

Visualization

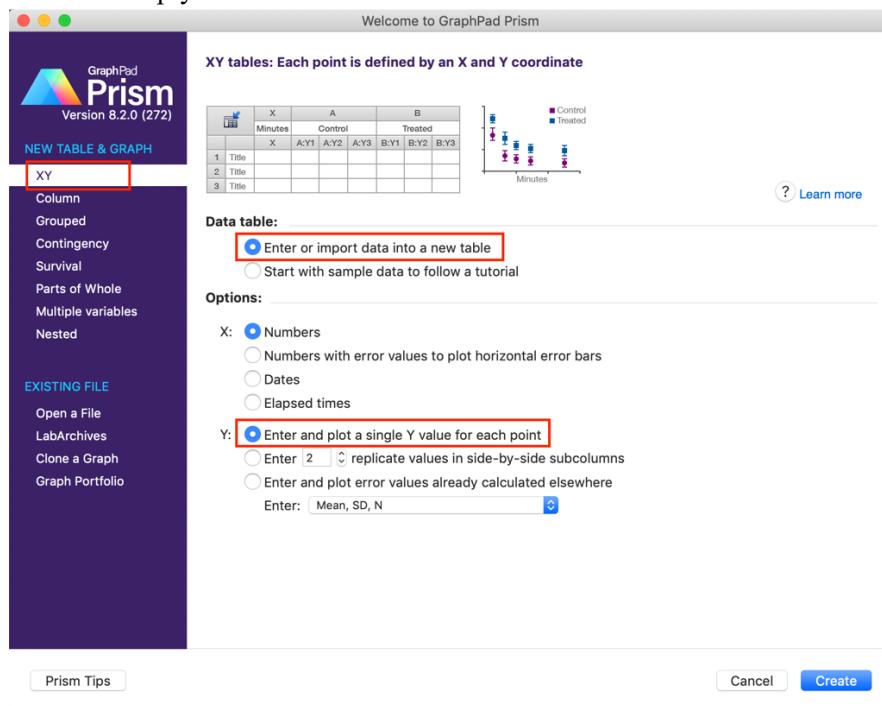
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Outline

1. How to create graphs in Prism
2. How to change a graph
3. Breaking Y axis into two segments
4. Graphing Example: XY plots – Nonlinear curve fitting
5. 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:



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

DATA SET 1

	Heart Rate	
	Treatment 1	Treatment 2
0	24	25
1	25	12
2	24	13
3	26	14
4	27	17
5	28	30
6	40	21
7	25	14
8	30	10

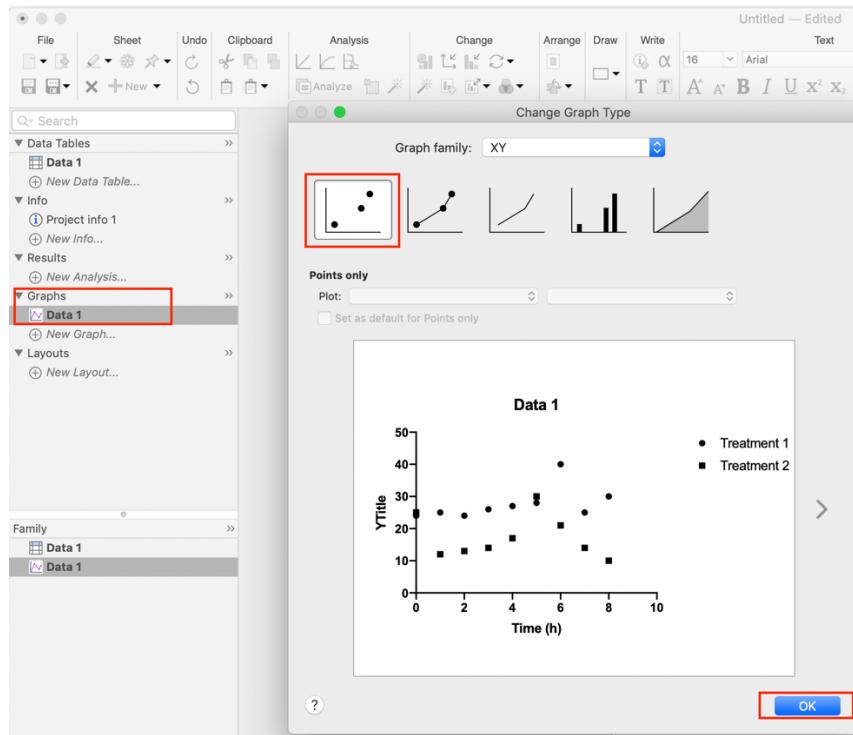
DATA SET 2

	Control	Treated	Treated+Antagonist
54	87	45	
23	98	39	
45	64	51	
54	77	49	
45	89	50	
47		55	

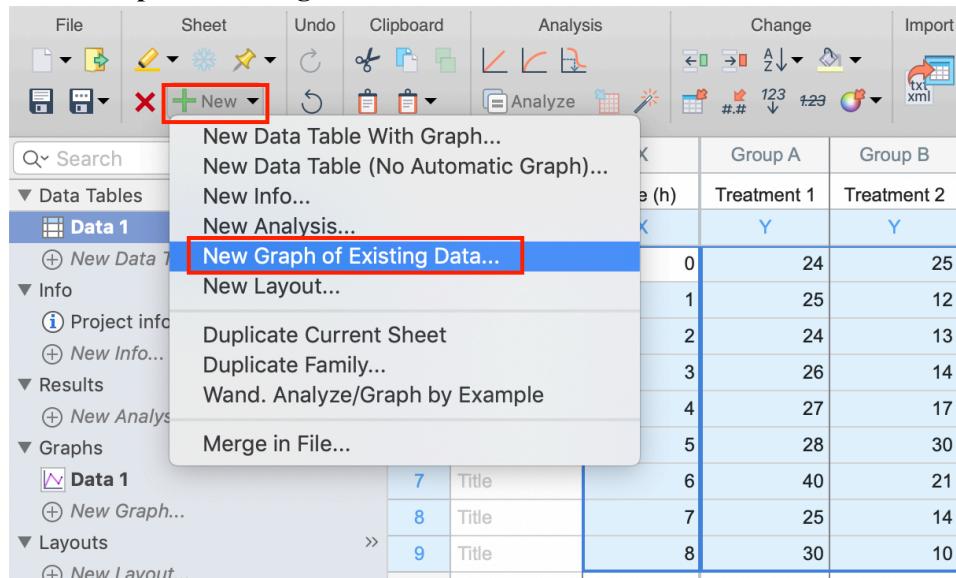
Go back to the Prism file and paste.

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



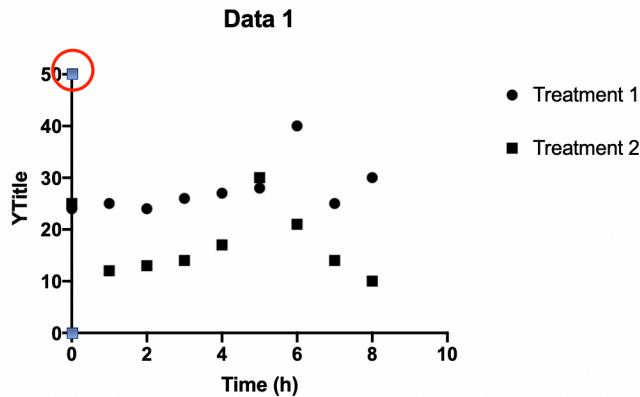
If you would like to create a new graph of existing data, **click the “New” button and choose “New Graph of Existing Data”**



