Supplemental Information

Supplemental Figure Legends

Figure S1. The carton of BiLC vectors. Genes were cloned onto N terminus of GlucN (A) or GlucC (B), and also C terminus of GlucN (C) or GlucC (D). The vectors termed as PB1/PB1c/PB2-GN (A), PA/PB2n/PB2-GC (B), GN-PB1/PB1c/PB2 (C) and GC-PA/PB2n/PB2 (D), respectively.

Figure S2. The structure model of PA-PB1 interaction detected by BiLC. (A), Structure model of Influenza A virus polymerase and positions of PA-C terminus (PA-Cter, Red), PB1-N terminus (PB1-Nter, Blue), PB1-C terminus (PB1-Cter, Blue) and PB2-N terminus (PB2-Nter, Red), as indicated. (B), The structure model of GN-PB1 and PA-GC. GN and GC fused with N terminus of PB1 and C terminus of PA respectively by a soft linker. (C), Cartoon of the BiLC competition assay. PA and PB1 can compete to bind to GN-PB1 and PA-GC, respectively, to disrupt the interaction between GN-PB1 and PA-GC.

Figure S3. Location of the selected mutation residues on PAc-PB1n complex (A) or PB1c-PB2n complex (B) as indicated. PAc and PB2n are in red, PB1n and PB1c in blue.

Figure S4. The flow chart and BiLC assay of PB1c-PB2n interaction. (A), Flow chart of experiments. (B), Structure of PB1c-PB2n complex. PB1c is in blue, PB2n in yellow. (C), BiLC results for PB1c-PB2n interaction. PB1c and PB2n were fused with GN and GC respectively and co-expressed in 293T cells. 24HPT, luminescence from *Gaussia* luciferase was detected with microplate reader. (D), Stable cell line named PB1c-PB2n BiLC cells expressing GN-PB1c and GC-PB2n was constructed with Teton system. The cell was induced with doxycycline at indicated concentrations for 24 hours. Then luminescence from *Gaussia* luciferase was detected with microplate reader. (E), Time course of the PB1c-PB2n BiLC cells induced with 200ng/ml doxycycline.

Figure S5. The inhibitory function of small molecules to PB1c-PB2n interaction detected by BiLC assay (*$p<0.05$). GN-PB1c and GC-PB2n were co-expressed in 293T cells and then the cells were treated with DMSO or Molecule #1-8 for 24 hours at indicated concentrations. Then luminescence from *Gaussia* luciferase was detected with microplate reader.

Figure S6. The inhibitory function of small molecules to IAV-Luc virus replication (*$p<0.05$). A549 cells were pretreated with DMSO or Molecule #1-8 at indicated concentrations for 2 hours. Then the cells were infected

with IAV-Luc virus at MOI 0.01 for 1 hour. 24 hours post infection, luminescence from cell supernatant was detected with microplate reader.

Figure S7. The cytotoxicity of Molecule #5. (A), Structure of Molecule #5. (B), One representative plaque assay result was shown. Figure legends were as same as that in Figure 3E. (C), A549 cells were treated with DMSO, 0.1% Triton X-100 and Molecule #5 at 10 μM for 24 hours and stained with trypan blue solution (0.4%) for 5 min. Trinton X-100 damaged cells were used as positive control.

Figure S8. The interaction between PA and PB2 detected by BiLC assay. PB2 was cloned on N or C terminus of GN, named as PB2-GN or GN-PB2 as shown in Figure S1A, C. Then it was co-expressed with PA-GC or GC-PA, respectively. 24 hours post transfection, luminescence from cell supernatant was detected with microplate reader. Protein expression levels were detected by western blot as indicated.

Figure S9. The assembly of influenza virus polymerase. The interactions between PA and PB2 in presence of PB1 (A), PB1 and PB2 in presence of PA (B), and PA and PB1 in presence of PB2 (C) were detected 24 hours post transfection. Luminescence from cell lysates was detected with microplate reader.

Supplemental Figures

Figure S1.

