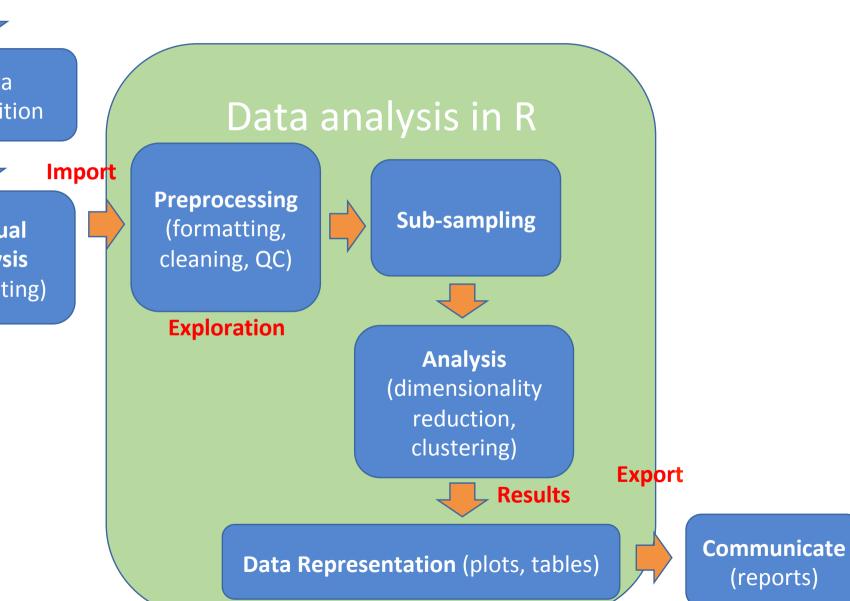
Generate HTML or PDF reports

Planning and Preparation Data acquisition Manual analysis (QC, gating)

Simplified workflow presented in day 1 and 2



If a data analysis project involves many steps and various plots, one easy and practical way to bundle and organize all steps of analysis together is to use R markdown files to generate PDF or html reports.

Document format that **combines R (or other) code with Markdown**, a lightweight markup language.

Integrate text, code, and the output of code (such as tables and figures) in a single document.

Create:

- Documents: HTML, PDF, Word
- Interactive documents: html, shiny apps
- Presentations
- eBooks
- Websites
- Package vignettes



Key components:

- Markdown: format text using plain text characters. Use Markdown to create headers, lists, links, and other formatting elements.
- R Code Chunks: include executable R code chunks. These chunks are enclosed by three backticks (```) and specified with the {r} tag. When generating (i.e. "knitting") the document, these code chunks are executed, and the results are inserted into the final document. Code chunks can be hidden or shown in the final document
- Output Formats: supports various formats, including HTML and PDF. The
 desired output format is specified in the document's metadata. When you
 "knit" the document, R Markdown generates the final document in the
 specified format.
- YAML Metadata: The metadata at the beginning of the R Markdown document is typically written in YAML format. This metadata includes information such as the document title, author, date, and output format.

RStudio facilitates the creation of R Markdown documents thanks to the rmarkdown, knitr and pander packages. The "scripts" now have .Rmd extension.

