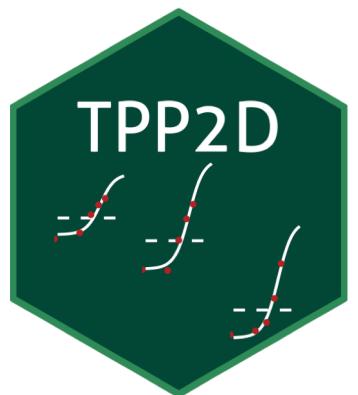


# Robust small molecule-protein interaction inference reveals unknown drug off-targets

Nils Kurzawa

PhD Student, Savitski Lab, EMBL Heidelberg



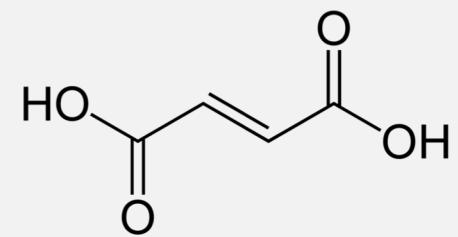
@nils\_kurzawa

nkurzaw

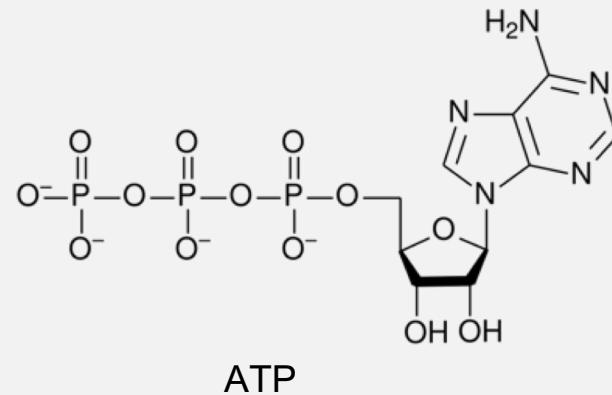
09/12/2019

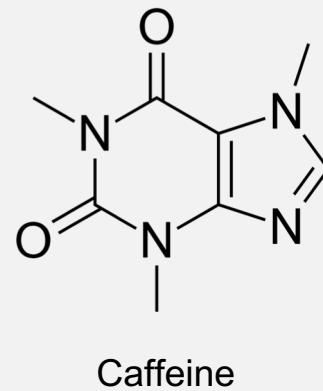
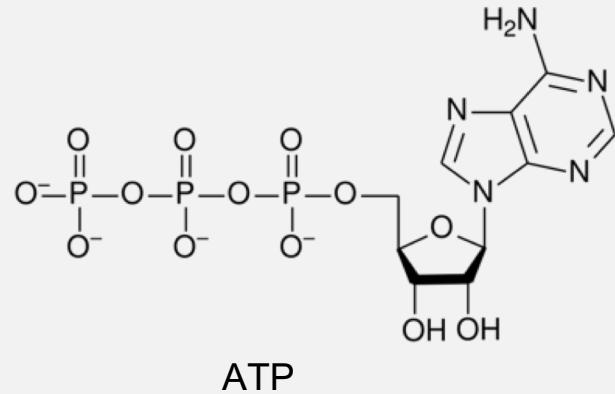
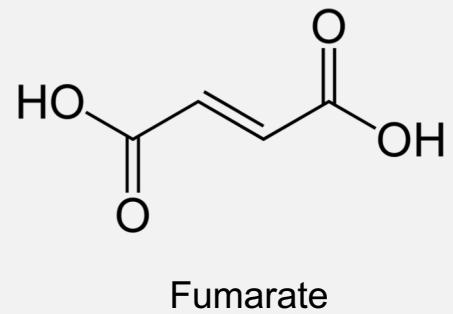




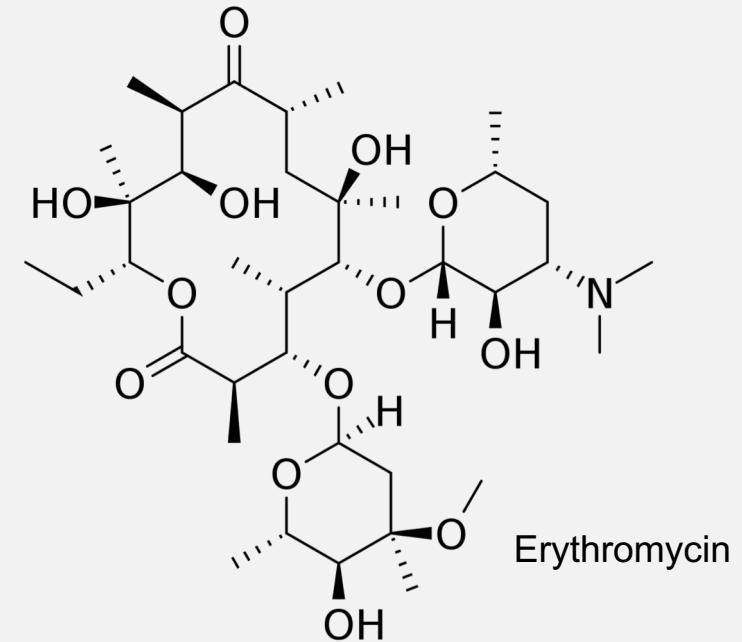
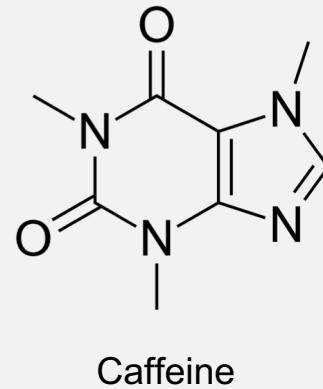
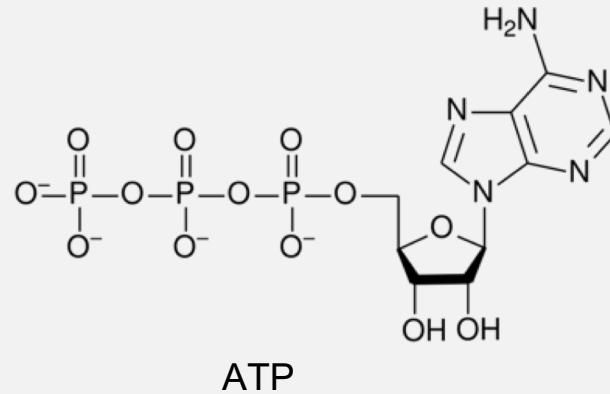
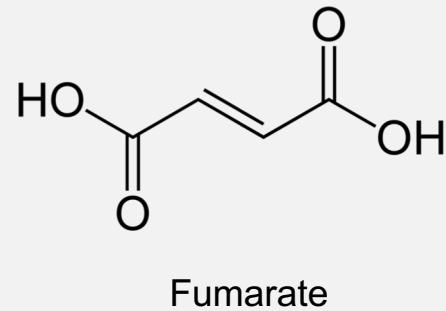
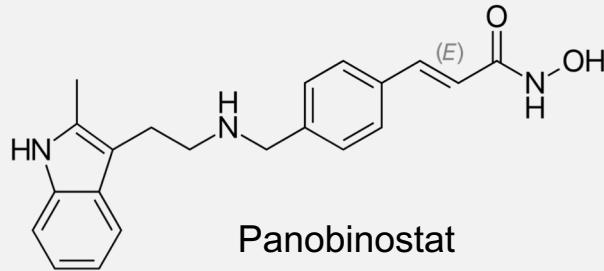


Fumarate

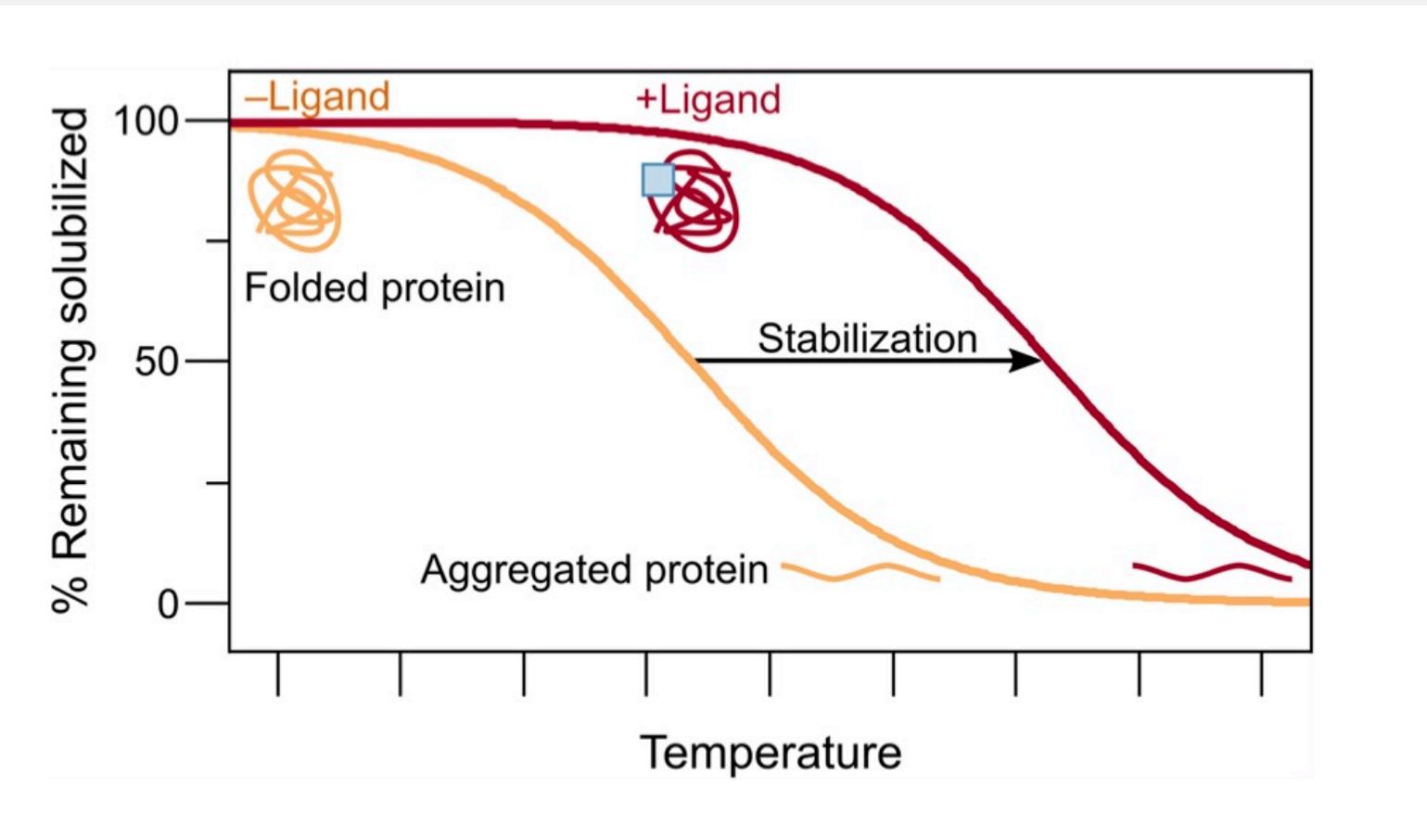




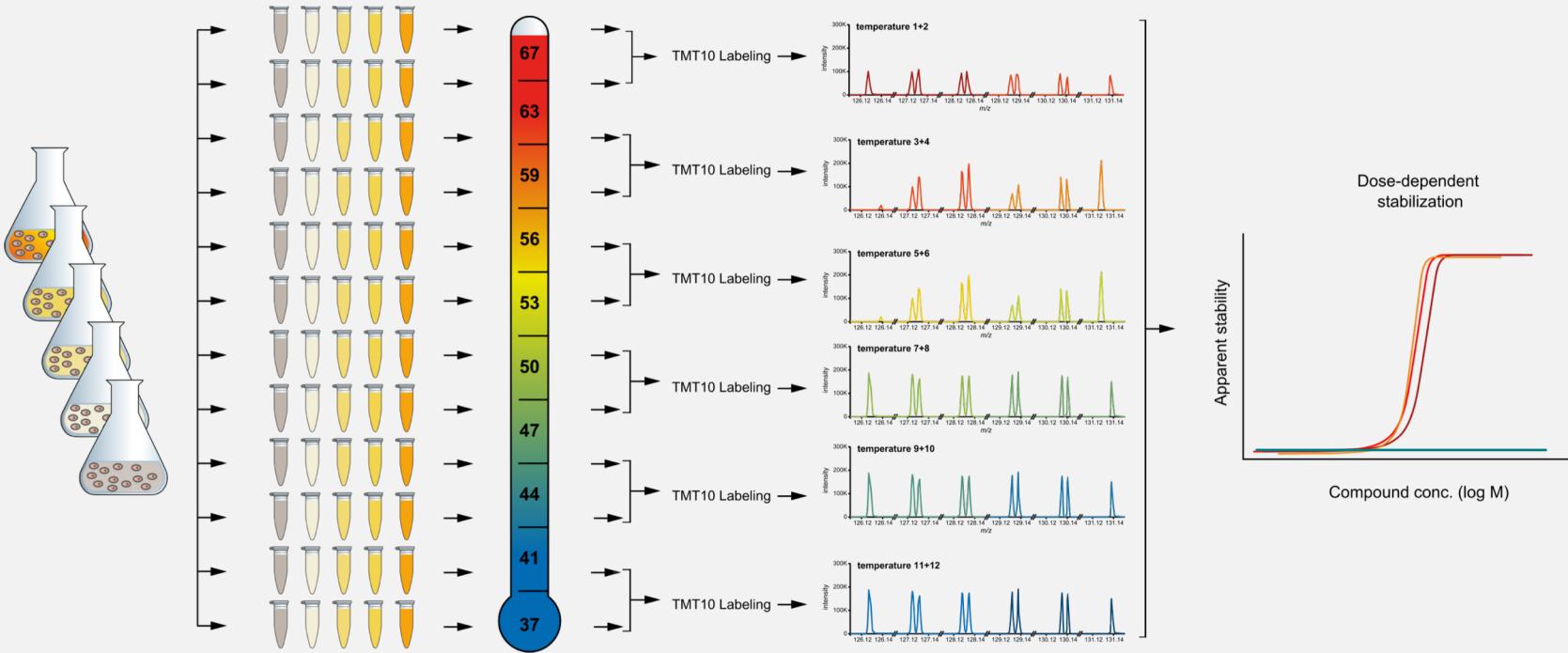
# Which proteins interact with certain small molecules?