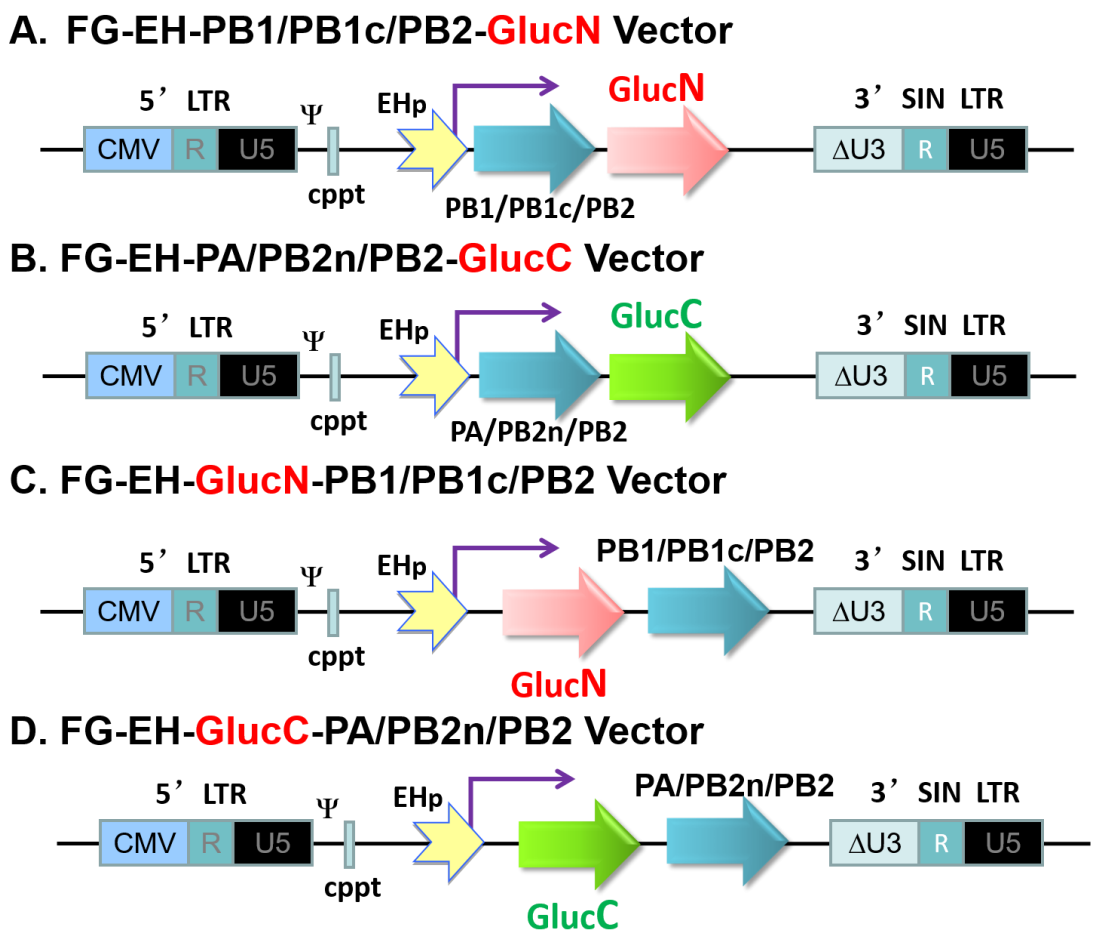


Figure S2.

