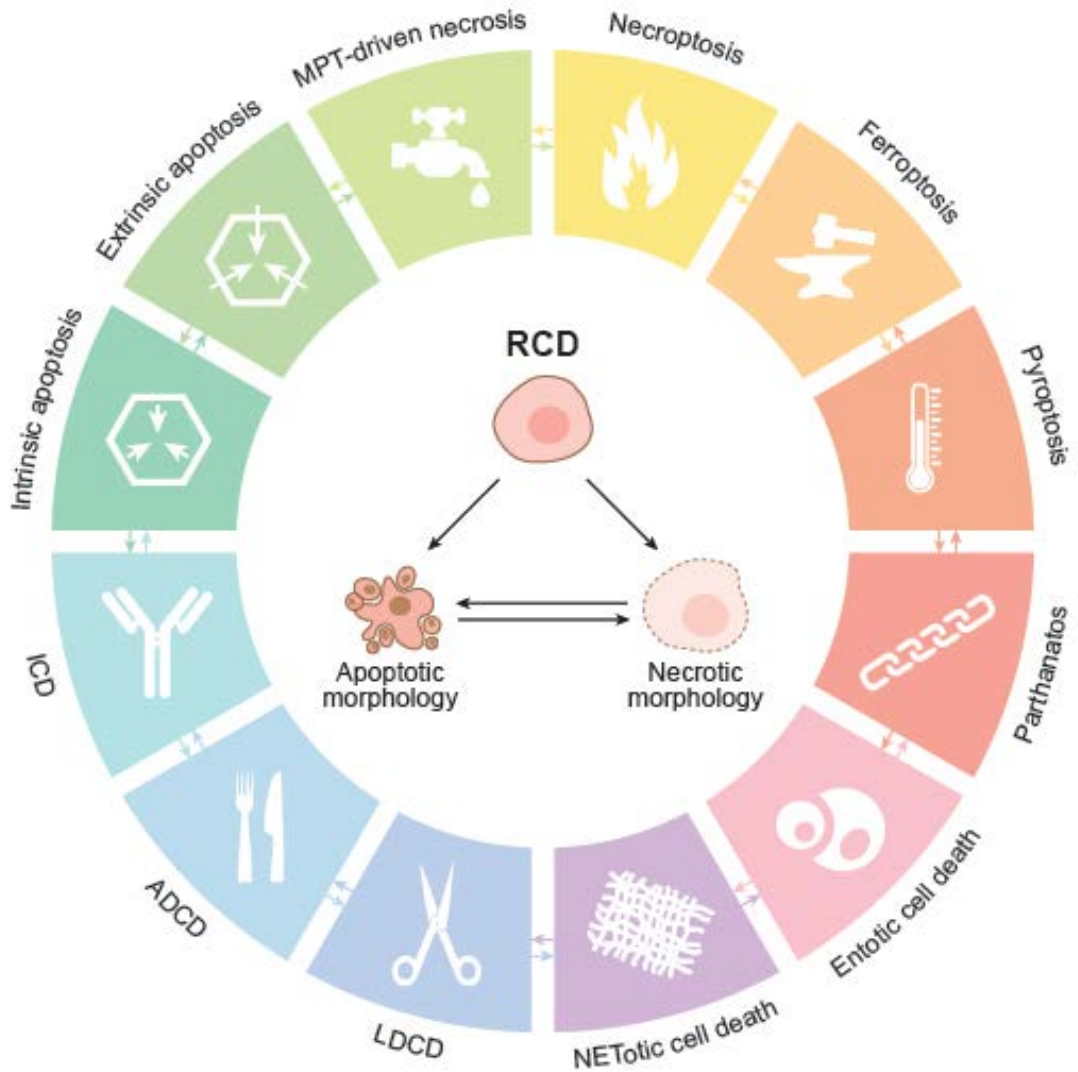
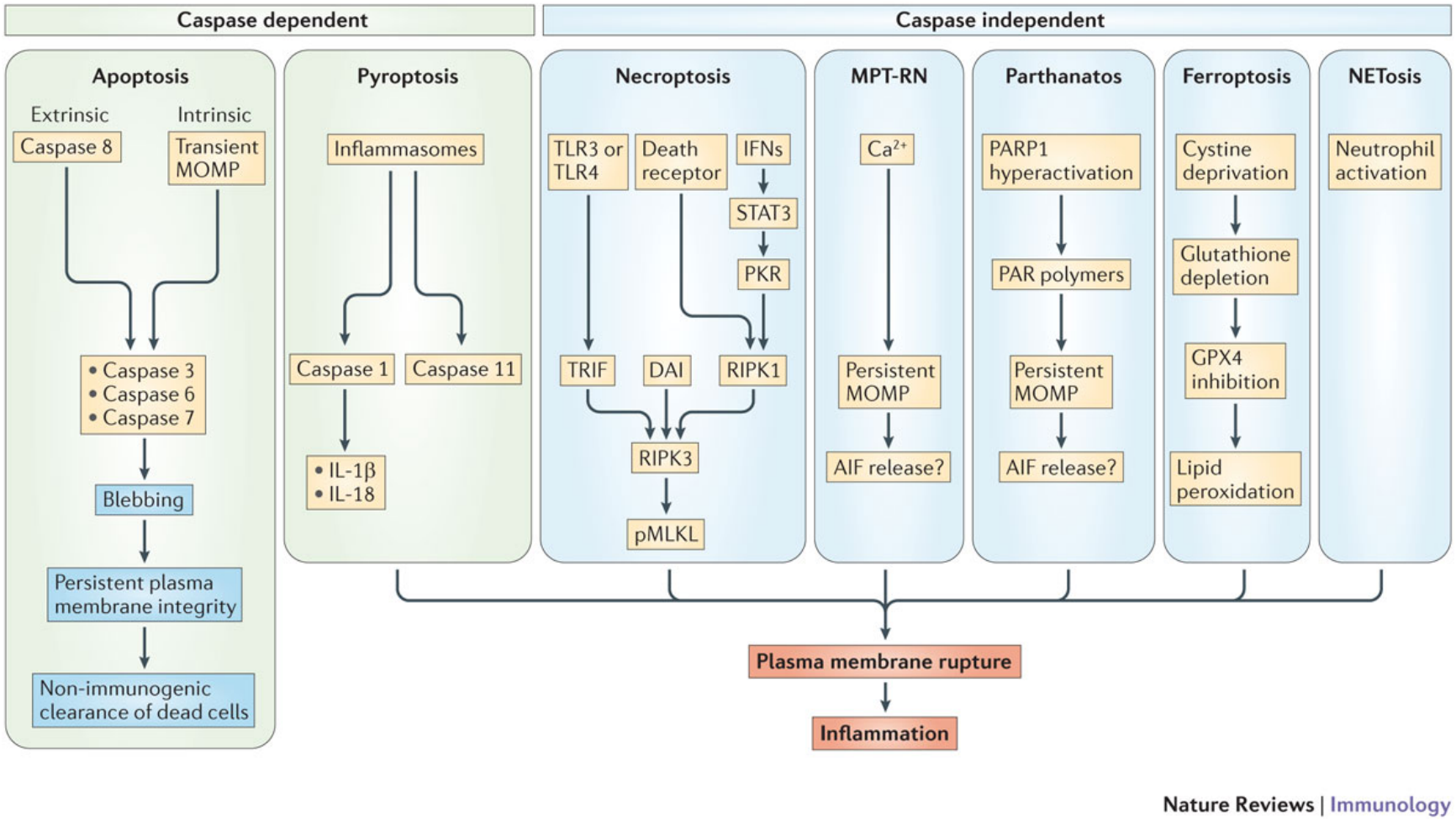
MCBD Lecture: Cell Death (15/10/21)



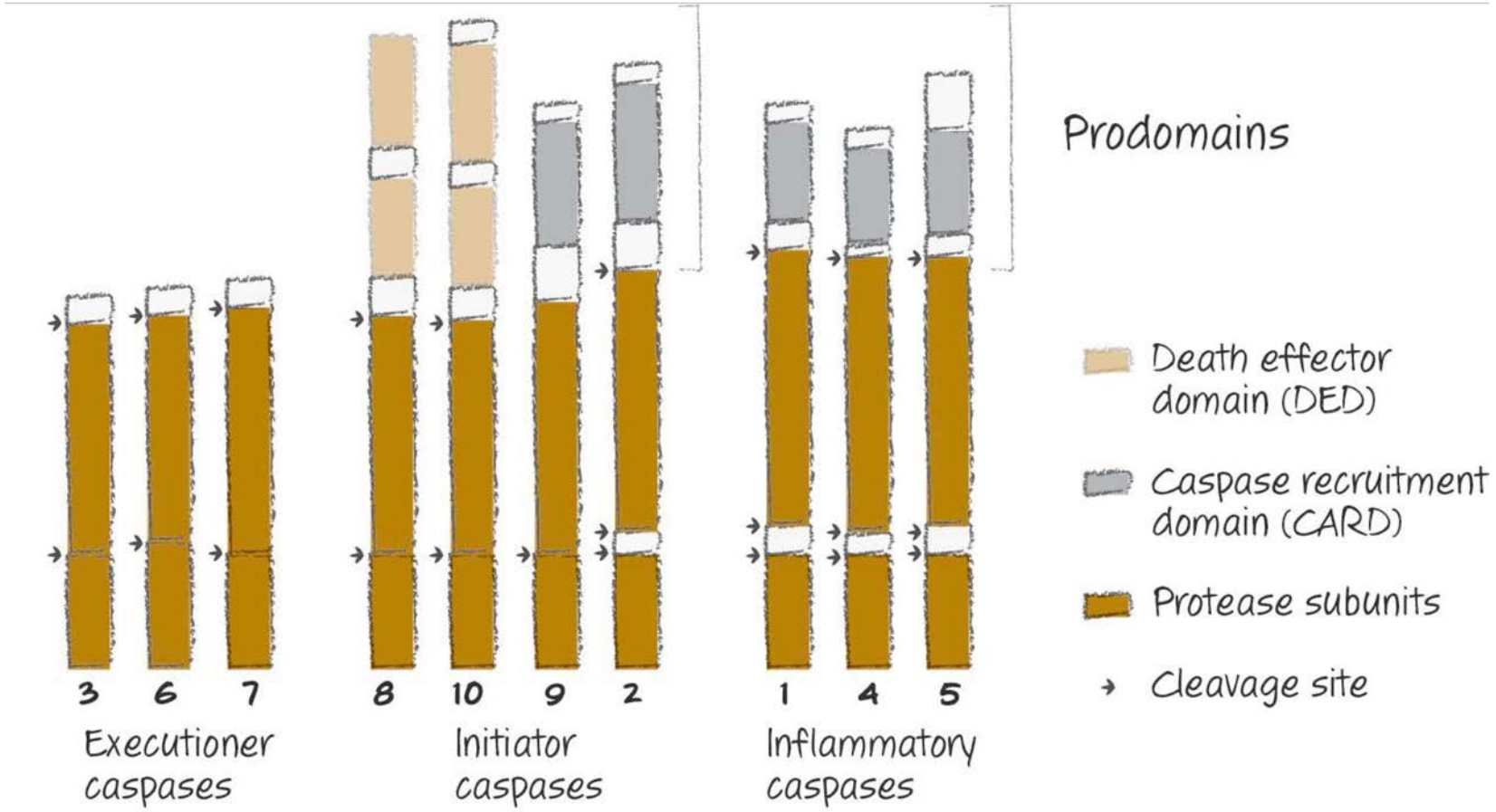
Galluzzi et al. CDD, 2018

Caspases in cell death and inflammation



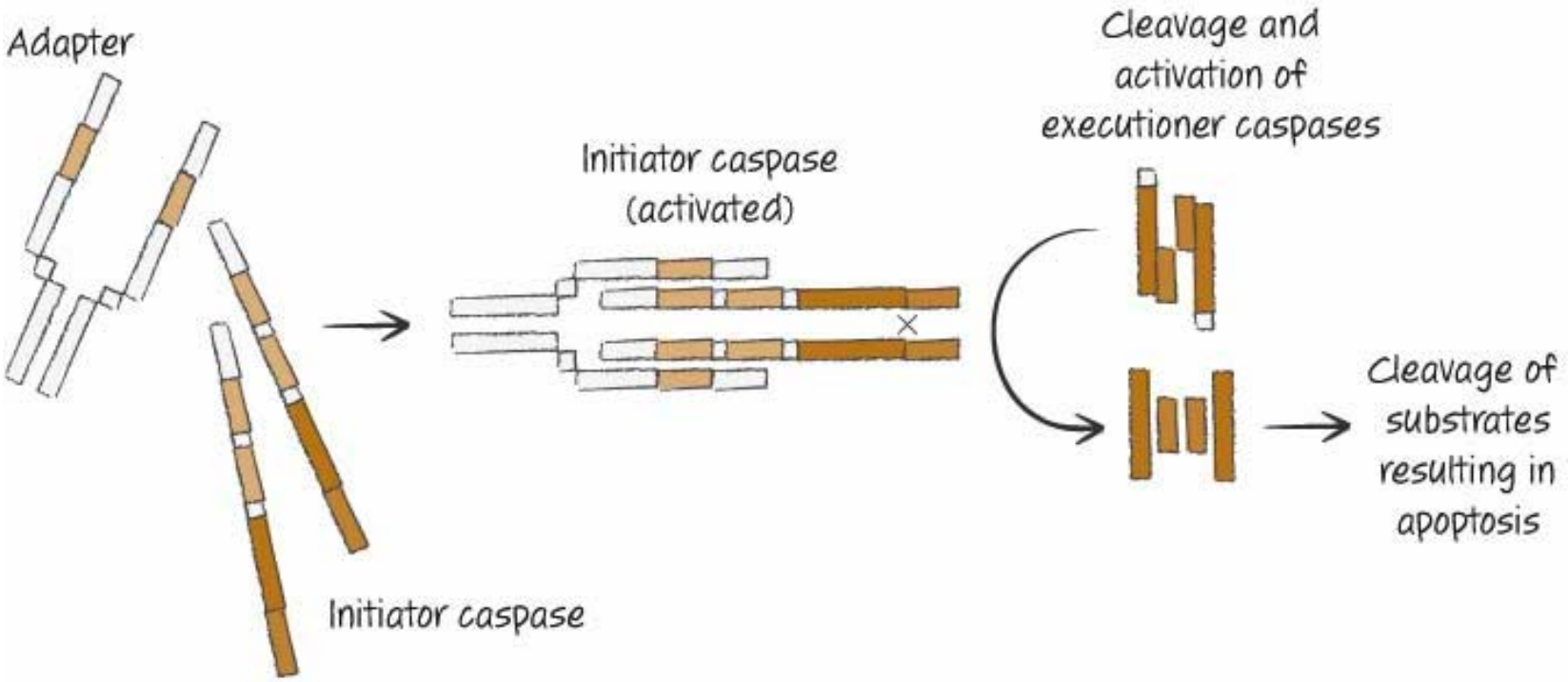
A. Linkermann et al. Nature Reviews Immunology 14, 759–767 (2014)

