Functional interrogation of Toxcast database for DILI-associated differences in Troglitazone vs Rosiglitazone Maleate

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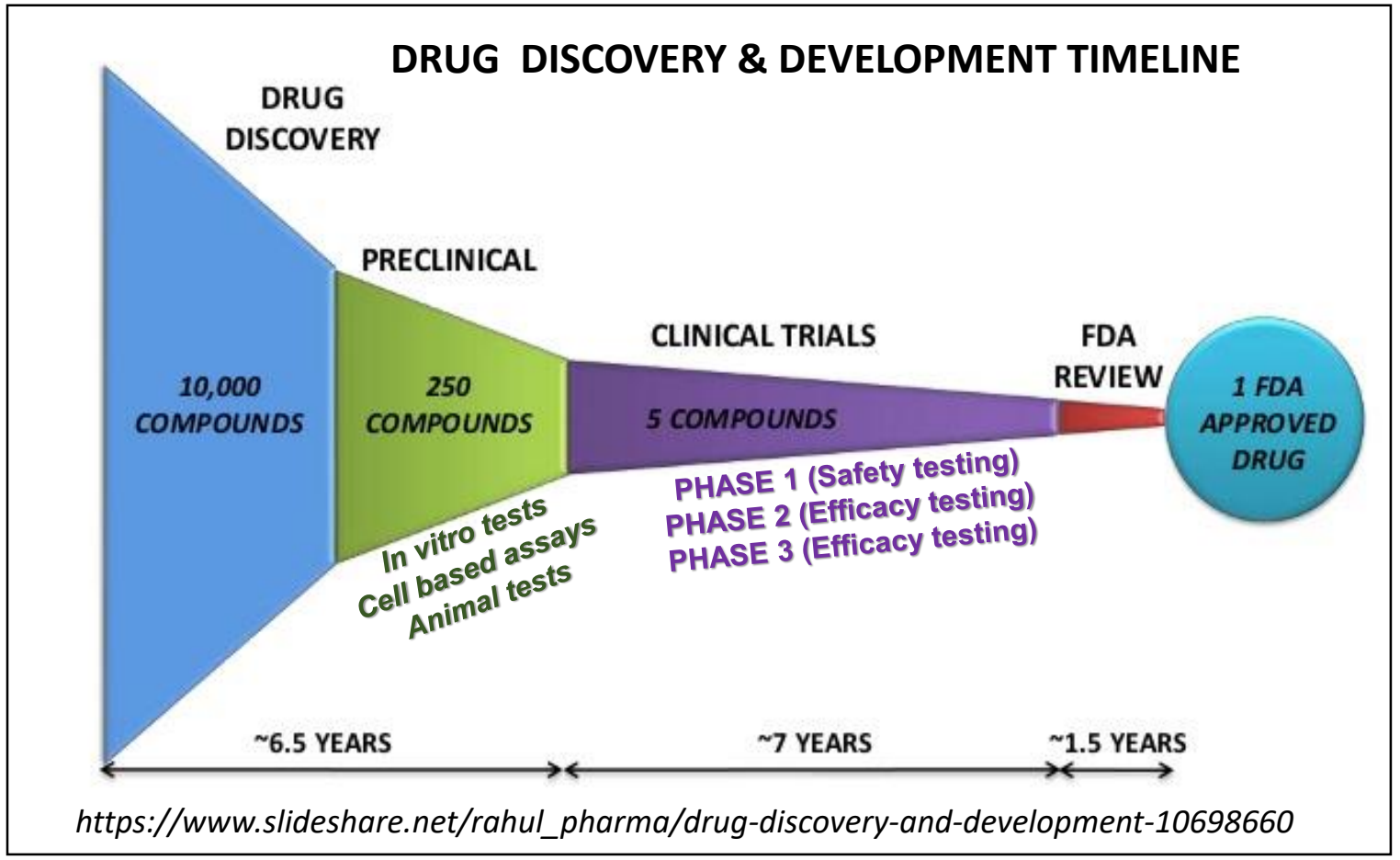
How to better leverage preclinical data for eliminating bad drugs from going into clinical trials/market ?

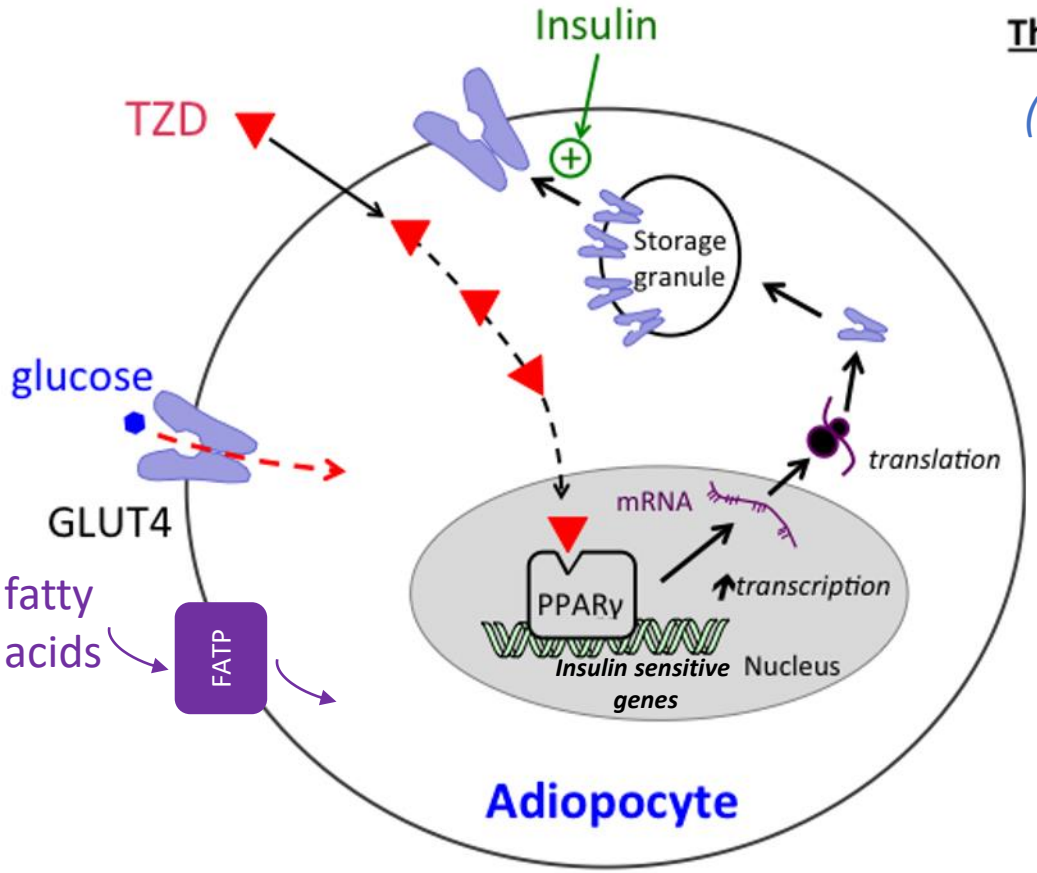


https://www.slideshare.net/rahul_pharma/drug-discovery-and-development-10698660

EBTC GOAL

Leverage "preclinical data" for identifying drugs with potential toxicities to liver

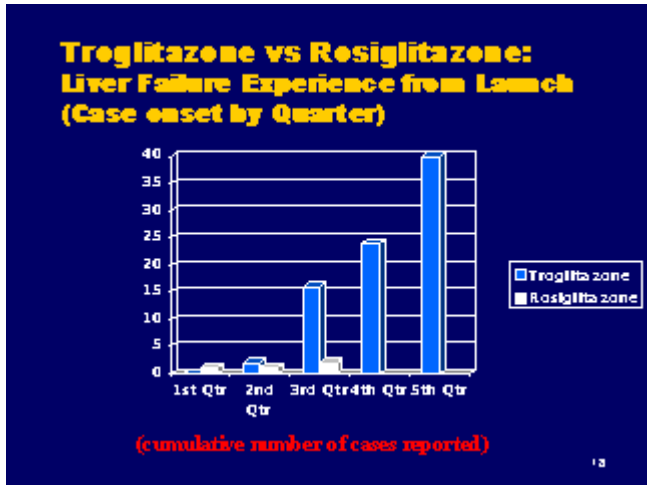
Anti-diabetic TZD drugs, Troglitazone and Rosiglitazone, stimulate insulin function by targeting PPARγ



Thiazolidinediones (TZDs):

(In market since 1999) **Rosiglitazone (maleate)*** – Target: PPARγ

(withdrawn in year 2000) **Troglitazone** **– Target: PPARγ > PPARα



** "lessDILI" drug (LTKB)*

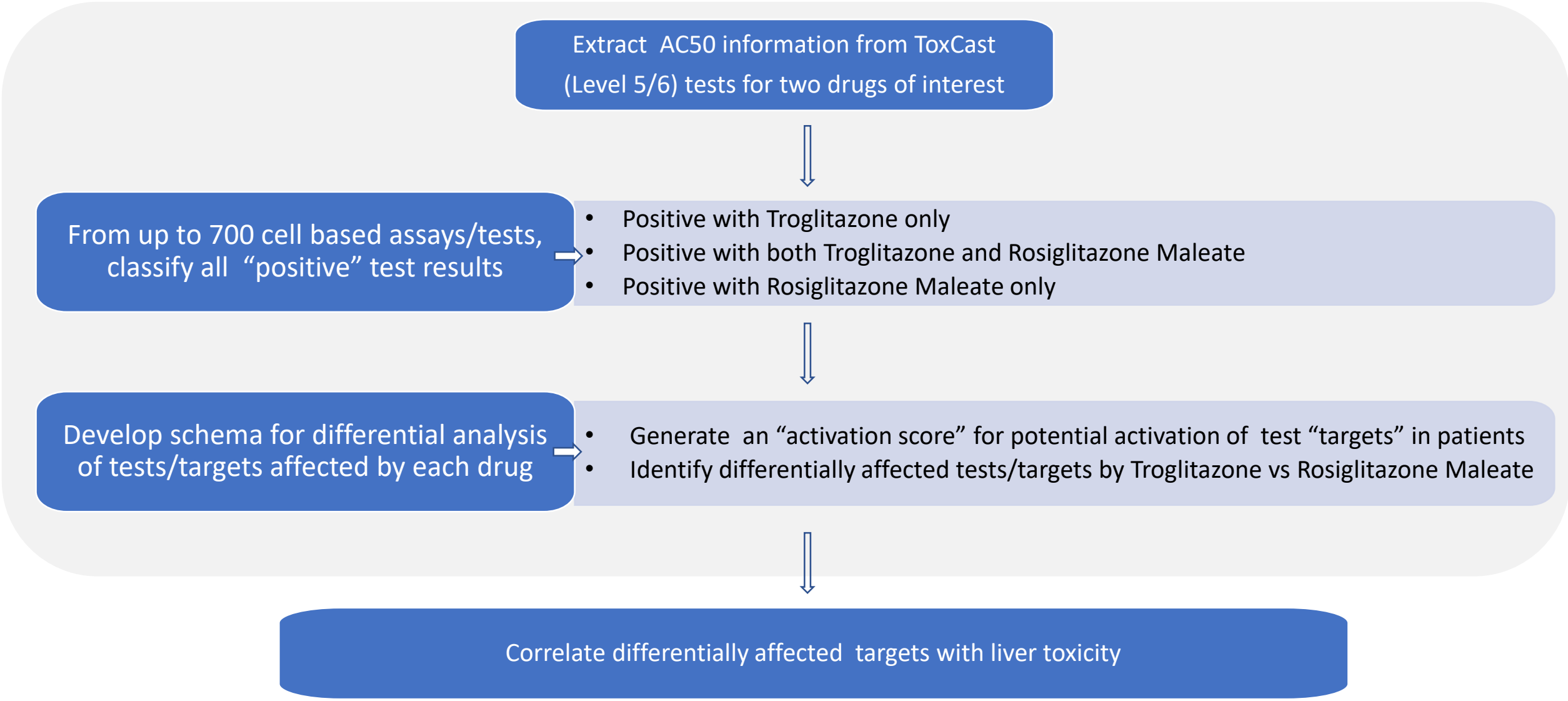
***"mostDILI" drug (LTKB)*

https://www.fda.gov/ohrms/dockets/ac/00/slides/3615s1a/sld018.htm

Can we leverage preclinical data (cell-based assays/test results) for Troglitazone to understand potential basis for liver toxicity?