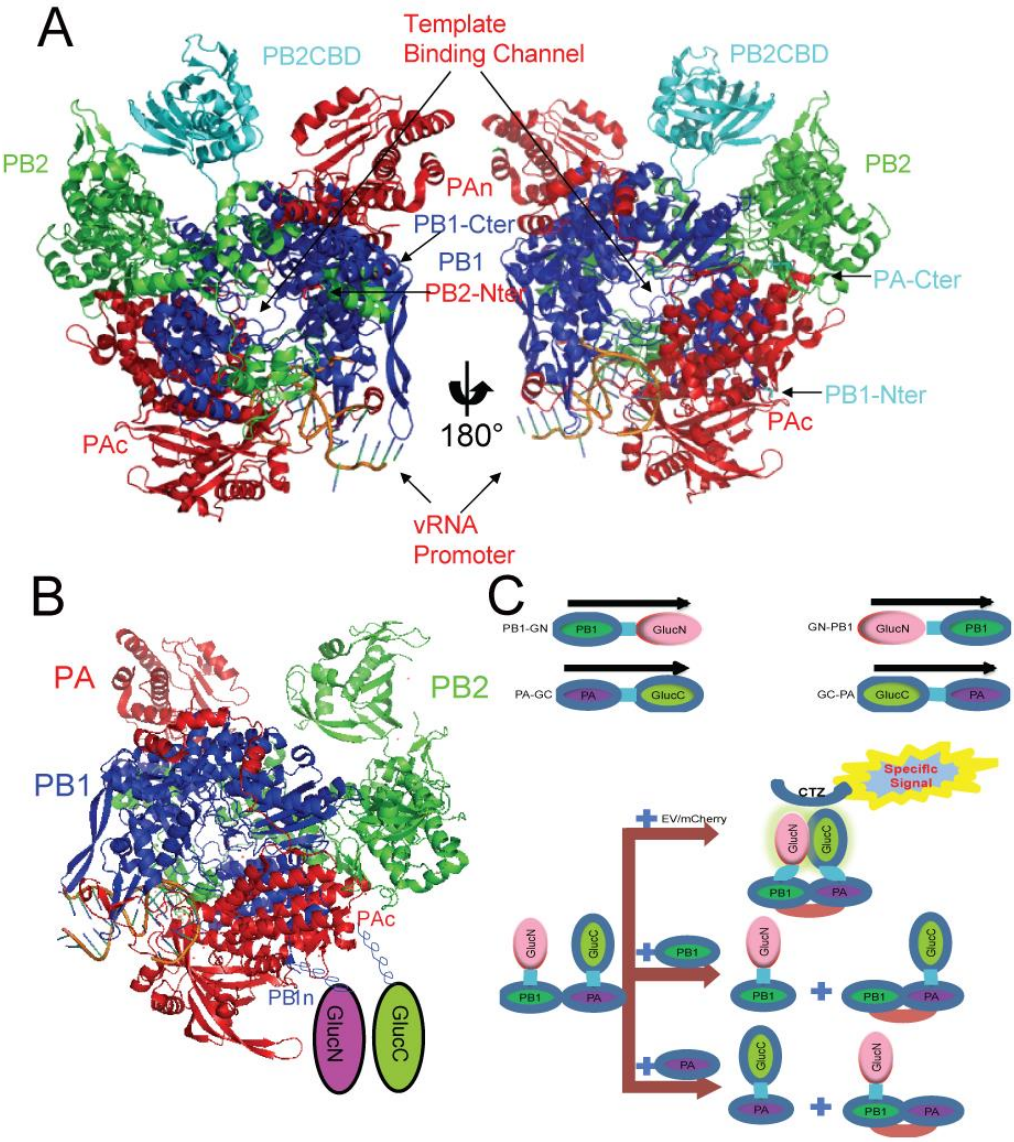

A
Template
Binding Channel
PB2CBD
PB2CBD
PB2
PB2
PAn
PB1-Cter
PB1
PB2-Nter
PA-Cter
PB1-Nter
PAc
PAc
180°
vRNA
Promoter
B
PA
PB2
PB1
PAc
PB1n
GlucN
GlucC
C
PB1-GN
GlucN
PB1
PA-GC
PA
GlucC
GN-PB1
GlucN
PB1
GC-PA
GlucC
PA
Specific
Signal
CTZ
EV/mCherry
GlucN
GlucC
PB1
PA
PB1
PA
GlucN
GlucC
PB1
PA
PB1
PA
GlucC
PA
GlucN
PB1
PA

Figure S3.

