

Sorting Cytokines Using Regression Methods and Decision Trees in Immunology Studies

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Outline

- 1 Background**

- 2 LPS Stimulation of Mouse Macrophages *In Vitro***

- 3 Serum from Healthy Humans Challenged with LPS**

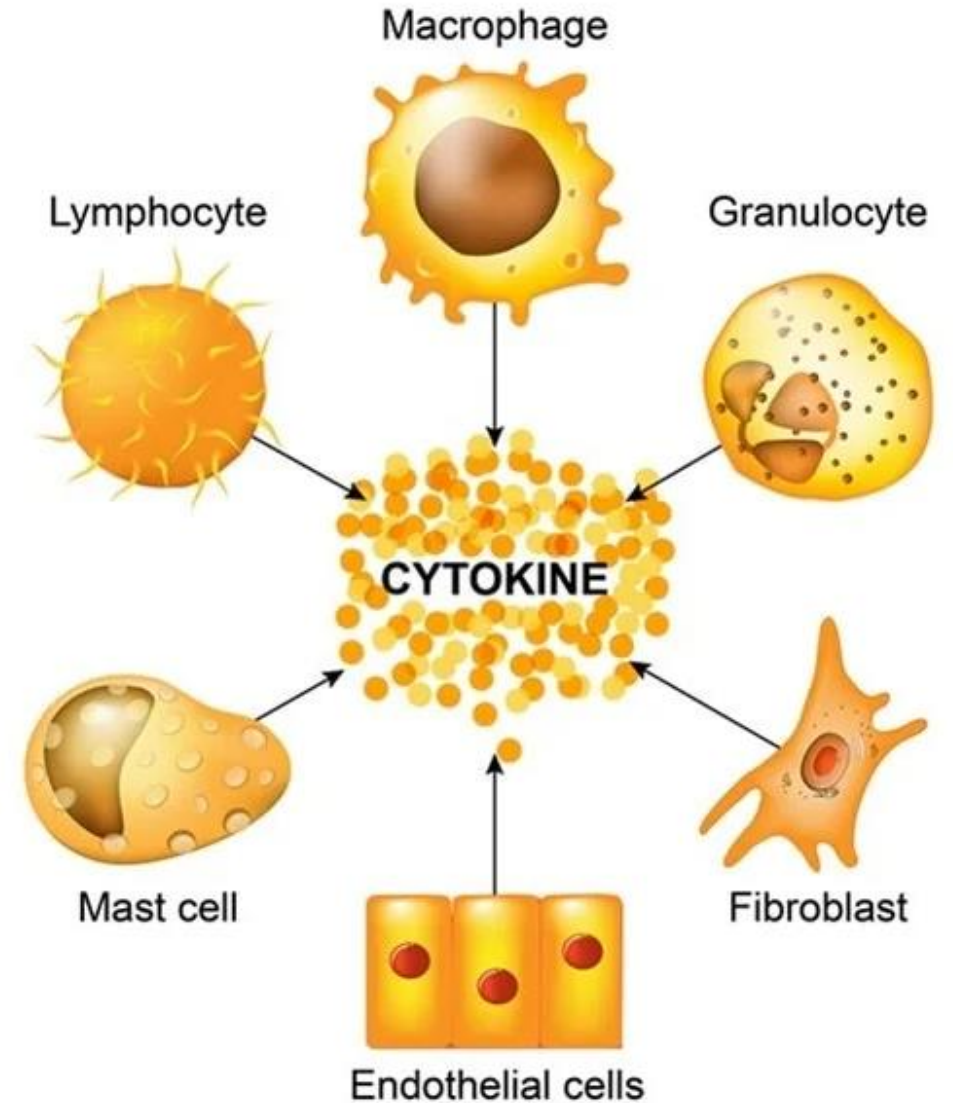
- 4 *Ex Vivo* Stimulation of Healthy Human Blood with LPS or TNF- α**

- 5 Conclusion**

Background

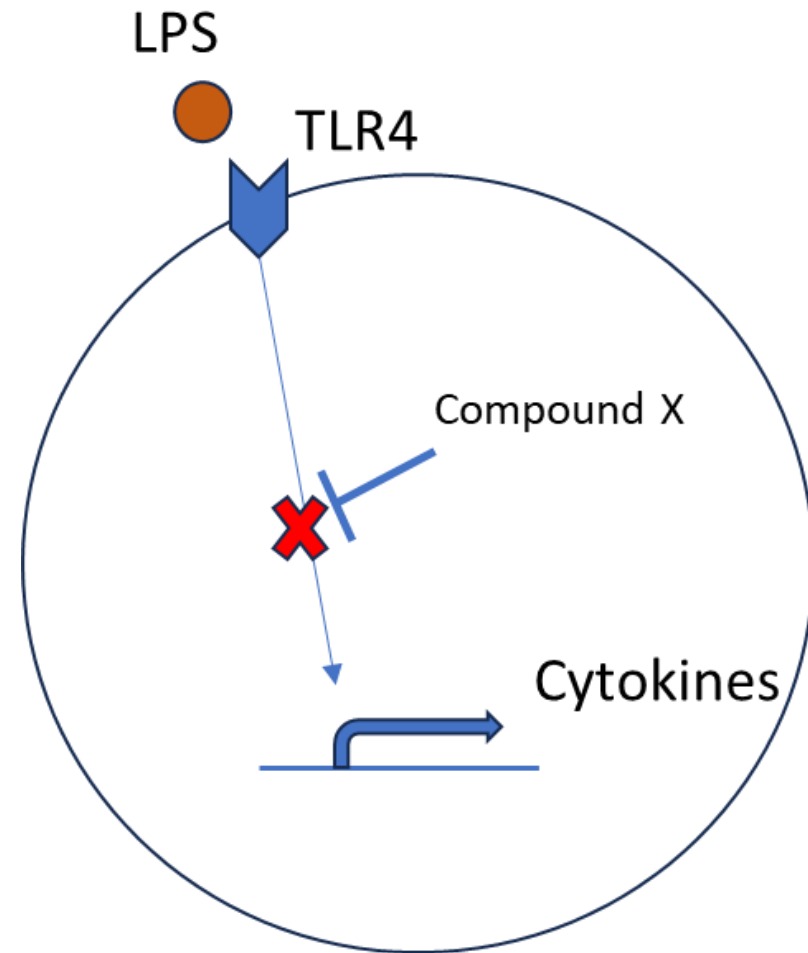
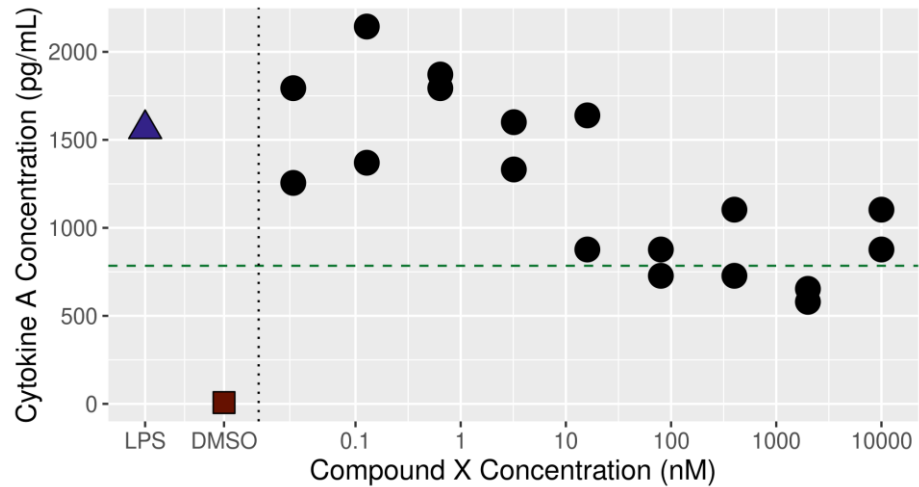
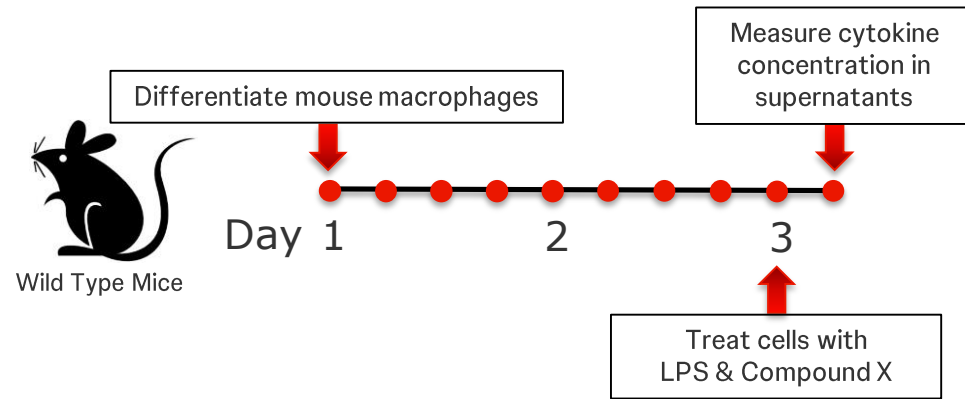
Cytokines and Why They Matter

- ❑ Cytokines are an integral part of the immune system
- ❑ Cytokines are proteins that act as “messengers” for the immune system
- ❑ The “messages” from certain cytokines (e.g., IL-6, TNF- α) can induce an unwanted pro-inflammatory response
- ❑ Stimulating and inhibiting the production or secretion of the correct cytokines is a common goal in immunology experiments



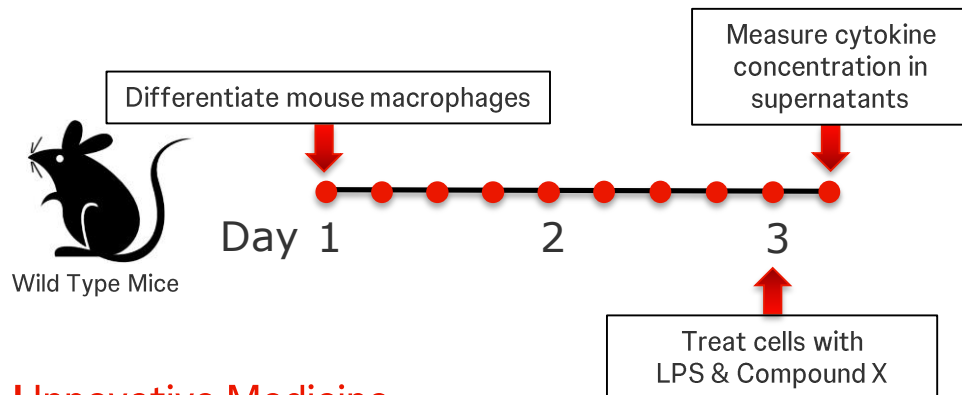
LPS Stimulation of Mouse Macrophages *In Vitro*

