

Table S1. MIC values of all tested antifungals for individual isolates.

Isolate	MIC (mg/L)					5FC	MGX
	AMB	FLC	VRC	AFG			
B12406	1	4	0.06	0.25		0.25	0.004
B15223	2	>128	16	8		0.125	0.004
B19460	2	32	0.25	0.125		0.125	0.008
B19547	2	32	0.25	0.125		0.125	0.004
B19617	2	32	0.125	>8		0.125	0.004
B19837	2	32	0.125	>8		0.125	0.004
B19618	2	32	0.25	>8		0.125	0.004
B17040	2	>128	2	0.06		0.25	0.016
B17041	2	>128	2	0.125		0.25	0.03
B17073	2	>128	2	>8		0.25	0.03
B17201	2	>128	2	>8		0.25	0.03
B18560	2	64	0.25	0.06		0.125	0.004
B18845	2	64	0.25	0.25		0.125	0.004
B18841	2	64	0.25	2		0.125	0.008
B18843	2	64	0.5	0.5		0.125	0.004
B12692	2	>128	2	0.5		0.25	0.03
B12694	2	>128	2	0.25		0.125	0.03
B12663	2	>128	2	8		0.25	0.03
B12664	2	>128	2	0.125		0.25	0.03
B12688	2	>128	2	>8		0.25	0.03
B20931	2	128	1	0.06		0.25	0.008
B21040	2	64	1	0.03		0.25	0.004
B21041	2	128	1	0.03		0.25	0.004
B21042	2	128	1	0.125		0.25	0.008
B21043	2	128	1	0.03		0.25	0.008

Isolates in bold were used in checkerboard assays. Resistance (red) and susceptibility (green) according to tentative CDC *C. auris* breakpoints. Grey background for inconclusive AMB MIC₉₀. 5FC, 5-flucytosine; AFG, anidulafungin; AMB, amphotericin B; FLC, fluconazole; MGX, manogepix; VRC, voriconazole

Table S2. MIC and FICI values for the combination with 5-flucytosine.

Anidulafungin + 5-flucytosine												
Strain	1st repeat				2nd repeat				3rd repeat			
	MIC ^a [mg/L]				MIC ^a [mg/L]				MIC ^a [mg/L]			
AFG	5FC	AFG/5FC	FICI ^b	AFG	5FC	AFG/5FC	FICI ^b	AFG	5FC	AFG/5FC	FICI ^b	
B19460	0.25	0.125	0.06/0.03	0.48/SYN	0.5	0.125	0.125/0.03	0.49/SYN	>0.5 ^c	0.25	0.25/0.06	0.49/SYN
B19618	>32 ^c	0.125	16/0.03	0.49/SYN	>32 ^c	0.25	4/0.125	0.56/IND	8	0.125	0.06/0.125	1.01/IND
B17040	0.5	0.25	0.25/0.016	0.56/IND	0.125	0.25	0.008/0.0125	0.56/IND	2	0.5	0.06/0.06	0.15/SYN
B17041	0.25	0.25	0.06/0.125	0.69/IND	0.25	0.25	0.06/0.125	0.74/IND	0.5	0.125	0.25/0.06	0.98/IND
B18560	0.5	0.125	0.06/0.06	0.60/IND	>0.5 ^c	0.125	0.06/0.03	0.30/SYN	>0.5 ^c	0.125	0.125/0.06	0.61/IND
B18843	4	0.125	1/0.03	0.49/SYN	4	0.125	2/0.06	0.98/IND	4	0.125	2/0.06	0.98/IND
B12694	0.5	0.25	0.06/0.06	0.36/SYN	0.25	0.25	0.06/0.03	0.36/SYN	0.5	0.25	0.06/0.03	0.24/SYN
B12663	4	0.25	2/0.125	1.00/IND	32	0.25	8/0.03	0.37/SYN	8	0.25	4/0.06	0.74/IND
B12664	0.5	0.125	0.5/0.06	0.98/IND	0.5	0.125	0.125/0.06	0.73/IND	0.5	0.25	0.06/0.125	0.62/IND
B20931	0.5	0.25	0.06/0.016	0.18/SYN	0.06	0.25	0.002/0.125	0.53/IND	0.125	0.25	0.06/0.03	0.60/IND
B21040	0.06	0.25	0.001/0.25	1.02/IND	0.016	0.25	0.016/0.008	1.03/IND	0.06	0.25	0.03/0.016	0.56/IND
Amphotericin B + 5-flucytosine												
Strain	1st repeat				2nd repeat				3rd repeat			
	MIC ^a [mg/L]				MIC ^a [mg/L]				MIC ^a [mg/L]			
AMB	5FC	AMB/5FC	FICI ^b	AMB	5FC	AMB/5FC	FICI ^b	AMB	5FC	AMB/5FC	FICI ^b	
B19460	2	0.25	1/0.125	1.00/IND	2	0.5	0.016/0.5	1.01/IND	2	0.5	0.016/0.5	1.01/IND
B19618	2	0.5	1/0.008	0.52/IND	2	0.5	1/0.25	1.00/IND	2	0.5	0.03/0.5	1.02/IND
B17040	2	0.5	1/0.25	1.00/IND	2	1	0.016/1	1.01/IND	4	1	0.016/1	1.00/IND
B17041	2	0.5	1/0.25	1.00/IND	2	0.5	1/0.125	0.75/IND	2	1	1/0.5	1.00/IND
B18560	2	0.25	0.0016/0.25	1.01/IND	2	0.25	0.016/0.25	1.01/IND	2	0.5	0.016/0.5	1.01/IND
B18843	2	0.25	1/0.03	0.62/IND	2	0.5	0.016/0.5	1.01/IND	2	0.5	1/0.25	1.00/IND
B12694	2	>0.5 ^c	0.125/0.5	0.56/IND	2	>0.5 ^c	0.25/0.5	0.63/IND	4	>0.5 ^c	2/0.5	1.00/IND
B12663	2	1	0.016/0.5	0.51/IND	2	1	0.016/1	1.01/IND	4	1	2/0.25	0.75/IND
B12664	2	0.5	1/0.125	0.75/IND	2	0.5	1/0.125	0.75/IND	4	0.5	2/0.06	0.62/IND
B20931	0.06	0.5	0.5/0.25	1.00/IND	1	0.5	0.5/0.25	1.00/IND	2	0.5	1/0.016	0.53/IND
B21040	1	0.5	0.5/0.06	0.62/IND	1	0.5	0.5/0.125	0.75/IND	1	0.5	0.5/0.25	1.00/IND
Voriconazole + 5-flucytosine												
Strain	1st repeat				2nd repeat				3rd repeat			
	MIC ^a [mg/L]				MIC ^a [mg/L]				MIC ^a [mg/L]			
VRC	5FC	VRC/5FC	FICI ^b	VRC	5FC	VRC/5FC	FICI ^b	VRC	5FC	VRC/5FC	FICI ^b	
B19460	0.5	0.125	0.004/0.125	1.01/IND	0.25	0.125	0.008/0.125	1.03/IND	0.25	0.125	2/0.125	8.00/ANT
B19618	0.5	0.125	0.008/0.125	1.02/IND	0.25	0.25	1/0.125	4.50/ANT	0.25	0.125	1/0.125	5.00/ANT
B17040	2	0.25	0.016/0.25	1.01/IND	2	0.25	0.016/0.25	1.01/IND	4	0.5	0.016/0.5	1.00/IND
B17041	2	0.125	0.03/0.125	1.02/IND	2	0.25	1/0.125	1.00/IND	4	0.25	2/0.016	0.56/IND
B18560	0.5	0.125	0.002/0.125	1.00/IND	0.25	0.125	1/0.06	4.48/ANT	0.5	0.25	0.002/0.25	1.00/IND
B18843	0.5	0.125	0.004/0.125	1.01/IND	0.5	0.25	2/0.125	4.50/ANT	0.5	0.25	2/0.125	4.50/ANT
B12694	2	0.25	0.016/0.25	1.01/IND	2	0.25	0.016/0.25	1.01/IND	2	0.25	0.016/0.25	1.01/IND
B12663	2	0.25	0.008/0.25	1.00/IND	1	0.25	0.008/0.25	1.01/IND	2	0.25	0.008/0.25	1.00/IND
B12664	2	0.125	1/0.016	0.63/IND	2	0.25	0.016/0.25	1.01/IND	2	0.25	0.016/0.25	1.01/IND
B20931	2	0.125	1/0.016	0.63/IND	1	0.125	0.008/0.125	1.01/IND	1	0.25	0.008/0.25	1.01/IND
B21040	1	0.125	0.008/0.125	1.01/IND	1	0.25	0.008/0.25	1.01/IND	1	0.25	0.008/0.25	1.01/IND