2. How 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.

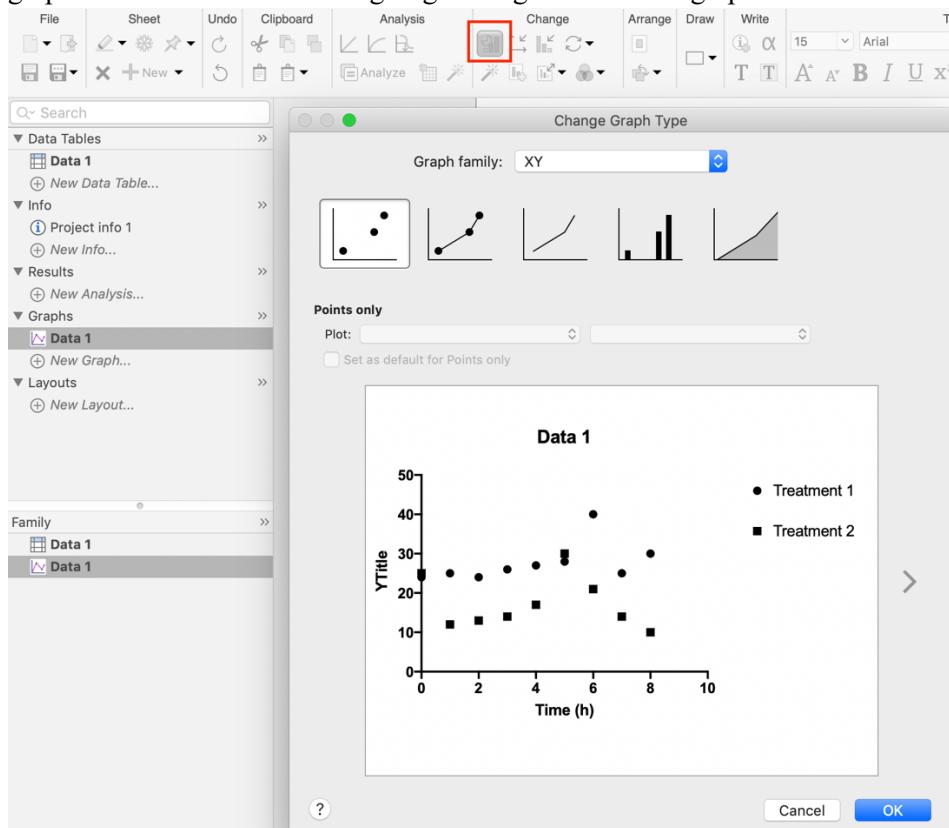


2) Shortcut Menus

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.

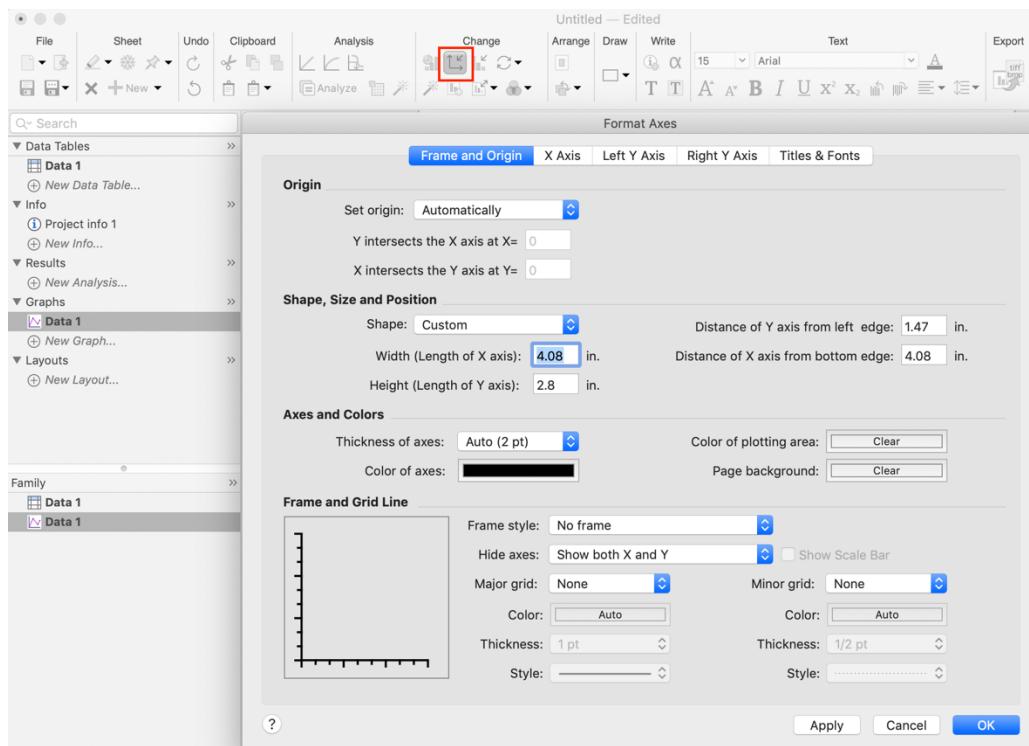
3) Change Graph Type dialog - 1st button in Change Section

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



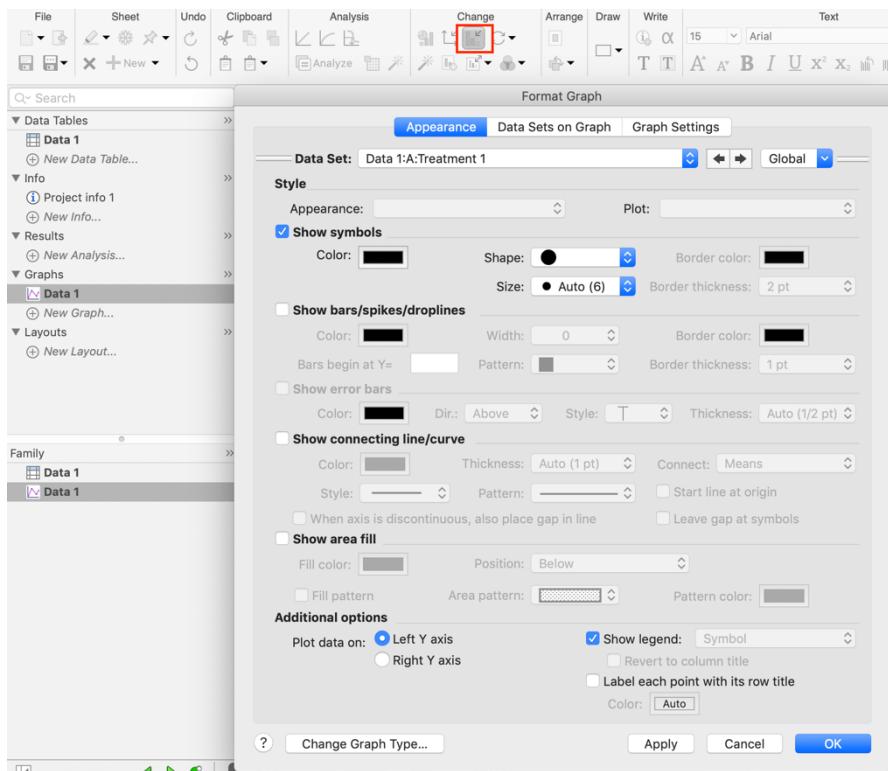
4) Format Axes Dialog – 2nd button in Change Section

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.



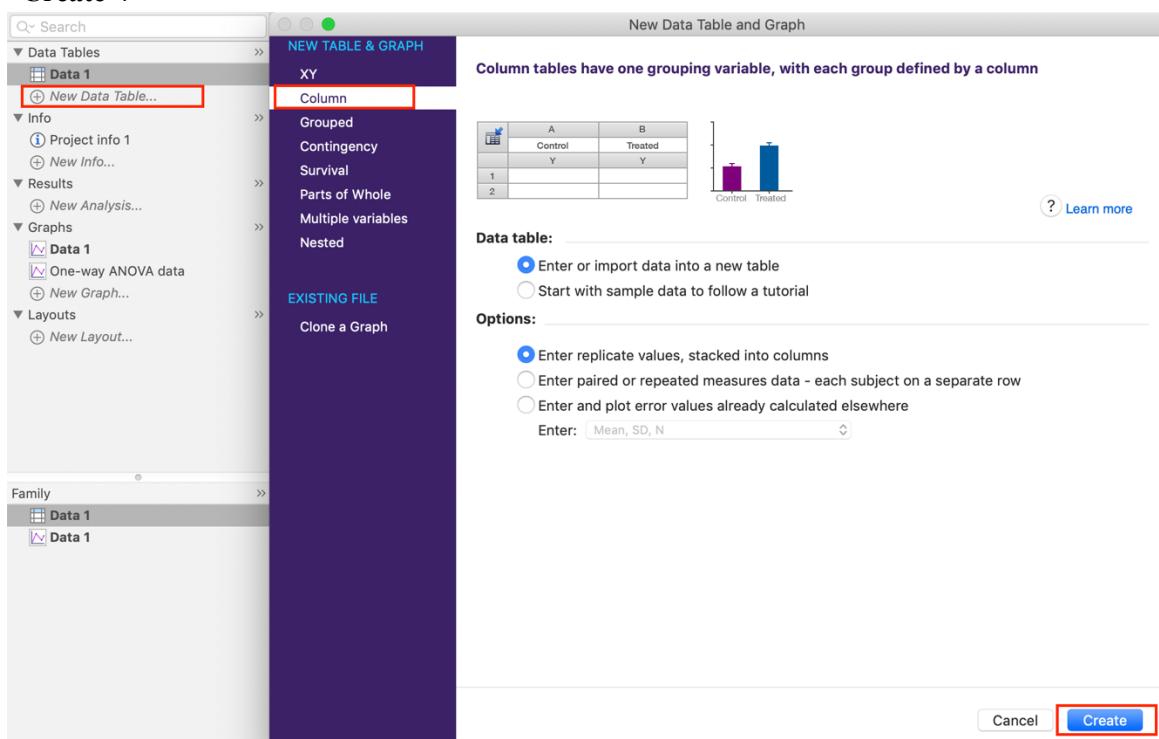
5) Format Graph dialog – 3rd button in Change Section

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.



