

## SUPPLEMENTARY DATA

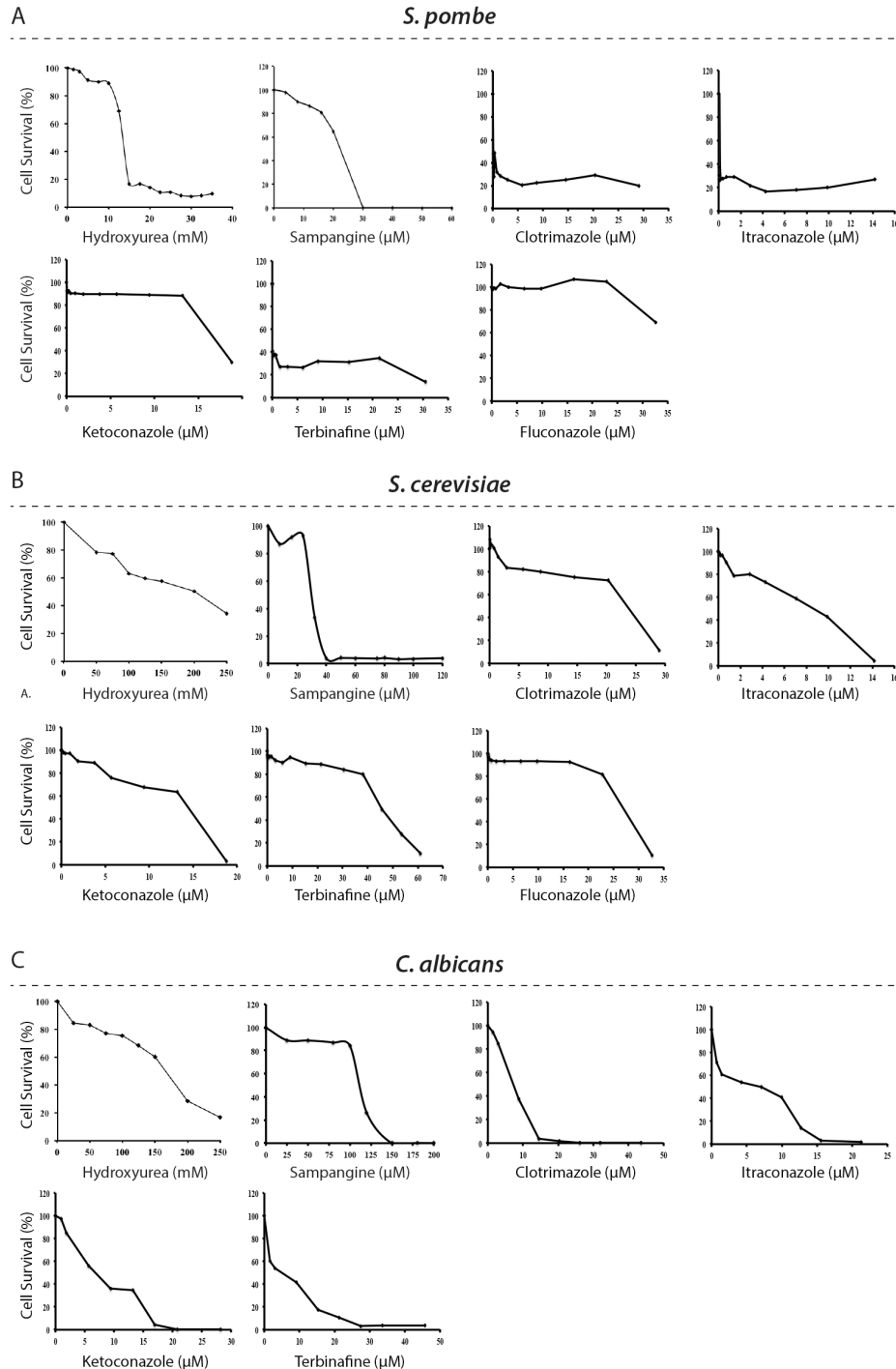
### Novel Cell-Killing Mechanisms of Hydroxyurea and the Implication Towards Combination Therapy for the Treatment of Fungal Infections

Amanpreet Singh, Ameeta Agarwal, and Yong-jie Xu

**Table S1. List of yeast strains used in this study.**

<b>Strain</b>	<b>Genotype</b>	<b>Source</b>
YJ150	<i>h<sup>-</sup> leu1-32 ura4-D18 ade6-M210 [LEU2 vector]</i>	Lab stock
YJ1261	<i>h<sup>-</sup> leu1-32 ura4-D18 ade6 [prom-Erg11-term-LEU2]</i>	Lab stock
YJ760	<i>h<sup>-</sup> Δrad3::ura4 leu1-32 ura4-D18 ade6 [LEU2 vector]</i>	Lab stock
APS184	<i>h<sup>+</sup> Δrad3::ura4 leu1-32 ura4-D18 ade6 [prom-Erg11-term-LEU2]</i>	This study
APS31	<i>h<sup>?</sup> hem13-1 cds1-6his2HA leu1-32 ura4-D18 ade6 [LEU2 vector]</i>	This Study
APS171	<i>h<sup>?</sup> hem13-1 cds1-6his2HA leu1-32 ura4-D18 ade6 [prom-erg11-term-LEU2]</i>	This Study
YJ1291	<i>h<sup>+</sup> erg11-1:ura4 cds1-6his2HA leu1-32 ura4-D18 ade6 [LEU2 vector]</i>	Lab stock
YJ1230	<i>h<sup>+</sup> erg11-1:ura4 cds1-6his2HA leu1-32 ura4-D18 ade6 [prom-Erg11-term-LEU2]</i>	Lab stock
SC5314	<i>Wild-type C. albicans strain</i>	Heitman lab
W303	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i>	Lab stock

**Figure S1.** The dose-dependent cytotoxic effects of HU, sampangine (SMP) and the indicated antifungal drugs on wild type *S. pombe*, *S. cerevisiae* and *C. albicans* measured by the 96-well plate assay. Logarithmically growing wild type *S. pombe* (A), *S. cerevisiae* (B) and *C. albicans* (C) cells were inoculated on 96 well plates at 3000 cells/well in YE6S, YPD and SDB medium, respectively. The drugs were added to each well at the indicated concentrations in the final volume of 200  $\mu$ l. The same amounts of carriers were added as control. Cells were incubated at 30°C for 48 days. The plates were scanned in a plate reader at A600. The graphs were made from values obtained from the plate reader using Microsoft excel. Each data points are the averages of readings of three separate wells.

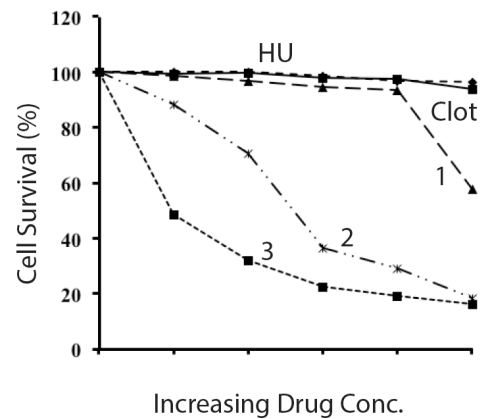


**Figure S2. The synergistic cytotoxic effect of HU and clotrimazole in *S. pombe*.** (A) Cell survival rate and the combination index (CI) values of the drug combinations 1 to 3 were determined by the 96-well plate assay and calculated using Chou-Talalay method as described in Materials and Methods. Numbers marked in bold indicate very strong synergism. (B) Graphical representation of cell-killing effect of HU, clotrimazole and various combinations of the two drugs. (C) Combination index plot. Fa values on the X-axis are the values of the cell growth inhibition at various CI values. CI values above the dash line, on the dash line, and below the dash line represent antagonistic, additive, and synergistic effect, respectively.

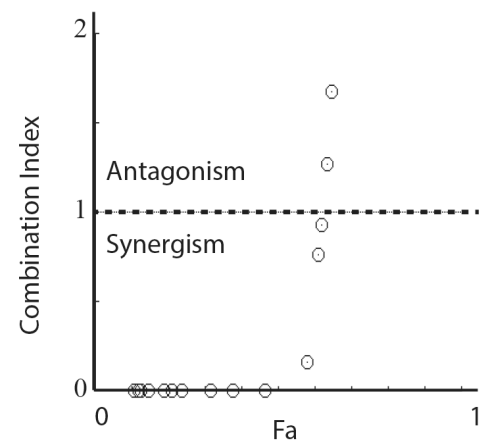
A.

