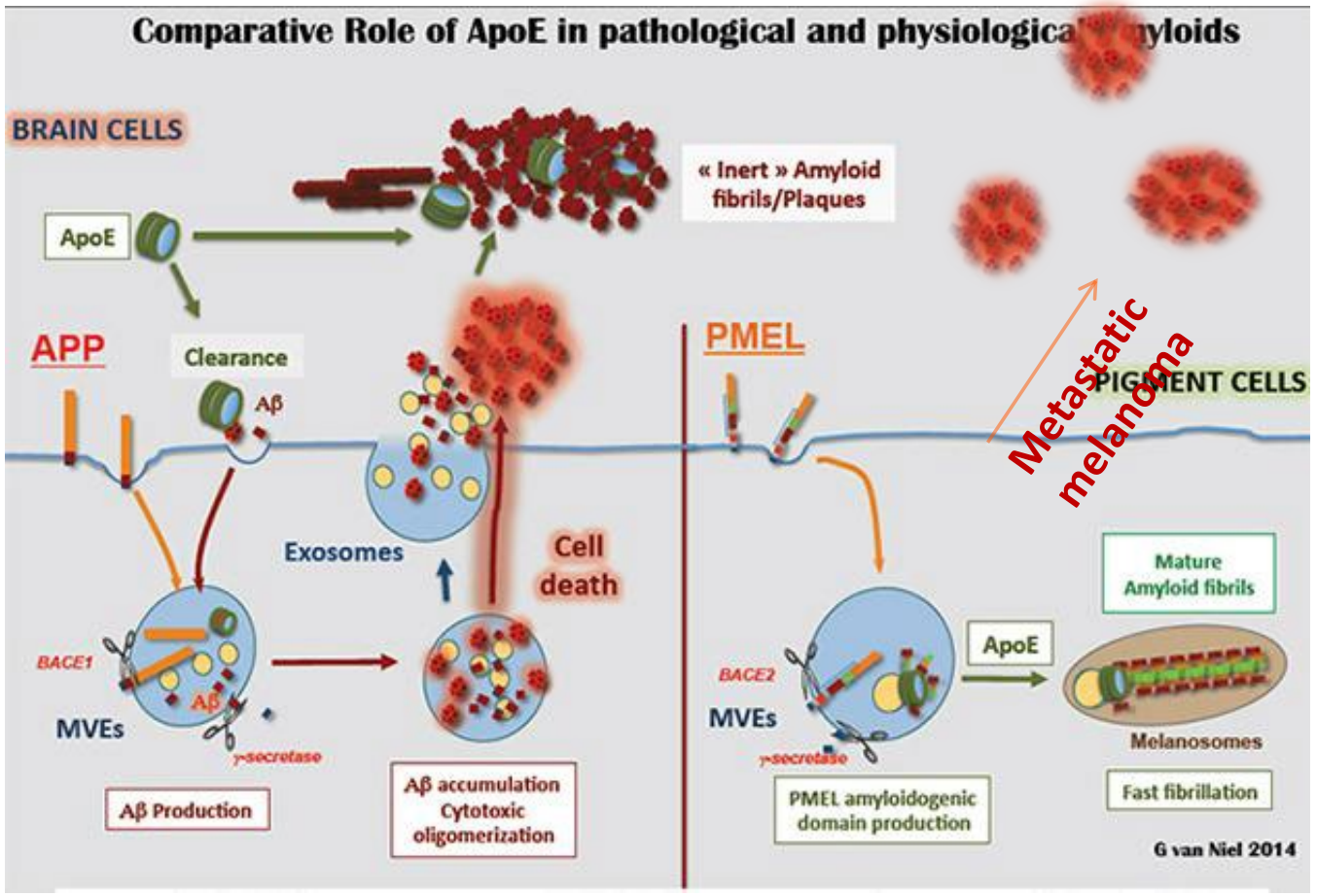
Primitive tumor



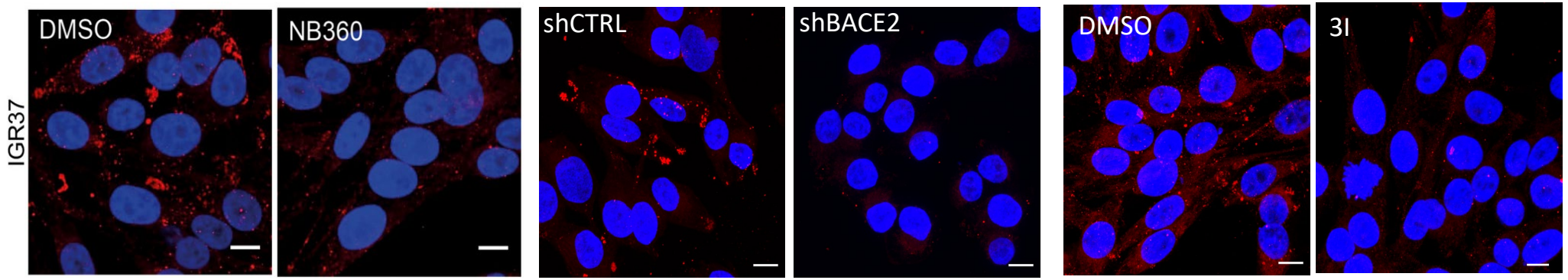
Brain metastasis

Lung metastasis

How are amyloids produced?



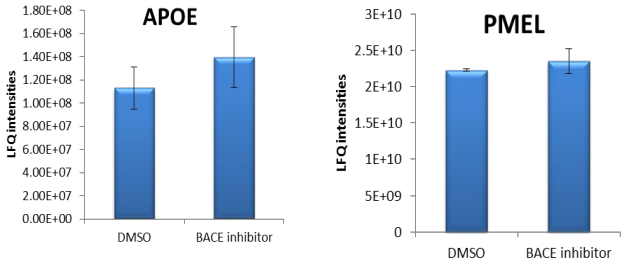
BACE2 produces amyloid aggregates in melanoma



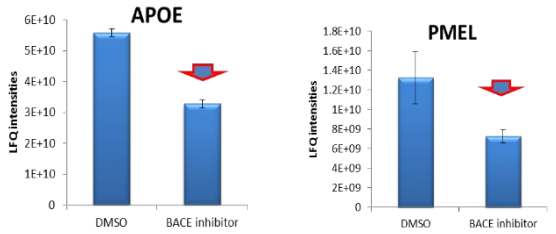
DAPI + Proteostat

DAPI + Proteostat

DAPI + Proteostat



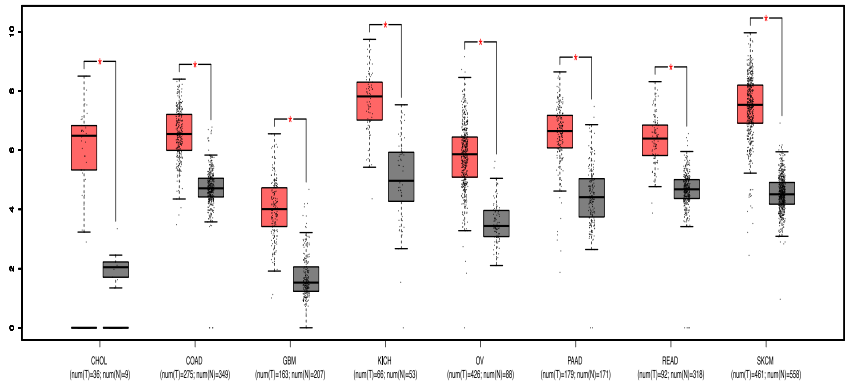
proteome

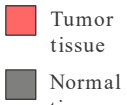


secretome

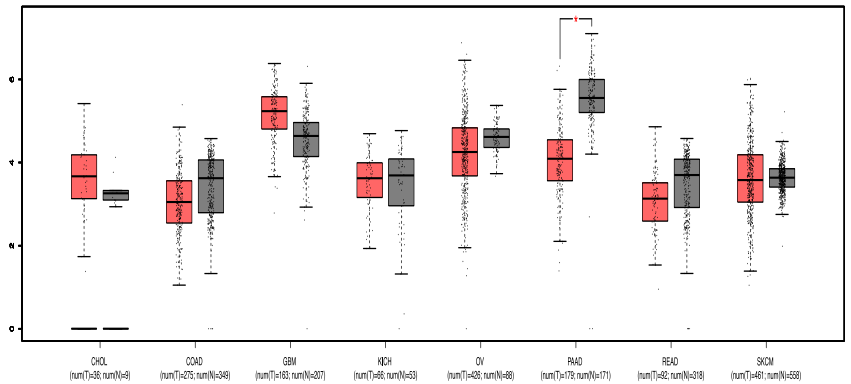
BACE2 in cancer

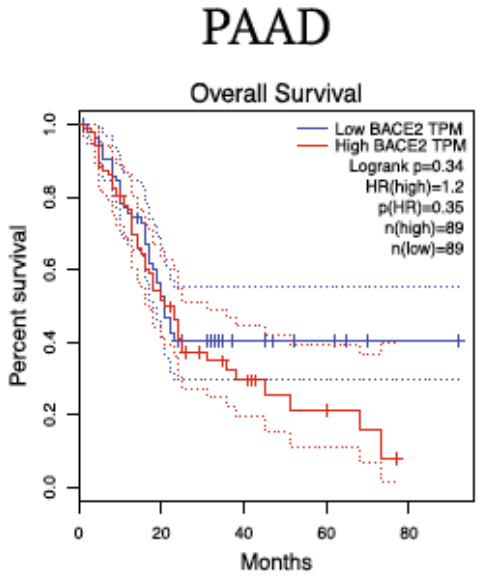
BACE2

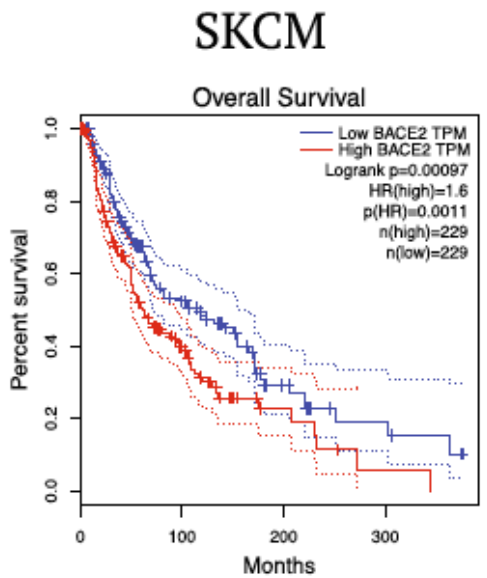




BACE1



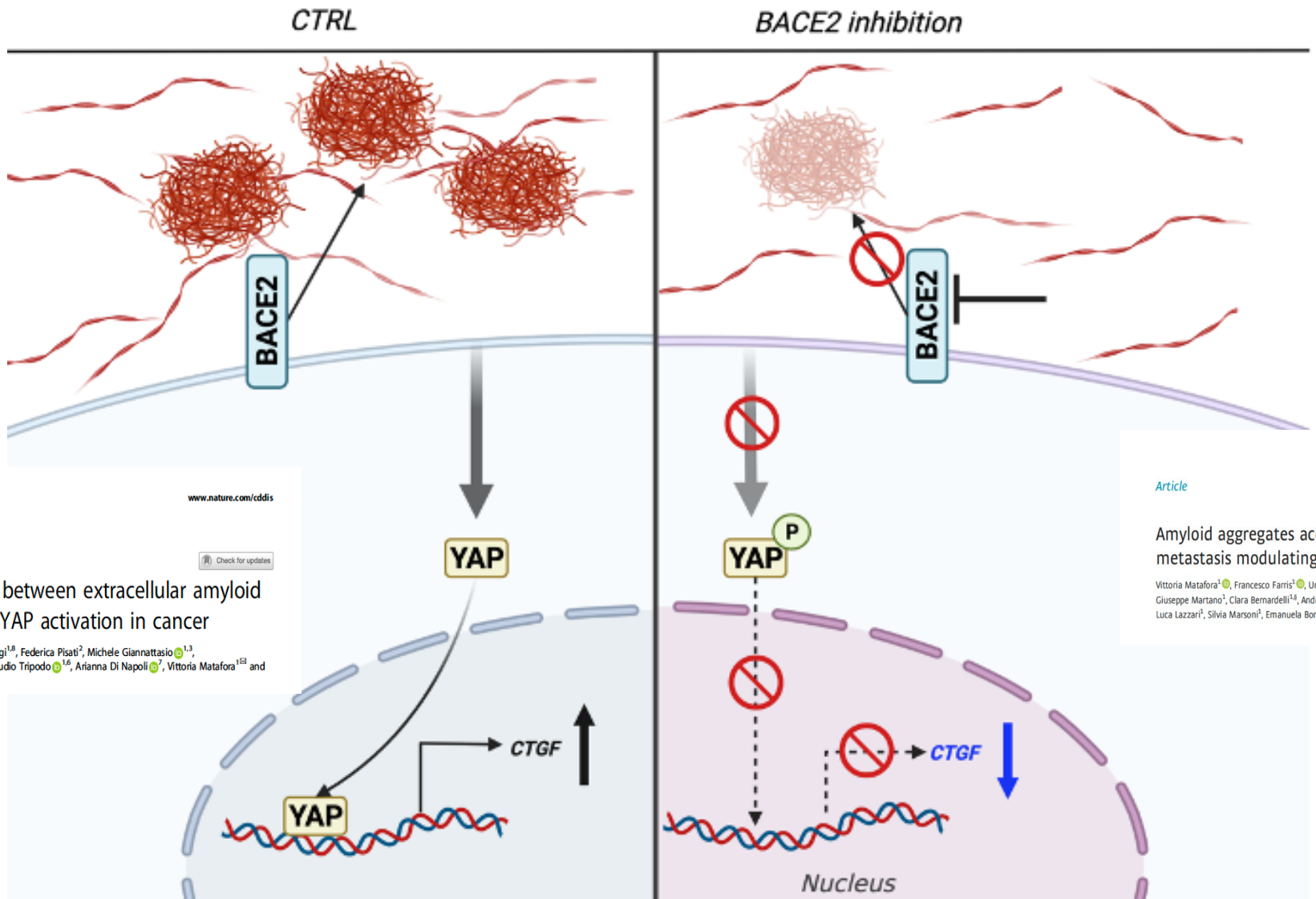




The emerging role of β-secretases in cancer

Farris *et al. Journal of Experimental & Clinical Cancer Research*
https://doi.org/10.1186/s13046-021-01953-3

