SUPPLEMENTARY INFORMATION:

Repurposing the antidepressant sertraline as SHMT inhibitor to suppress serine/glycine synthesis addicted breast tumor growth.

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Running title: Sertraline targets serine/glycine synthesis enzyme SHMT.

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This file includes:

SUPPLEMENTARY NOTE

A serine/glycine synthesis dependent lower eukaryotic yeast model

The yeast *Candida albicans*, the major fungal pathogen for humans, typically forms drug-tolerant biofilms on biotic surfaces such as the skin, the mouth, the human gastro-intestinal tract and genital area. To treat this type of mucosal fungal infection, azoles, such as miconazole, are currently used. In contrast to free-living *C. albicans* cells, the increasing tolerance of biofilm cells to azoles makes biofilm-associated infections hard to eradicate (1). Specifically, biofilm cells are up to thousand-fold more tolerant to miconazole than their planktonic counterparts (2).

Revisiting previously obtained transcriptome data of miconazole-induced tolerance pathways in *C. albicans* biofilm cells highlighted a biofilm-specific upregulation of genes involved in serine/glycine synthesis (*SHM2*) and one-carbon metabolism (*MET13, SAH1, GCV1, GCV2* and *MIS11*) (3). In support of these data, conditioned medium of *C. albicans* biofilms that were treated with a sublethal dose of miconazole contained an excess of serine and glycine as compared to conditioned medium of control cultures (Supplementary Fig. S1A). In this experimental set-up, there was no difference in reductive potential between miconazole-stressed *C. albicans* biofilm cells and control cells (Supplementary Fig. S1B). We therefore reasoned that miconazole-stressed *C. albicans* biofilm cells upregulate their serine/glycine synthesis as a tolerance mechanism against sublethal miconazole doses. Hence, miconazole-stressed *C. albicans* biofilms can serve as a valuable platform to screen for compounds targeting serine/glycine synthesis, which are expected to re-sensitize the biofilms to miconazole.

SUPPLEMENTARY MATERIALS AND METHODS

***C. albicans* yeast strain and chemicals.** *C. albicans* strain SC5314 (4) was grown routinely on YPD (1% yeast extract, 2% peptone and 2% glucose) agar plates at 30°C. RPMI-1640 (pH 7.0) with L-glutamine and without sodium bicarbonate was buffered with MOPS. 1000x stock solutions of miconazole (Sigma-Aldrich #3512) and sertraline (Sigma-Aldrich #S6319) were prepared in DMSO and stored at -20°C.

***C. albicans* serine and glycine medium measurements.** A *C. albicans* SC5314 overnight culture, grown in YPD, was diluted to an optical density (OD) of 0.1 (approximately 10^6 cells/ml) in RPMI-1640 and 2 ml of this suspension was added to the wells of a 6-well plate. After 1 hour of adhesion at 37°C, the medium was aspirated and biofilms were washed with PBS to remove non-adherent cells, followed by addition of 2 ml fresh RPMI-1640. Subsequently, the biofilms were grown at 37°C for 24 hours. After washing the biofilms with PBS, miconazole (75 µM) was added in RPMI-1640, resulting in a DMSO background of 0.2%. Next, biofilms were incubated at 37°C for an additional 24 hours. Finally, 1 ml of conditioned medium was collected and serine and glycine were quantified by cation-exchange chromatography on a Biochrom 30 analyzer (Biochrom, Cambourne, UK).

***C. albicans* metabolic activity assay.** Biofilms were grown in 6-well plates and treated in RPMI-1640 as described above. After washing with PBS, 2 ml Cell-Titer Blue (CTB; Promega #G8080) (5), diluted 1:100 in PBS, was added to each well. After 1 hour of incubation in the dark at 37°C, fluorescence was measured with a fluorescence spectrometer (Synergy Mx Multimode Microplate Reader; BioTek) at λ_{ex} of 535 nm and λ_{em} of 590 nm. Finally, the percentage of metabolically active biofilm cells was calculated as described before (6).

***C. albicans* nuclear membrane permeability assay.** Biofilms were grown in 96-well plates and treated with miconazole (75 µM) and/or sertraline (75 µM) in RPMI-1640. After washing with PBS, propidium iodide staining (Sigma-Aldrich) was performed as previously described (7).

Generation of Ba/F3 CRISPR-Cas9 RPL10 R98S mutant cells. Ba/F3 cells were electroporated (6 square wave pulses, 0.1ms interval, 175V) with a pX458 vector (a gift from Feng Zhang; Addgene plasmid #48138, RRID:Addgene_48138) containing an Rpl10-targeting sgRNA (5'-TGATACGGATGACATGGAAA-3') and a 117-nt donor oligo containing the R98S allele as well as 3 silent mutations to avoid re-recognition and cutting by the sgRNA-Cas9 complex (5'-CCAACAAATACATGGTAAAGAGTTGTGGCAAGGATGGCTTTCATATCCGAGTGAGGCTCCATCCTTTTCATGTAATCAGTATCAACAAGATGTTGTCCTGTGCTGGGGCTGACAGGT-3';
Integrated DNA Technologies). Following electroporation, cells were incubated for 24 hours in the presence of 10 µM SCR7 (Sigma-Aldrich #SML1546), followed by single cell sorting (BD FACS

Aria III) into ClonaCell™-TCS Medium (STEMCELL Technologies #03814). Outgrowing clones were expanded and screened for the desired modification using Sanger sequencing to confirm the Rpl10 status and to check for modification of relevant predicted off-target effects (Rpl10-ps3 and Rpl10l).

