Supplemental Methods and Results

Methods

Gene Expression Datasets

The gene expression datasets used in the study can be accessed on Gene Expression Omnibus (GEO) under the accession numbers GSE93311(prefrontal cortex, nucleus accumbens core and shell, bed nucleus of the stria terminalis, basolateral amygdala, central nucleus of the amygdala, and ventral tegmental area) and GSE93515 (ventral striatum). The methods for GSE93311 are described in detail below and on GEO. The methods for GSE93515 are described in detail on GEO and (Iancu *et al*, 2013).

Tissue Preparation for Gene Expression Analysis

Mice were decapitated, and their brains were quickly removed, flash frozen in liquid nitrogen, and stored at -80°C. Twenty micron coronal sections were prepared on a Microm HM550 cryostat (Thermo Fisher Scientific, Walldorf, Germany). Serial sections were collected and mounted on UV treated nuclease-free PEN membrane slides (Zeiss, Bernried, Germany). Slides were kept inside the cryochamber during the procedure and stored at -80°C until ready to use.

Staining Procedures

Sections were quickly transferred from -80°C to pre-cooled (4°C) 95% ethanol and incubated for 1 minute, followed by rehydration in pre-cooled (4°C) 70% Ethanol for 20 seconds. The slides were stained in 1% cresyl violet acetate (Sigma, St. Louis, MO, USA) in absolute ethanol for 40 sec and dehydrated in 70% ethanol for 5 seconds, 95% ethanol for 5 seconds then 100% ethanol for 15 seconds at room temperature. After air drying for 2 minutes, slides were placed on ice in a slide box with desiccant and immediately used for microdissection.

Laser Capture Microdissection (LCM)

Seven brain regions, including prefrontal cortex (PFC), nucleus accumbens core (AcbC), nucleus accumbens shell (AcbSh), bed nucleus of stria terminalis (BNST), basolateral amygdala (BLA), central nucleus of amygdala (CeA) and ventral tegmental area (VTA) were dissected in accordance with coordinates from the mouse brain atlas of Fanklin and Paxinos (2007) using the PALM MicroBeam system (Carl Zeiss MicroImaging, Bernried, Germany). Sections containing these brain regions were cut under 10x magnification, and transferred using ultra fine

forceps into a non-stick, nuclease-free 1.5 ml tube. Several sections were pooled and the tubes were kept on ice until dissections were completed.

RNA Extraction

Lysis solution from RNAqueous-Micro Kit (Ambion, Austin, TX, USA) was added to the microdissected samples. The samples were incubated at 42°C for 30 minutes and stored at -80°C until processed. RNA was isolated following the manufacturer's instructions for LCM samples, quantified using the Quant-iT RiboGreen RNA Assay Kit (Invitrogen, Carlsbad, CA, USA) and then qualified using the Agilent RNA 6000 Pico Kit (Agilent Technologies, Santa Clara, CA, USA). In our study, the average RNA integrity number was 6.77 ± 0.58, indicating that the samples were of good quality.

RNA Amplification and Hybridization

Total RNA was amplified using the Illumina TotalPrep-96 RNA Amplification Kit (Ambion, Austin, TX, USA). The cRNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Eilmington, DE, USA), and the size was analyzed using the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). Using 30 ng of RNA as input yielded at least 4 µg of amplified biotinylated cRNA, with most of the RNA distributed between 800 and 1500 nucleotides, indicating the expected yield and size. We sent 1.5 µg cRNA to The Keck Microarray Resource at Yale University where it was hybridized to Illumina Mouse WG-6 v2.0 Expression BeadChips (Illumina, San Diego, CA, USA).

Data Processing

The data were preprocessed using the Bioconductor lumi package in the R programming environment (Du *et al*, 2008). A total of 168 samples across seven brain regions were used for data analysis. Variance-stabilizing transformation and quantile normalization were applied (Lin *et al*, 2008). Outlier detection was performed within each brain region, and samples were considered as outliers when their distances to the average of all samples were larger than the threshold (2*median distances to the center). Two control samples (from AcbC and BNST) were removed. Variance-stabilizing transformation and quantile normalization were reapplied to the raw data of the remaining 166 samples. For each brain region, only genes that were significantly expressed (detection threshold 0.05) in at least 75% of samples were selected. Grubb's test ($P < 0.05$) was performed within groups to remove outliers for each gene.