Amyloid aggregates are new players in tumor microenvironment



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ARTICLE OPEN

Check for updates

Unveiling the mechanistic link between extracellular amyloid fibrils, mechano-signaling and YAP activation in cancer

Francesco Farris[1], Alice Elhagh[1], Ilaria Vigorito[1], Nicoletta Alongi[1,8], Federica Pisati[2], Michele Giannattasio[1,3], Francesca Casagrande[1,9], Lisa Veghini[4], Vincenzo Corbo[4,5], Claudio Tripodo[1,6], Arianna Di Napoli[7], Vittoria Matafora[1✉] and Angela Bachi[1✉]

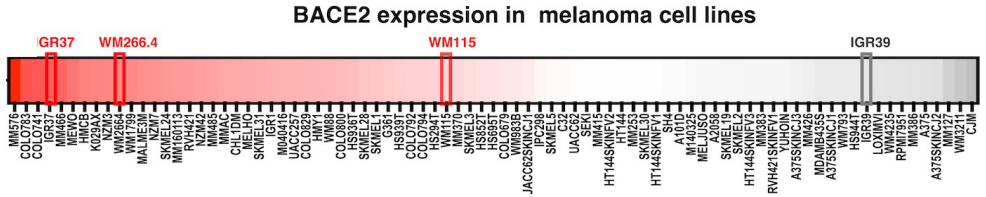
Article

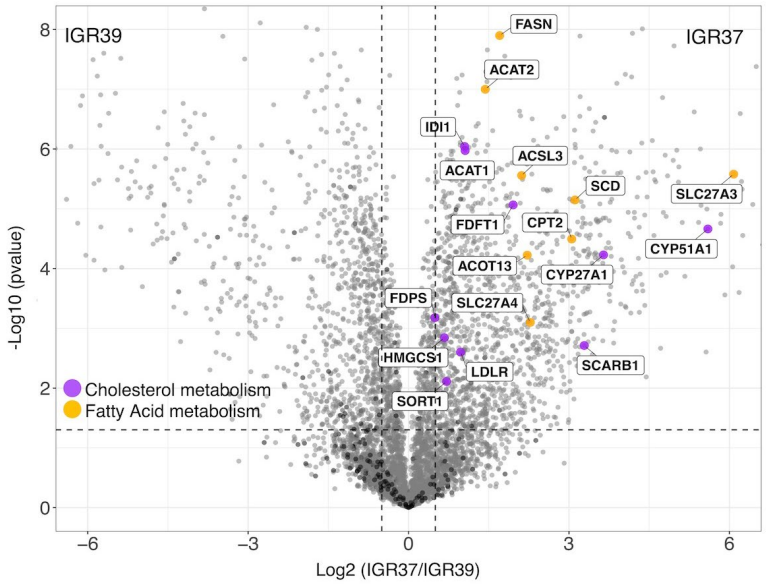
EMBO reports

Amyloid aggregates accumulate in melanoma metastasis modulating YAP activity

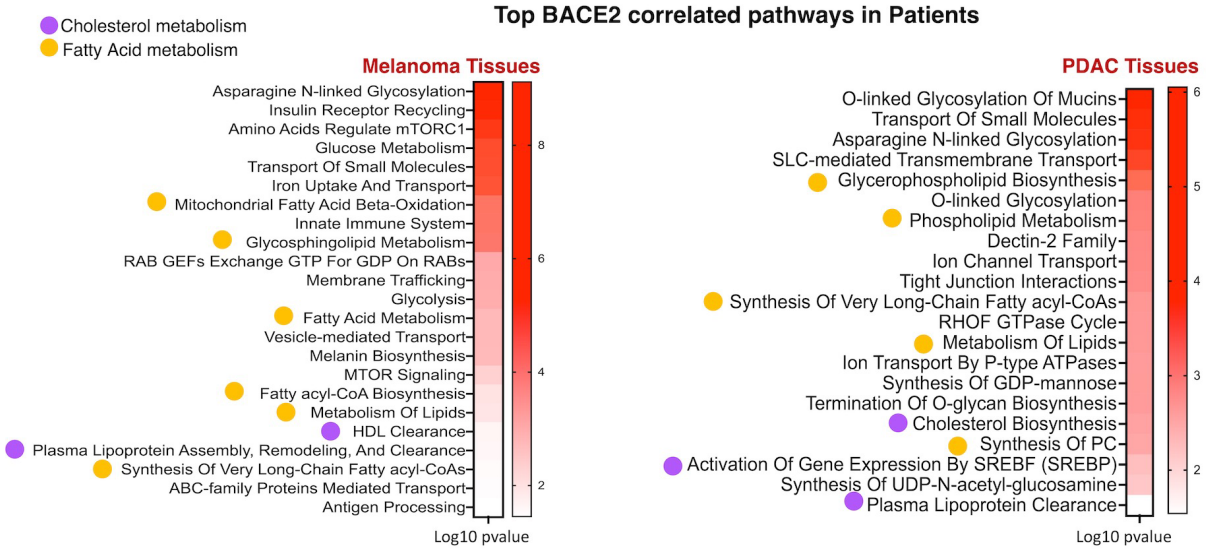
Vittoria Matafora[1], Francesco Farris[1], Umberto Restuccia[1,†], Simone Tamburri[1,‡], Giuseppe Martano[1], Clara Bernardelli[3,8], Andrea Sofia[1,2], Federica Pisati[1,3], Francesca Casagrande[1], Luca Lazzari[1], Silvia Marsoni[1], Emanuela Bonoldi[4] & Angela Bachi[1,*]

BACE2 in cancer is linked to lipid metabolism



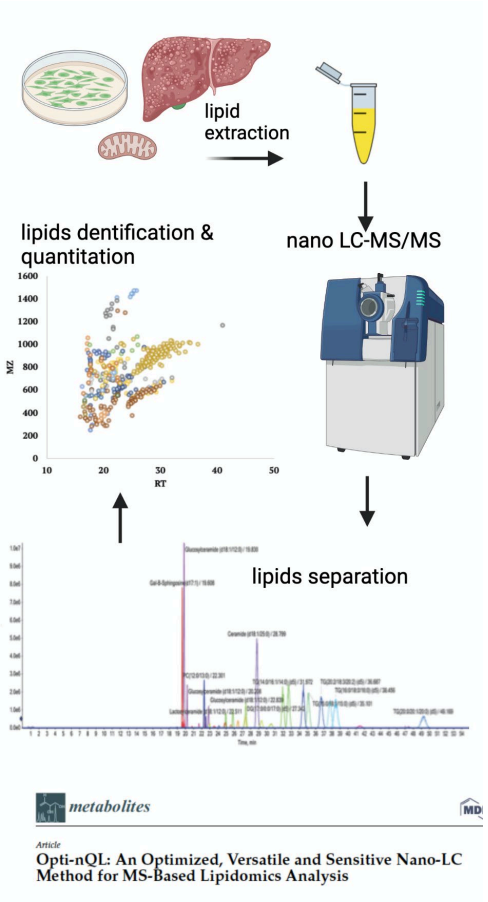


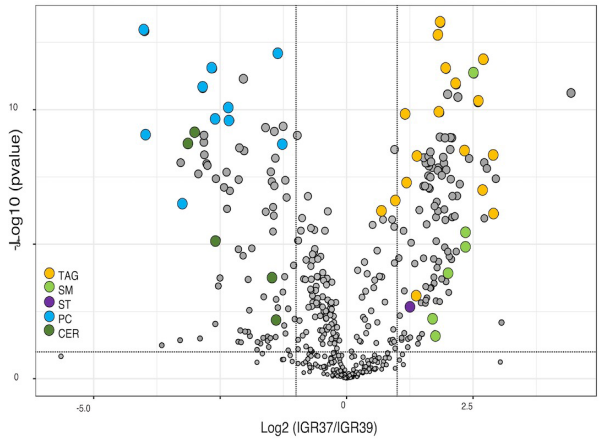
Gene Expression Profiling Interactive Analysis

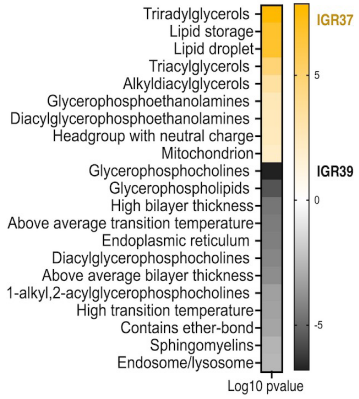


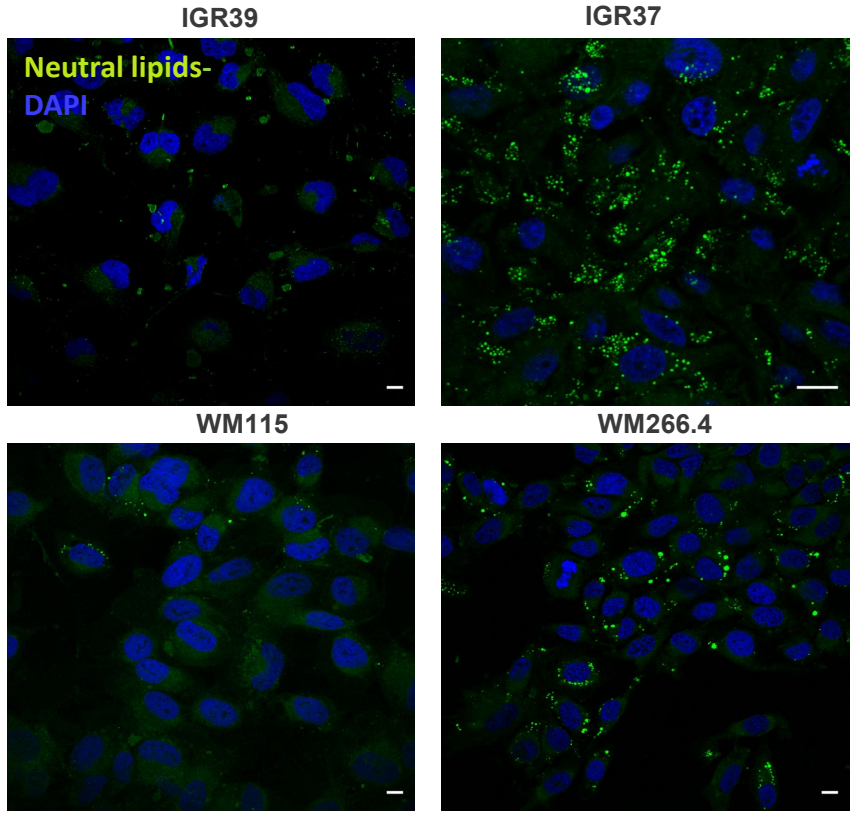
Unpublished data

BACE2 in cancer is linked to lipid metabolism



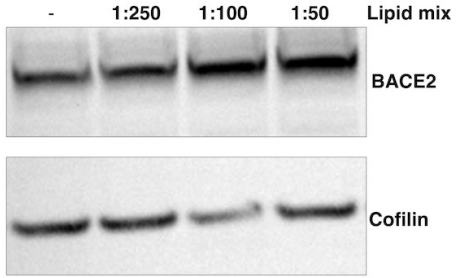


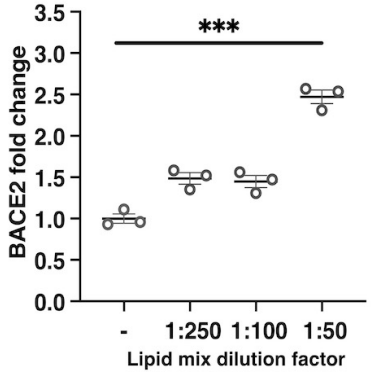


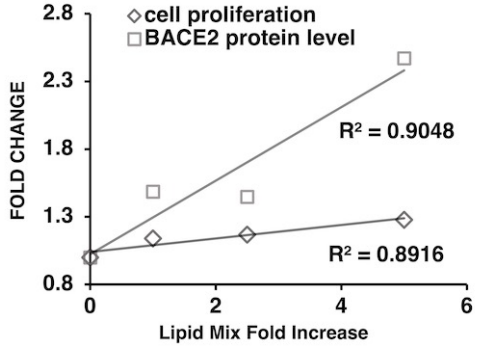


Unpublished data

Increasing lipid availabilty promotes BACE2 expression and cell proliferation



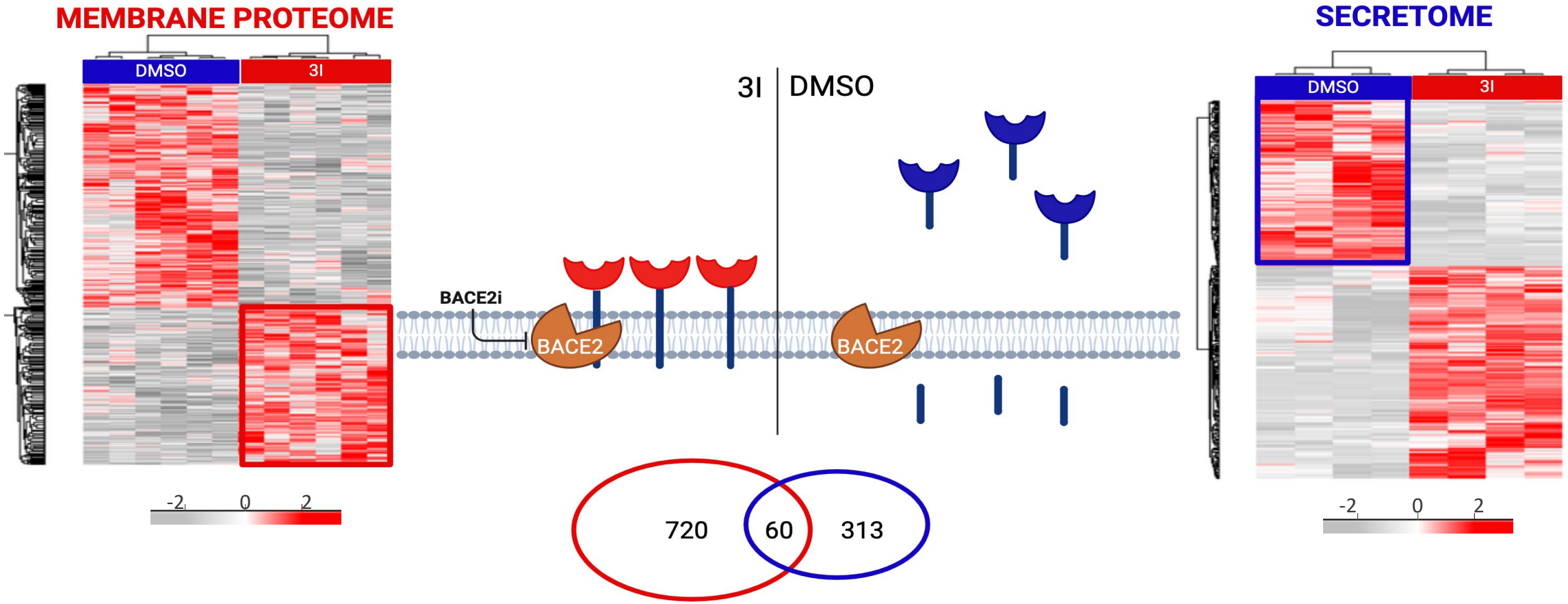




- High BACE2 cancers show an enhaced lipid metabilism
- Increasing extracellular lipids increases BACE2 levels
- More BACE2, higher proliferation of cancer cells