	HU (mM)	Clot ( $\mu$ M)	Cell Survival	CI Values
HU	5	0	0.0647	-
	7.5	0	0.635	-
	10	0	0.632	-
Clot	0	0.014	0.652	-
	0	0.020	0.651	-
	0	0.028	0.641	-
	0	0.034	0.638	-
	0	0.043	0.614	-
Combo 1	5	0.014	0.646	1.677
	5	0.020	0.635	1.272
	5	0.028	0.621	0.932
	5	0.034	0.612	0.766
	<b>5</b>	<b>0.043</b>	<b>0.378</b>	<b>5.43E-4</b>
Combo 2	<b>7.5</b>	<b>0.014</b>	<b>0.579</b>	<b>0.160</b>
	<b>7.5</b>	<b>0.020</b>	<b>0.464</b>	<b>4.18E-3</b>
	<b>7.5</b>	<b>0.028</b>	<b>0.239</b>	<b>2.51E-6</b>
	<b>7.5</b>	<b>0.034</b>	<b>0.19</b>	<b>3.36E-7</b>
	<b>7.5</b>	<b>0.043</b>	<b>0.12</b>	<b>7.1E-9</b>
Combo 3	<b>10</b>	<b>0.014</b>	<b>0.318</b>	<b>2.56E-5</b>
	<b>10</b>	<b>0.020</b>	<b>0.21</b>	<b>5.04E-7</b>
	<b>10</b>	<b>0.028</b>	<b>0.146</b>	<b>2.6E-8</b>
	<b>10</b>	<b>0.034</b>	<b>0.126</b>	<b>8.7E-9</b>
	<b>10</b>	<b>0.043</b>	<b>0.107</b>	<b>2.7E-9</b>

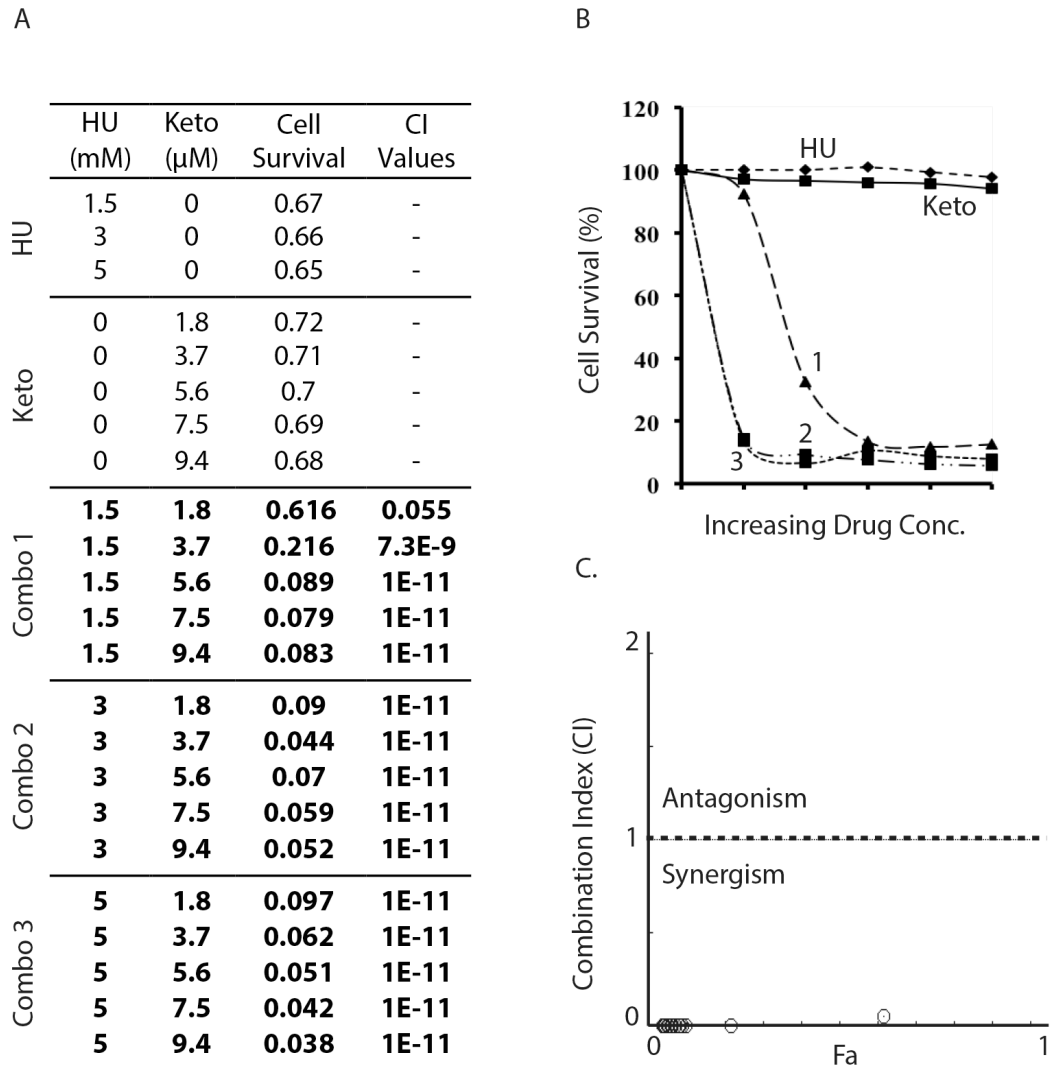
B.



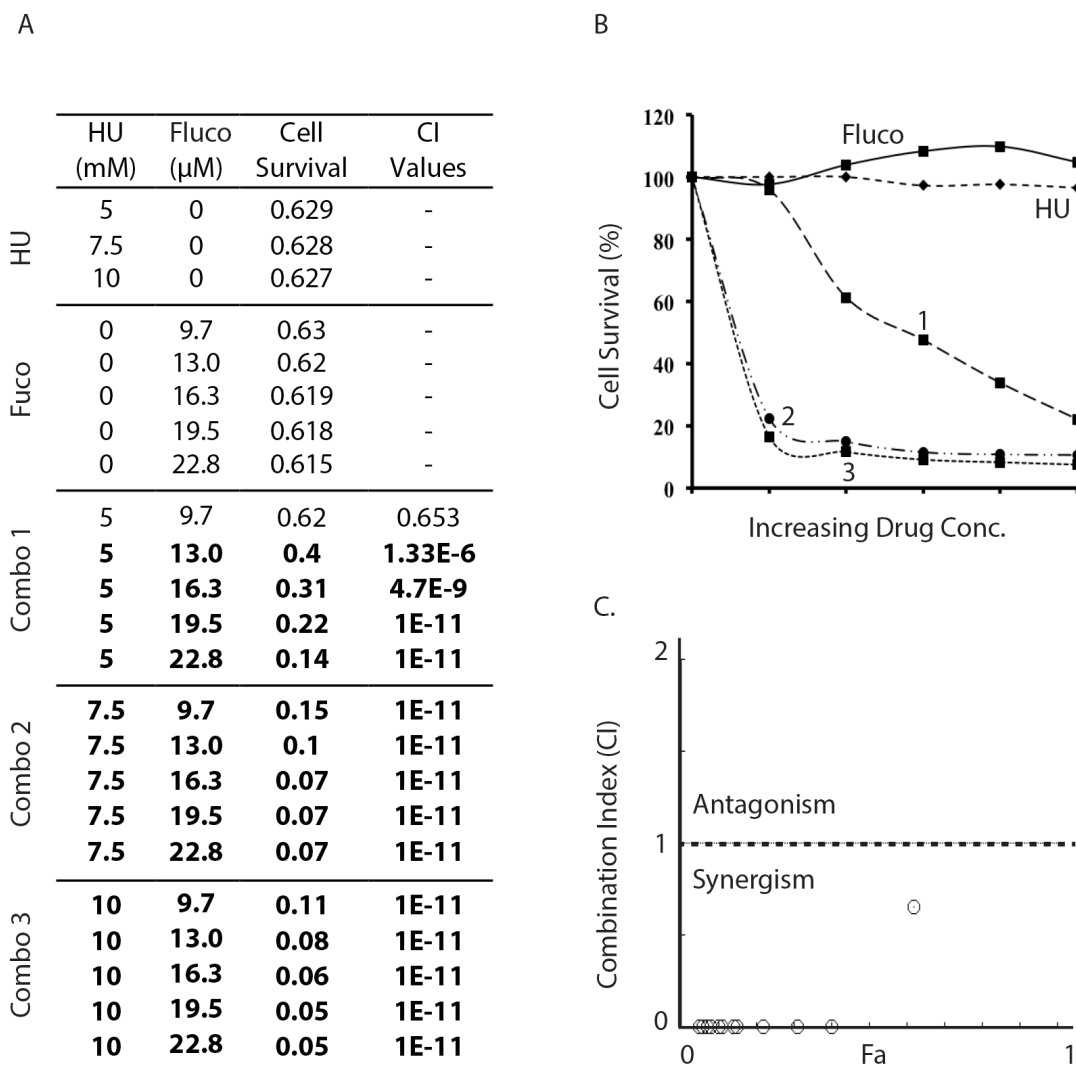
C.