^a90% growth inhibition endpoint for combinations with AMB. For all other combinations 50% growth inhibition endpoint was used.

^bMinimum FICI unless antagonism was observed.

^cMIC of single drug was rounded to the next highest two-fold dilution if 50% (90% for amphotericin B) growth inhibition was not reached at the highest concentration.

Table S3. MIC and FICI values for the combination with manogepix.

Anidulafungin + manogepix												
Strain	1st repeat				2nd repeat				3rd repeat			
	MIC ^a [mg/L]				MIC ^a [mg/L]				MIC ^a [mg/L]			
	AFG	MGX	AFG/MGX	FICI ^b	AFG	MGX	AFG/MGX	FICI ^b	AFG	MGX	AFG/MGX	FICI ^b
B19460	>0.5 ^c	0.008	0.016/0.004	1.02/IND	0.5	0.008	0.125/0.002	0.50/SYN	0.5	0.008	0.125/0.002	0.50/SYN
B19618	8	0.004	0.125/0.004	0.52/IND	>32 ^c	0.004	0.06/0.004	1.00/IND	8	0.008	0.125/0.004	0.52/IND
B17040	1	0.06	0.25/0.016	0.52/IND	0.5	0.03	0.03/0.008	0.33/SYN	0.5	0.03	0.008/0.016	0.55/IND
B17041	0.5	0.03	0.125/0.016	0.78/IND	0.25	0.03	0.03/0.016	0.65/IND	0.25	0.06	0.06/0.008	0.37/SYN
B18560	>0.5 ^c	0.008	0.03/0.002	0.28/SYN	>0.5 ^c	0.008	0.5/0.002	0.75/IND	>0.5 ^c	0.008	0.125/0.0005	0.19/SYN
B18843	4	0.004	2/0.0005	0.63/IND	4	0.008	1/0.004	0.75/IND	4	0.008	0.125/0.004	0.53/IND
B12694	0.5	0.03	0.25/0.004	0.19/SYN	0.25	0.016	0.03/0.008	0.62/IND	0.5	0.03	0.125/0.008	0.52/IND
B12663	>32 ^c	0.03	4/0.008	0.33/SYN	>32 ^c	0.03	4/0.008	0.33/SYN	8	0.03	0.06/0.03	1.01/IND
B12664	>0.5 ^c	0.06	0.03/0.016	0.30/SYN	0.25	0.06	0.002/0.03	0.51/IND	0.5	0.06	0.06/0.016	0.39/SYN
B20931	0.125	0.008	0.03/0.002	0.49/SYN	0.125	0.016	0.03/0.001	0.30/SNY	0.016	0.008	0.008/0.0005	0.56/IND
B21040	0.016	0.008	0.008/0.002	0.75/IND	0.016	0.008	0.008/0.004	1.00/IND	0.016	0.008	0.008/0.0005	0.56/IND

Manogepix + 5-flucytosine												
Strain	1st repeat				2nd repeat				3rd repeat			
	MIC ^a [mg/L]				MIC ^a [mg/L]				MIC ^a [mg/L]			
	5FC	MGX	5FC/MGX	FICI ^b	5FC	MGX	5FC/MGX	FICI ^b	5FC	MGX	5FC/MGX	FICI ^b
B19460	0.25	0.008	0.001/0.008	1.00/IND	0.125	0.004	0.001/0.004	1.01/IND	0.125	0.008	0.001/0.008	1.01/IND
B19618	0.25	0.004	0.125/0.002	1.00/IND	0.25	0.004	0.001/0.004	1.00/IND	0.125	0.004	0.002/0.004	1.02/IND
B17040	0.125	0.06	0.03/0.03	0.74/IND	0.25	0.03	0.002/0.03	1.01/IND	0.25	0.03	0.004/0.03	1.02/IND
B17041	0.25	0.06	0.125/0.008	0.63/IND	0.25	0.03	0.002/0.03	1.01/IND	0.25	0.06	0.125/0.03	1.00/IND
B18560	0.25	0.008	0.016/0.004	0.56/IND	0.25	0.008	0.001/0.008	1.00/IND	0.125	0.008	0.004/0.004	0.53/IND
B18843	0.25	0.004	0.125/0.016	4.50/ANT	0.25	0.008	0.001/0.008	1.00/IND	0.25	0.008	0.125/0.001	0.63/IND
B12694	0.25	0.03	0.001/0.03	1.00/IND	0.25	0.03	0.001/0.016	0.54/IND	0.25	0.06	0.001/0.03	0.50/SYN
B12663	0.25	0.03	0.002/0.03	1.01/IND	0.25	0.03	0.002/0.03	1.01/IND	0.25	0.06	0.002/0.03	0.51/IND
B12664	0.25	0.06	0.016/0.03	0.56/IND	0.25	0.03	0.008/0.016	0.57/IND	0.125	0.06	0.06/0.016	1.01/IND
B20931	0.25	0.008	0.002/0.008	1.01/IND	0.25	0.016	0.016/0.008	0.56/IND	0.25	0.016	0.002/0.016	1.01/IND
B21040	0.25	0.004	0.001/0.004	1.00/IND	0.25	0.008	0.008/0.004	0.53/IND	0.25	0.016	0.001/0.016	1.00/IND

^a90% growth inhibition endpoint for combinations with AMB. For all other combinations 50% growth inhibition endpoint was used.

^bMinimum FICI unless antagonism was observed.

^cMIC of single drug was rounded to the next highest two-fold dilution if 50% (90% for amphotericin B) growth inhibition was not reached at the highest concentration.

