
Mapping the stem cell landscape of mouse and human pituitary across key time points of life in both healthy and disrupted conditions

A Data Management Plan created using DMPonline.be

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Project abstract:

The pituitary positions at the regulatory summit of our endocrine system, accomplishing vital regulation of body development and physiology. The gland contains a population of stem cells whose molecular and functional passport remains blurred. In particular, their cellular complexity and evolution across key time points of life, as well as role(s) are unresolved. Moreover, the stem cells' behavior in disrupted pituitary is largely uncharted. Here, we tackle these important lacunas by constructing an inclusive single-cell transcriptome atlas of mouse pituitary and mapping its stem cell landscape at key endocrine stages, from neonatal maturation to aging-associated waning, in both healthy and injury- and tumorigenesis-disrupted conditions. Acquired datasets will be interrogated using cutting-edge bioinformatics to define age-allied stem cell complexities, dynamic trajectories and niche interactions, and will be integrated with human data to uncover translational correlates. Functional relevance will be assessed in vivo, and using organoids in vitro. Our study will comprehensively map the pituitary stem cell compartment, thereby creating a firm foundation for indepth deciphering their yet enigmatic role in pituitary biology and pathological stress across life. Moreover, it will open up translational perspectives toward regenerative therapy of life-acquired pituitary defects. Simultaneously, our study will provide a rich pituitary cellatlas resource for the research community.

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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
		Please choose from the following options: <ul style="list-style-type: none"> Generate new data Reuse existing data 	Please choose from the following options: <ul style="list-style-type: none"> Digital Physical 	Please choose from the following options: <ul style="list-style-type: none"> Observational Experimental Compiled/aggregated data Simulation data Software Other NA 	Please choose from the following options: <ul style="list-style-type: none"> .por, .xml, .tab, .cvs, .pdf, .txt, .rtf, .dwg, .gml, ... NA 	Please choose from the following options: <ul style="list-style-type: none"> <100MB <1GB <100GB <1TB <5TB <10TB <50TB >50TB NA 	
C57Bl6/J male mice	Dissect pituitary for downstream analyses, including: <ul style="list-style-type: none"> scRNA-seq from additional ages (pubertal and elderly age) Spatial transcriptomics Validation of transcriptomic findings Establish pituitary organoid cultures FACS sort mesenchymal cells (MCs) to establish assembloids 	Generate new data	Physical	NA	NA	NA	40 male mice
GhCre/+;R26iDTR/+ male mice	Dissect pituitary for downstream analyses, including: <ul style="list-style-type: none"> scRNA-seq from additional ages (pubertal and elderly age) Validation of transcriptomic findings Establish pituitary organoid cultures 	Generate new data	Physical	NA	NA	NA	80 male mice
Drd2-/- female mice	Dissect pituitary (tumor) for downstream scRNA-seq analyses (and validation of findings)	Generate new data	Physical	NA	NA	NA	20 female mice
Sox2CreERT2/+ mice	Breed animals with pituitary stem cell specific KO for Zeb2 gene (by crossing them with Zeb2fl/fl mice).	Generate new data	Physical	NA	NA	NA	80 mice
Zeb2fl/fl mice	Breed animals with pituitary stem cell specific KO for Zeb2 gene (by crossing them with Sox2CreERT2/+ mice). Assess the role of Zeb2 in pituitary stem cell behaviour.	Generate new data	Physical	NA	NA	NA	80 mice
Sox2eGFP/+ mice	FACS sort SOX2+ pituitary stem cells	Generate new data	Physical	NA	NA	NA	20 mice

Paraformaldehyde (PFA)- fixed pituitaries and derived organoids	Organoids and fixed samples are obtained as published in PMID: 35699412 and stored in designated storage spaces.	Generate new data	Physical	NA	NA	NA	<400 samples
RNA from pituitaries, organoids and assembloids	RNA samples are obtained from primary pituitary tissue as well as from sorted cells (MCs, SOX2+ cells) and organoids (and assembloids) at multiple passages. (Stored at - 80°C).	Generate new data	Physical	NA	NA	NA	<700 samples
cDNA from pituitaries, organoids and assembloids	cDNA samples are obtained from primary pituitary tissue as well as from sorted cells (MCs, SOX2+ cells) and organoids (and assembloids) at multiple passages. (Stored at - 20°C).	Generate new data	Physical	NA	NA	NA	<700 samples
Lab books	Notes on experiments, observations in the lab.	Generate new data	Physical	NA	NA	NA	<100 books
PCR results	Gel electrophoresis (gel image) obtained via Image Lab software.	Generate new data	Digital	Experimental	.tif	<1GB	NA
Light epifluorescence and confocal images	Images from (sections) of organoids, assembloids and/or pituitary tissue	Generate new data	Digital	Experimental	.tif, .lif, .lsm	<100GB	NA
RNA/DNA concentration/quality	Information obtained after RNA extraction via measurement with Nanodrop.	Generate new data	Digital	Experimental	.xlsx	<100MB	NA
RT-qPCR data/graphs	Data/graphs created via QuantStudio Real Time PCR software.	Generate new data	Digital	Experimental	xlsx, .eds, .pzfx	<100GB	NA
Sequencing data	scRNAseq, spatial transcriptomics	Generate new data Reuse existing data	Digital	Experimental	xlsx; .rds, .fastq, .fasta, .pdf	<1 TB	NA
Experimental analysis data and manuscripts	Analysis of obtained data summarized in presentations/excel/word files.	Generate new data	Digital	Experimental	.xlsx, .docx, .ppt	<100 MB	NA

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:

Use of published scRNA-seq datasets of mouse and human pituitary biopsies.

