Innate Immune Sensing in Aging: A double-edged sword

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Industrial PhD 2016-2020

PostDoc 2020-2023, ICMM

Research Scientist 2023-2024

PostDoc 2024-2025, BMI

Assistant Prof. 2025- BMI

Agenda

Introduction to innate immune signaling

- The cGAS-STING pathway (DNA)
- Rig-I/MAVS pathway (RNA)

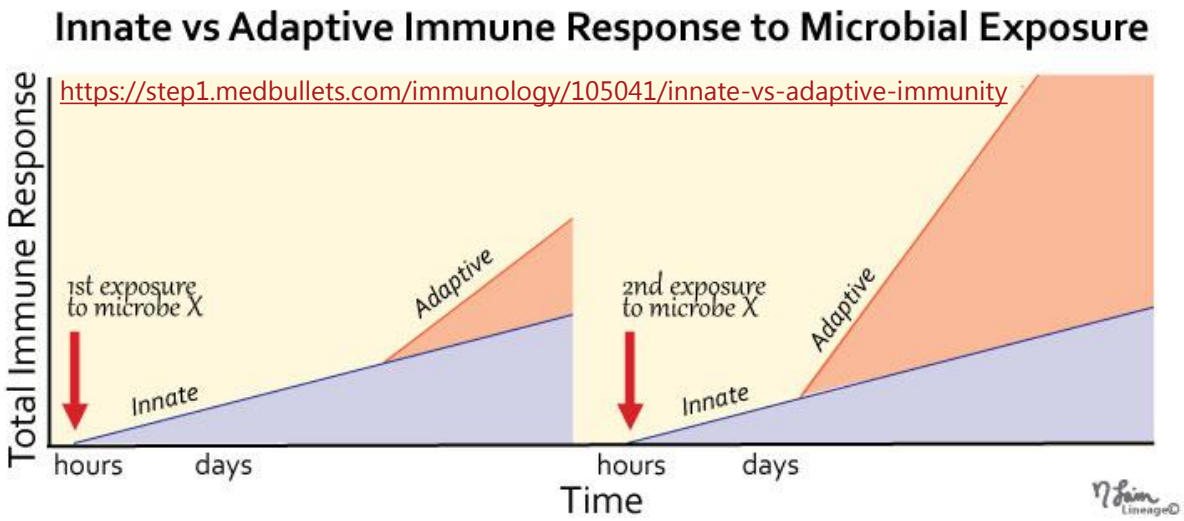
Study: The cGAS-STING signaling pathway is modulated by Urolithin A (UA)

Wrap up

Innate Immune Signaling

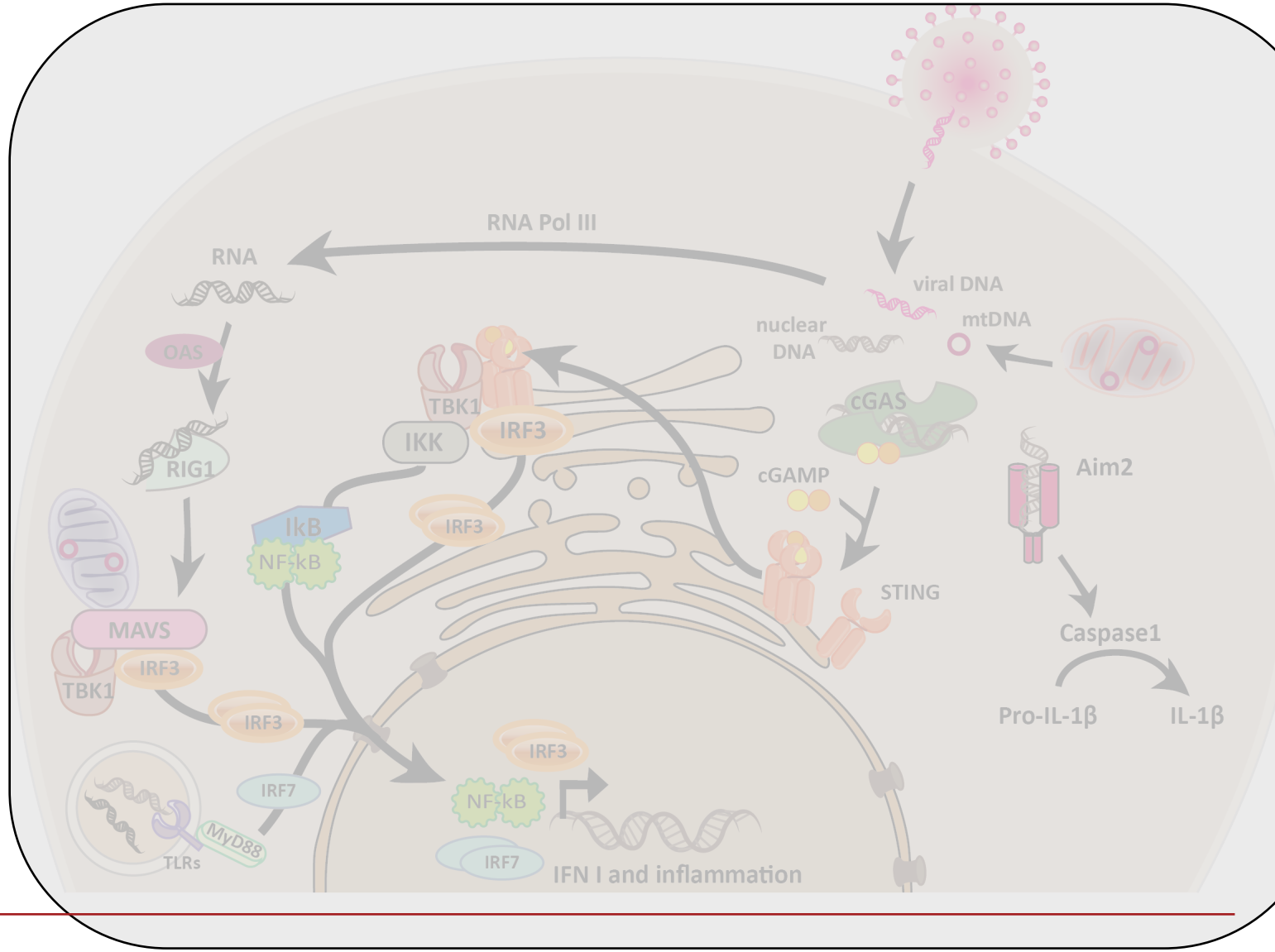
- Recognize specific pathogen- and damage-associated molecular signals
  - (PAMPs and DAMPs)
- Broad and non-specific
- Lack memory
- First line of defense
- Quick response

- Clear cells of danger
- Create an inflammatory environment which fine-tune the adaptive immune response



Innate Immune Signaling

Nucleic acids are potent PAMPs

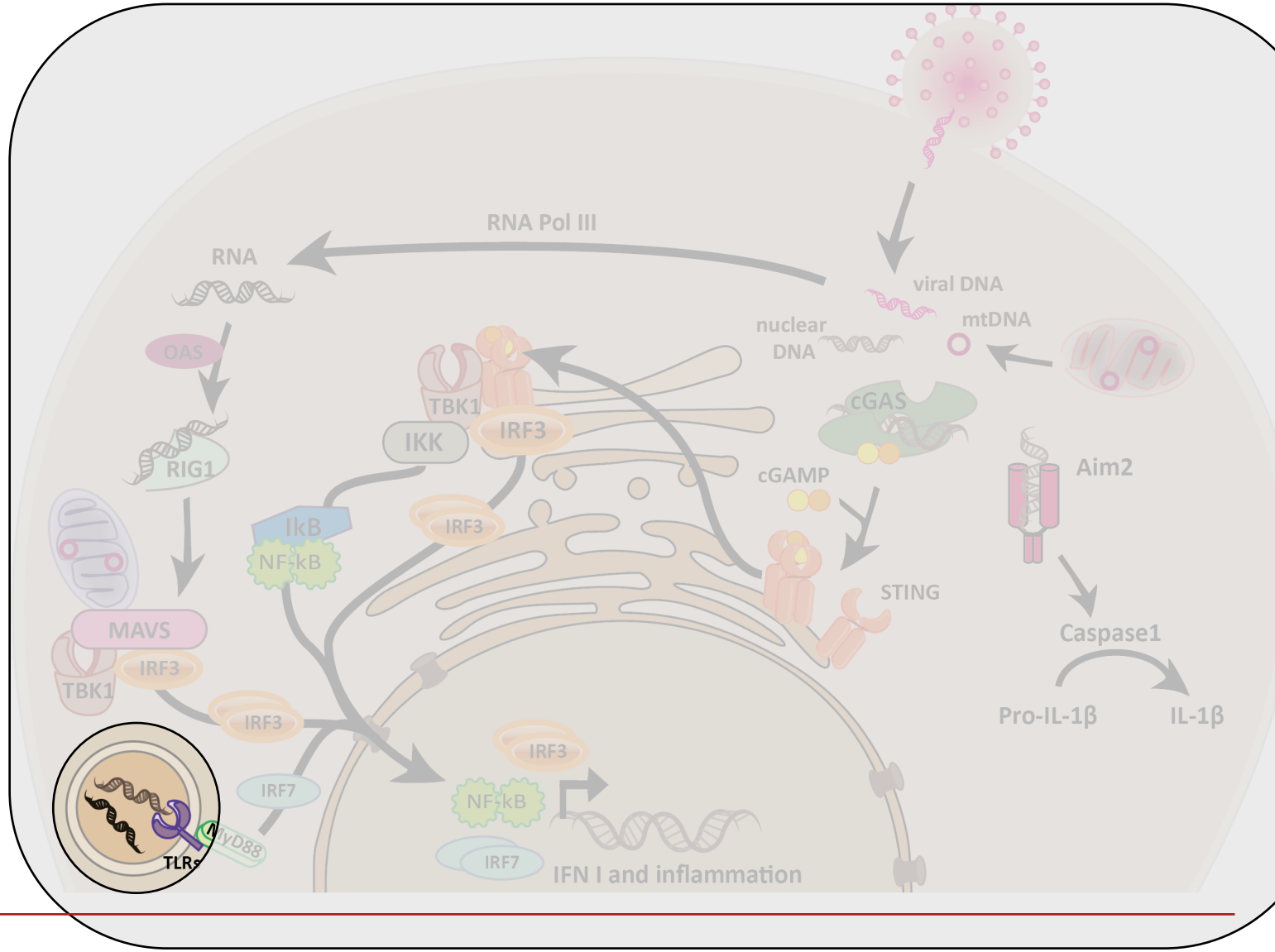


Innate Immune Signaling

Nucleic acids are potent PAMPs

Recognized by PRRs

- Plasma membrane and endosomes:
  - Toll-like-receptors (TLRs)
  - C-type lectin receptors (CLRs)



Innate Immune Signaling

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- Plasma membrane and endosomes:
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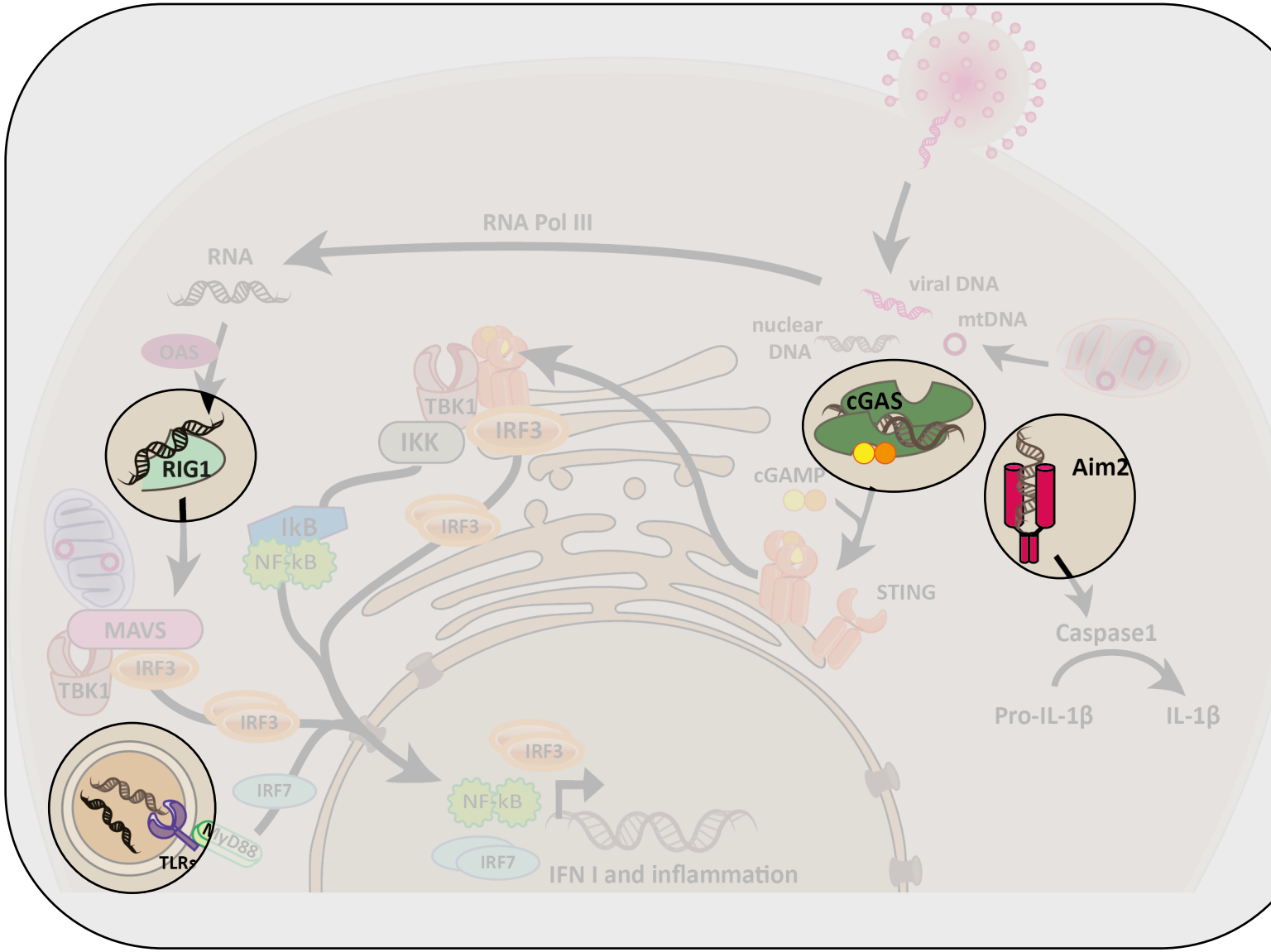
- Cytoplasm:
  - Retinoic acid-inducible gene-I (RIG-I)-like receptors
  - Absent in melanoma 2 (AIM2)-like receptors
  - Cyclic GMP-AMP synthase (cGAS)