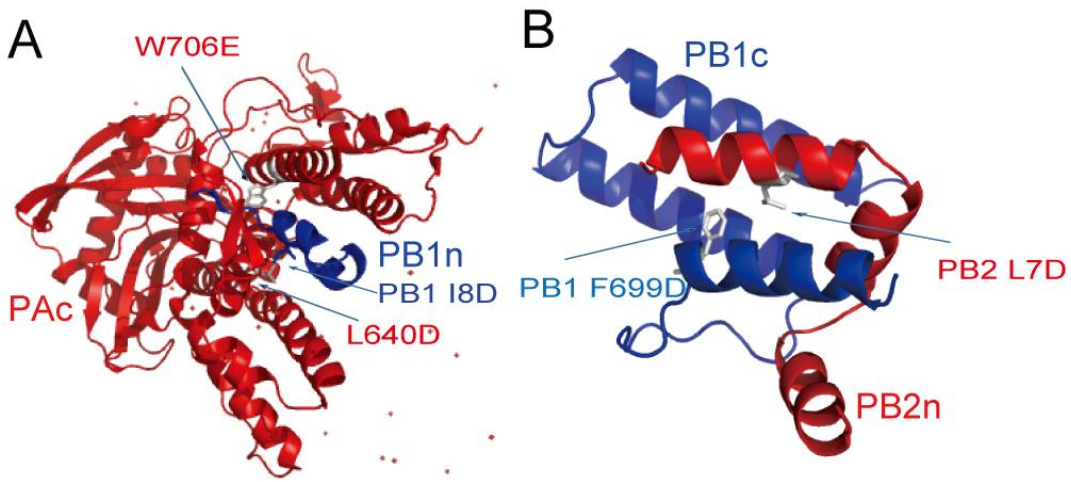


Figure S4.

