Supplemental Material

Figure S1.

A.) Anti-H3K27me3 antibody specifically recognizes trimethylation of Lysine 27 residue on histone H3.

B.) HCC1806 cells were allowed to recover overnight, then cells were treated with compounds and incubated for 3 days. After 3-day incubation, cells were fixed, stained with specific primary, secondary antibodies and DRAQ5 (to enable cellular identification and cell counting for toxicity), washed, and read on the Opera imager.

A

B

Day 1
Plate cells (5K, 384 well format, 24 hrs)

Day 2
Compound Treatment (72 hrs)

Day 5
Fixation (3.7% Formaldehyde, 15 mins)

Block/permeabilization
(Blocking solution, 1.5 hrs at RT)

1° antibody staining
(overnight at RT, then 5 washes)

Day 6
2° antibody with DRAQ5 staining
(2 hrs at RT, then 5 washes)

Read plates on the Opera
Figure S2.

A.) Numbers of hits and their associated targets identified from H3K27me3 phenotypic screen using BDCS.

B & C.) Binned analysis of H3K27me3 enhancers and suppressors based on how many compounds from each target were identified as hits in the screen.

<table>
<thead>
<tr>
<th></th>
<th>Hits</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>495</td>
<td>334</td>
</tr>
<tr>
<td><strong>Suppressors</strong></td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td><strong>Enhancers</strong></td>
<td>467</td>
<td>314</td>
</tr>
</tbody>
</table>

![Bar graph showing hit numbers per target for enhancers and suppressors]