Supplementary Information

Article

Hsp90-Mediated Multi-Drug Resistance in DNA Polymerase-Defective Strains of *Candida albicans*

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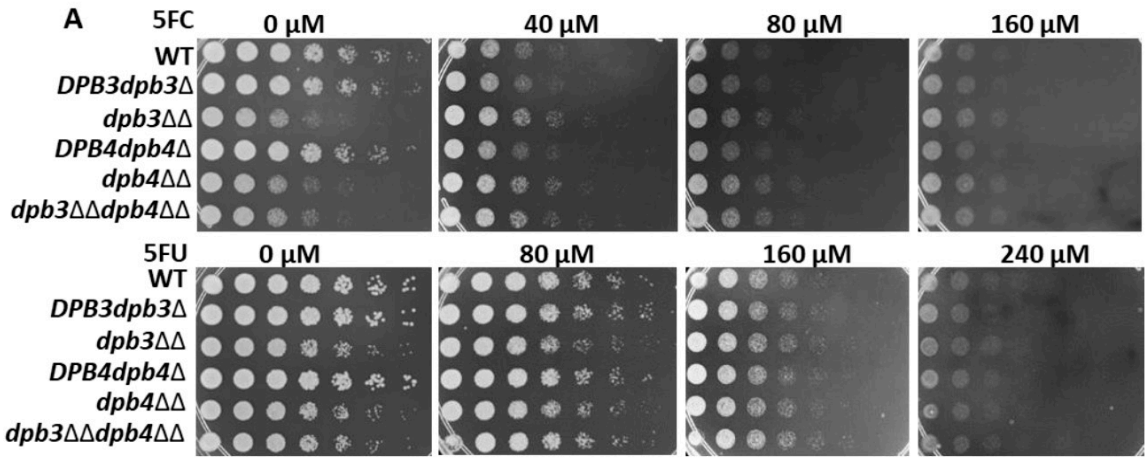
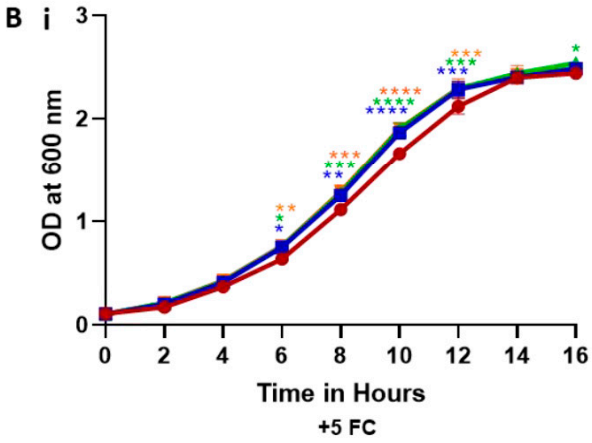
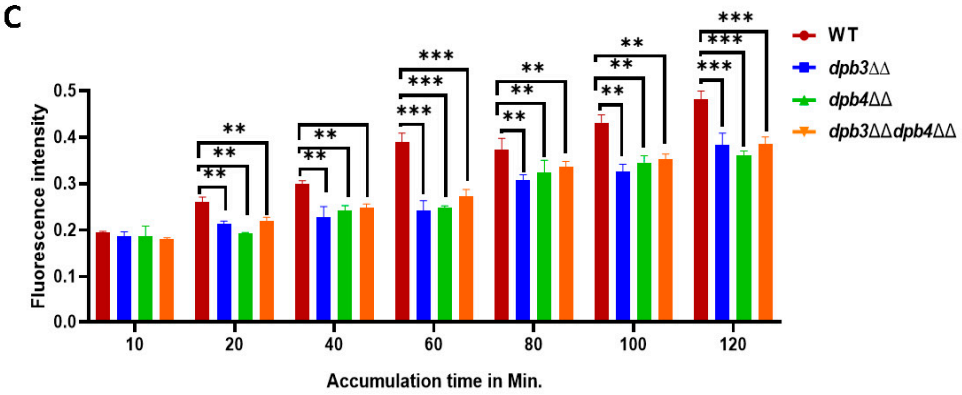
Running title: Role of Hsp90 in drug resistance

Keywords: DNA replication, DNA polymerase epsilon, *Candida*, Candidiasis, Hsp90, azoles, drug resistance, biofilm

Supplementary Figure S1: (A). Overnight cultures of WT, *DPB3dpb3*Δ, *dpb3*ΔΔ, *DPB4dpb4*Δ, *dpb4*ΔΔ, and *dpb3*ΔΔ*dpb4*ΔΔ strains were serially diluted and spotted on YPD plates without or with the indicated concentrations of 5FC and 5FU. All the plates were incubated at 30 °C for 48 hr and photographed. (**B**) Growth curve analyses of these strains were carried out in the presence of 5-FC (50 μM, i). The absorbance was measured at OD_{600} for 21 hr at a regular interval (Supp. Table -5) and was plotted using GraphPad Prism 8.0. **(C)** Drug accumulation assay was carried by adding berberine to 5 x 10^7 *C. albicans* cells in 1 x PBS and incubated at 30 °C in 200 rpm shaking condition. Cells were collected at regular interval and fluorescence was measured in an ELISA plate reader with the excitation and emission wavelength of 360 nm and 520 nm, respectively. Asterisks indicate (****P≤0.0001, ***P≤0.001, **P≤0.01, and *P<0.05) the statistically significant differences between the results of WT and mutant strains using a two-way ANOVA test.