Differential Expression Analysis

Differential expression analysis was conducted within each brain region using empirical Bayes moderated t-statistics from the Bioconductor limma package in R to compare the HS/Npt and High Drinking In The Dark mouse lines (Ritchie *et al*, 2015). Benjamini-Hochberg's false discovery rate (FDR) was calculated using the qvalue package to correct multiple tests (Storey, 2015). Probes were annotated using the annotation file from Illumina for Mouse WG-6 v2 BeadChips (revision 3). We used the illuminaMousev2.db R package to update the annotations to the most current mouse genome release, mm10 from UCSC genome browser, which is based on the Genome Reference Consortium GRCm38 mouse assembly through Ensemble (Barbosa-Morais *et al*, 2010).

Drinking in the Dark

Time course study for terreic acid: To determine whether terreic acid could reduce ethanol intake and BALs (as compared with vehicle) after 2 or 4 hours of ethanol drinking, we carried out a 4 day drinking in the dark (DID) schedule. A 20% ethanol solution (v/v in tap water) was offered for 2 hours on days 1, 2, and 3, and for either 2 or 4 hours on day 4 (beginning 3 hours after lights off as described in the main text). Mice were administered 0, 7.5, or 15 mg/kg terreic acid 30 minutes prior to ethanol access on day 4. At 2 hours, volumetric readings of ethanol sipper tubes were recorded and blood levels were determined for half of the mice from each dose group. At 4 hours, volumetric readings of ethanol sipper tubes were recorded and blood levels were determined for the other half of the mice in each dose group. A total of 12 HDID-1 mice per sex per dose per time point were used for this experiment.

Low dose terreic acid experiment: To determine whether 3.75 g/kg terreic acid could reduce ethanol intake and BALs (as compared with vehicle), we carried out a 2 day drinking in the dark (DID) schedule as described in the main text of the manuscript for terreic acid. A total of 12 HDID-1 mice per sex per dose were used for this experiment. Mice from this experiment were subsequently subjected to serial testing at a higher terreic acid dose as described in the main text (3 weeks of ethanol, water, and saccharin DID followed by locomotor activity monitoring; Fig 2).

