cancers

Supplementary Materials: Efficacy of HDAC Inhibitors Belinostat and Panobinostat against Cisplatin-Sensitive and Cisplatin-Resistant Testicular Germ Cell Tumors

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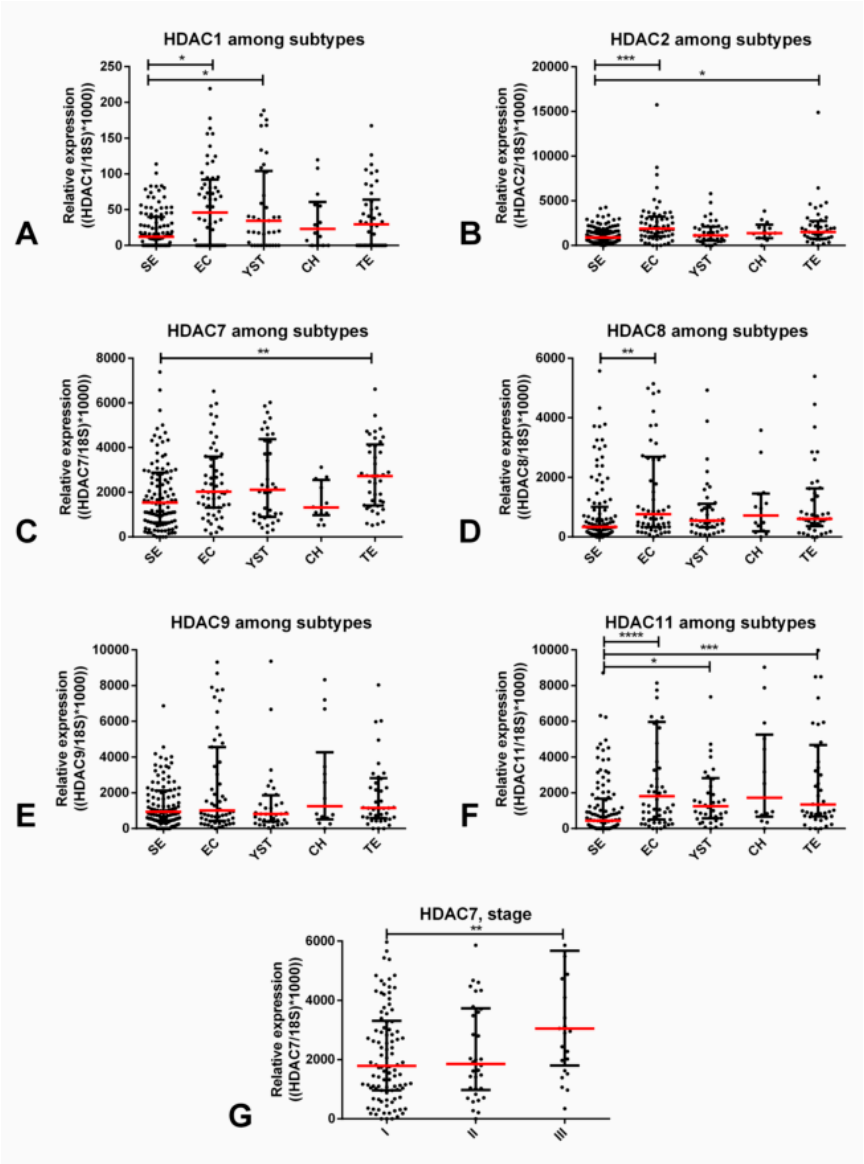
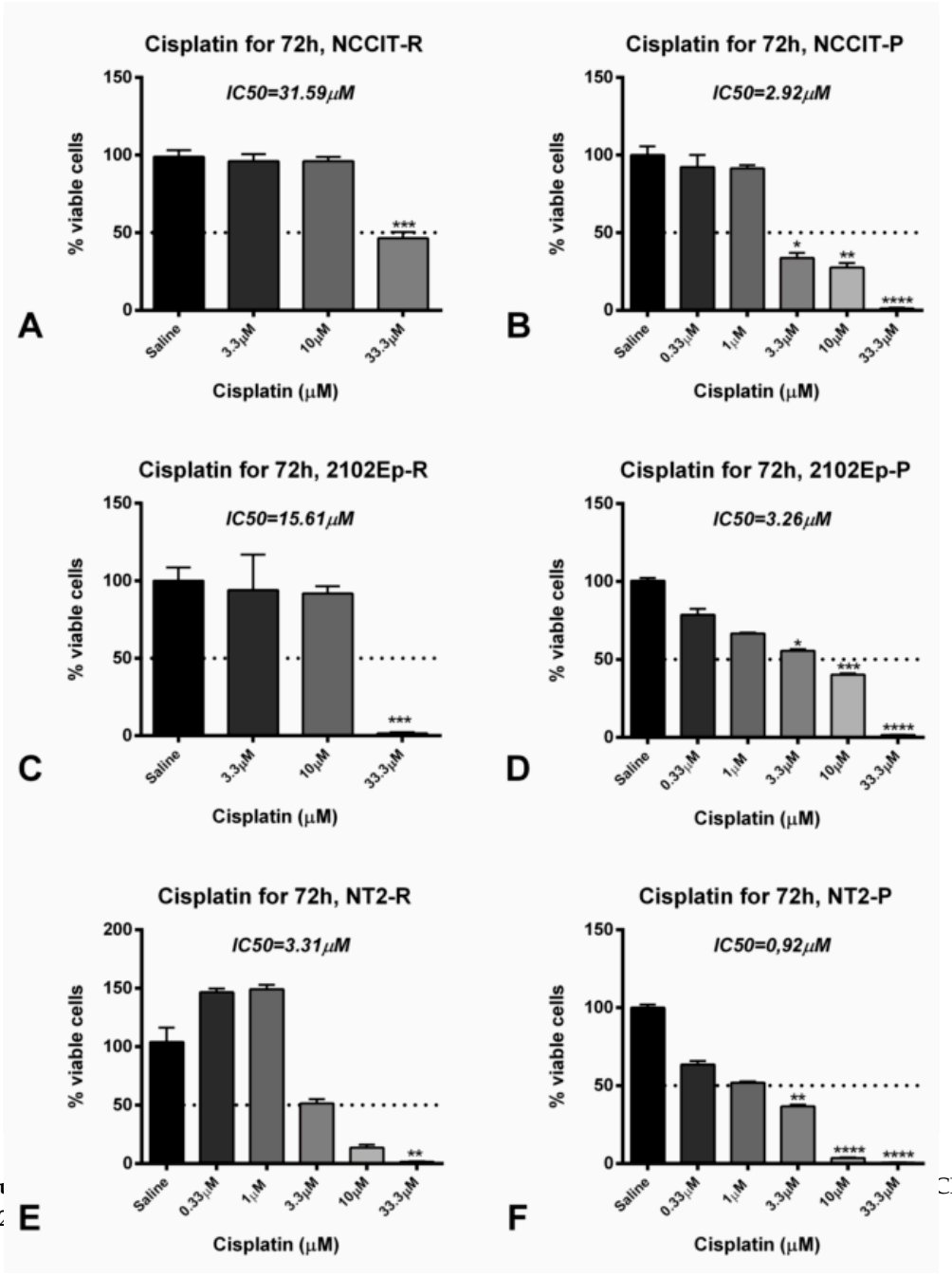