A
5FC
0 µM
40 µM
80 µM
160 µM
WT
DPB3dpb3Δ
dpb3ΔΔ
DPB4dpb4Δ
dpb4ΔΔ
dpb3ΔΔdpb4ΔΔ
5FU
0 µM
80 µM
160 µM
240 µM
WT
DPB3dpb3Δ
dpb3ΔΔ
DPB4dpb4Δ
dpb4ΔΔ
dpb3ΔΔdpb4ΔΔ


B i
OD at 600 nm
Time in Hours
+5 FC


C
Fluorescence intensity
Accumulation time in Min.
WT
dpb3ΔΔ
dpb4ΔΔ
dpb3ΔΔdpb4ΔΔ

Supplementary Table-S1: The growth curve analyses of WT, *dpb3*ΔΔ, *dpb4*ΔΔ, and *dpb3*ΔΔ*dpb4*ΔΔ strains were carried out without (**a**) and with fluconazole (**b**), amphotericin B (**c**), and caspofungin (**d**). The absorbance measured at different time points and statistical analysis has been given as tables.

(**a**) without any drugs

														P value		
	Time(hrs)	WT	Average	Average of averages ± SD	*dpb3*ΔΔ	Average	Average of averages ± SD	*dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*WT vs dpb3ΔΔ*	*WT vs dpb4ΔΔ*	*WT vs dpb3ΔΔ dpb4ΔΔ*
Set 1	0	0.101 0.098	0.0995		0.102 0.106	0.104		0.103 0.107	0.105		0.104 0.099	0.1015				
Set 2	0	0.106 0.107	0.1065	0.103±0.0035	0.103 0.105	0.104	0.104±0.001	0.105 0.108	0.1065	0.10575±0.0007	0.105 0.103	0.104	0.10275±0.0012	>0.9999	0.9983	>0.9999
Set 1	2	0.287 0.291	0.289		0.249 0.274	0.2615		0.243 0.265	0.254		0.253 0.268	0.2605				
Set 2	2	0.297 0.299	0.298	0.2935±0.0045	0.256 0.269	0.2625	0.262±0.005	0.256 0.234	0.245	0.2495±0.0045	0.239 0.271	0.255	0.25775±0.0027	0.3147	0.1058	0.2238
Set 1	4	0.801 0.789	0.795		0.613 0.587	0.6		0.574 0.581	0.5775		0.588 0.549	0.5685				
Set 2	4	0.79 0.799	0.7945	0.79475±0.00025	0.601 0.563	0.582	0.591±0.009	0.587 0.549	0.568	0.57275±0.004	0.578 0.594	0.586	0.57725±0.0087	<0.0001	<0.0001	<0.0001
Set 1	6	1.765 1.819	1.792		1.313 1.298	1.3055		1.265 1.297	1.281		1.265 1.285	1.275				
Set 2	6	1.762 1.815	1.7885	1.79025±0.0017	1.323 1.298	1.3105	1.308±0.025	1.198 1.331	1.2645	1.27275±0.008	1.242 1.235	1.2385	1.25675±0.0182	<0.0001	<0.0001	<0.0001
Set 1	8	2.734 2.845	2.7895		2.207 2.224	2.2155		2.259 2.249	2.254		2.291 2.285	2.288				
Set 2	8	2.66 2.785	2.7225	2.756±0.0335	2.195 2.203	2.199	2.20725±0.0082	2.276 2.109	2.1925	2.22325±0.030	2.327 2.212	2.2695	2.27875±0.0092	<0.0001	<0.0001	<0.0001
Set 1	10	3.119 3.167	3.143		2.632 2.597	2.6145		2.532 2.56	2.546		2.548 2.532	2.54				
Set 2	10	3.124 3.133	3.1285	3.13575±0.0072	2.532 2.589	2.5605	2.5875±0.027	2.514 2.479	2.4965	2.52125±0.024	2.538 2.434	2.486	2.513±0.027	<0.0001	<0.0001	<0.0001
Set 1	12	3.298 3.347	3.3225		3.218 3.279	3.2485		3.278 3.254	3.266		3.217 3.298	3.2575				
Set 2	12	3.314 3.321	3.3175	3.32±0.0025	3.198 3.288	3.243	3.24575±0.027	3.224 3.205	3.2145	3.24025±0.025	3.204 3.269	3.2365	3.247±0.0105	0.0033	0.0016	0.0038
Set 1	14	3.376 3.357	3.3665		3.31 3.317	3.3135		3.367 3.373	3.37		3.331 3.365	3.348				
Set 2	14	3.354 3.364	3.359	3.36275±0.0037	3.276 3.301	3.2885	3.301±0.0125	3.34 3.351	3.3455	3.35775±0.012	3.28 3.301	3.2905	3.31925±0.0287	0.0154	0.9903	0.111

