Supplementary Information

for

Predictive Modeling & Mechanistic Validation of Synergistic Pimodivir Combinations for Anti-influenza therapy via PB2cap Affinity Boost

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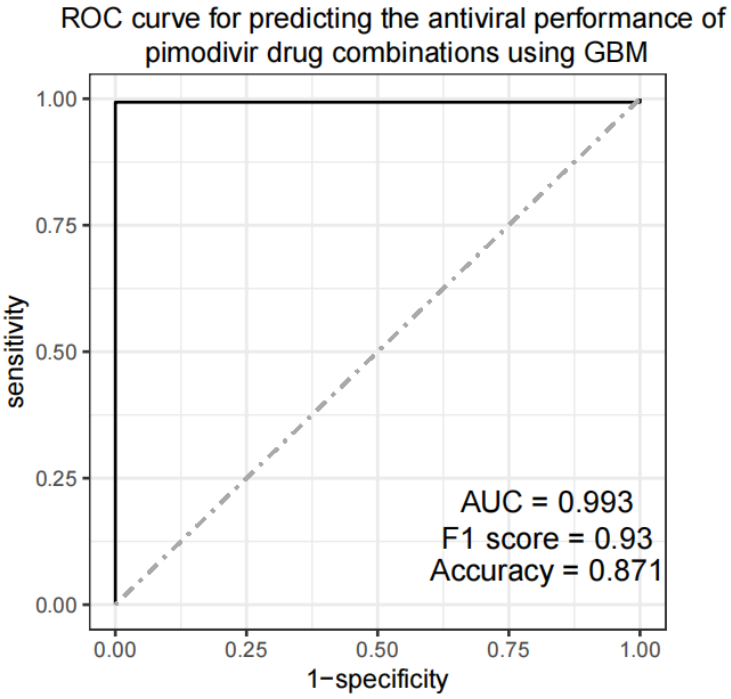
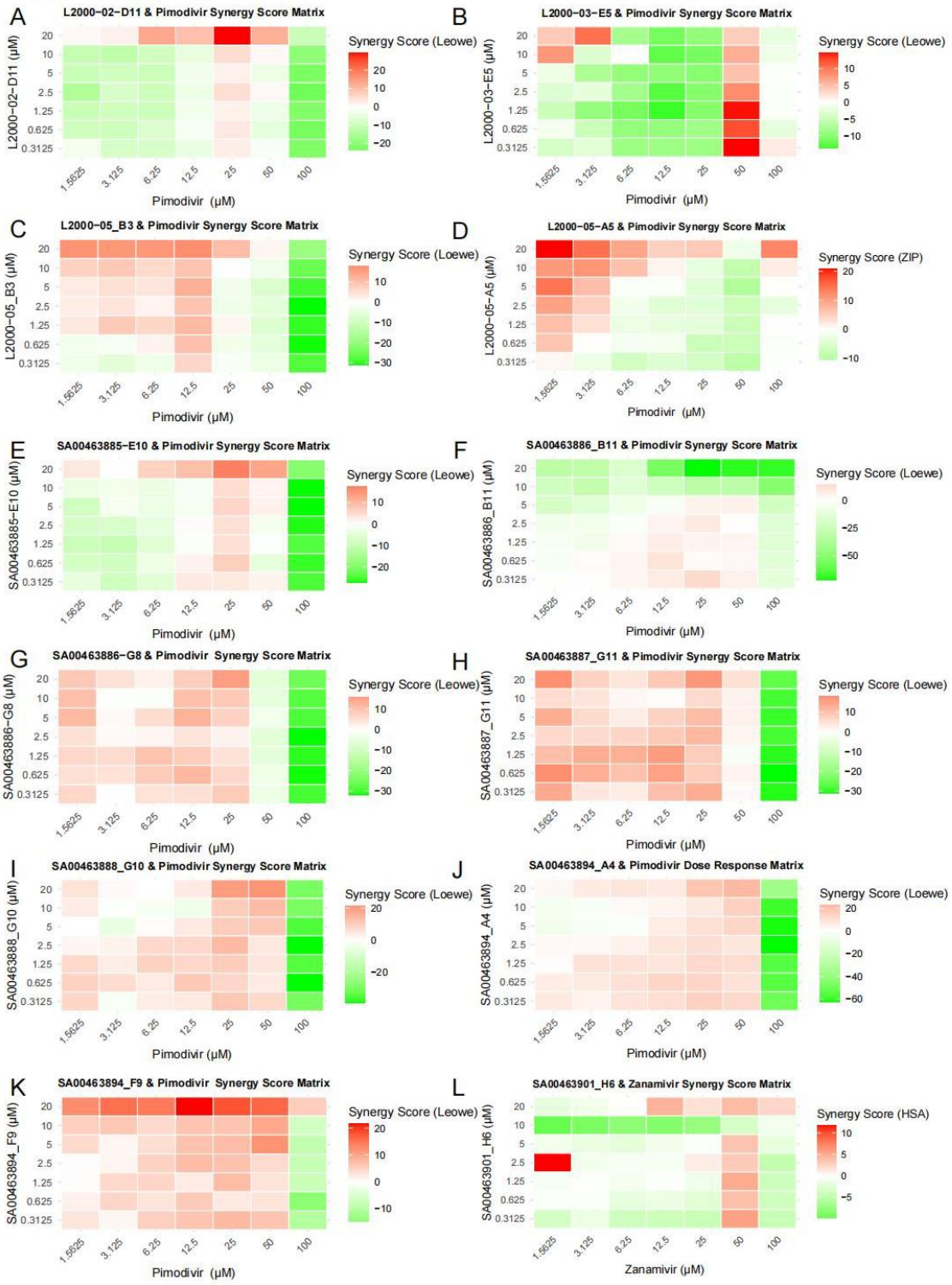
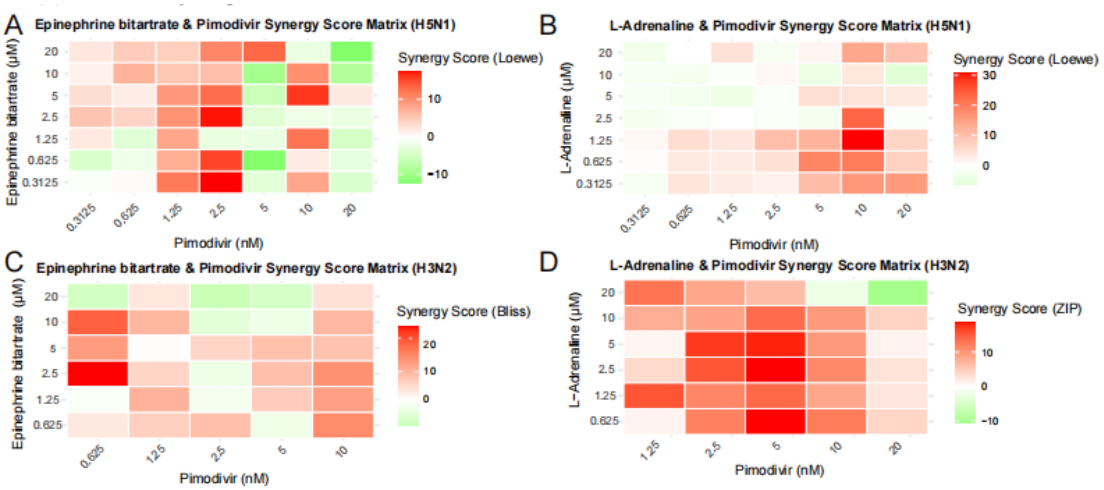