6) Rotating, flipping, reversing – 4th button in Change Section

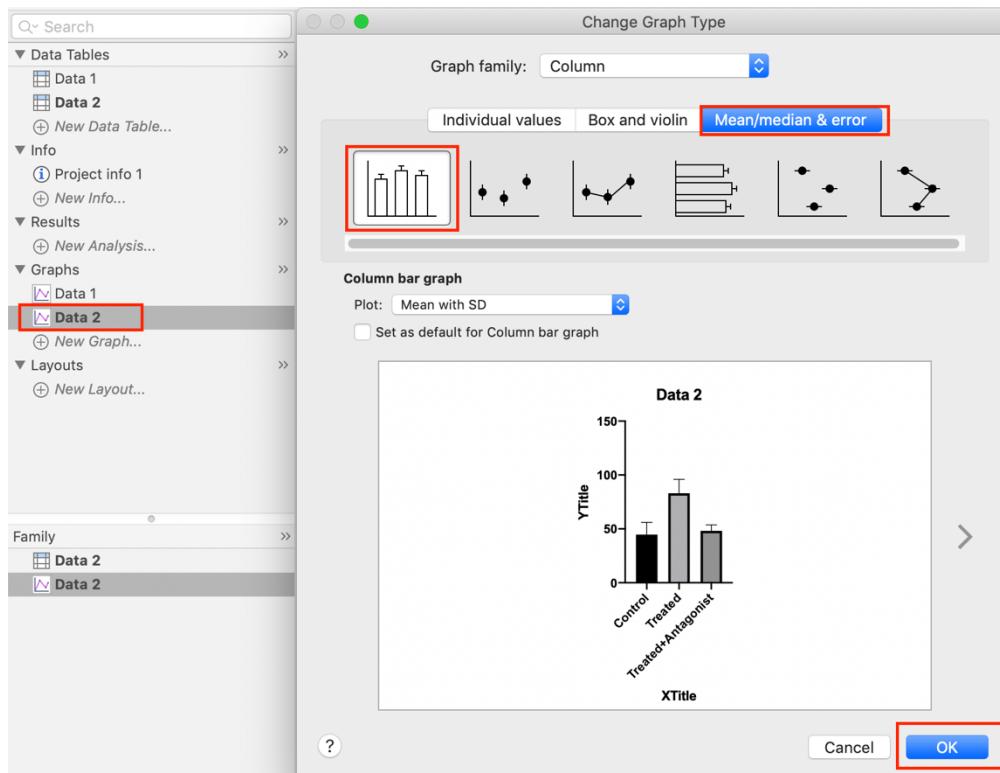
Create a new dataset by **clicking the “New Data Table...”** on the data tables list. Then choose **“Column”** data table and **“Enter or import data into a new table”**. Click **“Create”**.



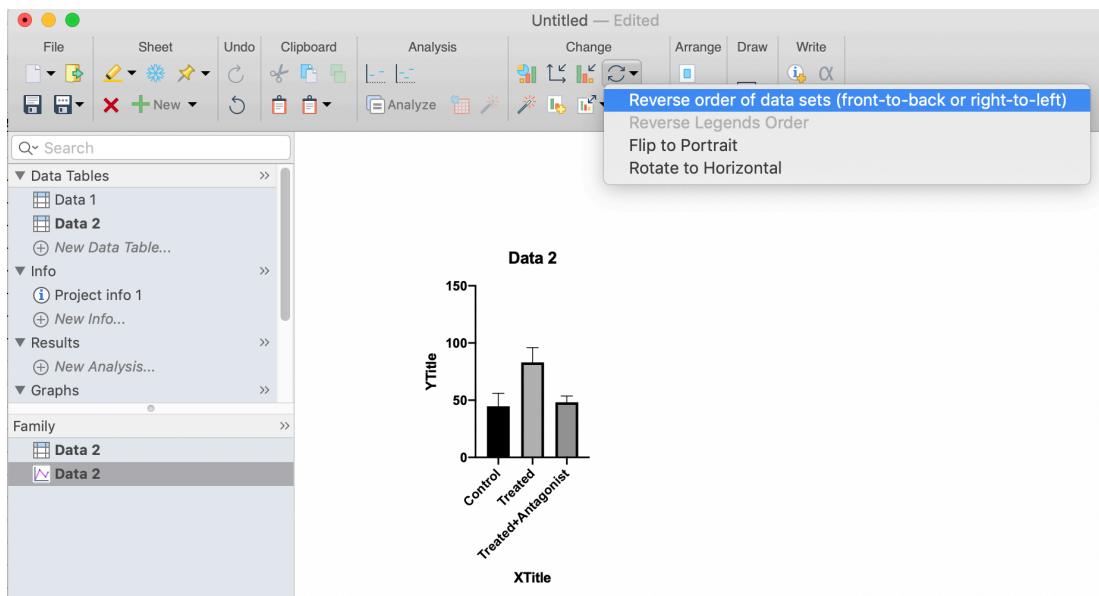
Go to the excel file “2.visualization.xlsx” and copy “DATA SET 2” then paste it to the empty Prism data table.

		Group A	Group B	Group C
		Control	Treated	Treated+Antagonist
	1	Y	Y	Y
	2	54	87	45
	3	23	98	39
	4	45	64	51
	5	54	77	49
	6	45	89	50
	7	47		55
	8			
	9			
	10			
	11			
	12			

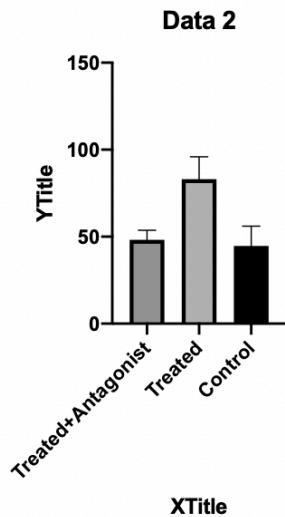
Click the graph “Data 2”. In the Change Graph Type dialog, choose “Mean/median & error” then the first bar chart then click “OK”.



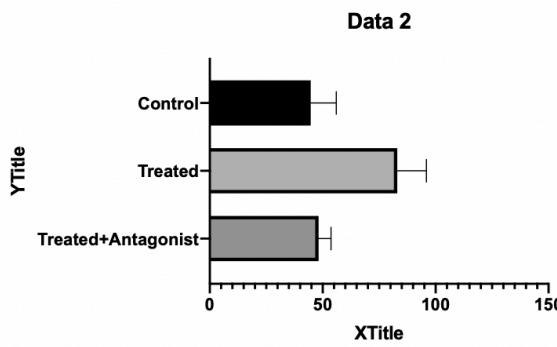
Click the Rotate/Flip button in the Change section of the Prism toolbar drops a menu with four choices. You can reverse the order of datasets on a graph, flip the page settings between landscape or portrait orientation, or rotate column graphs from vertical to horizontal.



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



Click “Rotate to Horizontal”:



7) Magic – Make Graphs Consistent – 5th button in Change Section

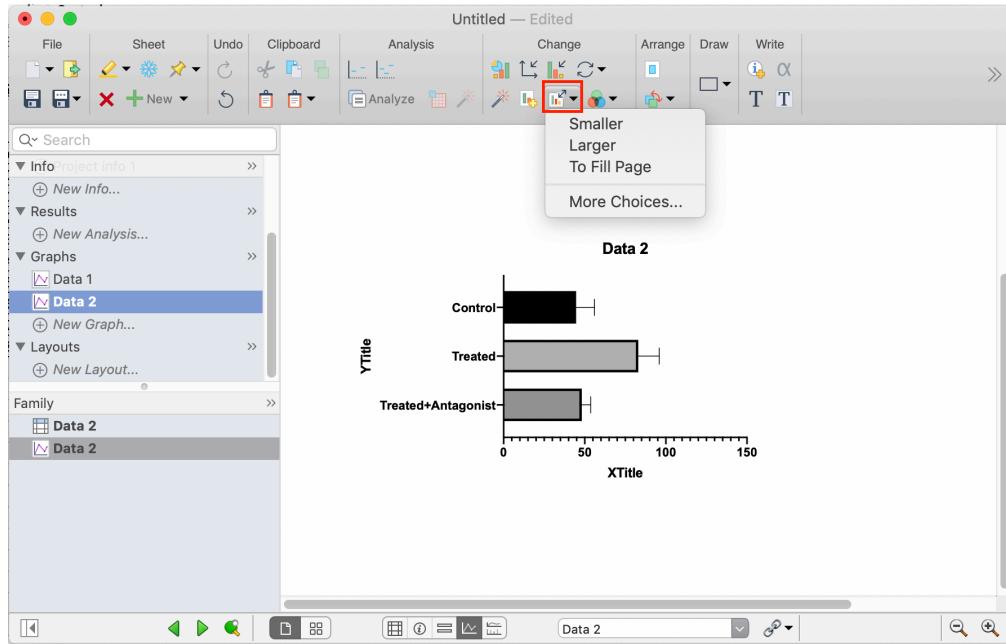
The Prism Magic tool makes one (or more) graph look like another. It is a huge time saver. More details could be found at [Prism Magic](#).