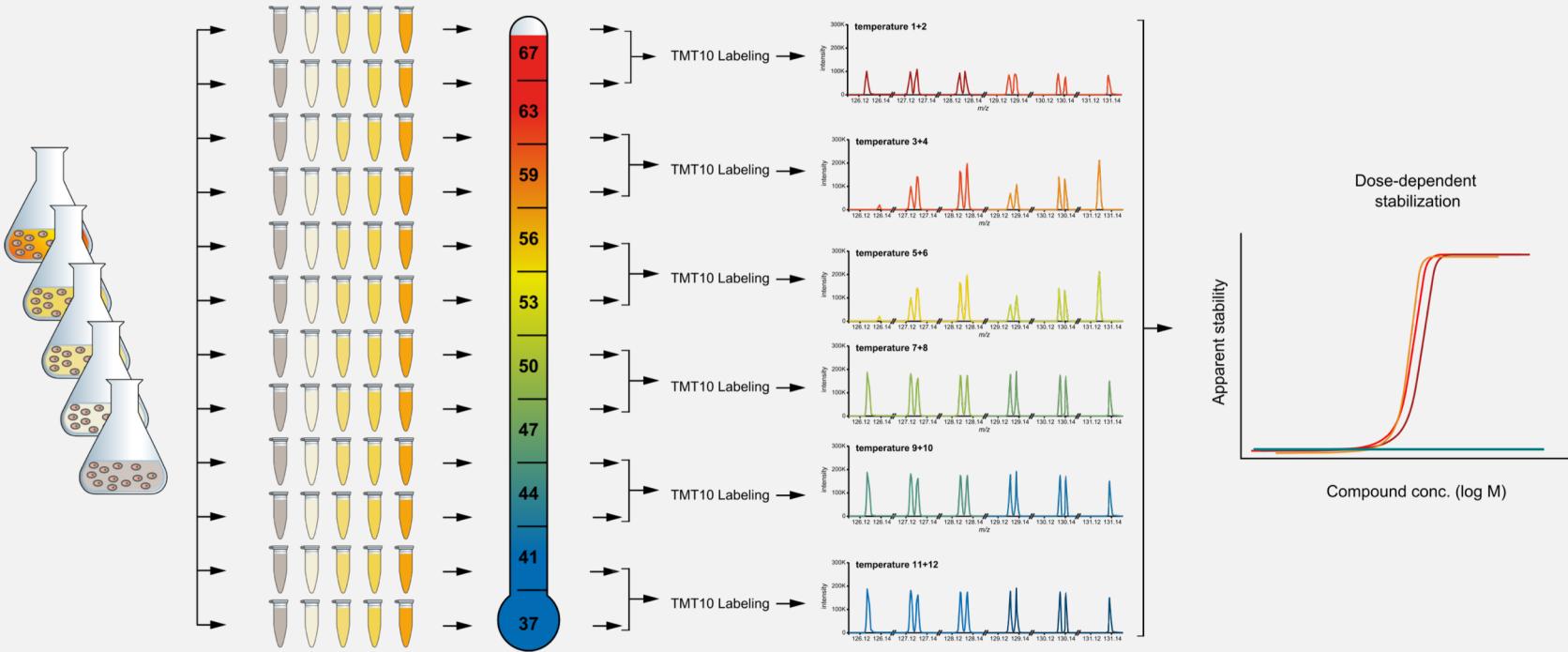
# The thermal shift assay



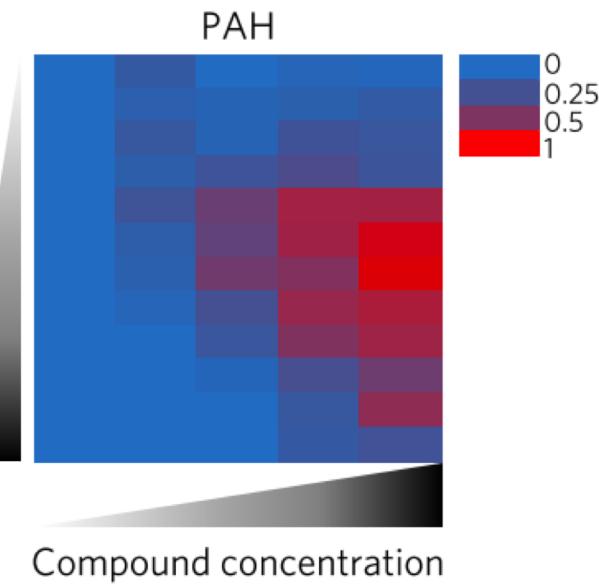
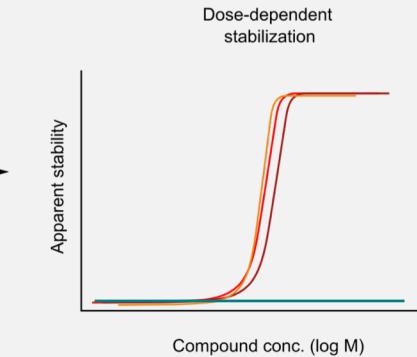
# Two dimensional thermal proteome profiling (2D-TPP)



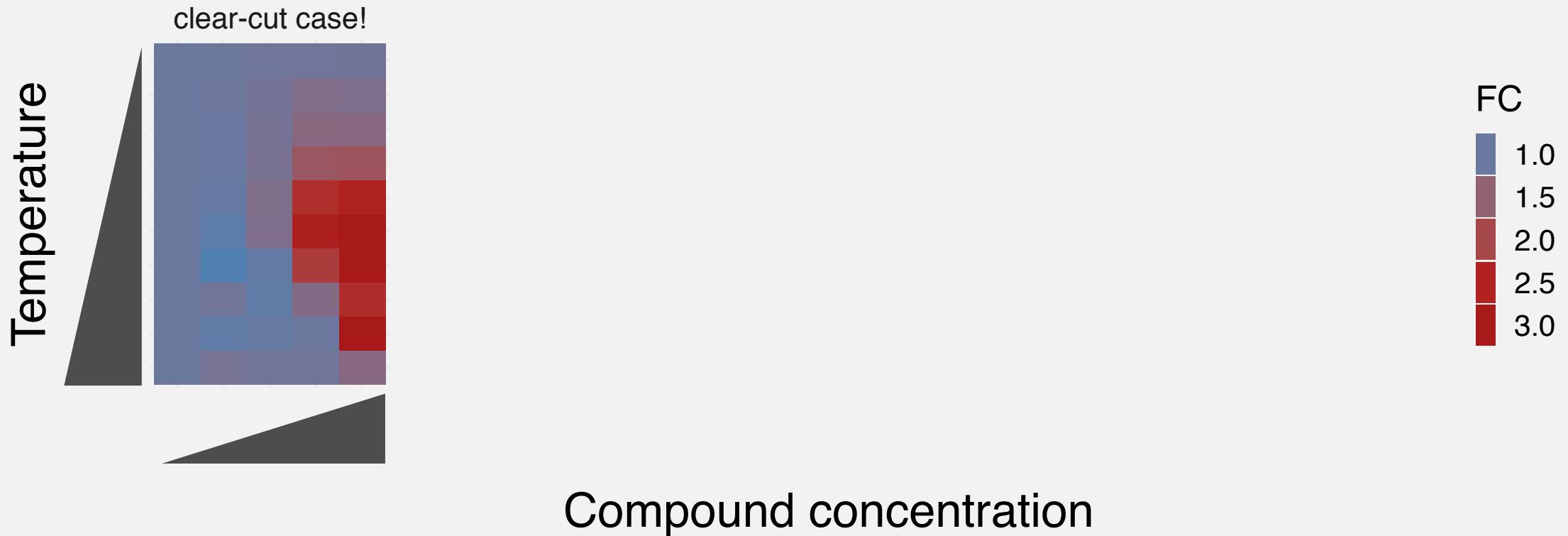
# Two dimensional thermal proteome profiling (2D-TPP)



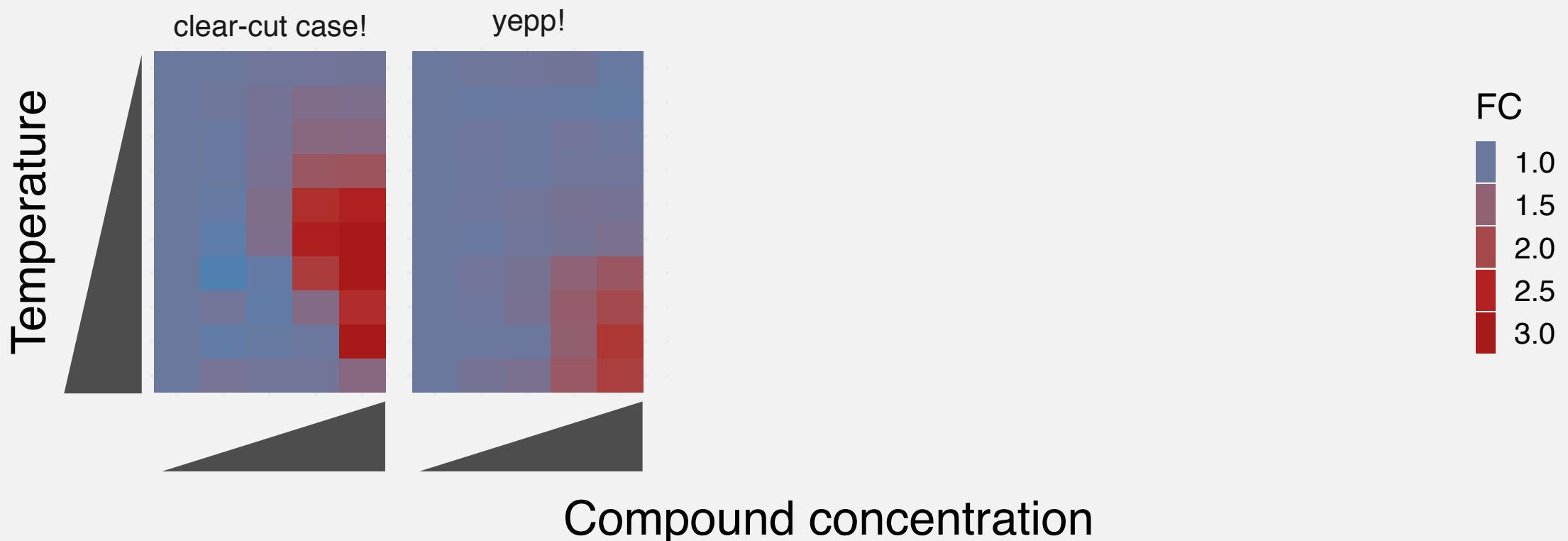
Panobinostat



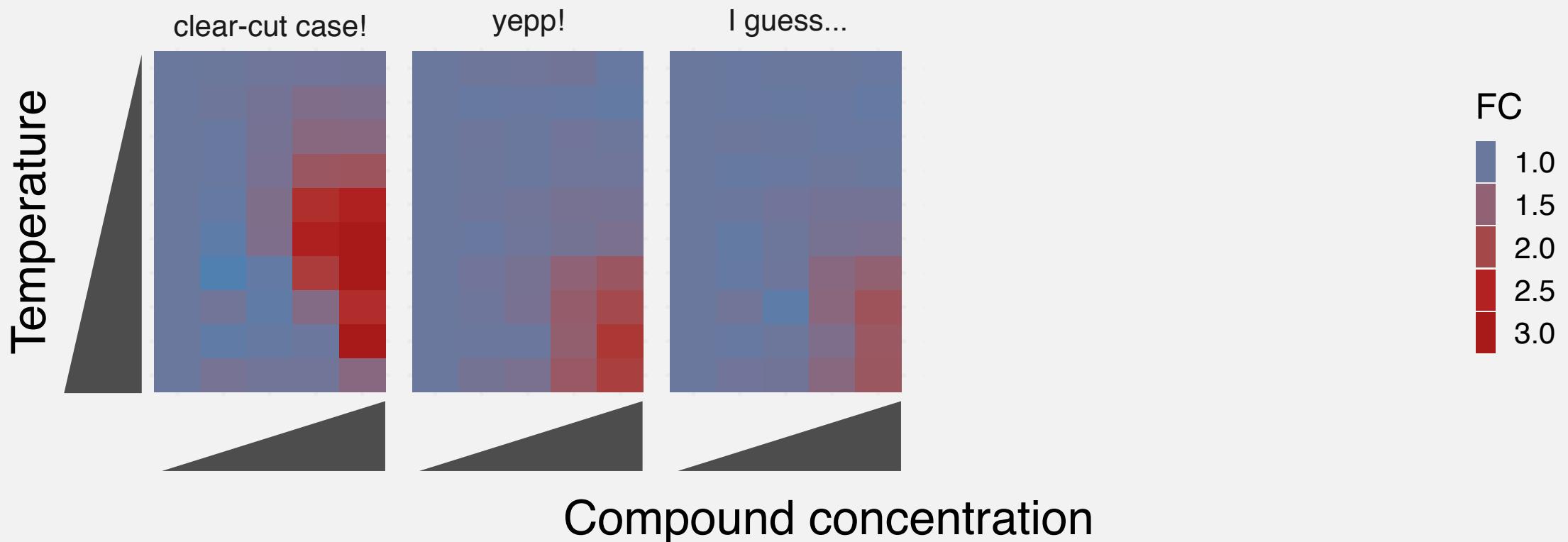
# When is a hit a hit?



