Supplemental Material

A Data-Driven Transcriptional Taxonomy of Adipogenic Chemicals to Identify White and Brite Adipogens

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| Chemical Name | Abbr. | CAS #   | Supplier                  | Catalog # | Max. Conc. Tested [M] | Max. Non-Toxic Conc. [M]* | PPARγ Ligand or Modifier | Purity | Reference                                |
|---------------|-------|---------|---------------------------|-----------|-----------------------|---------------------------|--------------------------|--------|------------------------------------------|
| 15-deoxy-Δ12,14-prostaglandin J2 | 15dPGJ | 87893-55-8 | Cayman Chemical Ultra Scientific | 18570 | 1x10-6                      | 1x10-6                    | Yes PPARγ ligand | > 95% | (Forman et al. 1995)                     |
| 2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl | PCB153 | 35065-27-1 | Tetrabromobenzotriazole | RPC-047 | 1x10-5                      | 1x10-5                    | No evidence               | NA*    | ---                                      |
| 2,2',5,5'-Diphenyltrichloroethane | PCB52  | 35693-99-3 | Sigma Aldrich             | 35599 | 1x10-5                      | 1x10-5                    | No evidence               | > 98% | ---                                      |
| 2,4,6-Tris(tert-butyl)phenol | TTBP   | 732-26-3 | Sigma Aldrich             | T49409 | 2x10-5                      | 2x10-5                    | Potential               | 98%   | (Auerbach et al. 2016)                    |
| 2-ethylhexanol | EthHex | 104-76-7 | Sigma Aldrich             | W315109 | 1x10-5                      | 1x10-5                    | No evidence               | > 99% | ---                                      |
| 3,3',4,4',5-Pentachloro-1,1'-biphenyl | PCB126 | 57465-28-8 | Ultra Scientific          | RPC-102 | 1x10-8                      | 1x10-8                    | No AhR<sup>b</sup> ligand, Reduces adipogenesis | NA     | (Gadupudi et al. 2015)                    |
| 3,3',5,5'-Tetrabromobisphenol A | TBBPA  | 79-94-7 | Sigma Aldrich             | 330396 | 1x10-5                      | 2x10-5                    | Yes PPARγ ligand | 97%   | (Riu et al. 2016)                        |
| 4,4'-Dichlorodiphenyldichloroethylene | DDE    | 72-55-9 | Sigma Aldrich             | 48679  | 1x10-5                      | 1x10-5                    | No ER ligand              | NA     | (Kim et al. 2016)                        |
| 4,4',5,6,7-Tetrabromobenzotriazol | DDT    | 50-29-3 | Sigma Aldrich             | 40124  | 1x10-5                      | 1x10-5                    | No ER ligand              | NA     | ---                                      |
| 9-cis-retinoic acid | 9cRA  | 5300-03-8 | Sigma Aldrich             | R4643  | 1x10-6                      | 1x10-6                    | Yes Activates PPARγ through RXR | > 98% | (Szeles et al. 2010)                     |
| All-trans retinoic acid | ATRA  | 302-79-4 | Sigma Aldrich             | R2625  | 2x10-6                      | 2x10-6                    | No RAR ligand, Reduces adipogenesis | > 98% | (Schwarz et al. 1997)                    |
| Benzyl butyl phthalate | BBzP  | 85-68-7 | Sigma Aldrich             | 36927  | 1x10-5                      | 1x10-5                    | Induces PPARγ target genes and 3T3 L1 adipogenesis | 98%   | (Yin et al. 2016)                        |
| Bisphenol A | BPA   | 80-05-7 | Sigma Aldrich             | 239658 | 1x10-5                      | 1x10-5                    | No ER ligand              | > 99% | (Molina-Molina et al., 2013)              |
| Bisphenol A diglycidyl ether | BADGE | 1675-54-3 | Sigma Aldrich             | D3415  | 1x10-5                      | 1x10-5                    | Yes Induces PPARγ target genes and 3T3 L1 and | NA     | (Chamorro-Garcia et al. 2012)            |
| Compound                        | Formulation | Source              | Catalog Number | Concentration | Adipogenesis | ER Ligand | PPARγ Ligand | β3 Agonist | GR Ligand | Potential Adipogenesis | Reference                        |
