SUPPORTING INFORMATION

Comparison of Approaches for Determining Bioactivity Hits from High-Dimensional Profiling Data

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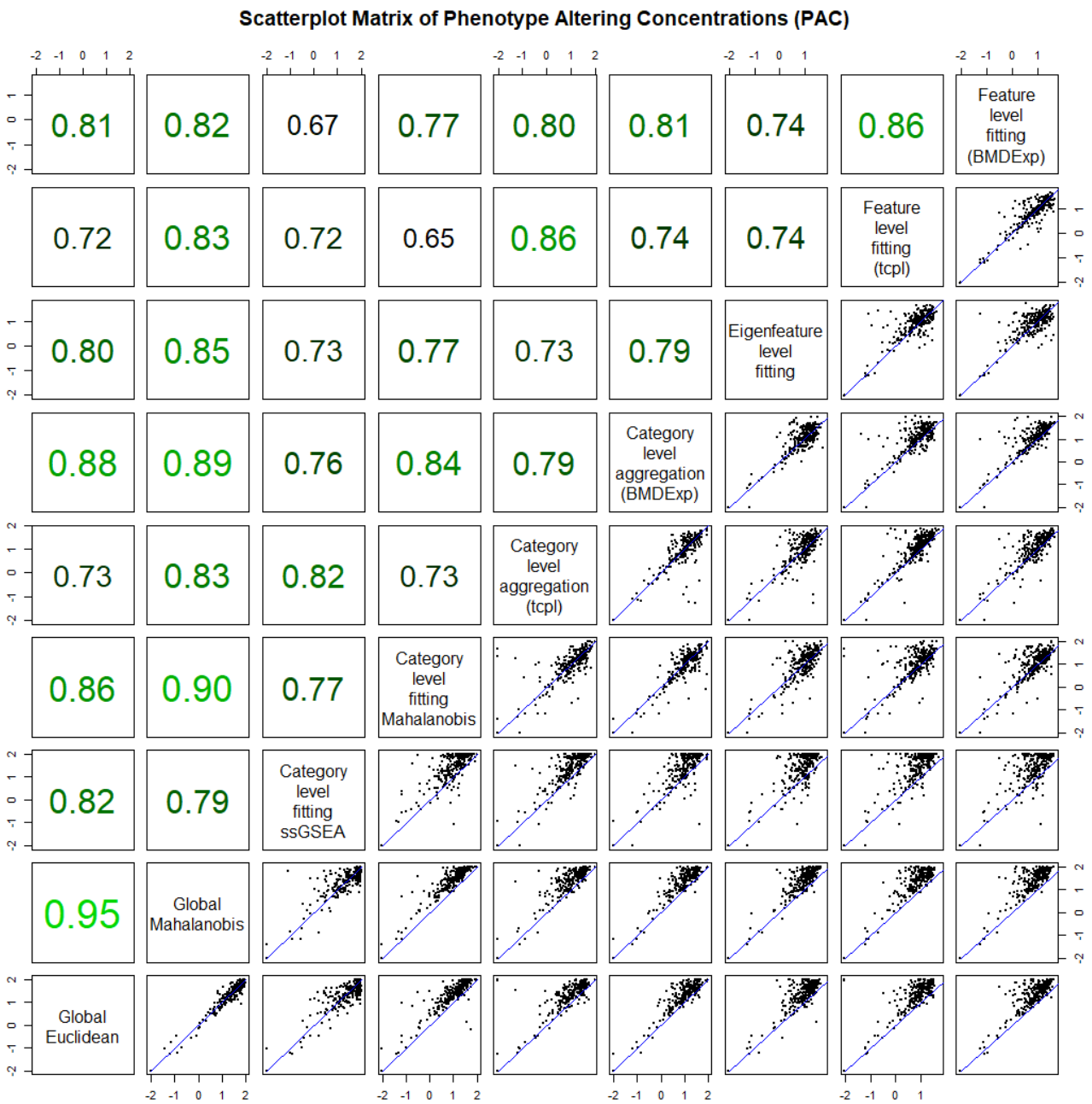
Approach	fixed parameters	tuned parameters	final choice
Feature-level fitting (BMDExp)	• all settings in the BMDExpress software were left unchanged relative to Nyffeler et al. 2020: • BMR = 1 • effect size prefilter • 4 models: Poly1, Poly2, Power, Hill	effect size threshold: 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4	1.75
Feature-level fitting (tcplfit2)	• BMR = 1 • effect size prefilter • 9 models: Poly1, Poly2, Power, Hill, Exp2-Exp5, constant	effect size threshold: 1, 1.2, 1.4, 1.5, 1.6, 1.7, 1.75, 1.8, 2, 2.5, 3	none (=1)
		hit call threshold: 0 - 1	0.95
Category-level aggregation (BMDExp)	• see under Feature-level fitting (BMDExp) • >= 30% of features affected per category	effect size threshold: 1, 1.349, 1.5, 1.6, 1.75, 2	1.75
Category-level aggregation (tcplfit2)	• see under Feature-level fitting (tcplfit2) • >= 30% of features affected per category	effect size threshold: 1, 1.2, 1.4, 1.5, 1.6, 1.7, 1.75, 1.8, 2, 2.5, 3	none (=1)
		hit call threshold: 0 - 1	0.95
