

MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES CODING WORKSHOP

Presents

Data visualization using antiviral drug-repurposing
results from CLUE

INSTRUCTED BY
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INFORMATION FOR PARTICIPANTS

**All workshops are being recorded and posted to the
[MMID Coding Workshop - YouTube](#)**

Question and Answer period will not be recorded.

Last 3 Workshops...

- Introduction to R (Rstudio, R scripts, R markdown)
 - Grace E. Seo
- Tidy data in R (Transforming and combining data)
 - Molly Pratt
- Visualizing data using R (ggplot2)
 - Samantha Lee

LEARNING OBJECTIVES

- 1. Understand what drug repurposing analyses are and how to use it as a tool for host-pathogen data***
- 2. Create a basic plot in R to visualize CLUE results***
- 3. Refine R plot components for specific colours, shapes and sizes***
- 4. Combine multiple plots in R and output as a file to the desktop***
- 5. Learn how to produce a similar graph in Tableau***

LEARNING OBJECTIVES

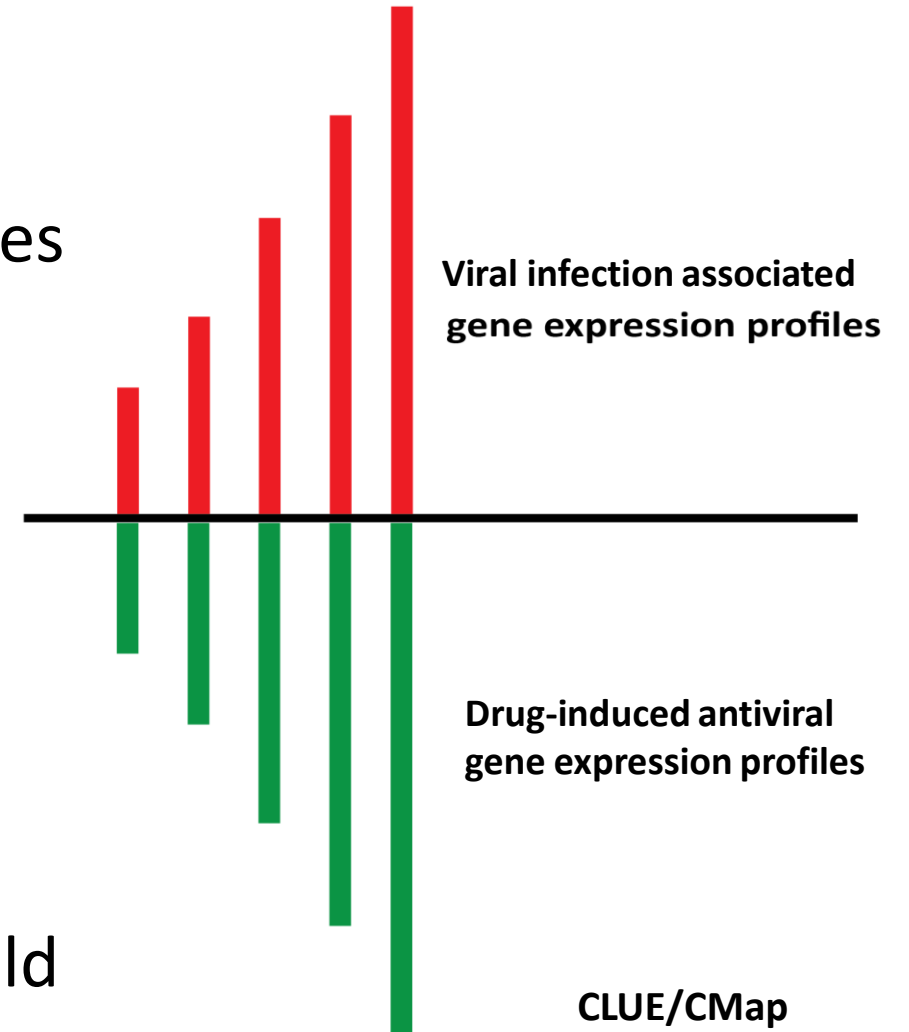
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Drug Repurposing Analyses

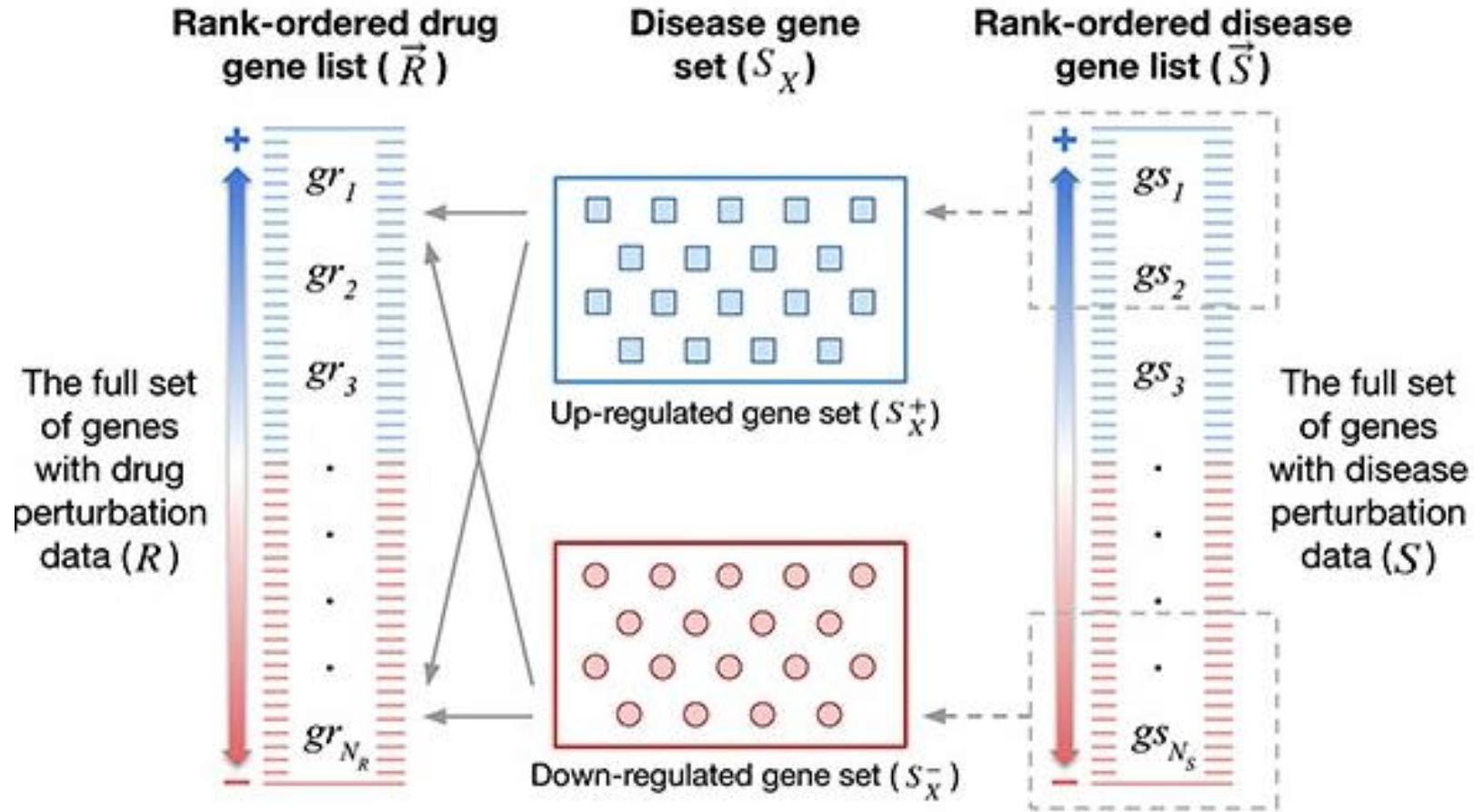
Purpose: Identify potential small molecules that have the opposing gene expression profiles to the profile that you have used as input

Proof: inclusion of genetic evidence in drug development pipelines has been shown to increase success by at least two-fold

Gene expression: is determined by z-score, fold change, etc



A. Gene expression signatures



[Front Immunol.](#) 2019; 10: 60.

Published online 2019 Jan 29. doi: [10.3389/fimmu.2019.00060](https://doi.org/10.3389/fimmu.2019.00060)

PMCID: PMC6361841

PMID: [30761132](https://pubmed.ncbi.nlm.nih.gov/30761132/)

Repurposing of Drugs as Novel Influenza Inhibitors From Clinical Gene Expression Infection Signatures

[Andrés Pizzorno](#),^{1,2,†} [Olivier Terrier](#),^{1,*†} [Claire Nicolas de Lamballerie](#),^{1,3} [Thomas Julien](#),^{1,4} [Blandine Padey](#),^{1,4} [Aurélien Traversier](#),¹ [Magali Roche](#),³ [Marie-Eve Hamelin](#),² [Chantal Rhéaume](#),² [Séverine Croze](#),⁵ [Vanessa Escuret](#),^{1,6} [Julien Poissy](#),⁷ [Bruno Lina](#),^{1,6} [Catherine Legras-Lachuer](#),^{3,8} [Julien Textoris](#),^{9,10} [S](#) and [Manuel Rosa-Calatrava](#)^{1,4,*}

Review Article | [Published: 04 March 2019](#)

Drug repurposing for antimicrobial discovery

[Maya A. Farha](#) & [Eric D. Brown](#) 

[Nature Microbiology](#) **4**, 565–577 (2019) | [Cite this article](#)

5273 Accesses | **83** Citations | **578** Altmetric | [Metrics](#)

Research | [Open Access](#) | [Published: 24 September 2021](#)

Clinical connectivity map for drug repurposing: using laboratory results to bridge drugs and diseases

[Qianlong Wen](#), [Ruoqi Liu](#) & [Ping Zhang](#) 

[BMC Medical Informatics and Decision Making](#) **21**, Article number: 263 (2021) | [Cite this article](#)

897 Accesses | **1** Altmetric | [Metrics](#)

CLUE

- Cloud-based infrastructure
- Houses the CMap data
- Gene expression information for over 30,000 perturbagens



History

Access past Queries you have run on the clue system



Command

Rapidly look up up perturbagens of interest in the Touchstone (L1000) and Proteomics (GCP, P100) databases.



Touchstone

Explore connectivities between signatures from ~3,000 drugs and genetic loss/gain of function of ~2,000 genes that make up the CMap touchstone (reference) database



Data Library

Explore datasets available through clue.io including L1000 cohorts and related perturbational information



Cell App

Explore our collection of ~3000 cell lines and their annotation



Repurposing

Explore our collection of ~5000 drugs and tool compounds to find potential drug repurposing opportunities to improve disease treatments



Morpheus

Explore, analyze, and annotate heat maps. Choose an existing dataset or upload your own data (for example, gene expression or connectivity scores)



Proteomics Query

Query your own P100 or GCP proteomic data against a reference database



ListMaker

Make lists of genes, compounds, cell lines, and pharmacologic classes then apply those lists site-wide to customize your analysis.



Metadata Browser

CLUE Metadata browser

Four types of CMap Queries

1. Gene expression

- Up and down regulated genes

2. Cell viability

- Up and down regulated cell sets

3. Proteomics – Phosphorylation

- GCT file

4. Proteomics – Histone Modifications

- GCT file

Query

Query CMap for reference perturbation signatures most similar (or dissimilar) to your samples.