|--------------------------------|-------------|---------------------|----------------|---------------|--------------|-----------|--------------|------------|-----------|------------------------|----------------------------------|
| Bisphenol S                    | BPS         | Sigma Aldrich       | 43034          | 1x10-5        | 1x10-5       | No        | Yes          | No         |          | No                     | (Molina-Molina et al., 2013)     |
| Candesartan                    | Cande       | Sigma Aldrich       | SML0245        | 2x10-5        | 4x10-6       | Yes       |              | No         |          | Yes                    | (Erbe et al. 2006)               |
| CL 316,243                     | CL316       | Sigma Aldrich       | C5976          | 5x10-6        | 5x10-6       | Yes       |              | No         |          | Yes                    | (Erbe et al. 2006)               |
| Corticosterone                 | Corti       | Sigma Aldrich       | 27840          | 2x10-6        | 2x10-6       | Yes       |              | No         |          | Yes                    | (Auerbach et al. 2016)           |
| Cyazofamid                     | Cyazo       | Sigma Aldrich       | 33874          | 4x10-5        | 2x10-5       | Potential |              | NA         |          | No                     | (Auerbach et al. 2016)           |
| d-cis,trans-Allethrin          | Allet       | Sigma Aldrich       | 33396          | 2x10-5        | 1x10-5       | Potential |              | NA         |          | No                     | (Auerbach et al. 2016)           |
| Dexamethasone                  | Dex-SP      | Sigma Aldrich       | D1159          | 2x10-7        | 2x10-7       | No        |              | No         |          | No                     | (Feige et al. 2007)              |
| Di(2-ethylhexyl) phthalate     | DEHP        | Sigma Aldrich       | 36735          | 1x10-5        | 1x10-5       | Yes       |              | No         |          | Yes                    | Hiromori et al., 2009            |
| Dibutyltin                     | DBT         | Sigma Aldrich       | 205494         | 2x10-7        | 2x10-7       | Yes       |              | No         |          | Yes                    | (Zhang et al., 2019)             |
| Diisononyl phthalate           | DINP        | Sigma Aldrich       | 376663         | 1*10-5        | 1*10-5       | Yes       |              | No         |          | Yes                    | (Temkin et al. 2016)             |
| Dioctyl sulfosuccinate sodium  | DOSS        | Sigma Aldrich       | 323586         | 5x10-6        | 5x10-6       | Potential |              | No         |          | Yes                    | (Cano-Sancho et al. 2017)        |
| Diphenyl phosphate             | DiPhPho     | Sigma Aldrich       | 850608         | 1x10-5        | 1x10-5       | Potential |              | No         |          | Yes                    | (Auerbach et al. 2016)           |
| Ethylene brassylate            | EtBra       | Sigma Aldrich       | W354309        | 1x10-5        | 1x10-5       | No        |              | No         |          | Yes                    | (Auerbach et al. 2016)           |
| Fenthion                       | Fenth       | Sigma Aldrich       | 36552          | 4x10-5        | 4x10-5       | Potential |              | No         |          | Yes                    | (Auerbach et al. 2016)           |
| Firemaster 550                 | FM550       | Gift from Heather Stapleton, Duke | 10 ug/ml | 10 ug/ml |               | Yes       |              |            |          | Yes                    | (Pillai et al. 2014)             |
| Fludioxonil                    | Fludi       | Sigma Aldrich       | 46102          | 2x10-5        | 2x10-6       | Potential |              |            |          | Yes                    | (Auerbach et al. 2016)           |
| Honokiol                       | Honok       | Sigma Aldrich       | H4914          | 2x10-5        | 4x10-6       | Yes       |              | No         |          | Yes                    | (Atanasov et al. 2013)           |