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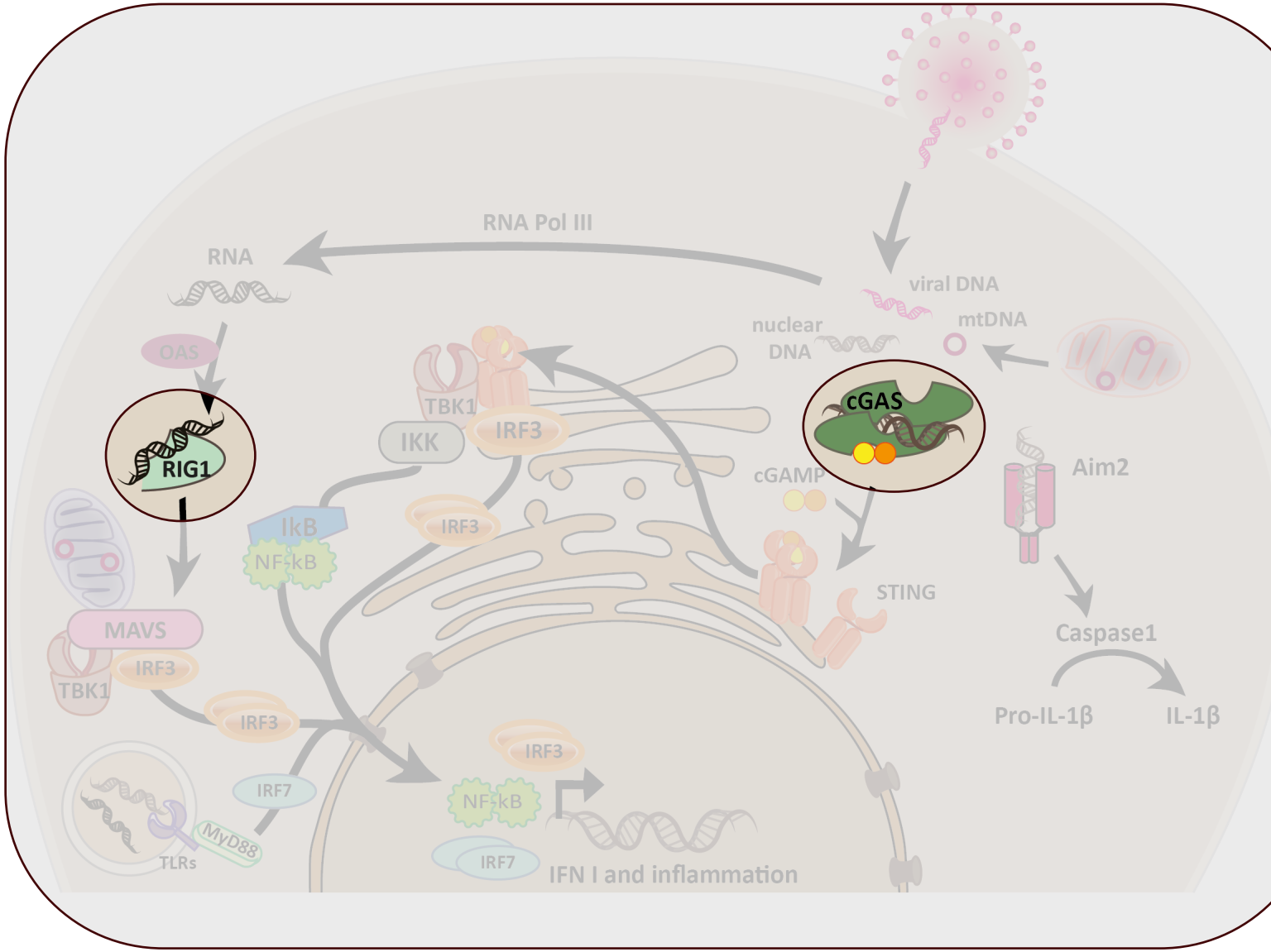
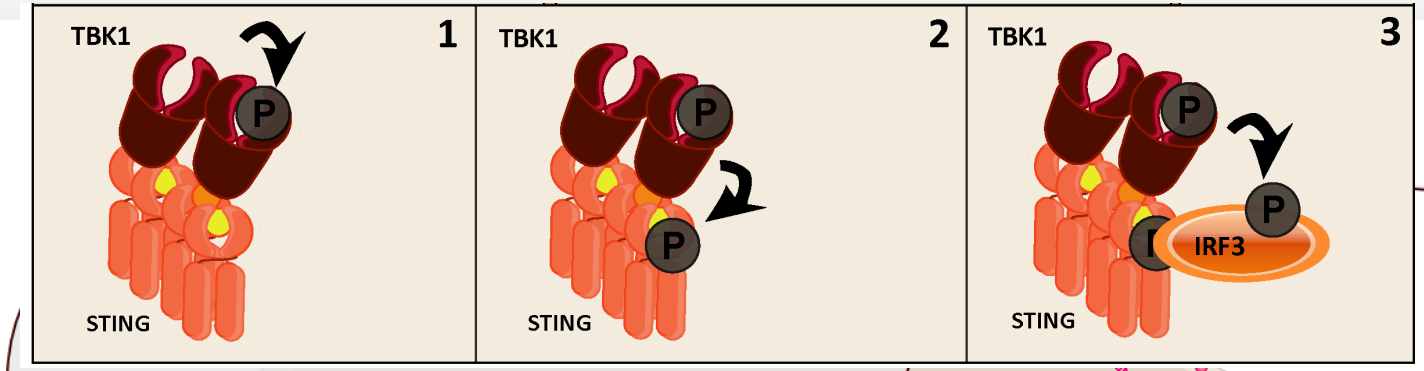


cGAS-STING

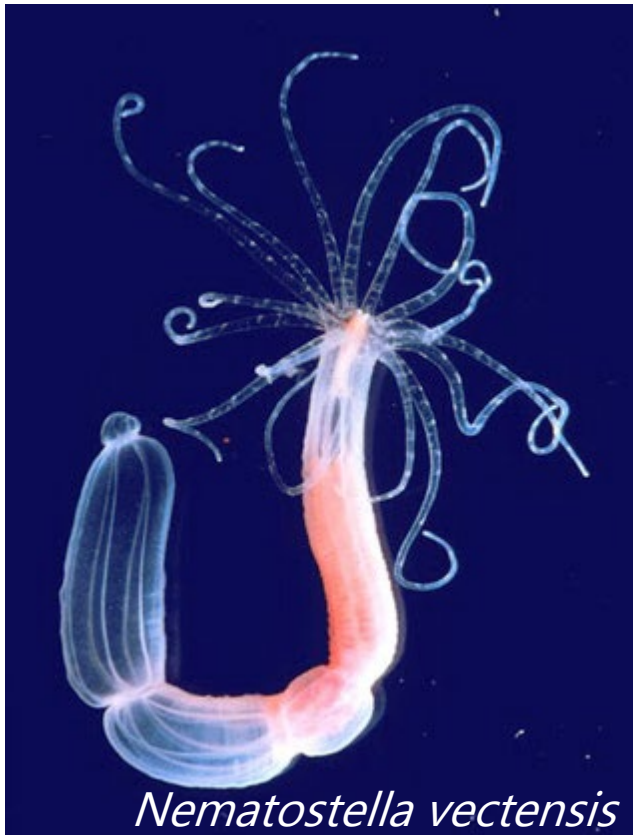
- Activated by cytoplasmic dsDNA.
- Does not discriminate between self- and foreign DNA



cGAS-STING signaling

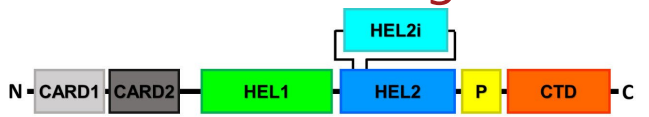
Additional functions

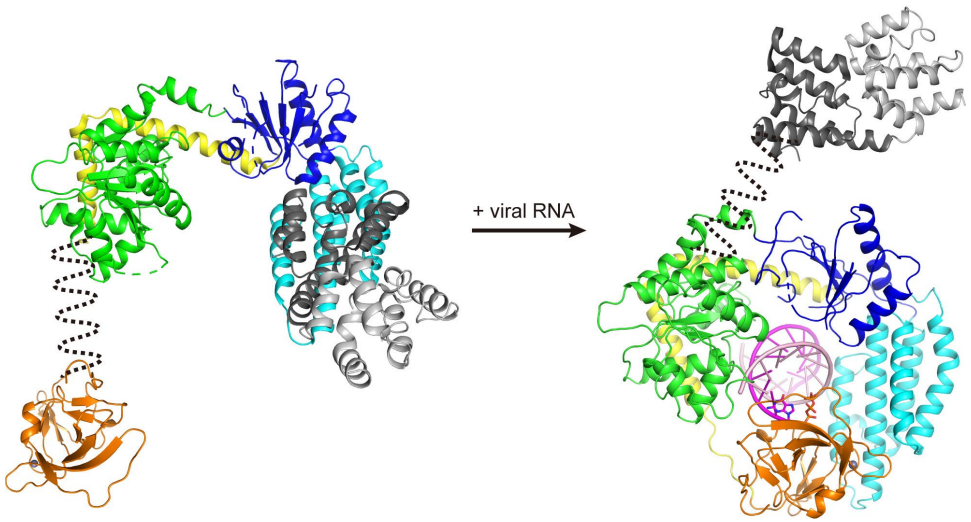
- cGAS-STING proteins found in evolutionarily distant organisms
- STING in autophagy (*Gui et al 2019*)

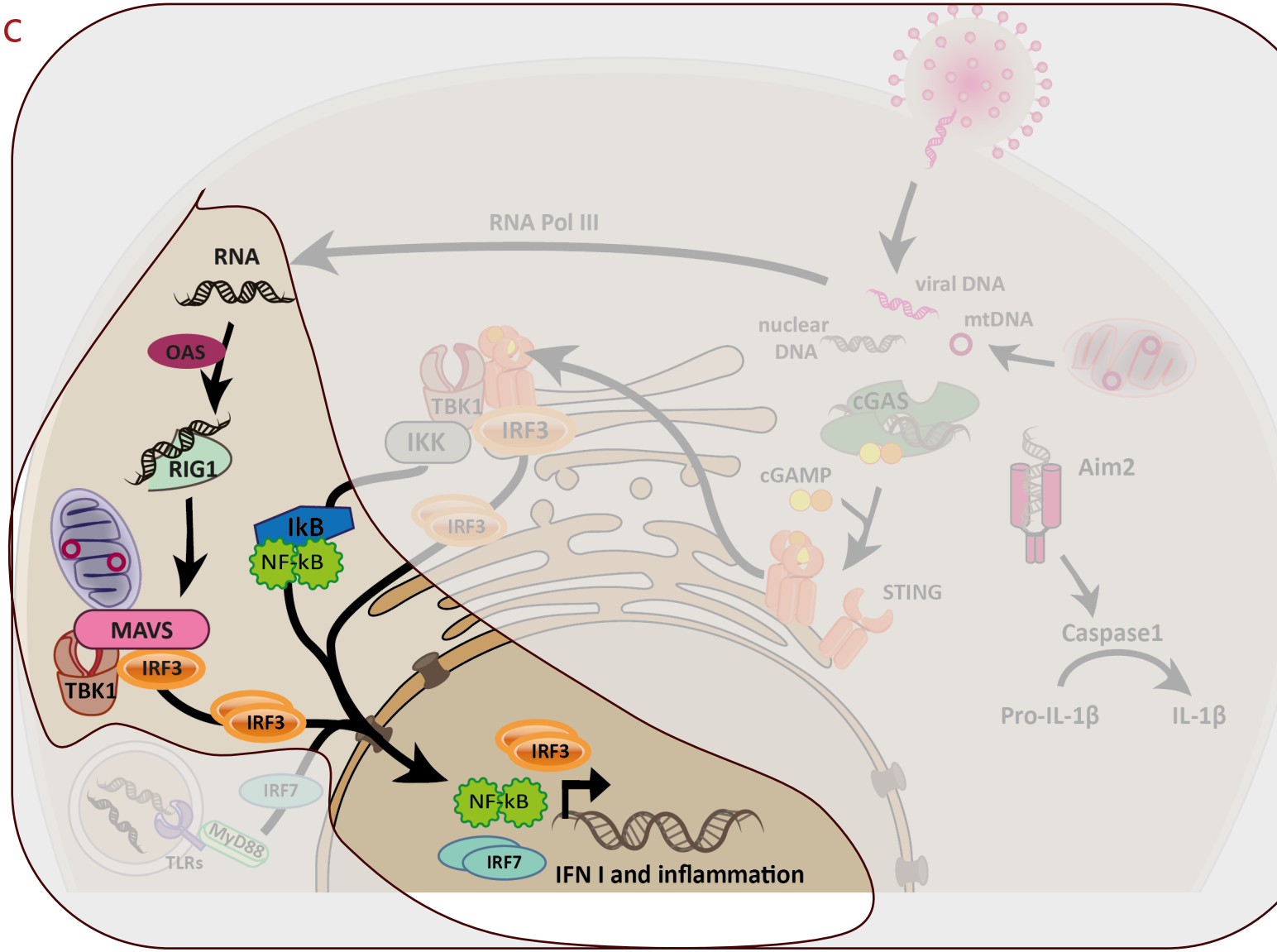


Rig-I/MAVS signaling

- RIG-I-like receptors are cytoplasmic RNA helicases detecting cytoplasmic RNA
- RIG-I binds relatively short 5′-ppp and blunt 5′-end RNA (dsRNA)
- OASes bind dsRNA → 2′, 5′-oligoadenylate → RNaseL activation → cleavage of RNA







