Instructions for writing the diploma thesis in Immunology

In your diploma thesis, you should present and document your diploma project in the correct way and style similar to a scientific manuscript. The aim is to present your project together with a sufficiently rich introduction, a clear result section and a complex discussion of your results in the light of other published observations.

The thesis must be written by yourself. Please be aware that your thesis will be tested for plagiarism by Turnitin software comparing it to the so far defended thesis in the repository, scientific papers, or book chapters. The thesis can be submitted in Czech, Slovak, or English. Clearly, English is the preferred language of communication in the scientific community. The thesis should be formally correct, easy to follow and carefully proofread (please use text editor-embedded spell-checkers or other available tools). The text itself should be written in an easily readable font (like Times New Roman, with a decent size – 12 is recommended), with a spacing of 1.5, please set the margins at least 2 cm from the right and 2.5 cm from the left and please mind the orientation of the odd/even pages. The thesis should be printed double-sided and bound using hard binding.

The thesis should contain all mandatory parts: Title page, Declaration, Acknowledgments, Abstract (EN), Abstrakt (CZ), Table of Contents, List of Abbreviations, Literature Overview, Thesis Aims, Material and Methods, Results, Discussion, Summary, References.

Title page
The Title page has the mandatory format, please follow the Dean's regulation No. 22/2017 pg. 11 for details.

Declaration
According to the Dean regulation, you should use and sign the following statement in Czech:

Prohlašuji, že jsem závěrečnou práci zpracoval/a samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.
V Praze, DD.MM.RRRR Jméno + Podpis

Here is the English transcript of the declaration:
I hereby declare that my thesis represents my own original research work. Wherever the contribution of others is involved, every effort is made to indicate this clearly including reference to the literature. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma.
Prague, DD.MM.YYYY Name + Signature

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Acknowledgments
Well, this is entirely up to you. People usually acknowledge their thesis supervisor, colleagues from the laboratory and their families.

Abstract (CZ + EN)
The abstract has to be written both in English and Czech/Slovak, irrespective of the language of the thesis. It has to be self-standing. Thus, the background of the work should be outlined, the aims of the thesis should be explicitly spelled out and results should be summarized. The abstract should end with a statement concerning the significance of your results and overall conclusion. Maximum is a single A4 page for each variant. The English and Czech/Slovak versions are supposed to mirror each other as much as possible.

Table of Contents
Please use the numbering of chapters and subchapters.

List of Abbreviations
Please include all nonstandard abbreviations and their explanation. You do not have to include well-known abbreviations like ELISA, FACS, DNA, RNA or gene names, etc. Please use the official gene IDs, not the aliases.

Literature Overview
It is useful to start with a short (max. one page) general introduction showing the broader long-term perspective of your research topic and continue with narrowing down the text to a specific topic and question of your thesis. Please do not include textbook knowledge (anticipate the reader is your fellow student at the end of the Master's program). The main volume of your thesis should clearly describe the current knowledge in your specific field and ideally reading your literature overview should be sufficient for the reader to get oriented in your specific field. The review should cover all important publications in the field published so far, mainly from the last 5 years, maximum 10 years. The text should prove that you are capable of the synthesis of information from several relevant sources, so please use solely primary citations. Also, it should be clear from the literature overview that you have a deep and up-to-date knowledge of your research field. The text has to be supported with citations in the proper format (please do NOT add citations manually and use dedicated citation managers). The in-text citations should follow the Name et al, YEAR format (for example, you can use the APA 7Th variant of Vancouver style). Sometimes it is more useful to use a figure, scheme, or illustration to highlight your point. All figures need to be referred to in the text (Fig. No) and positioned conveniently close to the referenced text. Please mind the copyright, if you are using the figure from the publication you need to obtain permission from the publisher and cite the original paper. Thus, it is highly recommended to use schemes and illustrations made by yourself.

Thesis Aims

You should describe your central hypothesis with the specific aims that you are going to test in your thesis. This can even be in the form of short statements (bullet points) or questions. Keep in mind that each hypothesis/aim must be finally discussed using current literature in the Discussion section and concluded in the Summary section.