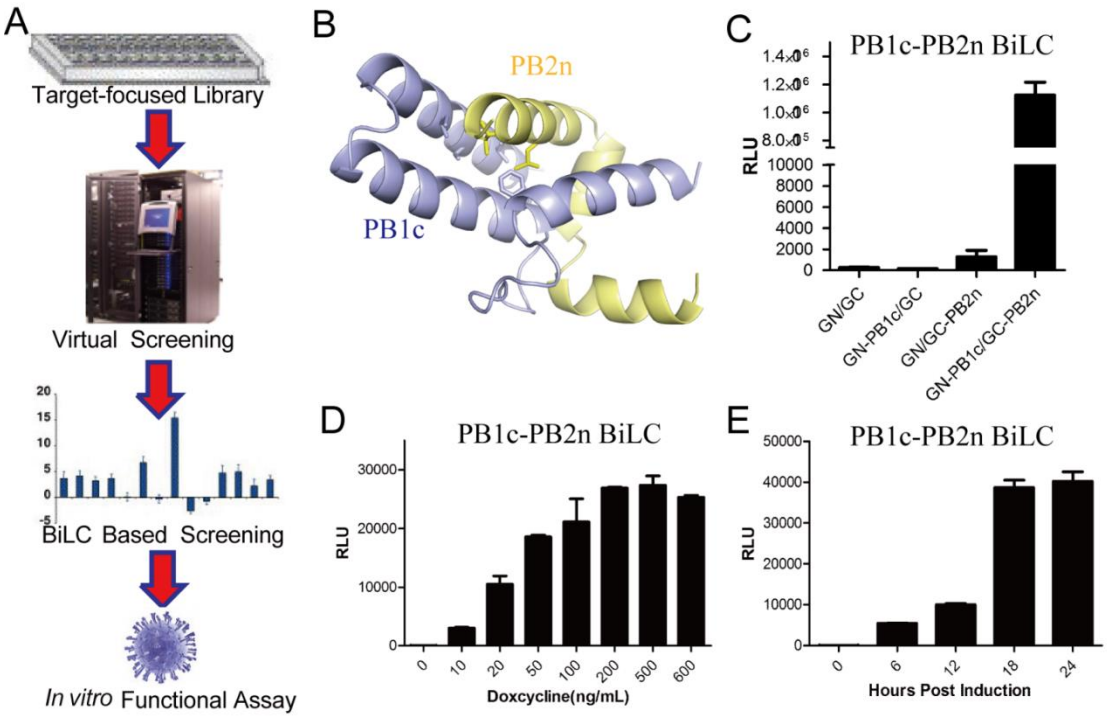

A
Target-focused Library
Virtual Screening
BiLC Based Screening
In vitro Functional Assay
B
PB2n
PB1c
C
PB1c-PB2n BiLC
RLU
GN/GC
GN-PB1c/GC
GN/GC-PB2n
GN-PB1c/GC-PB2n
D
PB1c-PB2n BiLC
RLU
Doxcycline(ng/mL)
E
PB1c-PB2n BiLC
RLU
Hours Post Induction

Figure S5.

