

Visualization

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Outline

1. How to create graphs in Prism
2. Eight ways to change a graph
3. Rotating, flipping, reversing
4. Changing a graph's shape and size
5. Breaking Y axis into two segments
6. Overlaying two column graphs
7. Graphing Example: XY plots – Nonlinear curve fitting
8. Exporting images from Prism

1. How to create graphs in Prism

When you create a new data table, Prism automatically creates a linked graph. For example, let's create an empty XY table in Prism:

Welcome to GraphPad Prism

XY tables: Each point is defined by an X and Y coordinate

	X	A			B		
	Minutes	Control			Treated		
	X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title						
2	Title						
3	Title						

NEW TABLE & GRAPH

XY

Column

Grouped

Contingency

Survival

Parts of Whole

Multiple variables

Nested

EXISTING FILE

Open a File

LabArchives

Clone a Graph

Graph Portfolio

Data table:

☒ Enter or import data into a new table

☐ Start with sample data to follow a tutorial

Options:

X: ☒ Numbers

☐ Numbers with error values to plot horizontal error bars

☐ Dates

☐ Elapsed times

Y: ☒ Enter and plot a single Y value for each point

☐ Enter 2 replicate values in side-by-side subcolumns

☐ Enter and plot error values already calculated elsewhere

Enter: Mean, SD, N

Prism Tips

Cancel Create

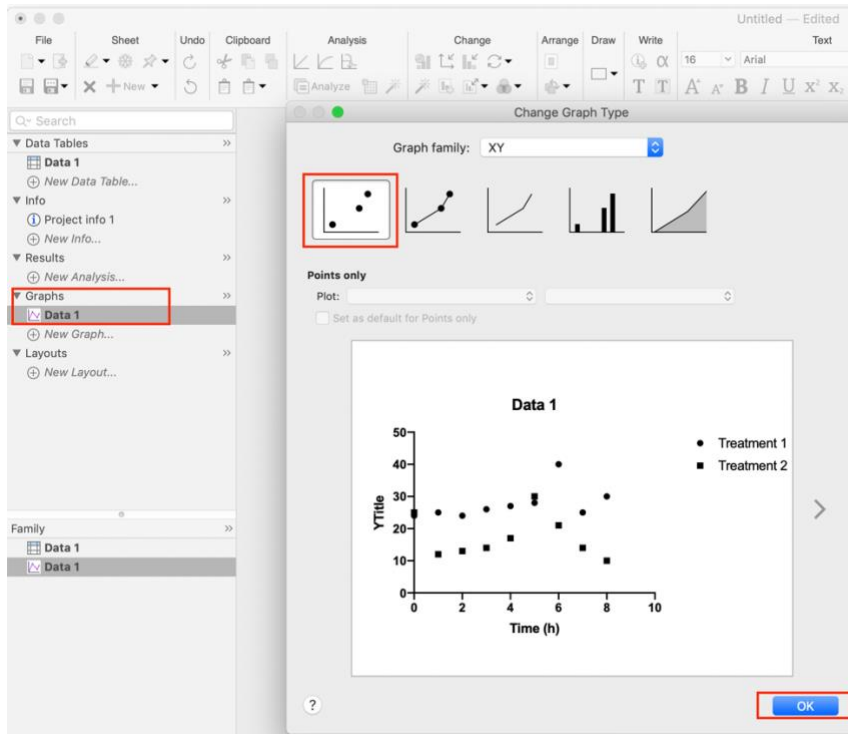
Then Go to the excel file “2.visualization.xlsx” and copy “DATA SET 1”

DATA SET 1			DATA SET 2		
Heart Rate					
Time (h)	Treatment 1	Treatment 2	Control	Treated	Treated+Antagonist
0	24	25	54	87	45
1	25	12	23	98	39
2	24	13	45	64	51
3	26	14	54	77	49
4	27	17	45	89	50
5	28	30	47		55
6	40	21			
7	25	14			
8	30	10			

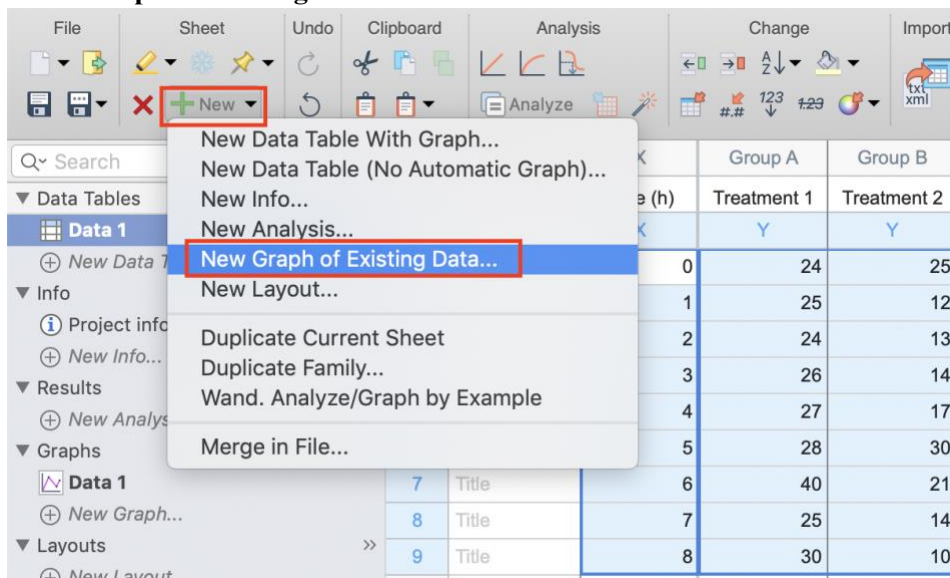
Go back to the Prism file and **paste**.

Table format:		X	Group A	Group B
	XY	Time (h)	Treatment 1	Treatment 2
1	Title	0	24	25
2	Title	1	25	12
3	Title	2	24	13
4	Title	3	26	14
5	Title	4	27	17
6	Title	5	28	30
7	Title	6	40	21
8	Title	7	25	14
9	Title	8	30	10

Prism automatically creates a linked graph. **Click “Data 1”** and a dialog will pop up where you can choose exactly the kind of graph you want. Choose the default one and **click “OK”**



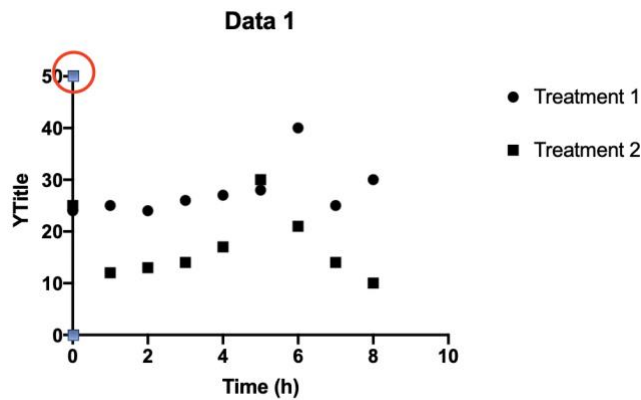
If you would like to create a new graph of existing data, click the “New” button and choose “New Graph of Existing Data”



2. Eight ways to change a graph

1) Direct Manipulation

Click on an axis to select it. Knobs appear on both ends. Move the mouse over the right knob of the X-axis or the top knob of the Y-axis. Drag the axis to make it longer or shorter. Or grab the middle of the axis and move the entire graph around the page.

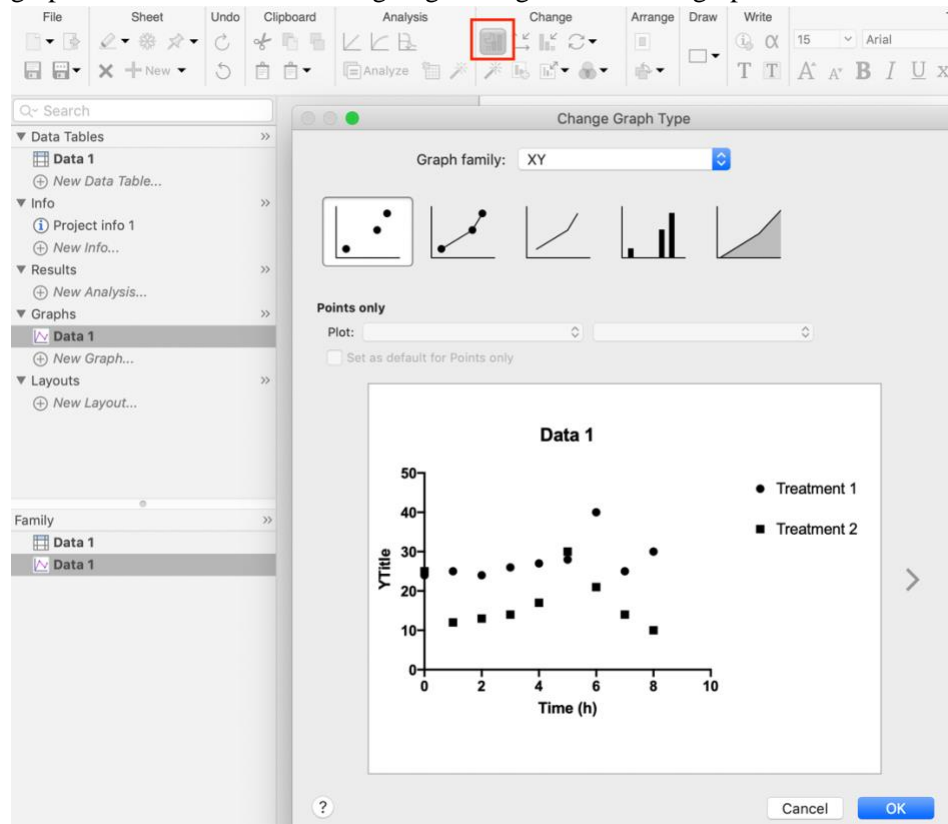


2) Shortcut Menus

Move the mouse over a symbol, title, axis, etc, and right click (control click with Macs). The shortcut menu will give you many choices to format the object.

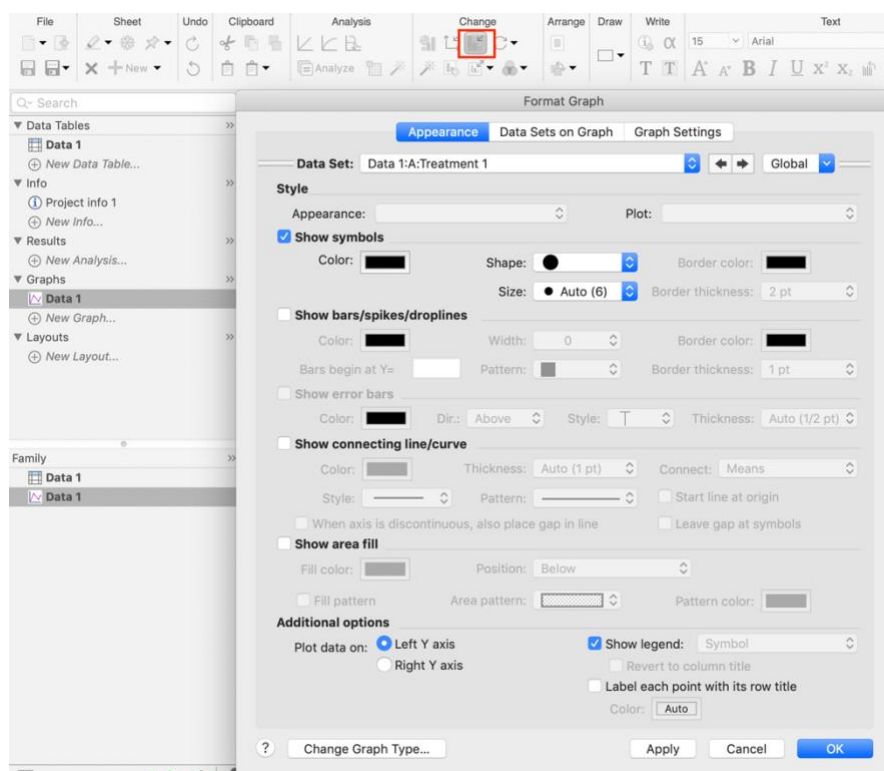
3) Change Graph Type dialog

Click the “Change Graph Type” button then it pops up the same dialog you used to create the graph. This is useful for making large changes in how the graph looks