Supplementary Figure 1: Performance of the model in predicting the antiviral efficacy of antiviral drug combinations. ROC curve, F1 score, accuracy illustrating the predictive performance of a GBM model estimating the antiviral efficacy of Pimodivir combinations.

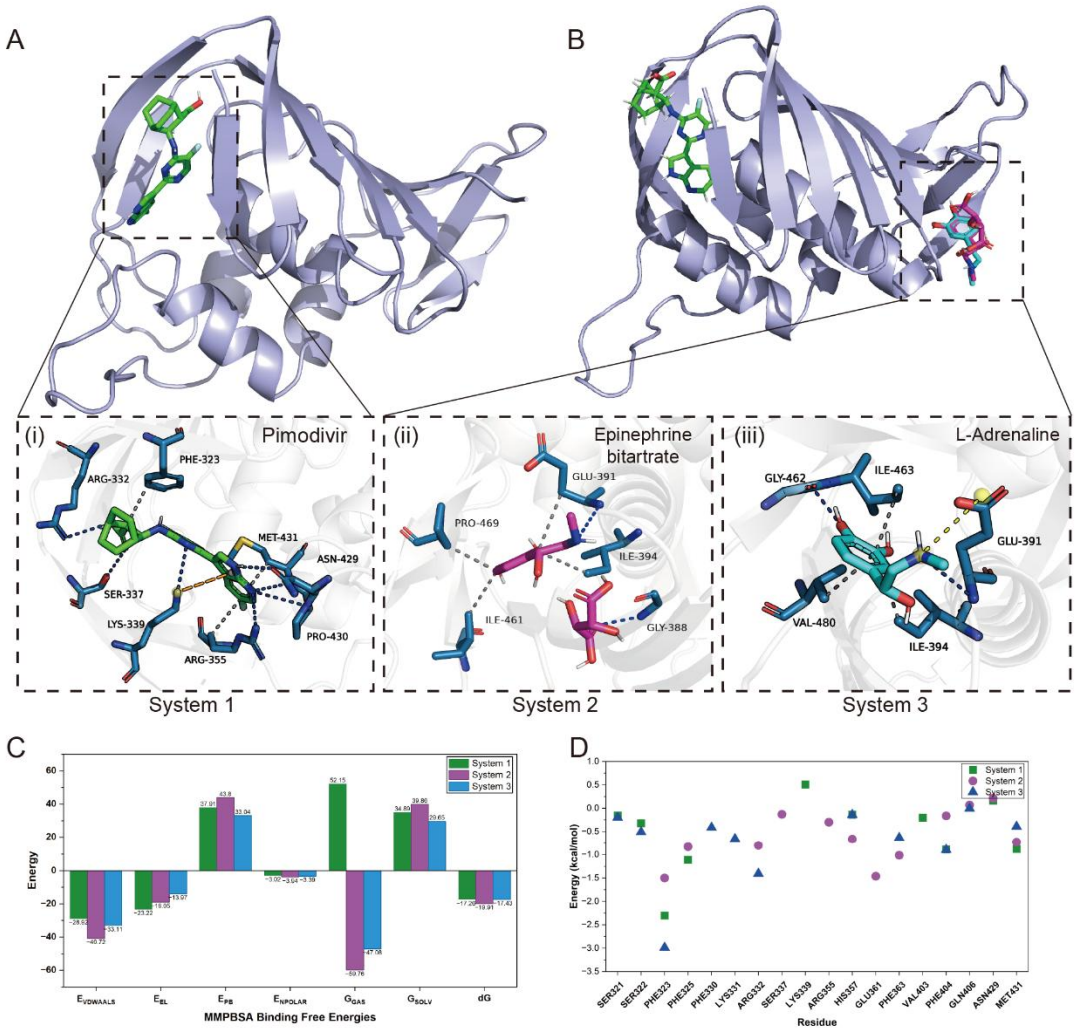


Supplementary Figure 2: Synergistic effects of drug combinations against H1N1 virus. A-L. Heatmaps of synergy scores obtained from cell viability values of H1N1 virus-infected cells treated with drug combinations at "checkerboard" concentrations in vitro. Specifically, the panels depict the following: (**A**) Loewe scores of Pimodivir with L2000-02_D11. (**B**) Loewe scores of Pimodivir with L2000-03_E5. (**C**) Loewe scores of Pimodivir with L2000-05_B3. (**D**) ZIP scores of Pimodivir with L2000-05_A5. (**E**) Loewe scores of Pimodivir with SA00463885_E10. (**F**) Loewe scores of Pimodivir

with SA00463886_B11. (**G**) Loewe scores of pimodivir with SA00463886_G8. (**H**) Loewe scores of pimodivir with SA00463887_G11. (**I**) Loewe scores of Pimodivir with SA00463888_G10. (**J**) Loewe scores of Pimodivir with SA00463894_A4. (**K**) Loewe scores of Pimodivir with SA00463894_F9. (**L**) HSA scores of Zanamivir with SA00463901_H6.