Note that choosing 'Latest' from the query parameters section below, will run the query against our [beta dataset](#) released on (Dec 17, 2020)

1) Name your query

Please note that names must contain only alphanumeric characters. Any non-alphanumeric characters will be stripped.

2) Query parameters

3) Load a collection of Entrez Gene IDs from Listmaker for up-regulated gene sets (and optionally a collection for down-regulated gene sets). At any time you may choose an [example](#) to fill in the boxes for the individual query.

UP-regulated genes

Enter 10-150 genes for optimal results.

DOWN-regulated genes (optional)

Please note that 150 is a technical limit.

Enter gene IDs or Entrez gene symbols by pasting a list or dragging a plain text file

- Invalid gene *Not valid HUGO symbol or Entrez ID, not used in query*
- Valid gene *Valid HUGO symbol or Entrez ID and part of BING space, used in query*
- Valid but not used in query *Valid HUGO symbol or Entrez ID not part of BING space, not used in query*

More information can be found in [this Connectopedia article](#)

4) Review and submit. Only valid genes will be used in your query.

 OPEN ACCESS  PEER-REVIEWED

RESEARCH ARTICLE

Gene Expression Signature-Based Screening Identifies New Broadly Effective Influenza A Antivirals

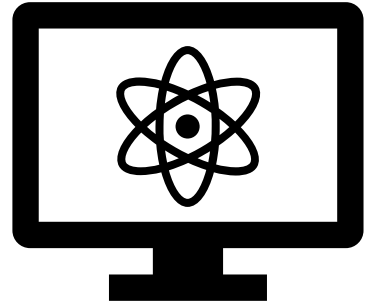
Laurence Josset , Julien Textoris, Béatrice Lloriod, Olivier Ferraris, Vincent Moules, Bruno Lina, Catherine N'Guyen, Jean-Jacques Diaz, Manuel Rosa-Calatrava 

Published: October 4, 2010 • <https://doi.org/10.1371/journal.pone.0013169>

Method: Used viral-infected human cells to obtain a list of differently expressed genes (via fold change)

Hypothesis: Perturbagens in CMap with an opposite gene expression to input may be candidate antivirals

Drug Repurposing Files to Visualize



Josset *et al.*, in supplementary table 5 have a list of up and down regulated genes that was input into the CLUE Query box

- As this experiment was done in a previous version of CLUE, two other down regulated genes had to be put in as requirements have changed for input

I am using an arbitrary cut off: **0.77**

****This is just for learning purposes, not to replicate this study's results****



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Query CMap for reference perturbation signatures most similar (or dissimilar) to your samples.

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UP-regulated genes

Enter 10-150 genes for optimal results.

- ☒ ETV3
- ☒ ABTB2
- ☒ SLC2A2
- ☒ PPFIBP1
- ☒ ICAM1
- ☒ MEF2D
- ☒ HYAL4
- ☒ LPAR1

DOWN-regulated genes (optional)

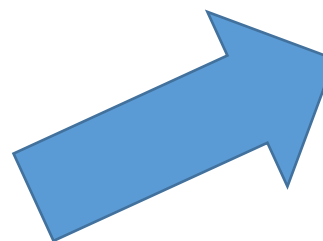
Please note that 150 is a technical limit.

- ☒ FOSB
- ☒ KPNA6
- ☒ HSPG2
- ☒ CAPN1
- ☒ PPP1R14D
- ☒ PNPLA6
- ☒ DNMT1
- ☒ NOP16

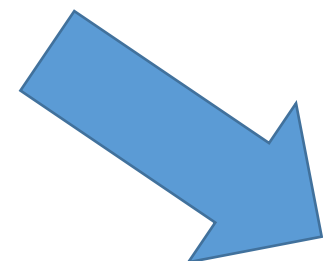
- ☒ Invalid gene *Not valid HUGO symbol or Entrez ID, not used in query*
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GSEA Results

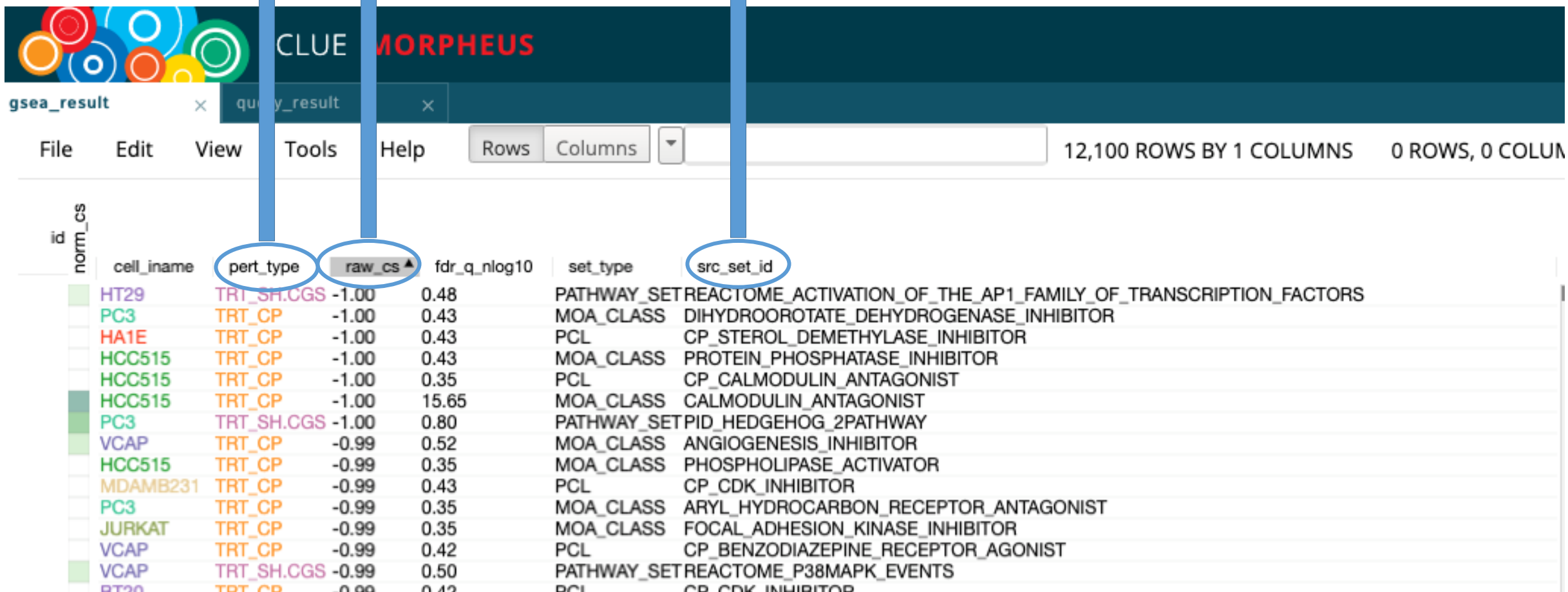


Query Results

Perturbagen type

Median connectivity score

Mechanism of action



The image shows the CLUE MORPHEUS software interface. At the top, there is a header bar with the logo (colored circles) and the text "CLUE MORPHEUS". Below the header, there are tabs for "gsea_result" and "query_result". A menu bar includes "File", "Edit", "View", "Tools", and "Help". To the right of the menu, there are buttons for "Rows" and "Columns", and a status bar indicating "12,100 ROWS BY 1 COLUMNS" and "0 ROWS, 0 COLUMNS". The main area displays a table with the following columns: "id", "norm_cs", "cell_name", "pert_type", "raw_cs", "fdr_q_nlog10", "set_type", and "src_set_id". Three blue arrows point from labels above to specific columns: "Perturbagen type" points to "pert_type", "Median connectivity score" points to "raw_cs", and "Mechanism of action" points to "src_set_id". The table contains data for various cell lines and perturbations.

	id	norm_cs	cell_name	pert_type	raw_cs	fdr_q_nlog10	set_type	src_set_id
	HT29		TRT_SH.CGS	-1.00	0.48		PATHWAY_SET	REACTOME_ACTIVATION_OF_THE_AP1_FAMILY_OF_TRANSCRIPTION_FACTORS
	PC3		TRT_CP	-1.00	0.43		MOA_CLASS	DIHYDROOROTATE_DEHYDROGENASE_INHIBITOR
	HA1E		TRT_CP	-1.00	0.43		PCL	CP_STEROL_DEMETHYLASE_INHIBITOR
	HCC515		TRT_CP	-1.00	0.43		MOA_CLASS	PROTEIN_PHOSPHATASE_INHIBITOR
	HCC515		TRT_CP	-1.00	0.35		PCL	CP_CALMODULIN_ANTAGONIST
	HCC515		TRT_CP	-1.00	15.65		MOA_CLASS	CALMODULIN_ANTAGONIST
	PC3		TRT_SH.CGS	-1.00	0.80		PATHWAY_SET	PID_HEDGEHOG_2PATHWAY
	VCAP		TRT_CP	-0.99	0.52		MOA_CLASS	ANGIOGENESIS_INHIBITOR
	HCC515		TRT_CP	-0.99	0.35		MOA_CLASS	PHOSPHOLIPASE_ACTIVATOR
	MDAMB231		TRT_CP	-0.99	0.43		PCL	CP_CDK_INHIBITOR
	PC3		TRT_CP	-0.99	0.35		MOA_CLASS	ARYL_HYDROCARBON_RECEPTOR_ANTAGONIST
	JURKAT		TRT_CP	-0.99	0.35		MOA_CLASS	FOCAL_ADHESION_KINASE_INHIBITOR
	VCAP		TRT_CP	-0.99	0.42		PCL	CP_BENZODIAZEPINE_RECEPTOR_AгонIST
	VCAP		TRT_SH.CGS	-0.99	0.50		PATHWAY_SET	REACTOME_P38MAPK_EVENTS
	BT20		TRT_CP	-0.99	0.43		PCL	CP_CDK_INHIBITOR

Perturbagen

Perturbagen type

Mechanism of action

Median connectivity score

<

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Explanation of Libraries

dplyr: A Grammar of Data Manipulation

- Core package of the tidyverse

snakecase: Convert Strings into any Case

- A string is an array of 1 or more characters (ex: words)

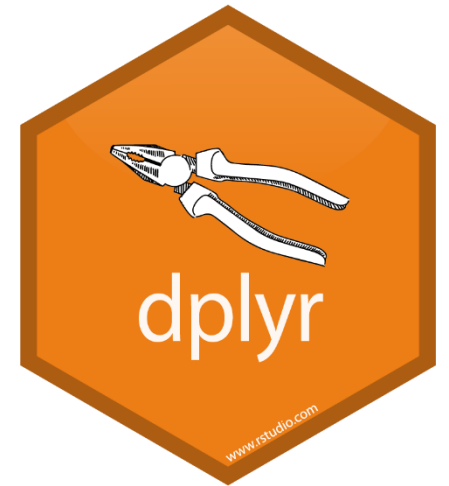
ggplot2: Main data visualization package in R

plotly: Helps make interactive figures/graphs in R

cowplot: Add on to ggplot2

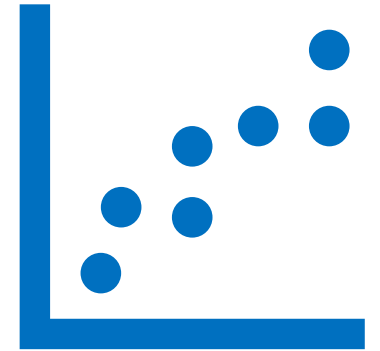
- To make figures publication ready
- Combine different types of plots

readxl: Reads in Excel files (.xls, .xlsx)



Purpose of making a scatterplot

- To show different types of perturbagens
- To show median connectivity score range
- To show in blue/red colours, which perturbagens show a highly dissimilar expression profile and a highly similar expression profile



Going over to RStudio...