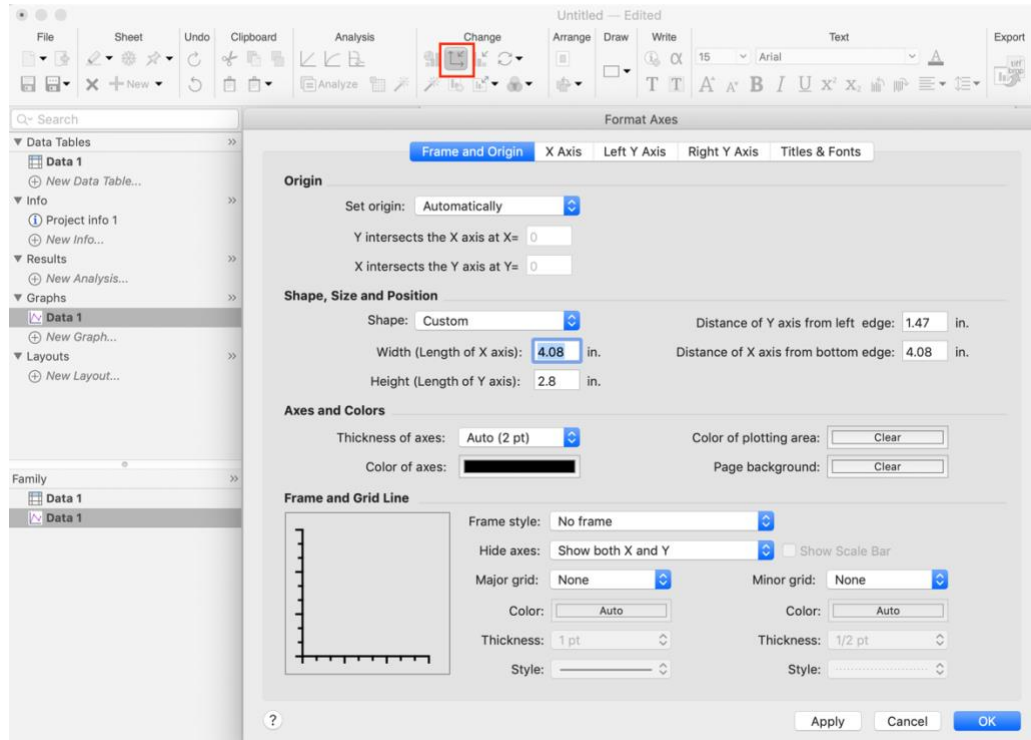
4) Format Graph dialog

Click the **“Format Graph”** button in the Change section of the Prism toolbar to change symbols, bars, error bars, area fills, legends, etc. OR **double click anywhere on the graph** (except the axes) to bring it up.



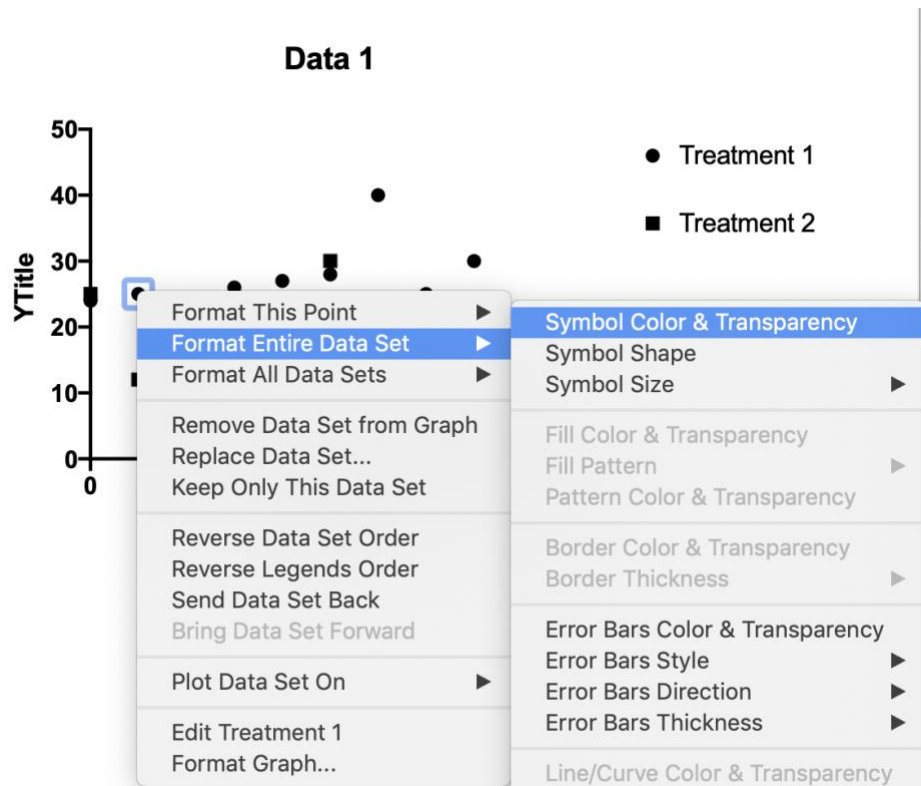
5) Format Axes Dialog

Click the **“Format Axes”** button in the Change section of the Prism toolbar to change the range, interval, scale, numbering, etc. of the axes. OR **double-click on an axis** to bring it up.

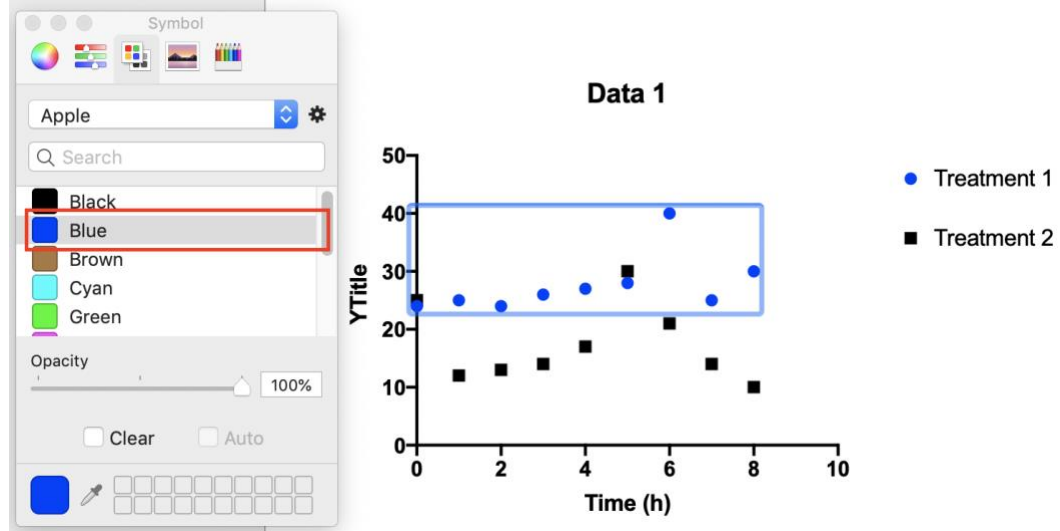


- 6) Select a range of points on a data table, and change how corresponding points are graphed. Select a cell or a block of cells, right (control) click, and choose Format Points. You'll be able to change the color, size, symbol, border, etc. of the points or bars that correspond to the selected cells.

For example, **right click the first dot** of treatment 1. Choose **“Format Entire Data Set”** then **“Symbol Color & Transparency”**.

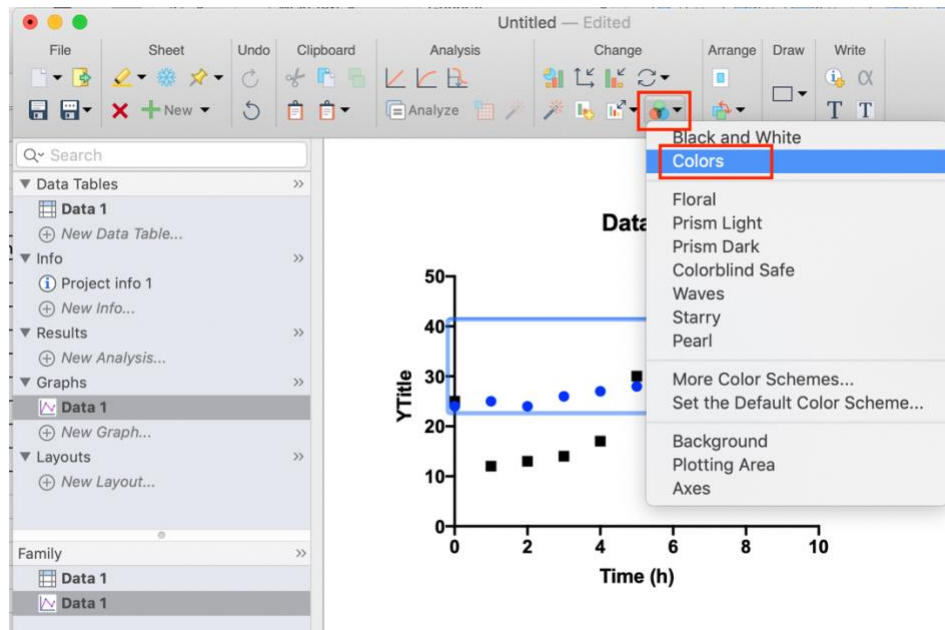


If we **choose “Blue”** from the Symbol dialog, all the dots of treatment 1 will become blue.

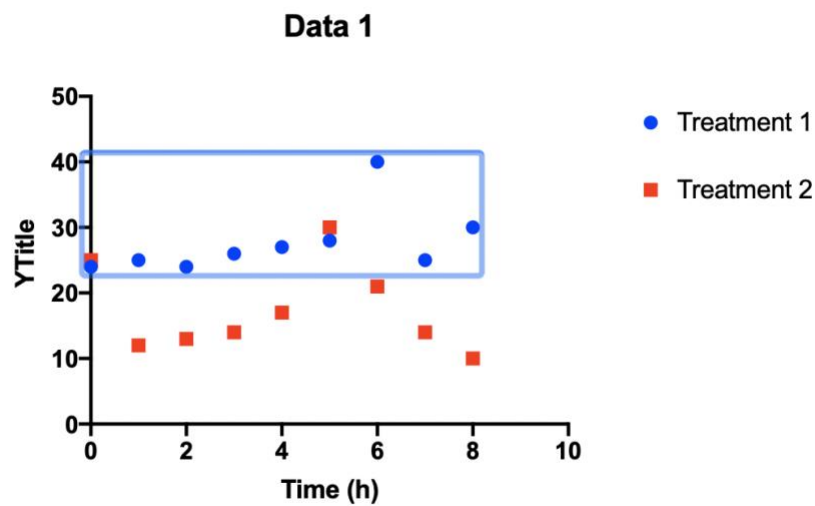


7) Color Schemes

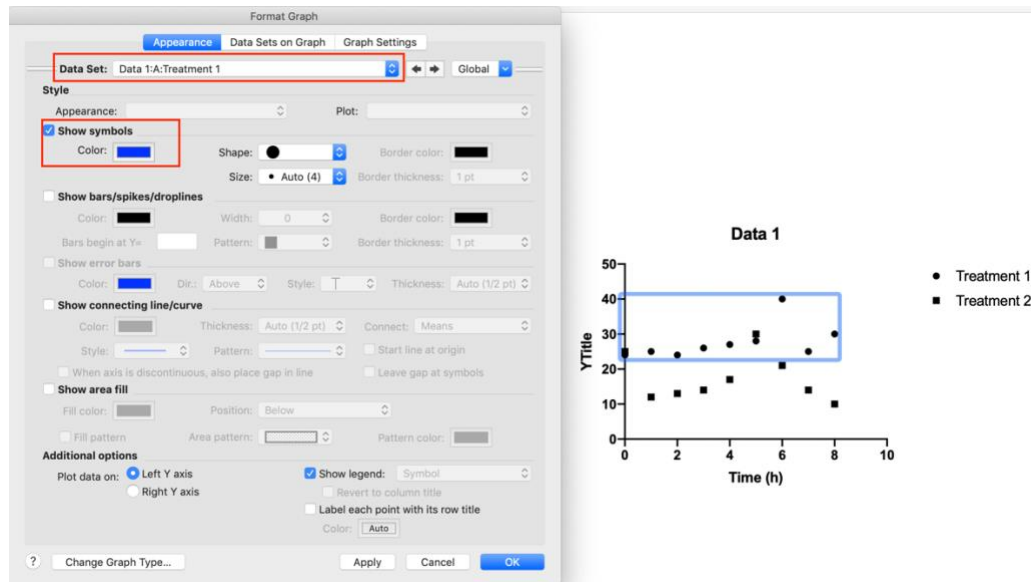
Click the color scheme in the Change section of the Prism toolbar to change all colors at once



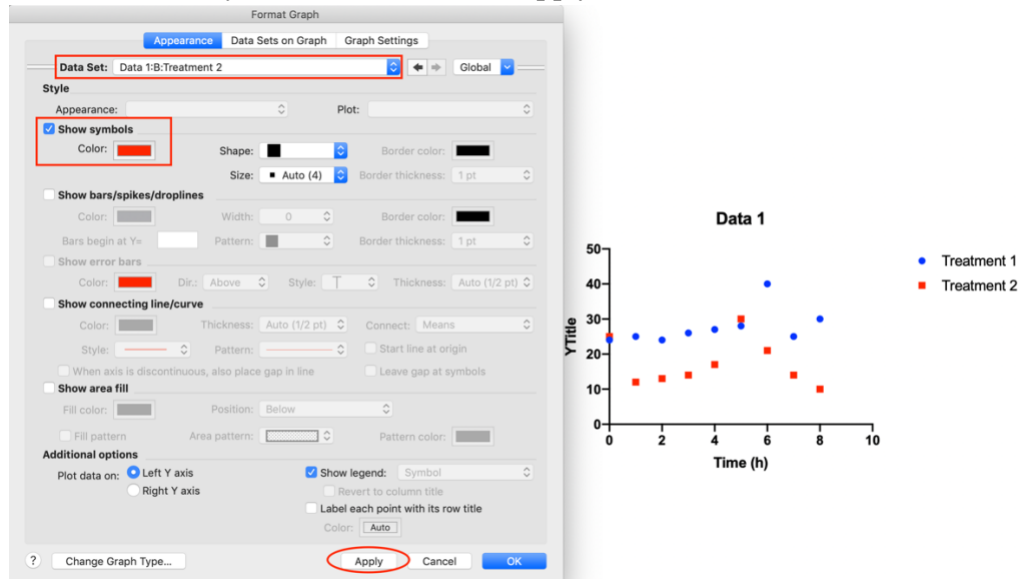
Then Prism assigns different colors to different treatment groups respectively.