Supplementary Figure 3: Synergistic effects of drug combinations against H5N1 and H3N2 viruses. A - D. Heatmaps of synergy scores obtained from cell viability values of H5N1 (A and B) and H3N2 (C and D) virus-infected cells treated with drug combinations at "checkerboard" concentrations in vitro. Specifically, the panels depict the following: (**A**) Loewe scores of Pimodivir with L2000-03-A10 (Epinephrine bitartrate) against H5N1. (**B**) Loewe scores of Pimodivir with L2000-03-B10 (L-Adrenaline) against H5N1. (**C**) Loewe scores of Pimodivir with L2000-03-A10 (Epinephrine bitartrate) against H3N2. (**D**) Loewe scores of Pimodivir with L2000-03-B10 (L-Adrenaline) against H3N2.



Supplementary Figure 4: Underlying mechanism of the PB2cap conformational change-induced binding enhancement. (A) Molecular docking of Pimodivir bound to the cap-binding domain of PB2 protein (PDB ID: 5EG8). **(B)** Molecular docking of (ii) Epinephrine bitartrate and (iii) L-adrenaline with PB2cap. **(C)** Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) binding free energy analysis. **(D)** MM/PBSA residue energy contribution heatmap of potential PB2 residues affected by conformational change.

Supplementary Table 1 Table of 147 candidate drugs screened by 5 models.

	Drug_id		Drug_id		Drug_id
1	L2000-05_B3	41	L2000-05_F5	81	SA00463903_D11
2	L2000-09_D5	42	SA00463897_E3	82	SA00463899_C5
3	L2000-09_E5	43	L2000-06_D7	83	SA00463894_A4
4	L2000-05_G3	44	SA00463904_B4	84	L2000-05_C8
5	L2000-09_A7	45	L2000-09_H6	85	L2000-09_C4
6	L2000-09_A6	46	SA00463904_A2	86	SA00463901_D10
7	SA00463902_D5	47	SA00463904_B3	87	L2000-03_F11
8	SA00463897_H5	48	L2000-09_D7	88	L2000-09_G5
9	L2000-05_A5	49	L2000-09_C7	89	SA00463894_C8
10	L2000-01_E9	50	SA00463903_B9	90	SA00463903_F6
11	SA00463897_H11	51	SA00463904_B8	91	SA00463898_E10
12	SA00463896_G11	52	SA00463886_E6	92	SA00463900_G3
13	L2000-02_D11	53	L2000-05_D5	93	SA00463885_F9
14	SA00463904_A7	54	SA00463894_E7	94	SA00463888_D2
15	SA00463885_F11	55	SA00463886_F10	95	SA00463887_B2
16	L2000-09_H7	56	SA00463886_H8	96	SA00463899_E6
17	SA00463887_F3	57	SA00463899_B7	97	SA00463894_G4
18	L2000-06_C4	58	SA00463900_D10	98	SA00463887_H9
19	SA00463896_F10	59	SA00463899_A4	99	L2000-09_H2
20	L2000-09_A5	60	L2000-09_E4	100	SA00463902_B5
21	L2000-09_H4	61	L2000-05_H6	101	SA00463898_B11
22	L2000-06_B4	62	L2000-09_F2	102	SA00463899_E3
23	SA00463896_G3	63	SA00463900_D4	103	L2000-03_A10
24	L2000-03_F8	64	SA00463888_H7	104	SA00463903_C10
25	SA00463902_E7	65	SA00463899_D10	105	SA00463888_A6
26	L2000-05_H8	66	SA00463903_F4	106	SA00463888_G2
27	SA00463900_H6	67	SA00463903_G4	107	SA00463903_C3
28	SA00463902_B2	68	SA00463902_C5	108	SA00463886_B11
29	L2000-02_H8	69	L2000-04_C10	109	L2000-05_D11
30	L2000-03_E5	70	SA00463899_C6	110	L2000-06_F2
31	L2000-09_C5	71	L2000-06_F4	111	SA00463886_A11
32	SA00463900_H10	72	SA00463900_D3	112	SA00463894_D3
33	SA00463896_F6	73	SA00463903_E2	113	L2000-04_E11
34	L2000-09_B7	74	SA00463887_G11	114	SA00463902_A3
35	L2000-09_E2	75	SA00463888_G10	115	SA00463903_A3
36	L2000-06_A2	76	SA00463886_G8	116	SA00463901_B5
37	L2000-09_G2	77	SA00463899_D6	117	SA00463894_G2
38	SA00463904_B10	78	SA00463888_D10	118	L2000-03_B10
39	L2000-09_F5	79	SA00463904_A8	119	SA00463903_C5
40	SA00463888_A7	80	SA00463885_A7	120	SA00463885_A2