Gas chromatography–mass spectrometry (GC-MS). Polar metabolites were derivatized and measured as described before (8,9). In brief, polar metabolites were derivatized with 20 mg/ml methoxyamine in pyridine at 37°C for 90 minutes and subsequently with N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide, with 1% tert-butyldimethylchlorosilane at 60°C for 60 minutes. Metabolites were measured with a 7890A GC system (Agilent Technologies) combined with a 5975C Inert MS system (Agilent Technologies). One microliter of samples was injected in split mode (ratio 1 to 3) with an inlet temperature of 270°C onto a DB35MS column. The carrier gas was helium with a flow rate of 1 ml/min. For the measurement of polar metabolites from the deuterated serine labeling experiment, the GC oven was set at 100°C for 1 minute and then increased to 105°C at 2.5°C/min and with a gradient of 2.5°C/ min finally to 320°C at 22°C/min. The measurement of metabolites has been performed under electron impact ionization at 70 eV using a selected-ion monitoring (SIM) mode. For the measurement of polar metabolites from the $^{13}C_6$-glucose labeling experiment, the GC oven was held at 100°C for 3 minutes and then ramped to 300°C with a gradient of 2.5°C/min. The mass spectrometer system was operated under electron impact ionization at 70 eV and a mass range of 100–650 a.m.u. was scanned. Mass distribution vectors were extracted from the raw ion chromatograms using a custom Matlab M-file (MATLAB, RRID:SCR_001622), which applies consistent integration bounds and baseline correction to each ion (10). Moreover, data were corrected for naturally occurring isotopes (11). For metabolite levels, arbitrary units of the metabolites of interest were normalized to glutaric acid, an internal standard, and protein content.

Liquid chromatography–mass spectrometry (LC-MS). A liquid chromatography, Dionex UltiMate 3000 LC System (Thermo Scientific), coupled to mass spectrometry (MS), a Q Exactive Orbitrap (Thermo Scientific), was used for the separation of polar metabolites. A volume of 15 µl of sample was injected and metabolites were separated on a C18 column (Acquity UPLC HSS T3 1.8 µm 2.1x100 mm) at a flow rate of 0.25 ml/min, at 40°C. A gradient was applied for 40 minutes (solvent A: 0 H2O, 10 mM Tributyl-Amine, 15 mM acetic acid – solvent B: Methanol) to separate the targeted metabolites (0 min: 0% B, 2 min: 0% B, 7 min: 37% B, 14 min: 41% B, 26 min: 100% B, 30 min: 100% B, 31 min: 0% B; 40 min: 0% B). The MS operated in full scan in negative mode (m/z range: 70-1050 and 300-800 from 8 to 25 min) using a spray voltage of 4.9 kV, capillary temperature of 320°C, sheath gas at 50.0, auxiliary gas at 10.0. Data was collected using the

Xcalibur software (Thermo Xcalibur, RRID:SCR_014593) and analyzed with Matlab (MATLAB, RRID:SCR_001622) using the same procedure as described above for the analysis of GC-MS data.

SUPPLEMENTARY TABLES

Table S1. List of yeast re-sensitizing agents.

Re-sensitizing capacity	Compound	Clinical use
HIGH (>90%)	Clioquinol	Antifungal, antiprotozoal
	Benzalkonium chloride	Disinfectant, antimicrobial, antifungal
	Dequalinium chloride	Antiseptic, disinfectant, bacteriostatic
	Methylbenzethonium chloride	Antiseptic, disinfectant, antimicrobial
	Pyrvinium pamoate	Anthelmintic
	Hexachlorophene	Disinfectant, antibacterial, antifungal
	Dichlorophene	Antifungal, antimicrobial, anticestodal
	Bithionate sodium	Anthelmintic
	Alexidine hydrochloride	Antimicrobial
	Gentian/Crystal violet	Antibacterial, antifungal, anthelmintic
INTERMEDIATE (50-90%)	Thimerosal **(sulfur groups)**	Antiseptic, antifungal
	Phenylmercuric acetate	Preservative, disinfectant
	Nitroxoline	Antibacterial
	Chloroxine	Antibacterial
	Piroctone olamine	Antifungal, anti-dandruff shampoo
	Ciclopirox olamine	Antifungal
	Pyrithione zinc **(sulfur groups)**	Fungistatic, bacteriostatic
	Broxyquinoline	Antiprotozoal
	Iodoquinol	Treatment of amoebiasis
	Evans blue	Viability assay dye
	Artemisinin	Antimalarial
	Artenimol	Antimalarial
	Abamectin	Insecticide, anthelmintic
LOW (<50%)	Suloctidil **(sulfur groups)**	Vasodilator
	Isosorbide mononitrate	Treatment of heart related chest pain
	Sodium nitroprusside	Antihypertensive
	Temozolomide	Oral chemotherapy drug
	Folic acid	Vitamin B_9
	Bupropion	Antidepressant
	Sulfamethoxypyridazine	Antibacterial
	Oxantel pamoate	Anthelmintic
	Tioconazole **(sulfur groups)**	Antifungal
	Butoconazole **(sulfur groups)**	Antifungal
	Flucytosine	Antifungal
	Diacetamate	Analgesic, anti-inflammatory drug
	Artemether	Antimalarial
	Artesunate	Antimalarial
	Orbifloxacin	Antibacterial
	Salinomycin	Antibacterial
	Methenamine	Hardening component, binder
	Candicidin	Antifungal
	Warfarin	Anticoagulant, blood thinner
	Carvedilol phosphate	Antihypertensive
	Acipimox	Lipid-lowering agent
	Merbromin	Antiseptic
	Phentermine	Treatment of obesity
	Malathion **(sulfur groups)**	Insecticide
	Methylene blue **(sulfur groups)**	Treatment of methemoglobinemia, dye
	Sulfanilate zinc **(sulfur groups)**	Antibacterial

Table S2. Computational docking and ligand efficiency scores of sertraline to SHMT1. A list of 6392 FDA approved molecules was docked into the active pocket of SHMT1 to compare with sertraline's docking and ligand efficiency score. The antifolate drugs pemetrexed and lometrexol, that are part of the list of FDA approved drugs, are used as a reference for SHMT inhibition.