Category-level fitting (Mahalanobis distance)	• retain enough principal components to cover > 90% of variance • consider only curves with a positive 'top'	BMR: 1, 1.349, 1.5, 2, 3	1
		hit call threshold: 0 - 1	0.8
Category-level fitting (ssGSEA)		normalize scores across test samples and categories: T/F	TRUE
		use rank-order vs effect sizes	use rank-order
		BMR: 1, 1.349, 1.5, 2	1.349
		hit call threshold: 0 - 1	0.5
Global Euclidean distance	• consider only curves with a positive 'top'	BMR: 1, 1.349, 1.5, 2	1
		hit call threshold: 0 - 1	0.2
Global Mahalanobis distance	• retain enough principal components to cover > 95% of variance • consider only curves with a positive 'top'	BMR: 1, 1.349, 1.5, 2	1
		hit call threshold: 0 - 1	0.2
Signal strength overall (F)		signature threshold: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6	1.5
		SS measure: Euclidean norm, Manhattan norm, # of affected features	Euclidean norm
Signal strengh plate-wise (F)		signature threshold: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6	2.25
		SS measure: Euclidean norm, Manhattan norm, # of affected features	Euclidean norm
Profile correlation (F)		signature threshold: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6	1.75
		Correlation measure: Pearson, cosine similarity, Jaccard similarity, Jaccard p-value	Pearson