OR the color options could also be made at the Format Graph dialog: **Double-click** any point in the graph then it pops up a “Format Graph” window. Change the “color” under the “Show symbols” to **blue**.



Select “**Data 1:B:Treatment 2**” from the drop-down list of “Data Set”. Change the “color” under the “Show symbols” to Red. Click “Apply”.



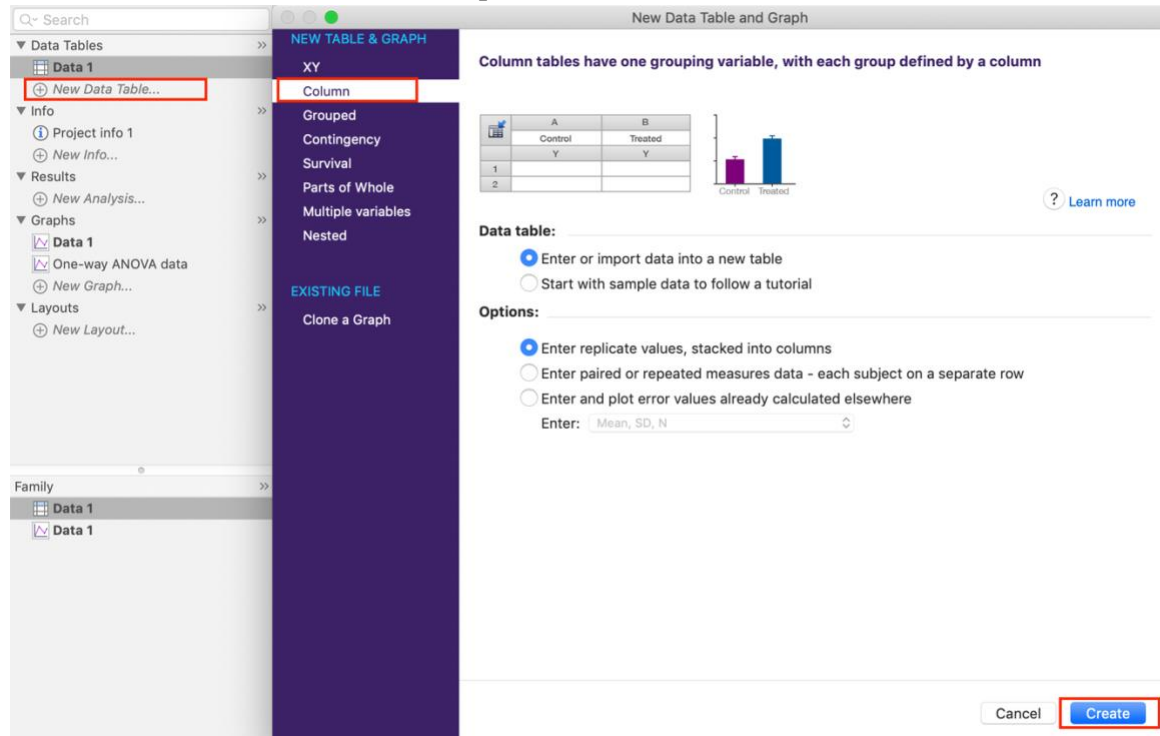
Obviously, the plot on the right assigned different colors to different treatment groups respectively.

8) Magic – Make Graphs Consistent

The Prism Magic tool makes one (or more) graph look like another. It is a huge time saver. More details could be found at [Prism Magic](#).

3. Rotating, flipping, reversing

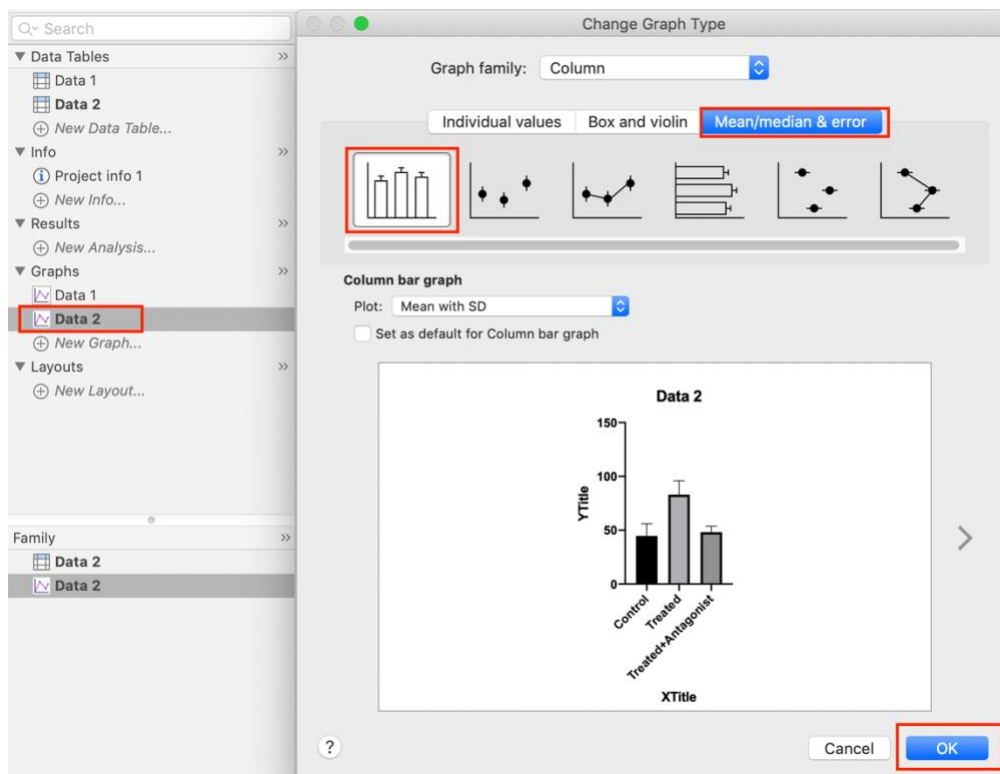
Create a new dataset by clicking the “New Data Table...” on the data tables list. Then choose “Column” data table and “Enter or import data into a new table”. Click “Create”.



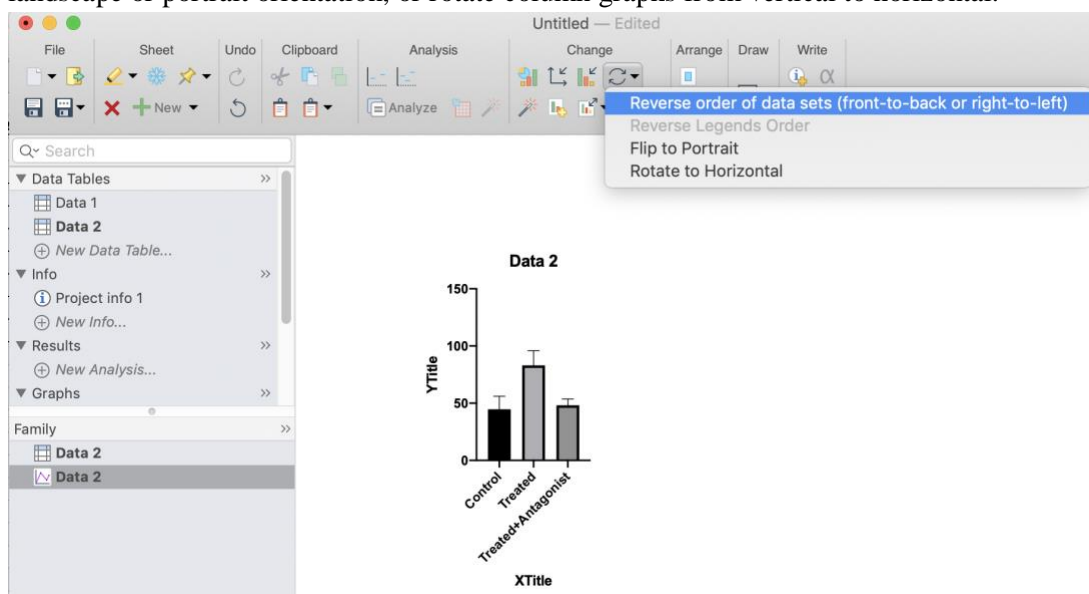
Go to the excel file “2.visualization.xlsx” and copy “DATA SET 2” then paste it to the empty Prism data table.

Search		Group A	Group B	Group C
Data Tables		Control	Treated	Treated+Antagonist
Data 1		Y	Y	Y
Data 2	1	54	87	45
New Data Table...	2	23	98	39
Info	3	45	64	51
Project info 1	4	54	77	49
New Info...	5	45	89	50
Results	6	47		55
New Analysis...	7			
Graphs	8			
Data 1	9			
Data 2	10			
New Graph...	11			
Layouts	12			
New Layout...				

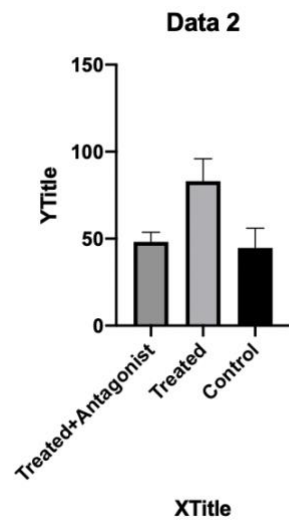
Click the graph “Data 2”. In the Change Graph Type dialog, choose “Mean/median & error” then the first bar chart then click “OK”.



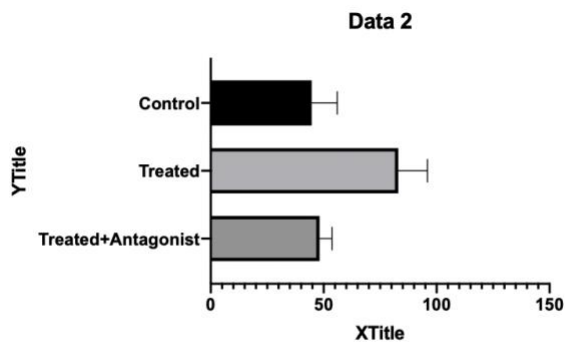
Click the Rotate/Flip button in the Change section of the Prism toolbar drops a menu with four choices. You can reverse the order of datasets on a graph, flip the page settings between landscape or portrait orientation, or rotate column graphs from vertical to horizontal.



Click “Reverse order of data sets (front-to-back or right-to-left)”:

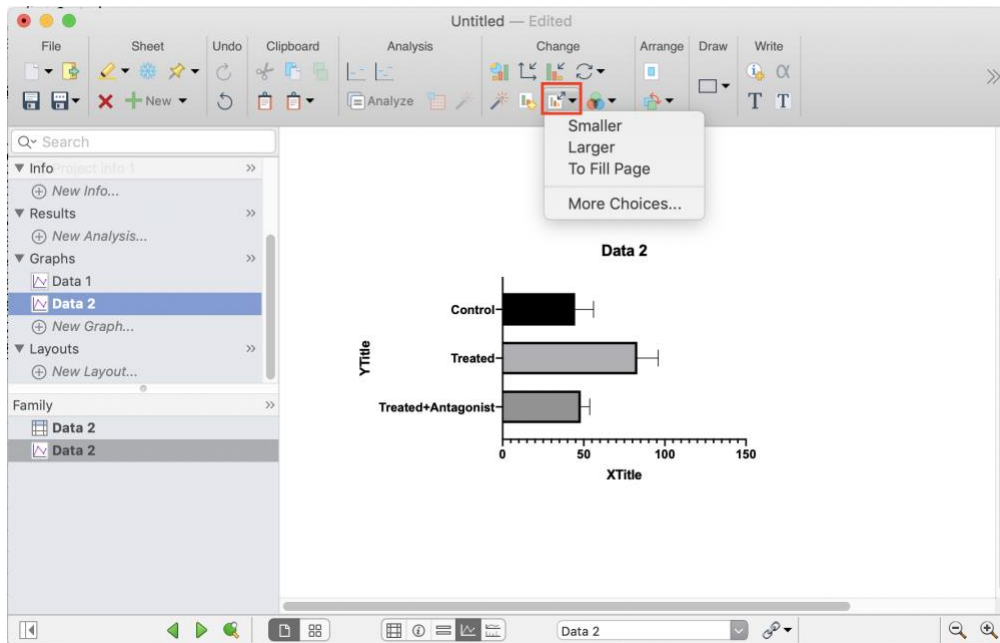


Click “Rotate to Horizontal”:

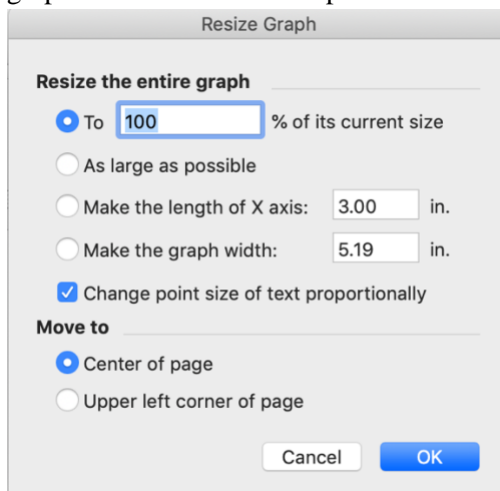


4. Changing a graph’s shape and size

Click the Resize Graph button in the Change section of the Prism toolbar. This button enlarges or shrinks both the graph and also the graph's symbols and fonts. In contrast, resizing by stretching an axis doesn't change symbol or font size.

