Methods

[Note: This work has not yet been published. Additional details are available upon request.]

Cell Culture:

WA09 (H9) human embryonic (hES) stem cells were licensed from WiCell. The cells were passaged and maintained on Matrigel (Corning) in mTeSR1 medium (StemCell Technologies) as cell aggregates in 6-well dishes. Cells were monitored daily and differentiated cells removed by aspiration. For the studies, cells were dissociated using TrypLE™ (Life Technologies) into single cell suspensions and seeded onto Matrigel in 96-well dishes at ~$2X10^5$ cells/well. For gene expression profiling, the following day cells were treated with chemicals at various concentrations for 24 hours in three 96-well plates (*i.e.*, biological replicates). An additional white-wall plate of cells was used to assess viability for each chemical treatment using the CellTiter-Fluor Viability assay (Promega) according to manufacturer's directions. For HDAC assays, cells were refed the day following seeding. Two days after seeding, cells were treated with chemicals at various concentrations in duplicate wells of duplicate 96-well white-wall plates. Acetylated Fluor de Lys® Substrate was added to the wells of one plate. Following 4 hours of chemical exposure, the HDAC assay plate was processed using the HDAC Fluorimetric Cellular Activity Assay Kit (Enzo Life Sciences) according to the manufacturer's instructions with the exception that the volume of developing reagent was increased to account for chemical treatment in more volume than recommended for use with ½-volume microplates. A deacetylated substrate was spiked into some wells following the developer reaction to check for interference of the fluorescence signal by the test chemical. The other plate was used to assess viability for each chemical treatment using the CellTiter-Fluor Viability assay.

RNA Sequencing:

At time of harvest medium was removed and cells were lysed with Qiazol (Qiagen) and stored at -80°C. Cells were pooled from 6 wells and mRNA was prepared using miRNeasy Mini Kit (Qiagen) with on-column DNase treatment. RNA integrity was verified using an Agilent Bioanalyzer 2100 and the Agilent 6000 Nano Kit. All NGS libraries were generated using the TruSeq® Stranded Total RNA HT Kit with Ribo-Zero Gold (Illumina) and 700 ng of total RNA. An aliquot of each cDNA library was checked for quality and mean fragment size via the Agilent 2100 Bioanalyzer with the Agilent DNA 1000 kit. The cDNA library was quantified using the Qubit™ 3.0 Fluorometer and the Qubit™ dsDNA BR Assay Kit (Invitrogen). The KAPA Library Quantification Kit for Illumina (KAPA Biosystems) in conjunction with the ViiA™ 7 Real-Time PCR System (LifeTech) was used to confirm the presence of adapter–ligated libraries. Libraries were pooled, denatured, neutralized, and diluted in HT1 buffer (Illumina) to 1.5 pM. The libraries (3 biological replicates per dose) were sequenced on the NextSeq™ 500 System (Illumina) using a High Output flow cell (Illumina) at 1x76 cycles for >20M reads per sample.

Bioinformatics:

Reads were aligned to the reference genome (GRCh38/v84) using Star (2.5) with quantMode on Ensembl GTF annotation (GRCh38v75). The table of counts was then imported in R and genes within a group with a total count less than one read per million reads were eliminated. The EdgeR (3.12.1) package was used for the analysis by normalizing with TMM (embedded in EdgeR) and calculating differentially expressed genes (DEGs) using the generalized linear models. Additional details regarding bioinformatic analyses can be provided upon request.