LPS Stimulation Mouse Macrophage *In Vitro* Assay



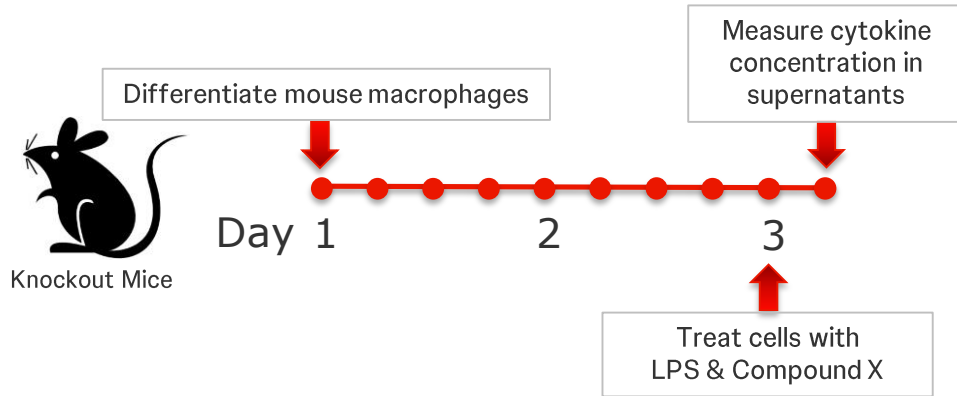
LPS Stimulation Mouse Macrophage *In Vitro* Assay

