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Supplemental Material

On the Utility of ToxCastTM and ToxPi as Methods for Identifying New Obesogens

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Figure S1. Regression analysis of activation and antagonism curves from Figures 1 and 4.

Data points are averages of triplicate transfections (3 biological replicates). Data are depicted as fold induction over vehicle (0.05% DMSO) controls \pm S.E.M. EC₅₀ and IC₅₀ values were obtained using nonlinear regression, variable slope in GraphPad Prism 5.0 Spirodiclofen did not plateau, therefore, it was constrained at the top dose.

Figure S2. Schematic of adipogenesis assays. Top. 3T3-L1 cells were seeded at 2×10^4 cells per well in 12-well plates. After 48 hours, cells were exposed to the adipogenic cocktail MDI (isobutyl-methylxanthine, dexamethasone, and insulin) for 2 days. Induction media was removed and cells were exposed to ToxCast chemicals (or controls) during 5 days replacing the media every 2 days. **Bottom.** 8×10^4 cells/well mBMSCs were maintained as subconfluent monolayers in basic medium. Once confluent, mBMSCs were induced to differentiate with adipogenic cocktail and ToxCast chemicals (or controls) during 14 days replacing the media every 3 days.

Figure S3. ToxPi chemical pyridaben inhibits adipogenesis in 3T3-L1 preadipocytes.

Adipogenesis was induced in cells according to Supplemental Material, Figure S2. Lipid accumulation was assessed by measuring the percent of surface area in each well covered by Oil Red 0 positive cells using Image J software. One-way ANOVA was conducted for

pyridaben treatment groups and DMSO vehicle (VEH), followed by Dunnett's post-hoc test: *** $P \leq 0.001$ compared to DMSO. Unpaired t-test was conducted for the positive control Rosiglitazone (ROSI) versus DMSO vehicle: ### $P \leq 0.001$.

Figure S4. Attagene identifies a suspiciously large number of RXR-selective chemicals.

Phase II, release 2014 (Filer et al. 2014) datasets (gain AC_{50} values) were obtained for Attagene agonist assays on RXR α and RXR β . Chemicals scoring $AC_{50} \leq 10 \mu\text{M}$ for each assay were incorporated in the Venn diagrams, created by BioVenn (Hulsen et al. 2008). The Venn diagram reveals 100 RXR β -selective and 30 RXR α -selective chemicals, with only 22 chemicals activating both receptor subtypes.

Figure S5. True positives and false positives are lost when employing ToxCast Phase II new models.

(A) **Green triangles:** ToxPi Phase I rankings (based solely on AC_{50} values). **Blue dots:** ToxPi Phase II rankings using the Phase I chemical library are based solely on AC_{50} values. **Squares:** ToxPi Phase II rankings using Phase I chemical library were constructed by first removing chemicals with low Z-scores, and then correcting the magnitude each Pi slice by adding the Z-score to the negative log (AC_{50}). Differences between triangles and dots are due to discrepancies between Phase I and Phase II assays on the same chemical. Differences between squares and dots are attributed to the Z-score correction. (B) **Circles/dots:** Raw Z-score values derived from ToxPi Phase II data using Phase I chemical library. **Triangles:** Z-score corrected AC_{50} values derived from ToxPi Phase II data using Phase I chemical library. All true PPAR γ activators rank significantly lower in both raw Z-score and Z-score corrected AC_{50} values compared to atrazine and quinclorac. However, atrazine is not a PPAR γ activator in Cos7 transient transfection assays (Supplemental Material, Figure S7).

Figure S6. ToxPi regeneration using Phase II, 2014 dataset and Phase I chemical library.

Adipogenesis ToxPi diagrams where slice size (magnitude) represents the activity of a ToxCast chemical in a particular assay or collection of assays (Supplemental Material, Table S1). Highest scoring ToxPi chemicals are predicted to be obesogenic. (A) Top scoring Phase II ToxPi chemicals with Z-score correction. The magnitude of the Pi slice is determined by adding the Z-score to the negative log (AC_{50}). (B) Top scoring Phase II ToxPi chemicals without Z-score correction, based solely on AC_{50} values.

Figure S7. Phase II ToxCast chemical activity on PPAR γ and RXR α .