Loading in the Libraries

```
#Load in the libraries
library(dplyr) #install.packages("dplyr")
library(readxl) #install.packages("readxl")
library(snakecase) #install.packages("snakecase")
library(ggplot2) #install.packages("ggplot2")
library(cowplot) #install.packages("cowplot")
library(plotly) #install.packages("plotly")
```

Make a scatterplot of sheet 1 results

Step 1. Read in Excel file and see what data looks like

```
#Step 1. Read in Excel File
clue_results<-read_excel("/Users/msarvis/Desktop/MMID/mmid_workshop.xls", sheet=1) %>%
  rename_with(to_snake_case)

#See what it looks like
glimpse(clue_results)
```

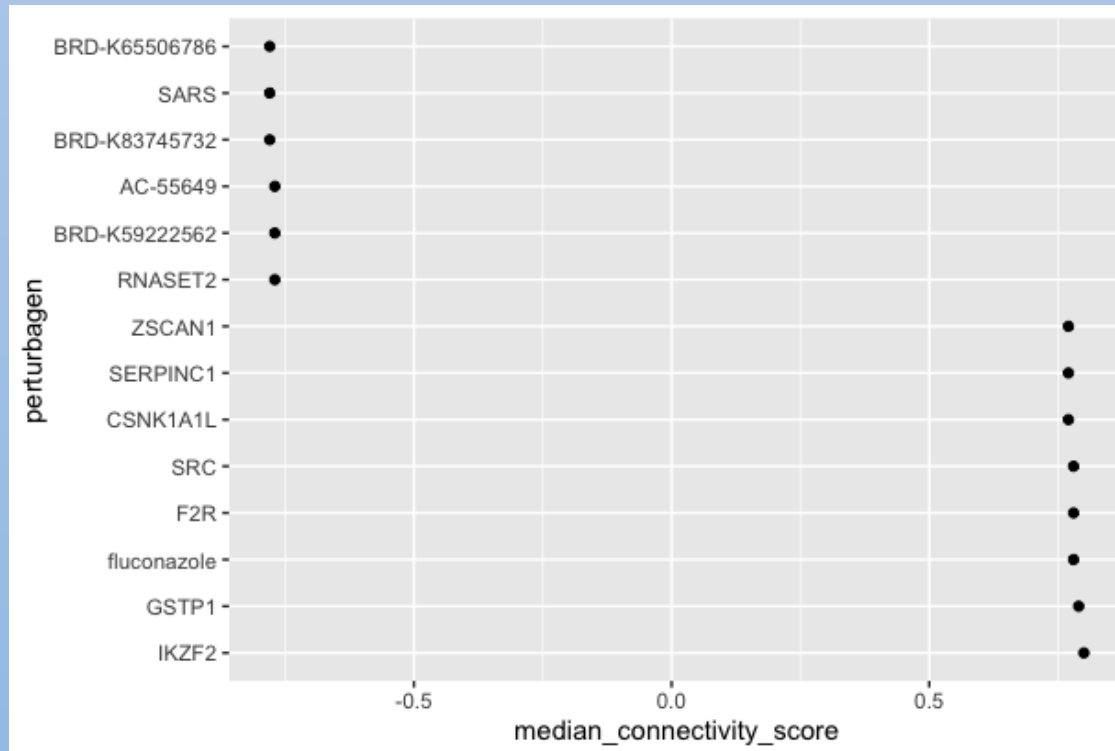
Step 2. Change the perturbagen names into a factor and give it levels

```
clue_results$perturbagen <- factor(clue_results$perturbagen, levels = clue_results$perturbagen)
```

Step 3. Create a basic scatterplot with ggplot2

```
#Step 2. Create a basic plot
basic_plot<-ggplot(clue_results, aes(x=median_connectivity_score, y=perturbagen)) +
  geom_point()

#View the plot
basic_plot
```



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Step 4. Change the colour of the perturbagens based on their connectivity score

```
colours <- ifelse(clue_results$median_connectivity_score < 0, "blue", "red")
```

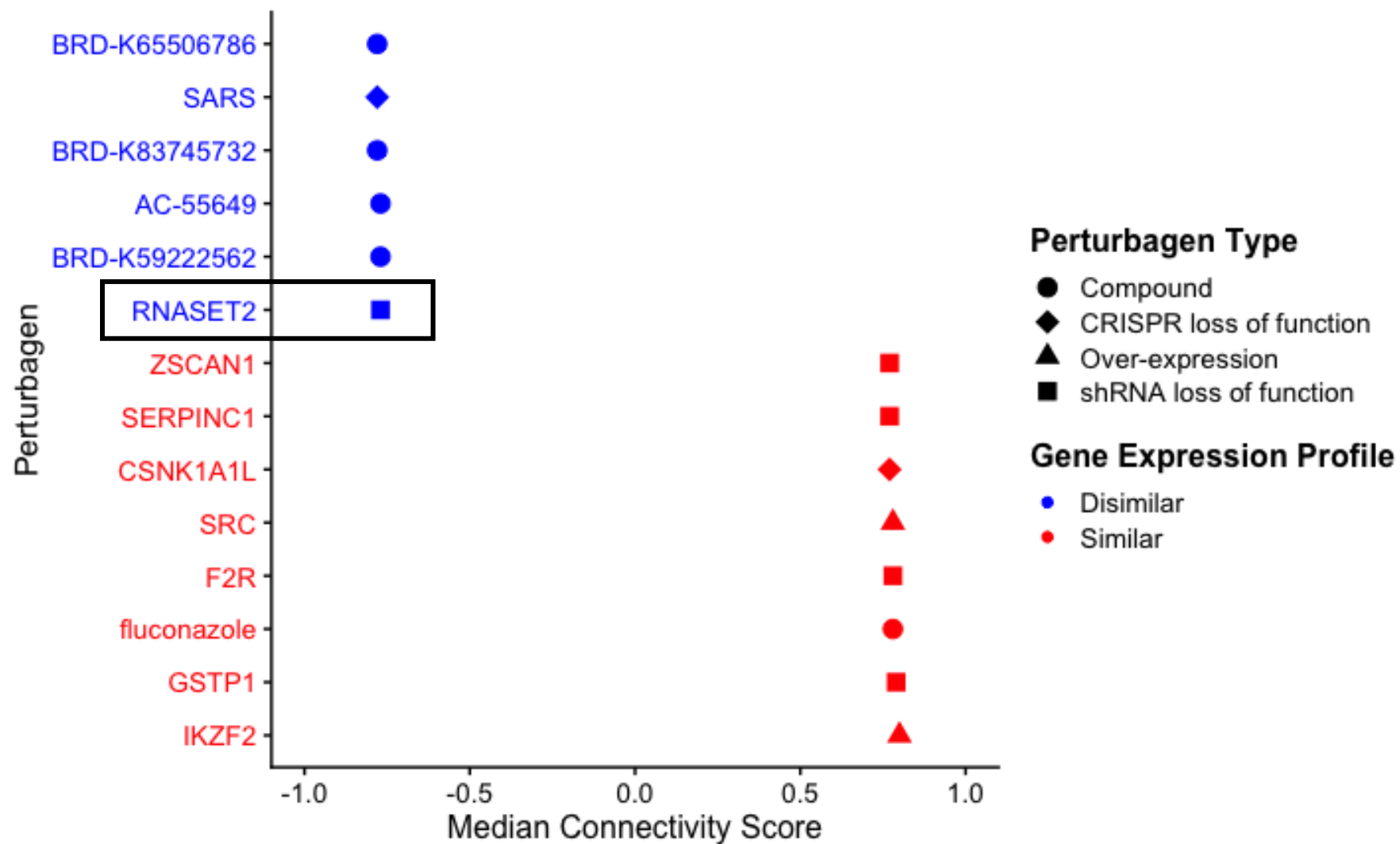
Step 5. Adding in details for colour, shape and sizes

```
drug_repurposing_plot<-basic_plot + geom_point(aes(colour=gene_expression_profile, shape= perturbagen_type, size= perturbagen_type)) +  
  theme_cowplot(12) + ggtitle("Top Antiviral Drug Repurposing Results")+  
  xlab("Median Connectivity Score") +ylab ("Perturbagen") + xlim(-1,1) +  
  theme(plot.title = element_text(size=20, hjust = 0.5), axis.text.y = element_text(hjust = 1, colour=colours),  
    legend.title = element_text(face = "bold")) +  
    scale_shape_manual(values= c(19,18,17,15))+  
    scale_color_manual(values = c("Disimilar" = "blue", "Similar" = "red")) +  
    scale_size_manual(values=c(3,4,3,3)) + guides(shape=guide_legend("Perturbagen Type")) +  
    guides(size=guide_legend("Perturbagen Type")) +  
    guides(color=guide_legend("Gene Expression Profile"))
```


```
ggplotly(drug_repurposing_plot)
```

***Bonus: See it interactively!**

Top Antiviral Drug Repurposing Results



What can we learn from the scatterplot?

frontiers
in Immunology

[Front Immunol.](#) 2020; 11: 1554. PMCID: PMC7438567
Published online 2020 Aug 13. doi: [10.3389/fimmu.2020.01554](https://doi.org/10.3389/fimmu.2020.01554) PMID: [32903619](https://pubmed.ncbi.nlm.nih.gov/32903619/)

RNase T2 in Inflammation and Cancer: Immunological and Biological Views

[Lei Wu](#),^{1,2,†} [Yanquan Xu](#),^{2,†} [Huakan Zhao](#),^{1,2,†} and [Yongsheng Li](#)^{1,2,*}

▶ [Author information](#) ▶ [Article notes](#) ▶ [Copyright and License information](#) [Disclaimer](#)



([10](#), [11](#)). Following tissue damage, RNase T2 is secreted and participates in **resistance against RNA viruses** or functions as an alarm signaling molecule to regulate the host immune response and contributes to tissue remodeling and repair ([12](#), [13](#)).

The **antiviral activities of RNase T2** have also been reported ([3](#), [62](#)). Elevated levels of extracellular RNase T2 expression resulted in increased resistance to Cucumovirus and Virgaviridae infection in plants ([62](#)). In

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Purpose of making a bar plot

- Compare between similar and dissimilar perturbagen types
 - How many perturbagens are in each category
 - Which perturbagen types are the most/least common
 - How the perturbagen types differ between expression profiles



Going over to RStudio...