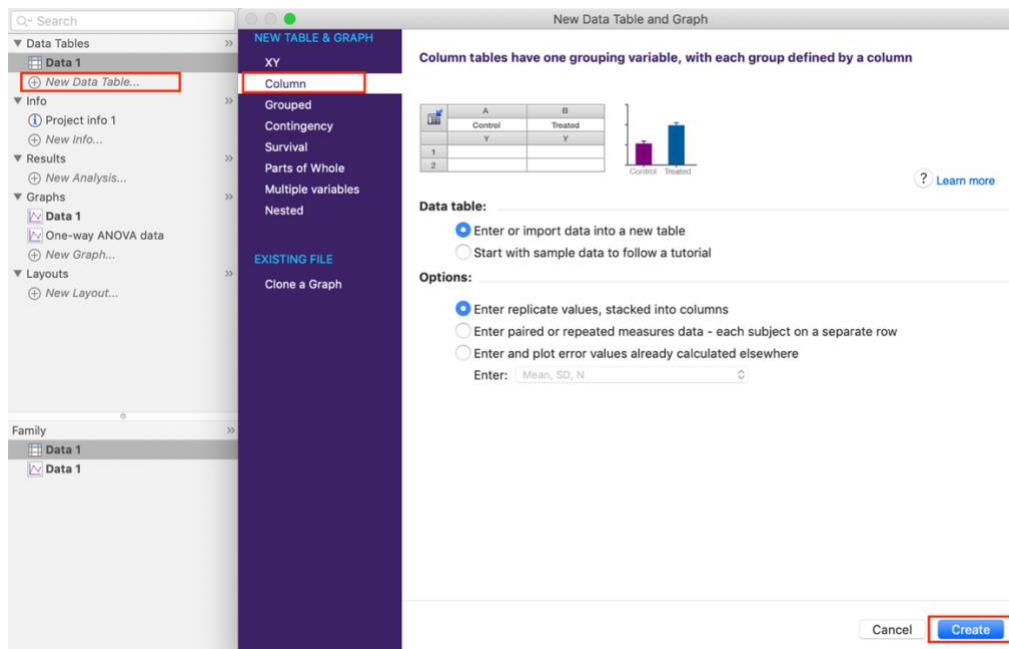


If you click More Choices, a dialog lets you pick the exact size you want and lets you resize the graph without changing font size. This can be useful when the journal specifies both the exact graph size it wants and the point size of fonts.



5. Breaking Y axis into two segments

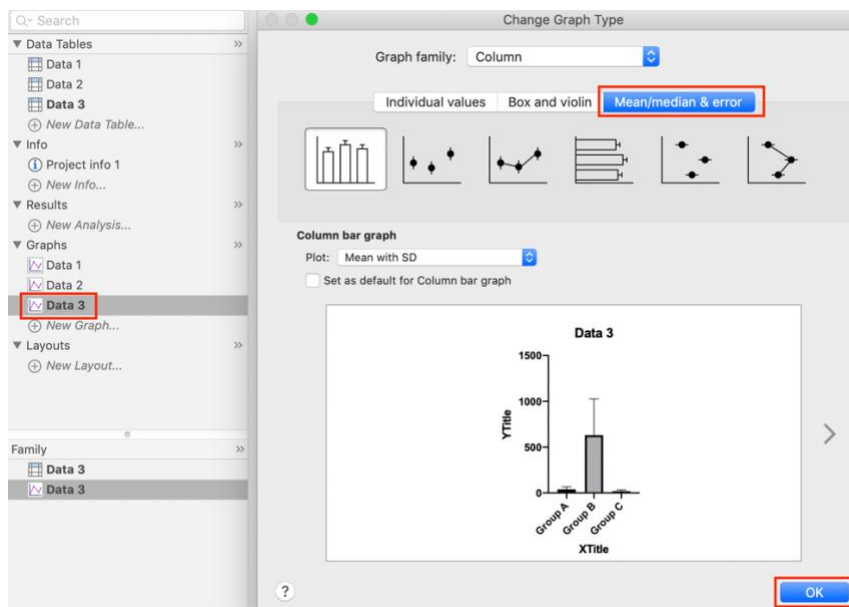
Create a new dataset by **clicking the “New Data Table...”** on the data tables list. Then **choose “Column”** data table and **“Enter or import data into a new table”**. Click **“Create”**.



Go to the excel file “2.visualization.xlsx” and copy “DATA SET 3” then paste it to the empty Prism data table.

	Group A	Group B	Group C
Group A	Y	Y	Y
1	10	50	40
2	30	800	25
3	50	900	15
4	25	1000	10
5	80	400	5

Similarly, click the graph “Data 3”. In the Change Graph Type dialog, choose “Mean/median & error” then the first bar chart then click “OK”.

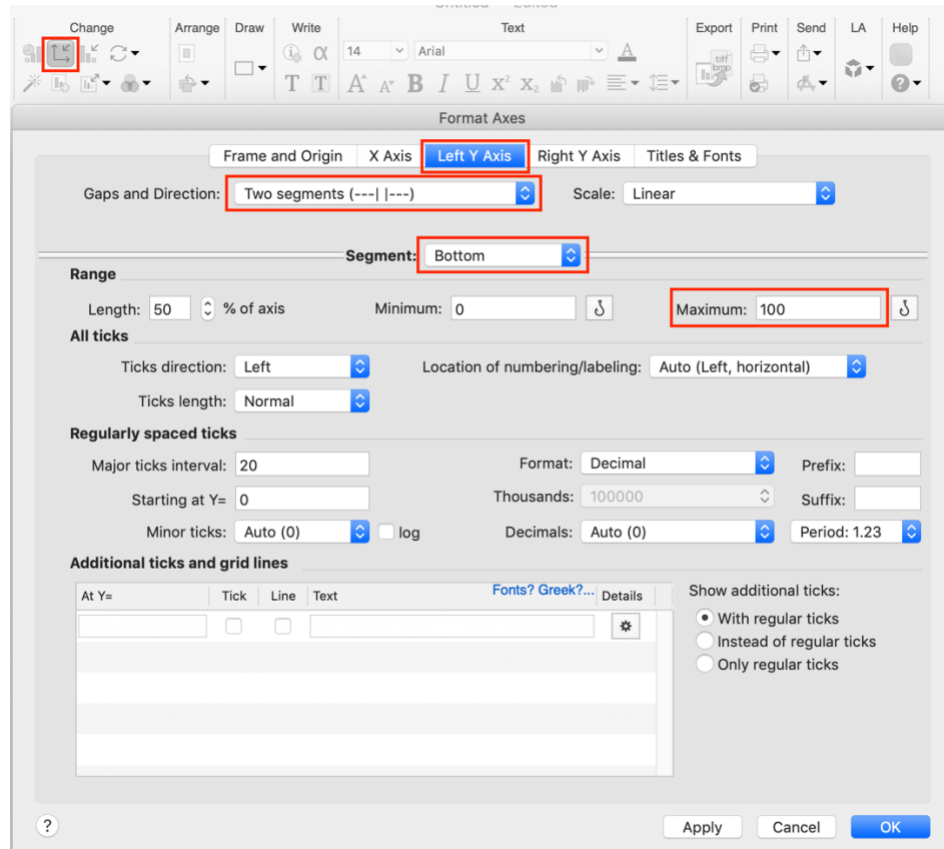


Apparently, Group B has a much higher distribution than the other 3 groups, which makes it not clear to see the boxplots of the other 2 groups. Therefore, we split the Y axis into two parts.

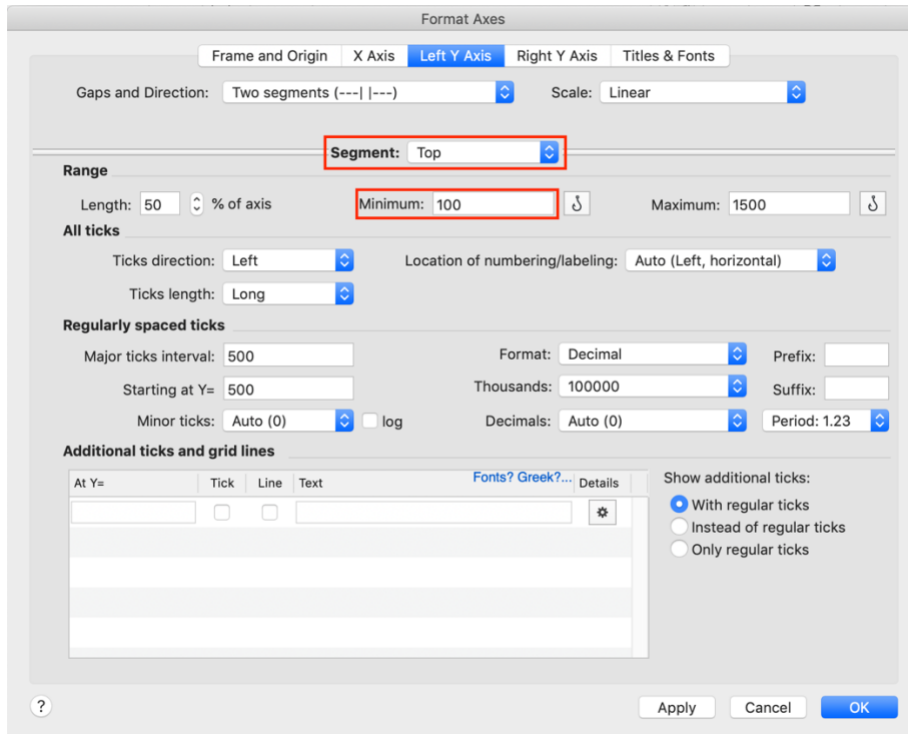
Click the **“Format Axes”** button in the Change section of the Prism toolbar. In the Format Axes dialog, click **“Left Y axis”**, then choose **“Two segments (---| ---)”** for “Gaps and Direction”.

You could also split Y axis into three segments here, which depends on your need.

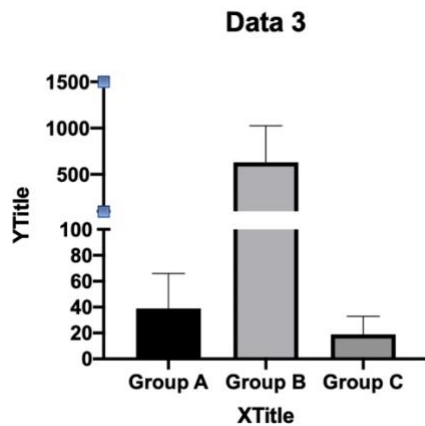
Then define the range for bottom segment and top segment. For the bottom segment, choose **“Bottom”** then assign the **Maximum as 100**.



For the top segment, choose **“Top”** then assign the **Minimum as 100**.