Results

Supplementary Figure 1

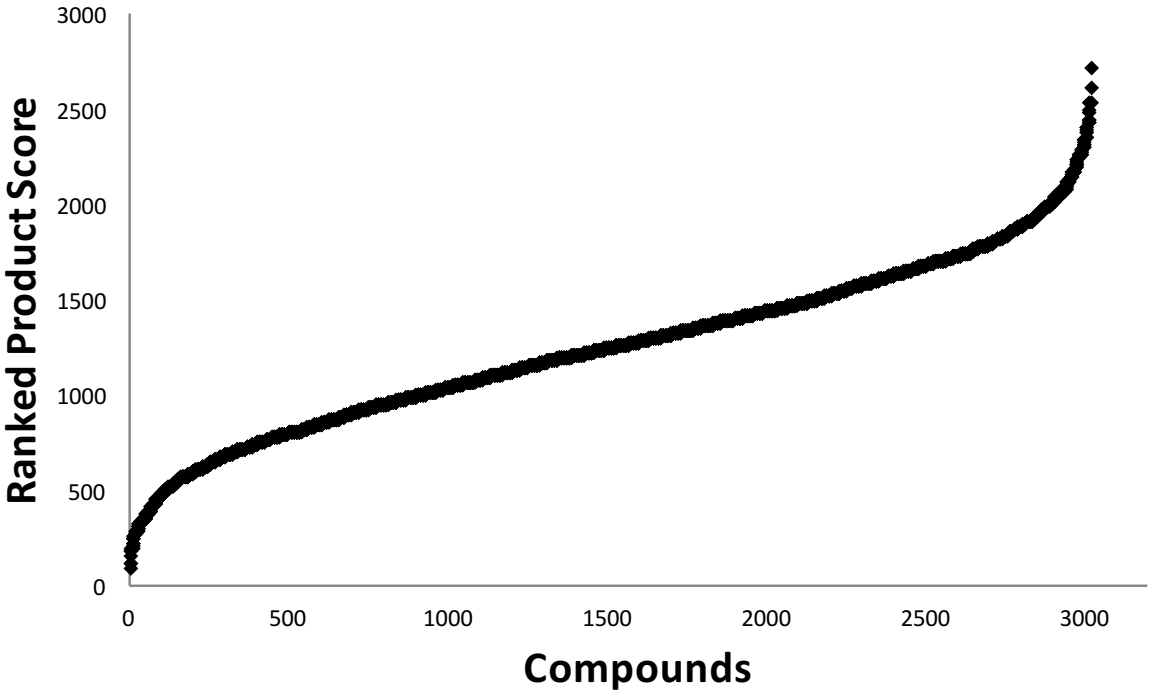


Figure S1. Prioritizing Compounds Based on Rank Product Score. Thirty-two input queries were submitted to LINCS-L1000 using the sig_gutc tool via C3 (Compute Connectivity on the Cloud, see Methods). The sig_gutc tool reports a summary connectivity score (collapsing across doses, time points, and cell lines) between more than 3 000 chemical compounds in the LINCS-L1000 touchstone dataset (comprised of the most reproducible signatures for well-characterized compounds) and the input signature. Each compound had 32 scores – one for each input query. To identify compounds that consistently had negative scores across the most brain areas irrespective of the query parameter settings, we rank ordered compounds according to a modified rank order product score (Breitling *et al*, 2004; Fortney *et al*, 2015). Each LINCS-L1000 query produced a list of drugs with a corresponding connectivity score. We ranked the scores for each query in increasing order (smallest to largest) using a competitive ranking scheme, i.e., drugs with the same score were assigned the same rank. To calculate the rank product score, we multiplied the assigned ranks for each drug across all of the 32 LINCS-L1000 queries and raised to the power 1/32. Since drugs were ranked in increasing order, and we were interested in negative connectivity scores, the drugs with the lowest rank product were of interest. Pergolide, BRD- K14355517, and terreic acid were the top 3 hits.

Time course study for terreic acid: We used the drinking in the dark assay to determine whether terreic acid could reduce ethanol intake and BALs (as compared with vehicle) after 2 or 4 hours

of ethanol drinking. Two-way ANOVA of ethanol intake and BAL data collected from mice after 2 hours of DID did not reveal a significant effect of sex or a dose x sex interaction, therefore we collapsed the data on sex and performed a one-way ANOVA. Results show that terreic acid significantly reduces 2 hour ethanol intake (**Fig S2a**; $F(2,141)=11.6$, $P<0.0001$) and BAL (**Fig S2b**; $F(2,69)=6.6$, $P<0.01$). To pursue dose comparisons, we performed Tukey's post-hoc analysis and found that both 7.5 and 15 mg/kg terreic acid reduced 2 hr ethanol intake ($P=0.001$ and $P<0.0001$, respectively) and BALs ($P<0.05$ and $P<0.01$, respectively) as compared with vehicle treated mice.

Two-way ANOVA of ethanol intake and BAL data collected from mice after 2 hours of DID did not reveal a significant effect of sex or a dose x sex interaction, therefore we collapsed the data on sex and performed a one-way ANOVA and used Tukey's post-hoc to test for differences in dose response (reported in Fig S1 caption). Terreic acid also significantly reduced 4 hour ethanol intake (**Fig S1c**; $F(2,70)=6.6$, $P<0.01$) and BAL (**FigS1d**; $F(2,65)=4.9$, $P<0.01$). n=12 HDID-1 mice per sex per dose per time point. Notably, terreic acid more robustly reduced intake and BAL in a 2 hr DID assay than in a 4 hr DID assay.