Step 6. Create a bar plot to compare the different gene expression profiles

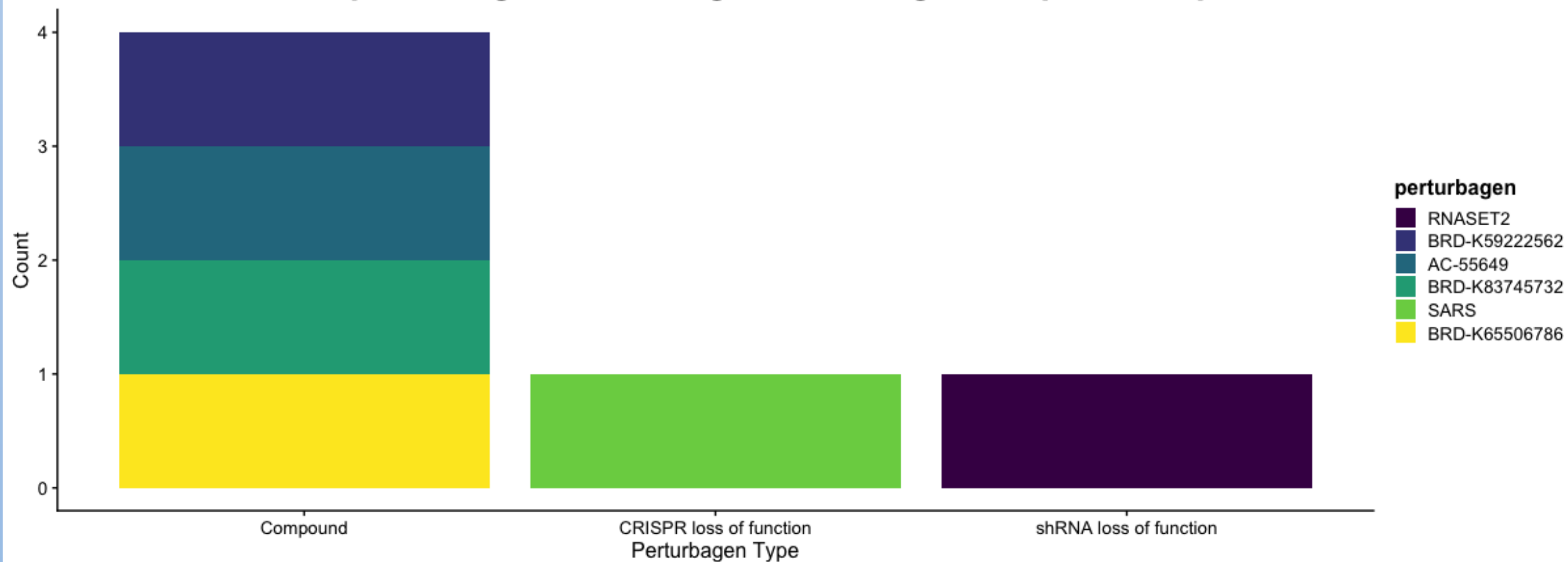
```
#a. Filter results to perturbagens inducing dissimilar (and similar) gene expression profiles (i.e. blue and red perturbagens)
disimilar_perturbagens<-clue_results %>% filter(median_connectivity_score<0)
similar_perturbagens<-clue_results %>% filter(median_connectivity_score>0)

#b. Make a basic bar graph
basic_bar_graph<-ggplot(disimilar_perturbagens, aes(x=perturbagen_type, fill=perturbagen)) +
  geom_bar()

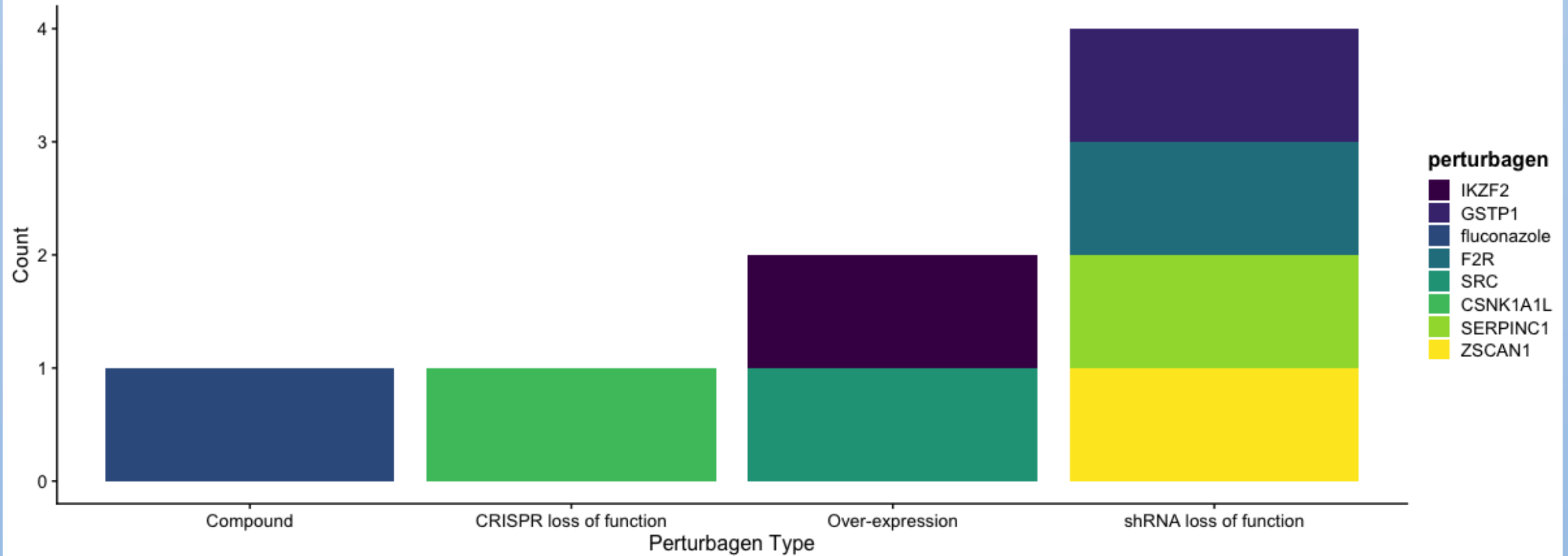
#c. Add in different elements you want to have
drug_repurposing_bar_plot_dissimilar<-basic_bar_graph + geom_bar() + theme_cowplot(12) +
  ggtitle("Breakdown of perturbagens inducing dissimilar gene expression profiles") +
  xlab("Perturbagen Type") + ylab ("Count") +
  theme (plot.title = element_text(size=20,hjust = 0.5),
        legend.title = element_text(face = "bold")) +
  scale_fill_viridis_d()

#Bonus: See it interactively
ggplotly(drug_repurposing_bar_plot_dissimilar)
```

Breakdown of perturbagens inducing dissimilar gene expression profiles



Breakdown of perturbagens inducing similar gene expression profiles



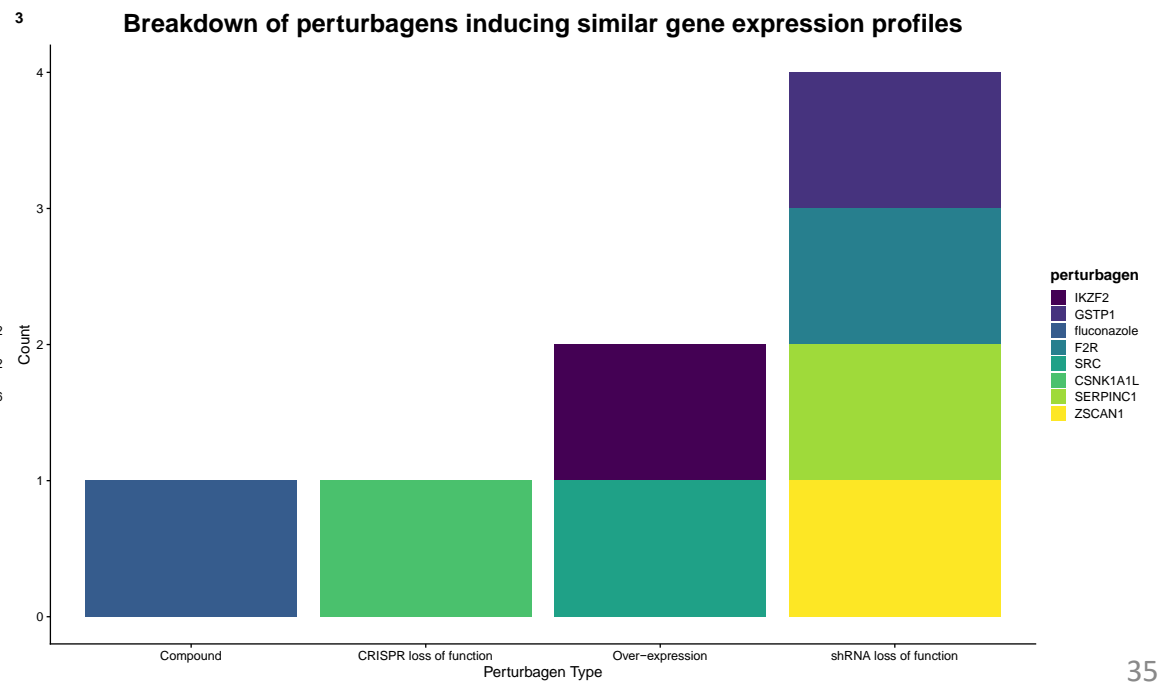
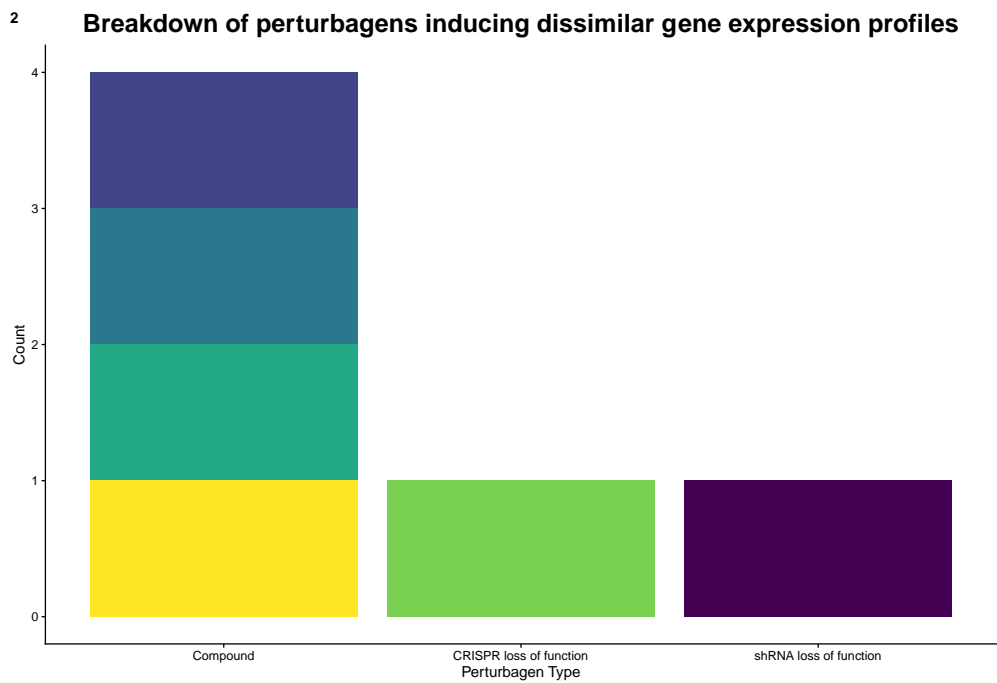
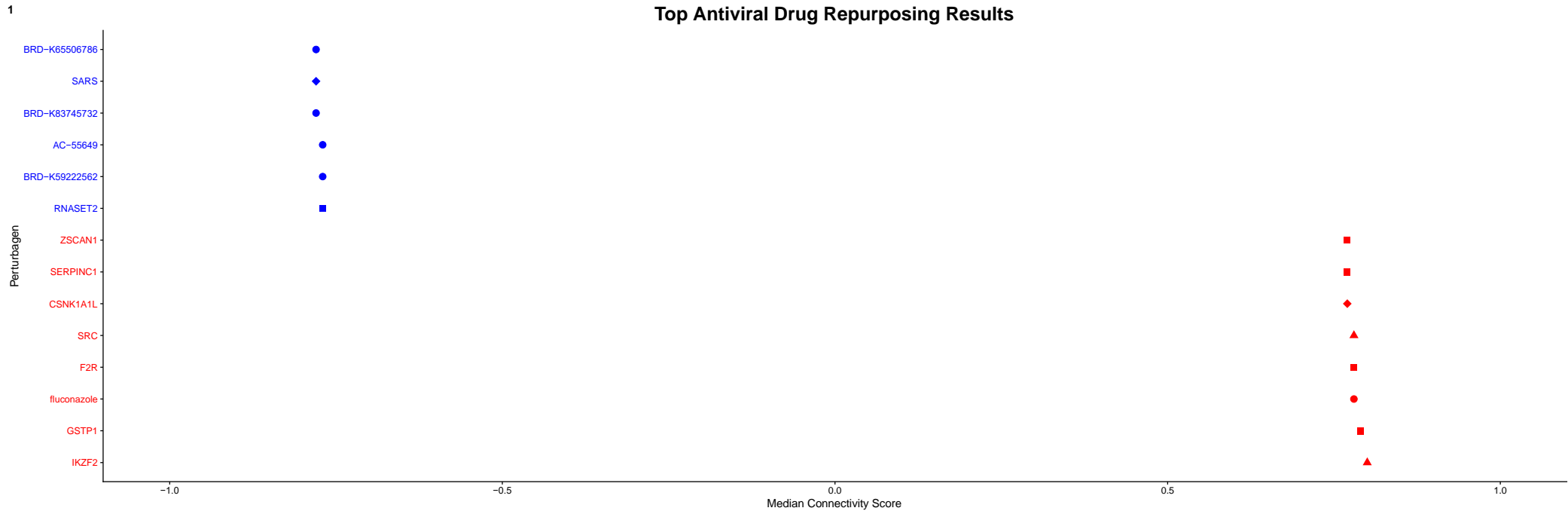
What can we learn from the bar plot?

