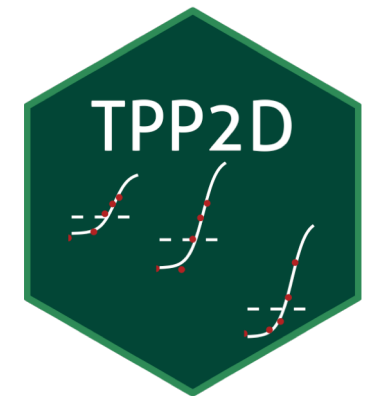


# Robust small molecule-protein interaction inference reveals unknown drug off-targets

Nils Kurzawa

PhD Student, Savitski Lab, EMBL Heidelberg

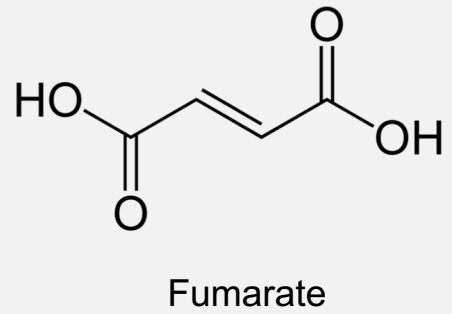
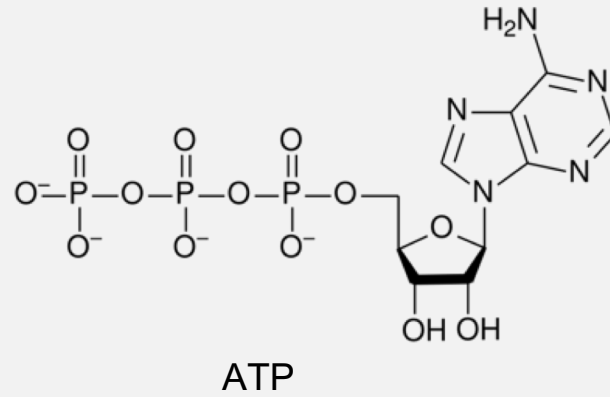


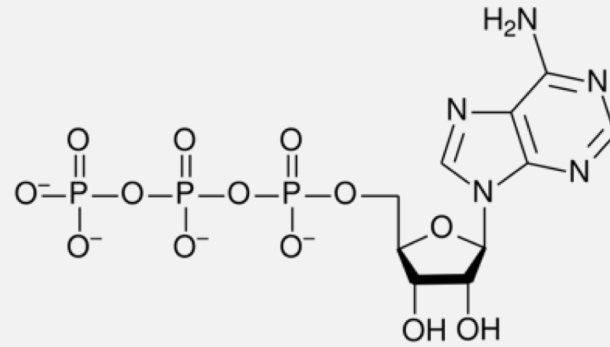
@nils\_kurzawa 

nkurzaw 

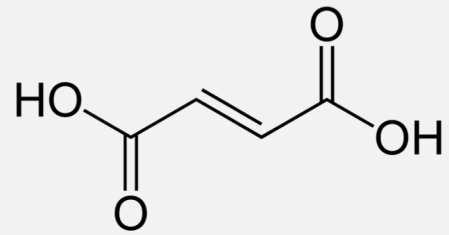
09/12/2019



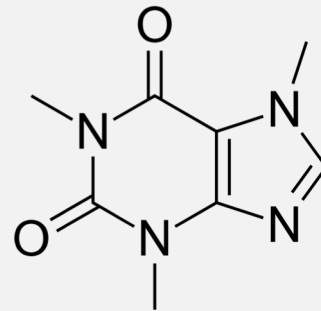




ATP

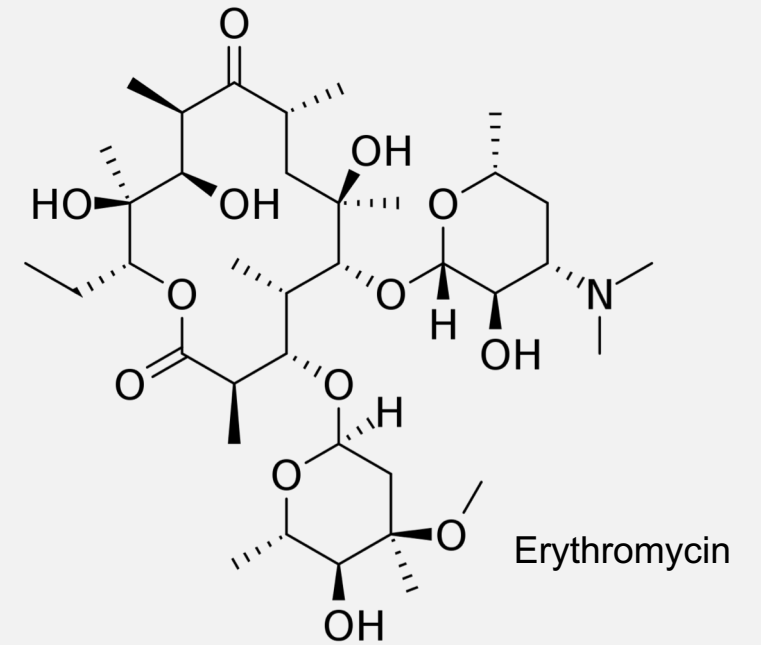
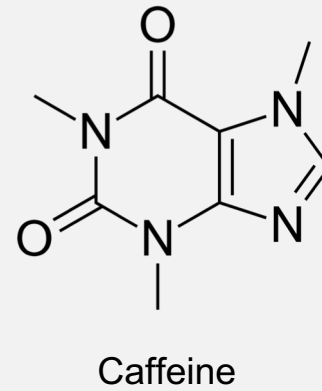
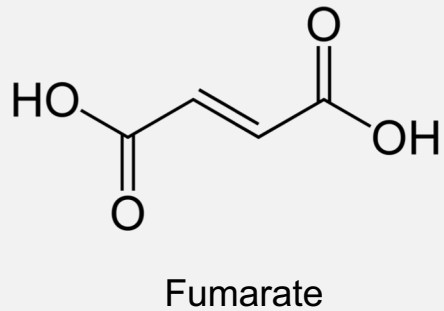
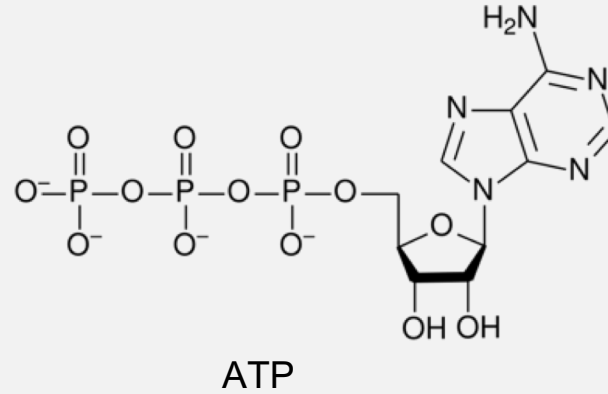
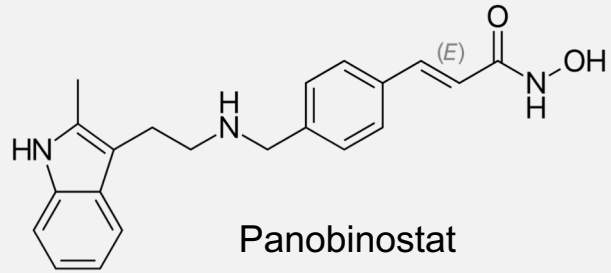


Fumarate

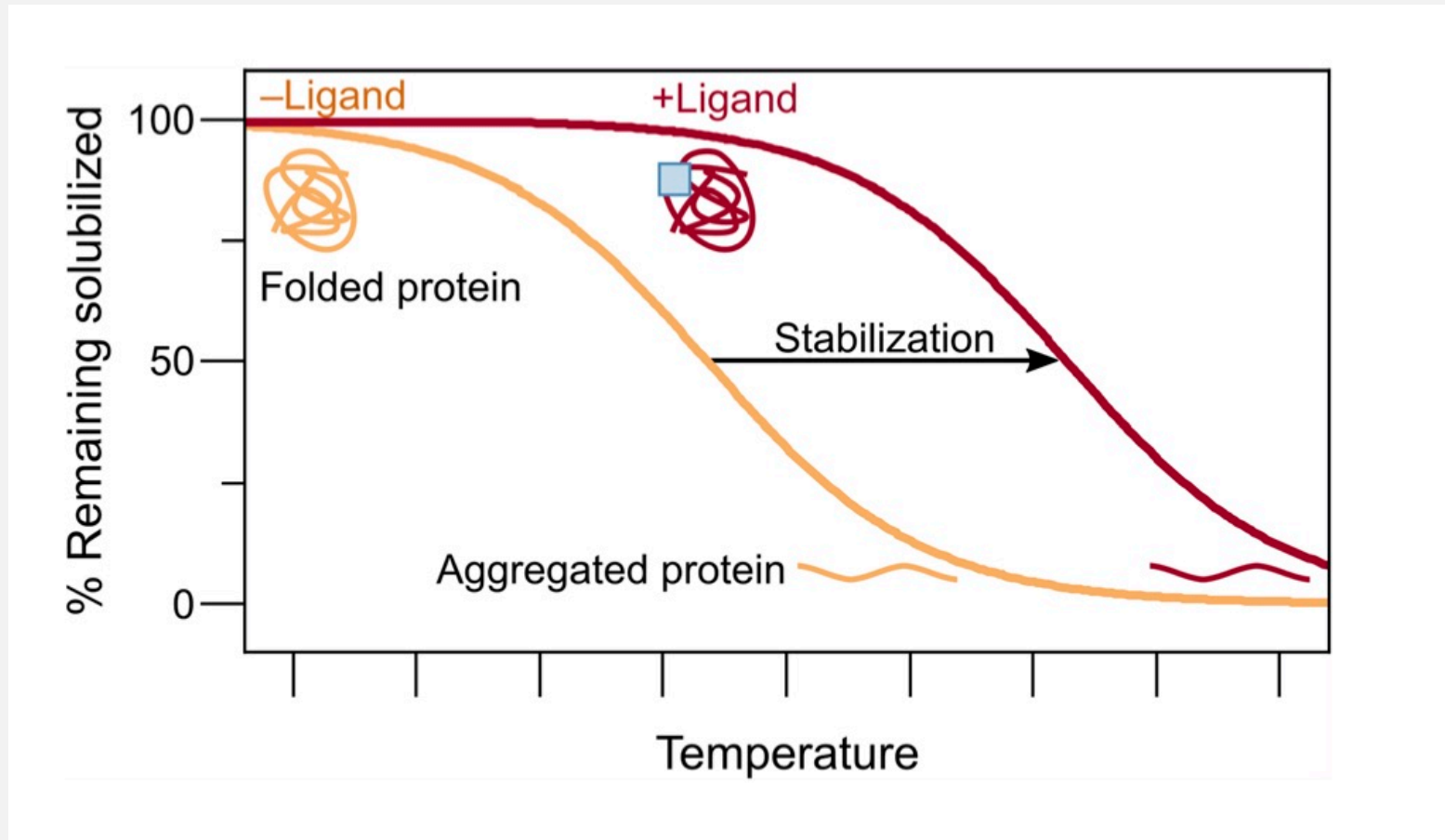


Caffeine

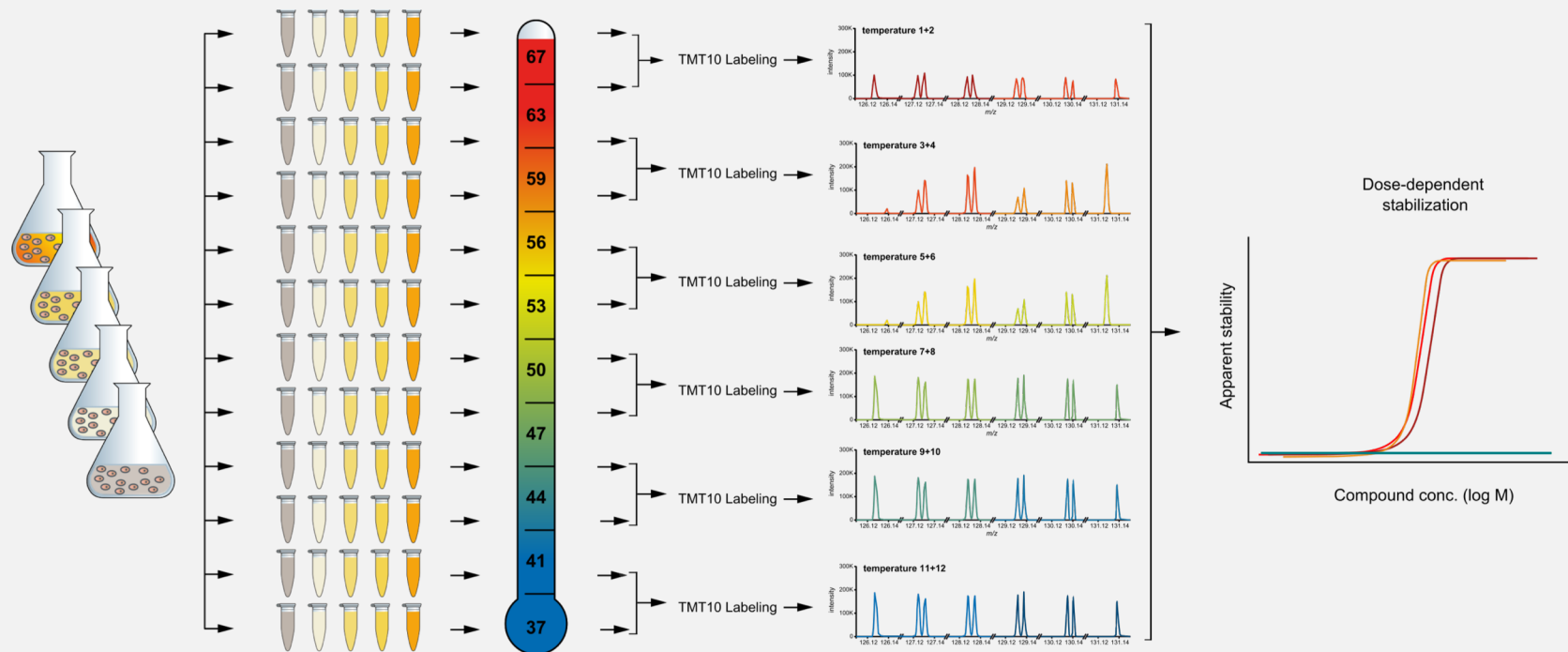
# Which proteins interact with certain small molecules?