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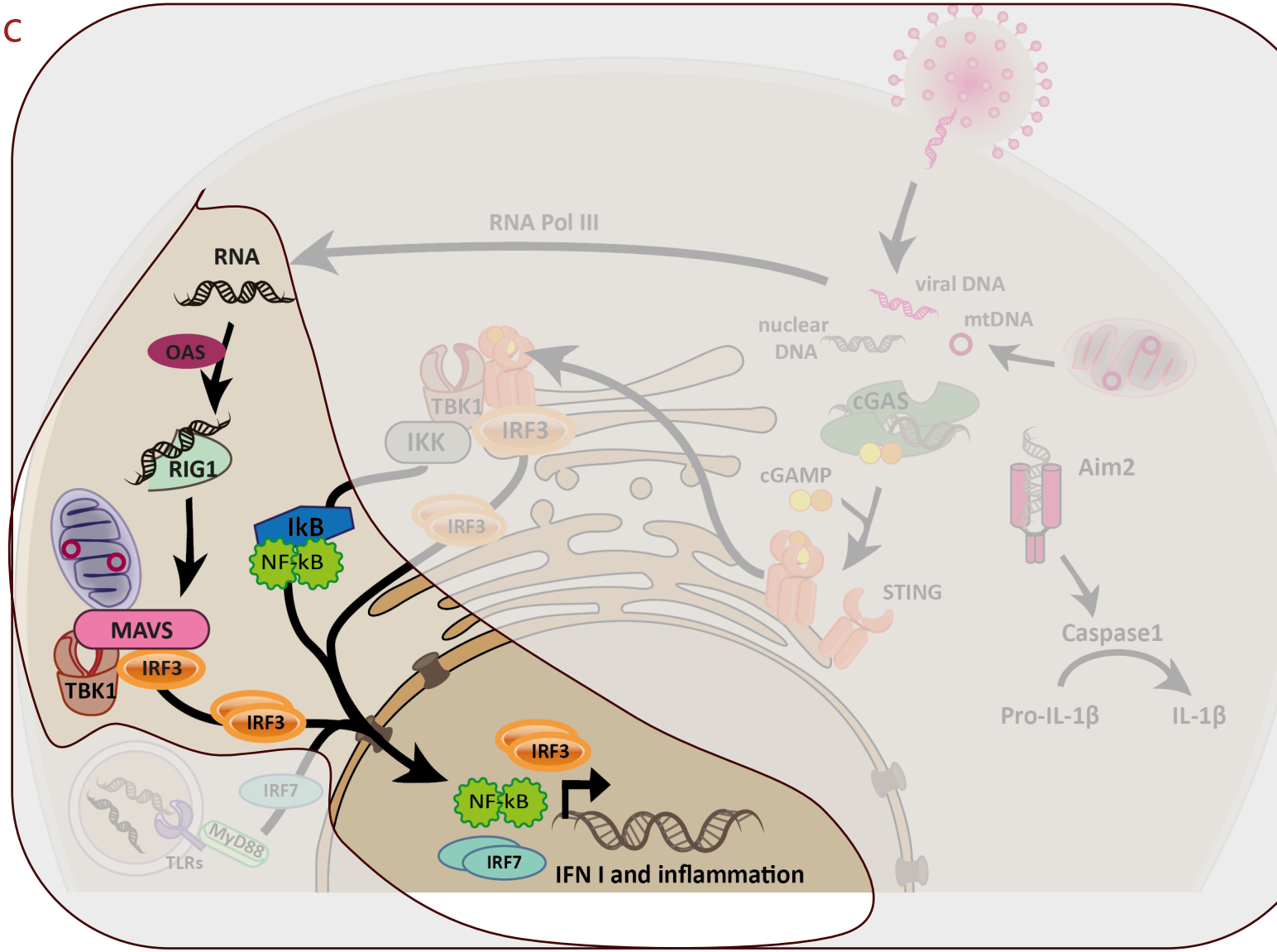
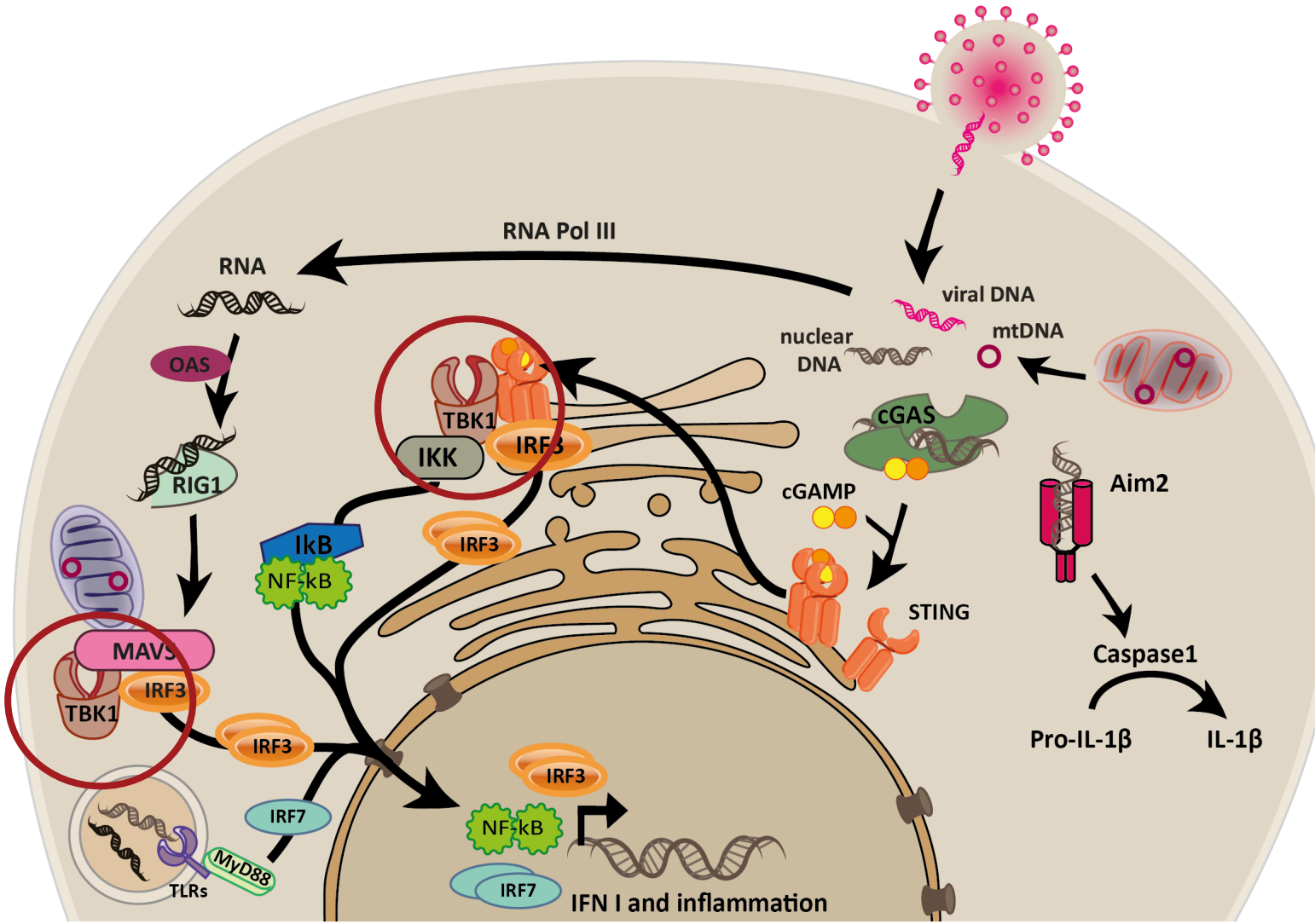
- Relocalization Mitochondrial antiviral-signaling (MAVS) protein
- MAVS aggregation → TBK1 recruitment



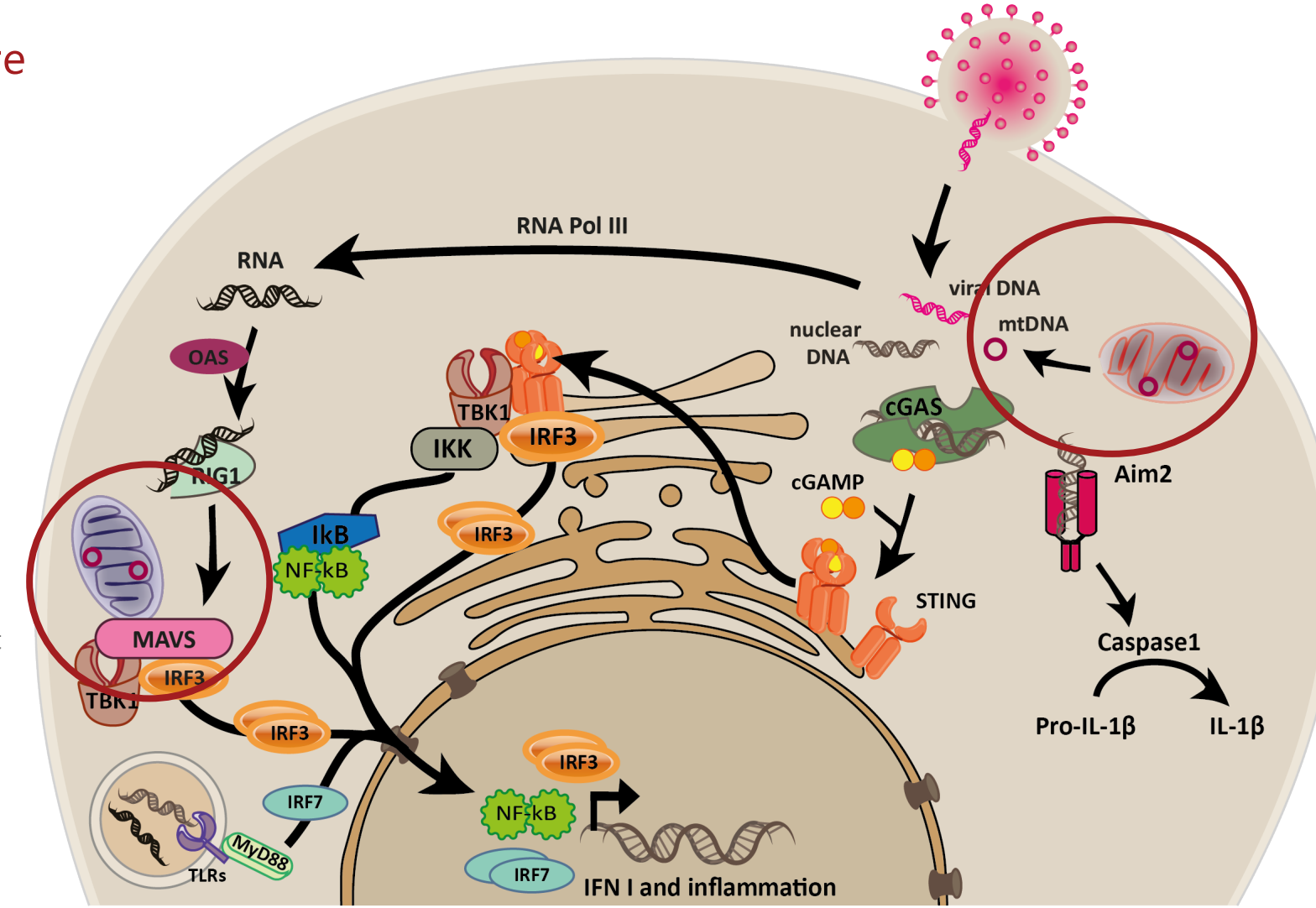
TBK1 in DNA and RNA sensing

- Key protein in innate immune signalosomes
- Implicated in regulation of apoptosis
- Autophagy and mitophagy induction via Optineurin and p62 interaction



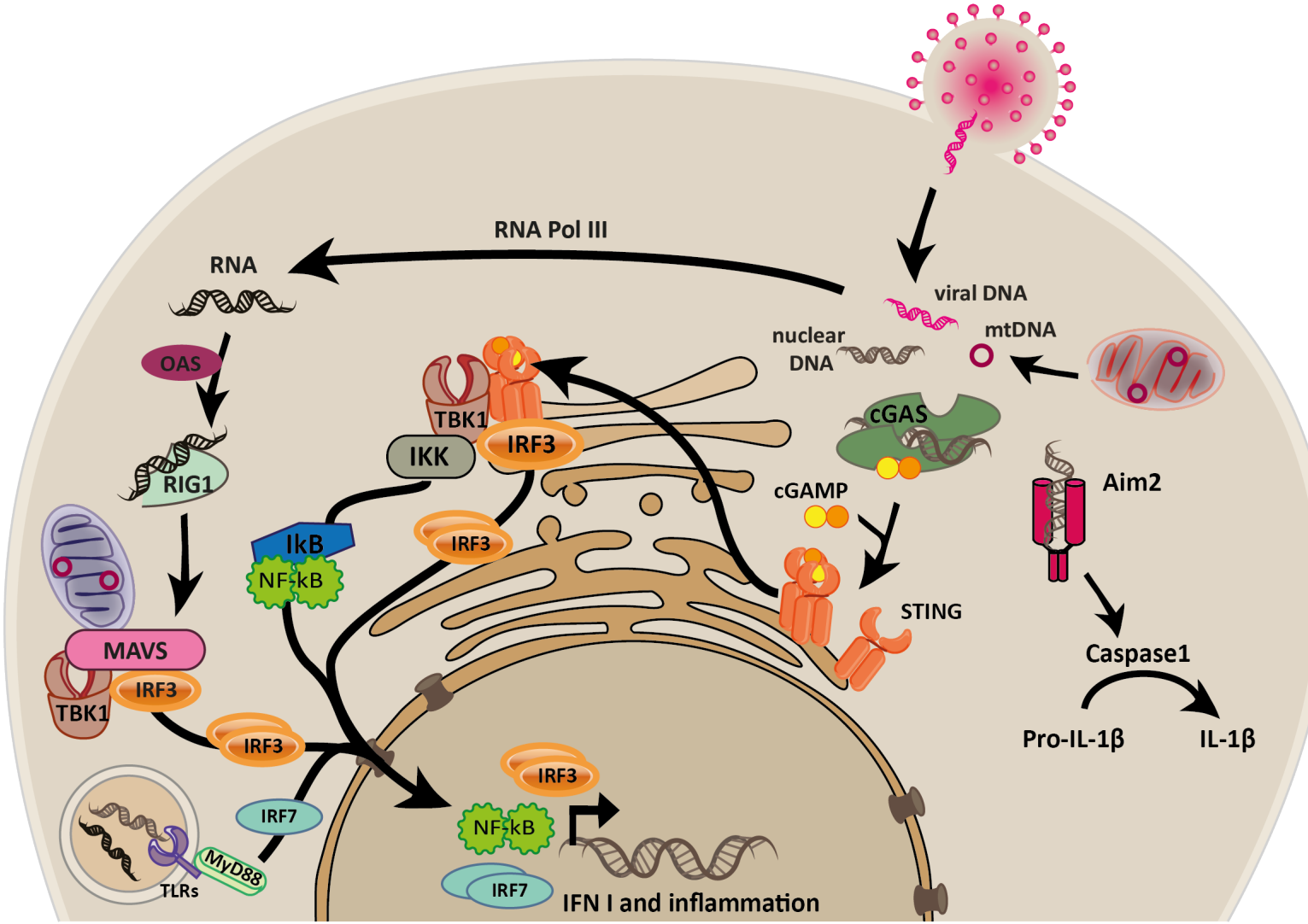
Mitochondria in DNA and RNA sensing

- Effective immune functions require energy
  - Mitochondrial MAVS coordinates glucose metabolism and RIG-I-like signaling (He et al 2023, Nature)
- Sufficient MAVS activation is dependent on mitochondrial health
  - Excessive fission and fusion inhibits signaling (Huang et al 2021 & Shi et 2014)
  - Membrane potential required for efficient type 1 IFN response (Koshiba et al 2011)
- mtDNA/mtRNA released upon compromised mitochondrial health



Cross-talk between DNA and RNA-sensing

- RNA pol III transcribe AT-rich dsDNA into 5´ppp-RNA
- Sting has been shown to interact with MAVS in the Rig-I/MAVS signaling pathway
- Interaction between Aim2, STING and NLRP3 (not shown)



Modulation of DNA- and RNA sensing is gaining interest

Defective DNA- or RNA sensing

- Autoimmune inflammatory diseases (Type 1 interferonopathies)
  - STING-associated vasculopathy with onset in infancy (SAVI)
  - TBK1 dysregulation associated with susceptibility to HSV1 infection, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia

- Cancer
  - Chemotherapy/radiation leads to DNA damage and release of DNA/RNA species → DNA- and RNA-sensing dependent cytokine production. Immune evasion.
  - STING and RIG-I agonists in clinical trials
    - Limited efficacy with monotherapy
    - Better in combination with fx PD-1 therapy
    - Validated the biology: pharmacological trigger of innate immune defense

ELSEVIER

Mechanisms of Ageing and Development

Volume 217, February 2024, 111897

The cGAS-STING signaling pathway is modulated by urolithin A

H.B. Madsen [a], J-H. Park [b], X. Chu [b], Y. Hou [b d], Z. Li [a], L.J. Rasmussen [a], D.L. Croteau [b c], V.A. Bohr [a] [b] ✉, M. Akbari [a e] ✉

ORIGINAL RESEARCH article

Front. Aging Neurosci., 27 November 2024

Sec. Cellular and Molecular Mechanisms of Brain-aging

Volume 16 - 2024 | https://doi.org/10.3389/fnagi.2024.1503336

Urolithin A and nicotinamide riboside differentially regulate innate immune defenses and metabolism in human microglial cells

Helena Borland Madsen[1] Claudia Navarro[1] Emilie Gasparini[1]

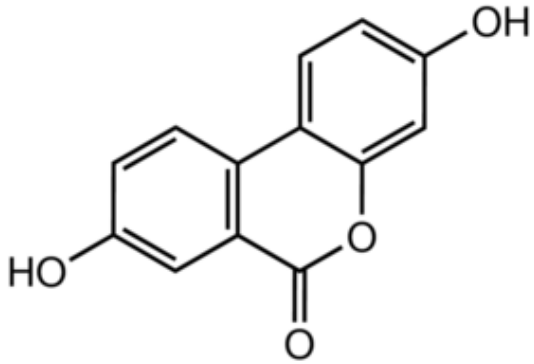
Jae-Hyeon Park[2]

Zhiquan Li[1]

Deborah L. Croteau[2,3] Vilhelm A. Bohr[1,2*]