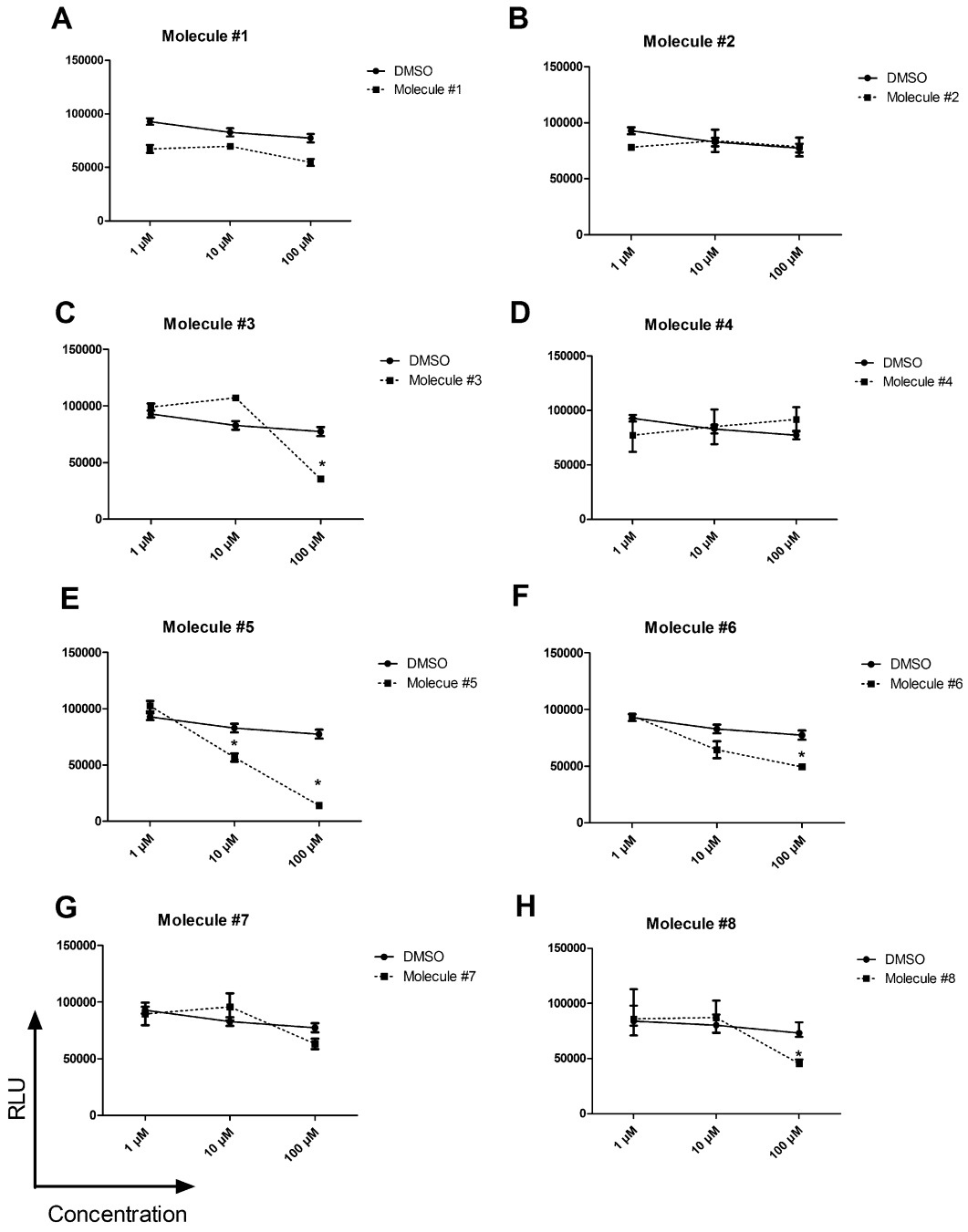

A
Molecule #1
150000
100000
50000
0
1 µM
10 µM
100 µM
DMSO
Molecule #1
B
Molecule #2
DMSO
Molecule #2
C
Molecule #3
DMSO
Molecule #3
*
D
Molecule #4
DMSO
Molecule #4
E
Molecule #5
DMSO
Molecue #5
*
*
F
Molecule #6
DMSO
Molecule #6
*
G
Molecule #7
DMSO
Molecule #7
H
Molecule #8
DMSO
Molecule #8
*
RLU
Concentration

Figure S6.