Figure S1. Transcript levels of several HDACs across individual TGCT subtypes, assessed in our patient cohort. (**A**) HDAC1, (**B**) HDAC2, (**C**) HDAC7, (**D**) HDAC8, (**E**) HDAC9, (**F**) HDAC11. (**G**) Differential expression of HDAC7 across disease stage. Bars and red dashes represent median and interquartile range. Abbreviations: HDAC—histone deacetylase; SE—seminoma; YST—yolk sac tumor; CH—choriocarcinoma; TE—teratoma; EC—embryonal carcinoma. .*?; **?; ***?, ****?



Figu CIT, NT2

Figure S3. Demonstration of cisplatin resistance and sensitivity in the cell lines used in the study. Cell viability after 72 h of exposure to cisplatin in NCCIT-R (**A**), NCCIT-P (**B**), 2102Ep-R (**C**), 2102Ep-P (**D**), NT2-R (**E**), and NT2-P (**F**). .*?; **?; ***?, ****?

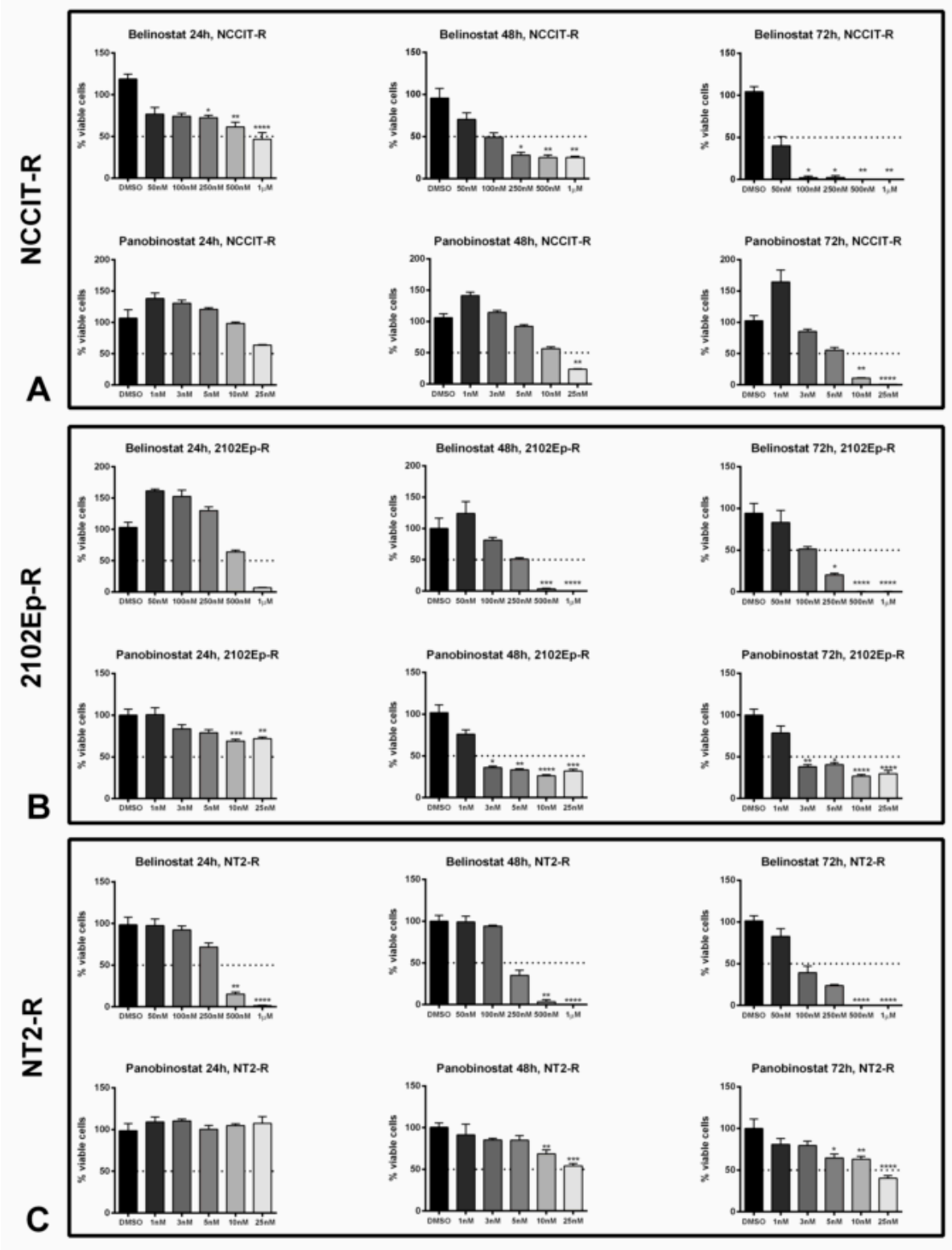


Figure S4. Barplots with cell viability studies after treatment with several doses of belinostat and panobinostat, per time point (24, 48, and 72 h), across cisplatin-resistant cell lines. (**A**) NCCIT-R; (**B**) 2102Ep-R; (**C**) NT2-R. .*?; **?; ***?, ****?

