Supporting Information

Optimization of high-throughput methyltransferase assays for the discovery of small molecule inhibitors

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Table of Contents

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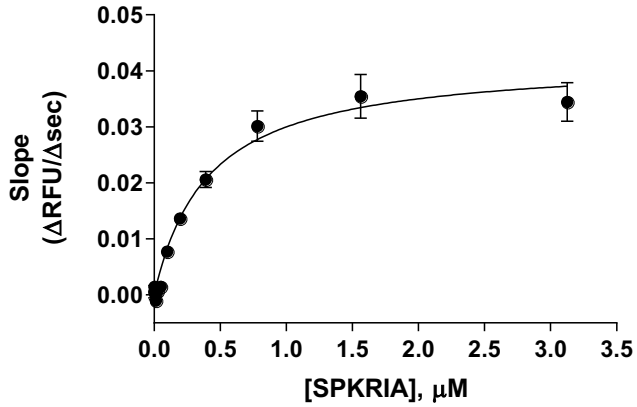

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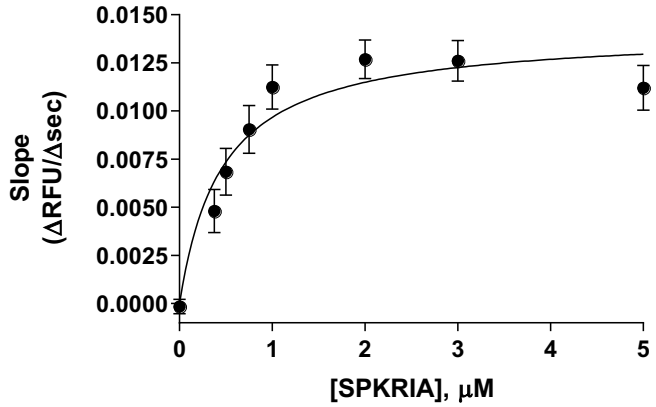

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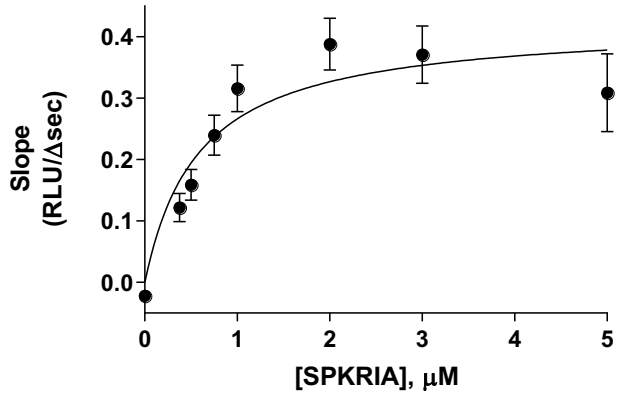
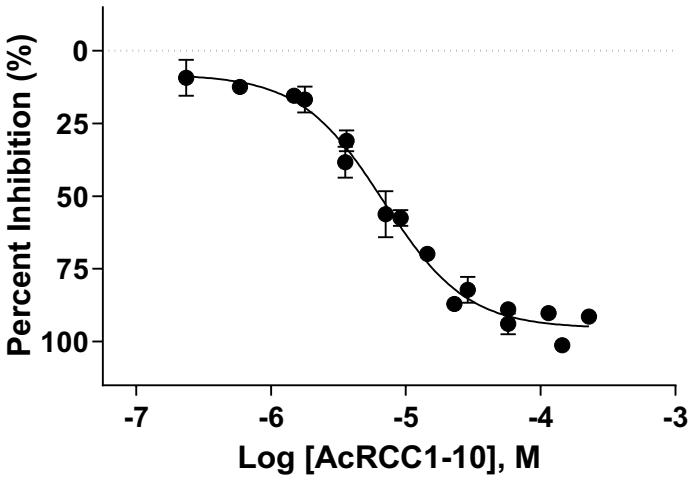


Figure S1. K_m determination for peptide substrate SPKRIA. A) 384-well plate format in SAHH-ThioGlo3 assay. B) 1536-well plate format in SAHH-ThioGlo3 assay. C) 1536-well plate format in MTase-Glo assay.

