GRASP55 and UPR Control Interleukin-1β Aggregation and Secretion

Proteostasis and protein secretion
17-18 October 2019 | Madrid, Spain

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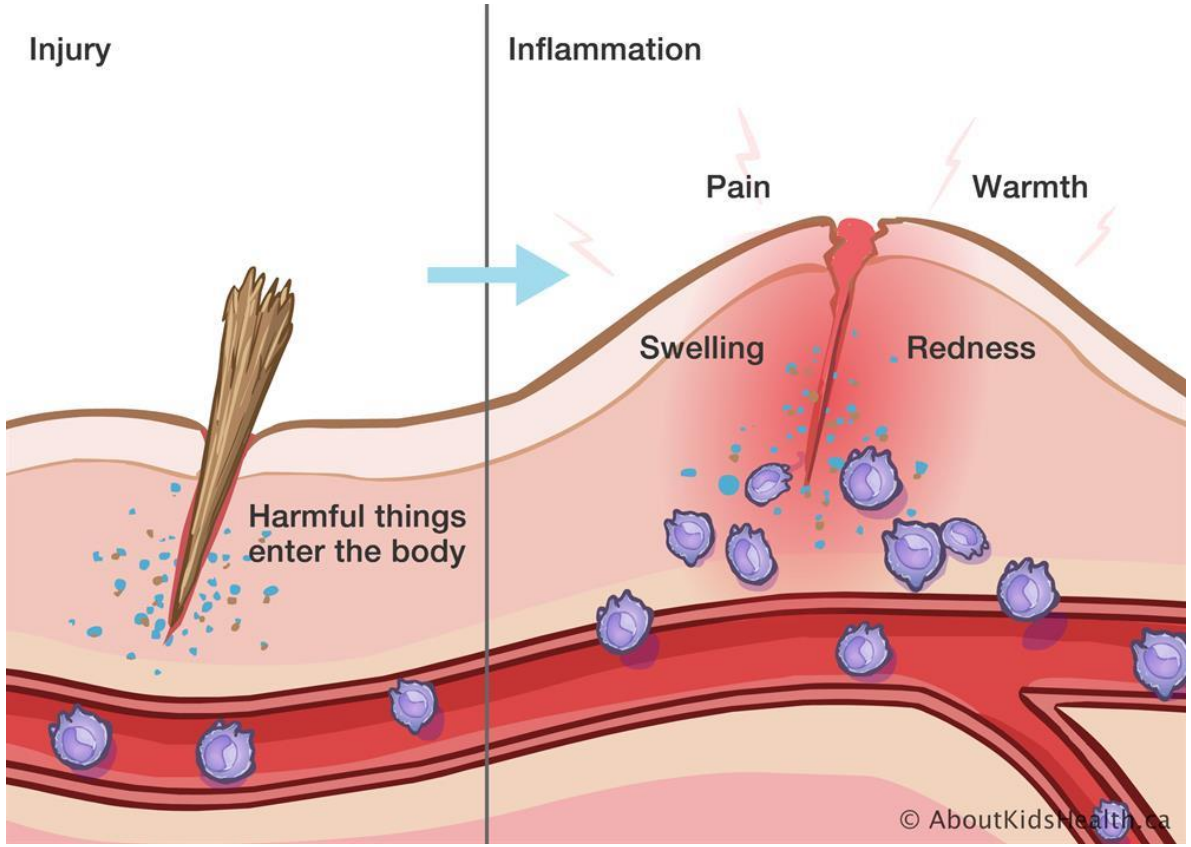
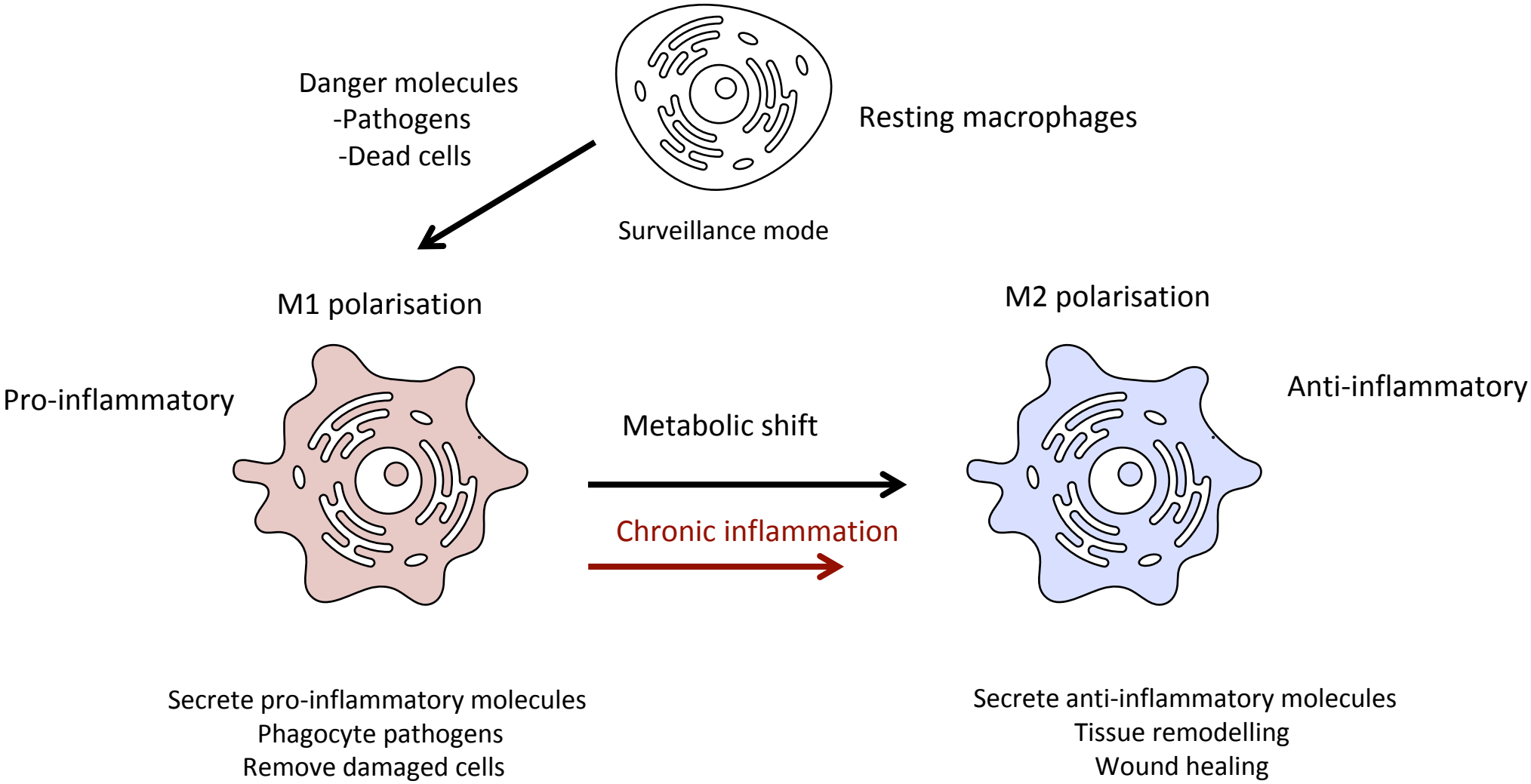
Inflammation- why should we study it?