8) Data Sets on Graph – 6th button in Change Section

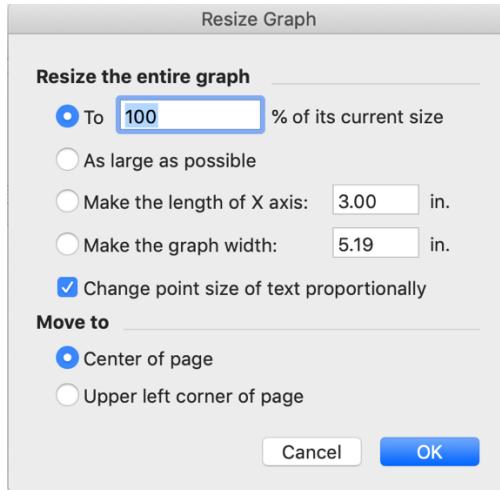
It is the same with the 3rd button in change section “Format Graph”

9) Changing a graph's shape and size – 7th button in Change Section

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.

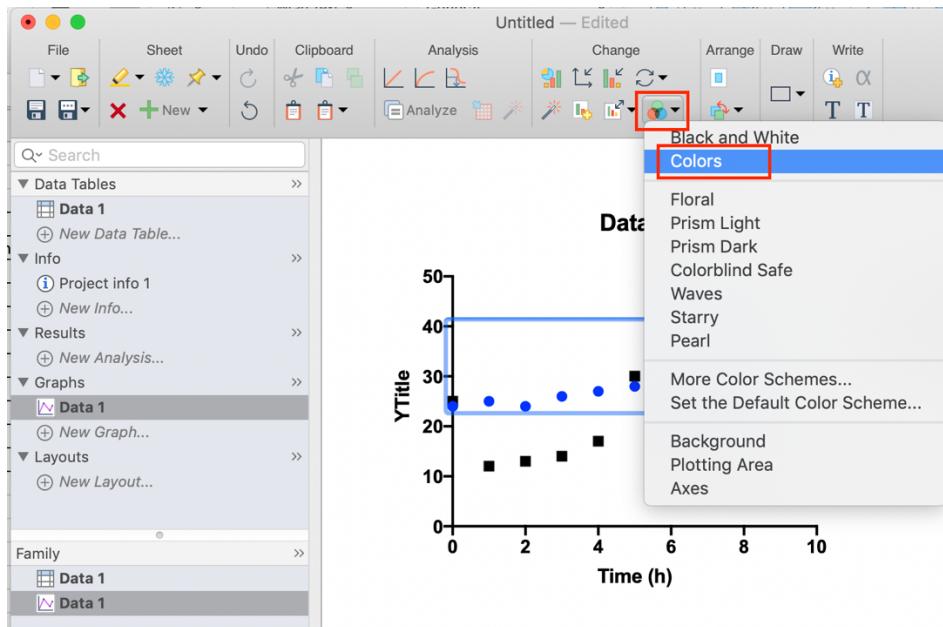


If you click More Choices, a dialog lets you pick the exact size you want and lets you resize the graph without changing font size. This can be useful when the journal specifies both the exact graph size it wants and the point size of fonts.



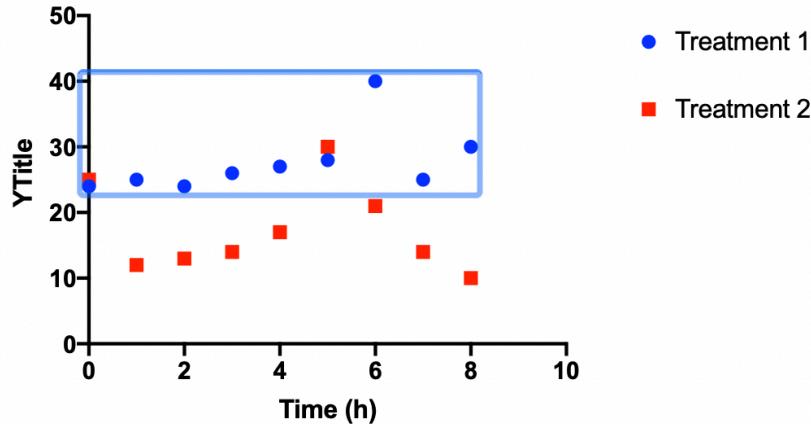
10) Color Schemes – 8th button in Change Section

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

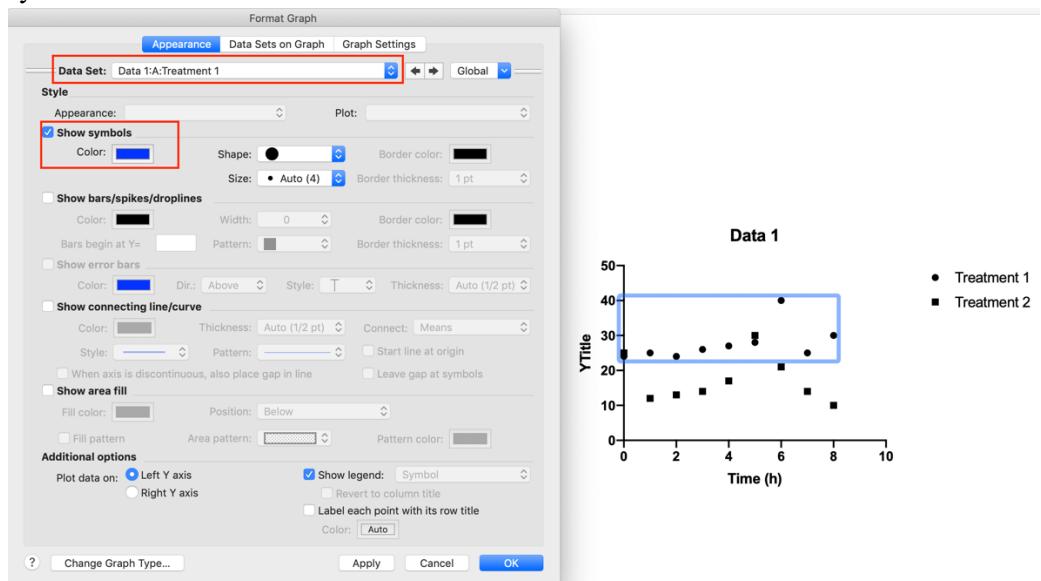


Then Prism assigns different colors to different treatment groups respectively.

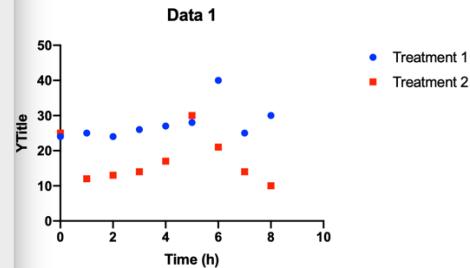
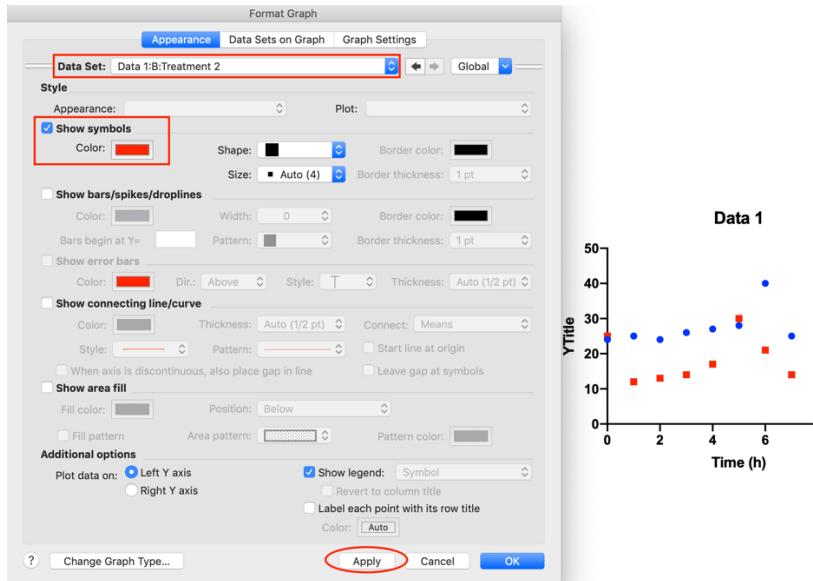
Data 1



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**.