A.



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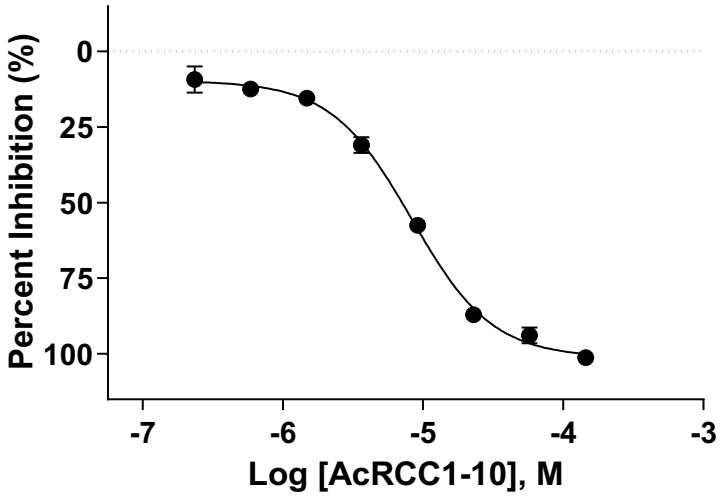
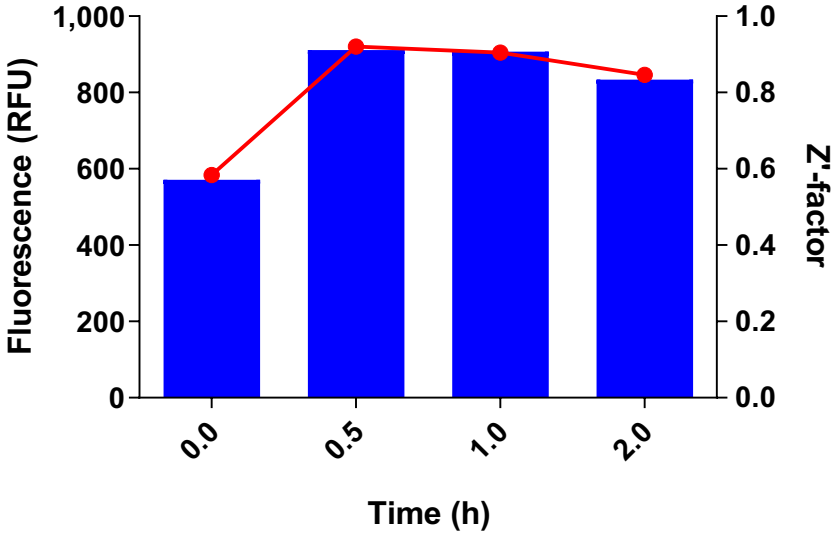


Figure S2. AcRCC1-10 control titration in 1536-well format (30 mins RT incubation). A) In SAHH-ThioGlo3 assay under the condition of 125 nM NTMT1, 1 μM RCC1-6, 100 μM SAM, 10 μM SAHH, and 15 μM ThioGlo3. B) In MTase-Glo assay (125 nM NTMT1, 1 μM RCC1-6, 100 μM SAM)

A.