Unpublished data

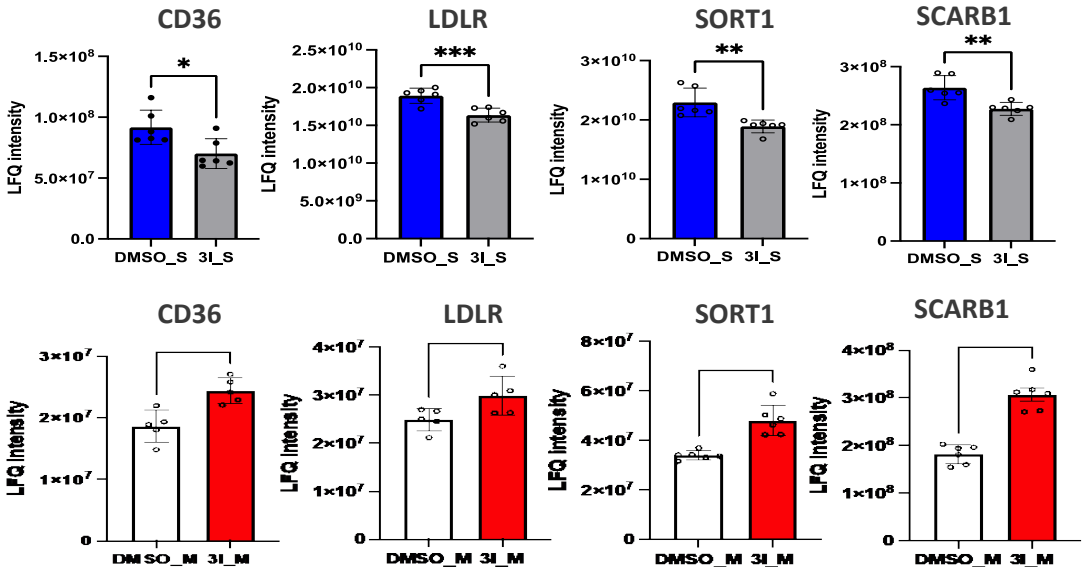
What is the molecular link between lipid metabolism and BACE2 activity?