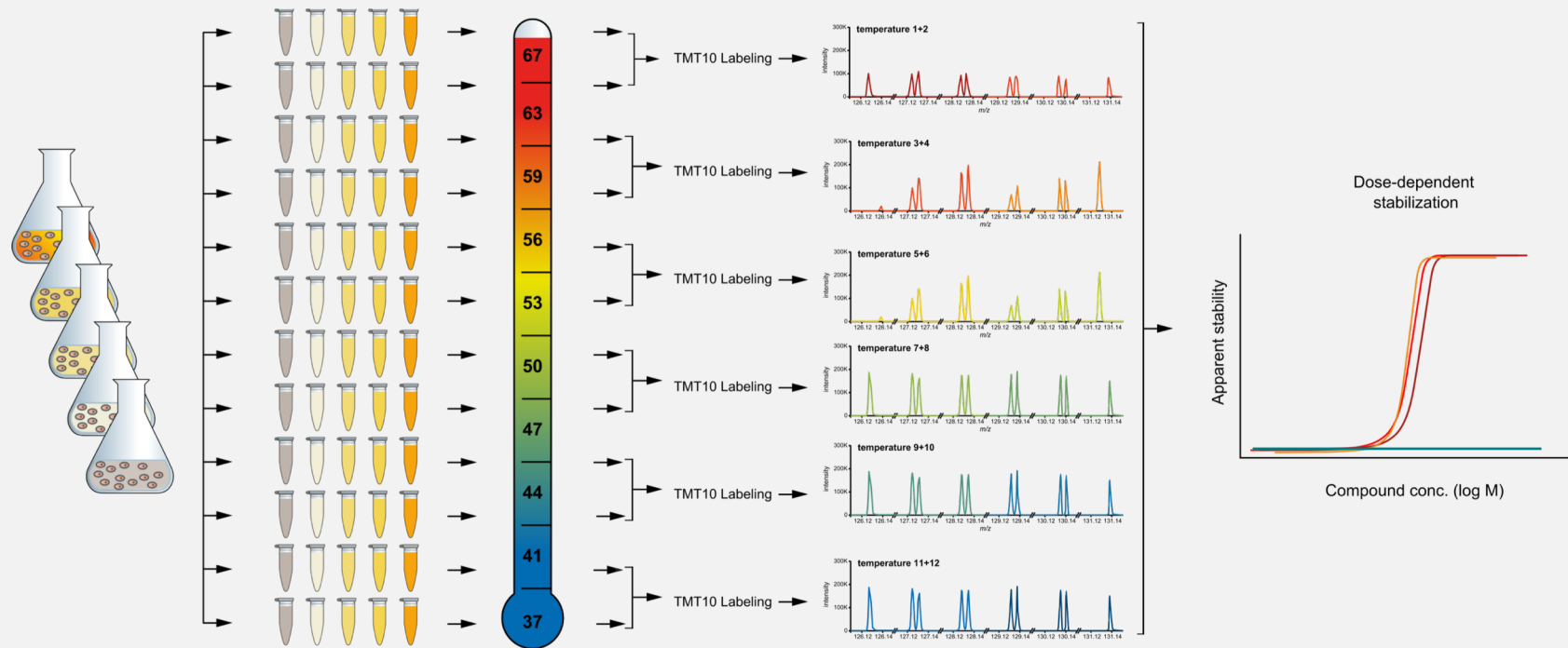
# The thermal shift assay



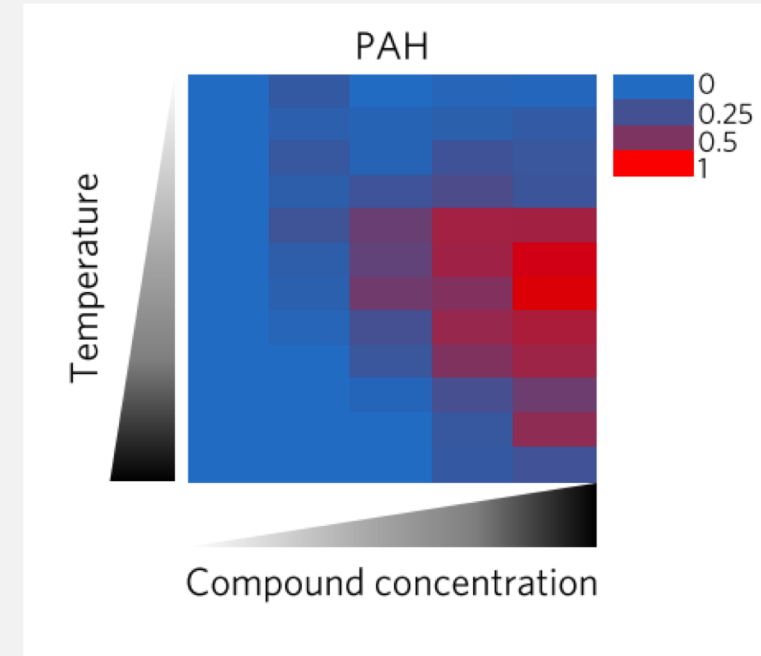
# Two dimensional thermal proteome profiling (2D-TPP)



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

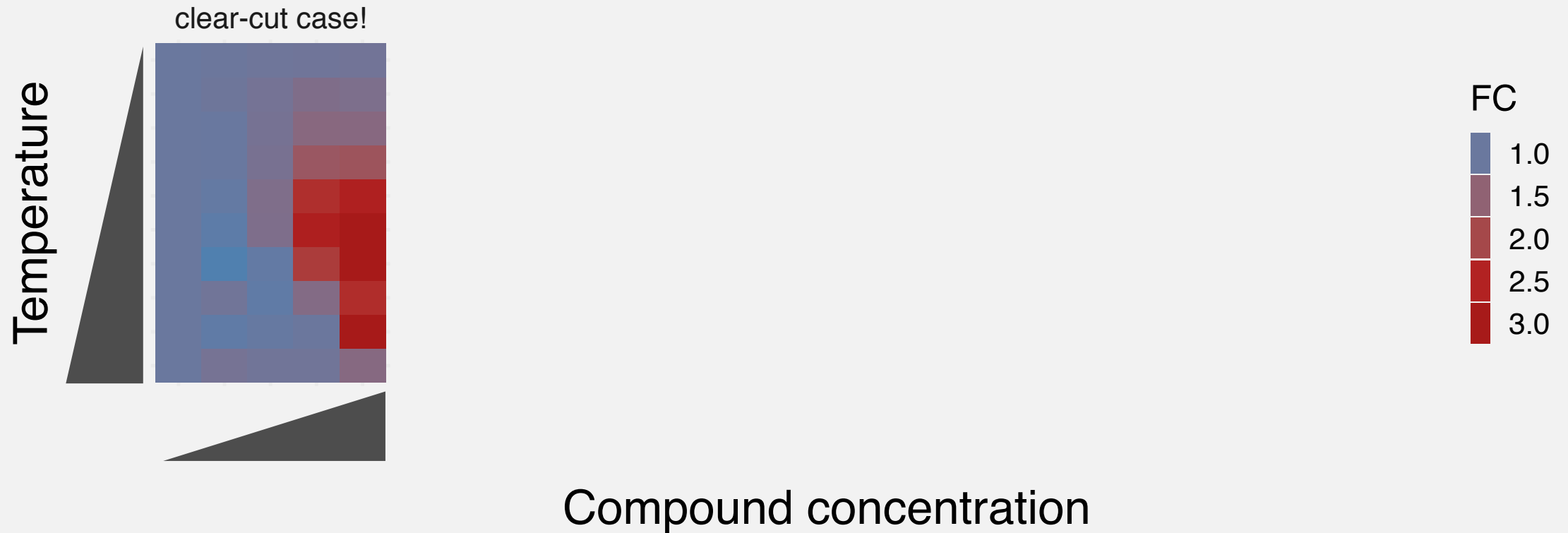


Panobinostat

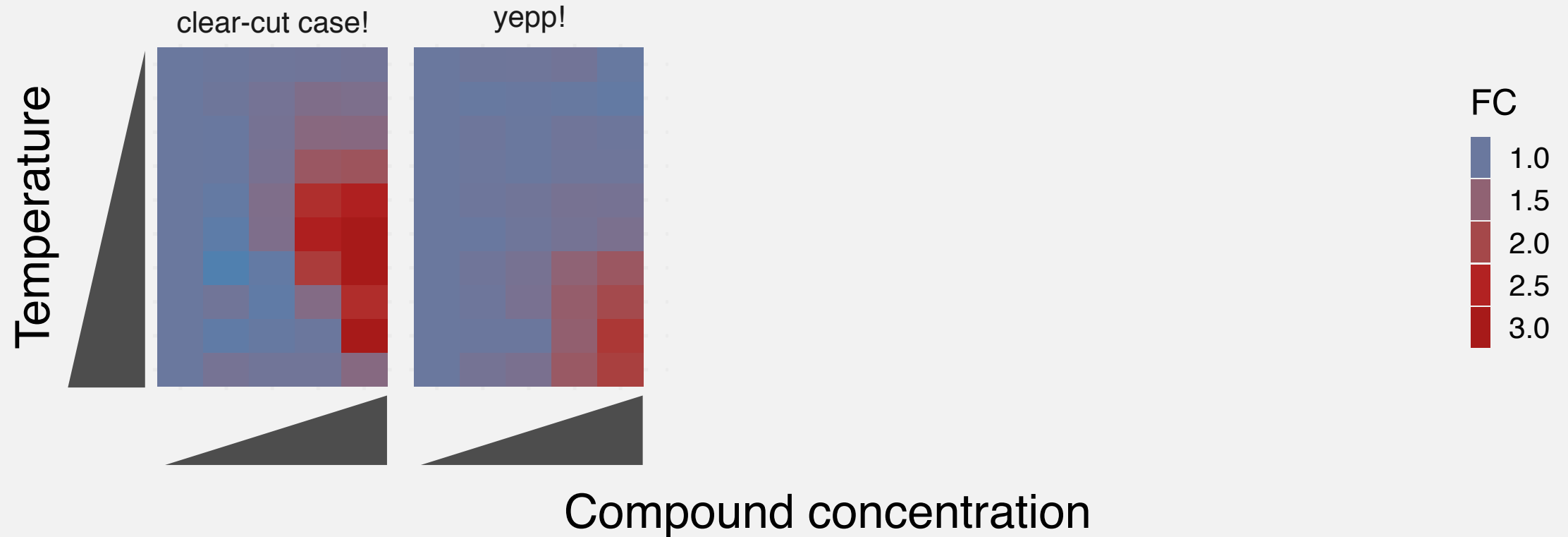




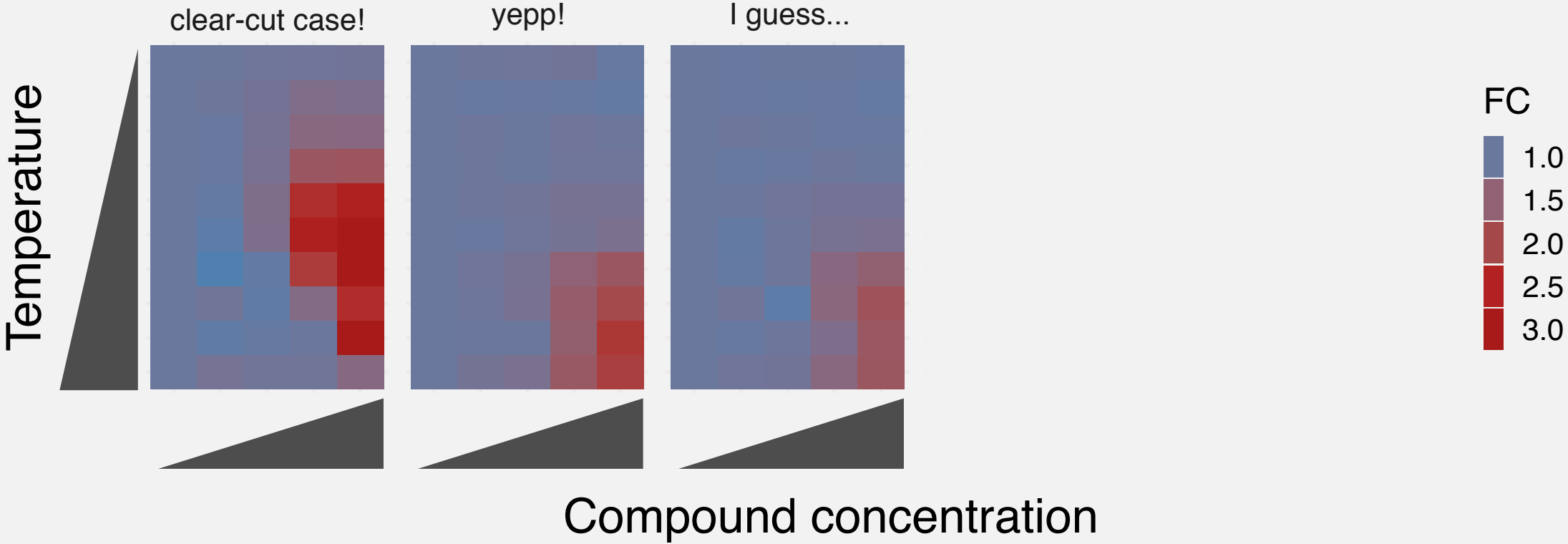
# When is a hit a hit?