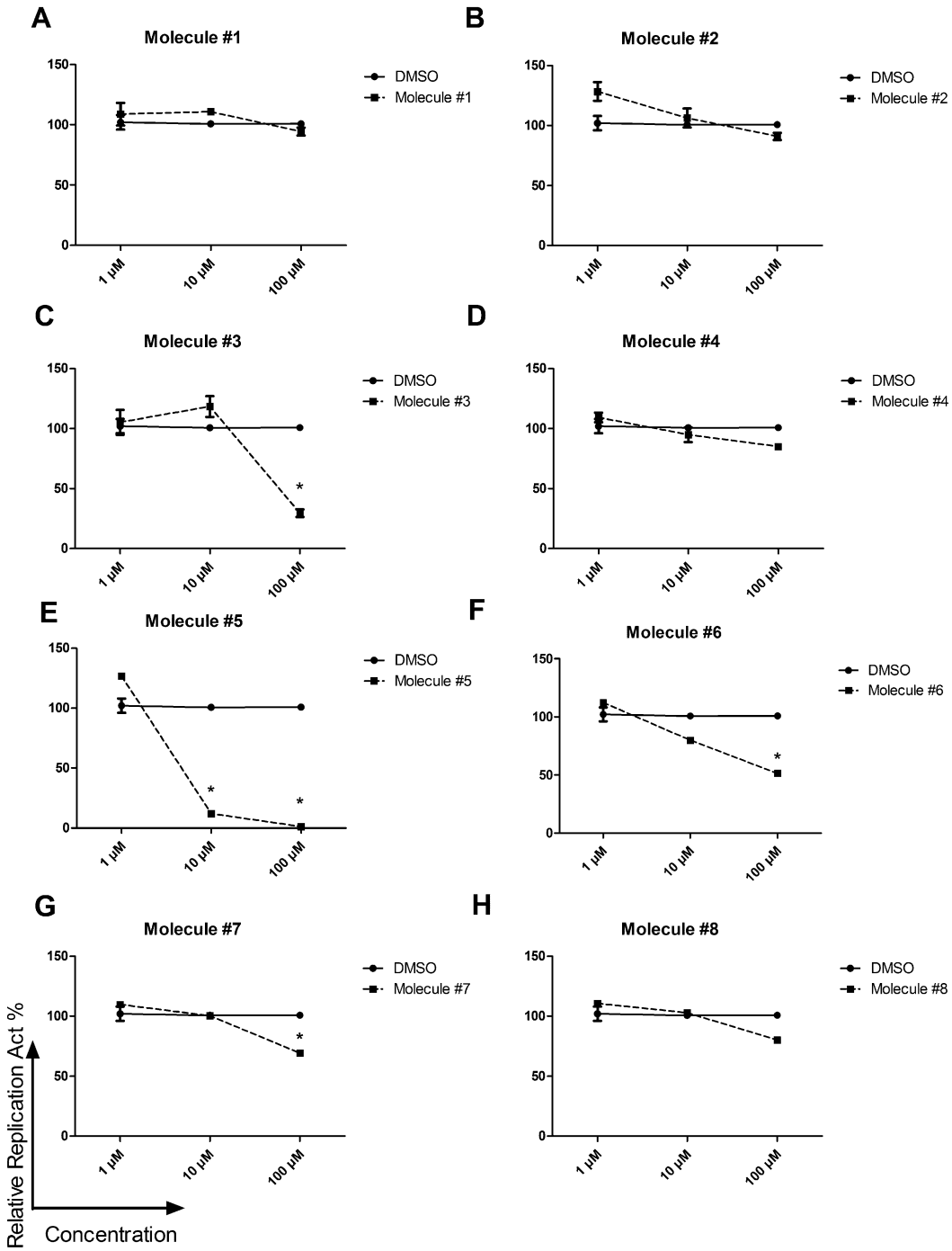

A
Molecule #1
B
Molecule #2
C
Molecule #3
D
Molecule #4
E
Molecule #5
F
Molecule #6
G
Molecule #7
H
Molecule #8
DMSO
Molecule #1
Molecule #2
Molecule #3
Molecule #4
Molecule #5
Molecule #6
Molecule #7
Molecule #8
150
100
50
0
1 µM
10 µM
100 µM
*
Relative Replication Act %
Concentration

Figure S7.

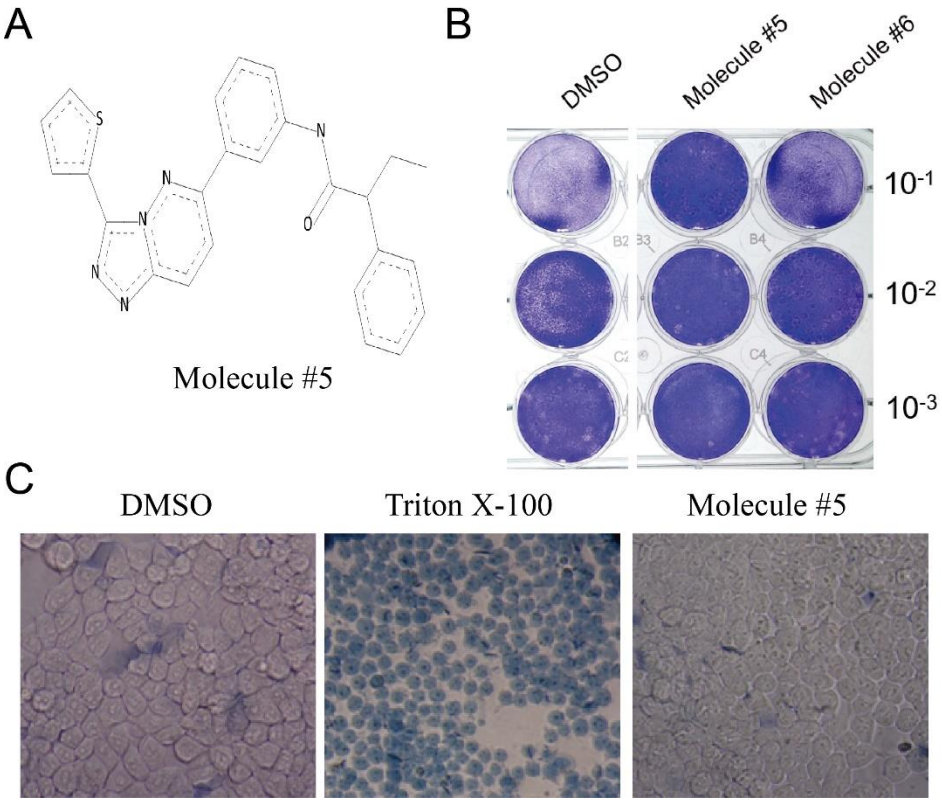

A
S
N
N
N
N
O
Molecule #5
B
DMSO
Molecule #5
Molecule #6
10-1
10-2
10-3
C
DMSO
Triton X-100
Molecule #5

Figure S8.