(b) with fluconazole

														P value		
	Time(hrs)	WT	Average	Average of averages ± SD	*dpb3*ΔΔ	Average	Average of averages ± SD	*dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*WT vs dpb3ΔΔ*	*WT vs dpb4ΔΔ*	*WT vs dpb3ΔΔ dpb4ΔΔ*
		0.103			0.109			0.108			0.11					
Set 1	0	0.105	0.104		0.104	0.1065		0.103	0.1055		0.108	0.109				
		0.1		0.1015±0.	0.105		0.10475±	0.105		0.1045±0.	0.108		0.108±0.0			
Set 2	0	0.098	0.099	002	0.101	0.103	0.0017	0.102	0.1035	001	0.106	0.107	01	0.995	0.9961	0.9635
		0.843			0.853			0.873			0.869					
Set 1	6	0.901	0.872		0.886	0.8695		0.923	0.898		0.914	0.8915				
		0.891		0.1015±0.	0.876		0.8765±0.	0.854		0.89675±	0.871		0.88675±			
Set 2	6	0.886	0.8885	008	0.891	0.8835	007	0.937	0.8955	0.001	0.893	0.882	0.004	0.9925	0.65	0.9635
		0.984			1.246			1.259			1.263					
Set 1	9	1.031	1.0075		1.273	1.2595		1.293	1.276		1.301	1.282				
		0.976		0.1015±0.	1.239		1.25475±	1.276		1.27875±	1.259		1.28025±			
Set 2	9	0.996	0.986	010	1.261	1.25	0.004	1.287	1.2815	0.002	1.298	1.2785	0.001	<0.0001	<0.0001	<0.0001
		1.143			1.286			1.339			1.373					
Set 1	12	1.053	1.098		1.353	1.3195		1.326	1.3325		1.254	1.3135				
		1.159		0.1015±0.	1.312		1.324±0.0	1.359		1.3305±0.	1.369		1.33±0.01			
Set 2	12	1.117	1.138	02	1.345	1.3285	04	1.298	1.3285	002	1.324	1.3465	6	<0.0001	<0.0001	<0.0001
		1.273			1.473			1.456			1.473					
Set 1	15	1.148	1.2105		1.398	1.4355		1.432	1.444		1.429	1.451				
		1.265		0.1015±0.	1.457		1.43575±	1.476		1.44625±	1.487		1.45875±			
Set 2	15	1.225	1.245	0172	1.415	1.436	0.0002	1.421	1.4485	0.002	1.446	1.4665	0.007	<0.0001	<0.0001	<0.0001
		1.253			1.523			1.514			1.541					
Set 1	18	1.283	1.268		1.489	1.506		1.462	1.488		1.483	1.512				
		1.298		0.1015±0.	1.559		1.525±0.0	1.545		1.50975±	1.562		1.52225±			
Set 2	18	1.304	1.301	0165	1.529	1.544	19	1.518	1.5315	0.021	1.503	1.5325	0.0102	<0.0001	<0.0001	<0.0001
		1.334			1.643			1.673			1.694					
Set 1	21	1.303	1.3185		1.632	1.6375		1.612	1.6425		1.623	1.6585				
		1.319		0.1015±0.	1.679		1.66425±	1.698		1.6555±0.	1.65		1.652±0.0			
Set 2	21	1.354	1.3365	009	1.703	1.691	0.026	1.639	1.6685	013	1.641	1.6455	065	<0.0001	<0.0001	<0.0001
		1.394			1.743			1.798			1.763					
Set 1	24	1.383	1.3885		1.714	1.7285		1.698	1.748		1.713	1.738				
		1.379		0.1015±0.	1.734		1.73625±	1.81		1.7685±0.	1.798		1.76175±			
Set 2	24	1.391	1.385	0017	1.754	1.744	0.0077	1.768	1.789	0205	1.773	1.7855	0.023	<0.0001	<0.0001	<0.0001

(**c**) with amphotericin b

														P value		
	Time(hrs)	WT	Average	Average of averages ± SD	*dpb3*ΔΔ	Average	Average of averages ± SD	*dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*WT vs dpb3ΔΔ*	*WT vs dpb4ΔΔ*	*WT vs dpb3ΔΔ dpb4ΔΔ*
		0.097			0.117			0.096			0.112					
Set 1	0	0.1	0.0985		0.105	0.111		0.104	0.1		0.108	0.11				
		0.104		0.101 ±	0.105		0.10725±	0.103		0.10325±	0.105		0.108±			
Set 2	0	0.103	0.1035	0.002	0.102	0.1035	0.003	0.11	0.1065	0.003	0.107	0.106	0.002	0.9949	0.9997	0.9929
		0.125			0.187			0.127			0.15					
Set 1	3	0.121	0.123		0.165	0.176		0.133	0.13		0.157	0.1535				
		0.135		0.1315 ±	0.191		0.17875±	0.145		0.1425±0.	0.164		0.1605±			
Set 2	3	0.145	0.14	0.009	0.172	0.1815	0.002	0.165	0.155	012	0.171	0.1675	0.007	0.3411	0.9738	0.7008
		0.15			0.268			0.181			0.182					
Set 1	6	0.158	0.154		0.26	0.264		0.185	0.183		0.176	0.179				
		0.176		0.16725 ±	0.271		0.272±	0.211		0.20275±	0.245		0.2175±			
Set 2	6	0.185	0.1805	0.013	0.289	0.28	0.008	0.234	0.2225	0.019	0.267	0.256	0.038	0.0073	0.5635	0.2942
		0.218			0.406			0.258			0.272					
Set 1	9	0.211	0.2145		0.389	0.3975		0.261	0.2595		0.263	0.2675				
		0.23		0.22825 ±	0.398		0.4075±	0.382		0.3115±0.	0.298		0.299±			
Set 2	9	0.254	0.242	0.013	0.437	0.4175	0.01	0.345	0.3635	052	0.363	0.3305	0.031	<0.0001	0.0381	0.0897
		0.325			0.634			0.647			0.81					
Set 1	12	0.365	0.345		0.589	0.6115		0.741	0.694		0.912	0.861				
		0.372		0.364 ±	0.687		0.64225±	0.679		0.69475±	0.789		0.8235±			
Set 2	12	0.394	0.383	0.019	0.659	0.673	0.030	0.712	0.6955	0.0007	0.783	0.786	0.037	<0.0001	<0.0001	<0.0001
		0.772			1.152			1.156			1.214					
Set 1	15	0.662	0.717		1.231	1.1915		1.197	1.1765		1.452	1.333				
		0.757		0.72675 ±	1.257		1.2345±	1.324		1.24375±	1.317		1.34225±			
Set 2	15	0.716	0.7365	0.0009	1.298	1.2775	0.043	1.298	1.311	0.067	1.386	1.3515	0.009	<0.0001	<0.0001	<0.0001
		1.432			2.532			2.432			2.887					
Set 1	18	1.398	1.415		2.641	2.5865		2.394	2.413		2.485	2.686				
		1.454		1.4155 ±	2.567		2.56525±	2.367		2.41925±	2.798		2.672±			
Set 2	18	1.378	1.416	0.0005	2.521	2.544	0.021	2.484	2.4255	0.006	2.518	2.658	0.014	<0.0001	<0.0001	<0.0001
		2.841			2.846			2.848			2.881					
Set 1	21	2.732	2.7865		2.883	2.8645		2.832	2.84		2.848	2.8645				
		2.654		2.7665 ±	2.798		2.848±	2.859		2.854±0.0	2.889		2.86575±			
Set 2	21	2.839	2.7465	0.02	2.865	2.8315	0.016	2.877	2.868	14	2.845	2.867	0.001	0.0432	0.0279	0.0113
		2.885			2.863			2.863			2.893					
Set 1	24	2.739	2.812		2.841	2.852		2.889	2.876		2.849	2.871				
		2.898		2.85025 ±	2.863		2.86075±	2.898		2.884±0.0	2.895		2.877±			
Set 2	24	2.879	2.8885	0.038	2.876	2.8695	0.008	2.886	2.892	08	2.871	2.883	0.006	0.977	0.6002	0.7468