Signal strength overall (E)	• no signature threshold	SS measure: Euclidean norm, Manhattan norm, # of affected features	Euclidean norm
Signal strength plate-wise (E)	• no signature threshold	SS measure: Euclidean norm, Manhattan norm, # of affected features	Euclidean norm
Profile correlation (E)	• no signature threshold	Correlation measure: Pearson, cosine similarity, Jaccard similarity, Jaccard p-value	cosine similarity

Supporting Information Table S1. List of fixed parameters and tunable parameters for each approach. The last column indicates the final choice of the tunable parameter to the left.

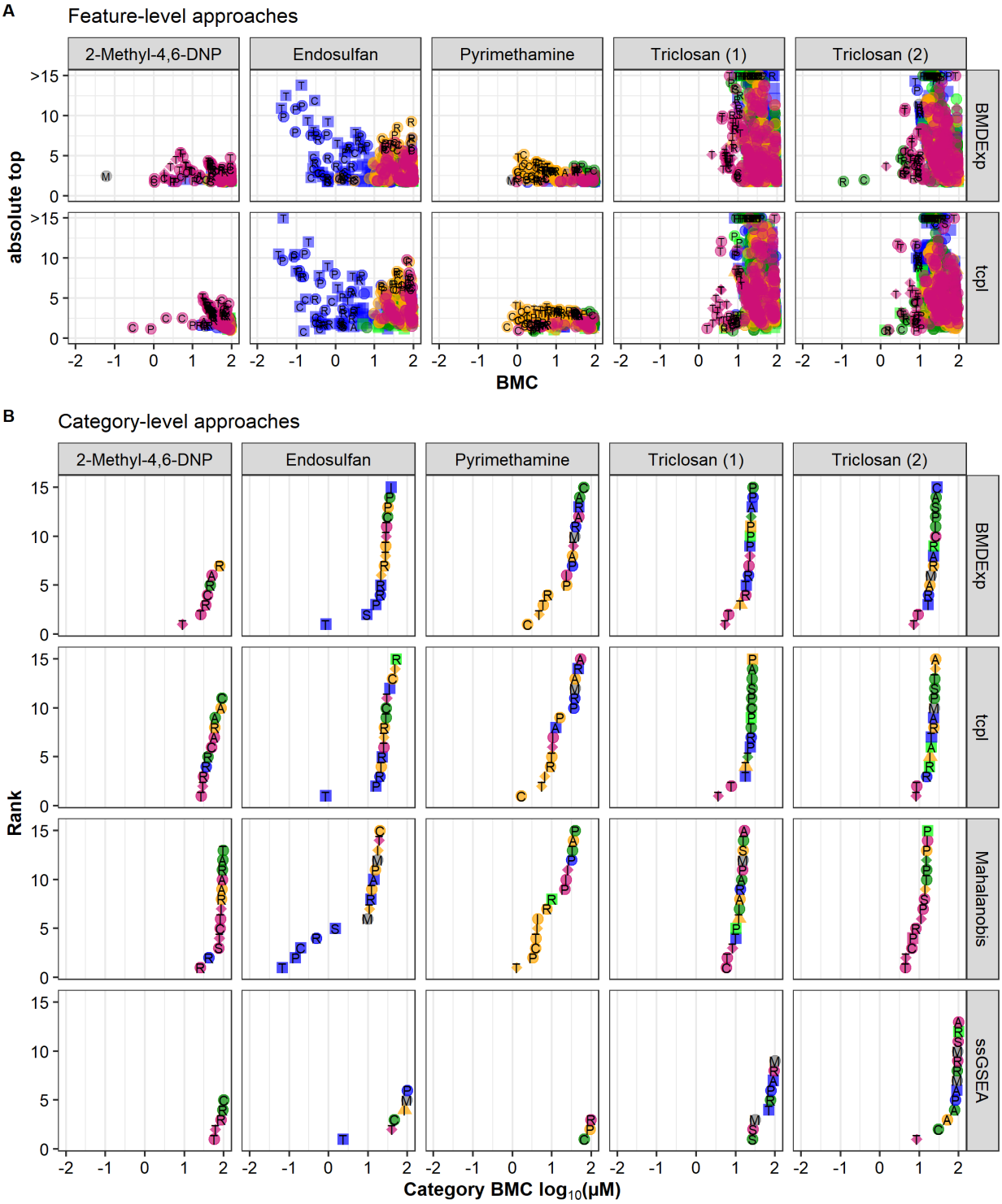
Approach	**Bioactive criteria (hit call)**	**PAC**
Feature-level fitting (BMDExp)	number of valid BMCs > 90th percentile of null chemicals	5th percentile
Feature-level fitting (tcplfit2)		
Category-level aggregation (BMDExp)	≥ 1 affected category	median BMC of most potent category
Category-level aggregation (tcplfit2)		
Category-level fitting (Mahalanobis distance)	≥ 1 affected category	BMC of most potent category
Category-level fitting (ssGSEA)		
Global Euclidean distance	valid BMC	BMC
Global Mahalanobis distance		
Signal strength overall (F)	SS > 90th percentile of null chemicals	-
Signal strength overall (E)		
Signal strengh plate-wise (F)	SS > 90th percentile of null chemicals	
Signal strengh plate-wise (E)		
Profile correlation (F)	Cor > 90th percentile of null chemicals	
Profile correlation (E)		

Supporting Information Table S2. Definition of hit call and PAC for each approach.