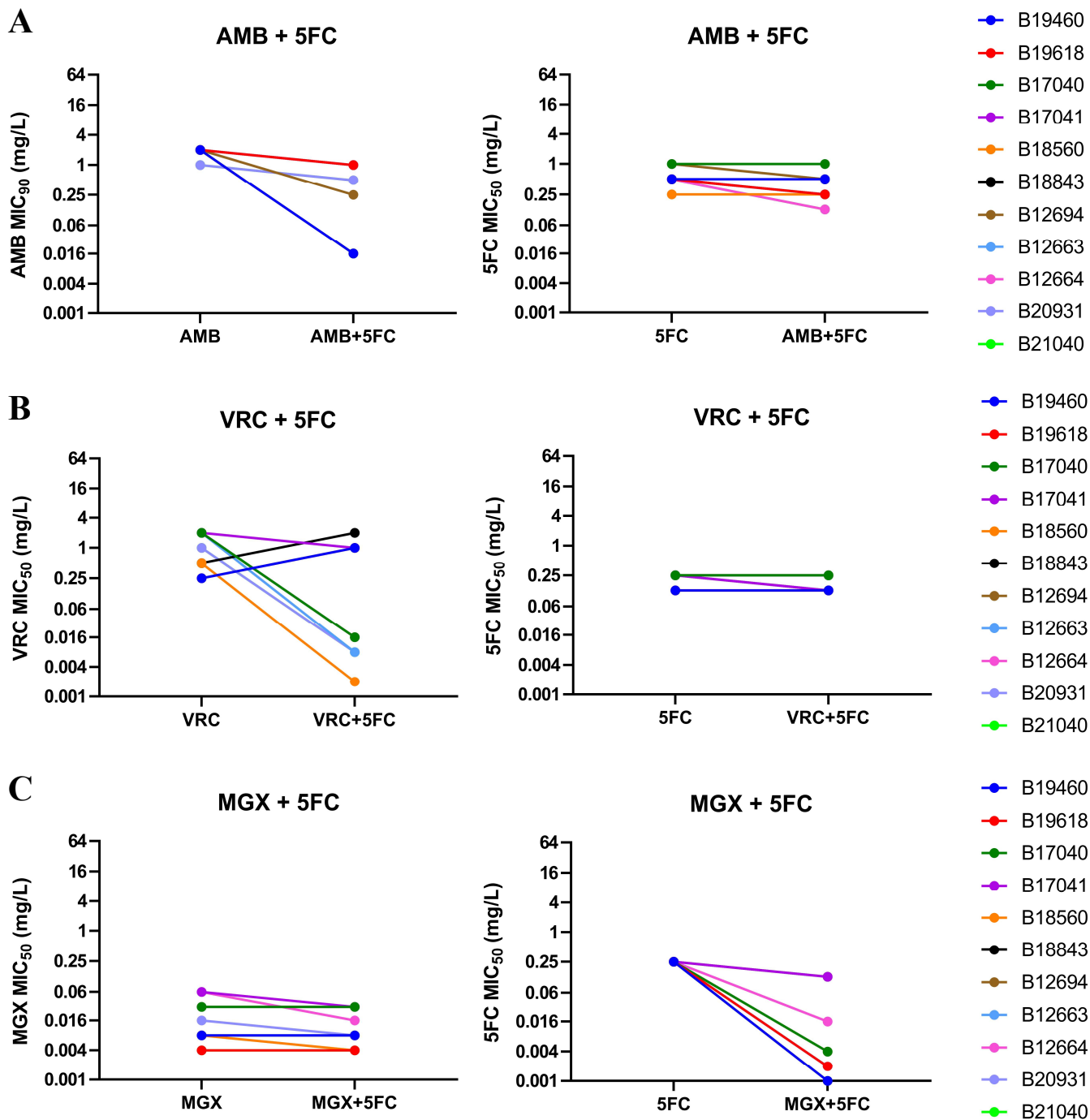


Figure S1. Changes in MIC values due to antifungal combinations for 11 *C. auris* isolates.

MIC values for 11 *C. auris* isolates in combinations of amphotericin B (A), voriconazole (B) and manogepix (C) with 5-flucytosine compared to the antifungals in monotherapy as determined by checkerboard assays. Symbols represent median values of three independent experiments. 5FC, 5-flucytosine; AMB, amphotericin B; MGX, manogepix; VRC, voriconazole.