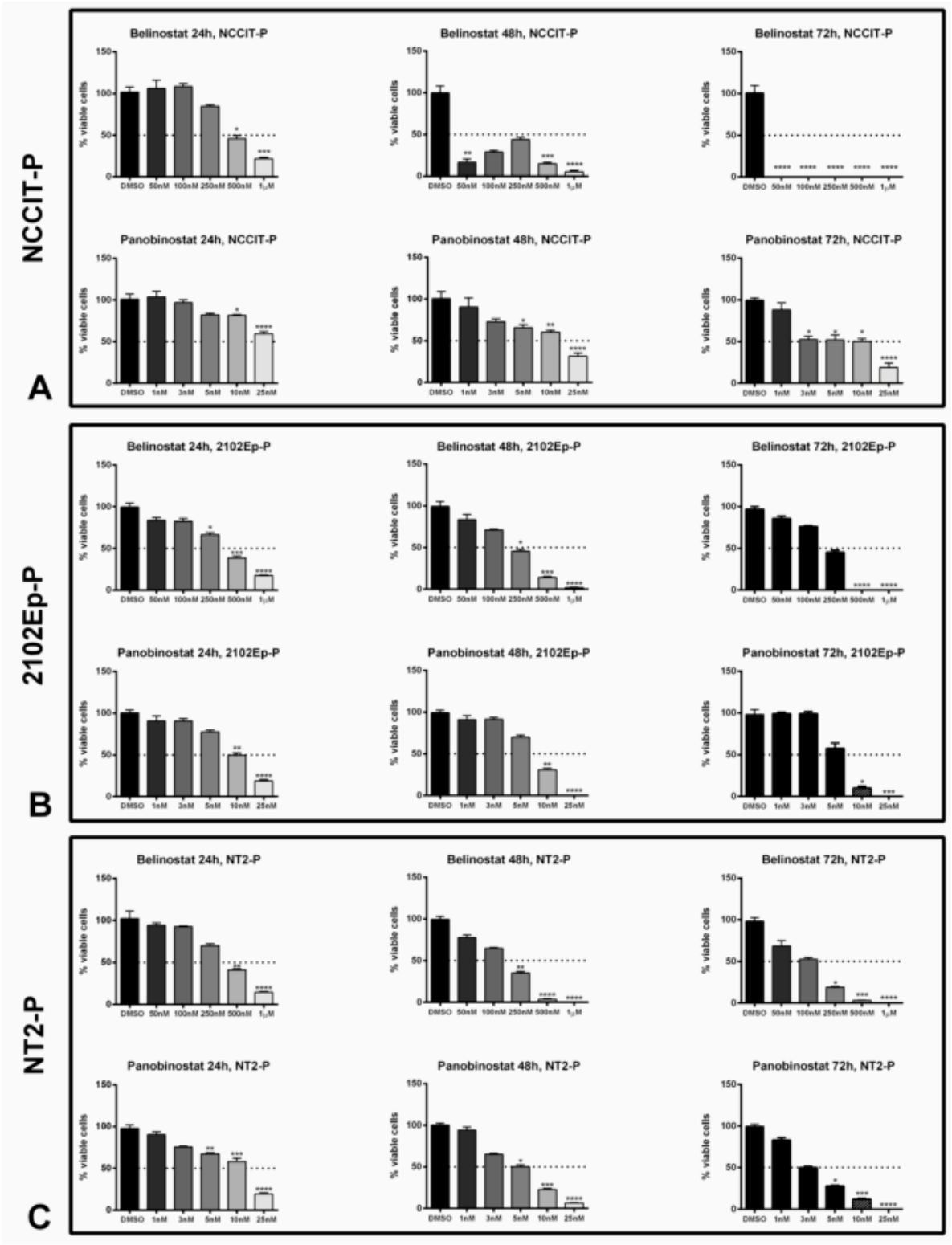


Figure S5. Barplots with cell viability studies after treatment with several doses of belinostat and panobinostat, per time point (24, 48, and 72 h), across cisplatin-sensitive cell lines. (**A**) NCCIT-P; (**B**) 2102Ep-P; (**C**) NT2-P. .*?; **?; ***?, ****?