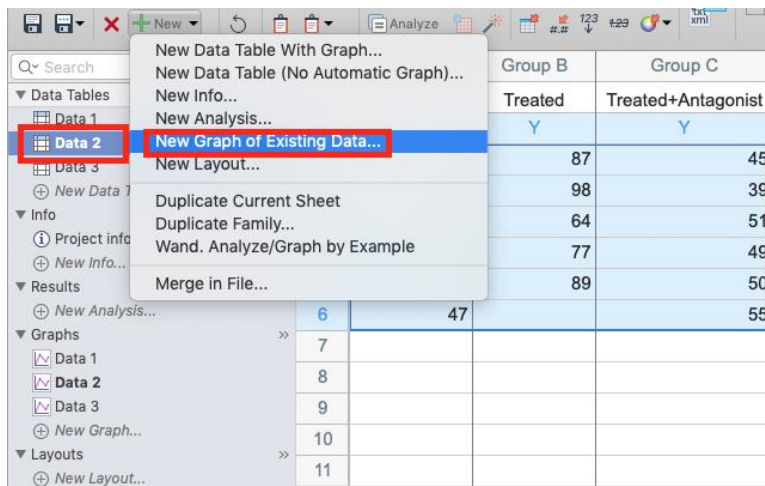
The customized plot will be like this:



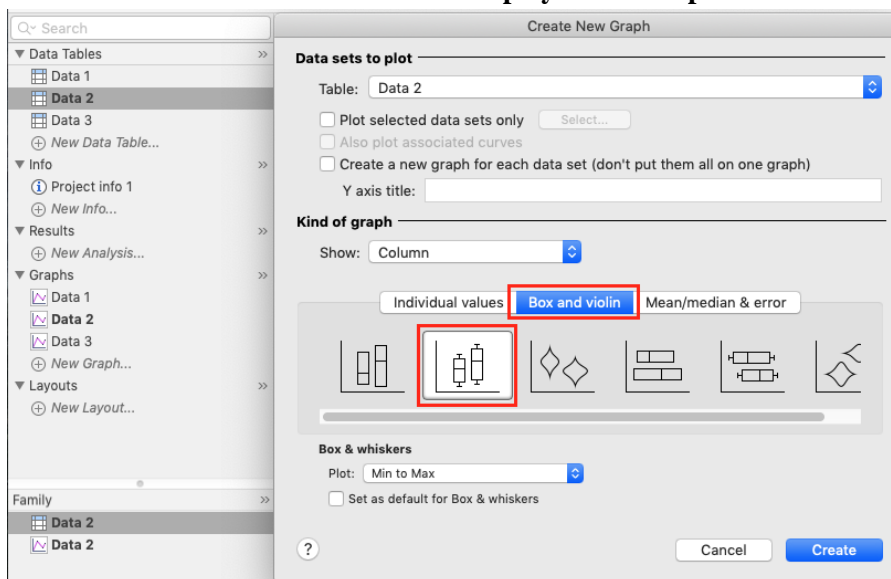
6. Prism layout and overlaying two column graphs

Prism page layouts let you combine several graphs on a single page, along with data or results tables, text, drawings, and imported images. Graphs can be from one or several Prism projects.

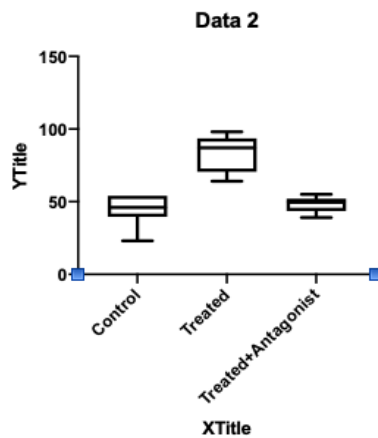
Let's go back to Data 2, click "+ New" then create "New Graph of Existing Data..."



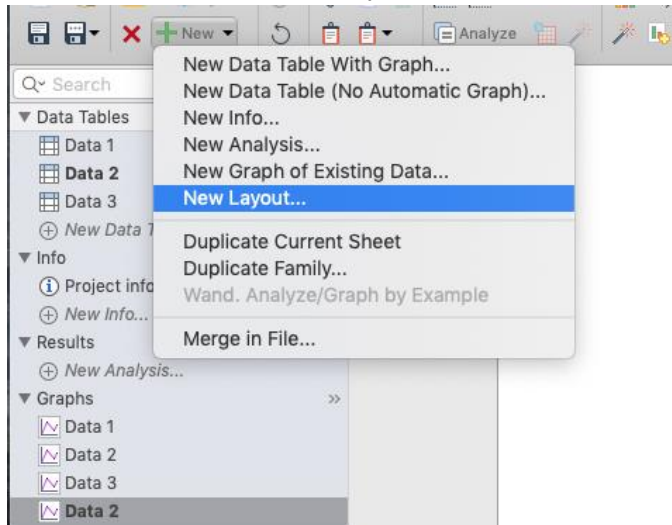
Choose “Box and violin” then the 2nd display of the boxplot:



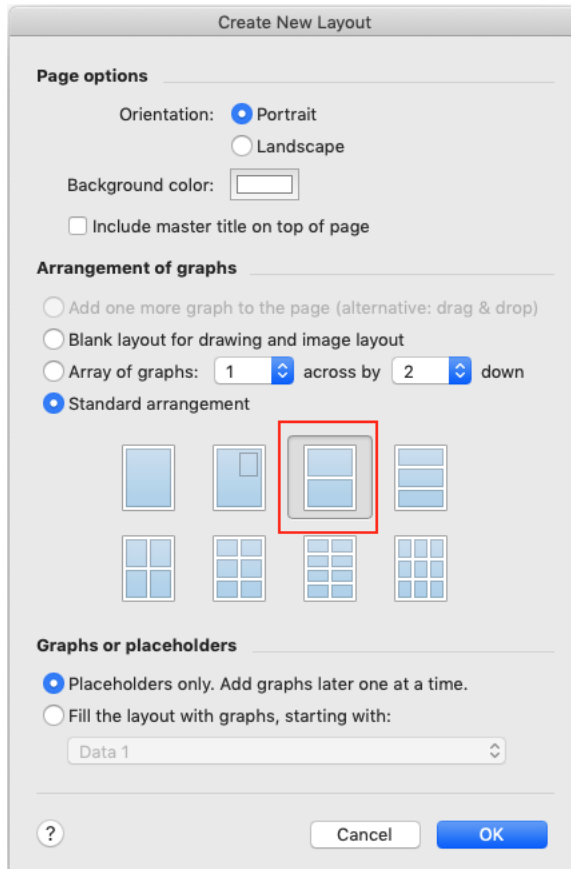
Then the plot will be like this:



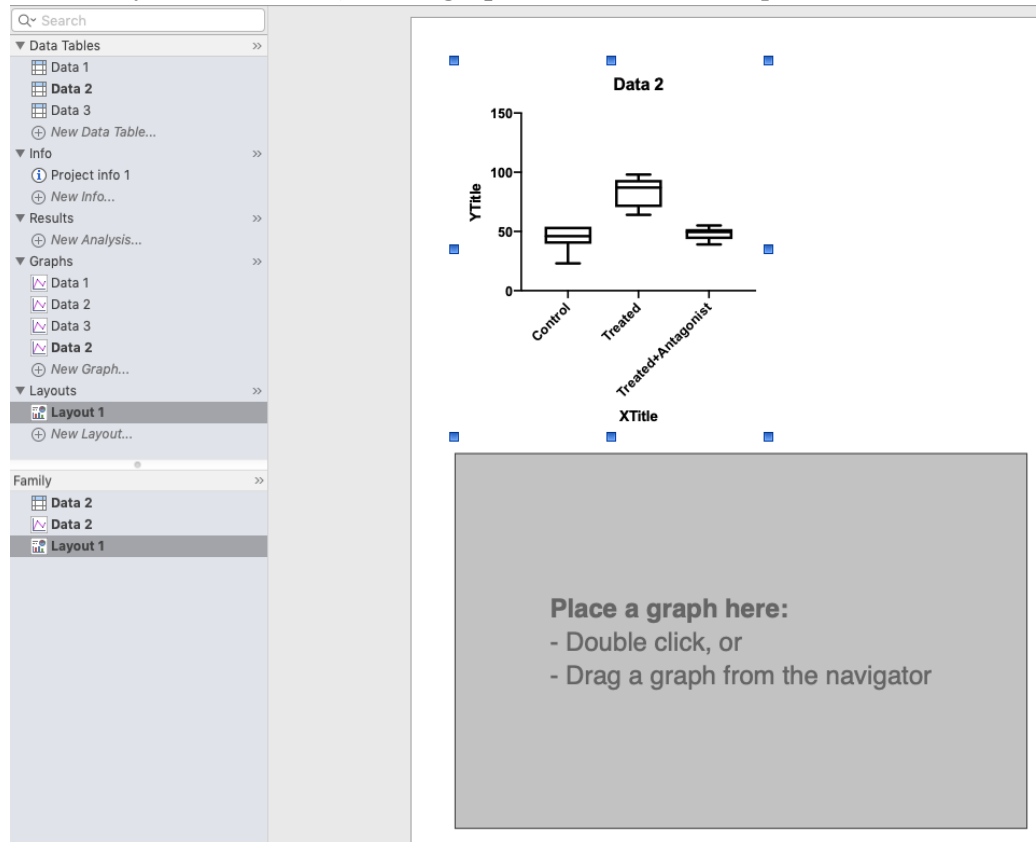
If individual data points inside the box plots are needed, we could overlap two plots together.
Click “+ New” then “New Layout...” to create a new layout:



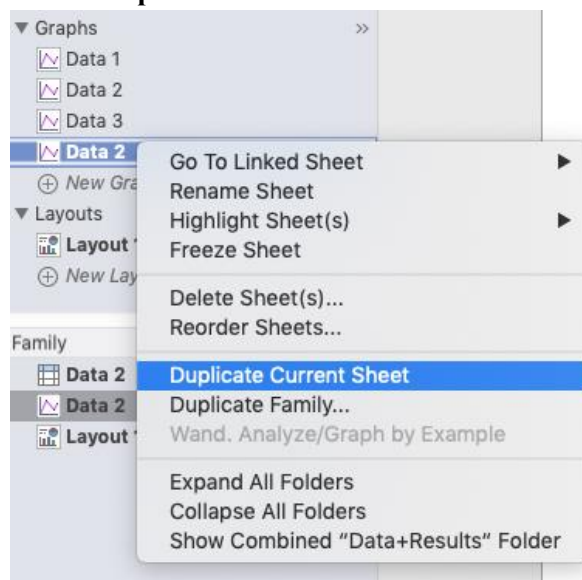
In the Create New Layout dialog, **choose the arrangement with two graphs placed vertically.**



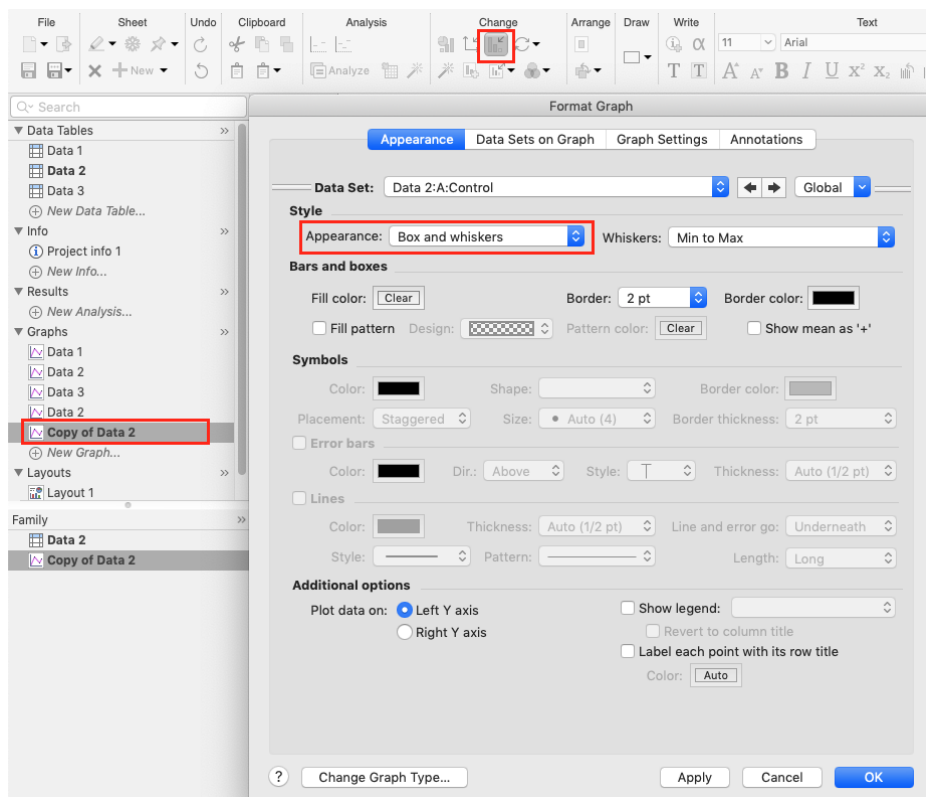
Click “Layout 1” then drag the 4th graph “Data 2” into the top window.