The ability of a graded dose series of ToxCast chemicals to activate (A) GAL4-mPPAR γ or (B) GAL4-hRXR α was tested in transiently transfected Cos7 cells. (A, B) Data points are averages of triplicate

transfections (3 biological replicates). Cytotoxicity, as measured by decreased β -galactosidase activity was observed at 33 μ M and 100 μ M for triclosan. Data are depicted as fold induction over vehicle (0.05% DMSO) controls \pm S.E.M. (A) ToxCast chemicals were tested in 3-fold serial dilutions from 100 μ M through 0.137 μ M, with the final data point being 0.05% DMSO. Rosiglitazone (A) and AGN1934204 (B) serve as a positive control activators and were tested in 10-fold serial dilutions.

Figure S8. Linear regression analysis of ToxCast Phase I and Phase II assays on the same endpoint (same chemical/assay pair).

Table S1. 16 assays were used to construct the adipogenic ToxPi models shown in Figure 3.

16 assays from Attagene, NovaScreen, and NCGC were incorporated into ToxPi models (Figure 3, Supplemental Material, Table S3). These 16 assays were chosen because they were relevant to the biological process of adipogenesis. All information provided in this table is derived from ToxCast_Phase_1_Assays_20110110.txt at

http://epa.gov/ncct/toxcast/data_archive.html (2011 release; Knudsen et al. 2011).

ATG_RXR α _TRANS, ATG_GR_TRANS, ATG_GRE_CIS, and NCGC_GR_Agonist assays showed no activation by any of the 320 ToxCast chemicals. The leftmost column indicates the assay or collection of assays contributing to a particular slice. For example, the PPAR γ slice in Figure 3A is comprised of three assays: ATG_PPAR γ _TRANS, NCGC_PPAR γ _Agonist, and NVS_NR_hPPAR γ .

Table S2. List of top scoring chemicals in ToxCast PPAR γ assays. AC₅₀ values (μ M) from 2 PPAR γ agonist assays (Attagene-ATG, NCGC), 1 PPAR γ direct binding assay (Novascreen-NVS), and 1 PPAR γ antagonist assay (NCGC/Tox21) are shown here, ranked on the Attagene assay -- 2011 release (Knudsen et al. 2011). GSID = DSSTox chemical identifier; CASRN = CAS Registry Number; NA = not active.

Table S3. 24 top, medium, low (zero/negative) scoring chemicals obtained by ToxPi analysis. Data directly correspond to ToxPi diagrams shown in Figure 3. Assay descriptions are available in Supplemental Material, Table S1. AC₅₀ values are expressed in μ M. Inactive chemicals were not active in any of the ToxCast assays for adipogenesis, feeding behavior, islet cell function, or insulin sensitivity. ATGC = Attagene cis-FactorialTM assay; ATGT = Attagene trans-FactorialTM assay; NCGC = GeneBLAzer agonist assay; NVS = Novascreen direct binding assay; NA = not active.

Table S4. Primer sequences

Table S5. Re-evaluation of PPAR γ activators using Phase II data for three ToxCast PPAR γ assays. AC₅₀ values (μ M) and Z-scores from ToxCast 2014 release (Filer et al. 2014) is shown. (A) The Phase I chemical library was ranked based solely on AC₅₀ values (μ M) of the Attagene and Tox21/NCGC PPAR γ assays and NovaScreen PPAR γ direct binding assay. (B) Chemicals were first filtered by removing those with cytotoxicity Z-scores less than 3 (U.S. EPA 2014) and then ranking them based on their Z-score + negative log (AC₅₀). NA = not active.

Table S6. Continuation of Table 1: Comparison of results in Figures 1 and 2 versus ToxCast assay data. Column 1 is a list of chemicals used in our PPAR γ activation/antagonism assays and adipogenesis assays. AC₅₀ values (μ M) from ToxCast 2011 (Knudsen et al. 2011) and 2014 (Filer et al. 2014) releases are shown. ATG = Attagene FactorialTM PPAR γ agonist assay; NVS = NovaScreen PPAR γ direct binding assay; NCGC = GeneBLAzer PPAR γ agonist or antagonist assay; NA = not active.

Table S7. Continuation of Table 2: Comparison of results in Figures 3-7 versus ToxCast Phase I assay data. Column 1 is a list of the ToxPi chemicals (Figure 3) used in PPAR γ or RXR α activation assays (Figure 4) and adipogenesis assays (Figures 5-7). AC₅₀ values (μ M) from ToxCast 2011 (Knudsen et al. 2011) and 2014 (Filer et al. 2014) releases are shown. ATG = Attagene FactorialTM agonist assay; NVS = NovaScreen direct binding assay; NCGC = GeneBLAzer agonist or antagonist assay; NA = not active.

References