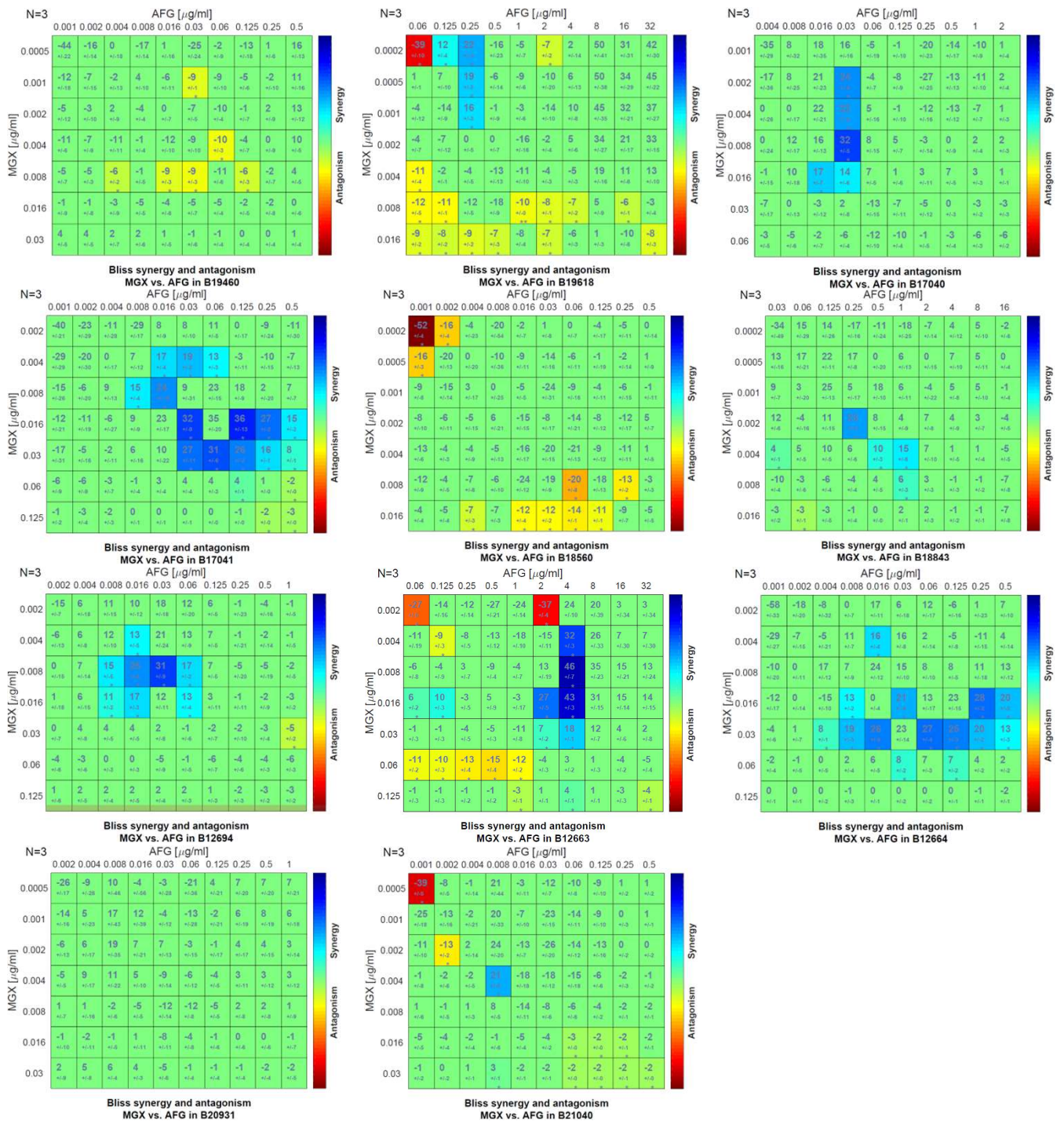


Figure S2. Matrix synergy plots for anidulafungin combined with manogepix.

The interactions of anidulafungin with manogepix were measured using checkerboard assays and analysed with Combenefit (n=3). The plots show the interaction score of each combination, the standard deviation (smaller font size) and their statistical significance (*P≤0.05; **P ≤ 0.001; ***P<0.0001. AFG, anidulafungin; MGX, manogepix.

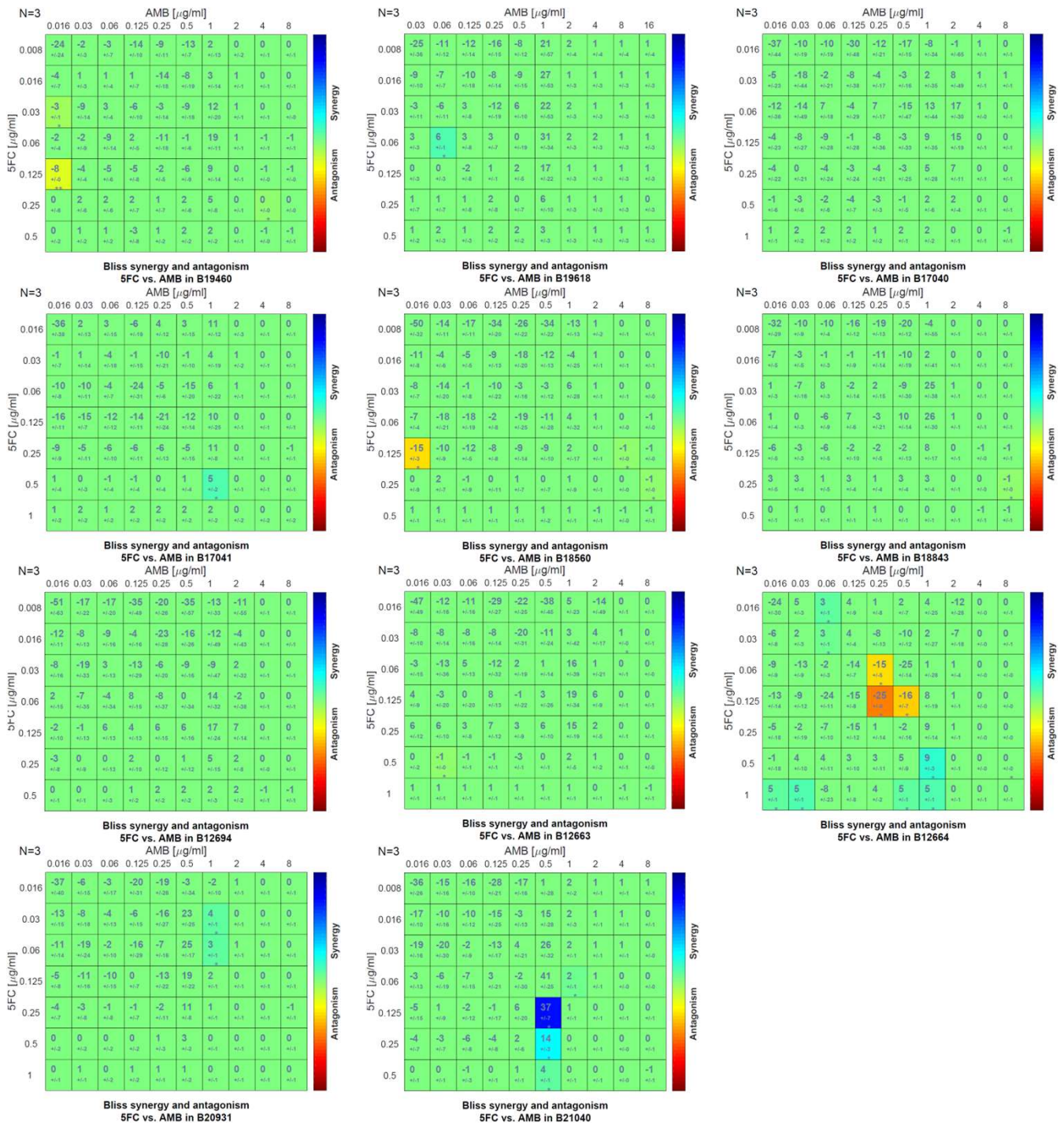


Figure S3. Matrix synergy plots for amphotericin B combined 5-flucytosine.

The interactions of amphotericin B with 5-flucytosine were measured using checkerboard assays and analysed with Combenefit (n=3). The plots show the interaction score of each combination, the standard deviation (smaller font size) and their statistical significance (*P<0.05; **P<0.001; ***P<0.0001. 5FC, 5-flucytosine; AMB, amphotericin B.