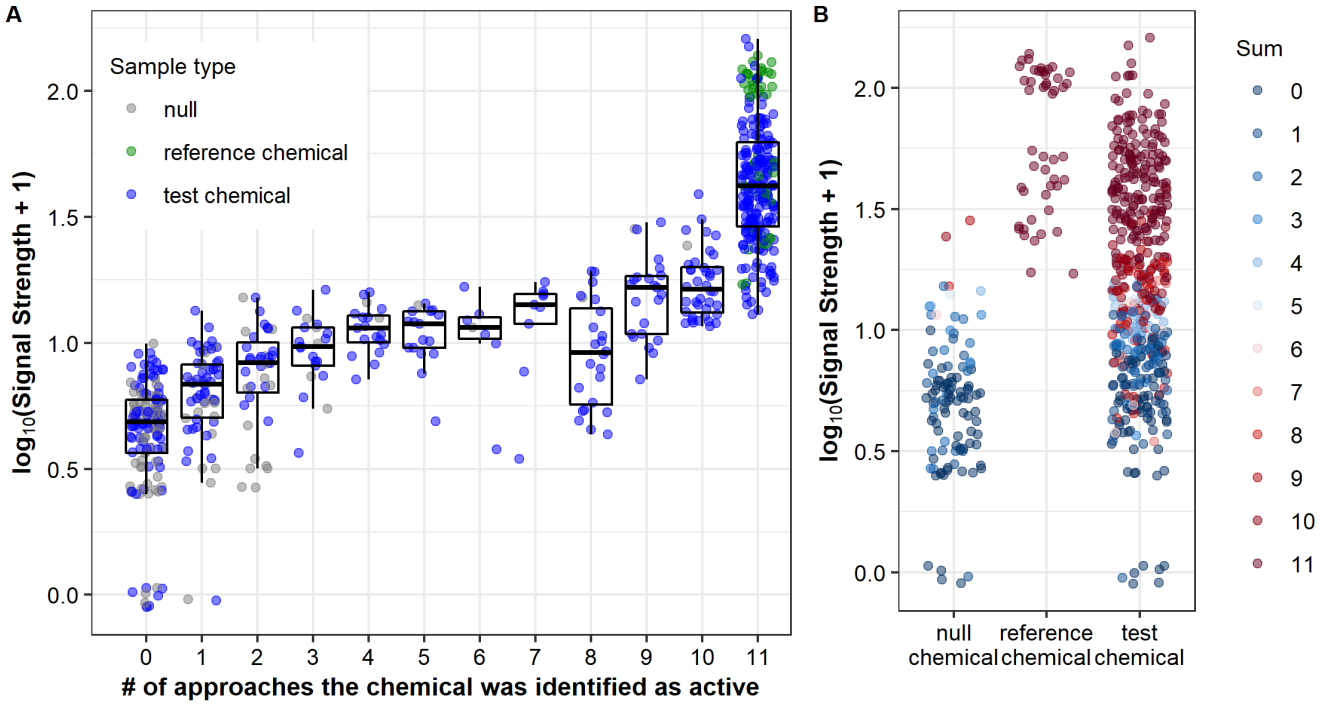
Supporting Information Fig. S1. Pairwise comparison of phenotype altering concentrations for test chemicals (n=475).

For each pair of multi-concentration approaches, PACs are shown for each chemical that was identified as active with both approaches (lower right panels). Concentrations are displayed as $\log_{10}(\mu M)$. The blue line is the identity line. Pearson correlation is illustrated in the upper left panels.