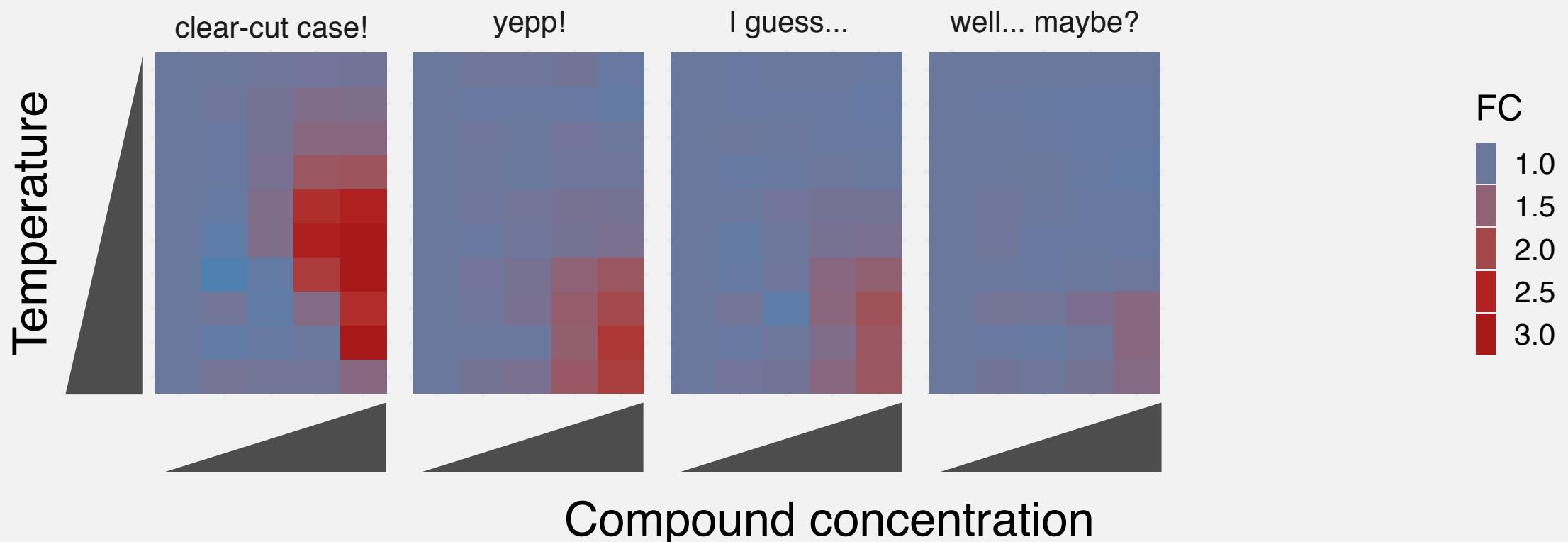
# When is a hit a hit?



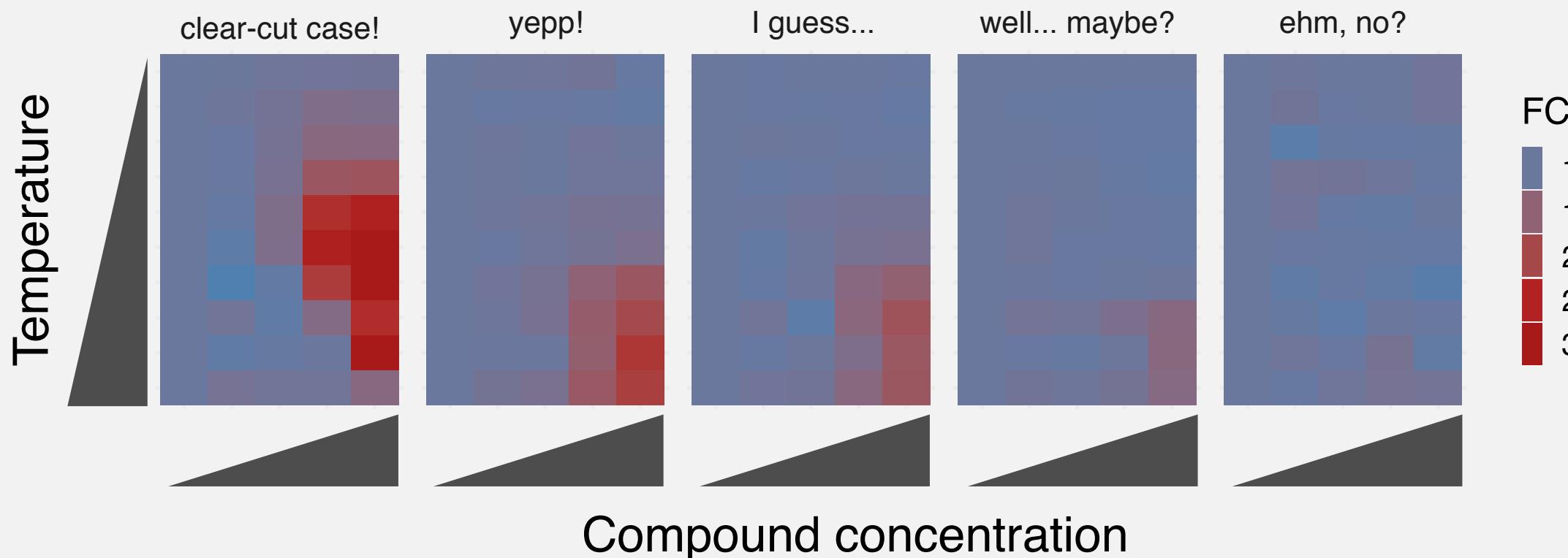
# When is a hit a hit?



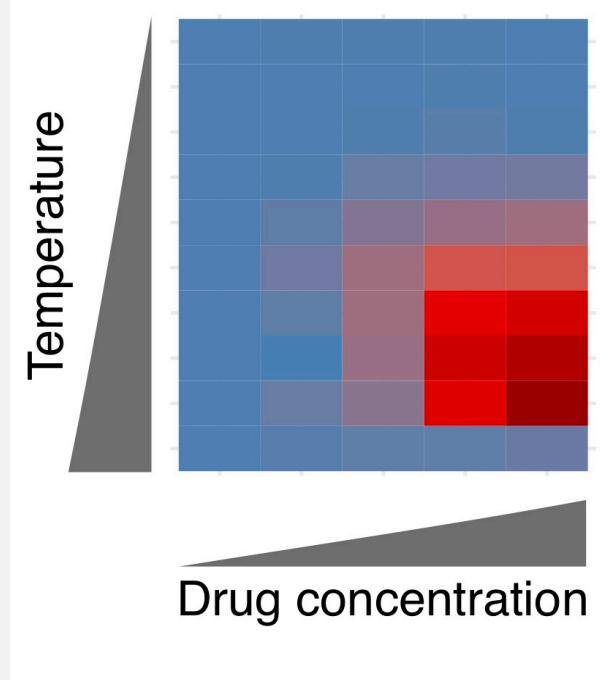
# When is a hit a hit?



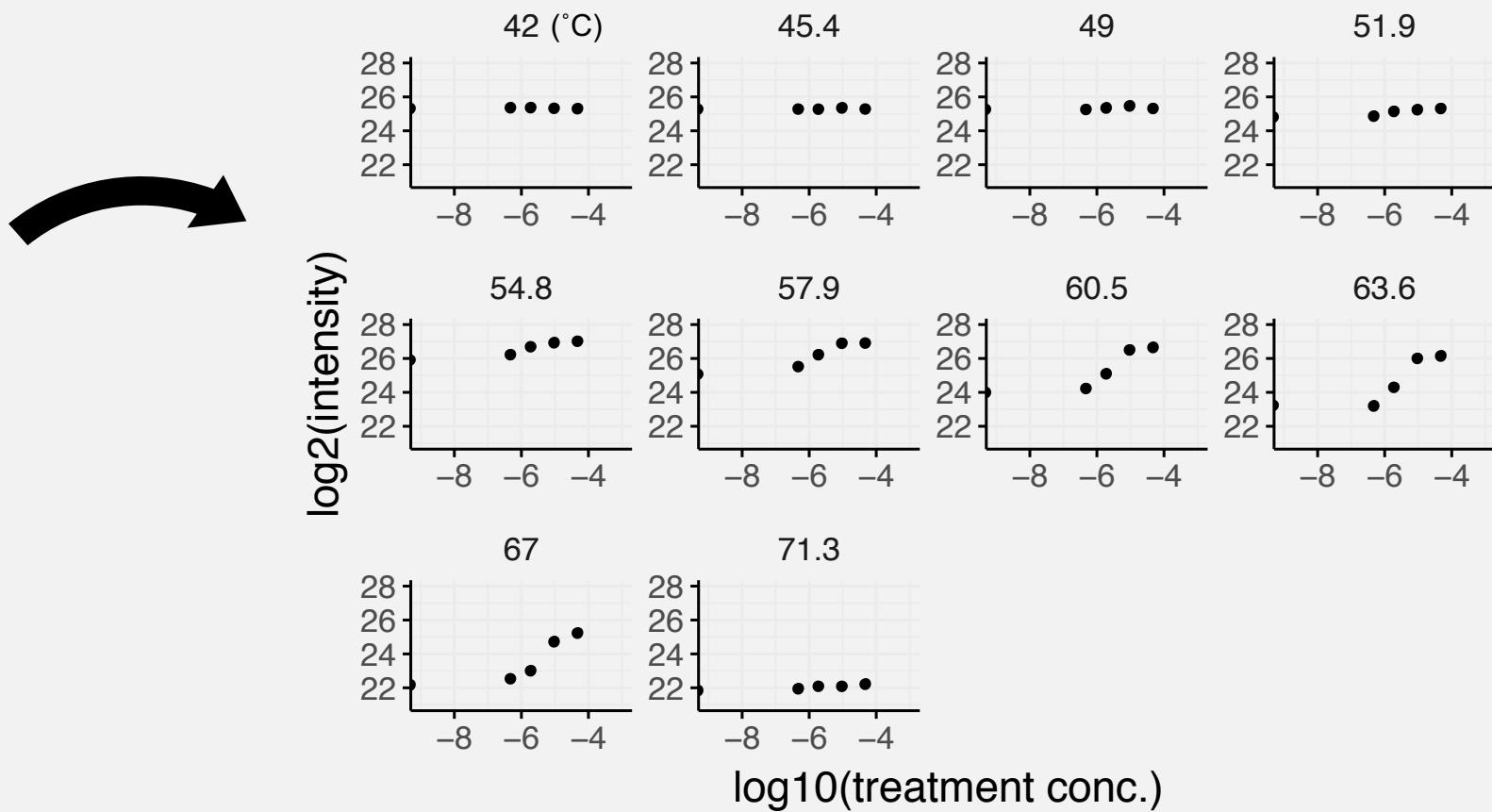
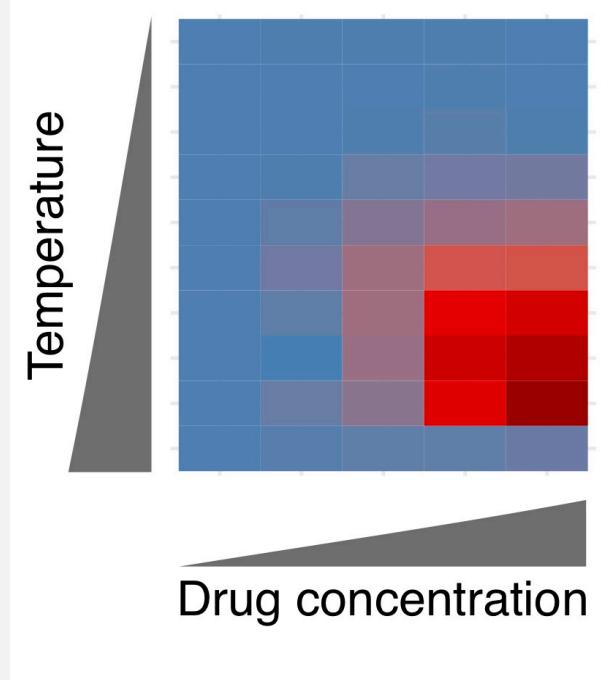
# When is a hit a hit?