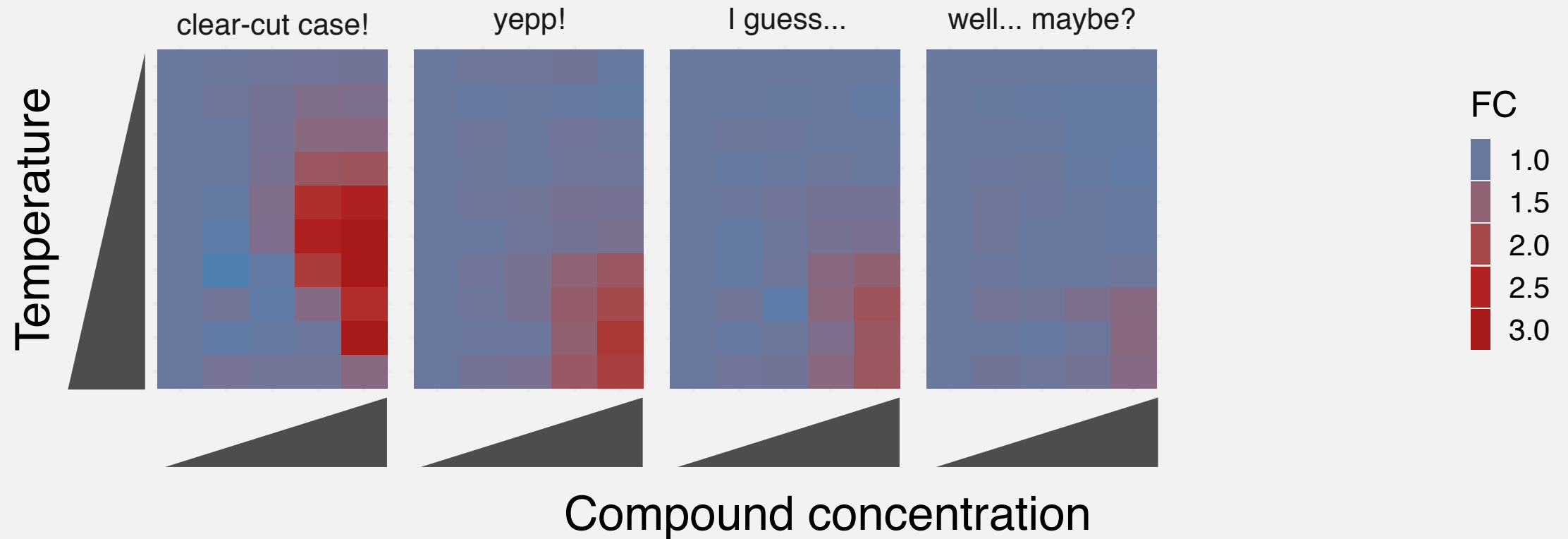
# When is a hit a hit?



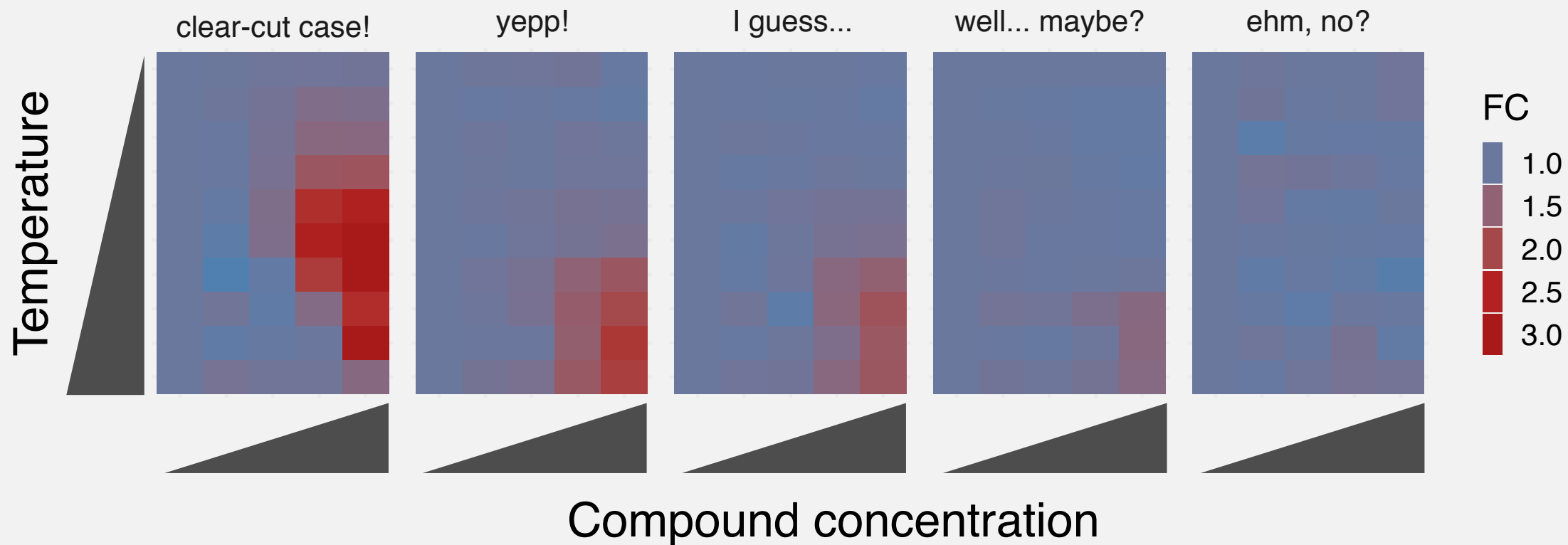
# When is a hit a hit?



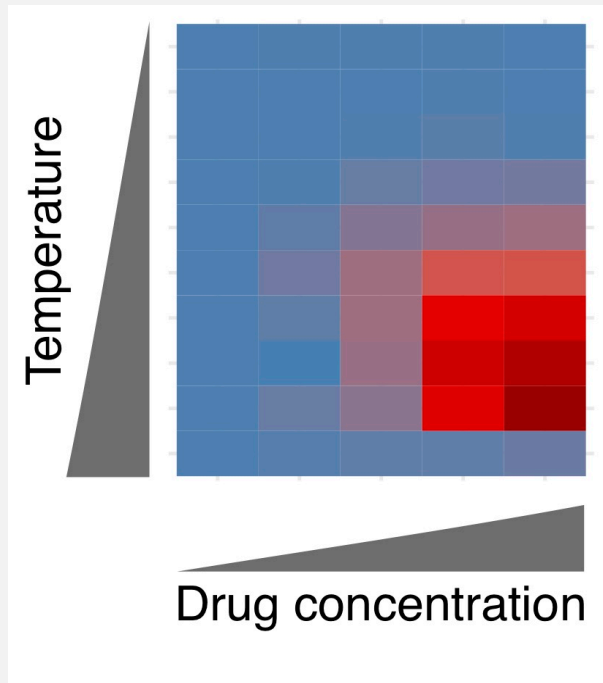
# When is a hit a hit?



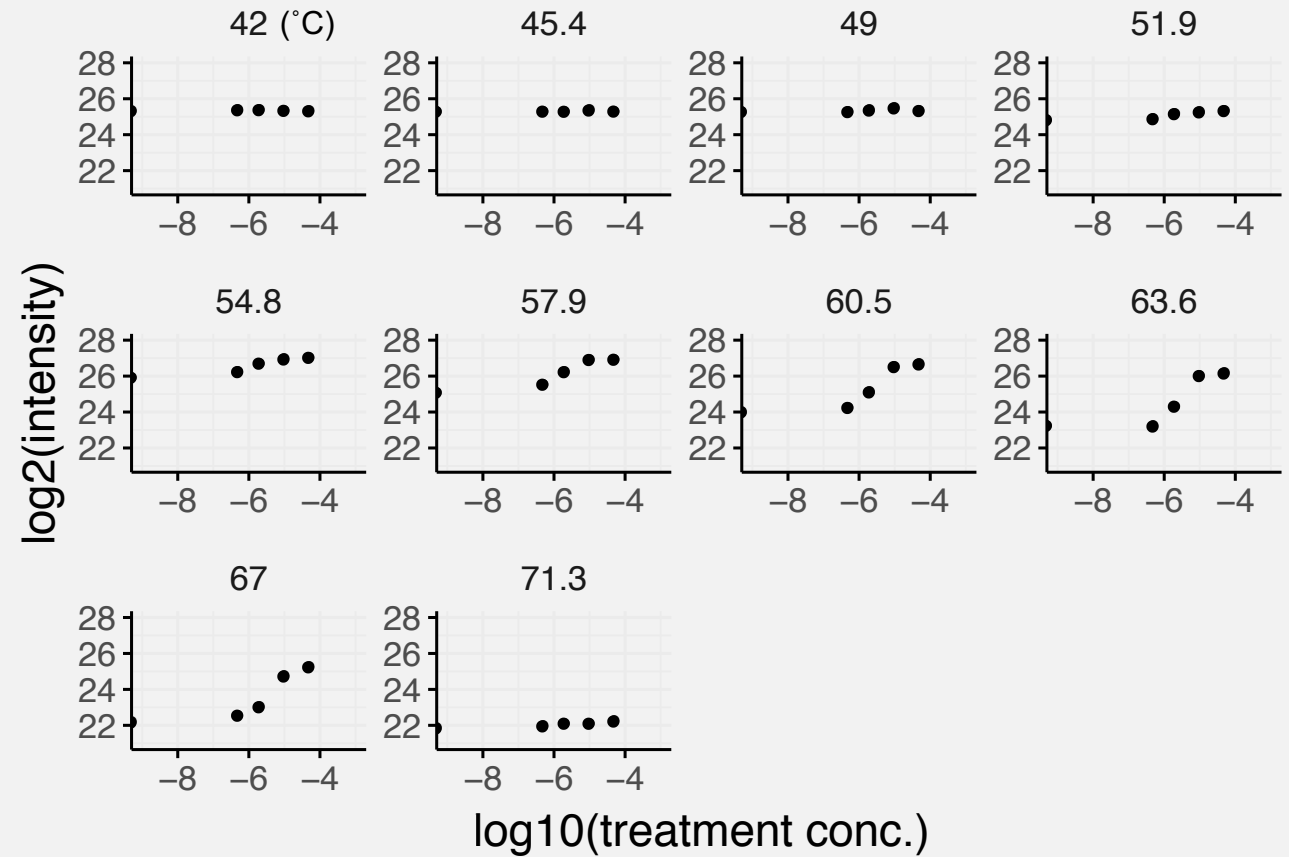
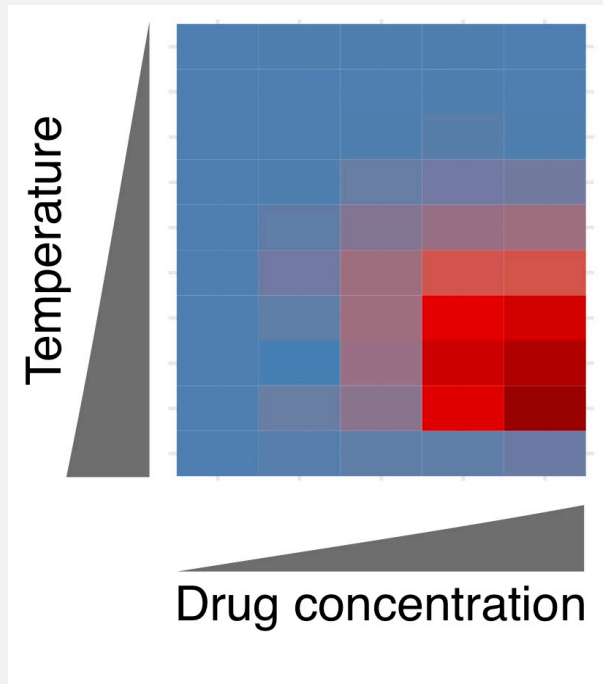
# When is a hit a hit?