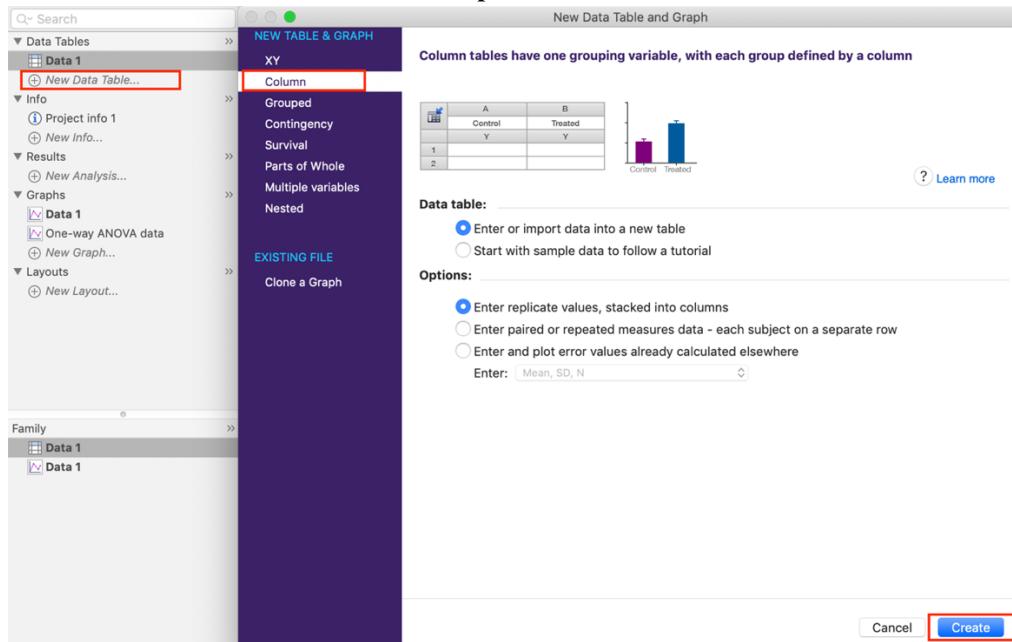
Select “**Data 1:B:Treatment 2**” from the drop-down list of “Data Set”. Change the “color” under the “Show symbols” to **Red**. Click “**Apply**”.



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

3. 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.

Search

▼ Data Tables >>

- Data 1
- Data 2
- Data 3**
- + New Data Table...

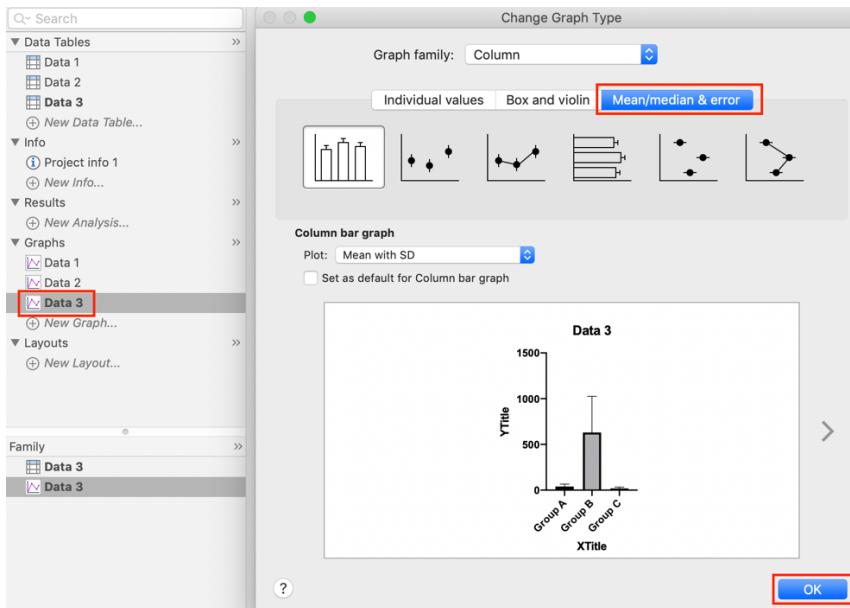
▼ Info >>

- Project info 1
- + New Info...

▼ Results >>

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

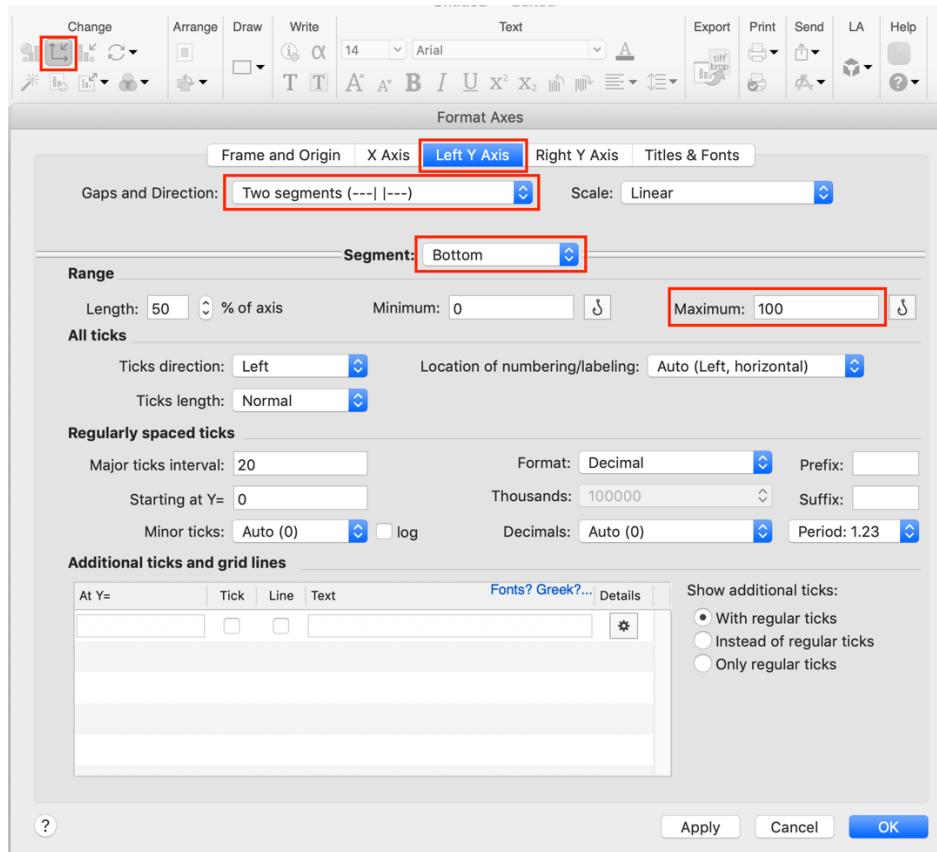
Similarly, click the graph “Data 3”. In the Change Graph Type dialog, choose “Mean/median & error” then the first bar chart then click “OK”.



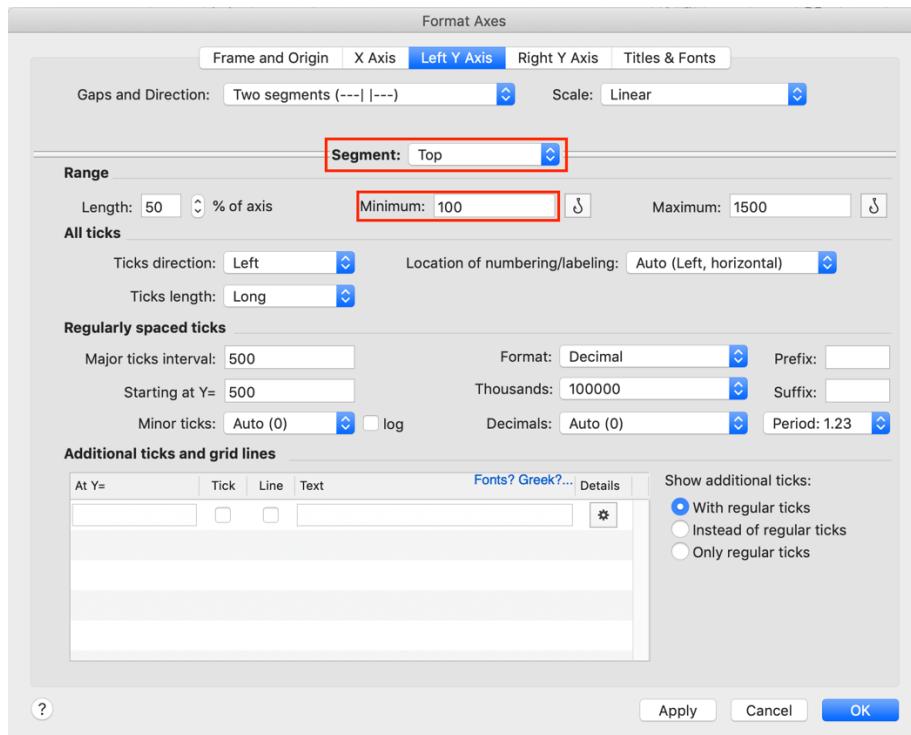
Apparently, Group B has a much higher distribution than the other 3 groups, which makes it not clear to see the boxplots of the other 2 groups. Therefore, we split the Y axis into two parts.