Compound	Docking score	Ligand Efficiency
Sertraline	61.49	3.07
Pemetrexed[a]	79.29	2.55
Lometrexol[a]	81.86	2.55

[a] *Confirmed antifolate drugs with SHMT inhibitory activity.*

Table S3. Computational docking and ligand efficiency score of sertraline to SHMT2. A list of 6392 FDA approved molecules was docked into the active pocket of SHMT2 to compare with sertraline's docking and ligand efficiency score. The antifolate drugs pemetrexed and lometrexol, that are part of the list of FDA approved drugs, are used as a reference for SHMT inhibition.

Compound	Docking score	Ligand Efficiency
Sertraline	51.34	2.56
Pemetrexed[a]	68.48	2.20
Lometrexol[a]	72.25	2.25

[a] *Confirmed antifolate drugs with SHMT inhibitory activity.*

SUPPLEMENTARY FIGURES

Figure S1

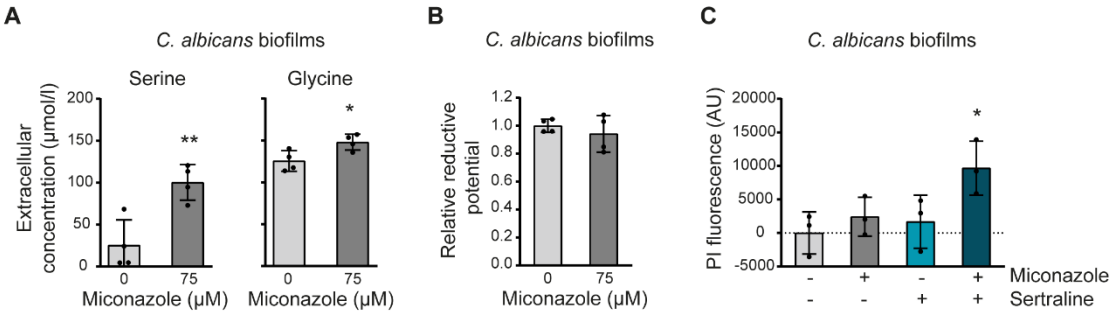

Figure S1. Sertraline re-sensitizes *Candida albicans* biofilms that upregulate their serine/glycine synthesis as a tolerance mechanism against sublethal antifungal stress. (A) Serine (left) and glycine (right) extracellular concentrations (µmol/l) of *C. albicans* biofilms treated with control (DMSO) or miconazole (75 µM) for 24 hours (n = 4, Student's t-test). **(B)** Reductive potential, measured with Cell-Titer Blue, of miconazole-stressed (75 µM) *C. albicans* biofilm cells and control cells (DMSO) (n = 4, Student's t-test). **(C)** Cell death, measured using propidium iodide (PI), of *C. albicans* biofilms treated with control (DMSO), miconazole (75 µM), sertraline (75 µM) or a combination of both (n = 3, Two-way ANOVA, Dunnett's multiple comparisons test). While 75 µM of miconazole or sertraline monotherapy was not significantly affecting cell death, sertraline was able to re-sensitize biofilm cells to miconazole's activity, resulting in approximately 4-fold increased cell death as compared to single drug treatment. In **(A-C)** data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$.

Figure S2

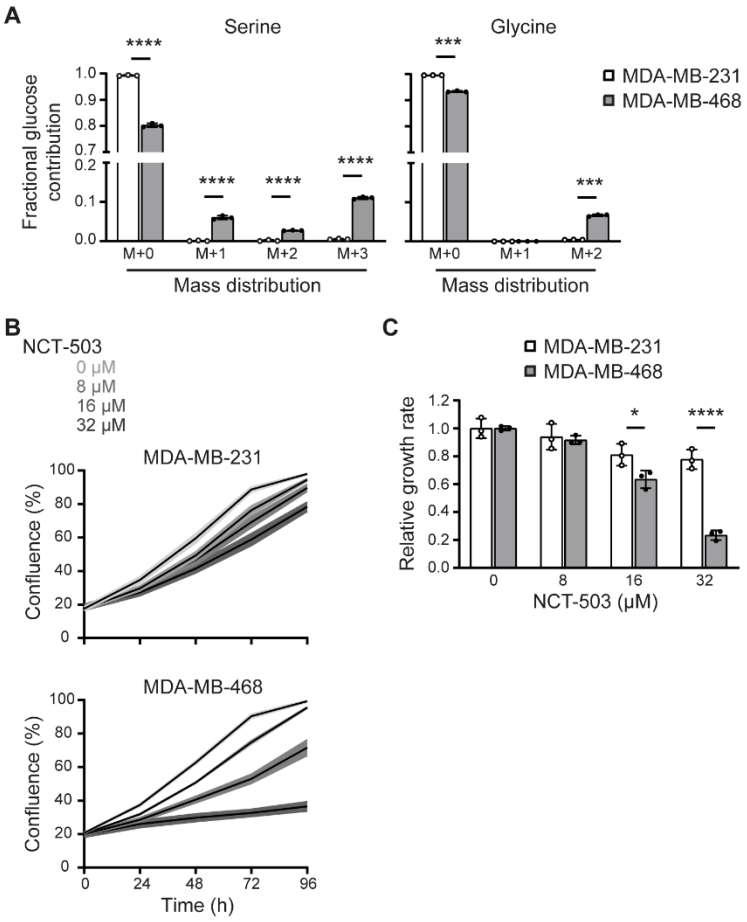

Figure S2. MDA-MB-231 and MDA-MB-468 distinguish in being serine/glycine uptake versus serine/glycine synthesis addicted, respectively. (A) Carbon incorporation from $^{13}C_6$-glucose into serine and glycine in MDA-MB-231, a serine/glycine uptake dependent BRCA cell line, and MDA-MB-468, a serine/glycine synthesis addicted BRCA cell line (n = 3, Multiple t-test). **(B)** Proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-231 (upper) and MDA-MB-468 (lower) cells upon treatment with indicated concentrations of NCT-503. One representative result of three biological replicates, containing each at least three technical replicates, is shown. **(C)** Quantification of (B). Data are presented as growth rate relative to the control treatment (n = 3, Two-way ANOVA, Sidak's multiple comparisons test). In **(A-C)** data are presented as mean ± SD. *p < 0.05, ***p < 0.001, ****p < 0.0001.