| LG100268                       | LG268       | Sigma Aldrich       | SML0279        | 1x10-7        | 1x10-7       | Activates PPARγ through RXR |            |          | Yes                    | (Cesario et al. 2001)           |
| LG100754                       | LG754       | Tocris              | 3831           | 2x10-7        | 2x10-7       | Yes       |              |            |          | Yes                    | (Cesario et al. 2001)           |
| Name                                           | Abbreviation | CAS Number     | Supplier       | IC50 (M)    | Effect                        | Notes                                      |
|------------------------------------------------|--------------|----------------|----------------|-------------|-------------------------------|--------------------------------------------|
| Magnolol                                       | Magno        | 528-43-8       | Sigma Aldrich  | M3445       | 2x10-5 2x10-5                 | Activates PPARγ through RXR               |
| MCC-555                                        | MCC555       | 161600-01-7    | Sigma Aldrich  | SML0896     | 5x10-6 5x10-6                 | Yes PPARγ ligand                          |
| Melengestrol acetate                           | Melen        | 2919-66-6      | Sigma Aldrich  | 73248       | 2x10-5 2x10-5                 | Yes PPARγ ligand                          |
| Mono-(2-ethyhexyl) tetrabromophthalate         | METBP        | Synthesized by Asis Chemical |           | 1x10-5 1x10-5 | Activates PPARγ reporter and induces adipogenesis in multipotent stromal cells | 95% (Fakhurudin et al., 2010) |
| Mono(2-ethylhexyl) phthalate                   | MEHP         | 4376-20-9      | Sigma Aldrich  | CDS01060 8  | 1x10-5 1x10-5                 | Yes PPARγ ligand                          |
| Monobenzyl phthalate                          | MBzP         | 2528-16-7      | Sigma Aldrich  | 89505       | 1x10-5 1x10-5                 | Yes PPARγ reporter                        |
| Mono-n-butyl phthalate                        | MBuP         | 131-70-4       | Sigma Aldrich  | 30751       | 2x10-5 2x10-5                 | Yes PPARγ reporter and induces 3T3 L1 adipogenesis | 95% (Watt and Schlezinger 2015) |
| n-Butylparaben                                 | BuPara       | 94-26-8        | Sigma Aldrich  | 54680       | 2x10-5 2x10-5                 | Activates PPARγ reporter and induces 3T3 L1 adipogenesis | > 98% (Hurst and Waxman 2003) |
| N-nitro-2-imidazolidinimine                   | Imida        | 138261-41-3    | Sigma Aldrich  | 37894       | 1x10-5 1x10-5                 | No evidence                               |
| nTZDpa                                         | nTZDpa       | 118414-59-8    | Sigma Aldrich  | SML0616     | 1x10-6 1x10-6                 | Yes PPARγ ligand                          |
| Perfluorooctanesulfonic acid                   | PFOS         | 2795-39-3      | Sigma Aldrich  | 77282       | 4x10-5 4x10-5                 | Potential NA                              |
| Perfluorooctanoic acid                         | PFOA         | 335-67-1       | Sigma Aldrich  | 33824       | 1x10-5 1x10-5                 | Potential                                 |
| Pioglitazone hydrochloride                     | Piogl        | 112529-15-4    | Sigma Aldrich  | E6910       | 1x10-5 1x10-5                 | Yes PPARγ ligand                          |
| Prallethin                                     | Prall        | 23031-36-9     | Sigma Aldrich  | 32917       | 1x10-5 1x10-5                 | Potential                                 |
| Pregnenolone 16α-carbonitrile                  | Pregn        | 1434-54-4      | Sigma Aldrich  | P0543       | 1x10-5 1x10-5                 | No PXR ligand                             |
| Propylparaben                                  | ProPara      | 94-13-3        | Sigma Aldrich  | P53357      | 1x10-5 1x10-5                 | Activates PPARγ reporter and              |

