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Agonist-specific Regulation of Aryl Hydrocarbon Receptor Target Genes in a Xenopus laevis Cell Line

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Agonist-specific regulation of aryl hydrocarbon receptor target genes in a *Xenopus laevis* cell line

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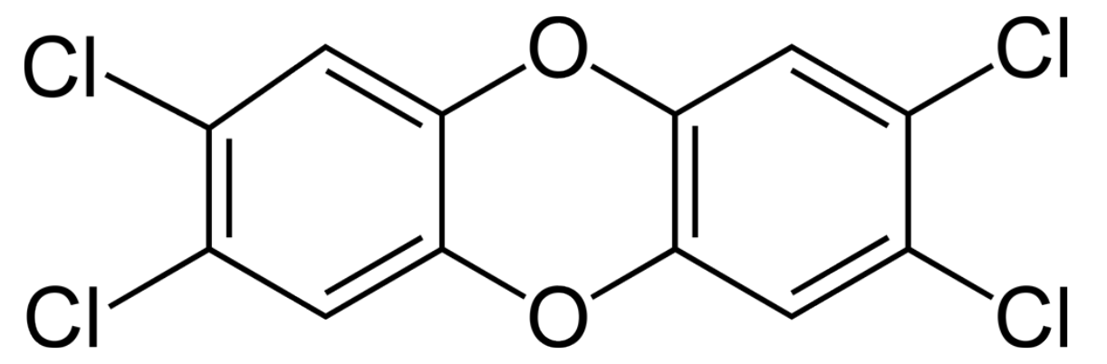
Kenyon College Summer Science Scholars 2016

