Function of PERK in T cell metabolic reprogramming: deciphering its role as ER stress sensor vs its novel role as coordinator of membrane contact sites

A Data Management Plan created using DMPonline.be

Creators: Blanca Felipe Abrio, Patrizia Agostinis

Affiliation: KU Leuven (KUL)

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Principal Investigator: Patrizia Agostinis

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Project abstract:

Metabolic reprogramming plays a crucial role in T cell development, differentiation, and acquisition of effector functions. Inadequate metabolic fitness of T cells is a critical factor limiting the efficacy of immunotherapy for cancer therapy, highlighting the need for studies to improve our understanding of T cell metabolic regulation. Growing evidence indicates that metabolism is regulated at highly specialized sites of close appositions between the endoplasmic reticulum (ER) and other organelles, called membrane contact sites (MCS). However, both the key molecular effectors of MCSs and their role in the metabolism and function of T lymphocytes remain elusive. Our lab revealed that PERK, a member of the unfolded protein response (UPR) elicited by loss of ER homeostasis, has additional (UPR-independent) tethering functions, endorsing this protein kinase with the ability to coordinate MCSs. Our preliminary data show that PERK shapes T cell metabolism and effector functions. Here, I will use state of the art molecular/cellular biology tools, a recently developed microfluidic system, and transgenic mice with a specific deletion of Perk in T cells as in vivo models to study the role of PERK in T cell metabolic reprogramming and anti-tumor immunity, and decipher the mechanistic underpinnings related to its role as coordinator of MCSs or the UPR. Exploring the role of PERK as a relevant metabolic checkpoint in T cells may open new perspectives for T cell-based therapies.

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FWO DMP (Flemish Standard DMP)

1. Research Data Summary

List and describe all datasets or research materials that you plan to generate/collect or reuse during your research project. For each dataset or data type (observational, experimental etc.), provide a short name & description (sufficient for yourself to know what data it is about), indicate whether the data are newly generated/collected or reused, digital or physical, also indicate the type of the data (the kind of content), its technical format (file extension), and an estimate of the upper limit of the volume of the data.

				Only for digital data	Only for digital data	Only for digital data	Only for physical data
Dataset Name	Description	New or reused	Digital or Physical	Digital Data Type	Digital Data format	Digital data volume (MB/GB/TB)	Physical volume
Western blot images	Scan of a western blot	Generate new data	Digital	Experimental	.tiff .jpeg .gel files	<100GB	
Microscopy images	Images acquired with different kind of microscopes and analyzed with Imaris or ImageJ software	Generate new data	Digital	Experimental	.czi .tiff .imaris	<5TB	
Microscopy movies	Movies acquired with different kind of microscopes and analyzed with Imaris or ImageJ software	Generate new data	Digital	Experimental	.czi .tiff .imaris	<5TB	
Experimental read outs	Raw data generated on equipment such as Flex station, plate reader and Seahorse XFe24 analyzer	Generate new data	Digital	Experimental	.pda .txt .asyr .xlsx	<1GB	
Flow cytometry	Raw data acquired with different kinds of flow cytometers and analyzed with Flowjo software	Generate new data	Digital	Experimental	.fcs	<100GB	
RNA-seq	Sequencing data generated by Novogene company	Generate new data	Digital	Experimental	.txt .xlsx	<100MB	
Metabolomics	Metabolites quantification obtained through mass spectrometry	Generate new data	Digital	Experimental	.bmp .jpg .xlsx	<1GB	
Mouse models	Data related to mice strains used for experiments and stored in LAIS system	Generate new data	Digital	Experimental	NA	NA	
Plasmids	Database containing the information (construct name, origin, description) of the plasmids used in the project	Generate new data	Digital	NA	.doc .xlsx	<100GB	
Chemicals	Database containing the information (name, provider, catalog number, waste category) of the chemicals used in the project	Generate new data	Digital	NA	.doc .xlsx	<100GB	
Antibodies	Database containing the information (target, source, provider, catalog number, application) of the antibodies used in the project	Generate new data	Digital	NA	.doc .xlsx	<100GB	
Cell lines	Database containing the information (name, cell type, origin) of the cell lines used in the project	Generate new data	Digital	NA	.doc .xlsx	<100GB	
Analyzed data	Processed and analyzed data	Generate new data	Digital	Experimental	.xlsx	<1GB	
Graphs and statistics	Graphical representations and statistical analysis performed with GraphPad prism software	Generate new data	Digital	Experimental	.pzfx	<100GB	

If you reuse existing data, please specify the source, preferably by using a persistent identifier (e.g. DOI, Handle, URL etc.) per dataset or data type:

NA

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? Describe these issues in the comment section. Please refer to specific datasets or data types when appropriate.

