2025 Helmholtz – OCPC – Programme

for the involvement of postdocs in bilateral collaboration projects

PART A

Title of the project:

Uncover functional networks in MHC-I quality control upon pathogenic infections

Helmholtz Centre and/or institute:

Helmholtz Centre for infection research, HZI Braunschweig, Germany

Project leader:

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Department: (at the Helmholtz centre or Institute)

Immune Signalling Group

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Description of the project (max. 1 page)**:**

This project aims to dissect the molecular mechanisms governing non-conformational MHC-I surface delivery and quality control (Fig. 1A) using a genome-wide FACS-based CRISPR-Cas9 screen, a technology already established in my lab (Fig. 1B). By employing cutting-edge screening techniques and advanced immunological assays, the project will map the network of proteins interacting with IRGQ and its paralogs, potentially identifying novel therapeutic targets for immune modulation.

In wild-type (WT) cells, misfolded MHC-I is efficiently retained or recycled, resulting in minimal surface expression of non-conformational MHC-I, as detected by the HC10 antibody. In contrast, IRGQ knockout (KO) cells exhibit restored HC10 surface staining, indicating disrupted quality control. To identify key regulators of this pathway, cells will be stained with HC10 and W6/32 (which detects properly folded MHC-I), followed by FACS sorting of populations where HC10 recycling is rescued in IRGQ KO cells. A preliminary screen has already identified proteins involved in vacuolar membrane trafficking and N-linked glycosylation—both critical processes for MHC-I quality control and surface expression (Fig. 1C).

To validate these findings, the identified candidate proteins will be subjected to individual CRISPR knockouts and overexpression studies to determine their role in the MHC-I quality control pathway. Functional comparisons with IRGQ will help delineate distinct and overlapping regulatory mechanisms. To further refine the understanding of IRGQ-dependent and -independent pathways,

a second comparative CRISPR screen will be performed in IRGQ KO cells, specifically targeting proteins essential for recycling misfolded MHC-I molecules from the plasma membrane.
For promising candidates emerging from these screens, their signaling mechanisms and contributions to MHC-I quality control and surface expression will be thoroughly investigated. Given that many intracellular pathogens have evolved sophisticated strategies to manipulate MHC-I trafficking to evade immune detection, understanding these regulatory networks is crucial for developing targeted therapeutic interventions. Several bacterial and viral pathogens interfere with MHC-I antigen presentation to escape CD8+ T cell-mediated immunity, thereby promoting chronic infection or immune evasion in the context of cancer. This project will assess whether the identified candidates play a role in these pathogen-driven immune evasion mechanisms by examining how their modulation affects antigen presentation and immune recognition during infections.
To achieve this, functional studies will explore how candidate proteins influence MHC-I recycling and degradation pathways upon infection with intracellular pathogens, such as *Salmonella* or *Listeria*, which are known to disrupt host vesicular trafficking. Additionally, we will evaluate whether loss or overexpression of these candidates alters pathogen survival, inflammatory responses, and CD8+ T cell activation. Given that some viruses encode proteins specifically targeting MHC-I for degradation or mislocalization, this study may also uncover host factors that restrict or enhance viral immune evasion.
By conducting the first genome-wide screen specifically targeting MHC-I quality control and recycling, this project will not only uncover novel functional networks regulating MHC-I folding and trafficking but also provide critical insights into pathogen-host interactions. Understanding how pathogens manipulate these pathways could lead to the identification of new targets for host-directed therapies to combat infectious diseases and improve immune-based treatments for cancer. Furthermore, these findings may have broader implications for autoimmunity and inflammatory diseases, where dysregulation of MHC-I processing contributes to aberrant immune responses. The results of this study will significantly advance our understanding of MHC-I biology and open new avenues for therapeutic interventions in immune-related disorders and infectious diseases.

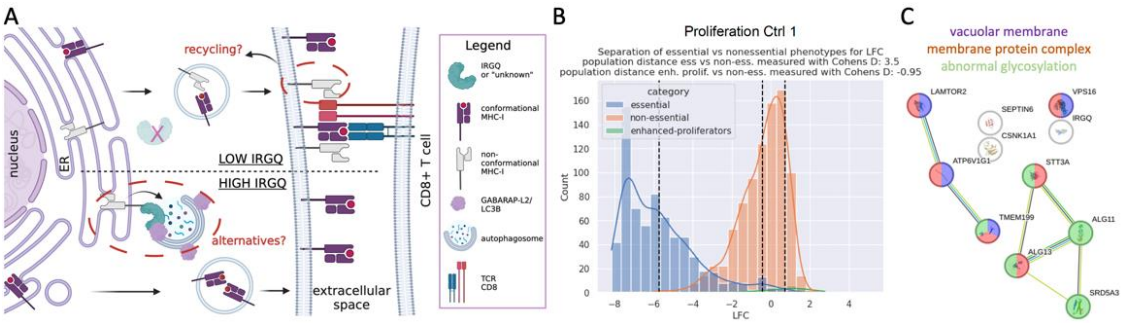

Fig. 1: Functional networks in MHC-I quality control. (A) Schematic of a genome-wide FACS-based CRISPR-Cas9 screen to identify proteins involved in non-conformational MHC-I surface delivery and recycling. (B) Quality control of the CRISPR/Cas9 screen to monitor the drop-out of essential genes. (C) Enriched genes of cells stained positive for HC10.
Project relevant publication: https://www.cell.com/cell/pdf/S0092-8674(24)01148-6.pdf

Description of existing or sought Chinese collaboration partner institute (max. half page)**:**

There is no established collaboration yet, but I am keen to establish partnerships with Chinese research institutes that share an interest in uncovering novel immune signalling pathways in response to pathogenic infections. My research focuses on host-pathogen interactions, particularly the regulation of autophagy, inflammation, and mitochondrial dynamics during bacterial infections. A collaboration with a Chinese institute specializing in immunology, microbiology, or infection biology would provide valuable opportunities for knowledge exchange, complementary expertise, and access to diverse experimental models. By joining forces, we can deepen our understanding of innate immune mechanisms and develop innovative strategies to combat infectious diseases.

Required qualification of the postdoc:

- PhD in Biochemistry, Cell Biology or Immunology
- Experience with data analysis (R-studio or python), common biochemical techniques (Western Blot, qRTPCR, FACS), cell culture, bacterial or viral infection models
- Additional skills in mouse work would be desirable but are not mandatory
- Language requirement: fluent in English (written and spoken)