Wild Type Mice & Simultaneous

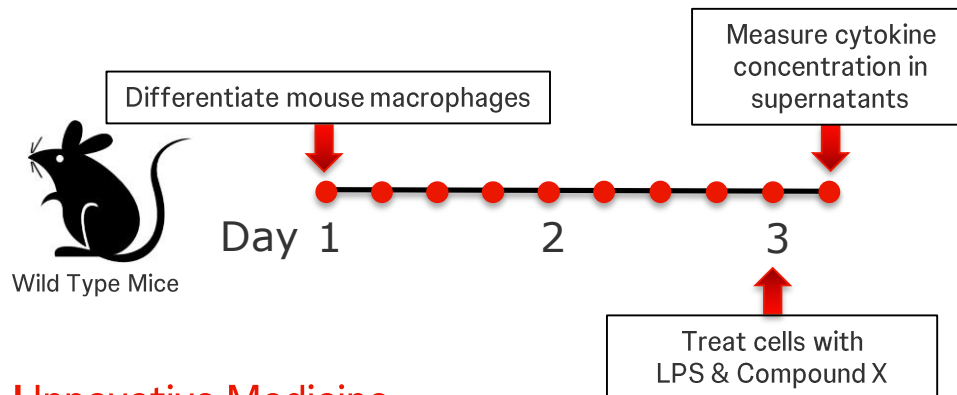


LPS Stimulation Mouse Macrophage *In Vitro* Assay

Knockout Mice & Simultaneous

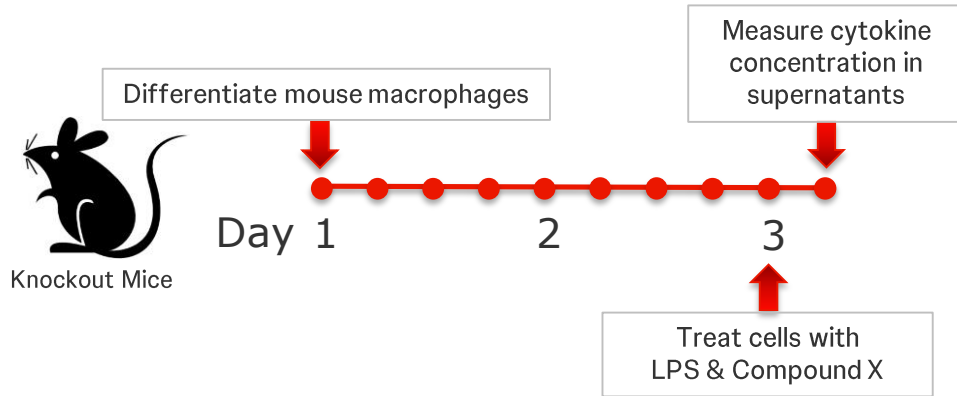


Wild Type Mice & Simultaneous

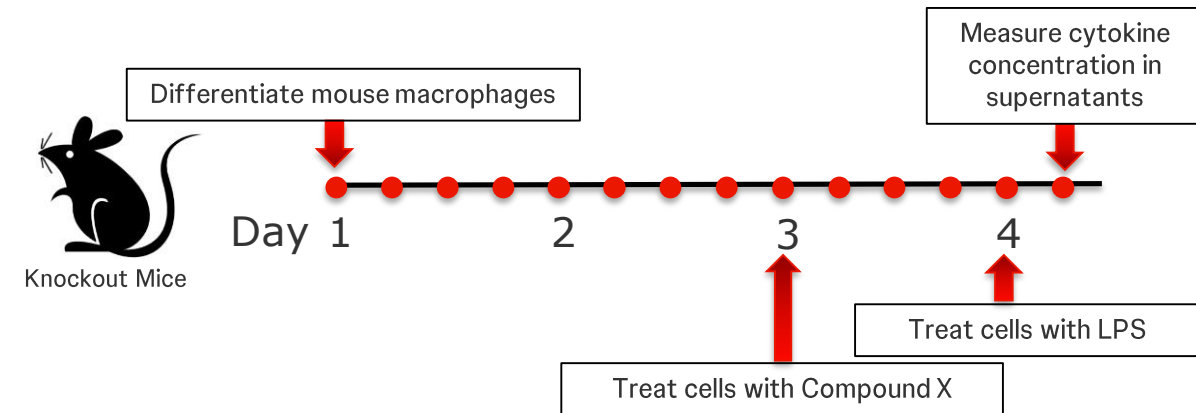


LPS Stimulation Mouse Macrophage *In Vitro* Assay

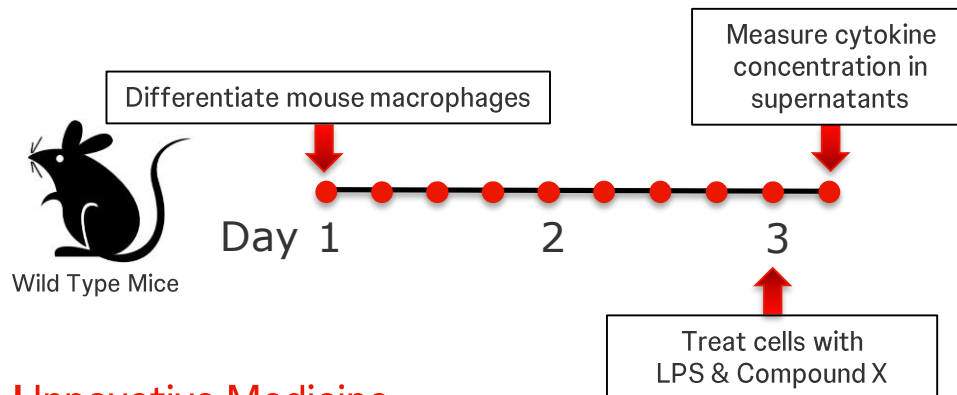
Knockout Mice & Simultaneous



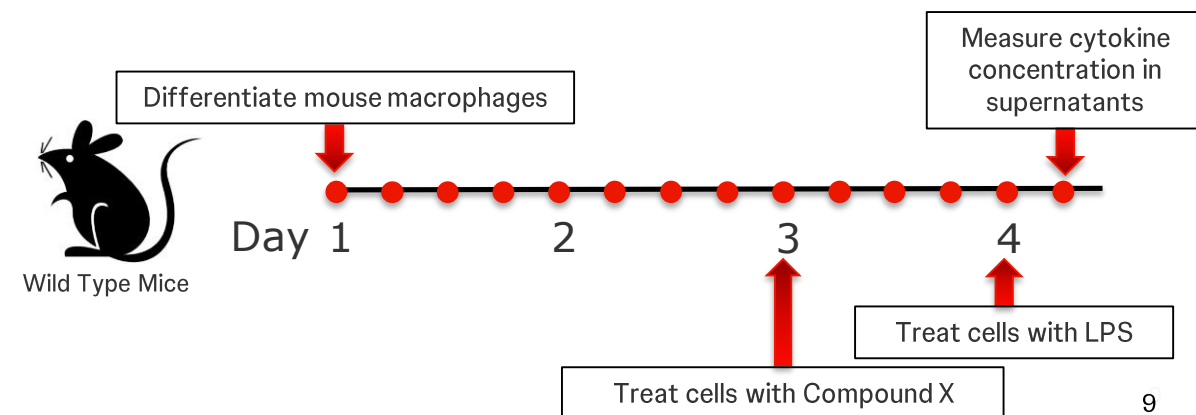
Knockout Mice & Preincubation



Wild Type Mice & Simultaneous



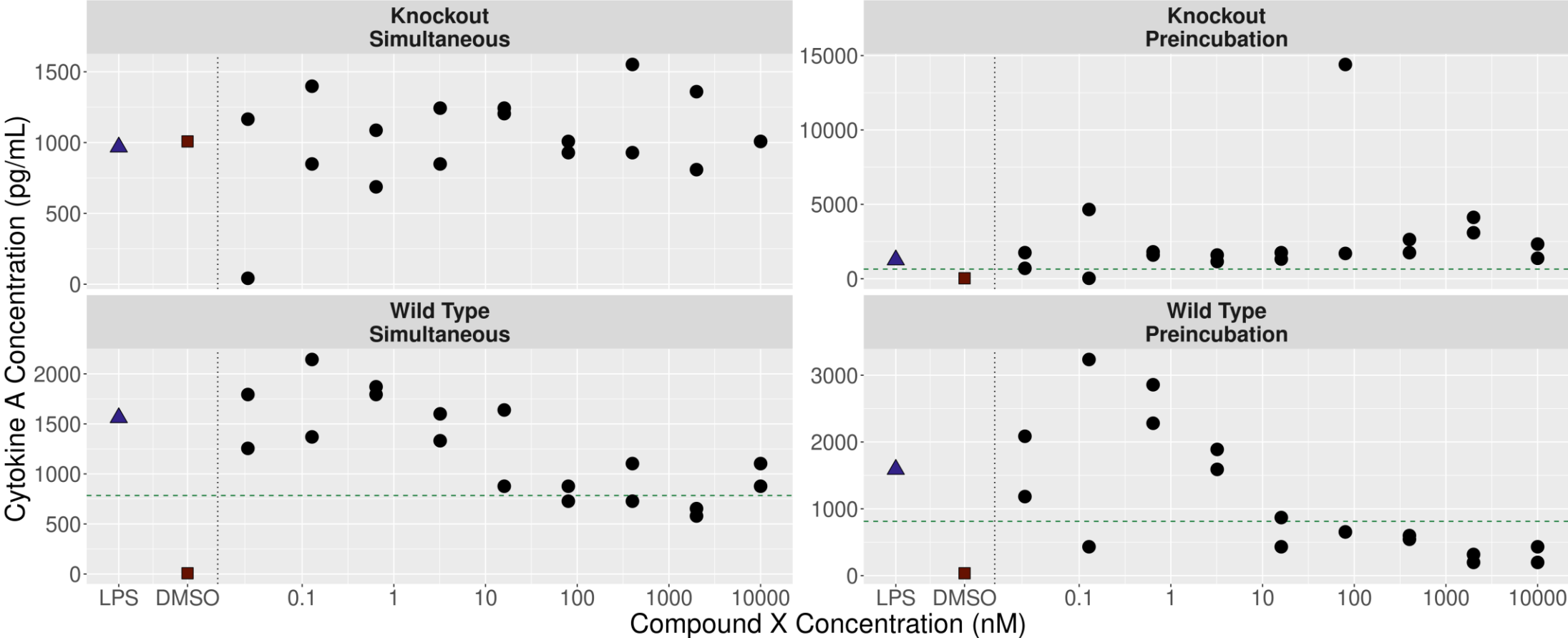
Wild Type Mice & Preincubation



Examine Concentration-Response Relationships

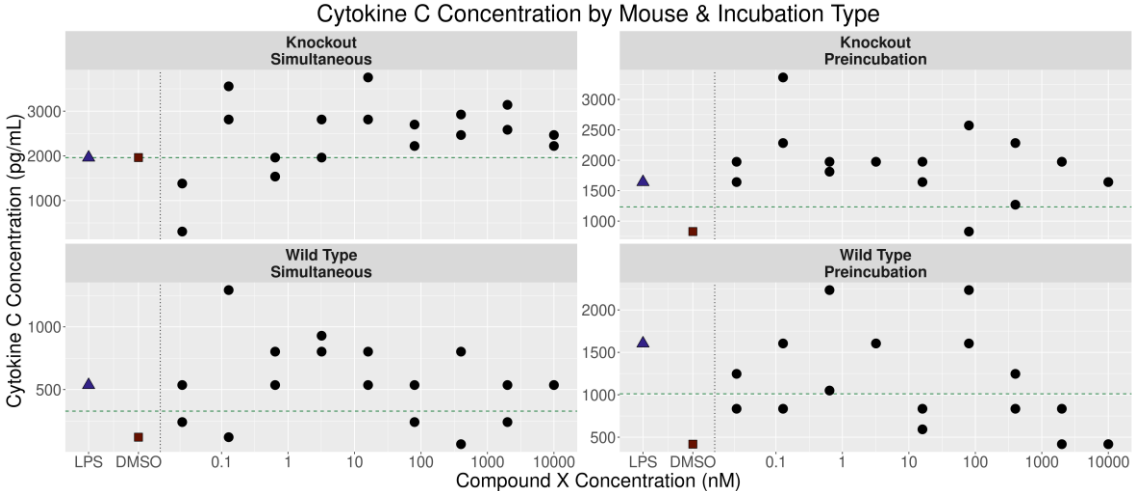
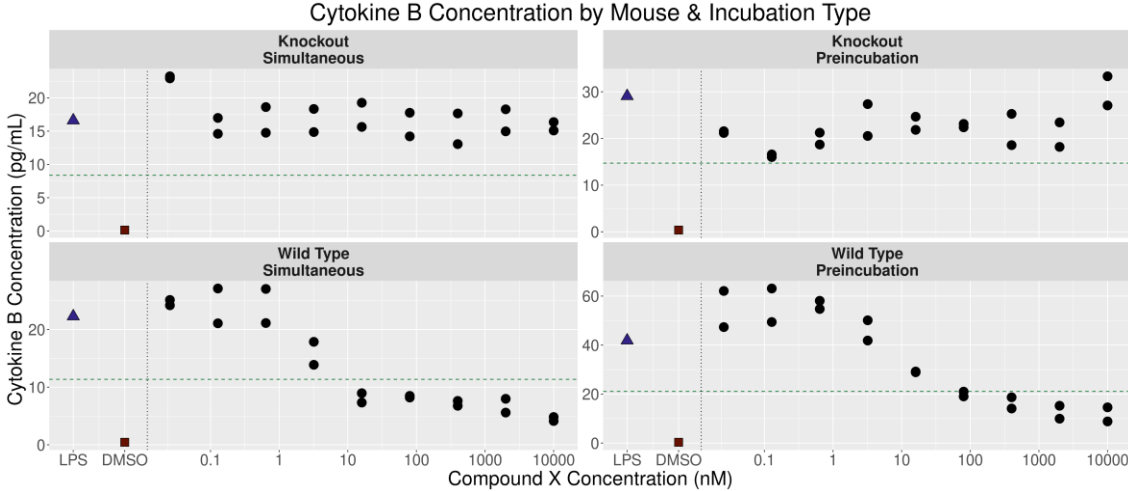
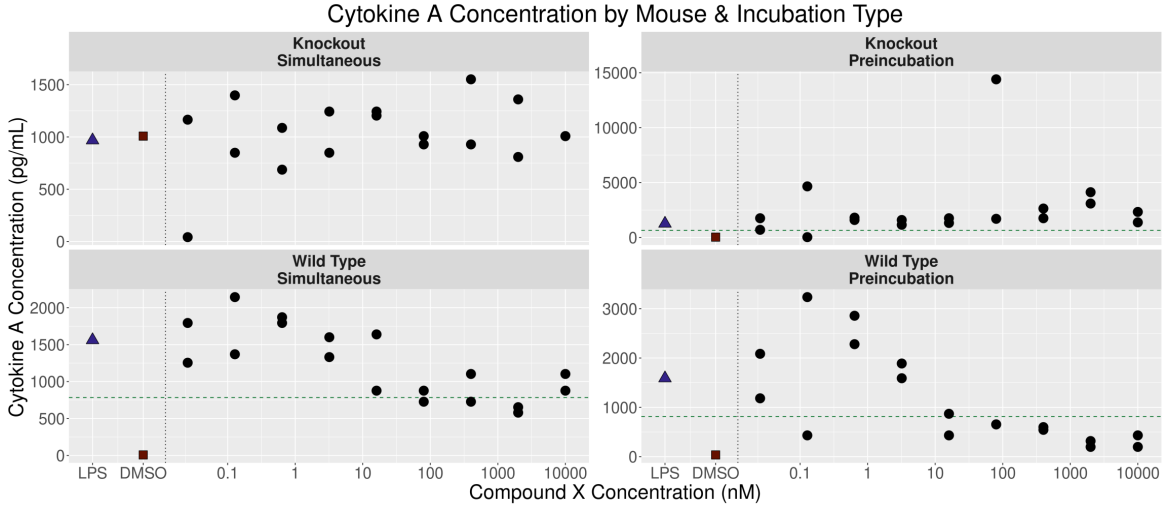
For Each Assay Condition

Cytokine A Concentration by Mouse & Incubation Type



Examine Concentration-Response Relationships

For Each Assay Condition & Cytokine Combination



Cytokine Classification Solution

How can the concentration-response relationship be classified for each assay condition and cytokine combination?

Fit a robust 4 parameter logistic regression model or robust linear regression model

(1) Check if the associated slope is negative; if slope comes from linear model check that it also has an associated p-value less than .05

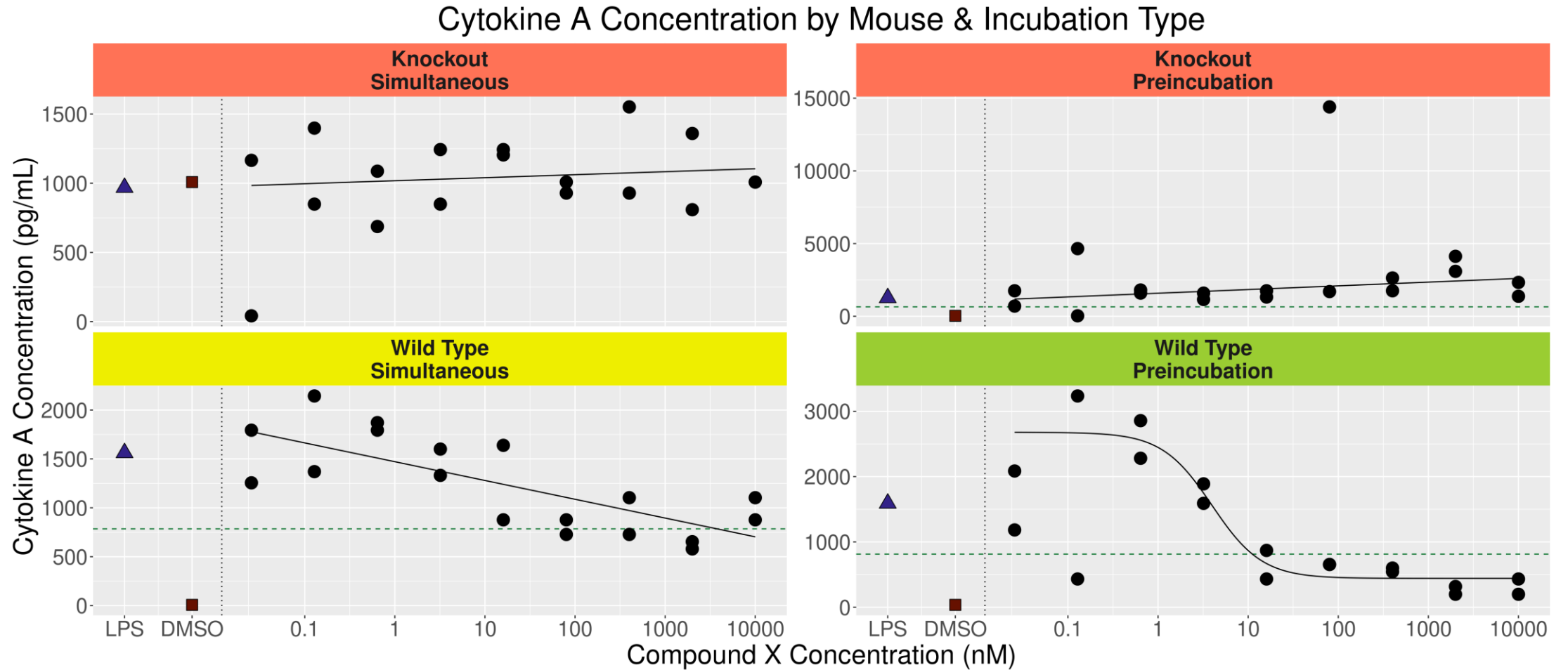
(2) Check whether the “bottom” parameter estimate is below center of the LPS/DMSO window

If (1) is false:
No Inhibition

If (1) is true but
(2) is false:
Partial Inhibition

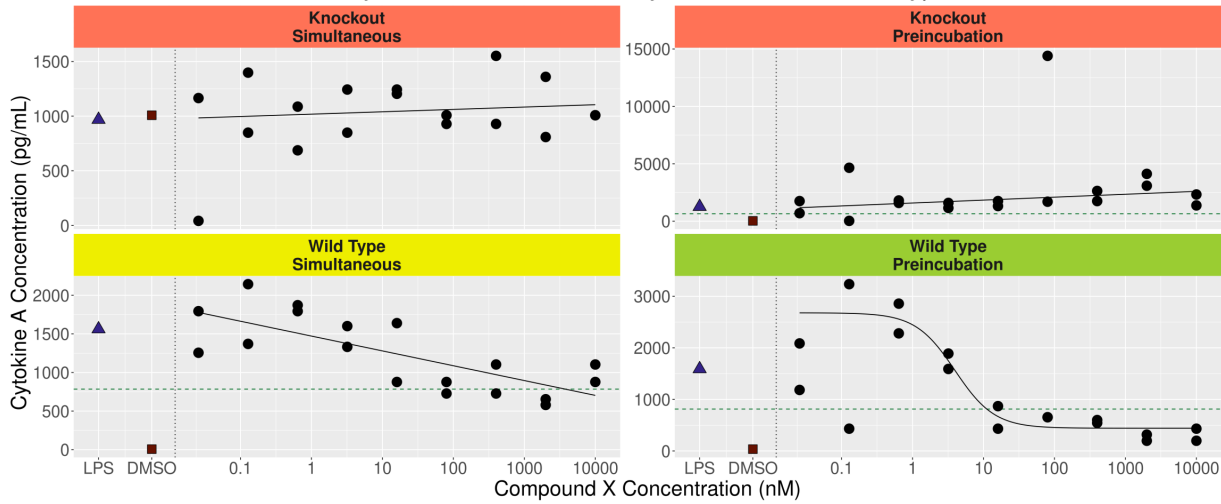
If (1) and (2) are
both true:
Inhibition