# How to analyze 2D-TPP datasets with false discovery rate control?

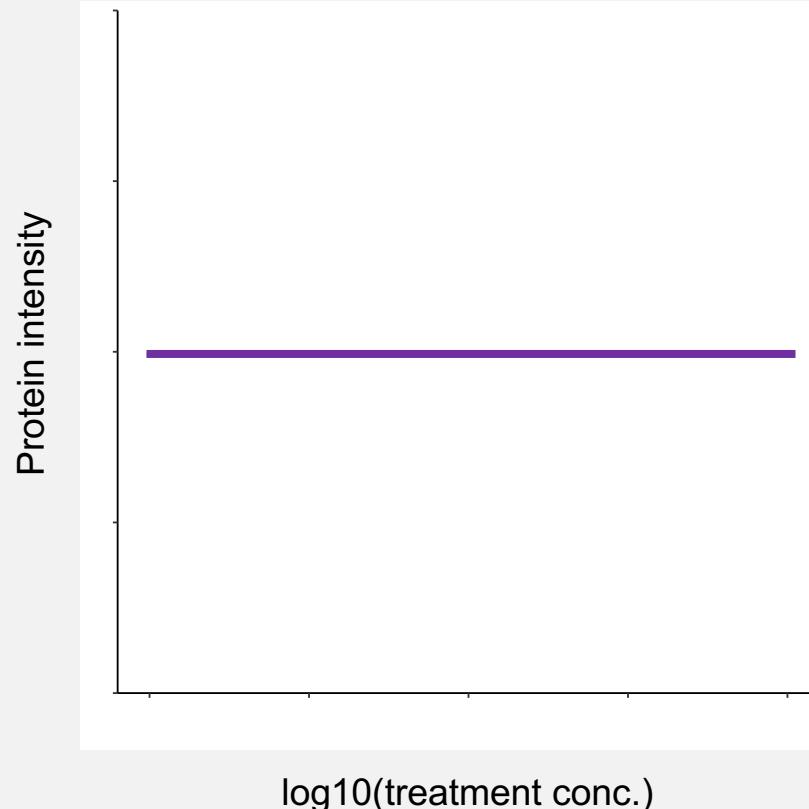


# How to analyze 2D-TPP datasets with false discovery rate control?



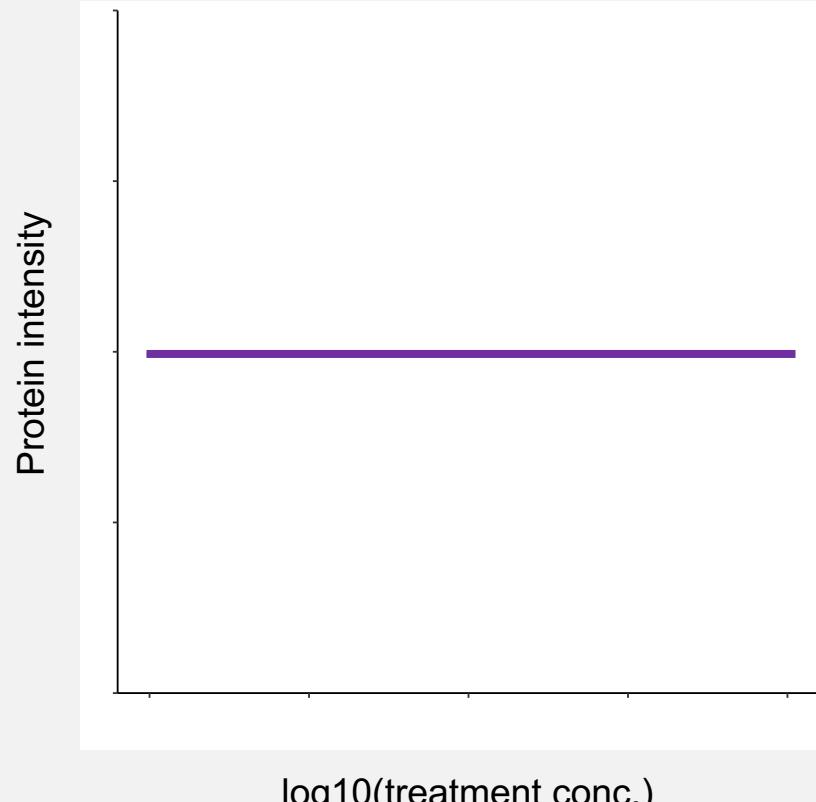
# A functional analysis approach for 2D-TPP data

**Null model:** protein remains unaffected by treatment

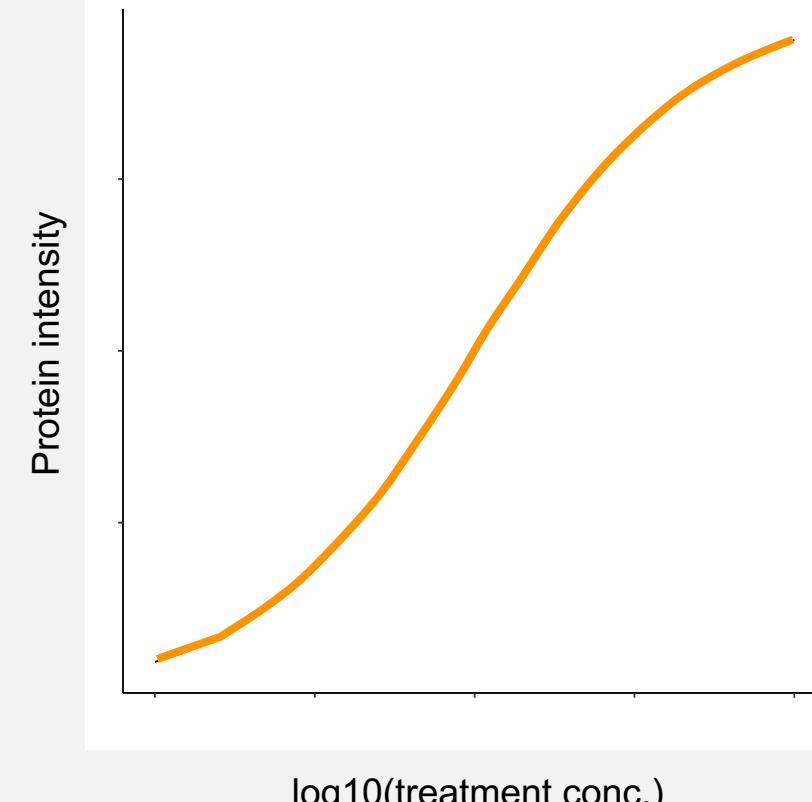


# A functional analysis approach for 2D-TPP data

**Null model:** protein remains unaffected by treatment

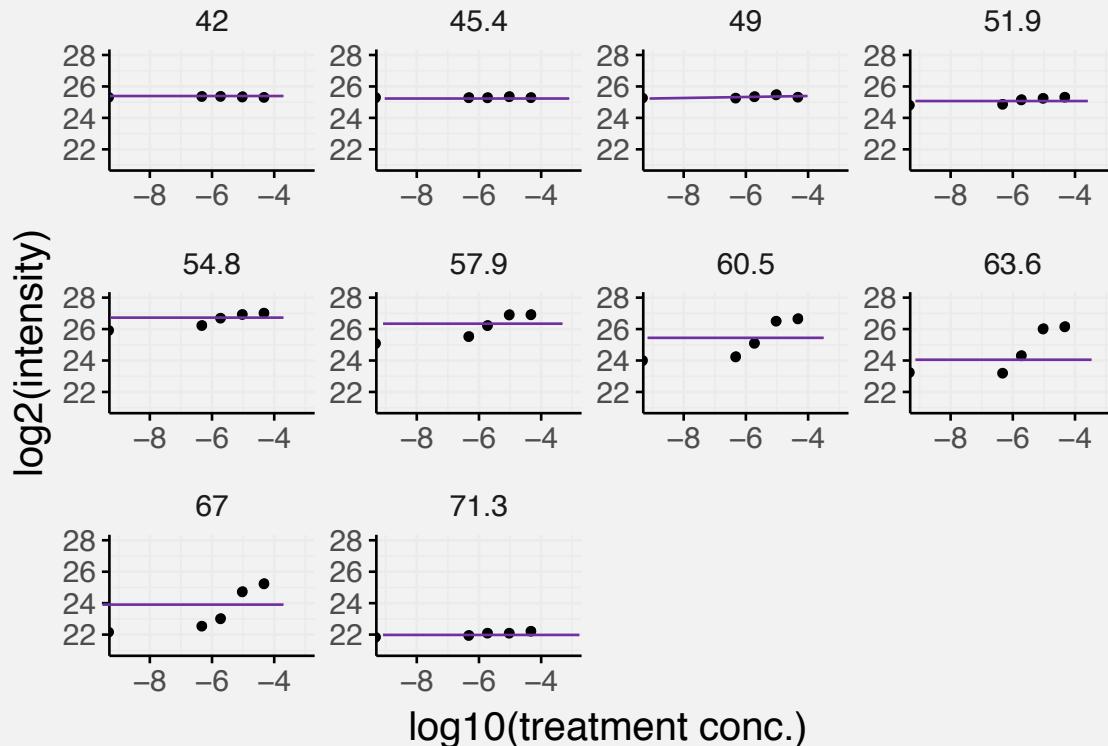


**Alternative model:** protein stability is affected by treatment

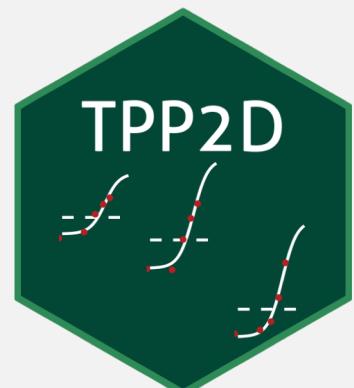


# Constructing null and alternative models

**Null model:** protein remains unaffected by treatment

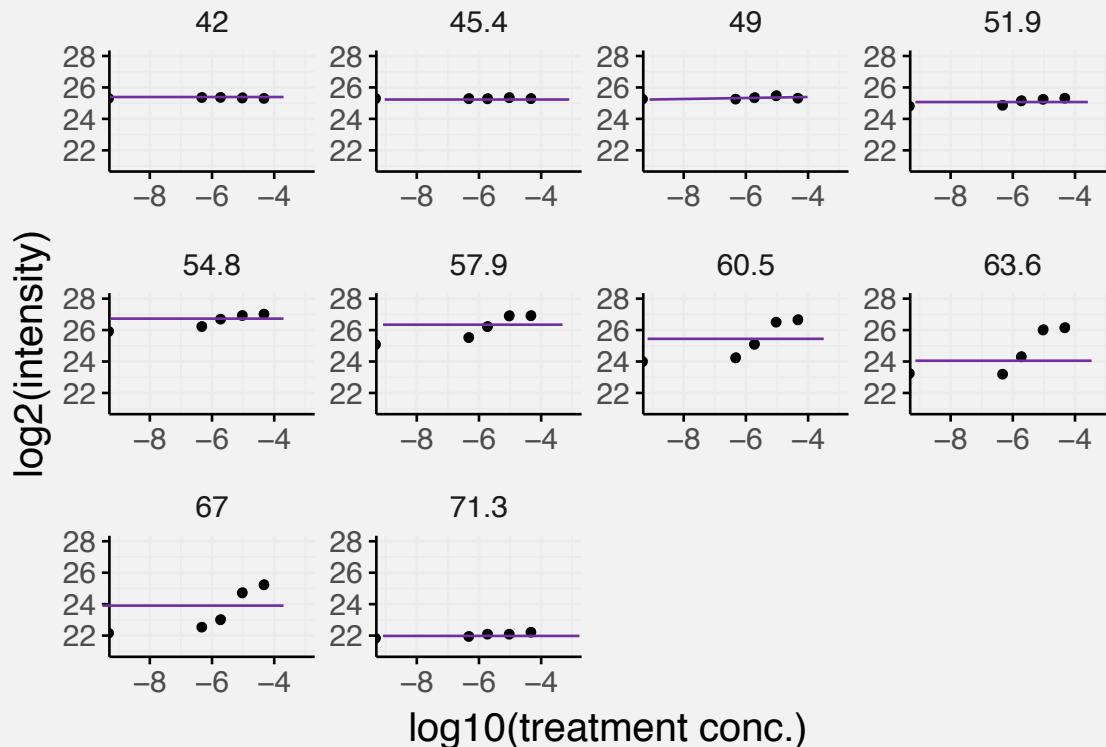


**Alternative model:** protein stability is affected by treatment

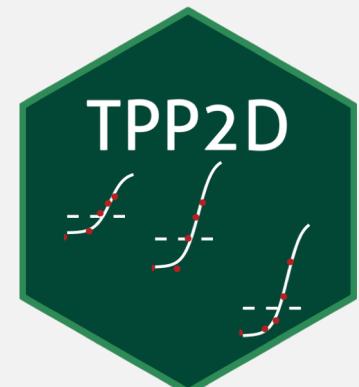
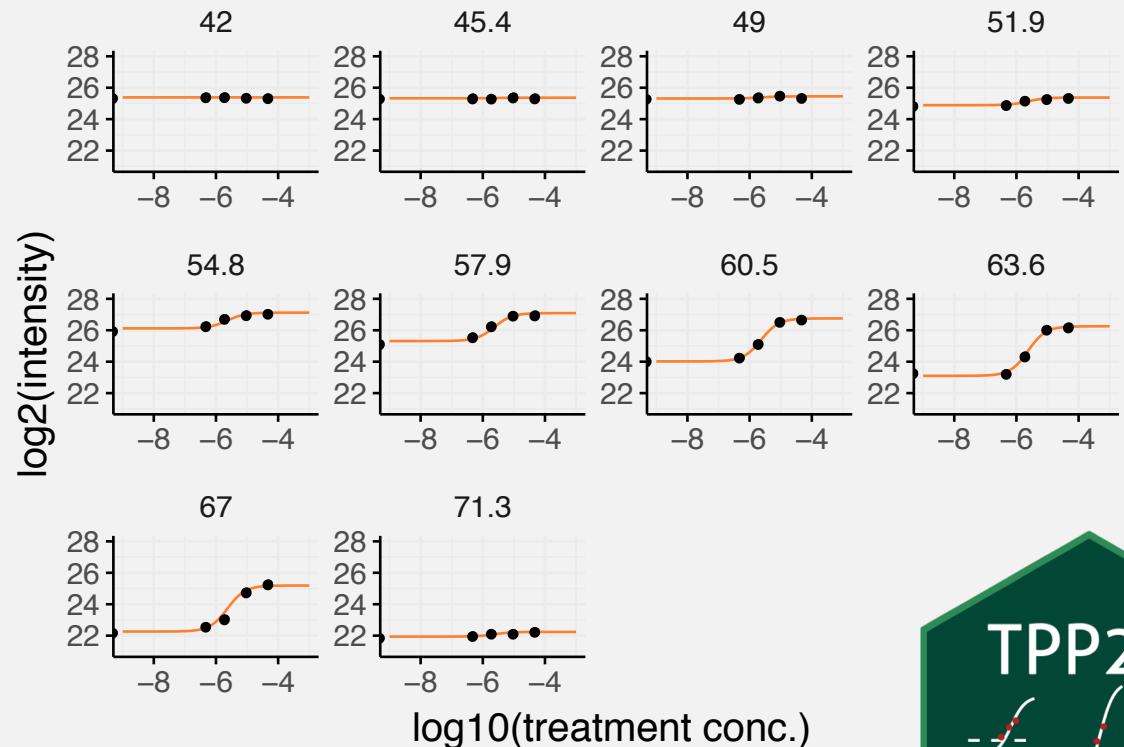