Caspases show conserved structural features



Caspases: cytein-dependent aspartate-directed proteases

Activation of caspases follows conserved rules



Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 1, Figure 4

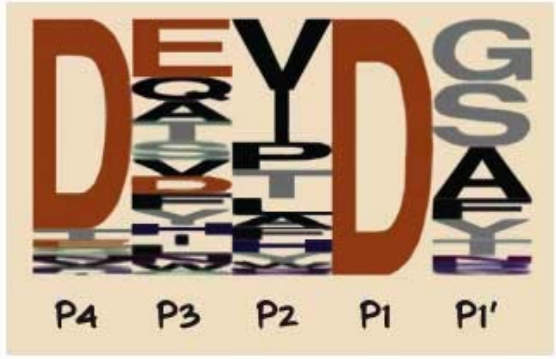
Caspases: cytein-dependent aspartate-directed proteases

Different caspases prefer different peptide sequences, at least in vitro

Caspase-1	WEHD
Caspase-2	VDQQD
Caspase-3	DELD
Caspase-4	LEVD
Caspase-5	(W/L) EHD
Caspase-6	(T/V) QVD
Caspase-7	DEVD
Caspase-8	LETD
Caspase-9	LEHD

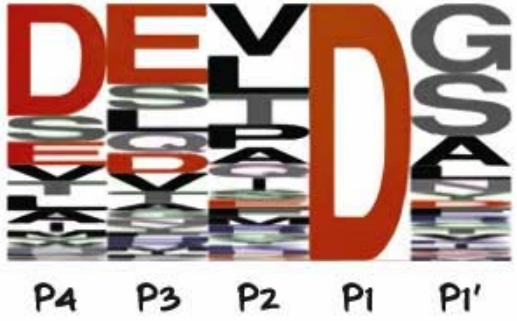
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 7

Peptide library



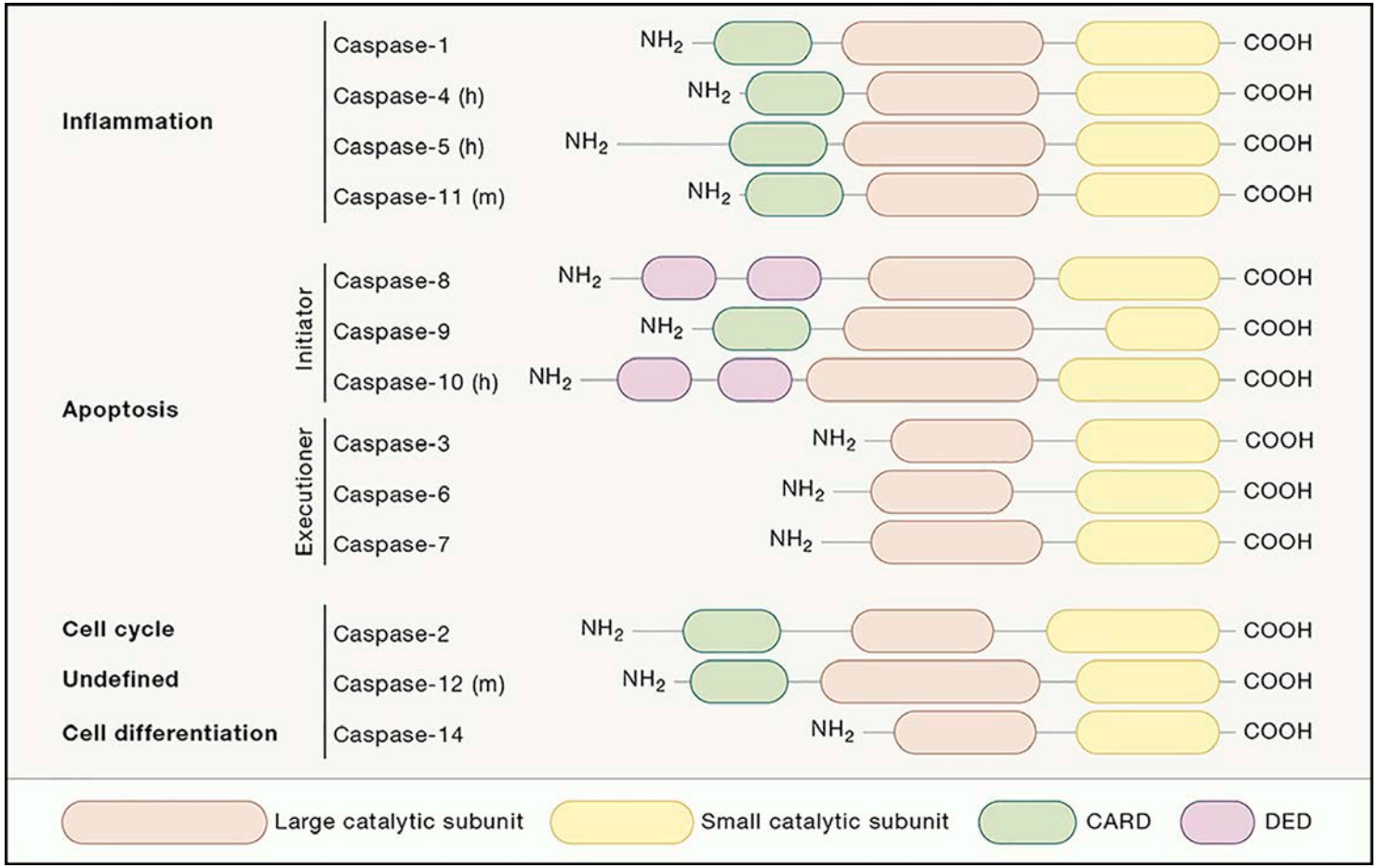
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 9

Proteom analysis



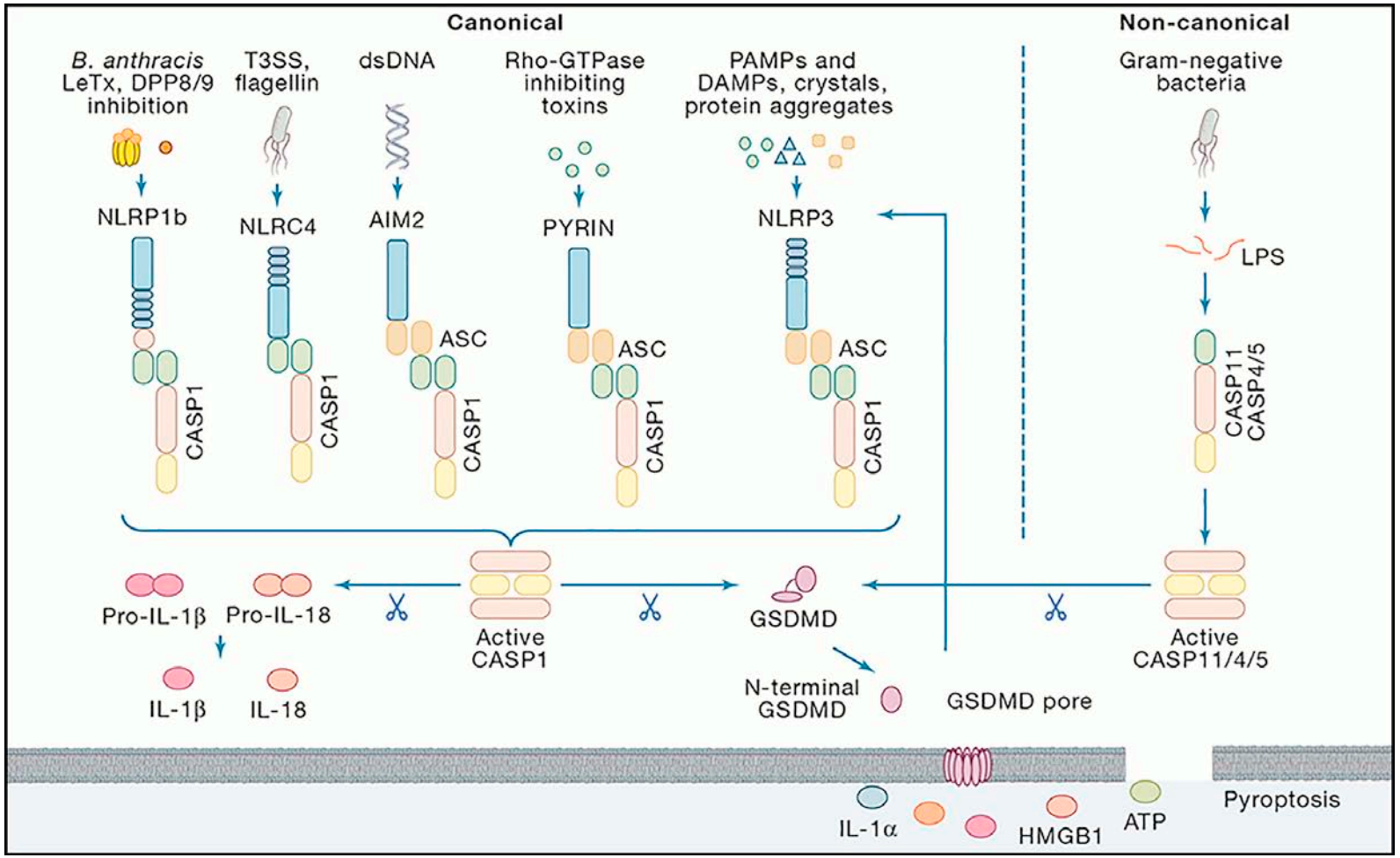
Means to an End, ©2011 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 10