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

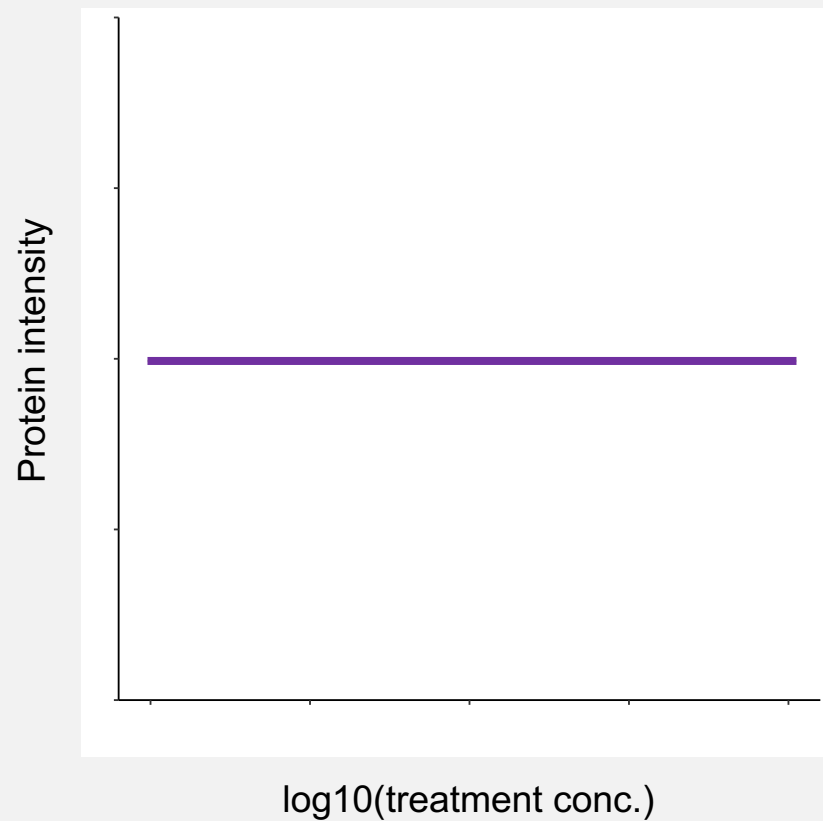


# How to analyze 2D-TPP datasets with false discovery rate control?



# A functional analysis approach for 2D-TPP data

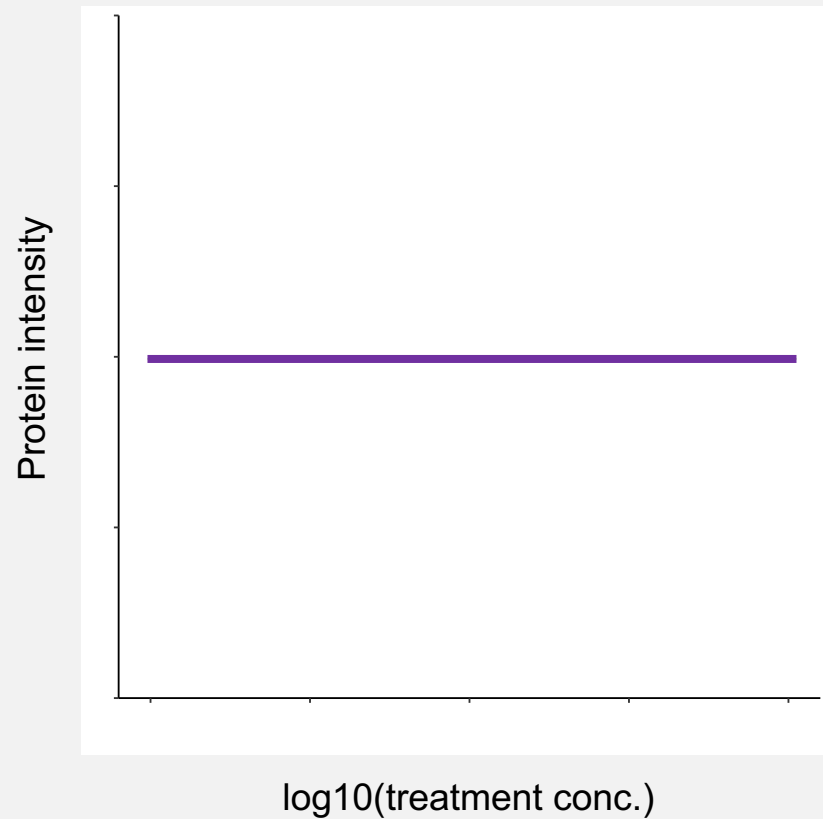
**Null model:** protein remains unaffected by treatment



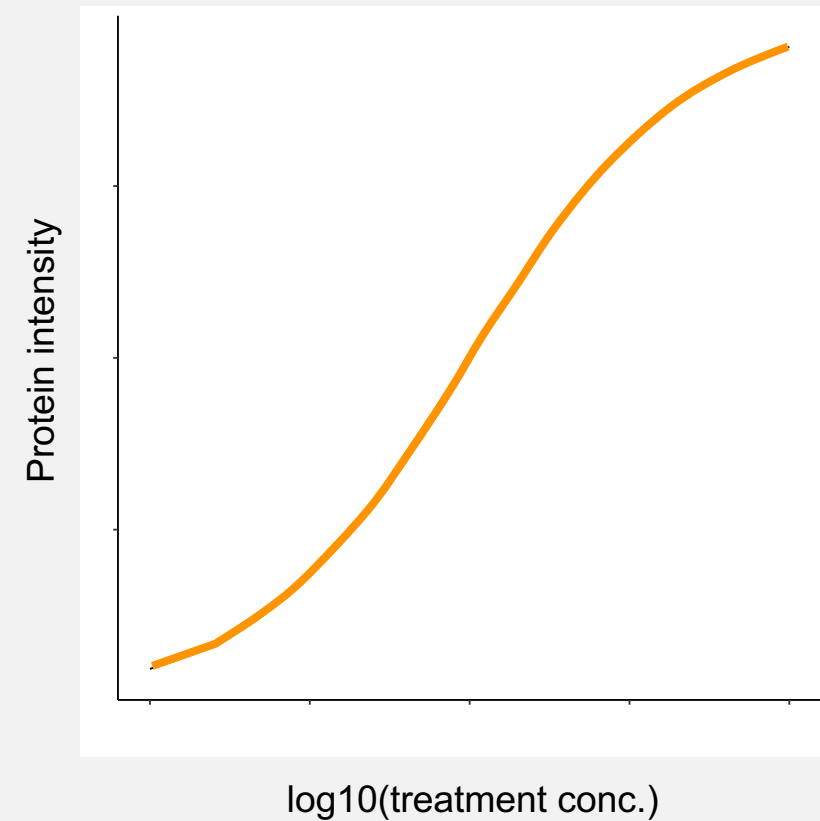


# A functional analysis approach for 2D-TPP data

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



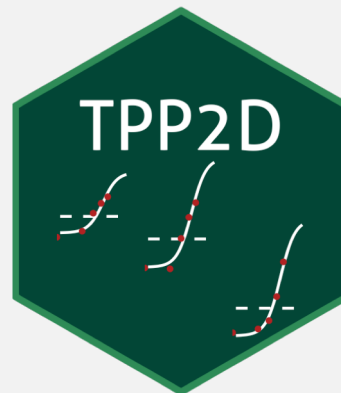
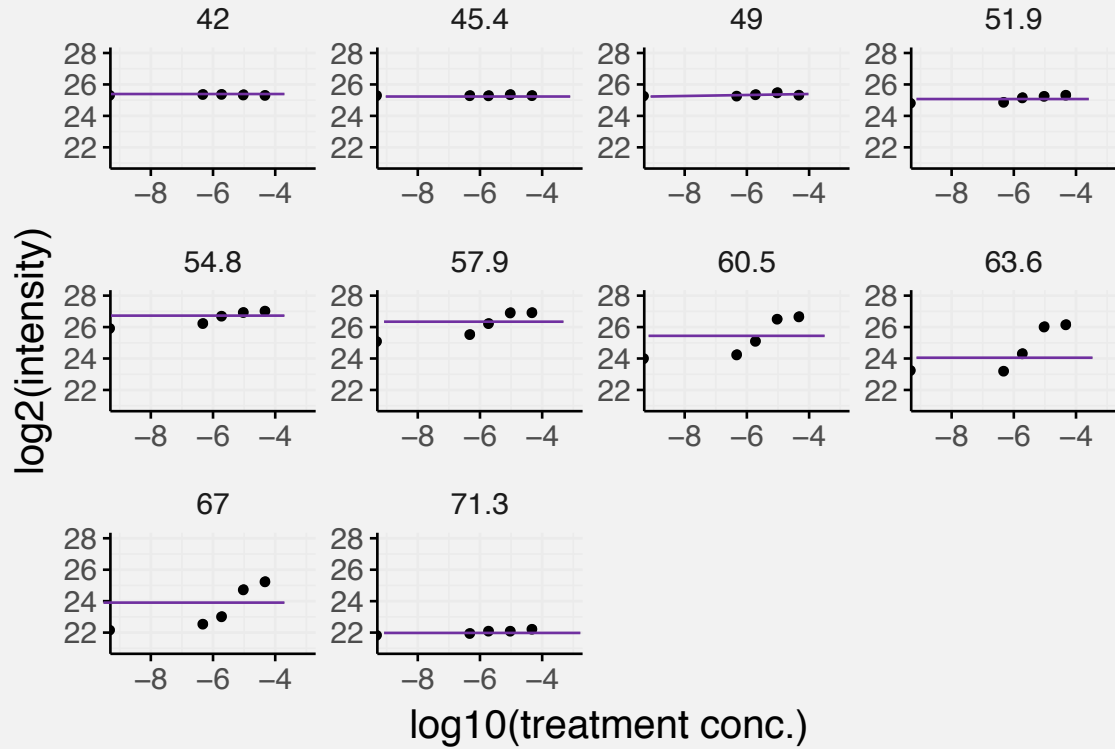
**Alternative model:** protein stability is affected by treatment



# Constructing null and alternative models

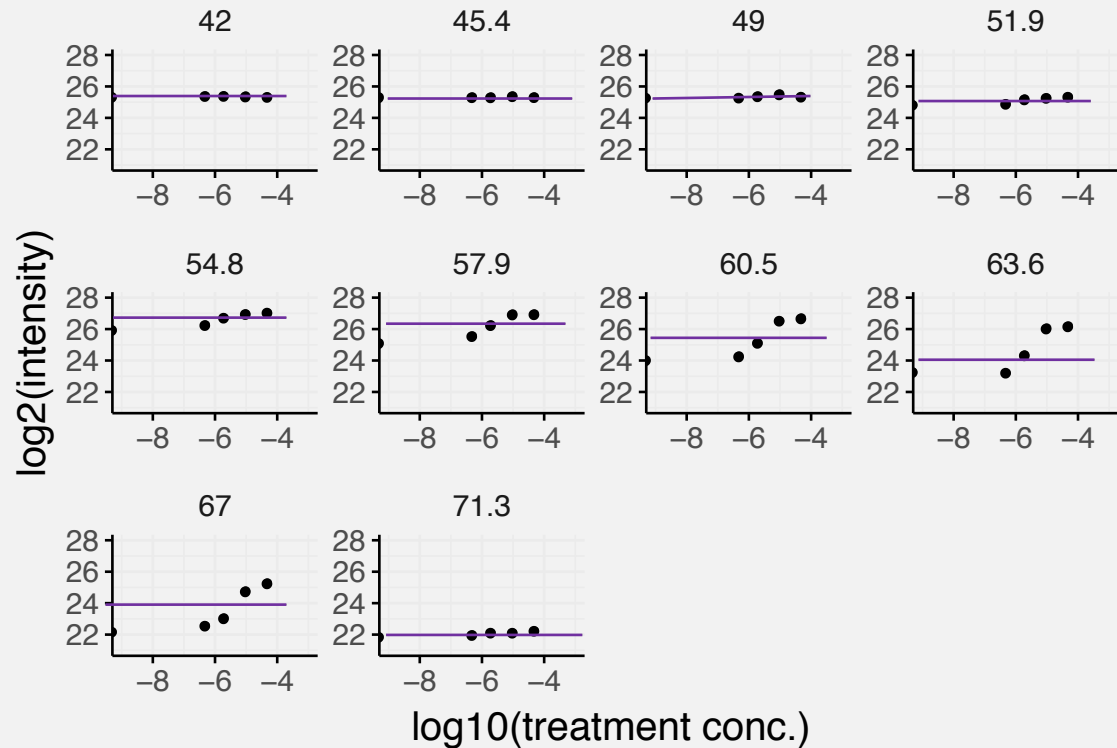
**Null model:** protein remains unaffected by treatment

