Supplementary Data

A high-throughput radioactivity-based assay for screening SARS-CoV-2 nsp10-nsp16 complex

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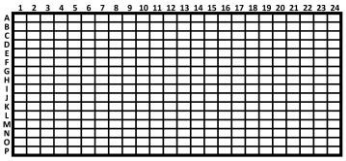
Supplementary Table 1. Screening chemical probes 6

Supplementary Scheme 1. Assay workflow.

1- Dispense 8 µL of the starting **reaction mixture** (including, Tris-HCl (pH 7.5), KCl, $MgCl_2$, Triton-X-100, BSA, DTT, nsp10-nsp16 complex, and RNA) into the wells of 384-plates using a liquid-handling robot.



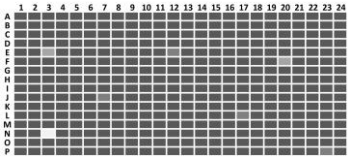
2- Add 1 µL of the **10X compounds** using the liquid-handling robot.

3- Incubate the mixtures at the room temperature for 15 min.
4- Start the reactions by adding 1 µL of **10X ^{3}H-SAM** solution in each well using the liquid-handling robot.

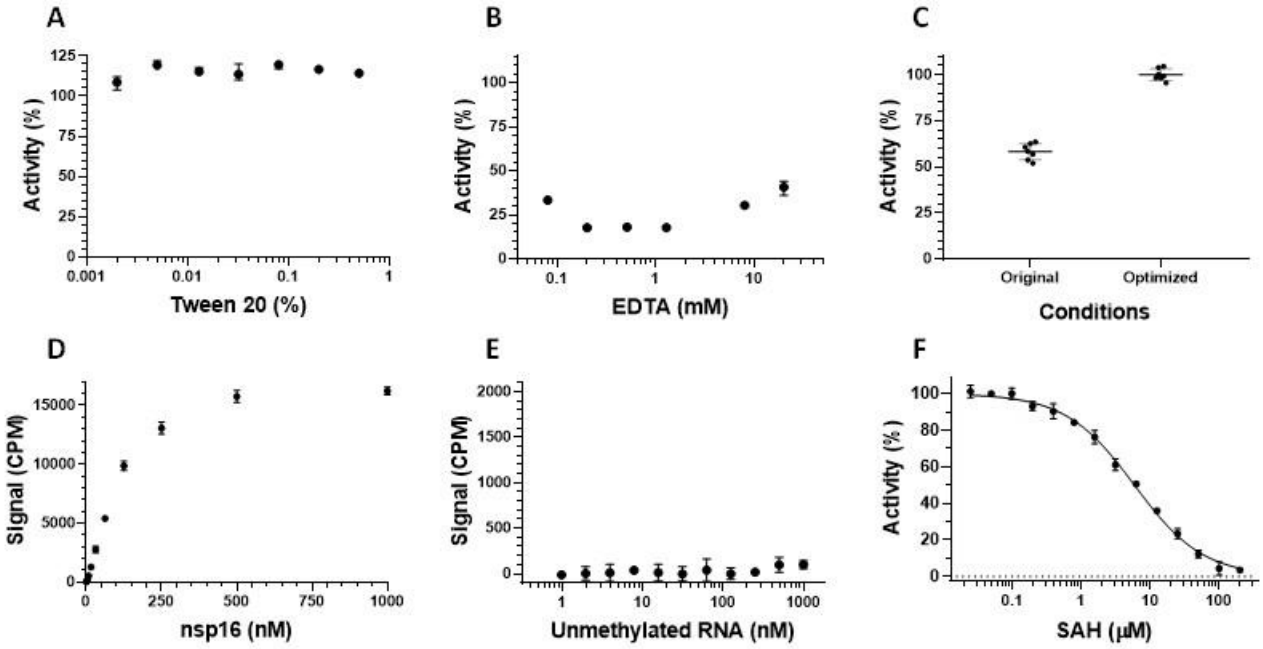
5- Incubate the mixtures at the room temperature for 30 min to allow the enzymatic reaction to progress:

N7-meGpppACCCCC-biotin + SAM $\xrightarrow[\text{inhibitors}]{\text{nsp10-nsp16}}$ **N7-meGpppA$_{2'\text{Ome}}$CCCCC-biotin + SAH**