https://cran.r-project.org/web/packages/rmarkdown/index.html

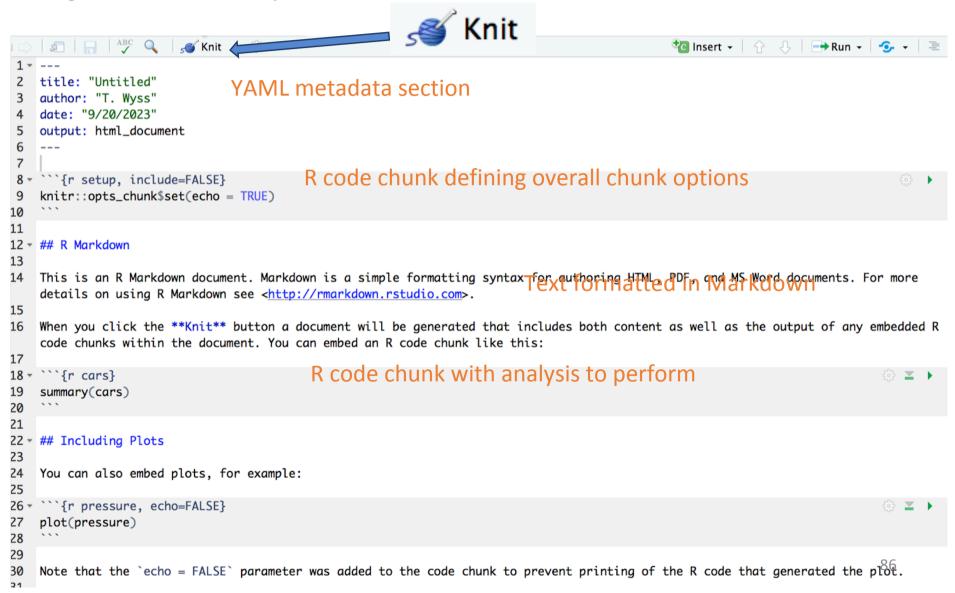
https://cran.r-project.org/web/packages/knitr/index.html

Create a .Rmd template within RStudio:

File > New File > R Markdown:

```
⇒ 🔊 📄 🕍 🔍 🧳 Knit 🔻 🛞 🕶
                                                                                             🛂 Insert 🗸 🔐 🕒 📑 Run 🗸 💁 🔻 🗏
   title: "Untitled"
                               YAMI metadata section
    author: "T. Wyss"
    date: "9/20/2023"
    output: html_document
6
                                         R code chunk defining overall chunk options
    ```{r setup, include=FALSE}
 knitr::opts_chunk$set(echo = TRUE)
10
11
12
 ## R Markdown
13
 This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML PDF, and MS Word documents. For more
 details on using R Markdown see http://rmarkdown.rstudio.com.
15
 When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R
 code chunks within the document. You can embed an R code chunk like this:
17
 R code chunk with analysis to perform
    ```{r cars}
18
    summary(cars)
20
21
22 - ## Including Plots
23
24
    You can also embed plots, for example:
25
    ```{r pressure, echo=FALSE}
 ∰ ¥
 plot(pressure)
28
29
 Note that the `echo = FALSE` parameter was added to the code chunk to prevent printing of the R code that generated the plot.
21
```

Knit: generate the final report



Generate a new file with .html extension saved by default in the same folder as where the .Rmd file is located.

### Untitled

T. Wyss 9/20/2023

#### R Markdown

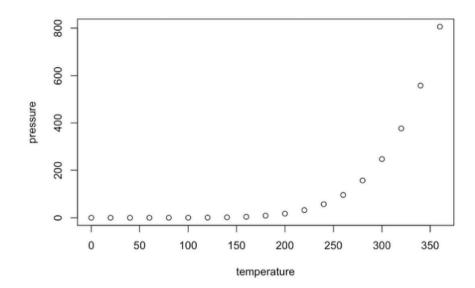
This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com.

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```
speed dist
Min. : 4.0 Min. : 2.00
1st Qu.:12.0 1st Qu.: 26.00
Median :15.0 Median : 36.00
Mean :15.4 Mean : 42.98
3rd Qu.:19.0 3rd Qu.: 56.00
Max. :25.0 Max. :120.00
```

### **Including Plots**

You can also embed plots, for example:



Note that the echo = FALSE parameter was added to the code chunk to prevent printing of the R code that generated the plot.

## YAML metadata section

```
1 ---
2 title: "R Markdown Example"
3 output: html_document
4 date: '2023-09-19'
5 ---
```

- This section must begin with a line containing just three dashes -- and must close with a line containing just three dashes ---
- Parameters come in key:value pairs
- The key:value pairs are separated by colons (not equals signs!)
- The order of the parameters (if not indented) does not matter
- Basic parameters are pre-selected, but we can change them (for example, if we wish a "pdf\_document" instead of "html\_document")

### Customize the YAML metadata section