Caspases in control of cell death, inflammation & more

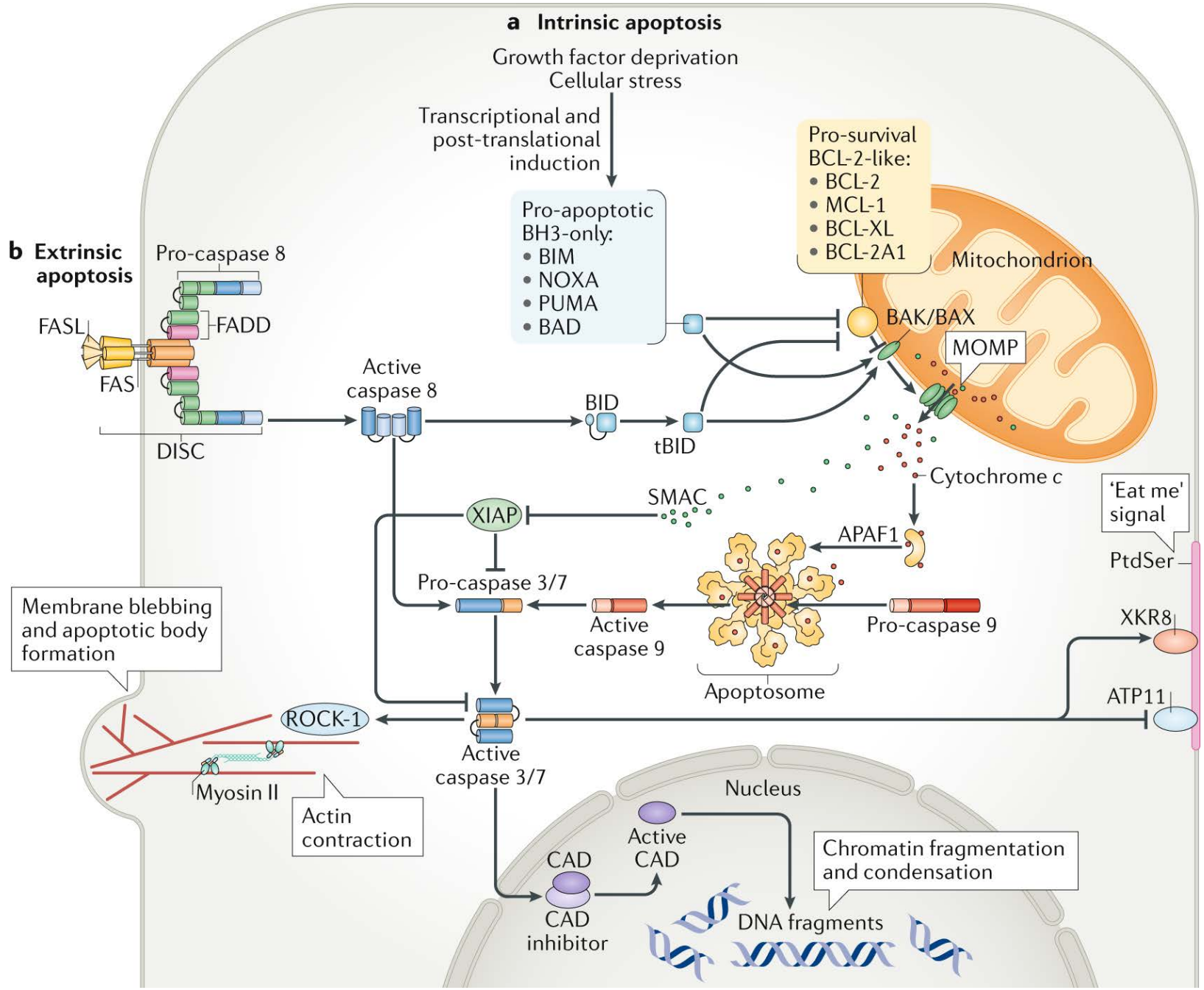


Opdenbosch & Lamkanfi, Immunity, 2019

Caspases in control of cell death and inflammation

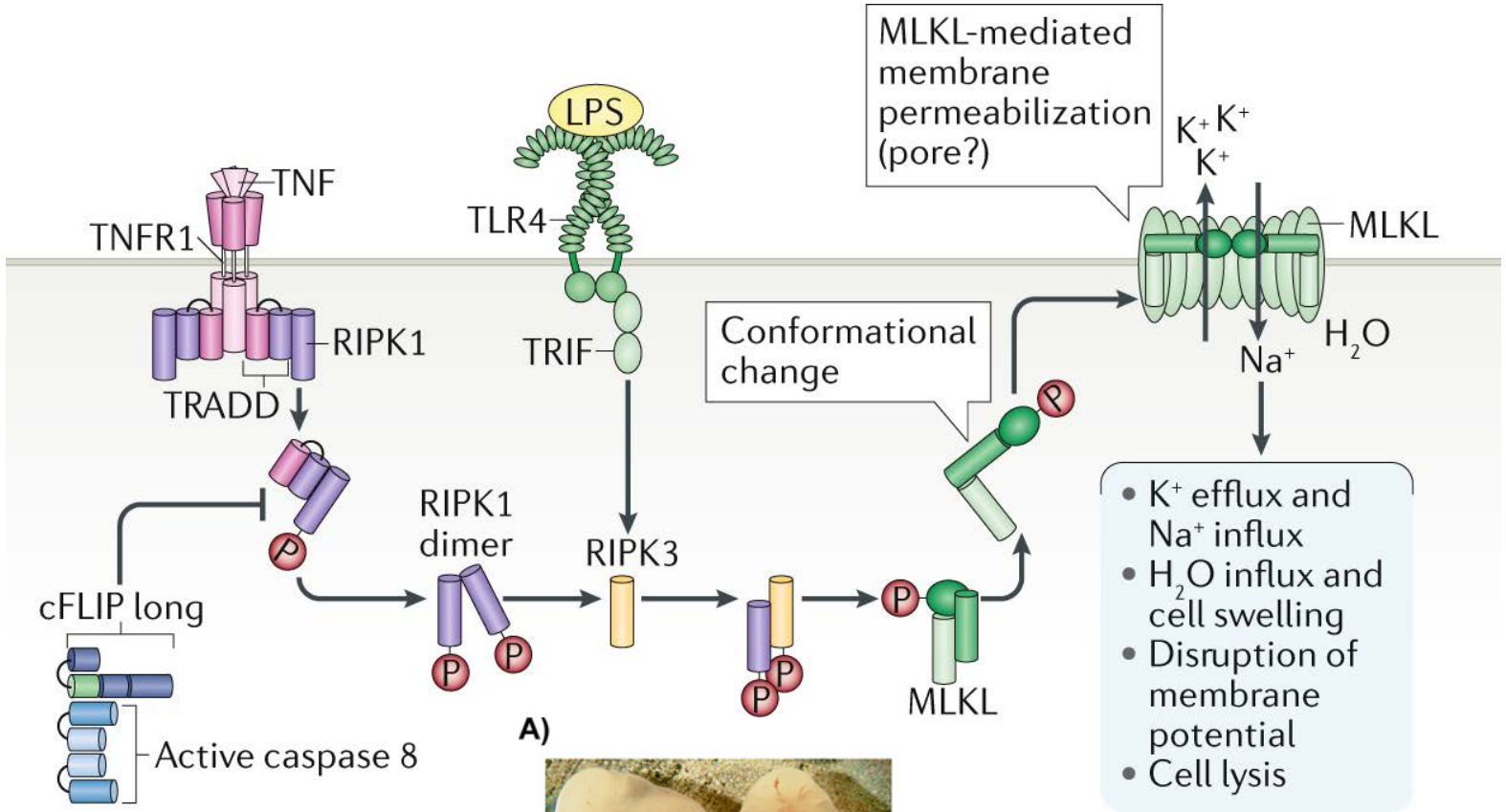


Opdenbosch & Lamkanfi, Immunity, 2019

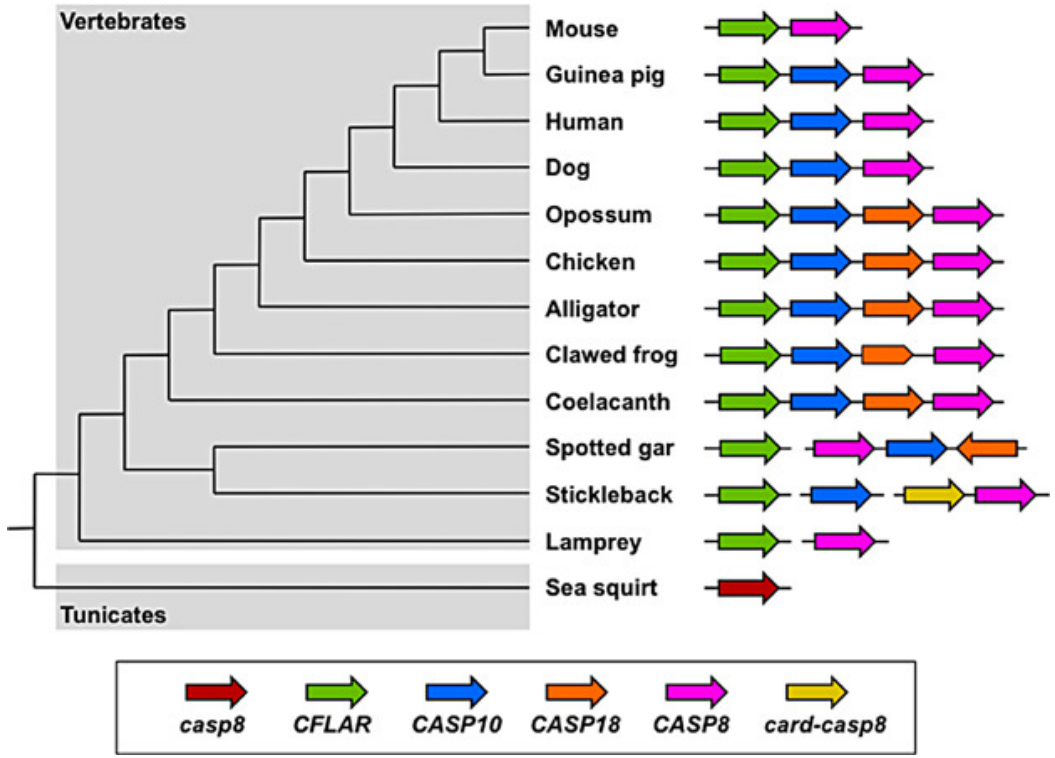


Bedoui, Herold & Strasser, NRMCB, 2020

CASPASE-8 prevents necrotic cell death

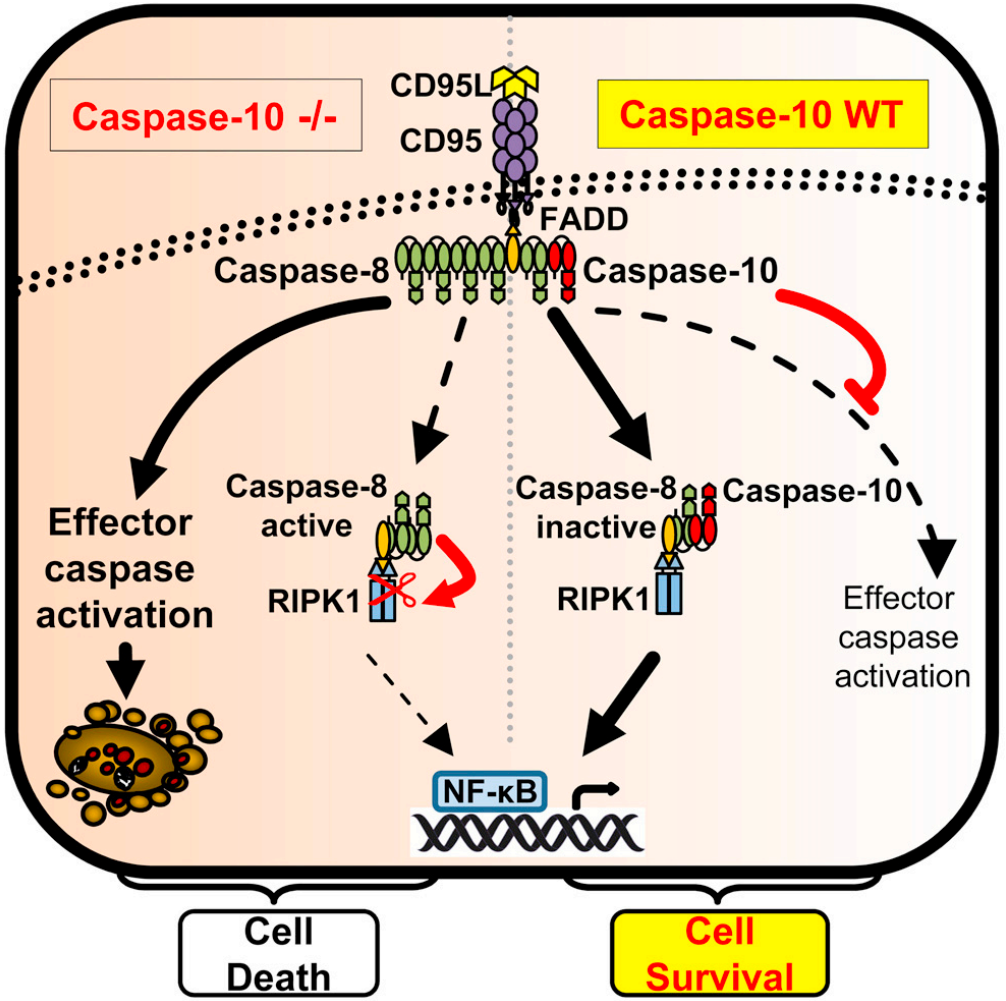


CASP-8 PREVENTS NECROPTOSIS, WHY DO WE NEED CASP-10



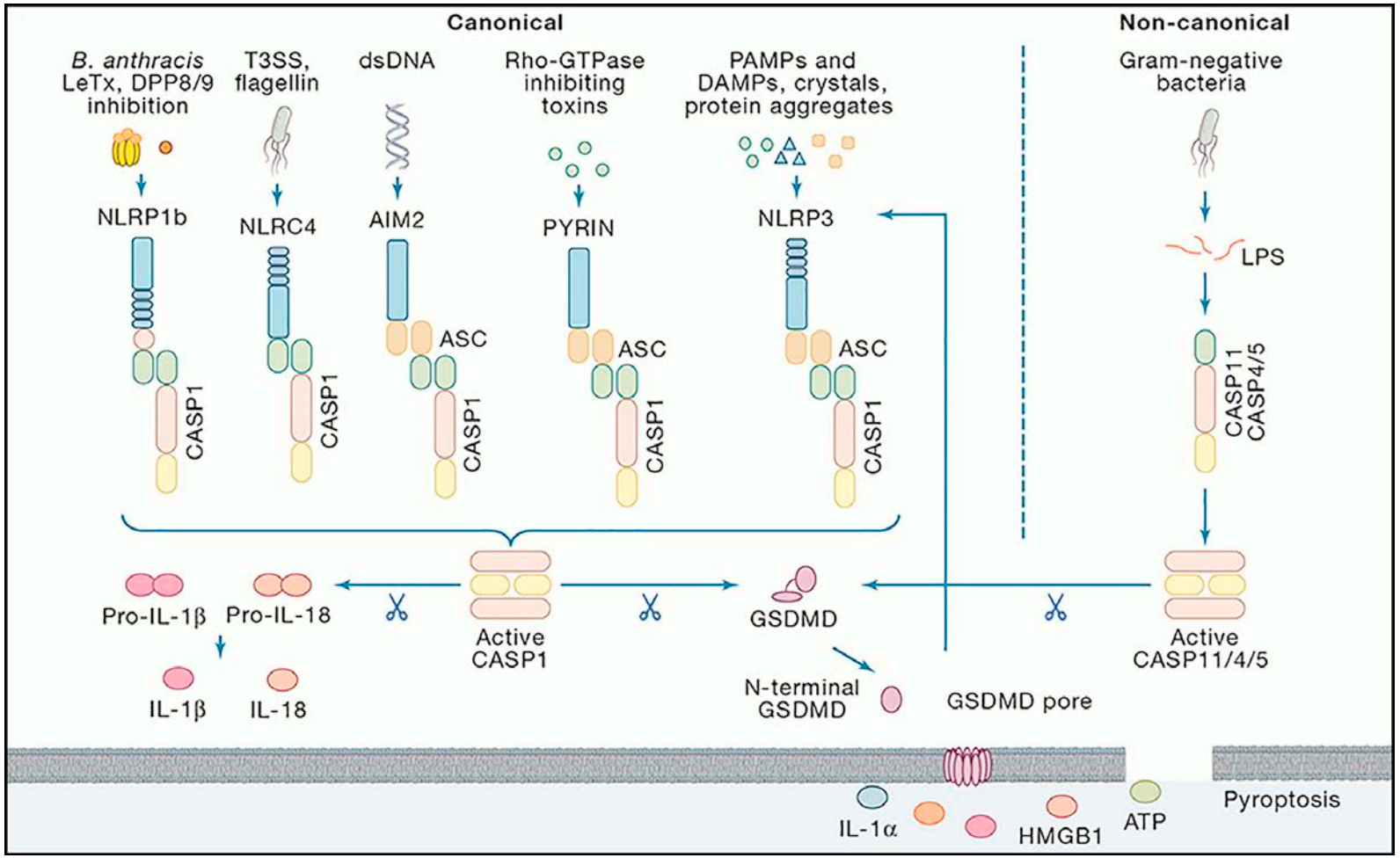
BioEssays, Volume: 37, Issue: 7, Pages: 767-776, First published: 22 May 2015

CAN CASP-10 SUBSTITUTE FOR CASP-8 IN DEVELOPMENT? ALPS-PATIENTS DO CARRY MUTATIONS IN CASP-8 or CASP-10



Horn et al. Cell Reports, 2017

Caspases in cell death and inflammation



Opdenbosch & Lamkanfi, Immunity, 2019

Article

Control of gasdermin D oligomerization and pyroptosis by the Ragulator-Rag-mTORC1 pathway

Charles L. Evavold,[1,7,*] Iva Hafner-Bratkovič,[1,2,3,7] Pascal Devant,[1] Jasmin M. D'Andrea,[4,5] Elsy M. Ngwa,[1] Elvira Boršić,[2] John G. Doench,[6] Martin W. LaFleur,[4,5] Arlene H. Sharpe,[4,5,6] Jay R. Thiagarajah,[1] and Jonathan C. Kagan[1,8,*]