- **Dissimilar** gene expression profile perturbagens
 - Most common: compound
 - Less perturbagens (n=6)
 - 3 types of perturbagens
- **Similar** gene expression profile perturbagens
 - Most common: shRNA loss of function
 - More perturbagens (n=8)
 - 4 types of perturbagens



Step 7. Combine the plots together and save to your computer desktop

```
bottom_row <- plot_grid(drug_repurposing_bar_plot_dissimilar, drug_repurposing_bar_plot_similar,  
                        labels = c('2', '3'), label_size = 12)  
plot_grid(drug_repurposing_plot, bottom_row,  
          labels = c('1', ''), label_size=12, ncol = 1)  
  
ggsave("/Users/msarvis/Desktop/MMID/drug_repurposing.pdf", height=15, width=25, units='in', dpi=300)
```



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RStudio (ggplot2)



- More control
- Can save to desktop
- Can do data analysis
- Can be interactive with Plotly or Shiny



- Need to code

Vs



Tableau



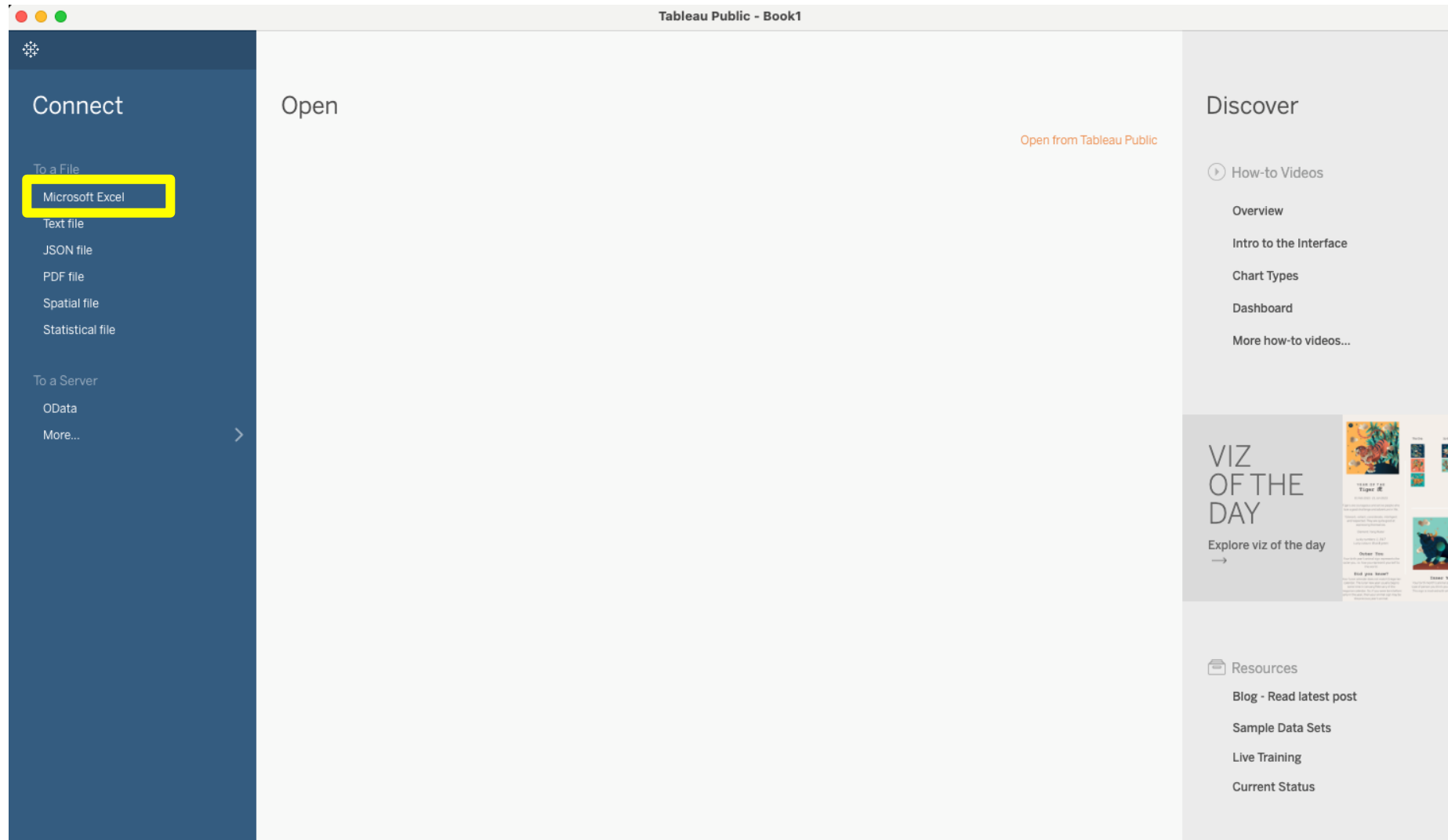
- Artistic freedom
- Dashboard is interactive
 - Less coding



- Less control
- Only saves to online website
- Limited capabilities with free version

Going over to Tableau...

1. Import your Excel file



<https://public.tableau.com/en-us/s/>

Enter an email and the app will install! Also make an account to save visualizations.

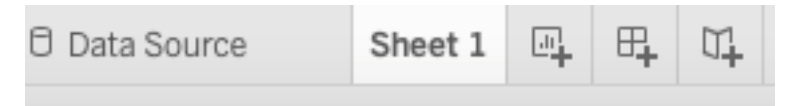
2. Put in your sheet and look at potential options

The screenshot shows the Tableau Public interface. On the left, the 'Connections' pane lists 'mimid_workshop' as a Microsoft Excel data source. Below it, the 'Sheets' pane shows 'mimid_workshop' and a 'New Union' option. The main workspace displays a message: 'Need more data? Drag tables here to relate them. [Learn more](#)'. At the bottom, a data table is shown with the following structure:

Type	Field Name	Physical T...	Rem...
Abc	Perturbagen	mimidwork...	Pertur...
#	Median connectivity score	mimidwork...	Media...
Abc	Filter type	mimidwork...	Filter t...
Abc	Perturbagen type	mimidwork...	Pertur...
Abc	Gene expression profile	mimidwork...	Gene ...

The data table below shows 14 rows of data with columns: Perturbagen, Median connectivity score, Filter type, Perturbagen type, and Gene expression profile.

Perturbagen	Median connectivity score	Filter type	Perturbagen type	Gene expression profile
IKZF2	0.80000	antiviral_article_results	Over-expression	Similar
GSTP1	0.79000	antiviral_article_results	shRNA loss of function	Similar
fluconazole	0.78000	antiviral_article_results	Compound	Similar
F2R	0.78000	antiviral_article_results	shRNA loss of function	Similar
SRC	0.78000	antiviral_article_results	Over-expression	Similar
CSNK1A1L	0.77000	antiviral_article_results	CRISPR loss of function	Similar
SERPINC1	0.77000	antiviral_article_results	shRNA loss of function	Similar
ZSCAN1	0.77000	antiviral_article_results	shRNA loss of function	Similar
RNASET2	-0.77000	antiviral_article_results	shRNA loss of function	Disimilar
BRD-K59222562	-0.77000	antiviral_article_results	Compound	Disimilar
AC-55649	-0.77000	antiviral_article_results	Compound	Disimilar