Material and Methods

The Material and Methods section should be written in sufficient detail, so the experienced reader must be able to repeat what you have done (please see the minimal reporting requirements for frequently used methods below). All essential materials should be described, including the supplier and catalog number. In the case of plasmids, cell lines, or genetically manipulated animals generated previously, a reference to the original paper should be provided and/or the original research lab credited. If the method itself was used previously in the same way you can refer to the description elsewhere, however, you should describe the method in sufficient detail so that the reader is not forced to look up essential parts in the reference. When using some commercial kit or reagent supplied with the protocol that was strictly followed, it is suggested to state that this method was done using a particular reagent following the manufacturer's recommendation and the principle of the assay must be shortly summarized. If applicable, you should clearly state if any aspect of your work needs a special ethical permit and provide the approval number (e.g. work with experimental animals, use of the material of human origin, work with human embryonic stem cells, or informed consent from patients). When using human material, please provide anonymized clinical and demographic data, which are then used for statistical evaluation. The inclusion and exclusion criteria for the study using human material must be clearly stated.

Results

The Results section needs to be clearly organized and follow the logical structure of your project. Please reflect the feedback you received during the seminar in this chapter. In each subpart, you have to clearly write, why this particular experiment was done, what was its experimental setup (what method and samples were used) and what did you observe and where it is shown. Obviously, given the restricted time for the work on the diploma thesis, it is not uncommon that not all the measurements are provided in a sufficient number of replicates or that some data are rather preliminary. Although this is not permitted in scientific publications, in the case of a diploma thesis this is fine, when appropriately discussed. You have to be aware of the limitations of such measurements when you are analyzing, discussing, summarizing, or putting your data in a broader context. Also, please pay attention to negative and positive controls for your experiments. You do not have to present only discoveries, most of the experimental data could be referred to as "negative" results. The diploma thesis should teach you good laboratory practice and how to design and interpret experiments in a scientific fair way. Please, do not hesitate to present "negative" data, if produced correctly. You

should also focus on a solid statistical evaluation of your data in comparison to an already published one.

Each figure should have its number (Fig. No), representing the order of appearance in the text. The figure itself should be placed close to its reference text and have to be accompanied by a figure legend. Figure legend should be self-standing, with the first line working as the heading. Data should be presented including mean or median, standard error bars and appropriate statistical tests. This information has to be described in each particular figure legend, including the number of replicates (n=?). Y-values are better shown in the same scaling to ensure that figures are easily comparable when depicting similar phenomena. Please prefer the use of absolute rather than relative (after normalization to 100 %) data whenever possible.

In general, data should be presented together with (at least one) representative figure depicting the raw data of a particular measurement. For example, it is not acceptable to provide just a graph plot summarizing the whole experiment without showing representative FACS plots. Please also avoid using bar graphs, use dot plots, violin plots, or similar instead.

The result part should refer to your own data. Of course, science is collaborative and as such, you might continue someone's project, you share your data and other colleagues share their data with you. Acknowledge the author(s) and fairly attribute any data which are not yours in the thesis. It is one of the most important good practices in science, you should learn by heart.

Minimal reporting requirements for data presentation

PCR, quantitative PCR, PCR-based methods (genotyping, cloning, amplifications)

Please provide the primer sequences and describe the conditions used for PCR in the methods section. If you are using classical gel-based PCR you should provide gel photos with markers and indicate the samples and controls position. For quantitative PCR, please indicate what housekeeping gene(s) were used for normalization, and how the housekeeping genes were selected, and please highlight the method used for quantification. Indicate if the absolute or relative quantification method was used, and provide your reasoning for the selection of the method.

Bulk RNA-sequencing

Please highlight the pipeline used for the preparation of sequencing libraries, the conditions and material used for sequencing libraries and the programs or scripts used for data analysis. Data should be presented as Volcano plots (fold-change versus p-value). The list of top up-regulated and downregulated genes should be provided (this can be a thesis supplement). It

is not acceptable to show just a heat map of selected genes, although this might be useful to illustrate a particular effect and needs to be accompanied by the data specified above.

Single-cell RNA-sequencing

Please highlight the pipeline used for the preparation of sequencing libraries, the conditions and material used for libraries sequencing and the programs or scripts used for data analysis. Please clearly indicate and show genes used to describe/annotate a particular cluster. The list of differentially expressed genes together with Volcano plots should be provided when comparing different conditions (please see above).