(Activates PPARγ reporter and induces 3T3 L1 adipogenesis)
| Chemical             | Chemical Source   | CAS No. | T0070907 (ppm) | T0901317 (ppm) | T1317 (ppm) | Tungs (ppm) | Resolvin-E1 (ppm) | TBT (ppm) | Protectin D1 | Quinoxyfen | Resolvin | Rosiglitazone | Sodium tungstate | Sodium arsenite | SR1664 | Telmisartan | Tonalide | Tributyl phosphate | Tributyltin |
|----------------------|-------------------|---------|----------------|---------------|-------------|-------------|-------------------|-----------|---------------|------------|----------|---------------|-----------------|----------------|---------|-------------|----------|-------------------|-------------|
|                      |                   |         |                |               |             |             |                   |           |               |            |          |               |                 |                |         |             |          |                   |             |
| Tributyl phosphate   | Aldrich           | 126-73-8| 2x10^-5        | 2x10^-5       | 2x10^-5     | 8x10^-8     | 8x10^-8          | PPARγ/RXR ligand | Yes          | Quinoxyfen | 124495-18-7 | Sodium tungstate | 7784-46-5     | 122320-73-4 | S26948 | T0901317    | T0070907 |                    |             |
|                      |                   |         |                |               |             |             |                   |             |               |            |          |               |                 |                |         |             |          |                   |             |
| Sodium tungstate     | Aldrich           | 10213-10-2 | 2x10^-5       | 2x10^-5       | 2x10^-5     | 2x10^-5     | 4x10^-5          | 8x10^-6    | Yes          |            |          |               | Sodium arsenite | Tungs (ppm)    | SR1664 | T0901317    | T0070907 | Tributyl phosphate |             |
| Sodium arsenite      | Aldrich           | 7784-46-5 | 2x10^-5        | 2x10^-5       | 2x10^-5     | 2x10^-5     | 2x10^-5          | 2x10^-5    | No           |            |          |               |                                          |                |
| SR1664               | Aldrich           | 1338259-05-4 | 1x10^-6       | 1x10^-6       | 1x10^-6     | 1x10^-6     | 1x10^-6          | 1x10^-6    | Yes          |            |          |               |                                          |                |
| T0901317             | Aldrich           | 293754-55-9 | 1x10^-6       | 1x10^-6       | 1x10^-6     | 2x10^-6     | 2x10^-6          | 2x10^-6    | No           |            |          |               |                                          |                |
| T0070907             | Aldrich           | 313516-66-4 | 2x10^-5       | 2x10^-5       | 2x10^-5     | 2x10^-5     | 2x10^-5          | 8x10^-6    | Yes          |            |          |               |                                          |                |
| Tebuconazole         | Aldrich           | 107534-96-3 | 2x10^-5       | 2x10^-5       | 2x10^-5     | 2x10^-5     | 8x10^-6          | 8x10^-6    | No           |            |          |               |                                          |                |
| Telmisartan          | Aldrich           | 144701-48-4 | 2x10^-5       | 2x10^-5       | 2x10^-5     | 2x10^-5     | 2x10^-5          | 2x10^-5    | Yes          |            |          |               |                                          |                |
| Tesaglitazar         | Aldrich           | 251565-85-2 | 5x10^-6       | 5x10^-6       | 5x10^-6     | 5x10^-6     | 5x10^-6          | 5x10^-6    | Yes          |            |          |               |                                          |                |
| Tolyfluanid          | Aldrich           | 731-27-1   | 2x10^-7        | 2x10^-7       | 2x10^-7     | 2x10^-7     | 2x10^-7          | 2x10^-7    | No           |            |          |               |                                          |                |
| Teralide             | Aldrich           | 21145-77-7   | 4x10^-6        | 4x10^-6       | 4x10^-6     | 4x10^-6     | 4x10^-6          | 4x10^-6    | Potential    |            |          |               |                                          |                |
| Tributyl phosphate   | Aldrich           | 126-73-8   | 2x10^-5        | 2x10^-5       | 2x10^-5     | 2x10^-5     | 2x10^-5          | 2x10^-5    | Yes          |            |          |               |                                          |                |
| Tributyltin          | Aldrich           | 1461-22-9   | 8x10^-8        | 8x10^-8       | 8x10^-8     | 8x10^-8     | 8x10^-8          | PPARγ/RXR ligand | 96%          |                         |            |          |               |                                          |                |
| Chemical Name | CAS Number | Supplier | Sigma Aldrich | Concentration | Maximal Concentration | PPARγ Activity |
|---------------|------------|----------|---------------|---------------|-----------------------|----------------|
| Triflumizole  | 68694-11-1 | Sigma Aldrich | 32611 | 2x10-5 | 2x10-5 | Yes Activates PPARγ reporter and induces 3T3 L1 adipogenesis |
| Triphenyl phosphate | TPhP | Sigma Aldrich | 241288 | 2x10-5 | 1x10-5 | Yes PPARγ ligand | > 99% |
| Triphenyl phosphate | TPhPhi | Sigma Aldrich | T84654 | 1x10-5 | 1x10-5 | Potential | 97% |
| Triphenylphosphine oxide | TPhPhOx | Sigma Aldrich | T84603 | 1x10-5 | 1x10-5 | Potential | 98% |
| Triphenyltin | TPhT | Sigma Aldrich | 245712 | 8x10-8 | 8x10-8 | Yes PPARγ/RXR ligand | 98% |
| Tris(1,3-dichloro-2-propyl) phosphate | TDCPP | Sigma Aldrich | 32951 | 2x10-5 | 2x10-5 | Potential | NA |
| Tris(1-chloro-2-propyl) phosphate | TCCP | Sigma Aldrich | 32952 | 1x10-5 | 1x10-5 | Potential | NA |
| Troglitazone | Trogl | Sigma Aldrich | T2573 | 5x10-6 | 5x10-6 | Yes PPARγ ligand | > 98% |