Figure S3

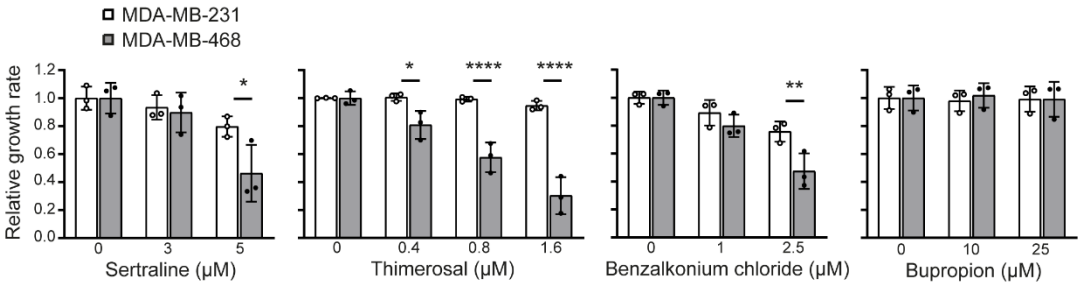

Figure S3. Sertraline, thimerosal and benzalkonium chloride selectively decrease MDA-MB-468 cell proliferation. Quantification of proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-231 (white) and MDA-MB-468 (grey) cells upon treatment with indicated concentrations of sertraline, thimerosal, benzalkonium chloride and bupropion (left to right). Data are presented as growth rate relative to the control treatment (mean ± SD). *p < 0.05, **p < 0.01, ****p < 0.0001, Two-way ANOVA, Sidak's multiple comparisons test.

Figure S4

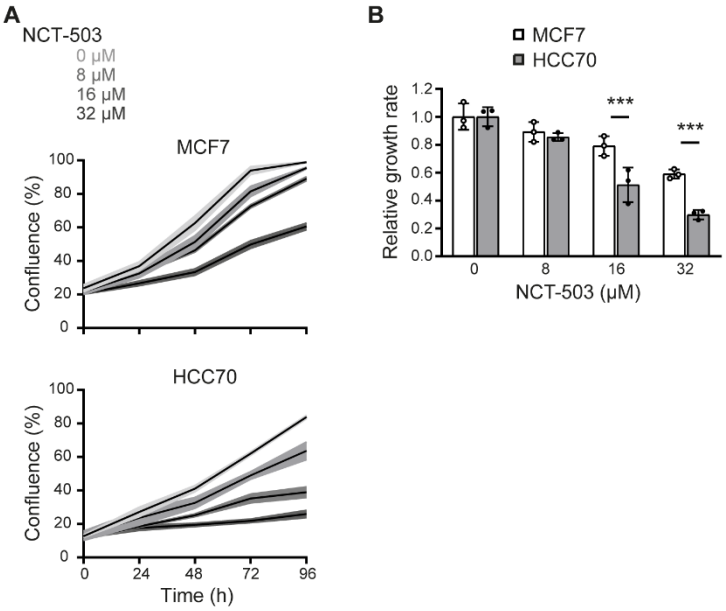

Figure S4. NCT-503 inhibits proliferation of HCC70 more than of MCF7. (A) Proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MCF7 (upper), a serine/glycine uptake dependent BRCA cell line, and HCC70 (lower), a serine/glycine synthesis addicted BRCA cell line, cells upon treatment with indicated concentrations of NCT-503. One representative result of three biological replicates, containing each at least three technical replicates, is shown. **(B)** Quantification of (A). Data are presented as growth rate relative to the control treatment (n = 3, Two-way ANOVA, Sidak's multiple comparisons test). In **(A-B)** data are presented as mean ± SD. ***p < 0.001.