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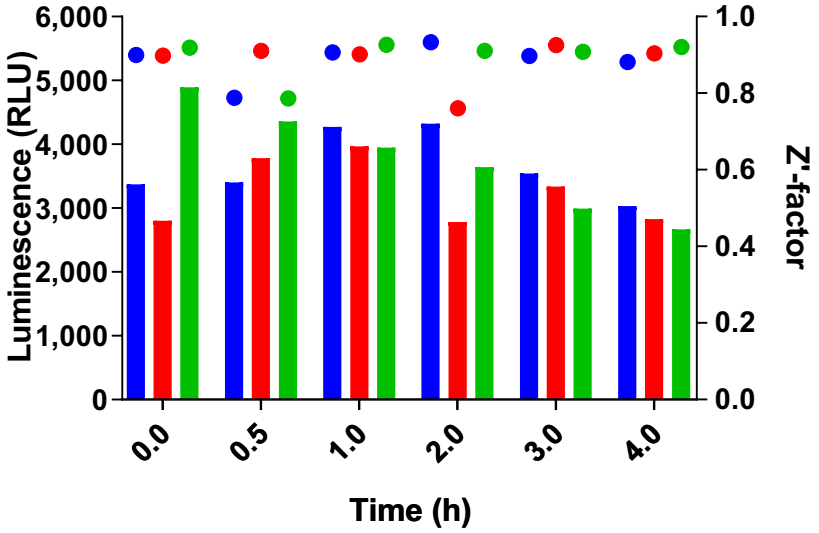


Figure S3. Reagent stability. A) Reagent stability (2h) of ThioGlo3 in 1536-well format. Blue bars are the fluorescent signal, red circles are Z'-factor value. B) Reagent stability (4h) of MTase-Glo in 1536-well format. Different color represents different day of preparation. Circle represents Z'-factor value.

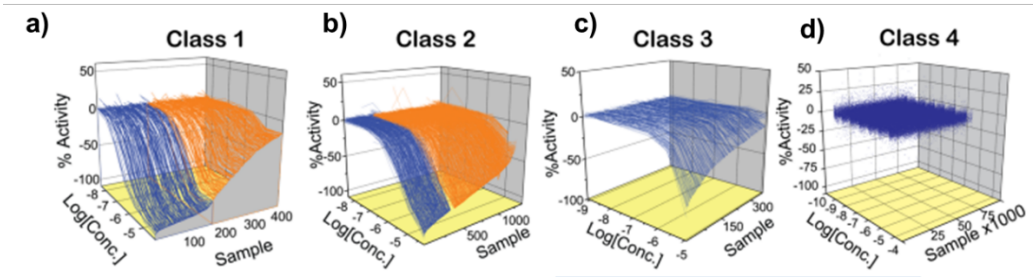


Figure S4. Example curves highlighting qHTS curve classification criteria. Lines connecting titration data corresponding to inhibitory compounds are shown. (a) Classes 1.1 (blue; >80% efficacy) and 1.2 (orange; ≤80% efficacy) inhibitors display full and partial activity, respectively, with r2 ≥0.9. (b) Incomplete curves for inhibitors having AC_{50} values within and beyond the tested titration range are Classes 2.1 (blue; >80% efficacy, r2 >0.9) and 2.2 (orange; ≤80% efficacy, r2 <0.9), respectively. (c) Incomplete inhibitory (blue) curves that show weak activity and poor fits are Class 3. (d) inactive compounds are Class 4.