-Acute pro-inflammatory response

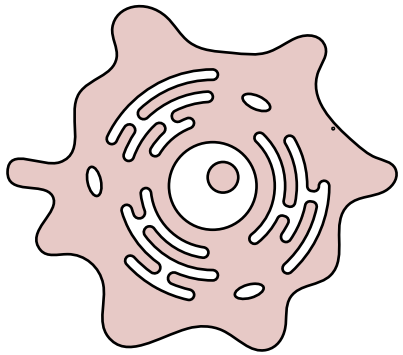
Beneficial

Macrophages-mediators of inflammation



Macrophages-pro-inflammatory phenotype

M1 polarisation



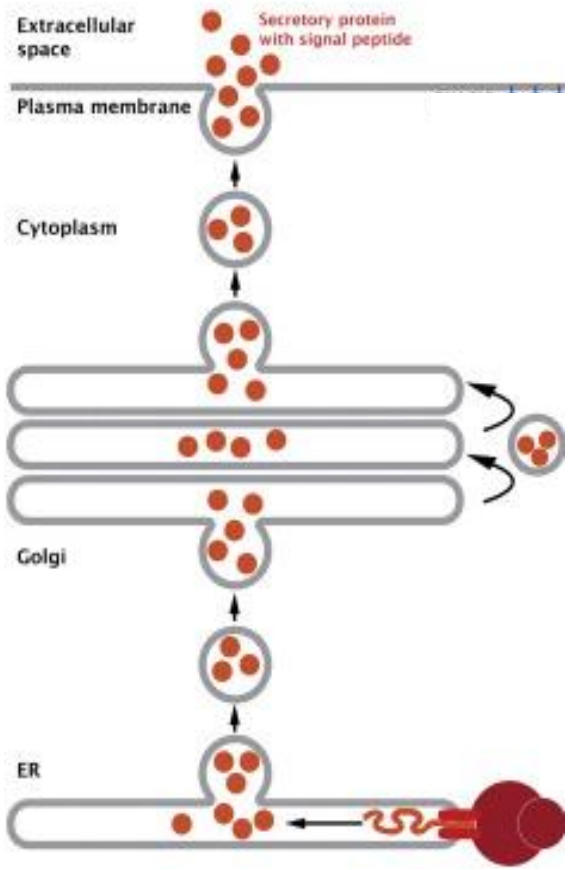
Pro-inflammatory

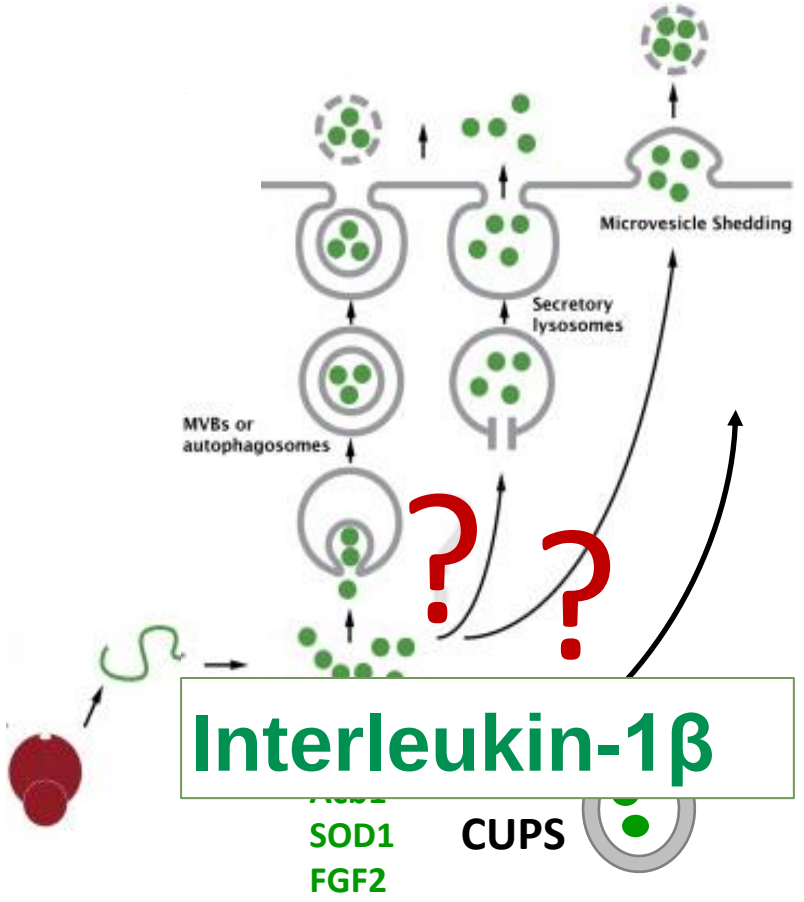
- Secrete pro-inflammatory molecules (cytokines, chemokine)
- Phagocyte pathogens (bacteria, viruses)
- Remove damaged cells

Cytokines like **IL-1α, IL-1β**, IL-6, IL-8, **IL-18,**

Secreted by - the conventional ER-to-Golgi pathway
- the **unconventional secretory pathway**

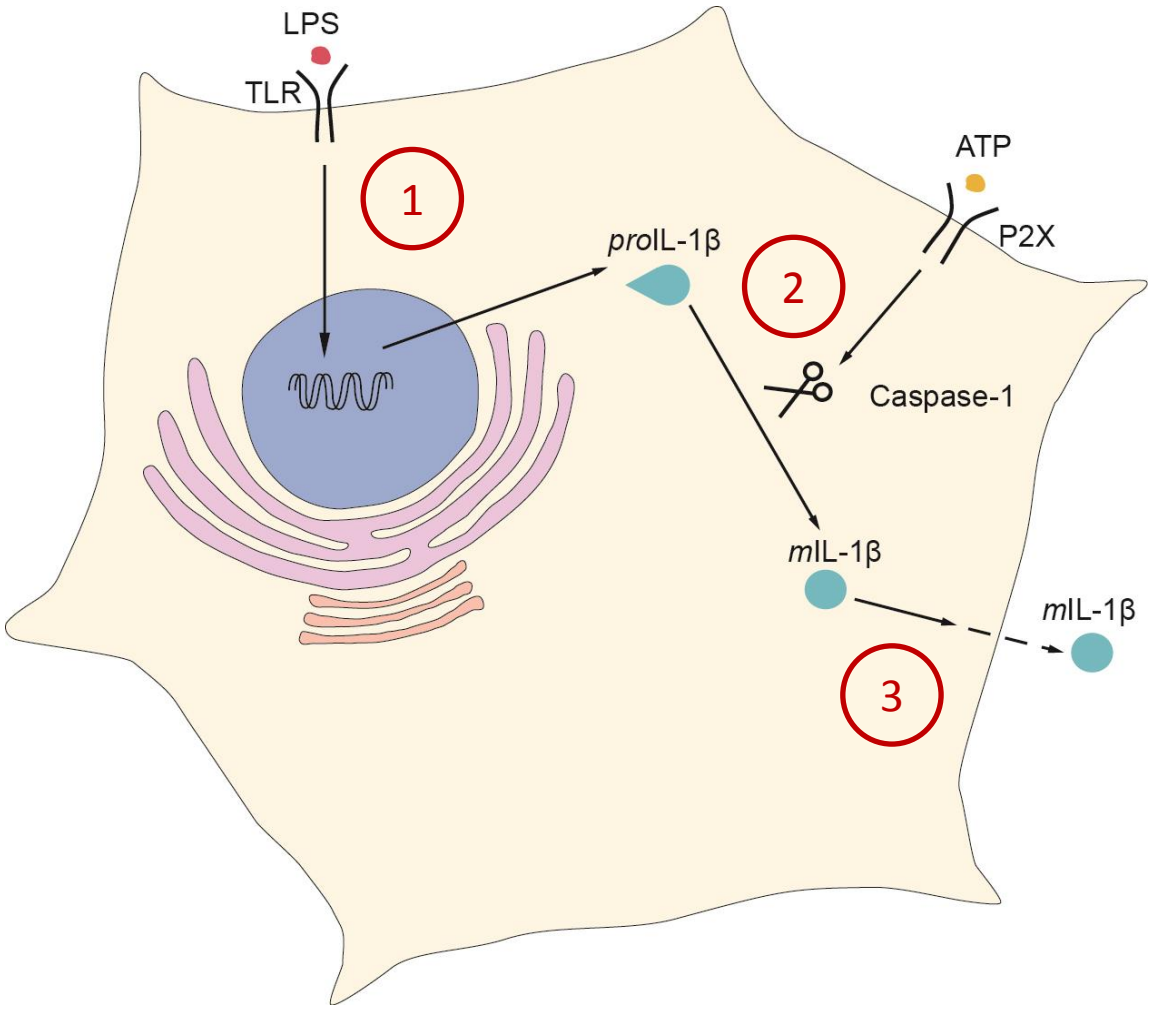
Protein Secretion from Eukaryotes





Adapted from: Steringer, J et al, *J.Mol.Biol.* (2015)

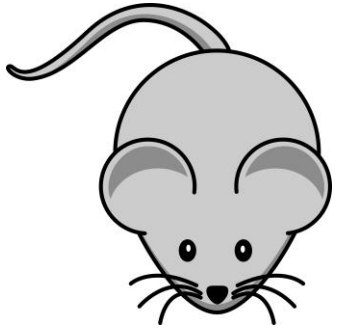
The pathway of IL-1β synthesis and export



Three step process:

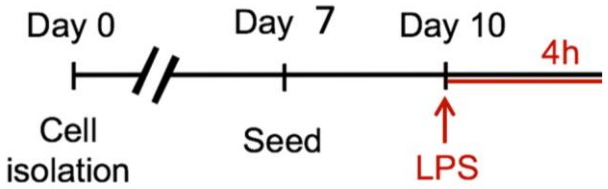
1. Synthesis of *pro*IL-1β
2. Proteolysis to *m*IL-1β
3. Export of *m*IL-1β to the extracellular space