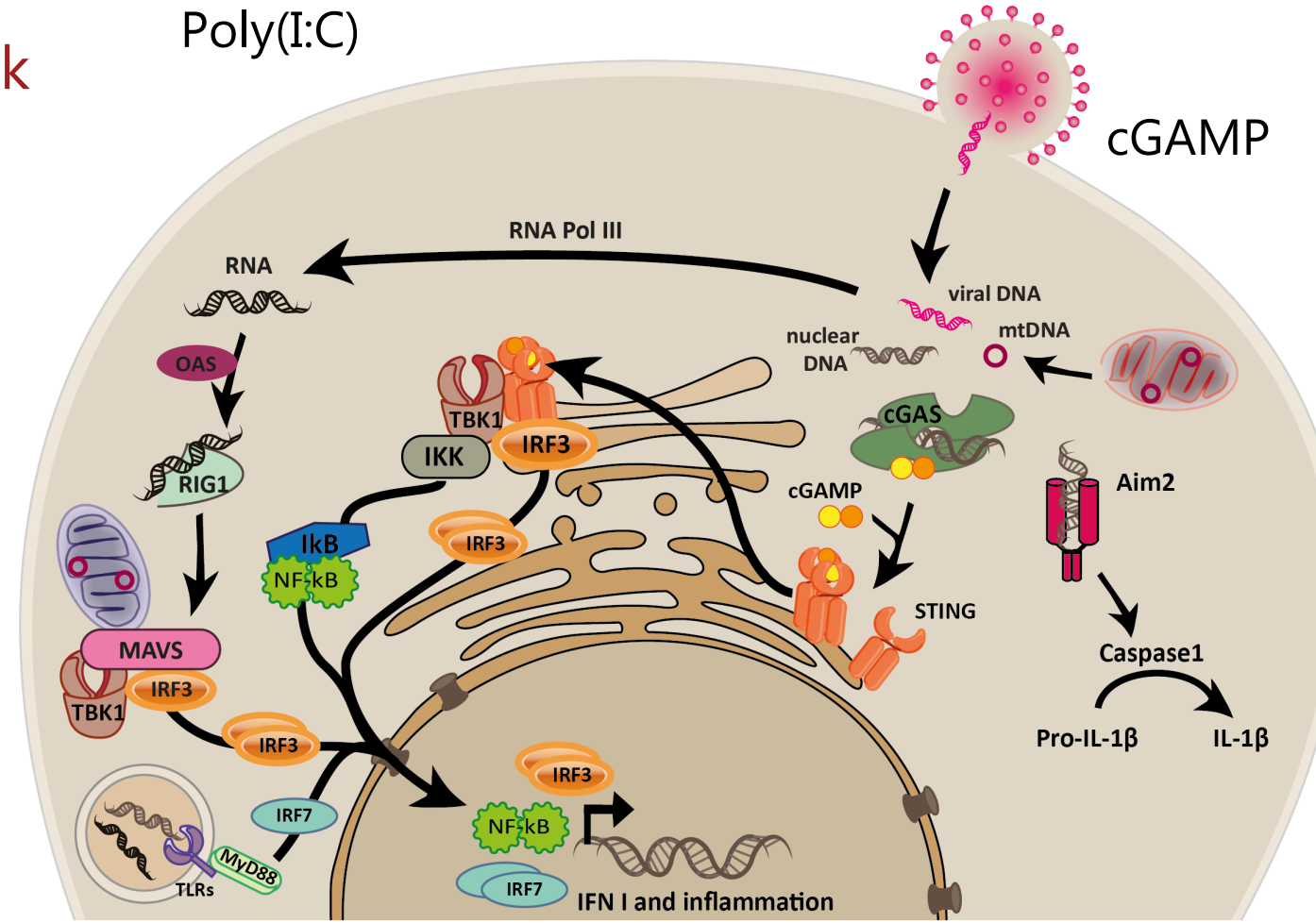
Urolithin A (UA)

- Gut microbial metabolite produced from ellagic acid
- Found in Nuts and fruits
- Known to have anti-inflammatory effects
- Stimulates mitophagy



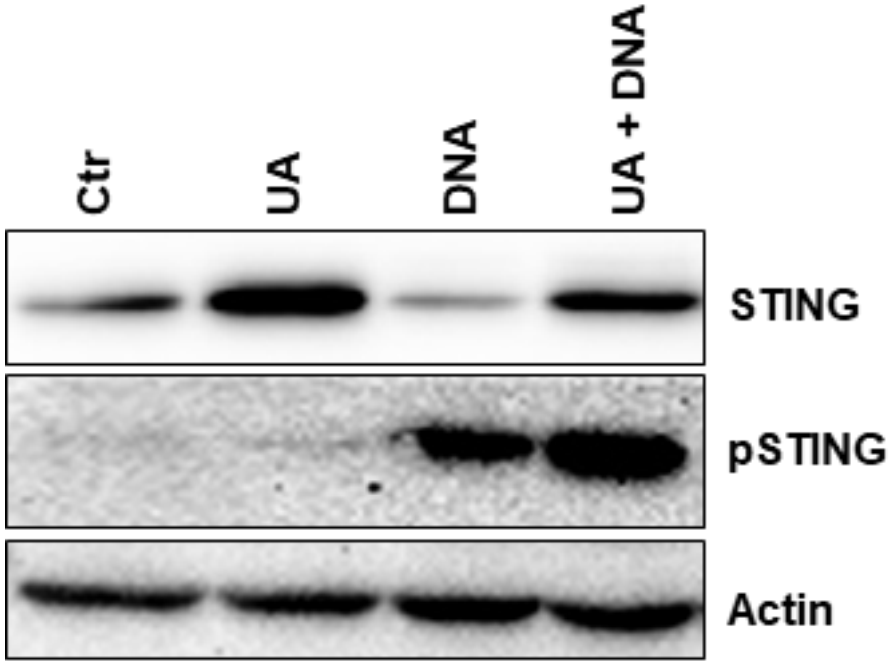
Experimental Setup

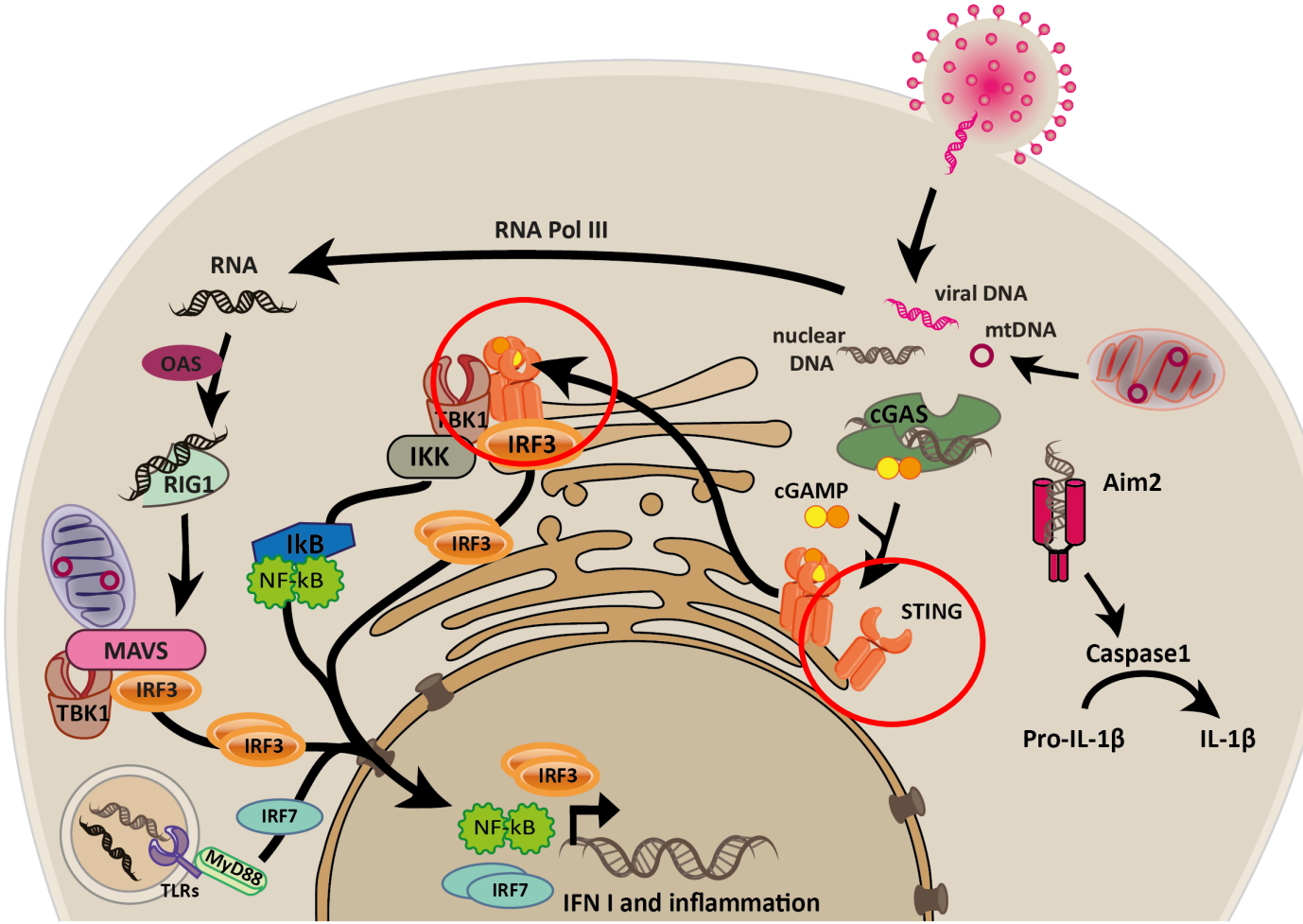
- HMC3 Human microglia cell line
- Treated with 10µM UA for 1 week
- Investigated anti-inflammatory properties by stimulating DNA- and RNA-sensing pathways