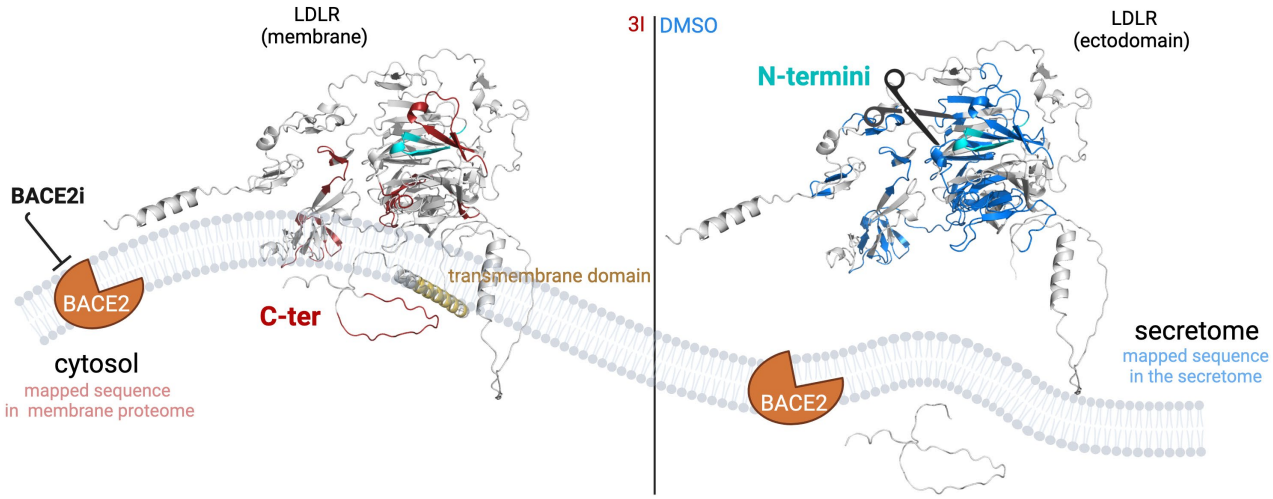


Unpublished data

BACE2 regulates lipid transporters ectodomain shedding

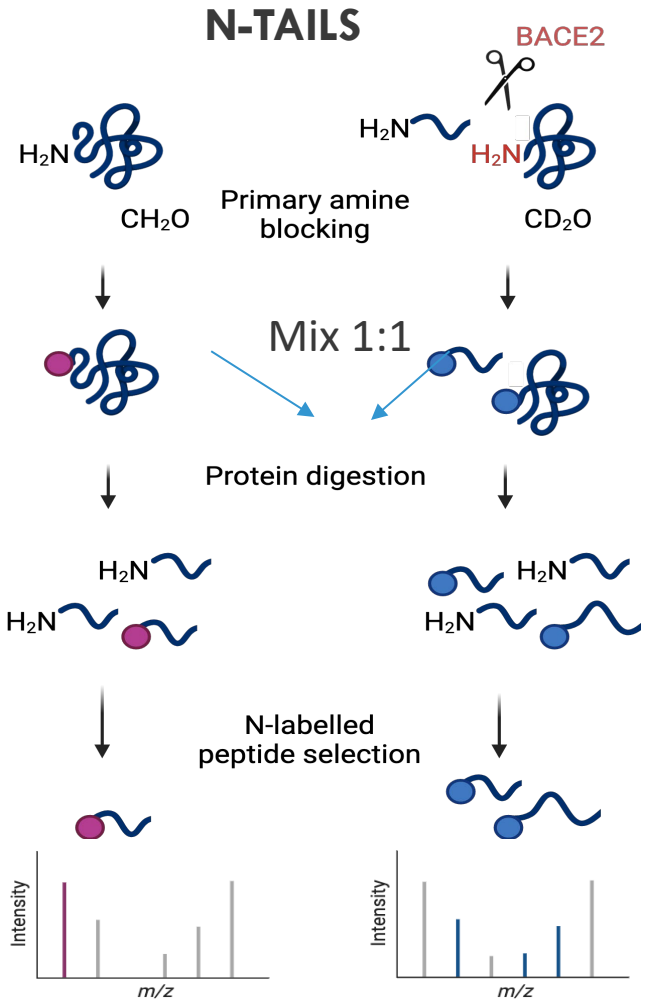
LIPID TRANSPORTERS

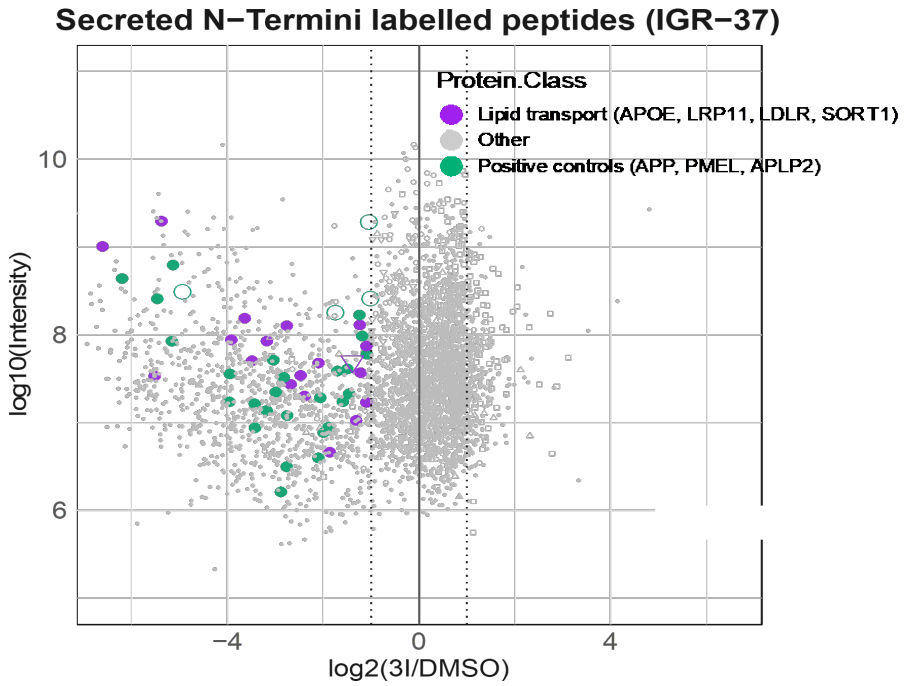


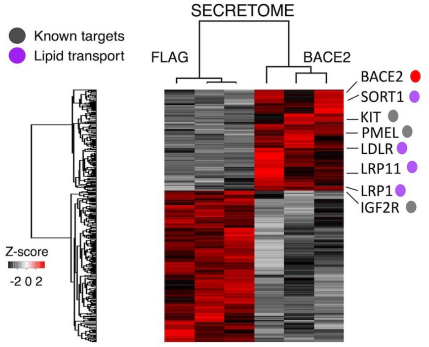


Unpublished data

BACE2 regulates lipid transporters ectodomain shedding



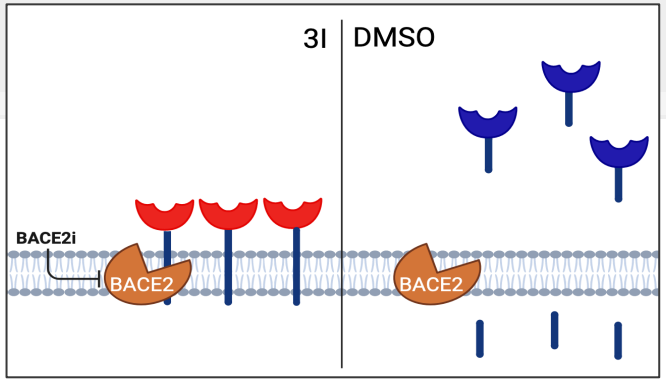




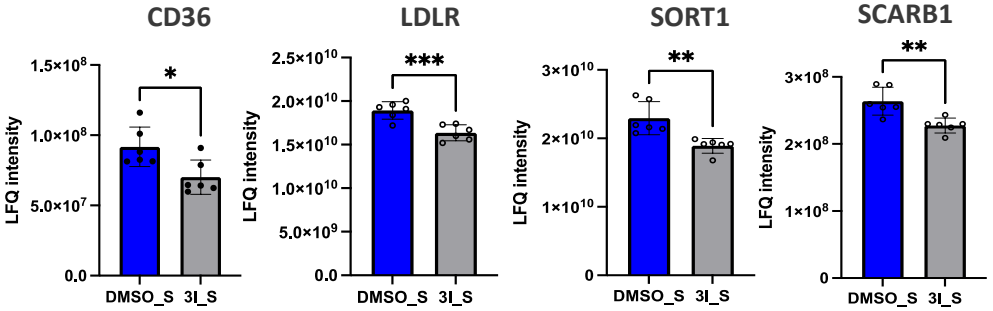
BACE2 overexpression in HEK

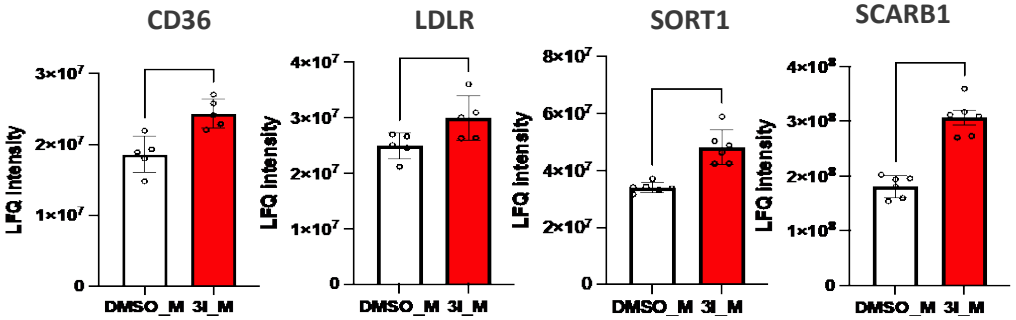
Unpublished data

BACE2 specifically acts on lipid transporters

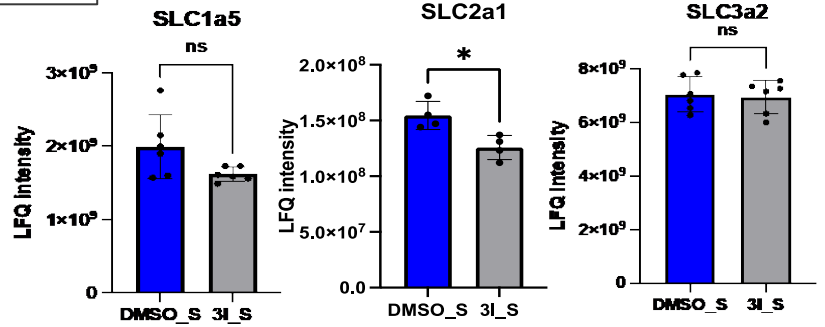


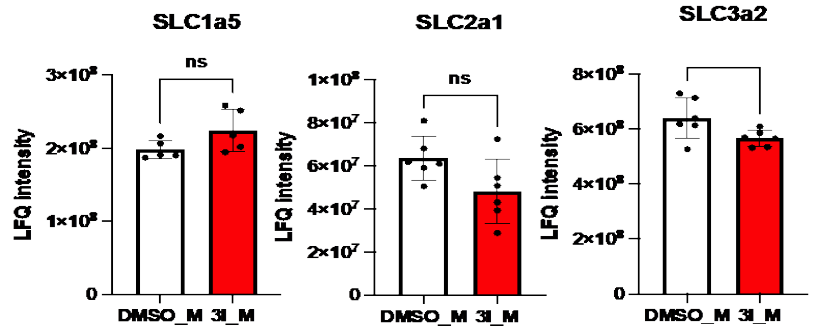
Lipid Transporters





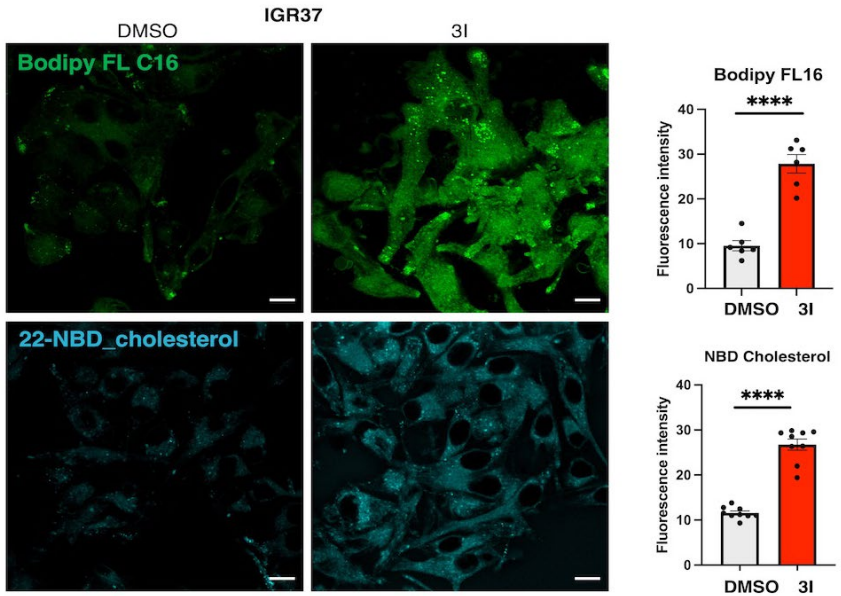
AA and Glucose Transporters

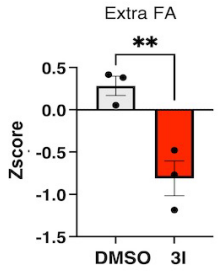


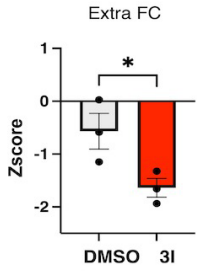


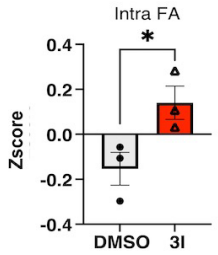
Unpublished data

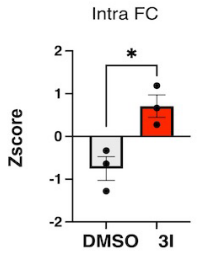
BACE2 regulates lipid transporters activity and lipid uptake











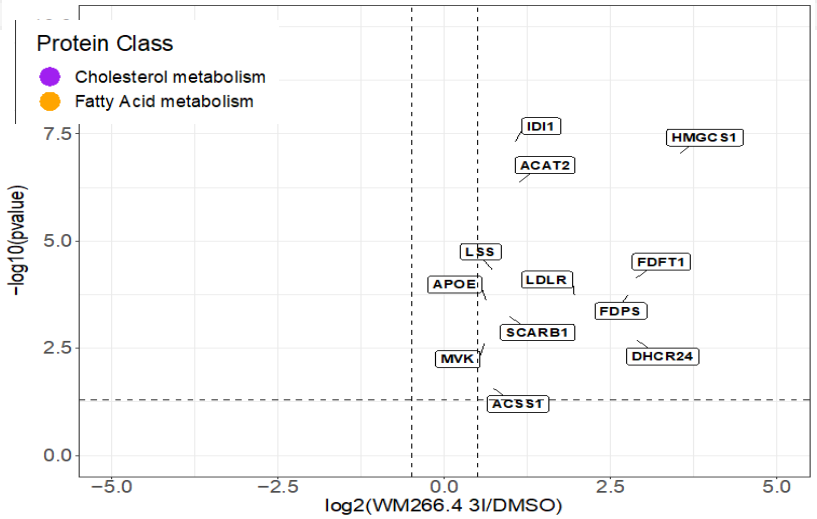
Unpublished data

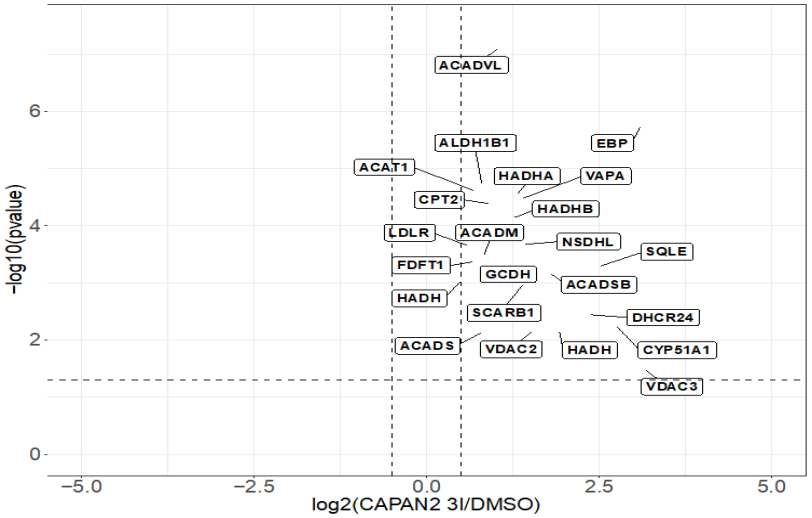
Which are the functional consequences of modifying lipid uptake in cancer cells?

- High BACE2 cancers show an enhaced lipid metabilism
- Increasing extracellular lipids increases BACE2 levels
- BACE2 tunes lipid influx by modulating lipid transporters activity
- More BACE2, higher proliferation of cancer cells

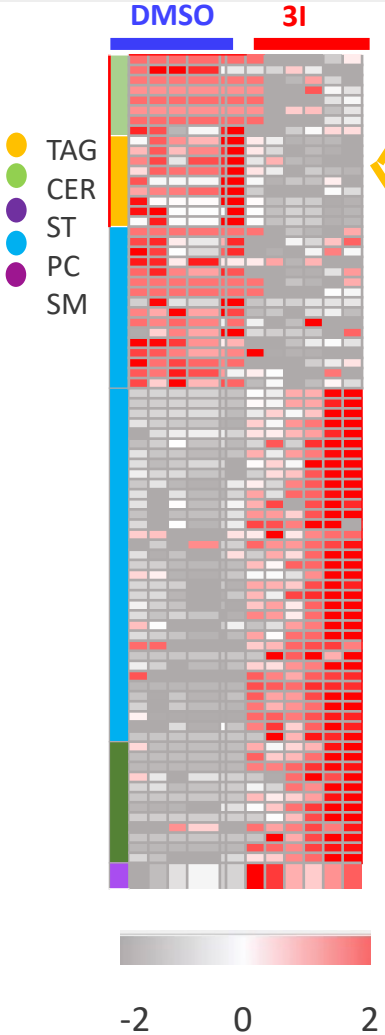
BACE2 affects lipid metabolism and proliferation

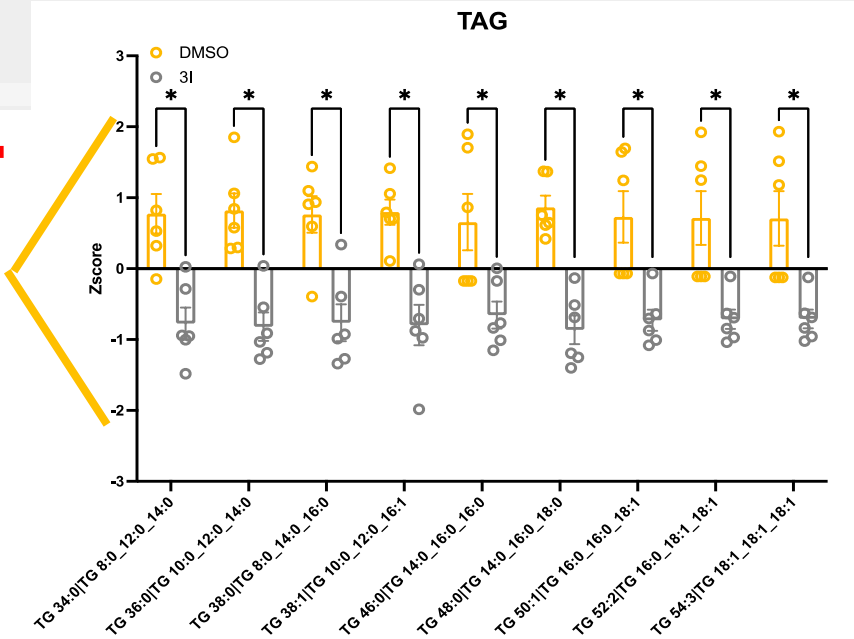
PROTEOMICS

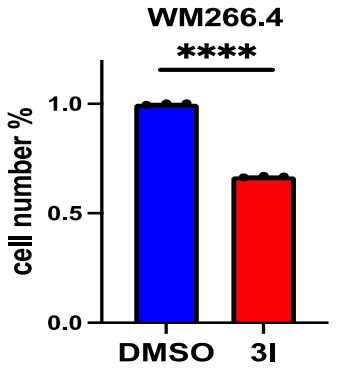




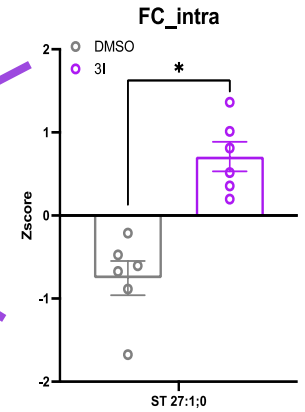
LIPIDOMICS

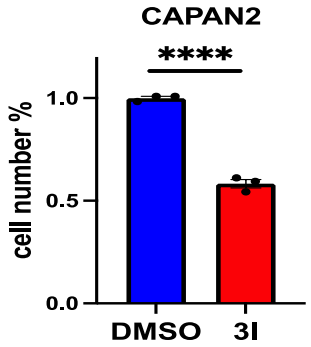






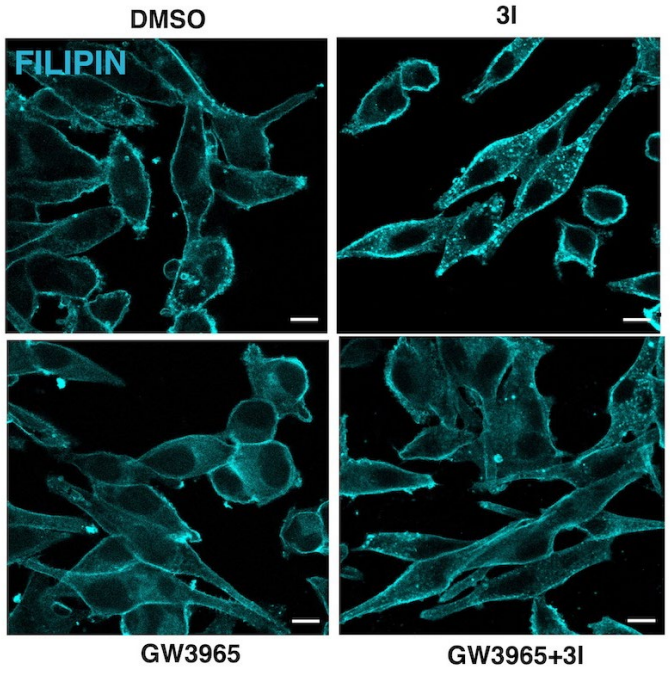
Free cholesterol

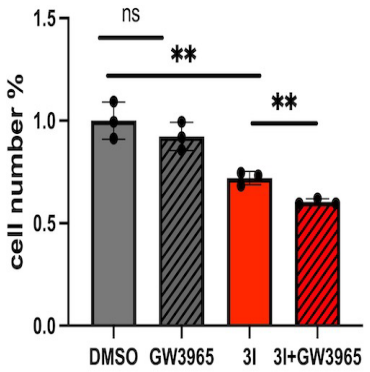


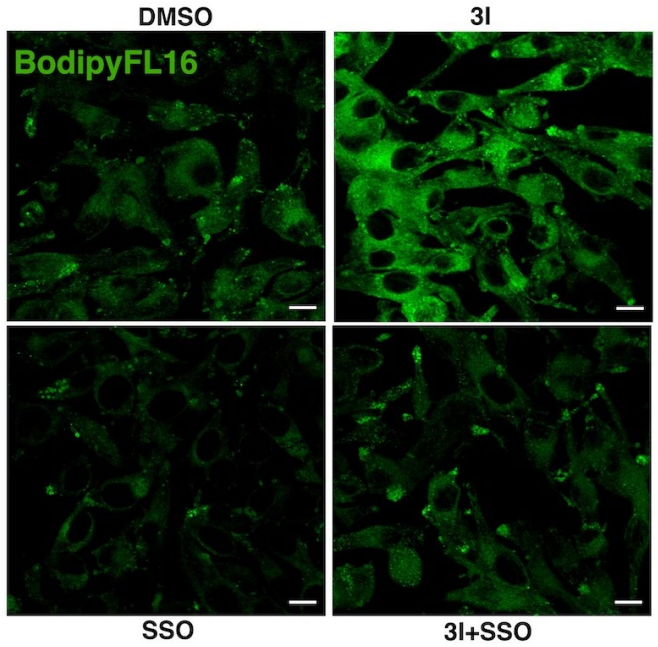


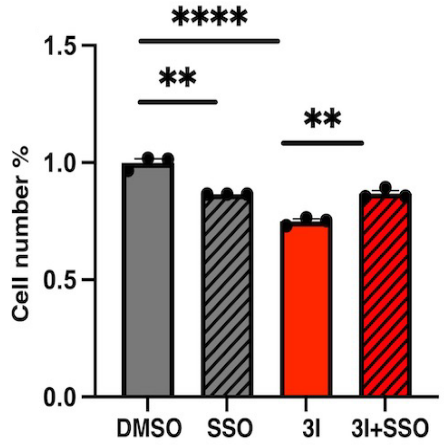
Unpublished data

Which lipids interfere with cell proliferation?