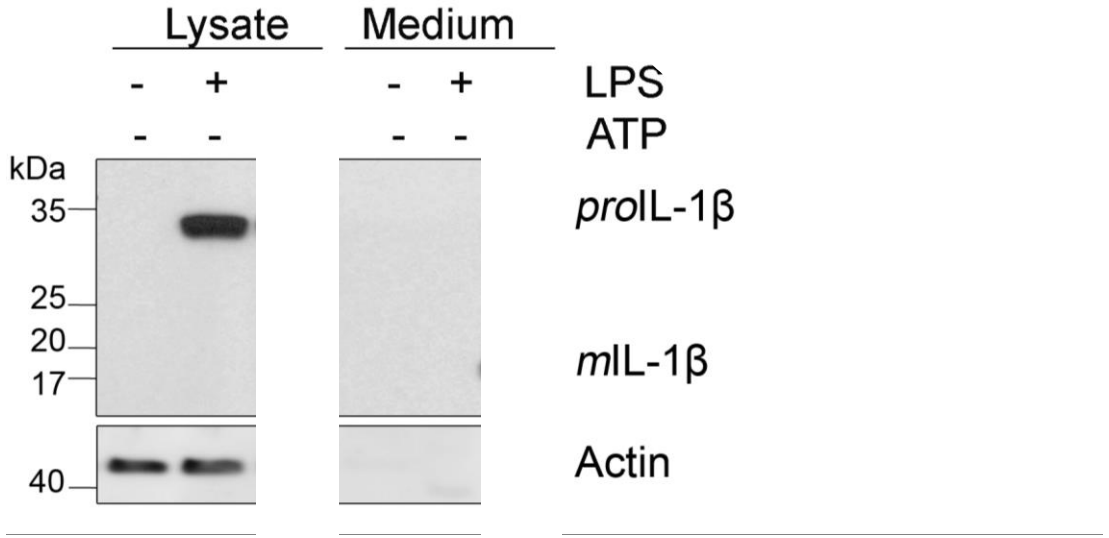
Measuring secretion of endogenous interleukin-1β from murine macrophages



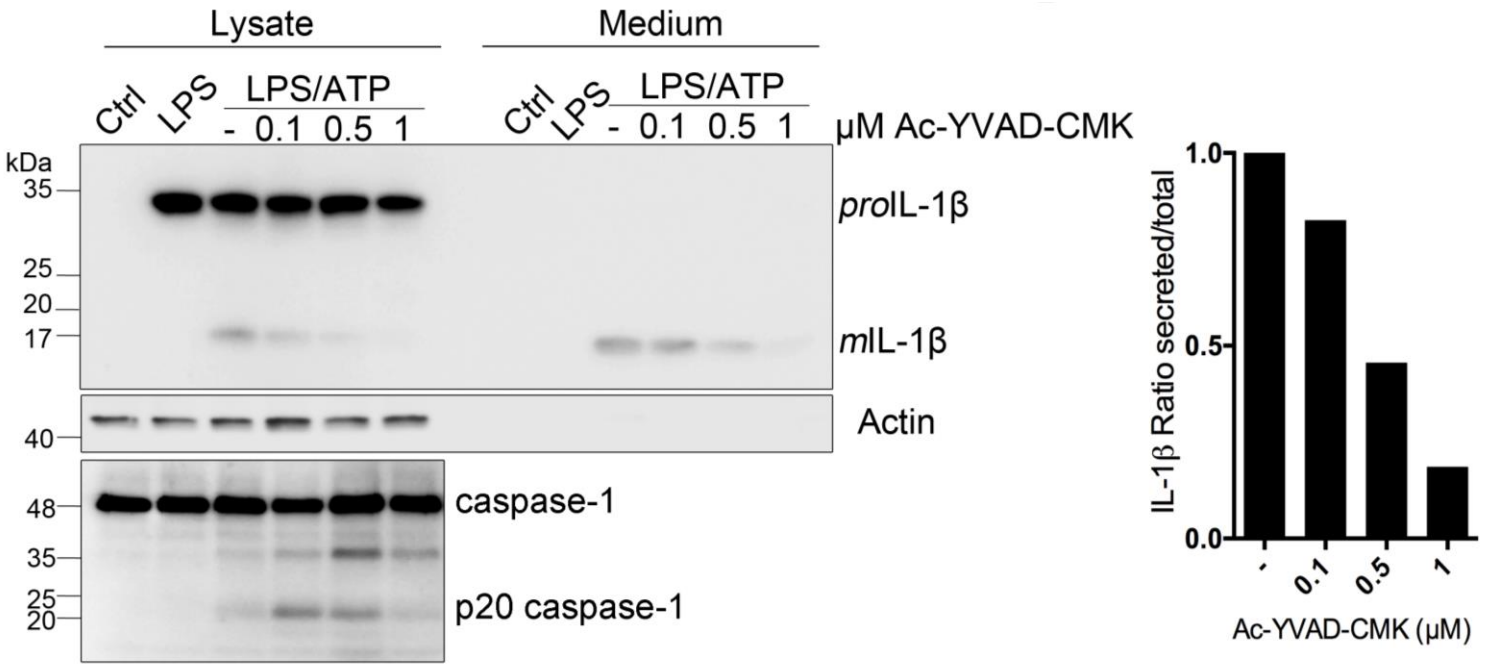
Bone marrow → Bone marrow-derived macrophages

Peritoneal fluid → Peritoneal macrophages





Caspase-1 inhibitors block IL-1β secretion from stimulated macrophages



GRASPs-Key proteins for Unconventional Protein Secretion

GRASPs were defined as Golgi structural proteins with peripheral location

Peripheral Golgi protein shown previously to be required for the unconventional secretion.

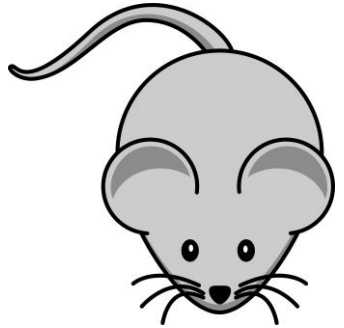
-GrpA- *D. discoideum* Kinset et al. 2007

-dGRASP - *D. melanogaster* Schotman et al. 2008

-Grh1- *S. cerevisiae* Bruns et al. 2011, Cruz-Garcia 2014, Curwin et al. 2016, Cruz-Garcia 2018

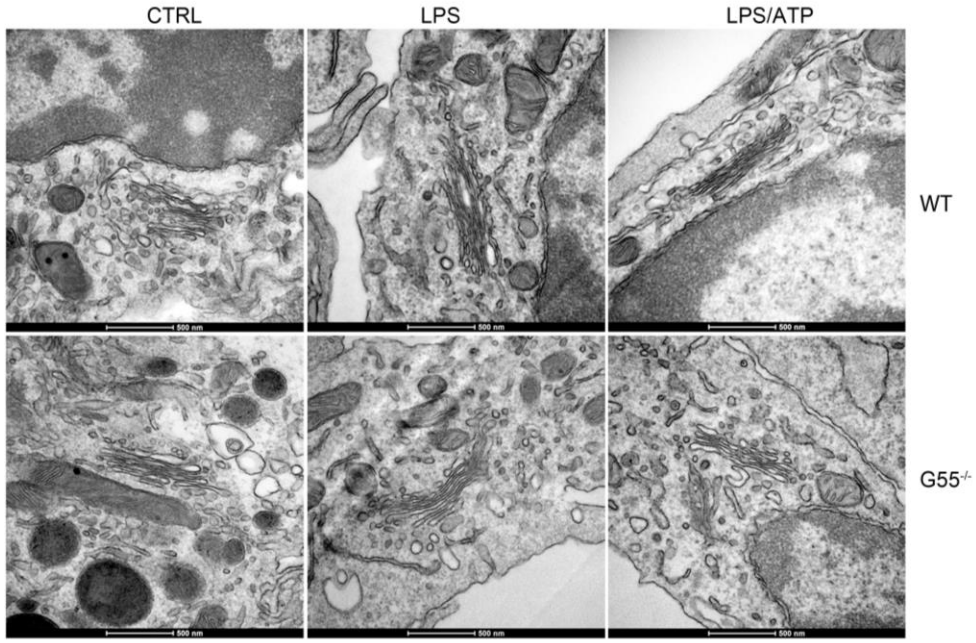
-GRASP55/65 – mammals Dupont N et al. 2011, Zhang M et al. 2015

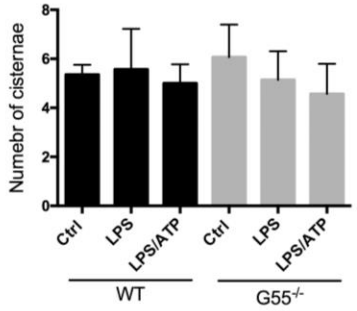
GRASP55 knockout mouse

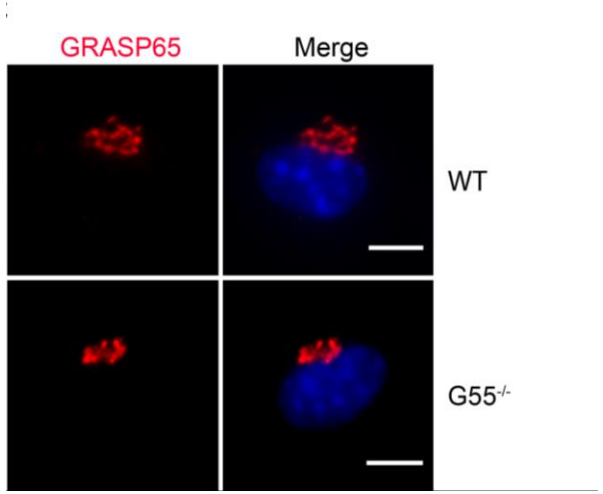


Viable with no major defects

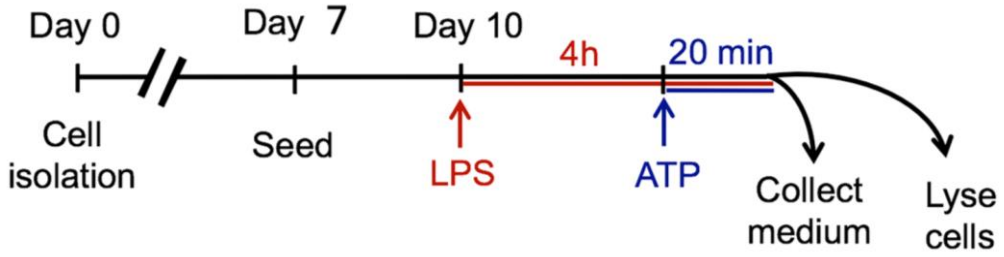
Deletion of GRASP55 does not disrupt Golgi stacking in macrophages