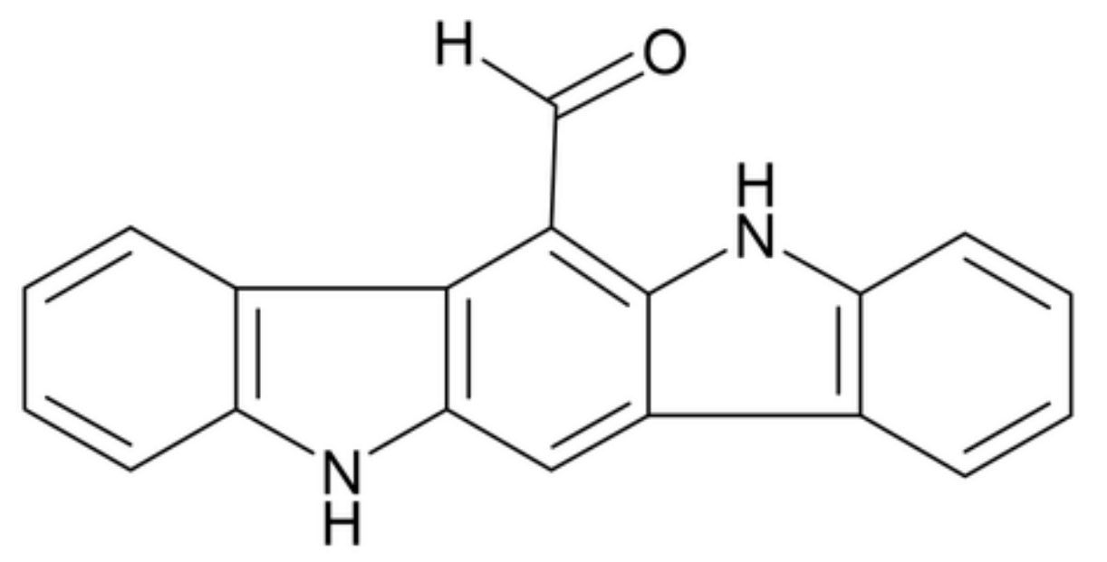
Abstract

In vertebrates, the activation of the aryl hydrocarbon receptor (AHR) by toxic contaminants such as 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) induces the Cytochrome P450 1A (*CYP1A*) family of genes . The endogenous AHR agonist 6-formylindolo (3,2-*b*) carbazole (FICZ) also induces *CYP1A* strongly, but is not toxic. In order to discern the underlying mechanism for this difference in toxicity, we sought to determine whether FICZ and TCDD alter the expression of the same genes outside of the *CYP1A* family. We hypothesized that FICZ and TCDD exposure would induce unique sets of gene targets, suggesting selective modulation of gene expression as an underlying mechanism for toxicity. We treated the *Xenopus laevis* cell line, XLK-WG with TCDD or FICZ concentrations corresponding to the the EC50 for *CYP1A6* induction. RNAseq analysis of transcribed RNA revealed 162 genes that were commonly induced or repressed by both agonists, 65 transcripts uniquely altered by TCDD treatment, and 235 genes that responded only to FICZ exposure. We next sought to verify these results using a second method, quantitative RT-PCR (qPCR). Surprisingly, our qPCR results contradicted our original RNAseq findings for several target genes. While genes induced or repressed by both compounds, such as *SYNE1* and *HAS2.L*, matched their trend in the RNAseq data, genes previously exhibiting agonist-dependent expression changes, such as *NOV.L* and *TIPARP.L*, responded similarly to TCDD or FICZ treatment. In the future, we hope to find the cause for this discrepancy in these data, ultimately determining the actual prevalence of selective modulation in the AHR pathway.

Background



Chemical structure of exogenous AHR agonist 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD)
[https://upload.wikimedia.org/wikipedia/commons/8/84/2,3,7,8-TCDD-2D-skeletal.png]



Chemical structure of endogenous AHR agonist 6-formylindolo (3,2-*b*) carbazole (FICZ)
[http://www.stressmarq.com/wp-content/uploads/SIH-383_6-Formylindolo3-2-bcarbazole_Chemical_Structure.png]

- TCDD and FICZ induce *CYP1A* family genes through the ligand-activated transcription factor AHR. The genes targeted by AHR are crucial in phase 1 and 2 detoxification reactions and other important developmental functions.
- Though both are AHR agonists, TCDD is toxic and FICZ is not.
- To uncover potential mechanisms for toxicity, we aim to determine whether TCDD and FICZ selectively modulate gene expression