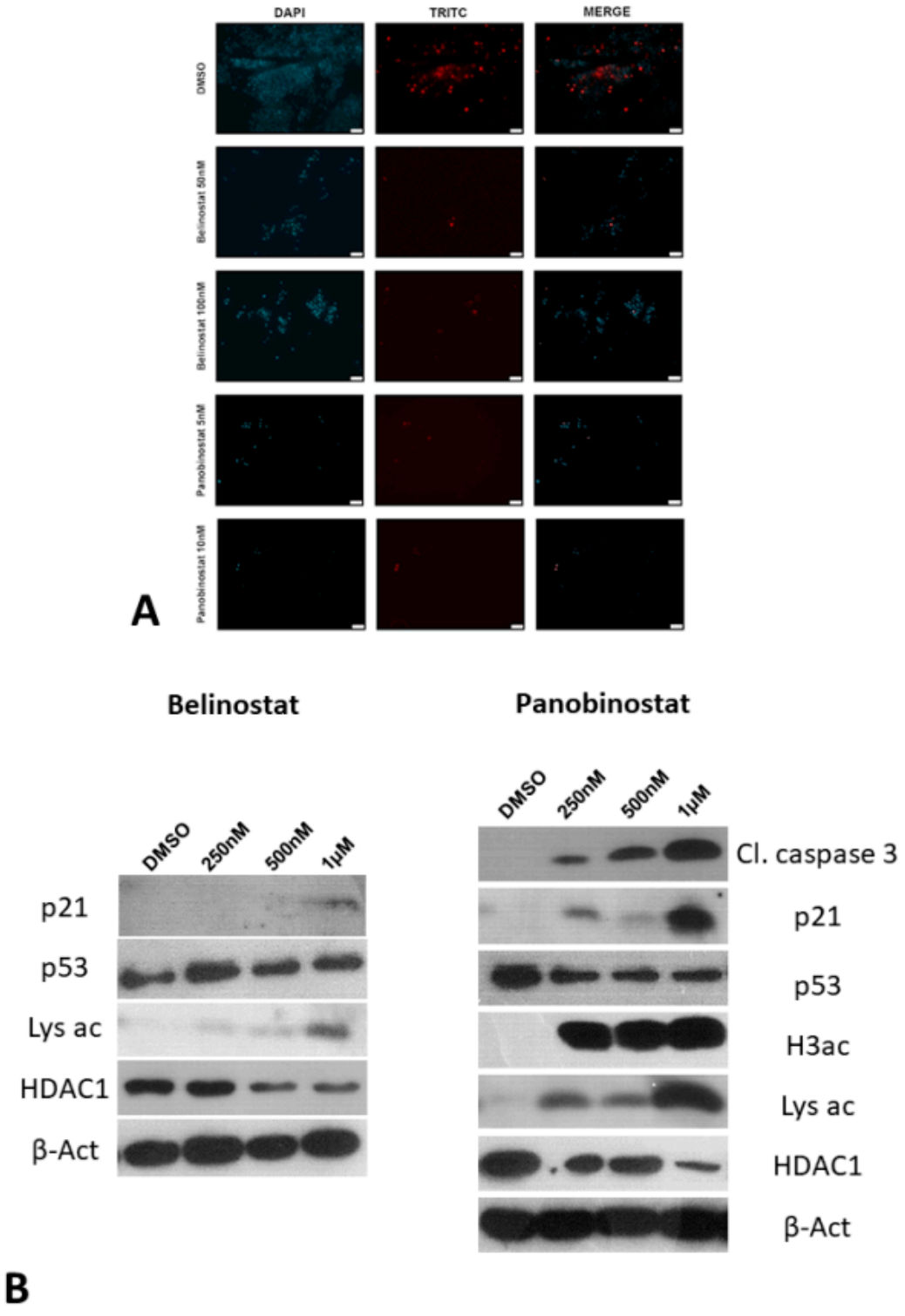


Figure S6. Effect of treatment of the NCCIT-R cell line with belinostat and panobinostat on cell cycle, apoptosis, and acetylation. (**A**) Effect of treatment with belinostat (50 and 100 nM) and with panobinostat (5 and 10 nM) for 72 h on Ki67 staining index; (**B**) Western blot validation of specific targets related to cell cycle (p21, p53), apoptosis (cleaved caspase 3) and acetylation (lysine acetylation, histone H3 acetylation, HDAC1) after treatment for 24 h with belinostat and panobinostat. Experiments were performed in triplicates. Beta-actin is presented as normalizer. scale bars??