http://tmedweb.tulane.edu/pharmwiki/doku.php/thiazolidinediones & www.diabetesincontrol.com (Handbook of Diabetes, 4th edition excerpt)

High level project workflow

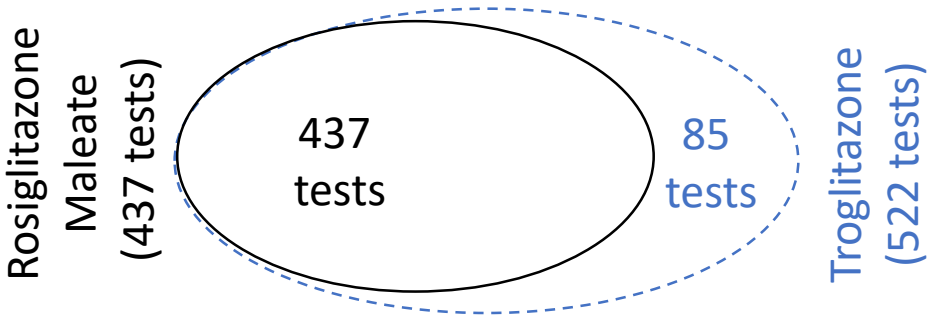


Higher # of “positive” tests for Troglitazone relative to Rosiglitazone Maleate

Workflow

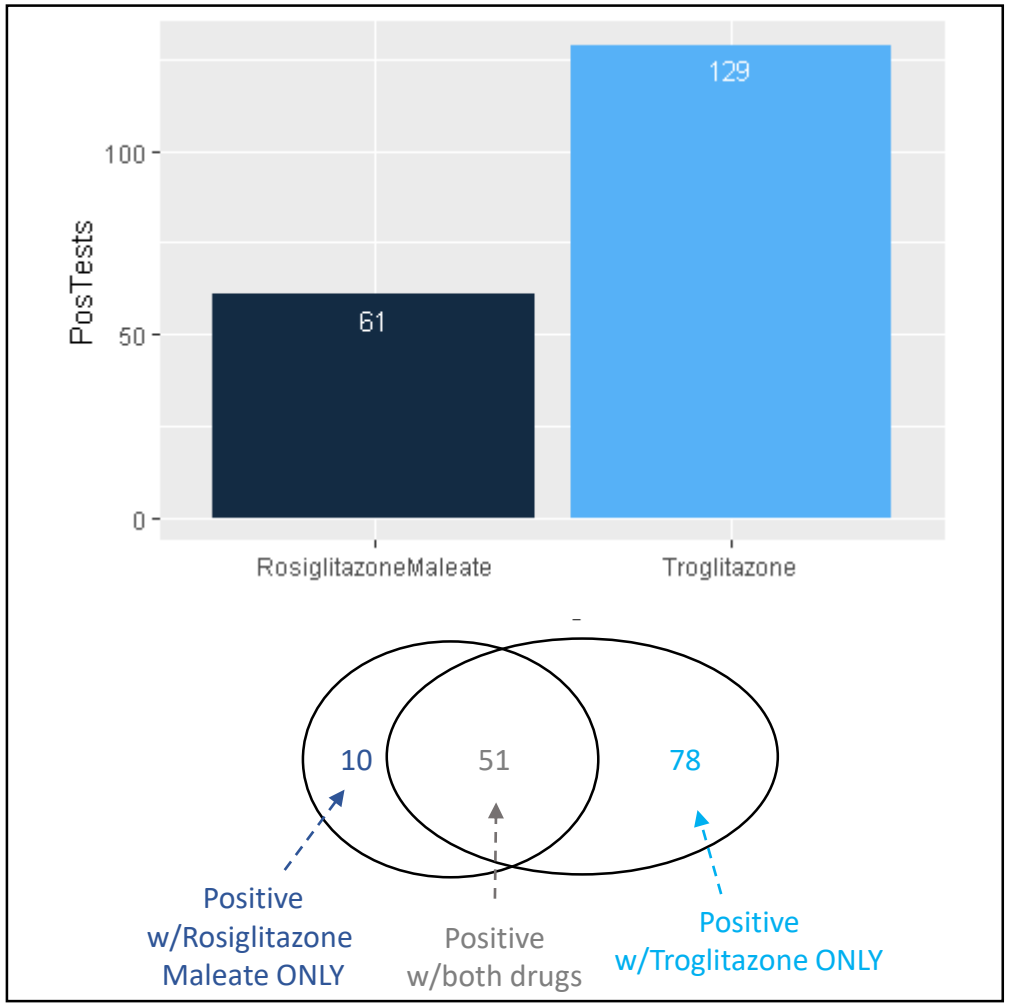
Tox21 dataset

For chosen drugs, identify *all tests with results*
(i.e., “**AC50”** ≠ NA & “**AC50”** <=10^6)



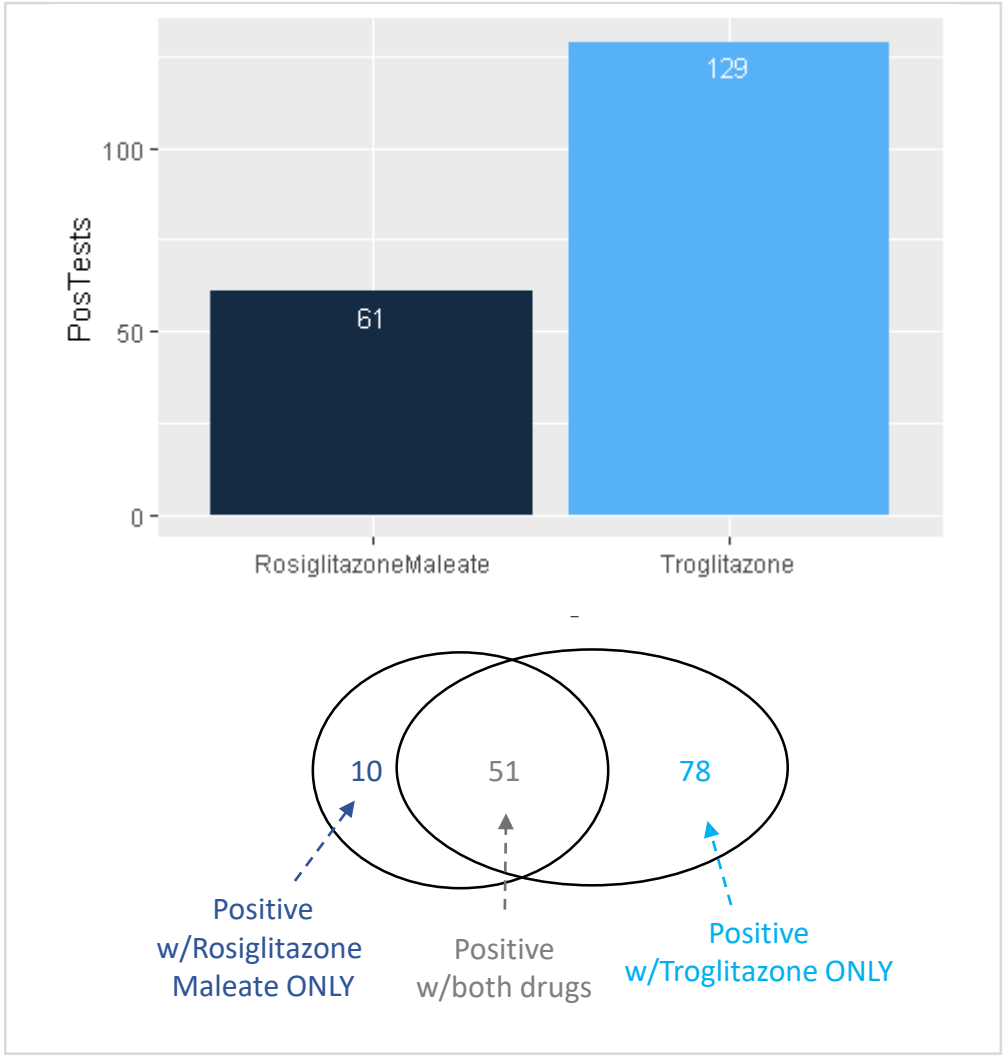
From common (437) tests, identify “positives”
(i.e., those with **“AC50”** < 10^6)