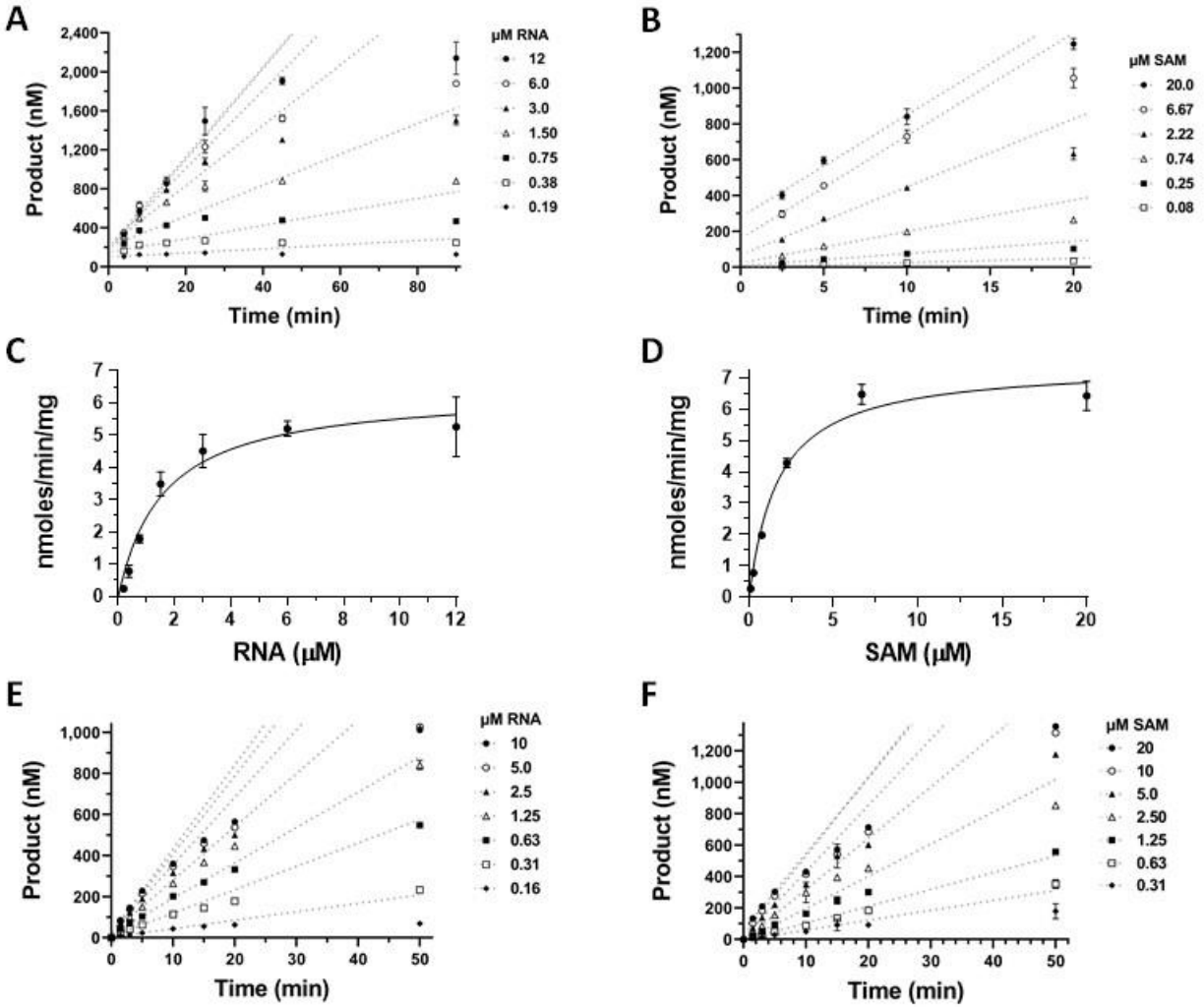
6- Stop the reactions and transfer the contents into SPA plates. Measure the signals.