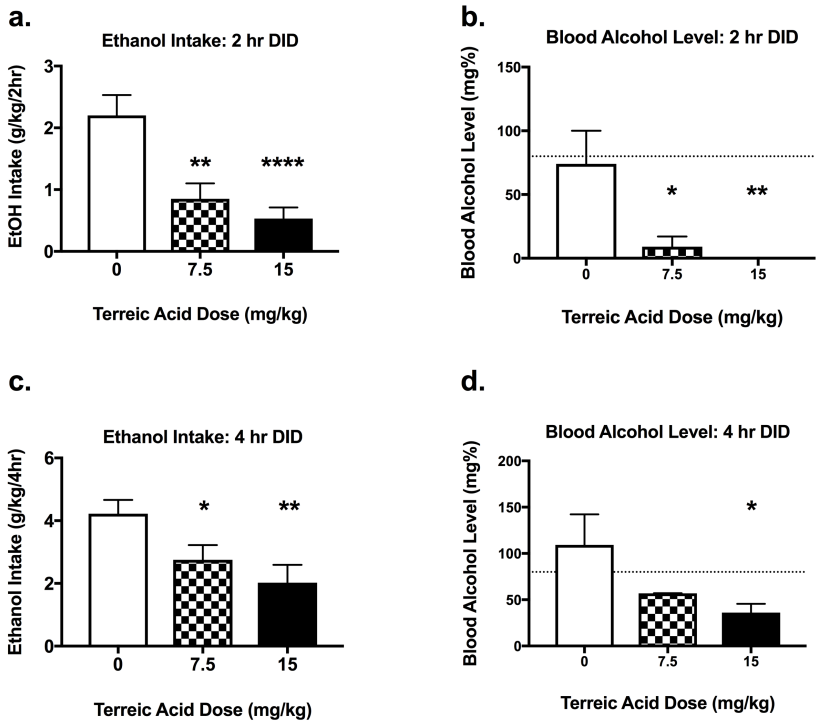
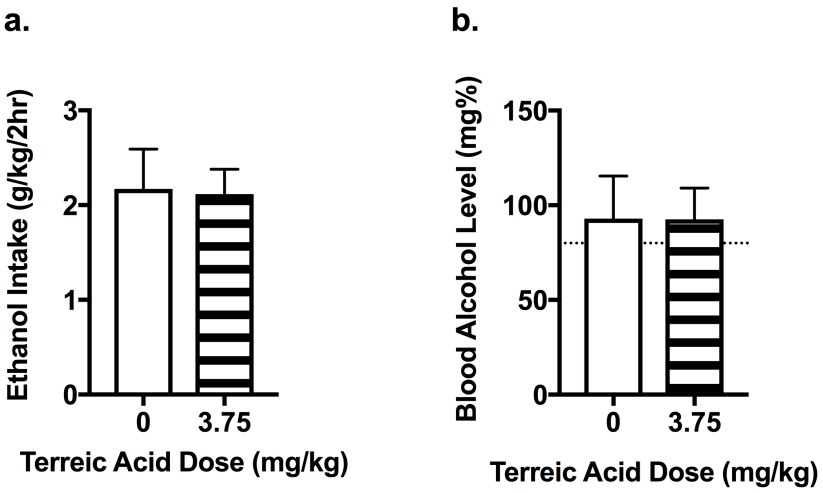


Figure S2. Terreic acid significantly reduces binge-like alcohol drinking and BAL in a 2 hour, but less robustly in a 4 hour, limited access DID schedule. Terreic acid reduced a) ethanol intake and b) BAL in a 2 hour DID session. Terreic acid also significantly reduced c) ethanol intake and d) BAL in a 4 hour DID session. Dashed line at y=80 in b) and d) represents the NIAAA definition of intoxication. Significant Tukey's post hoc results (as compared with 0 mg/kg dose) are represented by *=P<0.05, **=P<0.01, ****=P<0.0001.

Low dose terreic acid experiment: To determine whether 3.75 g/kg terreic acid could reduce ethanol intake and BALs (as compared with vehicle), we carried out a 2 day drinking in the dark (DID) schedule as described in the main text of the manuscript for terreic acid. n=12 HDID-1 mice per sex per dose. Mice from this experiment were subsequently subjected to serial testing at a higher terreic acid dose as described in the main text (3 weeks of ethanol, water, and saccharin DID followed by locomotor activity monitoring; Fig 2).

Figure S3. 3.75 mg/kg terreic acid does not significantly reduce a) binge-like alcohol drinking or b) BAL in a 2 hour limited access DID schedule.



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