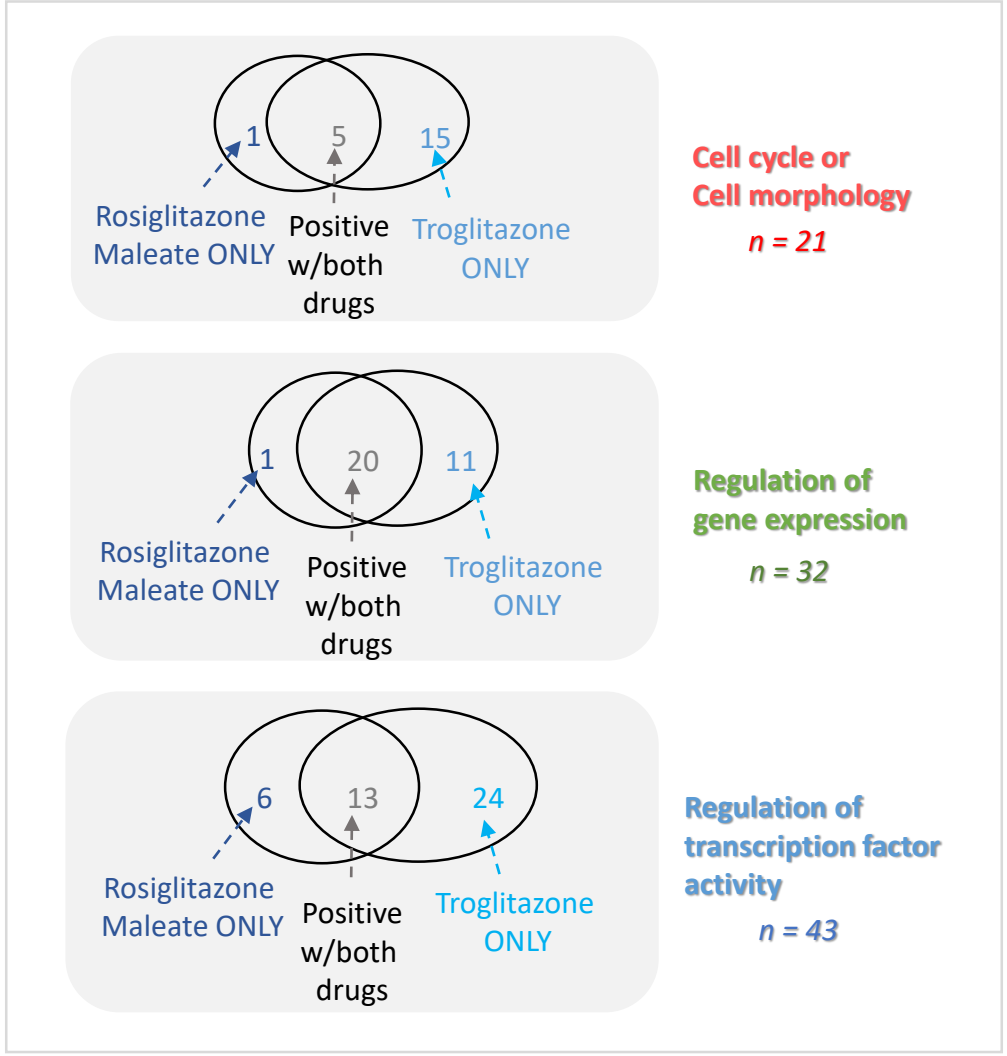
Results



Venn diagrams not drawn to scale

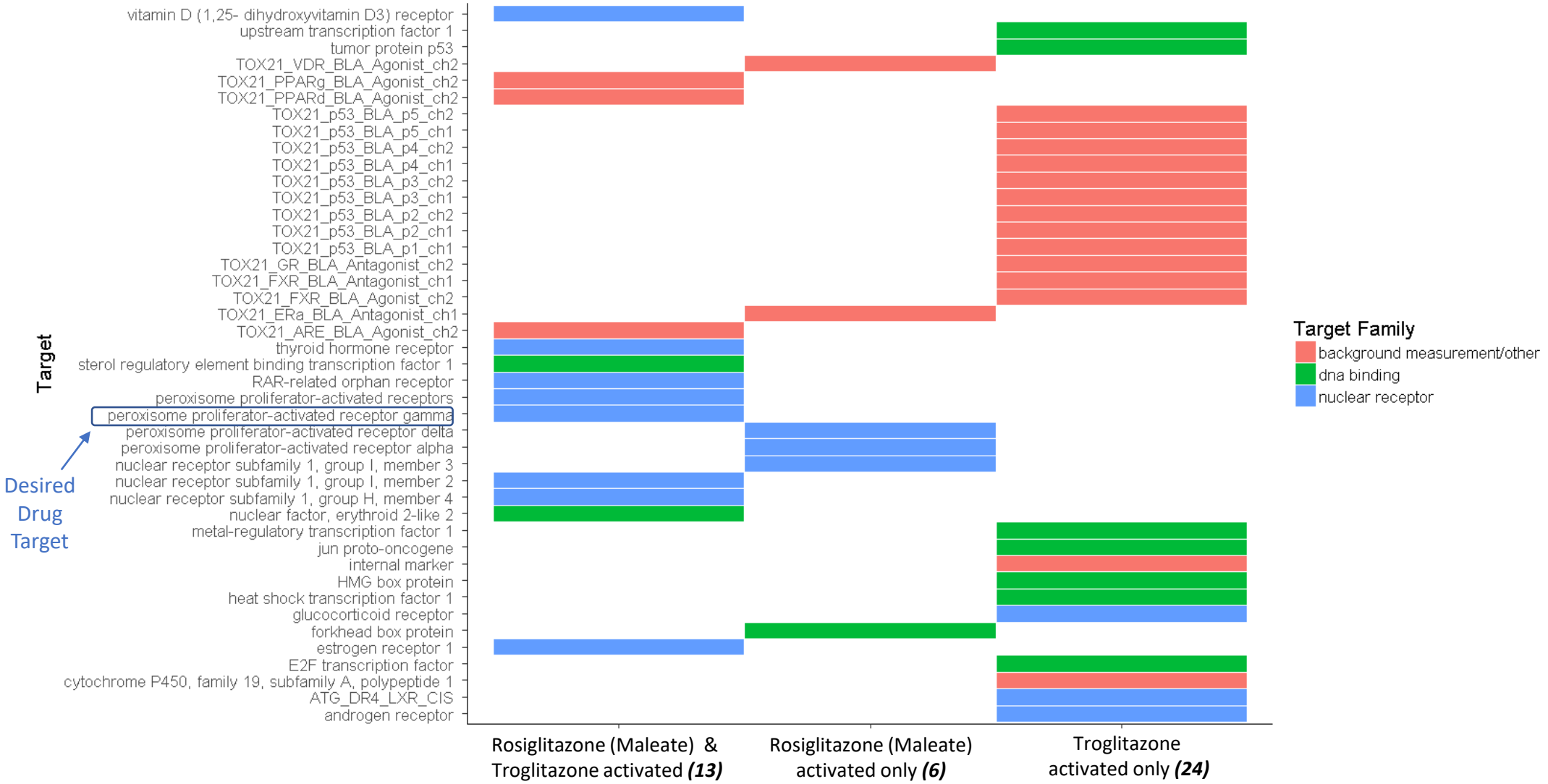
Troglitazone yields higher # of positive tests across biological processes





Venn diagrams not drawn to scale

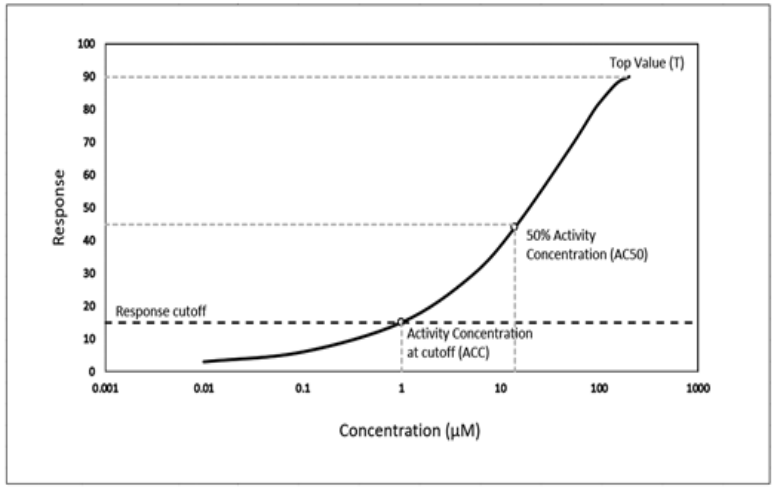
Troglitazone uniquely activates additional targets across target family of transcriptional regulators

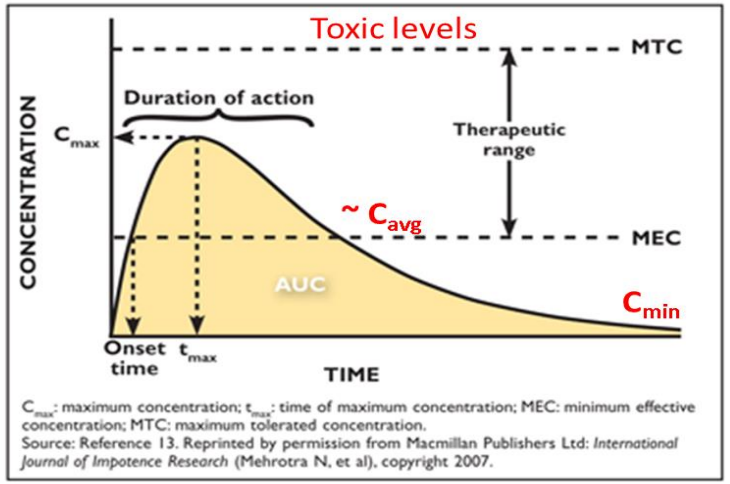


Stratification of targets' activation potential in humans using *Normalized Activation Score (NAS)*

C_{max} (of drug in humans) is > AC50 (for any in vitro/cellular assay) → ***higher potential for activation (of assay target)***

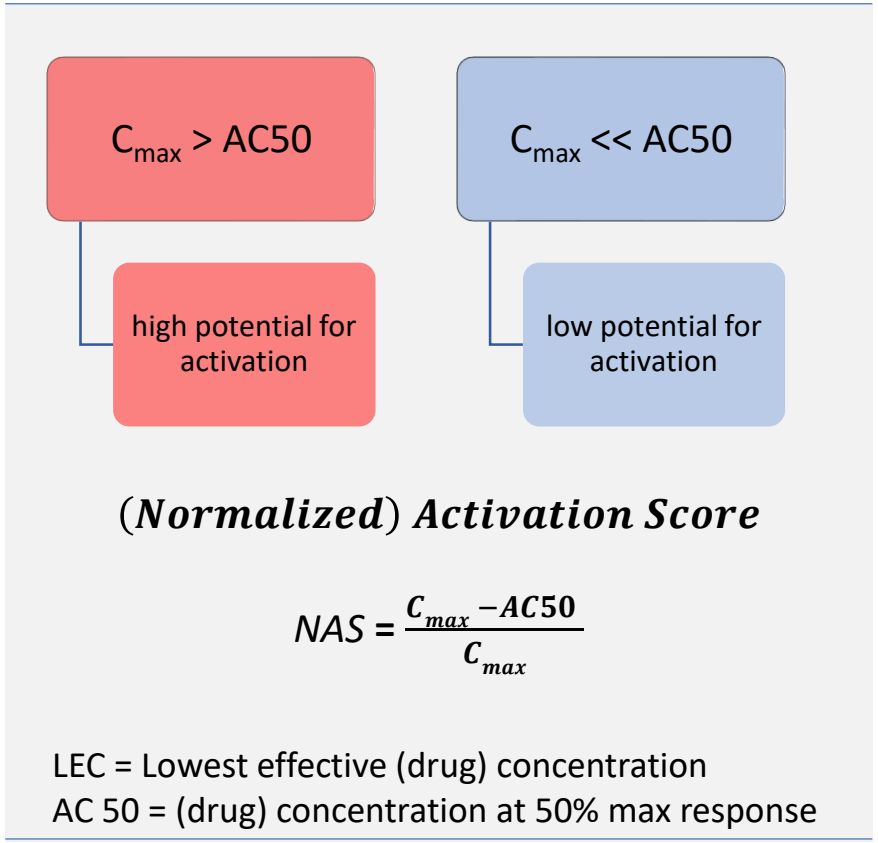
C_{max} (of drug in humans) << AC50 (for any in vitro/cellular assays) → ***lower potential for activation (of assay target)***





C_{max}: maximum concentration; t_{max}: time of maximum concentration; MEC: minimum effective concentration; MTC: maximum tolerated concentration.
Source: Reference 13. Reprinted by permission from Macmillan Publishers Ltd: *International Journal of Impotence Research* (Mehrotra N, et al), copyright 2007.

Schema for stratification of targets' activation potential in humans

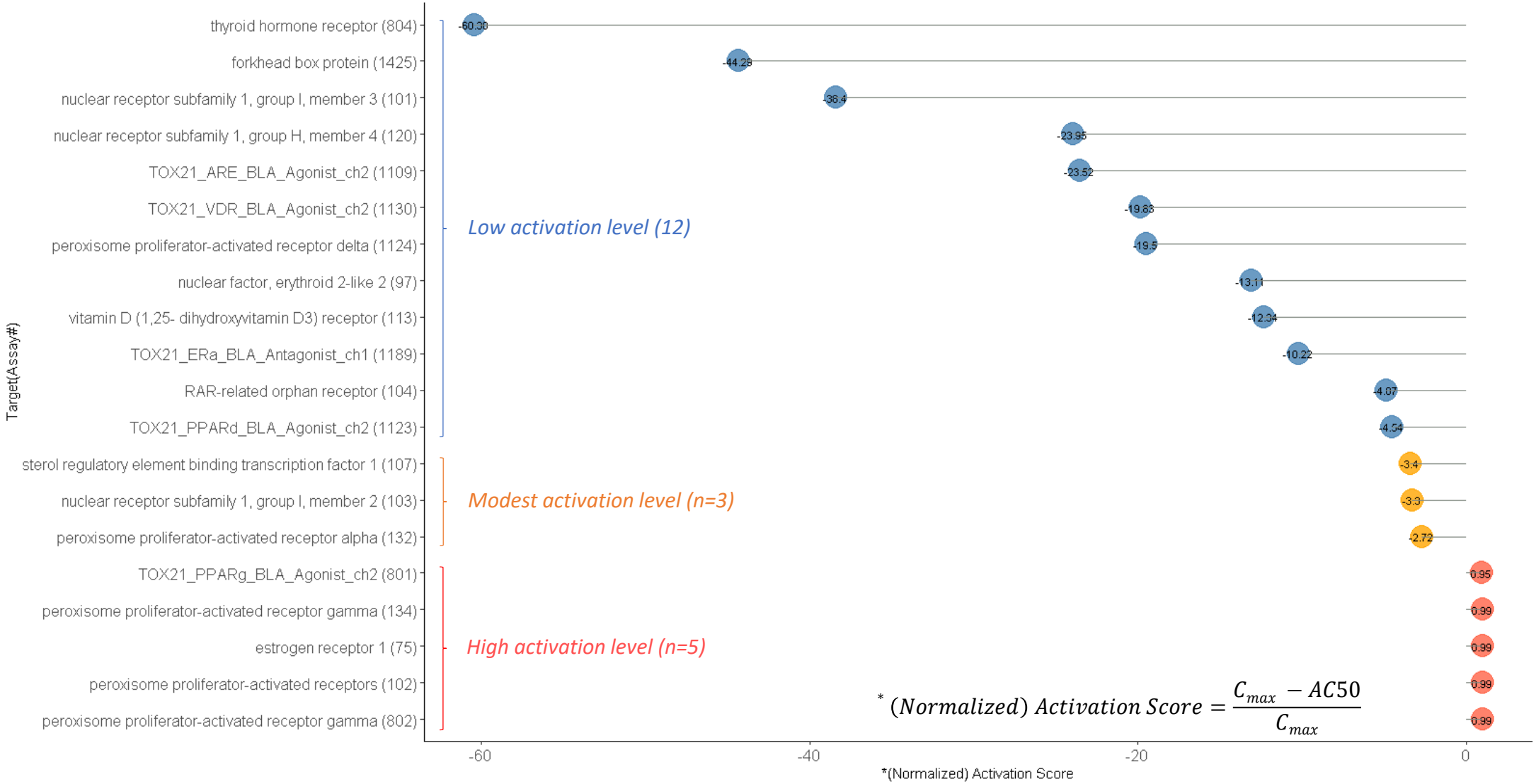


Normalized activation score (NAS) level	Indication
NAS>=0	C_{max} > EC50; highest activation potential
0>NAS>-4	C_{max}< EC50; modest activation potential
NAS<-4	C_{max}<<< EC50; lowest activation potential