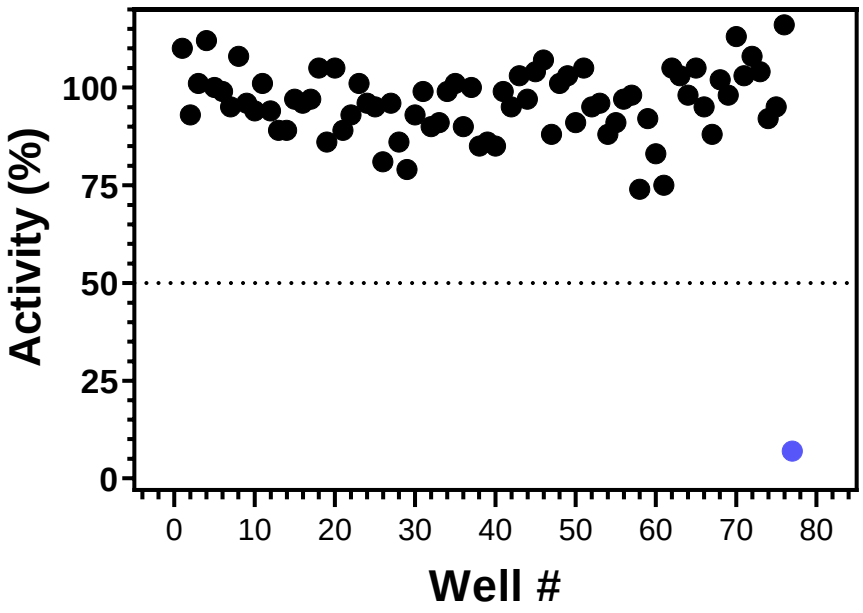
7- Calculate the percentage inhibition using Excel or GraphPad



Supplementary Figure 1. Assay optimization. (A) Effect of Tween-20 on activity of nsp10-nsp16 complex. **(B)** The inhibitory effect of EDTA on nsp10-nsp16 at various concentrations. **(C)** Comparison of nsp10-nsp16 MTase activity in the original buffer (50 mM Tris pH 8.0, 1 mM $MgCl_2$, and 5 mM DTT) versus the optimized buffer condition (50 mM Tris pH 7.5, 100 mM KCl, 1.5 mM $MgCl_2$, 5 mM DTT, 0.01% BSA, 0.01% Triton X-100), in the presence of 2 μM RNA substrate, 5 μM SAM (16% ^{3}H-SAM), and 250 nM nsp16. **(D)** MTase activity at various concentrations of nsp10-nsp16 complex using N7-meGpppACCCCC RNA (Cap-0). **(E)** The N7-unmethylated RNA is not a substrate for nsp10-nsp16 complex; here reactions were performed in the presence of 125 nM nsp10-nsp16, 5 μM SAM and varying concentrations of N7-unmethylated RNA substrate (0.97 nM to 1 μM) for 30 minutes. **(F)** SAH inhibited nsp10-nsp16 with an IC_{50} value of 5.9 ± 0.6 μM (Hill Slope: -0.9). The values in panel **C** are from seven independent experiments (n=7). All other experiments were performed in triplicate (n=3).



Supplementary Figure 2. Linearity of initial velocities at 125 and 250 nM of nsp10-nsp16 complex. The initial velocities were determined at 250 nM of nsp10-nsp16 complex using (**A**) various concentrations of RNA (as indicated on the plot) and fixed SAM concentration (6 µM), and (**B**) varying concentrations of SAM and fixed RNA concentration of 5.6 µM under the optimized condition. The first 10 minutes linear initial velocities from A and B were used to calculate the K_m values for (**C**) RNA substrate and (**D**) SAM. The linearity of the initial velocities was also assessed at 125 nM of nsp10-nsp16 complex (**E and F**). The velocities at various concentrations of (**E**) RNA and (**F**) SAM are shown up to 50 minutes. The linear portion of the initial velocities from E and F are re-plotted and shown in Figure 2A and 2B, respectively. All experiments were performed in triplicate (n=3)



Supplementary Figure 3. Screening of 76 chemical probes against nsp10-nsp16 complex. A collection of 76 chemical probes for epigenetic targets including 20 methyltransferase inhibitors were screened at 50 μM against nsp10-nsp16 under the optimized screening assay conditions (i.e., 0.8 μM RNA, 1.7 μM SAM, and 125 nM nsp16 enzyme). The corresponding percentage activity data for each probe is shown on the graph with a black dot. SAH was used at the same concentration as a control (blue dot). Please note that the dotted line marks the 50% activity threshold.

Supplementary Table 1. 76 chemical probes were screened against nsp10-nsp16 complex. 76 chemical probes including 20 methyltransferase inhibitors were screened against SARS-CoV-2 nsp10-nsp16 using the optimized HTS assay. The observed percentage of activity of nsp10-nsp16 in the presence of each of these compounds (at 50 μM) is presented. The list of compounds (available at https://www.thesgc.org/chemical-probes), and their specific protein targets are provided. Negative control analogues of the chemical probes are specified with "Negative Ctrl" under the "Specific Targets" column.