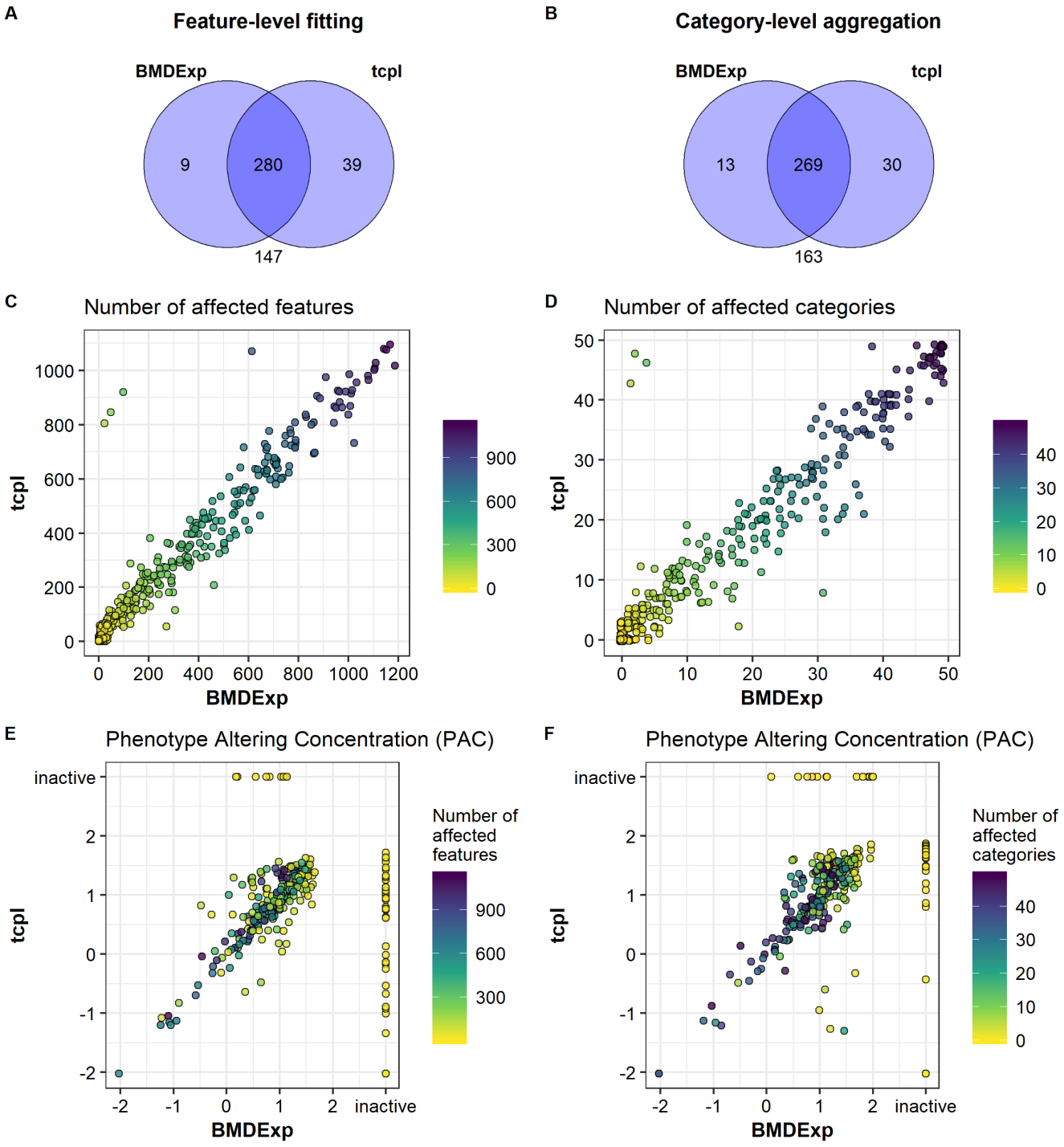


Supporting Information Fig. S2. Comparison of Bioactivity Profiles Across Feature- and Category-Based Approaches for a subset of test chemicals.

(A) Potency-magnitude plots for both feature-level approaches (BMDExpress and *tcplfit2*). For each chemical x feature, the BMC and the absolute effect size (i.e., 'top') is shown. Features are only displayed if they had a BMC. (B) Accumulation plots for all category-based approaches. Category BMCs were ranked by potency. Categories are only displayed if they had a BMC, and 15 categories at maximum. In both (A) and (B), features and categories, respectively, were coded with respect to shape/fluorescent channel (color), feature type (letter) or cellular compartment (shape) as indicated in Figure 5.



Supporting Information Fig. S3. Correlation of signal strength and the number of approaches a chemical was identified as active. The number of approaches a chemical was identified as active was derived from the same 11 approaches as in Figure 3. Signal strength was calculated using a signature threshold of 1.5 and using the Euclidean norm. Signal strength is displayed on a $\log_{10}$ scale.



Supporting Information Fig. S4. Comparison of approaches fit with tcplfit2 and BMDExpress for (A, C, E) feature-level approaches and (B, D, F) category-aggregation approaches.

(A, B) Venn diagram of the number of chemicals identified as active with each approach.

(C, D) Number of affected features (C) / categories (D) for each chemical (n=475) with each approach. The color code corresponds to the geometric mean of the number of affected features/categories in both approaches.

(E, F) Phenotype altering concentration (PAC) for chemicals that were identified as active with at least one approach. The color code corresponds to the geometric mean of the number of affected features/categories in both approaches