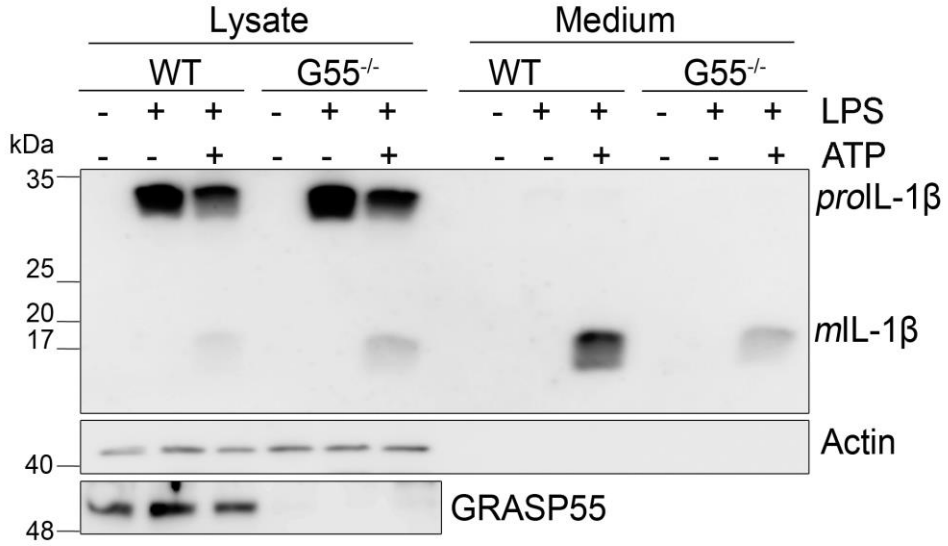


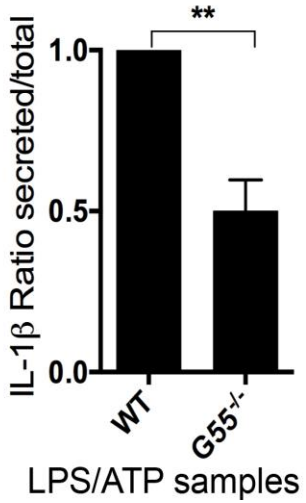


GRASP55 is required for secretion of *m*IL-1β



Secreted IL-1β **Intracellular IL-1β**

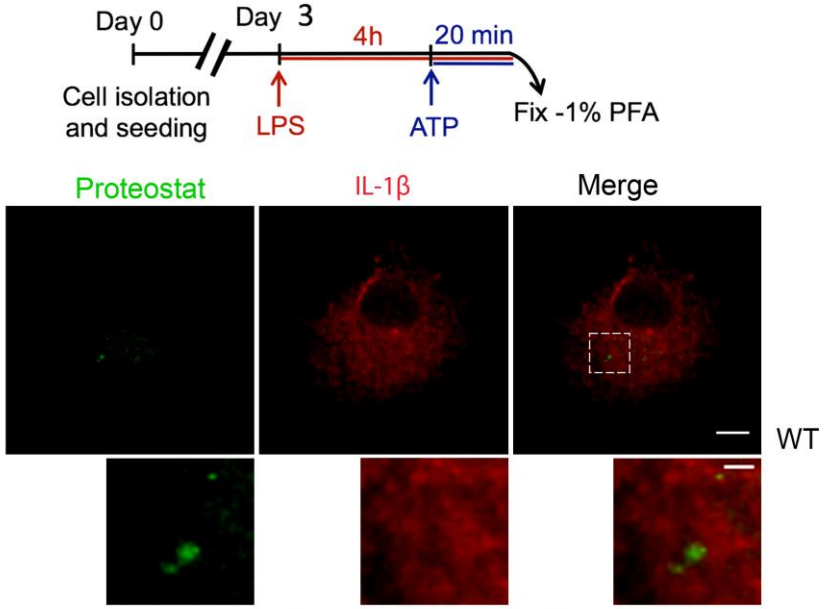


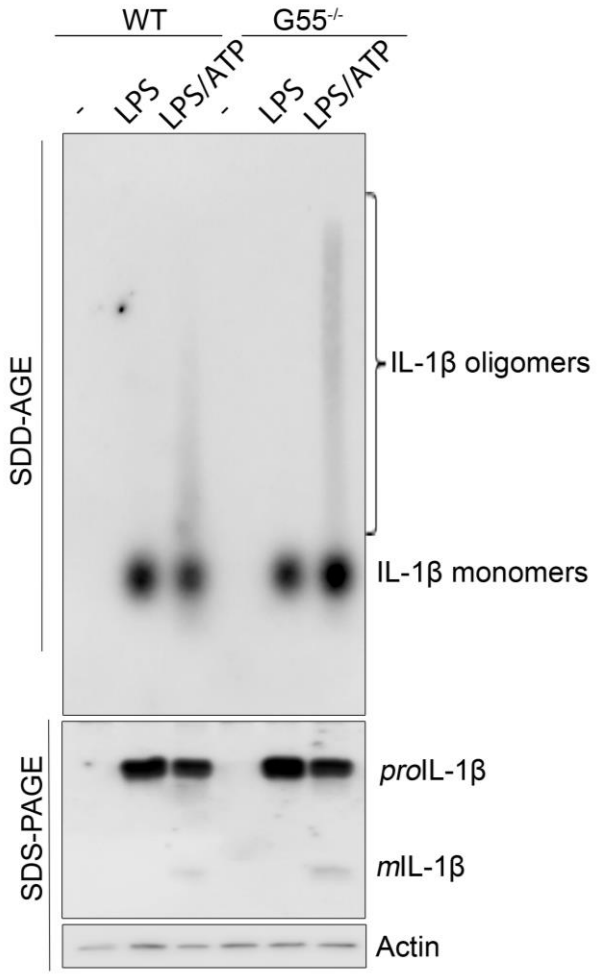


-Reduced secretion ~50%

-Accumulation of intracellular *m*IL-1β

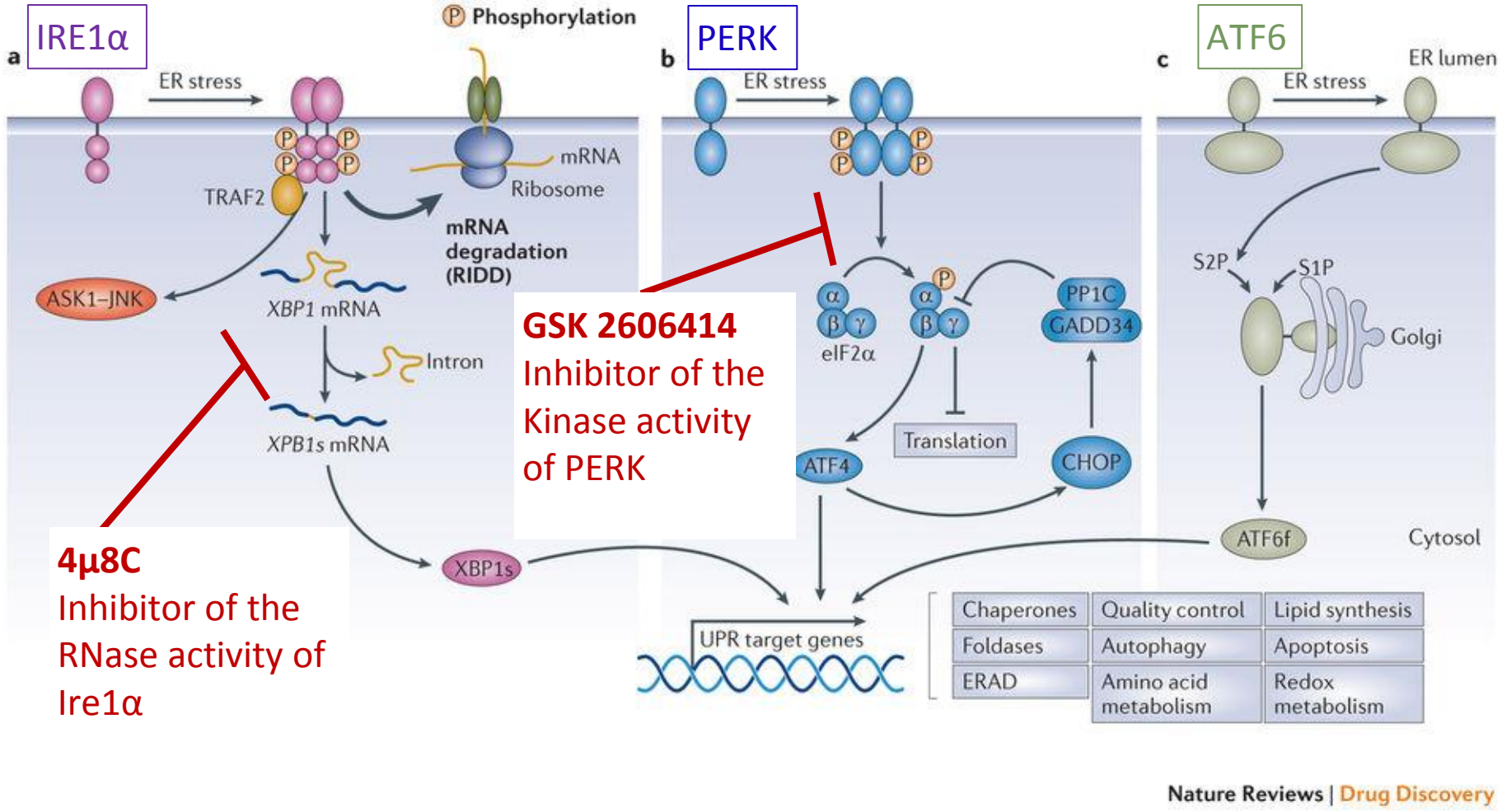
GRASP55 deletion promotes IL-1β aggregation