Cytometry

The representative full gating strategy should be provided for each type of experiment (including FSC/SSC, singlets, live/dead cells discrimination). Showing the fluorescence minus one control for setting up your gates and appropriate compensation is essential. Justification of the panel design should contain the selection of particular Negative controls (including isotype if needed). Especially in the case of rare populations, showing the back-gating (projection of your population of interest to all parent flow cytometry panels) might be important to prove your point. FACS plots should be correctly transformed and presented together with a full description of the x/y-axis including the name of the detected marker, fluorophore used for its visualization and if it was stained intracellular, e.g. CD4-FITC, Gata3-PE (ICS). If an unsupervised method is used for data analysis, the script and the parameters of the algorithm should be shown in the Methods section and titration and compensation data using concatenated single-stained controls must be shown in the Supplement. The list of antibodies used must be provided in the Methods section.

Microcopy

Microscopic images should contain scale bars. Please use representative images clearly describing reality. It is advisable to use several images depicting a particular phenomenon. It is advisable to include image analysis and statistics. Similar to cytometry, the appropriate controls and details of the analysis (including scripts) should be added to the Supplement. Please mind the co-localization controls and spectral overlaps.

Western blots

The uncropped gels should be used in figure panels. Please indicate clearly the sizes of proteins based on the marker. Please provide the loading controls and densitometry of the blots with statistics.

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Protein quantification (ELISA, bead assays or mass spectrometry)
Provide a calibration curve for a quantitation, including the commercial kits. For each analyte, find a normal physiological value in the literature or document your results from an appropriate size of a control cohort.

Discussion
This is the crucial part of your thesis, as it is supposed to show how you are able to analyze and interpret your data in light of current knowledge in your field. In this section, you are supposed to fully interpret and discuss your results. Please avoid repetition of results description from the previous chapter(s), you should rather interpret your results in the context of your hypothesis and in relation to what has been described previously in the literature. You can also discuss the additional experiments that should be done based on your results in order to test the hypothesis fully. Also, if you encounter any methodological problems and you can suggest other approaches that might test your scientific question better, this is the right place to discuss this issue.

Summary
A brief summary of the experimental part of your thesis. This part should reflect your thesis aims and how you were able to test your hypothesis. You can also outline what should be the future direction of this research project.

References
Please provide the list of references generated by the automatic reference manager. We highly recommend using the APA 7Th variant of the Vancouver style of citations. Please provide the exported list of references in a searchable format (e.g. RDF, JSON, CSV, BIB, RIS, XML) as a supplement to the diploma thesis and uploaded it directly to the SIS system together with the thesis. This can be directly exported from the citation manager that you are using.

Finalizing your thesis
The cover for your thesis can be prepared well in advance and has to follow Dean regulation No. 22/2017. The binding itself can be done directly by the Library of Chemical Sciences, Faculty of Sciences, Charles University, Hlavova 8, room 102k, Albertov, Prague 2, or basically in each copy center.

You have to also upload the thesis in SIS using the PDF/A format and bring two hard copies of your thesis to the departmental secretariat. Please watch carefully the website of the faculty and department for details.

Thesis defense

Please prepare a 20-minute-long presentation summarizing your diploma thesis. You should provide a sufficient introduction (background information), describe your thesis aims, results and summarize your talk. The presentation and subsequent discussion can be held in Czech, Slovak or English. Clearly, English is the preferred language of communication in the scientific community. You should expect that the majority of the auditorium did not read your thesis. Also, keep in mind that some kind of data presentation works well on paper but might be problematic when presented in the auditorium. Do not use too much text in your presentation, use figures and schemes instead, for figures please use fonts that are large enough. Your presentation should explain what the audience can see on the slides and how do you interpret your data. Please add citations directly to each slide.

After your presentation, your supervisor and thesis reviewer will both read their evaluation reports. You should prepare extra slides for reviewers' comments and use them when discussing points raised by the reviewer. As the last point, the discussion will be opened for the committee members and you can expect additional questions focusing on the essential aspects of your thesis.

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