*NA = Not Available

a Toxicity was assessed by microscopic inspection. The concentration was reduced by two fold until a non-toxic, maximal concentration was identified. Maximal concentrations were determined in an N=4.

b “Yes” indicates there is experimental evidence of modification of PPARγ activity, including PPARγ binding assays, coactivator recruitment or computational modeling (definitive ligands), PPARγ-driven reporter assays (at least 25% of the rosiglitazone-induced maximum), expression of PPARγ target genes and/or differentiation of 3T3 L1 or multipotent stromal cells into adipocytes in the absence of a known PPARγ ligand. Chemicals that changed the expression of PPARγ (e.g., PCB126 and DDE) were not considered to be ligands or modifiers. “No” indicates that the chemical was chosen based on the fact that it is known to be a specific ligand of another receptor. “No evidence” indicates that this chemical has not been tested for PPARγ activation but is structurally dissimilar to known classes of PPARγ ligands. “Potential” indicates that the chemical was identified in other screening approaches or by the ToxPi designed to identify chemicals in the ToxCast dataset that have potential to be PPARγ ligands/modifiers.

c Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor
**Table S2. Mouse (M) and human (H) primer sequences for reverse transcription qPCR.**

| GENE | SYMBOL | FORWARD | REVERSE | ANNEALING TEMP. °C |
|------|--------|---------|---------|-------------------|
| M-Rn18s | GTAACCGTTGAACCCCATATT | CCATCCACATCGTAGTCG | 55 |
| M-B2m | CTGCTACGTAACAGTTCCACC | CATGATGCTGATCATGTCGG | 55 |
| M-Cidec | AGGCCCTGTCGTGTAACGTCA | CAGTAGCTGCGACCTACT | 55 |
| M-Cidea | TGCTCTCTCTGTATCGCCCAGT | GCCGTGAGAATCTCAGTC | 55 |
| M-Elovl3 | TCGCGTTCTCATGAGTCTT | GACCTGATGCAACCCTATGA | 55 |
| M-Fabp4 | AGGCGCACCACATGACATCAG | TTTCATACCACTTCAGAC | 55 |
| M-Plin1 | GGGACCTGATGAGTCTTCC | GTATTGAAGGCGGATCTTTE | 55 |
| M-Pgc1a | AACAAGCAGCTGGTCGTACCTC | TTACTGAAGGTGCACATT | 55 |
| M-Pperg2 | TGGGTGAAACTCTTGGAGATTC | AATTTCTTGTGAAGTGTCA | 55 |
| M-Rip140 | AGAACGCACACAGGTGCACT | GCAGTAGCTGAGAACCTT | 55 |
| M-Adipoq | GCCGCTGCTGCCCACCATCAG | TTTCATACCACTTCAGAC | 55 |
| M-Ucp1 | ACTGCGCACTCCCTCCATT | CTTTCATACCACTTCAGAC | 55 |
| M-Aca2 | TAACGGGCTGCGCTACTTCA | AGGGGATGAACTTTGCTT | 55 |
| H-RPL27 | GTGAAATGTCCTGATACATCACC | TCAACTGACTTGACCGCC | 58 |
| H-B2M | GCTATCCAGCCTAGCTCAGA | CACAGGCAGCCAGCTACT | 58 |
| H-CIDEA | GGGATAAGGCTGGTCCTGATT | TCAATTCCCTTGCCAGGGTT | 55 |
| H-CIDEA | GGGACCTGTTAAGGTCTTCC | GTATTGAAGGCCGGAATCTTTE | 55 |