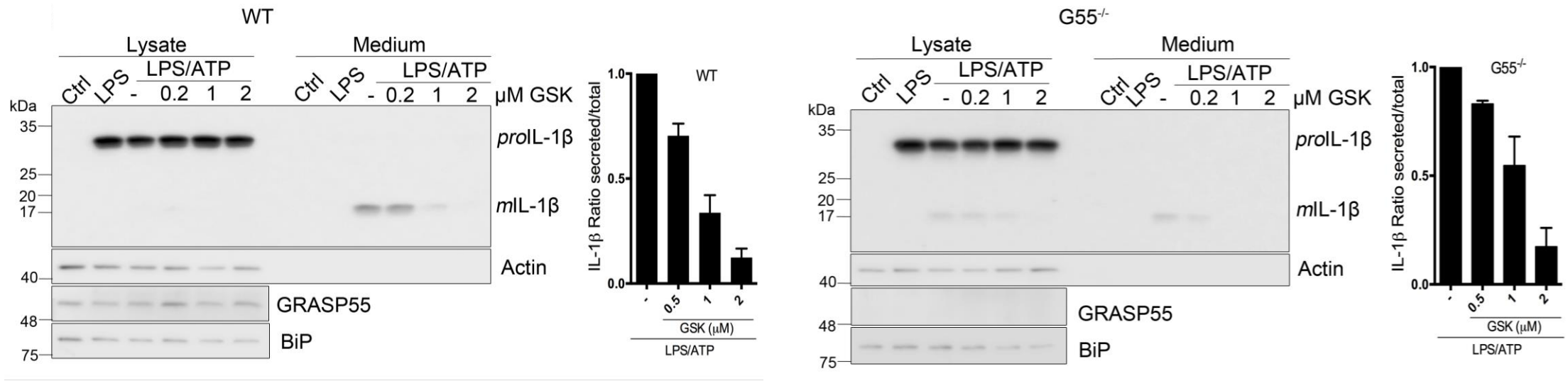
Could the formation of aggregates be correlated with cellular stress response pathways like UPR?

UPR signaling could have a contribution to this process?



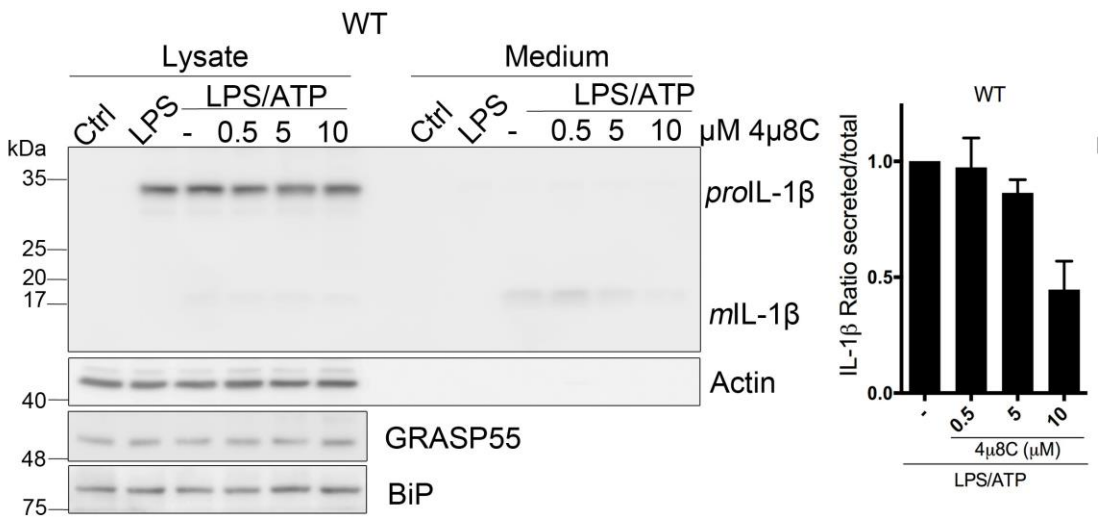
Adapted from: Hetz et al, *Nat. Rev. Drug Discov.* (2013)

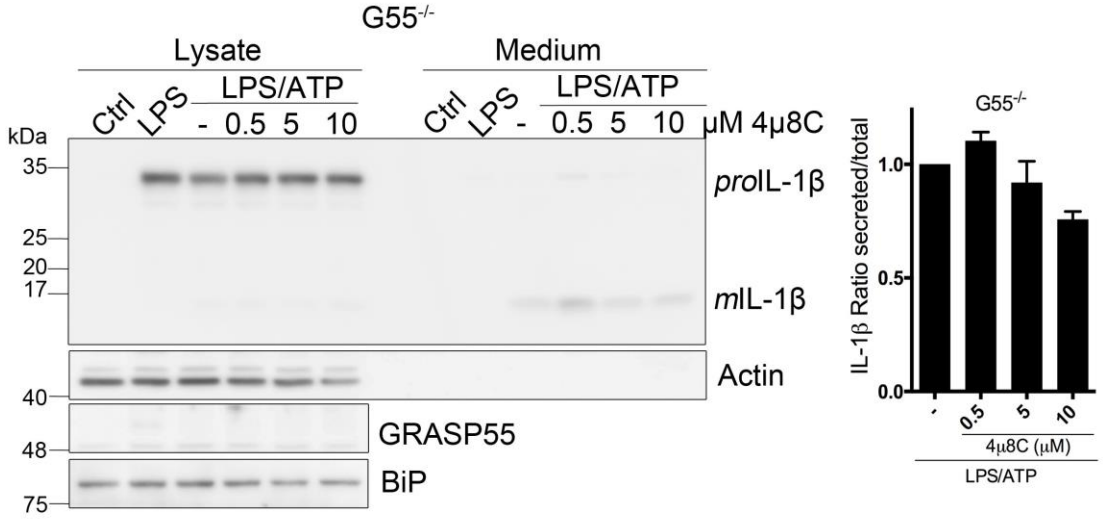
PERK pathway controls *pro* to *m*IL-1β proteolysis



-concentration dependent reduction of IL-1β secretion
-reduction of the pro to mIL-1β cleavage