- Yes, animal data

The following ethical files partly cover this and all experimental approaches performed in mice are approved by the Animal Ethics Committee of KUL. ECD files: P185/2020 and P013/2021

Will you process personal data? If so, briefly describe the kind of personal data you will use in the comment section. Please refer to specific datasets or data types when appropriate.

- No

Does your work have potential for commercial valorization (e.g. tech transfer, for example spin-offs, commercial exploitation, …)? If so, please comment per dataset or data type where appropriate.

- Yes

We do not exclude that the proposed work could result in research data with potential for tech transfer and valorization. Ownership of the data generated belongs to KU Leuven and VIB in accordance with the framework agreement of both institutes. VIB has a policy to actively monitor research data for such potential. If there is substantial potential, the invention will be thoroughly assessed, and in a number of cases the invention will be IP protected (mostly patent protection or copyright protection). As such the IP protection does not withhold the research data from being made public. In the case a decision is taken to file a patent application it will be planned so that publications need not be delayed.

Do existing 3rd party agreements restrict exploitation or dissemination of the data you (re)use (e.g. Material/Data transfer agreements/ research collaboration agreements)? If so, please explain in the comment section to what data they relate and what restrictions are in place.

- No

No third-party agreement restricts dissemination or exploitation of the data or strains generated from this project. In particular, existing agreements between VIB and KU Leuven do not restrict publication of data.

Are there any other legal issues, such as intellectual property rights and ownership, to be managed related to the data you (re)use? If so, please explain in the comment section to what data they relate and which restrictions will be asserted.

- No

There is no IP on the generated strains that would prevent us from storing the strains,
performing the anticipated experiments or publishing the results.

2. Documentation and Metadata

Clearly describe what approach will be followed to capture the accompanying information necessary to keep data understandable and usable, for yourself and others, now and in the future (e.g., in terms of documentation levels and types required, procedures used, Electronic Lab Notebooks, README.txt files, Codebook.tsv etc. where this information is recorded).

Data will be generated following standardized protocols. Metadata will be documented by the research and technical staff at the time of data collection and analysis, by taking careful notes in the electronic laboratory notebook (E-notebook) and in hard copy lab notebooks that refer to specific datasets.
Cryotubes of biological samples (cell lines) stored at -80°C or liquid nitrogen will be labelled with a reference that links to an entry in the database.
All datasets will be accompanied by a README.txt file containing all the associated metadata (see more details below).
The data will be generated following standardized protocols. Clear and detailed descriptions of these protocols will be stored in our lab protocol database, and published along with the results.

Will a metadata standard be used to make it easier to find and reuse the data? If so, please specify (where appropriate per dataset or data type) which metadata standard will be used. If not, please specify (where appropriate per dataset or data type) which metadata will be created to make the data easier to find and reuse.

- No

Metadata will include the following elements:
• Title: free text
• Creator: Last name, first name, organization
• Date and time reference
• Subject: Choice of keywords and classifications
• Description: Text explaining the content of the data set and other contextual information
needed for the correct interpretation of the data, the software(s) (including version number) used to produce and to read the data, the purpose of the experiment, etc.
• Format: Details of the file format.
• Resource Type: data set, image, audio, etc.
• Identifier: DOI (when applicable)
• Access rights: closed access, embargoed access, restricted access, open access.
Additionally, we will closely monitor MIBBI (Minimum Information for Biological and Biomedical Investigations) for metadata standards more specific to our data type.
For specific datasets, additional metadata will be associated with the data file as appropriate.
The final dataset will be accompanied by this information under the form of a README.txt
document. This file will be located in the top level directory of the dataset and will also list the contents of the other files and outline the file-naming convention used. This will allow the data to be understood by other members of the laboratory and add contextual value to the dataset for future reuse.

3. Data storage & back-up during the research project

Where will the data be stored?