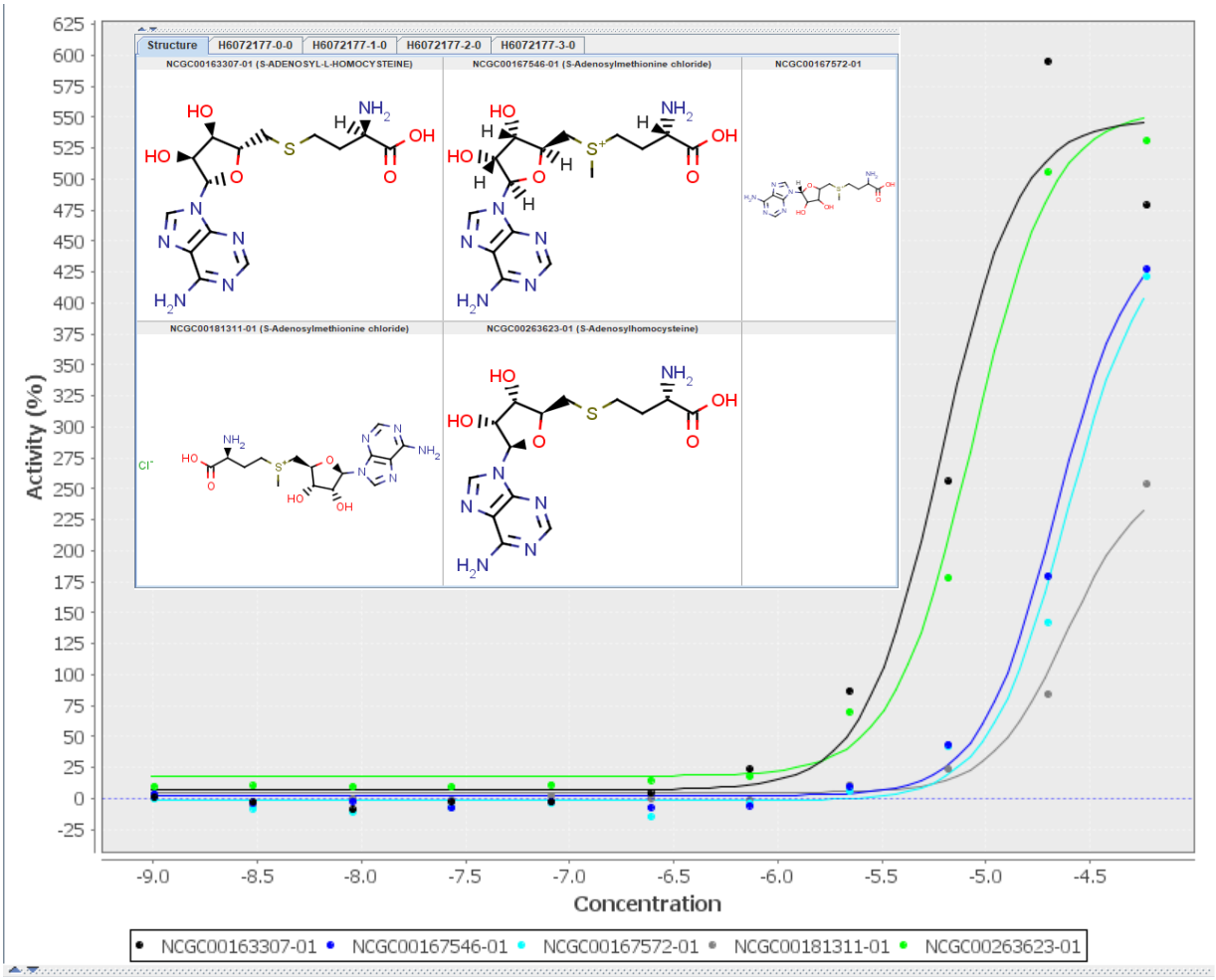
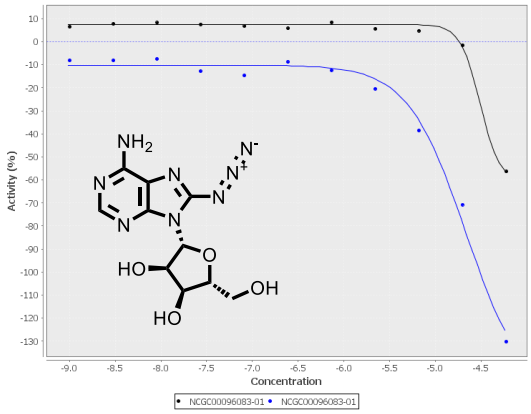


Figure S5. Positive curve classes from SAH mimetics in SAHH-ThioGlo3 and MTase-Glo assay formats.

A.