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

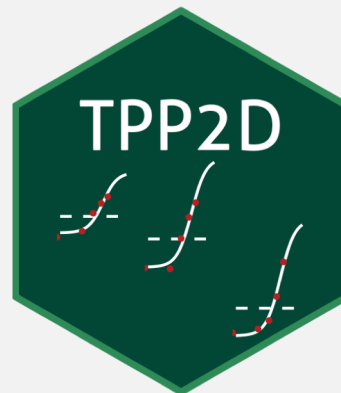
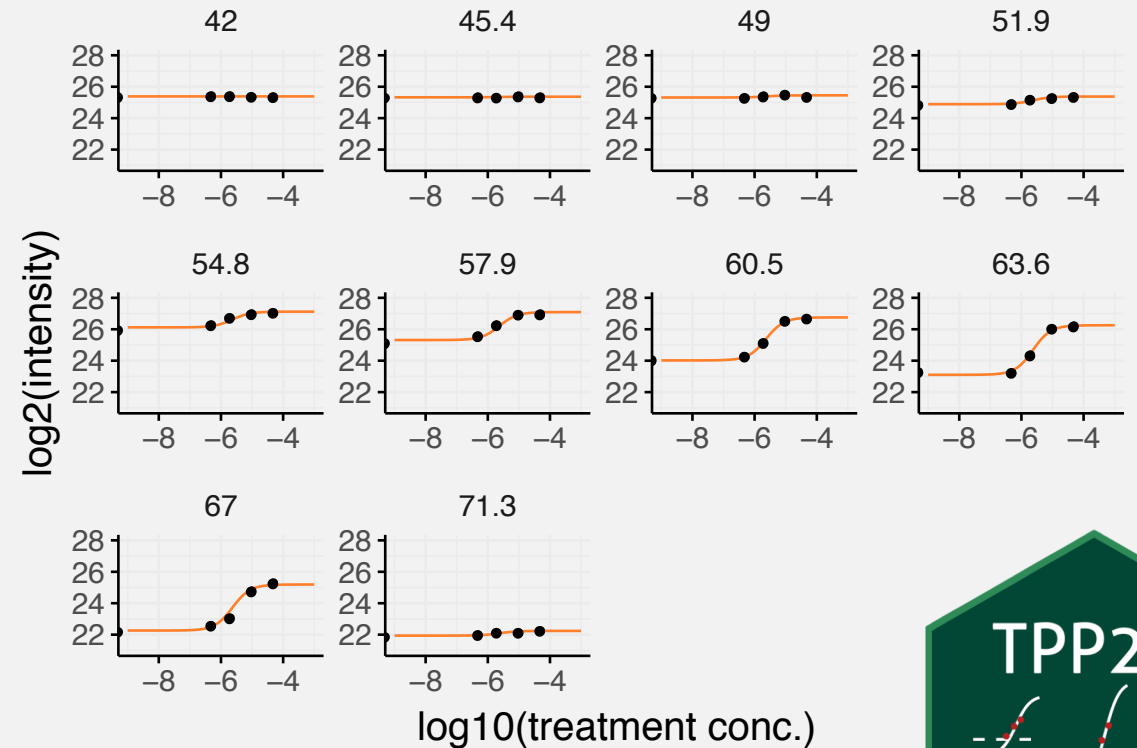


# Constructing null and alternative models

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

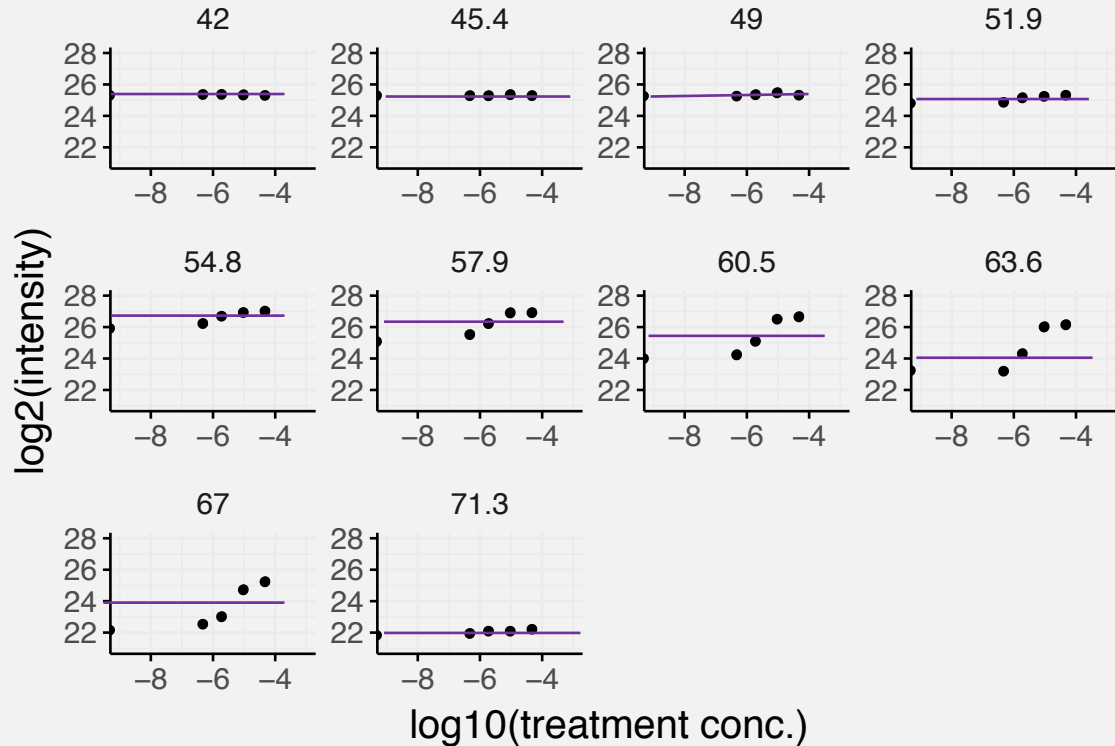


**Alternative model:** protein stability is affected by treatment

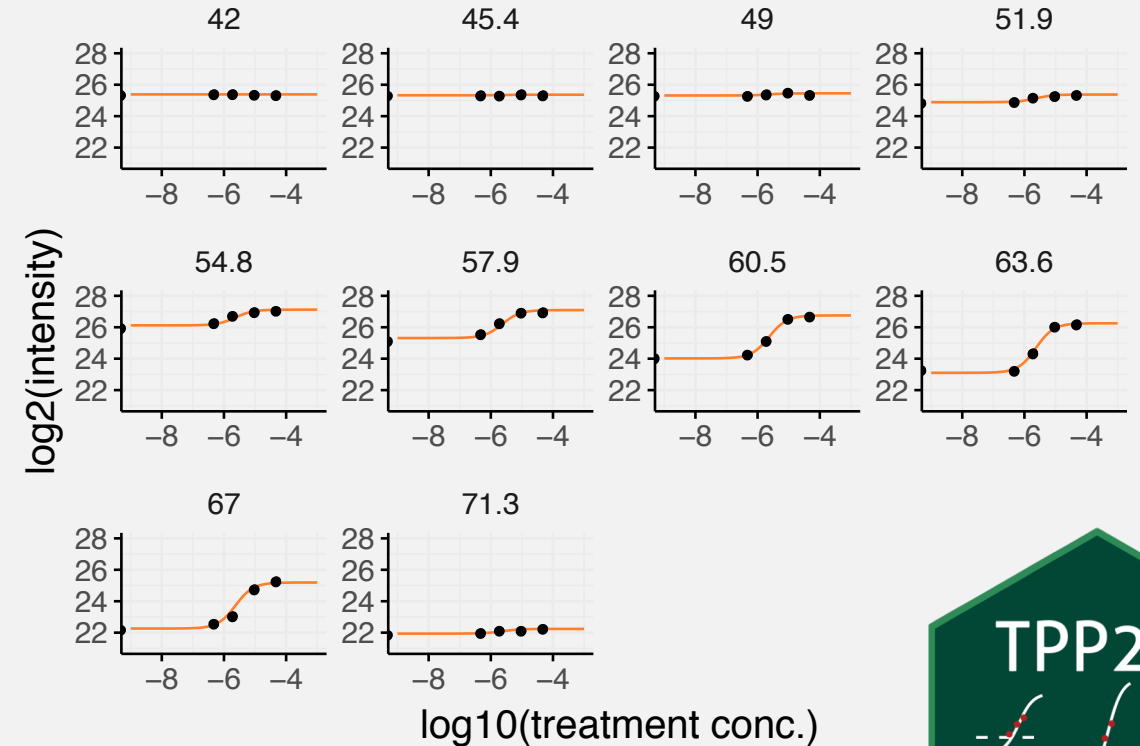


# Constructing null and alternative models

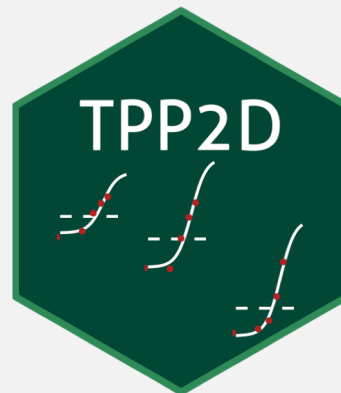
**Null model:** protein remains unaffected by treatment



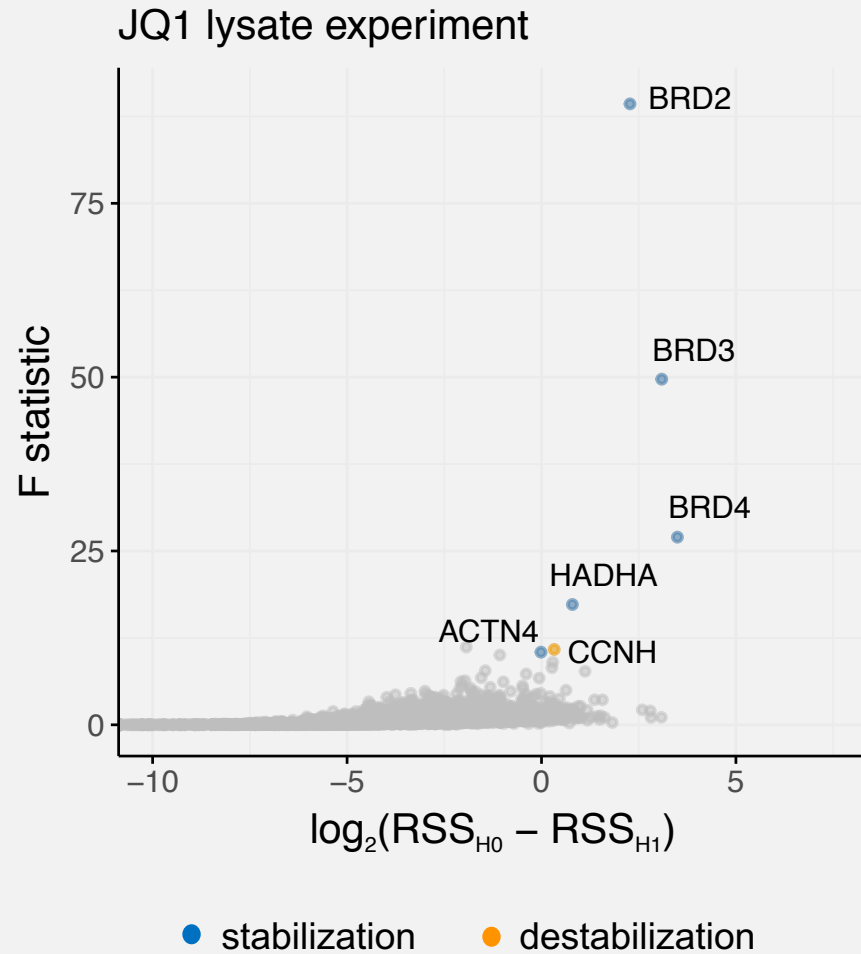
**Alternative model:** protein stability is affected by treatment



