



1 **Supplementary Materials**



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3 Figure S1. Combined treatment with gamitrinib and HDAC inhibitors elicits synergistic reduction in 4 cellular proliferation of GBM cells. (A) T98G cells were treated with gamitrinib (GTPP) and

5 panobinostat or the combination of both for 72h. Thereafter, cellular viability and statistical analysis

- 7 treatment with vehicle, panobinostat, gamitrinib or the combination for 72h in the indicated GBM
- 8 cells (n=3); (C) U251 and GBM14 cells were treated with gamitrinib and romidepsin for 72h.
- 9 Thereafter, cellular viability and statistical analysis were performed. Isobolograms are shown; (D)
- The graphs show cellular viability data following treatment with vehicle, romidepsin, gamitrinib orthe combination for 72h in the indicated GBM cells (n=4). Shown are means and SD. ANOVA was
- 12 used for statistical analysis. ****p<0.001.



14 Figure S2. Combined inhibition of TRAP1 and HDACs enhanced cell death in GBM cells. (A) U87

- 15 and LN229 cells were treated with the indicated concentration of gamitrinib, panobinostat, or the
- 16 combination of both for 48h. Thereafter, cells were labeled with annexin/propidium iodide (PI) dye
- 17 and analyzed by multi-parametric flow cytometry. Shown are representative flow plots; (B) The
- 18 graphs show apoptotic cells of gamitrinib, panobinostat, or the combination of both in U87, LN229,
- 19 and T98G (n=3). Shown are means and SD. ANOVA was used for statistical analysis. ****p<0.001.



Figure S3. Combined inhibition of TRAP1 and HDACs enhanced cell death in GBM cells. (A) The graphs show sub G1/G0 of U87 and LN229 cells treated with the indicated concentrations of gamitrinib and panobinostat (n=3); (B) U251 and GBM43 cells were treated with the indicated concentration of gamitrinib, panobinostat or combination of both for 48h. Thereafter, cells were labeled with propidium iodide (PI) dye and analyzed by flow cytometry. Shown are representative flow plots; (C) The graphs show sub G1/G0 of U251 and GBM43 cells treated with the indicated

- 27 concentrations of gamitrinib and panobinostat/romidepsin (n=3); (D) The graphs show sub G1/G0 of
- 28 U87 and LN229 cells were treated with the indicated concentration of gamitrinib, romidepsin, or
- 29 combination of both (n=3); (E) U251 and GBM43 cells were treated with the indicated concentration
- 30 of gamitrinib, romidepsin, or combination of both for 48h. Thereafter, cells were labeled with
- propidium iodide (PI) dye and analyzed by flow cytometry. Shown are representative flow plots; (F)
 The graph shows sub G1/G0 of GBM43 cells treated with the indicated concentrations of gamitrinib,
- 33 romidepsin, or combination of both (n=3). Shown are means and SD. ANOVA was used for statistical
- 34 analysis. ***/****p<0.001.



Figure S4. Combined inhibition of TRAP1 and HDACs enhanced activation of a cell death with apoptotic features, including cleavage of caspases. (A, B) U87, LN229, and T98G cells were treated with the indicated concentration of gamitrinib, panobinostat, or combination of both. Thereafter, cells were labeled with TMRE dye and analyzed by flow cytometry (n=3). Shown are means and SD. ANOVA was used for statistical analysis; (C, D) Standard western blots of cell lysates of U251 treated with gamitrinib, panobinostat/romidepsin, or the combination of both for 24h and 48h. Actin is used

- 42 as a loading control. FL: full length, CF: cleaved fragment; (E) The graphs show sub G1/G0 of U87
- 43 and T98G cells treated with the indicated concentrations of gamitrinib and panobinostat in the
- 44 presence or absence of zVAD (n=3); (**F**, **G**) U87 cells were transfected with non-targeting (siNT) or
- 45 TRAP1 specific siRNA (siTRAP1) and following 48h treated with panobinostat for 24h. Thereafter,
- 46 cells were labeled with TMRE dye and analyzed by flow cytometry (n=3). Shown are means and SD.
- 47 Statistical significance was determined by two-tailed Student's t-test. **p<0.01, ****p<0.001.