Inhibition of IRE1α blocks IL-1β secretion

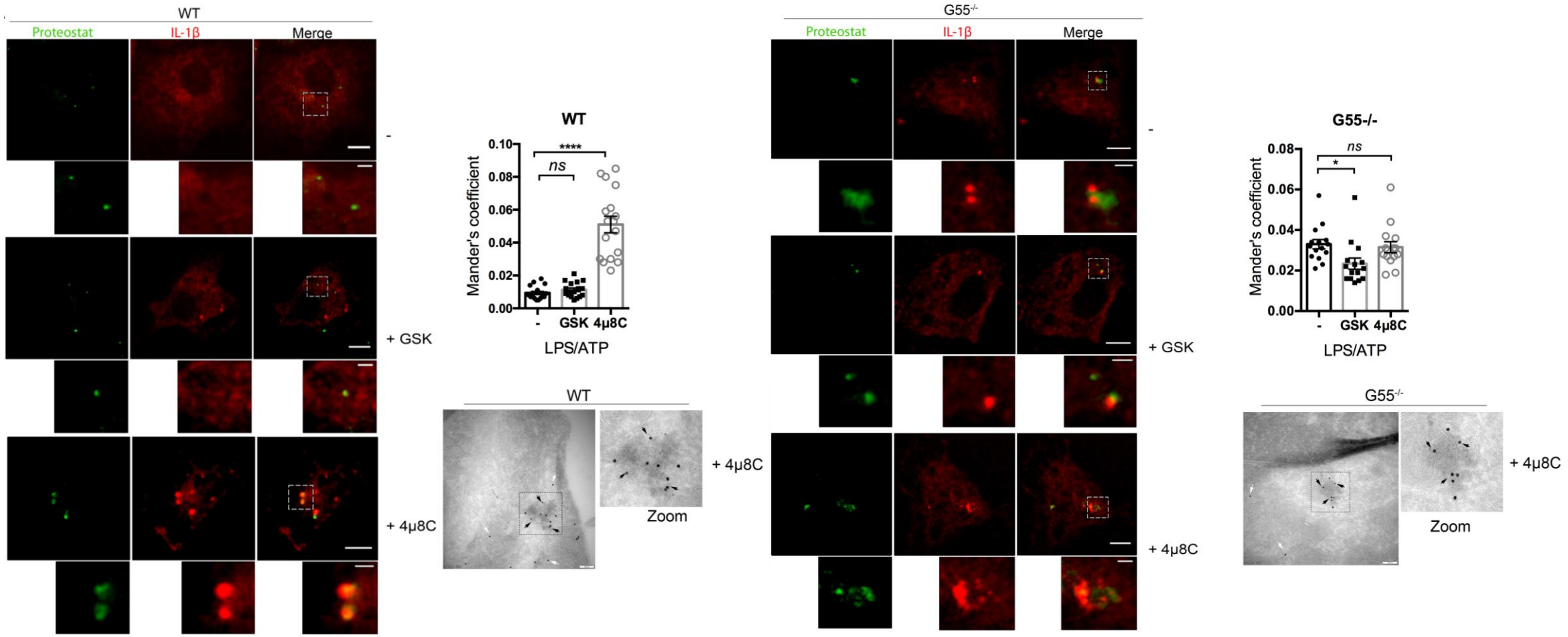




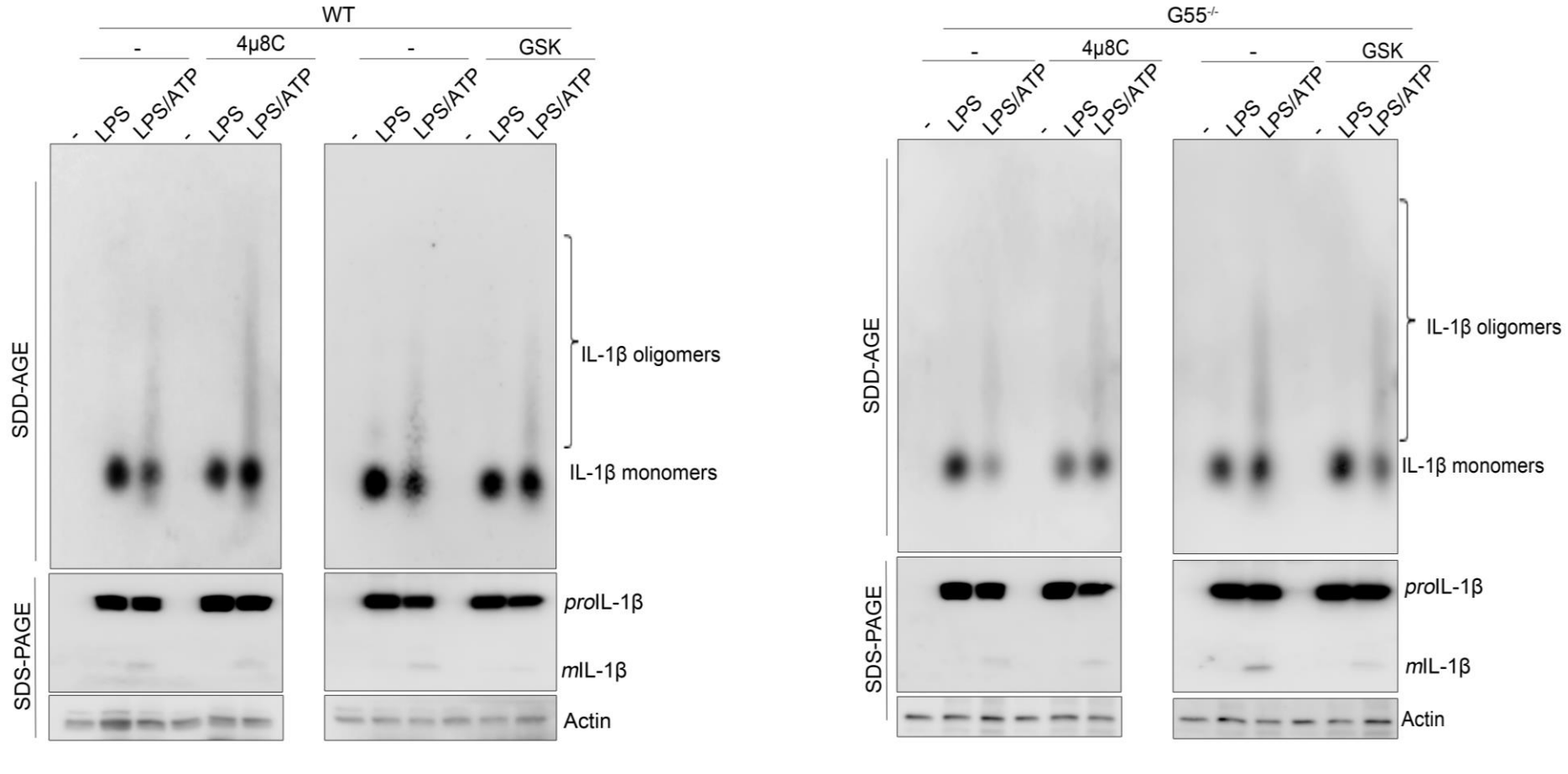
-concentration dependent reduction of IL-1β secretion
-small accumulation of mIL-1β intracellularly

-IL-1β secretion is not significantly reduced

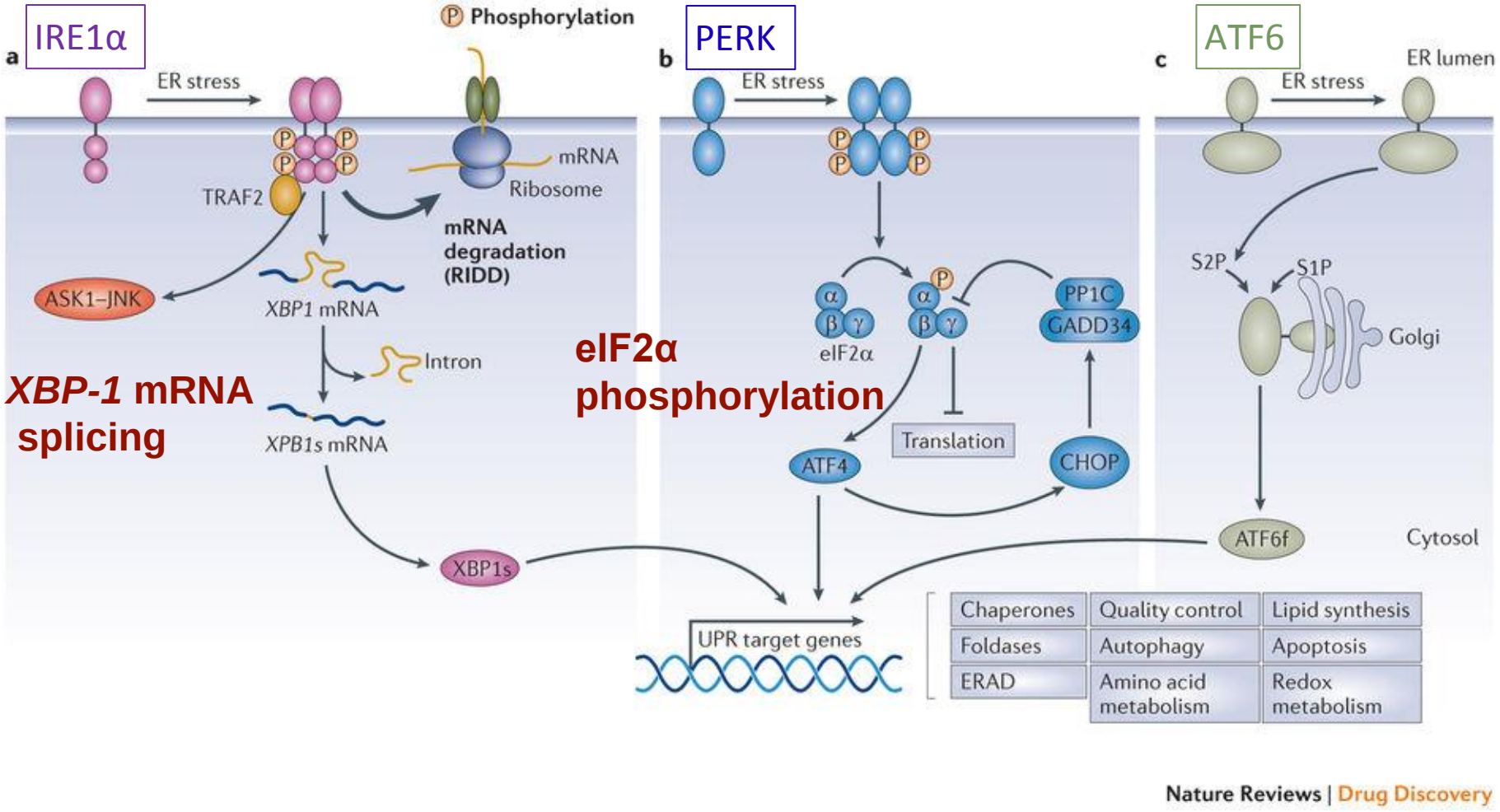
IRE1α but not PERK inhibition induces IL-1β aggregation