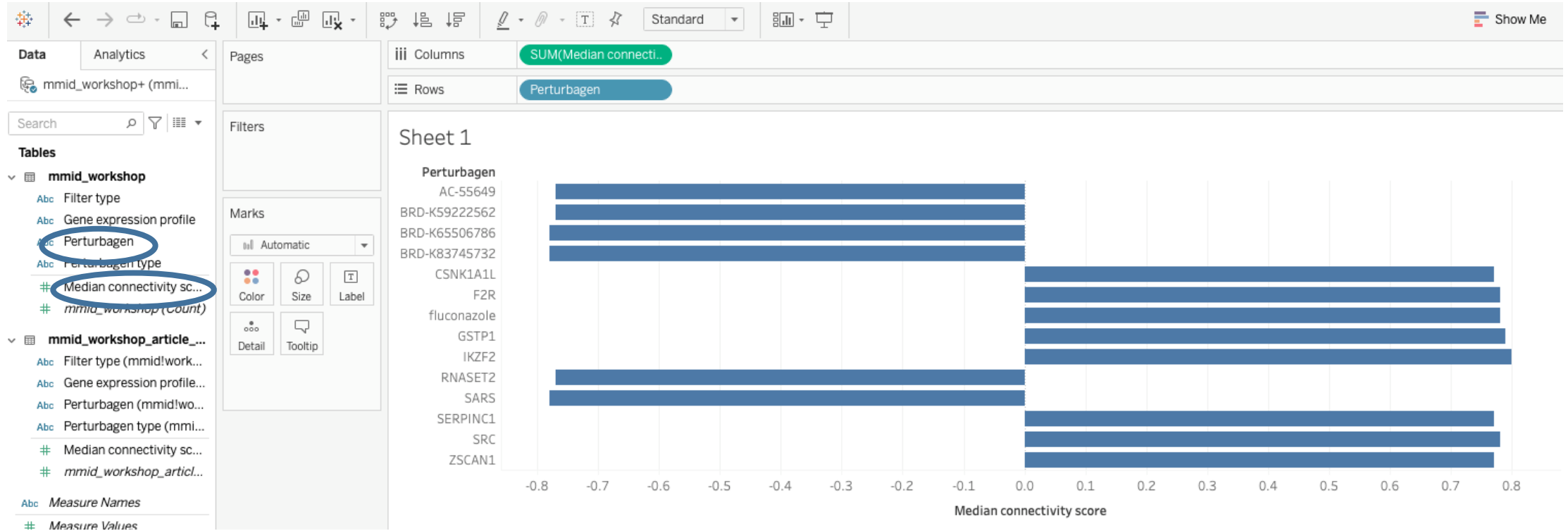
Where
you load
in data

Work on data
visualizations

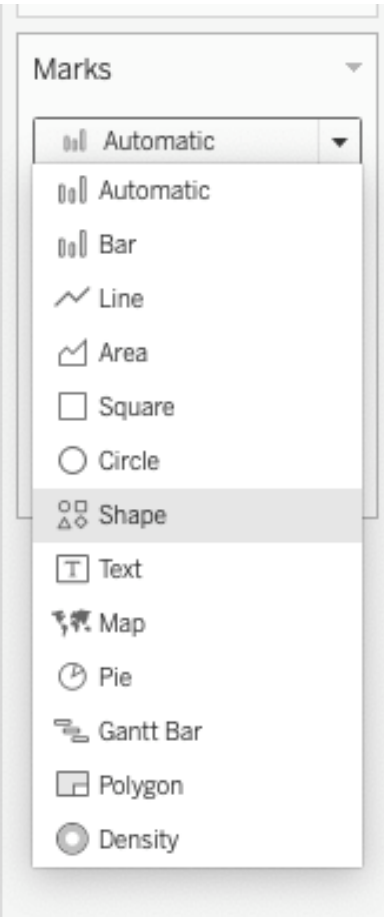
Combine all
visualizations
into a
dashboard

Combine
dashboards
into a
storyboard

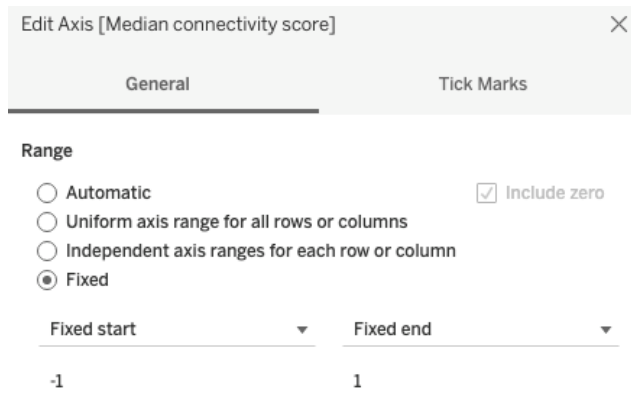
3. Create a scatterplot of results in Worksheet 1



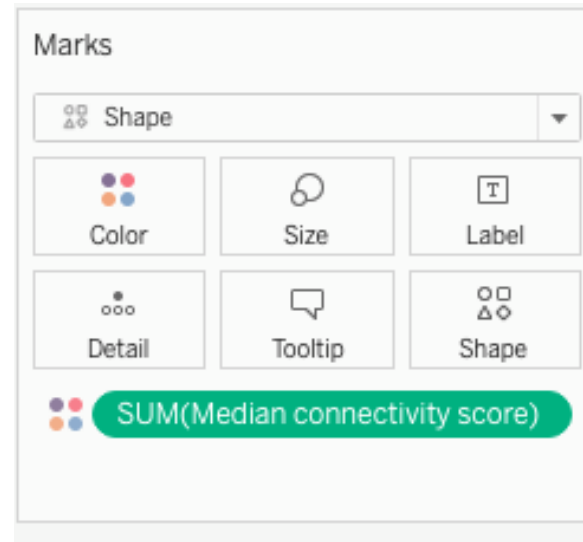
Add in colours, shapes, etc



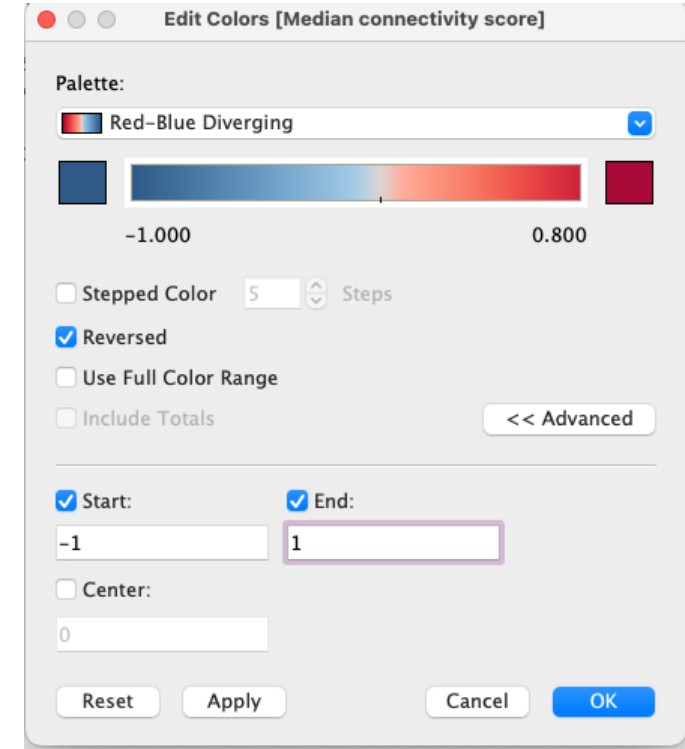
a. Choose graph type



b. Make x axis limits and rename axis



c. Make colours based on median connectivity score

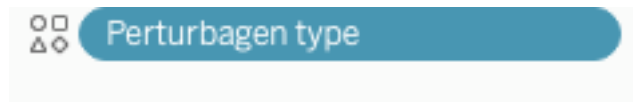


d. Make negative colours blue and positive colours red

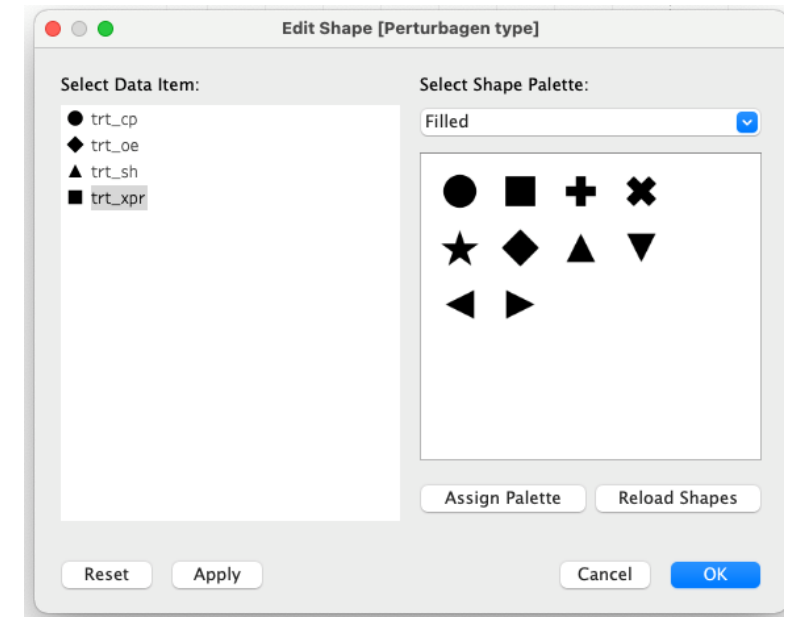
Add in colours, shapes, etc

Median connectivity score ★ ≡

e. Sort so that the connectivity scores are ordered as you like



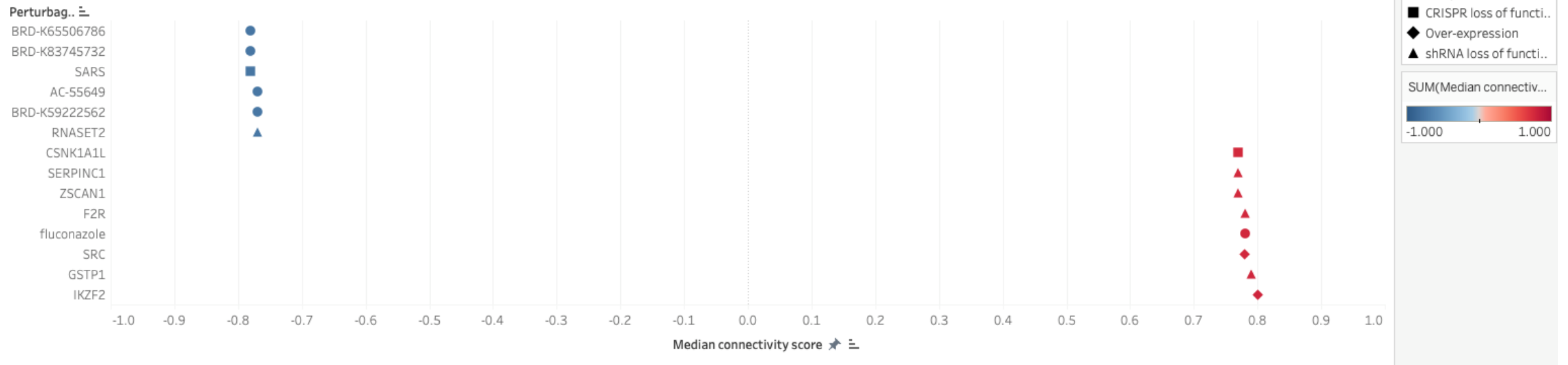
f. Make perturbagen type a specific shape



g. Select specific shapes for each perturbagen type

Final Scatterplot!

Top Antiviral Drug Repurposing Results



4. Create bar charts of data

Columns

Perturbagen type

Marks

Square

Automatic

Bar

Line

Area

Square

Circle

Shape

Text

Map

Pie

Gantt Bar

Polygon

Density

a. Create square graph with only one column value

Marks

Square

Color

Size

Label

Detail

Tooltip

Perturbagen

b. Make perturbagen a square colour

Filter [Perturbagen]

General

Wildcard

Condition

Top

None

By field:

Median connectivity score

Sum

>

0

Range of Values

Min:

Max:

Load

By formula:

Reset

Apply

Cancel

OK

c. Filter perturbagens in the bar graph by connectivity score

Filters

Perturbagen

Action (Perturbag..