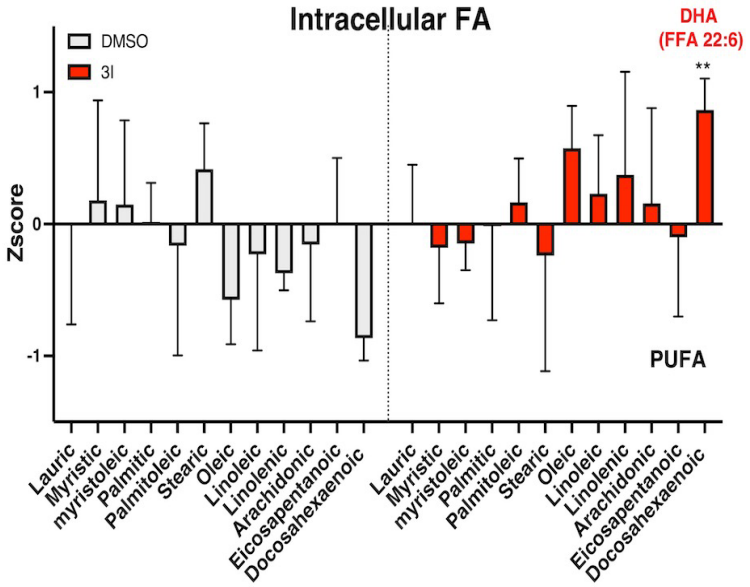


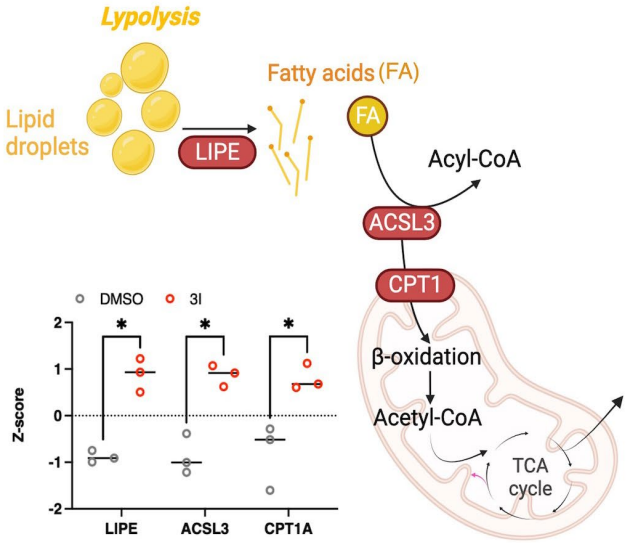


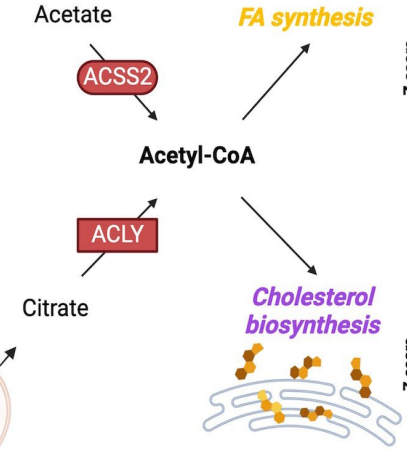
FA influx is a major contributor to the anti-proliferative effects of BACE2 inhibition.

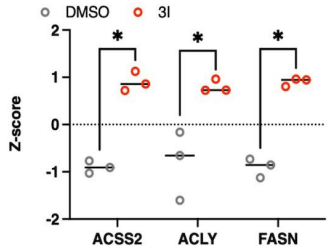
Unpublished data

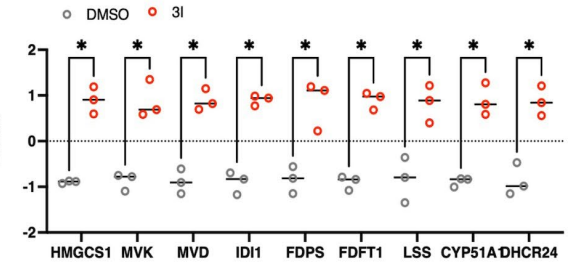
BACE2 increases intracellular PUFA and activates PPRα









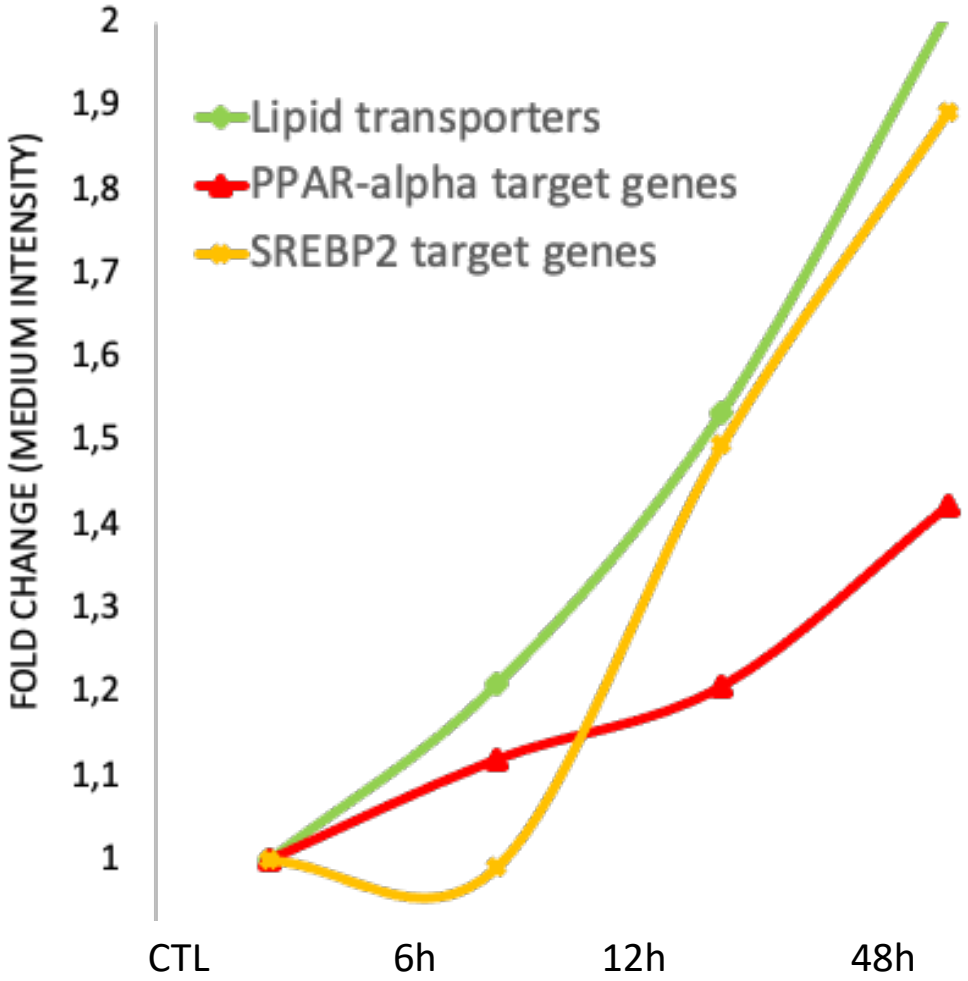


PPRα target genes

Unpublished data

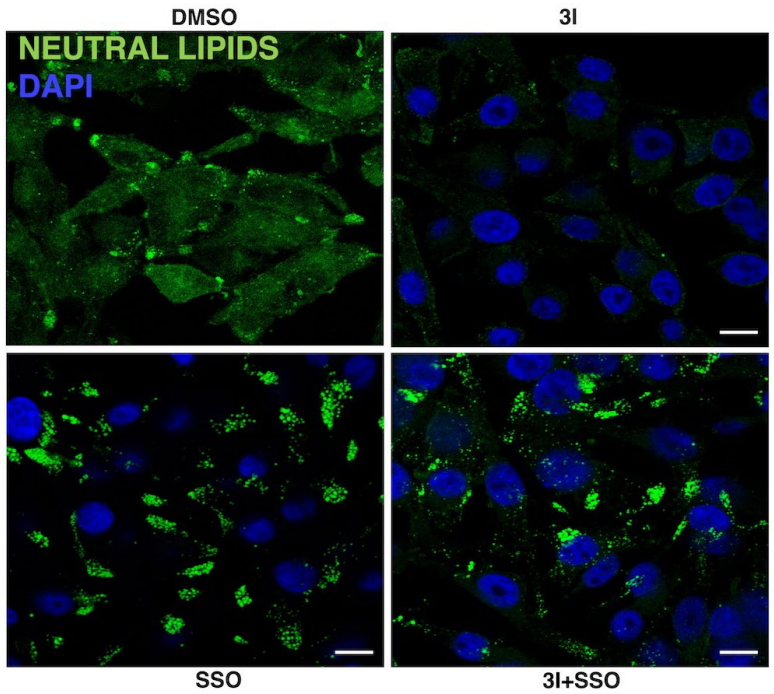
Lipid transporters accumulation is faster than lypolisi and steroidogenesis

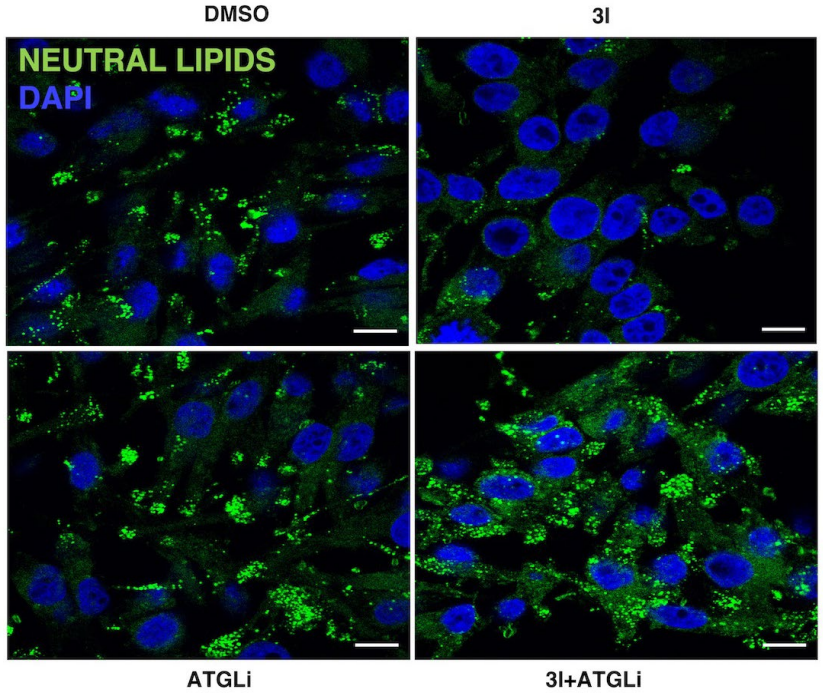
Gene name	DMSO	6h	12h	48h
SORT1				
CD36				
PTGFRN				
LDLR				
LRP1				
SCARB2				
SCARB1				
CD63				
LIPE				
LIPA				
CPT2				
ACSL1				
LSS				
CYP51A1				
FDFT1				
DHCR24				
DHRS4				
HMGCS1				
SREBF2				

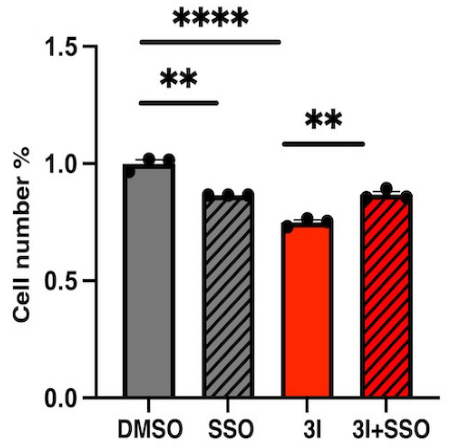


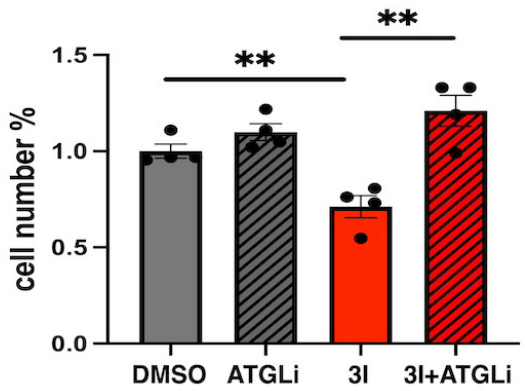
Proteomics analysis

Blocking lipolysis rescues the proliferation impairment induced by BACE2 inhibition