**Table S3. Metabolic parameters included and excluded in human transcriptome analysis.**

| PARAMETERS INCLUDED | PARAMETERS EXCLUDED |
|---------------------|---------------------|
| Fat free mass %     | Body mass index (kg/m2) |
| Fasting Plasma parameters | Waist-to-hip ratio |
| Free fatty acid (mmol/l) | Waist circumference (cm) |
| Total triglycerides (mmol/l) | Hip circumference (cm) |
| LDL cholesterol (mmol/l) | Plasma total fatty acids (mmol/l) |
| HDL cholesterol (mmol/l) | Plasma total cholesterol (mmol/l) |
| Adiponectin (ug/ml) | Matsuda composite insulin sensitivity index |
| Glucose (mmol/l) | HOMA-IR |
| Insulin (mU/l) | Systolic blood pressure (mm Hg) |
| Proinsulin (pmol/l) | Diastolic blood pressure (mm Hg) |
| Glycated HbA1c (%) | Glomerular filtration rate |
| High sensitivity C-reactive protein (mg/l) | |
| Interleukin-1 receptor antagonist (pg/ml) | |
Excel File 1. Detailed results from random forest classification.

Excel File 2. Detailed annotation of clustering results for individual modules.

Excel File 3. Supporting numerical data from all cell culture experiments.

Excel File 4. Detailed results of partial correlation analysis of clinical measurements and projections of chemical taxonomy gene signatures onto human adipose gene expression.
Figure S1. Differentiation and dosing protocols for 3T3-L1 cells (A) and primary human preadipocytes (B). DM, Differentiation medium; MM, Maintenance medium; AM, Adipocyte medium.
Figure S2. Correlation of lipid accumulation with Cidec, Fabp4, and Plin1 expression in differentiated and treated 3T3-L1 pre-adipocytes. Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail for 10 days. During differentiation, cells were treated with vehicle (Vh, 0.1% DMSO, final concentration) or test chemical (Table S1). On days 3, 5, and 7 of differentiation, the medium was replaced and the cultures re-dosed. Following 10 days of differentiation and dosing, cells were analyzed for lipid accumulation by Nile Red staining (Data are from Figure 1) and gene expression by 3’DGE. Each point represents the mean data for each chemical, (n=2-4). The least squares linear model estimate is shown in blue.
Figure S3. Lipid accumulation in differentiated and treated 3T3-L1 pre-adipocytes in the absence of dexamethasone.

Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail for 10 days, with the exception of using no dexamethasone. During differentiation, cells were treated with vehicle (Vh, 0.2% DMSO, final concentration), rosiglitazone (positive control, 100 nM) or test chemical (Table S1). On days 3, 5, and 7 of differentiation, the medium was replaced and the cultures re-dosed. Following 10 days of differentiation and dosing, cells were analyzed for lipid accumulation by Nile Red staining. A) Nile Red
staining induced by individual chemicals. Nile Red fluorescence was normalized by subtracting the fluorescence measured in naïve pre-adipocyte cultures within each experiment and reported as “Naïve Corrected RFU.” Data are presented as mean ± SE (n=4). Statistically different from Vh-treated (highlighted in green) (*p<0.05, ANOVA, Dunnett’s).

**B)** Correlation between lipid accumulation induced in 3T3 L1 cells differentiated in the presence (data are from Figure 1) and absence of dexamethasone. Numerical data are provided in **Excel File 3**. Pearson’s r = 0.5429 (p<0.0001).
Figure S4. Lipid accumulation in differentiated and treated OP9 pre-adipocytes.