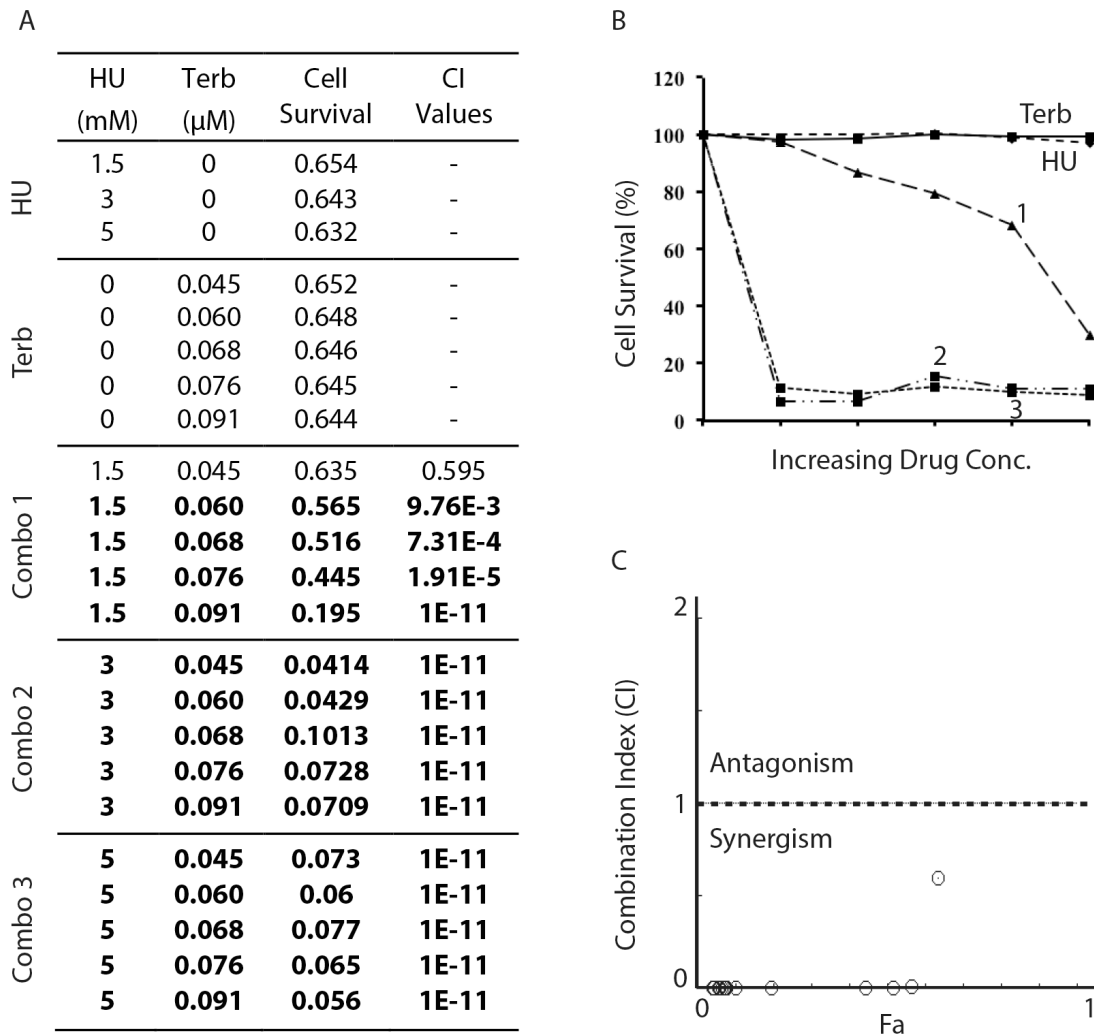
**Figure S3. The synergistic cytotoxic effect of HU and ketoconazole in wild type *S. pombe*.**  
See Figure S2 for detailed description.



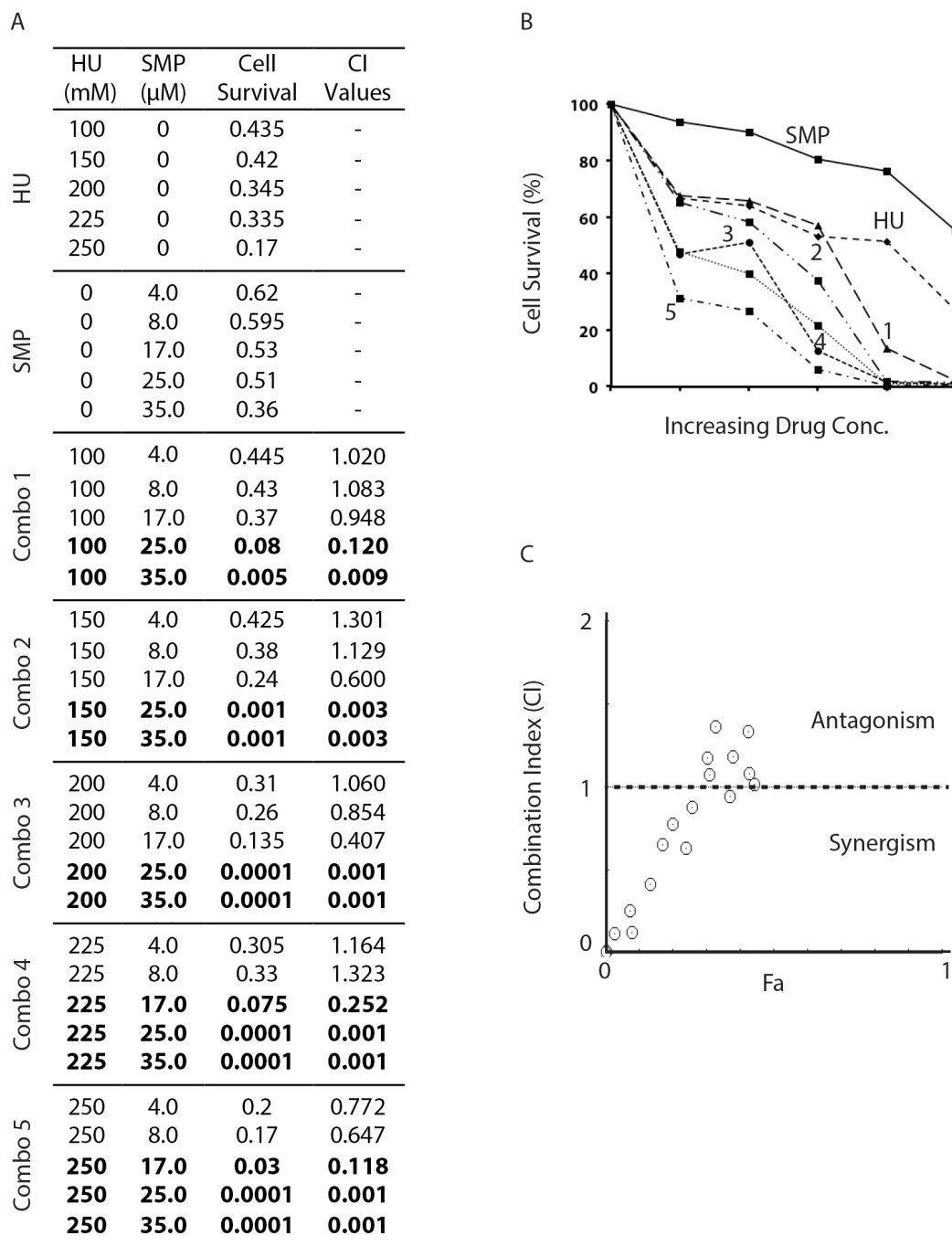
**Figure S4. The synergistic cytotoxic effect of HU and fluconazole in wild type *S. pombe*.**