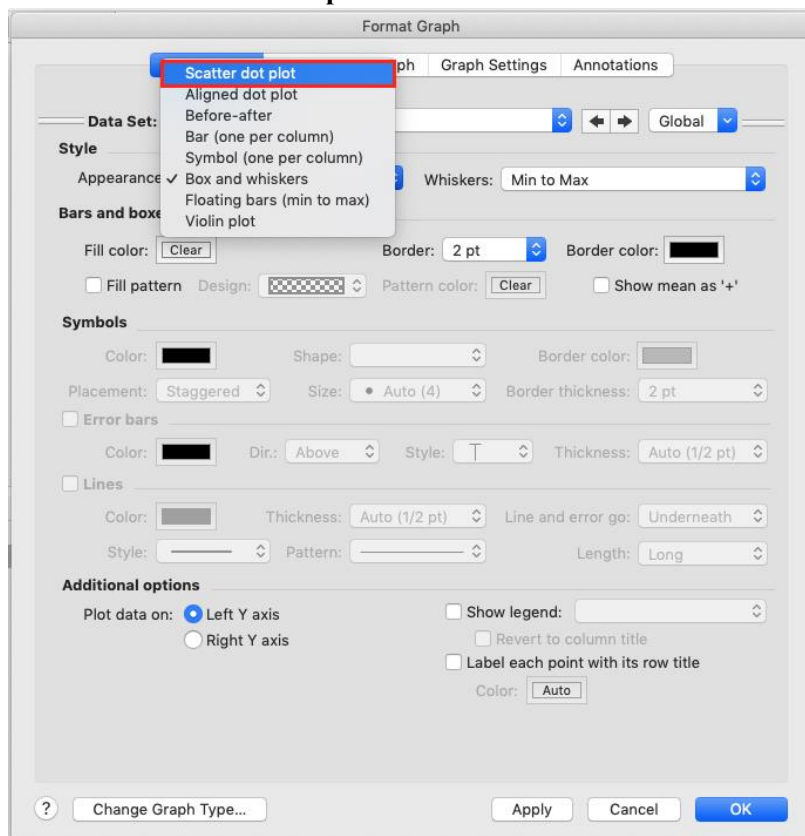
Then we create another scatter plot for the data points (dots). Click the boxplot “Data 2” then select “Duplicate Current Sheet”.



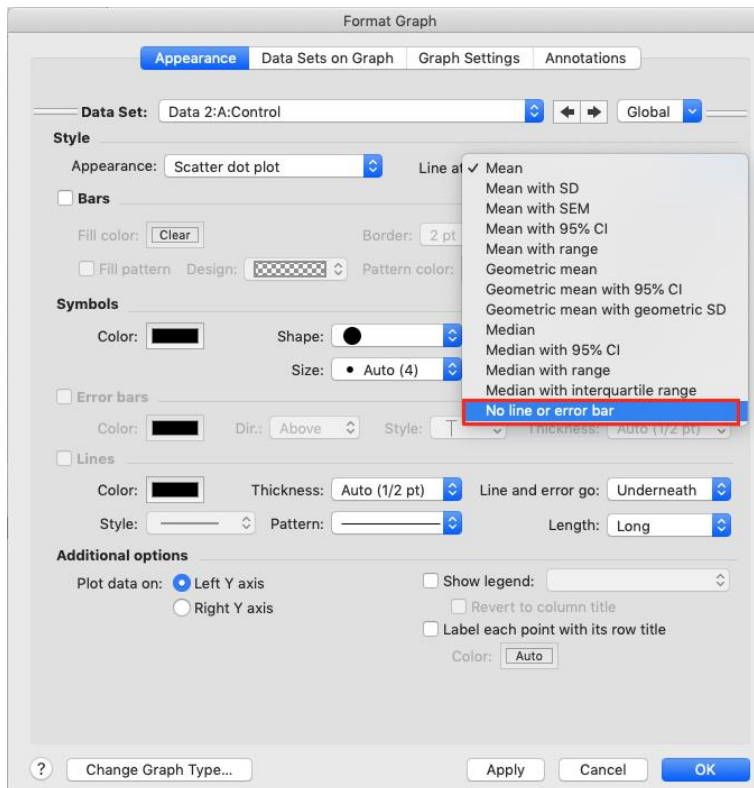
Click the duplicated graph “Copy of Data 2” then Click the “Format Graph” button in the Change section of the Prism toolbar. In the Format Graph dialog, click the Appearance.



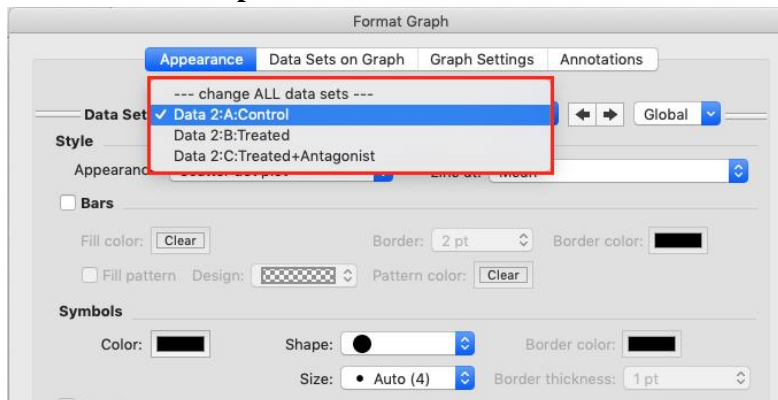
Then choose “Scatter dot plot”.



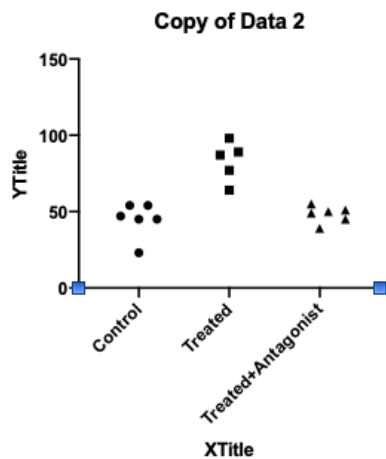
Then choose “No line or error bar”.



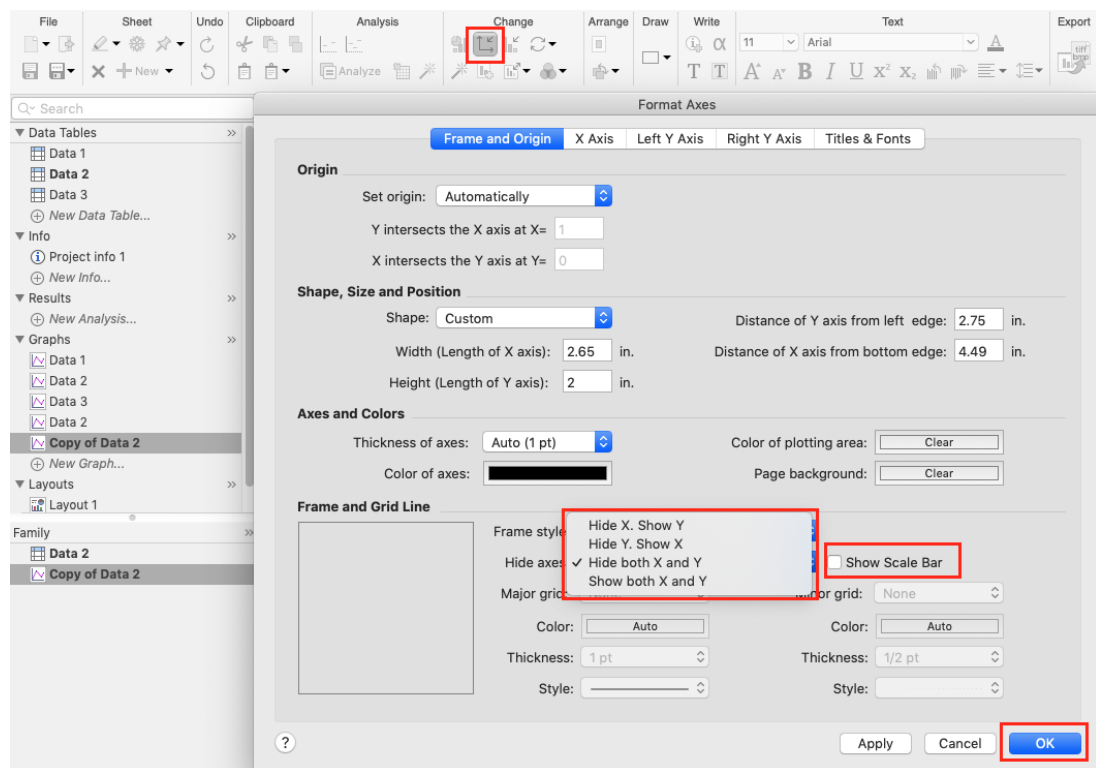
Choose the same options for all data sets. Then click “OK”.



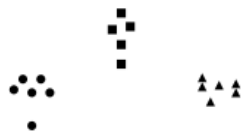
Then the plot will be like this:



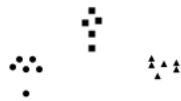
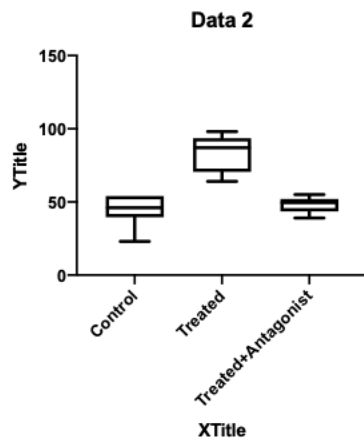
Next, let's remove the frames of this plot. Click the **“Format Axes”** button in the Change section of the Prism toolbar. In the Format Axes dialog, choose **“Hide both X and Y”** then uncheck **“Show Scale Bar”**. Click **“OK”**.



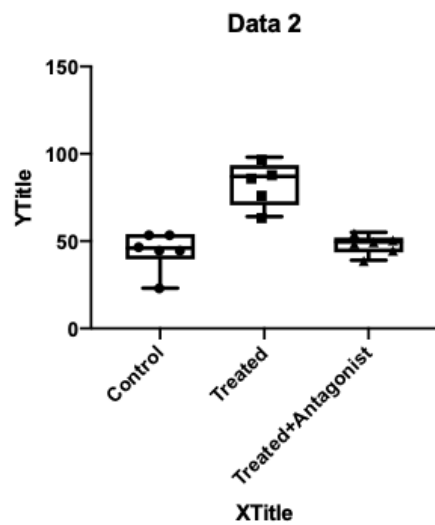
Manually delete the main title and axes titles. Then plot will be like this:



Drag this plot to the “Layout 1”. The layout 1 will be like this:

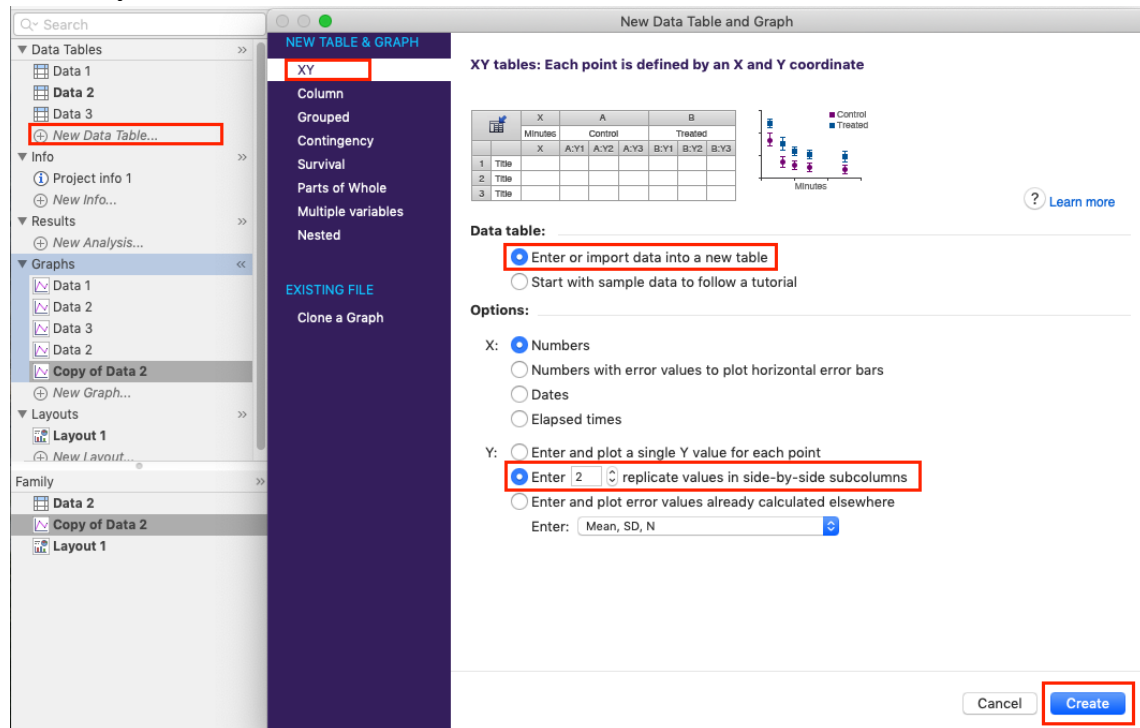


Drag the scatter plot to overlap the boxplot and the final plot will be like this:



7. Graphing Example: XY plots – Nonlinear curve fitting

Create a new dataset by clicking the “New Data Table...” on the data tables list. Then choose “XY” data table and “Enter or import data into a new table” then “Enter 2 replicate values in side-by-side subcolumns”. Click “Create”.