(**d**) with caspofungin

														P value		
	Time(hrs)	WT	Average	Average of averages ± SD	*dpb3*ΔΔ	Average	Average of averages ± SD	*dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*WT vs dpb3ΔΔ*	*WT vs dpb4ΔΔ*	*WT vs dpb3ΔΔ dpb4ΔΔ*
		0.103			0.109			0.108			0.11					
Set 1	0	0.105	0.104		0.104	0.1065		0.103	0.1055		0.108	0.109				
		0.1			0.095		0.1035±0.	0.102		0.10425±	0.106		0.1065±0.			
Set 2	0	0.108	0.104	0.104	0.106	0.1005	003	0.104	0.103	0.0012	0.102	0.104	002	>0.9999	>0.9999	0.9997
		0.401			0.489			0.494			0.473					
Set 1	8	0.451	0.426		0.501	0.495		0.521	0.5075		0.513	0.493				
		0.389		0.409±0.0	0.459		0.4835±0.	0.51		0.5115±0.	0.495		0.49675±			
Set 2	8	0.395	0.392	17	0.485	0.472	011	0.521	0.5155	004	0.506	0.5005	0.003	0.1307	0.0258	0.063
		0.529			0.612			0.701			0.663					
Set 1	11	0.583	0.556		0.678	0.645		0.604	0.6525		0.698	0.6805				
		0.536		0.5515±0.	0.673		0.658±0.0	0.698		0.665±0.0	0.678		0.68725±			
Set 2	11	0.558	0.547	004	0.669	0.671	13	0.657	0.6775	12	0.71	0.694	0.006	0.02	0.0127	0.0028
		0.649			0.794			0.789			0.732					
Set 1	15	0.701	0.675		0.801	0.7975		0.768	0.7785		0.791	0.7615				
		0.689		0.67825±	0.768		0.79725±	0.81		0.79825±	0.836		0.78925±			
Set 2	15	0.674	0.6815	0.003	0.826	0.797	0.0002	0.826	0.818	0.019	0.798	0.817	0.027	0.0088	0.0082	0.015
		0.695			0.973			0.943			0.994					
Set 1	18	0.723	0.709		1.003	0.988		0.964	0.9535		0.892	0.943				
		0.736		0.73475±	0.954		0.982±0.0	1.056		1.01875±	1.106		0.99525±			
Set 2	18	0.785	0.7605	0.025	0.998	0.976	06	1.112	1.084	0.065	0.989	1.0475	0.052	<0.0001	<0.0001	<0.0001
		0.789			1.121			1.09			1.121					
Set 1	21	0.793	0.791		1.187	1.154		1.132	1.111		1.136	1.1285				
		0.81		0.80375±	1.168		1.173±0.0	1.21		1.1575±0.	1.231		1.1845±0.			
Set 2	21	0.823	0.8165	0.012	1.216	1.192	19	1.198	1.204	046	1.25	1.2405	056	<0.0001	<0.0001	<0.0001
		0.851			1.246			1.269			1.314					
Set 1	24	0.893	0.872		1.339	1.2925		1.204	1.2365		1.273	1.2935				
		0.865		0.8835±0.	1.321		1.31425±	1.325		1.28575±	1.336		1.31625±			
Set 2	24	0.925	0.895	011	1.351	1.336	0.021	1.345	1.335	0.049	1.342	1.339	0.022	<0.0001	<0.0001	<0.0001

Supplementary Table-S2: Using TEM, the cell wall structure of WT and *dpb3*ΔΔ*dpb4*ΔΔ cells was observed and thickness of the cell wall was measured. The average of cell wall thickness of three different cells was considered. The statistically significant differences between the results of WT and mutant strains was analyzed using unpaired t test.