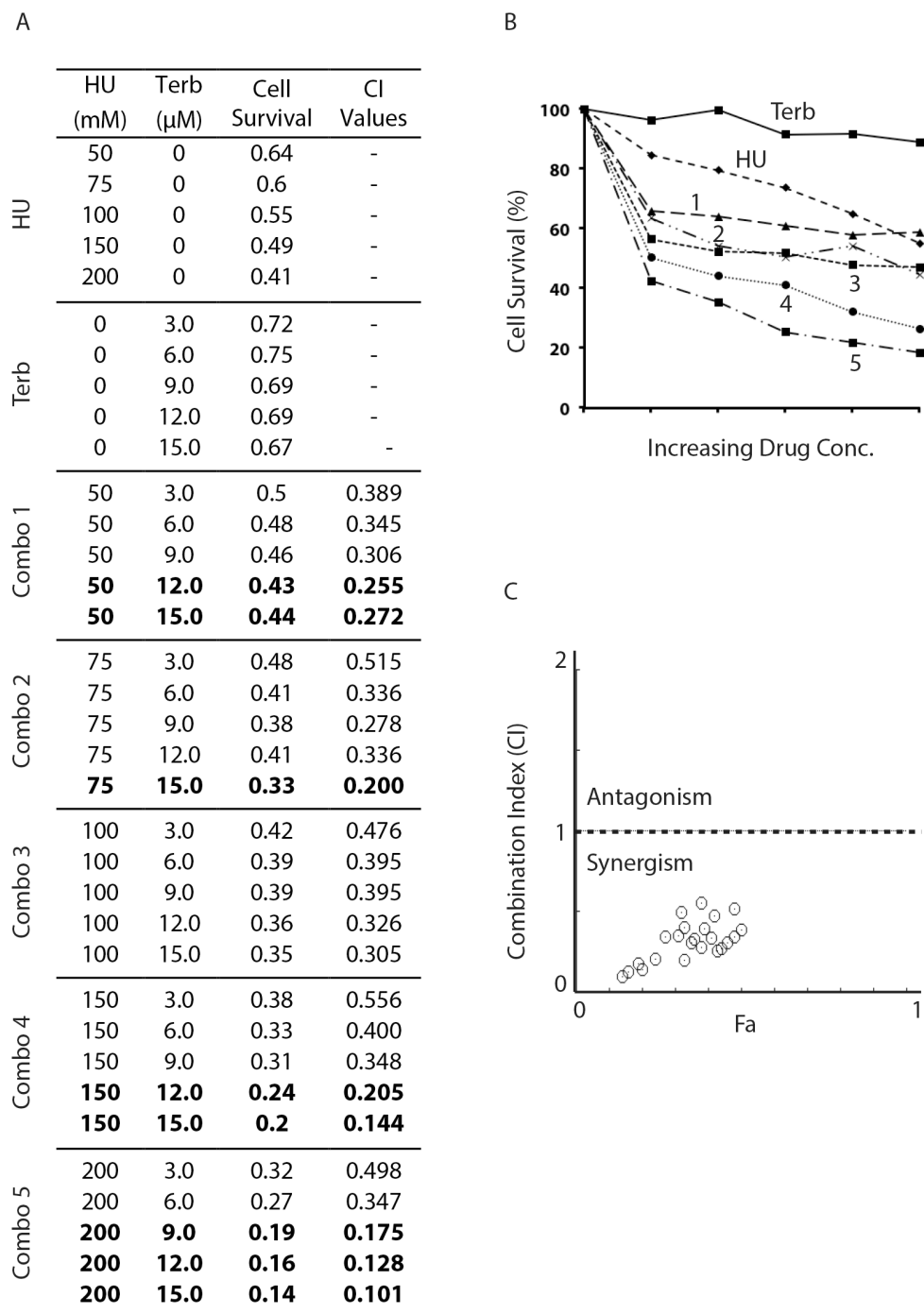
**Figure S5. The synergistic cytotoxic effect of HU and terbinafine in wild type *S. pombe*.**



**Figure S6. The synergistic cytotoxic effect of HU and Sampangine in wild type *S. cerevisiae*.**  
The experiment was carried out similar to that in *S. pombe* as described in Figure S2.