Figure S5

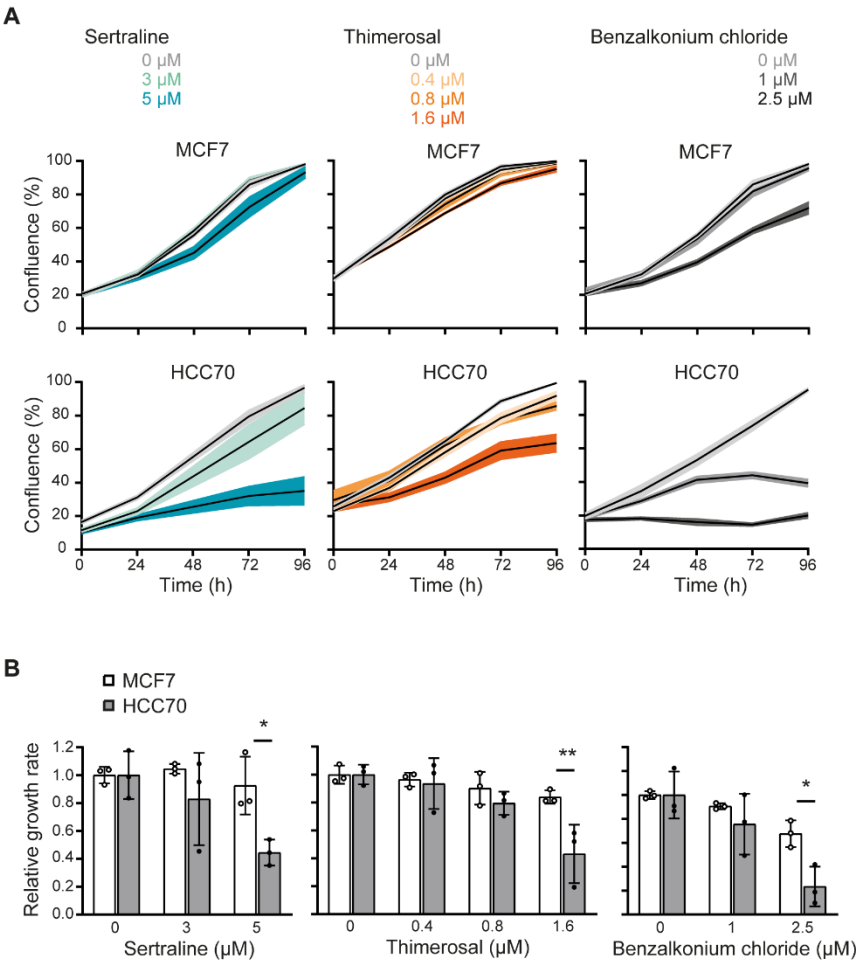

Figure S5. Sertraline, thimerosal and benzalkonium chloride selectively impair HCC70 cell proliferation. (A) Proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MCF7 (upper) and HCC70 (lower) cells upon treatment with indicated concentrations of sertraline, thimerosal, benzalkonium chloride (left to right). **(B)** Quantification of (A). Data are presented as growth rate relative to the control treatment (n = 3, Two-way ANOVA, Sidak's multiple comparisons test). In **(A-B)** data are presented as mean ± SD. *p < 0.05, **p < 0.01.

Figure S6

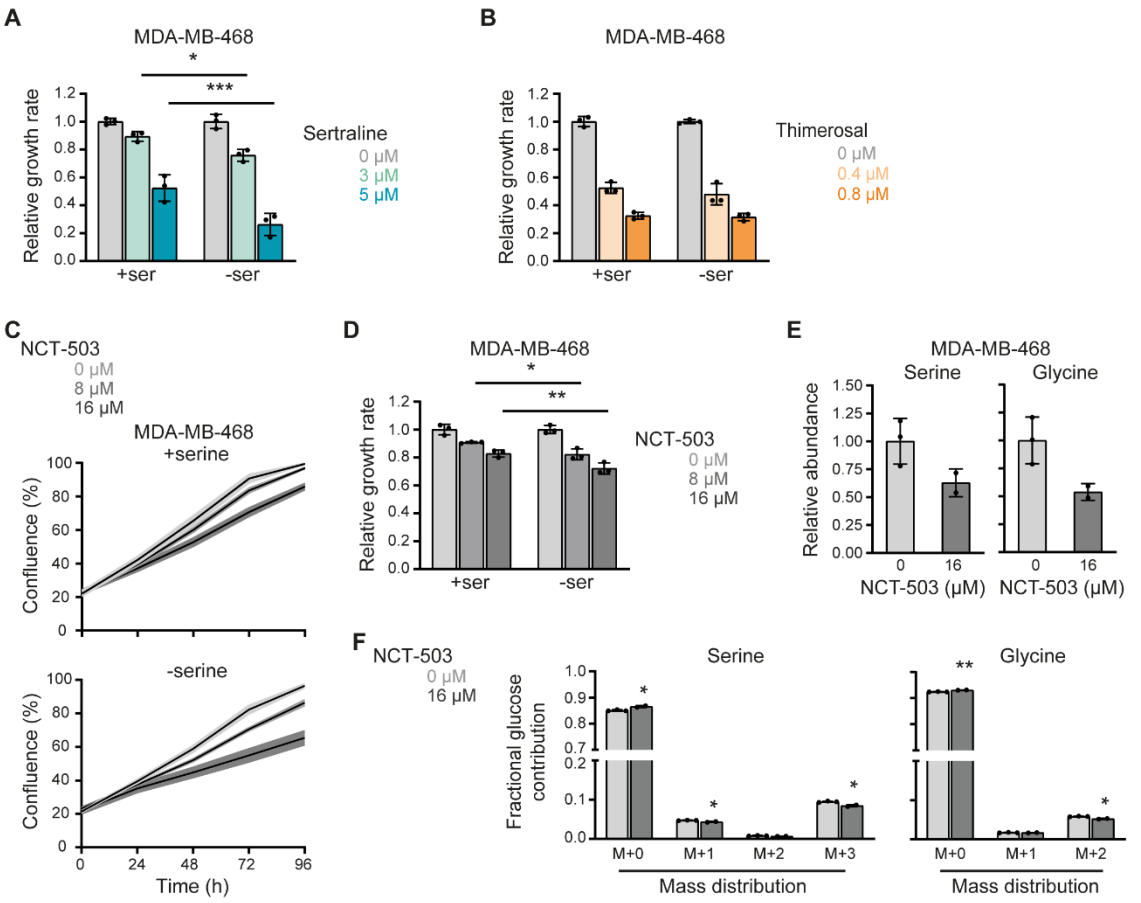

Figure S6. Sertraline and thimerosal act as NCT-503. (A-B) Quantification of proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-468 cells cultured in DMEM with or without serine (400 µM) and treated with indicated concentrations of sertraline (A) or thimerosal (B). Data are presented as growth rate relative to the control treatment (n = 3, Two-way ANOVA, Sidak's multiple comparisons test). **(C)** Proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-468 cells cultured in DMEM with or without serine (400 µM) and treated with indicated concentrations of NCT-503. One representative result of three biological replicates, containing each at least three technical replicates, is shown. **(D)** Quantification of (C). Data are presented as growth rate relative to the control treatment (n = 3, Two-way ANOVA, Sidak's multiple comparisons test). **(E)** Relative abundance of intracellular serine and glycine in MDA-MB-468 cells treated with control (DMSO) or NCT-503 (16 µM) for 24 hours (n = 2-3, Student's t-test). **(F)** Serine and glycine mass distribution showing the fractional glucose contribution of each mass upon treatment of MDA-MB-468 cells with

control (DMSO) or NCT-503 (16 μM) (n = 2-3, Multiple t-test). In (A-F) data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure S7