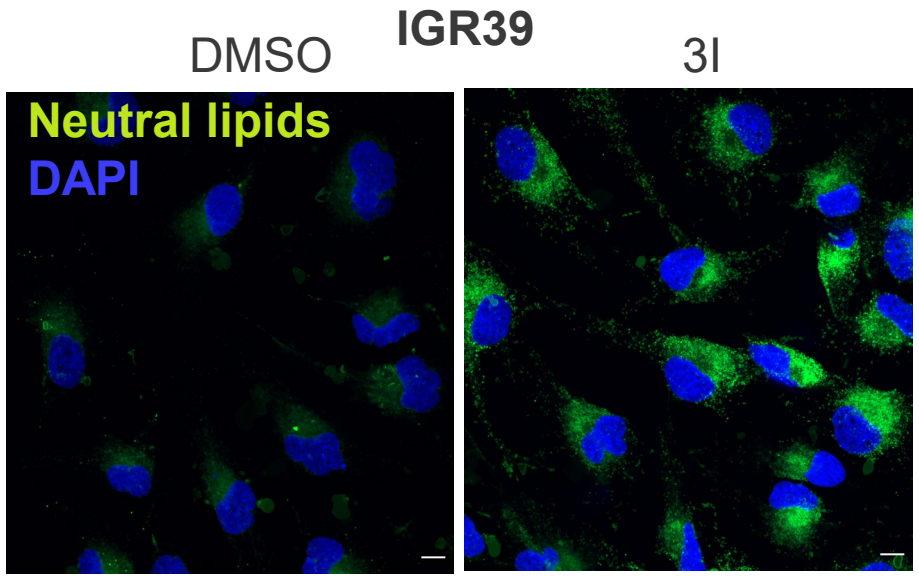


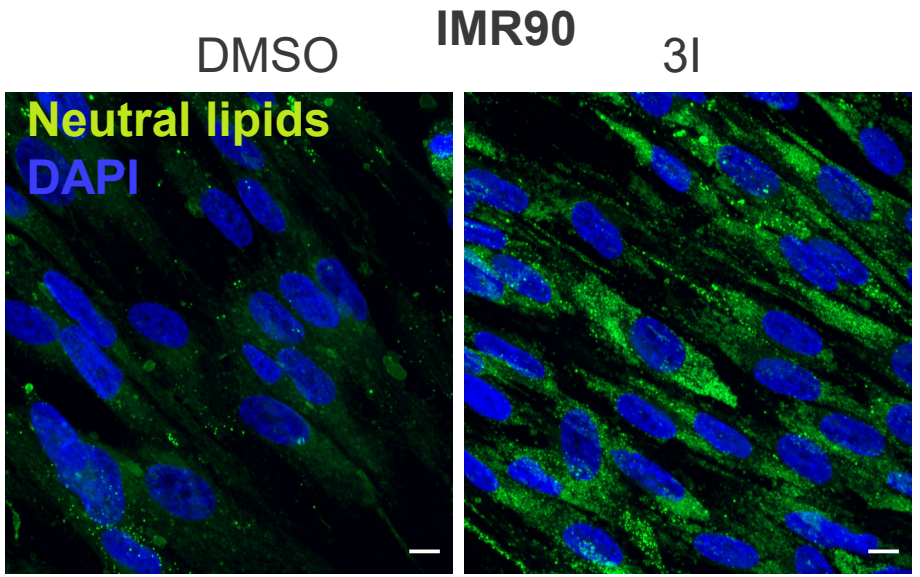


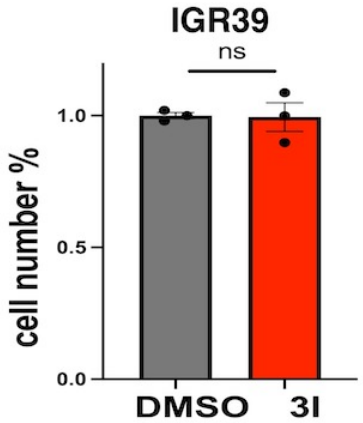


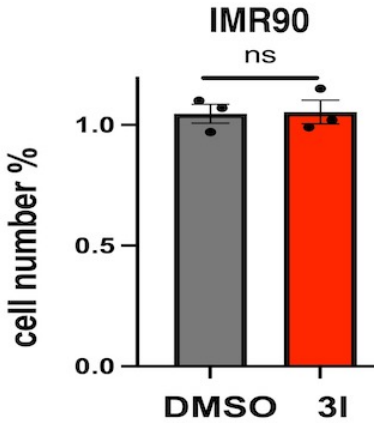
Unpublished data

The lipid-proliferation link is specific for high BACE2 cancer cells

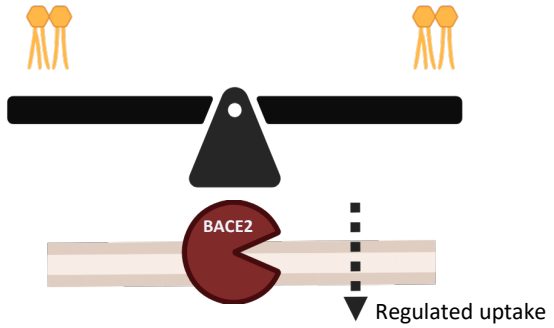


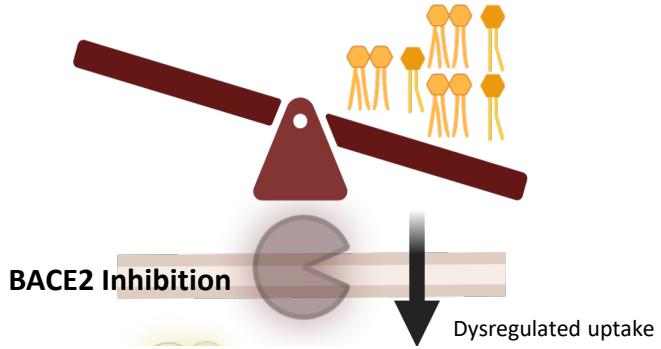






BACE2 is a lipid sensor and gatekeeper

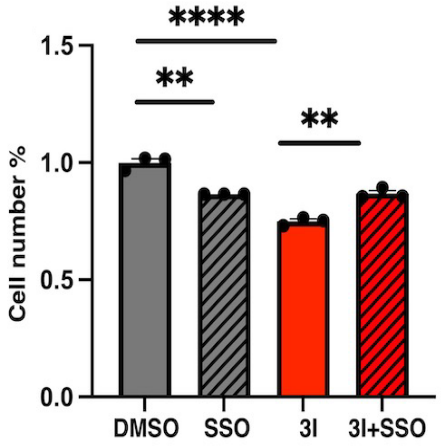




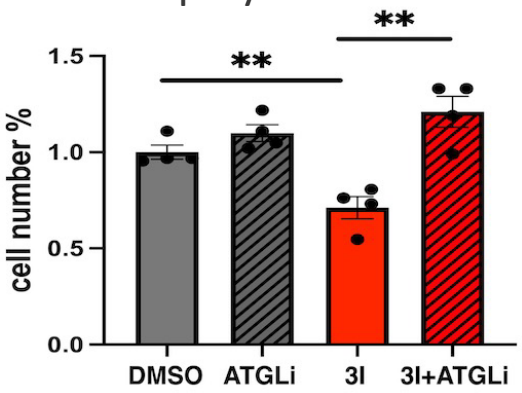
Highly proliferative cells

Proliferation impairment

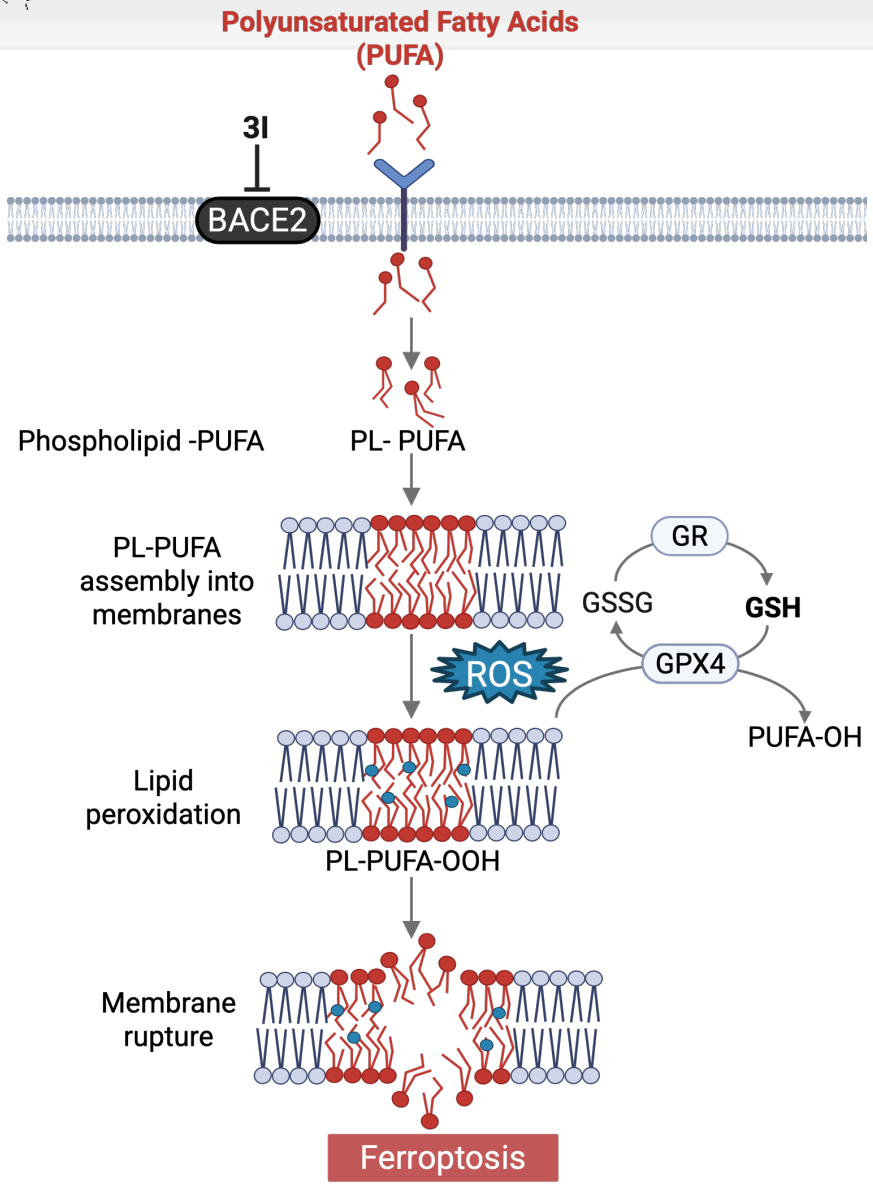
Lipid uptake



LDs lipolysis



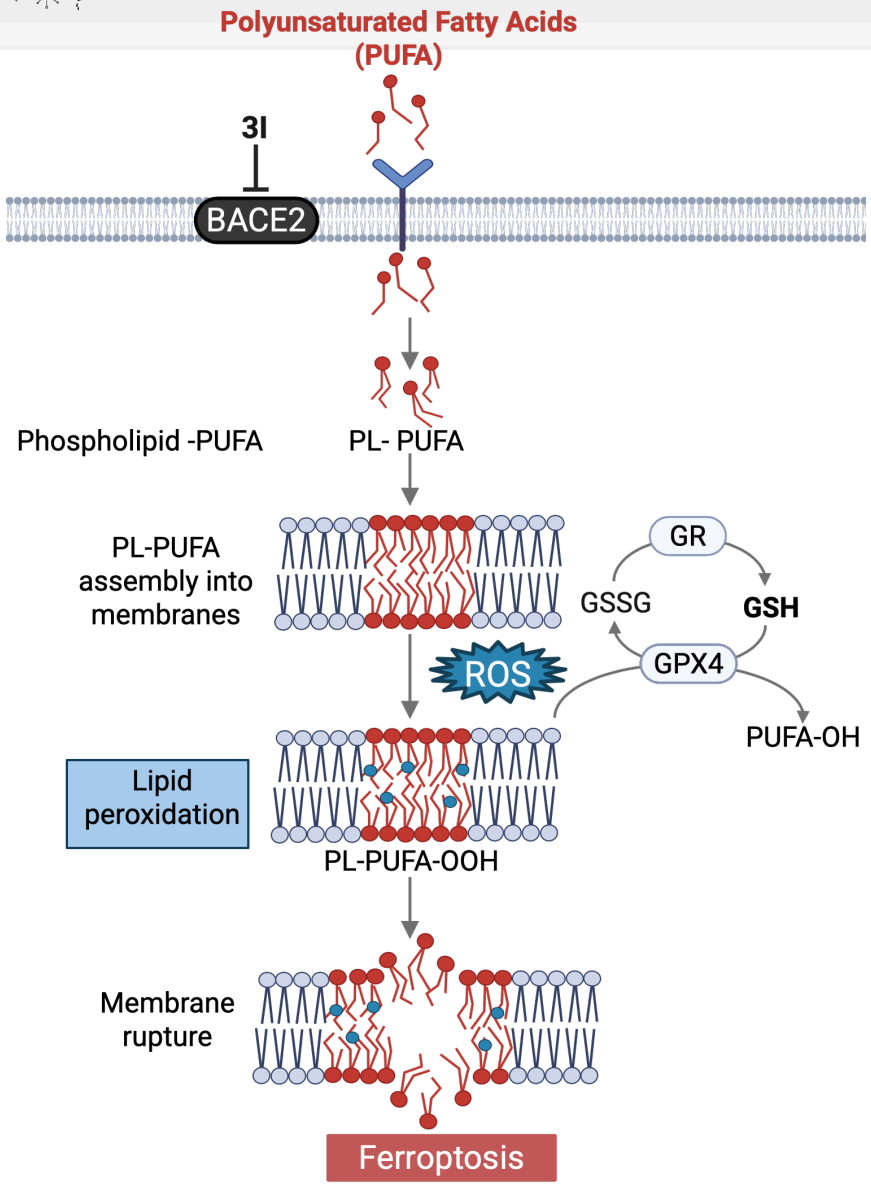
How lipid overload impairs cell proliferation?