Cytokine Classification Solution

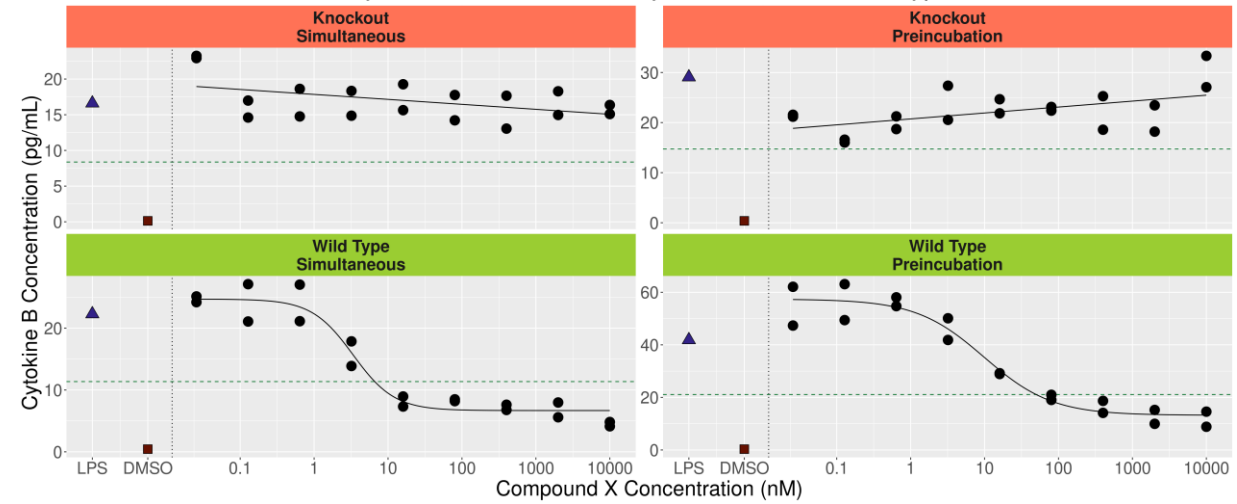


Cytokine Classification Solution

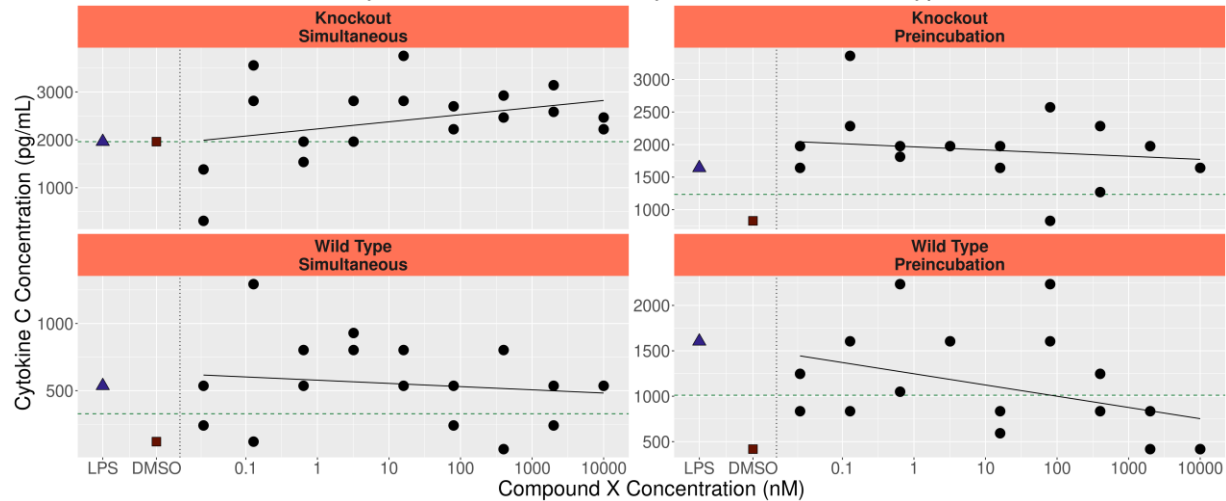
Cytokine A Concentration by Mouse & Incubation Type



Cytokine B Concentration by Mouse & Incubation Type

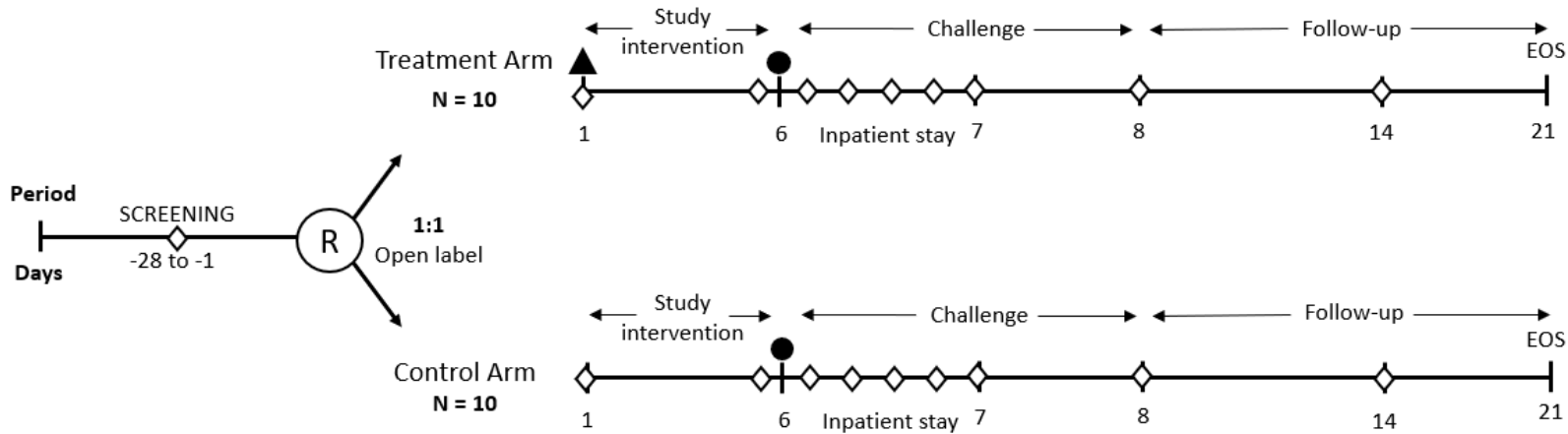


Cytokine C Concentration by Mouse & Incubation Type



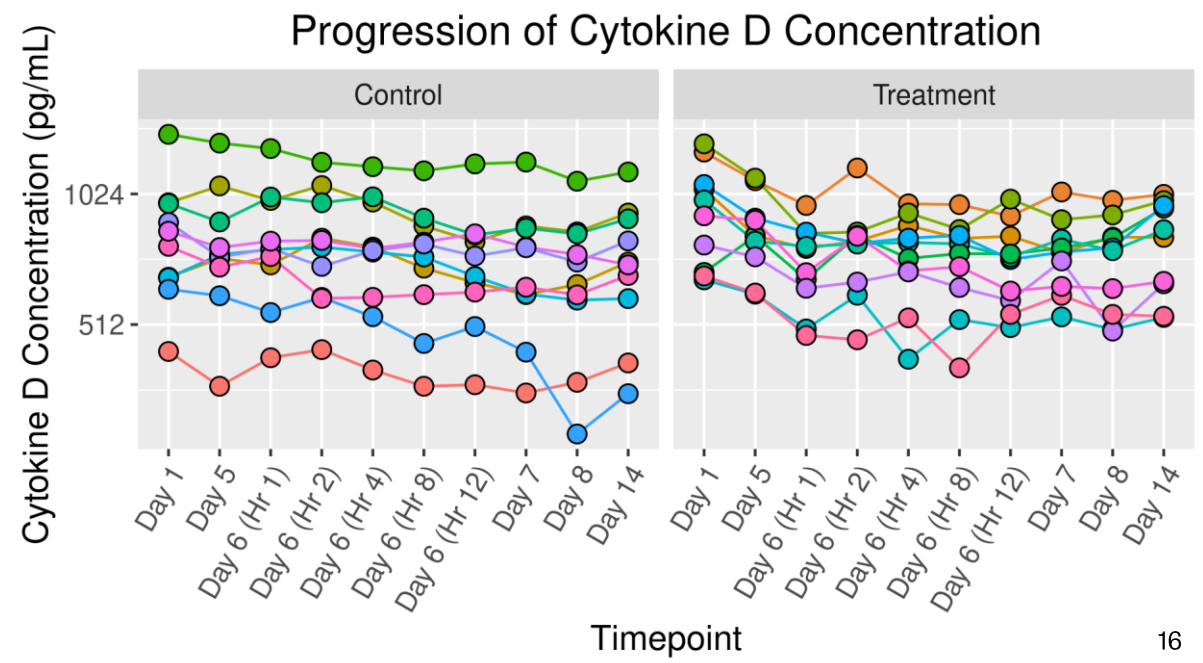
Serum from Healthy Humans Challenged with LPS

Serum from Healthy Humans Challenged with LPS

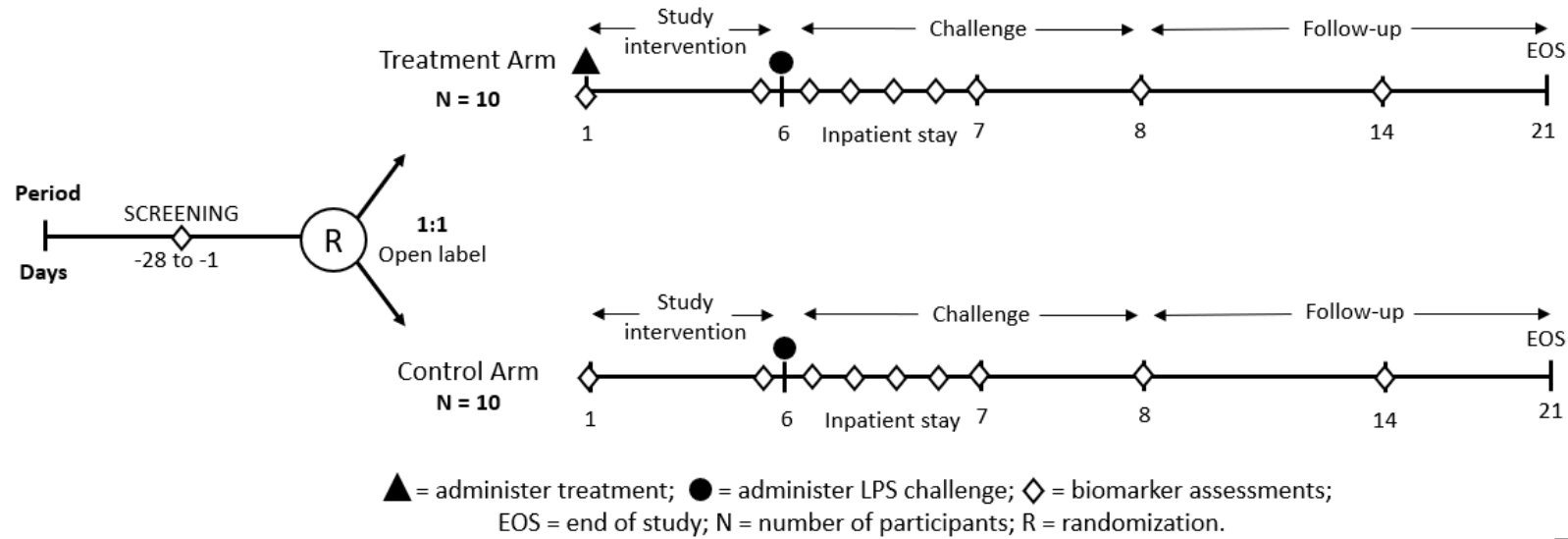


▲ = administer treatment; ● = administer LPS challenge; ◇ = biomarker assessments; EOS = end of study; N = number of participants; R = randomization.