For the 437 "common tests" between Rosiglitazone Maleate and Troglitazone :

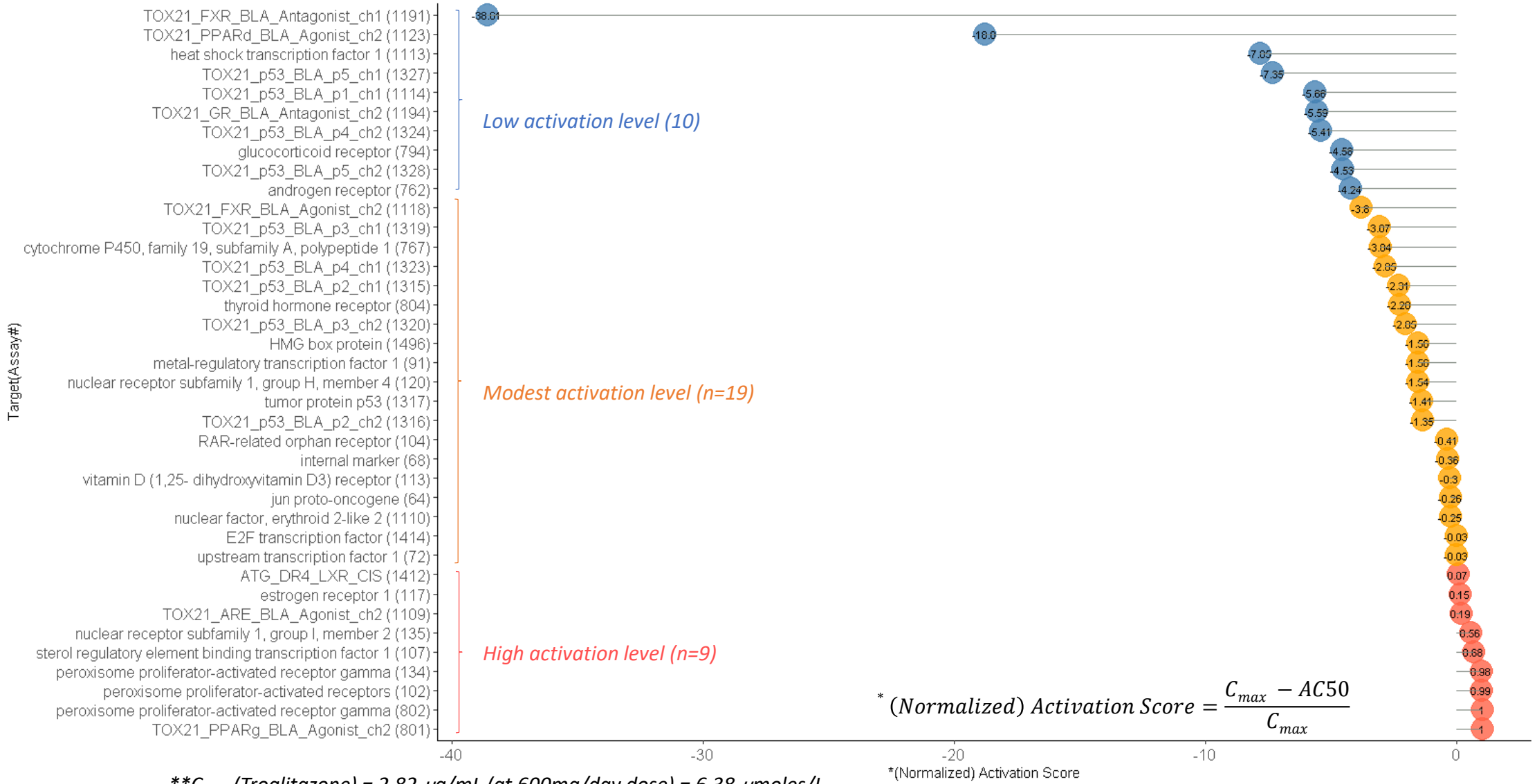
→***NAS*** *values were generated and compared across corresponding biological processes*

NAS stratified putative transcriptional regulatory targets of Rosiglitazone Maleate in humans



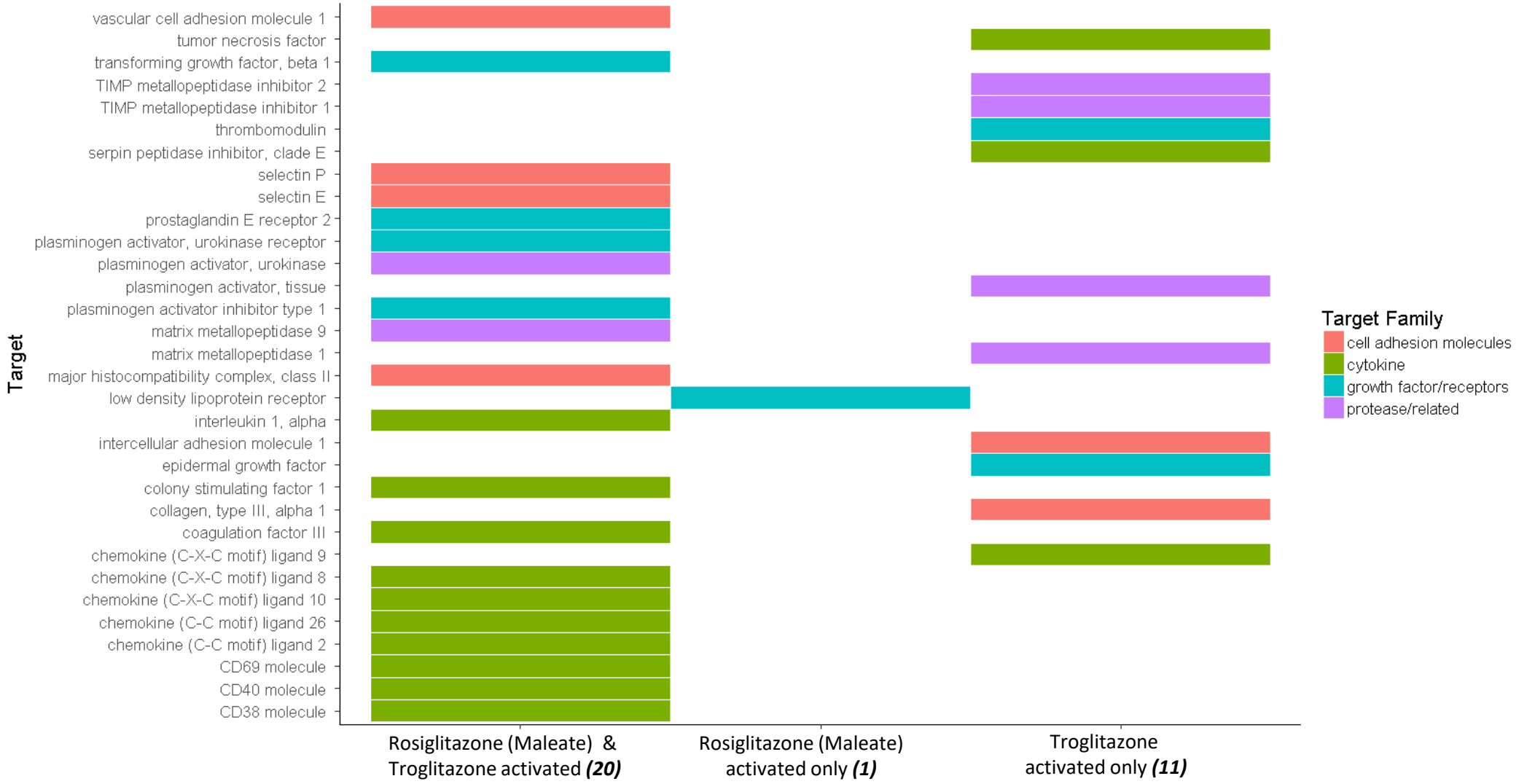
***C_{max} (Rosiglitazone Maleate) = 598 ng/mL (at 8mg/day dose) = 1.26 µmoles/L*

NAS stratified putative transcriptional regulatory targets of Troglitazone in humans

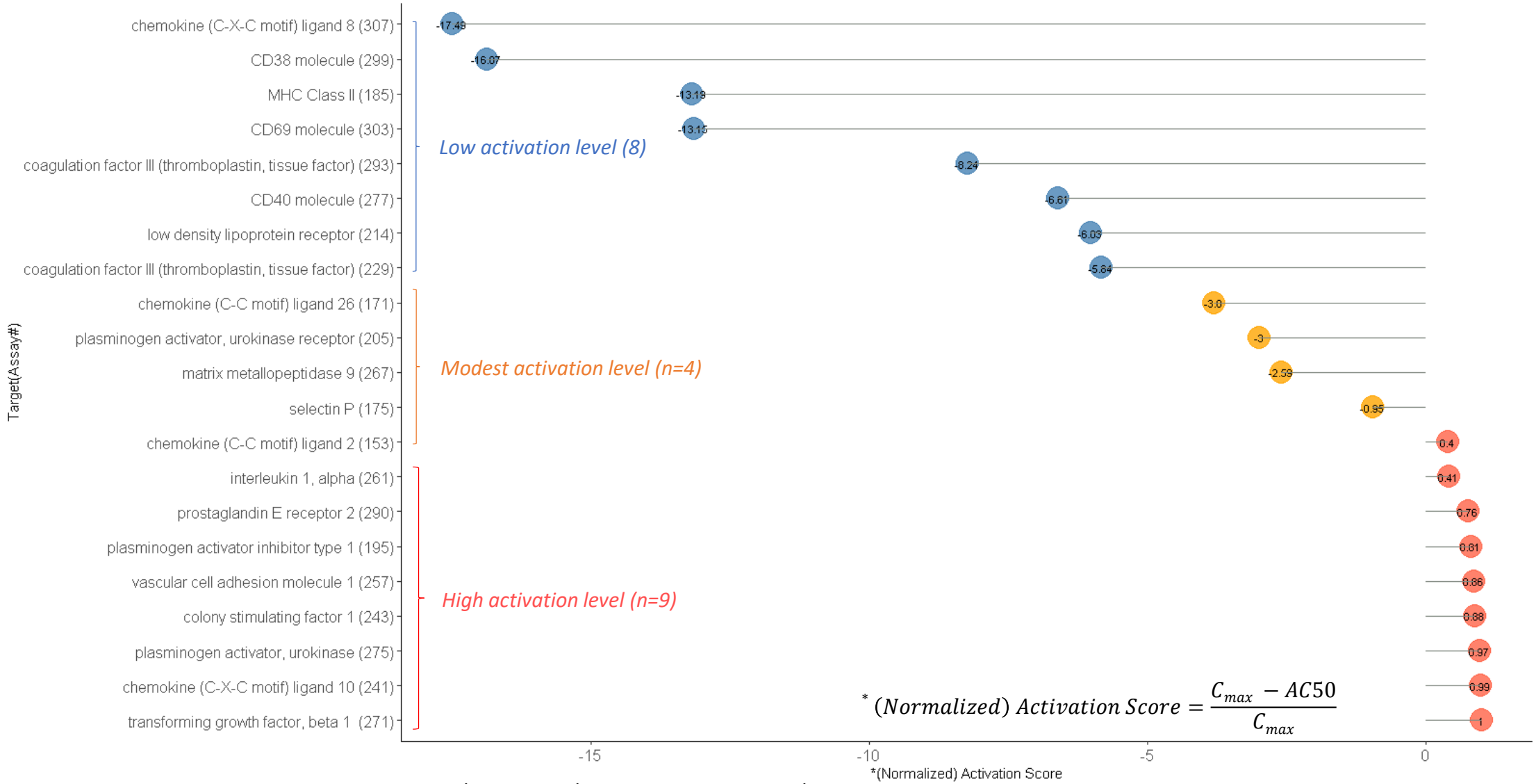


**C_{max} (Troglitazone) = 2.82 μg/mL (at 600mg/day dose) = 6.38 μmoles/L

Troglitazone uniquely activates additional targets across target family of gene expression regulators