Size

Label

d. Increase the square sizes

T

Perturbagen

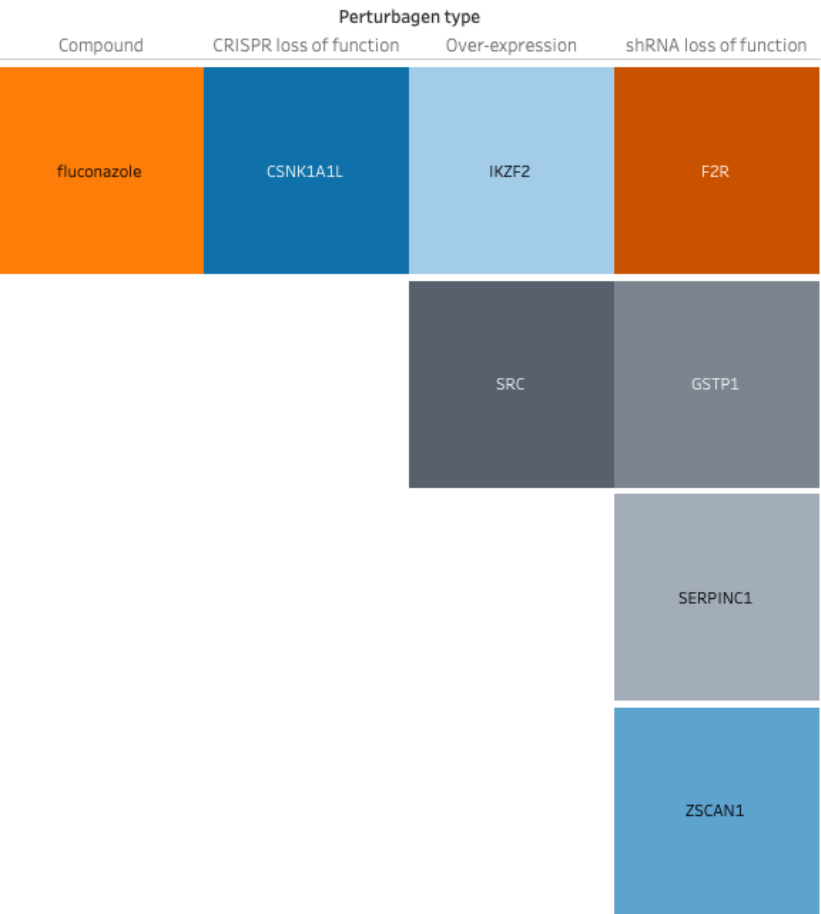
e. Write the perturbagen names on the graph

Bar charts produced in Tableau

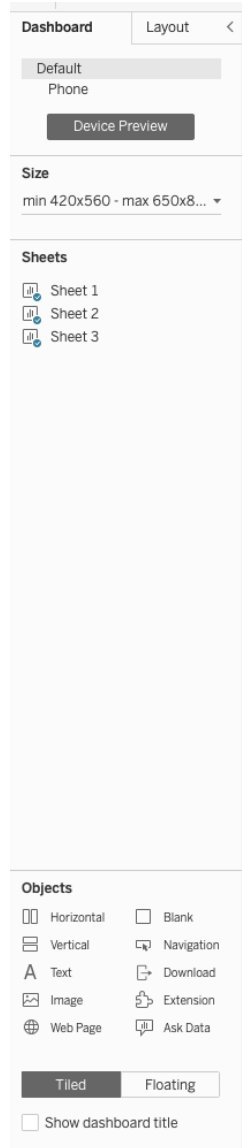
Breakdown of perturbagens inducing dissimilar gene expression profiles



Breakdown of perturbagens inducing similar gene expression profiles

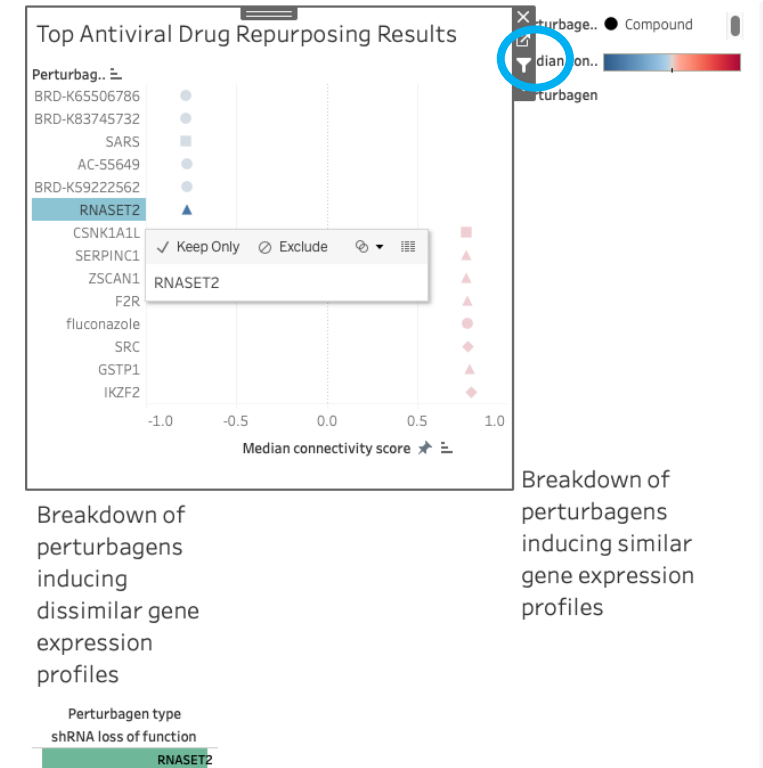


5. Combine all graphs on Dashboard



a. Different layouts you can have as well as objects you can insert (i.e. hyperlink, extension, etc)

Interactive element!

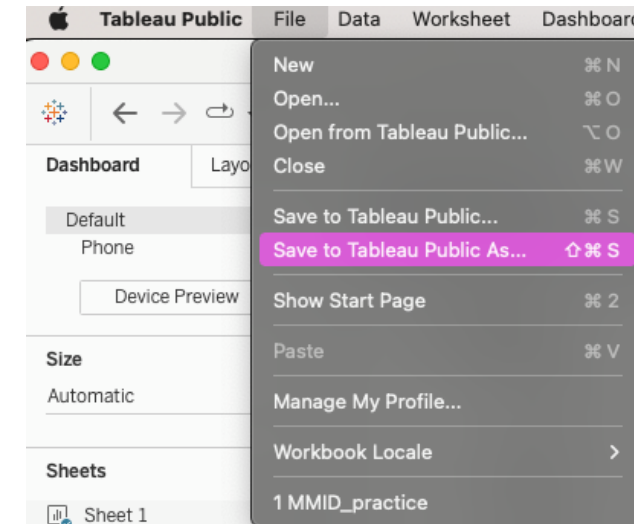
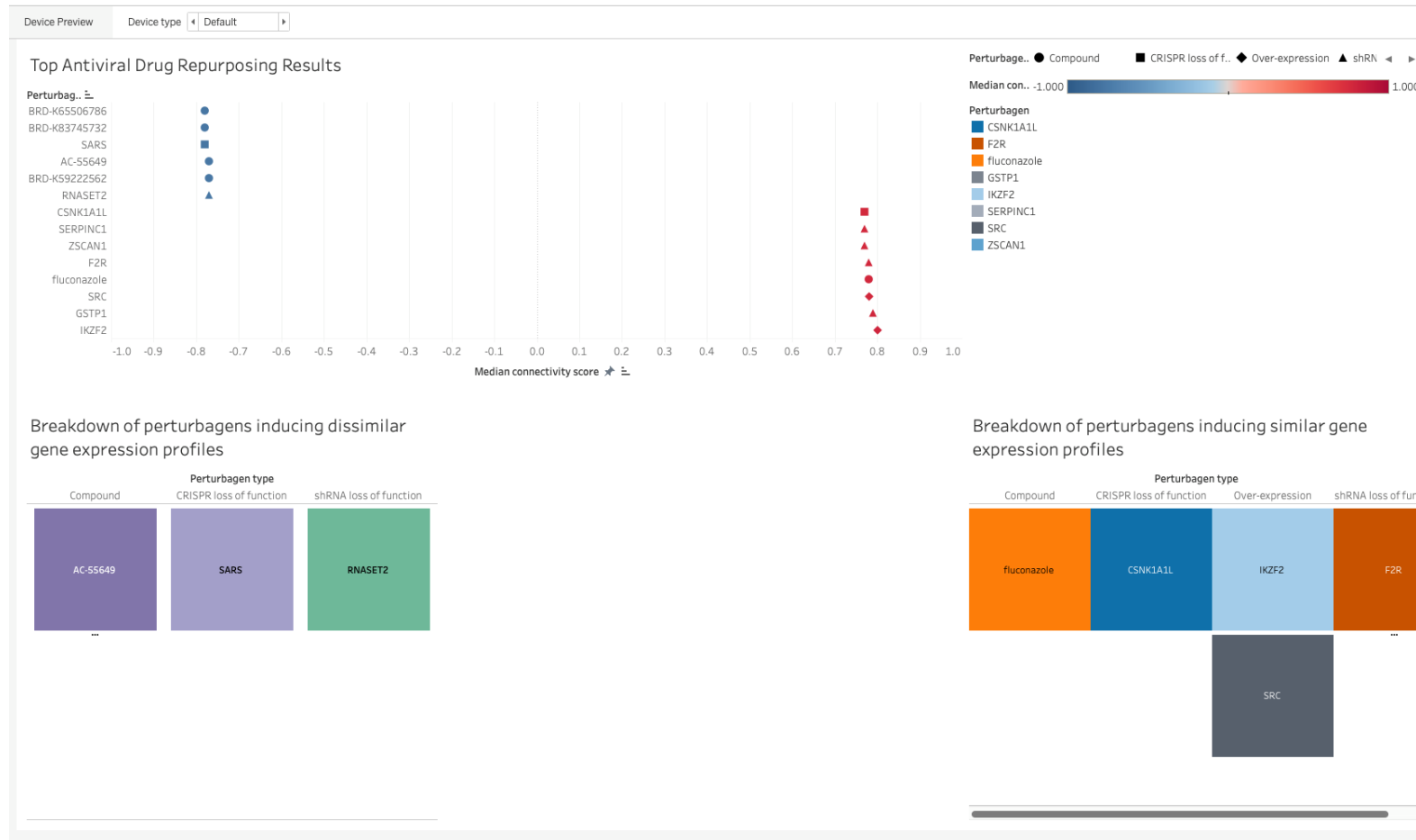


Breakdown of perturbagens inducing dissimilar gene expression profiles

Breakdown of perturbagens inducing similar gene expression profiles

b. Clicking the filter button on one graph and clicking on a perturbagen, can bring that one up on another graph