121	SA00463902_C7				
122	L2000-05_E5				
123	L2000-05_A2				
124	SA00463901_H5				
125	SA00463899_A9				
126	SA00463894_F9				
127	SA00463901_G10				
128	SA00463888_F2				
129	SA00463898_A8				
130	SA00463894_A7				
131	SA00463888_F6				
132	L2000-05_G7				
133	SA00463897_C8				
134	SA00463897_D11				
135	SA00463899_B3				
136	SA00463899_B8				
137	L2000-03_B5				
138	SA00463901_H6				
139	L2000-02_E5				
140	SA00463885_E10				
141	SA00463885_E9				
142	L2000-03_F10				
143	SA00463903_E5				
144	SA00463894_E10				
145	SA00463901_A7				
146	L2000-09_G6				
147	L2000-02_E7				

Supplementary Table 2 Table of predicted performance of antiviral drug combinations

Drug	Baloxavir	Favipiravir	Laninamivir	Naproxen	Oseltamivir	Peramivir	Pimodivir	Zanamivir
Best model	GBM	GBM	Stepwise +GBM	GBM	GBM	Lasso + GBM	GBM	Enet(α=0.1)
AUC	0.506	0.573	0.657	0.587	0.560	0.648	0.993	0.595
F1 score	0.293	0.6	0.939	0.775	0.770	0.207	0.930	0.749
Accuracy	0.639	0.429	0.884	0.633	0.626	0.116	0.871	0.599
Precision	0.297	0.429	0.884	0.633	0.626	0.116	1	0.599

Supplementary Table 3 Table of Drug Characteristics

Feature Name	Feature Length	Specific features included
physical and chemical properties	21	Molecular weight, number of heavy atoms, number of hydrogen bond acceptors, number of hydrogen bond donors, lipid solubility coefficient, number of rotating single bonds, electrical absorption, logarithmic oil-water partition coefficient (LogP), molecular weight (Molar Refractivity (MR)), electrical absorption, interatomic distance descriptor, AMR (LogP*MR), number of stereogenic centers of atoms, aromaticity, number of chiral centers, double bonding number of stereogenic centers, aromaticity, number of chiral centers, number of double bonds, number of stereoisomers, number of aromatic rings, degree of unsaturation, charge
MACCS	167	
avalon	512	
topological fingerprint	2048	
ECFP	4096（4*1024）	The radii of the fingerprints are 0, 2, 4, 6
FCFP	3072（3*1024）	The radii of the fingerprints are2、4、6
LECFP	2048（2*1024）	The radii of the fingerprints are4、6
LFCFP	2048（2*1024）	The radii of the fingerprints are4、6

Reference

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4. Yuan S, Chu H, Zhao H, Zhang K, Singh K, Chow BKC, et al. Identification of a small-molecule inhibitor of influenza virus via disrupting the subunits interaction of the viral polymerase. Antiviral Res. 2016;125:34–42.