Mouse: <https://doi.org/10.7554/eLife.75742>, <https://doi.org/10.1073/pnas.2100052118>

Human: <https://doi.org/10.1038/s41467-020-19012-4>, <https://doi.org/10.1016/j.celrep.2022.110467>

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

ECD reference number: P153/2018

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.

- No

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

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

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).

- Documentation of the mice: in an excel file the following information will be noted for every mouse: cage number, date of birth, sex, derived from which breeding couple, genotype, sacrifice date, used in which experiment.
- Daily lab activities are recorded in detail in the lab book.
- For documentation of microscopy images (of organoid cultures) the following information will be noted: date, experimental condition, passage of organoid culture, number of days in culture, magnification used. Images will be saved on the shared drive of the lab and KU Leuven OneDrive in a designated folder of the experiment. Within the experiment folder, additional folders are labelled in a clearly structured way (according to different experimental conditions or different timepoints within the experiment). The setup of an experiment is written down in the lab book. A meta data file, generated by the microscope software, is saved automatically together with the image.
- For RNA and cDNA concentration and quality measurements using Nanodrop: 260/230 and 260/280 ratios (quality measure) and concentrations are written down in lab book and later transferred manually to an excel file where all previous RNA/cDNA measurements are stored. Date of measurement together with name of the sample is included.
- For RT-qPCR data: excel file containing sample setup, raw data, results, melt curve data are given the name: "date, RT-qPCR_experiment name". The RT-qPCR data is saved in a "RT-qPCR folder" within the folder of the specific experiment, together with the template of the RT-qPCR plate set-up. Name of the template file: "date, RT-qPCR_experiment name_layout". Graphs from the data are made using Graphpad Prism (.pzfx file). File is named: "date, Graphs_experiment name", and saved in the same folder.
- Methodology and protocols for RNA extraction, cDNA preparation, immuno-histochemistry stainings, organoid culture, medium preparation... are all included in the lab book and stored on KU Leuven OneDrive in a designated folder. In the table of contents of the lab book the page number of each protocol included in the lab book can be found.

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

Currently, metadata standards are not implemented in the research group. Metadata are generated during microscopy, RT-qPCR analyses and from sequencing data. In general, to make the data easy to find, a personal folder on the shared drive of the lab and OneDrive is made and is further subdivided a clearly structured way (e.g. specific folders for different experiments). In the lab book a description of every experiment can be found including all the experimental conditions

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

Where will the data be stored?

- During the research: Digital data are stored on the shared drive of the lab (KU Leuven; with automatic back-up) and a copy is stored on the KU Leuven OneDrive. Copies can be made on the applicants personal OneDrive.
- Physical samples and biospecimens are stored in the restricted-access cool room/fridges (4°C) or freezers (-20°C or -80°C) or liquid nitrogen container of the research group.
- All data stored in the lab books remain available in the host lab even after departure of the applicant.
- After the research: The digital data are stored on the shared server of the lab, and on the storage space of the Flemish Super Computer VSC (for the large scRNA-seq data). After completing the study, all data are uploaded to a repository to be determined (e.g. archive space of the VSC) and placed under embargo for five years

How will the data be backed up?

Digital data are stored on the shared server of the lab (KU Leuven) and on the KU Leuven OneDrive, which both have automatic back-up.

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

The KU Leuven One Drive provides 2 TB (or 2000 GB) of storage which will be sufficient storage for the project.

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

Access to the shared drive of the lab and KU Leuven OneDrive are secured by a 2-step authentication process with personal log-in (personal u-number and password) and activation of the multifactor authenticator app provided by the KU Leuven. There is also a password on the personal computer of the applicant. Physical data is securely stored in the lab and offices that are only accessible through a badge system.

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

As long as the data does not exceed the 2 TB of storage of the KU Leuven OneDrive, no additional costs for data preservation are expected. If the storage capacity unexpectedly exceeds 2 TB, KU Leuven provides a large volume storage for research data in a cost-efficient manner: 104,42 euro/TB/year (to be purchased in blocks of 5 TB).

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...).

After the research, all digital data are stored on the shared server of the lab, and on the storage space of the Flemish Super Computer VSC (for the large RNA-seq data). After completing the study, all data are uploaded to a repository to be determined (e.g. archive space of the VSC) and placed under embargo for five years. RNA degenerates within a period of five years making the samples unusable after that period. Therefore, it is possible that physical samples containing RNA are not kept for the expected 5 year period after the end of the project.

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

After the research, all digital data are stored on the shared server of the lab, and on the storage space of the Flemish Super Computer VSC (for the large RNAseq data). After completing the study, all data are uploaded to a repository to be determined (e.g. archive space of the VSC) and placed under embargo for five years.

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

As long as the digital data does not exceed the 2TB of storage of the KU Leuven OneDrive, no additional costs for data preservation are expected.

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

The following datasets will be made available:

- Fluorescence/brightfield images
- RT-qPCR data
- scRNA-sequencing datasets

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

NA

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.

The obtained data (fluorescence/brightfield images, qPCR data) in the project will be made available through publications and the PhD Thesis. The scRNA-sequencing data will be made available on ArrayExpress after publication.

When will the data be made available?

Upon publication of the research results.

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

Data can be requested after signing a data sharing agreement (Attribution 4.0 International (CC by 4.0)). Public availability after publishing the data will also depend on the journals policy (postpublication data repository).

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?

There are currently no expected costs for data sharing.

6. Responsibilities

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

Designated PhD student

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

Designated PhD student

Who will manage data preservation and sharing?

The professor, Prof. Dr. Hugo Vankelecom

Who will update and implement this DMP?

The designated PhD student during the research project. The professor, Prof. Dr. Hugo Vankelecom will take over once the PhD is finished. The promotor, prof. Dr. Hugo Vankelecom, bears the end responsibility of updating & implementing this DMP.

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