Approach & Results

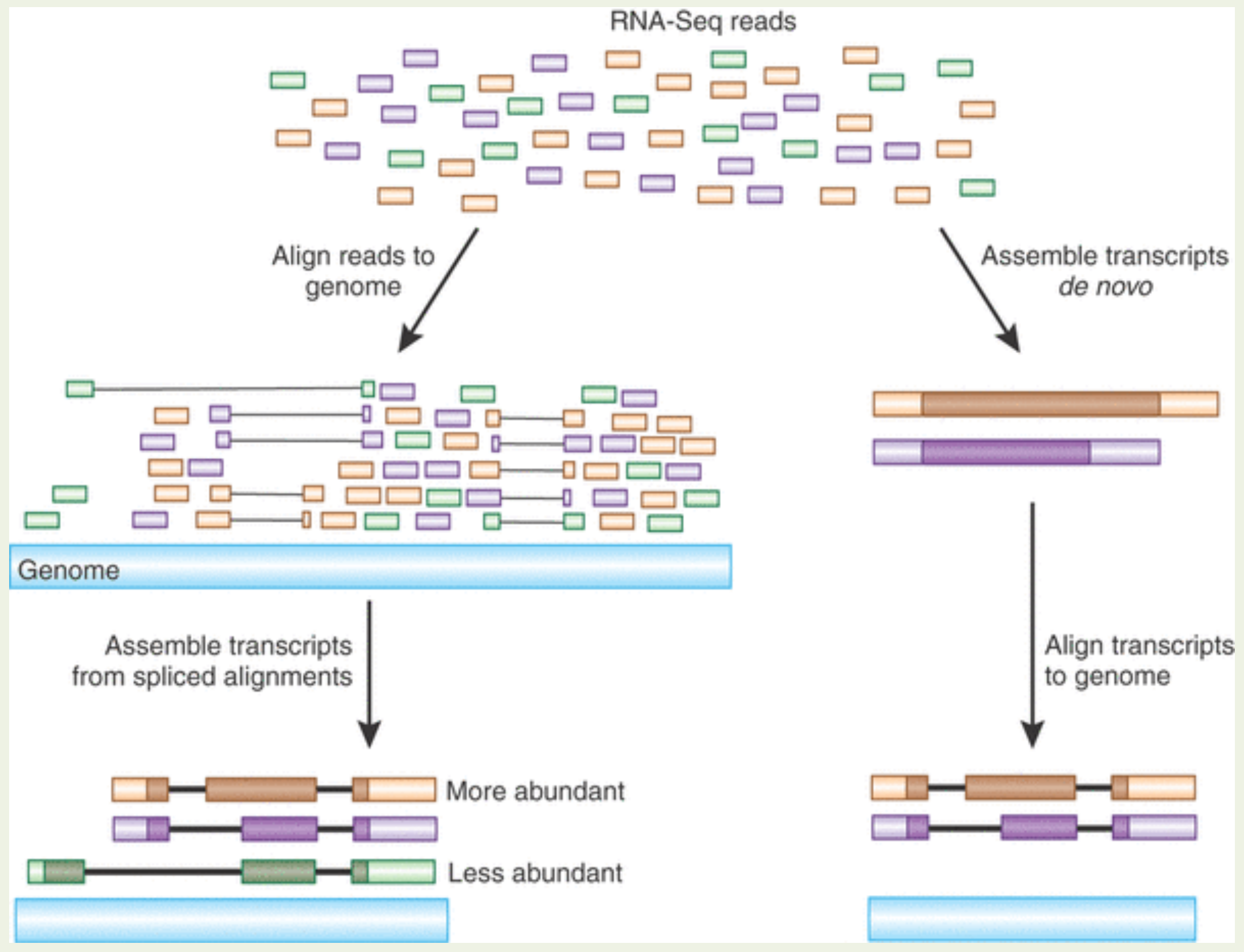
RNAseq

Treatment of Cells: *Xenopus laevis* kidney cells (XLK-WG) were treated with 175 nM TCDD or 0.2 nM FICZ, concentrations corresponding to the EC50 for *CYP1A6* induction.

RNA Extraction: Total RNA was extracted from the cells using RNEasy RNA Extraction kit (Qiagen) for use in RNAseq analysis to determine gene expression.

Sequencing & Assembly: Sequencing was performed by Cofactor Genomics through the Illumina platform. Sequences were analyzed using Trinity de novo transcript assembly and Tuxedo mapping to Xenopus laevis genome 7.0 (Neel Aluru, WHOI).

RNAseq Analysis



[http://www.nature.com/nbt/journal/v28/n5/images_article/nbt0510-421-F1.gif]