5. Combine all graphs on Dashboard



c. Save dashboard to Tableau public account

Tableau vs RStudio Drug Repurposing Graphs

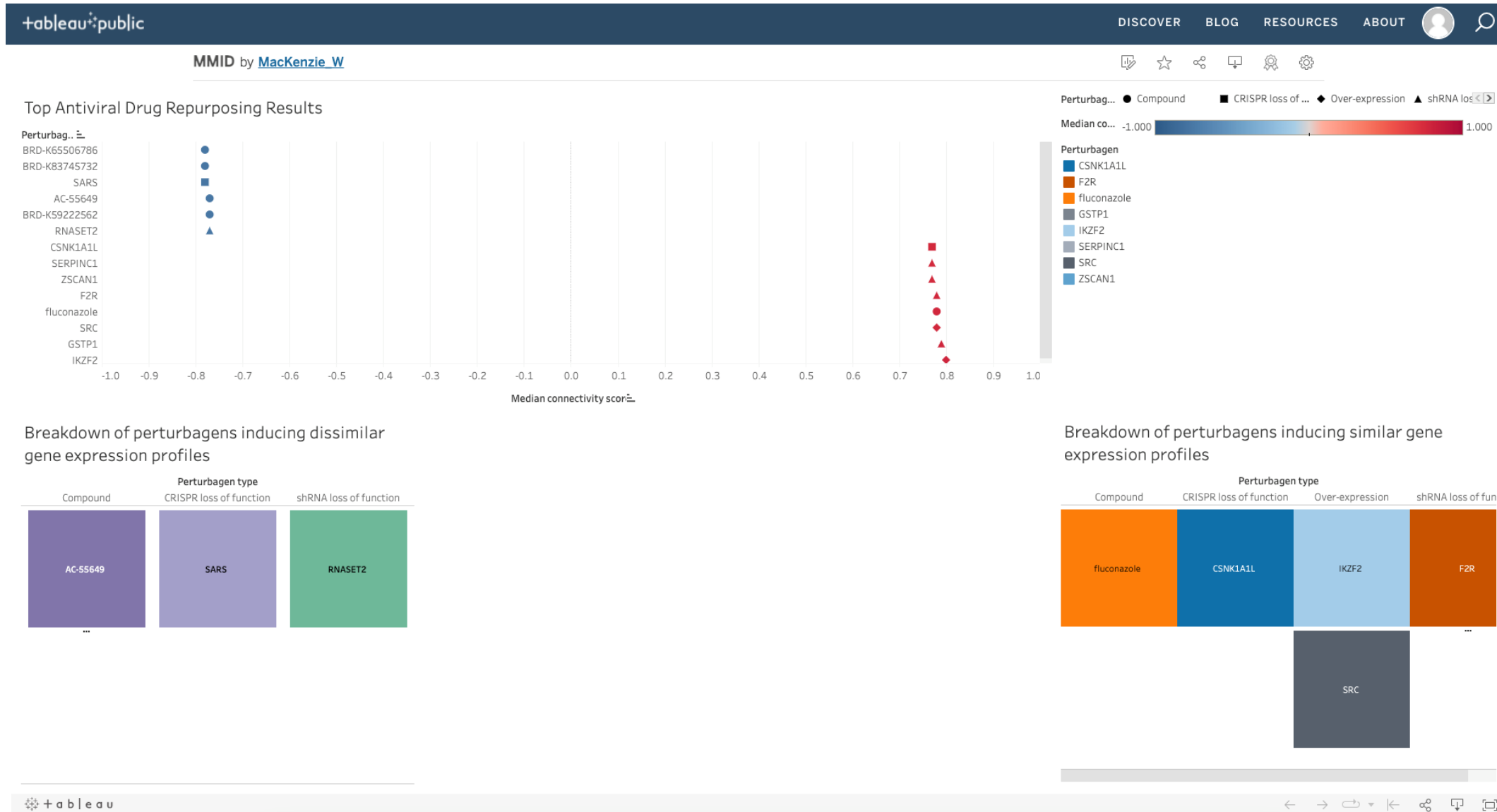
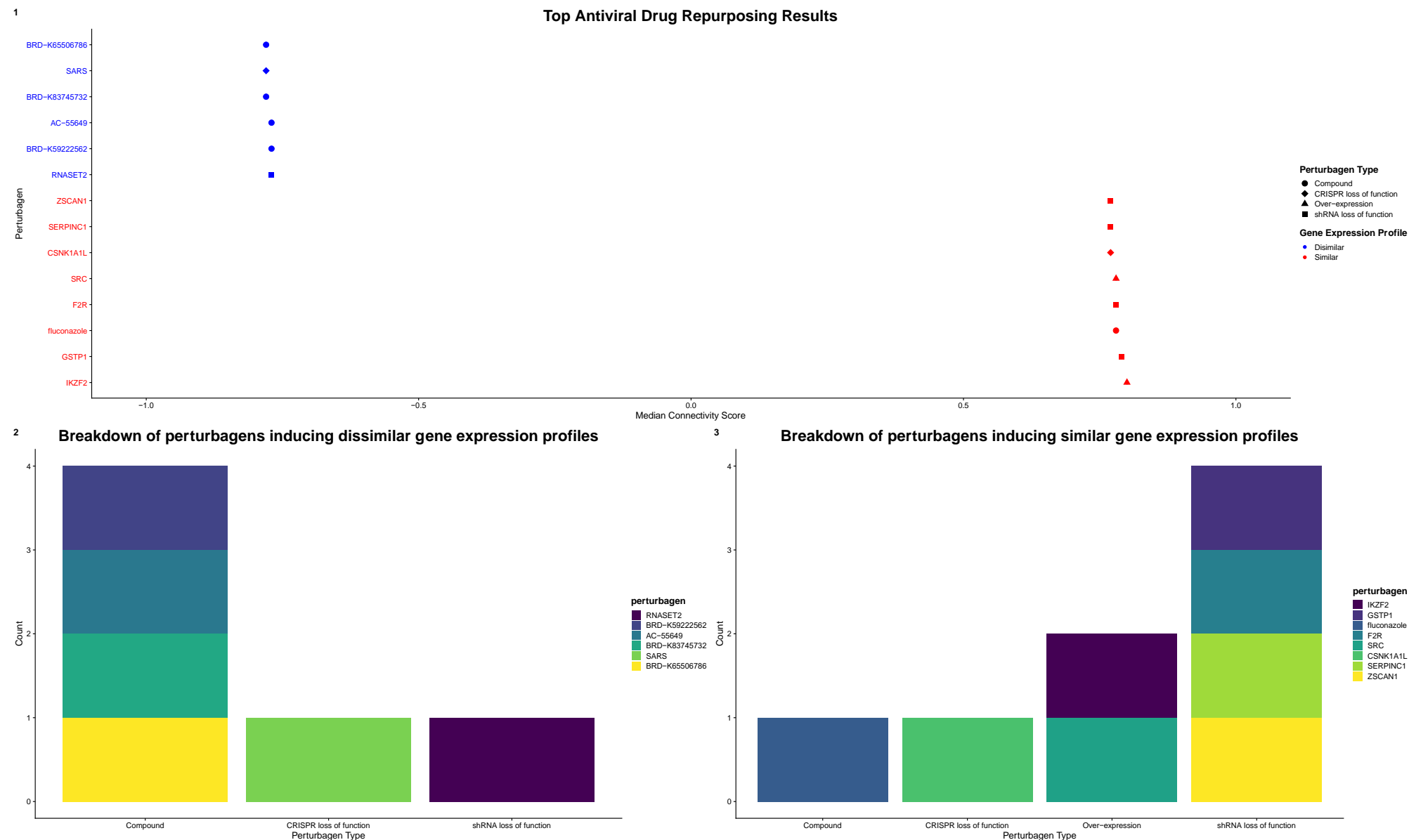


Tableau vs RStudio Drug Repurposing Graphs

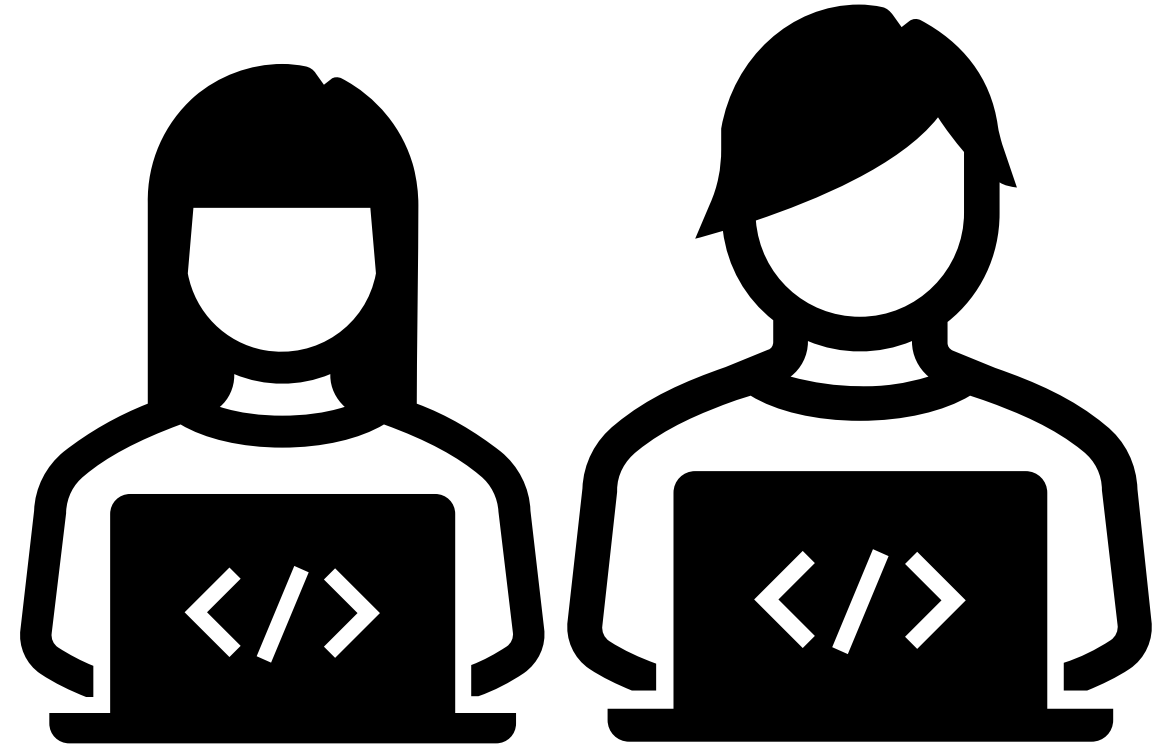


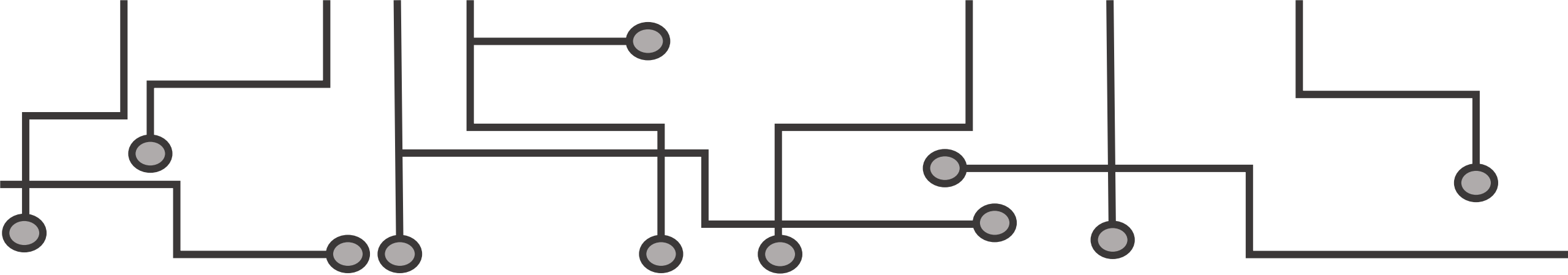
HELPFUL RESOURCES

- [CLUE/CMap](#)
- [ggplot2](#)
- [ggplot2/tidyverse](#)
- [cow_plot](#)
- [Tableau](#)

Videos

- [Drug Repurposing Hub at Broad Institute](#)
- [CMap Analysis](#)





THANK YOU FOR ATTENDING!
The Q&A Session will now begin.

Please make sure to fill out the [Exit Survey](#)
We value your feedback!

More questions? Please email us at
mmid.coding.workshop@gmail.com or post them to the workshop [slack channel](#)