[1]Division of Gastroenterology, Boston Children's Hospital and Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA
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[4]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA
[5]Evergrande Center for Immunological Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA
[6]Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA 02142, USA
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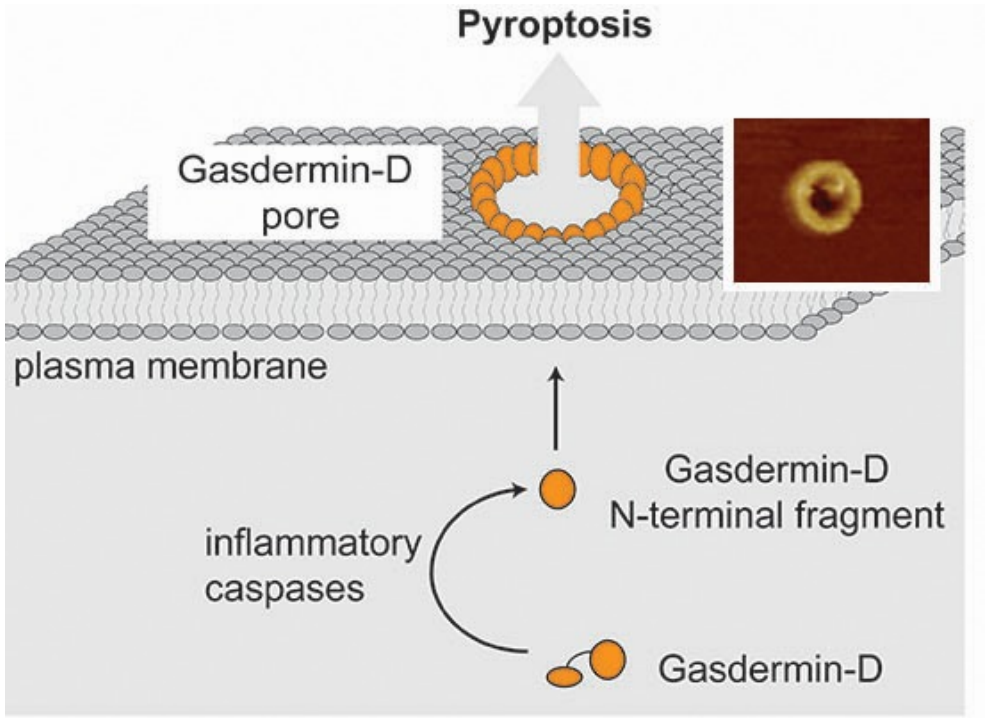
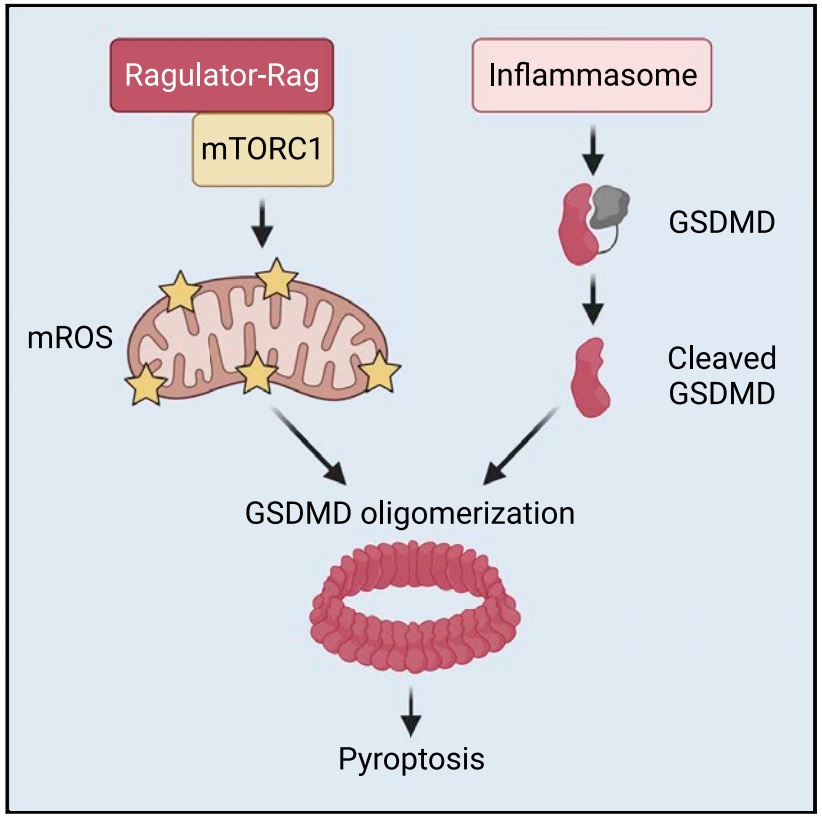
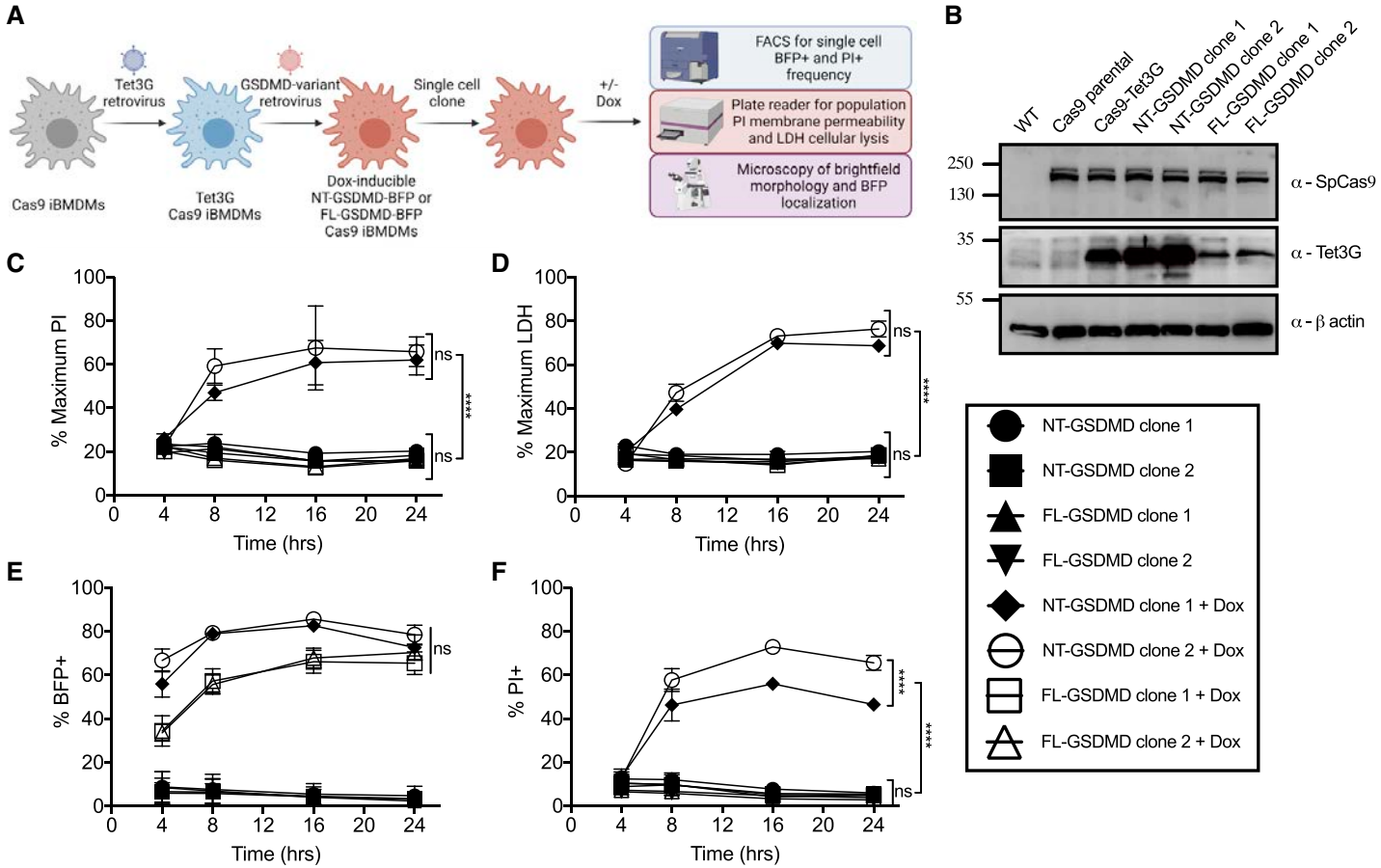
*Correspondence: charles.evavold@childrens.harvard.edu (C.L.E.), jonathan.kagan@childrens.harvard.edu (J.C.K.)
https://doi.org/10.1016/j.cell.2021.06.028

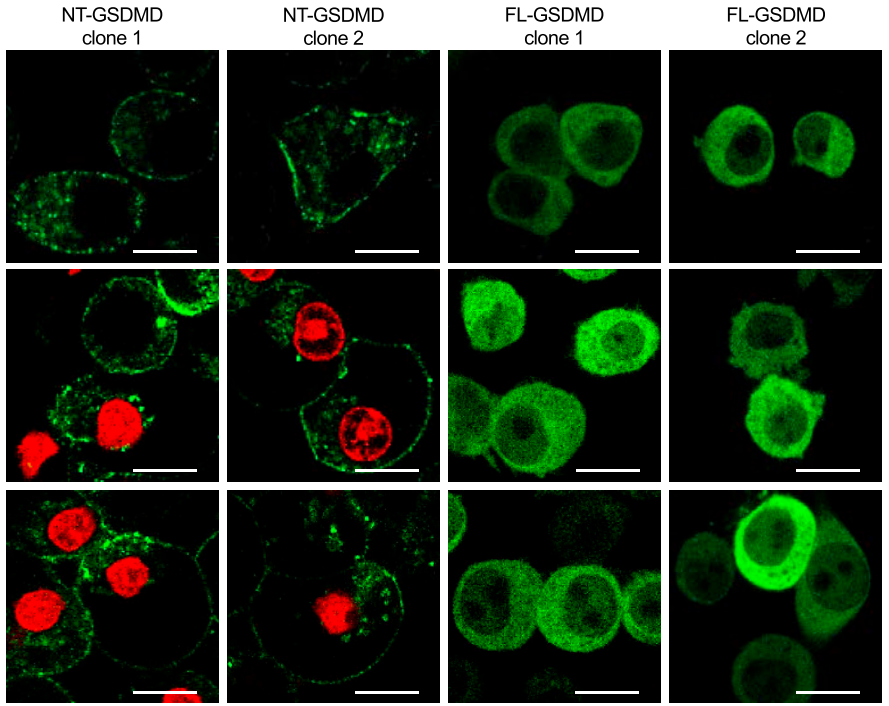


Graphical abstract

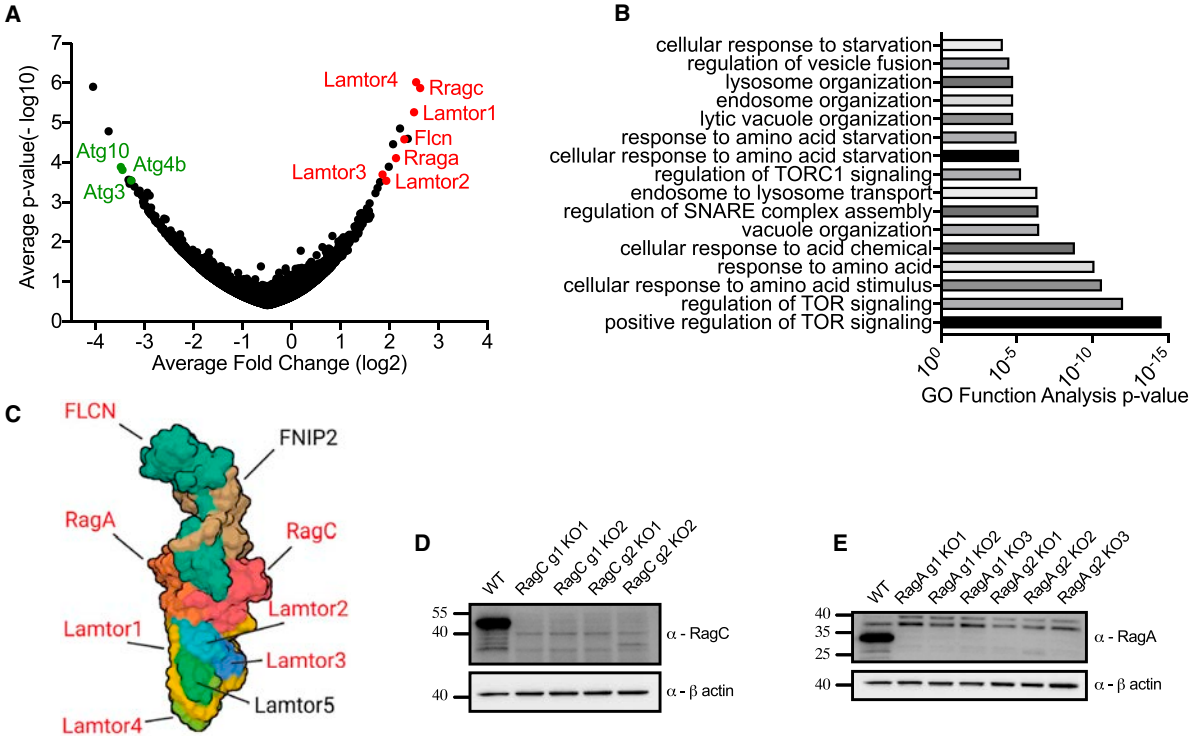


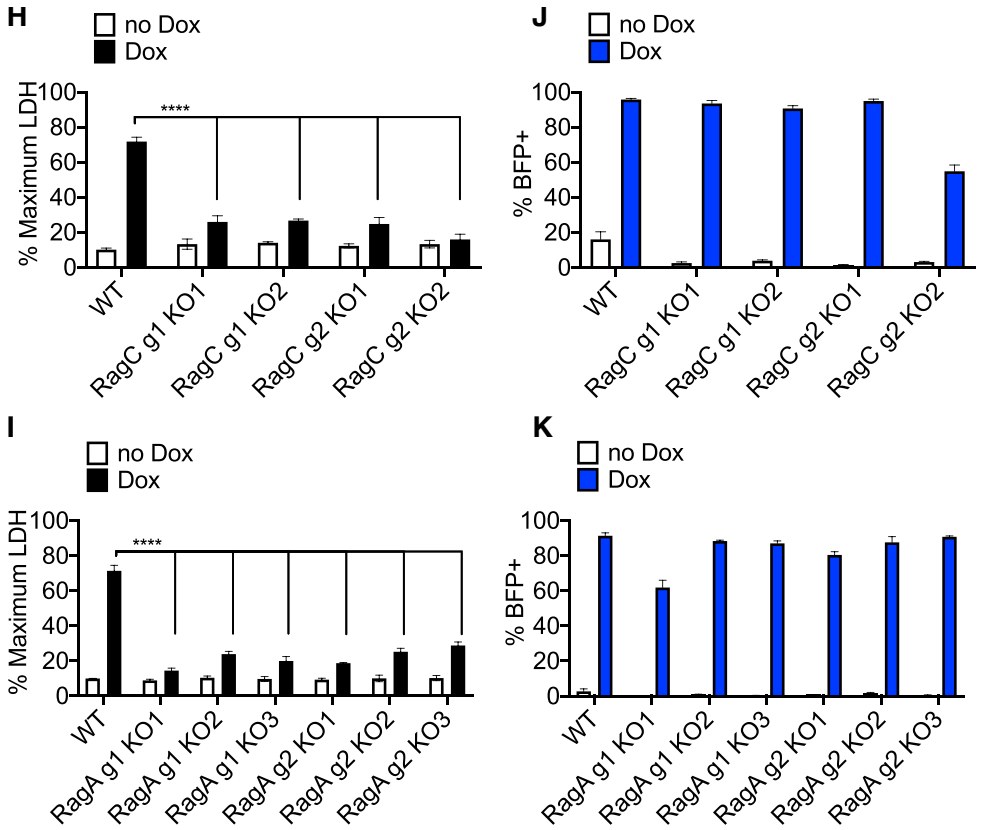
Forward genetic screen identifies new regulators of GSDMD activity in iBDMCs