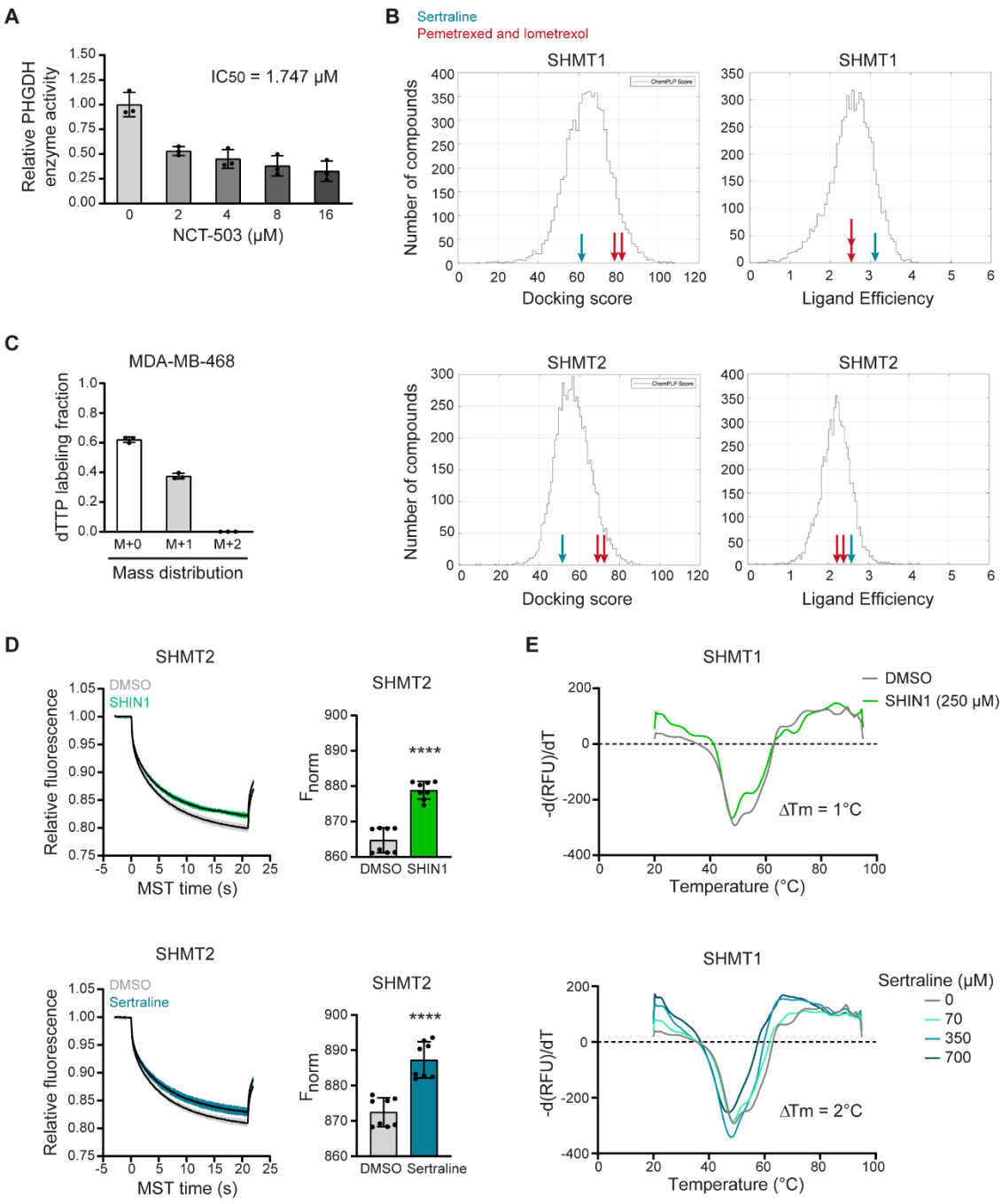

Figure S7. Sertraline is a dual SHMT1/2 inhibitor. (A) PHGDH *in vitro* enzymatic assay, measuring PHGDH activity upon addition of indicated concentrations of NCT-503. Values are presented relative to the control (n = 3). **(B)** Histograms showing the docking scores (left) and ligand efficiencies (right) of 6392 FDA approved molecules docked into the active pocket of SHMT1

(upper) and SHMT2 (lower). The SHMT inhibiting antifolates, pemetrexed and lometrexol, are indicated with red arrows to compare with sertraline (blue arrow). **(C)** Deuterium label (^2H) incorporation into thymidine (dTTP) in MDA-MB-468 cells incubated with [2,3,3-^2H]-serine (deuterium labeled serine) for 48 hours. Only M+1 dTTP (SHMT2), and no M+2 dTTP (SHMT1), was detected (n = 3). **(D)** MST curves (left) and normalized fluorescence data at 5 seconds (F_{norm}) for SHMT2 bound by 500 µM SHIN1 (upper) or 730 µM sertraline (lower). Error bars represent the standard deviation of the measurements of two independent repeats (n = 2), containing each at least four technical replicates. **(E)** Melting temperature (Tm) curves demonstrating destabilization of SHMT1 upon binding of SHIN1 (upper) and sertraline (lower). One representative result of two biological replicates is shown. In **(A, C and D)** data are presented as mean ± SD. ****p < 0.0001.