NAS stratified putative gene expression regulatory targets of Rosiglitazone Maleate in humans



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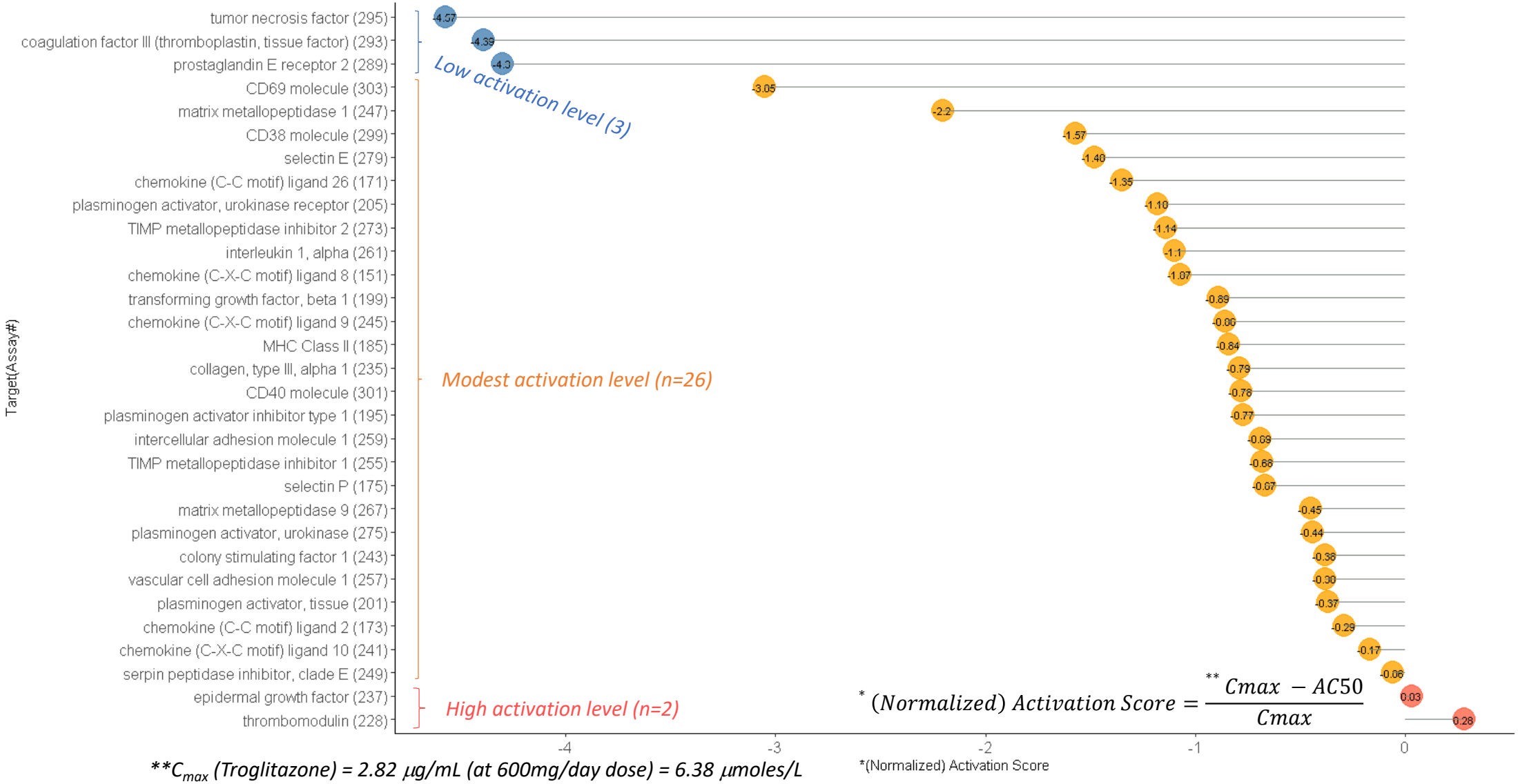
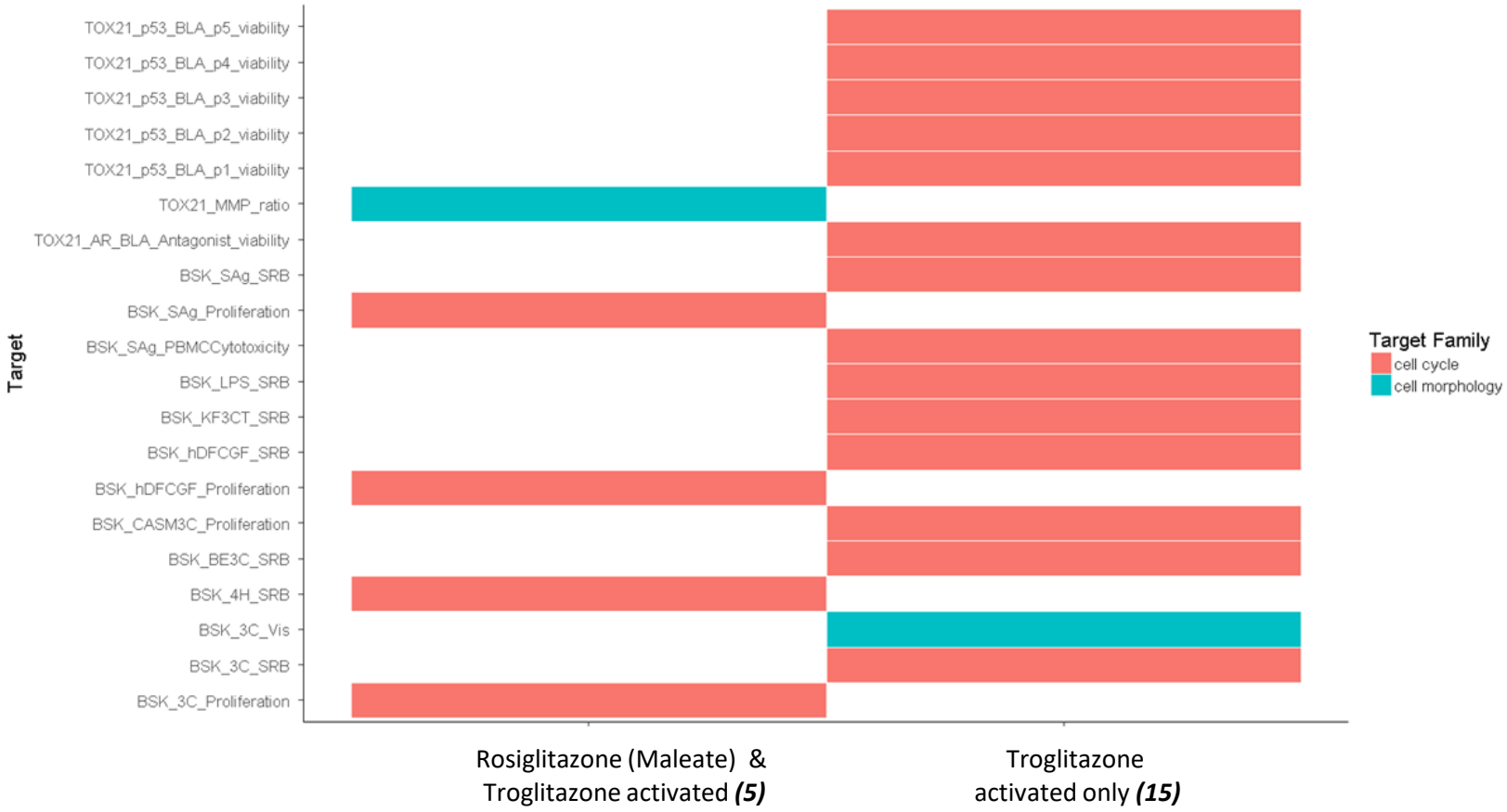
NAS stratified putative gene expression regulatory targets of Troglitazone in humans



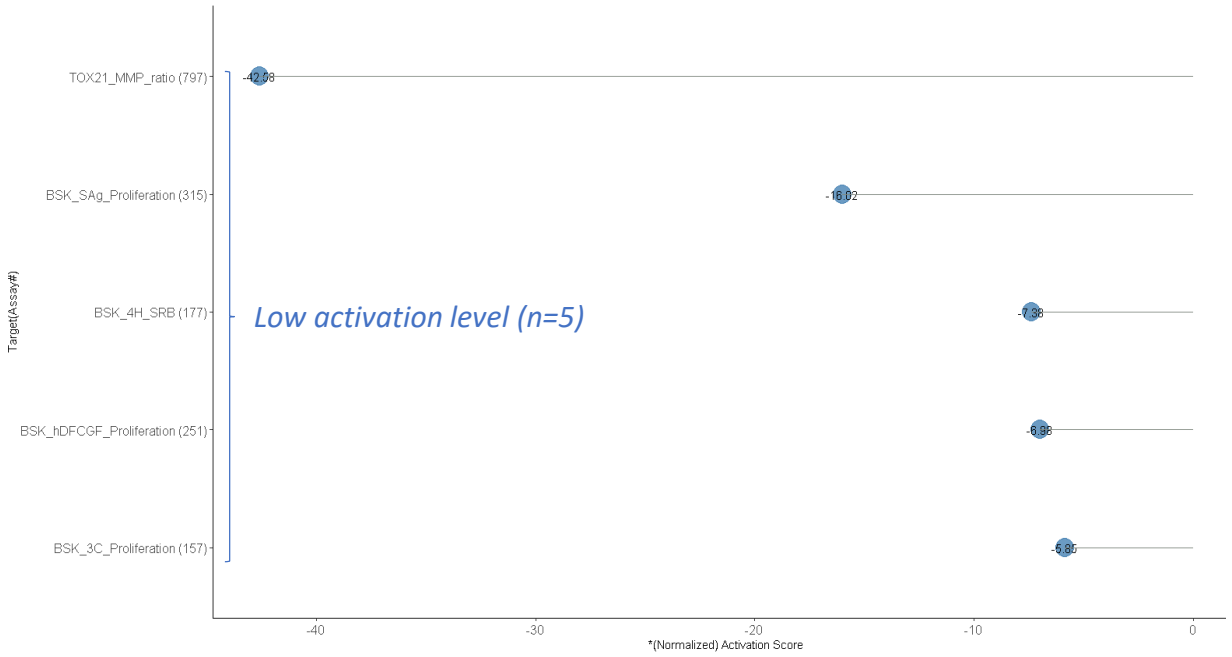
***C_{max} (Troglitazone) = 2.82 μg/mL (at 600mg/day dose) = 6.38 μmoles/L*