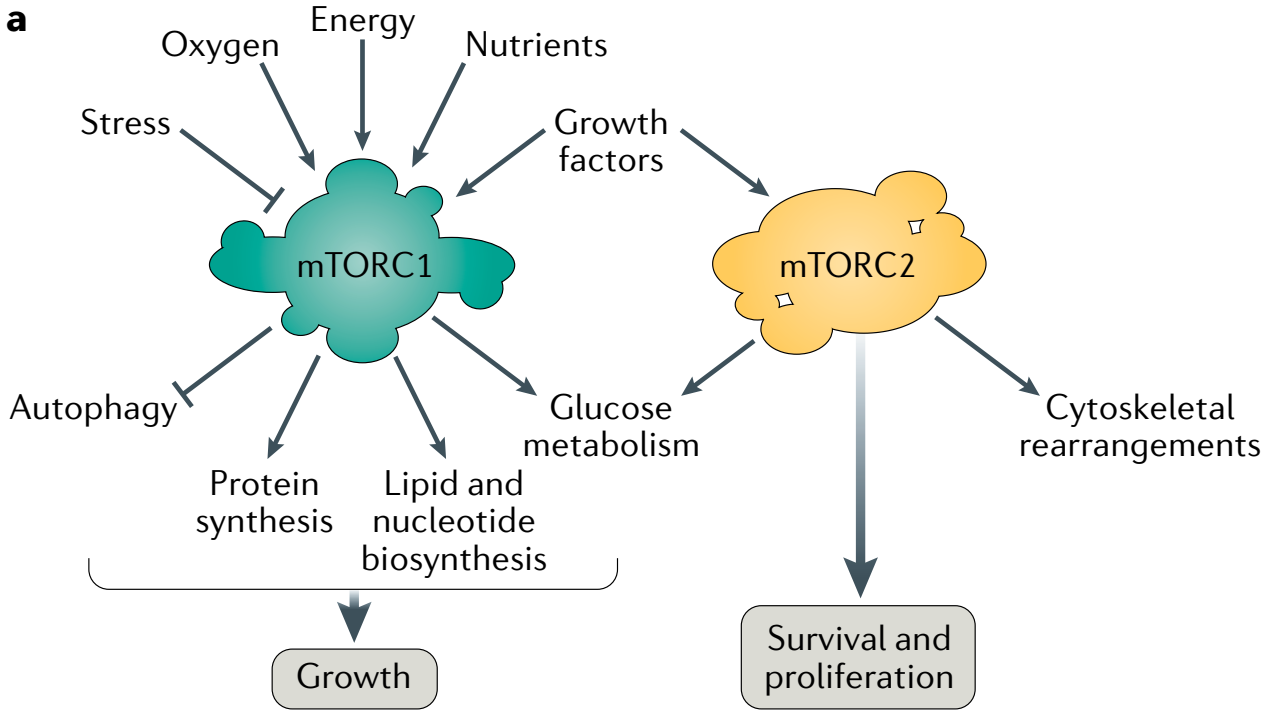


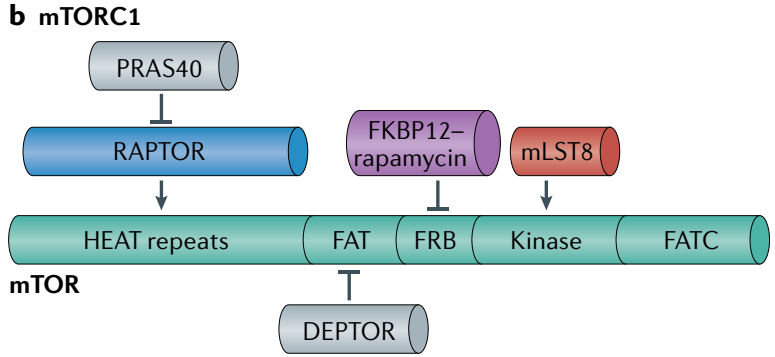
Forward genetic screen identifies the RAG-mTORC1 pathway as a modifier of GSDMD activity in iBDMCs

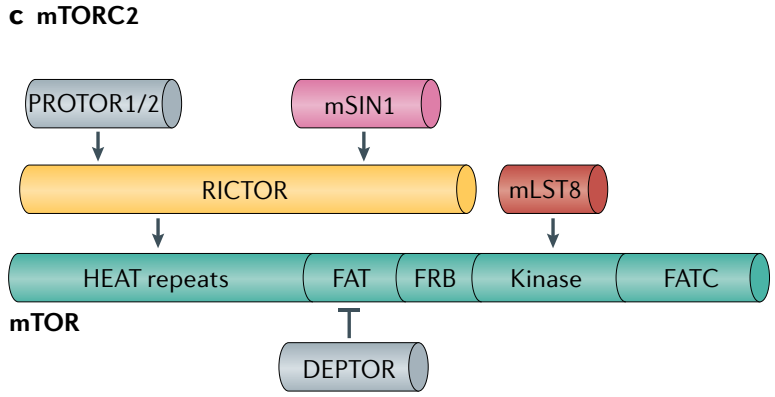




mTORC1 – a master regulator cell growth and metabolism

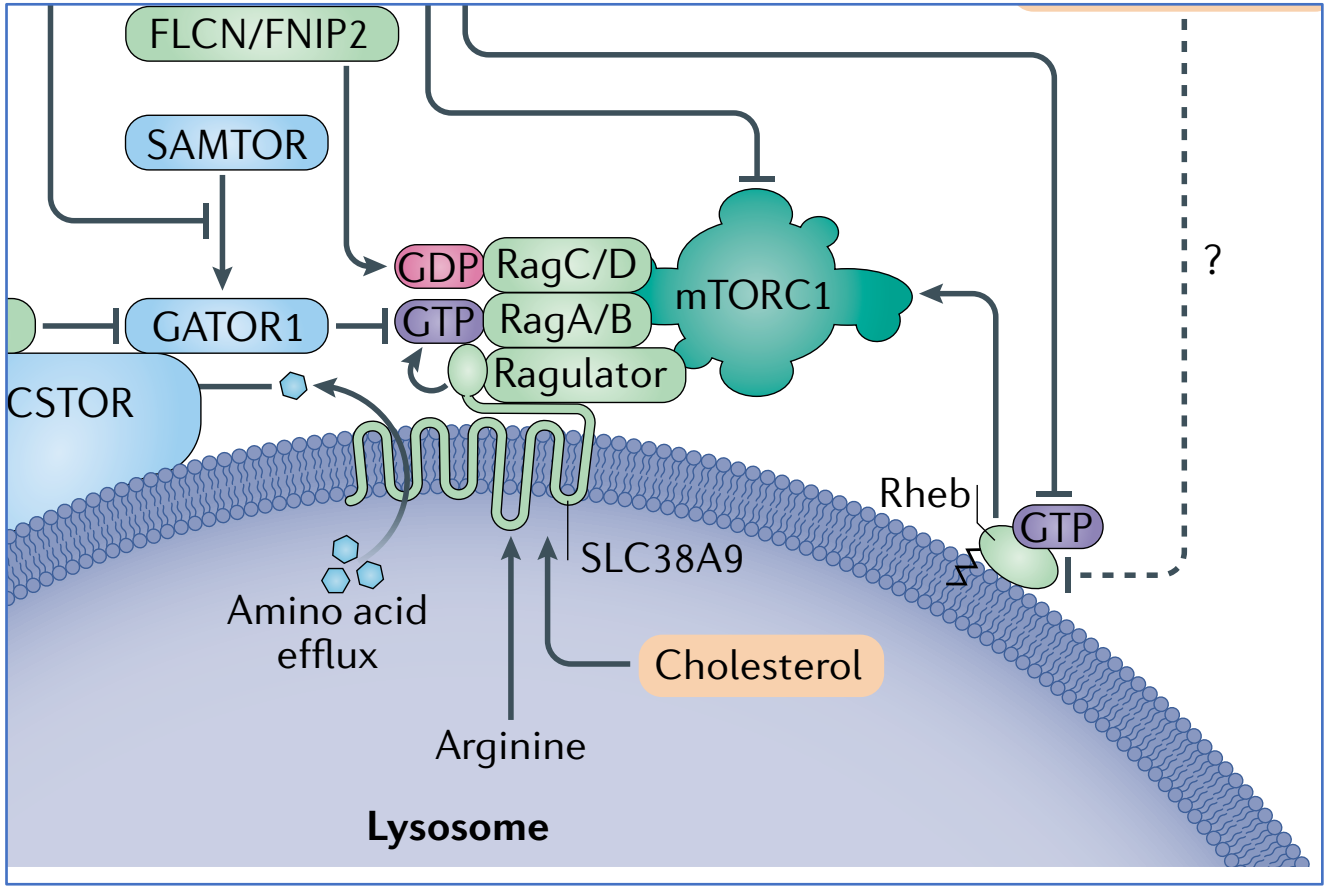


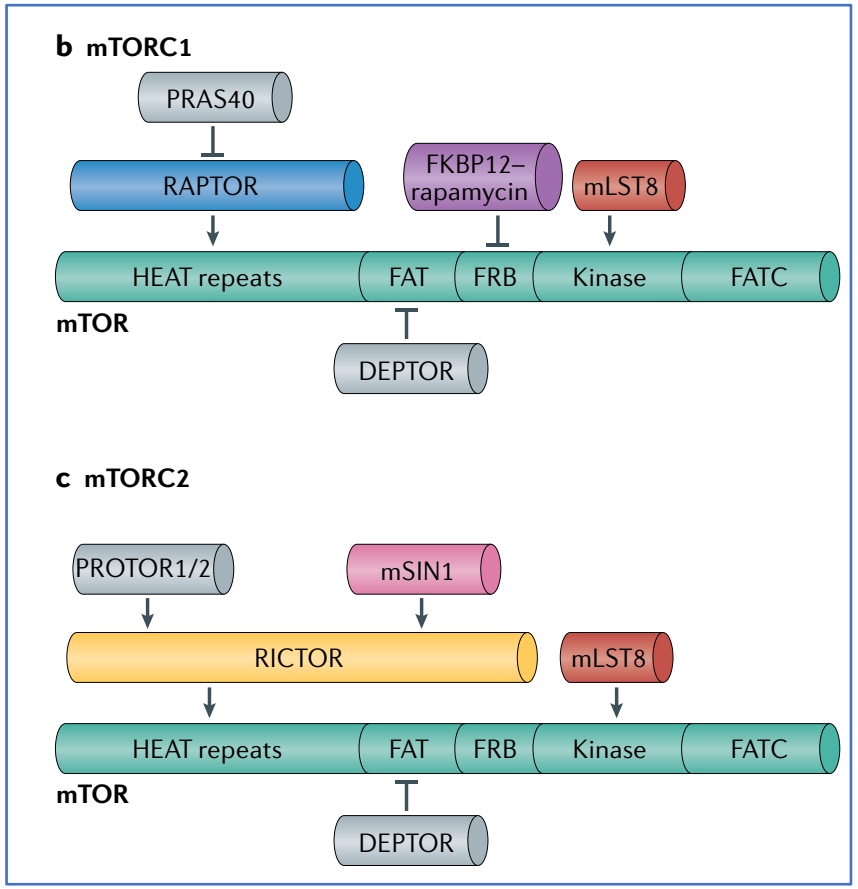




Grace Y. Liu[1,2,3,4] and David M. Sabatini[1,2,3,4]*

RAGs tether mTORC1 to lysosomes





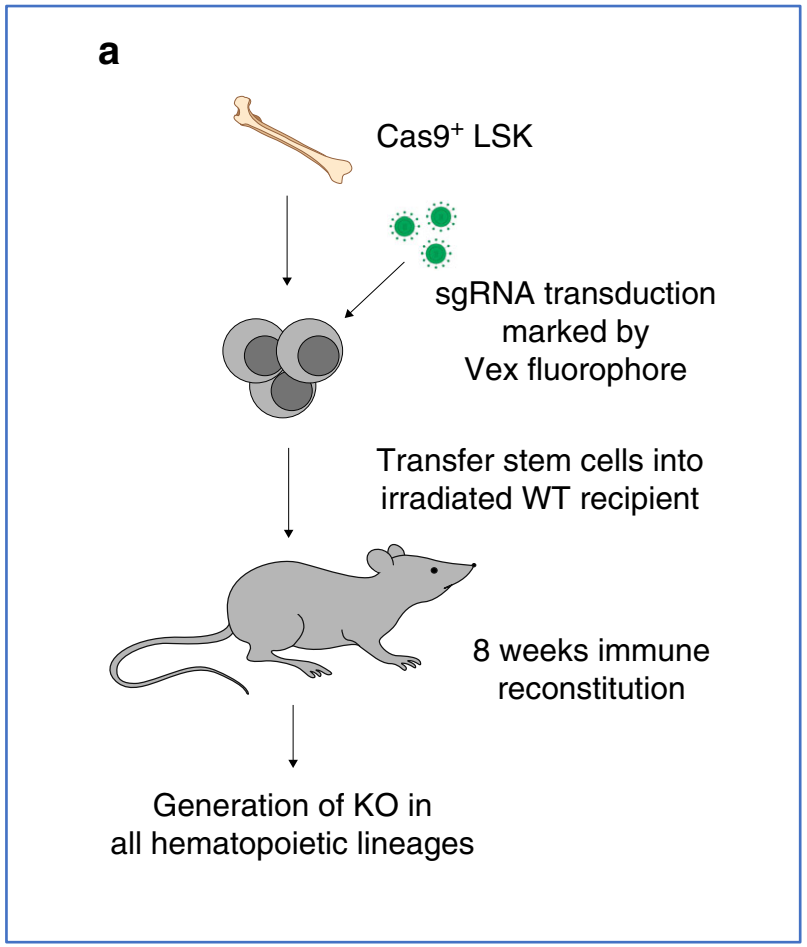
Grace Y. Liu[1,2,3,4] and David M. Sabatini[1,2,3,4]*