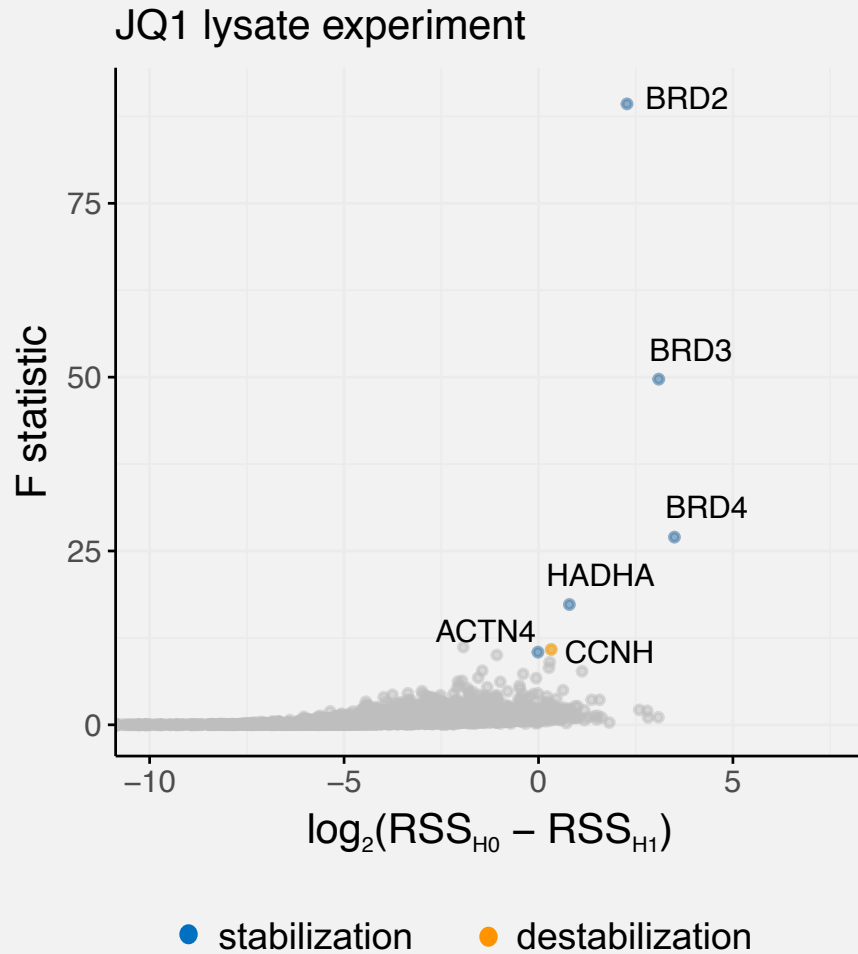
$$F = \frac{RSS^0 - RSS^1}{RSS^1}$$



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

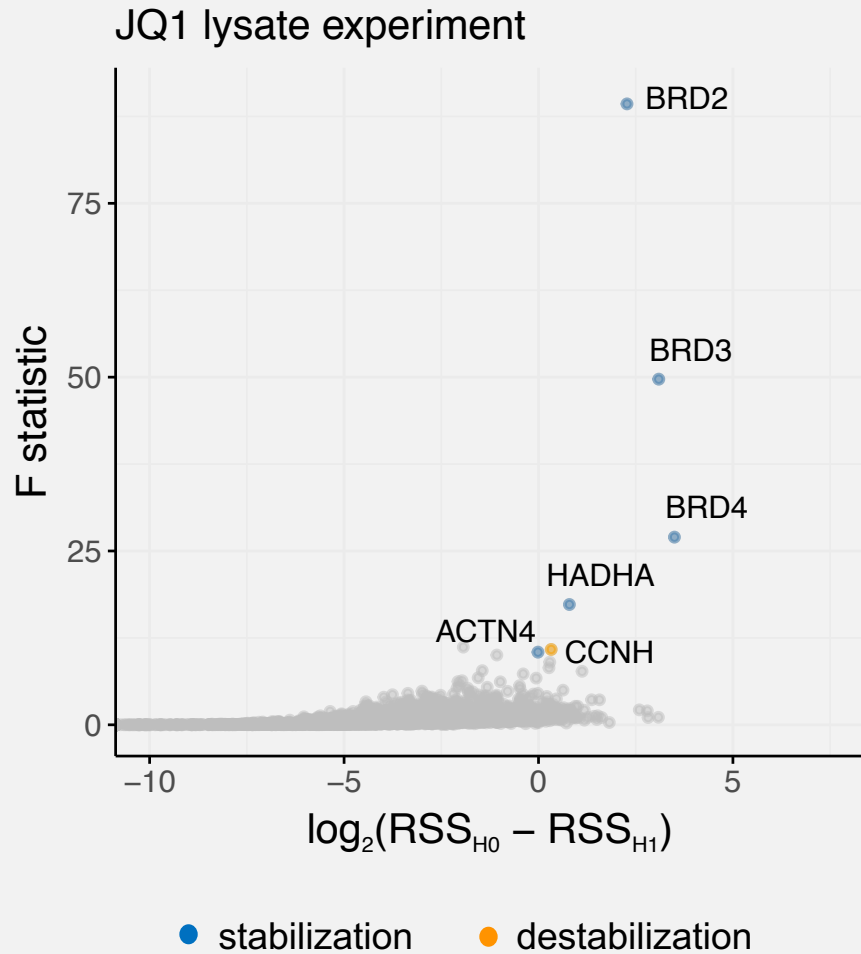


# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1



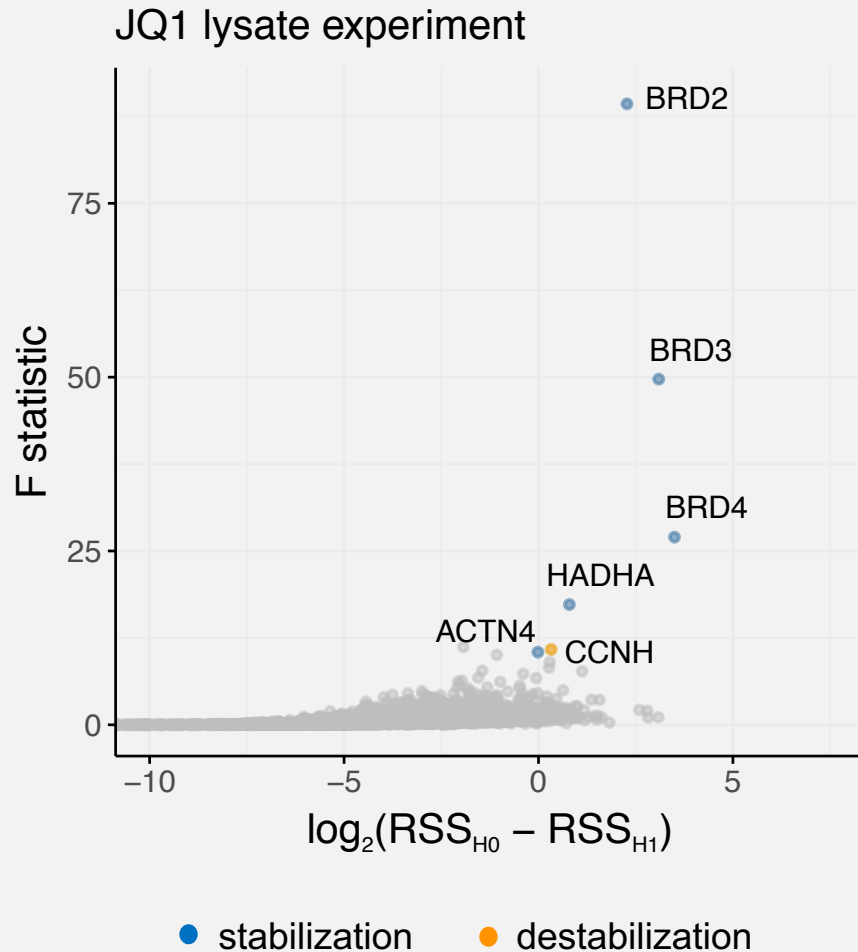
- Previously detected targets are found: BRD2-4 and HADHA

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

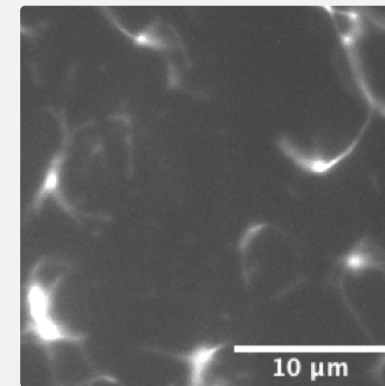


- Previously detected targets are found: BRD2-4 and HADHA
- New off-target found: ACTN4

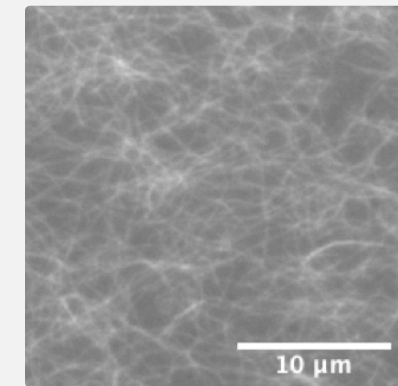
# $\alpha$ -Actinin 4 revealed as off-target of the BET-inhibitor JQ1



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



control



JQ1 treatment



Sristhi Dar



# Acknowledgements

## **EMBL Heidelberg:**

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André Mateus  
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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**



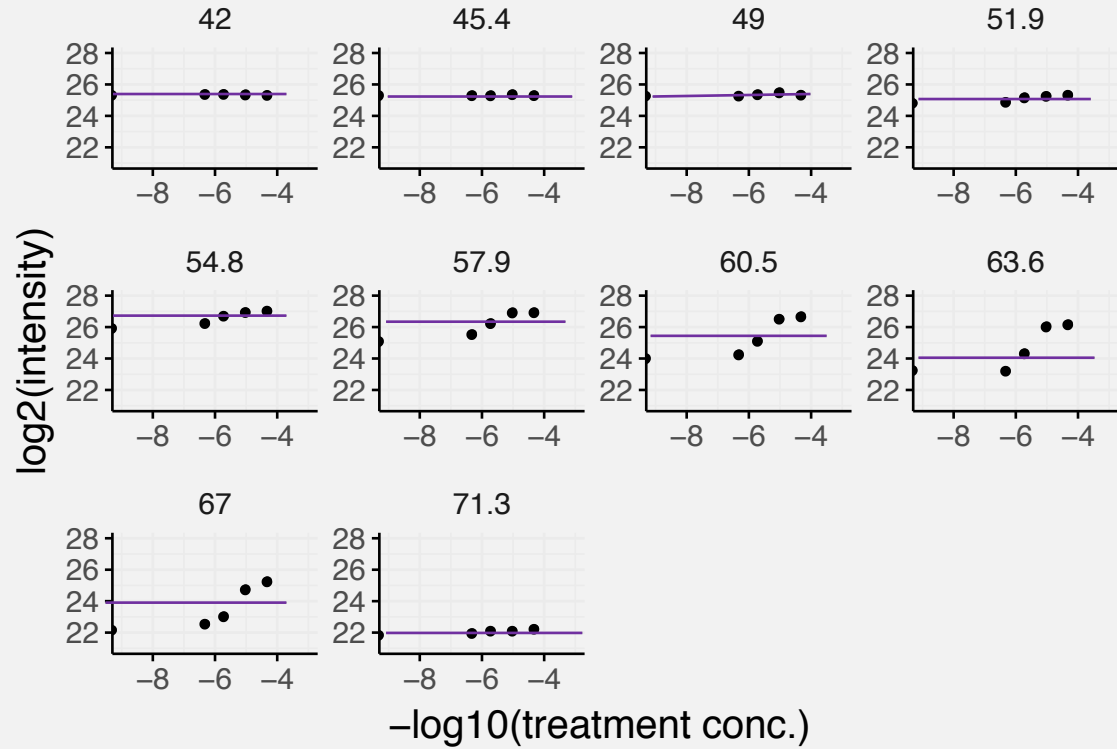


**Thank you!**



09/12/2019

# Constructing an H1 model (treatment effect)

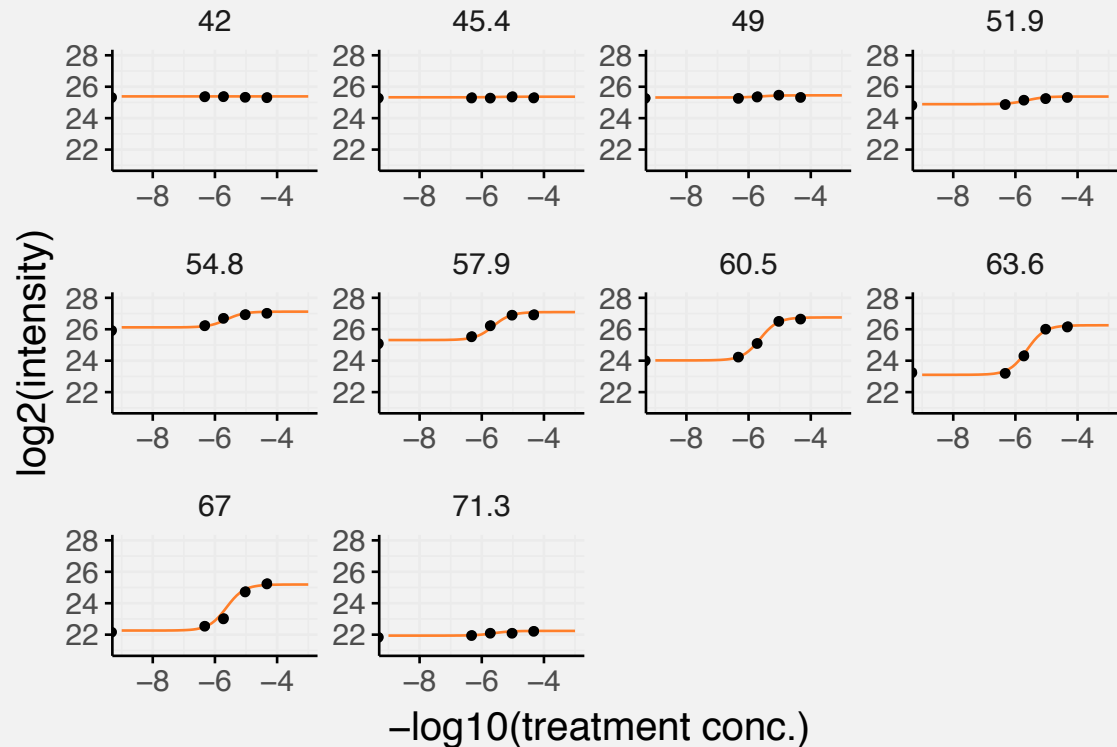


$$y_{i,t} = \beta_{i,t}^0 + \epsilon_{i,t}$$

$y_{i,t}$ : log<sub>2</sub> intensity for protein  $i$  at temperature  $t$

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

# Constructing an H1 model (treatment effect)



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

$y_{i,t}(c)$ : log2 intensity for protein i at temperature t, at concentration c

$\beta_{i,t}^0$ : concentration-independent intercept parameter for protein i at temperature t (value y will take for c = 0)