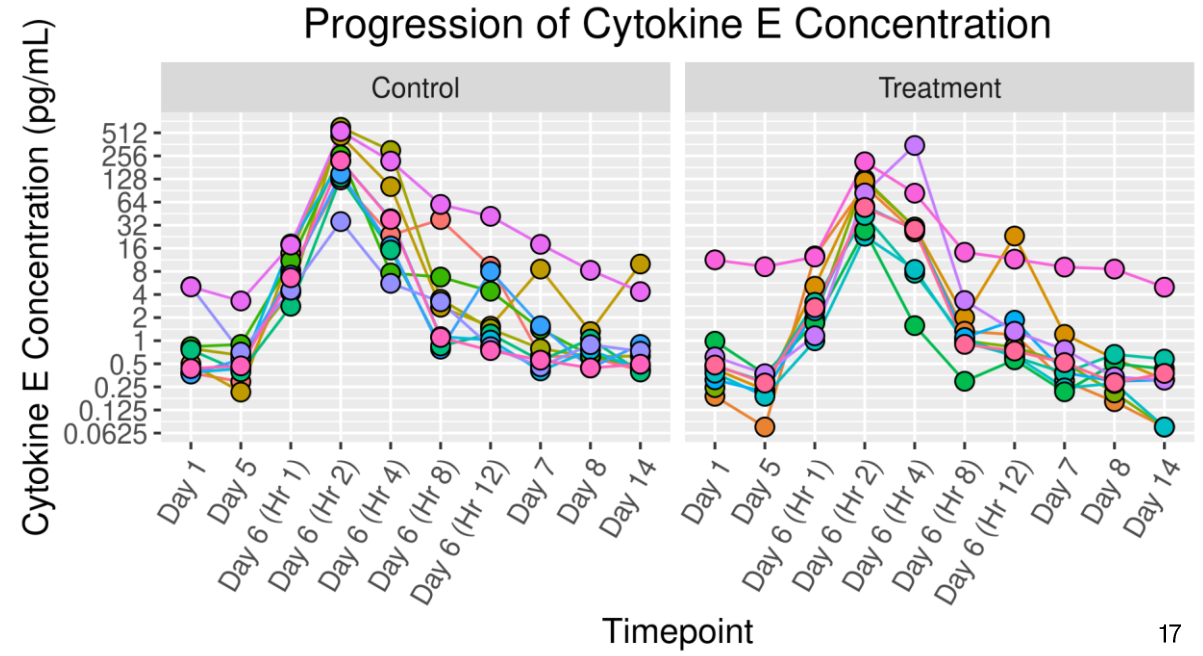
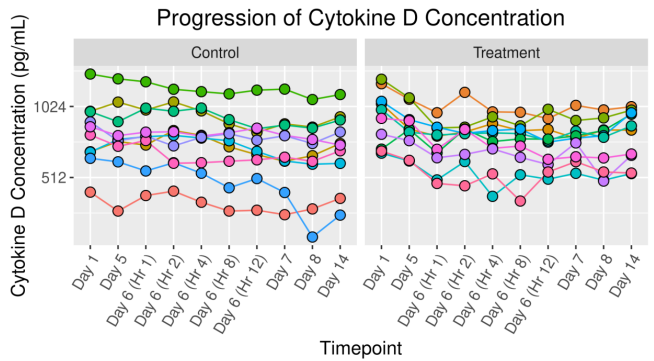
- ❑ Do we observe a difference in the concentration of certain cytokines?
- ❑ Different in what way?



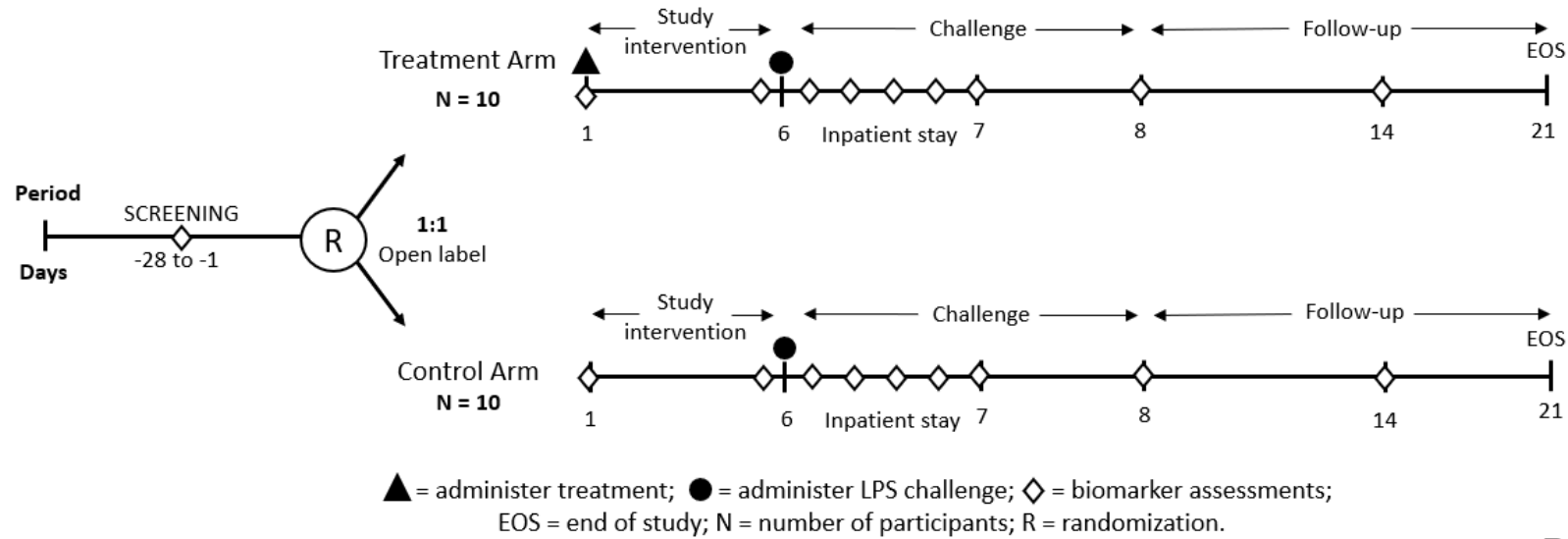
Serum from Healthy Humans Challenged with LPS



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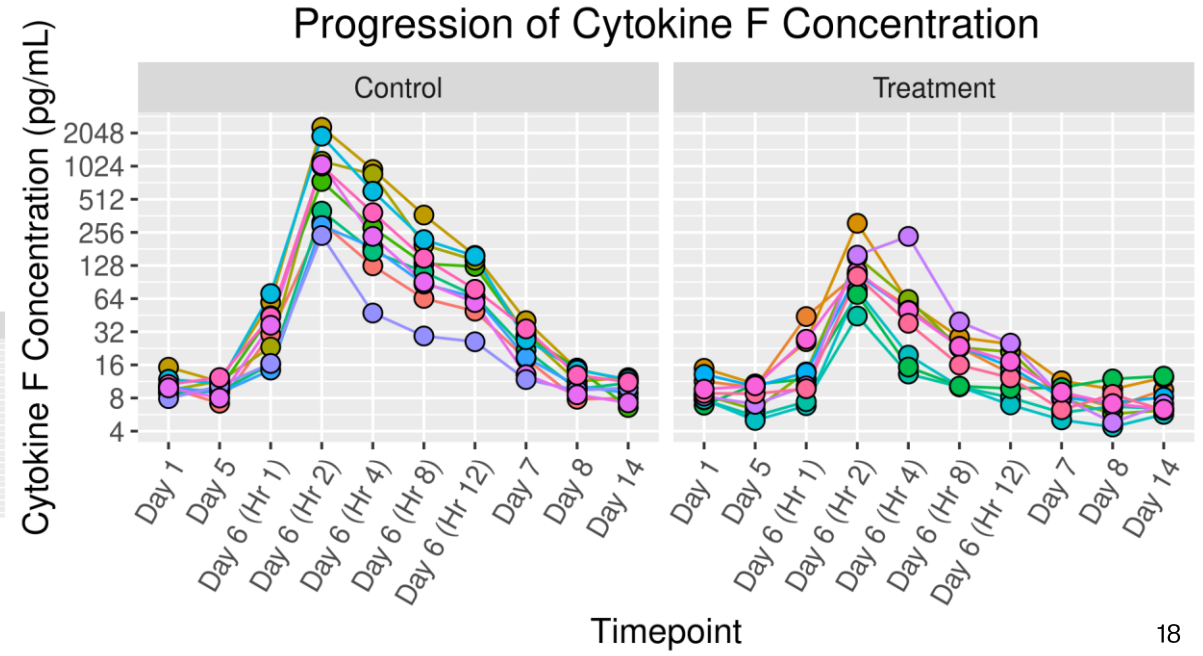
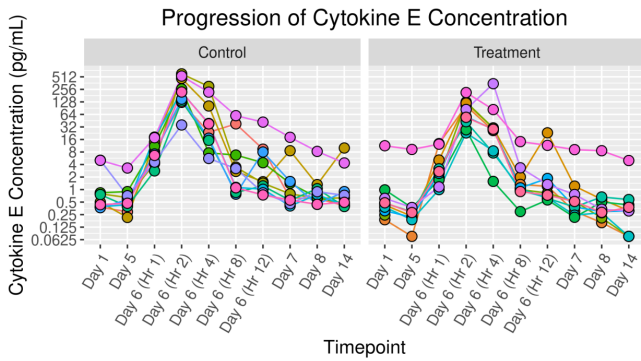
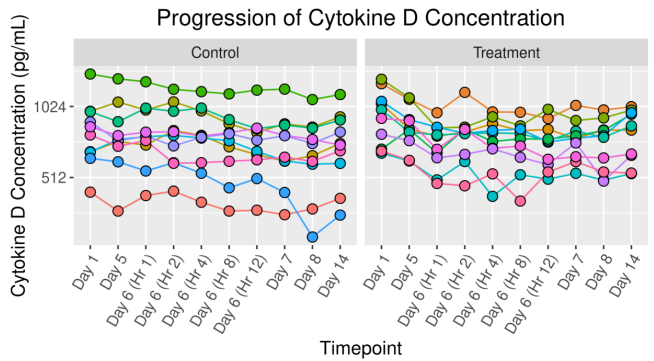