IRE1α inhibition induces IL-1β aggregation

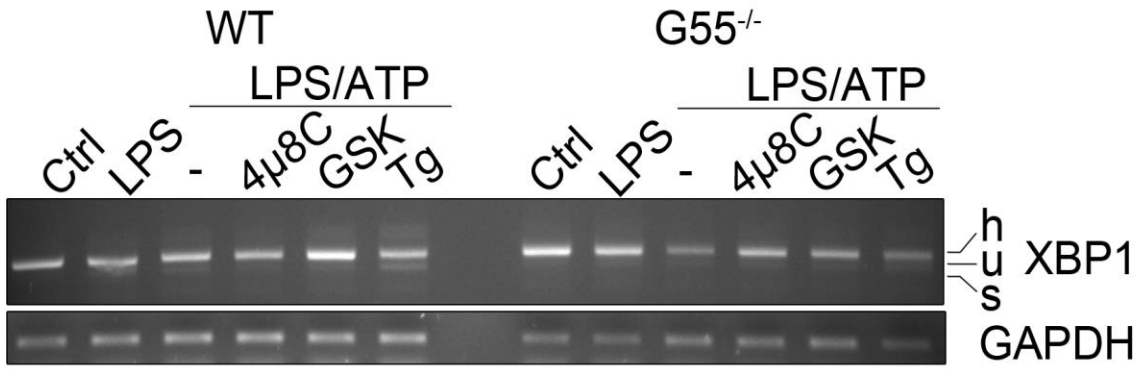


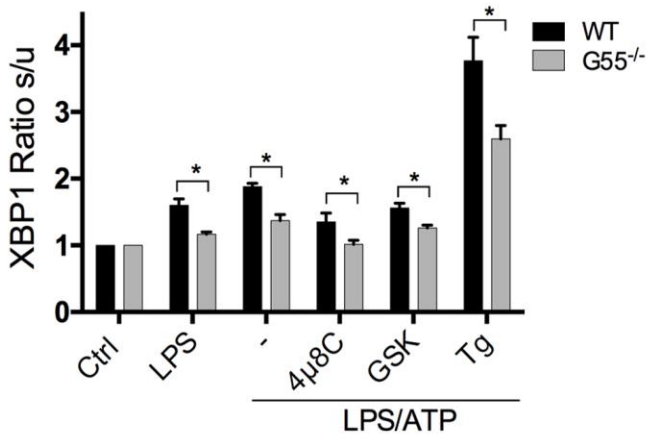
Assess UPR activation in stimulated macrophages



Adapted from: Hetz et al, *Nat. Rev. Drug Discov.* (2013)

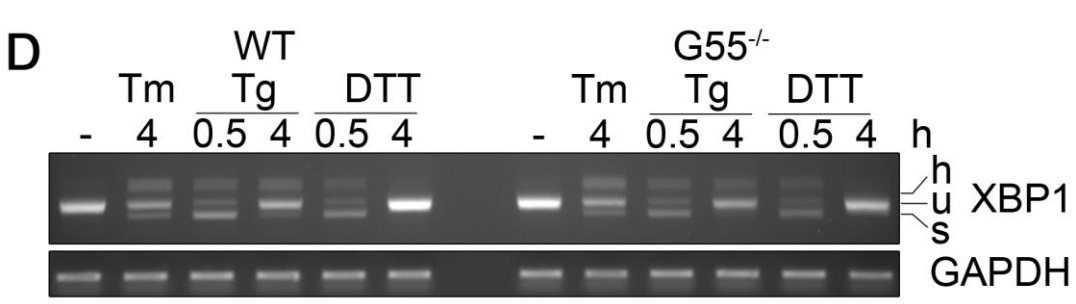
IRE1α- mediated *XBP-1* splicing is reduced in GRASP55$^{-/-}$ stimulated macrophages

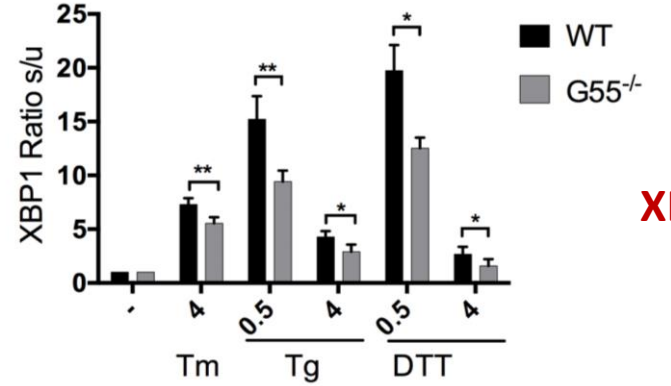




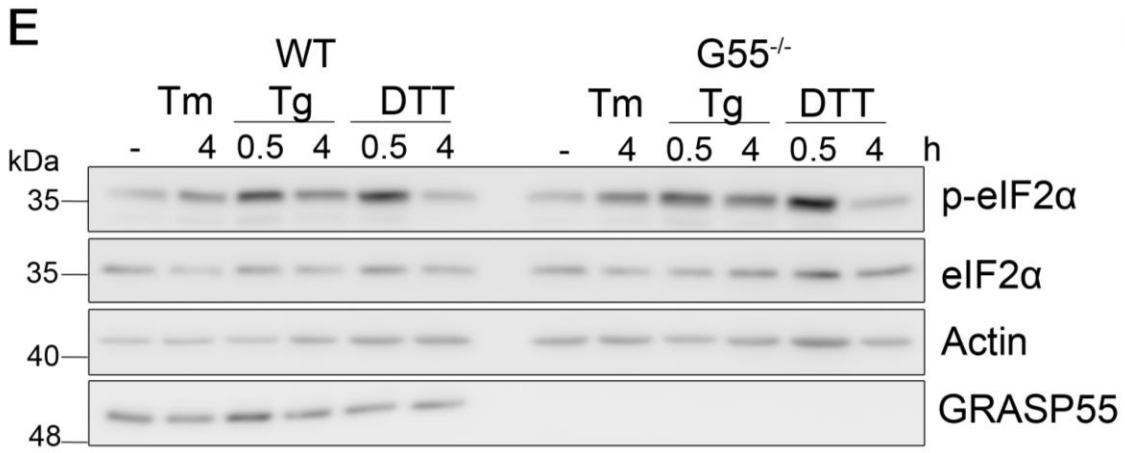
Is this specific for macrophages stimulated to secrete pro-inflammatory cytokines?

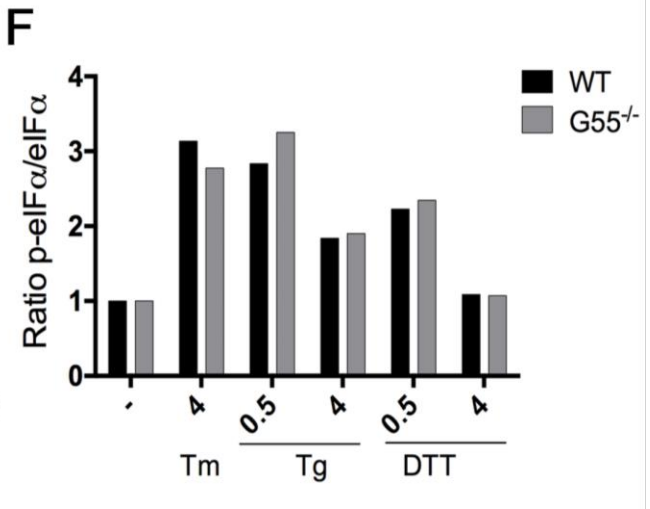
Deletion of GRASP55 impairs *XBP-1* mRNA splicing under stress conditions