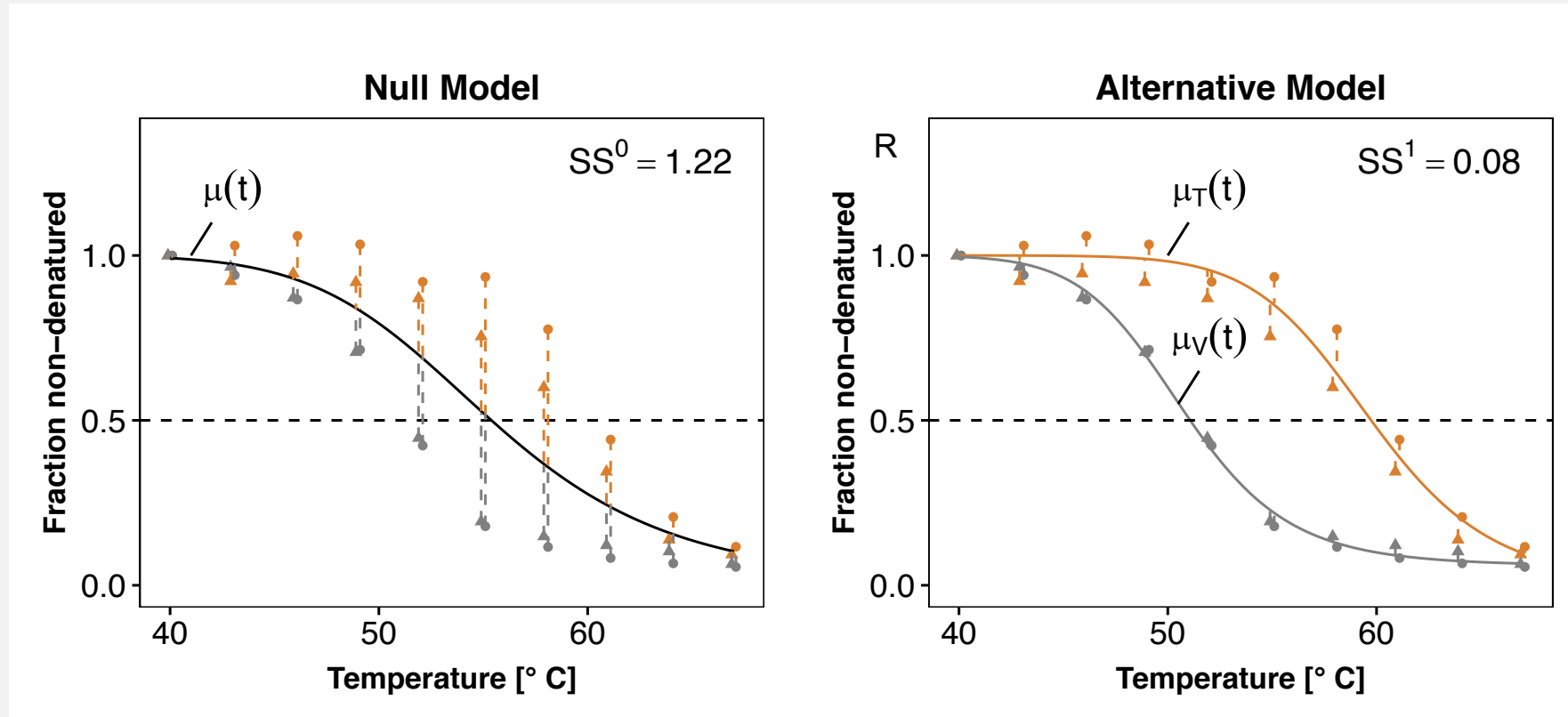
$\delta_i^{\max}$ : maximal stabilization

$\alpha_{i,t}$ : parameter indicating how much relative stabilization happens at temperature t

$\kappa_i$ : slope factor

$\xi(t)_i$ : linear function describing decline of the pEC50 with increasing temperature

# Functional analysis of TPP melting curves: NPARC



SS: sum of squared errors



Karsten Bach

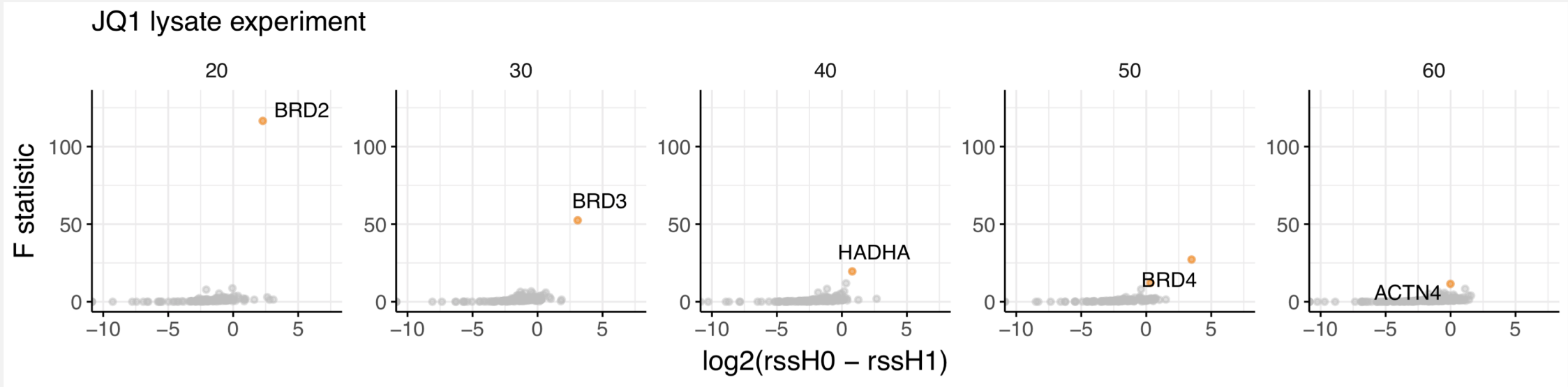


Dorothee Childs

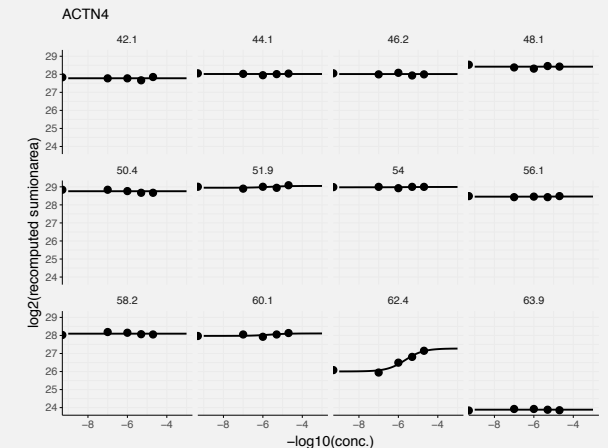


Holger Franken

# Method performance on JQ1 lysate dataset

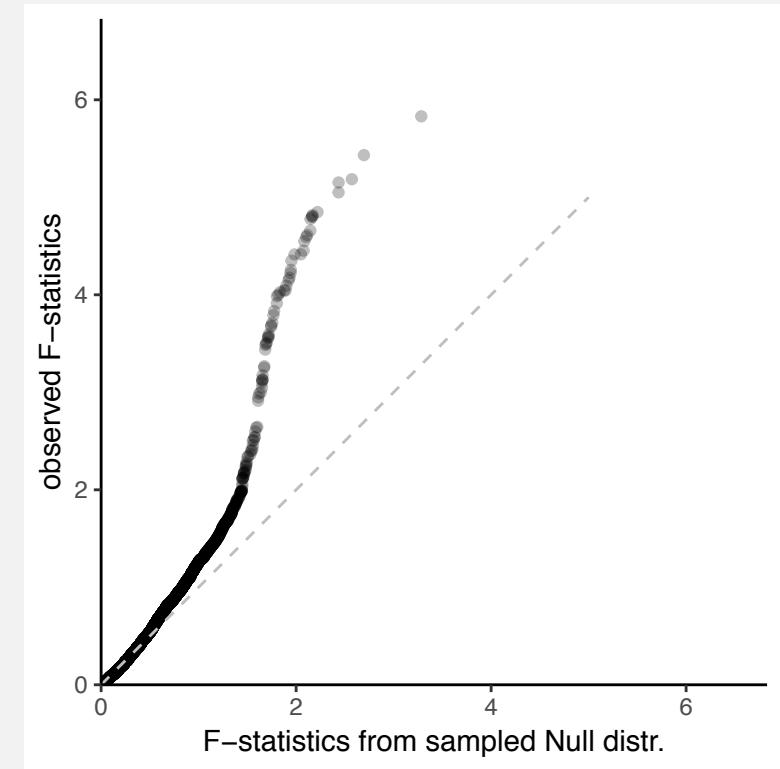


- We currently follow up on ACTN4 as a potential off-target of JQ1
- effects of JQ1 on actin bundle formation have been observed, but were attributed to transcriptional changes via BRDs Qu et al. 2018, Cell Death Discovery



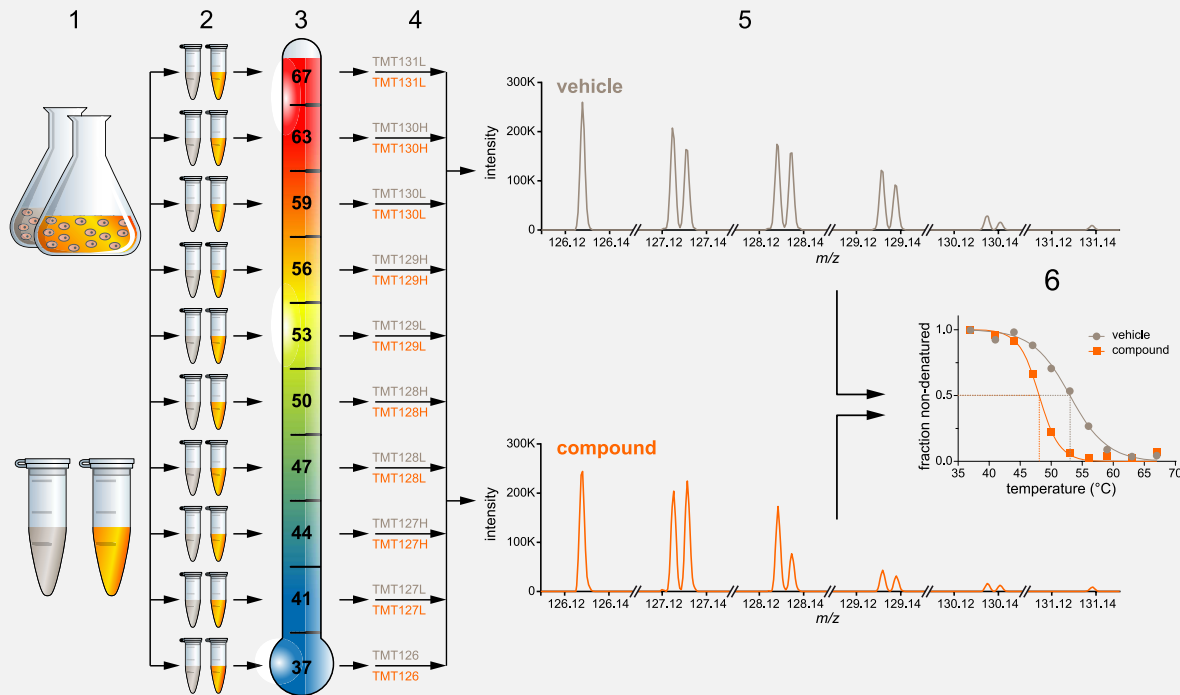
# Controlling FDR

- 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 H0 model for every protein
  - Resample residuals from H0 10 times per protein, fit H1 and compute F statistics
  - Repeatedly (**B** times) do this and jointly rank results with those from true dataset
  - Compute FDR: 
$$\text{FDR}_\theta = \frac{\pi_\theta \sum_b^B \#\{F_i^{0,b} \geq \theta\}}{\#\{F_i \geq \theta\}}$$