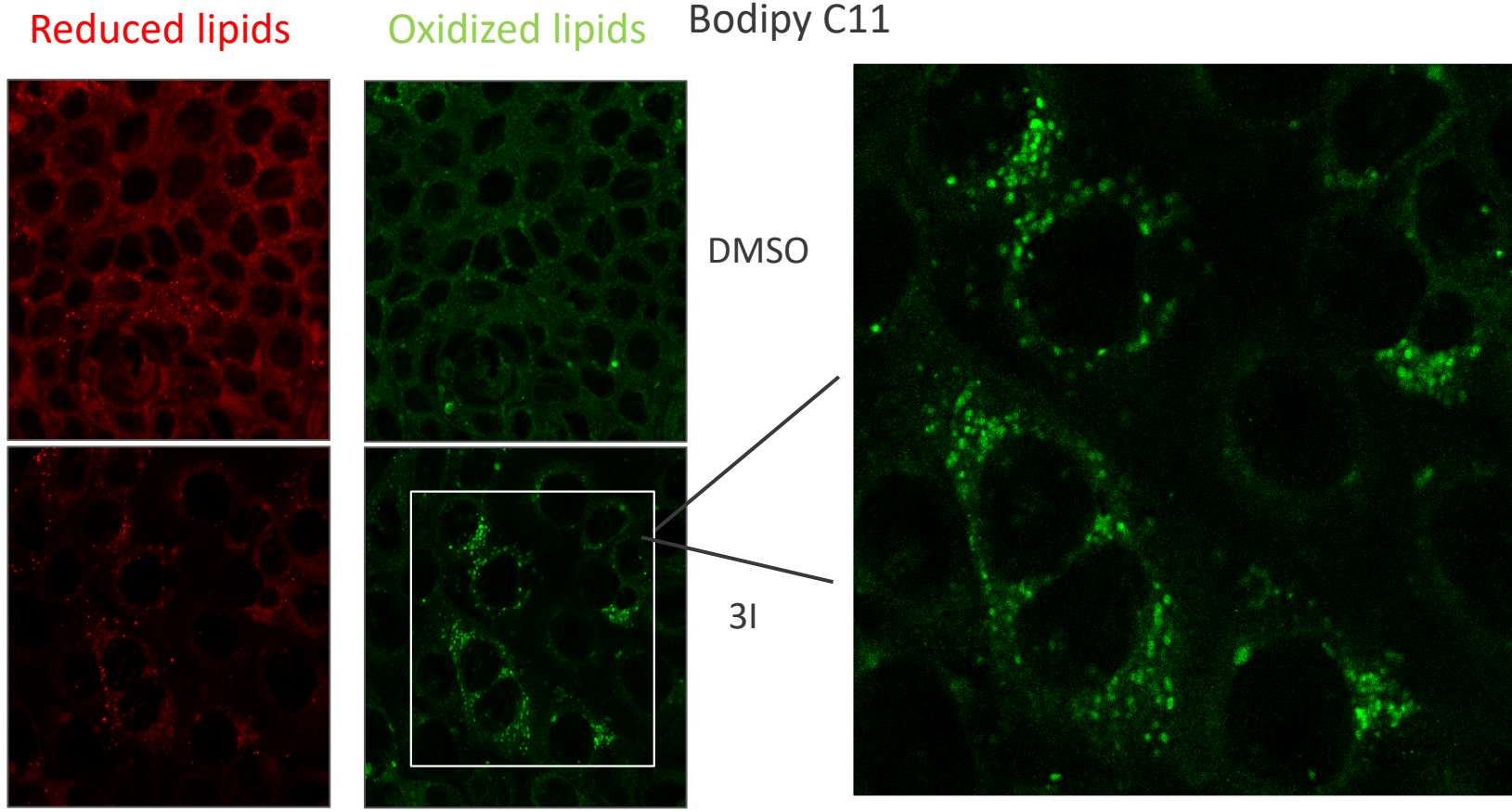


The antioxidant capacity is compromised

Unpublished data

Does BACE2 inhibition induce ferroptosis?

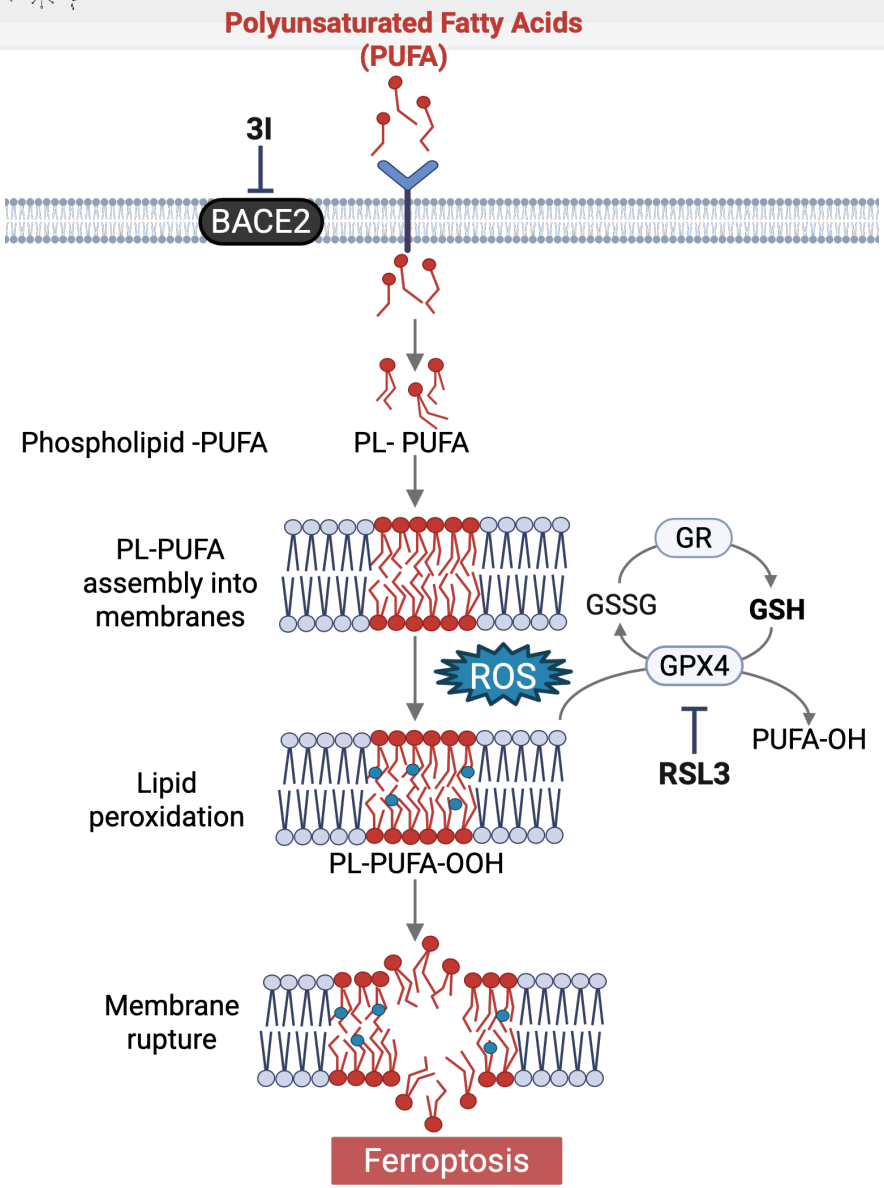




Lipid oxidation is increased upon BACE2 inhibition

Unpublished data

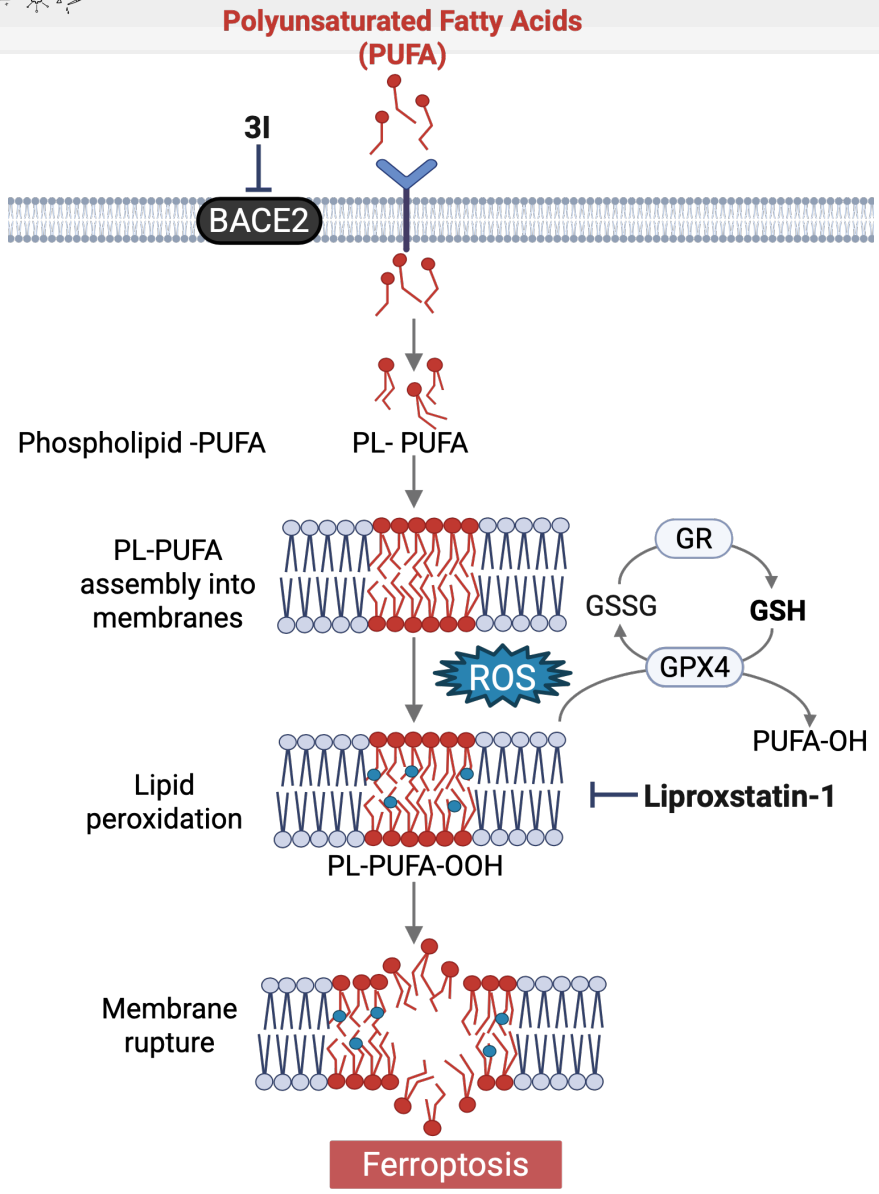
Does BACE2 inhibition induce ferroptosis?



RSL3 exacerbates decreased proliferation induced by BACE2 inhibition

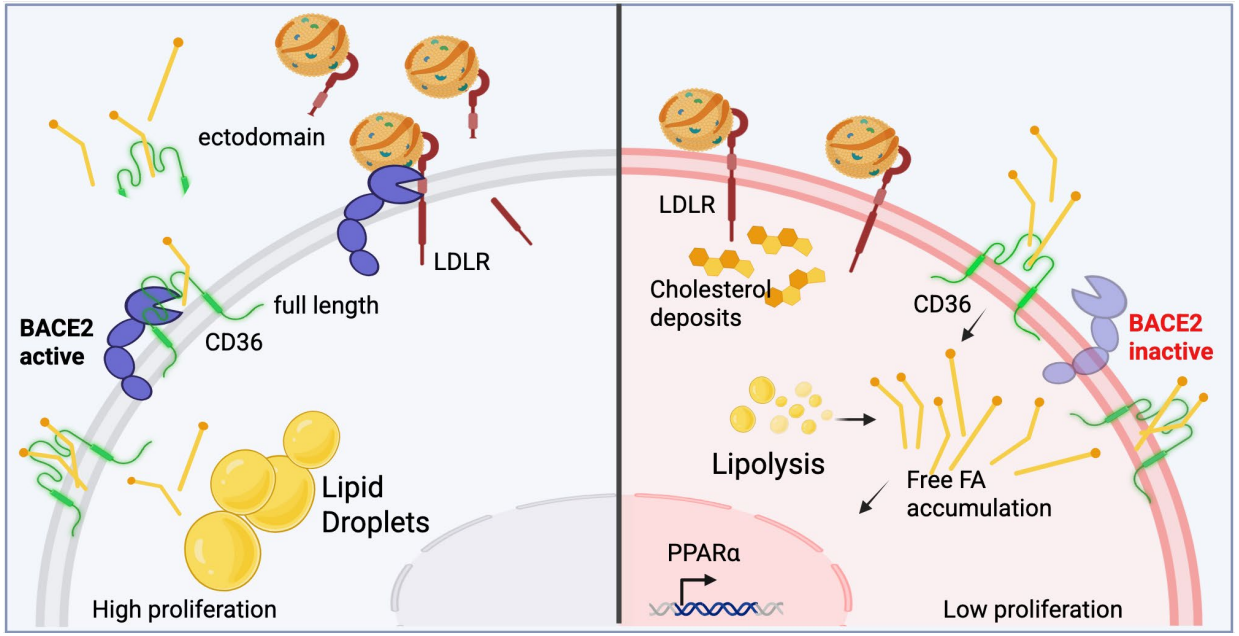
Unpublished data

Does BACE2 inhibition induce ferroptosis?