Cell cycle/Cell morphology targets

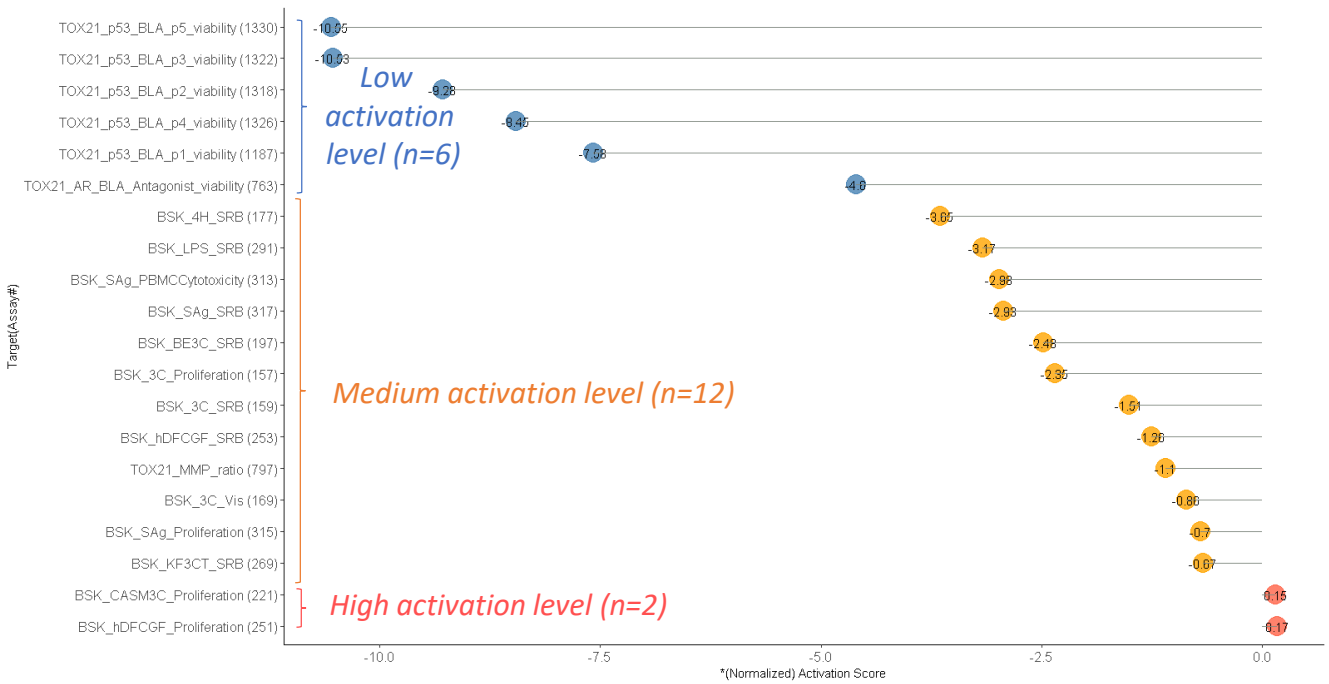


NAS stratified putative cell cycle/morphology targets of Rosiglitazone Maleate and Troglitazone in humans

Rosiglitazone Maleate



Troglitazone



$$^{*}\,(Normalized)\ Activation\ Score = \frac{C_{max} - AC50}{C_{max}}$$

***C_{max} (Rosiglitazone Maleate) = 598 ng/mL (at 8mg/day dose) = 1.26 µmoles/L*

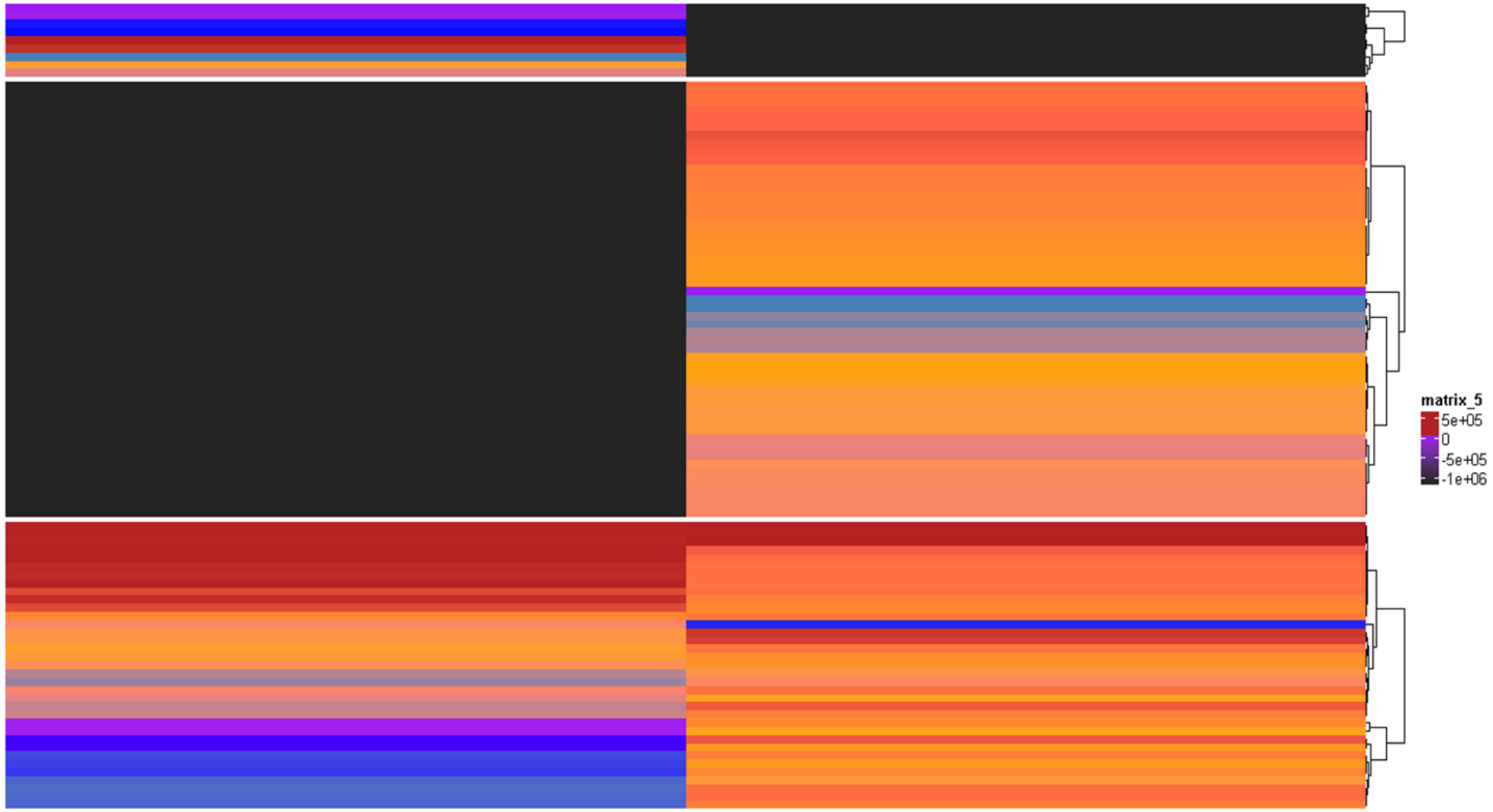
***C_{max} (Troglitazone) = 2.82 µg/mL (at 600mg/day dose) = 6.38 µmoles/L*

NAS based "clustering" of all tests

Tests affected by Rosiglitazone (Maleate) only

Tests affected by Troglitazone only

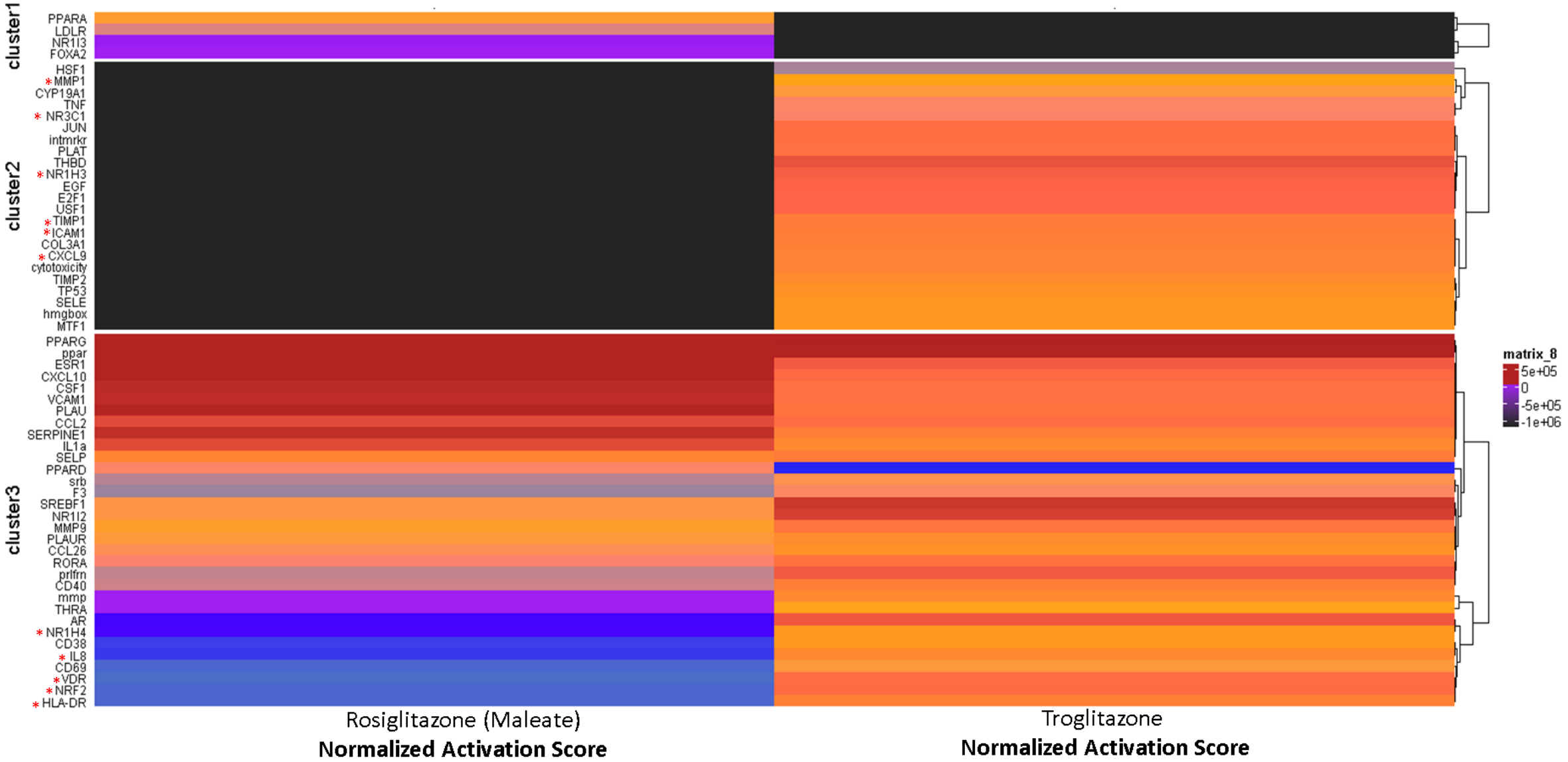
Tests affected by both drugs



Rosiglitazone (Maleate)
Normalized Activation Score

Troglitazone
Normalized Activation Score

NAS based "clustering" of all Targets/Pathways



** Implicated in liver injury/repair pathways* -

Duarte S et al., 2015; Saiman Y & Friedman SL., 2013; Bohm F et al., 2010; Rudraiah S et al., 2016, Zimmerman HW et al., 2011,Gomex-Ospina N et al., 2016, Jadeja RN et al., 2016, Zuniga S et al., 2011,Tan W et al., 2017

Summary/Conclusions

Normalized activation score (NAS) stratifies molecular actions of drugs

- Targets/pathways with NAS > 0 expected to have highest potential for activation in patients and represent potential non-target effects.