ARTICLE

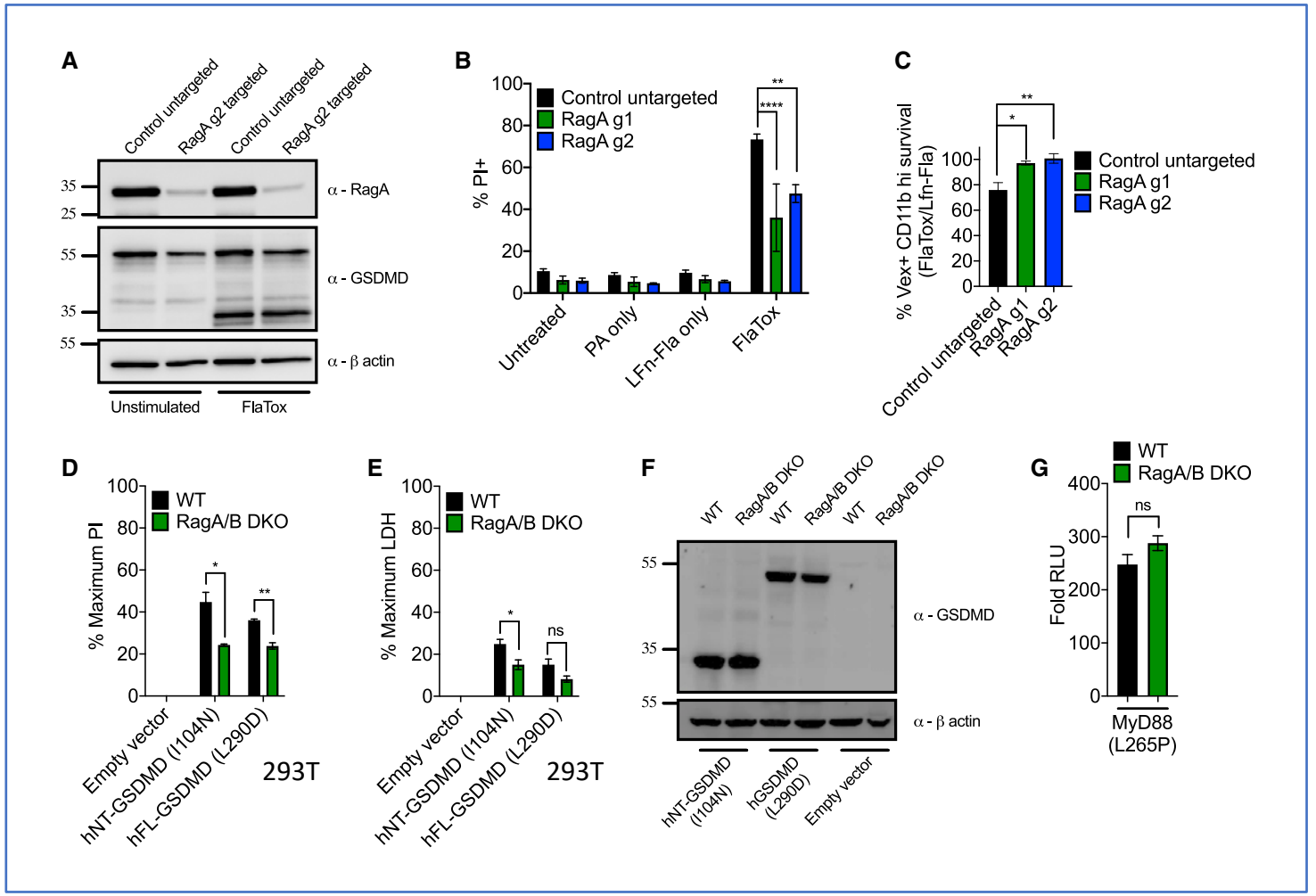
https://doi.org/10.1038/s41467-019-09656-2 OPEN

A CRISPR-Cas9 delivery system for in vivo screening of genes in the immune system

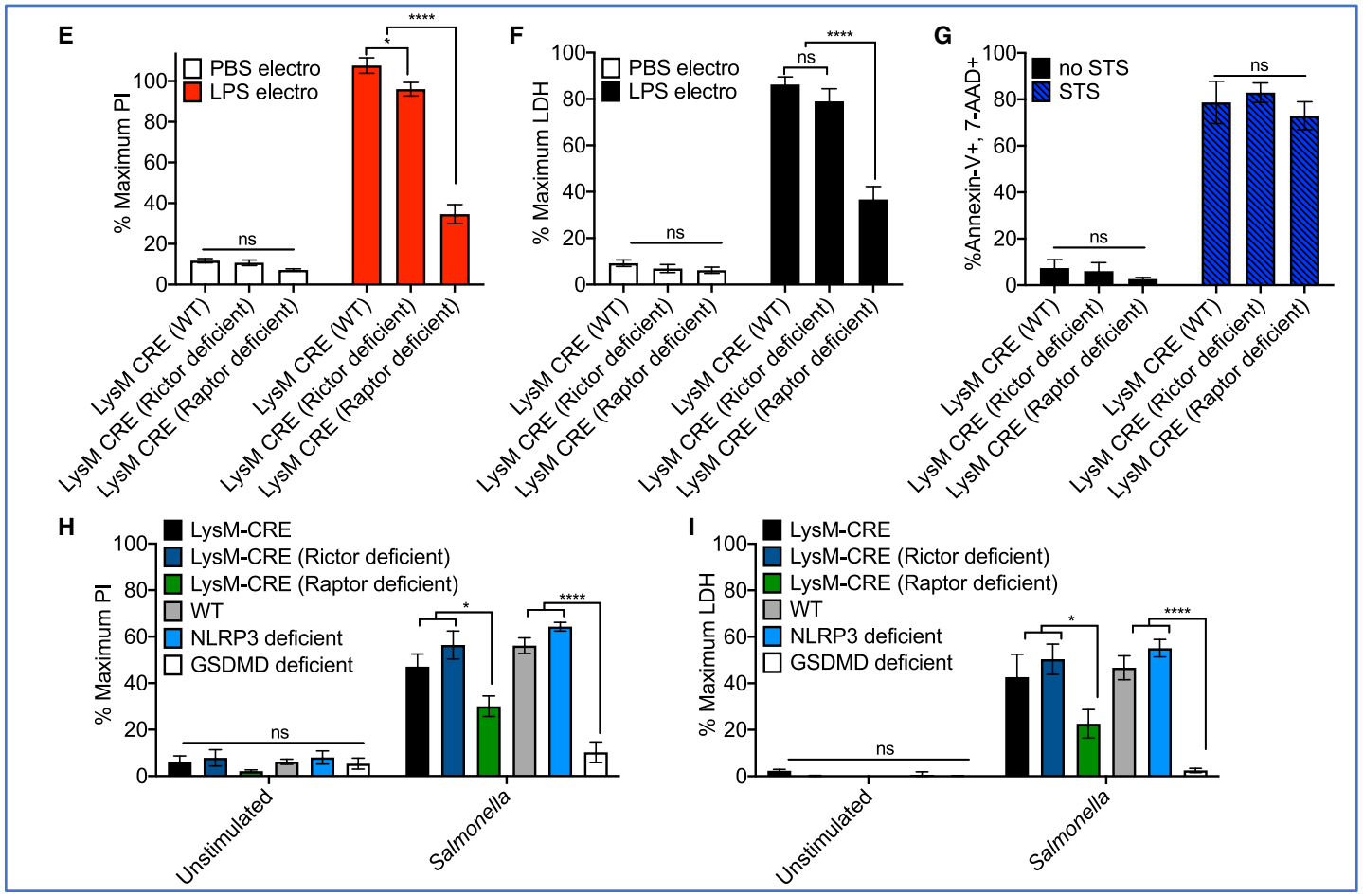
Martin W. LaFleur[1,2,3], Thao H. Nguyen[1,3], Matthew A. Coxe[1,3], Kathleen B. Yates[2,4], Justin D. Trombley[1,3], Sarah A. Weiss[2], Flavian D. Brown[1,2,3], Jacob E. Gillis[1,3], Daniel J. Coxe[5], John G. Doench[4], W. Nicholas Haining[2,4] & Arlene H. Sharpe[1,3,4]