```
1 - ---
 title: "Flow cytometry data analysis"
 author: "Data analysis: tania.wysslozanohoyos@unil.ch"
 date: "Analysis started on 01/11/2023"
 5
 output:
 HTML output format
 html_document:
 6
 Include table of content
 toc: true
 Up to which header level to include in the table of content
 8
 toc_depth: 3
 number_sections: true Whether to number the sections in the document according to header level
 9
 toc_float:
10
 Whether to create a table of content that stays on the side when scrolling down
11
 collapsed: true
 the html document
12
 code_folding: hide
 Whether to show the code chunks as collapsible buttons
13 - ---
...
 38 -
 39
 40 - # Comparison of PP-specific LECs to lymph node LECs
 41
 Single cells were initially OC'ed and filtered (see report
 17_Silvia_scRNAseq_072022_1.html), and clustered, followed by differential
 gene expression analysis (see report 17_Silvia_scRNAseq_072022_2.html).
208
 ## Correlation of the transcriptomes of PP/gut LECs with the ones of LN LECs
209 -
210
 Next, the gene expresssion of each cell was downloaded from GEO, accession
211
 [GSE145121](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE145121), and
 re-processed (see report 17_Silvia_scRNAseq_072022_GSE145112.html). The
 89
 authors did not provide LEC cell type annotations, so we generated clusters
 for their data, and used the cluster ID of the Xiang et al LECs that we
```

### Customize the YAML metadata section:

# Floating Table Of Content (TOC) with numbered sections Code folding buttons

### 1 Comparison of PP-specific LECs to lymph node LECs

- 1.1 Correlation of the transcriptomes of PP/gut LECs with the ones of LN LECs
- 1.2 Combined UMAP of Silvia's LECs with the LN LECs of Xiang et al
- 2 Stratification of general (gut+PP) LECs according to Agp1 and Ccl21a

# 1 Comparison of PP-specific LECs to lymph node LECs

Single cells were initially QC'ed and filtered (see report 17\_Silvia\_scRNAseq\_072022\_1.html), and clustered, followed by differential gene expression analysis (see report 17\_Silvia\_scRNAseq\_072022\_2.html).

Data was analyzed with R version 4.1.2 (2021-11-01). Most analysis steps were performed using the Seurat package for R (v4.3.0).

Code

A single-cell transcriptomic analysis of the mouse LN lymphatic vasculature is provided by Xiang et al 2020. First, a list of markers from Figure 8 was used to explore their expression level in the PP/Gut LECs.

As a reminder, these are the general (gut + PP) LECs and the PP-specific LECs sub-clustered:

Code

### Text

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see <a href="http://rmarkdown.rstudio.com">http://rmarkdown.rstudio.com</a>.

When you click the \*\*Knit\*\* button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

- Free text (like in a word document)
- New lines: enter two spaces\*at the end of the previous line and then hit return.

### Cases:

- Underscores (\_text\_) or single asterisk (\*text\*) to italicise
- Double asterisks (\*\*text\*\*) for bold text
- Back-ticks (`code`) to display text as code

# Titles and headers

Different header levels are established with different numbers of hash symbols at the start of a new line.