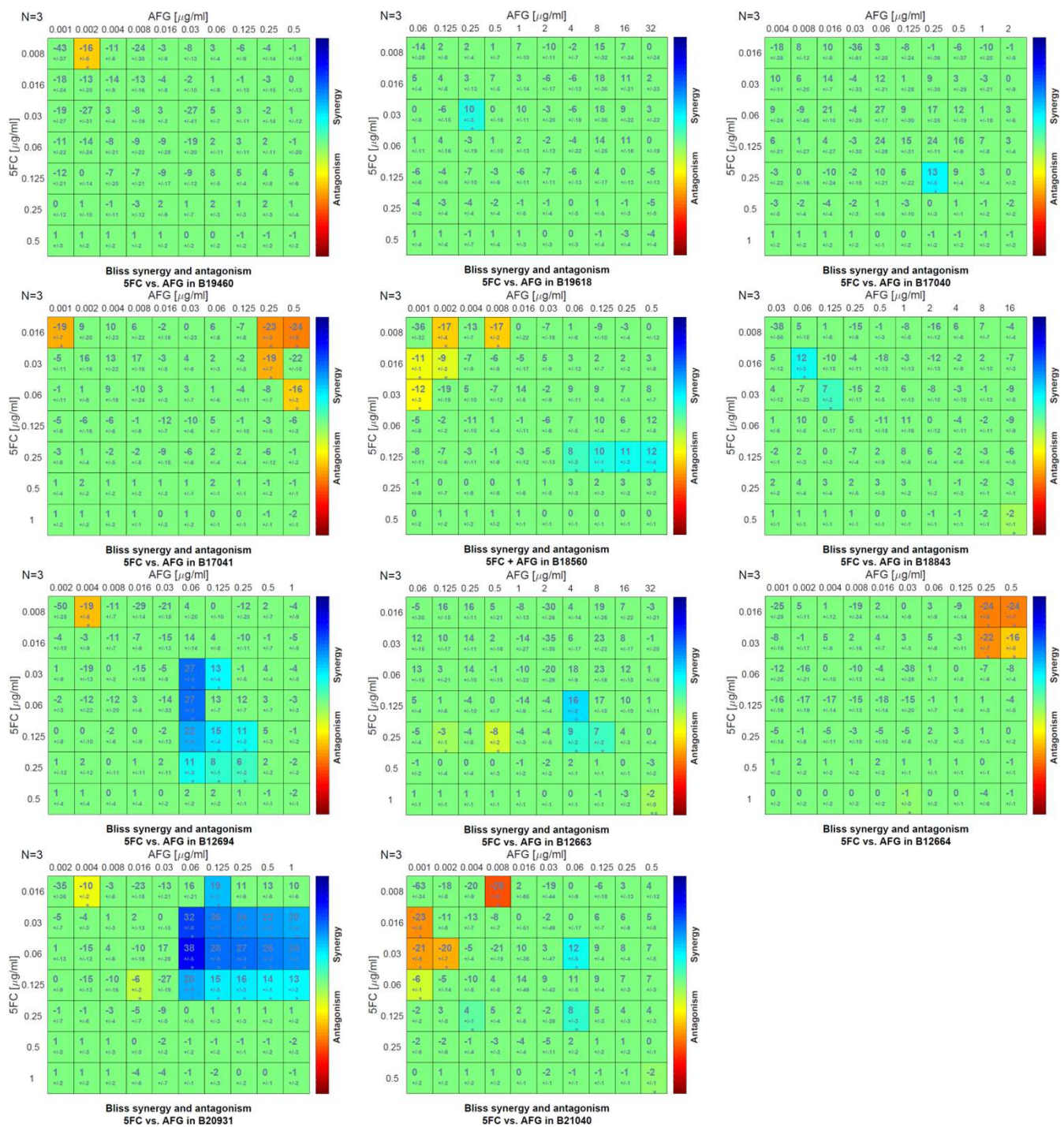


Figure S4. Matrix synergy plots for anidulafungin combined 5-flucytosine.

The interactions of anidulafungin with 5-flucytosine were measured using checkerboard assays and analysed with Combenefit (n=3). The plots show the interaction score of each combination, the standard deviation (smaller font size) and their statistical significance (* $P \leq 0.05$; ** $P \leq 0.001$; *** $P < 0.0001$). 5FC, 5-flucytosine; AFG, anidulafungin.

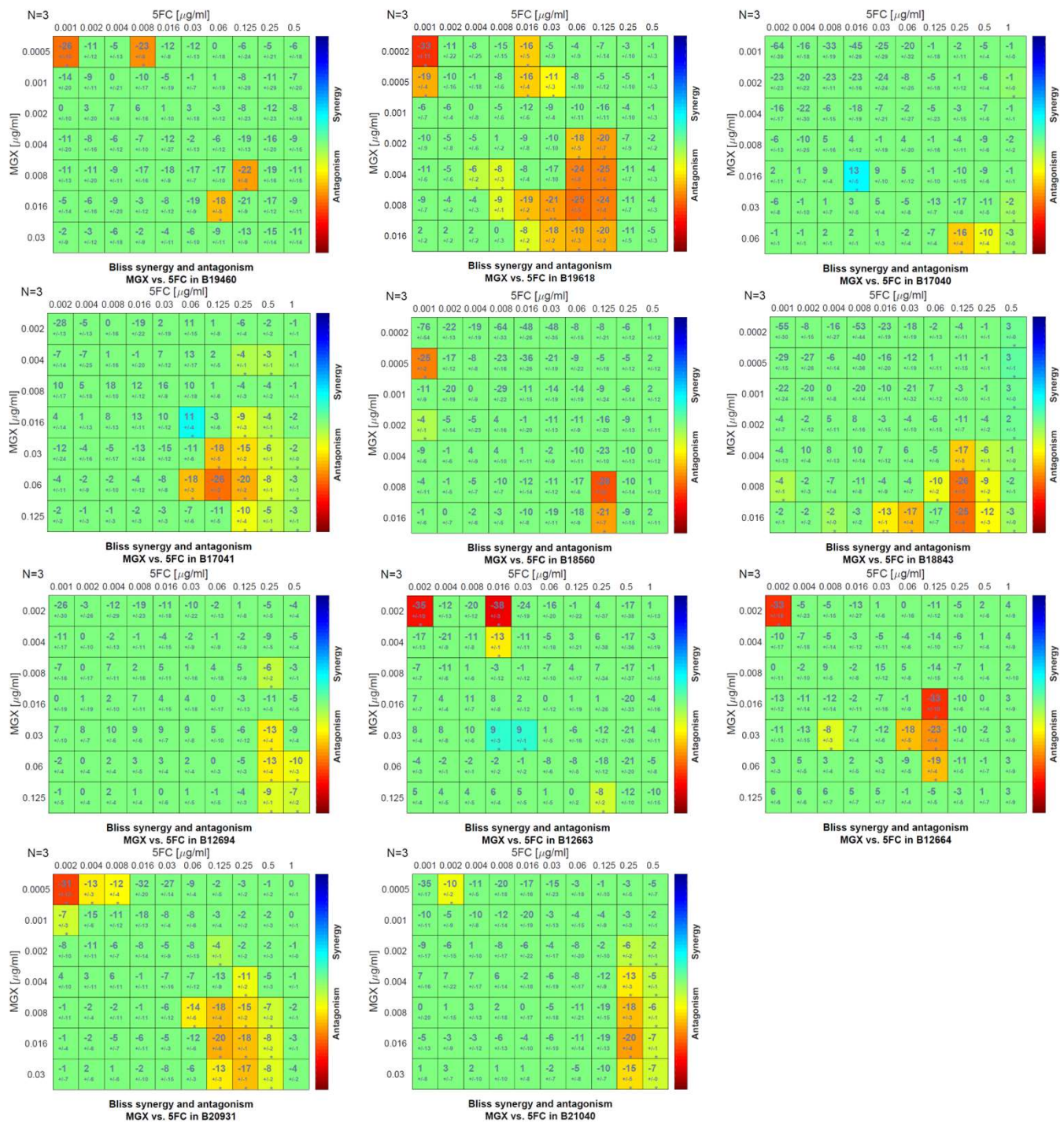


Figure S5. Matrix synergy plots for manogepix combined with 5-flucytosine.

The interactions of manogepix with 5-flucytosine were measured using checkerboard assays and analysed with Combeneft (n=3). The plots show the interaction score of each combination, the standard deviation (smaller font size) and their statistical significance (*P<0.05; **P<0.001; ***P<0.0001). 5FC, 5-flucytosine; MGX, manogepix.