Digital files will be stored on KU Leuven servers.
- Omics data: omics data generated during the project will either be stored on KU Leuven servers or on The Flemish Supercomputer Centre (VSC), initially in the staging area and later in the archive area.
- Vectors: As a general rule at least two independently obtained clones will be preserved for each vector, both under the form of purified DNA (in -20°C freezer) and as a bacteria glycerol stock (-80°C). All published vectors and the associated sequences will be sent to the non-profit plasmid repository Addgene, which will take care of vector storage and shipping upon request.
- Cell lines: Newly created human cell lines will be stored locally in the laboratory in liquid nitrogen storage and will be deposited in the UZ Leuven-KU Leuven Biobank. Other human cell lines will be stored locally in liquid nitrogen cryostorage of the laboratory when actively used for experiments. Animal cell lines will be stored in liquid nitrogen cryostorage of the laboratory.
- Bacterial and yeast strains will be stored in a -80°C freezer in the lab of Patrizia Agostinis. Costs are covered by general lab expenses.
- Genetically modified organisms: Mice will be maintained in facilities of the Laboratory Animal Center of KU Leuven, which applies Standard Operation Procedures concerning housing, feeding, health monitoring to assure consistent care in accordance with European and national regulations and guidelines. All animals will be registered in the Leuven Animal Information System (LAIS) database, along with corresponding genotyping information, ethical approval documents and animal provider receipts.
- Other biological and chemical samples: storage at 4°C and/or as frozen samples in cryovials as appropriate.
- Algorithms, scripts and softwares: All the relevant algorithms, scripts and software code driving the project will be stored in a private online git repository from the GitHub account of the department (https://github.com/vibcbd).

How will the data be backed up?

KU Leuven drives are backed-up according to the following scheme:
- data stored on the “L-drive” is backed up daily using snapshot technology, where all incremental changes in respect of the previous version are kept online; the last 14 backups are kept.
- data stored on the “J-drive” is backed up hourly, daily (every day at midnight) and weekly (at midnight between Saturday and Sunday); in each case the last 6 backups are kept.
- data stored on the digital vault is backed up using snapshot technology, where all incremental changes in respect of the previous version are kept online. As standard, 10% of the requested storage is reserved for backups using the following backup regime: an hourly backup (at 8 a.m., 12 p.m., 4 p.m. and 8 p.m.), the last 6 of which are kept; a daily backup (every day) at midnight, the last 6 of which are kept; and a weekly backup (every week) at midnight between Saturday and Sunday, the last 2 of which are kept.
- All omics data stored on the Flemish Supercomputer Centre (VSC) will be transferred on a weekly basis to the archive area which is backed up.
Incremental backups are done daily from one 20 TB QNAP NAS to a second 20 TB QNAP NAS.

Is there currently sufficient storage & backup capacity during the project? If yes, specify concisely.
If no or insufficient storage or backup capacities are available, then explain how this will be taken care of.

- Yes

There is sufficient storage and back-up capacity on all KU Leuven servers:
- the “L-drive” is an easily scalable system, built from General Parallel File System (GPFS) cluster with NetApp eseries storage systems, and a CTDB samba cluster in the front-end.
- the “J-drive” is based on a cluster of NetApp FAS8040 controlers with an Ontap 9.1P9 operating system.

How will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

Both the “L-drive” and “J-drive” servers are accessible only by laboratory members, and are mirrored in the second ICTS datacenter for business continuity and disaster recovery so that a copy of the data can be recovered within an hour.
Access to the digital vault is possible only through using a KU Leuven user-id and password, and user rights only grant access to the data in their own vault. Sensitive data transfer will be performed according to the best practices for “Copying data to the secure environment” defined by KU Leuven. The operating system of the vault is maintained on a monthly basis, including the application of upgrades and security patches. The server in the vault is managed by ICTS, and only ICTS personnel (bound by the ICT code of conduct for staff) have administrator/root rights. A security service monitors the technical installations continuously, even outside working hours.

What are the expected costs for data storage and backup during the research project? How will these costs be covered?

Each year €738 will be charged from our ICT service for the use of 5 TB on the L-drive (long term storage) and €51,9 will be charged each year for the use of 100 GB of the J-drive (short term storage). Back-up service is included in the price. For the K-drive (data archive) storage space of 1 TB is foreseen and will cost €128 each year, this is also expandable in blocks of 100 GB. These costs were foreseen in the application and if more the lab budget will be used to cover these expenses.

4. Data preservation after the end of the research project

Which data will be retained for at least five years (or longer, in agreement with other retention policies that are applicable) after the end of the project? In case some data cannot be preserved, clearly state the reasons for this (e.g. legal or contractual restrictions, storage/budget issues, institutional policies...).

The minimum preservation term of 5 years after the end of the project will be applied to all datasets. All datasets will be stored on the university's central servers with automatic back-up procedures for at least 10 years, conform the KU Leuven RDM policy. The costs (€156 per TB per year for “Large volume-storage”) will be covered by the lab.

Where will these data be archived (stored and curated for the long-term)?