Unpublished data

Conclusions and perspectives



- BACE2 regulates lipid transporters shedding and lipid uptake in cancer and normal cells
- BACE2 regulates intra and extracellular lipid content
- Manipulating lipid fluxes affects cancer cells viability inducing a lipid overload mediated toxicity
- Lipid metabolism could be targeted in BACE2 over-expressing tumors
- BACE2 in combination with ferroptosis inducers to enhance the anti-tumoral effects

- How is the specificity for lipid transporters regulated?
- How is BACE2 expression regulated?
- Can we exploit BACE2 modulation to rewire immune/stromal cells metabolism or reprogram their behavior in cancer TME ?

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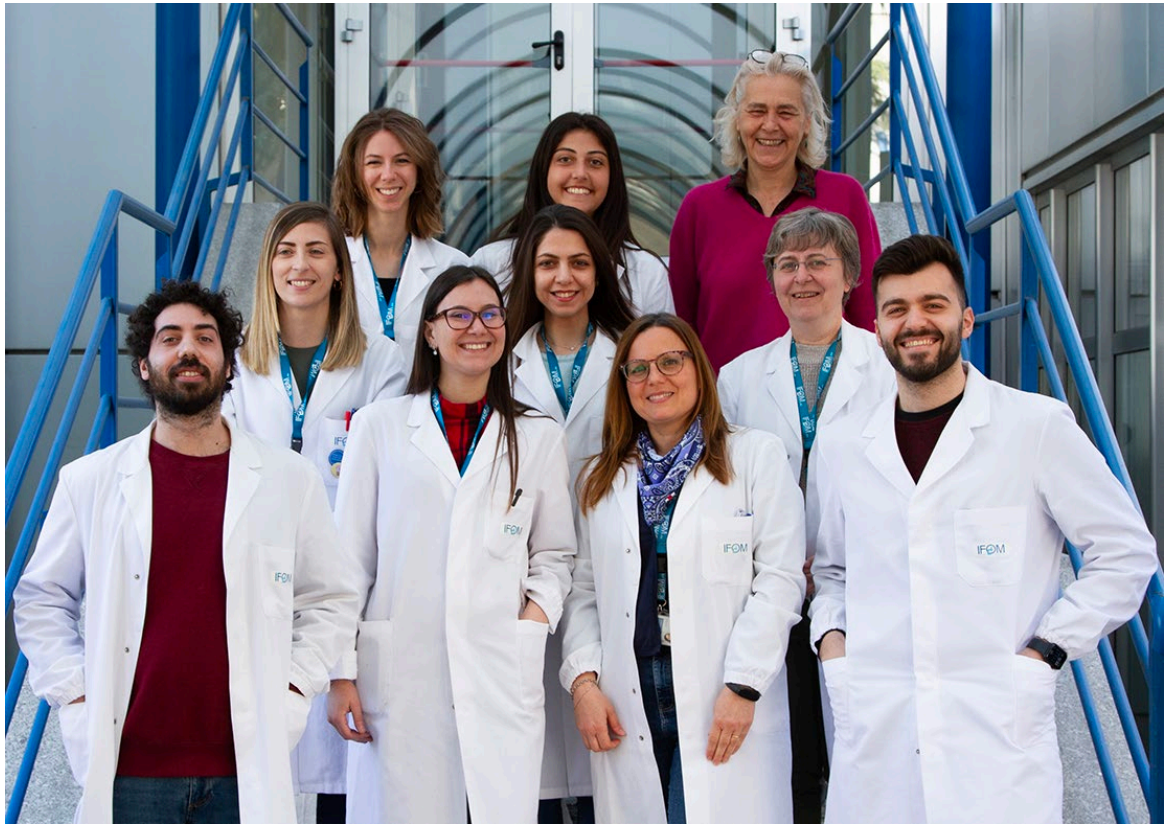
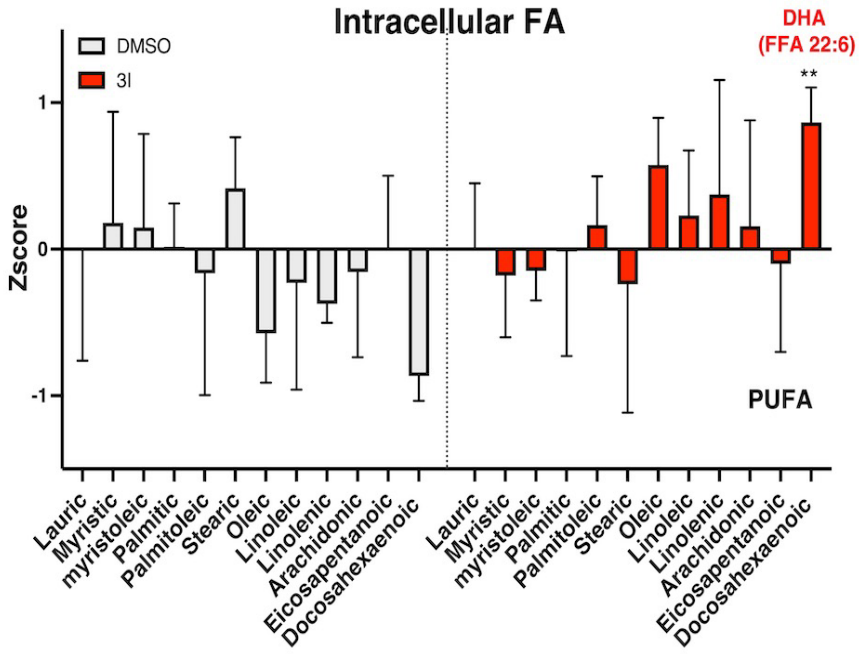
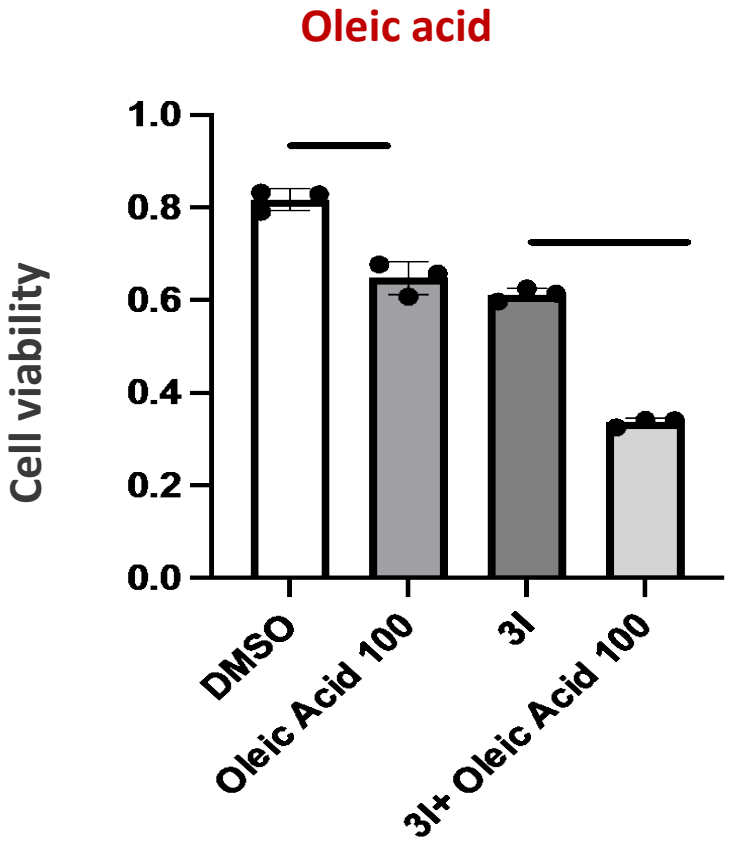


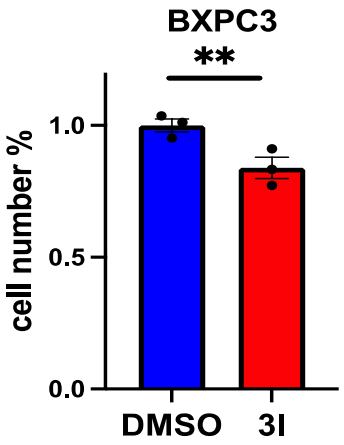
BACE2 increases intracellular PUFA which are toxic for the cells

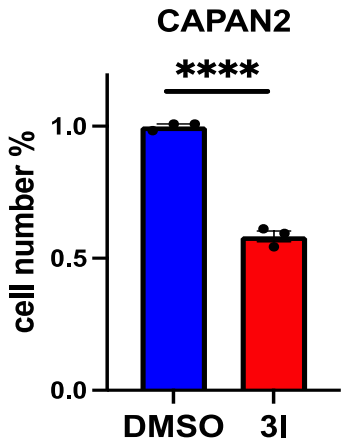


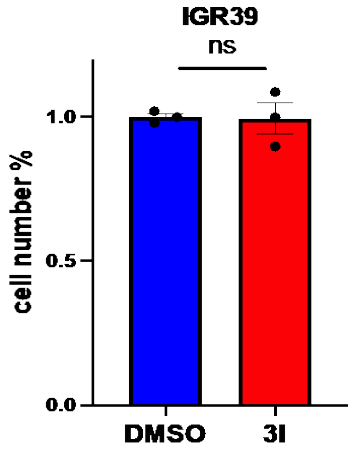


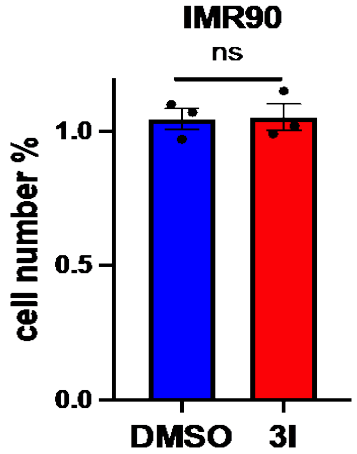
Unpublished data

The lipid - proliferation link is specific for high BACE2 cancer cells









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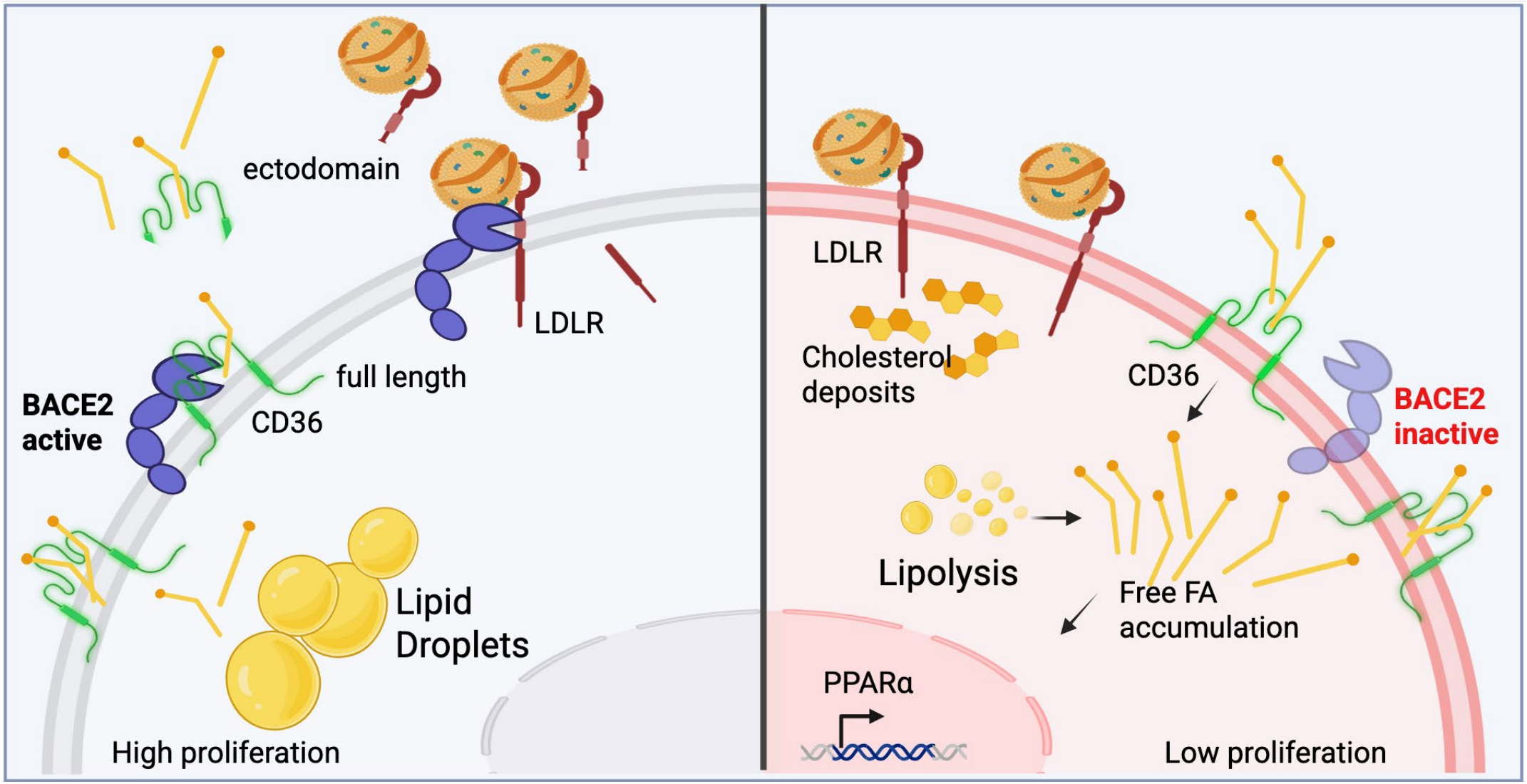
Conclusions



Is there a direct link between lipids metabolism and BACE2 activity?