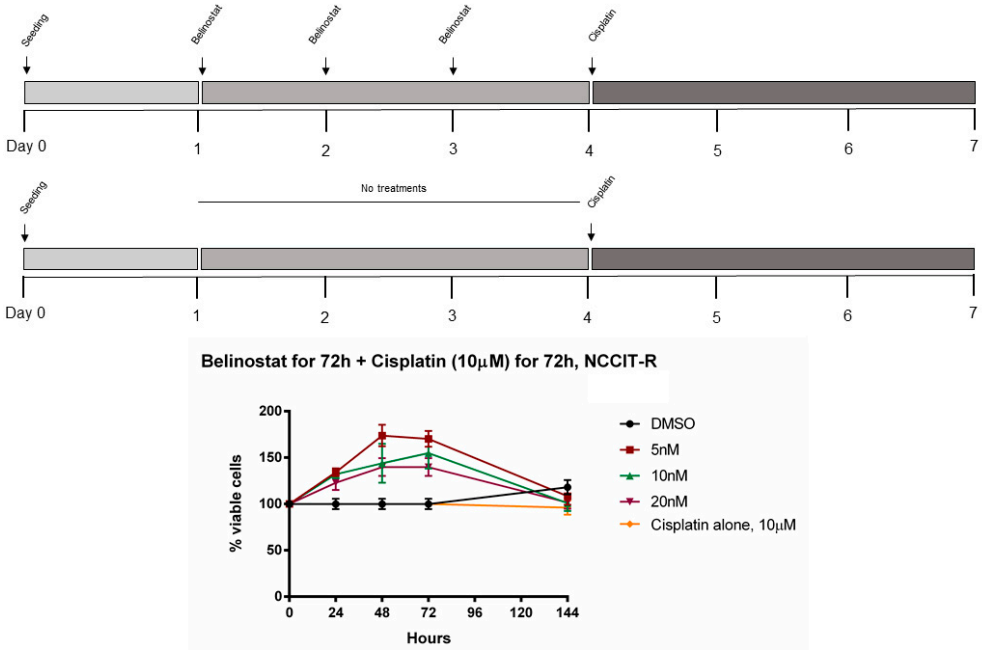


Figure S7. Effect of pre-treatment with non-toxic low nanomolar concentrations of belinostat on sensitivity to cisplatin. (**A**) Timeline of the experiment, with daily belinostat treatments for three days vs absence of treatment, followed by exposure to cisplatin 10 μM for 72 h; (**B**) Respective viability curves across the time of the experiment. Abbreviations: DMSO—dimethyl sulfoxide.