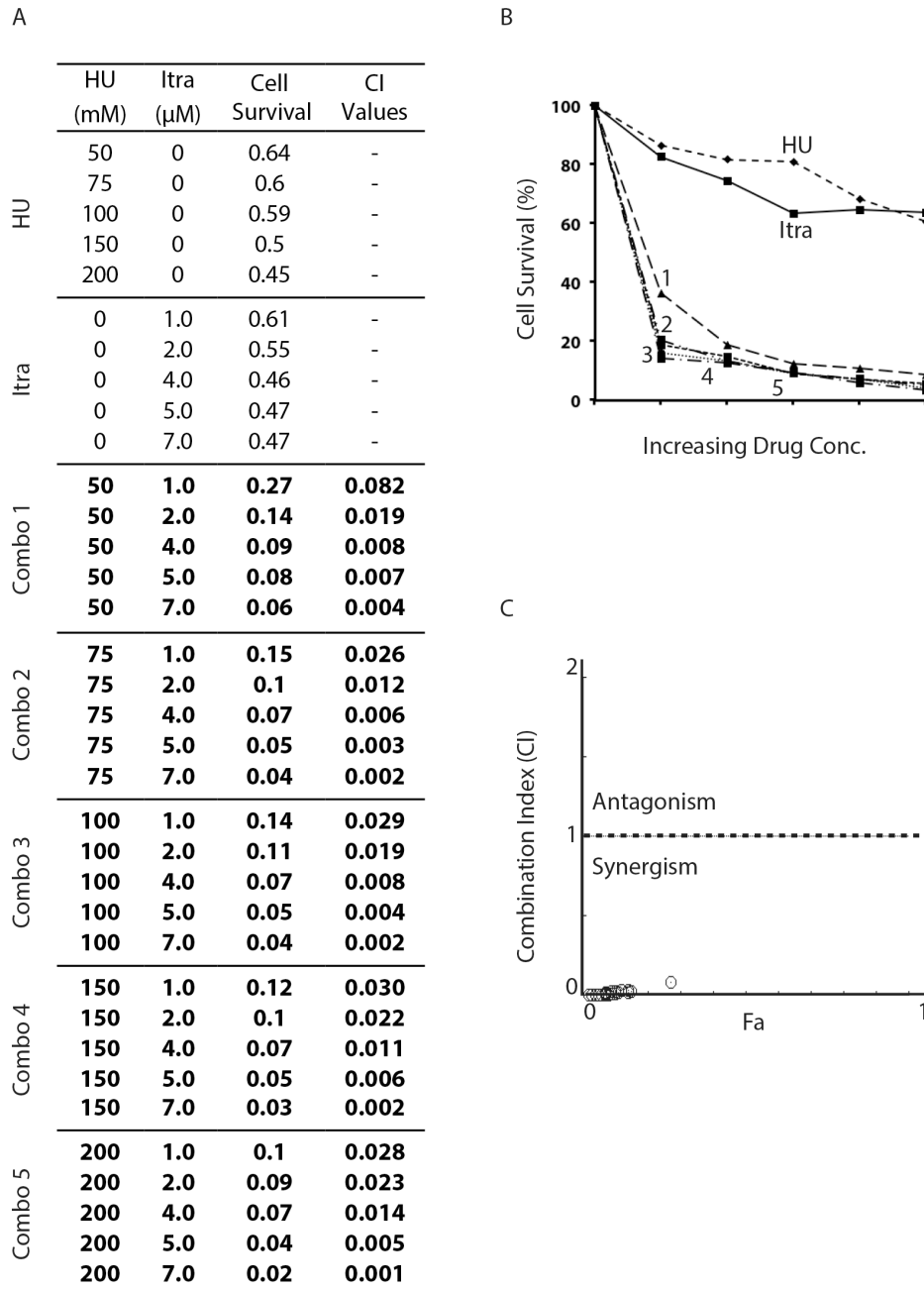


**Figure S7. The synergistic cytotoxic effect of HU and terbinafine in wild type *S. cerevisiae*.**



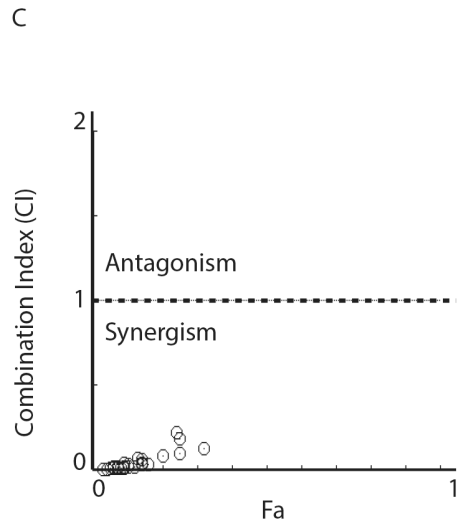
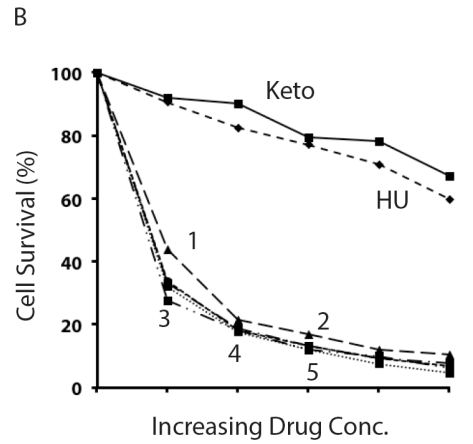