Serum from Healthy Humans Challenged with LPS



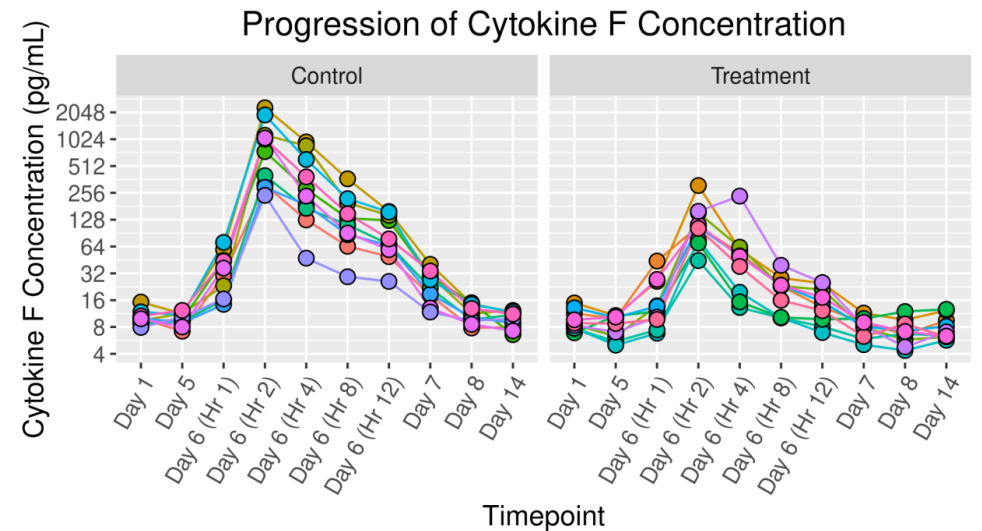
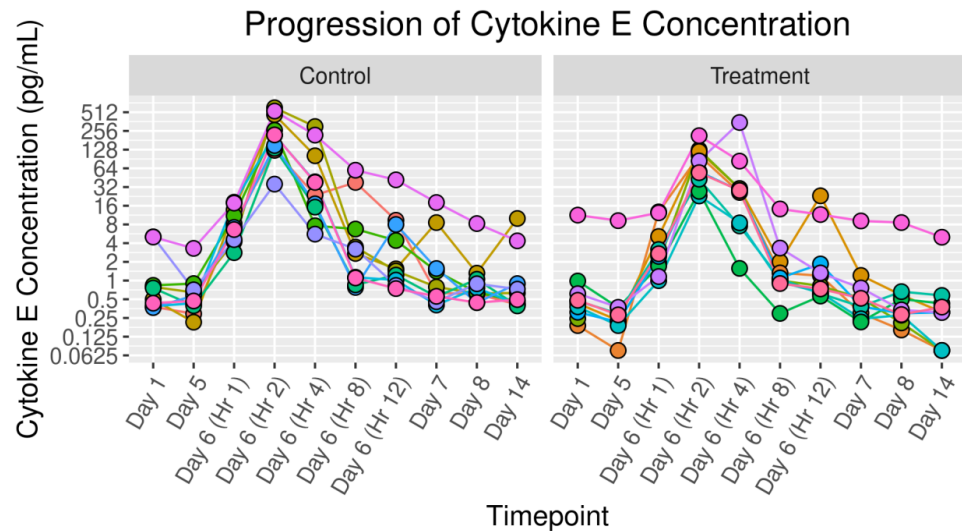
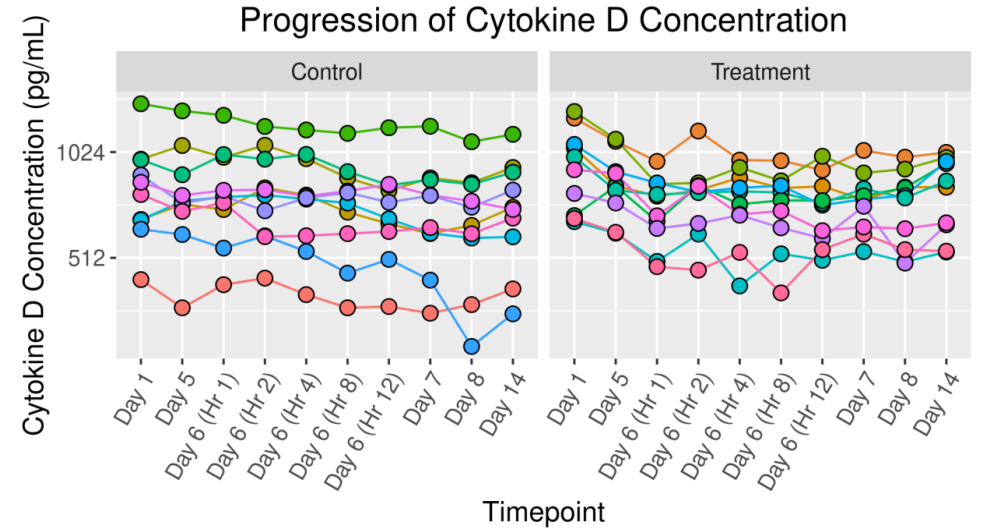
- Do we observe a difference in the concentration of certain cytokines?
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Cytokine Classification Problem

❑ Can this intuition be translated into an algorithm for cytokine classification?



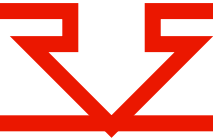
Cytokine Classification Solution

How can the repeated-measures relationship be classified for each cytokine?

Fit a mixed-effects linear model and extract the estimated marginal (EM) means from the model results



Within the Control group, check the timepoint with the peak positive difference from the Day 1 timepoint and
(1) if this difference is significant ($p < .05$)



Check the timepoint with the peak positive difference between Treatment and Control arm and if this
(2) difference is significant ($p < .05$)

If (1) is false:
**No Stimulation,
No Inhibition**

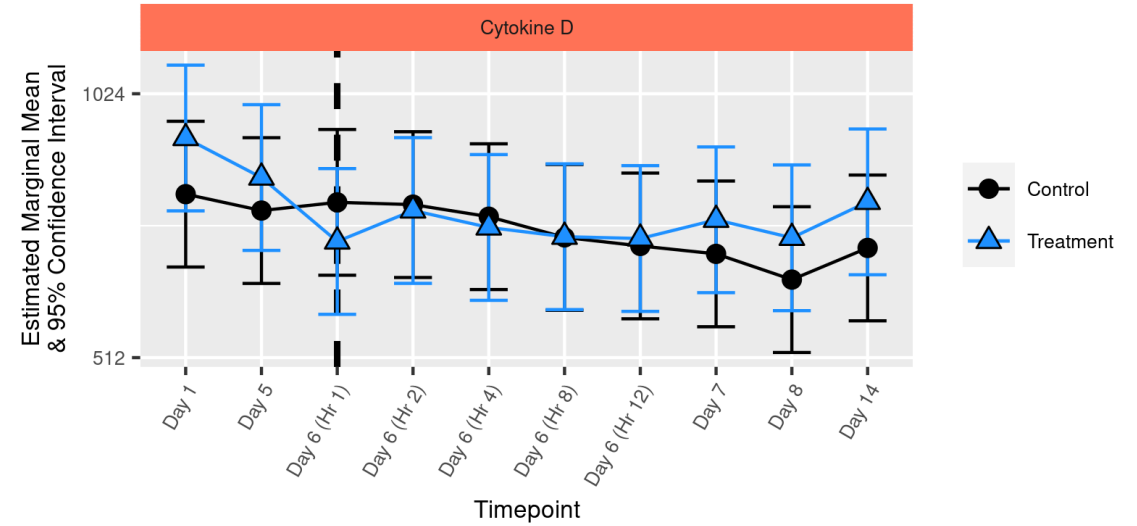
If (1) is true but (2) is false:
**LPS Stimulation,
No Inhibition**