XBP-1 splicing

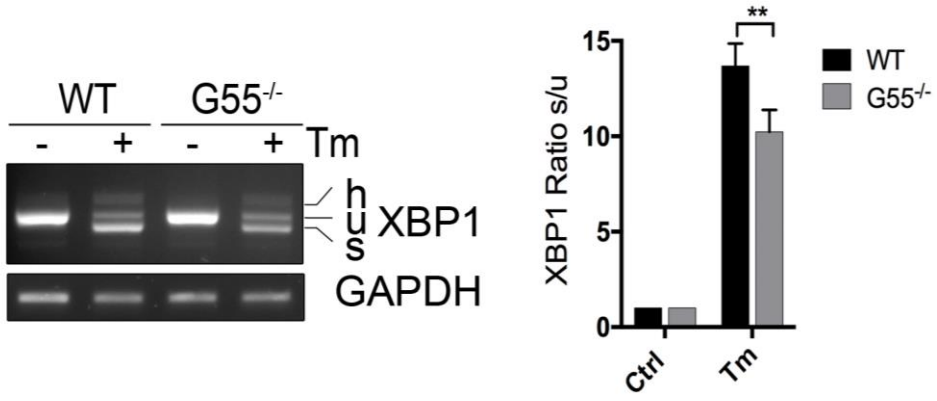




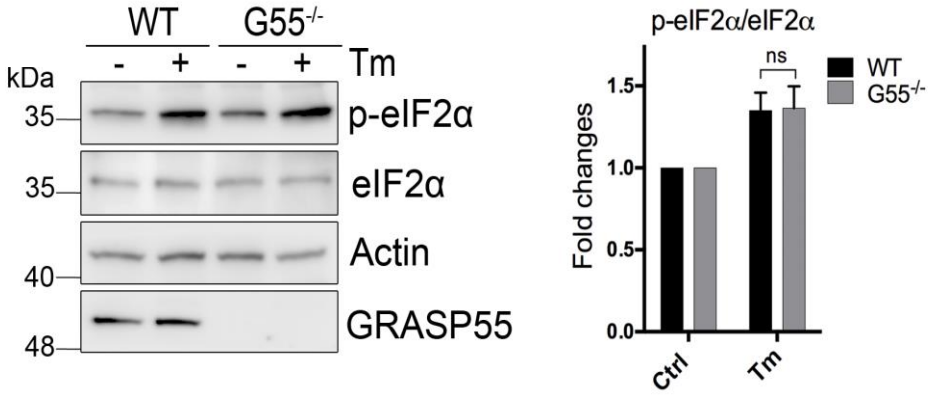
p-eIF2 level

Is is lineage specific?

GRASP55 modulates IRE1α/XBP-1 signaling under stress conditions in MEFs

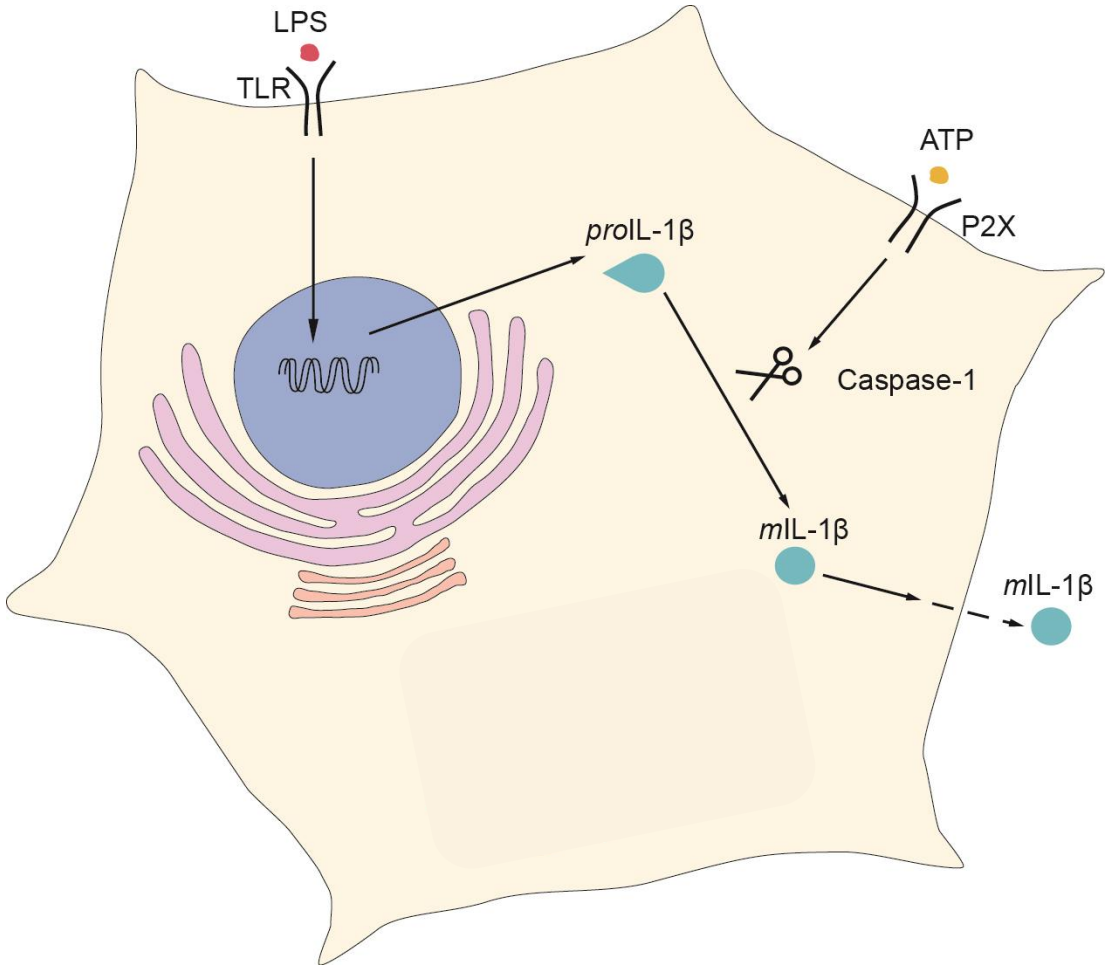


XBP-1 splicing



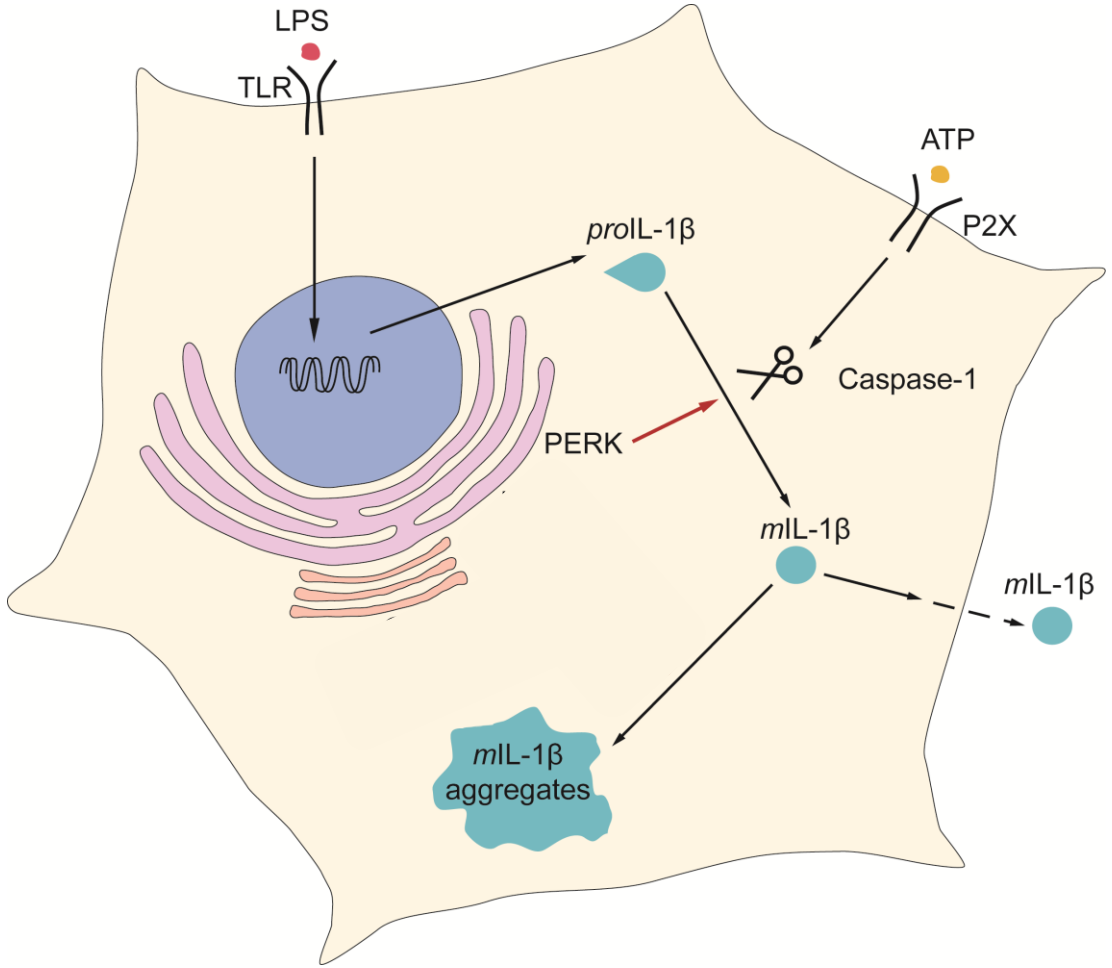
p-eIF2 level

*m*IL-1β has the propensity to aggregate if it is not efficiently secreted



Chiritoiu et al, *Dev Cell*, 2019

GRASP55 and UPR signaling (IRE1α and PERK) control IL-1β secretion and aggregation



Chiritoiu et al, *Dev Cell*, 2019

Acknowledgements:

Vivek Malhotra

Nathalie Brouwers
Ombretta Foresti
Ishier Raote
Gerard Cantero
Amy Curwin
Carla Burballa

David Cruz
Abira Ganguly
Cristian Butnaru
Julien Villeneuve
Anupama Ashok

Thank you!