**Figure S8. The synergistic cytotoxic effect of HU and itraconazole in wild type *S. cerevisiae*.**

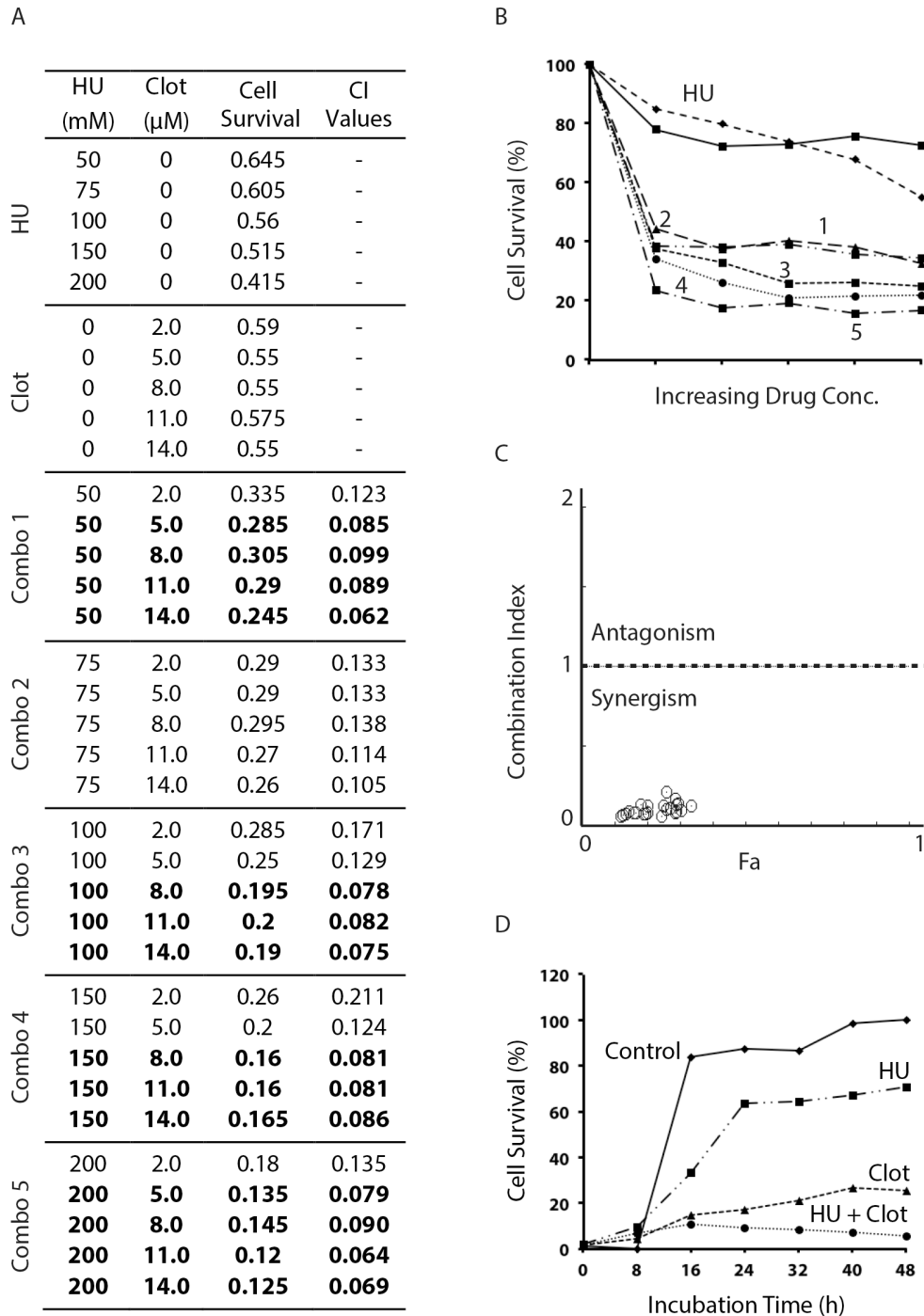


**Figure S9. The synergistic cytotoxic effect of HU and ketoconazole in wild type *S. cerevisiae*.**

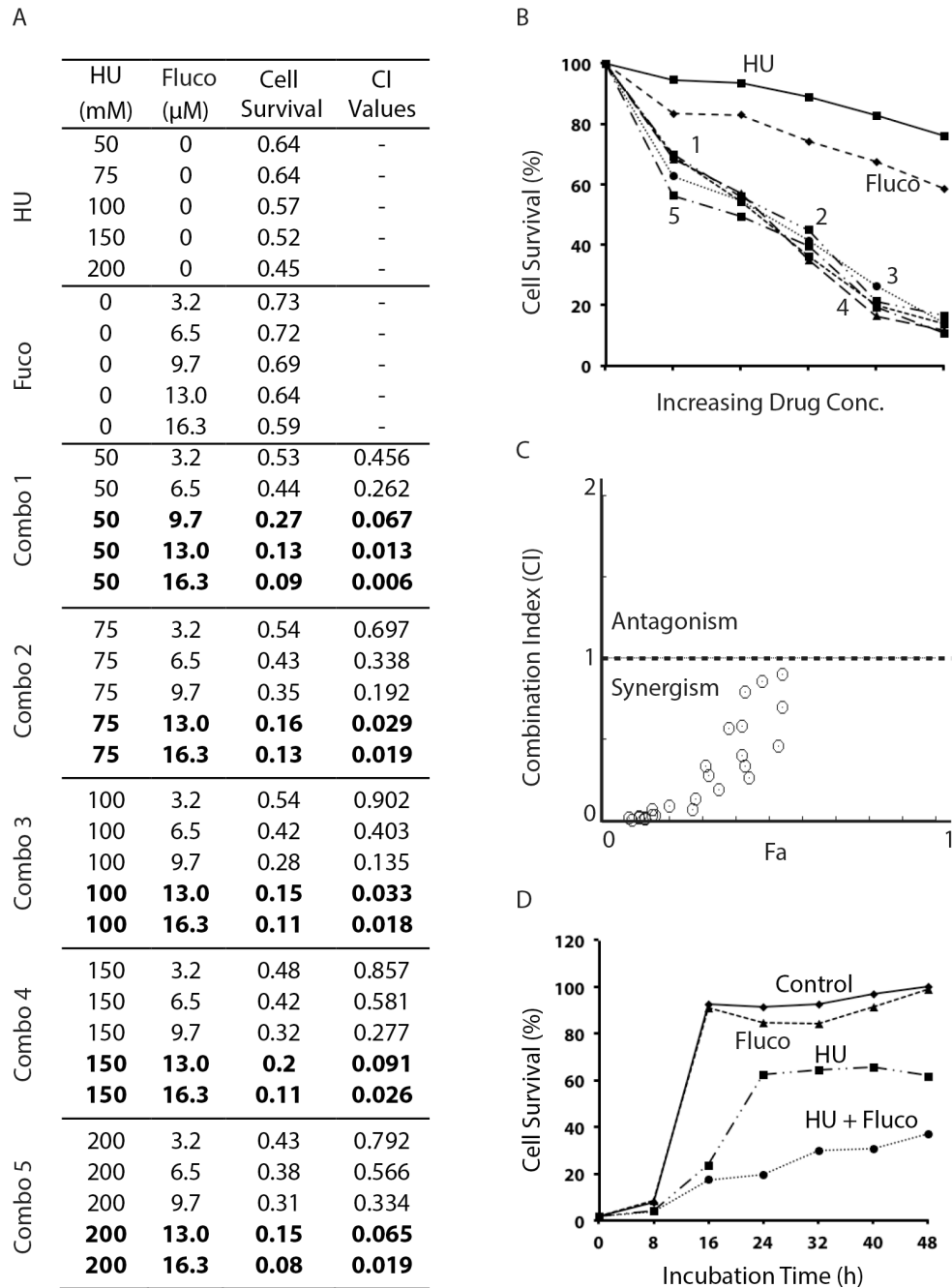
	HU (mM)	Keto ( $\mu$ M)	Cell Survival	CI Values
HU	50	0	0.67	-
	75	0	0.61	-
	100	0	0.57	-
	150	0	0.53	-
	200	0	0.44	-
Keto	0	1.8	0.68	-
	0	3.7	0.67	-
	0	5.6	0.59	-
	0	7.5	0.58	-
	0	9.4	0.50	-
Combo 1	50	1.8	0.32	0.126
	<b>50</b>	<b>3.7</b>	<b>0.16</b>	<b>0.031</b>
	<b>50</b>	<b>5.6</b>	<b>0.12</b>	<b>0.019</b>
	<b>50</b>	<b>7.5</b>	<b>0.09</b>	<b>0.011</b>
	<b>50</b>	<b>9.4</b>	<b>0.08</b>	<b>0.010</b>
Combo 2	<b>75</b>	<b>1.8</b>	<b>0.25</b>	<b>0.099</b>
	<b>75</b>	<b>3.7</b>	<b>0.14</b>	<b>0.033</b>
	<b>75</b>	<b>5.6</b>	<b>0.1</b>	<b>0.018</b>
	<b>75</b>	<b>7.5</b>	<b>0.07</b>	<b>0.010</b>
	<b>75</b>	<b>9.4</b>	<b>0.06</b>	<b>0.008</b>
Combo 3	<b>100</b>	<b>1.8</b>	<b>0.2</b>	<b>0.080</b>
	<b>100</b>	<b>3.7</b>	<b>0.14</b>	<b>0.042</b>
	<b>100</b>	<b>5.6</b>	<b>0.09</b>	<b>0.019</b>
	<b>100</b>	<b>7.5</b>	<b>0.07</b>	<b>0.012</b>
	<b>100</b>	<b>9.4</b>	<b>0.04</b>	<b>0.005</b>
Combo 4	150	1.8	0.25	0.185
	<b>150</b>	<b>3.7</b>	<b>0.14</b>	<b>0.061</b>
	<b>150</b>	<b>5.6</b>	<b>0.1</b>	<b>0.034</b>
	<b>150</b>	<b>7.5</b>	<b>0.07</b>	<b>0.018</b>
	<b>150</b>	<b>9.4</b>	<b>0.05</b>	<b>0.010</b>
Combo 5	200	1.8	0.24	0.223
	<b>200</b>	<b>3.7</b>	<b>0.13</b>	<b>0.069</b>
	<b>200</b>	<b>5.6</b>	<b>0.09</b>	<b>0.036</b>
	<b>200</b>	<b>7.5</b>	<b>0.06</b>	<b>0.018</b>
	<b>200</b>	<b>9.4</b>	<b>0.03</b>	<b>0.005</b>



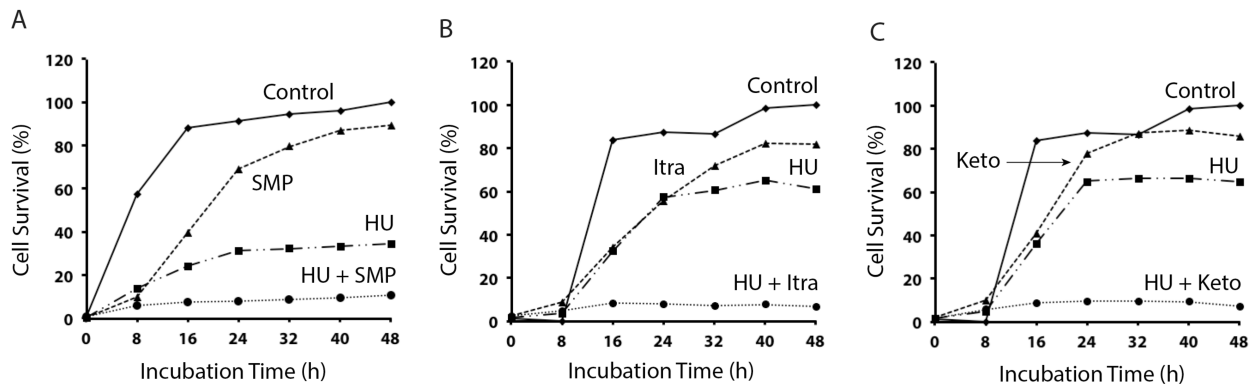
**Figure S10. The synergistic cell-killing effect of HU and clotrimazole on wild type *S. cerevisiae*.** The experiment was carried out similar to that in *S. pombe* as described in Figure S2. D is the time course study of the combination of 200 mM HU and 14  $\mu$ M clotrimazole when used alone or in combination (HU+Clot).



**Figure S11. The synergistic cytotoxic effect of HU and fluconazole in wild type *S. cerevisiae*.** D is the time course study of the combination of 200 mM HU and 16.3  $\mu$ M fluconazole when used alone or in combination (HU+Fluco).



**Figure S12. Time course study of the synergistic cytotoxic effects of HU in combination with SMP, itraconazole or ketoconazole in *S. cerevisiae*.** Logarithmically growing wild type *S. cerevisiae* was inoculated in YPD medium on 96 well plates at 3000 cells/well. 250 mM HU and 35  $\mu$ M SMP (**A**), 200 mM HU and 7  $\mu$ M itraconazole (**B**), or 200 mM HU and 9.4  $\mu$ M ketoconazole (**C**) were added to the cell cultures either alone or in drug combinations. Plates were incubated at 30°C and scanned at the indicated time points.

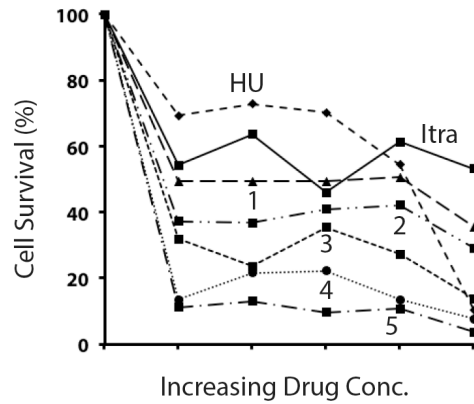


**Figure S13. The synergistic cell-killing effect of HU and itraconazole on wild type *C. albicans*.** D is the time course study of the combination of 200 mM HU and 7  $\mu$ M itraconazole when used alone or in combination.