UA affects the cGAS-STING pathway

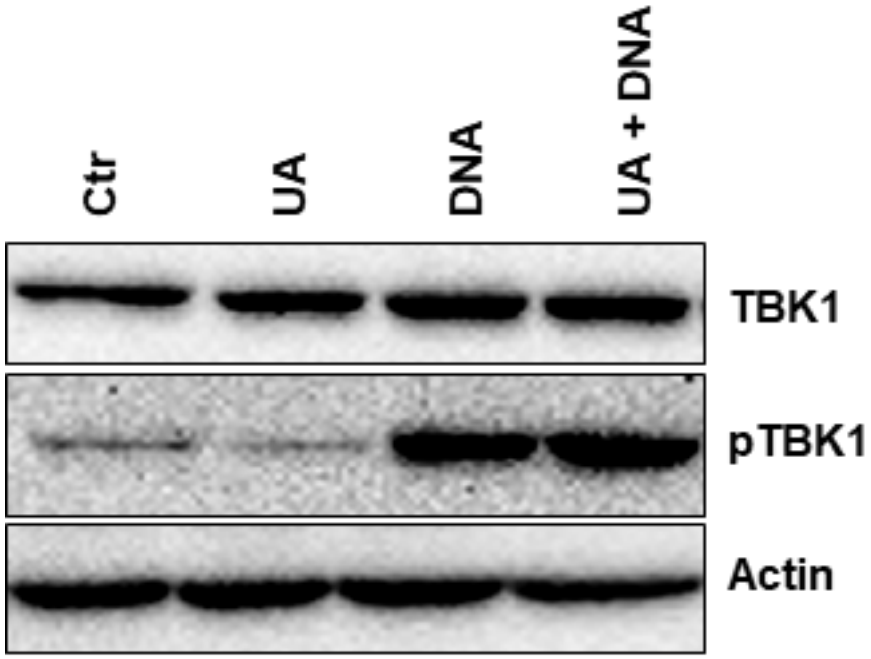
- STING

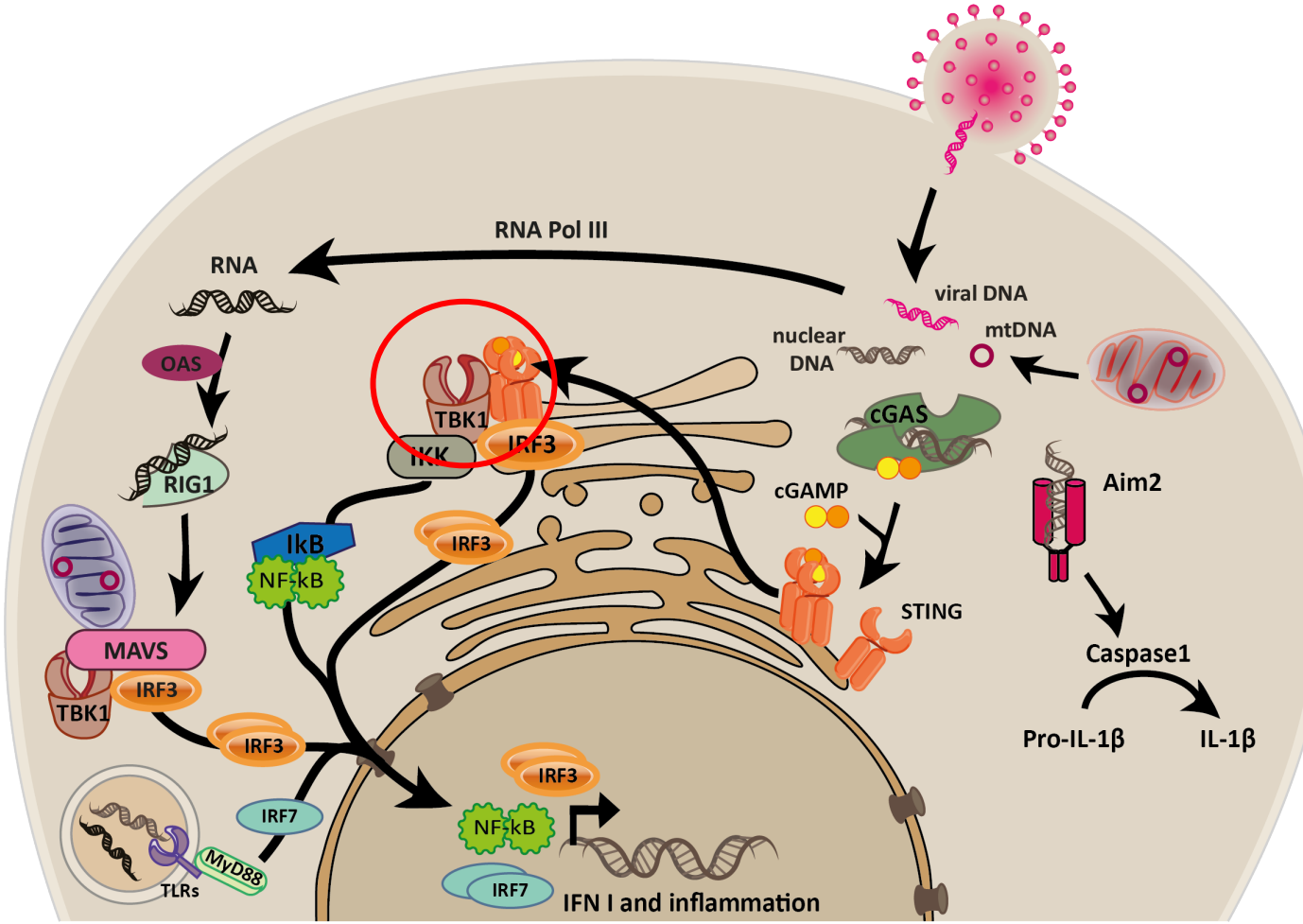




UA affects the cGAS-STING pathway

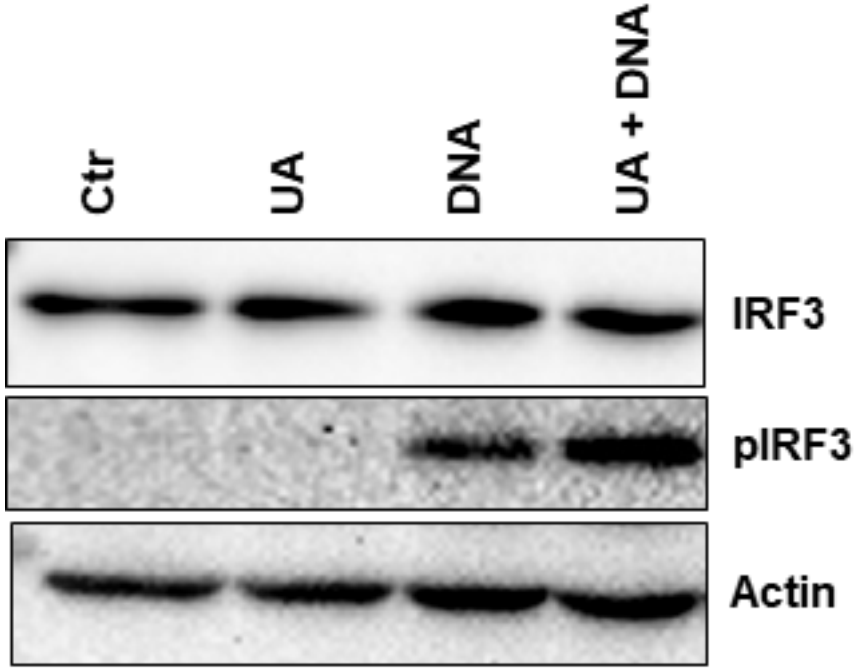
- TBK1

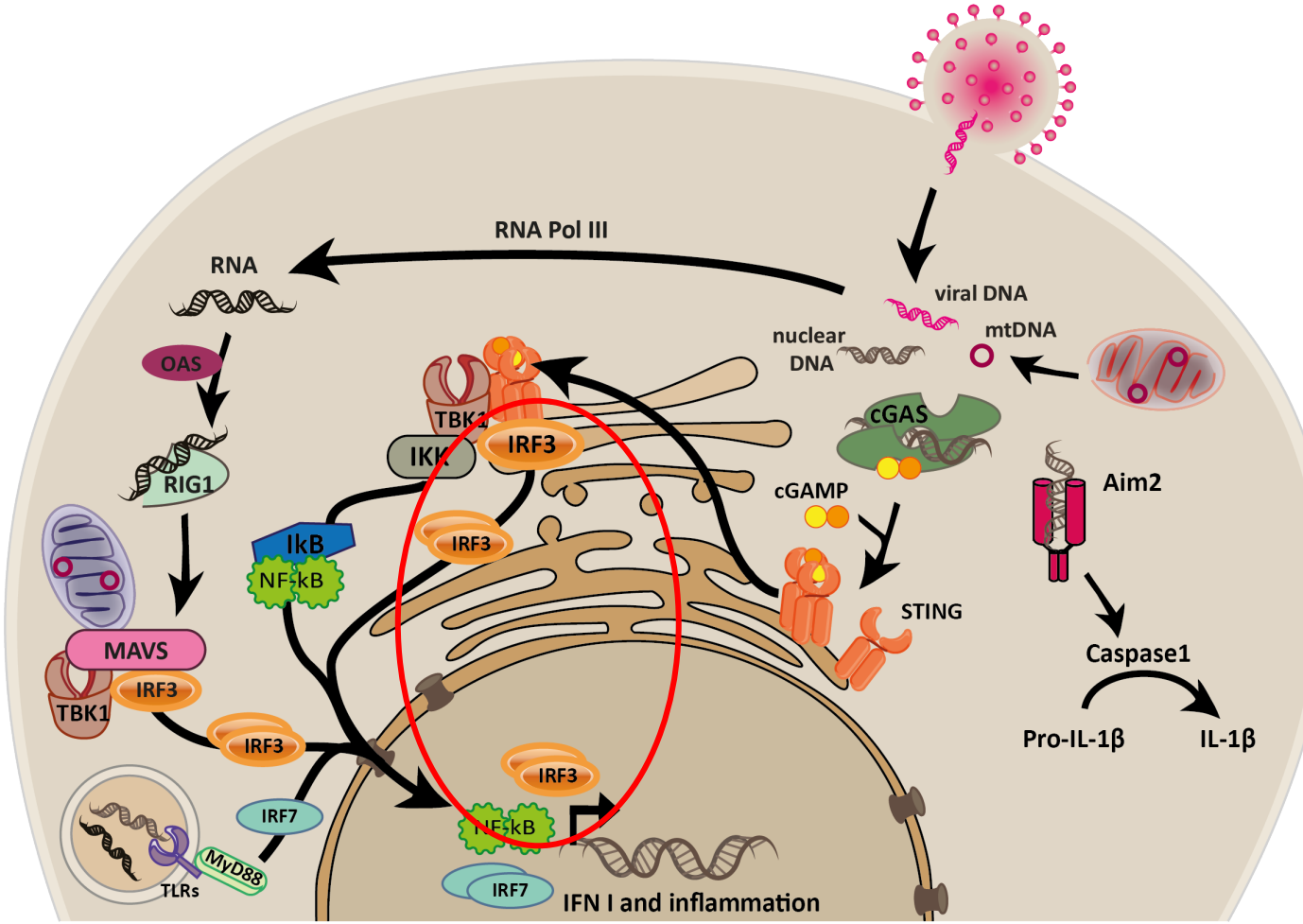




UA affects the cGAS-STING pathway

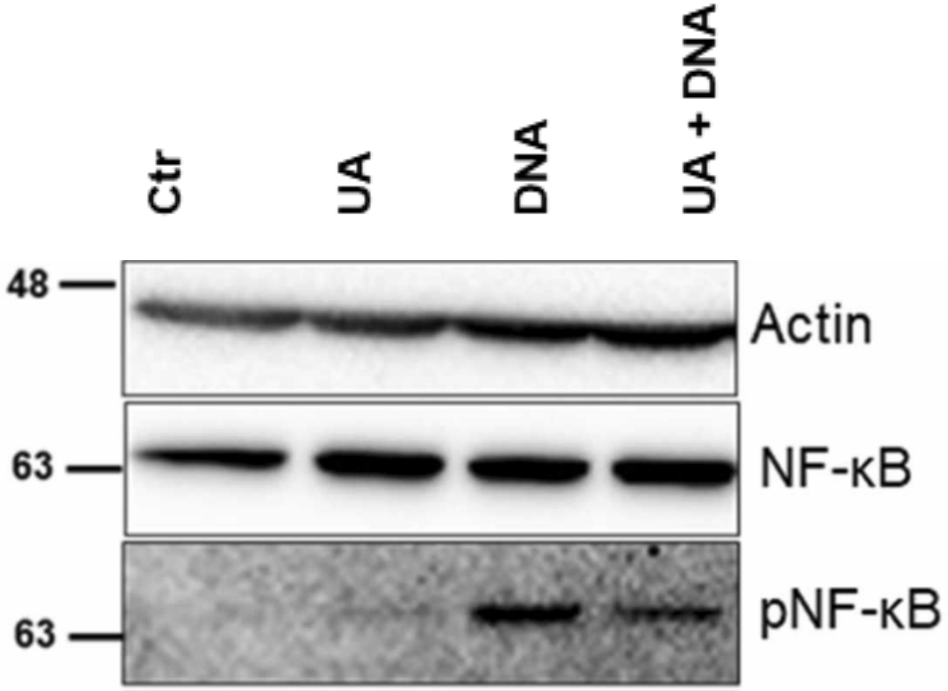
- IRF3

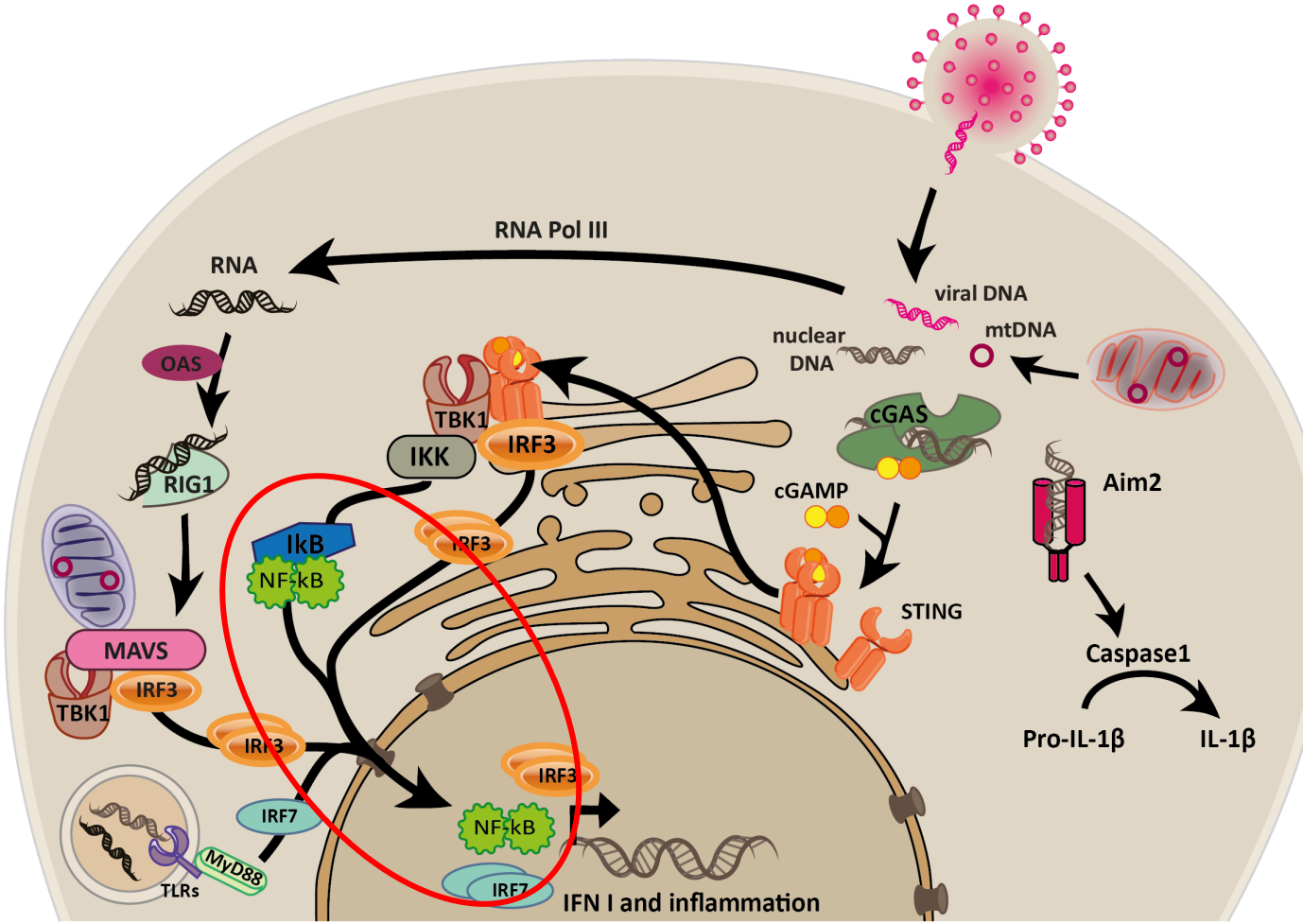




UA affects the cGAS-STING pathway

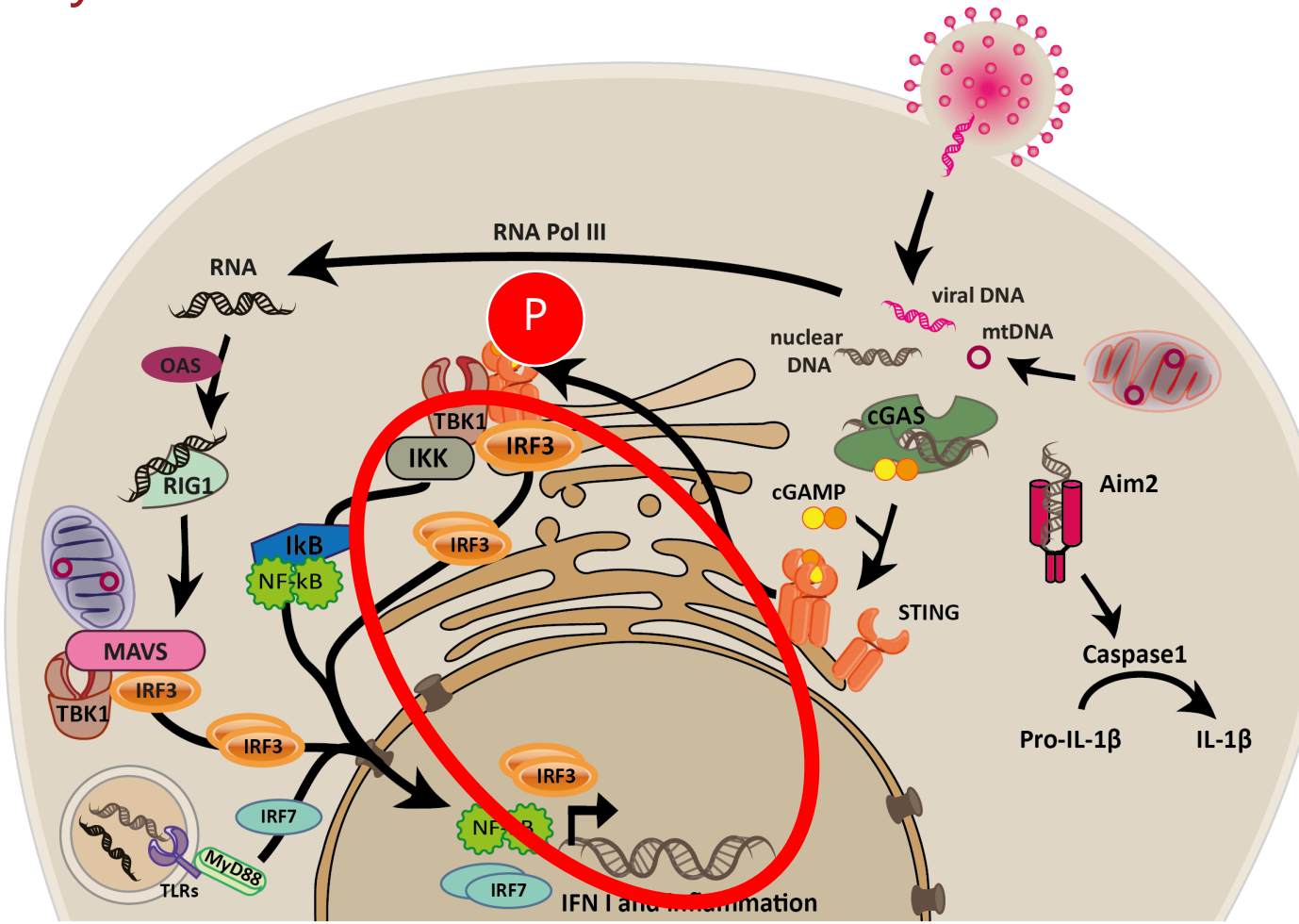
- NF-kB





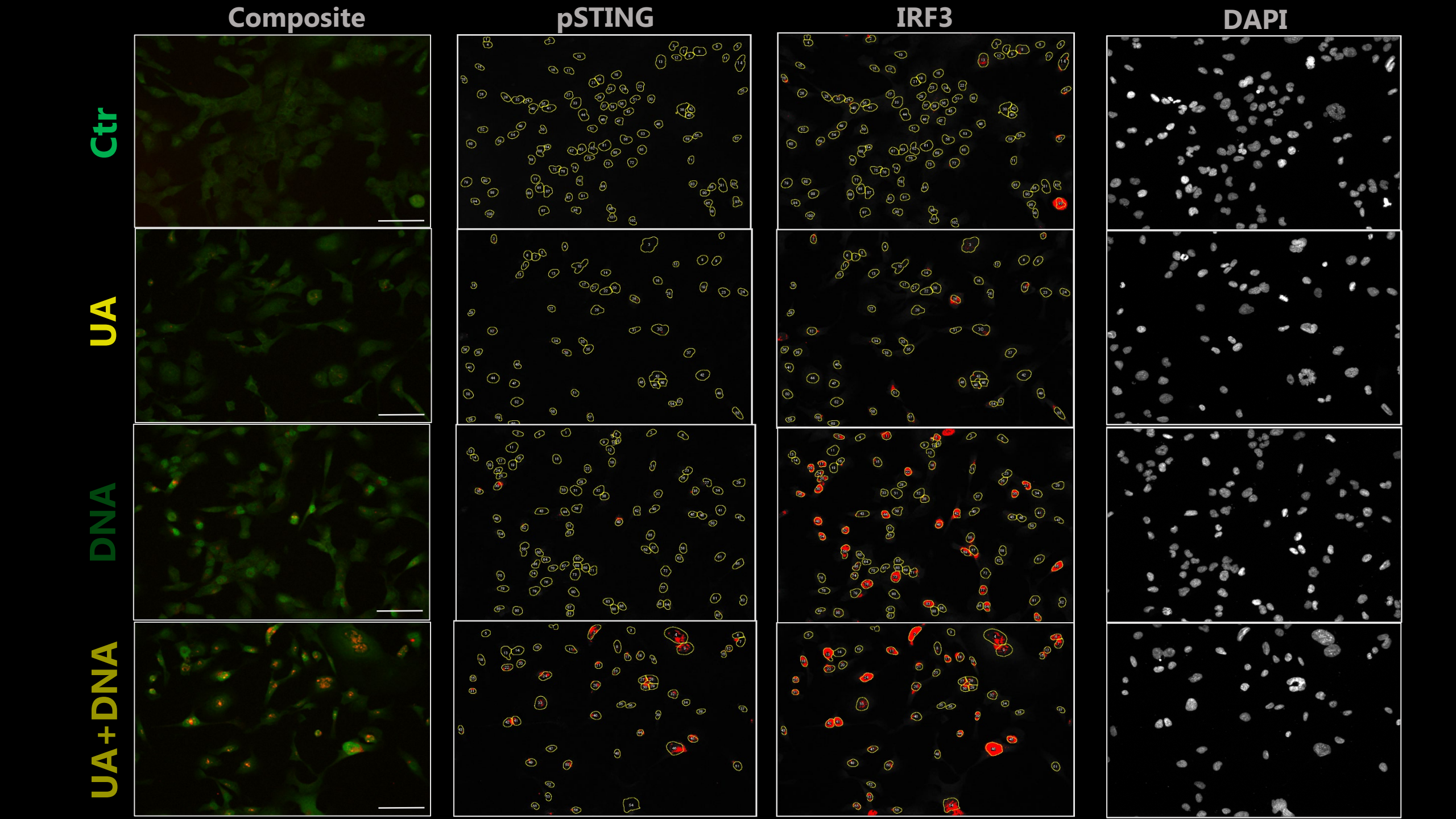
UA affects the cGAS-STING pathway

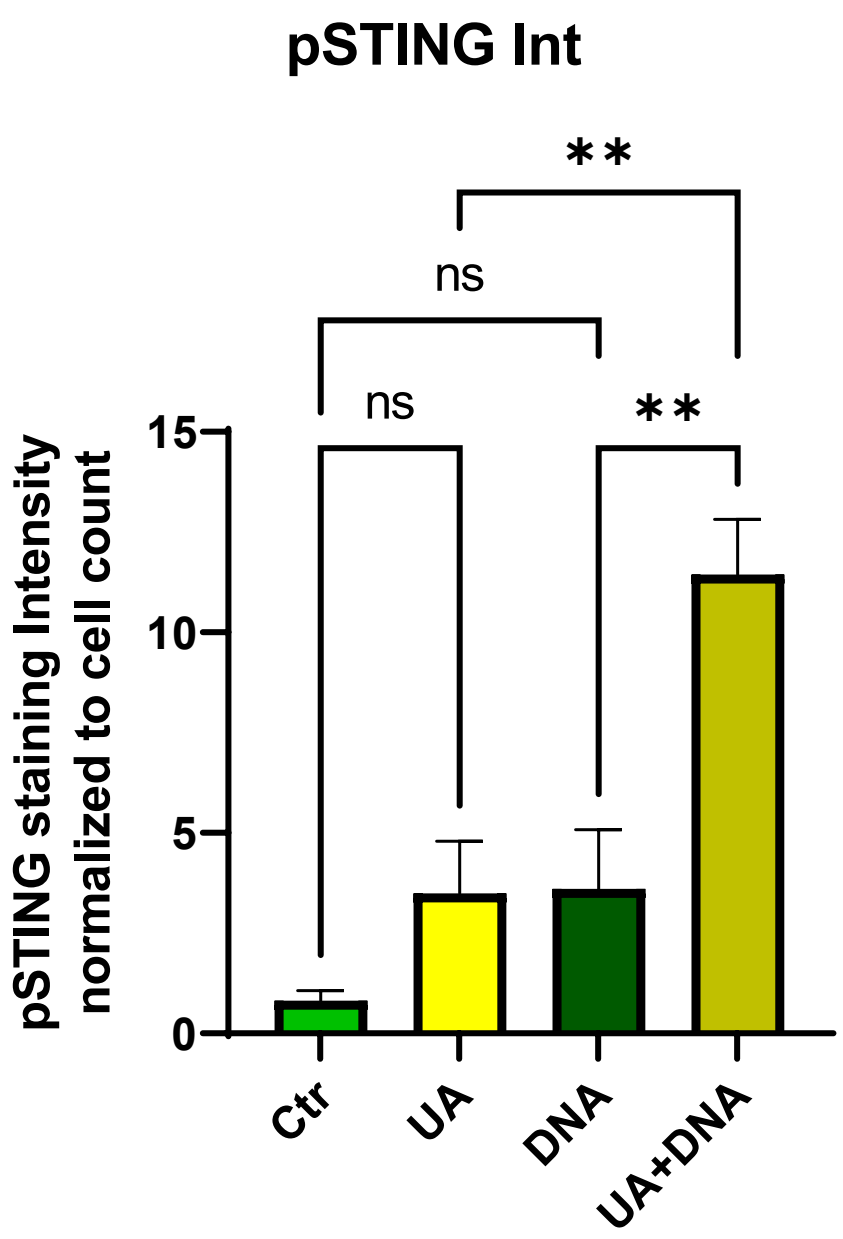
- ImageJ macro developed to quantify the IRF3 nuclear translocation and pSTING

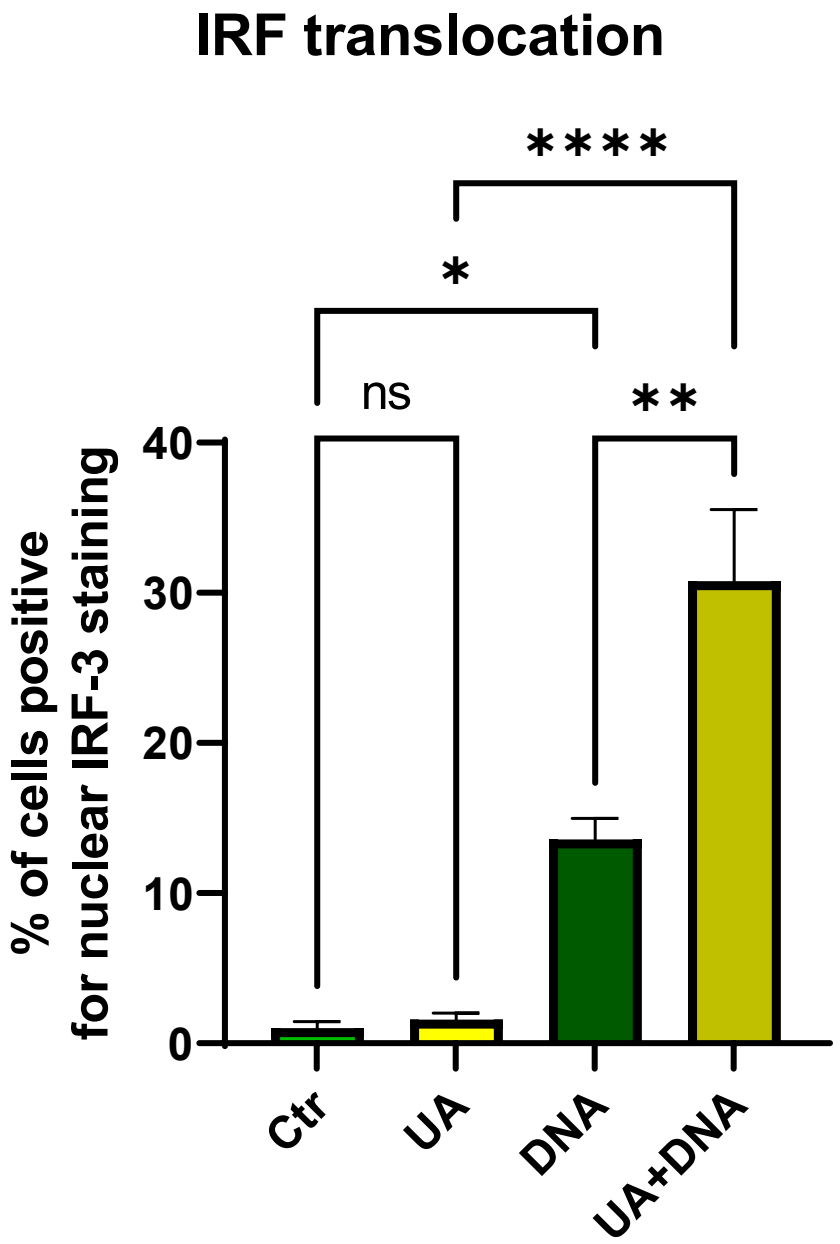