Figure S8

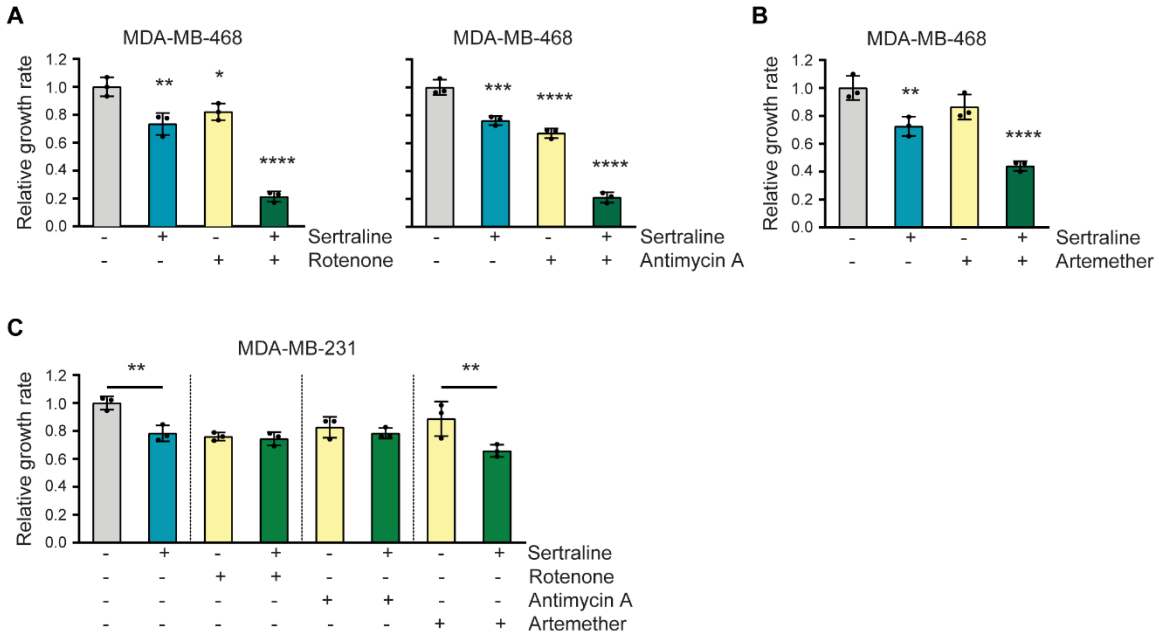

Figure S8. Combining sertraline with drugs causing general mitochondrial dysfunction further decreases proliferation of MDA-MB-468 cells. (A-B) Quantification of proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-468 cells upon treatment with sertraline (5 µM) (blue) and/or (green/yellow) rotenone (50 nM) (A), antimycin A (50 nM) (A) or artemether (80 µM) (B) (n = 3, Two-way ANOVA, Dunnet's multiple comparisons test). **(C)** Quantification of proliferation during 96 hours, as determined by real-time monitoring of cell confluence (%), of MDA-MB-231 cells upon treatment with sertraline (5 µM) (blue) and/or (green/yellow) rotenone (50 nM), antimycin A (50 nM) or artemether (80 µM) (n = 3, One-way ANOVA, Sidak's multiple comparisons test). In **(A-C)** data are presented as growth rate relative to the control treatment (mean ± SD). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure S9