Troglitazone associated targets are correlated with DILI risk

- Correlation with Troglitazone-associated targets (higher NAS for Troglitazone vs Rosiglitazone Maleate) with role in liver injury/repair pathways

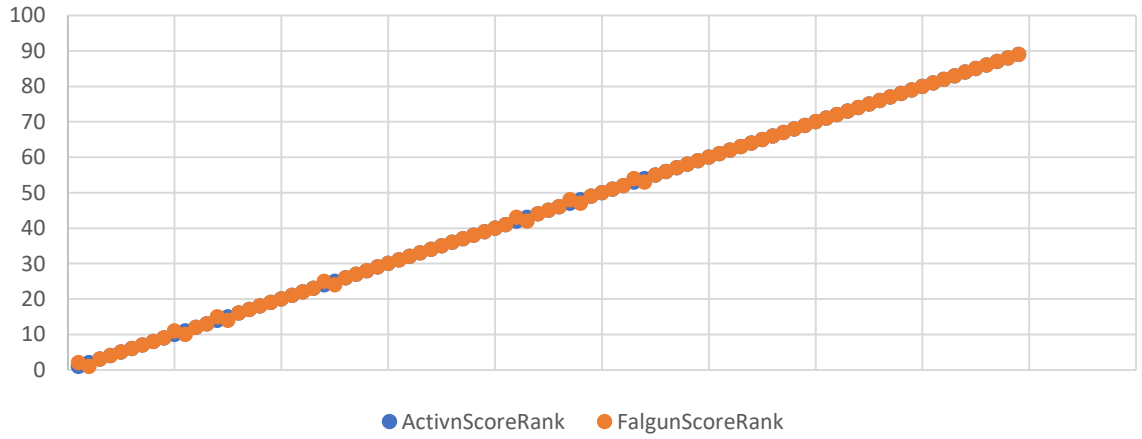
Next step: harness ToxCast for Liver Toxicity modeling

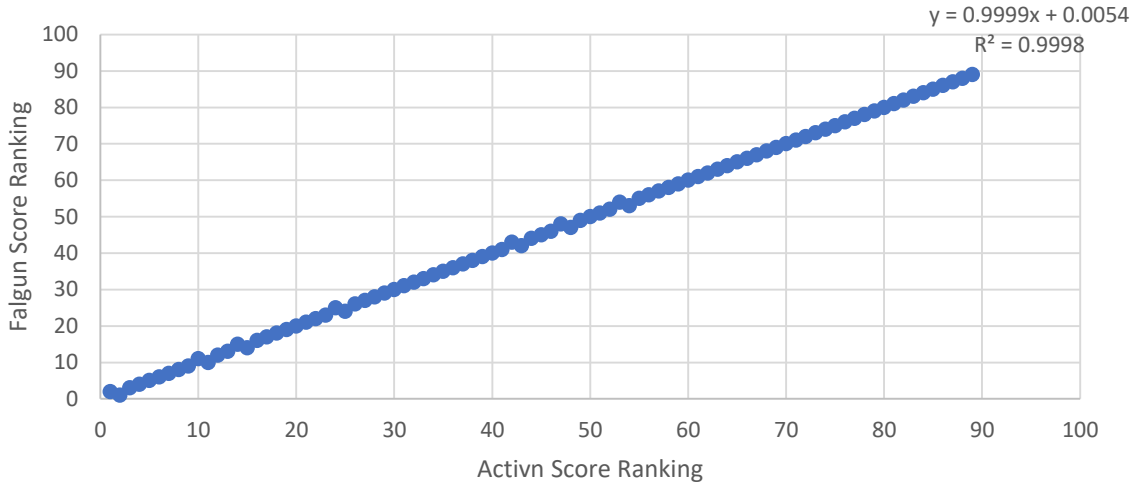
- *Integrate ToxCast targets/pathways data with known predictors of liver toxicity for potentially enhanced models of liver toxicity (initiated)*

Appendix

- “mostDILI” risk for Troglitazone-associated vs Rosiglitazone associated targets
- Comparison of target rankings for activation potential using NAS vs Falgun Shah score
- Count of positive tests for each biological process in Troglitazone vs Rosiglitazone (3 slides)
- Pharmacological activity/targets by DILI class from ToxCast database (3 slides)

Normalized Activation Score* performs nearly identically to Falgun Score** for stratification of Troglitazone affected targets/proteins (n = 89)

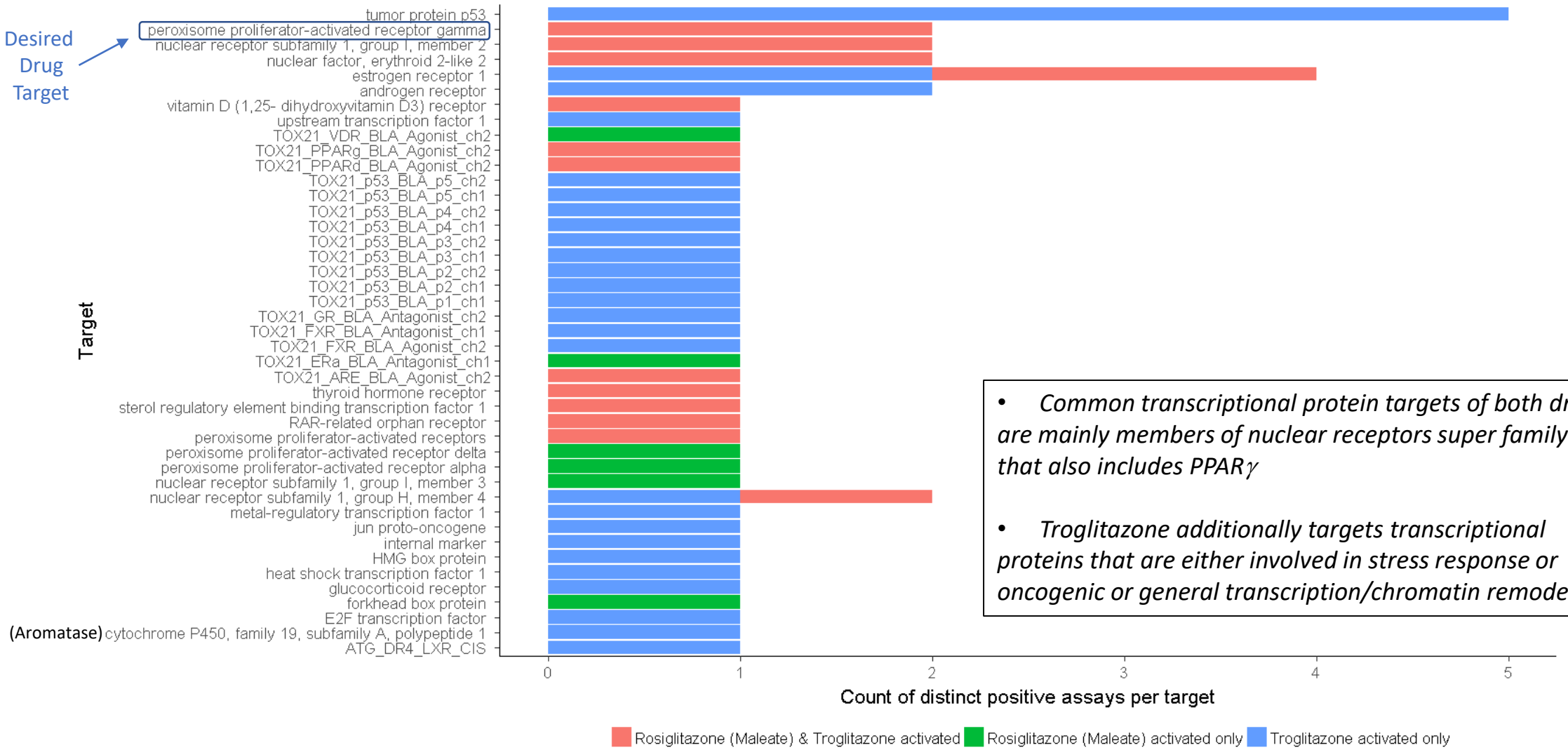




*Normalized Activation Score = (Cmax – EC50)/Cmax
**Falgun Score = Cmax/EC50 (Shah F et al., 2015 Toxicol Sci)

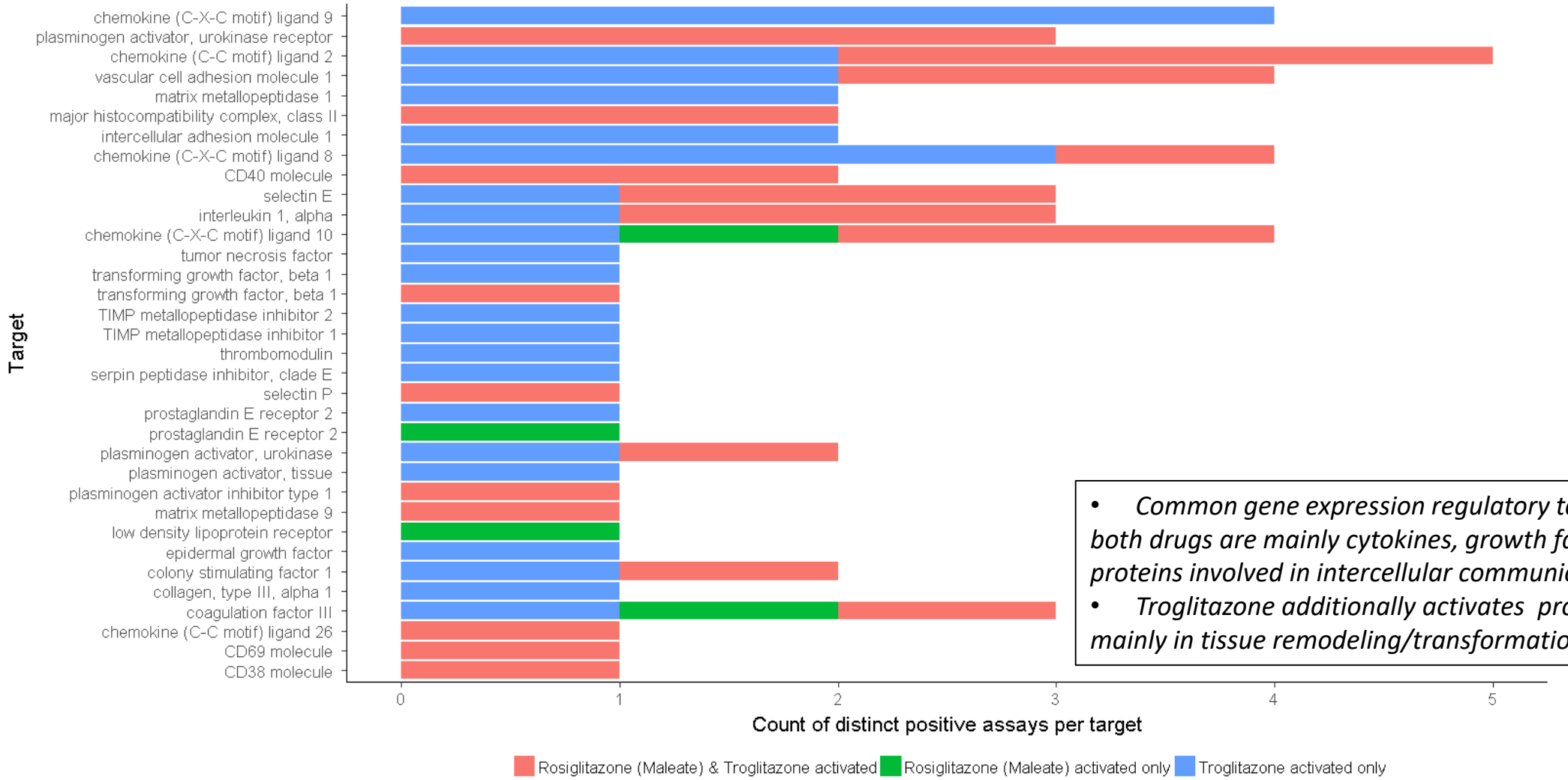
Activation level	Normalized Activation score	Falgun Score (C_{max}/AC50)
High activation	1 to ~0	~300 – ~1
Medium activation	0 to -4.0	1 - ~0.20
Low activation	Less than - 4.0	Less than 0.20