If (1) and (2) are both true:
**LPS Stimulation,
Inhibition by Treatment**

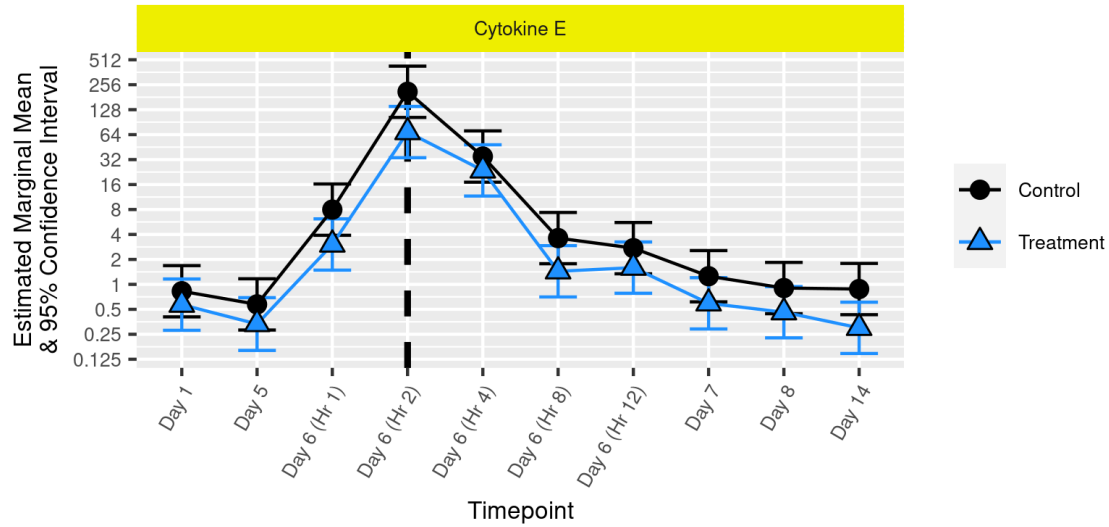
Cytokine Classification Solution



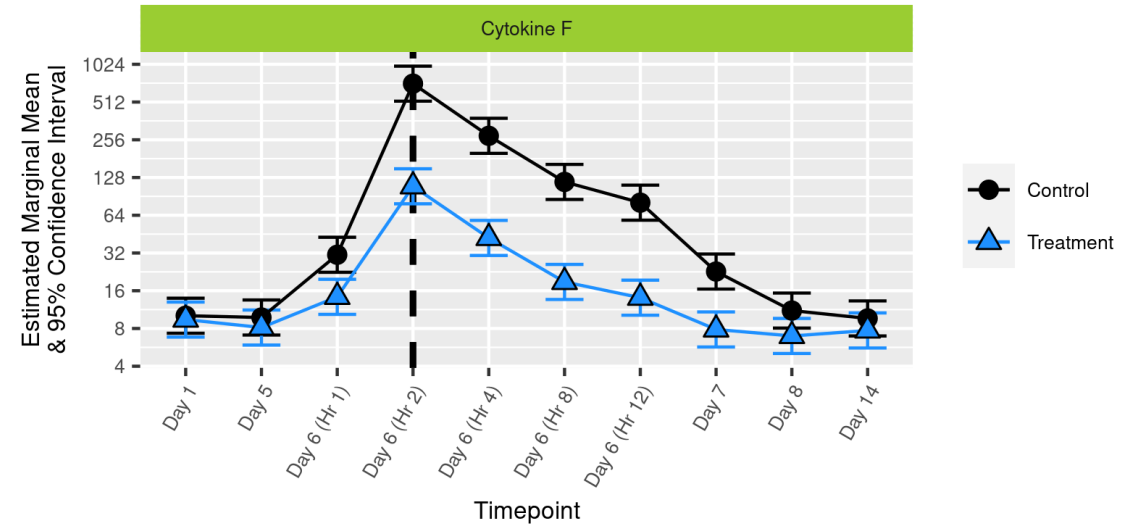
Estimated Marginal Means by Timepoint



Estimated Marginal Means by Timepoint

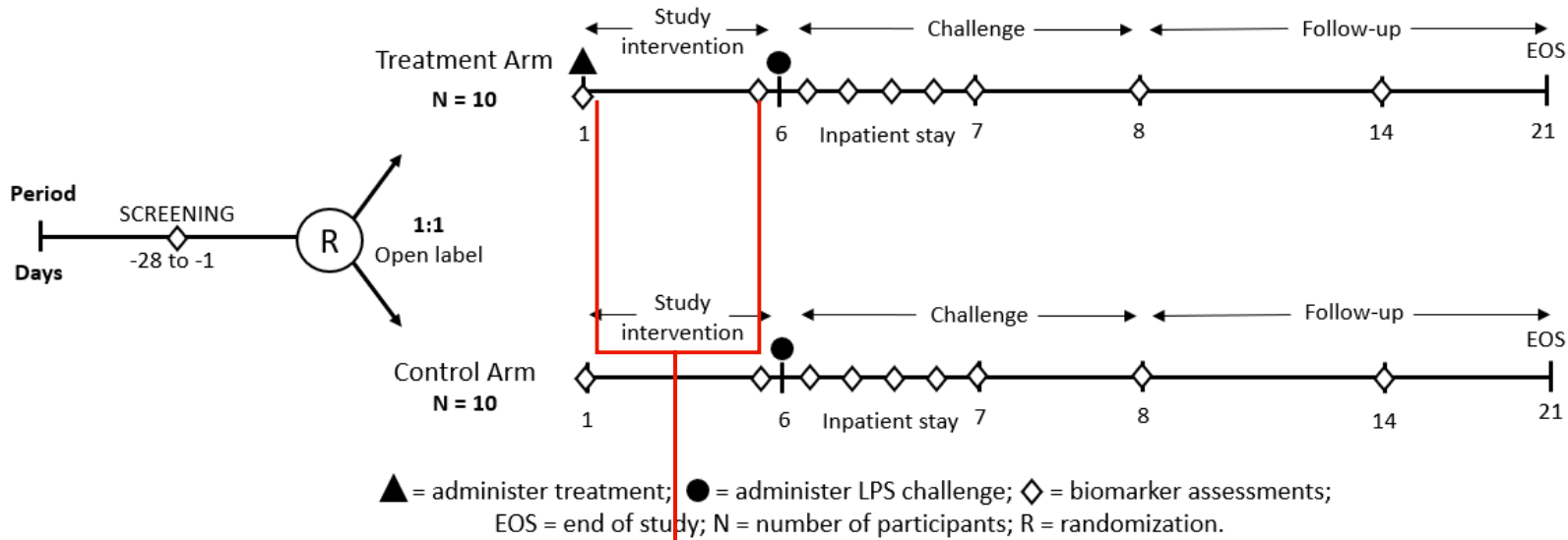


Estimated Marginal Means by Timepoint



Ex Vivo Stimulation of Healthy Human Blood with LPS or TNF- α

Ex Vivo Stimulation of Healthy Human Blood



Period	Screening	Study Intervention Admin	Challenge								Follow-up		
			1	2								3	4
Visit	Screening	1	2								3	4	
Visit Schedule	Day -28 to Day -1	Day 1	Day 6								Day 7	Day 8	Day 14
Visit Hour			Pre-challenge	0	1	2	4	8	12	24	48		
Visit Window			± 1 day [†]								± 3 hours	± 3 days	
Study Procedure													
Biomarkers ¹													
		X ⁿ	X ⁿ										
		X ⁿ	X ⁿ										



Control



LPS



TNF- α

Ex Vivo Stimulation of Healthy Human Blood

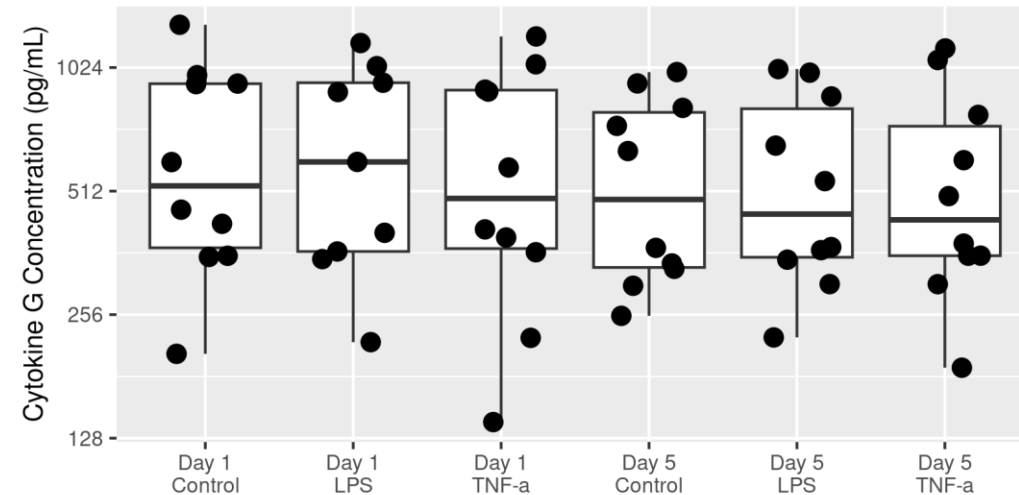
Period	Screening	Study Intervention Admin	Challenge								Follow-up		
			2								3	4	
Visit	Screening	1									3	4	
Visit Schedule	Day -28 to Day -1	Day 1	Day 6								Day 7	Day 8	Day 14
Visit Hour			Pre-challenge	0	1	2	4	8	12	24	48		
Visit Window			± 1 day [†]								± 3 hours	± 3 days	
Study Procedure													
Biomarkers[†]													
		X ⁿ	X ⁿ										
		X ⁿ	X ⁿ										



Do we observe a difference in the concentration of certain cytokines using this stimulation system?

Different in what way?

Cytokine G Concentration by Timepoint and Challenge



Ex Vivo Stimulation of Healthy Human Blood

Period	Screening	Study Intervention Admin	Challenge								Follow-up	
			2								3	4
Visit	Screening	1									3	4
Visit Schedule	Day -28 to Day -1	Day 1	Day 6						Day 7	Day 8	Day 14	
Visit Hour			Pre-challenge	0	1	2	4	8	12	24	48	
Visit Window			± 1 day [†]									
											± 3 hours	± 3 days
Study Procedure												
Biomarkers¹												
		X ⁿ	X ⁿ									
		X ⁿ	X ⁿ									



Control



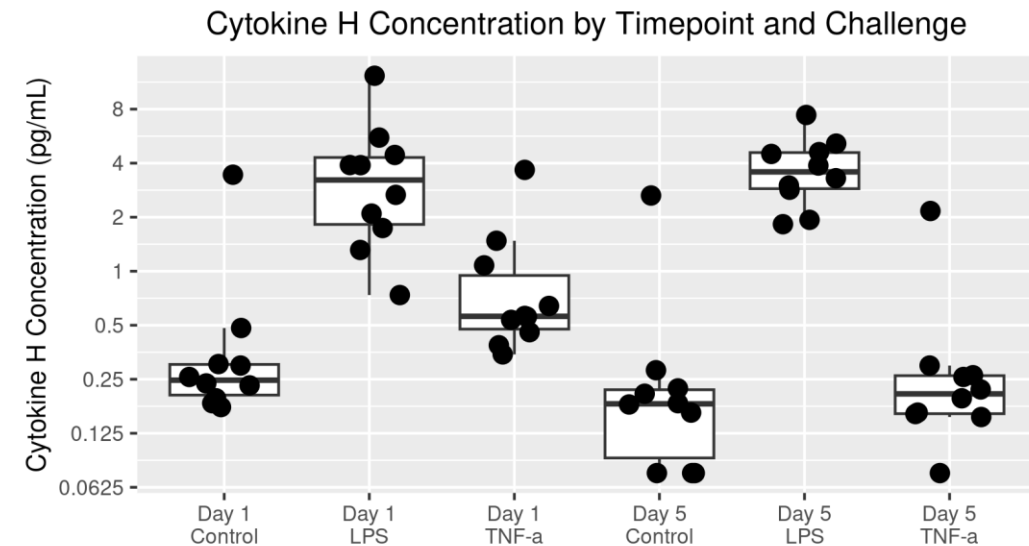
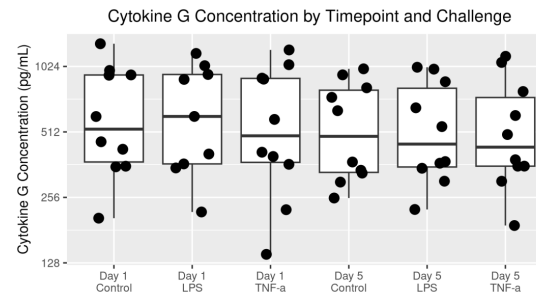
LPS



TNF- α

❑ Do we observe a difference in the concentration of certain cytokines using this stimulation system?

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Ex Vivo Stimulation of Healthy Human Blood

Period	Screening	Study Intervention Admin	Challenge								Follow-up	
											3	4
Visit	Screening	1	2								Day 8	Day 14
Visit Schedule	Day -28 to Day -1	Day 1	Day 6					Day 7				
Visit Hour			Pre-challenge	0	1	2	4	8	12	24	48	
Visit Window			± 1 day [†]								± 3 hours	± 3 days
Study Procedure												
Biomarkers¹												
TruCulture (LPS <i>ex vivo</i> stimulation)		X ⁿ	X ⁿ									
TruCulture (TNF α <i>ex vivo</i> stimulation)		X ⁿ	X ⁿ									



Control



LPS

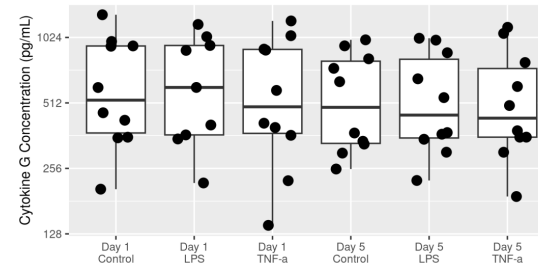


TNF- α

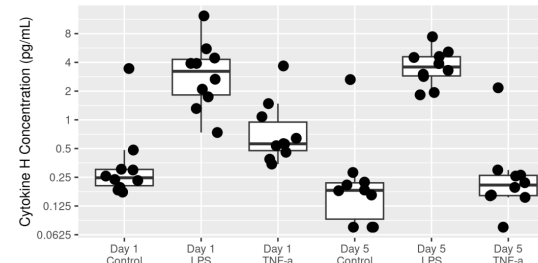
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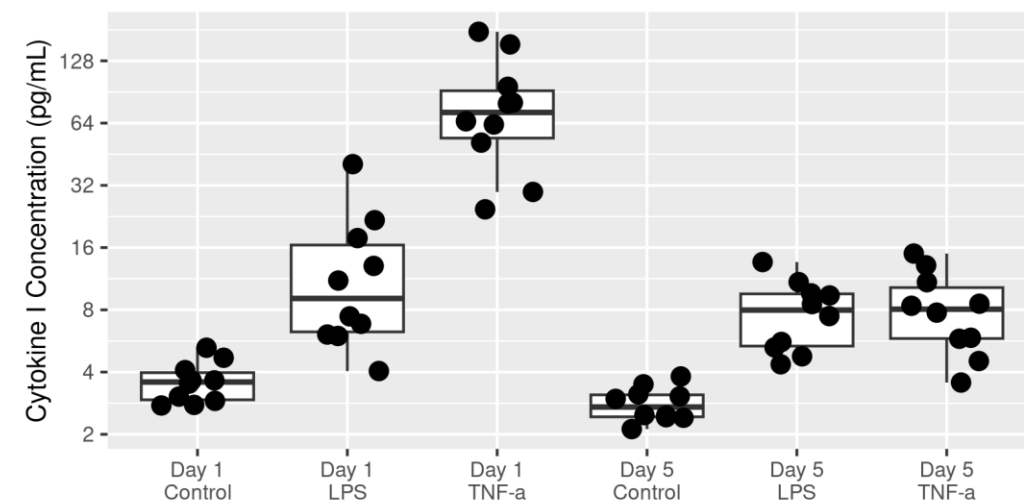
Cytokine G Concentration by Timepoint and Challenge