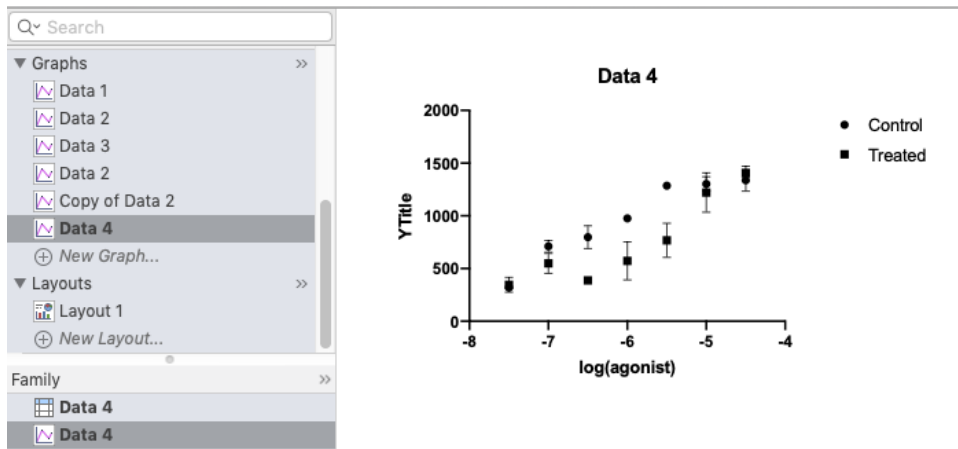


Go to the excel file “2.visualization.xlsx” and copy “DATA SET 4” then paste it to the empty Prism data table.

Table format:		X	Group A		Group B	
XY		log(agonist)	Control		Data Set-B	
		X	A:Y1	A:Y2	B:Y1	B:Y2
1	Title	-7.5	341	298	295	395
2	Title	-7.0	671	752	616	481
3	Title	-6.5	874	721	362	412
4	Title	-6.0	1000	951	444	700
5	Title	-5.5	1305	1265	882	652
6	Title	-5.0	1254	1351	1354	1089
7	Title	-4.5	1265	1411	1452	1354

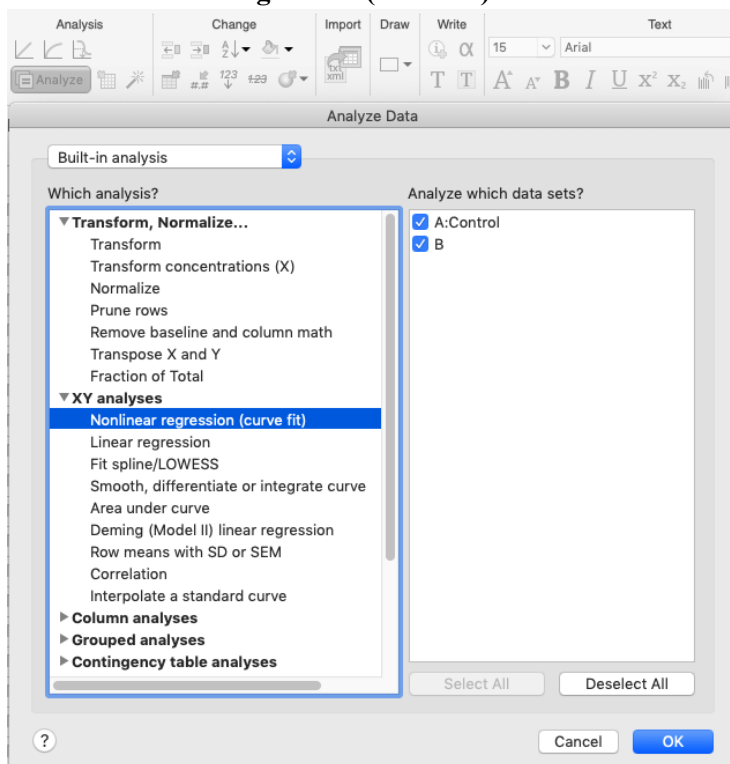
This is also the sample data "Dose-response: EC50 shift by global fitting" provided in the set of pharmacology tutorials. In this dataset, the X values are the logarithm of the concentration of agonist. The Y values are responses, in duplicate, in two conditions. Our goal is to compute the ratio of the two EC50 values.

Click “Data 4” under the Graphs section, the automatically generated plot will be like this:

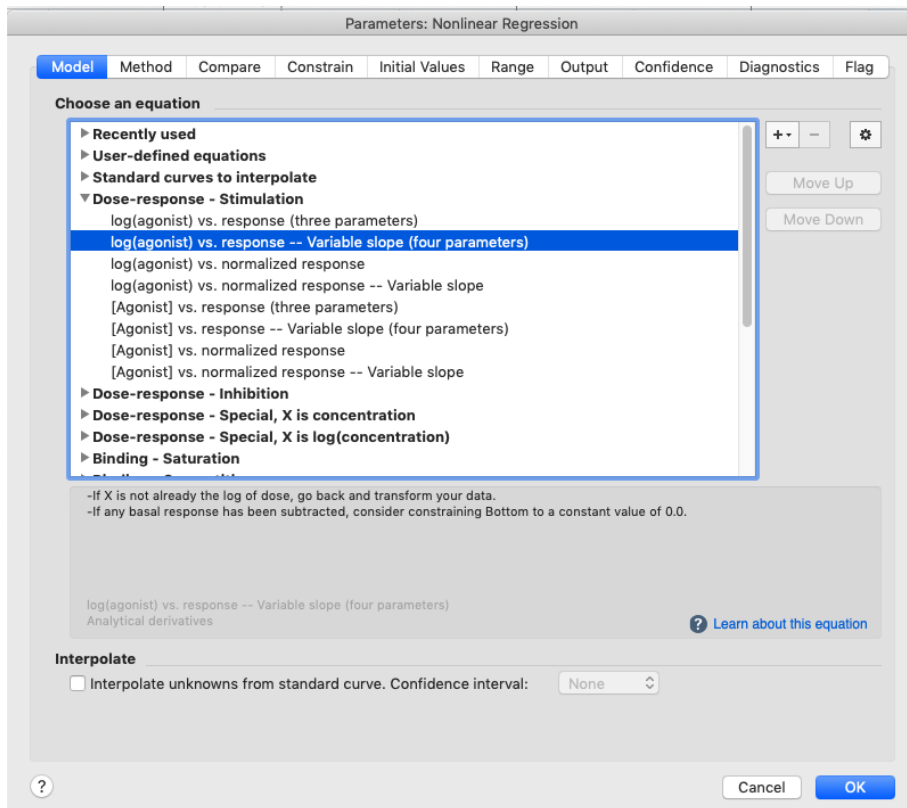


Why there is no error bar for some dots? Because if the error bar would be shorter than the size of the symbol, Prism simply won't draw it, even if the symbol is clear. [How to see the error bar](#).

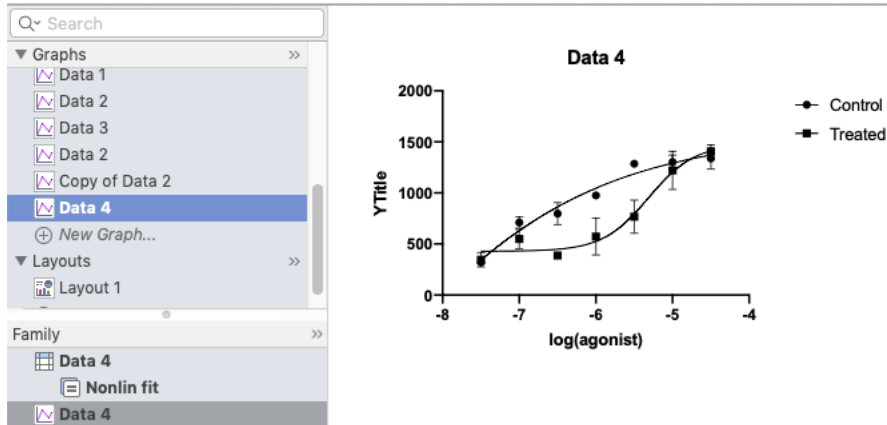
Let's do a nonlinear curve fitting on the data. Click **“Analyze”**. In the Analyze Data dialog, select **“Nonlinear regression (curve fit)”** under the **“XY analyses”**



In the following Parameters: Nonlinear Regression dialog, choose **“log(agonist) vs. response – Variable slope (four parameters)”** under **“Dose-response - Simulation”**. Click **“OK”**.



Then we can see the curves superimposed on the graph.



Or we can see the analysis results by clicking “Nonlin fit of Data 4”.

Q Search		Table of results	
Data Tables + New Data Table... Info Project info 1 + New Info... Results Nonlin fit of Data 4 + New Analysis... Graphs Data 1 Data 2 Data 3 Data 2 Copy of Data 2 Data 4 + New Graph... Layouts Layout 1 + New Layout... Family Data 4 Nonlin fit Data 4		Nonlin fit Table of results	
		A	B
		Control	Treated
		Y	Y
1	log(agonist) vs. response -- Variable slope (four parameter	Ambiguous	
2	Best-fit values		
3	Bottom	~ -3763	428.4
4	Top	1580	1478
5	LogEC50	~ -9.299	-5.312
6	HillSlope	0.2894	1.441
7	EC50	~ 5.028e-010	4.875e-006
8	Span	~ 5343	1049
9	95% CI (profile likelihood)		
10	Bottom	(Very wide)	??? to 550.6
11	Top	???	1221 to ???
12	LogEC50	(Very wide)	-5.618 to ???
13	HillSlope	???	0.2230 to ???
14	EC50	(Very wide)	2.408e-006 to ???
15	Goodness of Fit		
16	Degrees of Freedom	10	10
17	R squared	0.9501	0.9209
18	Sum of Squares	87904	169889
19	Sy.x	93.76	130.3
20			
21	Number of points		
22	# of X values	14	14
23	# Y values analyzed	14	14

The control results are labeled ambiguous. This means that Prism is unable to find a unique curve through the data. Lots of other sets of parameter values would lead to curves that fit just as well. The default nonlinear function used do not define a bottom plateau for the control (circles) data set, so its best-fit value is ambiguous. The EC50 is the concentration that gives a response halfway between the bottom and top plateaus of the curve. If the bottom is ambiguous, so is the EC50.

In order to get much better results from this data set, you could assume that that the top and bottom plateaus, and the slope, are the same under control and treated conditions. In other words, you assume that the treatment shifts the EC50 but doesn't change the basal response, the maximum response, or the Hill slope.

Return to the nonlinear regression dialog by **clicking the button in the upper left of the results table**. Go to the constraints tab and choose to share the value of Bottom, Top, and HillSlope. When you share these parameters, Prism fits the data sets globally to find one best-fit value for Bottom, Top and HillSlope (for both data sets) and separate best-fit values for the logEC50.

Q~ Search

▼ Data Tables

- Data 1
- Data 2
- Data 3
- Data 4
- New Data Table...

▼ Info

- Project info 1
- New Info...

▼ Results

- Nonlin fit of Data 4
- New Analysis...

▼ Graphs

- Data 1
- Data 2

Table of results

Nonlin fit
Table of results

Parameters: Nonlinear Regression

Model Method Compare **Constrain** Initial Values Range Output Confidence Diagnostics Flag

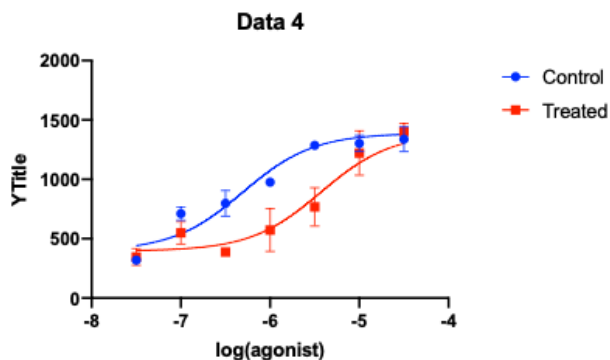
Parameter Name	Constraint Type	Value	Hook
Bottom	Shared value for all data sets		
Top	Shared value for all data sets		
LogEC50	No constraint		
HillSlope	Shared value for all data sets		

Then the fit in the result table is no longer labeled 'ambiguous' and the CIs are much tighter.