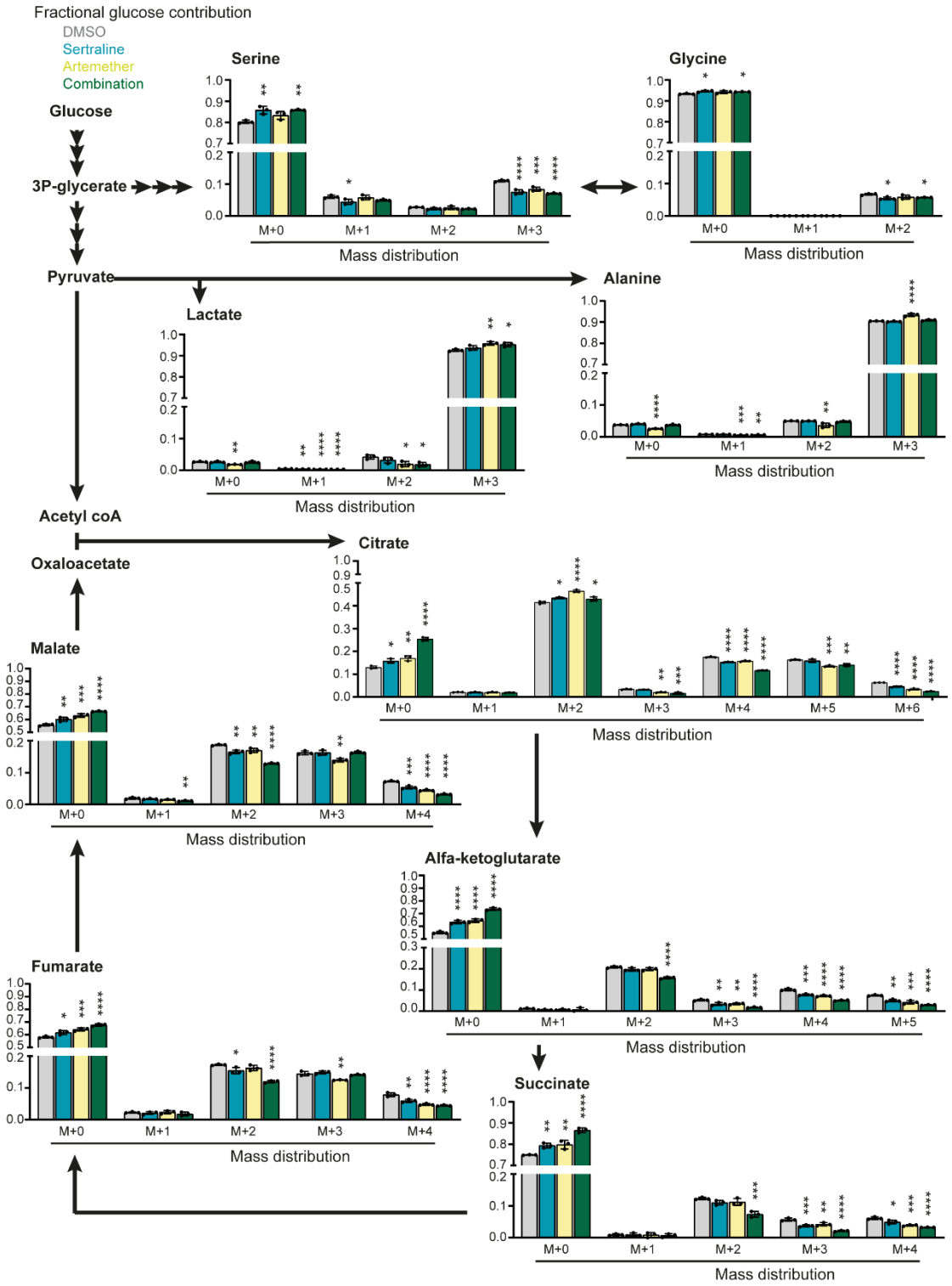

Figure S9. The artemether-sertraline combination further metabolically disrupts cancer cells. Carbon incorporation from $^{13}C_6$-glucose into downstream metabolites showing that both

compounds work together in reducing the proliferation of MDA-MB-468 by decreasing both the amount of labeled TCA cycle metabolites and the flux through serine/glycine synthesis. The effect on serine/glycine synthesis is due to sertraline rather than artemether (blue = sertraline, yellow = artemether, green = combination) (n = 3, Two-way ANOVA, Dunnett's multiple comparisons test). Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S10

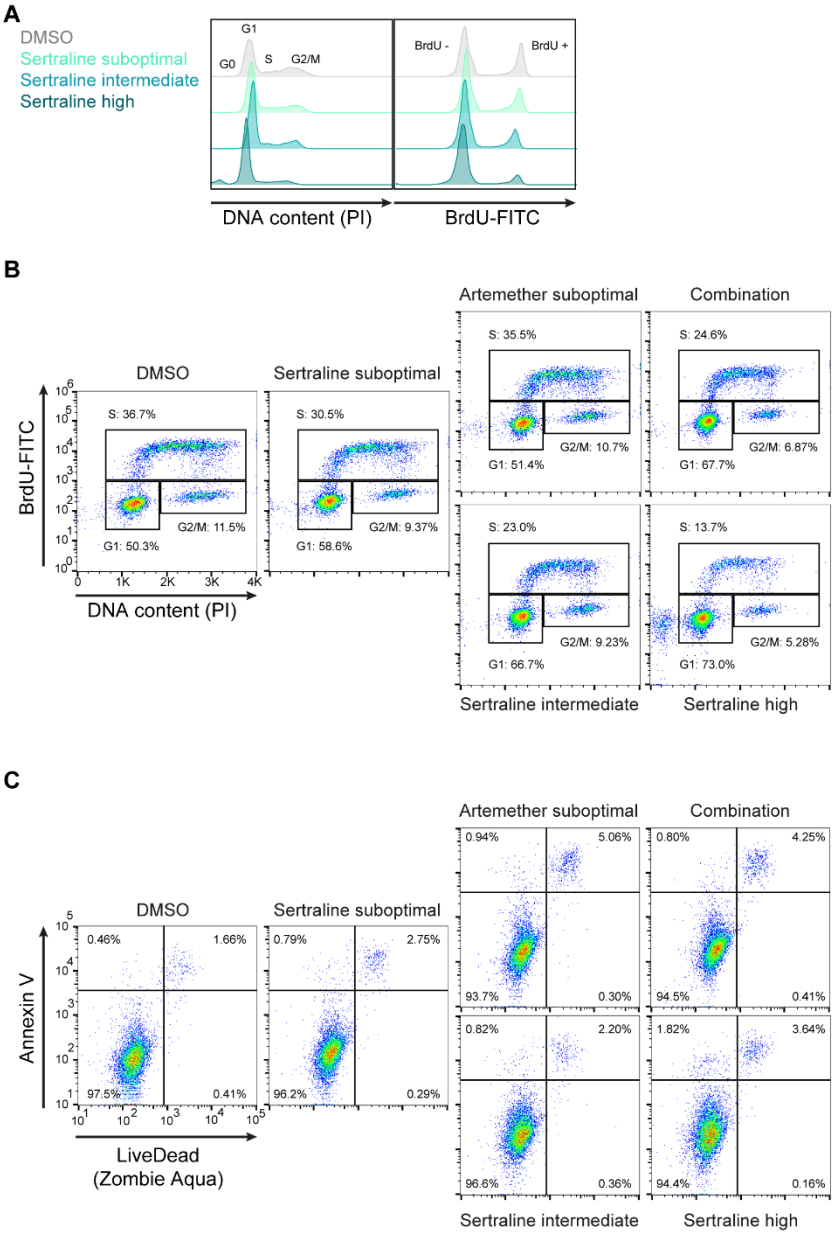

Figure S10. The sertraline-artemether combination causes G1-S cell cycle arrest. (A) Histograms showing PI cell cycle analysis (left) and BrdU incorporation (right) of MDA-MB-468 cells treated with DMSO or sertraline (sub-optimal = 5 µM; intermediate = 7.5 µM; high = 10 µM) for 24 hours. One representative result of three biological replicates is shown. **(B)** Representative FACS dot-plot for BRDU-PI cell cycle analysis of MDA-MB-468 cells treated with DMSO, sertraline (sub-optimal = 5 µM; intermediate = 7.5 µM; high = 10 µM) and/or artemether (80 µM) for 24 hours and incubated with BrdU for 1 hour. Cells were gated on viable cells, including the single cells. **(C)**

Representative FACS dot-plot for Annexin V (PE) – Zombie Aqua (eFluor506) cell viability staining of MDA-MB-468 cells treated with DMSO, sertraline (sub-optimal = 5 µM; intermediate = 7.5 µM; high = 10 µM) and/or artemether (80 µM) for 24 hours. Cells were gated on viable cells, including the single cells.

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