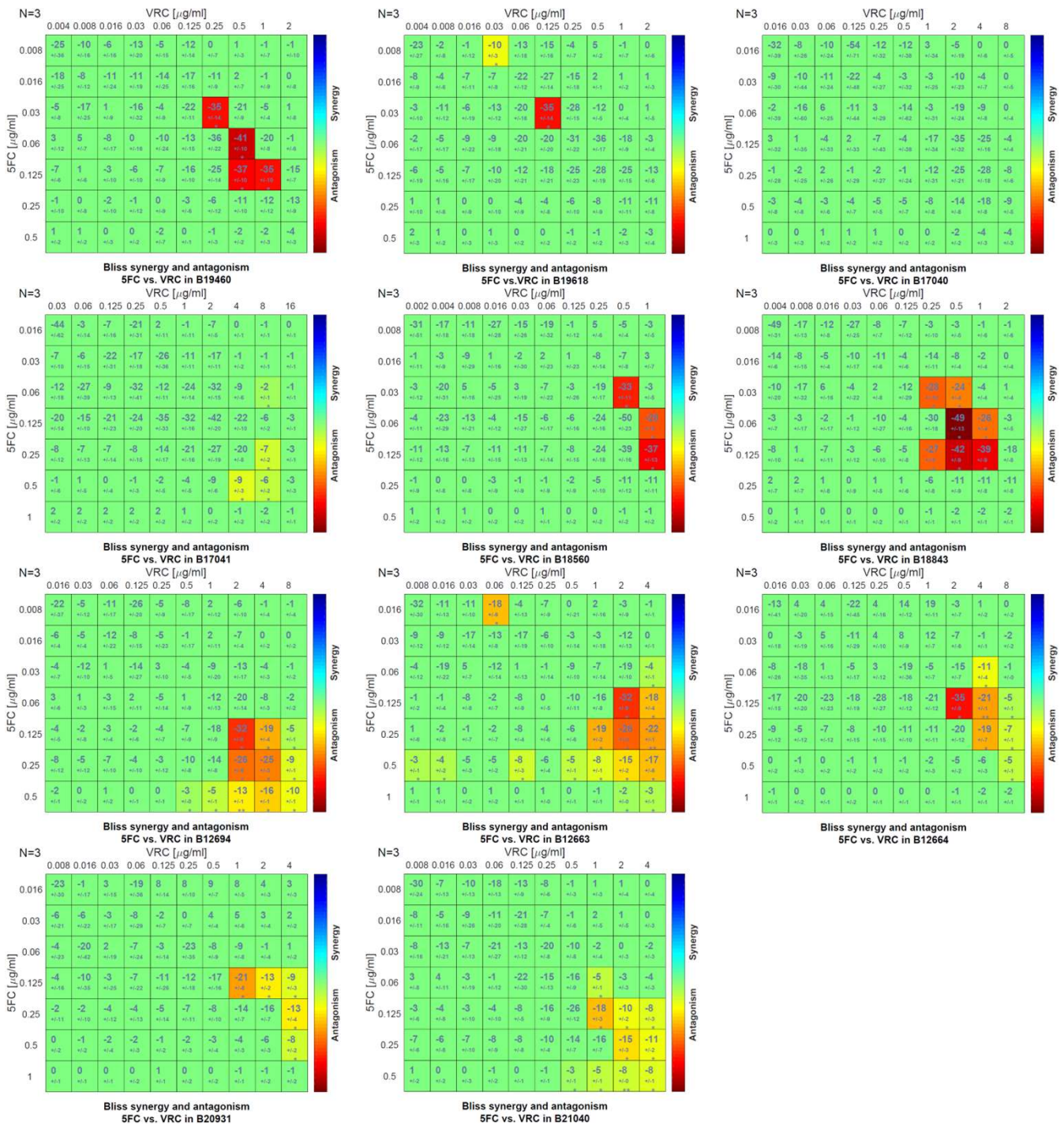


Figure S6. Matrix synergy plots for voriconazole combined with 5-flucytosine.

The interactions of voriconazole with 5-flucytosine were measured using checkerboard assays and analysed with Combeneft ($n=3$). The plots show the interaction score of each combination, the standard deviation (smaller font size) and their statistical significance (* $P \leq 0.05$; ** $P \leq 0.001$; *** $P < 0.0001$). 5FC, 5-flucytosine; VRC, voriconazole.

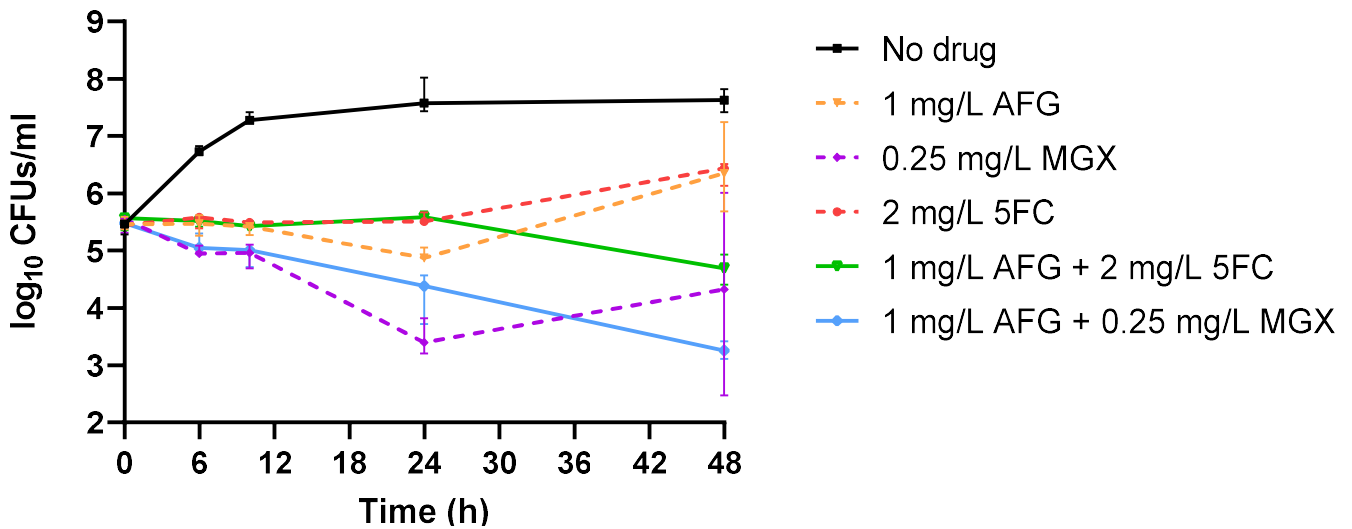


Figure S7. Time-kill curves for anidulafungin in combination with manogepix or 5-flucytosine against *C. auris*.