UA affects the cGAS-STING pathway

- Macro identifies each nuclei, then measures intensity of IRF3.
- Then measures intensity of pSTING in red chanel and normalizes this to nuclear count.

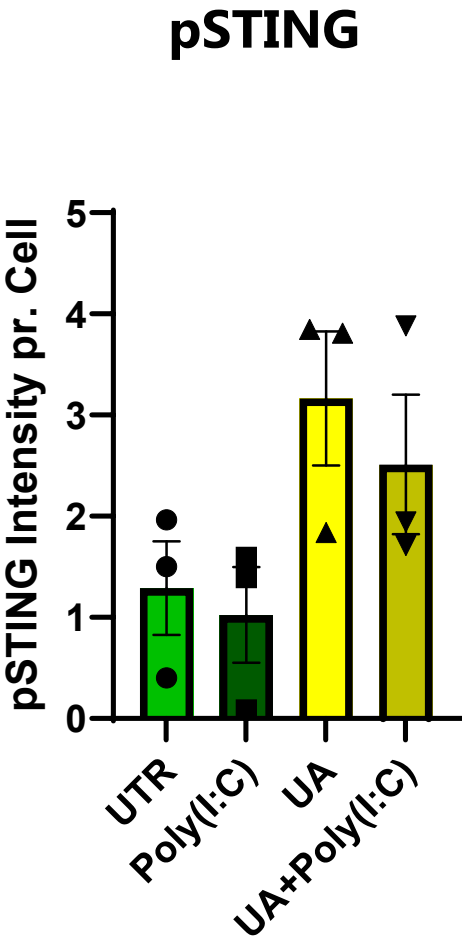

Composite
pSTING
IRF3
DAPI
Ctr
UA
DNA
UA+DNA

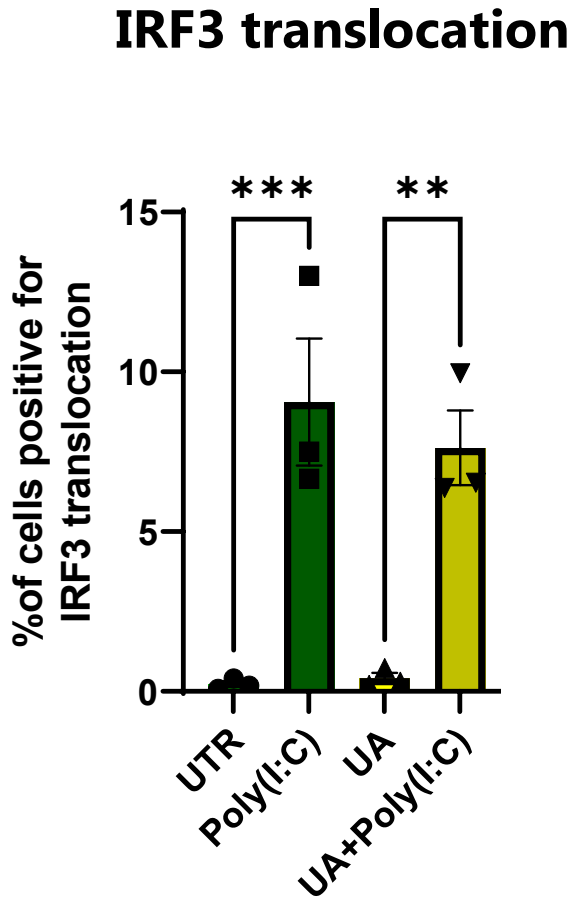

pSTING Int
**
ns
ns
**
pSTING staining Intensity
normalized to cell count
15
10
5
0
Ctr
UA
DNA
UA+DNA


IRF translocation
****
*
ns
**
% of cells positive
for nuclear IRF-3 staining
40
30
20
10
0
Ctr
UA
DNA
UA+DNA

UA does not affect the RNA-sensing pathway

- Treatment with the dsRNA mimic poly(I:C)
  - Does not activate STING (control)
  - No effect of UA on poly(I:C) stimulation