							P value
	WT	**Average**	**SD**	***dpb3ΔΔdpb4ΔΔ***	**Average**	**SD**	**WT Vs *dpb3ΔΔdpb4ΔΔ***
	78.99			208.05			
	90.57			156.55			
TEM	89.06	86.20667	5.140054	167.98	177.5267	22.08192	0.00469306

Supplementary Table-S3: Mean fluorescence intensity of aniline blue, concanavaline A, CFW stained cells of WT and *dpb3*ΔΔ*dpb4*ΔΔ strains was determined using flow cytometry. Data were represented as mean and Standard deviation from triplicate values. The statistically significant differences between the results of the WT and mutant was determined using one way ordinary ANOVA (Dunnett's multiple comparison test).

													P value		
	WT	Average	SD	*dpb3ΔΔ*	Average	SD	*dpb4ΔΔ*	Average	SD	*dpb3ΔΔdpb4ΔΔ*	Average	SD	WT Vs *dpb3ΔΔ*	WT Vs *dpb4ΔΔ*	WT Vs *dpb3ΔΔdpb4ΔΔ*
	113			131.94			138			146.69					
Aniline	117			131.04			135			147.39					
blue	115	115	1.632993	130.96	131.3133	0.444322	137	136.6667	1.247219	147.27	147.1167	0.30565	0.0018	0.0006	0.0001
	698			785			790			958					
Concanav	593			893			834			896					
aline A	550	613.6667	62.16287	915	864.3333	56.81158	867	830.3333	31.54186	884	912.6667	32.4277	0.002	0.0049	0.0006
	666			969			871			1088					
	689			1067			976			925					
CFW	712	689	18.77942	987	1007.667	42.59369	1011	952.6667	59.48856	1055	1022.667	70.36255	0.0007	0.0024	0.0005

Supplementary Table-S4: The growth curve analyses of *dpb3*ΔΔ*dpb4*ΔΔ strain (**a**) without and with geldanamycin alone (107nM), fluconazole alone (6 µM), and a combination of both of the drugs, (**b**) without and with geldanamycin alone (107nM), amp B alone (10 nM), and a combination of both of the drugs, (**c**) without and with trichostatin A alone (0.8 µM), fluconazole alone (6 µM), and a combination of both of the drugs, and (**d**) without and with trichostatin A alone (0.8 µM), ampB alone (107nM), and a combination of both of the drugs. The absorbance measured at different time points and statistical analysis has been given as tables.

(a)

															P value
	Time(hrs)	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ+Geldanamycin	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+ FLC*	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+ FLC+Geldanamycin*	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+FLC vs dpb3ΔΔ dpb4ΔΔ+FLC+ Geldanamycin*
Set 1	0	0.094	0.0955	0.0995±0.004	0.094	0.0955	0.0995±0.004	0.098	0.0995	0.10375±0.004	0.098	0.0995	0.10275±0.003	>0.9999
		0.097			0.097			0.101			0.101			
Set 2	0	0.102	0.1035		0.103	0.1035		0.106	0.108		0.105	0.106		
		0.105			0.104			0.11			0.107			
Set 1	3	0.351	0.355	0.35175±0.003	0.358	0.3535	0.35425±0.007	0.318	0.322	0.3275±0.005	0.281	0.311	0.313±0.002	0.7981
		0.359			0.349			0.326			0.341			
Set 2	3	0.345	0.3485		0.345	0.355		0.321	0.333		0.265	0.315		
		0.352			0.365			0.345			0.365			
Set 1	6	1.277	1.2715	1.27775±0.006	1.254	1.2595	1.25975±0.0002	0.899	0.8825	0.875±0.007	0.762	0.7635	0.76225±0.001	<0.0001
		1.266			1.265			0.866			0.765			
Set 2	6	1.289	1.284		1.275	1.26		0.876	0.8675		0.751	0.761		
		1.279			1.245			0.859			0.771			
Set 1	9	2.349	2.4085	2.408±0.005	2.412	2.383	2.4075±0.024	1.576	1.5845	1.58025±0.004	1.368	1.363	1.36325±0.0002	<0.0001
		2.468			2.354			1.593			1.358			
Set 2	9	2.339	2.4075		2.523	2.432		1.54	1.576		1.358	1.3635		
		2.476			2.341			1.612			1.369			
Set 1	12	2.692	2.6175	2.6155±0.002	2.654	2.633	2.64075±0.007	2.401	2.433	2.4465±0.013	1.475	1.503	1.50075±0.0022	<0.0001
		2.543			2.612			2.465			1.531			
Set 2	12	2.701	2.6135		2.665	2.6485		2.445	2.46		1.485	1.4985		
		2.526			2.632			2.475			1.512			
Set 1	15	2.742	2.6925	2.694±0.015	2.778	2.7605	2.741±0.019	2.635	2.6165	2.63425±0.017	1.543	1.5775	1.568±0.0095	<0.0001
		2.643			2.743			2.598			1.612			
Set 2	15	2.754	2.6955		2.745	2.7215		2.692	2.652		1.521	1.5585		
		2.637			2.698			2.612			1.596			
Set 1	18	2.979	2.9165	2.9075±0.009	2.954	2.8945	2.859±0.035	2.739	2.78	2.78025±0.0002	1.731	1.7715	1.787±0.0155	<0.0001
		2.854			2.835			2.821			1.812			
Set 2	18	2.912	2.8985		2.812	2.8235		2.765	2.7805		1.795	1.8025		
		2.885			2.835			2.796			1.81			
Set 1	21	2.987	2.9775	2.97225±0.005	2.896	2.9325	2.9345±0.002	2.854	2.876	2.87475±0.001	2.052	2.032	2.027±0.005	<0.0001
		2.968			2.969			2.898			2.012			
Set 2	21	2.976	2.967		2.923	2.9365		2.835	2.8735		2.042	2.022		
		2.958			2.95			2.912			2.002			
Set 1	24	2.982	2.9835	2.97975±0.003	2.879	2.9315	2.94425±0.012	2.929	2.9305	2.9445±0.014	2.265	2.2395	2.29375±0.0542	<0.0001
		2.985			2.984			2.932			2.214			
Set 2	24	2.987	2.976		2.956	2.957		2.949	2.9585		2.375	2.348		
		2.965			2.958			2.968			2.321			