C. auris isolate B17041 was grown in RPMI 2%G-MOPS cultures with and without anidulafungin, manogepix or 5-flucytosine at 8x MIC₅₀ in monotherapy and in combination for 48 h at 37 °C. At each timepoint, aliquots were diluted, plated onto agar plates and incubated for up to 48 h at 37 °C to determine CFUs/ml. Datapoints represent median \pm range from three independent experiments. Anidulafungin, 5-flucytosine and manogepix in monotherapy showed regrowth at 48h after an initial fungistatic effect in the first 24 h. Both anidulafungin combinations achieved fungistatic activity over the complete 48 h with a reduction in CFUs/ml of 2.2 log₁₀-fold and 0.8 log₁₀-fold compared to the starting inoculum for combinations with manogepix and 5-flucytosine, respectively. The decline in CFUs/ml for the combination treatments is unlikely to be caused by the formation of cell aggregates as aggregation was also observed for anidulafungin and manogepix in monotherapy which did not result in a reduction in CFUs/ml. No antifungal treatment resulted in fungicidal activity, defined as ≥ 3 log₁₀ reduction in CFU/ml compared to the starting inoculum. 5FC, 5-flucytosine; AFG, anidulafungin; MGX, manogepix.

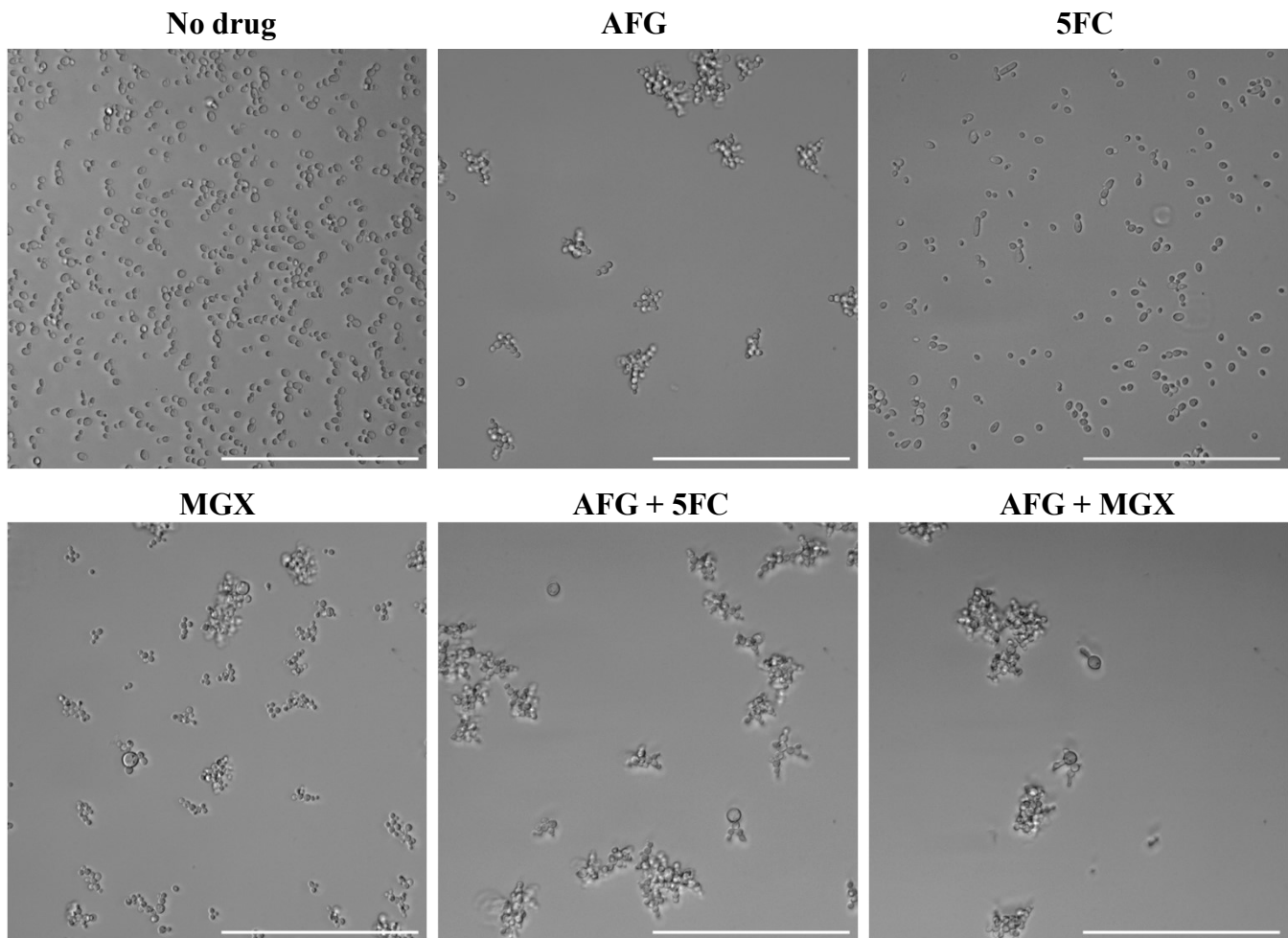
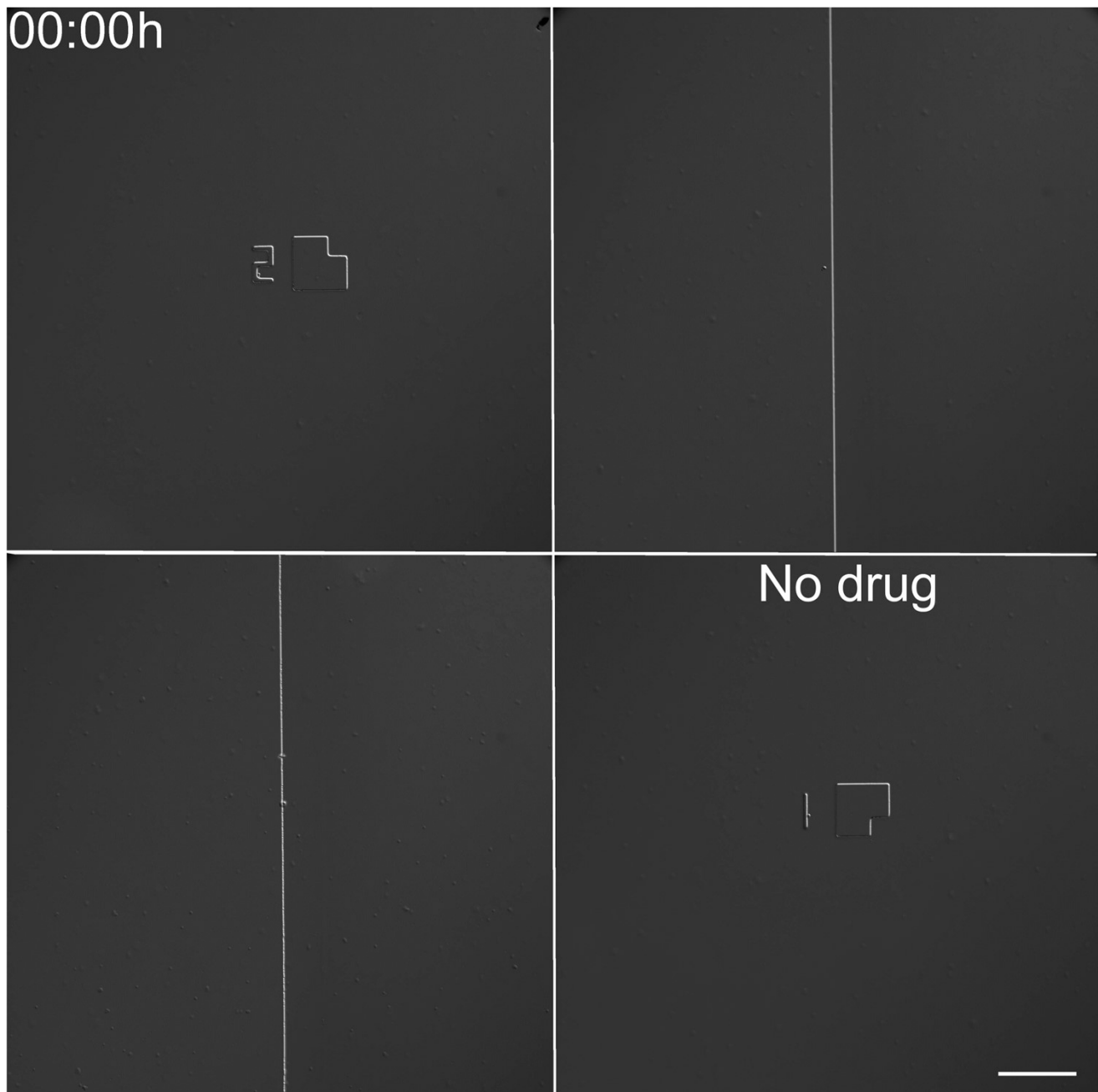