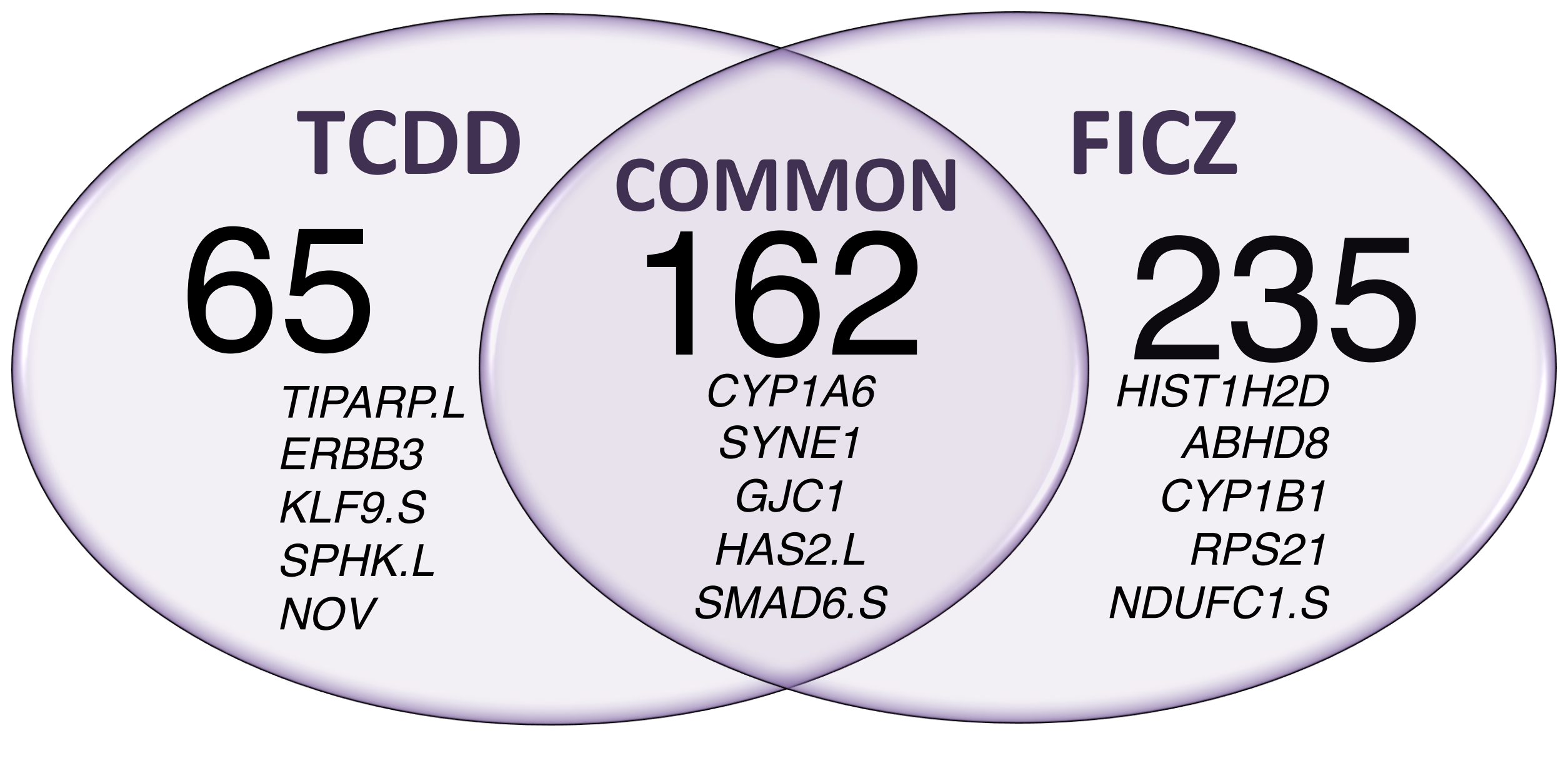


Figure 1. Agonist-dependent AHR gene targets. RNAseq analysis of the the transcribed genes extracted from each treatment group revealed 162 genes that were commonly induced or repressed by both agonists, 65 transcripts uniquely altered by TCDD treatment, and 235 genes that responded only to FICZ exposure.

Quantitative PCR

cDNA synthesis: RNA extracted from treated cells was reverse transcribed using the TaqMan Reverse Transcription kit (Applied Biosystems).

qPCR: cDNA was amplified using Power SYBR Green Master Mix (Applied Biosystems). To find the fold change induction or repression of target genes, the $\Delta\Delta$Ct value was calculated and data was normalized to a DMSO control group.