Confluent OP9 cells were differentiated using a standard hormone cocktail for 10 days, with the exception of using 125 nM dexamethasone. During differentiation, cells were treated with vehicle (Vh, 0.2% DMSO, final concentration), rosiglitazone (positive
control, 100 nM) or test chemical (Table S1). On days 3, 5, and 7 of differentiation, the medium was replaced and the cultures re-dosed. Following 10 days of differentiation and dosing, cells were analyzed for lipid accumulation by Nile Red staining. **A)** Nile Red staining induced by individual chemicals. Nile Red fluorescence was normalized by subtracting the fluorescence measured in naïve pre-adipocyte cultures within each experiment and reported as “Naïve Corrected RFU.” Data are presented as mean ± SE (n=4). Statistically different from Vh-treated (highlighted in green) (*p<0.05, ANOVA, Dunnett’s).  

**B)** Correlation between lipid accumulation induced in 3T3 L1 cells differentiated in the presence of dexamethasone (data are from Figure 1) and in OP9 cells. Numerical data are provided in **Excel File 3**. Pearson’s r = 0.5768 (p<0.0001).
Figure S5. Performance comparison of random forest methods.
Boxplots of performance estimates for repeated 10-fold cross validation for each of the four random forest methods considered for classifying PPARγ activity modifying compounds from high-throughput gene expression profiles of chemically treated 3T3 L1 cells. Abbreviated metrics shown include: area-under the curve (AUC), balanced accuracy (Bal. Acc.), F1-score (F1). Besides AUC, for all performance metrics besides, an appropriate classification threshold for predicting PPARγ activity modifying compound labels in each test set was estimated based on out-of-bag voting of their corresponding training set. Sensitivity and specificity are the proportions of identified known PPARγ activity modifying compounds and known non-PPARγ activity modifying compounds, respectively, out of the total number of each label in the data. Bal. Acc. is the mean of sensitivity and specificity. Precision is the proportion of accurately identified known PPARγ activity modifying compounds out of all predicted PPARγ activity modifying compounds. F1 is the harmonic mean of sensitivity and precision. Finally, AUC is the integral of sensitivity and specificity across every possible classification threshold. The distribution of each method/metric combination is based on 10 repetitions of cross validation (i.e. N=10). The full set of performance estimates for each repetition, performance metric, and classification procedure considered are shown in Excel File 1 (Performance Comparison). The midline, box limits, and whiskers show the median, upper/lower quartile, and minimum/maximum of each distribution cutoff at a distance of 1.5 * the interquartile range from their closest box limit, respectively. Individual points indicate values which extend beyond the whisker limits. The horizontal gap in each plot indicates that, while the range of possible estimates for each performance metric is between 0.0 and 1.0, all estimates fell between 0.45 and 1.0.
Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail for 10 days. During differentiation, cells were treated with 0.1% DMSO, final concentration (vehicle), test chemical, or were untreated.
naive). On days 3, 5, and 7 of differentiation, the medium was replaced and the cultures re-dosed. Following 10 days of differentiation and dosing, cells were analyzed for gene expression by 3’DGE. The labels, “Yes”, “No”, and “Potential”, indicate test chemicals predetermined to be known PPARγ activity modifying compounds, known non-PPARγ activity modifying compounds, and Potential PPARγ activity modifying compounds, respectively, based on previous studies (See Table S1). *Rpl13* and *Cidec* demonstrated the greatest predictive value for classifying PPARγ activity modifying compounds based on their Gini importance estimates from random forest modeling (Breiman 2001). Each point indicates sample-specific expression values, normalized by batch correction and Trimmed Mean of M-values (TMM) transformation, performed in R (v 3.4.3) using *ComBat* (v 3.26.0) (Leek et al. 2012) and *limma* (v 3.34.9) (Ritchie et al. 2015), respectively. The range and mean expression of the biological replicates of each treatment is indicated by a vertical and short horizontal line, respectively. Mean expression across all vehicle samples is shown as a horizontal line spanning the plot. Exposures which have statistically significant different means from vehicle (F statistic, FDR Q-value < 0.05) are highlighted with an asterisk. For test chemicals sample sizes range from N=2-4. For vehicle and naive sample sizes are N=25 and N=15, respectively.