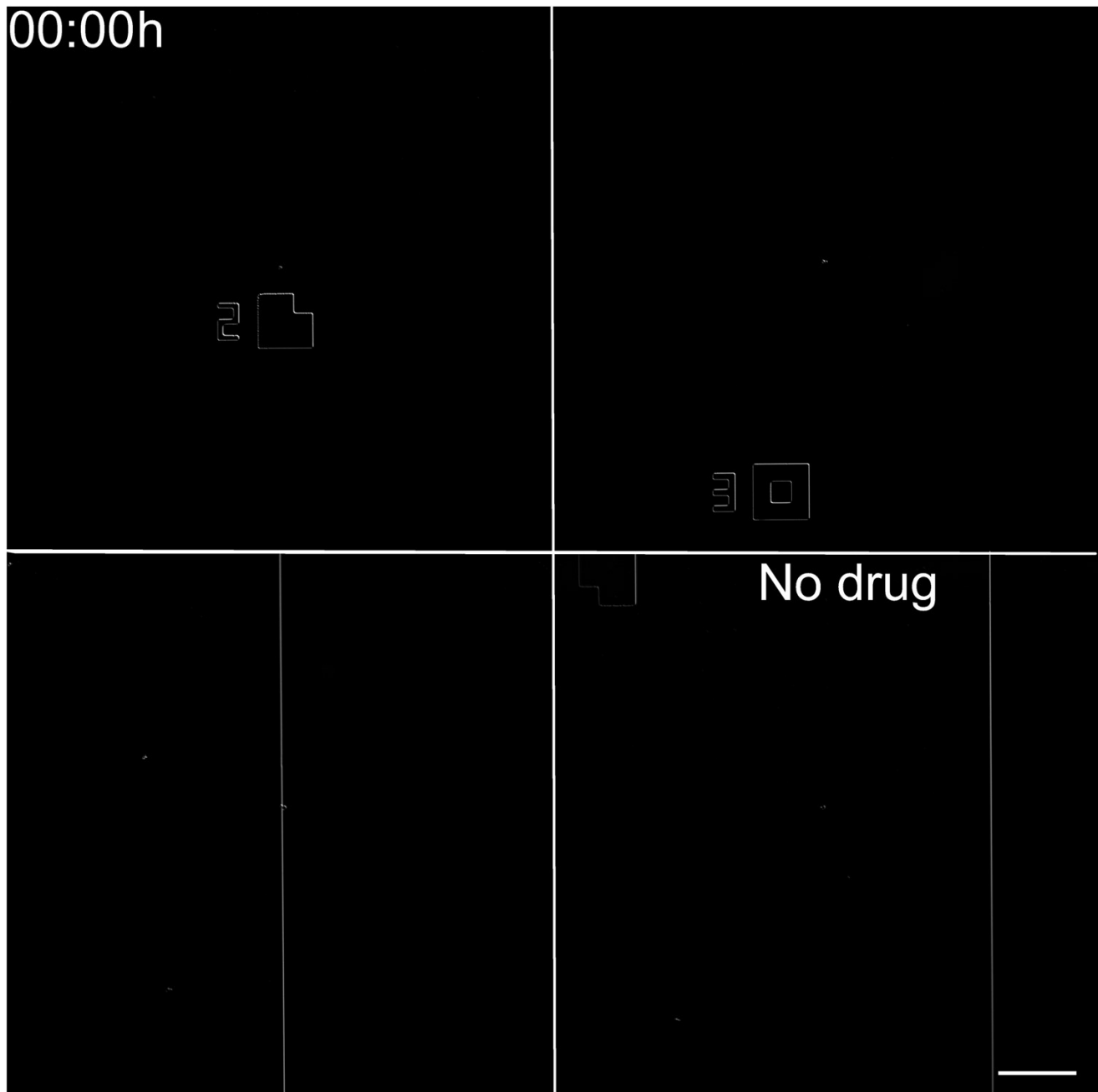
Figure S8. *C. auris* cell morphology after 24 h exposure to anidulafungin in combination with manogepix or 5-flucytosine.

Transmitted light images of *C. auris* B12663 cells grown in RPMI 2%G-MOPS with and without anidulafungin, manogepix or 5-flucytosine at 1x MIC₅₀ in monotherapy and in combination for 24 h at 37 °C. Under exposure to anidulafungin, manogepix and both combinations the cells displayed a rounder morphology with the formation of aggregates, while 5-flucytosine treatment resulted in a more elongated phenotype. Additionally, enlarged, round cells were observed in the presence of manogepix and both combinations. Scale bar: 100 µm. 5FC, 5-flucytosine; AFG, anidulafungin; MGX, manogepix.



Movie S1. Exposure of *C. auris* to anidulafungin and 5-flucytosine for 24 h.

Microfluidics DIC imaging of *C. auris* B12663 cells grown in the presence of RPMI 2%G-MOPS for 4 h, followed by further RPMI 2%G-MOPS or treatment with anidulafungin and 5-flucytosine alone or in combination at their MICs for 16 h. Scale bar: 100 μ m. 5FC, 5-flucytosine; AFG, anidulafungin.



Movie S2. Exposure of *C. auris* to anidulafungin and manogepix for 24 h.

Microfluidics DIC imaging of *C. auris* B12663 cells grown in the presence of RPMI 2%G-MOPS for 4 h, followed by further RPMI 2%G-MOPS or treatment with anidulafungin and manogepix alone or in combination at their MICs for 16 h. Scale bar: 100 μm . AFG, anidulafungin; MGX, manogepix.