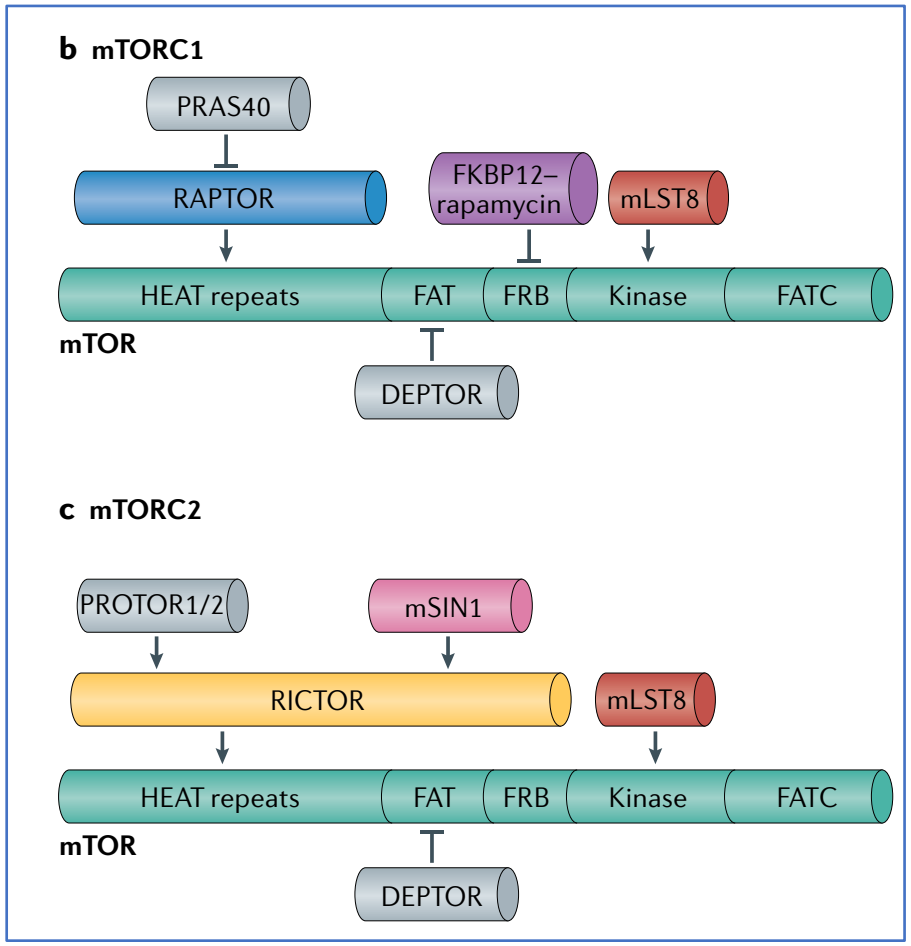


Hit validation in primary murine BMDCs and human HEK cells

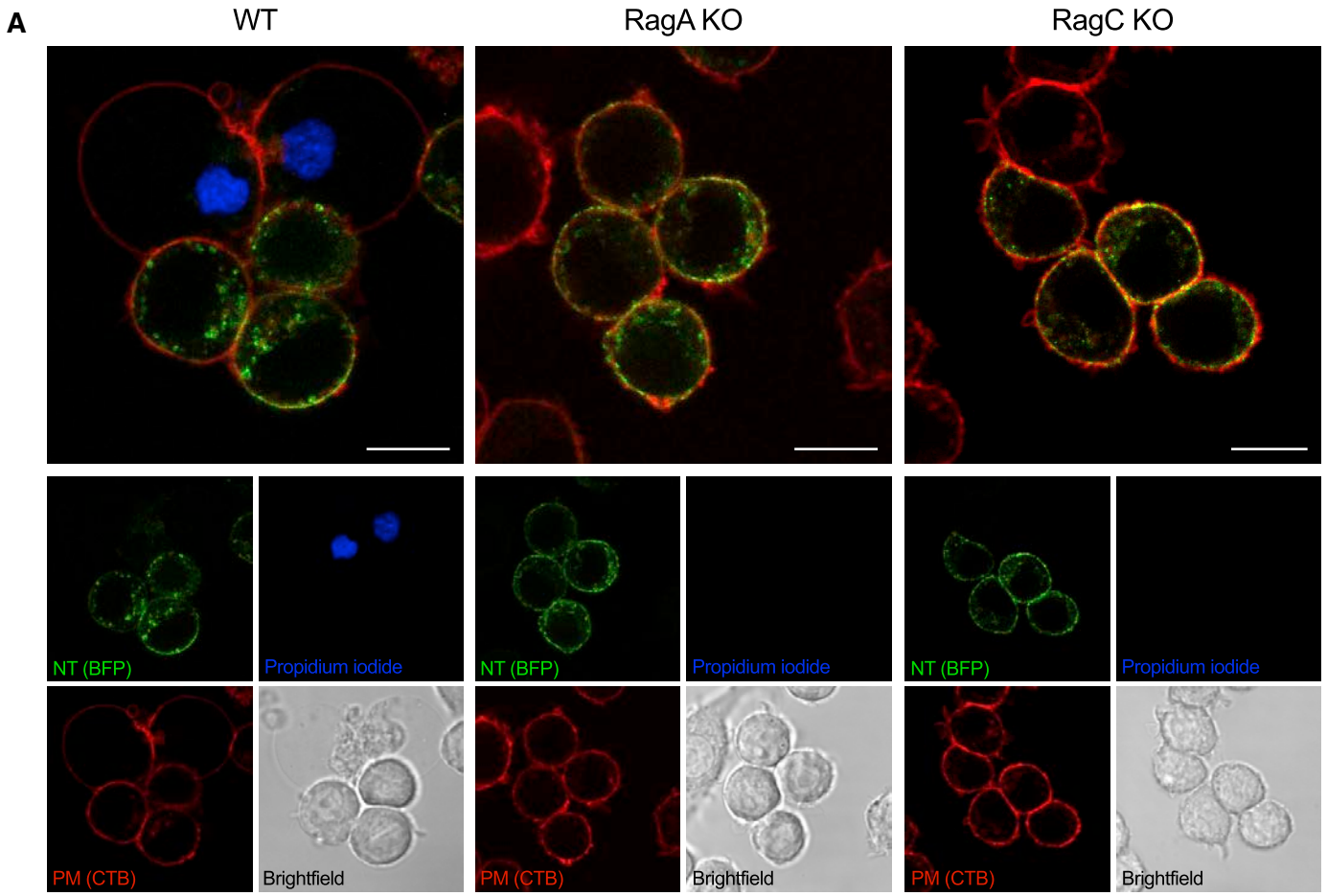


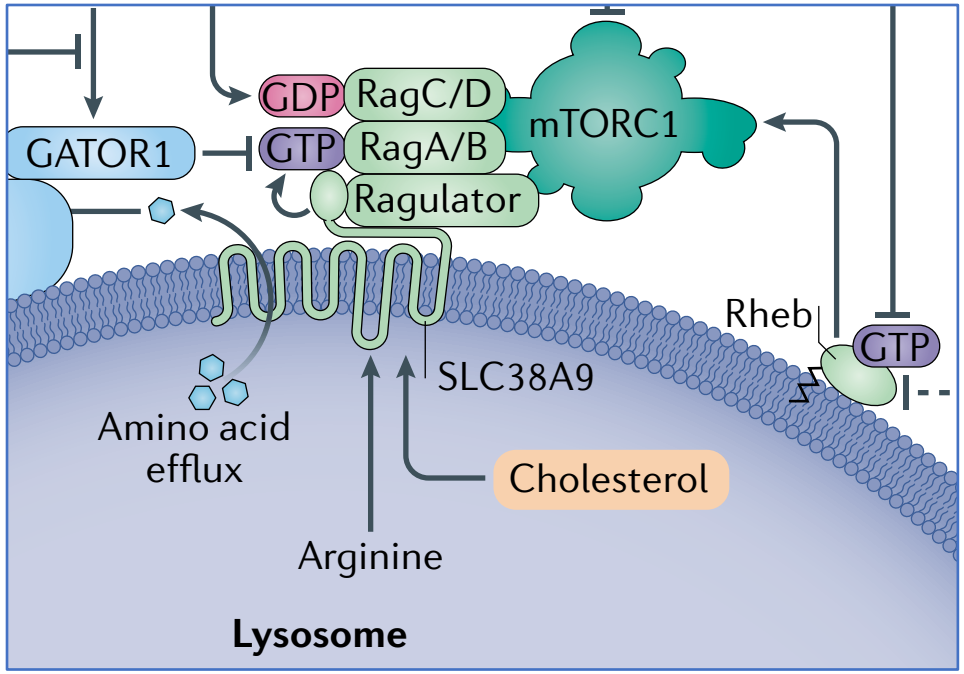
mTOR in the context of mTORC1 promotes pyroptosis in primary BMDCs



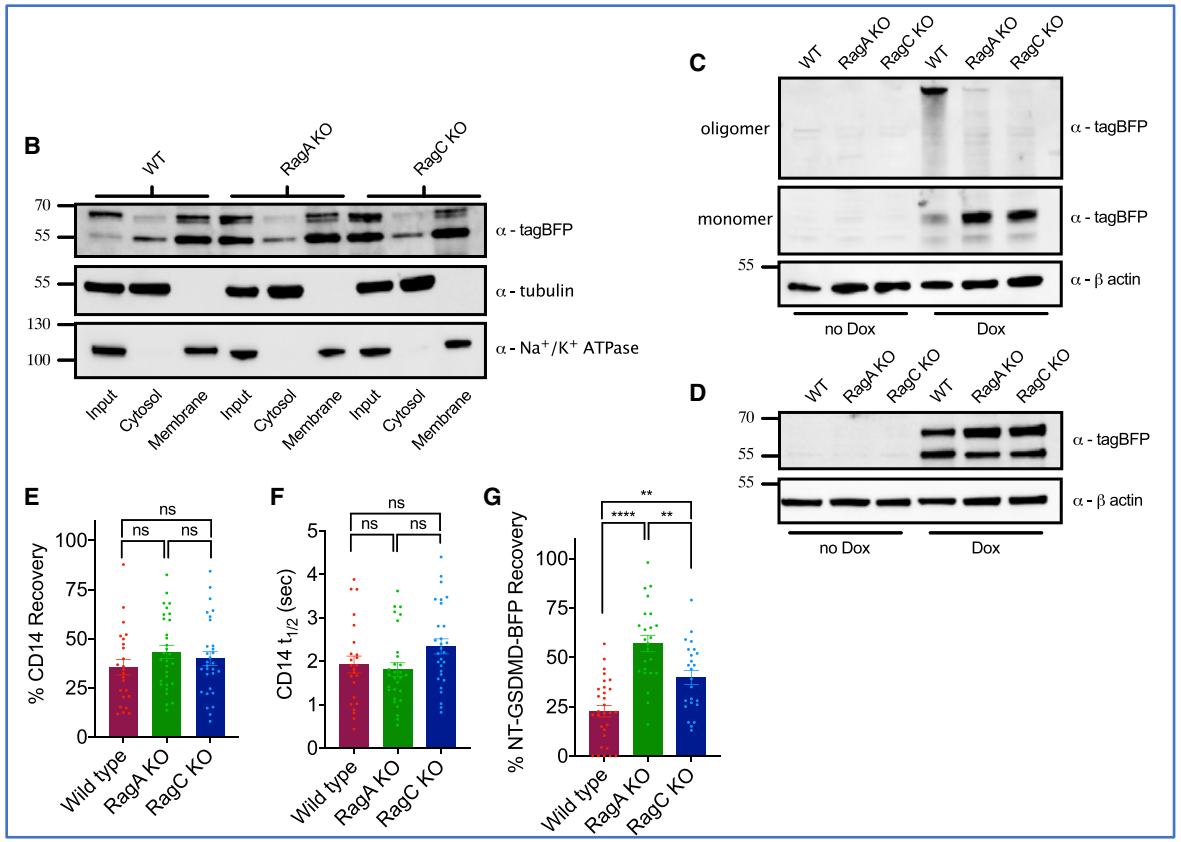


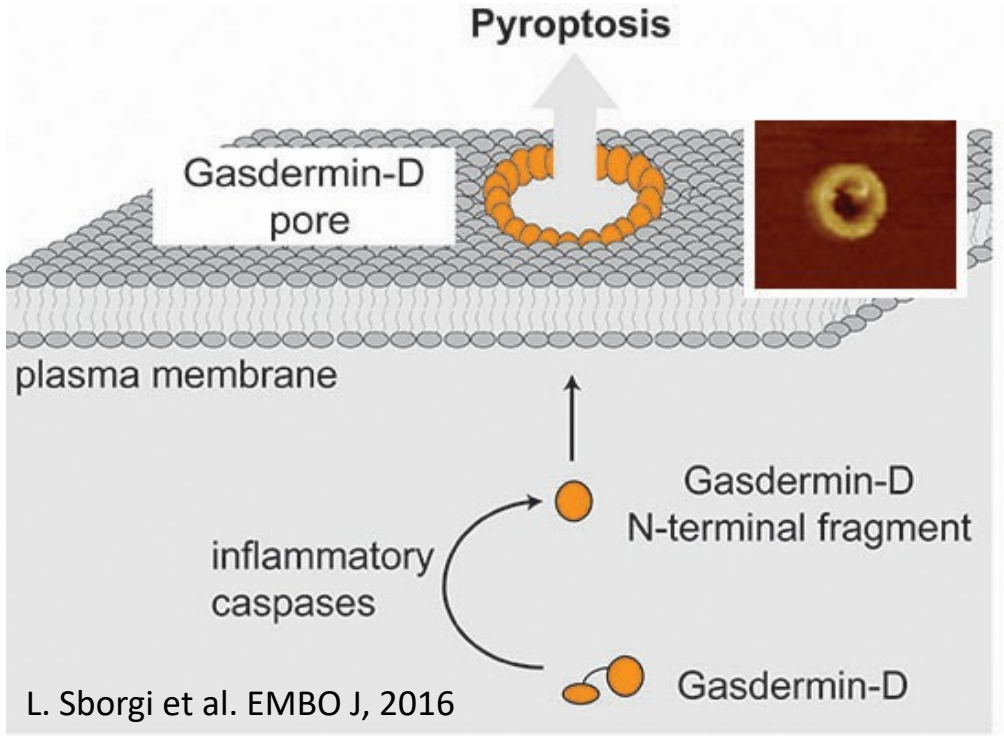
Loss of mTORC1 activity does not affect membrane recruitment of NT-GSDMD





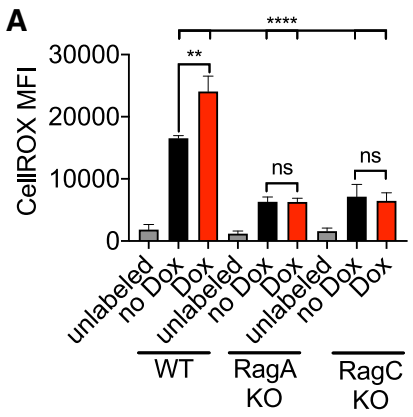
Loss of mTORC1 activity does not affect membrane recruitment of NT-GSDMD

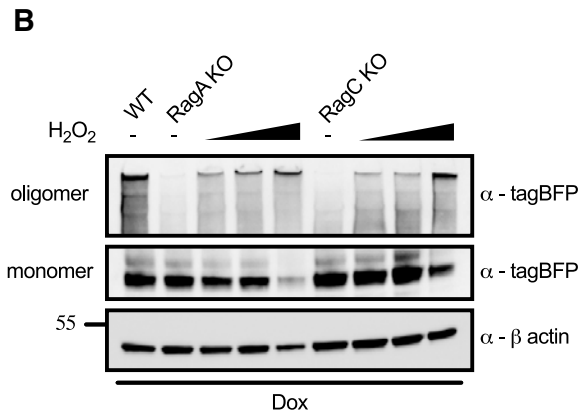


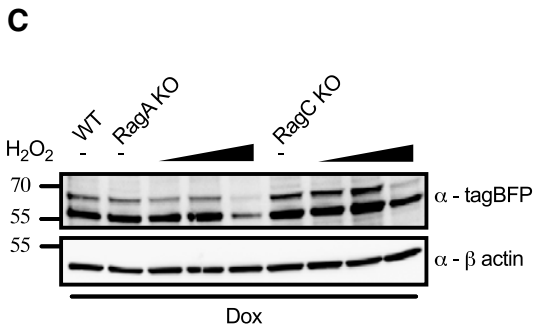


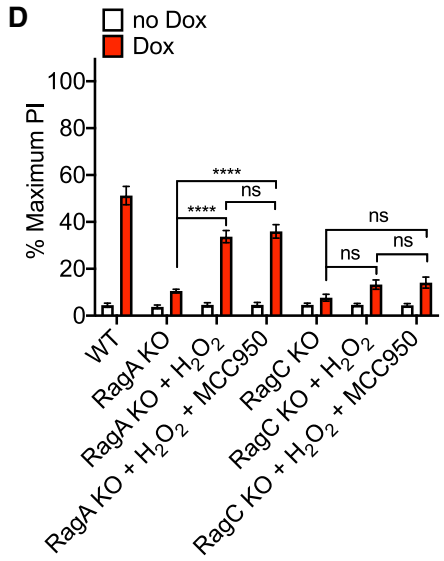
L. Sborgi et al. EMBO J, 2016

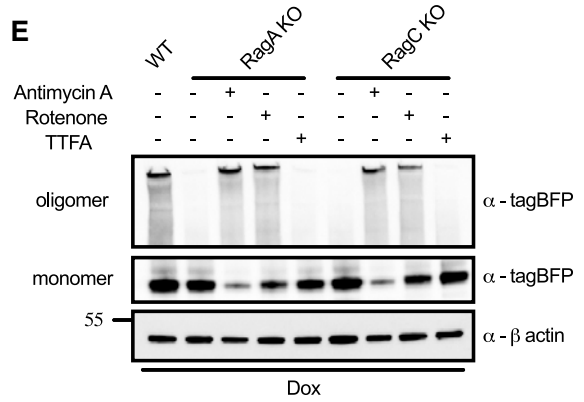
Loss of mTORC1 activity does affect cellular ROS levels