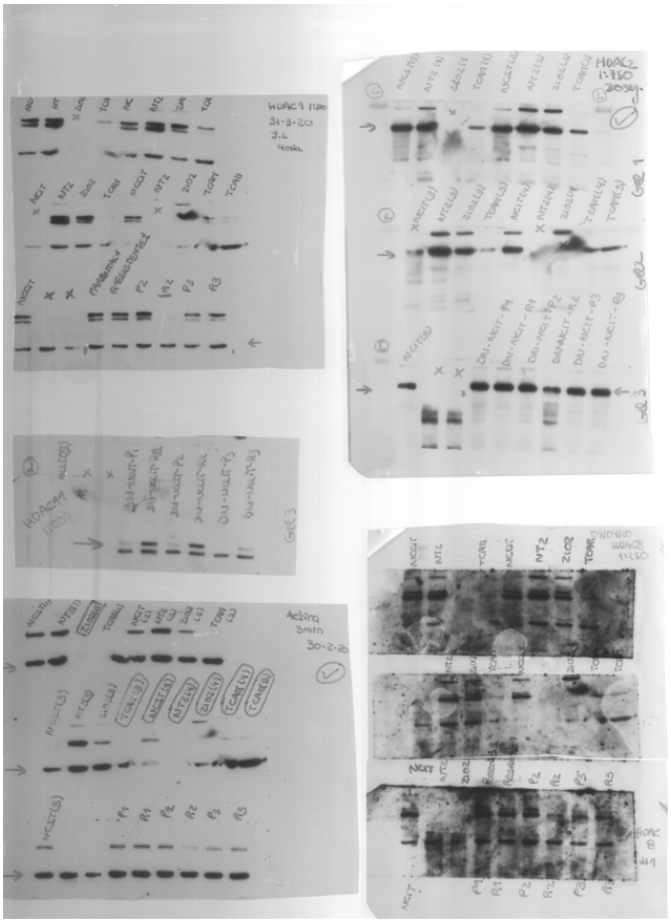


Figure S8

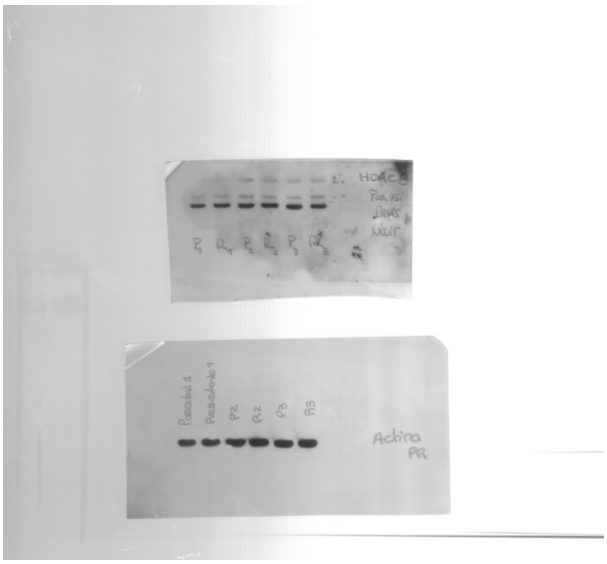


Figure S9

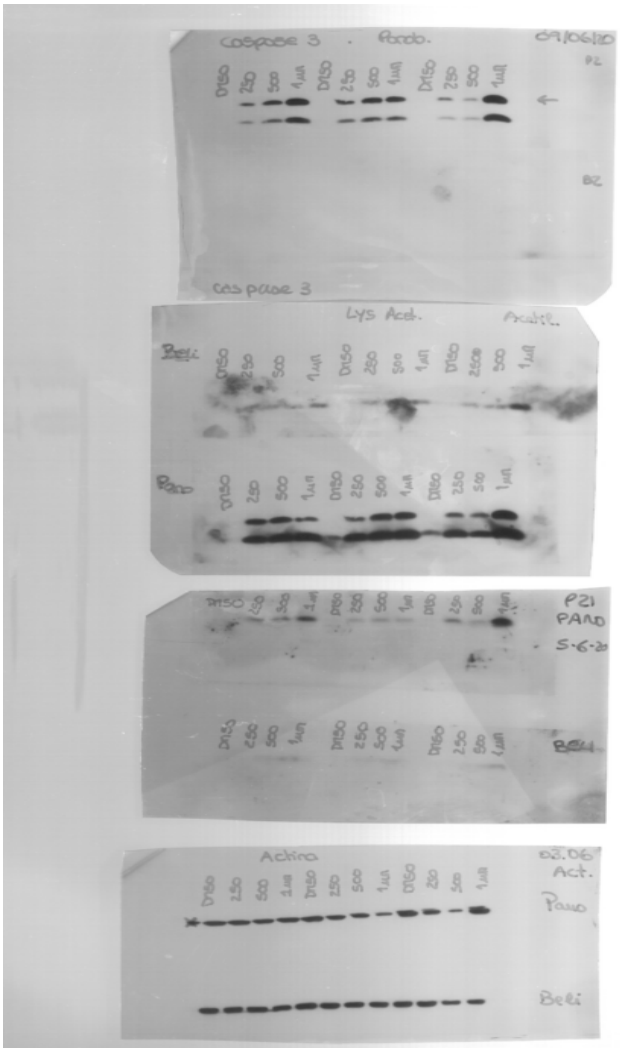


Figure S10

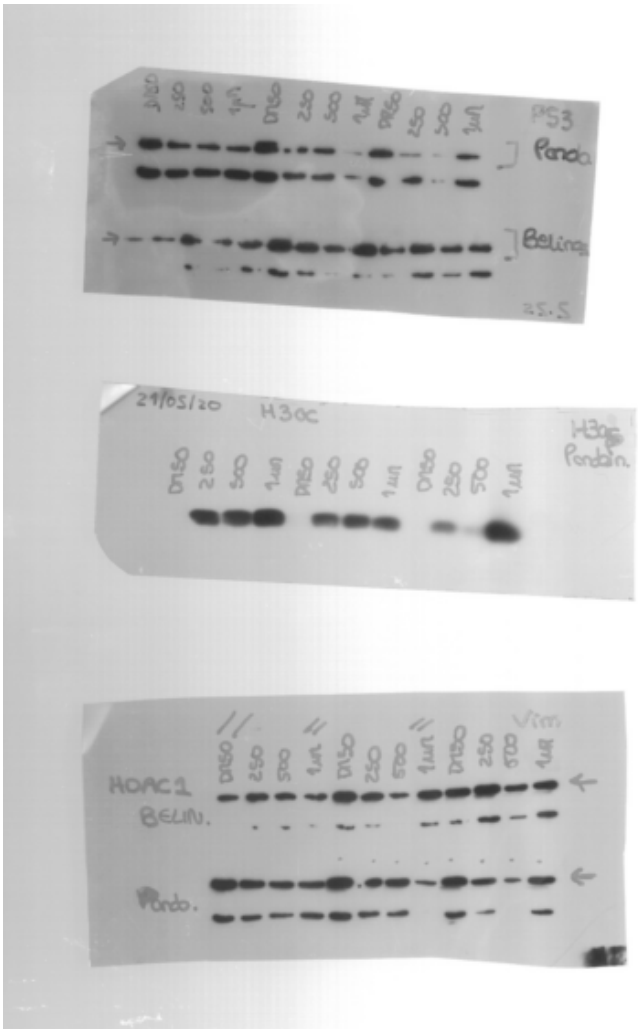


Figure S11

Table S1. Clinicopathological features of the study cohort.

Variables	Primary TGCT Cases (*n*, %)
Histologic subtypes – TGCT patients (*n*, %)	
Pure seminoma	84/161 (52.2)
Pure embryonal carcinoma	11/161 (6.8)
Pure postpubertal-type teratoma	4/161 (2.5)
Mixed tumor	62/161 (38.5)
Histological subtypes – individual components (*n*, %)	
Seminoma	109/261 (41.8)
Embryonal carcinoma	56/261 (21.5)
Postpubertal-type yolk sac tumor	38/261 (14.5)
Choriocarcinoma	15/261 (5.7)
Postpubertal-type teratoma	43/261 (16.5)
Stage (*n*, %)	
I	102/161 (63.4)
II	34/161 (21.1)
III	25/161 (15.5)
IGCCCG Prognostic Group, for metastatic patients (*n*, %)	
Good	45/59 (73.8)