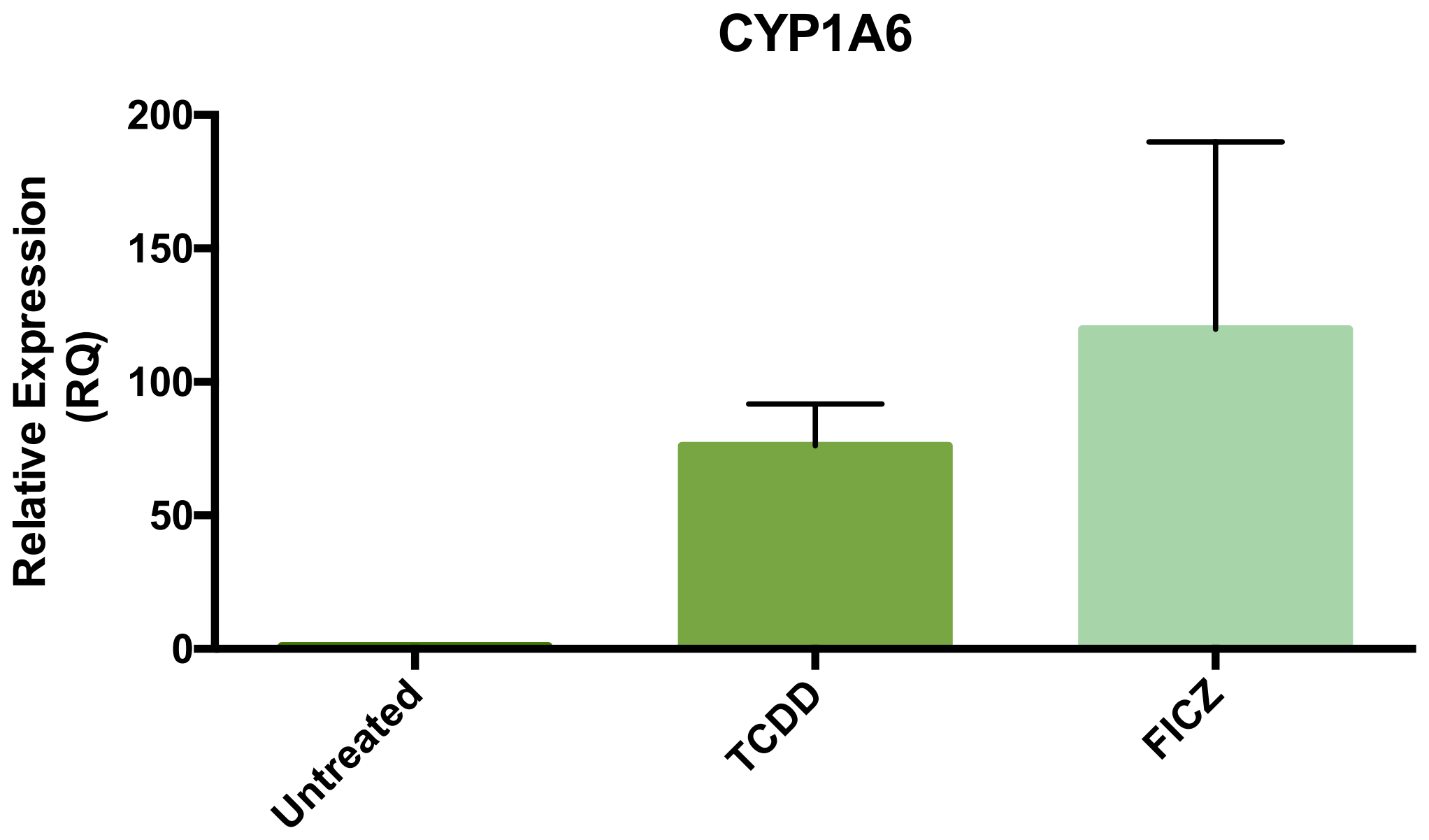


Figure 2. Relative gene expression of *CYP1A6*. Treatment with TCDD resulted in a 76.06 fold change induction of *CYP1A6*, while FICZ treatment resulted in a fold change of 119.7 (n=3, one-way ANOVA p=0.0464). Error bars = SEM.