(b)

														P value
	Time(hrs)	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ+Geldanamyin	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+* AmpB	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+ AmpB+Geldanamycin*	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+*AmpB *vs dpb3ΔΔ dpb4ΔΔ+*AmpB *+Geldanamycin*
Set 1	0	0.103	0.104	0.10275±0.001	0.094	0.0955	0.0985±0.003	0.113	0.111	0.1085±0.002	0.108	0.1135	0.1085±0.005	0.9999
		0.105			0.097			0.109			0.119			
Set 2	0	0.103	0.1015		0.098	0.1015		0.105	0.106		0.103	0.1035		
		0.1			0.105			0.107			0.104			
Set 1	3	0.279	0.2865	0.29775±0.011	0.321	0.3095	0.323±0.013	0.156	0.1645	0.16775±0.003	0.179	0.172	0.172	0.9983
		0.294			0.298			0.173			0.165			
Set 2	3	0.321	0.309		0.332	0.3365		0.186	0.171		0.179	0.172		
		0.297			0.341			0.156			0.165			
Set 1	6	1.162	1.1535	1.154±0.005	1.177	1.1665	1.1685±0.002	0.417	0.411	0.411	0.377	0.366	0.366	0.3693
		1.145			1.156			0.405			0.355			
Set 2	6	1.174	1.1545		1.169	1.1705		0.417	0.411		0.377	0.366		
		1.135			1.172			0.405			0.355			
Set 1	9	2.245	2.283	2.283	2.349	2.3085	2.30625±0.002	0.857	0.798	0.798	0.677	0.6655	0.6655	0.0007
		2.321			2.268			0.739			0.654			
Set 2	9	2.254	2.283		2.354	2.304		0.857	0.798		0.677	0.6655		
		2.312			2.254			0.739			0.654			
Set 1	12	2.554	2.576	2.576	2.592	2.5675	2.5695±0.002	1.713	1.622	1.622	1.387	1.3995	1.3995	<0.0001
		2.598			2.543			1.531			1.412			
Set 2	12	2.568	2.576		2.549	2.5715		1.713	1.622		1.387	1.3995		
		2.584			2.594			1.531			1.412			
Set 1	15	2.745	2.771	2.782±0.011	2.742	2.6925	2.70925±0.016	2.759	2.7575	2.7575	2.383	2.401	2.401	<0.0001
		2.797			2.643			2.756			2.419			
Set 2	15	2.784	2.793		2.754	2.726		2.759	2.7575		2.383	2.401		
		2.802			2.698			2.756			2.419			
Set 1	18	2.875	2.8935	2.91725±0.023	2.949	2.9015	2.95375±0.052	2.854	2.8595	2.8595	2.654	2.646	2.646	<0.0001
		2.912			2.854			2.865			2.638			
Set 2	18	2.898	2.941		2.958	3.006		2.854	2.8595		2.654	2.646		
		2.984			3.054			2.865			2.638			
Set 1	21	2.974	2.9695	3.0025±0.033	2.987	2.9775	3.0385±0.061	2.845	2.8785	2.928±0.049	2.832	2.8385	2.909±0.07	0.8818
		2.965			2.968			2.912			2.845			
Set 2	21	3.012	3.0355		3.098	3.0995		2.951	2.9775		2.985	2.9795		
		3.059			3.101			3.004			2.974			

(c)