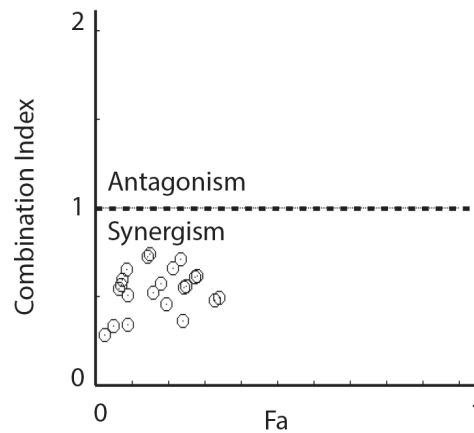
A

	HU (mM)	Itra ( $\mu$ M)	Cell Survival	CI Values
HU	50	0	0.485	-
	75	0	0.470	-
	100	0	0.465	-
	150	0	0.365	-
	200	0	0.07	-
Itra	0	1.0	0.36	-
	0	2.0	0.425	-
	0	4.0	0.31	-
	0	5.0	0.41	-
	0	7.0	0.355	-
Combo 1	50	1.0	0.33	0.483
	50	2.0	0.33	0.483
	50	4.0	0.33	0.483
	50	5.0	0.34	0.498
	50	7.0	0.24	0.362
Combo 2	75	1.0	0.25	0.562
	75	2.0	0.245	0.553
	75	4.0	0.275	0.612
	75	5.0	0.28	0.622
	75	7.0	0.195	0.457
Combo 3	100	1.0	0.215	0.660
	100	2.0	0.16	0.522
	100	4.0	0.235	0.711
	100	5.0	0.18	0.572
	100	7.0	0.09	0.341
Combo 4	150	1.0	0.09	0.512
	150	2.0	0.145	0.726
	150	4.0	0.15	0.745
	150	5.0	0.09	0.512
	150	7.0	0.05	0.340
Combo 5	200	1.0	0.075	0.600
	200	2.0	0.085	0.655
	200	4.0	0.065	0.543
	200	5.0	0.07	0.572
	<b>200</b>	<b>7.0</b>	<b>0.025</b>	<b>0.284</b>

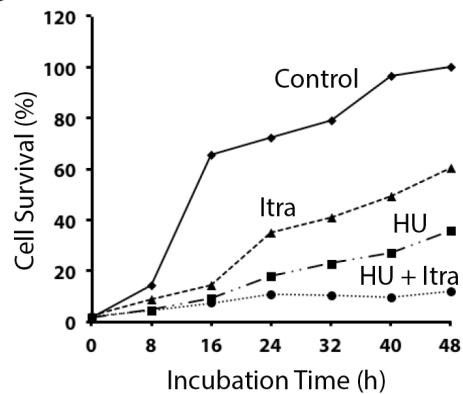
B



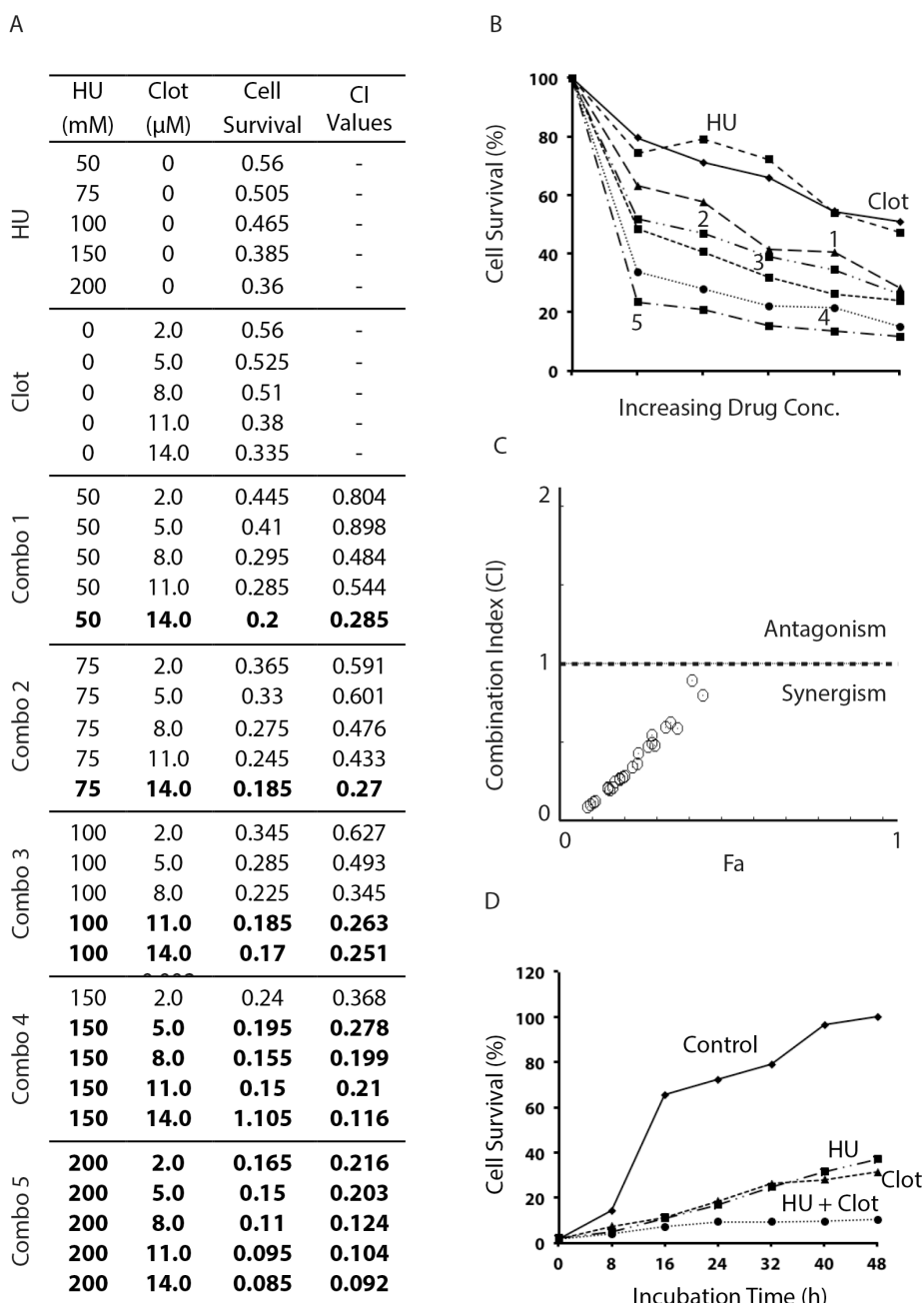
C



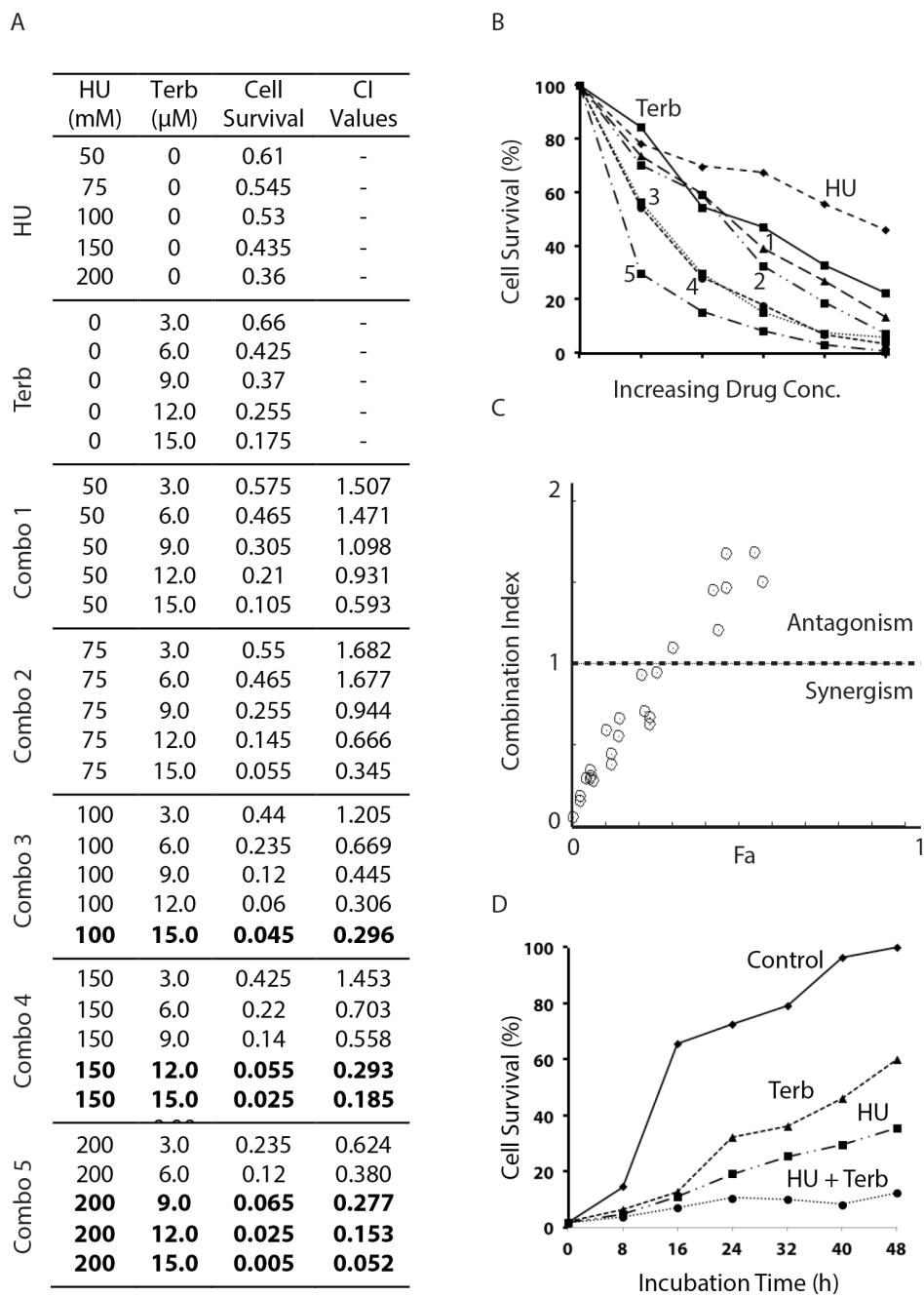
D



**Figure S14. The synergistic cytotoxic effect of HU and clotrimazole on wild type *C. albicans*.** D is the time course study of the combination of 200 mM HU and 14  $\mu$ M clotrimazole when used alone or in combination.