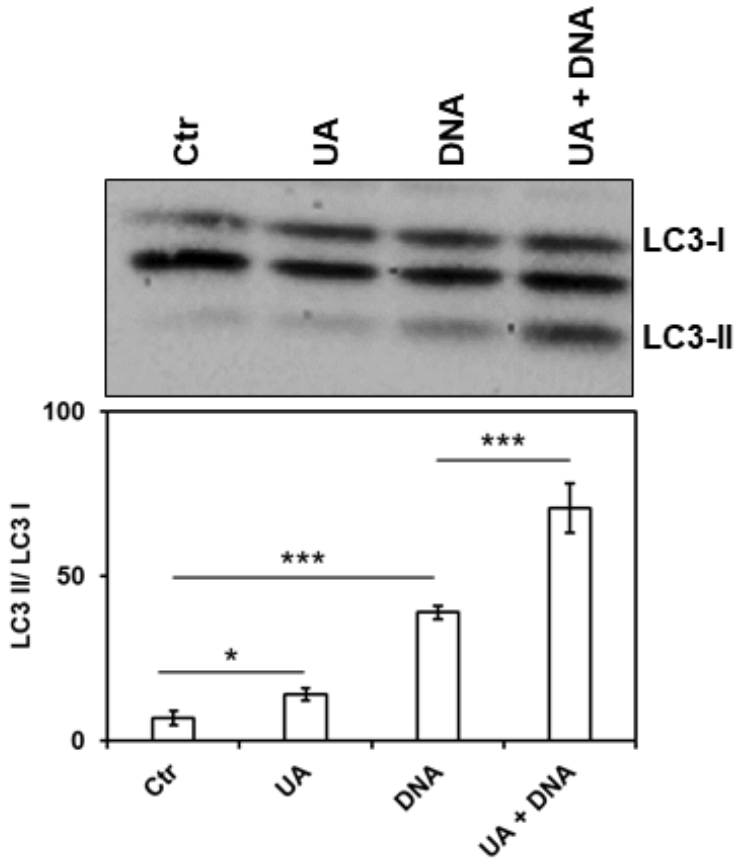


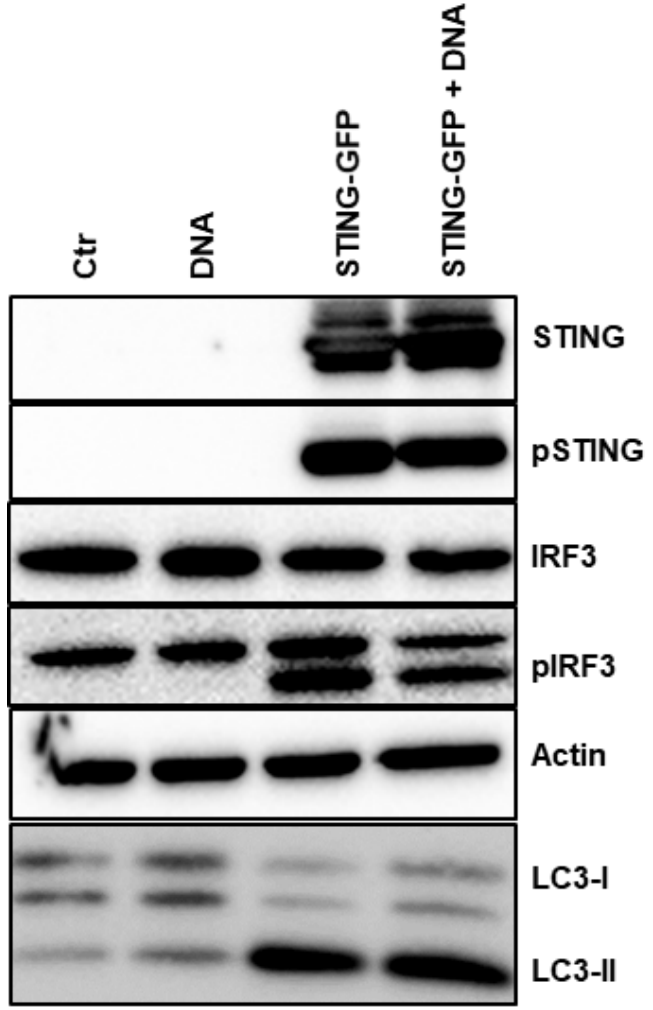


UA increases LC3-mediated degradation

UA increases LC3b lipidation

Increasing STING, increases LC3b lipidation

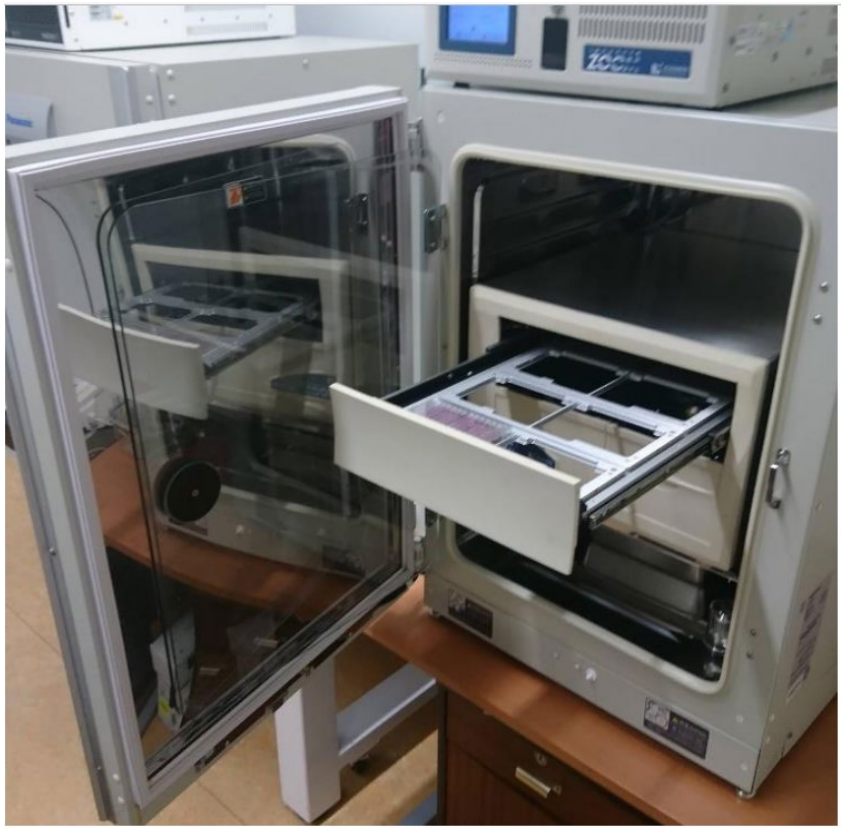




UA increases LC3-mediated degradation

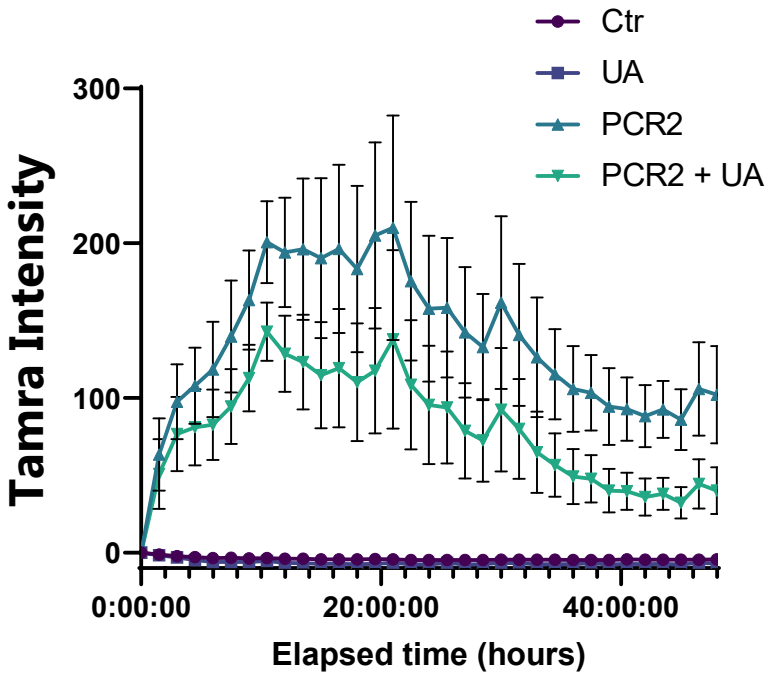
TAMRA-tagged dsDNA degradation assay

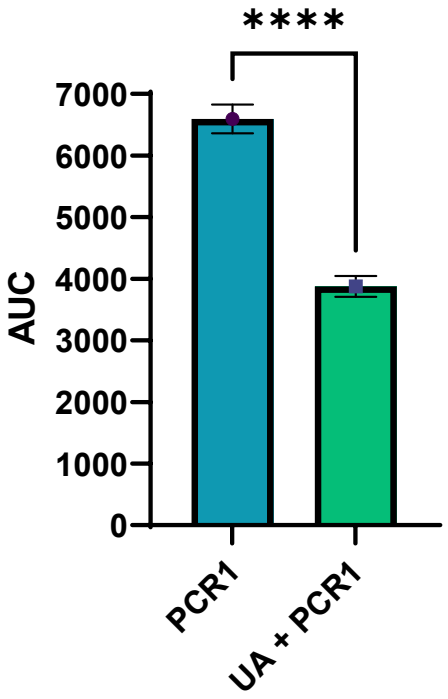
- HMC3 cells transfected with TAMRA-tagged DNA
- Fluorescence monitored over time in live cells



UA increases LC3-mediated degradation

Degradation of cytosolic DNA increased by UA

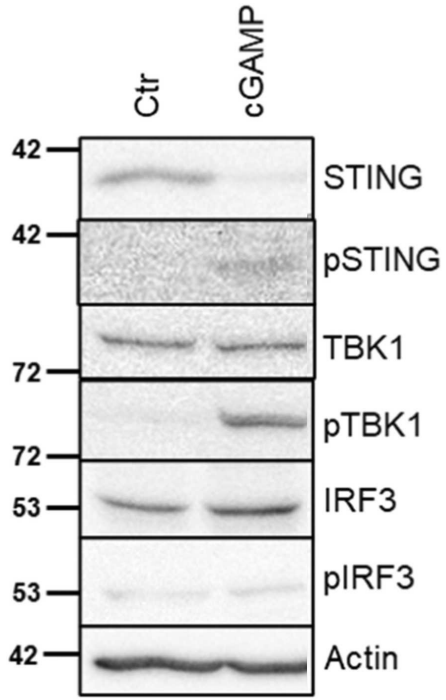




UA treatment improves STING signaling in a cancer cell line

HCT116 DNA repair deficient colon cancer cell line

- <100-fold elevated mutation rate and genome instability
- cGAMP to target STING directly



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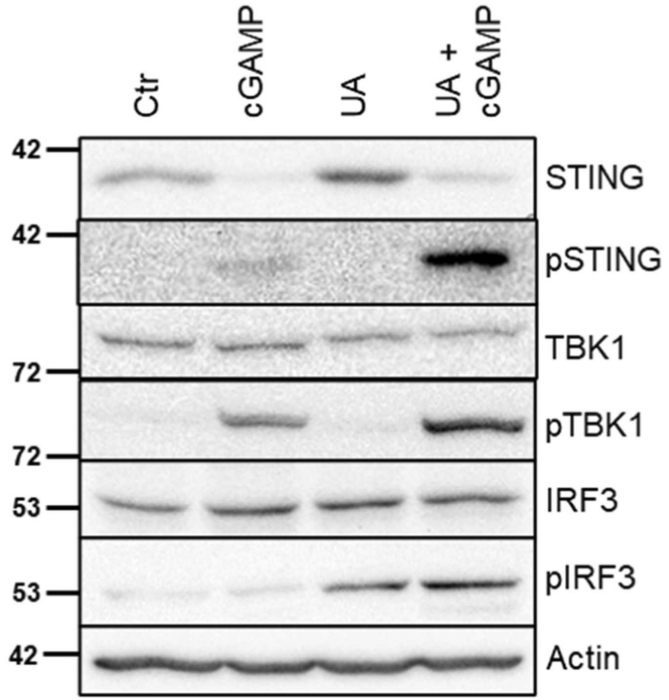
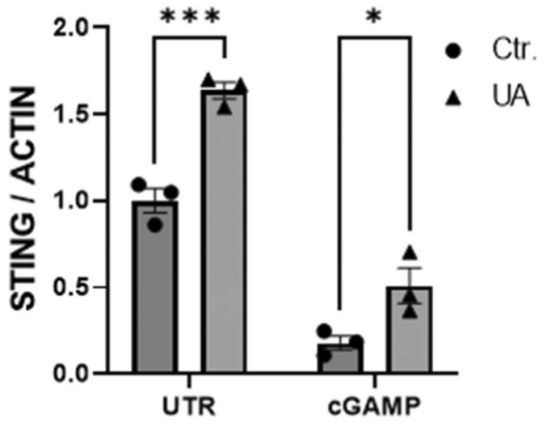
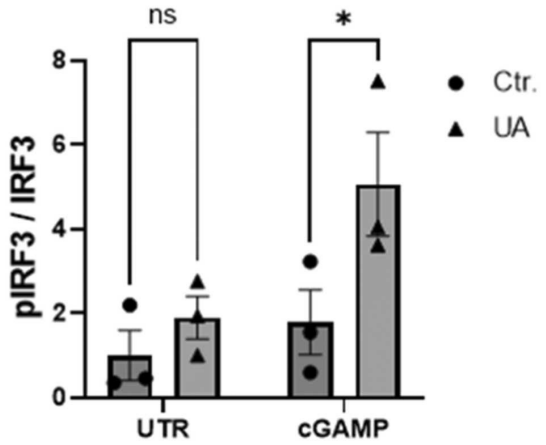
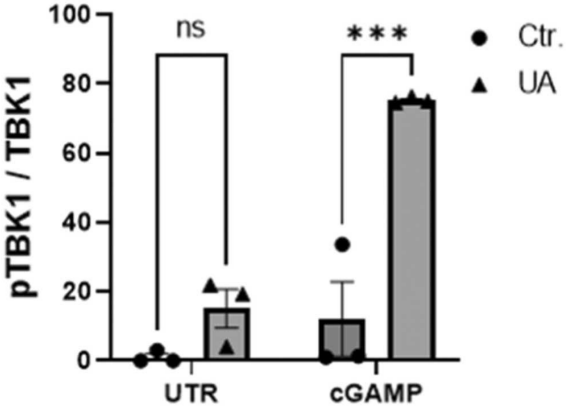
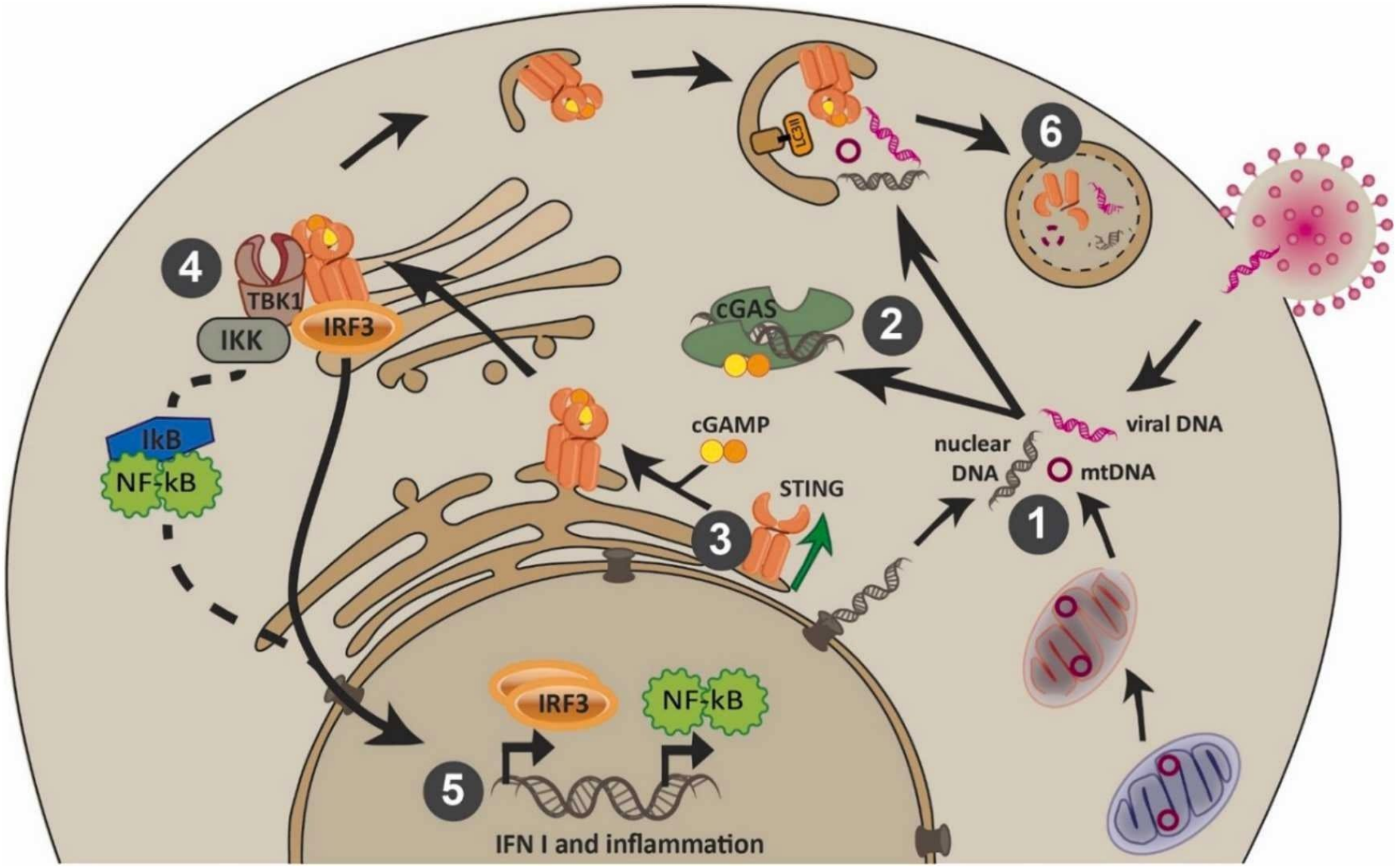