Cytokine H Concentration by Timepoint and Challenge



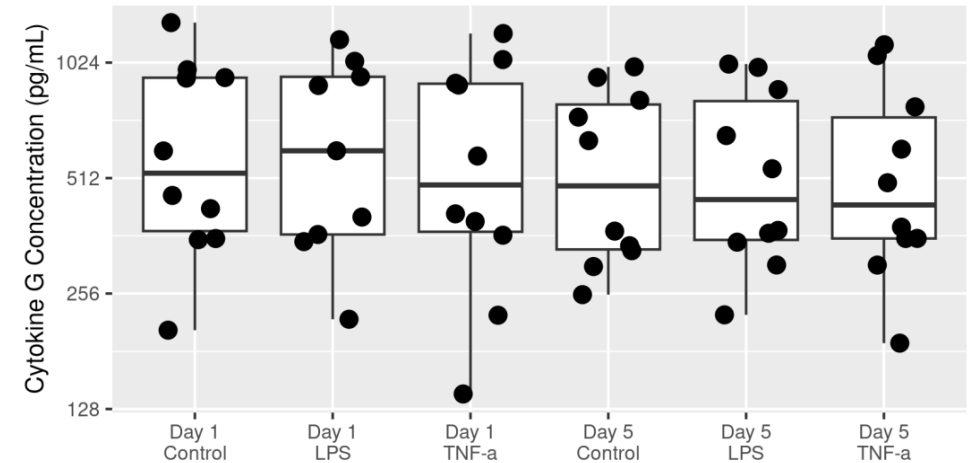
Cytokine I Concentration by Timepoint and Challenge



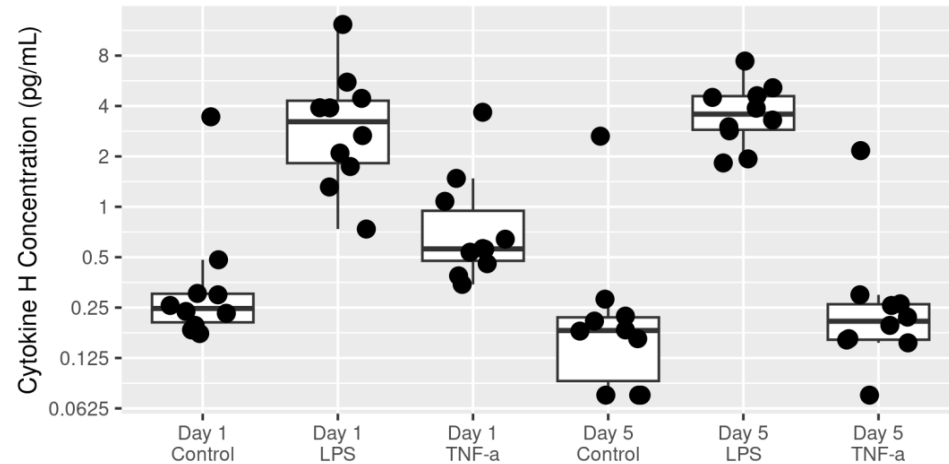
Cytokine Classification Problem

❑ Can this intuition be translated into an algorithm for cytokine classification?

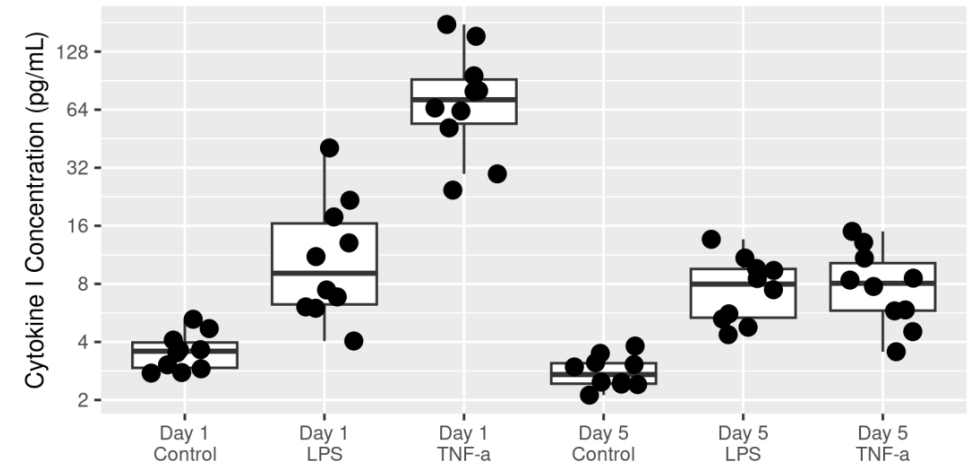
Cytokine G Concentration by Timepoint and Challenge



Cytokine H Concentration by Timepoint and Challenge

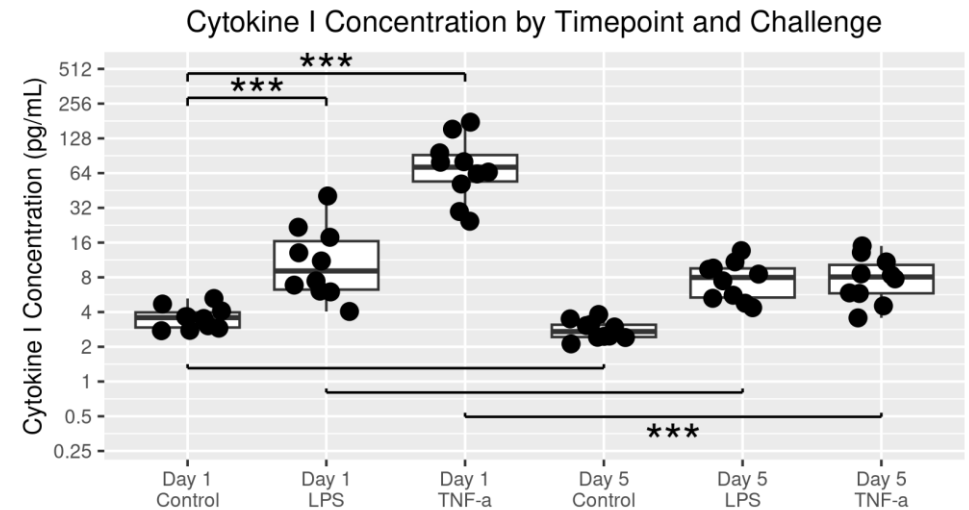
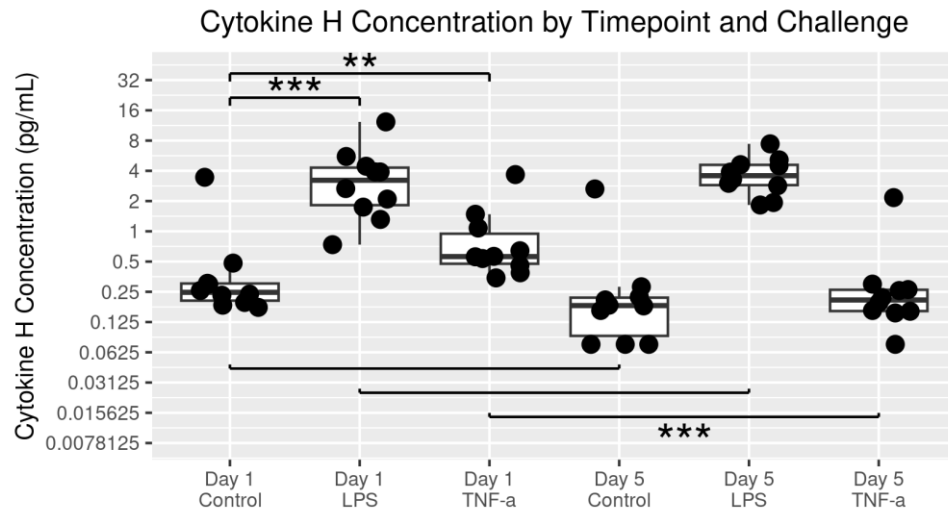
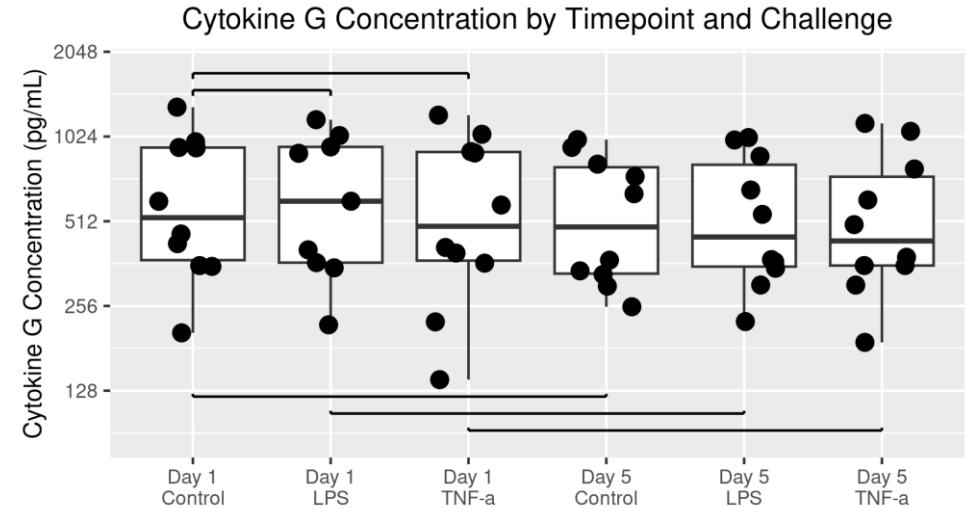


Cytokine I Concentration by Timepoint and Challenge



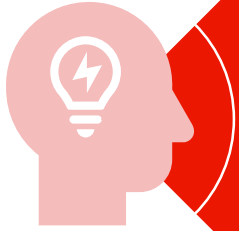
Cytokine Classification Solution

- ❑ Fit a mixed-effects linear model, examine EM means & classify via:
 - ❑ LPS or TNF- α stimulation on day 1?
 - ❑ Difference between day 5 and day 1 for any group?



Conclusion

Conclusion



Creative solutions are needed to identify differential human cytokine responses to immune challenges



Classification algorithms have limitations (e.g., defining the “right” classes, “problem” items that don’t fit in classes, etc.) but can still be useful



Biological relevance matters more than statistical significance, so effective collaboration with scientists is the most important factor

Thank you



If you have more questions, please contact:
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tbeaver2@jnj.com

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