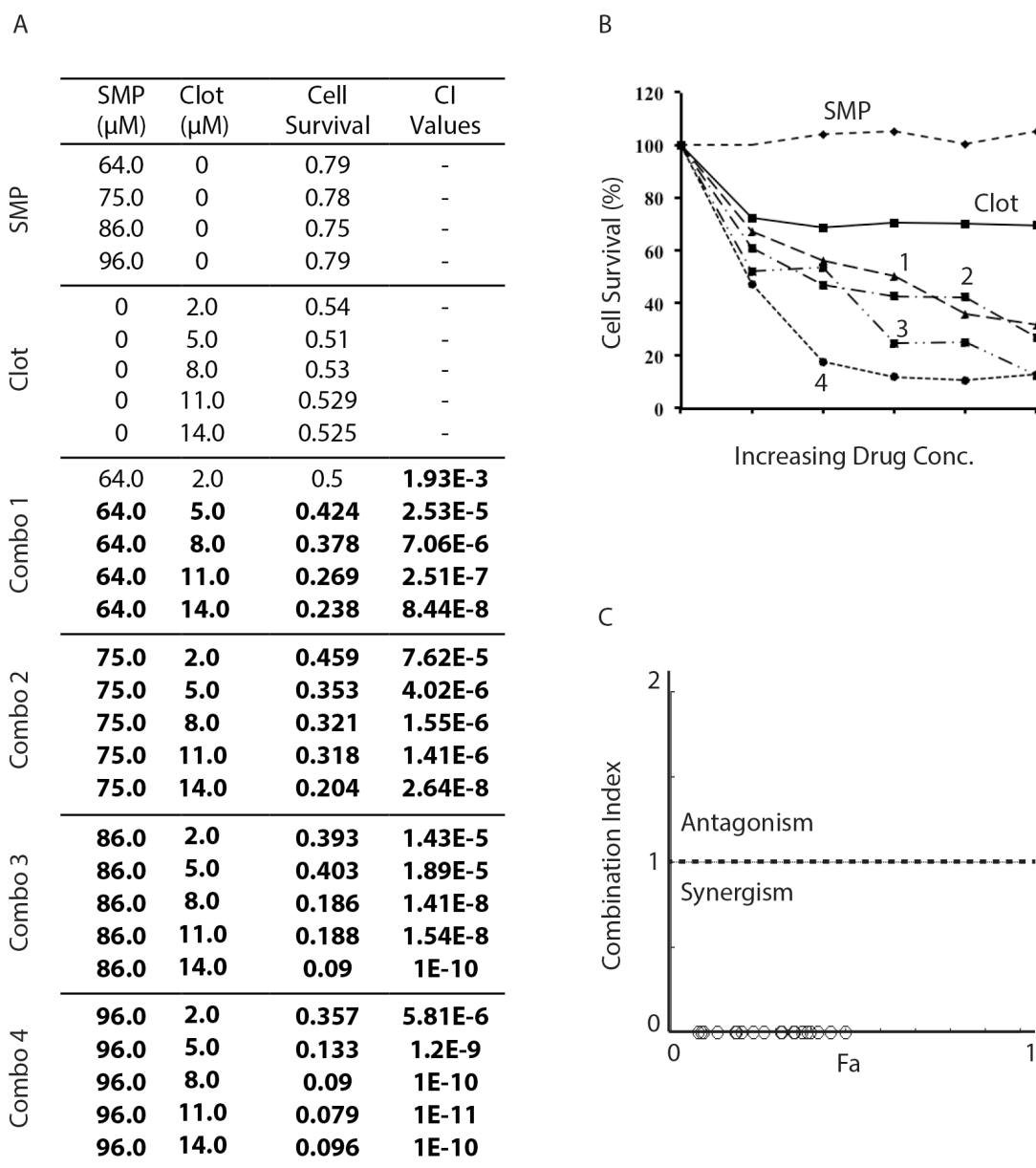


**Figure S15. The synergistic cell-killing effect of HU and terbinafine on wild type *C. albicans*.** The experiment was carried out similar to that in *S. pombe* as described in Figure S2. D is the time course study of the combination of 200 mM HU and 15  $\mu$ M terbinafine when used alone or in combination.





**Figure S16. The significant synergistic cell-killing effect of Sampangine and clotrimazole on wild type *C. albicans*.** The experiment was carried out similar to that in *S. pombe* as described in Figure S2.

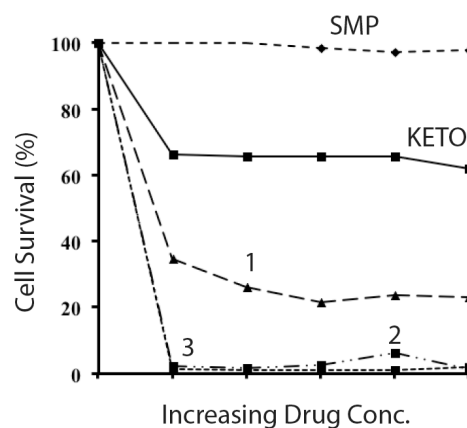


**Figure S17. The significant synergistic cell-killing effect of Sampangine and ketoconazole on wild type *C. albicans*.**

A

	SMP ( $\mu$ M)	Keto ( $\mu$ M)	Cell Survival	CI Values
SMP	64.0	0	0.79	-
	75.0	0	0.78	-
	86.0	0	0.77	-
Keto	0	1.8	0.53	-
	0	3.7	0.52	-
	0	5.6	0.51	-
	0	7.5	0.5	-
	0	9.4	0.49	-
Combo 1	<b>64.0</b>	<b>1.8</b>	<b>0.28</b>	<b>3.68E-3</b>
	<b>64.0</b>	<b>3.7</b>	<b>0.21</b>	<b>1.43E-3</b>
	<b>64.0</b>	<b>5.6</b>	<b>0.17</b>	<b>7.53E-4</b>
	<b>64.0</b>	<b>7.5</b>	<b>0.18</b>	<b>8.93E-4</b>
	<b>64.0</b>	<b>9.4</b>	<b>0.18</b>	<b>8.93E-4</b>
Combo 2	<b>75.0</b>	<b>1.8</b>	<b>0.017</b>	<b>2E-6</b>
	<b>75.0</b>	<b>3.7</b>	<b>0.013</b>	<b>1E-6</b>
	<b>75.0</b>	<b>5.6</b>	<b>0.021</b>	<b>3.33E-6</b>
	<b>75.0</b>	<b>7.5</b>	<b>0.049</b>	<b>2.9E-5</b>
	<b>75.0</b>	<b>9.4</b>	<b>0.0111</b>	<b>6.73E-7</b>
Combo 3	<b>86.0</b>	<b>1.8</b>	<b>0.009</b>	<b>5E-7</b>
	<b>86.0</b>	<b>3.7</b>	<b>0.007</b>	<b>2E-7</b>
	<b>86.0</b>	<b>5.6</b>	<b>0.009</b>	<b>4.56E-7</b>
	<b>86.0</b>	<b>7.5</b>	<b>0.006</b>	<b>1.66E-7</b>
	<b>86.0</b>	<b>9.4</b>	<b>0.0165</b>	<b>2.08E-6</b>

B



C