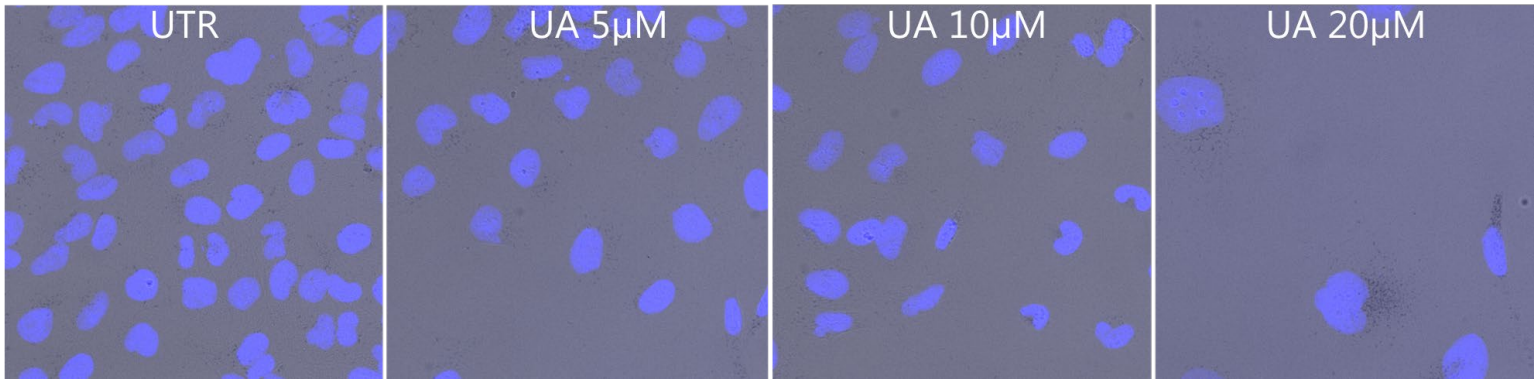


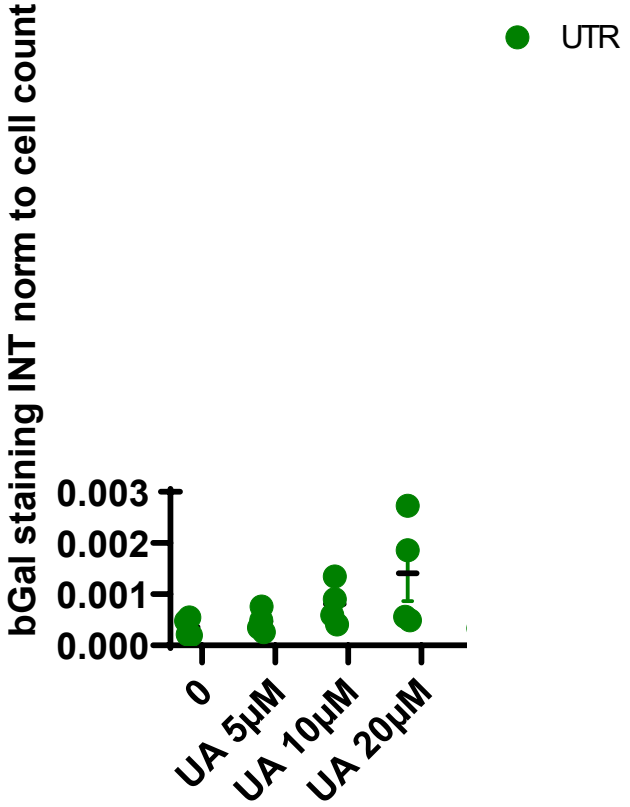



4
TBK1
IKK
IRF3
IkB
NF-kB
cGAS
2
cGAMP
STING
3
nuclear
DNA
viral DNA
mtDNA
1
6
IRF3
NF-kB
5
IFN I and inflammation

Senescence and UA

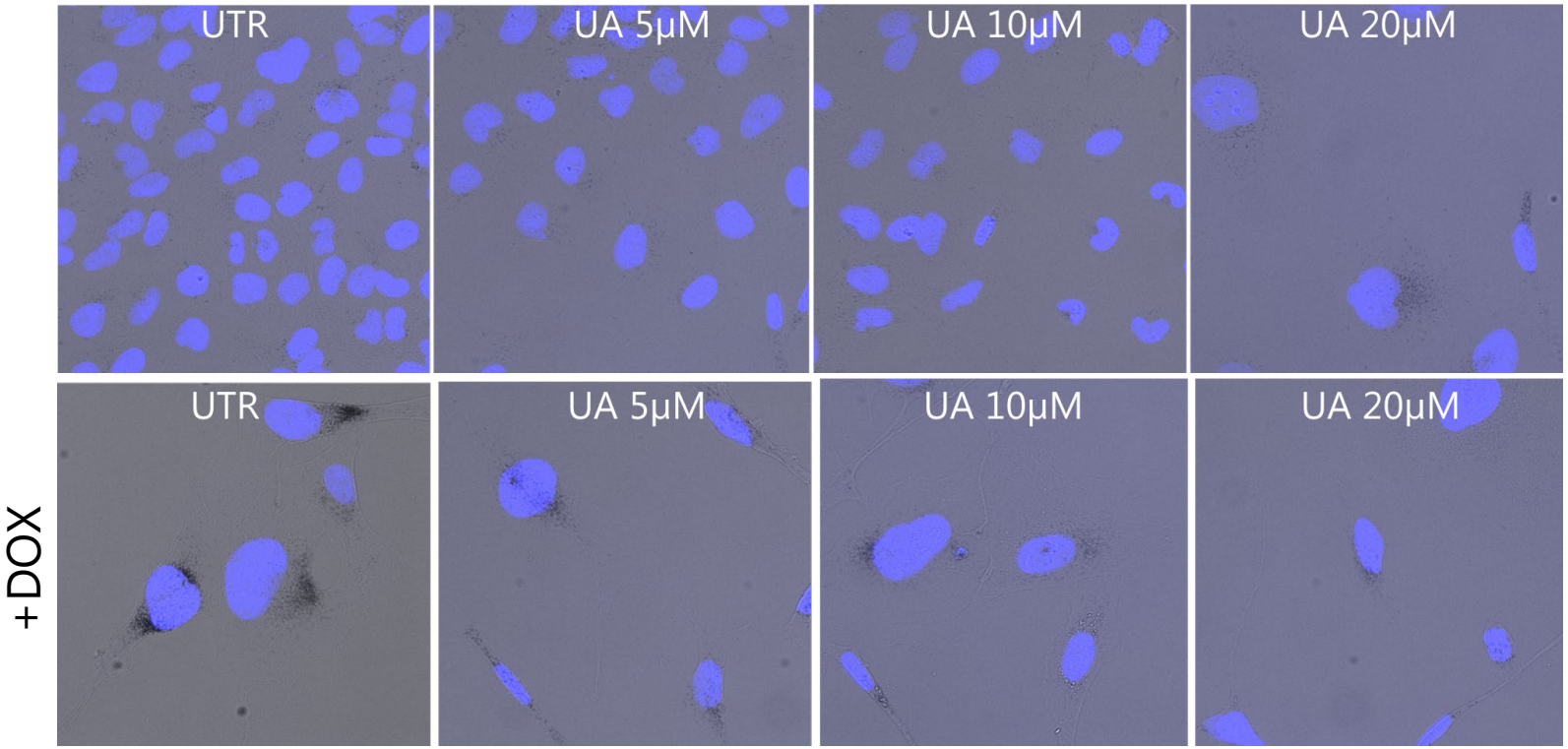
- Senescence-associated β-galactosidase assay
- UA does not significantly induce senescence…

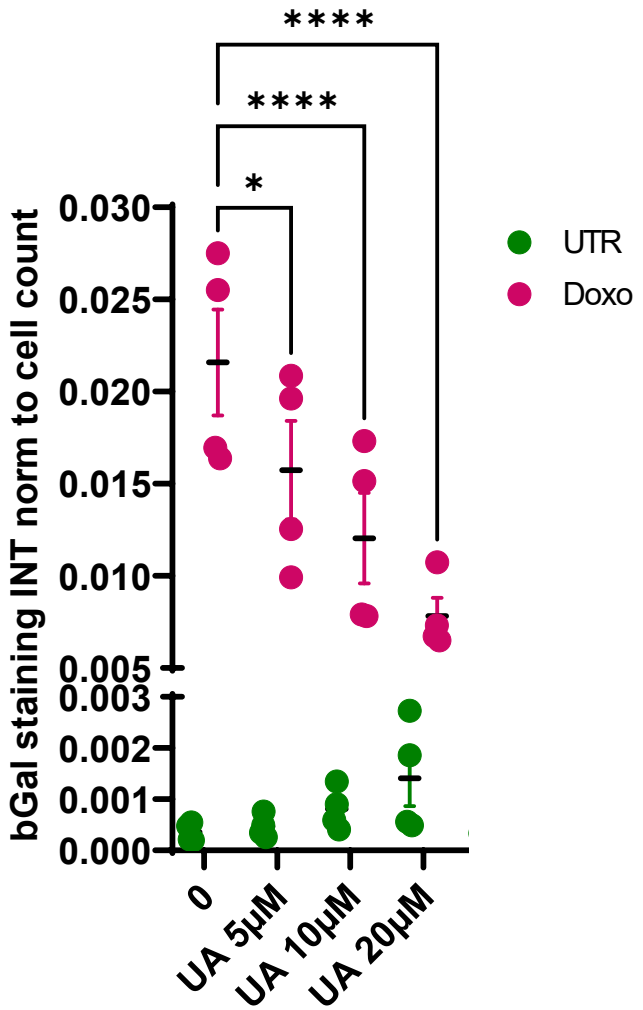




Senescence and UA

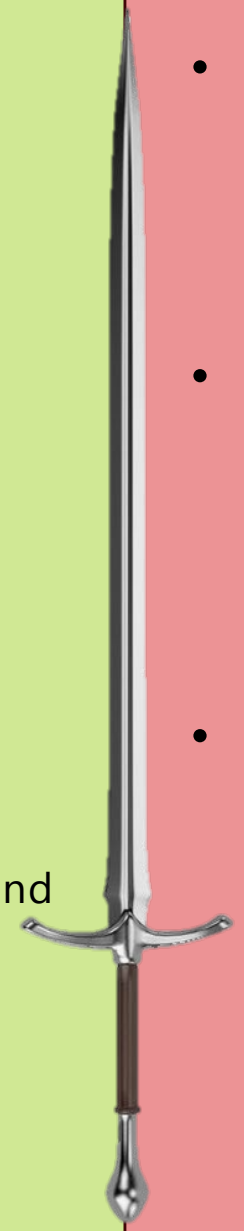
- Senescence-associated β-galactosidase assay
- UA does not significantly induce senescence…
- UA decreases doxorubicine (DOX)-induced senescence





Innate immunity in Aging

- Anti-tumorigenic
  - Depending on Tumour type and stage!
    - Short-term acute inflammation → cancer suppressor
    - Long-term chronic inflammation (SASP) → beneficial to tumor microenvironment, may promote metastasis
- Pathogen control
  - Aging immune system relies heavily on innate sensors, as adaptive immunity declines.
- Tissue homeostasis
  - Acute activation can help clear damaged cells and stimulate tissue repair by mobilizing immune responses.

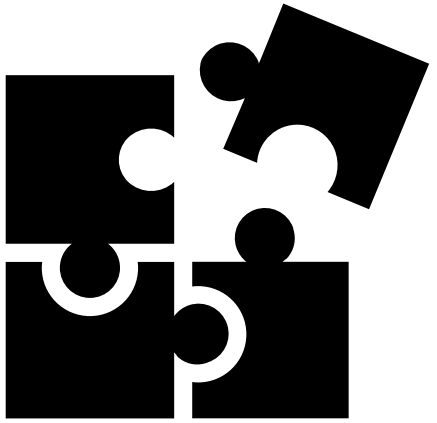


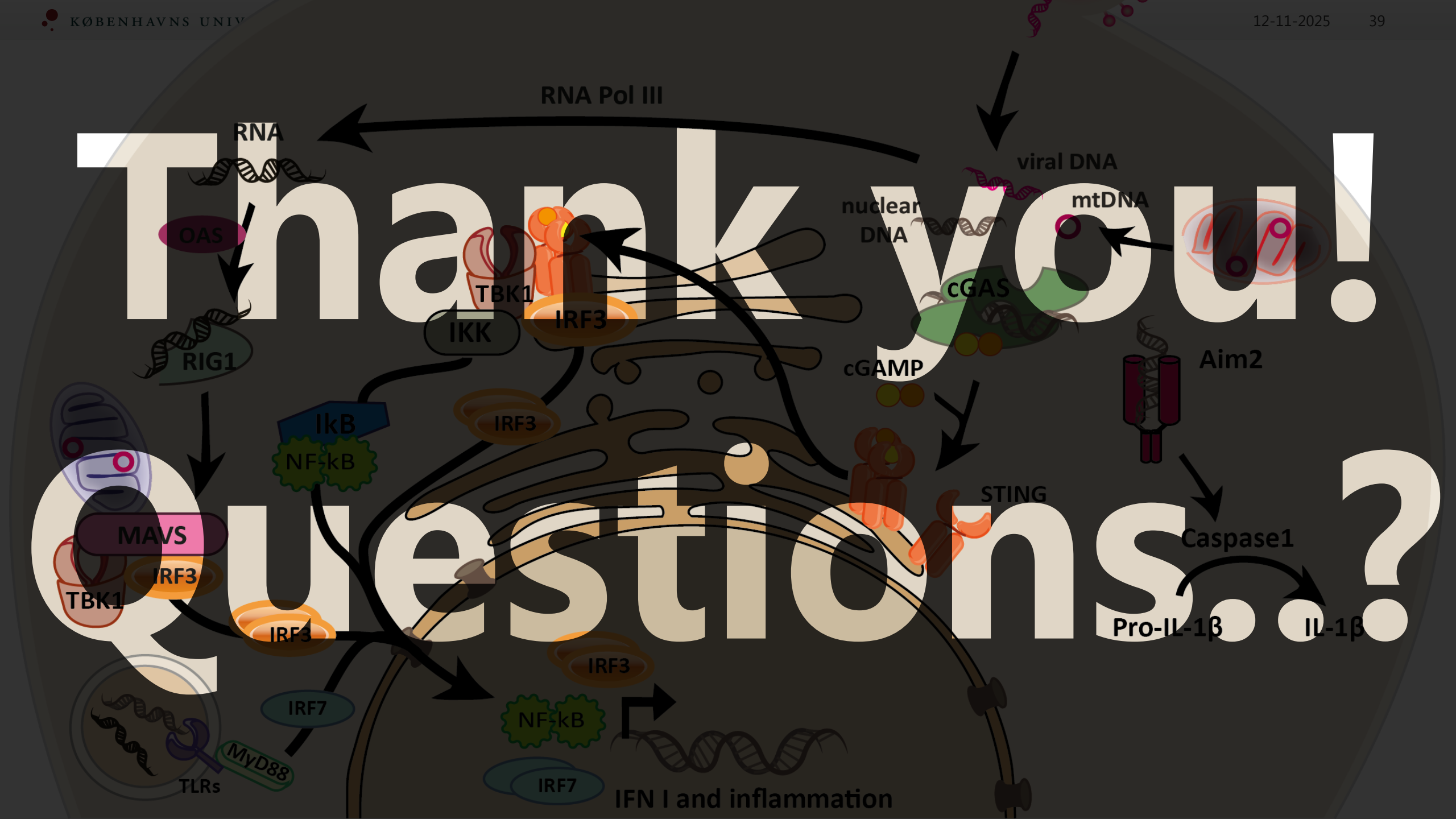
- Chronic inflammation (inflammaging)
  - In aged cells, persistent DNA damage, mitochondrial dysfunction, and micronuclei leakage causes chronic cGAS–STING activation → tissue damage.
- Neurodegeneration
  - Chronic STING signaling in microglia and neurons → sustained neuroinflammation, synaptic dysfunction, and cognitive decline. Instead of clearing damage, the system becomes self-perpetuating and toxic.
- Stem cell exhaustion & impaired regeneration
  - Chronic STING activation depletes stem cell niches by promoting senescence and inflammatory signaling, impairing tissue renewal in muscle, blood, and brain.

Take-home messages

- DNA- and RNA- signaling pathways are complex and not yet fully established
- Sting is upregulated by UA
  - UA increases cGAS-STING signaling upon stimulation (dsDNA/cGAMP)
  - UA increases lc3-mediated degradation, perhaps via STING-mediated autophagy
  - UA concentration and context matters

- DNA- and RNA sensing needs to be tightly regulated…





Thank you!

Questions...?