Table of results

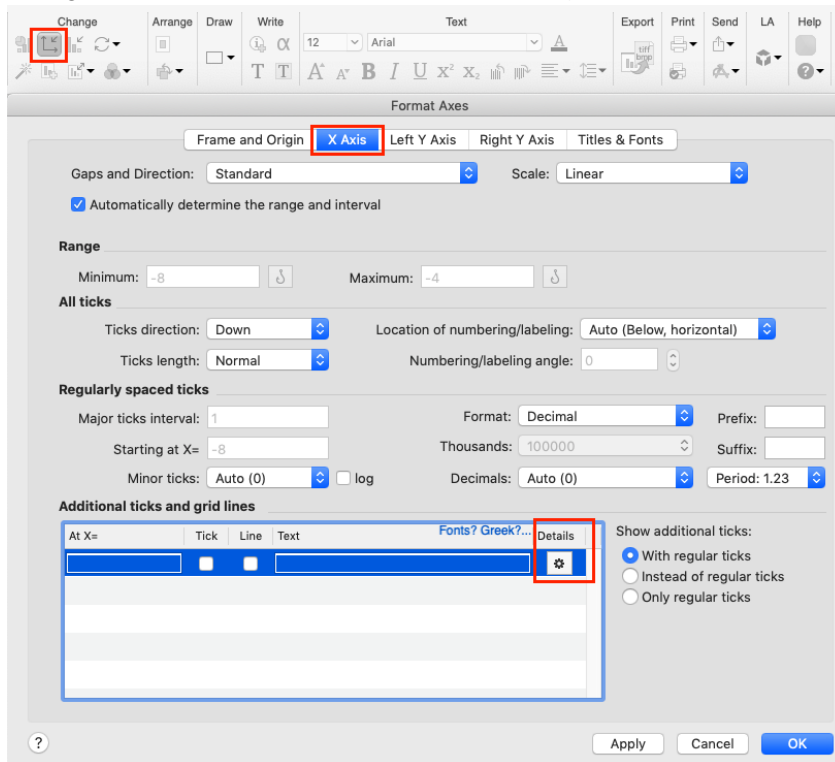
Nonlin fit Table of results		A	B	C
		Control	Treated	Global (shared)
		Y	Y	Y
1	log(agonist) vs. response -- Variable slope (four parameters)			
2	Best-fit values			
3	Bottom	395.5	395.5	395.5
4	Top	1388	1388	1388
5	LogEC50	-6.304	-5.429	
6	HillSlope	1.099	1.099	1.099
7	EC50	4.961e-007	3.720e-006	
8	Span	992.1	992.1	992.1
9	95% CI (profile likelihood)			
10	Bottom	214.9 to 511.8	214.9 to 511.8	214.9 to 511.8
11	Top	1263 to 1594	1263 to 1594	1263 to 1594
12	LogEC50	-6.627 to -5.978	-5.711 to -5.178	
13	HillSlope	0.6051 to 1.911	0.6051 to 1.911	0.6051 to 1.911
14	EC50	2.360e-007 to 1.053e-006	1.944e-006 to 6.639e-006	
15	Goodness of Fit			
16	Degrees of Freedom			23
17	R squared	0.9148	0.8999	0.9136
18	Sum of Squares	150099	214987	365087
19	Sy.x			126.0
20	Constraints			
21	Bottom	Bottom is shared	Bottom is shared	
22	Top	Top is shared	Top is shared	
23	HillSlope	HillSlope is shared	HillSlope is shared	
24				
25	Number of points			
26	# of X values	14	14	
27	# Y values analyzed	14	14	

Assign different colors to different groups.

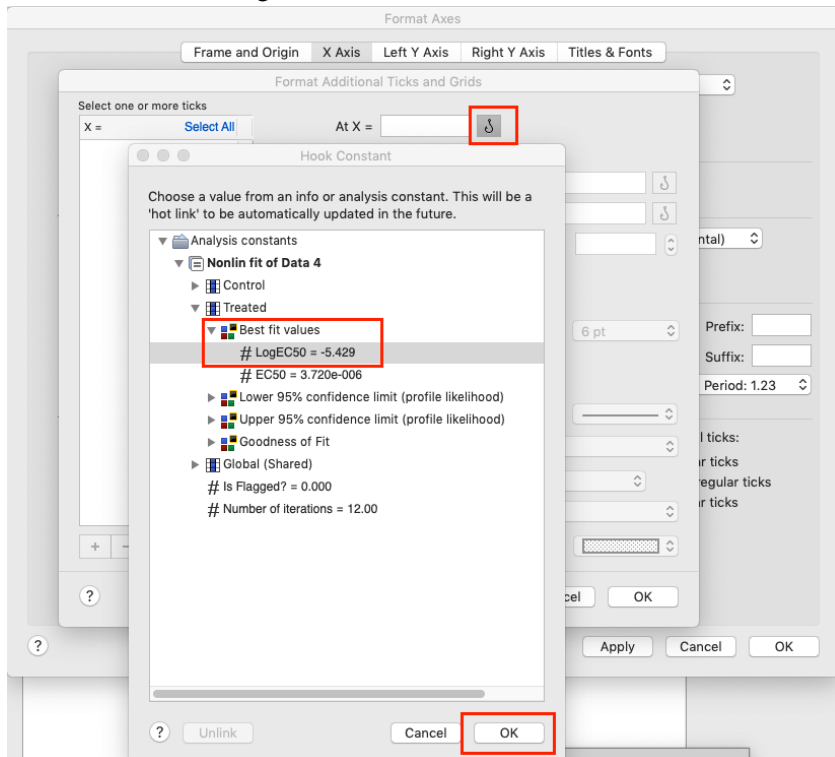


Adding additional ticks and grid lines

Click the “Format Axes” button in the Change section of the Prism toolbar. In the Format Axes dialog, choose **X Axis** tab then click the **setting button** of additional ticks and grid lines.



In Format Axes dialog, click the **hook** then select the “Best fit values” – “#LogEC50 = -5.429”



Then it goes back to the Format Additional Ticks and Grids dialog. **Define the Text Line 1 as “IC50”** and **check “Show Grid Line”**

Format Additional Ticks and Grids

Select one or more ticks

X = At X =

☒ Show Text

Text Line 1:

Text Line 2:

Location: Angle:

Offset: in.

☒ Show Tick

Size: Thickness:

Direction:

☒ Show Grid Line

Thickness: Style:

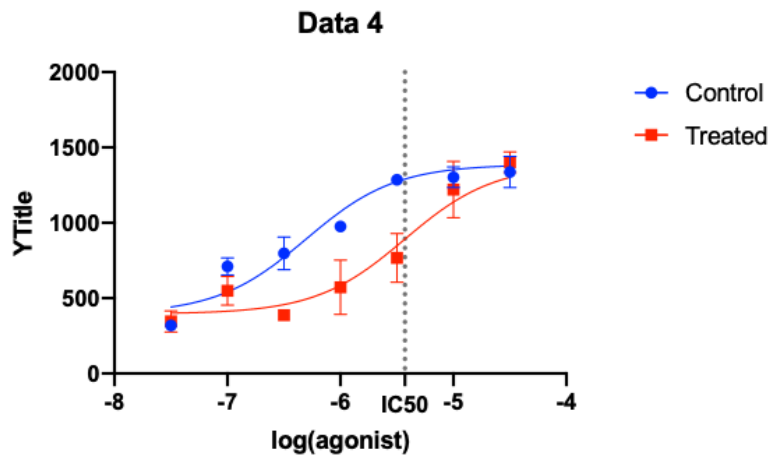
Color:

☐ Fill (shade) between this tick and X =

Fill Color:

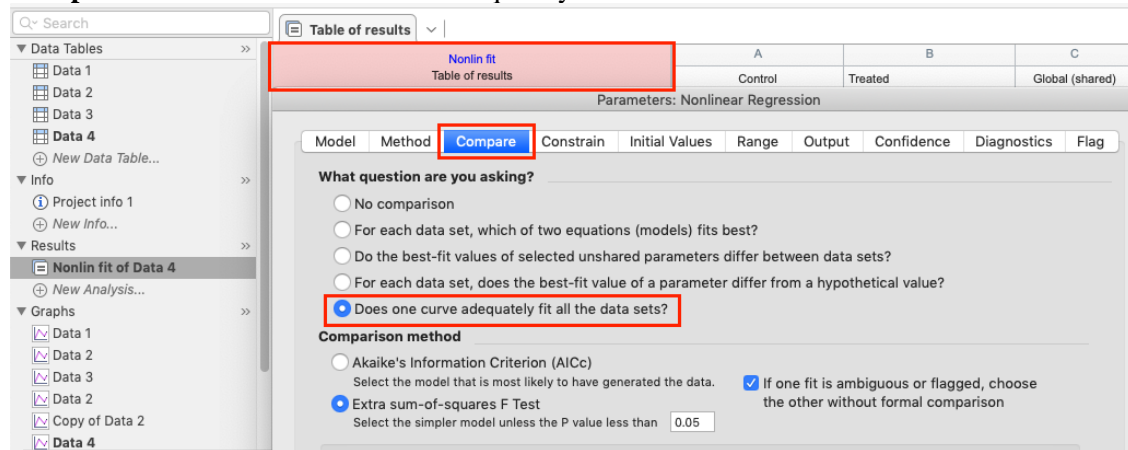
☐ Fill pattern Color: Pattern:

Then the final plot will be like this:



Statistically compare the two logEC50 values

Go back to the parameters dialog for nonlinear regression and **go to the Compare tab**. Check the option to test whether one curve adequately fits all the data sets.



Prism will now fit the data two ways. The 1st is the same as before, fitting a separate IC50 for each data set where three parameters were already shared but one wasn't. The 2nd fit shares all the parameters where all four parameters are shared, so Prism fits one curve through all the data, ignoring which treatment group they are in. The results are shown at the top of the results sheet.

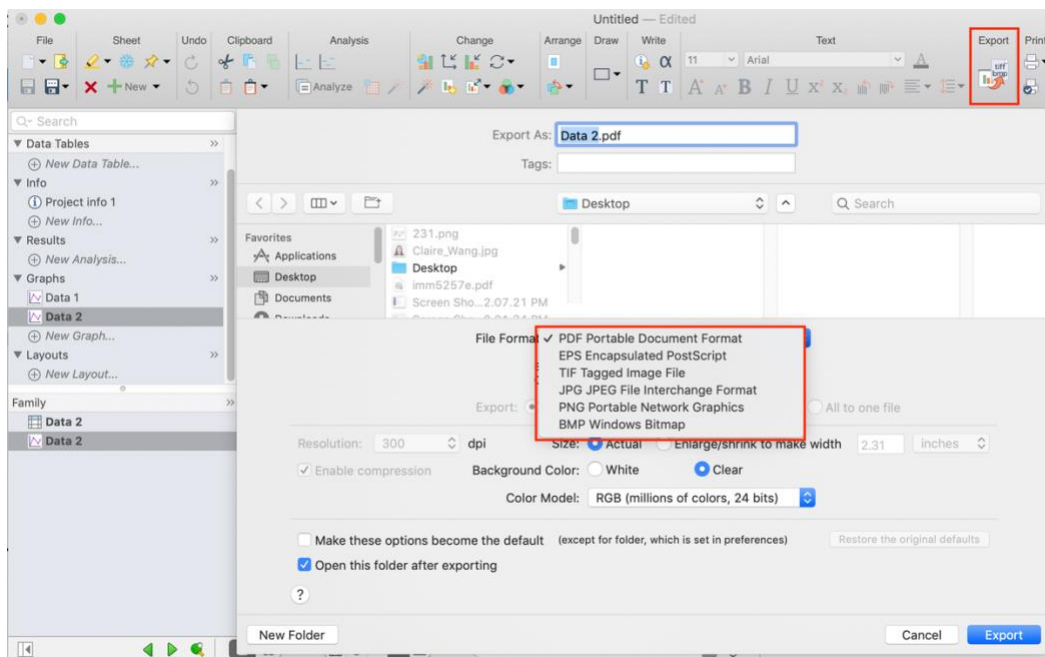
Nonlin fit Table of results		A	B	C
		Control	Treated	Global (shared)
		Y	Y	Y
1	Comparison of Fits			
2	Null hypothesis			LogEC50 same for all data sets
3	Alternative hypothesis			LogEC50 different for each data set
4	P value			<0.0001
5	Conclusion (alpha = 0.05)			Reject null hypothesis
6	Preferred model			LogEC50 different for each data set
7	F (DFn, DFd)			33.55 (1, 23)

The P value is tiny, so we reject the null hypothesis that the two IC50 values are identical in the population, and instead conclude that the two IC50 values are different.

8. Exporting images from Prism

- 1) If your goal is to send graphs to a colleague who doesn't own Prism, consider sending a Prism file. He or she can then open the file using Prism itself, or the free Prism trial (which expires in 30 days but then becomes a free Prism viewer that never expires).
- 2) If your goal is to place graphs into a Word document or PowerPoint presentation, you can **copy-and-paste** or **use the one-click send buttons** rather than exporting.
- 3) If you do want to export Prism images. **Click the Export button** on the Prism toolbar to bring up the Export Graph dialog. Alternatively, **drop the File menu and choose Export**. You can also use the Send-to-PowerPoint or Send-to-Word buttons. Or the Print button.

Choose the [export format](#), and also choose the location and name of the exported file.



The comparison between PDF and TIFF/TIF formats:

Export Formats	PDF	TIFF / TIF
Advantages	File can be stretched to any size with no loss of quality	Tend to be trouble-free when submitted to journals
Disadvantages	<ol style="list-style-type: none"> 1. Few Windows programs import pdf images 2. Few Journals accept them. 	You need to choose resolution and size

Conclusion

It is really complicated to create a graph template to be applied to all situations. It is better to think about what you would like to include in your graph and how you would like to present them. The best way might be checking how other people present their results in this area.

If you are still have any question about visualization, please feel free to contact me (qinlu.wang@nih.gov) or our BCBB (bioinformatics@niaid.nih.gov)

Reference:

[GraphPad Prism Tutorial 2 - Making XY Graphs](#)
[Graphs](#)