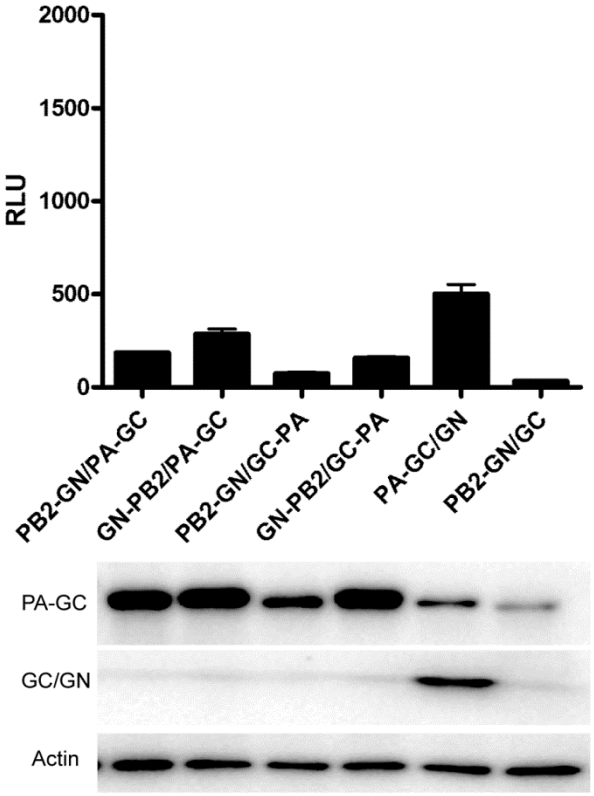
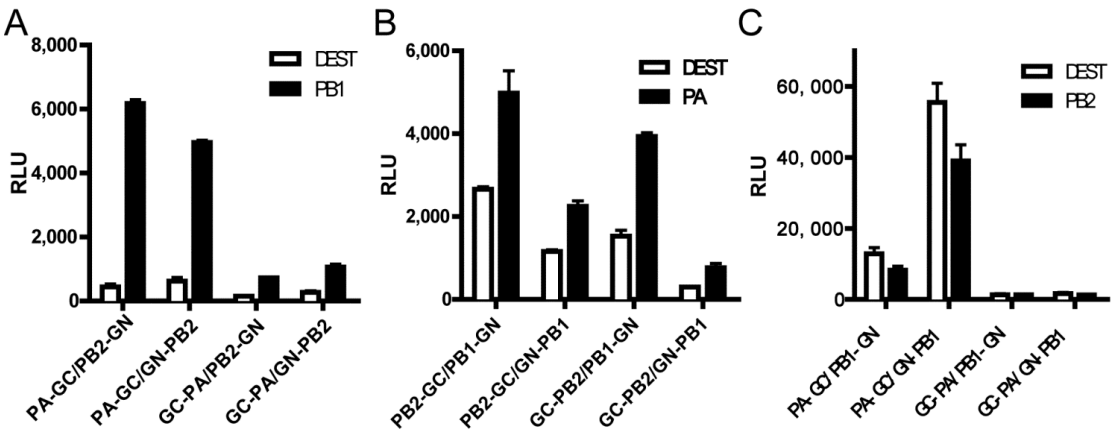

2000
1500
1000
500
0
RLU
PB2-GN/PA-GC
GN-PB2/PA-GC
PB2-GN/GC-PA
GN-PB2/GC-PA
PA-GC/GN
PB2-GN/GC
PA-GC
GC/GN
Actin

Figure S9.


A
8,000
6,000
4,000
2,000
0
RLU
DEST
PB1
PA-GC/PB2-GN
PA-GC/GN-PB2
GC-PA/PB2-GN
GC-PA/GN-PB2
B
6,000
4,000
2,000
0
RLU
DEST
PA
PB2-GC/PB1-GN
PB2-GC/GN-PB1
GC-PB2/PB1-GN
GC-PB2/GN-PB1
C
60, 000
40, 000
20, 000
0
RLU
DEST
PB2