														P value
	Time(hrs)	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ+ Trichostatin A	Average	Average of averages ± SD	*dpb3ΔΔdpb4 ΔΔ+*FLC	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+* FLC+Trichostatin A	Average	Average of averages ± SD	*dpb3ΔΔdpb4 ΔΔ+*FLC *vs dpb3ΔΔdpb4 ΔΔ+*FLC+Trichostatin A
		0.094			0.094			0.098			0.098			
Set 1	0	0.097	0.0955		0.097	0.0955		0.101	0.0995		0.101	0.0995		
		0.101		0.0995±0.	0.103		0.09975±	0.104		0.10225±	0.103		0.10225±	
Set 2	0	0.106	0.1035	004	0.105	0.104	0.004	0.106	0.105	0.0027	0.107	0.105	0.0027	>0.9999
		0.351			0.358			0.298			0.318			
Set 1	3	0.359	0.355		0.349	0.3535		0.312	0.305		0.352	0.335		
		0.326		0.35025±	0.346		0.35675±	0.305		0.309±0.0	0.298		0.32075±	
Set 2	3	0.365	0.3455	0.004	0.374	0.36	0.003	0.321	0.313	04	0.315	0.3065	0.004	0.7994
		1.277			1.25			0.887			0.8			
Set 1	6	1.266	1.2715		1.255	1.2525		0.872	0.8795		0.849	0.8245		
		1.253		1.268±0.0	1.267		1.25775±	0.887		0.8895±0.	0.845		0.84325±	
Set 2	6	1.276	1.2645	03	1.259	1.263	0.005	0.912	0.8995	01	0.879	0.862	0.01	0.0161
		2.349			2.442			1.572			1.046			
Set 1	9	2.468	2.4085		2.361	2.4015		1.589	1.5805		1.03	1.038		
		2.365		2.4095±0.	2.456		2.4185±0.	1.567		1.57525±	0.995		1.024±0.0	
Set 2	9	2.456	2.4105	001	2.415	2.4355	017	1.573	1.57	0.005	1.025	1.01	05	<0.0001
		2.692			2.633			2.398			1.243			
Set 1	12	2.543	2.6175		2.646	2.6395		2.375	2.3865		1.305	1.274		
		2.569		2.604±0.0	2.622		2.63875±	2.354		2.378±0.0	1.23		1.269±0.0	
Set 2	12	2.612	2.5905	13	2.654	2.638	0.007	2.385	2.3695	08	1.298	1.264	08	<0.0001
		2.742			2.756			2.621			1.413			
Set 1	15	2.783	2.7625		2.749	2.7525		2.612	2.6165		1.498	1.4555		
		2.698		2.736±0.0	2.747		2.75925±	2.594		2.6155±0.	1.435		1.45625±	
Set 2	15	2.721	2.7095	26	2.785	2.766	0.006	2.635	2.6145	001	1.479	1.457	0.001	<0.0001
		2.979			2.971			2.723			1.543			
Set 1	18	2.854	2.9165		2.821	2.896		2.814	2.7685		1.598	1.5705		
		2.865		2.90025±	2.845		2.8765±0.	2.745		2.7695±0.	1.495		1.562±0.0	
Set 2	18	2.903	2.884	0.016	2.869	2.857	019	2.796	2.7705	001	1.612	1.5535	01	<0.0001
		2.987			2.883			2.872			1.725			
Set 1	21	2.968	2.9775		2.959	2.921		2.895	2.8835		1.754	1.7395		
		2.956		2.97225±	2.798		2.90675±	2.869		2.87875±	1.684		1.72475±	
Set 2	21	2.978	2.967	0.005	2.987	2.8925	0.014	2.879	2.874	0.004	1.736	1.71	0.004	<0.0001
		2.982			2.983			2.934			1.845			
Set 1	24	2.985	2.9835		2.98	2.9815		2.941	2.9375		2.056	1.9505		
		2.998		2.98075±	2.896		2.96325±	2.975		2.959±0.0	1.864		1.93725±	
Set 2	24	2.958	2.978	0.002	2.994	2.945	0.018	2.986	2.9805	21	1.984	1.924	0.021	<0.0001

(d)

														P value
	Time(hrs)	*dpb3*ΔΔ *dpb4*ΔΔ	Average	Average of averages ± SD	*dpb3*ΔΔ *dpb4*ΔΔ+ Trichostatin A	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+* AmpB	Average	Average of averages ± SD	*dpb3ΔΔ dpb4ΔΔ+* Amp B+Trichostatin A	Average	Average of averages ± SD	*dpb3ΔΔdpb4 ΔΔ+*AmpB *vs dpb3ΔΔdpb4 ΔΔ+*AmpB+Trichostatin A
		0.103			0.104			0.113			0.112			
Set 1	0	0.105	0.104		0.107	0.1055		0.109	0.111		0.107	0.1095		
		0.102		0.102±0.0	0.106		0.105±	0.105		0.10925±	0.109		0.10825±	
Set 2	0	0.098	0.1	02	0.103	0.1045	0.0005	0.11	0.1075	0.001	0.105	0.107	0.001	0.9997
		0.279			0.311			0.186			0.213			
Set 1	3	0.294	0.2865		0.287	0.299		0.186	0.186		0.231	0.222		
		0.267		0.28175±	0.279		0.29375±	0.175		0.18625±	0.225		0.2275±	
Set 2	3	0.287	0.277	0.0047	0.298	0.2885	0.0052	0.198	0.1865	0.0002	0.241	0.233	0.005	0.021
		1.162			1.143			0.417			0.4			
Set 1	6	1.145	1.1535		1.161	1.152		0.405	0.411		0.418	0.409		
		1.156		1.1575±0.	1.136		1.153±	0.456		0.42475±	0.412		0.421±	
Set 2	6	1.167	1.1615	004	1.172	1.154	0.001	0.421	0.4385	0.013	0.454	0.433	0.012	0.9881
		2.245			2.319			0.857			0.657			
Set 1	9	2.321	2.283		2.351	2.335		0.739	0.798		0.677	0.667		
		2.227		2.30125±	2.359		2.318±	0.816		0.79525±	0.631		0.66225±	
Set 2	9	2.412	2.3195	0.0182	2.243	2.301	0.017	0.769	0.7925	0.002	0.684	0.6575	0.004	<0.0001
		2.554			2.585			1.713			1.385			
Set 1	12	2.598	2.576		2.661	2.623		1.531	1.622		1.384	1.3845		
		2.532		2.5655±0.	2.543		2.61675±	1.726		1.6455±0.	1.298		1.3635±	
Set 2	12	2.578	2.555	010	2.678	2.6105	0.006	1.612	1.669	023	1.387	1.3425	0.021	<0.0001
		2.745			2.839			2.759			2.401			
Set 1	15	2.797	2.771		2.787	2.813		2.756	2.7575		2.349	2.375		
		2.812		2.779750.	2.822		2.81075±	2.746		2.75±0.00	2.359		2.383±	
Set 2	15	2.765	2.7885	0087	2.795	2.8085	0.002	2.739	2.7425	7	2.423	2.391	0.008	<0.0001
		2.875			2.889			2.854			2.654			
Set 1	18	2.912	2.8935		2.854	2.8715		2.865	2.8595		2.685	2.6695		
		2.865		2.88525±	2.872		2.875±	2.895		2.87125±	2.662		2.668±	
Set 2	18	2.889	2.877	0.0082	2.885	2.8785	0.003	2.871	2.883	0.011	2.671	2.6665	0.0015	<0.0001
		2.974			2.878			2.845			2.845			
Set 1	21	2.965	2.9695		2.884	2.881		2.912	2.8785		2.865	2.855		
		2.945		2.96225±	2.915		2.89325±	2.875		2.8845±0.	2.912		2.877±	
Set 2	21	2.965	2.955	0.0072	2.896	2.9055	0.012	2.906	2.8905	006	2.886	2.899	0.022	0.9179