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.

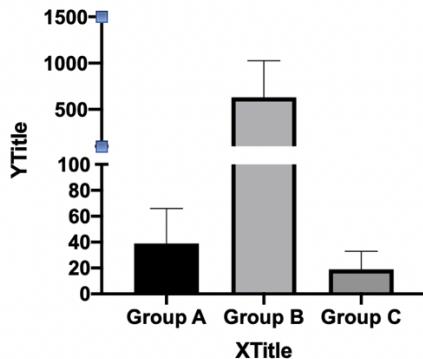


For the top segment, choose “Top” then assign the Minimum as 100.



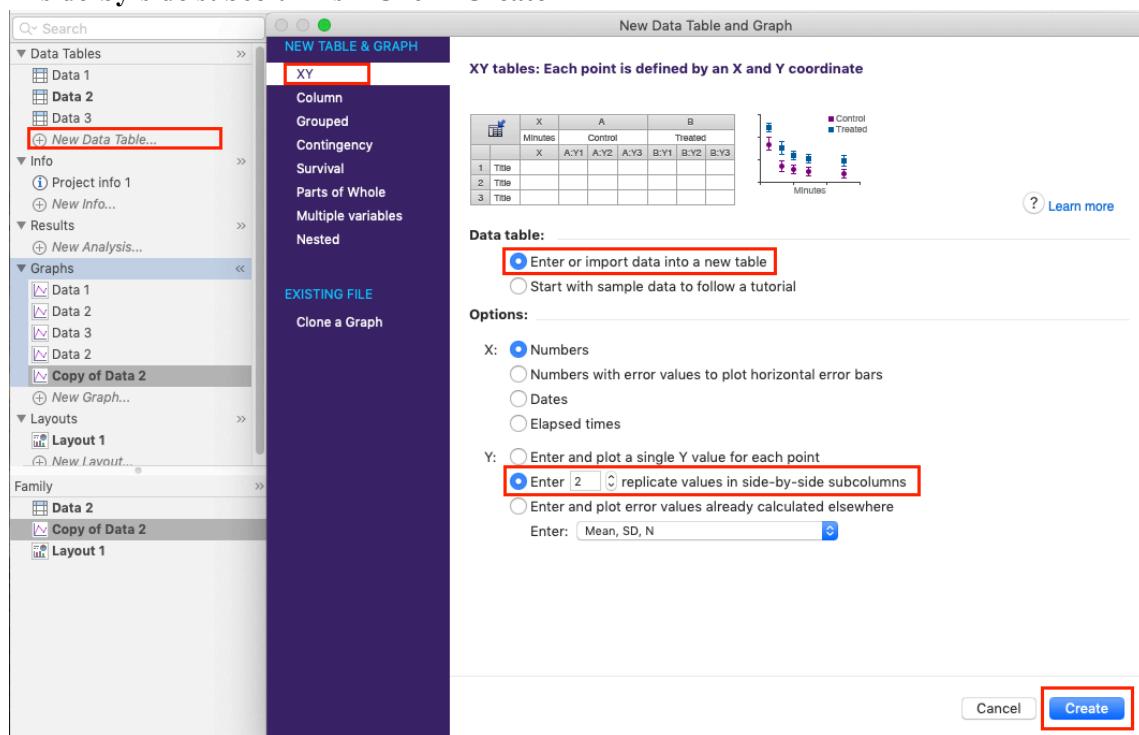
The customized plot will be like this:

Data 3



4. 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.

Q Search

▼ Data Tables >>

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

▼ Info >>

- Project info 1
- + New Info...

▼ Results >>

- + New Analysis...

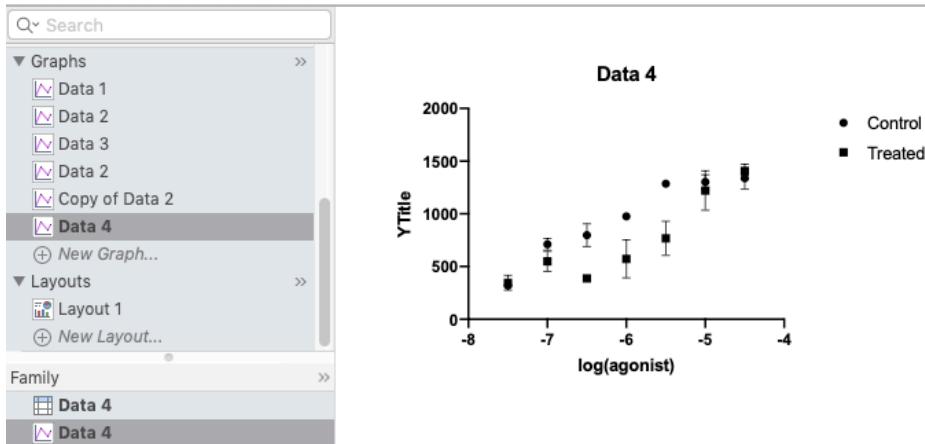
▼ Graphs >>

Table format: XY

	X	Group A		Group B	
		log(agonist)	A:Y1	A:Y2	B:Y1
1	Title	-7.5	341	298	295
2	Title	-7.0	671	752	616
3	Title	-6.5	874	721	362
4	Title	-6.0	1000	951	444
5	Title	-5.5	1305	1265	882
6	Title	-5.0	1254	1351	1354
7	Title	-4.5	1265	1411	1452

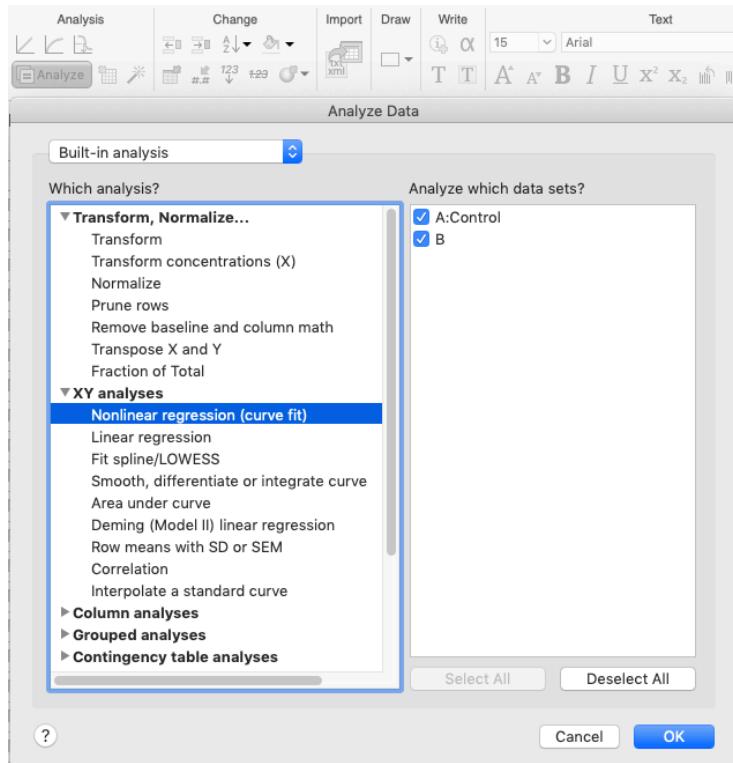
This is also the sample data "Dose-response: EC50 shift by global fitting" provided in the set of pharmacology tutorials. In this dataset, the X values are the logarithm of the concentration of agonist. The Y values are responses, in duplicate, in two conditions. Our goal is to compute the ratio of the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