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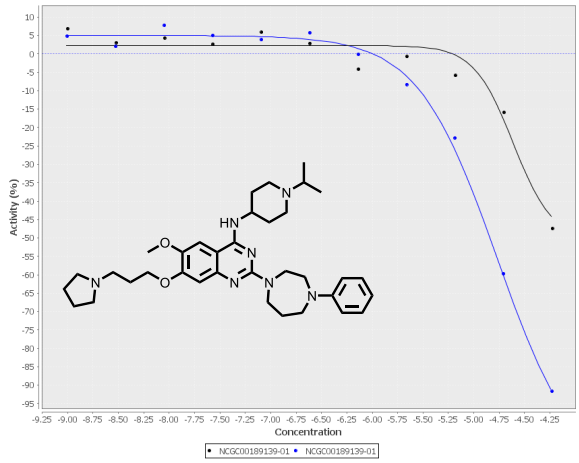

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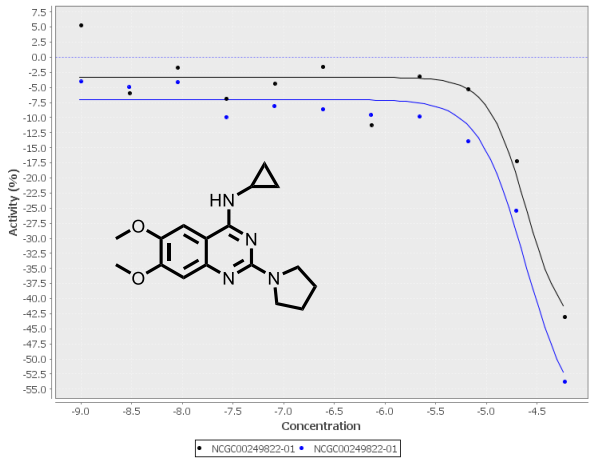
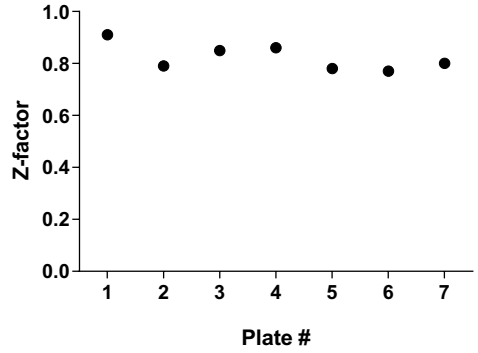


Figure S6. Three active hits identified in ThioGlo3 (blue line) and MTase-GLo (black line) assays. A) NCGC00096083 titrations. B) NCGC00189139 titrations. C) NCGC00249822 titrations.

A.



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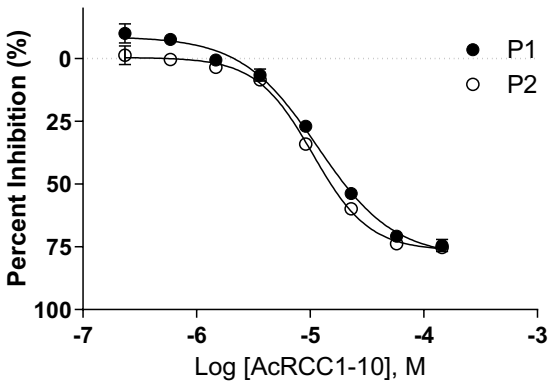

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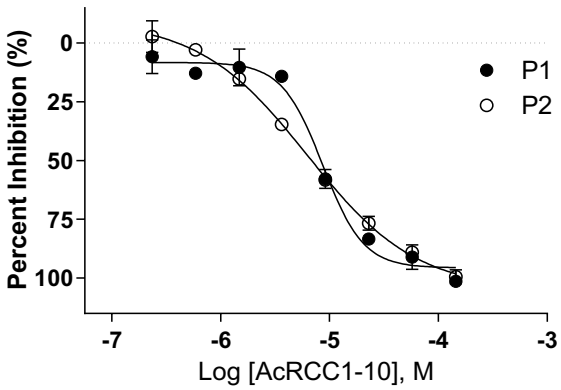

Figure S7. qHTS of LOPAC^{1280} library. A) SAHH-ThioGlo3 assay performance. B) Intraplate control titration of AcRCC1-10 in SAHH-ThioGlo3 assay. C) MTase-Glo™ intraplate control titration of AcRCC1-10.