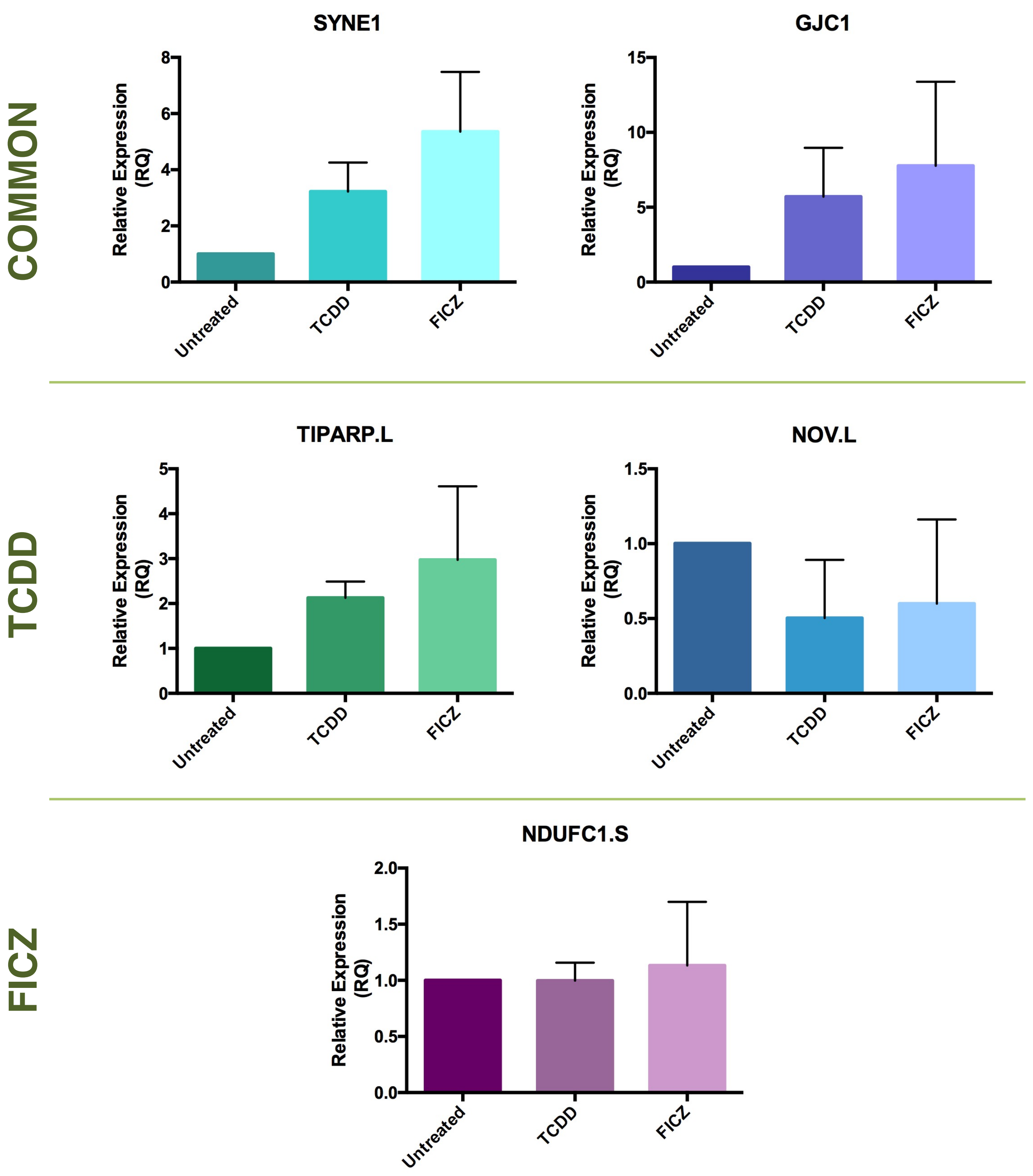


Figure 3. Relative gene expression of AHR targets. Treatment with TCDD resulted in induced gene expression in *SYNE1* (p=0.0071), *GJC1* (p=0.0464), and *TIPARP1.L* (p=0.0464), approximately no change in *NDUFC1.S* (p=0.8197) and repressed gene expression in *NOV.L* (p=0.3000). FICZ treatment resulted identical trends in gene expression despite RNAseq predictions of agonist-dependent gene expression (for all samples, n=3, p-values represent one-way ANOVA). Error bars=SEM.

Conclusions

- **Genes marked in RNAseq as commonly induced in both TCDD and FICZ treatment groups held their gene expression trends in qPCR analysis.** *CYP1A6, SYNE1*, and *GJC1* all showed an increase in gene exxpression when treated with either TCDD or FICZ
- **Agonist-dependent AHR gene targets identified in RNAseq data showed non-unique gene expression trends in qPCR analysis**. *TIPARP.L*, identified as a gene induced exclusively in TCDD treatment groups, showed increased expression in both TCDD and FICZ treatment groups in qPCR analysis. Similarly, RNAseq data identified *NOV.L* as a gene repressed exclusively in TCDD treatment groups. However, qPCR identified that this repression was present in both treatment groups. *NDUFC1.S*, suspected to be repressed under FICZ treatment in RNAseq data, showed an insignificant change in gene expression in both treatment groups
- **Selective modulation may not be a potential explanation for toxicity in cells.** Further research can reveal the cause for discrepancies in RNAseq and qPCR data.

Acknowledgements

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