# Constructing null and alternative models

**Null model:** protein remains unaffected by treatment

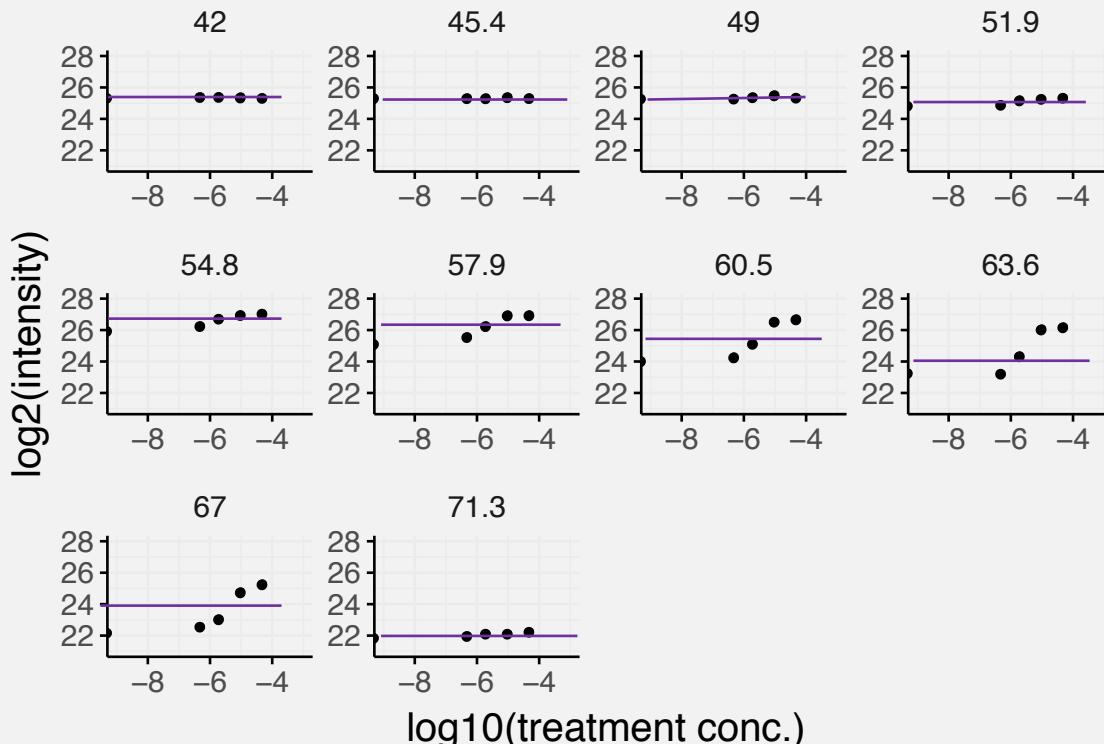


**Alternative model:** protein stability is affected by treatment

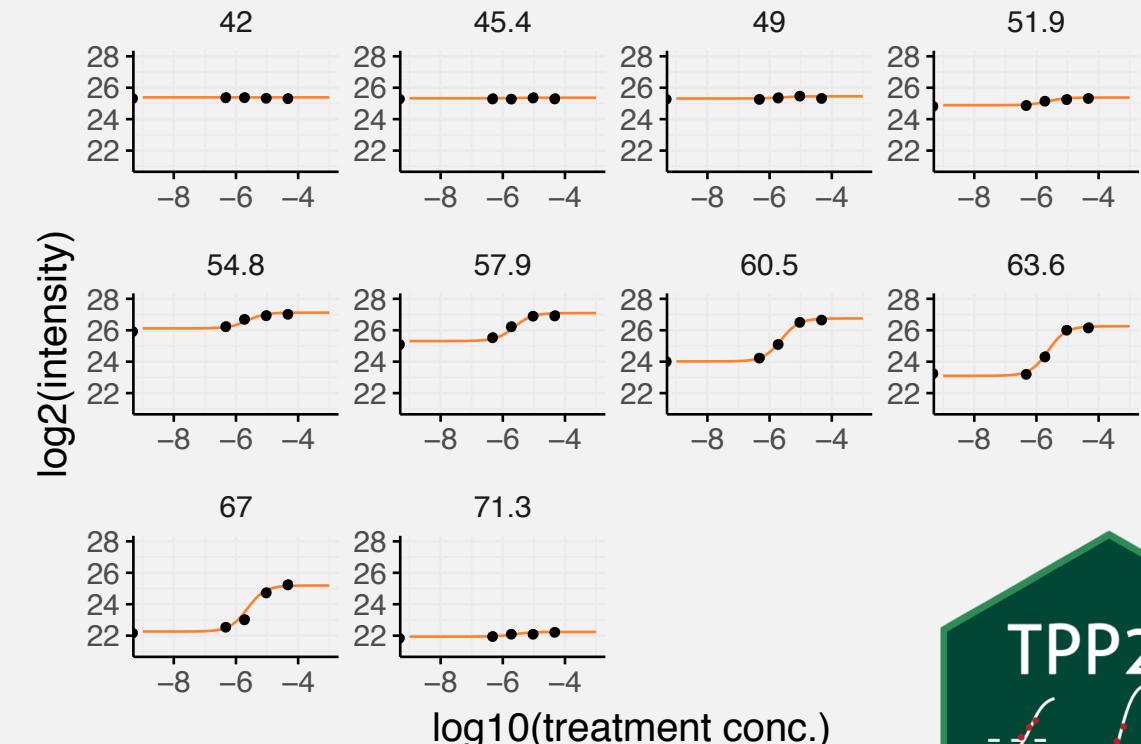


# Constructing null and alternative models

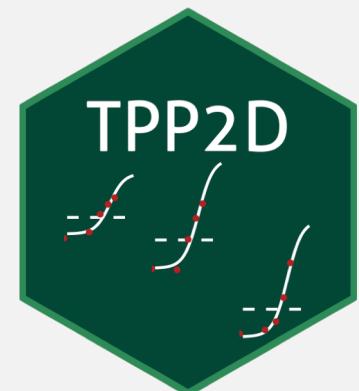
**Null model:** protein remains unaffected by treatment



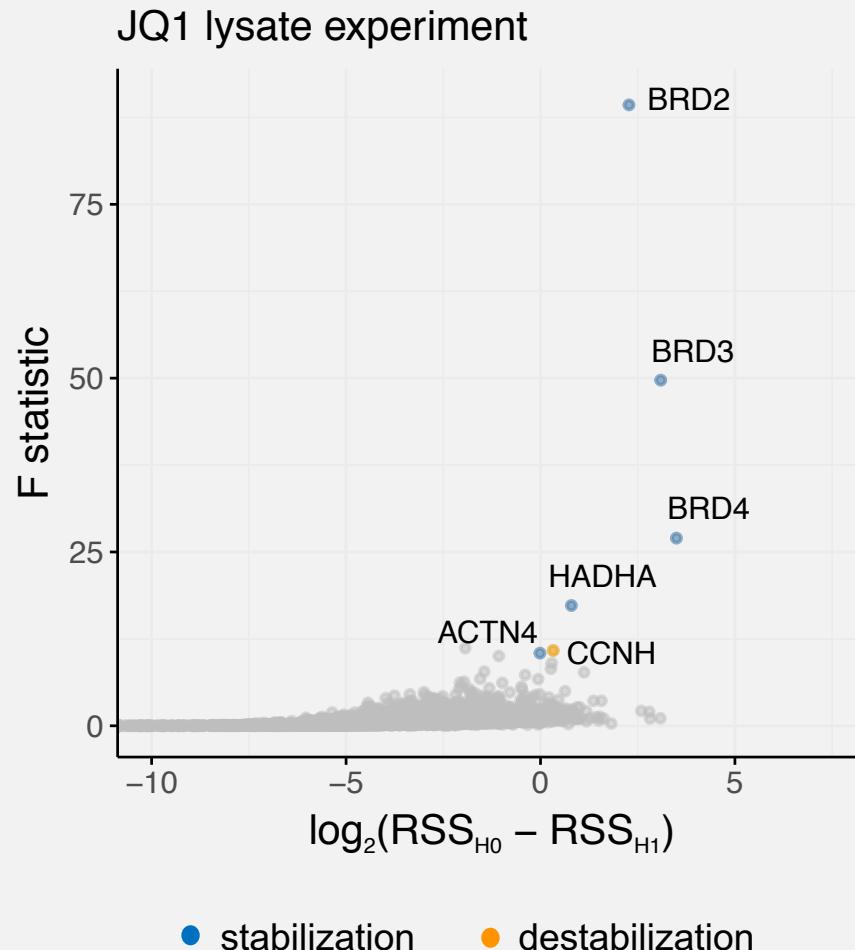
**Alternative model:** protein stability is affected by treatment