Figure S7. Cell number analyses in the differentiated and treated 3T3-L1s. Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail with 1 nM dexamethasone (A) and 250 nM dexamethasone (B) for 10 days. During differentiation, cells were treated with Vh (0.1% DMSO, final concentration), rosiglitazone (Rosig, 200 nM (A), 1 μM (B)), roscovitine (Rosco, 4 μM), 15dPGJ2 (500 nM (A), 1 μM (B)), TBBPA (20 μM) and TPhP (10 μM). On days 3, 5, and 7 of differentiation, the adipocyte maintenance medium was replaced and the cultures re-dosed. Cells were incubated for a total of 10 days of differentiation. To assess cell number, cells were stained with Janus green stain. Absorbance in experimental wells was normalized by dividing by the absorbance measured in naïve pre-adipocyte cultures within the experiment and reported as “Relative Cell Density.” Numerical data are provided in Excel File 3. Data are presented as means ± SE (n=8). Statistically different from Vh-treated (**p<0.01, ANOVA, Dunnett’s).
Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail with 250 nM dexamethasone for 10 days. During differentiation, cells were treated with vehicle (Vh, 0.2% DMSO, final concentration), rosiglitazone (Rosig, 200 nM), roscovitine (Rosco, 4 μM), 15dPGJ2 (500 nM), TBBPA (20 μM), TPhP (10 μM) and quinoxyfen (10 μM). On days 3, 5,
and 7 of differentiation, the adipocyte maintenance medium was replaced and the cultures re-dosed. Following 10 days of differentiation and dosing, cells were analyzed for gene expression by RT-qPCR. (A) Genes related to white adipogenesis. (B) Genes related to brite adipogenesis. Gene expression levels were normalized to the geometric mean of the expression levels of $B2m$ and $Rn18s$ and expressed as "Relative Expression" in comparison to naïve, pre-adipocyte cultures using the Pfaffl method. Numerical data are provided in Excel File 3. Data are presented as mean ± SE of n=6 independent experiments. Statistically different from Vh-treated (highlighted in green) (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ANOVA, Dunnett’s).
Figure S9. Spearman correlation analysis of lipid accumulation (Nile Red) and gene expression. Confluent 3T3 L1 cells were differentiated using a standard hormone cocktail with 1 nM dexamethasone for 10 days and analyzed for adipocyte differentiation by staining for lipids with Nile Red (shown in Figures 6 and 11) and gene expression (shown in Figures 7 and 11).
**Figure S10.** Cell number analyses in the differentiated and quinoxyfen and tonalide treated 3T3-L1s and human primary preadipocytes. (A) Confluent 3T3 L1 cells were differentiated using a standard human adipocyte hormone cocktail for 10 days. During differentiation, cells were treated with Vh (0.1% DMSO, final concentration), rosiglitazone (Rosig, 200 nM), quinoxyfen (Quino, 10 μM) or tonalide (Tonal, 4 μM). On days 3, 5, and 7 of differentiation, the adipocyte maintenance medium was replaced and the cultures re-dosed. Cells were incubated for a total of 10 days of differentiation. Data are presented as means ± SE (n=8). (B) Confluent primary human preadipocytes were differentiated using a standard hormone cocktail for 14 days. During differentiation, cells were treated with Vh (0.1% DMSO, final concentration), rosiglitazone (Rosig, 4 μM), quinoxyfen (Quino, 4 μM) or tonalide (4 μM). On days 3, 5, 7, 10, and 12 of differentiation, the medium was replaced and the cultures re-dosed. Following 14 days of differentiation and dosing, cultures were analyzed for relative cell density using JANUS Green staining. Absorbance in experimental wells was normalized by dividing by the absorbance measured in naïve pre-adipocyte cultures within the experiment and reported as “Relative Cell Density.” Data are presented as mean ± SE (n=3, each n is from adipocytes from an individual). Statistically different from Vh-treated (**p<0.01, ANOVA, Dunnett’s).
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