Compound	Specific Targets	% activity @ 50 µM
UNC1215	L3MBTL3	103
BSP	pan-Bromodomain	105
UNC0638	EHMT2 (G9a), EHTM1 (GLP)	102
UNC0737	EHMT2 (G9a), EHTM1 (GLP) (Negative Ctrl)	113
A-395	EED	100
A-395N	EED (Negative Ctrl)	99
TP-064	CARM1 (PRMT4)	98
TP-064N	CARM1 (PRMT4) (Negative Ctrl)	105
MS023	Type I PRMTs (PRMT1,3,4,6,8)	99
MS094	Type I PRMTs (PRMT1,3,4,6,8) (Negative Ctrl)	103
UNC1999	EZH2	108
UNC2400	EZH2 (Negative Ctrl)	104
PFI-2	SETD7	105
(S)-PFI-2	SETD7 (Negative Ctrl)	110
SGC-CBP30	CREBBP, EP300	103
A-366	EHMT2 (G9a), EHMT1 (GLP)	112
OICR-9429	WDR5	103
OICR-0547	WDR5 (Negative Ctrl)	101
NVS-PAK1-1	PAK1	107
GSK864	Mutant isocitrate dehydrogenase 1	81
BI-9321	NSD3	97
BI-9466	NSD3 (Negative Ctrl)	105
SGC707	PRMT3	105
XY1	PRMT3 (Negative Ctrl)	116
PFI-3	SMARCA2, SMARCA4, PBRM1 (PB1)	95
GSK-J1	KDM6B (JMJD3), KDM6A (UTX), KDM5B (JARID1B)	96
A-485	p300, CBP	95
A-486	p300, CBP (Negative Ctrl)	108
SGC0946	DOT1L	97
SGC0649	DOT1L (Negative Ctrl)	91
UNC0642	EHMT2 (G9a), EHTM1 (GLP)	98
GSK343	EZH2	93
L-Moses	KAT2B (PCAF), KAT2A (GCN5)	100
GSK484	PAD-4	101
BAY-876	GLUT1	97
BAY-588	GLUT1 (Negative Ctrl)	94
PFI-5	SMYD2	88
NVS-CECR2-1	CECR2	104
OF-1	BRPF1, BRD1 (BRPF2), BRPF3	88

Compound	Specific Targets	% activity @ 50 µM
IOX1	pan-2-OG	99
I-BRD9	BRD9	79
LP99	BRD9, BRD7	85
NI-57	BRPF1, BRD1 (BRPF2), BRPF3	97
I-CBP112	CREBBP, EP300	93
GSK-LSD1	KDM1A (LSD1)	86
GSK2801	BAZ2A, BAZ2B	89
BAZ2-ICR	BAZ2A, BAZ2B	96
PFI-4	BRPF1B	96
A-196	SUV420H1/H2	93
A-197, SGC2043	SUV420H1/H2 (Negative Ctrl)	101
GSK591	PRMT5	96
SGC2096	PRMT5 (Negative Ctrl)	98
IOX2	pan-2-OG	90
PFI-1	BRD2, BRD3, BRD4, BRDT (BET)	91
JQ1	BRD2, BRD3, BRD4, BRDT (BET)	91
TP-472	BRD9, BRD7	88
MS049	PRMT4 (CARM1), PRMT6	95
BAY-299	BRD1, TAF1	96
TP-238	CECR2, BPTF (FALZ)	95
LLY-507	SMYD2	90
BAY-598	SMYD2	89
BAY-369	SMYD2 (Negative Ctrl)	94
BI-9564	BRD9, BRD7	86
GSK6853	BRPF1	95
SGC6870	PRMT6	83
SGC6870N	PRMT6 (Negative Ctrl)	75
MRK-740	PRDM9	86
MRK-740-NC	PRDM9 (Negative Ctrl)	85
SGC3027	PRMT7	74
SGC3027N	PRMT7 (Negative Ctrl)	92
UNC6934	NSD2-PWWP1	92
UNC7145	NSD2-PWWP1 (Negative Ctrl)	95
LLY-283	PRMT5	99
LLY-284	PRMT5 (Negative Ctrl)	101
BAY-6035	SMYD3	89
BAY-444	SMYD3 (Negative Ctrl)	101
SAH (Control)	-	7