

May 06, 2025

Version 1

# Protocols for "Mitochondrial Anchored Protein Ligase MAPL is an inflammatory rheostat that regulates immune signalling and cell death" V.1

 [Nature Cell Biology](#)

DOI

[dx.doi.org/10.17504/protocols.io.8epv5x266g1b/v1](https://dx.doi.org/10.17504/protocols.io.8epv5x266g1b/v1)

mai.nguyen<sup>1,2</sup>, Jack Collier<sup>1,2</sup>, Olesia Ignatenko<sup>1,2</sup>, Sidong Huang<sup>3</sup>, Michel Desjardins<sup>4</sup>, heidi.mcbride<sup>1,2</sup>

<sup>1</sup>Department of Neurology and Neuroscience, Montréal Neurological Institute, McGill University, Montréal, Canada;

<sup>2</sup>Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA;

<sup>3</sup>Department of Biochemistry, McGill University, Montréal, Canada;

<sup>4</sup>Département de pathologie et biologie cellulaire, Université de Montréal, Montréal, Canada



Lilia Rodriguez

Université de Montréal

## Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

[Create free account](#)

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5x266g1b/v1>

External link: <https://doi.org/10.1038/s41556-025-01774-y>

**Collection Citation:** mai.nguyen , Jack Collier, Olesia Ignatenko, Sidong Huang, Michel Desjardins, heidi.mcbride 2025.

Protocols for "Mitochondrial Anchored Protein Ligase MAPL is an inflammatory rheostat that regulates immune signalling and cell death". **protocols.io** <https://dx.doi.org/10.17504/protocols.io.8epv5x266g1b/v1>

**Manuscript citation:**

Mai Nguyen, Jack J. Collier, Olesia Ignatenko, Genevieve Morin, Vanessa Goyon, Alexandre Janer, Camila Tiefensee Ribeiro, Austen J. Milnerwood, Sidong Huang, Michel Desjardins, Heidi M. McBride (2025) MAPL regulates gasdermin-mediated release of mtDNA from lysosomes to drive pyroptotic cell death. *Nature Cell Biology* doi: [10.1038/s41556-025-01774-y](https://doi.org/10.1038/s41556-025-01774-y)

**License:** This is an open access collection distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this collection and it's working**

**Created:** September 09, 2023

**Last Modified:** May 06, 2025

**Collection Integer ID:** 87577

**Keywords:** ASAPCRN, pyroptosis, pyroptosis through an inflammatory pathway, induced pyroptosis, pyroptosis, outer mitochondrial membrane sumo, genetic deletion of mapl, mitochondrial dna, inflammatory cell death, mitochondria, inflammatory pathway, mapl overexpression, involving mitochondria, placing mapl, inflammatory cell death in primary macrophage, multiple parkinson, mapl, cell death, neurodegeneration in vivo, lysosome, neurodegeneration, release of mtdna, mtdna, roles in cancer, immune signalling, related gene

**Funders Acknowledgements:**

**Aligning Science Across Parkinson's (ASAP)**

Grant ID: ASAP 000525

## Abstract

Cell death is inhibited in cancers but increased in neurodegeneration, highlighting that its regulation is essential for human health. MAPL is an outer mitochondrial membrane SUMO ligase with roles in cancer and neurodegeneration *in vivo*, yet how MAPL controls cell death remains unclear. Combining genome-wide screening and cell biological approaches, we found that MAPL induces pyroptosis through an inflammatory pathway involving mitochondria and lysosomes. MAPL overexpression promotes mitochondrial DNA (mtDNA) trafficking in mitochondrial-derived vesicles (MDVs) to lysosomes, which are permeabilised in a process requiring gasdermin pores. This triggers release of mtDNA into cytosol, activating the DNA sensor cGAS, which is required for cell death. Additionally, multiple Parkinson's disease-related genes, including VPS35 and LRRK2, also regulate MAPL-induced pyroptosis. Consistent with our findings, genetic deletion of MAPL, LRRK2 or VPS35 inhibited inflammatory cell death in primary macrophages, placing MAPL and the mitochondria to lysosome pathway at the nexus of immune signalling and cell death.

## Troubleshooting

## Files

Q SEARCH



Culturing of cell lines.docx

DOWNLOAD ↓



Generation of Bone Marrow derived macrophages.docx

DOWNLOAD ↓



Transfection and generation of stable cell lines.docx

DOWNLOAD ↓



siRNA transfection.docx

DOWNLOAD ↓



Blue native PAGE to evaluate NLRP3 oligomer.docx

DOWNLOAD ↓



Cells lysates SDS-PAGE and immunoblotting.docx

DOWNLOAD ↓



Pyroptotic cell death .docx

DOWNLOAD ↓



Detection of extracellular cytokine.docx

DOWNLOAD ↓



Amplification of adenovirus.docx

DOWNLOAD ↓

## Protocol

NAME

CRISPR-Cas9 Screening Protocol for Gene Perturbation Analysis

VERSION 1

CREATED BY



Lilia Rodriguez  
Université de Montréal

OPEN →



Immunofluorescence.docx

DOWNLOAD ↓



Image processing and quantification.docx

DOWNLOAD ↓



Live cell microscopy.docx

DOWNLOAD ↓



RNA isolation from tissues with TRIzol.docx

DOWNLOAD ↓



TEM for monolayer cells.docx

DOWNLOAD ↓



Isolation of cytosolic DNA.docx

DOWNLOAD ↓



Immunoisolation of lysosomes.docx

DOWNLOAD ↓