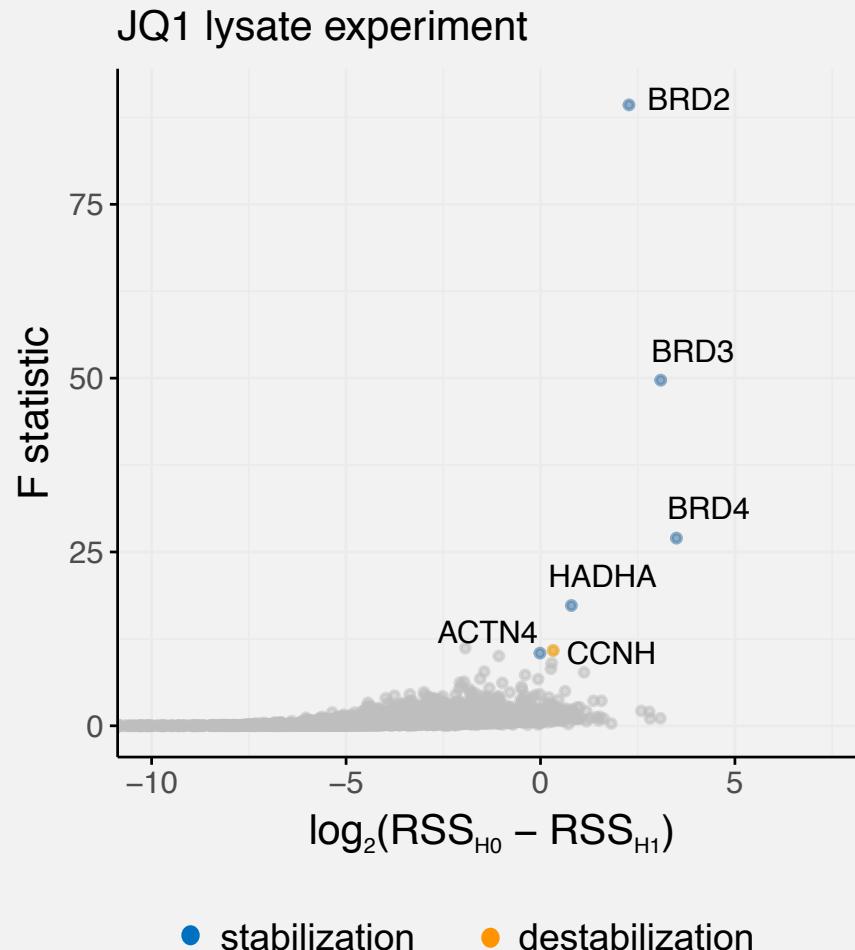
$$F = \frac{RSS^0 - RSS^1}{RSS^1}$$



# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1

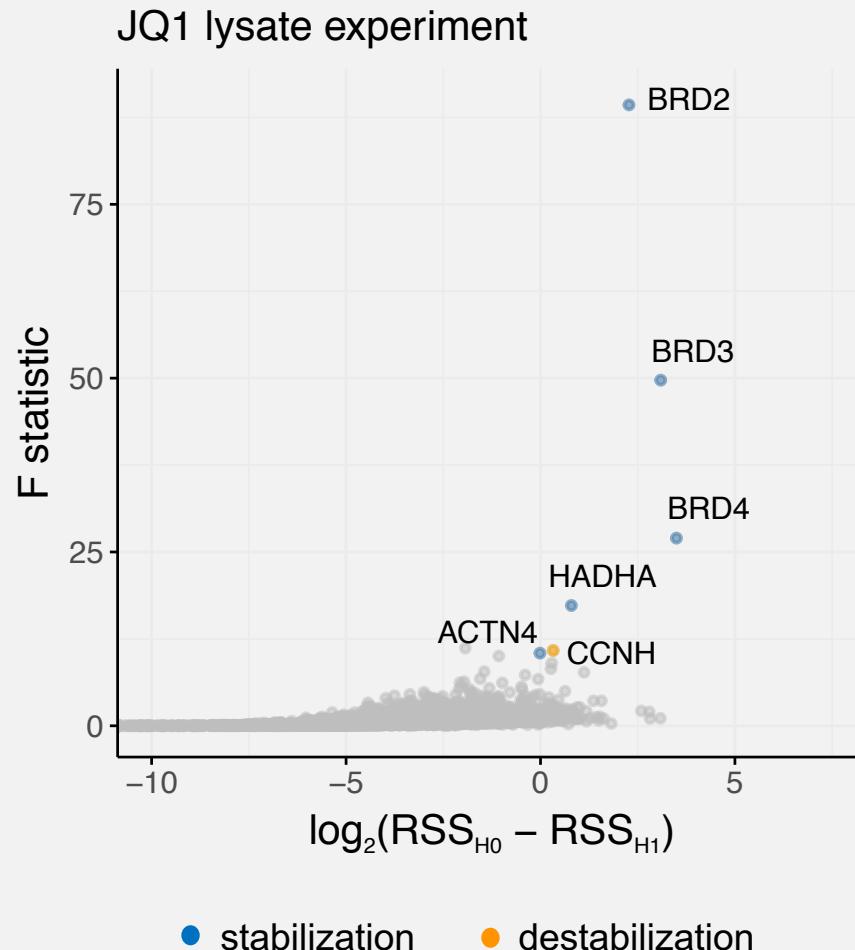


# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1



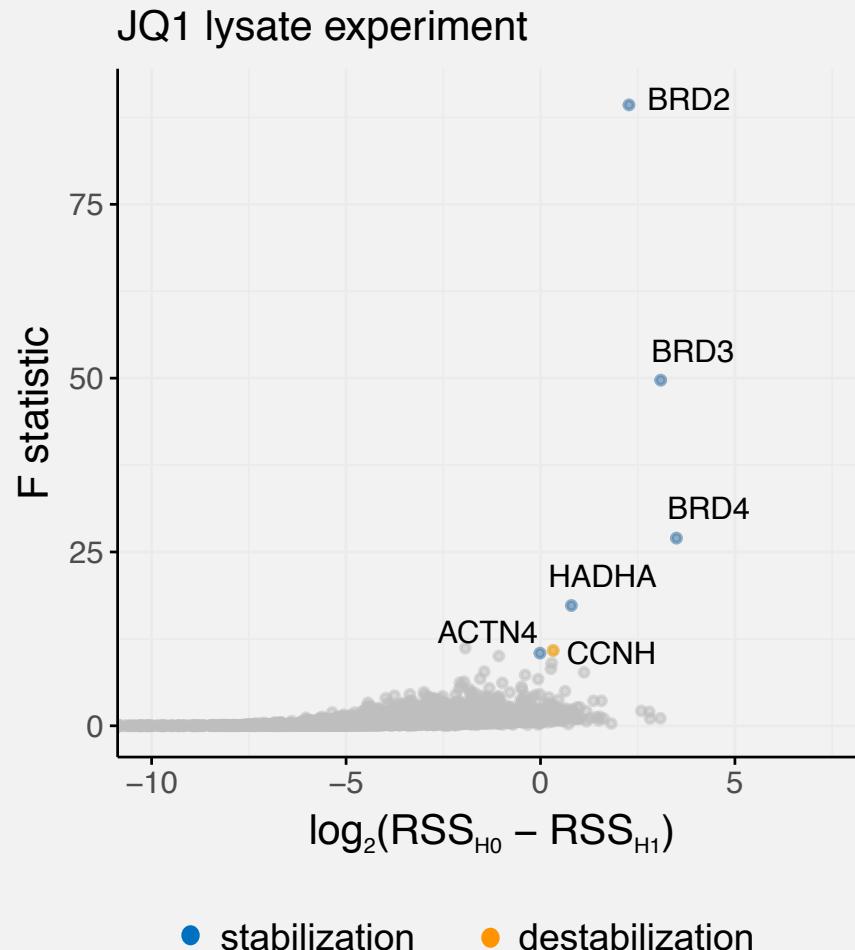
- Previously detected targets are found: BRD2-4 and HADHA

# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1

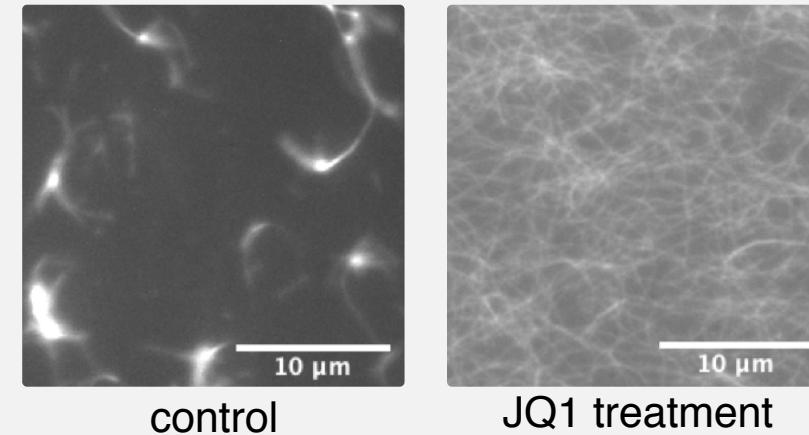


- Previously detected targets are found: BRD2-4 and HADHA
- New off-target found: ACTN4

# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1



- Previously detected targets are found: BRD2-4 and HADHA
- New off-target found: ACTN4



Sristhi Dar

# Acknowledgements

## EMBL Heidelberg:

Misha Savitski  
Wolfgang Huber  
Srishti Dar  
Sindhuja Sridharan  
Isabelle Becher  
André Mateus  
Britta Velten  
Dorothee Childs

## CRUK Cambridge:

Karsten Bach

## ZMBH Heidelberg:

Simon Anders

## Cellzome, GSK:

Marcus Bantscheff  
Jessica Perrin  
Thilo Werner  
Holger Franken  
Carola Doce  
Maria Fälth-Savitski

**All Savitski and  
Huber Group  
members at EMBL**

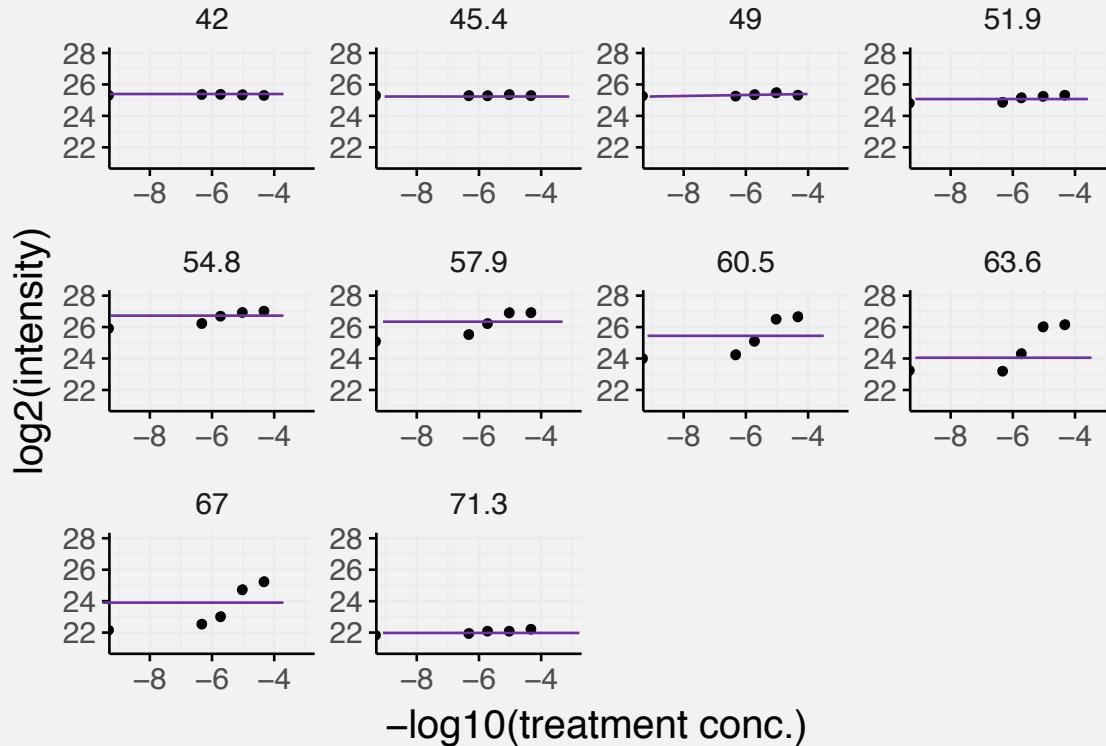




**Thank you!**



# Constructing an H1 model (treatment effect)

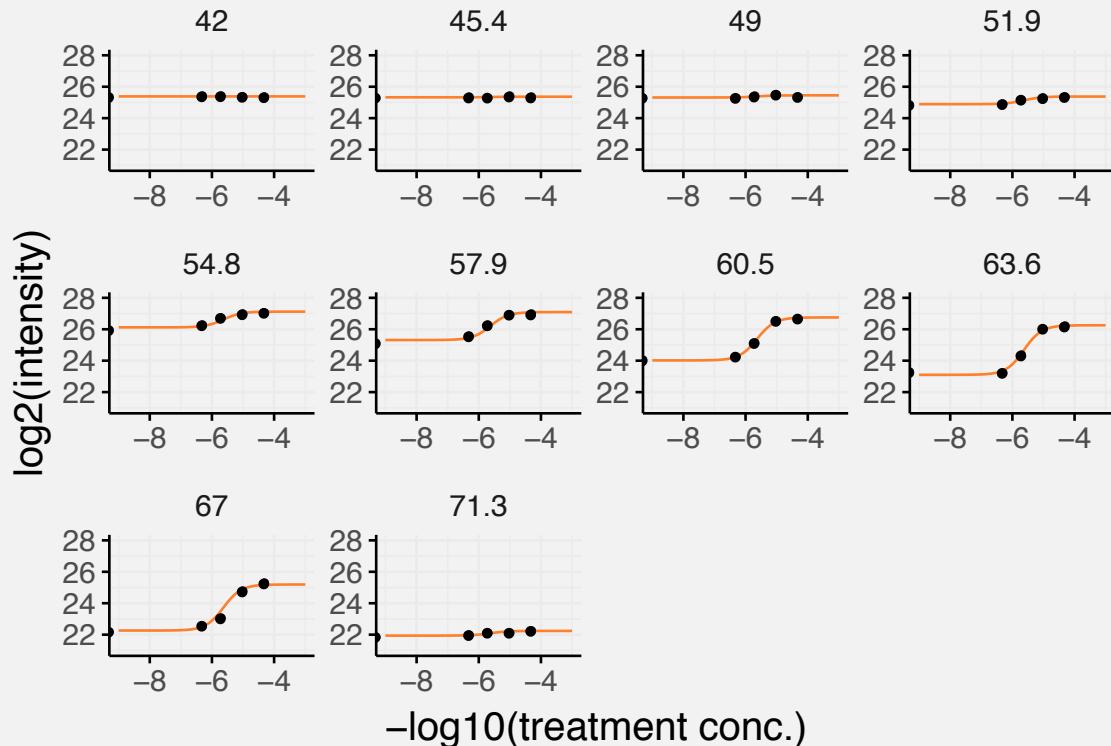


$$y_{i,t} = \beta_{i,t}^0 + \epsilon_{i,t}$$

$y_{i,t}$ :  $\log_2$  intensity for protein  $i$  at temperature  $t$

$\beta_{i,t}^0$ : concentration-independet intercept parameter for protein  $i$  at temperature  $t$