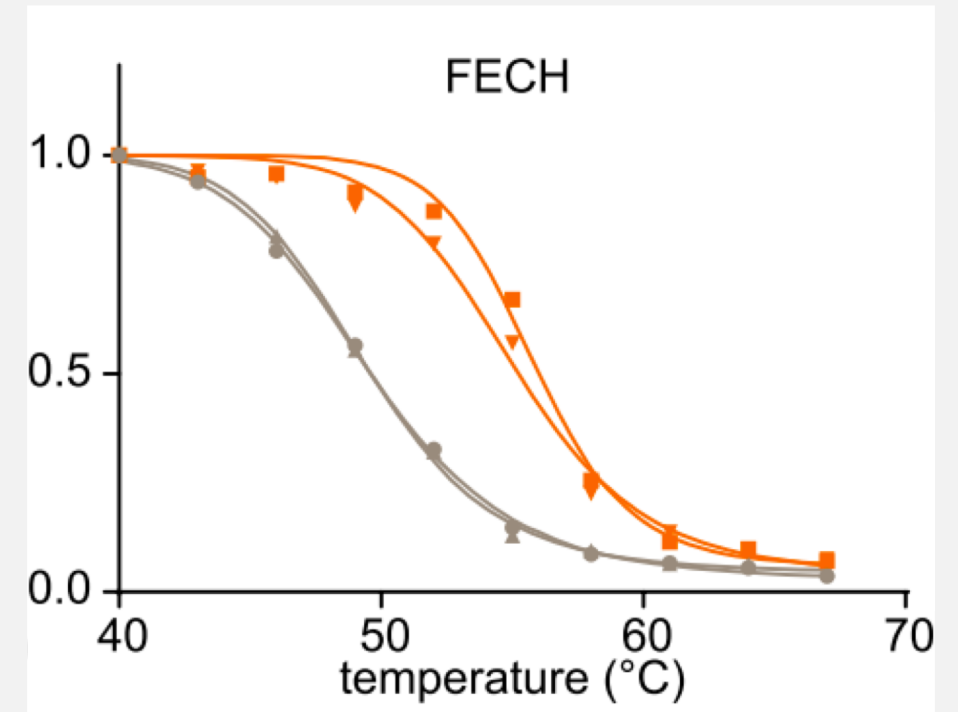




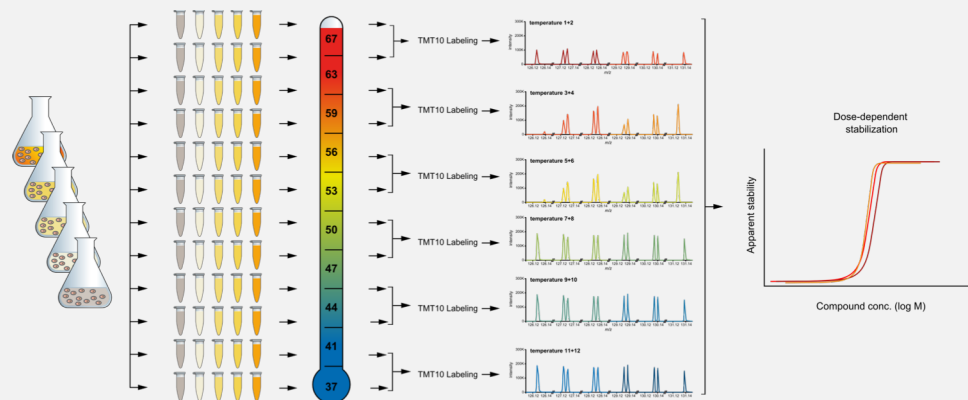
# Thermal proteome profiling (TPP)



Vemurafenib

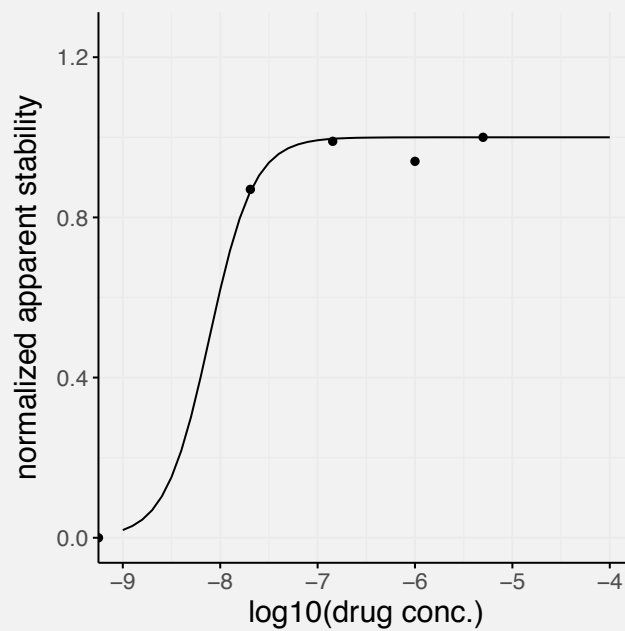


# 2D-TPP data analysis

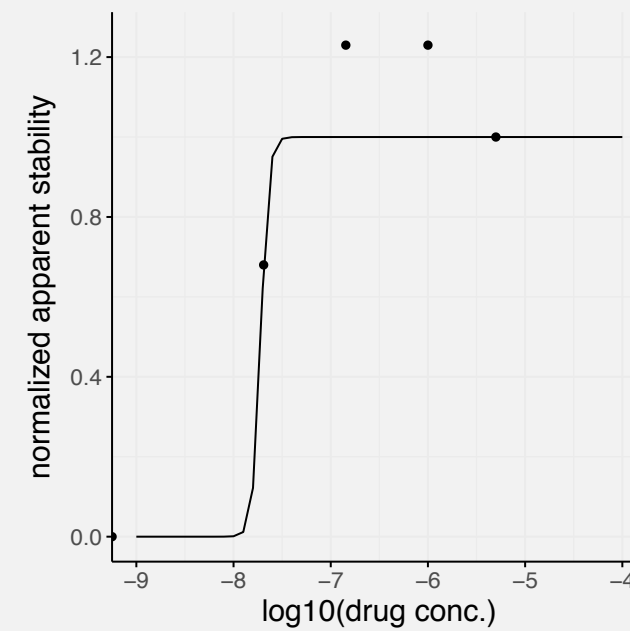


Cells treated with Panobinostat

HDAC2, 51.9 °C

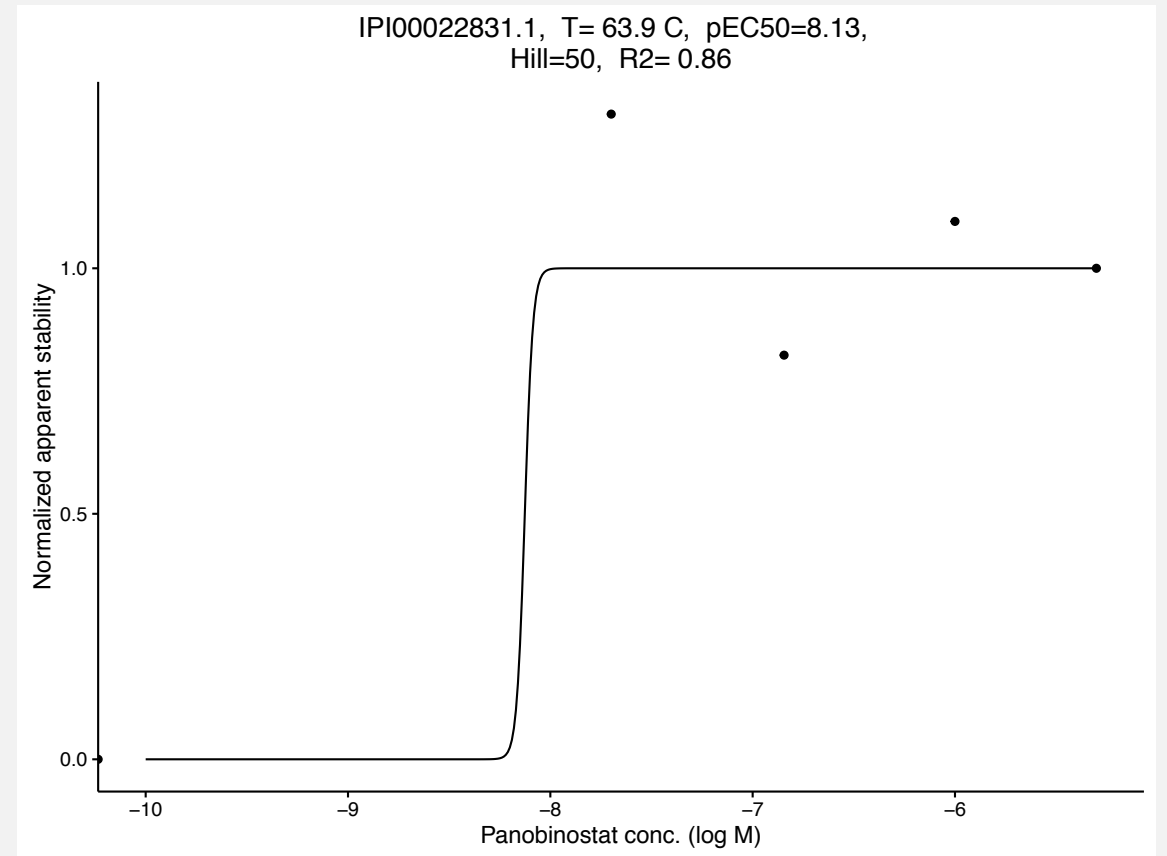


HDAC2, 54.0 °C



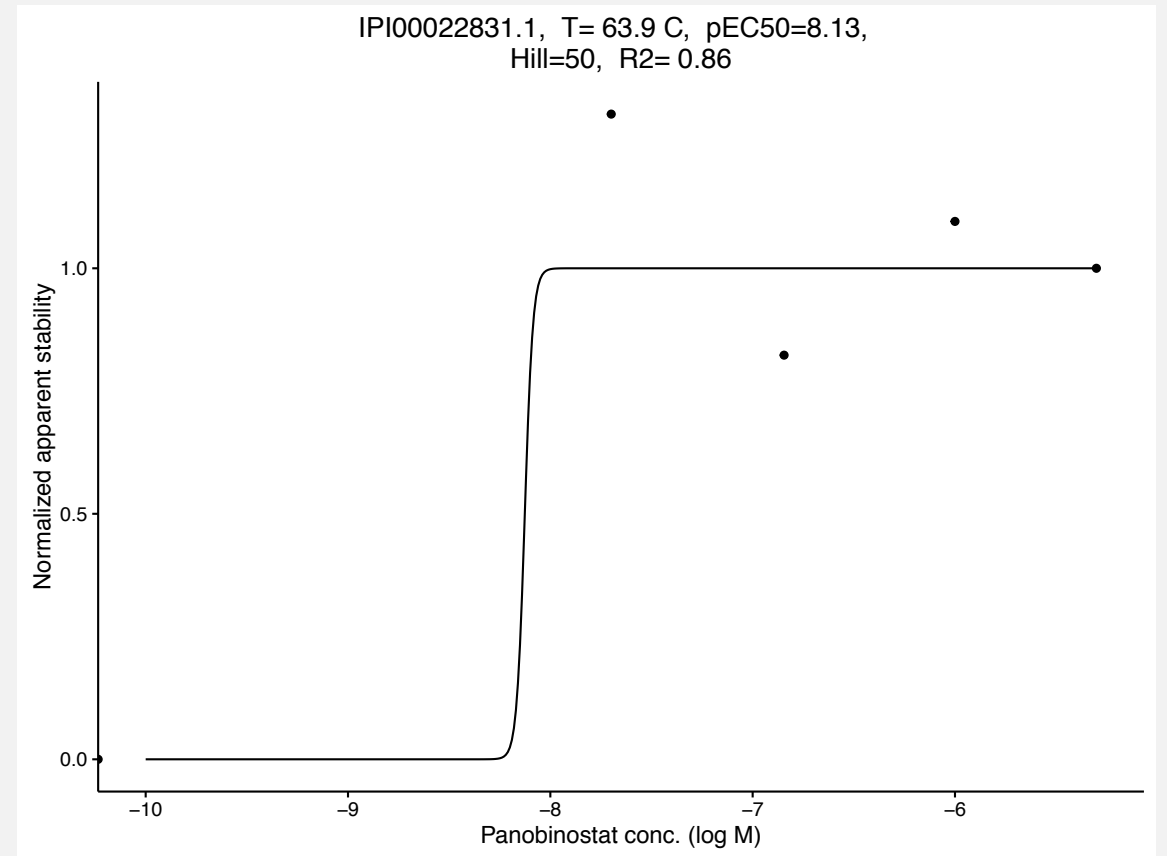
# 2D-TPP data analysis: what's the matter?

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

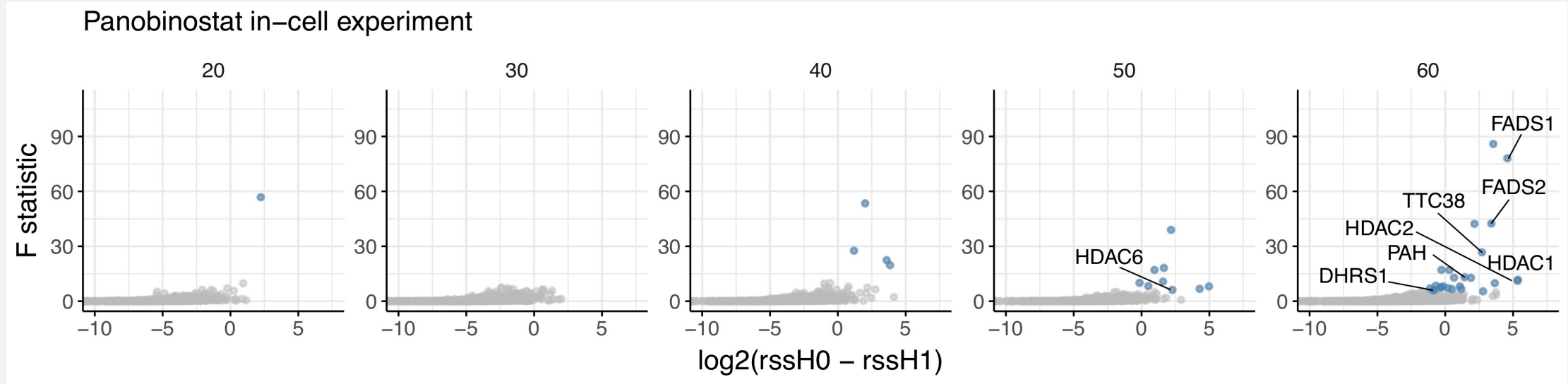


# 2D-TPP data analysis: what's the matter?

- 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