Figure S5. The combination treatment of gamitrinib and HDAC inhibitors modulates the expression
of the Bcl-2 family of proteins in GBM cells. (A) The graph shows the Noxa/Mcl1 ratio in U87, T98G,

51 and U251 cells treated with gamitrinib, panobinostat, or the combination of both; (**B**) T98G cells were

52 treated with gamitrinib, panobinostat, or the combination for 7h and 24h. The whole cell lysates were

53 subjected to protein capillary electrophoresis. Vinculin serves as loading control; (C) The graph

54 shows the Noxa/Mcl1 ratio in T98G cells treated with gamitrinib, panobinostat, or the combination

Cells 2020, 9, x FOR PEER REVIEW

of both; (**D**, **E**) Standard western blots of cell lysates of U251 treated with gamitrinib, panobinostat/romidepsin, or the combination of both for 24h and 48h. Actin is used as a loading control; (**F**) The graph shows the Noxa/Mcl1 ratio in GBM14, GBM43, and U251 cells treated with gamitrinib, romidepsin, or the combination of both.



59

60 Figure S6. The combination treatment of gamitrinib and HDAC inhibitors interferes with GBM cell

61 OXPHOS (oxidative phosphorylation) complexes. (A) The graphs show the expression of OXPHOS

- 62 complex in U87 and LN229 cells treated with with gamitrinib, panobinostat, or the combination of
- 63 both for 24h (n=2); (**B**, **C**) U87 and LN229 cells were treated with gamitrinib, panobinostat, or the
- 64 combination for 24h. The whole cell lysates were subjected to protein capillary electrophoresis.
- 65 Vinculin serves as loading control (n=3). Shown are means and SD (fold change (FC) over control).



- 67 Figure S7. Combined treatment with gamitrinib (GTPP) and HDAC inhibitor, panobinostat, elicits
- no detectable organ toxicity in vivo. (A, B) GBM12 and GBM43 cells were implanted into the subcutis

- 69 of immunocompromised Nu/Nu mice. After the tumors were established, randomization was
- 70 assigned to four treatment groups: vehicle, gamitrinib (3 mg/kg), panobinostat (5 mg/kg), and
- 71 combination treatment of both. Shown are the tumor of four treatment groups on the last day; (C)
- 72 Shown is the H&E staining of the vehicle and gamitrinib and panobinostat combination treatment in
- 73 different organelle of GBM12 cells in (A).
- 74

75 Uncropped blot and capillary electrophoresis images

Figure 2D

U87



Figure 2D



T98G

Figure 2G





Figure 3A



U87

Figure 3A







12

GTPP (µM) 0 3 0 3 Pb (µM) 0 0 0.2 0.2

1 0.67 0.66 0.58

230

180

116

66

Mcl-1

1 0.47 0.58 0.65

GTPP (µM) 0 3 0 3 Pb (µM) 0 0 0.2 0.2

	230- 180-
Vinculin	116
	66-
	40
	10

Figure 3B

GBM14



Figure 3B







U87, 7h



U87, 24h



T98G, 7h



T98G, 24h

Figure 5D

U87



Figure 5D

LN229



Figure 5E



Figure S4C

U251, 24h



Figure S4C



U251, 48h

GTPP (µM) 0 3 0 3 GTPP (µM) 0 3 0 3 Pb (nM) 0 0 50 50 Actin 42-1 2.09 3.5 18



Figure S4D



U251, 24h

Figure S4D



U251, 48h

Figure S5B



1 0.76 1.2 0.49

12-

Noxa

12

T98G, 7h

Figure S5B



T98G, 24h

1 1.25 1.11 0.4

12-

Noxa

12

Figure S5D





GTPP (µM) 0 3 0 3 Pb (nM) 0 0 50 50



GTPP (µM) 0 3 0 3 Pb (nM) 0 0 50 50



GTPP (µM) 0 3 0 3 Pb (nM) 0 0 50 50

GTPP (µM) 0 3 0 3 Pb (nM) 0 0 50 50



Figure S4D



U251, 48h

Figure S5E

U251, 24h



Figure S5E



U251, 48h

Figure S6B





U87

37 of 38

Figure S6B



LN229