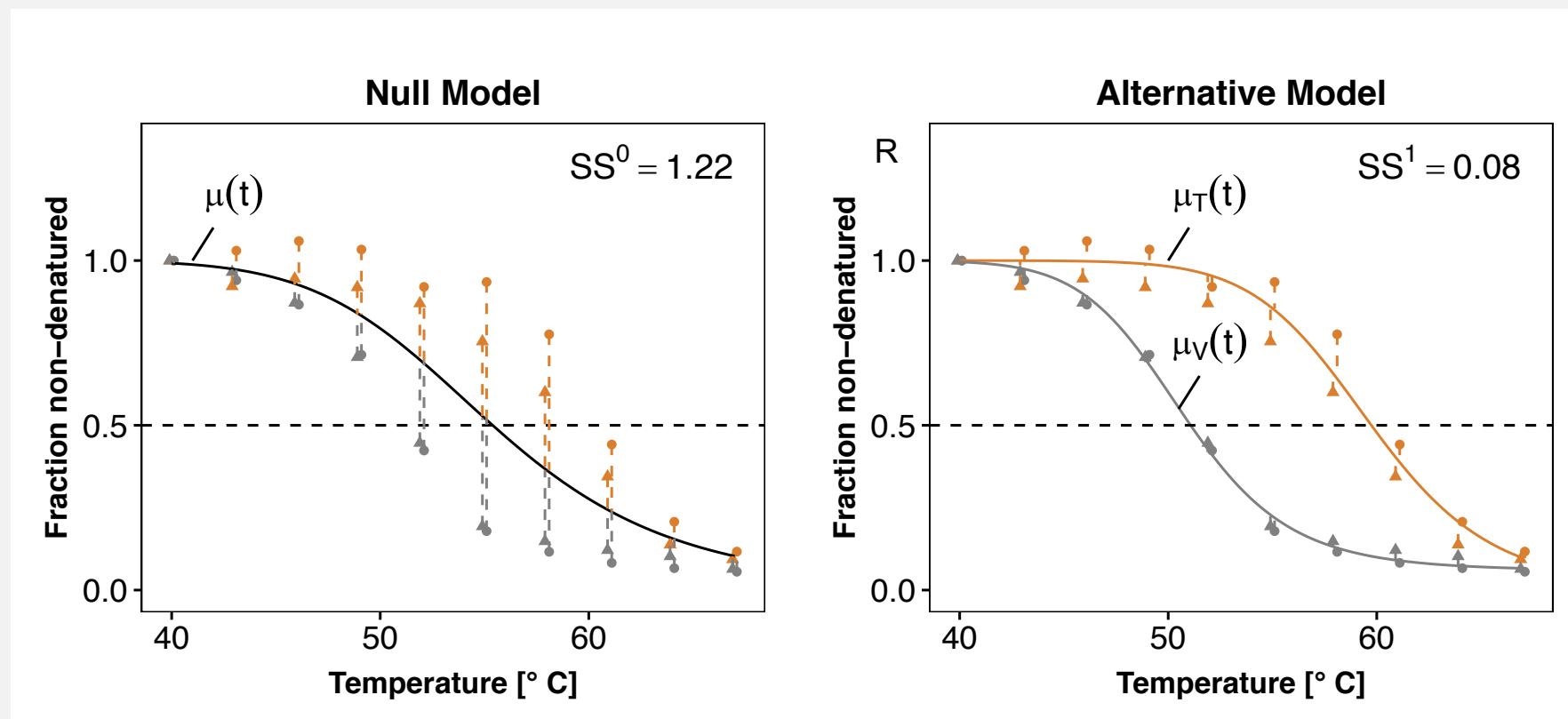
# Constructing an H1 model (treatment effect)



$$y_{i,t}(c) = \beta_{i,t}^0 + \frac{\alpha_{i,t} \delta_i^{\max}}{1 + \exp(\kappa_i(c - \xi(t)_i))} + \epsilon_{i,t,c}$$

$y_{i,t}(c)$ : log<sub>2</sub> intensity for protein  $i$  at temperature  $t$ , at concentration  $c$   
 $\beta_{i,t}^0$ : concentration-independet intercept parameter for protein  $i$  at temperature  $t$  (value  $y$  will take for  $c = 0$ )  
 $\delta_i^{\max}$ : maximal stabilization  
 $\alpha_{i,t}$ : parameter indicating how much relative stabilization happens at temperature  $t$   
 $\kappa_i$ : slope factor  
 $\xi(t)_i$ : linear function describing decline of the pEC<sub>50</sub> with increasing temperature

# Functional analysis of TPP melting curves: NPARC



SS: sum of squared errors



Karsten Bach

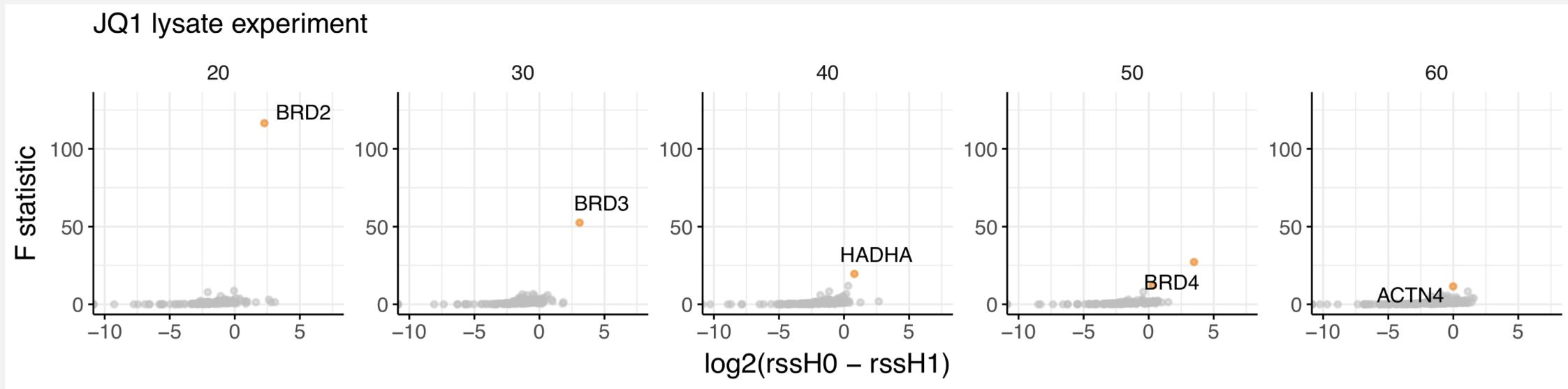


Dorothee Childs

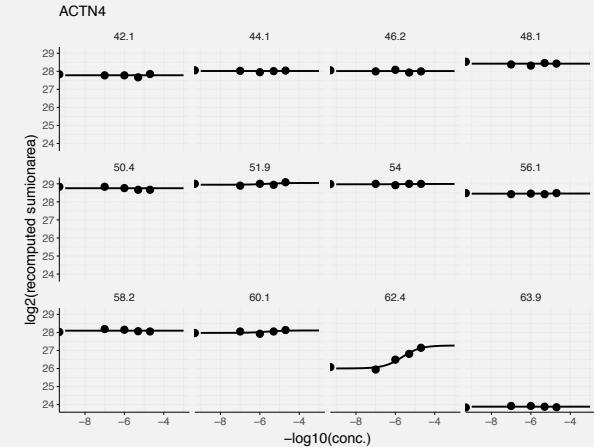


Holger Franken

# Method performance on JQ1 lysate dataset

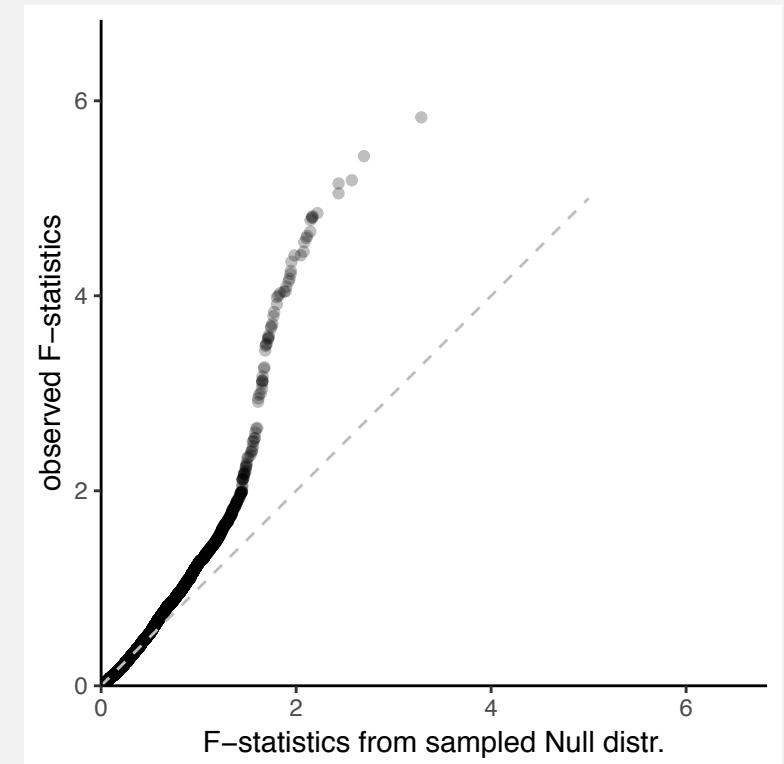


- We currently follow up on ACTN4 as a potential off-target of JQ1
- effects of JQ1 on actin bundle formation have been observed, but were attributed to transcriptional changes via BRDs Qu et al. 2018, Cell Death Discovery

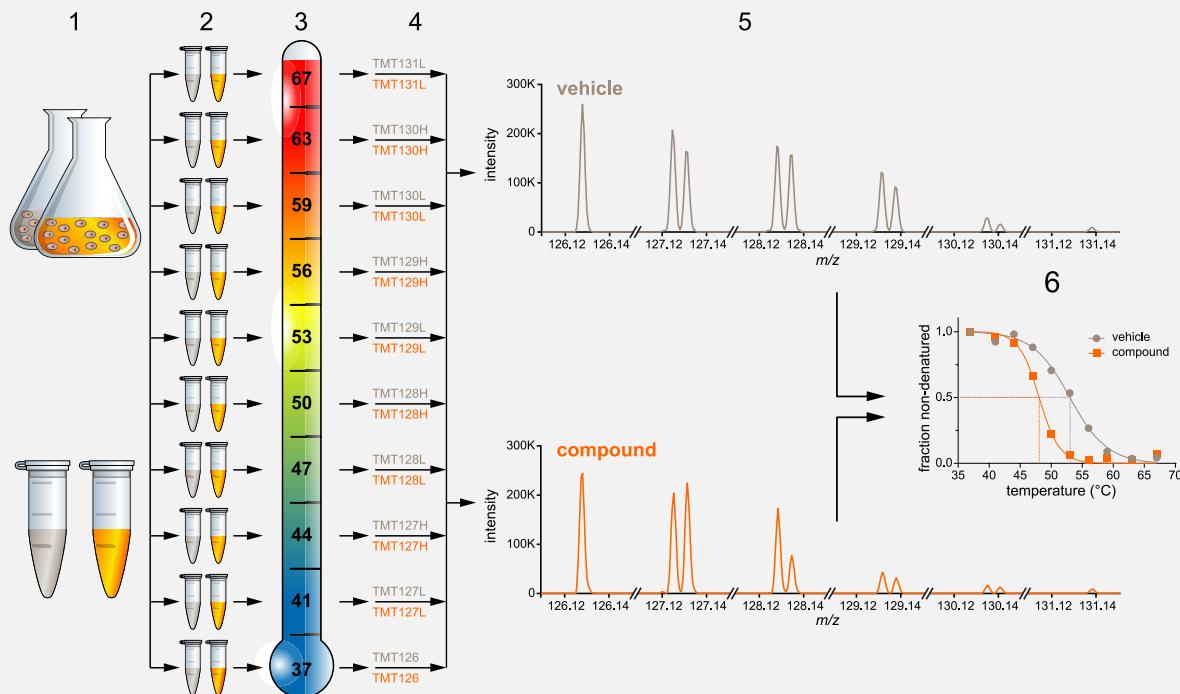


# Controlling FDR

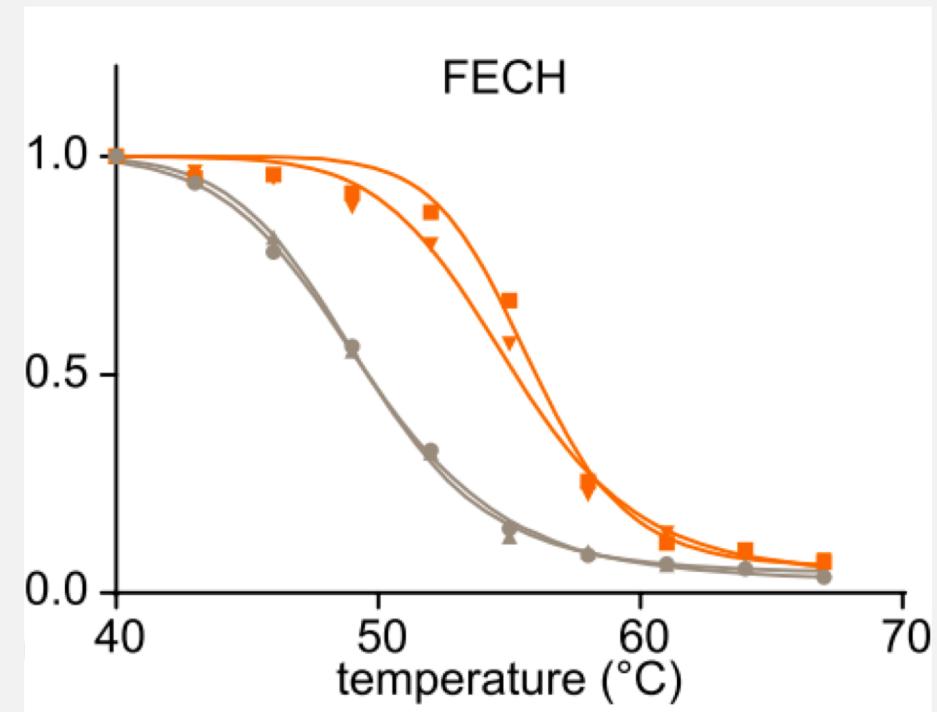
- Past experience: F statistic does not lead to valid p-values in melting curve/dose-response setting
- because residuals are correlated and heteroscedastic
- Approach: bootstrapping null distribution:
  - Fit H<sub>0</sub> model for every protein
  - Resample residuals from H<sub>0</sub> 10 times per protein, fit H<sub>1</sub> and compute F statistics
  - Repeatedly (**B** times) do this and jointly rank results with those from true dataset
- Compute FDR: 
$$FDR_{\theta} = \frac{\pi_{\theta} \sum_b^B \#\{F_i^{0,b} \geq \theta\}/(B)}{\#\{F_i \geq \theta\}}$$