Intermediate	8/59 (14.3)
Poor	6/59 (11.9)
Variables	Metastatic cases (*n*, %)

Histologic subtypes (*n*, %)	
Seminoma	1/14 (7.1)
Embryonal carcinoma	3/14 (21.5)
Yolk sac tumor	2/14 (14.3)
Teratoma	8/14 (57.1)

Abbreviations: TGCT–testicular germ cell tumors; IGCCCG–International Germ Cell Cancer Collaborative Group.

Table S2. Antibodies used in the study.

Antibody	Clone/Ref, Species	Vendor	Dilution
HDAC1	5C11, mouse	Sigma-Aldrich	1:500
HDAC2	HDAC2-62, mouse	Sigma-Aldrich	1:750
HDAC8	2F4, mouse	Novus-Biologicals	1:250
HDAC11	D5I8E, rabbit	Cell Signaling	1:500
Acetylated lysine	#9441, rabbit	Cell Signaling	1:500
H3ac	Polyclonal, rabbit	Merk, millipore	1:1000
p53	OP43, mouse	Oncogene science	1:1000
p21	SX118, mouse	Pharmingen	1:250
Ki67	MIB-1, mouse	DAKO	1:200
Cleaved caspase 3	Polyclonal, rabbit	Abcam	1:500
β-actin	AC-15, mouse	Sigma-Aldrich	1:10000