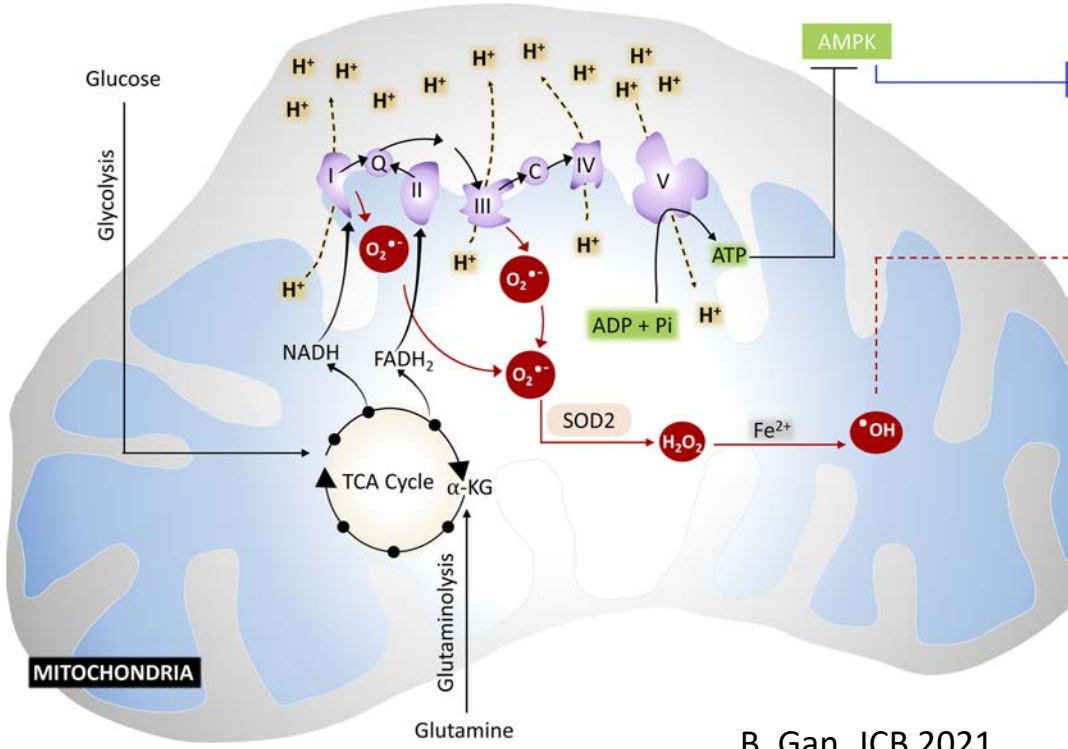






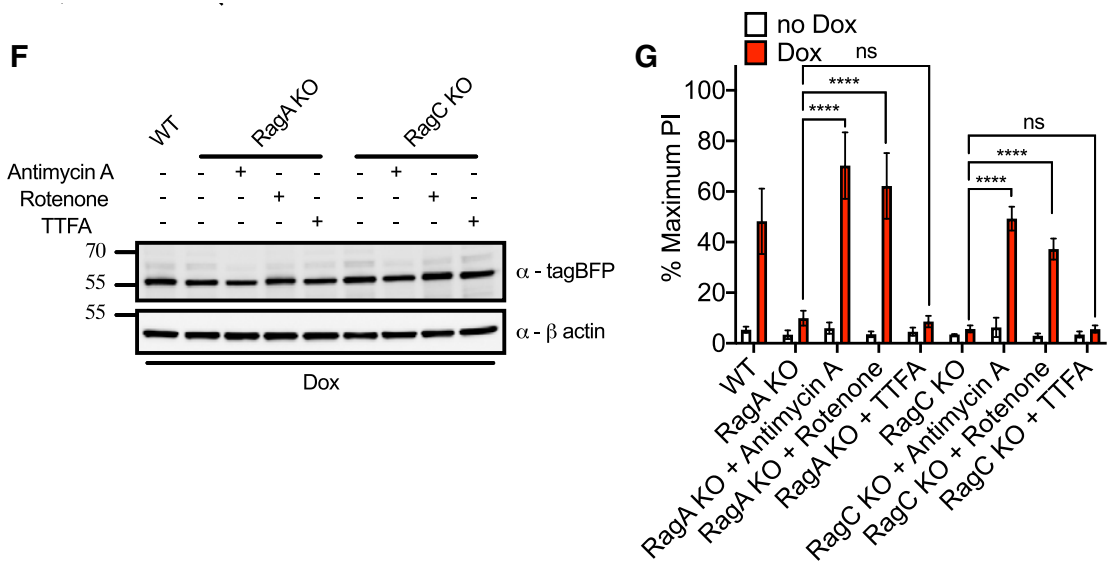


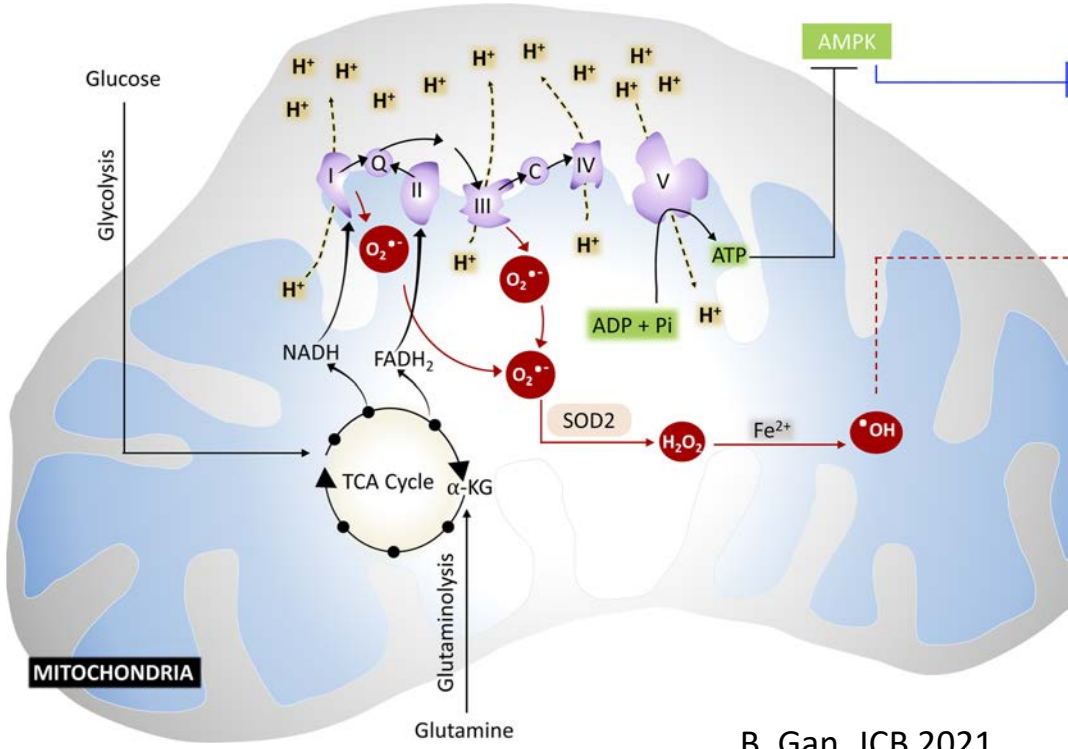




B. Gan, JCB 2021

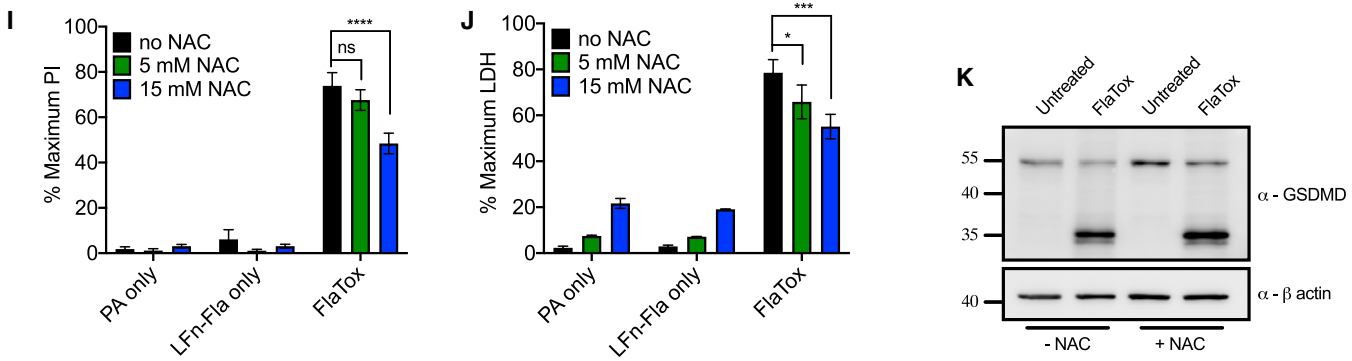
Increased ROS levels promote pyroptosis in RagA/C KO cells

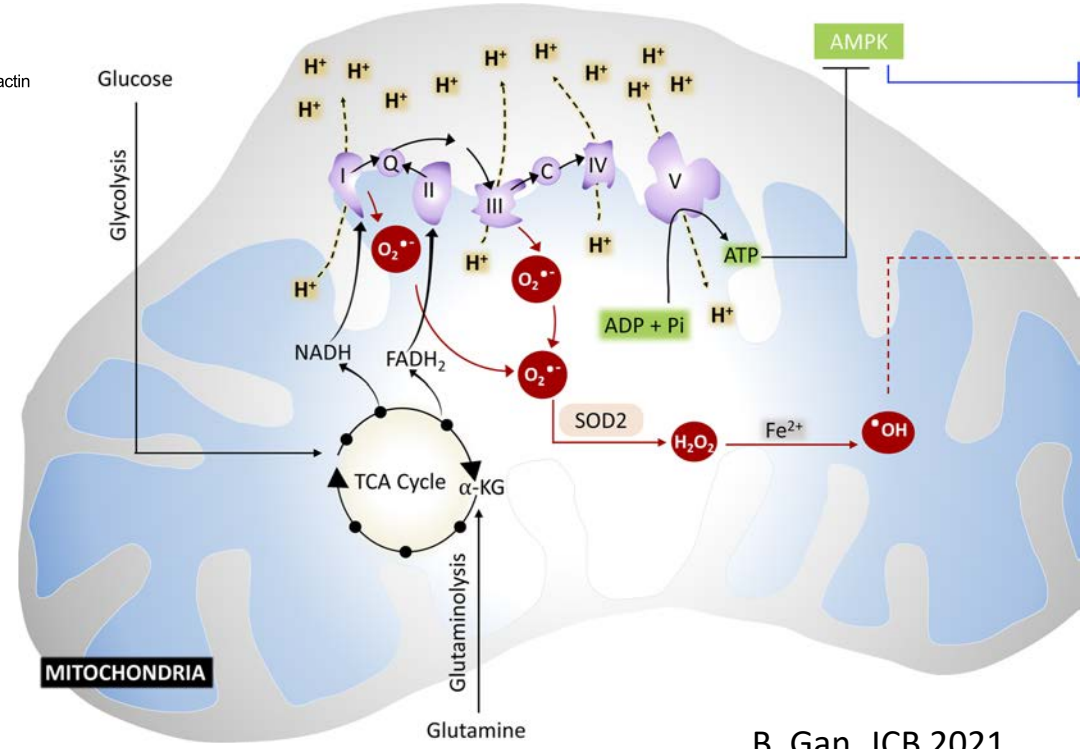




B. Gan, JCB 2021

ROS scavenging reduces pyroptosis w/o affecting processing

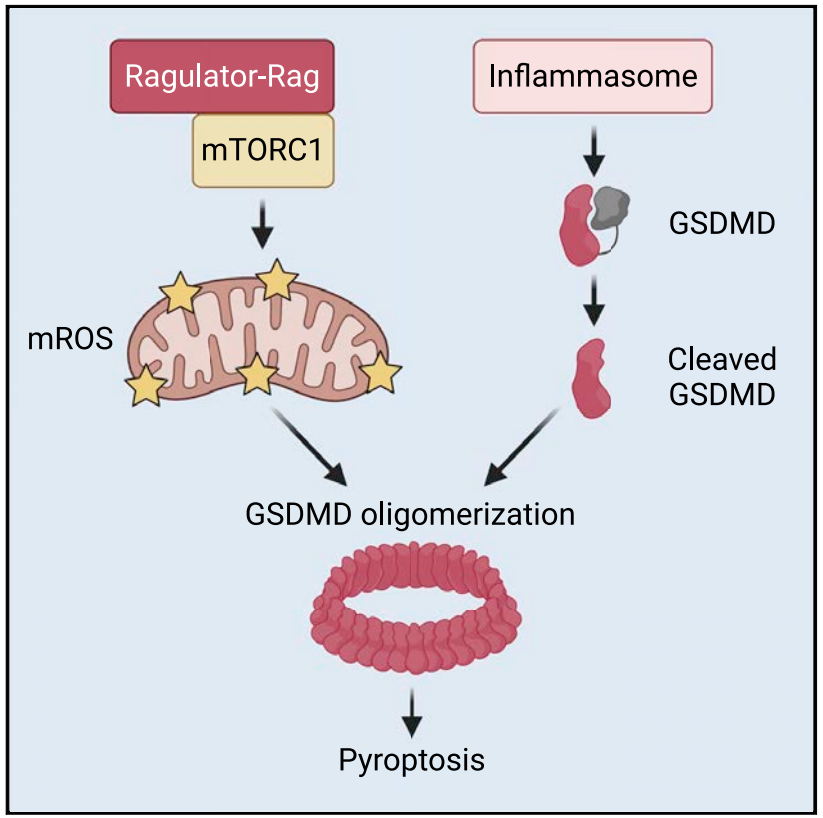




B. Gan, JCB 2021

Summary & open questions.....

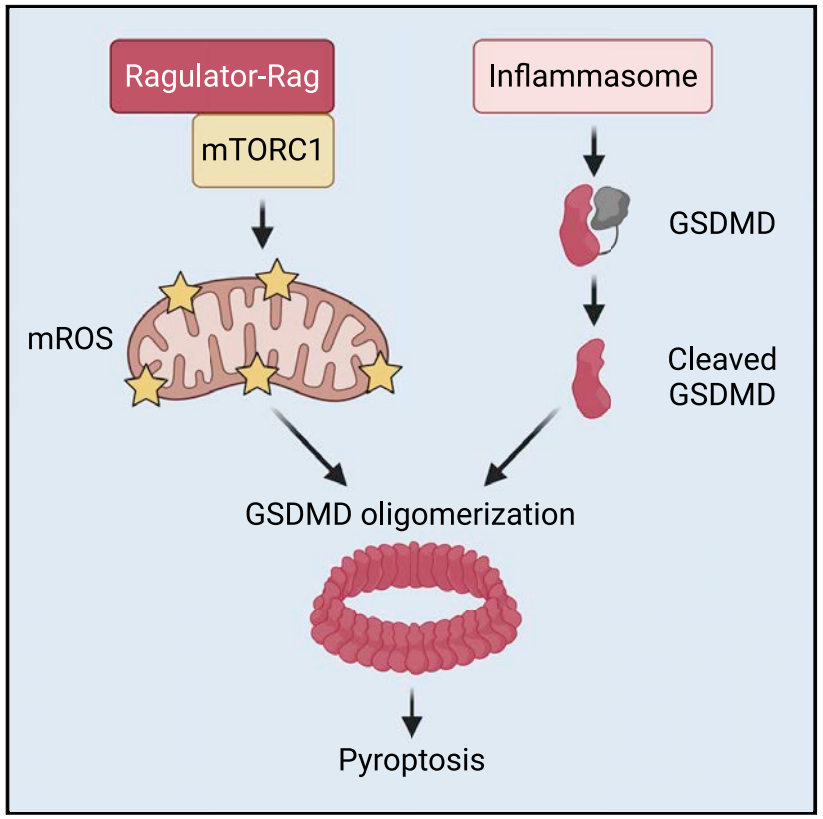
Graphical abstract



- The Ragulator-Rag-mTORC1 pathway is required for pyroptosis induced by gasdermin D
- Ragulator-Rag promotes gasdermin D oligomerization but not membrane localization
- Ragulator-Rag promotes reactive oxygen species (ROS) production in macrophages
- ROS promotes gasdermin D oligomerization, pore formation, and pyroptosis

Summary & open questions.....

Graphical abstract



- How does mTORC1 affect mito-ROS levels or respiration?
- How does ROS facilitate pore formation/oligomerization?
- Is this a direct effect on the NT-GSDMD protein -> how, M/C?
- May this be indirect, related to oxidation of membrane lipids by ROS?