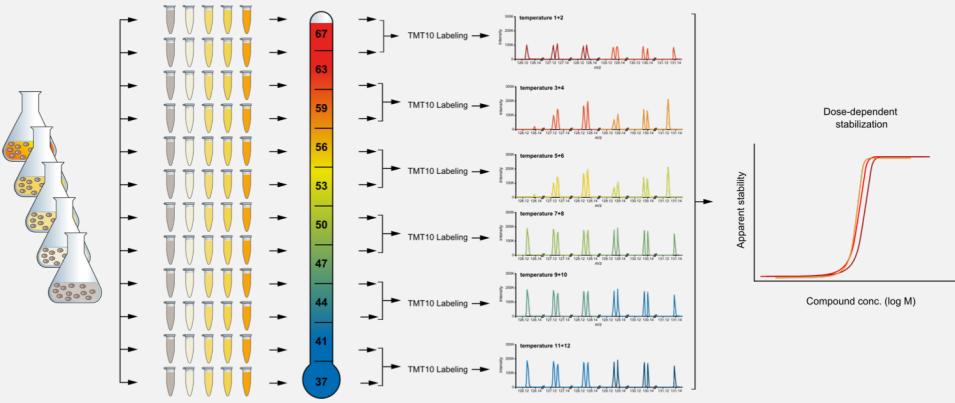
# Thermal proteome profiling (TPP)



Vemurafenib

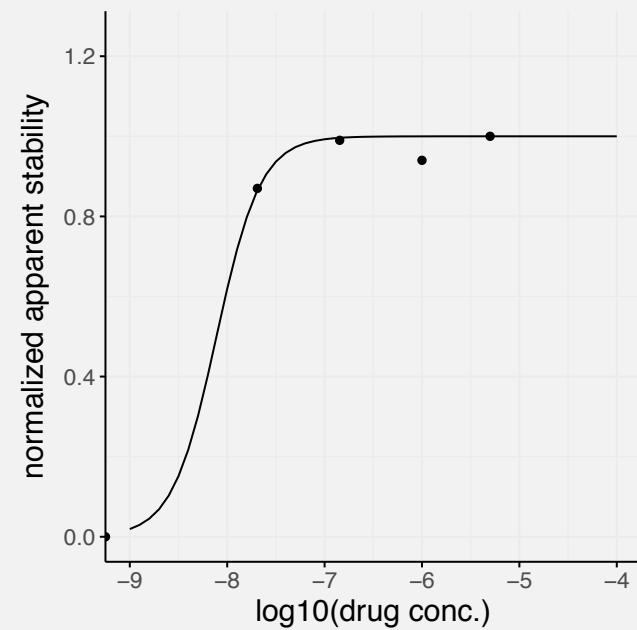


# 2D-TPP data analysis

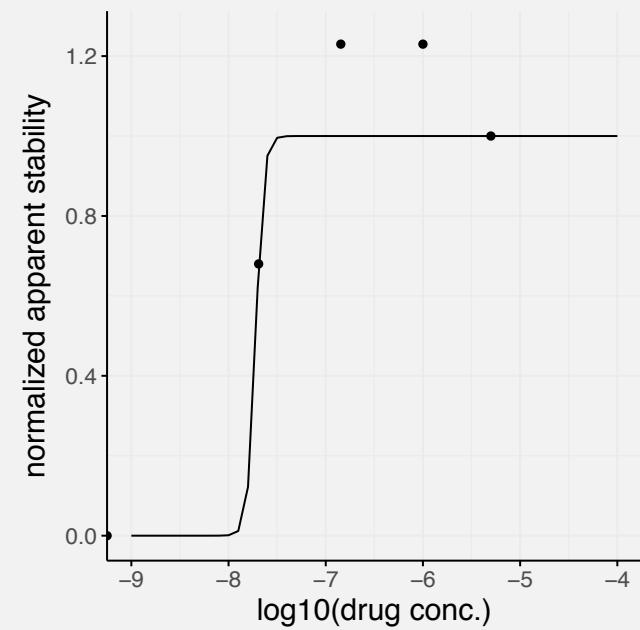


Cells treated with Panobinostat

HDAC2, 51.9 °C

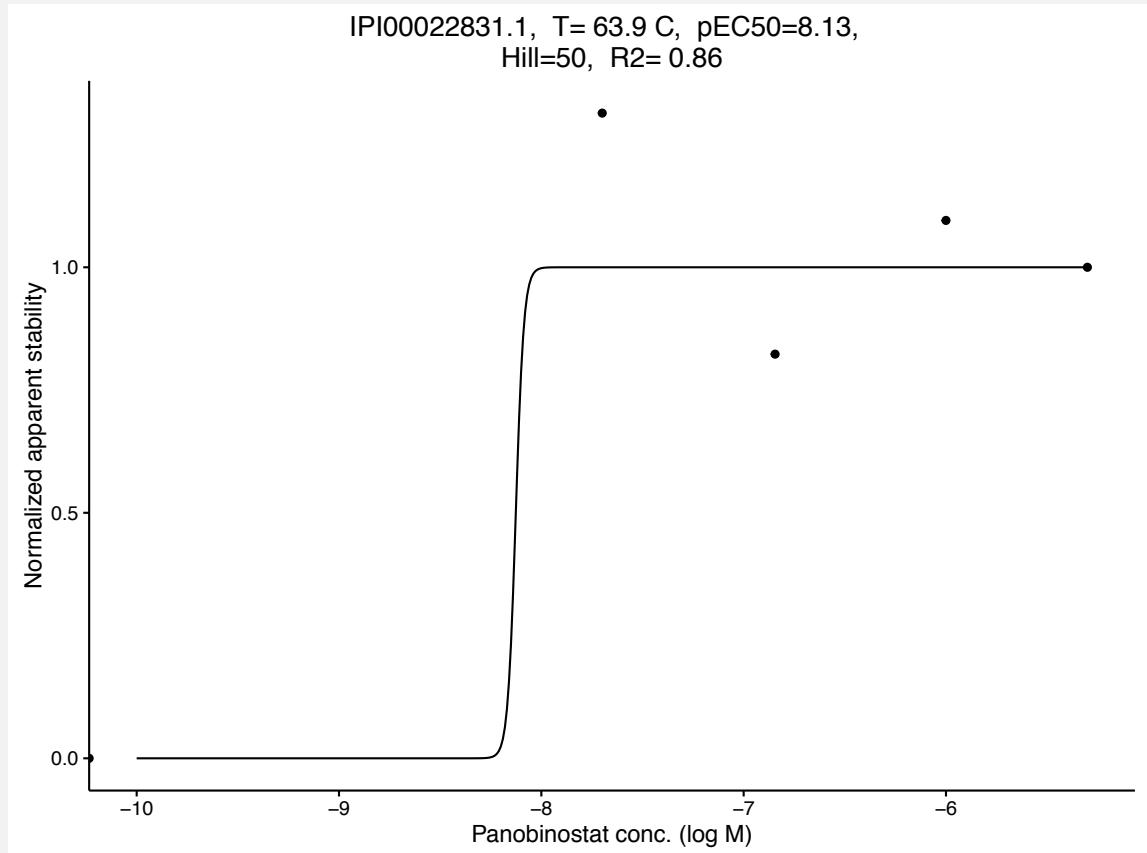


HDAC2, 54.0 °C



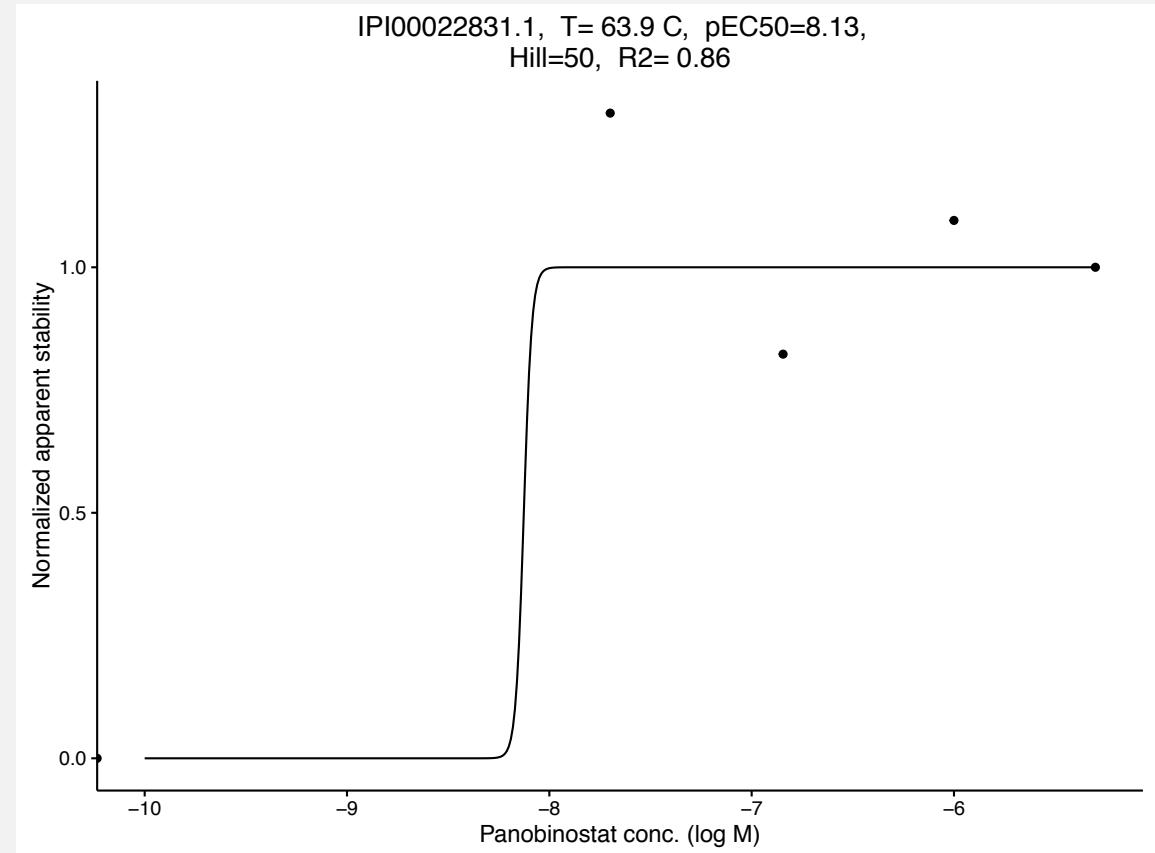
# 2D-TPP data analysis: what's the matter?

- Fitting dose-response models per temperature can be misleading
- Hits defined by manual thresholds

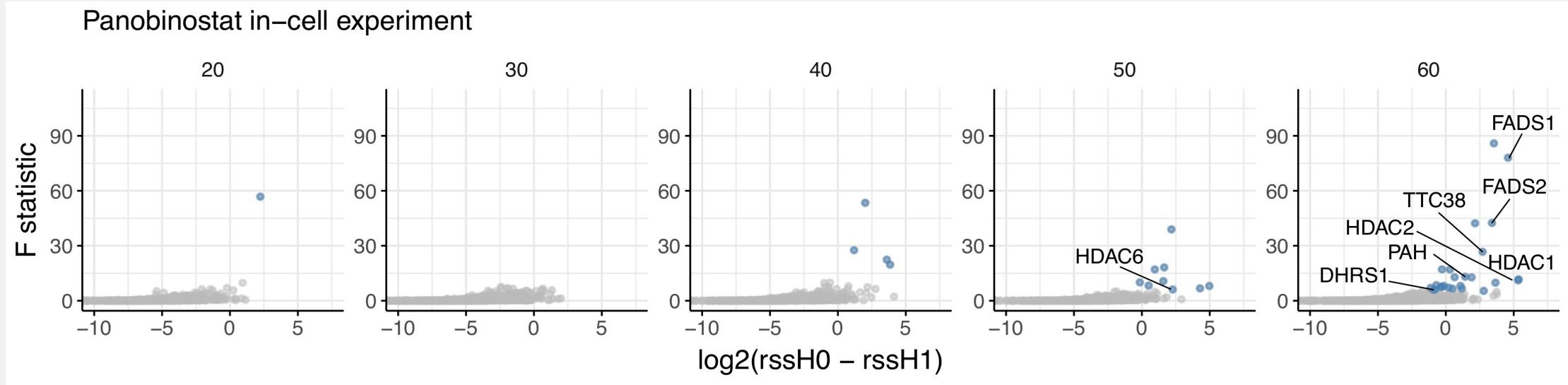


# 2D-TPP data analysis: what's the matter?

- Fitting dose-response models per temperature can be misleading
  - Hits defined by manual thresholds
  - No false discovery rate (FDR) control
- For experiments with several (expected) Targets, FDR estimation is crucial!



# Method performance on Panobinostat in-cell dataset



- Previously detected targets are found: HDAC1,2 and 6 and off-targets FADS1, 2, TTC38 and PAH
- New potential off-target found: DHRS1