As a general rule, datasets will be made openly accessible, whenever possible via existing platforms that support FAIR data sharing (www.fairsharing.org), such as MIFlowCyt (https://fairsharing.org/3518) for flow cytometry, OME (https://fairsharing.org/FAIRsharing.cq8tg2) for microscopy and ARRIVE (https://arriveguidelines.org/) for animal experiments, at the latest at the time of publication.
For all other datasets, long term storage will be ensured as follows:
-Digital datasets: files will be stored on the “L-drive”.
-Tissue samples: Tissues will be stored locally in the laboratory.
-Omics data: datasets will be stored on the “L-drive” or, for larger datasets, on the Vlaams Supercomputer Centrum.
-Vectors: As a general rule at least two independently obtained clones will be preserved for each vector, both under the form of purified DNA (in -20°C freezer) and as a bacteria

glycerol stock (-80°C).
-Cell lines: human cell lines will be stored in the UZ Leuven Biobank (-80°C). Animal cell lines will be stored in liquid nitrogen cryostorage of the laboratory.
-Other biological and chemical samples: storage at 4°C and/or as frozen samples in cryovials as appropriate.
- Following publication, the results associated with each study will also be deposited in the Dryad repository, where they will be preserved indefinitely.

What are the expected costs for data preservation during the expected retention period? How will these costs be covered?

Each year €128 will be charged from our ICT service for the use of 1 TB on the k-drive (long term storage), back-up service is included in the price. These costs were foreseen in the budget request of the application and if more, the lab budget will be used to cover these expenses.

5. Data sharing and reuse

Will the data (or part of the data) be made available for reuse after/during the project? In the comment section please explain per dataset or data type which data will be made available.

- Yes, in an Open Access repository
- Other, please specify:

Upon request by email.
Participants to the present project are committed to publish research results to communicate them to peers and to a wide audience. All research outputs supporting publications will be made openly accessible. Depending on their nature, some data may be made available prior to publication, either on an individual basis to interested researchers and/or potential new collaborators, or publicly via repositories (e.g. negative data).
We aim at communicating our results in top journals that require full disclosure upon publication of all included data, either in the main text, in supplementary material or in a data repository if requested by the journal and following deposit advice given by the journal. Depending on the
journal, accessibility restrictions may apply.
Biological material will be distributed to other parties if requested.

If access is restricted, please specify who will be able to access the data and under what conditions.

N/A

Are there any factors that restrict or prevent the sharing of (some of) the data (e.g. as defined in an agreement with a 3rd party, legal restrictions)? Please explain in the comment section per dataset or data type where appropriate.

- No

Where will the data be made available? If already known, please provide a repository per dataset or data type.

Data submission wizards such as EMBL-EBI (www.ebi.ac.uk/submission) will be used to choose the right archive for the data generated in this project. In case there is not good disciplinary repository KU Leuven RDR (www.kuleuven.be/rdm/en/rdr/) will be used.

When will the data be made available?

Upon publication of the research results
As a general rule all research outputs will be made openly accessible at the latest at the time of publication. No embargo will be foreseen unless imposed e.g. by pending publications, potential IP requirements – note that patent application filing will be planned so that publications need not be delayed - or ongoing projects requiring confidential data. In those cases, datasets will be made publicly available as soon as the embargo date is reached.

Which data usage licenses are you going to provide? If none, please explain why.

Creative Commons Attribution-NonCommercial-ShareAlike (CC-BY-NC-SA) will be used unless the final repository used suggests another usage license.

Do you intend to add a PID/DOI/accession number to your dataset(s)? If already available, you have the option to provide it in the comment section.

- Yes

What are the expected costs for data sharing? How will these costs be covered?

It is the intention to minimize data management costs by implementing standard procedures e.g. for metadata collection and file storage and organization from the start of the project, and by using free-to-use data repositories and dissemination facilities whenever possible. Data management costs will be covered by the laboratory budget. A budget for publication costs has been requested in this project.

6. Responsibilities

Who will manage data documentation and metadata during the research project?

Metadata will be documented by the research and technical staff at the time of data collection and analysis, by taking careful notes in the electronic laboratory notebook (E-notebook) that refer to specific datasets. The data will be reviewed by the principal investigator.

Who will manage data storage and backup during the research project?

The research and technical staff will ensure data storage and back up, with support from Raf De Coster for the KU Leuven drives.

Who will manage data preservation and sharing?

The PI is responsible for data preservation and sharing, with support from the research and technical staff involved in the project, and from Raf De Coster for the KU Leuven drives.

Who will update and implement this DMP?

The PI bears the end responsibility of updating and implementing this DMP.