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

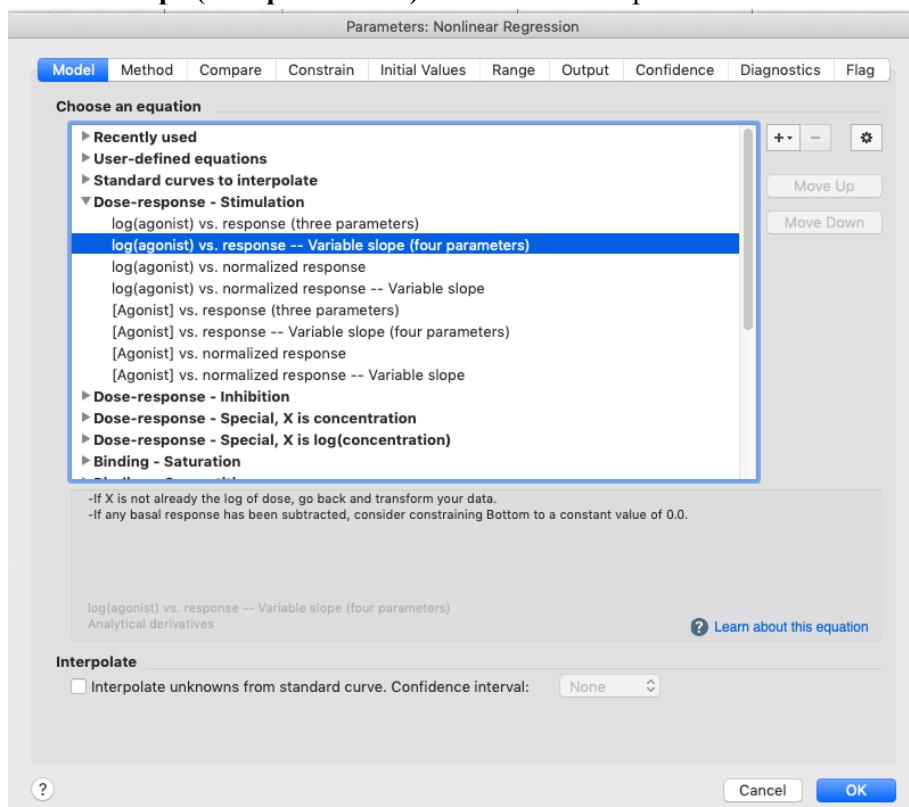


Why there is no error bar for some dots? Because if the error bar would be shorter than the size of the symbol, Prism simply won't draw it, even if the symbol is clear. [How to see the error bar](#).

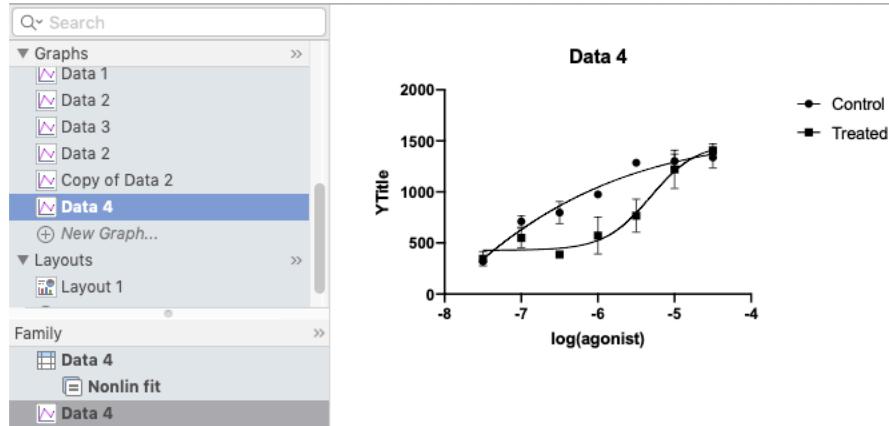
Let's do a nonlinear curve fitting on the data. Click “Analyze”. In the Analyze Data dialog, select “Nonlinear regression (curve fit)” under the “XY analyses”



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”.

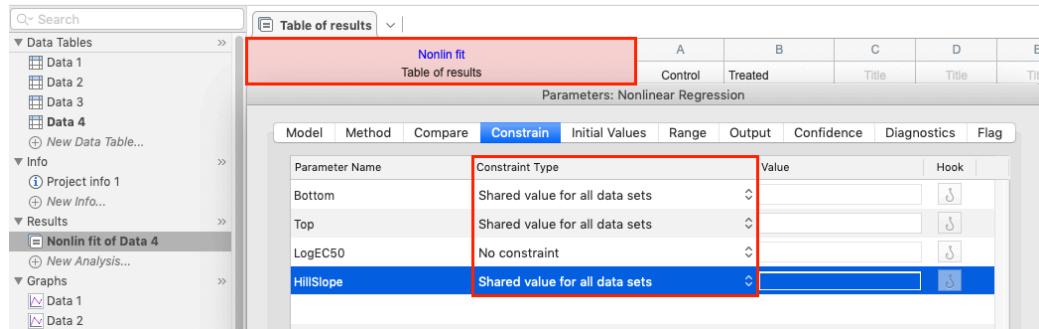
	A	B
	Control	Treated
1	log(agonist) vs. response -- Variable slope (four parameter)	Ambiguous
2	Best-fit values	
3	Bottom	~ -3763
4	Top	1580
5	LogEC50	~ -9.299
6	HillSlope	0.2894
7	EC50	~ 5.028e-010
8	Span	~ 5343
9	95% CI (profile likelihood)	
10	Bottom	(Very wide)
11	Top	??? to ???
12	LogEC50	(Very wide)
13	HillSlope	??? to ???
14	EC50	(Very wide)
15	Goodness of Fit	
16	Degrees of Freedom	10
17	R squared	0.9501
18	Sum of Squares	87904
19	Sy.x	93.76
20		130.3
21	Number of points	
22	# of X values	14
23	# Y values analyzed	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.

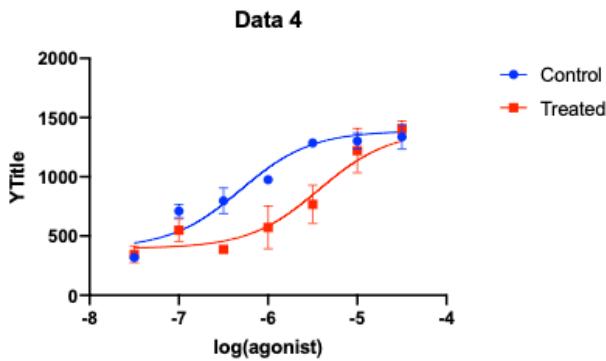
Return to the nonlinear regression dialog by **clicking the button in the upper left of the results table**. Go to the constraints tab and choose to share the value of Bottom, Top, and HillSlope. When you share these parameters, Prism fits the data sets globally to find one best-fit value for Bottom,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



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

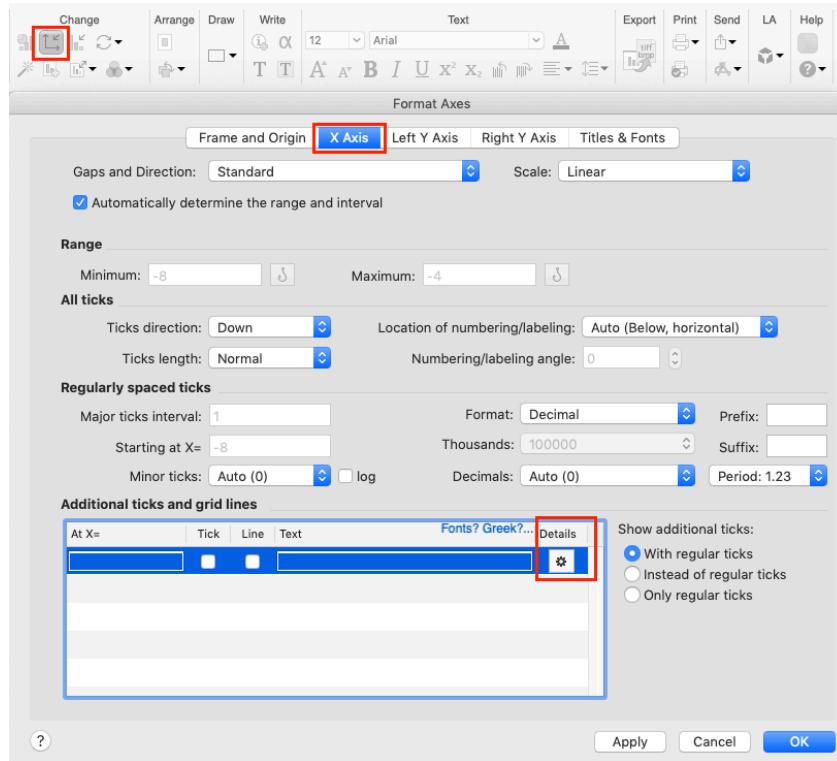
	Nonlin fit Table of results	A	B	C
		Control	Treated	Global (shared)
1	log(agonist) vs. response -- Variable slope (four parameters)			
2	Best-fit values			
3	Bottom	395.5	395.5	395.5
4	Top	1388	1388	1388
5	LogEC50	-6.304	-5.429	
6	HillSlope	1.099	1.099	1.099
7	EC50	4.961e-007	3.720e-006	
8	Span	992.1	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.