```
First-level heading / title

Second level heading

Third-level heading
```

This is different than in a code chunk, in which hashes are used for commenting!

# Bullets and numbering

Include a space between the asterisk and your bullet text Finish the previous sentence, enter two spaces, hit return twice, and then start the bullets

Use asterisks (\*) to create a bullet list.

# Bullet 1 (followed by two spaces and Enter/Return)
# Bullet 2 (followed by two spaces and Enter/Return)
# Sub-bullet 1 (followed by two spaces and Enter/Return)
# Sub-bullet 2 (followed by two spaces and Enter/Return)
# Sub-bullet 2 (followed by two spaces and Enter/Return)

Sub-bullets work the same way but are indented (tab)

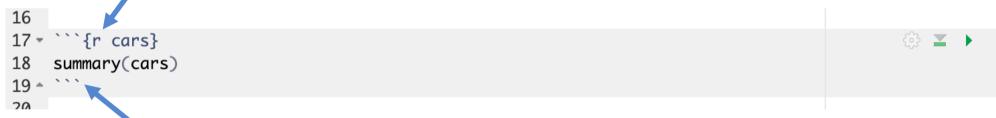
After each bullet enter two spaces and then hit return

# Code chunks

- This is where you load packages, import data, and perform the actual data processing, analysis and visualization.
- There may be many code chunks

Each chunk is opened with a line that starts with three back-ticks, and curly brackets that contain parameters for the chunk ({ }).

Here, there is a name for the chunk which is cars



The chunk ends with three more back-ticks

### Create a new chunk:

- type the starting and ending lines yourself, or
- use the keyboard shortcut "Ctrl + Alt + i" (or Cmd + Shift + r in Mac), or
- click the green 'insert a new code chunk' icon



# Code chunk options

- A chunk header must be written in one line
- Always start with 'r'
- After the 'r' you can **optionally** write a chunk "name" (**which has to be unique** across the whole Rmd file).
- Other options are written as tag=value, such as:
  - eval = FALSE to not run the R code
  - echo = FALSE to not print the chunk's R source code
  - warning = FALSE to not print warnings produced by the R code
  - message = FALSE to not print any messages produced by the R code
  - include = either TRUE/FALSE whether to include chunk outputs (e.g. plots)
    or not
  - fig.align = "center" adjust how a figure is aligned across the page

More about chunk options in <a href="https://yihui.org/knitr/options/">https://yihui.org/knitr/options/</a>

# Global options

- Applied to all chunks present in the Rmd file
- Set up within the very first R code chunk using the opts\_chunk
- For instance, below, the global options set with opts\_chunk\$set() are:
  - echo=TRUE : print the R source code
  - include=TRUE: include
  - warning=FALSE: do not print warning messages
  - message=FALSE: do not print any message (eg verbose output of some functions)
- opts\_knit\$set() can be used to set the folder where to save plots or look for data.

It changes the working director for all code chunks

# In-text R code

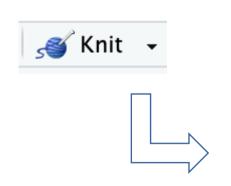
- Short R code commands within back-ticks
- Within the back-ticks, begin the code with "r" and a space, so RStudio knows to evaluate the text within the back-ticks as R code
- Example:

```
16
 `{r cars}
 summary(cars)
19 -
20
 The dimension of the cars dataset is `r dim(cars)`
22
 dist
 speed
 Median:15.0
 Median : 36.00
 :15.4
 : 42.98
 Mean
 Mean
 3rd Qu.:19.0
 3rd Qu.: 56.00
 Max.
 :25.0
 Max.
 :120.00
```

The dimension of the cars dataset is 50, 2

# Producing the document

- Knitting a file is how we generate the output file from the R markdown file
- RStudio will show you the progress within an 'R Markdown' tab near your R console
- The document will automatically open when complete
- The document will be saved in the same folder as your R markdown script, and with the same file name



### R Markdown Example

2023-09-19

#### R Markdown

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1st Qu.:12.0 1st Qu.: 26.00
Median :15.0 Median : 36.00
Mean : 15.4 Mean : 42.98
3rd Qu.:19.0 3rd Qu.: 56.00
Max. :25.0 Max. :120.00
```

The dimension of the cars dataset is 50, 2

# Let's practice – 11 : Rmarkdown

In this exercise we will create an .Rmd file, and modify it to include the code for analysis and results of a small pipeline with dimensional reduction plots of the **FR\_FCM\_Z3WR** data.

Create a new .Rmd in which you will:

- Modify the YAML metadata section to include a table of content with numbered sections and code folding buttons
- 2) Write a paragraph with a level-1 header, that describes the content of the document
- 3) Create code chunks that will run and print the output of the following analysis:
- 4) Knit the document to an html report and admire it in your web-browser!

# Please write wish-list for day 5 in the google doc

# Thank you for your attention!

https://agora-cancer.ch/scientific-platforms/translational-data-science-facility/

Any questions? Contact us! tds-facility@sib.swiss