Count of positive tests per transcriptional targets for each drug



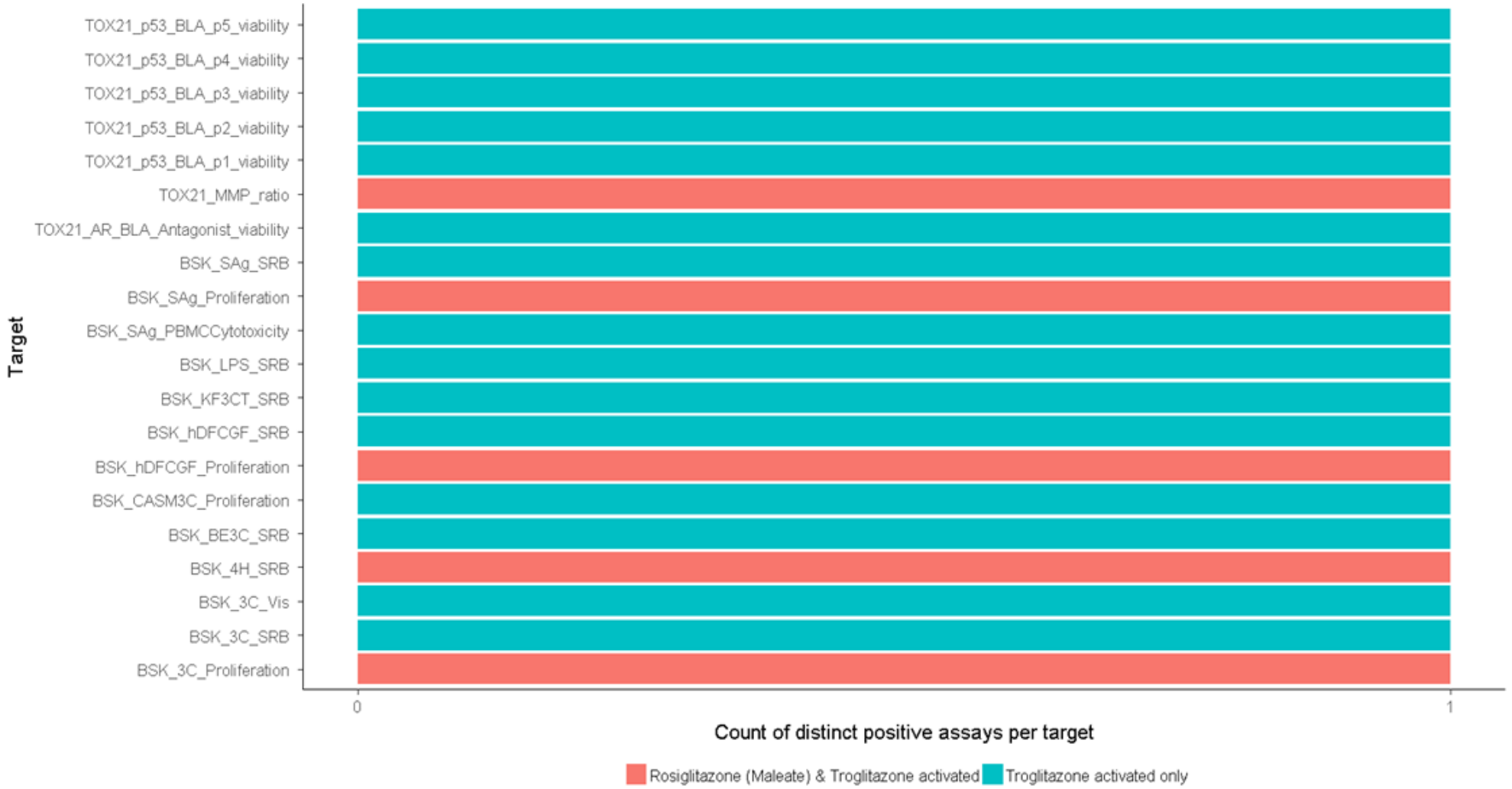
- *Common transcriptional protein targets of both drugs are mainly members of nuclear receptors super family – that also includes PPARγ*
- *Troglitazone additionally targets transcriptional proteins that are either involved in stress response or oncogenic or general transcription/chromatin remodelling*

Count of positive assays for gene expression regulatory targets per drug

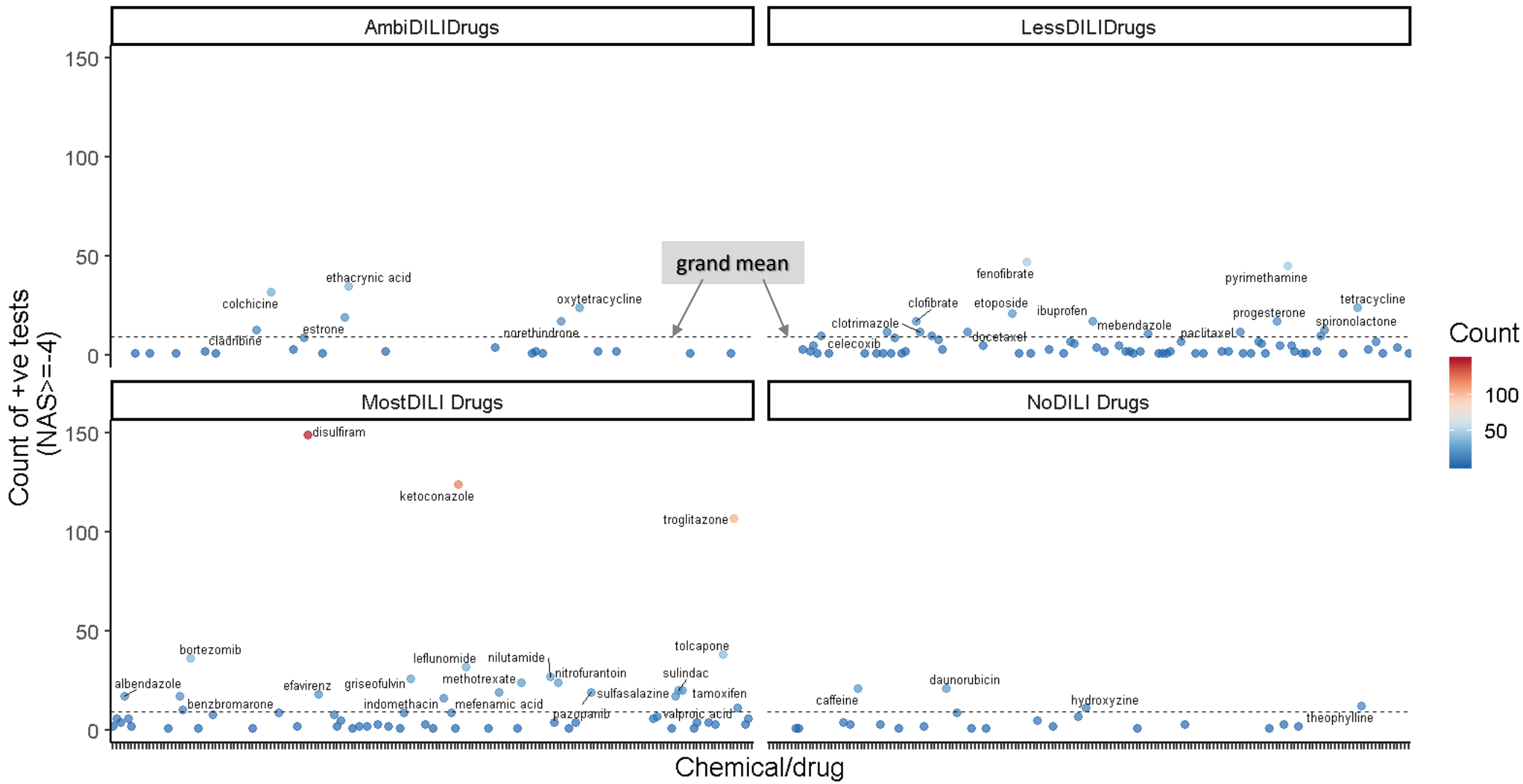


- *Common gene expression regulatory targets of both drugs are mainly cytokines, growth factors, and proteins involved in intercellular communications*
- *Troglitazone additionally activates proteins involved mainly in tissue remodeling/transformation*

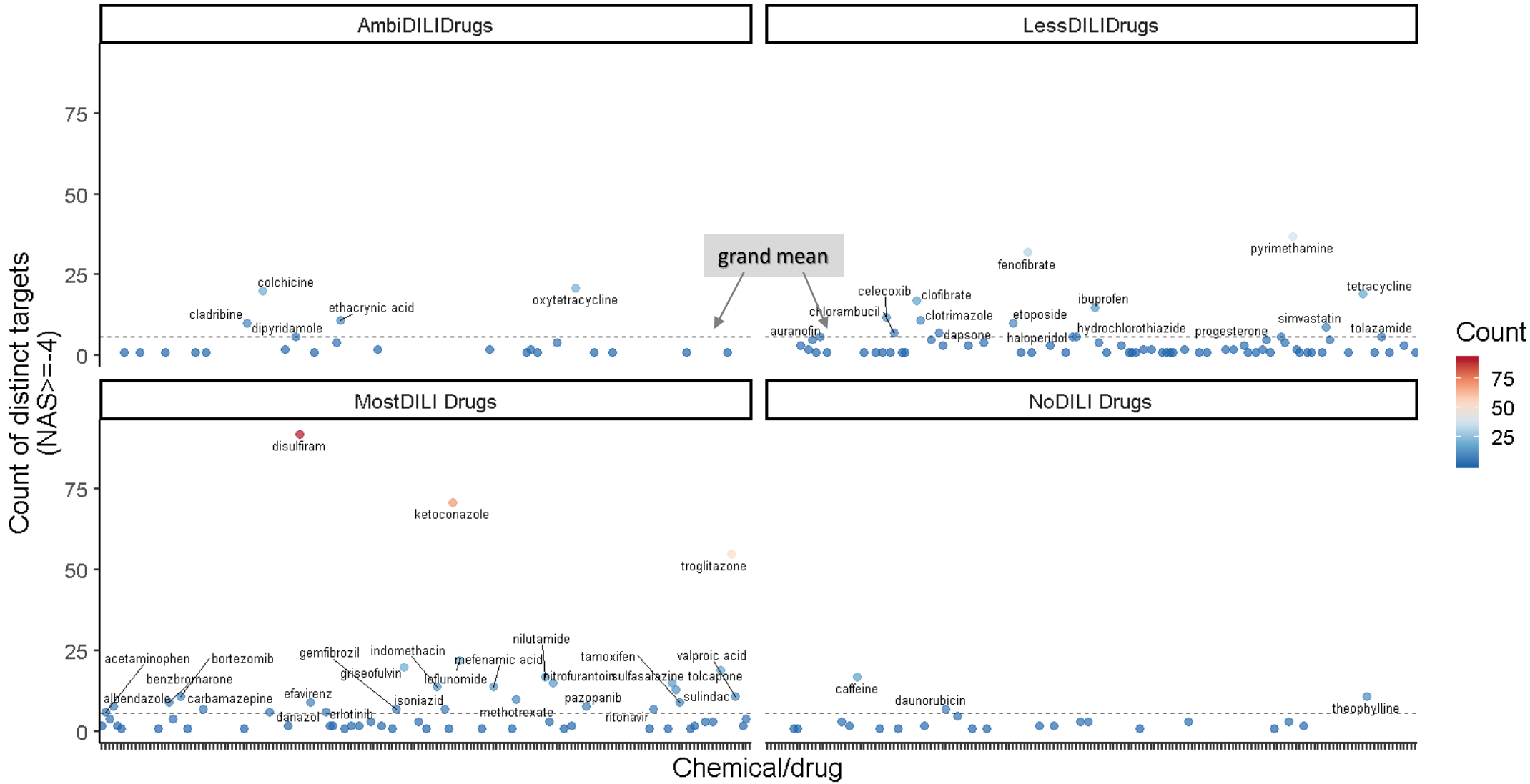
Count of positive assays for cell cycle/cell morphology targets per drug



Subset of drugs are more pharmacologically active in each DILI class



Subset of drugs are more pharmacologically active towards distinct* molecular targets



Highly reactive targets/pathways by DILI class