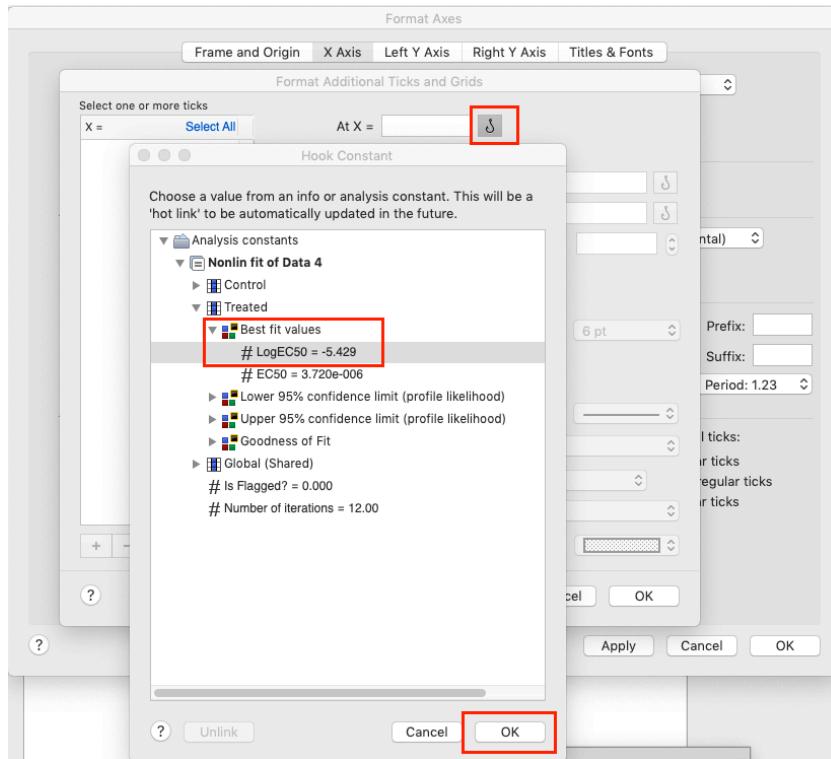


Adding additional ticks and grid lines

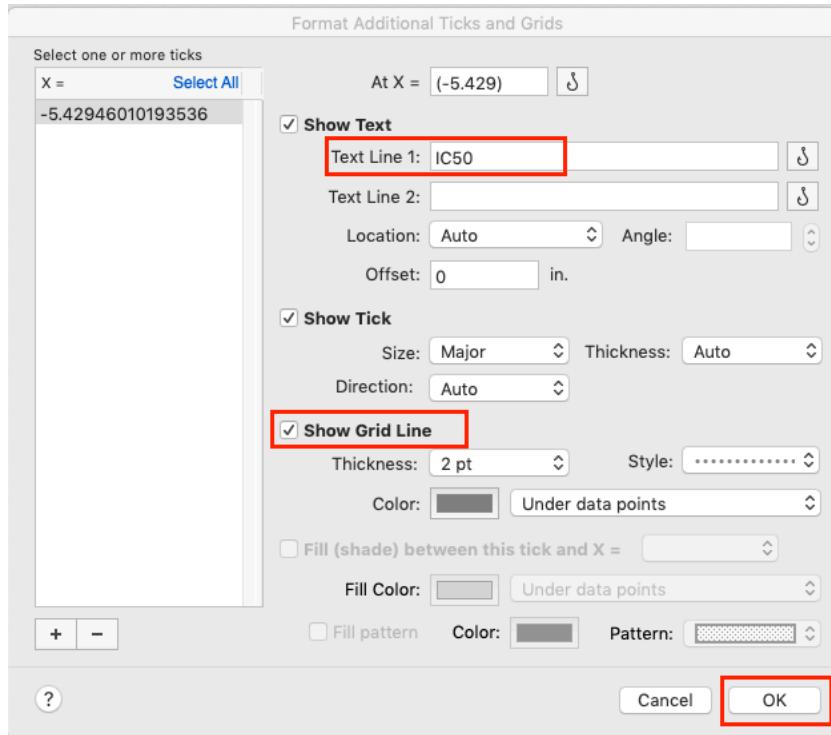
Click the “Format Axes” button in the Change section of the Prism toolbar. In the Format Axes dialog, choose X Axis tab then click the setting button of additional ticks and grid lines.



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

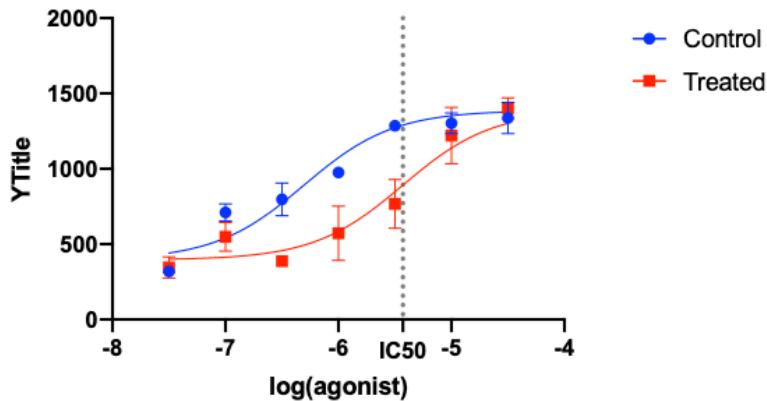


Then it goes back to the Format Additional Ticks and Grids dialog. **Define the Text Line 1 as “IC50” and check “Show Grid Line”**



Then the final plot will be like this:

Data 4



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.

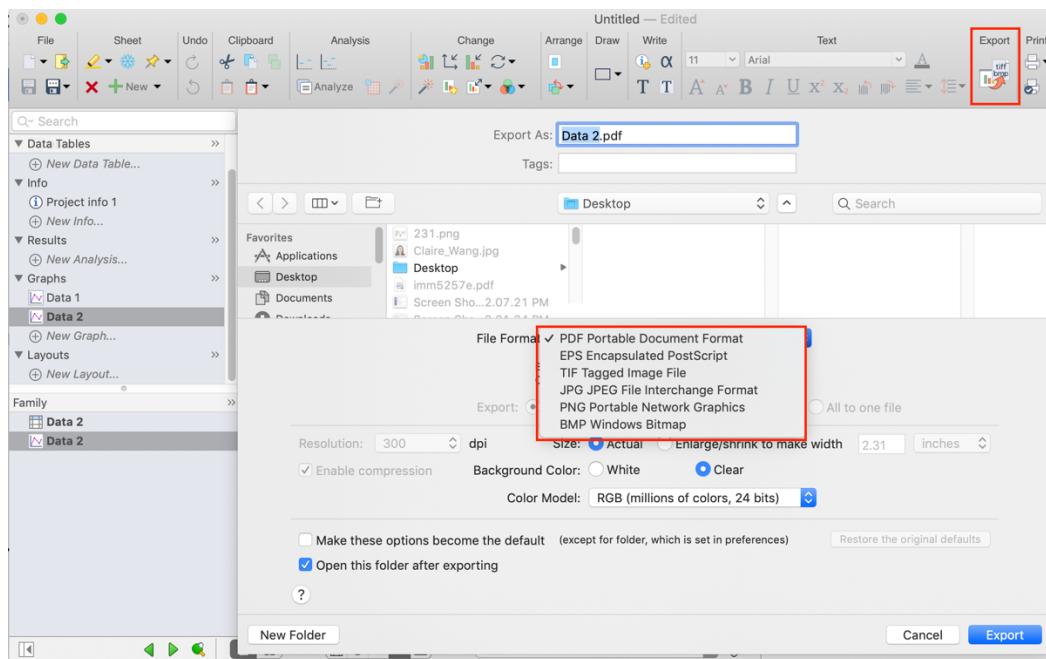
Nonlin fit		A	B	C
	Table of results	Control	Treated	Global (shared)
		Y	Y	Y
1	Comparison of Fits			
2	Null hypothesis			LogEC50 same for all data sets
3	Alternative hypothesis			LogEC50 different for each data set
4	P value			<0.0001
5	Conclusion (alpha = 0.05)			Reject null hypothesis
6	Preferred model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

5. 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	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 (bioinfomatics@niaid.nih.gov)

Reference:

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