Supplementary Table-S5: The growth curve analyses of WT, *dpb3*ΔΔ, *dpb4*ΔΔ, and *dpb3*ΔΔ*dpb4*ΔΔ strains were carried out with 5FC. The absorbance measured at different time points and statistical analysis has been given.

														P value		
	Time(hrs)	WT	Average	Average of averages ± SD	*dpb3* ΔΔ	Average	Average of averages ± SD	*dpb4* ΔΔ	Average	Average of averages ± SD	*dpb3* ΔΔ *dpb4* ΔΔ	Average	Average of averages ± SD	*WT vs dpb3ΔΔ*	*WT vs dpb4ΔΔ*	*WT vs dpb3ΔΔ dpb4ΔΔ*
Set 1	0	0.111	0.1095	0.1085±0.001	0.103	0.106	0.105±0.001	0.108	0.1035	0.10525±0.001	0.106	0.1035	0.1035	0.9994	0.9996	0.9985
		0.108			0.109			0.099			0.101					
Set 2	0	0.106	0.1075		0.103	0.104		0.106	0.107		0.103	0.1035				
		0.109			0.105			0.108			0.104					
Set 1	2	0.171	0.1715	0.1705±0.001	0.198	0.2035	0.20025±0.003	0.203	0.2045	0.21125±0.006	0.2	0.205	0.214±0.009	0.7917	0.6051	0.5576
		0.172			0.209			0.206			0.21					
Set 2	2	0.169	0.1695		0.205	0.197		0.215	0.218		0.231	0.223				
		0.17			0.189			0.221			0.215					
Set 1	4	0.376	0.371	0.369±0.002	0.398	0.404	0.41125±0.007	0.403	0.4095	0.4185±0.009	0.417	0.4145	0.42775±0.013	0.5791	0.4581	0.3238
		0.366			0.41			0.416			0.412					
Set 2	4	0.359	0.367		0.421	0.4185		0.424	0.4275		0.436	0.441				
		0.375			0.416			0.431			0.446					
Set 1	6	0.62	0.6305	0.63625±0.005	0.733	0.737	0.74925±0.012	0.743	0.741	0.7565±0.015	0.754	0.751	0.76475±0.013	0.0185	0.0116	0.0067
		0.641			0.741			0.739			0.748					
Set 2	6	0.609	0.642		0.754	0.7615		0.765	0.772		0.785	0.7785				
		0.675			0.769			0.779			0.772					
Set 1	8	1.108	1.1075	1.11425±0.006	1.231	1.243	1.25175±0.008	1.243	1.25	1.27125±0.021	1.265	1.2785	1.2885±0.01	0.0036	0.0009	0.0003
		1.107			1.255			1.257			1.292					
Set 2	8	1.119	1.121		1.256	1.2605		1.284	1.2925		1.285	1.2985				
		1.123			1.265			1.301			1.312					
Set 1	10	1.666	1.6945	1.66175±0.032	1.889	1.845	1.86325±0.018	1.823	1.848	1.89375±0.045	1.823	1.898	1.90525±0.007	<0.0001	<0.0001	<0.0001
		1.723			1.801			1.873			1.973					
Set 2	10	1.56	1.629		1.858	1.8815		1.934	1.9395		1.878	1.9125				
		1.698			1.905			1.945			1.947					
Set 1	12	2.089	2.0665	2.12075±0.054	2.234	2.2275	2.2845±0.057	2.249	2.254	2.29175±0.037	2.226	2.2355	2.29875±0.063	0.0006	0.0003	0.0002
		2.044			2.221			2.259			2.245					
Set 2	12	2.12	2.175		2.324	2.3415		2.361	2.3295		2.345	2.362				
		2.23			2.359			2.298			2.379					
Set 1	14	2.344	2.3625	2.3975±0.035	2.412	2.3665	2.40475±0.038	2.412	2.4035	2.44175±0.038	2.379	2.362	2.4260±0.064	0.9955	0.5448	0.811
		2.381			2.321			2.395			2.345					
Set 2	14	2.487	2.4325		2.408	2.443		2.523	2.48		2.487	2.49				
		2.378			2.478			2.437			2.493					
Set 1	16	2.412	2.405	2.44±0.035	2.432	2.4865	2.4855±0.001	2.512	2.522	2.542±0.02	2.462	2.454	2.4875±0.033	0.5237	0.0363	0.4905
		2.398			2.541			2.532			2.446					
Set 2	16	2.512	2.475		2.456	2.4845		2.557	2.562		2.511	2.521				
		2.438			2.513			2.567			2.531					